Operation of the ACEA NovoCyte Flow Cytometer

Version "0.9" – 11 Aug 2015

I. Room access

a. You must have a key to access BSRB 4661 during regular work hours.

i. Interim solution as of mid-Aug 2015: as soon as possible, I will obtain a "checkout" key that non-BSRB personnel can obtain from me by signing a sheet when they take it and when they return it.

ii. Long term solution possibilities

1. Re-key the door (all users will therefore need a new key, not just those outside 4th floor BSRB)

2. Install electronic ID badge reader at door (may be $$$)

b. You must have, IN ADDITION to the key, for working late and on the weekends:

i. Card access to the BSRB building

ii. Card access to the West end of the 4th floor (reader 5037-4C60 BSRB W Wing Lab NE entrance)

II. Startup, fluidics check, QC

a. Fill sheath if 1/3 full or less using the Fisher "NERL" cubitainer located next to the sink. Users are responsible for filling sheath. If you fill the tank with sheath, run "prime" cycle. Takes 17 min.

b. Empty waste if 2/3 full or more into the provided 15 gal poly barrel beneath the table. It's on rollers. Do NOT dump waste down the sink. Users are responsible for emptying waste into barrel.

i. After emptying waste, add ~200 ml bleach to the container (beneath sink)

c. If instrument is OFF: push power button – instrument will be ready in **3 min.**

d. QC: done by Peter or Nadeem 2x or 3x per week.

i. Note: If QC fails, instrument will "lock up" and NOT allow anyone to run samples until QC passes.

e. Turn on computer

i. Launch "NovoExpress" software

ii. Choose your name from the list. If you don't see it and want your own profile, contact me (pouillet@med.umich.edu)

iii. No passwords for individuals.

III. Hardware

a. You can bring 12x75 mm tubes or 96-well plates.

b. For tubes, use the special rack designed to fit in the "NovoSampler." It can hold 24 tubes at a time.

c. 96-well plates are subject to the same volume limitations as the BD LSRII, meaning:

- **i.** Whatever volume you tell the instrument to aspirate, it will aspirate an **additional 20 ul**. (The NovoCyte is "greedy.")
- **ii.** A certain amount of each well in a 96-well plate is considered "dead volume," i.e. you pipetted 200 ul into the well, but as far as the instrument is concerned, you only have ~170 ul. This dead volume is greater for flat-bottom plates. Don't use them.
- **iii.** Therefore if you pipetted 200 ul into your sample well and run it twice at 50 ul,
  1. The volumes removed are 70 & 70 = 140 ul (not 100!)
  2. The remaining volume is closer to 30 ul, not 60! You will suck air into the instrument even if you drop the volume aspirated to 25! (25+20 extra = 45 ul).
- **iv.** Plan ahead so that you have "enough" cells. "Enough" cells is defined as the quantity and concentration that gets you enough information to effectively complete your experiment. While this is ultimately up to you and your lab, we recommend 30,000 events per sample.
- **v.** You need to think in advance how many cells in your sample are contained in what volume. If you have 500,000 cells in 250 ul in the well, then you have 2000 cells per ul and in theory you should aspirate at least 15 ul to obtain 30,000 events. In practice I recommend taking twice that to begin with, particularly if this is a new experiment with cell types with which you are unfamiliar.
- **vi.** The NovoSampler can move sample through the instrument at continuously variable rates measured in microliters per minute. However, there are three standard sample flow rate settings: Slow (14 ul/min), Medium (35 ul/min), and Fast (66 ul/min). Continuous adjustment of the sample flow rate from 5 ul /min to 120 ul/min is possible. NOTE that the core stream diameter will increase from 4.6 microns to 22.7 microns, respectively, under these settings.
- **vii.** If you have a concentrated sample, use a speed that does not cause a measured event rate greater than **3000** events/sec. This is a "guesstimate." Currently I cannot find a maximum recommended event rate from ACEA for the NovoCyte.
- **viii.** If you have a dilute sample, increase the speed to save time.
- **ix.** If you **suck air** into the instrument because you misjudged the amount of sample in your well, you must **prime** the instrument, and it takes **much longer** on the NovoCyte than it does on the LSRII (**17 min!**).
- **x.** Caution: Note that ACEA does not recommend MIXING samples if you have >200 ul in a well of a 96-well plate. The method employed by the NovoCyte is to shake the plate at high frequency. At liquid volumes >200 ul, microdroplets can cross-contaminate other wells.

d. **Nice feature** of the NovoCyte is that the dynamic range of the instrument's plots is 7.2 log decades. What this means is that the range is so broad, **voltage adjustments of your populations are no longer necessary**. Just "load & go!"

