TIRF Labs, Inc.

Application Note

Lightguide-based Total Internal Reflection Fluorescence and Shallow Angle Fluorescence Microscopy (SAFM) - Novel Methods for Cell Biology Studies

TIRF Labs offers add-on accessories for novel methods of lightguide-based Total Internal Reflection Fluorescence Microscopy (lg-TIRFM) and Shallow Angle Fluorescence Microscopy (SAFM) designed as factory-aligned systems. These systems are exceptionally well-suited for multicolor TIRF and SAFM experiments for cell biology studies. (See also TIRF Labs' brochure ***Compare TIRF Geometries.***)

Figs. 1 and 2 show images of human embryonic kidney cells HEK293 obtained by lg-TIRF and SAFM. Figs. 3 and 4 on page 2 illustrate the principles of lg-TIRFM and SAFM. Both methods are performed on the same add-on microscopy accessory platform shown in Fig. 5. It takes no time to install/uninstall lg-TIRF. Switching between TIRF and SAFM requires a simple operation of installing an optical block. lg-TIRFM and SAFM are compatible with dry, water- and oil-immersion objectives.

Fig. 1 demonstrates the surface selectivity and multicolor capabilities of lg-TIRFM and SAFM. In TIRF mode (left diagrams), only a thin layer (0.2 micron) of the specimen next to the surface is excited and fluoresces. Fluorophores that are farther away from the surface are not excited, and therefore, do not fluoresce. SAFM mode provides excitation of a thicker layer - up to 10 micron. Because the nuclei are farther than 0.2 micron, but closer than 10 micron from the surface, TIRF does not detect, but SAFM does reveal the nuclei labeled by DAPI. Red color images in upper diagrams correspond to the emission of red fluorescent protein dsRED produced in kidney cells transfected with a plasmid containing the cDNA carrying an endoplasmic reticulum retention signal.

Unlike in through-objective TIRFM with excitation wavelengths longer than 380 nm, the entire range from UV to near IR (190-2000 nm) is available for the excitation with lg-TIRFM and SAFM. In experiments shown, the nuclei were labeled with DAPI - blue fluorescent dye that passes through cell membranes and selectively binds to double-stranded DNA. When bound to dsDNA, DAPI excitation/emission maxima are 358 nm/461 nm. The necessity of UV excitation prohibits usage of through-objective TIRFM for detection of DAPI. lg-TIRFM and SAFM are free from this disadvantage.

The kidney cells were studied 48 hrs after transfection with plasmid. The cells were transfected also with a plasmid containing the cDNA for human caveolin fused to GFP. Fig. 2 shows triple-color SAFM applied for imaging the blue nucleus stained with DAPI, the red plasma membrane labeled by FM-4-64 probe, and green caveolin-GFP. Rapid switching between different wavelengths (without the necessity for realignment to each color) allows for merging multicolor images to reveal the spatial distribution of respective components within the cell.

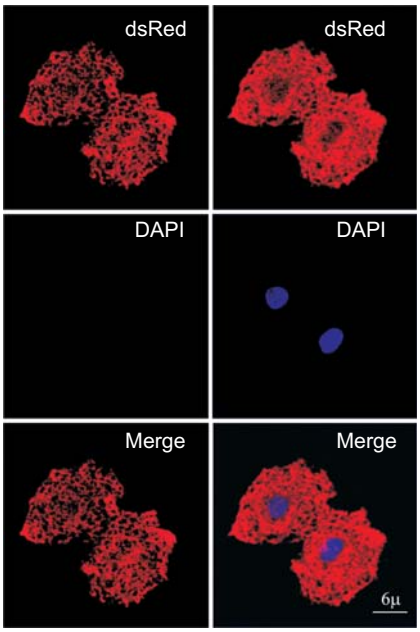

Figure 1. lg-TIRFM and SAFM images of human embryonic kidney cells HEK293 transfected with a plasmid containing the cDNA for producing a red fluorescent protein dsRED for carrying an endoplasmic reticulum retention signal. Excitation/emission maxima for detection of dsRED were 556/586 nm. The nuclei were labeled with blue fluorescent stain DAPI; excitation/emission maxima were 358 nm/461 nm. Objective was Olympus Plan-Apo-N oil-immersion 60X NA 1.45, Olympus CCD camera XM10, integration time 1s, Cell-M software. [Cell Calcium, 45 (2009) 439; BBA,1801 (2010) 147; J. Virol. 2013, 87(21):11894-907; Cell Calcium, 54, 3, (2013):246-56.]

