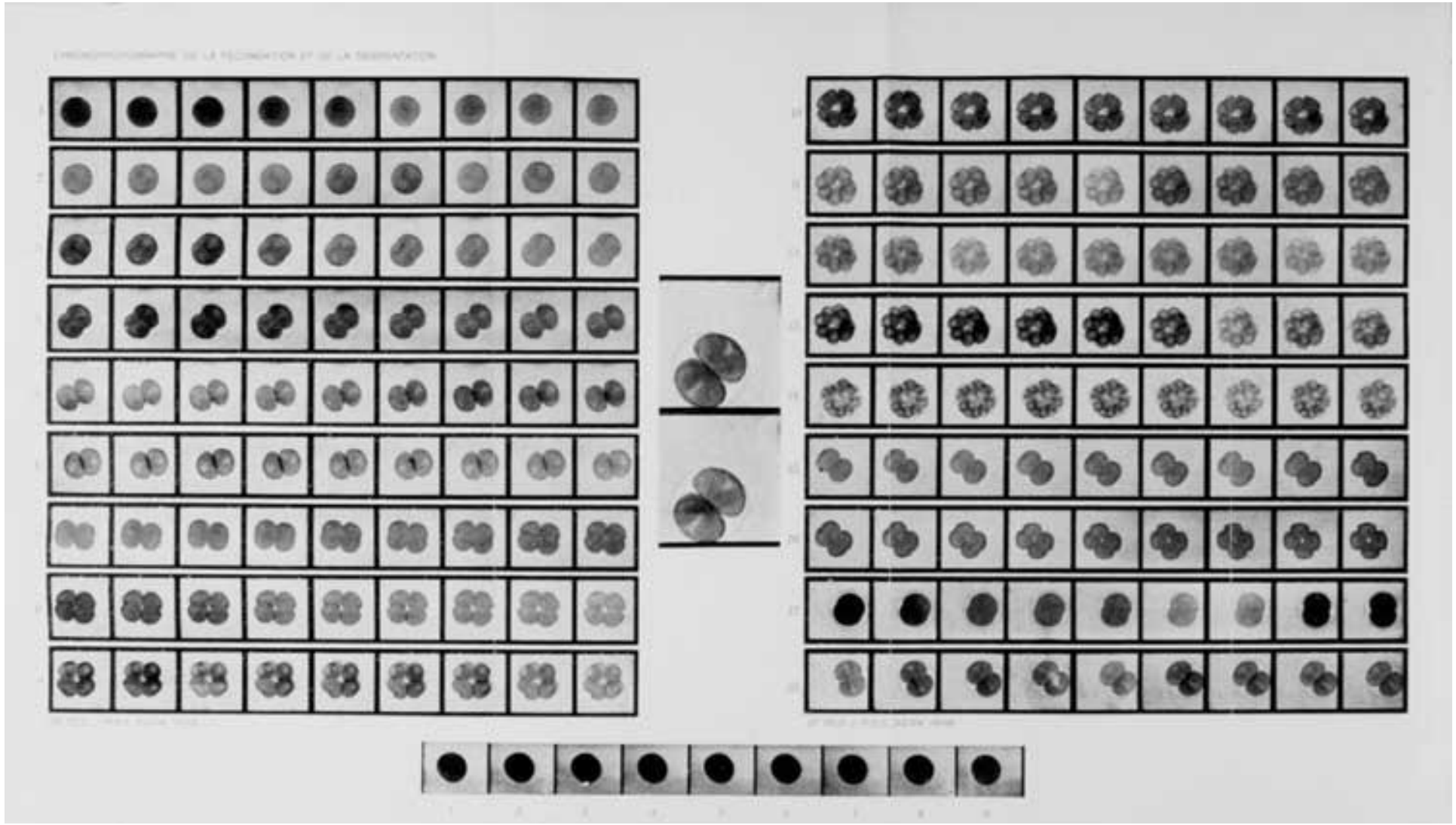
Live cell imaging in SUPERRESOLUTION

IMG
Light Microscopy Core Facility

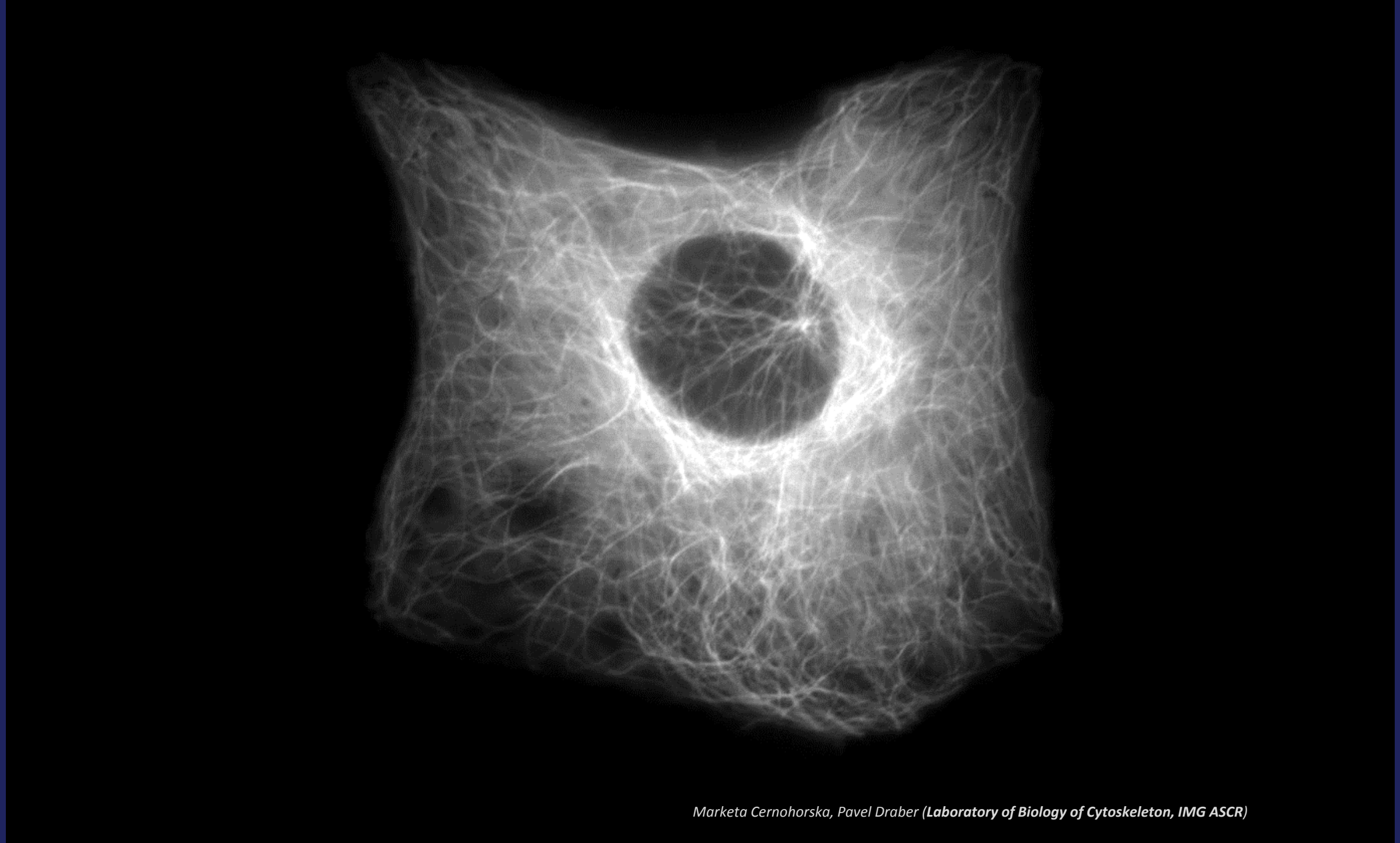
SLM 2017

Ivan Novotny



***Fertilization and Development of the Sea Urchin Egg* by Julius Ries, filmed in Paris in 1907**. One of the earliest time-lapse microcinematographic films ever made.

Hannah Landecker, Nature Methods 6, 707 - 709 (2009)



*Marketa Cernohorska, Pavel Draber (**Laboratory of Biology of Cytoskeleton, IMG ASCR**)*

Live cell imaging in SR

- Essential equipment for live cell imaging
- Visualization of structures in cells using the fluorescence microscopy
- Which methods in SR field for live cell imaging?
- Improve the imaging quality

