WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY

Newsletter Volume 6, issue 4 April 2020

Minimal Information about a Flow Cytometry Experiment: MIFlowCyt Standards

Over the last few years, the ability to reproduce published data has been demonstrated in many cases to be very difficult in part due to a lack of essential experimental details in the published papers. As grant funding agencies and journals start to ask for more detailed information, it seems like this is a good time to discuss the Minimal Information about a Flow Cytometry Experiment (MIFlowCyt) standards developed by the International Society for the Advancement of Cytometry (ISAC) and endorsed by the Data Interoperability Steering Committee of the Division of Allergy, Immunology, and Transplantation within the National Institute of Allergy and Infectious Diseases (NIAID).

What is the Purpose of MIFlowCyt Standards?

The purpose of the MIFlowCyt standards is to provide a framework for recording and reporting information about a flow cytometry experiment. It is basically a way to promote consistent annotated information about the biological and technical issues surrounding a flow experiment. MIFlowCyt does this by specifying requirements for data content in a standardized format.

Who came up with the MIFlowCyt Standards?

An international group of flow cytometry experts got together along with bioinformaticians, computational statisticians, software developers, instrument manufacturers, and clinical and basic research scientists to develop these standards.

Inside this Issue

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2160 HSCN

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Hours of operation:
9:30 am to 5:00 pm, M-F

After hours access is available for experienced users with prior approval from Dr. Kathy Brundage

Contact Dr. Brundage at:
kbrundage@hsc.wvu.edu

Why do we need reporting standards?

There are many reasons to have flow cytometry reporting standards. First, with high throughput, high multi-parameter flow cytometry data being generated on a daily basis the computational means for handling and analyzing the data is having a hard time keeping up. Second, the lack of standardization in reporting limits collaborations and independent validation. Third, standardization makes meta-analysis possible. Finally, lack of a standard reporting framework minimizes the value of existing flow cytometry data due to the poor annotation of the data.

What are the MIFlowCyt Standards?

The MIFlowCyt Standards are clearly laid out in a 2008 Cytometry Part A paper by Lee et al (Lee, JA, et al. MiFlowCyt: The Minimum Information About a Flow Cytometry Experiment. Cytometry PartA Vol.73A pp926-930). The paper gives a nice overview of the standards and why each type is included. In the paper's supplemental data, the authors give an example experiment, descriptive outline of the standards and a required info summary. This paper with supplements will be added to the WVU Flow Cytometry & Single Cell Core Facility website under the Useful Links tab for easy access.

The table below shows the different components that make up the MIFlowCyt Standards. The standards are grouped into 4 catagories; Experimental Overview, Flow Samples, Data Analysis, and Instrument Details. In the paper and supplemental materials the authors use the words **shall, shall if relevant,** and **should** to denote whether a standard is **an absolute requirement (shall), not generally applicable but maybe in some cases (shall if relevant), or recommended but not required (should)**.

Experiment Over-view	**Purpose/goal/hypothesis** **Experimental Variables** **Conclusions** **Quality Control**
Flow Samples	**Material** **Source/Organism/location** **Treatment** **Reagent/analyte/detector/reporter**
Data Analysis	**List-mode data** **Compensation** **Gating** **Descriptive statistics**
Instrument Details	**Instrument Identification** **Fluidics Configuration** **Optical Configuration** **Electronic Configuration**

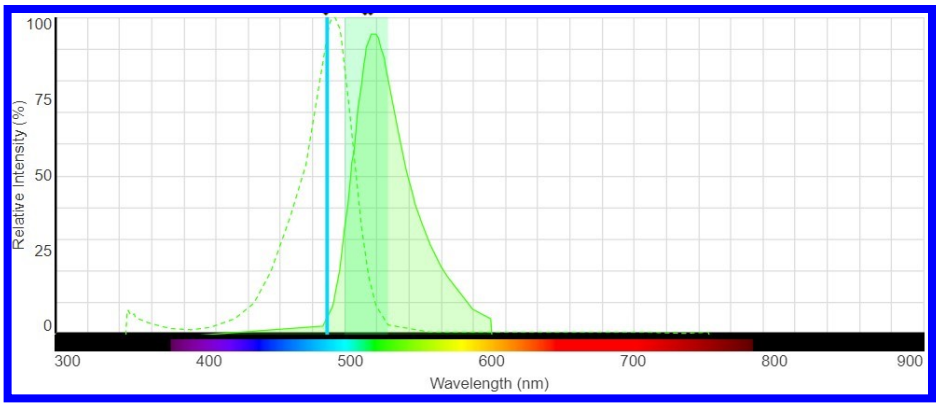
The Experimental Overview component is designed to explain the specific purpose or objective of the experiment. It shall include a summary of the experimental design, results and interpretation of the results. Under the Quality Control subsection, information about replicates, calibrations, control assays etc shall be included.

