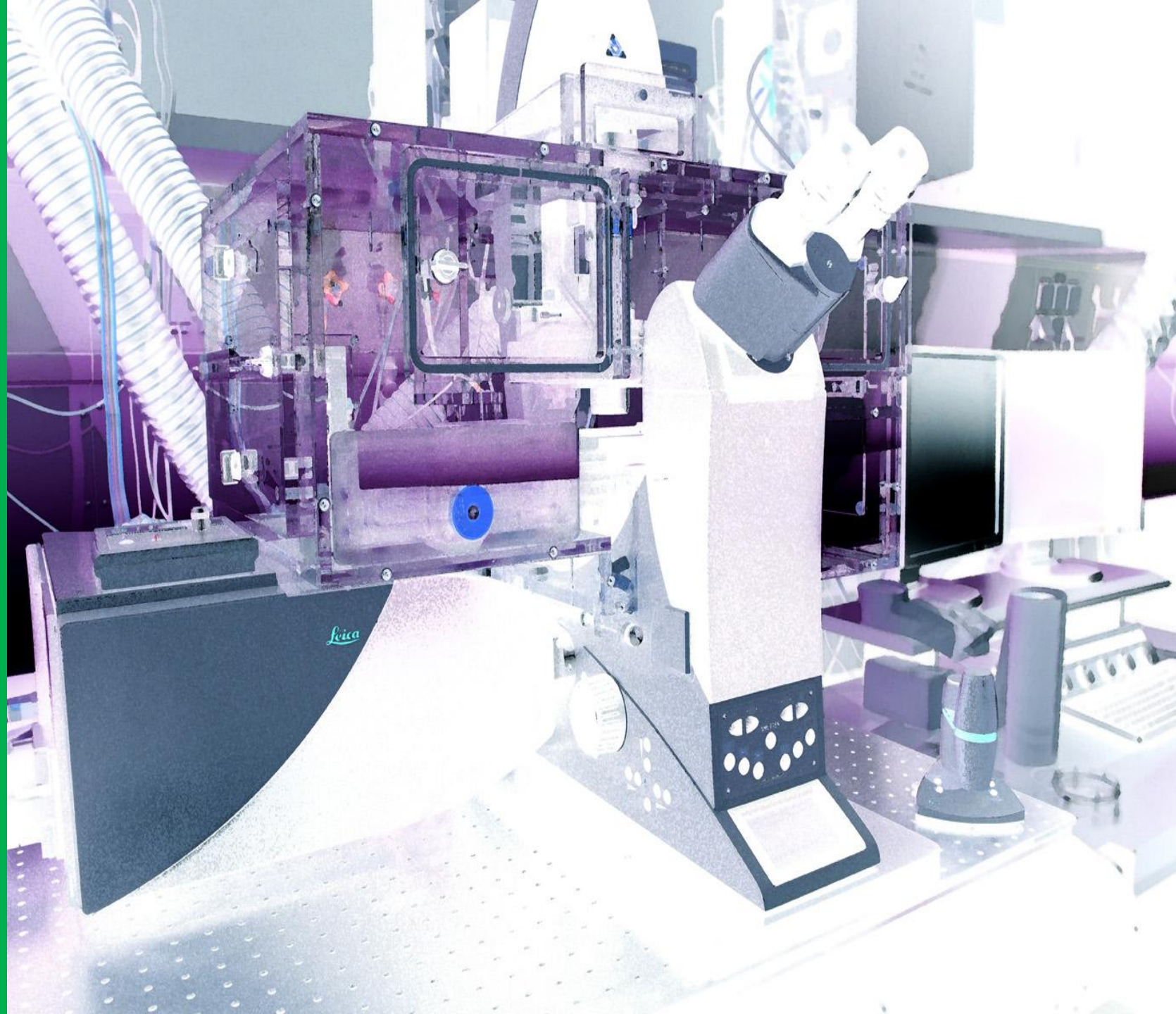
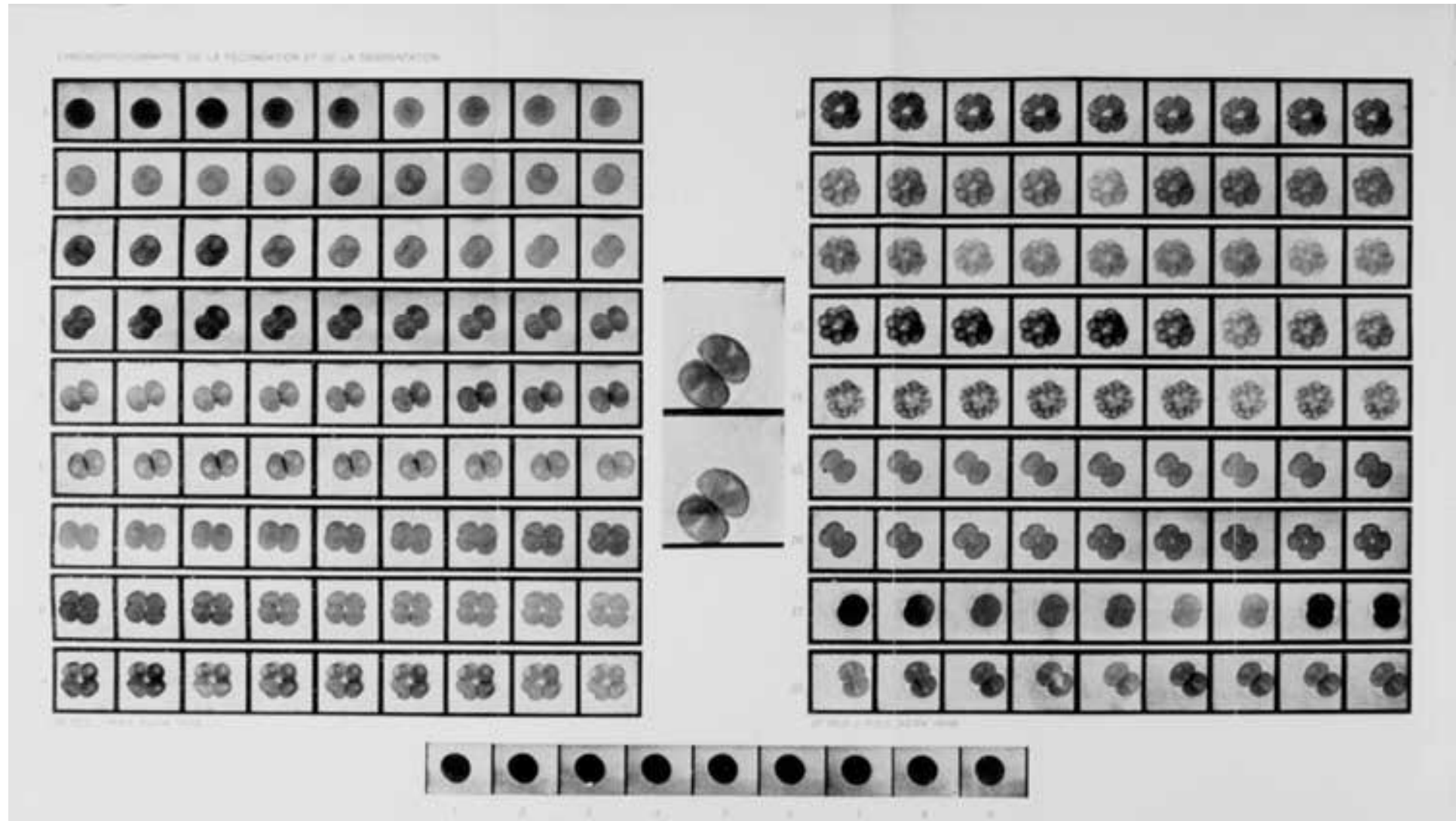


