

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



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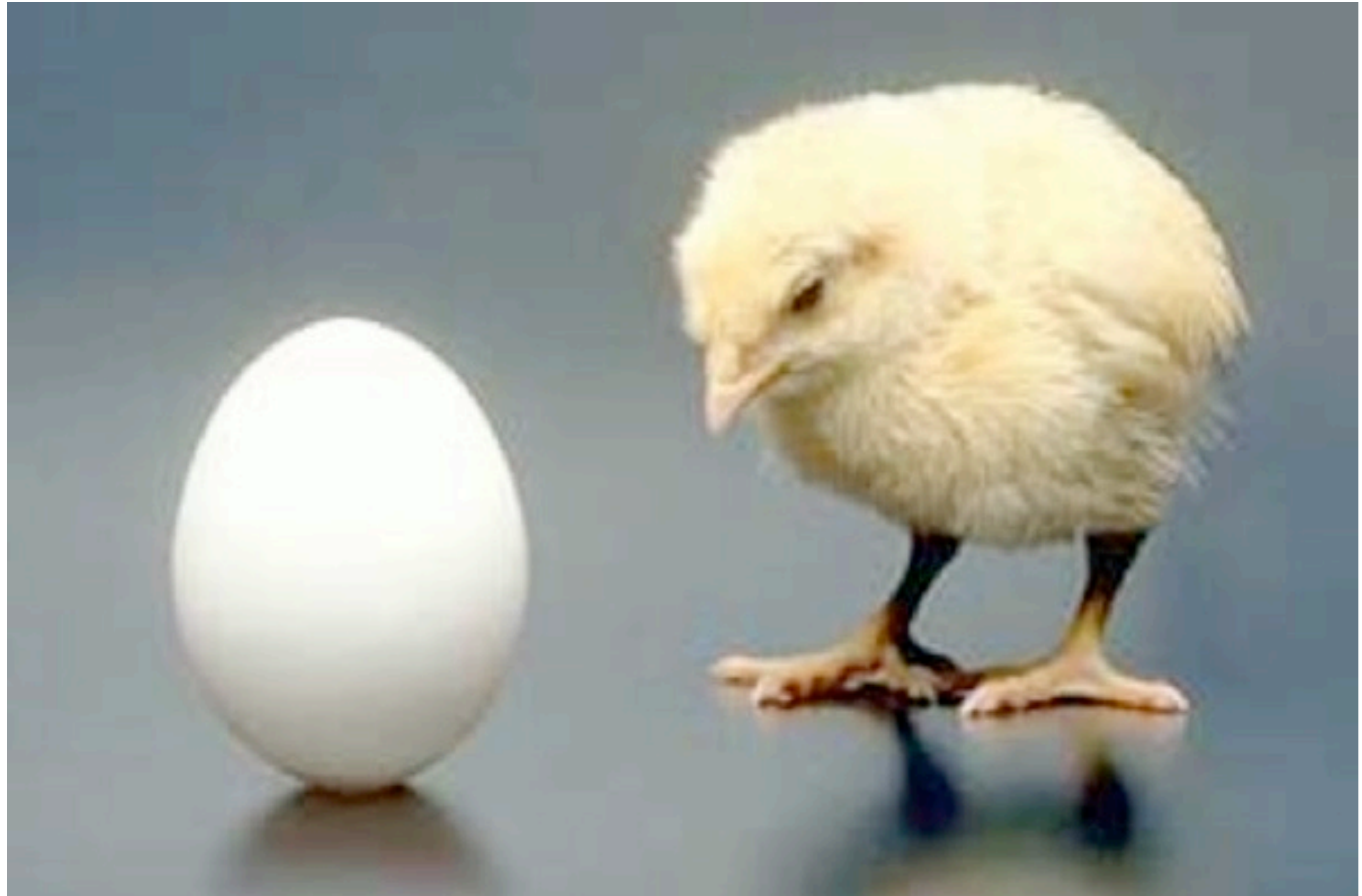
The mechanism of mRNA transport and local translation in *Drosophila*

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

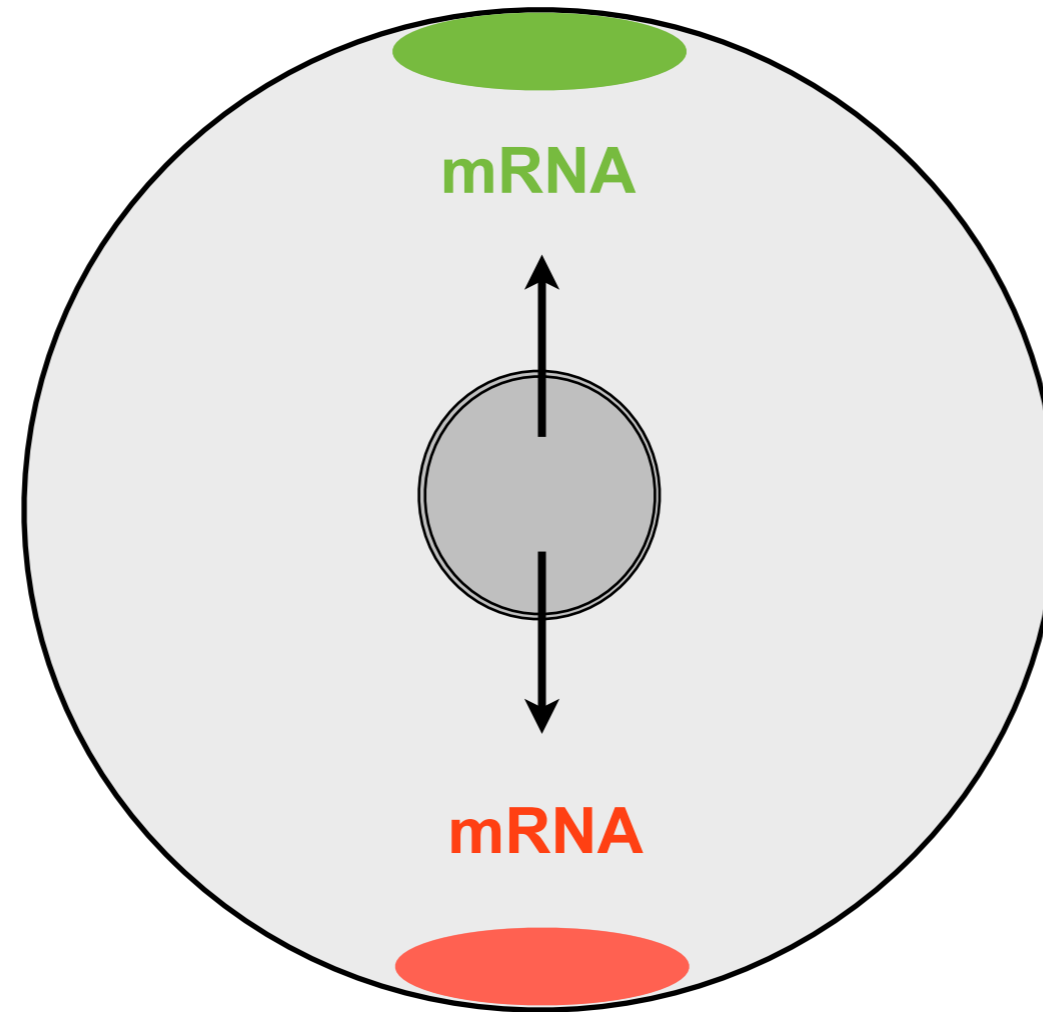
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How do you make a chicken from an egg?



