Network Biology Collaborative Centre

Sinai Health | Lunenfeld-Tanenbaum Research Institute

NBCC High-Content Screening Guide

The Network Biology Collaborative Centre (nbcc.lunenfeld.ca) at the LTRI provides access to cutting-edge high-throughput imaging equipment, as well as powerful automated image and data analysis tools. This guide provides information on the imaging applications we offer and the instrumentation that is available for analysis.

I. Who we are

The NBCC High-Content Screening node is overseen by LTRI's Senior Scientist and NBCC co-Director Dr. Laurence Pelletier who is an expert in microscopy and high-throughput applications. The facility is managed by Dr. Monica Hasegan who has a PhD in Physics, and strong expertise in advanced imaging, instrumentation, and data processing and analysis. Director of Operations Dr. Karen Colwill provides administrative and logistical support.

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II. How we help

The NBCC provides full support for your imaging needs from initial study concept to manuscript preparation. We work with you on experimental design, guide you to the appropriate imaging platform, provide training and assisted use on our scopes, develop customized imaging assays and pipelines, and assistance with image and data preparations for presentations and publications.

III. Applications

A. High-Content Imaging

High-Content Screening is automated image acquisition and analysis in high-throughput. It enables fast testing of many biological conditions including arrayed genetic and chemical screens, while ensuring reproducibility and objective quantification of images. A wide variety of different applications can be accommodated including cell counting and morphology, colony growth tracking, drug screening, cell viability and proliferation assays, protein translocation and co-localization assays. Assays can be performed in multi-well format on fixed or live cells in monolayers or organoids. Time-lapse imaging for multiple plates using plate scheduler modules is available on the BioPipeline (44 plates) and the Incucytes (6 plates).

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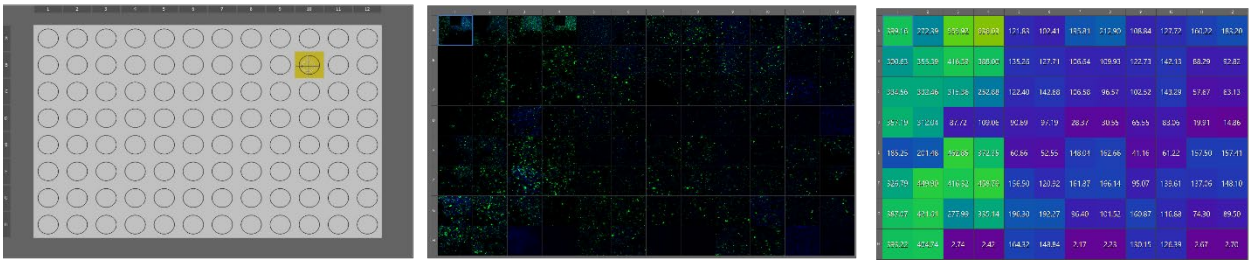

Figure 1. Plate scanning using the Nikon BioPipeline (left panel), image visualization for a whole plate (middle panel), and measurement visualization of the plate (right panel).

B. High-Content Data Analysis

We have several tools for automated image analysis. They include ones which can be used without extensive training to create simple analysis algorithms (Celígo analysis, Incucyte analysis, and Columbus), as well as software for generation of highly customized algorithms.

NIS-Elements HC (Nikon): NIS-Elements HC is a total acquisition-to-analysis solution for high-content imaging applications. It streamlines high-speed, automated multi-well plate acquisition, data review, analysis and management of multiple plate experiments. Multiple scans can be analyzed simultaneously during the imaging phase or post-acquisition on offline stations. Heat maps of wells/plates, sample images, binary masks, assay results, sample labels and other metadata are centralized for quick filtering, gating and drilling down to cellular detail. NIS-Elements offers histogram, scatterplot, bar chart, XY line, classification and gating functions. Easily navigate within the Plate View and export to Excel or bitmap.

Celígo analysis (Nexcelom Bioscience): This built-in image analysis tool allows on-the-fly analysis of acquired images. Recommended applications include label-free cell growth tracking, cell cycle analysis, cell viability and apoptosis, transfection optimization, multiplex fluorescent expression assays, and many others. Once image acquisition is complete, data can be re-analyzed at an offline workstation.

