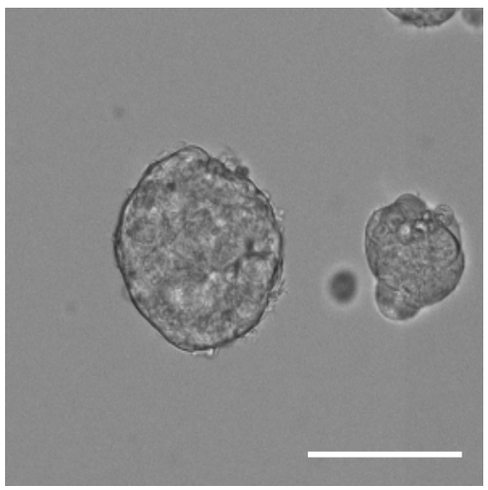
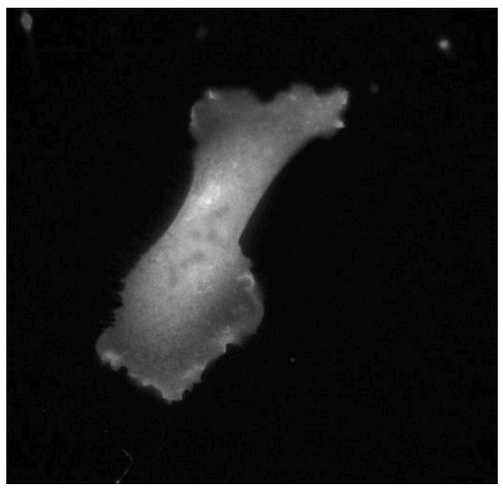
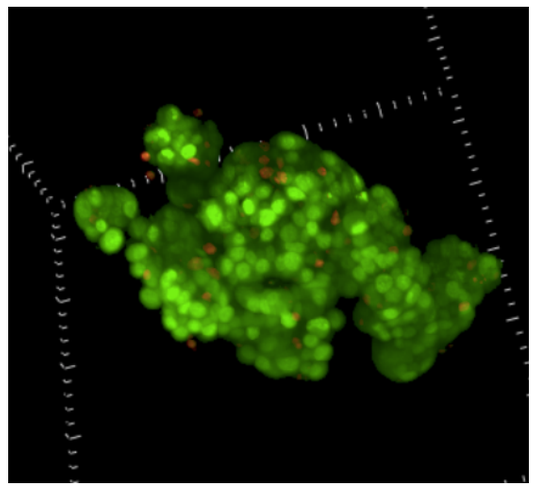
Live-cell Imaging: Liven up your data!

Nadia Halidi

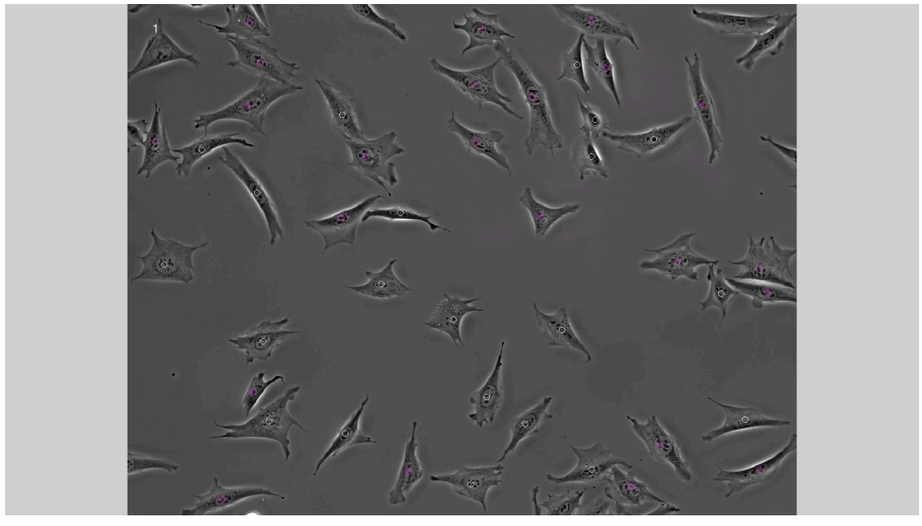
Imaging Facility Manager
Micron Advanced Bioimaging Unit



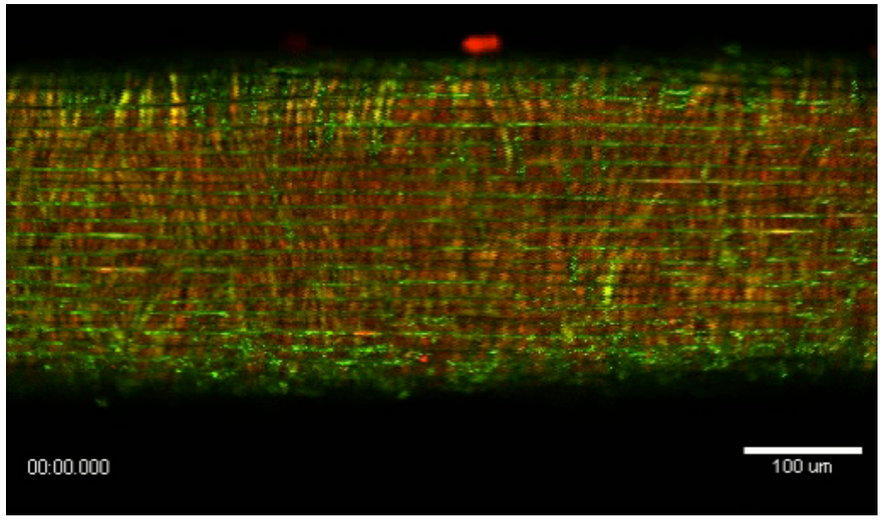




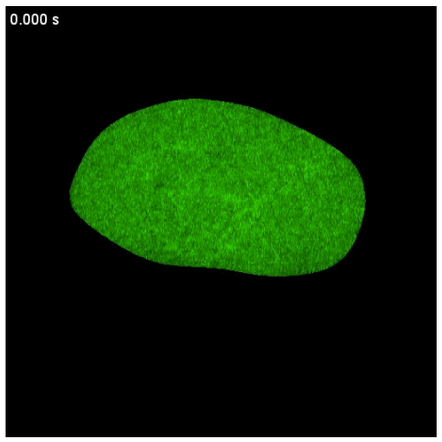
Live-cell imaging - Why?



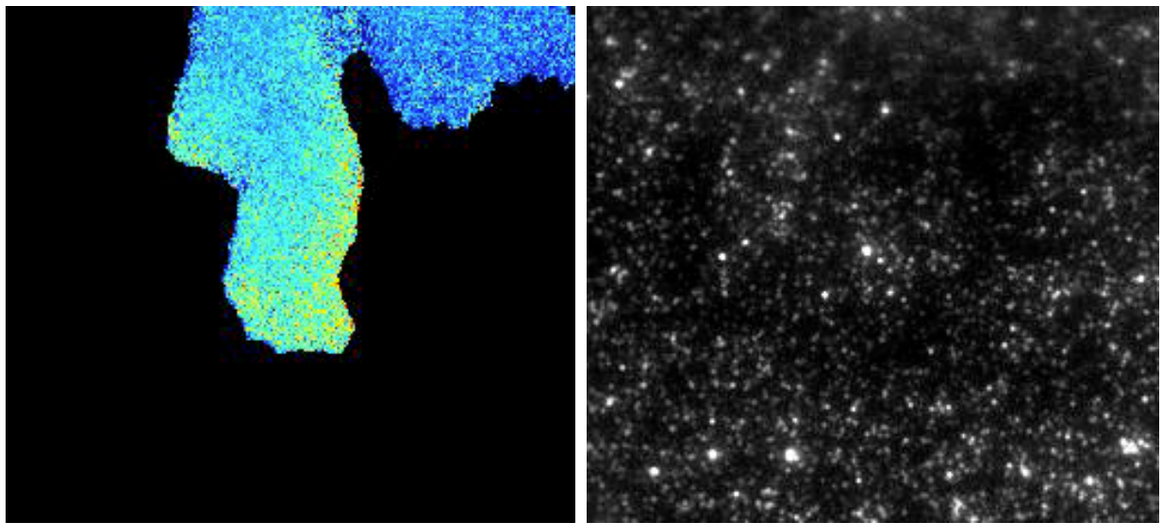
Tracking cell migration on substrates
(*Halidi, unpublished data*)



Calcium wave propagation in an arterial strip
(*Seppey et al. 2010*)



FRAP in U2OS cells transfected with GFP-tagged MLLT1
(*Moustakim et al. 2018*)



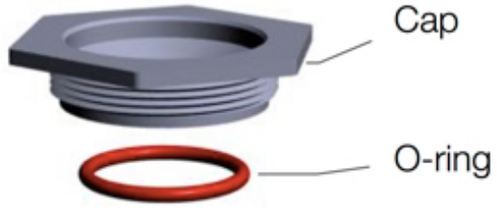
Simultaneous FRET-based biosensors activity and
traction force microscopy (*Halidi, unpublished data*)

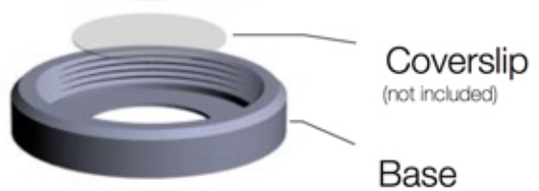
Track cellular and sub-cellular processes in real time

