Imaging systems in assay screening

Peter Ramm

High-throughput screening laboratories continuously seek higher throughput, lower cost and compound conservation. In an attempt to approach these goals, improvements in traditional detection instruments (e.g. scintillation counters, fluorescence plate readers, luminometers) are being made. A more radical alternative is the use of image-based detection methods, which have the advantage that large numbers of wells can be quantified in a single detection procedure (favouring miniaturization) and that faint luminescence or scintillation assays can be accomplished very rapidly. However, image-based screening is a very new technology and must prove itself before being broadly accepted. In this review, the technology of a commercial instrument for image-based screening (the LEADseeker™ from Amersham Pharmacia Biotech, Amersham, UK) is described, and its performance summarized with scintillation, luminescence, fluorescence and absorbance signals.

Massive increases in the availability of both compounds and targets have led high-throughput screening (HTS) laboratories to seek new technologies that yield both increased throughput (e.g. 100,000 samples per day) and lower costs. Screening at very high sample rates is often termed ultra-high-throughput screening (UHTS). UHTS can be implemented around standard screening instrumentation, by use of efficient assay protocols and multiple instrument stations to relieve bottlenecks. Recent developments in robotic and workstation equipment have allowed the implementation of this strategy, albeit with significant complexity and cost[1]. An alternative approach is to combine new detection technologies with the miniaturization of assay formats, so that assay-throughput rises while microplate, reagent and library usage are minimized (Table 1).

The practical advantages of moderate miniaturization (assays conducted in a few microlitres) are compelling, in that the assay chemistry is still familiar while the savings in library components and disposables are substantial. These advantages have driven the development of new detection instrumentation, suitable for miniaturized samples. Some of the new instruments are modifications of traditional luminometers, fluorescence plate readers and scintillation counters, whilst other detection instruments incorporate imaging components.

The specific role of an imaging detector is to increase detection throughput, conveniently and reliably, while yielding performance that approaches that of non-imaging detection methods. Although this review will focus on detection by imaging, there is much more to UHTS than the detector. A complete UHTS system includes novel detection methods, optimized assays, and sample preparation, dispensing and containment components.

Assay imaging instruments first appeared approximately three years ago[2] and although they were useful for assay prototyping and feasibility studies, they were not easily applied to practical screens. However, that situation is currently changing as the technology of image-based screening matures. A second generation of instruments is appearing that integrates an improved level of function

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Table 1. Summary of reagent conservation achieved in moving a scintillation proximity HIV-RT (reverse transcriptase) assay from 96- to 384- and 1536-well formats

	96-well plate	384-well plate	1536-well plate	Reduction (1536 : 96)
Template/primer	10 μl	5 μl	1 μl	21-fold
[^{3}H]dTTP	438 nCi	220 nCi	42 nCi	10-fold
Enzyme	0.3 units	0.2 units	0.0008 units	375-fold
SPA beads	500 μg	100 μg	20–40 μg	12-fold
Assay volume	150 μl	50 μl	8 μl	19-fold
IC_{50}	0.2 μM	0.3 μM	0.1 μM	

The 96-well condition used a standard scintillation counter and blue-emitting beads. All other conditions were imaged with LEADseeker (APBiotech, Amersham, UK), using streptavidin-coupled yttrium oxide red-emitting beads.

with automation and with the infrastructure and support mechanisms required by screeners. Among today's better-known imagers are the LEADseeker system from Amersham Pharmacia Biotech (APBiotech, Amersham, UK), the Fluorescence-Imaging Plate Reader (FLIPR) from Molecular Devices (Sunnyvale, CA, USA), and the ArrayScan from Cellomics Inc. (Pittsburgh, PA, USA).

Components for image-based screening

Imaging devices can be broadly categorized as scanners or area imagers. Scanners serially pass an illumination beam and a photodetector over each point in the specimen. Laser scanners built around confocal microscope optics[3] are particularly appropriate in low-volume fluorescence applications, such as fluorescence correlation spectroscopy and discrete cell scans, whilst large-area fluorescence scanners of the type used for molecular biology, such as the FluorImager (Molecular Dynamics, Sunnyvale, CA, USA), can be used to image thin, clear-bottom-well plates. However, these scanners are not designed for plate imaging and their performance with plates is not well characterized.

An area imaging system detects the entire specimen in parallel and does not perform a serial scan of the area. Most area imagers use charge-coupled device (CCD) cameras as image formation devices, which are attached to microscopes (micro-area imaging) for low-throughput discrete cell screens[4–7], and to lenses for lower resolution work (macro-area imaging, Fig. 1). Most macro-area imaging of well plates has been applied to low-throughput luminescence (for early examples, see Refs 8–12), though fluorescence imaging has also been reported[2,13]. Macro-area imaging has potential advantages:

- Because the entire specimen is imaged simultaneously, the detection process can be very rapid
- Images of entire well plates can be acquired in rapid sequence, this facilitating dynamic assays
- Areas containing cells can be detected (on the basis of intensity) independent of surrounding background areas
- Given an appropriate optical system, any excitation and emission wavelengths can be selected
- Luminescence, fluorescence and scintillation assays can be imaged
- Free (e.g. gels, fluidic microchips) or fixed (e.g. well plates) format specimens can be imaged.

