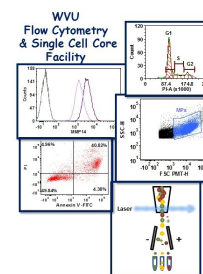


WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY



What's New in 2016:



Figure 1. C1 Single Cell Auto Prep System from Fluidigm.

As 2015 ends and 2016 begins, the WVU Flow Cytometry Core Facility is undergoing a few changes. First and foremost is the purchase of a new piece of equipment for the facility. Thanks to the generosity of the WVU CTSI (Dr. Sally Hodder) the facility now has a C1 Single Cell Auto Prep System from Fluidigm (Figure 1, discussed in detail below). The purchase of the C1 instrument has expanded the facility's single cell analysis capabilities into the field of genomics. By combining flow cytometry and the C1 instrument researchers now have the ability to dissect all aspects of cells from the phenotypic/functional level down to the genomic level. To acknowledge the extension of the facilities single cell capabilities, the facilities name has changed to "WVU Flow Cytometry & Single Cell Core Facility" aka WVU FCSCCF. Even though the name has changed the overall goal of the facility remains the same to provide state of the art technologies, instrumentation and expertise to further the research aims of WVU researchers.

New Instrumentation: C1 Single Cell Auto Prep System

December 2015 saw the arrival in the facility of Fluidigm's C1 Single Cell Auto Prep System (Figure 1). This instrument is a microfluidics device that allows the users to perform genomic analysis on single cells. The C1 system uses an integrated fluidic circuit (IFC) chip to isolate 96 individual cells. While in the IFC chip, RNA/DNA/microRNA is extracted from the single cells followed by amplification

Inside this Issue

1	What's New in 2016
1	New Instrumentation: C1 Single Cell Auto Prep System
2	Instrument list
3	Analysis tips
3	New Users Guide
3	Fee Schedule
4	From the Web
4	Upcoming Holidays & Events

Facility Location:
2160 HSCN
Phone: 304-293-6273
e-mail: flowcore@hsc.wvu.edu

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

After hours access for experienced users by prior approval from Kathy Brundage

Contact Kathy at:

kbrundage@hsc.wvu.edu

and cDNA generation (if needed). The resulting products can be sequenced or used in qPCR. Figure 2 shows a typical workflow.

A major advantage of the Fluidigm system is the use of microfluidics and nanoliter-scale reactions to maximize precision and minimize reagent usage while multiplexing the reactions. The IFC chip enables users to load samples and reagents into a relatively small number of wells (between 12 and 192, depending on configuration), and the C1 controller does the rest. There are a large collection of IFC chips that are or will soon be compatible with the C1 system making this instrument platform extremely flexible. The workflow can be coupled with next-generation sequencers like the Illumina MiSeq in the WVU Genomics Core Facility and the Illumina HiSeq in our partner facility at Marshall University to provide genome-scale data and analysis. If a researcher wants to perform targeted gene expression analysis and already has a panel of genes of interest, then the C1 can be used to isolate cells, lyse, and perform the reverse transcription. The C1 then performs a targeted pre-amplification reaction. The resulting pre-amplified cDNA is ready for real-time qPCR analysis.

Along with the C1 system, the facility also has a Miltenyi gentleMACS tissue dissociator to assist with making single cell suspensions from tissues and tumors.

If you are interested in exploring further the uses of the C1 system and how it may impact your research contact the WVU Flow Cytometry & Single Cell Core Facility for a consultation.

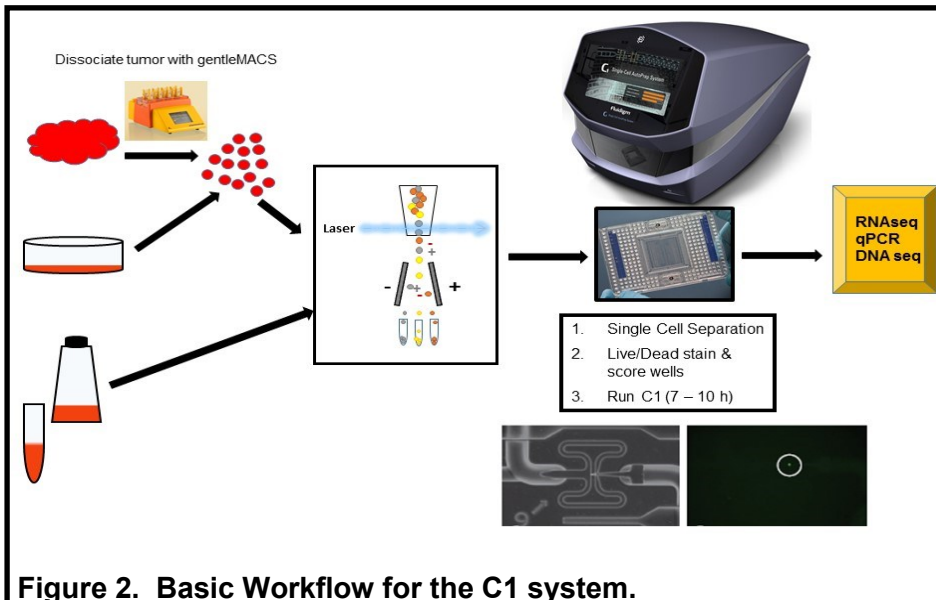


Figure 2. Basic Workflow for the C1 system.

