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a. REPORT UU	b. ABSTRACT UU	c. THIS PAGE UU			19b. TELEPHONE NUMBER 213-821-5720

RPPR Final Report

as of 05-Mar-2019

Agency Code:

Proposal Number: 70347EGDRP

Agreement Number: W911NF-17-1-0076

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Report Date: 16-Apr-2018

Date Received: 21-Jan-2019

Final Report for Period Beginning 17-Jan-2017 and Ending 16-Jan-2019

Title: Decoding the Principles of Emergence and Resiliency in Biological Collective Systems - A Multi-scale Approach

Begin Performance Period: 17-Jan-2017

End Performance Period: 16-Jun-2019

Report Term: 0-Other

Submitted By: Paul Bogdan

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STEM Degrees: 0

STEM Participants: 5

Major Goals: Microbial communities are known to display complex, collective behaviors. However, the underlying principles as to how these behaviors arise despite uncertainty in molecular and cellular components of the system remains unclear. Consequently, we examine the robustness of collective behaviors in microbial communities, using pattern formation of wild coliform bacteria as a model system. Many coliform bacteria naturally form complex dynamic patterns that arise from the combination of chemotaxis, nutrient degradation, and the exchange of amino acids between cells. Using both quantitative experimental methods and several theoretical frameworks, we dissect bacterial pattern formation at multiple scales, from the molecules to individual cells to self-organizing populations. By comparing pattern formation from multiple wild isolates, we attempt to identify universal principles that govern robust, collective behaviors in biological systems.

Towards this end, we adopt a multiscale approach combining experimental and theoretical approaches for the following research goals:

- (1) We develop a mathematical framework for characterizing and classifying the irregularity in microbial pattern formation and validate it against experimental measurements.
- (2) We determine the variability of protein copy numbers in living cells and develop a computational framework for measuring and predicting how noise in cellular components affects the overall system-level behavior.
- (3) In measurements of individual cells, we analyze behaviors such as chemotactic response, signaling potential, and swimming speed to predict how single-cell heterogeneity contributes to complex, collective behavior.
- (4) We develop a mathematical and experimental framework for identifying the single-cell functional states and quantify the cell-to-cell communication that lead to complex pattern formation. We define an information theoretic inspired framework for measuring how cell processing and cell-cell communication contribute to the degree of emergence, self-organization and robustness.
- (5) We propose a combined mathematical and experimental framework for investigating the robustness of pattern formation when two populations of pattern forming bacteria coexist in the same space.

This project combines experimental tools including the tools of synthetic biology, fluorescence and brightfield microscopy at multiple length and time scales, and microfluidic functional assays of single-cell behavior with theoretical tools including agent-based models, non-equilibrium master equations, nonparametric statistics, systems of coupled partial differential equations, and novel analytical methods to predict and control the behavior of collective systems.

In this performance period, we did not modify or made changes in the approach or methods.

Accomplishments: In this performance period (January 1st, 2017 – January 16th, 2019), the major activities and specific objectives accomplished are as follows:

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1. We developed a mathematical framework to characterize the emergence and self-organization of microbial communities from sparse spatio-temporal time-lapse imaging data. From microbial communities to cancer cells, many such complex collectives are said to possess emergent and self-organizing behavior. However, we lack a universal and rigorous mathematical framework to quantify the degree of emergence and self-organization of biological swarms especially when considering that only sparse spatiotemporal macroscopic data is available due to technological and scientific challenges. To overcome these challenges (e.g., spatiotemporal chemoattractant concentrations, cell swimming trajectories), we propose a multi-fractal inspired framework for quantifying the degree of emergence and self-organization from time-lapse imaging with low resolution in time (several minutes between two images) and space (tens of micrometers). Emergence describes the rate of change of the probability distribution characterizing the aggregation process and can be used to detect if the emergent phenomena moves the community into a state of low energy, the case of *Enterobacter cloacae*, or maximum energy. We also establish mathematical connections between the proposed emergency metric and the free energy concept in statistical physics. The self-organization metric we define shows the degree of similarity (order) between different regions of the environment and the synchronization of the location of new aggregates with the previous ones. More precisely, as the experiment advances, the new *Enterobacter cloacae* aggregates align their location with one of the old aggregates across all the regions of the petri dish suggesting an increase in self-organization. The benefits of this pioneering quantification strategies of emergence and self-organization consist of being able to discern when complex collectives (collective systems) display an intelligent behavior, categorize their swarming behavior with reference systems, compare and contrast them for the purpose of selecting the optimal swarms or for optimizing intelligent autonomous swarms.

2. We isolated and characterized 11 wild strains of bacteria capable of forming complex, dynamic patterns. For each strain, time-lapse revealed emergent patterns in the form of swarm rings and spots that appear due to the coordinated movement of large populations of cells. We characterized the differences in these strains through whole genome sequencing and measurements of motility and chemotaxis at the single-cell level. Genome sequencing revealed little variation in the presence and absence of the approximately 50 genes associated with microbial pattern formation, despite striking differences in the emergent patterns formed by each strain. Analysis of the single-cell swimming trajectories revealed heterogeneity in motility characteristics, including the distribution of swimming speeds, tumbling frequencies, and turning angle preferences. Surprisingly no one single-cell characteristic was correlated with variability in emergent properties such as the velocity of the swarm ring or spot distributions and sizes. These results indicate that the emergent properties of the system are not strongly determined by a single characteristic of individual agents in the system, but instead many parameters of individual agents collectively contribute to variability observed in population-level collective behavior. We have begun to develop modeling approaches to further examine the interplay between single-cell motility and chemotactic behavior and macroscale collective motion.

3. Our latest experiments have examined spot formation within very large populations of cells over distances of 20 cm. These experiments revealed variability in behavior both at the single-cell level (cells switch between a motile and non-motile state) and at the macroscale-level (some spots migrate several millimeters across the plate and merge with other spots). This merging behavior of spots appears to be unique to the species of bacteria we have isolated, and should serve as a new model system for collective migration of cells and biological active matter. We have been developing models that account for such stochasticity at both length scales to establish the mechanisms for spot nucleation, spot movement, and spot merging. In addition, we are analyzing whether spot migration may be optimizing the distribution of cells over the entire plate. This aspect of the project will extend beyond the project period, but should result in an additional publication towards the end of 2019.

4. Interfacing complex collective biological systems with cyber platforms and engineering their dynamics and behavior is an open grand challenge. One such complex collective systems is represented by human brain for which developing brain machine interfaces will allow to harvest information from the (physical) brain through sensing mechanisms, extract information about the underlying processes, and decide/actuate accordingly to guide and control complex engineered systems such as swarms of aerial and ground vehicles. Nonetheless, the brain interfaces are still in their infancy, but reaching to their maturity quickly as several initiatives are released to push forward their development (e.g., Neuralink by Elon Musk and 'typing-by-brain' by Facebook). State-of-the-art EEG-based non-invasive brain interfaces entail a highly skilled neuro-functional approach and evidence-based a priori knowledge about specific signal features and their interpretation from a neuro-physiological point of view. By building on mathematical concepts developed in this project, we propose new models that equip us with a fractal dynamical characterization of the brain processes. The model parameters can be seen as explainable from a system's perspective and used for classification using machine learning algorithms and/or actuation laws obtained via control system's theory. We evaluated our approach on real EEG-datasets to assess and validate the proposed methodology. The classification accuracies are high even with few training samples. This mathematical model was exploited to detect and predict the time point of contacting a viral infection from only 200 samples of physiological processes with a prediction accuracy of approximately 95%.

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Training Opportunities: This grant supported the training and professional development of the following students:

1. One graduate student, Valeriu Balaban from PI Bogdan's group working on developing mathematical approaches and codes, testing the proposed mathematical and algorithmic tools on synthetic and experimental case studies in collaboration with PI Boedicker's group, and summarizing our research results in several technical reports and a published Nature Scientific Reports manuscript. Valeriu Balaban has learned not only basic concepts of computer vision for processing and preparing the time lapse imaging data of microbial communities for the subsequent mathematical analysis, but also gained a solid background in fractal theory, statistical physics, information theory, nonlinear dynamics, and statistical machine learning. Working in close collaboration with PI Boedicker's group also offered unique opportunities for learning not only theoretical concepts related with synthetic and system biology, but also to test his background thanks to this project. Valeriu Balaban was in part supported by the USC fellowship.
2. Another graduate student, Xiaokan Guo from PI Boedicker's group was supported by this grant. Xiaokan has recently graduated with his PhD and is now working at a Biotech/Pharmaceutical company in China. As part of this project he developed new research skills to experimentally measure the chemotactic behavior and motility of individual bacterial cells. These experiments also enabled him to sharpen his image analysis skills, as he adapted and developed image analysis algorithms to extract single-cell parameters from movies of cell motility. He has also benefitted from interactions with the Bogdan group, introducing him to several new analytical tools and new perspectives in biophysics.
3. Another graduate student, Gaurav Gupta from PI Bogdan's group contributed to our project research discussions, developed numerous mathematical derivations in order to determine the right mathematical expressions for quantifying the degree of emergence and self-organization in microbial communities, and extended a number of mathematical ideas into new models for characterizing, modeling and analyzing the dynamics of complex collective biological systems such as the brain-in-action. Thanks to this unique project, Gaurav Gupta has learned concepts from statistical physics (free energy, entropy), fractal theory, and fractional calculus and was able to make a number of novel contributions to the field of system identification and machine learning under unknown unknowns. Gaurav Gupta was supported by this grant and by DARPA Young Faculty Award of PI Bogdan. The mathematical approaches and algorithms we developed in this project will be applied, tested and evaluated in a number of DoD problems such as the viral prediction and gene expression dynamics modeling in the DeepPurple biochronicity program.
4. This grant also offered the opportunity to train another PhD student, Panagiotis Kyriakis. Panagiotis has learned about multi-fractal analysis and statistical machine learning while developing and adapting the theory we constructed in this project to analyze MRI data and quantify the degree of mild cognitive impairment and Alzheimer disease. He obtained a number of promising results and we are currently improving them.
5. This grant previously supported an undergraduate student, Sean Lim. Although his salary was not directly paid from this award, funds were used for experimental supplies. He worked closely with both the Bogdan group and Xiaokan Guo. As part of this project he has learned many new laboratory skills, including single-cell characterization, microscopy, and gene sequencing. He is now attending medical school.

PIs Bogdan and Boedicker interacted with the above mentioned students through one-on-one meetings as well as bi-weekly project meetings where we discussed the challenges we faced, proposed solutions and guidelines and planned our research. We have maintained a Google doc and an overleaf account to summarize our research progress and worked collaboratively on one published manuscript on quantifying the emergence and self-organization of microbial communities. Other manuscripts are under preparation. We are currently planning our research activity to summarize our new research results and plan collaborative submissions in Spring 2019.

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Results Dissemination: During January 2017 – January 2019, the research results and accomplishments in this grant have been disseminated as follows:

1. PI Bogdan was invited and gave a Seminar Lecture entitled “Analytical Tools for Cyber-Physical Systems: Lessons Learned from Complex (Biological) Systems” in the Decision and Control Laboratory (DCL) at Georgia Tech, on November 10th, 2017.
2. PI Bogdan was invited and gave a Seminar Lecture entitled “Decoding the Laws of Emergence and Self-Organization in Complex Collectives: Lessons Learned from Biological Systems,” in the Department of Electrical and Computer Engineering (ECE) Seminar, Carnegie Mellon University, on February 1st, 2018.
3. PI Bogdan was invited and gave a Seminar Lecture entitled “Decoding the Laws of Emergence and Self-Organization in Complex Collectives: Lessons Learned from Biological Systems,” in the Department of Computational and Molecular Biology (DCMB) Seminar, University of Michigan, September 12th, 2018
4. The research accomplishments on mining complex dynamics of collective biological systems such as the human brain were summarizing in a manuscript that was presented and published in the Proceedings of the International Conference on Cyber-Physical Systems (ICCPs) part of the CPS Week taking place in Porto, Portugal in April 2018. Additional research results on mining the hidden dimensions of biological systems such as the brain of rodents and humans were summarized in a new scholar article that was accepted and will be published in the Proceedings of the International Conference on Cyber-Physical Systems (ICCPs) taking place in Montreal, Canada, in April 2019.
5. Additional research presentations of this work were discussed during the organized DARPA YFA visits at Lawrence Livermore National Laboratory and USMA.
6. PI Boedicker gave an invited talk at the Marine Biological Lab at Woods Hole entitled “Signal exchange in bacterial communities”. Boedicker also gave an invited talk at the Society for Industrial Microbial and Biotechnology entitled “Engineering Bacteria to Synthesize Nanomaterials” that mentioned our work on large-scale bacterial pattern formation.

We summarize below the citations of accepted papers, where the above-mentioned research accomplishments were described (we only summarize the new publications since last submitted report):

- 1) Valeriu Balaban, Sean Lim, Gaurav Gupta, James Boedicker and Paul Bogdan, “Quantifying emergence and self-organisation of *Enterobacter cloacae* microbial communities,” *Nature Scientific Reports* 8, August 2018.
- 2) Hana Koorehdavoudi, Paul Bogdan, Guopeng Wei, Radu Marculescu, Jiang Zhuang, Rika Wright Carlsen, and Metin Sitti, “Multi-fractal characterization of bacterial swimming dynamics: a case study on real and simulated *Serratia marcescens*,” *Proceedings of the Royal Society of London A: Mathematical, Physical and Engineering Sciences*, July 2017.
- 3) G. Gupta, S. Pequito, and P. Bogdan, “Re-thinking EEG-based non-invasive brain interfaces - modeling and analysis” *Proceedings of the International Conference on Cyber-Physical Systems (ICCPs), CPS Week, Porto, Portugal, April 2018.*
- 4) Gaurav Gupta, Sergio Pequito and Paul Bogdan, “Dealing with Unknown Unknowns: Identification and Selection of Minimal Sensing for Fractional Dynamics with Unknown Inputs,” *Proceedings of the American Control Conference (ACC)*, June 2018.
- 5) Mahboobeh Ghorbani, Edmond Jonckheere and Paul Bogdan, “Gene Expression Is Not Random: Scaling, Long-Range Cross-Dependence, and Fractal Characteristics of Gene Regulatory Networks,” *Frontiers in Physiology*, vol. 9, October 2018.
- 6) Vasileios Tzoumas, Yuankun Xue, Sérgio Pequito, Paul Bogdan and George J. Pappas, “Selecting Sensors in Biological Fractional-Order Systems,” *IEEE Transactions on Control of Network Systems*, vol. 5, no. 2, pp. 709-721, June 2018.
- 7) Xian Li, Karthi Duraisamy, Paul Bogdan, Janardhan Rao Doppa and Partha Pratim Pande, “Scalable Network-on-Chip Architectures for Brain-Machine Interface Applications,” in *IEEE Transactions on Very Large Scale Integration (VLSI) Systems*, vol. 26, no. 10, pp. 1895-1907, Oct. 2018.
- 8) Ruochen Yang*, Gaurav Gupta* and Paul Bogdan, “Data-driven Perception of Neuron Point Process with Unknown Unknowns,” to appear in the *Proceedings of the International Conference on Cyber-Physical Systems (ICCPs)*, April 2019.

The PDF files of these publications are attached at the end of this report.

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Honors and Awards: 1) Boedicker has been awarded the NSF CAREER Award for work involving computation and signaling and microbial communities, a project that developed from work done as part of this DARPA award as well as Boedicker's DARPA YFA research.
2) Bogdan has been awarded the 2017 Defense Advanced Research Projects Agency (DARPA) Young Faculty Award for research activities that spur from this project and in particular for developing mathematical models, analysis and control algorithms for time varying complex networks. The research results have been significantly enriched by the interactions with the DoD researchers and program officers as their feedback and research questions made us think and come up with new solutions that could open new fields in observability of fractal processes or quantification of emergence, self-organization and complexity not only in biology but also in social and economic / financial sciences.
3) Bogdan has been awarded the Okawa Foundation Award in Information and Telecommunications, July 2017.
4) Bogdan has received the Engineers' Council Outstanding Engineering Merit Award for "fundamental contributions to the design of cyber-physical systems and design methodologies for manycore platforms", February 2018.
5) Bogdan has been elevated at the IEEE Senior Member level for his pioneering contributions to studying complex systems.
6) Bogdan has been awarded the IEEE Council on Electronic Design Automation (CEDA) Ernest S. Kuh Early Career Award for "outstanding contributions to defining and engineering autonomy in complex cyber-physical systems, and particularly for pioneering research on mathematical-based on-chip reconfigurable interconnect synthesis, self-optimizing, self-programming and autonomous computing systems", November 2018.

Protocol Activity Status:

Technology Transfer: We did not have any technology transfer during this reporting period.

PARTICIPANTS:

Participant Type: PD/PI

Participant: James Boedicker

Person Months Worked: 12.00

Funding Support:

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

Participant Type: PD/PI

Participant: Paul Bogdan Bogdan

Person Months Worked: 12.00

Funding Support:

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

Participant Type: Graduate Student (research assistant)

Participant: Valeriu Balaban

Person Months Worked: 12.00

Funding Support:

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

Participant Type: Graduate Student (research assistant)

Participant: Xiaokan Guo

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as of 05-Mar-2019

Person Months Worked: 12.00

Funding Support:

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

Participant Type: Graduate Student (research assistant)

Participant: Gaurav Gupta

Person Months Worked: 12.00

Funding Support:

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

Participant Type: Undergraduate Student

Participant: Sean Lim

Person Months Worked: 12.00

Funding Support:

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

Participant Type: Graduate Student (research assistant)

Participant: Panagiotis Kyriakis

Person Months Worked: 6.00

Funding Support:

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

Participant Type: Co PD/PI

Participant: Cauligi Raghavendra

Person Months Worked: 6.00

Funding Support:

Project Contribution:

International Collaboration:

International Travel:

National Academy Member: N

Other Collaborators:

ARTICLES:

RPPR Final Report as of 05-Mar-2019

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published

Journal: Nature Scientific Reports

Publication Identifier Type: DOI

Publication Identifier: 10.1038/s41598-018-30654-9

Volume: 8

Issue: 12416

First Page #:

Date Submitted: 1/21/19 12:00AM

Date Published: 8/20/18 9:00PM

Publication Location: London, UK

Article Title: Quantifying emergence and self-organisation of Enterobacter cloacae microbial communities

Authors: Valeriu Balaban, Sean Lim, Gaurav Gupta, James Boedicker and Paul Bogdan

Keywords: Microbial communities, multi-fractal analysis, emergence, self-organization, complexity, complex collectives

Abstract: We lack universal mathematical tools for analysing the collective behaviour of biological swarms. To address this, we propose a multifractal inspired framework to measure the degree of emergence and self-organisation from scarce spatial (geometric) data and apply it to investigate the evolution of the spatial arrangement of Enterobacter cloacae aggregates. In a plate of semi-solid media, Enterobacter cloacae form a spatially extended pattern of high cell density aggregates. These aggregates nucleate from the site of inoculation and radiate outward to fill the entire plate. Multifractal analysis was used to characterise these patterns and calculate dynamics changes in emergence and self-organisation within the bacterial population. In particular, experimental results suggest that the new aggregates align their location with respect to the old ones leading to a decrease in emergence and increase in self-organisation.

Distribution Statement: 1-Approved for public release; distribution is unlimited.

Acknowledged Federal Support: Y

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published

Journal: 2018 Annual American Control Conference (ACC)

Publication Identifier Type: DOI

Publication Identifier: 10.23919/ACC.2018.8430866

Volume: 2018

Issue:

First Page #: 2814

Date Submitted: 1/21/19 12:00AM

Date Published: 7/1/18 7:00AM

Publication Location: Milwaukee, WI

Article Title: Dealing with Unknown Unknowns: Identification and Selection of Minimal Sensing for Fractional Dynamics with Unknown Inputs

Authors: Gaurav Gupta, Sergio Pequito and Paul Bogdan

Keywords: Spatio-temporal complexity, fractional dynamics, complex biological systems, brain activity mining, hidden dimensions of complex biological systems

Abstract: This paper focuses on analysis and design of time-varying complex networks, making the following novel contributions: (i) we present an alternating scheme that enables to determine the best estimate of the model parameters and unknown stimuli; (ii) we provide necessary and sufficient conditions to ensure that it is possible to retrieve the state and unknown stimuli; and (iii) upon these conditions we determine a small subset of variables that need to be measured to ensure that both state and input can be recovered, while establishing sub-optimality guarantees with respect to the smallest possible subset. Finally, we present several pedagogical examples of the main results using real data collected from an EEG wearable device.

Distribution Statement: 1-Approved for public release; distribution is unlimited.

Acknowledged Federal Support: Y

RPPR Final Report as of 05-Mar-2019

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published

Journal: Proceedings of the 9th ACM/IEEE International Conference on Cyber-Physical Systems (ICCPS)

Publication Identifier Type: DOI

Publication Identifier: doi.org/10.1109/ICCPS.2018.00034

Volume: 2018

Issue:

First Page #: 275

Date Submitted: 1/21/19 12:00AM

Date Published: 5/1/18 7:00AM

Publication Location: Piscataway, NJ, USA

Article Title: Re-thinking EEG-based non-invasive brain interfaces: modeling and analysis

Authors: Gaurav Gupta, Sergio Pequito and Paul Bogdan

Keywords: Spatio-temporal complexity analysis, hidden dimensions of biological systems, brain activity mining

Abstract: We propose to leverage new time-varying complex network models that equip us with a fractal dynamical characterization of the underlying processes. Subsequently, the parameters of the proposed complex network models can be explained from a system's perspective, and, consecutively, used for classification using machine learning algorithms and/or actuation laws determined using control system's theory. Besides, the proposed system identification methods and techniques have computational complexities comparable with those currently used in EEG-based brain interfaces, which enable comparable online performances. Furthermore, we foresee that the proposed models and approaches are also valid using other invasive and non-invasive technologies. Finally, we illustrate and experimentally evaluate this approach on real EEG-datasets to assess and validate the proposed methodology. The classification accuracies are high even on having less number of training samples.

Distribution Statement: 1-Approved for public release; distribution is unlimited.

Acknowledged Federal Support: Y

Publication Type: Journal Article

Peer Reviewed: Y

Publication Status: 1-Published

Journal: Frontiers in Physiology

Publication Identifier Type: DOI

Publication Identifier: 10.3389/fphys.2018.01446

Volume: 9

Issue:

First Page #: 1446

Date Submitted: 1/21/19 12:00AM

Date Published: 11/1/18 7:00AM

Publication Location:

Article Title: Gene Expression Is Not Random: Scaling, Long-Range Cross-Dependence, and Fractal Characteristics of Gene Regulatory Networks

Authors: Mahboobeh Ghorbani, Edmond Jonckheere and Paul Bogdan

Keywords: gene expression, gene regulatory network, fractals, dynamics, entropy

Abstract: Here, we report the scaling properties of gene expression time series in *Escherichia coli* and *Saccharomyces cerevisiae*. Unlike previous studies, which report the fractal and long-range dependency of DNA structure, we investigate the individual gene expression dynamics as well as the cross-dependency between them in the context of gene regulatory network. Our results demonstrate that the gene expression time series display fractal and long-range dependence characteristics. In addition, the dynamics between genes and linked transcription factors in gene regulatory networks are also fractal and long-range cross-correlated. The cross-correlation exponents in gene regulatory networks are not unique. The distribution of the cross-correlation exponents of gene regulatory networks for several types of cells can be interpreted as a measure of the complexity of their functional behavior.

Distribution Statement: 1-Approved for public release; distribution is unlimited.

Acknowledged Federal Support: Y

RPPR Final Report as of 05-Mar-2019

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published

Journal: IEEE Transactions on Control of Network Systems

Publication Identifier Type: DOI

Publication Identifier: 10.1109/TCNS.2018.2809959

Volume: 5

Issue: 2

First Page #: 709

Date Submitted: 1/21/19 12:00AM

Date Published: 6/1/18 7:00AM

Publication Location:

Article Title: Selecting Sensors in Biological Fractional-Order Systems

Authors: Vasileios Tzoumas, Yuankun Xue, Sérgio Pequito, Paul Bogdan and George J. Pappas

Keywords: Biological complexity, fractional dynamics

Abstract: We focus on sensor selection, that is, determine the minimum number of state variables that need to be measured, to monitor the evolution of the entire biological system, that is, all state variables, when modeled by discrete-time fractional-order systems (DTFOS) that are subject to modeling errors, process, and measurement noise. These systems are particularly relevant while modeling the spatiotemporal dynamics of processes in which the impact of long-range memory cannot be properly modeled by multivariate autoregressive integrative moving-average models. Therefore, the DTFOS enable a unified state-space framework to model the evolution of several biological (e.g., stem cell growth and bacteria evolution) and physiological signals (e.g., electroencephalogram and electromyogram).

Distribution Statement: 1-Approved for public release; distribution is unlimited.

Acknowledged Federal Support: Y

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published

Journal: IEEE Transactions on Very Large Scale Integration (VLSI) Systems

Publication Identifier Type: DOI

Publication Identifier: 10.1109/TVLSI.2018.2843282

Volume: 26

Issue: 10

First Page #: 1895

Date Submitted: 1/21/19 12:00AM

Date Published: 10/1/18 7:00AM

Publication Location:

Article Title: Scalable Network-on-Chip Architectures for Brain-Machine Interface Applications

Authors: Xian Li, Karthi Duraisamy, Paul Bogdan, Janardhan Rao Doppa and Partha Pratim Pande

Keywords: Fractional dynamics, exascale manycore architecture for fractional models

Abstract: Understanding the dynamics and functionality of the human brain and its relationship with different physical entities has proven to be extremely useful in many applications including disability therapy and designing the next-generation user-interfaces. Communication between the brain and external hardware using neural stimulation and recordings has also been demonstrated recently. Such systems are usually analyzed by employing the brain-machine-body interface (BMBI) model. We propose a machine-learning-inspired wireless network-on-chip (WiNoC)-based manycore architecture for handling the compute- and communication-intensive nature of the BMBI applications. The experimental results show that, compared with the traditional wireline mesh NoC, WiNoC achieves up to 55% savings in energy delay product for a system size of 1024 cores.

Distribution Statement: 1-Approved for public release; distribution is unlimited.

Acknowledged Federal Support: Y

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In this section, we briefly summarize our research strategy and accomplishments:

A) Wild bacterial strains were isolated from freshwater sources in Los Angeles on plates selective for bacteria related to *Escherichia coli*. Isolated strains were screened for collective pattern formation at centimeter length scales (see Figures 1 and 2 below).

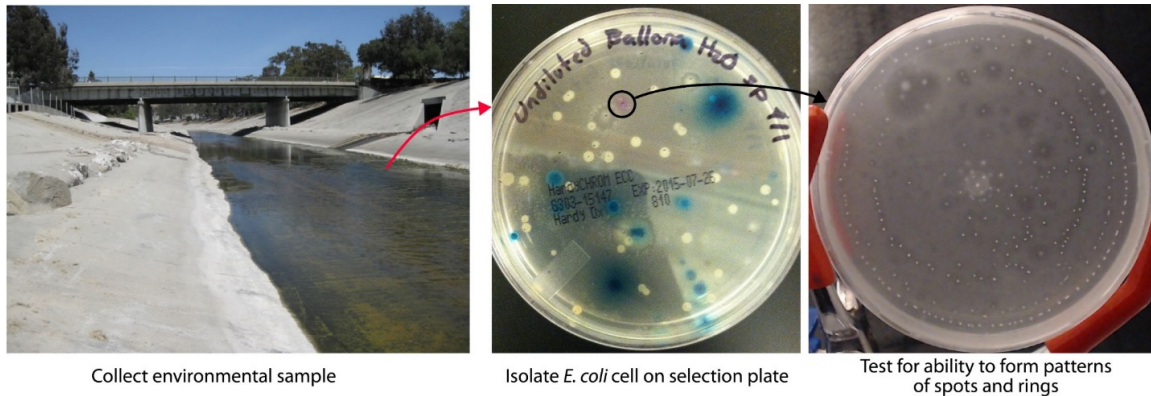


Figure 1. Main strategy: Wild bacterial strains were isolated and studied for pattern formation.

To screen for collective pattern formation, wild strains were placed at one end of a rectangular plate (see left hand side, lanes are 3 cm wide) filled with semi-soft agar. Over time, many strains formed complex patterns, including a swarm ring or a band (as shown in the middle rectangle of Figure 2), which radiated towards the opposite edge of the plate and spots of high cell density appeared (as shown on the right hand side panel of Figure 2).

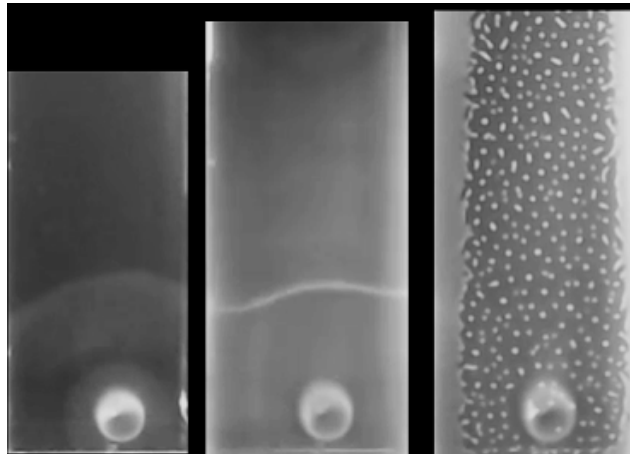


Figure 2. Pattern formation: Bacteria expand forming first a front wave and later on aggregates that move in space.

The emergent behaviors of each strain were measured, revealing variability in the collective pattern formation. We focused on two types of bacteria, seven different isolated of *Enterobacter cloacae* and four strains of *Aeromonas hydrophila*. The velocity of the high-density band of cells (indicated with arrows on top image, lane width is 3 cm), was variable for the wild isolates. Subsequent analysis of these strains attempted to connect this variability in the collective dynamics of each strain with variability in the genomic content and the single-cell characteristics. Figure 3 summarizes the front / band propagation and their band speeds across several bacteria types and strains. For completeness, we also analyzed their genomic structure. The genomes of our eleven isolates were sequenced. Alignment of the 16S rRNA sequences revealed that closely related strains had similar band speeds, suggesting that accumulated mutation in the genome modulated collective behaviors such as band formation and dynamics.

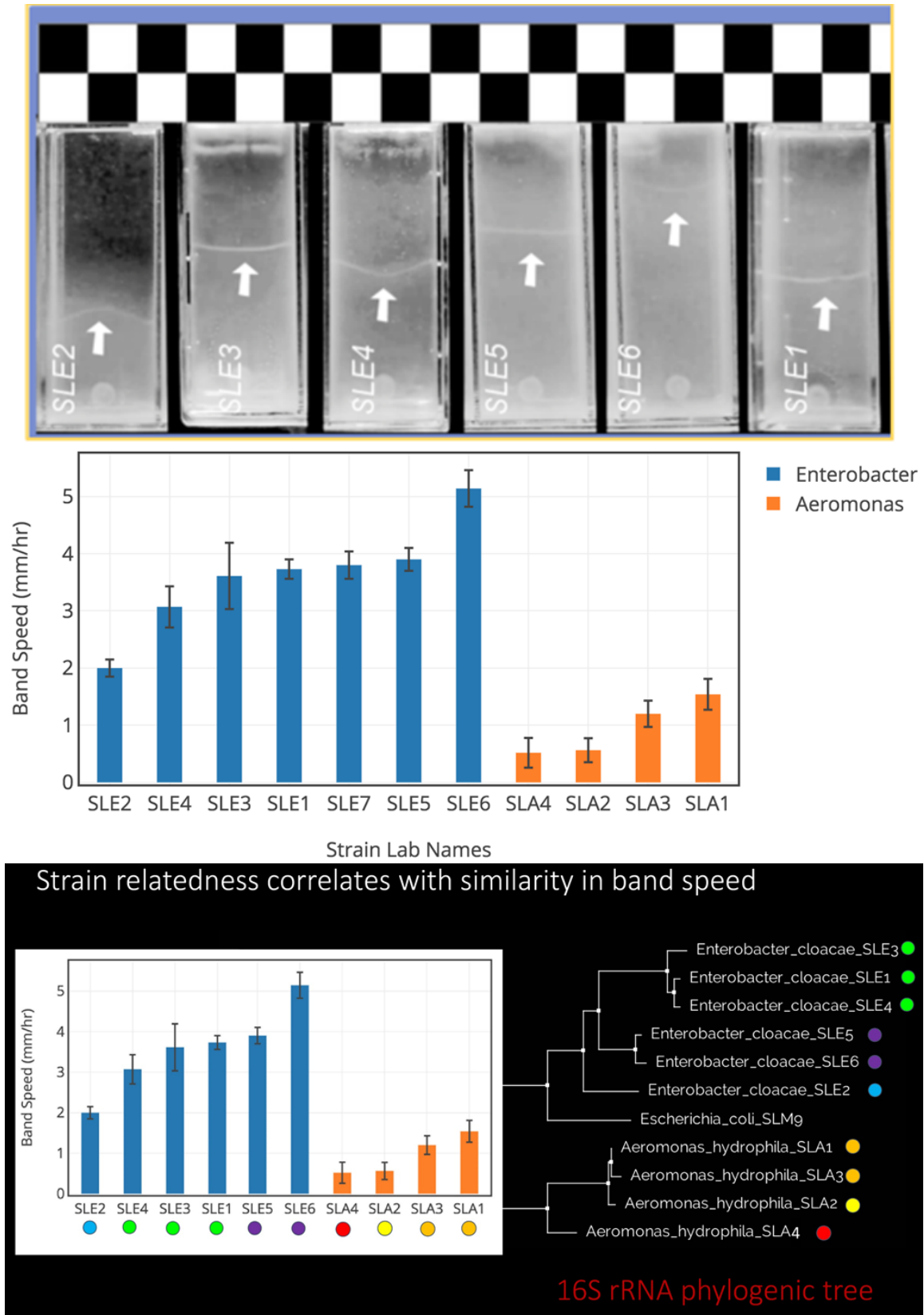


Figure 3. Analysis of band speed: Front formation (top) and band speed (bottom) for various bacteria types and strains.

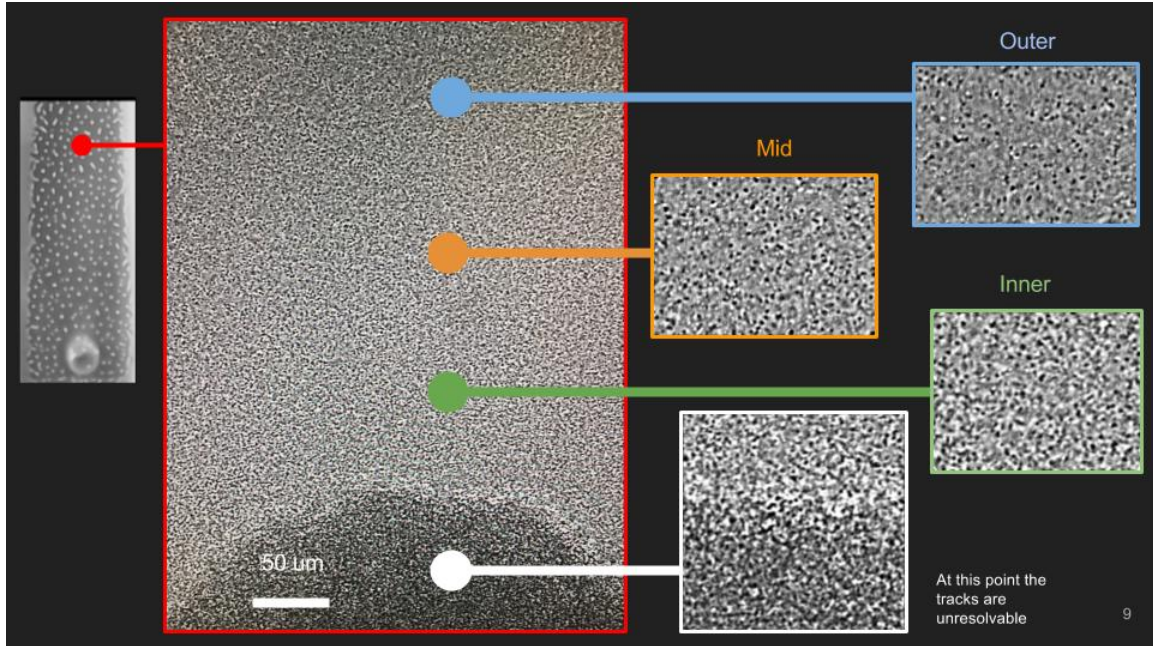


Figure 4. Extraction of single cell swimming trajectories from various regions of a bacterial aggregate.

Analysis of single-cell swimming trajectories within collective patterns in the semi-soft agar assay. Cells were found to have higher velocity in the vicinity of the high cell density spots that formed on plates of *Enterobacter cloacae*. We noticed consistent differences in speed values for the three regions across all bacteria types and strains (see the two plots in Figure 5). All these analysis results have been summarized or are going to be summarized in our manuscripts.

Figure 6 shows the characterization of the single-cell swimming characteristics of a wild *Enterobacter cloacae* strain and an *Aeromonas hydrophila* strain. Despite large difference in swimming speeds and tumbling angles, both strains formed similar emergent patterns.

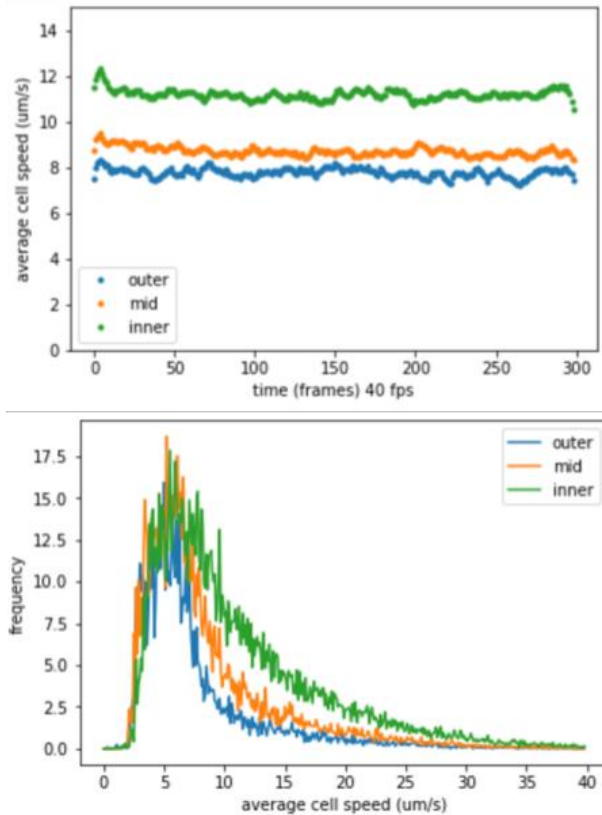


Figure 5. Cell speed for the three regions considered in the proximity of a bacterial aggregate.

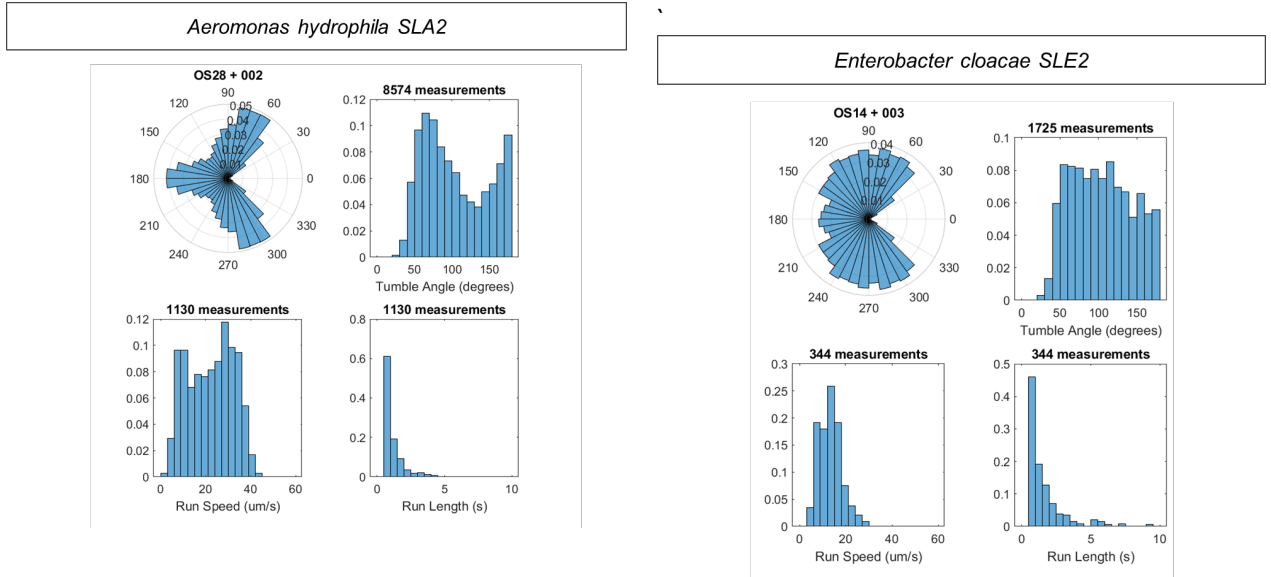


Figure 6. Characterization of the single-cell swimming characteristics of an *Aeromonas hydrophila* and a wild type *Enterobacter cloacae*.

When two band-forming strains were mixed together, the new collective behavior was revealed. The band speed switched from slow to fast. The time of the speed switch depended on the ratio of strains inoculated into the dish.

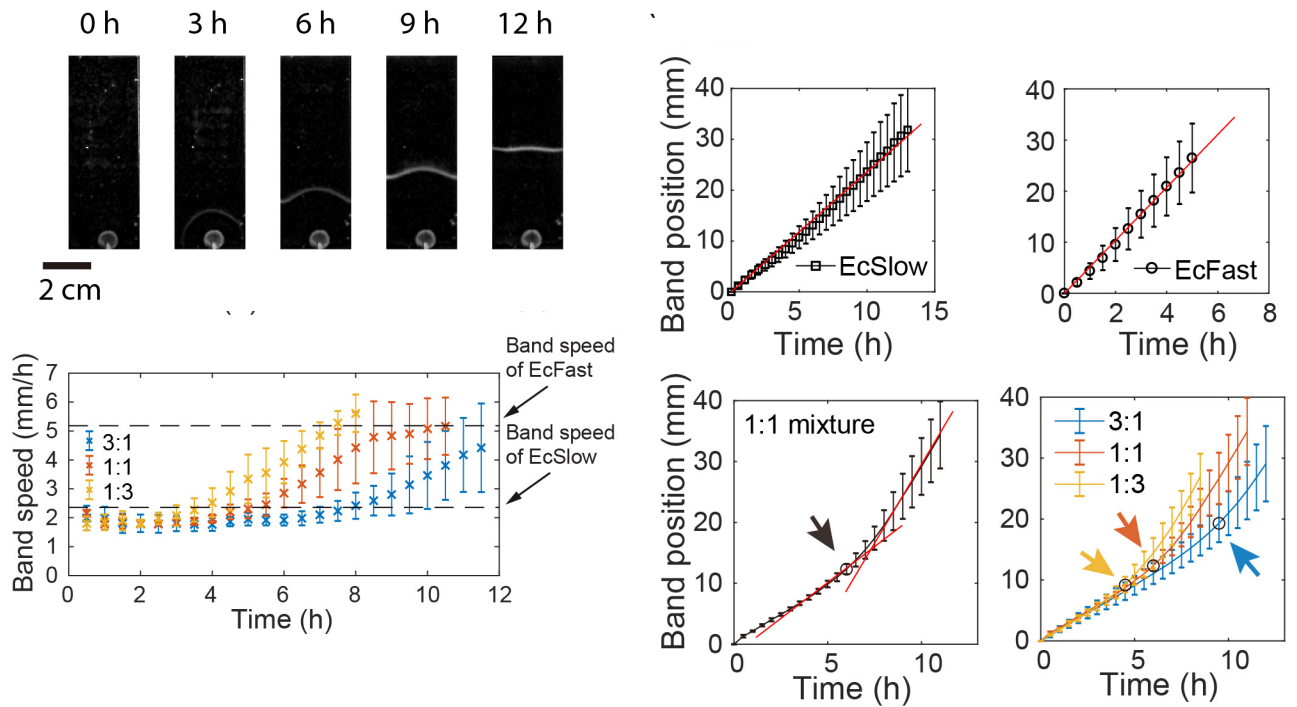


Figure 7. Analysis of two band-forming strains when mixed together shows that the band speed switched from slow to fast.

The speed switching behavior of the band observed at the single-cell level was analysis as shown in Figure 8. The switch in speed was controlled by a drastic change in the composition in the band from 90% EcSlow to 90% EcFast, revealing that band composition was the control parameter for this sudden change in collective behavior.

We have also initiated the analysis of aggregations patterns over large petri dishes. Figure 9 shows large arrays of spots emerging on 20 cm square culture dishes (small segment of the full pattern shown). After formation, aggregation spots migrated on the plate for several hours. During migration many spots merge together, a feature that seems to be unique to our experimental system. Current

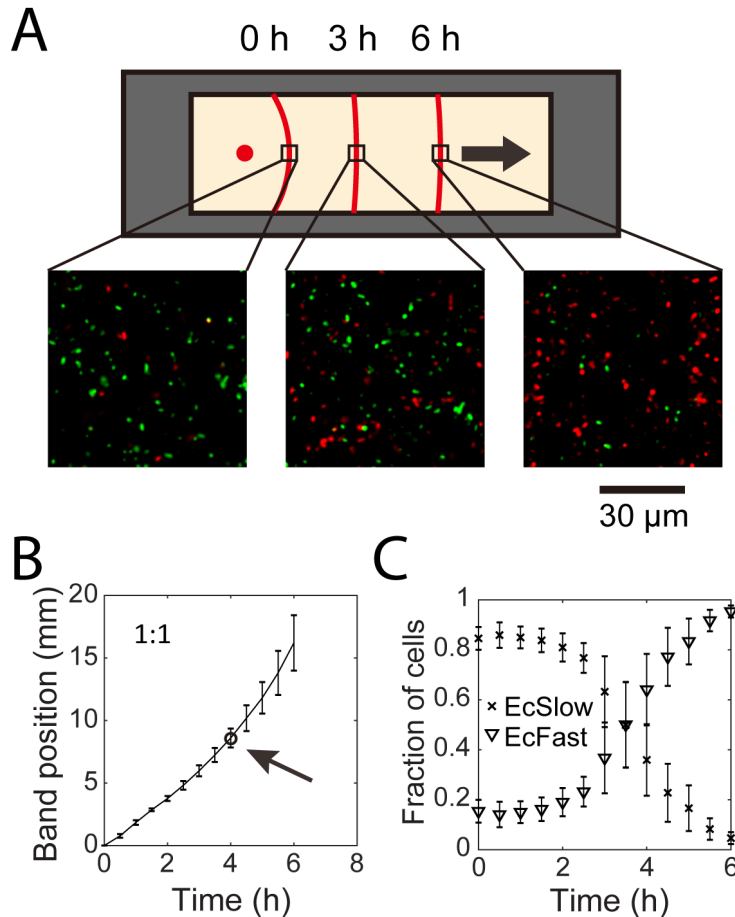


Figure 8. Speed switching behavior of the band analysis.

