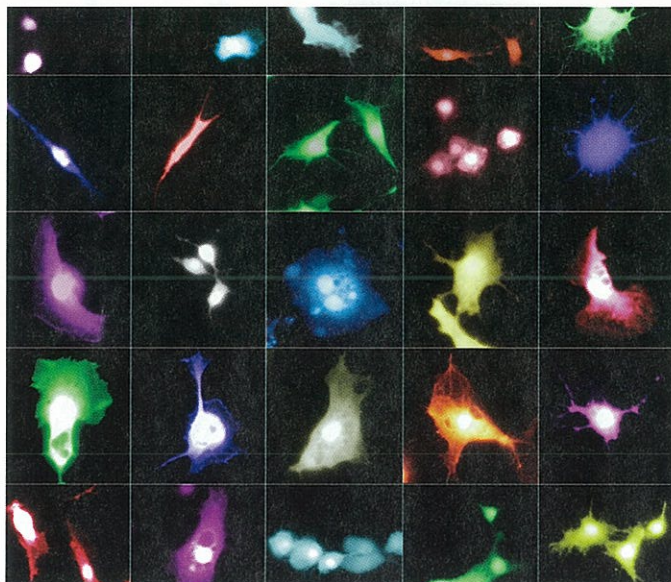


AUGUST 2007

Lab Tools



Seeing Faster, Seeing Smarter

Image analysis software offers more screening possibilities than ever, so how should you power your next screen?

By Ewen Callaway

Obtaining rich information such as morphology, protein colocalization, or nuclear translocation from high-throughput cell screens has largely been a matter of “tech-” nology – that is, put a lab tech or a graduate student in charge of screening thousands of images visually. High-throughput microscopy, however, has broken several barriers in the last few years by automating image analysis to assess thousands of different biological conditions. But, all that power doesn’t come cheaply. Systems can cost as much as a million dollars, not including the software. Moreover, users will need to make a significant investment in data storage, as a large screen could amass five terabytes of data.

More than a dozen companies offer systems that combine automated microscopy with proprietary image analysis software – or what Jason Swedlow, a cell biologist at the University of Dundee in Scotland, calls “high-throughput screening in a kit.” On the opposite end of the software spectrum is a custom-designed image analysis tool engineered for a specific lab’s needs. “There’s a range of sophistication and along with it goes a range of complexity,” says Swedlow. “No single tool does everything,” he adds. Nevertheless, as some of the packages are costly, collectors will be few.

Drosophila BG-2 cells from Chris Bakal’s RNAi screen for genes involved in determining cell shape.

As for most big-ticket items, Swedlow recommends seeing a demonstration of a package and talking with other scientists before plunking down hundreds of thousands of dollars.

Here, we present a guide to the range of products available and how they might fit the needs of most projects.

Off-The-Shelf: The IN Cell 3000 Analyzer

User: Nathalie Aulner, research scientist, Columbia University

Problem: Screening of a chemical library of 100,000 compounds for drugs that block nuclear translocation of NF- κ B in mammalian cell lines, such as HUVEC and HeLa.

Solution: Aulner performs her screen with GE Healthcare’s IN Cell 3000 Analyzer, a package that combines a confocal microscope with the company’s proprietary analysis software.

Pros:

- Seamless integration exists between experiment set-up, image acquisition, and analysis.
- Data analysis can often be done “on-the-fly” as a screen runs.
- Software provides predefined experiments, such as cell-cycle analysis, dendrite outgrowth, and micronuclei formation.
- Tech support is available.
- Simple user interface.

Cons:

- If your experiment doesn’t fit into one of the predefined modules, you’re out of luck or forced to design custom experimental parameters.
- Many commercial packages have trouble with smaller, clumpy, and highly confluent cells, such as *Drosophila*.
- Costly annual license fees, from less than \$10,000 to \$30,000.

The bottom line:

A confocal system such as the one Aulner uses can cost as much as \$1 million. Off-the-shelf software is a good place to start with such systems. Most academic core-screening facilities have one or two platforms that will breeze through a chemical library screen for drugs that promote apoptosis, or even a genome-wide RNAi screen for changes in subcellular protein localization.

Mix-and-Match: MetaXPress

User: Kaylene Simpson, postdoc, Harvard Medical School

Problem: Genome-wide RNAi screen to examine cell motility in a breast cancer cell line. Simpson scratches the surface of a confluent well, then measures cell migration by quantifying the area that remains empty.

Solution: Simpson began her experiment using off-the-shelf software on her core facility's microscope. Because the bundled software wasn't suited to run automated analysis, she began using MetaXPress, a software package that Molecular Devices makes. This one-two punch matches the high resolution of one manufacturer's microscope with the image analysis tools of another.

Pros:

- More powerful and flexible than sticking with the program that comes with your microscope.