Whatever the potential advantages, high-throughput imaging systems for screening have been slow to appear, this slowness resulting from the special difficulties of imaging well plates. One of the problems is that the plates are large while each discrete well is small and imaging a large area made up of small, dark targets requires highly specialized camera/lens systems. Furthermore, the delivery of uniform

Figure 1. *The area-imaging components of the LEADseeker (APBiotech, Amersham, UK), including a large telecentric lens and a cooled charge-coupled device (CCD) camera (mounted above the lens). The lens incorporates an epi-illumination mechanism, fed by a fibre-optic 'tail'.*

excitation illumination over the whole area is difficult, and requires unique optics. Finally, the walls of the well interfere with the collection of light, and with the delivery of fluorescence excitation to the well contents. These problems therefore create a far more complex situation than is encountered in most quantitative imaging applications.

Luminescence detection is much simpler, in that only the collection of light is required. Fluorescence imaging is more complex than luminescence, because both delivery and collection of light are potential sources of error, delivery being the greater problem. Methods for excitation delivery include transillumination (usually with some type of fibre-optic plate), large dichroic mirrors inserted in the optical path, lateral illumination sources spaced around a lens and (as in the LEADseeker) epi-illumination from within the lens. Transillumination, while simple to implement, fails because it generates high levels of background fluorescence. The other illumination methods have the advantage that excitation is directed away from the collection optics, thereby lowering background levels. However, these methods do not deliver perfectly even light over a large area, and this variation in light delivery interacts with the well-plate geometry (that is, entry of excitation into deep wells is affected by incidence angle, which will vary between wells).

Given these problems (and others), assay imaging instruments usually require high levels of operator interaction and require careful, and often tedious, external calibration. In the context of practical screening, prototypes are available but the reliable equipment necessary for high throughput remains elusive. Fortunately, advances in imaging technology have been rapid, as will be discussed in more detail. Data will be presented supporting the view that the problems of imaging both luminescent and fluorescent assays have been minimized and, in the opinion of the authors, imaging is no longer the major limiting factor in image-based screening. Rather, problems with assay preparation for miniaturization, dispensing or reformatting and specimen handling are presenting greater challenges to the creation of practical screening systems.

An integrated solution

The diverse nature of the problems in image-based screening favours the success of an integrated system-approach. APBiotech and Imaging Research Inc. (Ontario, Canada) have been developing the LEADseeker system as an integrated and optimized system for a variety of screening tasks. The major components of this system are:

- A cooled CCD camera
- A telecentric lens with integral epifluorescence illumination
- Software which calibrates, quantifies and reports
- A new generation of scintillating beads for proximity assays
- Validated chemistries for luminescence and fluorescence assays
- Liquid–plate handling and automation
- Service–support infrastructure.

Cameras

A CCD imager uses only a small section of the detection surface for any one well. For example, each well of a 384-well plate occupies only approximately 0.25% of the total detector surface, and it is hard to achieve high sensitivity in such small areas of the photodetector. It is possible to make the specimen so bright that sensitivity is not a major issue. For example, each well could be illuminated with an intense laser beam, so that the resulting fluorescence is easy to detect and this strategy has been successfully adopted in some instruments (e.g. FLIPR). However, limitations of this technique are that the instrument is only suitable for fluorescence or other bright assays, that the dependent variable (e.g. cell viability) may be affected by the intense illumination, and that an assay must use fluors matching the laser wavelengths.

Another strategy, used in the LEADseeker, is to make the detector so sensitive that it can achieve a usable signal under difficult conditions. This approach requires specialized cameras, but suits a wide variety of applications, including imaging of luminescence and of very dim fluorescence [including methods using minimal excitation intensity, cells which are weak expressers of labels such as green fluorescence protein (GFP), and fluorescence resonance energy transfer (FRET)]. A particular benefit of using a highly sensitive system is that adequate excitation can be achieved using broad-band lamps (e.g. halogen, mercury, xenon) with wavelength selection using filters.

Optics

Light must be transmitted from the wells to the detector and image-based screening systems accomplish this with fibre-optics placed in contact with clear-bottom plates, or with the use of lenses. Fibre-based collection optics have not yet appeared in commercial screening instruments, though they are in use and development at some sites (e.g. at Packard Instruments, Meriden, CT, USA and Aurora BioSciences, LaJolla, CA, USA).

The majority of image-based screening systems are lens-based. Lenses must be able to peer into all the wells on a plate, without geometric distortion, and they need to

gather light efficiently. If the same lens is to be used for fluorescence imaging, two additional requirements arise; the lens must be able to filter collected light so that it passes only specific emissions and the lens should allow the use of an excitation source.

