

High-Content Screening - Getting Started Guide

The following information is a guide to setting up your experiments for processing on our instruments. If you require additional information or explanation, please contact our manager Monica Hasegan (hasegan@lunenfeld.ca). There are no bad questions, and it is always better to ask them before you have your sample(s) ready.

I. Sample Preparation

- After fixation/staining is completed, wells of a plate must be filled with PBS. Wells must be 50-80% full (e.g. if well volume is 200 ul, it must be filled with 100 - 160 ul of PBS).
- Plates need to have clean bottom surfaces free of scratches, dust etc. Certain plates have a removable sticker at the bottom. Please do not remove the sticker prior to imaging.
- Before bringing the plates to the facility, they must be sealed either with aluminum foil seals or with Parafilm. Plates must then be placed in Ziploc® resealable bag(s)/container.
- The objectives are air or water objectives, so surface of plates/slides should be oil-free.
- Please do not write anything on **top of the plate's lid or on the bottom within the well area.**

II. Plate/Slide Selection

- All our high-content platforms can image a large variety of multi-well plates (from 6-well plates, to 12, 24, 48, 96, 384, 1536) that fit SBS specifications (i.e. same size as a regular 6-well plate). Currently there are over 100 plate types in the instruments' database and new plate types can be added upon request.
- Some Incucyte assays may require specific types of plates.
- The plates are not supplied by the facility and need to be bought by the user after consulting with the facility manager for the most appropriate one for their project goals.
- It is important to specify what kind of plates (brand and catalogue number) are going to be used in an experiment.
- Some manufacturers supply technical drawing of the plates, which are very useful to generate a new plate profile.
- Please remember that use of high-resolution objectives requires special thin-bottom plates. Cell growth area can be either made of glass or special plastic.

- Regular 1x3" glass slides as well as LabTek Chambered Coverglass can be imaged on the IN Cell 6000 and the BioPipeline, however it is not recommended to use the slides for high-throughput applications.

III. Live Imaging

- IN Cell 6000, BioPipeline and Incucyte allow for long-term live cell imaging. A heated stage and a heated lid/environment with CO₂ supply keep cells in incubator-like conditions.
- For the IN Cell 6000: Heated lid fits any SBS format plate. It is recommended to use gas-permeable membranes for imaging longer than 4 hours. Membranes are available at the Centre.
- Celigo is not equipped with an environmental chamber to keep cells in the optimal conditions, however temperature inside the instrument is around 37°C, thus minimizing stress on live cells.
- Brief information on all cell lines used for live imaging must be submitted to maintain an inventory list at the facility.