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Final Report: Multiscale Models and Measurements of Muscle Forces (Conference and Symposia grants)

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6. AUTHORS
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14. ABSTRACT
Muscle is active, regulated, soft matter that generates force complex interactions among millions of molecular motors organized in a highly structured compliant lattice of protein filaments. We pursued experimental tests to determine whether there are conditions under which key assumptions underlying models of force generation are violated (e.g. no radial motion, uniform strain, no viscous dynamics). Our objectives were to (1) examine the interplay between changes in axial and radial dimensions of muscle and the controlled activation of crossbridges and (2) to determine if axial and radial strain non-uniformities arise in impulsive length changes applied to active

15. SUBJECT TERMS
muscle contraction, molecular motors, x-ray diffraction

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a. REPORT	b. ABSTRACT	c. THIS PAGE			Thomas Daniel
UU	UU	UU	UU		19b. TELEPHONE NUMBER 206-543-1659

Report Title

Final Report: Multiscale Models and Measurements of Muscle Forces (Conference and Symposia grants)

ABSTRACT

Muscle is active, regulated, soft matter that generates force complex interactions among millions of molecular motors organized in a highly structured compliant lattice of protein filaments. We pursued experimental tests to determine whether there are conditions under which key assumptions underlying models of force generation are violated (e.g. no radial motion, uniform strain, no viscous dynamics). Our objectives were to (1) examine the interplay between changes in axial and radial dimensions of muscle and the controlled activation of crossbridges and (2) to determine if axial and radial strain non-uniformities arise in impulsive length changes applied to active muscle. Using workloop experiments on isolated muscle fibers in conjunction with high-speed laser and X-ray diffraction methods, we asked whether there is appreciable radial motion in the spacing of contractile filaments and whether crossbridges influence that motion. In addition, using rapid length changes applied to active muscle we also ask if there are strain non-uniformities that propagate as traveling viscoelastic waves at the cellular level. These experiments will reveal whether (a) unexplored physical properties contribute to muscle force generation and (b) a new model of the physics and energetics of force generation in living systems is required.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

- 2014 Molecular determinants of force generation in muscle: new directions for motor molecules. Keynote Speaker for the Graduate Symposium. Molecular Biology and Biochemistry Department, Simon Fraser University.
- 2014 Molecular determinants of force generation in muscle: new directions for motor molecules. Department of Mechanical Engineering, Johns Hopkins University.

Number of Presentations: 2.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Book

TOTAL:

Received Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

- Simon Sponberg, the postdoc associated with this research effort, was recently appointed Assistant Professor of Physics at the Georgia Institute of Technology effective Aug 1, 2014
- Gideon Dunster (graduate student joining the team) received the Univeristy of Washington Top Scholar Award for graduate recruiting.
- Tom Daniel received a Guggenheim Award for 2014-2015. That award will help fund his activities associated with this research effort.
- Tom Daniel was appointed co-Director of the University of Washington Institute of Neuroengineering, a program supported by a 7.2 M$ gift from the Washington Research Foundation

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Gideon Dunster (on fellowship)	0.00	
Joe Powers (on fellowship)	0.00	
FTE Equivalent:	**0.00**	
Total Number:	**2**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Simon Sponberg	0.50
FTE Equivalent:	**0.50**
Total Number:	**1**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Thomas Daniel	0.00	
FTE Equivalent:	**0.00**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:..... 0.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

Approach

Since the original proposal was funded about one year ago, several factors contributed to a change in our approach. We had initially intended to perform the quick release experiments using a new high-speed X-ray detector that was to be running at the Argonne National Labs (BioCAT Sector). However, because that detector was not functional for our planned trip, we chose instead to focus on workloop methods to explore the interplay between changes in length and radius of intact muscles under controlled activation. That approach, as noted below, proved to be extremely informative. We are now positioning ourselves to work towards the second objective. We anticipate completing this work (as a no cost extension) within about 6 months. Below we explain the detailed approach that allow us to achieve our objectives.