MMIB 2025

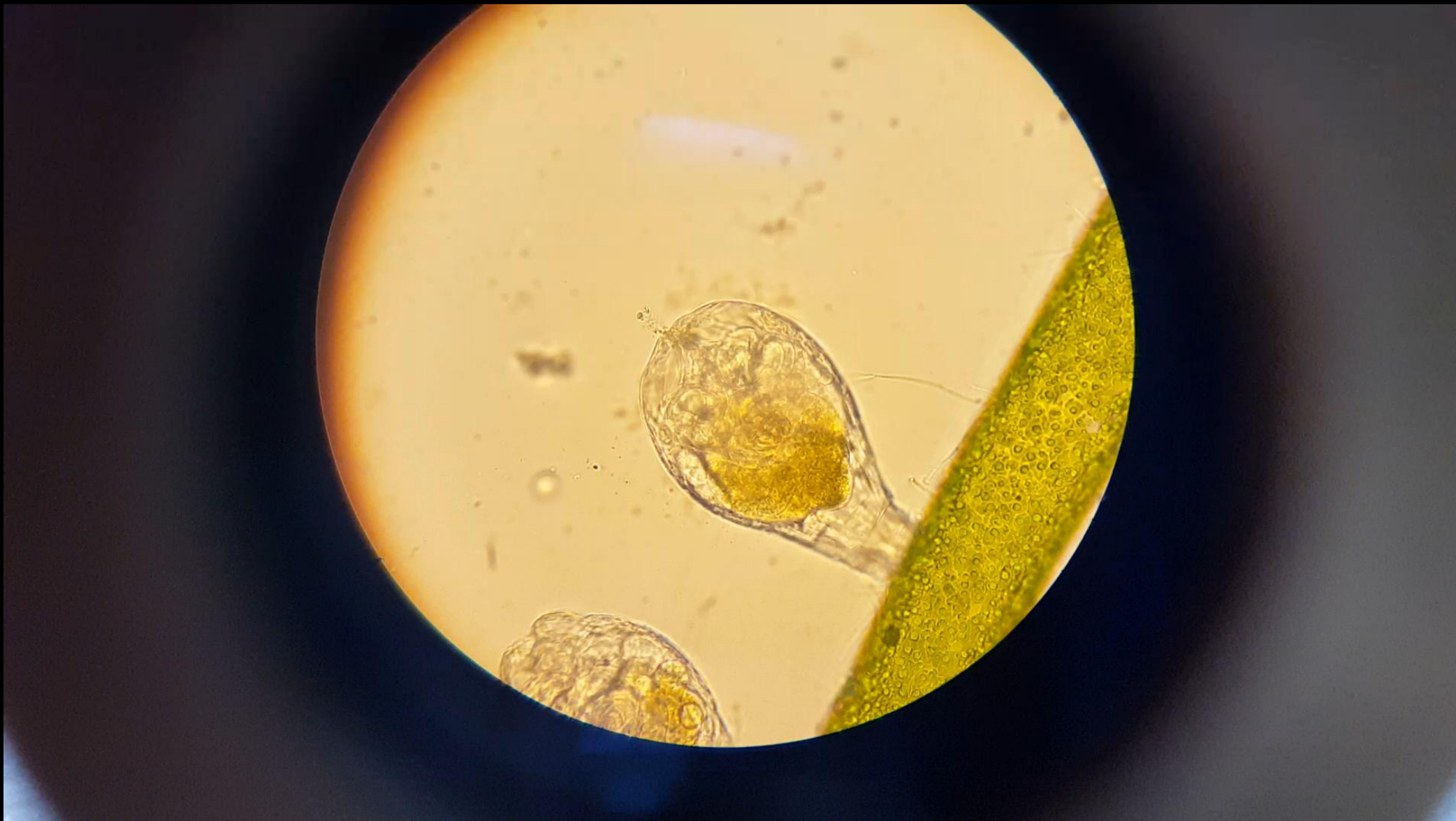
Live cell imaging

Ivan Novotny
LMCF IMG CAS

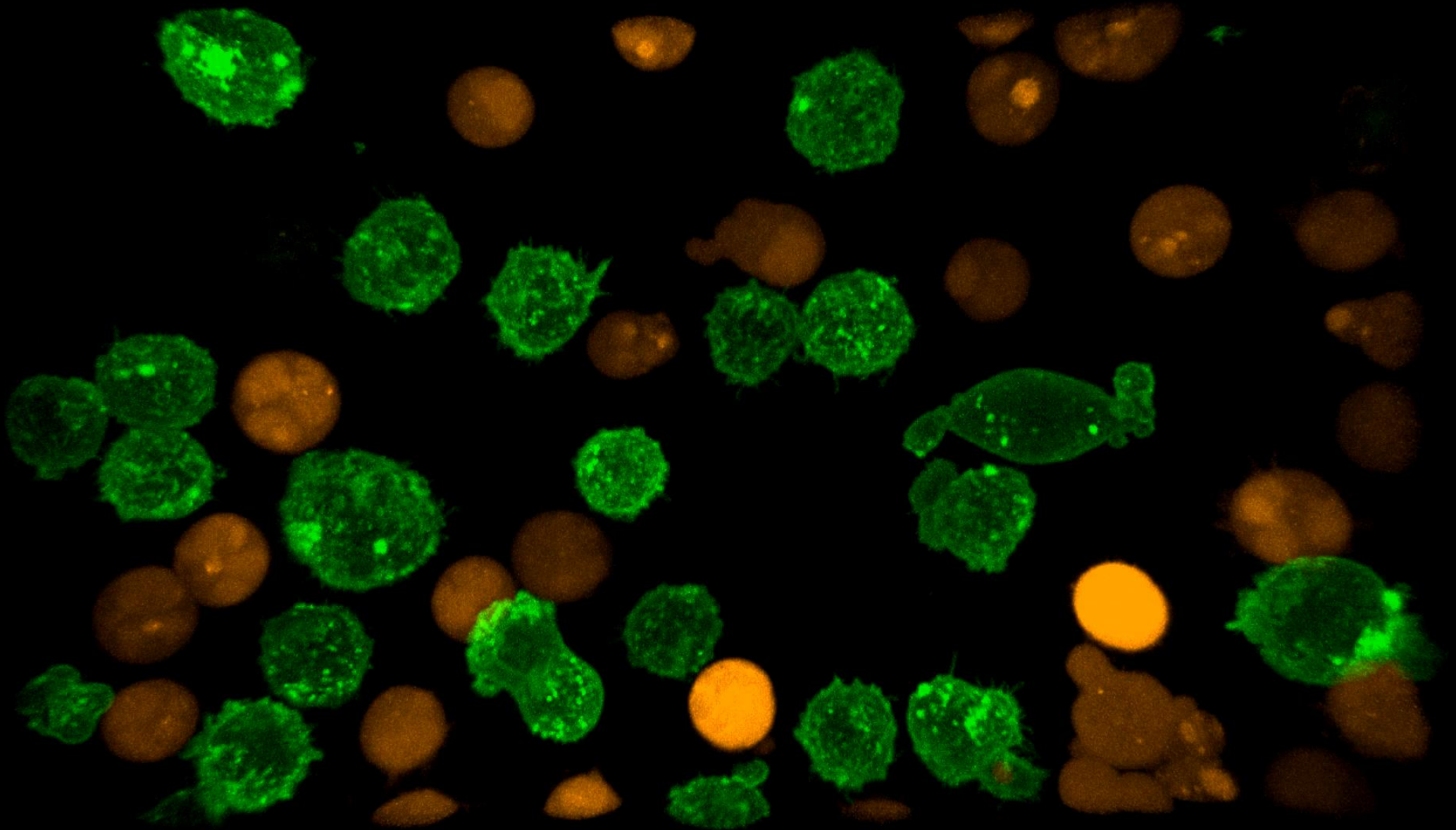




Fertilization and Development of the Sea Urchin Egg by Julius Ries, filmed in Paris in 1907. One of the earliest time-lapse microcinematographic films ever made.



