



## Microliter Sample Fluorescence using Hellma® TrayCells

### Introduction

Many biological samples require measurements with sample volumes in the microliter range because of high cost and low availability. With a Hellma® TrayCell (Fig. 1), a HORIBA Scientific spectrofluorometer, such as a FluoroMax® or a member of the Fluorolog® series, can be used to measure sample volumes as small as 1  $\mu$ L. The Hellma® TrayCell<sup>1</sup> is fiber-optic-based and specifically designed for UV-visible absorbance measurements of biological samples with ultra-small volumes.



Fig. 1. Hellma® TrayCells.

### Experiment and Results

To measure the fluorescence from this TrayCell, our fluorescence cuvette hold-

er is modified with a reflectance mirror positioned in the light path to direct the fluorescence from the TrayCell into the emission monochromator and then the detector (Fig. 2). This modification is designed for conventional fluorescence measurements in right-angle mode only.

With this setup in a Fluorolog®-3-22 modular spectrofluorometer, 1  $\mu$ L of aqueous fluorescein at several concentrations (pH 10) was measured and the result is shown in Fig. 3. The excitation was set to 450 nm with a bandpass of 5 nm on both excitation and emission monochromators and a Hellma® TrayCell with a 0.2 mm cap.

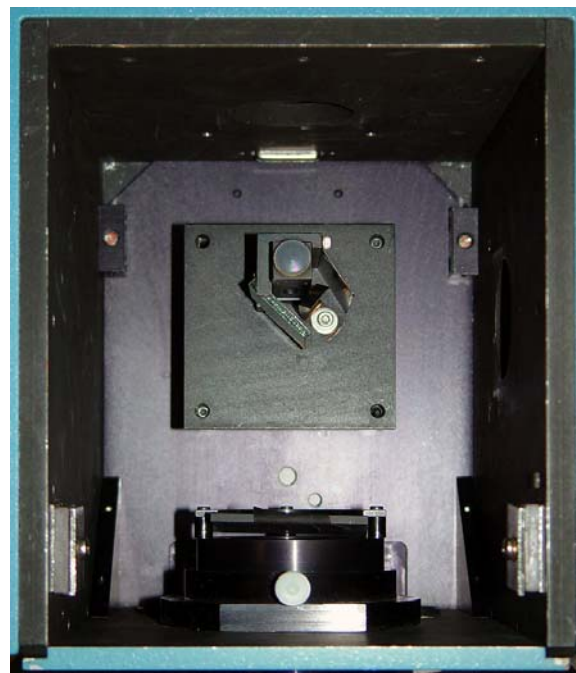
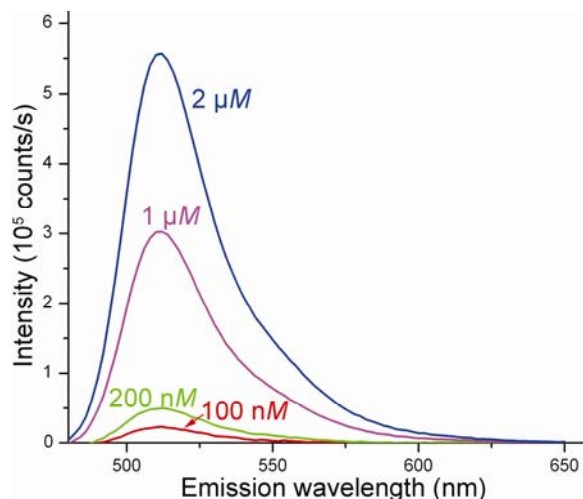


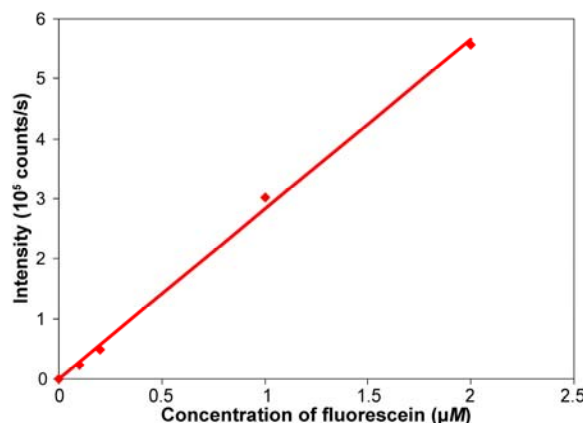
Fig. 2. Hellma® TrayCell mounted in a HORIBA Scientific spectrofluorometer's sample compartment.

