Detectors in Light Microscopy: Instrumentation Aspect

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www.embl.de/almf

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Overview

- ALMF presentation
- Fluorescence microscopy
- Instrumentation
- Application examples
- Detector wish list

ALMF presentation

Advanced Light Microscopy Facility (ALMF)

Head of Facility

Rainer Pepperkok

Sabine Reither

Beate Neumann

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Yury Belyaev

Volker Hilsenstein

Christian Tischer

Marko Lampe

High-Throughput Microscopy

Advanced Light Microscopy

EMBL

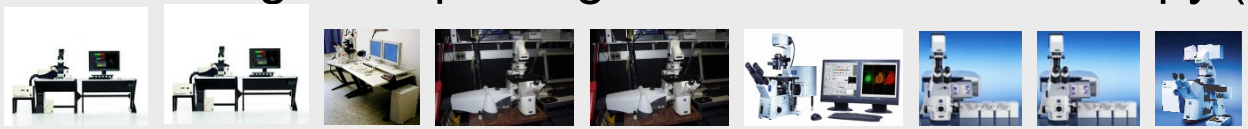
ALMF

ALMF missions

- Train and support users
- Develop new application protocols
- Organize and teach microscopy courses at all levels
- Design, set-up, test and offer state-of-the-art equipment
- Host and support short and long-term visitors

Available equipment in the ALMF

Laser scanning and spinning disk confocal microscopy (9x)



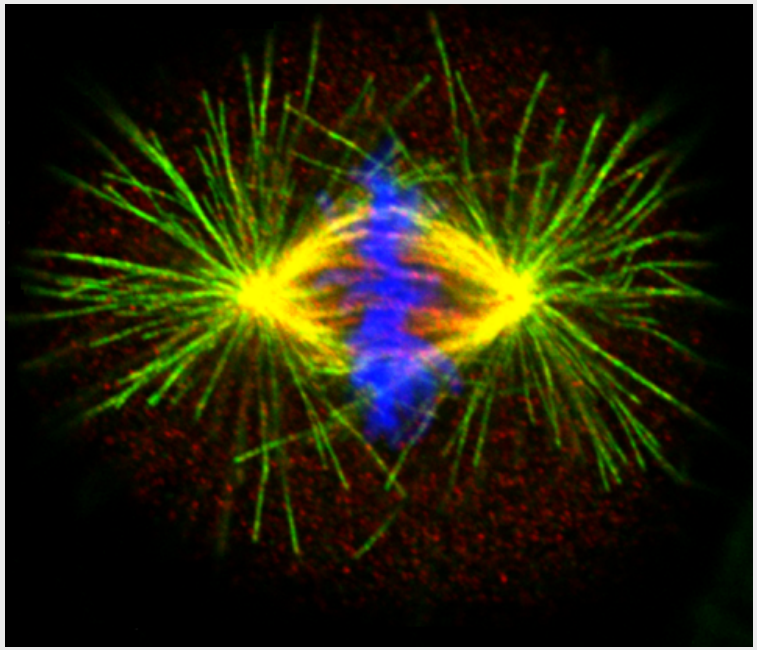
Widefield microscopy including GSD and deconvolution (9x)

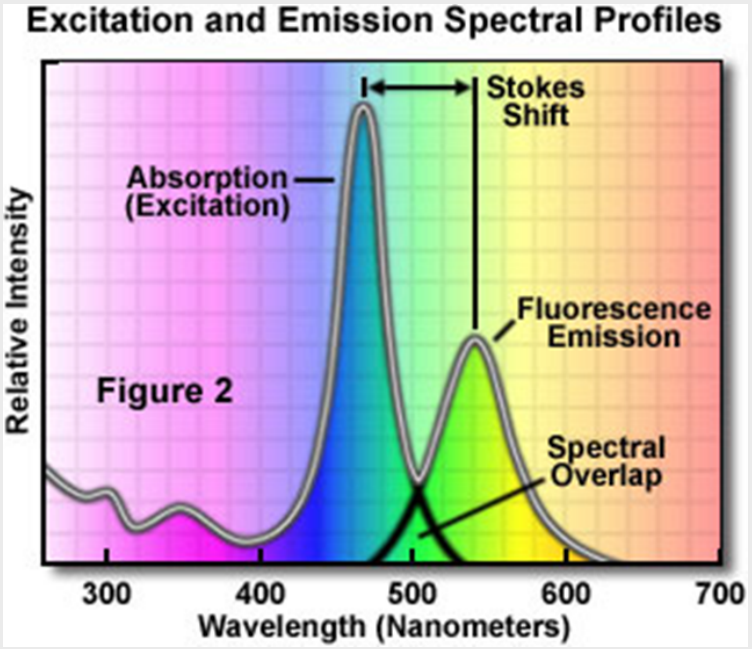
High-throughput microscopy (9x)

