

Microscope Adapter

Introduction

For convenient and reliable examination of fluorescent samples under a microscope, HORIBA Jobin Yvon offers a fiber-optic accessory, the Microscope Adapter. The accessory brings the excitation light from a FluoroMax® or Fluorolog® to the microscope and returns the sample's luminescence to the spectrofluorometer to be recorded and analyzed. Most microscopes, including Nikon, Olympus, Zeiss, and Leica, are compatible with the Microscope Adapter.

Equipment

The Microscope Adapter consists of 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 placed in the spectrofluorometer's sample compartment, to align the excitation and emission fiber-optics in place (Fig. 2). The sample-compartment adapter includes mirrors to direct the excitation beam into the excitation bundle, and the emission beam from the bundle into the excitation monochromator.

Included with each fiber-optic bundle are fittings to attach the bundles to the microscope.

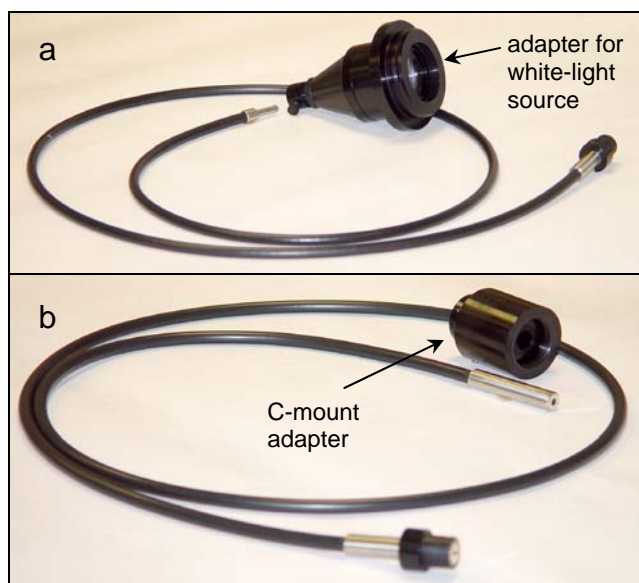


Fig. 1. (a) Excitation and (b) emission fiber-optic bundles.

The excitation bundle attaches to the mount for the white-light source, while the emission bundle terminates in a standard C-mount adapter.

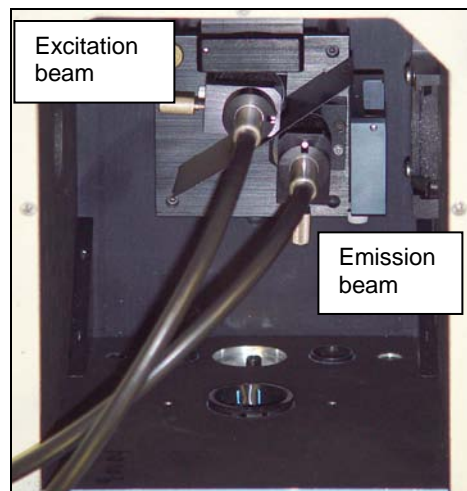


Fig. 2. Sample-compartment adapter with both fiber-optic bundles inserted.

Set-up

Setting up the accessory is simple. The excitation fitting is placed into the back 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 top of 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.

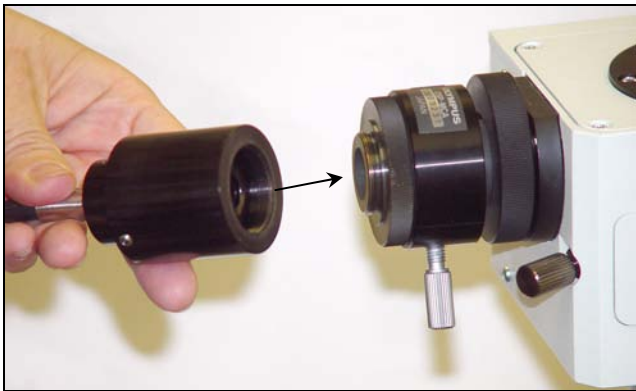


Fig. 4. Attaching the emission fiber-optic bundle to the microscope's C-mount.

