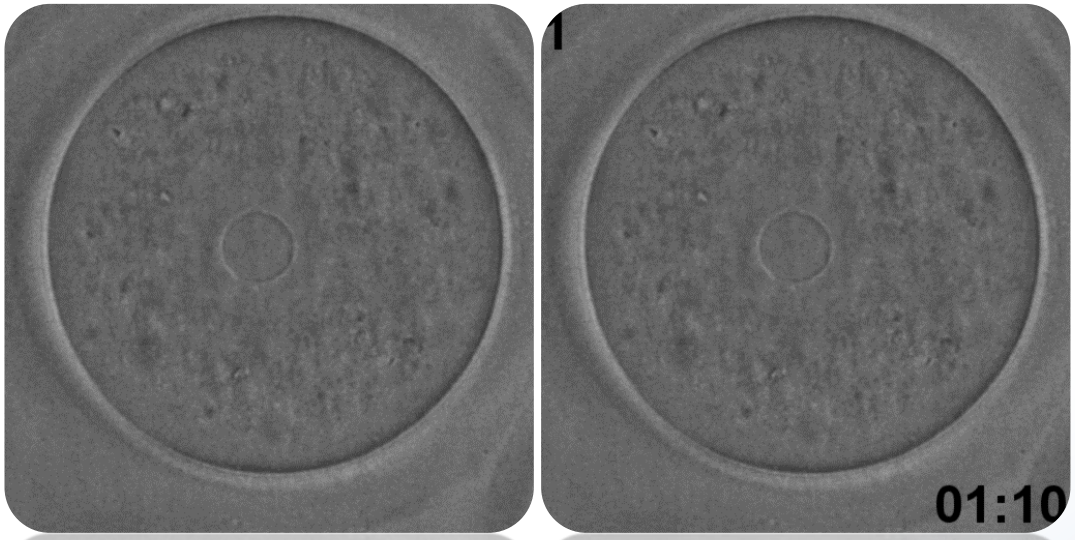
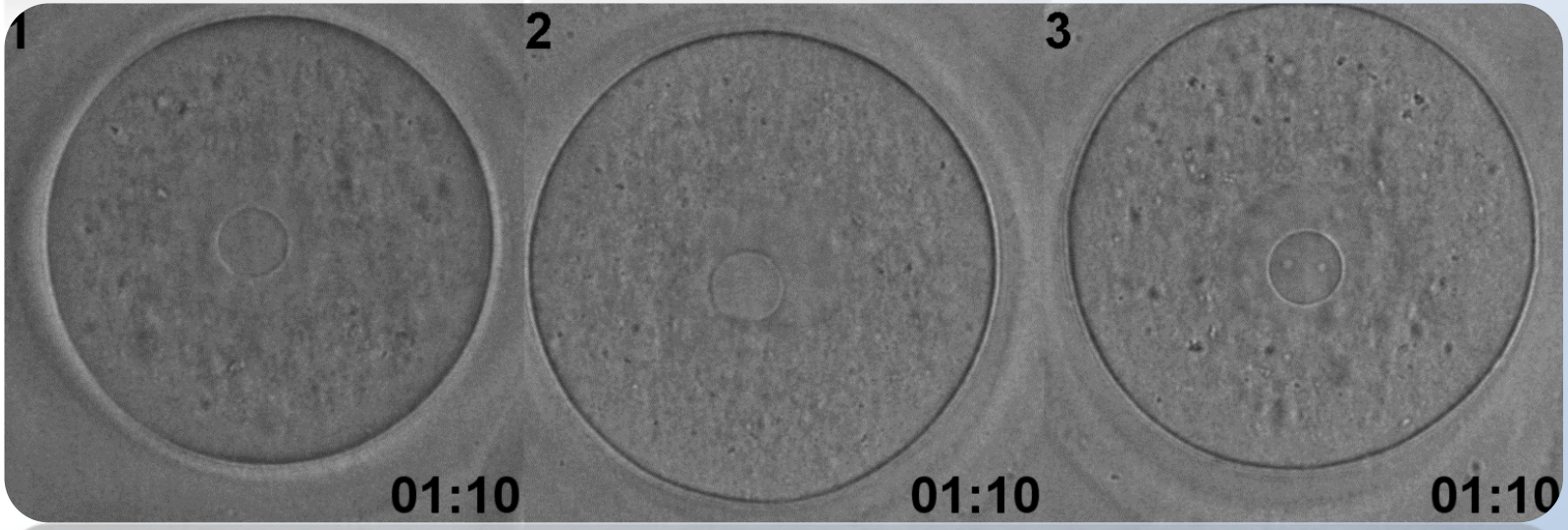
LIGHT MICROSCOPY TECHNIQUES FOR LIVE CELL IMAGING

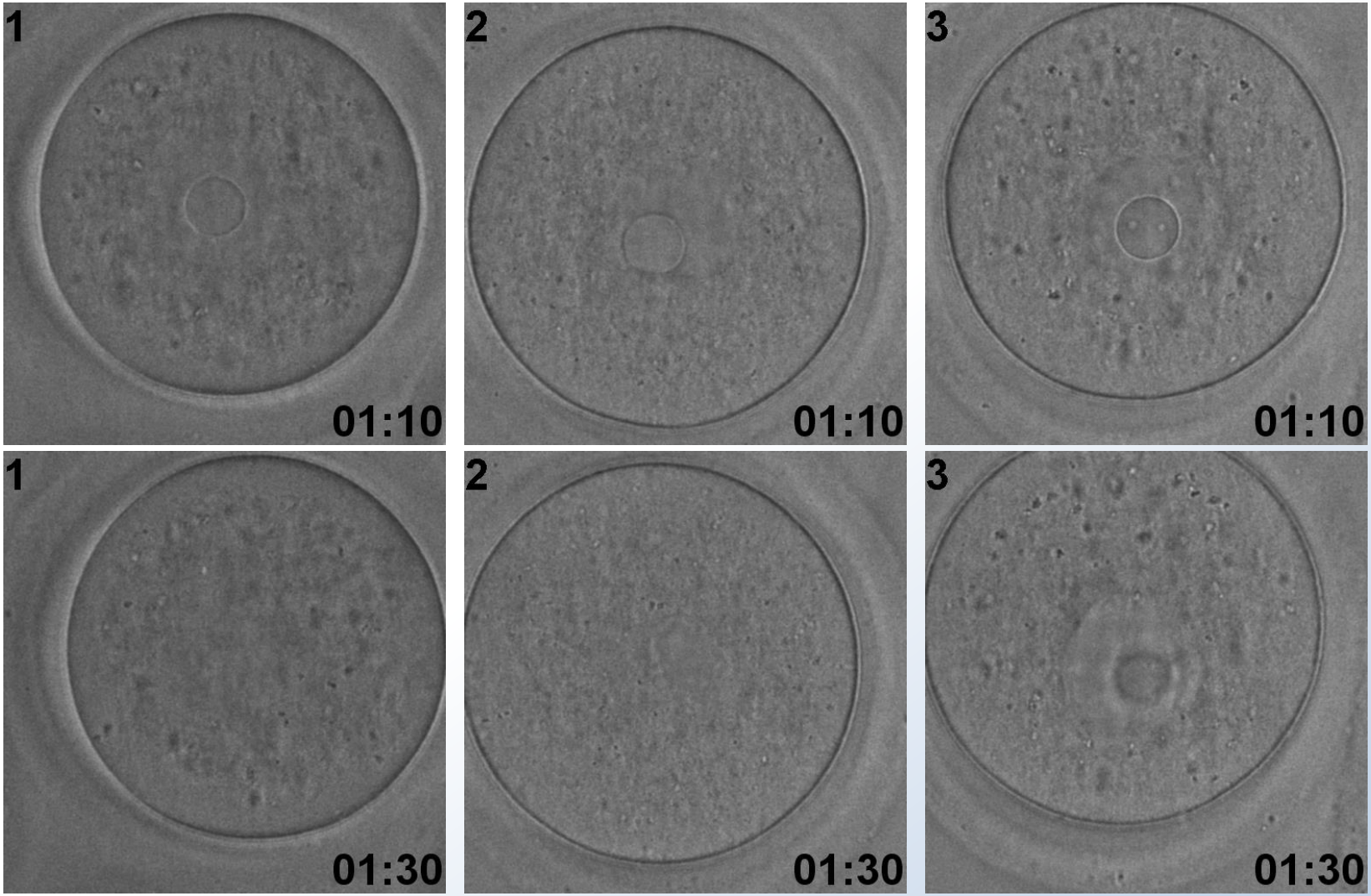


Mouse oocyte – Bright-field imaging

Time hh:mm

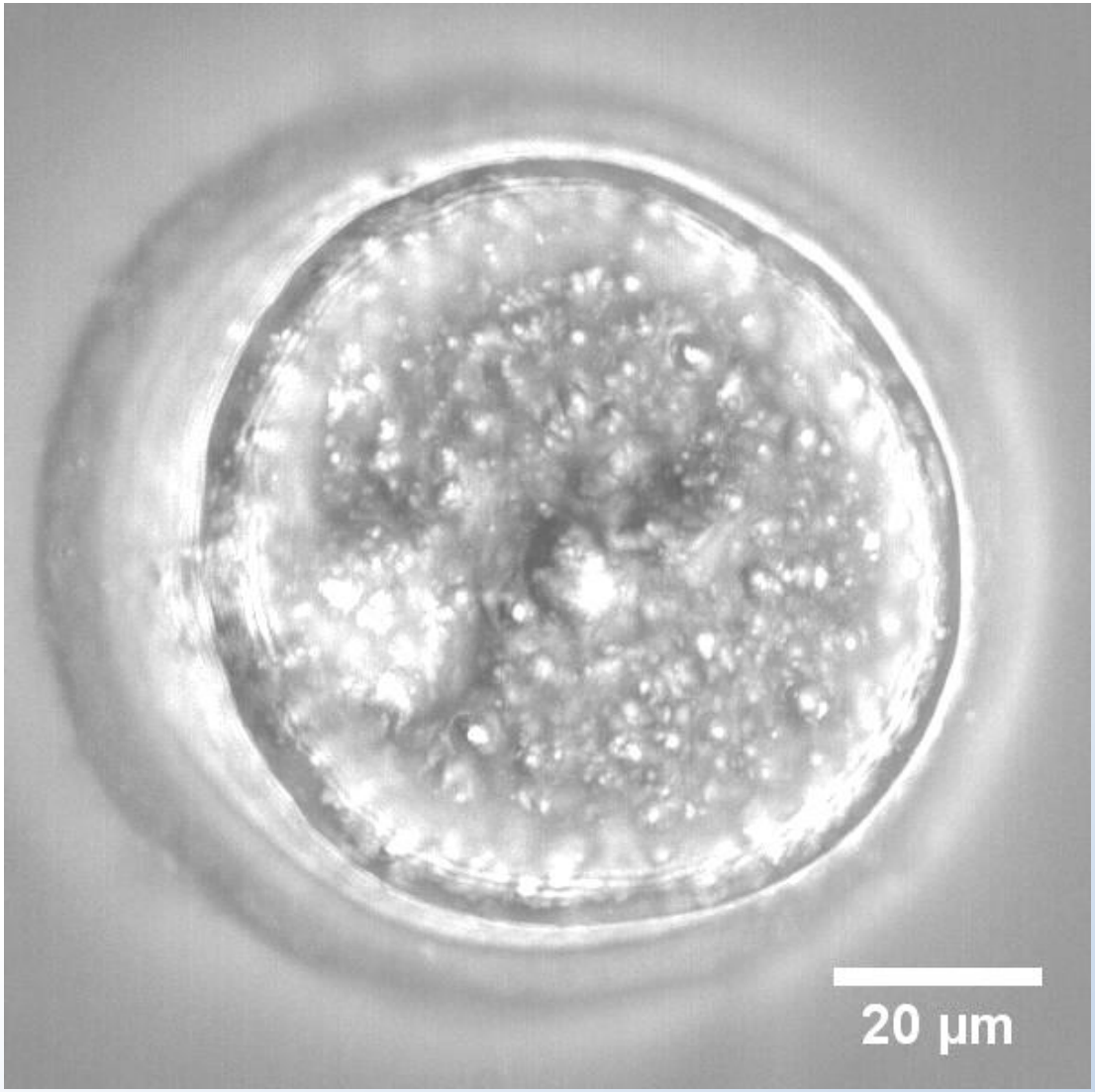


LIGHT MICROSCOPY TECHNIQUES FOR LIVE CELL IMAGING



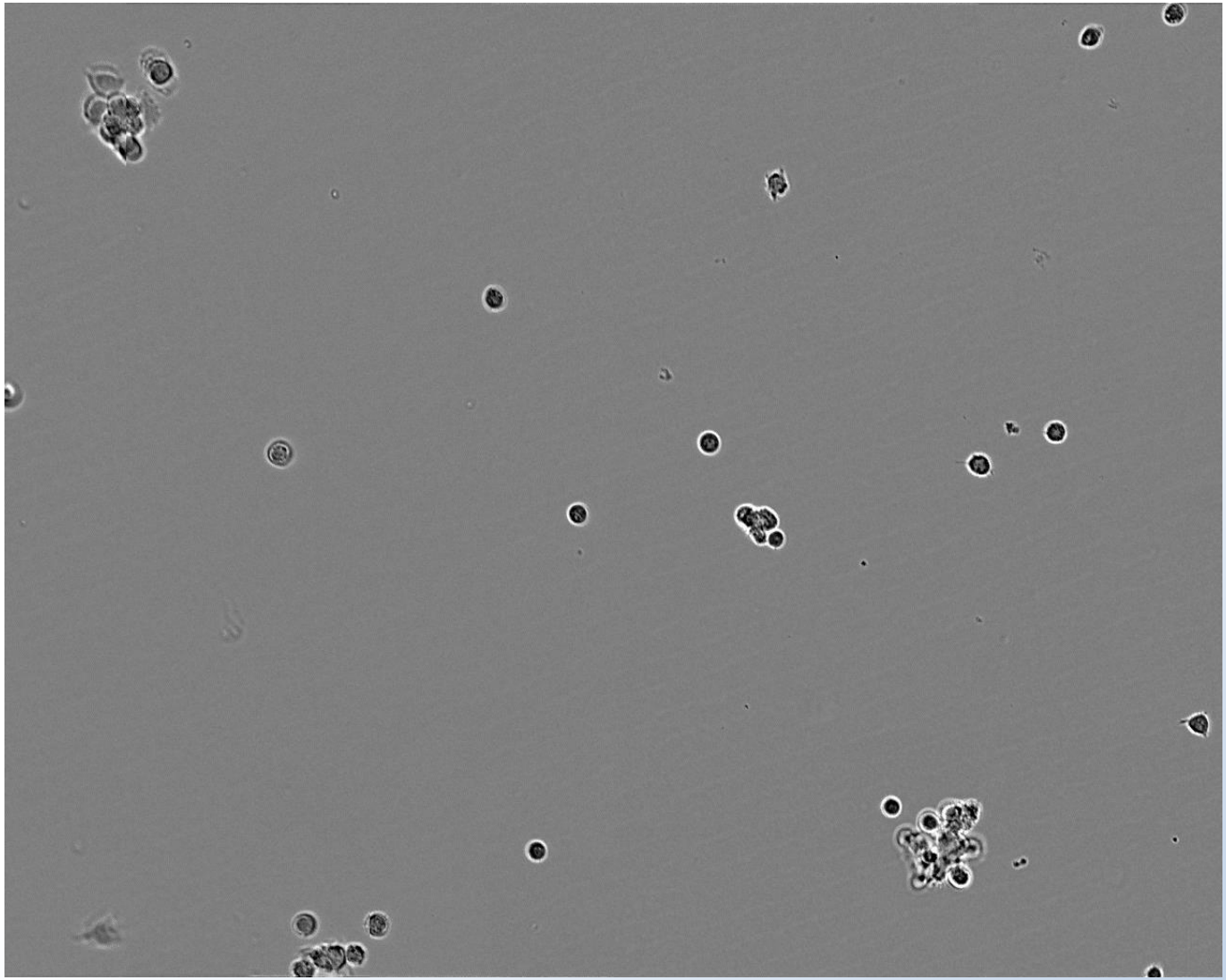
Timing of meiotic resumption

LIGHT MICROSCOPY TECHNIQUES FOR LIVE CELL IMAGING



Early mouse embryos - Bright-field imaging

LIGHT MICROSCOPY TECHNIQUES FOR LIVE CELL IMAGING

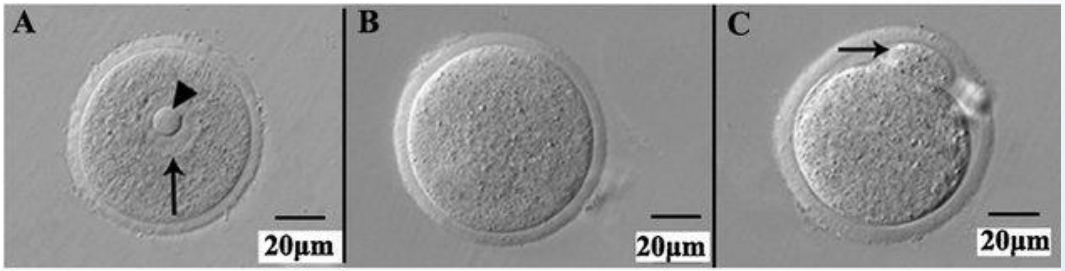


Mouse fibroblasts- Bright-field imaging

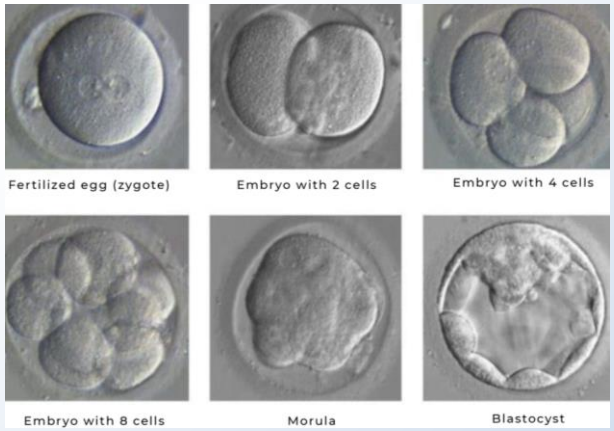
LIGHT MICROSCOPY TECHNIQUES FOR LIVE CELL IMAGING

Advantages

- **study of cellular events, timing**
  - germinal vesicle (**A**), meiotic resumption (**B**), extruding the first polar body (**C**)