Image restoration and analysis workstations (3x)

Fluorescence microscopy

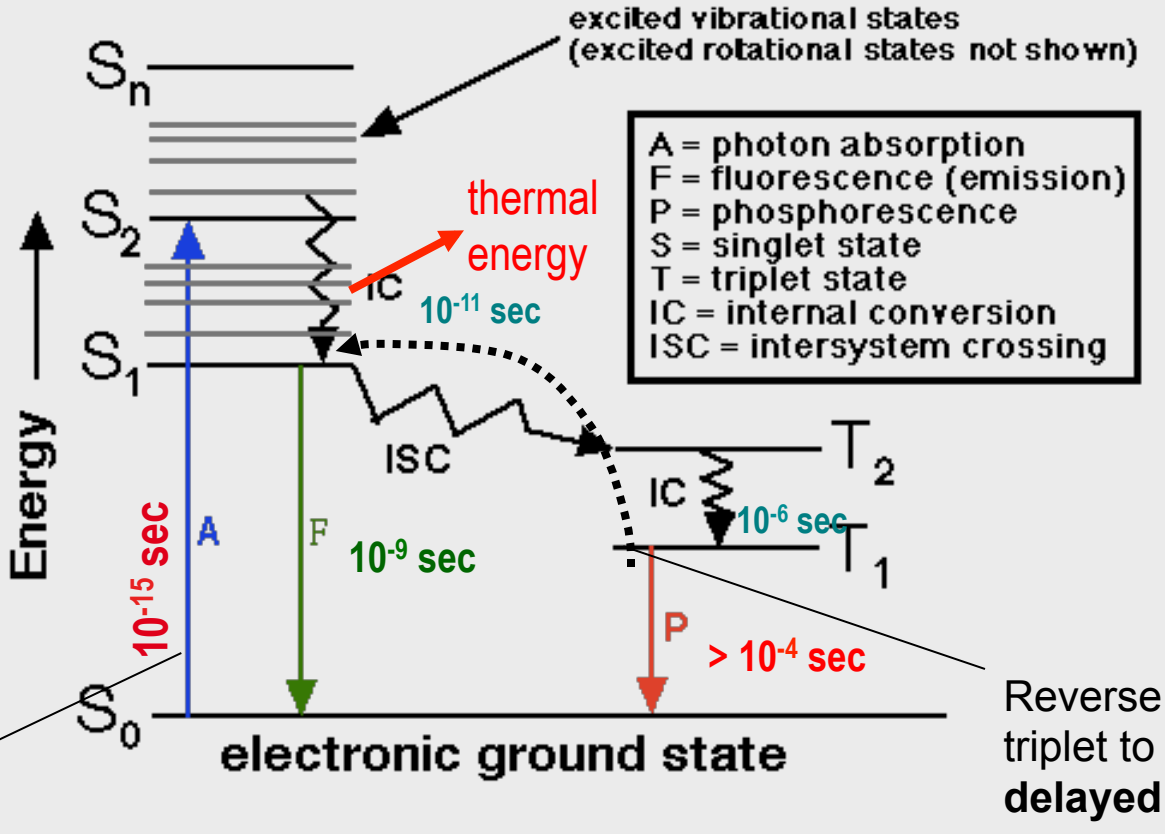
Fluorescence microscopy





- Specificity (molecules can be specifically labelled)
- Sensitivity (single molecule detection is possible)
- Can report on the environment of the labelled molecule

