Research part 2 - Optical trapping, holographic light-sheets and super-resolution microscopy

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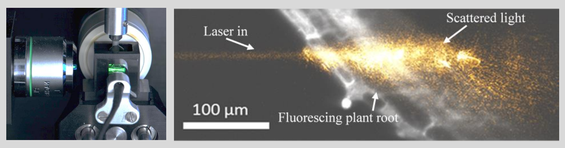
Laser scanning microscopy

Light-sheet microscopy with self-reconstructing laser beams



Fahrbach, Simon, Rohrbach, Nature Photonics (2010)
Fahrbach & Rohrbach, Nature Communications, (2012)

Background: In light-sheet microscopy only a slice at position $\mathbf{y}_s$ of a thick object $f(\mathbf{r})$ is illuminated from the side with a laterally scanned illumination beam $h_{ill}(\mathbf{r}\text{-}\mathbf{x}_s)$ that is in the focal plane of the objective lens (OL). The line confocal image $b(\mathbf{r})$ is

$$\int_{-x_m}^{x_m} \iiint f(\mathbf{r}-\mathbf{y}_s)\cdot\left(h_{ill}(\mathbf{r}-\mathbf{x}_s)*\mathrm{rect}\left(\frac{x-x_s}{d_{slit}}\right)\right)\cdot h_{det}(\mathbf{z}_s-(\mathbf{r}-\mathbf{x}_s))\,d^3\mathbf{r}\,dx_s$$

Problem: Thick, scattering media make the illumination beam scattering along the propagation direction z and thus degrading image quality. An ideal and an unwanted image are generated:

$$b(\mathbf{r})=\left(\left(h_{inc}(\mathbf{r})+h_{sca}(\mathbf{r})\right)\cdot f(\mathbf{r})\right)*h_{det}(\mathbf{r})=b_{ideal}(\mathbf{r})+b_{ghost}(\mathbf{r})$$

Approach: Self-reconstructing Bessel beams, generated by a computer hologram (SLM) allow flexible control of the 3D phase:

$$b_{ghost}(\mathbf{r})=\left(\left(|E_{sca}|^2+2|E_{holo}|\cdot|E_{sca}|\cdot\cos\left(\phi_{holo}(\mathbf{r})-\delta\phi(\mathbf{r})\right)\right)\cdot f(\mathbf{r})\right)*h_{det}(\mathbf{r})$$

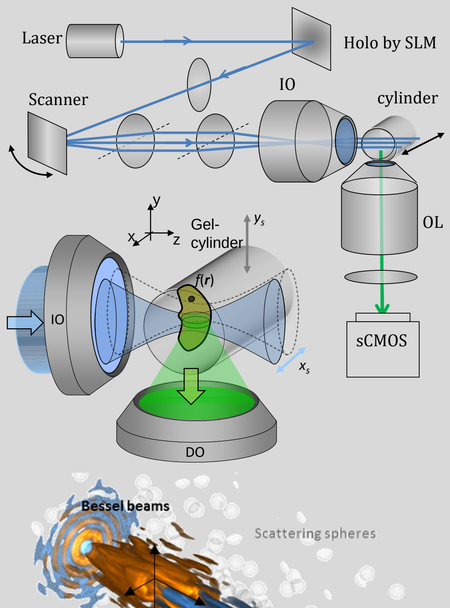
Self-healing photons in the Bessel beam's ring system result in an unexpected strong interference in the Bessel beam center leading to 50% increase of ballistic photons in propagation direction.

