

Authors: Karla Balaa &
Emmanuel Fort
Langevin Institute, ESPCI, Paris, France



Surface Plasmon enhanced TIRF imaging

Abstract:

Total Internal Reflection Fluorescence microscopy (TIRFM) is a powerful technique for imaging dynamic membrane events in living cells.

However the information gained from TIRFM may be limited by the intensity of the fluorescence signal and by background noise emanating from the inner part of the cell. Here we describe how Surface Plasmon Mediated Fluorescence Microscopy (SPMFM) can enhance TIRFM by increasing the fluorescence signal 5-6 fold and by reducing background noise dramatically. The imaging configuration is that for TIRFM except that the coverglass is coated on one side with a nanometre film of silver. For SPMFM (as in TIRFM), a high numerical aperture (N.A.) objective is essential for achieving required illumination angles

Introduction

TIRFM is used widely to study dynamic events close to the membrane of living cells. As with all fluorescence imaging techniques, information may be limited by the intensity of the fluorescence signal and /or background noise.

In TIRFM a laser beam passes through a high numerical aperture (N.A.) objective and undergoes total internal reflection when reflected from a high-refractive medium (e.g., glass) into a low-refractive medium (e.g. cell/water). By using a high N.A. objective, the laser beam can leave the front optical plane of the objective at a supercritical angle to result in total internal reflection. This produces an electromagnetic evanescent wave which penetrates the cell membrane adjacent to the coverglass and excites fluorophores within an ultra thin optical section of ~100 nm, thereby reducing background noise from out-of-focus fluorescence. However, because of a partial loss of light confinement due to light scattering in the cell, excited fluorophores in the inner part of the cell may contribute to background noise and compromise sensitivity. Here we describe how the fluorescence signal in TIRFM can be enhanced, and the background noise simultaneously reduced, with a new technique known as Surface Plasmon-Mediated Fluorescence Microscopy (SPMFM)¹.

Technology

Surface plasmons are oscillations of free electrons at the surface of a metal film, which propagate along the surface creating an associated evanescent electromagnetic field. Surface plasmons cannot be excited directly with incident light from the same side. However it is possible to excite surface plasmons propagating on the opposite side efficiently with plane polarized light. To achieve this, light must be directed at the metal from the medium with the higher refractive index and at a precise angle (the surface plasmon angle). Strong coupling occurs when the phase velocity of the plane polarised light matches that of the surface plasmon (the Kretschmann-Raether configuration). Surface plasmon excitation is associated with a sharp decrease in the reflected light, a phenomenon commonly used in Surface Plasmon Resonance (SPR) biosensors. The evanescent electromagnetic field associated with the surface plasmon on the sample side is very

similar to that created in TIRFM. It has the same penetration depth for the same incident angle. However, the intensity of this field can be much greater in the presence of a metallic thin film when excited at the correct angle. In the case of a 40 nm-thick silver thin film excited at 532 nm, intensity is increased 13 fold. Fluorophores near the metallic surface consequently receive much more light.

The metallic thin film also changes fluorophore emission dramatically and, hence, the ability to detect emitted photons. Collection efficiency varies with fluorophore-metal distance. For very short distances (<10 nm), fluorescence is quenched. For larger distances (>150 nm), most of the emitted fluorescence is reflected by the metal (>90%). The detection efficiency is high for intermediate distances only (10-50 nm) and can reach values as high as 50 % of the whole fluorescence emission (for silver thin film). The mechanism is similar to that for excitation; near-field components of the fluorophore emission couple to the surface plasmon to be converted to light on the glass side of the metal film. The molecular detection efficiency at large distances is, therefore, low and is useful in reducing background fluorescent noise from the inner part of the cell. The presence of the metallic thin film acts as a strong distance-dependent filter at the detection level, effectively selecting fluorophores at the correct distance for live membrane observation. Indeed, the basal membrane cell is estimated to lie within this range. The quenching of fluorescence at very short distances is also an advantage as it reduces noise from fluorescent molecules adhering to the glass surface.

When comparing the overall yield in surface plasmon-enhanced TIRFM compared with standard TIRFM, SPMFM provides a 5-6 fold signal at a fluorophore surface distance of 20 nm in the case of a 45 nm thick silver thin film (for isotropically distributed fluorophores with a typical fluorescence yield).

