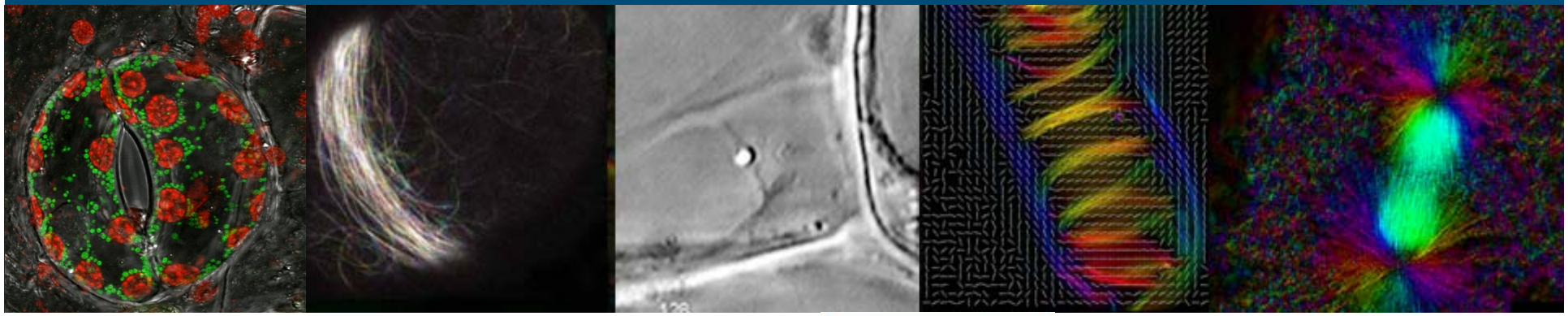
Applications of spinning disk confocal

Norbert de Ruijter
Cell Biology PSG - WLMC



WAGENINGEN UNIVERSITY
WAGENINGEN UR

WLMC
Wageningen Light Microscopy Center

13 June 2013

Outline

- Compare principle of Point scanner (CLSM) versus Spinning Disk Confocal (CSDM)
- Equipment
- SD Yokogawa Set-up with FRAP-PA (Roper)
- SD Yokogawa Set-up with 37°C-CO_2 (Andor)
- Applications / Examples

Spinning Disk Microscopy allows Live Cell Imaging

MT (red) polymerization drives tubular extension from ER (green) , Akhmanova et al, 2011.

The Challenge of Prolonged Live Cell Imaging

Key Parameters (XYZT imaging)

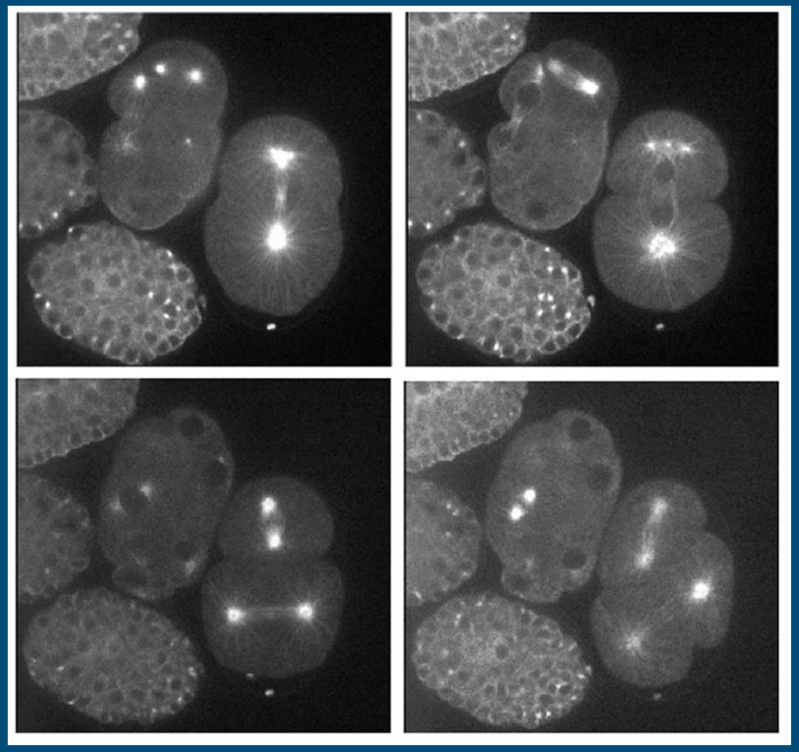
Prevent drift (XYZ)

Temporal resolution (frame rate)

Spectral resolution

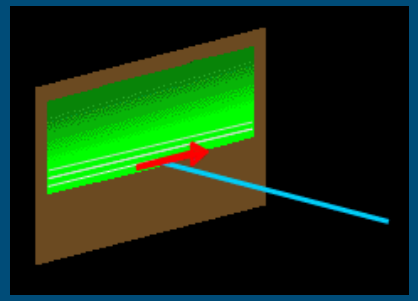
Low photobleaching

Low phototoxicity



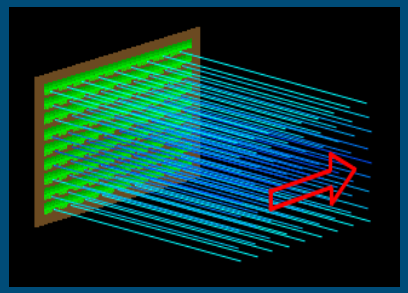
Confocal Laser scanning vs Spinning Disk Confocal

Single beam (point scanning)

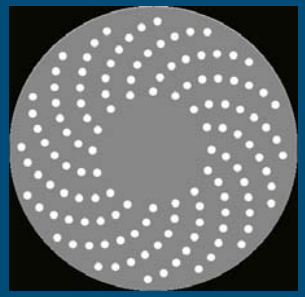


moving the spot of the light

Multiple beams



Nipkow Disk



moving the pinholes creates many spots /time

Disadvantages:

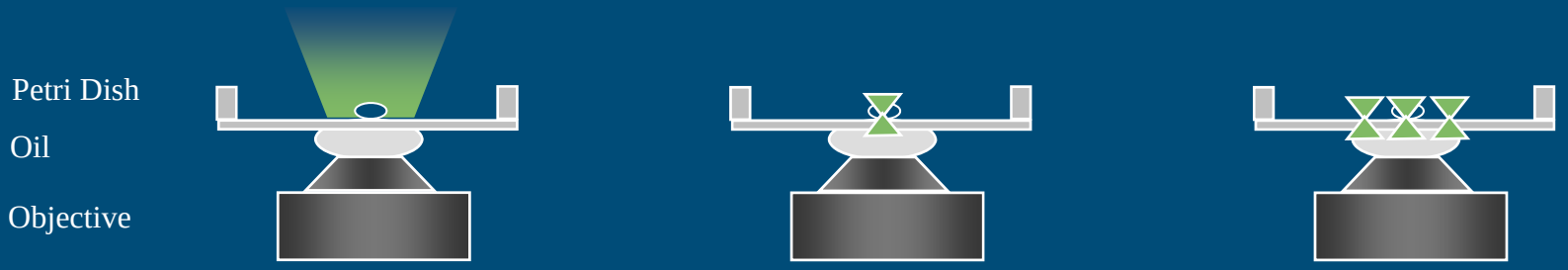
Relative slow image acquisition
High level of photobleaching and phototoxicity

