Image Scanning Microscopy:
from forward to inverse problem formulations

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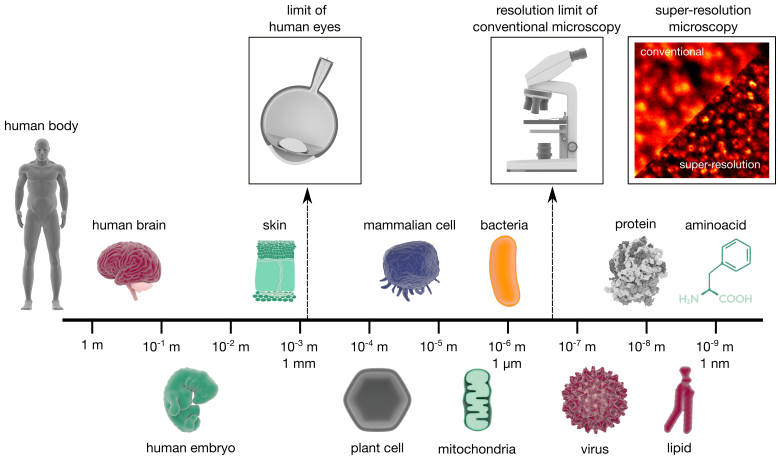
Outline

Why Microscopes? Magnified images of objects at the nanoscale



Microscope features?

- High contrast
- High resolution

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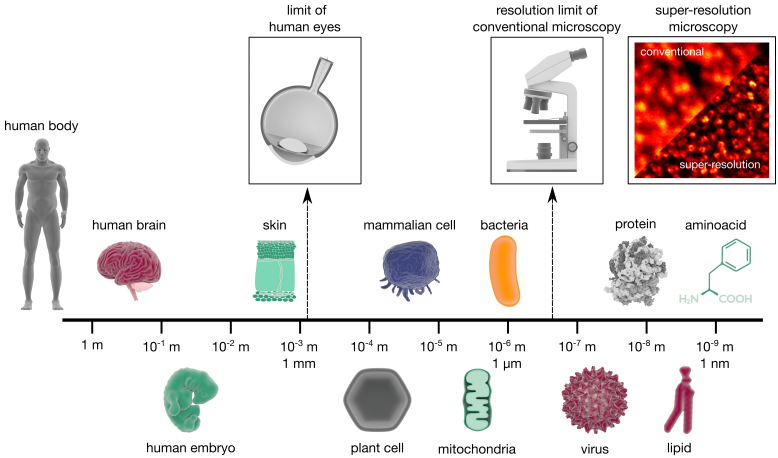

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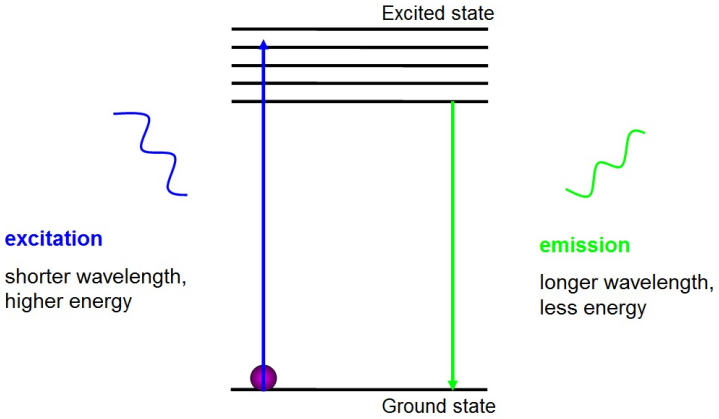
Fluorescence as a way to increase contrast

Fluorescence: emission of light by a compound which absorbed light.



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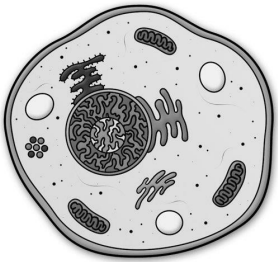

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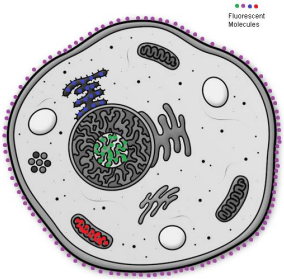

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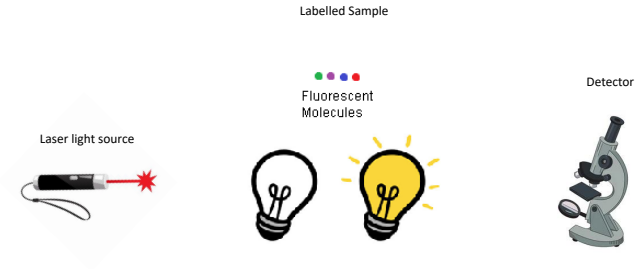

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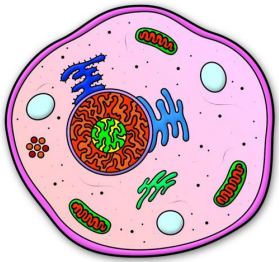

Why using fluorescence?

