Deep-Tissue Super-Resolution Microscopy using Single-Scattering Accumulation Algorithm

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Abstract

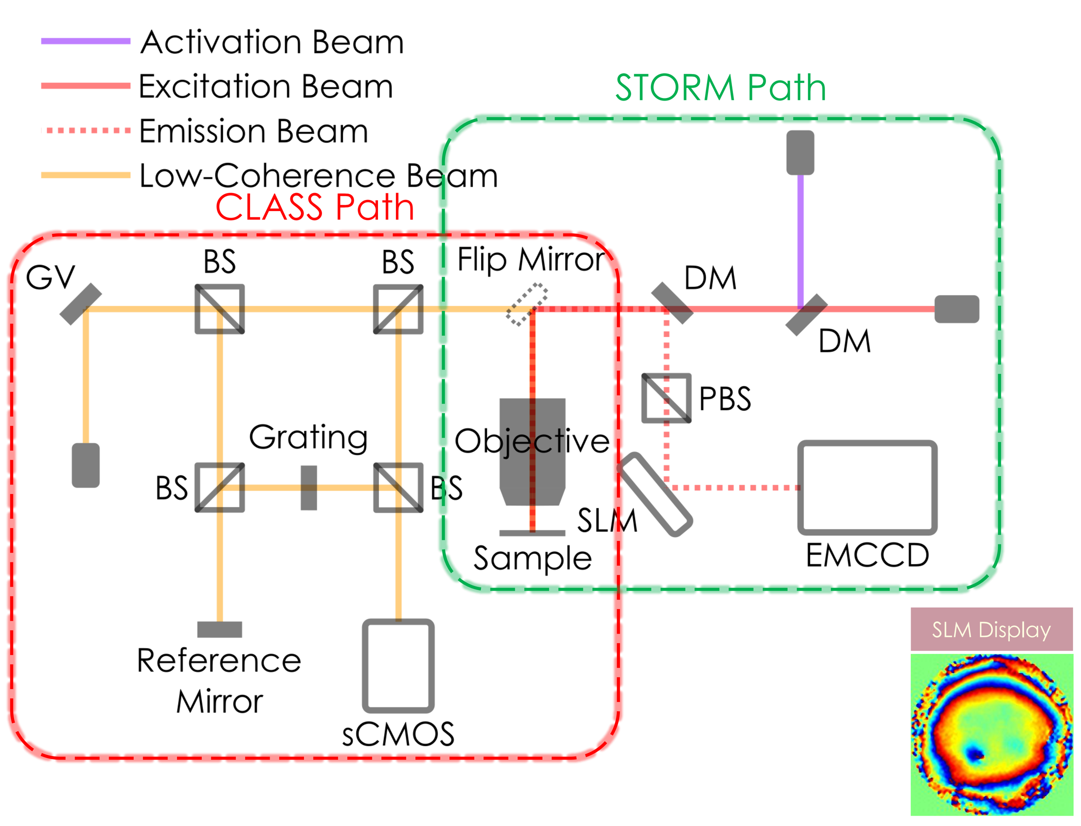
Single-molecule localization microscopy has enabled the resolving of biomolecules well beyond the diffraction-limited spatial resolution. However, its imaging depth has been too shallow to investigate thick biological tissues. A sample-induced aberration is one of the major reasons for this limitation. It gives rise to the blur and distortion of single-molecule emission images, impairing the extremely sensitive single-molecule localization process. To resolve this issue, we combined the closed-loop accumulation of single scattering (CLASS) microscopy with stochastic optical reconstruction microscopy (STORM). CLASS microscopy identifies the sample-induced aberration based on elastic backscattering, which is then applied to a spatial light modulator in the excitation beam path of STORM for aberration correction. We demonstrated super-resolution imaging of cell microtubules under artificially generated severe aberration consisting of Zernike modes higher than mode 20 and the STORM imaging of neurons in 100µm-thick mouse brain tissue at the depth of ~70µm.