The Flow Samples components contains the information about the samples, including sample source, sample type, any treatment they under went and how they were prepared. Critical for interpretation, a list of reagents including the protein being detected and fluorescent label antibody used (include clone name, company and fluorescent label) shall be included. Also in this section, a description of process being evaluated i.e. apoptosis, cell cycle shall be included.

The third category is Data Analysis. For this category, FCS file type as well as the type of compensation performed shall be described. A table of the compensation matrix is also required. A clear description of the gating strategy going all the way back to the all events shall also be included.

The final category is Instrument Details. In this category the instrument manufacturer and model number is sufficient unless the instrument has been uniquely modified. For the WVU FCSCCF, the LSRFortessa (analytical flow cytometry) is a Special Order Research Product (SORP) so the components of this category will have to be included. The FACSAria III (sorter) on the other hand is not a SORP instrument so only the manufacturer and model number is required. To make things easy for users of the core, a standard Instrument Details document for the LSRFortessa and FACSAria III will be put together. Users will be able to find the pdf for each instrument on the flow core website.

Meet the Fluorochrome: Dylight 488



Excitation max

485 nm (Blue laser)

Emission max

518 nm (same detector as the one used for FITC)

Type of Fluorochrome: Sulfonated fluorescein
Characteristics: Brighter than Alexa 488 and FITC
More photo stable than FITC

Upcoming Holidays & Events

April 2020	Coronavirus Restrictions	Facility Closed
May 12, 2020	Election Day Holiday	Facility Closed
May 25, 2020	Memorial Day Holiday	Facility Closed
June 19-23, 2020	Kathy out of the Lab	Facility Open for Experienced Users No sorting

New User Guide

Hands-on training for LSRFortessa, C1 Single Cell Auto Prep System, NanoSight NS300 and Zetasizer Nano Z is **mandatory** for all new users and must be scheduled by consultation with the facility director.

Training will initiate with user's first experiment. Due to the complexity of the instruments and software, facility staff will fully assist with the acquisition of the first dataset and will continue with additional assistance on a "needs" basis until users are comfortable operating the instrument on their own. Sorting on the FACSAria is by facility staff only.

Note to users:

Please remember to acknowledge the support of the HSC Research Office and NIH grants that support the WVU Flow Cytometry & Single Cell Core in all your publications. The grant numbers are listed below:

TME CoBRE grant: P20GM121322
WVCTS grant: GM104942 important if you used the Miltenyi AutoMACS pro (installed 6/29/18)
WV InBRE grant: GM103434
Fortessa S10 grant: OD016165
NanoSight NS 300 use Stroke CoBRE grant GM109098 and WVCTS grant GM104942
ZetaSizer NanoZ use Stroke CoBRE grant GM109098 and WVCTS grant GM104942

Flow Cytometers in the Facility

FACSAria III Cell Sorter

Operator: Facility Staff

Lasers:

- 488 nm solid state
- 561 nm solid state
- 633 nm solid state
- 407 nm solid state

Detection Parameters:

- Forward Scatter
- Side Scatter
- Simultaneous detection of 13 fluorochromes

Applications:

- Cell Sorting (Aseptic)
- Single Cell Sorting
- Cell Phenotyping
- Cell Viability
- Cell Cycle Analysis

LSR Fortessa

Operator: User of Facility Staff

Lasers:

- 405 nm solid state
- 488 nm solid state
- 561 nm solid state
- 628 nm solid state

