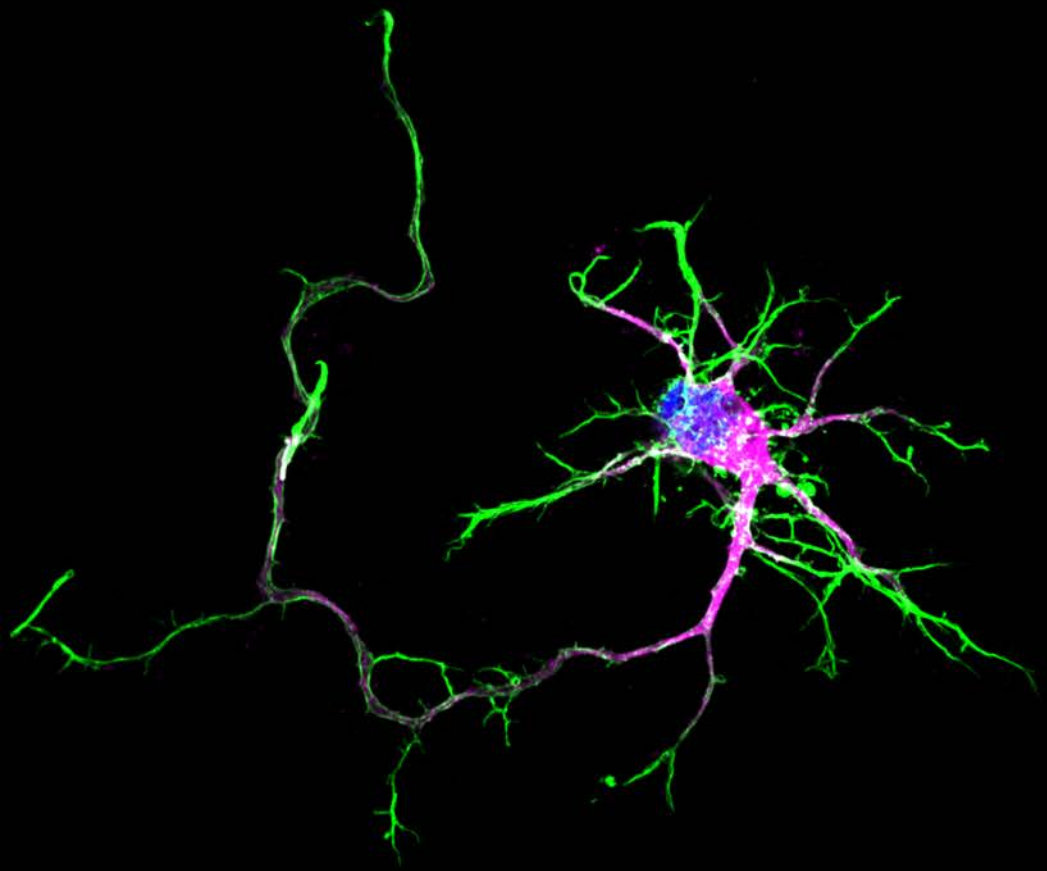
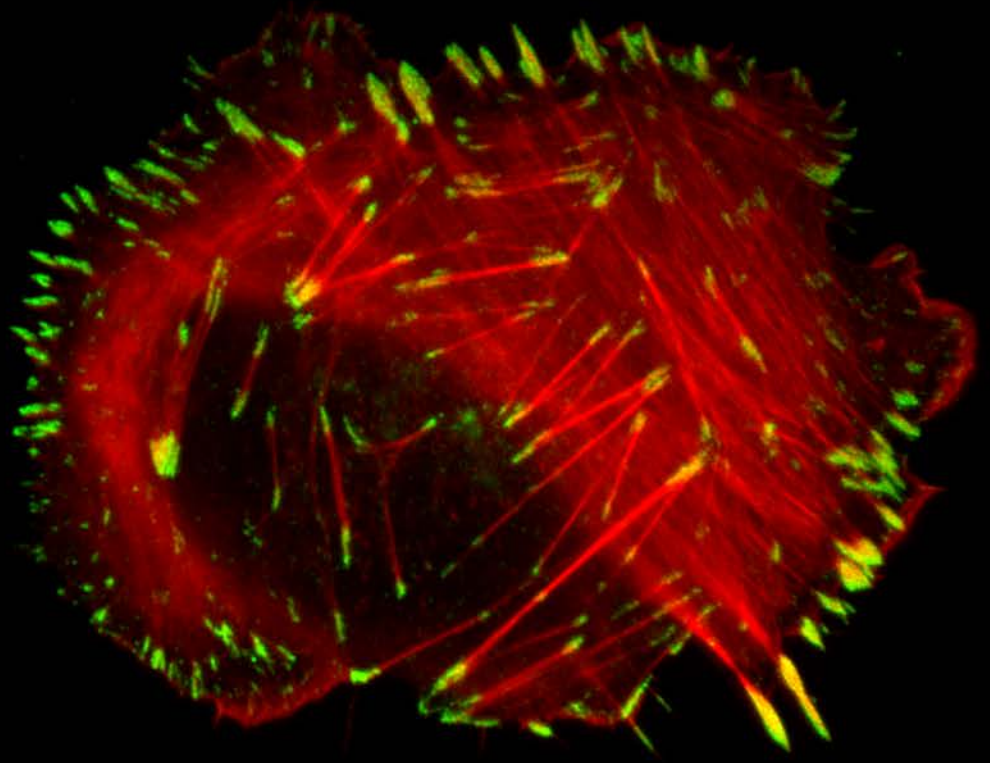
The (my) Bio-Imaging toolbox





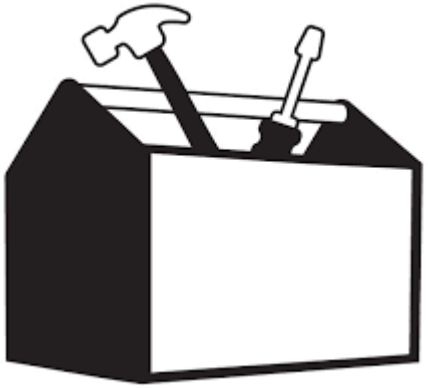
- Crash course –

Guillaume Jacquemet
@guijacquemet

Fair Warning

This is a crash course and...

... I will most likely crash too



This is a toolbox, by no mean a comprehensive lecture

First and most important tool:

- Interact with your colleagues

- Help from the Facilities

- Help from social media

However some questions are too technicals / at the forefront of research

Use social media to ask experts:

- Very large number of microscopists on twitter

- I now get most of my advices from there:

  - objectives to buy / new dye for STED / software / new Fps New technologies.

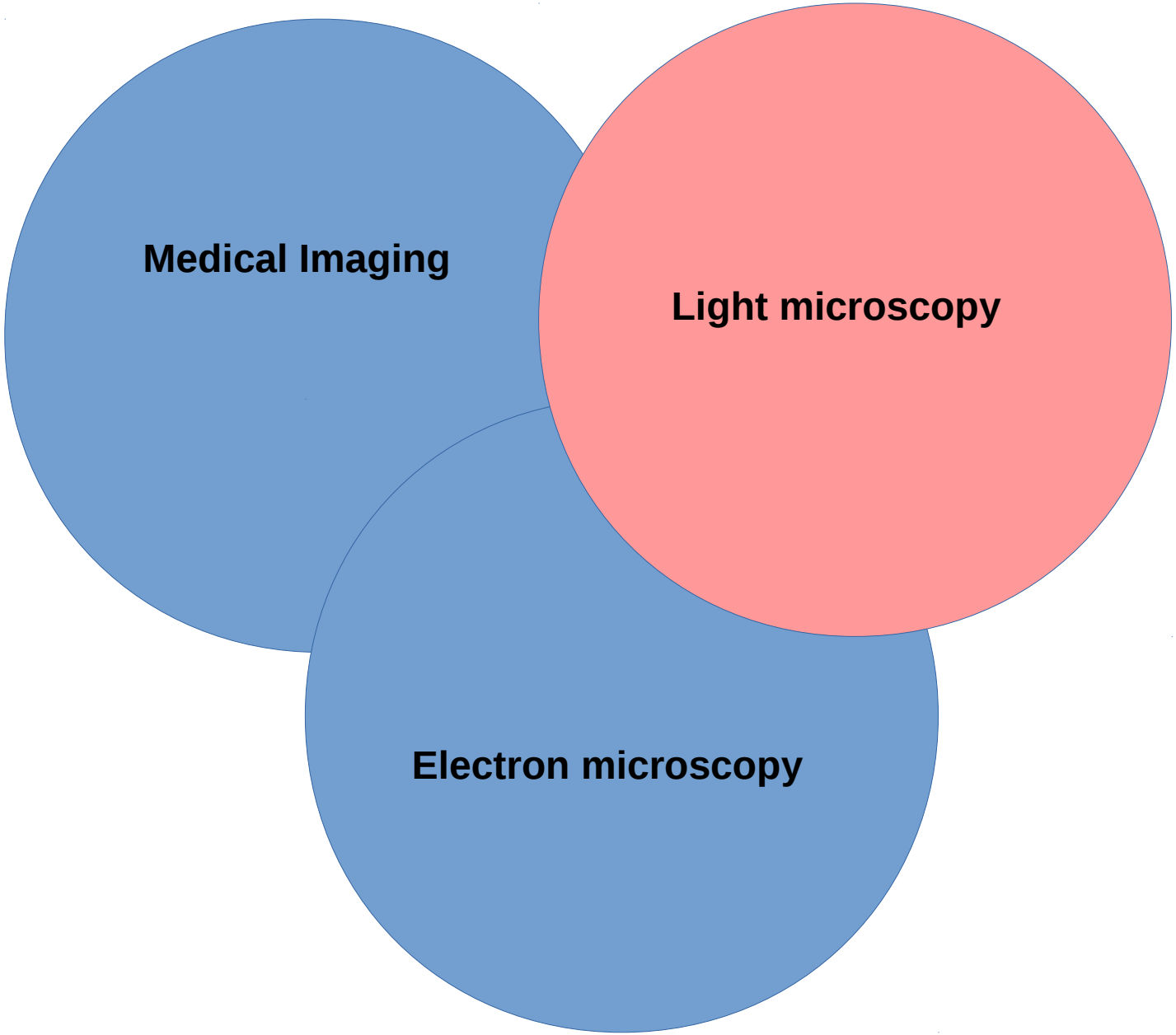
Outline

- Microscopy 101

- Embrace the future !

- Basic Theory
- Microscopes & hardware
- Sample preparation
- Live cell imaging
- Data analysis and softwares

Imaging techniques



Scale and Resolution

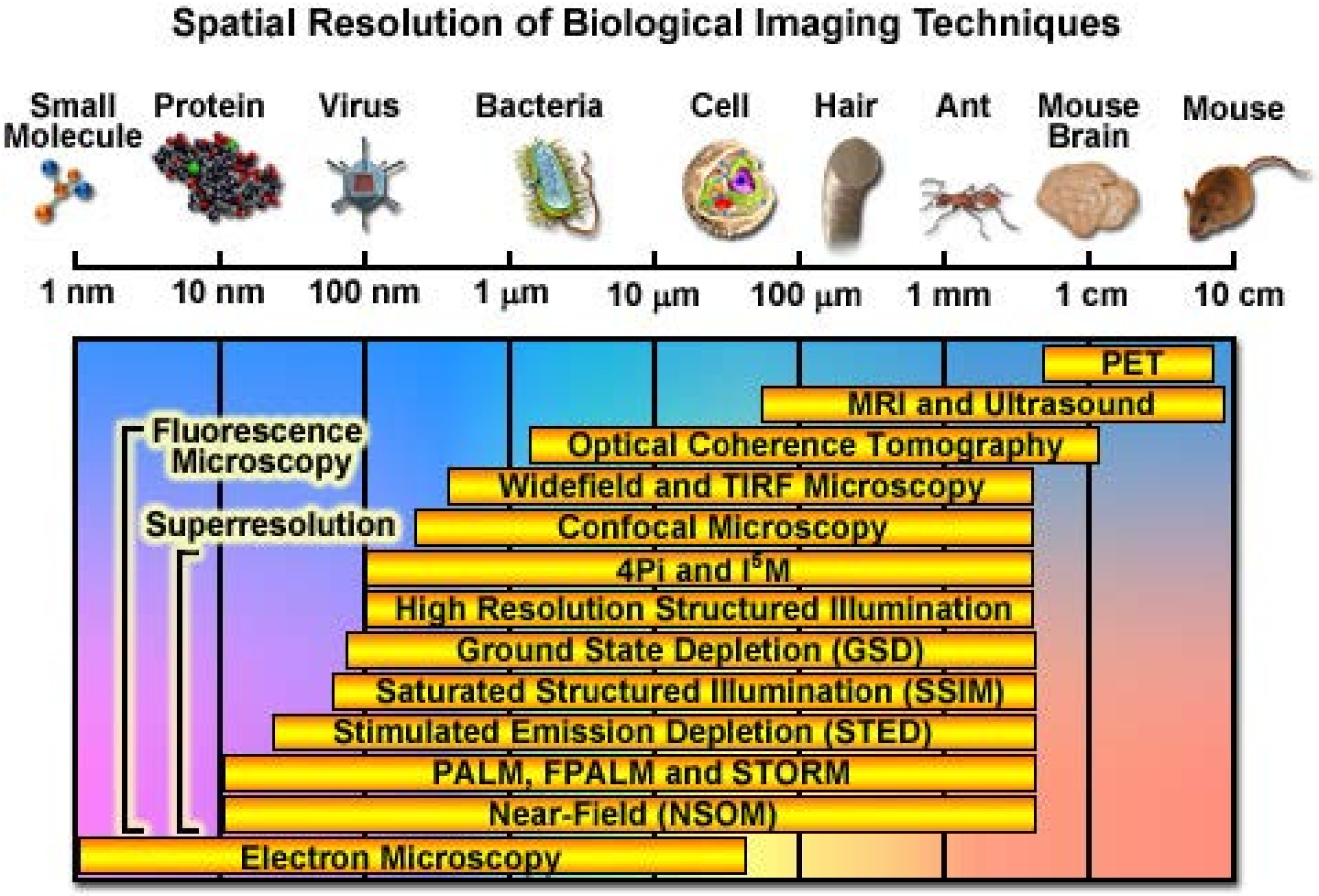
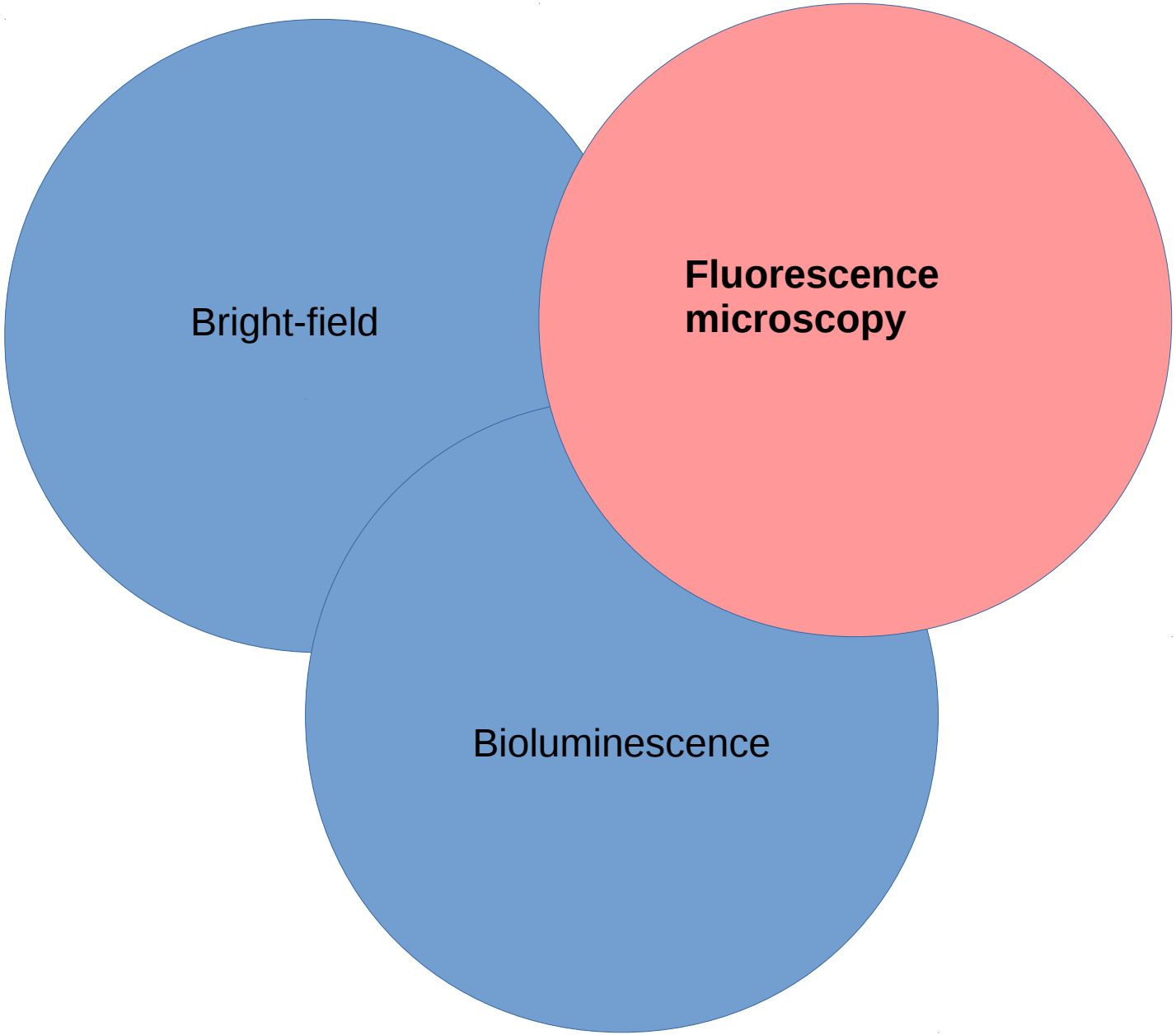


Figure 1

Light microscopy



Bright-field microscopy

Bright-field microscopy

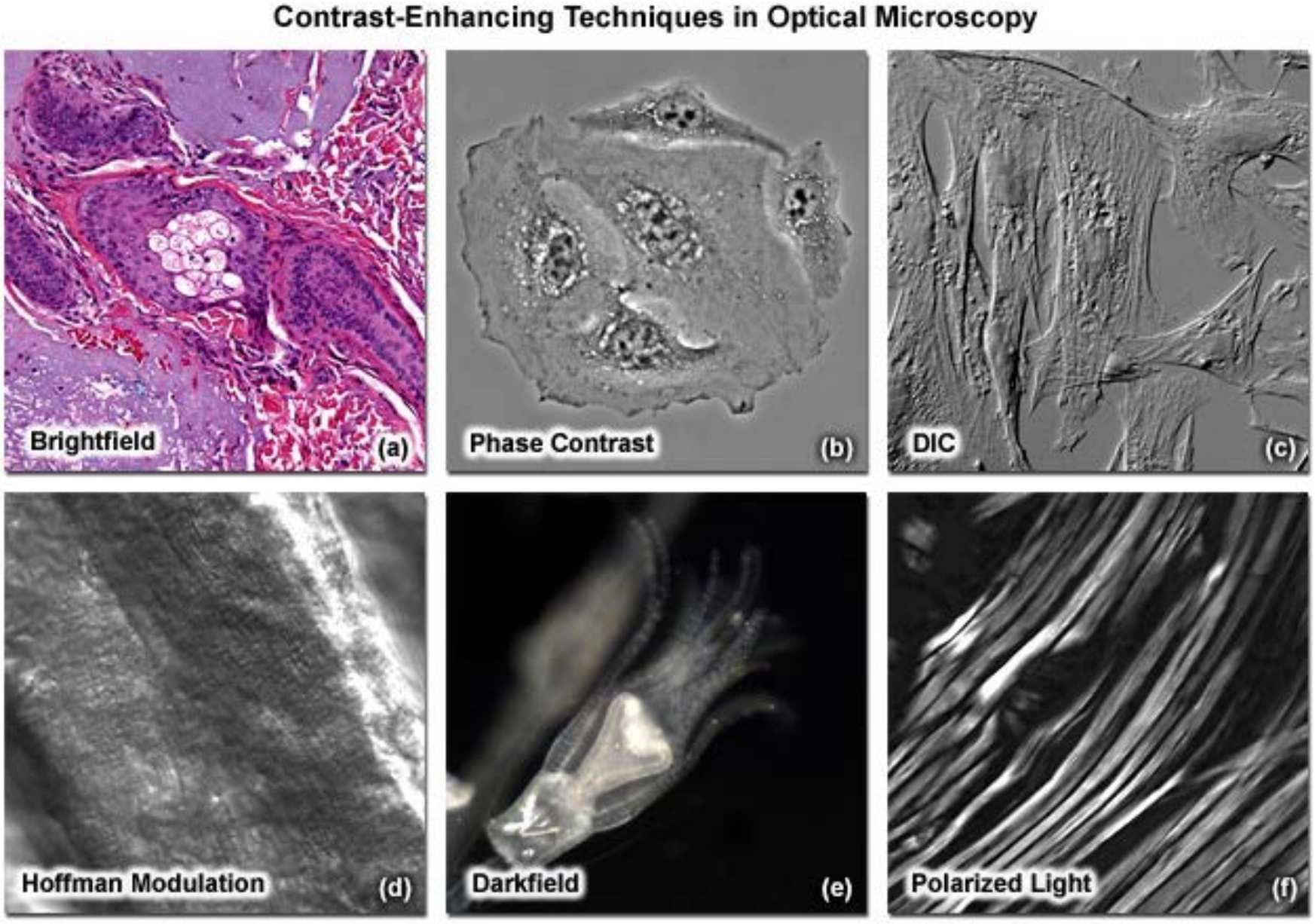


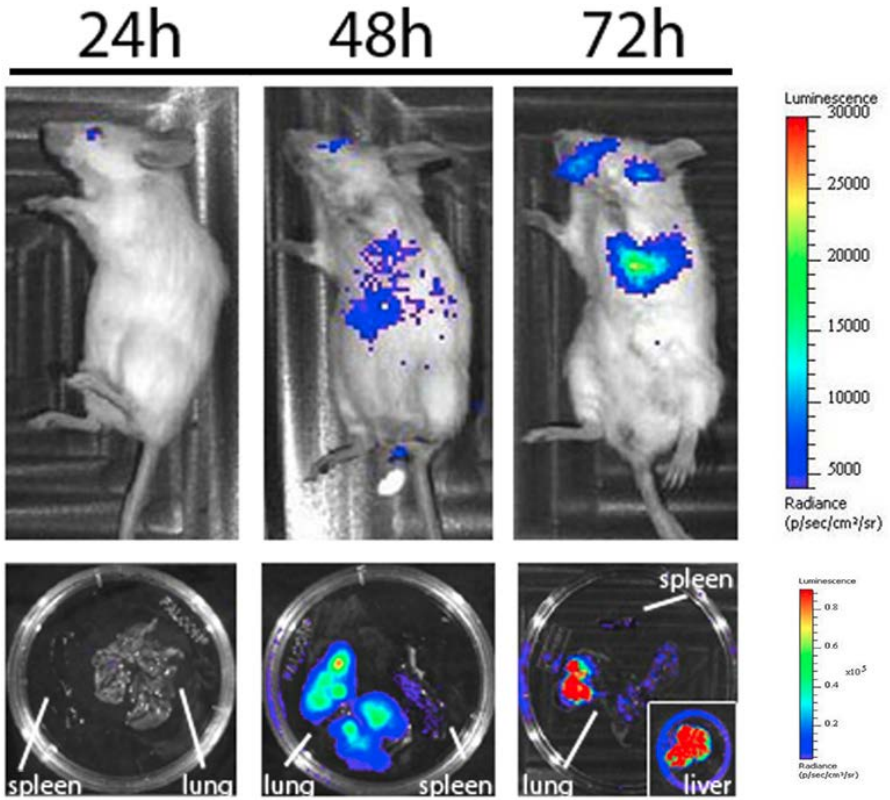
Figure 1

Bio-luminescence Microscopy

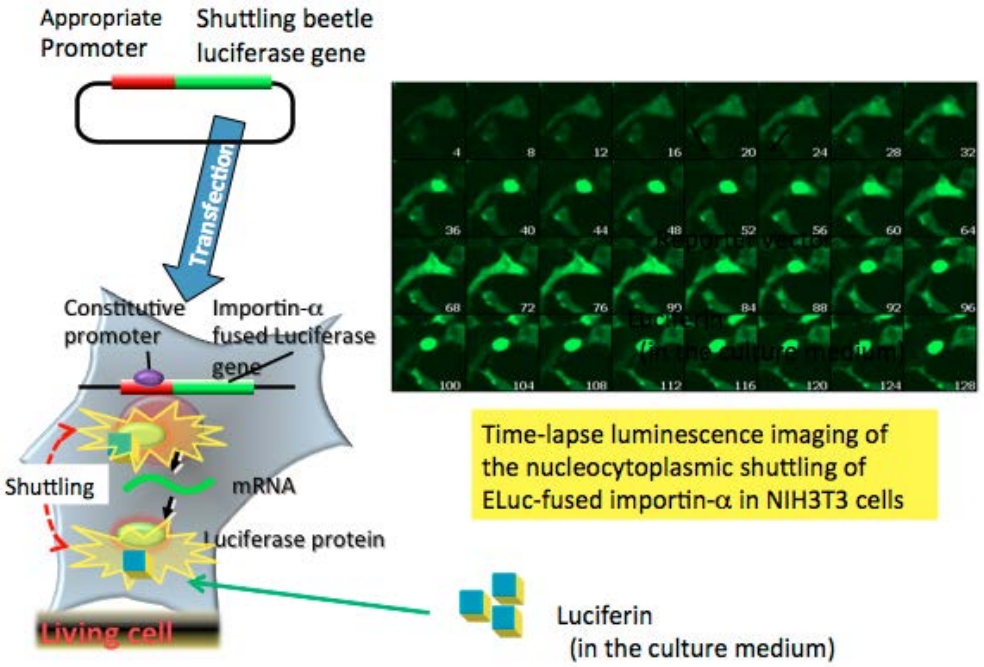
Luciferase + substrate = Light

Bio-luminescence Microscopy

In vivo imaging



Cellular reporters



Advantages

- Not Toxic !!
- Very long term imaging
- Light until substrate run out

Limitations

- Poor resolution
- Everything shine at once
- One color
- Expensive detection system

Embrace the future

ARTICLE

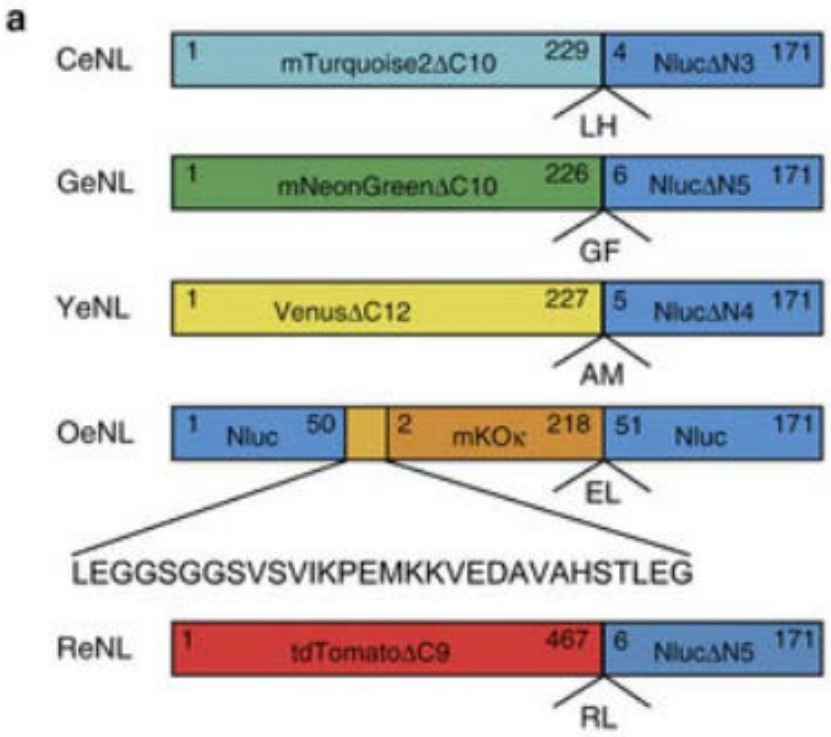
Received 12 Mar 2016 | Accepted 26 Oct 2016 | Published 14 Dec 2016

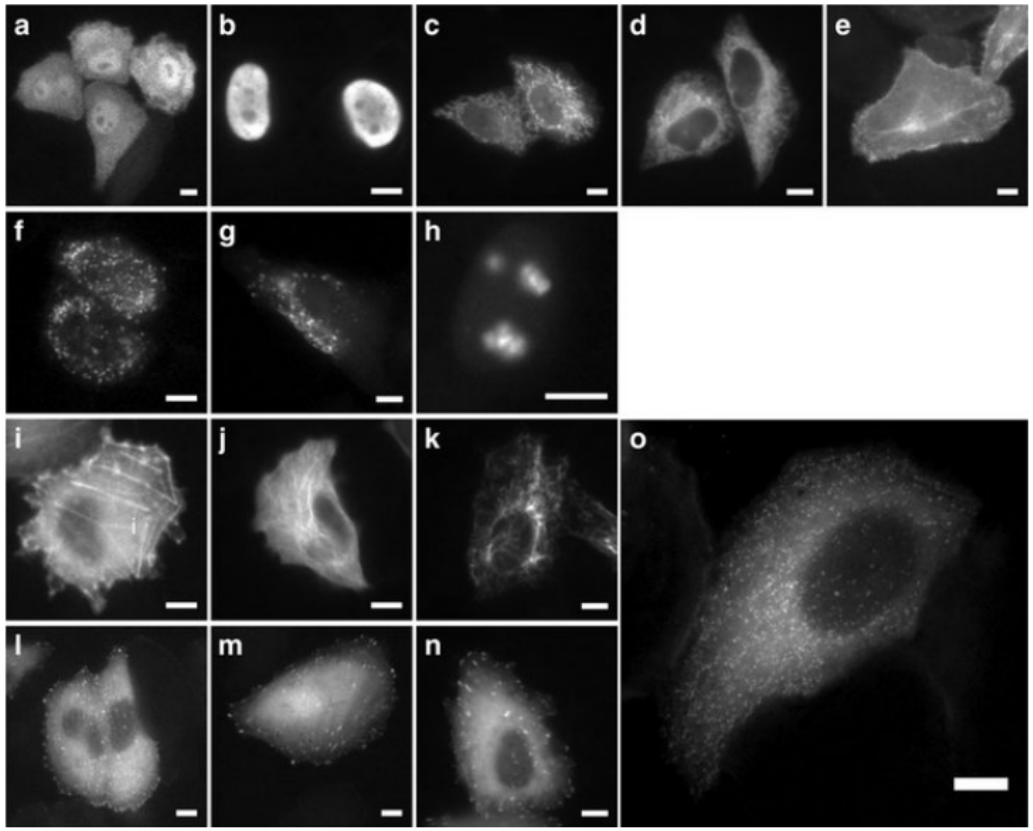
DOI: 10.1038/ncomms13718 OPEN

Five colour variants of bright luminescent protein for real-time multicolour bioimaging

Kazushi Suzuki[1], Taichi Kimura[2], Hajime Shinoda[1], Guirong Bai[3], Matthew J. Daniels[4], Yoshiyuki Arai[1,2,3], Masahiro Nakano[1,2,3] & Takeharu Nagai[1,2,3]

