

# Microplate readers revisited: the new Tandem Technology

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The human genome was successfully deciphered 5 years ago (1) and revealed about 30,000 genes. Thus it has become apparent that an ever increasing pool of potential therapeutic targets is now available. Researchers in life sciences and pharmaceutical companies have therefore set out to better understand the basic mechanisms underlying acute and chronic diseases such as Alzheimer's, Parkinsonism, Diabetes, and Chronic Pain.

The study of reporter genes, protein-protein binding, DNA content, and the incorporation of high throughput strategies in the primary stages of target validation has been the means of carrying out this research. Robotics, liquid handling, automation, and the miniaturization of microplates have increased the use of microplate readers in basic research and drug development. Together with the implementation of 384- and 1536-well plates, assay development is now a key factor in streamlining assays for a particular target. Thus kinase profiling, SNP genotyping, protein-protein binding, the

study of GPCRs, and cell-based assays are getting increasingly refined and more sophisticated.

The advancement of microplate reading technology has shown that multidetection readers are the best choice when it comes to using a single reader for a variety of proven assays. Recent reports suggest that absorbance measurements and FRET-based assays are widely used and will continue to be amongst other top notch applications (2,3). The challenge then was to develop multidetection readers that provide all modes of measurement for FI (fluorescence intensity), FP (fluorescence polarization), FRET (fluorescence resonance energy transfer), TRF (time-resolved fluorescence), luminescence, BRET (bioluminescence-RET), and UV/Vis full spectrum absorbance to meet the needs in research labs and biotech companies.

Microplate readers can typically be broken down into two classes – filter based and monochromator based. It is a fact that optical filters provide the best sensitivity in microplate readers. Advanced

optics, photomultiplier tubes (PMT), light guides, and their sophisticated combination provide an excellent platform for fluorescence, luminescence, and time-resolved fluorescence assays. In comparison to monochromators, filters offer the following advantages: more light transmission and excellent blocking of undesired wavelengths, higher sensitivity, precise control over transmitted peak shape, fast switching to other wavelengths when more than one filter pair is employed, and lower cost than monochromators. BMG Labtech's FLUOstar OPTIMA / POLARstar OPTIMA readers are

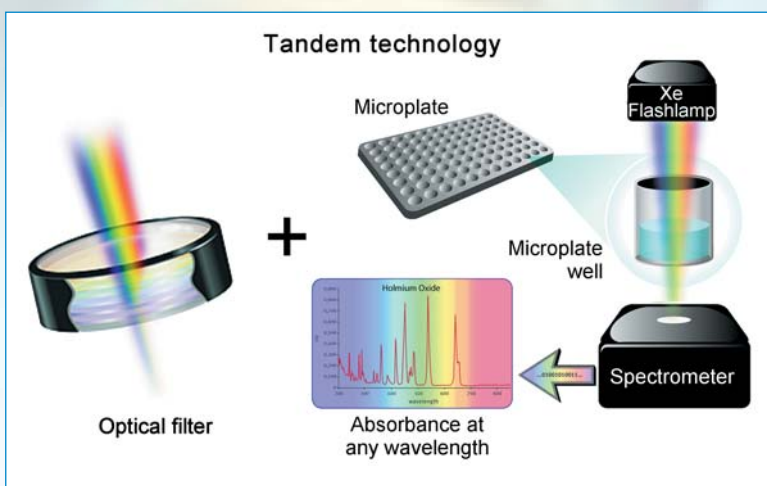


Figure 1 – The combination of an ultra-fast UV/Vis absorbance spectrometer and high quality optical filters, including light guides and photomultiplier tubes (PMT) make the concept of the Tandem Technology

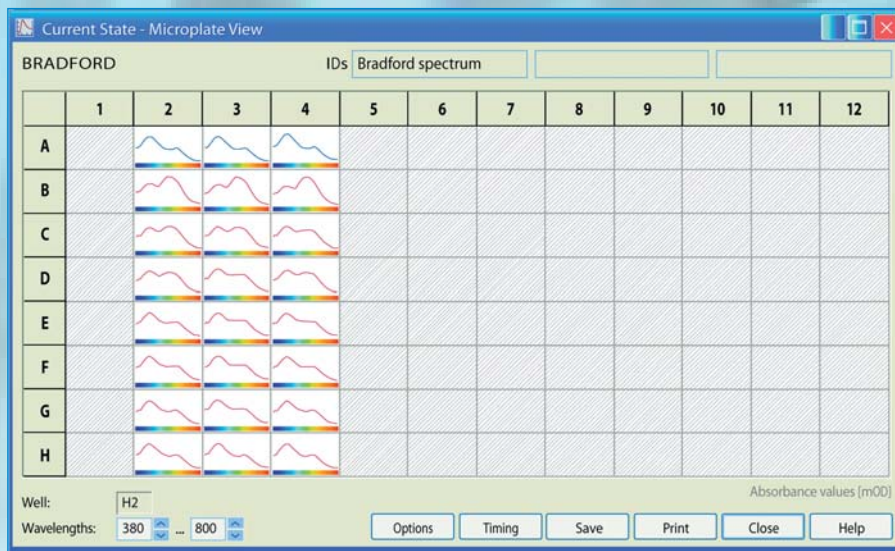


Figure 2 – Shown is the current state window of a spectral scan (380-800 nm) for the Bradford assay. Standards in red, blanks in blue ( $n=3$ ).