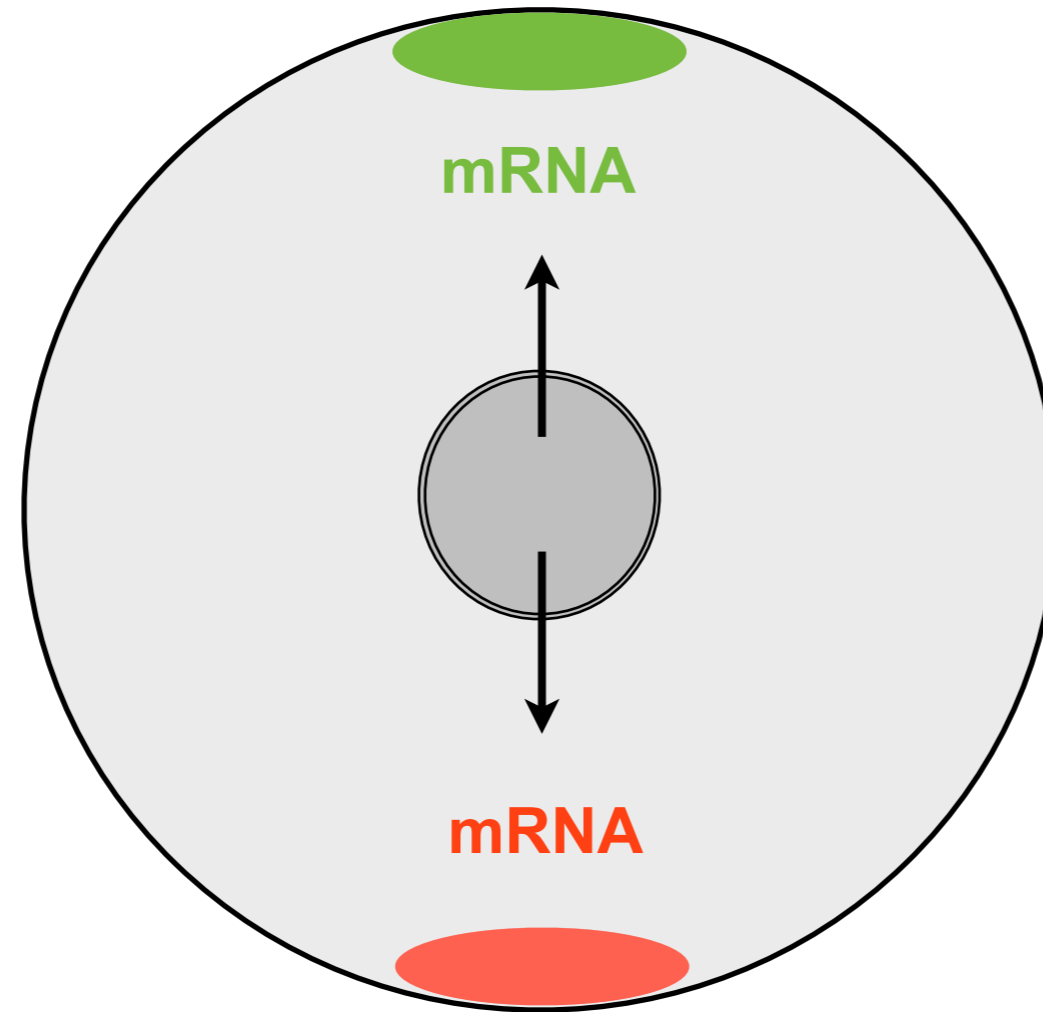
Establishing the animal body axes



**mRNA transport
localised translation**



Establishing the animal body axes

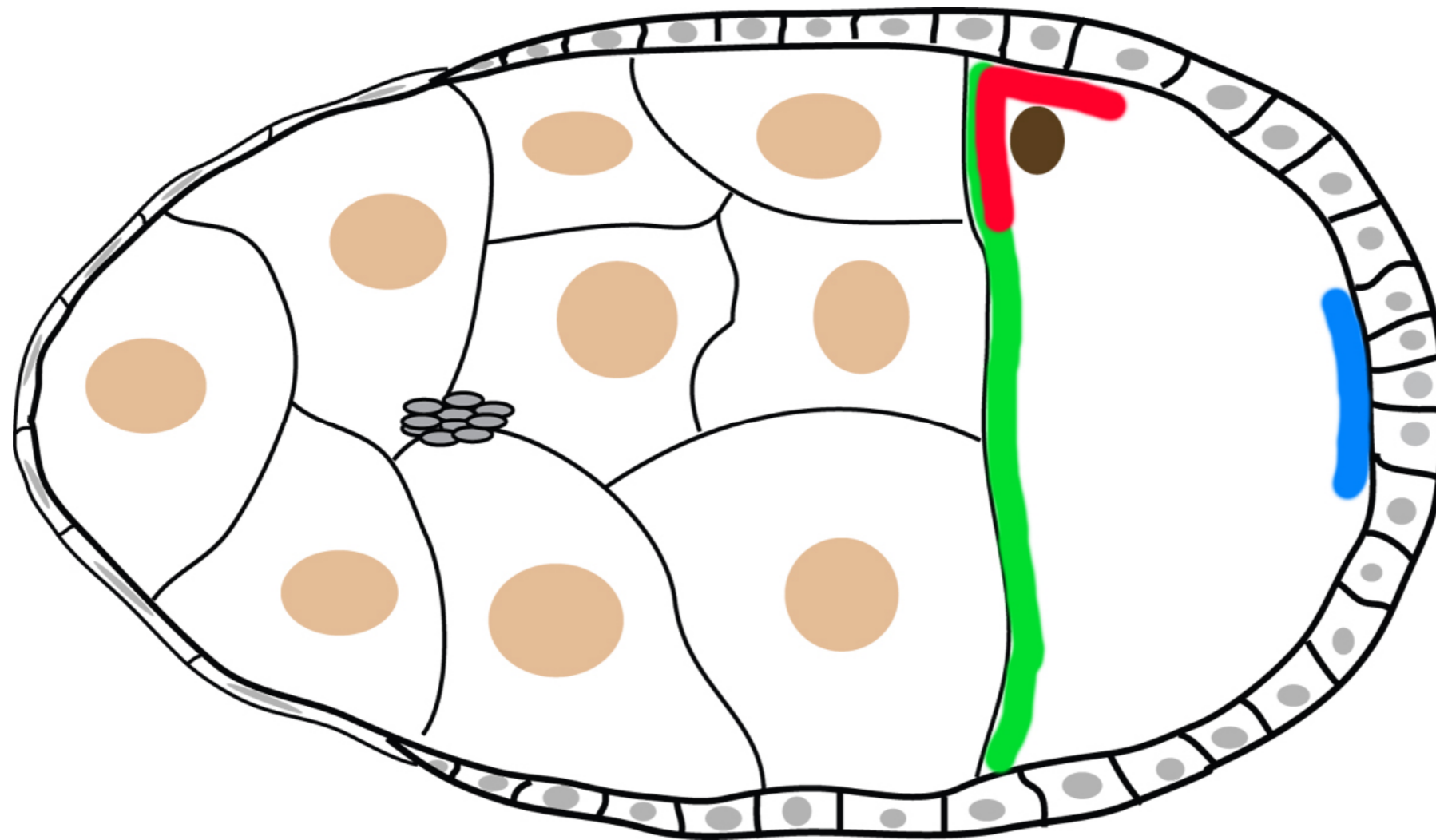


**mRNA transport
localised translation**



Drosophila oocyte

gurken (TGF- α) mRNA

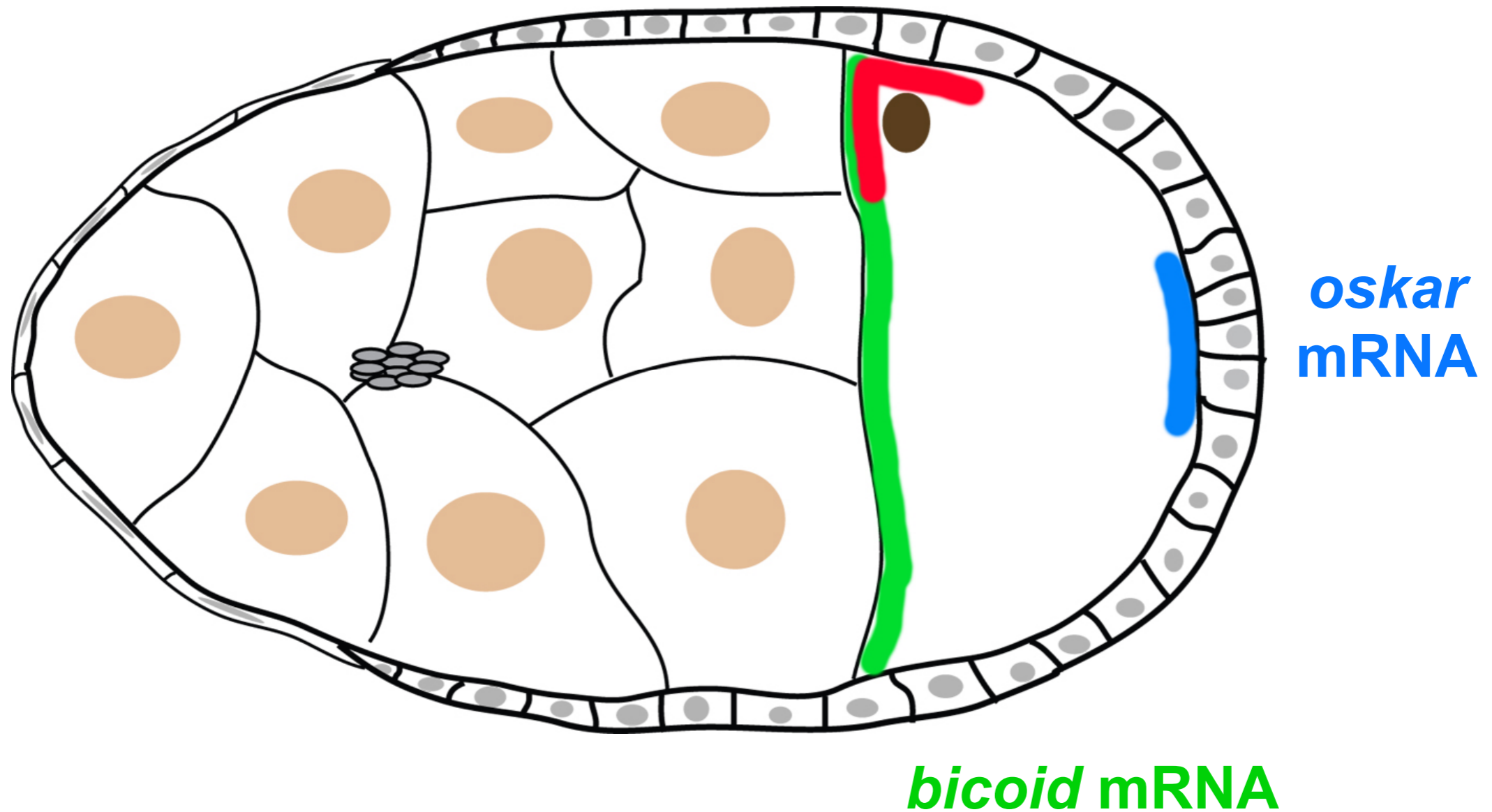


oskar
mRNA

bicoid mRNA

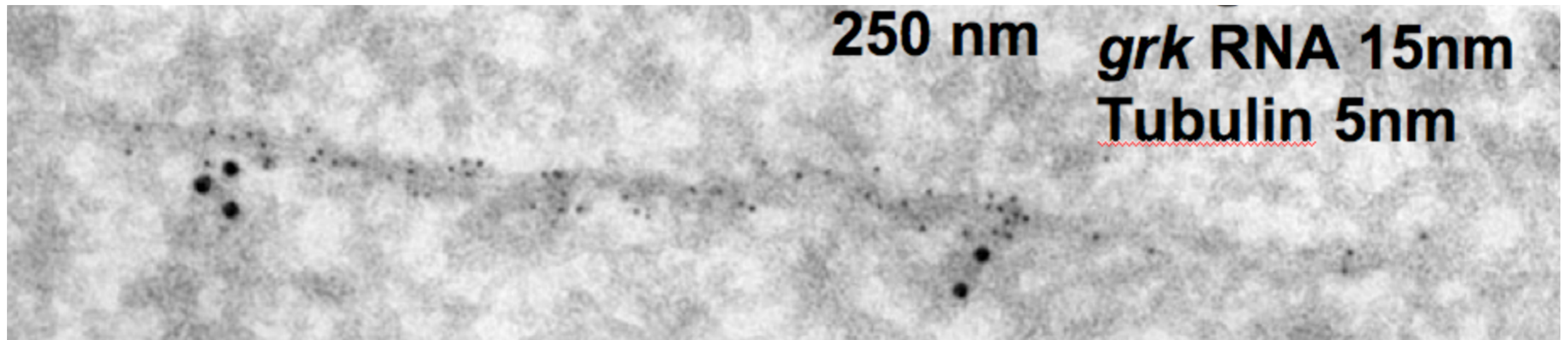
Drosophila oocyte

gurken (TGF- α) mRNA

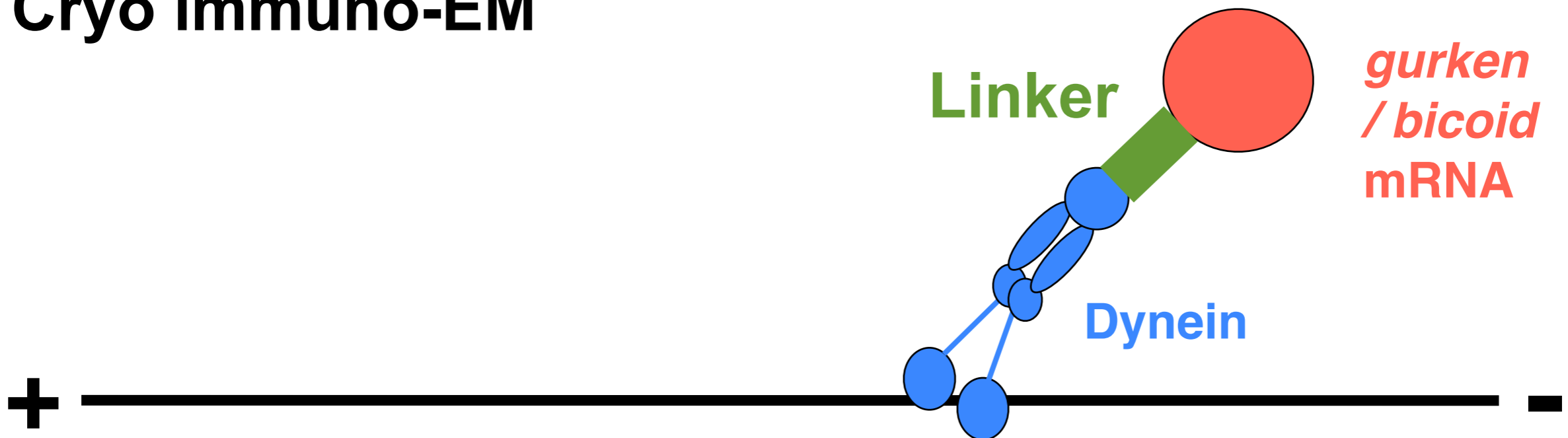


**How are mRNAs sorted to different destinations?
How are they translationally regulated?**

RNA transport by Dynein along microtubules



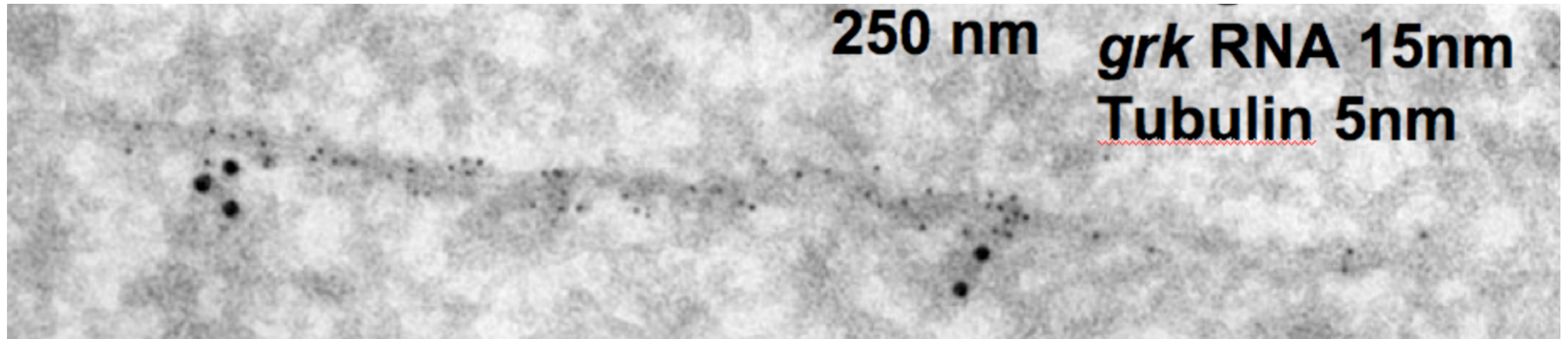
Cryo immuno-EM



Wilkie and Davis, Cell 2001
Delanoue and Davis, Cell 2005

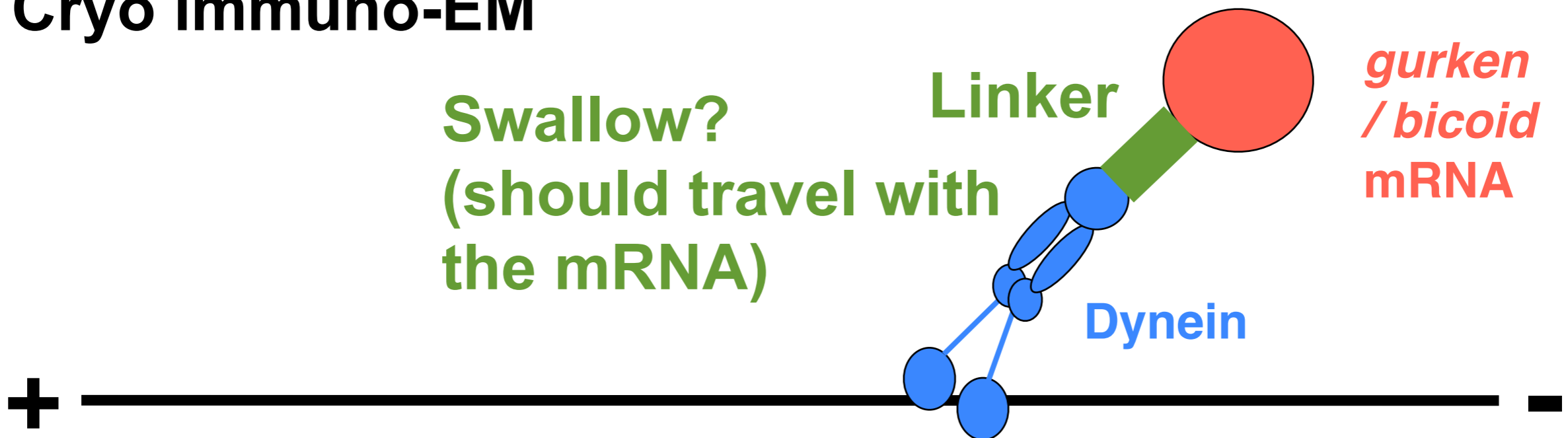
Delanoue et al, Dev Cell 2007
(collaboration with
Catherine Rabouille, Utrecht)

RNA transport by Dynein along microtubules



Cryo immuno-EM

Swallow?
(should travel with
the mRNA)



