Supporting information

Direct Imaging of Transmembrane Dynamics of Single Nanoparticles with Darkfield Microscopy: Improved Orientation Tracking at Cell Sidewall

Dong Xu, Yan He*, and Edward S. Yeung

State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, College of Biology

Hunan University, Changsha, 410082, P. R. China.

E-mail: yanhe2021@gmail.com

SUPPLEMENTARY NOTES

AuNR absorption probability function under darkfield illumination. When an oscillating-dipole-like AuNR is being perturbed by an external electric field, its excitation probability function P_a is determined by the product of the incoming electric field polarization vector $\hat{\varepsilon}$ and the dipole orientation vector $\hat{\mu}_a$,

$$P_a(\hat{\mu}_a, \hat{\varepsilon}) \equiv \left|\hat{\mu}_a, \hat{\varepsilon}\right|^2$$

$$= (\varepsilon_x \mu_{ax} \cos\delta_x + \varepsilon_y \mu_{ay} \cos\delta_y + \varepsilon_z \mu_{az} \cos\delta_z)^2$$

$$+(\varepsilon_x \mu_{ax} \sin\delta_x + \varepsilon_y \mu_{ay} \sin\delta_y + \varepsilon_z \mu_{az} \sin\delta_z)^2$$

where $\mu_{ax} = \sin\theta\cos\phi$, $\mu_{ay} = \sin\theta\sin\phi$, and $\mu_{az} = \cos\theta$.

For single-beam-inclined illumination with **linear polarized** electric field with **polarization direction** at azimuth angle γ and polar angle ξ,

$$\varepsilon_x = \sin\xi\cos\gamma, \quad \varepsilon_y = \sin\xi\sin\gamma, \quad \varepsilon_z = \cos\xi, \quad \delta_x = \delta_y = \delta_z,$$

$$P_a(\hat{\mu}_a, \hat{\varepsilon}) = (\sin\theta\cos\phi\sin\xi\cos\gamma + \sin\theta\sin\phi\sin\xi\sin\gamma + \cos\theta\cos\xi)^2.$$

For single-beam-inclined illumination with **unpolarized** electric field with **propagation direction** at polar angle γ and azimuth angle ξ,

$$P_a(\hat{\mu}_a, \hat{\varepsilon}) = 1 - (\sin\theta\cos\phi\sin\xi\cos\gamma + \sin\theta\sin\phi\sin\xi\sin\gamma + \cos\theta\cos\xi)^2$$

$$= 1 - [\sin\theta\sin\xi\cos(\phi - \gamma) + \cos\theta\cos\xi]^2$$

In the case of conventional darkfield microscopy, the annular inclined illumination can be decomposed into a mixture of unpolarized incident light with γ ranging from 0° to 360° and ξ ranging from 64.5° to 90° (for illumination with oil-type darkfield condenser of NA 1.20 ~ 1.43 at glass/water interface of refractive index n_1/n_2= 1.52/1.33). So we have,

$$P_a(\hat{\mu}_a, \hat{\varepsilon}) = \frac{\int_{1.125}^{1.571}\int_{0}^{2\pi}[1-[\sin\theta\sin\xi\cos(\phi-\gamma)+\cos\theta\cos\xi]^2]d\gamma d\xi}{\int_{1.125}^{1.571}\int_{0}^{2\pi}d\gamma d\xi}$$

$$= 0.936 - 0.404\sin^2\theta$$

According to this equation, P_a is a function only depends on θ. Consequently, $\sin^2\theta$ as well as the θ value of the AuNR can be obtained from the sum intensity,