FRET between luciferase and fluorescent proteins = multi color **luminescence**





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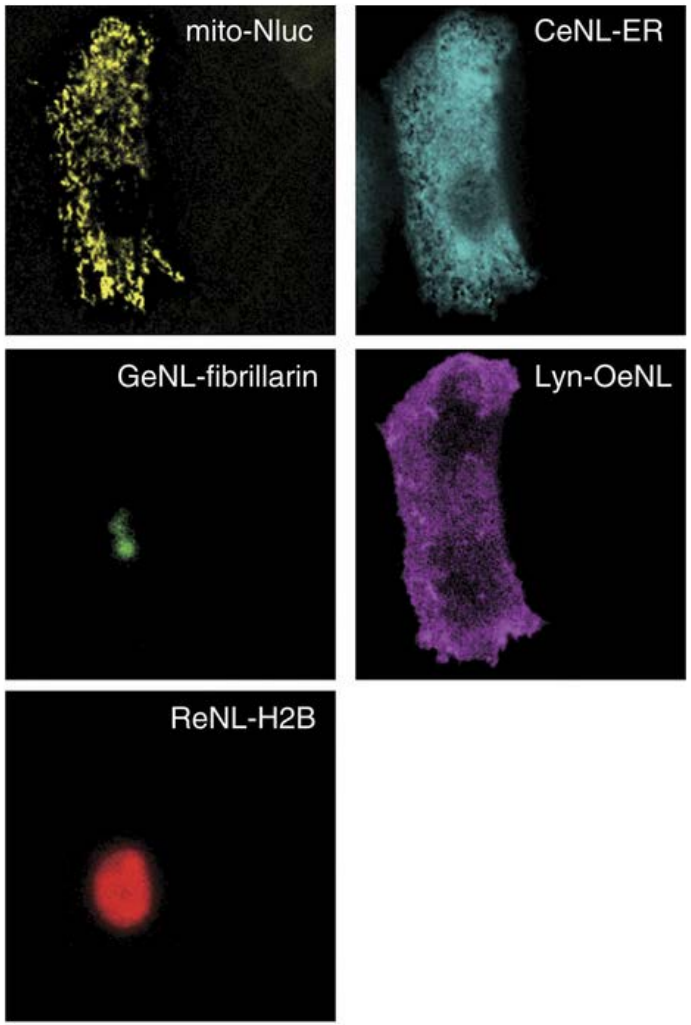
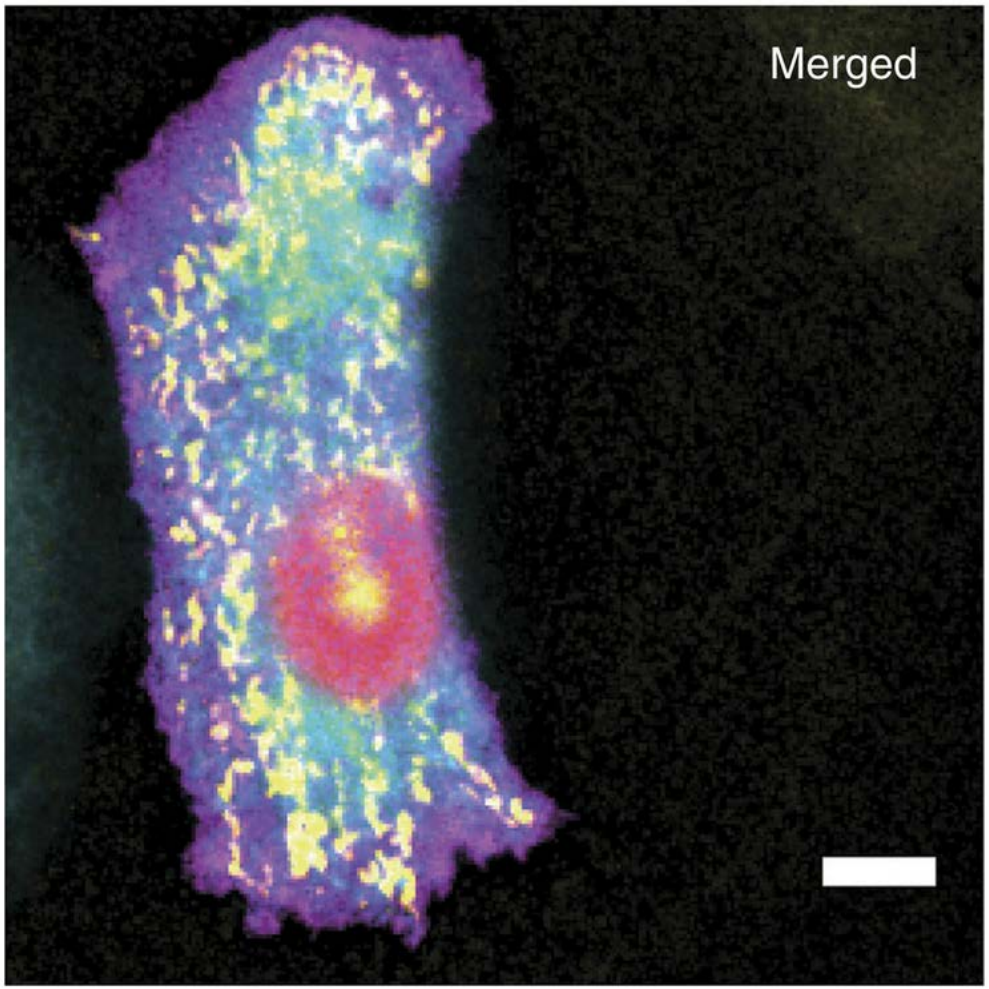
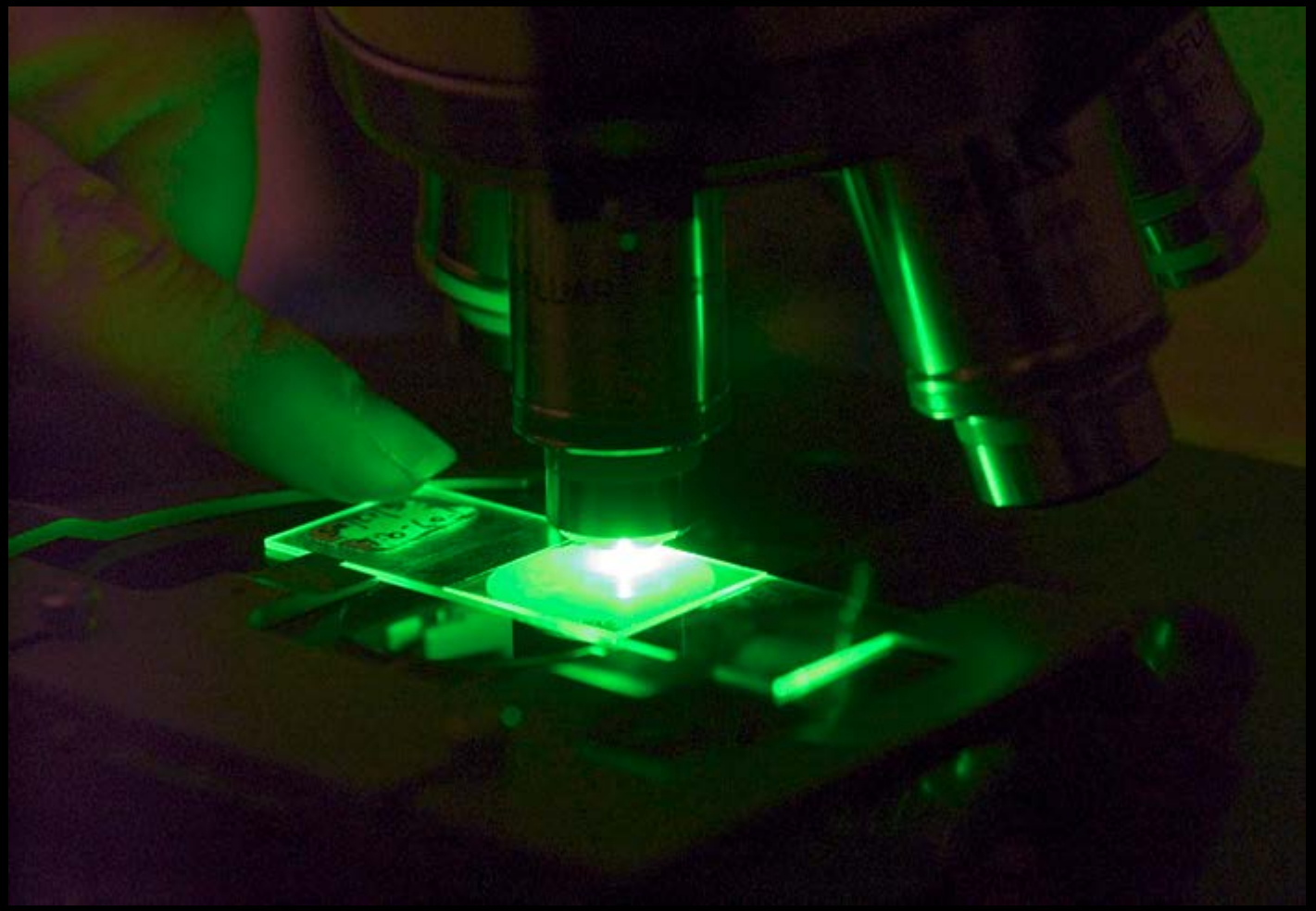
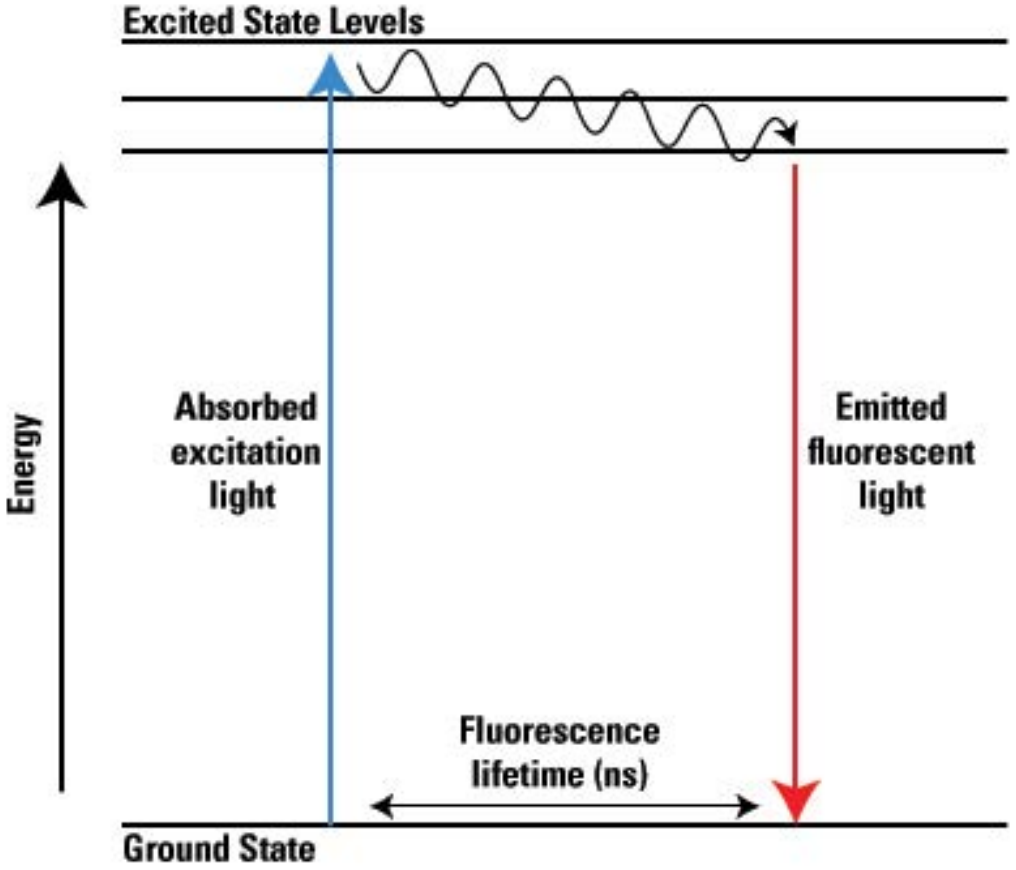


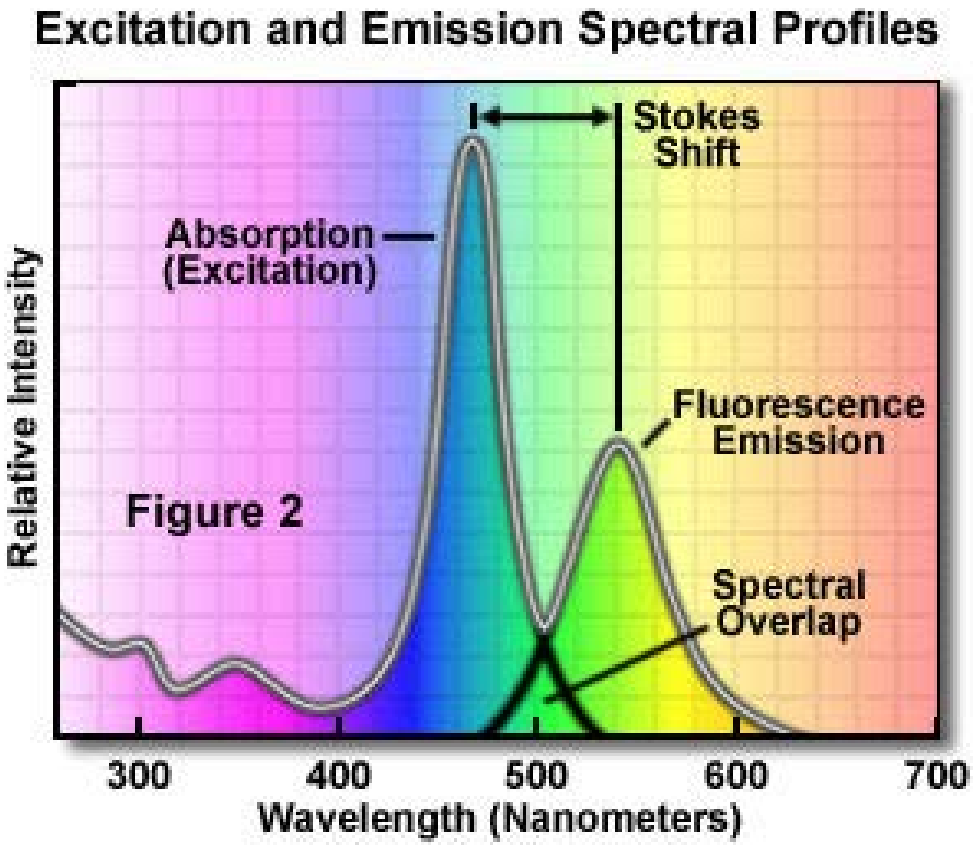


Fluorescence microscopy



Fluorescence microscopy must know I: How it works





Fluorescence microscopy must know II : The law

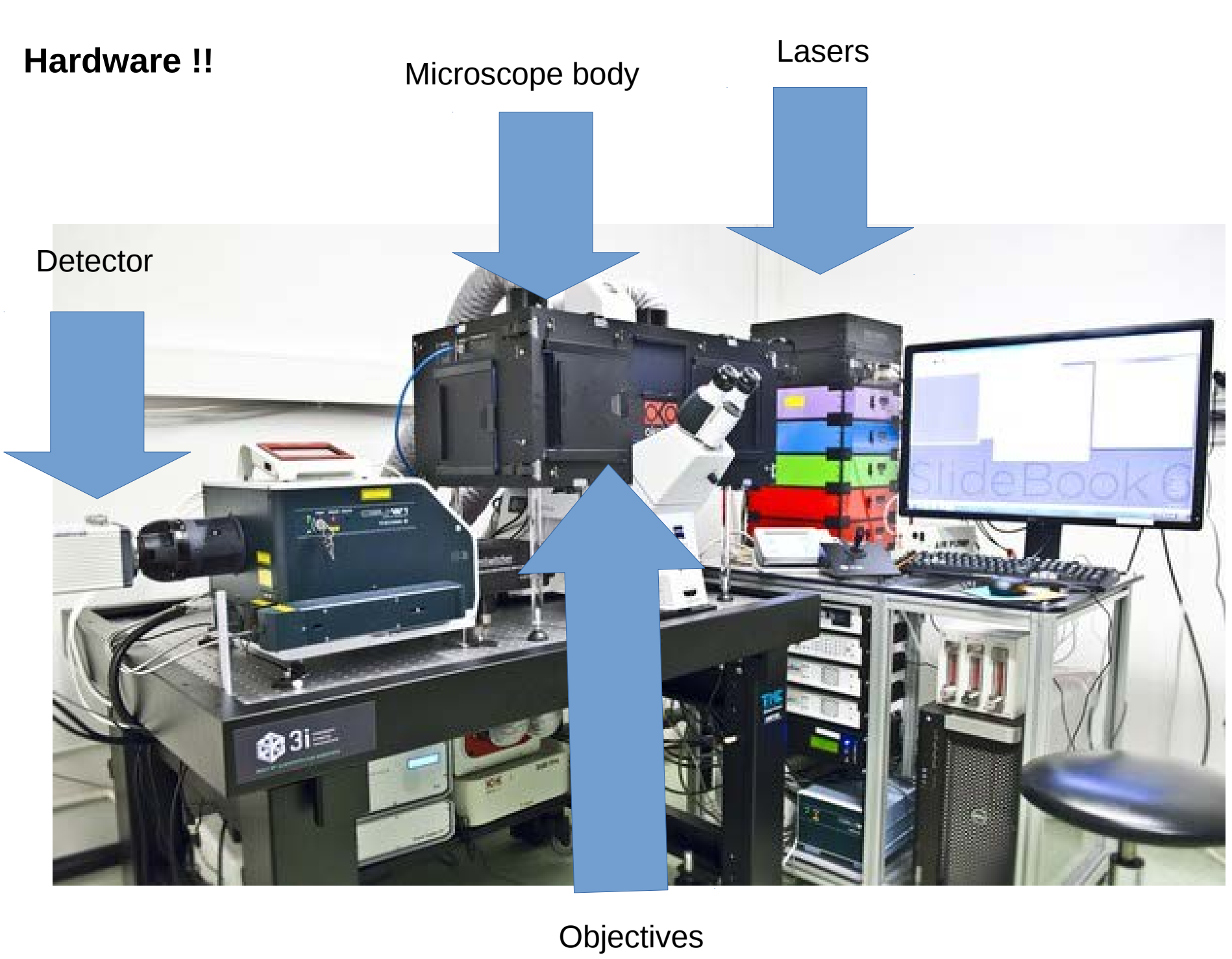
Abbe Resolution x,y = λ/2NA

λ : wavelength (fluorescence)

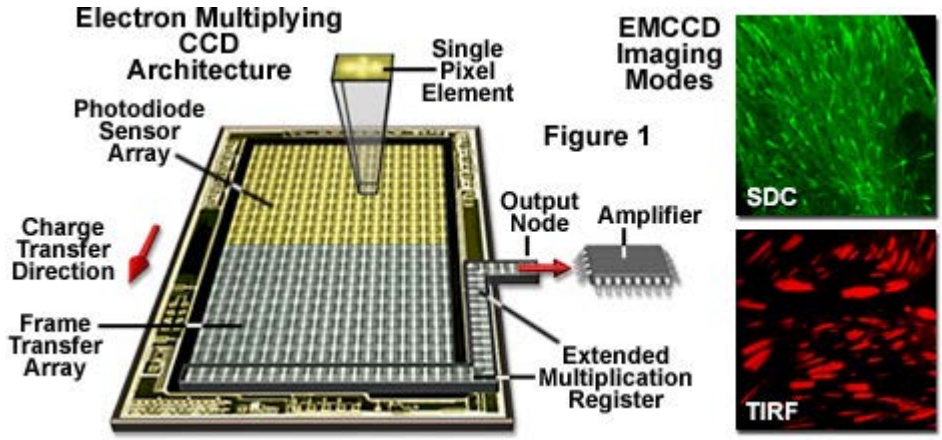
NA: numerical aperture of objective

- small wavelength (blue and green) result in higher resolution
- Use high NA objective !!!

Hardware !!


Microscope body
Lasers
Detector
Objectives
3i
SlideBook 6

Detection systems: Cameras vs detectors



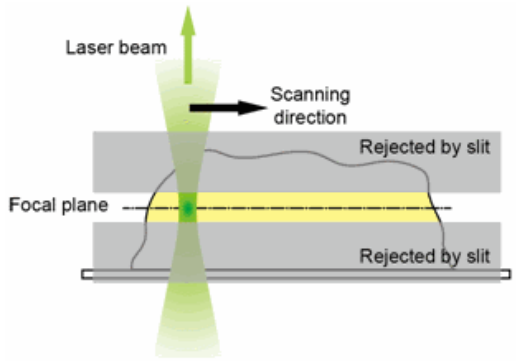
Camera

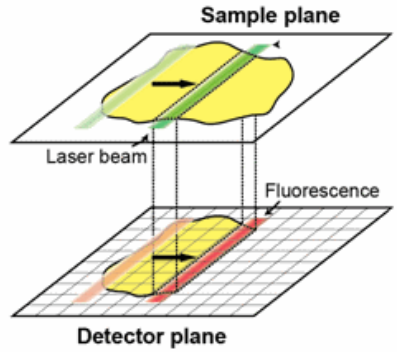
- **ORCA camera:** Not very sensitive, Large field of view, large dynamic range

- **EMCCD camera:** Very sensitive, small field of view, small dynamic range

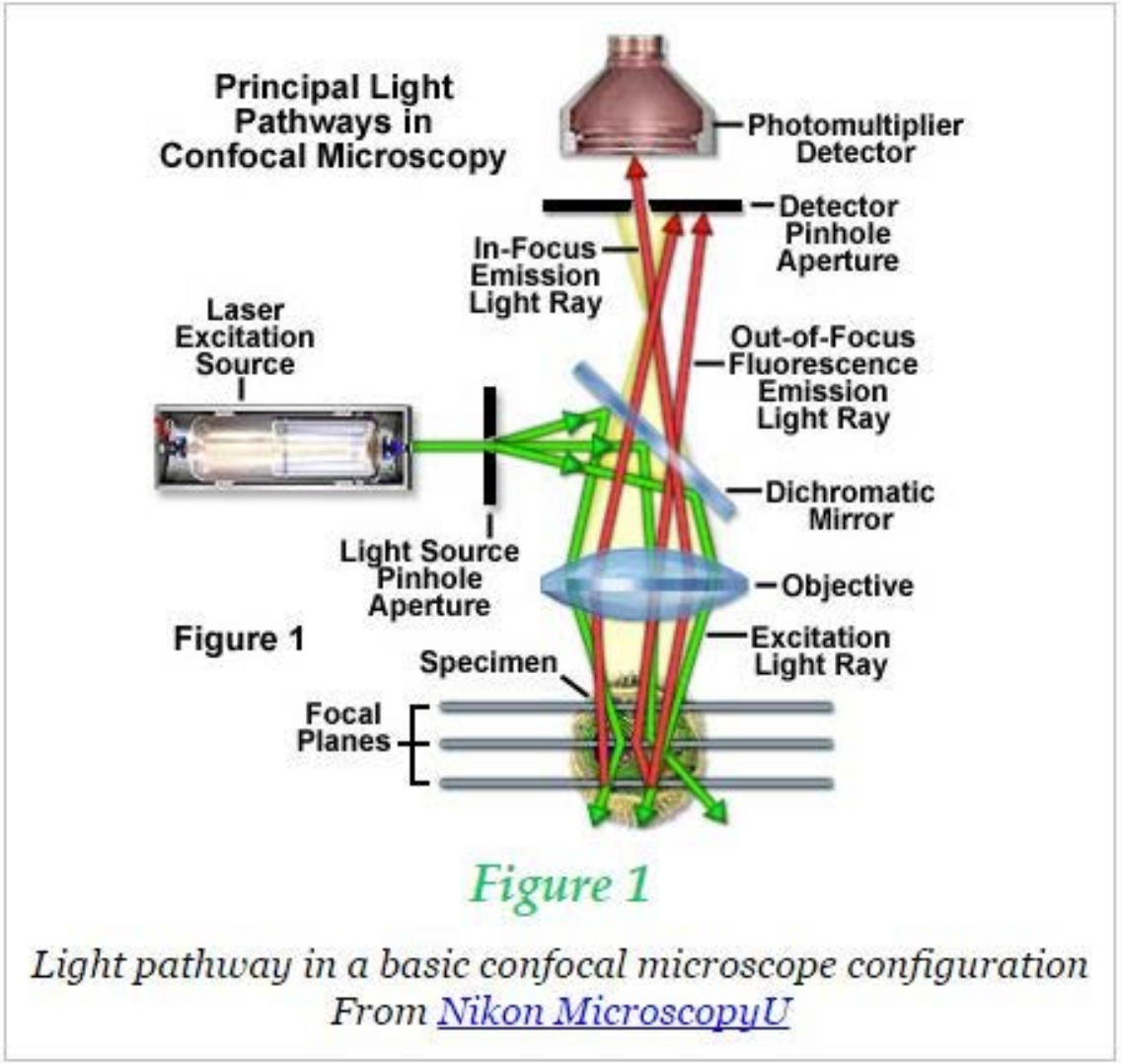
Detectors: Line based scanning detection system

- Very sensitive and tunable zoom, slow, noisy



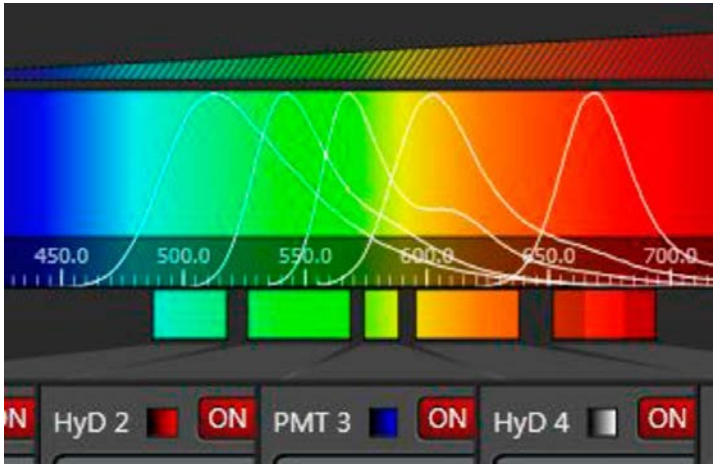


Confocal microscope (LSM 780)



- Adjustable Pinhole
to remove out of focus light

- Tunable spectra detection

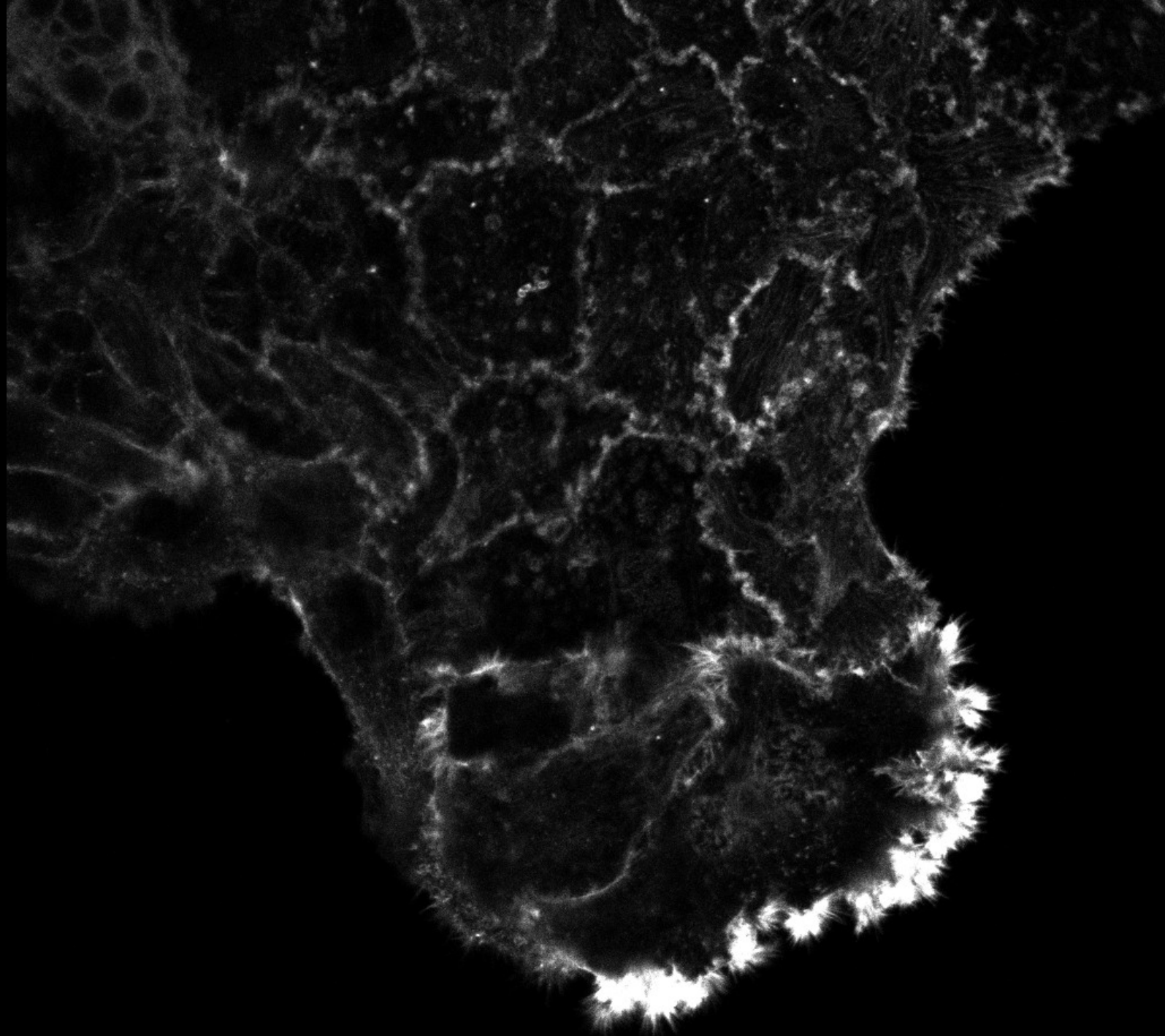


- Scanning-based detection
    - Sensitive
    - Tunable zoom

- Slow

https://www.microscopyu.com/techniques/confocal

Confocal



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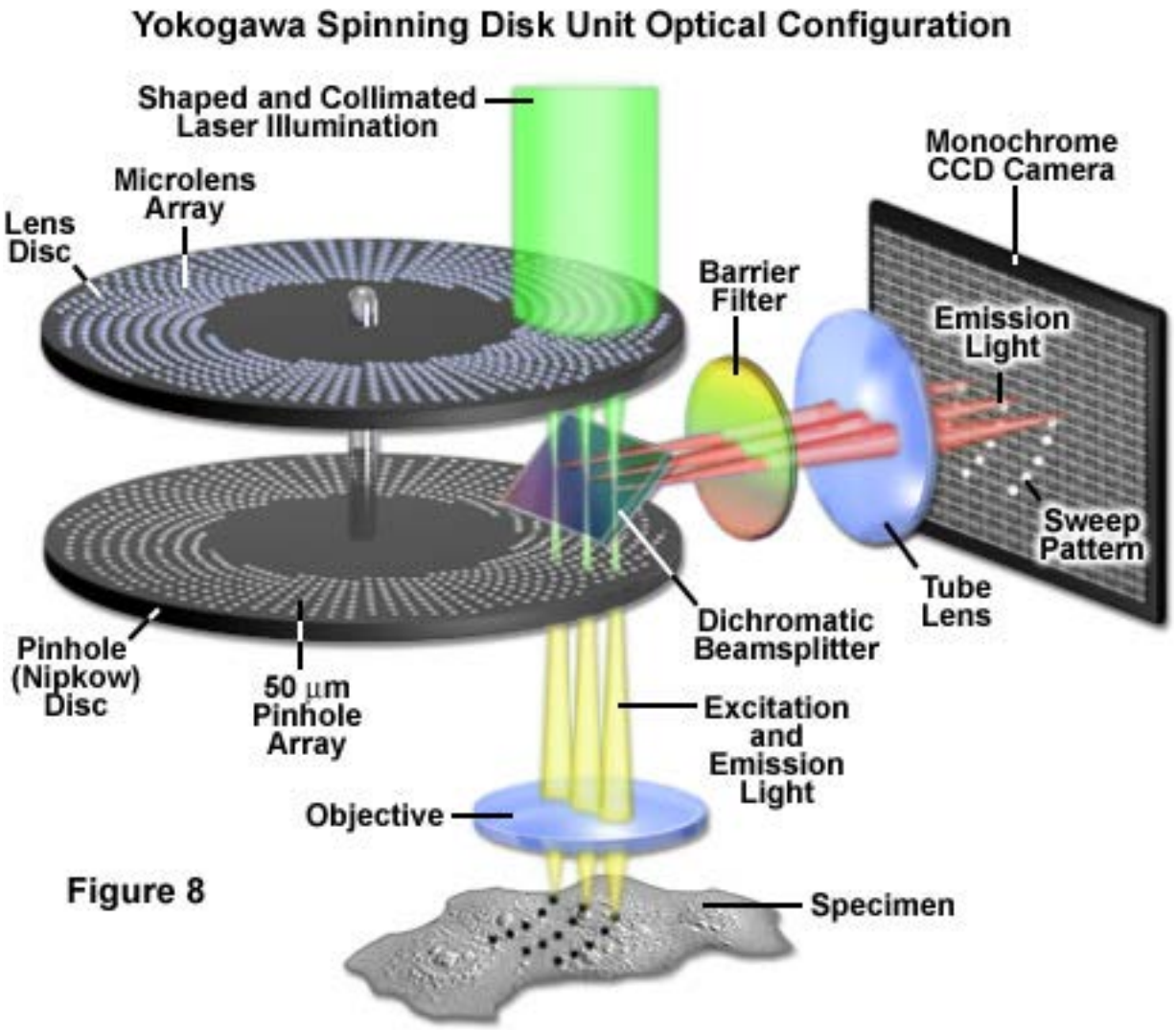
Spinning disk Confocal microscope



- Fixed pinhole

- Fixed spectra detection (filter set)

