The mechanism of mRNA transport and local translation in *Drosophila*

ILAN DAVIS ilan.davis@bioch.ox.ac.uk
Department of Biochemistry, the University of Oxford

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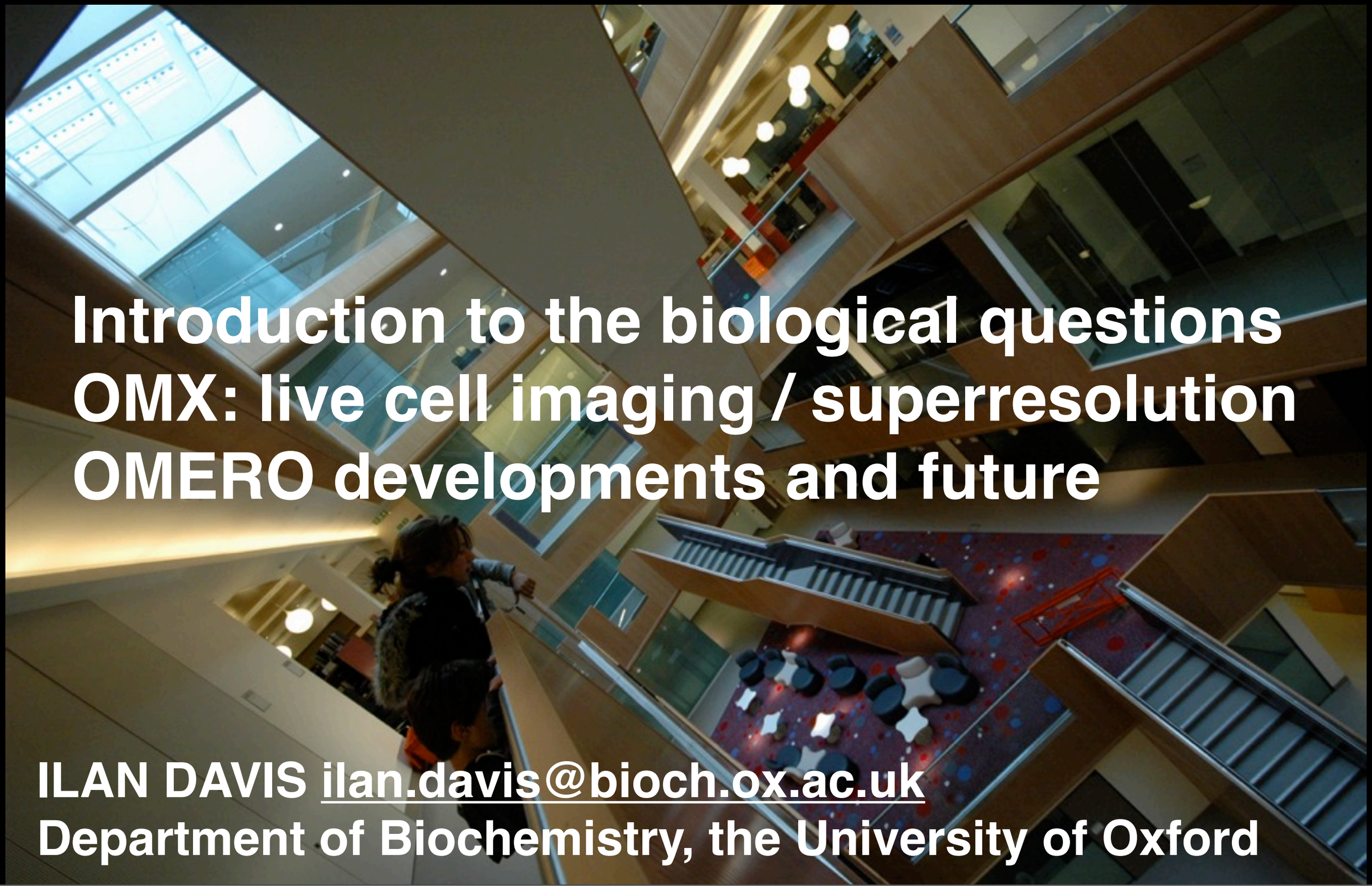

Introduction to the biological questions
OMX: live cell imaging / superresolution
OMERO developments and future

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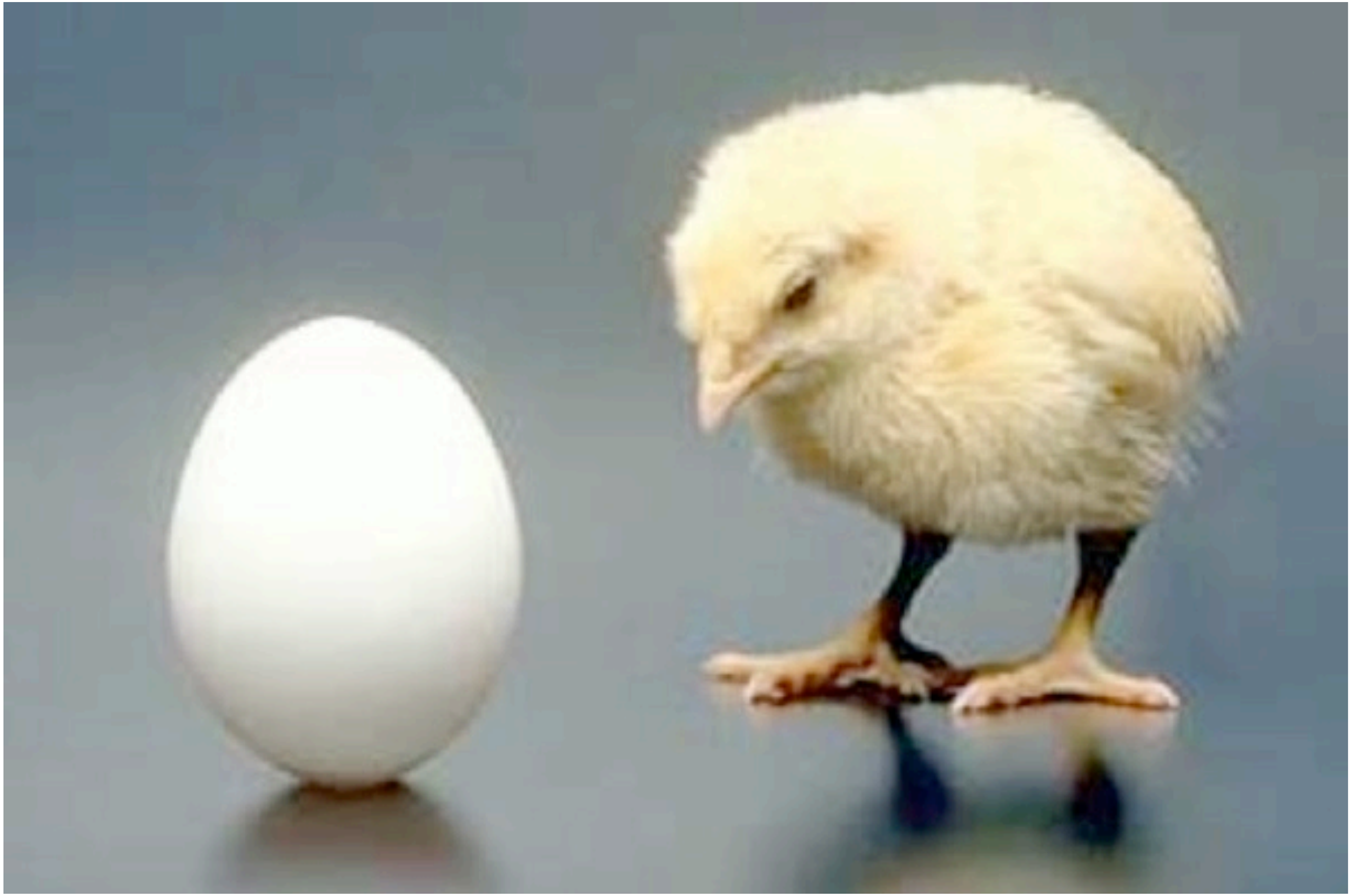
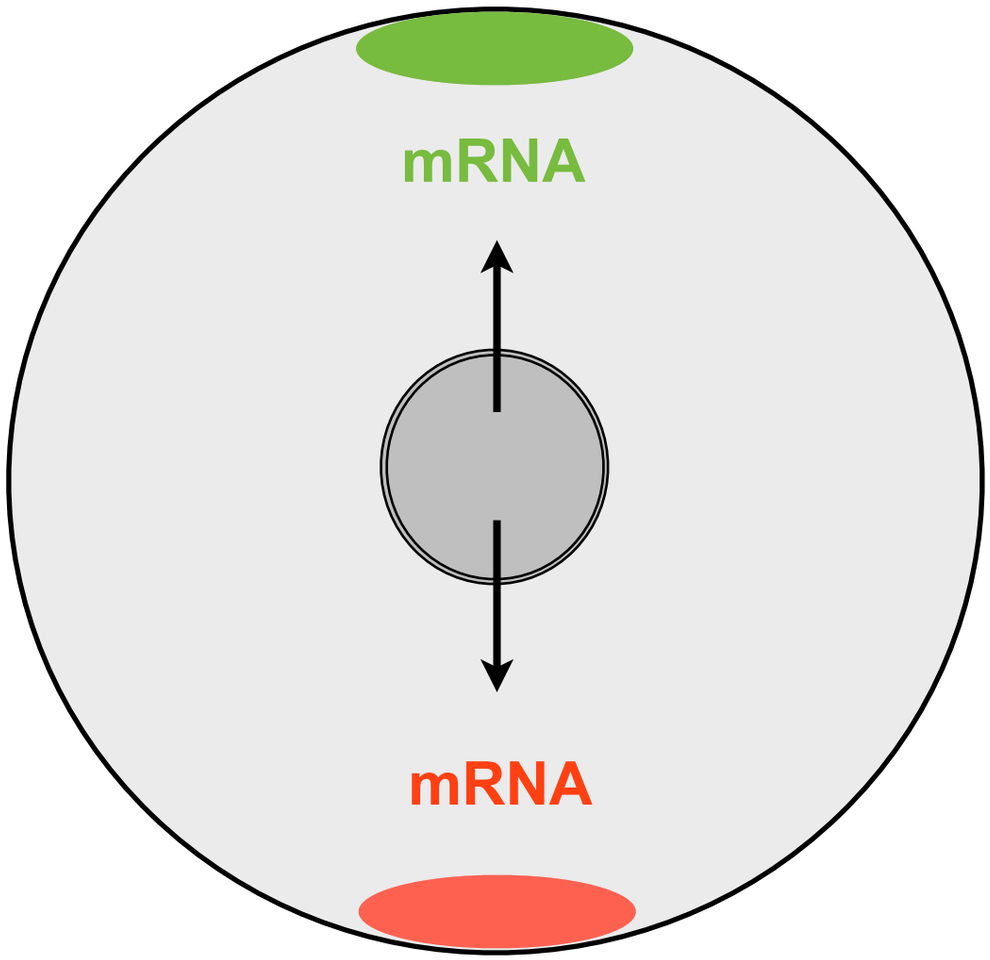
How do you make a chicken from an egg?

