MICRON Advanced Microscopy Course

Welcome and key things to remember

Ilan Davis, November 2020

THANKS to Nadia and Carina
And to all the lectures and contributors

Strategic Awards:
2010-2021

Medical Research Council

MRC led advanced microscopy technology grant 2014-2019

Micron OXFORD

http://www.micronoxford.com

Advanced microscopy course

Fire escapes

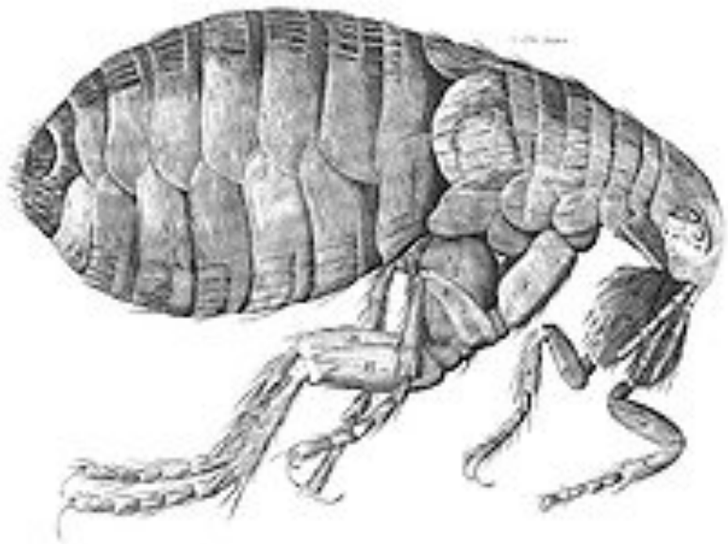
Lectures: https://micronoxford.com/micron-microscopy-course-1

There is no
Free lunch !!!

Giant leap


Basic
Microscopy
Advanced
Microscopy
History of the Micron advanced
Microscopy course

The importance of microscopes



1st century AD, glass was invented by the Romans - who then discovered crude lenses.
13th century spectacle makers were producing lenses to be worn as glasses.
1609 Galileo Galilei perfected the first device known as a microscope.
Zaccharias Janssen and Hans Lipperhey first compound microscope
Later in the 16th century, Anton van Leeuwenhoek began polishing and grinding lenses

Milestones in the history of microscopy

http://www.nature.com/milestones/milelight/pdf/milelight_timeline.pdf

http://www.nature.com/milestones/milelight/pdf/milelight_all.pdf

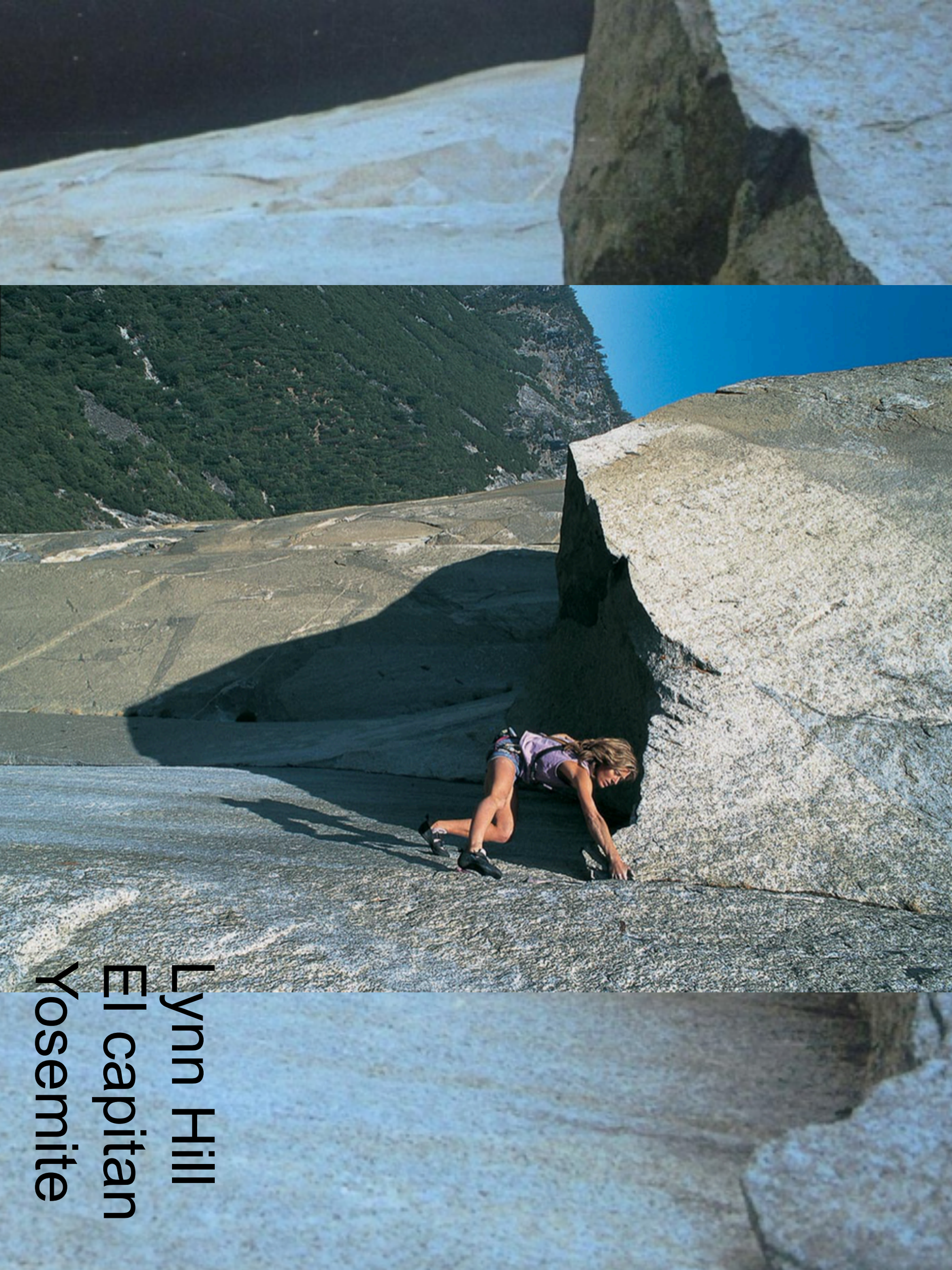
1st century AD, glass was been invented by the Romans - who then discovered crude lenses.