Membranes, gels and tissue cultures are flat, and can be imaged with standard or, sometimes, confocal optics. Wells, however, are more difficult to image, because they have depth. Standard lenses view lateral wells at an angle, so that the walls of the well interfere with imaging. This type of geometric distortion is termed 'parallax error'. Telecentric lenses overcome this problem by directly viewing all the wells, and although they are currently widely used in well-lit industrial applications, they have lacked the sensitivity and size format for assay imaging. Recently, APBiotech, Wallac Oy (Turku, Finland) and Affymax (Santa Clara, CA, USA) have introduced telecentric lenses designed for imaging, which claim both high sensitivity and large telecentric fields of view.

Gathering light. Telecentricity, high collection efficiency, flat focus in the field of view, spatial homogeneity, high resolution and colour correction are not easily combined in a single optic. Therefore, large-aperture optical designs incorporate compromises, and optical systems designed for assay imaging vary in their performance with dim assays. A general requirement is that the sensitivity (low noise, efficient) of the detection system to variations in the assay is adequate and uniform over the entire plate, including the brightest and dimmest wells. Therefore, testing and specification of the LEADseeker is carried out by running assays at all positions on the plate, including in the centre and in the corners.

A potential problem is that any camera/lens system exhibits varying collection efficiency over the surface of a well plate, being more sensitive at the centre of the plate than at the edges (vignetting). Moderate vignetting (e.g. LEADseeker is about 10% more sensitive at the centre) can be modelled on a well-to-well basis and can then be corrected using the appropriate software. However, software correction presupposes that none of the wells are dim enough to stress the capabilities of the detector. Otherwise, there can be unpredictable interactions between the location of a well and the sensitivity of the assay at that location (corner wells will be less sensitive). The LEADseeker overcomes vignetting in two ways:

- The optical system is sensitive enough to ensure that even the dimmest wells do not overstretch the system
- Vignetting is minimal.

Illumination. The LEADseeker is essentially a fluorescence macroscope and, although as with fluorescence microscopes, monochromatic excitation must be applied and the emission filtered, illumination–emission optics used in microscopes do not scale to large areas. Rather, macrofluorescence imaging of well plates presents novel problems. For example, illumination methods that might be applicable to large flat specimens (e.g. total internal reflectance, evanescent wave) are not appropriate to the geometry of well plates. Furthermore, transillumination, which is good at getting light into wells for absorbance assays, generates high background light levels with fluorescence. Hence, large dichroic mirrors mounted between the lens and the plate have been adopted by at least one manufacturer (Wallac with their ViewLux), but the performance of such a system remains to be proven.

To minimize background levels of light and to provide a uniform illumination, LEADseeker uses a fibre-optic excitation source within the collection optics (epi-illumination). Because the illumination is directed away from the collecting lens, background levels are minimal. Furthermore, the internal illumination source achieves acceptably homogenous illumination even with deep wells, this being a key factor in fluorescence-plate imaging. LEADseeker's epi-illumination optics achieve coefficients of variation (CVs) of within a few percent (as discussed later) and these findings compare well with results obtained with standard fluorescence plate readers. In contrast, imagers incorporating other illumination sources usually present highly variable incidence angles to the wells, resulting in some wells receiving far more excitation light than others. If the light delivered to some wells is much less than that delivered to others, assay sensitivity in the darker wells can be severely compromised.

Software

Non-imaging detectors can be internally calibrated (the detector is referenced to an internal absolute standard), and do not need to make simultaneous measurements from more than one target or detector, the detector moving from well to well, making a discrete measurement at each point. Under these conditions, the detection process is relatively straightforward. By contrast, area-imaging systems are not internally calibrated and must detect light from an entire well plate in a single acquisition. This process is subject to various errors, and requires innovative correction/calibration functions within the system software. For example, LEADseeker software includes functions that correct both for differences in illumination–collection efficiency between wells and for

camera errors including dark noise, hot/cold pixels and cosmic ray events.

The software must also include functions for automated well detection, some informatics capabilities, an industrial-quality user interface and interfaces to automated equipment. Therefore, the software component of an image-based screening system requires at least as much effort as the physical components. Most of the screening imagers are very new, and immature software remains a limiting factor in their operation.

Dispensing, plate handling and automation

Achieving high throughput requires the integration of an imaging system with suitable automation components. Ideally, the imaging components should be part of a flexible tool kit and to date, LEADseeker has been integrated within automation systems from Beckman Coulter (Fullerton, CA, USA) and Carl Creative Systems (Torrance, CA, USA). However, more work needs to be carried out to enable the imaging system to be interfaced to almost any other equipment already on the screening line.