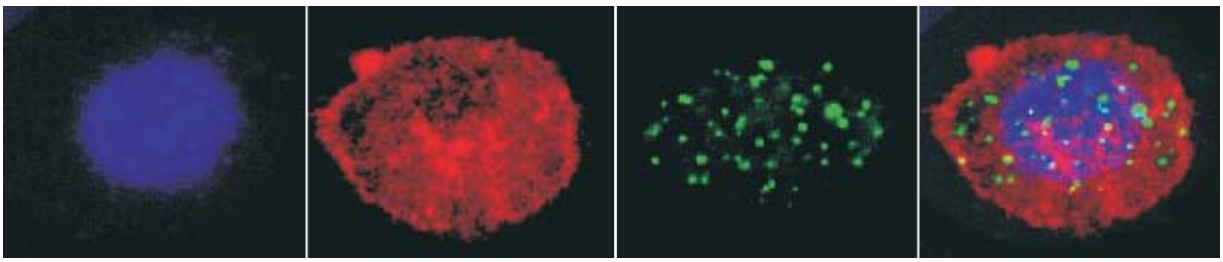

Figure 2. Triple-color SAFM images of human embryonic kidney cells HEK293 transfected with a plasmid containing the cDNA for human caveolin fused to GFP, excitation 488 nm, emission 510 nm. The nuclei were labeled with blue fluorescent stain DAPI; excitation/emission maxima were 358/461 nm. Plasma membranes were labeled with Molecular Probes FM-4-64 marker, 556/586 nm. Objective was Olympus Plan-Apo-N oil-immersion 60X NA 1.45, Olympus CCD camera XM10, integration time 1s, Cell-M software.

Multicolor lg-TIRFM and SAFM for Cell Biology Studies

Lightguide-based Total Internal Reflection Fluorescence (lg-TIRFM) and Shallow Angle Fluorescence Microscopy (SAFM)

Fig. 3 illustrates the principle of lightguide-based Total Internal Reflection Fluorescence Microscopy (lg-TIRFM), and Fig. 4 - Shallow Angle Fluorescence Microscopy (SAFM). In TIRF mode only a thin layer (0.2 micron) of the specimen next to the surface is excited by the evanescent wave and fluoresces. Fluorophores that are farther away from the surface are not excited, and therefore, do not fluoresce. In SAFM mode, there are beams of light that enter the solution phase and propagate along the surface of lightguide at shallow angles. A thicker layer of the specimen - up to 10 microns- is excited and fluoresces. Both lg-TIRFM and SAFM methods are performed on the same add-on accessory system shown in Fig. 5. Switching from SAFM to TIRFM requires a simple operation of installing an optical block on the top of TIRF lightguide. lg-TIRFM and SAFM are exceptionally flexible systems - they can be used with dry, water- or oil- immersion objectives. (See also TIRF Labs' brochure ***Compare TIRF Geometries.***) lg-TIRFM and SAFM are compatible with virtually any illuminator. TIRF Labs offers multicolor illuminators that provide rapid switching between different wavelengths. There is no necessity for realignment to different colors, which allows for rapid acquisition and merging images in multicolor experiments. Visit our website www.tirf-labs.com for details and see articles in Cell Calcium, 45 (2009) 439; BBA,1801 (2010) 147; Sensors 2012, 12, 1800-1815; Science Signaling, 2012, 5(219), ra29, J. Virology 87, No 21 (2013) 11894, Cell Calcium, 54, 3, (2013):246-56.].

