Nanoscopy

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SoSe 2015

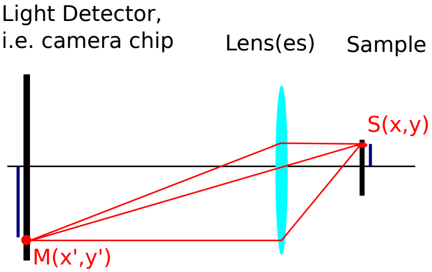
Literature

- Optics in Fourier space
  Introduction to Fourier optics
  Goodman, Joseph W.
  maybe take the 2005 3. edition, not 1968
- Light sources and microscopy design:
  Zeiss campus website
  http://zeiss-campus.magnet.fsu.edu

The simplified view of a microscope

$$M(x', y') = I \cdot S(x, y)$$

- $M(x', y')$ the light intensity measured (by some means) at position x', y' on the image plane.
- I the light intensity of the illumination.
- $S(x, y)$ the samples response to the illumination.
- This is completely idealized. Question is: Where does it need to be more realistic?

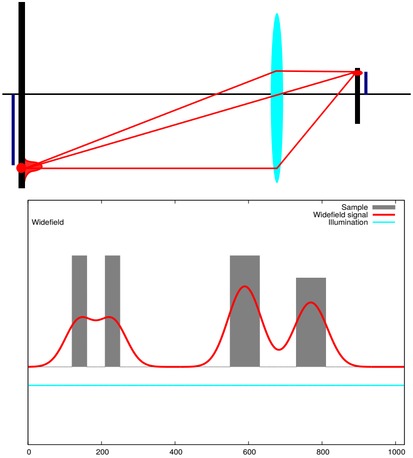


Points S(x,y) on the sample plane are mapped to measurement M(x', y') on the image plane.

Resolution and point-spread functions

$$M(x,y) = \mathrm{PSF} * (I \cdot S(x,y))$$

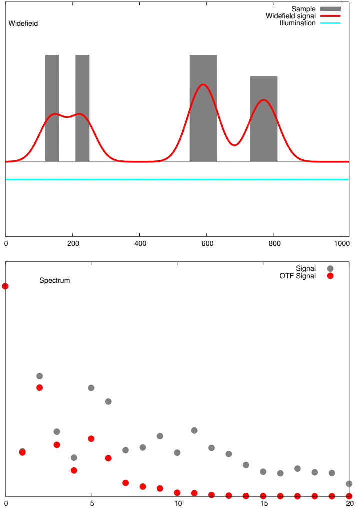
- **Diffraction limit:** Point-like emitter is spread to a distribution. Bringing points closer together, they can no longer be distinguished.
- Described by the **point-spread function (PSF)**. Optical systems fold the signal with their PSF when measuring.



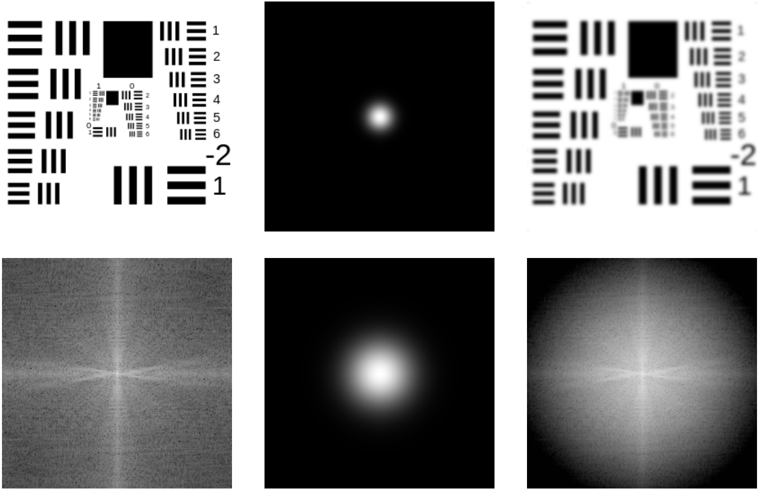
OTFs and Fourier space

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

- The PSF in Fourier space is called **Optical Transfer Function (OTF)**.
- Folding becomes multiplication in Fourier space.
- **OTF** directly links to resolution, chose any for a resolution limit:
  - OTF has regions where it is zero, thus projects to a subspace.
  - Light is quantized.
  - **Relevant:** Noise overlays the dampened high frequencies.



