

Five Reasons Why You Need Imaging on Your Multimode Plate Reader

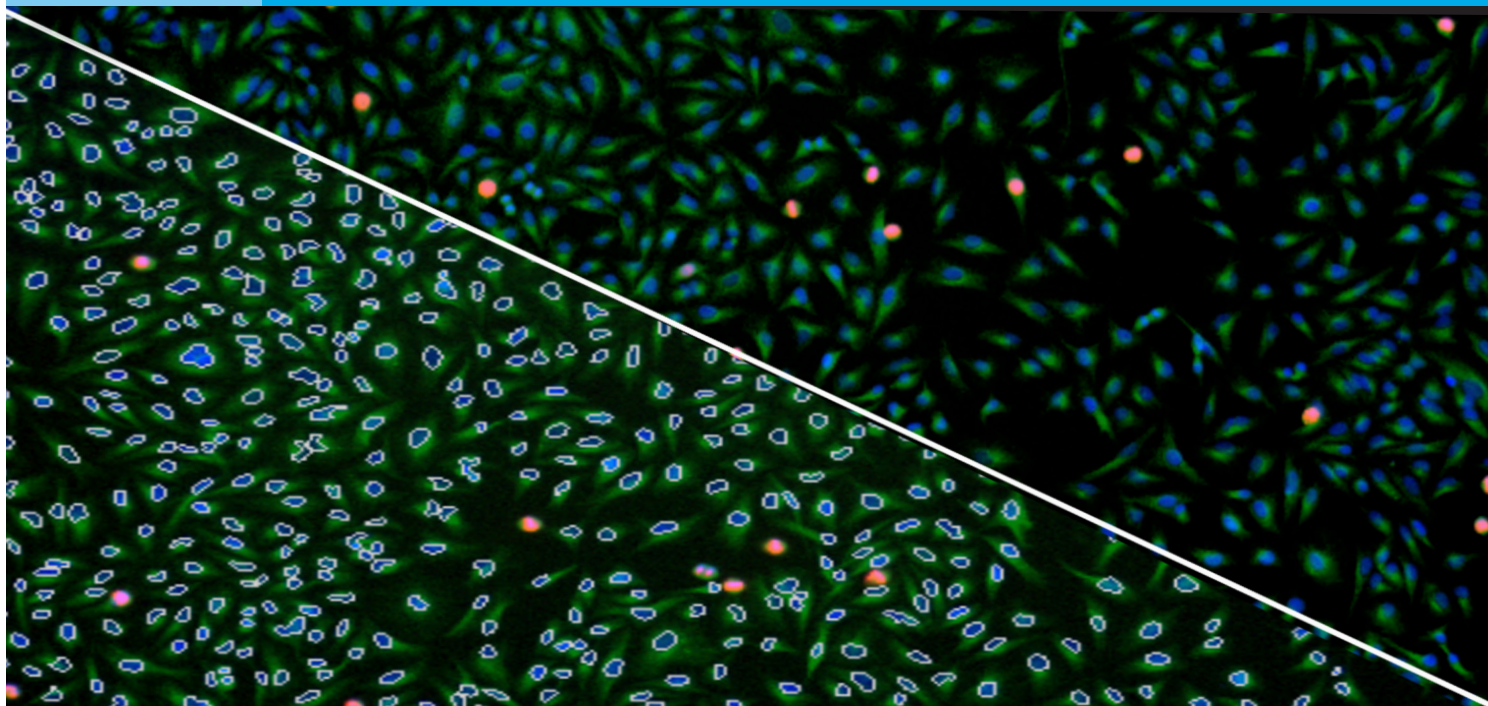


Figure 1. Automated image analysis (white masks in the bottom left) on the EnSight Multimode Plate Reader differentiates non-dividing HeLa cells (blue) from mitotic HeLa cells (red) based on their nucleus staining.

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To Image or Not to Image...That is the Question.

Plate readers first became available about 30 years ago and, during that time, market demand has driven instrument manufacturers to progress from producing single-mode plate readers to those with multiple modes of detection. This technology change may have been driven by pharmaceutical and biotechnology companies, but these tools are also the mainstay of scientific research in academic laboratories.

Multimode plate readers offering multiple detection modes, such as luminescence and fluorescence in addition to the usual absorbance mode characteristic of traditional spectrophotometers or ELISA plate readers, have become very popular. They offer flexibility, cost savings and a reduction in bench space needed, together with the convenience of employing only one instrument versus having to purchase

multiple dedicated instruments. Multimode plate readers can truly be called the workhorses for everyday screening of compounds using both biochemical and cell-based assays.