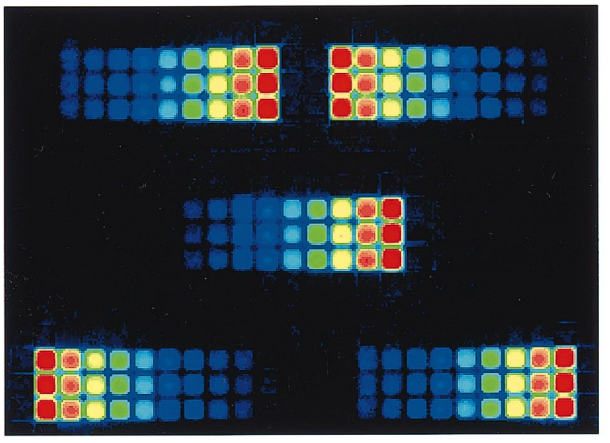
Some applications of assay imaging

Scintillation proximity

The scintillation proximity assay (SPA; Ref. 14) is detected very slowly in 384-well scintillation counters (the authors are unaware of any application of scintillation counters to SPA in a more highly miniaturized format). Although this makes the SPA an excellent candidate for miniaturization by imaging, this technology has not been available because the SPA is too dark for most cameras.

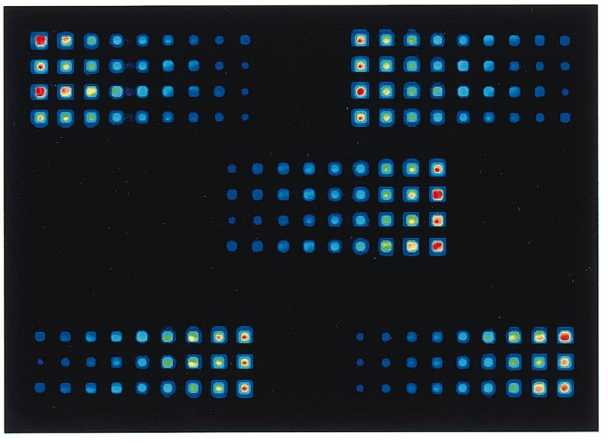
Some attempts to overcome the low signal levels have used a contact imaging approach, in which a camera is coupled to the specimen with a fibre-optic bundle instead of a lens [e.g. Hooper, C. *et al.* (1995) First Annual Conference of the Society for Biomolecular Screening]. Although this type of imager benefits from the high-collection efficiency of the fibre-optic bundle, there are also several disadvantages. For example, a highly tapered fibre-optic bundle would be required to image an entire well plate onto the much smaller surface of a CCD. Unfortunately, this tapering sacrifices collection efficiency and therefore, to reduce this effect, less tapering is used and the fibre-optic bundle views only part of the plate. This means that imaging of the plate must be acquired in several scans, introducing significant complexity (moving the plate under the detector), errors (differences across multiple acquisitions), and precluding the use of the system with assays that change over time. A second disadvantage of the fibre-optic input is its very limited depth of field. It is efficient

384



DPM (X 1000)	100	50	25	12.5	6.25	3.1	1.6	0.8	0.25	0
CV (%)	14	14	10	11	10	11	13	9	13	12
SNR	5338	2737	1496	654	339	161	81	36	13	

1536



DPM (X 1000)	100	50	25	10	5	2	1	0.5	0.25	0
CV (%)	4	5	3	6	7	5	6	7	14	6
SNR	4589	2245	1056	418	211	87	46	23	15	

Figure 2. *LEADseeker (APBiotech, Amersham, UK) images of 384- and 1536-well plates containing replicate dilution series of scintillating beads (yttrium oxide red-emitting beads, 200 or 50 μg beads per well, respectively). The test wells are distributed over the entire plate, to provide a realistic test of system sensitivity. Coefficients-of-variation (CV) and signal-to-noise ratios (SNR) are given below each image. These dilutions span the range over which most scintillation assays (including receptor binding) are conducted.*