Concept

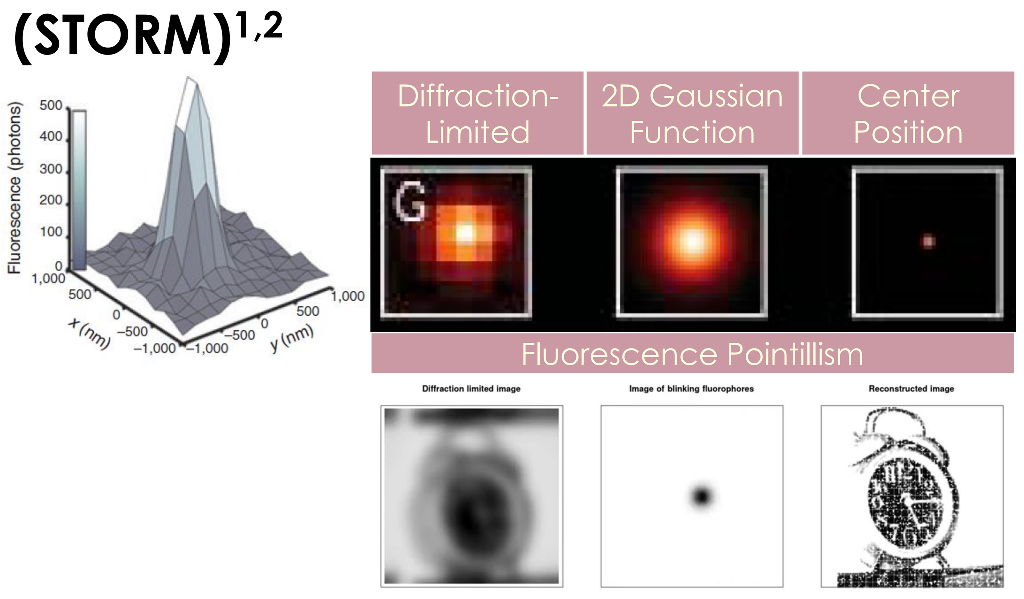
Adaptive optics-assisted super-resolution microscopy

- CLASS (closed-loop accumulation of single scattering) + STORM (stochastic optical reconstruction microscopy)
- Correcting CLASS-calculated sample-induced aberration ▶ obtaining deep-tissue STORM images
- The goal is to obtain STORM images of neurons in a mouse brain or an intact animal (e.g., zebrafish) at the depth of ~100µm

Principle



Stochastic optical reconstruction microscopy (STORM)[1,2]



Collective accumulation of single-scattered waves (CASS) & Closed-loop accumulation of single scattering (CLASS)[3,4]

$E(\mathbf{r};\mathbf{k}_n^{\mathbf{in}},\tau_0) = E_S(\mathbf{r}) + E_R(\mathbf{r})e^{-i\mathbf{k}_n^{\mathbf{in}}\cdot\mathbf{r}}$ [interference of two beams (E-field)]

where $\mathbf{r} = (x,y)$ (coordinates in camera-detected image) and $\mathbf{k}_n^{\mathbf{in}}$ representing nth scan angle

$I(\mathbf{r};\mathbf{k}_n^{\mathbf{in}},\tau_0) = |E(\mathbf{r};\mathbf{k}_n^{\mathbf{in}},\tau_0)|^2 = |E_S(\mathbf{r})|^2 + |E_R(\mathbf{r})|^2 + E_S(\mathbf{r})E_R(\mathbf{r})\left(e^{i\mathbf{k}_n^{\mathbf{in}}\cdot\mathbf{r}} + e^{-i\mathbf{k}_n^{\mathbf{in}}\cdot\mathbf{r}}\right)$ [camera-detected image (intensity)]

Taking Fourier transform and using the convolution theorem,

$\mathcal{F}\{|E_S(\mathbf{r})|^2 + |E_R(\mathbf{r})|^2\} = [|E_S(\mathbf{r})|^2 + |E_R(\mathbf{r})|^2]\mathcal{F}\{1\} = [|E_S(\mathbf{r})|^2 + |E_R(\mathbf{r})|^2]\delta(\mathbf{k})$