We use femto-second laser pulses or alternatively a second STED beam to suppress the unwanted fluorescence from the Bessel ring system. The two illumination sectioned Bessel beams read:

$$h_{ill}^{EX}(\mathbf{r})=|E_{EX}(\mathbf{r})|^2 \qquad h_{ill}^{ST}(\mathbf{r})=|E_{ST}(\mathbf{r})|^2$$

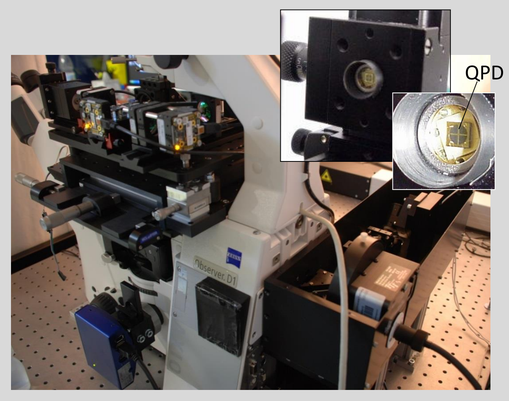
$$E_{EX}(x,y,0)=J_0(k_0\mathrm{NA}\,r)*\mathrm{sinc}(k_0\mathrm{NA}\sin(\beta_{EX}/2)\cdot y)$$

$$E_{ST}(x,y,0)=J_0(\alpha k_0\mathrm{NA}\,r)*\mathrm{sinc}(\alpha k_0\mathrm{NA}\sin(\beta_{ST}/2)\cdot y)*\left(\frac{2i}{y}\right)$$



Optical trapping and tracking

Time-multiplexed trapping & position and shape tracking



Background: Particle positions **b**(t) inside the optical trap can be tracked in 3D with nanometer precision at 2 MHz using back focal plane (BPM) interferometry. Interference signals of unscattered and scattered fields of the trapping laser are recorded with quadrant photodiodes (QPDs).

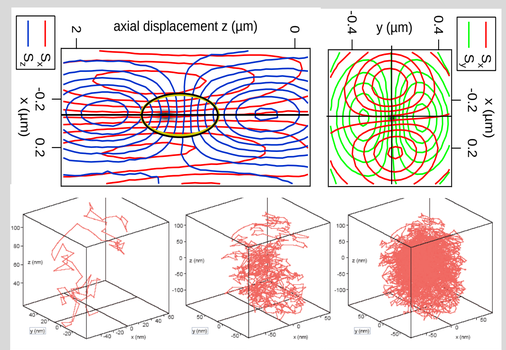
$$I(k_x,k_y,\mathbf{b})\approx\left(I_i+I_s(\mathbf{b})\right)+2\sqrt{I_i\cdot I_s(\mathbf{b})}\cdot\cos\left(\Delta\phi_s(\mathbf{b})\right)$$

$$S_j(\mathbf{b})=\iint I(k_x,k_y,\mathbf{b})\cdot stp\left(k_0NA-|\mathbf{k}|\right)\cdot\begin{pmatrix}2\,stp(k_x)-1\\2\,stp(k_y)-1\\stp(\frac{1}{2}k_0NA-|\mathbf{k}|)\end{pmatrix}dk_xdk_y$$

3 position signals $S_x(t)=(S_1+S_2)-(S_3+S_4)$, $S_y(t)=(S_1+S_3)-(S_2+S_4)$, $S_z(t)=(S_1+S_2+S_3+S_4)$ are autocorrelated in time to obtain the local optical potential curverture and local friction factors.

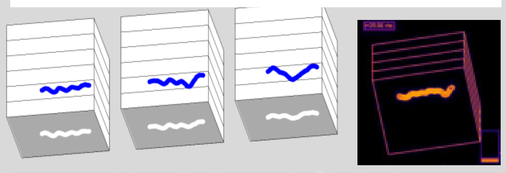
Problem: Fast 3D tracking of several particles or tracking the shape of more complex trapped objects is very difficult.

Approach: Using a high gradient optical point trap, which is time multiplexed by an AOD, we are able to trap and track up to 20 particles in parallel and calibrate them within seconds. This requires only a single QPD!