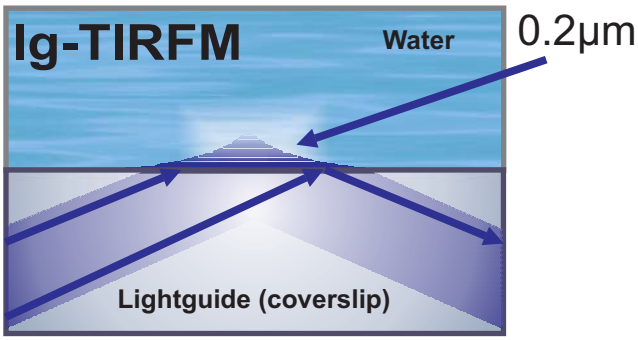

Figure 3. Exponentially decayed evanescent wave excites an approximately 0.2 micron layer of the specimen next to lightguide (coverslip).

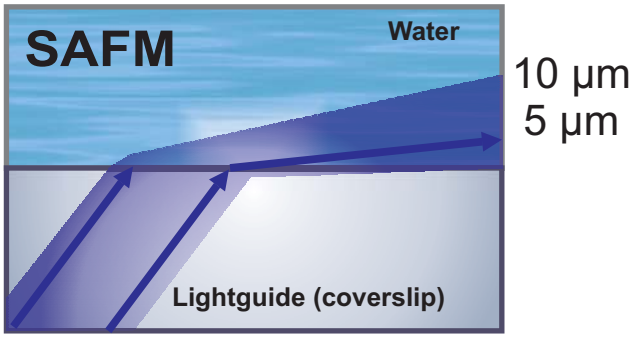

Figure 4. Shallow angle beam propagating along the surface of lightguide excites a 5-10 micron layer of the specimen.

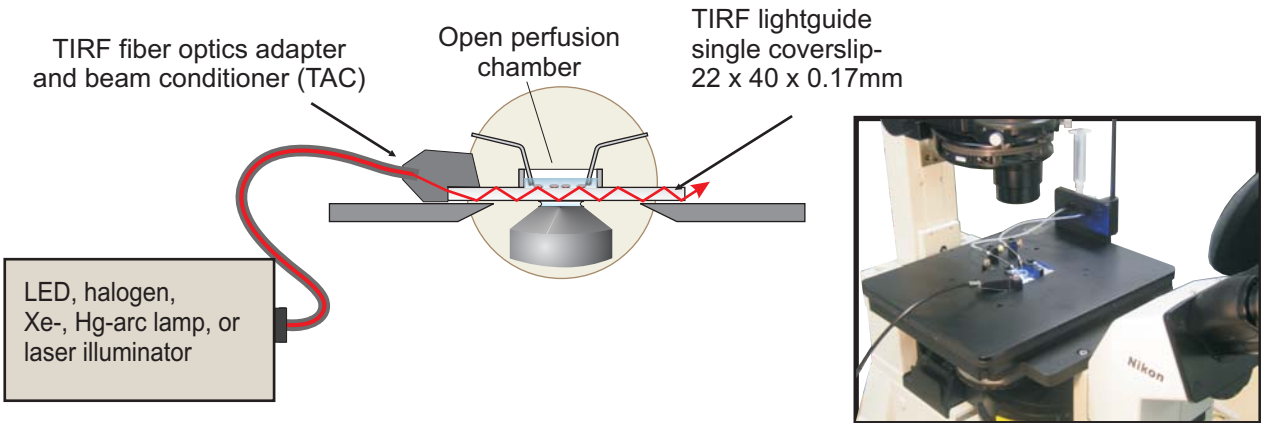

Figure 5. Schematics and photograph of lg-TIRFM / SAFM add-on system. The photo shows the model equipped with a pre-installed open perfusion chamber. Removable open perfusion chamber, closed flow cells and heated chambers are available as options. The photo illustrates lg-TIRFM platform adapted to the manual XY-translation stage of inverted Nikon Eclipse TE2000 microscope. Smaller and larger platforms are also available that fit to window that holds SBS 96-well plate, motorized Ludl and Prior stages, manual and motorized stages of Nikon, Olympus, Zeiss, and Leica.

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