With the number of choices available in the market nowadays, selecting a new plate reader can be a daunting task. Researchers, particularly those running cell-based assays, want to extract as much information as possible from every single well of their assay. Others may want a reader that they can configure or upgrade to ensure it will accommodate an increasingly broad range of assays as the needs of their labs change and evolve over time. And others may want to leverage the value of taking an orthogonal approach, comparing and combining results from different assays, all read on a single instrument.

One of the newest features that has become available on multimode plate readers in recent times is imaging technology – the ability to generate one or more images per well of 96- or 384-well microplates. There are multiple cellular applications that are a perfect match for fast, image-based cytometry on a multimode plate reader:

- **Assay Quality** – Assess cell-based assay quality to reveal cell-seeding errors, improper liquid handling and bacterial contamination.
- **Cell Health and Toxicity** – Detect toxic effects such as the loss of membrane integrity, cell shrinkage and nuclear fragmentation.
- **Transfection Analysis** – Determine transfection efficiencies and expression levels of proteins.
- **Cell Migration** – Analyze cell motility and wound healing capabilities of cells.
- **Viral Infection** – Determine infection rates and viral toxicity.
- **Proliferation Assays** – Perform growth curves and examine cell proliferation.

Drawing on its many years of experience in both cellular imaging, with the High Content Imaging instruments Opera® and Operetta®, and multimode detection readers, such as the EnVision®, EnSpire®, VICTOR™ and new EnSight™ system, PerkinElmer has compiled a list of the top five reasons why having imaging capability on a multimode plate reader can benefit your laboratory:

1. Seeing Is Believing

Quality Control at Your Fingertips

Detecting cell mishandling errors early on in the experimentation process can save valuable time and resources later – and keep the stress levels of research scientists down. When you're working with cells, you have the advantage of being able to visually inspect the subject of your research. Using imaging on a multimode plate reader such as the PerkinElmer's EnSight system allows you to easily pre-screen your samples for cell-seeding errors, bacterial contamination or compound aggregation, and to check whether the cells are in good condition (Figure 2).

For example, stains and other chemicals might affect the health of the cells, but may not be detected. Troubleshooting problems before they happen is vital to ensure experiments do not have to be repeated due to data errors. With the additional data procured from an imaging device, the researcher can feel confident in proceeding with the experiment.

Even if cell mishandling errors occur and cause the cell number to be highly variable between samples, the quality of assay data can be improved by normalization using imaging data. Normalization of data is a common approach where researchers adjust results of conventional cell-based assays to the amount of cell material present in the samples. Normalization methods include the determination of protein content, DNA concentration or cell number of the assay sample, but often require additional sample preparation. Determining the cell number or confluency of cells automatically in the same assay plate using imaging enhances your assay quality without extra effort.

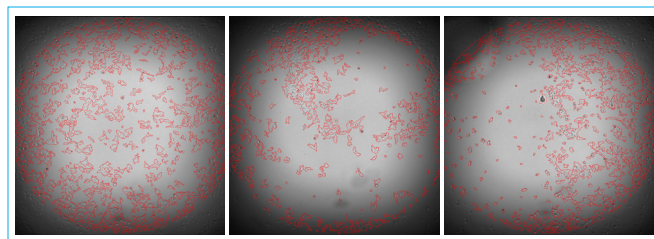


Figure 2. Brightfield images and automated confluency analysis of HeLa cells reveal cell handling issues. Correct cell handling (left) results in a confluency of 31.8%, whereas incorrect cell seeding (center, confluency: 20.5%) and improper liquid handling (right, confluency: 25.2%) lead to a decrease in cell confluency indicated by the red analysis mask.

2. Take a New Direction

Orthogonal Approaches for New Insights

By taking an orthogonal approach - comparing and combining results from different assays and using more than one parameter - researchers can gain additional data to confirm findings or add further depth of understanding. Orthogonal approaches are often achieved by examining cells from different angles using several assay formats. However, in many cases, assay kits include reagents which cannot be combined with each other, due to biochemical interference or cell lysis, which makes it impossible to analyze the cells using two assays, either together or sequentially, in the same plate. As a consequence, several plates have to be prepared and handled identically to ensure consistent assay conditions between the different assays. Using imaging as a non-invasive readout that leaves cells intact can replace certain assay formats, and you can be secure in the knowledge that your assay is reproducible as you're using the same sample for different readouts. Furthermore, since imaging enables several analysis parameters per well, orthogonal data can be obtained even without using other assay technologies.