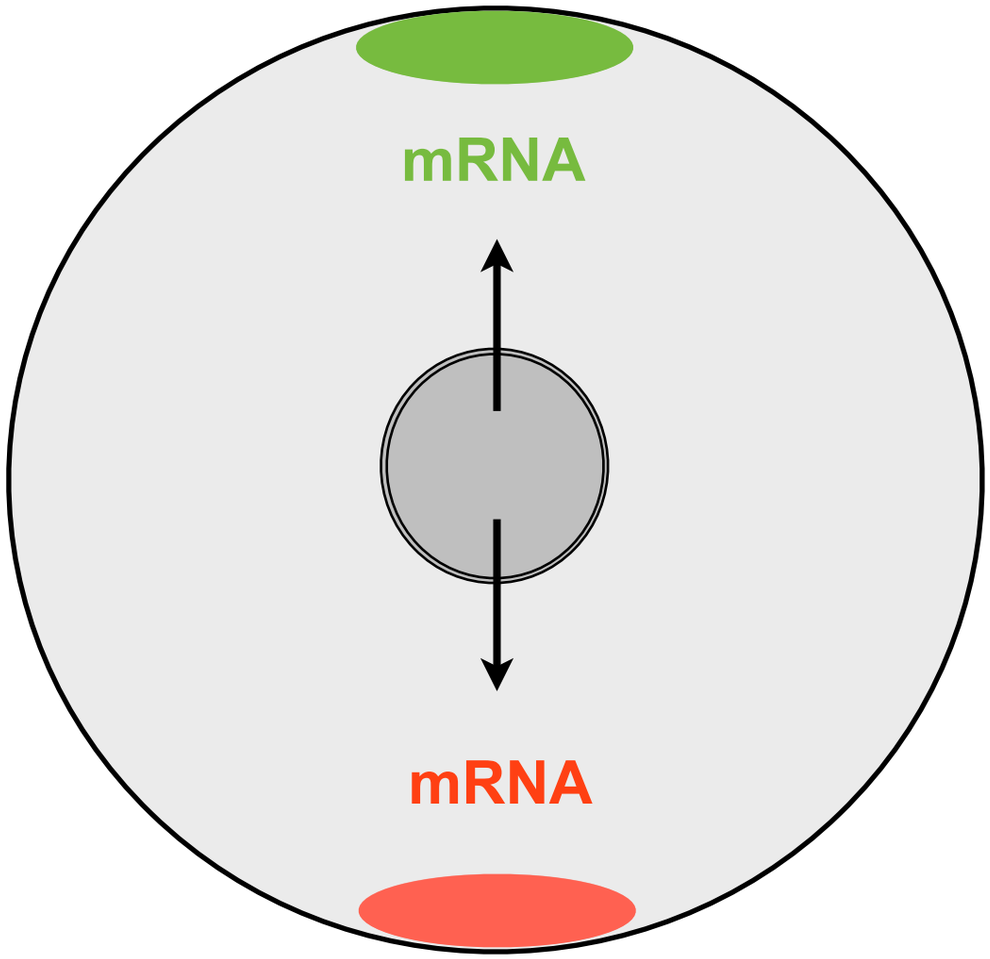


Establishing the animal body axes



mRNA transport
localised translation

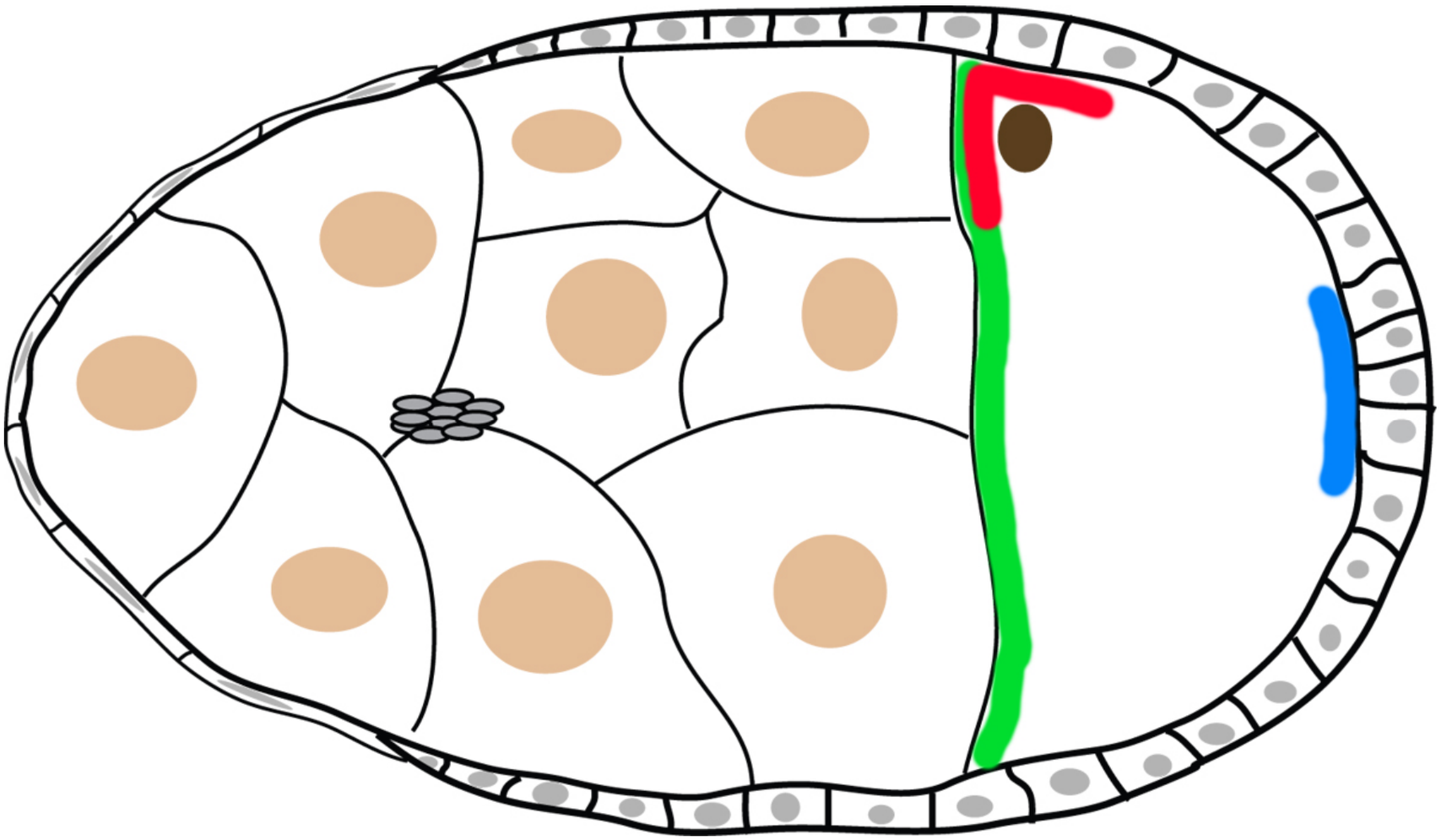
Establishing the animal body axes



mRNA transport
localised translation

Drosophila oocyte

gurken (TGF-α) mRNA

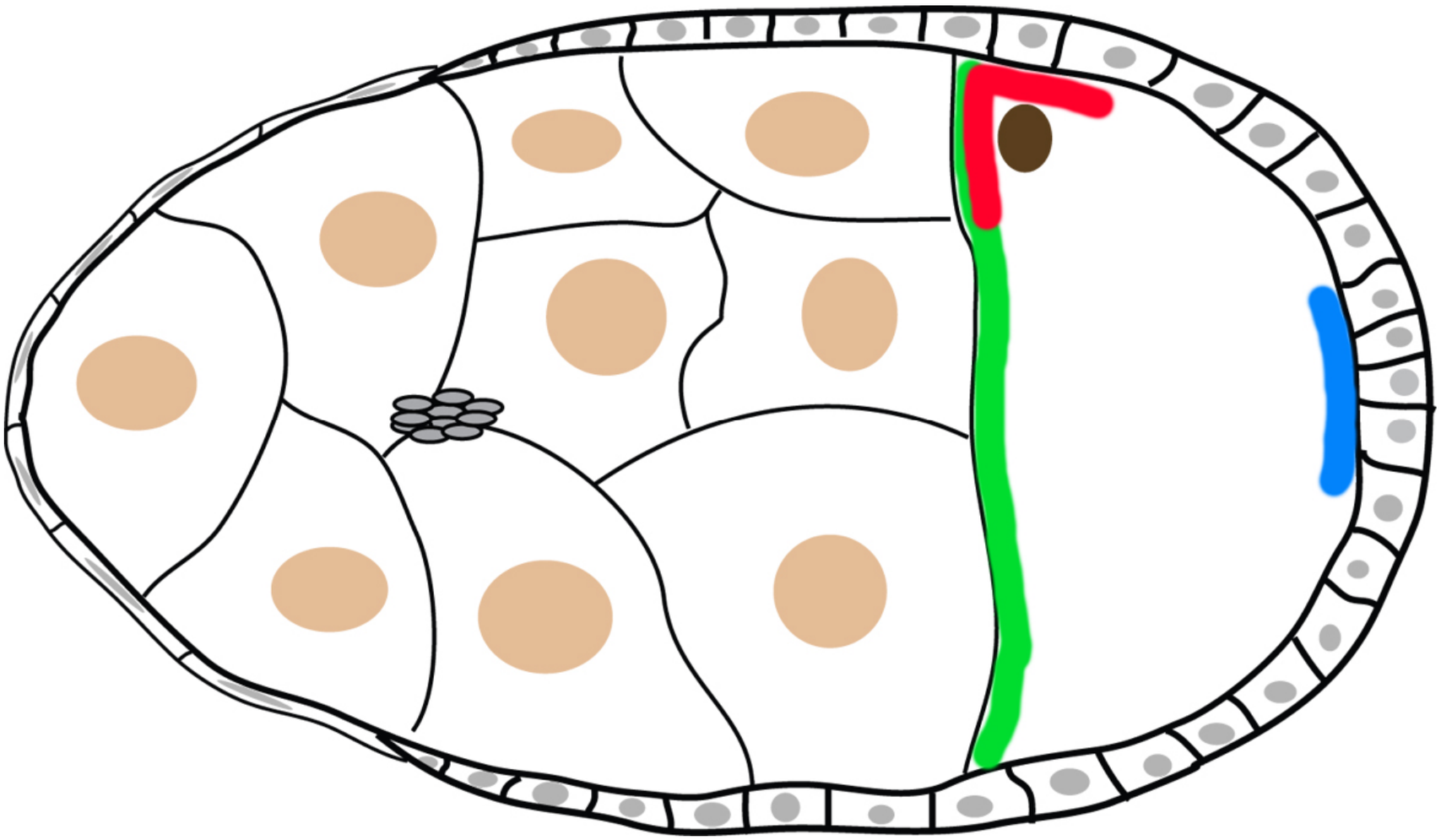


oskar mRNA

bicoid mRNA

Drosophila oocyte

gurken (TGF-α) mRNA

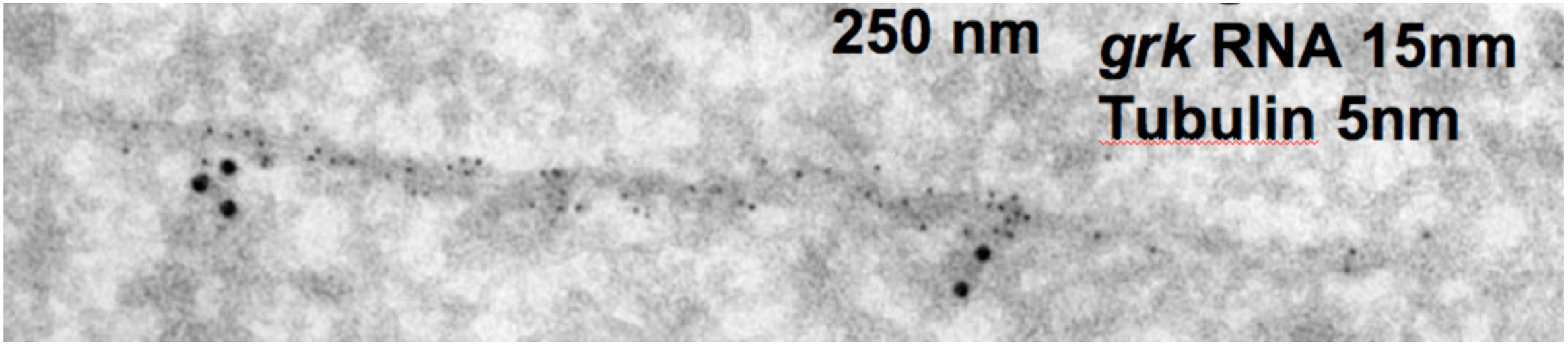


oskar mRNA

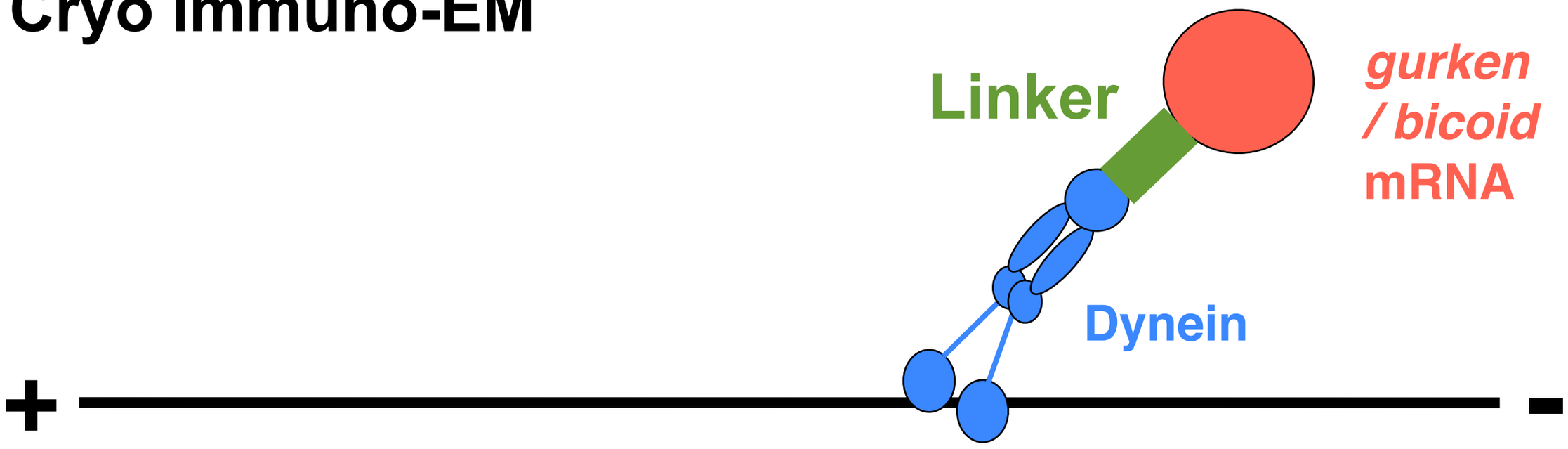
bicoid mRNA

How are mRNAs sorted to different destinations?
How are they translationally regulated?

