

### Preparation of samples for automatic slide scanner

The system moves slides robotically so there are several very strict instructions for preparation of the slides. Slides which are not of the standard outlined below will not be accepted for scanning.

- Use only superfrost slides, e.g., [Thermo Fisher Superfrost Plus](#)
- Slides should be whole, without chips or cracks.
- Coverglass should not protrude over the edges of the slide.
- Slides should be clean, without any mounting medium residues; these can be removed with kimwipes and ethanol, while gently immobilizing the coverglass.
- Slides should be dry and the coverglass should sit firmly; only compounds that solidify while drying should be used.
- Slides with nail polish will not be accepted.
- Samples should sit in the middle of the slide, with some space to the edges and at least 10mm to the label.
- If you mark one of the slices please mark with an uninterrupted circle in permanent marker.
- The scanner accepts slides of maximum 1.4mm thickness including sample, mounting medium and coverglass.

Slide Dimension	Min (mm)	Max (mm)
Width	25	26
Length	75	76
Thickness	0.9	1.2

If in doubt, you may use our caliper to measure thickness.

#### Fluorescence

The system has filter sets to image fluorophores of the DAPI, CFP, Cy2, Cy3, Cy5, Cy7 families. This is a widefield microscope system so thin sample slices (under 40 $\mu$ m) should be used. Hard set mounting only should be used and left to dry for one or two nights in room temperature before transferring to 4°C. It is preferable to schedule imaging a few days after preparation, without putting the slides in the fridge. If the slides were in the fridge, take them out a few hours before imaging.

#### Data and Backup

A multi-file with proprietary metadata and indexes is created for each slide. The image can only be opened and navigated when there is access to both the .mrxs file and the directory, in the same folder and under the same name.

Every night the local computer is automatically backed up. Each group has a directory under the PI's name and an email message will be issued if the quota is reached. The local computer and the BCF server should only be used as a transient backup for the data. Raw data files should be saved and backed up in your own computers.

If your quota is reached please either delete your data or contact [Maya Holdenberger](#).

#### Data analysis

Geometric measurements and annotations can be done with [Slide viewer](#). The images can be exported to common file types for image analysis with software such as Image Pro, FIJI, or Imaris. We recommend that you set up a consultation meeting with one of our staff to explore quantification and create an automated working protocol.