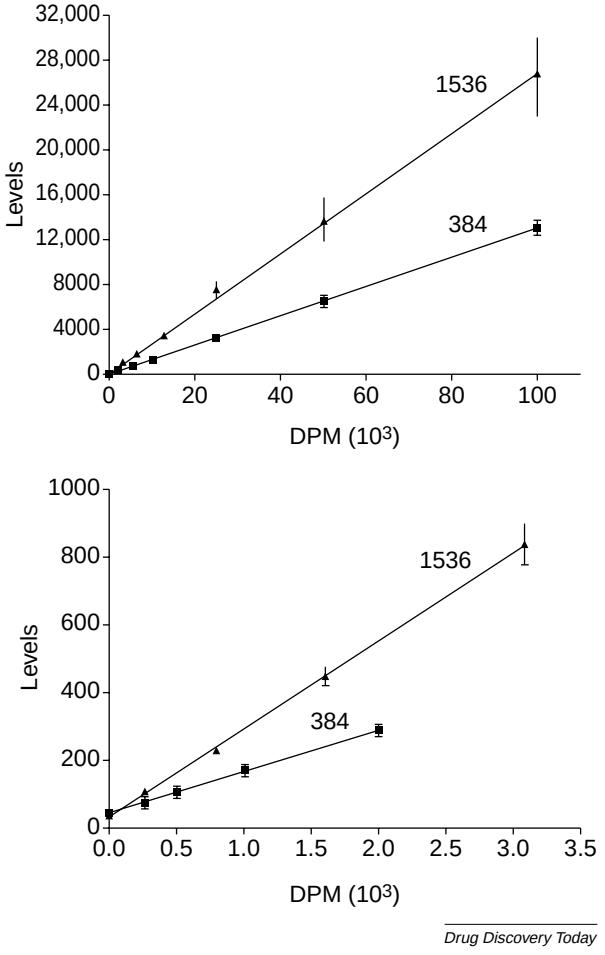


Figure 3. *Data from the images in Fig. 2 (15 replicates/value, ±SD) with full-range data at the top and a detailed view of extremely dim data at the bottom. Image luminescence values are expressed as gray levels. Levels are linearly spaced measurement intervals, which span the dynamic range of the detection system with 16-bit precision (65,536 discrete intervals). Levels from the imaging system are similar to relative luminescence units, counts and other uncalibrated measurements obtained from other detection devices. Typical scintillation counters (assume a counting efficiency of 25–50%) are usable down to approximately 500 DPM ≈ 125–250 CPM. The imaging system maintains good linearity and sensitivity to at least that point. Note that the 1536-well plate format is brighter than the 384-well plate format, even though it contains smaller quantities of the assay components. This illustrates that optimized-well geometry (in this case, concentrating light emission into smaller areas) can compensate for other factors that might reduce assay sensitivity.*

in acquiring signals from areas very close to its input, but is relatively inefficient if the signal originates from more distant areas of the well. To overcome this problem, special plates with thin, clear bottoms are used. The signal is also improved if it originates from the bottom of the wells, although this is not always possible.

An alternative to contact imaging is to take advantage of improvements in CCD detectors, lenses and in scintillating beads. For example, cameras incorporating thinned, back-illuminated CCDs offer higher quantum efficiencies than previous devices. Furthermore, new cooling technologies (using a circulating refrigerant) allow convenient operation of cryogenic cameras (cooled to liquid nitrogen temperatures) without the tedious refilling and maintenance associated with nitrogen cooling. Another new technology that contributes to improved detection is the use of new scintillating beads (imaging beads) that are brighter than the older beads and emit in the red wavelengths. LEADseeker performance with (red) imaging beads in 384- or 1536-wells is at least as effective as a scintillation counter reading the older (blue) SPA beads in 96- or 384-wells (Figs 2,3). The main difference is that the imaging system detection time is less than ten minutes to achieve >10:1 signal-to-noise ratio with a 250 disintegrations-per-minute signal (384- or 1536-well plates) compared to several hours in a 384-well scintillation counter.

An issue that arises when detecting very dark signals is that white plates are used to improve signal levels. However, white plates can phosphoresce to varying degrees, depending on the plate composition. With signals as dark as SPA, this plate phosphorescence can have a major impact on assay sensitivity, and so plates are usually given a long period (hours) of dark adaptation prior to reading. To overcome this problem, the LEADseeker incorporates a filter that selectively transmits bead signals, while removing 99% of the plate phosphorescence, enabling plates to be read immediately. It can be concluded that an assay imaging-system is capable of conducting miniaturized scintillation assays, and should exceed the performance of scintillation counters in this application.

Chemiluminescence and bioluminescence

There are many descriptions of area imaging as applied to luminescent assays on flat membranes[15–18], and many CCD-based commercial systems which claim luminescence imaging capabilities. Generally, these systems are used for flat specimens with assay imaging mentioned as a potential application of the system. Despite these advertised possibilities, the literature describing imaging of well-plate luminescence assays is rather sparse[19], demonstrating the difficulties of

using general-purpose low-light imagers for high-throughput screening of wells. By contrast, luminescence assays are relatively easy to perform with an optimized area imaging system and both chemiluminescent and bioluminescent assays produce good images using the LEADseeker (Fig. 4).

Fluorescence

Fluorescence imaging of macro specimens[20–24] has been dominated by low-cost commercial systems using gas-discharge light boxes. These systems image large, bright areas using a wide variety of CCD cameras. However, the transillumination optics generate high levels of background light, wavelength selection is limited, camera sensitivity is usually poor, and severe geometric error is seen in imaging wells. Newer, and more costly, systems (e.g. the Fuji LAS-1000, Wallac Arthur, Ref. 25) are characterized by the use of more sensitive cameras and by multimodal facilities for indirect illumination (typically edge or dorsal). These devices have been designed for molecular biology and use with well plates has not been documented, although problems with light delivery and collection can be envisaged.

