Electronic Supplementary Material (ESI) for Nanoscale.

Electronic supplementary information (ESI) to Two-Color Dark-Field (TCDF) microscopy for metal nanoparticles imaging inside cells

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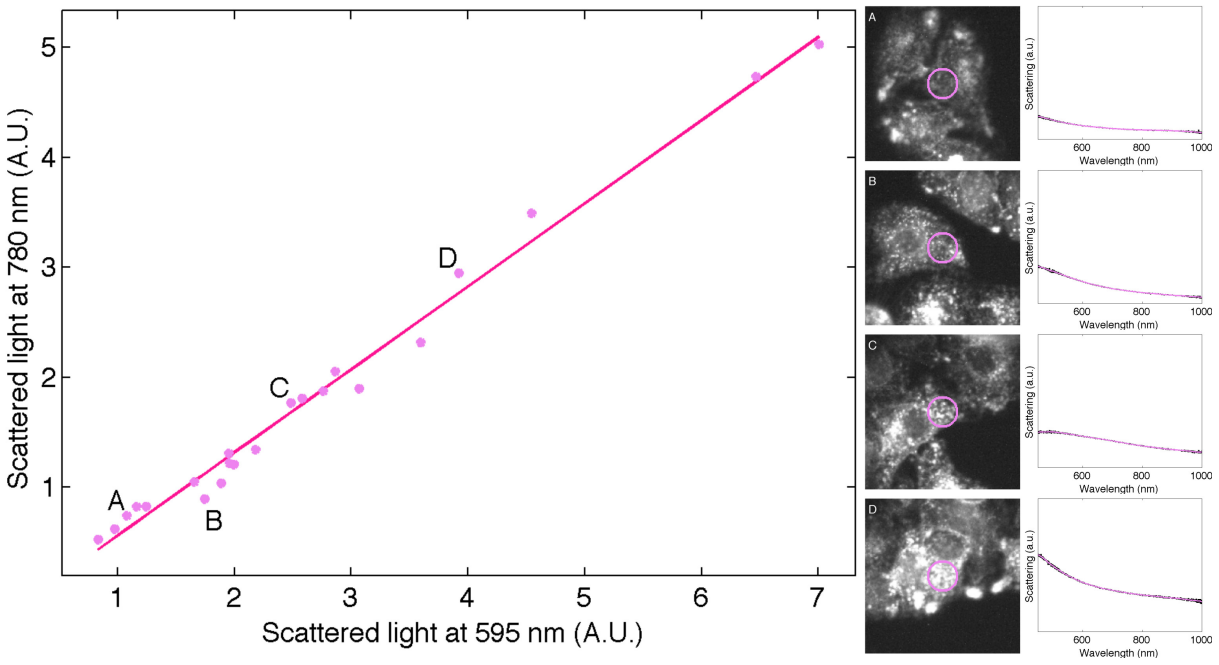

Figure S1: Scattering spectra of A495 cells and the relationship between scattered intensity at 780 nm and 595 nm. Intensities of scattered light from cells at 780 nm and 595 nm are not equal but they are related by a constant factor. This is crucial for our technique to work, as such a difference can be compensated simply by adjusting light sources intensity. Scattering spectra from cells were acquired for several cells and different organelles inside them. Graph shows data taken and linear fit, as well as four examples of the detection area in the cell and corresponding acquired spectrum.