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



**Richard
Parton**

**Timothy
Weil**



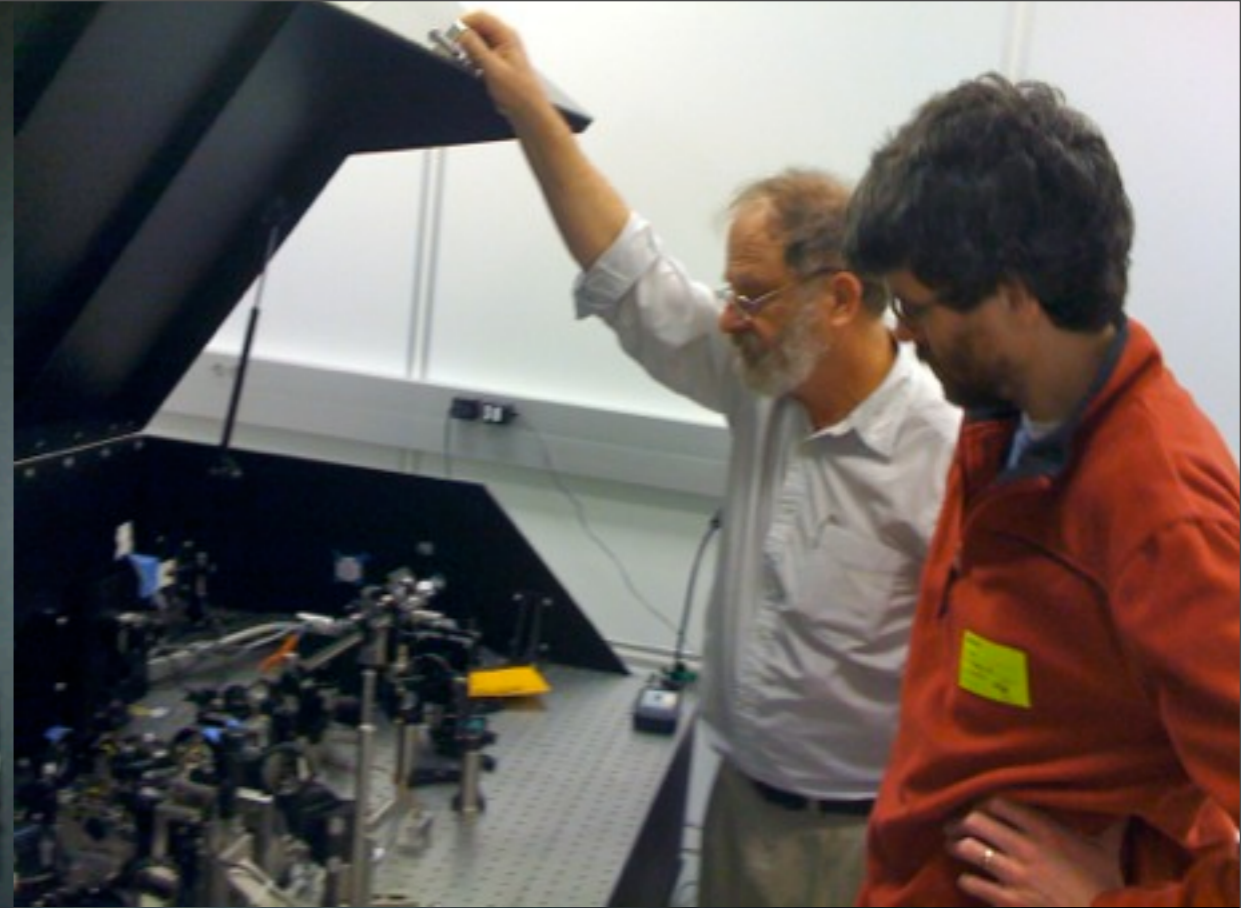
David Agard



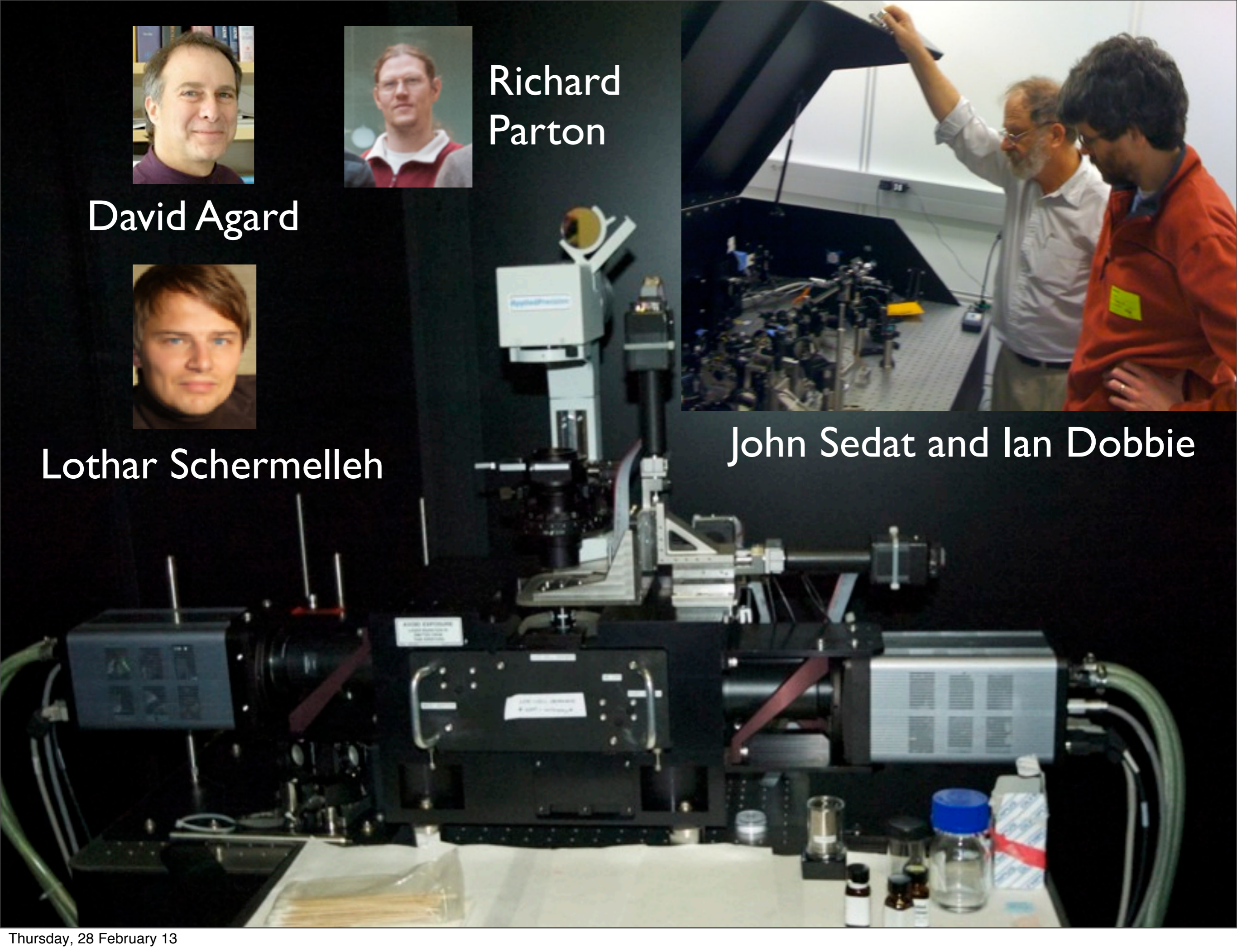
Richard Parton



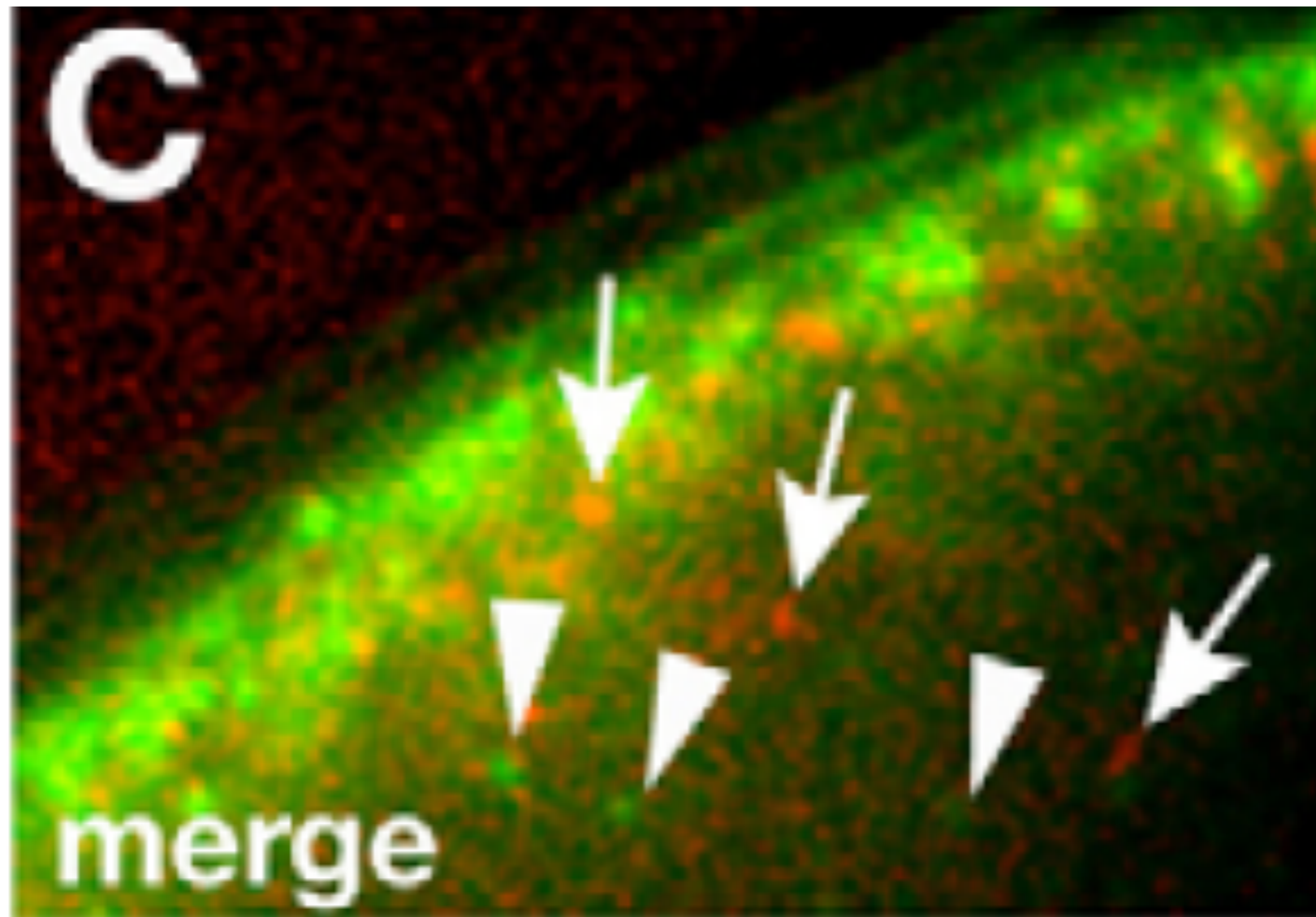
Lothar Schermelleh



John Sedat and Ian Dobbie



Swallow does not link *bcd* mRNA to Dynein



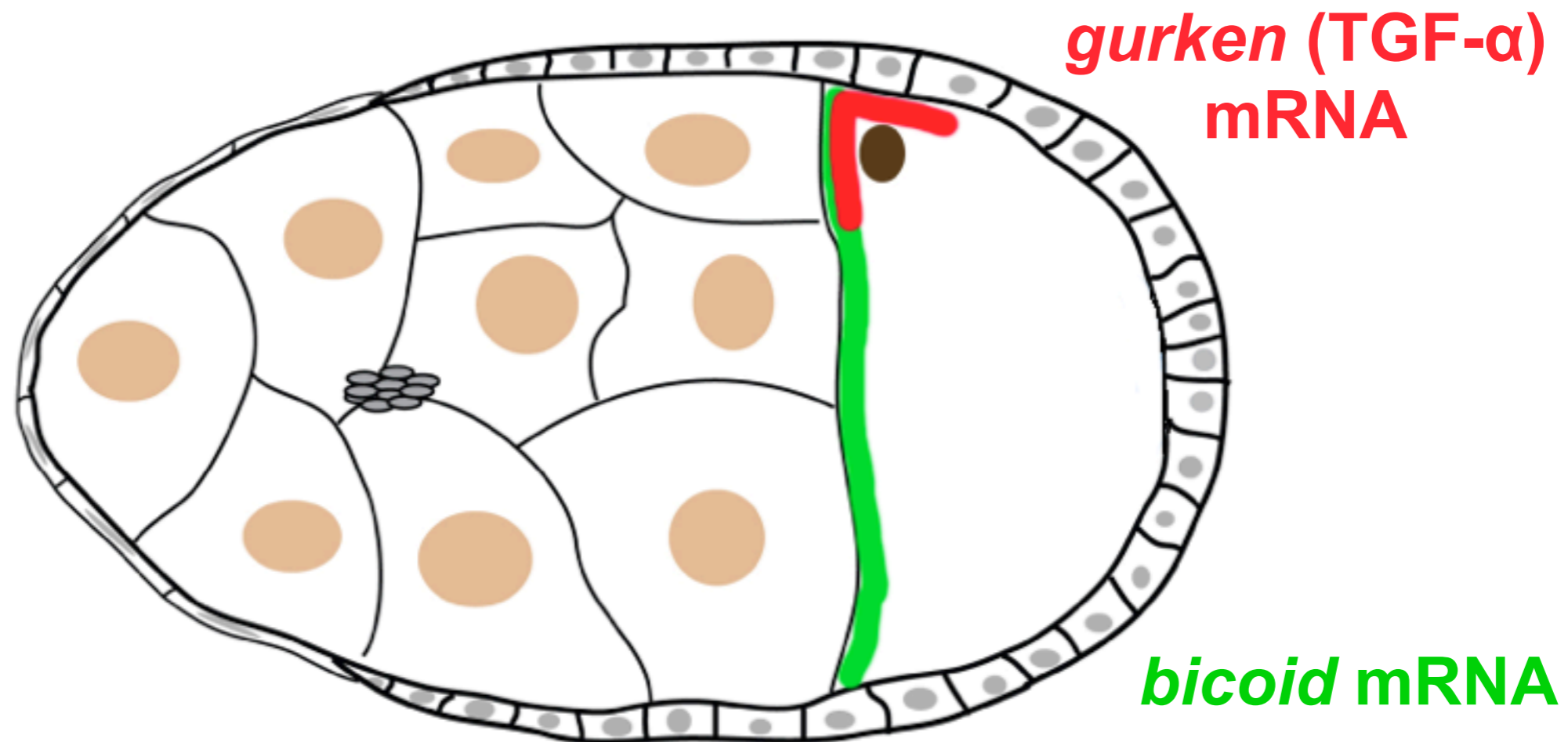
Swallow protein

grk MS2 GFP* tagged mRNA

Weil et al, Development 2010

Collaboration with Catherine Rabouille, Ubrecht
and Liz Gavis, Princeton

Drosophila oocyte

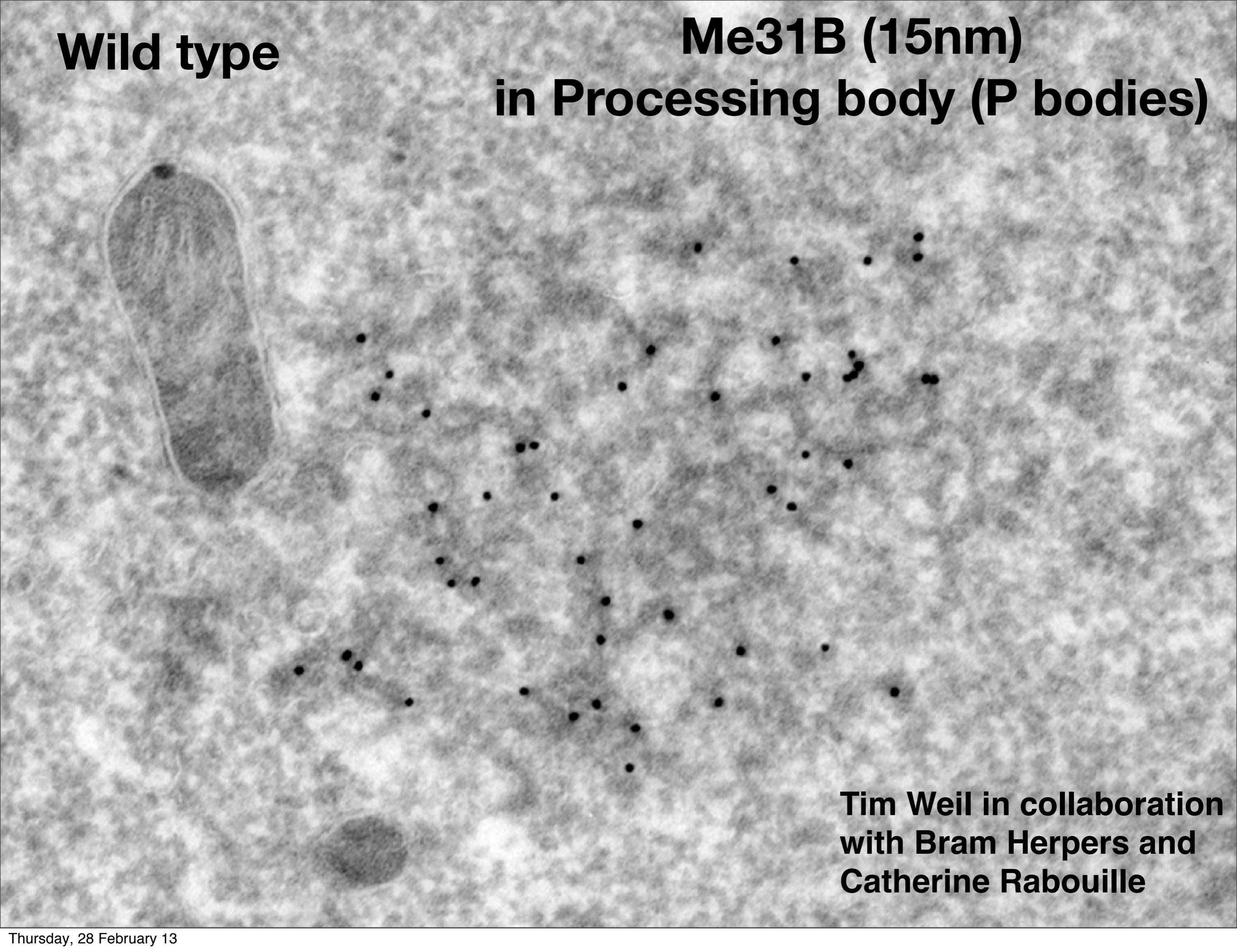


How are *gurken* and *bicoid* mRNA differentially translationally regulated?