Overview

- Sample preparation (mounting, staining, media)
- Choosing a microscope (inverted vs upright)
- Maintaining live cells on the microscope stage
- Efficiency of detection
- Photobleaching & Phototoxicity
- Data processing and analysis through examples

Sample preparation: Mounting options





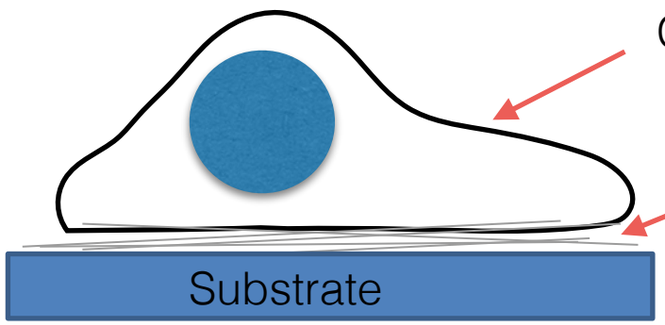
airekacells.com

Fluorodish.com

Thin coverslip #1.5 thickness or 170 um

Sample preparation: Mounting options

Extracellular matrix (ECM) proteins coating and coated plates

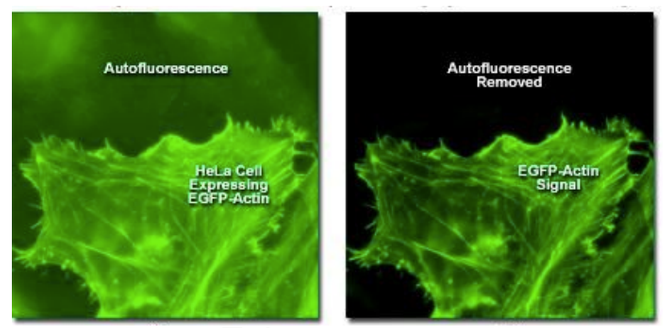


ECM: e.g. fibronectin, collagen, laminin

Glass, plastic, or silicon substrates of variable stiffness

Media options and considerations

- Avoid media w/ autofluorescence properties (phenol red, serum proteins)
- CO_2-dependent media —> requires CO_2 in the atmosphere
- CO_2-independent media —> requires buffers e.g. HEPES



Zeiss campus

Sample preparation: Staining options

- Fluorescent protein tags
- Fluorescently tagged ligands
- Fluorescent antibodies to extracellular epitopes
- Cell permeant small molecule fluorophores (e.g. dyes, DNA stains)

Brightness

Photostability

Fluorescent proteins: mTagBFP2, EGFP, tdTomato, iRFP, cerulean, citrine, mcherry, mKate2

Multi-color experiments: DAPI (Alexa 405, Alexa/Atto 488, Alexa 568, Alexa 647)

Live cell nuclear dyes: Hoechst, SYBR safe DNA stain (replacing Ethidium Bromide)

Cell tracker dyes, Vybrant DiI, CM-DiI, DiO and DiD cell-labeling

Choosing a microscope

Inverted

Which one to use?

Upright

Things to considerations

- Samples mounted on a multi-well plate
- Samples won’t grow on glass bottom dishes
- Sample thickness
- Need access to samples (e.g. addition of drugs, inhibitors)
- Environmental control is important
- Location of what we want to detect (adhesion sites —> TIRF)

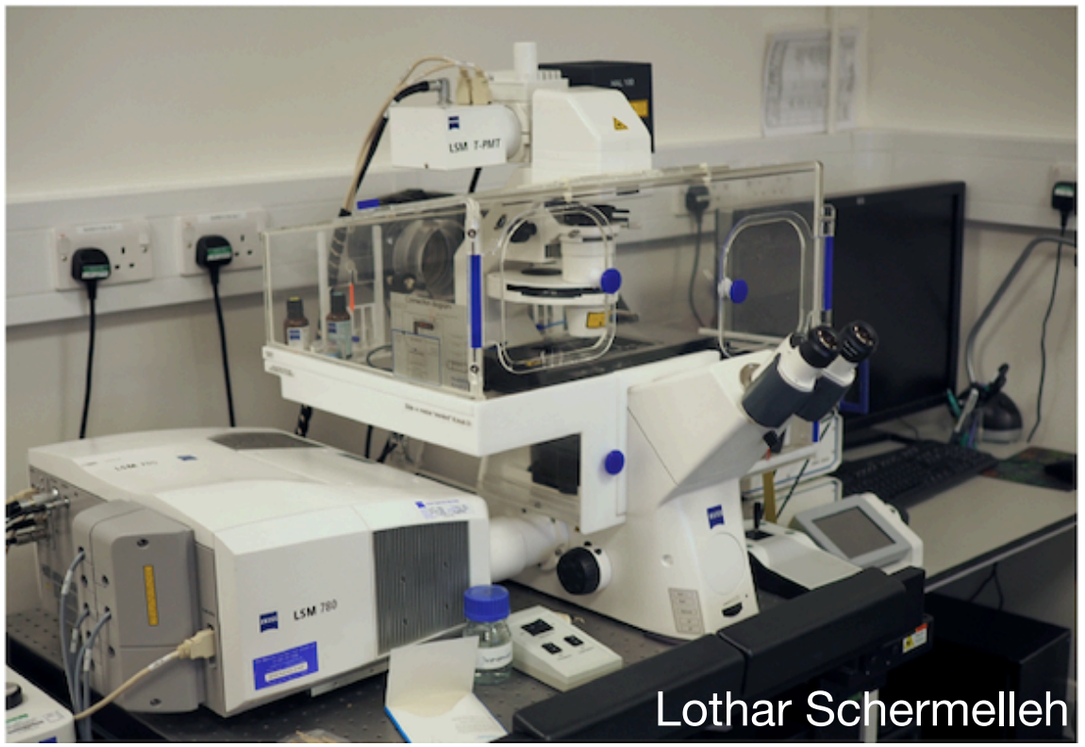
Maintaining live cells on the microscope stage

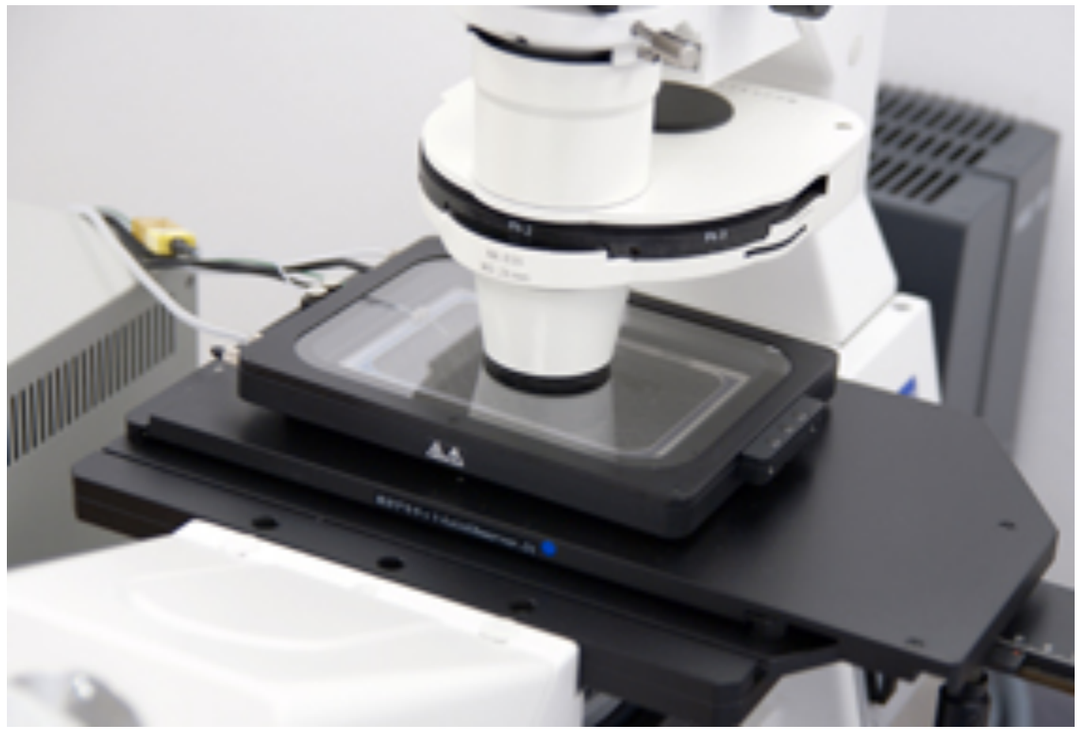
Environmental control:

Temperature

CO_2

Humidity





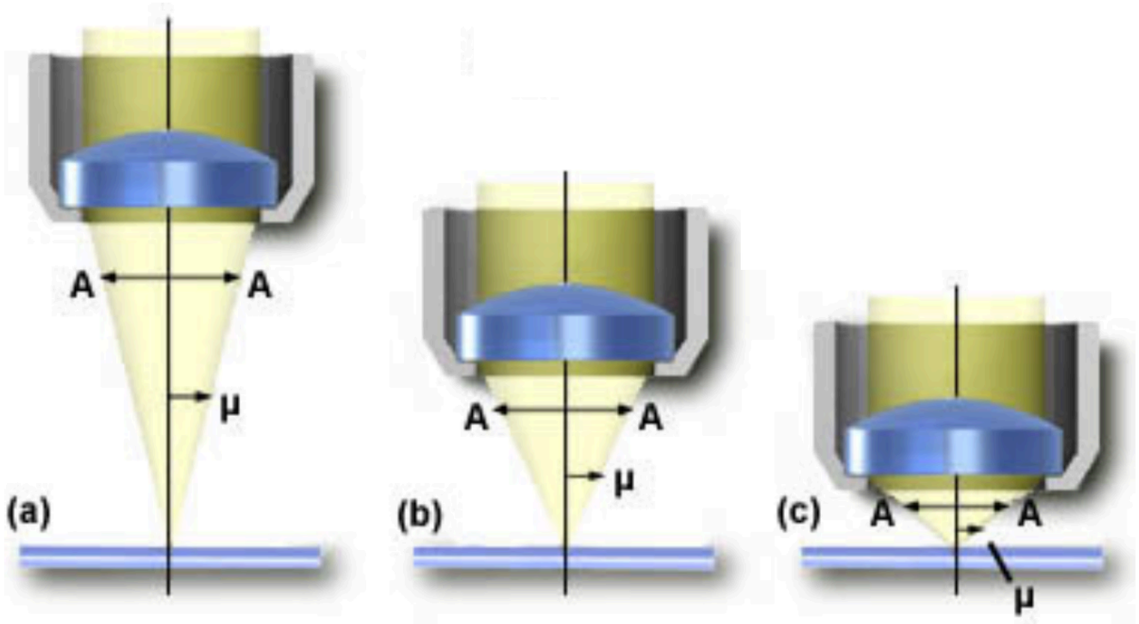
Ready to image? … Wait!

The efficiency of detection depends mainly on:

1. The objective
2. The filter set
3. The detector

Efficiency of detection: The objective

- The objective determines the special resolution and controls the amount of light gathered from the specimen. (look for NA)



Numerical aperture (NA) = n **x** sin μ

(a) μ = 7° NA = 0.12
(b) μ = 20° NA = 0.34
(c) μ = 60° NA = 0.87

https://micro.magnet.fsu.edu/

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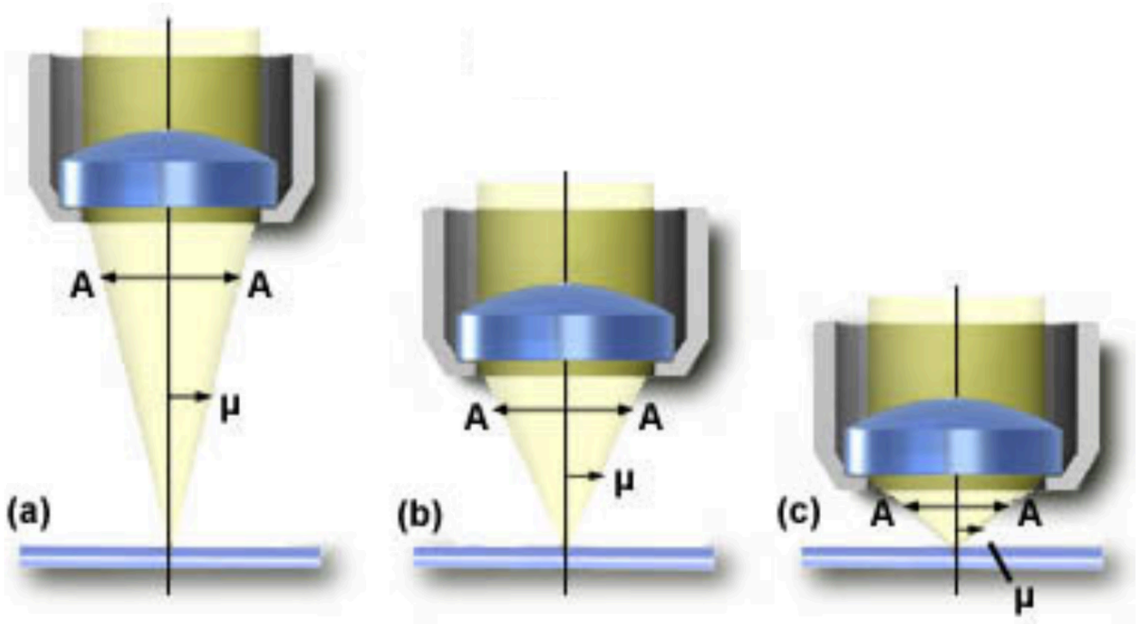

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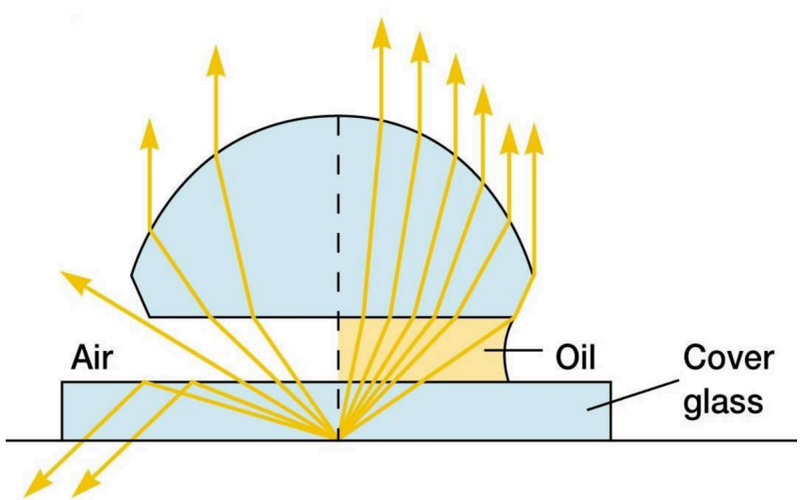
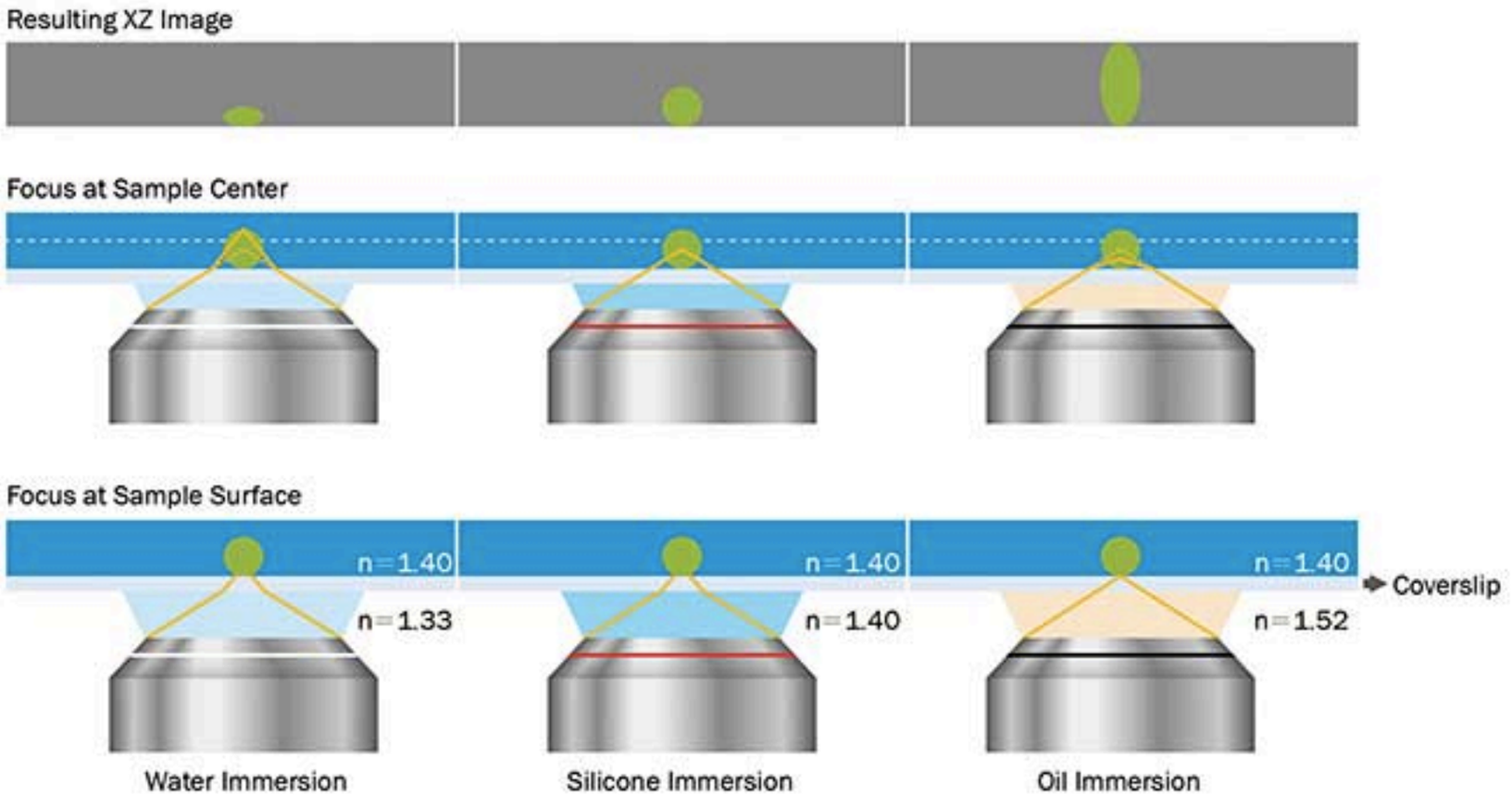