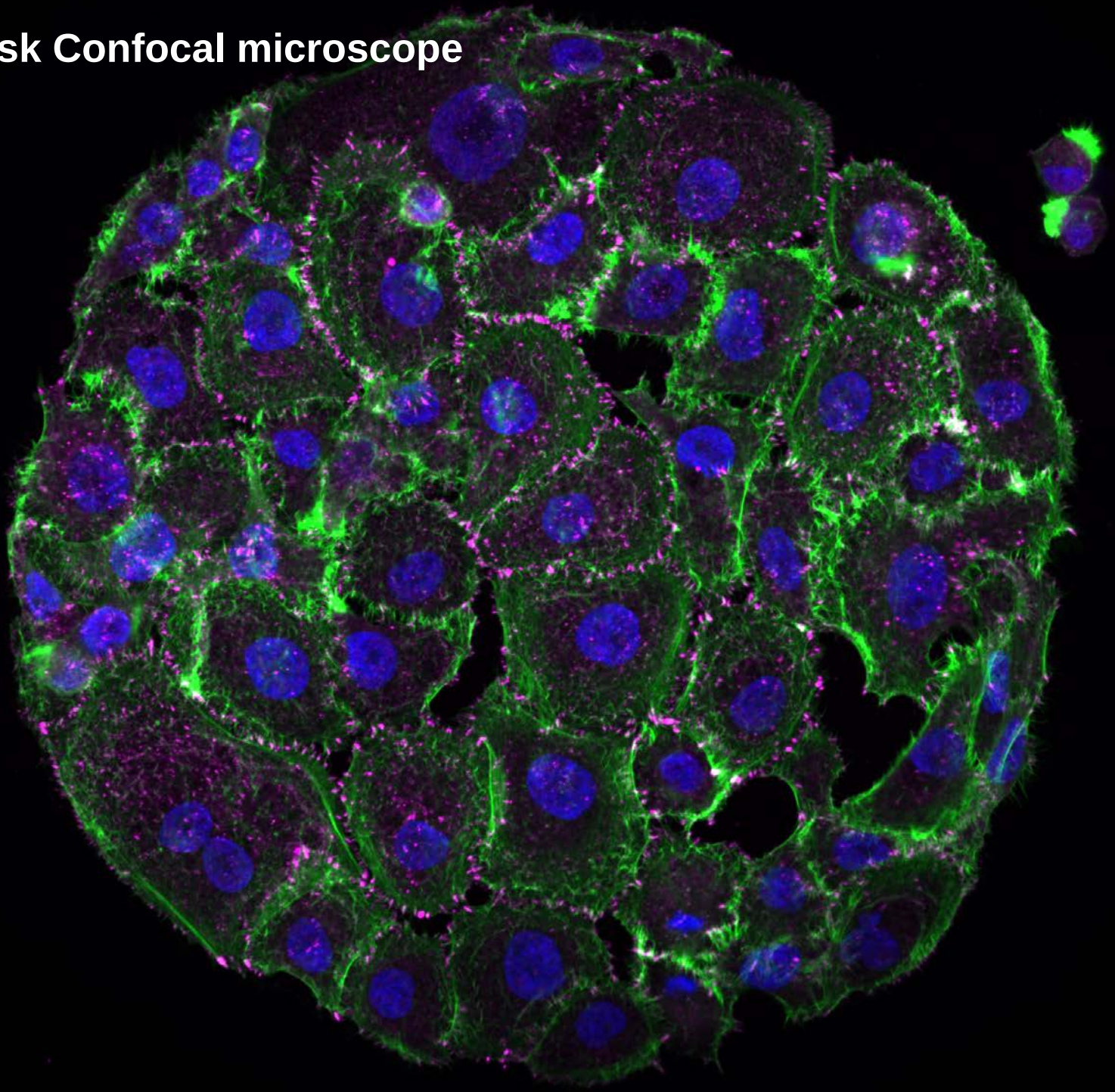
- Camera based detection
  - Fixed pixel size
  - Good quality images

- Very fast

http://zeiss-campus.magnet.fsu.edu/articles/spinningdisk/introduction.html


Spinning disk Confocal microscope
@guijacquemet

Spinning disk Confocal microscope



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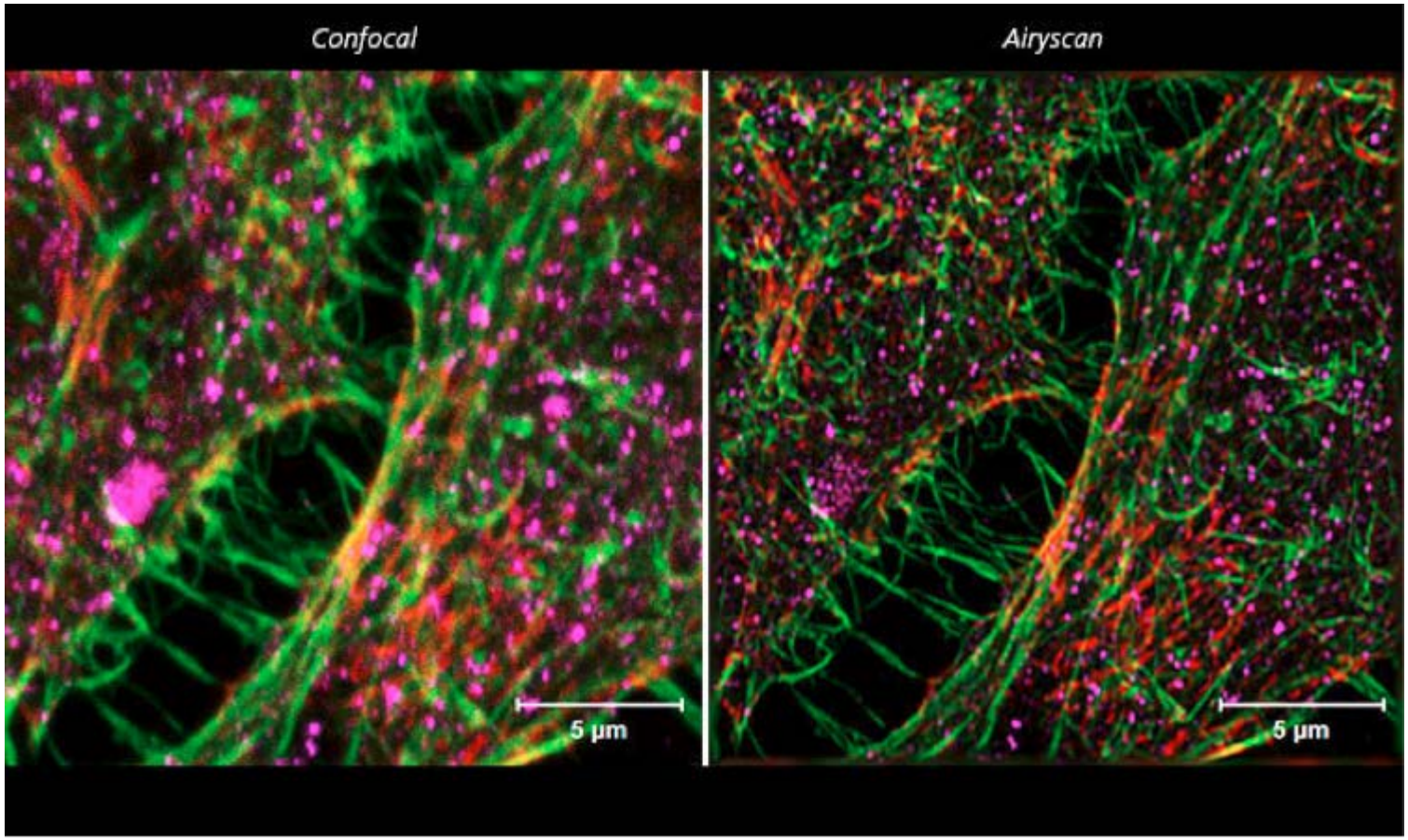
Confocal airyscan (LSM 880 + airyscan)

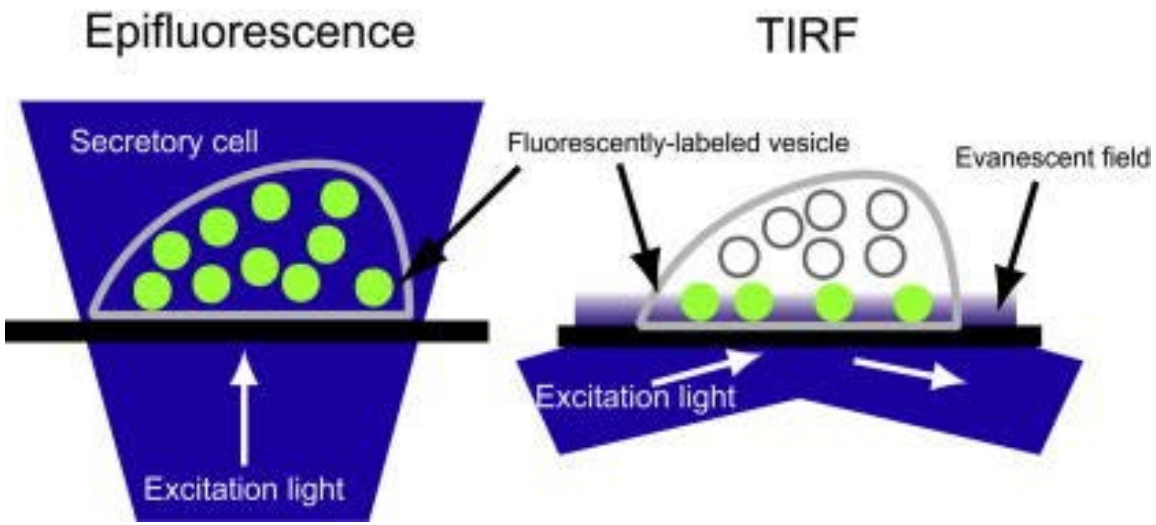
- Coming soon to Turku / CIC

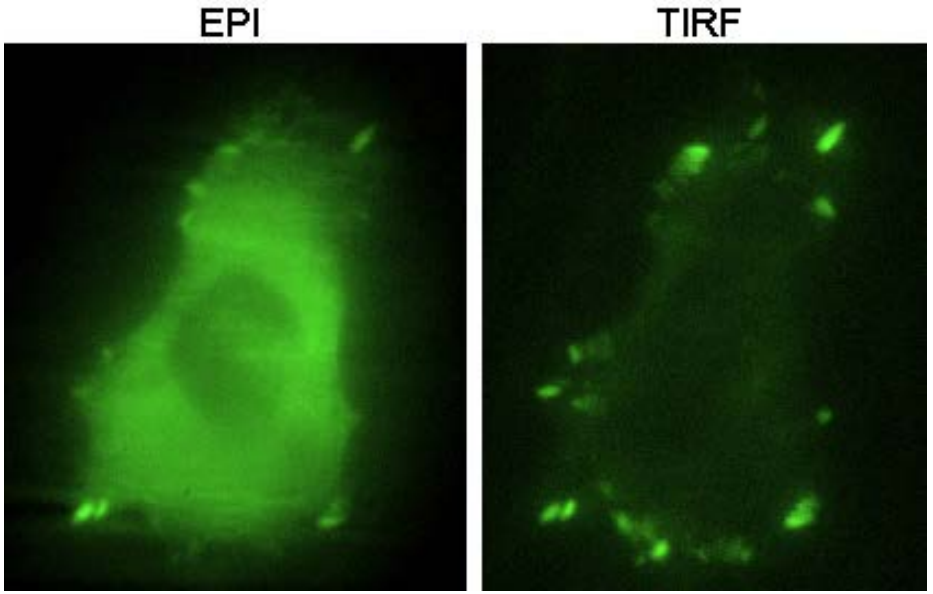
- Confocal, but much faster and higher resolution



http://bitesizebio.com/product_article/zeiss-airyscan-a-brave-new-microscopy-world-with-sharper-confocal-resolution/

TIRF microscope





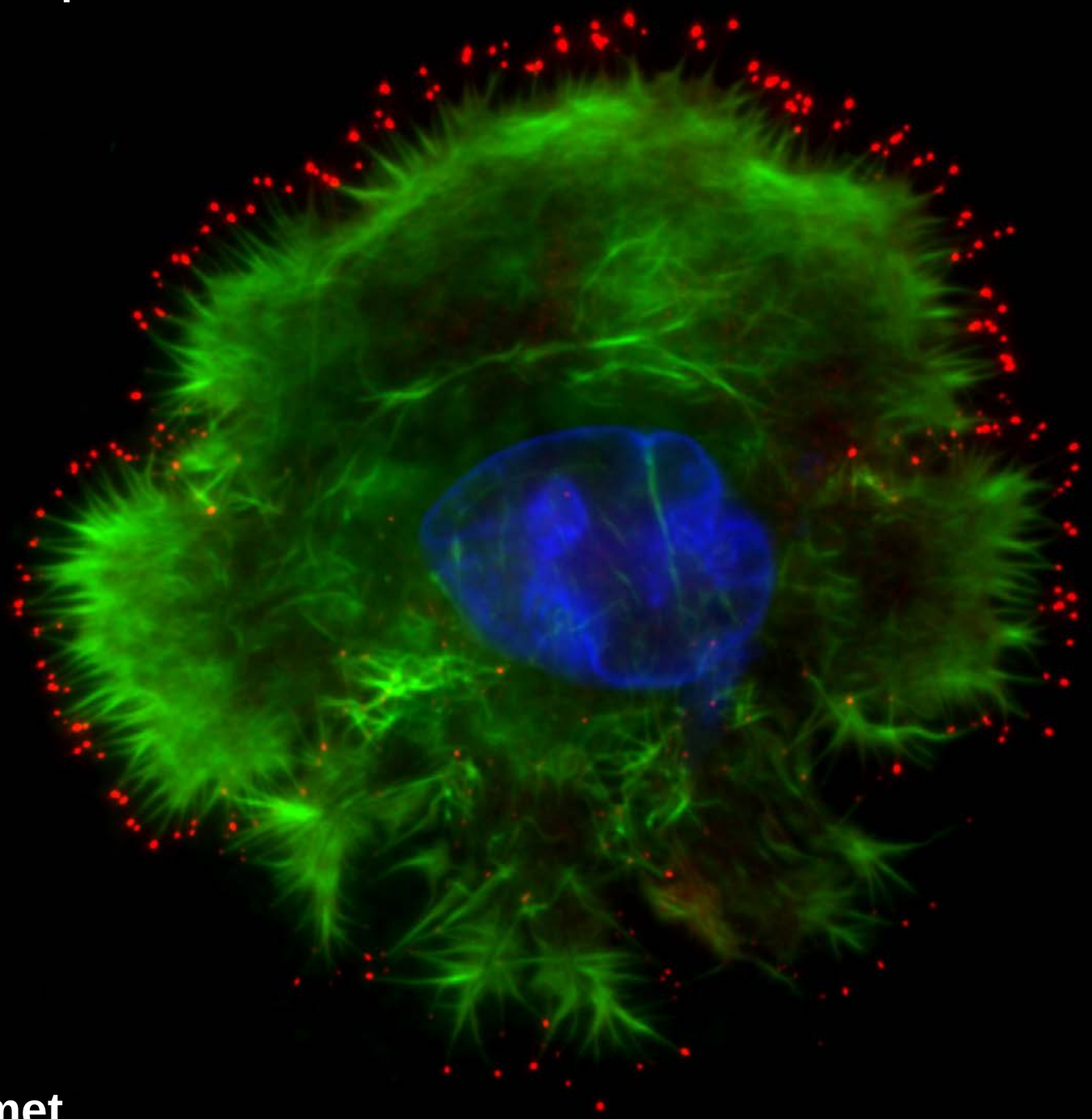
Focal adhesion

- 200 nm from the glass
- Very low light
- Camera based detection
- Fast

Great for live imaging

https://www.microscopyu.com/techniques/fluorescence/total-internal-reflection-fluorescence-tirf-microscopy

TIRF microscope



@guijacquemet

High content microscope → Screening platform

Contact: Michael Courtney

Pathway855 (BD)

Pathway855 High-Content imager (BD)

Our BD Pathway855 imager with on-stage fluidics is integrated with an automated incubator (capacity 42 plates) and ambient storage (capacity 120 plates/tip-racks). Addition items from our original systems have now arrived and these are currently (Jan/Feb 2016) being reintegrated.

The Pathway 855 imager has the following features:

Environmentally controlled chamber with thermostatting of entire optical path

Fast 100nm resolution xy positioning of objective for correction-free montaging

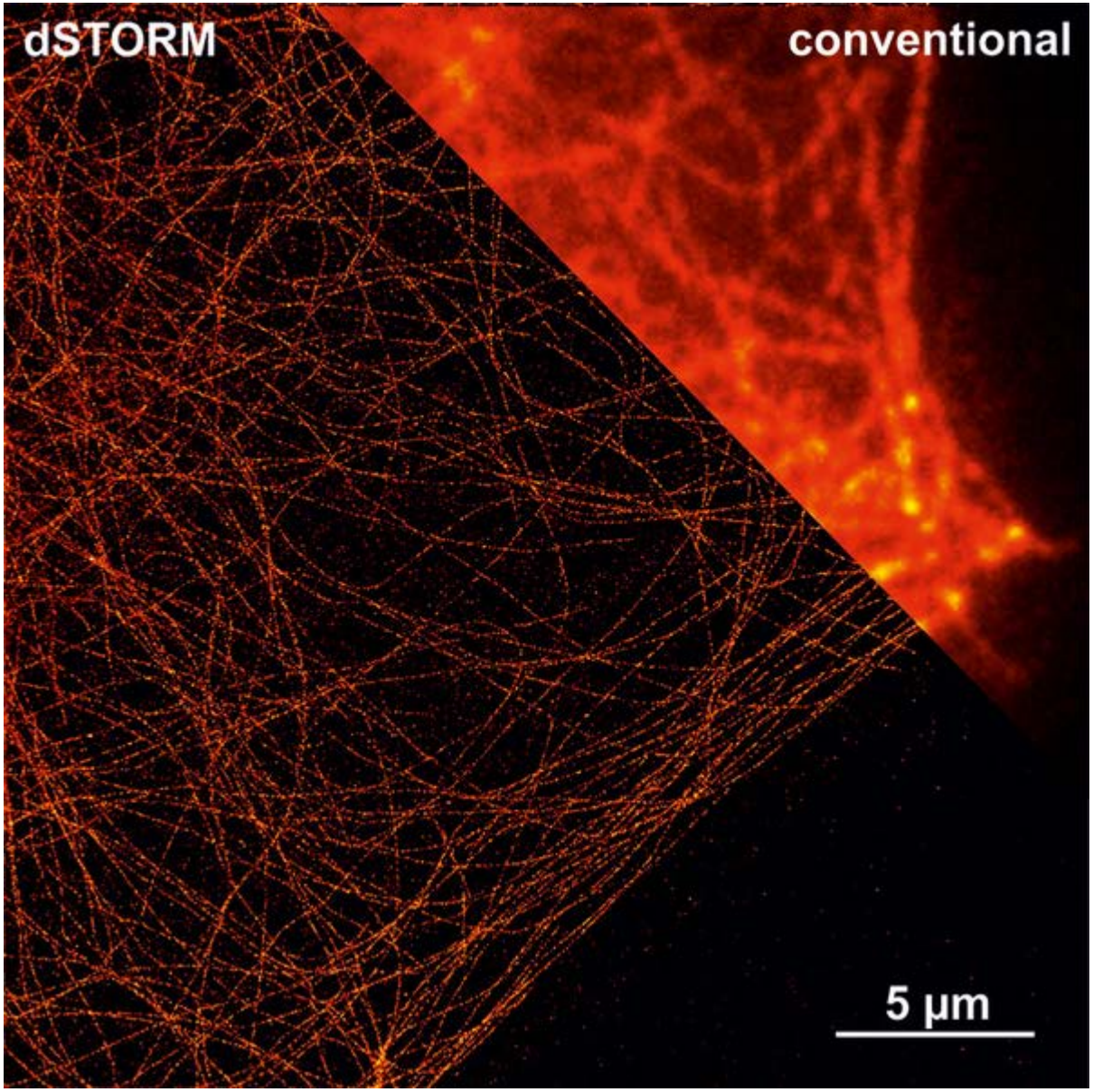
Reliable fast laser focus and 50nm resolution z positioning

Automated reagent addition from reagent plate with mixing while imaging

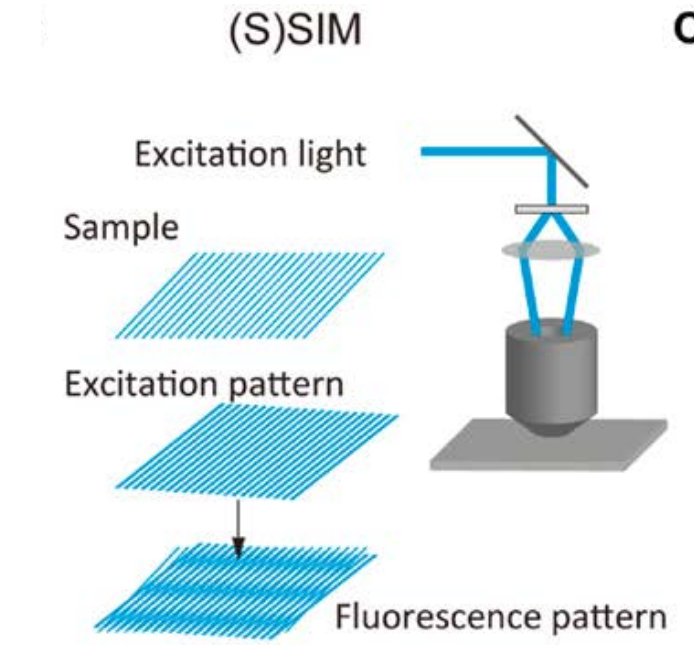
Dual, continuous spectrum calibrated excitation sources

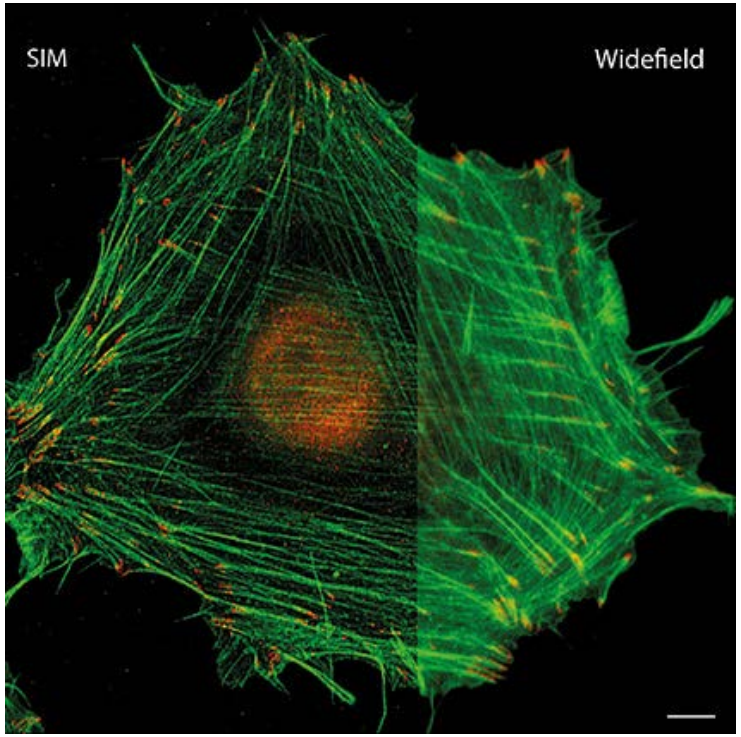
http://www.btk.fi/research/research-groups/courtney/hca-and-htm-at-the-university-of-turku/

Super resolution microscopes

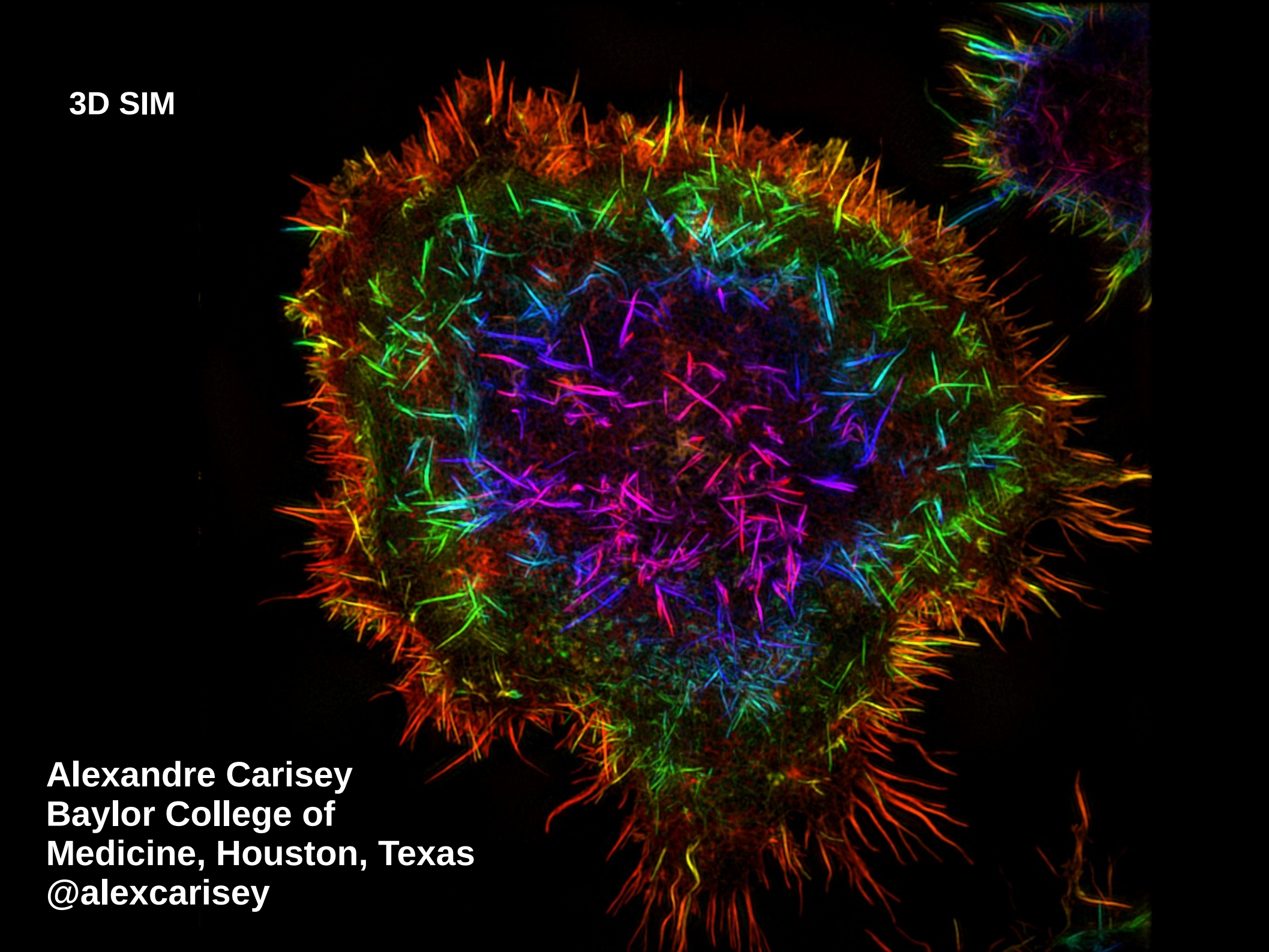


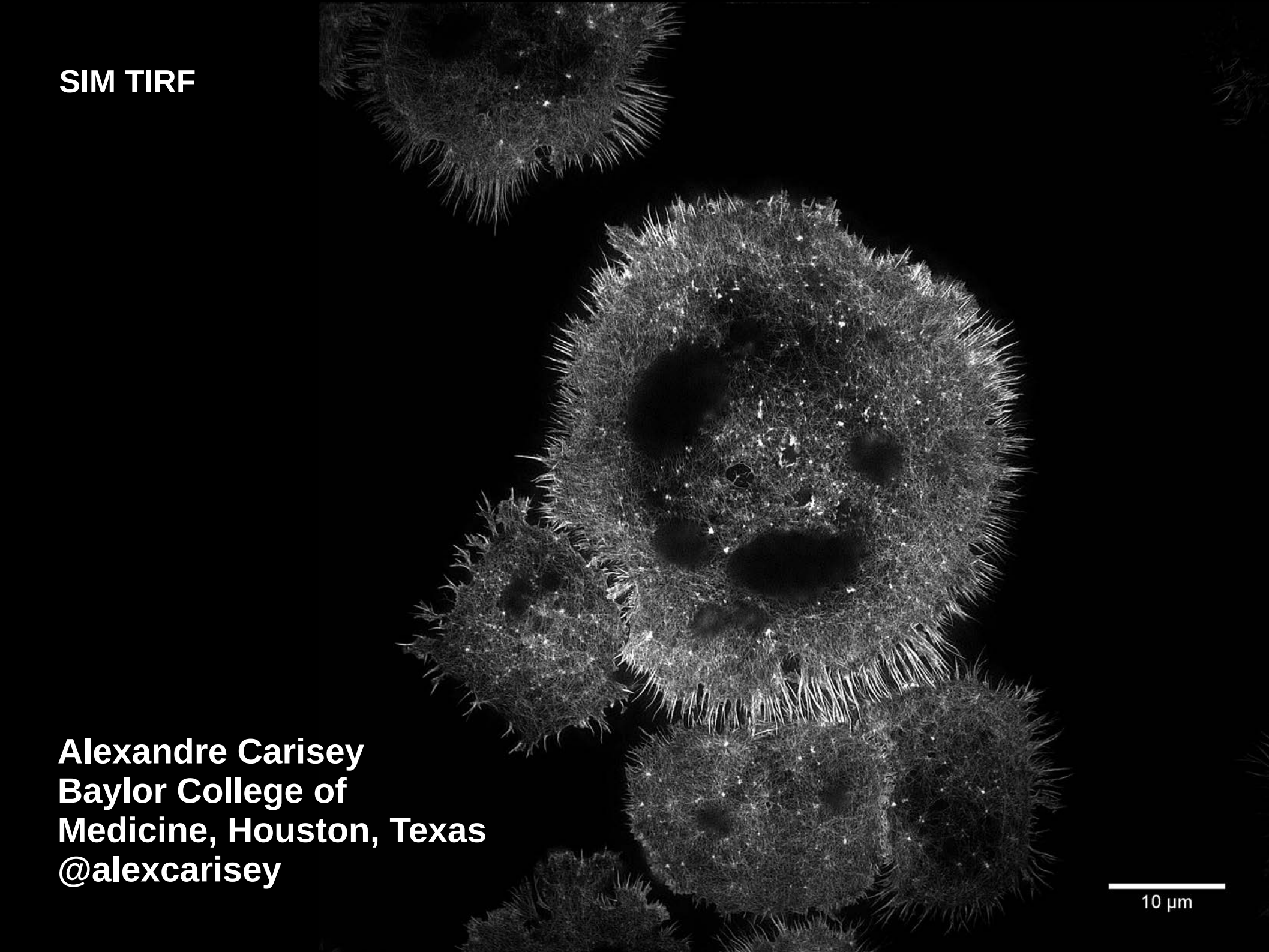
SIM microscope (Now available in CIC)

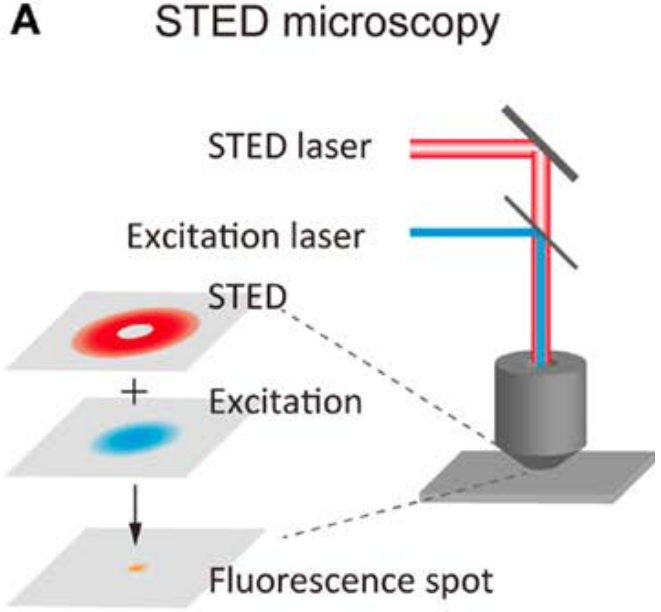




- Wide-field with grids
- around 100 nm resolution
- Live and Fixed samples
- any labels
- relatively fast (great for live)

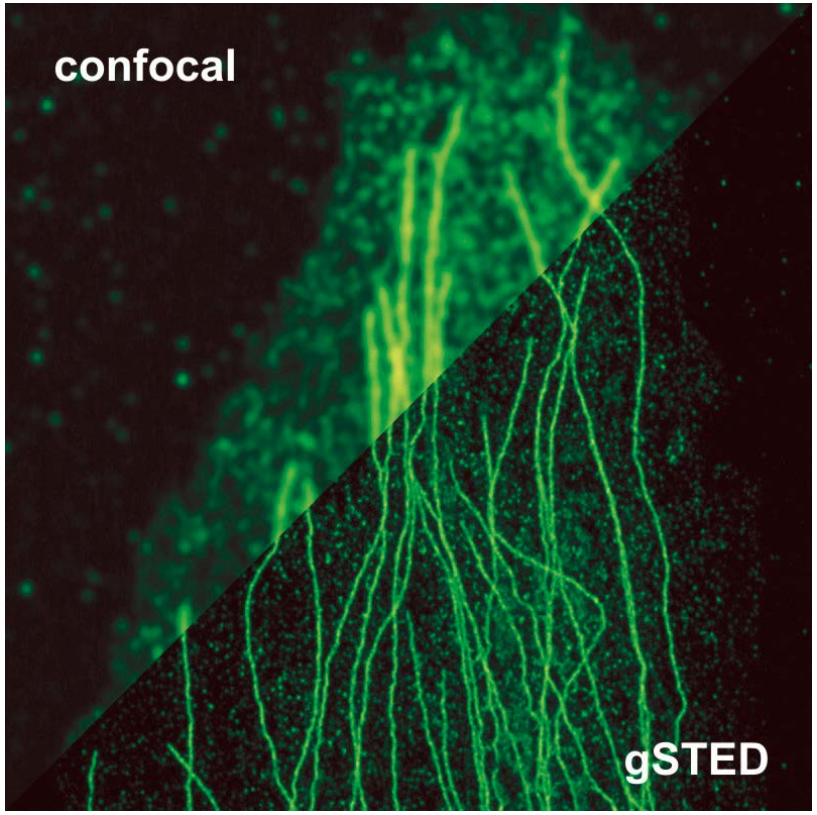

3D SIM
Alexandre Carisey
Baylor College of
Medicine, Houston, Texas
@alexcarisey


SIM TIRF
Alexandre Carisey
Baylor College of
Medicine, Houston, Texas
@alexcarisey
10 µm



Available here in turku

- Confocal with one extra laser
- around 50 nm resolution
- Live and Fixed samples
- Specific labels (multicolor difficult)
- Slow and high toxicity