Live cell imaging

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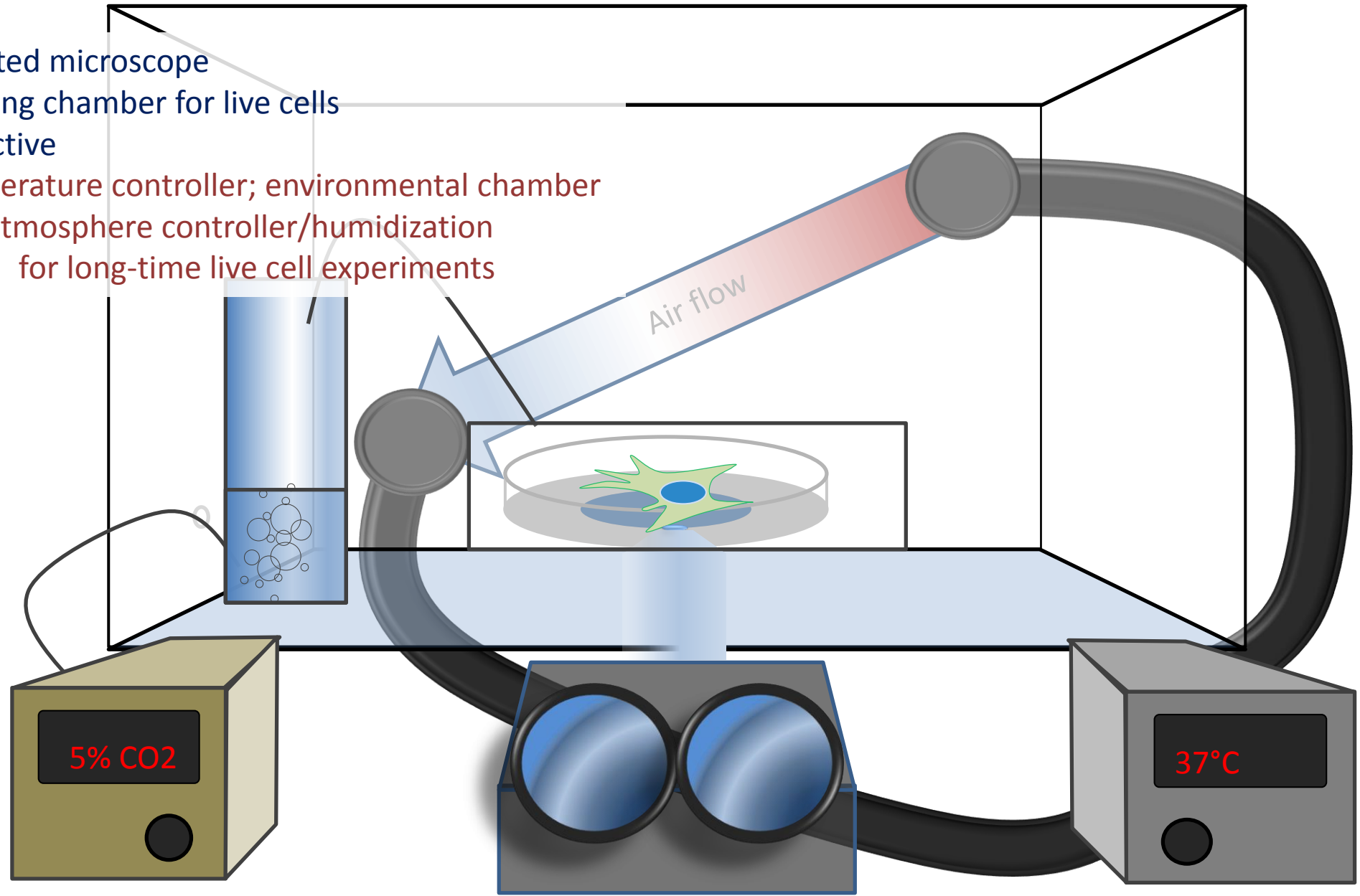
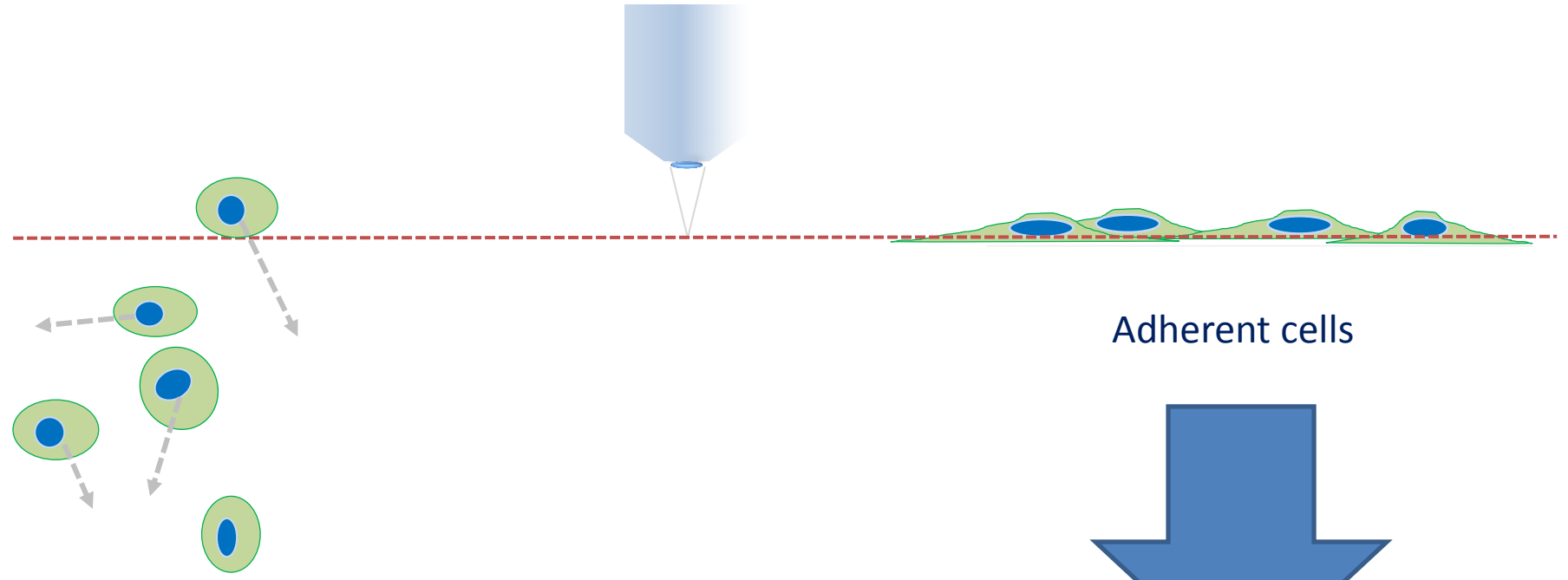
Live cell imaging – essential equipment

- Inverted microscope
- Imaging chamber for live cells
- Objective
- Temperature controller; environmental chamber
- CO_2 atmosphere controller/humidization
  for long-time live cell experiments



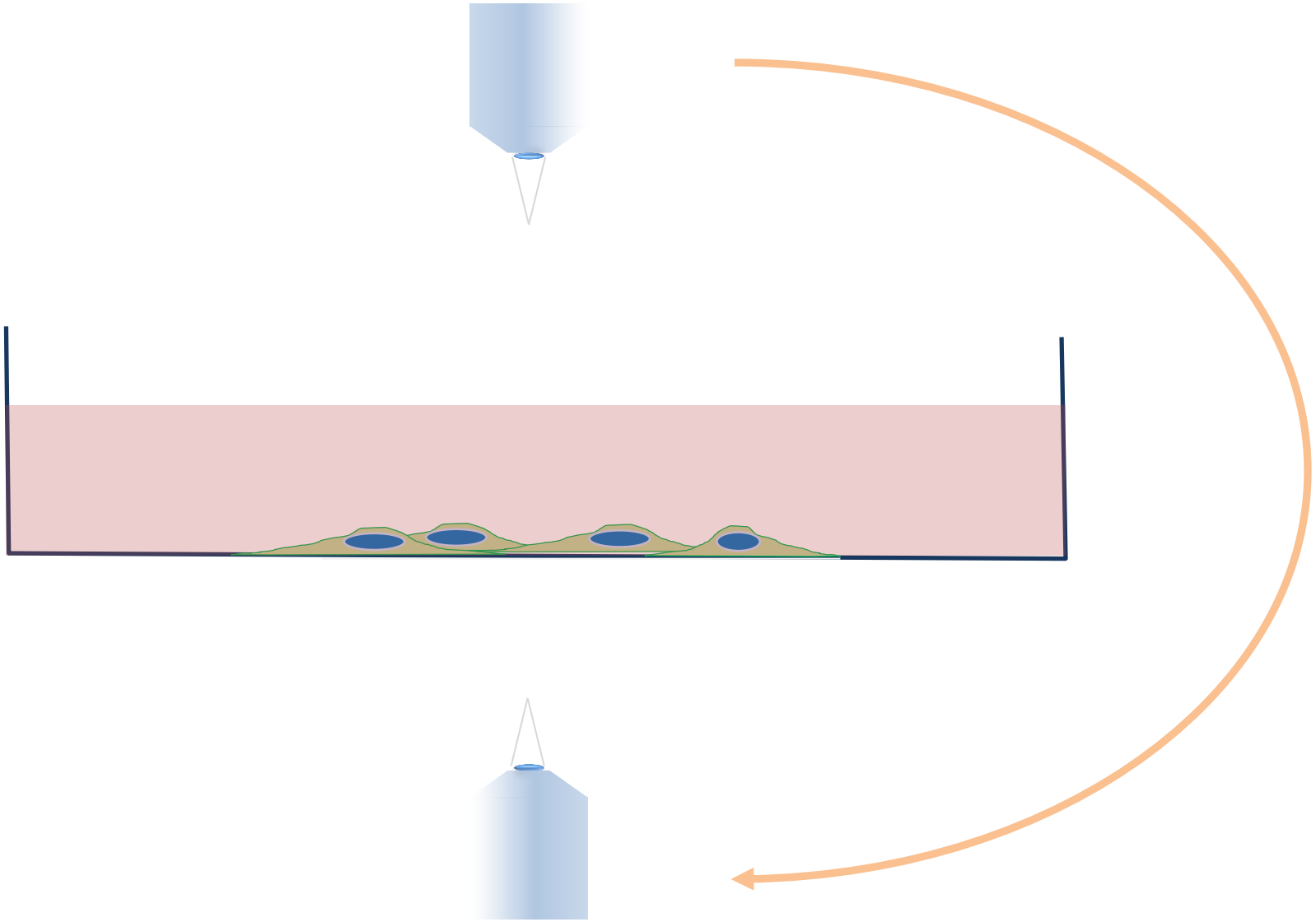
Live cells cultivation & observation



Cells in suspension

Adherent cells

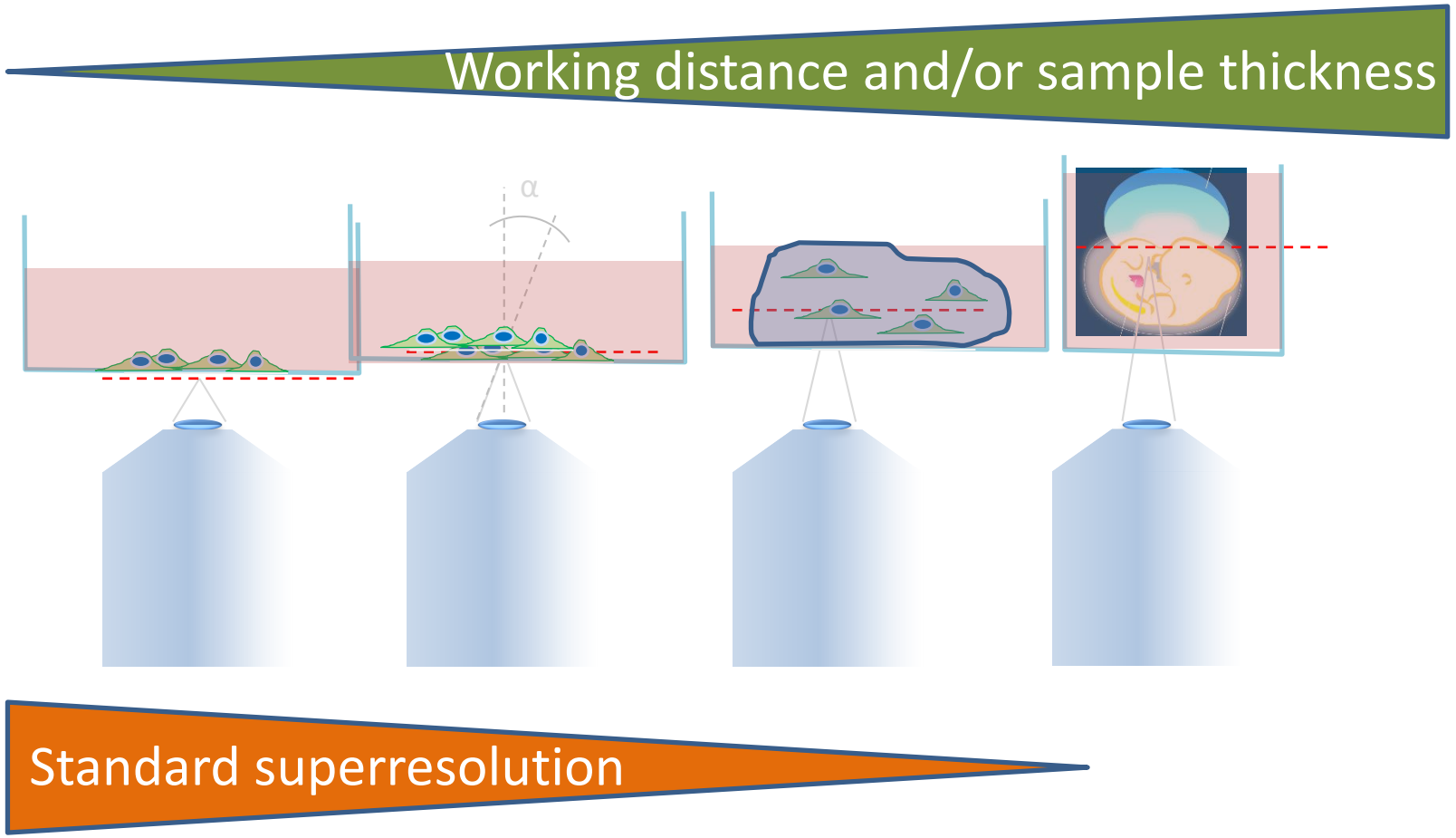
Cultivation & observation



Upright microscope

Inverted microscope

Which superresolution method?