Workloop methods:

- We use flight muscle from the large hawkmoth Manduca sexta
- Intact muscles are isolated by dissection and attached to an Aurora Instruments muscle lever system that provides both length control and force measurement (Figure 1).
- Muscle temperature is controlled by a temperature-bath with a saline drip
- Muscle activation was controlled by an A-M Systems stimulator with up to 10 different phases of activation.
- The entire system was placed into the X-ray beam at the Argonne National Labs BioCAT facility with two time-synched detectors (Figure 1) coupled to a computer controlled high speed shutter upstream of the sample. One detector provided diffraction images of radial filament spacing (order 40 nm) and the other detector, placed at a greater radial distance, provided diffraction images of the axial twist of thick and thin filaments (order 5 nm). Images are captured every 8 ms.
- Muscles were oscillated for 200 cycles and images were captured either every 8 ms (phase locked and phase averaged to each cycle) or every 11 ms providing a precession of images through the cycles. In either case, we summed images that correspond to identical phases within each cycle.
- By this scheme we can examine in real time the radial and axial protein deformations and how crossbridges influence those dynamics. To our knowledge this is the first demonstration of this multiscale process: any similar study had used chemically activated skinned muscle fibers.

Step changes in length

In experiments planned for December 2014, we will repeat the protocol outlined above but will use extremely rapid length changes. The rapid length change experiment is the single most commonly deployed experimental approach that seeks to probe, using high-speed force and length measurement, details of the mechanochemistry of molecular motors in muscle. That approach, however, would be strongly challenged if there were significant strain wave propagation resulting from rapid motion. Thus our second key objective is to use the technology that we have developed to examine that possibility as follows:

- We will use X-ray diffraction as above to obtain nm scale imaging of both axial and radial protein geometry. Using this method, we have been able to resolve radial spacing changes of thick filaments (to a few nm) and axial spacing of the helical repeat of thick filaments (to a few Angstroms). We will repeat step length changes and sample X-ray diffraction images at different positions along the muscle fiber.
- At the same time that we acquire high-speed X-ray diffraction images we will also acquire high-speed laser diffraction images that arise with the interaction of the laser with Z-disk spacing. This provides a measure of the local strain at the sub-micrometer scale. We will use analog optical tracking of the diffraction primaries (UDT silicon detectors) to get extremely high temporal resolution. As with the X-ray system, this will be done at different positions along the muscle fiber.
- We can also use high speed video acquisition methods to simultaneously measure fiber length changes so that we have centimeter scale measures of the dynamics.
- We are also simultaneously measuring force at the fixed end of the fiber using a silicon strain gauge force transducer..

Technology Transfer

- We have continued and built up our collaboration with Dr. Tom Irving at the BioCAT Beamline of the Synchrotron at the Argonne National Labs.
- As a consequence of our efforts new collaborations have been initiated with Dr.Peko (Anette) Hosoi and Dr. Jose Alvarado at the Massachusetts Institute of Technology.

Project Summary - Grant # W911NF-13-04354
Reporting Period: September 2013 – August 2014

Multiscale Physics and the Dynamics of Muscle

Thomas Daniel
Department of Biology
University of Washington, Seattle, WA 98195-1800

Abstract

Muscle is active, regulated, soft matter that generates force complex interactions among millions of molecular motors organized in a highly structured compliant lattice of protein filaments. Muscle contractility is a problem of multi-scale physics, spanning scales that occur at few nanometers to those that characterize entire muscles and moving creatures. In addition, fluid dynamic, chemical, elastic and inertial processes all potentially contribute to the emergent dynamics of the interaction of myriad molecular motors that generate force and consume energy. We pursued experimental tests to determine whether there are conditions under which key assumptions underlying models of force generation are violated (e.g. no radial motion, uniform strain, no viscous dynamics). Our objectives were to (1) examine the interplay between changes in axial and radial dimensions of muscle and the controlled activation of crossbridges and (2) to determine if axial and radial strain non-uniformities arise in impulsive length changes applied to active muscle. Using workloop (sinusoidal length change with controlled activation timing) experiments on isolated muscle fibers in conjunction with high-speed laser and X-ray diffraction methods, we ask whether there is appreciable radial motion in the spacing of contractile filaments in intact muscles undergoing active shortening and whether crossbridges influence that motion. In addition, using rapid length changes applied to active muscle we also ask if there are strain non-uniformities that propagate as traveling viscoelastic waves at the cellular level. These experiments will reveal whether (a) previously unexplored physical properties contribute to muscle force generation and (b) a new model of the physics and energetics of force generation in living systems is required. We are focusing on preliminary experiments that leverage recent advances in high-speed detectors and a unique muscle preparation.