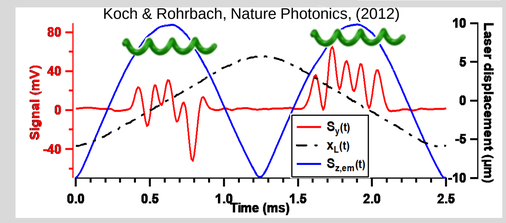
Similarly, by sweeping the point trap along a line x_L, we can generate an extended optical potential, which distributes optical forces at positions **b** smoothly along a more complex shaped object c(x), such as a helical bacterium.

$$\mathbf{F}_{opt}(\mathbf{b})=\frac{\alpha n}{2c}\frac{2}{T_s}\int_0^{T_s/2}P(\mathbf{r}_L)\int(\nabla I_i)(\mathbf{r}-\mathbf{r}_L)\cdot\delta(\mathbf{r}-\mathbf{b}-\mathbf{c}(x))\,d^3r\,dt$$



The tracking of a shape c(x) enables the generation of super-resolved 3D movies of a deforming helical bacterium at 1000 Hz (see movie on homepage).

$$\mathbf{S}(\mathbf{c}(x_L))\sim\iint\left(k_xx_L+k_yR_H\cos(k_Hx_L)-ak_zR_H\sin(k_Hx_L)\right)\mathbf{H}\,d^2k$$



Fast, label-free super-resolution microscopy by Rotating Coherent Scattering (ROCS)

Background and Problem: Living cells are highly dynamic systems with cellular structures being often below the optical resolution limit. Super-resolution microscopes, usually based on fluorescence cell labeling, are too slow to resolve small, dynamic structures.

$$f(\mathbf{r},t)=\sum_j f_j(\mathbf{r}-\mathbf{r}_j(t))=-\sum_j\alpha_j\cdot s_j(\mathbf{r}-\mathbf{r}_j(t))$$

Approach: We use a new label-free microscopy, which generates thousands of super-resolved, high contrast images at 100 Hertz and without any post-processing. ROCS is based on oblique sample illumination from direction $k_{iz} = k_0 \cdot \sin\theta$ with (ε partially) coherent light:

Incident field: $E_i(\mathbf{r},\phi,\theta,\varepsilon)=E_0\cdot e^{-i\varphi(\varepsilon)}\cdot e^{-i\mathbf{k}_i\mathbf{r}}$ and field on camera: $E_{cam}(\mathbf{r},\phi,q,\varepsilon)=\left[E_i(\mathbf{r},\phi,\varepsilon)\left(q+f(\mathbf{r})*g(\mathbf{r})\right)\right]*h_c(\mathbf{r})$

By circulating an incident laser beam by 360° during one image acquisition $\phi\in[0,2\pi]$, object information is amplified by multiple interference patterns (mixed coherence).

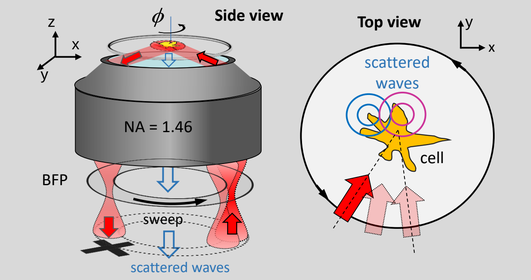
Darkfield ROCS image of j scattering structures $I(r,\varepsilon)$ =:

$$\int_0^{2\pi}\left|\sum_j E_{cam}(\mathbf{r},\phi)\right|^2 d\phi=\underbrace{\sum_j A_j^2}_{\text{incoherent image}}+\underbrace{\gamma(\varepsilon)\cdot2\sum_{j\neq k}A_jA_k\int_0^{2\pi}\cos\left(\Delta\varphi_{jk}(\phi)\right)d\phi}_{\text{super-resolving interference pattern}}$$

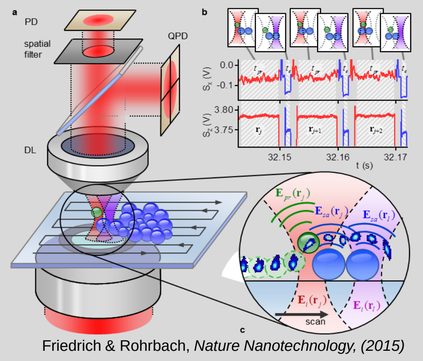
(fields at camera from direction ϕ)

Even without devonvolution, 150 nm small structures are separable by local destructive interferences, which are controlled by the degree of coherence $\gamma(\varepsilon)$.

ROCS images local changes in refractive index through scattered laser light and has revealed unexpected cellular details and dynamic processes.



