Innovations

A complete vision
Imaging Research, Inc.

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More chemicals, more drug targets and smaller testing wells are here. But someone has to determine the results of all those assays. Measuring one assay at a time may not be fast enough, and that is where Imaging Research Inc. (IRI; St Catherines, Canada) steps in. Their giant lens captures the image of an entire screening plate, and proprietary software defines the wells and the individual assay results.

From tissues to Tundra

Peter Ramm of Brock University (St Catherines, Canada) is the founder of IRI, and the early incarnation of the company reflected his research in neuroscience. Thus one aim of the IRI imaging system was to visualize the binding of labeled ligands to large brain sections. IRI developed imaging systems and software for the quantitative analysis of these images, but the imaging optics remained somewhat conventional.

In the hardware area, Ramm realized that there was a new opportunity. "We were aware of changes in the priorities of the pharmaceutical companies," he says. "Screening hadn't required imaging, but with these large libraries imaging seemed a reasonable approach to increase throughput." And IRI's experience in imaging entire brain sections could help their attempts to image entire assay plates. The Tundra system was born.

Big optics

Tundra is not a microscope. The combination of its key components — the custom-made 'Borealis' lens and a charge coupled device (CCD) — end up being slightly demagnifying. The CCD converts incoming photons into electrons, and is cooled to reduce spontaneous activity, and thinned to increase sensitivity.

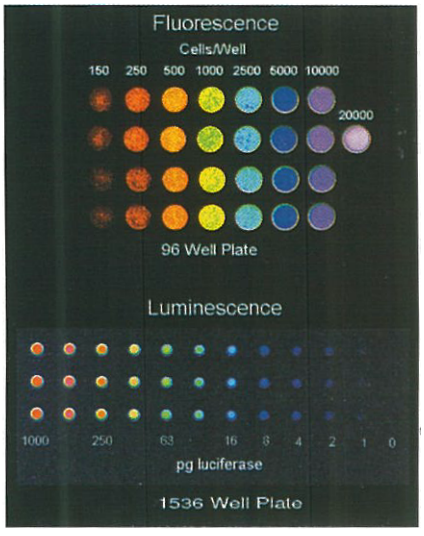
But the pride of the Tundra is the lens. The Borealis measures 12 cm in diameter, includes 13 separate lenses, and weighs in at 40 pounds. The combination of its size and large aperture means that it collects far more light than a normal lens. The Borealis is also telecentric, meaning that it can peer directly into all the wells of a plate. Normal lenses suffer from parallax, with light being distorted (the round tops of distant wells become ovals) and lost (well walls shade the contents of wells).

All fluorescence detectors fight against background. The microscope solution had been to make lenses epifluorescent, but before Borealis no epifluorescent macrolens was available. Without epifluorescence, the excitation light comes from one side and the filtered emission light is collected on the other. Unfortunately the filters are imperfect and the emission light can be a thousandfold weaker than the excitation light. "It's like seeing a star against the daylight sky," says Ramm. "But in epifluorescence you shine the excitation light out of the lens, away from the detector, and see only the emitted light coming back. Now you're looking at the stars against the night sky."

IRI has sold more than 20 Tundra units at over $200,000 each. A recent Tundra customer was the Institute for Chemistry and Cell Biology at Harvard Medical School (Boston, Massachusetts). Randy King, an independent fellow at the Institute, was impressed by the Tundra. "It's juiced at both the light-gathering end and the camera end to be as sensitive as you can get right now," he says. "There's a big gap between them and what else is out there."

Most drug companies are in the process of converting their screening format from 96- to 384-well plates,

Figure 1



The IRI system can measure fluorescence (top), luminescence (bottom) and absorbance. Image courtesy of IRI.

but King believes that the Tundra will handle his 6144-well plates quite happily. Based on the pixel density of the CCD, this is approximately the limit for the current system, although Ramm says that IRI is considering a high resolution system to look at smaller areas of genome microarrays.

On June 19, Amersham Pharmacia Biotech Ltd (APB) bought a controlling interest in IRI, and Tundra became a part of Leadseeker, a new screening package. Leadseeker, to be formally launched in September, will be considerably more expensive than Tundra, and will include fluidic dispensing and robotic delivery of plates. Leadseeker assays have been designed by IRI and APB.

One of the new assays in Leadseeker's repertoire will be the scintillation proximity assay (SPA), which is proprietary to APB. In SPA, radioactive ligands bind to proteins on scintillant-containing beads, causing very low levels of light to be emitted. SPA is a homogeneous assay, valuable because chemicals can usually be tested without any washing or filtering steps. Adding SPA capability would, says King, "be a pretty impressive feat."

