Writing a Helios Grant Application

This document provides general guidelines for writing a Helios™ instrument grant application. The guidelines are broken into sections that correspond to the structure of a National Institutes of Health (NIH) Shared Instrumentation Grant (SIG). Because mass cytometry is breakthrough technology, applicants should place special emphasis on two areas: First, demonstrate a clear understanding of the unique, research-advancing capabilities the platform provides. Second, outline a rational infrastructure and plan for successfully integrating the platform into the research goals of the institution. It is generally expected that all of the proposed research projects include a high-dimensional panel. Therefore, start the process at least six months before the deadline. As with any grant process, it is important to start early and review the funding opportunity announcement (FOA), to follow the grant application guide carefully, and to use your own words.

Justification of Need for Helios

For this section, it is important to articulate the unique technological attributes of the Helios platform and how they translate into research-advancing experiments that cannot be done with existing technologies. It is important to convey that your users currently do not have access to the technology at or near your institution. It is also useful to indicate how you will employ mass cytometry along with other techniques (such as flow cytometry, magnetic enrichment, and quantitative PCR) to which you already have access in order to achieve the aims in the proposal. Example text:

> Cells express a complex array of components that indicate physiological status, phenotype, and functional capability. Therefore, platforms that provide multiparametric cellular analysis are uniquely suited to …
>
> The Helios platform is uniquely capable of simultaneously resolving more than 40 parameters on a per-cell basis at high acquisition rates, providing an unparalleled ability to phenotypically and functionally profile single cells from normal and diseased states. Mass cytometry employs element-tagged probes that are measured on the Helios instrument, which resolves isotopes of different masses with minimal signal overlap over an extended mass range. Furthermore, mass cytometry uses rare earth elemental tags that provide similar signal intensities and are not naturally found in cells, avoiding measurement of background cellular signal. All of these attributes simplify large-panel experimental design and thus uniquely enable high-dimensional cytometry experiments not possible using other methods.

> Acquisition of the Helios mass cytometry platform will uniquely position our researchers to advance the understanding of … Conditions pertaining to our studies include …

The following publications and reviews provide relevant discussions of the unique benefits of mass cytometry technology in a variety of research areas. A complete bibliography of mass cytometry-related publications can be found here.

Reviews and Methods

- Baca, Q. et al. “The road ahead: implementing mass cytometry in clinical studies, one cell at a time.” *Cytometry B* (2016): doi:10.1002/cyto.b.21497.
- Baumgart, S. et al. “OMIP-034: comprehensive immune phenotyping of human peripheral leukocytes by mass cytometry for monitoring immunomodulatory therapies.” *Cytometry A* (2016): doi:10.1002/cyto.a.22894.
- Leelatian, N. et al. “Single cell analysis of human tissues and solid tumors with mass cytometry.” *Cytometry B Clinical Cytometry* (2016): doi:10.1002/cyto.b.21481
- Spitzer, M.H. and Nolan, G.P. “Mass cytometry: single cells, many features.” *Cell* 165 (2016): 780–791.
- Diggins, K.E., Ferrell, P.B. and Irish, J.M. “Methods for discovery and characterization of cell subsets in high dimensional mass cytometry data.” *Methods* 82 (2015): 55–63.
- Leipold, M.D. et al. “Multiparameter phenotyping of human PBMCs using mass cytometry.” *Methods in Molecular Biology* 1343 (2015): 81–95.
- Tanner, S.D., Baranov, V.I., Ornatsky, O.I. et al. “An introduction to mass cytometry: fundamentals and applications.” *Cancer Immunology, Immunotherapy* 62(5) (2013): 955–65.
- Bendall, S. and Nolan, G.P. “From single cells to deep phenotypes in cancer.” *Nature Biotechnology* 30(7) (2012): 639–47.

