



Fluorescence Micromerements using Fiber-Optic Adapters for Microscopes

Introduction

For convenient and reliable examination of fluorescent samples under a microscope, HORIBA Scientific offers a fiber-optic adaptor, the Microscope Adapter. The accessory brings excitation light from a FluoroMax[®] or Fluorolog[®] to the microscope and returns the sample's luminescence to the spectrofluorometer to be analyzed.

With our Microscope Adaptor, measurements can be recorded on microscopic samples, preserving all scan-types from the spectrofluorometer (e.g., excitation, emission, and excitation-emission matrix scans), for $\lambda = 240\text{--}1700$ nm with appropriate optics and detectors. This *Technical Note* describes setting up the accessory for Fluorolog[®] and FluoroMax[®], plus some uses, such as measurement of quantum dots (QDs) on wafers, matrix scanning from cloth, mapping of microspheres, and wide-field fluorescence imaging.

Equipment

The Microscope Adapter includes an excitation fiber-optic bundle, bringing the excitation beam to the microscope (Fig. 1a), an emission fiber-optic bundle, returning the emitted fluorescence to the spectrofluorometer (Fig. 1b), and an adapter within the spectro-fluorometer's sample compartment, to fix the excitation and emission fiber-optics in place (Fig. 2). The sample-compartment adaptor includes mirrors to direct the excitation beam into the excitation bundle,

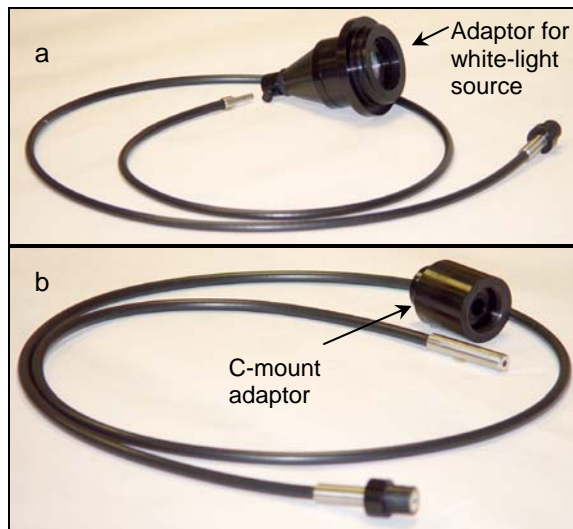


Fig. 1. (a) Excitation and (b) emission fiber-optic bundles.

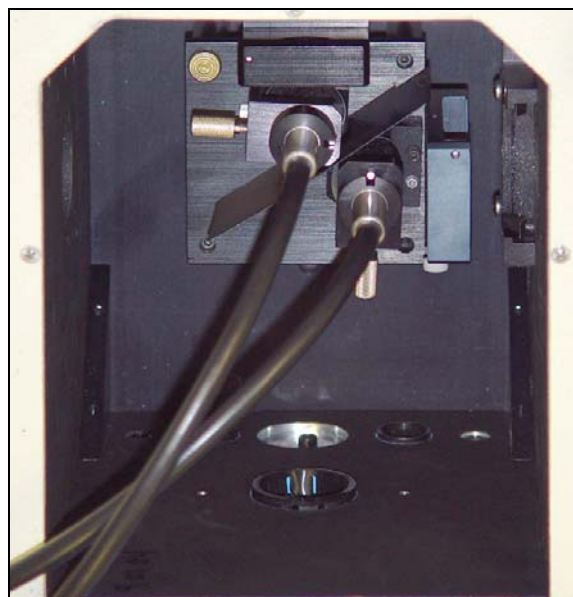


Fig. 2. Sample-compartment adaptor with both fiber-optic bundles inserted.

and the emission beam from the bundle

into the emission monochromator.

With each fiber-optic bundle are fittings to attach the bundles to the microscope. The excitation bundle attaches to the white-light source's mount, while the emission bundle terminates in a standard C-mount adapter.

Set-up

Setting up the accessory is simple. The excitation fitting is placed into the port for the white-light source of the microscope (Fig. 3). This brings the excitation beam from the excitation monochromator directly to the sample on the microscope's stage. The emission bundle is attached to the C-mount on the microscope (Fig. 4), directing sample fluorescence back into the spectrofluorometer.



Fig. 3. Attaching the excitation fiber-optic bundle to the microscope's white-light source.

The standard HORIBA Scientific cuvette-holder is removed from the spectrofluorometer, and the sample-compartment adaptor is inserted, fixed firmly in place with thumbscrews. Alignment of the fiber-optic bundles occurs via alignment pins and slots (Fig. 2). Fig. 5 shows a fully set-up Olympus



Fig. 4. Attaching the emission fiber-optic bundle to the microscope's C-mount.