Approach

Since the original proposal was funded about one year ago, several factors contributed to a change in our approach. We had initially intended to perform the quick release experiments using a new high-speed X-ray detector that was to be running at the Argonne National Labs (BioCAT Sector). However, because that detector was not functional for our planned trip, we chose instead to focus on workloop methods to explore the interplay between changes in length and radius of intact muscles under controlled activation. That approach, as noted below, proved to be extremely informative. We are now positioning ourselves to work towards the second objective. We anticipate completing this work (as a no cost extension) within about 6 months. Below we explain the detailed approach that allow us to achieve our objectives.

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In experiments planned for December 2014, we will repeat the protocol outlined above but will use extremely rapid length changes. The rapid length change experiment is the single most commonly deployed experimental approach that seeks to probe, using high-speed force and length measurement, details of the mechanochemistry of molecular motors in muscle. That approach, however, would be strongly challenged if there were significant strain wave propagation resulting from rapid motion. Thus our second key objective is to use the technology that we have developed to examine that possibility as follows:

- We will use X-ray diffraction as above to obtain nm scale imaging of both axial and radial protein geometry. Using this method, we have been able to resolve radial spacing changes of thick filaments (to a few nm) and axial spacing of the helical repeat of thick filaments (to a few Angstroms). We will repeat step length changes and sample X-ray diffraction images at different positions along the muscle fiber.
- At the same time that we acquire high-speed X-ray diffraction images we will also acquire high-speed laser diffraction images that arise with the interaction of the laser with Z-disk spacing. This provides a measure of the local strain at the sub-micrometer scale. We will use analog optical tracking of the diffraction primaries (UDT silicon detectors) to get extremely high temporal resolution. As with the X-ray system, this will be done at different positions along the muscle fiber.
- We can also use high speed video acquisition methods to simultaneously measure fiber length changes so that we have centimeter scale measures of the dynamics.
- We are also simultaneously measuring force at the fixed end of the fiber using a silicon strain gauge force transducer..

Relevance to Army

The research we propose falls squarely in interests outlined in the Broad Agency Announcement in section 1.3 on Complex Dynamics and Systems. Through an initial experimental approach to force generation by muscle, we will be examining potential cooperative interactions (or potentially destructive interactions) that combine nano-scale fluid dynamic processes and elastic wave propagation. Moreover, the coupling between these purely physical processes may profoundly influence the dynamics of motor molecules in ways that have never been considered before.

Accomplishments for Reporting Period

We are just completing our first year of funding and have made

- We have the first real-time measurements of both radial and axial changes in filament geometry (Figure 2).
- We have established preliminary evidence that crossbridge recruitment strongly modulates the radial dynamics of thick and thin filaments in active muscle (Figures 3 and 4)
- We have new results that indicate we can measure intensity peaks associated with the twist of myosin to within about 0.5% (Figure 5). This means that we can see exceedingly tiny changes in the pitch of the thick filament helix in real time – providing for the first time measures of thick filament stretching during muscle contraction.
- We have completed construction of a new apparatus for measuring simultaneous force, length and, with laser diffraction, z-disk spacing that can reveal wave propagation at the scale of micrometers in our lab.
-

Collaborations and Technology Transfer

- We have continued and built up our collaboration with Dr. Tom Irving at the BioCAT Beamline of the Synchrotron at the Argonne National Labs.
- As a consequence of our efforts new collaborations have been initiated with Dr.Peko (Anette) Hosoi and Dr. Jose Alvarado at the Massachusetts Institute of Technology.

