Nanoscopy

Marcel, Wolfgang

Biomolecular Photonics, Bielefeld University

SoSe 2015

CC BY SA

1. Overview of super-resolution techniques

2. Structured illumination
   - Variants of confocal microscopy
   - Background measurement by light modulation
   - SIM for lateral resolution enhancement

3. SIM microscope setup

CC BY SA

Overview: Superresolution Microscopy

Techniques that allow resolution beyond the Abbe limit

$$M_{l,\kappa}(x,y) = \int_{S_z} \mathrm{PSF}(z) * \left(I_l(x,y,z) \cdot S(x,y,z,\kappa) \right) \mathrm{d}z$$

- **Start today**: *Influence the illumination*: **Structured illumination microscopy (SIM)**
  Use multiple sets l of $I_l(x,y,z)$, where $I_l(x,y,z)$ varies along x, y, z. If now $M_l(x,y)$ and $I_l(x,y,z)$ is known, solve for $S(x,y,z)$.
  SIM denotes a specific technique and the general concept.
- After the holiday break: *Use (and sometimes influence) the sample response*:
  Localization Microscopy
  Add some *property* κ to the sample, so its response to illumination can change. This can be switching the fluorophore (e.g. STED) or a stochastic blinking process (STROM, dSTROM).
  Localization microscopy is a somewhat vague term.

CC BY SA

SIM

Structured illumination microscopy

CC BY SA

What is SIM

SIM denotes a specific technique and the general concept.

General SIM

All methods where $I(x, y) \neq$ const., especially

- Multi-spot confocal scanning[a]
- Digital background reduction, e.g. *Zeiss Apotome*
- **Resolution:** At most confocal, i.e. $\sqrt{2}$

[a] Standard confocal scanning: SIM in principle, but usually not called SIM

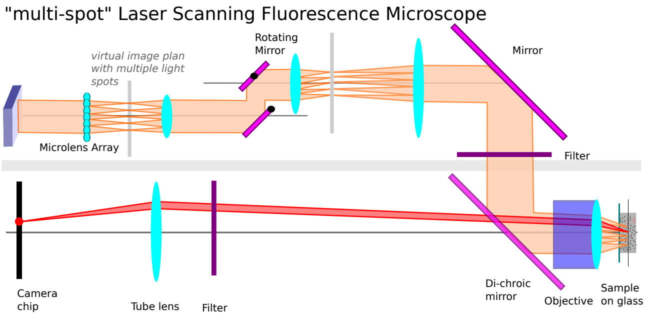
SIM for lateral resolution enhancement

- Lateral (2D) and lateral + axial (3D) light modulation
- with multiple known illumination pattern
- Digital reconstruction of frequency components
- **Resolution:** Usually factor of 2.

CC BY SA

Multi-spot laser-scanning

Idea: Speed up confocal scanning by using multiple spots at once.



- Spot generation: Micro-lens array, SLM[1] devices
- Spot detection: Multi-PMT array (few spots), camera (more noise)
- Pinhole: digital (post-processing), second synced micro-lens array.
- System very fast, $\sqrt{2}$ confocal improvement, two-photon application.

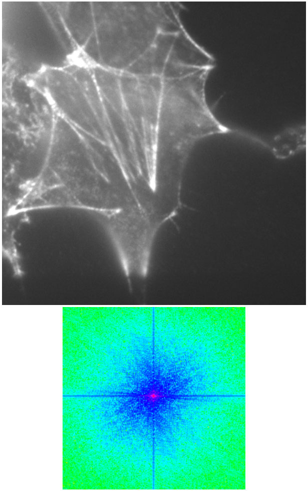
[1]Spatial light modulators

CC BY SA

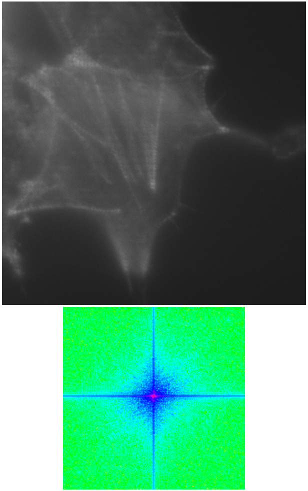
Background measurement by light modulation

Examples for background from earlier in this lecture.
Now: How to obtain those.