RNA transport by Dynein along microtubules



Cryo immuno-EM

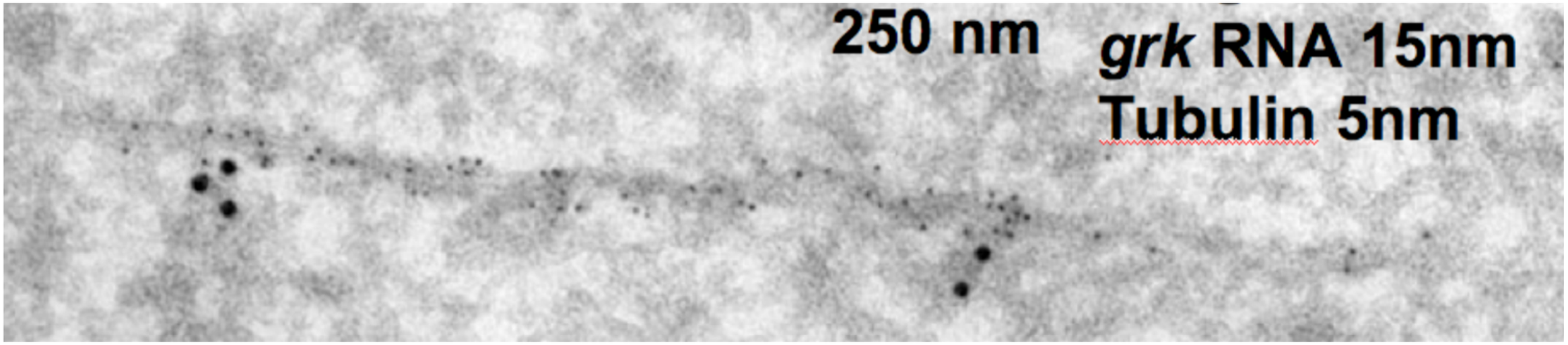


Wilkie and Davis, Cell 2001

Delanoue and Davis, Cell 2005

Delanoue et al, Dev Cell 2007 (collaboration with Catherine Rabouille, Utrecht)

RNA transport by Dynein along microtubules



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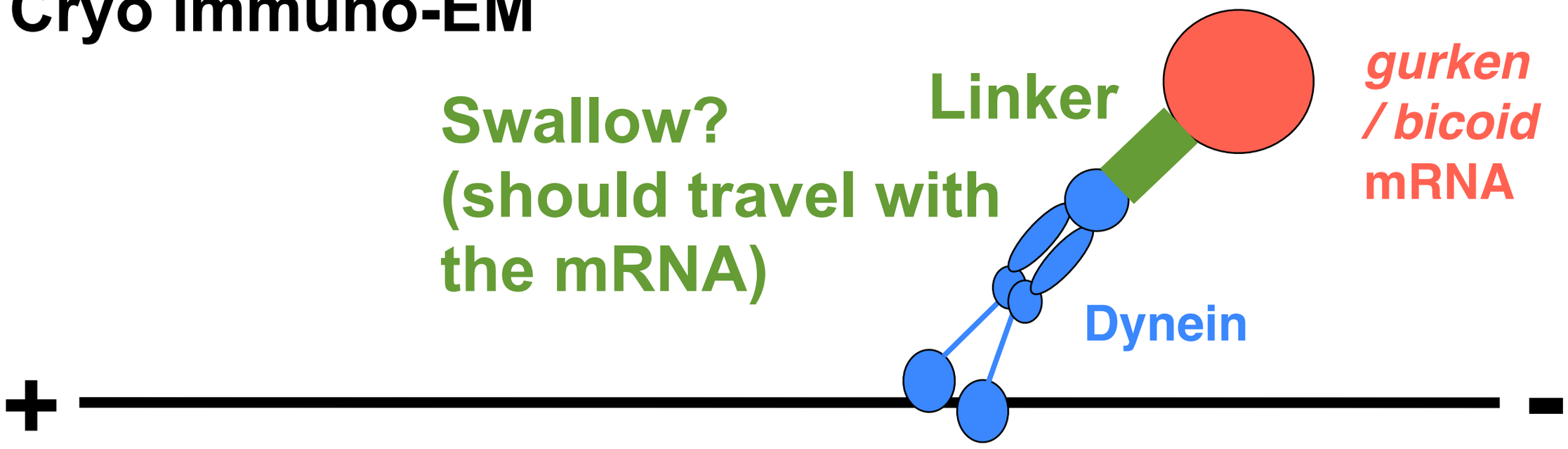


Wilkie and Davis, Cell 2001

Delanoue and Davis, Cell 2005

Delanoue et al, Dev Cell 2007
(collaboration with
Catherine Rabouille, Utrecht)

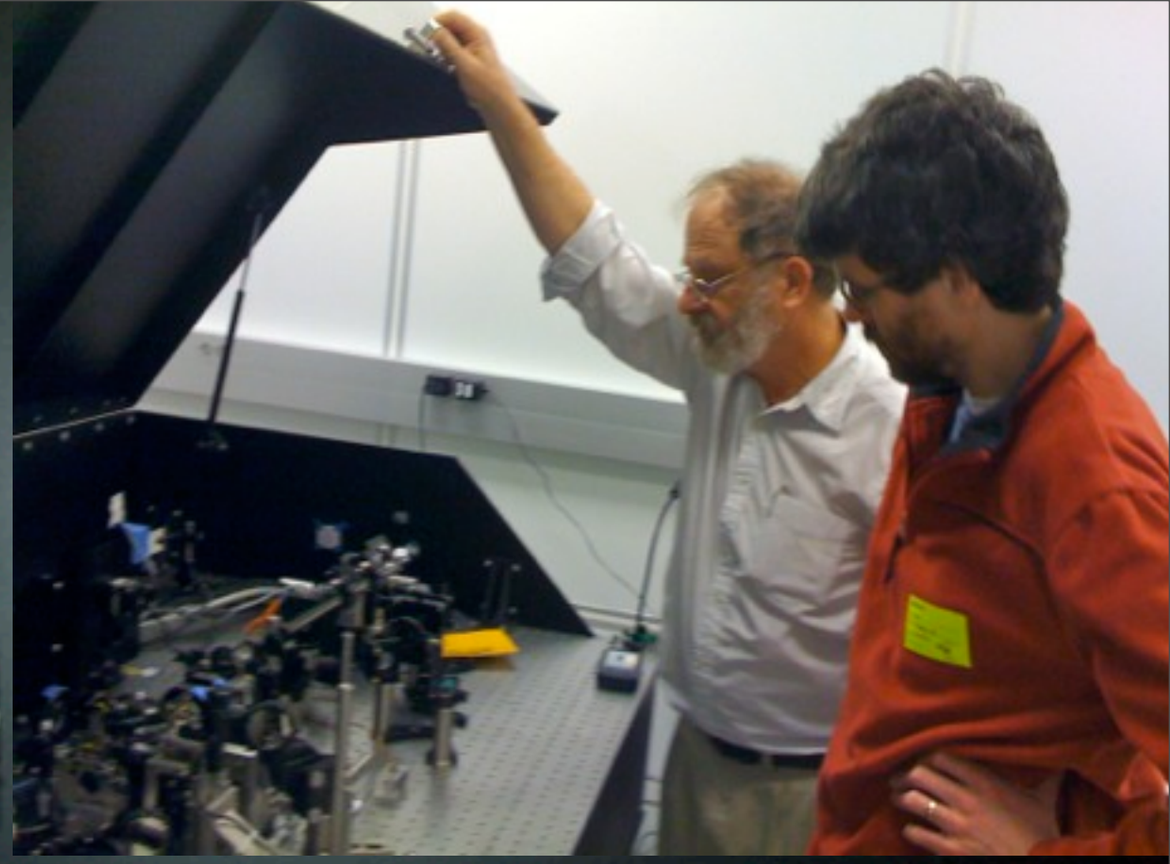
Space and Time

Higher resolution and faster

David Agard

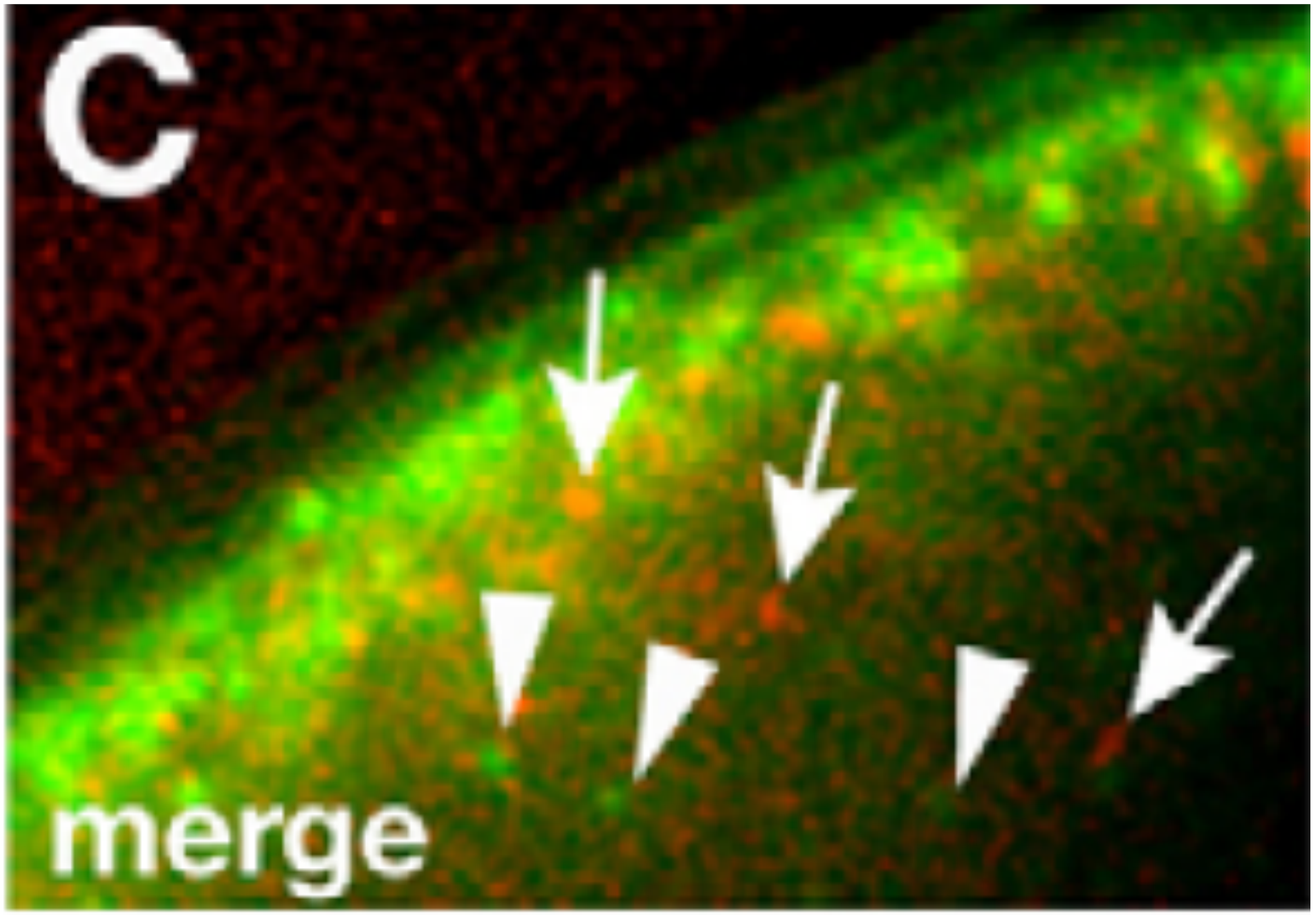
Richard Parton

Lothar Schermelleh



John Sedat and Ian Dobbie

Swallow does not link *bcd* mRNA to Dynein

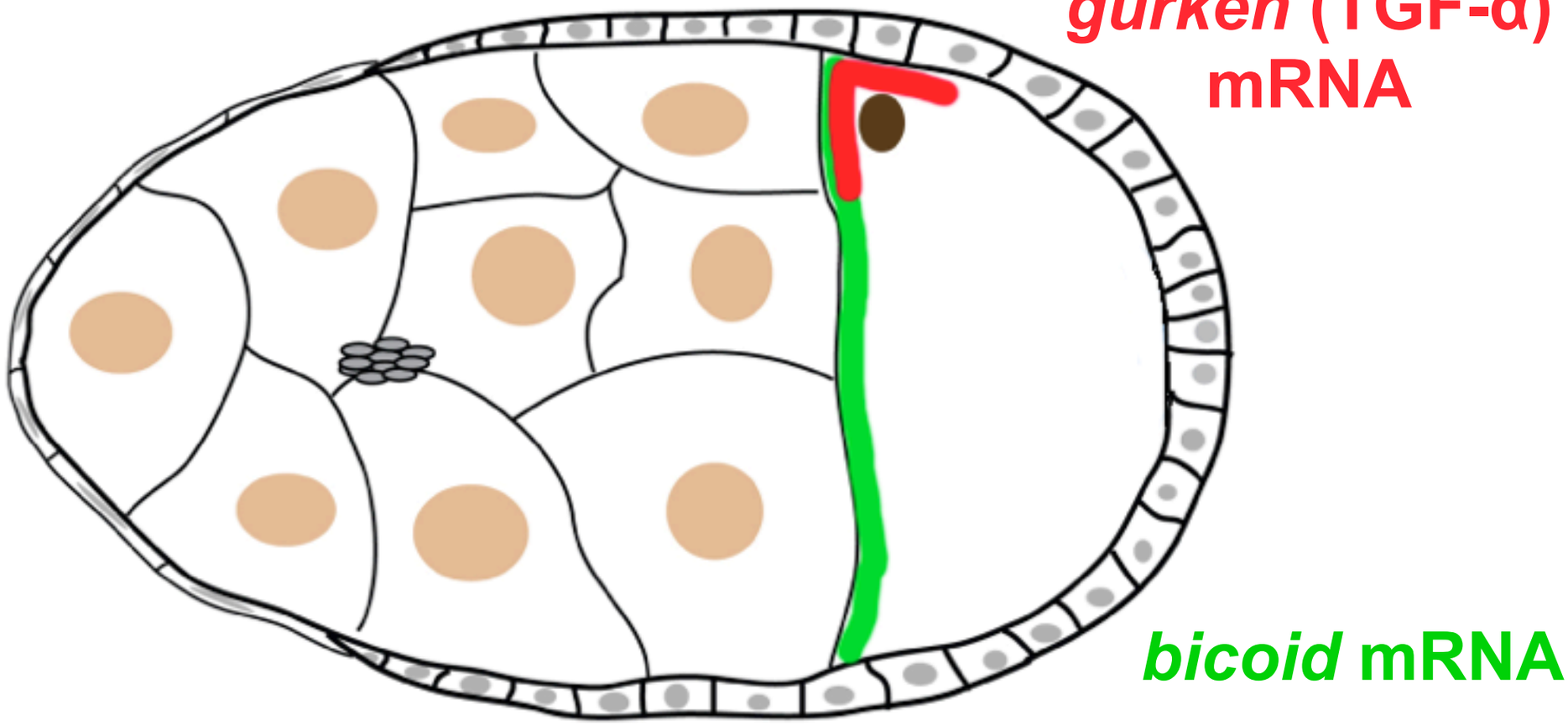


Swallow protein

***grk* MS2* GFP tagged mRNA**

Weil et al, Development 2010
Collaboration with Catherine Rabouille, Ubrecht and Liz Gavis, Princeton