  - embryo developmental stages

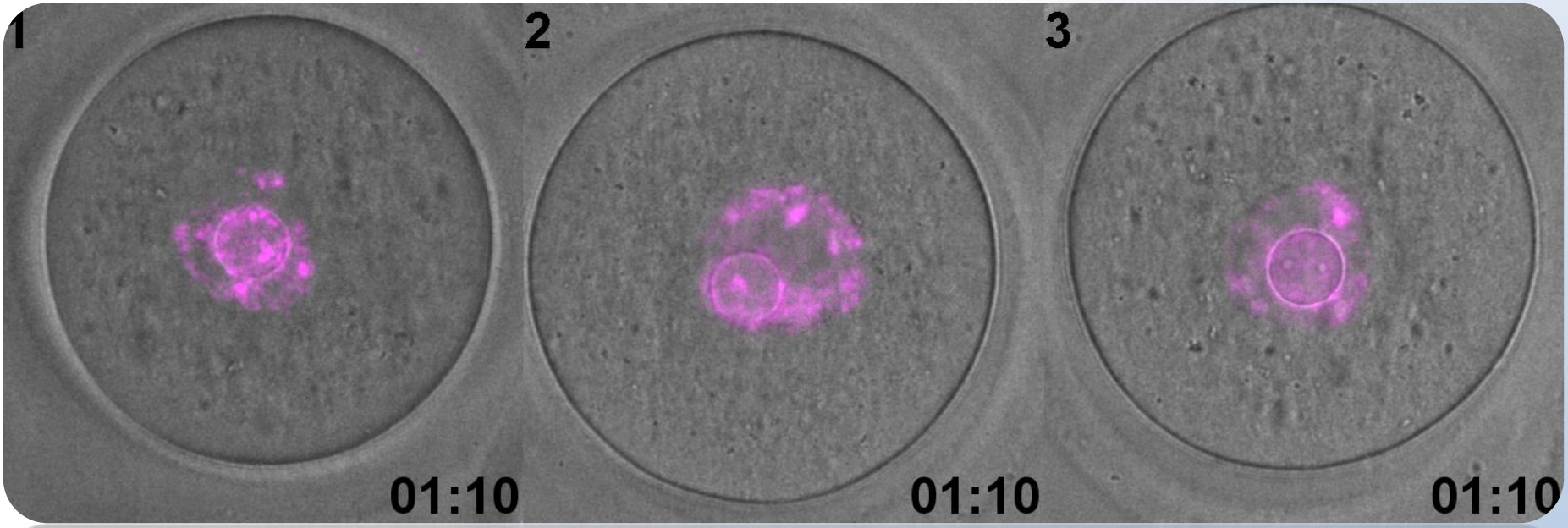


  - involvement of **signaling pathways**, proteins,… in these processes

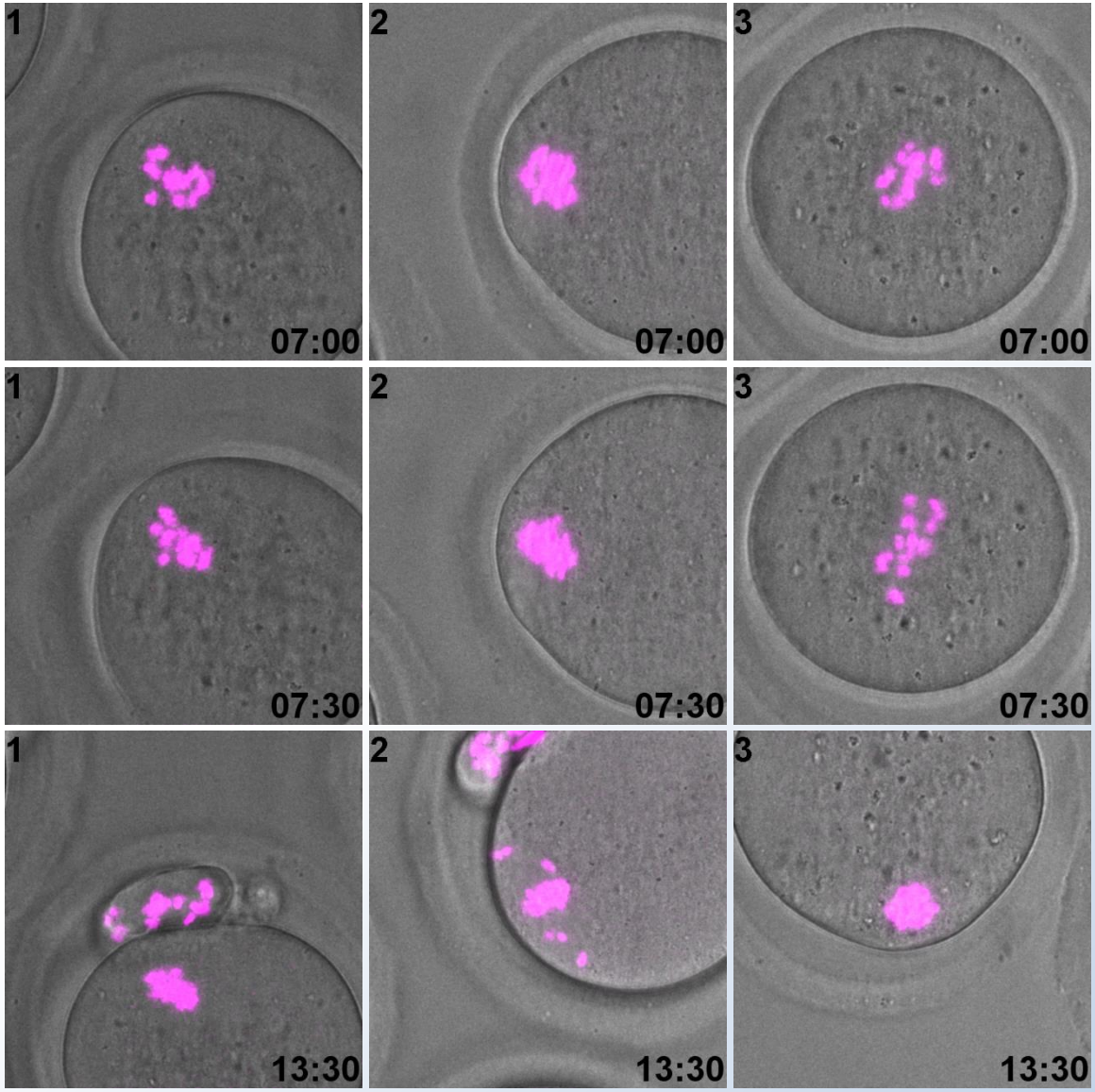
LIGHT MICROSCOPY TECHNIQUES FOR LIVE CELL IMAGING

Limitations

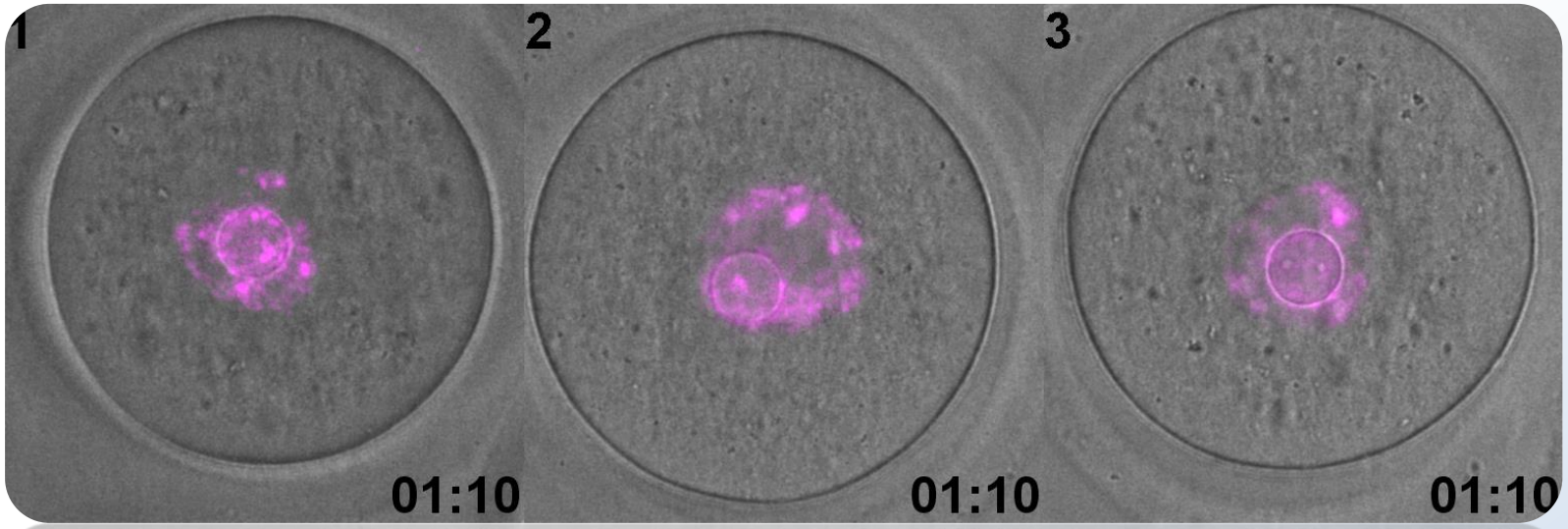
- optical resolution, low contrast
- culture conditions, pH, CO_2 level, O_2 level, oocyte and early embryo handling
- **subcellular events – fluorescence live-cell imaging**



FLUORESCENCE MICROSCOPY TECHNIQUES FOR LIVE CELL IMAGING



FLUORESCENCE MICROSCOPY TECHNIQUES FOR LIVE CELL IMAGING



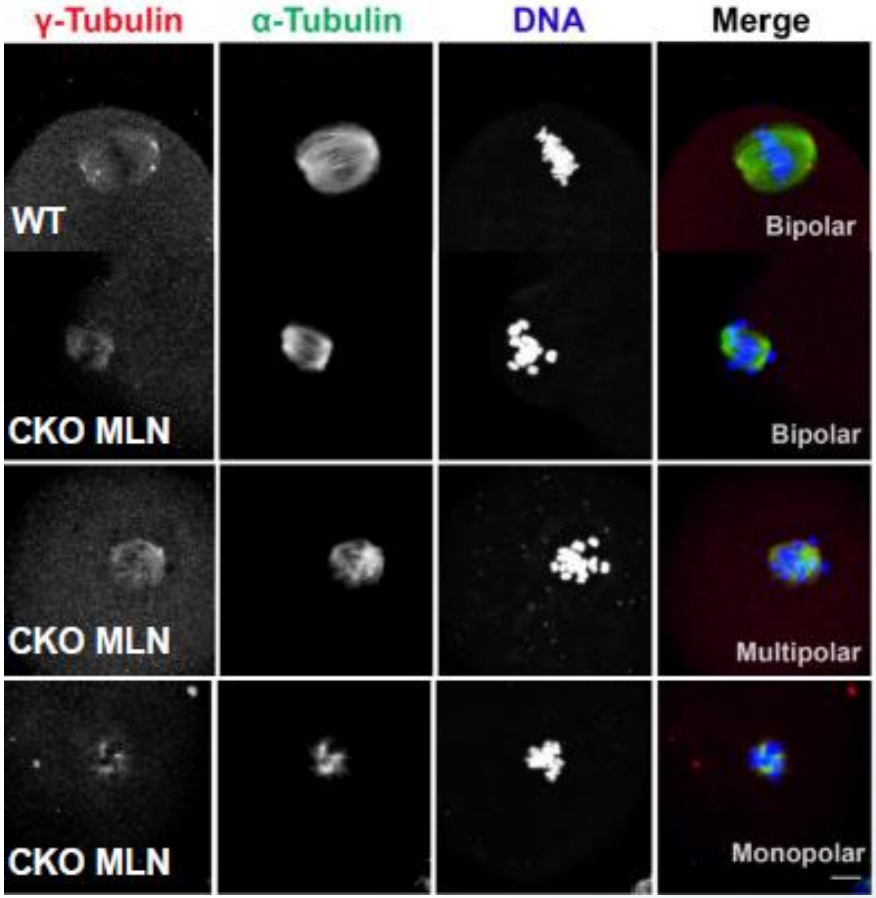
Advantages

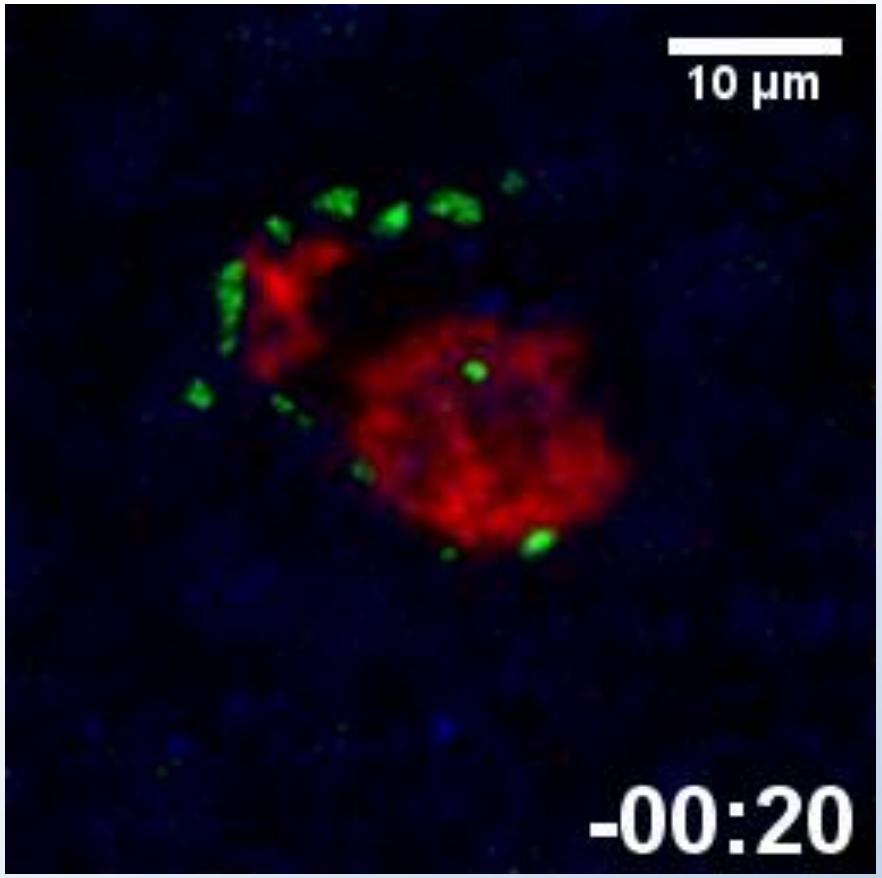
- study of **subcellular events**
- **image analysis** – qualitative and quantitative information

Limitations

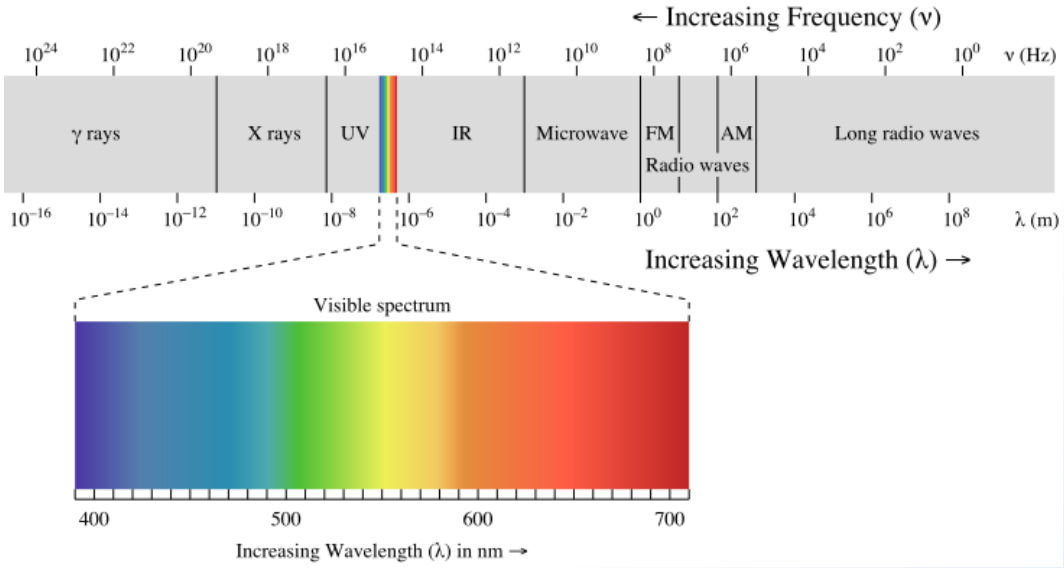
- culture conditions, pH, CO_2 level, O_2 level, oocyte and early embryo handling
- **phototoxicity,** photobleaching,...

IMMUNOFLUORESCENCE VS FLUORESCENCE LIVE CELL IMAGING

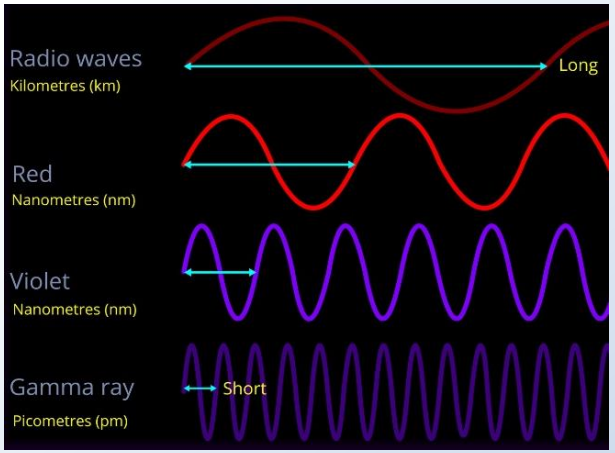




FLUORESCENCE MICROSCOPY



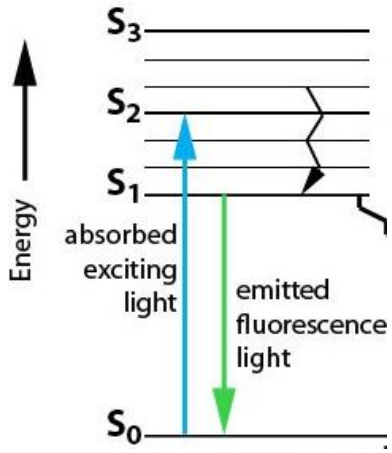
Philip Ronan, https://en.wikipedia.org/wiki/Visible_light_communication



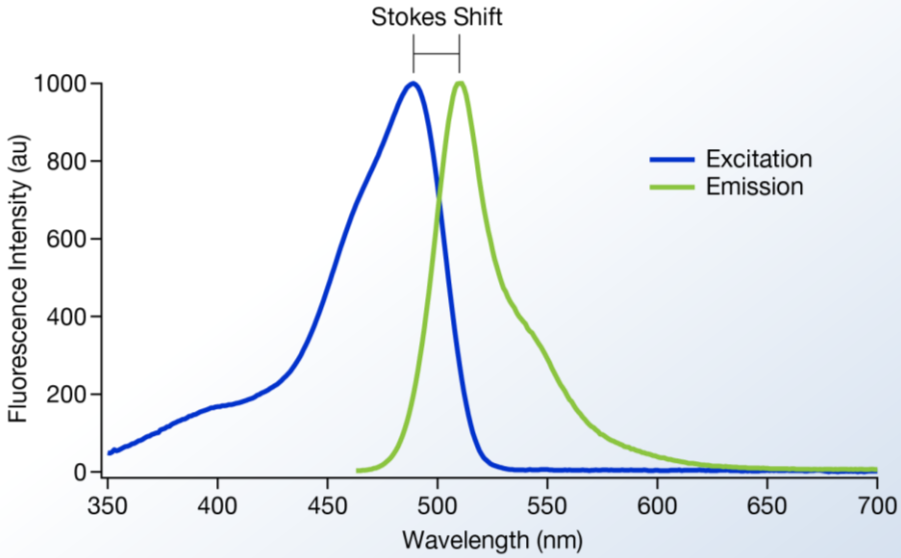
https://lightcolourvision.org/resource-library/comparing-wavelengths-radio-gamma/

- As you move **from violet to red**, the wavelength increases and **energy decreases**

FLUORESCENCE MICROSCOPY



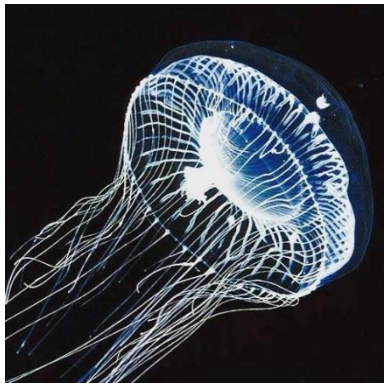
https://gauravtiwari.org/jablonski-diagram/



https://www.scientifica.uk.com/learning-zone/widefield-fluorescence-microscopy

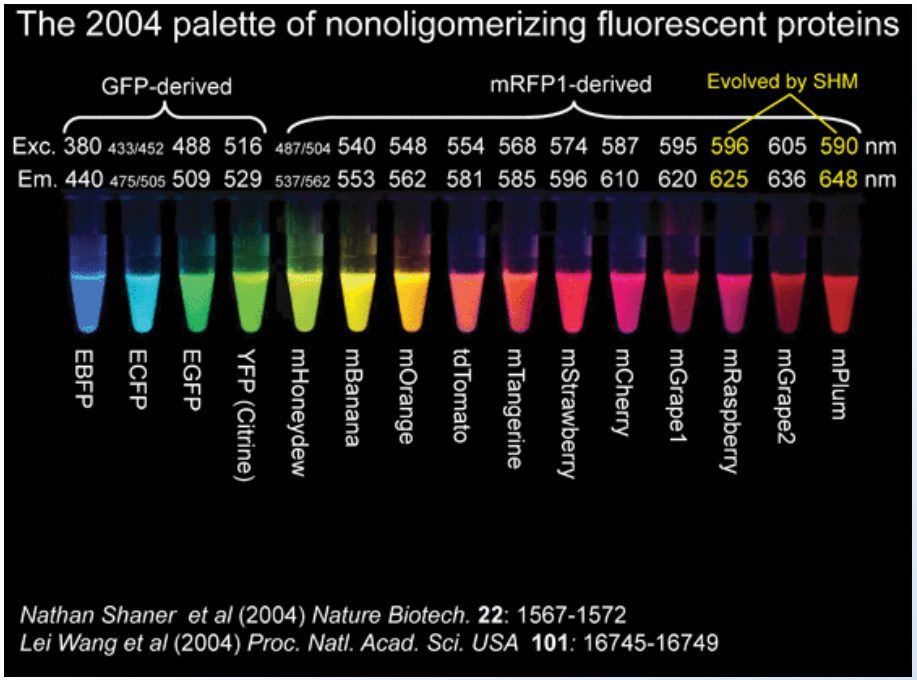
- The range of wavelengths that a fluorophore can absorb and emit are known as **excitation and emission spectra**