Surface scanning with optically trapped probes



Background: Photonic Force Microscopy (PFM) is a competing technique to Atomic Force Microscopy (AFM), where the thin tip of a mechanical cantilever is scanned across the surface to obtain a high-resolution height profile. With a much higher sensitivity and an easily adjustable trapping stiffness, the PFM can generate surface height profiles using an optically trapped probe.

Problem: The interferometric tracking signals of the probe's axial displacements are massively distorted by phase disturbances of the surface structure.

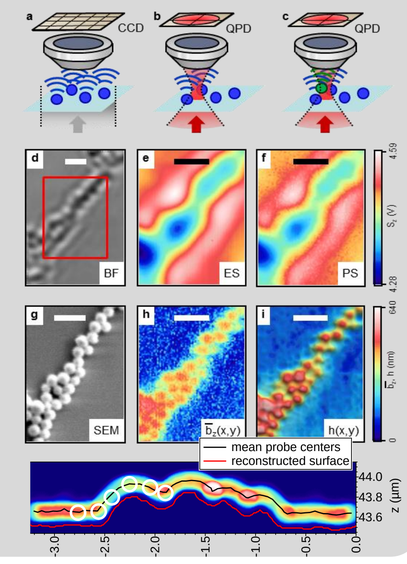
Approach: We use two optical laser foci that scan side by side across the structured surface: one with a 190nm small optically trapped probe and the other focus without a particle. First, we subtract both interferometric signals from each other, i.e. take the difference between the three beam interference and the two beam interference intensitymeasured in the back focal plane with a quadrant photo diode

$$\mathbf{b}_{pr}=\mathbf{g}^{-1}\cdot\iint_{A(QPD)}\Big(\underbrace{\left|\tilde{\mathbf{E}}_i+\tilde{\mathbf{E}}_{pr}+\tilde{\mathbf{E}}_{sa}\right|^2}_{\text{beam 1: probe scan}}-\underbrace{\left|\tilde{\mathbf{E}}_i+\tilde{\mathbf{E}}_{sa}\right|^2}_{\text{beam 2: empty scan}}\Big)\cdot\mathbf{H}\;dA$$

Second, we extract the high energy fluctuations of the probe define contact with surface structure after convolution of the position histogram $p(\mathbf{r})$ with the probe's shape function $s(\mathbf{r})$:

$$O(x,y,h(x,y))=\mathrm{p}(\mathrm{r})*s(\mathbf{r})=p_0\cdot\left(\exp\left[-\frac{1}{k_BT}\left(W_S(\mathbf{r})+W_{opt}(\mathbf{r})\right)\right]*s(\mathbf{r})\right)$$

By this we can recover the surface height profile h(x,y) with a resolution beyond the optical diffraction limit.



Super-resolution structured-illumination fluorescence microscopy

Background: Super-resolution (SR) microscopes require significantly more illumination photons than conventional microscopes. This leads to long acquisition times and/or enhanced fluorescence bleaching making SR imaging difficult for dynamic processes in biology.

Approach: Two counter-propagating evanescent waves generate structured illumination patterns with a fringe period of $P = \pi/k_{ev} = 170$ nm, which are phase shifted by $\phi_m = 0, \pm2\pi/3$

$$I_{ev,\alpha}(\mathbf{r}_\perp,z,\phi_m)=I_0(\mathbf{r}_\perp)\cdot\left(1+C(\mathbf{r}_\perp)\cdot\cos\left(2\,\mathbf{k}_{ev,\alpha}\cdot\mathbf{r}_\perp+\phi_m\right)\cdot\exp\left(-\frac{z}{d(\theta_i)}\right)\right.$$