- High contrast.
- Hih specificity

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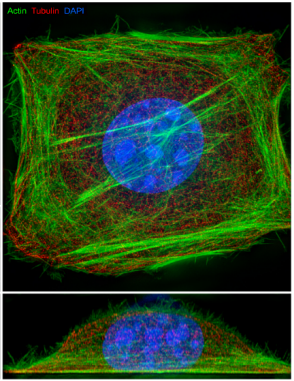

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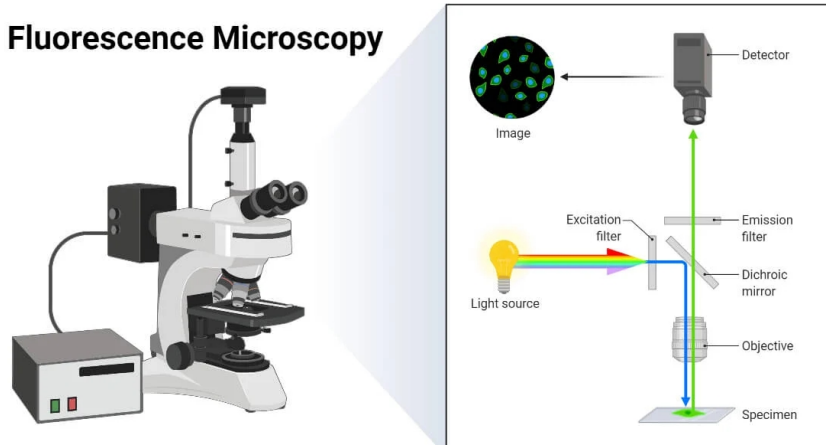
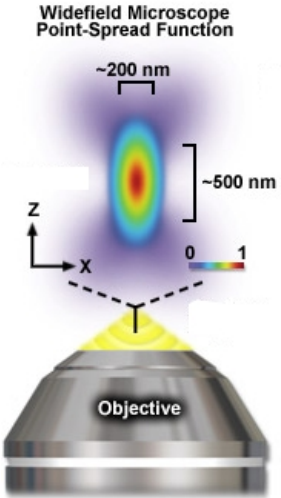
Fluorescence microscope



Point Spread Function (PSF)



Lens cannot focus emitted light into an infinitely small point

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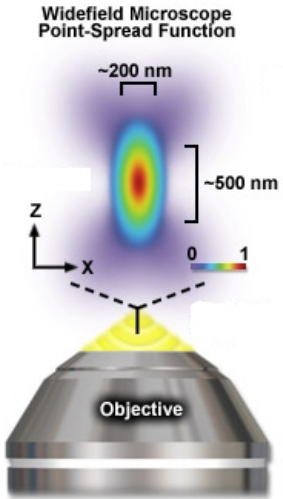

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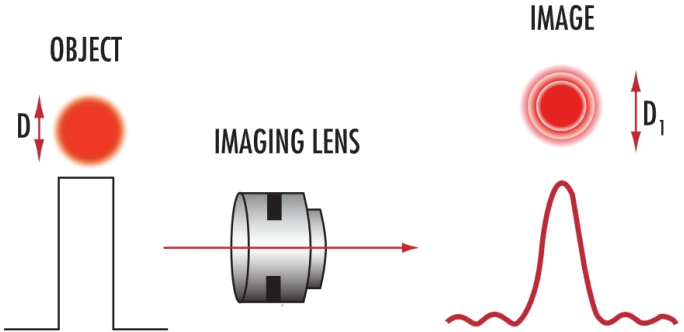
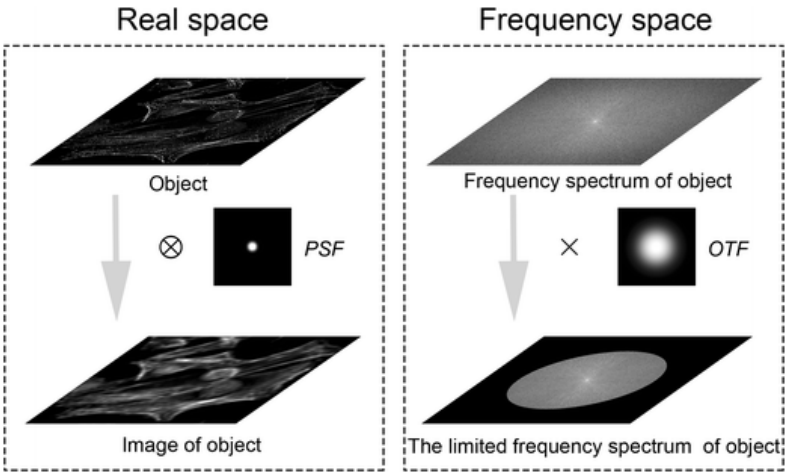