Resulting Journal Publications During Reporting Period

- We are still in our first year of funding and are preparing a manuscript for submission on the data that are summarized above.

Graduate Students and Postdoctoral Trainees Involved During Reporting Period

- Dr. Simon Sponberg was supported as a postdoctoral trainee during this reporting period.
- Mr. Gideon Dunster will be joining our research group this coming autumn as a first year rotation graduate student.

Awards, Honors and Appointments

- Simon Sponberg, the postdoc associated with this research effort, was recently appointed Assistant Professor of Physics at the Georgia Institute of Technology effective Aug 1, 2014
- Gideon Dunster (graduate student joining the team) received the Univeristy of Washington Top Scholar Award for graduate recruiting.
- Tom Daniel received a Guggenheim Award for 2014-2015. That award will help fund his activities associated with this research effort.
- Tom Daniel was appointed co-Director of the University of Washington Institute of Neuroengineering, a program supported by a 7.2 M$ gift from the Washington Research Foundation

FIGURES

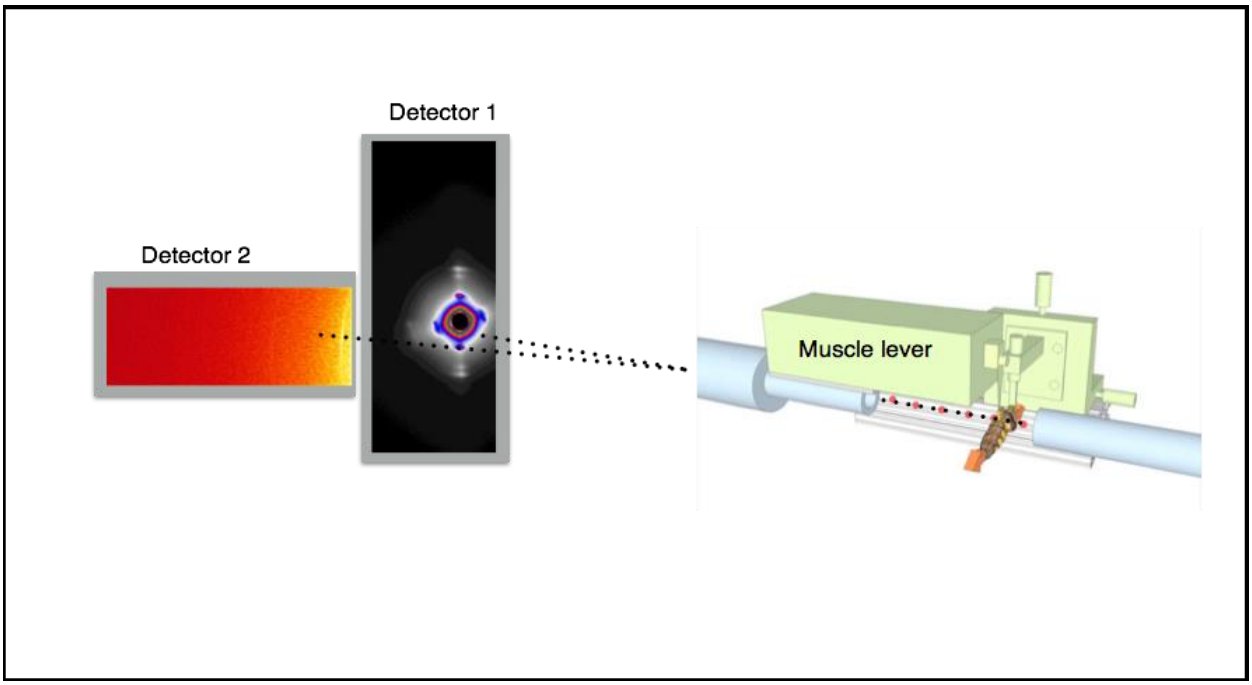

Figure 1. A diagram of the set up used to acquire X-Ray diffraction images in the Argonne National Lab BioCAT Beamline. A moth thorax is placed directly in the X-ray beam and mounted to a muscle lever that controls length and reports force. The X-ray is diffracted by the electron distribution of the proteins that constitute muscle. Detector1 is positioned to acquire diffraction images associated with the radial spacing of thick and thin filaments (on the order of 40 nm). Detector2 is positioned to the side of Detector1 to acquire diffraction images associated with the much smaller spacing of the helical arrangement of thick and thin filaments.