<sup>1</sup> Hellma USA, 80 Skyline Drive, Plainview, NY 11803



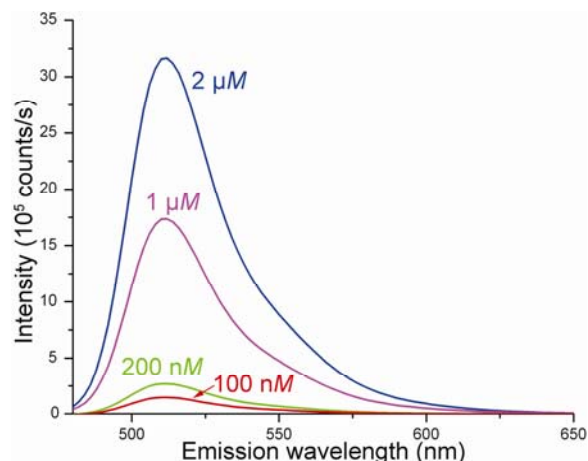
**Fig. 3.** Aqueous fluorescein (pH 10),  $\lambda_{excitation} = 450 \text{ nm}$ , bandpass = 5 nm excitation and emission, using a Hellma<sup>®</sup> cell with 0.2 mm cap with 1  $\mu\text{L}$  sample volume. The  $\lambda_{peak} = 512 \text{ nm}$ .

Fig. 4 shows the calibration curve of four concentrations of this basic aqueous fluorescein solution.

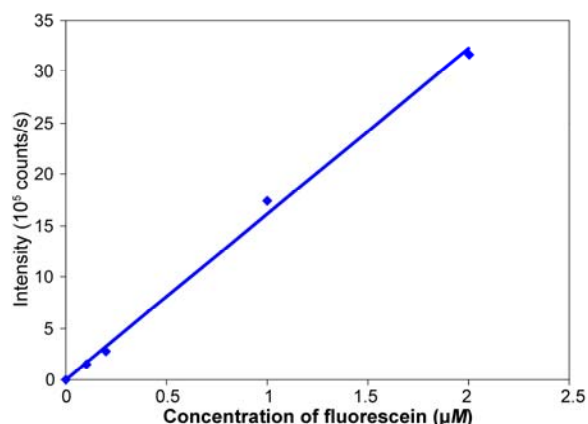


**Fig. 4.** Calibration curve for 0.2 mm cap Hellma<sup>®</sup> TrayCell.  $R^2 = 0.9977$ .

With the Hellma<sup>®</sup> 1 mm-cap TrayCell, a similar emission spectrum—but with five times higher intensity—was observed (Fig. 5). Fig. 6 presents the calibration curve for these four concentrations.



**Fig. 5.** Basic aqueous fluorescein in water with Hellma<sup>®</sup> 1 mm cap and similar parameters to Fig. 3. The  $\lambda_{peak} = 512 \text{ nm}$ .



**Fig. 6.** Calibration curve for 1 mm cap Hellma<sup>®</sup> TrayCell.  $R^2 = 0.9971$ .

## Conclusions

HORIBA Scientific spectrofluorometers can be used with high sensitivity to measure conventional fluorescence from expensive or rare samples with volumes as small as 1  $\mu\text{L}$  using Hellma<sup>®</sup> TrayCells.

Hellma<sup>®</sup> is a registered trademark of Hellma USA. Please contact us for compatibility with fluorescence accessories.

Copyright © 2010 HORIBA Scientific