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Resonant dielectric multilayers improving fluorescence imaging

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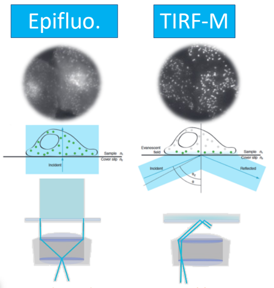
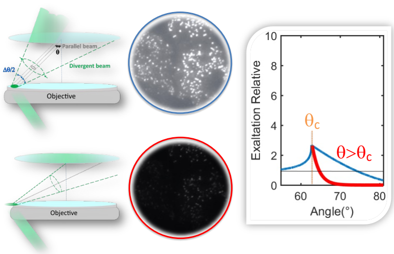
In thin films-based nanophotonics, field enhancement and confinement are classically used for sensing or imaging applications. In the framework of imaging, surface plasmon resonances have been used both in a prism-based configuration [1], and in an objective-based configuration [2]. Large fluorescence 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, biocompatibility limitations and high quenching effects by the coupling fluorophores/metals. Capitalizing upon strong and sharp optical resonances supported by Bloch surface waves in resonant dielectric multilayers, refs. [3 – 4] have introduced such concept for fluorescence imaging. We present dedicated dielectric multilayers (DM) optimized to be resonant under TIRF-microscopy constraints. By investigating the DM enhancement and transmission, we could predict and measure, with a good agreement, a fluorescence signal enhanced by a factor of 3 for both fluorescent model beads and virus-like particles [5].

CONTEXT
TIRF-M for viral imaging

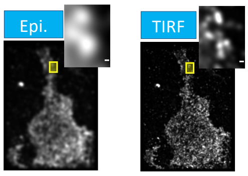
- Great axial resolution ideal for membrane events imaging
- Simple optical configuration
- Easily accessible to non-opticians
- Widely used in bio labs
- Weak contrast versus weak signal



Axial resolution improved by TIRF-M
Mattheyses et al., J Cell Sci, 2010



Technological barriers for imaging and monitoring viral budding and assembly using TIRF-M



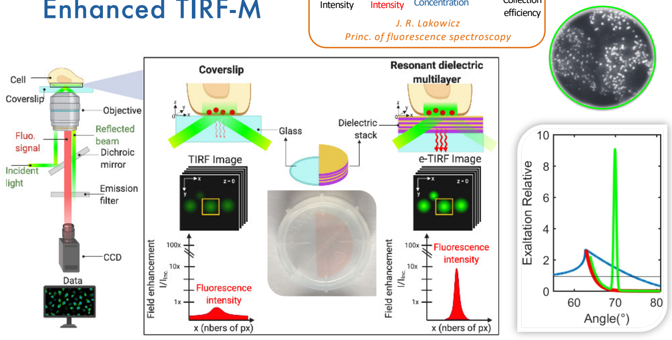
HIV-1 Gag assembly by epifluorescence versus TIRF-M
K. Inamdar, et al., Viruses, (2019)

CONCEPT
Enhanced TIRF-M

$$I_{FLUO} = I_{exc} \cdot \frac{N}{V} \cdot \varphi . \varepsilon . E$$

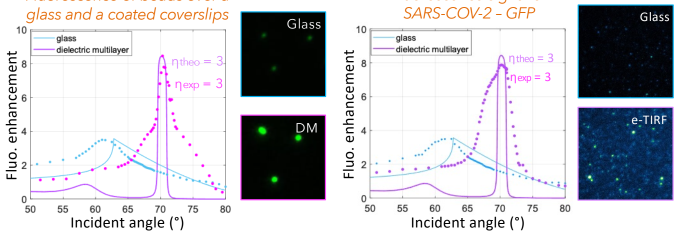
Fluorescence Intensity; Excitation Intensity; Concentration; Photo-physics; Collection efficiency

