Start-up and shutdown of Quanteon/Advanteon for experienced users

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1. Check the **<u>sheath fluid</u>** container, the **<u>NovoRinse</u>** solution container and the **<u>NovoClean</u>** solution container to make sure there are enough reagents inside. If the reagent level is below 1/3 capacity on the container, add more reagent to the containers.

2. Check the **<u>Waste</u>** container. If the waste container is more than 2/3 capacity, empty the waste container, and add around 200 mL of **bleach** to the waste container.

3. Gently press the power switch on the front of the Quanteon/Advanteon flow cytometer to turn on the instrument. The power switch light should turn green, indicating that the instrument has entered the initialization process.

4. Turn on the computer, the passwords for the computers were labelled at the bottom of the screen. When ready, double-click the NovoExpress software icon on the desktop to start. When prompted, select your username and log in.

5. The initialization process takes several minutes. After completion, the status bar will display ● *Ready*.

6. Instrument Quality Control

   6.1 Prepare QC beads for QC test:

   Check from the fridge whether there is still some QC beads solution ready for use.

   If there is not enough QC beads solution (less than 50 μL), please prepare it by yourself.

   i) Label a 12×75 mm tube for the QC test with "<u>QC beads, lot number and date</u>". Add 0.5 mL dilution buffer (**0.4 mL NovoFlow and 0.1 mL NovoRinse**) into the tube.

   ii) Take the NovoCyte QC Particles out from the fridge. Invert the bottle a few times by hand and vortex the bottle to mix the particles thoroughly.

   iii) Add one drop of the QC particles into the flow tube with dilution buffer.

   iv) Put the NovoCyte QC Particles stock bottle back to the fridge immediately.

   v) Vortex the test tube to mix the QC particles thoroughly.

   vi) Place the tube onto the sample holder of the instrument from Advanteon BVYG (A1 position in the rack from Quanteon/Advanteon BVR).

6.2 QC test: Click *Instrument* → *QC Test* to initialize the QC test.

6.3 Check the QC particles Lot ID which is consistent with the QC bottle you collected.

6.4 Click *Next* and then click *Run.* The instrument will collect the electronic noise, optical noise and events. This step takes about 4 minutes.

6.5 After the data collection is completed, click the *Report* button to view the test results.

The QC test provides a result for each tested parameter. There are three possible results.

> ➤ *Pass*: The parameter meets performance requirements.

> ➤ *Failed*: The parameter does not meet the performance requirements.

> ➤ *Acceptable*: The parameter does not meet the factory calibration requirements, but the use of the instrument does not affect experimental results.

6.6 **Troubleshooting on the QC test fails**:

Possible Causes	Recommended Solutions
Last user might not wash the instrument properly	Try to run a routine wash step using **Bleach**, **Rinse** and **MQ water** for 5 mins/tube, and then re-run the QC test.
QC particles are expired or not stored appropriately	Try to prepare a new tube of QC particles and mix them well to run the QC test again.
Flow cell is dirty	Try to execute *Rinse (30 s)* or *Unclog (2 mins)* function.
Air bubbles exist in the flow chamber	Try to execute *Debubble (5 mins)* function.
Flow cell is dirty	Try to execute *Cleaning (13 mins)* function.

7. Instrument Shutdown

7.1 Turn off the NovoCyte Quanteon/Advanteon instrument.

Gently press the power switch on the front panel of the instrument. During the shutdown process, the power switch light will be flashing green, and the instrument will automatically perform the shutdown cleaning process. After the shutdown process is completed, power is automatically turned off and the power switch light goes out.

7.2 Turn off the computer.

7.3 When the power is automatically turned off and the power switch light goes out, please fill in the NovoFlow buffer (dilute 100× stock solution with autoclaved MQ water).

7.4 Empty the waste container and add around 200 mL bleach into it.

Appendix 1: Add Instrument Reagents and Empty waste during your operation

1. Make sure the instrument is in *Ready* status.

2. Disconnect the tubing from the corresponding container by gently pressing the metal clip of the quick coupler on the cap.