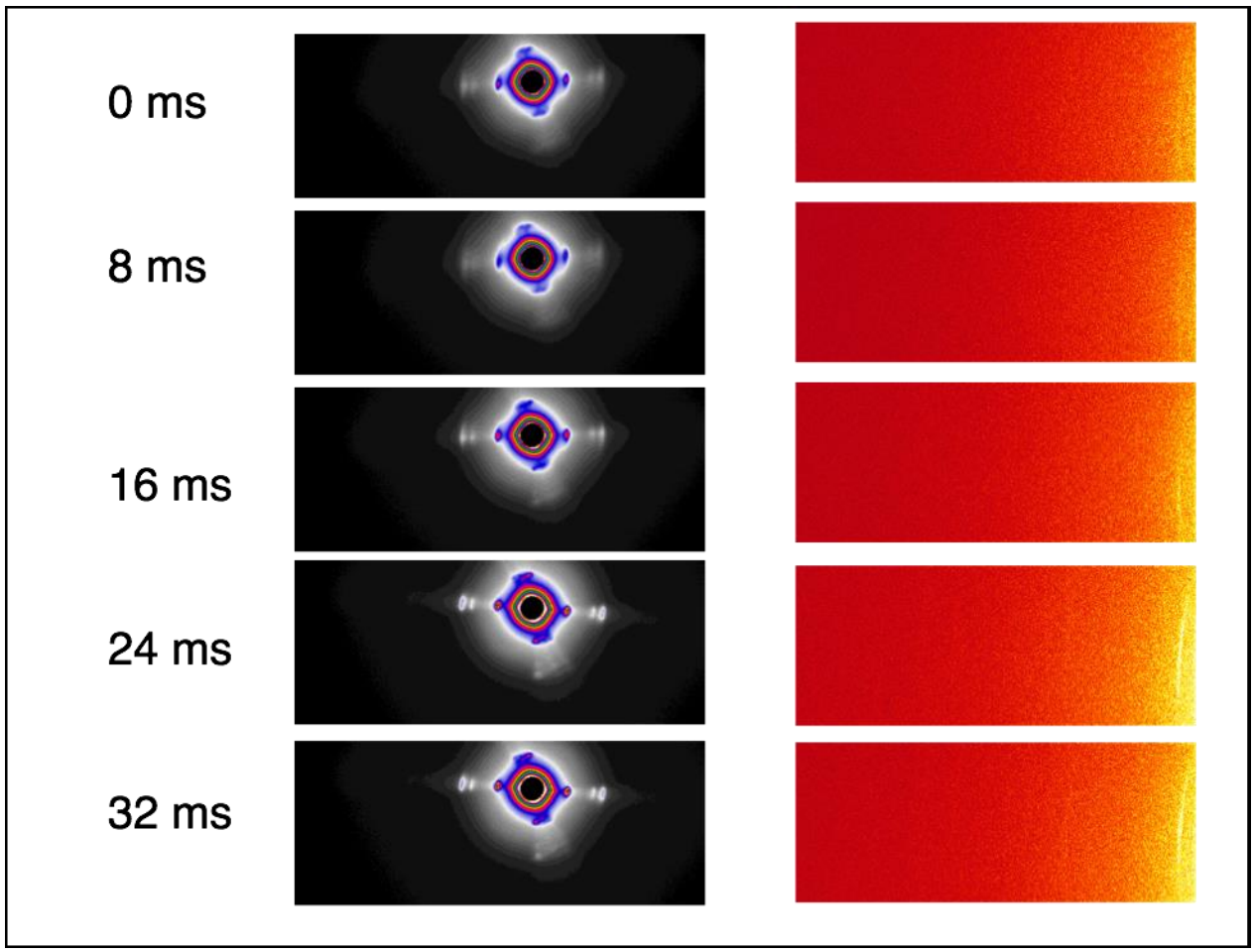

Figure 2. Five successive images (every 8 ms) from each detector. These are the first such images from dual detectors, time synched and acquiring information about active length changes. Figures 3 and 4 below show additional information about these images.