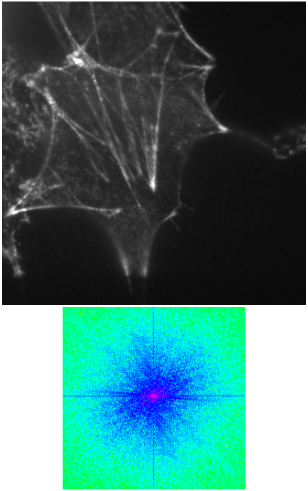
Full Image



Background

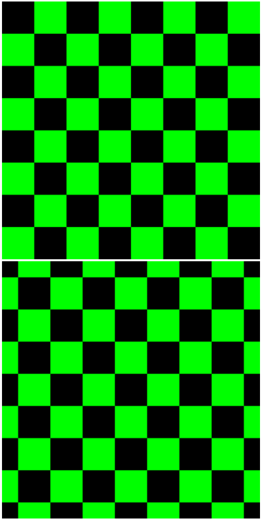


Full Image - Background



Background reduction by light modulation

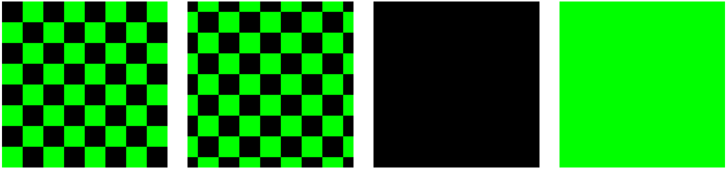
Idea: Measure background contributions, subtract them from image



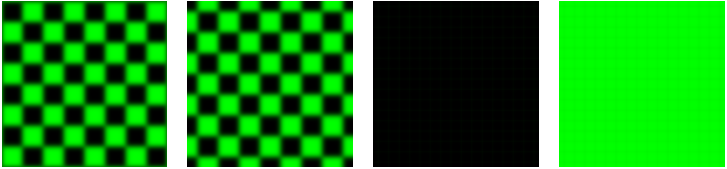
- Vary $I(x, y)$ in the focal plane
- Choose e.g. a checkerboard-pattern
  $I(x, y)/I_0 =$
  $1 + \text{sgn}(\sin(2\pi \cdot \kappa \cdot x + \phi_x)) \cdot \text{sgn}(\sin(2\pi \cdot \kappa \cdot y + \phi_y))$
- Set the pattern spacing frequency κ close to the resolution limit
- Take multiple measurements, each time shifting the pattern by varying ϕ_x, ϕ_y.
- **Important:** There are better algorithms to do this!

Simulation: Illumination in the focal plane and out-of-focus

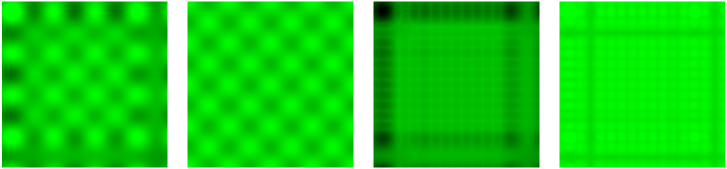
Illumination Pattern: (Pos. 1, . . . , Pos. 6, . . . , Minimum, Maximum)



Pattern in focal plane: (Pos. 1, . . . , Pos. 6, . . . , Minimum, Maximum)