3. Move the NovoFlow, NovoClean or NovoRinse container from the fluidics station onto the bench. Unscrew the container cap and add more reagents to it. Move the Waste container from the fluidics station to the sink, empty the waste container and add around 200 mL bleach into it.

4. Screw the container cap back onto the container.

5. Reconnect the quick coupler on the cap and gently place the container onto the fluidics station. Be careful to prevent any twisting or kinking of the tubing.

6. If the instrument is in *Ready* status, click the *Instrument → Fluidics Maintenance → Priming* on the NovoExpress Software to prime the fluidic system before running any samples.
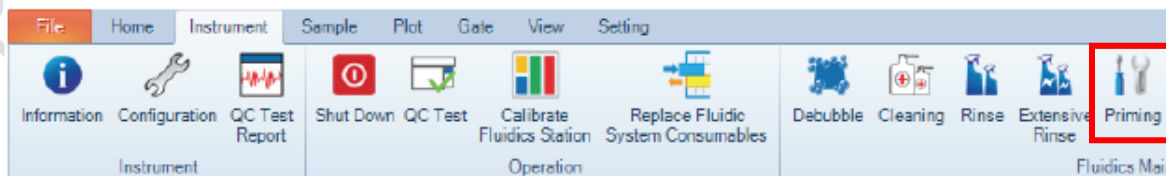
Appendix 2: Fluidic system consumables

The NovoCyte flow cytometer monitors the accumulated running time of the fluidic system consumables to ensure the consumables are changed in a timely manner for optimal flow cytometry results. When the accumulated running time is reached, the NovoExpress software will prompt a message to remind the user to replace the consumables (Figure 1). Similar message will be prompted when the last replacement is 60 days ago as shown in Figure 2.
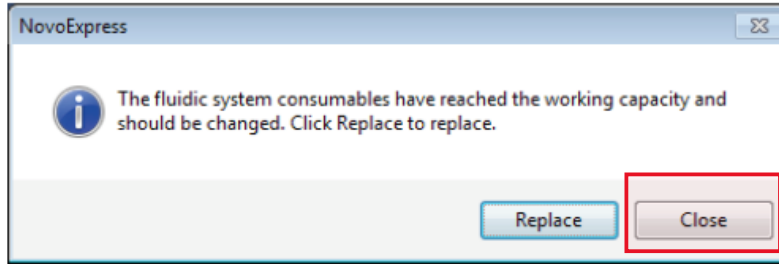

Figure 1. NovoExpress software message for replacing fluidic system consumables when accumulated running time is reached
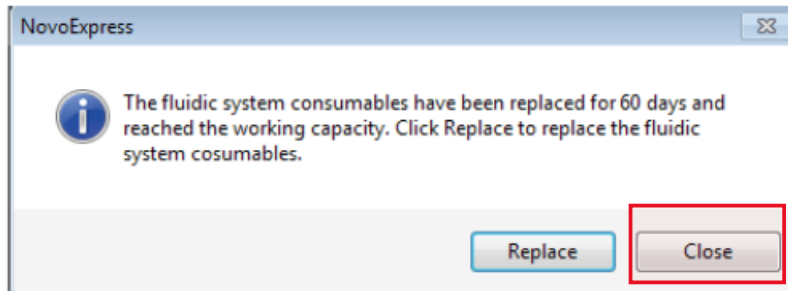

Figure 2. NovoExpress software message for replacing fluidic system consumables when last replacement in 60 days ago

If you see the above messages, please click "*Close*" and leave a message to me. Thanks!

Appendix 3: on Message ID 0x010F

| 0x010F | *Sheath filter is clogged. Please replace the sheath filter and run the Priming procedure* | Sheath in-line filter is clogged |

If you see the above message, please try to execute "*Unclog*" function from the NovoExpress Software.