13th century when spectacle makers were producing lenses to be worn as glasses.

MILESTONES TIMELINE

1595	Invention of the microscope (Milestone 1)	Zaccharias Janssen Galileo
1858	First histological stain (Milestone 2)	
1871	Synthesis of fluorescein (Milestone 2)	
1873	Diffraction limit theory (Milestone 3)	Ernst Abbe 1880 Agust Kohler
1911	First fluorescence microscopy (Milestone 4)	
1929	First epifluorescence microscope (Milestone 4)	1931 EM Ernst Ruska
1935	Phase contrast microscopy (Milestone 5)	1932 Fritts Zernike
1939	Polarization microscopy (Milestone 6)	
1942	Immunofluorescence (Milestone 7)	
1955	Differential interference contrast (Milestone 8)	Normaski
1961	Concept of confocal microscopy (Milestone 9)	Marvin Minsky
1967	The dichroic mirror (Milestone 4)	
1972	Fluorescence correlation spectroscopy (Milestone 10)	
1976	FRAP (Milestone 10)	
	FRET (Milestone 11)	
1980	Calcium probes (Milestone 12)	
1981	Video-enhancement differential interference contrast (Milestone 8)	
	TIRF microscopy (Milestone 13)	
1983	Deconvolution microscopy (Milestone 14)	Agard and Sedat
1987	Realization of confocal microscopy (Milestone 9)	AFM Calvin Quate
1990	Two-photon microscopy (Milestone 15)	
1993	Light sheet microscopy (Milestone 16)	Ernst Stelzer
	Single molecule microscopy (Milestone 17)	
1994	GFP (Milestone 18)	Martie Chalfie, Roger Chen
1997	Fluorescent protein-based biosensors (Milestone 19)	
1999	Red fluorescent proteins (Milestone 20)	Roger Chen
2000	Breaking the diffraction limit: STED (Milestone 21)	Stefan Hell
2002	Photoactivatable fluorescent proteins (Milestone 20)	Jennifer Lippincott-Schwartz
2006	Breaking the diffraction limit: PALM/STORM (Milestone 21)	

2000 3D-SIM
Mats Gustafsson
John Sedat



Lynn Hill
El capitan
Yosemite

PSF: Don’t miss the point

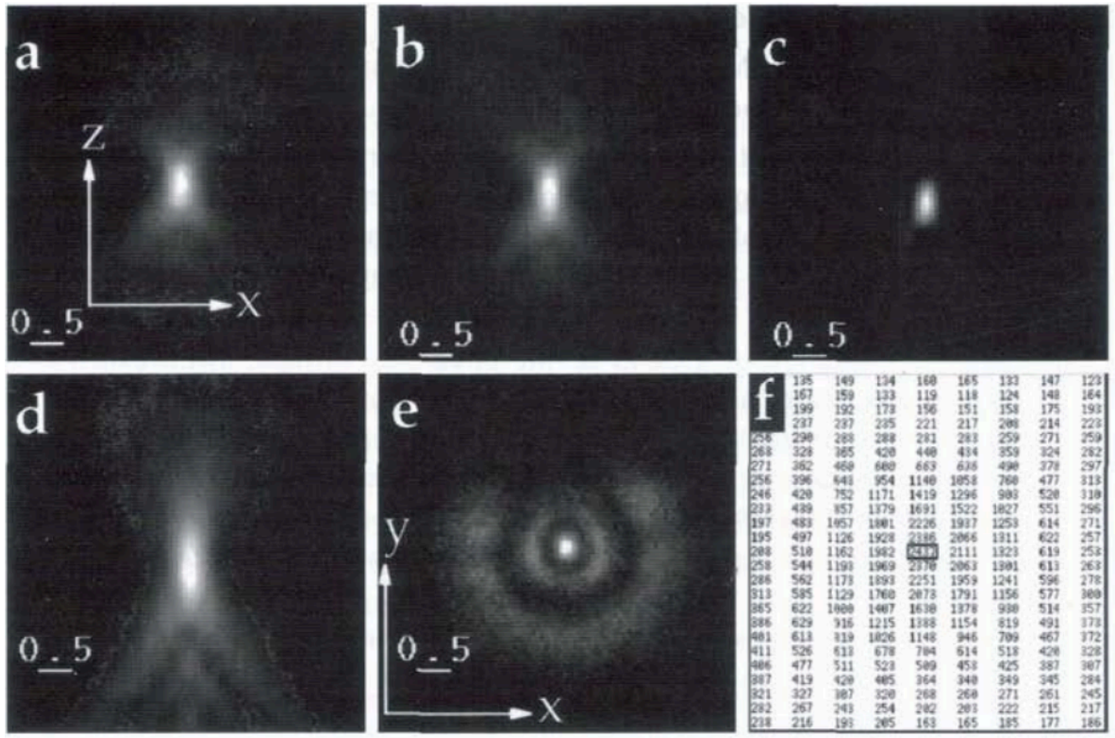


Figure 3. Examples of PSF of 0.1 μm fluorescent beads mounted in glycerol and imaged