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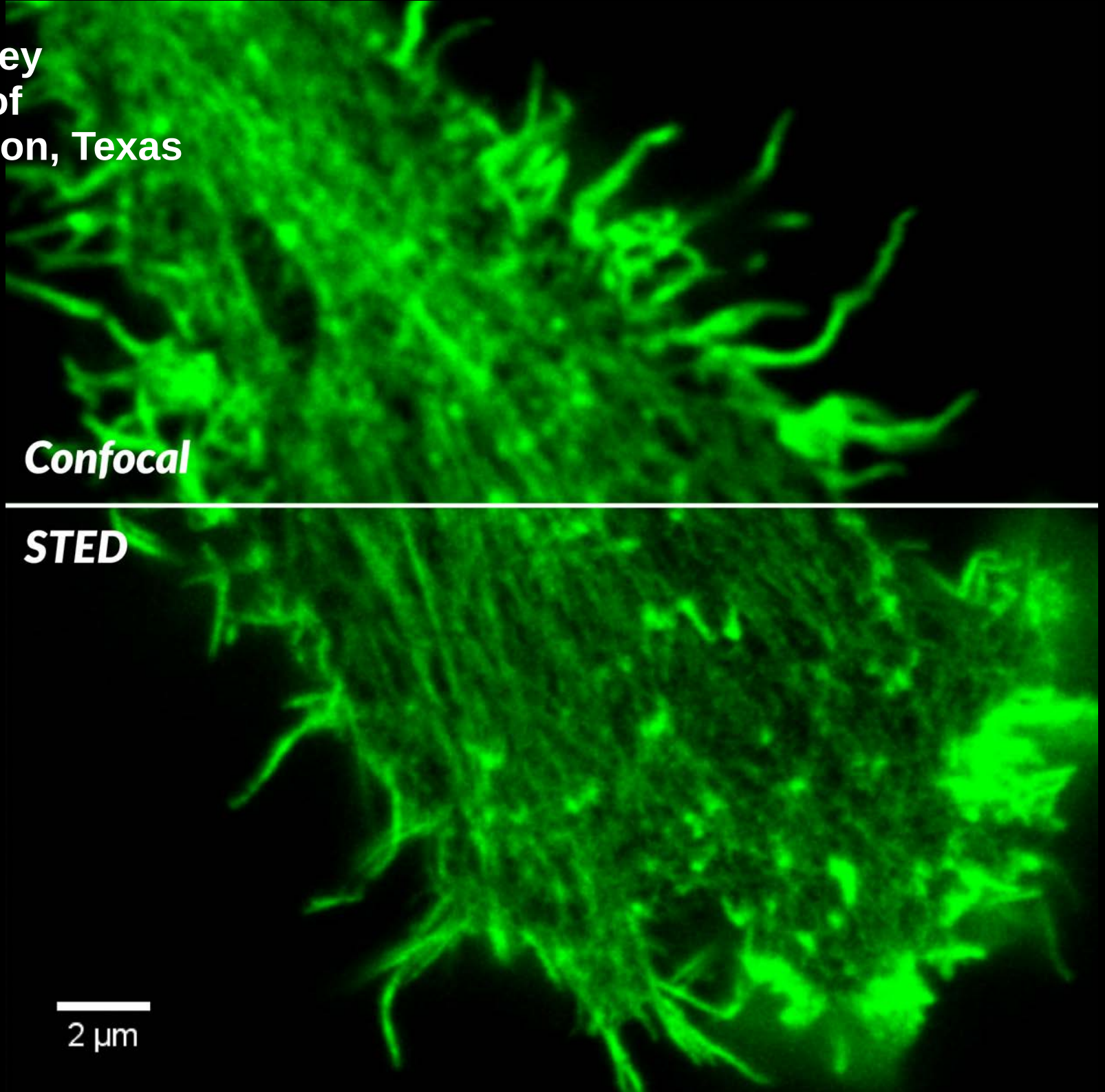
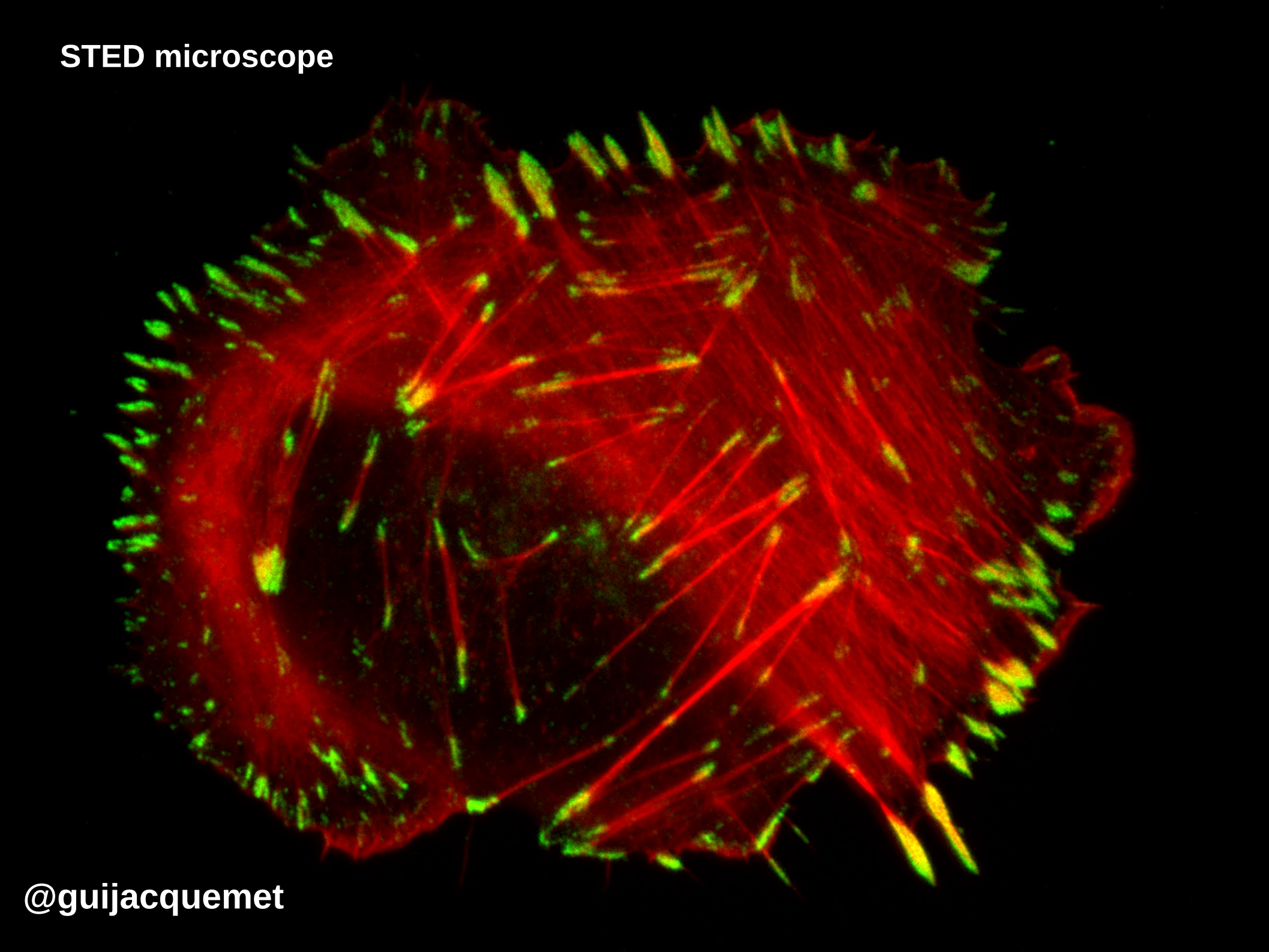
@alexcarisey

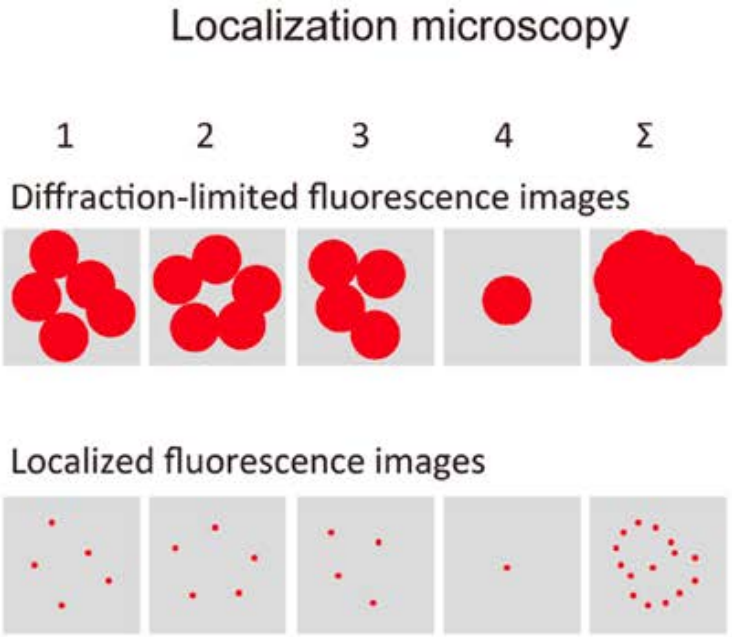


STED microscope

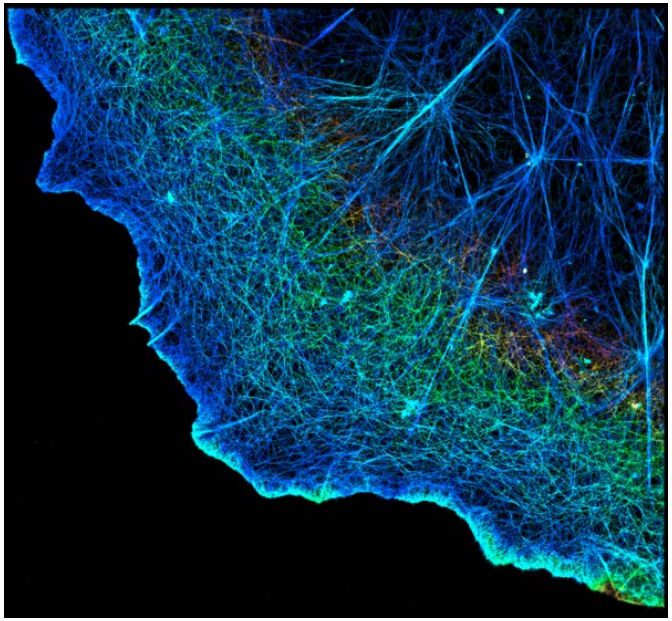


@guijacquemet

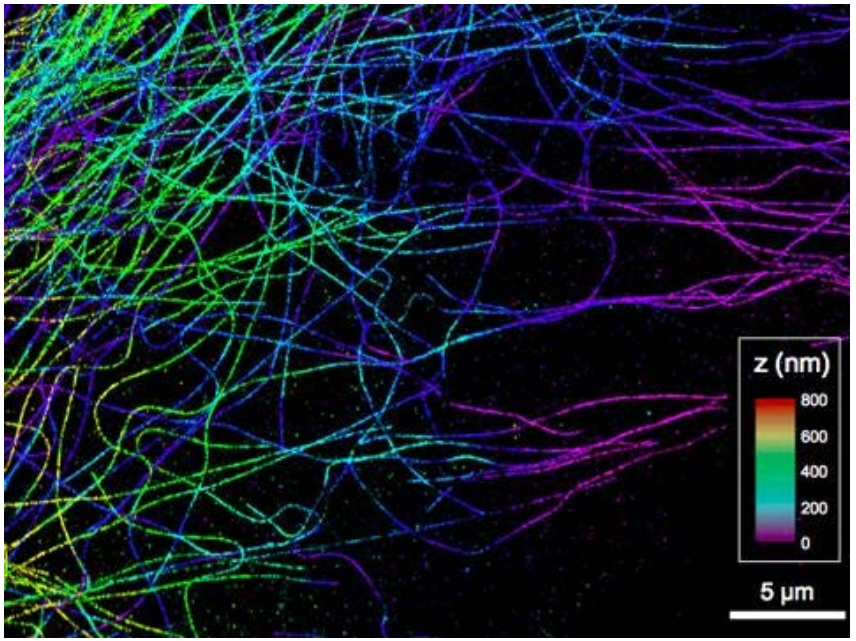
STORM / IPALM microscope (localization microscopy)



- 20-30 nm resolution
- fixed samples (Live possible)
- Specific labels (blinking) and buffers
- Very very Slow

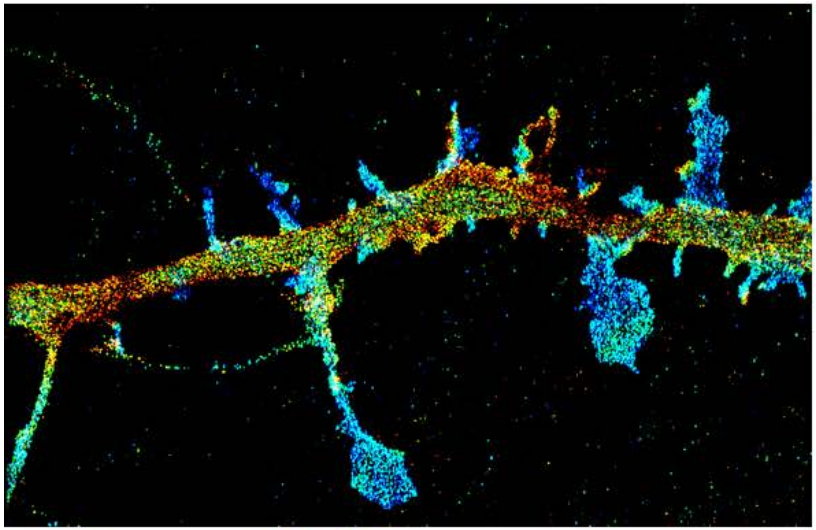


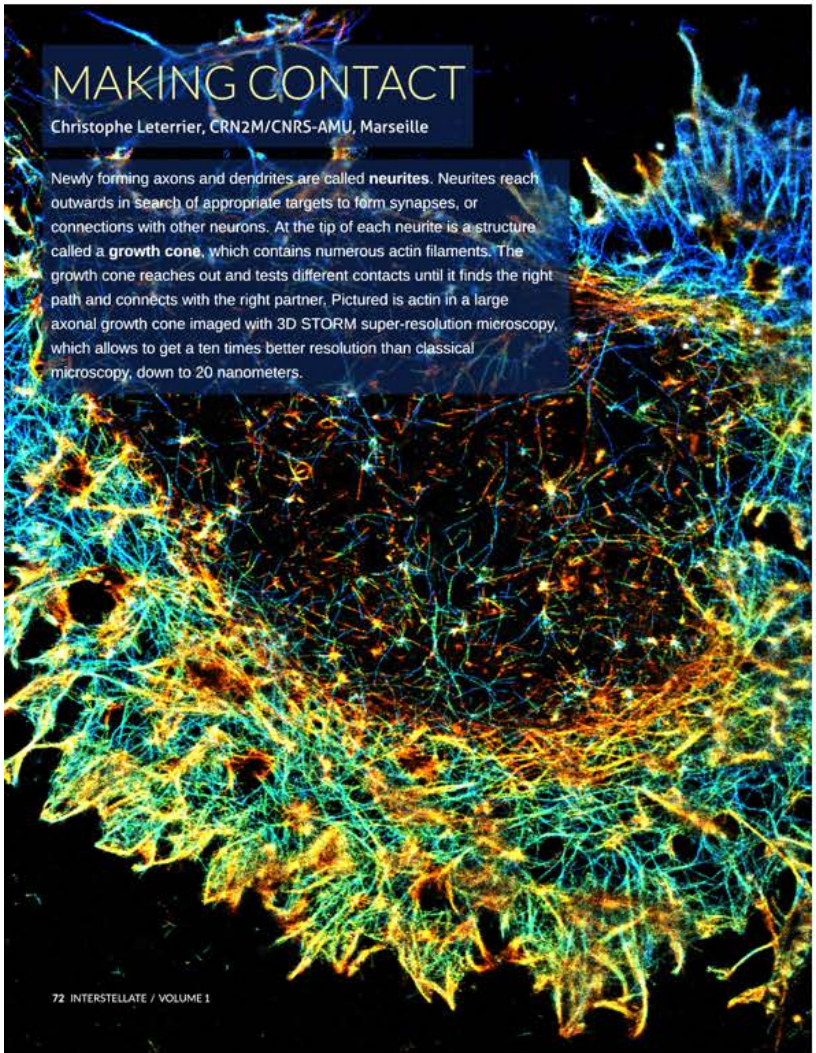
Zhuang Research Lab

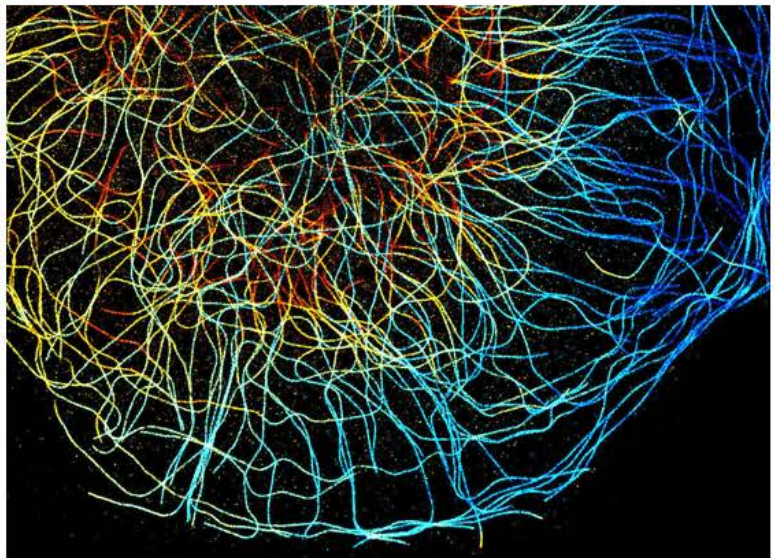


http://www.morrellonline.com/n-storm.html

Christophe Leterrier
CNRS-AMU NICN, Marseille
@christlet







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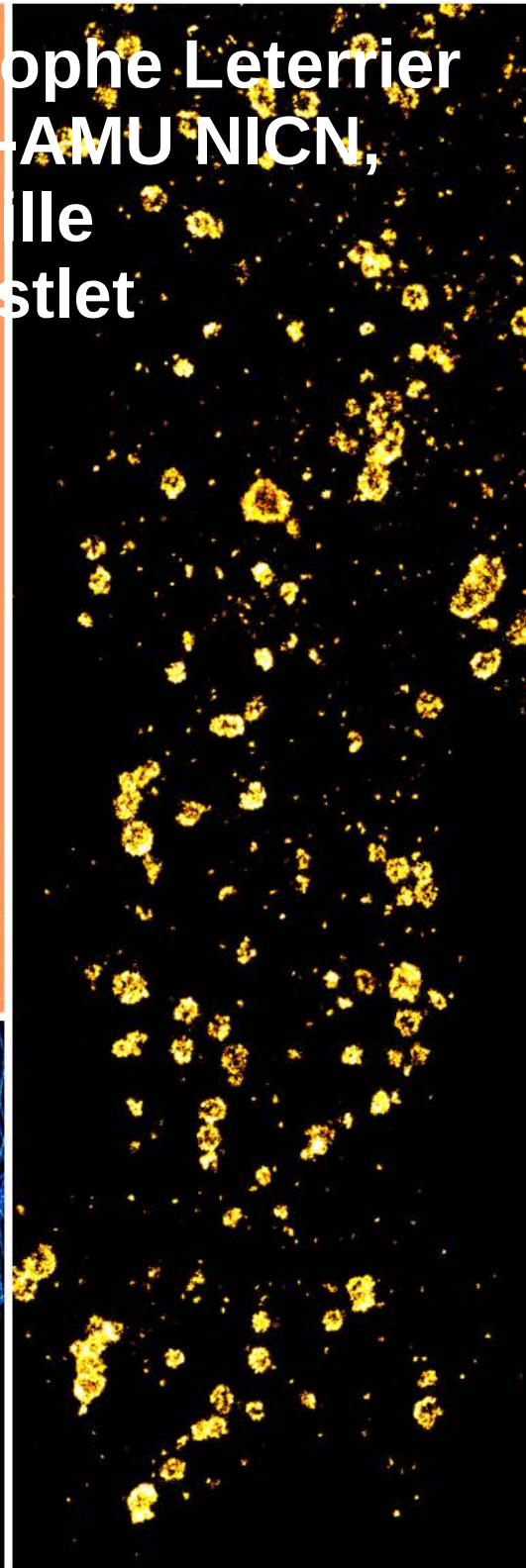
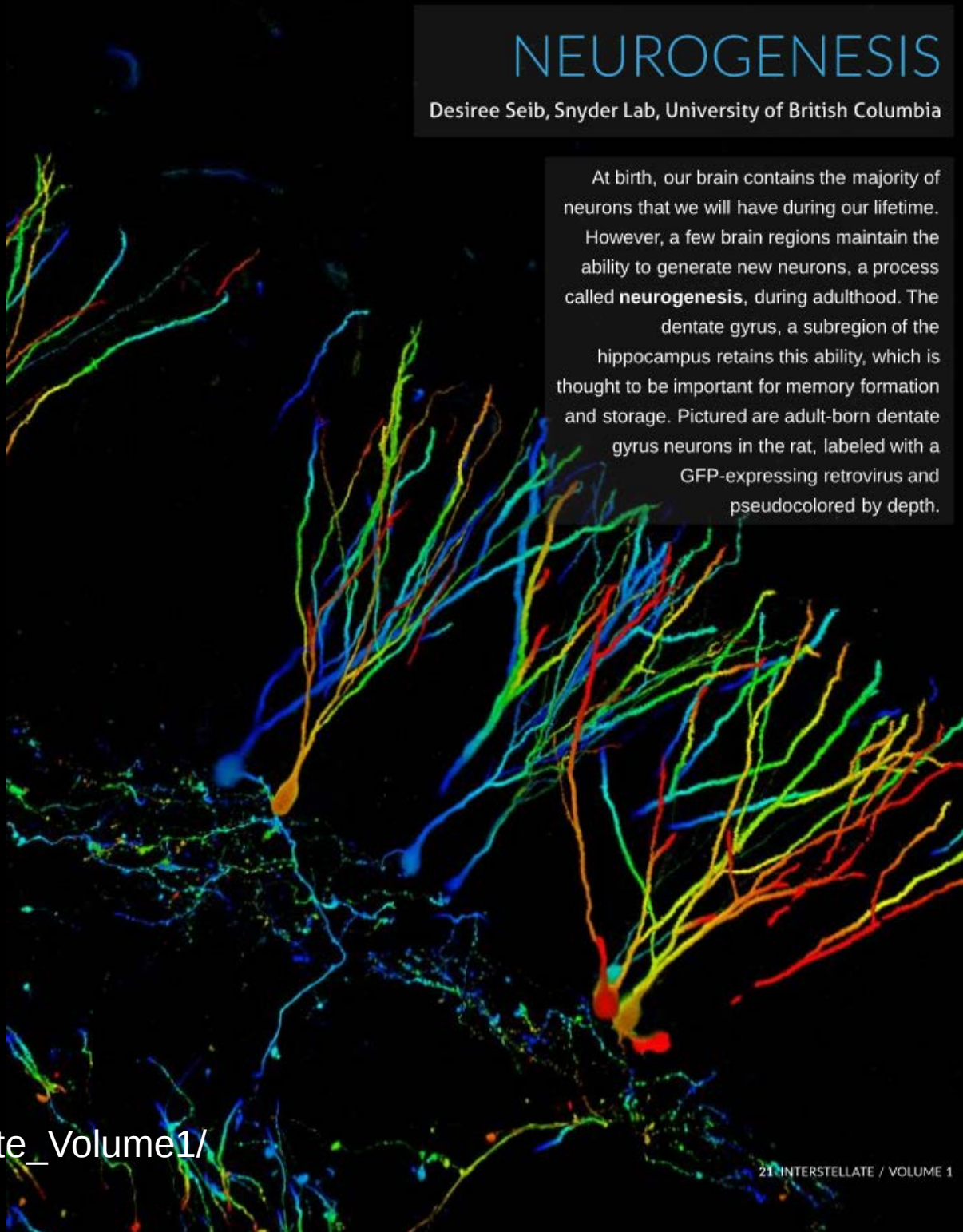
CNRS-AMU NICN, Marseille
@christlet

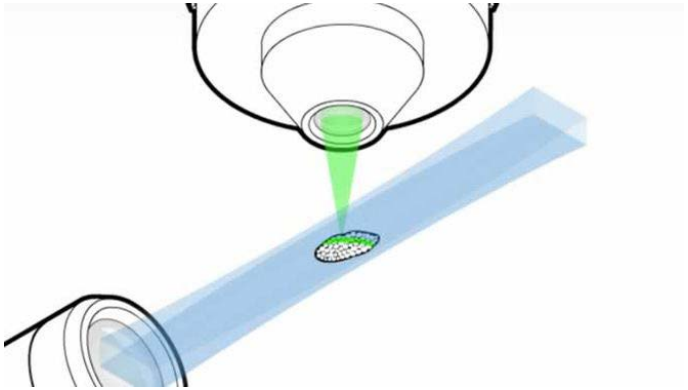


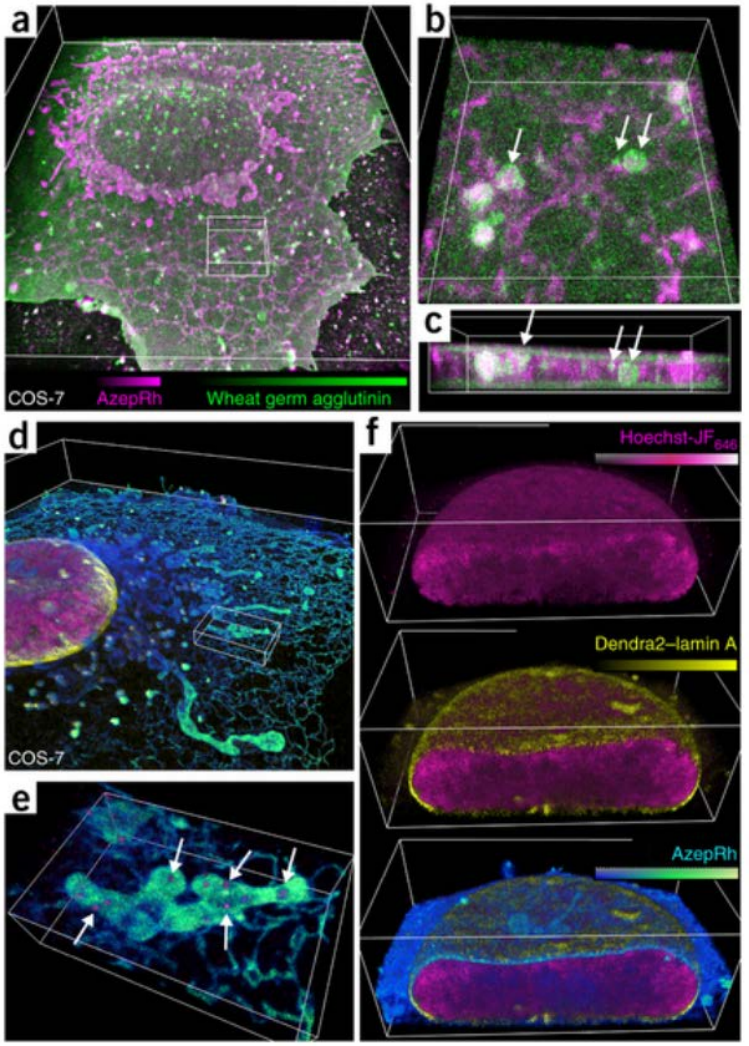
Interstellate Project



http://pub.lucidpress.com/Interstellate_Volume1/

The Betzig revolution I ：Lattice light sheet





Lattice light-sheet microscopy: Imaging molecules to embryos at high spatiotemporal resolution

Bi-Chang Chen[1,*,†], Wesley R. Legant[1,*], Kai Wang[1,*], Lin Shao[1], Daniel E. Milkie[2], Michael W. Davidson[3], Chris Janetopoulos[4], Xufeng S. Wu[5], John A. Hammer III[5], Zhe Liu[1], Brian P. English[1], Yuko Mimori-Kiyosue[6], Daniel P. Romero[7], Alex T. Ritter[8,9], Jennifer Lippincott-Schwartz[8], Lillian Fritz-Laylin[10], R. Dyche Mullins[10], Diana M. Mitchell[11,‡], Joshua N. Bembenek[11], Anne-Cecile Reymann[12,13,§], Ralph Böhme[12,13], Stephan W. Grill[12,13,§], Jennifer T. Wang[14], Geraldine Seydoux[14], U. Serdar Tulu[15], Daniel P. Kiehart[15], Eric Betzig[1,||]

High density labeling lattice light sheet
(Nature Methods 2015)

High-density three-dimensional localization microscopy across large volumes

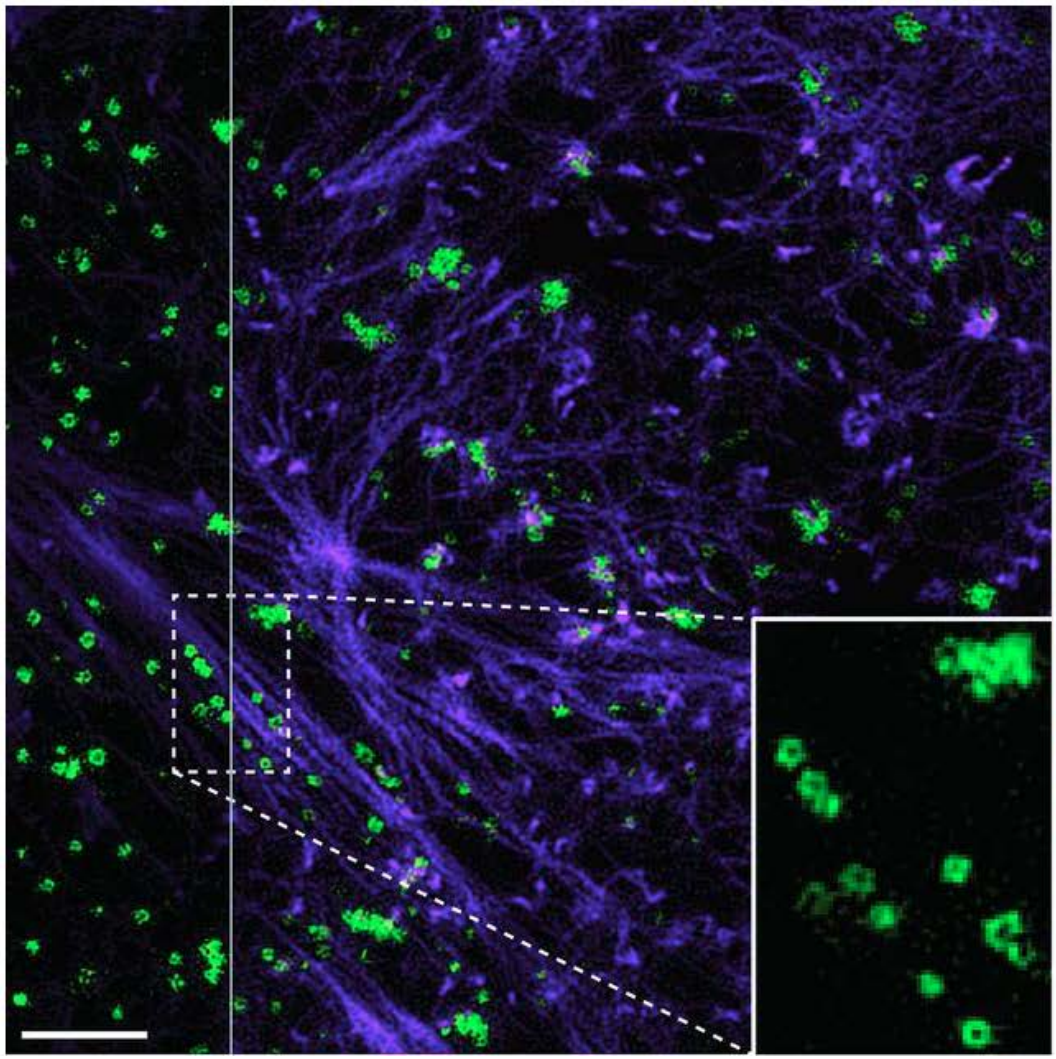
Wesley R Legant, Lin Shao, Jonathan B Grimm, Timothy A Brown, Daniel E Milkie, Brian B Avants, Luke D Lavis & Eric Betzig