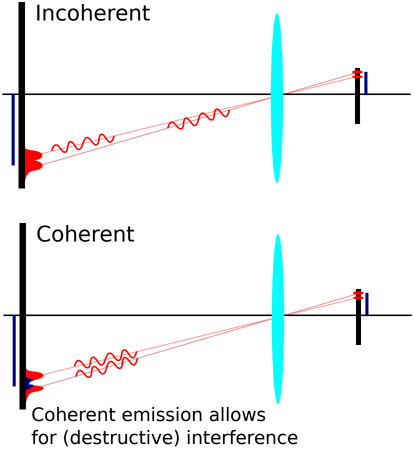
Fourier space for images



Base image: Wikipedia / Wikimedia: USAF-1951

Digression: Coherence and (destructive) interference

- **Wave-nature of light** gives the Abbe limit: Interference while traversing the optical system.
- Usual approach: Point source, obtain point-spread function, folding by PSF.
- Multiple point sources: There is a difference between coherent and incoherent emission: Destructive interference
- Equations thus far: **Intensities** add up, thus **no interference**.

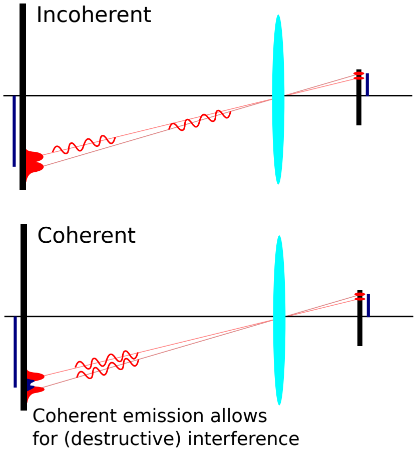


Coherent or incoherent emission

- This

$$M(x, y) = \mathrm{PSF} * (I \cdot S(x, y))$$

  implies adding up intensities. Or does it? Complex valued I, S, PSF...
- **Coherence**: Does the sample preserve the phase of incoming light?
- No problem for **fluorescence**, even with coherent (laser) illumination. **Fluorescence** lifetime in order of nanoseconds, destroys coherence.
- For other materials and processes, a closer look might be needed (e.g., stimulated coherent emission).



More detail: Ideal PSFs/OTFs and approximation

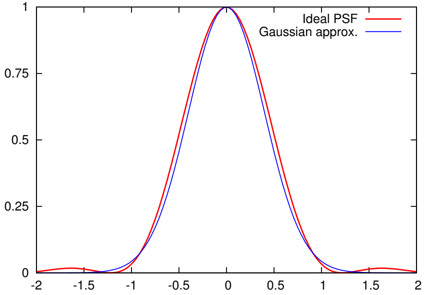
$$\tilde{M}(k_x, k_y) = \mathrm{OTF}(k_x, k_y) \cdot \left(I\tilde{S}(k_x, k_y)\right)$$

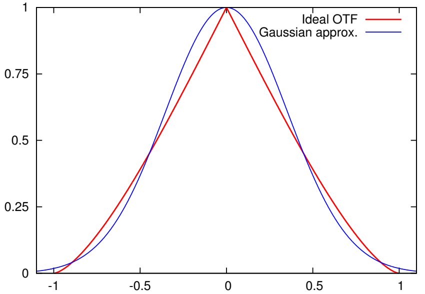
Ideal means a circular aperture in the Fourier plane, otherwise perfect optical system. Then:

$$\mathrm{PSF}(x) = \frac{2J_1(x)}{x}^2$$

$$\mathrm{OTF}(k) = \frac{2}{\pi}\left(\arccos(|k|) - |k|\sqrt{1-k^2}\right)$$

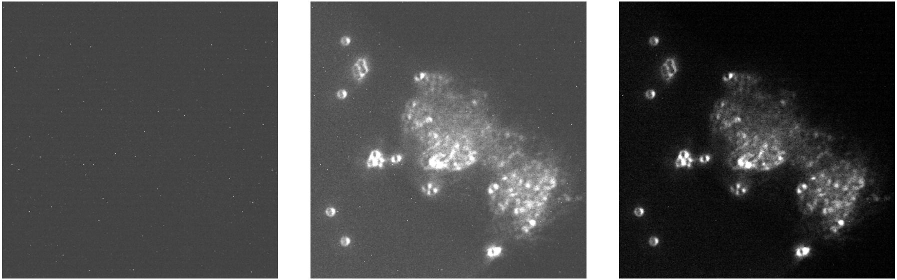
Gaussian approximation not too bad for PSF, and Fourier transform gets much easier. But: Gaussian function does not fall off to zero!