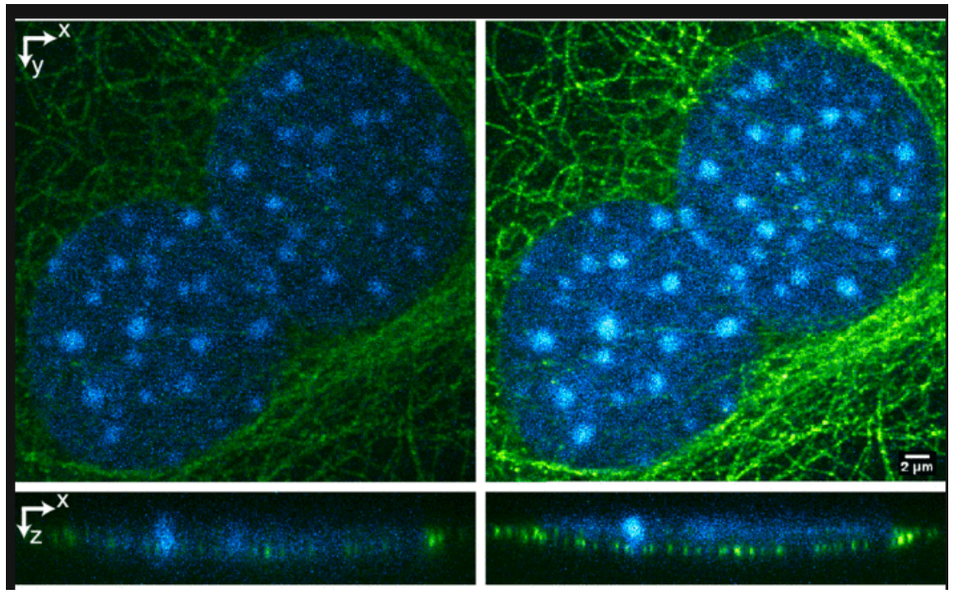
Visualizing fluorescence in *Drosophila*—optimal detection in thick specimens

ILAN DAVIS 2000

Reference: Chapter 6 in *Protein Localization by Fluorescence Microscopy - a practical approach*. Editor: V.J. Allan, OUP, 2000. pp. 133-162
ISBN (Pbk) 0-19-963740-7 ISBN (Hbk) 0-19-963741-5

No spherical aberration 

Spherical aberration 

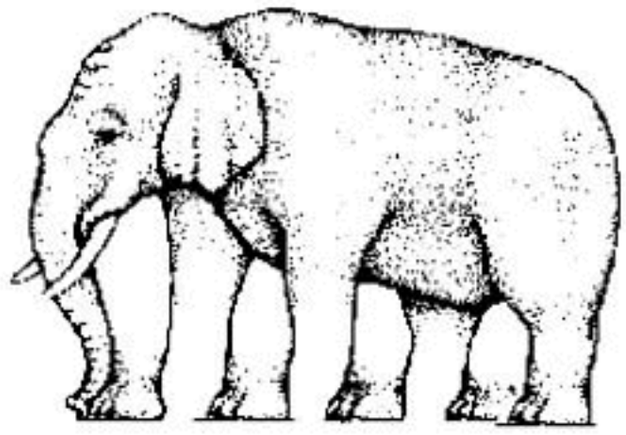


Automated spherical aberration correction in scanning confocal microscopy

Review of Scientific Instruments **85**, 123706 (2014); https://doi.org/10.1063/1.4904370