Research Area Applications

- Guilliams, M. et al. “Unsupervised high-dimensional analysis aligns dendritic cells across tissues and species.” *Immunity* 45 (2016): 669–684.
- Kay, A.W. et al. “Application of mass cytometry (CyTOF) for functional and phenotypic analysis of natural killer cells.” *Methods in Molecular Biology* 1441 (2016): 13–26.

- Lowther, D.E. et al. “PD-1 marks dysfunctional regulatory T cells in malignant gliomas.” *Journal of Clinical Investigation Insight* 1 (2016): doi:10.1172/jci.insight.85935DS1.
- Mingueneau, M. et al. “Cytometry by time-of-flight immunophenotyping identifies a blood Sjogren's signature correlating with disease activity and glandular inflammation.” *Journal of Allergy and Clinical Immunology* 137 (2016): 1,809–1,821.
- Van Unen, V. et al. “Mass cytometry of the human mucosal immune system identifies tissue- and disease-associated immune subsets.” *Immunity* 44 (2016): 1,227–1,239.
- Hansmann, L. et al. “Mass cytometry analysis shows that a novel memory phenotype B cell is expanded in multiple myeloma.” *Cancer Immunology Research* 3 (2015): 650–660.
- Levine, J.H. et al. “Data-driven phenotypic dissection of AML reveals progenitor-like cells that correlate with prognosis.” *Cell* 162 (2015): 184–197.
- Nair, N., Mei, H.E., Chen, S.Y. et al. “Mass cytometry as a platform for the discovery of cellular biomarkers to guide effective rheumatic disease therapy.” *Arthritis Research and Therapy* 17 (2015): 127.
- Zunder, E.R. et al. “A continuous molecular roadmap to iPSC reprogramming through progression analysis of single-cell mass cytometry.” *Cell Stem Cell* 16 (2015): 323–337.
- Becher, B. et al. “High-dimensional analysis of the murine myeloid cell system.” *Nature Immunology* 15 (2014): 1,181–1,189.
- Feinberg, H.G. and Nolan, G.P. “Mass cytometry to decipher the mechanism of nongenetic drug resistance in cancer.” *Current Topics in Microbiology and Immunology* 377 (2014) 85–94.
- Newell, E.W. and Lin, W. “High-Dimensional Analysis of Human CD8 T Cell Phenotype, Function, and Antigen Specificity.” *Current Topics in Microbiology and Immunology* 377 (2014): 61–84.

Justification of Need for Premium Cytobank

The high-dimensional datasets generated by the Helios system are best analyzed with software solutions that provide easy-to-use tools to interpret and present multiparametric analysis. Premium Cytobank (premium.cytobank.org/cytobank/login) is our preferred third-party data management and analysis solution. If you choose to use Premium Cytobank, either include it in the cost of the grant

proposal and add justification or demonstrate that there are institutional funds and support available to acquire and maintain this software package. The Premium Cytobank platform enables users to manage and analyze the large amounts of mass cytometry data on the web. An overview of the platform is available on the Cytobank website (support.cytobank.org/hc/en-us/articles/206337537-The-differences-between-Basic-Cytobank-Premium-Cytobank-and-Enterprise-Cytobank).

Premium Cytobank has tools designed specifically to handle the three layers of analysis common to high-dimensional mass cytometry experiments. These include raw data plotting tools such as histograms (and histogram overlays), dot plots, and gating; dimensionality reduction algorithms critical to rendering multidimensional data in two-dimensional space (for example, SPADE and viSNE); and statistical representations of multiple samples such as heat maps and dose response curves. Furthermore, Premium Cytobank provides a centralized, secured, annotated database of all recorded mass cytometry data and facilitates internal and multisite collaborations. Premium Cytobank meets the data sharing requirements outlined in the NIH Grants Policy Statement, November 2016. Availability of Research Results: Publications, Intellectual Property Rights, and Sharing Biomedical Research Resources by providing sharing containers to make published data and results available to the community.

