Nanoscopy

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1. SIM reconstruction algorithm
   - Image formation in SIM
   - Fourier space decomposition
   - Measurement with structured illumination
   - Illumination in Fourier space
   - Illumination parameter estimation
   - Fourier space shifts and separation

2. Summary and Outlook

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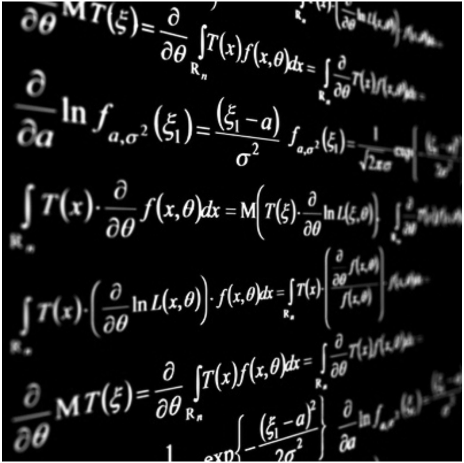
Sorry, some math...



Math (from here)

Image formation in SIM

$$M_l(x,y) = \int_{S_z} \mathrm{PSF}(x,y,z) * (I_l(x,y,z) \cdot S(x,y,z))\,\mathrm{d}z \quad (1)$$

$$I(x,y,z) = \mathrm{PSF}_{\mathrm{ex.}}(x,y,z) * I'(x,y,z) \quad (2)$$

- Interested in: **Sample response** (fluorescence density) $S(x,y,z)$.
- Limited by: PSF, i.e. Abbe limit lateral, background axial
- All SIM approaches:
  - Obtain $S(x,y,z)$
  - by combining multiple measurements M_l
  - for different illuminations $I_l(x,y,z)$
- **Keep in mind**: Illumination also limited by $\mathrm{PSF}_{\mathrm{ex.}}$.

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Solve for $S(x, y, z)$

$$M_l(x,y) = \int_{S_z} \mathrm{PSF}(x,y,z) * \left(I_l(x,y,z) \cdot S(x,y,z)\right) \mathrm{d}z \tag{3}$$

$$I(x,y,z) = \mathrm{PSF}_{\mathrm{ex.}}(x,y,z) * I'(x,y,z) \tag{4}$$

- Convolution integral: No direct solution for $S(x, y, z)$
- Iterative solvers possible, but that is more advanced
- Direct solution
  - possible for specific forms of $I_l(x, y, z)$
  - by decomposition in Fourier space

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Decomposition in Fourier space

$$\tilde{M}_l(k_{x,y}) = \int_{S_z} \mathrm{OTF}(k_{x,y,z}) \cdot \left(\tilde{I}_l(k_{x,y,z}) * \tilde{S}(k_{x,y,z})\right) \mathrm{d}z \tag{5}$$

$$\tilde{I}(k_{x,y,z}) = \mathrm{OTF}_{\mathrm{ex.}}(k_{x,y,z}) \cdot \tilde{I}'(k_{x,y,z}) \tag{6}$$

- In Fourier space: $I(k_{x,y,z})$ folds with $S(k_{x,y,z})$
- Assume $I(k_{x,y,z}) = \sum_i \delta_i(k_{x,y,z})$
- Then M_l given by
  - Finite sum of shifted copies of $S(k_{x,y,z})$
  - Each shift position given by $k_{x,y,z}$ in $\delta_i(k_{x,y,z})$
- Collect as many M_l as there are $\delta_i(k_{x,y,z})$
  $\rightarrow$ directly solvable for $S(k_{x,y,z})$
- 2D SIM: 2 peaks from sin, 1 peak from DC

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Step 1: Measurement for structured illumination

Regular illumination pattern

Use

$$I_l(x, y) = \frac{I_0}{2} \cdot [1 + \sin((2\pi \cdot p + \phi)/\kappa)]$$

where

$$p = x \cdot \cos(\alpha) + y \cdot \sin(\alpha)$$

Multiple measurements

- Use multiple angles α for illumination
  Typically 3 or 4, evenly spaced
- For each angle, illuminate with (at least) 3 phases $\phi = \frac{0}{3}\pi, \frac{2}{3}\pi, \frac{4}{3}\pi$
  - Phases not evenly spaced: loss of SNR
  - More than three phases: Reconstruction gets over-defined, but still possible
- Number of angles: Full resolution enhancement along each angle α.