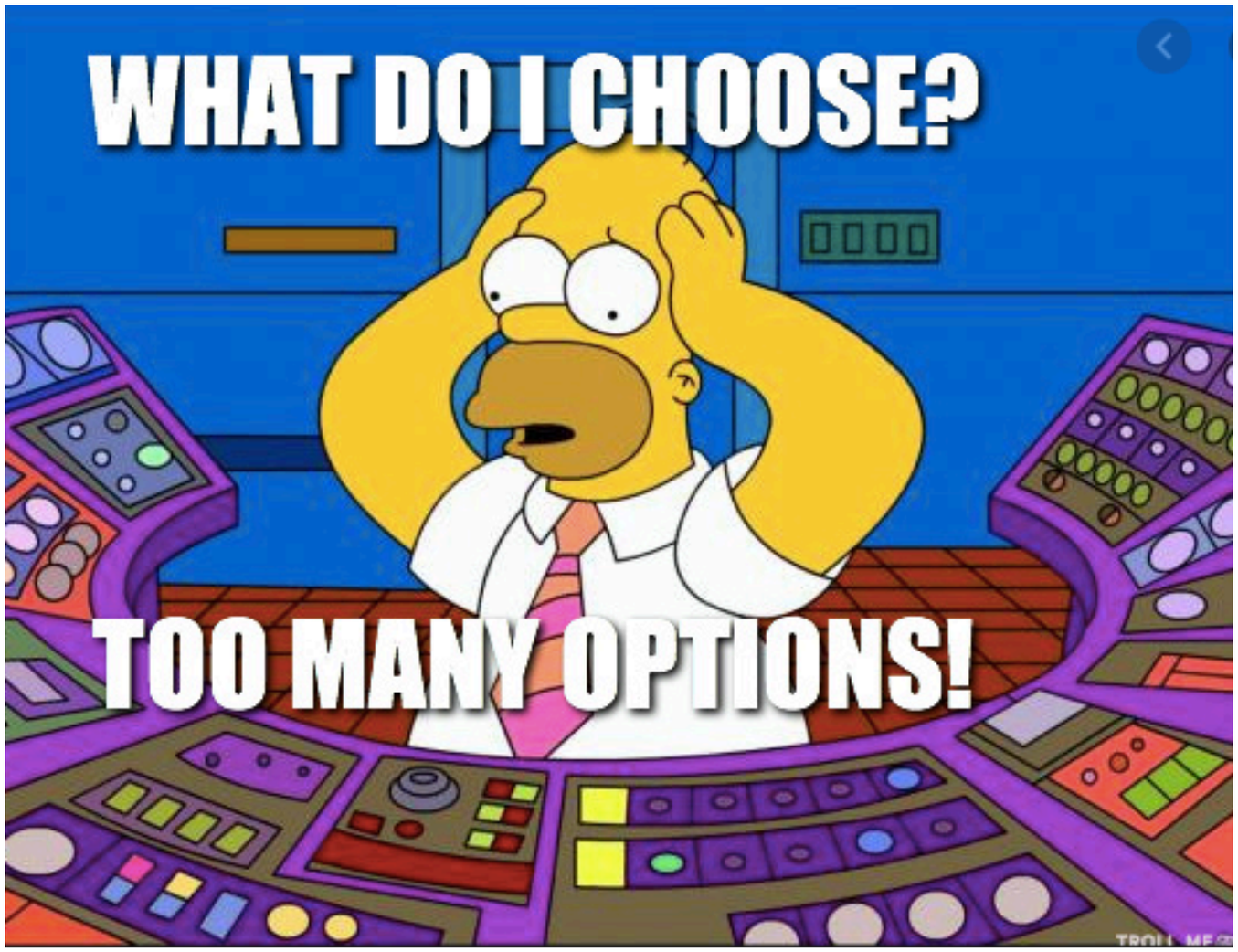
H. W. Yoo[1,2, a)], M. E. van Royen[3], W. A. van Cappellen[3], A. B. Houtsmuller[3], M. Verhaegen[1], *and* G. Schitter[2]

Seeing is not believing:
Quantitate before you believe



Negative controls are essential to
Discount autofluorescence / cross reaction of antibodies

