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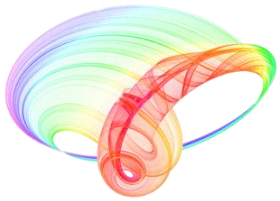

PHOTONICA2021

VIII International School and Conference on Photonics

& HEMMAGINERO workshop

23 - 27 August 2021,

Belgrade, Serbia

Editors

Mihailo Rabasović, Marina Lekić and Aleksandar Krmpot

Institute of Physics Belgrade, Serbia

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Pixel categorization based on resonance energy transfer between fluorescent molecules: a pathway towards localization of functionalized metal nanoparticles in individual cells by fluorescence microscopy

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Molecular resonance energy transfer (RET) is a basis of a half-century-old tool known as the molecular ruler, used for inference of mutual distances of two spectrally-coupled fluorophores usually relevant in biological contexts [1]. RET influences not only the absolute intensities of fluorescence signals of the molecular pair, but also their other optical characteristics, e.g. the valence electrons' excited states. On the other hand, metal-based hybrid nanostructures show great promise as future multifunctional nanoplatforms, due to their superior sensitivity to external perturbations [2]. The metal components of the nanostructures influence the optical properties of fluorescent molecules in the vicinity, similarly as bulk metals do, which often presents a drawback. Here we want to demonstrate how this particular feature in metal-based nanosystems can be used as an advantage point in conventional biophysical imaging if the beneficial underlying physical mechanisms are identified [3].

In this talk, we will first explain the influence of gold nanoparticles on resonance energy transfer between tryptophan, essential amino acid, and riboflavin, vitamin B2, in an aqueous environment. We will show that the relative change of the signal in the tryptophan spectral channel is more pronounced when the biomolecules are attached to the nanoparticles. Afterward, we will demonstrate how time-lapse fluorescence imaging of nanoparticle-incubated liver cancer cells can be used for inferring subcellular areas of nanoparticle accumulation. By exposing the incubated cell to continuous UV radiation, the photobleaching of the fluorescent molecules in the sample occurs. Being sensitive to the electronic structure of the fluorophore, the photobleaching rates of different subareas in the cell show distinctive trends, depending on the chemical composition. As the nanoparticles promote RET between tryptophan and riboflavin, the nanostructures exhibit a particular photobleaching trend compared to the cells' endogenous fluorescent species [4]. Thus, by separating areas of fluorescence images into different classes based on their photobleaching rates, i.e. grouping the pixels of the same signal trends, we are able to infer the preferential accumulation sites of bifunctionalized gold nanoparticles by using fluorescence microscopy of lower lateral resolution than the size of the nanoparticles. In this way, we demonstrate a potent auxiliary detection modality of metal-based hybrid nanostructures in optical imaging.

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