Advantages:

- High frame rates
- Increased illumination time per pixel
- Little photobleaching / photodamage
- Allows prolonged imaging

Fluorescence Illumination

Illumination in widefield microscopy and confocal microscopy:

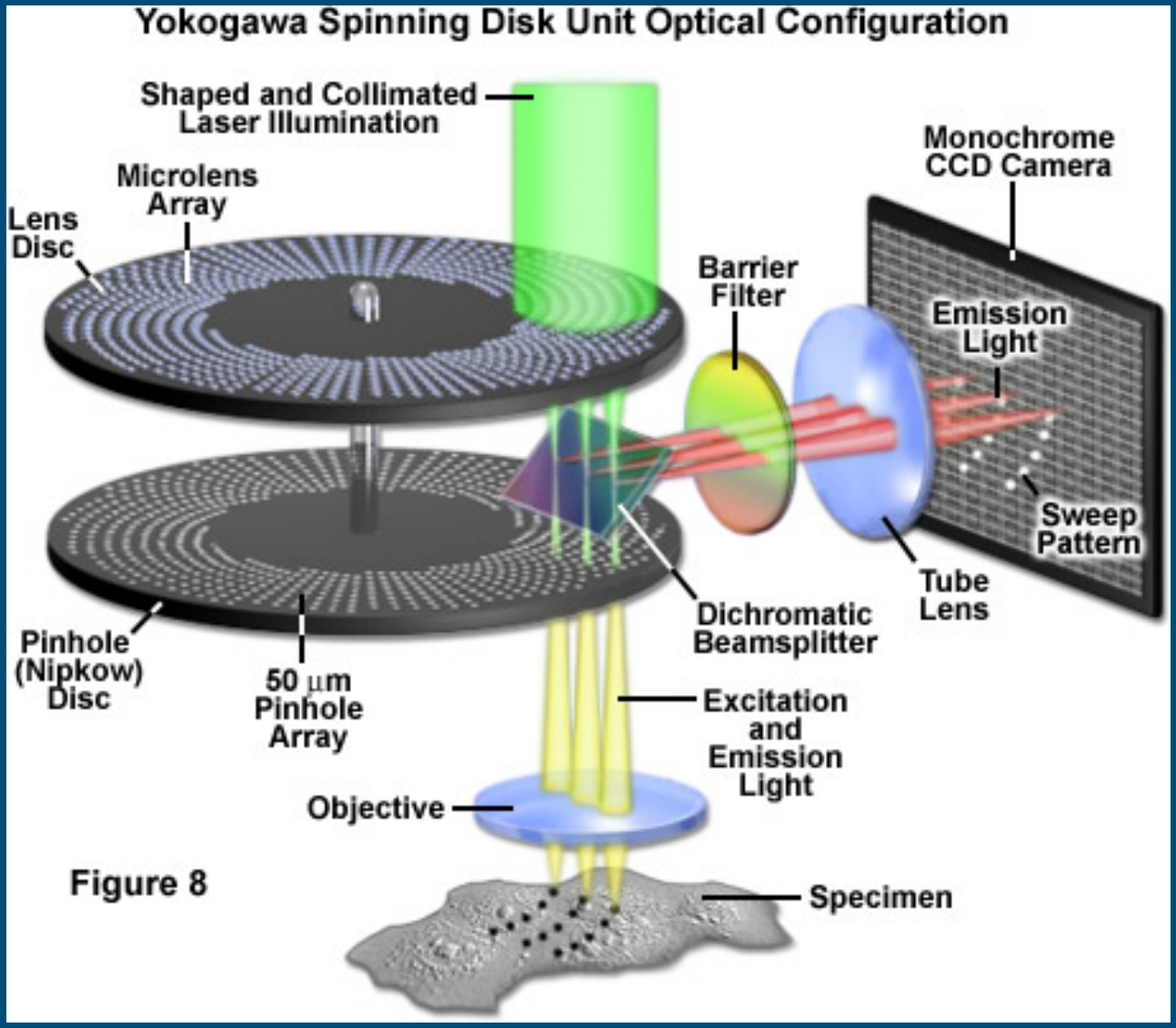


Wide Field

Laser Scanning

Spinning Disk

Spinning disc microscopy



Dual Spinning Disk Technology

Spinning disc microscopy (SDM)

Imaging speed of CLSM is limited by scan speed of laser focus.

In SDM a pattern of multiple focal points sweeps the sample. For this, a rotating Nipkow disk with tiny holes is imaged on the sample. The holes take the function of the pinhole in the CLSM.

A disk with microlenses focuses the excitation beam on to the holes in the Nipkow disk (to prevent massive loss of excitation light).

Emission light is imaged onto a CCD camera with Quantum Efficiency (QE) up to 90%. The imaging speed of SDM is limited by the readout time of the camera (up to 1000 frames/s).

Each location on the sample is illuminated ±1000 times per second with a laser excitation intensity that is a 1000 times lower than in CLSM.

Low excitation intensity combined with high-QE light registration gives mild imaging conditions that decrease **photobleaching**.

