Electronic supplementary information.

Cost-effective side-illumination darkfield nanoplasmonic markers microscopy.

Mengjiao Qi, Cecile Darviot, Sergiy Patskovsky, * and Michel Meunier

Engineering Physics Department, Ecole Polytechnique de Montréal, Laser Processing and Plasmonics Laboratory, Montréal, Québec, H3C 3A7, Canada. E-mail: sergiy.patskovsky@polymtl.ca

FIGURES.

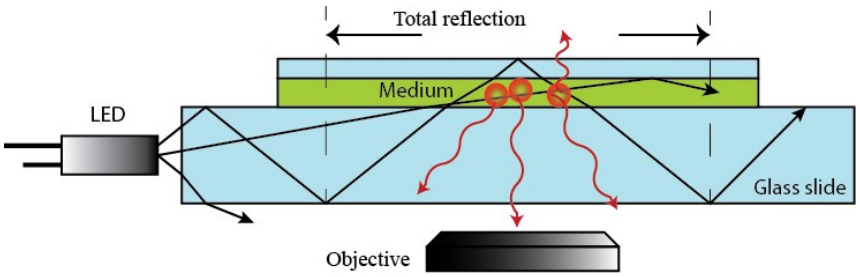

Fig.1 Schematics of darkfield side-illumination. Medium illumination angles are limited by angle of internal reflection.

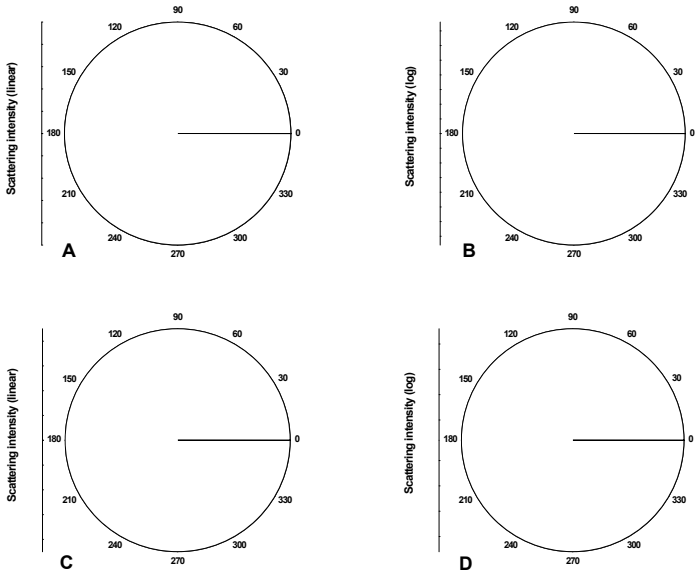

Fig.2 Scattering Intensity dependence on the scattering angle for 80nm Au NP (A – linear, B- log) and 10um size single cell (C- linear, D – log).

Two main factors explain the lower contrast of conventional dark-field microscopy for Plasmonic NPs imaging as compared to the side-illumination method.

First, typical dark-field microscopy requires to exclude the unscattered beam from the image. To provide these conditions, in the case of conventional dark-field microscopy with Nikon condenser (0.8-0.9 NA) such as the one considered in this work, the NA of the microscope objective will need to be limited to 0.7NA. In the case of side-illumination dark-field (Fig.1) method, the incident light angular range lies between the total internal reflection of the glass/air and glass/medium interfaces. And, as we see from the figure 1, side-illumination approach provides more favorable illumination and rejection condition than conventional dark-field even for objectives with the similar NA.

Second, in conventional dark-field microscopy, the detected scattered light will be limited to the forward scattered light with angular properties dependent on the scattering object, in our case NPs and NPs-Cell complex. Side-illumination provides a combination of forward and backscattering illumination (Fig.1) where backscattering enhance NPs-cell contrast due to the dominate Rayleigh scattering of NPs and highly directional forward diffusion of cells (Fig.2) [1,2].

Another point that improves NPs imaging contrast and resolution is the use of discrete LEDs with much narrower spectral range that Halogen lamp illumination and intensity maximum close to the NPs scattering peaks.

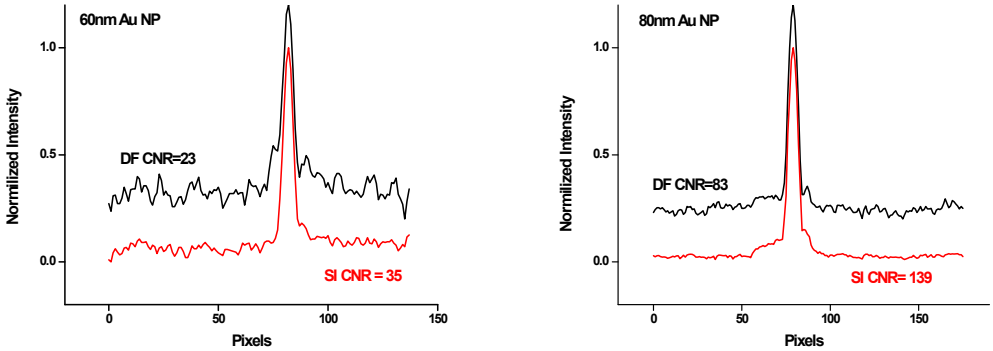

Fig.3 Experimental CNR for 60nm and 80nm gold nanoparticles.

In the Fig.3 we show quantitative analysis of the contrast-to-noise ratio (CNR) for the two plasmonic gold NPs 60nm and 80nm detected by conventional dark-field and side-illumination methods. Side-illumination approach provides CNR up to 1.5 times higher than conventional dark-field.
Contrast-to-noise ratio CNR = $(S_A - S_B)/\sigma_0$ where S_A and S_B are signal intensities for NPs peak position S_A and mean background level S_B in the region of interest and σ_0 is the standard deviation of the pure image noise[3].

1. Meyer, R. A.; Brunsting, A., Light scattering from nucleated biological cells. *Biophys J* **1975,** *15* (3), 191-203.
2. Kinnunen, M.; Karmenyan, A., Overview of single-cell elastic light scattering techniques. *J Biomed Opt* **2015,** *20* (5).
3. Welvaert, M.; Rosseel, Y., On the Definition of Signal-To-Noise Ratio and Contrast-To-Noise Ratio for fMRI Data. *Plos One* **2013,** *8* (11).