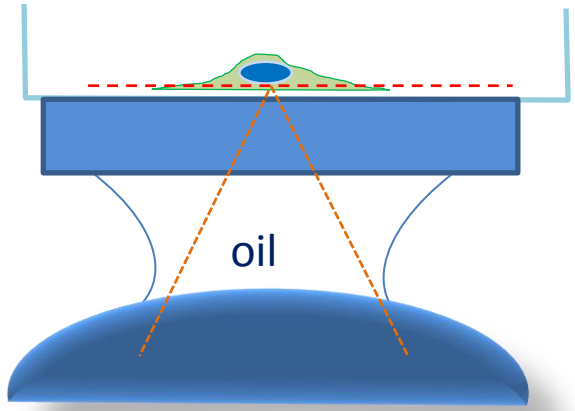


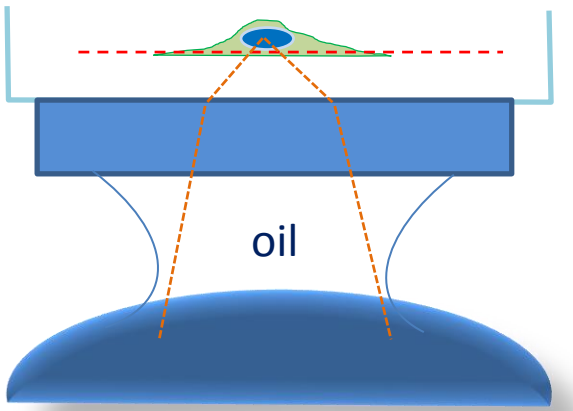
STED	**STED**	**STED**
SIM	**SIM**	SIM
SMLM	SMLM	SMLM

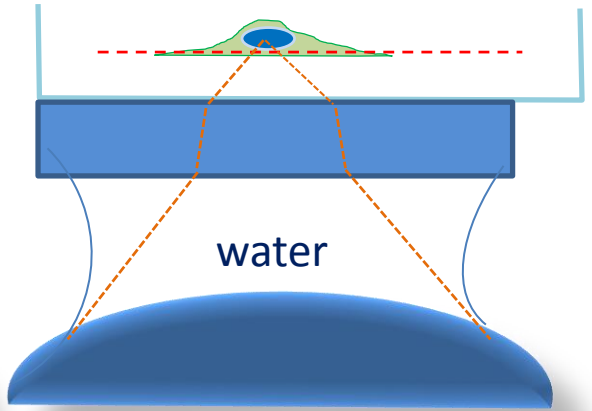
Lens immersion

Attached to the cover glass

Longer path through water-like environment





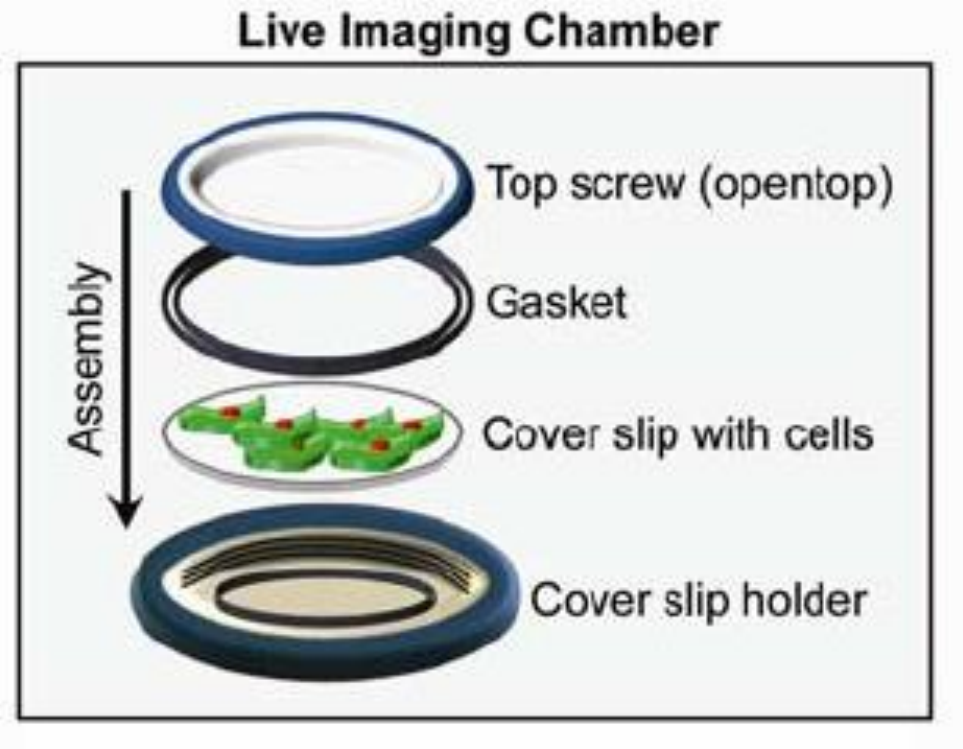


STED
SIM
SMLM

STED
SIM?
SMLM

Dishes/chambers for live cell imaging (and cultivation)

Imaging glass-bottom chambers

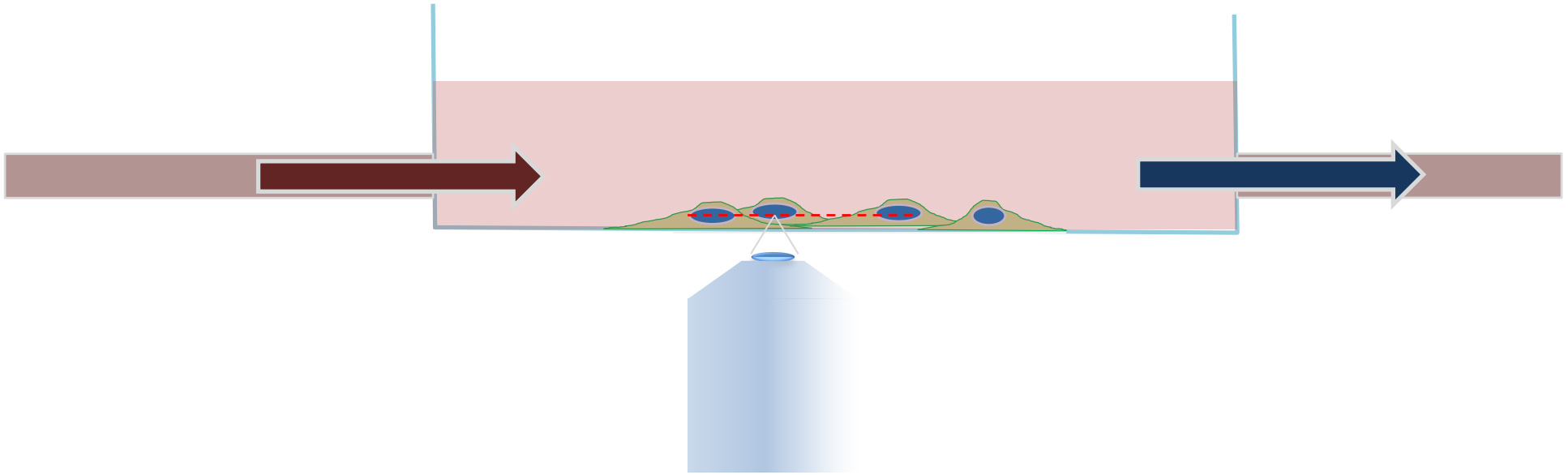


GLASS THICKNESS!!! – use High precision bottom-glass

1.5# = 170 µm ± 5 µm

Dishes/chambers for live cell imaging

Perfusion chamber – the medium is exchanged during the time-lapse experiment



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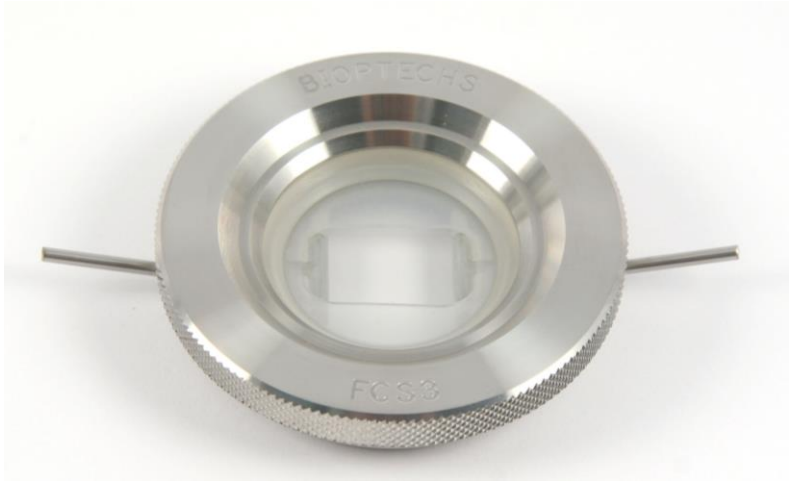

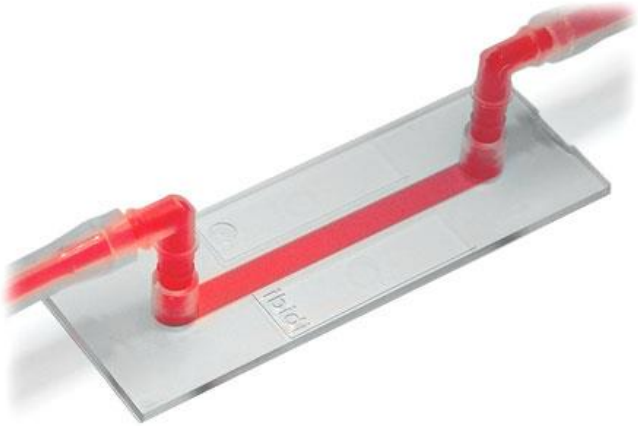

Live cell imaging – essential equipment

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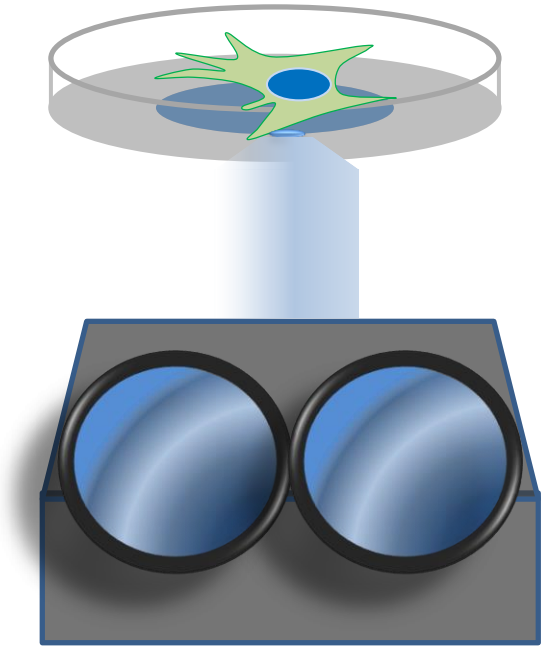