Andor spinning disk system

Nikon Ti Eclipse

PFS-3 far red -150 µm

ASI piezo-stage xyz

Diode BF/UV

CO2-temp stage incubator

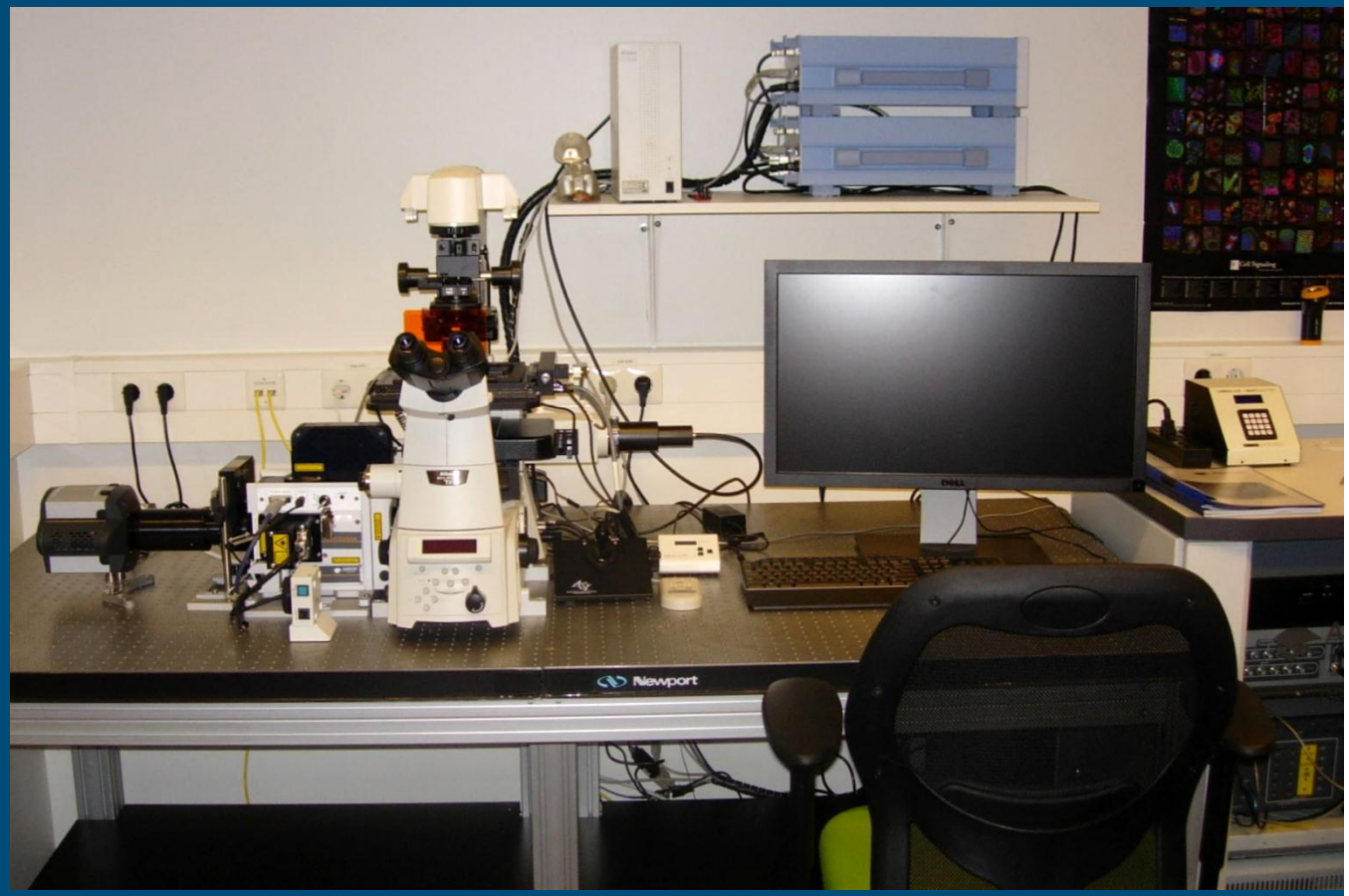
Andor Camera iXON888

CSU-X1 Yokogawa SD

Rotr emission filterwheel

By-pass DIC

MetaMorph control



Options on Andor Spinning Disk confocal

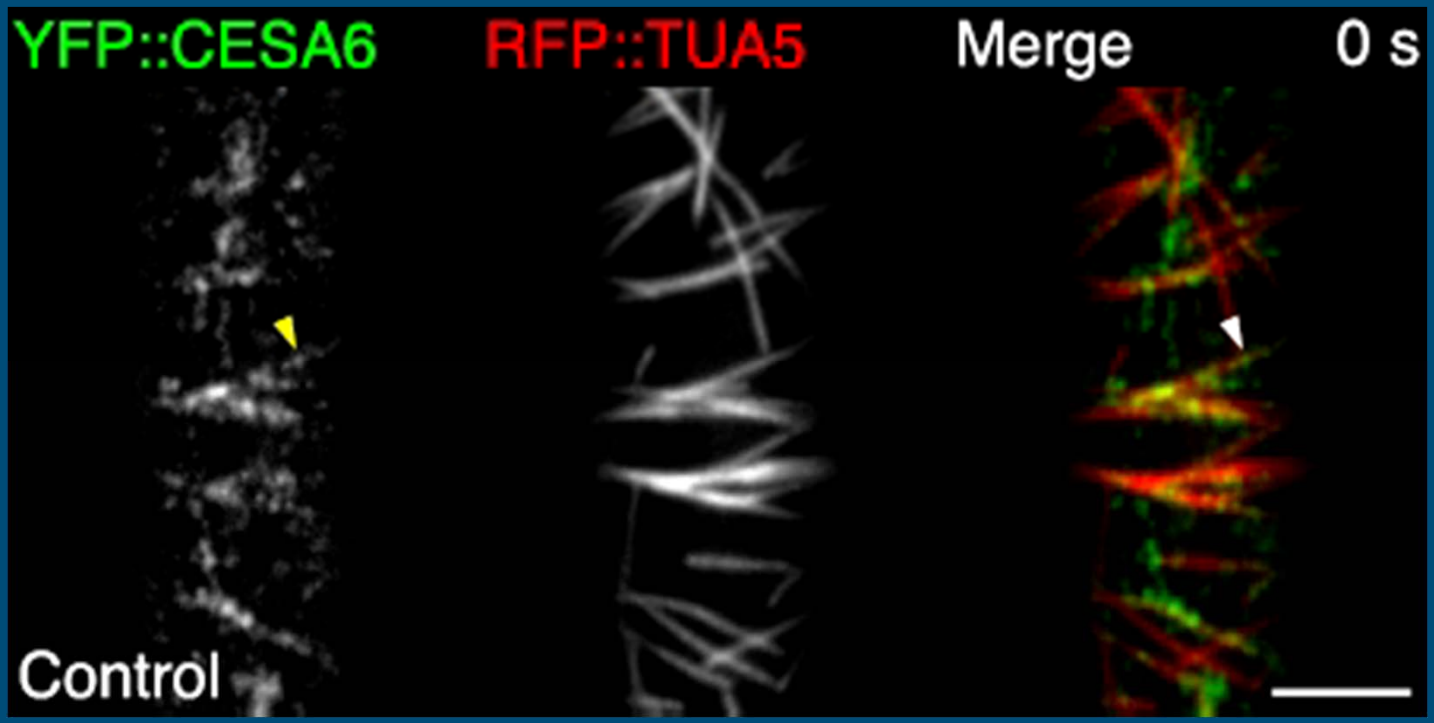
Available lasers from Andor-Laser combiner:

- diode CW (100 mW): **405 nm**
- DPSS CW (50 mW): **488 nm**
- DPSS CW (50 mW): **561 nm**
- diode CW (100 mW): **633 nm**

Available filtercubes in emission path:

quad BP: 440/40 nm, 521/21 nm, 607/34 nm, 700/45 nm
DAPI: 447/60 nm
GFP: 525/30 nm
RFP: 607/36 nm
Cy5: 685/40 nm

