**Starting up Helios system**

*The backflush and heating up system (#1,2) can be done ahead (1-2 hours) of turning on the plasma. This ensures good washing of the system*

**1.Backflushing the system:**

* **Sample probe line/Sample line:**  Fill a syringe with MilliQ water and attach the luer adapter and square connector nut. Disconnect the blue ferrule of the PSI sample line from the grounding nut. Connect the free end of the PSI sample line to the syringe. Open the handle of the Sample Loader and place a clean 5 mL tube into the sample holder/use the old tube that was there. Using the syringe, carefully inject water through the luer adapter. Steady pressure will be required to create adequate flow into the tube. Continue to push until a few droplets have come out of the sample probe line.
* **Grounding Nut :**  Connect the syringe with a luer adapter to the back side of the grounding nut. Gently, inject water through the grounding nut, the flush direction should be toward the instrument-opposite of sample flow- to remove debris. The stream should be straight; if bent, continue flushing until it straightens.
* **Sample capillary:** connect to syringe with luer and square connector nut and push the water through.

**2.Heating the system :**

* Fill 5 mL filter-capped FACS tubes with fresh Tuning Solution, Washing Solution, MilliQ water, and 3% Nitric Acid. Do not fill with pipettors, only by pouring.
* Log In in the Fluidigm software as administrator, no password.
* At the bottom of the screen, turn on the Heater. Open Control panel and Status panel.
* Turn on argon and turn sample introduction on. Place the capillary and let fresh wash solution drip. *This step helps to wash the system before startup, to reduce clog possibility.*
* Check PSI. Should be 3.5-4.3 psi range. If much higher- try to troubleshoot:

First, disconnect the nebulizer capillary from the grounding nut and check the pressure. Without a nebulizer capillary line attached, the pressure should be 2.7-2.9 psi. If it is OK-the problem in capillary, backflush it again and connect and run nitric acid for 3-5 min.

If the pressure is high without capillary: Backflush PSI Sample line again from the blue ferrule, then try to run nitric for 5 -10 min. If not, disconnect the PSI Sample line from the connection to bottom of the flow cell and backflush the PSI Sample line to remove the clog. Assemble all back, run nitric again and then put a Wash Solution tube.

**3.Plasma Start Up**

**Nebulizer:**

* Wash the nebulizer - inspect the 5% citranox and MilliQ water in the tubs for particles. If cloudy or visible particles, dump the affected solution and refill from the stock containers.

Next, connect the tubing syringe to the nebulizer and backflush from the gas port (sidearm with plastic connecter) and sample port (vertical, smaller port) with 5% citranox. Then, fill tubing syringe with 5% citranox and forward flush through the gas port only. Observe the spray: it should be a single, solid stream with no angle to it.

If there is an angle to it, repeat citranox backflushing a few times then inspect under the microscope to check for debris.

Then, repeat backflushing and forward flushing with MilliQ water to remove the citranox. Use the syringe to try to pull any excess water from the gas port.
* Connect the nebulizer to the gas line (be sure to tighten it!) and press “Nebulizer gas ON” on the Control panel. This should remove excess water from the tip of nebulizer as a stream.
* Connect capillary to the sample port of the nebulizer.
* Press sample introduction ON, Neb Gas ON, and observe the spray against a dark surface (works well with blue nitrile gloves). The spray should be fog-like, and evaporate almost as fast as it’s generated.

If you notice larger droplets or uneven sample flow, this can be a sign of a partially clogged nebulizer and you may need to repeat the backflushing.
* Turn off sample introduction and Neb gas. Insert the nebulizer into the nebulizer adaptor port in the spray chamber. Loosen the nebulizer adaptor port a half-turn counter-clockwise, insert the nebulizer into the nebulizer adaptor port until it reaches a hard stop with the gas port arm against the adapter port screw, and tighten clockwise a quarter-turn to secure the nebulizer into the adapter. Make sure that it is snug so that there will be a good seal and the nebulizer won’t slip out during your run.

**Plasma ignition:**

* Verify that the system is almost heated up (>180C).