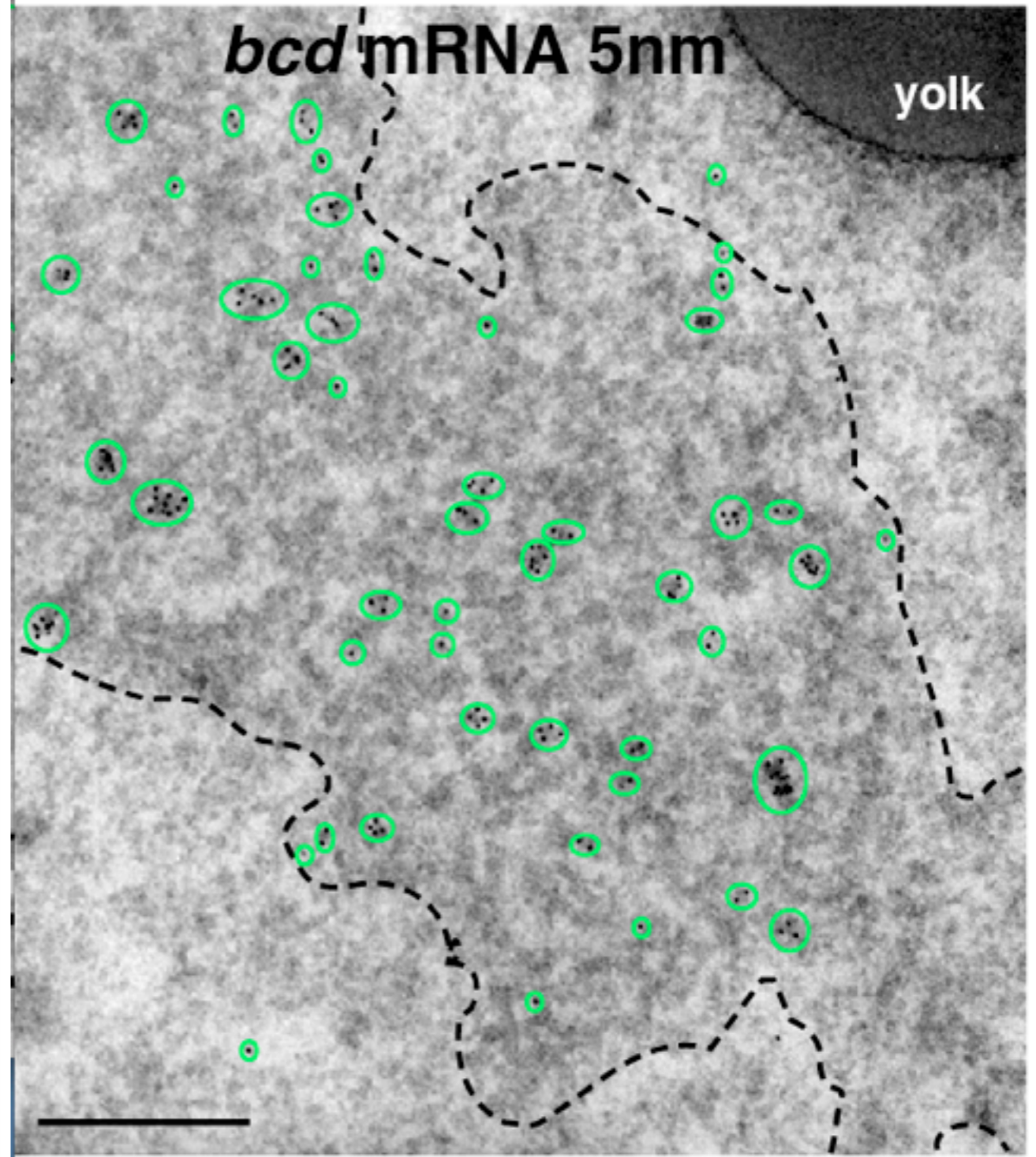
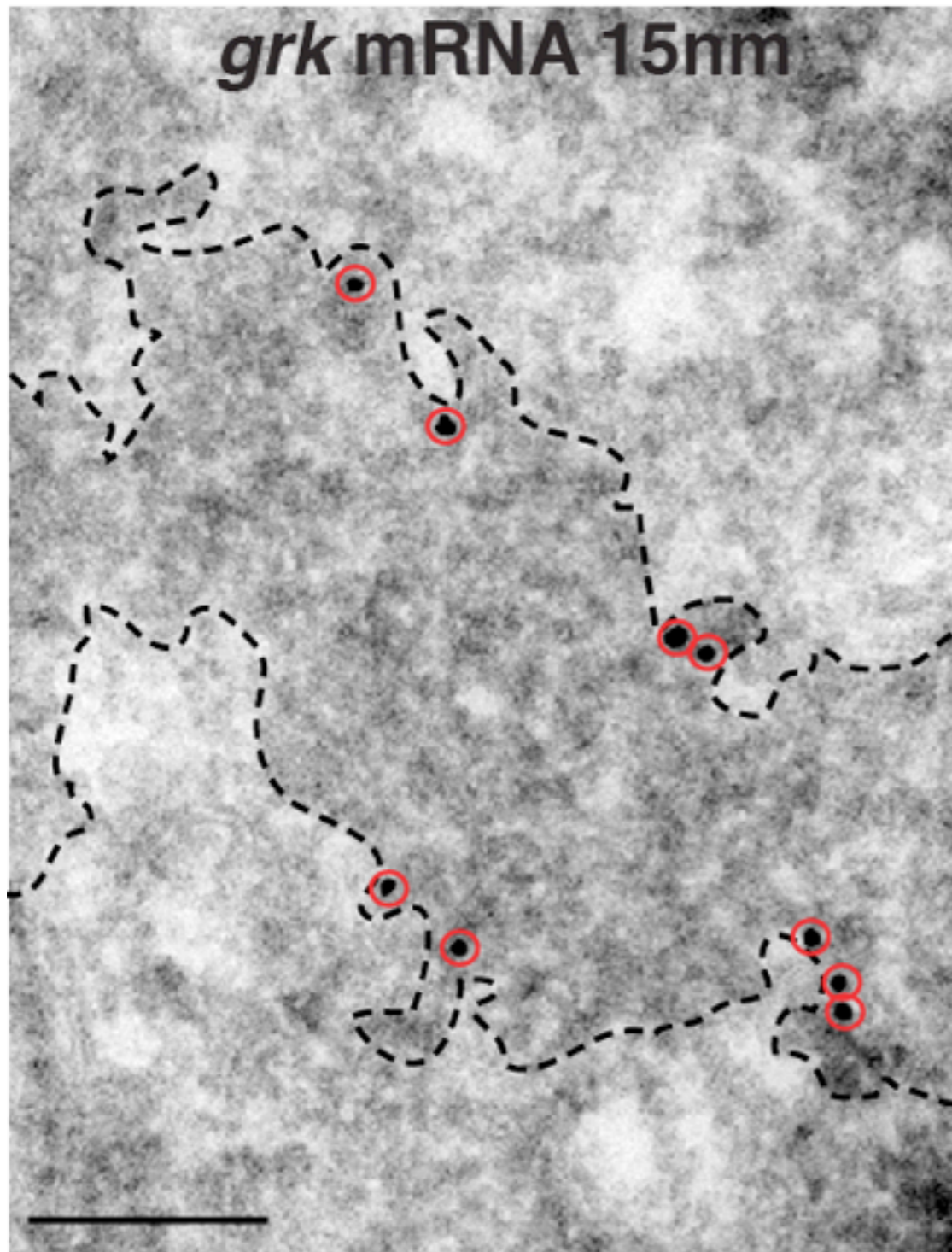
Weil, Parton et al, Nature Cell Biol. 2012
(collaboration with the Rabouille lab)

Wild type

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

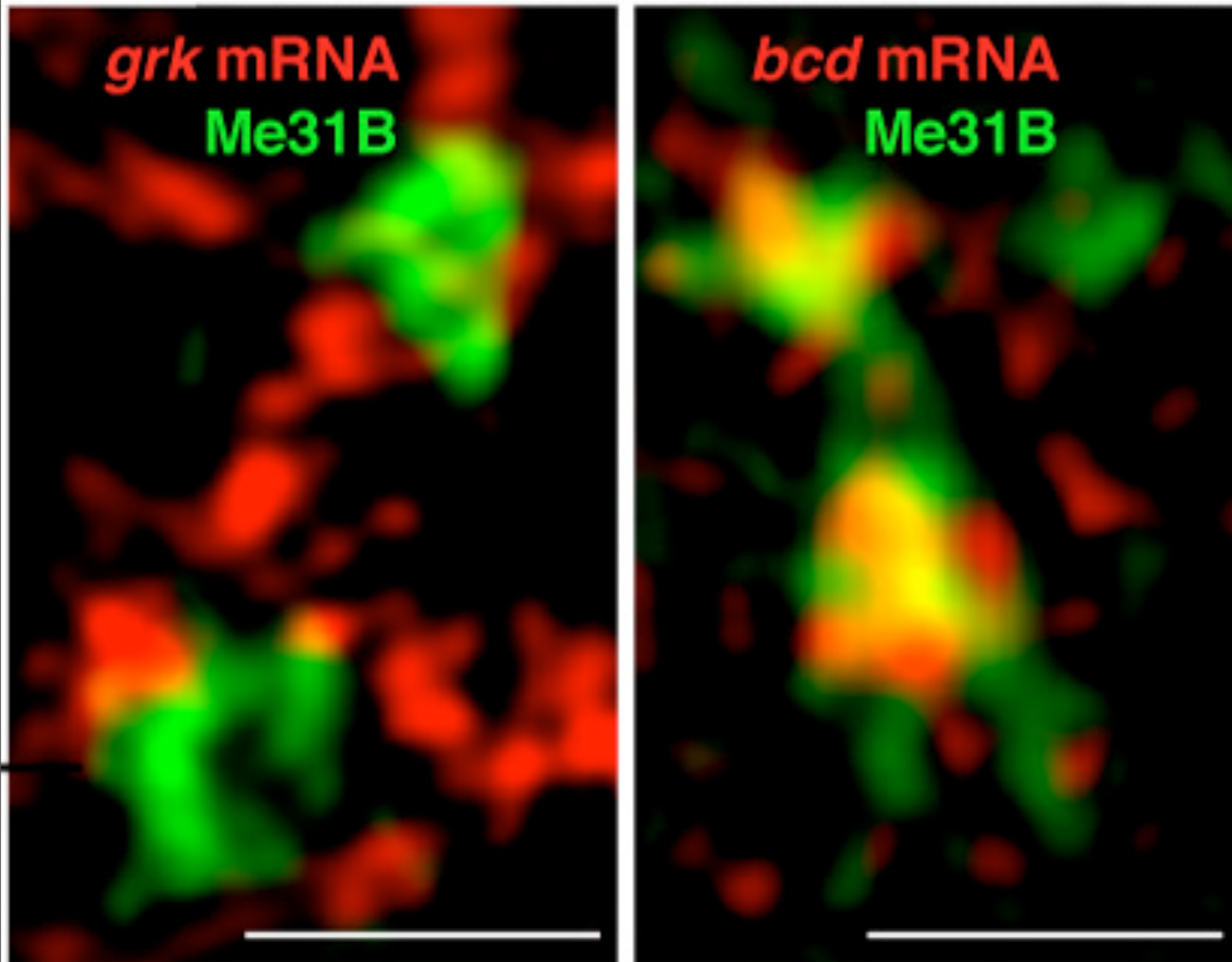


**Tim Weil in collaboration
with Bram Herpers and
Catherine Rabouille**



Tim Weil

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



**Structured
Illumination**

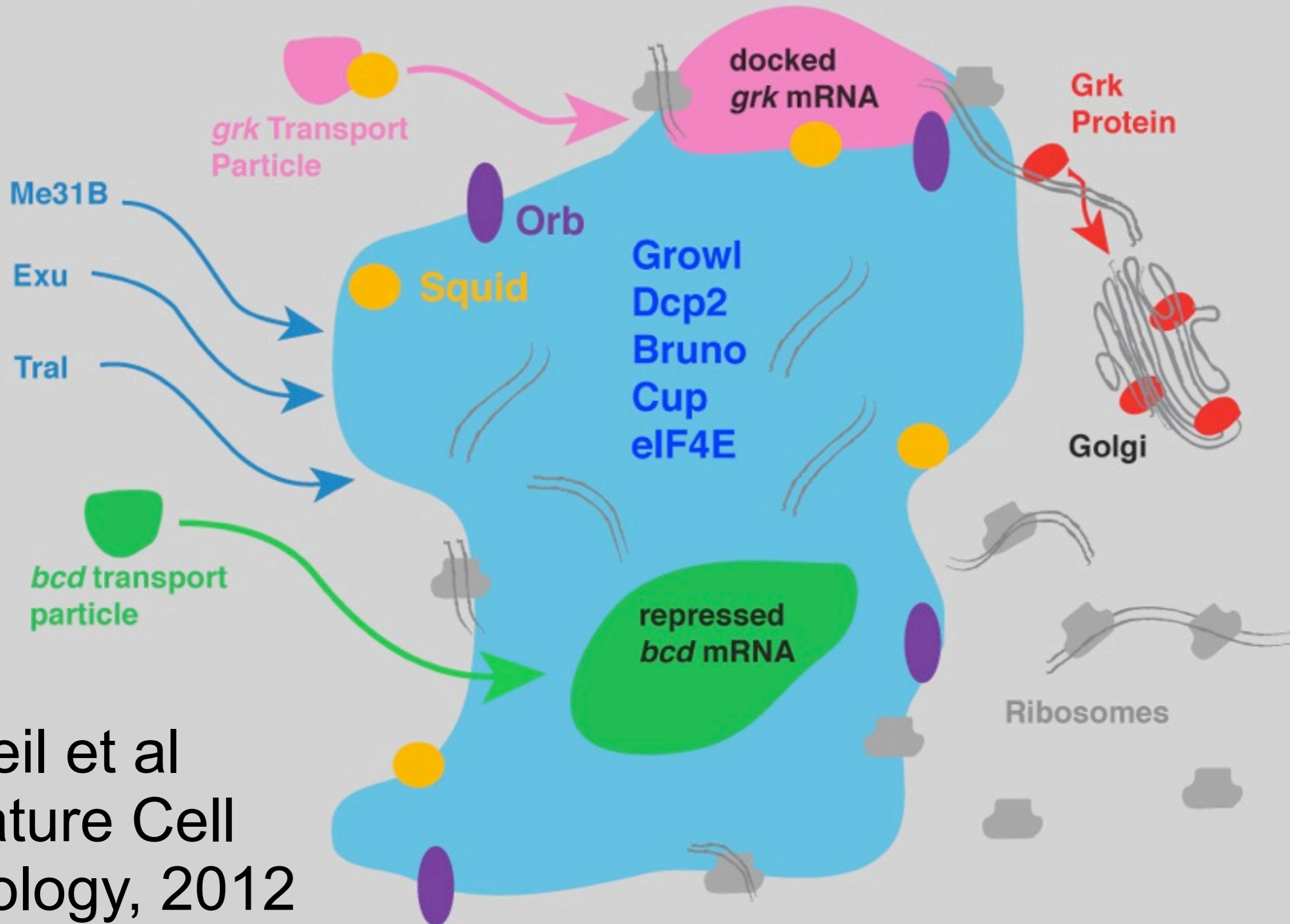
**twice
conventional
resolution in
x,y and z.**