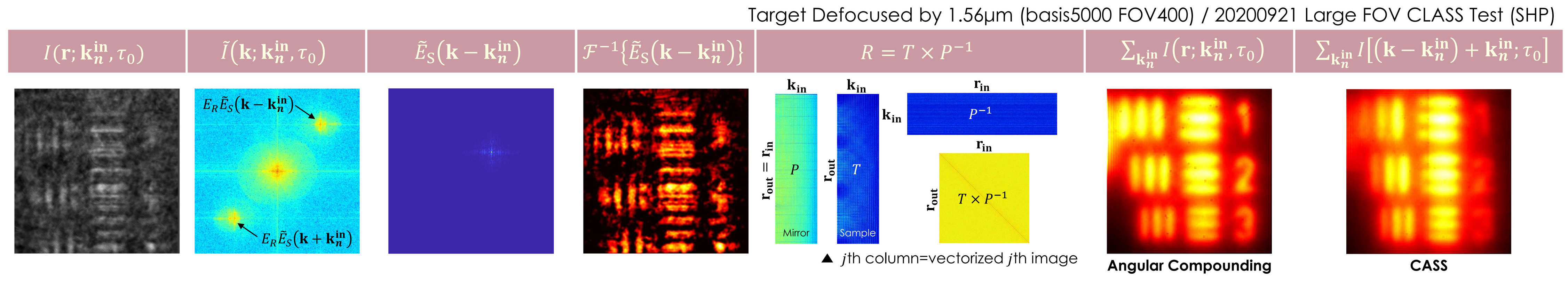
$\mathcal{F}\left\{E_S(\mathbf{r})\left[E_R(\mathbf{r})e^{\pm i\mathbf{k}_n^{\mathbf{in}}\cdot\mathbf{r}}\right]\right\} = \tilde{E}_R(\mathbf{k})\iint d^2\mathbf{r}\left[E_S(\mathbf{r})e^{\pm i\mathbf{k}_n^{\mathbf{in}}\cdot\mathbf{r}}\right]e^{-i\mathbf{k}\cdot\mathbf{r}} = \tilde{E}_R(\mathbf{k})\iint d^2\mathbf{r}\left[E_S(\mathbf{r})e^{-i(\mathbf{k}\mp\mathbf{k}_n^{\mathbf{in}})\cdot\mathbf{r}}\right] = \tilde{E}_R(\mathbf{k})\tilde{E}_S(\mathbf{k}\mp\mathbf{k}_n^{\mathbf{in}})$

Then,

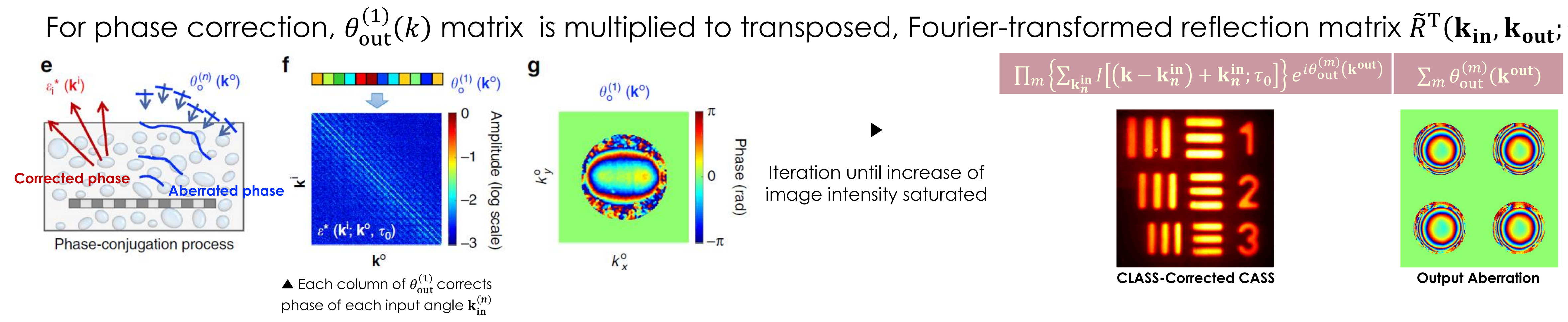
$\mathcal{F}\{I(\mathbf{r};\mathbf{k}_n^{\mathbf{in}},\tau_0)\} \equiv \tilde{I}(\mathbf{k};\mathbf{k}_n^{\mathbf{in}},\tau_0) = [|E_S(\mathbf{r})|^2 + |E_R(\mathbf{r})|^2]\delta(\mathbf{k}) + \tilde{E}_R(\mathbf{k})[\tilde{E}_S(\mathbf{k}-\mathbf{k}_n^{\mathbf{in}}) + \tilde{E}_S(\mathbf{k}+\mathbf{k}_n^{\mathbf{in}})]$ (Fourier-transformed image)

