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# Resonant dielectric multilayers improving fluorescence imaging

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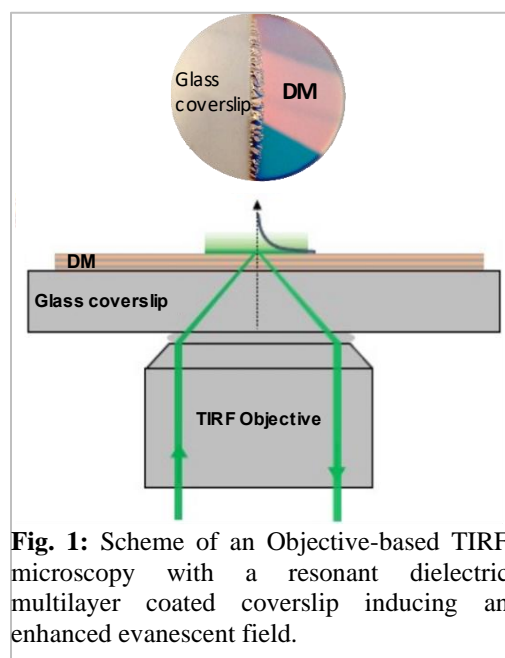
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**Abstract:** We present dedicated dielectric multilayers (DM) optimized to be resonant under TIRF-microscopy constraints. By investigating DM enhancement and transmission, we could predict and measure a fluorescence signal enhanced by a factor of 3.

## 1. Introduction

In thin films-based nanophotonics, field enhancement and confinement are classically used for sensing or imaging application. In the framework of imaging, surface plasmon resonances have been used, a prism-based configuration [1], and in an objective-based configuration [2]. Large fluorescence signal enhancements were evidenced over model fluorescent molecules and fluorescently marked cells respectively. However, the use of metallic thin films implies limitations in terms of illumination conditions or due to quenching effects by the coupling fluorophores/metals. Capitalizing upon strong and sharp optical resonances supported by Bloch surface waves in resonant dielectric multilayers (DM), [3 – 4] have introduced such concept in fluorescence imaging.



**Fig. 1:** Scheme of an Objective-based TIRF microscopy with a resonant dielectric multilayer coated coverslip inducing an enhanced evanescent field.

In Total Internal Reflection Fluorescence microscopy (TIRF-M), these resonant effects can act on the local evanescent field involved to improve the fluorescence signal and limit the background noise. Indeed, the major drawbacks in TIRF-M are: 1- its angular divergence leading to an illumination mixing propagative and evanescent waves and 2- the strong evanescent intensity decrease as function of the incident angle. All these degrade TIRF-M performances in terms of fluorescence intensity and signal to noise ratio. To overcome these issues, a possible solution is to locally enhance the evanescent field intensity. However, theoretical and experimental disagreements are usually reported with DM. We thus introduced an innovative resonant DM adapted to the Total Internal Reflection Fluorescence microscopy (TIRF-M) constraints [5]. We present here the numerical and experimental results obtained in all aspects from optimisation, fabrication, to TIRF-M implementation.

## 2. Material and methods

### 2.1. Experimental set-up

The experimental setup is based on a commercial microscope (Nikon Eclipse Ti) supplied with a 100x 1.49 NA objective and a coupled to 561 nm TE-polarized laser (See Fig. 1). In this inverted configuration, the fluorescence emission is collected through the objective before being directed to an EM-CCD camera and recorded via the implemented MetaMorph software. The laser excitation residual intensity is also filtered thanks to dichroic mirror and a bandpass filter. As schematized in Fig. 1, the excitation beam is focused at the back focal plane of the objective, with an incident angle controlled by tuning the radial position of the focused beam on the back focal plane. With this system, the incident angle at the coverslip free interface can be changed from 0° to 81°.

### 2.2. Dielectric multilayers optimization

The DM were optimized using the absorption-based optimization reported in [6 – 9]. The main inconvenient here is the resulting strong and sharp resonance that is not adapted to TIRF-M application. Therefore, we developed a

dedicated optimization method in order to consider the intrinsic angular divergence and the fluorescence collection through the objective (i.e. DM transmission efficiency). We optimized these dielectric multilayers for a TE – polarized laser of 561 nm at an incident angle of 68°. In our optimization, we specified the spectral (excitation:  $\lambda_{exc}=561$  nm and emission:  $\lambda_{em}$  ranging from 550 to 700 nm) and angular (beam incident angle:  $\theta_{inc}=68^\circ$  and photons collection angle:  $\theta_{coll}$  ranging from 0 to 81°) ranges together with a maximization of the electric field at the free interface of the thin film stack in contact with the biological medium.