**Tim Weil and
Richard Parton**

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

P body assembly

Translational regulation



Weil et al
Nature Cell
Biology, 2012

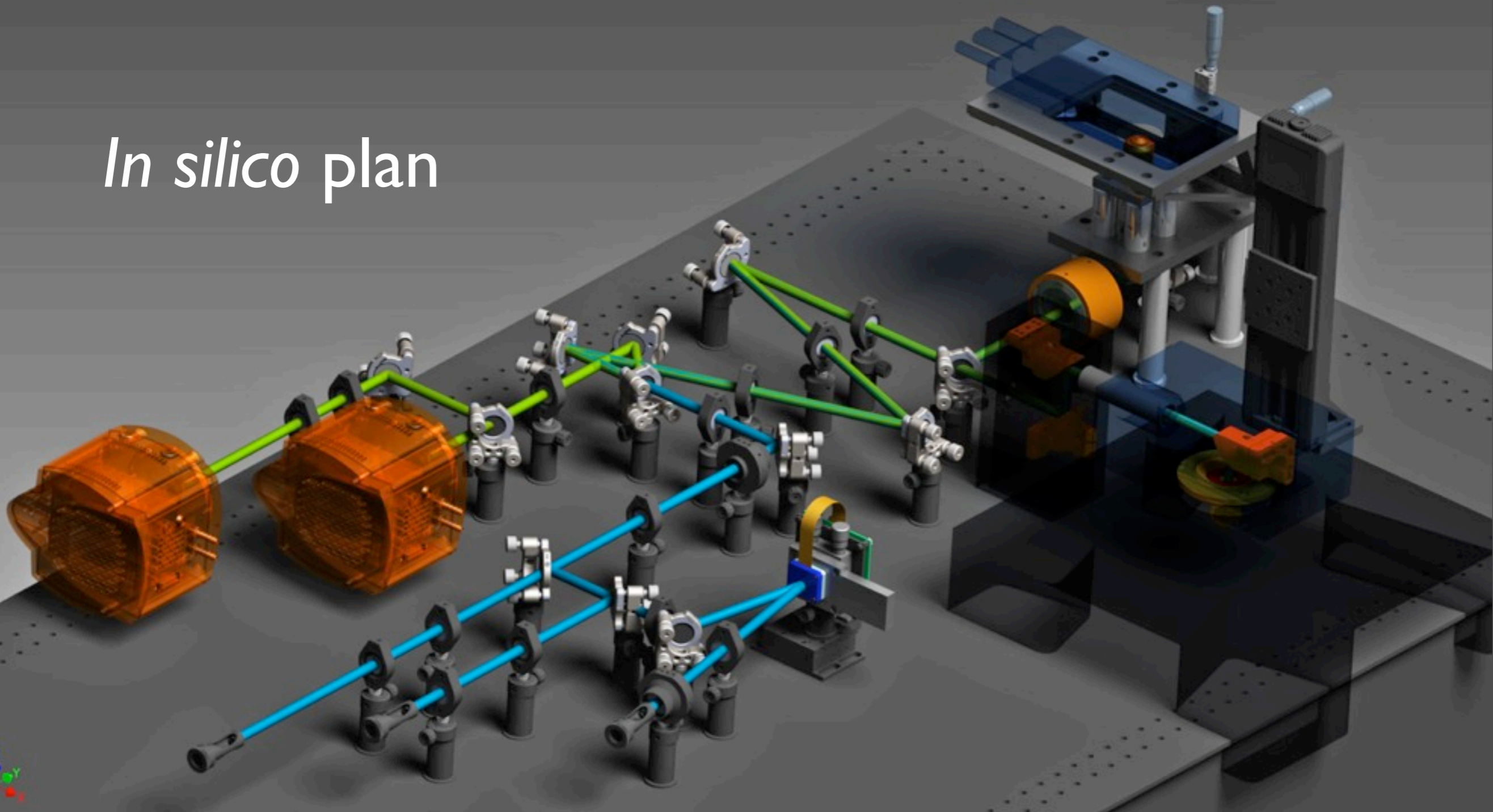


Further development: OMX-T

Rainer Kauffman
and Ian Dobbie

Based on John Sedat's
design

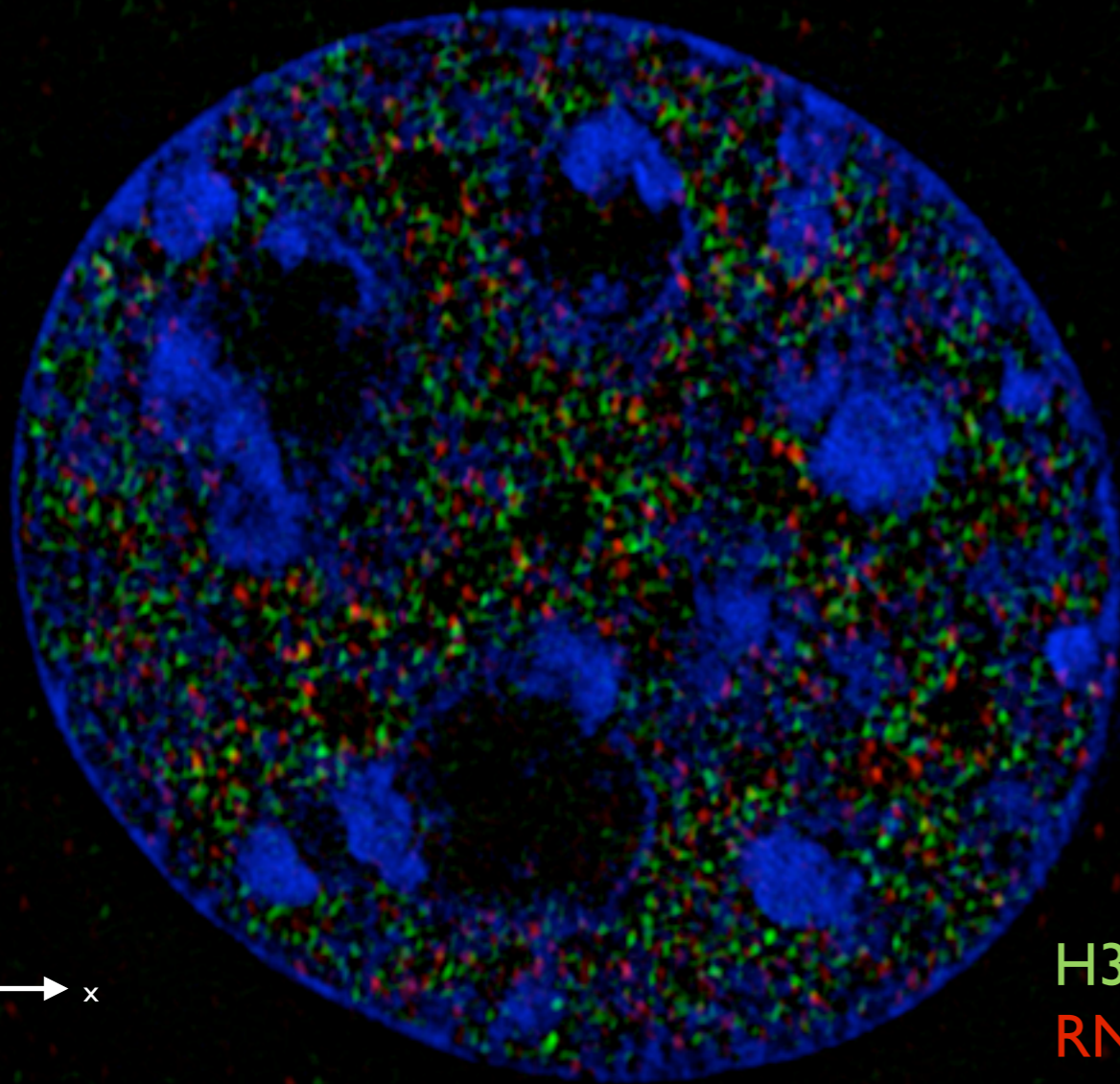
In silico plan



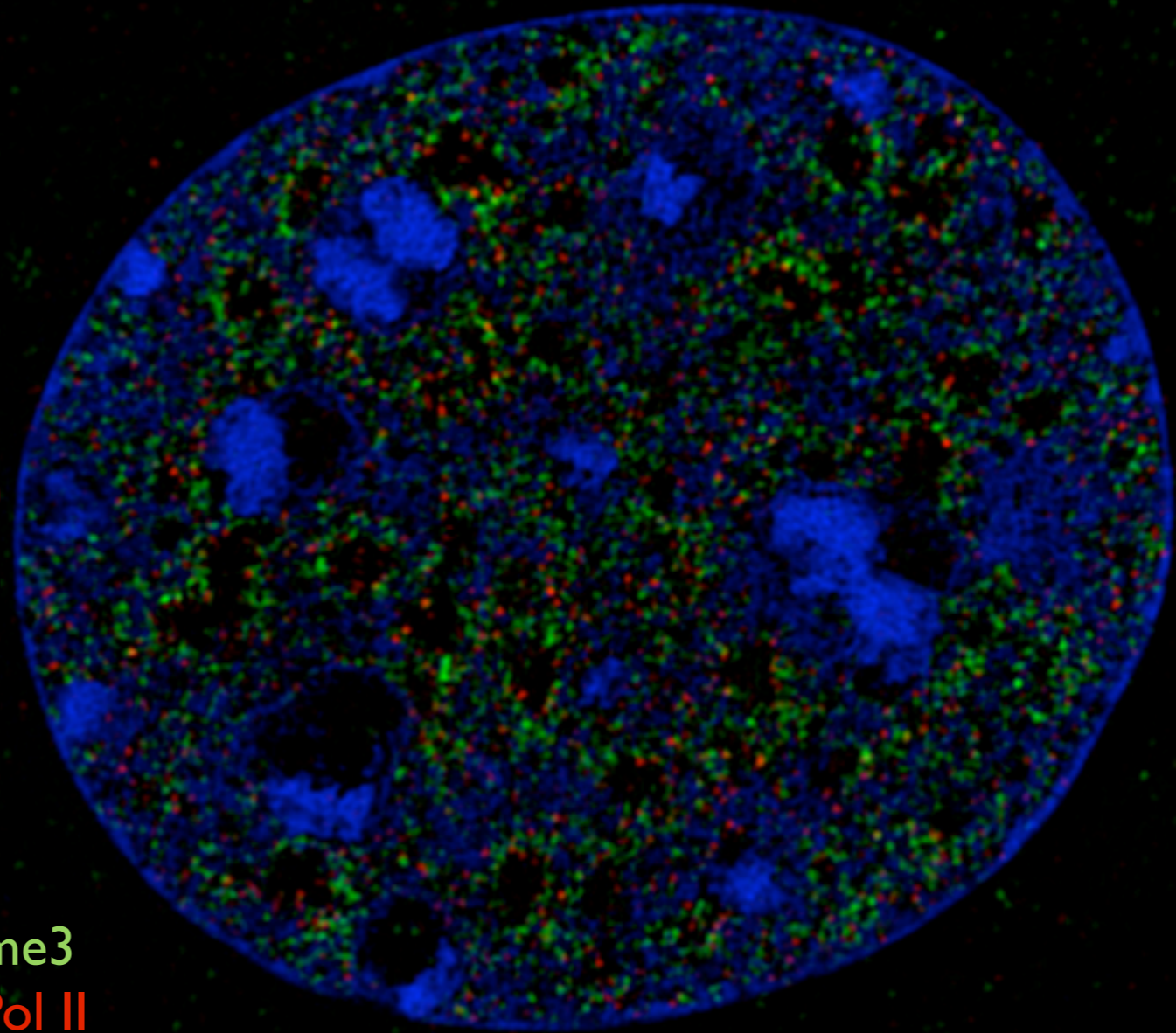
Quality control: SI-Reconstruction artifacts