Photon budget: every photon counts

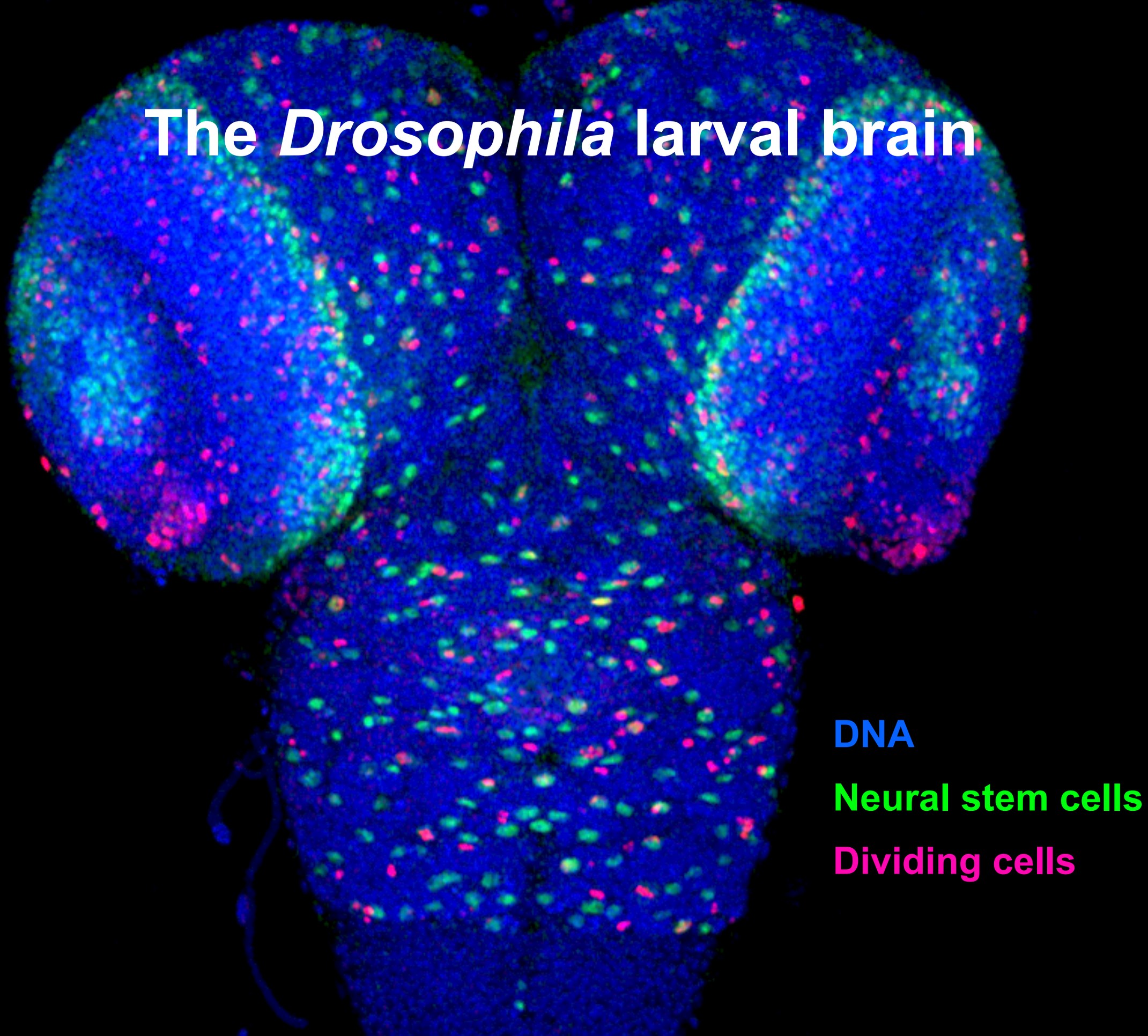


Signal : noise ratio

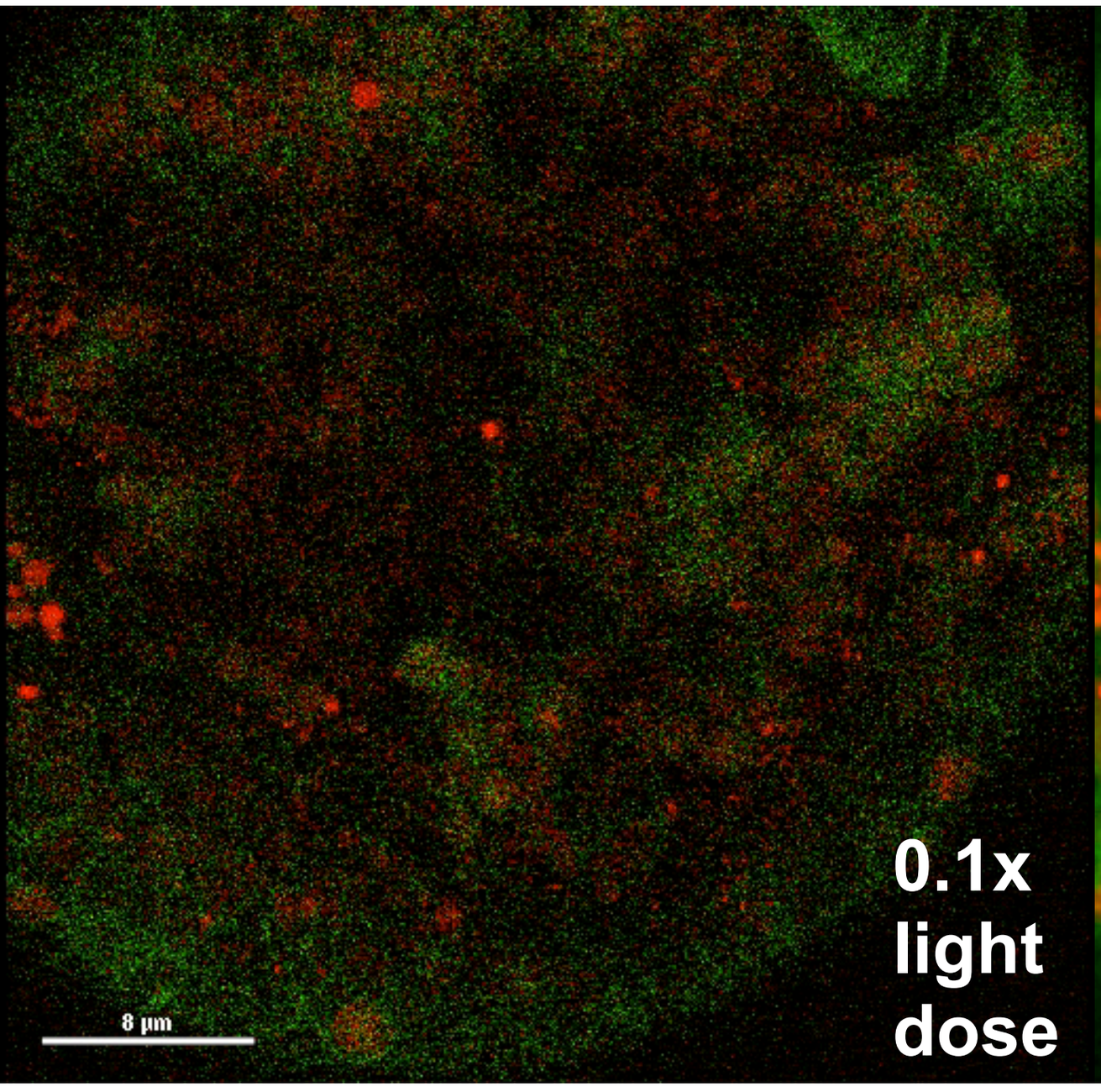
$$n / \sqrt{n}$$

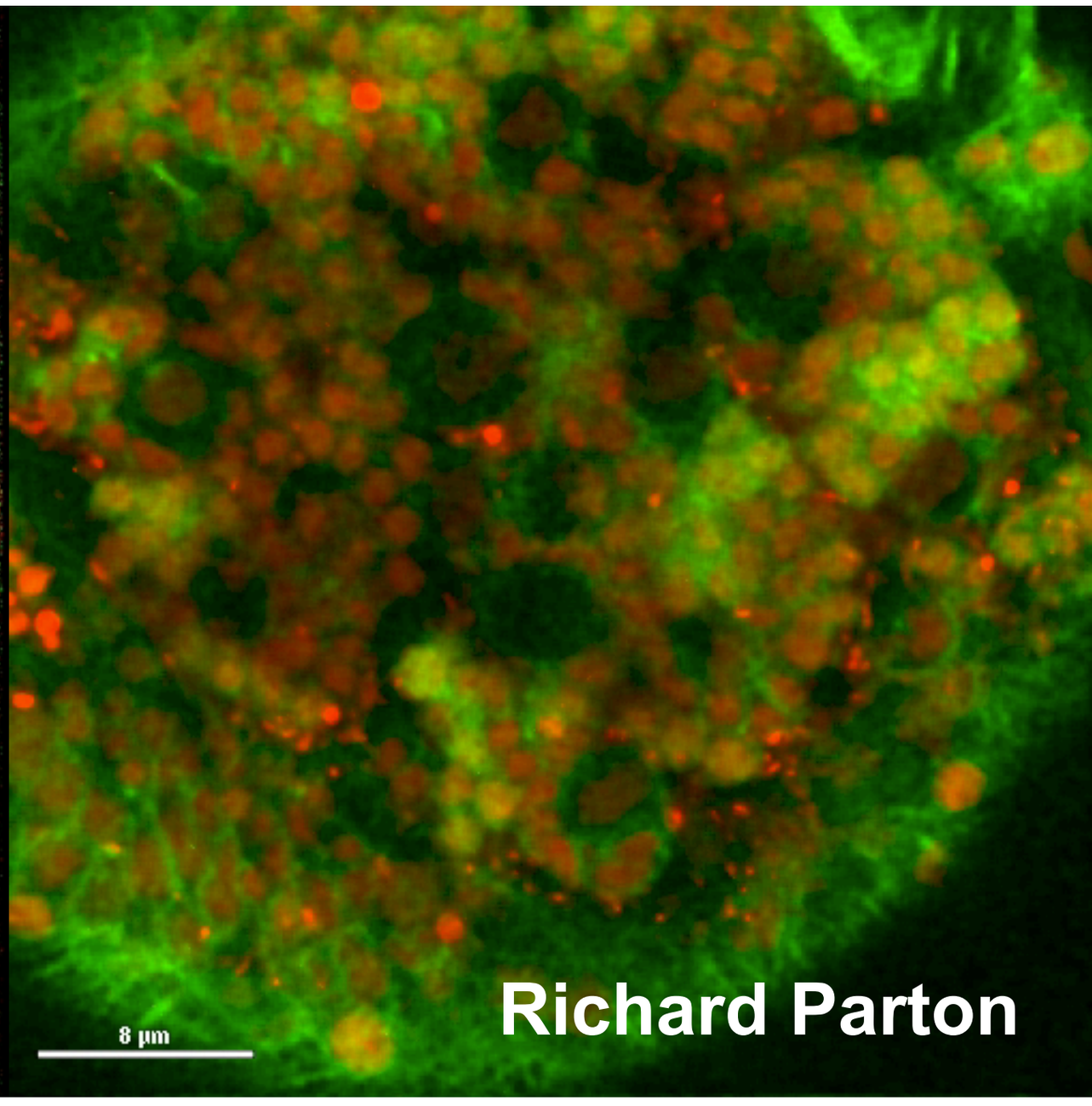