Noise: Camera noise, photon shot noise

"Camera" noise



Noise/dark frame (left), full signal (middle), signal with darkframe substraction (right) [1]

- **Noise** arises as the camera (mis)counts the number of photons. **Thermal and quantum effects** distort the number of photo-converted electrons.
- **Thermal and quantum effects** distort the amplification and conversion from electron charge to a digital value.

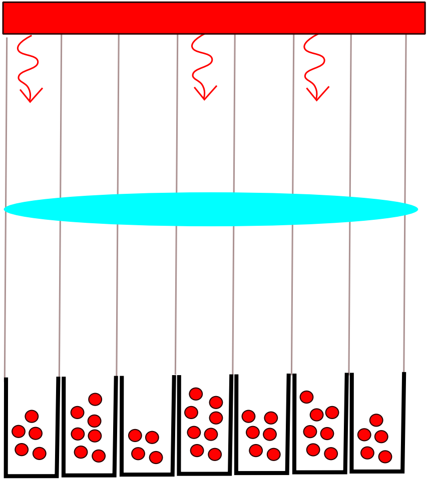
- **Cooling** helps with some of the thermal effects.
- **Dark frame substration** (below)
- **"Better" electronics** helps, see lecture on camera types and trade-offs (noise, speed, price).

[1] Images provided by Christian Pilger

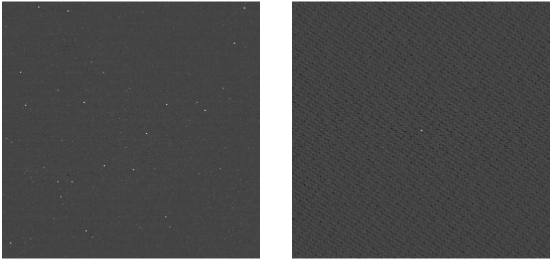
Photon shot noise

- Evenly fluorescent surface:
  N photons / sec.
- Fluorescence is random, i.e. emission probability distribution is constant
- For a finite measurement: not every point will receive the same amount of photons
- Poisson distribution: Noise scales with $\mathrm{SNR} = \frac{N}{\sqrt{N}} = \sqrt{N}$
- This problem is fundamental: Only solution is to increase N, i.e. higher intensity
- **Good cameras** are limited by shot noise (not only for microscopy)

Fluorescent surface, evenly distributed fluorescence probability



Noise in Fourier space

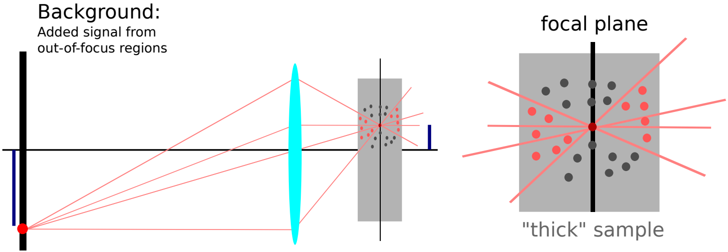


Noise in spatial (left) and frequency (right) domain

$$M(x,y) = (\mathrm{PSF} * (I \cdot S(x,y))) + N(x,y)$$

- $N(x,y)$ is random: Distribution influenced by various factors.
- Each pixels might be different, e.g. different average and variation.
- In first approximation there should be no correlation between pixels. Spectral distribution can be used to check.
- **High-frequency filtering** thus will eliminate some of it.
- **Camera characteristics** can be measured quite extensively.

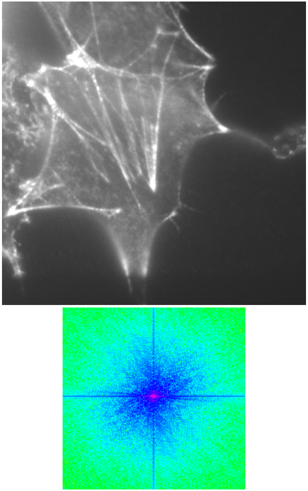
Background



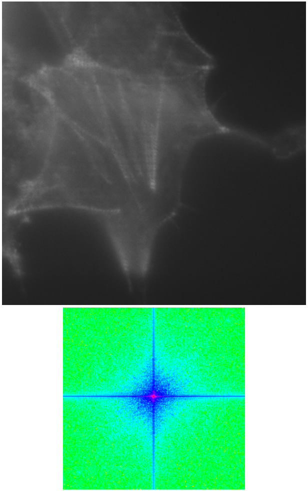
- Samples are often not flat, but somewhat thick.
- Image plane (photons stopped by camera chip) sets a fixed sample focal plane
- Pixels collect additional light from **out-of-focus contributions**