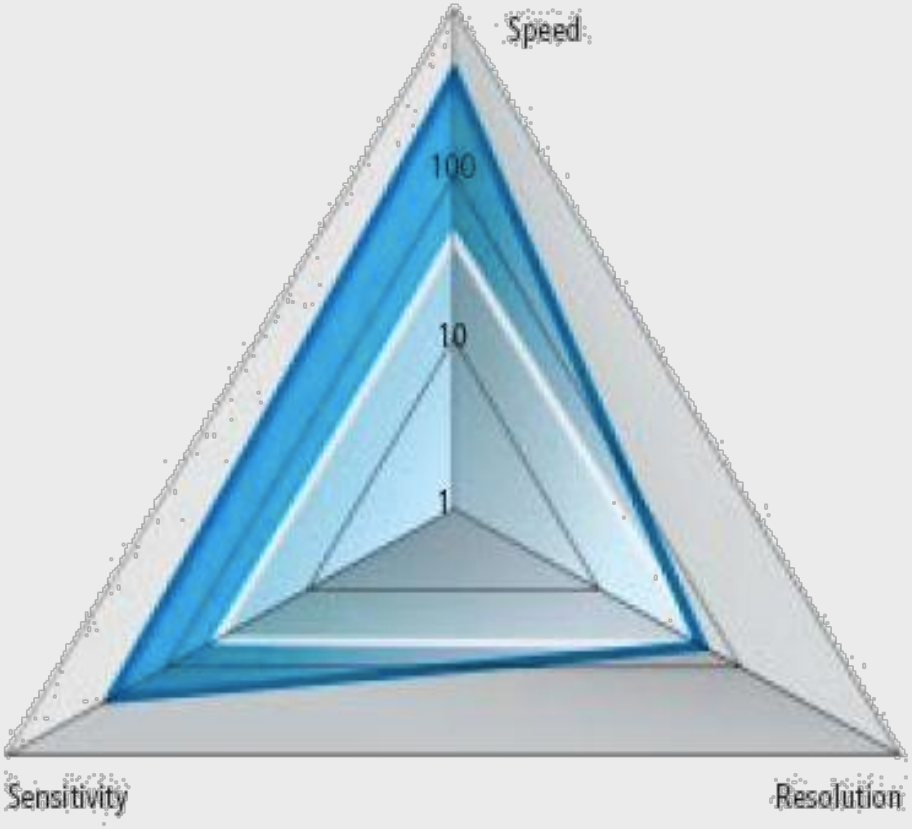
Jablonski diagram



Reverse transition from triplet to singlet, causing **delayed** fluorescence

Frank-Condon principle:
Absorption is fast enough to prevent any change in configuration of the fluorochrome molecule