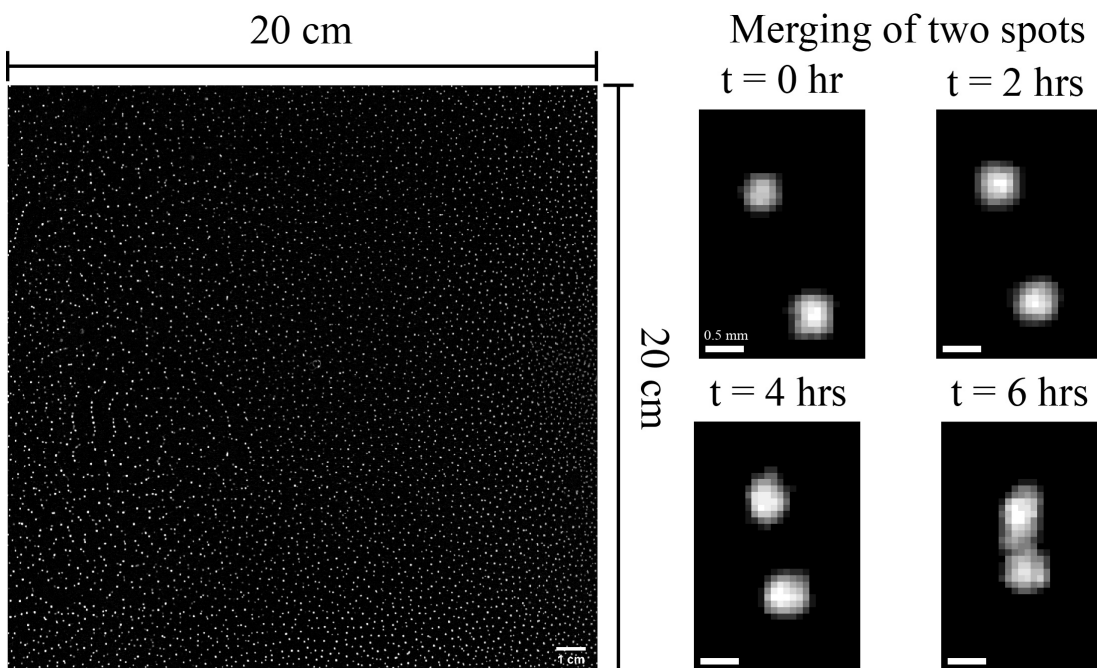


Figure 9. Large arrays of spots emerged on 20 cm square culture dishes.

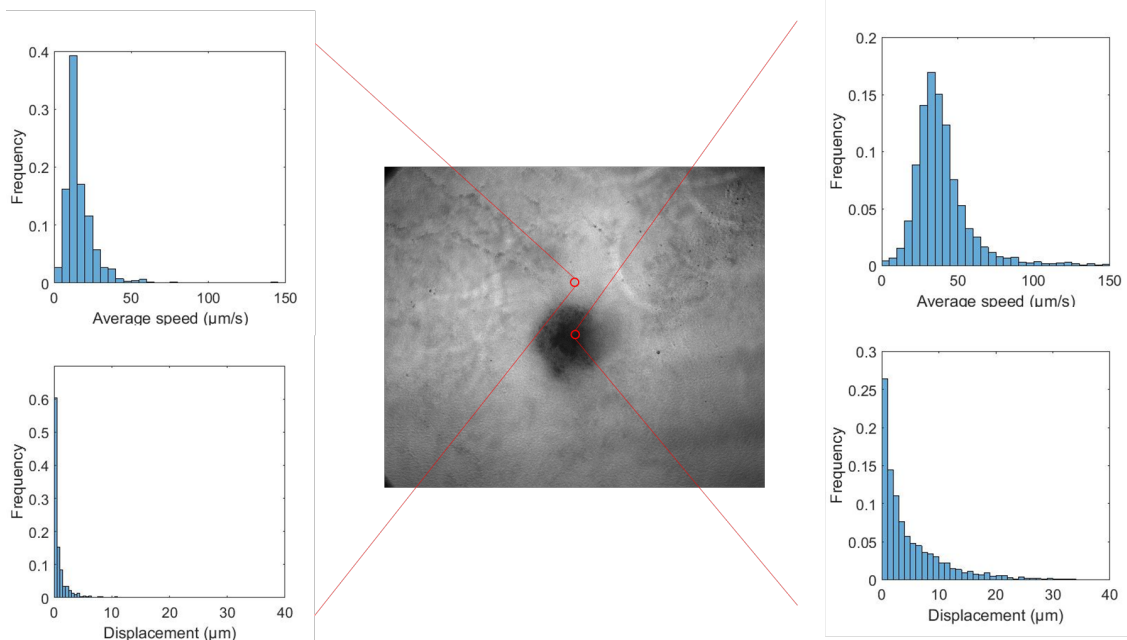


Figure 10. Cell movement analysis of single cells reveals heterogeneous across space.

work, through modeling and experiments, is trying to explain how such coordinated movement of millions of cells emerges from the rules that govern single-cell motility. We are currently quantifying the degree of emergence and self-organization of these larger microbial communities.

Figure 10 shows a preprocessing of microscopic movies of cell movement at multiple positions within the dish which reveals single-cell dynamics differed inside and outside of an aggregation spot.

Figure 11 shows the probability distribution for the distances between the aggregates at the beginning and at the end of the experiment. Figure 11(a) shows the comparison to a uniform random distribution, the aggregates at the start of the experiment match and have the same average distance as the uniform random, the peaks match approximately. However, at the end of the experiment, the distribution

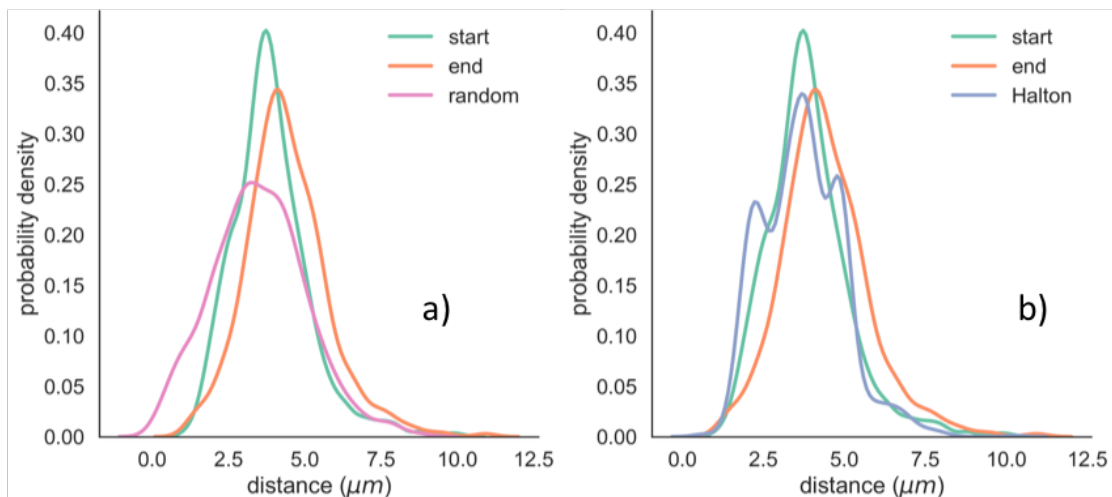


Figure 11. Probability distribution for the distances between the aggregates at the beginning and at the end of the experiment.

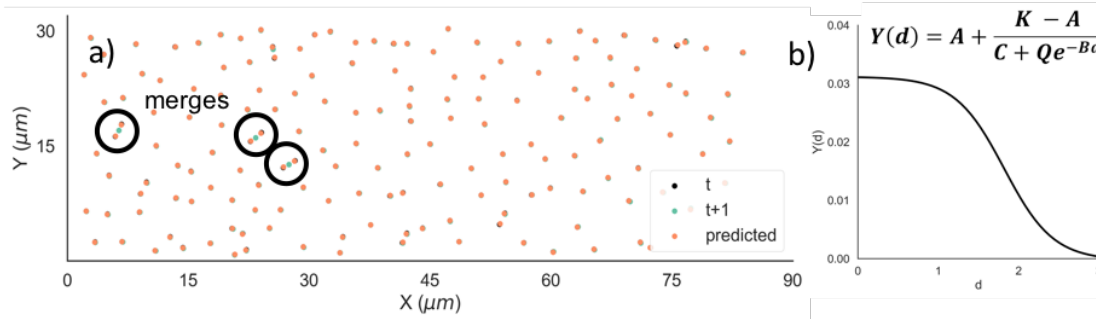


Figure 12. Agent based modeling of the interaction between aggregates. a) The spatial distribution of the aggregates. Aggregates exhibit little to no movement the majority of time, except the few cases when they merge and travel more. b) The fitted generalized logistic function used for modeling the attraction and the repulsion forces between the aggregates. This fit has the highest error for aggregates that merge hinting to non-deterministic behavior of this phenomenon.

shifted to the right suggesting increase in distance between the aggregates. Figure 11(b) shows the comparison with the deterministic Halton sequence of generating spatially distributed aggregates which produces the same average distance as the aggregates at the beginning, but as previously, it does not fit the distribution at the end of the experiment.

Figure 12 shows a comparison between the bacteria aggregation formation and a generalized agent-based model that uses the parametric model from Figure 12(b). The fitted generalized logistic function used for modeling the attraction and the repulsion forces between the aggregates. This fit has the highest error for aggregates that merge hinting to non-deterministic behavior of this phenomenon.

Figure 13 summarizes a new approach we developed to compute the multi-fractal properties and quantify the degree of emergence and self-organization from small petri dishes. Figure 13(a) summarizes the mapping of the aggregation pattern using a space covering Hilbert curve. Figure 13(b) shows the evolution of the one-dimensional pattern resulted from the mapping of aggregation spots. Figure 13(c) summarizes the multifractal spectrum for the pattern from panel 13(b). Figure 13(d) shows the shifted multi-fractal spectrum that reveals the evolution of the right part indicating a slight decrease in diversity for the less common patterns.

The variety of collective motions attracts the attention of scientists as they form and evolve without a leader, exhibit interactions over large distances compared to the size of the agents, and which can suddenly turn a complex and chaotic motion into an organized system. This sudden change in motion dynamics represents a phase transition. Phase transitions, e.g., the freezing of water when the temperature drops, have a control parameter that define when the transition happens. In case of water the control parameter is the temperature. For bacteria, the control parameter could be the lack of food which changes the chemical environment and thus through chemotaxis alters the dynamics of the bacteria that cluster together to form aggregates. These aggregates move, repelling from each other, or merge together and as a consequence aggregates optimize the distance between them as shown in Figure 11(b).

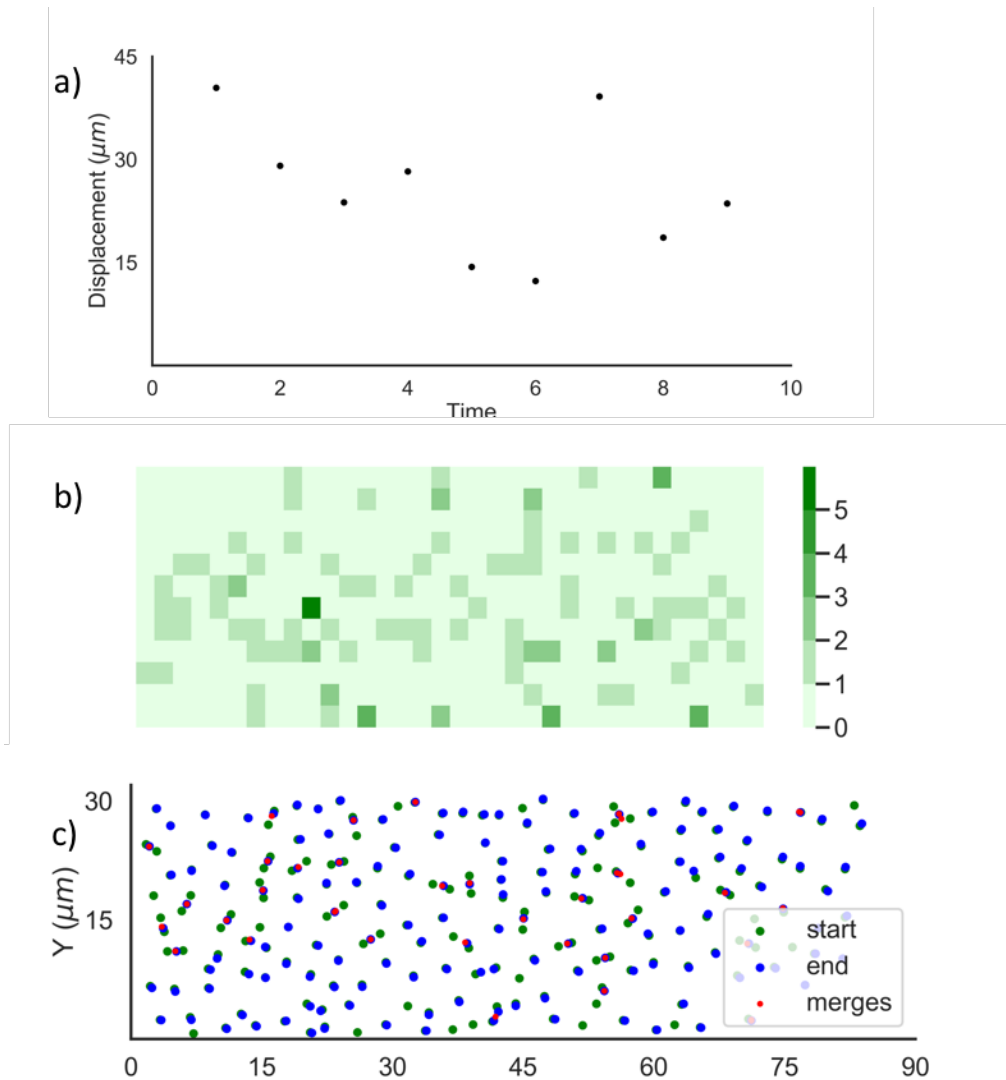


Figure 14. Displacement of bacteria aggregates. a) Total movement of the aggregates between two time points. Aggregates travel more at the beginning of the investigation period (few hours after they formed) compared to the end (just before disintegration). b) Histogram of the rectangular plate where the aggregates traveled the most. Distance shown in micrometers. Aggregates at the edge travel less compared to aggregates in the center of the plate. c) The position of the bacteria aggregates at the start and end of the experiment along with the location where the aggregates merged. Most of the merges occur in the center of the plate where they were also moving more active.

1. In the first few hours after the aggregates form their position changes most with a linear decline as time passes as shown in Figure 14(a). However, right before they disintegrate, the total displacement of the aggregates (the sum of individual aggregates displacement) increases again. The high displacement at the beginning can be viewed as a rearrangement to optimize the distance between them as shown in Figure 11(b) producing a better space coverage than human designed algorithms.

2. The agar, the medium in which bacteria are placed, is a porous medium that might limit the movement of bacteria in some locations and thus influence the aggregate formation. Figure 14(b) shows a heat map of the rectangular plate where the aggregates moved the most. The uniform distribution of the regions with high activity shows no faults in the agar medium. However, as expected, due to border effects, the aggregates

formed at the edges did not travel as much as the aggregates formed in the middle. Moreover, the aggregates at the edge were less influence by the chemogradients generated by the other aggregates as they had fewer neighbors and thus repositioned less compared the ones in the center.

During the experiment, some aggregates stay in the same place where they were formed initially, some move repelling from their neighbors, and some attract and merge. These dynamics are shown in Figure 14(c). The majority of merging events take place in the center of the plate as expected with only a few near the edges. This can be explained by the fact that a high density of aggregates in the center generates stronger chemical gradients that make two aggregates attract or repel. We are performing additional experiments to shed more light on the complexity of aggregates formation and dynamics by also quantifying the multi-fractal characteristics.

C) Many complex biological systems including the human brain, biological swarms, gene regulatory networks or protein-protein interaction networks, display complex interdependent non-stationary and long-range memory dynamics. While the mathematics of such time varying complex networks currently ignores the fractal and long-range dependence characteristics, what makes their analysis even harder is that we often have available only partial observations either in space (in terms of number of state variables that we can measure or know about) and time (only few measurements can be obtained due to time, technological and economic costs). Specifically, complex networks such as the brain, whose nodes will dynamically evolve according to a fractal order dynamical model, are often observed locally. Meaning that some of the dynamics assessed by the models are not only due to the local interaction, but might be constrained by unknown sources, i.e., stimuli that are external to the considered network. Similar theoretical problems appear in system identification problems concerning the gene regulatory networks or protein-protein interaction networks in biological cells or in describing the dynamics of autonomous complex swarms subject to environmental cues.

With these challenges in mind, we developed a mathematical model that enables us to account for the existence of both fractional dynamics and unknown stimuli, and determine the model that best captures the local dynamics under such stimuli. Note that this enhances the analysis of these systems once we have an additional feature (i.e., the stimuli) that can be the main driver of a possible abnormal behavior of the complex system / network. In addition, these mathematical problems are fundamental for designing efficient brain machine interfaces that aim to harvest information from the (physical) brain through sensing mechanisms, extract information about the underlying processes, and decide/actuate accordingly. To make the discussion more concrete, we summarize in equation (1) the mathematical model:

$$\begin{aligned}\Delta^\alpha x[t+1] &= Ax[t] + Bu[t] \\ y[t] &= Cx[t],\end{aligned}\tag{1}$$

where $x[t]$ is the state vector characterizing the brain activity dynamics as a collection of nodes in the time-varying complex network, α is a vector of corresponding fractional-order coefficients for each node in the network and $y[t]$ is the observed signal vector. The system matrices A, B and C are spatial-coupling, input and observation matrices, respectively. The fractional differential order α can be either a real or an integer number and thus can encode either the long-range memory (long-range dependence or non-Markovian) or the short-range memory (Markovian) properties in the dynamical system

model. Without loss of generality, we assume that the sensors are dedicated to each node, and therefore the observation matrix is the identity matrix. However, the proposed mathematical framework can also consider other B matrices.

The brain activity mining and interpretation process consists of: (i) Collecting the EEG data from the dedicated sensors, (ii) Estimating the model parameters from the EEG data; and (iii) Making predictions using the estimated model parameters. In this setup, the monitoring of brain activity is obtained through EEG sensing around the motor cortex of the brain. The second step is non-trivial due to the presence of unknown inputs in equation (1). With the assumption of known input matrix B as it depends on the experimental realization and fractional-order using well-known wavelet approach, we proposed an inspired Expectation-Maximization (EM) algorithm to jointly estimate the coupling matrix A and the inputs u . This EM-inspired algorithm is summarized as follows: The initial point of the algorithm $A^{(0)}$ is estimated by least squares, i.e.,

$$A^{(0)} = \arg \min_A \|Z - XA\|_2^2, \quad (2)$$

where Z is constructed by expanding the fractional order operator in equation (1) as $z[k] = \Delta^\alpha x[k]$ and truncating the expansion to some finite value. The expectation step of the algorithm works to estimate the unknown inputs. The unknown unknowns do not act on each sensor and are mainly sparse. Using this intuition, we have enforced the Laplace prior on the inputs u to write the E-step as

$$u[k] = \arg \min_u \|z[k] - A^{(l)}x[k] - Bu[k]\|_2^2 + \lambda \|u\|_1, \quad (3)$$

where λ is a parameter used to make a trade-off between sparsity and error. The inputs are estimated at each time point k taken into consideration. With the estimation of inputs in the E-step, the remaining part is the update of spatial coupling matrix, which is executed in the M-step as follows:

$$A^{(l+1)} = \arg \min_A \|\tilde{Z} - XA\|_2^2, \quad (4)$$

where $\tilde{Z} = Z - UB$ and $U = [u[1], u[2], \dots, u[K]]^T$. We showed that the above-mentioned algorithm is convergent. It is observed that the convergence is fast for the

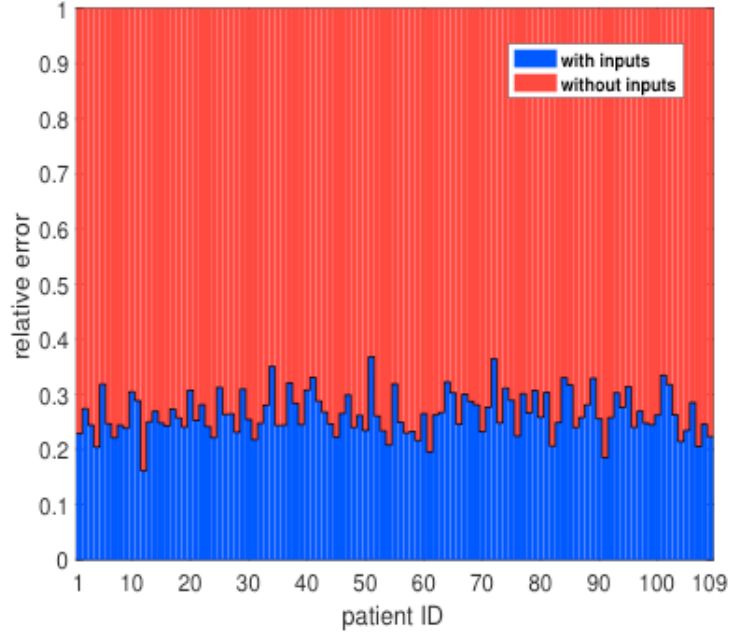


Figure 15. Time-varying complex networks subject to unknown stimuli. Error ratio when making predictions using a fractional-order dynamical model with and without inputs across 109 individuals. The fractional-order dynamical model with unknown inputs fits the EEG data much better than the case of no inputs.

EEG signals we analyzed and even one or two iterations are sufficient for major mean squared error reduction.

We investigated the benefits of the above-mentioned mathematical formalism by considering an EEG dataset representing the human brain activity and report the error ratio with and without inputs in Figure 15. It can be observed that the error (square root of mean squared error) for the case when the unknown inputs are included is less than one-third of the error when inputs are not considered. Moreover, the parameters of the proposed model can be seen as explainable from a system's perspective, and, subsequently, used for classification using machine learning algorithms and/or actuation laws determined using control system's theory. Besides, our proposed system identification methods and techniques have computational complexities comparable with those currently used in EEG-based brain interfaces, which enable comparable online performances. Our proposed mathematical models and algorithm are also valid using other invasive and noninvasive technologies. The classification accuracies as reported in the attached manuscript are high even on having less number of training samples.

D) We are analyzing the ADNI brain datasets to quantify the degree of mild cognitive impairment and Alzheimer disease progression through the above multi-fractal analysis.

Students

Number of students receiving STEM degrees during the reporting period: 0

Number of undergraduate and graduate STEM participants during the reporting period: 4

Products

Valeriu Balaban, Sean Lim, Gaurav Gupta, James Boedicker and Paul Bogdan, "Quantifying emergence and self-organisation of *Enterobacter cloacae* microbial communities," *Nature Scientific Reports* 8, August 2018.

Hana Koorehdavoudi, Paul Bogdan, Guopeng Wei, Radu Marculescu, Jiang Zhuang, Rika Wright Carlsen, and Metin Sitti, "Multi-fractal characterization of bacterial swimming dynamics: a case study on real and simulated *Serratia marcescens*," *Proceedings of the Royal Society of London A: Mathematical, Physical and Engineering Sciences*, July 2017.

G. Gupta, S. Pequito, and P. Bogdan, "Re-thinking EEG-based non-invasive brain interfaces - modeling and analysis" *Proceedings of the International Conference on Cyber-Physical Systems (ICCPS)*, CPS Week, Porto, Portugal, April 2018.

Gaurav Gupta, Sergio Pequito and Paul Bogdan, "Dealing with Unknown Unknowns: Identification and Selection of Minimal Sensing for Fractional Dynamics with Unknown Inputs," *Proceedings of the American Control Conference (ACC)*, June 2018.

Mahboobeh Ghorbani, Edmond Jonckheere and Paul Bogdan, "Gene Expression Is Not Random: Scaling, Long-Range Cross-Dependence, and Fractal Characteristics of Gene Regulatory Networks," *Frontiers in Physiology*, vol. 9, October 2018.

Vasileios Tzoumas, Yuankun Xue, Sérgio Pequito, Paul Bogdan and George J. Pappas, "Selecting Sensors in Biological Fractional-Order Systems," *IEEE Transactions on Control of Network Systems*, vol. 5, no. 2, pp. 709-721, June 2018.

Xian Li, Karthi Duraisamy, Paul Bogdan, Janardhan Rao Doppa and Partha Pratim Pande, "Scalable Network-on-Chip Architectures for Brain-Machine Interface Applications," in *IEEE Transactions on Very Large Scale Integration (VLSI) Systems*, vol. 26, no. 10, pp. 1895-1907, Oct. 2018.

Ruochen Yang*, Gaurav Gupta* and Paul Bogdan, “Data-driven Perception of Neuron Point Process with Unknown Unknowns,” to appear in the *Proceedings of the International Conference on Cyber-Physical Systems* (ICCPS), April 2019.

Sean Lim, Xiaokan Guo, and James Boedicker, Connecting single-cell properties to collective behavior in multiple wild isolates of the *Enterobacter cloacae* complex, in revision at PLoS ONE.

Xiaokan Guo, Sean Lim, Paul Bogdan, and James Boedicker, Collective behavior for a two-strain microbial community, submitted.

SCIENTIFIC REPORTS

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Quantifying emergence and self-organisation of *Enterobacter cloacae* microbial communities

Valeriu Balaban¹, Sean Lim², Gaurav Gupta¹, James Boedicker² & Paul Bogdan¹

From microbial communities to cancer cells, many such complex collectives embody emergent and self-organising behaviour. Such behaviour drives cells to develop composite features such as formation of aggregates or expression of specific genes as a result of cell-cell interactions within a cell population. Currently, we lack universal mathematical tools for analysing the collective behaviour of biological swarms. To address this, we propose a multifractal inspired framework to measure the degree of emergence and self-organisation from scarce spatial (geometric) data and apply it to investigate the evolution of the spatial arrangement of *Enterobacter cloacae* aggregates. In a plate of semi-solid media, *Enterobacter cloacae* form a spatially extended pattern of high cell density aggregates. These aggregates nucleate from the site of inoculation and radiate outward to fill the entire plate. Multifractal analysis was used to characterise these patterns and calculate dynamics changes in emergence and self-organisation within the bacterial population. In particular, experimental results suggest that the new aggregates align their location with respect to the old ones leading to a decrease in emergence and increase in self-organisation.

Collective behaviour in multi-agent systems attracts significant attention due to their ability to synchronise their actions and to self-organise in the absence of a global controller. Often observed in microbial communities, this behaviour drives single cells to work together to reach goals they could not reach on their own. *Dictyostelium discoideum* cells usually migrate alone, but under limited food resources, use chemotactic intercellular signalling to form clusters of cells in preparation to reproduce¹. *Vibrio fischeri* inoculate in the light producing organs of several marine species with whom they develop symbiotic relationships. These cells after reaching a population threshold produce light and help the marine animal camouflage². The chemotaxis-based group migration of cancerous cells (not as individuals) increases the likelihood of metastasis³. From interacting microbial communities to cancer populations, there is a need for analysing such complex collectives exhibiting emergent properties.

Unlike prior efforts to analyse the properties of individual swimming bacteria^{4–6}, this work studies the collective behaviour of aggregate formation, as aggregation is an emergent property of the group and not of the individual cells. However, macroscopic analysis of these biological multi-agent systems must deal with data that is limited in temporal and spatial resolutions. We propose to overcome the lack of detailed spatiotemporal information by using images to quantify the dynamic geometry that unfolds as the individual cells sense the environment, communicate with one another, and decide to join or leave specific aggregation groups over time. More precisely, we do not seek to track individual cell-to-cell communication and cellular decision-making events, but rather quantify the higher-order spatial correlations that build up the geometrical patterns through a multifractal formalism. Towards this end, we develop analytical methods based on multifractal analysis to characterise the emergent properties of complex biological patterns. Compared to earlier works on emergence^{7–11} and self-organisation^{11–14}, current framework analyses the process across multiple observation scales and captures the variations across regions with similar properties. We apply it to aggregate formation in *Enterobacter cloacae* for which the incomplete measurement of cell metabolism, sensitivity level to chemoattractants, and local concentration of several chemoattractants prevent a rigorous analysis of the collective behaviour at the microscale.

Enterobacter cloacae cells when inoculated in soft agar medium form complex spatiotemporal patterns, shown in Fig. 1a, similar to those observed in *Escherichia coli* and *Salmonella enterica*^{15,16}. These patterns result from the interactions of individual cells and represent examples of emergent biological behaviour. To analyse these

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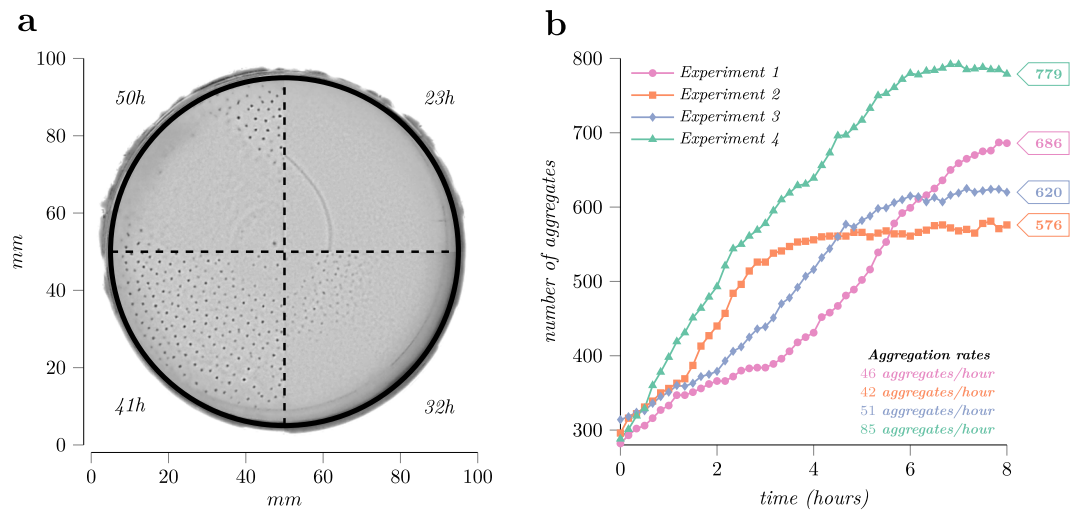


Figure 1. The growth phases of the microbial community along with the aggregation rates for the four experiments. **(a)** The development of the *Enterobacter cloacae* aggregation pattern. During the first stage bacteria form a band that traverses the plate, the 23h mark. Next, starting from the site of inoculation at the centre of the plate, a pattern of aggregates forms radiates outward, 32 h and 41 h sectors. Towards the end, aggregates disintegrate as shown in the 50h time mark. **(b)** The evolution of the number of aggregates during the investigation interval. The interval starts when plates form 300 aggregates and spans the next eight hours, the period of time needed for full coverage of the plate.

patterns, we set up four experiments placing a bacteria colony of about 10^6 cells in the centre of a Petri dish from which in the next 50 hours a pattern of aggregates grows over the entire plate. Figure 1a and Supplementary Video S1–S4 illustrate the time-lapse of the experiments which show that cells first generate a ring of high cell density, known as the swarm band, which traverses the plate for 12–14 hours. After the band expansion stage, within the 20 to 30 hours into the experiment, dense millimetre-scale groups of nearly immobilised cells, the aggregates, form at the bottom of the migration medium starting from the centre and evolving towards the edge. Several hours later, these aggregates disintegrate equalising the distribution of bacteria cells across the plate.

For four replicate experiments, we quantified the emergence and the self-organisation from the evolution of the aggregation pattern. In such complex, dynamic patterns, it is unclear to what degree the location of new aggregates is random or whether the aggregation pattern has order which extends over length scales much larger than the size of an individual aggregate. In what follows, we describe the new mathematical framework based on multifractal analysis and then use it to quantify the degree of emergence and self-organisation in collective microbial communities from image snapshots. The analysis of emergence and self-organisation was used to analyse the spatial properties of the aggregation pattern over multiple scales it unfolds covering the plate.

Results

Multifractal analysis. The fundamental feature of fractal objects is self-similarity across scales. Namely, any part of the fractal under any magnification exhibits similar properties as the initial fractal¹⁷. For example, in terms of shape, branches connected to the tree trunk present similar properties as ones connected to other branches. In other words, solely from the shape, the branch itself cannot be differentiated from the magnification of one of its parts.

The fundamental measure for self-similar objects is the fractal dimension. This dimension D defines how volume V scales with the object linear size l , i.e., $V \sim l^D$. To better explain this, we express the volume in terms of box counting. In this case, to calculate the volume, we cover the fractal object with hypercubes of dimension d and edge length l and multiply the number of hypercubes containing part of the object with the volume of a hypercube. Mathematically this is expressed as $V(l) = \lim_{l \rightarrow 0} N(l)l^d$, where $V(l)$ is the volume of the fractal, $N(l)$ is the number of hypercubes that cover the fractal, and l^d is the volume of the hypercube. Since we are interested in the scaling behaviour of the volume with respect to the linear scale and since $N(l) \sim V(l)$, in what follows, instead of $V(l)$, we will analyse how $N(l)$ varies with l . As example, a segment of length 1 can be covered with $N(l) = 1/l$ one-dimensional hypercubes (segments) of length l resulting in $V(l) \sim l^{-1}$ and therefore the fractal dimension is $D = 1$. Next, we follow the same steps for a surface of unit area and find that $N(l) = l^{-2}$. Both objects, the line and the plane, are not fractals as their volume is related to their linear size by an integer exponent.

More formally, the fractal dimension using the box counting approach is defined for an object as follows:

$$D = \lim_{l \rightarrow 0} \frac{\log(N(l))}{\log(1/l)} \quad (1)$$

This formula, when applied to previous non-fractal objects, yields the same result, which for these objects is equal to their Euclidian dimension. For fractal objects, however, the result is a real (non-integer) number. To

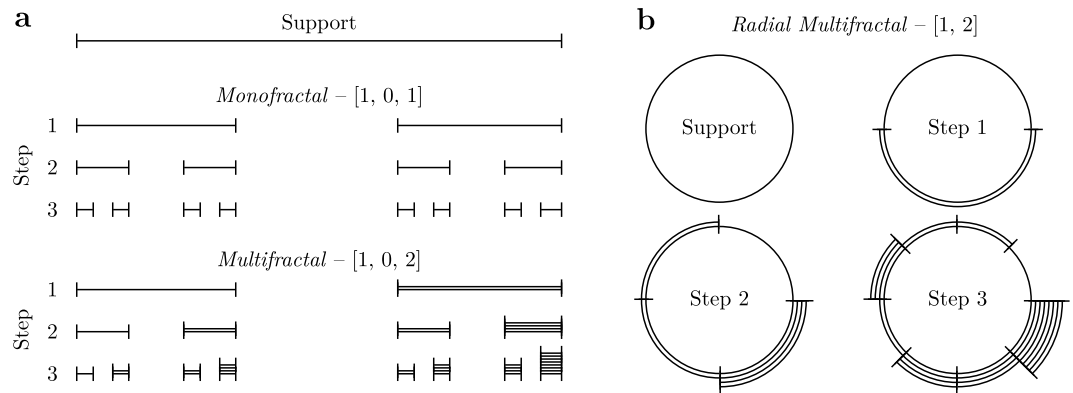


Figure 2. Multifractal construction steps on linear and radial support. **(a)** Construction steps for the Cantor set, the monofractal, and the multifractal version of it. Multifractals, in contrast to monofractals, have at least two distinct growth rates which generate segments of different weights. **(b)** The same construction steps applied on circular support but at each step split into smaller sectors.

illustrate this, we will use the expression (1) to compute the fractal dimension of the Cantor set fractal shown in Fig. 2a top. To construct this fractal, we divide a segment of length 1 into three equal parts and remove the middle one. We repeat the procedure for each newly formed segment. Now, in order to compute the fractal dimension of the Cantor set, we count the number of segments of length $l = 3^{-n}$ that cover the object, where n is a natural number. Since for any n , only $2/3$ of segments cover a part of the fractal, we find that $N(l) = 2^n$ and substituting in expression (1), we obtain $D = \log(2)/\log(3) \approx 0.63$. As we can see, the obtained fractal dimension is less than 1 since it covers less space than a line, but greater than 0 since it is more than just a point.

However, in nature, most of the fractal-like objects contain regions with approximately similar scaling rates, but not strictly the same as fractals require. To analyse these objects, it is natural to consider them as a set of fractals to accommodate for the difference in the observed scaling behaviour. These objects with multiple scaling rates are named multifractals and the ones with one scaling rate monofractals. Both types of fractals are constructed in the same way, the only difference are the properties of the initial set. For monofractals, the scaling rates in the initial set are either zero or one, whereas multifractals have no such restrictions. Figure 2 illustrates the first three construction steps for monofractals and multifractals. First, the algorithm divides the support into subintervals and assigns a weight to each subinterval based on the corresponding rate¹⁷. Next, previously formed subintervals are considered as the new support and the procedure repeats. Since both fractal objects shown in Fig. 2a have three scaling rates, the segments at each step are divided into three with the middle one removed since its rate is zero. In the monofractal, all segments have the same weight, however, in the multifractal example, the right (in Fig. 2a) part has double the weight of the parent segment weight since the last scaling rate is two. Moreover, as shown in Fig. 2b, fractals can be constructed also on circular support. In this case, the initial set contains no zeros, and thus no segments are removed. If we randomly distribute points in each sector based on its weight, we can generate spatial patterns that resemble the bacteria aggregation process but it is not identical. The Supplementary Fig. S6 shows the results of such simulations including also the initial set from above. As it can be seen, the multifractal results closely match the pattern shown in Fig. 1a, but not the monofractal because of the large empty sectors corresponding to the zero scaling rate.

On the basis that multifractals contain multiple scaling rates that cannot be encoded into a single number, the fractal dimension has to be extended to a function. As a result, the previous relationship $N(l) \sim l^{-D}$ augments to $N_\alpha(l) \sim l^{-f(\alpha)}$ where $f(\alpha)$ is the multifractal spectrum, a function of the crowding index α . The crowding index, also called Hölder exponent, groups regions with the same scaling exponent and maps to one fractal dimension. To simplify the quantitative analysis, in what follows, we normalise the support to have unit volume, and thus, the measure covered by the hypercubes becomes a probability as all sum to one. The crowding index is now expressed as $p_i(l) \sim l^\alpha$, where $p_i(l)$ is the probability of the hypercube of linear size l with index i . Multiple regions can be characterised by the same α , case in which, N_α counts all the hypercubes that cover these regions, and thus $f(\alpha)$ the fractal dimension of this subset of hypercubes. In general, the fractal spectrum is a single-humped function with $\max f(\alpha) = D$, where D is the fractal dimension of the object with the same initial set, but will all non-zero rates equal to 1. Note, the multifractal spectrum for a monofractal is a point since all regions have one scaling rate and thus are identified by single crowding index α and a single fractal dimension $f(\alpha) = D$.

To better grasp the meaning of α and $f(\alpha)$ let us look at the scaling properties of the leftmost and rightmost segments of the multifractal shown in Fig. 2a. From the figure, we see that these two segments have at each step the lowest and respectively the highest weight as indicated by the number of lines composing the segments, and thus correspond to $\alpha_{\min}$ and $\alpha_{\max}$, respectively. Moreover, since both at each step correspond to only one segment, $N_{\alpha_{\min}}(l) = N_{\alpha_{\max}}(l) = 1, \forall l > 0$, they have a zero fractal dimension $f(\alpha) = 0$ as $l^0 = 1, \forall l > 0$, Fig. 3b. Since other crowding indices are covered by more than one hypercube they have a non-zero fractal dimension and generate the humped shape of the spectrum.

The computation of the multifractal spectrum $f(\alpha)$ for an object has two steps, (1) calculate the generalised dimension for multiple linear sizes l to estimate the scaling properties of regions, and (2) obtain the multifractal

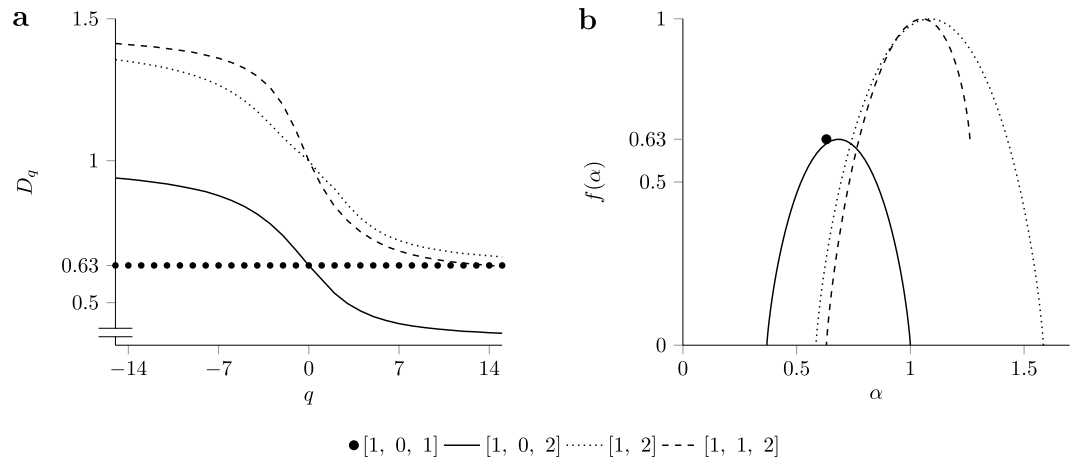


Figure 3. (a) The generalised dimension D_q is a decreasing function for multifractals and constant for monofractals. (b) The multifractal spectrum computed from the generalised dimension. Monofractals have the spectrum concentrated to a point whereas for multifractals the spectrum is a single-humped function.

spectrum from the Legendre transformation of the generalised dimension^{17–19}. To obtain the generalised dimension D_q , first, we compute the Renyi entropy using the probabilities $p_i(l)$ of the hypercubes, and then divide the entropy by $\log(l)$ to adjust the measure to the observation scale. Of note, despite its name, the generalised dimension is a scale adjusted entropy and not a fractal dimension defined as

$$D_q = \frac{1}{q-1} \lim_{l \rightarrow 0} \frac{\log(\sum_{i=1}^N p_i(l)^q)}{\log(l)} \quad (2)$$

where the sum is over hypercubes of non-zero probability. The generalised dimension for the objects shown in Fig. 2a as a function of order q (i.e., between -15 to 15) is shown in Fig. 3a. The order q has the role of an adjustable magnifying glass that selects only regions with specific properties. Negative values of q give more weight to low probabilities whereas positive values of q to high probabilities. This adjustable entropy allows us to capture the scaling behaviour. As Fig. 3a shows, the monofractal produces a straight line whereas the multifractal a monotonic decreasing function capturing the diversity of the scaling exponents.

Next, applying the Legendre transformation to D_q , the multifractal spectrum $f(\alpha)$ is recovered as $f(\alpha_q) = f_q$ from

$$\alpha_q = \frac{\partial[(q-1)D_q]}{\partial q} \quad (3)$$

$$f_q = q\alpha_q - (q-1)D_q \quad (4)$$

Figure 3b shows the multifractal spectrum obtained using the above transformation for four multifractal objects. The maximum value of the spectrum indicates how much the fractal fills the space, i.e., less empty regions, the higher the maximum. Fractal objects with a zero in the initial set, namely $[1, 0, 2]$, have the spectrum maximum lower than the Euclidian dimension of the support as part of it is not covered by the fractal. Further, the width of the spectrum depicts variability (diversity) across regions as the limits, α_{min} and α_{max} , correspond to the regions with the highest and the lowest scaling exponent, and thus, the larger the difference between these values, the wider the spectrum. When multiple regions correspond to α_{min} or α_{max} , the matching fractal dimension $f(\alpha_{max})$ or $f(\alpha_{min})$ becomes non-zero, as illustrated by the right side of the^{1,2} spectrum shown with dashed lines in Fig. 3b. For this spectrum, the limit value $f(\alpha_{min}) \approx 0.63$ is same as the fractal dimension of the Cantor set, since the two ones in the initial set contribute in this case.

