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Imaging Mass Cytometry Compensation Slide Preparation

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Michelle Daniel[1], Marda Jorgensen[2]

[1]University of Zürich; [2]University of Florida

Human BioMolecular Atlas Program (HuBMAP) Method Development Community
Tech. support email: **Jeff.spraggins@vanderbilt.edu**

Marda Jorgensen

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We use this protocol and it's working

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Abstract

This SOP describes the preparation of compensation slides with single antibodyconjugate spots that can be used to estimate mass channel spillover for IMC. Spillover varies from metal lot to lot due to varying impurities. Once a spillover matrix for a specific set of antibody-conjugates has been generated, the experiment of interest, in which the same antibody conjugates have been used, can be spillover-corrected.
It is crucial that this procedure is performed for the exact same antibody conjugates that are used to pipette the antibody panel for sample staining.
The original publication can be viewed here:
“Compensation of Signal Spillover in Suspension and Imaging Mass Cytometry” by S. Chevrier, HL Crowell and VRT Zanotelli et al., Cell Systems, 2018

Guidelines

AirLab Web: database that holds all information on antibody clones, Lot numbers and antibody panel information.

Materials

MATERIALS

- Ultrapure Agarose **Invitrogen - Thermo Fisher Catalog #**16500100
- Metal Tagged Antibodies as used to compensate
- Trypan Blue Stain 0.4% **Invitrogen - Thermo Fisher**
- Hot Plate
- Microwave
- Microscopy Slides Superfrost Plus **Catalog #**10.0344.01
- Beaker
- Airlab

Troubleshooting

Generation of agarose coated slides

1 1. Prepare 2% agarose in ddH2O in small beaker and melt, e.g. in a microwave.
2. Preheat microscopy slide to 70°C on a hot plate.
3. Distribute ca. 350-400 µl of liquid agarose over the slide until it is covered.
4. Let the agarose dry for at least 30 min.

Note

At the end of this section the agarose coat will be very thin, homogeneous and barely visible.
These slides can be stored until further usage at room temperature in dry conditions.

Generation of antibody conjugate dilutions

2 1. Pipette an array of 0.3 µl trypan blue spots onto the agarose coated slide. The spots should be
well separated from each other. Prepare as many trypan blue spots as the number of antibodyconjugated
mass-channels used in the study to be compensated (e.g. for every antibody).
2. In AirLab open the relevant panel and print it out.
3. Retrieve all listed antibody conjugates from the 4 °C fridge and keep them on ice.
4. For each antibody conjugate, pipette 0.2 µL onto the trypan blue spot on the agarose coated
slide. Note the exact location of each conjugate on the slide and make sure that no material bleed
over occurs. For simplicity, it may be preferable to sort the antibodies according to the mass of
the conjugated metal.
5. Let the slide dry for at least 1h.
6. Store the slide at room temperature in a dedicated box for measurement.

Note

In this step the metal spots are created on the agarose coated slide. The metal is diluted in the
blue organic dye “trypan blue”. This should help to locate the metal spots on the spillover slide.