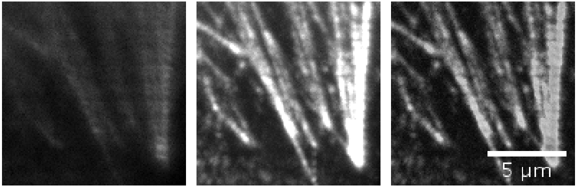


Pattern out-of-focus: (Pos. 3, . . . , Pos. 8, . . . , Minimum, Maximum)

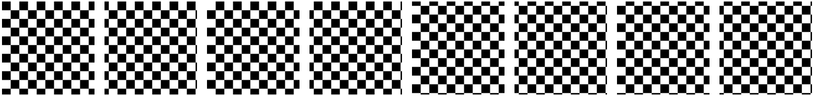


Example measurement

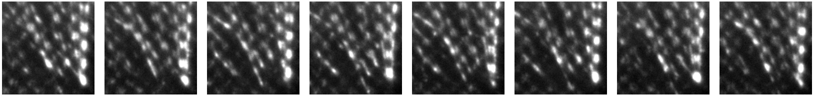
Min, Max, Result



Illumination Pattern:



Focal plane measurement:



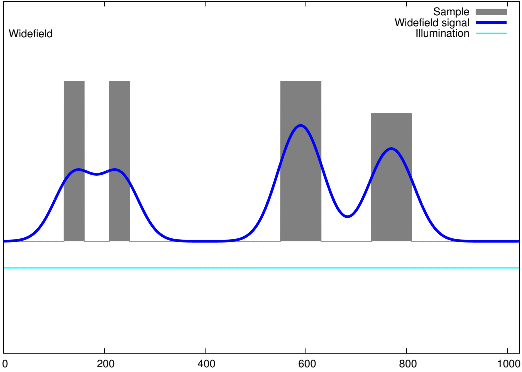
SIM for lateral resolution enhancement

Resolution enhancement through SIM

CC BY SA

Structured illumination: Starting from wide-field…

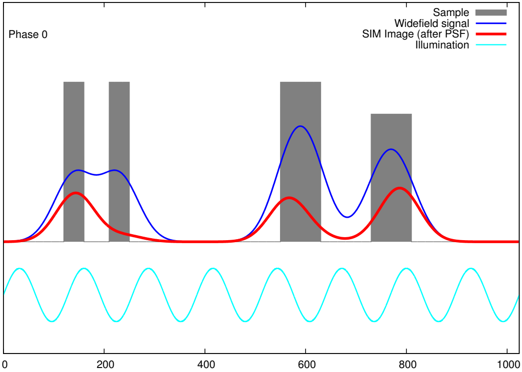
- Resolution limited by diffraction limit
- OTF cuts away high frequencies, projection to subspace
- What happens the illumination I is modulated along x, y?



Widefield image

Structured illumination: image acquisition

- Modulate the illuminating light with $I(x) = I_0 \cdot (1 + \sin(2\pi\kappa x + \phi))$
- Use a modulation wavelength k near the diffraction limit
- Shift the phase ϕ ...

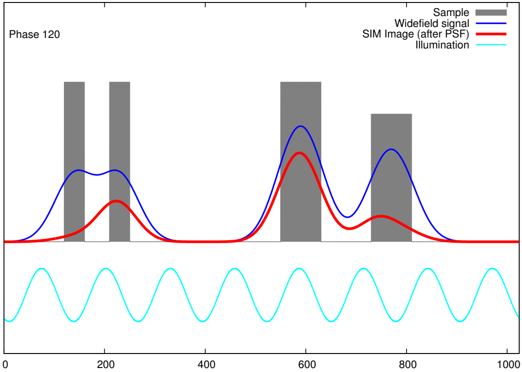


Phase $\phi = \frac{0}{3}\pi$

CC BY SA

Structured illumination: image acquisition

- . . . and gather additional information about the sample. . .