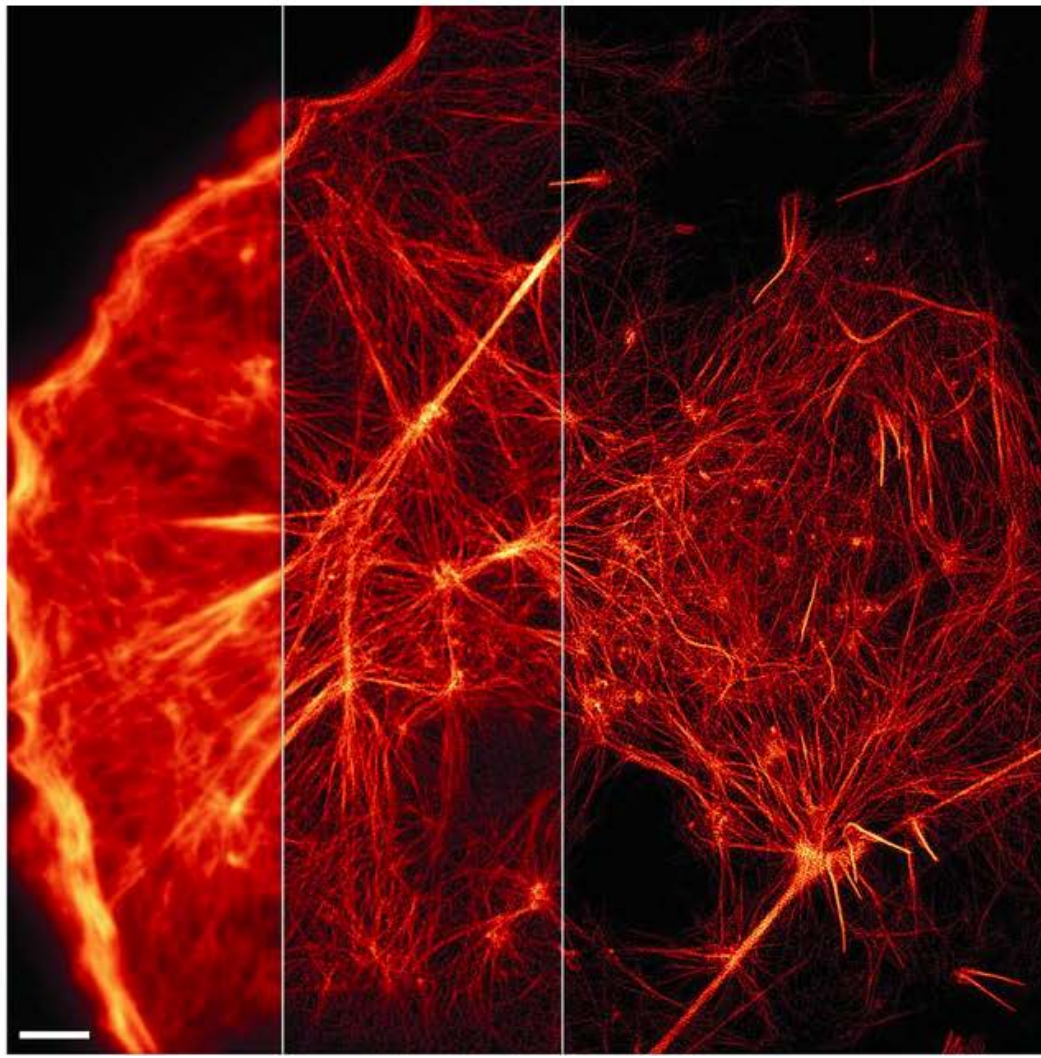
The Betzig revolution II

High NA SIM TIRF Science 2015

Extended-resolution structured illumination imaging of endocytic and cytoskeletal dynamics

Dong Li[1], Lin Shao[1], Bi-Chang Chen[1,*], Xi Zhang[2,3], Mingshu Zhang[2], Brian Moses[4], Daniel E. Milkie[4], Jordan R. Beach[5], John A. Hammer III[5], Mithun Pasham[6], Tomas Kirchhausen[6], Michelle A. Baird[5,7], Michael W. Davidson[7], Pingyong Xu[2], Eric Betzig[1,†]

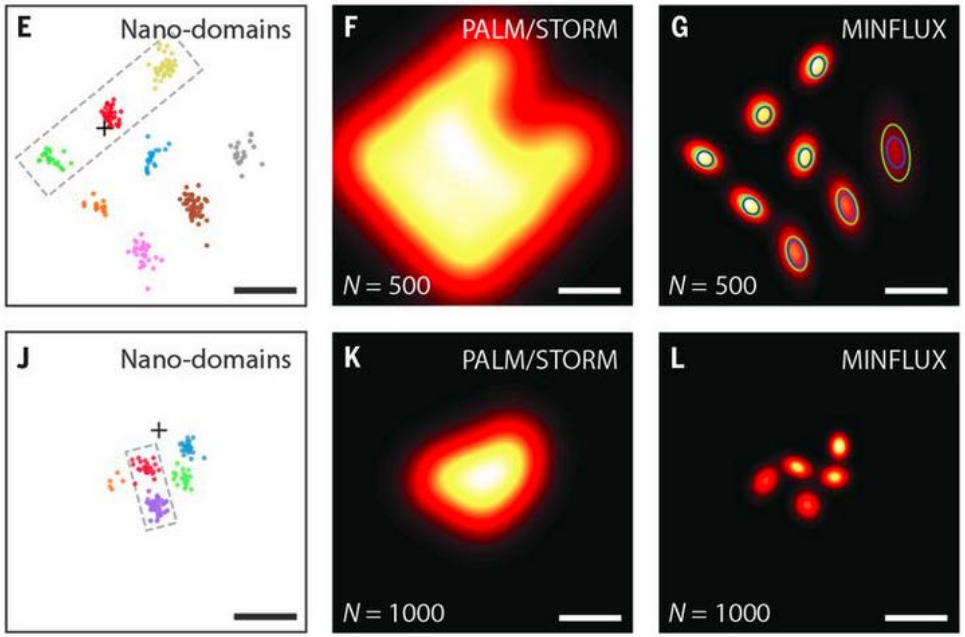




Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes

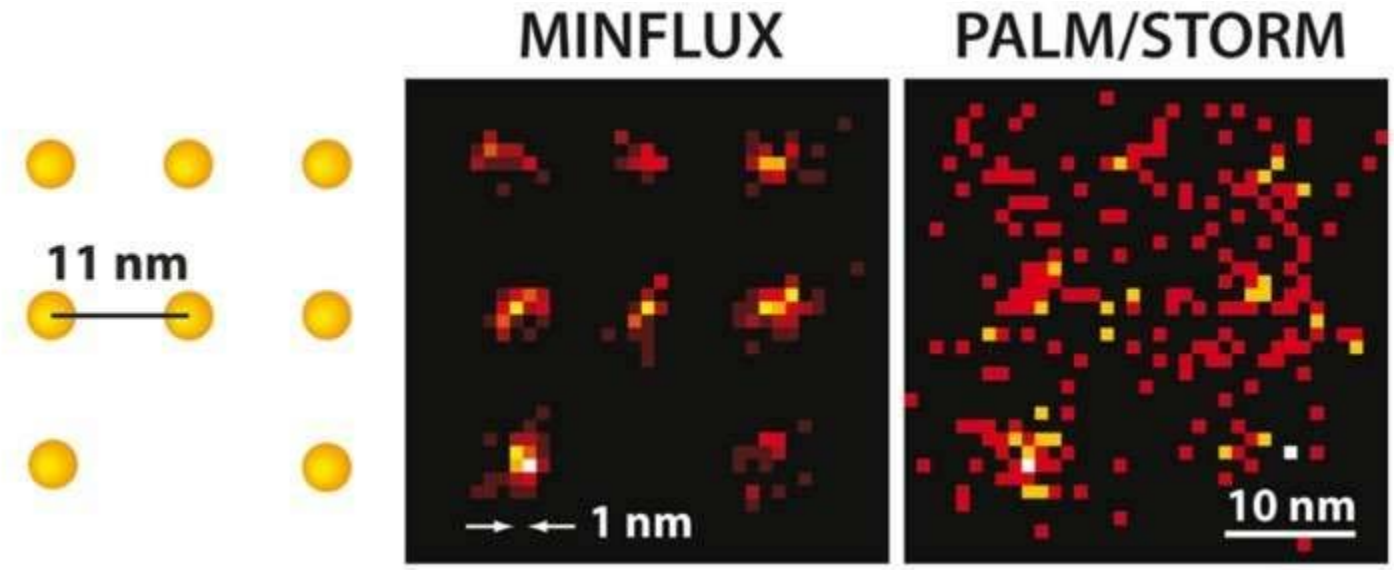
Science 10 Feb 2017:
Vol. 355, Issue 6325, pp. 606-612
DOI: 10.1126/science.aak9913

Francisco Balzarotti[1,*,†], Yvan Eilers[1,*], Klaus C. Gwosch[1,*], Arvid H. Gynnå[2], Volker Westphal[1], Fernando D. Stefani[3,4], Johan Elf[2], Stefan W. Hell[1,5,6,†]



- 1- 10 nm resolution

- Combination of STED and STORM



Web lectures

Ibiology Microscopy courses
https://www.youtube.com/watch?v=EAdEZzY0R6Y&list=PLQFc-Dxlf4pSHREZvz41xHFSEp65iNkBL

Fluorescence microscopy
https://www.youtube.com/watch?v=AhzhOzgYoqw

Confocal
https://www.youtube.com/watch?v=YRQsjPAx9UU

Choosing the right microscope
https://www.youtube.com/watch?v=01v2kR8dlnQ

Two Photon
https://www.youtube.com/watch?v=CZifB2aQDDM

Super resolution
https://www.youtube.com/watch?v=w2Qo__sppcI

Betzig lecture
https://www.youtube.com/watch?v=2R2ll9SRCeo

Web resources

Nikon resources
https://www.microscopyu.com/

Zeiss resources
http://zeiss-campus.magnet.fsu.edu/index.html

Olympus resources
http://olympus.magnet.fsu.edu/index.html

Leica resources
https://www.leica-microsystems.com/science-lab/

Accessing Microscopes

- CIC in Turku

- Collaborations

Apply to use microscope !!!!

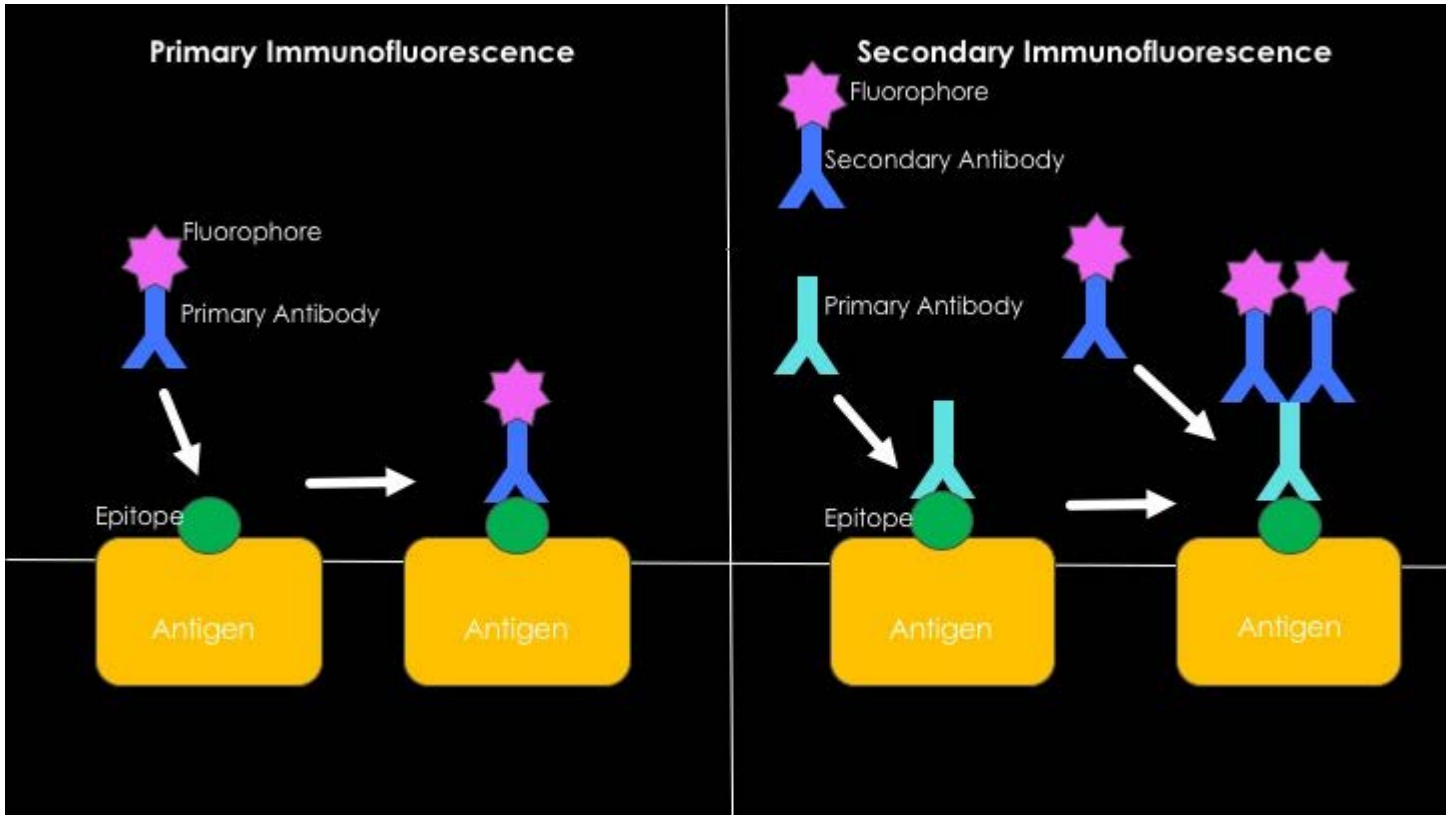
- Eurobio-imaging network (http://www.eurobioimaging.eu/

https://www.eurobioimaging-interim.eu/locations-and-technologies.html)

- Janelia Farm visiting program (https://www.janelia.org/you-janelia/visiting-scientists)

Sample preparation (Immuno-labelling)

The basics


Primary Immunofluorescence
Fluorophore
Primary Antibody
Epitope
Antigen
Antigen
Secondary Immunofluorescence
Fluorophore
Secondary Antibody
Primary Antibody
Epitope
Antigen
Antigen

Sample preparation (fixed)

My advices:

- **Validate your antibodies / reagents**
- **Do not prepare IF sample like you prepare a western blot**
- **Choose your fixative wisely**
- **Fixation will always create artefacts**

Choice of fixation:

	Fixative	Effect	Advantages	Disadvantages
Chemical crosslinkers	Formaldehyde	Crosslink proteins via their free amino groups	Preserves well cellular morphology. Good for already present fluorescent proteins.	Antigens might also be crosslinked
	Glutaraldehyde		Preserves well cellular morphology. Good for already present fluorescent proteins.	Antigens might also be crosslinked High autofluorescence
Organic solvents	Methanol	Fixation by dehydrogenation and protein precipitation. Cells will simultaneously become permeabilized.	Good preservation of cellular architecture. Faster procedure in comparison to chemical crosslinkers.	Strong negative effect on many epitopes. Not suitable for fluorescent proteins. Soluble and lipid components are getting lost.
	Acetone		Less damaging to epitopes. Faster procedure	Not suitable for fluorescent proteins. Soluble and lipid components are getting lost.

Tab. 2: Fixation reagents.

- But also the concentration and timing matter !!!

http://www.leica-microsystems.com/science-lab/how-to-prepare-your-specimen-for-immunofluorescence-microscopy/

Blocking or not blocking ??

- BSA, Horse serum, donkey serum, gelatin ?

- If your antibody is good, blocking is useless and may increase background

- Try without blocking first

Article | OPEN

Non-specific binding of antibodies in immunohistochemistry: fallacies and facts

Igor Buchwalow, Vera Samoilova, Werner Boecker & Markus Tiemann

Scientific Reports **1**, Article number: 28 (2011)
doi:10.1038/srep00028
Download Citation

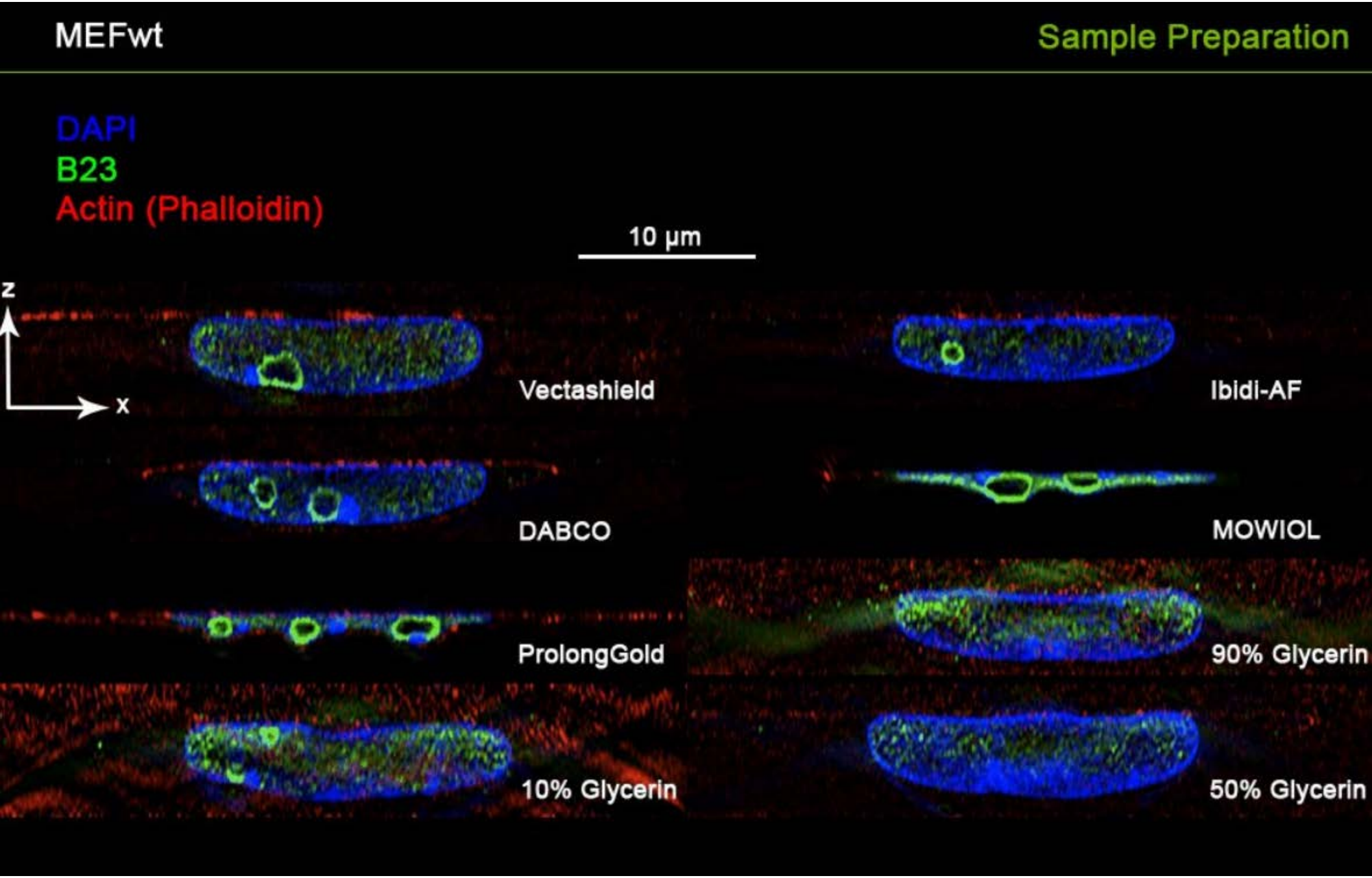
Biological techniques | Imaging | Medical research

Received: 13 April 2011
Accepted: 16 June 2011
Published online: 01 July 2011

Classical immunofluorescence protocol

- Fixation with 4 % PFA for 10 min
- Permeabilization with 0.5% triton (PBS) for 3 min
- Blocking with 1M glycin for 1h
- Primary antibody for 25 min
- 3 PBS washes
- Secondary antibody for 25 min
- 3 PBS washes
- Image / or mount.
- Best to dry samples O/N at 4C before mounting

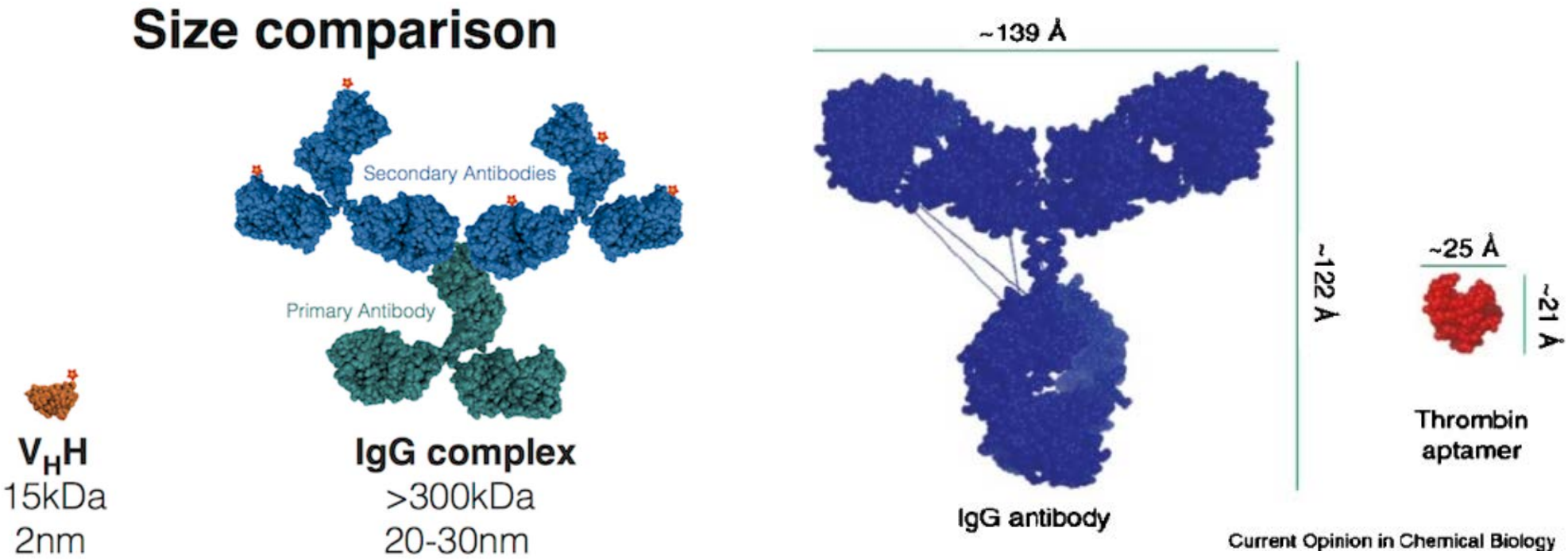
The choice of mounting media (z resolution) : Credit : A. Maiser. LMU. DE (via @patrina_pellett)




The Future

Replacing antibodies: Nanobodies, fab fragments and Aptamers

- Antibodies are very large



STED resolution around 40 – 50 nm

STORM resolution around 20 – 30 nm

http://www.chromotek.com/about-us/the-alpaca-antibody-advantage/

Very rapid and easy detection of GFP proteins using EM

GBP-APEX

GBP = anti GFP nanobody

APEX = Peroxydase

Resource

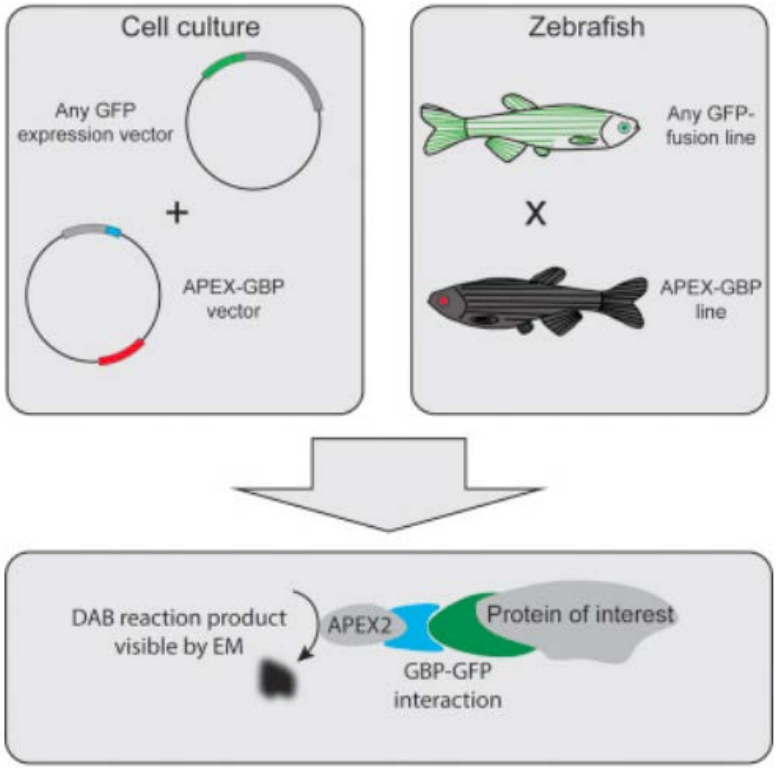
Modular Detection of GFP-Labeled Proteins for Rapid Screening by Electron Microscopy in Cells and Organisms

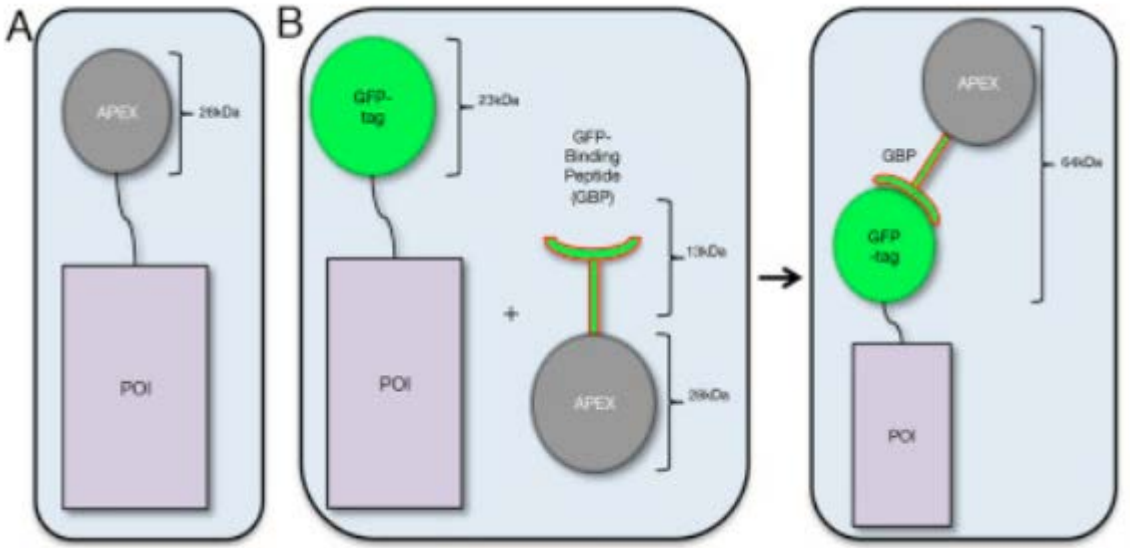
Nicholas Ariotti[1,3], Thomas E. Hall[1,3], James Rae[1], Charles Ferguson[1], Kerrie-Ann McMahon[1], Nick Martel[1], Robyn E. Webb[2], Richard I. Webb[2], Rohan D. Teasdale[1], Robert G. Parton[1,2],

[1] Institute for Molecular Bioscience, University of Queensland, QLD 4072, Australia

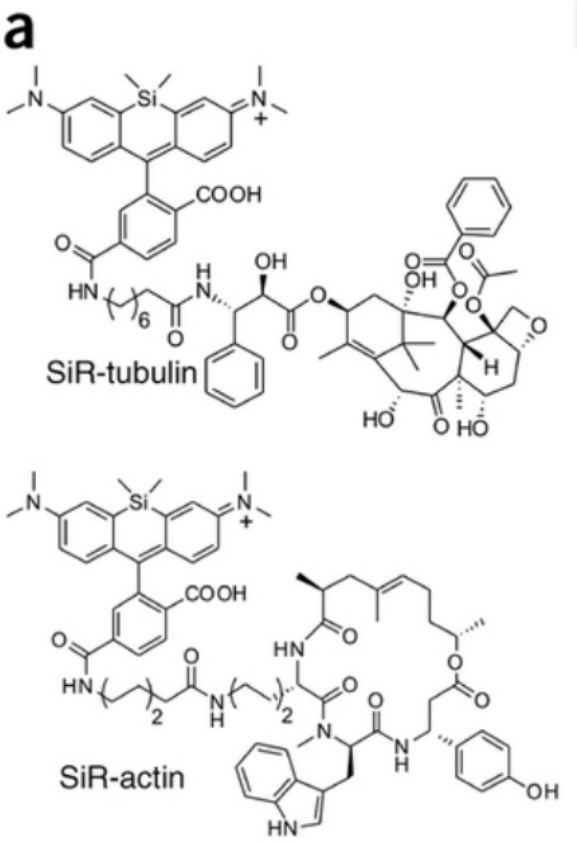
[2] Centre for Microscopy and Microanalysis, University of Queensland, Brisbane, QLD 4072, Australia

Received 14 May 2015, Revised 16 September 2015, Accepted 19 October 2015, Available online 12 November 2015

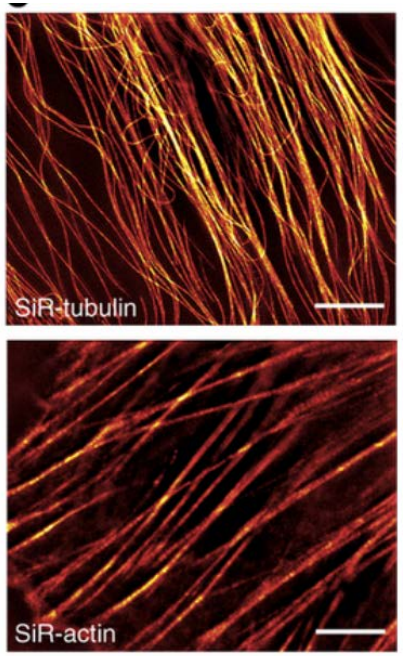




Chemicals to label cellular structures



- Cytoskeleton Kit (SiR-Actin and SiR-Tubulin)
- SiR-Actin Kit
- SiR-DNA Kit
- SiR-Lysosome Kit
- SiR-Tubulin Kit
- SiR700-Actin Kit
- SiR700-DNA Kit
- SiR700-Lysosome Kit
- SiR700-Tubulin Kit



SiR–Hoechst is a far-red DNA stain for live-cell nanoscopy

Gražvydas Lukinavičius, Claudia Blaukopf, Elias Pershagen, Alberto Schena, Luc Reymond, Emmanuel Derivery, Marcos Gonzalez-Gaitan, Elisa D'Este, Stefan W. Hell, Daniel Wolfram Gerlich & Kai Johnsson

http://www.cytoskeleton.com/live-cell-reagents/spirochrome

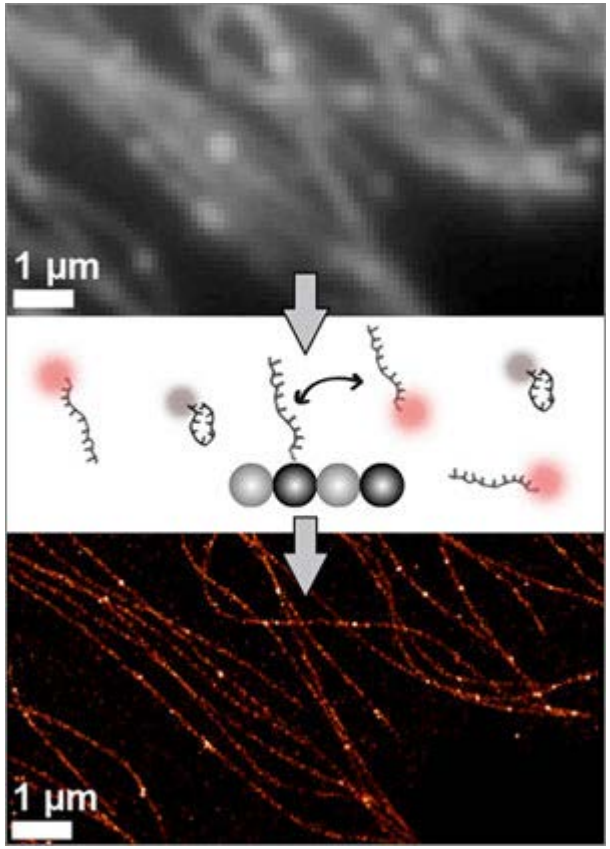
Fluorogenic Probes for Multicolor Imaging in Living Cells

Gražvydas Lukinavičius, Luc Reymond, Keitaro Umezawa, Olivier Sallin, Elisa D'Este, Fabian Göttfert, Haisen Ta, Stefan W. Hell, Yasuteru Urano, and Kai Johnsson

Fluorogenic probes for live-cell imaging of the cytoskeleton

Gražvydas Lukinavičius, Luc Reymond, Elisa D'Este, Anastasiya Masharina, Fabian Göttfert, Haisen Ta, Angelika Güther, Mathias Fournier, Stefano Rizzo, Herbert Waldmann, Claudia Blaukopf, Christoph Sommer, Daniel W Gerlich, Hans-Dieter Arndt, Stefan W Hell & Kai Johnsson

The future: DNA- PAINT and exchange-Paint



Antibody (or protein) couple with DNA strand

Complementary DNA strand coupled with fluorophore

- Very High density

- Never bleach (almost) due to binding unbinding rate Of the DNA

- Exchange Paint

Multiplexed 3D cellular super-resolution imaging with DNA-PAINT and Exchange-PAINT