Time-lapse experiment



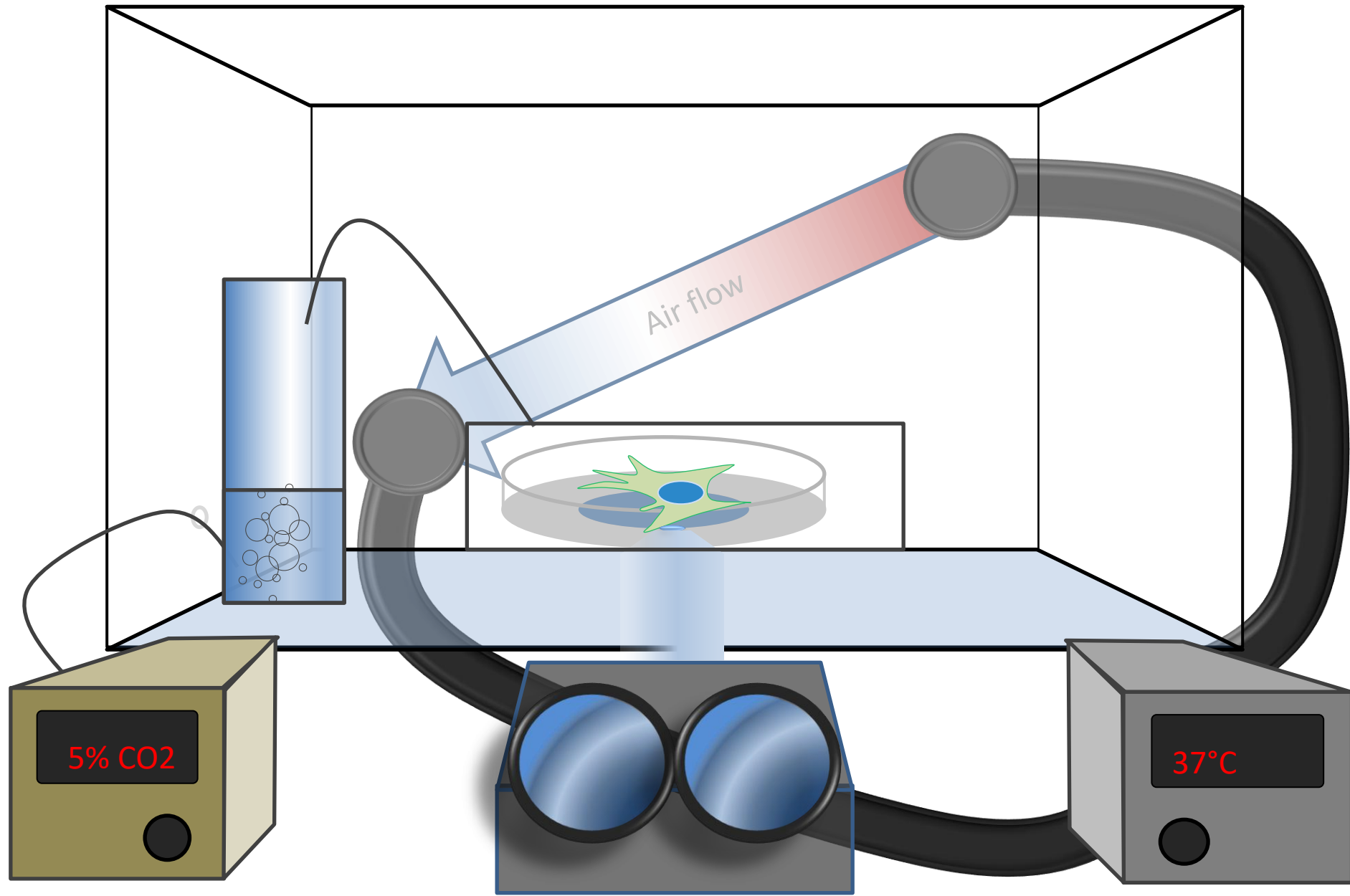
Live cell imaging

- Essential equipment for live cell imaging
- Visualization of cell structures in fluorescence microscopy
- Which methods use live cell imaging – the main purpose
- How to improve a quality of the imaging

