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13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.					
14. ABSTRACT Howard University's newly acquired Fluorescence Activated Cytometric Sorter (FACS) has been integrated into the new flow cytometric core facility. The instrument became available and open to the research community on 4/20/2015. Currently, there are 11 researchers that have gone through instrument training (i.e. antibody panel setup and sample preparations). In the three months it has been active, six Howard University researchers have used the instrument, with three using the equipment twice per week. The instrument has been integrated with the Accuri C6 Flow Cytometric Analyzer and Life Technologies Countess Automated Cell Counter as auxiliary equipment.					
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					19b. TELEPHONE NUMBER 202-806-6783

Report Title

Final Report: Howard University Flow Cytometric Sorter For Research and Education

ABSTRACT

Howard University's newly acquired Fluorescence Activated Cytometric Sorter (FACS) has been integrated into the new flow cytometric core facility. The instrument became available and open to the research community on 4/20/2015. Currently, there are 11 researchers that have gone through instrument training (i.e. antibody panel setup and sample preparations). In the three months it has been active, six Howard University researchers have used the instrument, with three using the equipment twice per week. The instrument has been integrated with the Accuri C6 Flow Cytometric Analyzer and Life Technologies Countess Automated Cell Counter as ancillary supportive instrumentation. Briefly, researchers first use the Countess to determine the concentration of their samples; the countess has interchangeable LED light cubes for visualizing all the fluorescent detection channels that are available on the purchased FACS instrument. Next, the cells are sorted; results have yielded consistently > 98% purity. Populations are further confirmed using the Accuri C6 (that was bundled with the purchase of the FACS instrument). Current researchers using the instrument have sciences that include bone reformation (Dazhi Yang), microbial ecologies (Patrick Ymele-Leki), cell cycle and cancer onset (Sharma Sudha), immunotherapies and antigen presentation biomaterials (M. Lipscomb, Kunle Kassim), and gene therapies (K. Washington).

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

Number of Presentations: 0.00

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

Graduate Students

<u>NAME</u>	<u>PERCENT_SUPPORTED</u>
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

<u>NAME</u>	<u>PERCENT_SUPPORTED</u>
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	National Academy Member
Michael Lipscomb	0.00	
FTE Equivalent:	0.00	
Total Number:	1	

Names of Under Graduate students supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
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

<u>NAME</u>
Total Number:

Names of personnel receiving PHDs

<u>NAME</u>
Total Number:

Names of other research staff

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
FTE Equivalent:	
Total Number:	

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

1. Forward:

This award provided Howard University with a state-of-the-art, cutting-edge flow cytometric sorter. As of this date, it is the only flow cytometric sorter on Howard University's campus. The instrument was a pivotal means in the isolation and purification of cell populations for intricate in vitro and in vivo studies. The results of which will bring about significant research and broader impacts in the STEM and biomedical research enterprises.

2. Table of Contents:

None.

3. List of Appendixes, Illustrations, and Tables:

None.

4. Statement of the Problem Studies:

Biomedical research is predicated on the ability to study the intricacies and dynamic processes of living systems. The approach can be applied in vivo, ex vivo, or in vitro settings. However, a significant problem in the study of cellular life is manifested by the inability to study individual cell groups or unique populations. Instead, we are left with a heterogeneity composition that circumvents the ability to delineate the cause vs. effect – because it then becomes implausible to resolve variables (or experimental groups) from constants (or controls). However, flow cytometry is a powerful platform for fluorescence measurement of numerous parameters of individual (single) biological particles, cells, or subcellular components (1-4). Flow cytometric sorting utilizes the exact same principals as an analyzer, but adds the ability to isolate and collect specific cells based on user defined multiparameter settings (5-7). Thus, flow cytometric sorting addresses a significant problem in biomedical research by allowing the researcher to isolate a homogenous pot of cells for detailed studies. These studies may allow the individual to (i) genetically or proteomically alter the expression profile; (ii) evaluate response to specific drug targets or biochemical properties; and/or (iii) unique interactions with infectious agents or cancerous cells, to name a few. Each of these can be investigated in vitro or applied in vivo upon transplantation into animal models. Results can then define molecular, cellular, and physiological underpinnings as a potential clinical-relevant treatment, therapy, or delineation of mechanism.