A point source is not imaged as a point, but as a "blob" called Point Spread Function (PSF)

PSF & OTF

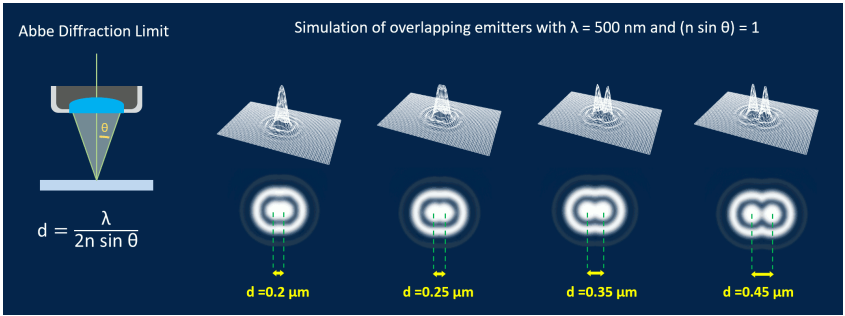

Real space
Frequency space
Object
PSF
Image of object
Frequency spectrum of object
OTF
The limited frequency spectrum of object

Resolution: the Abbe diffraction limit

The minimal distance d to resolve two point sources is given by

$$d = \frac{\lambda}{2NA} = \frac{\lambda}{2nsin(\theta)} \approx 200 - 250\,\text{nm}$$

where λ is the emission wavelength, NA is the numerical aperture, n is the refractive index.



Need of image reconstruction pipelines

To acquire more information, introduce extra information: time / space.

Processing steps are needed!

Modern algorithms: analyse datasets coming from advanced microscopy measurements!

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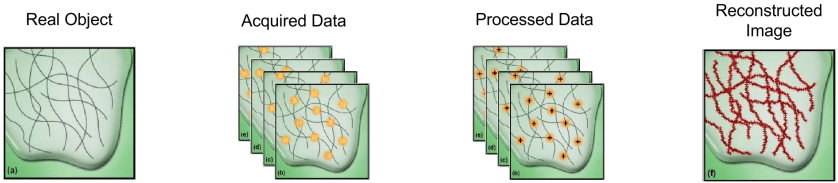
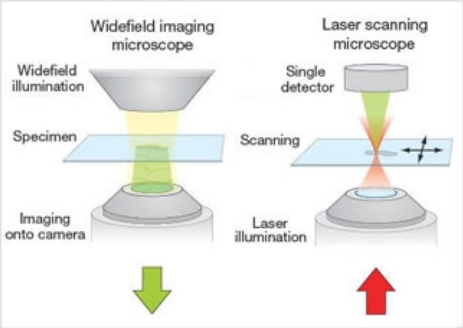


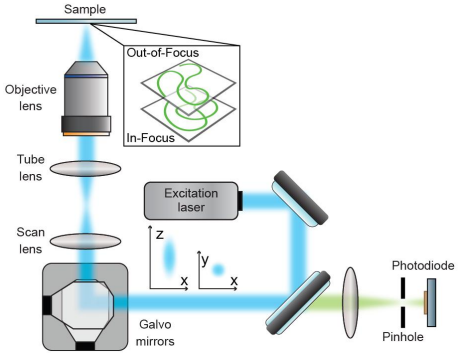
Modern algorithms: analyse datasets coming from advanced microscopy measurements!

Widefield VS Confocal



Confocal Laser Scanning Microscope (CLSM)

setup.pdf



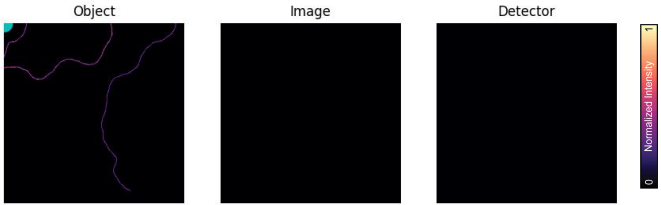
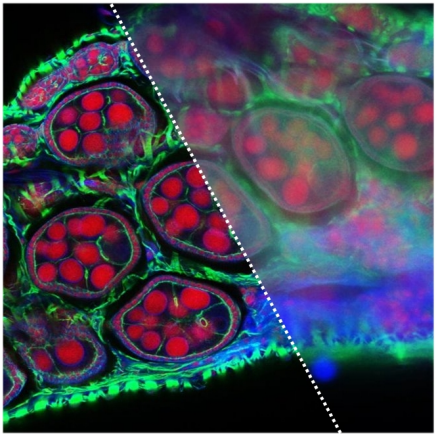


Image is formed point-by-point.
The single element detector collects a single intensity value (total photon count).