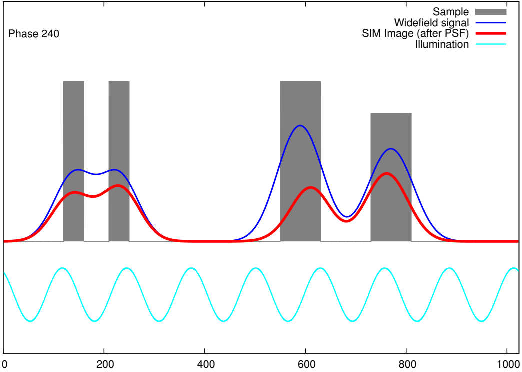


Phase $\phi = \frac{2}{3}\pi$

CC BY SA

Structured illumination: image acquisition

- ... by collecting images at three phases.
- In principle, any three phases will do (thats because any three span the same Fourier subspace).
- In reality, they should be $\frac{2}{3}\pi$ apart. (thats because measurements have errors and noise).

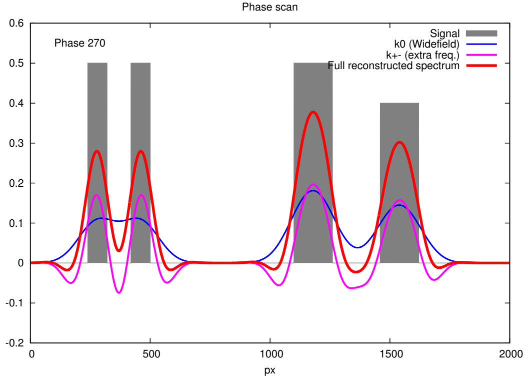


Phase: $\phi = \frac{4}{3}\pi$

CC BY SA

Structured illumination: image reconstruction

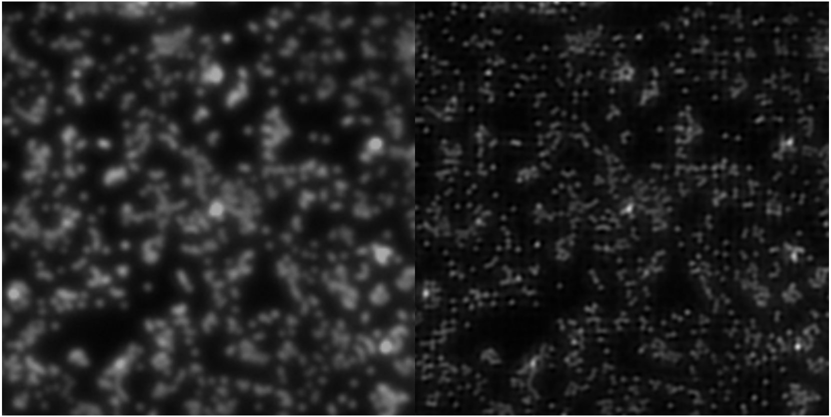
- Recombine three images ($\phi_{0,1,2} = \frac{0}{3}\pi, \frac{2}{3}\pi, \frac{4}{3}\pi$) to one with higher resolution
- Separate contribution through M^{-1}, where
$$M = \begin{pmatrix} 1 & \frac{1}{2}e^{i\phi_0} & \frac{1}{2}e^{-i\phi_0} \\ 1 & \frac{1}{2}e^{i\phi_1} & \frac{1}{2}e^{-i\phi_1} \\ 1 & \frac{1}{2}e^{i\phi_2} & \frac{1}{2}e^{-i\phi_2} \end{pmatrix}$$
- Shift contributions to new position $\pm k_0$ in Fourier space