Pretty pictures
Are not the
only choice

The *Drosophila* larval brain



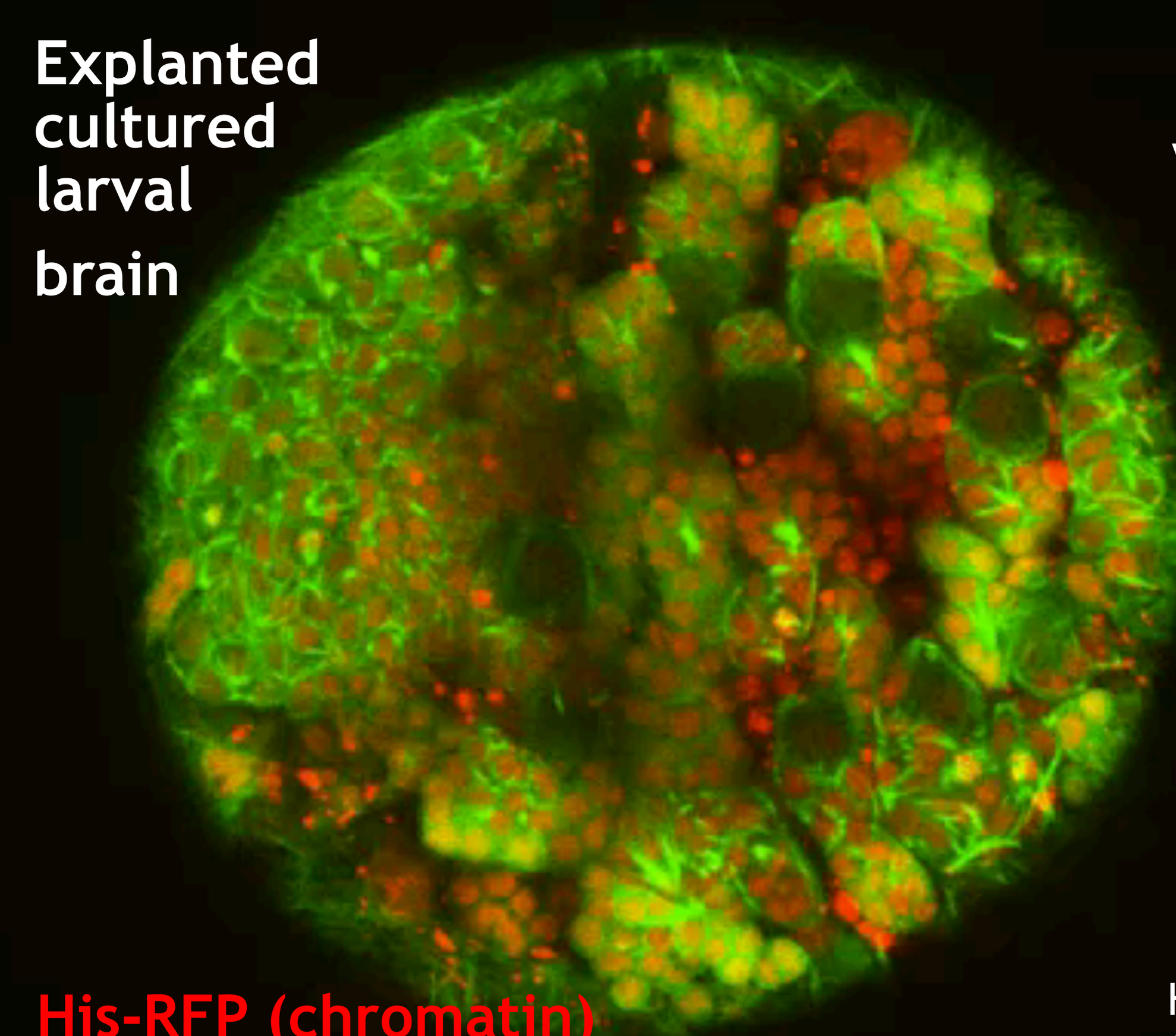
Patch-based denoising





Charles Kervrann, Jérôme Boulanger