- It is possible to ignite plasma below 180C, but you will not be able to start sample introduction until above 180C. It is not recommended that the nebulizer is inserted into the spray chamber port without liquid introduction, as this can “bake” material onto the tip of the nebulizer and cause clogging. Therefore, do not start plasma until the Heater has reached >180C.

* Click the **Start** button in the bottom panel of the Helios software.
* When the insert nebulizer dialog box appears, click **OK**.
* This will turn on the heater and begin to heat up as well as turning on the plasma in the system. The **Start** button will turn yellow after a few seconds.
* The start up process takes about 3 min. You can hear a pop and see a flash of light (look on spray chamber connection with injector) when plasma ignites.
* *Please ensure that the instrument is not left unattended during the plasma startup sequence. User intervention may be required to address instrumentation issues.*
* When the plasma startup has completed, a pop-up appears. Click **OK**.
* When the heater reaches approximately 200C the Startup button will turn green, Ready will appear, and the temperature field will turn blue when it has reached 200C.
* After ignition, the plasma will need ~20 minutes to reach full stability and temperature. Start sample introduction again and continue running Wash Solution.

**Tuning:**

* Open “Rain plot” window and click sample introduction on. Run 1-2 min then preview as TOF/Masses to see the metal signals. Check contaminations-Ba138, I127,(Pb208?) Run 5 min, preview.
- you should also see the Zirconium (Zr) impurities in the Wash solution, near the left side of the screen in TOF mode. These should be relatively unbroken.

If too contaminated with metals like Pb, I, or Ba, run 3% nitric. After 10-15 min total, switch to Tuning Solution and run for 5min. Preview again to see the Tuning metals. Again, the Tuning Solution metal streaks should be solid/unbroken, and quite dark relative to all the other streaks.
* Click the **Tune** tab in the menu panel section of the software. Make sure that “Full Tuning Protocol” is selected. Click preview in the **Tuning Acquisition** window to ensure that tuning solution is injected properly before starting the tuning protocol The Masses Per Reading “rainbow” plot window will appear in the workspace with Preview . Run for 20 seconds or until the lines have stabilized to a plateau. Take a look on the lines, if they are too wavy it might be a nebulizer clog/gas seal issues. Maybe the signal level too low (usually the numbers should be close to previous day/run from the notebook).
* Click **Record** anytime during Preview to start the Tuning protocol.
* A progress bar appears to indicate the progress of Tuning procedures. The Full Tuning protocol takes approximately 15-17 min.
* Click **OK** in the dialog box that appears when the Tuning procedure (calibration) is complete.
* Click the **Results** button at the bottom of Tuning Sequence to access the Results tab in the Tuning Manager window to access the report generated at the end of the tuning procedure.
* Preview the report. If PASSED, check the parameters-Tb/Tm if they are in +/-10% range of the previous run.

Tb/Tm Signal Intensity failure:
HIMC standard (not software failure): Tm and/or Tb more than 10% lower than previous Tuning - run tuning again.
Software standard: Tb <700K Dual. The HIMC considers this to be far too low.

Software failure:
%RSD - probably clog in nebulizer. Try again or/and replace new nebulizer.
Oxide ratio failed - “Software bug”, just run again.
Dual slopes - usually fails early in the Tuning protocol steps. This is usually caused by very low signal intensity due to Detector Voltage, Current, Neb Gas, or Makeup Gas being very different than before.

Makeup Gas and Nebulizer Gas values should be pretty similar to values from previous Tuning (0-0.02 difference). If the difference is very large (>0.02 difference NG, >0.05 difference MG), then there is probably a seal problem somewhere and it should be corrected before running samples (even if Tuning passed).

**4. Sample running:**

* If Tuning passed, put Washing Solution in the PSI and run for 10 min or until the Tb/Tm streaks are gone when you run Preview.
* Open your template, define file storage directory, name, cell events to be collected.
* Then run 5-10 min of MilliQ water to wash out the Wash solution before running samples. The Zr streaks should be gone before you start running your samples.

The system is ready!!