Drosophila oocyte

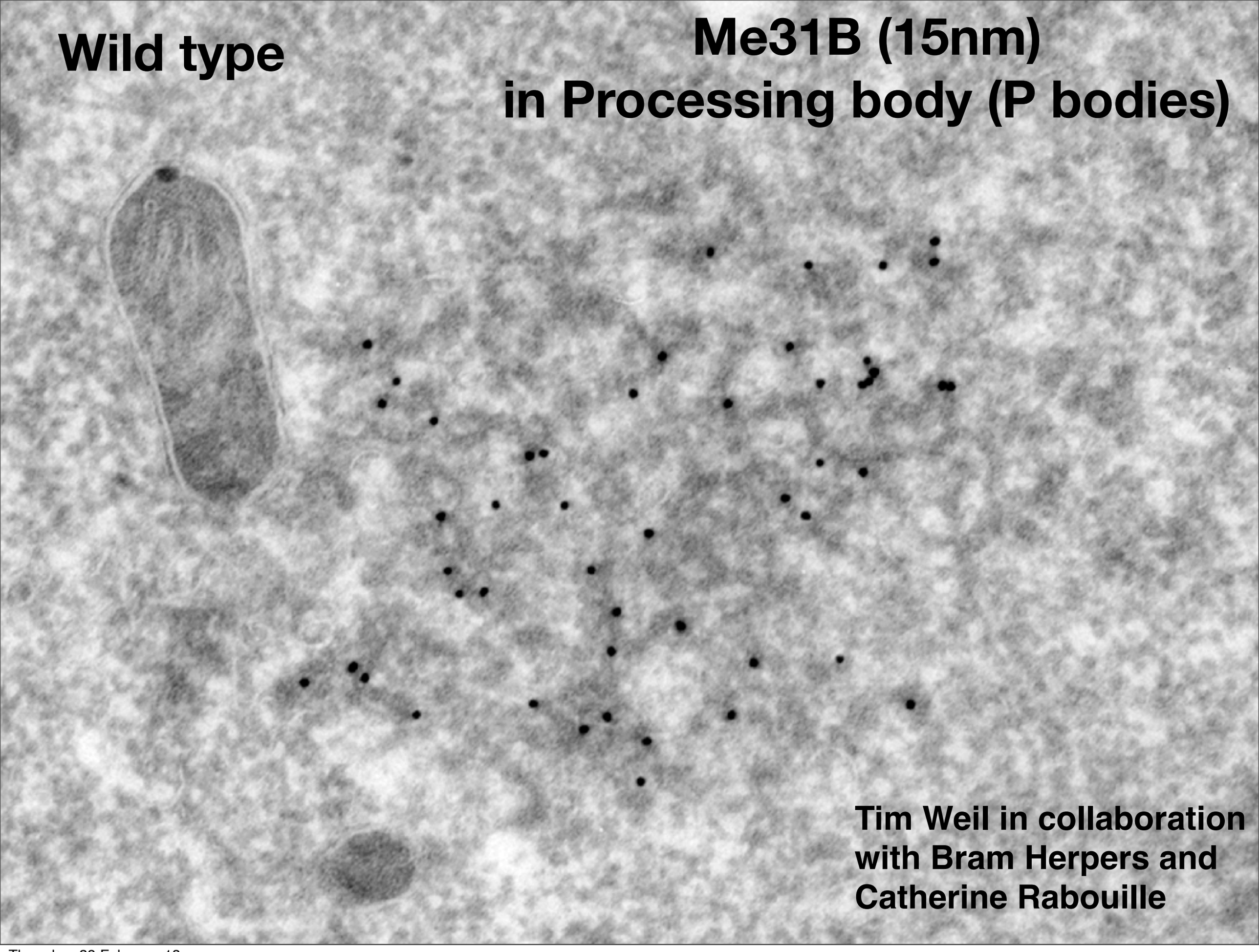


How are *gurken* and *bicoid* mRNA differentially translationally regulated?

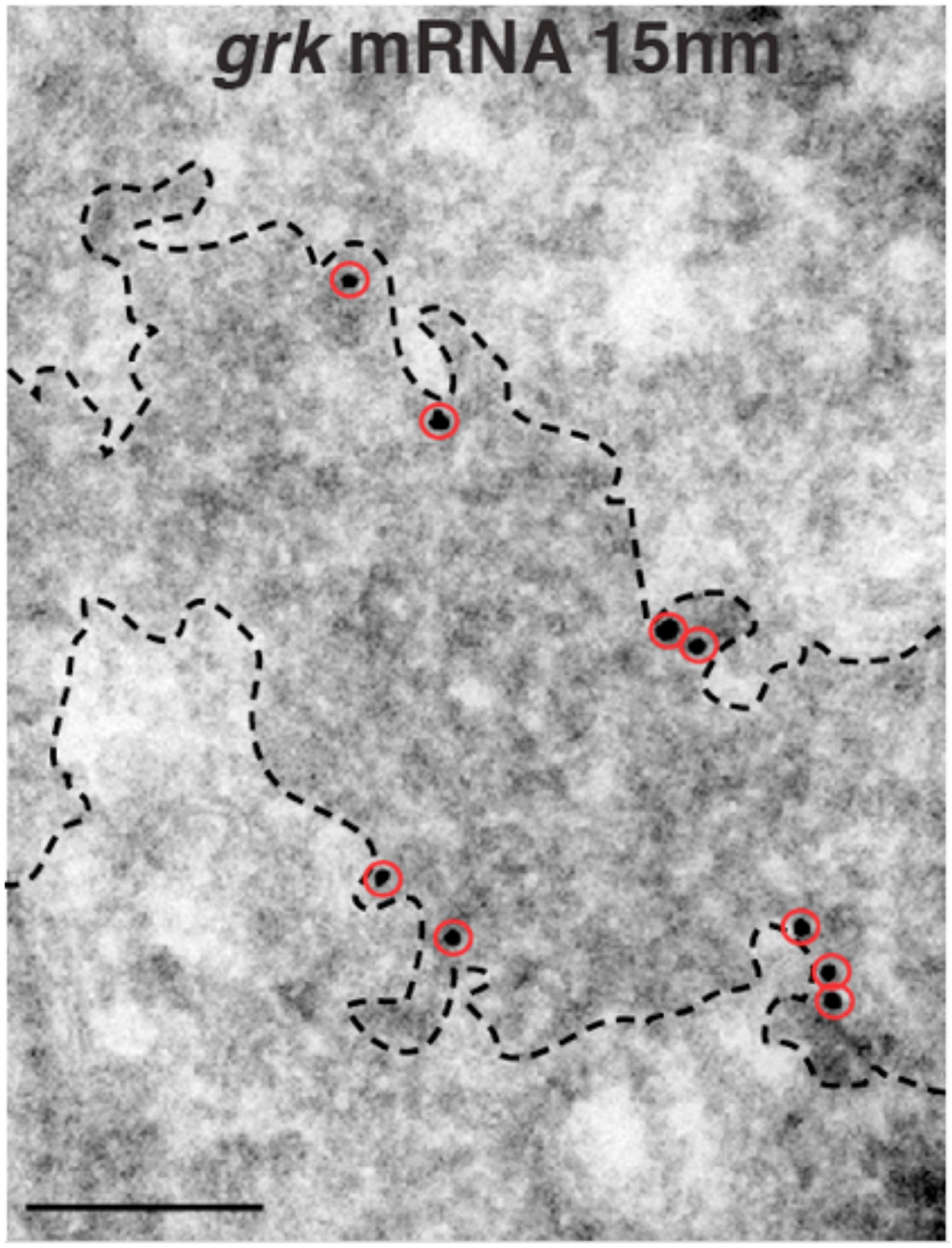
Weil, Parton et al, Nature Cell Biol. 2012
(collaboration with the Rabouille lab)

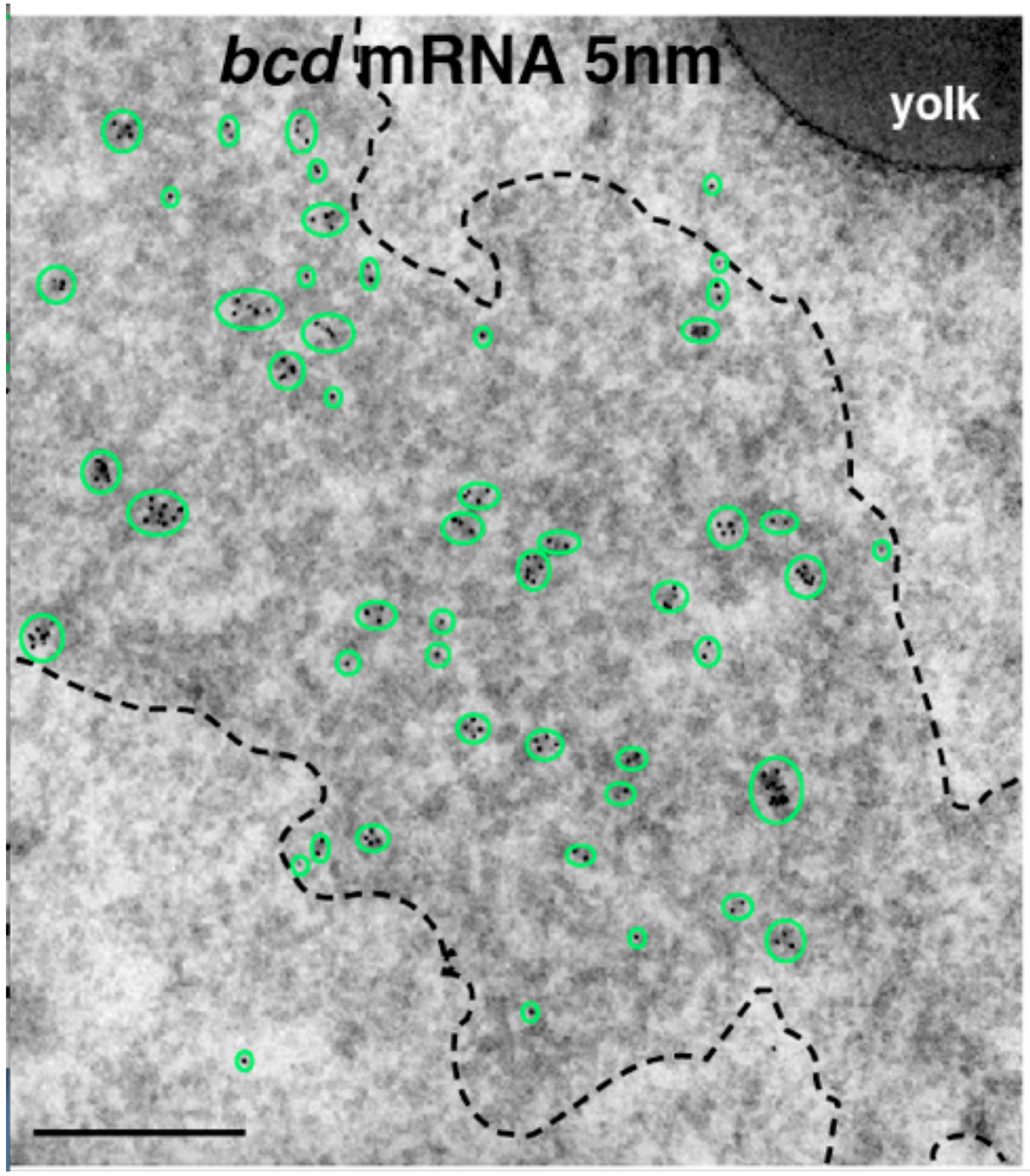
Wild type

Me31B (15nm)
in Processing body (P bodies)



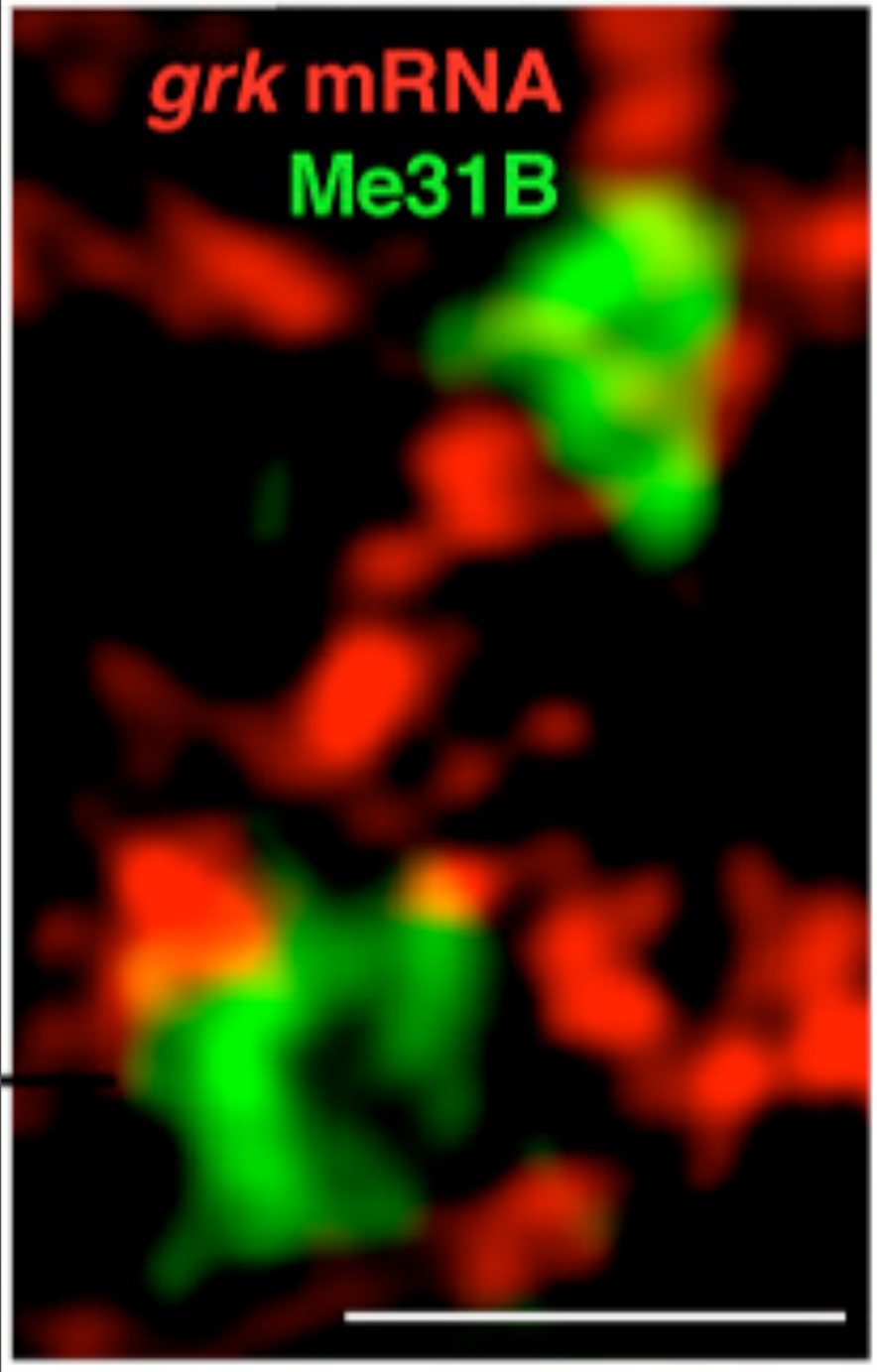
Tim Weil in collaboration
with Bram Herpers and
Catherine Rabouille