To log in and reserve a flow cytometer, C1, AutoMACS, MSD or analysis computer, please point your browser to the following URL



<https://cores-wvu.mis.vanderbilt.edu/login.cfm>

Instruments in this facility

FACSAria Cell Sorter/Analyser

Operator: Facility Staff

Lasers: 488 nm Sapphire(SS)
633 nm HeNe
407 nm Violet(SS)

Detection Parameters:

Forward Scatter, Side Scatter, simultaneous detection of up to 11 fluorochromes

Applications:

Cell Sorting (Aseptic)
Cell phenotyping
Cell Viability
FISH, FRET, SPA

FACSCalibur Analyser

Operator: User

Lasers: 488 nm Argon
633 nm Red Diode

Detection Parameters:

Forward Scatter, Side Scatter, simultaneous detection of up to 4 fluorochromes

Applications:

Cell phenotyping
Cell cycle analysis

AutoMACS Magnetic

Bead separator

Operator: User

Application:

single marker (extracellular) sorting, depletion sorting

LSR Fortessa

Operator: User

Lasers: 405 nm OBIS LX
488 nm Sapphire (SS)
561 nm Sapphire (SS)
628 nm OEM

Detection Parameters:

Forward Scatter, Side Scatter, simultaneous detection of up to 12 fluorochromes

Applications:

Cell phenotyping
Cell Viability
Cell Cycle analysis
FISH, FRET, SPA

Analysis Tips:

Since early October, the flow cytometry analysis software FCS Express 5 from De Novo Software has been available for download by all WVU Flow Cytometry & Single Cell Core Facility. users A key feature of this version of the software is that we have an internet dongle and three site licenses. With the internet dongle users can now download the software to any and as many PC computers with an internet connection as they wish and perform data analysis outside the WVU Flow Cytometry & Single Cell Core Facility.

To take advantage of the internet dongle feature:

1. Contact the Flow Cytometry Core via email (kbrundage@hsc.wvu.edu or flowcore@hsc.wvu.edu)
2. You will be sent a user login, password, configuration file and step by step downloading instructions

If you prefer to continue doing your analysis in the facility, there are two computers available for you to use.

Upcoming FCS Express 5 Software Webinars

February 17 (Wednesday)	2–3pm Erma 201	Regression Analysis, Generating Bar Graphs and Pie Charts
-------------------------	----------------	---

REMINDER: GOOD NEWS FOR WVU INVESTIGATORS WHO WOULD LIKE TO SORT

The HSC Research Office has generously provided the facility with some funds to subsidize/co-pay users' sorting costs. From Sept 2015 through Feb 2016, the cost of sorting for a WVU researcher is \$15/hour.

Fee Schedule (2015-2016)

	User Operated Analyzer	Facility Operated Analyzer	FACSAria Sorting	AutoMACS	MSD Sector Imager 2400	User Operated C1	Facility Operated C1	gentleMACS Octo Dissociator
WVU User	\$33/h	\$50/h	\$74/h	\$14/use	\$10/use	No Cost	No Cost	\$10/sample
Non-WVU User	\$49.50/h	\$75/h	\$111/h	\$21/use	\$15/use	\$75/plate	\$225/plate	\$15/sample

New User Guide

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

Sorting as well as data acquisition on FACSAria is by facility staff only.

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.

From the Web:

In a December issue of The Scientist, there was an article (<http://www.the-scientist.com//?articles.view/articleNo/44593/title/Free-Flow/>) which may be of interest to those who would like to explore other flow cytometry data analysis softwares besides the FCS Express 5 that we have in use in our facility. In the article, the author reviews several free and open-source alternative flow cytometry data analysis software programs including Bioconductor, CytoFlow, Single Cell Debarcoder and Cytospec. The common denominator with these software packages is that they tend to be not as polished as the commercial software packages and usually require the user to have experience in writing computer scripts. They can also be buggy and slower to respond than commercial software. So the question is why would you want to use them? The answer is that sometimes there are data problems that commercial software can not handle. Open source alternatives allow the user to have more flexibility in handling the data. So if you are the adventurous type give one of them a try!!!

Upcoming Holidays & Events

February 10, 2016	Fluidigm C1 Single Cell System	1– 2 pm HSCN 2157
February 17,2016	FCS Express 5 Software Webinar	2–3 pm BMRF 201
February 19 - 26, 2016	Kathy out of Lab	Facility Open Analyzers available No sorting
March 25, 2016	Spring Holiday	Facility Closed

Note to users:

Please acknowledge the WVU Flow Cytometry Core Facility when reporting your flow cytometry data, using the appropriate phrase(s):

FACSAria users: Flow Cytometry experiments were performed in the West Virginia University Flow Cytometry & Single Cell Core Facility, which is supported by the National Institutes of Health equipment grant number RR020866 and the Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant numbers P30GM103488 (CoBRE) and P20GM103434 (INBRE).

LSRFortessa users: Flow Cytometry experiments were performed in the West Virginia University Flow Cytometry & Single Cell Core Facility, which is supported by the National Institutes of Health equipment grant number S10OD016165 and the Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant numbers P30GM103488 (CoBRE) and P20GM103434 (INBRE).

C1 System users: Experiments were performed in the West Virginia University Flow Cytometry & Single Cell Core Facility, which is supported by the Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant numbers U54GM104942 (CTR), P30GM103488 (CoBRE) and P20GM103434 (INBRE).