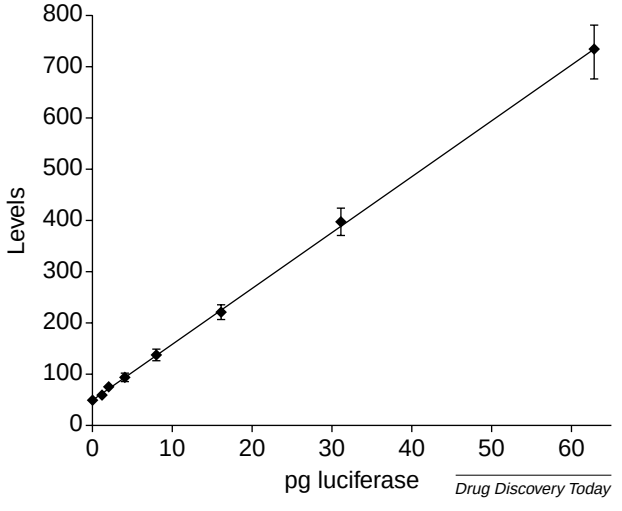


Figure 4. *LEADseeker (APBiotech, Amersham, UK) imaging of luciferase enzyme dilutions (8 μl per well, three replicates, ±SD) using the LucLite (Packard Instrument Co., Meriden, CT, USA) assay reagents and protocol, in a white 1536-well plate (Greiner Laboratories, Frickenhausen, Germany). Images were created after a two-minute exposure, in a dark-adapted plate, using a noncryogenic (−40°C) charge-coupled device (CCD) camera. Even under these suboptimal conditions (cryogenic cameras are much more sensitive), less than 10 pg of luciferase can be easily discriminated from background.*

Table 2. Fluorescein detection with the LEADseeker (APBiotech, Amersham, UK) and a large-area scanning laser (Fuji FLA-2000, FujiFilm, Tokyo, Japan) shows low and comparable coefficients of variance (CVs; n = 3 replicates per data point)

	Concentration (nM)					
	500	**250**	**100**	**50**	**25**	**10**
384-well plates						
LEADseeker [CV (%)]	2.1	2.2	2.2	2.2	3.2	3.1
FLA-2000 [CV (%)]	6.3	5.6	5.3	5.7	6.0	6.1
1536-well plates						
LEADseeker [CV (%)]	4.9	3.4	5.1	3.1	1.8	3.1
FLA-2000 [CV (%)]	2.0	2.3	3.9	2.3	2.3	2.3

There have been attempts to screen with macro-laser imagers, such as the Molecular Dynamics FluorImager/Storm (large fluorescence scanners, usually applied to electrophoresis gels). However, these instruments lack the depth of focus and interface to automation components necessary to allow convenient application to most screening tasks. However, even standard laser imagers can approximate the performance of camera-based devices when used with thin, clear-bottom plates and with assays that concentrate the signal at the laser-focus plane (Table 2). Under these conditions, the confocal properties (shallow depth of field) of a scanning laser can have advantages. In the future, macro-scanning laser instruments will be adapted to assay detection. However, at the moment, scanning lasers are represented by confocal microscopes and are limited to near-micro assays[26], to discrete cell assays, and to various microvolume detection tasks (fluorescence correlation spectroscopy and other single molecule detection methods).

With CCD camera-based systems, the major problem in macro-assays is the delivery of excitation to the contents deep in the wells. Because of this limitation, there is little data to support the use of macro-imaging in fluorescence screens[27,28]. LEADseeker accomplishes illumination delivery via the integral epi-illuminator, and the system is competent with a broad variety of fluors (including UV, see Fig. 5). As more assays are verified within the platform, high-throughput fluorescence screens are becoming practical.

One interesting aspect of imaging systems is the potential to conduct time-resolved assays over an entire plate. A LEADseeker variant incorporating a cooled, intensified, gated camera has been used to perform time-resolved fluorescence (TRF)-detection of Europium in 384- and 1536-well plates (Fig. 6). The gain in sensitivity using this