John Sedat : Carlton et al. (2010) PNAS 107: 16016

Explanted cultured larval brain



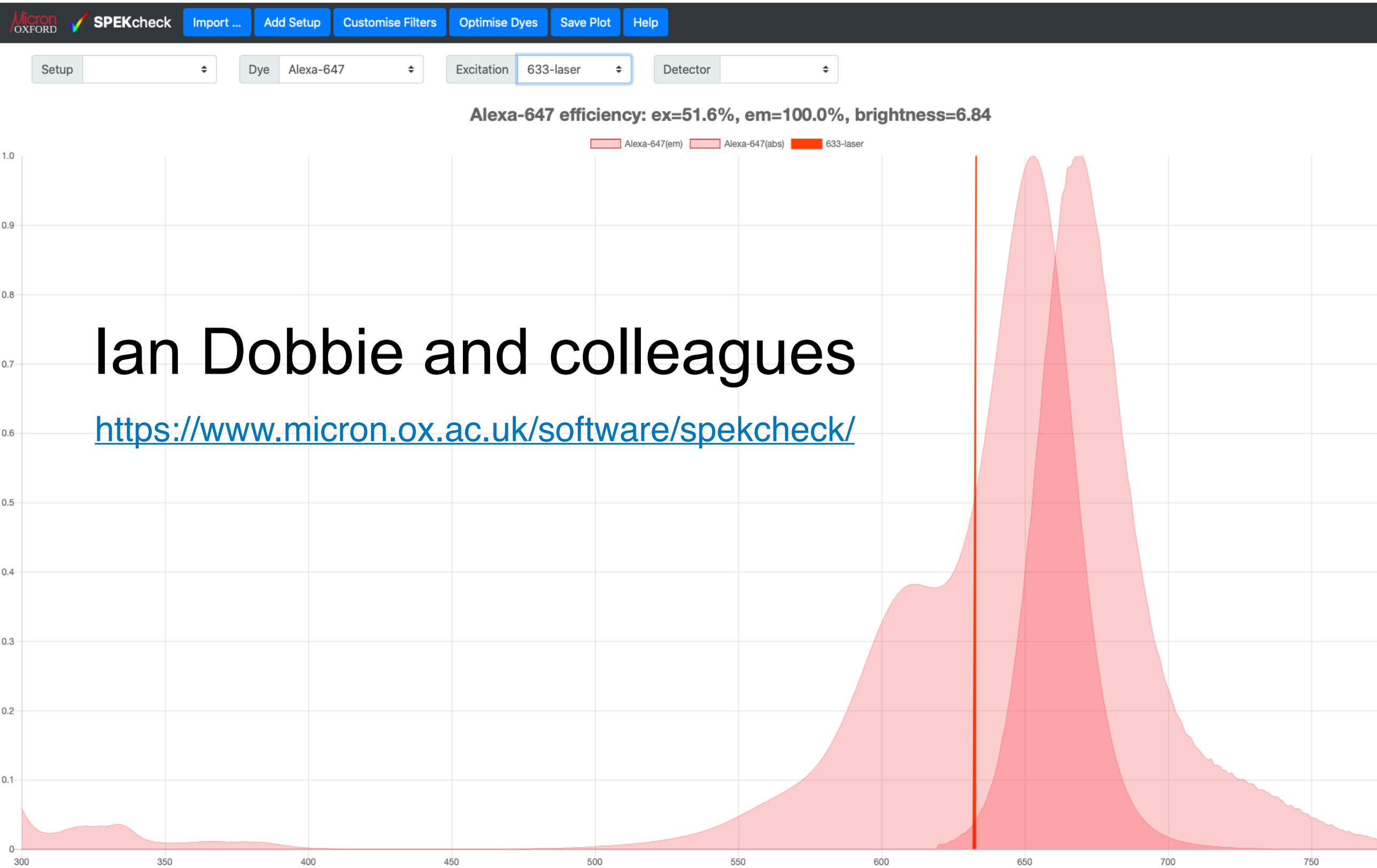
10x 3D volumes / hr
15 Zs / stack
15 hours total
(1 Z section displayed)