Eternal triangle of compromise

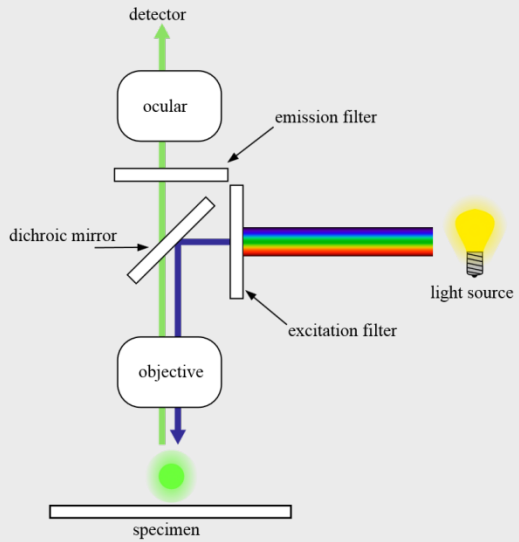


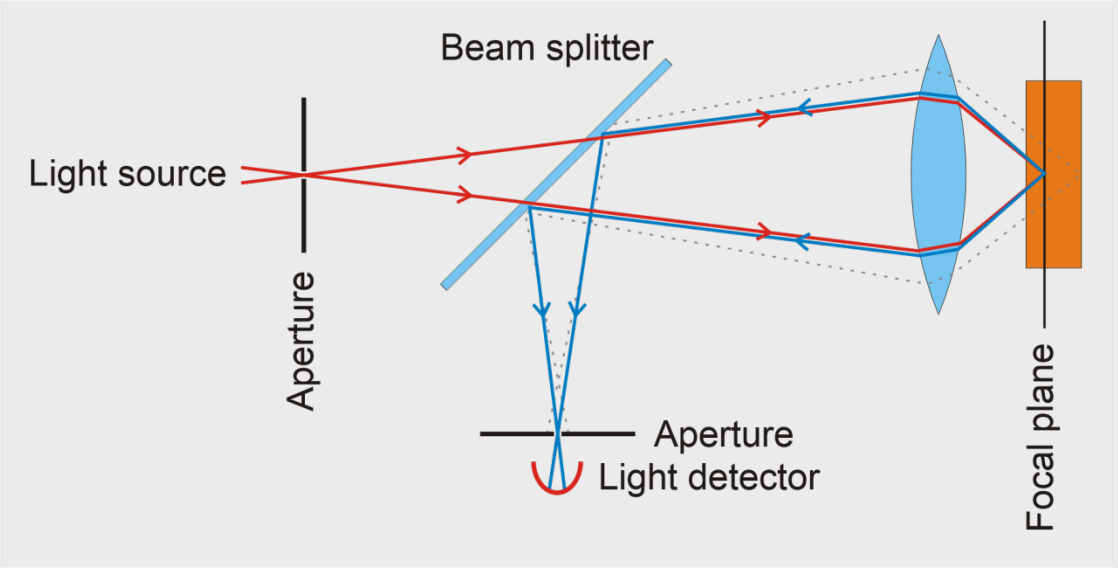
Trade-off among resolution, sensitivity and speed

Picture adapted from www.zeiss.de

Instrumentation

Wide field vs confocal microscopy





Wide field microscopy:

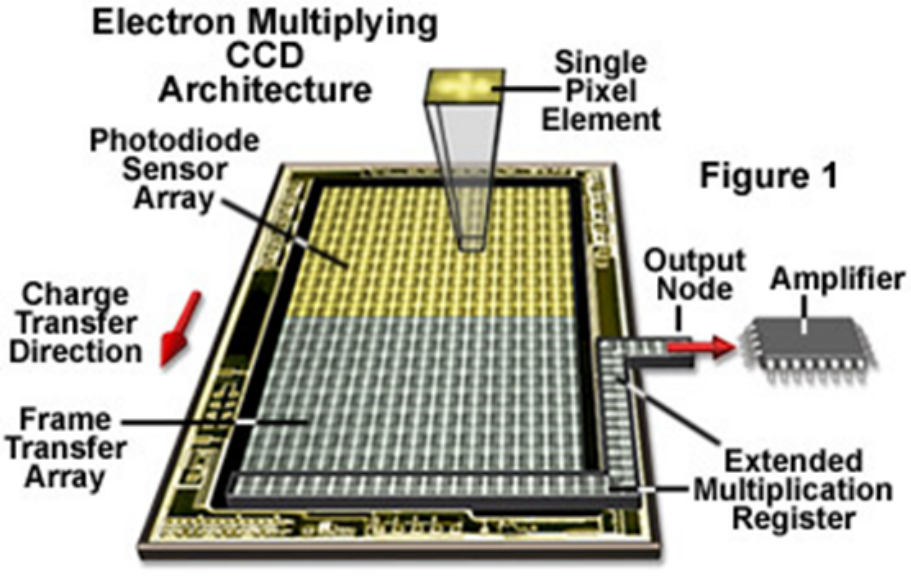
- Fast and long-term time-lapse of thin samples
- Fast ~ 30 fps
- ~10000 photons/pixel

Confocal microscopy:

- Imaging for high resolution 3D rendering
- Slow ~ 1 fps
- ~10 photons/pixel

Adapted from www.wikipedia.org

Cameras (CCD, EMCCD, sCMOS)