### 2.3. Dielectric multilayers deposition

The designed DM substrates were fabricated plasma assisted reactive magnetron sputtering method (Helios – Bühler/Leybold Optics). They are deposited on a 170  $\mu\text{m}$  thick glass coverslip (Menzel-Gräzer, VWR) adapted to objective-based TIRF-M. To obtain a direct comparison of the field enhancement between the DMs and a bare glass coverslip, the coatings were done over half of the glass surface (see figure 1). The resulting DM are made of alternating low ( $\text{SiO}_2$ ) and high ( $\text{Nb}_2\text{O}_5$ ) refractive indices materials with a last dissipating layer. Note that the refractive indices of each material are determined by spectrophotometry between 400 to 2400 nm over deposited single layers to be used in all numerical simulations (for structure optimizations to optical response predictions).

## 3. Results

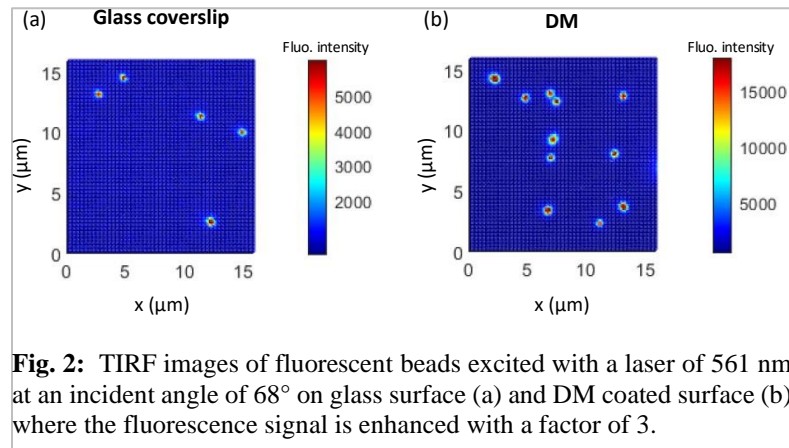
### 3.1. Numerical investigations

By numerical analysis, we estimated an electric field enhancement several hundred times higher than the one on glass surface under plane wave conditions. However, when using an objective-based TIRF-M, both the instrumental characteristics such as the beam angular divergence (acting on the excitation field enhancement) and the fluorescence collection scheme (linked to the transmission efficiency of the DM) has to be considered. Under such conditions, the field enhancement at the free interface is highly defaced and the transmission efficiency of the DM adds an additional decrease on the resulting fluorescence enhancement.

We developed numerical studies to predict the enhancement factor of fluorescence by taking into account these two issues. We estimated a loss of more than 95% in the enhancement factor of the fluorescence signal. However, when measuring the fluorescence signal over 200 nm beads, we still observed large disagreements between the predictions and the experiment. Another parameter of importance over such resonance is the extinction coefficient ( $k \sim 10^{-5}$ ) of the dissipative layer i.e. top layer. We numerically evidenced that a small variation of  $k$  will provokes a strong decrease of the field enhancement. Therefore, we tackled the problem differently and conceived DM with a dissipating top layer of measurable and reproducible  $k$  to fully control its absorption.

### 3.2. Experiment

With this improved design, a fluorescence enhancement of factor of 4 is predicted by taking account the beam divergence and the transmission efficiency. Experimental measurements, realized on a commercial TIRF-M using fluorescent 200 nm beads were then carried out as shown in Fig. 2. We compared beads fluorescence signals on the glass surface Fig. 2 (a) and the DM surface Fig. 2 (b). We measured a fluorescence signal 3 times higher with the DM together with an improvement of signal to noise ratio by a factor 3.



**Fig. 2:** TIRF images of fluorescent beads excited with a laser of 561 nm at an incident angle of 68° on glass surface (a) and DM coated surface (b) where the fluorescence signal is enhanced with a factor of 3.

## 4. Conclusion

We designed resonant dielectric multilayers coated coverslip adapted for TIRF imaging application by acting on the local excitation field in order to enhance the fluorescence intensity. These DM can be designed for any excitation wavelengths and incident angles which permit to consider illumination at larger incident angles already improving the

axial resolution. To consider the TIRF-M experimental constraints and get a good agreement between the predicted and measured fluorescence signal, we developed dedicated numerical analysis methods. We successfully demonstrated an increase of the fluorescence signal by a factor 4 with respect to classical glass surface, which is in agreement with the observed experimental enhancement factor of 3. We now pursue optimisation steps to achieve high fluorescence enhancement. This study will impact the field of fluorescence-based microscopies by improving the lateral resolution and speed of acquisition, allowing fast super-resolution imaging on simple microscopes.

## 5. Acknowledgements

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