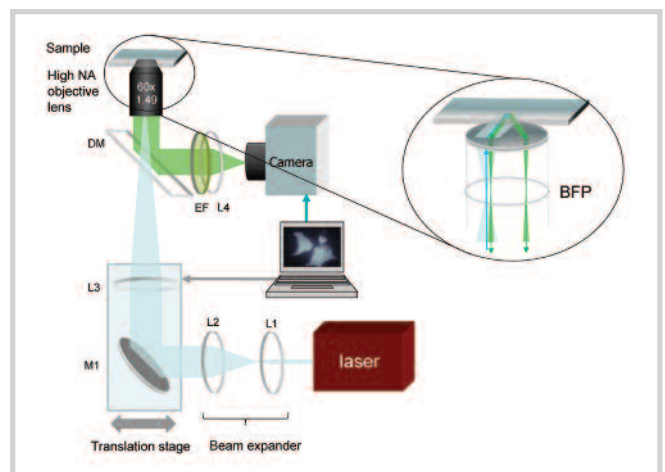


Figure 1: SPMFM on a standard objective-based TIRF microscope.

The excitation laser beam passes through a beam expander (lenses L1 and L2). Light is then directed (mirror M1) and focused (lens L3) on the back focal plane (BFP) of a Nikon Eclipse Ti inverted microscope. The mirror and focusing lens are mounted on a computer controlled micro-translation stage to tune the excitation beam incident angle. A sensitive EMCCD camera is used for fluorescence detection.

The incident p-polarized light reaches the sample plane with an adjustable angle (insert image). When this angle is equal to the surface plasmon angle, an intense evanescent field is created on the sample side.