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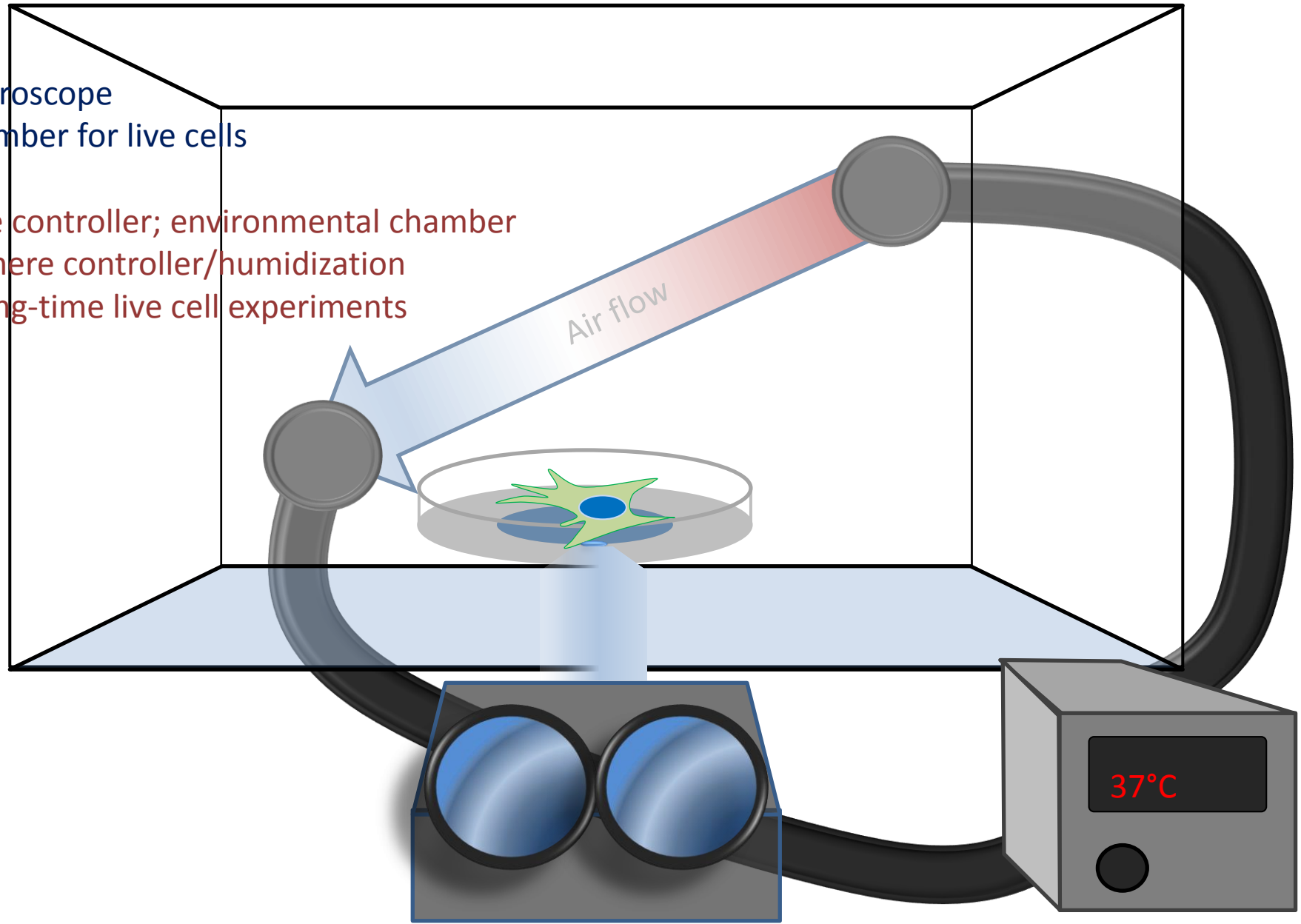

Plexiglas incubation chamber with chamber for CO2 atmosphere

Plexiglas incubation chamber + CO2

..or heated cell imaging chamber, CO2 atmosphere controller

..or heated cell imaging chamber + CO2

And heated objective – good idea

Live cell imaging – essential equipment

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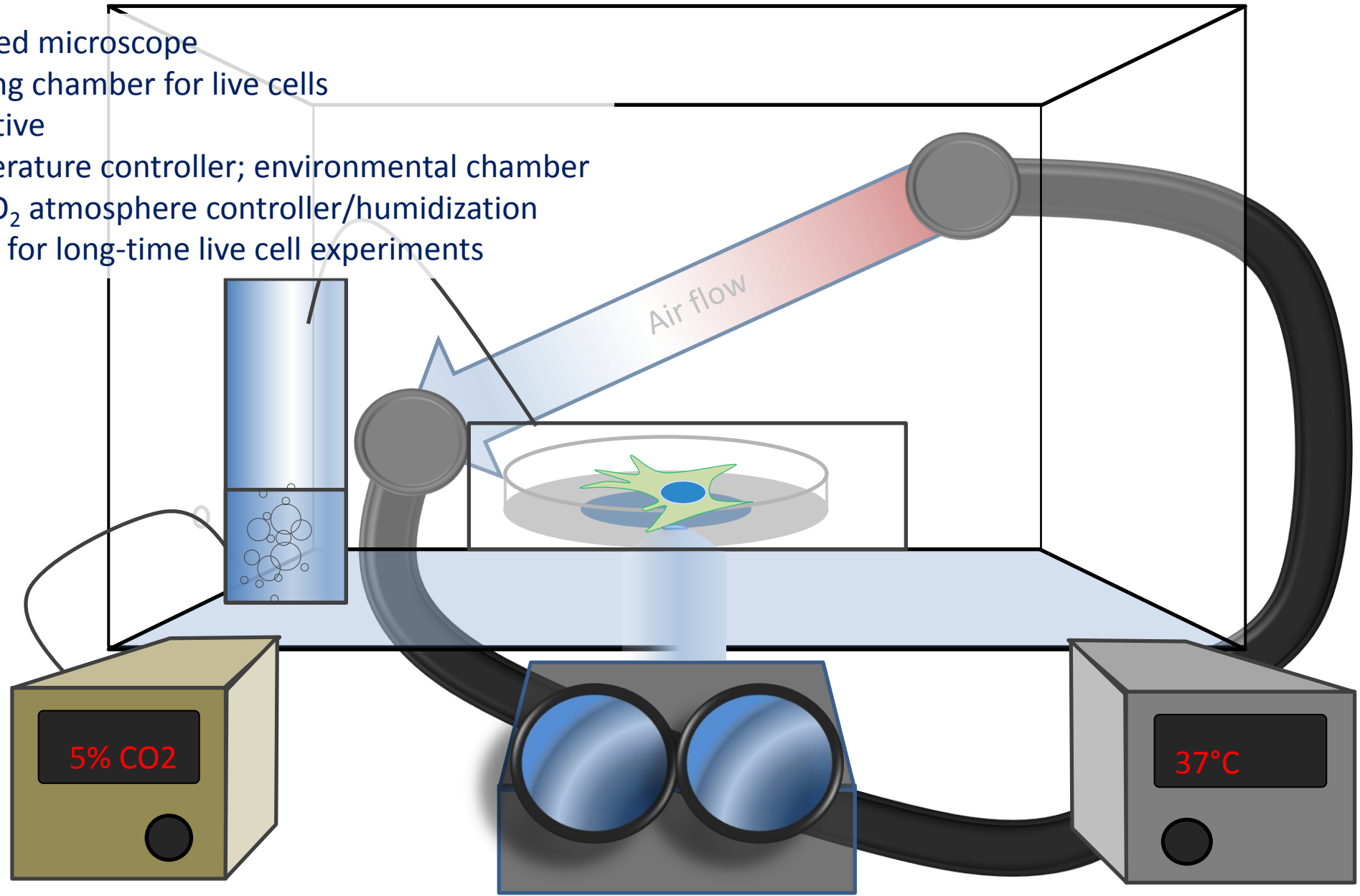

