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# Resonant multi-dielectric coverslip for enhanced total internal reflection fluorescence microscopy

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## Abstract

In this paper, we discussed how multi-dielectric coated coverslip can be designed to reach large optical field enhancement when working under proper illumination conditions and in total internal reflection. As objective-based total internal reflection fluorescence microscopy (TIRF-M) is also based on total internal reflection illumination, we propose to use the resulting large field enhancement supported in such coverslip to improve TIRF-M sensitivity by amplifying the collected fluorescence signal. We present here the optimization, realization and testing in TIRF-M of resonant coverslips designed to take into account the experimental constraints. The latter's are due to the inverted configuration of TIRF-M together with the use of a high numerical aperture objective. The challenge is therefore to find the best coating design compromising between the angular tolerance and the field enhancement. We will report here enhanced-TIRF-M imaging over model samples but the ultimate goal here is to be able to monitor and quantify dynamics of pathogens at the membrane of living cells. [1]

## Keywords:

TIRF Microscopy, Thin films, Field enhancement, Evanescent beam

## 1 Introduction

Total Internal Reflection Fluorescence microscopy also called TIRF microscopy is a technique that uses evanescent wave illumination to selectively excite fluorophores in a thin layer of the sample close to the cover slip. TIRF microscopy is particularly useful for studying processes that occur at the cell membrane. This technique, as its name suggests, is based on total reflection which occurs when the incident beam arrives at an angle greater than the critical angle so that it is mainly reflected when it encounters the interface between the cover slip and the biological sample. TIRF microscopy suffers from various limitations which can affect the optical contrast and the resolution of the obtained biological images. For example, the problem of uneven illumination, due to the beam divergence, can degrade the image quality. In fact, the evanescent beam reaches its high intensity when the sample is illuminated at the critical angle. Yet, the exciting beam is inherently divergent thus a part of the beam is totally reflected which leads to the essence of the evanescent beam and another part, i.e the rays coming at angles less than the critical angle, propagates through the sample. To overcome this issue, we can increase the incident angle to guarantee an even illumination of the whole sample. Unfortunately, this leads to a decrease of the evanescent beam's intensity which degrades again the image quality. Thin films suggest promising prospects for amplifying the sensitivity of TIRF imaging due to their extreme confinement field property at the interface. Thin films are thin layers of material with a thickness typically ranging from a few nanometers to a few micrometers. They are used to modify the behavior of light, by reflecting, transmitting, or absorbing it in specific ways. Hence dielectric multilayers can be used, due to their bio-compatibility property, to enhance locally the evanescent beam without introducing any modifications into the traditional TIRF microscope.

## 2 Results and discussions

In this work, the multilayer stack consists of alternating quarter wave layers of SiO<sub>2</sub>, the low refractive index material, and Nb<sub>2</sub>O<sub>5</sub>, the high refractive index material. The stack ends with an absorbing layer of SiO<sub>x</sub>. We actually replaced the last layer SiO<sub>2</sub> by SiO<sub>x</sub> to control the absorption. In fact, one major parameter used to control the exaltation of the field is the extinction coefficient  $k$  which is also the imaginary part of the refractive index of a given material. We can't determine experimentally values of  $k < 10^{-3}$  and thus we can't use SiO<sub>2</sub> as absorbing layer and control  $k$  (ref  $k(\text{SiO}_2) = 10^{-5}$ ). We shall use a material with a measurable  $k$ . We decided hence to modify chemically the last layer in order to preserve the bio compatibility property. We controlled the oxidization level of the ending layer while deposit to transition from SiO<sub>2</sub> to SiO<sub>x</sub>. The refractive index of this layer is then determined by spectrophotometry which leads to a value of  $n = 1.602 + i0.0032$  at 561nm. the exaltation is 5 times less important but its full width at half maximum is ten times greater.

**insert graphs**

## 3 Conclusion

In conclusion, resonant dielectric multi-layer stacks were designed to enhance the evanescent beam which leads to improving the sensitivity of TIRF Microscopy. We carried out numerical simulations to predict the optical response of the designed component. The increase of the value of the extinction coefficient of the top layer leads to a drop of the enhancement at the free interface but helps increase the angular tolerance at the resonance.

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## References

- [1] Anita Mouttou, Fabien Lemarchand, Cihan Koc, Antonin Moreau, Julien Lumeau, Cyril Favard, and Aude L Lereu. Resonant dielectric multilayer with controlled absorption for enhanced total internal reflection fluorescence microscopy. *Optics Express*, 30(9):15365–15375, 2022.