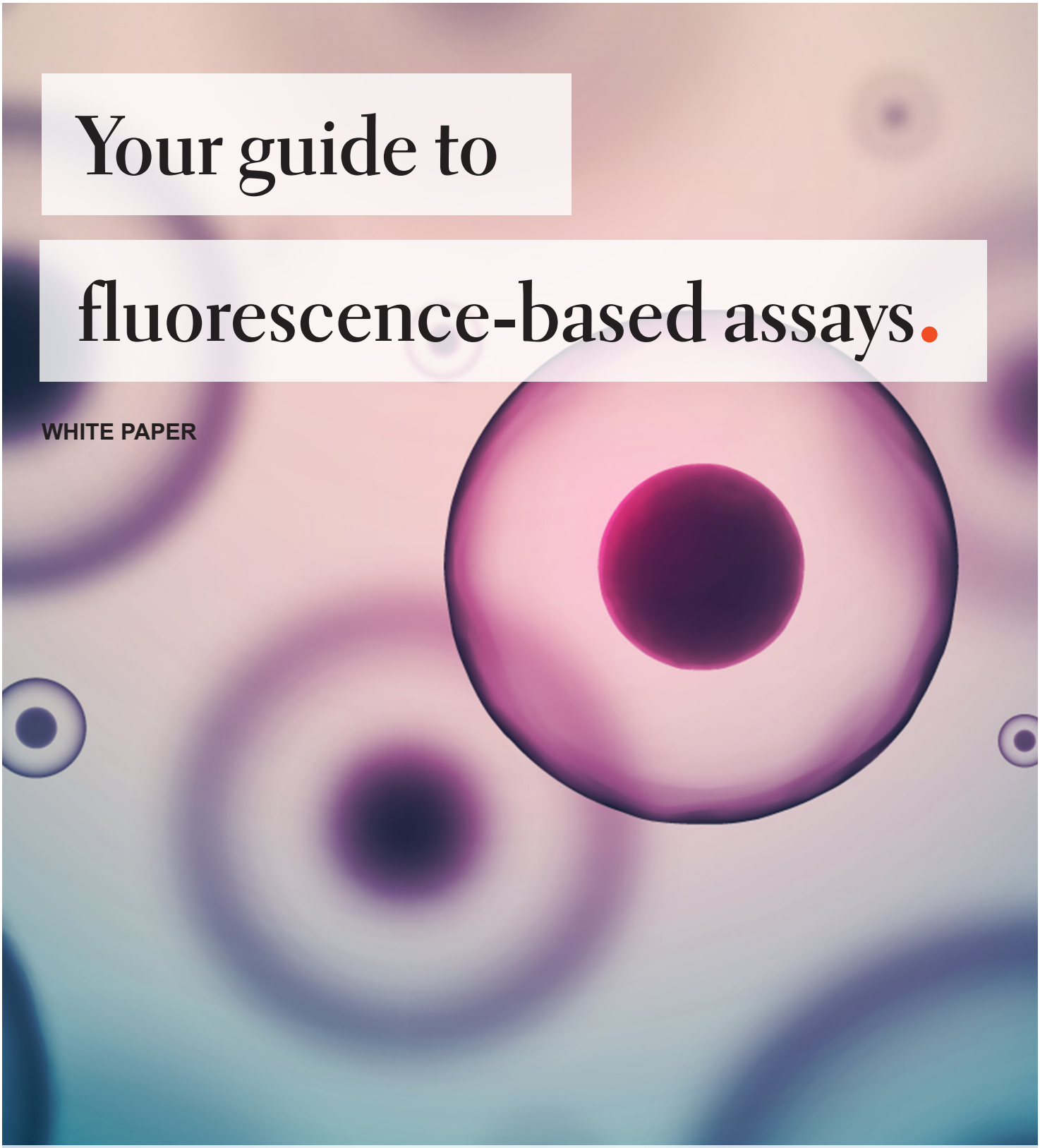
Your guide to fluorescence-based assays.

WHITE PAPER



A BRIGHTER FUTURE WITH FLUORESCENCE-BASED ASSAYS

Compared to many other detection technologies, fluorescence provides hard-to-beat performance and flexibility. Fluorescent labels are stable for months and deliver high sensitivity, and the diversity of the available dyes offers almost unlimited possibilities for assay design. Together with many other advantages, this makes fluorescence detection one of the safest and easiest ways for you to improve the quality and sensitivity of your assays.