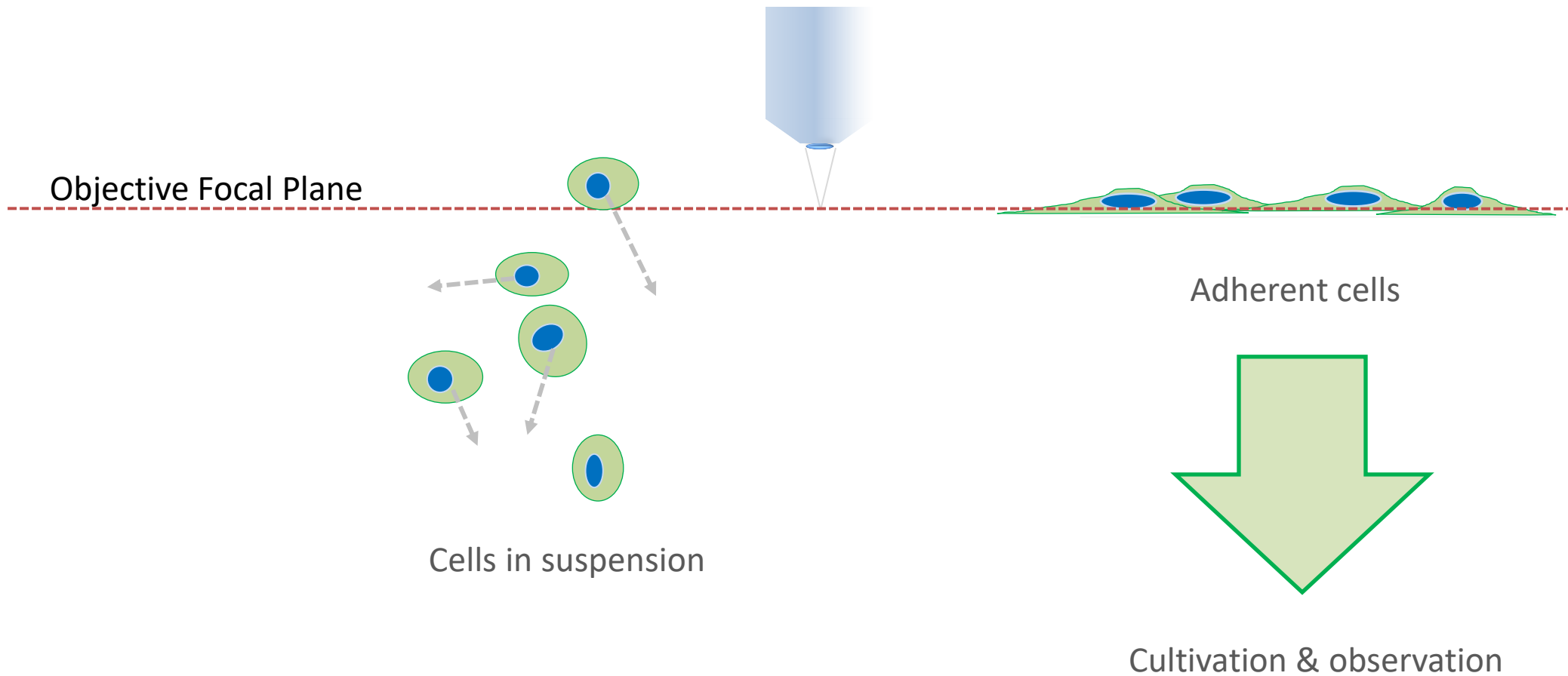
Live cell imaging

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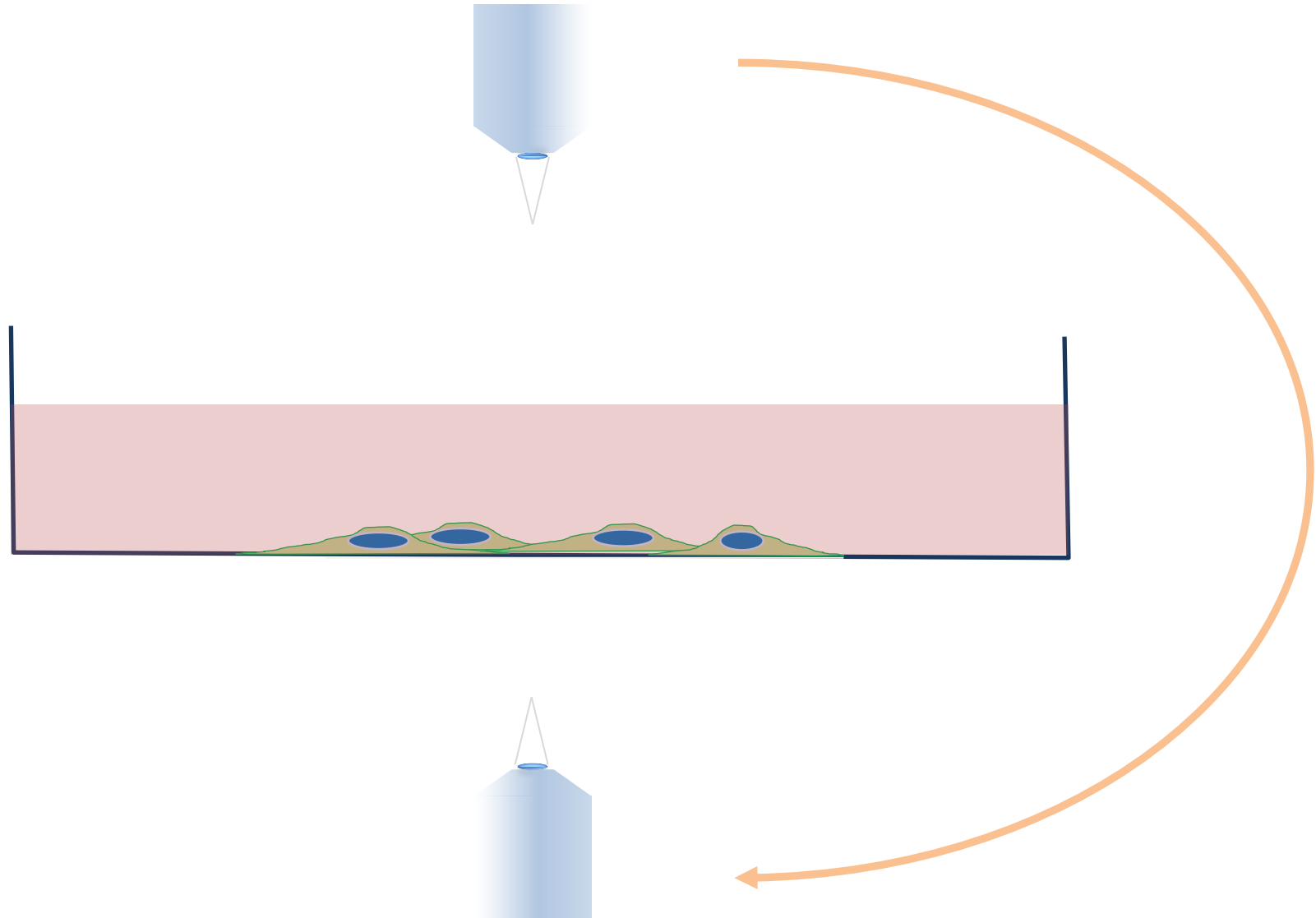
Essential equipment for live cells