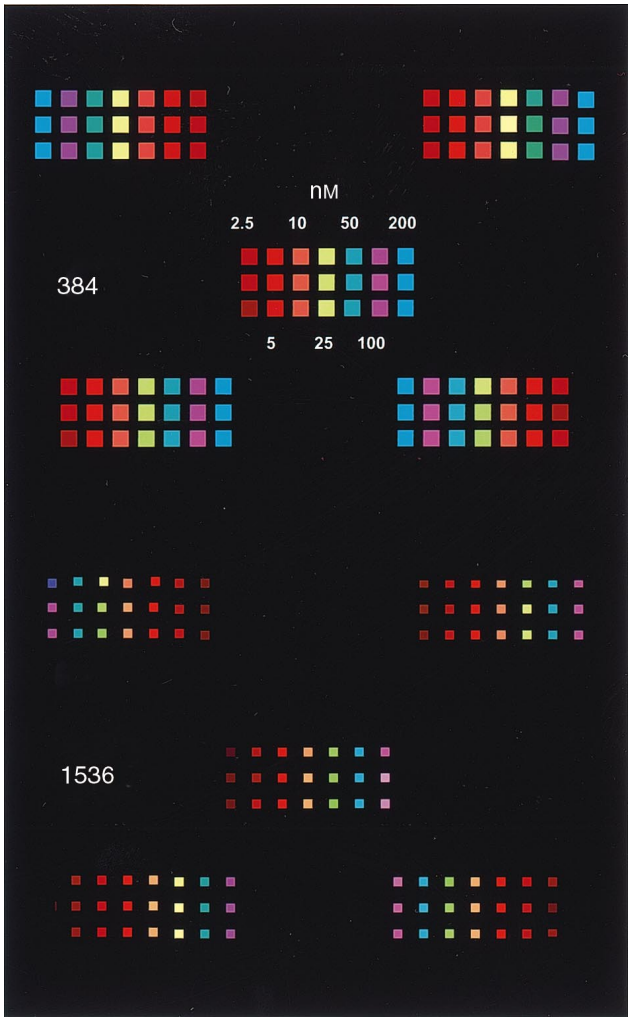


Figure 5. *LEADseeker (APBiotech, Amersham, UK) imaging of a fluorescein dilution series in 384- and 1536-well plates (black, clear bottom). The dilution series (100, 50, 25, 10, 5, 2.5, 0 nM) spans a range that is useful in many types of assays. Note that the system exhibits good uniformity over the entire plate area and illustrates the operation of a sophisticated correction algorithm that compensates for spatial variations in the delivery and collection of light.*

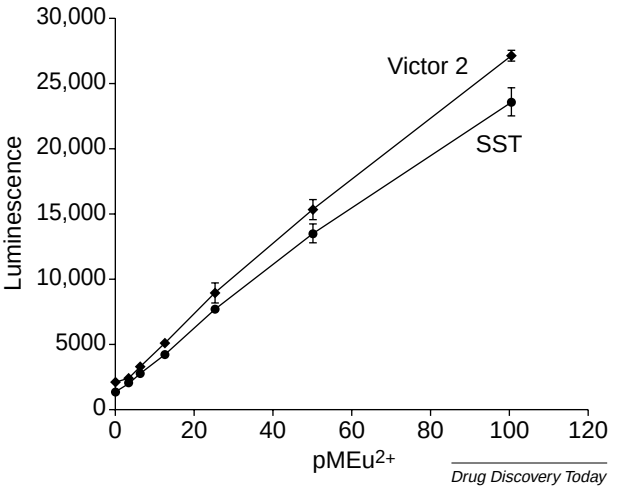


Figure 6. *Comparison of time-resolved data from a LEADseeker variant (APBiotech, Amersham, UK), and a Wallac Victor 2 fluorescence-plate reader (with time-resolved fluorescence attachments) in a 384-well plate format. Image luminance values are expressed as gray levels. Levels are linearly spaced measurement intervals that span the dynamic range of the detection system with 16-bit precision (65,536 discrete intervals). This Europium (Eu^{2+}) dilution series, including enhancer, shows clean data down to below 10 pM, more than a 2-log gain compared to prompt fluorescence. The correlation between the same data read by imaging and by the fluorescence plate reader is >0.999.*

system is two to three orders of magnitude, relative to the prompt detection of Europium, and system sensitivity approaches that of a TRF-capable fluorescence plate reader, such as the Wallac Victor 2 (Wallac Oy).

Absorbance

Of all the potential applications of an image-based screening system, absorbance assays are probably the simplest in that illumination is both simple and bright (Fig. 7). Plates can be illuminated using diffuse transillumination, providing plenty of light, therefore enabling the use of cameras and optics with relatively low sensitivity[29]. Given the relative ease with which an image-based absorbance reader could be implemented, it is somewhat surprising that screening-grade instruments have yet to appear, although it is expected that this situation will change in the near future.

Free-format assays

Regularly spaced samples (wells and dot blots within a grid) can be referred to as fixed-format assays, whereas irregularly spaced samples (e.g. electrophoretic separations, Fig. 8) can be termed free-format assays. Historically, free-format assays have used image-analysis systems, which can detect and quantify data at any position within a specimen. In fact, there are some types of screens (e.g. tissue culture lawns with bead-bound libraries, protein

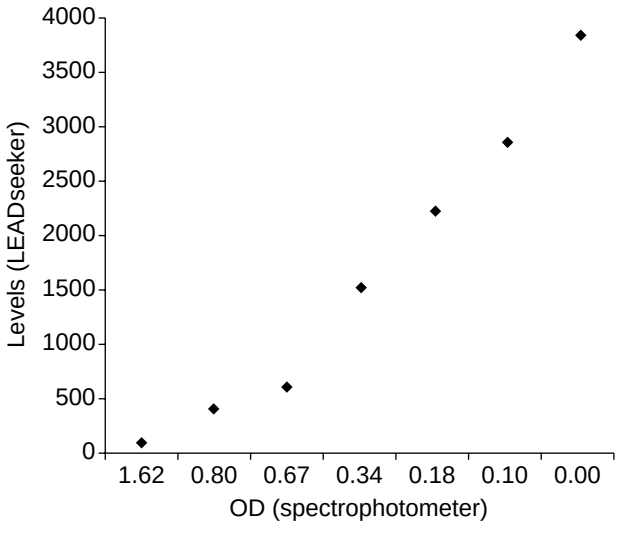


Figure 7. *Comparison of the results using imaging (LEADseeker, APBiotech, Amersham, UK) and a spectrophotometer (Beckmann, Fullerton, CA, USA) in a model absorbance assay. The imaging system viewed 2 μl of a yellow dye (fluorescein) in a 1536-well plate (black, clear-bottom), whilst the spectrophotometer applied white light and viewed absorbance at 490 nm in 1 ml cuvettes. Image luminance values are expressed as gray levels. Levels are linearly spaced measurement intervals that span the dynamic range of the detection system with 16-bit precision (65,536 discrete intervals). The imager used 485 nm filters on both excitation and emission. With both instruments, coefficients of variance (three replicates per concentration) were ≪1%, indicating excellent sensitivity. The imaging data also correlated well with data from the spectrophotometer (R_{xy} = 0.97).*

analyses with two-dimensional gels) in which the imaging system's ability to work with free-format specimens is very useful.