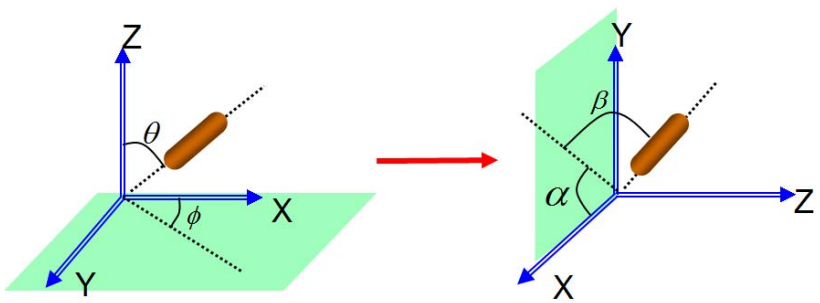
$$I_{sx} + I_{sy} \propto (0.936 - 0.404\sin^2\theta)\sin^2\theta,$$

or

$$\frac{I_m - I_b}{I_p - I_b} = \frac{0.936\sin^2\theta - 0.404\sin^4\theta}{0.532} = 1.759\sin^2\theta - 0.759\sin^4\theta$$

where $I_m = I_{sx} + I_{sy}$, I_p is the measured maximum sum value, I_b is the measured background. By definition, $\theta = \pi/2$ when $I_m = I_p$, so a scaling factor 0.532 is needed to balance the equation.

Coordinate transformation via matrix rotation



Z axis	$\cos\theta$		Y axis	$\cos\beta\sin\alpha$
X axis	$\sin\theta\cos\phi$	→	Z axis	$\sin\beta$
Y axis	$\sin\theta\sin\phi$		X axis	$\cos\beta\cos\alpha$

Calculated error of azimuth and polar angle determination. In our study, the azimuth angle ϕ is determined from

$$Y = f(I_x, I_y, I_b) = \frac{(I_x - I_b) - (I_y - I_b)}{(I_x - I_b) + (I_y - I_b)} = \frac{I_x - I_y}{I_x + I_y - 2I_b} = \cos(2\phi)$$

To calculate $\Delta\phi$ the standard way is to take the derivative of Y,

$$\Delta\phi = -\frac{\Delta Y}{4\sin\phi\cos\phi}$$

But that will lead to $\Delta\phi \to \infty$ at ϕ=0 or $\phi = \pi/2$, which is not reasonable. So we deduced the expression directly from the definition of measurement error,

$$\cos(2\phi + 2\Delta\phi) = Y + \Delta Y .$$

Since $Y = \cos 2\phi$, and $\cos 2\Delta\phi = 1 - 2\sin^2\Delta\phi$, we have

$$\cos 2\phi \times \sin^2\Delta\phi + \sin 2\phi \sin\Delta\phi \cos\Delta\phi + \Delta Y/2 = 0$$

Where the maximum relative error

$$\Delta Y / Y = \left|\frac{\partial \ln f(I_x, I_y, I_b)}{\partial I_x}\right| \Delta I_x + \left|\frac{\partial \ln f(I_x, I_y, I_b)}{\partial I_y}\right| \Delta I_y + \left|\frac{\partial \ln f(I_x, I_y, I_b)}{\partial I_b}\right| \Delta I_b$$

can be obtained from the standard deviations of the measured intensities, which is estimated to be about 10% under the cell-top observation mode and 3% under the side-wall observation mode when imaging the cell with our DFM system. The value of $\Delta\phi$ at different ϕ was obtained by solving the above equation on $\Delta\phi$ using MATLAB.

Similarly, the polar angle θ in our study was determined from

$$Y = f(I_m, I_p, I_b) = \frac{I_m - I_b}{I_p - I_b} = 1.759\sin^2\theta - 0.759\sin^4\theta$$

According to $\cos 2\theta = 1 - 2\sin^2\theta$, $\cos 4\theta = 2\cos^2 2\theta - 1$, and the definition of measurement error, we can get

$$Y = 0.595 - 0.5\cos 2\theta - 0.095\cos 4\theta ,$$

$$Y + \Delta Y = 0.595 - 0.5\cos 2(\theta + \Delta\theta) - 0.095\cos 4(\theta + \Delta\theta) , \text{ and}$$

$$\Delta Y = \cos 2\theta \sin^2 \Delta\theta + 0.5 \sin 2\theta \sin 2\Delta\theta + 0.190 \cos 4\theta \sin^2 2\Delta\theta + 0.095 \sin 4\theta \sin 4\Delta\theta$$

Where the error ΔY are obtained from the relative errors of intensity measurements,

$$\Delta Y = (\Delta Y / Y) \times Y$$

$$\Delta Y / Y = \left| \frac{\partial \ln f(I_m, I_p, I_b)}{\partial I_m} \right| \Delta I_x + \left| \frac{\partial \ln f(I_m, I_p, I_b)}{\partial I_p} \right| \Delta I_y + \left| \frac{\partial \ln f(I_m, I_p, I_b)}{\partial I_p} \right| \Delta I_b$$

The value of $\Delta\theta$ at different θ was obtained by solving the above equation on $\Delta\theta$ using MATLAB.