Combining imaging with other detection technologies on a plate reader can also enable researchers to get a more complete picture from their assay data. For example, the effect of test compounds on cell viability and proliferation is often determined using a classical endpoint assay such as intracellular ATP with a luminescent readout. However, ATP detection is not a "universal" readout and might not reflect some cell growth and toxicity mechanisms. Another potential pitfall is that active substances can sometimes interfere with the biochemistry of assay reagents and their effect may be masked. By using alternative assay technologies, such as image-based cytometry, compound effects can be investigated from different perspectives: you can combine brightfield and fluorescence well-imaging with luminescent ATP measurements to investigate cytotoxic effects from many angles - cell morphology, nuclear and cytoplasmic characteristics, as well as intracellular ATP – all in a single plate (Figure 3).

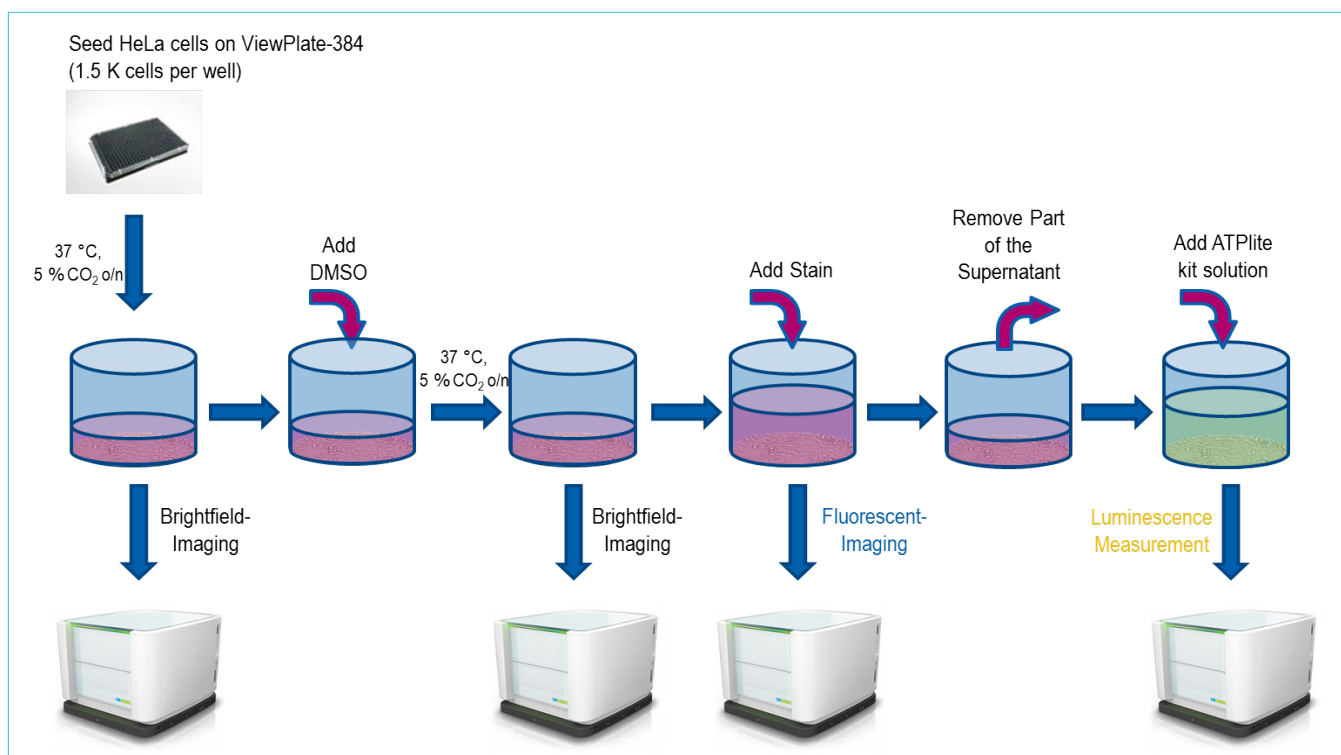


Figure 3. The workflow for multiparametric cytotoxicity analysis combines brightfield and fluorescent imaging with a luminescence readout to investigate different aspects of cytotoxicity in the same well.