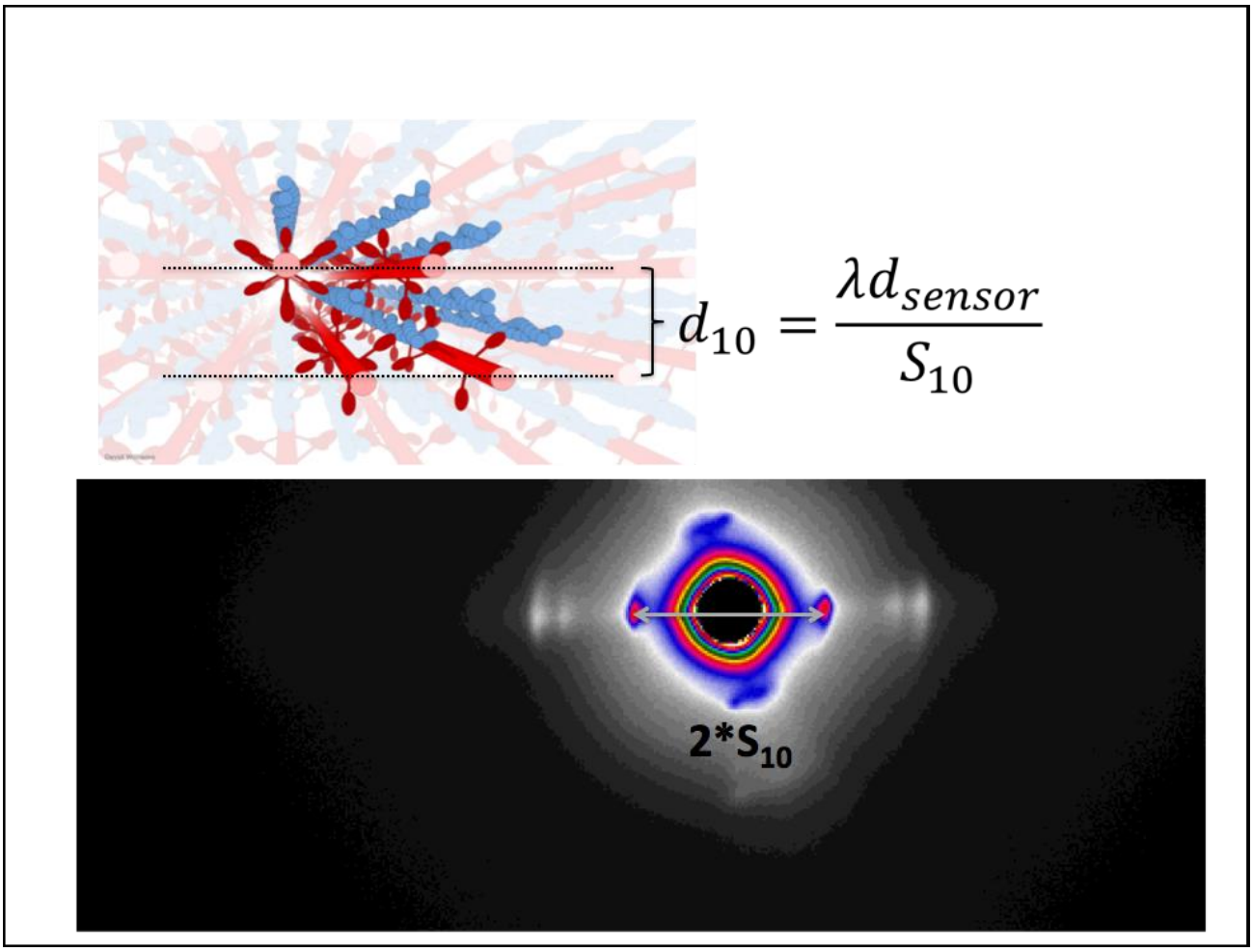

Figure 3. A detail view of one of image (it is comprised of the superposition of ~100 phase locked images). The $S_{1,0}$ spacing corresponds to the diffraction associated with the spacing of thick filaments (the $d_{1,0}$ spacing). We use Bragg's law along with the wavelength of the X-ray and the distance between the sample and the detector to compute the effective $d_{1,0}$ spacing.