SUPPLEMENTARY FIGURES AND CAPTIONS

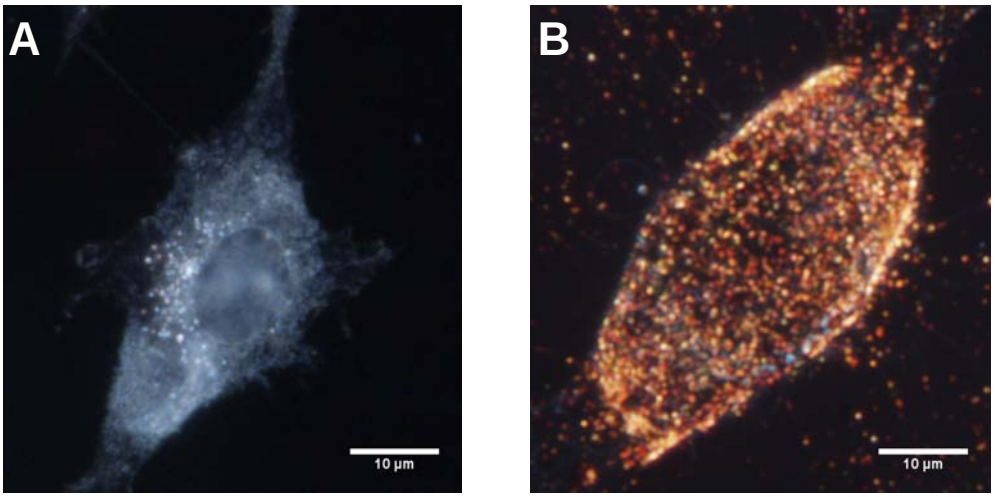


Figure S1. The as-prepared AuNRs can be readily internalized by the cells. (A) Darkfield image of a blank HeLa cell. (B) Image of a cell after incubating with MUTAB modified AuNRs for 30 min.

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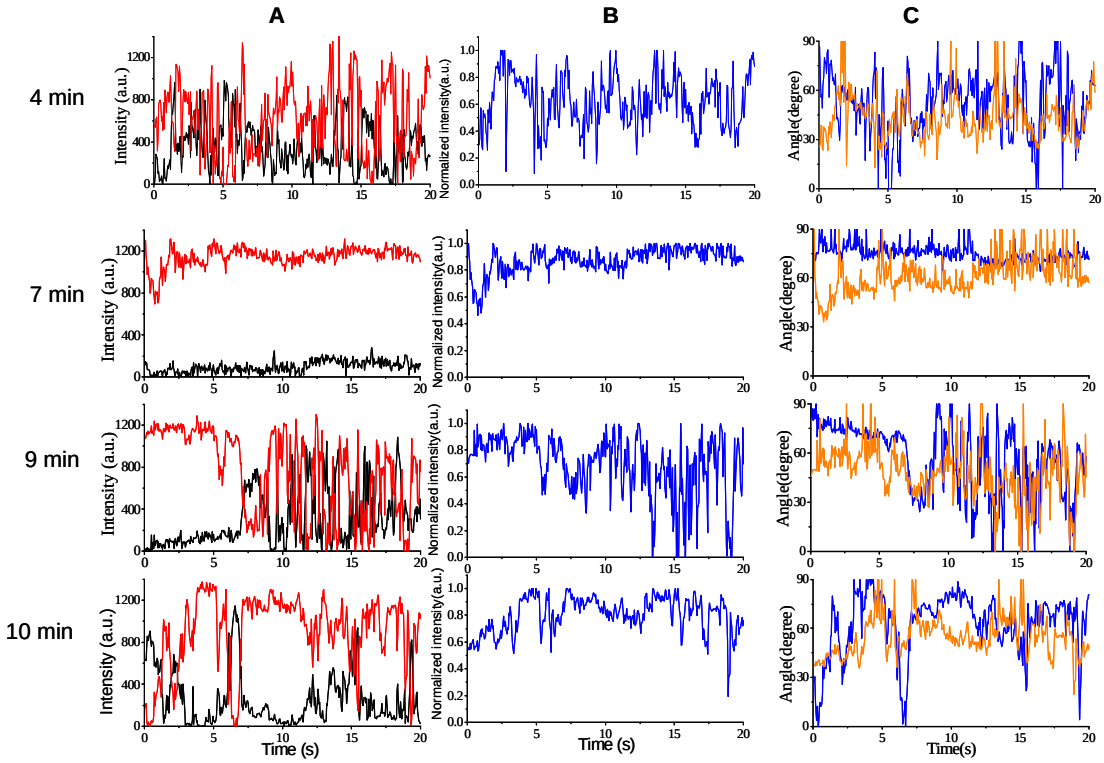