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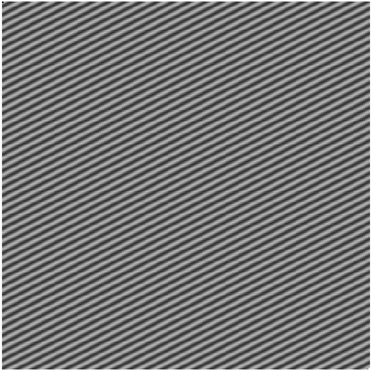
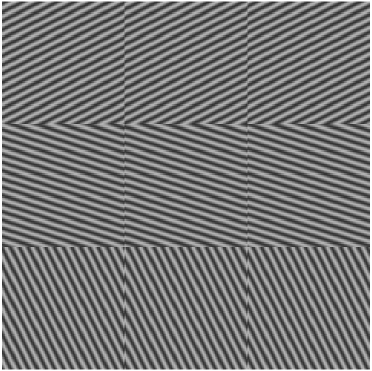
Step 1: Illumination pattern

Simulated illumination pattern





Left: First illumination pattern. Right: Montage of all illumination patterns

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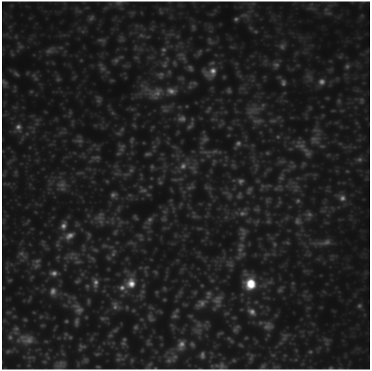
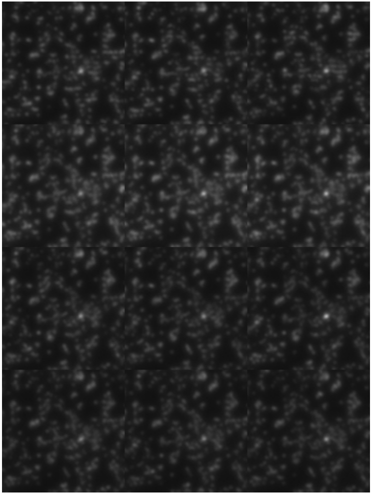
Step 1: Measurement (2D SIM)

Test surface dye-filled beads. Pattern spacing: 256 nm ($\sim$ 86% of resolution limit).





Left: First illumination pattern. Right: Cut-out, montage of all patterns

Any visible variation between the different patterns?

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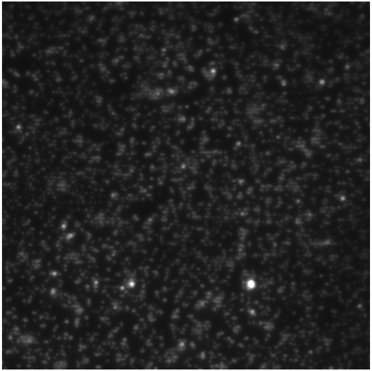
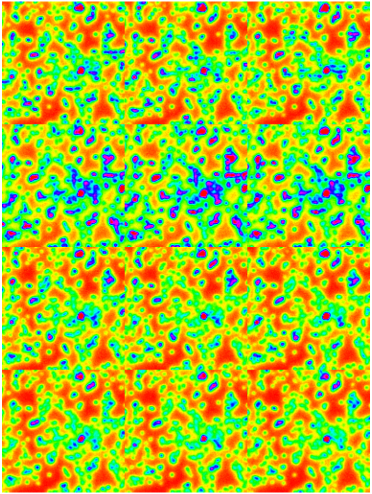
Step 1: Measurement (2D TIRF SIM)

Test surface dye-filled beads. Pattern spacing: 256 nm (at resolution limit).