Microscope construction & direction of observation



The concept of an inverted microscope

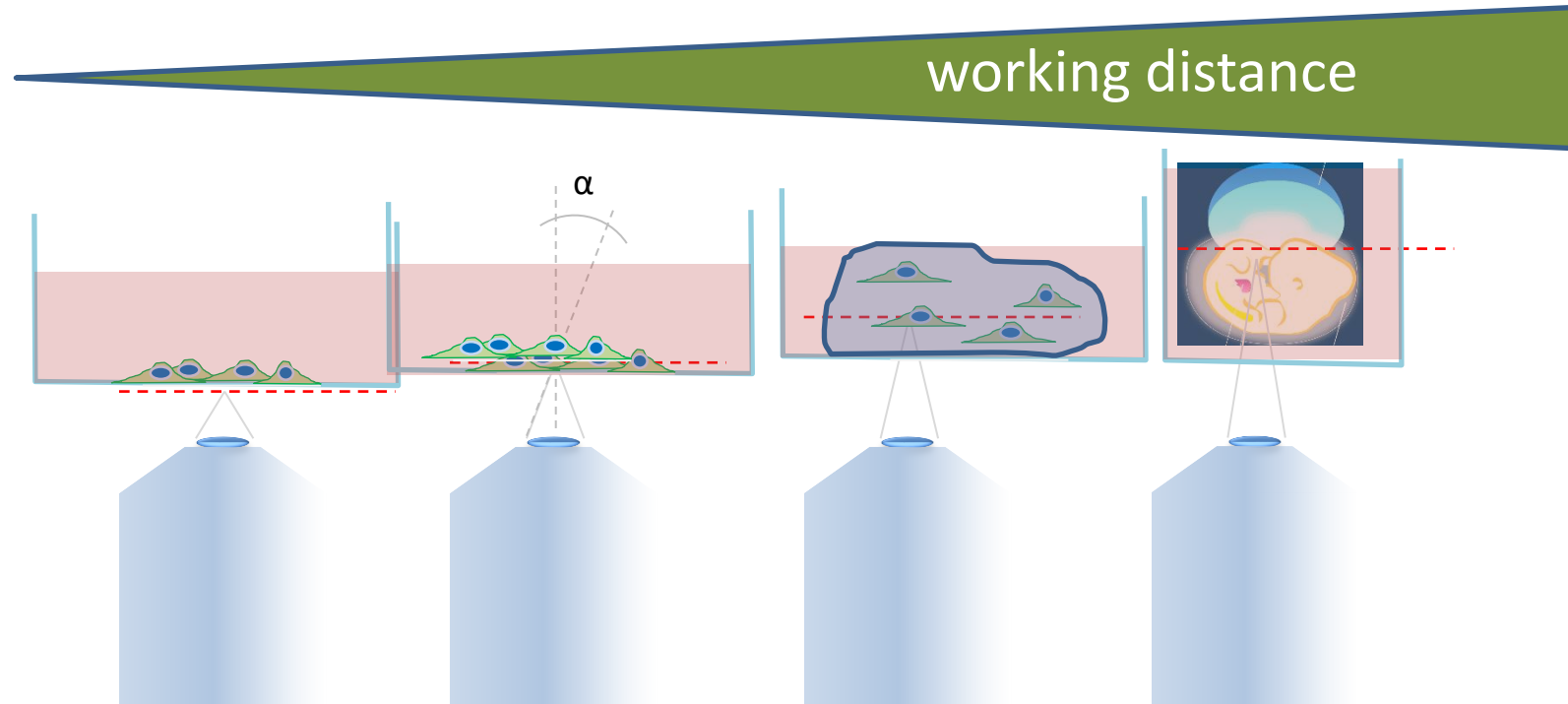


Upright microscope



Inverted microscope

Working distance & resolution and methods



$$NA = n \sin \alpha$$

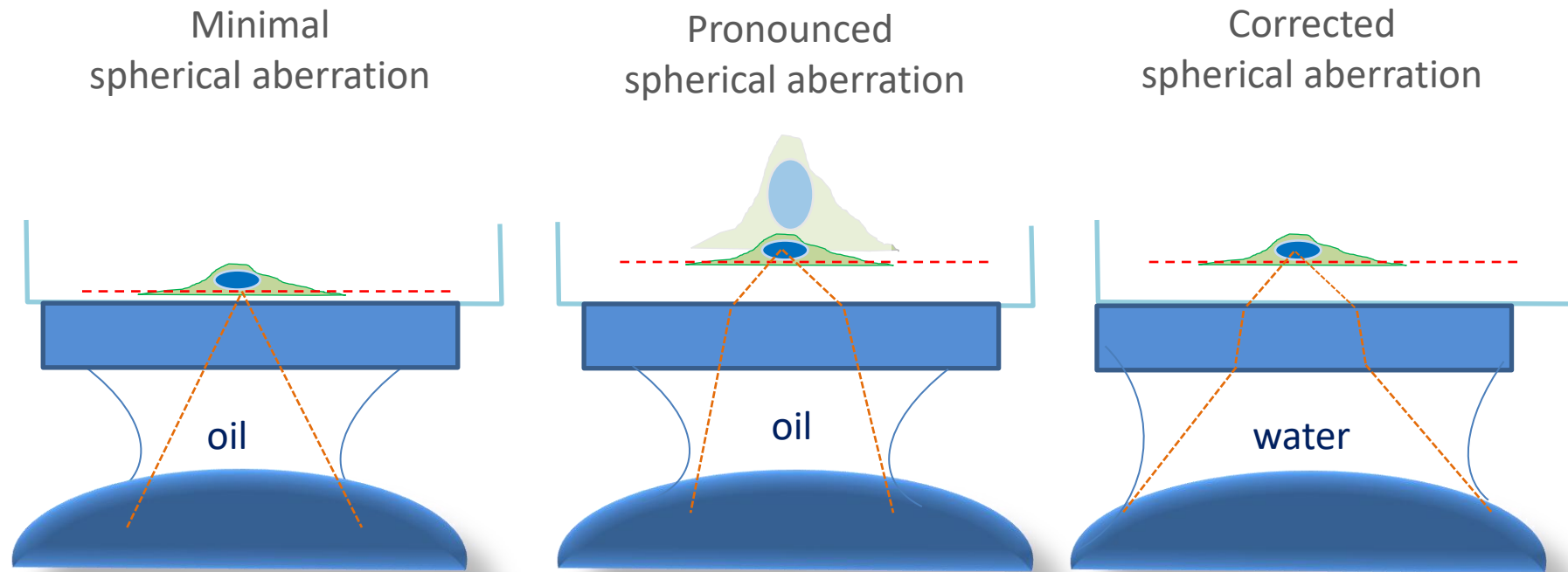
numerical aperture (resolution)