Fig. 5. Olympus BX51 microscope and Fluorolog[®] 3-22 with fiber-optic accessory.

BX51 microscope attached to the Fluorolog[®] 3-22 sample compartment.

We have created a custom pinhole-turret with six pinhole sizes, built for Olympus microscopes (both upright and inverted). The pinhole turret is immediately above the microscope's trinocular head in the emission light-path. The six pinholes (100–1000 μm) provide variable spatial resolution from 1–100 μm using 100 \times and 10 \times objectives. With this system, microscopic measurements with the desired spatial resolution, plus steady-state mapping, can be performed.

Results and discussion

Fig. 6 shows three emission spectra of three separate spots from CdSe QDs on a wafer. Optical resolution was 2 μm with a 50 \times objective and 100 μm pinhole in the emission path. A Xe lamp was the light source, the excitation monochromator was set to 370 nm, and emission was directed to the sample through a 370 nm dichroic mirror, then transmitted to the emission monochromator and R928P photomultiplier tube. Fluorescence at three different spots (each 2 μm dia.) was recorded: QD aggregates (red), a single QD (blue), and the bare wafer (green).

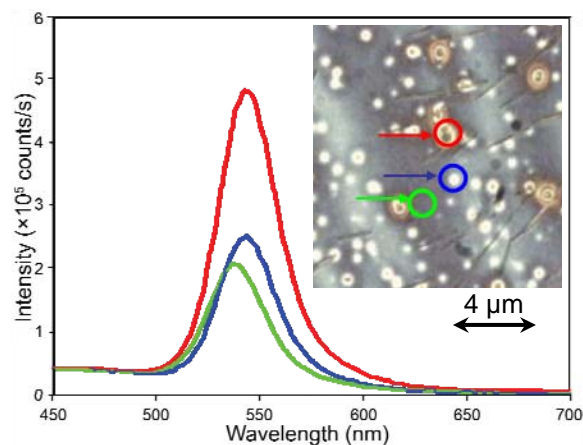


Fig. 6. Inset: CdSe QDs on a wafer. Plot: Emission spectra measured from several areas of interest: Red: CdSe QD aggregates; blue: single CdSe QD; green: bare wafer.

A matrix scan from a spot 50 μm in diameter on dyed cloth was also examined using this set-up (Fig. 7).

With an automated stage, steady-state mapping also can be done on a specific area. Fig. 8 shows a map of microspheres 15 μm in diameter. Pixel size was 2.5 μm with a 40 \times objective

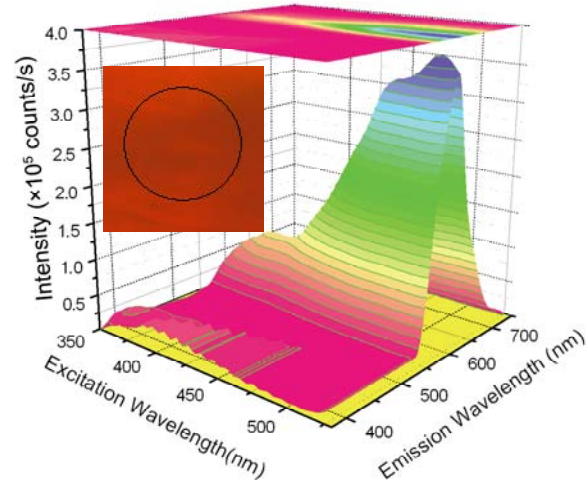


Fig. 7. Inset: image under the microscope of a cloth sample dyed pink. Plot: matrix scan from circled area, 50 μm in dia.

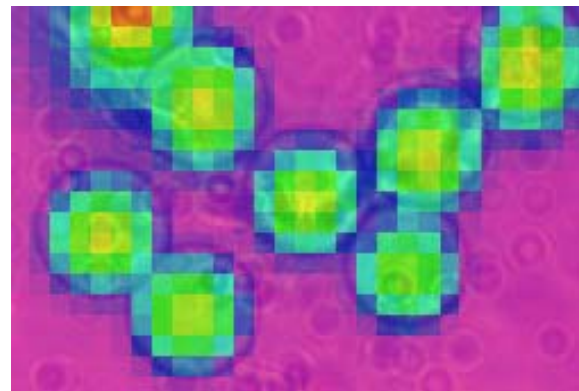


Fig. 8. Intensity map of beads 15 μm in diameter; $\lambda_{\text{exc}} = 370 \text{ nm}$ and $\lambda_{\text{em}} = 432 \text{ nm}$.

and a 100 μm pinhole in the emission light-path. The excitation monochromator was set to 370 nm, and the emission monochromator was parked at 432 nm. A dichroic beamsplitter directed the 370 nm excitation to the sample, and transmitted fluorescence at 432 nm to the emission light path. With this beamsplitter, many other measurements can be performed from 370–850 nm. The useful range can be extended into

