Getting ready for imaging mass cytometry

If you are **new to imaging mass cytometry**, you will need to:

- Register your project by submitting the Hyperion Registration Form.
- Request for a project meeting with the IMC Platform team to discuss your project.
- Attend the Introduction to Hyperion course (under *Training* tab) which includes information on how the technology works and getting started with validating a Hyperion panel.
- Attend a machine introduction (this will usually occur the first time you run samples on the Hyperion).

Planning and booking:

- Once the IMC Platform team receives a completed registration form, we will add you and your research group to the IRIS booking calendar so you have access to the service.
- Sign in to the IRIS booking calendar (under the *Reservation* tab).
- Please check the booking calendar at https://iris.science-it.ch for free slots and to request a booking (titled Service request in IRIS) as outlined in the *Reservation* tab.
- Long-term longitudinal experiments should be discussed in advance to guarantee instrument availability.
- Please place your Service request at least 5 working days in advance. The response time is generally within 24h.
- Cancellation is possible up to 24h before the booking free of charge. Cancellation is not possible less than 24h before the booking slot due to inability to recover fees.
- Time for sample acquisition will be calculated based on your number of samples, number of ROIs, and the size of the ROIs. Scan area timing is: ≥1 mm^2/2 hr (@200 Hz).

Sample preparation:

Part 1. Section and slide preparation (e.g., day 1)

- Cut at least two serial sections (one after another) of the same tissue block and mount them on two separate slides

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- Two sections minimum are required: one for fluorescence staining (to validate the target regions) and one for metal labeling for imaging mass cytometry. More can be cut as desired.
- Preferably 5um – 7um thickness, but can be thinner or thicker if necessary.
- Make sure FFPE tissues (also optimal for OCT) are cut onto charged slides. This ensures that the tissue stays adherent to the slide during antigen retrieval and staining.

Part 2. Staining of slide #1 with fluorophore-conjugated antibodies for validation of target areas (e.g., day 1-2)

- Fix (if required), block, and stain slide #1 with fluorophore-labeled antibodies against relevant targets (e.g., FITC-CD45, AF594-CD11b).
- Perform fluorescence imaging to a) determine that staining occurs successfully, and b) identify areas that can be targeted for imaging on the IMC.
- Take a photo (with a normal camera or smartphone) of the tissue section so that it can be marked up with regions for targeting.
- Take images with a microscope. The images so that the same region of tissue can be identified on slide #2.

Part 3. Staining of slide #2 with metal-conjugated antibodies for imaging mass cytometry (e.g., day 3)

- Fix (if required), block, and stain slide #2 with the metal antibody panel and then DNA intercalator.
- Air-dry completely.
- Take a photo of the whole slide so that it can be loaded into the IMC acquisition software.

Part 4. Imaging on the Hyperion (e.g., day 4)

- Come to the facility at your scheduled time.
- Bring with you:
  - Images of the slide in some accessible location (email, dropbox etc.)
  - Mark up of the tissues section illustrating where the ROI for ablation should be created.

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- A copy of the panel that you used for staining.

Quality control (QC):

- The IMC will be tuned before each new user.
- The use of reference samples on each slide is highly recommended to control for batch effects (day to day variations in sample preparation and staining procedure) and day-to-day variations of the instrument.

Data management

- For each acquisition, two files will be created: a TXT file, and a MCD file. These files will be transferred to your storage device. Please be aware that Hyperion MCD files are very large files therefore, they will be deleted from the computer hard drive 3 months after your sample acquisition.

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