where $\mathbf{k} = (k_x, k_y)$

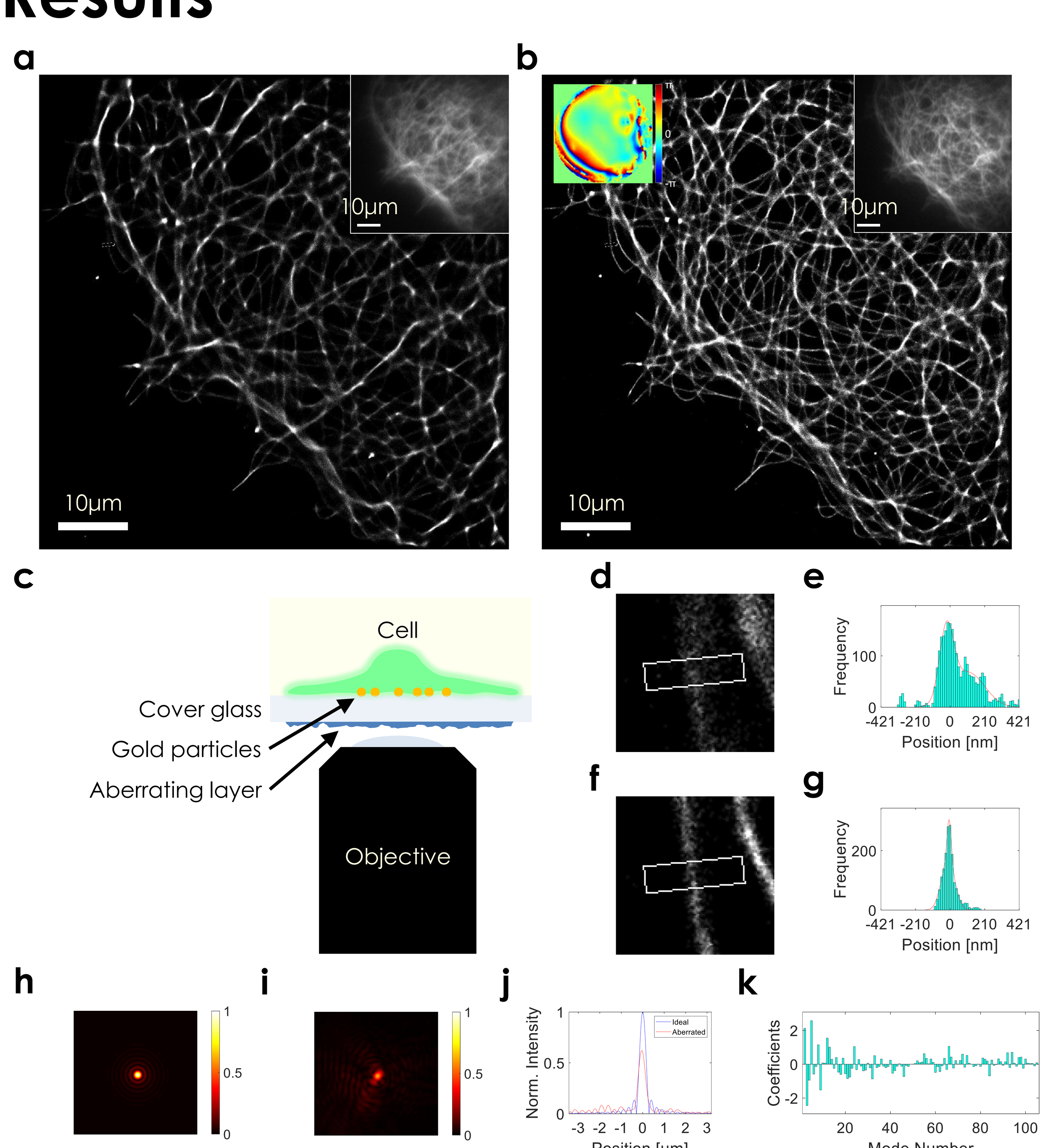
Target Defocused by 1.56µm (basis5000 FOV400) / 20200921 Large FOV CLASS Test (SHP)