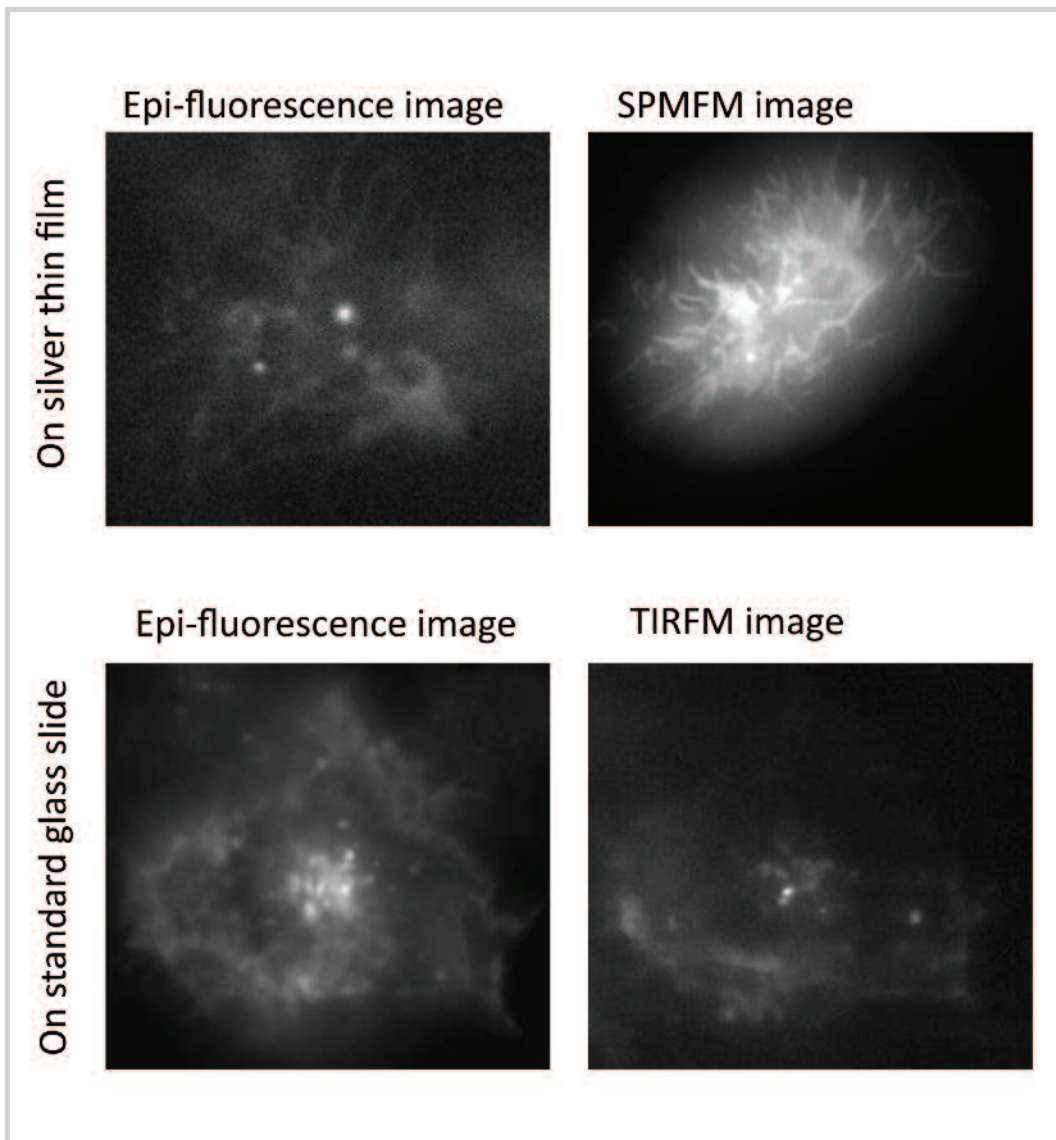


Figure 2: HEK cells transfected with mCherry on silver thin film (upper) and on a standard glass slide (lower) at a subcritical excitation angle (left) and at a supercritical angle corresponding to surface plasmon excitation of the silver (right).

Applications

SPMFM can be used to enhance the sensitivity of TIRFM applications (such as the observation of endo- and exocytosis, protein dynamics, cell-substrate interactions and signalling events). Figure 2 shows images of live Human Embryonic Kidney (HEK) cells transfected with mCherry (excitation at 530 nm; emission 580 nm). The images compare silver-coated glass (upper) and a standard glass slide (lower) using epi-fluorescence imaging at a subcritical incident angle (left) and a supercritical angle for surface plasmon excitation (right).

All images were captured under identical experimental conditions (exposure time, gain, laser power etc.) and no brightness or contrast correction was applied. The epi-fluorescence image with a silver thin film (top left) is darker than glass alone (lower left) because of low light transmission through the film. The SPMFM image appears very bright when the surface plasmon angle is achieved. This provides a straightforward way of adjusting and optimising the excitation light beam (difficult in standard TIRFM). The SPMFM image has clearly reduced background noise with high lateral resolution.

Conclusion:

SPMFM is a powerful technique with which to obtain highly sensitive, real time images of membrane events in living cells. The fluorescence signal is enhanced and background noise reduced dramatically in SPMFM compared with standard TIRFM.

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Endnote:

The authors welcome email enquiries for metal coated slides for anyone wishing to try this new imaging approach.



Emmanuel Fort



Karla Balaa

Authors' Background

Emmanuel Fort was appointed Associate Professor at the Paris Diderot University in 2000. He joined the Langevin Institute (City of Paris Industrial Physics and Chemistry Institution – ESPCI) in 2009. He is a founder and leader of the Applied Plasmonic Imaging Centre dedicated to applied plasmonic research in live science, spectroscopy and integrated nanophotonics for telecommunication and computing. Professor Fort's interests include surface nano-fabrication, plasmonic properties and plasmon-fluorophore coupling, fluorescence imaging, nanoplasmonic sensors for biomedical applications and in vivo imaging. He is also involved in studies on classical systems with particle-wave duality and quantum-like behavior.
Email emmanuel.fort@espci.fr

Karla Balaa received her Ph.D. degree from University of Nantes, France, in 2007, her Master's degree from the Lebanese University, Lebanon in 2003. She's currently a research engineer at the Applied Plasmonic Imaging Centre in Langevin Institute, ESPCI-ParisTech. Her research fields are plasmonics for biomedical applications and technological development in fluorescence imaging.
Email: karla.balaa@espci.fr

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2. Le Moal E., Fort E., Lévêque-Fort S., Cordelières F. P., Fontaine-Aupart M.P., Ricolleau C. Enhanced fluorescence cell imaging with metal-coated slides. Biophys J. 2007;92(6):2150-61.

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- The sensitivity of TIRFM can be enhanced by using surface plasmon properties of silver thin films
 - Surface plasmons efficiently transmit excitation and fluorescence light through the metallic thin film resulting in an enhanced signal and a drastic reduction in background noise
 - High 1.49 N.A. TIRF objectives are essential for SPMFM in enabling excitation of the surface plasmon above the critical angle
 - No specific additional equipment is required for SPMFM except a coverslip coated with a metallic thin film (preferably silver).
- For more information on Nikon TIRF systems go to:
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Asia

NIKON CORPORATION

6-3, Nishiohi 1-chome, Shinagawa-ku, Tokyo 140-8601, Japan
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Europe and Africa

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Laan van Kronenburg 2 1183AS Amstelveen The Netherlands
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NIKON INSTRUMENTS INC.

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