Efficiency of detection: The objective

- The objective determines the special resolution and controls the amount of light gathered from the specimen. (look for N.A.)
- Avoid refractive index mismatches between the sample and the immersion oil.



https://www.olympus-lifescience.com

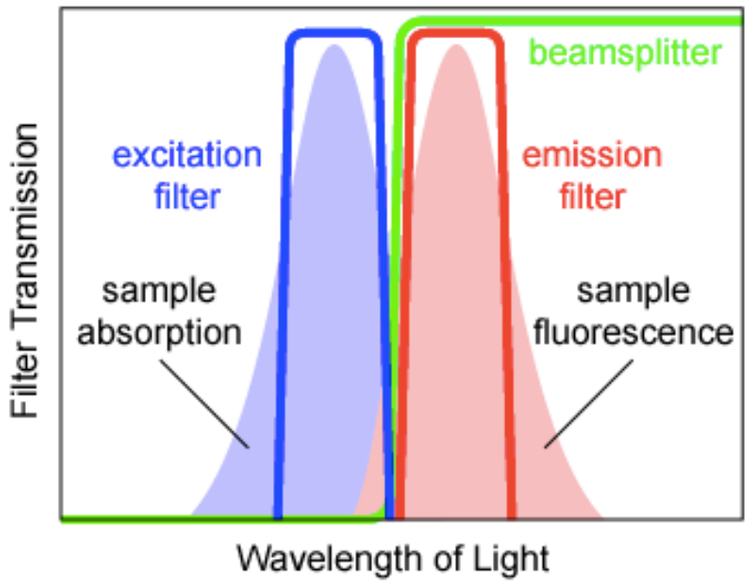
Efficiency of detection: The objective

- The objective determines the special resolution and controls the amount of light gathered from the specimen. (look for N.A.)
- Avoid refractive index mismatches between the sample and the immersion oil.
- Working distance
- Field of view
- Number of optical corrections in the lens design

https://www.olympus-lifescience.com

Efficiency of detection: The filter sets

- Know your fluorescent protein absorption and emission spectra
- What filters are there on the system (preferably narrow bandpass)



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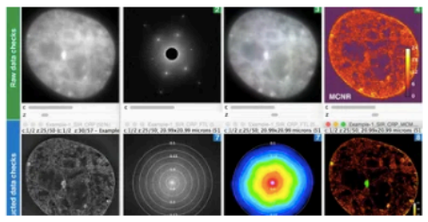
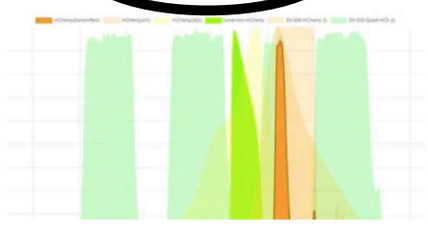
SIMcheck



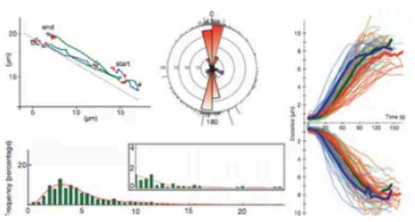
SIMcheck is a suite of ImageJ plugins enabling users to identify and avoid common problems with 3D-Structured Illumination Microscopy (3D-SIM)

SPEKcheck



Advanced fluorescence imaging methods require careful matching of excitation sources, dichroics, emission filters, detectors, and dyes to operate at

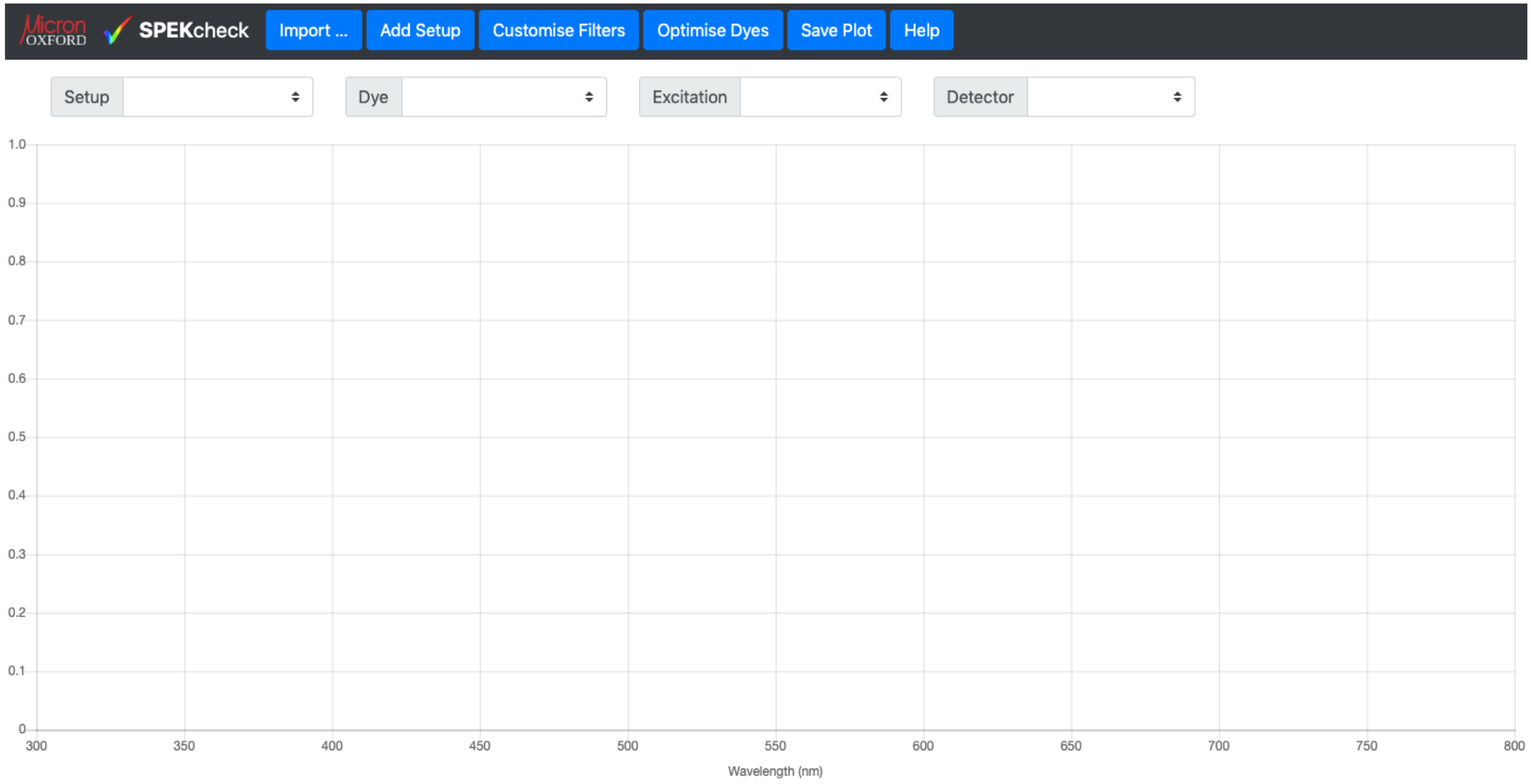
Particle Stats



The study of dynamic cellular processes in living cells is central to biology and is particularly powerful when the motility characteristics of

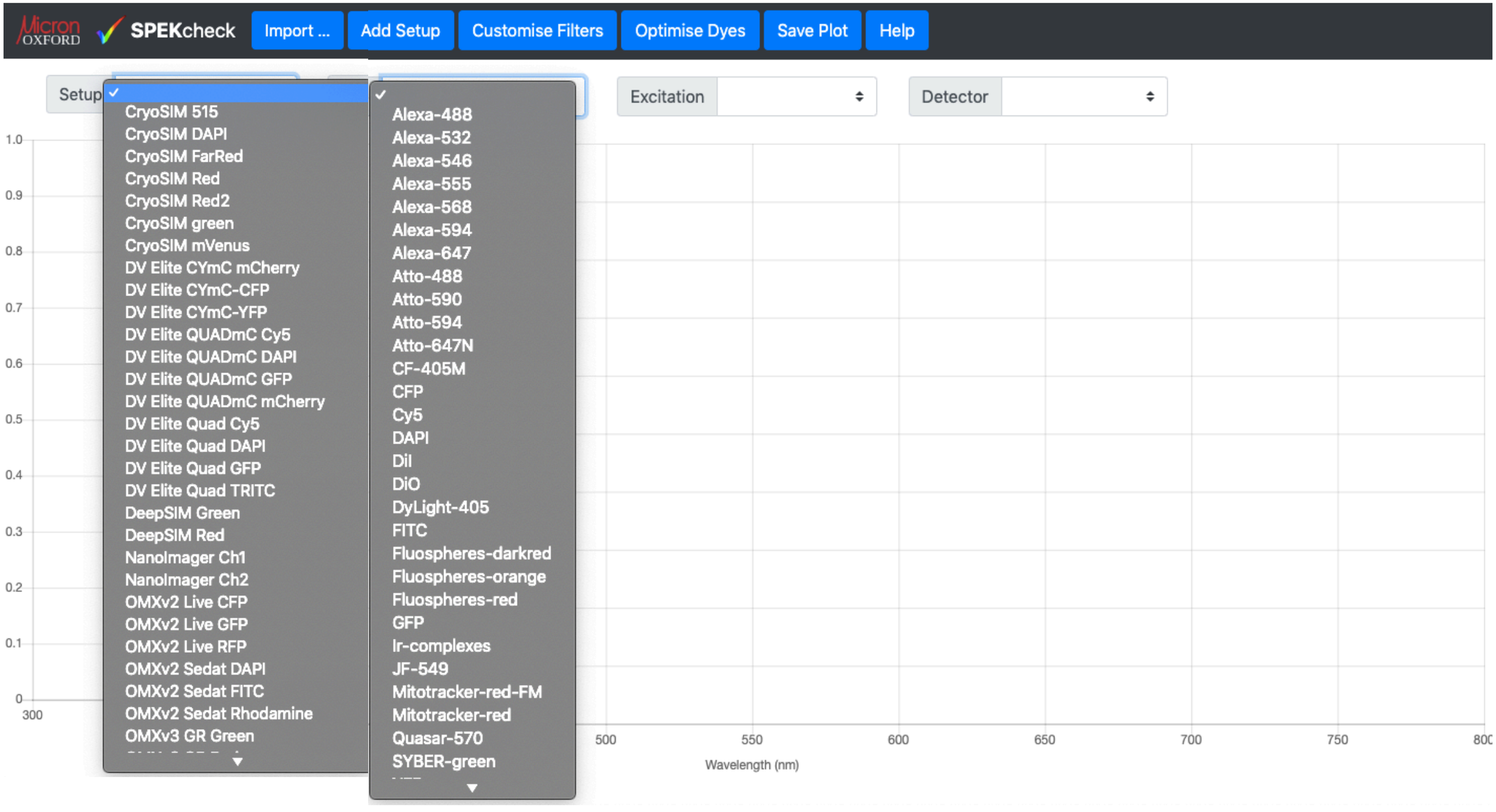
Efficiency of detection: The filter sets