Study dynamics of microtubules and cellulose synthase complexes at membrane

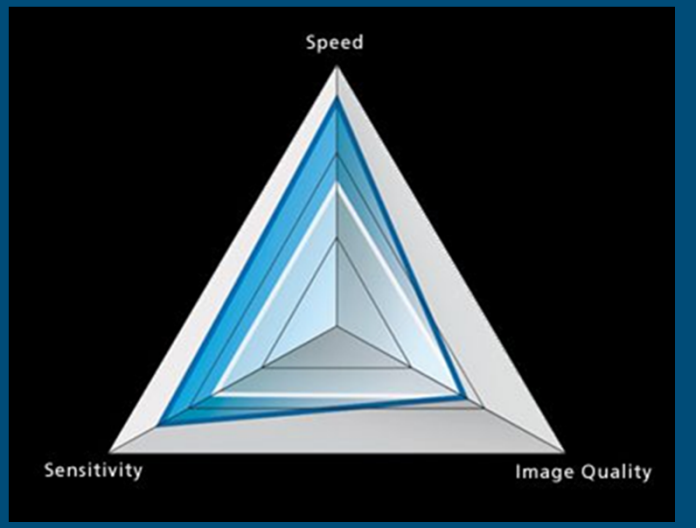


Specifications camera EMCCD IXON 3 - 888 Andor

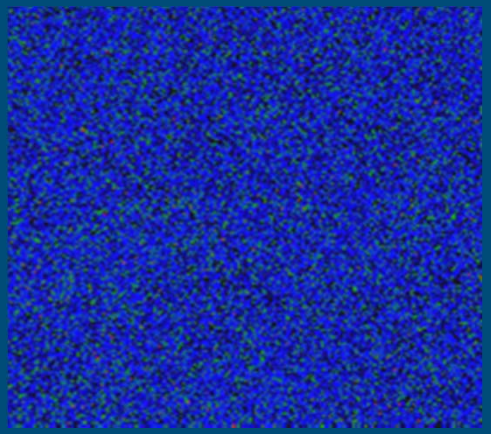
Most relevant: High Quantum Efficiency and Low Noise level

This Andor EMCCD camera with back-illuminated megapixels
combines large field of view, single photon detection capability and > 90% QE

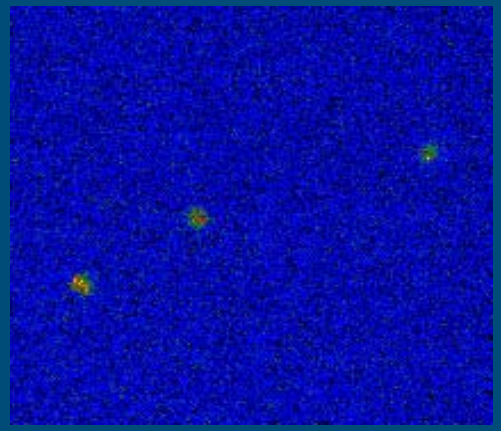
Active pixels:	1024 x 1024
Pixel size (W x H):	13 x 13 µm
Active area pixel well depth:	90,000 e-
Gain register pixel well depth	730,000 e-
Maximum readout rate	10 MHz
Frame rate	8.7 - 4,205 fps
Read noise	< 1e- with EM gain
Maximum cooling	-95°C