Lightsheet microscope

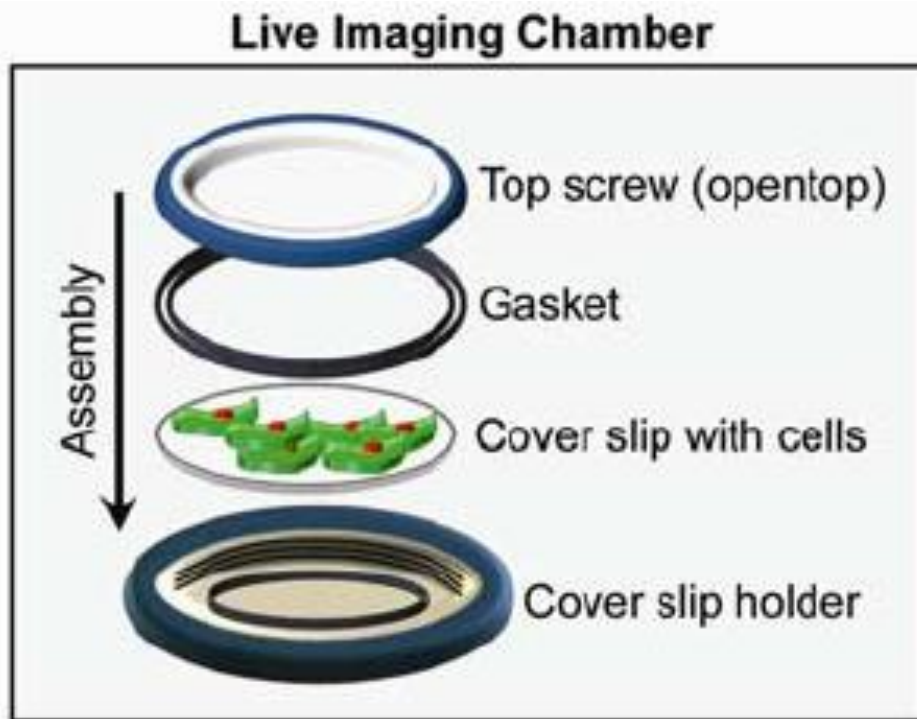
Confocal microscope

Superresolution microscopes

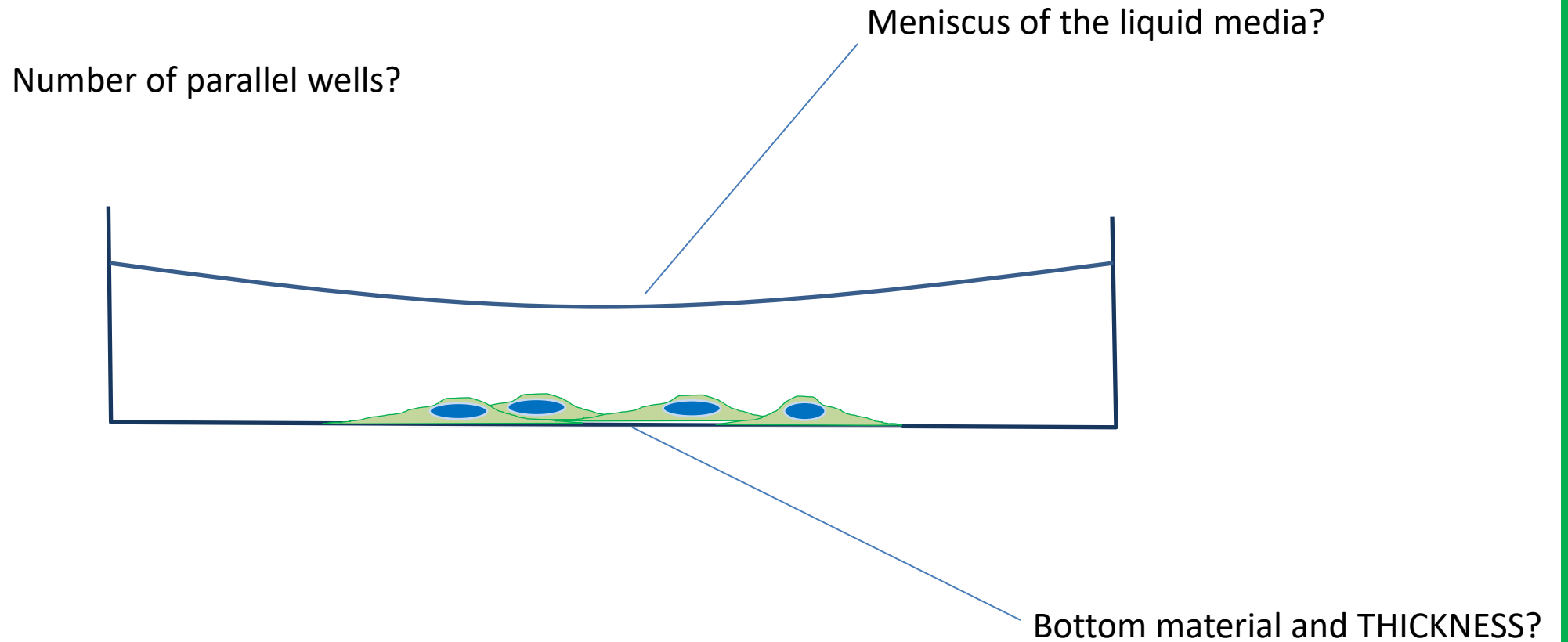
Spherical aberration in live cell imaging and Lens immersion



Dishes/chambers/plates... for live cell imaging (and cultivation)



Dishes/chambers/plates... what keep in mind



Dishes/chambers/plates... what keep in mind

Just common plastic for low resolution imaging



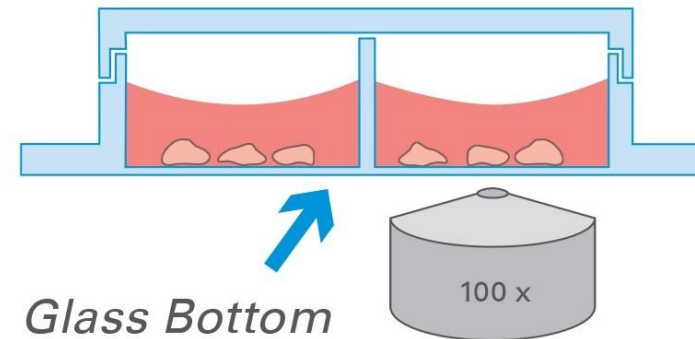