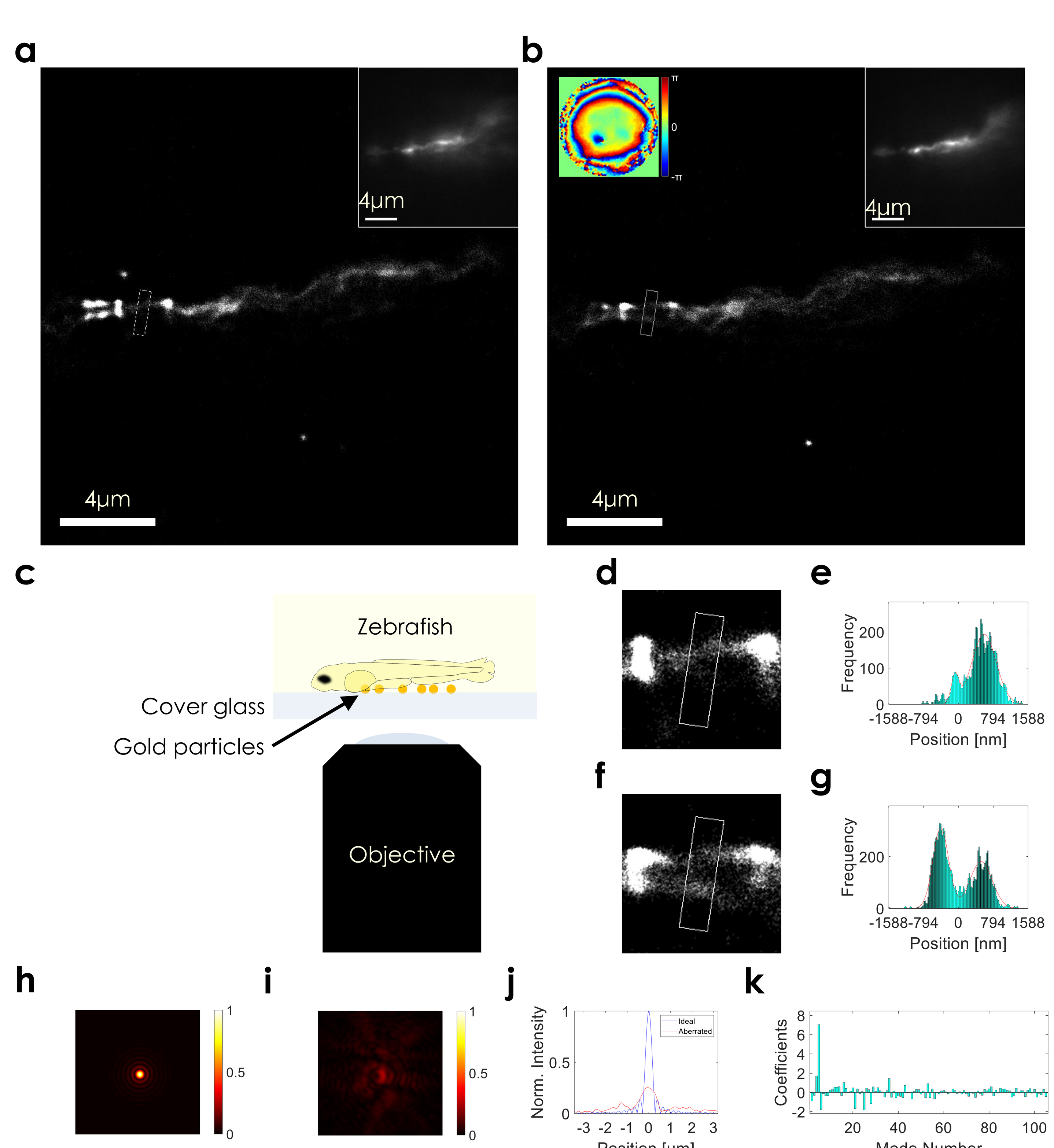


For phase correction, $\theta_{\mathrm{out}}^{(1)}(k)$ matrix is multiplied to transposed, Fourier-transformed reflection matrix $\tilde{R}^{\mathrm{T}}(\mathbf{k_{in}},\mathbf{k_{out}};\tau_0)$



Results





References

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