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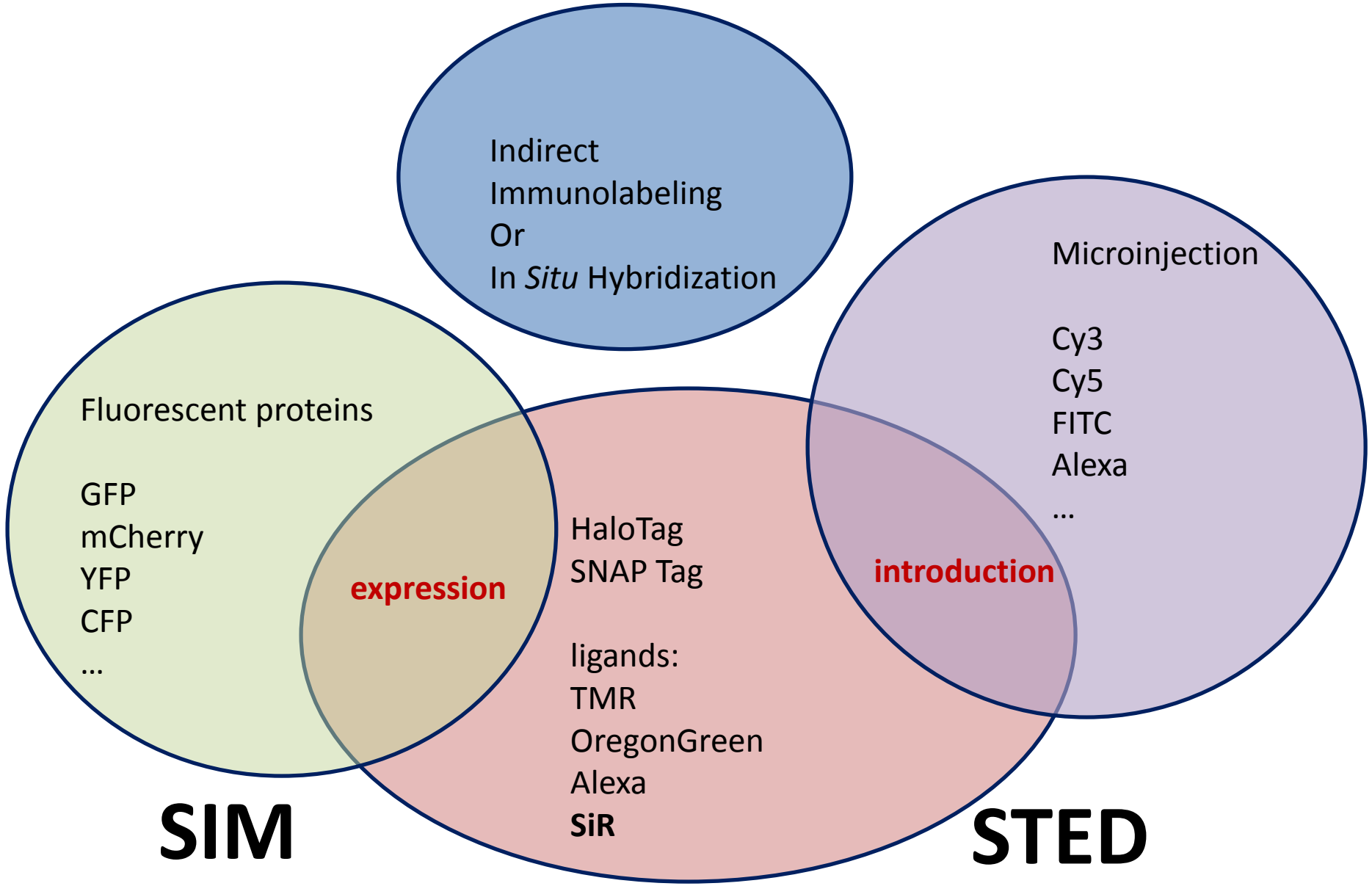
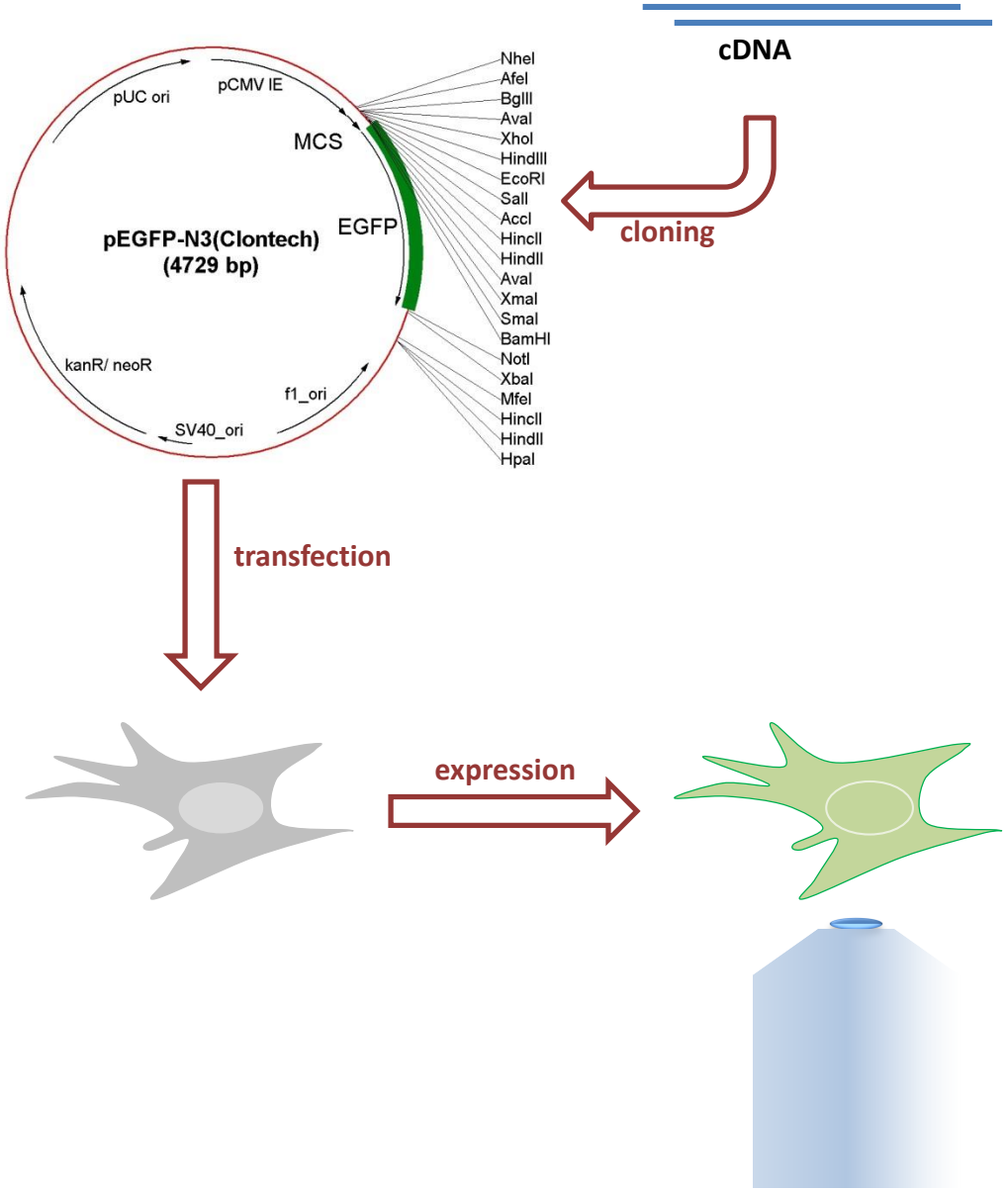
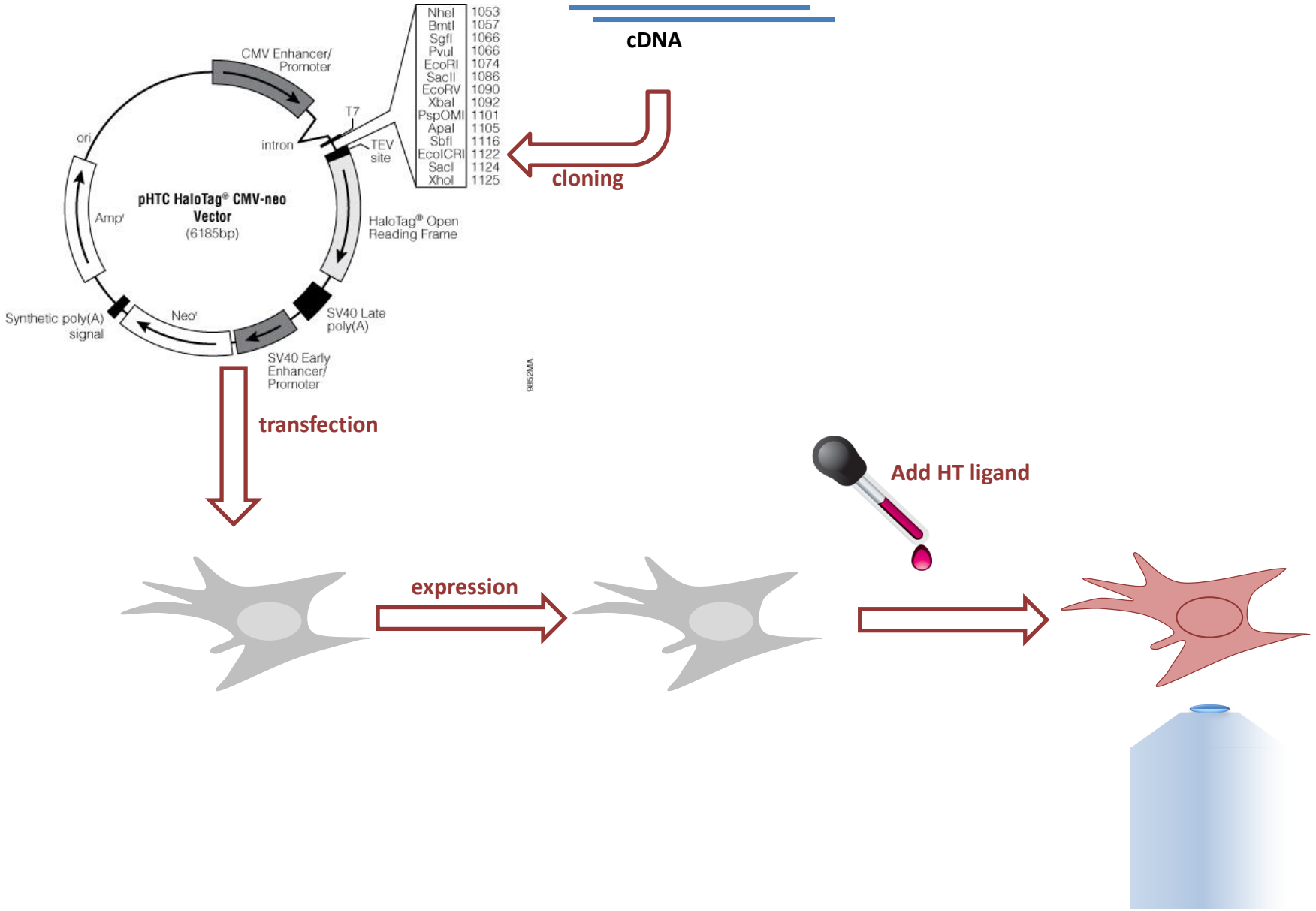
Visualization of cell structures in fluorescence microscopy