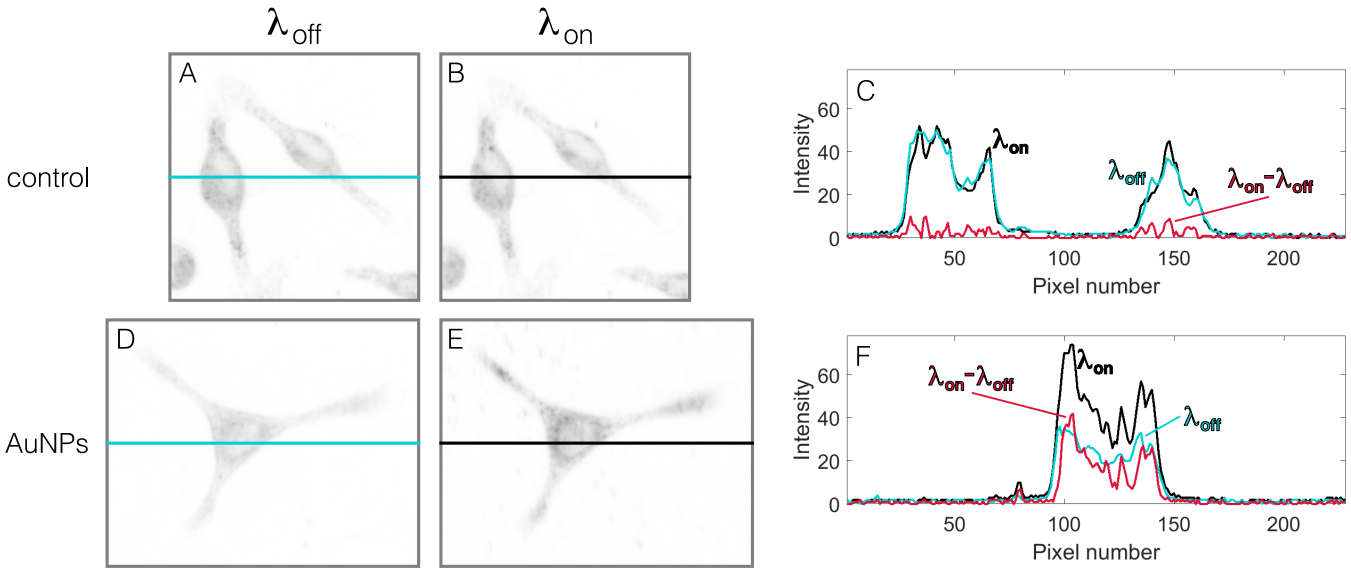

Figure S2: Images for each wavelength of cells presented in Figure 2. Images on-resonance (λ_{on}: B and E) correspond to DF images in Figure 2 (D and E, respectively). In spite of intensity of cells is very similar for control and AuNPs-loaded cells using DF microscopy (B vs. E), when comparing those to their off-resonance counterpart (λ_{off}: A and D), the presence of NPs becomes evident: intensity of images of control cell is very similar for both wavelengths (A vs. B), whereas it is not for AuNPs-loaded cell (D vs. E). Intensity profiles are also shown for control (C) and AuNPs-loaded (F) cells. Graph C exemplify how TCDF significantly reduces the scattering from the cell by subtracting the λ_{on} and λ_{off} images. Graph F illustrates how in the presence of NPs, such subtraction between the images, gives non-negligible signal. Although our method does not completely cancels the background, it is sufficient to carry out unequivocal discrimination between bare and loaded cells.

