NovoCyte Advanteon BVYG Standard Operation Protocol

Basic Operation

1. NovoExpress Software Log In

Log into NovoExpress software with your own login name and password. Make sure Auto Login is unchecked.

*Please contact Faculty Core Facility Staff to establish a new user account.

![Login window](image.png)
2. Compensation (Perform when needed)

Step 1. Set-up Compensation Controls
   a. In the Home tab of the Menu Bar, click the Auto Compensation button.
      ![Auto Compensation Button]

   b. Select Compensation on: Height, Parameter for calculation: Median and check the boxes of channels involved. Then click OK.
      ![Auto Compensation Options]

   ![Compensation Channels]
c. Compensation Control Specimen is created in the Experiment Manager panel with corresponding empty control samples (e.g. Unstained, B530 FITC, V445 Pacific Blue, and Y586 PE).

Step 2. Compensation Control Acquisition
a. Double Click your sample and let the red arrow point to it
b. Then click **Run** on the Cytometer Control Panel.

c. Repeat the steps a and b to the following single stained tubes.
d. After all controls have been acquired, the compensation matrix is calculated automatically.

Step 3. To view or adjust the compensation matrix, click the Compensation Matrix button in the Home tab of the Menu Bar and the Compensation Matrix window will show.

Step 4. Apply Compensation Matrix to Experiment Sample
   a. Click the New Sample(s) button to create a new sample (sample1) of Specimen 1.
   b. Drag the Compensation node under the Compensation Specimen and Drop over the desired sample.
   c. Repeat step a and b to create new sample of Specimen 2 if needed.
d. To fine tune the Compensation, click on the plot you want to adjust and click the
*Quick Compensation* button in the *Home* tab of the *Menu Bar* OR the *quick compensation icon* in the tool bar.

Scrollbars appear on any two parameters plots with fluorescent parameters opened on the workspace. Quickly adjust compensation by **dragging the scrollbar**.
e. To view or adjust the compensation matrix, click the **Compensation Matrix** button in the **Home** tab of the **Menu Bar** and the Compensation Matrix window will show.
3. **Sample setting without auto-compensation:**

Step 1. Set up new sample

a. Click the *New Sample(s)* button to create a new sample (sample1) of *Specimen 1*.

b. Double click *Sample 1* on the *Experiment Manager* until the red arrow is pointing to Sample 1.
Step 2. Select Channels
   a. Click on the “A” and “H” of the parameters panel in Cytometer setting to Select OR Unselect ALL.

   b. Check the box of A or H of the interested channels to select. Please always check A for FSC (H is checked by default).
Step 3. Conditions Setup

a. Set up the data recording stop conditions by checking the box next to the condition Events and/or Time and/or Volume. Acquisition will stop when ANY one of the selected condition(s) is fulfilled.

*Volume is compulsorily selected.

![Stop Condition](image)

Range of each conditions:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Events</td>
<td>1 – 10,000,000</td>
</tr>
<tr>
<td>Time</td>
<td>0-60 min; 0-59 Sec</td>
</tr>
<tr>
<td>Volume</td>
<td>5 – 5000 µL</td>
</tr>
</tbody>
</table>

b. Select flow rate by click the radio button of Slow (14 µL/min), Medium (35 µL/min), and Fast (66 µL/min) OR use the slider to adjust the flow rate from 5~120 µL/min.

* Current sample’s flow rate and the corresponding core diameter are shown in the bottom of the panel.

![Flow Rate](image)
c. Set the appropriate threshold by select the appropriate parameters and type in the appropriate number on the *Threshold* panel.

![Threshold panel](image)

<table>
<thead>
<tr>
<th>Cell Types</th>
<th>FSC-H Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell lines, larger than 20 μm in cell diameter</td>
<td>300,000~1,000,000</td>
</tr>
<tr>
<td>Cells lines, smaller than 20 μm in cell diameter</td>
<td>100,000~300,000</td>
</tr>
<tr>
<td>Fixed or un-fixed, freshly isolated cells (leukocytes, spleen cells, thymocytes)</td>
<td>50,000~200,000</td>
</tr>
<tr>
<td>Platelets</td>
<td>5,000~10,000</td>
</tr>
<tr>
<td>Bacteria</td>
<td>1,000~10,000</td>
</tr>
</tbody>
</table>
Step 4. Draw Plots

a. Click the icon of the interested **plot type** above the workspace

<table>
<thead>
<tr>
<th>Plot type</th>
<th>Number of parameters</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dot plot</td>
<td>2</td>
<td>The intensities of two parameters are represented by the coordinates of an event (one dot) on the plot.</td>
</tr>
<tr>
<td>Density plot</td>
<td>2</td>
<td>The intensities of two parameters are represented by the coordinates of an event (one dot) on the plot with colour-coded density display.</td>
</tr>
<tr>
<td>Contour plot</td>
<td>2</td>
<td>The intensities of two parameters are represented by the coordinates on the plot with contour line to show density.</td>
</tr>
<tr>
<td>Histogram plot</td>
<td>1 (x axis only)</td>
<td>The intensity of a parameter is represented along the x-axis, and the number of events at each intensity value is represented along the y-axis.</td>
</tr>
</tbody>
</table>
b. Create the following plots with the following sequence.
FSC-H VS SSC-H (Mother population of interest) >
FSC-H VS FSC-A (Single Cell Gate) >
Live-Dead VS SSC-A (if applicable) >
Fluorescence Plots (if applicable)
c. To change the parameters of a plot, **mouse over the axis label** and **right-click** to open the drop-down menu of parameters list. Select the parameter of interest.

d. **Right-click** within a plot to change the plot type if needed.
e. To copy all the settings and plots to other samples in Specimen 1, **drag Sample 1 and drop over Specimen 1** on the Experiment Manager.

f. Click **Paste to All**.
Step 5. Save Experiment  
   a. Click **File** on the **Menu** bar.