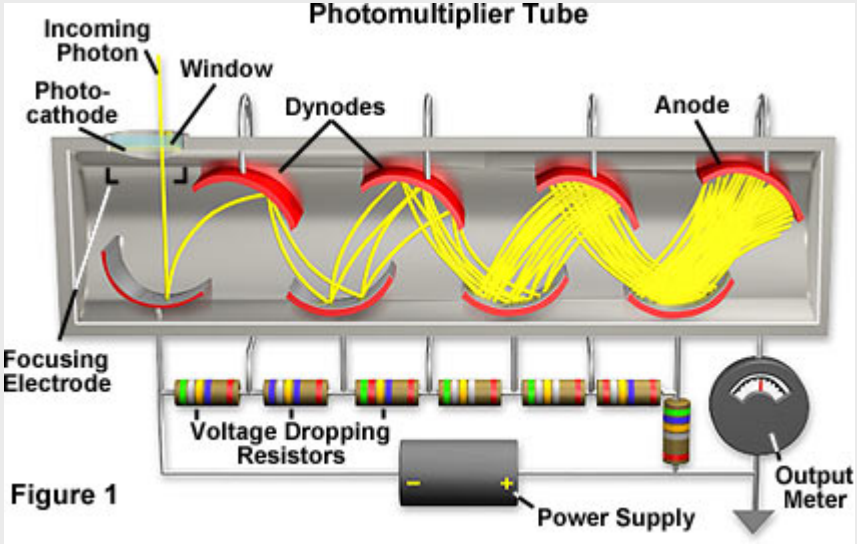


Widefield and spinning disk microscopy

EMCCD for single molecule applications, localization based super resolution

- Quantum efficiency up to 90%
- High dynamic range
- High linearity (accurate quantification)

Photomultiplier

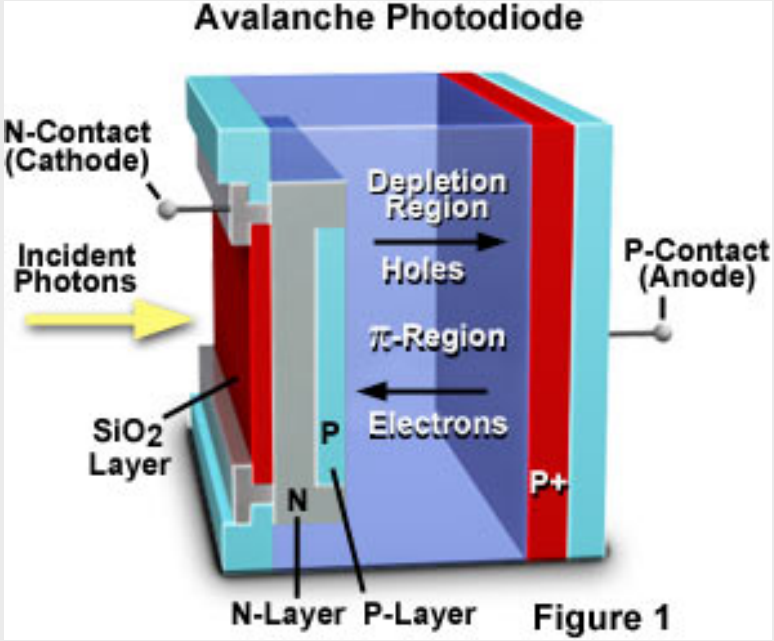


Laser Scanning Confocal microscopy

New HyD, GaAsP detectors for single photon applications, FCS, FLIM

- Quantum efficiency 20% - 50%
- High dynamic range
- Nonlinear gain on voltage (needs calibration)

