



Sample Analysis Report

Number 67

Fluorescence in Microvolumes: 30uL Volumes in 384 Well Microplates

Dr. Robert Keighley
Varian Limited 10 Mead Road, Yarnton Oxford. OX5 1QU

Introduction

The UV/Visible Spectrophotometer market has shown extraordinary growth in the analysis of smaller volume samples. In this sector it is now routine to perform analysis on samples of less than a single microliter.

The Cary Eclipse spectrofluorimeter offers a very sensitive yet fast and versatile platform in a rugged design. The typical Raman sensitivity (signal/noise) for water at 350 nm (ex) is approx 3500-4500.

With a very durable source lamp, which should typically last as long as the instrument, the ability to couple with high efficiency to environmental-light immune fiberoptics, and a high precision 96 or 384 well microplate reader, exceptionally fast scanning and strobed illumination the Eclipse has many advantages in applications in production or laboratory environments.

With the advent of new microplates from Porvair with reduced well height, which ideally suit the Eclipse wellplate optical design, smaller volumes can now be measured with ease. Should the need arise, a newly designed ultra-microvolume cell (Built by Starna Scientific Limited) allows precise and sensitive readings in volumes as small as 5 microlitres.

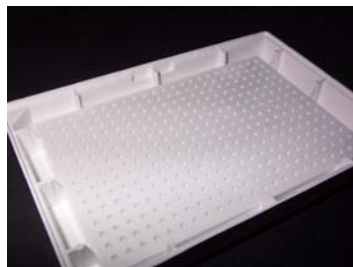
Instrumentation

All measurements were made using the Eclipse Fluorimeter (below) which was equipped with either a standard 96/384 well wellplate reader attachment or with the standard cell. The sample used was fluorescent ink diluted to give reasonable scale readings in a 3ml cuvette.

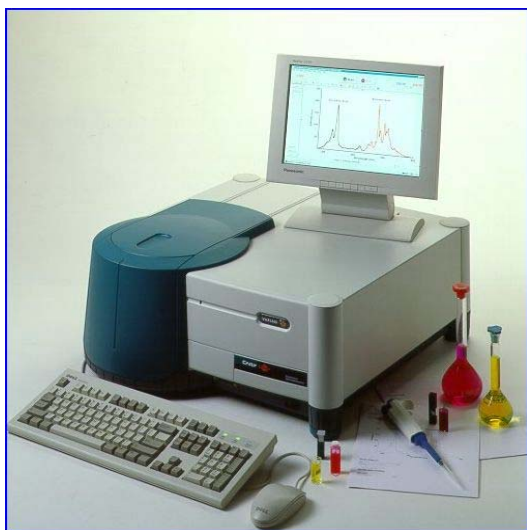


The 'plug and play' Eclipse Wellplate Reader features downward looking optics to give excellent sensitivity with the smallest of samples.

Conditions were adjusted to give reasonable scale readout. Comparative quantitation can be established by keeping the instrument conditions constant, or by a calibrated adjustment of conditions.



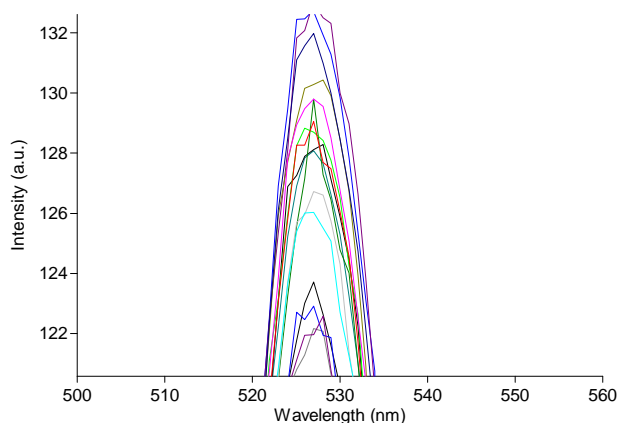
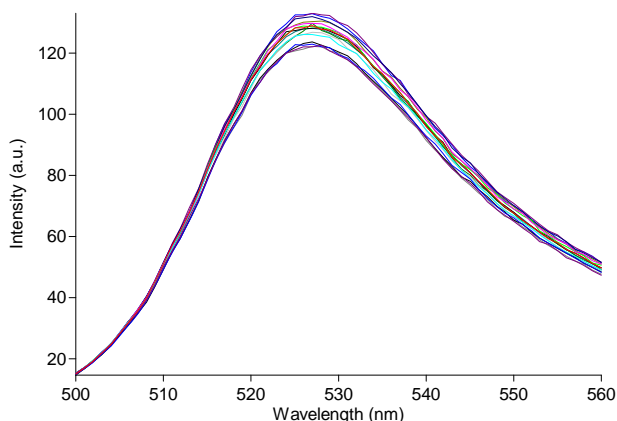
The Porvair 30uL shallow-well microplates are available in fluorescence grade white, black and clear designs. The design incorporates a shallow internal base yet it is stiff and strong enough for robotics applications. The wells hold just over 30uL to full and can be used with smaller volumes down to about 20uL.



The wellplate reader was aligned for optimal sensitivity in the shallow-well plates and the plate library updated with the dimensions of the plates. Setup conditions: PMT 600 Volts (medium) Slits 5nm filters auto open.