Research Projects

It is important to outline a set of projects that clearly link the unique benefits of mass cytometry to the proposed research and that are planned in sufficient detail to indicate a basic understanding of the practical considerations required for using the system for the research. The projects should not be achievable with existing or alternatively available instrumentation or methods. Most principal investigators (PIs) should currently have NIH grant funding. Proposed projects should have sufficient detail so that the need for a high-dimensional panel is clear. At least one project should have primary and simple proof-of-principle data collected on a Helios instrument to demonstrate that the applicant understands the sample preparation and processing workflow using the technology. The experiment should consist of one or two samples stained with a panel of metal-conjugated antibodies developed with support from a Fluidigm field applications scientist. Plan and coordinate such experiments with members of the Fluidigm account management and application support group several months before the grant deadline. There are multiple cytometry cores where samples from outside sources can be run, and Fluidigm sales can provide direction to these if needed.

Technical Expertise

It is important to demonstrate that the site has sufficient technical expertise (or definite plans to obtain the technical expertise) to ensure successful integration of the Helios platform into the facility and the research goals of the community. The placement facility ideally has a strong history of maintenance and administration of a variety of complex instrumentation. Mastery of the mass cytometry workflow includes proficiency in

- Conjugating antibodies to metals using Maxpar® labeling kits
- Multiparameter panel design
- Standard suspension-based cell staining techniques
- Instrument maintenance, calibration, and operation
- High-dimensional data analysis, interpretation, and presentation.

For core facilities, it is recommended to have a dedicated operator with flow cytometry, mass spectroscopy, and/or mass cytometry experience who will become highly trained in the daily operation and maintenance of the Helios instrument. We highly recommend involving individuals with bioinformatics expertise to help support high-dimensional analysis and interpretation. Highlight a proven track record of integrating complex instrumentation into the research community, and include training programs and strategies for introducing new technology. Include prior experience with validation of breakthrough technology.

Administration

This section should clearly describe the structure in place for oversight of the new system and outline plans for gradual integration of the Helios platform into the facility and research community. Because this is a new technology, particular emphasis should be devoted to methods for introducing the technology to the community, engaging new users and projects, and supporting the growth and expertise of maturing users. These activities have the overall aim of generating a growing set of users with proficiency in experimental design, data acquisition, and data analysis sufficient for peer-reviewed papers, grant submissions, and oral presentations. Describe your plans for the following administrative activities:

- Training
- Outreach
- Technical support
- Engaging new experiments and researchers
- Supporting experimental design concepts and analysis
- Data management
- Reagent generation

Financing

A proposed five-year financial plan for the Helios system should include expenses for labor, a service contract, consumables, argon gas cylinder rental and refills, analysis software, and data management.

Funds for any facility renovations to accommodate the instrument should also be identified. Contact your Fluidigm sales representative for more detailed information on installation requirements.

Projected revenue, typically derived from an hourly rate multiplied by projected use, should be included.

Institutional Commitment

It is important to show material institutional commitment to support the success of the Helios system, including historical support for other instruments, as well as funds budgeted for hiring of appropriate personnel, renovations, fulfillment of installation requirements, and service contract support. It is preferable to show strong institutional support for at least a two-year period. Any statements demonstrating institutional commitment to creating a center of excellence with the Helios platform are desirable.

CORPORATE HEADQUARTERS
7000 Shoreline Court, Suite 100
South San Francisco, CA 94080 USA
Toll-free: 866 359 4354 in the US and Canada
Fax: 650 871 7152
fluidigm.com

SALES
North America | +1 650 266 6170 | info-us@fluidigm.com
Europe/EMEA | +33 1 60 92 42 40 | info-europe@fluidigm.com
China (excluding Hong Kong) | +86 21 3255 8368 | info-china@fluidigm.com
Japan | +81 3 3662 2150 | info-japan@fluidigm.com
All other Asian countries | +1 650 266 6000 | info-asia@fluidigm.com
Latin America | +1 650 266 6000 | info-latinamerica@fluidigm.com