www.micron.ox.ac.uk/software/spekcheck/



Efficiency of detection: The filter sets

www.micron.ox.ac.uk/software/spekcheck/



Efficiency of detection: The detectors

Widefield & spinning disk confocal:

CCD

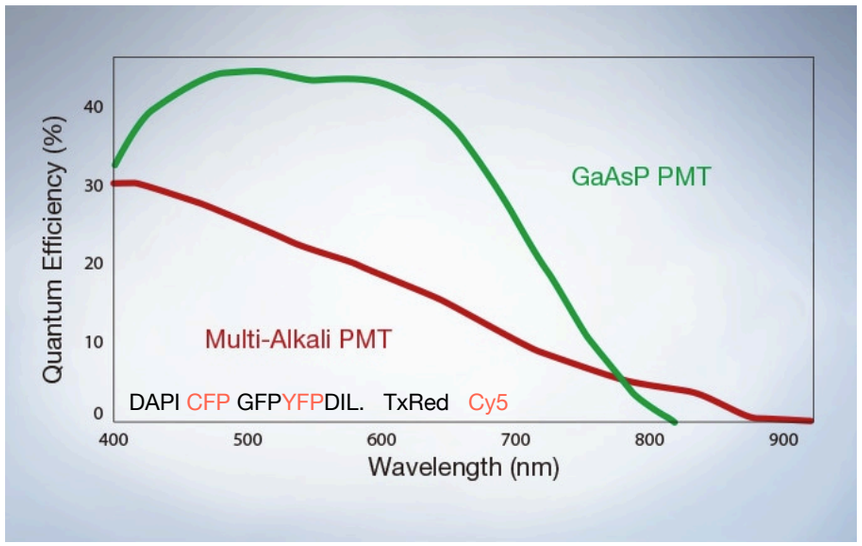
EMCCD

sCMOS

Point scanning confocal detectors

PMT

Gallium arsenide phosphide (GaAsP)



The final image always boils down to signal-to-noise!

Ready to image? … Wait!

The efficiency of detection depends mainly on:

1. The objective
2. The filter set
3. The detector

… but also

4. Correcting focus drift
5. Stage control
6. Imaging multi color w/ spectral detection & linear unmixing

Confocal, widefield or lightsheet

Widefield w/ deconvolution

Confocal for thick samples → spinning disk confocal

→ point scanning confocal

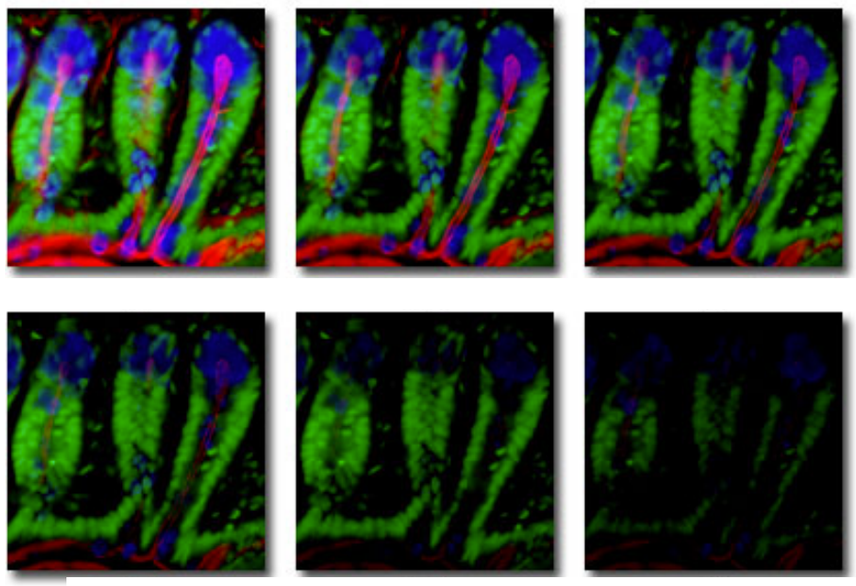
Lightsheet

Two-photon microscopy

Photobleaching & Phototoxicity

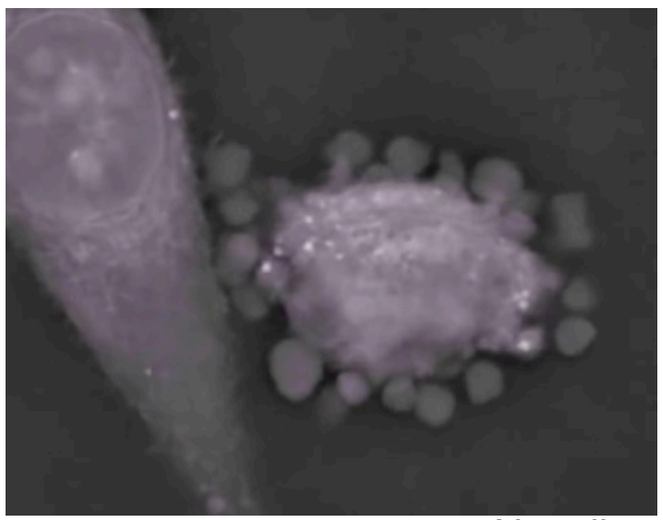
Micron OXFORD

Photobleaching —> dye not happy!

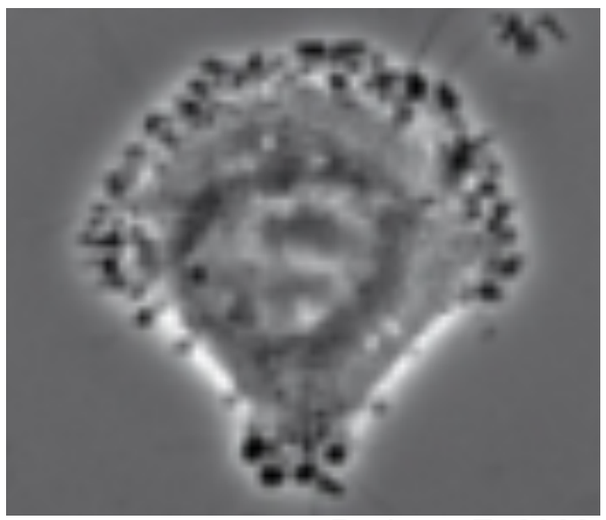


micro.magnet.fsu.edu

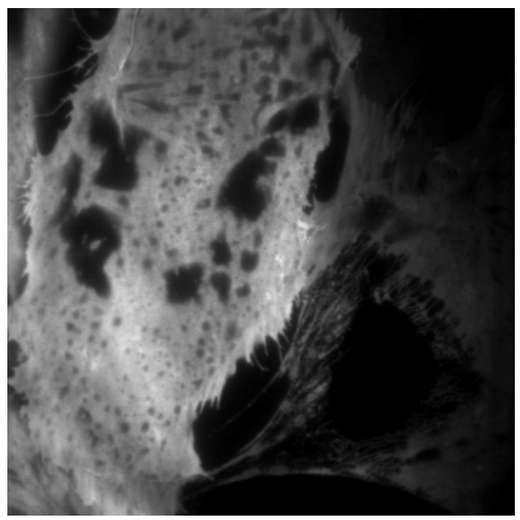
Phototoxicity —> cells not happy!