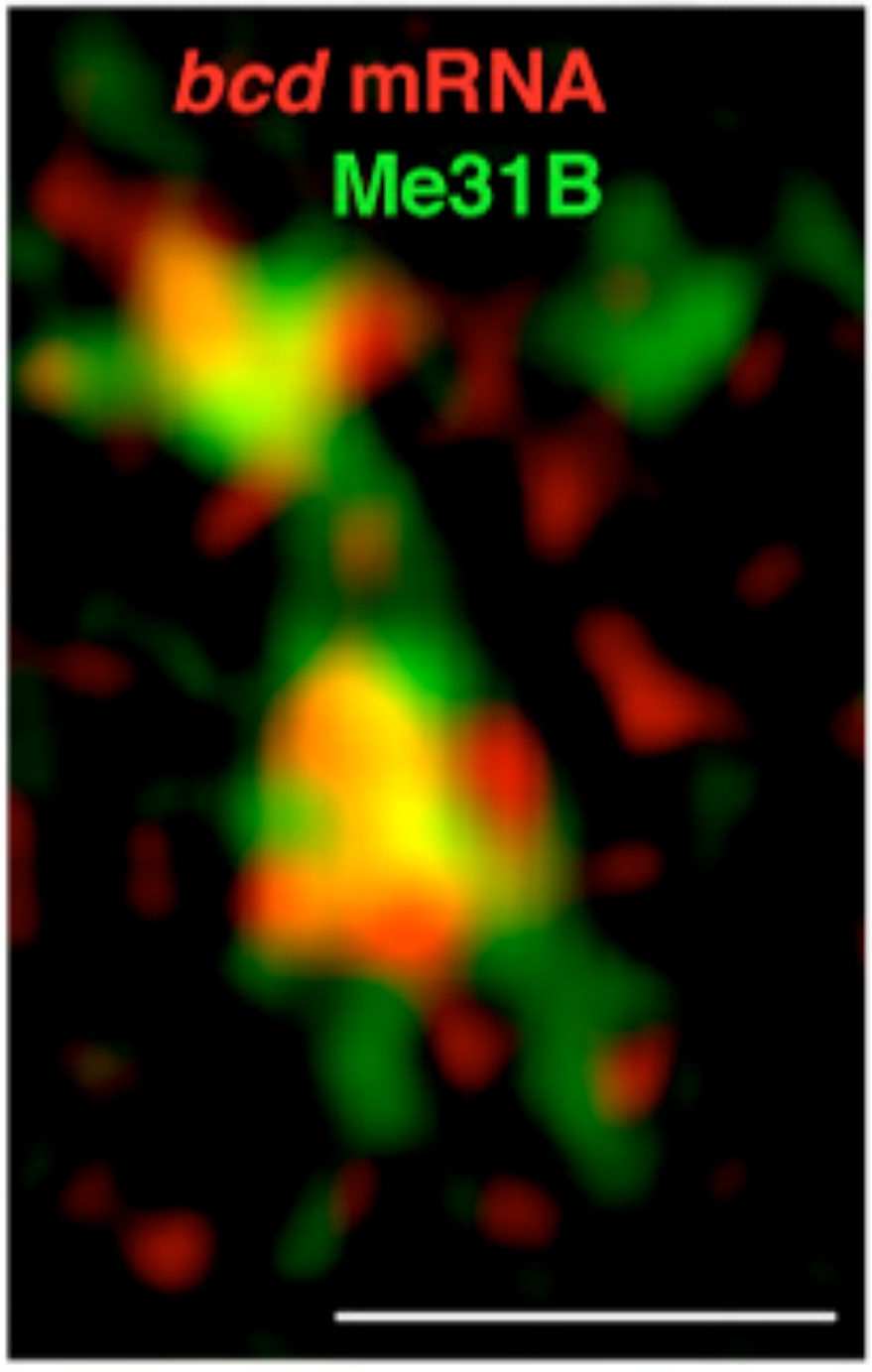




Tim Weil

grk mRNA is interdigitated with P bodies, but *bcd* mRNA is also localised within Me31B



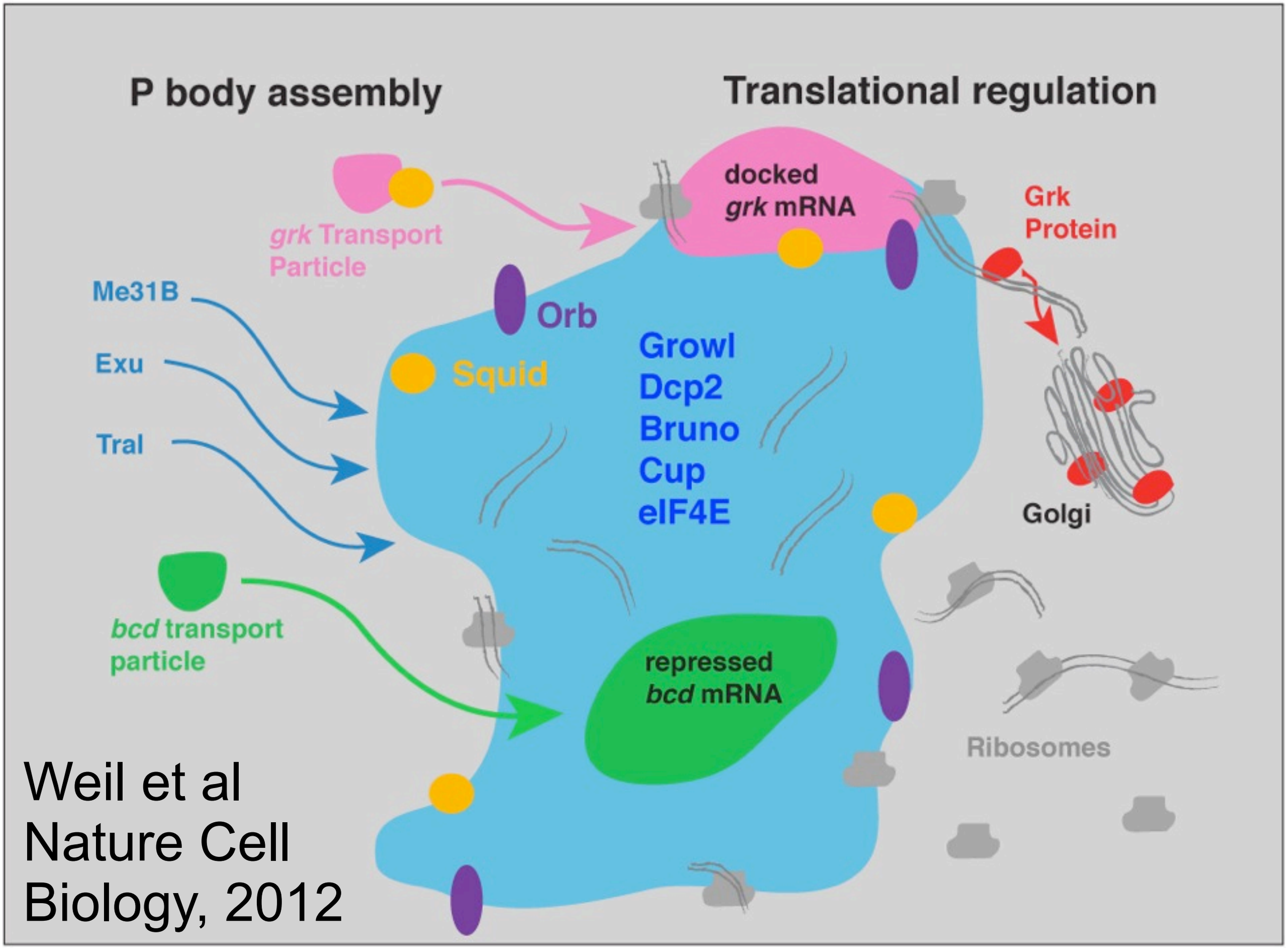


Structured Illumination

twice conventional resolution in x,y and z.