5. Summary of the most important results:

The equipment was installed in April 2015. During November 2014 – March 2015, a flow core cytometric facility was renovated to provide HVAC, electrical and plumbing needs, as well as bench layout casework. The equipment was further fitted with aerosol control units to maintain BSL2+ conditions, as well as outfitted with vacuum pumps and pressurized plumbing to optimally control fluidics and handling. Upon completion of the renovations, the unit was installed by certified technicians; all laser-alignment and optics were confirmed (albeit some misalignment did occur throughout the use; this was quickly rectified by the technical support teams). The instrument now contains 2 lasers (488nm and 640nm) with 4-2 color configuration (i.e. 530/40, 585/29, 692/40, 750 off the 488nm laser and 660/20 and 750 off the 640nm laser). This now provides the users with up to 8 total parameters (and 6 fluorescent parameters) for isolating sample populations. Additionally monies purchased a panel of antibodies, small benchtop centrifuges, vortexes and other ancillary devices to work in conjunction with the instrument. An online google shared drive has been created that automatically backs up and syncs all user data files, as well as integrating online calendar scheduling for user access and training. The scheduler has ensured there are no time conflicts with use of the instrument by the multiple users. In the last 3 months, 11 research laboratory users have been trained on the instrument. Predominately, the training included establishing antibody panels, familiarity with compensation, fluorescence, and sorting strategies, and preparation of samples for BSL2+ environment and biosafety hazards. Of the 11 users, 6 have used the system successfully to sort populations for their studies. Because we are still in the early phases, data sets have not been assembled into manuscripts for publications, as of yet. However, several isolated populations have been used in preliminary studies. Often, the success was low on the initial 1-2 sorting opportunities. However, adjusting parameters and optimizing conditions has led to an increase from 84% efficiency on average (over 13 sorts) to 98% efficiency upon the third (or greater) sorting events. This increase is both due to sample preparation as well as the operator (i.e. Lipscomb laboratory personnel) becoming more experienced and efficient with the sorting procedures. Three laboratories are actively using the sorter 1-2 times per week. 5 other laboratories have begun scheduling and working with the operators to assemble antibody panels for staining and sorting experiments. We expect that by October 1st, there will be approximately 1-2 sorts per day Monday through Friday. Data generated will be assembled in manuscripts, with the grant references as a funding source and the Lipscomb laboratory acknowledged (and or contributing author).

6. Bibliography

1. Watson, J. V. Flow cytometry in biomedical science. *Nature* 325, 741-742, doi:10.1038/325741a0 (1987).
2. Herzenberg, L. A., Parks, D., Sahaf, B., Perez, O. & Roederer, M. The history and future of the fluorescence activated cell sorter and flow cytometry: a view from Stanford. *Clinical chemistry* 48, 1819-1827 (2002).
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4. Brown, M. & Wittwer, C. Flow cytometry: principles and clinical applications in hematology. *Clinical chemistry* 46, 1221-1229 (2000).
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6. Cunningham, R. E. Overview of flow cytometry and fluorescent probes for flow cytometry. *Methods Mol Biol* 588, 319-326, doi:10.1007/978-1-59745-324-0_31 (2010).
7. Givan, A. L. Flow cytometry: an introduction. *Methods Mol Biol* 263, 1-32, doi:10.1385/1-59259-773-4:001 (2004).

7. Appendix

None.

Technology Transfer

Initiation of Phase Zero with Abiogenic Technologies LLC as a startup company based on access and research using the major research instrumentation equipment. Briefly, the equipment allows single cell isolation and purity of cell samples for seeding on newly developed biomaterials to serve as polymer nano-copolymers. Success of the latest datasets has been assembled into a new SBIR grant application for submission in September 2015. Continued access to the FACS instrument will provide the means to assemble key measurable outcomes to show success of the copolymer for in vivo studies.

Abstract:

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Scientific Progress and Accomplishments:

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7. Appendixes

None