When computing the multifractal spectrum for real-world data several aspects must be considered. The reader may observe that when we computed the fractal dimension of a surface we used square boxes, but when we generated patterns similar to ones of *Enterobacter cloacae* we used radial boxes. The reason is if we cover the plates with square boxes the majority will partially cover the aggregates due to the round shape of the plates as shown in Supplementary Fig. S1. To overcome this, we use radial boxes that do not suffer from this problem. After selecting the box shape, the next step is to compute the probabilities p_i for different box sizes. Since the expression for D_q contains a limit operator, we estimate its value from the slope of the numerator with respect to the denominator and as both contain logarithms the box sizes have to be selected on a logarithmic scale to evenly spread the points for a better linear fit. Moreover, the linear fit should be performed for each value of q with a range chosen such that the generalised dimension D_q saturates at the limits. In this case, extending the range of q has little effect as no more information about the distribution is gained. A multifractal spectrum generated by negative values of q

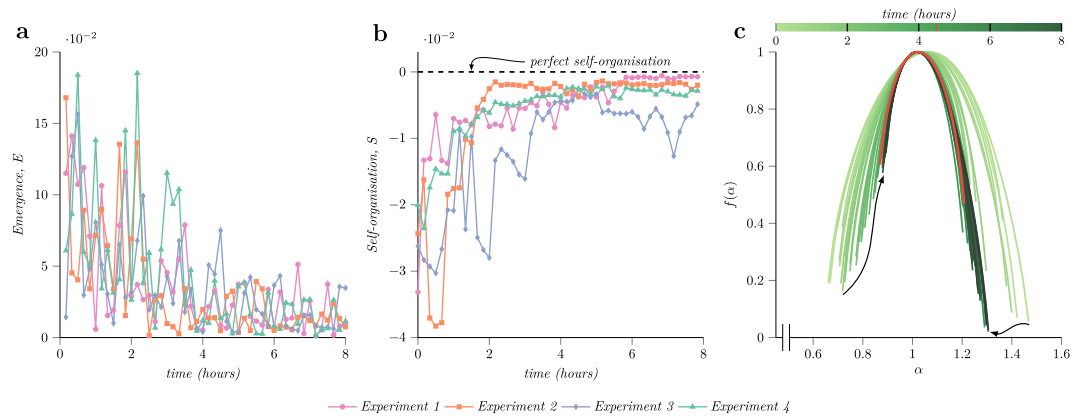


Figure 4. The computed emergence and self-organisation metrics and the evolution of the multifractal spectrum over time. **(a)** The emergence metric computed using equation (5), which has a decreasing trend as more aggregates form on the plate and saturates several hours before the plates are fully populated. **(b)** The self-organisation degree of *Enterobacter cloacae* microbial community computed using expression (6) indicating an increase in self-organisation as aggregates populate the plate. **(c)** The evolution of the multifractal spectrum for the experiment four which registered the highest growth rate. It shows the superposition of multifractal spectrum computed at each time point encoded as colour. The spectrum shown in orange represents the time when the spectrum converges to a constant shape after the first four hours of the experiment.

has to receive special attention since in this case probabilities of lower value receive more weight and thus have to be estimated precisely as the error is also amplified in this case.

The multifractal spectrum is the central part of any multifractal analysis and can be used to describe the group properties of interacting agents, such as bacteria aggregates. The multifractal analysis investigates the statistical scaling laws of complex fragmented geometrical objects which cannot be described by classic geometric methods¹⁸. Considering that microbial communities exhibit complex time-varying aggregation patterns, we employ the above-mentioned multifractal formalism to characterise the phase-space dimensionality and complexity of the observed dynamics. Consequently, interpreting the microbial community as an intelligent system driven by heterogeneous interactions meant to cooperate for achieving a collective goal allows us to develop two approaches for quantifying the instantaneous degree of emergence and self-organisation in collective systems.

Emergence quantification in collective microbial communities. Systems composed of interacting components have the value of the whole greater than the sum of the constituents due to the extra value created by the interactions which are not present when the constituents are considered separately. Furthermore, since part of the system properties resulted from local interactions they change dynamically over time. These properties are called emergent and their change over time is called emergence. Identically, the aggregates, that change in form (arrangement) and spatial (distance between aggregates) distribution over time, represent an emergent property since they result from the cell-to-cell interactions and not of individual cells. Consequently, given that generalised dimensions D_q characterises the distribution of the emergent property across multiple scales, we define in equation (5) the degree of emergence exhibited by a microbial community as

$$E = \int \frac{\partial D_q}{\partial t} g(q) dq \quad (5)$$

where E represents the emergence, D_q the generalised dimension, t represents time, and $g(q)$ a function that makes the integral finite. In this particular case, $g(q)$ was chosen to be $\partial \alpha(q)/\partial q$ which is non-zero for the range of q for which it selects regions with different scaling properties. An advantage of expression (5) is that by taking a definite integral over a particular range of q the emergence of specific regions in the system can be obtained. Notably, integrating over the negative values of q gives the emergence in regions with a small number of aggregates. Whereas integrating over the positive values of q computes the emergence in regions with a large number of aggregates. Consequently, this emergence formula quantifies the multi-scale nature of the aggregates distribution in space.

Figure 4a shows the degree of emergence computed from the patterns of *Enterobacter cloacae* aggregates extracted from time-lapse imaging of migrating bacteria. The integration of the generalised dimension was performed for $q \in [0, 10]$ and thus the results do not include the emergence of regions with a small number of aggregates. Negative q values were not considered to avoid misleading results since more data points are required to estimate precisely the scaling properties of these regions. The dots in Fig. 4a represent the degree of emergence between two consecutive image snapshots. All plates exhibited a similar trend, a sharp decrease in emergence followed by an asymptotic convergence to zero after the two hours mark relative to the investigation interval. Since the emergence conveys the change in multiscale entropies and the two hours time mark occurs before aggregates cover the entire plate, it indicates that the new aggregates formed after this time preserve the system entropy.

Quantifying self-organisation in microbial communities. Self-organisation, similar to emergence, denotes a collective behaviour and represents the ability of a group to drive the system towards an ordered state. During this transition, all group members, independently and in the absence of a centralised controller, adjust their actions to increase the order of the whole.

From the perspective of multifractal analysis, monofractals exhibit perfect order since all the regions have identical properties across all scales. On the other hand, in multifractal systems, regions have different properties that vary across the observation scales. Therefore, multifractals exhibit a lower degree of self-organisation. Systems maintain their degree of self-organisation when the emergence is zero since it quantifies the changes in system properties over time, and thus no changes in system properties imply no changes in self-organisation degree. The next expression is proposed for measuring the self-organisation degree of a system:

$$S = -\int (\alpha_0 - \alpha)^2 f(\alpha) d\alpha \quad (6)$$

where S represents the self-organisation, α_0 the principal crowding index obtained when $q=0$, and $f(\alpha)$ the fractal dimension associated with the respective crowding index. When applied to monofractals, this expression yields zero since α is always equal to α_0 as the spectrum is concentrated to a point. However, multifractals do not have a spectrum concentrated in a point, and therefore, the integral in this case would always be greater than zero, and because of the minus sign in front of the integral, would always yield a lower self-organisation degree compared to the monofractal case. Moreover, for multifractals the larger the variation of properties between regions that compose the system, the wider the multifractal spectrum and thus the lower the self-organisation.

The self-organisation properties of *Enterobacter cloacae* were analysed from the computed multifractal spectrum. Figure 4c and Supplementary Fig. S8 show the evolution of the multifractal spectrum. All experiments during this interval follow the same general pattern, the spectrum varies more in the first hours and then converges to a constant shape, which is an expected behaviour given the coupling between emergence and self-organisation. In experiment four, Fig. 4c, the spectrum converges after 4.5 hours to the shape showed in orange. The convergence of the multifractal spectrum indicates that the probability distribution of the locations of aggregates also converges. New aggregates appear at positions that preserve the current degree of self-organisation; the current distribution of aggregates dictates the location of new aggregates.

On the left part of the peak, the spectrum describes the properties of the regions with a large number of aggregates, whereas the right characterises the ones with a small number of aggregates. In Fig. 4c, the varying shifts upward of the left part of the multifractal spectrum $f(\alpha)$ suggest an increase in the number of regions with more aggregates and a decrease in variation of the scaling properties across these regions. However, the right half of the spectrum exhibits only an abrupt shift to the left and no organised movement over the vertical axis which implies that the number of regions that contain fewer aggregates decreased. Comparing the spectrum evolution shown in Fig. 4c with the theoretical experiment from Supplementary Fig. S7 hints that later aggregates follow a monofractal distribution whereas the first aggregates that appear follow a multifractal one.

Figure 4b shows the degree of self-organisation computed using equation (6). In case of this strain of bacteria, the distribution of the aggregates is described by a multifractal, and hence the self-organisation measure will be lower than zero. All four experiments display a rapid increase of self-organisation fuelled by the emergence at the beginning of the investigation interval which then saturates to a constant value several hours later. Although the experimental conditions were the same, the position of individual aggregates was non-deterministic, however, the collective properties of patterns formed were consistent. Moreover, by analysing Fig. 4b and Fig. 1b it can be seen that the aggregation rates and the number of aggregates show no correlation with the manifested emergence and self-organisation behaviour of the microbial communities.

Discussion

Collective behaviour refers to complex macroscopic dynamics of microbial communities exhibiting emergence and self-organisation properties without a global controller. Alternatively stated, the cognitive abilities and the adaptation to environmental changes are distributed among individuals forming the group^{20–25}. The emergent behaviour in systems ranging from microbial communities to carcinogenic systems and somatic cellular societies generates complex qualities not present at the individual level such as information generation, collective memory, and efficient cell-to-cell communication. Consequently, recognising the exhibited degree of collective intelligence highlights the importance of quantifying the capabilities of microbial communities to explore (emergence) and optimise (self-organisation) in changing environments. In many cases, detailed microscopic information about individual processing and cell-to-cell communication as well as molecular changes in the environment are difficult to record, hence, a multi-scale spatiotemporal methodology is required to quantify the emergence and self-organisation in spatially complex biological systems. Consequently, this paper presented a multifractal inspired framework for characterising the collective behaviour and investigating from a macro perspective the aggregation properties of *Enterobacter cloacae*.

The strategy for estimating the emergence from snapshots of complex systems dynamics improves prior efforts based on information theoretic concepts of quantifying emergence^{7–11} and self-organisation^{11–14}. For instance, in¹¹, the emergence is proportional to the information produced by complex systems, and self-organisation was defined as the increase in complexity in response to internal influences and excluding external ones. However, these metrics are hard to evaluate in practice since the analysed system may not be isolated from the environment, and the external influence may be unknown. Moreover, all previously proposed methods do not explicitly consider the scale of interaction, therefore when the system dynamics are influenced by phenomena operating at different scales special procedure must be applied when combining individual metrics computed at different scales. To overcome this challenge, equations (5) and (6) exploit the generalised dimension and the multifractal spectrum to characterise complex systems dynamics over multiple scales.

Using the proposed multifractal framework, it was found that emergence slowly decreases as the aggregates cover the plates, Fig. 4a. As emergence is proportional to the change in the generalised entropy, the positions of previously formed aggregates regulated the position of new ones such that the entropy remained constant. Since self-organisation is a reaction to changing environmental conditions, and given the increase of self-organisation metric, Fig. 4, *Enterobacter cloacae* microbial communities adapt the distribution of aggregates to resemble a monofractal distribution, similar densities at all scales and all regions to conform to environmental constraints. As the environmental conditions are set by the cells themselves and, at a larger extent, each aggregate through their production of chemoattractants, bacteria restrict their swimming behaviour into certain patterns. Moreover, from the marked spectrum it can be seen that the spatial distribution settles before the aggregates completely cover the plates, which suggest the presence of an optimum spatial arrangement. Similar to water freezing during which the molecules arrange themselves in a specific order as the temperature decreases, the *Enterobacter cloacae* self-organises in a monofractal resembling aggregation pattern as the nutrients level decrease across the plate. Alternatively, a widening of multifractal spectrum would indicate an increase in the diversity of the distribution among sectors and, thus, it would imply that multiple configurations of the aggregates position offer similar benefits in the environment lacking enough nutrients. The tendency of the aggregates formed by bacteria to obey a monofractal law rather than a multi-fractal one might imply a form of self-optimisation that is taking place within the community since the monofractal will enforce more exact repetition and symmetry in the system than a multifractal law.

The proposed mathematical framework can analyse multiple types of collective behaviour and discover universal laws exhibited by large communities when limited microscopic spatiotemporal information is available, i.e., no access to individual trajectories of microbial agents, agent interactions, or other molecular information that are difficult to monitor²⁶. In the future, these new metrics to quantify emergence and self-organisation that occurs over multiple scales enable new insights into the advantages of collective behaviour in biological systems. It is known that pattern formation is essential to many biological systems and has fitness advantages for populations of cells. For example, complex spatial arrangements of bacteria in biofilms result in increased resistance to antibiotics and other stresses^{27–30}. Specific structures also are likely to optimise efficiency of cellular communication³¹, although the relationship between emergence and multiscale communication in cellular communities is not yet clear. Quantifying the dynamics of emergence of such systems should uncover mechanisms that generate complex biological patterns and will potentially reveal collective properties may have been optimised to balance cooperative and competitive interactions between cells. Complex spatial structures are also observed in eukaryotic systems³², including tissues and tumours. Our analysis could generate insights into the collective behaviour in such cellular networks to aid in the development of therapeutic strategies to target cell-cell interactions and emergent properties. There is also interest in extending our ability to program synthetic biological systems over multiple lengths scales^{33,34}. Advanced analytical tools to analyse the multiscale patterns that occur in natural and synthetic biological systems, such as the methods reported here, will be needed to develop a more complete and predictive understanding of the mechanisms and consequences of collective behaviour in cellular networks.

Methods

Experimental setup. The motility plate is a 94 mm petri dish (Greiner Bio-One) containing 10 mL of motility gel, which is about 1.3 mm in thickness. Motility gel consisted of 0.26% agar, and M9 minimal salts (BD) supplemented with 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, 22 mM (0.4%) glucose, 3 mM sodium succinate, and 20 μ g/mL each of the amino acids histidine, methionine, threonine, and leucine³⁵. After being poured, the media was allowed to solidify for 1 hour on the benchtop. Afterward, the dishes were inoculated in the centre with bacteria culture and were sealed with parafilm. Growing at 25 °C, the bacterial community radiated outwards in collective motion from the inoculation centre and coalesced into patterns about 45 hours later.

Bacteria cultures. The pattern-forming strain was from a collection of bacteria isolated in a previous study³⁶. 16S rRNA sequencing revealed the strain to be most closely related to *Enterobacter cloacae*. For experiments, the strain was inoculated from frozen glycerol stocks and grown to saturation overnight in M9 salts medium supplemented with 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, and 0.25% glycerol as the carbon source³⁷. The strain was grown at 37 °C at 3 mL scale and shaken at 200 rpm. The next day, we diluted the suspension cultures to an optical density at 600 nm of 0.2. We inoculated 10 μ L of the culture (10^6 cells) on the motility plate at this density for each experiment.

Multifractal spectrum calculation from experimental data. In order to perform multifractal analysis, first, the position of aggregates on the petri dish has to be extracted using image processing methods. The current setup uses an LED panel to uniformly illuminate the plates from above and a camera that every 10 minutes takes an image of the plate from below. As a result of this setup, the bacteria aggregates identified by a high cell density block more light to reach the camera sensor and thus appear in the image as regions of dark pixels whereas regions with fewer bacteria correspond to brighter pixels. Since the bacteria aggregates appear as dark regions and thus correspond to groups of pixels with lower intensity values, their position can be detected using a local minima algorithm. Next, using the box-counting method with radial boxes the probabilities of individual boxes can be estimated using the extracted locations of the aggregates. These probabilities are computed for multiple linear scales l for the radial boxes, which are then substituted into equation 2 for computing the generalised dimension D_q . Subsequently, applying the Legendre transformation to D_q the multifractal spectrum is obtained. More information about this procedure is provided in Supplementary Materials.

Data availability. The jupyter notebook used to process the images of microbial communities images and compute the multifractal spectrum along with the introduced metrics will be available on the GitHub page.

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Author Contributions

S.L. and J.B. designed the biological experiments; V.B., S.L., G.G., J.B., and P.B. performed the research and wrote/revised the manuscript.

Additional Information

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Multi-fractal characterization of bacterial swimming dynamics: a case study on real and simulated *Serratia marcescens*

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To add to the current state of knowledge about bacterial swimming dynamics, in this paper, we study the fractal swimming dynamics of populations of *Serratia marcescens* bacteria both *in vitro* and *in silico*, while accounting for realistic conditions like volume exclusion, chemical interactions, obstacles and distribution of chemoattractant in the environment. While previous research has shown that bacterial motion is non-ergodic, we demonstrate that, besides the non-ergodicity, the bacterial swimming dynamics is multi-fractal in nature. Finally, we demonstrate that the multi-fractal characteristic of bacterial dynamics is strongly affected by bacterial density and chemoattractant concentration.

1. Introduction

The future potential of using bacteria for therapeutic purposes [1,2] and regenerative medicine makes the dynamics of such microswimmers highly attractive to study. As an example, to overcome the penetration limitation of cancer chemotherapy, Toley & Forbes suggest using motile bacteria which can migrate into solid tumours as carrier of the drug in chemotherapy [3]. In another example, Felfoul and his co-workers show that magnetotactic bacteria can be used to transport drug-loaded nanoliposomes into the oxygen-depleted hypoxic region of the tumour [4]. Considering such applications, significant research efforts have been focused on analysing and modelling bacterial swimming dynamics. Broadly speaking, the mathematical models used to describe the bacterial swimming dynamics can be classified into two categories. The first category is based on a *microscopic* (i.e. cell-level) view of bacterial swimming through a set of equations where each equation describes the state of a single agent [5–9]. The second category provides a *macroscopic* (i.e. population-level) view via continuum-based partial differential equations that capture the dynamics of population density over space and time, without considering the intracellular characteristics directly [10–18]. Among the present models, Schnitzer [19] uses the Smoluchowski equation to describe the biased random walk of the bacteria during chemotaxis to search for food. To focus on a detailed description of the motion taking place during one run interval of the bacteria, de Gennes [20] derives the average run length travelled by bacteria during one counterclockwise interval. Along the same direction, to consider the environmental condition affecting the biased random walk of bacteria, Croze and his co-workers [21] study experimentally and theoretically the effect of concentration of soft agar on chemotaxis of bacteria. To study the effect of obstacles (another environmental condition) on the motion of bacteria, Chepizhko and his co-workers study the motion of self-propelled particles in a heterogeneous two-dimensional environment and show that the mean square displacement of particles is dependent on the density of obstacles and the particle turning speed [22,23]. Building on these models, Cates [24] highlights that bacterial dynamics does not always obey detailed balance, which means it is a biased diffusion process depending on the environmental conditions. Moreover, Ariel and his co-workers focus on diffusion of bacteria and show that the bacteria perform super-diffusion during swarming on a surface [25]. To add to the current knowledge, in this paper, we take into account realistic conditions like volume exclusion (i.e. no two bacteria can occupy the same space at the same time), chemical interactions among bacteria (i.e. autochemotaxis happening when bacteria excrete the converted substrate succinate molecules in the environment into chemoattractant aspartate molecules [26]), obstacles (e.g. during the drug delivery task, the existing cells and biological residues act as obstacles and interfere with the swimming bacteria) and heterogeneous distribution of chemoattractant in the environment and identify fractal characteristics of single bacterium motion, which could have a fundamental impact on mathematical modelling of both single bacterium and population swimming dynamics.

We analyse the motion trajectories of *Serratia marcescens* [27,28]. Extracting single bacterium trajectories of motion from computer simulations for *S. marcescens* (figure 1*a,b* shows the different simulation set-ups that we used for the chemoattractant gradient in the environment) and applying single-cell visual tracking methods to real experiments on *S. marcescens* (figure 1*c*) reveals the fractal characteristics of bacterial motion.

To elucidate the complexity of the bacterial dynamics, we investigate the individual trajectories of simulated *S. marcescens* beside trajectories of real *S. marcescens* at multiple scales in space and time; this way, we show single bacterium motion has a phase transition from a super-diffusive behaviour to a normal diffusion and lastly to a sub-diffusive pattern based on bacterial density and chemoattractant distribution. We also show that bacterial motion exhibits a *multi-fractal* behaviour. The multi-fractal feature means that bacterial motion is self-similar, which is a concept dating back to the pioneering work of Kolmogorov to explain the chaotic behaviour [29,30]. Consequently, the multi-fractal formalism offers mathematical techniques to capture this nonlinear self-similarity in their motion.

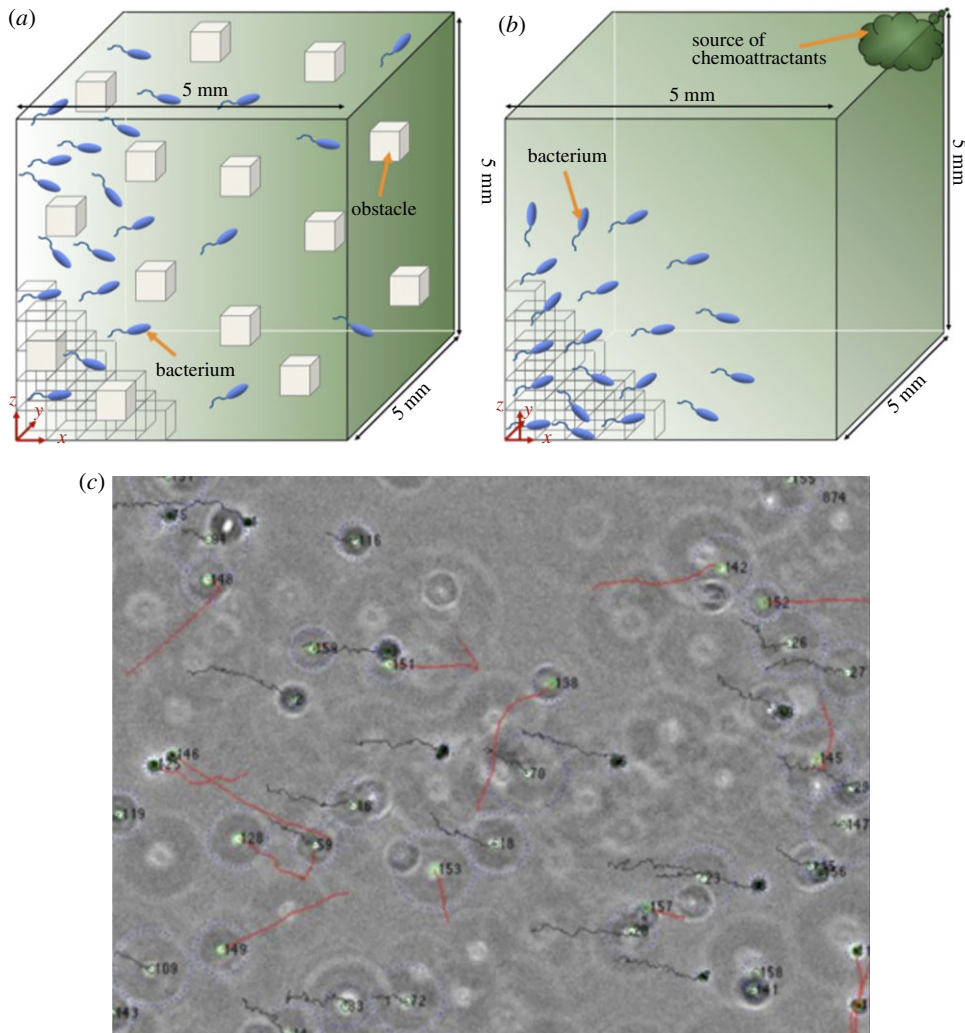


Figure 1. Simulation configuration of *S. marcescens* in BNSim (see §2) and single particle tracking of *S. marcescens* during experiments. (a) In case 1 for simulation in BNSim, a cubic environment with dimensions $5000 \times 5000 \times 5000 \mu\text{m}^3$ has been considered. The whole environment subdivided into 10^6 smaller cubes, each with a size of $50 \times 50 \times 50 \mu\text{m}^3$. We consider a linear gradient ($10^{-4} \text{ mM } \mu\text{m}^{-1}$) of chemoattractant (L-aspartate) only in the y -direction and no gradient in other directions. (b) In case 2 for simulation in BNSim, we consider the same configuration as in case 1 for bacteria and the environment. We only changed the gradient of chemoattractant in the environment. In this case, we consider a linear gradient of chemoattractant (L-aspartate) from the injection location to the targets, meaning that there is a linear gradient in 3 different dimensions. (c) Single particle tracking of *S. marcescens* in an *in vitro* environment with dimensions of $10000 \times 500 \times 150 \mu\text{m}^3$ with linear gradient ($10^{-4} \text{ mM } \mu\text{m}^{-1}$) of chemoattractant (L-aspartate) in the second direction (y -direction).

The remainder of this paper is organized as follows. In the first section of results, we investigate the swimming dynamics of simulated *S. marcescens*. We explore the second-order moment of motion using an ergodicity test under different conditions including bacterial density and chemoattractant distribution. Then, we explore the third-order moment of motion with a nonlinearity test. Next, we discuss that focusing on second- and third-order moments of a single bacterium's motion cannot capture the entire spatial and temporal complexity of the motion; therefore, we perform multi-fractal analysis which considers higher-order moments of their motion. We show that the simulated *S. marcescens* bacterial swimming displays multi-fractal

characteristics. In the last section of results, using experimental swimming trajectories of *S. marcescens*, we show that their motion is multi-fractal, validating our conclusions for simulated bacteria. Finally, the discussion section concludes the paper and outlines some future research directions.

2. Material and methods

(a) Experiments

Laboratory experiments were conducted to measure the swimming behaviour of live and motile *S. marcescens* under two conditions: (i) an isotropic environment (i.e. uniform distribution of substances) with dimension equal to $10\,000 \times 500 \times 150\,\mu\text{m}^3$ and (ii) a stable linear gradient of L-aspartate, which is a canonical chemoattractant for bacterial species such as *S. marcescens*.

(i) Bacterial culture

To conduct the experiments, *S. marcescens* (ATCC 274, American Type Culture Collection, Manassas, VA, USA) was cultured using established protocols [28,31–33]. The bacteria were first grown to reach the exponential state in their growing process (during this state, the number of bacteria doubles at an approximately constant rate, which is around every 20 min for *S. marcescens*) in a liquid culture (25 g of Difco LB Miller Broth and 1 l of deionized (DI) water, pH 7.0) on a shaker at 37°C for 3.5–4 h. Then, an aliquot of 2.0 μl of the liquid culture was placed on an agar plate (25 g of Difco LB Miller Broth, 6 g of Bacto Agar, 5 g of glucose, 1 l deionized water), and the agar plate was incubated at 30°C for 16–20 h. For the experiments, bacteria from the leading edge of the colony were extracted and then diluted in motility buffer (0.01 M KH_2PO_4 , 0.067 M NaCl, 0.1 mM EDTA, pH 7.0) to appropriate densities for the measurements. Subculturing the bacterial cells on an agar plate allows the most motile bacterial cells, which have been shown to be located along the leading edge of a spreading colony [28], to be selectively chosen for the study.

(ii) Experimental set-up

A three-channel microfluidic concentration gradient generator was fabricated to measure bacterial swimming parameters under chemotaxis and without chemotaxis; detailed descriptions of the device can be found in Zhuang *et al.* [27], and a figure of the device is shown in the electronic supplementary material, figure S10. The gradient generator consisted of three parallel microfluidic channels within an agarose gel. By controlling the concentration of the chemoattractant in the outer two channels of the device, a linear chemoattractant concentration profile could be generated by molecular diffusion in the middle channel in which the bacterial suspension was placed. The gradient generator was calibrated by observing the diffusion of 10^{-4} M fluorescein (Sigma-Aldrich Co.) in the device as described in Zhuang *et al.* [27] and Edwards *et al.* [28]. Fluorescence intensity profiles from these studies revealed that a stable linear gradient developed within 20 minutes. Given that the diffusion coefficient of L-aspartate is $9.0 \times 10^{-6}\,\text{cm}^2\,\text{s}^{-1}$ and the diffusion coefficient of fluorescein is about half that of L-aspartate [27], it can be concluded that about 10 min are required to develop a stable chemoattractant concentration gradient of L-aspartate and the gradient remains stable beyond 10 min. In our study, the bacterial swimming motion was measured after 15 min, ensuring a stable linear concentration gradient within a quiescent fluid environment.

(iii) Experimental conditions

Chemotaxis of *S. marcescens* was measured under an L-aspartate gradient of $0.2\,\text{mM}\,\text{mm}^{-1}$, with an average concentration of 0.1 mM. To measure the bacterial swimming behaviour in an isotropic environment, we used the same device but with a uniform distribution of motility buffer (without an L-aspartate gradient). For both cases, with and without the chemoattractant gradient, the

bacterial swimming parameters were studied for three different cell densities, which were 10^6 bacteria cm^{-3} , 10^7 bacteria cm^{-3} and 10^8 bacteria cm^{-3} . All the experiments were conducted at room temperature ($19\text{--}22^\circ\text{C}$).

(iv) Imaging and tracking

The bacteria were imaged with an inverted microscope ($40\times$ objective, Zeiss AxioObserver.A1, Carl Zeiss, Oberkochen, Germany), and videos were obtained at 88 frames per second with a CCD camera (Foculuc FO134SB). All video data were acquired at least $20\text{ }\mu\text{m}$ (approx. 20 body lengths) from any surface to minimize the wall effects on the bacterial swimming behaviour, and no obstacles hindered the movement of the bacteria (except for other bacterial cells). The bacteria were tracked in three dimensions by an in-house visual tracking program developed in Matlab (R2012a, The MathWorks, Inc, Natick, MA, USA). The tracking program extracts the x – y positions of bacteria in the video frames using image thresholding techniques, while the z -position was obtained by making use of the lens aberrations created by out-of-focus bacterial cells. This defocused optical tracking method has been applied by several groups to track the three-dimensional motion of bacteria and small particles [28,34–37]. A linear relationship exists between the radius of the aberration ring appearing around an out-of-focus cell and the cell's vertical distance (along z -axis) to the focal plane. The following equation exhibits this relation:

$$r = c_1|z| + c_0. \quad (2.1)$$

In the above equation, c_0 and c_1 were calibrated by performing an experiment in which the bacteria were fixed a known distance away from the focal plane. The variables were determined from Edwards *et al.* [28]. Therefore, the z -position of a bacterial cell could be determined by measuring the size of the aberration ring around the cell (for more details see Note 12 in the electronic supplementary material).

(b) Simulations

For completeness of our analysis, we used computer simulations of known mathematical models for the chemical pathways of *S. marcescens* to capture their dynamics through simulations in addition to experimental data. The main reason for using simulations beside real experiments is the ergodicity test. For performing the ergodicity test and measuring the time-averaged mean square displacement and the ensemble mean square displacement, we need at least 1000 trajectories of exactly the same bacteria starting with exactly the same initial condition. Owing to constraints on experimental conditions, we are not able to perform the same experiment 1000 times exactly with the same initial conditions to track exactly the same bacteria in all of them. To tackle this issue, we used BNSim [38], an open-source, parallel multi-scale stochastic modelling platform integrating various simulation algorithms with genetic circuits and a chemotaxis pathway modelled in a complex three-dimensional environment (for the detailed mathematical model of chemotaxis pathway, see Note 6 in the electronic supplementary material). Specifically, to simulate the chemoreceptors, we used the Monod–Wyman–Changeux model in which the receptor homodimers assemble into fully cooperative signalling teams that switch rapidly between active and inactive states [39]. The methylation kinetics is based on the celebrated Barkai and Leibler model for a near-perfect adaptation system [40,41]. For the signal transduction from chemoreceptor to the flagella motor regulator Yp, the concentration of phosphorylated CheYp is assumed to be proportional to the kinase activity without considering the nonlinear dependence [42]. Finally, we used a two-state model to describe the motor behaviour of bacteria, which sets the clockwise (CW) and counterclockwise (CCW) states in two potential wells [43,44]. The transition rates have been fitted to experimental data [27,28,45]. For the interactions among cells, we consider volume exclusion effects and also autochemotaxis (chemical interaction) between the bacteria inside the swarm in all of our simulations. Autochemotaxis happens when bacteria excrete the converted substrate

succinate molecules in the environment into chemoattractant aspartate molecules [26,46–48]. Once the succinate gets depleted, bacteria start consuming the chemoattractant aspartate excreted by themselves and they may detect each other's presence through this process [24]. Of note, this form of chemical interaction has been defined as *cue* in the chapter entitled Communication in bacteria by Diggle and his co-workers in [49]. (Note 10 in electronic supplementary material explains how autochemotaxis has been considered in the computational model).

We performed simulation for *S. marcescens* with two different speeds considering three different cases as follows. For some cases we used a higher swimming speed that is more appropriate to *Escherichia coli* than to *S. marcescens* (simulation cases 1 and 2). The simulation of the chemical pathway is the same for all the cases.

The simulations have been done for three different cases.

(i) Case 1

We used a cubic environment with dimension $5000 \times 5000 \times 5000 \mu\text{m}^3$ (figure 1a). The whole environment was subdivided into 10^6 smaller cubes, each with a size of $50 \times 50 \times 50 \mu\text{m}^3$. The rationale for using these small cubes was to capture bacterial interaction with nearby bacteria and the environment efficiently. The three-dimensional space is therefore tessellated in cubes, while a bacterium is a sphere with a radius of $1 \mu\text{m}$. We also used these smaller cubes to model obstacles in our simulations. The way we implement the obstacles in our simulation is by making these small cubes impenetrable; thus, when a bacterium approaches one of these cubes, it cannot continue swimming and so it has to choose a new direction for runs. Simulated bacteria had a constant run speed of $14 \mu\text{m s}^{-1}$ and a rotational diffusion constant of $0.062 \text{ rad}^2 \text{ s}$ [50,51]. After each tumble, cells were reoriented in a new direction that is randomly sampled from a gamma distribution with a scale parameter of 18.32 and a shape parameter of 4, matching the experimentally observed distribution of new run angle.

In this set of simulations, different population densities of $8 \times 10^2 \text{ bacteria cm}^{-3}$, $8 \times 10^3 \text{ bacteria cm}^{-3}$ and $8 \times 10^4 \text{ bacteria cm}^{-3}$ in the environment were considered. These population densities correspond to a total number of 10^2 bacteria, 10^3 bacteria and 10^4 bacteria, respectively. To study the effect of chemotaxis on the environment, we considered a steady-state linear gradient ($10^{-4} \text{ mM } \mu\text{m}^{-1}$) of chemoattractant (aspartate) in the y -direction. We simulated each bacterial density considering two different conditions: with the gradient of chemoattractant in the environment and without the gradient of chemoattractant in the environment.

Besides population density of bacteria, existence of obstacles will affect bacterial motion. In realistic scenarios of using bacteria for drug delivery *in vivo*, the swarm dynamics will be affected by collisions with existing obstacles. For instance, during the drug delivery task, the existing cells and biological residues act as obstacles and interfere with the swimming bacteria. To analyse the effect of obstacles on bacterial motion, we considered 0.01% of the small cubes uniformly distributed in the environment to be impenetrable (i.e. totally 10^4 number of cubes are impenetrable); thus, when the bacterium hits one of these cubes, it cannot continue to enter the cube and it will continue tumbling to choose a new direction for its run (Note 8 in electronic supplementary material explains how the obstacles have been applied to BNSim in more detail).

It is important to mention that the obstacles' effect on single bacterium motion is different from the impact of bacteria population density based on volume exclusion effects. Each bacterium tries to avoid collision with the obstacles around it, meaning that the obstacles affect single bacterium motion. On the other hand, bacteria population density also affects bacterium motion (directly and indirectly). This means each bacterium modifies its motion to preventing collision with other bacteria (directly). Moreover, the chemical interaction between the bacteria based on their population density will affect the motion of each bacterium (indirectly). In other words, autochemotaxis between the bacteria is dependent on bacteria population density. Therefore, it is important to investigate the effects of obstacles and bacterial density on single bacterium motion separately based on the differences in the nature of their effects.

(ii) Case 2

In this case, we had the same set-up for environment and simulated bacteria as for case 1. The only difference was in the population densities we simulated and the chemoattractant gradient in the environment.

In this case, we considered a wider range of population densities than in case 1, namely 8×10^2 bacteria cm^{-3} , 8×10^3 bacteria cm^{-3} , 8×10^4 bacteria cm^{-3} , 8×10^5 bacteria cm^{-3} and 8×10^6 bacteria cm^{-3} in the environment. These population densities correspond to the total number of bacteria, from 10^2 bacteria up to 10^6 bacteria in the simulations. The other significant difference between this case and case 1 was that we considered a steady-state radial gradient (10^{-4} mM μm^{-1}) of chemoattractant (aspartate) from the injection location towards the targeted region, namely $((x^2 + y^2 + z^2)^{0.5} \times 10^{-4} \text{ mM } \mu\text{m}^{-1})$. We simulated each bacterial density considering two different conditions: with the gradient of chemoattractant in the environment and without the gradient of chemoattractant in the environment.

(iii) Case 3

In this case of simulation, we used a different swimming speed for bacteria compared to that of cases 1 and 2. Moreover, we used a different dimension for the environments. We used a three-dimensional environment with dimensions $10\,000 \times 500 \times 150 \mu\text{m}^3$. The whole environment was subdivided into smaller cubes each with a size of $50 \times 50 \times 50 \mu\text{m}^3$. For the simulation, densities of 10^4 bacteria cm^{-3} , 10^5 bacteria cm^{-3} , 10^6 bacteria cm^{-3} and 10^7 bacteria cm^{-3} in the environment were considered. These population densities correspond to total numbers of 7 bacteria, 75 bacteria, 750 bacteria and 7500 bacteria, respectively. To study the effect of chemotaxis in the environment, we considered a steady-state linear gradient (10^{-4} mM μm^{-1}) of chemoattractant (aspartate) in the y -direction. We simulated each bacterial density considering two different conditions: with the gradient of chemoattractant in the environment and without the gradient of chemoattractant in the environment.

3. Results

(a) Investigation of simulated *S. marcescens* swimming dynamics

(i) Exploring the second-order moment of motion with the ergodicity test

The dynamics of a bacterial population is affected by numerous factors, such as the spatio-temporal gradient of chemoattractant within the environment [52,53], bacteria density, volume exclusion [54], the intensity of autochemotaxis [55] and saturation of bacteria receptors. One important consequence of the mentioned dependencies is that the collective motion of bacteria is non-ergodic in nature [56–58]. To investigate this, we performed an ergodicity-breaking test which checks that long-time averages differ from ensemble averages of bacterium displacement. Simply speaking, if the dynamics of a bacterium's motion is non-ergodic, the time-averaged mean square displacement (TAMSD) (i.e. $\overline{\lim_{T \rightarrow \infty} \delta^2(\Delta = t, T)} = (1/T - \Delta) \int_0^{T-\Delta} |r(t + \Delta) - r(t)|^2 dt$) is not equal to the ensemble mean square displacement (EMSD) (i.e. $\langle r^2(t) \rangle = \int r^2 P(r, t) d^3 r = \langle |r(t) - r(0)|^2 \rangle$). In mathematical terms, the system is non-ergodic if $\lim_{T \rightarrow \infty} \delta^2(\Delta = t, T) \neq \langle r^2(t) \rangle$ [59–61]. In the TAMSD formula, Δ and T represent the lag time and the overall measurement time, respectively. In the EMSD formula, $P(r, t)$ is the probability density function to find bacteria at position r at time t (in the TAMSD formula, the overline denotes the time average). It is important to mention that *in silico* simulation of bacteria provides the opportunity of performing the same simulation with exactly the same initial condition over 1000 times and of monitoring the behaviour of an identical bacterium in all simulations. This helps us to calculate the ensemble mean square displacement of a bacterium and to investigate the ergodicity test. Such analysis cannot be done for experiments *in vitro*, which is an advantage of *in silico* simulations to study the dynamics of bacteria.

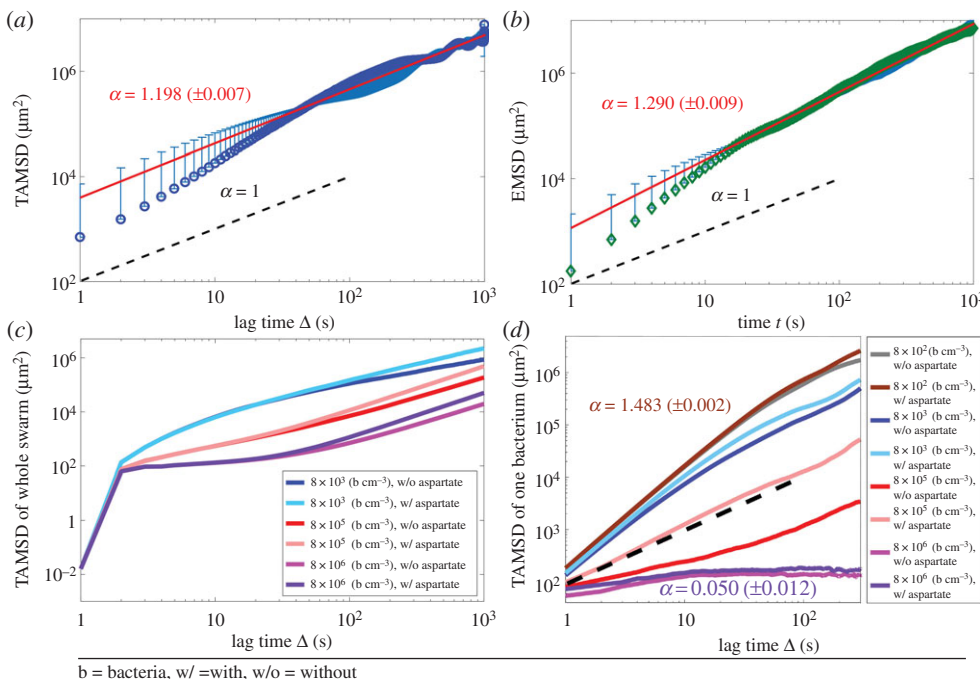


Figure 2. Mean square displacement (MSD) plots for simulated *S. marcescens* in BNSim. (a) Time-averaged MSD of one *S. marcescens* bacterium motion simulated in BNSim as a function of lag time Δ ($\overline{\delta^2(\Delta, T)} = (1/T - \Delta) \int_0^{T-\Delta} |r(t + \Delta) - r(t)|^2 dt$) for a density of 8×10^4 (bacteria cm^{-3}) in an environment with a linear gradient of chemoattractant and without obstacles. This plot shows super-diffusion behaviour ($\text{MSD}(t) = k_\alpha t^\alpha$, $1 < \alpha < 2$) (configuration case 1 in §2). (b) Ensemble average MSD of *S. marcescens* motion simulated in BNSim versus time t calculated from the formula ($\text{MSD}(t) = \langle |r(t) - r(0)|^2 \rangle$) over 100 trajectories for a density of 8×10^4 (bacteria cm^{-3}) with a linear gradient of chemoattractant and without obstacles. This plot also shows super-diffusion behaviour. The observed difference between these two plots (a,b) shows an ergodicity-breaking behaviour in the system and demonstrates the non-ergodicity of bacterial dynamics (configuration case 1 in §2). (c) The TAMSD of the centre of the whole population for *S. marcescens* motion simulated in BNSim shows the effect of population density and chemoattractant on the centre of the bacteria population motion (configuration case 2). (d) The TAMSD of one *S. marcescens* trajectory simulated in BNSim for different cases shows the effect of chemoattractant and density on single bacterium motion (configuration case 2 in §2). By increasing the population density, we observe a phase transient in bacterial behaviour from super-diffusion to normal diffusion and then sub-diffusion (configuration case 2 in §2).

Figure 2a–d summarizes the ergodicity investigations on the simulated bacterial swimming dynamics under various bacterial densities, chemoattractant gradient and autochemotaxis effects. For instance, figure 2a,b show the difference between TAMSD and EMSD plots for a simulated bacterium in a population of 8×10^4 bacteria cm^{-3} in an environment with a linear gradient of chemoattractant and without obstacles except other bacteria. More precisely, the TAMSD for this case when the lag time $t = 10$ (s) is $\overline{\delta^2}(t = 10, T = 1000) = 15\,631$ (μm^2), while the EMSD obtained over 100 trajectories for the same timing conditions is $\langle r^2(t = 10(\text{s}), T = 1000(\text{s})) \rangle = 12\,961$ (μm^2). The difference between the TAMSD and the EMSD in this case is 20%. The TAMSD for a lag time $t = 10$ s is independent of the initial time reference, which means that bacteria will move a distance equal to 125.02 (μm) on average. In contrast, the EMSD for the same time $t = 10$ (s) implies that bacteria will travel (on average) a distance of 113.84 (μm), which has a 10% difference. The significant difference between the TAMSD and the EMSD shows that, for simulated bacteria, the motion is non-ergodic and time-dependent [58]. Comparing the slopes of the lines fitted to both TAMSD and EMSD plots confirms that the single bacterium motion is non-ergodic (the slope of the line fitted to the TAMSD plot is $1.198 (\pm 0.007)$, while that for the EMSD plot is equal to 1.290

(± 0.009)). For completeness, we test all cases for different points in time (electronic supplementary material, table S2). The cases we analysed cover different bacterial densities and chemoattractant distribution in the environment. In all of these cases, the equality between the TAMSD and the EMSD does not hold; this confirms the non-ergodicity of the bacterial motion. In conclusion, the non-ergodicity finding holds for both case studies considered: (i) the effect of bacterial density and (ii) the effect of chemoattractant distribution in the environment.

The non-ergodicity of single bacterium motion follows anomalous diffusion behaviour based on EMSD plots. Generally speaking, the anomalous diffusion is a diffusion process characterized by a nonlinear relationship of the EMSD with time [62–66]. In contrast, a normal diffusion process has an EMSD that varies linearly with time. Figure 2*b* shows bacteria-driven motion displays a *super-diffusion* type of behaviour [67], which can be modelled using a power law form of the second moment with exponent ranging between 1 and 2 (i.e. $\langle \delta^2(\Delta, T) \rangle = K_\alpha \Delta^\alpha$, $1 \leq \alpha \leq 2$) for different bacterial density cases. The *super-diffusive* behaviour of non-ergodic bacterium motion cannot be modelled with a Brownian random walk with an average drift which has ergodic characteristics.

Figure 2*c,d* shows that the single bacterium trajectory and the movement of the centre of the group exhibit super-diffusion behaviour for some cases in our simulations. The super-diffusion behaviour predicts that its corresponding random walk is characterized by a stable distribution [68,69]. To further elucidate this behaviour of the simulated bacteria, we investigate the histogram of the distance travelled by bacteria $P(r, \tau)$ in a specific lag time τ (figure 3*a*). It can be observed that it exhibits a stable type of distribution (electronic supplementary material, table S4). The origin of this behaviour is the lack of nutrient concentration in our simulation model, which leads to a high level of noise in the methylation model. This noisy fluctuation in the methylation model is the reason for the power-law run-length distribution in simulated bacterium motion [68]. Figure 3*a* shows that the side peak of the bacterium displacement histogram $P(r, \tau)$ shifts towards the right-hand side and broadens with time. Based on the Generalized Central Limit Theorem, the histogram will converge to a stable distribution as the lag time τ increases [70,71].

