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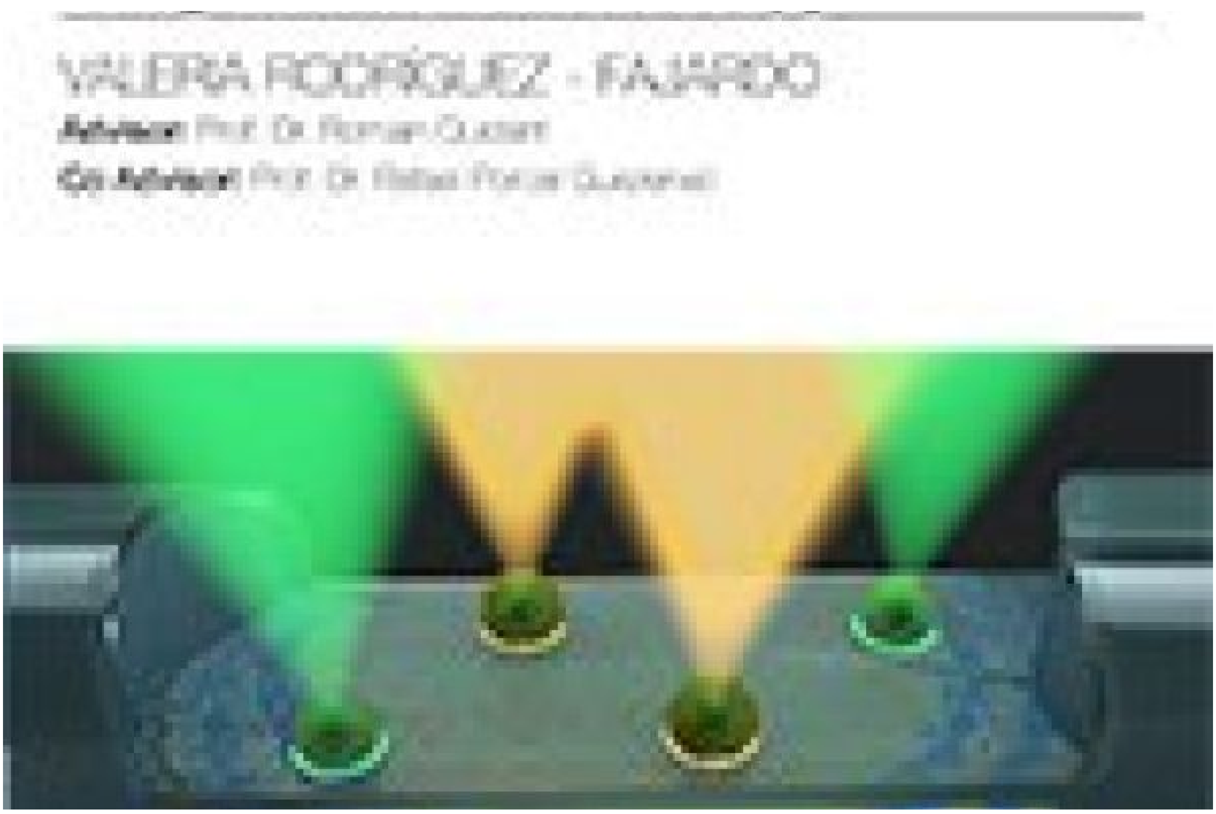

PhD Thesis Defense VALERIA RODRIGUEZ FAJARDO 'Novel Methods for Plasmonic Nanoparticles Imaging Inside Cells Using Dark-Field Microscopy'

VALERIA RODRIGUEZ-FAJARDO

February 22, 2018

Thursday February 22, 10:00. ICFO Auditorium

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Plasmon Nano-Optics

ICFO-The Institute of Photonic Sciences

The potential impact of noble metal nanoparticles (NPs) in diverse fields, particularly medicine, is tremendous. However, before NPs can be routinely used in clinical practices, it is

fundamental to comprehensively study their interaction with cells.
This study is non-trivial because such interaction is an extremely complex process that depends on multiple factors.

In order to use plasmonic NPs as biosensing probes or to investigate their interaction with biological specimens, it is necessary to detect them. The most frequently used methods are electron microscopy (SEM/TEM), two-photon luminescence (TPL) and dark-field (DF) microscopy. While the first one outperforms in terms of resolution, it requires intensive sample preparation and is highly invasive. Although TPL is capable of identifying NPs with good accuracy and contrast, measurements can be affected by high peak powers of pulsed illumination. Moreover, they require specialized equipment, and are limited by insufficient temporal resolution to follow the NP-cell interaction dynamics or track cells in flow. In this context, DF stands out as a great option. However, since the cell ?s scattering can be very high, DF alone is not reliable for detecting metal NPs immersed in cells.
In this thesis we present two alternative methods for imaging of plasmonic NPs embedded in cells, both based on DF microscopy. Scattering is very attractive for several reasons: acquisition time is not fundamentally limited; it is harmless for
the cells or plasmonic NPs; it does not suffer from blinking or bleaching; and its implementation is simple and does not require specialized elements. The key idea is removing the cell?s contribution to the total scattering by using the distinct
optical properties of cells and plasmonic NPs, thus overcoming the reliability issue of standard DF.

Polarization difference dark-field (PDDF) microscopy exploits the different responses of cells and gold nanorods (GNRs) to light?s polarization orientation. While scattering intensity of GNRs is highly dependent on it, cell?s is not. Therefore, by subtracting two images , one for each polarization direction, cells ? scattering is eliminated. We validated the concept using a phantom sample, and proved PDDF?s ability to discriminate GNRs-loaded cells from bare ones is higher than DFs. However,
its applicability is limited by two factors: it is only useful for asymmetric NPs, and it is not possible to carry out quantitative measurements.

Two-color dark-field (TCDF) microscopy uses the distinct optical properties of cells and plasmonic NPs on illumination wavelength. While NP?s scattering strongly changes with wavelength, cell?s does not. Hence, the subtraction of two images, one for each color, will cancel out cell?s scattering. Using phantom samples we first proved standard DF is not suitable for plasmonic NPs detection in environments with non-negligible scattering, whereas TCDF is capable of doing so. We carried out experiments on mammalian cells that show TCDF performs better than standard DF in terms of both specificity and sensitivity. Finally we demonstrated the potential of TCDF for long-term tracking of NPs in cells and cell screening in mixed populations under both static and flowing conditions.

Thanks to its robustness, fewer limitations and better performance, the use of TCDF is more convenient than PDDFs. We demonstrated TCDF is efficient and versatile: It works for adherent and suspension cells, under static and flowing conditions, and for diverse applications. Importantly, TCDF?s performance could be further improved by optimizing the optical setup and using more sophisticated calibration methods. TCDF is presented as a complement to existing methods (TPL and SEM/TEM), since it is more suitable for live-cell studies and performs better in terms of speed, although it is not as sensitive.

TCDF could be applied to other resonant NPs and systems, as long as their scattering properties significantly differ.

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