Tim Weil and Richard Parton

Model for differential translational regulation of *gurken* and *bicoid* mRNA



Weil et al
Nature Cell
Biology, 2012

Further development: OMX-T

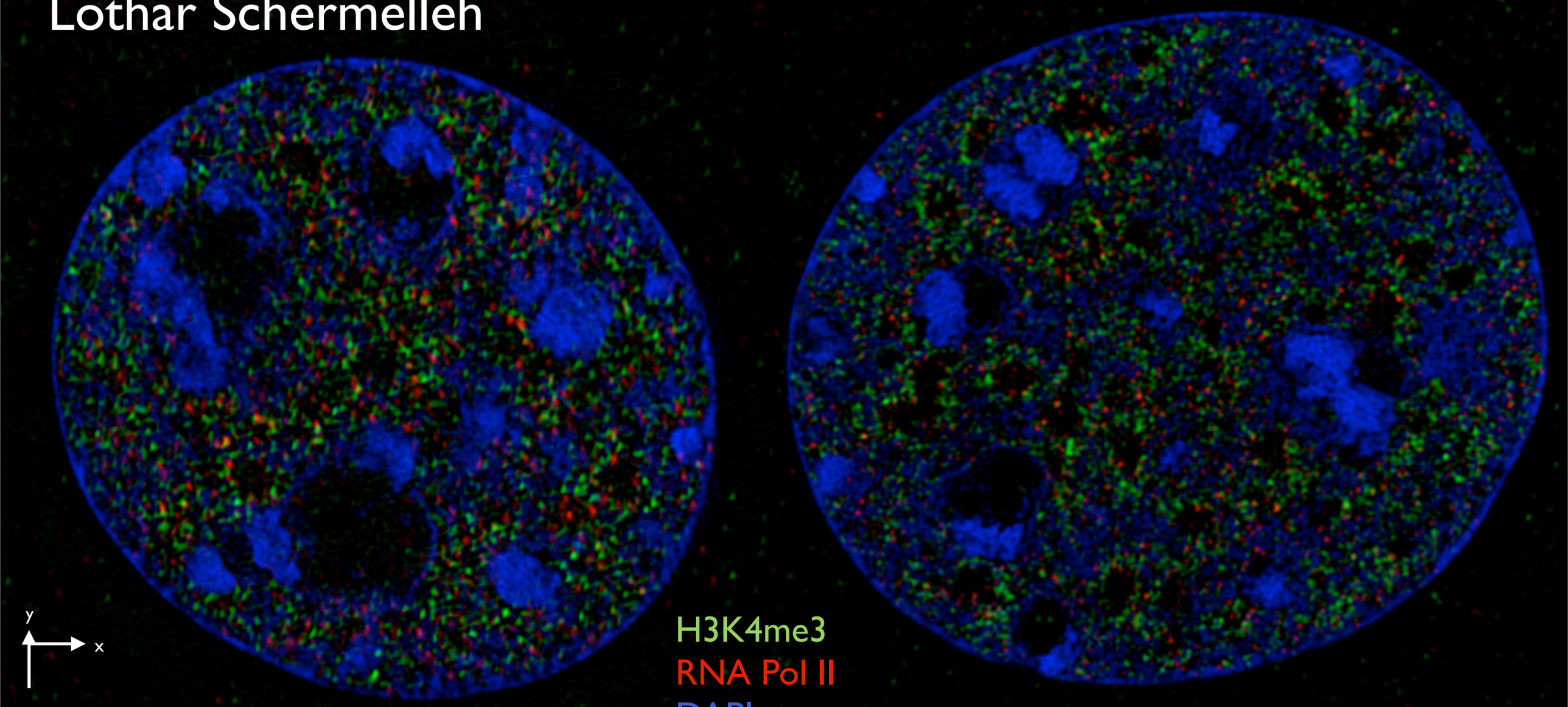
Rainer Kauffman
and Ian Dobbie

Based on John Sedat's design

In silico plan

Quality control: SI-Reconstruction artifacts

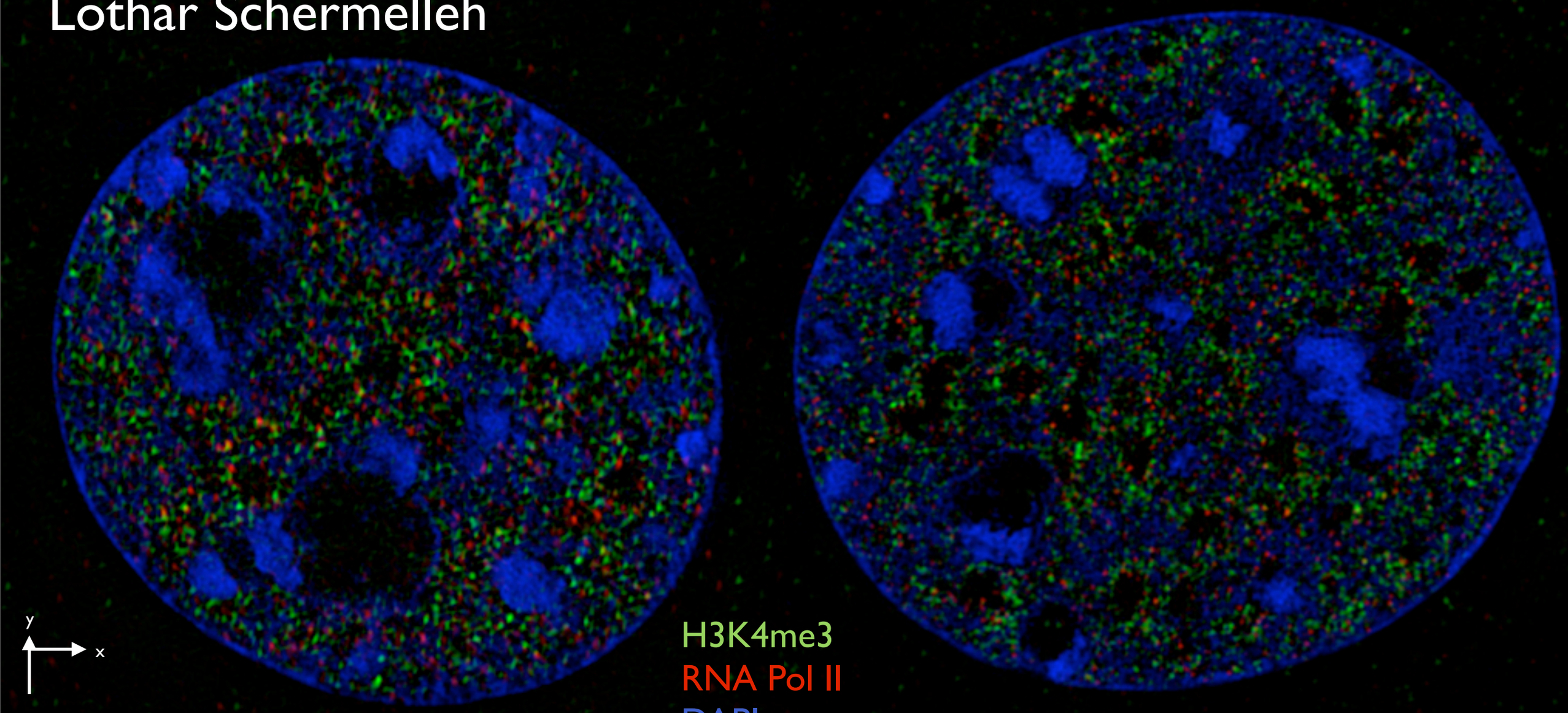
Lothar Schermelleh



Good SI reconstruction

Quality control: SI-Reconstruction artifacts

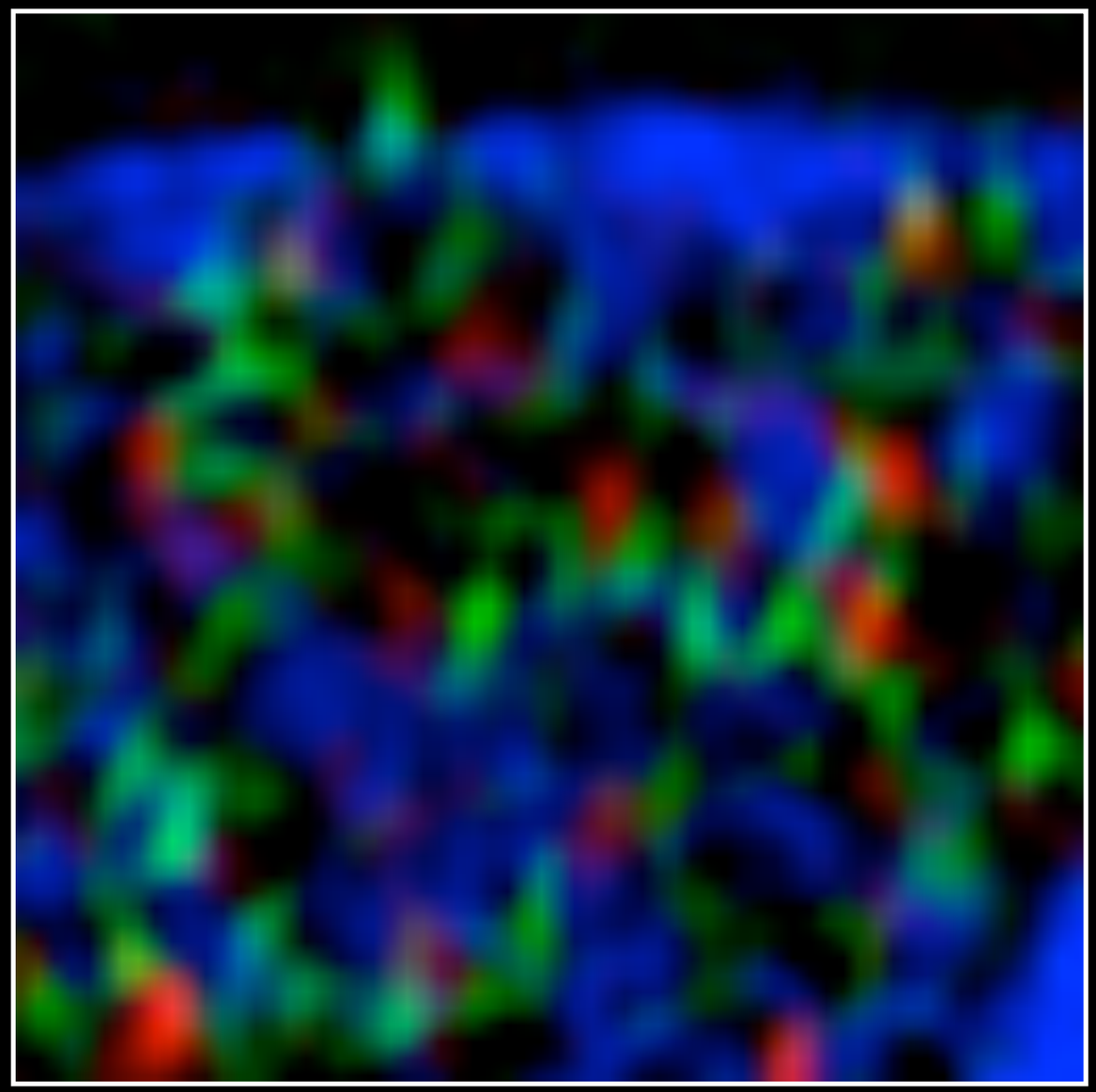
Lothar Schermelleh

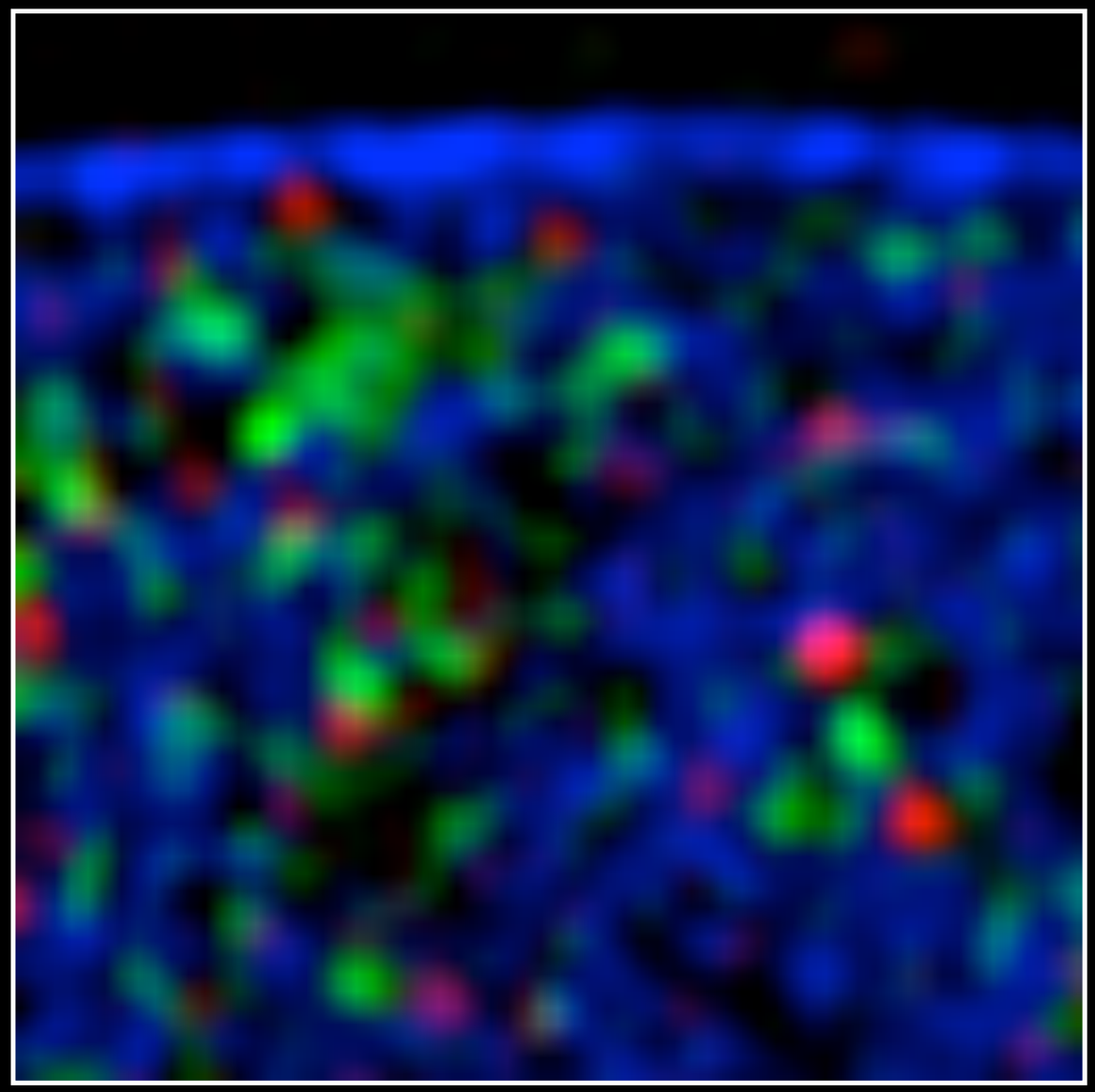