Detection Parameters:

- Forward Scatter
- Side Scatter
- Simultaneous detection of 17 fluorochromes

Applications:

- Cell phenotyping
- Cell Viability
- Cell Cycle analysis
- Apoptosis Assays

Other Instrumentation Available in the Facility

AutoMACS Pro Magnetic Bead separator

Operator: User

Application:

- Single extracellular marker cell sorting
- Depletion/negative cell sorting

gentleMACS Octo Dissociator with Heaters

Operator: User

Application:

- Dissociation of tissues into single cell suspension for culture or flow cytometry assays
- Homogenizes tissues for downstream molecular biology applications

C1 Single Cell Auto Prep System

Operator: User or Staff

Application:

Uses microfluidics, to separate cells into individual compartments, isolate RNA from the single cells, and generate cDNA for downstream genomic applications.

Downstream applications:

- RNA seq
- DNA seq
- PCR

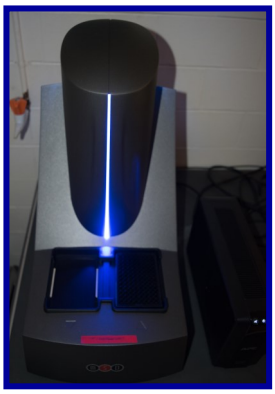
Format: 96 or 384 chambers per chip

MSD Multi-Array Platform

Operator: User

Applications:

- Detection of cytokines, cell signaling proteins
- Multiplexed assay design: (1-10 analytes/plate)
- Detection range: 1 – 10,000 pg/ml
- Sample volumes: 25 µl or less
- Assay Time: 4—6 hours depending on analytes being detected



Nanosight NS 300

Operator: User or Staff

Application:

- Determines the size and concentration of particles 10 nm to 1 microns in size
- Equipped with 4 lasers (405 nm, 488 nm, 532 and 642) to detect fluorescently labeled particles

Zetasizer Nano Z

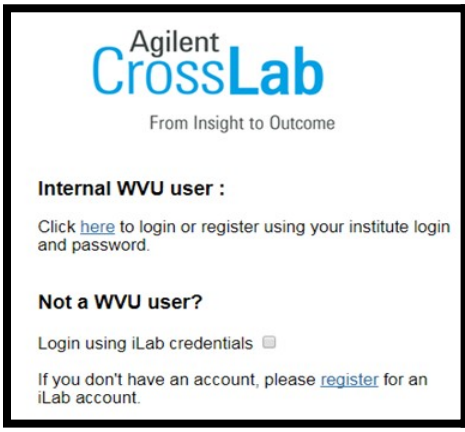
Operator: User or Staff

Application:

Measures the zeta potential of particles in a solution using laser Doppler micro-electrophoresis

Fee Schedule

Instrument	Operator	For WVU & NIOSH Users	For Non-WVU Users
AutoMACS Pro	Facility Staff or User	$4.50 / separation	$6.85 / separation
C1 Single Cell Auto Prep System	Facility Staff	$210/plate	$320/plate
	User	No Cost	$115/plate
FACSAria III	Analysis: Facility Staff	$52.50/h	$80/h
	Analysis: User	$34.65/h	$53/h
	Sorting	$77.70/h	$120/h
	Sorting Setup	$19.43/sort	$30/sort
gentleMACS	Facility Staff or User	$10.50/sample	$16/sample
LSRFortessa	Facility Staff	$52.50/h	$80/h
	User	$34.65/h	$53/h
MSD QuickPlex SQ120	Facility Staff or User	$10.50/h	$16/h
NanoSight NS300	Facility Staff	$61.00/h	$93/h
	User	$42.50/h	$65/h
Zetasizer Nano Z	Facility Staff	$25/sample + $52.50/h	$39/sample + $80/h
	User	$25/sample	$39/sample + $16/h



The facility uses iLAB scheduling/billing software from Agilent to manage the use of the facility's instrumentation. If you would like to use the instruments housed in the facility please use the URL shown below to register as a WVU User and to login to reserve an instrument.

https://wvu.corefacilities.org/account/login