CLSM: pros and cons



CLSM exploits a closed pinhole to achieve:

- ✔ Super-resolution
- ✔ Optical sectioning

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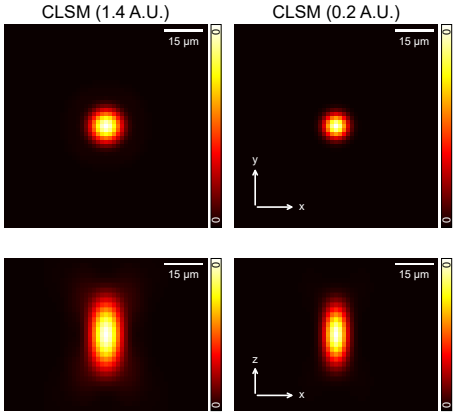

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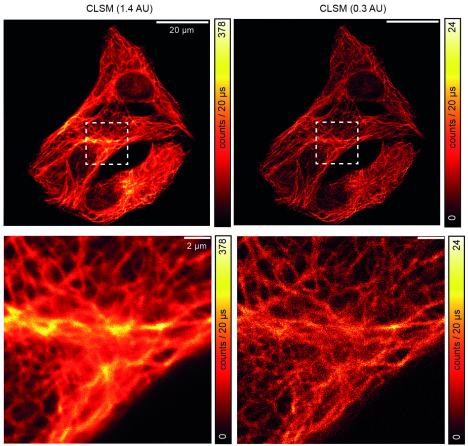

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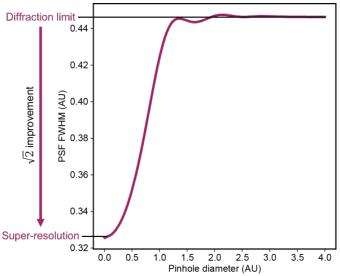

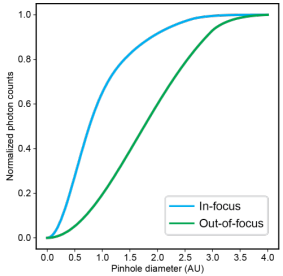

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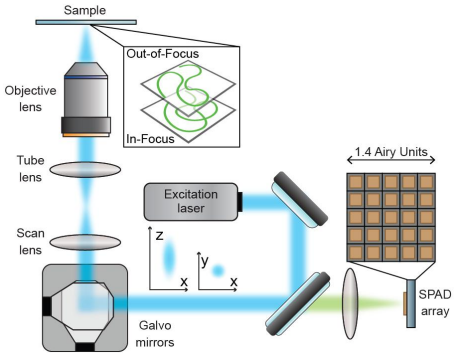
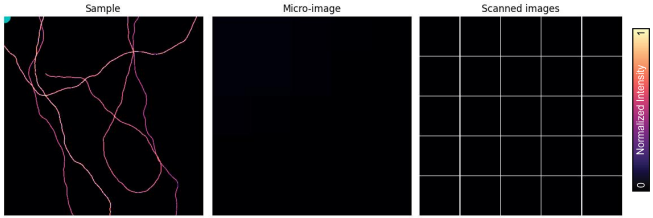
Image Scanning Microscopy (ISM)





ISM collects a 2D micro-image on the detector plane (x_d, y_d) for each scan point (x_s, y_s).
The 4D dataset is thus:

- A collection of (N_x, N_y) micro-images: $i(\boldsymbol{x}_s|\boldsymbol{x}_d)$
- A collection of N_{Ch} scanned images $i(\boldsymbol{x}_s|\boldsymbol{x}_d)$

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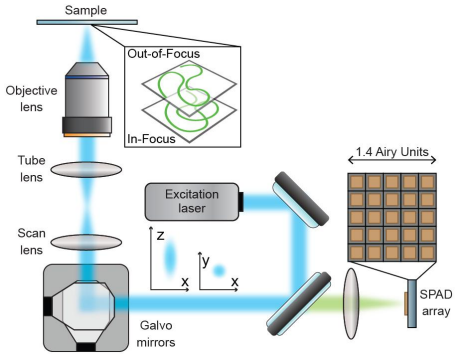

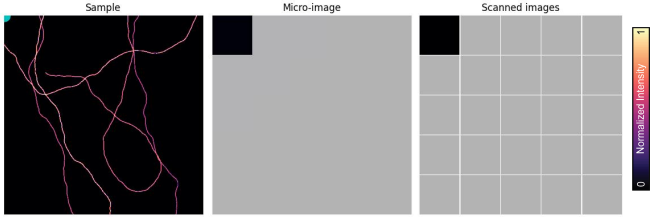

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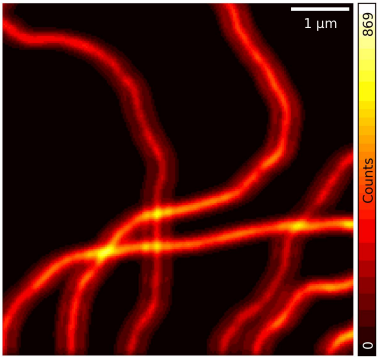
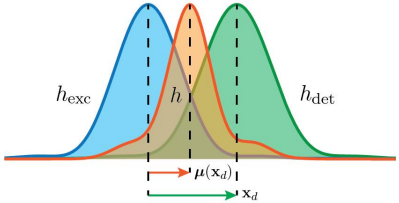
ISM PSFs

