Fastest Confocal at Vanderbilt

HTS/HCS Confocal Imaging
Stats

**Illumination**
- 4 laser sources (405, 488, 561, 640 nm)
- tunable UV/VIS (350 - 410 nm)
- TLED (brightfield)

**Objectives**
- 10 x Air
- 20 x Air
- 20 x Water Immersion*
- 40 x Air
- 40 x Water Immersion*

*Water-immersion objectives ensure the highest resolution of subcellular features, while air objectives offer exceptional working distance.

**Cameras**
- 3 Peltier-cooled high-resolution CCD cameras (1.3 MP, 12-bit)
- 1 non-confocal camera (used for UV/Vis)

**Other**
- Wide range of fluorescent emission filter combinations (450/50, 520/35, 540/75, 565/40, 585/40, 600/40, 690/50)
- Ultra-fast laser based autofocus (0.1s)
- Motorized stage and z-focus drive
- Accepts 96, 384, 1536 wellplates, microscope glass slides, and custom microfluidics
Opera 2.0 Upgrade: March 2012

- 4 times more computing memory
- New file-saving format
  - Allows for acquisition of unlimited number of image fields.
- Software upgrade
  - More robust autofocus allows for integration of lower magnification objectives.
  - More natural workflow.
  - Four-color overlay.
- Brightfield capability
  - Image tissue samples and cells that have not been fluorescently labeled, and protect delicate live cells.
  - Track cells inside microfluidics.

- Now up to 80,000 images in a single run
- At a rate of over 400 confocal images/min
David Cortez Lab: Whole-genome siRNA analysis screen to identify and validate novel replication stress response proteins
Rebecca Ihrie Lab: Interneurons that are progeny of the GFAP-positive stem cells (DCX, Beta-3 tubulin, nucleus).
Rebecca Ihrie Lab: GFAP-positive astrocytes and astrocyte-like stem cells.
19,000+ images* - Live/Dead stained Collagen-Fibroblast Construct - Jeff Davidson Lab -

*Visualized in 3D using Volocity
Actin-stained murine dermal fibroblasts – Jeff Davidson Lab (Rhodamine Phalloidin, Sytox Green, 20x air objective)
Diana Neely Lab – Primary human neurons

(Cy3, Alexa488, dyLight488, dyLight649)
Borden Lacy Lab – Effects of Clostridium difficile toxins

doi:10.1371/journal.ppat.1003072
Precise Zebrafish Embryo Wellplates

Conventional loose arrangement inside a 96-well plate bottom

VS.
Susan Opalenik - Murine Dermal Fibroblasts
Nuclei (Vybrant Dye Cycle), Membrane (Dil)

Susan Opalenik - Murine Dermal Fibroblasts
Nuclei (Vybrant Dye), Cytoplasm (Calcein AM)
Analyze Data at *Opera Speed*

**Columbus™ Analysis Software**
- Get started without extensive training!
- Batch analysis of complete multiwell plates.
- Comprehensive range of preconfigured analysis algorithm building blocks makes your data analysis quick and efficient.
- PhenoLOGIC machine-learning technology let’s you train the software to recognize and classify different populations and image regions using a point-and-click approach.
- Exact analysis summary statistics for multiple wells or entire screens.
- Web-enabled multi-user access allows everyone in your lab to access, visualize and analyze data.
- Quad-core 2.67 GHz processor, 8 GB RAM, and 4 TB (easily expandable) storage space on the Columbus server allow for high-speed processing.
- Can be used from remote desktops.

**High-Performance 3D Analysis Software**
- Rapid, interactive, high-resolution volume rendering of time resolved, multichannel 3D image sets
- Compare and relate cellular organelles within and between samples
- Perform morphological analysis
- Measure fluorescence localization and co-localization
- Measure distances within and between organelles
- Choose from automatic or manual tracking to characterize object movement
- Automatically measure or analyze multiple samples with batch processing
Columbus Screenshot – Building Scripts
Columbus Screenshot – Heatmaps and Results
PerkinElmer Columbus - Powerful HTS/HCS Image Analysis
Analyze whole wellplates in minutes!

Vaccinia infected cells (James Crowe lab)
David Cortez Lab - Whole-genome siRNA analysis

- Easily visualize your results in Columbus -
Volocity Screenshot – Cell Tracking in 3D
Volocity Screenshot – Cell Tracking inside Custom Microfluidics in 3D
Wellplates, Glass Slides & Custom Microfluidics

• Wide-range of commercial wellplates
  ✓ Glass or plastic bottom
  ✓ 12, 24, 96, 384, 1536-well
  ✓ We can add new plate definitions!

• Custom slide holder for two 2x3 slides

• Custom microfluidics in wellplate format
  ✓ Feature alignment with 96-well positions
  ✓ Small volume bioreactors
  ✓ Cell culture chambers with mixers, gradients, oxygen films, valves, cell traps, custom surface treatments, etc.
  ✓ Fully bio-compatible!
Fast!

Fast stage, fast autofocus, wellplate compatible

CONFOCAL

Minimize out-of-focus “haze”, get real intensity values!

Flexible

Multiple laser, camera, and filter selections

Cell Friendly

Minimal photo-bleaching with spinning disk and short exposure times

Automated

When did you last analyze 1.5 million images?

✓ More Elaborate Experiments!
✓ More Information!
✓ Greater Statistical Power!
✓ Faster Results!