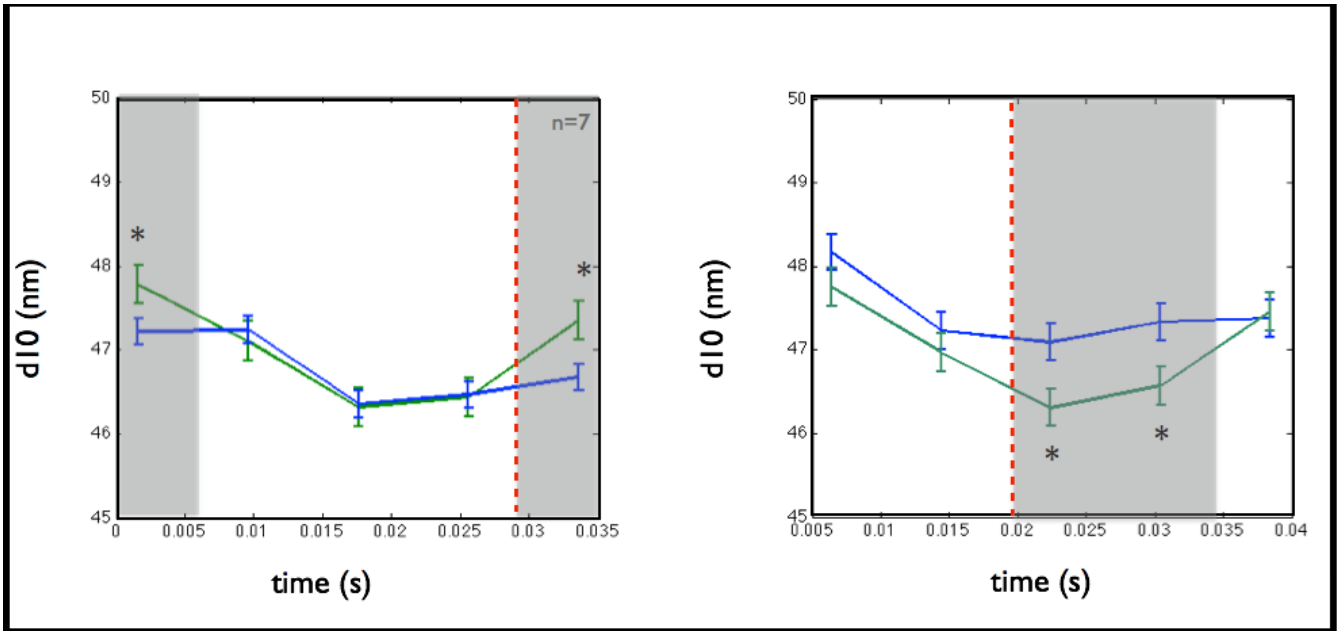

Figure 4. The $d_{1,0}$ spacing that we estimate (blue lines) from Bragg's law is plotted against time for two different activation phases for a muscle undergoing 25 Hz sinusoidal length change (0.04 second period). One starts late in the lengthening cylcle (0.03 seconds) and the other starts in the middle of the cycle (0.02 seconds). Crossbridge attachment times are approximately shown by the shaded regions. The green lines correspond to the $d_{1,0}$ spacing we would predict if the radial spacing between thick filaments reflected the reduction in radius that follows with increase length of a constant volume cell. These data are the first indications that (a) radial spacing varies considerably during axial lengthening and shortening of a muscle and (b) cross bridge recruitment apparently restricts radial thick filament motion.