Dishes/chambers/plates... what keep in mind

High quality plastic for high resolution imaging, with defined bottom thickness



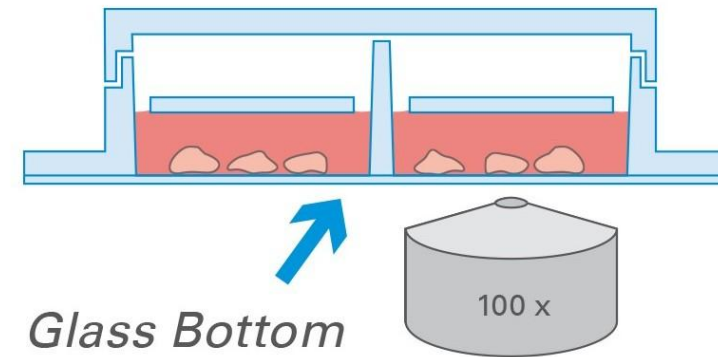
Dishes/chambers/plates... what keep in mind

High quality plastic with defined glass bottom thickness, NO solution for PH/DIC



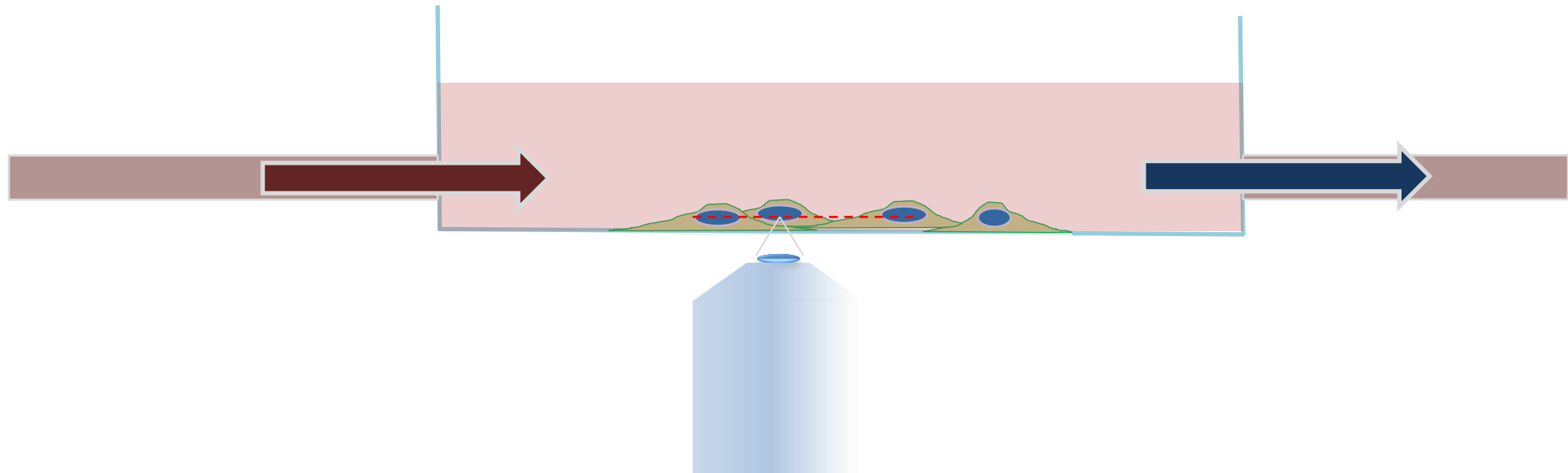
Dishes/chambers/plates... what keep in mind

High quality plastic with defined glass bottom thickness, feature for PH/DIC, NO MENISCUS!



