Sample Analysis Report

Number 68

Fluorescence in Microvolumes: A New 5uL Microcell for the Eclipse Fluorimeter.

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Introduction

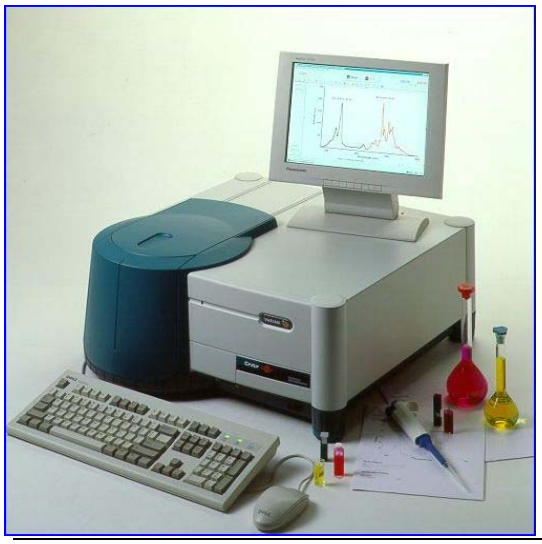
The UV/Visible Spectrophotometer market has shown extraordinary growth in the analysis of smaller volume samples. In this sector it is now routine to perform analysis on samples of less than a single microliter. (Sample Analysis reports 30,32,40, 41,48 *and others*)

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.

Starna Scientific Limited have adapted their patented lens cell to cover a fluorescence application thus allowing precise and sensitive readings in volumes as small as 5uL

Figure 1 The Varian Cary Eclipse Fluorimeter



Instrumentation

All measurements were made using the Eclipse Fluorimeter (below) which was equipped with a standard cell holder and a Starna 19.05F/L/Q/5/Z20 5ul microcell.

Figure 2 the standard Eclipse cell holder. The sample used was fluorescent ink diluted to give reasonable scale readings in a 3ml cuvette. The same holder is used for the microvolume cuvette.

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.

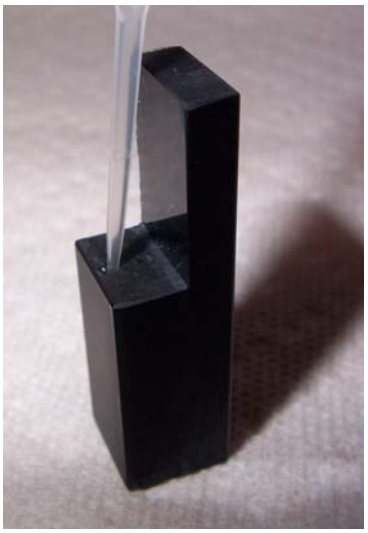


Figure 3 The Starna 5uL Microvolume cell has been designed to make loading and flushing simple, with a minimum dead volume. The cell design incorporates a front face lens which dramatically improves the sensitivity of the device.

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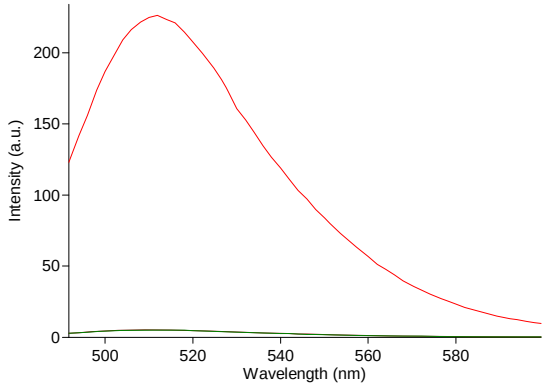
Alignment of the microcell was achieved by installing the cell with a moderately bright fluorophore in the standard cell holder and using the Align module in the software to

seek optimum signal by slowly adjusting the height and pitch of the cell.

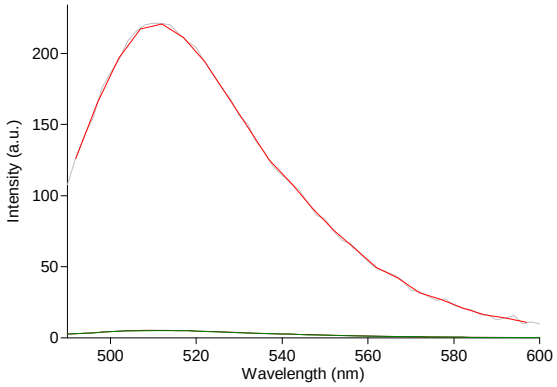
Results 1 :Microvolume Cell

A solution of fluorescent dye from a yellow highlighter pen was used for the experiment. A dilute solution was prepared and pipetted into a standard 1 cm square fluorescence cuvette. Prescan was used to determine the optimal wavelengths for excitation and emission. These wavelengths were then selected for the study.