Figure 2*c,d* shows the effect of population density on the TAMSD for different bacterial density cases. The TAMSD variation of the centre of the entire bacterial population as a function of time lag Δ (figure 2*c*) shows that increasing the bacterial density makes the population less motile. Volume exclusion effects and chemical interactions caused by autochemotaxis between bacteria are the main reasons of this behaviour (Note 7 in electronic supplementary material explains how volume exclusion effects have been considered in the computational model).

Figure 2*d* shows the TAMSD of a single bacterium, which is in agreement with the results obtained from the TAMSD of the entire swimming trajectory. One reason for this behaviour is that increasing their population causes a significant increase in the autochemotaxis (Note 10 in electronic supplementary material explains how autochemotaxis has been considered in the computational model). In turn, this may cause receptor saturation which can ‘confuse’ bacteria in choosing its preferred direction of motion in the environment; this means that their motion may become restricted due to their high accumulation in the environment. Hence, the TAMSD of bacterial motion decreases (for the same time interval) with the increase in bacterial density.

Figure 2*d* magnifies the volume exclusion effect for the case of 8×10^6 bacteria cm^{-3} (purple and magenta lines). As one can see, in this case, the bacterium is not able to move and therefore it remains in the neighbourhood of its original location. By increasing the bacterial population up to 8×10^5 bacteria cm^{-3} , we observe a phase transition in their motion from a super-diffusive behaviour to a normal diffusion and lastly to a sub-diffusive pattern (electronic supplementary material, table S3).

Besides the TAMSD plots, the displacement histogram $P(r, \tau)$ shows a higher probability for a longer displacement in the same time interval related to lower bacterial densities (figure 3*b*). When constraints like other bacteria in the environment limit a bacterium’s motion, then the bacterium tends to oscillate more and randomly move in the environment rather than moving directionally.

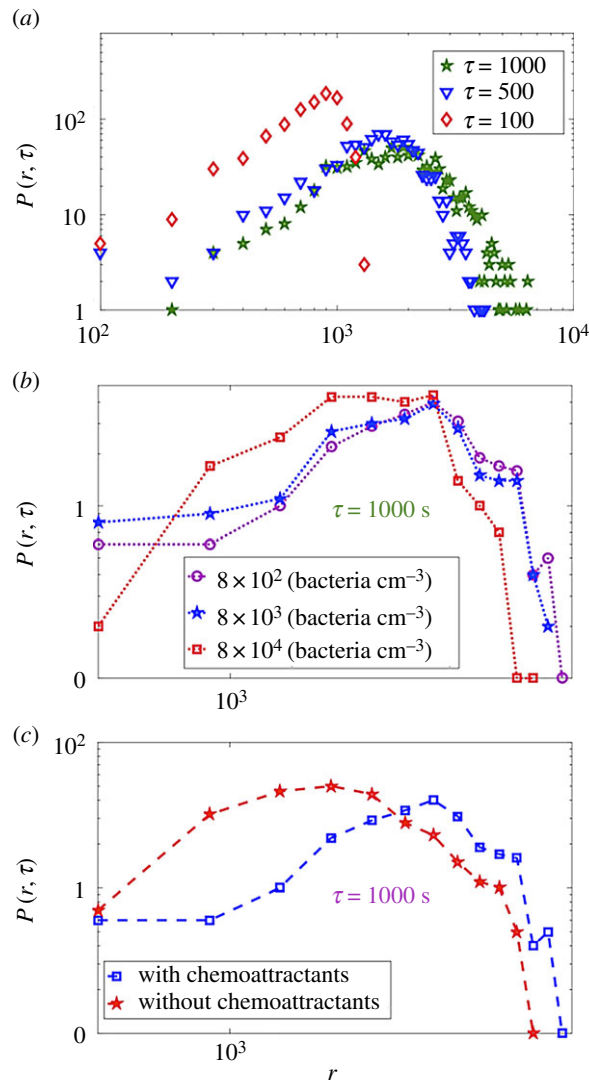


Figure 3. Effects of time, bacteria population density and chemoattractant on *S. marcescens* motion simulated in BNSim. (a) Effect of time on bacterial motion. Log–log plot of $P(r, \tau)$ as a function of distance r for different lag times $\tau = 100$ s, 500 s, 1000 s. This result is for 8×10^2 bacteria cm^{-3} in an environment without chemoattractant and obstacles. This plot shows that the bacteria move more with time and get further away from their initial locations. The peak of the plot decreases more (configuration case 1 in §2). This plot exhibits a stable type of distribution (electronic supplementary material, table S4). (b) Effect of density on bacterial motion. Log–log plot of $P(r, \tau)$ for lag time $\tau = 1000$ s compared for different bacterial densities simulated in BNSim in an environment with chemoattractant gradients and without obstacles. By increasing the bacterial population, their motion will be restricted and they are able to move less freely in the environment (configuration case 1 in §2). (c) Effect of chemoattractant on bacterial motion. Log–log plot of $P(r, \tau)$ for lag time $\tau = 1000$ s compared for the case with and without chemoattractant in the environment for the population of 8×10^2 bacteria cm^{-3} . By adding chemoattractant in the environment, the bacteria tend to move more and oscillate less, so they get further away from their initial conditions in the direction of increasing gradient of chemoattractant (configuration case 1 in §2).

We also study the effect of L-aspartate gradient on the TAMSD of bacterial motion trajectory. Figure 2c,d shows that by adding L-aspartate gradient (configuration case 2 in §2) to the environment, independent of bacterial density, the TAMSD will increase for both a single bacterium and an entire population for simulated bacteria. Moreover, figure 3c presents the

histogram of displacement of bacteria after lag time τ (i.e. $P(r, \tau)$) for the case with/without L-aspartate gradient in the environment. This figure shows that the probability of covering longer displacements (after the same time lag) by bacteria is higher in the case of L-aspartate gradient in the environment, meaning that bacteria tend to move directionally along a targeted direction and oscillate less when they sense a chemoattractant gradient in their environment while their instantaneous speed stays the same (figure 3c).

Up to this point, we focus on the non-ergodicity of single bacterium motion by investigating the second-order moment of their motion under different environmental condition. In what follows, we shift our focus to the third-order moment of their motion by performing a nonlinearity test.

(ii) Exploring the third-order moment of motion with nonlinearity test

Details about the first and second moments of the bacterial trajectory (corresponding to mean square displacement of bacterial motion) do not provide enough information regarding the complexity of their dynamics. Consequently, we analyse the higher-order moments of a bacterium's trajectory by investigating the skewness metric. Skewness represents the third-order moment of bacterium motion and helps to investigate whether or not the motion increments (i.e. changes in the spatial position of the bacterium in three-dimensional space between two different time instances) obtained from the bacterium trajectory obey a multivariable normal distribution in the three-dimensional environment. Non-zero skewness shows that the probability density function of motion increment does not follow the normal distribution and this is a signature of nonlinearity [72].

Using the Mardia method [72–74], we measured the multivariate skewness of our data for simulated bacteria (see electronic supplementary material, equation (2)). The non-zero highly variable skewness plots demonstrate that bacterial motion cannot be modelled by a multivariable normal distribution for all the cases under consideration (i.e. with and without chemoattractant and obstacles in the environment); this confirms the nonlinear behaviour (skewness plot in electronic supplementary material, figure S1).

To provide a more comprehensive analysis on the existence of nonlinear behaviour, we employed several statistical methods like Henze–Zirkler's multivariate normality test [75–77], Royston's multivariate normality test [78–80] and Doornik–Hansen omnibus multivariate normality test [81,82]. Electronic supplementary material, table S1 summarizes the results for these tests and further demonstrates the nonlinearity of simulated bacterium dynamics.

(iii) Exploring higher-order moment of motion using multi-fractal analysis

Traditional analysis of bacterial motion has focused on measuring the root mean squared fluctuations of the displacement and thus is limited to first- and second-order moment analysis. However, relying on the second-order statistics not only is inconsistent with the observed non-Gaussian behaviour, but also it does not capture the entire spatial and temporal complexity structure of the interactions among bacteria and the environment. Consequently, we employ concepts from statistics (e.g. higher-order moments) and fractal theory (e.g. multi-fractal spectrum, generalized Hurst exponent) to perform a multi-fractal [83,84] analysis and quantify the correlation structure and complexity of bacterium trajectories (see Note 5 in electronic supplementary material for more details). More precisely, we compute the generalized Hurst exponent as a function of the higher-order moment q and the multi-fractal spectrum. The generalized Hurst exponent of a bacterium trajectory measures the scaling and memory properties of the q th order moment of the distribution of fluctuations. For example, the generalized Hurst exponent for $q=2$ coincides with the autocorrelation function. In addition, the generalized Hurst exponent helps us distinguish between mono-fractal and multi-fractal behaviour. If the generalized Hurst exponent proves to be independent of the order q of the moments, then the bacterium motion can be classified as mono-fractal. In contrast, if the generalized Hurst exponent exhibits any dependence on order q , then the bacterium motion

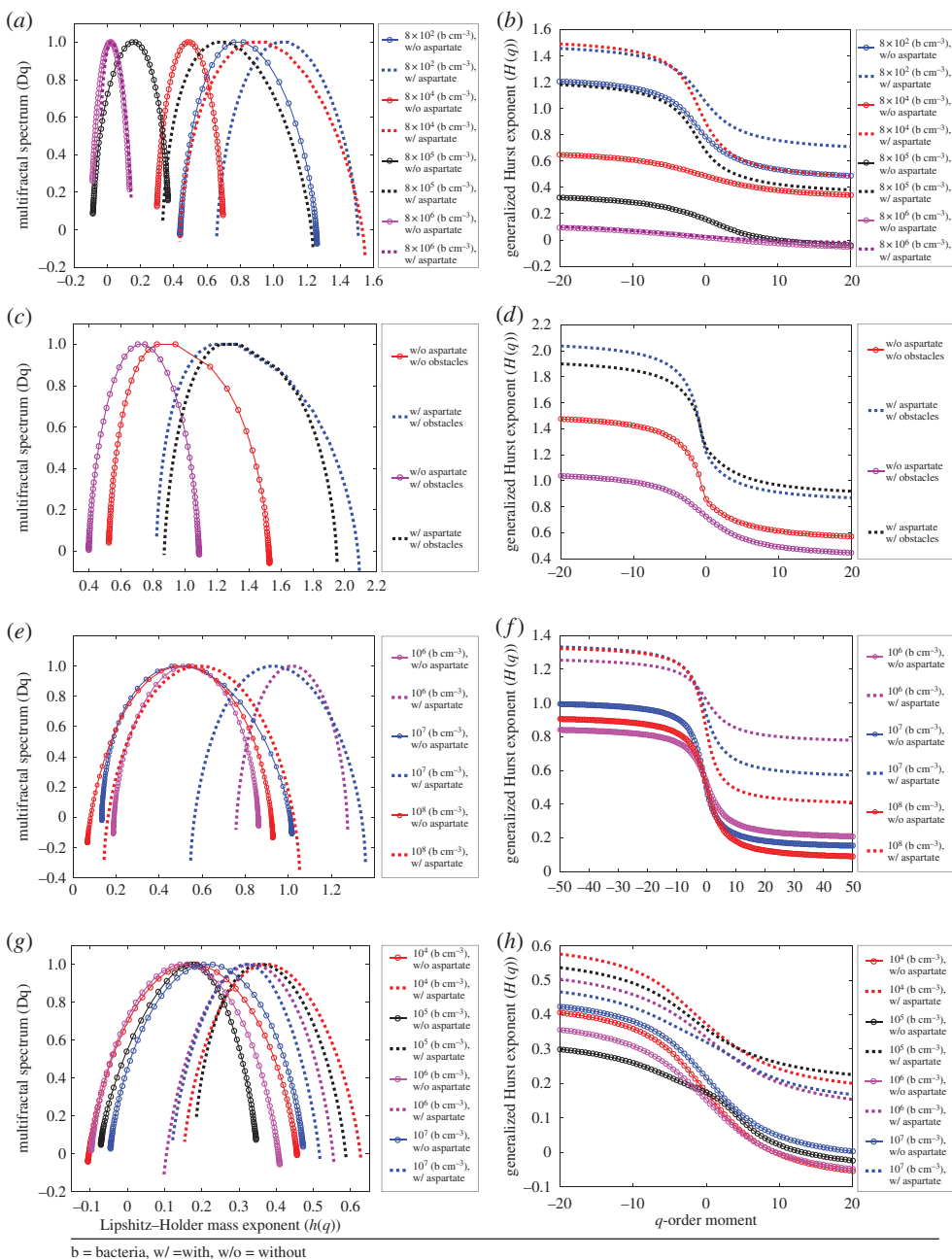


Figure 4. (Caption opposite.)

is regarded as multi-fractal. Alternatively, we can investigate the existence of a multi-fractal behaviour by analysing the wideness of the multi-fractal spectrum (i.e. $W = h(q)_{\max} - h(q)_{\min}$ estimating the range of present Holder exponents in the data). A wider multi-fractal spectrum shows that the multi-fractality degree is stronger, meaning that the motion structure is more complex and inhomogeneous. Therefore, a wider range of fractal exponents are needed to cover the complex structure of the bacterium motion. Another measure for multi-fractality degree is the most probable Holder exponent $h(q)$ corresponding to the peak of the multi-fractal spectrum (i.e. $h_0(q)$). Low $h_0(q)$ shows that the motion of bacteria is more correlated and regular in appearance,

Figure 4. (Opposite.) Multi-fractal analysis of motion for simulated *S. marcescens* in BNSim and *S. marcescens* *in vitro* experiments. (a) Multi-fractal spectrum of the simulated *S. marcescens* position in the direction of the linear gradient of chemoattractant for different cases with and without chemoattractant and different bacterial densities (configuration case 2 in §2). (b) Generalized Hurst exponent of the simulated *S. marcescens* position in the direction of the linear gradient of chemoattractant for different cases with and without chemoattractant and different bacterial densities (configuration case 2 in §2). (c) Multi-fractal spectrum of the simulated *S. marcescens* position in the direction of the linear gradient of chemoattractant with a density of 8×10^4 bacteria cm^{-3} for different cases with and without chemoattractant and obstacles in the environment (configuration case 1 in §2). (d) Generalized Hurst exponent of the simulated *S. marcescens* position in the direction of the linear gradient of chemoattractant with a density of 8×10^4 bacteria cm^{-3} for different cases with and without chemoattractant and obstacles in the environment (configuration case 1 in §2). (e) Multi-fractal spectrum of the experiments *in vitro* on *S. marcescens* position in the direction of the linear gradient of chemoattractant for different cases with and without chemoattractant and different bacterial densities. (f) Generalized Hurst exponent of the experiments *in vitro* on *S. marcescens* position in the direction of the linear gradient of chemoattractant for different cases with and without chemoattractant and different bacterial densities. (g) Multi-fractal spectrum of simulated *S. marcescens* position in the direction of the linear gradient of chemoattractant for different cases with and without chemoattractant and different bacterial densities (configuration case 3 in §2). (h) Generalized Hurst exponent of simulated *S. marcescens* position in the direction of the linear gradient of chemoattractant for different cases with and without chemoattractant and different bacterial densities (configuration case 3 in §2).

meaning that the motion is mono-fractal. Overall, multi-fractal analysis is an applicable tool to characterize the variability and heterogeneity in bacterium motion structure [85,86].

Figure 4*a–d* presents our results from the multi-fractal detrended fluctuation analysis [87,88] on motion trajectories of simulated *S. marcescens* in BNSim for different bacterial densities with and without the L-aspartate gradient in the environment (see simulation section in §2). Based on the simulation results (figure 4*a*), we observe that increase in the density of bacteria decreases the width of the multi-fractal spectrum (W) and decreases ($h_0(q)$) as two different measures of the degree of multi-fractality. For instance, in the case of chemoattractant in the environment, the wideness (W) of the multi-fractal spectrum is shrinking from $W = 0.851$ to $W = 0.182$ as the population of bacteria increases from 8×10^2 (bacteria cm^{-3}) to 8×10^6 (bacteria cm^{-3}). Similarly, we observe that the most probable Holder exponent $h_0(q)$ is shifting from 1.06 to 0.02 by increasing bacterial density from 8×10^2 (bacteria cm^{-3}) to 8×10^6 (bacteria cm^{-3}). The comparison shows that by increasing bacterial density, the width of the multi-fractal spectrum decreases; this implies that the bacterium motion dynamics transitions from a multi-fractal behaviour to a mono-fractal behaviour. Simply speaking, the bacterium oscillates more than exhibiting a directional motion. This transition translates into a reduction in $h_0(q)$. The lower $h_0(q)$ shows that the process is correlated, meaning that the underlying structure in bacterium motion becomes more regular by increasing the bacterial population. The increase in bacterial population makes their multi-fractal spectrum asymmetric, which captures the dominance of low- or high-fractal exponents with respect to medium fractal exponents. In the case of 8×10^6 (bacteria cm^{-3}) with chemoattractant in the environment, the right-skewed spectrum shows relatively strong weight for low-fractal exponents.

From figure 4*a,b*, we can conclude that adding chemoattractant to the environment has exactly the opposite effect on a single bacterium's motion compared to the effect of the increase in density. For example, we considered the two cases of bacterial population equal to 8×10^2 (bacteria cm^{-3}) without and with chemoattractant in the environment. The wideness (W) increases from 0.822 to 0.851 when we add chemoattractant to the environment. Similarly, the most probable Holder exponent $h_0(q)$ shifts from 0.78 to 1.06 by adding the chemoattractant. This implies that in an environment rich in chemoattractant, the multi-fractal spectrum related to a bacterium motion gets a wider shape with higher values of $h_0(q)$, and this is true for all the density cases we analysed except the highest density equal to 8×10^6 (bacteria cm^{-3}). In this case, because of the very high density of bacteria in the environment, the volume exclusion effects and the receptor saturations, there is no significant difference in $h_0(q)$ between the cases with and without

chemoattractant and both of them are mono-fractal. In other words, adding chemoattractant to the environment impacts bacterial motion in a way that makes their motion more directional and less oscillatory. Figure 4c,d demonstrates the effect of obstacles in the environment for simulated bacteria (Note 8 in the electronic supplementary material discusses the importance of studying the effects of obstacles and also how they are considered in the computational model). We compared the case of bacterial density 8×10^4 bacteria cm^{-3} without chemoattractant and with obstacles ($W = 1.004$ and $h_0(q) = 0.88$) with the case of the same density with chemoattractant gradient and without obstacles ($W = 0.6918$ and $h_0(q) = 0.72$). In an environment with obstacles, the multi-fractal spectrum shifts towards lower values of $h_0(q)$ and narrower spectrum widths. In such an environment, the bacterium has the tendency to oscillate more than to follow a directional motion.

(b) Investigation of real *S. marcescens* swimming dynamics through *in vitro* experiments

We extract the *S. marcescens* motion trajectories from real experiments by the single particle tracking method and analyse them. The results from skewness analysis show the same nonlinear behaviour for the bacterium motion (electronic supplementary material, figure S1). This is in agreement with the previously presented results for simulated *S. marcescens*, meaning that the motion of *S. marcescens* is highly nonlinear in the environment.

Figure 4e,f shows the multi-fractal analysis for motion trajectories of *S. marcescens* from experiments *in vitro*. The results elucidate that *S. marcescens* has a multi-fractal motion in these environments. For the same population density, by adding chemoattractant to the environment the spectrum moves towards the right, which is a sign of multi-fractal behaviour. We observe a similar but not identical multi-fractal behaviour from real *S. marcescens* compared to simulated *S. marcescens*. The reason the results do not match exactly is that for the simulated *S. marcescens* we have control over most of the internal and environmental parameters. In contrast, real *S. marcescens* like other biological systems in nature show a higher degree of adaptation such as adjustment to environmental conditions, self-organization and complexity in their behaviour. Another reason for the difference between the simulation and experiment could be attributed to other forms of interactions that happen in the biological world and are not captured in the current computational model. However, if someone discovers new forms of interaction, they could be included in the computational model. This remains for future work.

4. Discussion

Bacteria like *S. marcescens* perform an efficient diffusive search (based on the status of their receptor signals) to find food in a complex environment [27]. In this study, we investigate the fractal characteristics of the bacterium motion while swimming in a three-dimensional environment. We first analyse the second- and third-order moment of the single bacterium motion trajectory with the ergodicity and nonlinearity tests under different conditions including bacterial density and chemoattractant distribution. These tests help building the bases for higher-order moment analysis of single bacterium motion through the MF DFA method to capture the entire spatial and temporal complexity of single bacterium motion. Based on MF DFA analysis, we show that bacterial swimming dynamics has multi-fractal characteristics. Moreover, we study how bacterial density and complexity of the environment (i.e. obstacles and chemoattractant) influence the multi-fractal characteristics of bacterial swimming dynamics. Consequently, our results contribute to the current state of knowledge by demonstrating that bacterial dynamics exhibits multi-fractal characteristics.

The effect of chemoattractant is one of the main environmental conditions affecting the multi-fractal characteristics of bacteria. Multi-fractal analysis shows that in the presence of an L-aspartate gradient in the environment, the multi-fractal spectrum gets wider and moves towards the right, meaning that bacteria tend to move directionally and oscillate less compared to the case without a chemoattractant gradient being present in the environment.

We also consider the effect of bacterial density as another important factor that affects the multi-fractal characteristics of bacteria. Our results show that increasing the density of bacteria makes the multi-fractal spectrum of their motion shift towards the left and transit to a mono-fractal behaviour. Moreover, further studies would be needed to relate these multi-fractal properties with the observed dependency between shear viscosity and density of the motile bacteria [89].

Previous research has shown that bacterial colonies display spatial fractal properties [90]. Ben-Jacob & Levine studied the spatial fractal properties of *Paenibacillus dedritiformis* bacteria colonies under the effect of the antibiotic Septrin [91]. They show that, in some patterns, the members of the colony stay closer together and form large vortices, which can increase the colony's ability to dilute the antibiotic with the lubricating fluid secreted by individual microbes; on the other hand, in some patterns their colony organizes into narrow and straight branches. This pattern happens when the food is scattered in the environment and will maximize bacterial contact with the limited nutrients in their environment. It is essential to emphasize on the point that, in contrast to previous research, we investigate the multi-fractal characteristics of the bacterium motion while swimming in a three-dimensional environment in this study.

This multi-fractal characteristic of the bacterium dynamics can get affected by continuous interactions among bacteria within the population. Moreover, this multi-fractal dynamic characterization of bacterial motion can allow us to quantify the effects of molecular (drugs) and gene-based therapies by investigating whether or not cells exhibit a directed movement as a result of the detection and transduction of external stimuli or continue to perform random wanderings. Interestingly, stem cell dynamics also exhibit a rich multi-fractal behaviour, although stem cells do not use flagella or cilia for locomotion. This suggests that this mathematical framework can be possibly used for identifying universal mathematical characteristics for cell motion irrespective of the size, scale and nature of interactions [92].

A comprehensive understanding of individual and collective movement of cells can be important for developing mathematical models of cell growth that can abstract and account for biologically relevant features such as cell density and volume exclusion effects, mechanical forces acting on cells surface, environmental conditions (e.g. pH, temperature), chemical factors (e.g. chemo-attractants, chemo-repellents), as well as factors that affect the internal state (e.g. cell-cell communication, mitotic phase, cell health state, cell age). For instance, the above-mentioned mathematical characterization can help decide the type of dynamical models (short-term memory versus long-term memory), the level of detail at which individual cells must be described (how much of the internal state needs to be modelled), or the effect of various external stimuli on an individual cell and collections of cells.

Overall, this newly identified mathematical property can enable the microscopic description of the motion of individual cells through concepts and tools from non-equilibrium statistical physics (e.g. multi-fractal master equation). Integrating over the population density and the characterized effect of internal and external stimuli could enable us to construct macroscopic models and propose control strategies for the population of cells in complex environments to reach specific targets [93–95]. An equally important research problem is to understand not only how the multi-fractal features allow for a compact multi-scale mathematical description, but also how they enable and simplify the controllability of complex systems [96,97]. This is left for future work.

Data accessibility. Bacteria trajectory data (data are available in the following link: <https://drive.google.com/file/d/0B449CRbDSlIiM3JmUnBxSDg1OWs/view?usp=sharing>).

Authors' contributions. H.K., P.B., G.W., R.M., J.Z., R.W.C. and M.S. contributed to the discussion, design of the experiments and writing of the manuscript. H.K. and P.B. contributed to developing the statistical analysis of the biological and computational experimental data. H.K. prepared the figures, wrote their captions, and organized the information in both main text and electronic supplementary material. J.Z., R.W.C. and M.S. performed the biological experiments, and analysed and created the biological datasets. H.K., G.W. and R.M. contributed to the design of the computational experiments, saving and extracting the simulated datasets.

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Dealing with Unknown Unknowns: Identification and Selection of Minimal Sensing for Fractional Dynamics with Unknown Inputs

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Abstract—This paper focuses on analysis and design of time-varying complex networks having fractional order dynamics. These systems are key in modeling the complex dynamical processes arising in several natural and man made systems. Notably, examples include neurophysiological signals such as electroencephalogram (EEG) that captures the variation in potential fields, and blood oxygenation level dependent (BOLD) signal, which serves as a proxy for neuronal activity. Notwithstanding, the complex networks originated by locally measuring EEG and BOLD are often treated as isolated networks and do not capture the dependency from external stimuli, e.g., originated in subcortical structures such as the thalamus and the brain stem. Therefore, we propose a paradigm-shift towards the analysis of such complex networks under unknown unknowns (i.e., excitations). Consequently, the main contributions of the present paper are threefold: (i) we present an alternating scheme that enables to determine the best estimate of the model parameters and unknown stimuli; (ii) we provide necessary and sufficient conditions to ensure that it is possible to retrieve the state and unknown stimuli; and (iii) upon these conditions we determine a small subset of variables that need to be measured to ensure that both state and input can be recovered, while establishing sub-optimality guarantees with respect to the smallest possible subset. Finally, we present several pedagogical examples of the main results using real data collected from an EEG wearable device.

I. INTRODUCTION

A time-varying complex network with fractional dynamics has node activities over time influenced by its own history and also by activities of the other nodes. The systems with such attributes enables the modeling of coupled non-stationary processes that exhibit long-term memory [1]–[5]. A multitude of examples can be found in several application domains such as biology, and engineering, e.g. bacteria dynamics [6]–[8] and swarm robotics [9], [10], respectively. Nonetheless, their applicability becomes even more useful in the context of heterogeneous networks that interact geographically and across different time-scales, as it often occurs in the context of cyber-physical systems (CPS).

In the context of the present manuscript, we are motivated by the recent success of fractional order dynamics in modeling spatiotemporal properties of physiological signals, such as electrocardiogram (ECG), electromyogram (EMG), electroencephalogram (EEG) and blood oxygenation level dependent (BOLD) just to mention a few [11], [12]. Despite these modeling capabilities, there is one main limitation that continues to elude scientists and engineers alike. Specifically,

complex networks such as the brain, whose nodes will dynamically evolve using fractal order dynamics, are often observed locally. Meaning that some of the dynamics assessed by the models are not only due to the local interaction, but might be constrained by unknown sources, i.e., stimuli that are external to the considered network. Consequently, we propose a model that enables us to account for the existence of such unknown stimuli, and determine the model that best captures the local dynamics under such stimuli. Observe that this enhances the analysis of these systems once we have an additional feature (i.e., the stimuli) that can be the main driver of a possible abnormal behavior of the network [13].

In the context of neuroscience and medical applications, the ability to determine unknown inputs is crucial in the retrieval of the model under the so-called input artifacts [14] captured in the EEG readings due to a pulse that causes a higher variation of the potential than the baseline. Alternatively, the existence of a model to cope with the presence of unknown inputs enable the modeling of stimuli that are originated in the subcortical structures of the brain that are often neglected in the current EEG and functional magnetic resonance imaging (fMRI) that leverages the BOLD signals [15]. Thus, it is imperative to develop such framework, as well as tools that enable us to perform the analysis and design of the systems modeled by such setup, which we introduce in the current paper.

To the best of the authors' knowledge, such framework has not been advanced in the context of discrete-time fractional order dynamics. In the domain of continuous-time fractional dynamics, works like [16], [17] exist for the design of observer in the presence of unknown inputs. The closest work for the discrete-time case is [18], which does not consider the case of unknown inputs. Nonetheless, the usefulness of accounting for unknown inputs in the context of linear time invariant (LTI) systems is an old topic [19]–[24]. Specifically, the closest papers to the results proposed in this paper are as follows: observer with unknown inputs [20], delayed systems with unknown inputs [24], estimation of unknown inputs with sparsity constraints [25]. Notwithstanding, LTI systems are not good approximations for fractional order dynamical systems due to their limited memory capabilities. Yet, due to the numerical approximation of the highly nonlinear infinite dimension fractional order system used, we are able to obtain finite dimension closed-form description that will enable us to derive results alike those attained in the context of LTI systems.

The main contributions of the present paper are threefold: (i) we present an alternating scheme that enables to determine the best estimate of the model parameters and unknown stim-

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uli; and (ii) we provide necessary and sufficient conditions to ensure that it is possible to retrieve the state and unknown stimuli; and (iii) upon these conditions we determine a small subset of variables that need to be measured to ensure that both state and input can be recovered, while establishing suboptimality guarantees with respect to the smallest possible subset.

The remaining of the paper is organized as follows. Section II introduces the model considered in this paper and the main problems studied in this manuscript. Next, in Section III and IV, we present the solution to these problems. Finally, in Section V, we present an illustrative example of the main results using real EEG data from a wearable technology.

II. PROBLEM FORMULATION

In this section, we first describe the time-varying complex network model having fractional order dynamical growth under unknown excitations. Next, upon this model, we propose two main problems regarding analysis and design to be addressed in the present paper.

A. System Model

We consider a linear discrete time fractional-order dynamical model described as follows:

$$\begin{aligned}\Delta^\alpha x[k+1] &= Ax[k] + Bu[k] \\ y[k] &= Cx[k],\end{aligned}\quad (1)$$

where $x \in \mathbb{R}^n$ is the state, $u \in \mathbb{R}^p$ is the unknown input and $y \in \mathbb{R}^n$ is the output vector. Also, we can describe the system by its matrices tuple (α, A, B, C) of appropriate dimensions. In what follows, we assume that the input size is always strictly less than the size of state vector, i.e., $p < n$. The difference between a classic linear time-invariant and the above model is the inclusion of fractional-order derivative whose expansion and discretization [26] for any i th state ($1 \leq i \leq n$) can be written as

$$\Delta^{\alpha_i} x_i[k] = \sum_{j=0}^k \psi(\alpha_i, j) x_i[k-j], \quad (2)$$

where α_i is the fractional order corresponding to the i th state and $\psi(\alpha_i, j) = \frac{\Gamma(j-\alpha_i)}{\Gamma(-\alpha_i)\Gamma(j+1)}$ with $\Gamma(\cdot)$ denoting the gamma function.

Having defined the system model, the system identification i.e. estimation of model parameters from the given data is an important step. It becomes nontrivial when we have unknown inputs since one has to be able to differentiate which part of the evolution of the complex network is due to its intrinsic dynamics and what is due to the unknown inputs. Subsequently, one of the first problems we need to address is that of system identification from the data, as described next.

B. Data-driven Model Estimation

The fractional-order dynamical model takes care of long-range memory which often is the property of many physiological signals. The estimation of the spatiotemporal parameters when there are no inputs to the system was addressed

in [18]. But as it happens, ignoring the inputs inherently assume that the system is isolated from the external surrounding. Hence, for a model to be able to capture the system dynamics, the inclusion of unknown inputs is necessary. Therefore, the first problem that we consider is as follows.

Problem-1: Given the input coupling matrix B , and measurements of all states across a time horizon $[t, t+T-1]$ of length T , we aim to estimate the model parameters (α, A) and the unknown inputs $\{u[k]\}_t^{t+T-2}$.

Notice that this would extend the work in [18] to include the case of unknown inputs. In fact, we will see in Section III that the proposed solution would result in a different set of model parameters.

C. Sensor Selection

For the system model described by (1), where the system parameters were determined as part of the solution to the *Problem-1*, we consider that the output measurements are collected only by a subset of sensors. In numerous applications (for example physiological systems) it happens that the sensors are dedicated, i.e., each sensor capture an individual state [27], so the measurement model can be written as

$$y[k] = \mathbb{I}^S x[k], \quad (3)$$

where $\mathbb{I}^S$ is the matrix constructed by selecting rows indexed by set S of the $n \times n$ identity matrix I_n . As an example, if all sensors are selected, i.e., $S = [n] \equiv \{1, 2, \dots, n\}$, then $\mathbb{I}^S = I_n$. For selecting the best set of sensors S , with knowledge of the system matrices and the given observations, we would resort to the constraint of *perfect observability* that is defined as follows.

Definition 1 (Perfect Observability). *A system described by (1) is called perfectly observable if given the system matrices (α, A, B, C) and K observations $y[k], 0 \leq k \leq K-1$, it is possible to recover the initial state $x[0]$ and the unknown inputs $\{u[k]\}_{k=0}^{K-2}$.*

Subsequently, the second problem that we consider is as follows. **Problem-2:** Determine the minimum number of sensors S such that the system whose dynamics is captured by $(\alpha, A, B, \mathbb{I}^S)$ is perfectly observable from the collection of K measurements collected by a sub-collection of S dedicated outputs, i.e.,

$$\begin{aligned} \min_{S \subseteq [n]} |S| \\ \text{s.t. } (\alpha, A, B, \mathbb{I}^S) \text{ is perfectly observable.} \end{aligned} \quad (4)$$

In section IV, we will derive the mathematical formulation in terms of algebraic constraints of the perfect observability, which later be used to obtain a solution to (4).

III. MODEL ESTIMATION

We consider the problem of estimating α , A and inputs $\{u[k]\}_t^{t+T-2}$ from the given limited observations $y[k], k = [t, t+T-1]$, which due to the dedicated nature of sensing mechanism is same as $x[k]$ and under the assumption that the input matrix B is known. The realization of B can be application dependent and is computed separately using experimental data. For the simplicity of notation, let us

denote $z[k] = \Delta^\alpha x[k+1]$ with k chosen appropriately. The pre-factors in the summation in (2) grows as $\psi(\alpha_i, j) \sim \mathcal{O}(j^{-\alpha_i-1})$ and, therefore, for the purpose of computational ease we have limited the summation in (2) to the first J values, where $J > 0$ is sufficiently large. Therefore, $z_i[k]$ can be written as

$$z_i[k] = \sum_{j=0}^{J-1} \psi(\alpha_i, j) x[k+1-j], \quad (5)$$

with the assumption that $x[k], u[k] = 0$ for $k \leq t-1$. Using the above introduced notations and the model definition in (1), the given observations are written as

$$z[k] = Ax[k] + Bu[k] + e[k], \quad (6)$$

where $e \sim \mathcal{N}(0, \Sigma)$ is assumed to be Gaussian noise independent across space and time. For simplicity, we have assumed that each noise component has same variance, i.e., $\Sigma = \sigma^2 I$. Also, let us denote the system matrices as $A = [a_1, a_2, \dots, a_n]^T$ and $B = [b_1, b_2, \dots, b_n]^T$. The vertical concatenated states and inputs during an arbitrary window of time as $X_{[t-1, t+T-2]} = [x[t-1], x[t], \dots, x[t+T-2]]^T$, $U_{[t-1, t+T-2]} = [u[t-1], u[t], \dots, u[t+T-2]]^T$ respectively, and for any i th state we have $Z_{i, [t-1, t+T-2]} = [z_i[t-1], z_i[t], \dots, z_i[t+T-2]]^T$. For the sake of brevity, we would be dropping the time horizon subscript from the above matrices as it is clear from the context.

Since the problem of joint estimation of the different parameters is highly nonlinear, we proceed as follows: (i) we estimate the fractional order α using the wavelet technique described in [28]; and (ii) with α known, the z in (5) can be computed under the additional assumption that the system matrix B is known. Therefore, the problem now reduces to estimate A and the inputs $\{u[k]\}_t^{t+T-2}$. Towards this goal, we propose the following sequential optimization algorithm similar to an expectation-maximization (EM) algorithm [29]. Briefly, the EM algorithm is used for maximum likelihood estimation (MLE) of parameters subject to hidden variables. Intuitively, in our case, in Algorithm 1, we estimate A in the presence of hidden variables or *unknown unknowns* $\{u[k]\}_t^{t+T-2}$. Therefore, the ‘E-step’ is performed to average out the effects of unknown unknowns and obtain an estimate of u , where due to the diversity of solutions, we control the sparsity of the inputs (using the parameter λ'). Subsequently, the ‘M-step’ can then accomplish MLE estimation to obtain an estimate of A . The solution provided in [18] can be related to the proposed technique as follows.

Remark 1. *The solution to the system parameters (α, A) estimation without inputs [18] is a special case of the EM like approach proposed in the Algorithm 1.*

Proof. Upon setting $\{u[k]\}_t^{t+T-2} = 0$ in the E-step of the Algorithm 1, M-step would be the same at each iteration. Hence the algorithm stays at the initial point which is the solution in [18]. $\square$

It is worthwhile to mention the following result regarding EM algorithm.

Algorithm 1: EM algorithm

Input: $x[k], k \in [t, t+T-1]$ and B

Output: A and $\{u[k]\}_t^{t+T-2}$

Initialize compute α using [28] and then $z[k]$. For $l = 0$, initialize $A^{(l)}$ as

$$a_i^{(l)} = \arg \min_a \|Z_i - Xa\|_2^2$$

repeat

(i) ‘E-step’: For $k \in [t, t+T-2]$ obtain $u[k]$ as

$$u[k] = \arg \min_u \|z[k] - A^{(l)}x[k] - Bu[k]\|_2^2 + \lambda' \|u\|_1,$$

where $\lambda' = 2\sigma^2\lambda$;

(ii) ‘M-step’:

obtain $A^{(l+1)} = [a_1^{(l+1)}, a_2^{(l+1)}, \dots, a_n^{(l+1)}]^T$ where

$$a_i^{(l+1)} = \arg \min_a \|\tilde{Z}_i - Xa\|_2^2,$$

and $\tilde{Z}_i = Z_i - U b_i$;

$l \leftarrow l + 1$;

until until converge;

Theorem 1 ([30]). *The incomplete data (without unknown unknowns) likelihood $\mathbb{P}(z, x; A^{(l)})$ is non-decreasing after an EM iteration.*

Hence, the proposed algorithm being EM (detailed formulation in the Appendix I) has non-decreasing likelihood. Additionally, we have the following result about the incomplete data likelihood.

Proposition 1. *The incomplete data likelihood $\mathbb{P}(z, x; A^{(l)})$ is bounded at each iteration l .*

We can comment about the convergence of the Algorithm 1 as follows.

Lemma 1. *The Algorithm 1 is convergent in the likelihood sense.*

Proof. Using Theorem 1, Proposition 1 and Monotone Convergence Theorem, we can claim that the likelihood $\mathbb{P}(z, x; A^{(l)})$ will converge. $\square$

It should be noted that convergence in likelihood does not always guarantee convergence of the parameters. But as emphasized in [31], from numerical viewpoint the convergence of parameters is not as important as convergence of the likelihood. Also the EM algorithm can converge to saddle points as exemplified in [29].

IV. SENSOR SELECTION

Before defining the problem of sensor selection, we review the properties of fractional-order growth system with closed-form expressions for state vectors. Using the expansion of fractional order derivative in (2), we can write the state evolving equation as

$$x[k+1] = Ax[k] - \sum_{j=1}^{k+1} D(\alpha, j) x[k+1-j] + Bu[k], \quad (7)$$

where $D(\alpha, j) = \text{diag}(\psi(\alpha_1, j), \psi(\alpha_2, j), \dots, \psi(\alpha_n, j))$. Alternatively, (7) can be written as

$$x[k+1] = \sum_{j=0}^k A_j x[k-j] + Bu[k], \quad (8)$$

where $A_0 = A - D(\alpha, 1)$ and $A_j = -D(\alpha, j+1)$ for $j \geq 1$. With this definition of A_j , we can define the matrices G_k as follows [32]:

$$G_k = \begin{cases} I_n & k = 0, \\ \sum_{j=0}^{k-1} A_j G_{k-1-j} & k \geq 1. \end{cases} \quad (9)$$

Thus, we can obtain the following result.

Lemma 2 ([32]). *The solution to system described by (1) is given by*

$$x[k] = G_k x[0] + \sum_{j=0}^{k-1} G_{k-1-j} Bu[j]. \quad (10)$$

A. System Observability

To achieve perfect observability, i.e., to retrieve the initial state and the unknown inputs, we need system matrices and K observations. While any observation matrix C is sufficient for defining the perfect observability, if we set $C = \mathbb{I}_S$ as introduced in Section II, then K and S are intertwined. Simply speaking, by increasing K we will have more measurements acquired across time which could be used to compensate the number of measurements at each instance of time ruled by the set S of sensors used.

Given the K observations from $k = 0$ to $K - 1$, we can represent the initial state $x[0]$ and the unknown inputs using (10) by defining the following matrices

$$\Theta = [(CG_0)^T, (CG_1)^T, \dots, (CG_{K-1})^T]^T, \quad (11)$$

and

$$\Xi = \begin{bmatrix} 0 & 0 & \dots & 0 & 0 \\ CG_0 B & 0 & \dots & 0 & 0 \\ CG_1 B & CG_0 B & \dots & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ CG_{K-2} B & CG_{K-3} B & \dots & CG_0 B & 0 \end{bmatrix}, \quad (12)$$

where C and B are the observation and input matrices from (1) respectively, and G_k is as defined in (9). Having Θ and Ξ defined and using (1), we can write the initial state and inputs in terms of the observations as

$$Y = \Theta x[0] + \Xi U, \quad (13)$$

where $Y = [y[0]^T, y[1]^T, \dots, y[K-1]^T]^T$ and $U = [u[0]^T, u[1]^T, \dots, u[K-1]^T]^T$.

Using (13) and the Definition 1, a necessary and sufficient condition to attain the perfect observability is obtained as follows.

Proposition 2. *The system described by (1) is perfectly observable after K measurements if and only if $\text{rank}([\Theta \Xi]) = n + (K - 1)p$.*

Proof. The proof follows from re-writing equation (13) as

$$Y = [\Theta \Xi] \begin{bmatrix} x[0] \\ U \end{bmatrix}, \quad (14)$$

and, therefore, $x[0]$ and first $K - 1$ inputs from U can be recovered if and only if $\text{rank}([\Theta \Xi]) = n + (K - 1)p$. $\square$

Proposition 2 will be key to formulate the constraints in the sensor selection problem as detailed in the next section.

B. Sensor Selection Problem

Given the system matrices (α, A, B) and first K observations, the problem of sensor selection is defined as selecting the minimum number of sensors such that the system is perfectly observable. It can be mathematically written as

$$\min_{S \subseteq [n]} |S| \quad \text{s.t.} \quad \text{rank}([\Theta \Xi]|C = \mathbb{I}^S) = n + (K - 1)p \quad (15)$$

where $\text{rank}([\Theta \Xi]|C = \mathbb{I}^S)$ denotes the rank of $[\Theta \Xi]$ matrix when Θ and Ξ are constructed from (11) and (12) with $C = \mathbb{I}^S$. An analogous problem of sensor selection with no inputs is studied in [27] and it was shown to be NP-hard; hence, (15) is at least as computationally challenging since it contains the former as a special case when $B = 0$.

Consequently, we propose a sub-optimal solution to the above problem, while providing optimality guarantees within constant factor. For the discrete set $\Omega = [n]$, a function $f : 2^\Omega \rightarrow \mathbb{R}$ is called submodular if for all sets $S, T \subseteq \Omega$

$$f(S \cup T) + f(S \cap T) \leq f(S) + f(T). \quad (16)$$

Also, the marginal of an element a w.r.t. set S is defined as $f_S(a) = f(S \cup a) - f(S)$. Alternatively, a set function is referred as submodular if and only if it satisfies the diminishing returns property, i.e., $f_S(a) \geq f_T(a)$ for all $S \subseteq T \subseteq \Omega$ and $a \in \Omega \setminus T$ [33]. The monotone submodular functions have a remarkable property of performance through greedy selection within constant factor of the optimality [34].

With the motivation to borrow such attributes, let us define a discrete set function $f(S)$ as, $f(S) = \text{rank}([\Theta \Xi]|C = \mathbb{I}^S)$.

Theorem 2. *The discrete set function $f(S) = \text{rank}([\Theta \Xi]|C = \mathbb{I}^S)$ is submodular in S .*

Since f is submodular, we will be using a greedy selection of sensors to maximize the rank of $[\Theta \Xi]$; in other words, greedily select sensors such that $f(S) = n + (K - 1)p$. The sensor selection algorithm is described as Algorithm 2.

Lemma 3. *The complexity of Algorithm 2 with a total of n sensors and K length time horizon is $\mathcal{O}(n^5 K^3)$ i.e polynomial.*

Proof. With $\Omega = [n]$ the computation of $f_{S_G}(s)$ would require at most $\mathcal{O}(n^3 K^3)$. The algorithm being forward greedy selection has at most $n(n+1)/2$ executions and hence the complexity of the algorithm is at most $\mathcal{O}(n^5 K^3)$. $\square$

Therefore with Theorem 2 and Lemma 3, the Algorithm 2 provide a sub-optimal solution with optimality guarantees within constant factor to the NP-hard problem (15) in the polynomial order complexity.

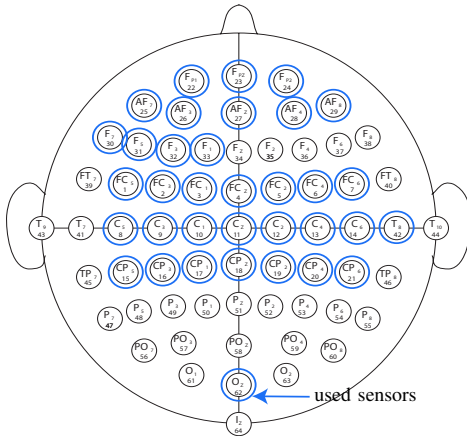


Fig. 1: Sensor distribution for the measurement of EEG. The channel labels are shown with their corresponding number.

Algorithm 2: Greedy Sensor Selection

Output: S_G
Initialize $S_G = \phi$;
repeat
 $s^* = \arg \max_{s \in \Omega \setminus S_G} f_{S_G}(s)$;
 $S_G \leftarrow S_G \cup s^*$;
until $f(S_G) = n + (K - 1)p$;

V. EXPERIMENTS

We demonstrate the application of the results derived in the previous sections on physiological signals. In particular we have taken a 64-channel EEG signal which records the brain activity of 109 subjects. The 64-channel/electrode distribution with the corresponding labels and numbers are shown in Figure 1. The subjects were asked to perform motor and imagery tasks. The data was collected by BCI2000 system with sampling rate of 160Hz [35], [36].