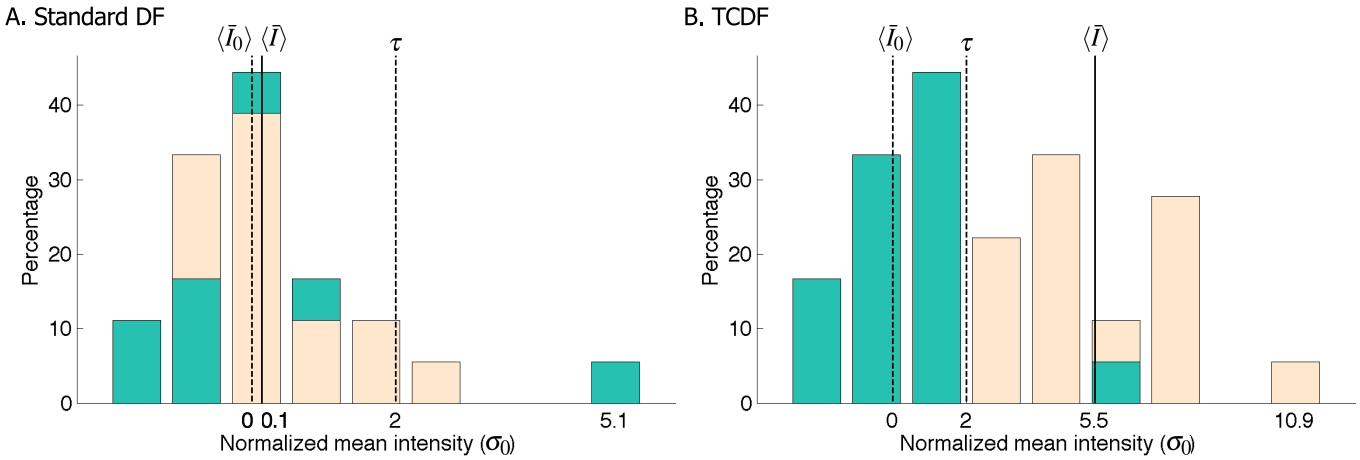

Figure S3: Distributions of cells mean intensity for control (green) and AuNPs-loaded (light orange) cells for A. standard DF and B. TCDF. Data shown correspond to 0 nm and 2 nM concentration of AuNPs during incubation, respectively, of one the experiment realizations. We normalized the data to the standard deviation of control distribution σ_0 and centered the data around the control sample average $\langle \bar{I}_0 \rangle$. We determine the threshold τ using only the data from the control sample as $\tau = \langle \bar{I}_0 \rangle + 2\sigma_0$. One can confidently state the cells in a sample were loaded with NPs if the contrast value ($C = \frac{\langle \bar{I} \rangle - \langle \bar{I}_0 \rangle}{\sigma_0}$) is greater than or equal to two; this is, if the average per sample ($\langle \bar{I} \rangle$) is separated from the average of control sample ($\langle \bar{I}_0 \rangle$) by more than twice the control sample's standard deviation (τ).

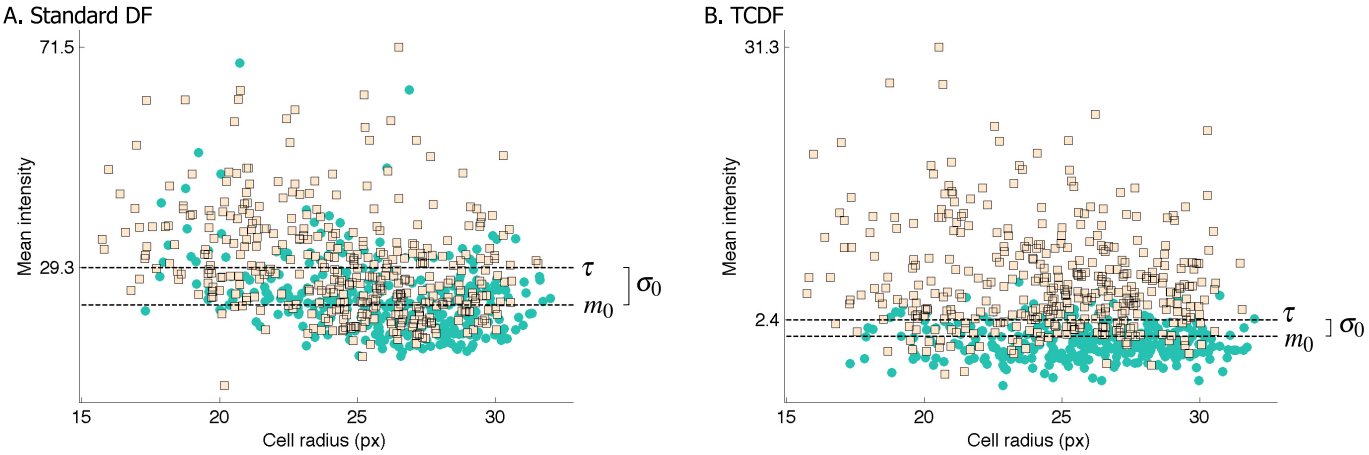

Figure S4: Scatter plots of cell's mean intensity and radius for control (green) and AuNPs-loaded (light orange) cells for A. standard DF and B. TCDF. Threshold τ was determined solely from data of the control sample as its mode value m_0 plus its standard deviation σ_0 ($\tau = m_0 + \sigma_0$). A cell was classified as control if its mean intensity value was below τ. Conversely, a cell was classified as loaded if its mean intensity value was above the threshold.