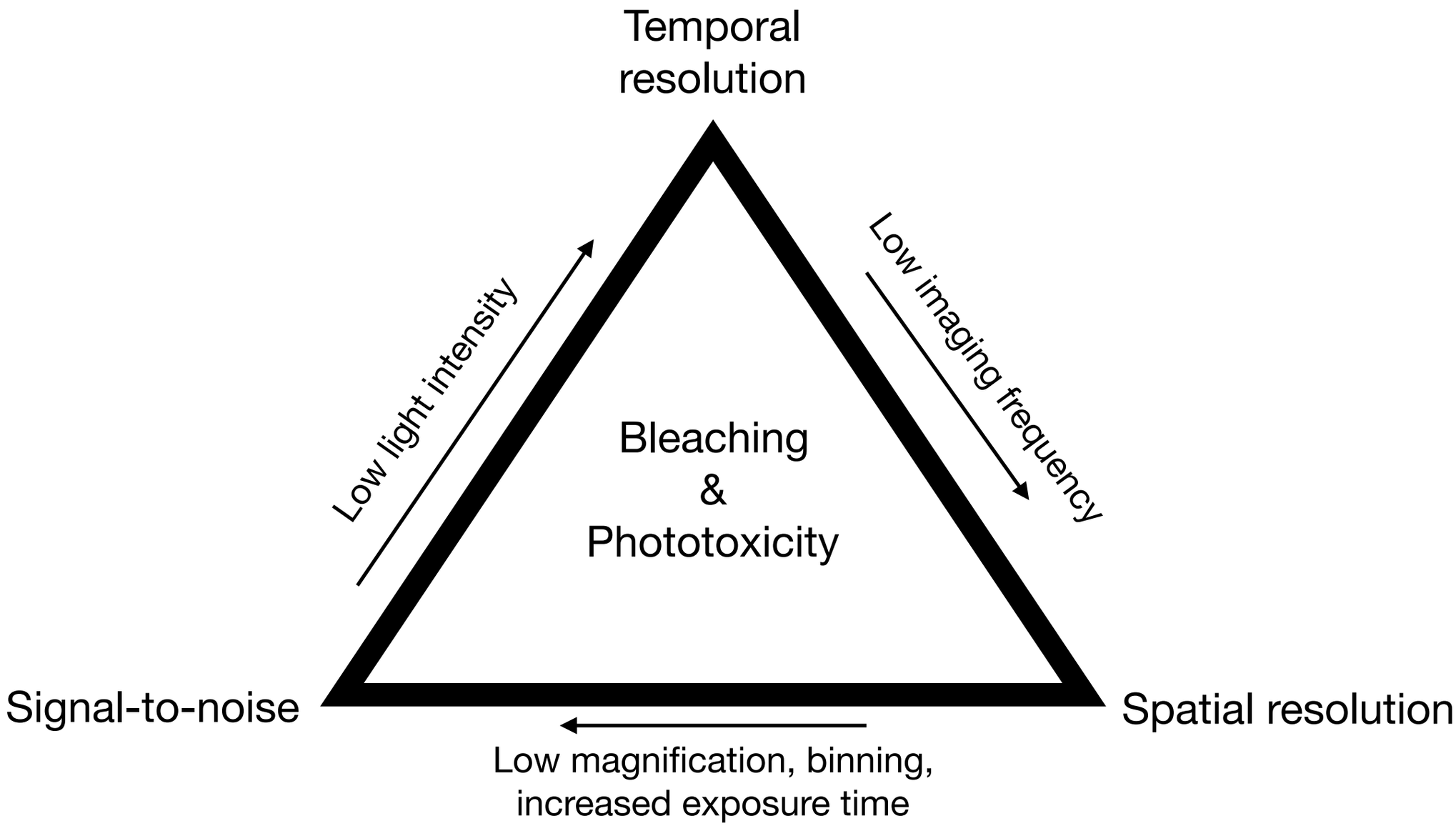
Nanolive



Derivery E, 2008



The iron triangle



Which microscope to use …again?

Widefield Deconvolution

- Collects Out-of-focus light -> Deconvolution
- Good signal-to-noise
- Z-sections requires post acquisition processing
- No Electronic zoom
- Good with point sources and weak signals
- Images could be deconvolve

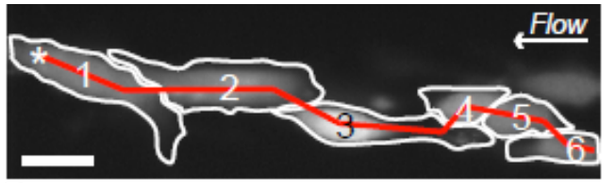
Confocal imaging

- Discards out-of-focus light
- Poorer signal-to-noise
- Immediate single z-sections
- Electronic zoom
- Good with diffuse and low contrast signal
- Skip lines <— be causes!
- Images could be deconvolve as well!

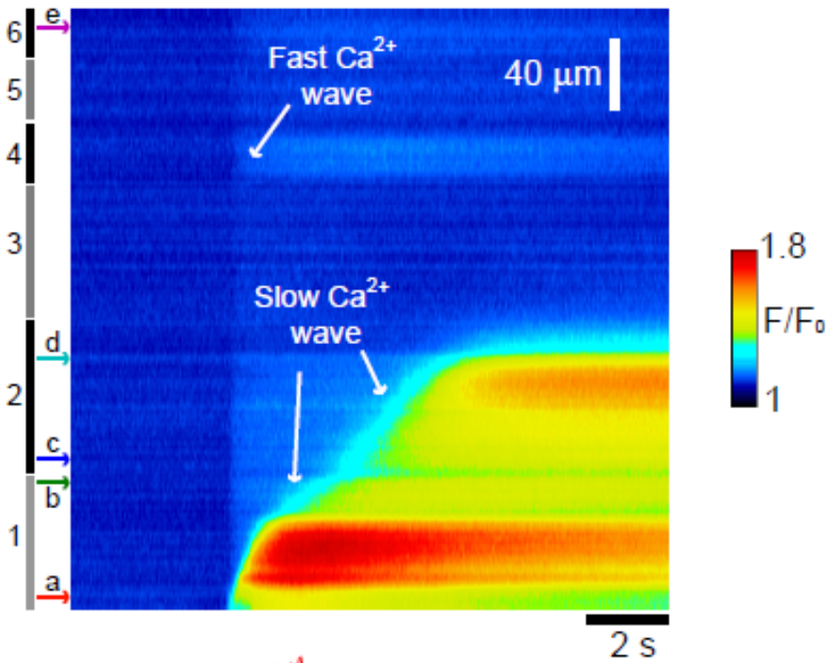
Data processing and analysis through examples

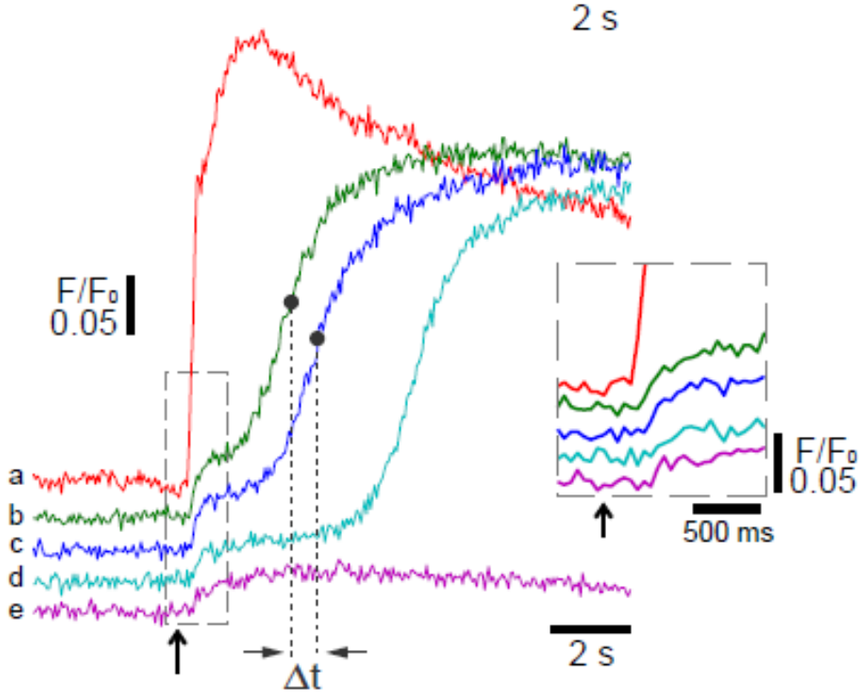
I have images! YAAAAY! …. so what now?

Fast dynamics:
Propagation of intercellular Ca^{2+} waves



Primary SMCs grown on µCP collagen lines.





