Sample Guide 2023

To better help prepare you for a successful imaging session, the staff at the BioImaging Shared Resource (BISR) have compiled this list of sample considerations and must-haves.

Sample preparation

- Samples for imaging must have a #1.5 coverglass (0.17mm thickness).
- Plastic-bottom chambers are not recommended.
- Consider any practical limitation your imaging may have on sample thickness
- All slides to be imaged must have a sealed coverglass. This could be with nail polish or a fully hardened mounting media**

Please discuss your probe choice with BISR staff before booking a session.

Acceptable chambers

- Standard microscopy slides (<u>EXAMPLE</u>) and #1.5 coverglass (<u>EXAMPLE</u>)
- Coverglass-bottomed 35mm petridishes (EXAMPLE)
- Coverglass-bottomed multi-well chambers (EXAMPLE)
- Coverglass-bottomed wellplate-format chambers (EXAMPLE)

If you have a sample prepared with another chamber, contact BISR staff to discuss possible limitations to your imaging.

Final preparation

- If you store your samples in the cold, be sure to let them come to room temperature before imaging and clean off any condensation that forms.
- ALWAYS clean your sample coverslips before imaging with either lens cleaner or 70% ethanol
- If your sample requires incubation, be sure to turn on the microscope incubation chamber at least 30 minutes before imaging to allow the system to reach equilibrium.

Tips and tricks

- To avoid antibody aggregates creating a speckled background in your image, try spinning down your antibody solutions before use.
- To help mitigate photobleaching of your fixed samples, consider a mounting media with an anti-fade component.
- For fixation, be sure your fixative is freshly made from concentrate or powder.
- If you image with an oil immersion objective and intend to image again in the future, be sure to clean your coverslip after imaging; before storage.
- For cold storage, we recommend storing slides flat in a box or cardboard holder, placed within two (2) sealable plastic bag to minimize condensation.

** Some recommended mounting media include <u>Fluoromount G</u>, <u>Prolong Glass</u>, or <u>Prolong Diamond</u> (with or without DAPI)