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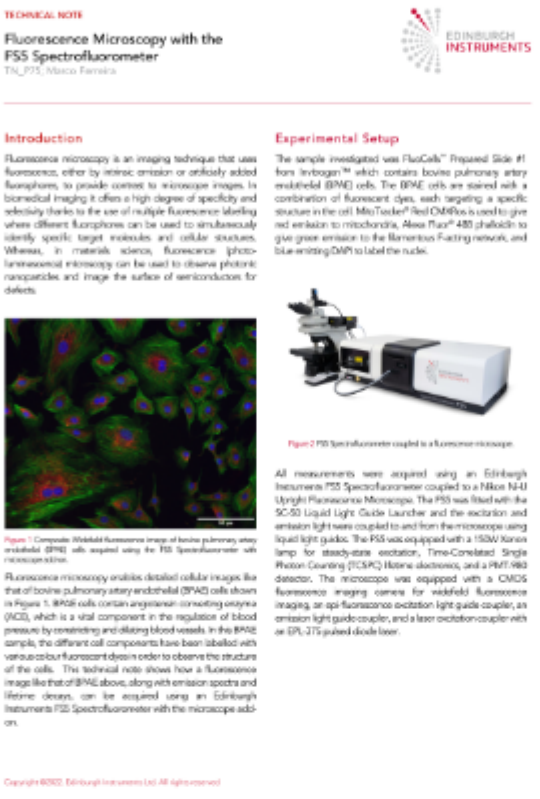
TECHNICAL NOTE

Fluorescence Microscopy with the FS5 Spectrofluorometer

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Introduction

Fluorescence microscopy is an imaging technique that uses fluorescence, either by intrinsic emission or artificially added fluorophores, to provide contrast to microscope images. In biomedical imaging it offers a high degree of specificity and selectivity thanks to the use of multiple fluorescence labelling where different fluorophores can be used to simultaneously identify specific target molecules and cellular structures. Whereas, in materials science, fluorescence (photo-luminescence) microscopy can be used to observe photonic nanoparticles and image the surface of semiconductors for defects.

Experimental Setup

The sample investigated was FluoCells™ Prepared Slide #1 from Invitrogen™ which contains bovine pulmonary artery endothelial (BPAE) cells. The BPAE cells are stained with a combination of fluorescent dyes, each targeting a specific structure in the cell. MitoTracker® Red CMXRos is used to give red emission to mitochondria, Alexa Fluor® 488 phalloidin to give green emission to the filamentous F-actin network, and blue-emitting DAPI to label the nuclei.

Figure 2 FS5 Spectrofluorometer coupled to a fluorescence microscope.

All measurements were acquired using an Edinburgh Instruments FS5 Spectrofluorometer coupled to a Nikon Ni-U Upright Fluorescence Microscope. The FS5 was fitted with the SC-50 Liquid Light Guide Launcher and the excitation and emission light were coupled to and from the microscope using liquid light guides. The FS5 was equipped with a 150W Xenon lamp for steady-state excitation, Time-Correlated Single Photon Counting (TCSPC) Hotime electronics, and a PMT-980 detector. The microscope was equipped with a CMOS fluorescence imaging camera for widefield fluorescence imaging, an epi-fluorescence excitation light guide coupler, an emission light guide coupler, and a laser excitation coupler with an EPL-375 pulsed diode laser.

Figure 1 Composite Widefield fluorescence image of bovine pulmonary artery endothelial (BPAE) cells acquired using the FS5 Spectrofluorometer with microscope add-on.

Fluorescence microscopy enables detailed cellular images like that of bovine pulmonary artery endothelial (BPAE) cells shown in Figure 1. BPAE cells contain angiotensin-converting enzyme (ACE), which is a vital component in the regulation of blood pressure by constricting and dilating blood vessels. In this BPAE sample, the different cell components have been labelled with various colour fluorescent dyes in order to observe the structure of the cells. This technical note shows how a fluorescence image like that of BPAE above, along with emission spectra and lifetime decays, can be acquired using an Edinburgh Instruments FS5 Spectrofluorometer with the microscope add-on.



## Spectrofluorometer Add-on for Widefield Fluorescence Microscopy

Fluorescence microscopy is an imaging technique that uses fluorescence, either by intrinsic emission or artificially added fluorophores, to provide contrast to microscope images. This technical note shows how fluorescence images along with emission spectra and lifetime decays, can be acquired using a Spectrofluorometer with the microscope add-on.

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