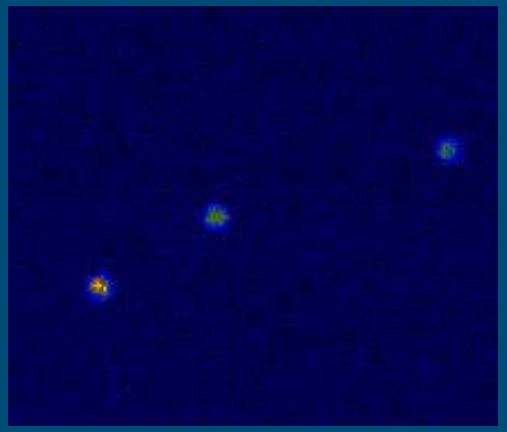
Effect of EMCCD Gain on S/N



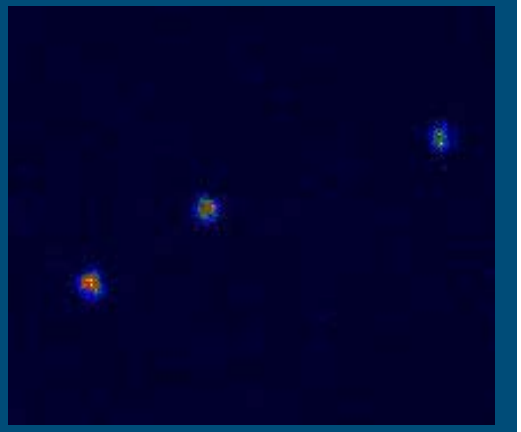
Gain x1



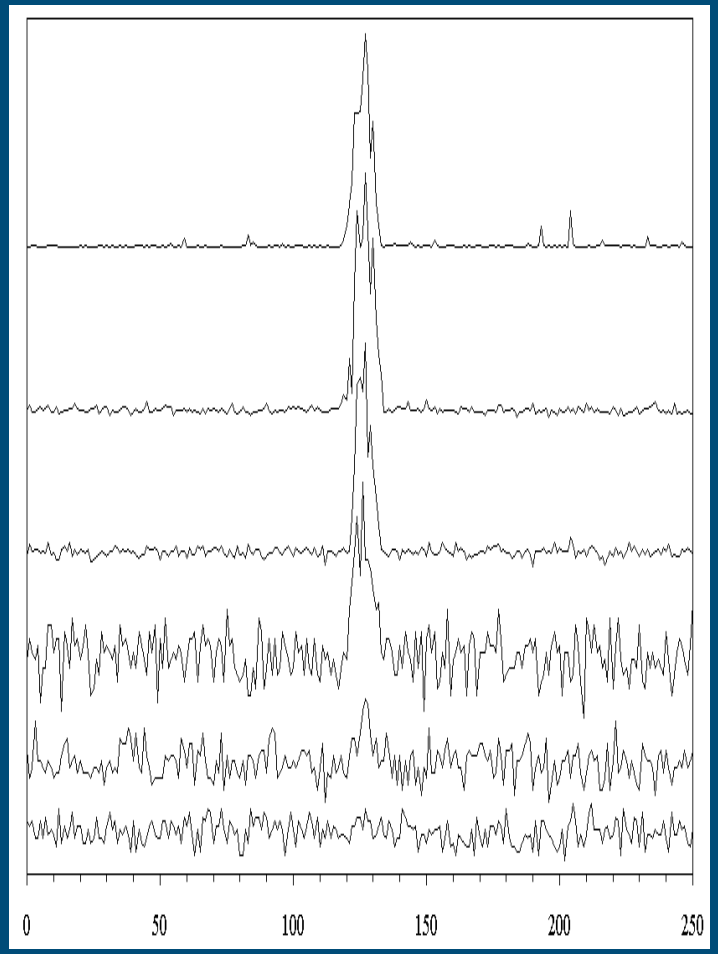
Gain x10



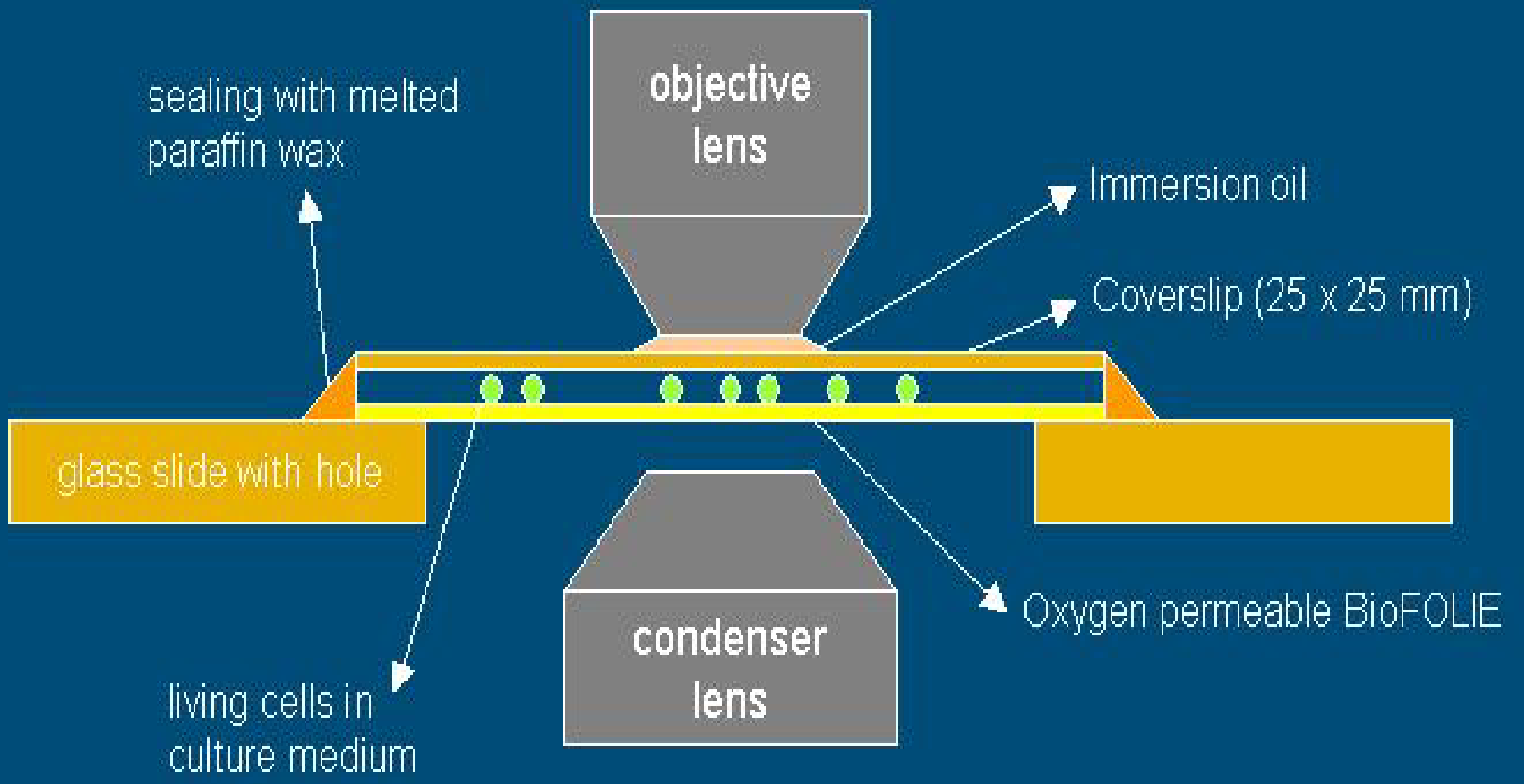
Gain x100



Gain x500



Life cell imaging in Biofoil slides



Stage top incubator (Tokai Hit)

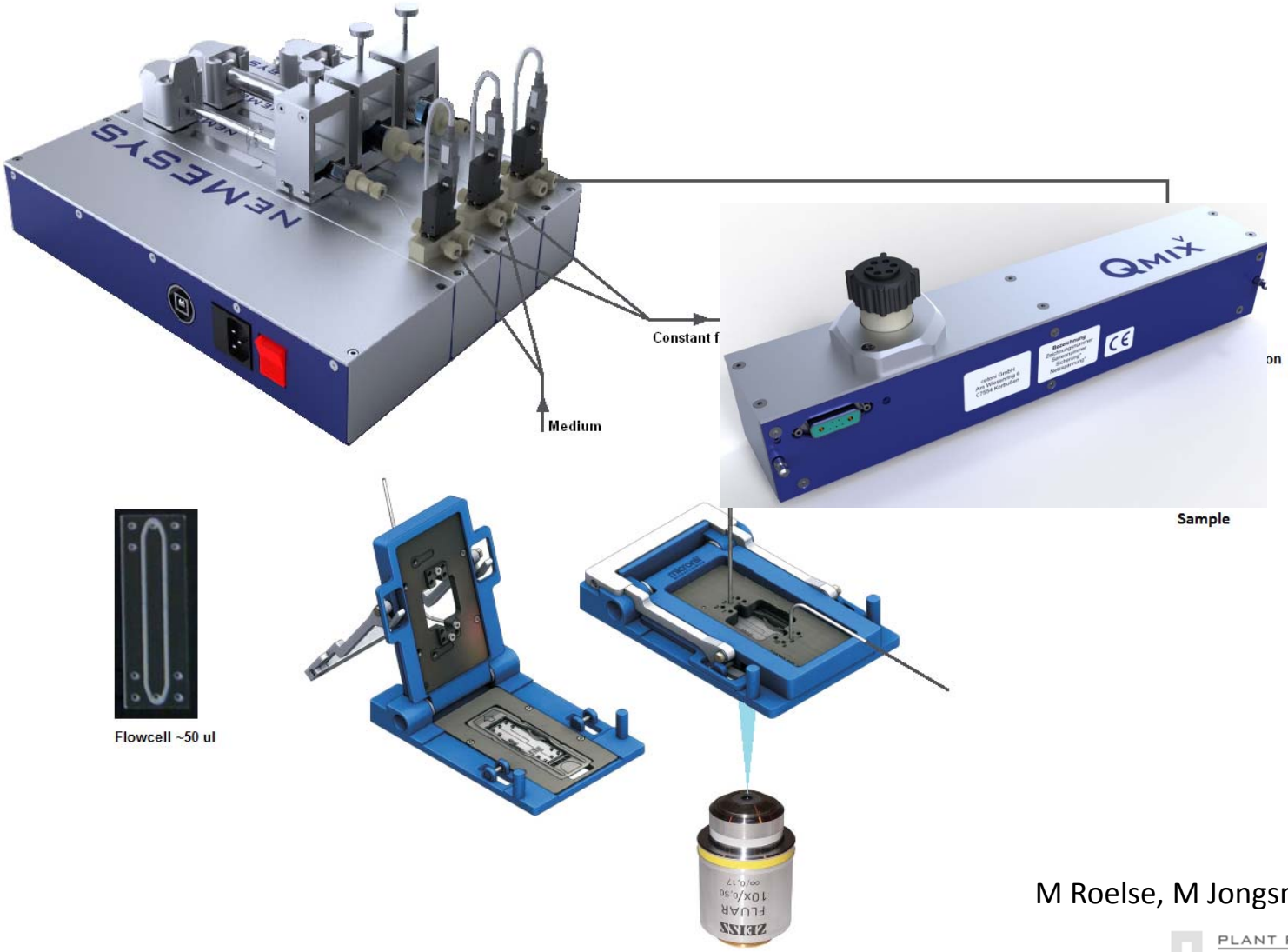
for motorized piezo controlled xyz stage (ASI)

Gas flow meter supplies premixed moisturized gas into Chamber Unit at stable 5% concentration and 37° C

Lens heater collar gives heat control for close-ups at 60-100x and OI or WI lenses.