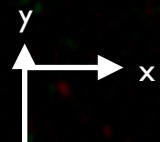
Lothar Schermelleh



H3K4me3
RNA Pol II
DAPI



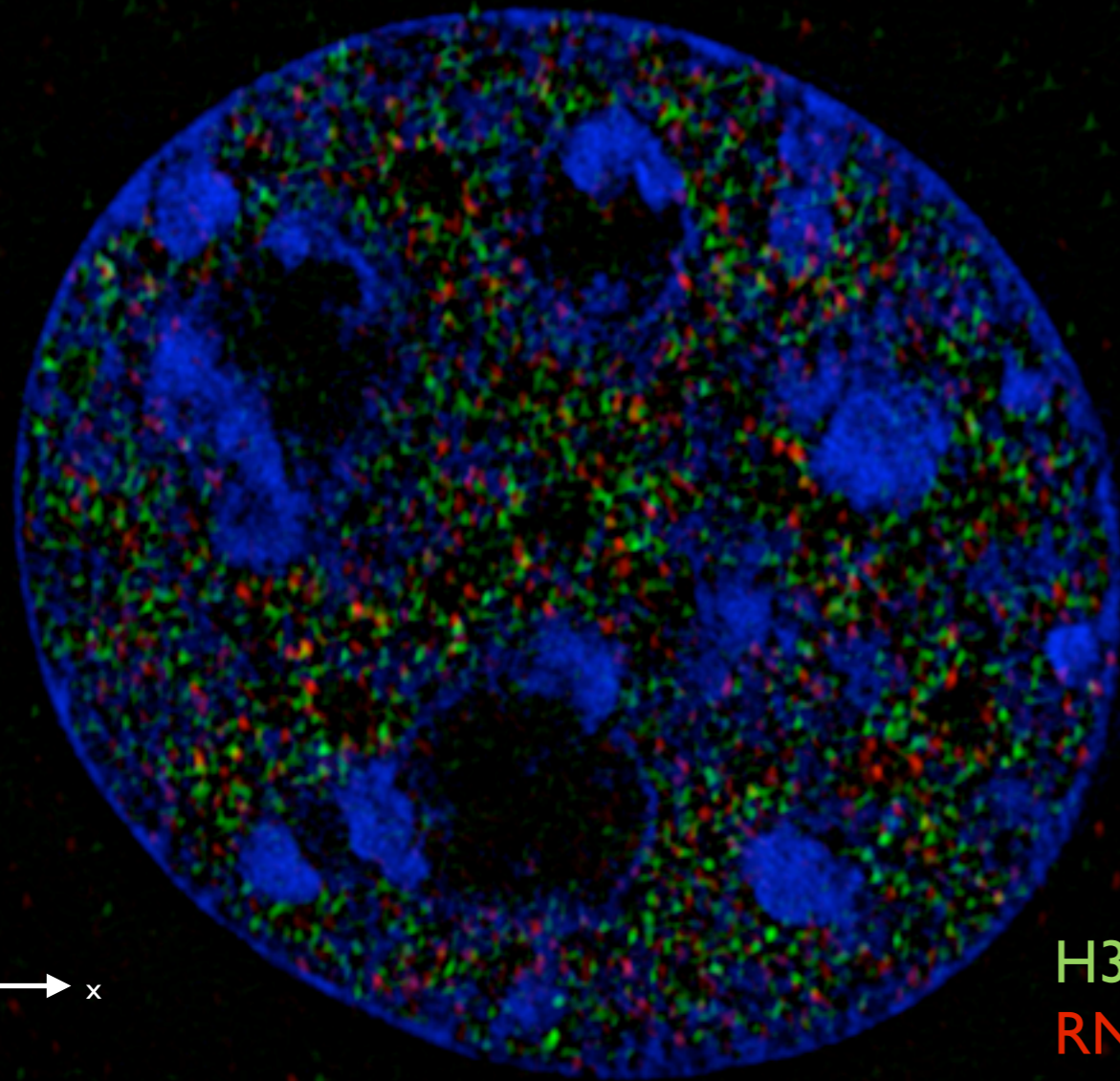
Good SI reconstruction



Quality control: SI-Reconstruction artifacts

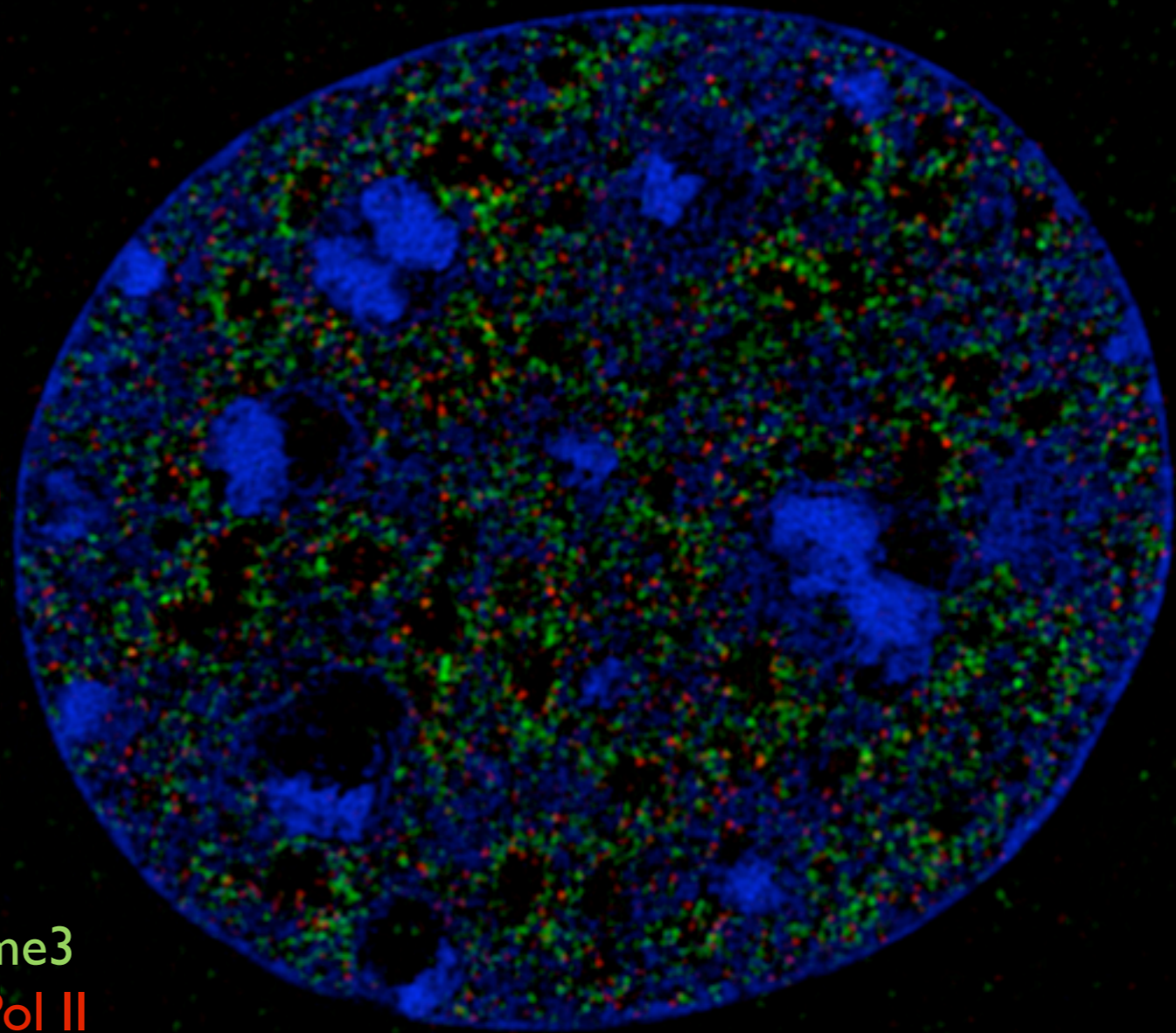


Lothar Schermelleh

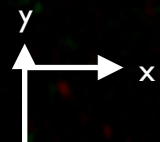


H3K4me3
RNA Pol II
DAPI

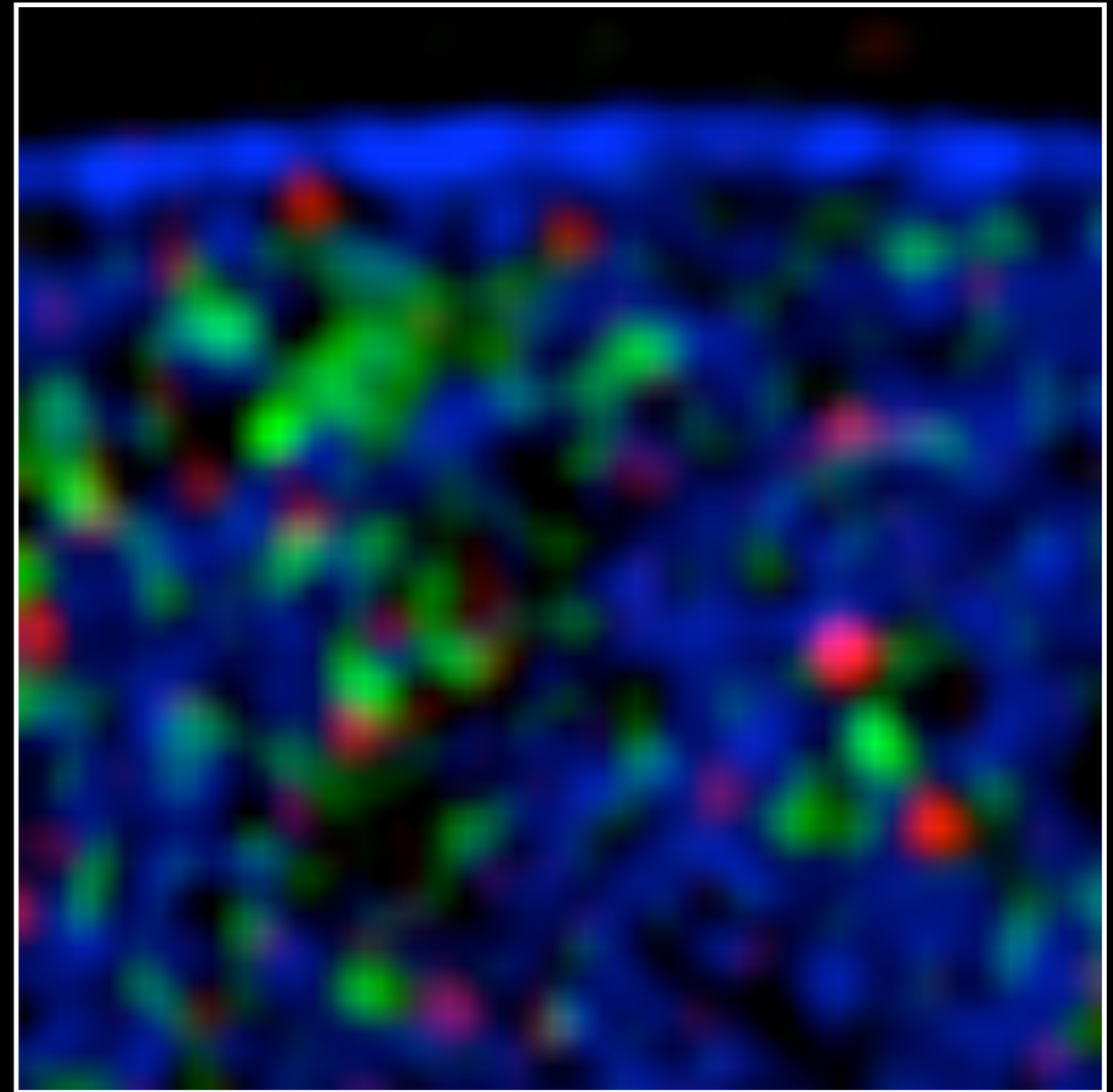
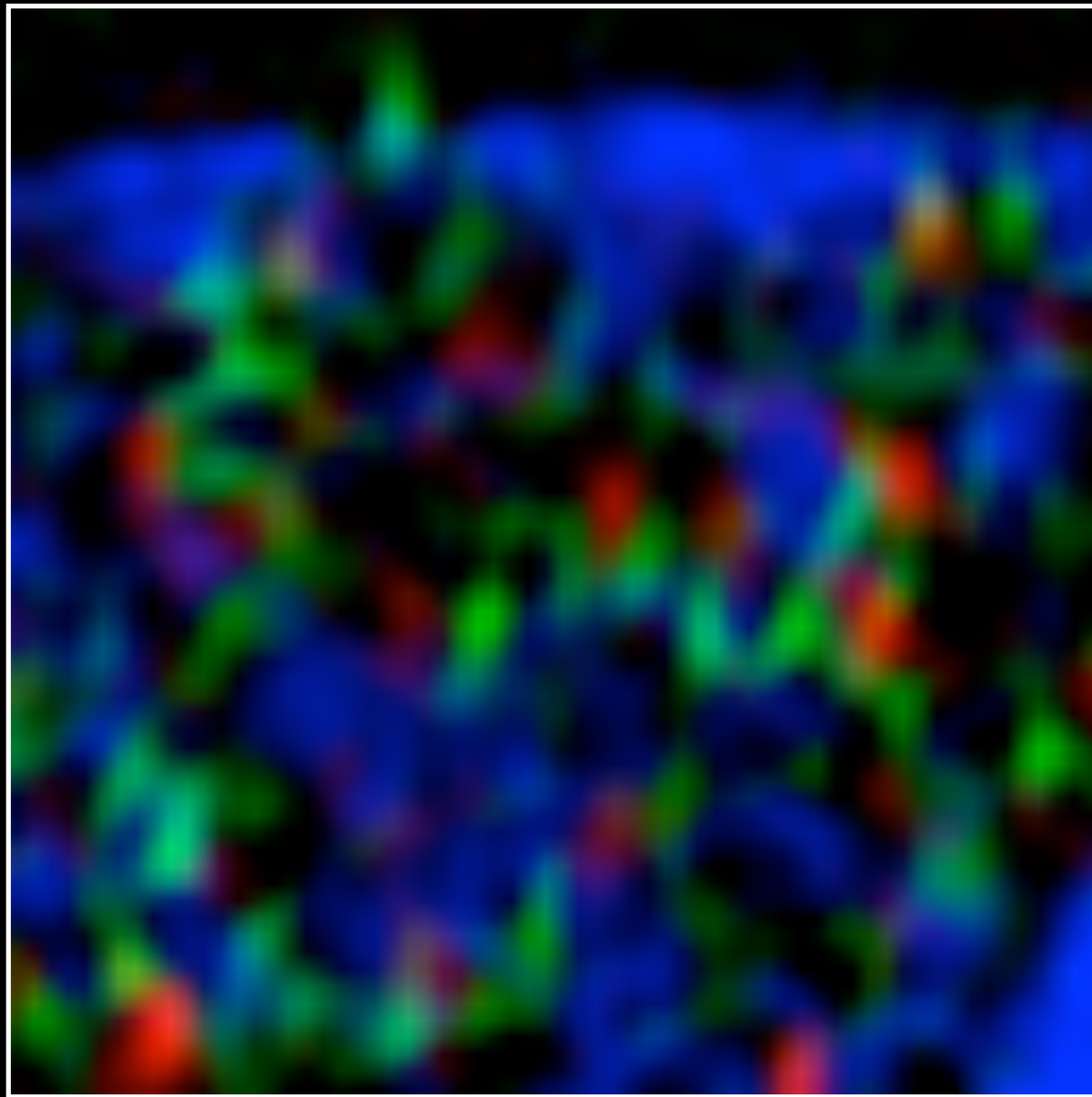
Bad SI reconstruction