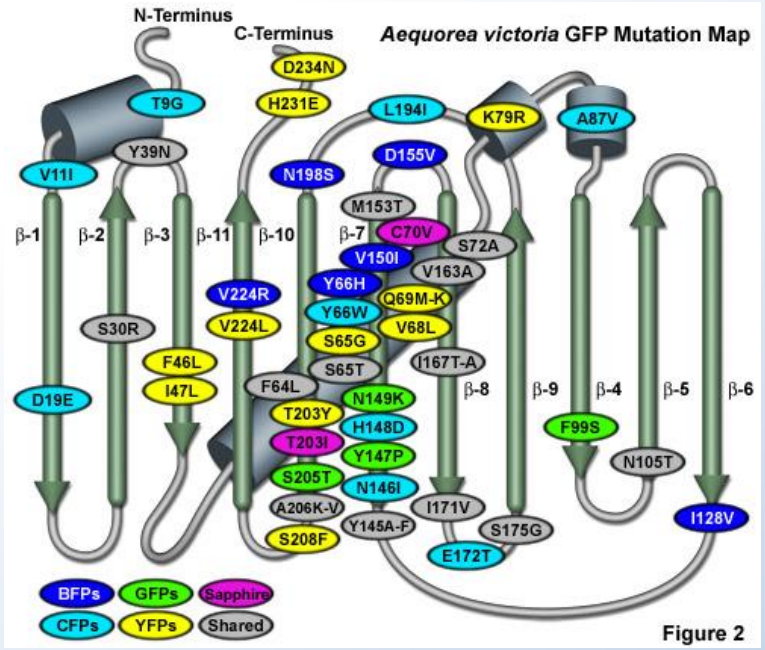
FLUORESCENCE PROTEINS



Aequorea victoria

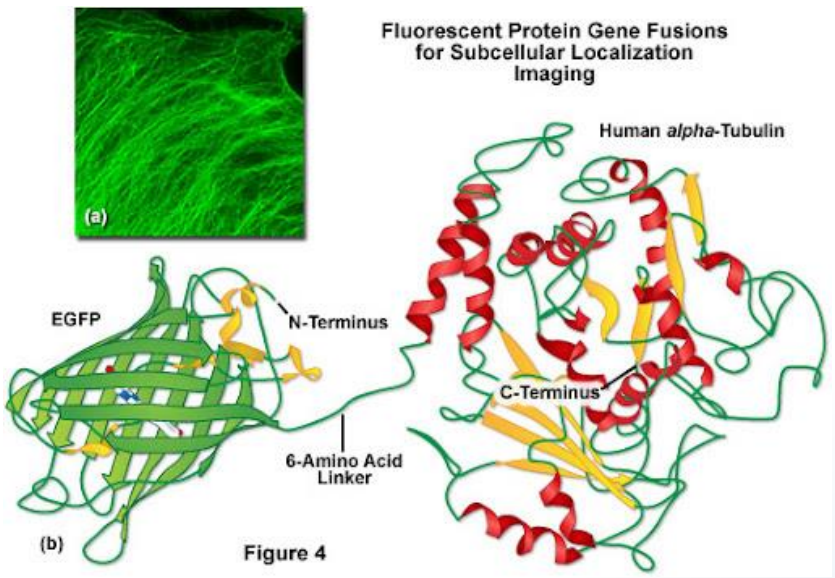
Discosoma sp.





http://zeiss-campus.magnet.fsu.edu/print/probes/fpintroduction-print.html

FLUORESCENCE PROTEINS

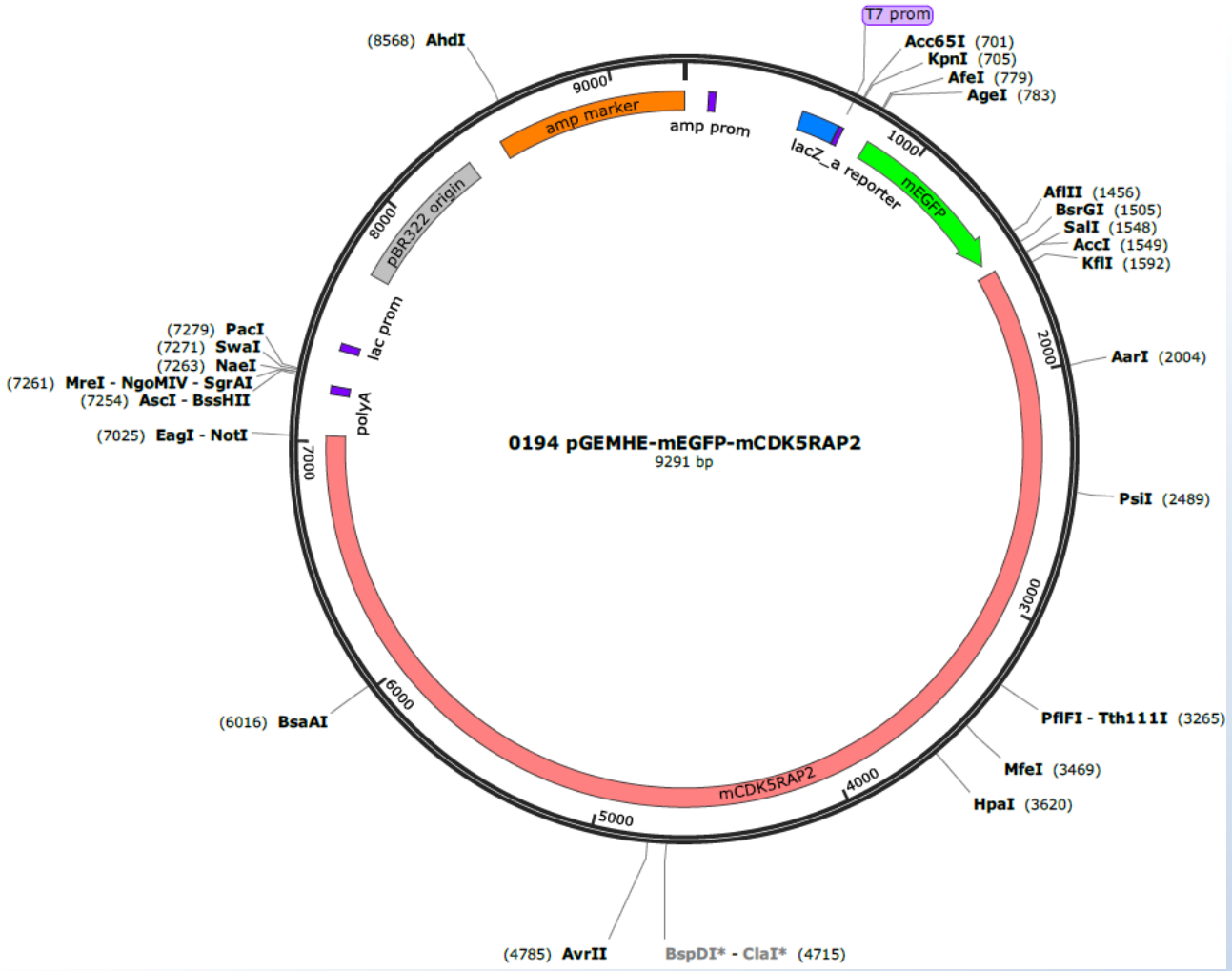


http://zeiss-campus.magnet.fsu.edu/print/probes/fpintroduction-print.html

Recombinant protein expression:

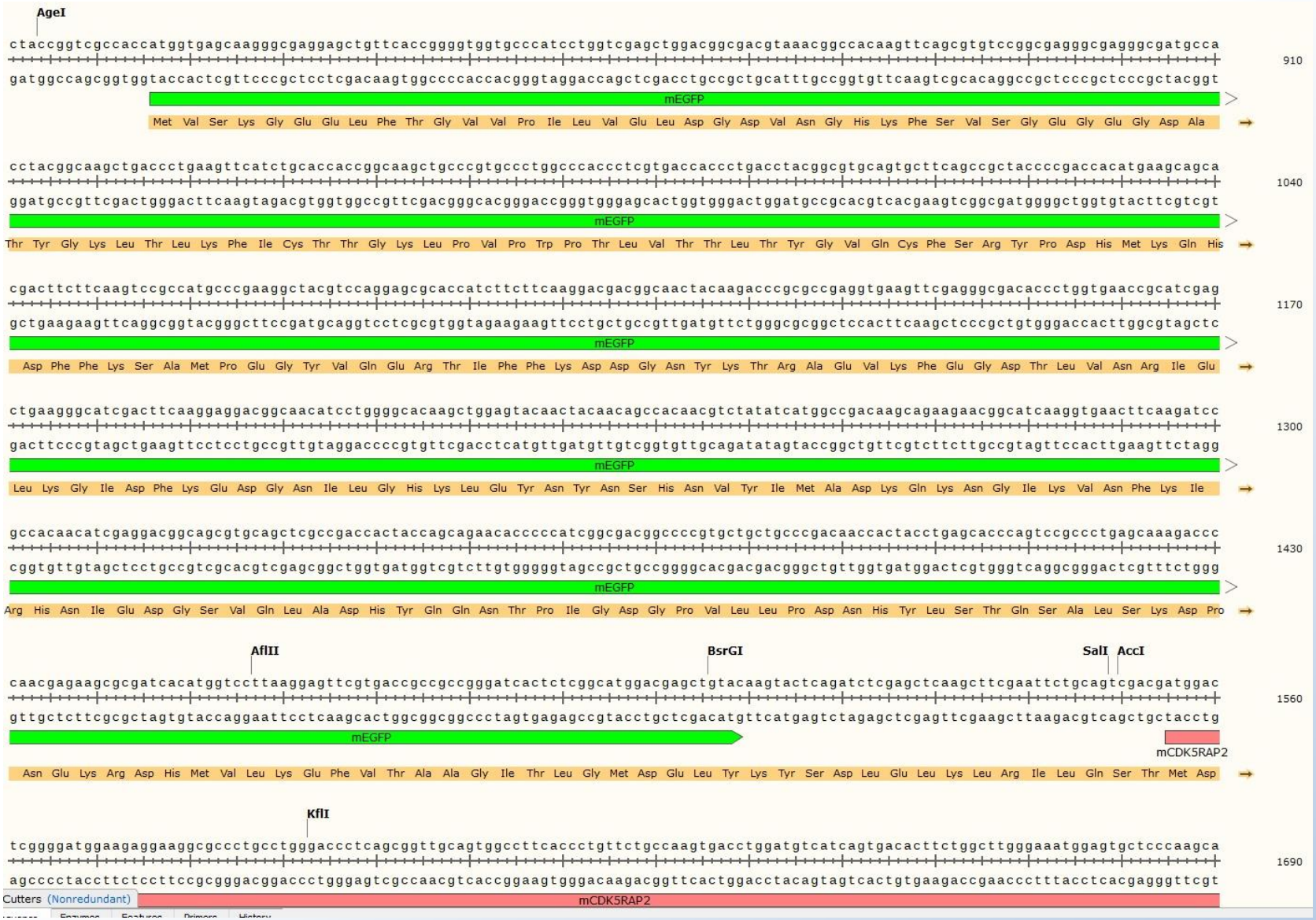
- transfection
- electroporation (DNA, mRNA, protein)
- microinjection (DNA, mRNA, protein)
- lentiviruses
- CRISPR/Cas9
- Transgenic models - CAG::H2B-EGFP mice

FLUORESCENCE PROTEINS

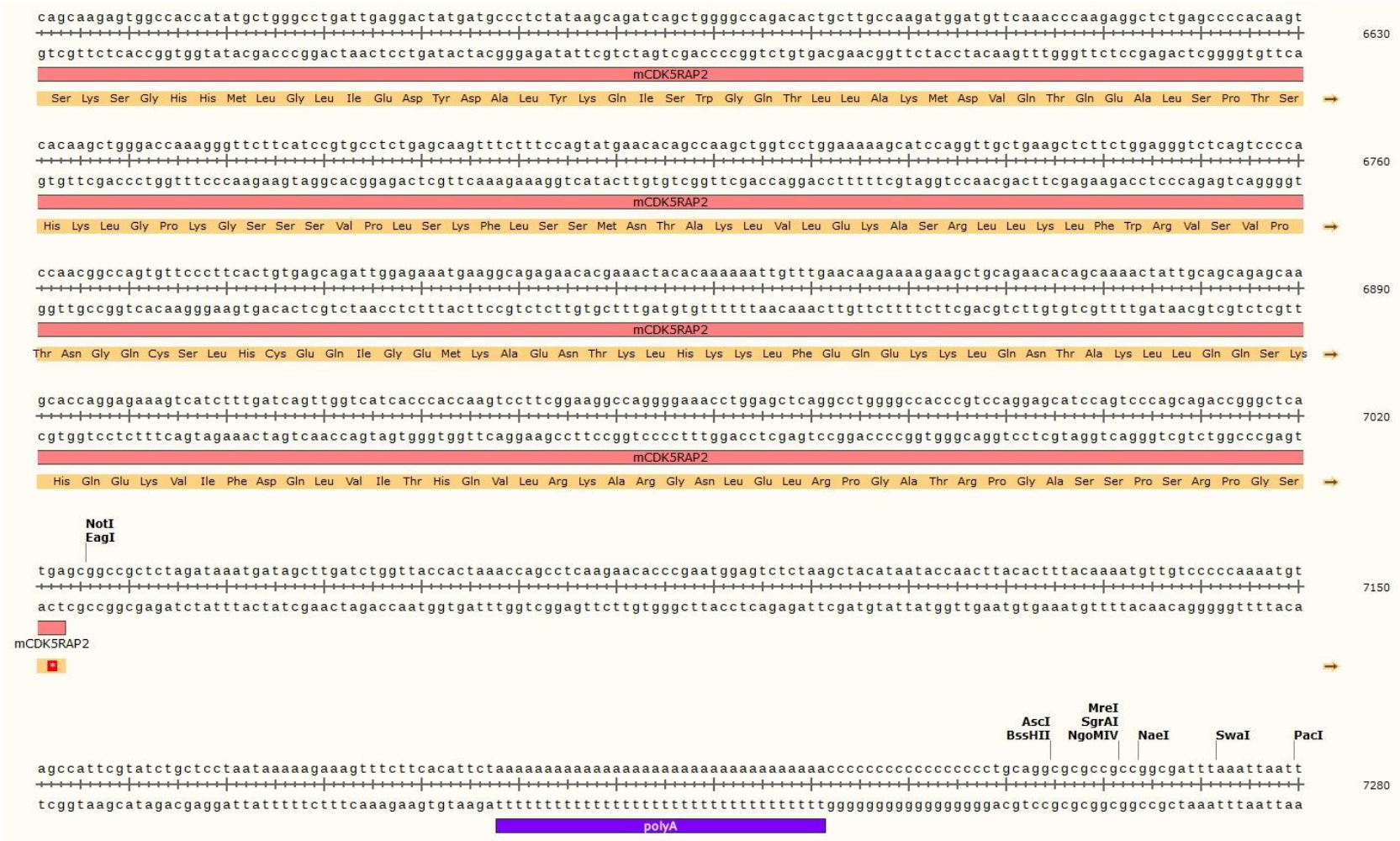


- **design of DNA plasmids, and possible modifications** (fluorescence markers, targeting, mutations, polyA tailing…)