The standard HORIBA Jobin Yvon cuvette-holder is removed from the spectrofluorometer, and the sample-compartment adapter is inserted, fixed firmly in place with thumbscrews. Alignment of the fiber-optic bundles is accomplished via alignment pins and slots (Fig. 2). A fully set-up Olympus microscope attached to the Fluorolog[®] sample compartment is shown in Fig. 5. A similar setup is shown using a Zeiss microscope (Fig. 6).

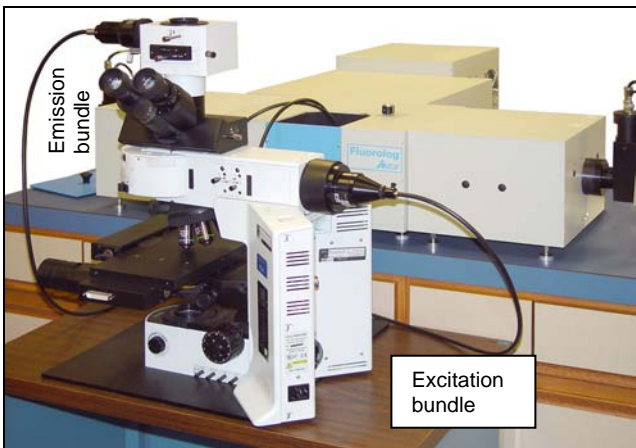


Fig. 5. Olympus microscope and instrument with fiber-optic accessory.

Results

A single crystal of glucose isomerase (Hampton Research, Aliso Viejo, CA) was examined under an Olympus BX51M microscope, connected to a Fluorolog[®]-3-221 spectrofluorometer via the Mi-

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(All HORIBA Jobin Yvon companies were formerly known as Jobin Yvon)

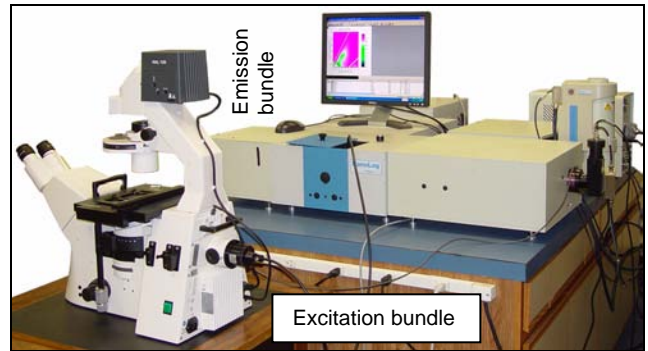


Fig. 6. Zeiss microscope and instrument with fiber-optic accessory.

croscope Adapter. Glucose isomerase is used in the food industry to convert glucose into fructose. The crystal was imaged as shown in Figure 7, and an emission spectrum (Fig. 8) was taken. Spectral or intensity mapping is also possible with the automated stage option.



Fig. 7. A glucose isomerase crystal (70 μm across) using different pinholes in the emission path, and a 10 \times objective.

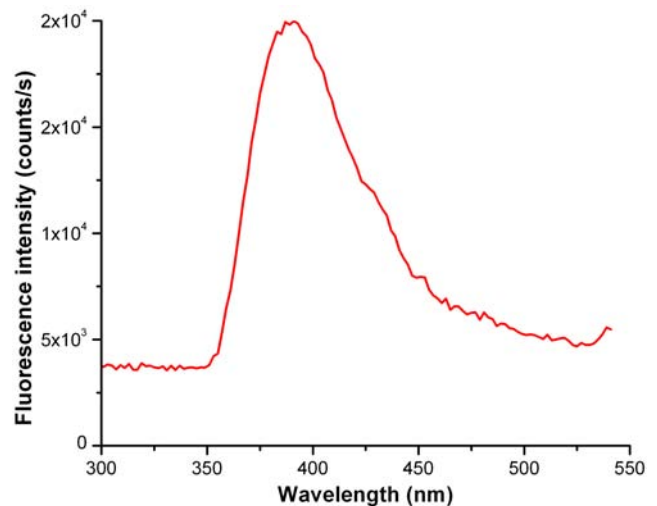


Fig 8. Emission spectrum of a crystal of glucose isomerase. Excitation = 280 nm, integration time = 0.2 s, step size = 1 nm, and bandpass = 8 nm for emission and excitation.

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