Avalanche photodiode

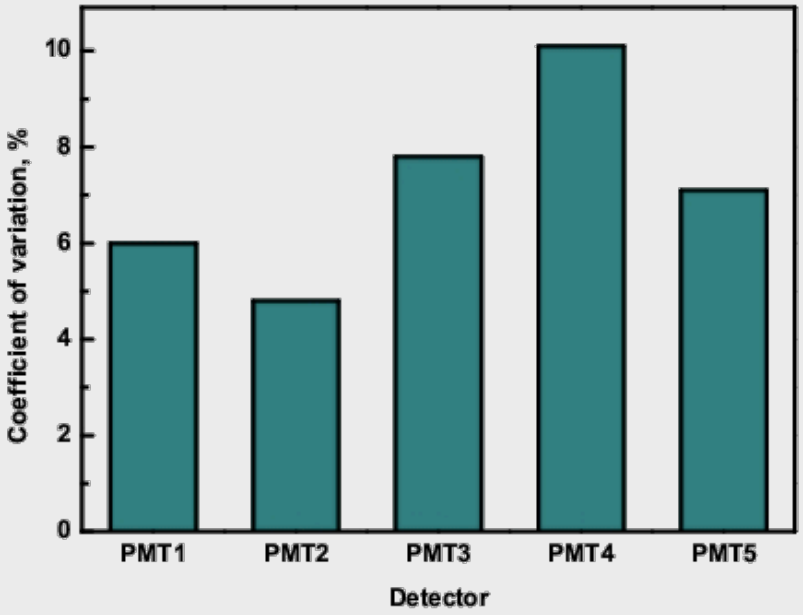


Laser Scanning
Confocal microscopy

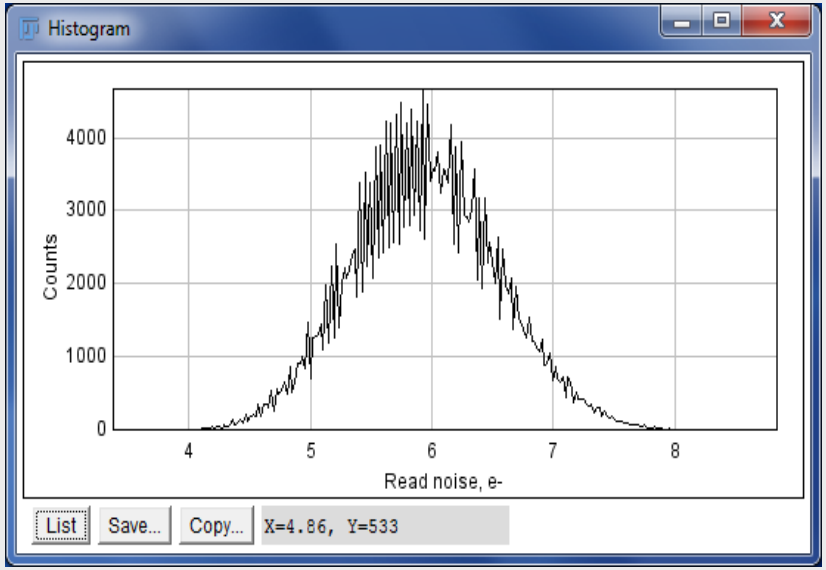
Imaging at very low fluorescence level, more often for single molecule/photon counting, FCS, FLIM

- Quantum efficiency up to 90%
- Low dynamic range
- Dark current might be issue

ImageJ macros for detectors evaluation



Comparison of PMTs in Leica SP5 confocal (505-530 nm range).

