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

1. Start FACSymphony A5 (ORAC)
    a. Turn on power to the cytometer (green button on right side of instrument)
    b. Open the UV laser control software (Coherent Connection) and turn on the UV (355 nm) laser. Set power to 60mW – see SOP for detailed instructions)
    c. On the FACSFlow Supply system, exchange the sheath (FACSFlow) for a new tank if sheath level is low (spare FACSFlow is found to the left of the instrument)
    d. Empty the waste tank if full or near full and add 500 mL of bleach before installing back on FACSFlow Supply system
    e. While machine is in RUN and MED, bleed any air out from the bubble filter. Allow the machine to run water for ~2 minutes
    f. The A5 takes ~20 minutes to warm up
2. Start the Cytoflex (The Hooded Claw)
    a. Turn on power to the cytometer (black power switch on back of instrument)
    b. Fill Sheath Fluid (only use Cytoflex Sheath Fluid or dH20 if no Cytoflex Sheath available – NO PBS!)
    c. Empty Waste Container if full and add 400 mL of Bleach.
    d. In the CytExpert software, click **Cytometer** → **Initialize**
    e. Select **Cytometer** → **System Startup Program** (8 minutes).
        i. In the software plate settings window, set wells A1 – A3 as empty wells and set wells B1 – B3 as DI water wells
        ii. Fill wells B1-B3 of the daily clean plate with DI water and load the plate on the instrument
        iii. Click **Start** in the plate settings window and click **OK** to confirm
3. Start the Aurora (Zen)
    a. Turn on power to the cytometer (round silver button on left side of instrument)
    b. Exchange sheath tank for a full tank (spare sheath tanks with MilliQ water are found to the right of the sink)
    c. Empty waste container if full, add 400 mL of bleach before reattaching
    d. Login into the SpectroFlo software and choose **QC & Setup** from the **Get Started** menu
    e. The Aurora takes ~20 minutes to warm up
    f. While warming up refill any empty sheath tanks with MilliQ water

While the analyzers warm up, begin starting up the Aria:

    a. Disconnect the waste tank and empty the waste in the sink while running water, taking care to keep the connectors from getting wet. Reconnect the empty waste tank.
    b. Detach the air (clear) and fluid (blue) lines from the EtOH tank and move them out of the way by draping to the right of the cart, spray with 70% ethanol
    c. Disconnect the sheath tank and replace with a new, full one
        - Detach the sensor from the cart (black cord leading from the sheath tank to the cart) and place the old, depleted sheath tank on the floor
        - Using the wrench, loosen the probe on the old tank and without touching it (in order to maintain sterility), spray with ethanol and wipe down with kim wipe, transfer it to the new tank

- Hand-tighten the nut that secures the sensor and then use the wrench to tighten (do not overtighten)
- Hand-tighten the vent

d. Spray the connector of the blue fluid line with EtOH and transfer it to the new sheath tank
e. Do the same with the connector on the air line
f. Turn on the machine's main power using the green button on left side of cytometer (ensuring the white "laser" button is also illuminated) and the power strip Cooling Unit. Turn the UV Laser power strip to the right of the instrument.
g. Open Coherent Connection
h. If all lasers do not show up on their own, "Scan for all lasers"
i. Once all three lasers are displayed, stop the 355nm UV laser, set the power to 50mW, and restart that specific laser
j. Open FACSDiva software and login as Administrator (no password required)
k. Fill a 5 mL FACS tube with 3 mL of sterile DI water using the syringes to the right of the instrument
l. Once sorter is connected, go to **Cleaning Modes** → **Clean Flow Cell** and run this function a total of 3 times
m. After cleaning the flow cell, open the Flow Cell Access door, remove the Q-tip, dry the area with another Q-tip, and insert the closed loop nozzle
n. Go to **Cytometer** → **Fluidics Start-Up**
    - Fluidics start up takes ~20 minutes
o. Fill up the 70% ethanol tank (70% ethanol can be found in the yellow flammables cabinet outside the core main entrance)
p. Fill up Aria sheath tank with PBS (from lab carboys), attach autoclave tape and autoclave (see Autoclave SOP)

Back to the analyzers:

NOTE: Always use beads from the working vial and not the backup vials. The working vials are in the first square compartment from the front in the drawer of the fridge

1. Using the BD CS&T beads (orange label, cat #642412), prepare the beads and start the QC procedure following the FACSymphony CS&T SOP
2. Using the Beckman Coulter beads (cat #B53230), prepare the beads and follow the Cytoflex QC and Standardization SOP
3. Using the SpectroFlo beads (cat #97-30310-01), prepare the beads and follow the QC & Setup SOP

After the Aria pauses the Fluidics Start-Up:

q. Visually inspect the 85-micron nozzle under microscope for blockages
    - If dirty, fill a FACS tube with 3 mL sterile DI water, cap tightly and sonicate for 30 sec.
    - Dump the tube of DI water containing the nozzle on the counter on a large Kimwipe, taking care to keep away from the sink

- Visually inspect the nozzle on the microscope again to confirm blockages are cleared
- Turn off microscope and discard Kimwipe and FACS tube in biohazard waste

r. Replace the closed loop with the clean 85-micron nozzle
s. Finish the Fluidics Start-Up
t. Close the Flow Cell Access door

Run CS&T (cat#655051) on the Aria ~ 1-1.5 hours and Accudrop (cat#345249) ~30 min. before first booked sort

Head to Winship C-5027

4. Start the Aurora (Avon)
    a. Turn on power to the cytometer (round silver button on left side of instrument)
    b. Exchange sheath tank for a full tank (spare sheath tanks with MilliQ water are found to the right of the sink)
    c. Empty waste container if full, add 400 mL of bleach before reattaching
    d. Login into the SpectroFlo software and choose **QC & Setup** from the **Get Started** menu
    e. The Aurora takes ~20 minutes to warm up
    f. While warming up refill any empty sheath tanks with MilliQ water

5. Start FACSymphony A3 (Liberator)
    a. Turn on power to the cytometer (green button on right side of instrument)
    b. Open the UV laser control software (Coherent Connection) and turn on the UV (355 nm) laser. Set power to 60mW – see SOP for detailed instructions)
    c. On the FACSFlow Supply system, exchange the sheath (FACSFlow) for a new tank if sheath level is low (spare FACSFlow is found to the left of the instrument)
    d. Empty the waste tank if full or near full and add 500 mL of bleach before installing back on FACSFlow Supply system
    e. While machine is in RUN and MED, bleed any air out from the bubble filter. Allow the machine to run water for ~2 minutes
    f. The A5 takes ~20 minutes to warm up

NOTE: Always use beads from the working vial and not the backup vials. The working vials are in the first square compartment from the front in the drawer of the fridge

1. Using the BD CS&T beads (orange label, cat #642412), prepare the beads and start the QC procedure following the FACSymphony CS&T SOP
2. Using the SpectroFlo beads (cat #97-30310-01), prepare the beads and follow the QC & Setup SOP