Left: First illumination pattern. Right: Cut-out, montage with spectrum LUT

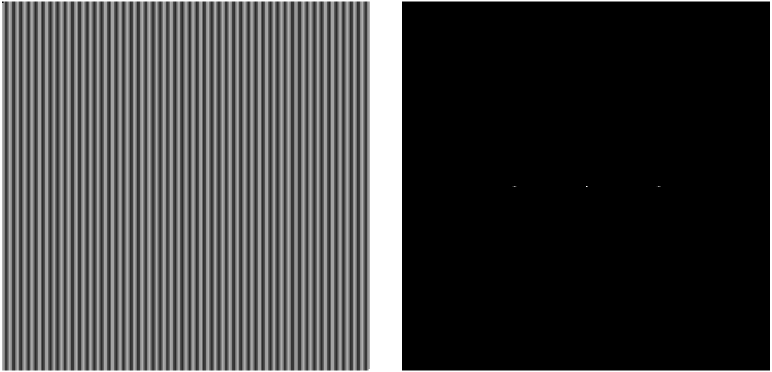
Look closely at the bright structures. . .

Step 2: Illumination in Fourier space

$$I_l(x, y) = \frac{I_0}{2} \cdot [1 + \sin((2\pi \cdot p + \phi)/\kappa)]$$

where

$$p = x \cdot \cos(\alpha) + y \cdot \sin(\alpha)$$



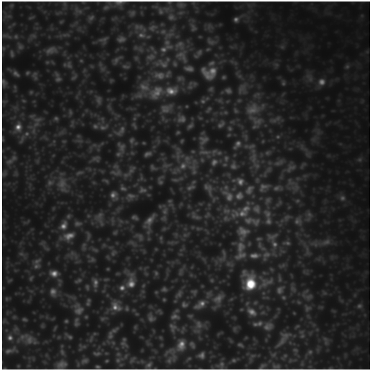
Left: Illumination pattern. Right: FFT / FHT power spectrum

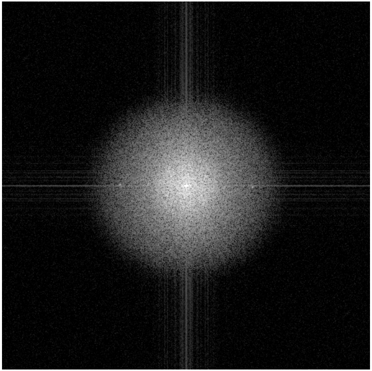
Three dots in FFT

Step 2: Illumination in Fourier space

$$\tilde{M}_l(k_x, k_y) = \mathrm{OTF} \cdot \left(\tilde{S}(k_x, k_y) * \tilde{I}_l(k_x, k_y) \right)$$

Test surface





Left: Illumination pattern. Right: FFT / FHT power spectrum

Fourier space reveals the illumination pattern[1].

[1] but only because $\kappa \sim 0.8\kappa_{\mathrm{max}}$

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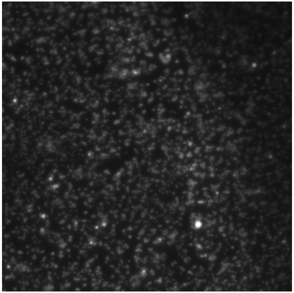
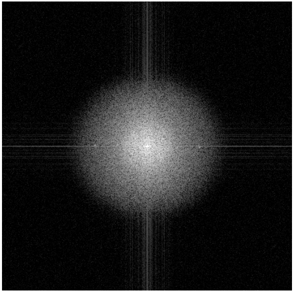
Step 2: Acquire parameters

- Three peaks in FFT power spectrum: Center "DC" and symmetric $\sin(2\pi \cdot p + \phi)$-contribution.
- Position directly translates to κ, α.
- The phase ϕ is contained in $z = r \cdot e^{i\phi}$, as the FFT yields a complex number. However, it is easily distorted by other structures in the sample.





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Step 3: Decompose the measurements

Measurement

Three phases ϕ_0, ϕ_1, ϕ_2 for illumination, each row in K represents one measurement:

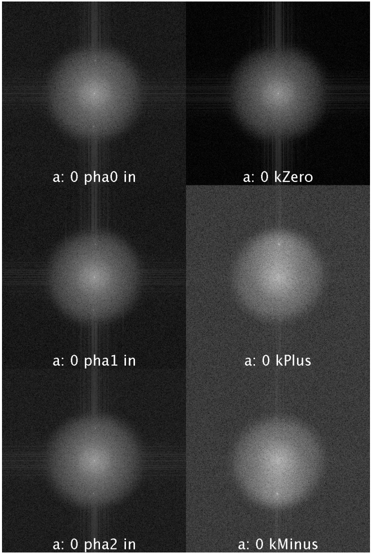
$$K = \begin{pmatrix} 1 & \frac{1}{2}e^{i\phi_0} & \frac{1}{2}e^{-i\phi_0} \\ 1 & \frac{1}{2}e^{i\phi_1} & \frac{1}{2}e^{-i\phi_1} \\ 1 & \frac{1}{2}e^{i\phi_2} & \frac{1}{2}e^{-i\phi_2} \end{pmatrix}$$

Columns: DC contributions, two symmetric contributions from the sinusoidal form.