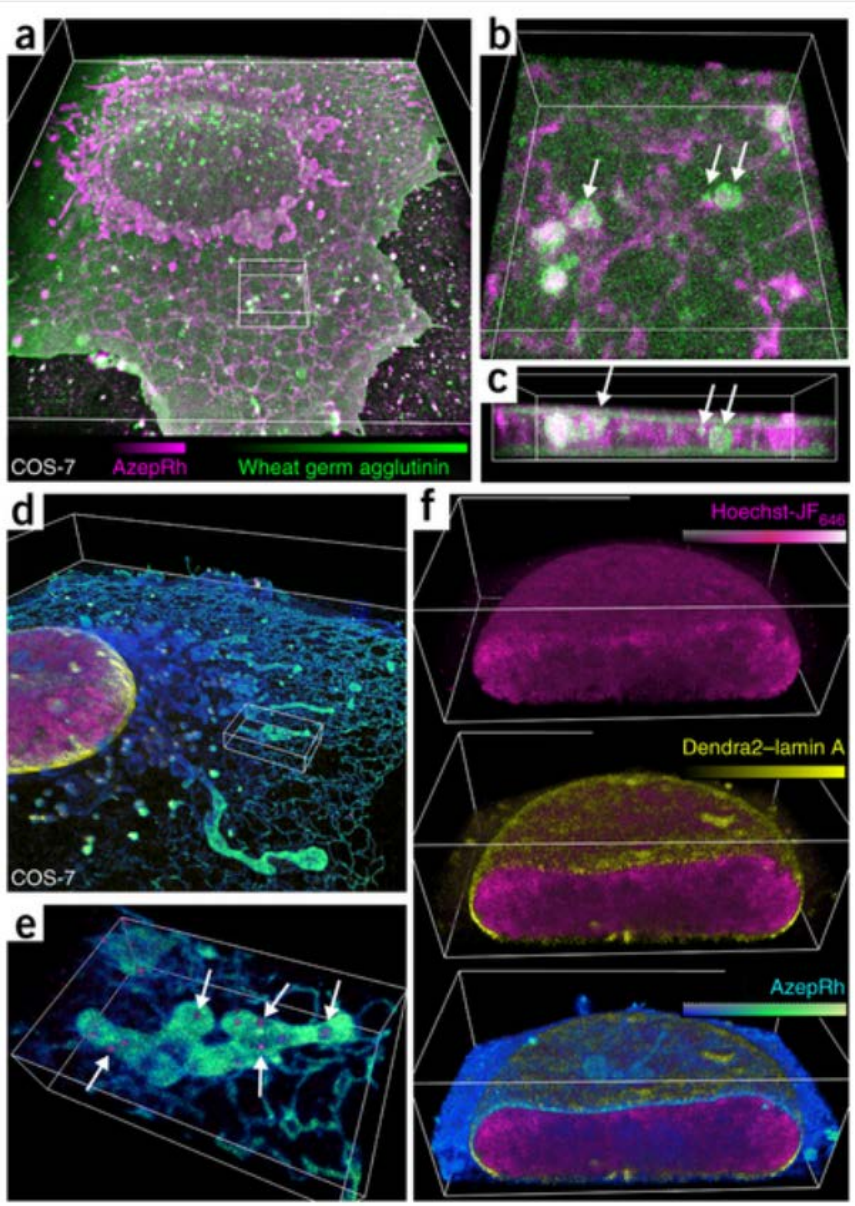
2014

Ralf Jungmann, Maier S Avendaño, Johannes B Woehrstein, Mingjie Dai, William M Shih & Peng Yin

DNA Paint application: high fluorophore density

DNA paint + lattice light sheet = Awesome

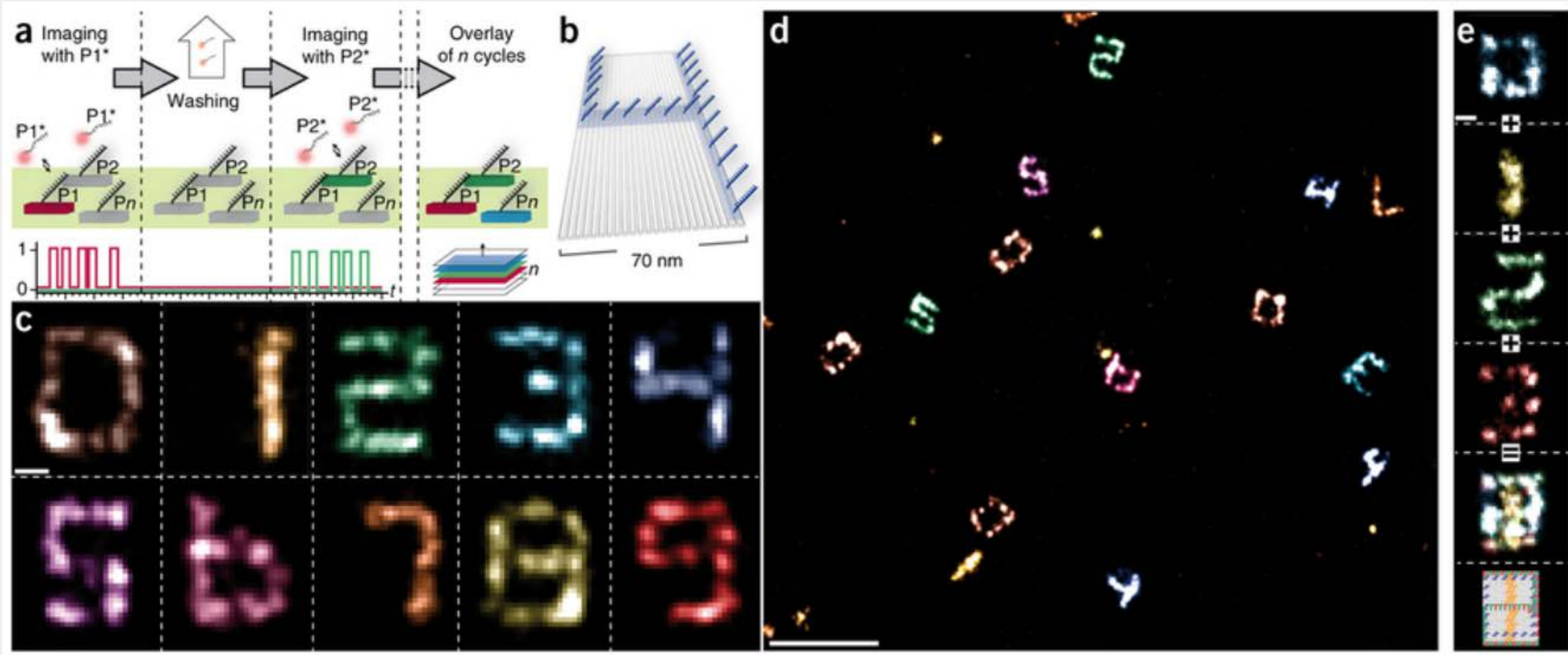
(and also a science paper)



High-density three-dimensional localization microscopy across large volumes

Wesley R Legant, Lin Shao, Jonathan B Grimm, Timothy A Brown, Daniel E Milkie, Brian B Avants, Luke D Lavis & Eric Betzig

The future: DNA PAINT and exchange-Paint



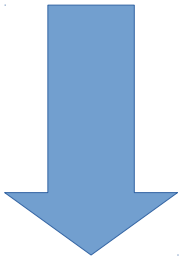
Multiplexed 3D cellular super-resolution imaging with DNA-PAINT and Exchange-PAINT

2014

Ralf Jungmann, Maier S Avendaño, Johannes B Woehrstein, Mingjie Dai, William M Shih & Peng Yin

Why is it the future ?

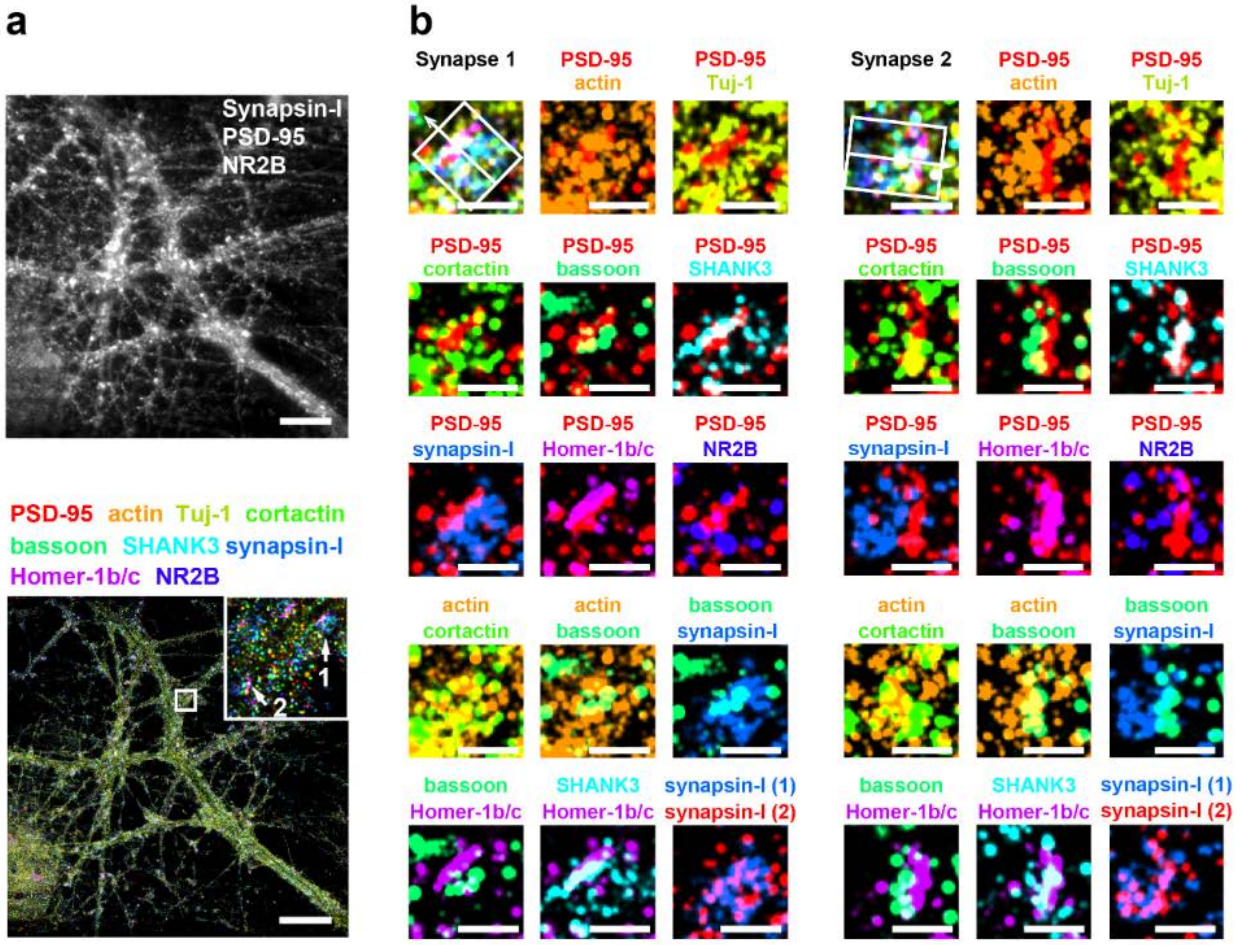
Imagine a world where all your primary antibodies are DNA labeled !



- Unlimited number of target can be imaged sequentially on the same sample

DNA Paint application: multi color imaging

13 color imaging using exchange PAINT



Multiplexed confocal and super-resolution fluorescence imaging of cytoskeletal and neuronal synapse proteins

Syuan-Ming Guo, Remi Veneziano, Simon Gordonov, Li Li, Demian Park, Anthony B Kulesa, Paul C Blainey, Jeffrey R Cottrell, Edward S Boyden, Mark Bathe

doi: https://doi.org/10.1101/111625

@eboyden3

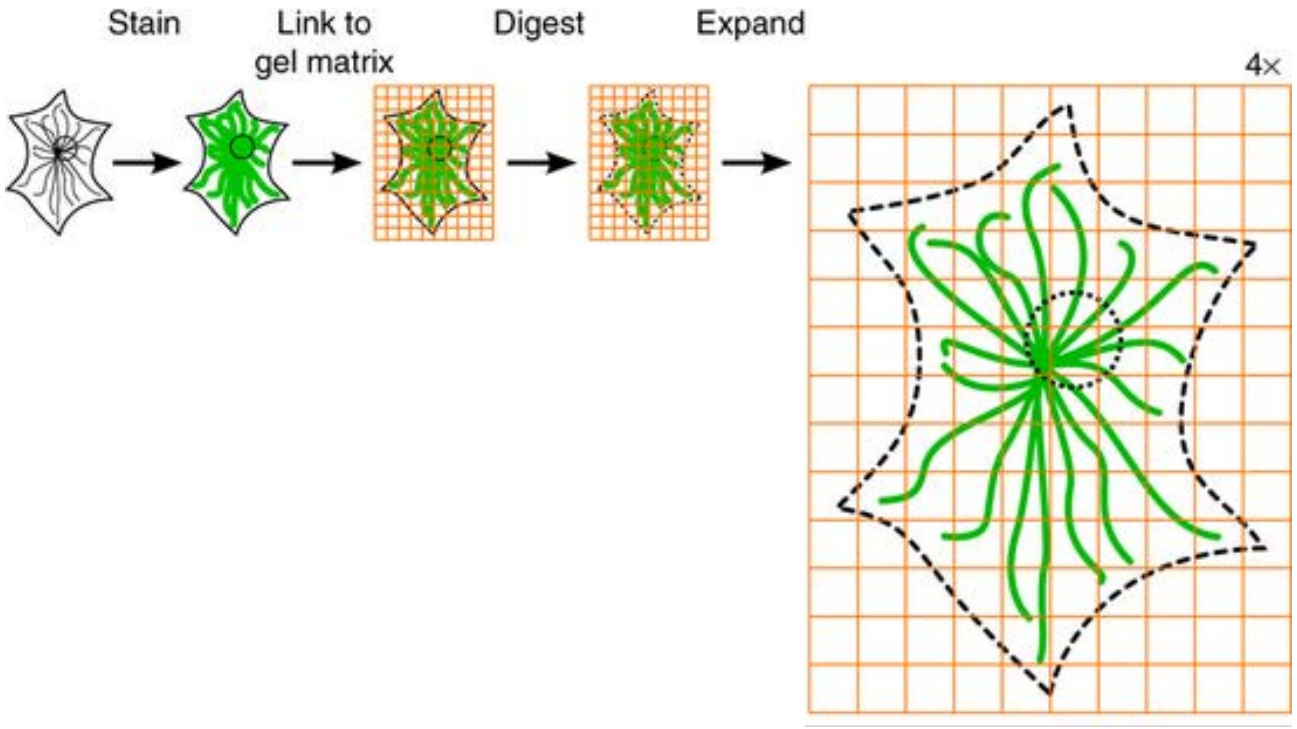
Expansion microscopy - Principle

Expansion microscopy

Fei Chen[1,*], Paul W. Tillberg[2,*], Edward S. Boyden[1,3,4,5,6,†]
+ See all authors and affiliations

Science 30 Jan 2015:
Vol. 347, Issue 6221, pp. 543-548
DOI: 10.1126/science.1260088

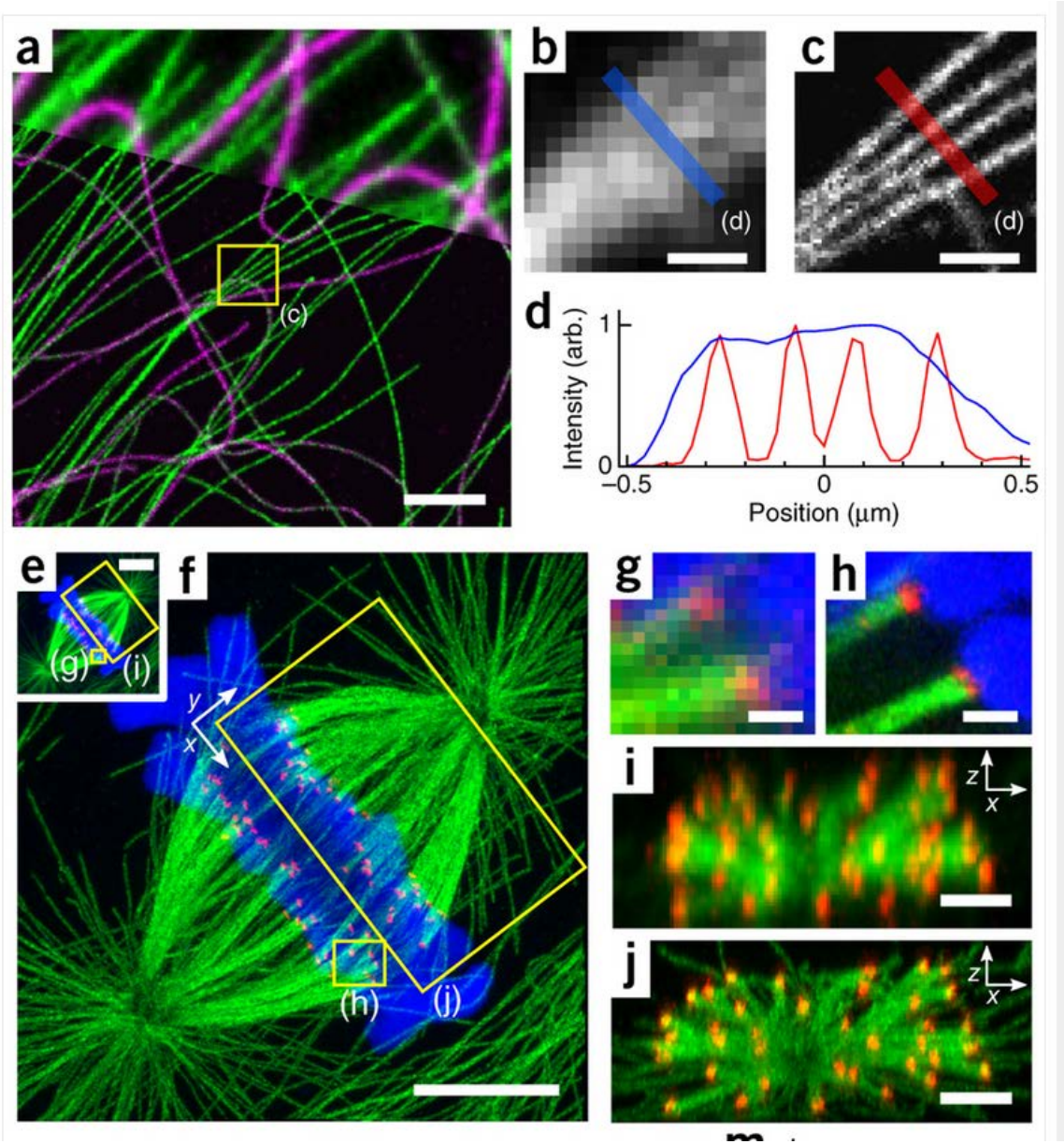
- Make the sample bigger (4.5x) !!



@eboyden3

Works - with cells

Resolution (~ 80 nm)
Using normal confocal



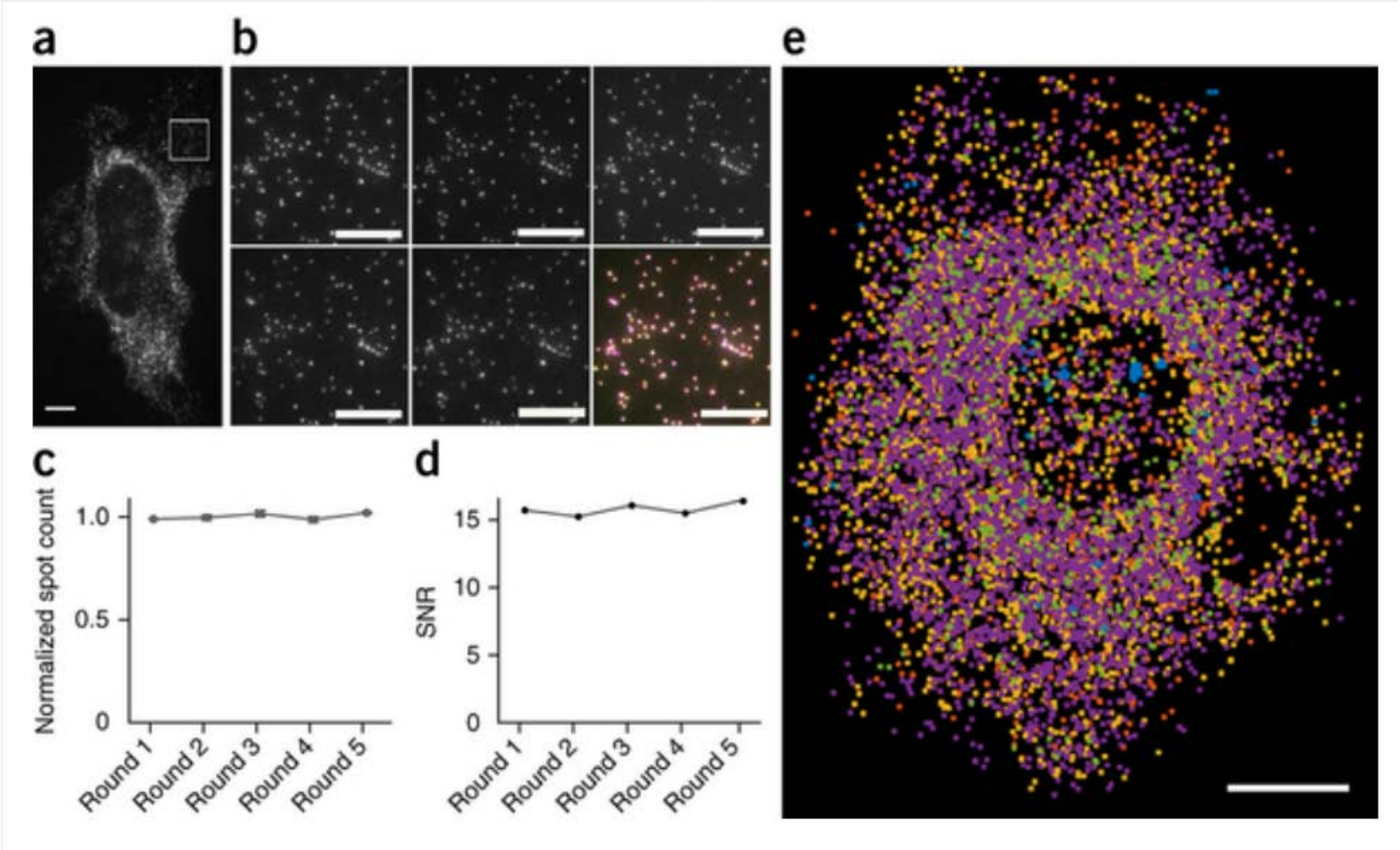
Expansion microscopy with conventional antibodies and fluorescent proteins

Tyler J Chozinski, Aaron R Halpern, Haruhisa Okawa, Hyeon-Jin Kim, Grant J Tremel, Rachel O L Wong & Joshua C Vaughan

Protein-retention expansion microscopy of cells and tissues labeled using standard fluorescent proteins and antibodies

Paul W Tillberg, Fei Chen, Kiryl D Piatkevich, Yongxin Zhao, Chih-Chieh (Jay) Yu, Brian P English, Linyi Gao, Anthony Martorell, Ho-Jun Suk, Fumiaki Yoshida, Ellen M DeGennaro, Douglas H Roossien, Guanyu Gong, Uthpala Seneviratne, Steven R Tannenbaum, Robert Desimone, Dawen Cai & Edward S Boyden

Expansion microscopy – RNA

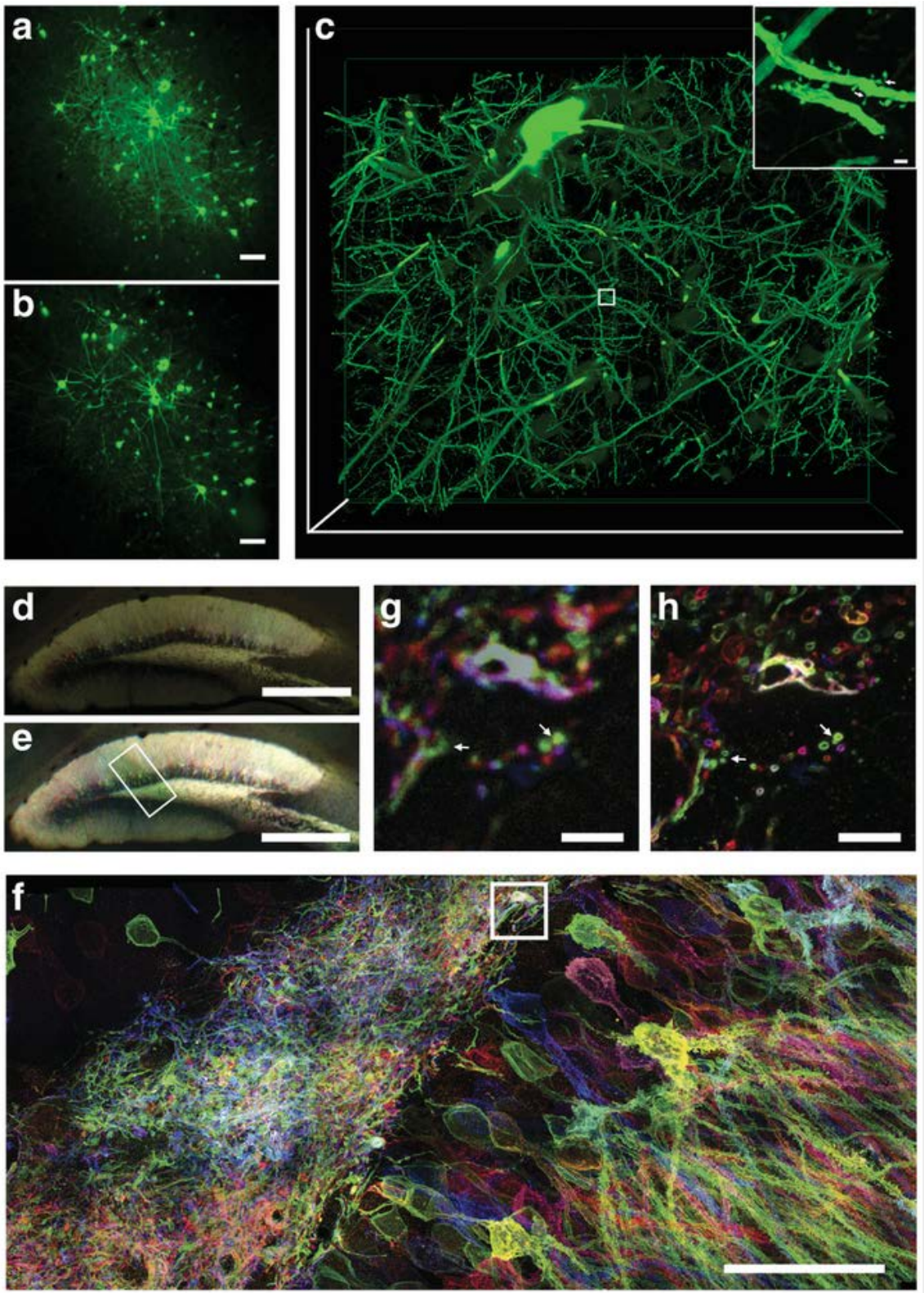


Nanoscale imaging of RNA with expansion microscopy

Fei Chen, Asmamaw T Wassie, Allison J Cote, Anubhav Sinha, Shahar Alon, Shoh Asano, Evan R Daugharthy, Jae-Byum Chang, Adam Marblestone, George M Church, Arjun Raj & Edward S Boyden

Expansion microscopy ExM

Works - with tissue sections
(Fresh and Paraffin)



Protein-retention expansion microscopy of cells and tissues labeled using standard fluorescent proteins and antibodies

Paul W Tillberg, Fei Chen, Kiryl D Piatkevich, Yongxin Zhao, Chih-Chieh (Jay) Yu, Brian P English, Linyi Gao, Anthony Martorell, Ho-Jun Suk, Fumiaki Yoshida, Ellen M DeGennaro, Douglas H Roossien, Guanyu Gong, Uthpala Seneviratne, Steven R Tannenbaum, Robert Desimone, Dawen Cai & Edward S Boyden

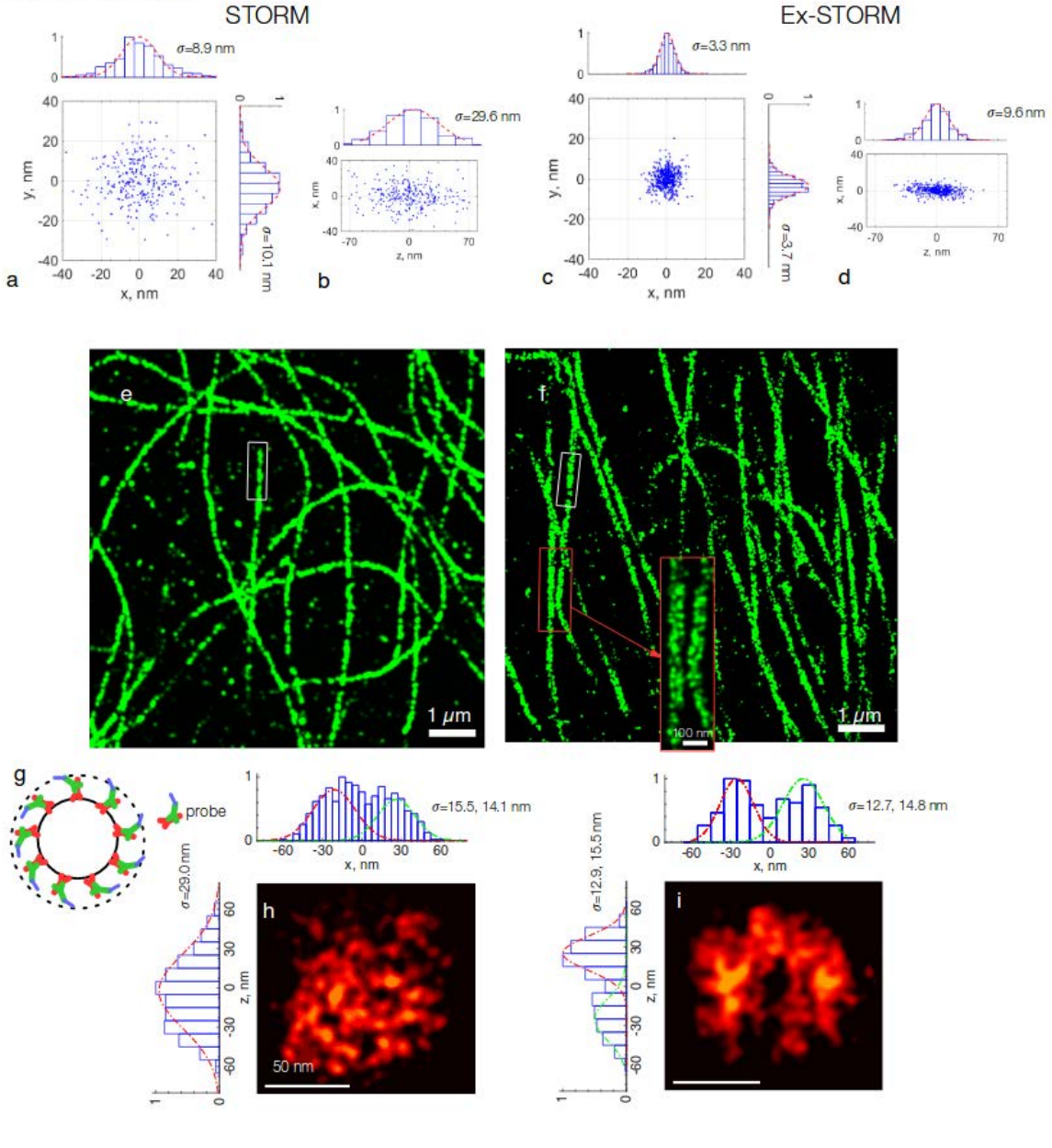
Works with Super-RES scopes

ExM-STORM: Expansion Single Molecule Nanoscopy

Hu Cang, Zhisong Tong, Paolo Beuzer, Qing Ye, Josh Axelrod, Zhenmin Hong

doi: https://doi.org/10.1101/049403

Fig. 2. Ex-STORM



Resources on Expansion microscopy:

- Listen to Ed Boyden: https://www.youtube.com/watch?v=NeIhXVEITHM

  https://www.youtube.com/watch?v=-o9-X8TvgFo&t=105s

  https://www.youtube.com/watch?v=ilNwzTIU770

  - Visit: Protocols, Papers, advices

  http://expansionmicroscopy.org/

  - ProExM video protocol
  https://www.youtube.com/watch?v=OksNCAJwxVI

  - Commercial company selling products

  http://www.extbio.com/

  - Visit Ed Lab

@eboyden3

Live cell imaging
and fluorescent proteins !