Strong, stable signal

Fluorescence has established itself as the major detection technology in the biosciences sector for many reasons. It does not require the addition of detection reagents, and can be read multiple times – essentially as often as the sample can withstand exposure to light. Some fluorescent dyes – such as the lanthanides – have special properties, including emission signals with long decay times. Measuring the emission of lanthanides in a time-resolved manner with a delay of a few hundred microseconds improves the signal-to-noise ratio and boosts sensitivity.

Fluorescently-labeled reagents are also highly stable, with a long shelf life that enables the efficient preparation of large, standardized batches that can be used for extended periods. This minimizes inter-assay reagent variability.

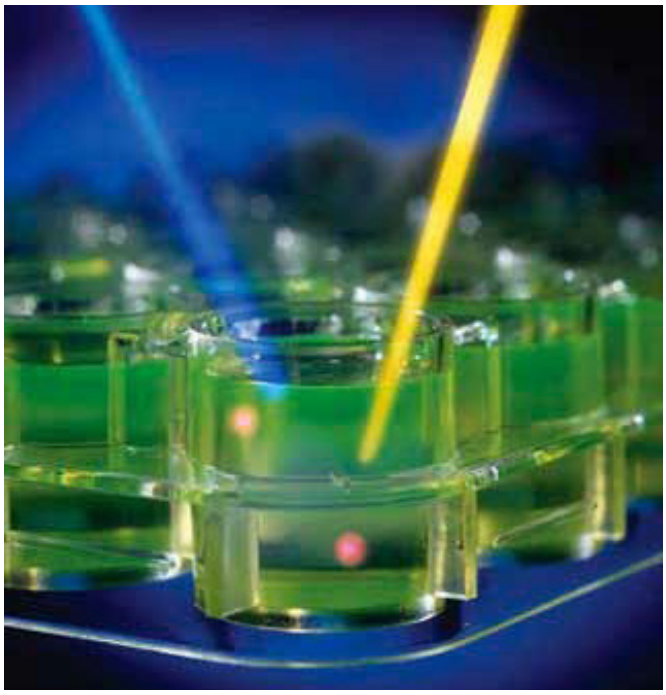


Figure 1: Fluorescence-based assays are stable, simple and sensitive

Seemingly endless possibilities

The enormous popularity of fluorescence-based detection has stimulated the development of a myriad of kits from a large number of suppliers, ensuring fast access to research tools. Fluorescent tags can be added to almost every biomolecule and used for many applications, such as identification, quantification or tracing biological interactions. Some suppliers even offer a choice of ready-to-use biomolecules labeled with a fluorophore. Other commercially available fluorescent molecules include nucleotides and enzyme substrates.

The development of molecular biology-based fluorescence labeling has revolutionized the study of gene expression. For example, green fluorescent protein (GFP) can be fused to proteins or used as a reporter to follow gene expression, enabling researchers to study processes in living cells that were previously invisible. GFP fusion is also becoming increasingly popular for protein biochemistry, to monitor protein production and purification without the need for chemical labeling.

Multiplex for normalization and multi-target identification

Using fluorescence detection, you can simultaneously measure a number of target molecules in a sample by labeling each one with a different fluorophore. This ability to multiplex makes fluorescence detection the technology of choice in many situations. For example, you can concurrently measure the number of live and dead cells in a sample by labeling one red and the other green. Multiplexing is powerful, because it allows researchers to interrogate multiple targets in cells of a given state. All you need is a reader that can flexibly accommodate a variety of detection settings, and deliver high sensitivity over the complete spectral range.

MASTER THE CHALLENGES OF CELL- BASED FLUORESCENCE ASSAYS

Cell-based assays are giving us deeper insight into cellular mechanisms in a true biological context. Fluorescence assays are playing a leading role, with applications ranging from cytotoxicity, proliferation, apoptosis and G protein-coupled receptor (GPCR) signaling assays to high throughput screening (HTS) in drug discovery.

When developing any cell-based fluorescence assay, you need to consider issues such as tight environmental control, avoidance of microplate edge effects, and cell density optimization. There are also issues specific to fluorescence assays to address, including the selection of dyes and optimization of the excitation and emission wavelengths. Finally, the cell culture environment itself also raises challenges, which the right approach can overcome.

