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Imaging Mass Cytometry Compensation Slide Acquisition

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1 Works for me dx.doi.org/10.17504/protocols.io.bf2jjqcn

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ABSTRACT

This SOP describes the slide acquisition for spill-over correction of Imaging Mass Cytometry experiments. The protocol makes use of the compensation slide, which was prepared previously following the SOP: "HuBMAP_Protocol_3_Compensation_Slide_Preparation.pdf" to measure each conjugated antibody individually and to record all observed spill-over. The resulting compensation matrix can be used to compensate data that has been recorded using the same antibody conjugates.

GUIDELINES

IMC: Imaging Mass Cytometer or Hyperion (Commercial name by manufacturer).
ROI: Region of interest

MATERIALS

NAME	CATALOG #	VENDOR
IMC (software 7.0 and higher)		Fluidigm
Compensation Slide		
Flat-bed scanner		

Set-Up

1 1. Attach the flat-bed scanner to a computer and take an image of the compensation slide.
2. Create a folder on the CyTOF computer on the "E" drive.
3. Save the image of the compensation slide in that folder with the same name as the compensation slide.
4. Insert the compensation slide into the Hyperion.
5. Under Data Acquisition create a new File ("Empty12AF").
6. Import the slide image.
7. Save the mcd file in the newly created folder.
8. Create a panorama across all the spots. Potentially, the spots may be hard to see in the actual camera view.

Requires a tuned and running IMC. For tuning see: dx.doi.org/10.17504/protocols.io.bf2gjqbw

Create ROIs

2 1. For each of the spots, create an ROI with the size of 10x200 (10 lines with 200 pixels).
2. Name each ROI with the metal name of the conjugate (e.g. "Gd156", "Ir191" etc).
3. Apply a panel with the same (or more) channels and settings as measured or will be measured for the experiment to be compensated. When creating the panel, make sure to actually select the correct metal isotopes as this is important for the spill-over matrix generation.
4. Check "Generate Text File".
5. Hit Run.

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