Decomposition

Invert[a] K to K^{-1}.
Via K^{-1}, get the DC contribution (wide-field) and higher frequencies.

[a] 3×3 matrix, otherwise Moore-Penrose pseudo inverse



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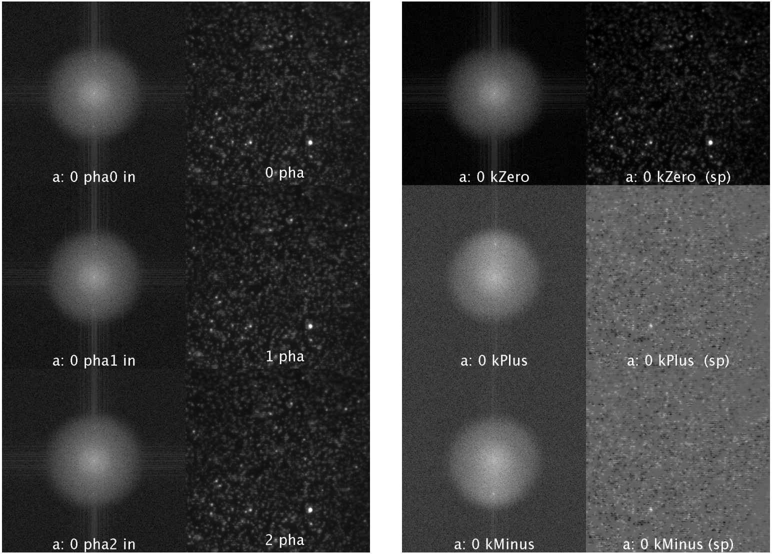
Step 3: Spatial results of decomposition



DC component reconstructs to an images with wide-field characteristics.
Sinusoidal components not yet meaningful, but: κ and α have not been used so far.

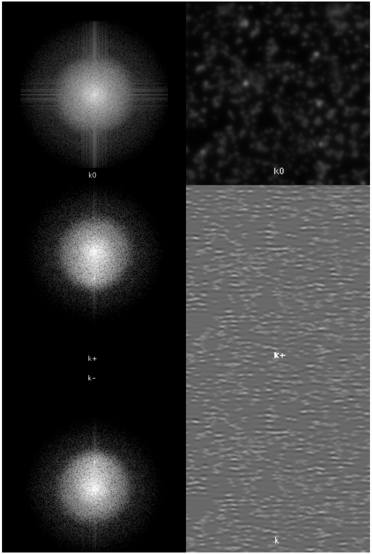
Step 3: Shifting frequency components

Compute shift

- DC component: Widefield, done.
- Two symmetric components: $\sin((2\pi \cdot p + \phi)/\kappa)$, where $p = x \cdot \cos(\alpha) + y \cdot \sin(\alpha)$
- Shift these contributions by $\pm\kappa, \alpha$

Result

- Pixels needed for shift: Resolution enhancement
- $+\kappa$ and $-\kappa$: contain the same information by definition
- Spatial transformation: Structure becomes apparent



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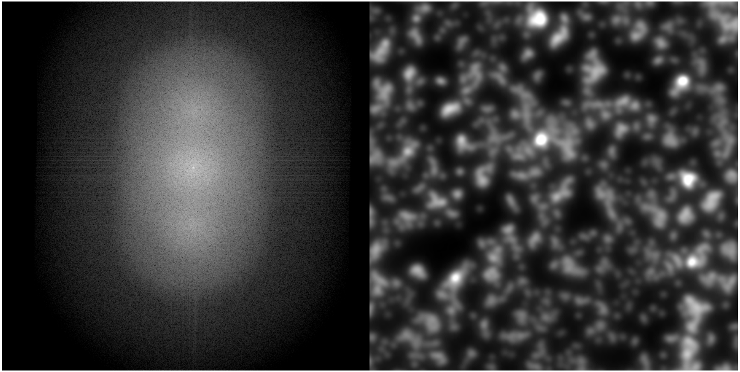
Step 3: Added frequency components



Add up the DC and shifted sinusoidal components. Result: Resolution-enhancement along illumination pattern direction α.