J. R. Lakowicz, Princ. of fluorescence spectroscopy



Acting on the local excitation field at high incident angles *e-TIRF-M - Brevet (2021)*

Viral imaging using e-TIRF-M

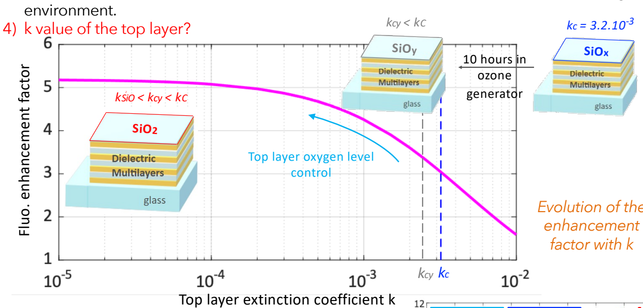


Demonstration of the sensitivity improvement with e-TIRF by a factor of 3 over both model beads and SARS-COV2-GFP virus-like particles. A good agreement is observed between numerical work and experiment.

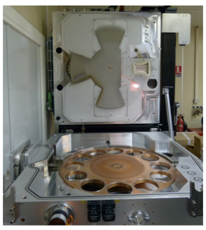
Resonant dielectric multilayer
Control of the absorption of the top layer

Optimization criteria: 1- Maximizing the field
2- Large angular acceptance
In accordance with $\Delta\theta_{TIRF}$ = 15 mrad
3- Optimizing the transmission

Numerical optimization with weighted targets to adapt the experimental conditions:
1) spectral model for resonances
2) broader angular model to numerically force the convergence on structures with good angular tolerance
3) field maximization at the free interface of the structure in contact with the biological environment.
4) k value of the top layer?

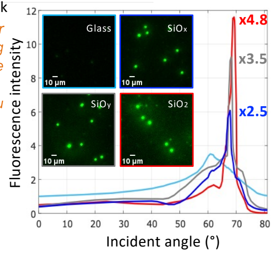


Evolution of the enhancement factor with k

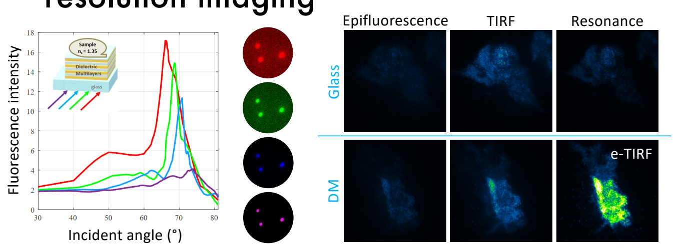


Deposition by plasma assisted magnetron sputtering - Helios. Oxygen level tunable

Fluorescence of beads for the 3 stacks evidencing the impact of k over the fluorescence enhancement. A plateau is reached with $k \geq 10^{-4}$.



Multi-color enhancement for super resolution imaging



Ongoing optimization of resonant DM to support obj-TIRF-adapted multi-resonances

Comparison between epifluo., TIRF and e-TIRF imaging of HIV-1Gag transfected cells

After sensitivity improvement, we aim at developing e-TIRF-M based super-resolution imaging to observe membranes mechanisms such as virus assembly and budding occurring the host cell membrane

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Modelling of light scattering in resonant multilayered stacks

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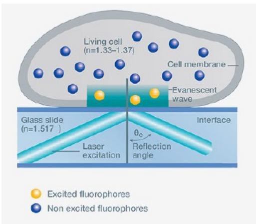
Abstract : The aim of our study is to estimate the scattering effect caused by surface roughness within a multilayer using Finite Element Method. The specific multilayers we examined were designed to enhance the optical resonance under total internal reflection (TIR) and thereby improve the sensitivity of fluorescence microscopy.

Context

Goal: Studying the dynamics of pathogens in TIRF-M.

Problem: Low sensitivity and lateral resolution of TIRF microscopes.

