CORRELATIVE LIGHT ELECTRON MICROSCOPY VIA FOUR WAVE MIXING IMAGING OF GOLD NANOPARTICLES

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Correlative Light Electron Microscopy (CLEM) is one of the most powerful imaging technologies as it combines the advantages of live cell imaging from light microscopy (LM) with the sub-nanometer spatial resolution of electron microscopy (EM) into one experiment. CLEM is however seriously hampered by the availability of robust probes. It is highly questionable whether most bimodal probes using both a fluorophore (for LM) and an electron-dense gold nanoparticle (for EM) attached to the protein of interest actually show the same protein pool. This is a serious drawback that needs to be addressed.

In this work, we show a novel CLEM workflow where we use only 1 probe; gold nanoparticles (NPs). To visualise the gold NP in LM in cells, we use a novel optical imaging technique recently developed in our lab, based on the nonlinear four wave mixing (FWM) response at the surface plasmon resonance of the gold NP [1]. This provides a highly sensitive, specific, and quantitative particle detection which does not rely on fluorescence readout, and is background-free even in highly scattering environments. As marker protein, we used the Epidermal Growth Factor (EGF) coupled to either 10 or 20 nm gold NPs, bound to the cell surface of HeLa cells grown on sapphire discs and subsequently high-pressure frozen. We then freeze substituted these samples in pure acetone to Lowicryl HM20 to be polymerized; 300nm sections were analysed by FWM microscopy and TEM and the images were registered together. We found a perfect match between gold particle signals from both modalities (see Figure), with mean position deviations in the 10nm range. In short, this novel CLEM method alleviates major issues around multimodal probes and generates a more reliable CLEM workflow with one to one translation of the markers. Notably, by visualising *single* NPs, it has the potential to follow individual (low abundance) receptor internalisation and place them in their cellular context.

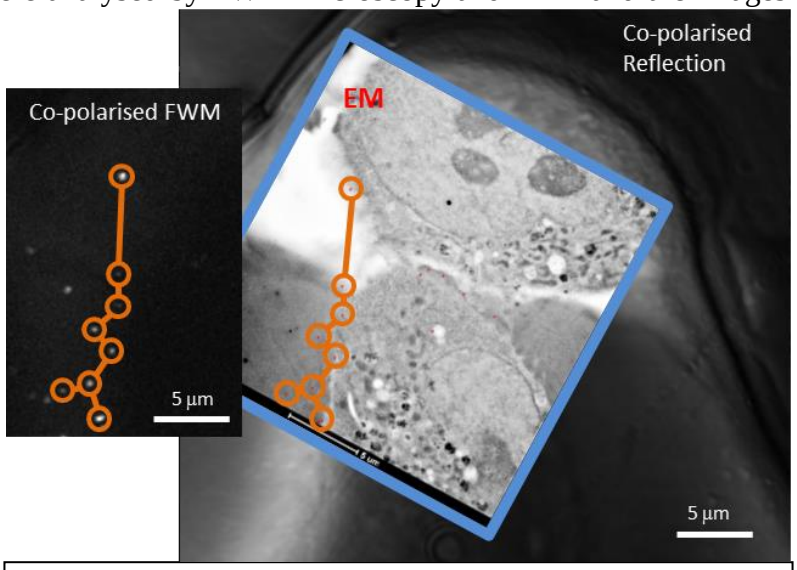

Figure. EGF coupled 20 nm gold particles bound to the surface of HeLa cells visualised using FWM microscopy (left) match perfectly (brown circles) with the gold particles as they are found inside the section in the TEM (right).

[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); 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).