Columbus (Perkin Elmer): Columbus is a universal image data storage and analysis system that enables access to stored images via a standard internet browser. The Columbus system allows scientists to remotely access, view, annotate, and analyze images. Columbus is based on an OMERO server and therefore supports a variety of existing microscope file formats. Integrated image analysis tools are intuitive and efficient. A high-performance computing server enables hyper-threading of image analysis. PhenoLOGIC™ machine learning technology provides an easy way to classify cells or subcellular regions within a given dataset.

Acapella (PerkinElmer): Acapella is a powerful tool for image analysis. It has a set of proprietary scripts for detection of typical cell shapes and sub-cellular organelles. This is

combined with a versatile programming environment to create customized algorithms for a variety of biological applications.

Matlab (Mathworks): Matlab is the most powerful application for advanced analysis of images. Flexible image and data handling allows highly specialized routines for image analysis, including complex image filtering and segmentation. Costing for this analysis will be determined based on the complexity of the analysis.

CellProfiler (open source): CellProfiler is an open-source cell image analysis software designed by Cimini Lab at the Broad Institute of MIT and Harvard. CellProfiler analysis pipelines can easily be built and customized using a series of image-processing modules, to determine any phenotypes of interest in your images. The measurement results can be exported to a spreadsheet or a database.

IV. Core Instrumentation

A. Nikon Biopipeline

The Nikon BioPipeline is a high-end, flexible, live and fixed sample high-content screening system. It is ideally suited for high-resolution screening assays such as detection of DNA damage foci, translocation assays, measuring of cilia lengths, co-localization studies, drug screenings or any other assay that needs to combine high-resolution with automated imaging. It is a line scanning confocal platform based on Nikon's Eclipse fully-motorized, inverted Ti2 microscope and the A1R-HD25 hybrid confocal system with the world's largest 25 mm field of view. It features two scanners: a low-noise galvano scanner, e.g. 10 frames per second (fps) at 512*512 pixels and a fast resonant scanner, e.g. 30 fps at the same field-of-view.

B. IN Cell 6000

Our facility has two IN Cell 6000 microscopes, equipped with 10x, 20x, 40x and 60x objectives. Both microscopes are also equipped with temperature and environmental controls, enabling live-cell screens.The IN Cell Analyzer 6000 is a high-end, laser-based, line-scanning confocal imaging platform. It utilizes a novel and proprietary optical engine to achieve both wide field and confocal imaging capabilities in one system. The Iris-like variable aperture and next-generation scientific CMOS camera enable high quality imaging at high speeds without compromising data quality. This instrument is designed for high-throughput imaging and assay development. It can be used for live cell and endpoint assays as well as 3-D imaging, co-localization studies and low signal assays.

C. Incucyte

We have 2 Sartorius Incucyte systems available in the Facility. Specific live-cell imaging modules include cell migration, cell-by-cell AI, organoids and spheroids.

S3: The Incucyte S3 is equipped with high-definition phase-contrast optics and two fluorescent wavelengths (red: ex565-605 nm, em625-705 nm; green: ex440-480 nm, em504-544 nm), and three objectives on an automated turret: 4x PLAN, 10x PLAN FLUOR, and 20x PLAN FLUOR. It can accommodate up to six microplates at a time.

SX5: The Incucyte SX5 Live-Cell Analysis System is the latest version of Incucyte technology. In addition to having the same green/red optical module as the S3, it has a secondary, swappable optical module with Green/Orange/NIR: Green (ex453–485 nm, em546–568 nm), Orange (ex648-674 nm, em494–533 nm), and NIR (ex576–639 nm, em685-756 nm).

D. Celígo

This instrument is ideal for up to 4-channel imaging of multi-well plates at 4x low-resolution. Proprietary F-theta lens installed on the cytometer provides great image flatness and contrast. Bright LED light-sources enable label-free brightfield imaging as well as acquisition of blue, green and red fluorophores. Uniform well illumination and large-chip CCD camera allow users to image entire wells at a high-speed. Built-in image analysis tools allow performing on-the-fly analysis in a variety of biological applications (cell/colony counting, migrations assays, expression analysis and many others).