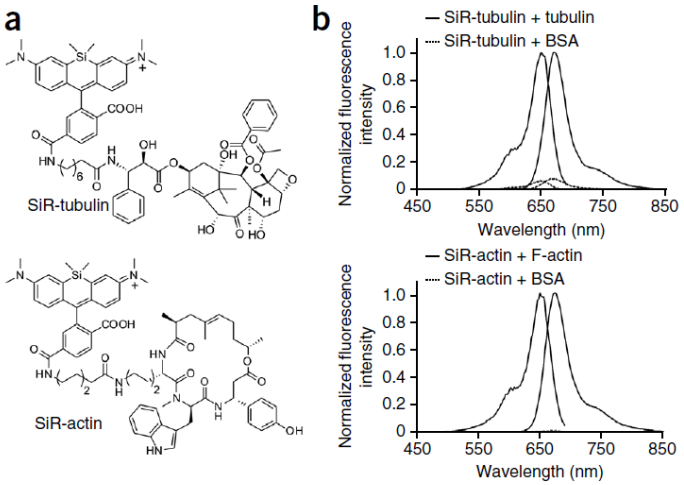
FLUORESCENCE PROTEIN PLAZMIDS

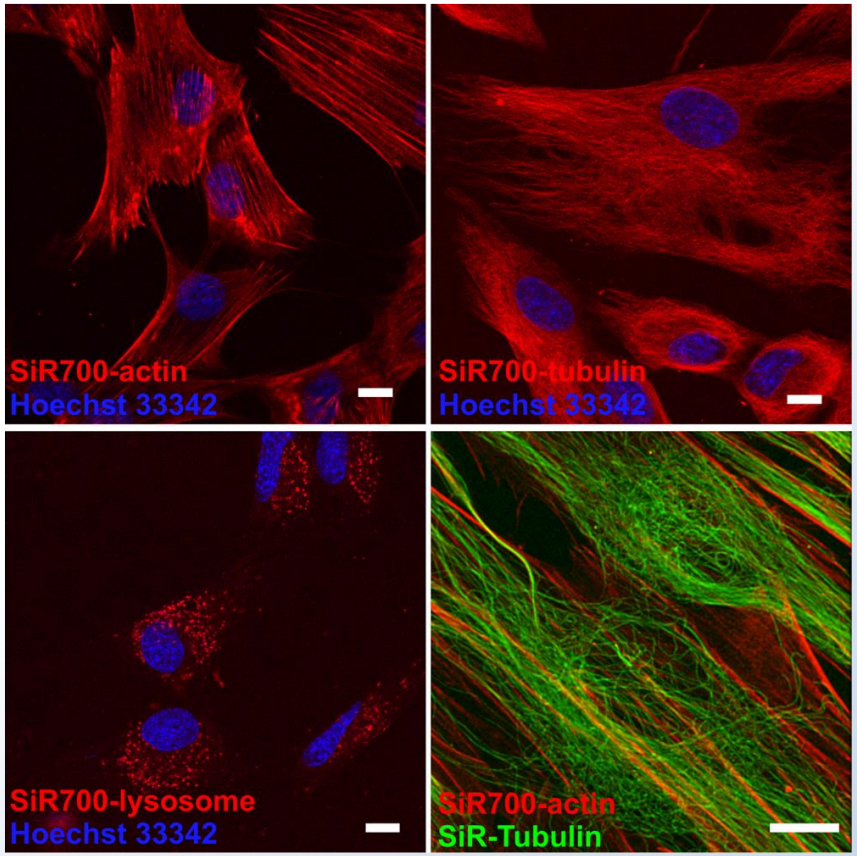


FLUORESCENCE PROTEIN PLAZMIDS



VITAL FLUORESCENCE PROBES – SILICON RHODAMINE (SIR) PROBES

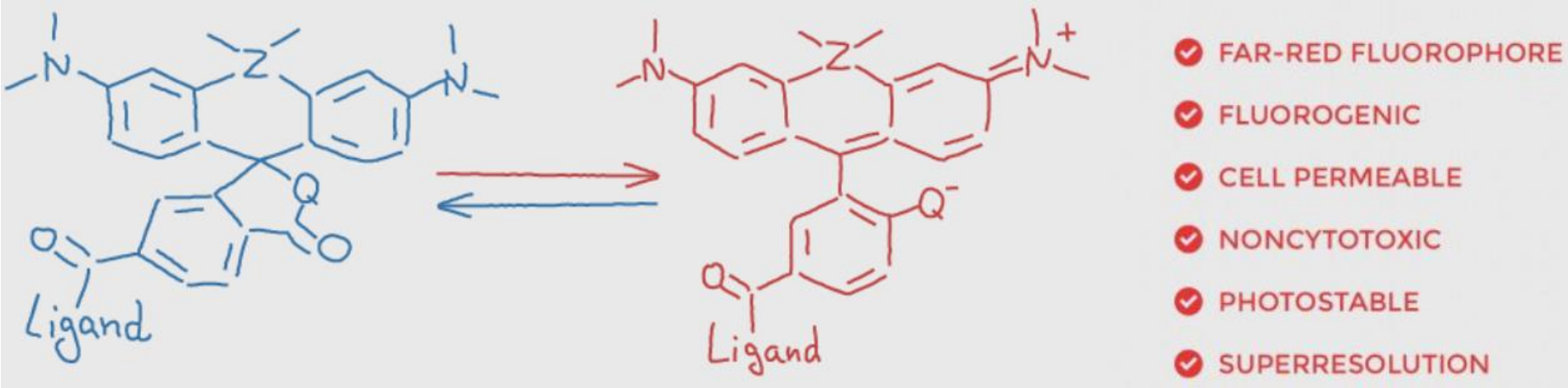




www.spirochrome.com

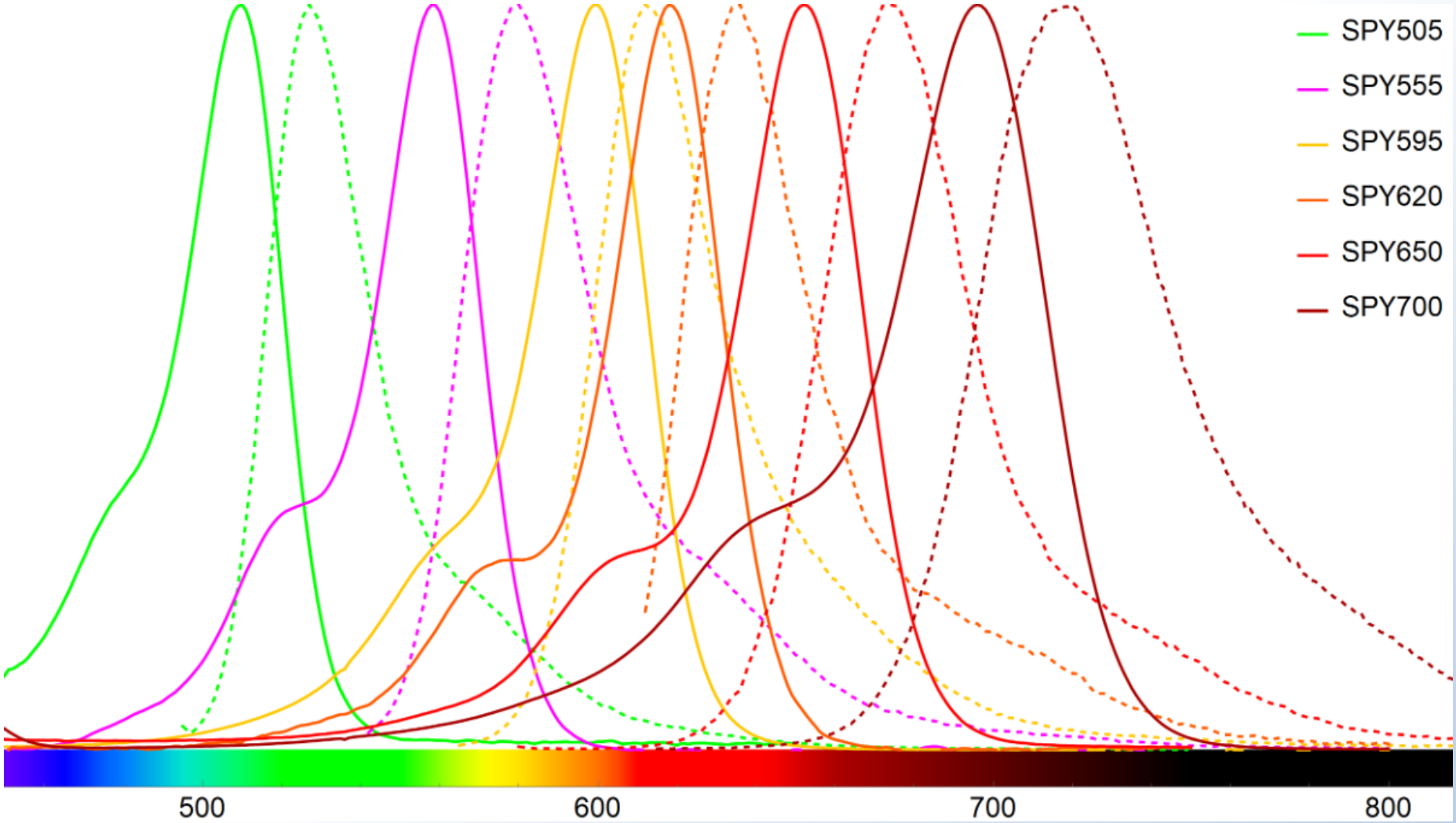
- **SiR-tubulin** microtubule binding drug Docetaxel
- **SiR-actin** F-actin binding natural product jasplakinolide
- **SiR-lysosome** cathepsin D binding natural product pepstatin A

VITAL FLUORESCENCE PROBES – SIR PROBES



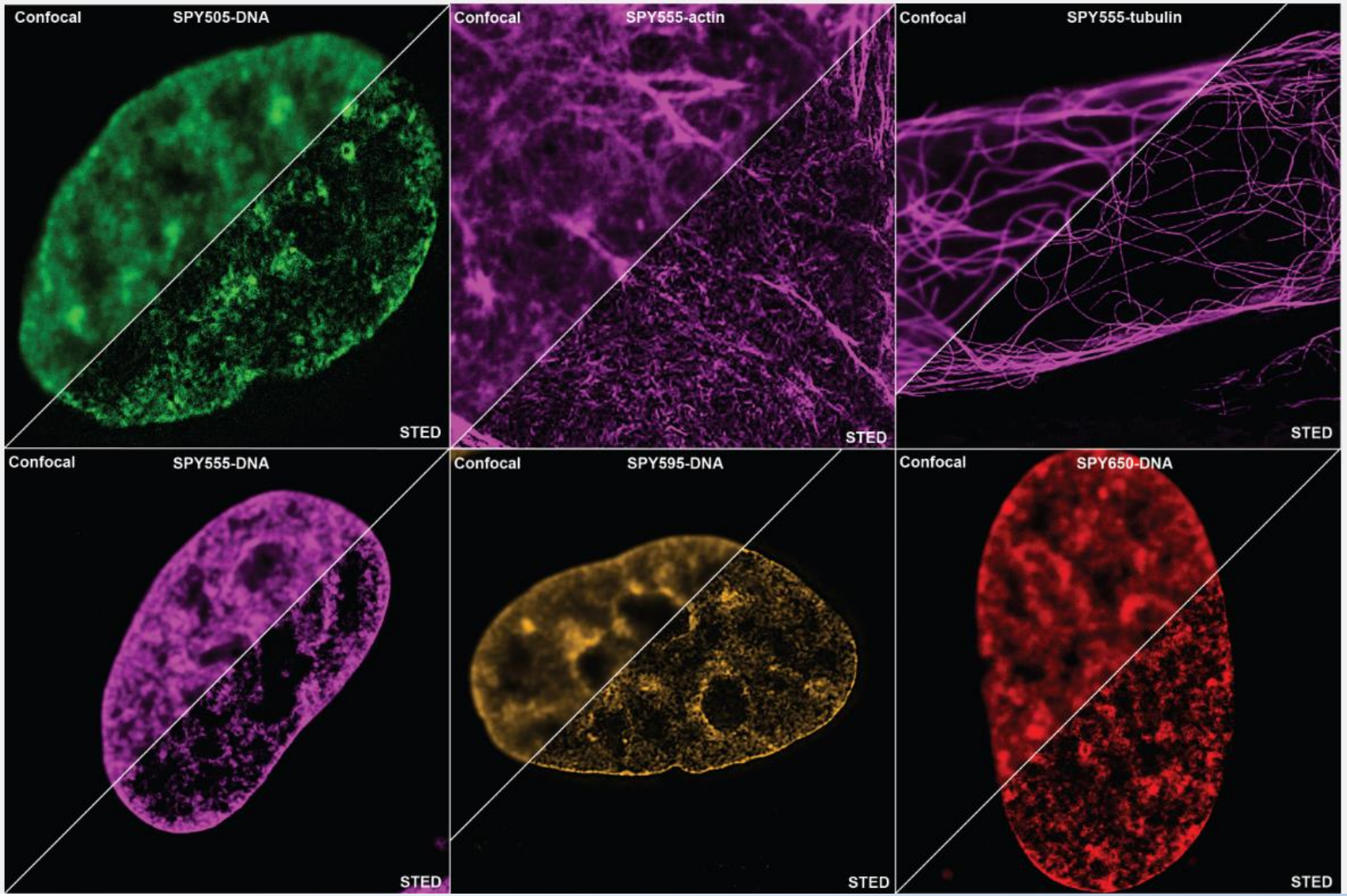
www.spirochrome.com

VITAL FLUORESCENCE PROBES – SPY™ PROBES



www.spirochrome.com

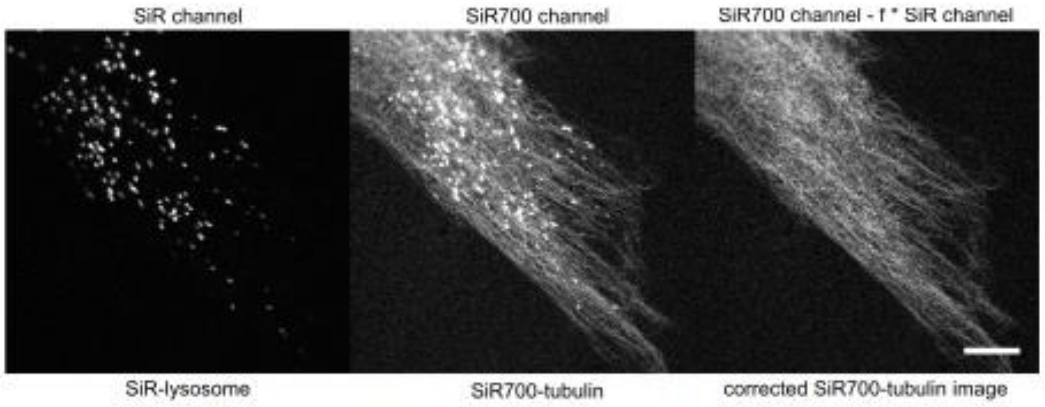
VITAL FLUORESCENCE PROBES – SPY™ PROBES



www.spirochrome.com

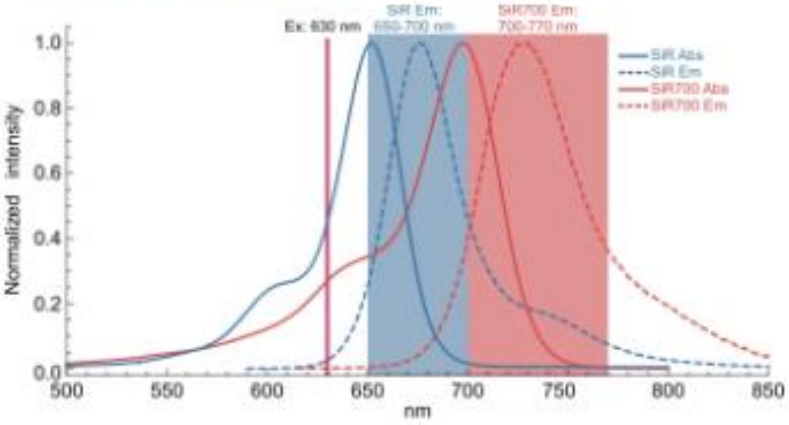
DUAL COLOUR IMAGING WITH SIR- AND SIR700-PROBES

Fluorophore	$\lambda_{abs\ (max)}$ (nm)	ε_{max} ($M^{-1} \cdot cm^{-1}$)	$\lambda_{em\ (max)}$ (nm)	lifetime (ns)	QY
SiR	652	100,000	667	2.7	0.4
SiR700	689	100,000	716	1.4	0.13



Example of bleed through removal by image subtraction one cells stained with SiR-lysosome and SiR700-tubulin. Scale bar 10 um.

Dual colour imaging examples



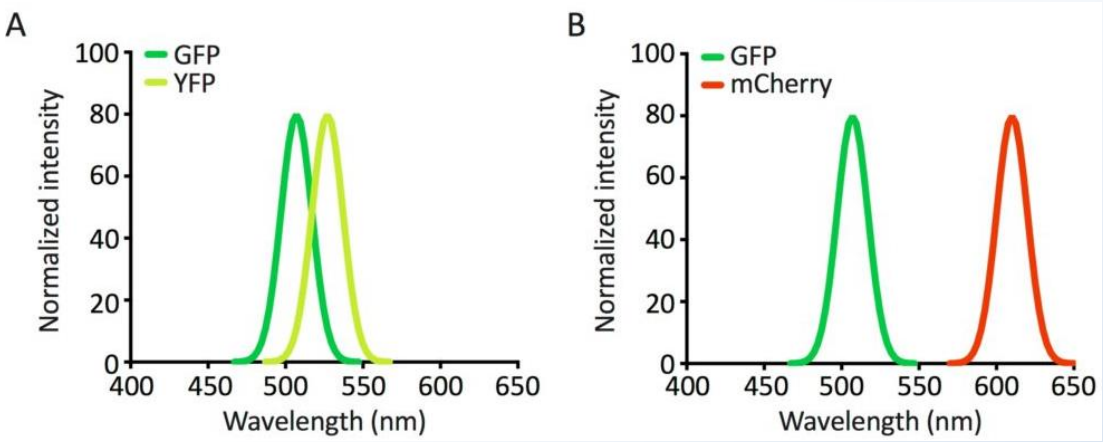
MULTICOLOUR IMAGING

Advantages

- two and more subcelullar structures, cell types (up to ten?)

Limitations

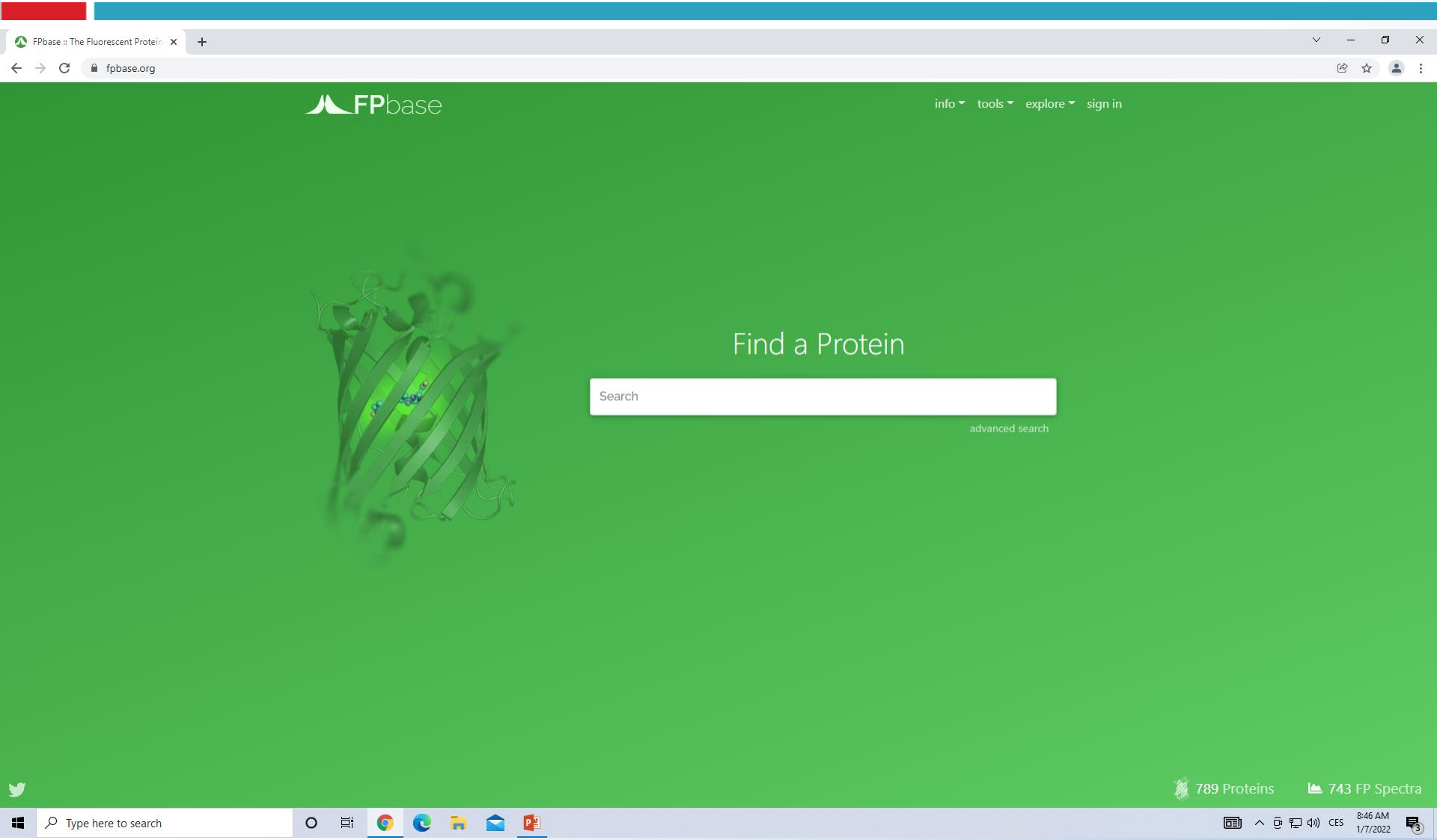
- spectral overlap



https://bitesizebio.com/33529/fluorescence-microscopy-the-magic-of-fluorophores-and-filters/

- **selection of fluorescence proteins**
  - EGFP, mCherry, far-red SiR
  - EGFP, EYFP, mCherry, SiR ?
- **phototoxicity**

FPBASE :: THE FLUORESCENT PROTEIN DATABASE



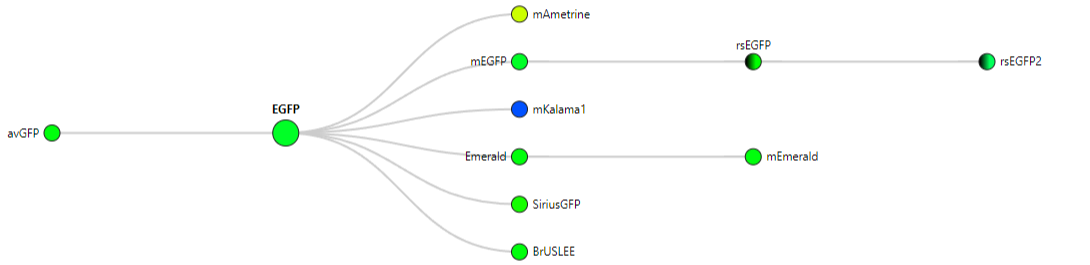
EGFP

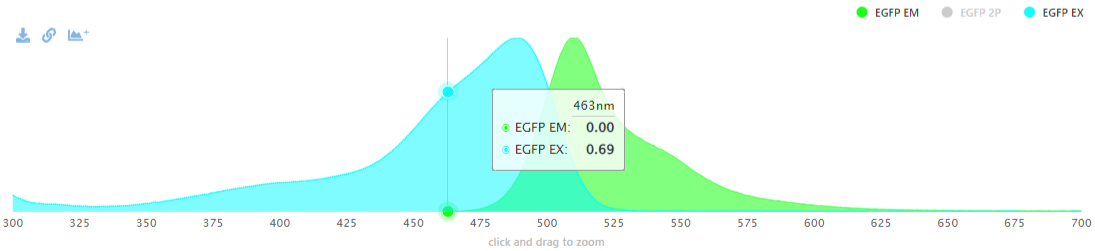
a.k.a. enhanced GFP, GFPmut1

similar: mEGFP

This protein has unmoderated changes. **Click here** to view the last moderated version of this protein from Feb 11, 2021 00:36:50. Or view the full **change history**

EGFP is a basic (constitutively fluorescent) green fluorescent protein published in 1996, derived from Aequorea victoria. It is reported to be a rapidly-maturing weak dimer with moderate acid sensitivity.