Alternative views

IRI is in a crowded market. The traditional detectors are scanners that use photomultiplier tubes (PMTs) to detect signal from a single well. Current examples include the Victor[2] ($23,500–$50,000 from EG&G Wallac, Gaithersburg, Maryland), and the Analyst, which also does fluorescence polarization (~$100,000 from LJL Biosystems, Sunnyvale, California). Drug screens are a constant trade-off between speed and sensitivity, and PMTs tend to be more sensitive but slower than CCDs.

Next year, Wallac will release the Viewlux, a CCD-based plate imager for time-resolved fluorescence. "When you move beyond the 384-well format you start to move beyond the capabilities of the PMTs," says John Westerfeld, Wallac product manager. "Sensitivity is a problem, and [the detection areas of the PMTs] start spilling over to adjacent wells."

One way to get around this density problem is to feed the signal to the PMTs using a fiber optic array. Aurora Biosciences Corporation (La Jolla, California) and its corporate partners use a fiber optic array of up to 192 elements to screen 3456-well plates. "There's no way that a telecentric lens system can be more sensitive than a fiber array that is designed for fluorescence detection, and is within a hundred microns of the source," says Harry Stylli, Aurora's vice president for screening technology.

Aurora uses both CCDs and PMTs, but Stylli believes that in the end CCDs will win out. "In the next generation the dominant [systems] will be camera-based. You can sell them for more, they are better in low light, they have the potential to image whole areas, and they can do imaging not just detection."

Molecular Devices Corporation (Sunnyvale, California) already has a plate imager on the market called FLIPR (fluorescent imaging plate reader). "It was designed to do a specific thing and to do it well," says Simon Pitchford, product marketing manager. He estimates that 80% of FLIPR machines are used for time-dependent detection of calcium levels, with most other assays involving pH or membrane polarization changes. All these assays involve dyes with relatively intense fluorescence, so FLIPR can get away with a regular sized lens.

FLIPR can deliver agonists or antagonists simultaneously to all wells of a 96-well plate, and excitation and imaging of a plate takes as little as one second. A laser is split into eight parallel scans for more rapid excitation, and a CCD camera takes an image of the entire plate. Pitchford claims that oblique illumination keeps the light at the base of the well, so background fluorescence is reduced. Imaging through a mask and from the bottom of the well reduces the problem of parallax.

The current version of FLIPR goes for ~$250,000, and has integrated fluid delivery. A 384-well version, to be released in September, will add robotics handling of the plates.

Carl Zeiss Jena GmbH (Jena, Germany) has increased throughput simply by using multiple detectors: 96 micro-objectives, which read out in parallel to a CCD. "The advantage of that approach is that you can use lenses with higher numerical aperture and so greater collection efficiency," says Martin Gluch, project leader for the Ultra-High-Throughput System at Zeiss. The unnamed and unpriced system, to be launched in August, can also handle 384- and 1536-well plates in multiple reads, and has integrated robotics and fluid handling.

More detailed information

The Zeiss system is designed to be used in combination with the High Content Screening (HCS) system from Cellomics Inc. (Pittsburgh, Pennsylvania). This system uses more conventional microscope optics to image a single well of a plate. The key to HCS is the software, which analyzes a statistically significant number of cells (typically 50–300) and reports on the movement of molecules (nuclear localization, receptor internalization) or cellular morphology changes (hypertrophy, apoptosis). It takes ~20 minutes to screen a 96-well plate, and so is best suited to analyzing the hits obtained using the faster Zeiss machine. In one day of screening, the Cellomics system should be able to profile the 1000 hits identified from the 100,000 chemicals screened by the Zeiss system.

EVOTEC BioSystems GmbH (Hamburg, Germany) gets its quantitative information by looking at the motion of single molecules. The EVOscreen uses fluorescence correlation spectroscopy (FCS) to determine whether a ligand is moving quickly (unbound) or slowly (bound to a large target). A laser is focused into a volume approximately the size of an *Escherichia coli* cell (1 fl or 10^{-15} l). For solutions in the micromolar to picomolar range, the number of molecules in this volume is small enough that the motion of single fluorescent molecules can be measured by looking at the molecules' photon showers, using avalanche photodiodes. The result is affinity data, and on and off rates, collected at the rate of one second per well.

Two EVOscreen systems have been sold for ~$15 million each, and once another two are sold the company will be devoted to in-house screening. The off-the-shelf Zeiss ConfoCor instrument has the same optics, but it lacks the EVOscreen detection and software units.

The EVOTEC system tests only small molecule binding events, but it has the advantage of always being ready for more miniaturization. "With conventional fluorescence you need to collect fluorescence from as many molecules as possible," explains Bjoern Lindemann, vice president for corporate communications at EVOTEC. "But with FCS we are looking at single molecules, so miniaturization is not a problem."

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