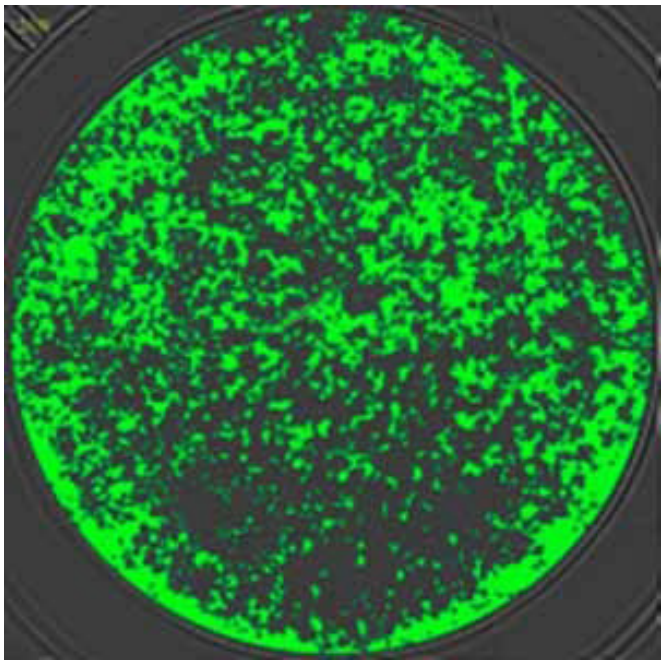


Figure 2: Cell-based fluorescence assays have revolutionized our view of cellular processes. This image shows automatic cell confluence determination using the Spark® multimode reader's integrated bright field optics and software-based labeling (green) of identified cells.

How to cope with autofluorescence background

Autofluorescence is a typical problem that needs to be addressed when developing a high performance, cell-based fluorescence assay. A common source of this background fluorescence is the phenol red dye used as a pH indicator in most cell culture media. One strategy to minimize phenol red's influence on cell-based assays with adherent cells is to take readings from the bottom, rather than the top, of the plate. This avoids light from the fluorophore having to travel through the medium to reach the detector.

Choosing the wrong plate can also lead to higher background. White plates increase the background in a non-time-resolved arrangement, because they reflect the light used for excitation, while clear plates allow crosstalk between neighboring wells. Therefore, black plates with a transparent bottom are always the first choice for cell-based assays with adherent cells, since they minimize background, inhibit crosstalk and allow bottom reading.

Advanced optics enable analysis of cell monolayers

Unlike detecting and measuring fluorescence in homogeneous liquid samples, cell-based assays generally involve analyzing adherent cells that are unevenly distributed across the bottom of the well. Achieving a robust, sensitive assay therefore means taking multiple measurements at different points across the entire well bottom. Achieving high assay sensitivity also depends on measuring only the signal from the thin monolayer of adherent cells. Focusing the emission detection on the well bottom maximizes the signal and minimizes the background fluorescence from, for example, the medium above the adherent cell monolayer. These requirements demand sophisticated optics that enable emission detection across the whole well bottom and can focus signal detection on a shallow depth within the total well height.

Signal intensities from cell-based assays obviously greatly depend on the cell number in each well. To ensure that experimental results can be compared, the cell number should be checked before starting the experiment and again before detecting the signal. This enables the signal to be normalized and easy identification of outliers caused by wells with deviating cell numbers.

For more information on measuring cell confluence to improve measurement consistency, see “Live cell imaging” lifesciences.tecan.com/live-cell-imaging.

Capabilities for demanding cell-based fluorescence assays

Implementing fluorescence detection is one of the easiest and safest ways to improve the quality and sensitivity of your assays. Tecan’s Spark multimode microplate reader can deliver the flexibility you need to develop a wide range of fluorescence-based biochemical and cell-based assays to meet your needs now and in the future.

About the author

Dr Stefan Haberstock studied biology at the Trinity College Dublin/Ireland and at the Gutenberg University of Mainz/ Germany where he received his diploma degree in 2008. In the following he joined the research group of Prof Dötsch at the Goethe University of Frankfurt/Germany and graduated with a PhD degree awarded for his research on the cell free expression of membrane proteins. In 2012 he joined Molecular Sensing Inc. supporting the commercialization of the innovative technology back scattering interferometry as application specialist. In 2013 he joined Tecan where he is part of the European sales development team in the life science area.

Spark multimode reader is for research use only.

Australia +61 3 9647 4100 **Austria** +43 62 46 89 330 **Belgium** +32 15 42 13 19 **China** +86 21 220 63 206 **France** +33 4 72 76 04 80 **Germany** +49 79 51 94 170 **Italy** +39 02 92 44 790 **Japan** +81 44 556 73 11 **Netherlands** +31 18 34 48 17 4 **Nordic** +46 8 750 39 40 **Singapore** +65 644 41 886 **Spain** +34 93 595 25 31 **Switzerland** +41 44 922 89 22 **UK** +44 118 9300 300 **USA** +1 919 361 5200 **Other countries** +41 44 922 81 11

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