capable of measuring all the assays described, and offering Simultaneous Dual Emission (SDE) for fast FRET, BRET, and FP measurements. However, full spectrum absorbance measurements cannot be done and hence a new concept was developed.

Here we introduce BMG Labtech's Tandem Technology, featured in the Omega microplate reader series (Figure 1). Tandem Technology is a combination of two technological concepts – the world's only ultra-fast UV/Vis full spectrum absorbance spectrometer built into a microplate reader, and high performance optical filters.

The ultra-fast UV/Vis absorbance spectrometer incorporates highly efficient

optical grating and a solid state array detector that allows measurement of light intensity throughout the UV and visible parts of the spectrum. For the first time ever, capturing full spectral absorbance at high speed from 220-850 nm in a multidetection microplate reader can be performed at a

resolution of 1 nm. Unlike a monochromator, the spectrometer allows you to capture the entire UV/Vis spectrum of a sample in approximately one second per well – no scanning needed. It is not necessary to select a particular wavelength to monitor because the entire spectra will be available anytime you wish. However, if you choose to reduce data set size up to eight discrete wavelengths can be selected and the data will be collected simultaneously, similar to a full spectrum scan.

Data analysis is performed with the newly released MARS (Microplate data Analysis and Reduction) Software. Standard curve and the concentration for unknown samples are calculated automatically. If the option "path length correction" is used, the measured data is

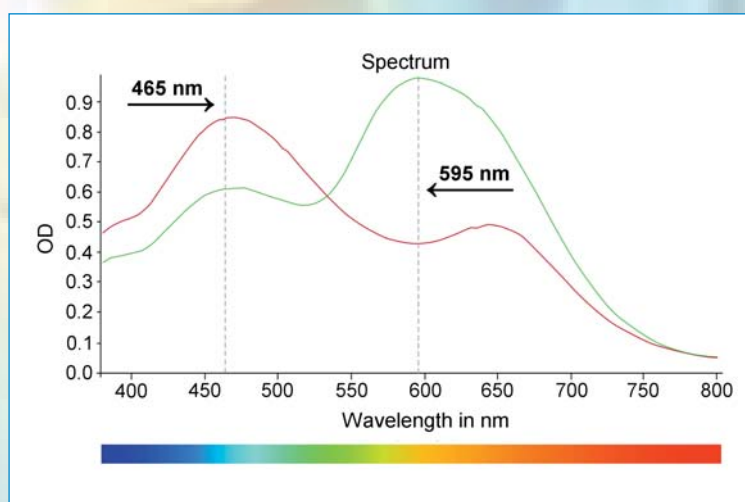


Figure 3 – A shift of the absorbance spectrum from 465 nm to 595 nm indicates a difference of unbound (red) and bound (green) protein Coomassie® Brilliant Blue