Reconstructed signal

CC BY SA

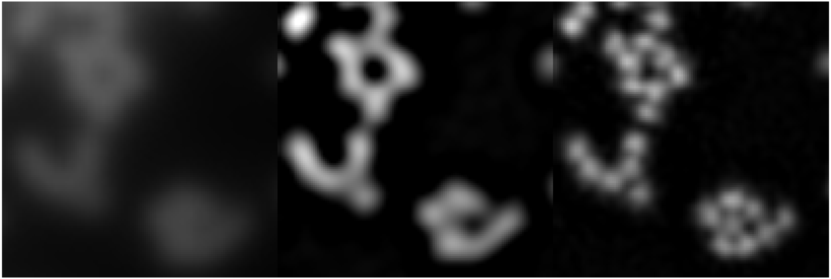
2D SIM reconstruction of test sample



- Sample: Closely-packed surface of dye-filled beads
- Widefield: Beads beyond the resolution limit
- SIM: Beads clearly visible
- Measurement on a simple 2D projection SIM (1.6× resolution)

CC BY SA

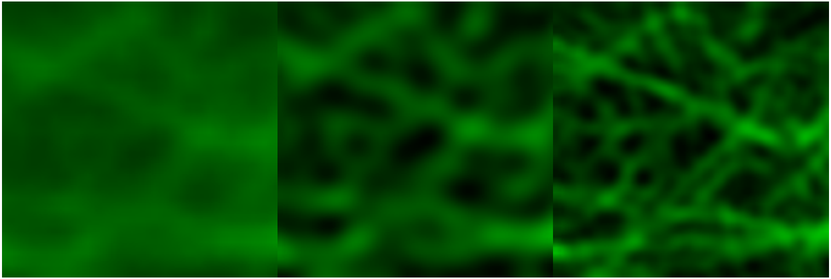
2D SIM reconstruction of test sample



Left to right: Wide-field, filtered wide-field, SIM reconstruction

- Tetraspeck 200nm dye-filled beads
- Area $5.6 \times 5.6 \mu\mathrm{m}$
- Measured on the OMX

SIM reconstruction of Actin



Left to right: Wide-field, filtered wide-field, SIM reconstruction

- Actin structure in an LSEC cell
- Area $5.6 \times 5.6 \mu\mathrm{m}$
- Measured on the OMX

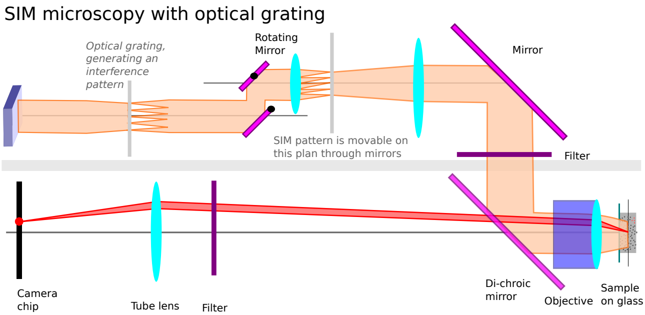
CC BY SA

SIM microscopes

Setup of a structured illumination microscope

CC BY SA

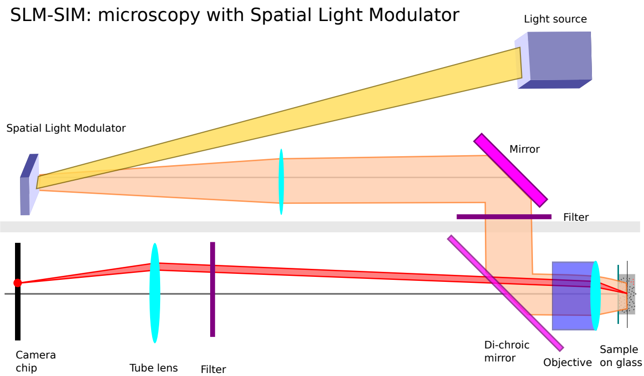
SIM setup with optical grating



- First design, around 2000
- Good modulation depth
- Rather complex, mechanical (moving mirrors) setup
- Used by the machine next door

CC BY SA

SIM setup with spatial light modulators



- SLMs widely available through projectors, etc.
- Almost free of moving components
- Very fast, low cost
- Used in low cost and/or high speed machines