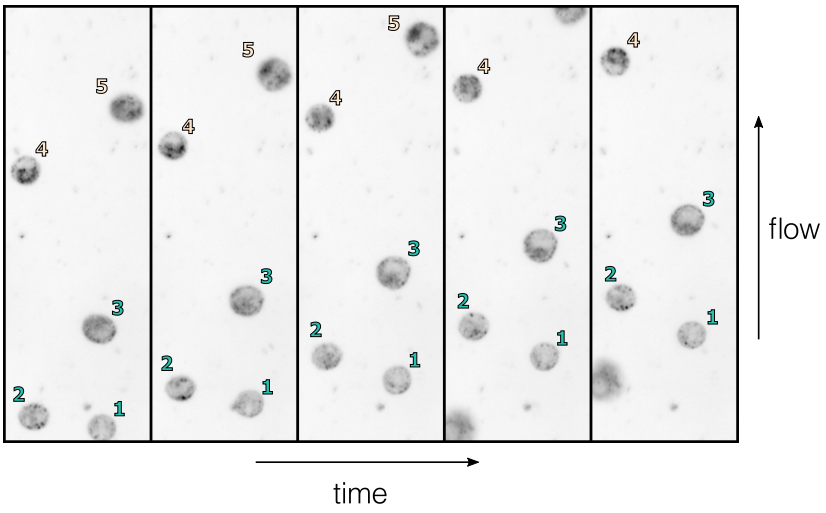

Figure S5: Example negative dark-field images of cells flowing through the microfluidic channel. Control and AuNPs-loaded cells are labeled with green and light orange numbers, respectively.

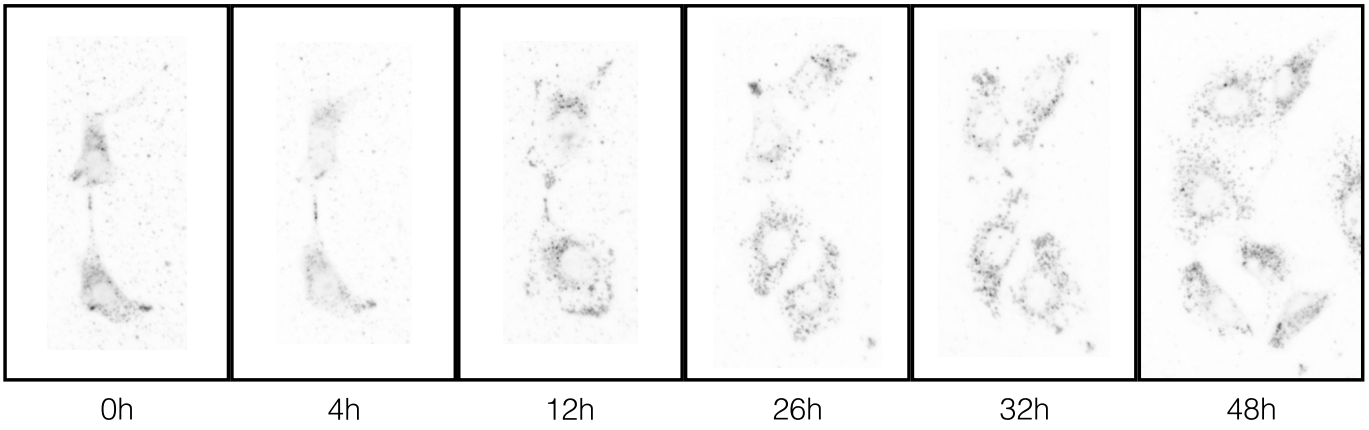

Figure S6: Dark-field negative images of a group of cells and their evolution over time. Dark-field illumination does not affect the cells health. Figure shows images of several AuNPs-loaded cells taken during 2 days. During data acquisition environment conditions for the cells were not controlled. Between each measurement cells were kept in the CO_2 incubator. It can be seen cells follow a normal trend for cell division, what implies their health is not compromised by the measurements.