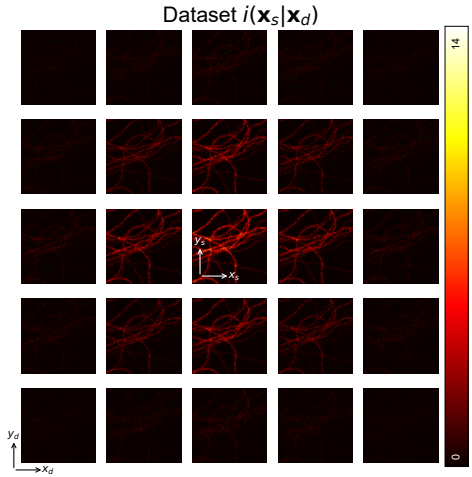


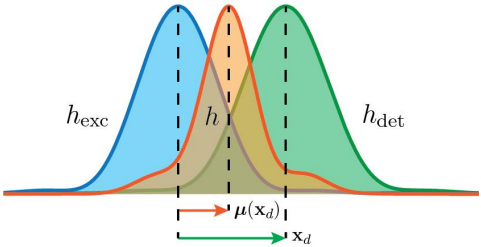


Each detector element $\boldsymbol{x}_d$ generates a scanned image with a different PSF:

$$h(\boldsymbol{x}_s|\boldsymbol{x}_d) = h_{\mathrm{exc}}(\boldsymbol{x}_s) \times h_{\mathrm{det}}(\boldsymbol{x}_s - \boldsymbol{x}_d)$$

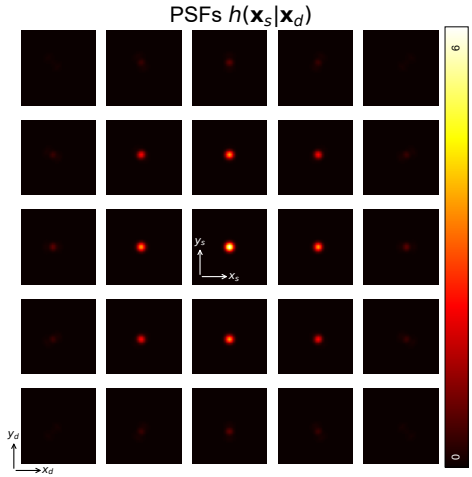
ISM PSFs

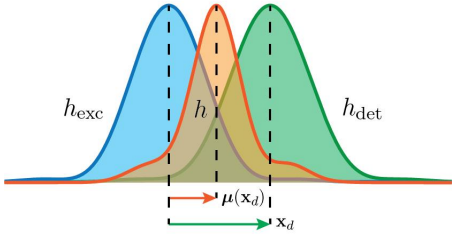




Each PSF is unique, so are the scanned images even though they represent the same sample $\rightarrow$ redundancy!

ISM PSFs



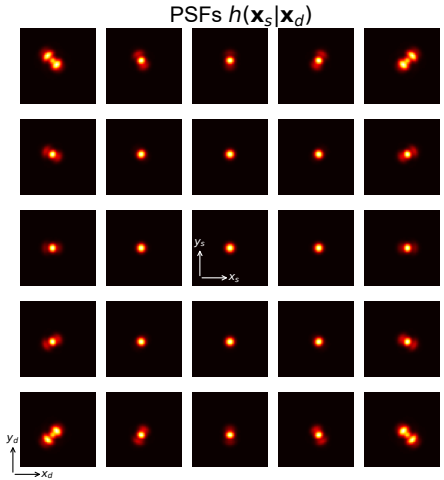


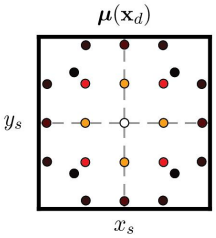
Each PSFs can be approximated as

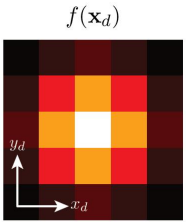
$$h(\boldsymbol{x}_s|\boldsymbol{x}_d) = h(\boldsymbol{x}_s - \mu(\boldsymbol{x}_d))$$

where $\mu(\boldsymbol{x}_d)$ is the *shift-vector* of the scanned image.