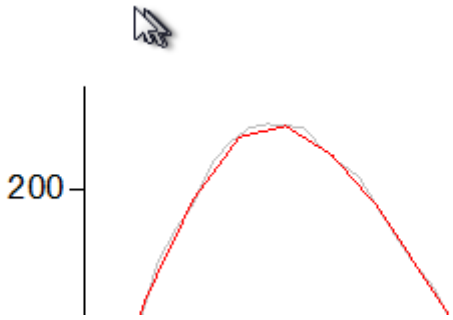
Scan 1 Shows the superimposed results of scanning a moderately bright sample (PMT set to 480V, 5nm slits) in full size (red)and 5uL (green)microvolume cuvette.



Scan 2 Shows the same results but with the microvolume trace multiplied by a factor of 42 using the onboard calculator software. (grey trace)

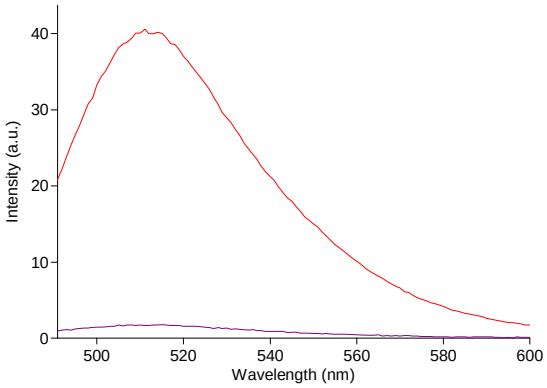


Scan 3 Detail from the apex showing superimposed full size cuvette (red)and scaled microvolume cuvette data.(grey)

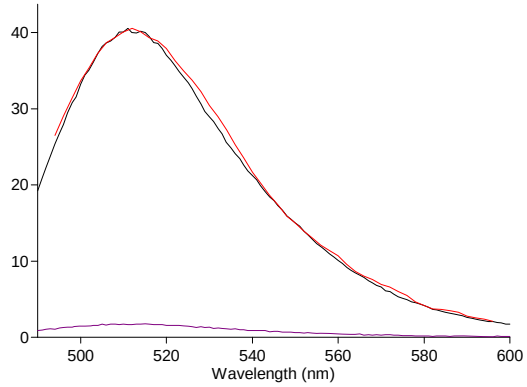


The correlation between the results show that at this dilution level and with this cell alignment there is no qualitative difference between the result quality (all data collected at the same scan speed) but that there is about a 40 fold reduction in ultimate sensitivity**.**

Scan 4 Dilute samples: Scan with diluted sample (Instrument now set to 870V PMT, 5nm slits) Full size cuvette(red) vs microvolume(purple)



Scan 5 Dilute samples Scan in microvolume cuvette rescaled (black) and superimposed on full volume scan (red) (factor x23)



The scans once more show excellent correlation even though the absolute reading for the microcell was in this case less than 3 AFU. The cell was removed and re-aligned between these sets of readings and it was possible to achieve about 5% full volume sensitivity with more dilute samples..

Discussion of Results:

The new Microcell provides a solution to customers where sample cannot be prepared in or diluted to larger volumes. The microcell avoids the need for dilution and keeps reagents costs to a minimum, providing analysis in volumes typically handled in molecular biology and forensics applications. The ability of the cell to provide good sensitivity even in very small volumes makes it ideally suited to proteomics applications, where the senisitivity of the cell is sufficiently high to measure native fluorescence of proteins prepared in microliter quantities. The design of the cell would also allow the majority of the sample to be recovered if required.

Although the device is less sensitive than full-sized cuvette readings, the difference is much less (about 5% of the sensitivity in full size cuvettes) than the loss in sensitivity which would be encountered diluting the sample to the required minimum volume (about 300uL) for full centimeter-square cuvettes. The efficiency is greater than that of a standard microcell of equivalent geometry due to the patented lens design on the cuvette front-face.

Fig 5 Front face of the microcell showing lens

These data are the actual untouched experimental results obtained on Thursday 15th November 2007

For further information, contact your local Varian Sales Office. 01865 291500

Cell (Part number 19.05F/L/Q/5/Z20/MC) Designed and Manufactured by Starna Scientific Limited 52-54 Fowler Road, Hainault Essex IG6 3UT. Subject to Patent Protection.. A cell was Kindly provided for evaluation by Mr Keith Hulme of Starna.