Interim result

- Sinusoidal pattern[2], 3 contributions in Fourier space
- Matrix inversion, decomposition and shift
- Resolution enhancement along the pattern direction
- **Number of phases:** At least 3, more lead to an over-defined matrix
- When measuring these 3 phases, κ and α have to be fixed, phases should be evenly spaced.
- **Number of angles:** Arbitrary, but usually 3 or 4 to fill the Fourier space.
- κ may change between angles α.
- **Resolution enhancement:** Directly given by pattern spacing κ.

[2] Other pattern: How does their Fourier transform look like?

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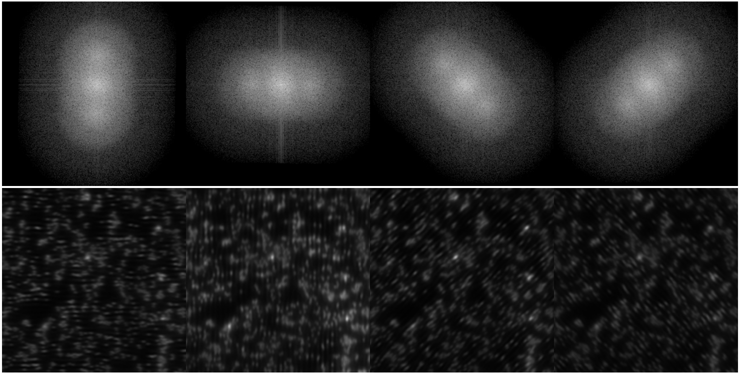
Step 4: Measure for multiple angles α



Three angles α. Resolution enhancement along each angle of the pattern. Next step: Combine these spectra.

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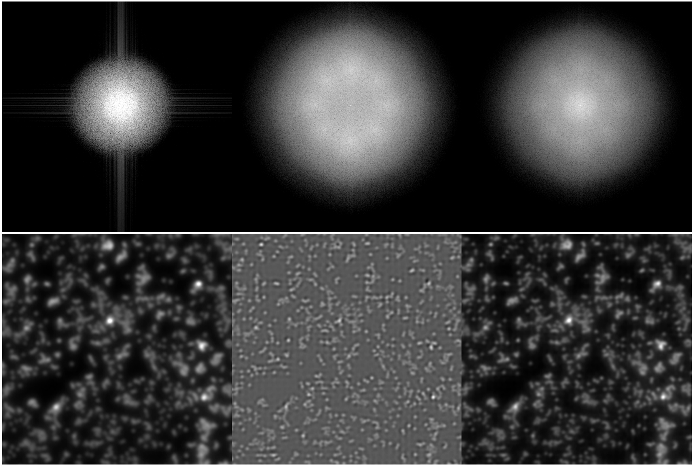
Step 4: Combine all angles

$$\tilde{M}_l(k_x, k_y) = \mathrm{OTF}(k_x, k_y) \cdot \left(\tilde{S}_l(k_x, k_y) * \tilde{I}(k_x, k_y) \right)$$



Low, high and all frequency components

Low frequency widefield, high frequency additional information, all frequencies combined. Close, but not quite right. What is missing?

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Step 5: OTF frequency filtering

$$\tilde{M}_l(k_x, k_y) = \mathrm{OTF} \cdot \left(\tilde{S}(k_x, k_y) * \tilde{I}_l(k_x, k_y) \right)$$

- So far, result looks better, but not quite right
- Transfer function: Not only limits resolution, but dampens high frequencies
- This leads to problems when shifting components around
- Solution: Divide by OTF in frequency domain.
- However: Low to zero regions in OTF will cause artifacts
- Full solution: frequency filtering

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Step 5: (modified/generalized) Wiener filter

$$\tilde{R}(k_x, k_y) = \sum_l \frac{OTF(k_x, k_y)\tilde{M}_l(k_x, k_y)}{\mathrm{OTF}^2(k_x, k_y) + \omega}$$