ISM Shift-vectors and Fingerprint







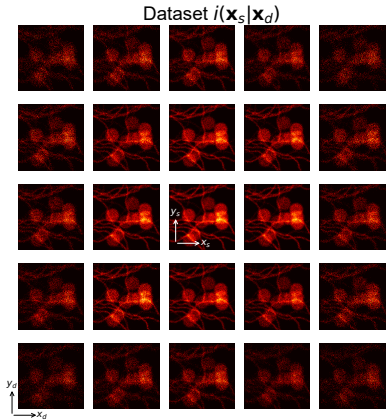
The shift-vectors $\mu(\boldsymbol{x}_d)$ are calculated as the shifts maximising similarities between images.

Fingerprint map $f(\boldsymbol{x}_d)$: sum of all the micro-images collected during the scan

ISM datasets

Main characteristics of ISM dataset:

- High dimensional data (4D or more)
- More spatial information encoded
- No read-out noise $\rightsquigarrow$ pure Poisson
- High temporal resolution



[A. Zunino et al., Nature Photonics volume 17 (2023)]

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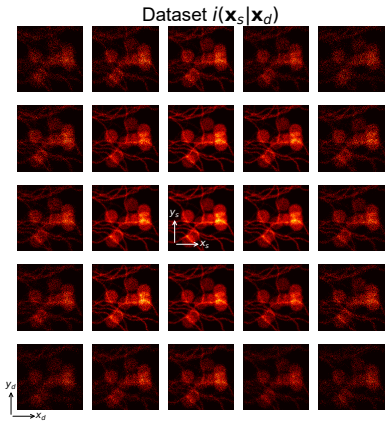

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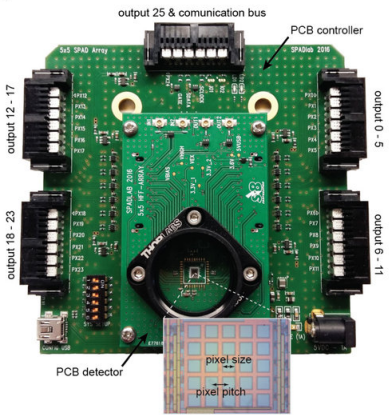

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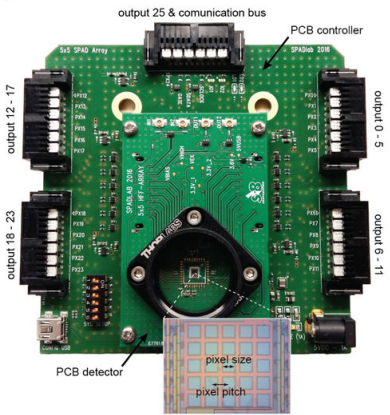

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Linear Space-Invariant (LSI) System

A map $S : f \mapsto g$ is a Linear Space-Invariant (LSI) System if:

1. **Linearity**

$$S[af_1 + bf_2] = a\,S[f_1] + b\,S[f_2]$$

2. **Shift-invariance**

$$S[f(x - x_0)] = g(x - x_0)$$

Any input function can be written as

$$f(x) = \int_{\mathbb{R}} f(\lambda)\,\delta(x - \lambda)\,d\lambda$$

Thus

$$g(x) = \int_{\mathbb{R}} f(\lambda)\,h(x - \lambda)\,d\lambda = (f * h)(x)$$

where $h(x) = S[\delta(x)]$ is the impulse response.

Image Formation in Microscopy

- A microscope is modeled as a **linear shift-invariant (LSI)** system.
- The observed image $\mathbf{y}[\mathbf{r}]$ is the convolution of the object's fluorophore distribution $\mathbf{u}[\mathbf{r}]$ and the system's **Point Spread Function (PSF)**.
- The PSF represents the response of the imaging system to a point source.

CLSM Forward Model

The forward model in confocal imaging is

$$\mathbf{y} = \mathcal{P}(\mathbf{h} * \mathbf{u}) = \mathcal{P}(\mathbf{A}\mathbf{u})$$

where:

- $\mathbf{y} \in \mathbb{N}^N$ is the measured image
- $\mathbf{u} \in \mathbb{R}_+^N$ is the unknown object
- $\mathbf{A} \in \mathbb{R}^{N \times N}$ is the Toeplitz matrix computing the convolution with the PSF h
- $\mathcal{P}$: Poisson noise

ISM Forward Model

The forward model in ISM imaging is

$$\mathbf{y}_d = \mathcal{P}(\mathbf{h}_d * \mathbf{u}) = \mathcal{P}(\mathbf{A}_d \mathbf{u}), \qquad d = 1, \dots, 25$$

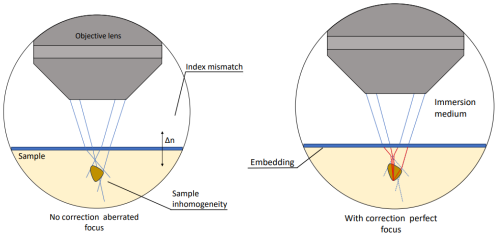
where:

- $\mathbf{y}_d \in \mathbb{N}^N$ is the measured image from detector d with $p = n_1 \times n_2$ pixels
- $\mathbf{u} \in \mathbb{R}_+^N$ is the unknown object
- $\mathbf{A}_d \in \mathbb{R}^{N \times N}$ is the Toeplitz matrix to compute the convolution with the PSF h_d
- $\mathcal{P}$: Poisson noise

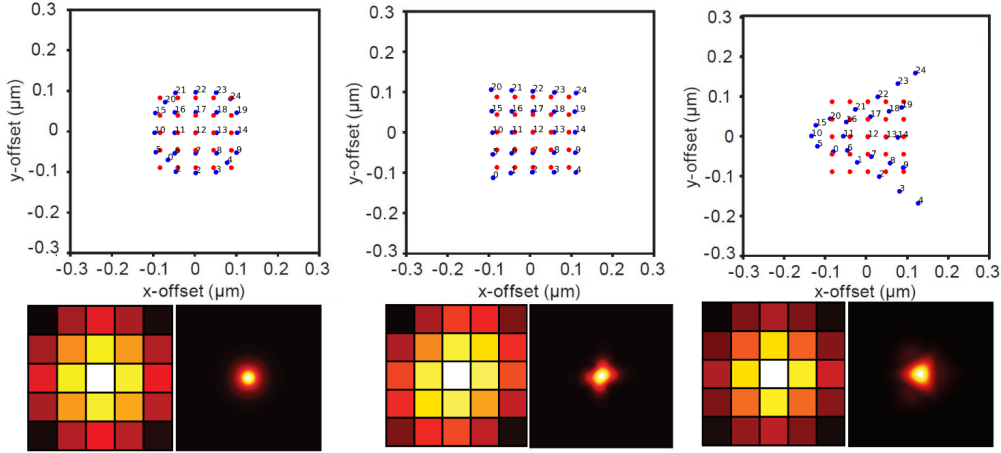
Common Sources of Aberrations

These effects distort the PSF shape:

- **Spherical Aberration:** Caused by refractive index mismatches between the immersion medium and the sample.
- **Astigmatism:** Often used intentionally in 3D super-resolution microscopy to encode depth.
- **Coma:** Results from misalignment of optical elements.



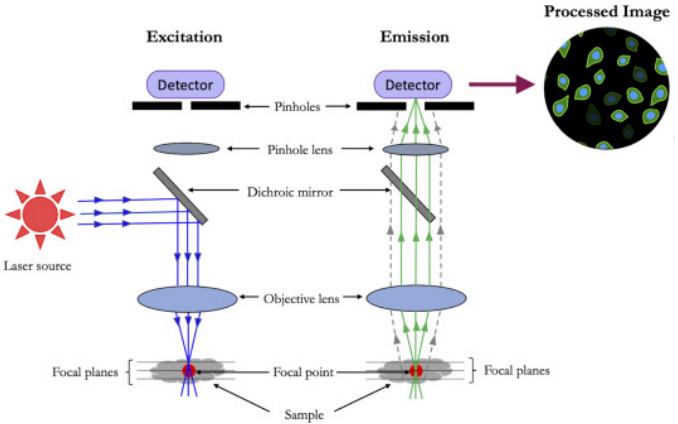
Aberrations examples on ISM



References

- [A. Zunino et al., *"Open-source tools enable accessible and advanced image scanning microscopy data analysis"*, Nature Photonics volume 17, pp 457–458 (2023)]
- [A. Zunino et al., *"Structured detection for simultaneous super-resolution and optical sectioning in laser scanning microscopy"*, Nature Photonics volume 19, pp 888–897 (2025)]
- [B. Richard and E. Wolf, *Electromagnetic diffraction in optical systems II*, Proceedings of the Royal Society of London. A. Mathematical and Physical Sciences (1959)]
- [Y. Liu, V. Stergiopoulou et al, *"Revisiting PSF models: unifying framework and high-performance implementation"*, arxiv DOI, (2025)]
- [F. Fersini et al, *Wavefront estimation through structured detection in laser scanning microscopy*, Biomed. Opt. Express (2025)]

Confocal Microscope



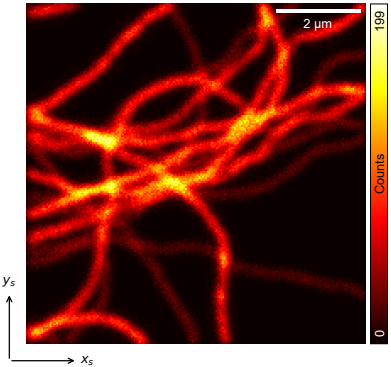
Photon resolved microscopy

photon-resolved microscopy
multi-parameter list of photons

Id	x_d	x_d	t_{abs}	τ
1	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
N	-	-	-	-

spatial tag — temporal tag

conventional microscopy
2D photon histogram

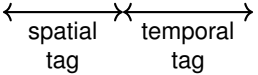


Photon resolved microscopy

photon-resolved microscopy

multi-parameter list of photons

Id	$\boldsymbol{x}_d$	$\boldsymbol{x}_d$	t_{abs}	τ
1	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
N	-	-	-	-