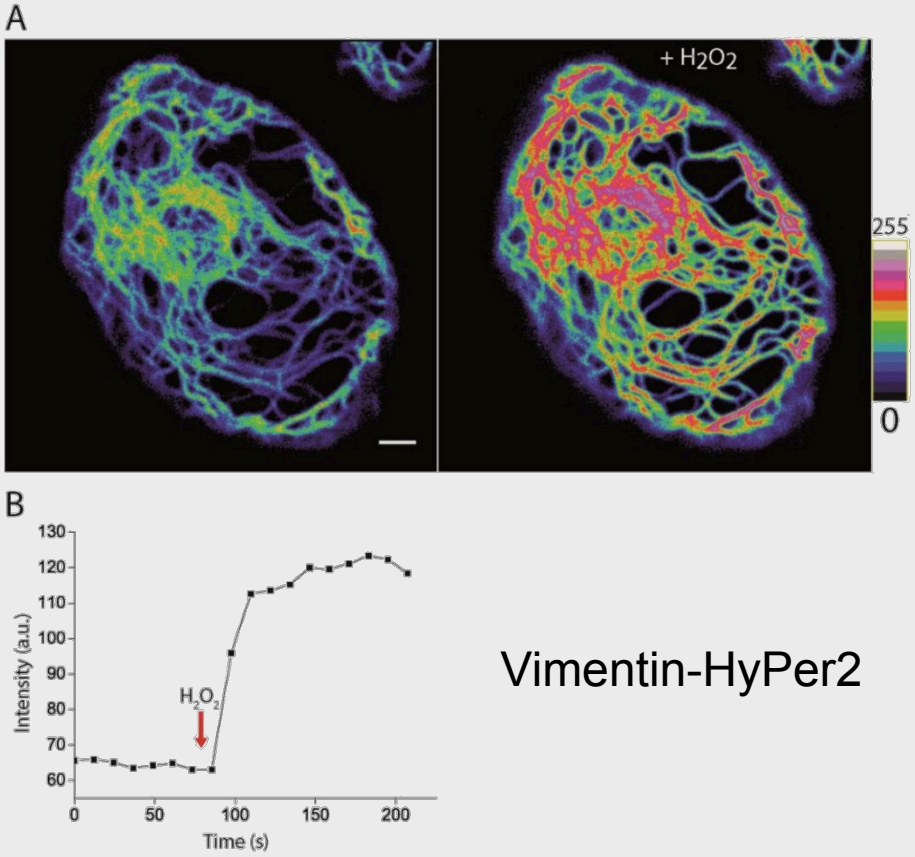


Read noise of CoolSNAP camera in (specs value of 6 e^-).

(www.embl.de/services/core_facilities/almf/services/downloads/index.html)

Application examples

Live-cell STED microscopy of biosensors



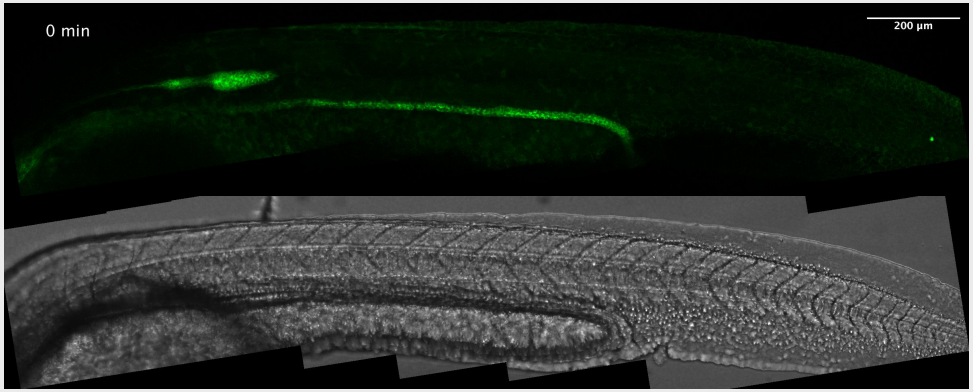
Vimentin-HyPer2

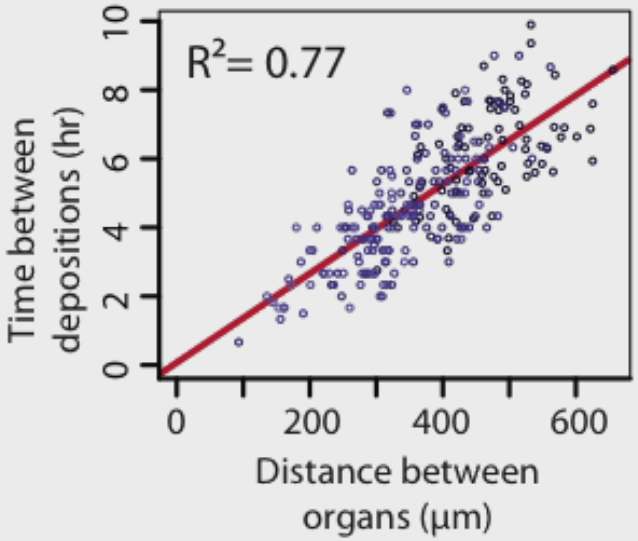
Microscope:
Leica SP8 STED 3X
Methods:
STED, deconvolution
Sample:
HeLa-Kyoto cells
Software:
ImageJ, Huygens

Study of H_2O_2 production within a living cell with high temporal and spatial resolution.