Oligomerization	Organism	Molecular Weight	Cofactor
Weak dimer	Aequorea victoria	26.9 kDa	-

compare

Type here to search
CES 8:47 AM 1/7/2022

Oligomerization	Organism	Molecular Weight	Cofactor
Weak dimer	Aequorea victoria	26.9 kDa	-

Attributes

FPbase ID: R9NL8

Ex λ	Em λ	EC (M^{-1} cm^{-1})	QY	Brightness	pKa	Maturation (min)	Lifetime (ns)
488	507	55,900	0.6	33.54	6.0	25.0	2.6

Edit States/Attributes

EGFP OSER Measurements

% Normal Cells	OSER/NE ratio	Cell Type	Reference
76.5 ± 6.9 (10000 cells)	-	HeLa	Cranfill et al. (2016)
76.5 ± 6.9 (10000 cells)	-	HeLa	Shaner et al. (2013)
76.5 ± 6.9 (10000 cells)	-	HeLa	Hoi et al. (2013)
-	3.89 ± 0.25 (50 cells)	U-2 OS	Costantini et al. (2012)

Photostability

$t_{1/2}$ (s)	Power	Light	Mode	In Cell	Fusion	°C	Reference
174.0		Arc-lamp	Widefield	×	none	23.0	Shaner et al. (2005)
50.1	1.5 (mW)	Laser	Point Scanning Confocal	Hela			Zhong et al. (2018)

A caution on interpretation of photostability measurements

Add photostability info

EGFP Sequence

EGFP was derived from avGFP with the following mutations: M1_S2insV/F64L/S65T/H231L

```
  1 MVSKGEELFT GVVPILVELD GDVNGHKFSV SGEGEGDATY GKLTLKFICT TGKLPVPWPT LVTTLTYGVQ CFSRYPDHMK QHDFFKSAMP
 91 EGYVQERTIF FKDDGNYKTR AEVKFEGDTL VNRIELKGID FKEDGNILGH KLEYNYNSHN VYIMADKQKN GIKVNFKIRH NIEDGSVQLA
181 DHYQQNTPIG DGPVLLPDNH YLSTQSALSK DPNEKRDHMV LLEFVTAAGI TLGMDELYK
```

GenBank: AAB02572 UniProtKB: C5MKY7 IPG: 928978

Structure

PDB ID 4EUL (1.35 Å)

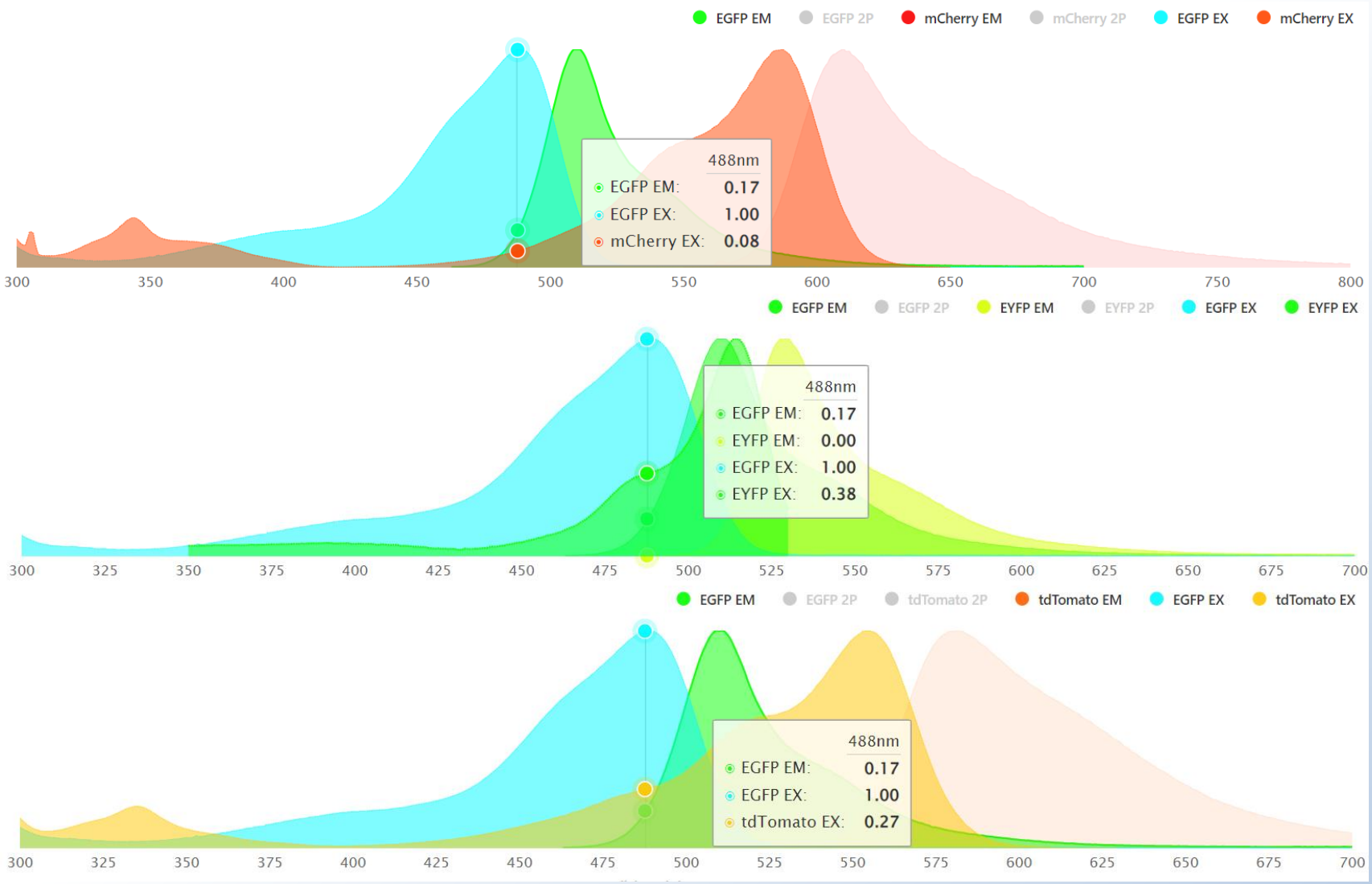
Crystal structure of enhanced Green Fluorescent Protein to 1.35A resolution

compare

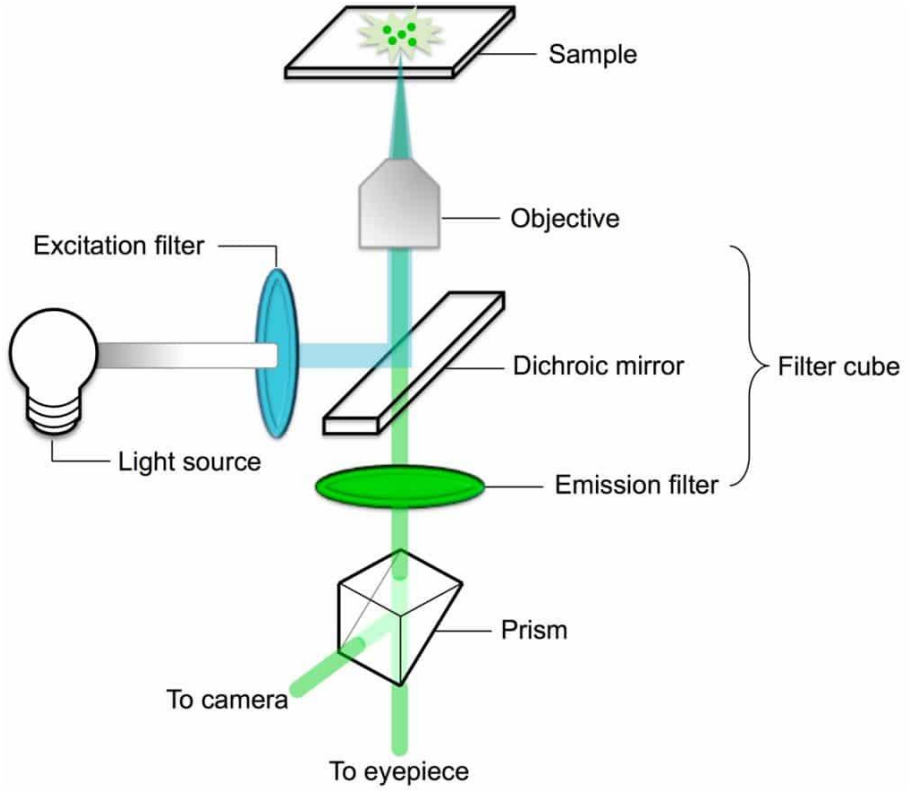
Type here to search

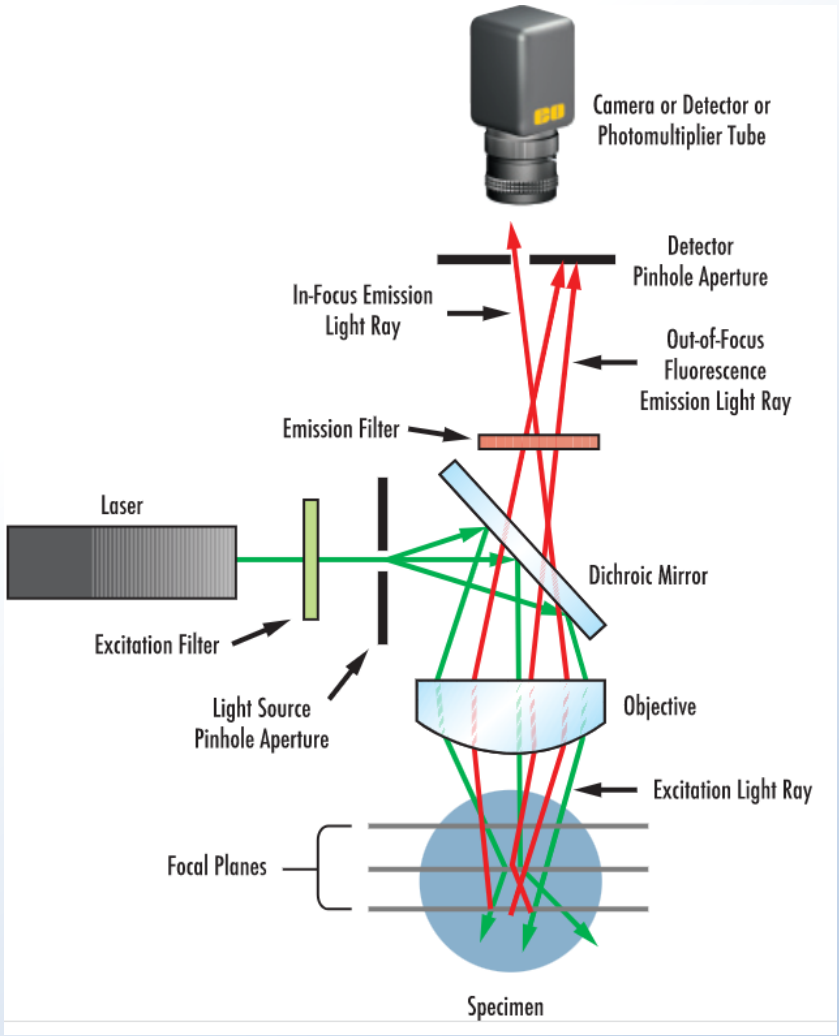
CES 8:47 AM 1/7/2022

SELECTION OF FLUORESCENCE PROTEINS FOR MULTICOLOUR IMAGING



WIDE-FIELD VS CONFOCAL MICROSCOPY

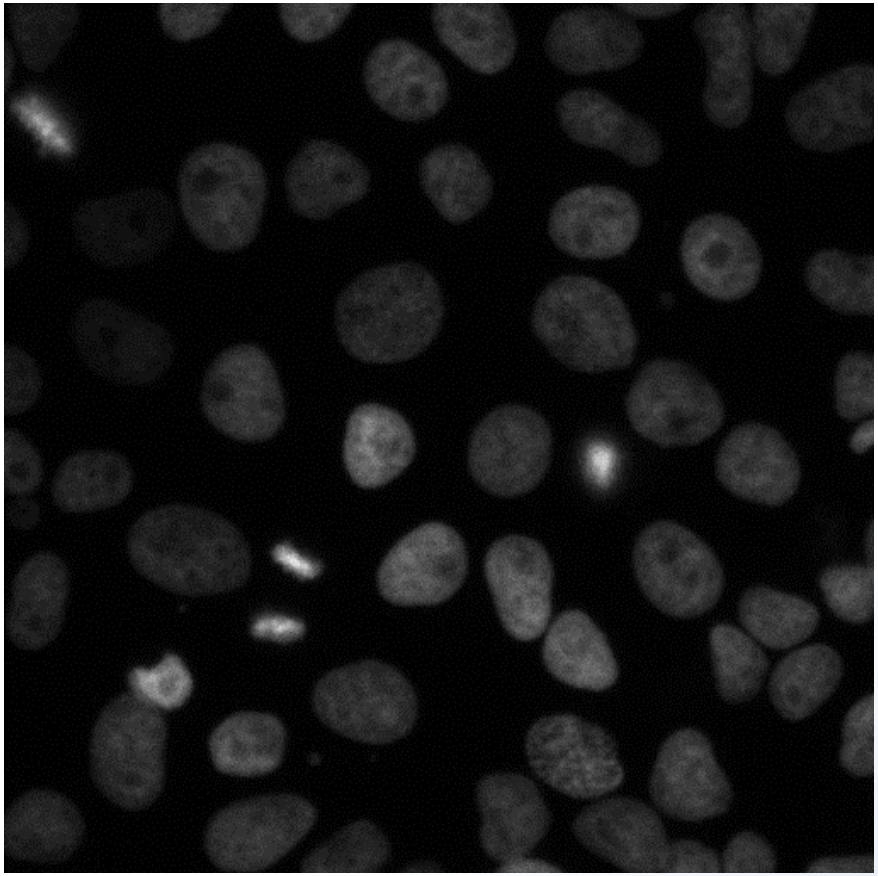




https://bitesizebio.com/33529/fluorescence-microscopy-the-magic-of-fluorophores-and-filters/

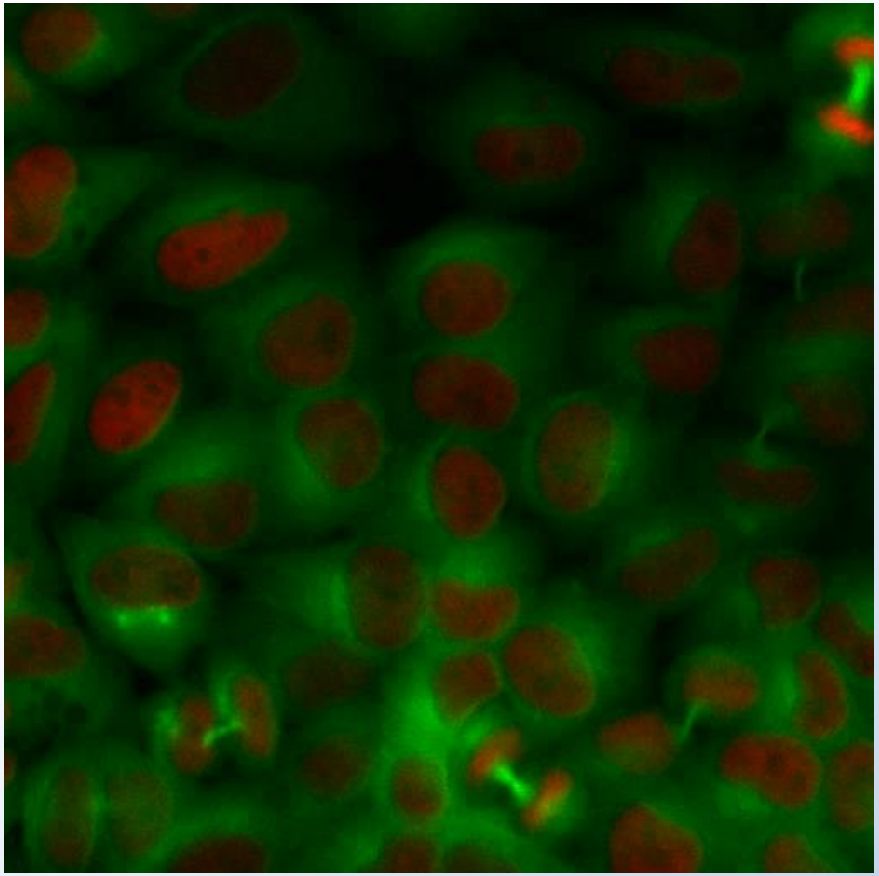
https://www.edmundoptics.eu/knowledge-center/application-notes/microscopy/confocal-microscopy/

CELL CYCLE ANALYSIS IN TRANFECTED SOMATIC CELLS



Confocal live-cell imging of HeLa cells

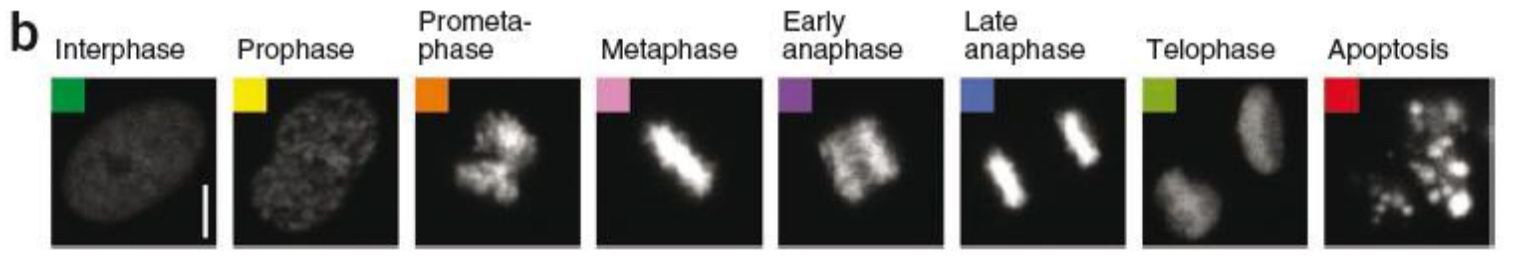
H2B-mCherry

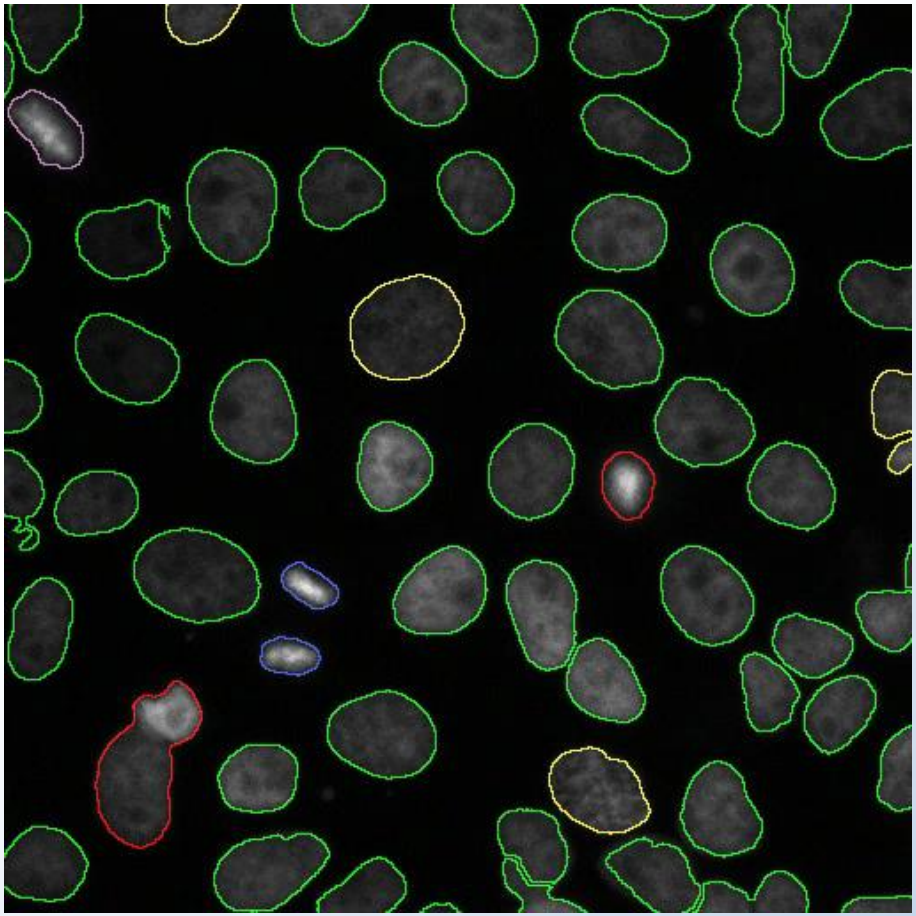


Confocal live-cell imging of HeLa cells

H2B-mCherry, alfa-tub-eGFP

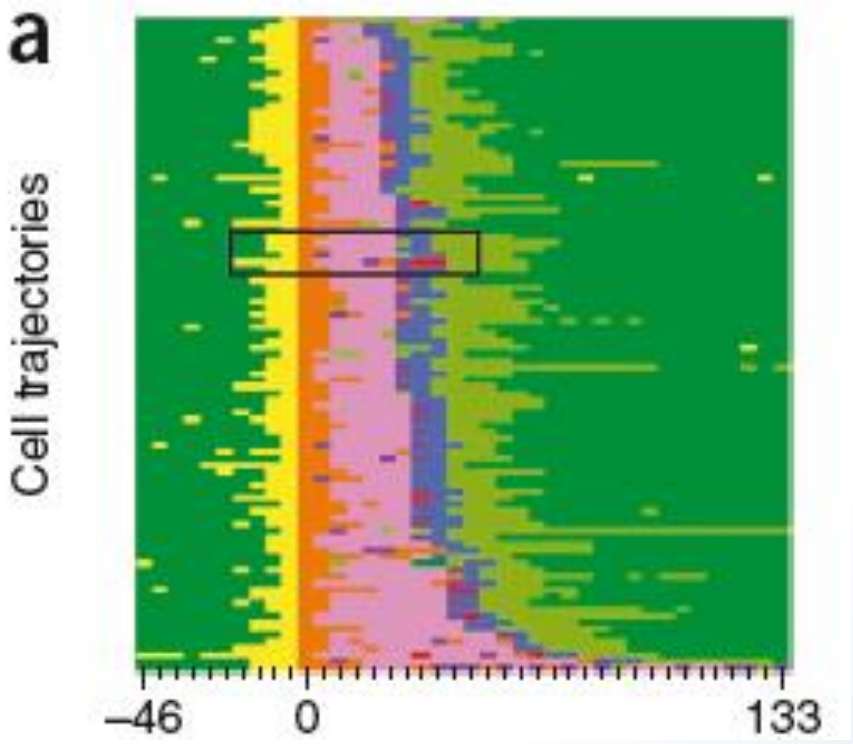
CELL CYCLE ANALYSIS IN TRANFECTED SOMATIC CELLS