Background: Examples

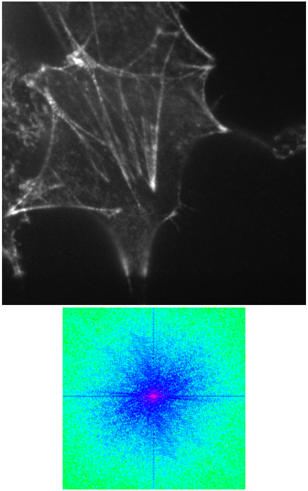
Full Image



Background



Full Image - Background

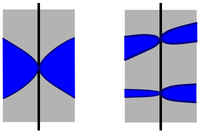


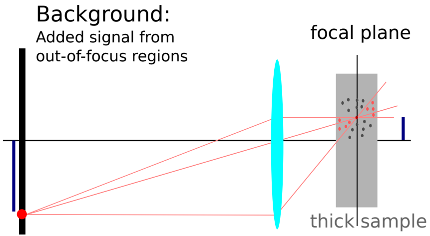
Background: 3D PSF

$$M(x,y) = \int_{S_z} \mathrm{PSF}(z) * (I \cdot S(x,y,z))\,\mathrm{d}z$$

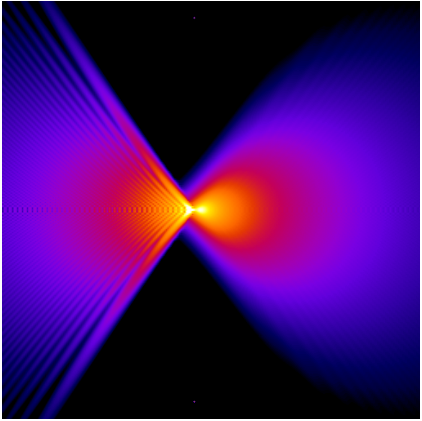
- 3D PSF (and sample) to account for these contribution
- z-component generally harder to calculate, but can be measured and/or simulated.
- **Important**: Axial vs. lateral resolution and improvement

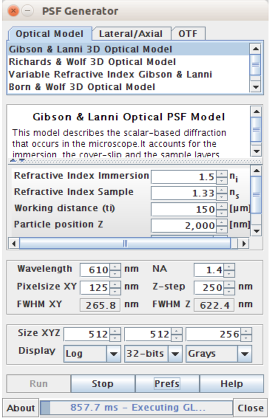
Sketch of 3D PSF





Background: Obtaining a PSF





ImageJ Plugin: PSF Generator

- Simulation: Software takes into account particle position, working distance, all refractive index changes between immersion medium, cover slip, sample.
- Measurement: Use single point-like emitters (reasonably smaller than resolution limit) and scan them through the focus.

Summary

What is background, what is noise

Noise is an uncertainty in measuring the number of photons on a pixel.
Background are photons picked up from (usually out-of-focus) positions on the sample that are of no interest to the measurement.

In common

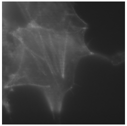
- Both add to the measurement.
- Both are **not signal**. Thats somewhat by definition, as in *not the signal we are interested in*.
- Sometimes low enough to ignore, sometimes so high the image is ruined.
- Some post-processing addresses both.

Differences

- Noise depend on
  - Intensity (photon shot-noise)
  - Camera quality
- Background depends on
  - the sample
  - the way it is illuminated
  - the way it is imaged
- Often, with $M = I \cdot S$, increasing I keeps noise constant, while background scales with I. In these cases: **More light improves SNR, but not SBR**.

Summary: Background and Noise

$$M(x,y) = \underbrace{\int_{S_z} \mathrm{PSF}(z)}\ast (I \cdot S(x,y,z))\,\mathrm{d}z + \underbrace{N(x,y)}$$



Background

- Unwanted out-of-focus contributions
- Background scales with illumination intensity, thus SBR (signal-background-ratio) uninfluenced by more light
- Improvements: Illumination, Deconvolution, . . .

Noise

- Measurement errors for camera photon count
- Noise is constant when illumination is increased, thus SNR (signal-noise-ratio) can be improved by more light
- Other improvements: Exposure time, camera type (with cost/speed/noise trade-off).