Start-up operations (check schedules first and modify startup accordingly):

1. Start Agilents (Quanteon 1,2 and Penteon)
   Daily –
   a. Check fluidics cart (NovoFlow, Waste, NovoRinse, NovoClean) and fill or empty as needed
   b. Turn on power to the cytometer (green button on left side of instrument)
   c. In NovoExpress software, go to **Instrument** tab and click **Fluidic Maintenance Sequences** → select **Startup/QC** (20min)
   d. While machines are cleaning make NovoCyte QC particles: 2 drops in 800uL PBS and 400uL of NovoRinse (working vial found in upper left pull out drawer in black fridge)
   e. After cleaning sequence is completed, click **QC test** → select the appropriate bead lot from the drop down menu
   f. After QC click "**Cleaning**"
   g. Close software and restart PC

   Monthly –

   a. Instead of **Startup/QC** select **Long Clean**
   b. After QC open "**Rainbow test**" experiment. Run single peak beads to test fluidic stability
   c. In the **Instrument** tab select "**Cleaning**"
   d. Close software and restart PC

2. Start the Aurora
   Daily –
   a. Fill sheath tank with MilliQ-water
   b. Empty waste container if full, add 400 mL of bleach before reattaching
   c. Replace water tube on SIT with Hellmanex 1:3
   d. Login into the SpectroFlo software and choose Acquisition from the Get Started menu
   e. Go to **Cytometer** tab and select "**Clean Flow Cell**"
      i. Tube 1 - Hellmanex 1:3
      ii. Wait 15 minutes before moving on to Tube #2
      iii. Tube 2 – MilliQ-water
   f. Select "**Default**" experiment
   g. Click "**Run**" - run MilliQ-water on high for 20 minutes

h. While water is running make tube of SpectroFlo beads: 1 drop of beads in 150uL of MilliQ-water (working vial found in red rack in butter drawer in door of black fridge)
i. After water – go to **QC & Setup** tab
j. Load tube of QC beads and hit "**Start**"
k. After QC, perform "**Fluidic Shutdown**" → found under **Cytometer** tab

Monthly –

a. Perform "**Long Clean**" under **Cytometer** tab
    a. Replace filter with bypass tubing (found in third drawer under Aurora)
    b. Remove tube from sheath container and take filter off the end of tube.
    c. Place sheath tube into 10% bleach solution (2L)
    d. Hit start in "**Long-Clean**" and follow software wizard instructions
    e. Go through entire "**Long Clean"** with bleach solution → do not switch to water
    f. After entire "**Long Clean**" is done. Put filter back on to end of sheath line and put back into sheath container.
    g. Repeat "**Long Clean**" but with all steps/fluids consisting of MilliQ-water
    h. After second "**Long Clean**" is finished, remove bypass tubing and reinstall the filter
b. Run a tube of MilliQ water (**Default** experiment) for 20 minutes on high
c. Perform QC per "**Daily**" protocol

3. Start the Attune

Daily –

a. Check fluids (Waste, Focusing fluid, Wash solution, Shutdown solution)
b. In AttuneNxT software, go to **Instrument** tab and click **Instrument Startup**
c. After startup, load a clean dry 96-well plate into autosampler and a tube of 3mL Attune Wash solution (squeeze bottle on top of Attune) onto the SIT.
d. Go to **Instrument** tab and select "**Quick Wash**"
e. While machine is cleaning make Attune Performance QC particles: 3 drops in 2mL of PBS (working vial found in upper left pull out drawer in black fridge)
f. After cleaning sequence is completed, click **Performance Test** → select the appropriate bead lot from the drop down menu
g. After performance test load a tube of 3mL Attune Wash solution, a clean dry 96-well plate into the autosampler and click "**Quick Wash**"
h. Close software and restart PC

4. Tyto (microfluidic sorter):
   Daily –
   a. Wipe down counters with 70% EtOH
   b. Restart computer

5. Bigfoot (droplet sorter in BSL2+ suite):
   If a sort is scheduled –
   a. Check bulk fluids (NERL sheath, Waste, MilliQ-water, cleaner, decontamination)
   b. Sonicate the nozzle needed for the first sort – at least 20min
   c. While nozzle is sonicating:
      i. clean salt out of flow chamber, off charge plates, pinhole and waste catcher with MilliQ-water
      ii. clean mirrors and back plate with Windex
      iii. wipe down all surfaces with 70% EtOH
   d. Install sonicated nozzle and click "**Startup**" in software
   e. Let sheath run for 30 minutes then complete startup steps (droplet maintenance, stream setup, QC and drop delay)

   If no sort is scheduled – every Monday, Wednesday and Friday
   f. Check bulk fluids (NERL sheath, Waste, MilliQ-water, cleaner, decontamination)
   g. Before clicking startup:
      i. clean salt out of flow chamber, off charge plates, pinhole and waste catcher with MilliQ-water
      ii. clean mirrors and back plate with Windex
      iii. wipe down all surfaces with 70% EtOH
   h. click "**Startup**" in software
   i. Let sheath run for 30 minutes then complete startup steps (droplet maintenance, stream setup, QC and drop delay)
   j. Shutdown ("**Quick**")