3. Results in Next to No Time

Rapid Well Imaging

With PerkinElmer's EnSight Multimode Plate Reader, imaging and analysis of a single-channel measurement of a 384-well microplate can be performed in less than five minutes, and for a single-channel protocol on a 96-well plate, measurements can be completed in less than two minutes. With such short imaging times disturbances to cells are minimized and a CO₂ gas module is not required, since cells are not sitting in the plate reader for a prolonged period.

Remember how much time you've spent looking down a microscope, examining your cells well-by-well? With short imaging times, measures such as cell viability or cell confluency can be quickly and accurately assessed for the whole plate. Time savings are amplified in applications which involve the analysis of multiple time points, as this can be carried out rapidly without any detrimental effects, using a single preparation of cells.

Table 1. Typical plate imaging and analysis times for a single-channel measurement on the EnSight Multimode Plate Reader (Cell count based on Hoechst stained nuclei at 100% and 1ms exposure time).

Throughput (Time Per Plate)	
96-well	< 2 minutes
384-well	< 5 minutes

4. Don't DIY

Automated vs. Manual Imaging and Analysis

Compared to manual imaging and analysis using a microscope, automated plate imaging gets you faster, more detailed and more accurate results.

Automating imaging and analysis avoids the manual observation and calculation of, for example, cell counting, normalization and confluency determination which can be very time-consuming and error-prone. With the EnSight system, this is done quickly and accurately for you by the software, as focusing is automated and imaging settings are set up for a whole plate rather than for individual samples.

If you are new to imaging, you might think that learning to set up and analyze experiments will require significant training. But with the right software to guide you through the process, you can quickly learn to generate and analyze your imaging data, and gain new insights from your results.

PerkinElmer's Kaleido™ data acquisition & analysis software includes pre-programmed image analysis methods so that you can get up and running without delay (Figure 4). You don't have to be an expert in image analysis and there is no need to develop your own image analysis methods. If your research requires a customized approach, then custom protocols can be developed that are specific for your image analysis needs. Images are automatically generated, analyzed during measurement time and data can be exported to a spreadsheet for further analysis.

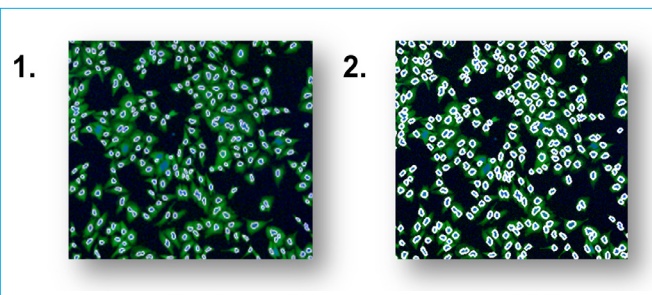


Figure 4. Example of automated image analysis. Cell nuclei are automatically detected via a nucleus stain (1) and a ring structure is used to analyze the intensity of a cytoplasm stain (2) while the plate is measured. White masks are provided by the Kaleido software to visualize the analysis method during analysis setup.

5. Do More for Less

Use Your Resources Efficiently

Broadening the detection capabilities of a multimode plate reader to include brightfield and fluorescence imaging brings all the benefits of these modes to the lab, whilst saving precious bench space, and eliminating the need for separate detection instruments. Upgradeable systems such as the EnSight from PerkinElmer allow researchers to add new modes and capabilities over time. Lab budgets are under increasing pressure these days so it may be less expensive to purchase an imaging module on a plate reader than make a separate investment in a dedicated imaging instrument. If there is a confocal microscope or high-content analysis system already in the laboratory, using the imaging capability on a multimode plate reader frees up these other devices for assays that need higher resolution or more detailed imaging.

Conclusion

There are many choices of multimode plate reader available in today's marketplace. Choosing a plate reader with an imaging mode brings many benefits to the research laboratory. Using imaging to help detect and avoid cell handling errors will pay dividends quickly by not having to repeat experiments. Researchers can start imaging and get high quality results in minutes with the right software. Having the ability to use one instrument for plate reading and imaging saves on the cost of purchasing multiple instruments, and saves precious bench space. PerkinElmer's EnSight Multimode Plate Reader, together with the pre-programmed image analysis methods of the Kaleido software makes the set up and running of imaging assays fast, straightforward and intuitive. Imagine what you could do with a plate reader with imaging in your lab.

Visit our website for more information: www.perkinelmer.com/EnSight

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