1. Ensure UV laser is powered to 60mW and instrument has warmed up for at least 20 min.
2. Replace sheath tank if fluid is low and discard waste if full or nearing full
3. Make up SpectroFlo beads (current lot #)
    a. Add 500 uL of PBS to FACS tube
    b. Vortex bead bottle and add 2 drops to FACS tube
4. In Diva, make a new experiment from a template:
    a. Open the NANT/ANBL folder in the Diva browser
    b. Go to **Experiment** (top menu bar) > **New Experiment**
    c. In the Experiment templates dialog, choose **Flow Core Contracts** tab
    d. Select the **NANT** template and click **OK**
5. Double click to open the experiment that was created in the Browser and rename it based on the tube labels (not the cytometer acquisition date):
    NANT: **CHLA-19-D1_07Jul2020**
    ANBL: **ANBL1821_852238_C2D1_21Nov2020**

    *Note:* If two separate samples are being run on the same day e.g., multiple ANBL1821 samples, multiple NANT2017 samples, or a combination of ANBL and NANT samples, then a second experiment will be created AFTER the first sample set is acquired (see step 12)

    *Note:* If running multiple samples on the same day: pre and post SpectroFlo bead files, single stain controls, unstained beads control, and the unstained cells control need only be run under the first experiment created; the files for these controls can then be copied and added to any additional experiment folders following acquisition

6. Rename the SpectroFlo specimen to reflect the date the experiment was acquired on the cytometer, i.e., not the collection date: **SpectroFlo_07Mar2020**
7. Rename the experimental specimen to reflect naming on the tube labels:
    a. The date should reflect the date on the tube label, not the date acquired on the cytometer
    NANT: **NANT2017_CHLA-19-D1_07Jul2020**
    ANBL: **ANBL1821_852238_C2D1_21Nov2020**
8. Rename the Control specimen to reflect the date on the tube label:
    NANT: **NANT2017_control_07Jul2020**
    ANBL: **ANBL1821_control_21Nov2020**
9. Adjust bead MFI (median fluorescence intensity) target values
    a. In the browser (left hand side of screen), expand the SpectroFlo specimen and select the arrow to the left of the "**pre**" tube
    b. Ensure you are looking at the "SpectroFlo" **Global worksheet**
    c. Confirm the correct bead lot and acquisition date is designated in the tube name
    d. Vortex the FACS tube with diluted beads and install on the SIP
        i. Set instrument to **RUN**
        ii. Adjust coarse flow rate to **MED**
        iii. Confirm fine adjust flow rate is set to **250**

iv. Select **Acquire** in acquisition dashboard

e. Adjust PMT voltages to achieve MFI in target range across all detectors for fluorochromes in the experiment
   i. **Record** the "Pre" tube under the SpectroFlo specimen and save a file of 10,000 total events
   ii. **After acquisition is finished, confirm all target MFIs are still within range. These are the voltages all remaining tubes will be recorded at.**
   iii. If any fluorescent channels are out of range, take note and make adjustments to voltages while acquiring the SpectroFlo beads again. **Record and append** over the original bead file and confirm all target MFIs are within range before moving on to compensation controls.
   iv. Check PMT voltages for consistency across all tubes in the experiment. All tubes in the experiment should have the same voltages as the SpectroFlo Pre bead file.

10. Record controls
    a. Expand the **Compensation Controls** specimen
    b. Switch to the **Normal Worksheet** view and navigate to the tab for the unstained control
    c. Record each control

    *Note*: All single stains are compensation **beads** except the BB515 single stain, collected in the B 515/20 detector, which is composed of **cells**
       i. Install the first compensation tube on the SIP and adjust the P1 gate in the FSC vs. SSC plot while acquiring to include the bead singlets.
       ii. Right click on the P1 gate and select **apply to all compensation controls**
       iii. Collect 10,000 events for the unstained bead control
       iv. Install the BB515 compensation tube on the SIP and readjust the P1 gate in the FSC vs. SSC plot while acquiring to include the lymphocytes. Confirm BB515 staining and collect 50,000 total events for BB515 single stain cell control
       v. Collect 10,000 total events for each single stain bead control
       vi. Install the FVS 620 bead single stain and adjust the P1 gate in the FSC vs. SSC plot while acquiring to include the bead singlets. Collect 10,000 total events in the FVS 620 single stain control tube
       vii. Switch back to the Global worksheet view and select the "Sample gates" worksheet
    d. Record 20,000 total events for the unstained cell control under the **Control** specimen
    e. Record the fully stained PMBC control sample until either the acquisition limit is reached or when the tube has a small volume remaining (~20 uL).

11. Record experimental samples

    **NOTE:** Cell numbers in tubes may be too low to acquire the full number of events set to record in each file. Run the tube and record the data until there is ~20 uL remaining.
    a. Record the 3 FMO tubes:
       i. CD161 BUV661
       ii. CD226 BUV493
       iii. CD314 BUV395
    b. Record the fully stained sample ("panel" tube)

12. If only one study is being run, skip to step 13. If samples from a second study are being run, create a new experiment:
    a. Right click on Experiment name
    b. Select **Duplicate without data**
    c. Rename the new experiment
    d. Rename the experimental specimen with the correct study name, sample ID, timepoint, and collection date based on tube labels
    e. Rename the control tube if an additional PMBC control sample is available
    f. Record PMBC control panel tube (if available), FMO tubes, and experimental sample
13. Switch back to the SpectroFlo worksheet, and record the SpectroFlo beads "post" file (10,000 events)
14. Clean the instrument by running and recording the following:
    i. Bleach tube (3 min. on HI)
    ii. Clenz tube (3 min. on HI)
    iii. Water tube (3 min. on HI)
15. Leave water tube on SIP and put instrument back to **LO** and **STANDBY**
16. Check the instrument schedule to determine whether to shut down the cytometer
17. To shut down, make sure water is on the SIP, instrument is in **STANDBY**, and press the green button on the right-hand side of the instrument
18. Export and transfer data
    a. Ensure the experiment is open (double-click to open the experiment – open book icon should be visible)
    b. Right-click on the experiment name and go to **Export** -> **FCS files**
    c. Confirm save location (Navigate to the D drive -> BDExport -> FCS)
    d. Click **OK** to export files
    e. If multiple patients were run on the same day, open any additional experiments run and export to D drive as in steps 1-4
    f. Navigate to the exported folders:
        i. Go to **Desktop** -> **FCS folder shortcut**
        ii. In the Windows file explorer, sort on the "Date Modified" option so that the most recently modified folders are at the top of the list
        iii. Your manually exported folders should appear at the very top
    g. Transfer each of the exported folders to OneDrive into the appropriate folder

       **NANT**: \Rae, Aaron - PWFC - Emory Pediatrics Winship Flow Core\PWFC - Flow Core Contract Work\
       **ANBL**: \Rae, Aaron - PWFC - Emory Pediatrics Winship Flow Core\PWFC - Flow Core Contract Work\

    h. Confirm all the files transferred
    i. Log out of OneDrive and close the web browser
    j. Log out of DIVA and log out of PPMS
    k. Fill out acquisition information for the samples in the Contract Work Billing Log, found here:

**\Rae, Aaron - PWFC - Emory Pediatrics Winship Flow Core\PWFC - Flow Core Contract Work\Contract Work Billing Log**

l. Make any notes about the sample prep or acquisition on the Information Log, found here:
**\Rae, Aaron - PWFC - Emory Pediatrics Winship Flow Core\PWFC - Flow Core Contract Work\Contract Sample Information Log**