A. Model parameters estimation

The parameters of the system model α and A , are estimated by the application of Algorithm 1. The performance of EM algorithm like any iterative algorithm is crucially dependent on its initial conditions. For the considered example of EEG dataset, it was observed that convergence of the algorithm is fast. Further, even a single iteration was sufficient to reach the point of local maxima of the likelihood. This shows that the choice of the initial point for EM algorithm is considerably good. The input coupling matrix B can be easily determined through experiments. The values predicted by the model in comparison with actual data are shown in Figure 2. The one step prediction follows very closely the actual data, but there is small mismatch in the five step prediction. The ratio of square root of mean squared error of the prediction by model with and without inputs [27] is shown in Figure 3 for total of 109 subjects. As observed, the error ratio is less than one-third in the case when unknown inputs is considered. Therefore, the fractional-order dynamical model with unknown inputs fits the EEG data much better than the case of no inputs. In the

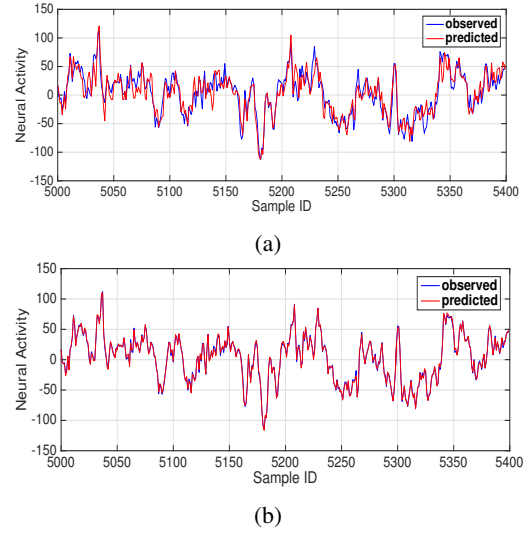


Fig. 2: Comparison of predicted EEG state for the channel C_1 using fractional-order dynamical model. The five step and one step predictions are shown in (2a) and (2b) respectively.

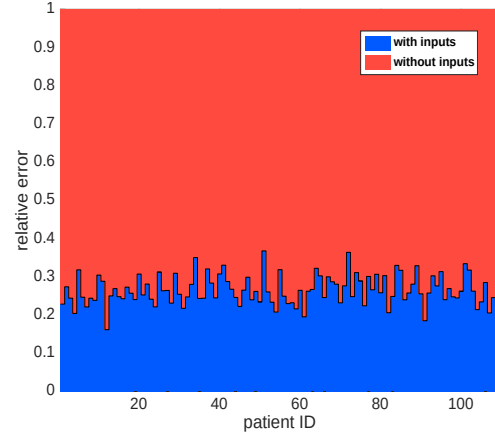


Fig. 3: Error ratio for prediction using fractional-order dynamical model with and without inputs.

next part, we will use these estimated parameters to compute the set of sensors for perfect observability.

B. Sensor selection

The estimated parameters are used to construct Θ and Ξ as written in (11) and (12) for the greedy sensor selection Algorithm 2. Upon the application of Algorithm 2, we found that roughly half of the sensors (35 out of 64) are sufficient enough to retrieve the initial state and unknown inputs uniquely. The selected sensors for a particular subject are marked in the Figure 1. Due to the large size and sparsity of $[\Theta \Xi]$ matrix, some values of the initial state may blow up due to the presence of very small eigenvalues. In such cases, we can first remove the unknown inputs from (13) by multiplying both sides by $W = I - \Xi(\Xi^T \Xi)^{-1} \Xi^T$, i.e., projecting signals into the orthogonal space of Ξ . We can then enforce the norm constraint of $x[0]$ into the least squares estimation of $x[0]$ or, in other words, use RIDGE regression

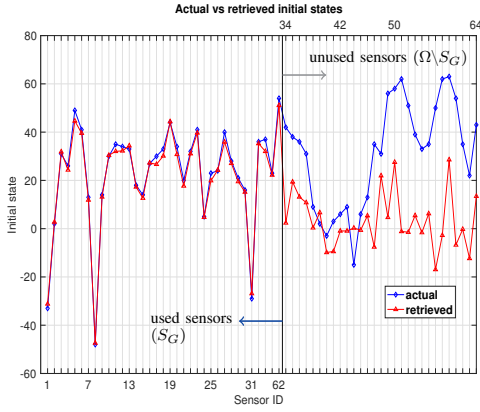


Fig. 4: Actual vs predicted initial state using only subset of sensors S_G , selected by the Algorithm-2.

[37], i.e.,

$$x[0] = \arg \min_x \|WY - W\Theta x\|_2^2 + \epsilon \|x\|_2^2,$$

where $\epsilon > 0$.

Upon the knowledge of the initial state, the unknown inputs are recovered in the similar fashion or by enforcing sparsity constraint. Figure 4 shows a comparison between the actual and retrieved initial states when using the sensor set S_G as output of the Algorithm 2. The retrieved initial states are close to the actual values provided they are estimated in the presence of numerical errors and sparsity.

The presented experimental results show how the proposed schemes are useful in mapping the complex dynamics of brain in the presence of unknown stimuli. The same framework can be easily applied to the study of other complex time evolving networks such as the physiological dynamics systems, for example BOLD signals, EMG, ECG etc.

VI. CONCLUSION

In this paper, we introduced the framework of discrete fractional order dynamical systems under unknown inputs. Also, we provided tools to perform analysis and design of such systems. Specifically, for the analysis, we presented an alternating scheme that enables to determine the best estimate of the model parameters and unknown stimuli. Also, we provided necessary and sufficient conditions to ensure that it is possible to retrieve the state and unknown stimuli. Furthermore, we enable the design of sensing capabilities of such systems, and provided a mechanism to determine a small subset of variables that need to be measured to ensure that both state and input can be recovered, while establishing sub-optimality guarantees with respect to the smallest possible subset.

Future research will focus on exploiting the structure of fractional order dynamical systems in the context of multi-case scenarios under quantitative description of the estimation quality of the states and inputs. Such extension will enable to determine the confidence in the model obtained that would permit formal claims about the mechanism underlying in the process under study. Additionally, some of

the algorithms need to be improved to be deployed in real-time applications when energy and computational resources are limited.

VII. ACKNOWLEDGMENT

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APPENDIX I EM FORMULATION

We present the detailed construction of EM like algorithm in this section. In our formulation, the observed (incomplete) data is x and z while u is the hidden data, therefore the complete data would be (z, x, u) . Let us consider, $\Sigma = \sigma^2 I$ and at the l th iteration we denote

$$u[k]^* = \arg \max_u \mathbb{P}(u|z[k], x[k]; A^{(l)}).$$

We can enforce Laplacian prior for $u[k]$ for sparsity (any other prior could also be used) such that $\mathbb{P}(u[k]) \propto \exp(-\lambda \|u[k]\|_1)$. Therefore, $u[k]^*$ is then derived as

$$\begin{aligned} u[k]^* &= \arg \max_u \log \mathbb{P}(u|z[k], x[k]; A^{(l)}) \\ &= \arg \max \log \mathbb{P}(u) + \log \mathbb{P}(z[k], x[k]|u; A^{(l)}) \\ &= \arg \max -\frac{1}{2\sigma^2} \|z[k] - A^{(l)}x[k] - Bu\|_2^2 - \lambda \|u\|_1. \end{aligned}$$

We have approximated the conditional distribution as $\mathbb{P}(u[k]|z[k], x[k]; A^{(l)}) \approx \mathbb{1}_{\{u[k]=\hat{u}[k]\}}$. In the final step of expectation, we can write

$$\begin{aligned} Q(A; A^{(l)}) &= \mathbb{E}_{A^{(l)}} [\log \mathbb{P}_c(z[k], x[k], u[k]|x[k], z[k]) \\ &= \mathbb{E}_{u[k]|z[k], x[k]; A^{(l)}} [\log \mathbb{P}(z[k], x[k], u[k]; A^{(l)})] \\ &= \mathbb{E}_{u[k]|z[k], x[k]; A^{(l)}} [\log \mathbb{P}(z[k], x[k]; A^{(l)})] \\ &\quad + \log \mathbb{P}(u[k]|z[k], x[k]; A^{(l)}) \\ &= \log \mathbb{P}(z[k], x[k]; A^{(l)}) \mathbb{1}_{\{u[k]=\hat{u}[k]\}}, \end{aligned}$$

where $\mathbb{P}_c$ is used to signify the likelihood of the complete data. For the Maximization step, we can simply write

$$\begin{aligned} A^{(l+1)} &= \arg \max_A Q(A; A^{(l)}) \\ &= \arg \max_A \log \mathbb{P}(z[k], x[k]; A) \mathbb{1}_{\{u[k]=\hat{u}[k]\}}, \end{aligned}$$

or in other words,

$$a_i^{(l+1)} = \arg \min_a \|\tilde{Z}_i - Xa\|_2^2,$$

where any k th component of $\tilde{Z}_i$ is $\tilde{Z}_{i,k} = z_i[k] - \hat{u}[k]^T b_i$.

APPENDIX II PROOF OF PROPOSITION 1

Proof. We show that the likelihood for incomplete (observed) data is bounded at each EM update step. Let us denote the likelihood of the observed data in relation to the parameter $A^{(l)}$ as

$$\mathbb{P}(A^{(l)}) = \mathbb{P}(z, x; A^{(l)}), \quad (17)$$

which is further written as

$$\begin{aligned}\mathbb{P}(A^{(l)}) &\propto \int \mathbb{P}(z, x|u; A^{(l)})\mathbb{P}(u)du \\ &= C \int \exp\left(-\frac{1}{2\sigma^2}\|z - A^{(l)}x - Bu\|_2^2\right) \\ &\quad \exp(-\lambda\|u\|_1) du \\ &\leq C \int \exp(-\lambda\|u\|_1) du \leq \mathcal{O}(1),\end{aligned}$$

where C is the normality constant. Therefore $\mathbb{P}(A^{(l)})$ is bounded for every iteration index $l \geq 0$. $\square$

APPENDIX III

PROOF OF THEOREM 2

Proof. For a given $n \times m$ matrix A , let $\mathcal{R}(S)$ denote the span of rows of A indexed by set S . Let $f(S)$ be the rank of matrix composed by rows indexed by set S , therefore $f(S) = |\mathcal{R}(S)|$. For given $\Omega = \{1, 2, \dots, n\}$, $S \subseteq T \subseteq \Omega$ and $a \in \Omega \setminus T$, we can write

$$\begin{aligned}|\mathcal{R}(T \cup a)| &= |\mathcal{R}(S \cup a)| + |\mathcal{R}(T \setminus S) \cap \mathcal{R}(S \cup a)^\perp| \\ &= |\mathcal{R}(S \cup a)| + |\mathcal{R}(T \setminus S) \cap \mathcal{R}(S)^\perp \cap \mathcal{R}(a)^\perp| \\ &\leq |\mathcal{R}(S \cup a)| + |\mathcal{R}(T \setminus S) \cap \mathcal{R}(S)^\perp| \\ &= |\mathcal{R}(S \cup a)| + |\mathcal{R}(T)| - |\mathcal{R}(S)|\end{aligned}$$

where the last equality is written using the fact that dimension of intersection of $\mathcal{R}(T \setminus S)$ and orthogonal space of $\mathcal{R}(S)$ are number of linearly independent rows in $\mathcal{R}(T)$ which are not in $\mathcal{R}(S)$ i.e. $|\mathcal{R}(T)| - |\mathcal{R}(S)|$. $\square$

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Re-thinking EEG-based non-invasive brain interfaces: modeling and analysis

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Abstract—Brain interfaces are cyber-physical systems that aim to harvest information from the (physical) brain through sensing mechanisms, extract information about the underlying processes, and decide/actuate accordingly. Nonetheless, the brain interfaces are still in their infancy, but reaching to their maturity quickly as several initiatives are released to push forward their development (e.g., NeuraLink by Elon Musk and ‘typing-by-brain’ by Facebook). This has motivated us to revisit the design of EEG-based non-invasive brain interfaces. Specifically, current methodologies entail a highly skilled neuro-functional approach and evidence-based *a priori* knowledge about specific signal features and their interpretation from a neuro-physiological point of view. Hereafter, we propose to demystify such approaches, as we propose to leverage new time-varying complex network models that equip us with a fractal dynamical characterization of the underlying processes. Subsequently, the parameters of the proposed complex network models can be explained from a system’s perspective, and, consecutively, used for classification using machine learning algorithms and/or actuation laws determined using control system’s theory. Besides, the proposed system identification methods and techniques have computational complexities comparable with those currently used in EEG-based brain interfaces, which enable comparable online performances. Furthermore, we foresee that the proposed models and approaches are also valid using other invasive and non-invasive technologies. Finally, we illustrate and experimentally evaluate this approach on real EEG-datasets to assess and validate the proposed methodology. The classification accuracies are high even on having less number of training samples.

Keywords—brain interfaces; spatiotemporal; fractional dynamics; unknown inputs; classification; motor prediction

I. INTRODUCTION

We have recently testimony a renewed interest in invasive and non-invasive brain interfaces. Elon Musk has released the NeuraLink initiative [1] that aims to develop devices and mechanisms to interact with the brain in a symbiotic fashion, thus merging the artificial intelligence with the human brain. The potential is enormous since it would ideally present a leap in our understanding of the brain, and an unseen enhancement of its functionality. Alternatively, Facebook just announced the ‘Typing-by-Brain’ project [2] that gathered a team of 60 researchers whose target is to be capable of writing 100 words per minute that contrasts with the state-of-the-art of 0.3 to 0.82 words per minute assuming an average of 5 letters per word. Towards this

goal, Facebook has invested in developing new non-invasive optical imaging technology that is five times faster and portable with respect to the one available on the market and would possess increased spatial and temporal resolution. Nonetheless, these are just some of the initiatives among others by some big Silicon Valley players that want to commercialize brain technologies [3].

Part of the motivation for the ‘hype’ in the use of neurotechnologies – both invasive and non-invasive brain interfaces – is due to their promising application domains [4]: (i) *replace*, i.e., the interaction of the brain with a wheelchair or a prosthetic device to replace a permanent functionality loss, (ii) *restore*, i.e., to bring to its normal use some reversible loss of functionality such as walking after a severe car accident or limb movement after a stroke, (iii) *enhance*, i.e., to enable to outperform in a specific function or task, as for instance an alert system to drive for long periods of time while attention is up; and (iv) *supplement* as in equipping one with extra functionality, as a third arm to be used during surgery. Notwithstanding, these are just some of the (known) potential uses of neurotechnology.

Despite the developments and promise of future applications of brain interfaces (some of which we cannot currently conceive), we believe that current approaches to both invasive and non-invasive brain interfaces can greatly benefit from cyber-physical systems (CPS) oriented approaches and tools to increase their efficacy and resilience. Hereafter, we propose to focus on non-invasive technology relying on electroencephalogram (EEG) and revisit it through a CPS lens. Moreover, we believe that the proposed methodology can be easily applicable to other technologies, e.g., electromagnetic fields (magnetoencephalography (MEG) [5], and the hemodynamic responses associated to neural activity, e.g. functional magnetic resonance imaging (fMRI) [6], [7], and functional near-infrared spectroscopy (fNIRS) [8]). Nonetheless, these technologies present several drawbacks compared to EEG, e.g., cost, scalability, and user comfort, which lead us to focus on EEG-based technologies. Similar argument can be applied in the context of non-invasive versus invasive technologies that require patient surgery.

Traditional approach to EEG neuroadaptive technology consists of proceeding through the following steps [9], [10]: (a) signal acquisition (in our case, measurement of

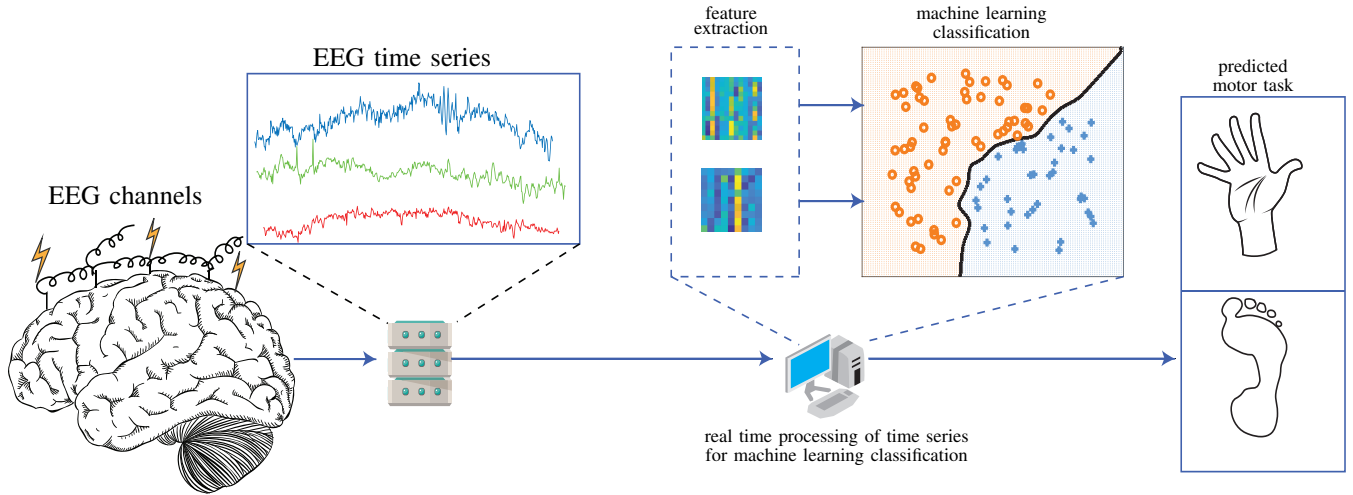


Figure 1: A systematic process flow of the Brain interface. The imagined motor movements of the subject are captured in the form of EEG time series which are then fed to the computational unit. A fractional-order dynamics based complex network model is estimated for the time series and the model parameters are used as features for machine learning classification. The output of classifier predicts various motor movements with certain confidence.

the EEG time-series); (b) signal processing (e.g., filtering with respect to known error sources); (c) feature extraction (i.e., an artificial construction of the signal that aims to capture quantities of interest); (d) feature translation (i.e., classification of the signal according to some plausible neurophysiological hypothesis); and (e) decision making (e.g., provide instructions to the computer to move a cursor, or a wheelchair to move forward) – see also Figure 1 for an overview diagram.

In this paper, we propose to merge steps (b) and (c) motivated by the fact that these often accounts for spatial or temporal properties, and are only artificially combined in a later phase of the pipeline, i.e., at step (d) of feature translation. First, we construct a time-varying complex network where the activity of nodes (or EEG signal) have long-range memory and the edges accounts for inter-node spatial dependence. Thus, we argue that the previous approach discards several spatial-temporal properties that can be weighted for signal processing and feature extraction phases. In other words, current EEG brain interfaces require one to have an understanding of the different regions of the brain responsible. For instance, regions for motor or visual actions, as well as artificial frequency bands that are believed to be more significant for a specific action (also known as *evidence-based*). Besides, one needs to understand and anticipate the most likely causes noise/artifacts in the EEG data collected and filter out entire frequency bands, which possibly compromises phenomena of interest not being available for post-processing. Instead, we propose a modeling capability to enable the modeling of long-range memory time-series that at the same time accounts for un-

known stimuli, e.g., artifacts or inter-region communication.

A. Related Work and Novel Contributions

We put forward that the ability to properly model the EEG time-series with complex network models, that account for realistic setups, can enable the brain interfaces methods to get transformed from detectors to decoders. In other words, we do not want to solely look for the existence of a peak of activity in a given band that is believed to be associated with a specific action. But we want to decompose the bunch of signals into different features, i.e., parameters of our complex network model, that are interpretable. Thus, allowing us to understand how different regions communicate during a specific action/task by representing them as nodes of the complex network and estimating the dependence via coupling matrix. The node activities are assumed to be evolving at different time-scales and in the general setup of presence of external stimuli which could either be unwanted noise or external process driving the system. In engineering, this will enable us to depart from a skill dependent situation to general context analysis, which will enhance the resilience of the approaches for practical nonsurgical brain interfaces. Besides, it will equip bioengineers, neuroscientists, and physicians with an exploratory tool to pursue new technologies for neuro-related diagnostics and treatments, as well as neuro-enhancement.

The proposed approach departs from those available in the literature, see [4], [9], [10] and references therein. In fact, to the best of authors' knowledge, in the context of noninvasive EEG-based technologies, [11] is the only existing work that explores fractional-order models in the context of single-channel analysis, which contrasts with the spatiotemporal

modeled leveraged in this paper that copes with multi-channel analysis. Furthermore, the methodology presented in this paper also accommodate unknown stimuli [12]. For which efficient algorithms are proposed and leveraged hereafter to simultaneously retrieve the best model that conforms with unknown stimuli, and separating the unknown stimuli from the time-series associated with brain activity. Our methods are as computationally efficient and stable as least-squares and spectral analysis methods used in a spatial and temporal analysis, respectively; thus, suitable for online implementation in nonsurgical brain interfaces.

The main contributions of the present paper are those of leveraging some of the recently proposed methods to develop new modeling capabilities for the EEG based neuro-wearables that are capable of enhancing the signal quality and decision-making. Furthermore, the parametric description of these models provides us with new features that are biologically motivated and easier to translate in the context of brain function associated with a signal characterization, and free of signal artifacts. Thus, making the brain-related activity interpretable, which leads to resilient and functional nonsurgical brain interfaces.

B. Paper Organization

The remaining of the paper is organized as follows. Section II introduces the complex network model considered in this paper and the main problem studied in this manuscript. Also we will see the description of the employed method for feature selection and then classification techniques. In Section III, we present an elaborated study on the different datasets taken from the BCI competition [13].

II. RE-THINKING EEG-BASED NON-INVASIVE BRAIN INTERFACES

Brain interfaces aim to address the following problem.

Is it possible to classify a specific cognitive state, e.g., motor task or its imagination, by using measurements collected with a specific sensing technology that harvest information about brain activity?

In the current manuscript, we revisit this problem in the context of brain-computer interfaces (BCI), when dealing with EEG-based noninvasive brain interfaces. Towards this goal, we review the currently adopted procedures for solving this problem (see Figure 1 for an overview), and propose a systems' perspective that enables to enhance the BCI reliability and resilience. Therefore, in Section II-A we provide a brief overview of the EEG-based technology and the connection with the brain-areas' function associated with studies conducted in the past. Next, in Section II-B, we introduce the spatiotemporal fractional model under unknown stimuli. This will be the core of the proposed approach in this manuscript to retrieve new features for classification, and, subsequently, enhancing brain interfaces capabilities. In Section II-C, we describe how to determine the system

model's parameters, and in Section II-D we describe how to interpret them for the classification task.

A. EEG-based Technology for Brain Interfaces: a brief overview

EEG enables the electrophysiological monitoring of space-averaged synaptic source activity from millions of neurons occurring at the neocortex level. The EEG signals have a poor spatial resolution but high temporal resolution, since the electrical activity generated at the ensemble of neurons level arrives at the recording sites within milliseconds. The electrodes (i.e., sensor) are placed over an area of the brain of interest, being the most common the visual, motor, sensory, and pre-frontal cortices. Usually, they follow standard *montages* – the International 10-20 system is depicted in Figure 2.

Most of the activity captured by the EEG electrodes is due to the interactions between inhibitory interneurons and excitatory pyramidal cells, which produces rhythmic fluctuations commonly referred to as *oscillations*. The mechanisms that generate those oscillations is not yet completely understood, but it has been already identified that some 'natural oscillations' provide evidence of activity being 'processed' in certain regions of the brain at certain 'frequencies'. Therefore, oscillatory behavior of human brain is often partitioned in bands (covering a wide range of frequencies decaying as $1/f$ in power): (i) δ -band (0.5-3Hz); (ii) θ -band (3.5-7Hz); (iii) α -band (8-13Hz); (iv) β -band (14-30Hz); and (v) γ -band (30-70Hz). Furthermore, there has been some evidence that activity in certain bands is associated with sensory registration, perception, movement and cognitive processes related to attention, learning and memory [14]–[16]. Notwithstanding, such associations are often made using correlation and/or coherence techniques that only capture relationships between specific channels. But such methods are not able to assess the causality between signals that enables forecasting on the signal evolution, captured by a model-based representation as we propose to do hereafter.

Different changes in the signals across different bands are also used to interpret the event-related potentials (ERPs) in the EEG signals, i.e., variations due to specific events – see [4] for detailed analysis. In the context of sensory-motor data used in the current manuscript to validate the proposed methodology, sensorimotor rhythms (SMRs) are often considered. These represent oscillations that are recorded over the posterior frontal and anterior parietal areas of the brain, i.e., over the sensorimotor cortices (see Figure 2). SMRs occur mainly in the α -band (for sensors located on the top of the motor cortices), and on beta and lower gamma for those on the sensorimotor cortices [17]. Consequently, these have been used as a *default feature* for classification of motor-related execution and only the imagination of performing a motor task. Notwithstanding, the spatiotemporal modeling is simultaneously captured through direct state-space modeling

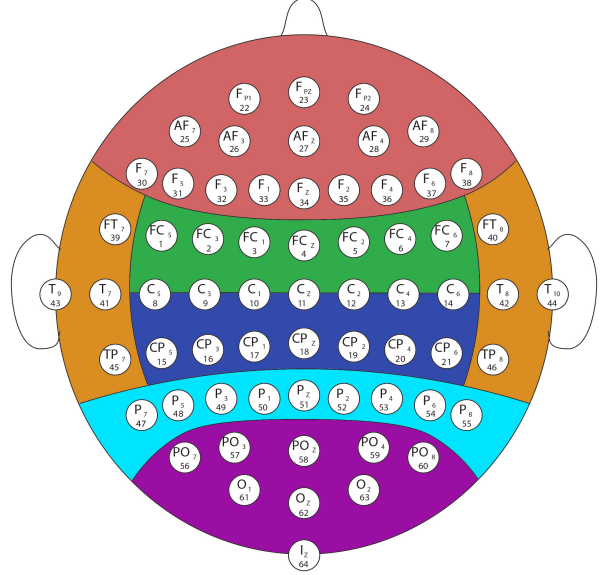
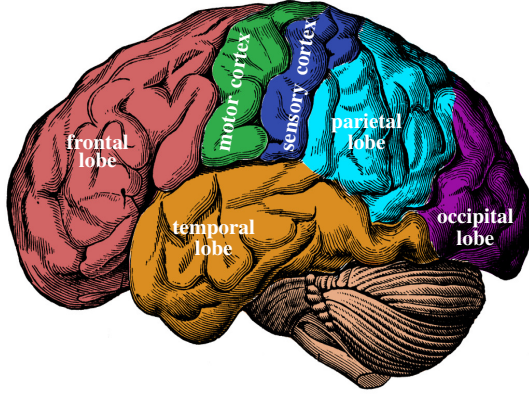


Figure 2: Description of the brain functional regions and their corresponding location with respect to the EEG sensor cap.

that enables the system's understanding of the dynamics of the underlying process. In addition, it provides a new set of attributes that can be used to improve feature translation, i.e., classification.

B. Spatiotemporal Fractional Model with Unknown Stimuli

A multitude of complex systems exhibit long-range (non-local) properties, interactions and/or dependencies (e.g., power-law decays in memories). Example of such systems includes Hamiltonian systems, where memory is the result of stickiness of trajectories in time to the islands of regular motion [18]. Alternatively, it has been rigorously confirmed that viscoelastic properties are typical for a wide variety of biological entities like stem cells, liver, pancreas, heart valve, brain, muscles [18]–[26], suggesting that memories of these systems obey the power law distributions. These dynamical systems can be characterized by the well-established mathematical theory of fractional calculus [27], and the corresponding systems could be described by fractional differential equations [28]–[32]. However, it is until recently that fractional order system (FOS) starts to find its strong position in a wide spectrum of applications in different domains. This is due to the availability of computing and data acquisition methods to evaluate its efficacy in terms of capturing the underlying system states evolution.

Subsequently, we consider a linear discrete time fractional-order dynamical model under unknown stimuli (i.e., inputs) described as follows:

$$\begin{aligned}\Delta^\alpha x[k+1] &= Ax[k] + Bu[k] \\ y[k] &= Cx[k],\end{aligned}\quad (1)$$

where $x \in \mathbb{R}^n$ is the state, $u \in \mathbb{R}^p$ is the unknown input and $y \in \mathbb{R}^n$ is the output vector. The differencing operator Δ is

used as the discrete version of the derivative, for example $\Delta^1 x[k] = x[k] - x[k-1]$. As evident, the difference order of 1 has only one-step memory, and hence the classic linear-time invariant models are not able to answer the long-range memory property of several physiological signals as discussed before. On the other hand, the expansion of fractional-order derivative in the discretized setting [33] for any i th state ($1 \leq i \leq n$) can be written as

$$\Delta^{\alpha_i} x_i[k] = \sum_{j=0}^k \psi(\alpha_i, j) x_i[k-j], \quad (2)$$

where α_i is the fractional order corresponding to the i th state and $\psi(\alpha_i, j) = \frac{\Gamma(j-\alpha_i)}{\Gamma(-\alpha_i)\Gamma(j+1)}$ with $\Gamma(\cdot)$ denoting the gamma function. We can observe from (2) that fractional-order derivate provide long-range memory by including all $x_i[k-j]$ terms. A quick comparison between the prediction accuracy of fractional-order derivative model and linear-time invariant model is shown in Figure 3. The fractional-order model can cope with sudden changes in the signals while the linear model cannot.

We can also describe the system by its matrices tuple (α, A, B, C) of appropriate dimensions. The coupling matrix A represents the spatial coupling between the states across time while the input coupling matrix B determines how inputs are affecting the states. In what follows, we assume that the input size is always strictly less than the size of state vector, i.e., $p < n$.

Having defined the system model, the system identification, i.e., estimation of model parameters, from the given data is an important step. It becomes nontrivial when we have unknown inputs since one has to be able to differentiate which part of the evolution of the system is due to its

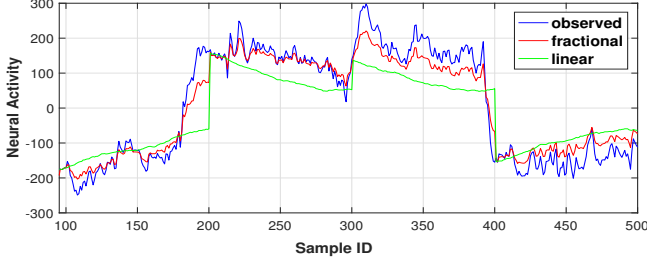


Figure 3: Comparison of observed experimental EEG data with the prediction from fractional-order model and linear-time invariant model.

intrinsic dynamics and what is due to the unknown inputs. Subsequently, the analysis part that we need to address is that of system identification from the data, as described next.

C. Data driven system identification

The problem consists of estimating α , A and inputs $\{u[k]\}_t^{t+T-2}$ from the given limited observations $y[k]$, $k = [t, t+T-1]$, which due to the dedicated nature of sensing mechanism is same as $x[k]$ and under the assumption that the input matrix B is known. The realization of B can be application dependent and is computed separately using experimental data – as we explore later in the case study, see Section III. For the simplicity of notation, let us denote $z[k] = \Delta^\alpha x[k+1]$ with k chosen appropriately. The pre-factors in the summation in (2) grows as $\psi(\alpha_i, j) \sim \mathcal{O}(j^{-\alpha_i-1})$ and, therefore, for the purpose of computational ease we would be limiting the summation in (2) to J values, where $J > 0$ is sufficiently large. Therefore, $z_i[k]$ can be written as

$$z_i[k] = \sum_{j=0}^{J-1} \psi(\alpha_i, j) x[k+1-j], \quad (3)$$

with the assumption that $x[k], u[k] = 0$ for $k \leq t-1$. Using the above introduced notations and the model definition in (1), the given observations can be written as

$$z[k] = Ax[k] + Bu[k] + e[k], \quad (4)$$

where $e \sim \mathcal{N}(0, \Sigma)$ is assumed to be Gaussian noise independent across space and time. For simplicity we would assume that $\Sigma = \sigma^2 I$. Also, let us denote the system matrices as $A = [a_1, a_2, \dots, a_n]^T$ and $B = [b_1, b_2, \dots, b_n]^T$. The vertical concatenated states and inputs during an arbitrary window of time as $X_{[t-1, t+T-2]} = [x[t-1], x[t], \dots, x[t+T-2]]^T$, $U_{[t-1, t+T-2]} = [u[t-1], u[t], \dots, u[t+T-2]]^T$ respectively, and for any i th state we have $Z_{i, [t-1, t+T-2]} = [z_i[t-1], z_i[t], \dots, z_i[t+T-2]]^T$. For the sake of brevity we would be dropping the time horizon subscript from the above matrices as it is clear from the context.

Since the problem of joint estimation of the different parameters is highly nonlinear, we proceed as follows: (i) we

estimate the fractional order α using the wavelet technique described in [34]; and (ii) with α known, the z in equation (3) is computed under the additional assumption that the system matrix B is known. Therefore, the problem now reduces to estimate A and the inputs $\{u[k]\}_t^{t+T-2}$. Towards this goal, we exploit the algorithm similar to expectation-maximization (EM) [35] from [12]. Briefly, the EM algorithm is used for maximum likelihood estimation (MLE) of parameters subject to hidden variables. Intuitively, in our case, in Algorithm 1, we estimate A in the presence of hidden variables or *unknown unknowns* $\{u[k]\}_t^{t+T-2}$. Therefore, the ‘E-step’ is performed to average out the effects of unknown unknowns and obtain an estimate of u , where due to the diversity of solutions, we control the sparsity of the inputs using the parameter λ' . Subsequently, the ‘M-step’ can then accomplish MLE estimation to obtain an estimate of A .

It was shown theoretically in [12] that the algorithm is convergent in the likelihood sense. It should also be noted that the EM algorithm can converge to saddle points as exemplified in [35]. The Algorithm 1 being iterative is crucially dependent on the initial condition for the convergence. We will see in Section III that the convergence is very fast making it suitable for online estimation of parameters.

Algorithm 1: EM algorithm

Input: $x[k]$, $k \in [t, t+T-1]$ and B

Output: A and $\{u[k]\}_t^{t+T-2}$

Initialize compute α using [34] and then $z[k]$. For $l = 0$, initialize $A^{(l)}$ as

$$a_i^{(l)} = \arg \min_a \|Z_i - Xa\|_2^2$$

repeat

(i) **‘E-step’:** For $k \in [t, t+T-2]$ obtain $u[k]$ as

$$u[k] = \arg \min_u \|z[k] - A^{(l)}x[k] - Bu[k]\|_2^2 + \lambda' \|u\|_1,$$

where $\lambda' = 2\sigma^2\lambda$;

(ii) **‘M-step’:**

obtain $A^{(l+1)} = [a_1^{(l+1)}, a_2^{(l+1)}, \dots, a_n^{(l+1)}]^T$ where

$$a_i^{(l+1)} = \arg \min_a \|\tilde{Z}_i - Xa\|_2^2,$$

and $\tilde{Z}_i = Z_i - Ub_i$;

$l \leftarrow l + 1$;

until until converge;

D. Feature Translation (Classification)

The unprocessed EEG signals coming from the sensors although carrying vital information may not be directly useful for making the predictions. However, by representing the signals in terms of parametric model (α, A) and the unknown signals as we did in the last section, we can

gain better insights. The parameters of the model being representative of the original signal itself can be used to make a concise differentiation.

The A matrix represents how strong is the particular signal and how much it is affecting/being affected by the other signals that are considered together. While performing or imagining particular motor tasks, certain regions of the brain gets more activated than others. Simultaneously, the inter-region activity also changes. Therefore, the columns of A which represent the coefficients of the strength of a signal affecting other signals can be used as a feature for classification of motor tasks. In this work, we will be considering the machine learning based classification techniques like logistic regression and Support Vector Machines (SVM) [36]. The other classification techniques c The choice of kernels would vary from simple ‘linear’ to radial basis function (RBF), i.e., $k(x_i, x_j) = e^{-\gamma(x_i - x_j)^2}$. The value of parameters of the classifier and possibly of the kernels are determined using the cross-validation. The range of parameters in the cross-validation are from $2^{-5}, \dots, 2^{15}$ for γ and $2^{-15}, \dots, 2^3$ for $C = 1/\lambda$, both in the logarithmic scale, where λ is the regularization parameter which appears in optimization cost of the classifiers [36].

III. CASE STUDY

We will now illustrate the usefulness of the fractional-order dynamic model with unknown inputs in the context of classification for BCI. We have considered two datasets from the BCI competition [13]. The datasets were selected on the priority of larger number of EEG

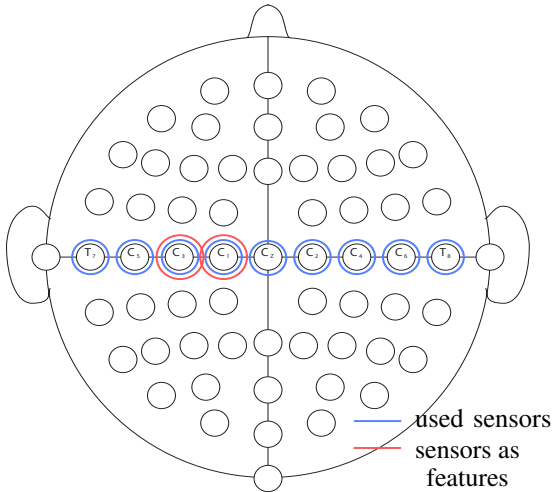


Figure 4: A description of the sensor distribution for the measurement of EEG. The channel labels for the selected sensors are shown for dataset-I.

channels and number of trials for training. The available data is split into the ratio of 60% and 40% for the purpose of training and testing, respectively.

A. Dataset-I

We consider for validation the dataset labeled ‘dataset IVa’ from BCI Competition-III [37]. The recording was made using BrainAmp amplifiers and a 128 channel electrode cap and out of which 118 channels were used. The signals were band-pass filtered between 0.05 and 200 Hz and then digitized at 1000 Hz. For the purpose of this study we have used the downsampled version at 100 Hz. The dataset for subject ID ‘al’ is considered, and it contains 280 trials. The subject was provided a visual cue, and immediately after asked to imagine two motor tasks: (R) right hand, and (F) right foot.

1) *Sensor Selection and Modeling*: To avoid the curse-of-dimensionality, instead of considering 118 sensors available, which implies the use of 118×118 dynamics entries for classification, only a subset of 9 sensors is considered. Specifically, only the sensors indicated in Figure 4 are selected on the basis that only hand and feet movements need to be predicted, and only a 9×9 dynamics matrix and 9 fractional order coefficients are required for modeling the fractional order system. Besides, these sensors are selected because they are close to the region of the brain known to be associated with motor actions.

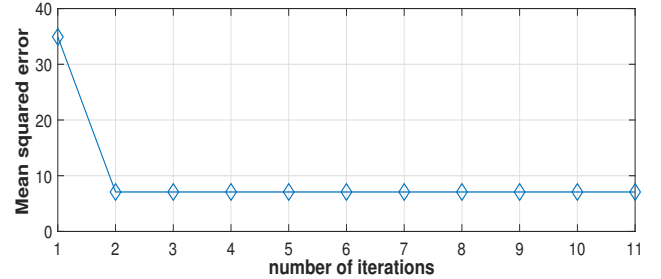


Figure 5: Mean squared error of Algorithm 1 as function of number of iterations for dataset-I.

2) *System Identification and Validation*: The model parameters (α, A) and the unknown inputs are estimated by using the Algorithm 1. As mentioned before, the performance of the algorithm being iterative is dependent on the choice of the initial conditions. For the current case, we have observed that the algorithm converges very fast, and even a single iteration is enough. The convergence of mean squared error in the M-step of Algorithm 1 for one sample from dataset-I is shown in Figure 5. This shows that the choice of initial conditions are fairly good. The one step and five step prediction of the estimated model is shown in Figure 6. It is evident that the predicted values for one step very closely follow the actual values. There are some

differences between the actual and predicted values for five step prediction.

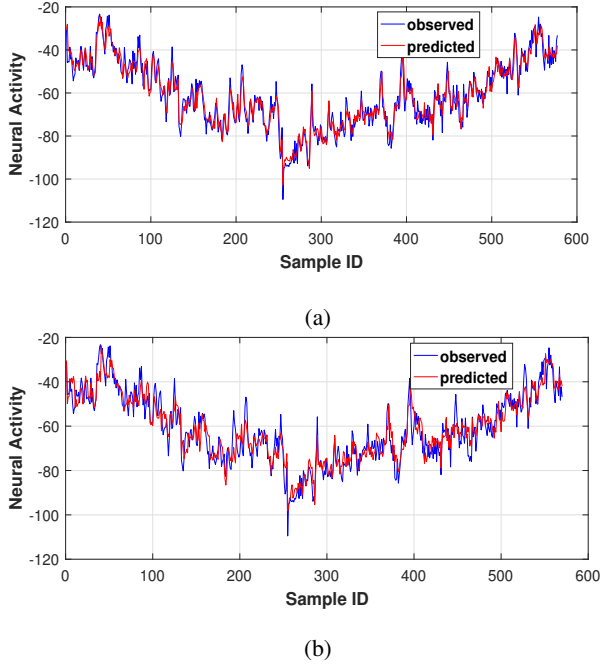


Figure 6: Comparison of predicted EEG state for the channel T_7 using fractional-order dynamical model with unknown inputs. The one step and five step predictions are shown in (a) and (b) respectively.

3) *Discussion of the results:* The most popular features used in the motor-imagery based BCI classification relies on exploiting the spectral information. The features used are the band-power which quantifies the energy of the EEG signals in certain spectrum bands [38]–[40]. The motor cortex of the brain is known to be affecting the energy in the bands namely, α and β as discussed in Section II. While it happens that unwanted signal energy is captured in these bands as well while performing the experiments, for example neck movement, other muscle activities etc. The filtering of these so called ‘unwanted’ components from the original signal is a challenging task using the spectral techniques as they often share the same band.

We used a different approach to deal with these unknown unknowns in Section II. The magnitude spectrum of the original EEG signal and on removing the estimated unknown inputs is shown in Figure 7. It should be observed that the original signal and the signal upon removing the unknown inputs have significant energy in the α and β bands. The unknown inputs behave similar to the white noise which is evident from their Gaussian probability distribution (PDF) as shown in Figure 8. The inputs are not mean zero but their PDF is centered around a mean value of approximately 58.

The model parameters (α, A) are jointly estimated with the unknown inputs using Algorithm 1, therefore the effect

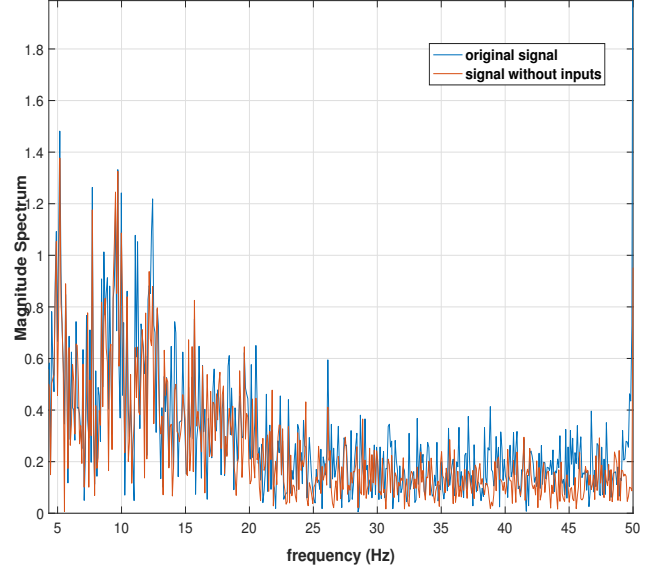


Figure 7: Magnitude spectrum of the signal recorded by channel T_7 with and without unknown inputs.

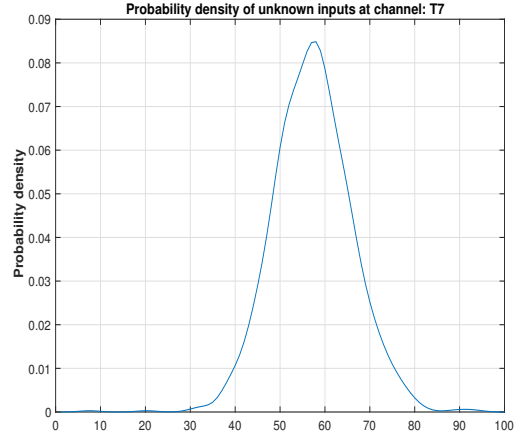


Figure 8: Probability density function of the unknown inputs estimated from the signal recorded by channel T_7 .

of the inputs is inherently taken care of in the parameters. The structure of matrix A for two different labels is shown in Figure 9. We have used the sensors C_3 and C_1 which are indexed as 3 and 4, respectively in Figure 9. It is apparent from Figure 9 that the columns corresponding to these sensors have different activity and hence deem to be fair candidates for the features to be used in classification. Therefore, the total number of features are $2 \times 9 = 18$.

B. Dataset-II

A 118 channel EEG data from BCI Competition-III, labeled as ‘dataset IVb’ is taken [37]. The data acquisition technique and sampling frequencies are same as in dataset of the previous subsection. The total number of labeled trials

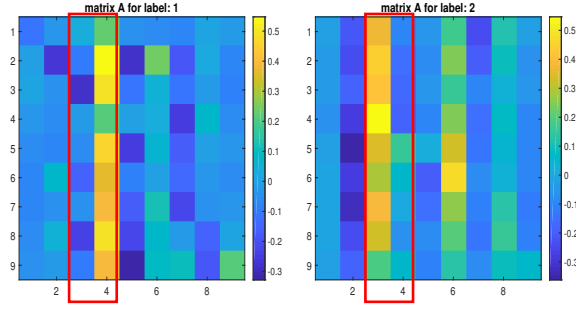


Figure 9: Estimated A matrix of size 9×9 for the dataset-I with marked columns corresponding to the sensor index 3 and 4 used for classification.

are 210. The subjects upon provided visual cues were asked to imagine two motor tasks, namely (L) left hand and (F) right foot.