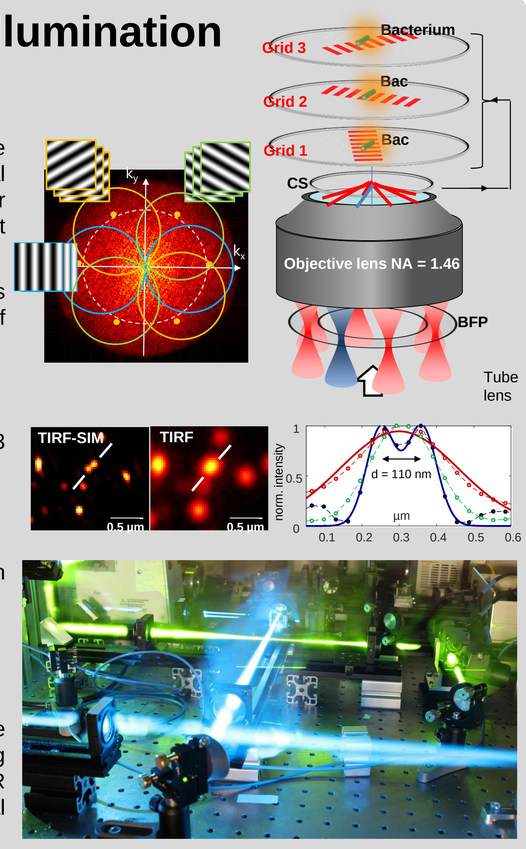
By combining 9 shifted, complex Fourier spectra $P_{m\alpha}(\mathbf{k})$ for 3 phases ϕ_m and 3 azimuthal angles α,

$$P_{m\alpha}(\mathbf{k})=2\pi\left[F(\mathbf{k})+\tfrac{C}{2}F(\mathbf{k}-\mathbf{k}_{0\alpha})e^{-i\phi_m}+\tfrac{C}{2}F(\mathbf{k}+\mathbf{k}_{0\alpha})e^{i\phi_m}\right]\cdot OTF(\mathbf{k})$$

double-color images with unique 110nm spatial resolution can be obtained simultaneously:

$$p_{SR}(\mathbf{r})\approx FT^{-1}\left[F(\mathbf{k})\cdot\sum_{m=-1}^{1}\sum_{\alpha=1}^{3}\left(2\pi\cdot OTF\left(\mathbf{k}+m\cdot\mathbf{k}_{0\alpha}\right)\right)\right]$$

By using a Michelson beam splitter and a retroreflector, we switch the illumination pattern within < 5ms, allowing acquisitions of 90 raw fluorescence images and nearly 10 SR images per second, allowing to observe dynamic biological processes.



Plasmonic coupling of two optically trapped particles

Background and Problem: Two small metallic particles diffusing inside an optical trap cannot be imaged or tracked separately. Therefore, the fast interaction between both particles is not resolvable. However, when the particles get into contact plasmonic coupling can be measured by a red shift of the resonant spectrum. The fields defining the scattering cross-section, become

$$\begin{pmatrix}E_{||s}(\theta,\omega)\\E_{\perp s}(\theta,\omega)\end{pmatrix}=\frac{e^{-ikr}}{4\pi r}\cdot ik^2V\cdot(1+k_f)\cdot\frac{\varepsilon_s(\omega)-\varepsilon_i}{\varepsilon_s(\omega)+k_f\varepsilon_i}\begin{pmatrix}\cos\theta & 0\\0 & 1\end{pmatrix}\begin{pmatrix}E_{||i}\\E_{\perp i}\end{pmatrix}$$

Approach: We combine 1 MHz 3D BFP-interferometry from the 1064nm trapping laser light with 500 Hz plasmonic spectrometry and analyse the dynamics of up to five 80nm silver spheres inside a single optical point trap.

$$S_j(\mathbf{b}_1,\mathbf{b}_2,\ldots)=\iint_{QPD}\left|\tilde{\mathbf{E}}_0+\tilde{\mathbf{E}}_1+\tilde{\mathbf{E}}_2+\ldots\right|^2\cdot H_j\;dk_xdk_y$$

From the correlations between spectral changes and the changes in the interferometric signals, we can recover the intermediate formation of dimers, trimers and other binding configurations.

$$CCF_{jk}(\tau)=\left\langle S_j(t)\cdot S_k(t+\tau)\right\rangle\approx\left\langle S_j\cdot S_k\right\rangle\cdot\exp\left(-\tau/\tau_{jk}\right)$$