   ![File Menu]

   b. Click **Save As**.

   ![Save As]

   c. Save the experiment (.ncf or .FCS) in the folder below.  
      **Computer** > **Experiment Data** (*D:* ) > **User** > **Department** > **YOUR FOLDER**

      Click **Save**.
4. **Data Analysis during acquisition**

Step 1. Set the appropriate display range of the plot.

a. Select the FSC-H Vs SSC-H plot (The colour of the header of the plot will be darker). Click **Auto range button** to optimize the data display range.

b. To fine tune the data display range, **click zoom in / zoom out buttons**.
c. **Drag** on the interested region on the plot if you click **zoom in**.

d. **Click within a plot if you click zoom out.** The range increases by 20% of the current range. Click repeatedly until the desired range is reached.

e. To change the scale of parameters, **right click on the coordinate label** to open and select the axis scaling (i.e. Linear, Log or Biexponential). Click **Setting** for more options.
f. If you cannot achieve a desirable range by using the plot range tools, **adjust the Gain** of the corresponding channels in Cytometer Control - Parameters.

To adjust photodetector gain of one parameter, **double click the current Gain number** of the specified parameter, the photodetector gain adjustment slider will show. **Drag the slider bar or directly enter the value** to change the photodetector gain.

*Gain can only be adjusted during acquisition.*
Step 2. Gating
   a. Draw Gates to gate out the target population on the FSC-H VS SSC-H plot with gating tools.

   Dot Plot, Density Plot, Contour Plot – All gates suitable
   Histogram Plot – Range / Bi-range gate suitable
b. To create *rectangular/elliptical/range/bi-range gate*, click the corresponding icon and drag in the plot to enclose the target population within the shape. Release the mouse button to create the gate.

To create *polygonal gate*, click the corresponding icon and left click in the plot to create the first vertex of the polygon. Click in a new location to create the second vertex of the polygon. Continue moving around the target population and creating vertices until the target population is enclosed. On the last vertex, double-click to complete the polygon and create the gate.

To create *quadrant gate*, click the corresponding icon and click in the plot to create the center of the quadrants and create the gate. As shown below, the center, endpoints, and lines of the quadrant gate can be moved to enclose the correct populations.
c. To create gate subpopulation, *right-click at the plot header* of a plot to display a drop-down menu. Select the mother gate and create a new gate for your target.

d. The *Gate Manager* panel displays all gates of the active sample in list mode or tree mode. It provides user interface to modify gate name, color and color precedence and also shows gate hierarchy and gate statistics.
Step 3. Statistics
   a. To edit statistics, click the button on the lower right corner of a plot to expand the plot and display the statistics chart first.

   b. **Right-click within the chart** and select the parameters to hide or display.
5. Data Export
Step 1. Export FCS file
a. Select the sample, specimen, group, or experiment file node with data to be exported in the Experiment Manager Panel. Right-click the node and select Export → Export to FCS Files. The Export Events window will open.

![Export Events window]

b. Choose “All” for the Gate option. Click “…” button next to entry box of Path. Select your saving destination in Experiment Data Drive (D:/) Experiment Data (D:) > user > Department > Your NAME

Select “FSC3.0” for Format and Click OK.
Step 2. Export PDF file (optional)
   a. To Export Plots and Statistics to a PDF, **double-click the Report node** in the Experiment Manager panel and Report Window will popup.

   ![Experiment Manager Panel](image)

   b. Click the **PDF** button of the tool bar.

   ![Report Window](image)

   Select your saving destination in Experiment Data Drive (D:/)
   D:/user/Your department/Your NAME

   c. Click **Save** buttons on the top left-hand corner of the window when you finish your experiment.
6. System Cleaning

a. Place tubes of at least 1mL of cleaning solution 1, 2 and 3 (FACS Clean, FACS Rinse, Milli-Q H₂O).
b. Click File on the Menu bar.

c. Click New > New from Experiment File.

d. If you haven’t stained with PI, then Select Desktop > clean.ncf. Then click OK

e. If you have stained with PI, then Select Desktop > clean-PI.ncf. Then click OK

f. Double click the sample 1, then Click “Run”
g. After running sample 1, double click the sample 2, then click “Run”
h. After running sample 2, double click the sample 3, then click “Run”

7. **Re-use Experiment as template**
   a. Click *File* on the *Menu* bar.

   b. Click *New > New from Experiment File*.

   c. Select your target experiement file (.ncf) . Then click *OK*

   d. Click *File > Save As* to save the new experiment.
8. NovoExpress Software Log out
   a. Click *File* on the *Menu* bar.

   ![File menu]

   b. Click *Logout*