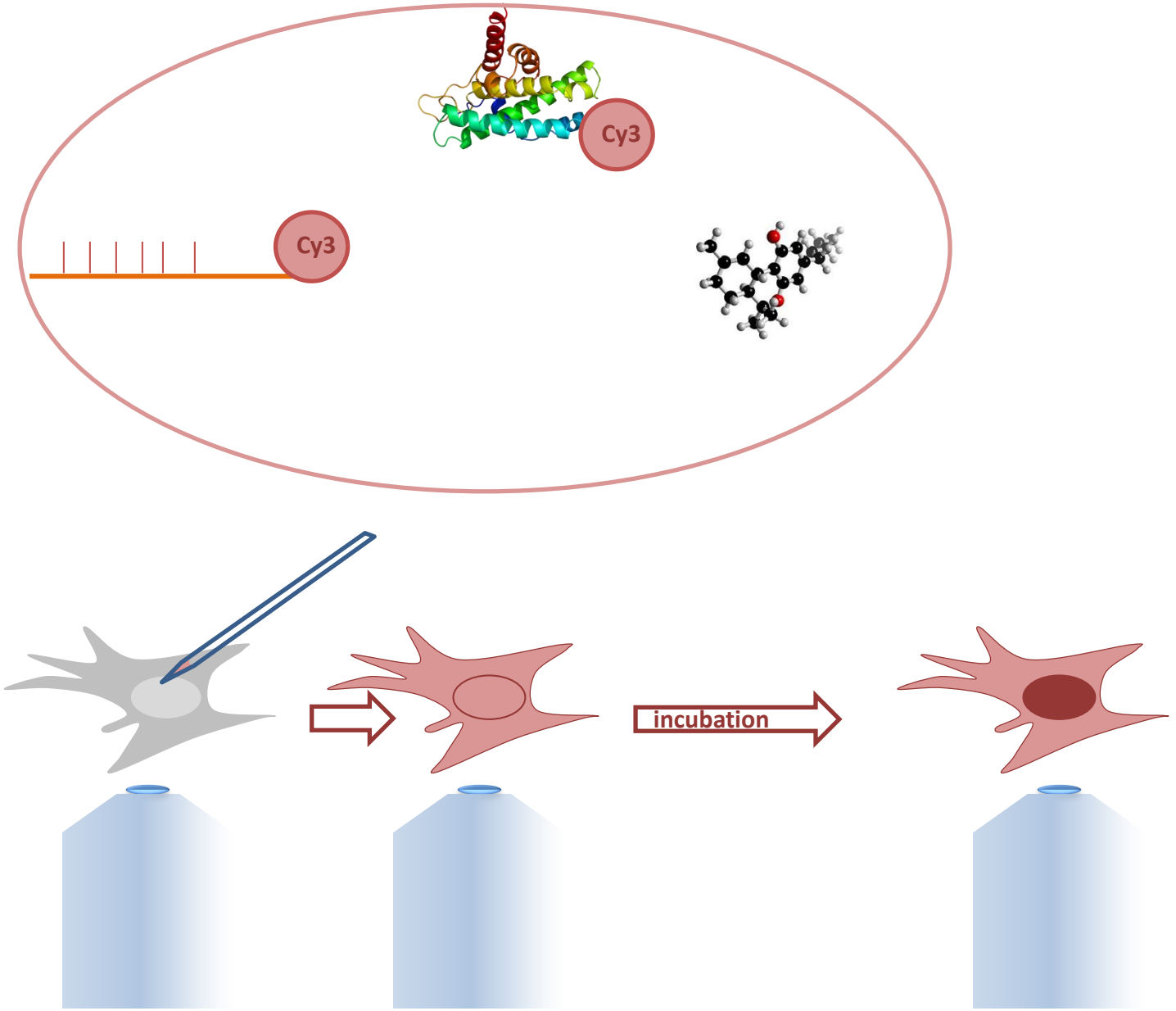
Fluorescent proteins



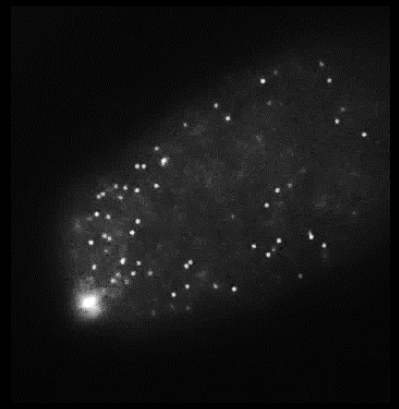
e.g. HaloTag system…



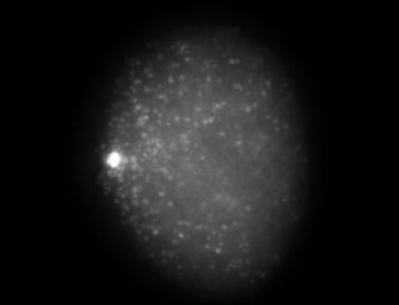
Microinjection



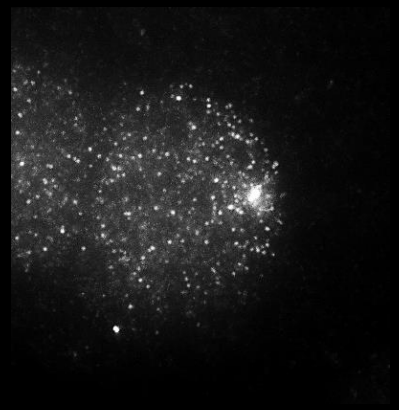
The same reporter, different visualization



GFP tagged protein of interest



HaloTagged protein of interest + TMR ligand



Microinjected Cy3 tagged protein

*Ivan Novotny, Edouard Bertrand (**Laboratory of RNA Biogenesis, IGMM CNRS**)*

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Live cell imaging – general rule:

The sample MUST NOT move within the single time-frame acquisition!

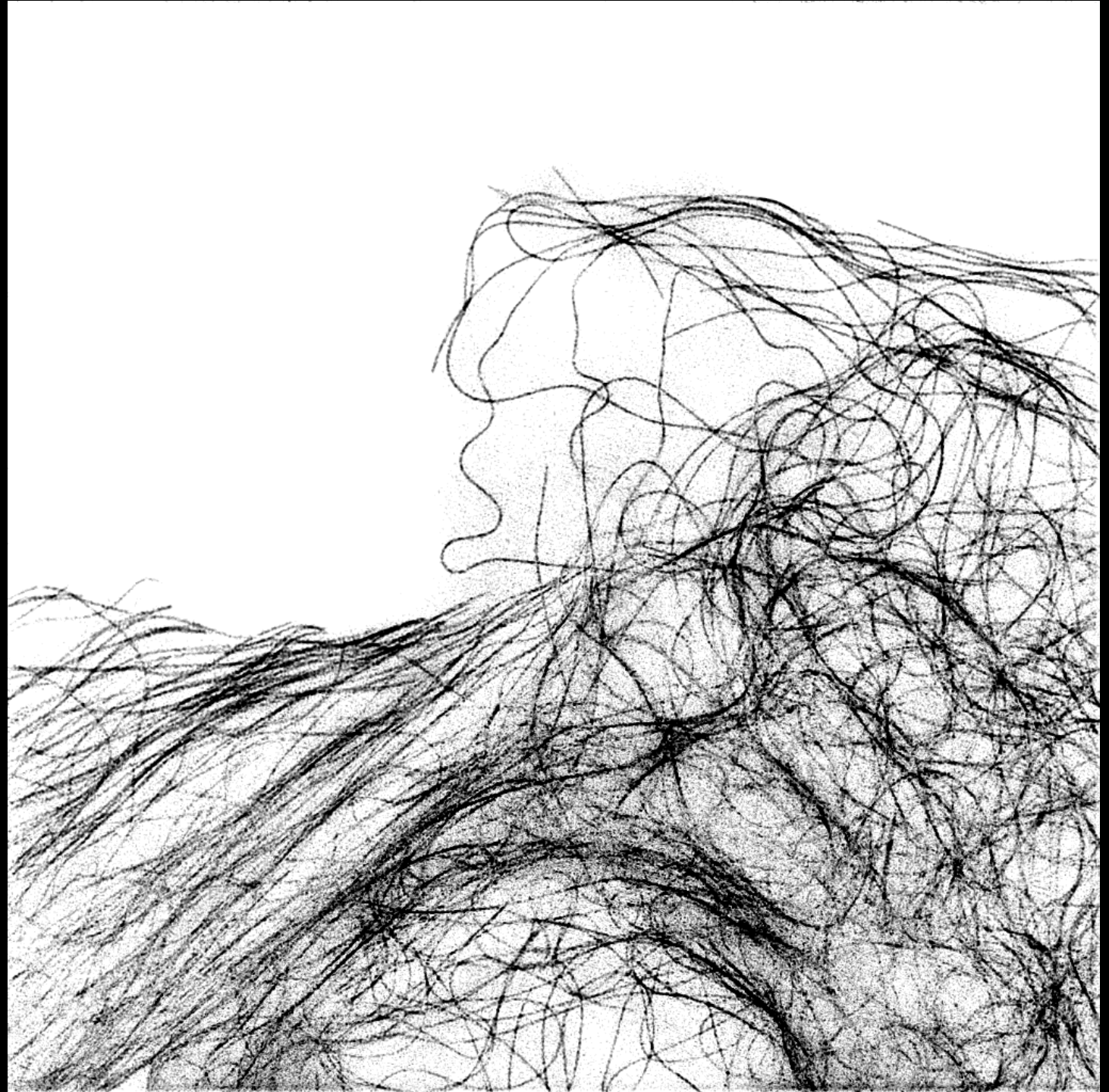
Live cell imaging - SIM

SIM Strengths

X-Y-Z resolution of 110 × 110 × 360 nm for 488nm excitation in the 3D mode; lateral resolution of 100 nm in the TIRF mode
No special sample preparation or fluorophore requirements
2-color 3D live cell imaging capability

SIM Limitations

Samples for 3D SIM should be thinner than 12 µm.
The SIM reconstruction - motion artifacts when **sample moves while the illumination pattern changes** more than 100nm per image.
Photobleaching and photo-toxicity can really restrict imaging time.



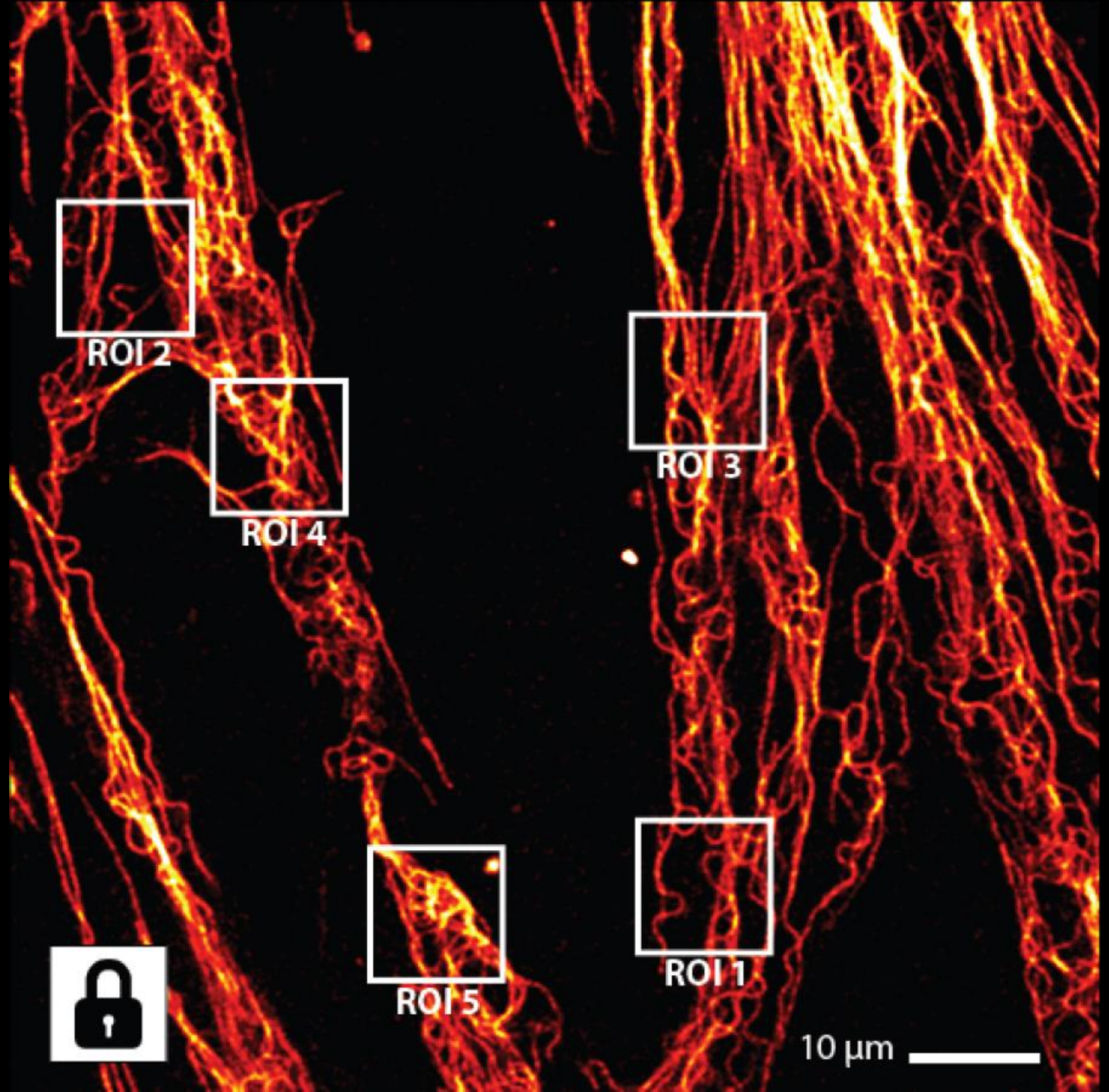
Live cell imaging - STED

STED Strengths

X-Y-Z resolution of 40 × 40× 300 nm with optimal settings
No mathematical reconstruction of the final image
Can go deeper to the specimen and acquire thicker z-stack (theoretically)

STED Limitations

Very high energy – influence the cell viability
Relatively slow acquisition
Limitation in fluorophores/depletion laser


ROI 2
ROI 4
ROI 3
ROI 5
ROI 1
10 µm

Live cell imaging – Localization..

Very limited, very high photo-toxicity and long time for one single time frame..

