Imaging YFP-expressing cells with the Sapphire FL

Introduction

Green fluorescent protein (GFP) and the related yellow fluorescent protein (YFP) are powerful tools frequently used as markers in cellular biology experiments. GFP was isolated from the jellyfish *Aequorea victoria*. Scientists discovered that the protein retains its fluorescence when expressed in cultured cells or model organisms such as mice and *C. elegans*.[1] This ability makes GFP a perfect marker to study gene expression, protein localization, or protein dynamics in vivo. Variants of GFP with different spectral properties have been introduced, including a red-shifted variant of GFP, called YFP.[2] Using GFP and YFP variants with appropriate excitation and emission maxima can allow two markers to be studied at once in living cells.[2]

Many applications have been developed to take advantage of GFP and YFP. For example, cells, such as tumor cells, can be engineered to express fluorescent proteins, allowing the location of those cells to be followed within an organism. To study protein dynamics, cells can be engineered to express fusion proteins in which GFP or YFP is attached to one end of a protein of interest; if the presence of the fluorescent protein does not affect structure or function, the expression, location, and metabolism of the protein of interest can be studied in real time.

Unlike other fluorescence detection methods like antibody staining or immunohistochemistry, using GFP or YFP as markers does not require additional components, such as substrates or enzymes. The fluorophores are generated within the cell, and cells or tissues do not need to be fixed or treated before imaging. The only requirement for detecting YFP or GFP expression is an imaging system or microscope equipped with the correct excitation and emission wavelengths. The ability to detect fluorescence in living cells or organisms allows dynamic processes to be observed without affecting physiology.

This application note demonstrates detection of YFP expressed in transformed cells using the Sapphire FL and 488 (YFP) Standard Optical Module.

Materials and Methods

HeLa cells were cultured in 24-well tissue culture plates. For transfection, cells were seeded at 50,000 cells per well at a viability was 93.6%. The cells were grown overnight and then transfected using the TransIT-HeLaMONSTER® Transfection Kit (Mirus Bio, part #MIR 2904) according to the manufacturer's instructions. Briefly, media was aspirated from the well, the cells washed with 1 ml sterile PBS, and 0.5 ml media with FBS added. Transfection mix was prepared by adding 1 µl pCMV6-AN-YFP Mammalian Expression Vector (Origene, part #PS100035), 3 µl TransIT, and 2 µl Monster

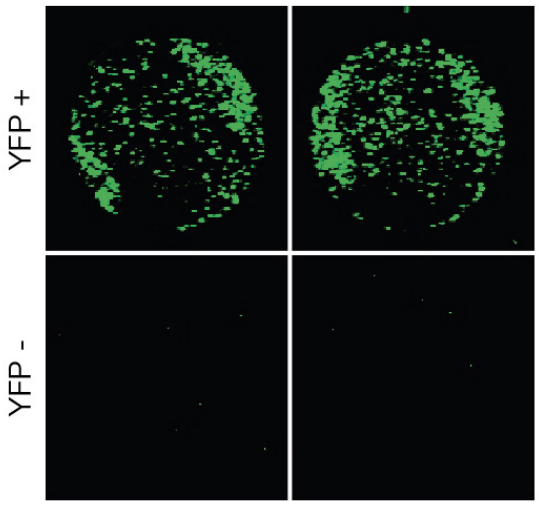

Figure 1. YFP-expressing HeLa cells imaged on the Sapphire FL using the 488 (YFP) Standard Optical Module. Fluorescence is displayed using green pseudo color. The top two wells contain transfected cells. The bottom two wells are negative controls.

reagent to 100 µl serum-free media. The transfection mix was incubated for 30 minutes at room temperature. 50 µl of the mix was added to the cells and the cells were incubated at 37 °C for 72 hours. The cells were then imaged.

Control cells were treated identically, but a YFP-expression vector was not included in the transfection mix.

The pCMV6-AN-YFP Mammalian Expression Vector drives expression of TurboYFP, which has excitation/emission maxima of 525 nm and 538 nm respectively. Imaging was conducted using the Sapphire FL with the 488 (YFP) Standard Optical Module. This module consists of a 488 nm laser and a 534/20 emission filter designed specifically for imaging YFP. The laser intensity setting was 10, and the resolution setting was 100 µm.

Results and Discussion

Figure 1 shows an image of transfected cells expressing YFP, captured on the Sapphire FL using the optical module designed for YFP detection. Strong fluorescent signal is detected in the two wells containing transfected cells while signal is absent from the two negative control wells.

The Sapphire FL is a perfect choice for imaging cells and model organisms expressing GFP or YFP. With its large, deep imaging area, the Sapphire FL can image a variety of sample types including tissue culture dishes and model organisms as well as flat samples such as slides and membranes. The instrument provides flexibility to image a wide range of fluorescent dyes and labels, with many available laser diodes that can be combined with emission filters to create modules for almost any fluorophore. For experiments involving YFP, Azure offers a Standard Module specifically designed to detect YFP fluorescence, which includes a combination of a 488 nm laser diode and a 534/20 emission filter. As shown in Figure 1, this module permits high-sensitivity detection of YFP expressed in transfected cells.

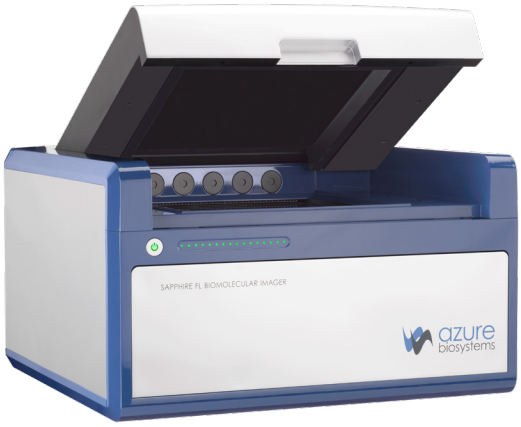

Figure 2. Sapphire FL is a laser scanner designed for multicolor fluorescent imaging, NIR fluorescent imaging, chemiluminescence, phosphor imaging, and more.

References

1. Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC. Green fluorescent protein as a marker of gene expression. *Science.* 1994;263(5148):802-805.
2. Baumann CT, Lim CS, Hager GL. Simultaneous visualization of the yellow and green forms of the green fluorescent protein in living cells. *J Histochem Cytochem.* 1998;46(9):1073-1076.

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