Mishina, N. M., et al. (2015). Nano Lett. 15(5): 2928-32

Organ patterning in zebrafish lateral line





Microscope:

PE Ultraview VoX, ERS

Methods:

Timelapse imaging with multi-positions

Sample:

Cldnb::lynGFP transgenic zebrafish embryos (28hpf to 48hpf)

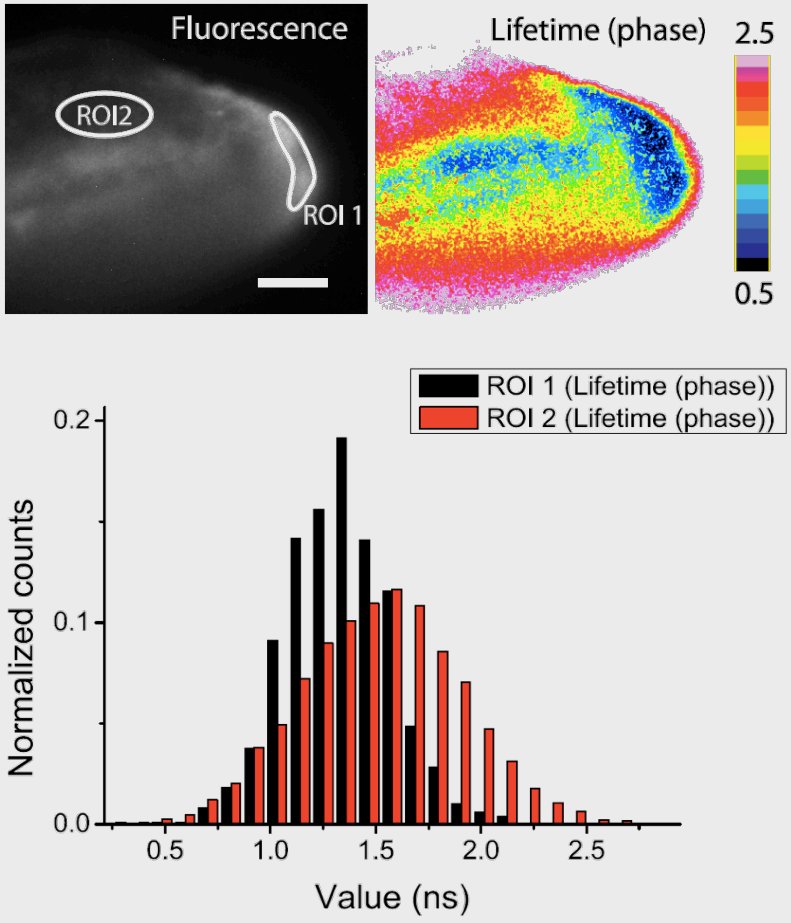
Software:

Volocity, Fiji, R

Organ deposition timing is the key determinant in organ patterning

Sevi Durdu, EMBL Heidelberg

Inflammation driven H_2O_2 production



Microscope:

Widefield Lambert LIFA

Methods:

FLIM

Sample:

Zebrafish larvae

Software:

LIFA, ImageJ

HyPer-3 genetically encoded sensor for *in vitro* and *in vivo* measurement of intracellular H_2O_2

Bilan, D. S., et al. (2012). ACS Chemical Biology 8(3): 535-542.

Detector wish list

Detector wish list

- Array (or line) detector with 1024x1024(128) pixels
- QE like EMCCD, i.e., 90% across full spectrum
- Micro time: sub-ns time resolution for FLIM-like applications
- Macro time: 100 000 frames/s readout time (full frame)
- ROI and/or random pixels readout
- Low readout and dark noise
- Pixel size like in state-of-the art sCMOS, i.e., 6.5 um
- Full visible spectrum and maybe NIR (400-1000 nm)

Acknowledgements

The ALMF Team

EMBL, Heidelberg

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