

Experiment Setup on Novocyte Opteon

The typical workflow for setting up an experiment on Novocyte Opteon is described in this guide.

1. Startup

Turn on Computer

- Login to the Operator account (no password).

Check the fluidics

- Empty the waste tank if almost full:
 - Disconnect tubing from tank.
 - Take the tank to the sink and empty it. Add **~300mL of bleach** to the tank.
 - Reconnect the waste tank to the instrument.
- Refill the sheath tank if nearly empty:
 - Disconnect tubing from sheath tank and carry it to sink.
 - Fill the sheath tank with **DI water**. The DI water supply is above the sink.
 - Reconnect the sheath tank to the instrument.



Turn on Opteon

- The Opteon is set to auto start at 8AM.
- If the device is not already on, press the **ON / OFF** button on the front panel.
- **Start NovoExpress** software and **login**. The staff can help with setting up your user ID.

Notes:

- The device needs about **10 minutes to warmup**.
- The status of the instrument can be viewed in the bottom left-hand corner of the software.
- Multiple instances of the software can be started at the same time, but only one can be connected to the instrument. The **Plate Manager** is missing if the cytometer is not connected.

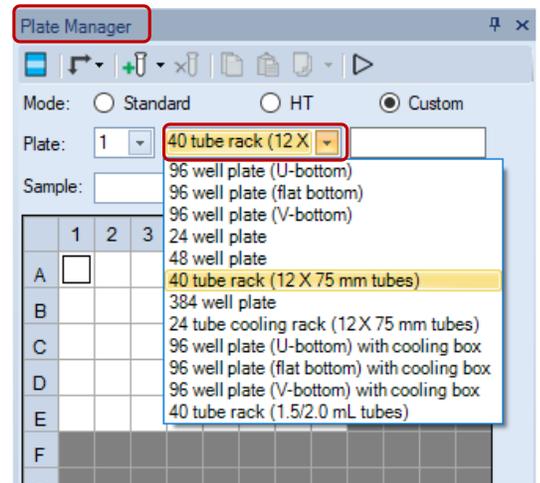
2. Setting Up an Experiment

Creating New Experiment

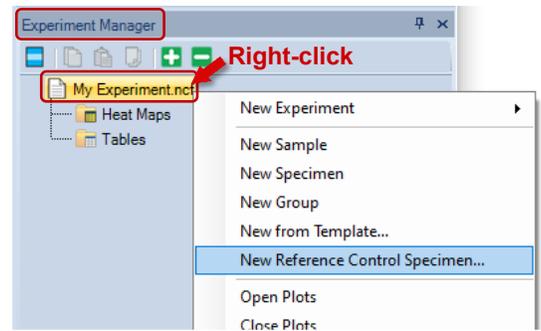
- NovoExpress will automatically open a **Blank Experiment**.
- Alternatively, Click **File** → **New** → **New Blank Experiment**
- To save the experiment, Click **File** → **Save As**, navigate to the desired file location, edit the file name and click **Save**.
- Go to **Plate Manager** and **select a plate** (96-well plate, 40 tube rack etc.)

Unmixing

- *Right-click* the experiment name in **Experiment Manager** and click **New Reference Control Specimen**.
- A new window will open:



- Make sure “**Auto Update Reference Spectra...**” is selected.
- Click **Edit** next to *Single Stained Controls*, select the fluorochromes you need to detect, then click **OK**.
- Additional **Unstained controls** can be added if needed.
- If necessary, modify the **Control type** and **Negative Population** for each single stained control.
- Add **Marker** names.
- Click **OK**.

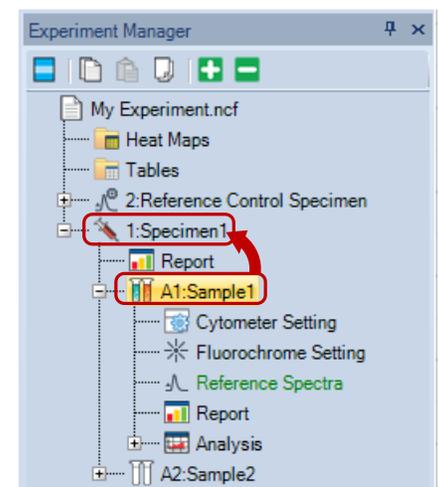
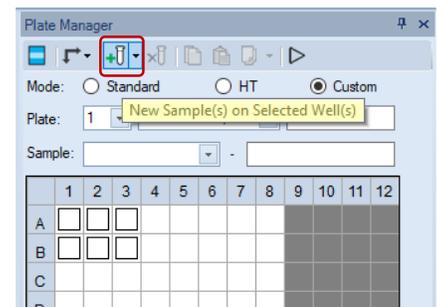