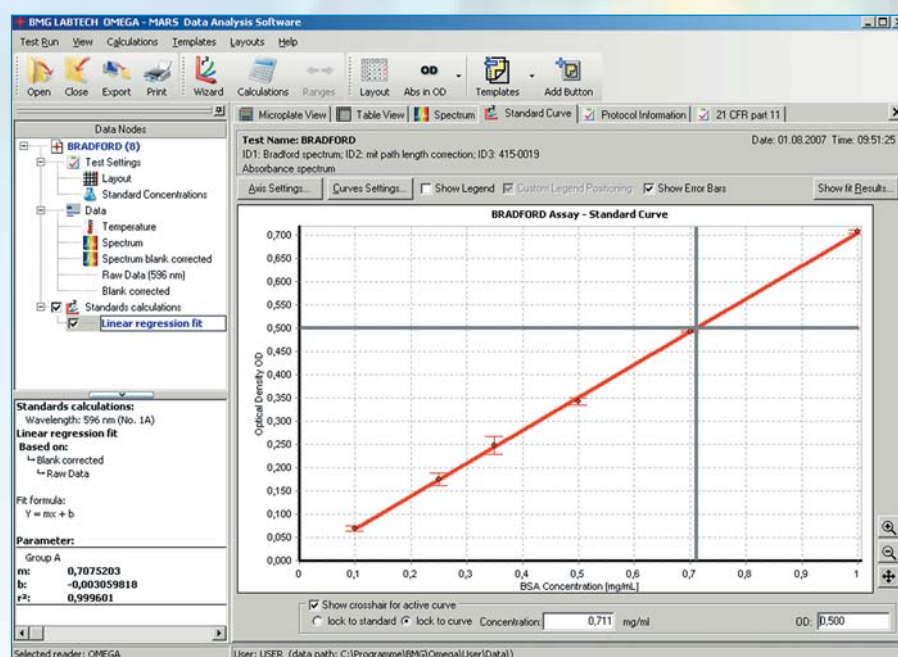


Figure 4 – Shown is the MARS software, depicting the standard curve of BSA protein concentration vs. Optical Density (OD). The crosshair was manually set to an OD=0.5 and the corresponding concentration was determined to be 0.711 mg/mL. The good quality of the fit is indicated by  $r^2=0.9996$

normalized to a path length of 1 cm, thereby allowing a direct comparison between absolute data obtained from a microplate reader and data obtained from a cuvette-based spectrometer.

The Tandem Technology was validated using the FLUOstar / POLARstar Omega readers. The Bradford Assay (protein binding) and the determination of DNA content ( $A_{260}/A_{280}$  ratio) were used to validate the absorbance capacity of the UV/Vis spectrometer.

1. The Bradford assay (4) is based on the binding of protein to a dye, leading to a shift in the absorbance maximum of the dye. The Bradford Reagent was purchased ready to use and a stock solution of bovine serum albumin (BSA) was diluted to 1 mg/mL and served as the protein standard. Bradford reagent, 290  $\mu$ L, was pipetted into a transparent 96-well microplate. 10  $\mu$ L of the protein dilution was added followed by mixing in the wells. The Bradford assay was successfully performed

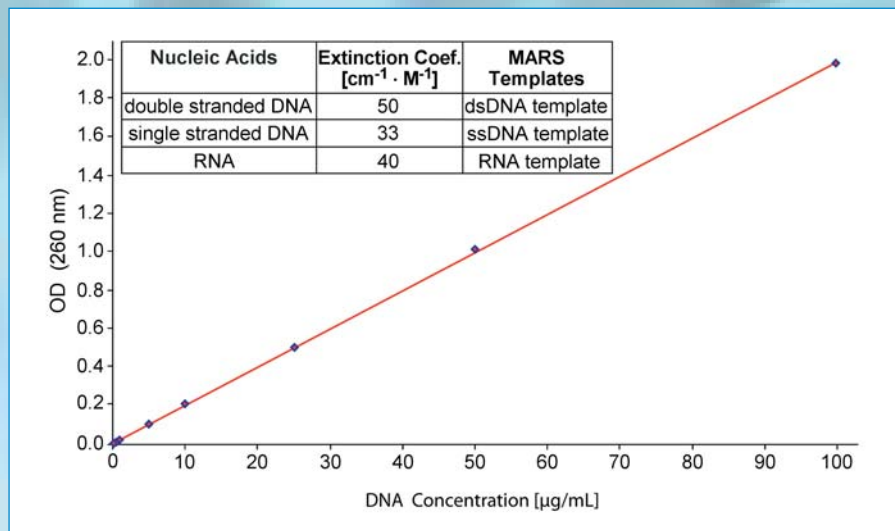


Figure 5 – A linear regression fit is shown of DNA concentration vs OD. A high linearity was obtained between 0.1 to 100 µg/ml, indicated by  $r^2=0.9998$ . The MARS software provides ready-to-use analysis templates for different nucleic acids

aliquots of distilled water served as a blank. Double stranded and single stranded DNA or RNA possess different extinction coefficients. The MARS software offers ready-to-use templates so data analysis for different nucleic acids was simply done by executing the appropriate template (Figure 5).

The built-in ultra-fast UV/Vis absorbance spectrometer in the Omega microplate series from BMG Labtech was successfully validated by performing two different assays. In conclusion, the Tandem Technology provides high quality data in a single multidetection microplate reader and offers maximum flexibility.

## REFERENCES

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according to the manufacturer’s protocol (Figure 2, Ref. 5). The protein assay is linear in the range of 0.1-1.4 mg/mL. Because of its homogeneous and fast nature, the Bradford assay is a preferred method to determine the protein concentration of samples (Figure 3). Data analysis and calculation of the standard curve was performed with the MARS software (Figure 4).

2. One of the most common methods for nucleic acid detection is the

measurement of solution absorbance at 260 nm ( $A_{260}$ ) since nucleic acids have an absorption maximum at this UV wavelength. DNA purity can be calculated by performing ratio absorbance measurements at  $A_{260}/A_{280}$ . DNA stock solution from calf thymus was taken and dissolved to yield concentrations in the range from 0.1 to 100 µg/mL. Four replicates of 350 µL aliquots of each standard were pipetted into a 96-well UV plate. Replicates of 350 µL