IV. Setting up your work area for a run

a. → NovoExpress operates a little differently if you're used to BD's FACSDiva software.

b. NovoExpress uses Experiments, Specimens, and Samples.

c.

V. **Fluorescence Compensation**

a. Fluorescence Compensation is a **separate training module** that must be specifically requested, for the following reasons:

   i. Many flow users elect to compensate their data back at their home lab using different software

   ii. Avoid "information overload" during training

b. Nonetheless, compensation is a **critical** aspect of flow and it must be understood if you are going to do >1 color experiments!

VI. When you are done with your run:

a. If someone has an appointment after you, run the "Rinse" cycle. Takes 2 min.

b. If you are the last user of the day: press the power button. Button will start to flash, indicating that the shutdown cleaning cycle has begun. Takes about 30 min. Instrument will shut itself off when done. So, shut down computer, push instrument power button, walk away (lock door behind you).

VII. **Explanation of the various cleaning/unclogging commands**

a. Debubble

   i. Use the *Debubble* function in the Fluidics Maintenance panel if bubbles are suspected to exist in the fluidics system. To use *Debubble,* place a test tube containing at least 1 mL of 70% **ethanol** in the sample holder. Click the *Debubble* function, and the sample injection tube will aspirate the ethanol to initialize the debubble process. The *Debubble* process takes approximately 3 min. **Always use at least 1 ml ethanol!**

b. Cleaning

   i. Uses "NovoClean" (bleach) and sheath fluid in sequence to flush the entire fluidics system. The *Cleaning* process takes about **14 minutes**.

c. Rinse

   i. Use the *Rinse* function to clean the fluidics system. Uses sheath fluid only. Takes 2 min.

d. Extensive Rinse

   i. Recommended that users run *Extensive Rinse* once a month. First flushes with NovoRinse (detergent), soaks the fluidic tubing for a certain period of time, then flushes the system with sheath fluid. Takes 12 min.

e. Priming

   i. *Priming* function should be used whenever one of the following situations occurs:

- Non-operation of the instrument for more than two days.
- Running out of sheath fluid during run
- After adding reagent to the reagent container
- Running out of sample, sucking air into the fluidics system.
- **Takes 17 min!**

f. Unclog

i. Run Unclog once a month as a preventive measure. Cleans out the **FLOW CELL**. *Unclog* uses "NovoClean" (bleach) to flush the flow cell under high pressure, then soaks the flow cell for a certain period of time, and then flushes with sheath fluid. Unclog takes **about 17 min**.

g. Backflush

i. When the sample injection probe is blocked, use *Backflush* to clear. This flushes the **sample injection probe** under high pressure using the sheath fluid in reverse of the normal sample flow direction, and the waste is aspirated by the SIP wash apparatus. *Backflush* takes approximately **3 min**.

VIII. **Setting up software for a run**

a. Upon login you will by default be presented with a new, **blank** experiment document.

b. You may open previously saved experiments but it is unlike FACSDiva in that you do not see multiple experiments in the data tree. It is advisable to have a few experiments saved as "templates" with the file extension .nct, then open one of these upon starting a new day's experiment.

c. Choose your sample source (24-well plate, 24-tube rack, 96-well plate, etc) – upper right corner

d. Set # mixing cycles (caution – see **III.d.x,** above) and number of wells between mixes

e. Set # rinsing cycles and number of wells between rinses (note: cannot be set to 0)

f. Choose the parameters for which you wish to collect data. Note that you will need to check both Height and Area if you wish to collect both. The NovoCyte will default to HEIGHT. Note that under "Parameters" in the Cytometer Setting window, note that the software calls the detector names column the "Alias" column. You can double-click on the alias for any detector and rename it as you see fit, e.g., "FITC" can be come "GFP."