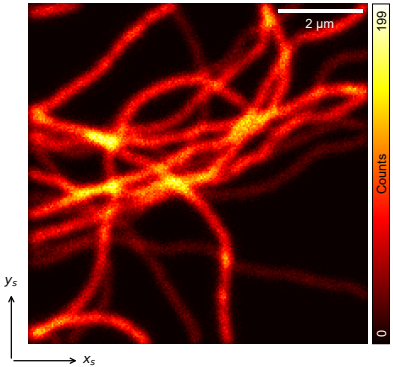
Photon resolved microscopy

photon-resolved microscopy
multi-parameter list of photons

Id	x_d	x_d	t_{abs}	τ
1	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
N	-	-	-	-

spatial tag | temporal tag

conventional microscopy
2D photon histogram



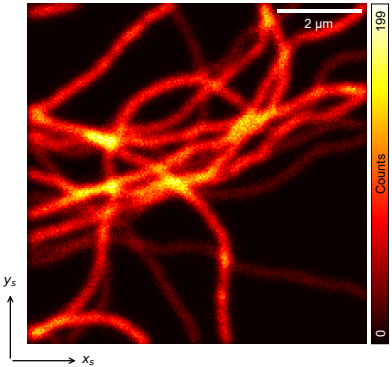
Photon resolved microscopy

photon-resolved microscopy
multi-parameter list of photons

Id	$\boldsymbol{x}_d$	$\boldsymbol{x}_d$	t_{abs}	τ
1	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
N	-	-	-	-

spatial tag | temporal tag

conventional microscopy
2D photon histogram



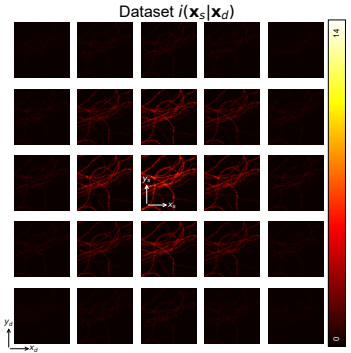
Photon resolved microscopy

photon-resolved microscopy
multi-parameter list of photons

Id	$\boldsymbol{x}_d$	$\boldsymbol{x}_d$	t_{abs}	τ
1	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
N	-	-	-	-

spatial tag | temporal tag

Image Scanning Microscopy
4D photon histogram



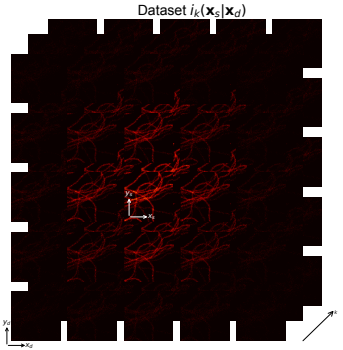
Photon resolved microscopy

photon-resolved microscopy
multi-parameter list of photons

Id	$\boldsymbol{x}_d$	$\boldsymbol{x}_d$	t_{abs}	τ
1	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
-	-	-	-	-
N	-	-	-	-

spatial tag | temporal tag

Image Scanning Microscopy
5D photon histogram



Lateral Resolution ($z = 0$)

The intensity PSF at the focal plane ($z = 0$) is determined by the circular aperture geometry. Using Bessel functions, the lateral intensity profile is:

$$h(r, 0) = \left| \int_0^1 J_0(k\mathrm{NA}r\rho)\rho d\rho \right|^2 = \left| \frac{J_1(k\mathrm{NA}r)}{k\mathrm{NA}r} \right|^2$$

- The first zero of $J_1(x)$ occurs at $x_0 \approx 3.8317$.
- Setting $k\mathrm{NA}r = x_0$ and solving for r gives the **Rayleigh Criterion**:

Lateral Limit

$$r_{\min} = 0.61 \frac{\lambda}{\mathrm{NA}}$$

Axial Resolution ($r = 0$)

Resolution along the optical axis (z) is defined by the depth of focus. Along the axis ($r = 0$), the integral simplifies to a sinc-squared function:

$$h(0, z) = \left(\frac{2n}{kz\mathrm{NA}^2} \right)^2 \sin^2 \left(\frac{kz\mathrm{NA}^2}{4n} \right)$$

- The first zero of the cardinal sine function occurs at π.
- Solving $\frac{kz\mathrm{NA}^2}{4n} = \pi$ for z gives:

Axial Limit

$$z_{\min} = \frac{2\lambda n}{\mathrm{NA}^2}$$

Note: Axial resolution scales with $1/NA^2$, making it much more sensitive to Numerical Aperture than lateral resolution.