For the purpose of this study, the aim of which was to establish well-well variation in results when using microvolumes, dispensing was using a 20-100uL pipette set to 30uL and used to fill 'random' wells in the plate as well as each corner well.

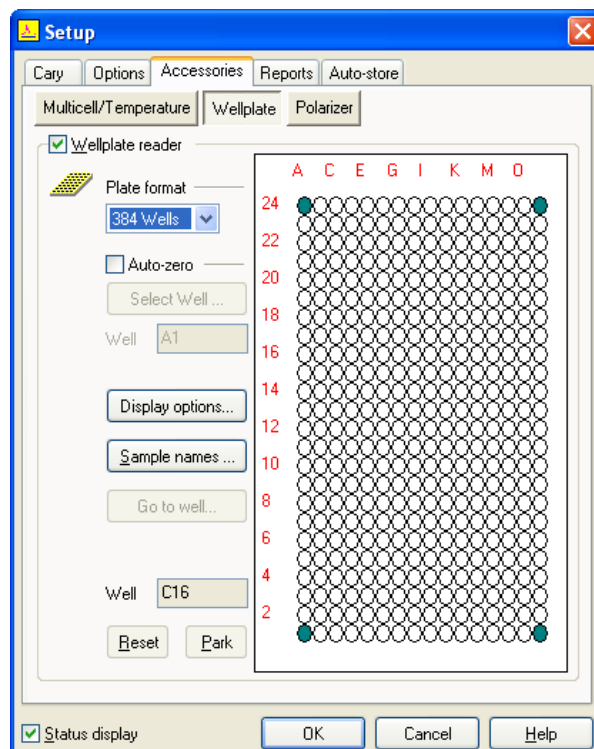
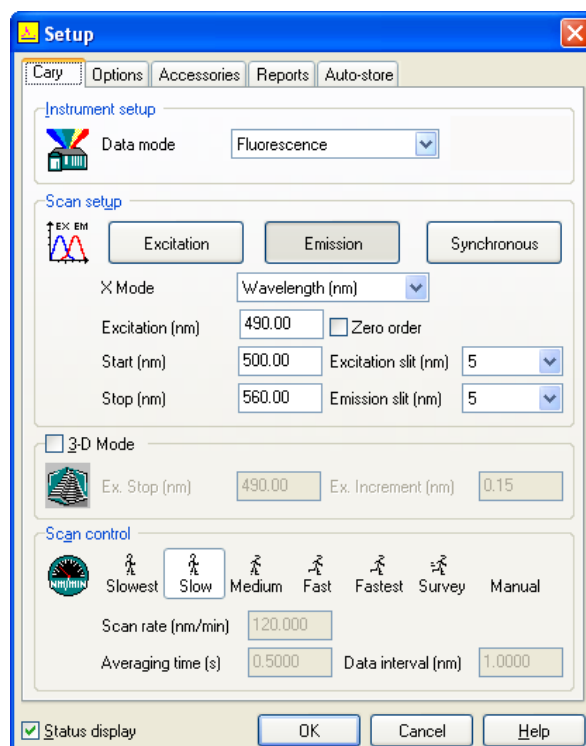
Results1 20 Wavelength scans taken at each corner of the plate and at random positions within the plate to establish well-well variations. Full image then detail.



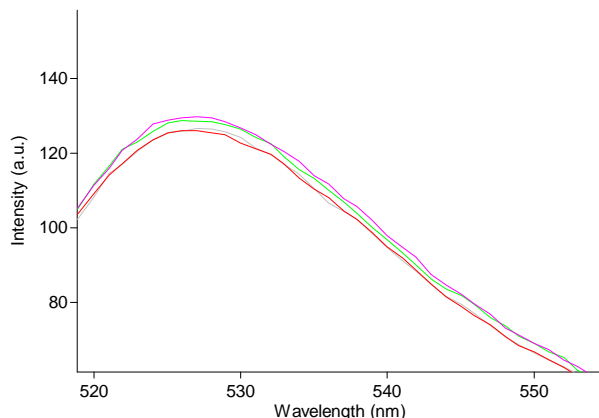
The scan shows that there is some slight well-well variation and that this variation is approximately +/- 5% of the total observed intensity. (126 afu +/- 6 afu) This observation corresponds well to a 'best case' variation of

about 2.5% in 96 well plates containing 400uL as the pipetting and meniscus position errors are of increased importance in these very small volumes.

Results 2 'Optimized Corners' readings taken at each of the four corners of the plate using a slower scan setting and more dilute sample



Results 2: Corner wells with slow scan (reduced scan range) and dilute sample (PMT 890 Volts, slits 5 nm)



Discussion of Results

This result indicates that the best well-well variance for this type of plate and 30uL samples will be in the order of +/- 5% however, the qualitative nature of the results indicate that there is no significant reduction in the quality of data compared to open beam measurements in a cuvette or using 96 wells with 12.5 times this volume.

Only the white variant of these new plates has been tested so far. This plate design creates the ability to measure fluorophores in smaller volumes than previously possible.

These data are the actual untouched experimental results obtained on Monday 5th November 2007

Porvair Plates Manufactured and distributed by: Porvair Sciences, Unit 6, Shepperton Business park, Govett Avenue, Shepperton, Middlesex, TW 17 8BA.

Plates for this study kindly supplied by Christine Southern at Porvair.