WVU FLOW CYTOMETRY & SINGLE CELL CORE FACILITY

Newsletter Volume 3, issue 4 April 2017

New Instrumentation Has Arrived!!! NanoSight NS300 & Zetasizer Nano Z

Exciting times in the WVU FCSCCF, after much wait the new NanoSight NS300 and Zetasizer Nano Z has been installed in the facility. Both instruments can be user operated. Training is now available for those who wish to use either instrument.

Malvern NanoSight NS300

The NanoSight N300 is designed to detect, size and count particles from 10 nm to 2 microns in size. In addition, the one in our facility comes with 4 lasers (405 nm, 488 nm, 532 nm and 642 nm) allowing for detection of fluorescently labeled particles. This instrument can be operated either by the facility staff or the user after appropriate training.

Key Points:

1. Particle concentration: The manufacturer recommends a particle concentration of 10^7—10^9 particles/ml

2. Sample size:
   A. Counting and sizing measurements: 500 µl to 1 ml
   B. Fluorescent measurements: 1 - 5 ml depending on how many different fluorescent molecules you are measuring

3. Length of time to run a sample depends on the number of runs you do per sample and the length of time of each run
   A. When counting and sizing only: 3 - 5 minutes
   B. Fluorescent measurements: 5 minutes/fluorochrome.

4. Cost of using the instrument:
   A. User operated: $42.50/hour
   B. Facility operated $61.00/ hour
   C. Training $61.00/hour

Inside this Issue

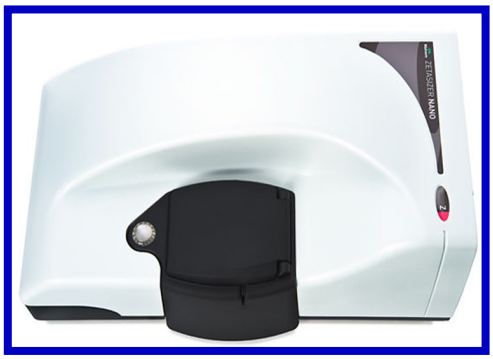
Facility Location:
2160 HSCN
Phone:304-293-6273
e-mail: flow-core@hsc.wvu.edu

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

Malvern Zetasizer Nano Z



The Zetasizer Nano Z is designed to measure the zeta potential (the charge on particles in a solution) via electrophoretic mobility of particles in a solution using Laser Doppler Micro-Electrophoresis. Like the NanoSight NS300, this instrument can be operated by the user after appropriate training.

Key Points:

1. Minimum recommended particle concentration depends on the particle size
   A. < 10nm particles: 0.5 mg/ml
   B. 10 nm—100 nm particles: 1 mg/ml
   C. 100 nm—1μm particles: 0.01 mg/ml
   D. >1 μm particles: 0.1 mg/ml

2. Sample size: 0.75 - 1 ml

3. Length of time to run a sample: approximately 6 minutes

4. Cost of using the instrument including the disposable folded capillary zeta cells:
   A. User operated: $25/sample
   B. Facility operated $25/sample + $52.50/hour

If you are interested in learning more about either instrument and have plans to use them in the future, you are encouraged to attend a seminar on May 23rd by the technical specialist from Malvern Instruments. Hope to see many of you there!!

New User Guide

Hands-on training for FACSCaliber, 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 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 FACSAria is by facility staff only.

Flow Cytometers in this 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)**
- **Cell phenotyping**
- **Cell Viability**
- **FISH, FRET, SPA**

LSR Fortessa

Operator: User

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 12 fluorochromes**

Applications:
- **Cell phenotyping**
- **Cell Viability**
- **Cell Cycle analysis**
- **FISH, FRET, SPA**

To log in and reserve any of the instrumentation in the facility, please point your browser to the following URL

CORES
Core Ordering & Reporting Enterprise System

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

Other Instrumentation Available in the Facility

AutoMACS 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

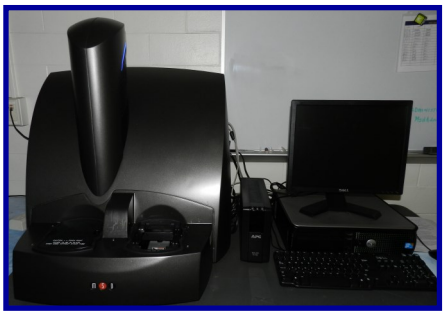
Format: 96 or 384 chambers per chip

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



Fee Schedule

	User Operated Analyzer	Facility Operated Analyzer	FACSAria Sorting	AutoMACS	MSD Sector Imager 2400	User Operated C1	Facility Operated C1	gentleMACS Octo Dissociator
WVU User	$34.65/h	$52.50/h	$77.70/h	$14.70/use	$10.50/use	No Cost	$210/h	$10.50/ sample*
Non-WVU User	$51.98/h	$78.75/h	$116.55/h	$22.05/use	$15.75/use	$112.50/ plate	$315/plate	$15.75/ sample*

*Only if facility tubes and filters are used. If you supply your own then there is no cost.

Data Analysis Tips: Linking Markers To Each Other

What does linking markers mean?

Linking markers together means that moving the end of one marker will automatically cause an end point on a different marker to update at the same time effectively allowing bifurcation of histogram plots.

How do I link markers?

1. Create two markers on a histogram, i.e. (-) cell population and (+) cell population
2. Right click on the plot containing the two markers and select "**Format**" then **"Markers"**
3. Choose one of the Markers and delete the field for "**High In Markers Option"**
4. Click on the **T** icon and choose "**Statistic**" from "**Select a Token Type**"
5. Click "**Insert**" and a Create Statistic dialog opens
6. Choose "**Statistic**" category on the left
7. From the "**Select a Marker**" drop down select the other Marker
8. Choose "**Low Bound or High Bound**" from the "**Statistics**" list depending which end you want to link
9. Click "**OK**"
10. Check the "**Locked"** box of the high bound for the first Marker in the "**Marker Options**"
11. Click "**Ok**" in the "**Formatting dialog"**

Now, you have two markers with locked borders at one end and non-locked boundaries that can still be moved independently at the other.

Upcoming Holidays & Events

May 8 - 9, 2017	Kathy out of the lab	Facility open; No sorting
May 23, 2017	NanoSight & Zetasizer Seminar	Time & Room to be announced
May 29, 2017	Memorial Day	Facility Closed
June 8 –19, 2017	Kathy out of the Lab	Facility open; No Sorting
June 27, 2017	Nanostrings Seminar	1 - 2 pm, Room 2157
July 4, 2017	4th of July	Facility Closed
July 27 - August 1, 2017	Kathy out of the lab	Facility open; No sorting

Note to users:

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

LSRFortessa users: Flow Cytometry experiments were performed in the West Virginia University Flow Cytometry & Single Cell Core Facility, which is supported by the following National Institutes of Health equipment grant S10OD016165 and the following Institutional Development Award (IDeA) grants U57GM104942 (WV CTSI), P30GM103488 (CoBRE) and P20GM103434 (INBRE).

For all users of the facility: Experiments were performed in the West Virginia University Flow Cytometry & Single Cell Core Facility, which is supported by the following National Institutes of Health and the Institutional Development Awards (IDeA) grants U57GM104942 (WV CTSI), P30GM103488 (CoBRE), P20GM109098 (Stroke CoBRE) and P20GM103434 (INBRE).