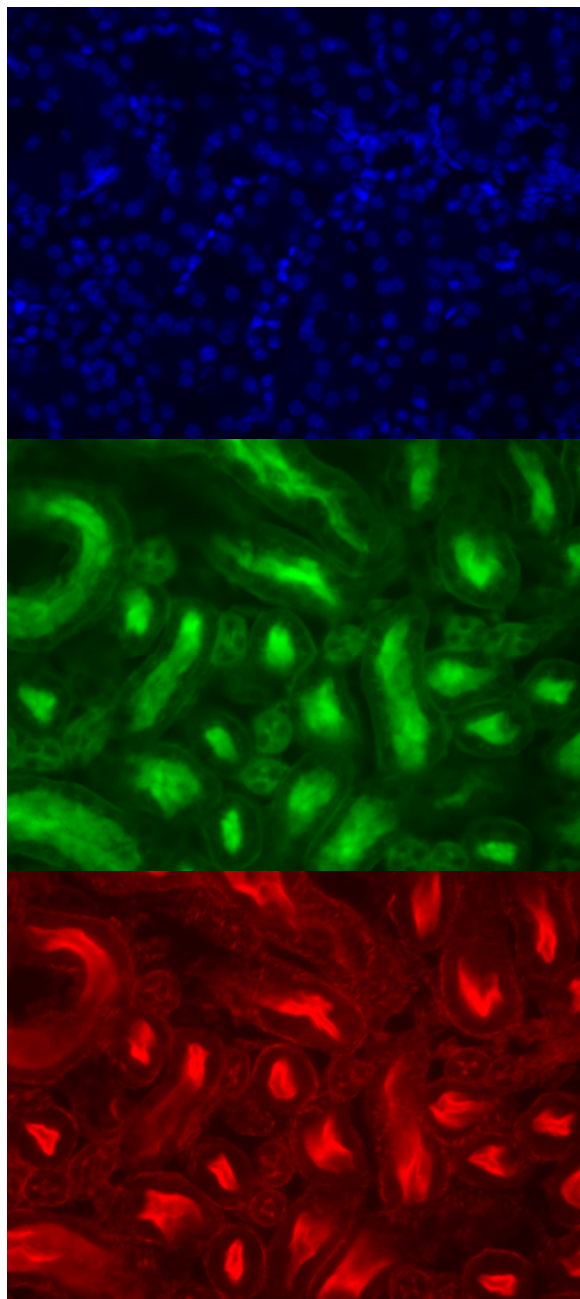


Fig. 9. Wide-field fluorescence images of mouse kidney. Top: $\lambda_{exc} = 350 \text{ nm}$; middle: $\lambda_{exc} = 450 \text{ nm}$; and bottom: $\lambda_{exc} = 550 \text{ nm}$.

the deep UV and near-IR.

With no pinhole, wide-field fluor-

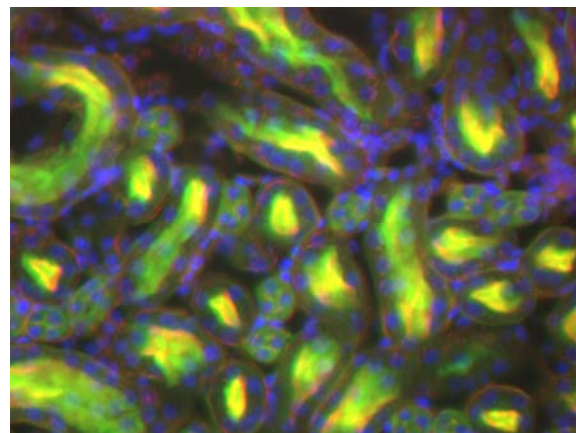


Fig. 10. Wide-field blue, green and red images (Fig. 9) merged together.

escence images can be captured digitally, with a Xe lamp as excitation, via the fiber-optic collimating lens adaptor. Figs. 9a–c were captured with a SPOT RT3 Monochrome (RT2500) fluorescence camera¹ at excitations 370 nm, 450, and 550 nm, respectively, from a mouse-kidney tissue slide². The camera was installed onto the second port of the pinhole-turret. The three images were merged (Fig. 10) with appropriate software.

Conclusions

With the use of this fiber-optic adaptor and the pinhole adaptor, many microscopic fluorescence measurements can be achieved. Most microscopes, including Nikon, Olympus, Zeiss, and Leica, are compatible with the Microscope Adapter.

¹ Diagnostic Instruments, Inc., 6540 Burroughs Street, Sterling Heights, MI 48314.

² Molecular Probes prepared slide #3, Invitrogen Corporation, 5791 Van Allen Way, Carlsbad, CA 92008.