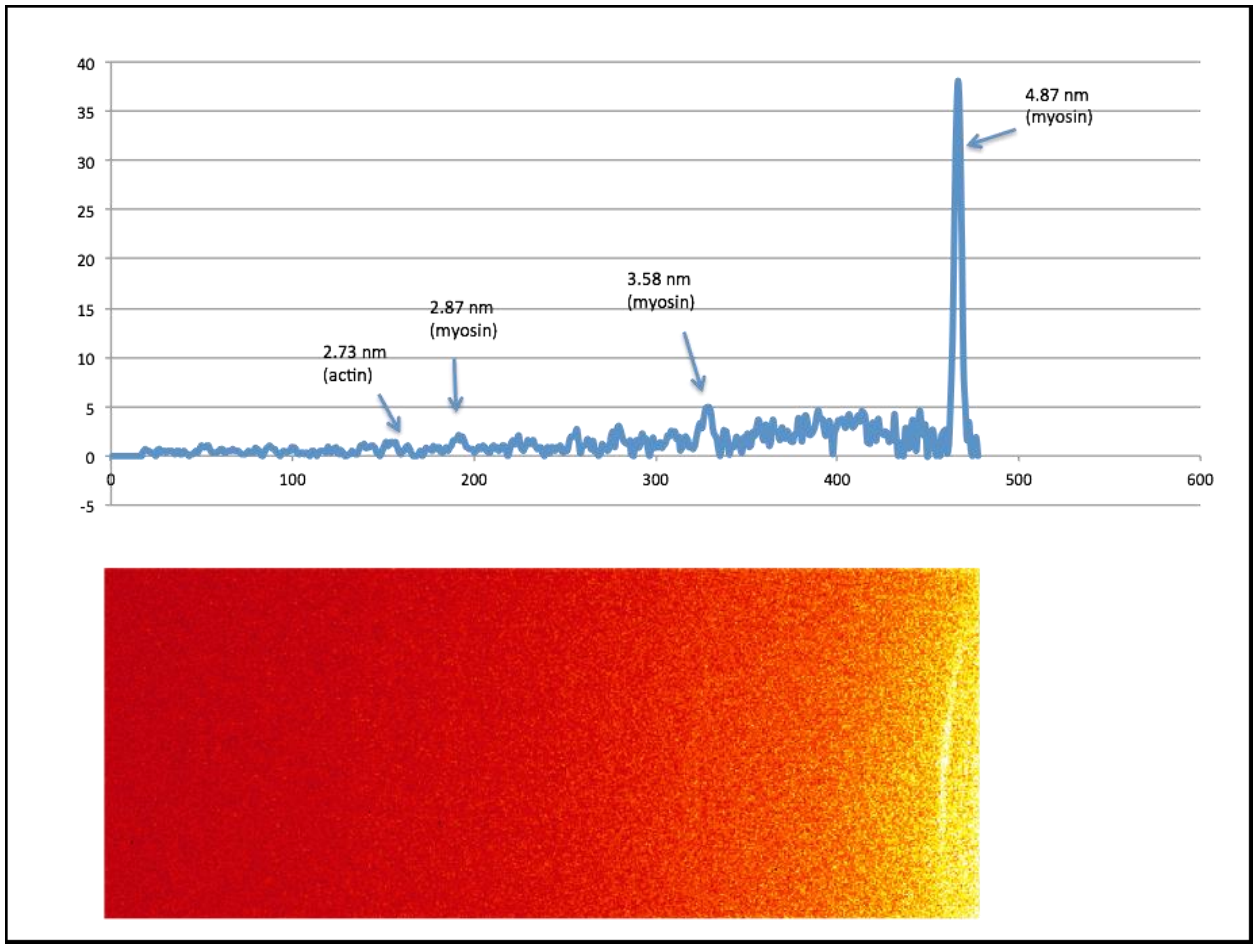

Figure 5. A detail view of one of image (it is comprised of the superposition of ~100 phase locked images) from Detector2 along with the intensity peaks (trend removed) corresponding to the thick filament helical pitch. We can localize the dominant peak to within about 0.5%. That provides a resolution of about 1 Angstrom in estimates for the pitch changes of myosin thick filaments.