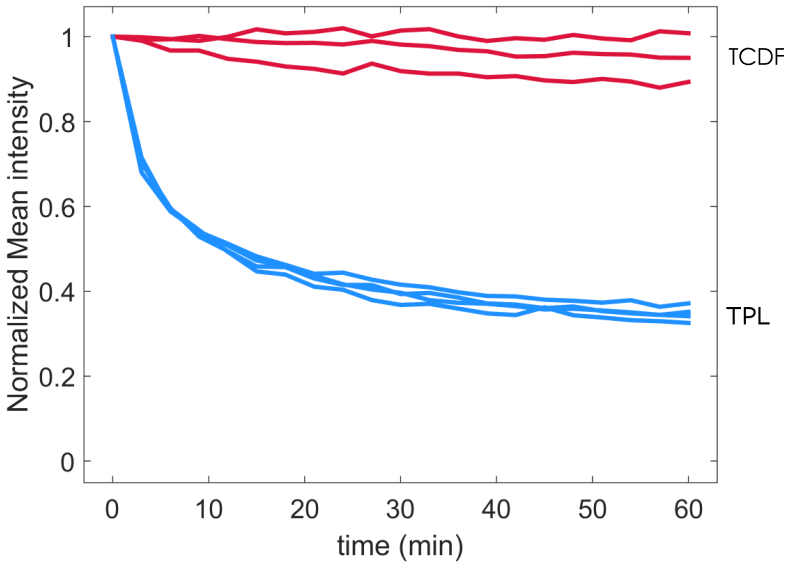

Figure S7: Mean intensity of TCDF and TPL along 1 hour. Metal nanoparticles are not affected by the scattering measurements. On the contrary, high peak intensities of TPL measurements can melt the nanoparticles, thus altering the signal intensity. This disadvantage of TPL can be overcome by decreasing the input laser intensity, but so does the signal collected, which in turn affects the method's sensitivity or time resolution.

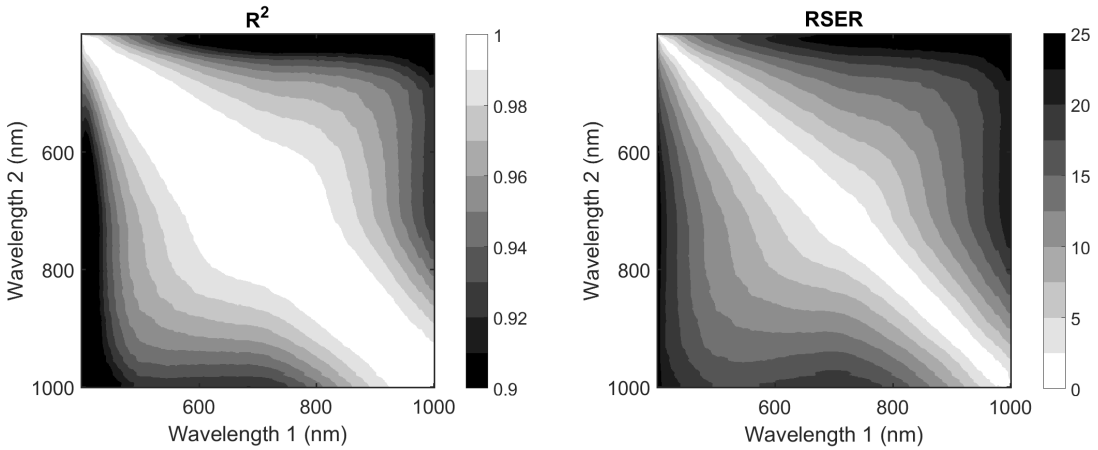

Figure S8: Evaluation of the fit goodness for different illumination wavelenght pairs. As for the case shown in figure S1, we fitted the scattering intensities for each wavelength pair using a linear model and evaluate how well this model fits the data. For this purpose, we used two parameters: the coefficient of determination ($R^2 = 1 - \left[\sum_{n=1}^{N}(y_n - f_n)^2\right] \Big/ \left[\sum_{n=1}^{N}(y_n - \bar{y})^2\right]$, with y_n is the value of the n-th data point, f_n its corresponding fitted value and $\bar{y}$ the data mean value) and the standard error of regression ($\text{SER} = \sqrt{\frac{1}{N-2}\sum_{n=1}^{N}(y_n - f_n)^2}$). The first one is a fairly common statistical value, that measures the percentage of the response variable variation, and it ranges from 0 to 1. The second one represents the average distance that the observed values fall from the regression line and uses the units of the response variable. Importantly, for a perfect fit $R^2 = 1$ and $\text{SER} = 0$. Since units for SER are different for each color pair, in order to compare how good are the fits, it is necessary to normalize it, therefore we defined $\text{RSER} = 100\frac{\text{SER}}{\bar{y}}$. Most R^2 values are above 0.9, which indicates the linear model describes well the data. This is reinforced by the parameter RSER, that for the major part of the analysed wavelength range is below 20%. In both cases the diagonal values correspond to a perfect fit, as expected considering in this case the wavelengths are equal. Although this case is not suitable for TCDF, it is useful to check the calculations. Ranges found for both R^2 and RSER demonstrate data fits well to a linear model for a broad range of wavelengths, therefore revealing TCDF would work for different illumination wavelength pairs, not only for the one we implemented.