Good SI reconstruction



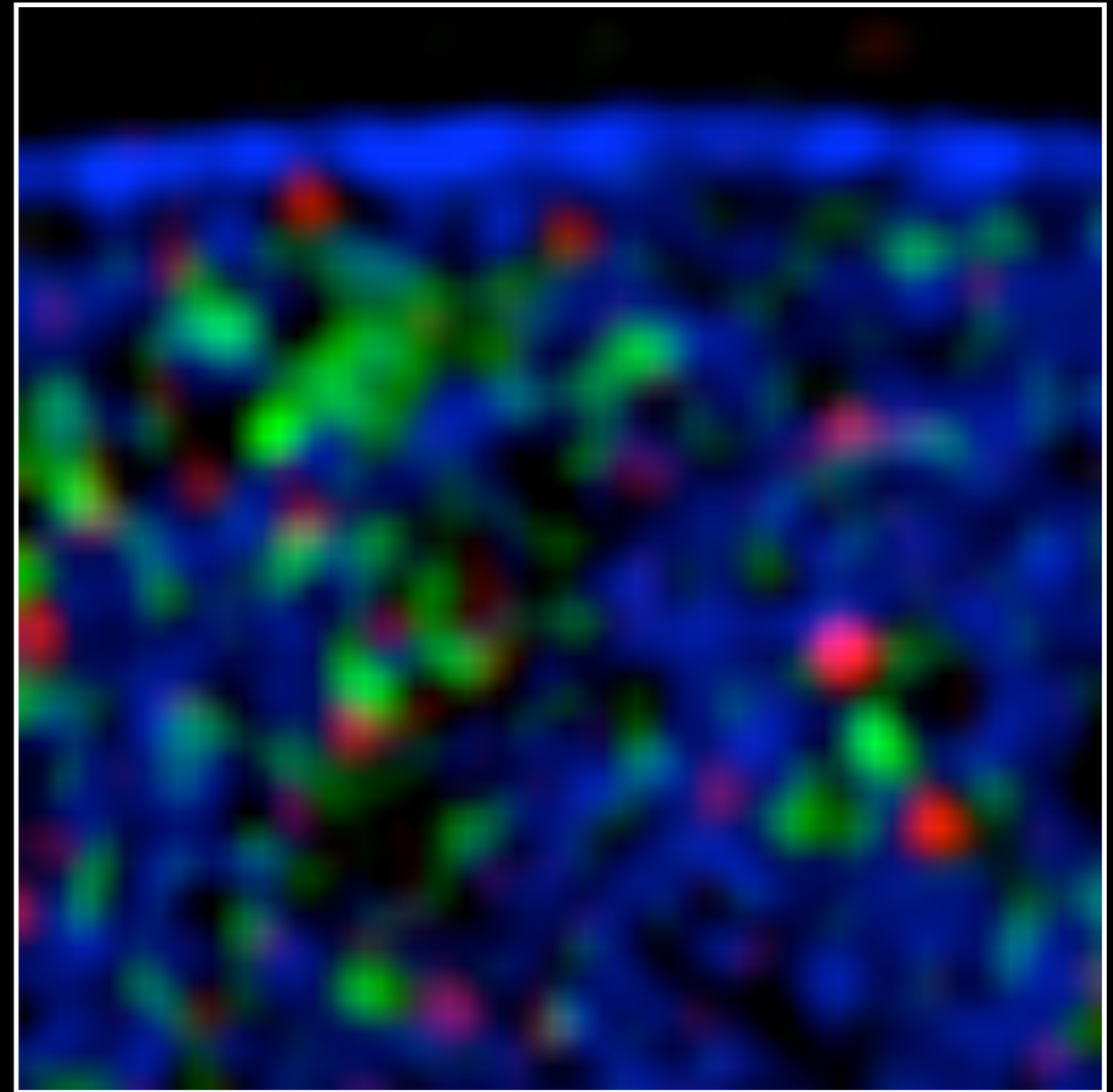
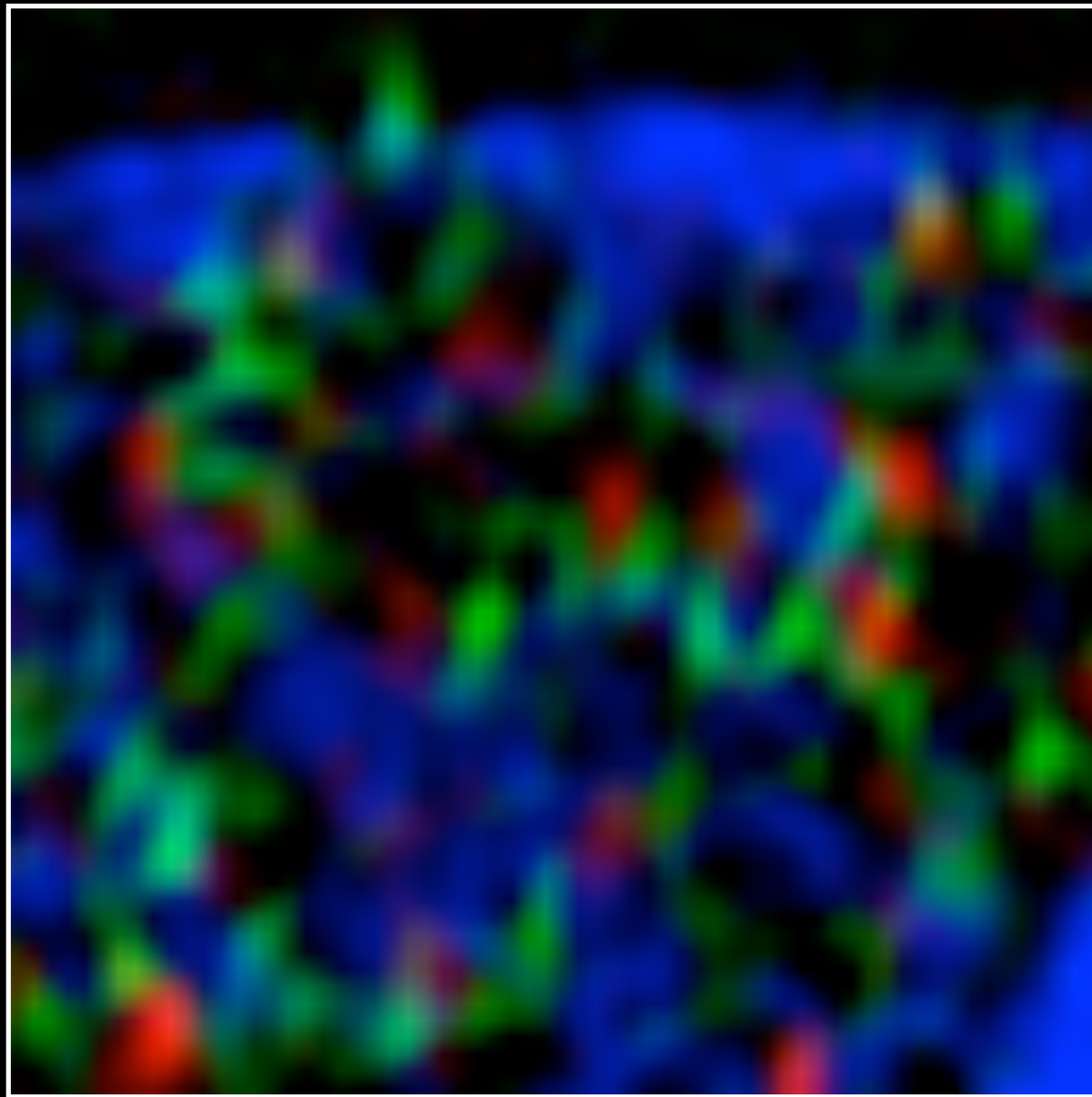
Quality control: SI-Reconstruction artifacts



H3K4me3
RNA Pol II
DAPI

Good SI reconstruction

Quality control: SI-Reconstruction artifacts



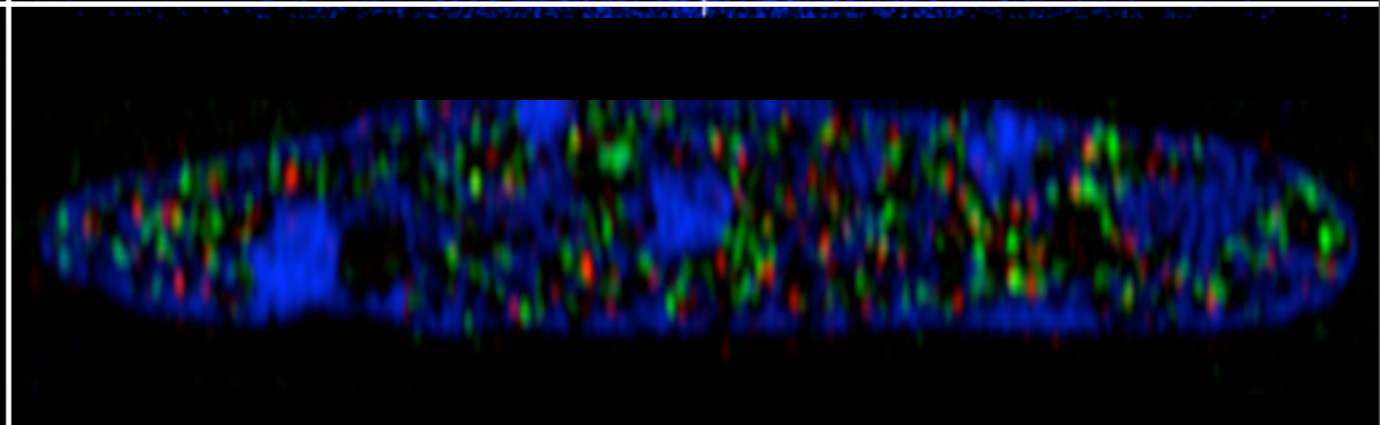
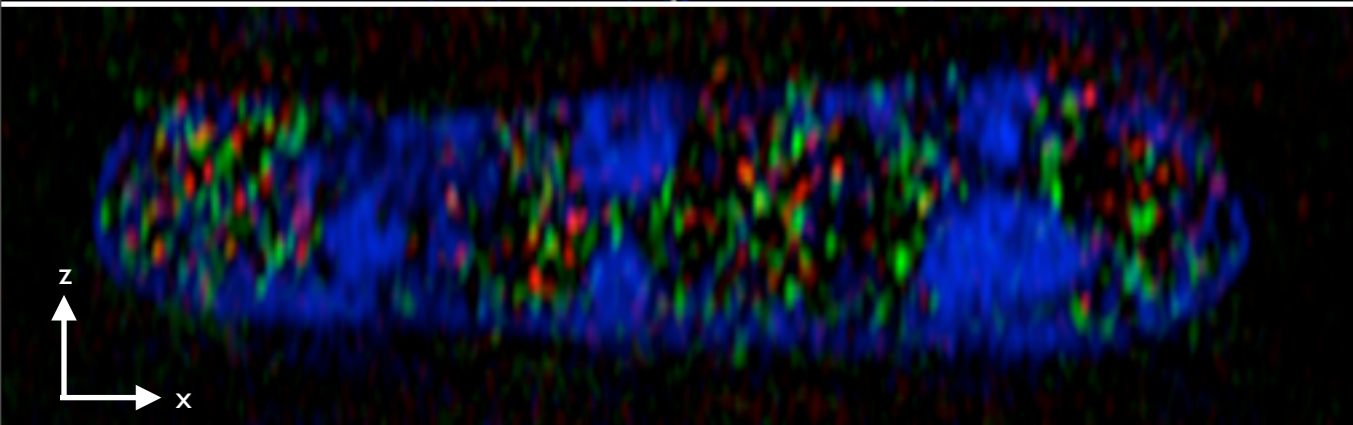
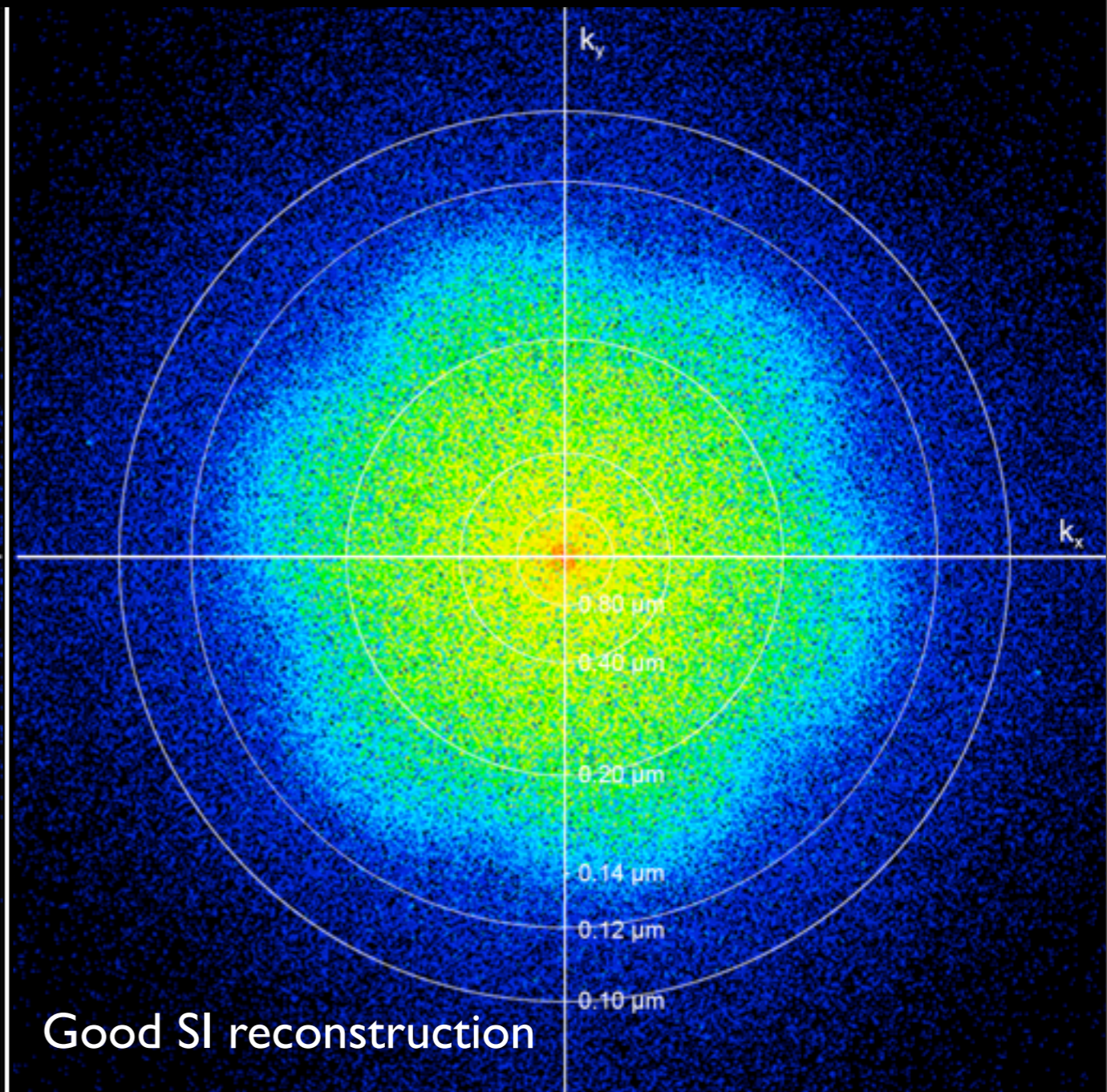
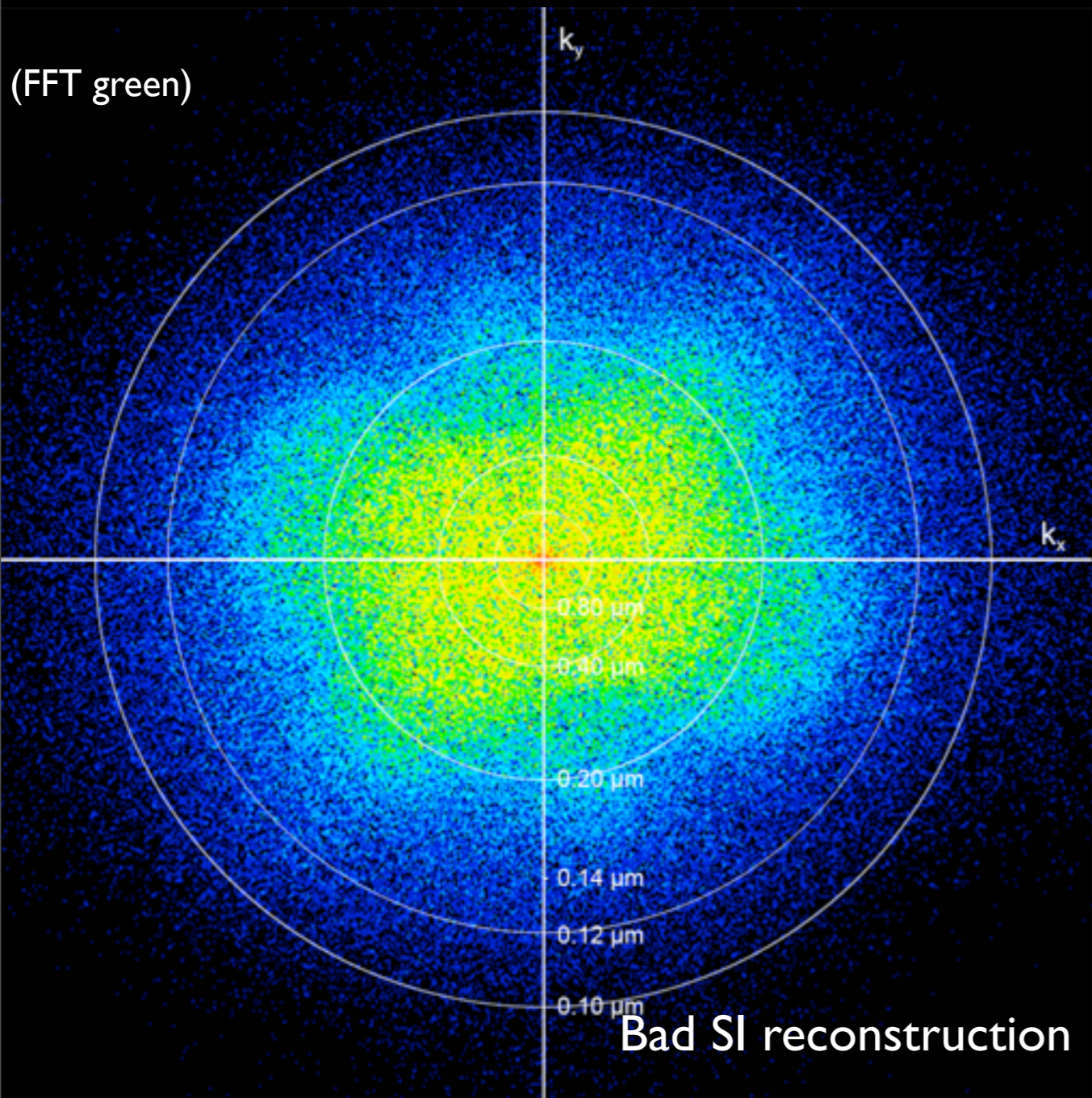
H3K4me3
RNA Pol II
DAPI