Bad SI reconstruction

Good SI reconstruction

Quality control: SI-Reconstruction artifacts

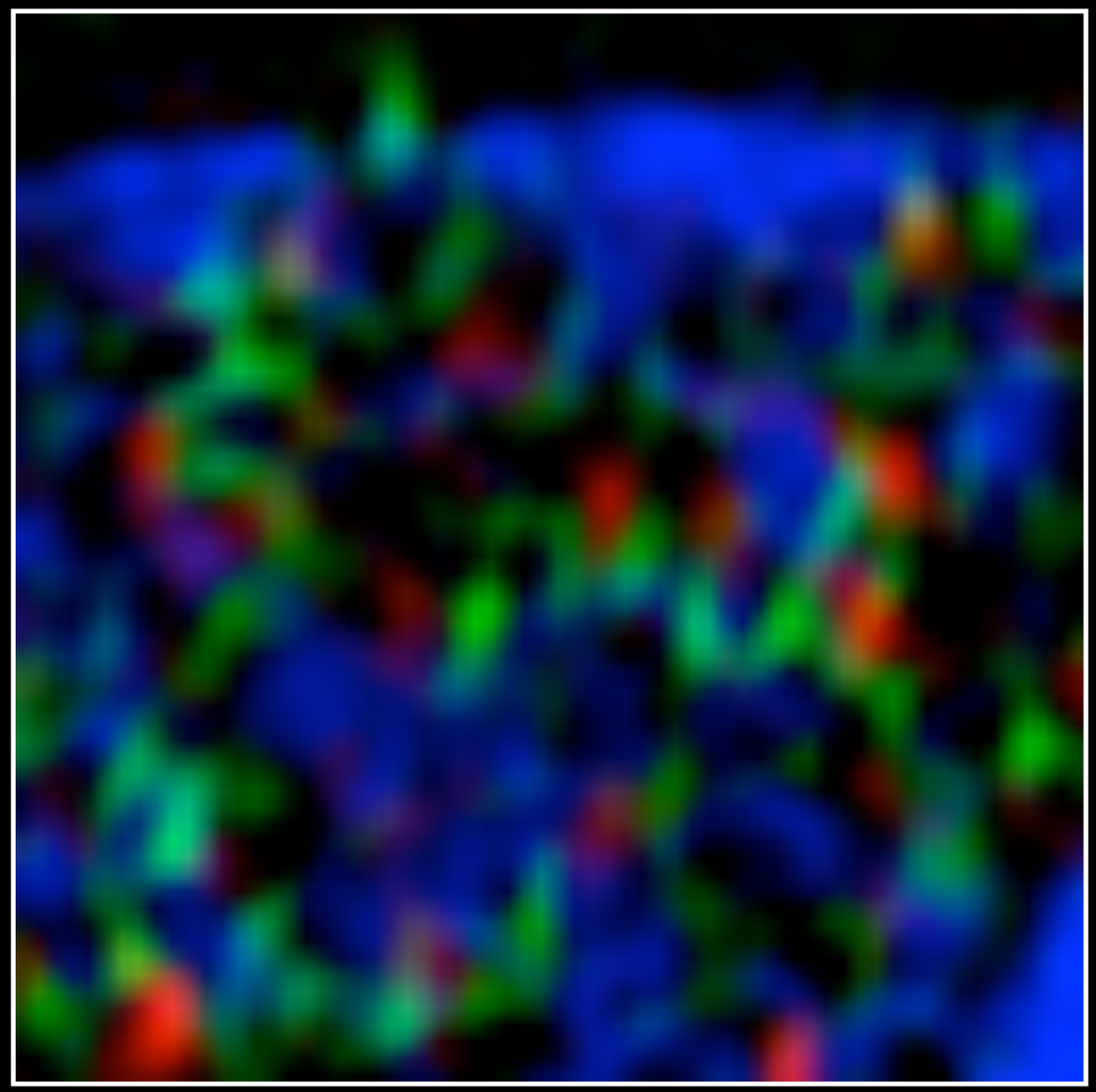


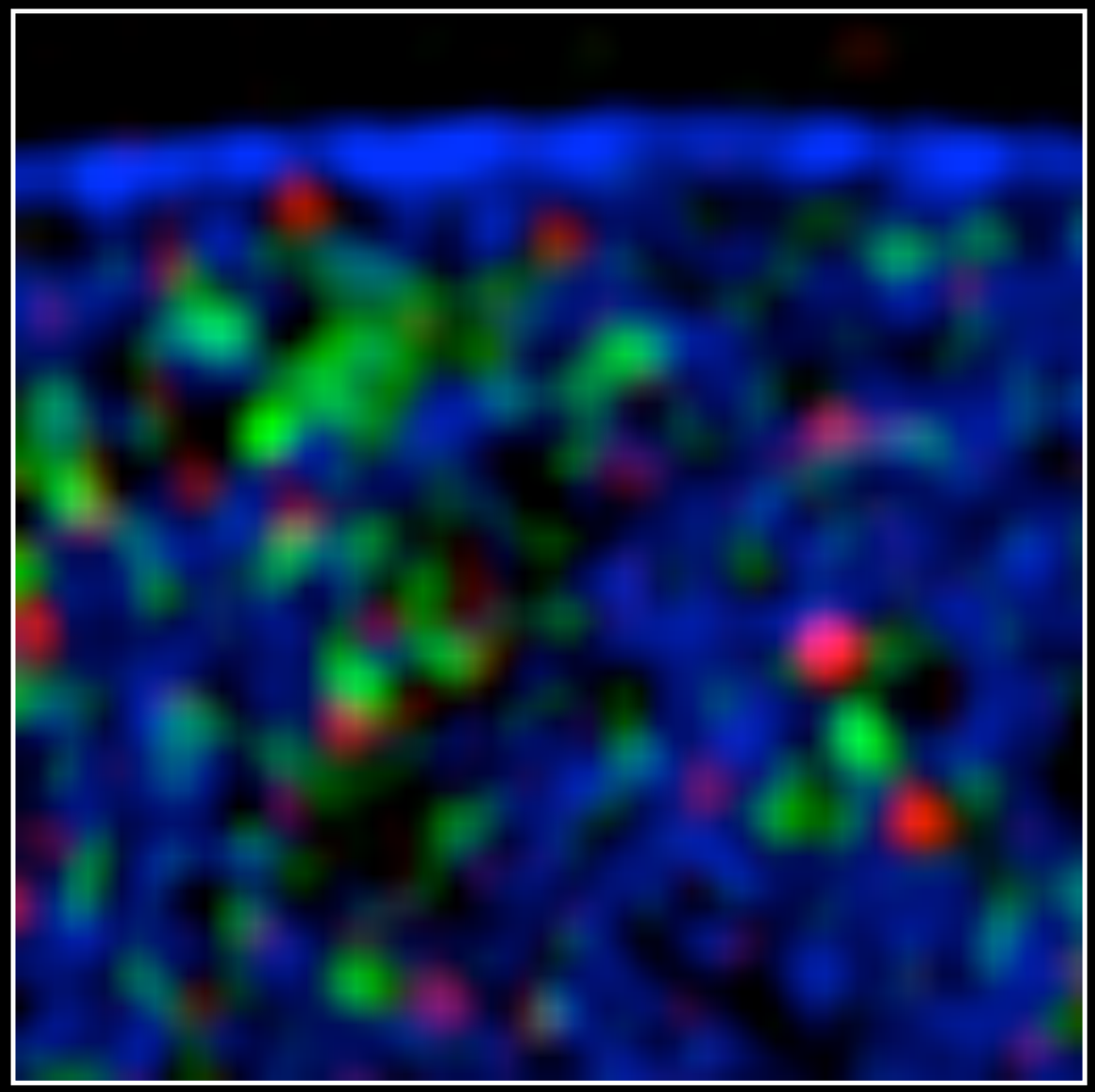


H3K4me3
RNA Pol II
DAPI

Good SI reconstruction

Quality control: SI-Reconstruction artifacts





H3K4me3
RNA Pol II
DAPI

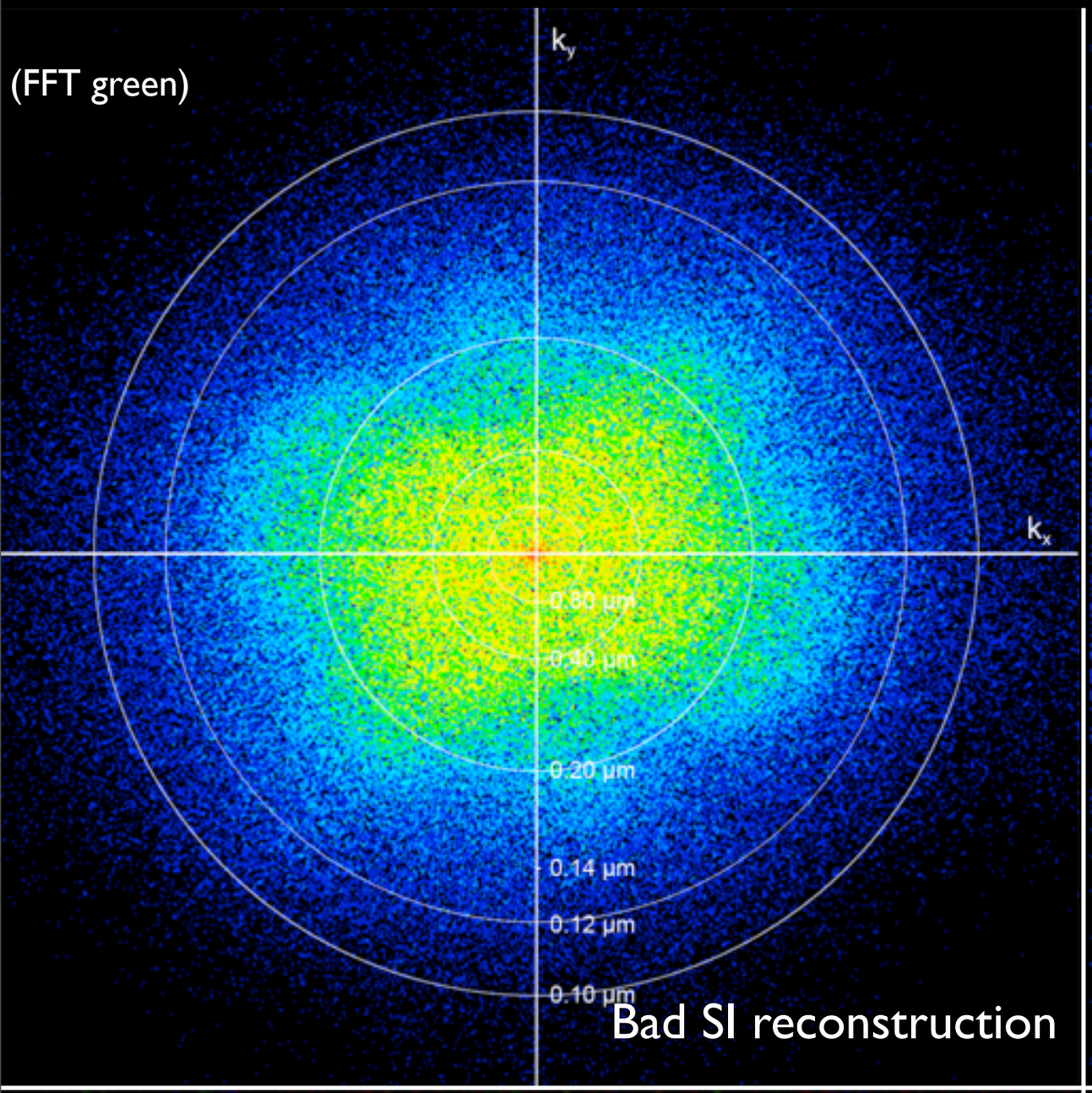
Bad SI reconstruction

Good SI reconstruction

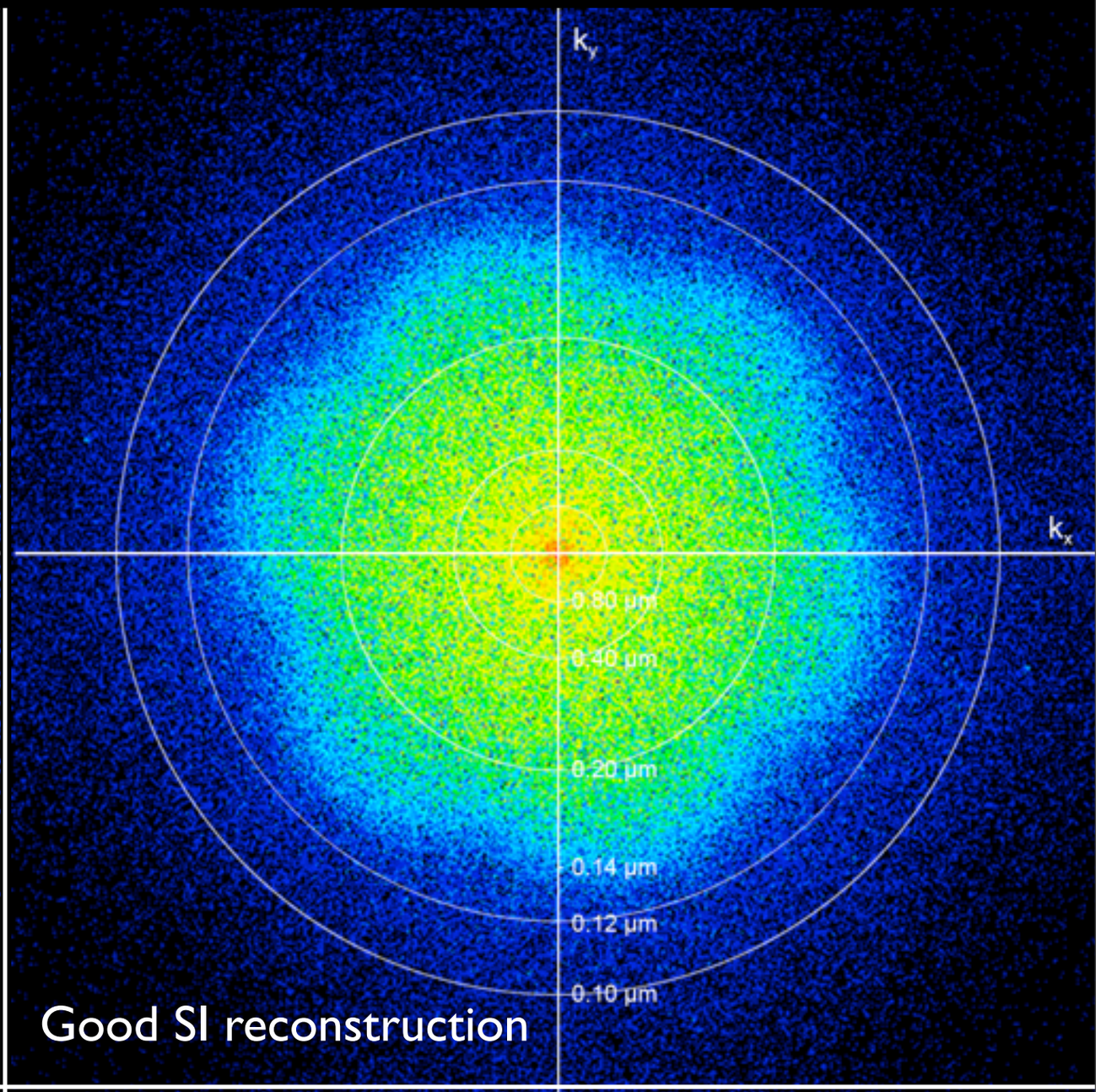
Lothar Schermelleh

Quality control by Fourier analysis

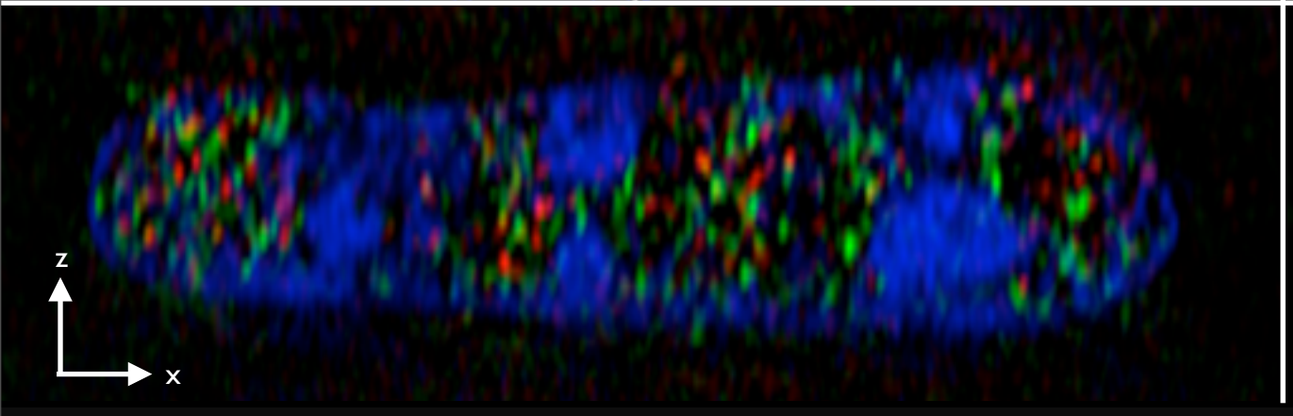
(FFT green)

