

Multidetection Microplate Readers: Filter-Based, Monochromator-Based. . .or Both?

by Xavier Amouretti

The appeal of multidetection microplate readers is easy to understand: they combine multiple measurement technologies such as absorbance, fluorescence, and luminescence into one compact instrument. This, along with both the extension of the microplate format into new applications and the continued development of new assay technologies, creates a powerful research tool. This observation is substantiated by the tremendous growth of the detection platform over the last several years, leading one to believe that the multidetection microplate reader will become the *de facto* choice for most researchers.

Given the great variations found in microplate-based assays, the promise of multidetection microplate readers is that they can accommodate new and unexpected user requirements and applications when they arise. This article reviews one of the most important decisions to be made when purchasing such a reader, that is, the type of wavelength selection system available: filter-based or monochromator-based. The choice has a fundamental impact on what reagent technology will be possible to perform on the instrument. The author reviews the main drivers behind the choice of filters versus monochromator systems and the significant benefits offered by the patent-pending Synergy™ 4 Multidetection Microplate Reader with Hybrid Technology (BioTek Instruments, Winooski, VT) (Figure 1).

Monochromator-based readers

Monochromators use diffraction gratings to physically separate the individual wavelengths present in the white light coming from the instrument's light source. A series of slits allow for selecting a specific wavelength to excite the sample. A similar system is used to clean the signal coming from the sample before it is measured. Monochromator-based readers offer several evident benefits (summarized in Table 1). First, they are very convenient to use. The wavelengths to be measured are easily selected through the software and are generally available in 1-nm increments. Manipulation and storage of accessories are



Figure 1 Synergy 4 multidetection microplate reader.

unnecessary, contrary to filter-based systems. Adjustments for new applications and wavelengths are relatively simple, without an increase in time or cost for extra optical elements.

Second, monochromators can run spectral scans that can be used to characterize new fluorophores or study spectral shifts in some assays. The usefulness of the scanning function, however, is limited by the fact that microplate readers are optically different from dedicated analytical cuvette-based spectrofluorometers. The 90° angle between the excitation beam and the emission channel found in cuvette-based readers (horizontal photometry) is impossible to achieve in a microplate reader (vertical photometry). For this reason, microplate readers exhibit higher background noise than cuvette-based readers and cannot provide the sensitivity and resolution expected from an analytical-grade instrument.

The third benefit of monochromator-based microplate readers—flexibility—is mostly perceived. Because these

Table 1 Main advantages of monochromator-based and filter-based microplate reader designs

Monochromator-based microplate readers		Filter-based microplate readers	
Feature	Benefit/application	Feature	Benefit/application
No need to purchase filters	Convenience, cost savings	Less expensive	Cost savings
Spectral scanning function	Characterization of new fluorophores, study of spectral shifts	More sensitive	Better results, cost savings on reagents
Full range of wavelength available	Convenience and flexibility	Bandwidth selection	Weak fluorescence, dyes with large Stokes' shifts
		Fast wavelength switching	Dual-wavelength assays with fast kinetics such as ion channel assays
		Injection system	Ion channel assays, flash ATP, flash luciferase assays
		Filtered luminescence	BRET, BRET2™ (PerkinElmer, Waltham, MA), Chroma-Glo™ (Promega Corp., Madison, WI)

microplate readers are often more expensive than filter-based microplate readers and allow reading at most wavelengths, it is assumed that they can run more assays than filter-based microplate readers. Further discussion will reveal that the contrary is true.

Filter-based readers

Optical filters are characterized with a central wavelength and a bandwidth. These two fixed parameters precisely define which wavelengths are going to the sample on the excitation side, and from the sample to the detector on the emission side. Filter-based microplate readers have a number of important advantages (see Table 1). First, they are typically less expensive than monochromator-based microplate readers. This comes from the fact that filter wheels are much less expensive parts than monochromators, and the light sources required to obtain the same level of sensitivity do not need to be as powerful.