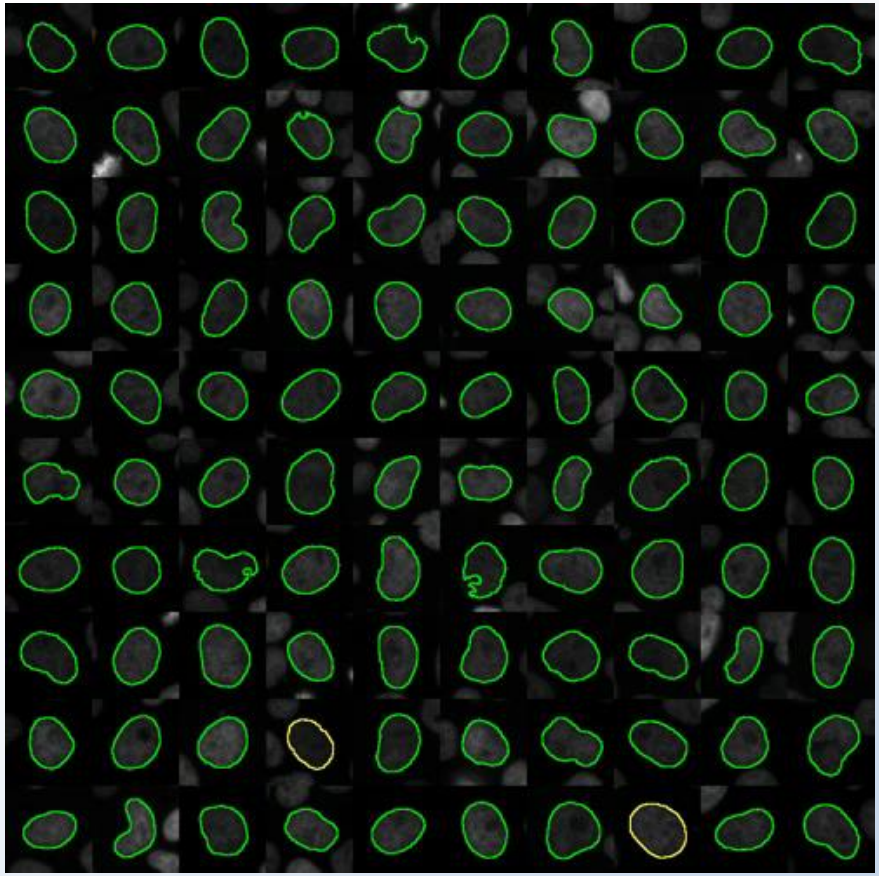


Nat Methods.
2010 Sep;7(9):747-54.

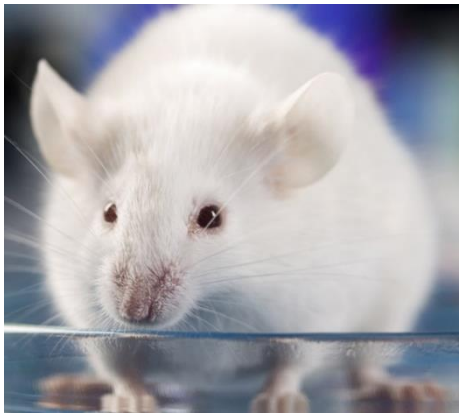
CELL CYCLE ANALYSIS IN TRANFECTED SOMATIC CELLS

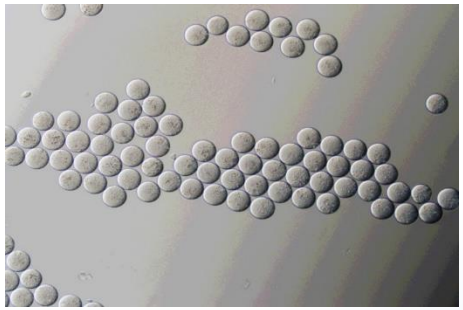


In silico synchronization



LIVE-CELL MICROSCOPY OF MOUSE OOCYTES





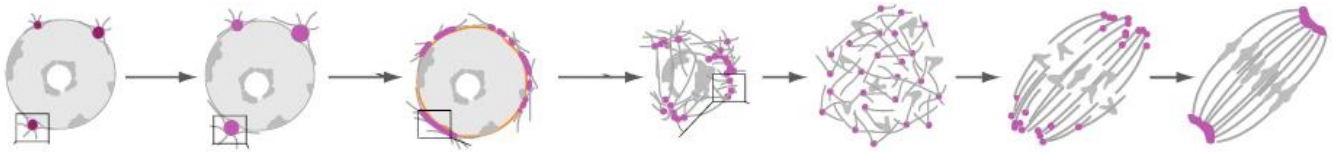
Advantages of mouse oocytes:

- ✓ spherical shape
- ✓ size
- ✓ optical transparency
- ✓ availability
- ✓ microinjection

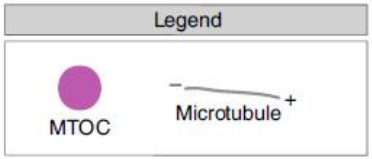
Live-cell experiment:

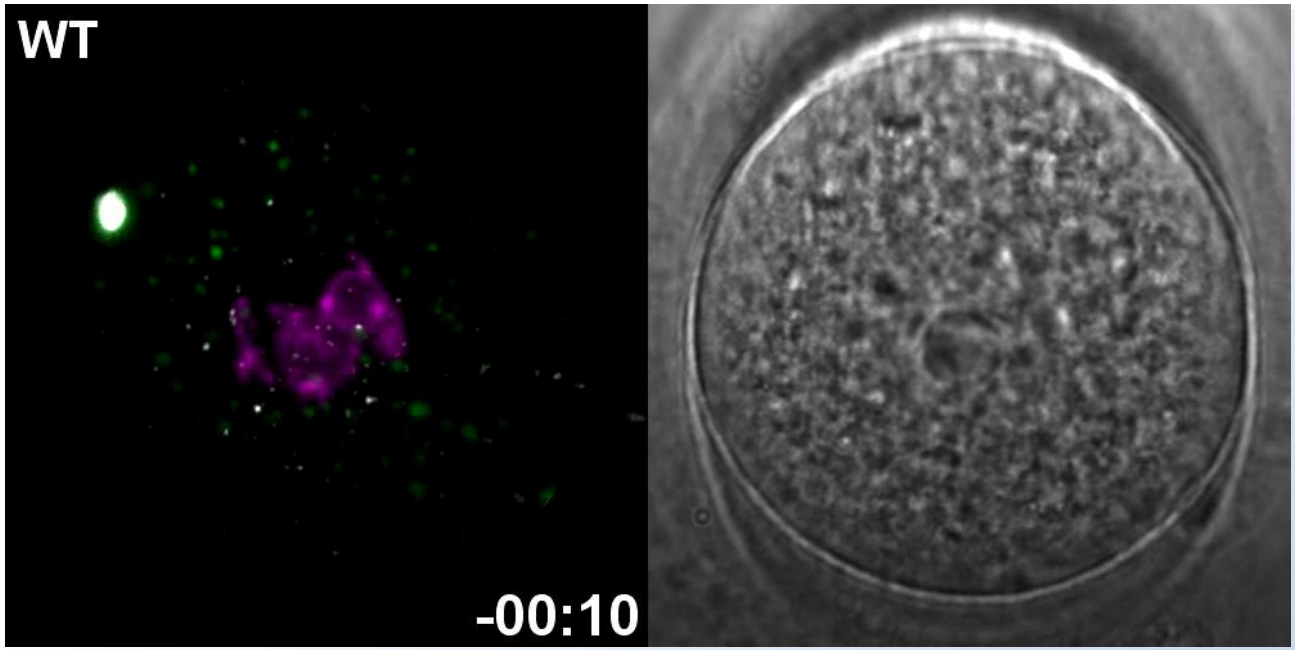
- ❑ 1.Isolation of mouse oocytes
- ❑ 2.mRNA microinjection
- ❑ 3. expression of marker
- ❑ 4. imaging

ACENTROSOMAL SPINDLE FORMATION IN MAMMALIAN OOCYTES



Clift, Schuh, 2015, Nat Commun., PMID: 26147444



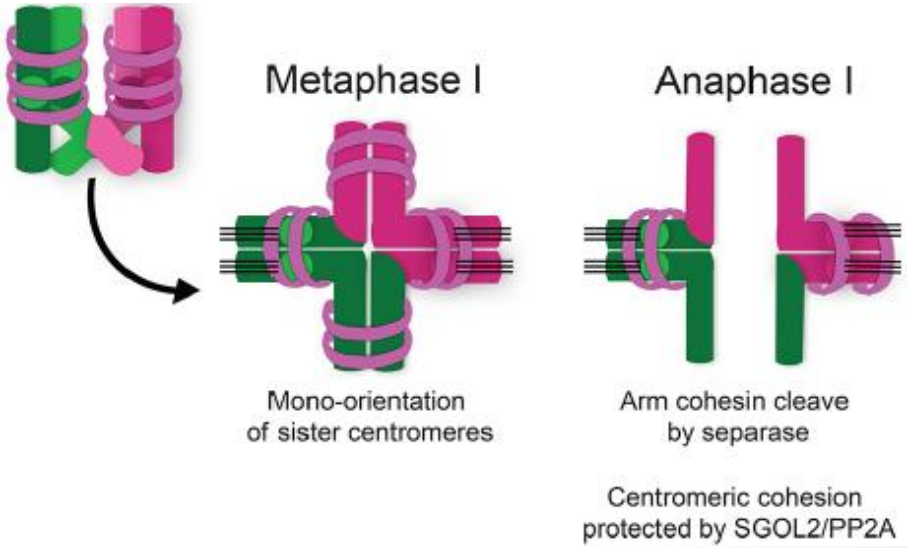


Light-sheet live-cell imaging

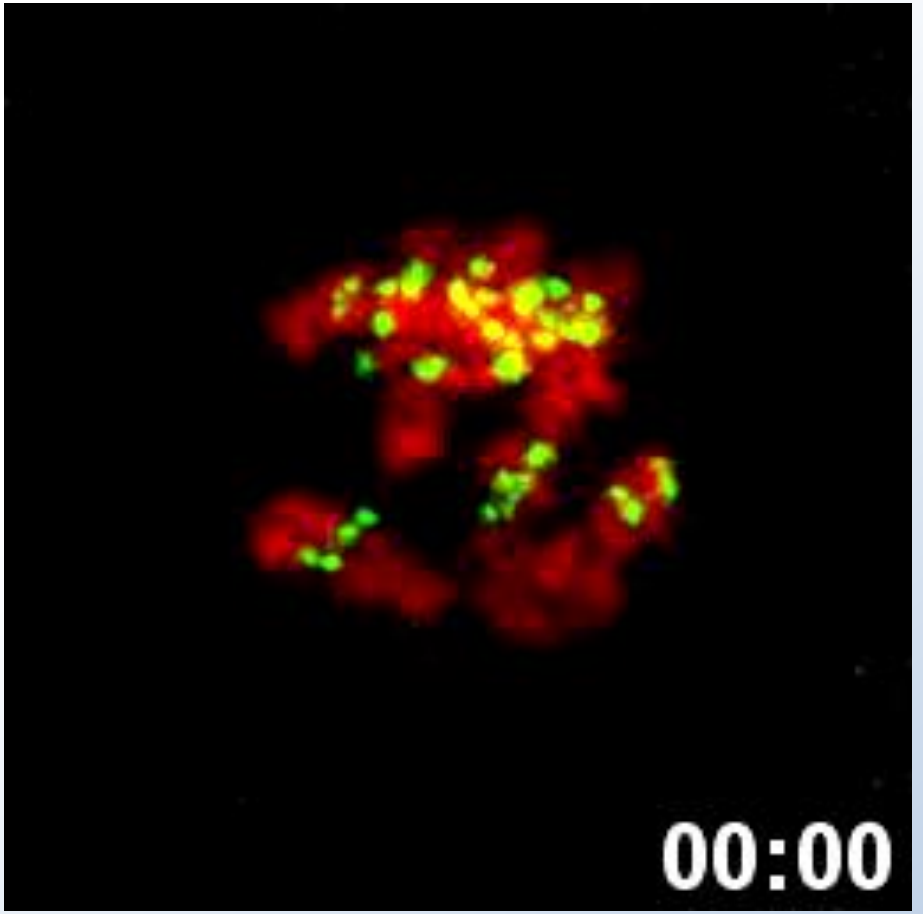
H2B-mCherry, SiR-tubulin, CDK5RAP2-eGFP

Time hh:mm after GVBD

HOMOLOGOUS CHROMOSOME SEGREGATION IN MAMMALIAN OOCYTES



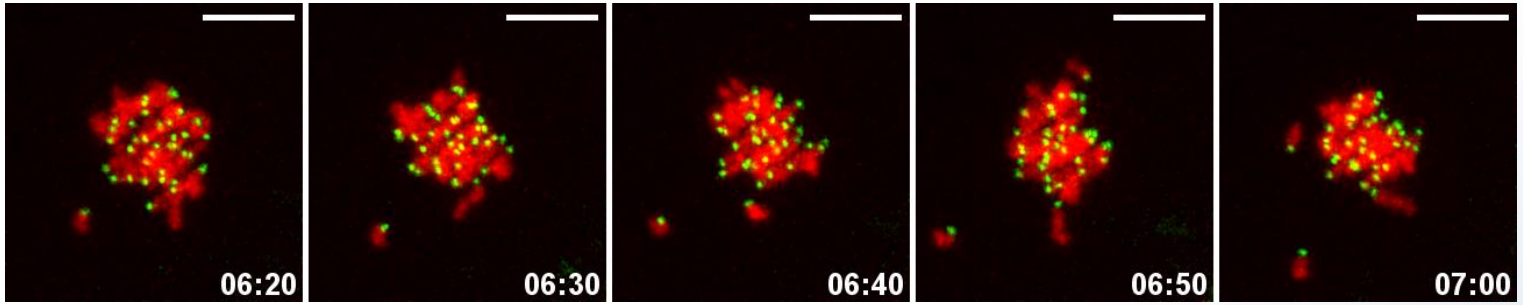
Beverley et al, 2021, Frontiers in Cell and Developmental Biology