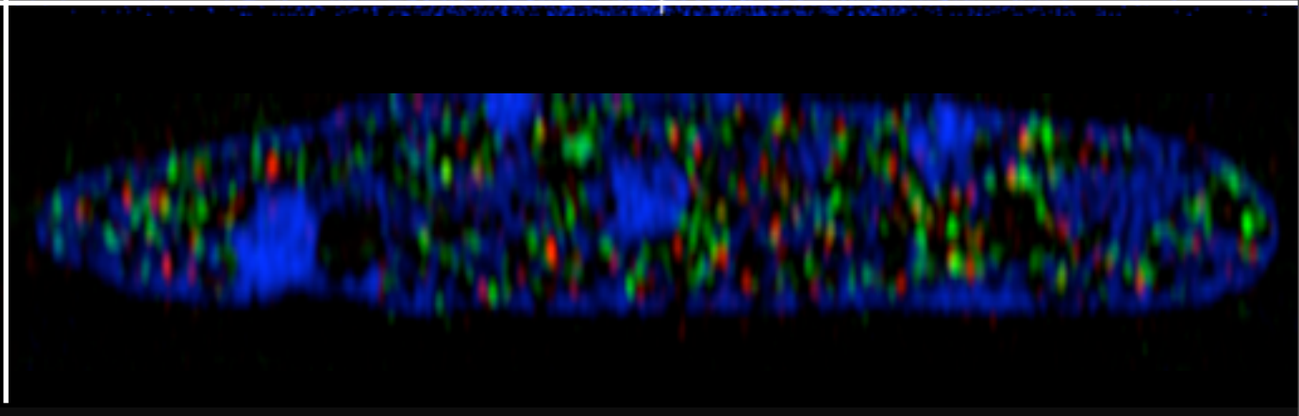


Bad SI reconstruction

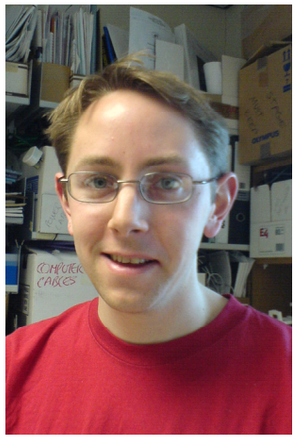


Good SI reconstruction



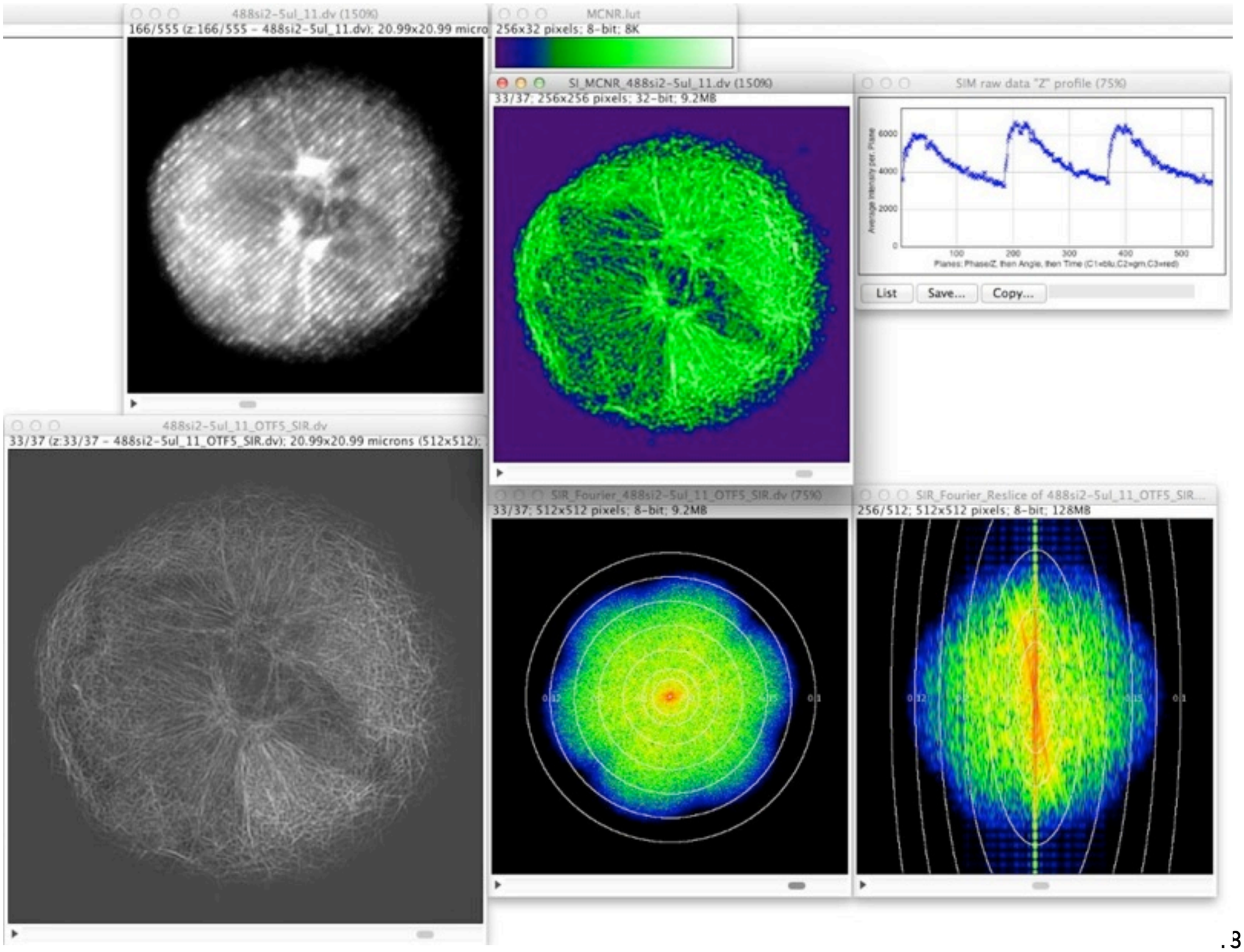


SIMCheck
(ImageJ plug in)

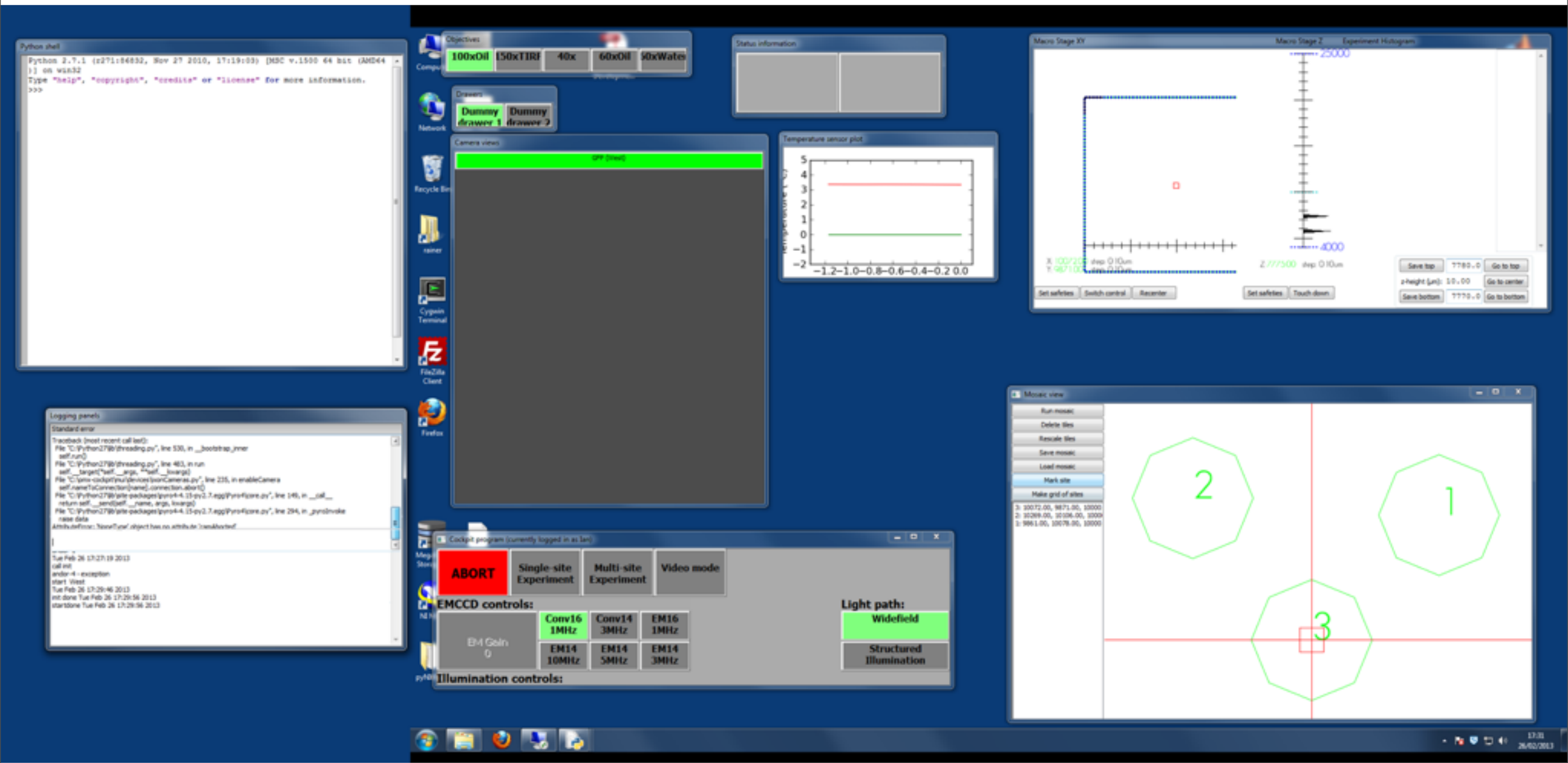


Graeme Ball

Lothar Schermelleh



Cockpit - from John Sedat



Douglas Russell

OMERO - Oxford Satellite

Use model: Lab data management and sharing with PI. Good practice. Open and transparent

Viewing, organizing and annotating Need to improve user experience

Integrating OMX workflows Acquisition and Importing, Preprocessing, Quality control, Image analysis (e.g. with Cockpit)

OMERO - integrating image analysis

Promises to be a cross-platform integrator of Matlab, Python, ImageJ algorithms

Simcheck

Denoising

New Deconvolution algorithms

DOUGLAS

Micron
OXFORD
wellcome trust
http://www.micronoxford.com/

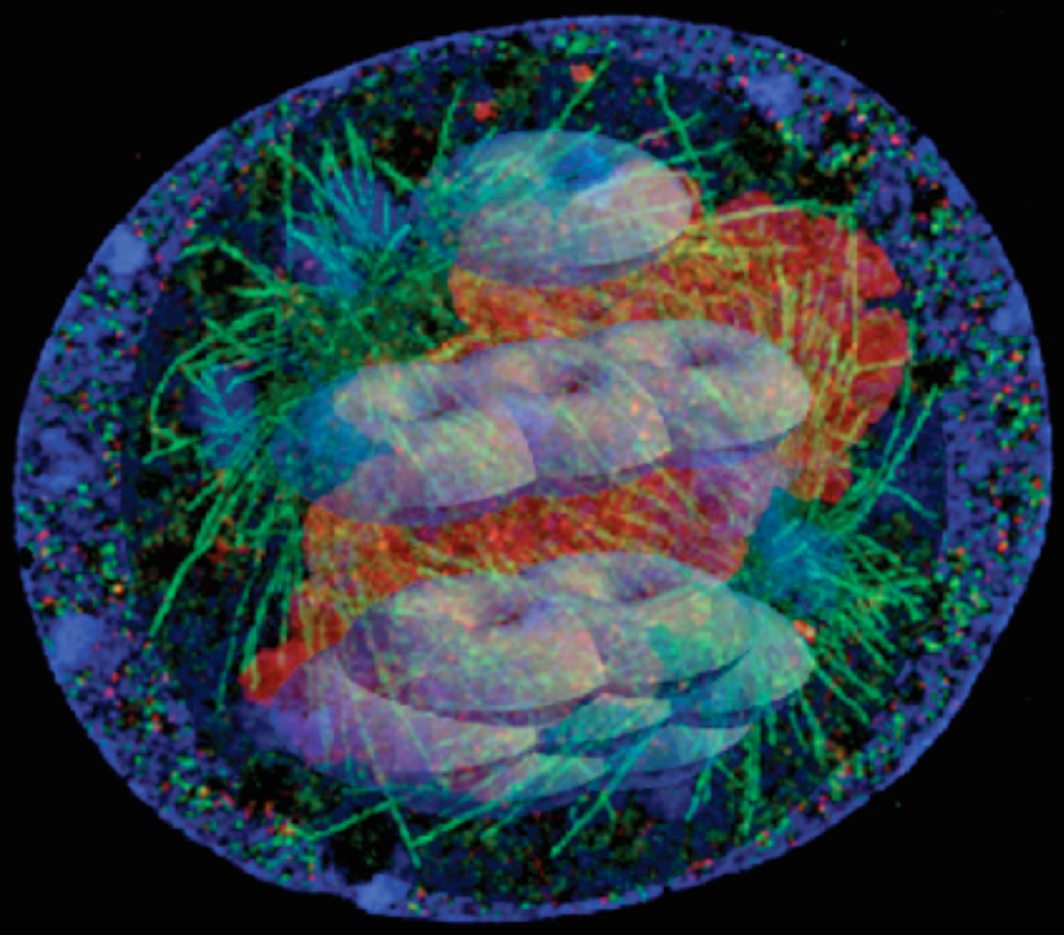
Nanoscopy Oxford

Super-resolution microscopy applied to biomedical research

Now recruiting six postdoctoral positions

Image analysis specialist

Correlative EM, X-ray and light microscopy specialist



Adaptive optics developer

Super-resolution software developer

Bespoke neuro-imaging developer

Saturated structured illumination developer

Acknowledgements

Reagents: Bloomington and other stock centres, flybase

Funding: wellcome trust MRC, BBSRC, John Fell Fund