i. To see which formal parameter label (BL1, VL2, RL1, etc) is associated with which filter, click the "instrument" tab, then the "configuration" button (icon = wrench)

g. Choose your Stop Conditions

i. # of events to collect

ii. Whether you wish have the # of events collected satisfy a gate to be counted

iii. # of microliters of sample to aspirate

iv. Either or both of the # events or # ul critera can be checked.

h. Choose your desired starting Flow Rate

i. Choose your Threshold. Default is 100,000. For small lymphocytes, you may want to try 50,000. For platelets, try 10,000. Bacteria would require 1000-10,000 AND SSC-H >5000 as a secondary threshold.

j. Draw plots. Draw gates and apply them.

k. Highlight wells in the plate icon and create tubes for all of these. Try to do it in such a way that you have only one **specimen** (syringe icon). This is my preferred approach; organizing your experiment into different specimens may be helpful to you. I have always found it simpler to lump controls and all samples (tube icons) under one "specimen" icon (syringe icon).

l. Click the floppy disk icon in upper left. The NovoExpressTM software automatically saves the data, along with the cytometer settings information, to the experiment file. However, data analysis, including all the plots and gates, fluorescence compensation, created Reports and Statistical Tables, etc., must be saved manually. To save, click *File* → *Save* from the menu or click the Save icon . Otherwise, when closing the NovoExpressTM software, a message will prompt users about saving the data.

m. Now comes the rub: making all these choices made in **c** through **j** apply across your entire experiment. It's different than in FACSDiva software. In FACSDiva the "worksheet" contained a set of plots and gates that was independent of your samples/tubes. In NovoExpress each tube can have its **own set** of plots, gates, compensation matrix, Stop Conditions, Flow Rates, and analysis parameters.

IX. Experiment Manager

a. The NovoExpress Software uses a hierarchical structure including groups, specimen, and samples to organize and manage experimental data.

- **i. Work List**: an optional way to view information on sample names and collection parameters. Anything entered in the work list is reflected in the Experiment Manager tree. This may be useful just because it seems easy in NovoExpress to end up with samples that have different collection params, i.e., you may have customized collection params in Sample 1 but you need to be careful that by Sample 7 you're still trying to collect 20,000 events (not 10,000) at a threshold of 10,000 (not 100,000), etc.
- **ii.** Similar to FACSDiva, the user has the option to organize his/her samples into Specimens, with each Specimen having Samples. Personally I don't need such organization, so I stick with just one sample.
- **iii.** The Sample icon is represented by two tubes. The fact that there are two instead of one means nothing. The Sample is the most basic organizational unit and contains sample data collection parameters, instrument settings, fluorescence compensation settings, reports, analyses, and data. Tubes without data will appear white ("empty"). Tubes with data will appear "full."
- **iv.** It is important to keep track of the various settings within each tube:
  1. Cytometer settings contain the sample parameters, the acquisition stop conditions, and the sample flow rate and threshold settings.
  2. Fluorescence compensation – the matrix for the sample.

3. Report – found at both the specimen and sample level. Can include plots and statistical analysis for all samples under the specimen. Reports under sample nodes can include plots and statistical analysis only for the sample.
4. Analysis – what plots and gates. This is one of the **most significant differences with FACSDiva**. Each sample tube has ITS OWN set of plots and gates, unless you carefully copy your set of plots'/gates to ALL Samples in your experiment.

X. Gating

a. is controlled more from the perspective of the gate than from the plot. If you want to apply a gate to a plot, don't click the plot. First click the Gate tab. Then click the gate name itself elsewhere in the plots window. Then click "Gating." (alternatively, click "Gate" tab first, then choose the gate of interest from the drop-down menu at far left. Choose the plot you want to apply it to. Observe for change.

XI. **How to use the integrated cell cycle analysis feature**

a. See Section 5.5.1 in the ACEA NovoExpress Software user guide.

XII. **How to perform absolute counts**