1) *Sensor Selection and Modeling:* Due to the small number of training examples, we have again resorted to select the subset of sensors for the model estimation as we did for the dataset-I in the previous section. Since the motor tasks were left hand and feet, therefore we have selected the sensors in the right half of the brain and close to the region which is known to be associated with hand and feet movements as shown in Figure 11. We will see in the final part of this section that selecting sensors based on such analogy helps not only in reducing the number of features, but also to gain better and meaningful results.

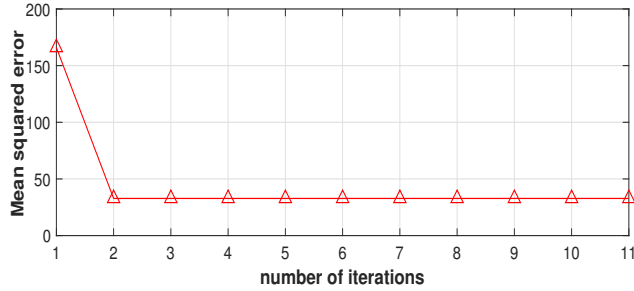


Figure 10: Mean squared error of Algorithm 1 as function of number of iterations for dataset-II.

2) *System Identification and Validation:* After performing the estimation of the model (α, A) and the unknown inputs using the subset of sensors, we can see the similar performance of the model on dataset-II as was in dataset-I. The convergence of mean squared error in the M-step of Algorithm 1 for one sample from dataset-II is shown in Figure 10. The one step and five step predictions are shown in Figure 12. The model prediction follows closely the original signal.

3) *Discussion of the results:* The spectrum of the original EEG signal at channel CFC_2 and its version with unknown

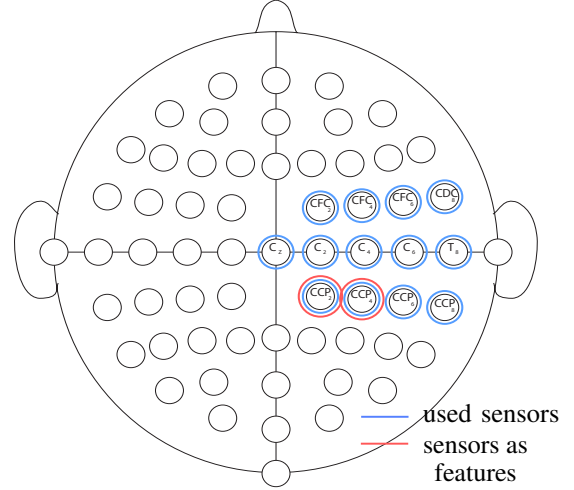
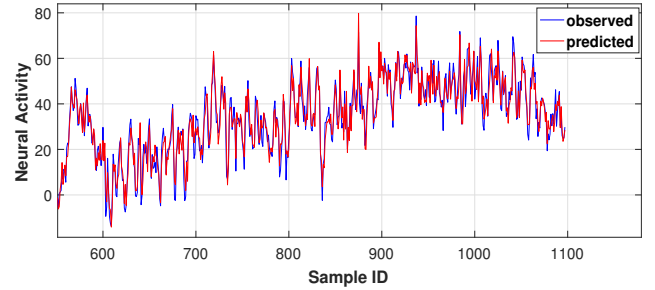
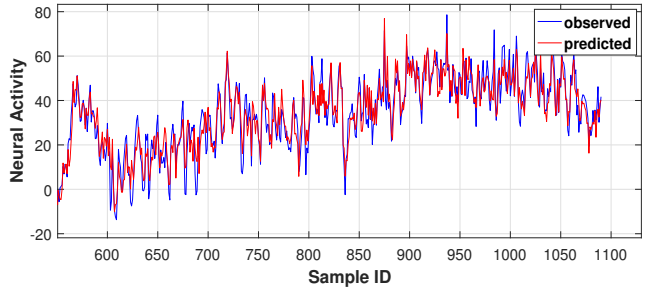


Figure 11: A description of the sensor distribution for the measurement of EEG. The channel labels for the selected sensors are shown for dataset-II.



(a)



(b)

Figure 12: Comparison of predicted EEG state for the channel CFC_2 using fractional-order dynamical model with unknown inputs. The one step and five step predictions are shown in (a) and (b) respectively.

inputs removed are shown in Figure 13. The spectrum shows peaks in the α and β bands. We witness a similar observation as before that both of the signals share the same band and hence making it difficult to remove the effects of the

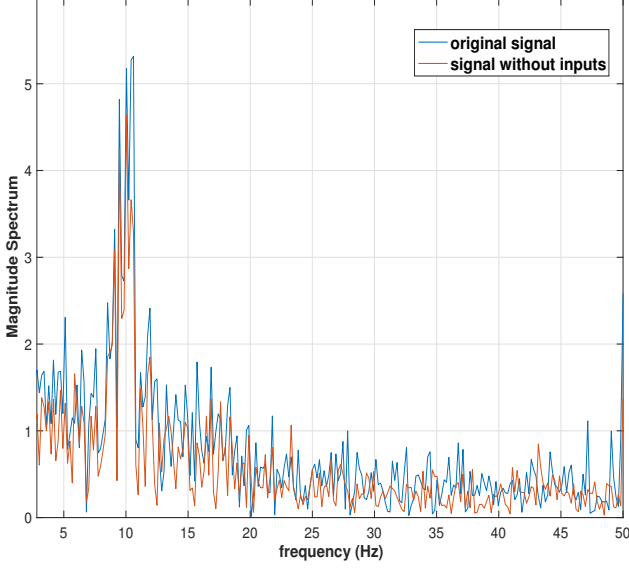


Figure 13: Magnitude spectrum of the signal recorded by channel CFC_2 with and without unknown inputs.

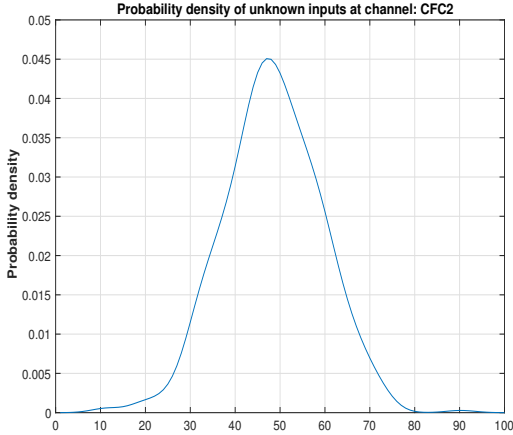


Figure 14: Probability density function of the unknown inputs estimated from the signal recorded by channel CFC_2 .

unwanted inputs. The unknown inputs resembles that of white noise and the PDF is close to Gaussian distribution with mean centered at around 48.

The estimated A matrix from Algorithm 1 is shown in Figure 15 for two different labels. Out of all 13 sensors, the sensors CCP_2 and CCP_4 which are indexed as 10 and 11 in the matrix have striking different activity. The columns corresponding to these two sensors seem good choice for being the features for classification. Therefore, the total number of features are $2 \times 13 = 26$ for this dataset. Next, we discuss the classification accuracy for both the datasets.

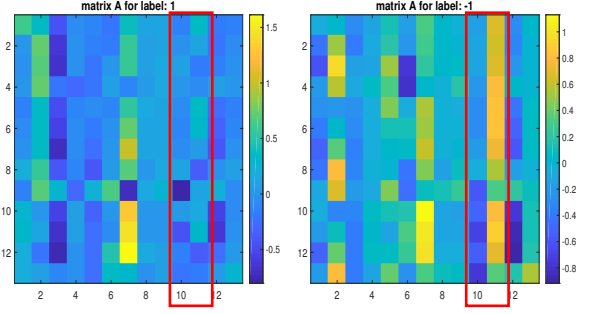


Figure 15: Estimated A matrix of size 13×13 for the dataset-II with marked columns corresponding to the sensor index 10 and 11 used for classification.

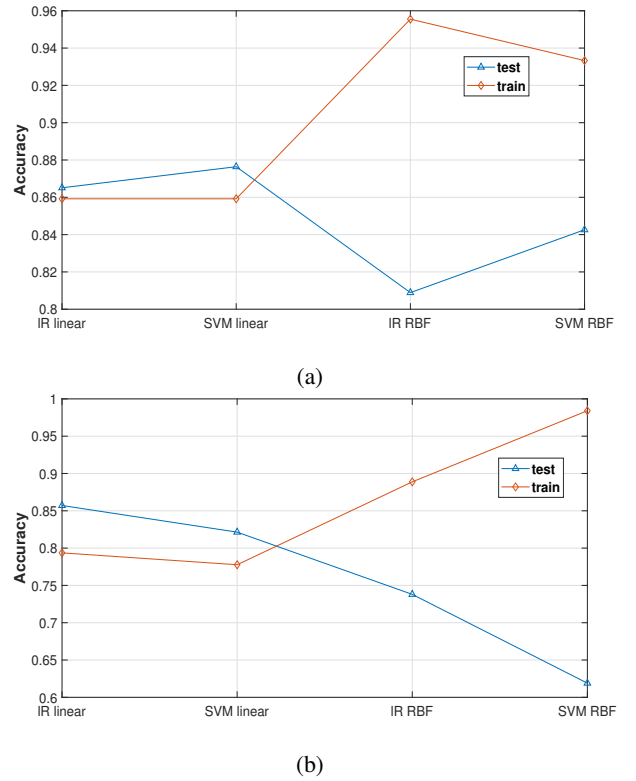


Figure 16: Testing and training accuracies for various classifiers arranged in the order of classification model complexity from left to right. The estimated accuracies for dataset-I and dataset-II are shown in (a) and (b) respectively.

C. Classification Performance

Finally, the performance of the classifiers using the features explained for both the datasets are shown in Figure 16. The classifiers are arranged in the order of complexity from left to right with logistic regression (IR) and linear kernel being simplest and SVM with RBF kernel being most complex. The performance plot parallels the classic machine learning divergence curve for both the datasets. The accuracy

for training data increases when increasing the classification model complexity while it reduces for the testing data. This is intuitive because a complex classification model would try to better classify the training data. But the performance of the test data would reduce due to overfitting upon using the complex models. We have very few training examples to build the classifier and hence such trend is expected. The performance of the classifiers for both the datasets are fairly high which reflects the strength of the estimated features. We can see a 87.6% test accuracy for dataset-I and 85.7% for dataset-II. While these accuracies depend a lot on the cross-validation numbers and other factors like choice of classifier which can be better tuned to get higher numbers.

For both the datasets we have seen that the proposed methodology efficiently extracts the features which serves as good candidate to differentiate the imagined motor movements. By implicitly removing the effects of the unwanted stimuli, the coefficients of the coupling matrix A are shown to be sufficient for discriminating relation between various EEG signals which are indicative of the motor movements. The testing accuracies are high which indicate the good quality of the extracted features.

IV. CONCLUSION

We have revisited the EEG-based noninvasive brain interfaces feature extraction and translation from a cyber-physical systems' lens. Specifically, we leveraged spatiotemporal fractional-order models that cope with the unknown inputs. The fractional-order models provide us the dynamic coupling changes that rule the EEG data collected from the different EEG sensors, and the fractional-order exponents capture the long-term memory of the process. Subsequently, unknown stimuli is determined as the external input that least conforms with the fractional-order model. By doing so, we have filtered-out from the brain EEG signals the unknown inputs, that might be originated in the deeper brain structures. The presence of unknown stimuli is possibly the result of the structural connectivity of the brain that crisscrosses different regions, or due to artifacts originated in the muscles (e.g., eye blinking or head movement). As a consequence, the filtered signal does not need to annihilate an entire band in the frequency domain, thus keeping information about some frequency regions of the signal that would be otherwise lost.

We have shown how the different features obtained from the proposed model can be used towards rethinking the EEG-based noninvasive interfaces. In particular, two datasets used in BCI competitions were used to validate the performance of the methodology introduced in this manuscript, which is compatible with some of the state-of-the-art performances while requiring a relatively small number of training points. We believe that the proposed methodology can be used within the context of different neurophysiological processes and corresponding sensing technologies. Future research

will focus on leveraging additional information from the unknown inputs retrieved to anticipate specific artifacts and enable the deployment of neuro-wearables in the context of real-life scenarios. Furthermore, the presented methodology can be used as an exploratory tool by neuroscientists and physicians, by testing input and output responses and tracking their impact in the unknown inputs retrieved by the algorithm proposed; in other words, one will be able to systematically identify the origin and dynamics of stimulus across space and time. Finally, it would be interesting to explore the proposed approach in the closed-loop context, where the present models would benefit from control-like strategies to enhance the brain towards certain tasks or attenuate side effects of certain neurodegenerative diseases or disorders.

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Gene Expression Is Not Random: Scaling, Long-Range Cross-Dependence, and Fractal Characteristics of Gene Regulatory Networks

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Gene expression is a vital process through which cells react to the environment and express functional behavior. Understanding the dynamics of gene expression could prove crucial in unraveling the physical complexities involved in this process. Specifically, understanding the coherent complex structure of transcriptional dynamics is the goal of numerous computational studies aiming to study and finally control cellular processes. Here, we report the scaling properties of gene expression time series in *Escherichia coli* and *Saccharomyces cerevisiae*. Unlike previous studies, which report the fractal and long-range dependency of DNA structure, we investigate the individual gene expression dynamics as well as the cross-dependency between them in the context of gene regulatory network. Our results demonstrate that the gene expression time series display fractal and long-range dependence characteristics. In addition, the dynamics between genes and linked transcription factors in gene regulatory networks are also fractal and long-range cross-correlated. The cross-correlation exponents in gene regulatory networks are not unique. The distribution of the cross-correlation exponents of gene regulatory networks for several types of cells can be interpreted as a measure of the complexity of their functional behavior.

Keywords: gene expression, gene regulatory network, fractals, dynamics, entropy

INTRODUCTION

Protein synthesis is fundamental for biological systems to perform a variety of functions. They control the organism's shape or can function as enzymes catalyzing specific metabolic pathways to regulate specific cellular processes. These cellular functions include responding to stimuli, transporting molecules and catalyzing metabolic reactions. In order to program cells for performing the desired functionality, one should regulate the protein synthesizing process. The process of protein synthesis from the activation of a specific gene is called gene expression (Lockhart and Winzeler, 2000; Teichmann and Babu, 2004; Huang et al., 2005; Düvel et al., 2010).

Gene expression (briefly shown in **Figure 1a**) is the process in which the genetic information of a cell causes a cell to generate a functional gene product and, finally, perform specific cell functions (Niedenthal et al., 1996). In other words, it is the process by which genotype information gives rise to phenotype (observable characteristics). It is a vital process, which causes cellular differentiation, morphogenesis, and the versatility and adaptability of any organism (O'Connor et al., 2010). Controlling the production process of the desired gene expression product (e.g., a protein) refers

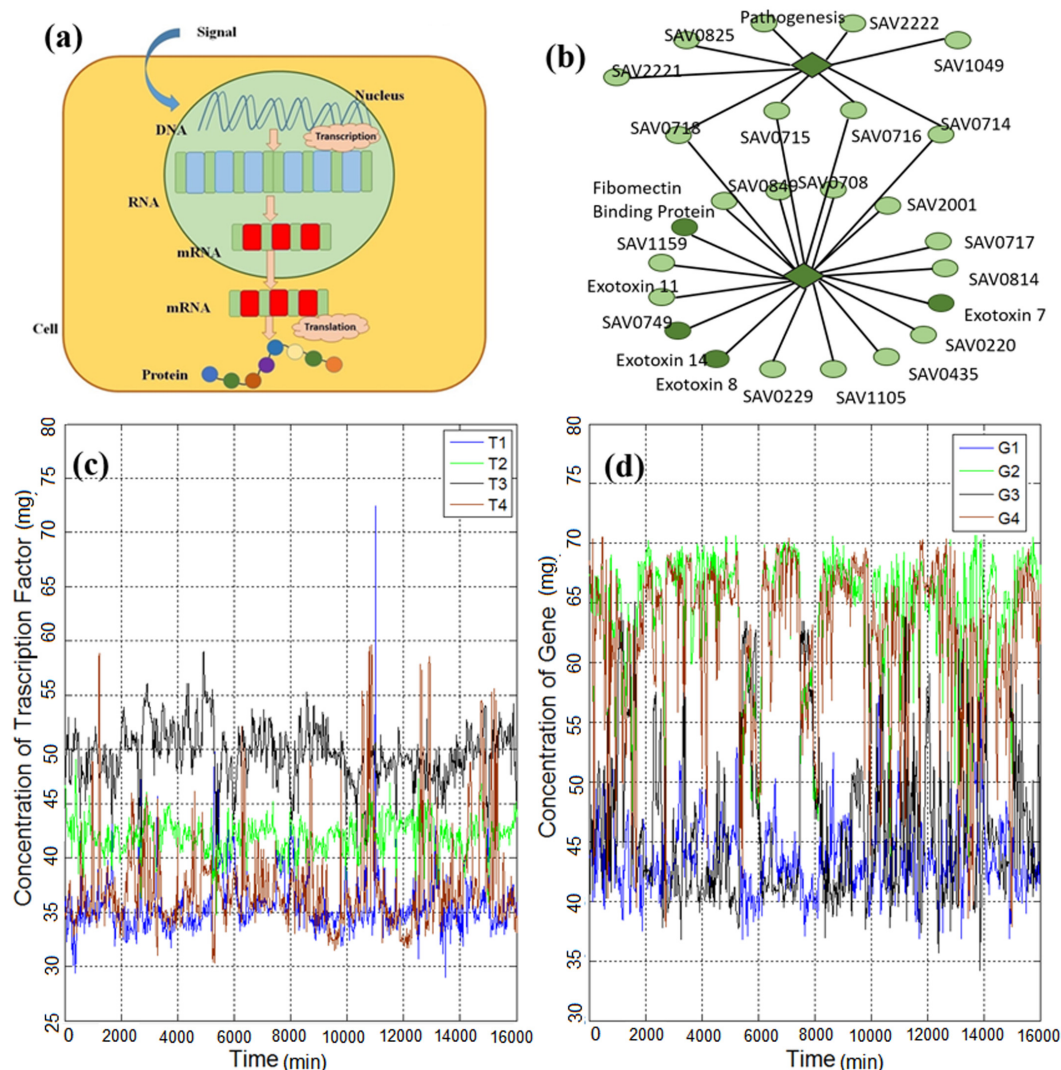


FIGURE 1 | (a) Gene expression process is initiated with the triggering of a gene in the DNA strand and continues with the generation of RNA, mRNA and, finally, the protein product. (b) Part of the gene regulatory network of *Escherichia coli* reported by Lockhart and Winzler (2000), which consists of genes and transcription factors (TFs). The diamonds represent TFs and the circles represent genes. (c) Time series of TFs of *E. coli* obtained from Marbach et al. (2012) and explained in the methods section. (d) Time series of genes of *E. coli* obtained from Marbach et al. (2012) and explained in the methods section.

to the regulation of the gene expression process. The regulation of gene expression controls the amount and timing of production of target proteins (Malone et al., 2009). Hence, investigating the dynamics of gene expression enables to understand the mechanisms driving biological organisms. This knowledge helps us from both scientific and engineering perspectives. It can be exploited to detect an anomaly or disease or to engineer cells for performing specific tasks (e.g., drug delivery for cancer treatment) as it is the target of synthetic biology.

The biophysical mechanism affecting the regulation process has been actively studied (Elf et al., 2007; Kolesov et al., 2007; Kuhlman and Cox, 2012; Bauer and Metzler, 2013; Pulkkinen and Metzler, 2013). For instance, searching for the target gene by the transcription factors (TFs) is discussed in Kolesov et al. (2007); Pulkkinen and Metzler (2013) and the diffusion process of

search for the target genes is studied in Elf et al. (2007); Bauer and Metzler (2013). Also, the spatial distribution of gene products is reported in Kuhlman and Cox (2012). However, these prior studies were not concerned with the mathematical characterization of the gene expression dynamics for several gene regulations in a network of genes. To identify the main mathematical characteristics of gene expression dynamics, we investigate individual and cross-dependent gene expression time series. First, we investigate the statistical properties of single (isolated) gene expression time series (shown in Figures 1c,d), and, then, we analyze the cross-correlation between pairs consisting of a gene and a TF in the gene regulatory network (Figure 1b). In contrast to the previous study (Tsuchiya et al., 2016) in which regulation of cell fate through genome-wide expression by temporal-spatial self-organization is considered,

here, we mainly analyze the temporal variability of individual genes. We then investigate the correlation of linked TF and genes. Moreover, we analyze the expression level of single cells without considering population effect (Tsuchiya et al., 2007).

The remaining of this paper is organized as follows: In the first part of the Results section, we present our findings on the individual and cross-dependence dynamics of gene expression time series. Then, we report the distribution of the cross-dependencies and a complexity quantification strategy for the gene expression networks. In the latest section of Results, we investigate whether the observed multifractality can be explained by a well-known model such as the Mandelbrot analytical cascade model. The Discussion section concludes our findings and outlines some future research directions. Lastly, the Methods section summarizes the mathematical strategies used for obtaining the findings reported here.

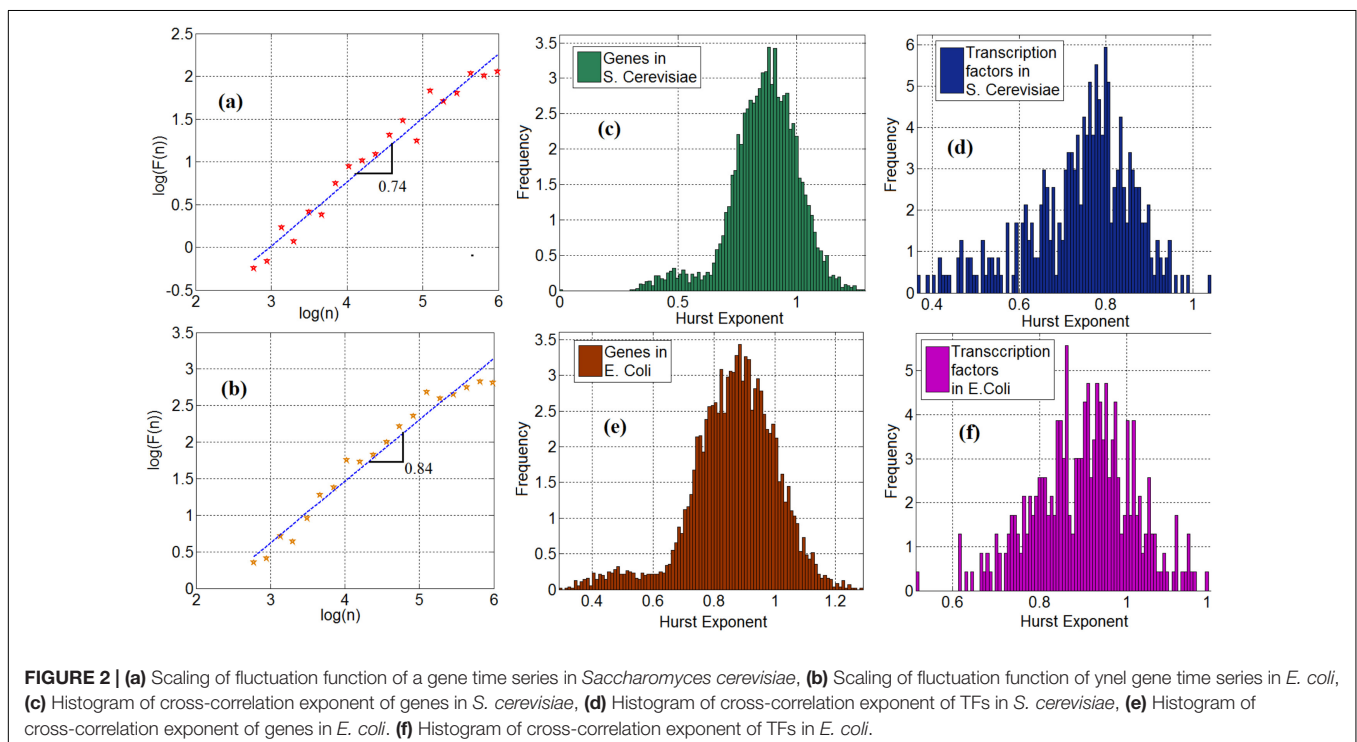
RESULTS

Gene Expression Dynamics Exhibits Long-Range Dependency and Multifractal Properties

We investigate the statistical properties of gene expression data and compute the Hurst exponents of gene expression time series in *Saccharomyces cerevisiae* (*S. cerevisiae*) and *Escherichia coli* (*E. coli*). **Figures 2a,b** show the log-log plot of the fluctuation function as a function of the scale for the time series of a TF (ynel) in *S. cerevisiae* and *E. coli*, respectively. In these plots, the slope of the curve represents the Hurst exponent. We observe

that 95 and 98% of the time series of genes from the *S. cerevisiae* and the *E. coli* gene expression networks, respectively, exhibit a long-range dependency property. More precisely, their Hurst exponent was greater than 0.5. To demonstrate this important property, **Figures 2c,e** show the histogram of the Hurst exponent of gene expression time in *S. cerevisiae* and *E. coli*, respectively. Generally speaking, a Hurst exponent that exceeds the 0.5 threshold value denotes a persistent (positively correlated) behavior in the sense that a high value is likely to be followed by another high value with nonzero probability. In addition, because the Hurst exponent for most of the genes is significantly higher than 0.5, the gene and TF time series cannot be regarded as a random process and modeled through Markovian formalism (Kantelhardt et al., 2001). This mathematical characteristic provides a clue as to how to construct stochastic models for gene expression processes, but this is left for future work. We observe the same property in the time series of TFs in *S. cerevisiae* and *E. coli*. More precisely, 97% of the TFs in *S. cerevisiae* and *E. coli* possess the long-range dependence property. The histogram of the Hurst exponent of TFs in *S. cerevisiae* and *E. coli* are shown in **Figures 2d,f** respectively.

Employing fractal analysis is also helpful to gain insight into other interesting properties. Here, we see a bimodal characteristic in the Hurst exponent distribution, shown in **Figures 2c–f**. This feature is especially visible in **Figures 2c,e** where the histogram of genes in *S. cerevisiae* and *E. coli* is presented. This may be explained by a possible bimodal diffusion potential, as in Muzychuk (2006). Since gene expression includes a diffusion process with multiple diffusion potentials (inside and outside the nucleus), this bimodality can be explained by non-equilibrium Brownian motion with multiple potential profiles. However,



further experimental studies are required to elucidate the nature and implications of these bimodal statistics.

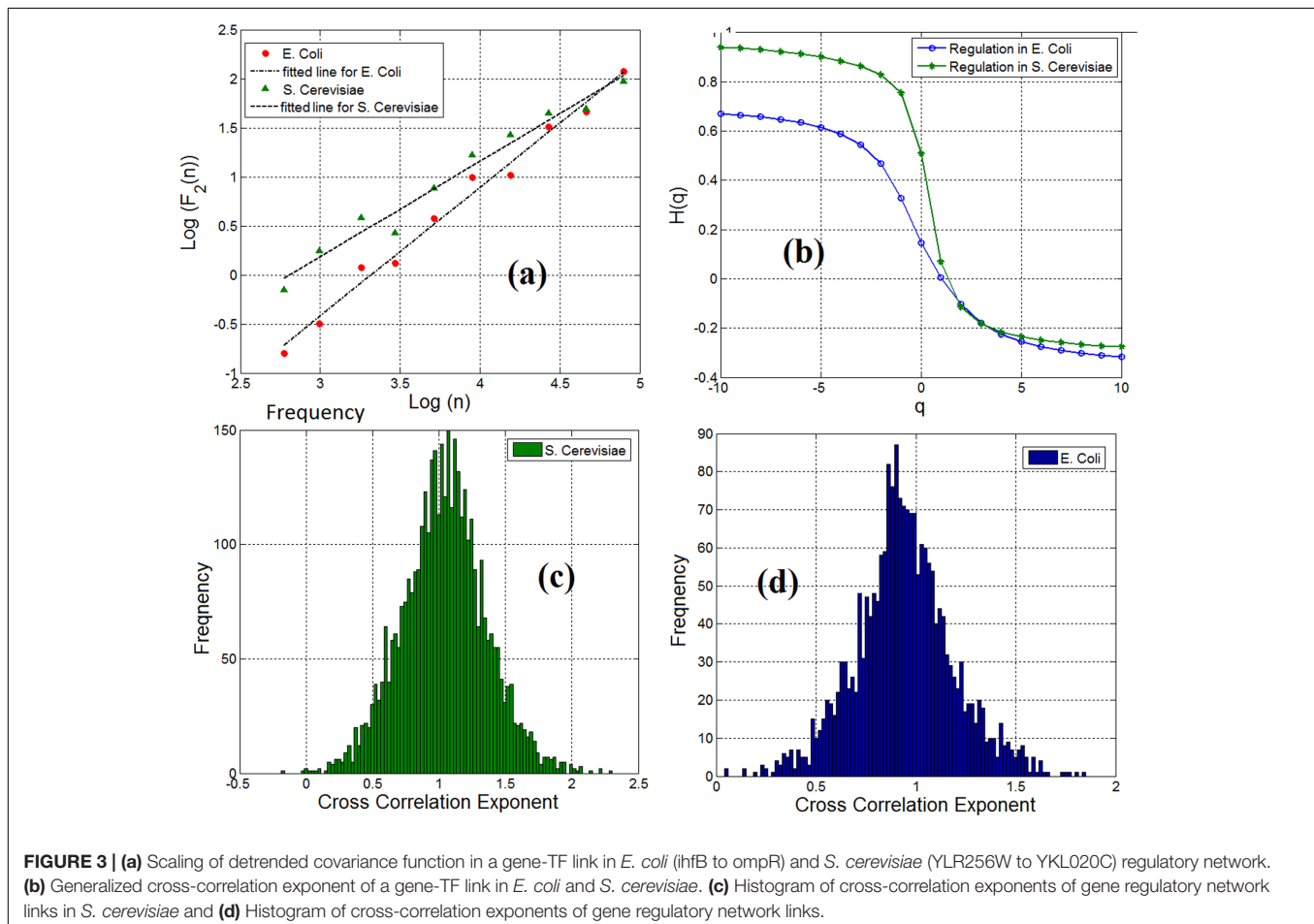
By employing the multifractal detrended fluctuation analysis (MFDFA) (Kantelhardt et al., 2002) (see Materials and methods section) to examine the multifractal characteristics of gene expression time series, we observe that both genes and TFs have pronounced multifractal properties. For monofractal behavior, the generalized Hurst exponent displays a linear dependency with the order q of the cross moments. Instead, if the generalized Hurst exponent exhibits a nonlinear dependency (such as the S-shape displayed in **Figure 3b**) as a function of the order q of the cross-moments, then the stochastic interdependence is considered to possess multifractal characteristics.

To provide a more in-depth report, we use the bootstrapping technique (Efron, 1982) to investigate the existence of the long-range dependence property, considering the limitations related to the length of the experimental time series. For every gene expression time series, we have sampled 10 randomized subintervals of the gene expression time series, each containing 90% of the ordered piece of the original time series. Then, we calculate the Hurst exponents for all the randomized versions. The difference between the percentage of the long-range dependency for the gene expression time series and the randomized versions was approximately 0.006 for *E. coli* and

0.0001 *S. cerevisiae*. We also investigate whether the observed Hurst exponent varies in different conditions. We observed that for time series of *E. coli*, the Hurst exponent varies in different acidic levels and osmotic stress level and we have reported them in **Supplementary Material**.

Time Series of Interactions Within the Gene Regulatory Networks Demonstrate Long-Range Cross-Correlation and Multifractal Properties

We analyzed the cross-correlation between linked pairs of genes and TFs in gene regulatory networks. By computing the cross-correlation exponent (Podobnik and Stanley, 2008), we noticed that 98% of the linked pairs of genes and TFs in gene regulatory network for *E. coli* and *S. cerevisiae* possess the long-range dependence property. **Figure 3a** shows the scaling of the detrended covariance function for a pair of gene and TF (link) in *E. coli* (ihfB to ompR) and *S. cerevisiae* (YLR256W to YKL020C). We have applied the multifractal detrended cross-correlation analysis for pairs of genes and TFs (links) in the gene regulatory network of *E. coli* and *S. cerevisiae* and found that there is a pronounced multifractal cross-correlation signature in these gene regulatory network links. **Figure 3b** shows the



generalized Hurst exponent $H(q)$ as a function of the order of the cross moments q in **Figure 3a**. For a mono-fractal behavior, the generalized Hurst exponent displays a linear dependency with the order q of the cross-moments. Instead, if the generalized Hurst exponent exhibits a nonlinear dependency (such as the S-shape displayed in **Figure 3b**) on the order q of the cross-moments, then the stochastic interdependence is considered to possess multifractal characteristics. In conclusion, the causal relationship between TFs and genes in gene regulatory networks was mainly also long-range dependent. The concentration level of a gene not only depends on the current concentration level of the linked TF but also on the previous values of that gene. This dependency obeys a power-law-like relationship.

The Distribution of Cross-Correlation Exponents of Pairs of Genes and Transcription Factors of Gene Regulatory Networks Has a Wide Range of Variation

Although we observe the fractal and long-range cross-correlation in linked pairs of genes and TFs in the gene regulatory networks, the cross-correlation exponents were not the same in all the links. We have shown the distribution of the cross-correlation exponents for pairs of genes and TFs in the *S. cerevisiae* and *E. coli* gene regulatory networks in **Figures 3c,d** respectively. Inspired by Shannon entropy (Shannon, 2001), we use this histogram for measuring the entropy, and hence, the information content of a gene regulatory network across different cell types for quantitative analysis and specification of gene regulatory networks. The computed Shannon sample entropy for *S. cerevisiae* and *E. coli* was 4.18 and 5.29, respectively. Consequently, we conclude that the gene expression network of *E. coli* has more complex dynamics than that of *S. cerevisiae*. Also, considering a static gene regulatory network and having traces of gene expression time series for a cell at different times, we can compute the cross-correlation exponents for the links at a different time. This can be useful to compare statistical properties and complexity of dynamics. Similarly, by having different time series of gene expression dynamics, we can compare normal vs. disease affected (for example cancer typed) cells.

Multifractal Characteristics of Interactions Within the Gene Regulatory Network Can Be Modeled by Random Cascades on Wavelet Dyadic Trees

We analyzed the multifractal property of the cross-correlation of pairs of genes and TFs in a gene regulatory network. We investigated whether the observed multifractality can be explained by the known analytical cascade models including the Mandelbrot bimodal cascade model (Mandelbrot et al., 1997) (see Materials and methods section) and the random cascades on wavelet dyadic trees (Arneodo et al., 1998). We observe deviations of the empirical spectrums from the Mandelbrot model and an approximate agreement to the random cascades on wavelet dyadic trees model.

Based on the range of the Holder exponent values in the multifractal spectrum, we observe that only 0.04 of the links in a gene regulatory network of *S. cerevisiae* and none of the links in the network of *E. coli* can be modeled by the Mandelbrot cascade model for multifractal spectrums (see Materials and methods section). We observe that even for the few links that we could find a closest Mandelbrot model spectrum, the deviation from the Mandelbrot model and the data we had for gene regulatory network was significant. We show two such samples in **Figure 4**. **Figure 4a** shows several multifractal spectrums for the links in *E. coli* gene networks. Note that the peak of the multifractal spectrum for these spectrums was lower than the value 1, which does not fit with the Mandelbrot Binomial Cascade Model (Mandelbrot et al., 1997). **Figure 4b** shows several multifractal spectrums for the links in *S. cerevisiae*. **Figure 4c** shows the closest Mandelbrot Model we could fit for the links in the *S. cerevisiae* gene regulatory model. There is a significant deviation between the Mandelbrot model and the spectrum from gene regulatory network data.

We also investigated the agreement between the observed multifractality of the cross-dependencies in the gene regulatory network of *S. cerevisiae* and *E. coli*, respectively, and a few well-known multifractal models such as the random cascades on wavelet dyadic trees (Arneodo et al., 1998). We investigated whether the log-normal W-cascade model can be fitted to the cross-dependencies (links) in the two above-mentioned

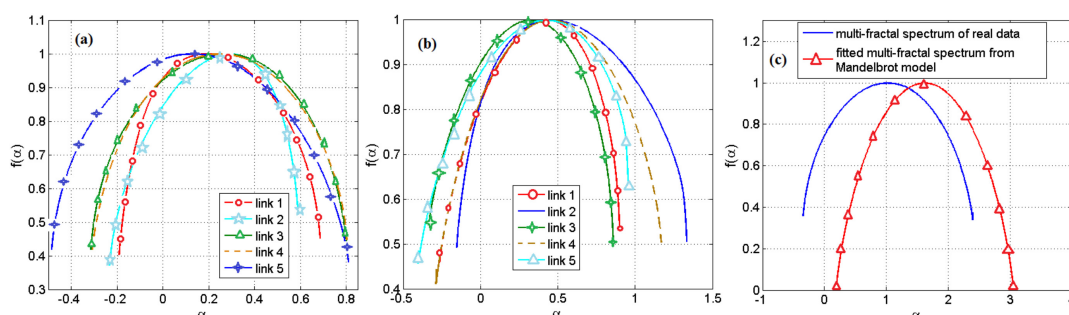


FIGURE 4 | (a) Multifractal spectrum of several randomly picked links of gene regulatory network of *E. coli*. **(b)** The multifractal spectrum of several randomly picked links of gene regulatory network of *S. cerevisiae*. **(c)** The multifractal spectrum of a link between the gene regulatory network of *S. cerevisiae* and the best spectrum from the Mandelbrot binomial model.

gene regulatory networks. We extracted the parameter of the estimated log-normal W-cascade model based on the peak of the empirical spectrum and the variation of the singularity spectrum (see the Methods section). We observed very similar spectrums for a significant number of links. We computed the overlapping area under the curve for both the estimated and the empirical multifractal spectrums. The ratio of the area of the overlapping fitted spectrum to the area of the empirical spectrum can be used to either accept or reject the postulated multifractal model as a good fitting for the empirically estimated multifractal spectrums. In this study, we used two threshold values of 70 and 75% for the ratio of mentioned areas. For the gene expression cross-dependencies in *E. coli*, we observed a 74 and 38% agreement between the postulated model and the empirically estimated multifractal spectrums when considering overlapping area ratio thresholds of 70 and 75, respectively. For the gene expression cross-dependencies in *S. cerevisiae*, we observed a 59 and 31% agreement between the postulated multifractal model and the empirically estimated multifractal spectrums when considering overlapping area thresholds of 70 and 75%, respectively. **Figures 5A,B** show a best fitting scenario between the postulated multifractal model and an empirically estimated spectrum for a cross-dependence in the gene regulatory network of *E. coli* and *S. cerevisiae*, respectively.

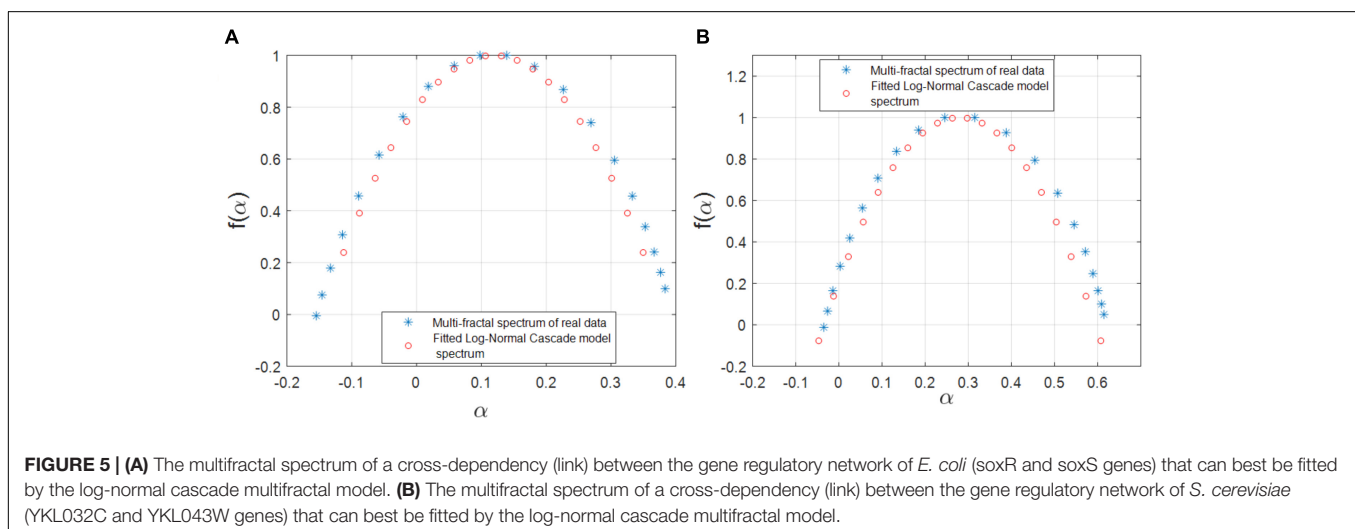
We also investigated the agreement between the empirical multifractal spectrums and the log-Poisson W-cascade model (Arneodo et al., 1998). We observed that the empirical multifractal spectrums could not be described by this cascade model since the second derivative of the mass exponent should follow a power law (see Methods section) while the empirical data has a significant deviation from a power law trend (see **Figure 6** in the Methods section).

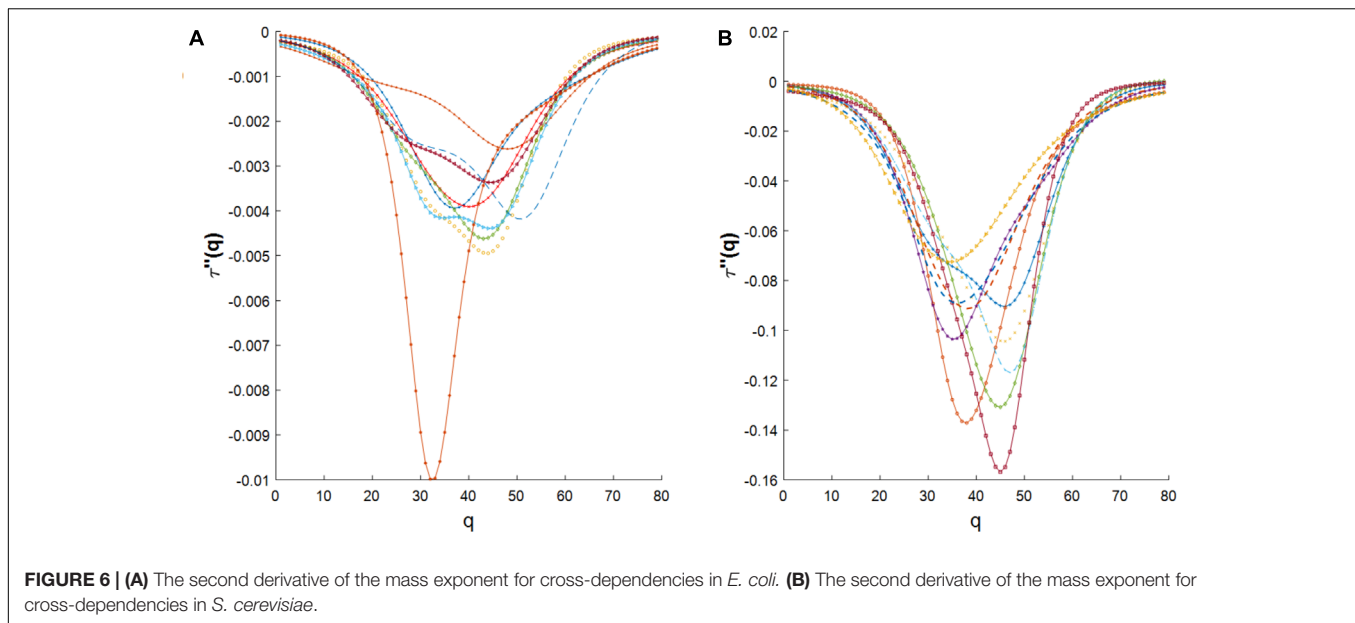
DISCUSSION

A genome expression vector is the most informative descriptor of a cell state, as the functional state of an organism is determined

largely by the pattern of expression of its genes (O'Connor et al., 2010). Gene expression is the process in which information from a gene is used to synthesize a functional gene product. It is the process in which the information flows within a complex biological system. As the search for patterns in nature and their interpretation is one of the main purposes of science, unveiling the DNA patterns in those sequences has become an exciting challenge to the present generation of biologists, statistical physicists, and information scientists. Toward this end, many researchers have studied the statistical properties of coding and non-coding segments of DNA sequences. They have reported interesting results showing fractal nature of coding DNA regions (Lockhart and Winzler, 2000; Teichmann and Babu, 2004; Huang et al., 2005; Düvel et al., 2010). However, these studies fail to address the dynamical properties of the biological systems. Since biological systems are dynamic, their study requires monitoring their activity at multiple time points.

To investigate the causal relations in gene expression, numerous biophysical mechanisms affecting the regulation process were studied in Elf et al. (2007), Kolesov et al. (2007), Kuhlman and Cox (2012), Bauer and Metzler (2013), Pulkkinen and Metzler (2013). It is demonstrated by several simulations that rapid and reliable gene regulation requires that the TF be close to their target site on DNA (Kolesov et al., 2007). In Pulkkinen and Metzler (2013), the authors use an explicit model for numerical analysis. The authors report that the observed variations in regulation efficiency are linked to the magnitude of the variation of TF concentration peaks as a function of binding site distance from the source. In Bauer and Metzler (2013), the authors have presented a semi-analytical model for the *in vivo* target search of the TFs within a diffusion framework. They have shown that alternating between three-dimensional bulk diffusion and one-dimensional sliding along the DNA contour can provide a quantitative approach to gene regulation in living bacteria cells. Their proposed model agrees with experimental findings regarding the mean search time of lac repressor in a living *E. coli*. In Elf et al. (2007), the authors have reported their observation of kinetics of the gene expression process





at the single-molecule level in living cells by labeling with a fluorescent protein, which agrees with 1D diffusion along DNA segments and 3D diffusion. In Kuhlman and Cox (2012), the authors test the expectation of previous theoretical models using high-throughput single-molecule microscopy to determine the average spatial distribution of lac repressor. Their finding shows inconsistency between expectations and experimental findings. They show that the gene products distribution is spatially inhomogeneous and dependent on the location of the repressor gene in bacteria and eukaryotes. However, they do not consider the gene expression dynamics from a network of genes perspective and do not account for cross-correlations and multiscale phenomena.