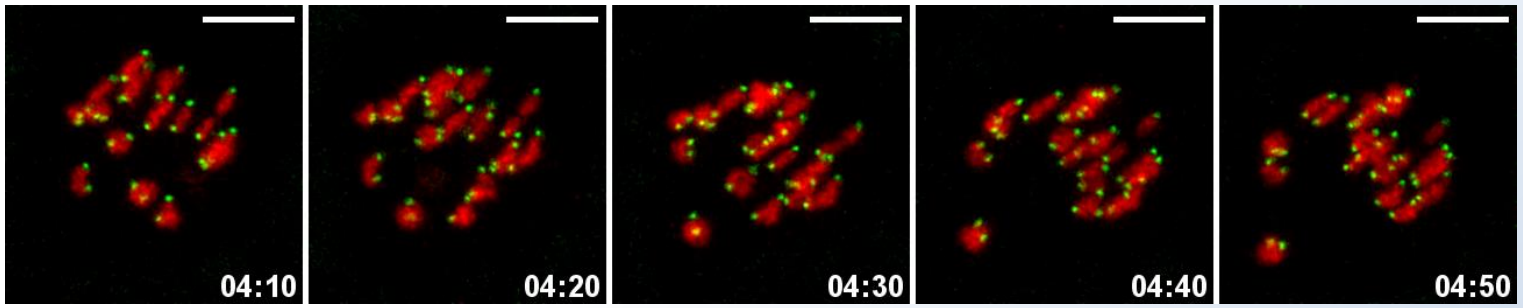
H2B-mCherry, CENP-C-2x mEGFP

Time hh:mm after meiotic resumption

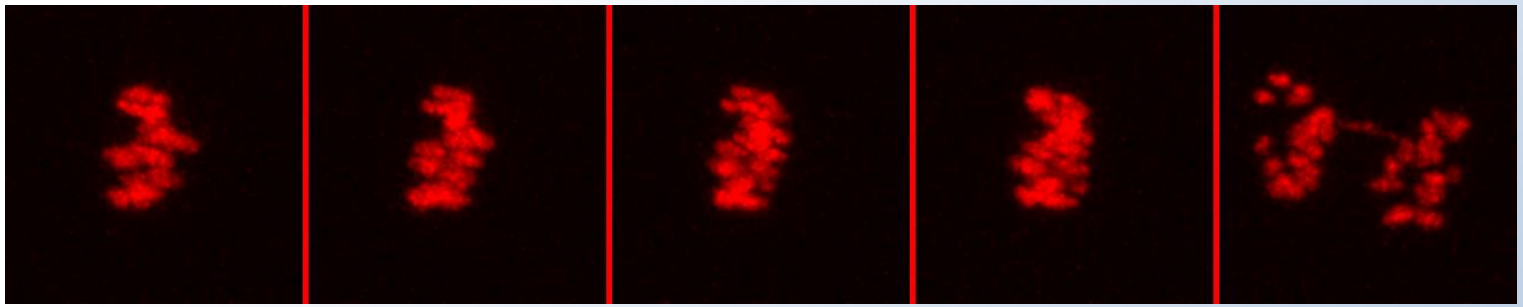
HOMOLOGOUS CHROMOSOME SEGREGATION IN MAMMALIAN OOCYTES



Chromosome fragments

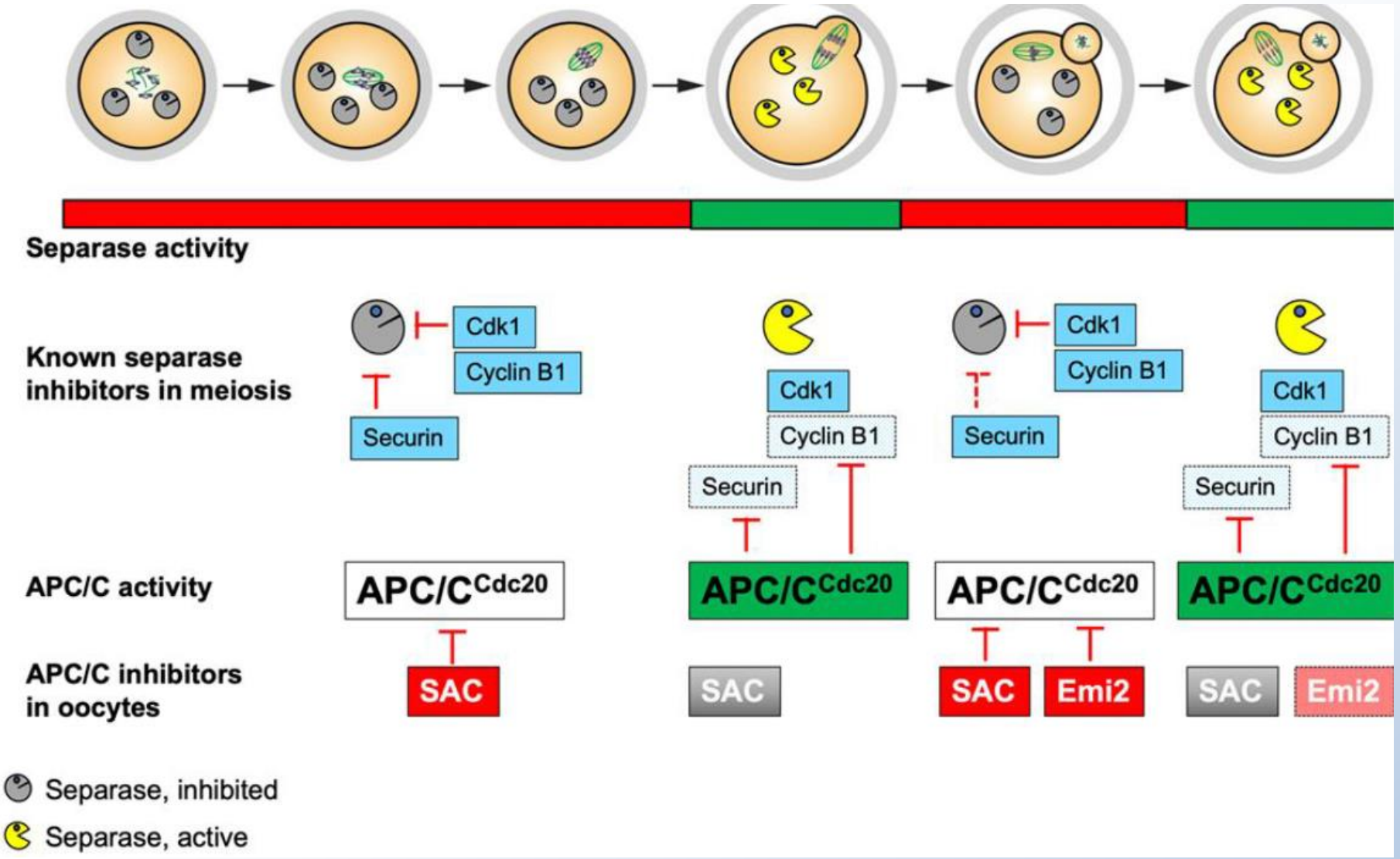


Premature separation of homologues chromosomes



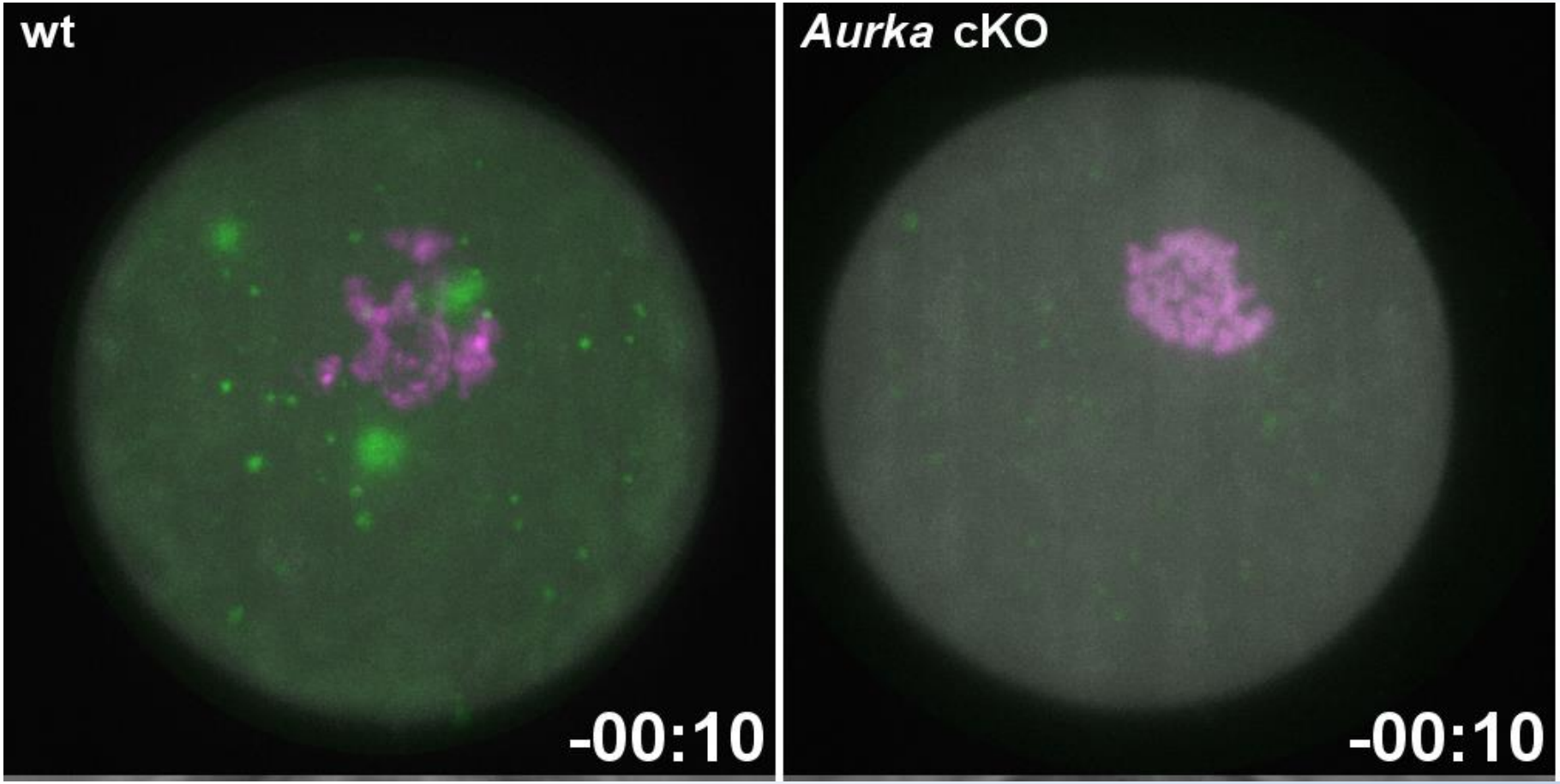
Anaphase bridges

APC ACTIVATION IN MAMMALIAN OOCYTES



Wassmann, 2022, Cells

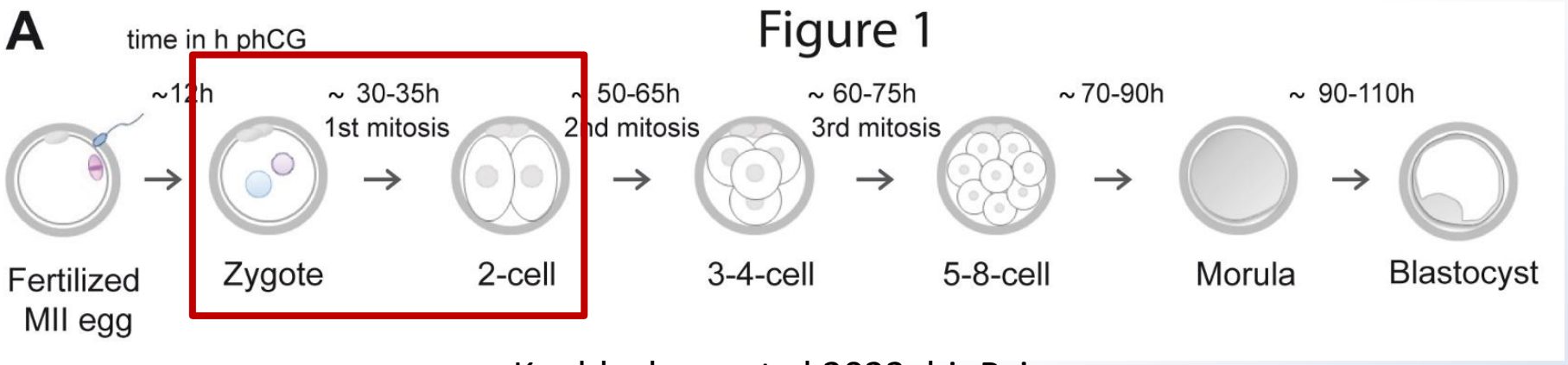
APC ACTIVATION IN MAMMALIAN OOCYTES



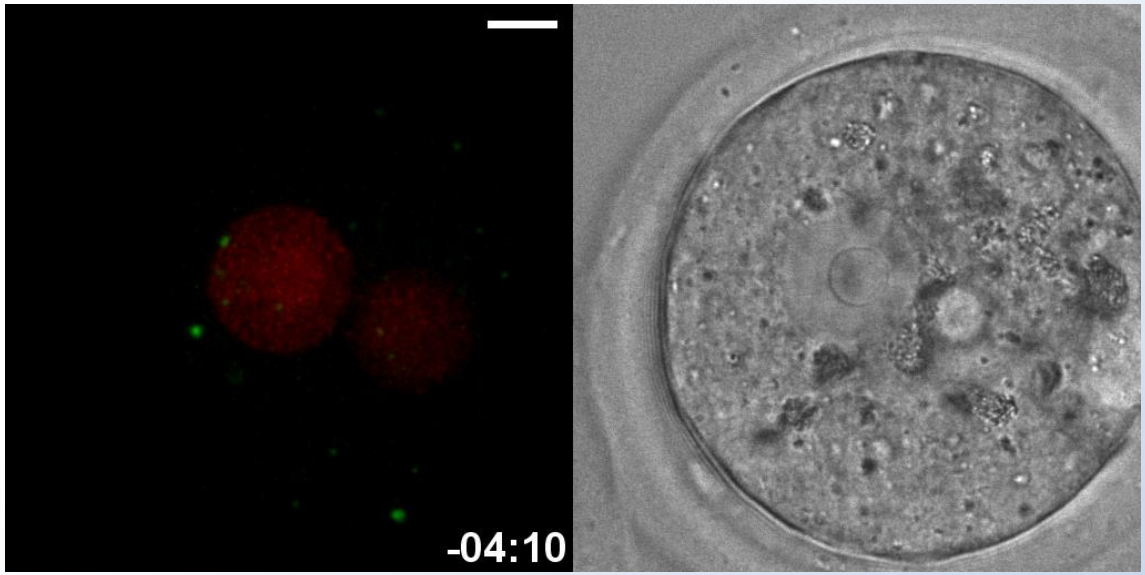
H2B-mCherry, SiR-tubulin, securin-eGFP

Time hh:mm after meiotic resumption

INITIAL EMBRYONIC CELL DIVISIONS IN MOUSE

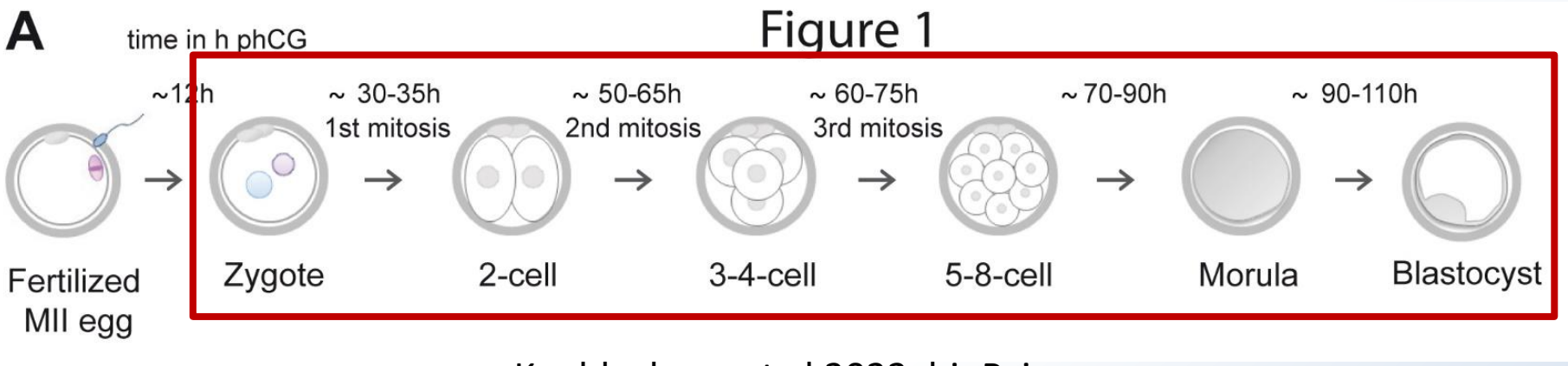


Knoblochova et al 2022, bioRxiv



Confocal live-cell imaging of chromosomes and MTOCs during 1st division in of mouse zygote - Drutovic, 2020, unpublished

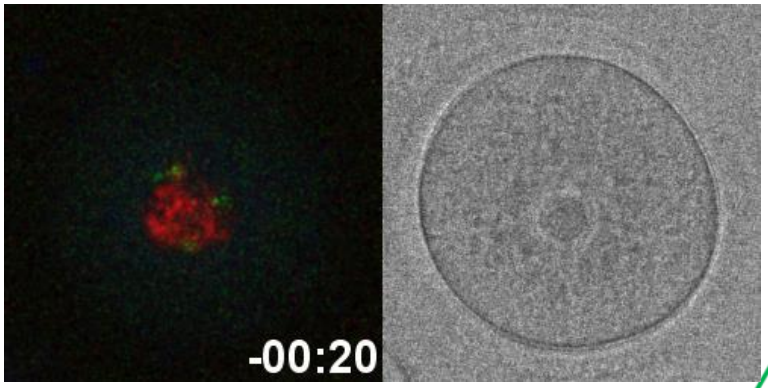
PREIMPLANTATION DEVELOPMENT



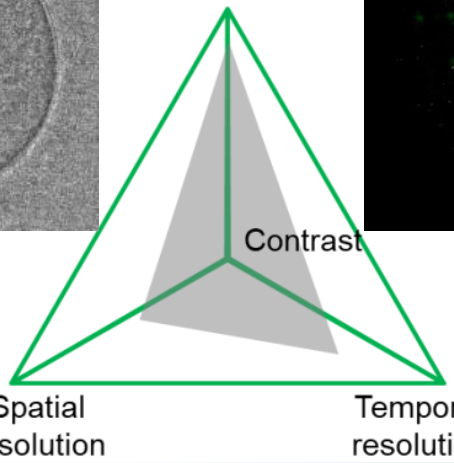
Knoblochova et al 2022, bioRxiv

„TRIANGLE OF FRUSTRATION“

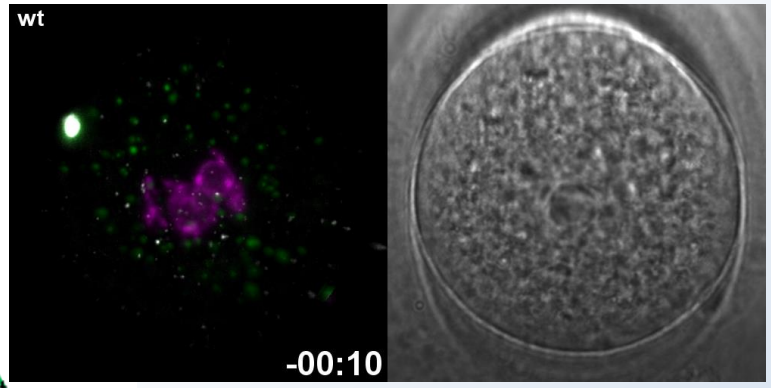
Confocal microscopy



Whole oocyte
14 optical sections
5 µm section thickness



Light-sheet microscopy

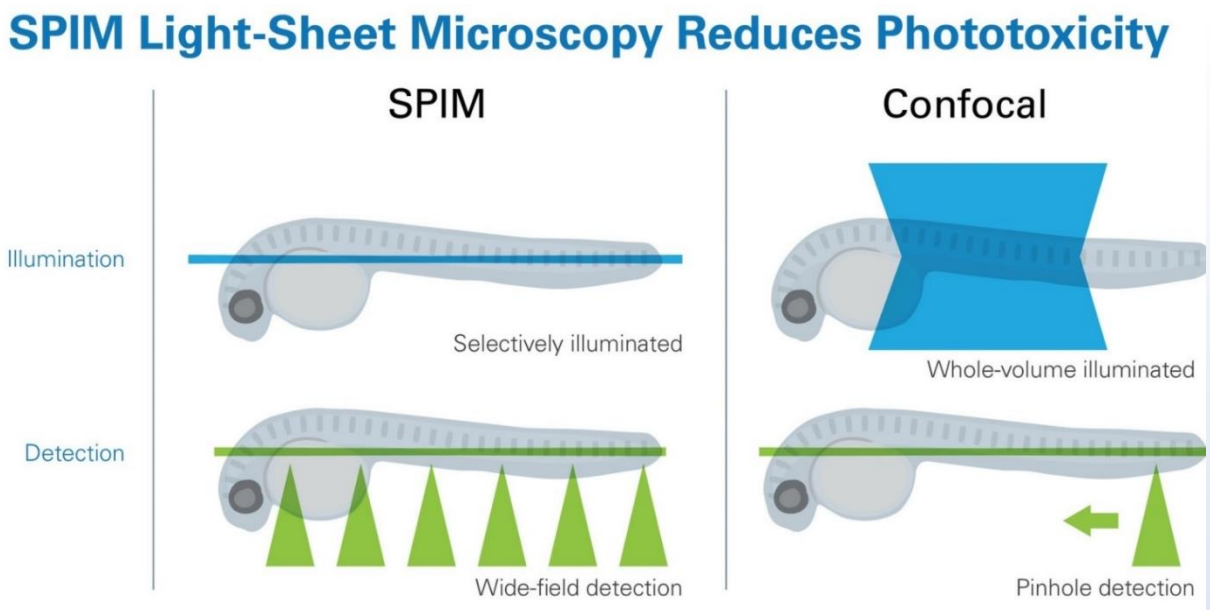


Whole oocyte
30 optical sections
2 µm section thickness

Spindle area
16 optical sections
2.5 µm section thickness

CONFOCAL VS SPIM MICROSCOPY

- **SPIM - Selective Plane Illumination Microscopy**

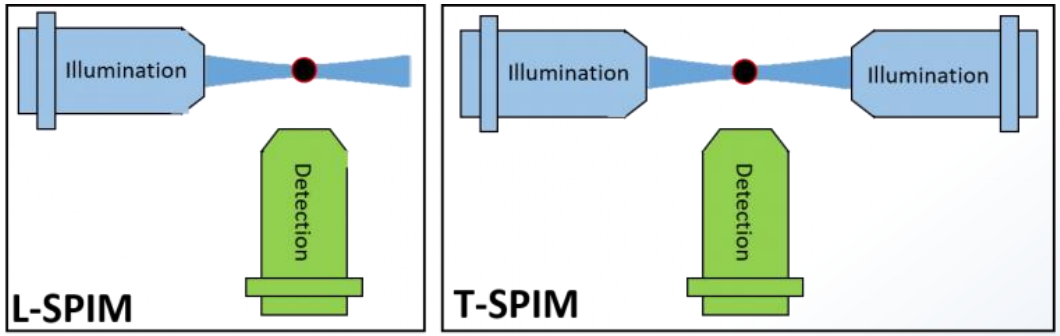


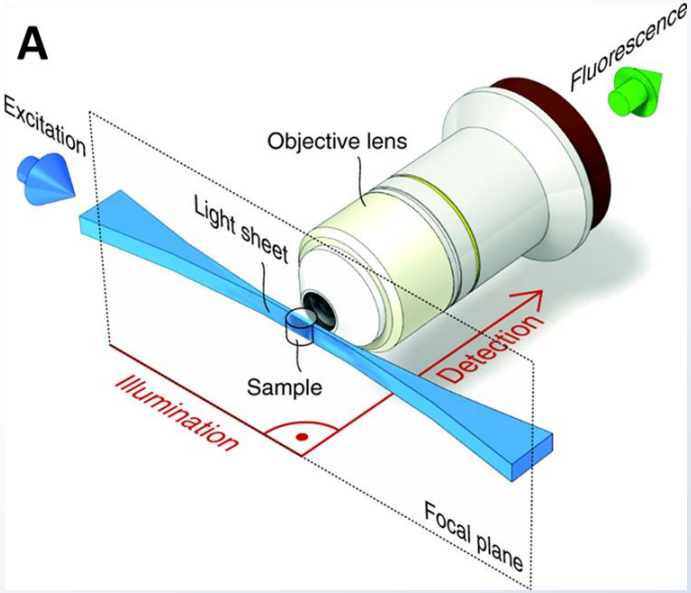
https://www.prnewswire.com/news-releases/bruker-acquires-emerging-light-sheet-microscopy-company-luxendo-300452724.html

Advantages of SPIM

- selective illumination
- high acquisition speed
- reduced phototoxicity
- increased signal-to-noise ratio

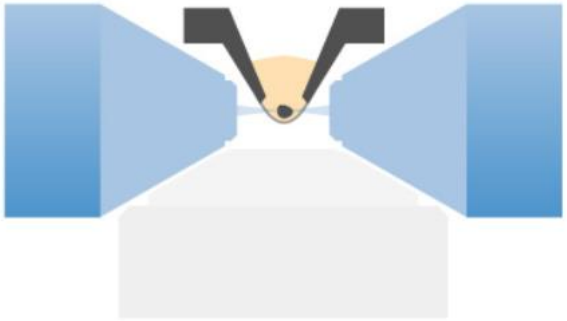
SPIM (SINGLE PLANE ILLUMINATION MICROSCOPY)