Fast Ca^{2+} wave → 2310 ± 210 µm/s

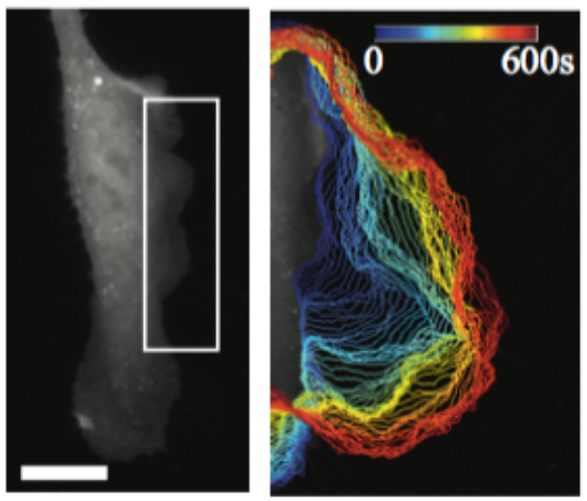
Slow Ca^{2+} wave → **1)** 19.8 ± 1.6 µm/s

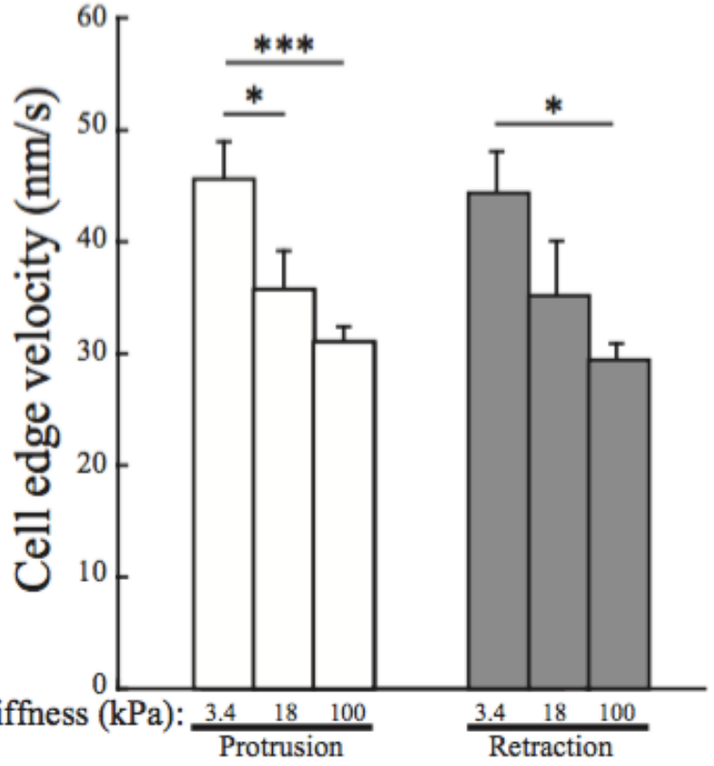
2) 21.4 ± 2.2 µm/s → 28%

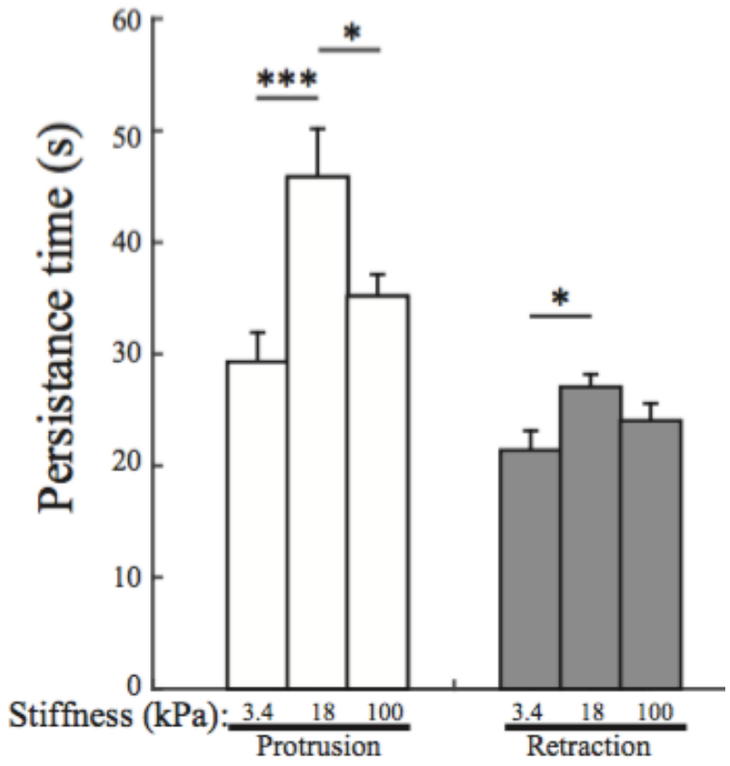
Transjunctional delay $\Delta t = 0.84 \pm 0.16$ s