To study the dynamic nature of gene expression processes, researchers must monitor activity levels of genes and TFs at multiple time points. The most informative source of information regarding gene expression activity is the gene expression time-series. With advances in gene array technology, the level of gene expression of thousands of genes (by providing the concentration level of gene expression products) can be measured simultaneously. By accessing a high-throughput data collection, a wide range of insights, such as characterizing the functions of specific genes, the relationships among these genes, and their regulation and coordination can be gained. These insights can also be used to understand the gene regulatory network as a complex network. There are many studies which try to infer the underlying gene regulatory network from empirical time series (Marbach et al., 2012). However, little is known about the mathematical characteristics of the gene expression dynamics from a complex systems perspective.

In this paper, we investigated the scaling properties of gene expression dynamics. Unlike previous work that demonstrated the fractal properties in DNA sequences (Peng et al., 1994; Arneodo et al., 1996; Zhang et al., 2011; Marbach et al., 2012), we investigate the dynamics of cross-dependencies

between genes and TFs within the gene regulatory networks. We show that the gene expression time series (which is the concentration of gene expression products in the process of gene expression) have fractal and long-range dependence properties in *E. coli* and *S. cerevisiae*. We also investigate the cross-correlation of gene-TFs, which are linked together in gene expression networks. We report the fractal and long-range cross-dependency of linked genes and TFs of gene expression networks in *E. coli* and *S. cerevisiae*. We also show that the multifractal nature of these cross-correlations cannot be modeled through a Mandelbrot binomial cascade model. In contrast, we found very good agreement between the empirical multifractal spectrum of the cross-dependencies in the gene regulatory networks and the log-normal W -cascade model. We suggest investigating more cascade models on empirical data (Bacry and Muzy, 2003; Chainais et al., 2005; Kiyono et al., 2007) as future work. In summary, there is a need for more advanced theoretical models that can capture the multifractality observed in this critical biological process. One possible method for modeling gene expression dynamics can exploit the multifractal Fokker-Planck formalism, as discussed in Xue and Bogdan (2017).

We also propose using the distribution of cross-correlation exponent of the links in gene regulatory network as a measure of the complexity for the regulatory networks. Having this complexity measure enables a quantitative descriptor for different cell types or to differentiate different cell fates when the system undergoes transitions. We report the distribution of cross-correlation exponent of links in regulatory networks of *E. coli* and *S. cerevisiae* as case studies. We suggest investigating this property on a wider range of biological systems when enough data sets are available. We also propose using this property as a network property in general. We propose using the distribution of cross-correlation exponents of gene-TF links in a complex network to measure the

complexity of the interactions in the network. Also, the computed cross-correlation exponents of a network can be used by other algorithms, such as those proposed in Anand and Bianconi (2009), Anand et al. (2011), Teschendorff and Enver (2017) for computing the entropy of a network. This has a variety of applications in distinguishing the different status of cells (e.g., healthy vs. disease affected states). This can reveal insightful results in many complex networks either in biology, social, financial and many other interesting examples of complex networks.

Our findings explain the inherent variability in gene expression processes, even among isogenic cells situated in an identical environment. Because of the long-range cross-dependency of a gene and its linked TF, the current concentration level of a gene depends both on current and previous values of its own and its linked TF. As explained in previous studies, this leads to phenotypic diversity, which can be helpful for surviving in an uncertain and fluctuating environment (Ji et al., 2013). Also, the endogenous cellular mechanism through positive and negative feedback controls variability in gene expression to prevent disruption from normal development. Hence, unlike the usual assumption about noise as a nuisance, variability in gene expression makes the population of cells more robust against environmental fluctuations. Interestingly, there are other examples in nature in which the presence of noise makes the system smarter. For example, in Maass (2014), they have shown how the presence of noise in a network of spiking neurons in the human brain enables probabilistic reasoning and creative problem-solving.

This study is the first to demonstrate the long-range dependency of gene expression dynamics. In contrast to previous studies (Bernaola-Galván et al., 1996), which have shown the long-range dependency for the structure of DNA, we investigate the dynamics of gene expression time series. Previous studies show that coding regions of DNA structure, which store the biological information for the gene expression process, possess the long-range dependency property (Bernaola-Galván et al., 1996). In contrast, our results report the same property in gene expression time series. Of note, these dynamics stem from the transformation of information from the structure to dynamics by producing gene products. This is an insightful empirical result that can trigger more studies on other examples from nature, as well as analytical and mathematical investigations. For example, investigating other processes that follow a rule from a static structure to generate dynamical products and process (such as the central dogma of biology) can be interesting. Lastly, mathematical and analytical investigation of the relation between structure and dynamics of processes are also fundamental in theory. It would be revealing to investigate how long-range dependency (and/or fractal/multifractal properties) evolves from structure to dynamics (and vice versa) in processes. Answering to the question of how long-range dependency transfers between structure and dynamics and how the degree of fractality/multifractality of structure and dynamics are like each other would have a huge impact on predicting the behavior of complex systems.

MATERIALS AND METHODS

We use the data set from the publicly available DREAM project¹ (Stolovitsky and Califano, 2007), which is for assessment of network inference methods. It is organized around annual challenges where the community of network inference experts is solicited to run their algorithms on benchmark data sets. The data is provided from Gene-expression microarray datasets for *E. coli* and *S. cerevisiae*². A compendium of microarray data is compiled for *E. coli*, where all chips are on the same Affymetrix platform, the *E. coli* Antisense Genome Array. In total, 805 chips with available raw data Affymetrix files were compiled. Completion of microarray normalization and filtering resulted in a total of 4,297 genes over the 805 microarrays. Also, a compendium of microarray data was compiled for *S. cerevisiae*, where all chips are on the same Affymetrix platform, the Affymetrix Yeast Genome S98 Array. Chips were downloaded from GEO (Platform ID: GPL). In total, 536 chips with available raw data Affymetrix files were compiled. The completion of microarray normalization and filtering resulted in a total of 5,667 genes over the 536 microarrays. Transcriptional interactions and, hence, gene regulatory networks for *E. coli* and *S. cerevisiae* are collected from strong experimental supports in Marbach et al. (2012). Known transcriptional interactions for *E. coli* are collected from manually curated Ecocyc (Gama-Castro et al., 2010) and RegulonDB (Keseler et al., 2010) databases. A gene regulatory network for *E. coli* is constructed from RegulonDB Release 6.8. Only transcriptional interactions with at least one strong piece of evidence were included (2,066 interactions). For *S. cerevisiae*, we use the network based on the most stringent thresholds from MacIsaac et al. in MacIsaac et al. (2006) compared to other studies (Hu et al., 2007; Abdulrehman et al., 2010). By varying the thresholds required for binding and evolutionary conservation of motifs, different versions of the network were obtained. Based on the most stringent thresholds, which includes only interactions with strong evidence of binding and a strongly conserved motif, the interactions in the regulatory network are obtained. There are 5950 time-series, each having 536 data points for *S. cerevisiae*, and 4511 time-series, each having 805 data points for *E. coli* expression series.

Noise in Gene Expression Time Series

Since DNA, RNA, and proteins involved in the gene expression process can be present and active at a few copies per cell, this process is sensitive to stochastic fluctuations (Raser and O'shea, 2005). The four most important sources of variation in gene expression dynamics include (i) the inherent stochasticity of biochemical processes that are dependent on the small number of molecules, (ii) differences in the internal states of cells, (iii) subtle environmental differences, and (iv) genetic mutations (Raser and O'shea, 2005). The existence of this variation causes genetically

¹<http://wiki.c2b2.columbia.edu/dream>

²<https://www.synapse.org/#!Synapse:syn278209/wiki/70349>

identical organisms with identical environmental exposure to varying in behavior and shape. The fluctuation in the gene expression process is inevitable and does not follow the law of mass action. Hence, in this study, we have investigated the process independently of assuming stationarity and the MFDFA method explained below is used.

The Hurst Exponent and Multifractal Detrended Fluctuation Analysis (MFDFA)

In this paper, we have used the MFDFA method for analysis of gene expression time series. This method, which is the extension of detrended fluctuation analysis (DFA) to extract the Hurst exponent (Kantelhardt et al., 2002), is introduced in Kantelhardt et al. (2002) for analysis of multifractal properties of nonstationary time series. Since we do not have the stationarity assumption for gene expression time series, MFDFA method is a suitable one for studying them. Scaling properties and long-range dependency of time series can be obtained by the DFA method. However, for time series with multifractal properties and different scaling exponents on different scaling regimes or different time intervals, it is essential to exploit the multifractal detrended fluctuation method (MFDFA) to reveal the multifractal property.

The MFDFA method consists of five steps to estimate the multifractal spectrum of a nonstationary time series. Similar to the DFA method, the *profile* of the time series is obtained first, which is determined by the integration of the difference of the time series with its average value ($\hat{x}$):

$$y(i) = \sum_{i=1}^k (x(i) - \hat{x})$$

Second, it divides the profile into non-overlapping segments (or scales ($n = N/s$), where s is the scale). For each of these boxes, a least squared local trend is fitted.

Third, it calculates the local trend within each segment. For each of these boxes, a least squared local trend is fitted. The value of the fitted time series obtained for boxes of length (n) is denoted by y_n .

Fourth, it computes the average of the fluctuation function over all segments to obtain the q th order fluctuation function.

$$F(s)_q = \sum_{k=1}^{Ns} ((y(i) - y_n(i))^2)^q / Ns$$

Finally, the Generalized Hurst exponent is estimated by fitting a linear line to the log-log plot of the $F(s)_q$ with respect to scale (s), according to the following equation:

$$F(s)_q = s^{H(q)}$$

The Hurst exponent is the value of the Generalized Hurst exponent ($H(q = 2)$), which is a special case and is used usually when one is interested only in analyzing the long-range dependency of a signal and not the multifractal chrematistics.

Finally, the multifractal spectrum of the multi-variable signal ($\alpha, f(\alpha)$) is estimated by the Legendre transform:

$$\tau(q) = H(q)^*(q - 1)$$

$$\alpha(q) = d\tau(q)/dq$$

$$f(\alpha) = q\alpha - \tau(q)$$

Detrended Cross-Correlation Analysis (DCCA)

This method is designed to investigate the power law cross-correlation between two time-series (Podobnik and Stanley, 2008). Similar to the DFA (discussed in the previous sub-section), which computes the scaling behavior of the auto-correlation function, the DCCA method computes the scaling behavior of the cross-correlation function between two time-series and analyzes its scaling behavior.

DCCA method first computes the integrated *profile* of each time series:

$$y_1(i) = \sum_{i=1}^k (x_1(i) - \hat{x}_1)$$

$$y_2(i) = \sum_{i=1}^k (x_2(i) - \hat{x}_2)$$

Second, both the entire time series is divided into non-overlapping intervals. Third, it computes the local trend in each interval for each time series ($y_{1,n}(i)$, and $y_{2,n}(i)$). Fourth, it calculates the covariance of the residual of profiles from local trends. It calculates the detrended covariance ($H(q)$) by summing over all segments of the nonstationary time series:

$$\hat{F}(s)_q = \sum_{k=1}^{Ns} ((y_1(i) - y_{1,n}(i))^2 (y_2(i) - y_{2,n}(i))^2)^q / Ns$$

The cross-correlation exponent (λ) is estimated by fitting a linear line to the log-log plot of the $\hat{F}(s)_q$ with respect to scale (s):

$$\hat{F}(s)_q = s^\lambda$$

The Mandelbrot Binomial Cascade Model

This model is proposed by Mandelbrot et al. (1997) to better explain an alternative for probability distribution for the erratic or fractal appearance of a probability measure. It starts with a probability measure (μ), which is self-similar:

$$\mu([a,b]) = m_0 \mu([2a,2b]) + m_1 \mu([2a-1,2b-1])$$

Once the unit interval $[0,1]$ is divided into two subintervals, m_0 mass is assigned to the left subinterval and $m_1 = 1 - m_0$ is assigned to the right subinterval. Repeating this step for each of the subintervals for n times will result in the Mandelbrot model with n iterations. Mandelbrot has proved that the limit behavior of this model when n is infinitely large (∞) can be best illustrated

by multifractal formalism. He formulated the $(\alpha, f(\alpha))$ spectrum on the basis of the parameters of the Mandelbrot Cascade model. We have compared the observed multifractality spectrum in gene expression time series to the closest one obtained by Mandelbrot cascade model.

Random Cascades on Wavelet Dyadic Trees

This model (Arneodo et al., 1998) is proposed to model multifractal objects. The notion of cascade here refers to a self-similar process whose properties are defined multiplicatively in different scales. In summary, in this model, the wavelet coefficients of a function are self-similar at different scales. Two types of W -cascades are proposed: Log-Normal W -cascades and Log-Poisson W -cascades. For the log-normal cascade (with μ and σ for parameters of the normal random variable), the following equation holds for singularity spectrum:

$$F(\alpha) = -\frac{\ln 2}{2\sigma^2}(\alpha + \mu/\ln 2)^2$$

For the Log-Poisson W -cascades, the following equation holds for the mass exponent:

$$\tau(q) = \frac{1}{\ln 2}(\lambda(1-\delta^q) - \gamma q) - 1$$

As can be seen, the second derivative of the mass exponent for Log-Poisson W -cascade model has the following equation:

$$\tau'(q) = \lambda/\ln 2(-\ln \delta \delta^q) - \gamma/\ln 2$$

$$\tau''(q) = -\lambda/\ln 2(\ln \delta)^2 \delta^q$$

We have reported the similarity of the multifractal spectrum of cross-dependencies in gene expression time series to the log-normal W -cascades model. Also, we have reported the disagreement of the multifractal spectrum of cross-dependencies

in gene expression time series to log-Poisson W -cascade model due to its deviation from power-law shape as shown in **Figure 6**.

Entropy and Entropy of a Network

Shannon entropy (Shannon, 2001) is a measure of the *unpredictability* of the state, or equivalently, of its *average information content*. Shannon defined the entropy of a discrete random variable X with possible values of $\{x_1, x_2, \dots, x_k\}$ and probability mass function $P(X)$ as:

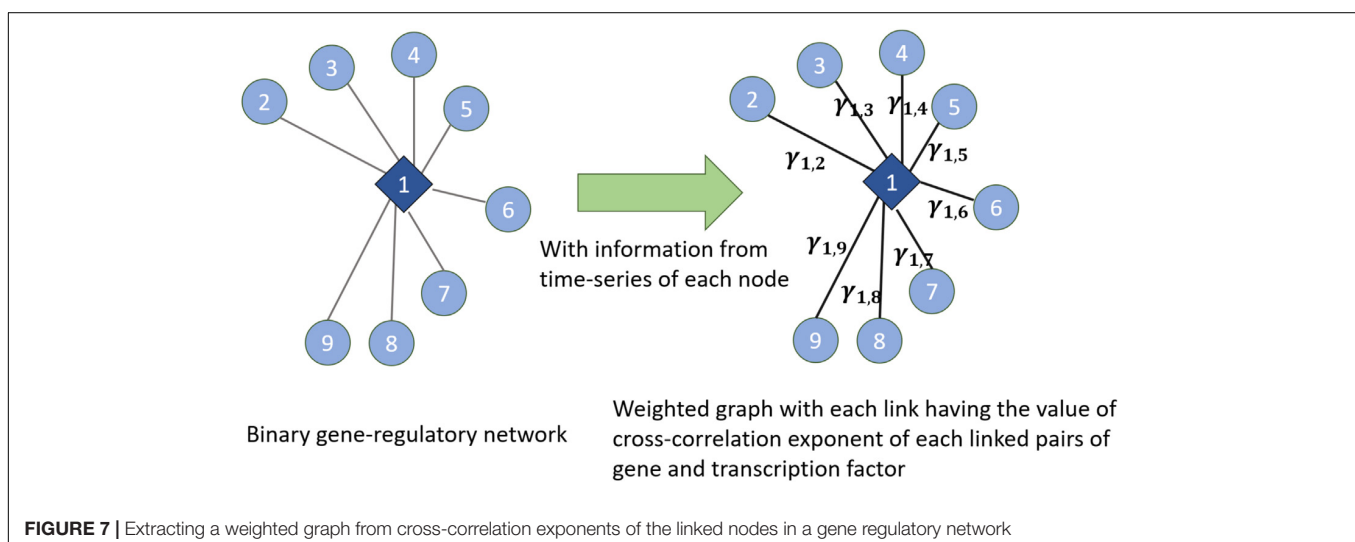
$$H(X) = E[I(X)] = E[-\log P(X)]$$

More explicitly, entropy can be written as:

$$H(X) = -\sum_{i=1}^n P(x_i) \log P(x_i)$$

Entropy is a measure of the unpredictability of the state or its average containing information. One example to illustrate is when there is no uncertainty and the random variables take only one value in which the value of the entropy will be zero. As the number of possibilities increases, the entropy increases as well.

We have used the notion of entropy in the context of networks. We consider the weight of the links in the network as the random variable and we discuss entropy of the weight of the weighted links. Given an undirected binary graph of gene regulatory networks and time series of genes and TFs in the network (which are the nodes in the gene regulatory network), we construct a weighted network (shown in **Figure 7**). In the constructed weighted network, the weight of each link is the *cross-correlation exponent* of the time series of two time-series linked together in the gene regulatory network. Then, in the new constructed weighted network, we consider the distribution of the weights of the links and entropy of them as a measure of the entropy of the network. Also, this weighted network can be used for other algorithms measuring the entropy of complex networks proposed in Anand and Bianconi (2009), Anand et al. (2011). **Figure 7** illustrates this method by showing how the weights are assigned



to each link. In this figure, $\gamma_{1,2}, \dots, \gamma_{1,9}$ are the cross-corrections of the time series of the TF and genes which are linked together in the gene regulatory network in the left part of the figure. Hence, this shows how knowing the existing interactions in the network and having the time series of each node's dynamics can lead us to know cross-correlation exponents and then assigning the concept of entropy to the network dynamics.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication. PB conceived the idea. MG, EJ, and PB discussed the research problem and approaches, identified analytical challenges and solutions. MG implemented the research discussions and performed the research.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphys.2018.01446/full#supplementary-material>

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Selecting Sensors in Biological Fractional-Order Systems

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Abstract—In this paper, we focus on sensor selection, i.e., determine the minimum number of state variables that need to be measured, to monitor the evolution of the entire biological system, i.e., all the state variables, when modeled by discrete-time fractional-order systems (DTFOS) that are subject to modeling errors, process and measurement noise. These systems are particularly relevant when modeling of spatiotemporal dynamics of processes in which the impact of long-range memory cannot be properly modeled by multivariate auto-regressive integrative moving-average models. Therefore, DTFOS enable a unified state-space framework to model the evolution of several biological (e.g., stem cell growth and bacteria evolution) and physiological signals (e.g., electroencephalogram and electromyogram).

Therefore, in this paper, we focus on the solution to four different (yet related) problems of sensor selection for DTFOS, that are motivated by constraints on the data acquisition that are enforced by the detrimental impact of the sensing mechanisms to the biological system, the cost of performing the measurements with the current sensing technology, or spatial constraints that limit the number of sensors that can be deployed. Towards determining the solution to these problems that we show to be NP-hard, we leverage the representation of the DTFOS to derive new objectives and conditions that, ultimately, enable us to efficiently approximate a solution to the different problems by exploiting the submodularity structure, which enables us to establish sub-optimality guarantees.

I. INTRODUCTION

A multitude of complex systems exhibits long-range (non-local) properties, interactions and/or dependencies (e.g., power-law decays in the weights of linear combination of past data) used to describe the biological system evolution. Example of such systems includes Hamiltonian systems, where the memory (i.e., dependence on the past data) is the result of stickiness of trajectories in time to the islands of regular motion [1]. Alternatively, it has been rigorously confirmed that viscoelastic properties are typical for a wide variety of biological entities like stem cells, liver, pancreas, heart valve, brain, muscles [1]–[3], [3]–[9], suggesting that the long-range memory of these systems obey the power law distributions. These dynamical systems can be characterized by the well-established mathematical theory of fractional calculus [10],

and the corresponding systems could be described by fractional differential equations [11]–[14], [42]. However, it is until recently that fractional order system (FOS) starts to find its strong position in a wide spectrum of applications in different domains due to the availability of computing and data acquisition methods to evaluate its efficacy in terms of capturing the underlying system states evolution.

Specifically, in [11], by the adoption of non-Gaussian statistical approaches, the authors identify the co-existence of fast and slow dividing subpopulations, and quiescent cells, in stem cells from three species. The mathematical analysis also shows that, instead of developing independently, stem cells exhibit a time-dependent fractal behavior as they interact with each other through molecular and tactile signals. In [12], the existence of a statistical fractal behavior and inadequacy of modeling blood glucose dynamics via linear state space models, is proved by the multi-fractal spectrum computed from the blood glucose time series of four individuals. A fractional order system model is alternatively proposed and evaluated to have superior regarding predictive power and controller synthesis. In [13], a multi-dimensional FOS is considered to capture the muscular dynamics in the process of forearm movement. The motivation comes from the power-law correlation decay as opposed to the exponential law, which is fundamentally assumed by the popular autoregressive moving average model. After the retrieval of the FOS model from the observations, it is shown that the model output is superior to ARMA to capture the observed spatial and temporal long-range dependence. In [42], a more comprehensive set of physiological processes (i.e., neural, muscular and vascular processes) are considered to study the minimal sensor placement problem in the context of the multi-dimensional FOS. The experimental results suggest that the adoption of FOS and the control theory developed based on it can help improve the design of efficient and reliable cyber-physical systems in the biomedical domain. In [14], the authors propose a statistical non-extensive causal inference framework and construct the generalized master equation (GME) to characterize the dynamics of complex systems that exhibit power-law spatiotemporal dependencies. The solution of the GME suggests a FOS be considered to capture the dynamical behaviors of the systems. In addition to the application of fractional order calculus to differentiable dynamical systems, very recent efforts have also been very successful to extend local fractional calculus to non-differentiable, irregular sets like fractals or fractal networks [15]–[18]. The fractality/multifractality of network, their characterization, computation, their influence on the dynamics of complex networked systems is attracting greater attention from a multi-disciplinary perspective. The possibility to extend the fractional to self-similar non-smooth objects is

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opening new frontiers in science. Non-linear analysis of data offers still unsolved analytical problems related not only to complex physics and abstract mathematical theories including fractals and fractional calculus [19].

Subsequently, because the current sensing technology is mainly digital, we focus on *discrete-time fractional-order systems* (DTFOS) [20], whose parameterization consists of a relatively small number of parameters, and the dynamics subject to modeling errors and external disturbances. Furthermore, in addition to modeling errors and external disturbances in the DTFOS dynamics, we also account for external disturbances in the sensing technology since our motivating technology, i.e., the EEG, uses sensing technology where noise commonly corrupts the collected data. Subsequently, in this paper, we propose to explore and exploit the trade-offs between the selected sensors and the capability to assess the process state over time, which we refer to as estimation performance since the state is obtained up to a confidence level subject to disturbance and noise. In other words, the combined effect of the modeling errors and external disturbances in the spatiotemporal dynamical processes requires the proper deployment of sensing technology that guarantees the best estimation performance (that is, the least estimation error) given the modeling errors' and external disturbances' characteristics.

In the last years, we have witnessed a growing interest on the trade-off between the number of used sensors and the degree of observability of linear time-invariant (LTI) systems [21]–[27], which are a particular case of DTFOS. In particular, this trade-off has been explored under the assumption that either the exact LTI system model is available, in which case one needs to ensure observability [21]–[27]. More recently, this interest has extended to deal with DTFOS, either when the models are exact [42], or in the context of structural observability [28]. Although ensuring observability is key towards the implementation of stable estimators, it does not explicitly explore the trade-offs between the chosen sensors and the quality of the state estimate and the model uncertainty, which is of utmost importance in biological settings, e.g., in EEG applications. These trade-off has been studied so far only for LTI systems, as we briefly review next. In [29], [30], the authors explore the trade-offs for LTI systems in the context of Kalman estimation. Specifically, in [29] the authors consider to place small numbers of sensors to optimize the resultant MMSE, and in [30] the author designs an output matrix with a desired norm that minimizes the MMSE.

In this paper, we extend the current literature to address the trade-off between the chosen sensors and the quality of the state estimate for the case of DTFOS with known parametric model and under possible uncertainties in the dynamics, as well as, noise in the measurements collected by the sensing technology. Specifically, we address the following problems: (i) determine the minimum number of sensors to ensure bounded process disturbance error within a prescribed threshold; (ii) determine the placement of a specified number of sensors to minimize the process disturbance error; (iii) determine the minimum number of sensors to ensure bounded state estimation error within a prescribed threshold; and (iv) determine the placement of a specified number of

sensors to minimize the state estimation error. It is worth noticing that among these four problems, the first couple of problems enforces the validity of the model by quantifying the uncertainty of the system's evolution, whereas the remaining two aim to determine the most likely state of the process across a time-window.

The main contributions of this paper can be cast in the following three domains:

Translational – it equips scientists (e.g., biologists and neuroscientists) and engineers alike with a unified framework to decide upon the sensor measurements to be considered to perform state estimation, i.e., to perform *sensor selection* to quantify uncertainty in the state and unknown disturbances and noises, in the context of fractional-order state-space representations capable of modeling spatiotemporal dynamics of processes in which the impact of long-range memory cannot be properly modeled by multivariate auto-regressive integrative moving-average models.

Theoretical – we propose to derive observability conditions that enable the quantification of the uncertainty of biological processes modeled by the proposed state-space representation, as well as identify the state variables that play a key role in monitoring the evolution of the dynamics while making the trade-off with the accuracy of the estimation. Specifically, we propose computationally efficient algorithms to provide sub-optimal solutions to the minimum number of variables that need to be measured (that is NP-hard), while establishing guarantees on the optimality gap.

Application – recently there is a renewed interest in neuro-wearable devices largely boosted by initiatives sponsored by either the Facebook that aims to use wearable devices to write 100 words per minute, and the NeuraLink by Elon Musk that aims to develop implantable brain-computer interfaces. Subsequently, we propose to revisit the neuro-wearables that rely on electroencephalogram, and determine the sensor location that seems to be the most effective with respect to a pre-specified number of sensors. In particular, we argue that for a variety of tasks the location of sensors currently used in such wearable devices is sub-optimal with respect to the proposed objectives that aim to ensure the quality of estimated state, process and measurement noise. Consequently, we conclude that at the light of this framework, some of the neuro-wearables should be re-designed to enhance dynamic systems properties such as observability.

In summary, our main contributions are as follows: (i) we formalize the sensor placement problems in context of four different (yet related) problems pertaining to sensor placement to minimize the process disturbance error and state estimation error; (ii) we show that these problems are NP-hard; (iii) we present approximation schemes for their solution that have provably optimal approximation performance; and (iv) we illustrate the proposed approaches using EEG signal data associated with a variety of tasks.

The remainder of this paper is organized as follows. In Section II, we provide our setup and problem formulation. In Section III, we present our main results. In Section IV, we illustrate how the main results can be applied in the context of real EEG signal data. Section V concludes the paper.

II. PROBLEM STATEMENT

In this section, we introduce the problems addressed in the present paper. First, we introduce the DTFOS model used in Section II-A, while revisiting some of its properties, and the best linear estimator for it in Section II-B. Then, in Section II-C, we introduce the optimal sensor placement problem for DTFOS, which seeks to determine the minimum collection of sensors that ensure a pre-specified estimation performance, or the configuration of a given number of sensors that attain the best process disturbance and estimate quality.

A. DTFOS Model

We consider the linear DTFOS described by

$$\begin{aligned}\Delta x_{k+1} &= Ax_k + w_k, \\ y_k &= Cx_k + v_k, \quad k = 0, 1, \dots\end{aligned}\quad (1)$$

where $x_k = [x_k^1, x_k^2, \dots, x_k^n]^\top \in \mathbb{R}^n$ ($n \in \mathbb{N}$) is the state vector, $y_k \in \mathbb{R}^c$ the measured output vector, w_k the process disturbance and v_k the measurement noise, and x_0 the initial condition. Additionally, $\Delta \equiv \text{diag}(\Delta_{k+1}^{\alpha_1}, \Delta_{k+1}^{\alpha_2}, \dots, \Delta_{k+1}^{\alpha_n})$ is the diagonal matrix operator, where $\Delta_{k+1}^{\alpha_i}$ is the discrete fractional-order difference operator such that

$$\Delta_{k+1}^{\alpha_i} x_{k+1}^i \equiv \sum_{j=0}^{k+1} (-1)^j \binom{\alpha_i}{j} x_{k-j+1}^i,$$

and $\binom{\alpha_i}{j} = \frac{\Gamma(\alpha_i+1)}{\Gamma(j+1)\Gamma(\alpha_i-j+1)}$, where $\alpha_i > 0$ is the *fractional-order exponent*, and $\Gamma(x) = \int_0^\infty t^{x-1} e^{-t} dt$ is the Gamma function. In summary, the matrix A captures the spatial coupling (i.e., dependency) of the process, whereas α_i capture the temporal dependency of the process associated with x_i .

Also, we notice that it is possible to provide a closed-form solution to (1), following [31], and which can be described as follows.

Lemma 1: For all $k \geq 1$, the solution to (1) is given by $x_k = G_k x_0 + \sum_{j=0}^{k-1} G_{k-1-j} w_j$, where

$$G_k \equiv \begin{cases} I, & k = 0 \\ \sum_{j=0}^{k-1} A_j G_{k-1-j}, & k \geq 1, \end{cases}$$

where $A_0 = A$, and A_j is a diagonal matrix whose i -th entry is $(-1)^j \binom{\alpha_i}{j+1}$. $\diamond$

In particular, Lemma 1 states that a linear DTFOS can be interpreted as a linear time-variant switching system, where transitions are known. Subsequently, we can develop a Kalman-like estimator for this process, which estimates' characterization is leveraged to study the trade-offs between performance of the estimator and a specified sensor placement.

B. Minimum Variance Linear Estimator

For any estimation horizon K (that is, k in (1) varies from 0 to K), we first present the minimum mean square linear estimator of $z_K \equiv (x_0^\top, w_0^\top, w_1^\top, \dots, w_{K-1}^\top)^\top$. This estimator is particularly useful in biological systems to assess the validity of the model, since a quantification of uncertainty

is obtained. To this end, we use the following common assumption.

Assumption 1: Let the initial condition be unknown and modeled by a random variable whose expected value is $\bar{x}_0$ and its covariance is $\mathbb{C}(x_0) \succ 0$. In addition, let the process disturbance w_k and the measurement noise v_k to be described by zero-mean random variables, whose covariance is described respectively by $\mathbb{C}(w_k) \succ 0$ and $\mathbb{C}(v_k) \succ 0$, for all $k \geq 0$, where $\mathbb{C}(v_k)$ is a diagonal matrix; that is, the measurement noises between any two sensors that correspond to two rows of C are uncorrelated. Furthermore, for all $k, k' \geq 0$ with $k \neq k'$, let the x_0 , w_k and v_k , as well as, the w_k , $w_{k'}$, v_k and $v_{k'}$ to be uncorrelated. $\circ$

Moreover, we consider the following notations: let the vector of measurements $y_{0:K} \equiv (y_0^\top, y_1^\top, \dots, y_K^\top)^\top$, the vector of process noises $w_{0:K-1} \equiv (w_0^\top, w_1^\top, \dots, w_{K-1}^\top)^\top$ and the vector of measurement noises $v_{0:K} \equiv (v_0^\top, v_1^\top, \dots, v_K^\top)^\top$. Notice that whereas the vector $y_{0:K}$ is known, the vectors $w_{0:K-1}$ and $v_{0:K}$ are not. Additionally, we refer to the interval $[0, K] \equiv \{0, 1, \dots, K\}$ as the *estimation horizon* of (1), and its *length* is $K + 1$.

Next, given an estimation horizon $[0, K]$, to derive the minimum mean square linear estimator of z_K , from (1) and Lemma 1, we have

$$y_{0:K} = \mathcal{O}_K z_K + v_{0:K}, \quad (2)$$

where $\mathcal{O}_K = [L_0^\top C^\top, L_1^\top C^\top, \dots, L_K^\top C^\top]^\top$ with the $n \times n(K+1)$ matrix $L_i = [G_i, G_{i-1}, \dots, G_0, \mathbf{0}]$, and $\mathbf{0}$ is the zero matrix with appropriate dimensions.

Thus, following similar steps to those performed for linear time-invariant systems [32], the minimum mean square linear estimate of z_K is given by

$$\hat{z}_K \equiv \mathbb{E}(z_K) + \mathbb{C}(z_K) \mathcal{O}_K^\top (\mathcal{O}_K \mathbb{C}(z_K) \mathcal{O}_K^\top + \mathbb{C}(v_{0:K}))^{-1} (y_{0:K} - \mathcal{O}_K \mathbb{E}(z_K) - \mathbb{E}(v_{0:K})),$$

where $\mathbb{E}(x)$ is the expected value of x , and $\mathbb{C}(x) \equiv \mathbb{E}([x - \mathbb{E}(x)][x - \mathbb{E}(x)]^\top)$ its covariance. Furthermore, the error covariance of $\hat{z}_K$ is given by

$$\begin{aligned}\Sigma_{\hat{z}_K} &\equiv \mathbb{E}((z_K - \hat{z}_K)(z_K - \hat{z}_K)^\top) \\ &= \mathbb{C}(z_K) - \mathbb{C}(z_K) \mathcal{O}_K^\top (\mathcal{O}_K \mathbb{C}(z_K) \mathcal{O}_K^\top + \mathbb{C}(v_{0:K}))^{-1} \mathcal{O}_K \mathbb{C}(z_K).\end{aligned}\quad (3)$$

In this paper, we capture the estimation performance of $\hat{z}_K$ with the metric $\log \det(\Sigma_{\hat{z}_K})$, which is proportional to the conditional entropy of z_K given the measurements $y_{0:K}$, and as a result, captures how well z_K is explained by $y_{0:K}$ [33, Proposition 2]. In particular, the metric $\log \det(\Sigma_{\hat{z}_K})$ captures the probability that the estimation error $\|z_K - \hat{z}_K\|_2^2$ is small. To explain this, consider the η -confidence ellipsoid of $z_K - \hat{z}_K$ [34]: The η -confidence ellipsoid is the minimum volume ellipsoid that contains $z_K - \hat{z}_K$ with probability η . Specifically, it is encapsulated by $\mathcal{E}_\epsilon(\hat{z}_K) \equiv \{z : z^\top \Sigma_{\hat{z}_K} z \leq \epsilon\}$, where $\epsilon \equiv F_{\chi_{n(K+1)}^2}^{-1}(\eta)$ and $F_{\chi_{n(K+1)}^2}$ is the cumulative distribution function of a χ -squared random variable with $n(K+1)$ degrees of freedom [35]. Therefore, the volume of $\mathcal{E}_\epsilon(\hat{z}_K)$ that

quantifies the estimation's error of $\hat{z}_K$ is given as follows:

$$\text{vol}(\mathcal{E}_\epsilon(\hat{z}_K)) \equiv \frac{(\epsilon\pi)^{n(K+1)/2}}{\Gamma(n(K+1)/2+1)} \det(\Sigma_{\hat{z}_K}^{1/2}). \quad (4)$$

Henceforth, if we consider the logarithm of (4), we obtain

$$\log \text{vol}(\mathcal{E}_\epsilon(\hat{z}_K)) = \beta + 1/2 \log \det(\Sigma_{\hat{z}_K}), \quad (5)$$

where β is a constant that depends only on $n(K+1)$ and ϵ , and, as a result, we refer to the $\log \det(\Sigma_{\hat{z}_K})$ as the *log det initial state-uncertainty estimation error of the minimum variance linear estimator* of (1).

Alternatively, we might be interested in determine the minimum variance linear estimator of $x_{0:K} \equiv (x_0, x_1, \dots, x_K)$, denoted by $\hat{x}_{0:K}$. To this end, the collection of measurements is given by $y_{0:K} = O_K x_{0:K} + v_{0:K}$, where O_K is the block diagonal matrix with diagonal elements $K+1$ copies of the matrix C . Subsequently, following similar steps to those in [36], the state estimation $\hat{x}_{0:K}$ error covariance is given by

$$\Sigma_{\hat{x}_{0:K}} = \mathbb{C}(x_{0:K}) - \mathbb{C}(x_{0:K})O_K^\top (O_K \mathbb{C}(x_{0:K})O_K^\top + \mathbb{C}(v_{0:K}))^{-1} O_K \mathbb{C}(x_{0:K}). \quad (6)$$

Besides, by proceeding similarly to the reasoning above, we can define the *log det batch-state estimation error of the minimum variance linear estimator* of (1) as follows:

$$\log \text{vol}(\mathcal{E}_\epsilon(\hat{x}_{0:K})) = \beta + 1/2 \log \det(\Sigma_{\hat{x}_{0:K}}). \quad (7)$$

C. Optimal Sensor Placement

Now, we introduce four different (yet, related) problems to assess the optimal sensor placement with respect to the log det of the initial state-uncertainty and batch-state estimation error of the minimum variance linear estimator of (1). Specifically, we propose for each to determine the placement of r sensors such that the overall estimation error is minimized, and determine a placement of sensors such that the estimation error satisfies a specified threshold.

Therefore, we propose to use the following sensor placement model: across the estimation horizon $[0, K]$, a *unique* subset of r sensors in (1) is placed and used, that corresponds to r of the c rows of C ($r \leq c$). In particular, for all $k \in [0, K]$ in (1),

$$y_k = SCx_k + v_k, \quad k \in [0, K], \quad (8)$$

where S is the sensor placement matrix (constant across the estimation horizon $[0, K]$); that is, it is a zero-one matrix such that $S_{ij} = 1$ if sensor j is placed (which corresponds to the j -th row of C), and $S_{ij} = 0$ otherwise. We assume that a sensor can be placed at most once, and as a result, for each i there is one j such that $S_{ij} = 1$ while for each j there is at most one i such that $S_{ij} = 1$. Hence, given a sensor selection matrix S , the indices of the rows of C that correspond to used sensors is denoted by $\mathcal{S}$, i.e., $\mathcal{S} \equiv \{j : \text{exists } i \text{ such that } S_{ij} = 1\}$.

Consequently, given the DTFOS in (1) and a finite estimation horizon $[0, K]$, we consider the following four problems:

Initial State-Uncertainty Estimation Error

(i) Provided a specified error threshold $R \in \mathbb{R}^+$, determine the *initial state-uncertainty minimal sensor placement problem* that is a solution to the following problem:

$$\begin{aligned} & \underset{S \subseteq \{1,2,\dots,c\}}{\text{minimize}} && |\mathcal{S}| \\ & \text{subject to} && \log \det(\Sigma_{\hat{z}_K}(\mathcal{S})) \leq R, \end{aligned} \quad (\mathcal{P}_1)$$

where $\det(\Sigma_{\hat{z}_K}(\mathcal{S}))$ is the determinant of $\Sigma_{\hat{z}_K}$ in (3) when O_K is replaced by $O_K(\mathcal{S})$, with explicit dependence on $\mathcal{S}$, and described by $O_K(\mathcal{S}) = [L_0^\top C(\mathcal{S})^\top, L_1^\top C(\mathcal{S})^\top, \dots, L_K^\top C(\mathcal{S})^\top]^\top$, and $C(\mathcal{S})$ denotes the rows of C with indices in $\mathcal{S}$;

(ii) Provided a maximum number r of sensors to be placed, determine the *initial state-uncertainty cardinality-constrained sensor placement problem for minimum estimation error* that consists of a solution to the following problem:

$$\begin{aligned} & \underset{S \subseteq \{1,2,\dots,c\}}{\text{minimize}} && \log \det(\Sigma_{\hat{z}_K}(\mathcal{S})) \\ & \text{subject to} && |\mathcal{S}| \leq r. \end{aligned} \quad (\mathcal{P}_2)$$

Batch-State Estimation Error

(iii) Provided a specified error threshold $R \in \mathbb{R}^+$, determine the *minimal sensor placement problem* that is a solution to the following problem:

$$\begin{aligned} & \underset{S \subseteq \{1,2,\dots,c\}}{\text{minimize}} && |\mathcal{S}| \\ & \text{subject to} && \log \det(\Sigma_{\hat{x}_{0:K}}(\mathcal{S})) \leq R, \end{aligned} \quad (\mathcal{P}_3)$$

where $\det(\Sigma_{\hat{x}_{0:K}}(\mathcal{S}))$ is the determinant of $\Sigma_{\hat{x}_{0:K}}$ in (6) when O_K is replaced by $O_K(\mathcal{S})$, which is the block diagonal matrix with diagonal elements K copies of the matrix $C(\mathcal{S})$, and $C(\mathcal{S})$ denotes the rows of C with indices in $\mathcal{S}$; and

(iv) Provided a maximum number r of sensors to be placed, determine the *cardinality-constrained sensor placement problem for minimum estimation error* that consists of a solution to the following problem:

$$\begin{aligned} & \underset{S \subseteq \{1,2,\dots,c\}}{\text{minimize}} && \log \det(\Sigma_{\hat{x}_{0:K}}(\mathcal{S})) \\ & \text{subject to} && |\mathcal{S}| \leq r. \end{aligned} \quad (\mathcal{P}_4)$$

◦

Problems $(\mathcal{P}_1) - (\mathcal{P}_4)$ address different problems that focus on different practical considerations. Specifically, $(\mathcal{P}_1)$ aims to determine the minimum number of sensors to ensure bounded process disturbance error within a prescribed threshold, which enables the minimization of the estimation of the uncertainty that drives the system; thus, equipping us with an uncertainty quantification of the process evolution. In contrast, $(\mathcal{P}_2)$ addresses the problem of determining the placement of a specified number of sensors to minimize the process disturbance error, which captures the situations where one has a budget on the available sensing technology, and wants to deploy the sensors to maximize the performance of the process captured by minimizing the system's uncertainty.

Problem $(\mathcal{P}_3)$ focus on determining the minimum number of sensors to ensure bounded state estimation error within a prescribed threshold, which might be related with the satisfaction

of some standard or accuracy required to have a sound estimate of the system's state. Finally, $(\mathcal{P}_4)$ targets the placement of a specified number of sensors to minimize the state estimation error when the number of sensing mechanisms is limited and one aims to minimize the system's state estimate uncertainty.

Notwithstanding, as it will become clear in the upcoming sections, the underlying optimization structure is similar, which enables us to study them in a unified fashion. Specifically, to address these problems, we will show that both $\log \det(\Sigma_{\hat{z}_K})$ and $\log \det(\Sigma_{\hat{x}_{0:K}})$ are *supermodular* and *non-increasing* (formally defined in Section III). As a consequence, approximation algorithms for these type of functions can be leveraged to provide approximate solutions to these problems with worst-case performance guarantees.

III. SENSOR PLACEMENT FOR DTFOS

We present the main results of the present paper. First, we show that $(\mathcal{P}_1)$ - $(\mathcal{P}_4)$ are NP-hard (Theorem 1), which implies that optimal polynomial solutions to these problems are unlikely to exist. Next, we propose polynomial algorithms (Algorithm 1 and 2) to obtain an approximate solution to these problems, while ensuring worst-case performance guarantees (Theorem 3 and 4). In more detail, in Theorem 2, we show that the constraint and objective function in $(\mathcal{P}_1)/(\mathcal{P}_3)$ and $(\mathcal{P}_2)/(\mathcal{P}_4)$, respectively, are supermodular. Thereby, greedy algorithms can be provided to approximate the solution to these problems while ensuring a worst case scenario bounded optimality gap. Finally, in Theorem 5, we provide a discussion on the fundamental limits on the state-uncertainty estimation error and batch-state estimation, while exploring the trade-off with problems' parameters.

We start by showing the computational complexity of our problems in the next result.

Theorem 1: The problems $(\mathcal{P}_1)$ - $(\mathcal{P}_4)$ are NP-hard. $\diamond$

Subsequently, we need to devise a strategy that approximates the solutions to the proposed problems. Towards this goal, consider the following definitions.

Definition 1: A function $h : 2^{[c]} \mapsto \mathbb{R}$ is *submodular*, where $[c] = \{1, \dots, c\}$, if for any sets $\mathcal{S}$ and $\mathcal{S}'$, with $\mathcal{S} \subseteq \mathcal{S}' \subseteq [c]$, and any $a \notin \mathcal{S}'$,

$$h(\mathcal{S} \cup \{a\}) - h(\mathcal{S}) \geq h(\mathcal{S}' \cup \{a\}) - h(\mathcal{S}').$$

A function $h : 2^{[c]} \mapsto \mathbb{R}$ is *supermodular* if $(-h)$ is submodular. $\diamond$

Definition 2: A function $h : 2^{[c]} \mapsto \mathbb{R}$ is a *non-increasing set function* if for any $\mathcal{S} \subseteq \mathcal{S}' \subseteq [c]$ it follows that $h(\mathcal{S}) \geq h(\mathcal{S}')$. Moreover, h is a *non-decreasing set function* if $(-h)$ is a non-increasing set function. $\diamond$

Furthermore, a function $h : 2^{[c]} \mapsto \mathbb{R}$ is submodular if, for any $a \in [c]$, the function $h_a : 2^{[c] \setminus \{a\}} \mapsto \mathbb{R}$ defined as $h_a(\mathcal{S}) \equiv h(\mathcal{S} \cup \{a\}) - h(\mathcal{S})$ is a non-increasing set function. This property is commonly referred to as the *diminishing returns property* [37].

Now, we show that the constraint and objective function of $(\mathcal{P}_1)/(\mathcal{P}_3)$ and $(\mathcal{P}_2)/(\mathcal{P}_4)$, respectively, are supermodular and non-increasing.

