Imaging CFP-expressing cells with the Sapphire FL

Introduction

Fluorescent proteins are used to measure the transcription of reporter genes or to detect and localize protein expression in living cells. Expression of a fluorescent protein in cultured cells or in cells of an animal or plant is achieved by transfecting the cells with a genetic construct encoding the protein. Often, the fluorescent protein is fused with a protein of interest to track the location and expression dynamics of that protein. An advantage of such protein trackers over other detection methods is that these fluorescent dyes are synthesized in situ by the transfected cells and allow tracking without the need for any additional cofactors or substrates. Using condition-specific promoters (for a given cell type, developmental stage, or other parameter) allows the location and/or timing of the expression of the protein to be controlled. Under a fluorescent microscope or fluorescent imaging system, the location of the protein can be visualized (1,2).

Fluorescent proteins are available with emission maxima across the visible spectrum (3). Green fluorescent protein (GFP), the original fluorescent protein purified from the jellyfish *Aequorea victoria*, is the most studied. Naturally occurring and engineered variants of GFP with different spectral or structural properties have been developed. GFP-related proteins can be found with emission spectra in the blue to yellow range of the spectrum, including cyan fluorescent protein (CFP).

Choosing which fluorescent protein marker to use for an experiment might depend on the filters available on the lab's imaging system, or on the desire to multiplex and the need to select fluorophores with non-overlapping excitation and emission spectra. There is some evidence that GFP can be more toxic to cells than CFP or yellow fluorescent protein (YFP), suggesting CFP or YFP might be better options when making stable clones for tracking cells in long-term in vivo studies (4).

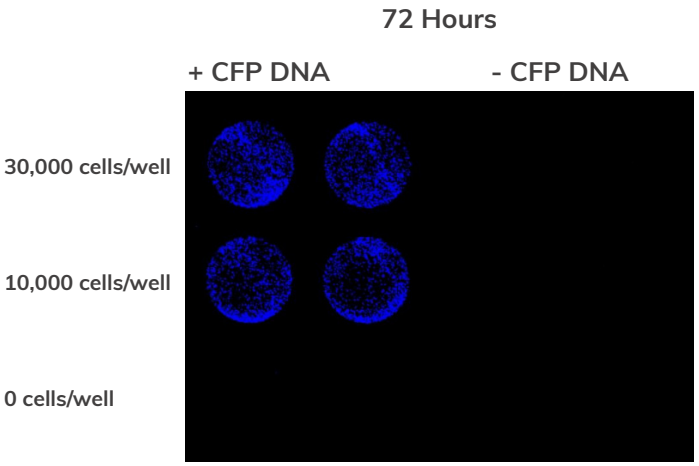

Figure 1. Cultured cells expressing CFP imaged on the Sapphire FL using the 450 Standard Optical Module.

This application note demonstrates imaging of CFP-expressing cultured cells with the Azure Biosystems Sapphire FL Biomolecular Imager using the Sapphire's 450 Standard Optical Module.

Materials and Methods

Transfection

HeLa cells were cultured in a T75 flask in EMEM media supplemented with 10% FBS until confluent. Cells were trypsinized to remove from the flask and quantified. For transfection, cells were plated in a 96-well tissue culture plate with black wells and a clear bottom. Cells were plated in quadruplicate at a density of 10,000 cells/well or 30,000 cells/well and incubated overnight. Cells in two wells of each cell density were transfected with a CFP-expressing vector (pCMV6-AC-mCFP, Origene, product number PS100044) using the TransIT-2020 transfection kit (Mirus Bio, product number 32104826) according to the manufacturer's

instructions. Cells were incubated with the transfection mix for 72 hours, and imaged at 24, 48, and 72 hours after transfection. At 72 hours, strong expression of CFP was detected.

Imaging

The cell culture plate was imaged on the Sapphire FL using the 450 Standard Optical Module which includes an excitation laser at 450 nm and a 494/34 nm emission filter. Focus type was "Plate", which is +3.00 mm above the imaging surface. Imaging was conducted with intensity level 5, quality set to "High", and 50-micron resolution.

Results

Figure 1 shows an image of CFP-expressing cells captured on the Sapphire FL using the 450 Standard Optical Module. Strong fluorescence is detected in the wells containing transfected cells, while no fluorescence background is detected in the negative control wells.

The Sapphire FL is an excellent choice for imaging cells and model organisms expressing CFP or other fluorescent reporter proteins. The imaging area of the Sapphire FL can image a variety of sample types including samples with depth such as tissue culture dishes, tissue samples, and small model organisms, as well as flat samples such as slides and membranes. With user-changeable optical modules, the Sapphire FL provides the flexibility to image a wide range of fluorescent dyes and labels. Standard modules such as the 450 Standard Optical Module are available for many commonly used fluorophores. In addition, custom laser diodes and emission filters can be combined to create modules for almost any fluorophore. As shown in Figure 1, the 450 Standard Optical Module with a 450 nm excitation laser and 494/34 nm emission filter results in strong, high-sensitivity detection of CFP expressed in transfected cells.

References

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