- The position of each reference control in the tube rack or plate is indicated in the **Plate Manager**, in the **Experiment Manager** and in the **Worklist**.
 - Place the reference control tubes in the 40-tube rack to match their position shown in the software.
 - Alternatively, you may rearrange the location of controls in the software:
 - Open the **Work List** (click the  icon in the top-left corner of **Plate manager** or **Experiment manager**) and type the desired location for each control.
 - Or rename the sample to match the position in the **Experiment Manager**
- **Load the rack or plate** onto the stage. The A1 position is marked on the stage.
- **Activate the first Reference Control** to be acquired by double-clicking the sample in the **Experiment Manager** (the red arrow indicates the active sample).
- In the **Cytometer Control**, Set the Flow Rate to **Slow** (14ul/minute).
- Select **Run, Single Well** or **Run Plate** to record controls. Click **Stop Single Well** during a plate run to skip to the next control.
- Under the **Unmix** tab, confirm that **Auto Update Mode** is highlighted.
- Double-click **Reference Spectra** under the Reference Control Specimen in the **Experiment Manager**.
 - The Fluorescent Spectra should be visible in the new window, which confirms that Unmixing was successful.
 - **If there are errors in unmixing calculations**, an error message will be displayed at the bottom of the window showing the reference spectra. *NO WARNING WILL POP UP ON THE SCREEN!*

Add Specimens and Samples

“*Specimen*” means a *group of samples*. The samples in a specimen *may or may not* have identical properties!

- Switch to the **Unmixed Worksheet** to view Unmixed data.
- In the **Plate manager**, *click and drag* to select wells, click the Tube icon  at the top of the **Plate Manager** to add **new samples to selected wells**.
- Samples can be easily repositioned in **Worklist**.
- In the **Unmixed workspace** add an FSC-SSC plot. Add more plots and gates if you wish.
- **Activate the first sample** (double-click) and click **Run**.
- Adjust the threshold, stop conditions, and flow rate in Cytometer Setting.
- **To apply the properties of a sample** (*Cytometer Setting, Fluorochrome Setting, Reference Spectra, Report, Analysis*) to all samples in a specimen, drag-and-drop **the icon of that sample** onto the icon of the **Specimen**.
- **Sample names may be copied from a spreadsheet** (Libre Office/Excel) and **pasted** into the **Plate Manager**.



3. Exporting

- To **export all FCS files** right-click on the Experiment Name and choose **Export FCS Files**.

- To **Save a Template**, right-click on an experimental **Sample** and choose **Export FCS Template**. This template will contain all sample properties without data. The template can be imported into a new experiment.

4. Repeating an Experiment

- Click on **File → New → New from Template →** Navigate to the Exported Specimen FCS Template File (.nct) from original experiment.
The imported sample template will contain Unmixing, stop conditions, Flow Rate, Threshold, etc.
- Click **File** and **Save As** to save the new experiment.
- In the **Worklist**, you may add or remove samples. You can also change the position of the samples.
- **Run samples.**
 - If addition of Single-Color Controls is required, previous Reference Controls can be added to re-Unmix with the new Reference Data:
 - Open the Unmix tab and choose New Reference Control Specimen.
 - At the top of the window that appears click the box, “**Import samples from FCS files**”
 - **Navigate** to the folder that contains Reference FCS files and choose the Raw files.
 - Add any new reference controls by clicking the Edit button in the Single Stained Control section.
 - Click **OK**.
- Click **Auto Update Mode** in the Unmix toolbar to automatically Unmix after collecting any new data.
- **Drag the updated Reference Spectra** onto the appropriate Specimen(s) to apply Unmixing to all the samples.

5. Cleaning

Each user must clean the instrument after finishing running samples.

- Select 2 empty wells in the **Plate Manager**. Right-click and select **Batch Creation of New Specimens**.
- Select Import Specimen Template.
- Navigate to find the **Cleaning Template** file located on the Desktop.
- A Specimen with 2 samples will appear in the Experiment Manager.
 - Load a tube with **~2 ml Contrad** and a tube with **~2 ml DI water** on the tube rack.
 - The position of each tube is indicated in the **Experiment Manager**.
 - Click Run Plate and then highlight both cleaning tubes.

6. Shutdown

- If you finish running samples **after 5 PM, check the iLab calendar** to see if you are the last user of the day.
- If you are the last user, shutdown the instrument:
- Press the **ON/OFF button on the front panel** to shut down the Opteon.
 - Alternatively, in the **Instrument** tab, click **Shut Down** and follow instructions. **DO NOT** check “**Clean sample injection probe**” box. The Flow Core staff is responsible of cleaning the sample injection probe.

A checklist containing the steps described above is available on the desktop of the PC connected to Opteon.