Figure S2. The time dependent variations of (A) two-spot intensities I_{sx} (black) and I_{sy} (red), (B) sum intensity, (C) azimuth angle (blue) and polar angle (orange) of the AuNR in the 4 videos in Movie 2.

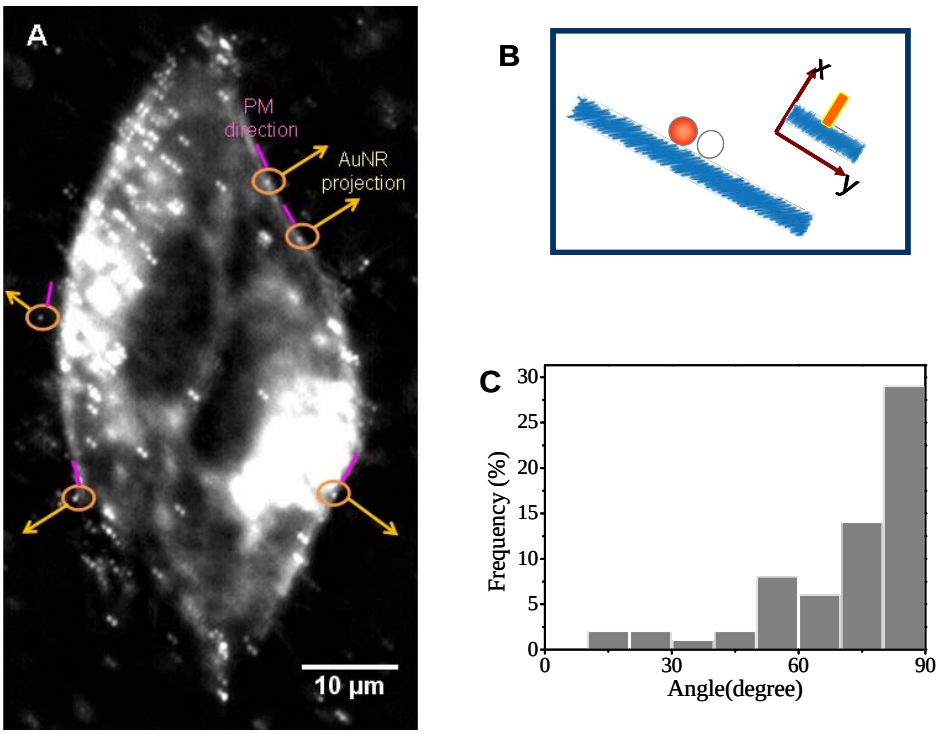

Figure S3. (A) Image of a cell showing the projections of AuNRs strongly confined by the PM and the directions of the PM defined by the scattering from intracellular components at different sites of the PM. So the angle, β_{prj}, can be measured directly. (B) As a special case, when only one of the two spots is lit, the projection of the AuNR must be parallel to either the x-axis or the y-axis. (C) Histogram of the β_{prj} value of 64 strongly confined AuNRs randomly picked from different cells. It exhibits only one major population narrowly distributed around 90°.