- Multiply by OTF and divide by OTF^2, thus: Result without frequency dampening
- Numerator: One contribution of OTF is multiplied in post-processing, one is inherent to the measurement.
- Parameter ω: Artificial high-frequency dampening.
  Dominates in regions of a low OTF, with quadratic response.
- Determining the OTF: Big difference between 2D and 3D:
  2D: Use any OTF (Gaussian, Bessel, . . .) with a somewhat matching FWHM
  3D: Measure a quite exact OTF along the axial direction.
- There are more involved filtering methods available (e.g. iterative ones like total variations)
  But: *No further information is generated by filtering.*

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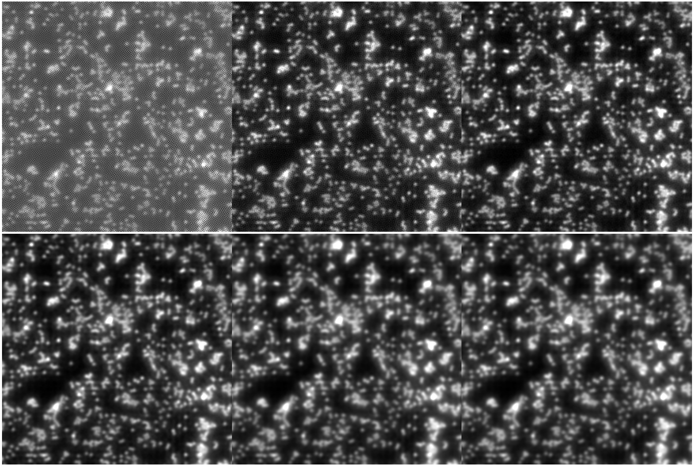
Step 5: Result of Wiener filtering



Reconstruction with different Wiener filter settings:

0.05, 0.1, 0.5, 2, 10, 50

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Step 5: Apotization

$$\tilde{S}'(k_x, k_y) = \text{APO} \cdot \sum_l \frac{OTF(k_x, k_y)\tilde{M}_l(k_x, k_y)}{\text{OTF}^2(k_x, k_y) + \omega}$$

- After the filtering step, $R(k_x, k_y)$ now has no "natural" dampening of higher frequencies.
- This leads to harsh contrasts that would not be obtained by a higher resolution microscope
- Fix: Multiply an apotization function APO to the result
- APO: An artificial OTF, dampening high frequency components the same way a microscope would
- FWHM of the APO: motivated by the higher resolution limit set through the original OTF, κ and (to a lesser degree) ω.
- In practice: Start with $\text{FWHM}_{\text{APO}} = 2 \cdot \text{FWHM}_{\text{OTF}}$, tweak ω and APO with lots of leeway.

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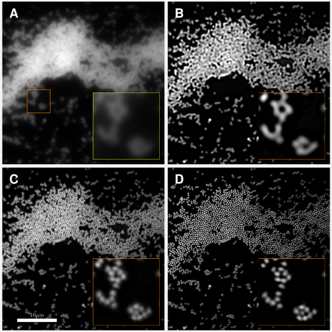
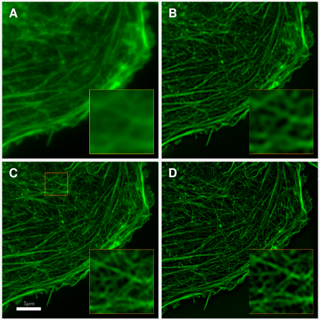
Step 5: Final result



200 nm Tetraspeck beads, excitation at 643 nm, measured on a DeltaVision OMX (GE LifeScience). Wide-field [A], filtered wide-field [B], single slice 2D SIM-reconstruction by our software [C], 3D SIM reconstruction of the same slice, by SoftWORX (Applied Precision/GE LifeScience) [D].



Wide-field [A], filtered wide-field [B], single slice 2D SIM reconstruction by our software [C], 3D SIM reconstruction of the same slice, by SoftWORX (Applied Precision/GE LifeScience) [D]. LSEC actin filaments are labeled with AlexaFluor 488 / Phalloidin, measured on a DeltaVision OMX (GE LifeScience).

Summary and Outlook

Algorithm presented today

- Sinusoidal form needed
- 2D since $\approx$ 2000,
  3D since $\approx$ 2008.
- In Bielefeld: OMX and self-made setup
- In development: Free, open-source reconstruction software

Outlook next lectures

- SIM: Extension to 3D
- SIM: Iterative solvers
- Localization microscopy

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