Watch Betzig Videos here:

https://vimeo.com/user33367262

Extended-resolution structured illumination imaging of endocytic and cytoskeletal dynamics

Dong Li[1], Lin Shao[1], Bi-Chang Chen[1,*], Xi Zhang[2,3], Mingshu Zhang[2], Brian Moses[4], Daniel E. Milkie[4], Jordan R. Beach[5], John A. Hammer III[5], Mithun Pasham[6], Tomas Kirchhausen[6], Michelle A. Baird[5,7], Michael W. Davidson[7], Pingyong Xu[2], Eric Betzig[1,†]

Live cell imaging, getting started

- Ask for help

- **Use the right media**

- optimize your right imaging condition

- **Choose the right Fluorescent proteins**

Remember the observer effect:

Observer effect refers to changes that the act of observation will make on a phenomenon being observed

Media for live cell imaging

- Try normal media + Hepes !! (CO_2 independent)

Too much autofluorescence → - Try imaging media (ie: molecular probe)

Phototoxicity problems → - Try anti- Fade media (ie ProLong Antifade Reagents for Live Cells)

For Single molecule tracking:

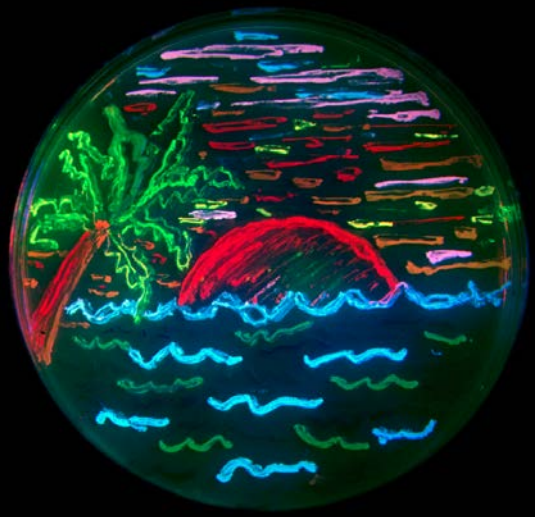
UV pre-bleaching media for single-molecule imaging (@mrpaulreynolds)

Anti-Fading Media for Live Cell GFP Imaging

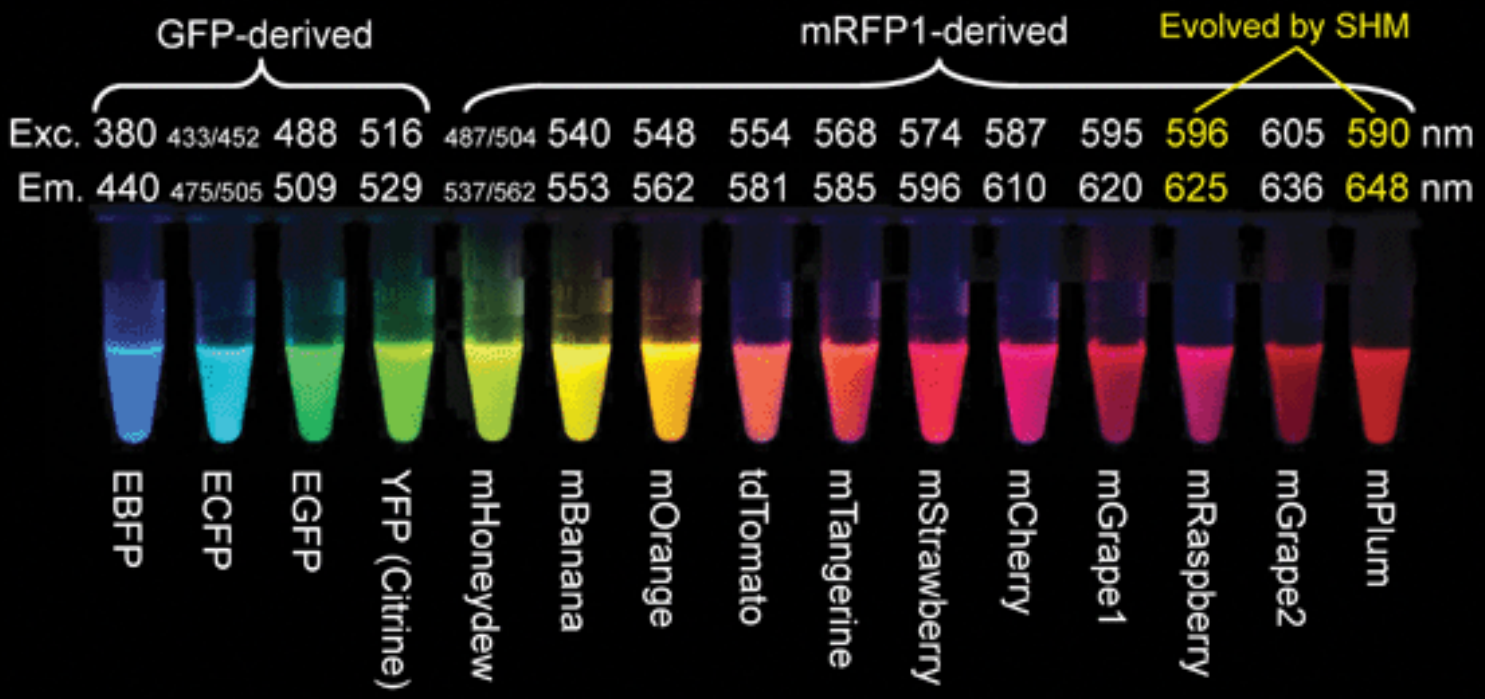
Alexey M. Bogdanov, Elena I. Kudryavtseva, Konstantin A. Lukyanov

Published: December 21, 2012 • http://dx.doi.org/10.1371/journal.pone.0053004

The wonderful world of FPs



The 2004 palette of nonoligomerizing fluorescent proteins



Nathan Shaner et al (2004) *Nature Biotech.* **22**: 1567-1572
Lei Wang et al (2004) *Proc. Natl. Acad. Sci. USA* **101**: 16745-16749

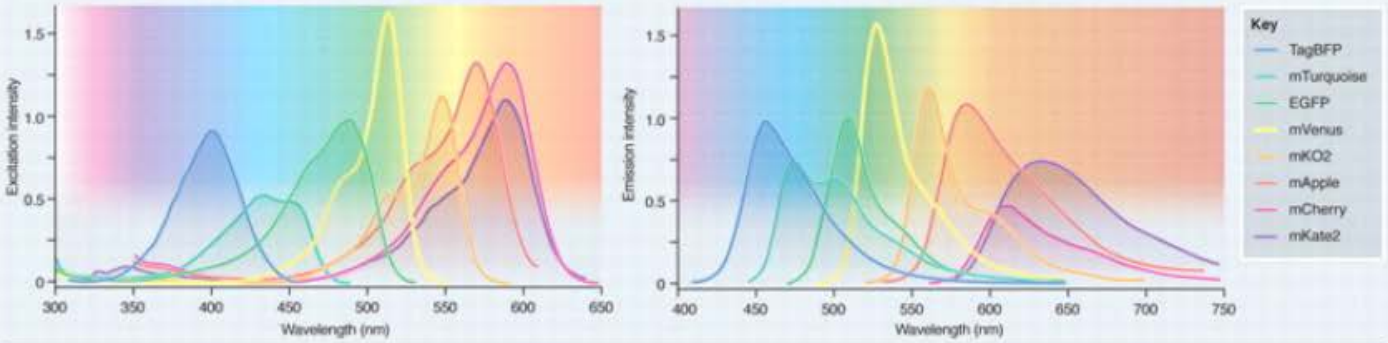
Journal of Cell Science
jcs.biologists.org

Fluorescent Proteins at a Glance

Gert-Jan Kremers, Sarah G. Gilbert, Paula J. Cranfill, Michael W. Davidson and David W. Piston

CHROMA

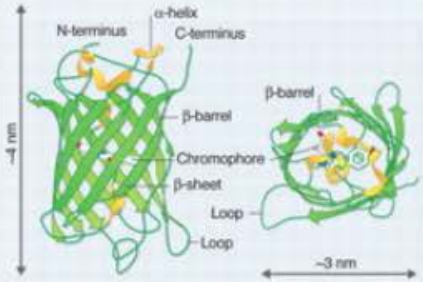
Excitation and emission spectral properties of the brightest fluorescent proteins



Fluorescent protein properties

Protein (acronym)	Excitation maximum (nm)	Emission maximum (nm)	Extinction coefficient $\times 10^{-3}$ (M^{-1} cm^{-1})	Quantum yield	Relative brightness (% of EGFP)
mTagBFP	399	456	52.0	0.63	98
mTurquoise	434	474	30.0	0.84	75
mEGFP	488	507	56.0	0.60	100
mVenus	515	528	92.2	0.57	156
mKO2	551	565	63.8	0.62	118
mApple	568	592	75.0	0.49	109
mCherry	587	610	72.0	0.22	47
mKate2	588	633	62.5	0.40	74

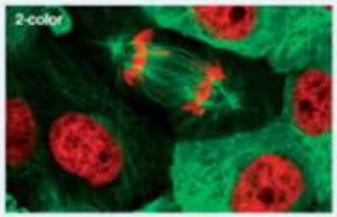
β-barrel motif



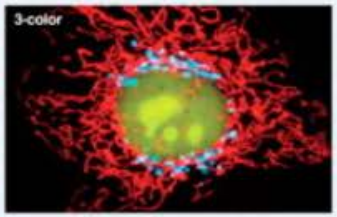
Critical mutations

Mutation	Properties
S30R	Increases folding rate, enhances protein stability
F64L	Accelerates chromophore formation
Q69M	Improves chloride and pH resistance, photostability and folding
S72A	Faster folding rate, stabilizes protein
S147P	Faster maturation rate, located near chromophore
N149K	Faster folding rate, stabilizes protein
V163A	Reduces hydrophobicity, no effect on folding rate
I167T	Reduced thermosensitivity, faster maturation rate

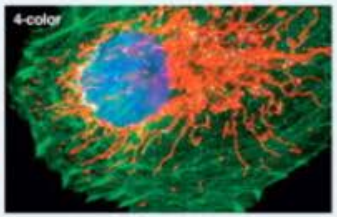
Multi-color imaging using fluorescent protein fusions



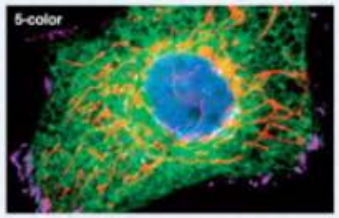
LLC-PK1 (pig kidney) cells expressing mEGFP fused to α-tubulin (green) and mApple fused to histone H2B (red)



HeLa (human carcinoma) cells expressing mTurquoise fused to a Golgi-targeting peptide (cyan), mVenus fused to a nuclear targeting signal (yellow), and mCherry fused to a mitochondrion-targeting peptide (red)

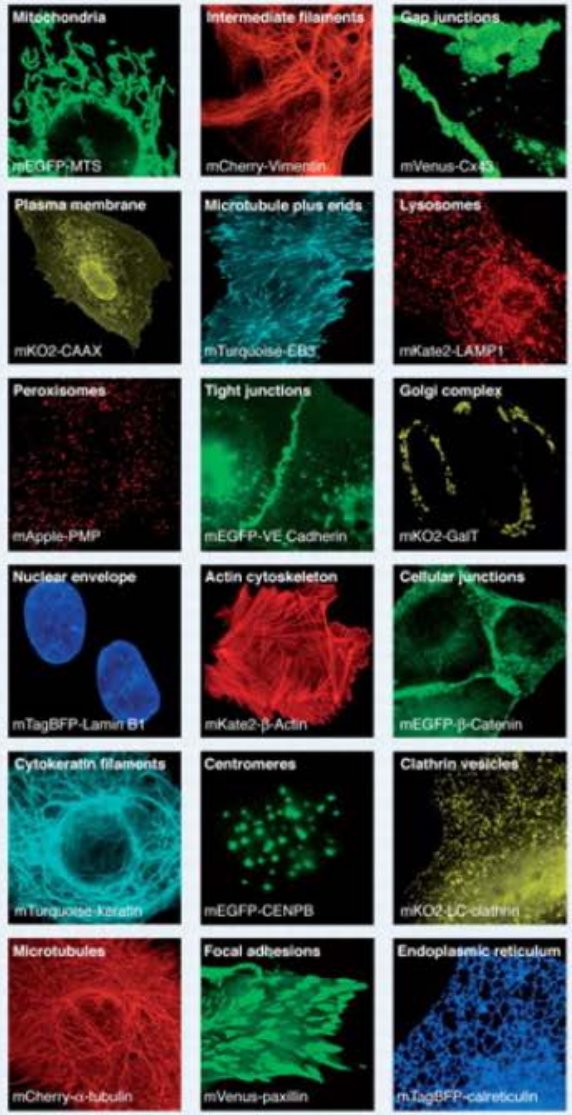


RK-13 (rabbit kidney) cells expressing mTagBFP fused to histone H2B (blue), mTurquoise fused to peroxisomal membrane protein (cyan), mEGFP fused to Lifeact (actin; green), and mCherry fused to pyruvate dehydrogenase (mitochondria; red)



HeLa cells expressing mTagBFP fused to histone H2B (blue), mTurquoise fused to peroxisomal membrane protein (cyan), mEGFP fused to calreticulin (ER; green), mKO2 fused to zyxin (focal adhesions; purple), and mKate2 fused to pyruvate dehydrogenase (mitochondria; red)

Fluorescent protein localization



Trends in Biochemical Sciences

Volume 42, Issue 2, February 2017, Pages 111–129

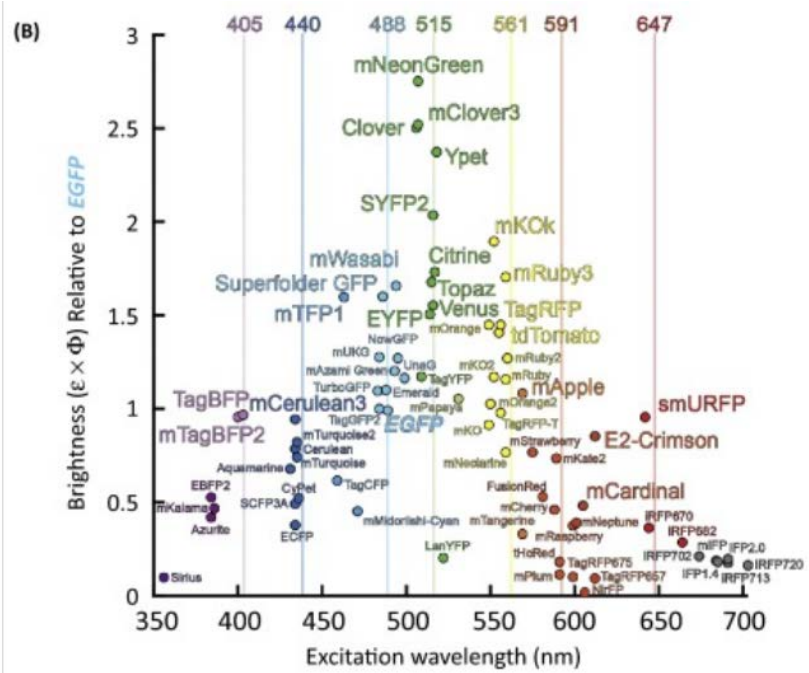
Feature Review

The Growing and Glowing Toolbox of Fluorescent and Photoactive Proteins

Erik A. Rodriguez[1], Robert E. Campbell[2], John Y. Lin[3], Michael Z. Lin[4, 5], Atsushi Miyawaki[6], Amy E. Palmer[7], Xiaokun Shu[8, 9], Jin Zhang[1],

Show more

http://dx.doi.org/10.1016/j.tibs.2016.09.010



http://www.sciencedirect.com/science/article/pii/S0968000416301736

NATURE METHODS | ANALYSIS

Quantitative assessment of fluorescent proteins

Paula J Cranfill, Brittney R Sell, Michelle A Baird, John R Allen, Zeno Lavagnino, H Martijn de Gruiter, Gert-Jan Kremers, Michael W Davidson, Alessandro Ustione & David W Piston

Affiliations | **Contributions** | **Corresponding author**

Nature Methods **13**, 557–562 (2016) | doi:10.1038/nmeth.3891

Received 18 March 2016 | Accepted 07 May 2016 | Published online 30 May 2016

doi:10.1038/nmeth.3891

How to choose your Fps (More than 400 available across the spectra)

- **Excitation & Emission** (ex/em) –-→ Color

- **Oligomerization**
Use monomeric proteins !!!!

- Oxygen

- **Maturation Time**
(superfolder GFP (sfGFP) and mNeonGFP can fold in <10min at 37°C,
mCherry takes ~15min, TagRFP ~100min and DsRed ~10hours)

- Temperature

- **Brightness**

- Photostability

- **pH Stability**

More info here: http://blog.addgene.org/which-fluorescent-protein-should-i-use

Spectra viewer : http://www.fpvis.org/ @scopekurt

What you most likely use:

- GFP: 1994
- EGFP : 1996
- emeraldGFP: 1999
- mCherry : 2004
- EYFP: 1997
- ECFP: 1997

We are in 2017...


The Future

The Blues

- mTurquoise2 (2012)

@joachimgoedhart

Structure-guided evolution of cyan fluorescent proteins towards a quantum yield of 93%

Joachim Goedhart, David von Stetten, Marjolaine Noirclerc-Savoye, Mickaël Lelimousin, Linda Joosen, Mark A. Hink, Laura van Weeren, Theodorus W.J. Gadella Jr & Antoine Royant

Nature Communications **3**, Article number: 751 (2012)
doi:10.1038/ncomms1738
Download Citation

Received: 14 October 2011
Accepted: 08 February 2012
Published online: 20 March 2012

- mCerulean3 (2011)

RESEARCH ARTICLE

An Improved Cerulean Fluorescent Protein with Enhanced Brightness and Reduced Reversible Photoswitching

Michele L. Markwardt, Gert-Jan Kremers, Catherine A. Kraft, Krishanu Ray, Paula J. C. Cranfill, Korey A. Wilson, Richard N. Day, Rebekka M. Wachter, Michael W. Davidson, Mark A. Rizzo

Published: March 29, 2011 • http://dx.doi.org/10.1371/journal.pone.0017896

The Greens

- mNeonGreen (2013)

@NathanShaner

A bright monomeric green fluorescent protein derived from *Branchiostoma lanceolatum*

Nathan C Shaner, Gerard G Lambert, Andrew Chammas, Yuhui Ni, Paula J Cranfill, Michelle A Baird, Brittney R Sell, John R Allen, Richard N Day, Maria Israelsson, Michael W Davidson & Jiwu Wang

Affiliations | **Contributions** | **Corresponding author**

Nature Methods **10**, 407–409 (2013) | doi:10.1038/nmeth.2413

Received 25 July 2012 | Accepted 19 February 2013 | Published online 24 March 2013

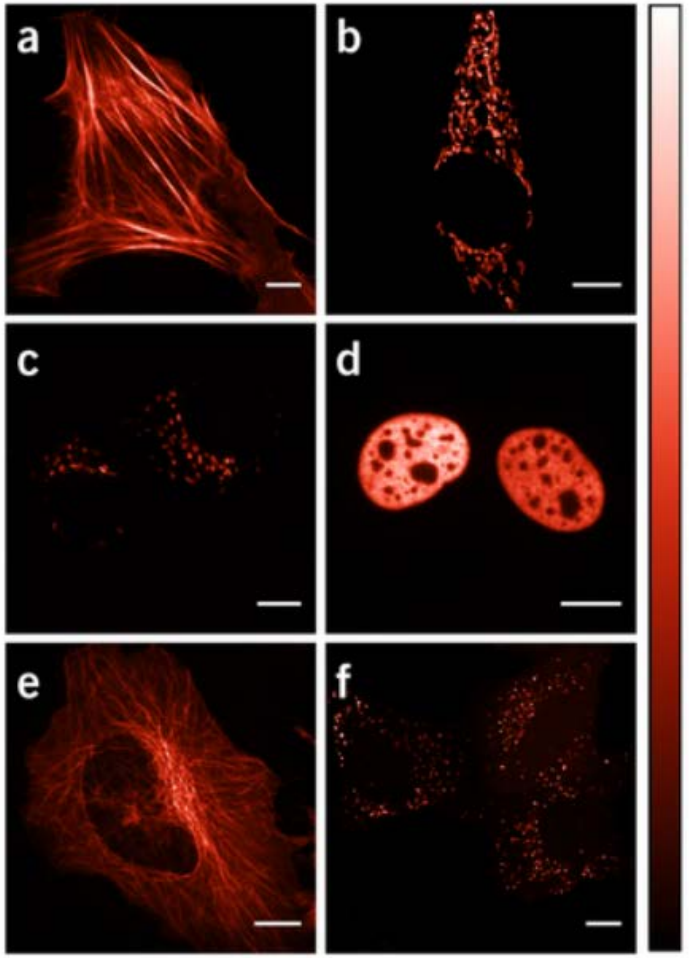
- mClover3 (2016)

Article | OPEN

Improving brightness and photostability of green and red fluorescent proteins for live cell imaging and FRET reporting

Bryce T. Bajar, Emily S. Wang, Amy J. Lam, Bongjae B. Kim, Conor L. Jacobs, Elizabeth S. Howe, Michael W. Davidson, Michael Z. Lin ✉ & Jun Chu ✉

The Reds : mScarlet



2016

mScarlet: a bright monomeric red fluorescent protein for cellular imaging

Daphne S Bindels, Lindsay Haarbosch, Laura van Weeren, Marten Postma, Katrin E Wiese, Marieke Mastop, Sylvain Aumonier, Guillaume Gotthard, Antoine Royant, Mark A Hink & Theodorus W J Gadella Jr

Nature Methods **14**, 53–56 (2017) | doi:10.1038/nmeth.4074
Received 29 July 2016 | Accepted 20 October 2016 | Published online 21 November 2016 | Corrected online **12 December 2016**

The Far REDs

-mIRFP670 (2016) : no biliverdin required

Article | OPEN

Bright monomeric near-infrared fluorescent proteins as tags and biosensors for multiscale imaging

Daria M. Shcherbakova, Mikhail Baloban, Alexander V. Emelyanov, Michael Brenowitz, Peng Guo & Vladislav V. Verkhusha

Nature Communications **7**,
Article number: 12405 (2016)
doi:10.1038/ncomms12405

Received: 27 December 2015
Accepted: 29 June 2016
Published online: 19 August 2016

-smURF (2016): very bright in presence of biliverdin (same as EGFP)

@erin_rod_phd

A far-red fluorescent protein evolved from a cyanobacterial phycobiliprotein

Erik A Rodriguez, Geraldine N Tran, Larry A Gross, Jessica L Crisp, Xiaokun Shu, John Y Lin & Roger Y Tsien

Nature Methods **13**, 763–769 (2016) | doi:10.1038/nmeth.3935
Received 07 December 2015 | Accepted 01 July 2016 | Published online 01 August 2016
| Corrected online **16 September 2016**
Corrigendum (October, 2016)

The one for super resolution (switchable)

Skylan (2015)

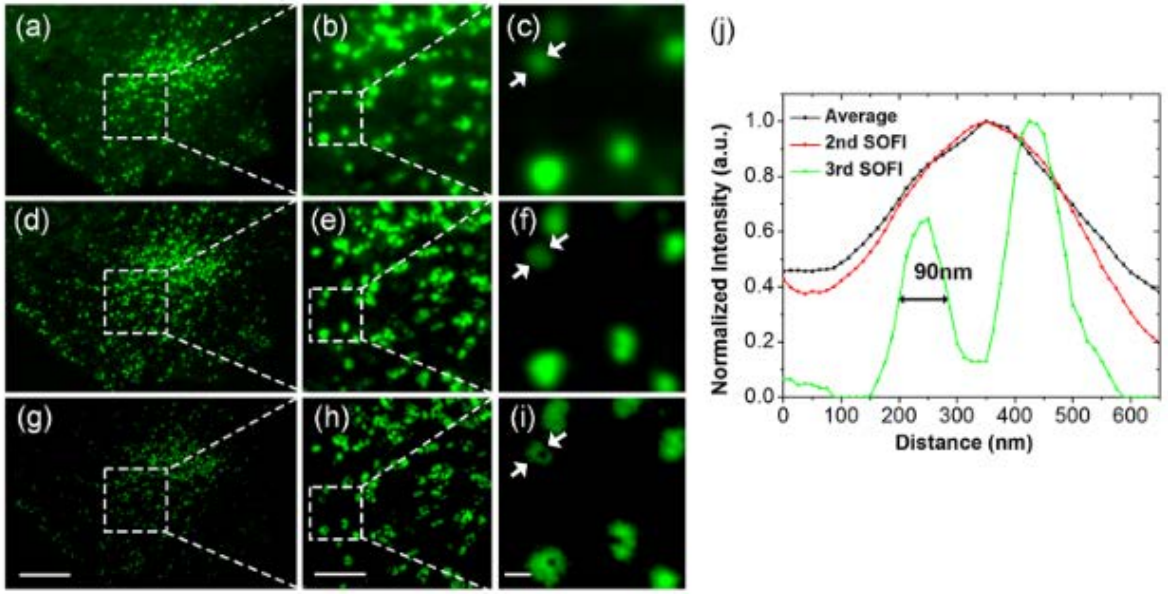


Figure 5. SOFI images of clathrin-coated pits (CCPs) in live U2OS cells. (a–c) TIRFM images of CCPs of living U2OS cells fused with Skylan-S under its optimal condition. (d–f) Second-order SOFI images. (g–i) Third-order SOFI images. (b,e,h) Zoomed-in views of the boxed regions in panels a, d, and g, respectively. (j) Intensity profiles of cross sections taken along the white arrows indicated in panels c, f, and i. The scale bars represent (a,d,g) 10 μm, (b,e,h) 3 μm, and (c,f,i) 500 nm.

Development of a Reversibly Switchable Fluorescent Protein for Super-Resolution Optical Fluctuation Imaging (SOFI)

Xi Zhang[†‡], Xuanze Chen[§‖], Zhiping Zeng[§], Mingshu Zhang[‡], Yujie Sun[‖], Peng Xi[*§], Jianxin Peng[*†], and Pingyong Xu[*‡]

[†] Institute of Entomology, School of Life Sciences, Central China Normal University, Wuhan 430079, China
[‡] Key Laboratory of RNA Biology, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China
[§] Department of Biomedical Engineering, College of Engineering, Peking University, Beijing 100871, China
[‖] State Key Laboratory of Biomembrane and Membrane Biotechnology, Biodynamic Optical Imaging Center (BIOPIC), School of Life Sciences, Peking University, Beijing 100871, China

ACS Nano, **2015**, *9* (3), pp 2659–2667
DOI: 10.1021/nn5064387
Publication Date (Web): February 19, 2015

The FRET Pairs

Blue - Yellow

- mTurquoise2 - mCitrine

Green- Red

MClover3 - mRuby3

Article | OPEN

Improving brightness and photostability of green and red fluorescent proteins for live cell imaging and FRET reporting

Bryce T. Bajar, Emily S. Wang, Amy J. Lam, Bongjae B. Kim, Conor L. Jacobs, Elizabeth S. Howe, Michael W. Davidson, Michael Z. Lin & Jun Chu

Red- Far Red

More info: www.mdpi.com/1424-8220/16/9/1488/pdf

The FP that resist to the cellular environment (PH / Oxidation)

Article

A palette of fluorescent proteins optimized for diverse cellular environments

Lindsey M. Costantini, Mikhail Baloban, Michele L. Markwardt, Mark Rizzo, Feng Guo, Vladislav V. Verkhusha & Erik L. Snapp

Nature Communications **6**, Article number: 7670 (2015)
doi:10.1038/ncomms8670
Download Citation

Fluorescent proteins | Organelles

Received: 09 October 2014
Accepted: 28 May 2015
Published online: 09 July 2015

Article | OPEN

Identification and Characterisation of a pH-stable GFP

Tania Michelle Roberts, Fabian Rudolf, Andreas Meyer, Rene Pellaux, Ellis Whitehead, Sven Panke & Martin Held

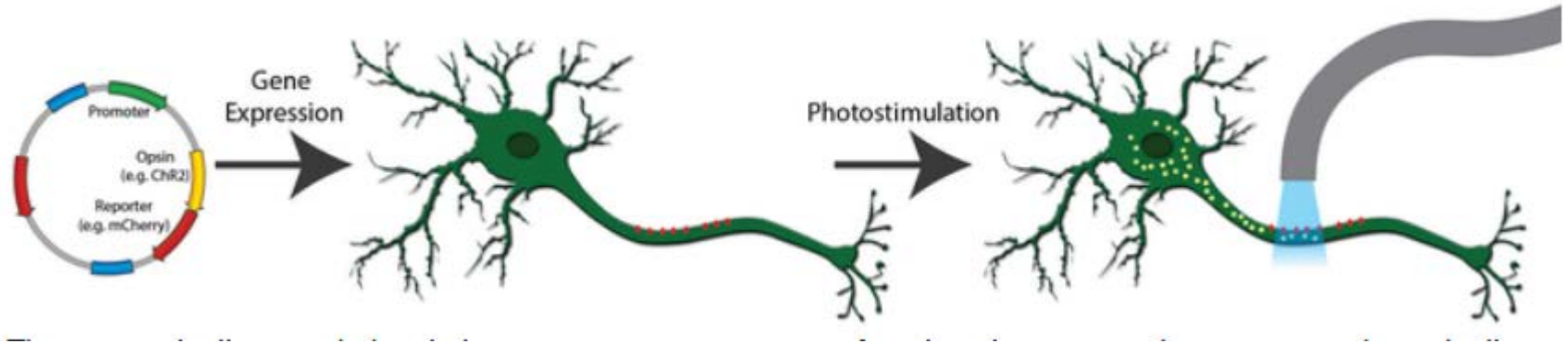
Scientific Reports **6**, Article number: 28166 (2016)
doi:10.1038/srep28166
Download Citation

Fluorescent proteins | Protein design

Received: 10 February 2016
Accepted: 01 June 2016
Published online: 21 June 2016

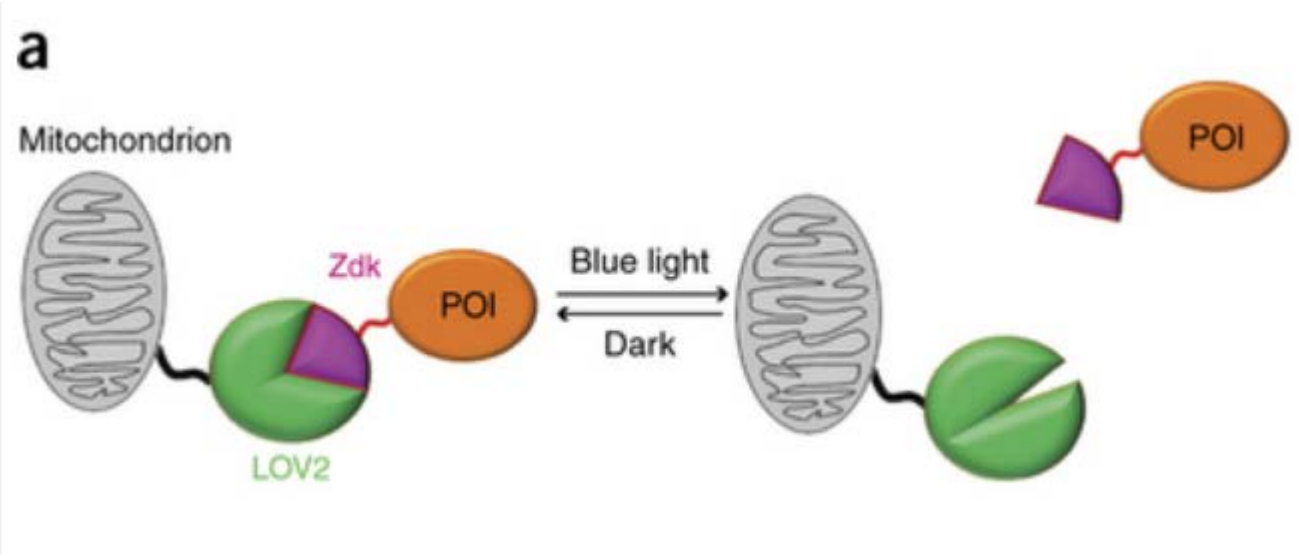
Optogenetics

Controlling cellular behavior with light



https://www.addgene.org/optogenetics/

The TRAPs (LOVTRAP)



LOVTRAP: an optogenetic system for photoinduced protein dissociation

Hui Wang, Marco Vilela, Andreas Winkler, Miroslaw Tarnawski, Ilme Schlichting, Hayretin Yumerefendi, Brian Kuhlman, Rihe Liu, Gaudenz Danuser & Klaus M Hahn

Affiliations | **Contributions** | **Corresponding authors**

Nature Methods **13**, 755–758 (2016) | doi:10.1038/nmeth.3926

Received 21 November 2015 | Accepted 16 June 2016 | Published online 18 July 2016

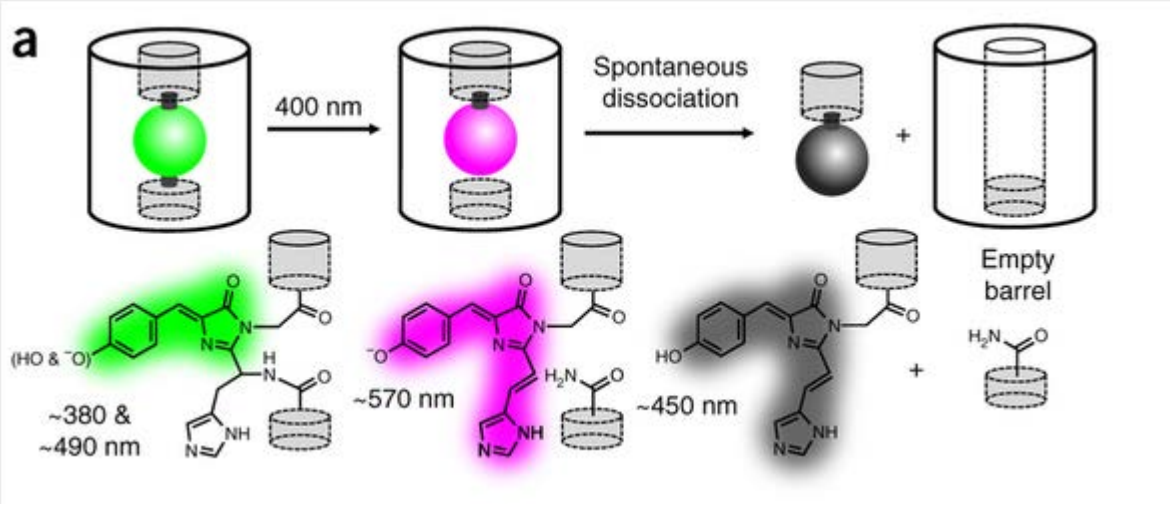
Optogenetic control with a photocleavable protein, PhoCl

Wei Zhang, Alexander W Lohman, Yevgeniya Zhuravlova, Xiaocen Lu, Matthew D Wiens, Hiofan Hoi, Sine Yaganoglu, Manuel A Mohr, Elena N Kitova, John S Klassen, Periklis Pantazis, Roger J Thompson & Robert E Campbell

Affiliations | **Contributions** | **Corresponding author**

Nature Methods (2017) | doi:10.1038/nmeth.4222

Received 23 September 2016 | Accepted 14 February 2017 | Published online 13 March 2017



And everything else...