Blinking dyes for live cells:
Oregon green
TMR
SiR/HMSiR

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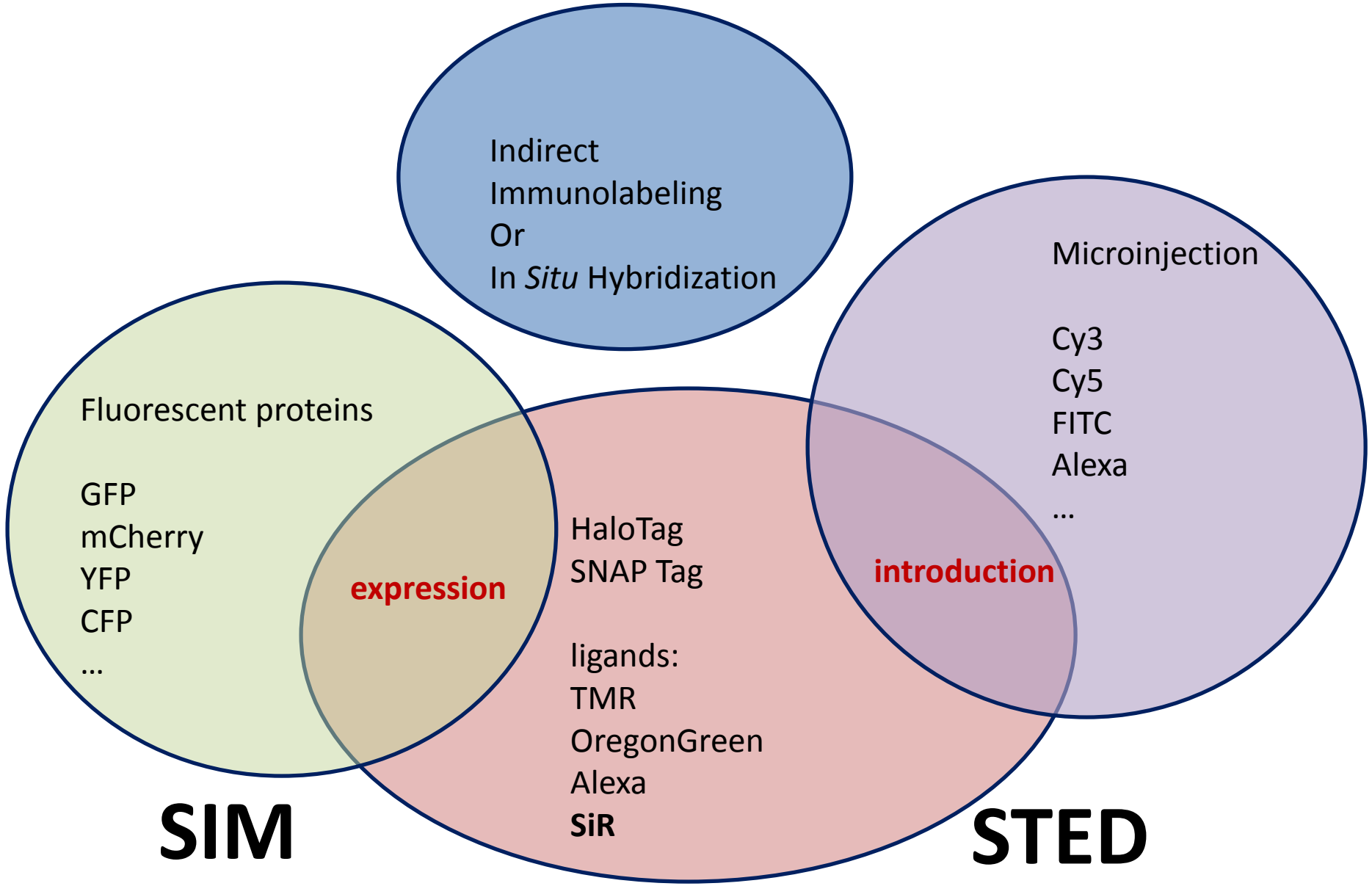
- Improve the imaging quality – HOW?? Dyes.

Visualization of cell structures in fluorescence microscopy



What would be real SR method, robust and suitable for most sample types --

Confocal = **STED**

Wide-spread method..

Lattice lightsheet in SIM mode

Nowadays very limited reachability

Keep cells alive as long as possible = use as far red as possible

Far-red dyes for live cell development

Silicon-rhodamines **- SiR** is a bright and far-red fluorophore with excitation and emission wavelengths around 650 and 670 nm, a spectral range where very little autofluorescence and phototoxicity

Live cell imaging - STED

SiR F-Actin labelling

Abs/Em: 652/674 nm

50 nmol SiR-actin Kit

SiR-DNA labelling

Abs/Em: 652/674 nm

50 nmol SiR-DNA Kit

SiR-Lysosome labelling

Abs/Em: 652/674 nm

50 nmol SiR-lysosome Kit

SiR-Microtubule labelling

Abs/Em: 652/674 nm

50 nmol SiR-tubulin Kit

SiR-Cytoskeleton Kit

Abs/Em: 652/674 nm

50 nmol SiR-actin +
50 nmol SiR-tubulin

https://spirochrome.com/products/

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Covalently Attach Fluorescent Labels or Affinity Tags to Membrane or Intracellular Proteins

- Cell permeant TMR, Oregon Green®, diAcFAM and Coumarin ligands readily cross the cell membrane for labeling intracellular proteins
- Cell impermeant tags include Alexa Fluor® 488 and 660 for quick cell surface labeling

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diAcFAM (1mM)	TMR (5mM)	Oregon Green® (1mM)	PEG-Biotin (5mM)
R110Direct™ (0.1mM)	TMRDirect™ (0.1mM)		