Anti z-axis drift, clear window, single and multiwell sample holders

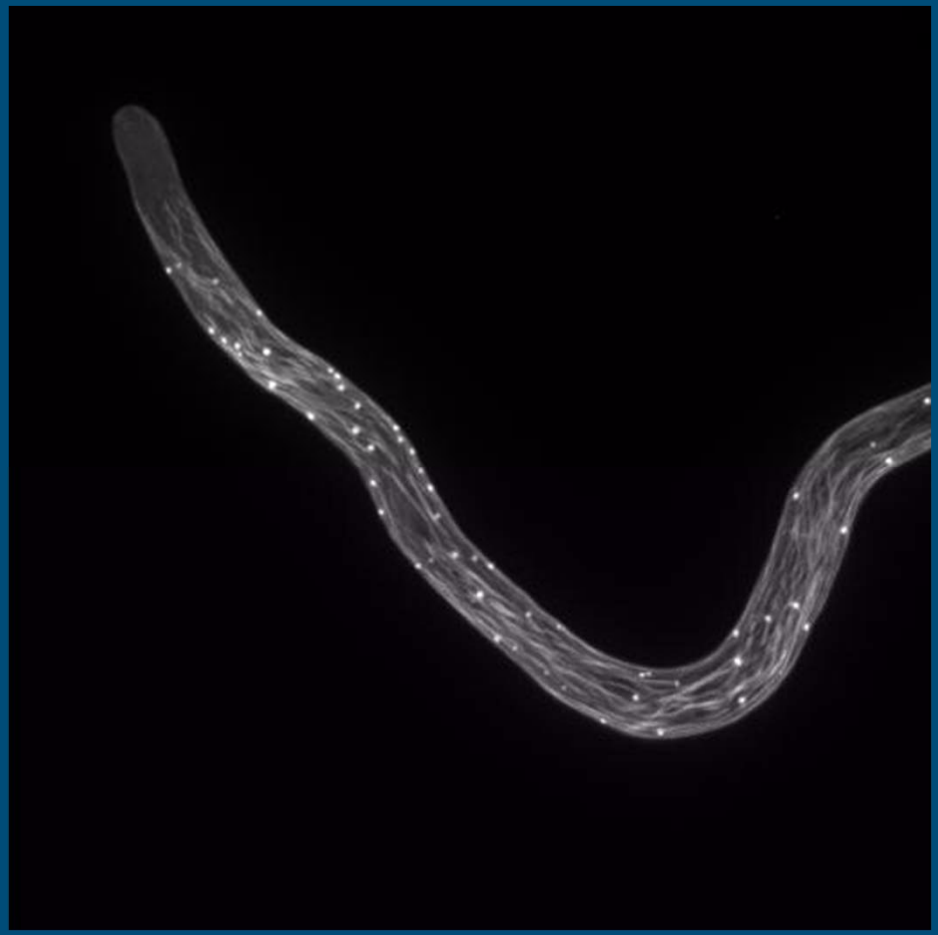
Microfluidic flow cells allow medium replacement, probe application during imaging



Micronit

M Roelse, M Jongsma, BioScience, PRI

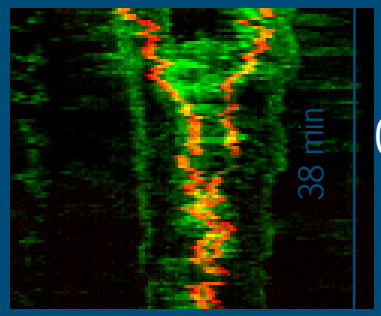
Study mode of action of a range of herbicides on actin or tubulin in oomycetes to find specific pathogen reduction



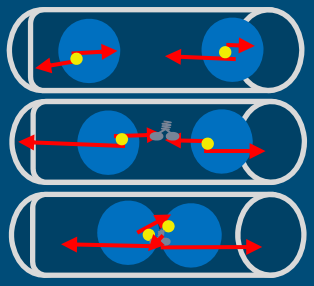
Actin in growing hyphe of *Phytophtera infestans by Life-act-GFP in SDCM (unpublished data Kiki Kots – Phytopathology-WUR)*