50GB Movie

His-RFP (chromatin)
Jup-GFP (microtubules)

Hailstone et al. (Davis)
eLife 2020

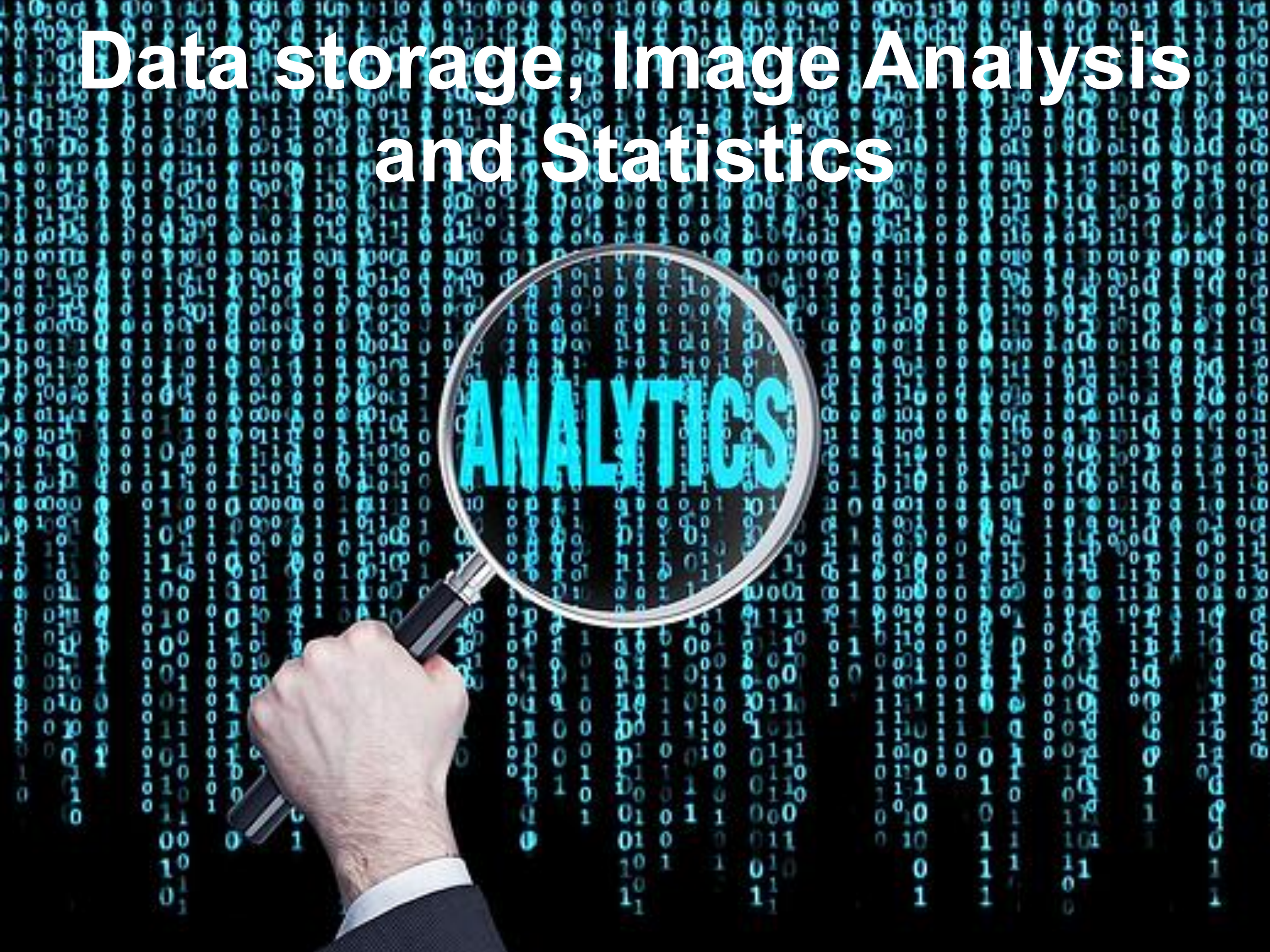
Light sources and filters: catch the wave



Ian Dobbie and colleagues

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

Data storage, Image Analysis and Statistics



If you get stuck - ask for help

Thanks for listening
Enjoy the course

Nirmal Purja