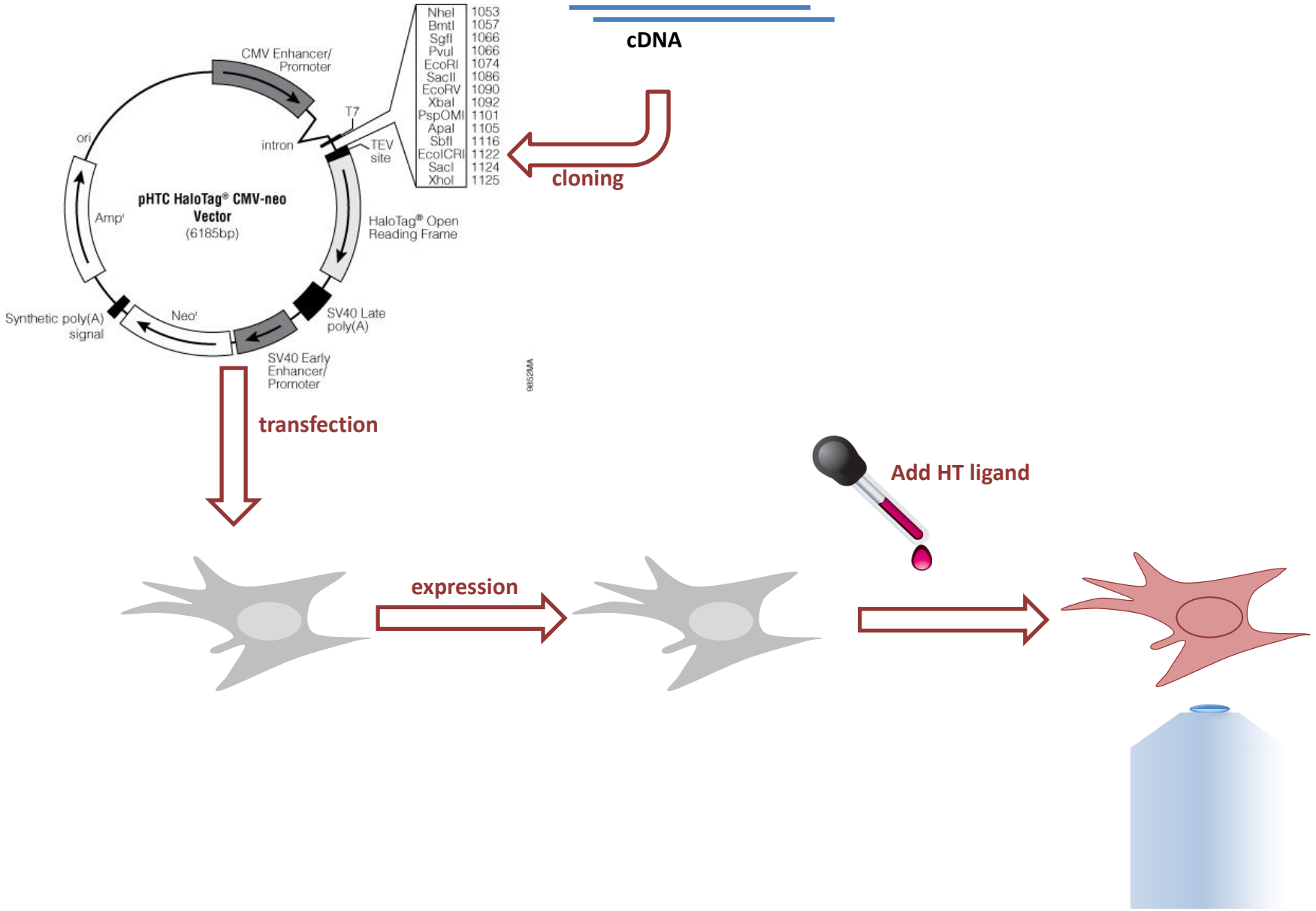
Size

15µl	30µl

Catalog number selected: **G8251**

Please Enquire

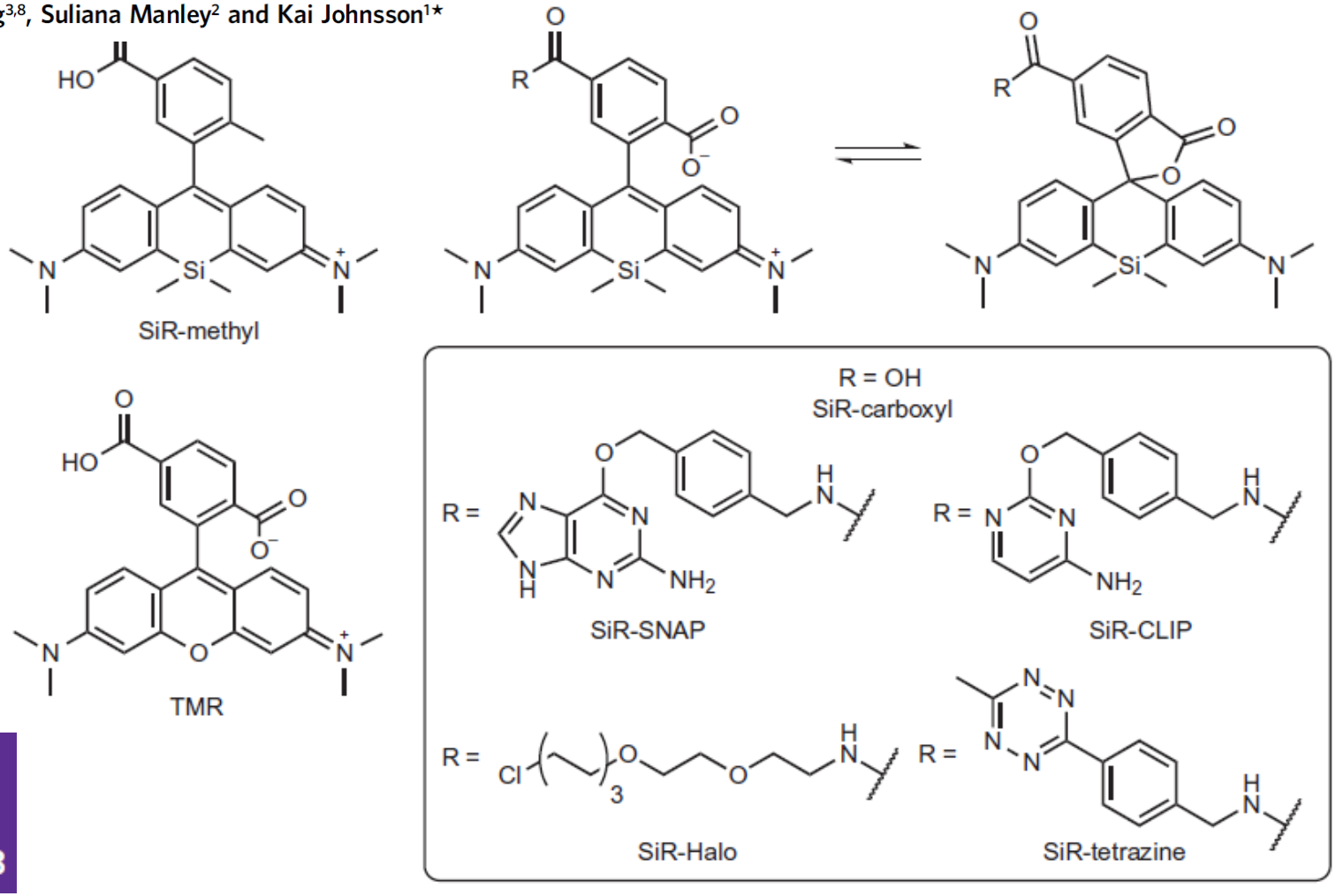
HaloTag system…



NATURE CHEMISTRY DOI: 10.1038/NCHEM.1546

A near-infrared fluorophore for live-cell super-resolution microscopy of cellular proteins

Gražvydas Lukinavičius[1†], Keitaro Umezawa[1†], Nicolas Olivier[2], Alf Honigmann[3], Guoying Yang[4], Tilman Plass[5], Veronika Mueller[3], Luc Reymond[1], Ivan R. Corrêa Jr[6], Zhen-Ge Luo[7], Carsten Schultz[5], Edward A. Lemke[5], Paul Heppenstall[4], Christian Eggeling[3,8], Suliana Manley[2] and Kai Johnsson[1]*



ARTICLES
PUBLISHED ONLINE: 6 JANUARY 2013

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- It is a cell-permeable substrate (also termed SiR-SNAP) based on 6-carboxy-tetramethylsiliconrhodamine
- It is suitable for standard Cy5 filter sets
- It has an excitation maximum at 645 nm and an emission maximum at 661 nm

Ordering Information

Catalog #	S9102S
Size	30 nmol
List Price	$307.00
Your Price	Sign In
Quantity	1

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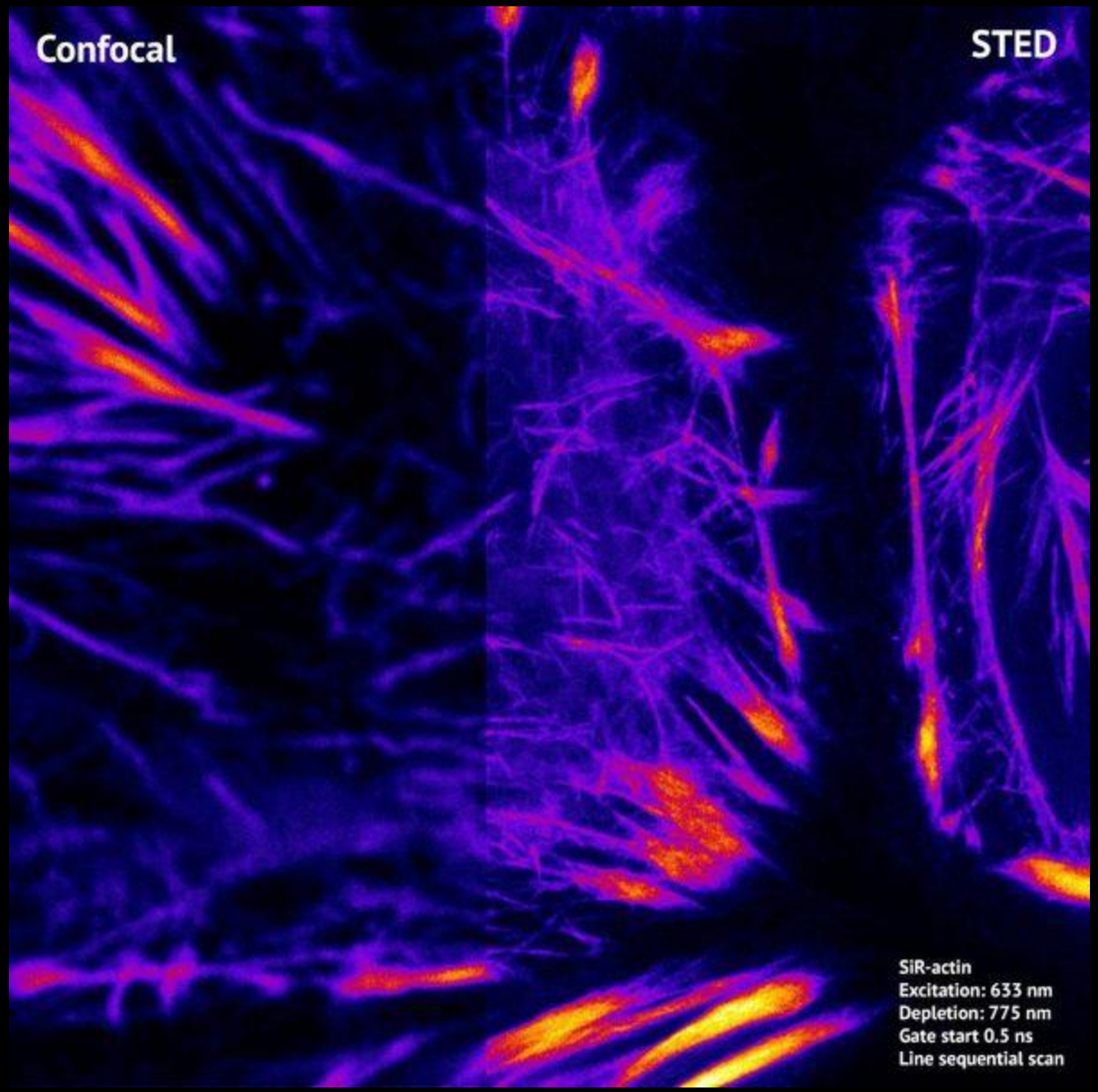
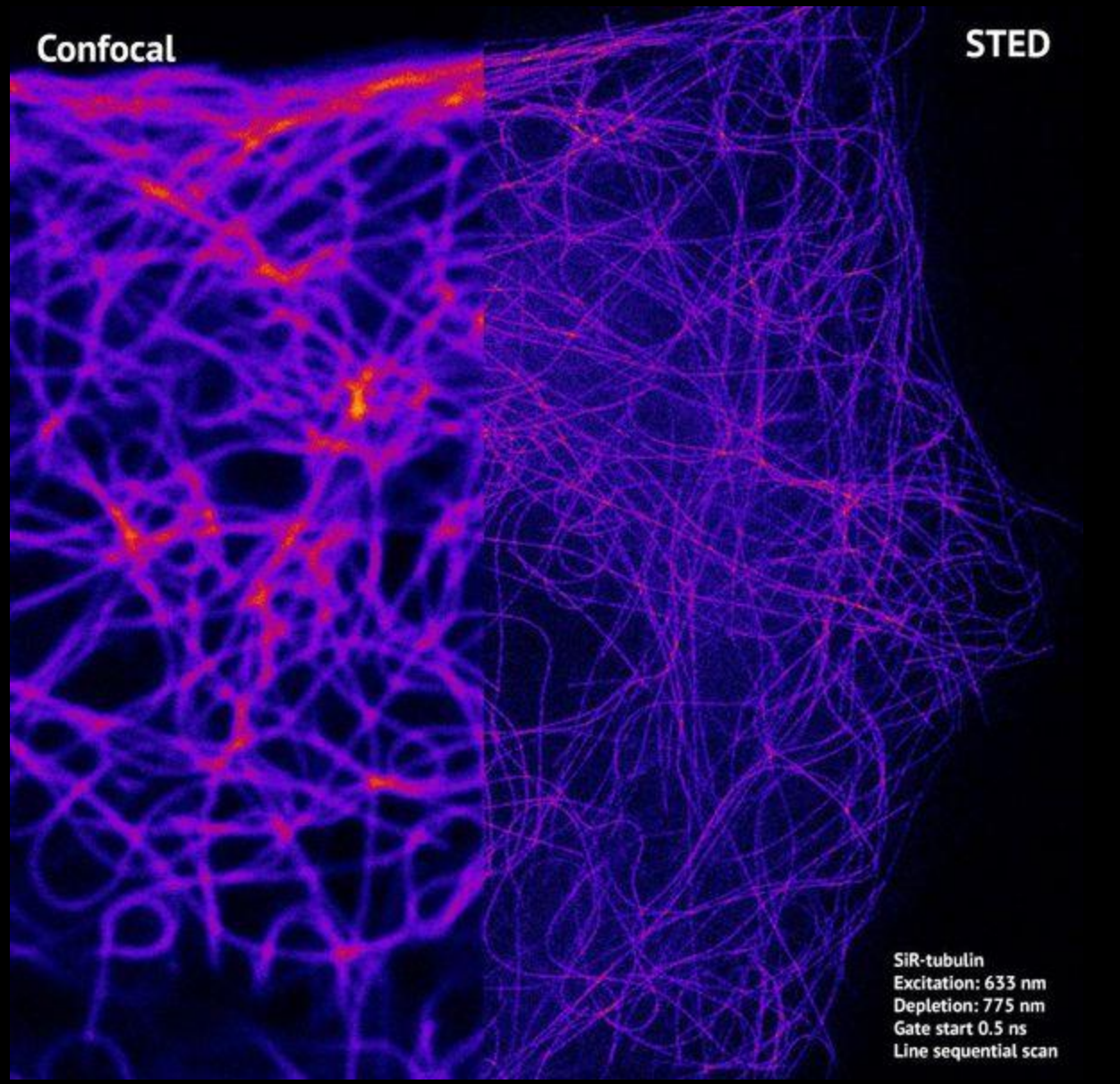
STED with 775 nm depletion laser + SiR





https://www.leica-microsystems.com/science-lab/a-bright-dye-for-live-cell-sted-microscopy/#fancybox-14918-3

Thank you for the attention!

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Many thanks for support:

Institute of Molecular Genetics, CAS

Czech-Bioimaging Infrastructure – LM2015062

“Centre of Model Organisms” OPPK (CZ.2.16/3.1.00/21547)

“Biomodels for health” (LO1419)

CZ.02.1.01/0.0/0.0/16_013/0001775 Modernizace a podpora výzkumných aktivit národní infrastruktury pro biologické a medicínské zobrazování Czech-BioImaging

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MINISTRY OF EDUCATION, YOUTH AND SPORTS

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