CELLULAR UPTAKE OF GOLD NANOPARTICLES STUDIED VIA CORRELATIVE FOUR-WAVE-MIXING IMAGING AND CONFOCAL FLUORESCENCE MICROSCOPY

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Gold nanoparticles (NPs) are promising drug delivery vehicles owing to their facile synthesis, simple bio-conjugation, and low toxicity. However, when embedded in highly heterogeneous and fluorescing environments such as cells and tissues, these NPs have to be large (typically >50nm diameter) to be distinguished optically against backgrounds via their linear scattering at the surface plasmon resonance (SPR). As a result, cell imaging protocols often adopt the use of fluorophore tags attached onto the NP, and assume that the fluorophore is a reliable reporter. Here, we present a study where small gold NPs (10nm to 40nm diameters) are visualised background-free inside HeLa cells using an innovative nonlinear microscopy contrast based on their SPR resonant transient four-wave-mixing (FWM), as demonstrated in [1,2]. Various fluorescently-labelled ligands were conjugated to the NPs and internalised through clathrin-mediated endocytosis. Their location was measured with confocal fluorescence and was correlated to that of the NPs from FWM. Surprisingly, even covalently attached fluorescently-labelled ligands exhibited very low co-localization coefficients (Pearson's & Mander's), highlighting the limitations of fluorescence tagging (see figure). This study opens new perspectives to the understanding of the cellular uptake of conjugated NPs and their ligands.

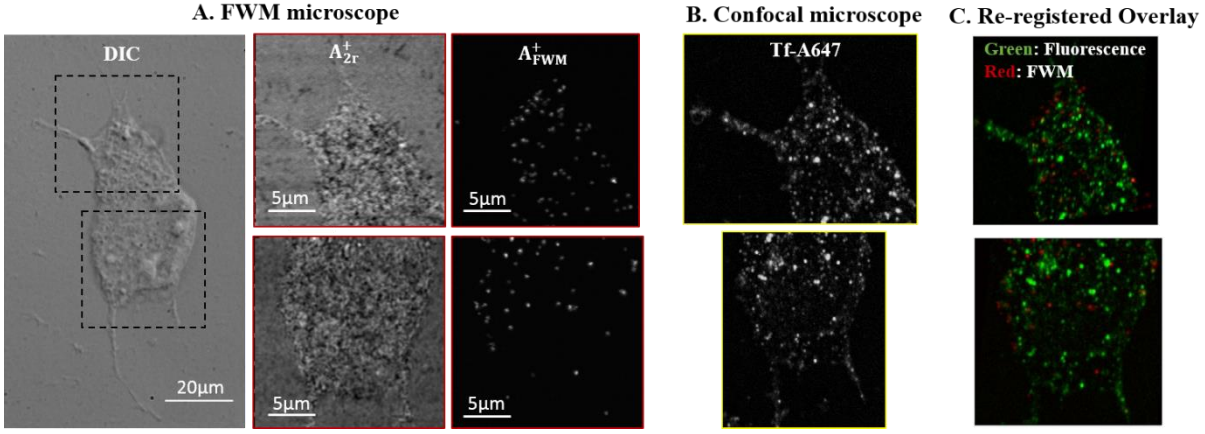

Fig. 1 A. Imaging on the FWM microscope; Differential interference contrast (DIC) image of the whole cell, FWM imaging of 40nm NPs in the two cell regions indicated, where the reflected probe field amplitude (A^+_{2r}) and the FWM amplitude (A^+_{FWM}) are shown. FWM amplitude is shown as a maximum intensity projection over 6µm z-stack while the reflection is shown on a single xy plane. **B. Imaging on the confocal microscope;** fluorescently labelled Transferrin (Tf) ligand with Alexa Fluor 647 is shown as a maximum intensity projection over the same cell regions. **C. False colour overlay of the two imaging modalities**.

[1] Francesco Masia, Wolfgang Langbein, Peter Watson, and Paola Borri, "Resonant four-wave mixing of gold nanoparticles for three-dimensional cell microscopy" *Optics Letters* **34**, 1816 (2009).
[2] F. Masia, W. Langbein, P. Borri, "Measurement of the dynamics of plasmons inside individual gold nanoparticles using a femtosecond phase-resolved microscope" *Phys. Rev. B* **85**, 235403 (2012).