Theorem 2: Let c be the number of rows of C , and $s_i \in \{0, 1\}$ be 1 if and only if i -th sensor (i -th row of C)

is placed, and $L_{0:K} \equiv [L_0^\top, L_1^\top, \dots, L_K^\top]^\top$. In addition, let $M^{(i)} \equiv C_{0:K}^\top I^{(i)} \mathbb{C}(v_{0:K})^{-1} I^{(i)} C_{0:K}$, where $C_{0:K}$ is the block diagonal matrix where each of its $K+1$ diagonal elements is equal to C , and $I^{(i)}$ is the diagonal matrix with $c(K+1)$ diagonal elements such that, for all $k \in [0, K]$, the $(kc+i)$ -th element is 1, and the rest of the elements are equal to zero. Then, given any finite estimation horizon $[0, K]$, the following two equalities hold:

$$\begin{aligned} \log \det(\Sigma_{\hat{z}_K}(\mathcal{S})) = \\ - \log \det \left(\sum_{i=1}^c s_i L_{0:K}^\top M^{(i)} L_{0:K} + \mathbb{C}(z_K)^{-1} \right), \end{aligned}$$

and

$$\log \det(\Sigma_{\hat{x}_{0:K}}(\mathcal{S})) = - \log \det \left(\sum_{i=1}^c s_i M^{(i)} + \mathbb{C}(x_{0:K})^{-1} \right).$$

Furthermore, both $\log \det(\Sigma_{\hat{z}_K}(\mathcal{S}))$ and $\log \det(\Sigma_{\hat{x}_{0:K}}(\mathcal{S}))$ are supermodular and non-increasing set functions with respect to the choice of the sensor set $\mathcal{S} \subset [c] = \{1, \dots, c\}$. $\diamond$

As consequence of Theorem 2, it follows that the functions exhibit the diminishing returns property, i.e., its rate of reduction with respect to newly placed sensors decreases as the cardinality of the already placed sensors increases. Therefore, some well known approximation schemes [38], [39] can be leveraged to obtain sub-optimal solutions to $(\mathcal{P}_1)$ - $(\mathcal{P}_4)$ with optimality guarantees.

In Algorithm 1 and Algorithm 2, we present strategies to approximate the solutions to $(\mathcal{P}_1)/(\mathcal{P}_3)$ and $(\mathcal{P}_2)/(\mathcal{P}_4)$, respectively. Specifically, in Algorithm 1, we provide an efficient algorithm for $(\mathcal{P}_1)/(\mathcal{P}_3)$ that returns a sensor set that satisfies the prescribed threshold and has cardinality up to a multiplicative factor from the minimum cardinality sensor sets that meet the same estimation bound. More importantly, this multiplicative factor depends only logarithmically on the problems' parameters. These properties and the time complexity are described in the following result.

Algorithm 1 Approximation Algorithm for $(\mathcal{P}_1)/(\mathcal{P}_3)$

Input: $h_\alpha(\mathcal{S}) = \log \det(\Sigma_\alpha(\mathcal{S}))$, where $\alpha \in \{\hat{x}_{0:K}, \hat{z}_K\}$ for $k \in [0, K]$, and a threshold R on the total estimation error incurred by $h_\alpha(\mathcal{S})$.

Output: Approximate solution $\mathcal{S}_\alpha$ for $(\mathcal{P}_1)/(\mathcal{P}_3)$.

```

 $\mathcal{S}_\alpha \leftarrow \emptyset$ 
while  $h_\alpha(\mathcal{S}_\alpha) > R$  do
     $a_i \leftarrow a' \in \arg \max_{a \in [c] \setminus \mathcal{S}_\alpha} (h_\alpha(\mathcal{S}_\alpha) - h_\alpha(\mathcal{S}_\alpha \cup \{a\}))$ 
     $\mathcal{S}_\alpha \leftarrow \mathcal{S}_\alpha \cup \{a_i\}$ 
end while

```

Theorem 3: Let a solution to $(\mathcal{P}_1)/(\mathcal{P}_3)$ be denoted by $\mathcal{S}_\alpha^*$, and the set obtained by Algorithm 1 be denoted by $\mathcal{S}_\alpha$. Moreover, denote the maximum diagonal element of $\mathbb{C}(x_0)$ and $\mathbb{C}(w_k)$, among all $k \in [0, K]$, as σ_0^2 and σ_w^2 , respectively. Then,

$$\log \det(\Sigma_\alpha(\mathcal{S}_\alpha)) \leq R, \quad (9)$$

and the optimality gap bounded as follows:

$$\frac{|\mathcal{S}_\alpha|}{|\mathcal{S}_\alpha^*|} \leq 1 + \log \left\{ \frac{\log \det(\Sigma_\alpha(\emptyset)) - \log \det(\Sigma_\alpha([c]))}{R - \log \det(\Sigma_\alpha([c]))} \right\} \equiv \eta \quad (10)$$

where $\log \det(\Sigma_{\hat{z}_K}(\emptyset)) \leq n(K+1) \log \max(\sigma_0^2, \sigma_w^2)$.

Furthermore, the time complexity of Algorithm 1 is $O(c^2(nK)^{2.4})$. $\diamond$

Therefore, Algorithm 1 returns a sensor set that meets the estimation threshold of $(\mathcal{P}_1)/(\mathcal{P}_3)$. Moreover, the cardinality of this set is up to a multiplicative factor of η from the minimum cardinality sensor sets that meet the same estimation bound. In other words, η is a worst-case approximation guarantee for Algorithm 1. Besides, η depends only logarithmically on the problems' parameters. Additionally, the dependence of η on n , R and $\max(\sigma_0^2, \sigma_w^2)$ is expected from a design perspective. Specifically, by increasing the state space size n , requesting a better estimation guarantee by decreasing R , or incurring a noise of greater variance, should all push the cardinality of the selected sensor set upwards.

Next, in Algorithm 2, we provide an efficient algorithm for $(\mathcal{P}_2)/(\mathcal{P}_4)$ that returns a sensor set of cardinality r , where r is chosen by the designer. In the next result, we provide optimality guarantees of the solution obtained with Algorithm 2, as well as the computational complexity incurred by the algorithm.

Theorem 4: Let a solution to $(\mathcal{P}_2)/(\mathcal{P}_4)$ be denoted by $\mathcal{S}_\alpha^*$, and the set obtained by Algorithm 2 be denoted by $\mathcal{S}_\alpha$. Then,

$$\frac{\log \det(\Sigma_\alpha(\mathcal{S}_\alpha)) - \log \det(\Sigma_\alpha(\emptyset))}{\log \det(\Sigma_\alpha(\mathcal{S}_\alpha^*)) - \log \det(\Sigma_\alpha(\emptyset))} \geq 1 - \frac{1}{e}, \quad (11)$$

where the approximation factor $1 - 1/e$ in (11) is the best one can achieve in polynomial time for this problem.

Furthermore, the time complexity of Algorithm 2 is $O(cr(nK)^{2.4})$. $\diamond$

Algorithm 2 Approximation Algorithm for $(\mathcal{P}_2)/(\mathcal{P}_4)$

Input: $h_\alpha(\mathcal{S}) = \log \det(\Sigma_\alpha(\mathcal{S}))$, where $\alpha \in \{\hat{x}_{0:K}, \hat{z}_K\}$ for $k \in [0, K]$, and a bound on the number r of sensors used to minimize $h_\alpha(\mathcal{S})$.

Output: Approximate solution $\mathcal{S}_\alpha$ for $(\mathcal{P}_2)/(\mathcal{P}_4)$.

```

 $\mathcal{S}_\alpha \leftarrow \emptyset, i \leftarrow 0$ 
while  $i < r$  do
     $a_i \leftarrow a' \in \arg \max_{a \in [c] \setminus \mathcal{S}_\alpha} (h_\alpha(\mathcal{S}_\alpha) - h_\alpha(\mathcal{S}_\alpha \cup \{a\}))$ 
     $\mathcal{S}_\alpha \leftarrow \mathcal{S}_\alpha \cup \{a_i\}, i \leftarrow i + 1$ 
end while
    
```

Notice that from Theorem 4 it follows the approximation quality depends on n , r and $\max(\sigma_0^2, \sigma_w^2)$ as expected from a design perspective. Specifically, by increasing the state space size n , requesting a smaller sensor set by decreasing r , or incurring a noise of greater variance should all push the quality of the approximation level downwards.

Limits on Selecting Sensors in DTFOS

Next, we provide explicit bounds on the variance of the state-uncertainty estimation error, while exploring the trade-off

with the following quantities: (i) the length of the estimation horizon $[0, K]$; (ii) the number of placed sensors r ; and (iii) the characteristics of the noises w_k and v_k . In particular, the next result imposes limitations on the assessment of the results that cannot be overcome.

Theorem 5: Let $\sigma_0^{(-1)} \equiv \max_{i \in [n]} [\mathbb{C}(x_0)^{-1}]_{ii}$, $\sigma_w^{(-1)} \equiv \max_{i \in [n(K+1)]} [\mathbb{C}(w_{0:K})^{-1}]_{ii}$, and $\sigma_v^{(-1)} \equiv \|\mathbb{C}(v_{1:K})^{-1}\|_2$. Also, denote by $\bar{L}$ the matrix $L_{0:K} L_{0:K}^\top$. Then, the following inequality holds for the variance of the error of the minimum variance estimator $\hat{z}_K$:

$$\text{tr}(\Sigma_{\hat{z}_K}) \geq \frac{n(K+1)}{r \sigma_v^{(-1)} \|C\|_2^2 \|\bar{L}\|_2 + \max\{\sigma_0^{(-1)}, \sigma_w^{(-1)}\}}. \quad (12)$$

In other words, for constant $\|C\|_2^2$ and $\|\bar{L}\|_2$, (12) implies that the state-uncertainty estimation error used to assess the validity of the model (1) is bounded by a quantity that decreases as the number of placed sensors r increases, and increases as the system's state size or the horizon K increases. Subsequently, it implies that $\text{tr}(\Sigma_{\hat{z}_K})$ can decrease only inversely proportional with the number r of placed sensors, and, as a result, increasing the number r to reduce the variance of the error of the minimum variance linear estimator is ineffective. Additionally, the bound in (12) increases linearly with the system's state size, which imposes additional fundamental limitations for large-scale DTFOS.

Lastly, we notice that similar arguments and fundamental bounds can be readily derived for the variance of the batch-state estimator error, i.e., $\text{tr}(\Sigma_{\hat{x}_{0:K}})$, by following the same steps as in [32].

IV. EEG SENSOR PLACEMENT

In this section, we propose to study $(\mathcal{P}_1)-(\mathcal{P}_4)$ in a real-world application setting collected by the BCI2000 system with a sampling rate of 160Hz [40]. Specifically, we consider 64-channel EEG data set which records the brain activity of 10 subjects (S001-S010) when they are performing motor and imagery tasks [41]. Each subject sits in front of a screen where targets might appear at the right/left/top/bottom side of the screen. Upon noticing the target, each subject is asked to open and close the corresponding fists or feet as a function of where the target appears. Each individual performed 14 experimental runs consisting of one minute with eyes open, one minute with eyes closed, and three two-minute runs of 4 interacting tasks with the target: (*Task 1*) open and close left or right fist as the target appears on either left or right side of the screen; (*Task 2*) imagine opening and closing left or right fist as the target appears on either left or right side of the screen; (*Task 3*) open and close both fists or both feet as the target appears on either the top or the bottom of the screen; and (*Task 4*) imagine opening and closing both fists or both feet as the target appears on either the top or the bottom of the screen.

First, we estimated the parameters of the DTFOS for the different tasks¹, which can be modeled by DTFOS as argued

¹The identification techniques used were introduced in [13], and the software implementation can be found at https://github.com/urashima9616/DFOS_Sensor_Selection.

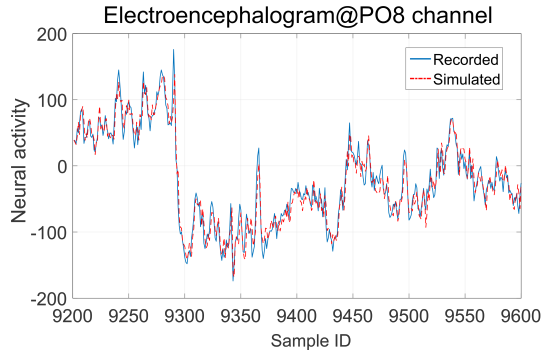


Fig. 1. EEG data recorded and the simulated using DTFOS at the EEG channel PO₈.

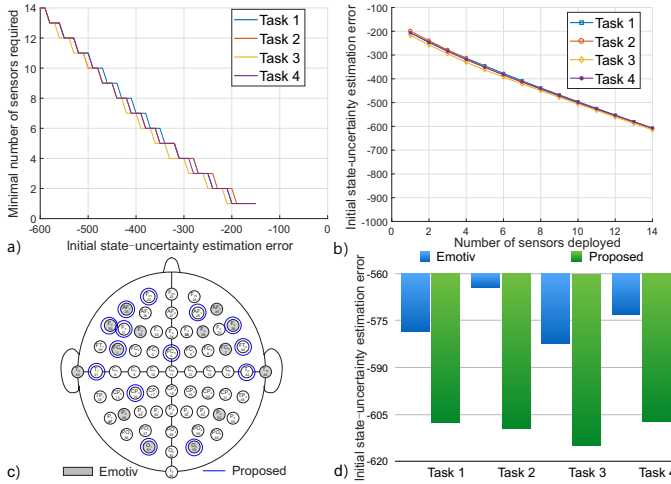


Fig. 2. (a) Minimal sensor placement to achieve a prescribed initial state-uncertainty estimation errors. (b) initial state-uncertainty log det errors achieved given different sensor budgets. (c) The 64-channel geodesic sensor distribution for measurement of EEG, where the sensors in gray represent those of the Emotiv EPOC and the ones in red are those returned by Algorithm 2 when solving (P_2) (that relieved to be the same for all 4 tasks), given the identified DTFOS and a deployment budget of 14 sensors. (d) initial state-uncertainty log det estimation errors associated with the highlighted sensor placements in (c).

in [42]. To illustrate the modeling capabilities of the proposed DTFOS model, in Figure 1 we contrast the recorded data at location PO₈ against the one simulated using the DTFOS identified. It is worth mention that similar performances are achieved across different channels, subjects and tasks. Besides, the fractional order exponents range from 0.34 to 1.04 across different tasks, which provides evidence that these could not be properly modeled by linear time-invariant systems – see [42] for further details. Lastly, we considered that the initial state, disturbance, and measurement noise follow a normal distribution with zero mean and covariance described by the identity matrix (both with appropriate dimensions).

Initial State-Uncertainty Estimation Error

First, we considered a single subject (S002), and determined the different DTFOS systems associated with the four different tasks. We applied Algorithm 1, with $\alpha = \hat{z}_K$ and $K = 7$, to solve (P_1) , and in Figure 2-(a) we plot the minimal number of sensors required as a function of

required initial state-uncertainty log det estimation error for the different tasks. The following observations are due: (i) given the same level of initial state-uncertainty estimation error required, the minimal number of sensors varies slightly when the subject is performing different tasks; and (ii) given a task, the initial state-uncertainty log det error exhibits supermodular properties (see Theorem 2).

To address (P_2) , i.e., to evaluate the achievable levels of the initial state-uncertainty estimation error given different sensor deployment budgets, we resorted to Algorithm 2 with $\alpha = \hat{z}_K$ and $K = 7$. In Figure 2-(b), we present the summary of the results, namely, the log det errors given different cardinality-constraints under the 4 tasks. It can be observed that the information gain, i.e., the improvement on estimation errors, is diminishing as the number of sensors used increases – as predicted by Theorem 4.

Additionally, we considered the deployment of 14 sensors, which is the same number of sensors available in some of the current EEG wearable technology, e.g., the Emotiv EPOC [43]. In Figure 2-(c), we report the sensor deployment returned by Algorithm 2 when solving (P_2) , which revealed to be the same across all 4 tasks (for the same individual). Specifically, we circled in blue the 14 sensors determined by our framework, whereas the Emotiv EPOC [43] sensors are colored in gray. From Figure 2-(c), we first notice that the sensor distribution pattern of Emotiv EPOC is symmetrical, whereas the Algorithm 2 places the sensors asymmetrically. Moreover, even though some of the locations are fairly close to each other (e.g., 41/43, 16/48, 40/38, 42/44, and 14/7), it turns out that only 5 out of 14 locations (i.e., 25, 29, 44, 48, 63) are identical, and Emotiv EPOC does not consider sensors 23, 4, and 18.

Subsequently, we assessed how the different sensor distributions, i.e., the proposed by our framework and the proposed by Emotiv EPOC, affect the estimation errors. In Figure 2-(d), we report the initial state-uncertainty estimation error across the different tasks. It is worth noticing that the sensors considered by our framework perform considerably better than the sensor distribution used by the Emotiv EPOC. Specifically, the log det estimation errors attained by the proposed sensor placement are smaller compared to those of Emotiv EPOC. In fact, it presents considerable gains across the different tasks, and, in particular, in Tasks 2 and 4 that require the use of imagination instead of the motor skills. Lastly, it is important to notice that the same sensor placement performs almost equally well across different tasks. Therefore, these results support the fact that from the point-of-view of an initial state-uncertainty estimation error using a model-based approach, the sensors' locations of the commercial EEG devices should be re-designed so to ensure better estimation performance.

Batch-State Estimation Error

Now, we address the batch-state estimation problems proposed in (P_3) – (P_4) . Towards this goal, we consider the DTFOS corresponding to the four different tasks for the same subject (S002). In particular, we obtain the solution to (P_3) by relying on Algorithm 1 with $\alpha = \hat{x}_{0:K}$ and $K = 7$, whose solutions

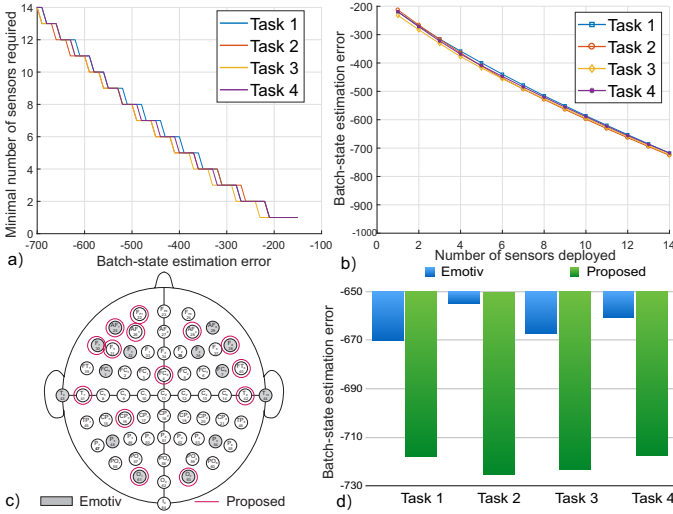


Fig. 3. (a) Minimal sensor placement to achieve a prescribed batch-state estimation errors. (b) batch-state log det errors achieved given different sensor budgets. (c) The 64-channel geodesic sensor distribution for measurement of EEG, where the sensors in gray represent those of the Emotiv EPOC and the ones in red are those returned by Algorithm 2 when solving (P_4) (that relieved to be the same for all 4 tasks), given the identified DTFOS and a deployment budget of 14 sensors. (d) batch-state log det estimation errors associated with the highlighted sensor placements in (c).

are found in Figure 3-(a) for several levels of batch-state estimation errors. From Figure 3-(a), we observe that for a specific level of batch-state estimation error the variation (across the different tasks) in the minimum number of sensors is minor. Moreover, we observe that the batch-state log det estimation error exhibits a diminishing returns property – as per Theorem 2.

Next, we use Algorithm 2 with $\alpha = \hat{x}_{0:K}$ and $K = 7$ to tackle problem (P_4) , i.e., to compute the achievable levels of the batch-state estimation error across several sensor placement budgets. The results are presented in Figure 3-(b), where we report the batch-state log det estimation errors given different cardinality-constraints across the 4 tasks. Similarly to the previous figure, we notice that the gain, i.e., the improvement on the estimation error, is diminishing as the number of sensors used increases (see Theorem 4).

Furthermore, using Algorithm 2 to solve (P_4) with a budget of 14 sensors, we obtained the sensor placement illustrated in Figure 3-(c). In Figure 3-(c), we circle in red the 14 sensors' placement found by our framework, whereas we depict the Emotiv EPOC [43] sensors in gray. Notice that the sensor placement obtained turned out to be the same across all 4 tasks for the same individual and it is asymmetrical, in contrast with the one of the Emotiv EPOC. Notably, only 5 out of 14 locations (i.e., 25, 29, 44, 48, 63) in both cases are the same, even though some of the sensor locations are close to each other (e.g., 41/43, 16/48, 40/38, 42/44, and 14/7). Moreover, the Emotiv EPOC does not consider the sensors 23, 4, and 18. In Figure 3-(d), we compared the batch-state estimation errors (across the different tasks) of the sensor deployment returned by our framework against the sensors placement of the Emotiv EPOC. We make the following observations: first, the sensors considered by our framework perform considerably better than the sensor distribution used by the Emotiv EPOC, especially

with respect to Tasks 2 and 4. Furthermore, it is worth mention that the same sensor placement returned by our framework performs almost equally well across the different tasks.

Assessment of Inter-subject variability

To assess how the inter-subject variability of brain dynamics affects the sensor selection under a fixed budget, we next consider a set of experiments where we solve P_2 and P_4 for 10 subjects across the four different tasks (Task 1-4). In particular, based on the identified DTFOS associated with the four tasks for a subject, we apply Algorithm 2 to obtain the placement of sensors given a deployment budget of 14 sensors by minimizing (i) initial state-uncertainty estimation error, and (ii) batch-state estimation error, respectively. In addition, we also identify the most voted 14 sensor locations based on the poll of sub-optimal solutions returned when solving P_2 and P_4 individually for all 10 subjects. The results are summarized in Figure 4-(a-d). We use heat maps to show the distribution of sensor locations returned by solving P_2 and P_4 individually in Figure 4-(a) and 4-(c), respectively. We can make the following remarks: (i) there exists a noticeable degree of inter-subject variabilities in the sensor deployment. This can be evidenced by the fact that the chosen sensors span over 40, 41, 46, 46 and 37, 43, 43, 46 different locations across the 4 different tasks when solving P_2 and P_4 , respectively. This suggests that the underlying brain dynamics are subject to remarkable individual heterogeneities even in response to the same set of tasks. Subsequently, the best-possible sensor schemes have to be designed to be individual-specific.

At the same time, there is good percentage of agreement and lower loss of performance when planning homogeneous commercial solutions. Specifically, sensors 1, 25, 29, 41, 42 are almost unanimously chosen by all 10 subjects, as result of solving both P_2 and P_4 . This strongly hinges that (for the proposed tasks) there seems to be some fundamental underlying dynamics that enables the state estimation and this subset of sensors are responsible for accessing them. To see this more clearly, we report the top voted 14 sensor locations as an average case and color them in descending order of consensus as a function of darkness in Figure 4-(b) and (d) – as comparison, we also report the sensor deployment proposed by Emotive EPOC with red circles in both geodesic maps. The following observations are due. First of all, notice that all 14 sensors are voted by at least 5 subjects while sensor 1, 25, 29, 41, 42 are chosen by at least 8 subjects. Secondly, when considering the minimization of batch-state estimation errors instead of initial state-uncertainty estimation errors, the sensor deployment can be very different. For instance, sensor 63 and 64 are critically important when solving P_4 (as 8 out of 10 subjects choose them) whereas their influence to the initial state-uncertainty estimation are out-weighted by other sensors. Third, similar with our previous case study, only 7/14 and 6/14 most voted sensor deployment by 10 subjects are identical to those proposed by Emotiv EPOC when solving P_2 and P_4 , respectively. This potentially suggests the need of the redesign of Emotiv provided the estimation performance we setup in our study, since under our proposed approach the most

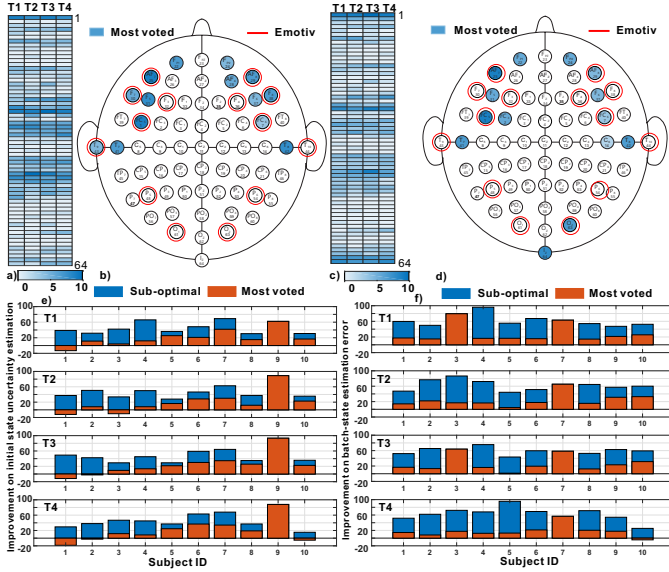


Fig. 4. (a-b) The 64-channel geodesic sensor distribution over 10 subjects under Task 1-4 and the most voted deployment given a 14-sensor budget by minimizing (a) the initial state-uncertainty estimation error and (b) batch-state estimation error. (c-d) The improvement on (c) initial state-uncertainty estimation error and (d) batch-state estimation error when (i) the sub-optimal 14-sensor deployment returned by Algorithm 2 individually (blue bar) and (ii) the most voted 14-sensor deployment by 10 subjects (red bar) are considered.

voted deployment and sub-optimal individual deployment (as returned by the algorithms proposed in this paper) achieve better performance. Specifically, we show the improvement on the log det estimation error of both initial state-uncertainty and batch state over the one by Emotiv EPOC when the most voted deployment (red bar) and the sub-optimal individual deployment (blue bar) are employed – see Figure 4-(e) and (f), respectively. The positive improvement suggests that our proposed deployment is better than that of Emotiv EPOC. We notice that the sub-optimal deployment returned by solving $\mathcal{P}_2$ and $\mathcal{P}_4$ individually improves the estimation error significantly in all cases, which is aligned with the results of our previous case study on a single subject. Overall, based on the aforementioned observations one could conclude that the sensors' locations of the commercial EEG devices could be re-designed to enhance both their initial state and batch-state estimation error performance.

Discussion of the results

We first notice that the proposed sensor locations seem to cope better with scenarios where the neuro-activation is not as well understood as the motor-related tasks (Tasks 1 and 3), e.g., imagining actions associated with Tasks 2 and 4. Additionally, we emphasize that the time-window considered was small ($K = 7$), since aimed to attain real-time estimation. As a result, it is expected to obtain even better performance results if we increase the size window. Besides, the cumulative error increases faster in the Emotiv EPOC.

Although in our case study we relied on EEG data, it is expected that the proposed problems have distinct value in different biological settings – as explained in the introduction. Specifically, in problems where signals are well modeled by

the proposed DTFOS, as it is the case of other physiological signals such as electromyograms (EMG) and electrocardiograms (ECG) [42].

V. CONCLUDING REMARKS

We considered biologically motivated discrete-time linear fractional-order systems and studied the trade-off between the sensor placement and the properties that pertain to Kalman-like filter performance. Specifically, we formalized the sensor placement problems in context of four different (but related) problems pertaining to the sensor placement to minimize the state-uncertainty and batch-state estimation error. We showed that these problems are NP-hard, and we presented polynomial approximation strategies for their solution that have sub-optimality guarantees.

Additionally, we explored the different problems in the context of real EEG data during a period of time where the individuals performed four different tasks. The results obtained support the capability of the proposed framework to deal with critical sensing deployment problems, and unveiled that the number and location of the sensors vary across tasks and subjects for the same experimental setup. Furthermore, we argue that these locations are not compatible with those used by state-of-the-art EEG wearables (e.g., Emotiv EPOC), which supports the need for further research and re-design of future EEG wearables that aim to attain a specified estimation performance for a given task.

Future research will consider the multi-scenario case, where the sensor placement has to consistently and reliably consider possible dynamics, e.g., multi-tasks simultaneously when EEG is considered. Additionally, we propose to validate the presented methodology when a large cohort of individuals and tasks is considered.

APPENDIX A PROOFS OF THE MAIN RESULTS

Proof of Theorem 1: We prove that $(\mathcal{P}_1)$ – $(\mathcal{P}_4)$ are NP-hard by focusing on the case where (i) the measurement matrix C is the identity matrix ($C = I$), (ii) the measurement noise v_k is Gaussian with zero mean and covariance the identity matrix ($\mathbb{C}(v_k) = I$), and (iii) $K = 0$, in which case $z_K = x_{0:K} = x_0$, and thus, $(\mathcal{P}_1)$ – $(\mathcal{P}_4)$ are equivalent to the following problem:

$$\begin{aligned} & \underset{\mathcal{S} \subseteq \{1,2,\dots,c\}}{\text{minimize}} && \log \det(\Sigma(\hat{x}_0)) \\ & \text{subject to} && |\mathcal{S}| \leq r. \end{aligned} \quad (13)$$

In more detail, we prove that the problem in (13) is NP-hard by proving that the following problem is equivalent to (13), and that it is NP-hard:

$$\begin{aligned} & \underset{\mathcal{S} \subseteq \{1,2,\dots,c\}}{\text{minimize}} && \mathbb{H}(x_0 | y_0(\mathcal{S})) \\ & \text{subject to} && |\mathcal{S}| \leq r, \end{aligned} \quad (14)$$

where $\mathbb{H}(x_0 | \mathcal{S})$ is the entropy of x_0 given the measurements $y_0(\mathcal{S})$ collected by the selected sensors in $\mathcal{S}$ at time $k = 0$.

Specifically, we prove that the problem in (14) is NP-hard by proving it is equivalent to the entropy maximization problem

$$\begin{aligned} & \underset{S \subseteq \{1,2,\dots,c\}}{\text{maximize}} && \mathbb{H}(y_0(S)) \\ & \text{subject to} && |S| \leq r, \end{aligned} \quad (15)$$

which we prove to have an equivalent instance to the NP-hard instance of the entropy maximization problem in [44].

To prove that the problems in (13) and (14) are equivalent, we use [33, Proposition 2], which implies for $K = 0$ that

$$\mathbb{H}(x_0|y_0(S)) = \frac{\log \det(\Sigma(\hat{x}_0))}{2} + \frac{n \log(2\pi e)}{2}, \quad (16)$$

where $\hat{x}_0$ is the minimum mean square estimator of x_0 given y_0 [45, Appendix E]. Therefore, (16) implies that minimizing the objective in (13) is equivalent to minimizing the objective in (14).

We next prove that the problems in (14) and (15) are equivalent. To this end, first observe that

$$\mathbb{H}(x_0|y_0(S)) = \mathbb{H}(x_0) + \mathbb{H}(y_0(S)|x_0) - \mathbb{H}(y_0(S)), \quad (17)$$

(we derive (17) using the conditional entropy chain rule [46]), as well as that:

- $\mathbb{H}(x_0)$ is constant with respect to S ; and
- $\mathbb{H}(y_0(S)|x_0)$ is constant for $C = I$, $\mathbb{C}(v_0) = I$, and for fixed $|S|$, (which is the case throughout this proof, since due to the monotonicity of the log det and the entropy, in all problems in (13), (14), and (15), it is $|S| = r$ for any S that solves (13), (14), and (15)), since if $y_0^{(i)}(S)$ ($x_0^{(i)}$, respectively) denotes the i -th element of $y_0(S)$ (x_0 , respectively), then

$$\begin{aligned} \mathbb{H}(y_0(S)|x_0) &\stackrel{(a)}{=} \sum_{i=1}^{|S|} \mathbb{H}(y_0^{(i)}(S)|x_0, y_0^{(1)}(S), \dots, y_0^{(i-1)}(S)) \\ &\stackrel{(b)}{=} \sum_{i \in S} \mathbb{H}(y_0^{(i)}(S)|x_0^{(i)}) \stackrel{(c)}{=} \frac{1}{2} \log(2\pi e) |S| \end{aligned}$$

In particular, equalities (a), (b) and (c) hold for the following reasons, respectively: (a) holds due to the conditional entropy chain rule [46]; (b) holds since for all $j \neq i$, $y_0^{(i)}$ given $x_0^{(i)}$ is independent of $x_0^{(j)}$ and $y_0^{(j)}$; and (c) holds since $y_0^{(i)}$ given $x_0^{(i)}$ is Gaussian with variance 1, since we consider the case where $\mathbb{C}(v_0) = I$.

Due to (17) and the latter two observations, we conclude that (14) is equivalent to (15).

Given the equivalence of (14) and (15), we conclude the proof by finding an instance for the problem in (13) that is equivalent to an instance for the problem in (15) that is NP-hard. In particular, consider Σ to be any $n \times n$ matrix that makes the entropy maximization problem in [44, Theorem 1] NP-hard: Σ is a positive definite symmetric with all the diagonal entries equal to $3n$, and all the off-diagonal entries equal to 0 or 1. The problem (15) is NP-hard if we can find an instance for the problem in (13) where $y_0(\{1, 2, \dots, c\})$ is a Gaussian random variable with covariance Σ . Indeed, let $\mathbb{C}(x_0)$ be any positive definite symmetric matrix with all diagonal entries equal to $3n - 1$, and all off-diagonal entries equal to 0 or 1 (Σ_0 is positive definite by construction, since it is both diagonally dominant, and as a result invertible, and

symmetric). For this selection of parameters, y_0 has covariance Σ ; the reason is threefold: (i) $y_0 = x_0 + v_0$, (ii) x_0 and v_0 are Gaussian with covariances Σ_0 and I , respectively, and (iii) x_0 and v_0 are uncorrelated; as a result, y_0 is Gaussian with covariance $\Sigma_0 + I = \Sigma$. ■

Proof of Theorem 2: In the following paragraphs, we only present the proof for $\log \det(\Sigma_{\hat{z}_K}(S))$, since the proof for $\log \det(\Sigma_{\hat{x}_{0:K}}(S))$ follows similar steps. In particular, we complete the proof for $\log \det(\Sigma_{\hat{z}_K}(S))$ in three steps: (i) we prove its closed formula; (ii) we show that it is non-increasing; and (iii) we prove that it is supermodular.

Given any finite estimation horizon $[0, K]$, we first prove that

$$\begin{aligned} \log \det(\Sigma_{\hat{z}_K}(S)) &= \\ &= -\log \det \left(\sum_{i=1}^c s_i L_{0:K}^\top M^{(i)} L_{0:K} + \mathbb{C}(z_K)^{-1} \right). \end{aligned} \quad (18)$$

In particular, as in the proof of Lemma 1 in [32], we let $S_{0:K}$ denote the block diagonal matrix with diagonal elements $K + 1$ copies of the sensor placement matrix S in (8). Then, using the Woodbury matrix identity [47, Corollary 2.8.8] at (3), we obtain $\Sigma_{\hat{z}_K}(S) = (\mathcal{O}_K^\top \Xi \mathcal{O}_K + \mathbb{C}(z_K)^{-1})^{-1}$, where $\Xi \equiv (S_{0:K} \mathbb{C}(v_{0:K}) S_{0:K}^\top)^{-1}$, and due to the definitions of $S_{0:K}$, $C_{0:K}$ and $\mathcal{O}_K = S_{0:K} C_{0:K} L_{0:K}$. Moreover, due to the definition of $S_{0:K}$, i.e., it contains block of matrices that are only zero or identity matrices, and because $\mathbb{C}(v_{0:K})$ is block diagonal, it follows that $\Xi = S_{0:K} \mathbb{C}(v_{0:K})^{-1} S_{0:K}^\top$, which can be verified by direct calculation. Overall, we obtain

$$\Sigma_{\hat{z}_K}(S) = (L_{0:K}^\top C_{0:K}^\top \Lambda \mathbb{C}(v_{0:K})^{-1} \Lambda C_{0:K} L_{0:K} + \mathbb{C}(z_K)^{-1})^{-1},$$

where $\Lambda \equiv S_{0:K}^\top S_{0:K}$. Now, since Λ and $\mathbb{C}(v_{0:K})^{-1}$ are block diagonal, and the blocks of Λ are either identity or zero matrices, $\mathbb{C}(v_{0:K})^{-1} \Lambda = \Lambda \mathbb{C}(v_{0:K})^{-1}$. Furthermore, the definition of $S_{0:K}$ implies that $\Lambda^2 = \Lambda$. Thus, it follows that

$$\Sigma_{\hat{z}_K}(S) = (L_{0:K}^\top C_{0:K}^\top \Lambda \mathbb{C}(v_{0:K})^{-1} C_{0:K} L_{0:K} + \mathbb{C}(z_K)^{-1})^{-1}. \quad (19)$$

For the last step, observe first that $\Lambda = \sum_{i=1}^c s_i I^{(i)}$, so

$$\begin{aligned} & L_{0:K}^\top C_{0:K}^\top \Lambda \mathbb{C}(v_{0:K})^{-1} C_{0:K} L_{0:K} \\ &= \sum_{i=1}^c s_i L_{0:K}^\top C_{0:K}^\top I^{(i)} \mathbb{C}(v_{0:K})^{-1} C_{0:K} L_{0:K} \end{aligned} \quad (20)$$

$$= \sum_{i=1}^c s_i L_{0:K}^\top M^{(i)} L_{0:K}, \quad (21)$$

where we derive (21) from (20) by using for $I^{(i)}$ the reverse steps to the ones we used for Λ to derive (19).

Next, to prove that $\log \det(\Sigma_{\hat{z}_K}(S))$ is a non-increasing set function in the choice of the sensors S , we follow similar steps to those in Theorem 2 in [32]. Specifically, consider $S \subseteq S'$, and observe that (18) and from [47, Theorem 8.4.9], $\Sigma_{\hat{z}_K}(S') \preceq \Sigma_{\hat{z}_K}(S)$, since $L_{0:K}^\top M^{(i)} L_{0:K} \succeq 0$ and $\mathbb{C}(z_K) \succ 0$. As a result, $\log \det(\Sigma_{\hat{z}_K}(S')) \leq \log \det(\Sigma_{\hat{z}_K}(S))$.

Finally, to prove that $\log \det(\Sigma_{\hat{z}_K}(S))$ is a supermodular set function, we prove that $-\log \det(\Sigma_{\hat{z}_K}(S))$ is submod-

ular. In particular, recall that a function $h : 2^{[c]} \mapsto \mathbb{R}$ is submodular if and only if, for any $a \in [c]$, the function $h_a : 2^{[c] \setminus \{a\}} \mapsto \mathbb{R}$, where $h_a(S) \equiv h(S \cup \{a\}) - h(S)$, is a non-increasing set function. Therefore, to prove that $h(S) = -\log \det(\Sigma_{\hat{z}_K}(S))$ is submodular, we may prove that the $h_a(S)$ is a non-increasing set function. To this end, we denote $\sum_{i=1}^c s_i L_{0:K}^\top M^{(i)} L_{0:K}$ in (18) by $M(S)$, and follow similar steps to those in the proof of Theorem 6 in [24]. Specifically, we note that

$$\begin{aligned} h_a(S) &= \log \det(M(S \cup \{a\}) + \mathbb{C}(z_K)^{-1}) - \\ &\quad \log \det(M(S) + \mathbb{C}(z_K)^{-1}) \\ &= \log \det(M(S) + M(\{a\}) + \mathbb{C}(z_K)^{-1}) - \\ &\quad \log \det(M(S) + \mathbb{C}(z_K)^{-1}). \end{aligned}$$

For $S \subseteq S'$ and $t \in [0, 1]$, define $\Phi(t) \equiv \mathbb{C}(z_K)^{-1} + M(S) + t(M(S') - M(S))$ and

$$g(t) \equiv \log \det(\Phi(t) + M(\{a\})) - \log \det(\Phi(t)).$$

Then, $g(0) = h_a(S)$ and $g(1) = h_a(S')$. Moreover, since

$$\frac{d \log \det(\Phi(t))}{dt} = \text{tr} \left(\Phi(t)^{-1} \frac{d\Phi(t)}{dt} \right)$$

(as in eq. (43) in [48]), it follows that

$$\dot{g}(t) = \text{tr} \left[((\Phi(t) + M(\{a\}))^{-1} - \Phi(t)^{-1}) F \right],$$

where $F \equiv M(S') - M(S)$. Furthermore, from [47, Proposition 8.5.5], we have that

$$(\Phi(t) + M(\{a\}))^{-1} - \Phi(t)^{-1} \succeq 0,$$

where $\Phi(t)$ is invertible since $\mathbb{C}(z_K)^{-1} \succ 0$, $M(S) \succeq 0$, and $M(S') \succeq M(S)$. Since also $F \succeq 0$, from [47, Corollary 8.3.6], it readily follows that

$$\lambda_{\max}[(\Phi(t) + M(\{a\}))^{-1} - \Phi(t)^{-1}] F \leq 0.$$

Thus, $\dot{g}(t) \leq 0$, and $h_a(S') = g(1) = g(0) + \int_0^1 \dot{g}(t) dt \leq g(0) = h_a(S)$, i.e., h_a is non-increasing. ■

Proof of Theorem 3: The proof of the theorem is attained in three main steps: (i) we prove (9); (ii) we prove (10); and (iii) we prove the computational complexity of Algorithm 1.

To prove (9), let $S_0, S_1, \dots$ be the sequence of sets selected by Algorithm 1 and l the smallest index such that $\log \det(\Sigma_{\hat{z}_K}, S_l) \leq R$. Therefore, S_l is the set that Algorithm 1 returns. To prove (9), we first observe that Theorem 2 implies that $\log \det(\Sigma_{\hat{z}_K}, S)$ is a supermodular and non-increasing. Then, from [49], we have that

$$\frac{l}{|S^*|} \leq 1 + \log \frac{\log \det(\Sigma_{\hat{z}_K}, \emptyset) - \log \det(\Sigma_{\hat{z}_K}, [c])}{\log \det(\Sigma_{\hat{z}_K}, S_{l-1}) - \log \det(\Sigma_{\hat{z}_K}, [c])}.$$

Now, l is the first time that $\log \det(\Sigma_{\hat{z}_K}, S_l) \leq R$, and as a result $\log \det(\Sigma_{\hat{z}_K}, S_{l-1}) > R$, and, as a consequence, we have that (10) holds. Furthermore, $\log \det(\Sigma_{\hat{z}_K}, \emptyset) = \log \det(\mathbb{C}(z_K))$, and from the geometric-arithmetic mean

inequality, we obtain that

$$\begin{aligned} \log \det(\mathbb{C}(z_K)) &\leq n(K+1) \log \frac{\text{tr}(\mathbb{C}(z_K))}{n(K+1)} \\ &\leq n(K+1) \log \frac{n(K+1) \max(\sigma_0^2, \sigma_w^2)}{n(K+1)} \\ &= n(K+1) \log \max(\sigma_0^2, \sigma_w^2). \end{aligned}$$

Finally, to prove the computational complexity of Algorithm 1, note that the `while` loop is repeated for at most c times. Moreover, the complexity to compute the determinant of an $n(K+1) \times n(K+1)$ matrix, using the Coppersmith-Winograd algorithm [50], is $O((nK)^{2.4})$, which is also the complexity incurred by the multiplication between such two matrices. Additionally, the determinant of at most $c+1$ matrices must be computed so that the

$$\arg \max_{a \in [c]} (\log \det(\Sigma_{\hat{z}_K}, S) - \log \det(\Sigma_{\hat{z}_K}, S \cup \{a\}))$$

can be computed. Also, $O(c)$ time is required to find a maximum element between c available. Therefore, the overall computational complexity of Algorithm 1 is dominated by $O(c^2(nK)^{2.4})$. ■

Proof of Theorem 4: In the following paragraphs, we complete the proof of the theorem in three steps: (i) we prove (11); (ii) we prove that the approximation factor $1 - 1/e$ in (11) is the best one can achieve in polynomial time for $(P_2)/(P_4)$; and (iii) we discuss the computational complexity of the algorithm.

To prove (11), we first observe that Theorem 2 implies that $\log \det(\Sigma_{\hat{z}_K}, S) - \log \det(\Sigma_{\hat{z}_K}, \emptyset)$ is a supermodular, non-increasing and non-positive set function. Consequently, the results from [49] can be invoked to obtain (11).

To prove that the approximation factor $1 - 1/e$ in (11) is the best one can achieve in polynomial time for $(P_2)/(P_4)$, we recall that in the worst-case $(P_2)/(P_4)$ are equivalent to the minimal observability problem (see proof of Theorem 1). Then, the result follows by noticing that the minimal observability problem has the same computational complexity as the set cover problem [21], which cannot be approximated in polynomial time with a factor better than $1 - 1/e$ [39].

Finally, the computational complexity of Algorithm 2 can be derived by following the same steps and reasoning as the one proposed in the proof of Theorem 3 to show the time complexity of Algorithm 1. ■

Proof of Theorem 5: Since the arithmetic mean of a finite set of positive numbers is at least as large as their harmonic mean, the following inequality holds:

$$\text{tr}(\Sigma_{\hat{z}_K}) \geq \frac{(n(K+1))^2}{\text{tr}(\sum_{i=1}^c s_i L_{0:K}^\top M^{(i)} L_{0:K} + \mathbb{C}(z_K)^{-1})}, \quad (22)$$

where we used the closed form for $\Sigma_{\hat{z}_K}$ proved in Theorem 2.

Furthermore, in the denominator of (22), for the first term it is $\text{tr}(\sum_{i=1}^c s_i L_{0:K}^\top M^{(i)} L_{0:K}) = \sum_{i=1}^c s_i \text{tr}(M^{(i)} L_{0:K} L_{0:K}^\top)$, where $\text{tr}(L_{0:K}^\top M^{(i)} L_{0:K}) \leq n(K+1) \|\tilde{C}\|_2^2 \|\mathbb{C}(v_{1:K})^{-1}\|_2 \|L_{0:K} L_{0:K}^\top\|_2$, since $\|I^{(i)}\|_2 = 1$, and for the second term it is

$\text{tr}(\mathbb{C}(z_K)^{-1}) \leq n(K+1) \max\{\sigma_0^{(-1)}, \sigma_w^{(-1)}\}$. Therefore,

$$\text{tr} \left(\sum_{i=1}^c s_i L_{0:K}^\top M^{(i)} L_{0:K} + \mathbb{C}(z_K)^{-1} \right) \leq$$

$$rn(K+1) \sigma_v^{(-1)} \|\bar{C}\|_2^2 \|\bar{L}\|_2 + n(K+1) \max\{\sigma_0^{(-1)}, \sigma_w^{(-1)}\}.$$

Hence, $\text{tr}(\Sigma_{\hat{z}_K}) \geq n(K+1)/(r\sigma_v^{(-1)} \|\bar{C}\|_2^2 \|\bar{L}\|_2 + \max\{\sigma_0^{(-1)}, \sigma_w^{(-1)}\})$. ■

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