The second benefit is sensitivity. All things being equal (quality of optical elements, power of light source, detector used), a filter-based microplate system will be more sensitive than a monochromator-based microplate system. Filter systems are more efficient at delivering light to the sample. They are also very efficient at providing correct light blocking between the excitation channel and the emission channel. Thus, the paradox is that filter-based microplate readers will usually be more sensitive than monochromator-based microplate readers for less money.

A third advantage of filter-based systems is bandwidth selection. A filter can be specifically tailored to a par-

ticular assay (or fluorophore) to obtain maximum sensitivity, and can have a bandwidth anywhere between 5 nm to more than 100 nm, selectable in 0.5–1 nm increments. Monochromator-based microplate readers come with a fixed or limited range of bandwidth selection. Given the fact that they measure in epifluorescence mode (not at a 90° angle, as found on cuvette-based readers), this bandwidth limitation is a problem with low-level fluorescence, where a large measurement bandwidth is necessary, such as AlphaScreen® assays (PerkinElmer), which require a strong excitation and the use of a 100-nm emission bandpass.

The next advantage is that filter wheels allow quick switching back and forth between two wavelengths. Typical assays where this is required are ratiometric fluorescent ion channel assays. In these assays, the signal needs to be monitored at two wavelengths, one being used to correct the other. The reactions are extremely fast, triggered by automated injection, and the assay kinetics must be monitored at two distinct wavelengths during the few seconds that follow reagent injection. Typically, filter sets can be switched back and forth in a fraction of a second. Many high-throughput microplate readers are equipped with dual-filter systems and detectors to read these types of assays. Monochromator microplate readers cannot run these ratiometric assays because of the time it takes to move the monochromator position from one wavelength to the other. A secondary consequence of this difference is that all filter-based multidetection microplate readers are available with reagent injectors to run these types of assays; most monochromator-based microplate readers are not. Very common assays such as flash luminescence (e.g., flash luciferase, or flash ade-

nosine 5'-triphosphate [ATP] assays) do require automatic injection.

Luminescence brings us to the final major advantage of filter-based systems: efficient light transmission. Because of the significant amount of light lost through dual-monochromator systems during wavelength selection, luminescence cannot be read with adequate sensitivity. As a consequence, light is typically channeled directly from the sample to the detector, without going through the monochromator system. This works very well for whole light assays such as glow ATP and glow luciferase assays, but does not allow for light filtration. Some key applications of luminescence involve the need for emission filtration: Bioluminescent Resonance Energy Transfer assays (BRET, BRET2) or Chroma-Glo assays are examples of dual-wavelength assays that cannot be run on monochromator-based microplate readers, unless they are equipped with a separate dedicated filter-based luminescence system.

The numerous advantages of filter-based systems over monochromator-based systems explains why, as previously mentioned, filter-based microplate readers are in practical terms more versatile than monochromator-based microplate readers, since they will allow the user to run more of the typical applications found in the microplate format.

Uniting wavelength selection systems

Hybrid Technology, introduced in the Synergy 4, is a significant step forward in the design of multidetection

microplate readers. By combining the benefits of both filter-based and monochromator-based fluorescence detection, it brings to laboratories a new level of flexibility and convenience. At a price similar to monochromator-only systems, the Synergy 4 bridges the gaps highlighted previously, and provides a design that covers virtually all microplate-based applications.

Summary

In the end, while it appears that multidetection microplate readers are purchased on the promise that they will meet future needs, both filter-only and monochromator-only microplate readers have important limitations in the type of assays they can perform. Filter-based microplate readers do not allow spectral scanning, and necessitate the use of specialized filters for each new fluorophore used. Monochromator-based microplate systems have weaknesses inherent to the nature of their optical design, which prevent users from running some of the common applications found in the microplate format. One microplate reader that unites the two technologies within a single footprint can truly satisfy current and future laboratory needs and applications.

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