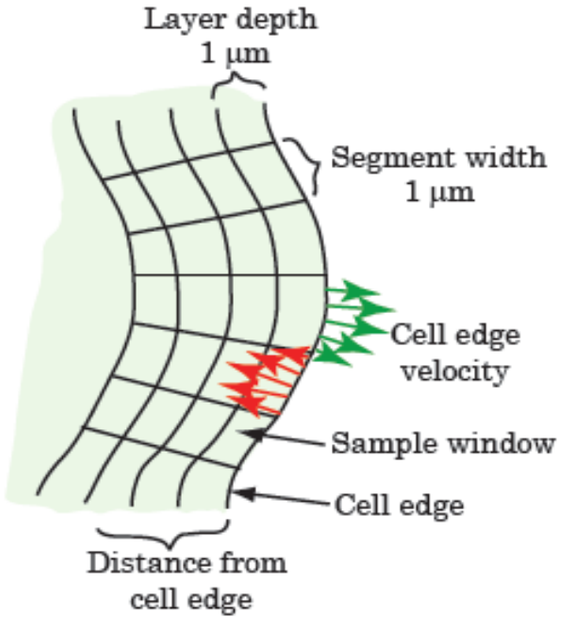
Halidi et al.Cell Calcium, 2011

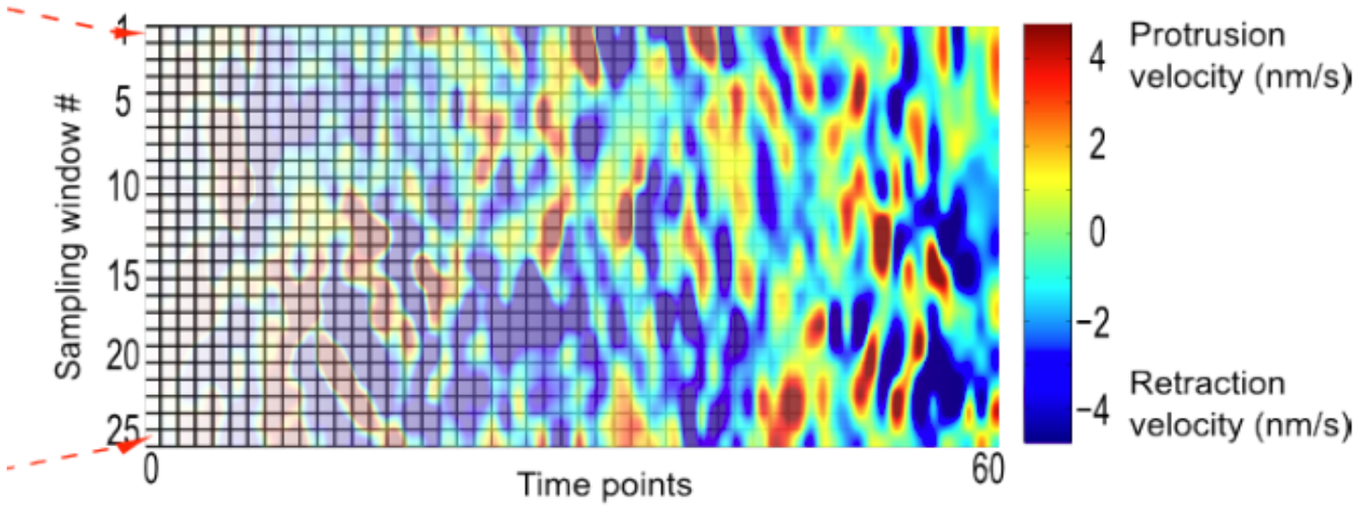
Morphodynamics





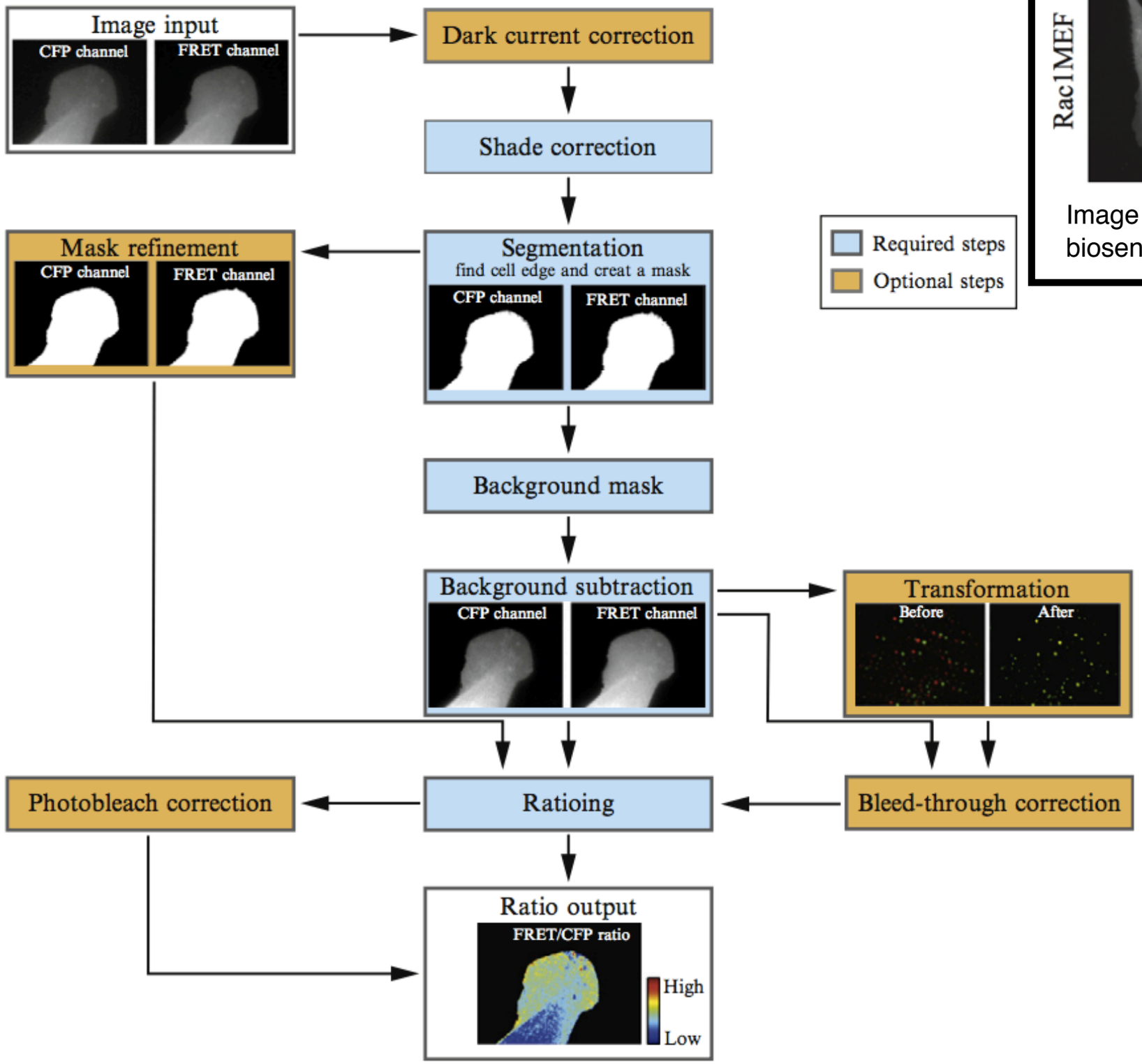






Halidi, unpublished data

FRET experiment



Required steps
Optional steps

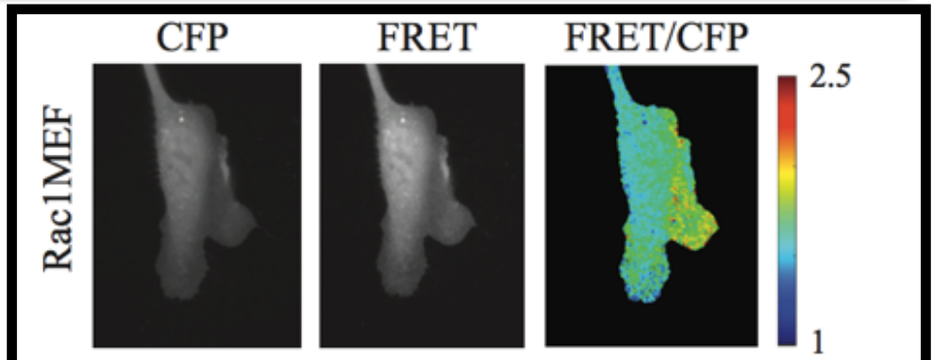


Image corrections and processing for FRET-based biosensor read- outs of signaling activities

Biosensors software downloadable:
http://www.utsouthwestern.edu/labs/danuser
Vilela et. al. 2013 Methods in Enzymology

FRAP experiments

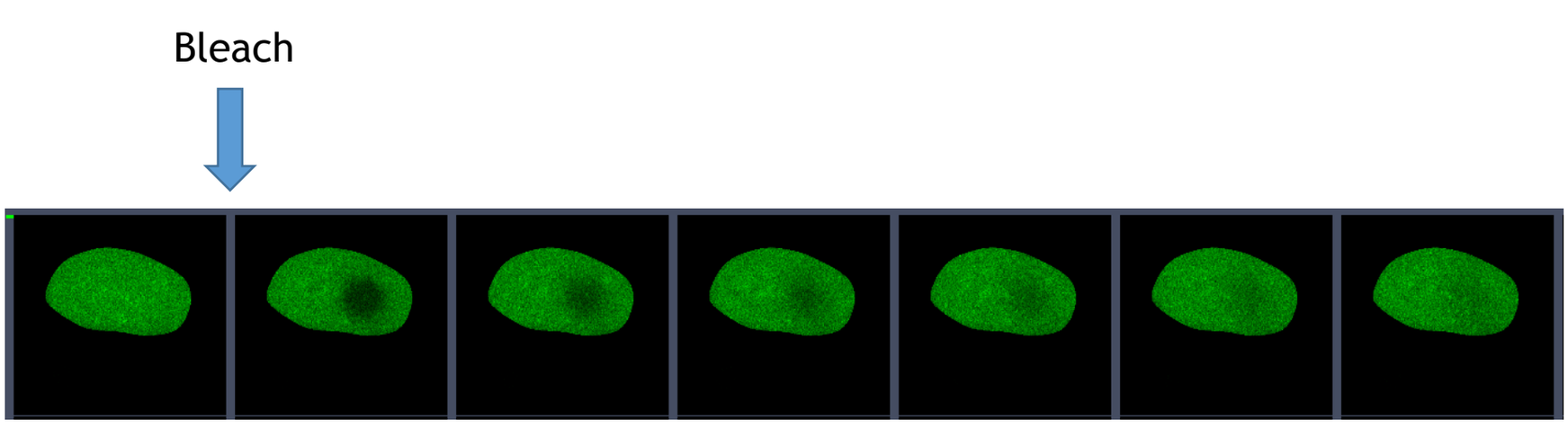
Acquire Images
In different Rois

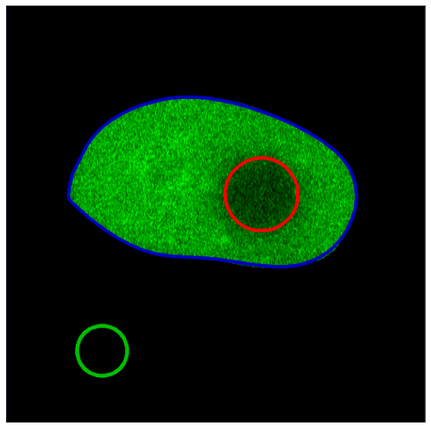
Visualize Raw Data

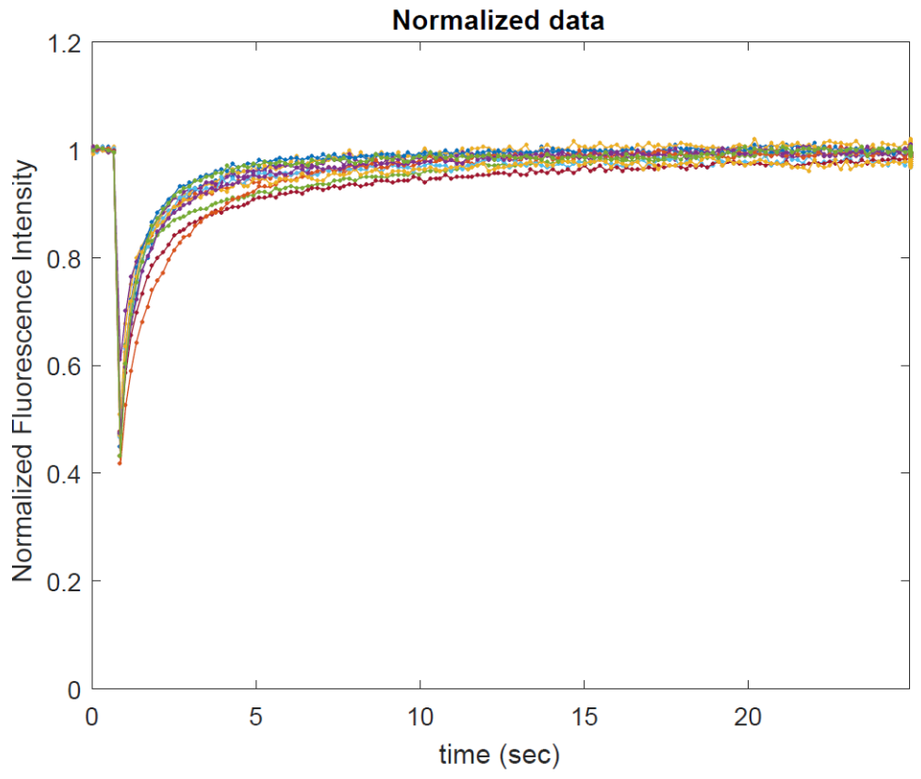
Correct for bg & bleaching

Normalize Data

Curve fitting







Moustakim et al. 2018

Instruments at Micron Imaging Facility

Fluorescence Systems

Widefield fluorescence microscopes
Personal DeltaVision
DeltaVision Core
DeltaVision Elite
DeltaVision Elite 37°C

Scanning confocal systems
ZEISS LSM 780
ZEISS LSM 880 inverted w/ Airyscan
ZEISS LSM 880 upright w/ Airyscan
Olympus FV1000
Olympus FV1200
Olympus FV3000

Spinning disk confocal systems
PerkinElmer UltraVIEW

Lightsheet systems
ZEISS lightsheet Z.1

Super-resolution Systems

Structured illumination
DeltaVision OMX V2
DeltaVision OMX V3

Photoactivated localization
Bespoke PALM/TIRF

Stochastic optical reconstruction
Nanoimager

Image Analysis Suite

OME database
Image processing and analysis softwares

FIJI/ImageJ	Imaris
Arivis	SoftWorx
MatLab	Volocity
Chromagnon	Zen blue

Thank you!

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