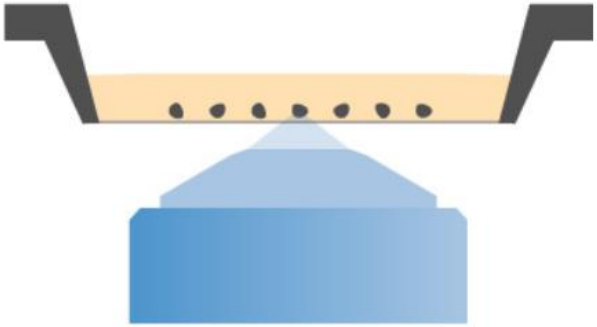


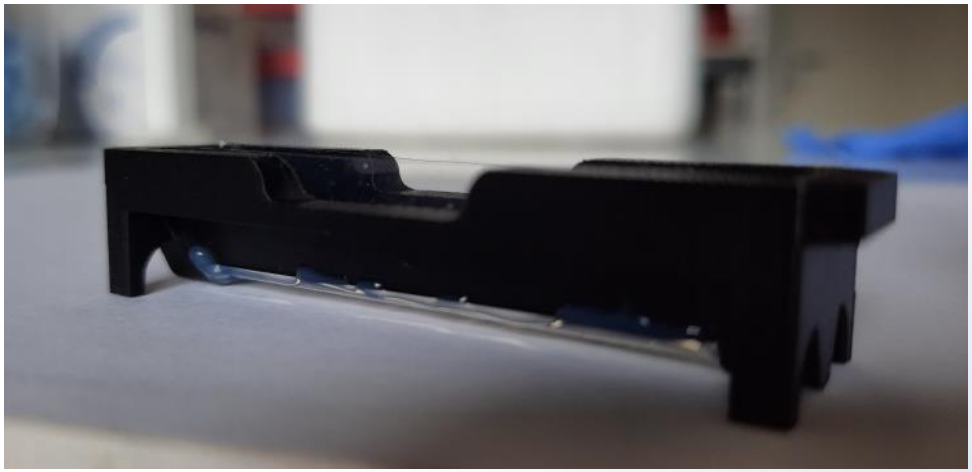


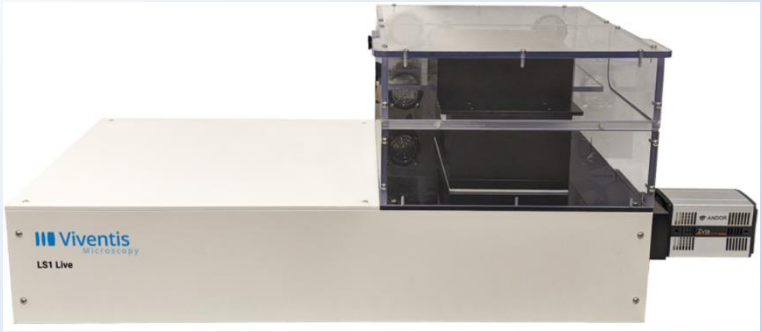
www.photometrics.com/learn/light-sheet-microscopy/introduction-to-light-sheet-microscopy4.html

VIVENTIS LS1 LIVE SPIM MICROSCOPY SYSTEM

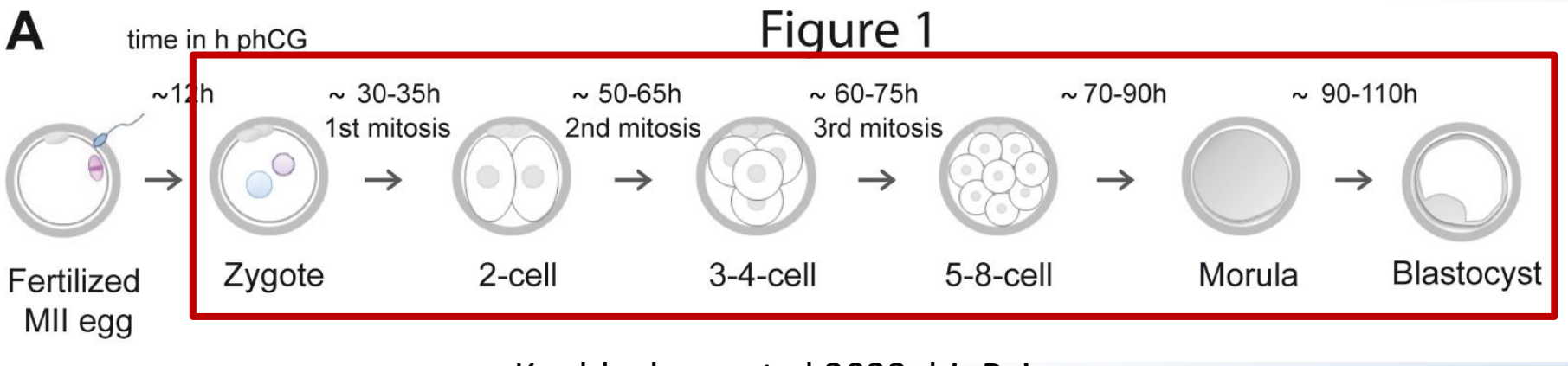




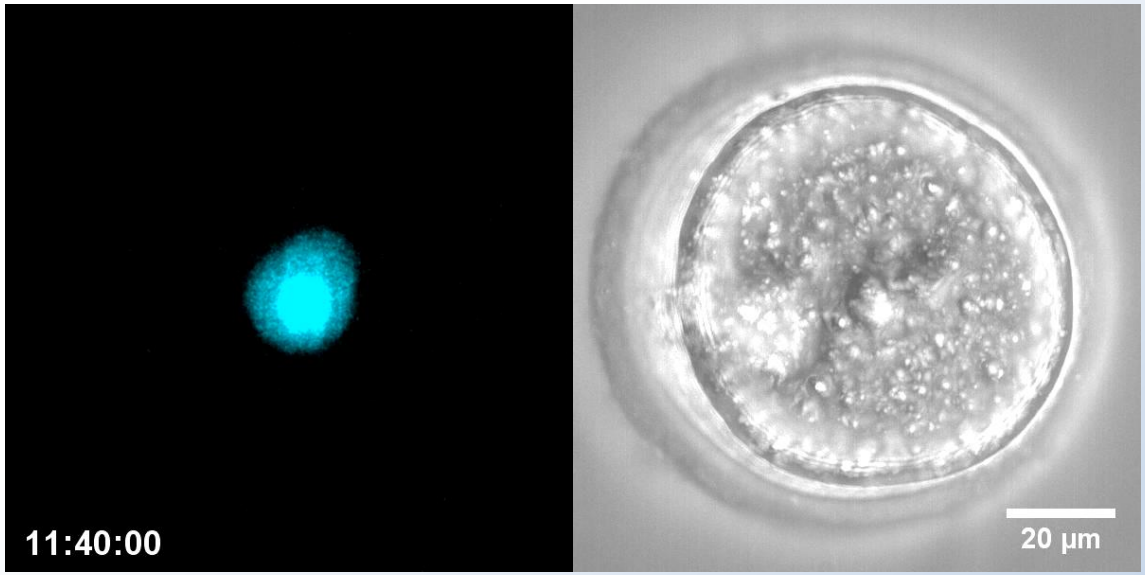




PREIMPLANTATION DEVELOPMENT



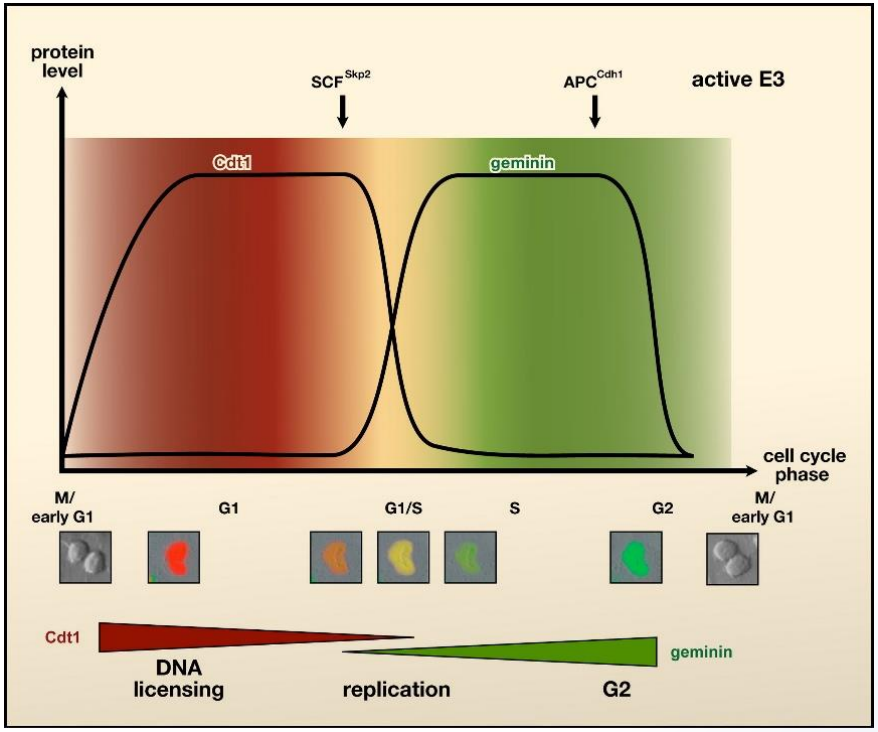
Knoblochova et al 2022, bioRxiv

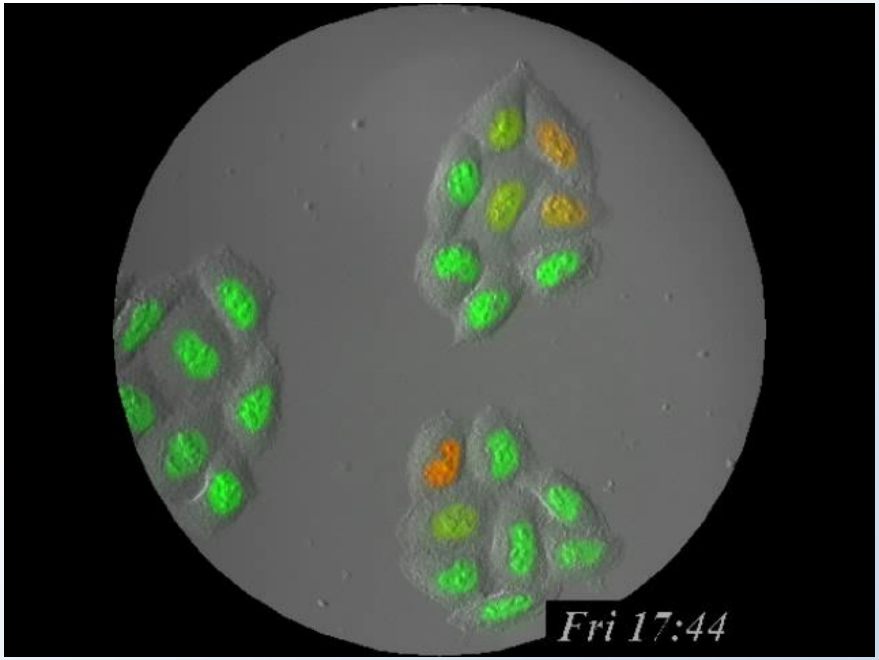


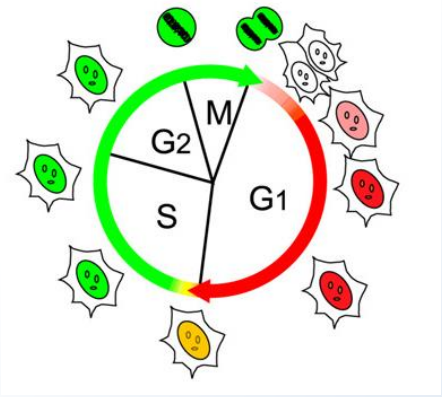
Light-sheet live-cell microscopy of preimplantation development

Chromosomes (cyan), brightfield, time after HCG stimulation

LENGTH OF INDIVIDUAL CELL CYCLE PHASES

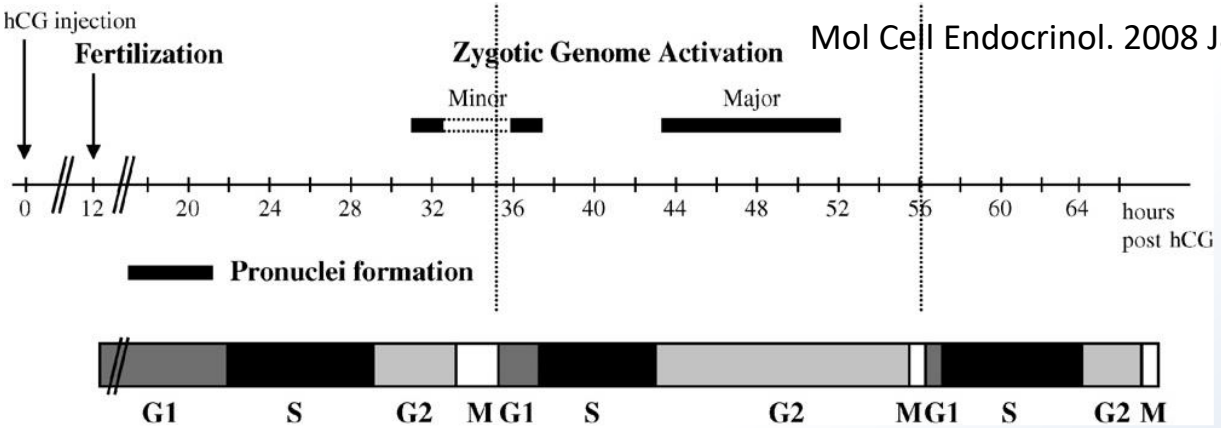




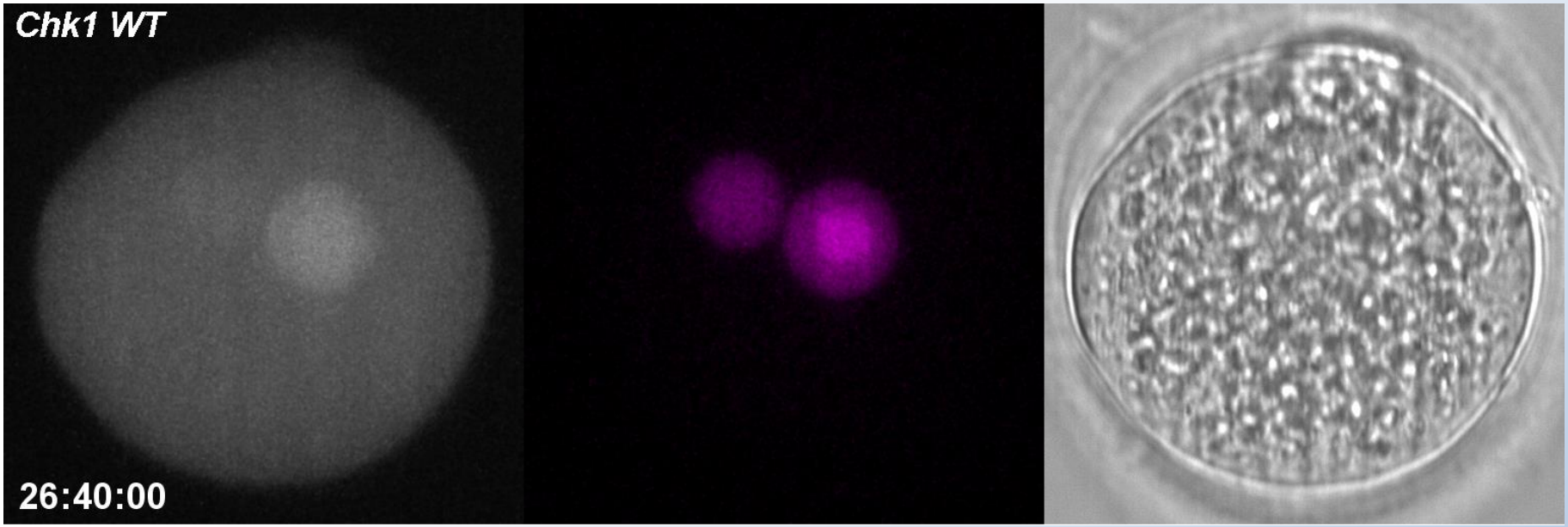


Cell. 2008 Feb 8;132(3):487-98.

VIVENTIS LS1 LIVE SPIM MICROSCOPY SYSTEM



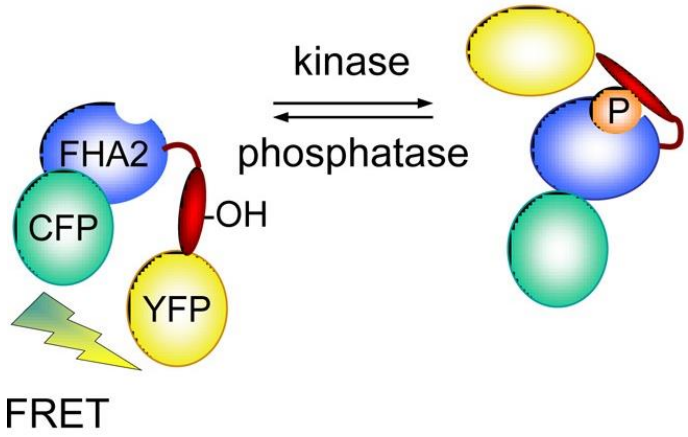
Mol Cell Endocrinol. 2008 Jan 30;282(1-2):78-86.

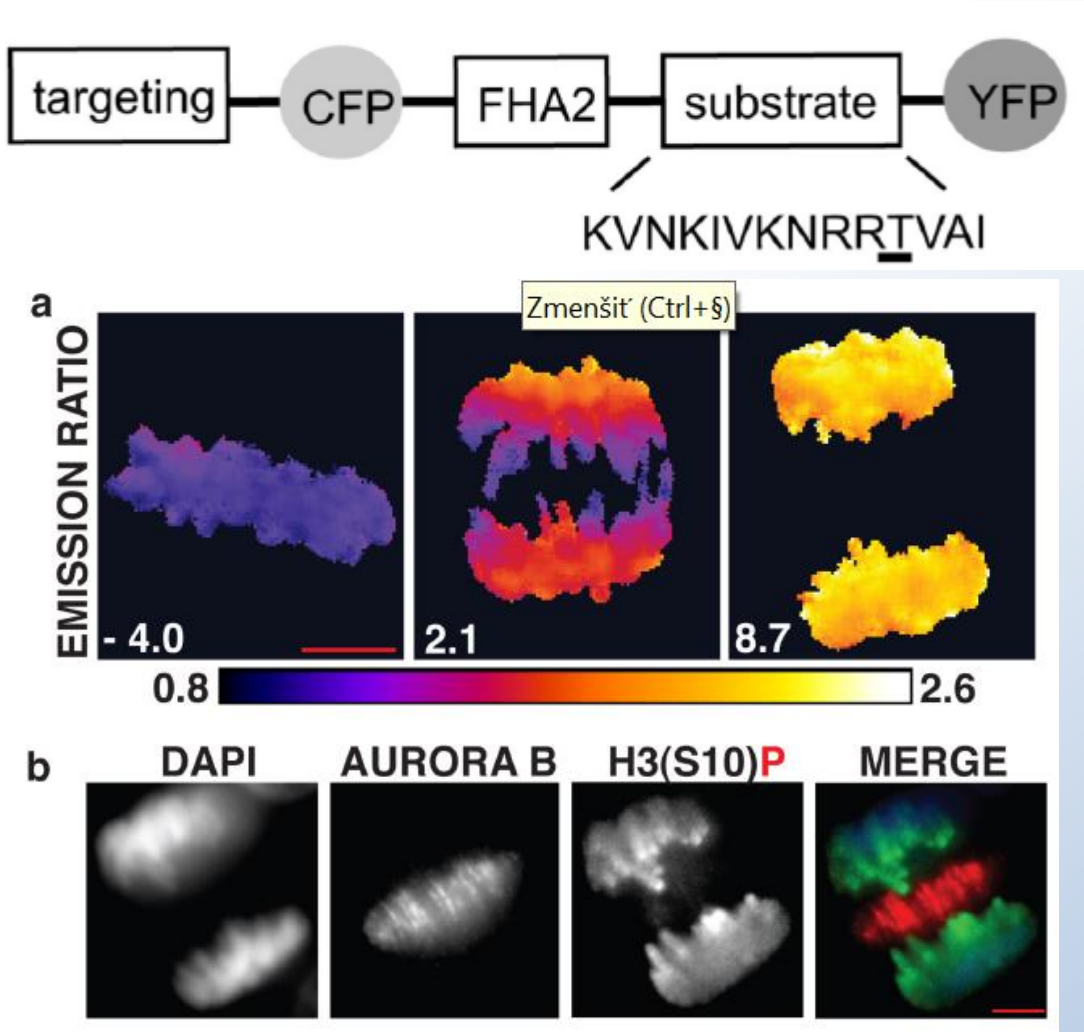


Cell cycle progression during pre-implantation mouse embryos development

H2B-mCherry, mCDT1-EYFP

SIGNALING PATHWAY ACTIVITY BY FRET BIOSENZORS





The anaphase phosphorylation gradient is observed for multiple Aurora B substrates

Fuller et, 2008, Nature

QUANTITATIVE IMAGE ANALYSIS