

May 08, 2020

## Imaging Mass Cytometer Tuning

DOI

<https://dx.doi.org/10.17504/protocols.io.bf2gjgbw>

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**DOI:** <https://dx.doi.org/10.17504/protocols.io.bf2gjgbw>

**Protocol Citation:** Michelle Daniel, Marda Jorgensen 2020. Imaging Mass Cytometer Tuning . **protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.bf2gjgbw>

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** May 05, 2020

**Last Modified:** October 02, 2020

**Protocol Integer ID:** 36648

**Keywords:** tuning of an imaging mass cytometer, imaging mass cytometer tuning, imaging mass cytometer, tuning process, tuning, imaging, adjustment of the laser energy, laser power optimization, detector voltage optimization, data acquisition, imc, sop in order, laser energy

## Abstract

This SOP describes the tuning of an Imaging Mass Cytometer (IMC). The tuning process encompasses parameters such as Interface alignment, gas flow rate optimizations, laser power optimization and detector voltage optimization. All these factors aim at having a machine that performs equally over time.

The machine needs to pass specifications listed in this SOP in order to be used for data acquisition.

Machines do not need to be tuned daily but at least twice per week and upon every startup.

Additionally, the adjustment of the laser energy is described in this SOP, which needs to be performed every 3 weeks.

## Materials

### MATERIALS

 IMC (software 7.0 and higher) **Fluidigm**

 3-metal tuning slide **Fluidigm**

## Before start

**Ensure a sufficient supply and pressure of argon and helium gases prior to starting.**

## Start plasma and insert tuning slide

- 1
  1. If the machine is running, check the "tuning log sheet.ods" and get the date of the last tuning. If the last tuning was done more than two days ago proceed to step 3.3.
  2. Check argon supply and pressure (5 bar). Make sure that one of the argon tanks has at least 25% which will last overnight. Get a new tank if required from the shop in the basement using the PanGas pay card.
  3. Check Helium pressure (2 bar). Check the Helium bottles in the cabinet. If the pressure drops below 100 bar open the second bottle and close the one that was open. Move the empty bottle in front of the room and hang a sign "Empty" on it. Inform Esther Danenberg or Stefanie Engler to order a new bottle.
  4. Open the CyTOF software on the computer and log in with your name and password.
  5. Insert the tuning slide into the Hyperion.
  6. Start plasma.
  7. Wait for at least 30 minutes until you proceed with tuning.

## Adjust Eref

- 2
  1. Confirm that "Adjust E-ref" needs to be performed. If not, proceed to tuning.
  2. Hit "Settings" under Manual Tune.
  3. In the camera window move to an empty region next to an ablated region (right side).
  4. Hit "Get Current Coordinate" in the Manual Tune Settings.
  5. Copy the values of the Ablation Energy Range to the tuning log sheet.
  6. Hit "Adjust Eref".
  7. Copy the new values of the Ablation Energy Range to the tuning log sheet.

### Note

This process needs to be done every 2-3 weeks. Therein the laser energy is adjusted to guarantee optimal ablation with respect to decreasing laser power output over time. Check the tuning "tuning log sheet.ods" on the IMC computer for the last time this was performed.

## Tuning

- 3
  1. Hit "Auto Tune" under Instrument Control.
  2. Hit "New" to create a new tuning template.
  3. In the camera window move to an empty space next to previously ablated regions (right side).
  4. Hit "Get Current Coordinate" in the Auto Tune window.
  5. Under Parameters check "QC Report", "HTI Current/Gases Calibration" and "Transient Calibration".
  6. Hit "Run".
  7. If tuning does not pass repeat one more time with the same parameters.
  8. If tuning does not pass again also check "Coarse XY Optimization" and "Fine XY Optimization".
  9. If tuning fails again contact Daniel Schulz or Esther Danenberg or Stefanie Engler.

#### Note

XY optimization does only need to be performed if the torch box of the machine has been opened or if tuning continuously fails.

## Check additional results if tuning passes

- 4
  1. If tuning passes under Results check the Transient Cross Talk 1. This value should be below 0.05. If this is not the case the Hyperion and the Helios are not well aligned. Align tubing. If you have not obtained training to align tubing contact Esther Danenberg, Stefanie Engler or Daniel Schulz.
  2. Check the detector voltage value and compare with previous tuning results. Large jumps (>50 V) in detector voltage indicate abnormal deterioration of the detector potentially due to sample/gas contaminations. Contact Daniel Schulz.
  3. Copy all the parameters required in the tuning log sheet from the Results to the tuning log sheet on the IMC computer.