Study pulling forces in yeast by MT motor proteins

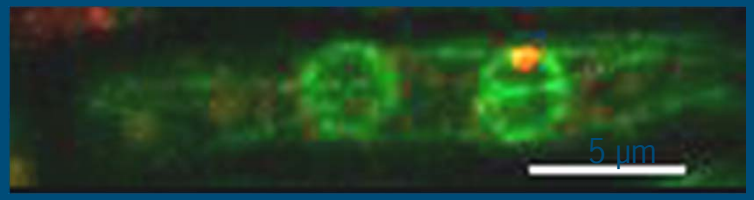
+ motor

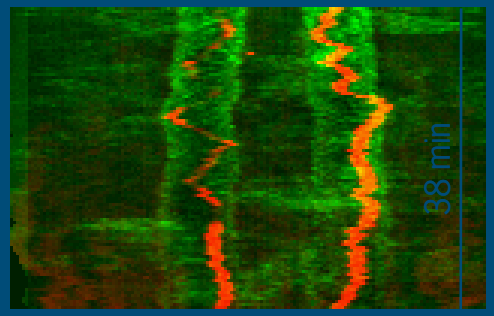


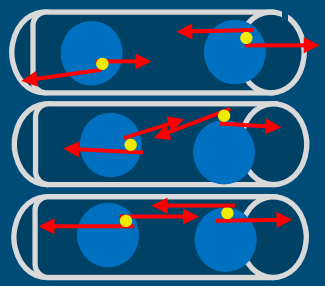
0.84 µm/min



- motor



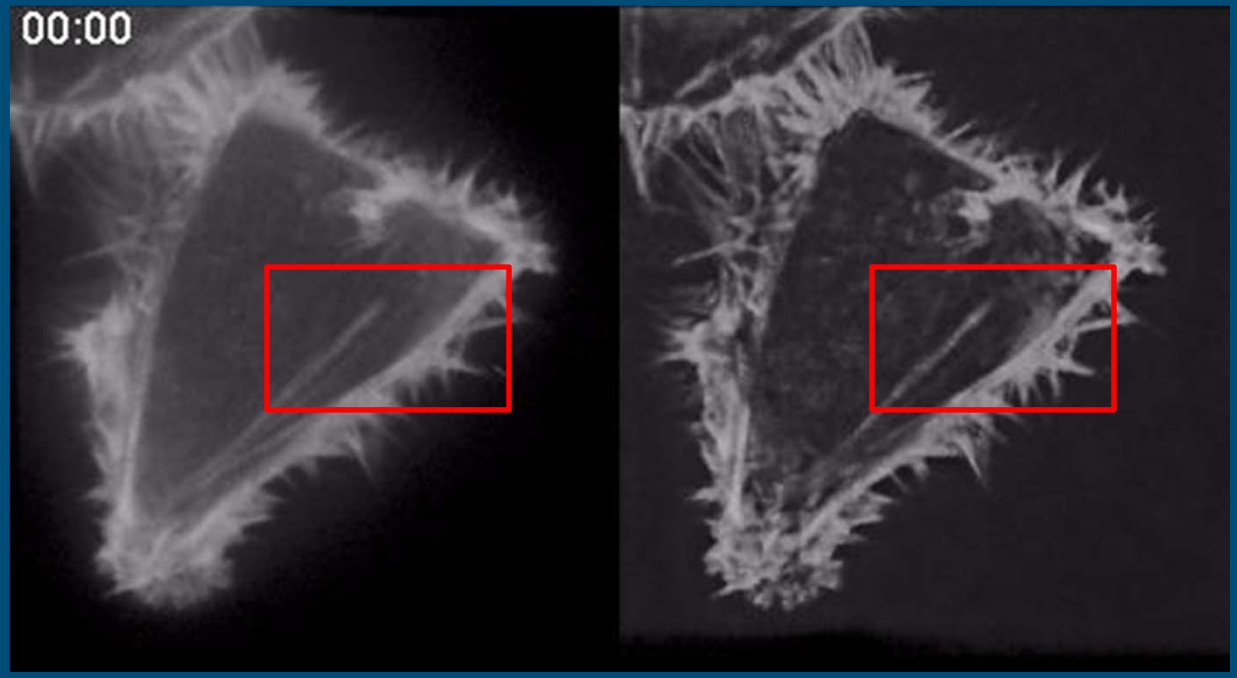


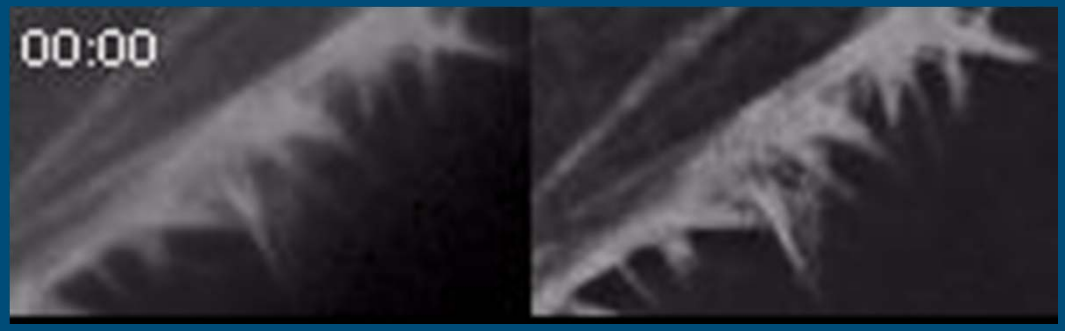


Motion of microtubule attachment sites (SPBs) is in agreement with pulling forces.

Courtesy Juliana Taepal, PhD at CLB

Study dynamics of filopodia on fibroblast surface as marker for cell vitality





Raw data Deconvoluted

20 slices projected, z=0,25um

3 sec interval, 10 timepoints (looped)

Courtesy Lieke Golbach, 2013

FRAPPA

Rapidly raster scans the sample, causing chemical changes (bleaching) to fluorescent dyes.

Mainly used for

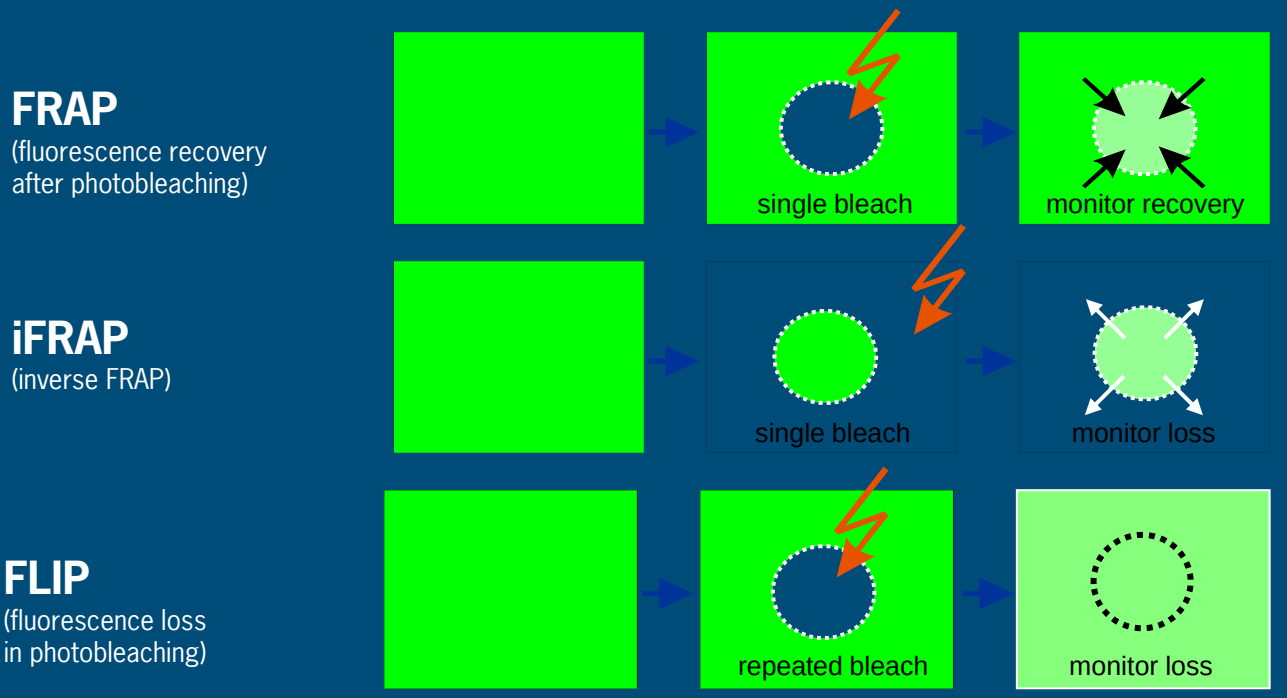
- FRAP – Fluorescent Recovery After Photobleaching
- PA – Photoactivation/Photoconversion