Cons:

- Data sharing between programs can be difficult. Users may have to acquire black and white images for each channel and then feed them into the second program. Often, multichannel images are in a proprietary file format, not the universal TIFF format.
- Difficult to keep image files organized and linked to an image's metadata describing channel, exposure, and magnification.
- Double the license fees.

The bottom line: Most users fall into this category, says Swedlow. Users shouldn't limit themselves to software from a microscope-maker, but a package such as MetaXPress can run another \$10,000, according to one core facility. Companies such as Definiens and Biolumigena make image analysis software as well. Moreover, once users analyze images and convert them into numbers, powerful data analysis packages such as Matlab and the open-source "R" can crunch the data.

Custom World: Customized image analysis software

User: Chris Bakal, postdoc, Harvard Medical School

Problem: Genome-wide RNAi screen in *Drosophila* cells to discover signaling pathways that determine cell shape.

Solution: After trying several commercial and open-source options, Bakal started working with a computational biology group to design customized image analysis software. "You realize right away that off-the-shelf software does a bad job recognizing cell boundaries and doesn't give you the output you want," he says. "You want to look at hundreds of variables instead of just a few."

Pros:

- The ultimate in flexibility and customization.

Cons:

- Requires extensive programming experience or a collaboration with another group that has it.

The bottom line: The available commercial and open-source tools will satisfy the majority of users, says Bernard Mathey-Prevot, who runs the *Drosophila* RNAi screening facility at Harvard. For users seeking to extract as much information as possible from their images, however, a custom algorithm may be the answer. For example, commercial software does a poor job of measuring cell texture or cell contour. Bakal wants to know as much about his cells as possible. "You don't know beforehand what's going to be important, so you try to capture everything," he says.

Live and Let Live : Pathway 855

User: Maria DeBernardi, deputy director, Integrated Imaging Center, Johns Hopkins University

Problem: Imaging real-time calcium flux in living, drug-treated cancer cells.

Solution: Becton Dickinson's all-in-one Pathway 855 system is one of a few commercial systems geared toward live-cell imaging. The instrument's software manages the whole process, from setup to analysis, and allows DeBernardi to analyze a cell individually over time to see how it responds to a drug.

Pros:

- Ease of use of a commercial system.
- Ability to analyze individual cells over time.

Cons:

- Limited analysis options.

The bottom line: With live time-lapse microscopy, "automated analysis becomes essential because the volume of data is too large," says Randy King, a cell biologist at Harvard Medical School who's working on an open-source imaging package for live cells. Systems from GE Healthcare, Cellomics, and Olympus are also capable of imaging live cells and all come with their own software.

Open Source: Cellomics + Cell Profiler

User: Scott Floyd, postdoc, Massachusetts Institute of Technology

Problem: Large-scale RNAi screen in tissue culture osteosarcoma cells looking for DNA damage and cell proliferation phenotypes.

Solution: Floyd uses a Cellomics scope to acquire images but turns off the analysis software. After collecting some 5,000 images in a single screen, he analyzes the data with Cell Profiler, one of the leading open-source tools.

Pros:

- User-friendly platform: "It looks like a Mac OS Window," says Floyd.
- Open-source platform allows other developers to contribute tools.

- System is compatible with a MySQL to link images and analysis in a database.
- Works with Matlab.
- It's free.

Cons:

- Online community is the main support system.
- Slow analysis - a large (10,000) set of images can take a couple days to analyze even with a computing cluster.

The bottom line:

"The open-source efforts are not mature, but maturing," says Swedlow. "None of them [is] a fully developed project yet." They require more patience and dedication from users, but the cost savings can be huge. "You can save a ridiculous amount of money by buying a plain old scope and using Cell Profiler," says Anne Carpenter, a computational biologist at the Broad Institute, who designed the open-source tool.

Selected Systems

Manufacturer	System	Cost
Applied Precision	cellWoRx	\$200,000
BD	Pathway 855	<\$400,000
Cellomics	ArrayScan	\$175,000-\$250,000
Evotec Technologies	Opera	\$1,000,000
GE Healthcare	In Cell 3000	\$800,000-\$1,000,000
Molecular Devices	ImageXpress Micro	\$250,000

Selected Software Packages

Manufacturer	Program	Annual license cost
BD	Pathway high-content imaging software	Included in cost of system
Biologene	CellMine	Company refused to divulge
Cellomics	BioApplication Image Analysis	\$20,000
Definiens	Cellenger	\$6,500+
Evotec Technologies	Acapella	\$30,000
Molecular Devices	MetaXpress	\$7,000 plus \$2,500 for each analysis module (e.g., cell cycle, dendrite outgrowth)

Further Reading

- R. Pepperkok, J. Ellenberg, "High-throughput fluorescence microscopy for systems biology," *Nat Rev Mol Cell Biol*, 7:690-6, 2006.
 C. Smith, M. Eisenstein, "Automated imaging: data as far as the eye can see." *Nat Methods*, 2:547-55, 2005.