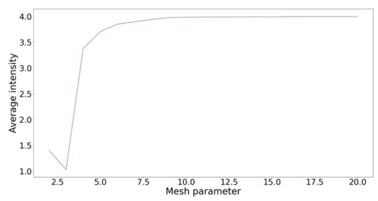
Solution: Developing micro to nano-structured optical components based on resonant multilayer stacks to improve TIRF microscopy sensitivity and lateral resolution.



TIRF microscopy

Finite Element Method

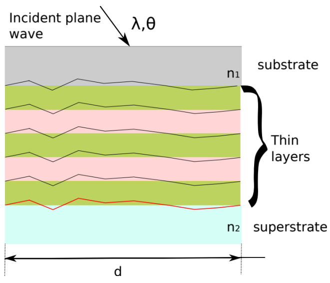
- Defining an accurate geometry of the real model.
- Adding perfectly matched layers (PML) to simulate the free space.
- Choosing the suitable mesh parameter.
- Periodic boundary conditions.
- Average intensity at the free interface $\int_\Gamma |E|^2 d\Gamma$



Convergence of the mesh parameter

Introduction

- Surface topography affects a surface's response to an excitation beam.
- Roughness refers to surface texture with high spatial frequency deviations.
- Roughness can introduce unwanted scattering and diffraction phenomena, parasiting the optical response of multilayer-based components.
- This is especially problematic for resonant components that operate at incident angles greater than the critical angle.



The geometry of the modeled multilayer stack with rough surfaces

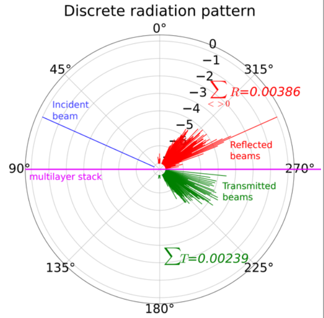
Results

Diffraction equation

m λ=d $n_1(\sin(\theta_0)+\sin(\theta_i))$ in Reflection

m λ=d $(n_1\sin(\theta_0)+n_2\sin(\theta_i))$ in Transmission

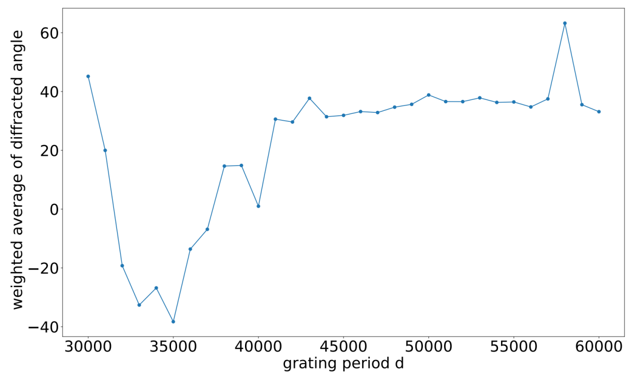
- m : diffraction order
- d : period of the grating
- θ_0: incident angle
- n_1 : refractive index of the substrate
- n_2: refractive index of the superstrate
- θ_i: ith diffraction angle



Angular distribution of the intensity (log): R is the reflection efficiency and T is the transmission efficiency

d-independence

- Comparison of discrete radiation patterns while increasing the period of the grating d.
- Beyond a certain value of d, the diffraction diagram remains unchanged.
- Introduction of another parameter to check this independence.
- The weighted average of diffracted angles expressed as: $\theta_m = \frac{\sum_{k\neq 0} R_k \theta_k}{\sum_{k\neq 0} R_k}$

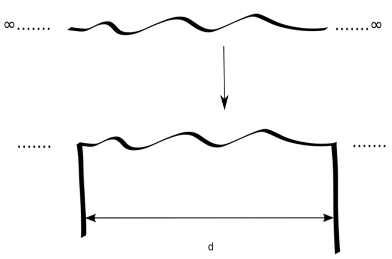


Convergence of the diffracted angle in regards to the period of the grating d

Numerical model

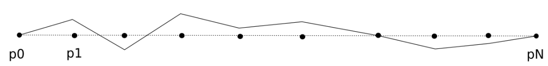
Periodicity

- To numerically study optical scattering, it is necessary to model a geometrically limited rough surface due to limited computing resources.
- To achieve this goal, it is necessary to limit the extent of our domain. We assume that the roughness has a periodicity of d.



