Imaging mCherry- and tdTomato-Expressing Cells with the Sapphire FL

Introduction

Fluorescent proteins have become indispensable tools in cell biology, enabling the visualization of gene expression, protein localization, and dynamic cellular processes in living cells.[1,2] While green fluorescent protein (GFP) and its derivatives remain among the most widely used reporters, red fluorescent proteins (RFPs) provide several complementary advantages. Their longer excitation and emission wavelengths reduce interference from endogenous cellular auto-fluorescence, experience less light scattering in biological tissues, and are well suited for imaging thicker samples.[1,2] In addition, their spectral separation from commonly used blue, cyan, and green fluorophores makes RFPs valuable components of multicolor imaging experiments, allowing multiple targets to be visualized simultaneously with appropriate optical filters and imaging conditions.[2,3]

Many of the red fluorescent proteins used today were developed through protein engineering of DsRed, a naturally occurring fluorescent protein isolated from the coral Discosoma sp.[4,5] Although DsRed offered bright red fluorescence, its tetrameric structure and slow chromophore maturation limited its utility as a genetically encoded reporter. Subsequent engineering efforts have produced a variety of improved RFPs with enhanced maturation rates, brightness, photostability, and oligomeric properties, expanding their usefulness across a wide range of biological applications.[2,5]

Among these engineered RFPs, mCherry and tdTomato have become two of the most widely adopted fluorescent reporters, although they were optimized for different experimental purposes.[5] mCherry is a rapidly maturing monomeric fluorescent protein that is well suited for fusion to proteins of interest, minimizing the risk of perturbing protein localization or function while providing reliable fluorescence in live-cell imaging experiments.[2,5] In contrast, tdTomato was engineered as a tandem dimer to maximize fluorescence intensity and is one of the brightest genetically encoded red fluorescent proteins available.[5] Its exceptional molecular brightness makes it particularly advantageous for applications requiring strong whole-cell labeling, reporter gene expression, or visualization of cells with relatively low expression levels. Together, these complementary properties have established mCherry and tdTomato as versatile tools for fluorescence microscopy, with both proteins serving as valuable red fluorescent reporters that can be readily combined with fluorophores across the visible spectrum in multiplex imaging applications.[2,3]

This application note demonstrates detection of mCherry and tdTomato expressed in transformed cells using the Sapphire FL and the 560 Standard Optical Module.

Materials and Methods

T75 flasks were coated with 10 mL of 0.1% Gelatin Solution (Millipore, PN ES-006-13) for 30 minutes at 37°C. The gelatin was then aspirated and the flasks were allowed to dry for 1 hour in the tissue culture hood. 1 mL of HL-1 Cells (8.79 x 10^5 cells/mL) were thawed in a 37°C water bath, then immediately placed in 14 mL of warmed HL-1 media. Media was changed daily by aspiration of the old media and replacement with 15 mL of fresh media warmed to 37°C. Briefly, on Day 5, cell viability was assessed by AO/PI assay and determined to have 93.4% viability. Cells were then plated at 1 x 10^5 cells/mL and incubated overnight.

Cells were transfected with pmCherry (pmCherry-N1 Vector, Clontech, PN 632523) and ptdTomato (ptdTomato-N1 Vector, Clontech, PN 632532) vectors using TransIT-X2 transfection reagent (Mirus, PN MIR 6004). Viability by AO/PI assay was determined to be 87.4% for pmCherry-transfected cells, 83.0% for ptdTomato-transfected cells, and 93.7% for untransfected control cells.

48 hours post-transfection, cells were seeded in 96-well plates at 391 – 50,000 cells/well in a two-fold dilution series, then incubated overnight. Plates were then imaged on the Sapphire FL at 100 µm resolution using a custom focus setting of 2.5 mm. The 560 Standard Optical Module (Azure Biosystems, PN IS4071), a 560nm laser with 665/150 nm emission filter, was used to image mCherry and tdTomato at Intensity 7 and 6, respectively.

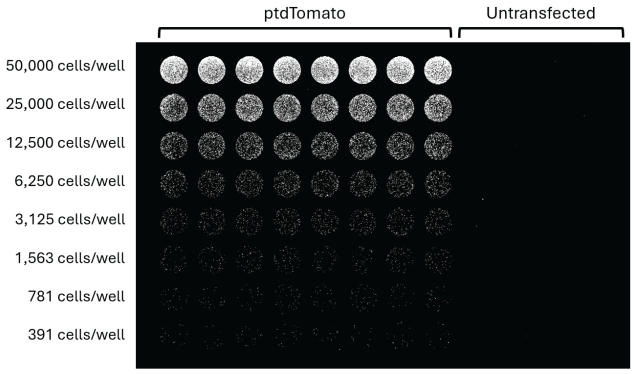

Figure 1. Cultured HL-1 cells transfected with ptdTomato or untransfected. Columns 1-8: cells expressing tdTomato. Columns 9-12: untransfected controls. Imaged at 100 µm resolution on the Sapphire FL using the 560 Standard Optical Module.

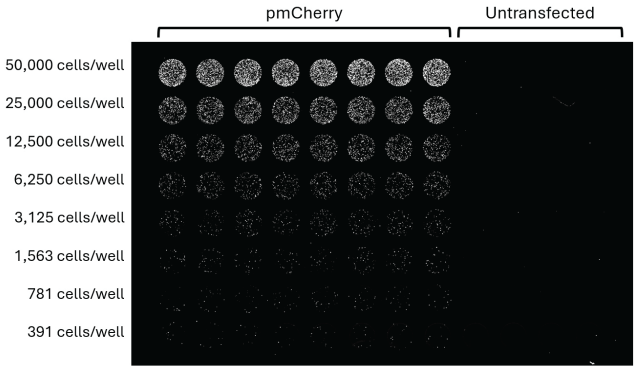

Figure 2. Cultured HL-1 cells transfected with pmCherry or untransfected. Columns 1-8: cells expressing mCherry. Columns 9-12: untransfected controls. Imaged at 100 µm resolution on the Sapphire FL using the 560 Standard Optical Module.

Results and Discussion

Figures 1 and 2 show images of tdTomato-expressing cells and mCherry-expressing cells, respectively, captured on the Sapphire FL using the 560 Standard Optical Module. Both red fluorescent proteins are strongly detected with minimal fluorescent background in the negative control wells. Red fluorescent proteins are widely used as reporters in cell-based applications, making the ability to detect fluorescent signal from proteins like tdTomato and mCherry crucial to many experiment workflows. The Sapphire FL is the only system in its class with a dedicated 560 nm laser, making it uniquely suited to imaging fluorophores in the red portion of the visible spectrum. User-exchangeable optical modules ensure optimal flexibility and compatibility with fluorophores across the spectrum, from UV to NIR. With a software-adjustable Z-plan and resolution options down to 5 µm, the Sapphire FL excels at imaging a wide variety of samples, including cells in multi-well plates, tissue slides, western blots, model organisms, and more.

References

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