FRAP + PA = FRAPPA

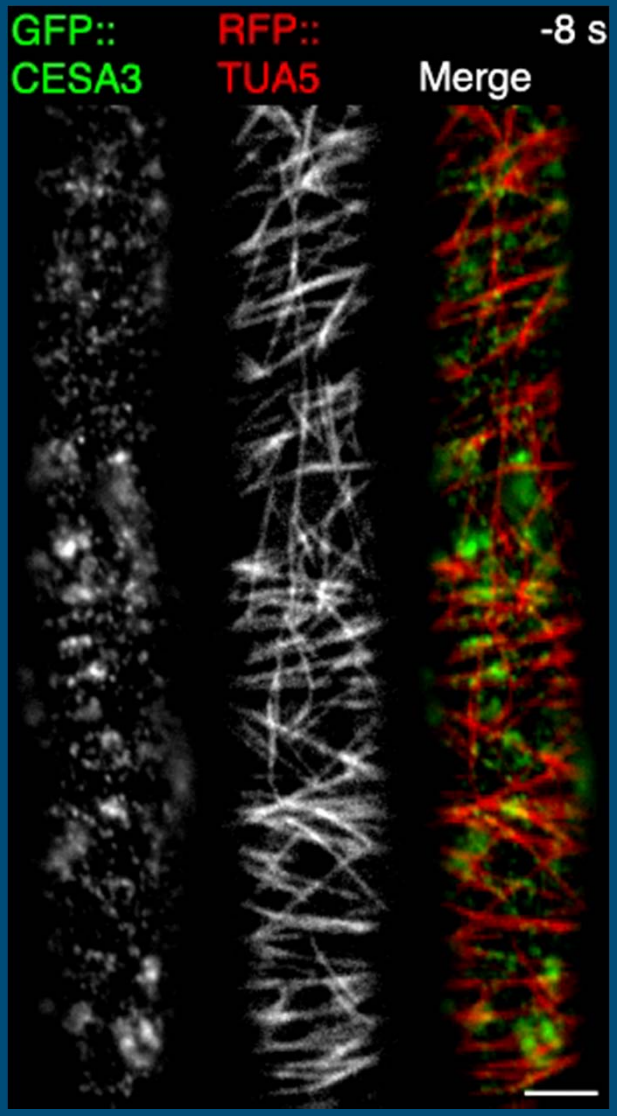
Used with the XD Spinning Disk, available on Roper SD

FRAP applications to score mobility

With high laser power: selectively and precisely bleach regions of interest
Analyze the recovery to gain insight into the dynamics of the labeled protein



Photobleaching to visualize delivery of GFP-labeled CESA complexes to the plasma membrane



A cell expressing GFP::CESA3 and mRFP::TUA5 was imaged in the plane of the plasma membrane. At t = 0 s, GFP is bleached. During the recovery, several CESA particles (arrowheads) are delivered to plasma membrane.

The majority of delivery sites are coincident with cortical microtubules.

A 2-frame running average was applied to the time series. Scale bar, 5 μm.

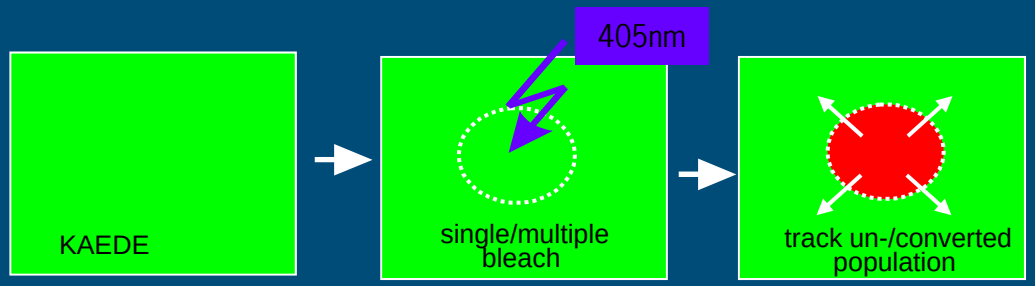
Gutierrez, Lindeboom, Paredez, Emons, Ehrhardt.
Nature Cell Biology 11, 797 - 806 (2009)

epidermal cells of the upper hypocotyl of Arabidopsis seedlings that were dark-grown for 3 days

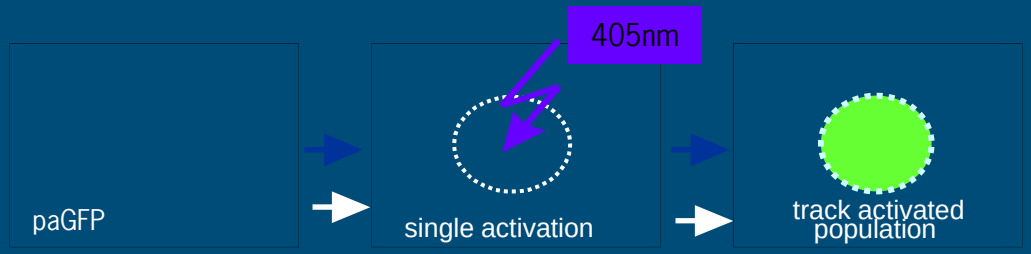
Currently used 405 nm applications

-Manipulation of fluorophores

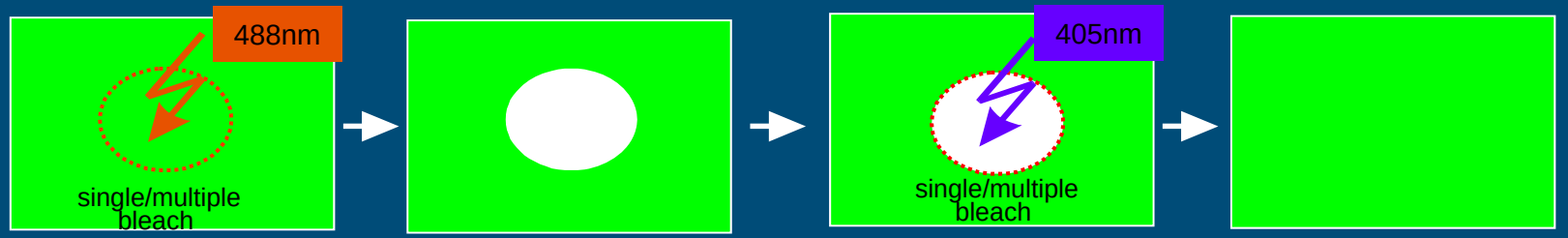
Photoconversion
(converting green chromophore
Kaede into red
with 405 nm)



Photoactivation
(activating paGFP
with 405 or 413 nm)



Photoswitching
(deactivate Dronpa with 488 nm
and activate with 405 or 413 nm)



Key: Spinning disk confocal imaging:

- Functions as a normal multi color confocal
- Provides the most gentle way for prolonged life imaging with minimal photodamage
- Ideal for imaging poor signals at high magnification

Thanks for your attention