Cell biophysics of fluorescent probes for super-resolution optical microscopy

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Superresolution microscopy

- **PALM** - photoactivation localization microscopy, ~20 nm lateral resolution, fixated samples only, low intensity staining sufficient

- **STORM** - stochastic optical reconstruction microscopy

- **STED** - stimulated emission depletion microscopy, ~60 nm lateral resolution, living cells, high intensity needed (expression high enough)

- **SIM** - structured illumination microscopy, ~ 100 nm lateral resolution, fixated samples only
PALM/STORM - Principle

• Only small fraction of fluorescence molecules population is activated

• The localization is calculated by mathematical algorithms

• Result: table of numbers with precise location of the fluorophore molecule
Photoactivation, photoconversion and photoswitching

**Photoactivation irreversible**

- **Dark**
- **Fluorescent**
- **Bleached**

**UV** → **VIS**

**Photoconversion irreversible**

- **Fluorescent 1**
- **Fluorescent 2**
- **Bleached**

**UV** → **VIS**

**Photoactivation reversible**

- **Dark**
- **Fluorescent**

**UV** ↔ **VIS**

**Photoconversion reversible**

- **Fluorescent 1**
- **Fluorescent 2**
Photoconversion and photoswitching

Modified from Hoppins et al., 2007
PALM/STORM
PALM/STORM
A. Löschberger, M. Sauer, University of Würzburg, Germany
PALM - Zeiss ELYRA P.1

td-EOS-TOMM20

80 nm
PALM - Double staining

By: T. Spacek
PALM/STORM

• Practical tips&tricks:

• Optimal for membrane bound or filamentar structures

• Acquisition of one image takes ~ 20 min

• Acquisition in TIRF mode

• No crop or rotation of the image

• One image ~ 1.5 GB

• Temperature stability is crucial

• Atto antibodies
PALM/STORM

• PROs
  • Highest lateral and axial resolution
  • No need for high expression of proteins

• CONs
  • Fixated samples only
  • Longer acquisition time
  • to find proper image for acquisition - tricky
STED - Principle

Modified from Udo J. Birk: Super-Resolution Microscopy
STED - Principle

\[ p_{STED} = \exp\left(-\frac{I_{STED}}{I_{sat}}\right) \]

\[ d_{STED} = \frac{\lambda}{2NA} \frac{1}{\sqrt{1 + I_{STED}/I_{sat}}} \]


Udo J. Birk: Super-Resolution Microscopy
STED - Principle

Udo J. Birk: Super-Resolution Microscopy
Brian R Patton et al 2015 Methods Appl. Fluoresc. 3 024002
STED - Principle

Udo J. Birk: Super-Resolution Microscopy
STED - Leica SP8

nuclear pore complex protein (nup153)

By: D. Smeets
Visualization of outer mitochondrial membrane

AKAP-eYFP
Calsequestrin

Anti-CSQ2, secondary Ab: DyLight550
rat ventricular myocyte

By: A. Zahradnikova
Calsequestrin

Anti-CSQ2, secondary Ab: DyLight550
rat ventricular myocyte

By: A. Zahradnikova
3D-STED

Z-resolution

T-tubules, mCling-ATTO635 Rat ventricular myocyte

By: A. Zahradnikova
STED - Leica SP8

confocal  STED

WFS1-GFP

10 μm
STED - Double staining

Tubulin

TOMM20

By: D. Smeets
STED

**PROs**

- Acquisition of living cells possible
- In one image STED/non STED
- Shorter acquisition time when compared to PALM/STORM

**CONs**

- Lower lateral and axial resolution when compared to PALM/STORM
- High expression of proteins necessary
- Autofluorescence
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Superresolution needs supereroptimization!