Bad SI reconstruction

Good SI reconstruction

Lothar Schermelleh

Quality control by Fourier analysis



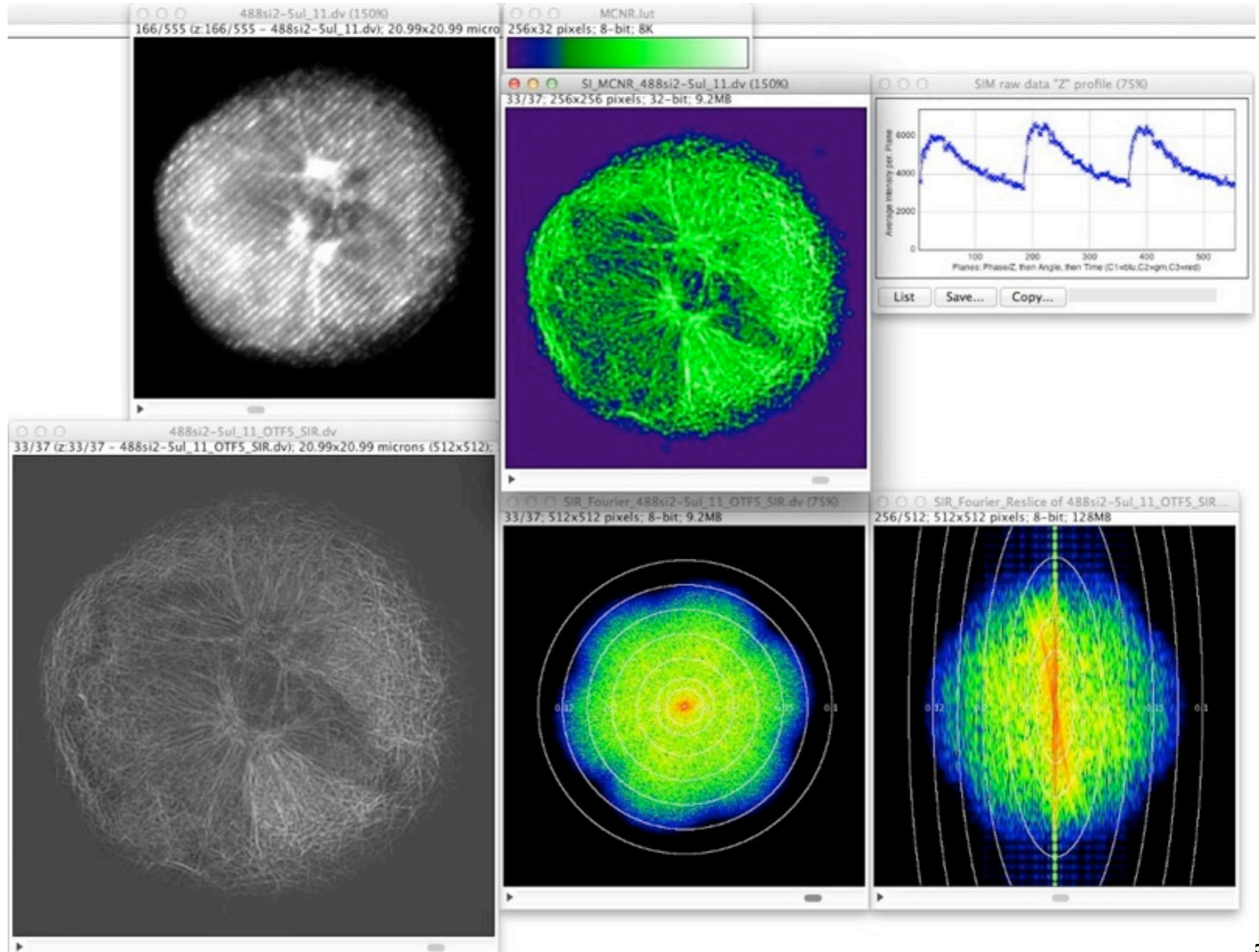
SIMCheck (ImageJ plug in)



Graeme Ball



**Lothar
Schermelleh**



Cockpit - from John Sedat

The screenshot displays the Cockpit software interface, which is a control panel for a microscope system. The interface is organized into several panels:

- Python shell:** A terminal window showing the Python 2.7.1 shell prompt and some system information.
- Objectives:** A panel with buttons for different objectives: 100xOil, 150xTIRF, 40x, 60xOil, and 50xWater.
- Drawers:** A panel with buttons for 'Dummy drawer 1' and 'Dummy drawer 2'.
- Camera view:** A large central area showing a camera feed, currently displaying a green bar with the text 'OFF (live)'. Below it is a 'Temperature sensor plot' showing a graph of temperature (°C) over time, with a red line at approximately 3.5°C and a green line at approximately -0.5°C.
- Macro Stage XY:** A panel showing a plot of the stage position (X and Y coordinates) and a histogram of the Z-axis position. The Z-axis has markers at 4000 and 25000. Below the plot are buttons for 'Set safeties', 'Switch control', and 'Recorder'.
- Logging panels:** A panel showing system logs and error messages, including a traceback for a 'NameError'.
- EMCCD controls:** A panel with buttons for 'EM Gain' (set to 0), 'Conv16 1MHz', 'Conv14 3MHz', 'EM16 1MHz', 'EM14 10MHz', 'EM14 5MHz', and 'EM14 3MHz'.
- Light path:** A panel with buttons for 'Widefield' (highlighted) and 'Structured illumination'.
- Mosaic view:** A panel showing a mosaic of three images labeled 1, 2, and 3. Image 3 is highlighted with a red box. The mosaic is overlaid with a red crosshair.

The interface also includes a sidebar with icons for 'Computer', 'Network', 'Recycle Bin', 'Home', 'Cyrus Terminal', 'FileZilla Client', and 'Firefox'. At the bottom, there is a taskbar with icons for 'Cockpit program (currently logged in as Jan)', 'Start', 'Run', 'Stop', and 'Exit'. The system tray shows the time as 17:31 on 26/02/2013.

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)**



**Douglas
Russell**

OMERO - integrating image analysis

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

Simcheck

Denoising

New Deconvolution algorithms

DOUGLAS

Micron
OXFORD

wellcometrust

<http://www.micronoxford.com/>

Nanoscopy Oxford



**Super-resolution microscopy
applied to biomedical research**

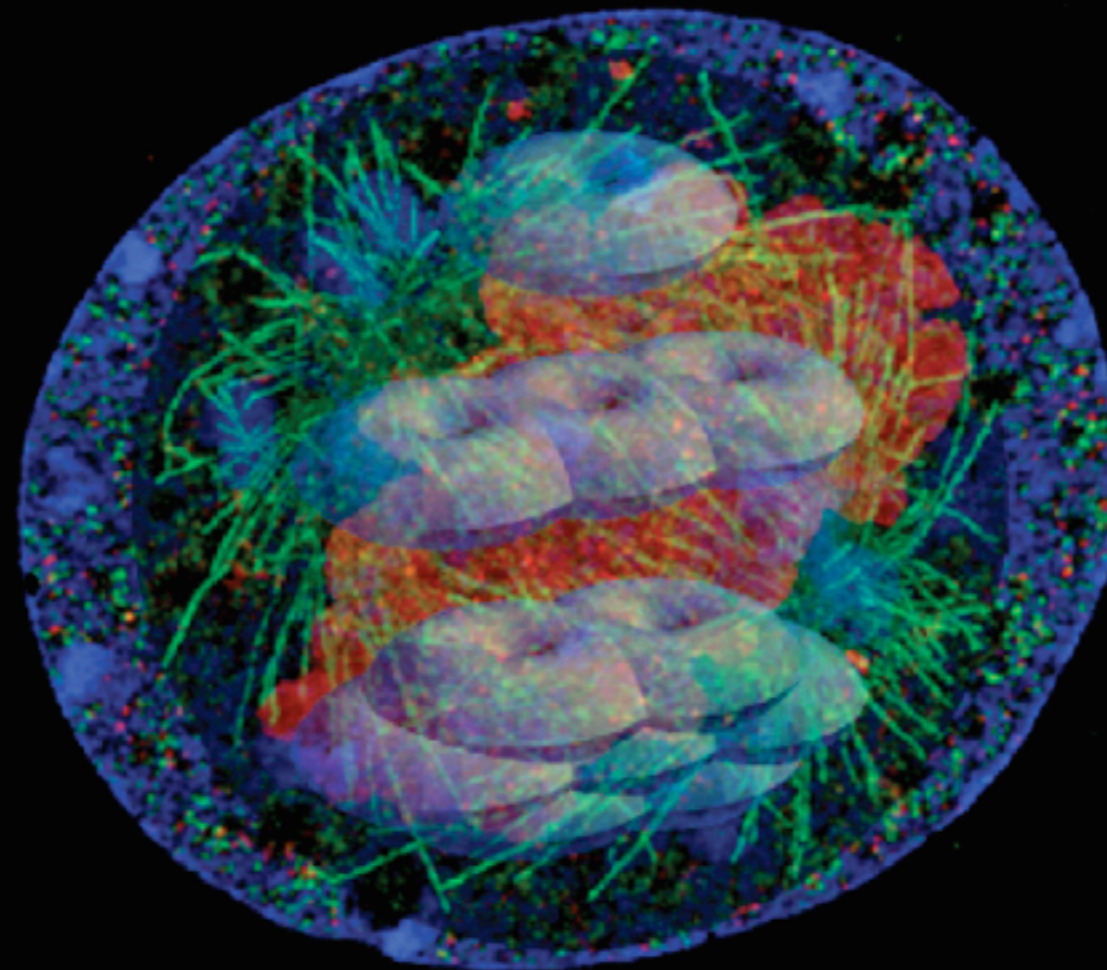
Now recruiting six postdoctoral positions

**Image analysis
specialist**

**Adaptive
optics
developer**

**Super-
resolution
software
developer**

Saturated structured illumination developer



**Correlative EM, X-ray
and light
microscopy
specialist**

**Bespoke
neuro-
imaging
developer**

Acknowledgements



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Tim Weil
Russell Hamilton
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Lu Yang
Kirsty Young
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James Halsted
Anna Hakes
Clare Bromley

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Carine Meignin
Veronique Van De Bor
Renald Delanoue
Nina McDougall
Alejandra Clark

Micron Oxford
Lothar Schermelleh
Ian Dobbie
Eva Wegel
Graeme Ball
Rainer Kaufmann

Collaborators
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John Sedat
David Agard
Hugo Bellen
David Finnegan

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Funding:

welcometrust

MRC, BBSRC, John Fell Fund