	FRET	Find FRET pairs and standards to study protein-protein interactions or conformational changes within a protein.
	Biosensors	Monitor small biomolecules or other physiological intracellular processes with genetically encoded fluorescent biosensors.
	Optogenetics	Use light to detect, measure, and control molecular signals, cells, or groups of cells with either actuators or sensors.
	Chemogenetics	Use small molecules to activate genetically engineered cellular receptors that affect signalling pathways within cells.
	Subcellular Localization	Determine where your protein of interest resides by using a well-characterized fluorescent fusion protein.
	*In Vivo* Imaging	Image with these powerful tools to study individual plasmids or protein-protein interactions in organs and whole mammals.
	Regulate Biological Activity	Use fluorescent proteins to modulate biological activity, like transcription.

https://www.addgene.org/fluorescent-proteins/

Getting fluorescent proteins

- **Ask**

- **Addgene** (https://www.addgene.org/)

  - Michael Davidson Fluorescent Protein Collection (over 3000 tagged proteins)

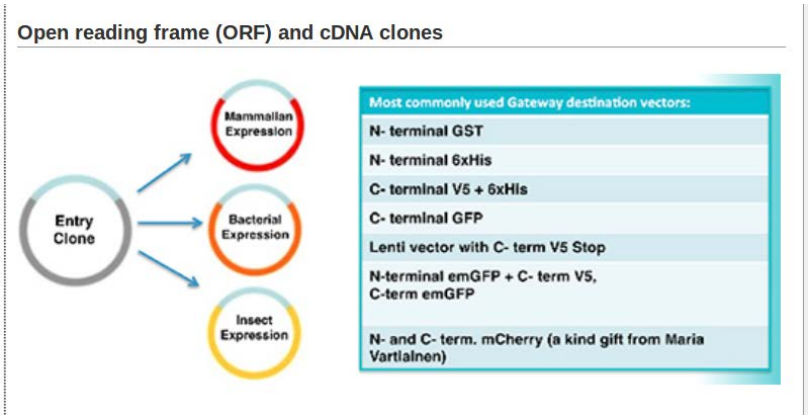
    https://www.addgene.org/fluorescent-proteins/davidson/

- **GBU unit in Helsinki**: Large ORF library accessible to research done in Finland

  - http://www.biocenter.helsinki.fi/bi/gbu/

  - Gateway entry clones

  - Cloning services (20 euro)



Allen Cell Collection: Human stem cells where the endogenous proteins are GFP tagged

http://www.allencell.org/cell-line-catalog

Allen Cell Collection available at Coriell

Cell Line ID	Protein	Gene name
AICS-0005	Paxillin	Paxillin (PXN)
AICS-0011	TOM20	Translocase of outer mitochondrial membrane 20 (TOMM20)
AICS-0012	Alpha-tubulin	Tubulin-alpha 1b (TUBA1B)
AICS-0013	LaminB1	Lamin B1 (LMNB1)
AICS-0017	Desmoplakin	Desmoplakin (DSP)

List of Cell Lines in Progress

Cell Line ID	Protein	Gene name	Structure	Tag Location	Fluorophore	Parental Line	Status
AICS-0010	Sec61-beta	Sec61 translocon beta subunit (SEC61B)	Endoplasmic reticulum	N-terminus	mEGFP	WTC	Final QC
AICS-0014	Fibrillarin	Fibrillarin (FBL)	Nucleolus	C-terminus	mEGFP	WTC	Final QC
AICS-0016	Beta-actin	Actin beta (ACTB)	Actin	N-terminus	mEGFP	WTC	Final QC
AICS-0020	Vimentin	Vimentin (VIM)	Intermediate filaments	N-terminus	mEGFP	WTC	Screening & QC
AICS-0022	LAMP1	lysosomal associated membrane protein 1 (LAMP1)	Lysosome	C-terminus	mEGFP	WTC	Design phase
AICS-0023	Tight junction protein ZO-1	Tight junction protein 1 (TJP1)	Tight junctions	N-terminus	mEGFP	WTC	Screening & QC
AICS-0024	Myosin IIB	Myosin heavy chain 10 (MYH10)	Myosin	N-terminus	mEGFP	WTC	Screening & QC
AICS-0025	beta-galactoside alpha-2,6-sialyltransferase 1	ST6 beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1)	Golgi	C-terminus	mEGFP	WTC	Pre-clonal edited
AICS-0029	LaminB1	Lamin B1 (LMNB1)	Nucleus	N-terminus	tdTomato	WTC	Screening & QC
AICS-0030	LC3	Microtubule associated protein 1 light chain 3 beta (MAP1LC3B)	Autophagosomes	N-terminus	mEGFP	WTC	Screening & QC
AICS-0031	Alpha-tubulin	Tubulin-alpha 1b (TUBA1B)	Microtubules	N-terminus	mtagRFP-T	WTC	Screening & QC
AICS-0032	Centrin	Centrin 2 (CETN2)	Centrosome	N-terminus	mtagRFP-T	WTC	Screening & QC
AICS-0036	GFP	(AAVS1-CAG-GFP)	Cytoplasm		mEGFP	WTC	Screening & QC

Software and data analyses

ImageJ / Fiji

Fiji is an image processing package—a "batteries-included" distribution of ImageJ, bundling a lot of plugins which facilitate scientific image analysis.

Download » Cite » Contribute »

NATURE METHODS | PERSPECTIVE

Fiji: an open-source platform for biological-image analysis

Johannes Schindelin, Ignacio Arganda-Carreras, Erwin Frise, Verena Kaynig, Mark Longair, Tobias Pietzsch, Stephan Preibisch, Curtis Rueden, Stephan Saalfeld, Benjamin Schmid, Jean-Yves Tinevez, Daniel James White, Volker Hartenstein, Kevin Eliceiri, Pavel Tomancak & Albert Cardona

Affiliations | **Corresponding authors**

Nature Methods **9**, 676–682 (2012) | doi:10.1038/nmeth.2019

Published online 28 June 2012

ImageJ / Fiji resources

http://wiki.cmci.info/documents/ijcourses#macro_programming_in_imagej

Basics

https://imagej.net/Category:Tutorials

http://imagej.net/Using_Fiji

DOI:10.5281/zenodo.51511

List of main plugins

http://imagej.net/List_of_update_sites

https://imagej.nih.gov/ij/plugins/

Scripting / write your own ImageJ-based software

DOI:10.5281/zenodo.30267

https://imagej.nih.gov/ij/developer/macro/macros.html

NanoJ- SRRF

- ImageJ based and Free

- Image with any microscope

- Around 80 nm Resolution

Article | OPEN

Fast live-cell conventional fluorophore nanoscopy with ImageJ through super-resolution radial fluctuations

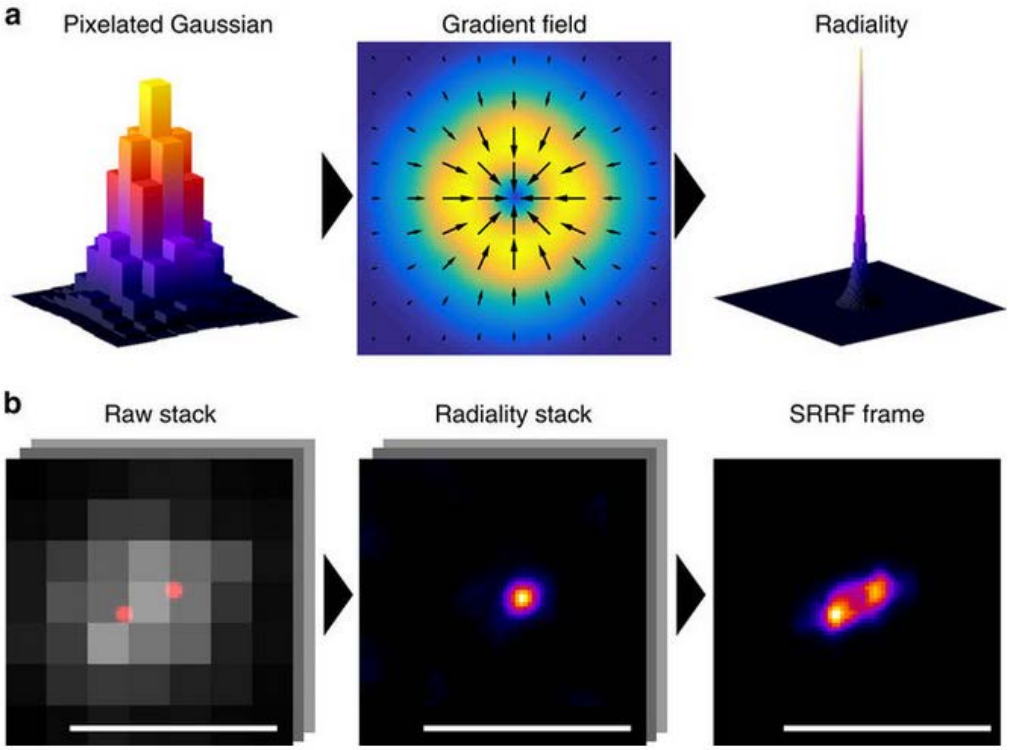
Nils Gustafsson, Siân Culley, George Ashdown, Dylan M. Owen, Pedro Matos Pereira & Ricardo Henriques ✉

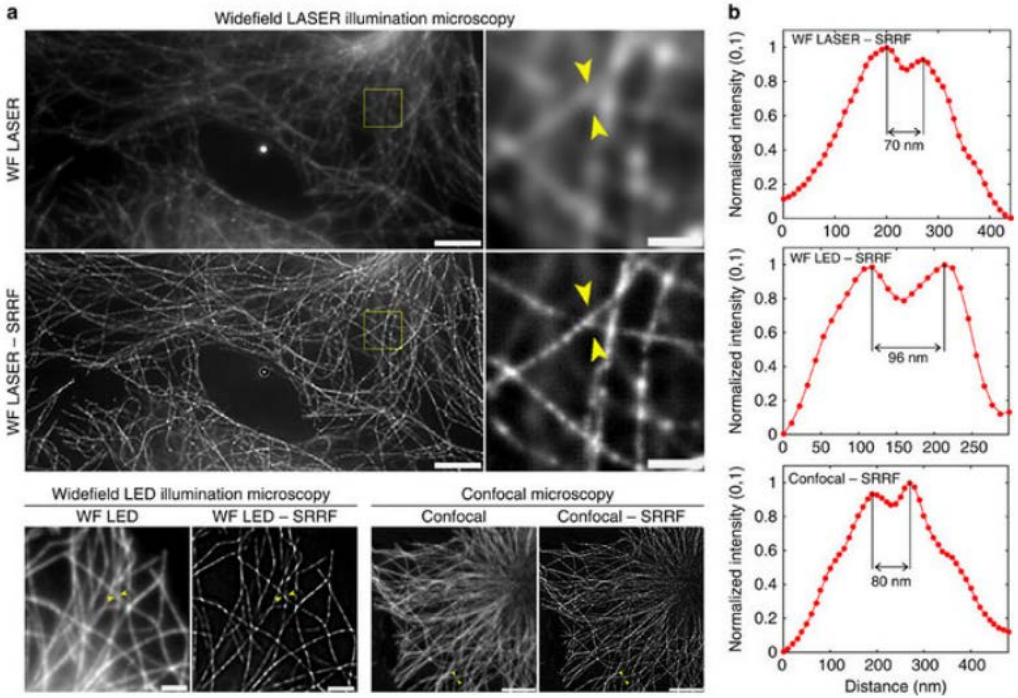
Nature Communications **7**,
Article number: 12471 (2016)
doi:10.1038/ncomms12471

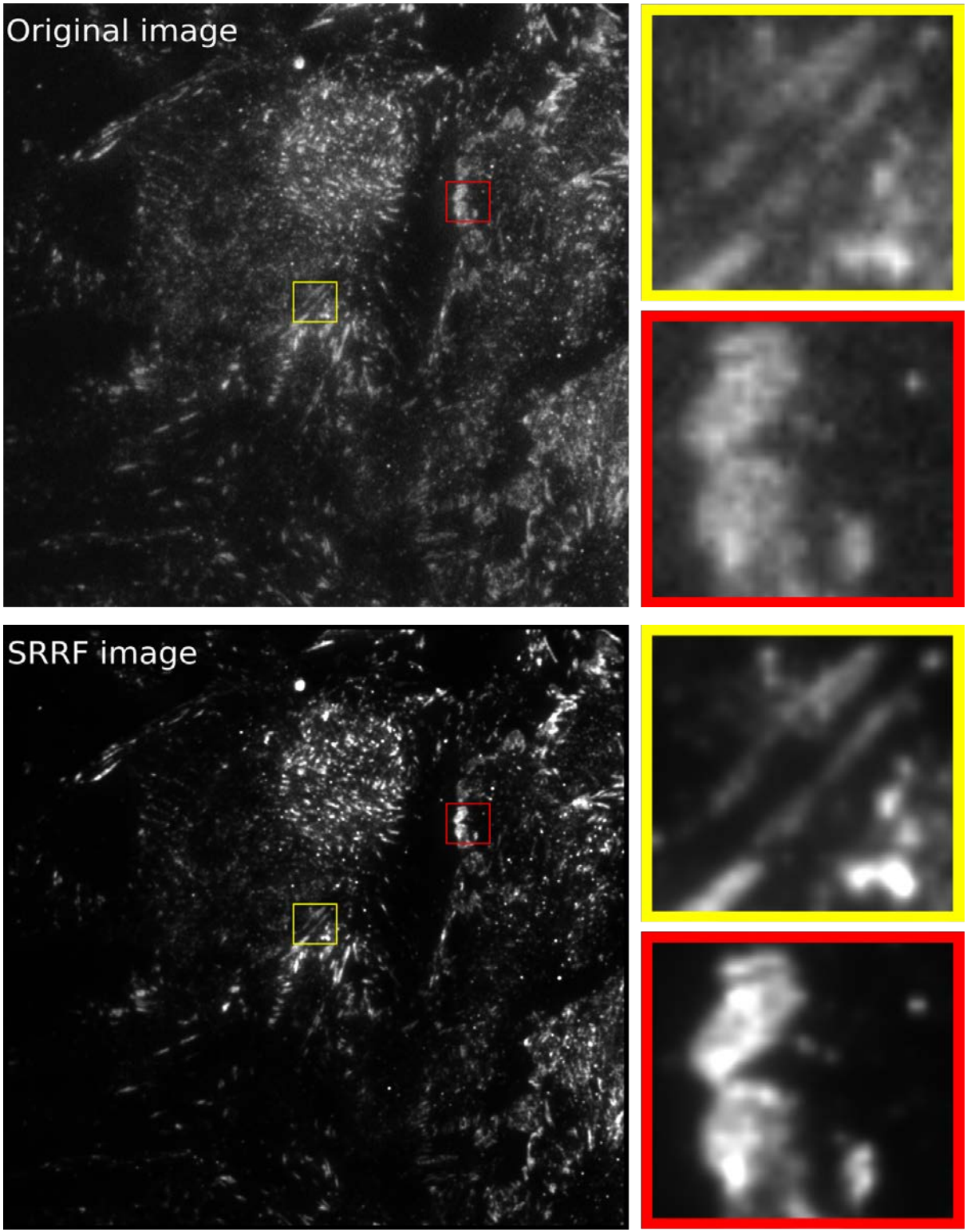
Received: 22 March 2016
Accepted: 05 July 2016
Published online: 12 August 2016

@HenriquesLab

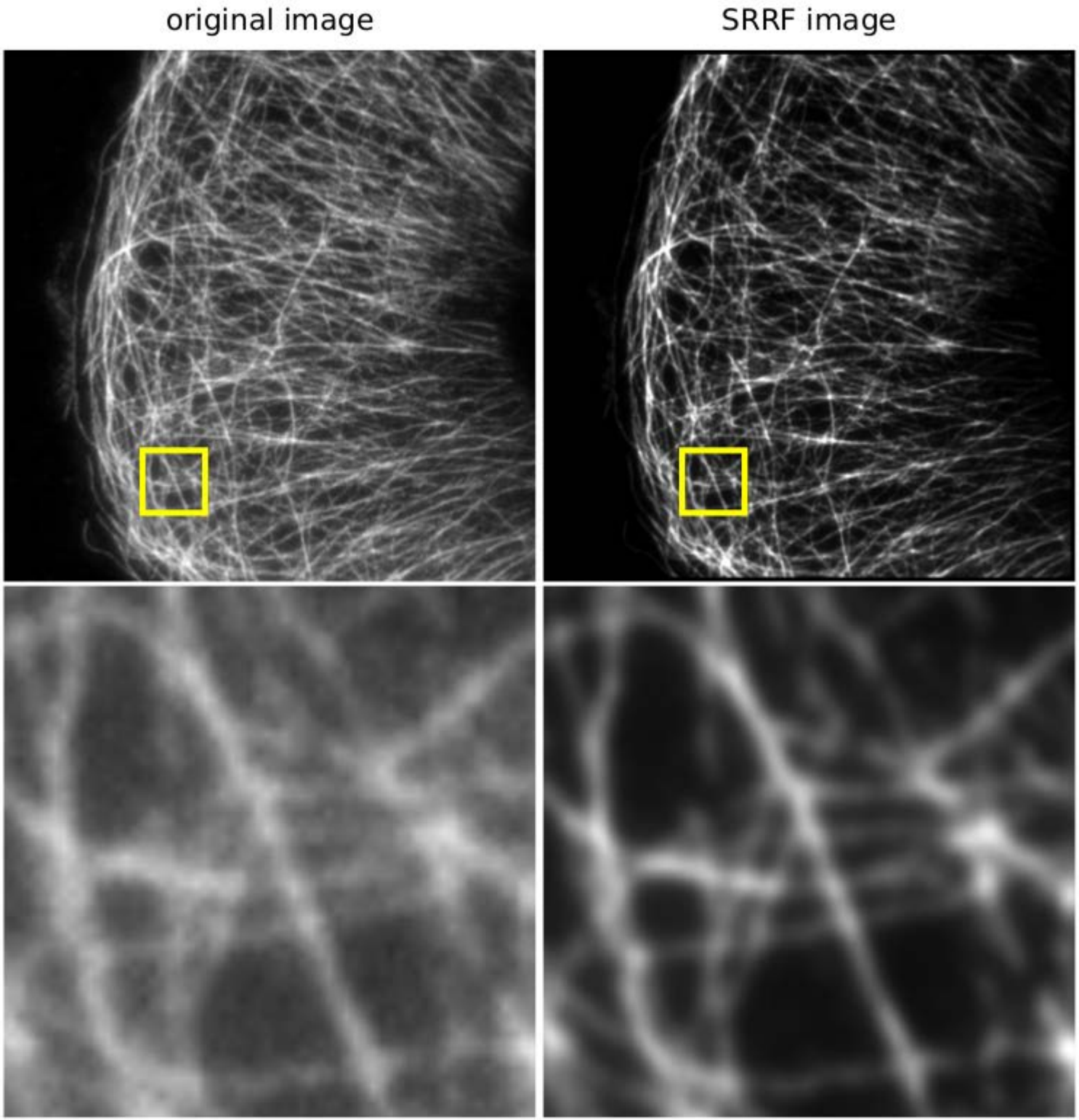
Figure 1: The SRRF algorithm.







@guijacquemet



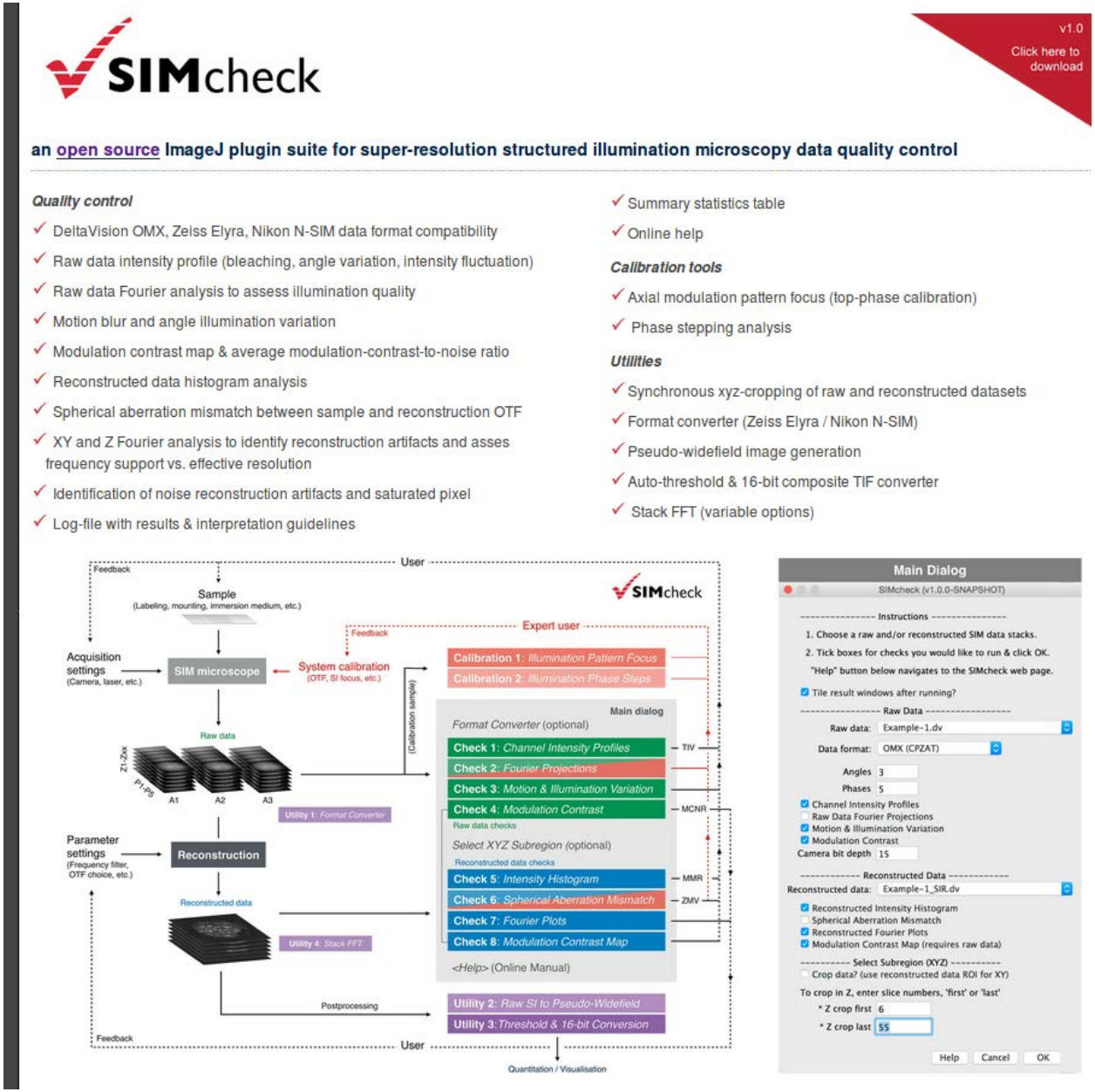
@guijacquemet

SIM check

ImageJ based

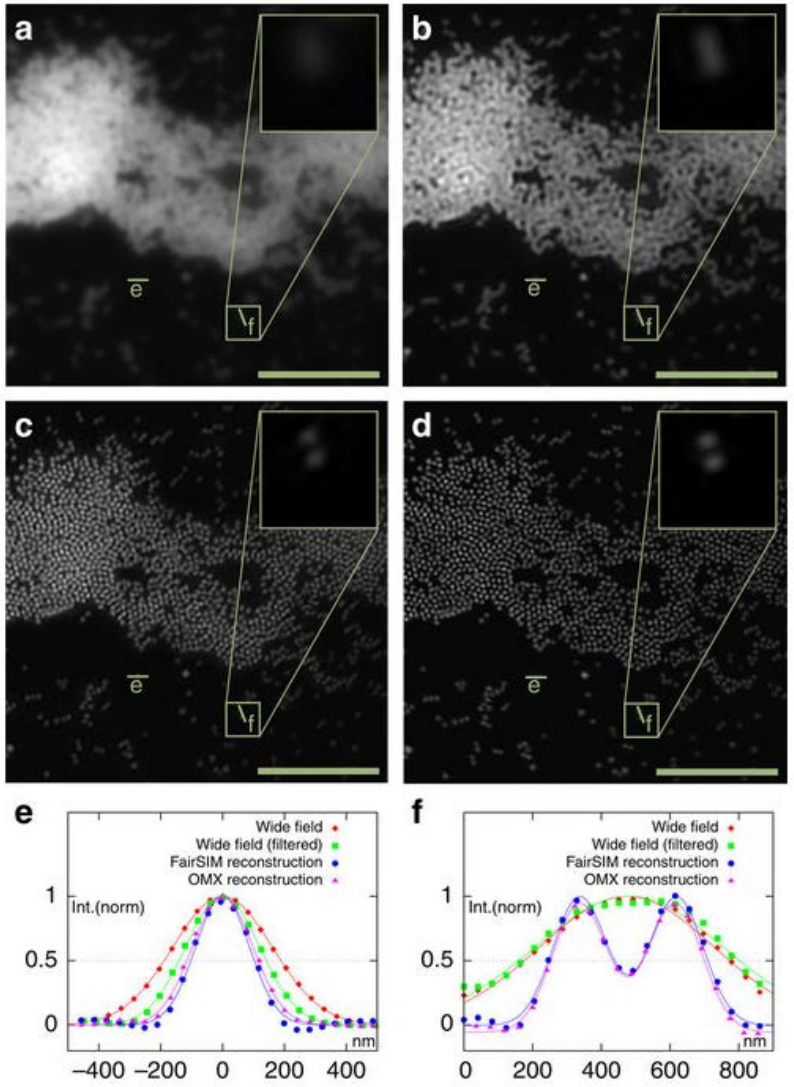
Free

Quality control for SIM data



FairSIM - ImageJ based

Figure 3: FairSIM reconstruction of data sets obtained on the G Healthcare DeltaVision|OMX.



Article | OPEN

Open-source image reconstruction of super-resolution structured illumination microscopy data in ImageJ

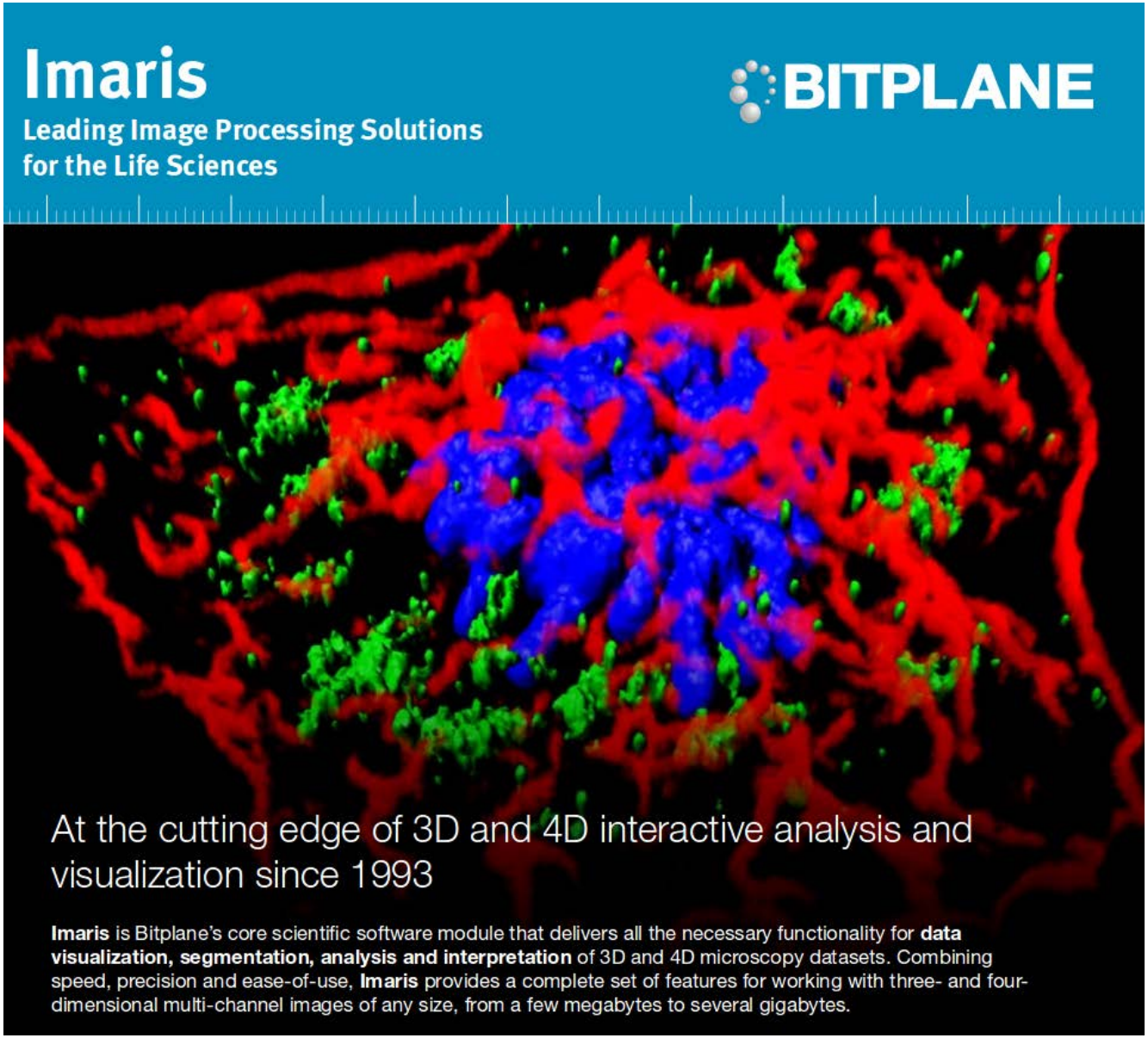
Marcel Müller ✉, Viola Mönkemöller, Simon Hennig, Wolfgang Hübner & Thomas Huser ✉

Nature Communications **7**,
Article number: 10980 (2016)
doi:10.1038/ncomms10980
Download Citation

Biophysics | Microscopy

Received: 16 October 2015
Accepted: 08 February 2016
Published online: 21 March 2016

Imaris 3D visualization software (CIC has a license)

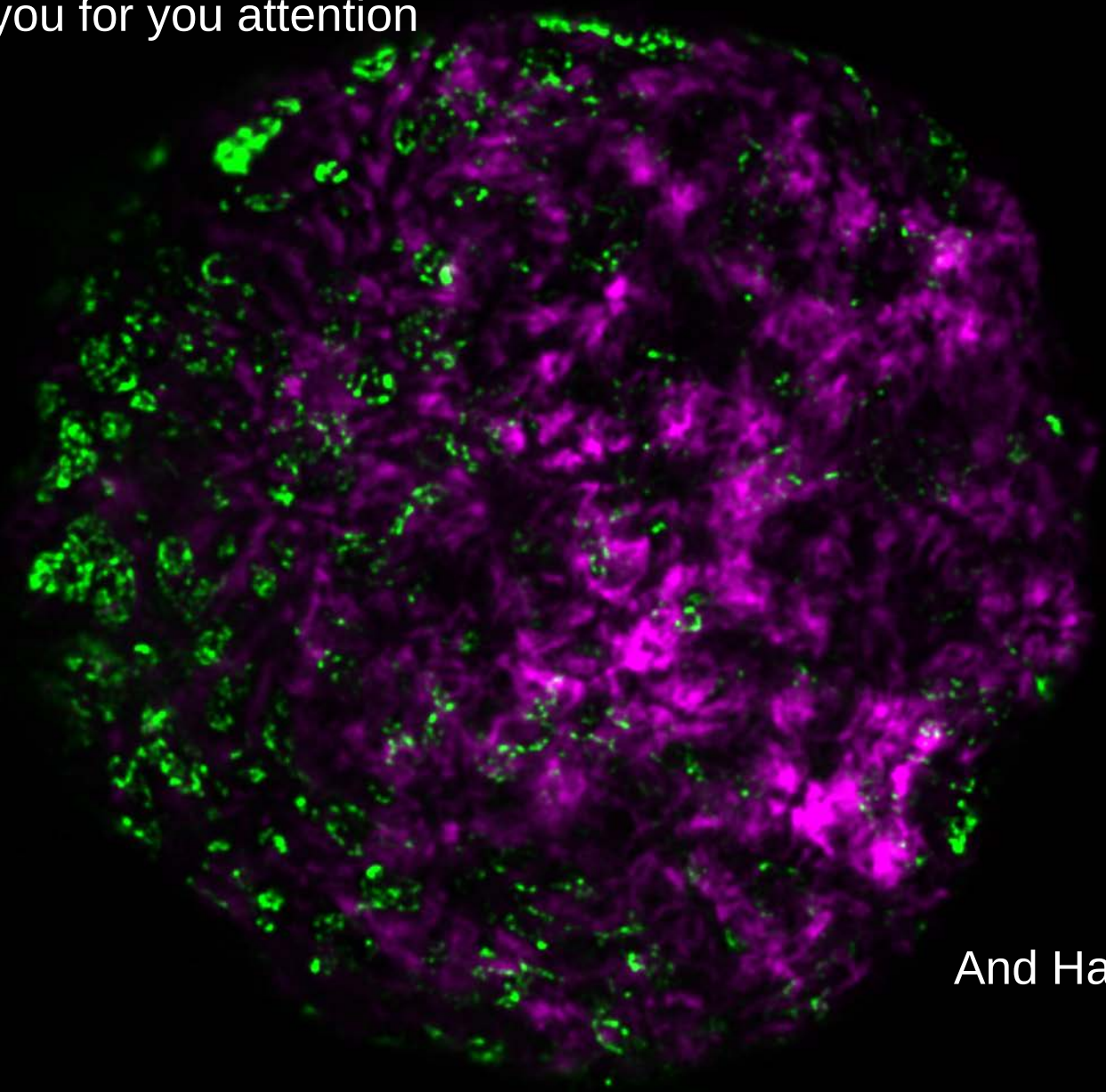


http://www.bitplane.com/imaris/imaris

And many others

- Cell Profiler
- BioImagexD
- MatLAB
- ...

Thank you for you attention



And Happy imaging