Conclusions

Image-based screening is a viable alternative to traditional detection technologies. Because the detection and analysis procedures can be very rapid, bright luminescence or fluorescence assays can be analyzed in less than one minute per plate (384- or 1536-well plates). By contrast, dark luminescence and scintillation assays require longer exposures (e.g. five minutes per plate), suggesting a conservative throughput of about 30 plates per hour for

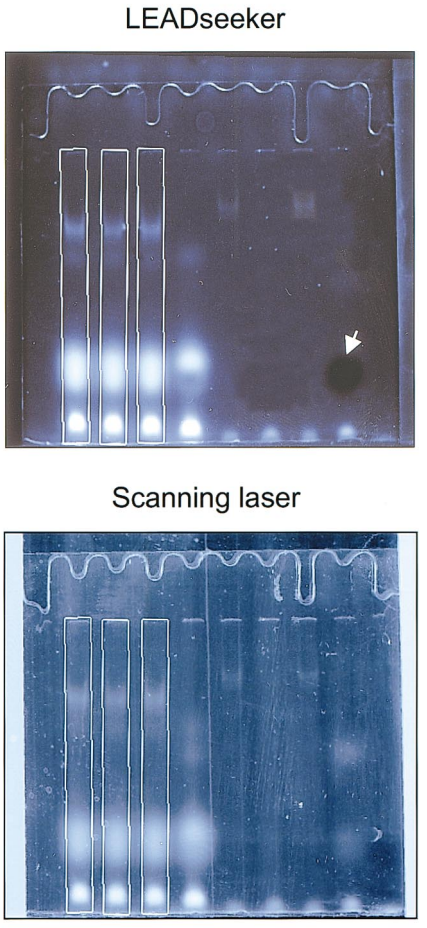


Figure 8. *Free-format imaging. Fluorescein-labelled gel-shift assay imaged with a LEADseeker (APBiotech, Amersham, UK; 480 nm excitation, 535 nm emission), and with a Fuji FLA-2000 (FujiFilm, Tokyo, Japan) scanning laser imager (437 nm excitation, 520 nm emission). The gel is held between glass plates. Note that the LEADseeker shows little reflectance or fluorescence from the glass to the left and right of the gel matrix. This indicates good rejection of out-of-band luminescence. The LEADseeker also shows a dark area at the bottom right (see the arrow), that is an artifact within the gel. This artifact emits nothing at the very tight emission window of the LEADseeker (±5 nm), although it does emit light at other wavelengths. The FLA-2000 images high levels of reflected excitation from the glass around the specimen, as well as nonspecific fluorescence from the artifact at the bottom right of the gel. This relatively high level of nonspecific background results from suboptimal excitation at the 437 nm laser line and from the broader emission filter of the scanner.*

brighter assays, and 6–12 plates per hour with darker assays. These are realistic expectations for a LEADseeker system equipped with an automation peripheral and it would appear that, as far as the detection and analysis process is concerned, ultra-high throughput is attainable.

While these data are promising, the detection/analysis components are only part of a complete system for UHTS. Plate formulations must be optimized for imaging (e.g. low fluorescence backgrounds) to yield the best detection performance and dispensing and reformatting equipment must be adapted to the high-density plate formats. Furthermore, assays need to be verified in miniaturized formats, standard protocols developed and new applications evaluated. For example, with the use of appropriate enzymes and fluorescent-labelled oligonucleotides, an imaging system might be able to analyze mRNA by multiplex RT-PCR or analyze multiplex single-nucleotide polymorphisms.

With all of these demands, implementation of a practical system for image-based screening requires considerable resources. The recent commitment of leading suppliers to image-based screening programmes will accelerate new developments in this rapidly expanding field, and gives early adopters confidence that the technology is here to stay.

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Corrigendum

Please note three corrections to the article *High-throughput screening: new frontiers for the 21st century* by Randy Bolger published in *Drug Discovery Today* (1999) issue 6, 251–253. Firstly in line 4, the author intended to say that the conference took place from 1 to 3 March 1999 instead of 1998. Secondly, under the heading 'Critical evaluations of current technologies', in the last sentence of paragraph 3, the author would like to highlight that the statement 'LANCE, an improved version of HTRF' is inaccurate, and thirdly, HTRF is a registered trademark of Packard Instrument Company (Meriden, CT, USA).

The author would like to apologize for these inaccuracies and for any misunderstandings these have created for the readers.

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