From an infinite surface to a periodic one: The introduction of diffraction gratings

Rough surface



- Sampling the surface
- Adding different heights to the distinct points.
- Measuring the roughness of a microscopic slide and using the values as a reference.
- Replicating the same roughness pattern within the different layers of the stack.
- Modelling the response of the obtained diffraction grating using Finite Element Method.

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SINGLE STEP PLASMA ETCHING OF NB_2O_5/SIO_2 MULTILAYER STACKS

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Structures formed by using multiple layers of alternating dielectric materials with high and low refractive indices find their applications in various optical devices including optical filters, sensors, Bragg reflectors and laser devices. While most filters are generally uniform in the transverse direction, the structuring of the filters at the micron scale represents unique opportunities especially for multispectral imaging or microscopy applications. To achieve such micrometric structures, the plasma etching of the multilayer stack is a critical process step and the challenge is to optimize etching recipe providing a single step etch solution for both dielectric materials.

This paper presents the dry etching characteristics of Nb_2O_5/SiO_2 multilayer stacks. Dry etching was achieved by using a combination of C_4F_8, O_2 and Ar gases in an inductively coupled plasma (ICP)-reactive ion etching system [1]. Ni electroplated layer was used as a hard mask. This required a process flow composed of several technological steps. In order to reduce the number of technological steps, another process flow was designed by using chromium as an etch mask. Another process was developed using a combination of C_2F_6, O_2 and Ar gases in a capacitively coupled plasma - reactive ion etching (CCP-RIE) system. The recipe was optimized to achieve a high etch rate, good selectivity, and anisotropic etch profile (close to 90°).

The structures fabricated were optically characterized using the system described in ref. [2]. Mapping of the local transmission in the spectral bandpass of the filter (e.g. 770 nm) of several consecutive pixels is shown in Figure 1c. All coated and uncoated regions of the pixelated filter transmit 100% of the light, except a narrow region surrounding each pixel with size of 1-2 µm, confirming that diffraction effects on the side of each pixel is minimum. Applications for multispectral imaging and microscopy will be presented.

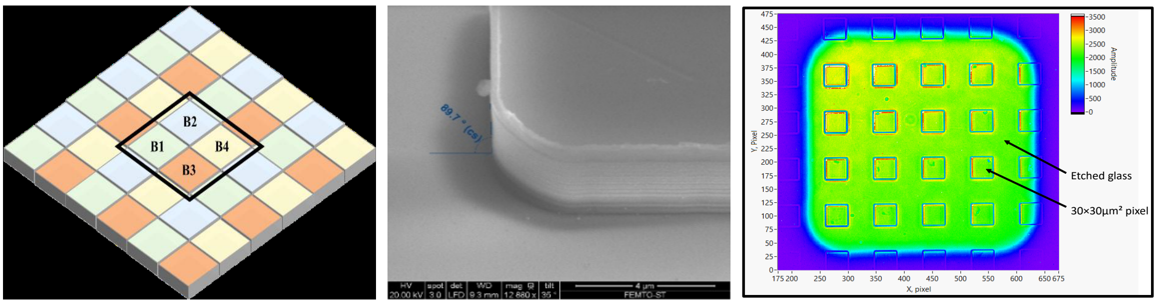

Figure 1: a) Example of 2×2 pixelated filter. b) SEM cross section of etched Nb_2O_5/SiO_2 multilayer. c) Transmission at 770 nm of a pixelated filter.

References:

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