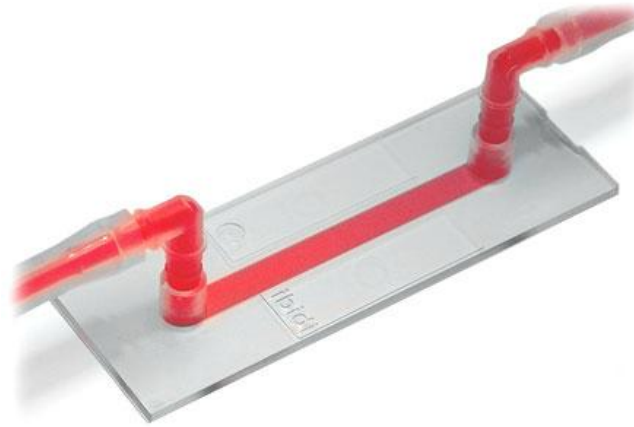
Dishes/chambers/plates... what keep in mind

Perfusion chamber? – the medium is exchanged during the experiment



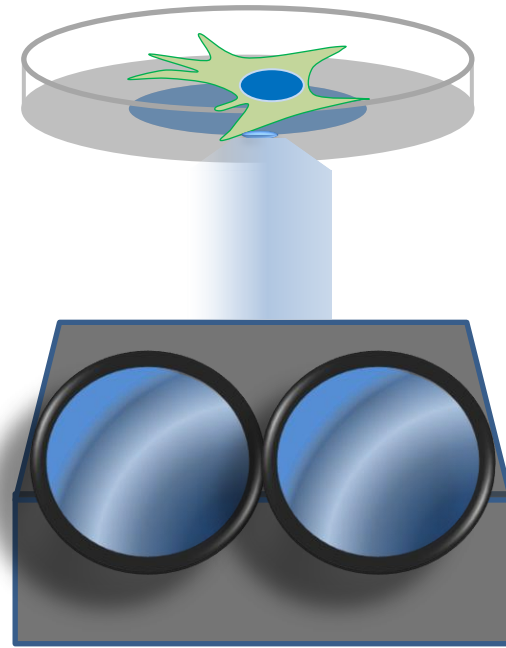
Dishes/chambers/plates... what keep in mind

Imaging glass-bottom perfusion chambers

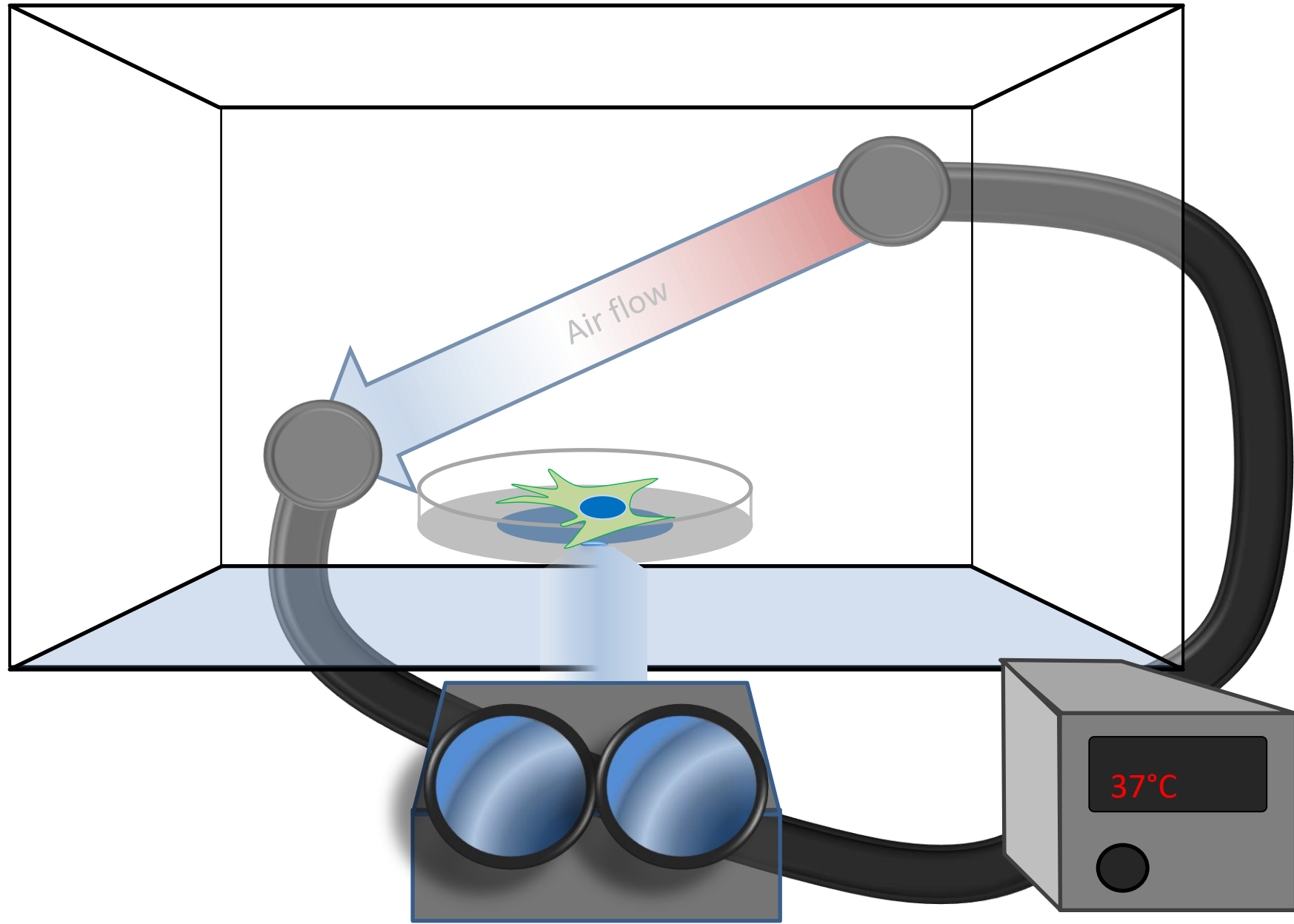


Live cell imaging – essential equipment

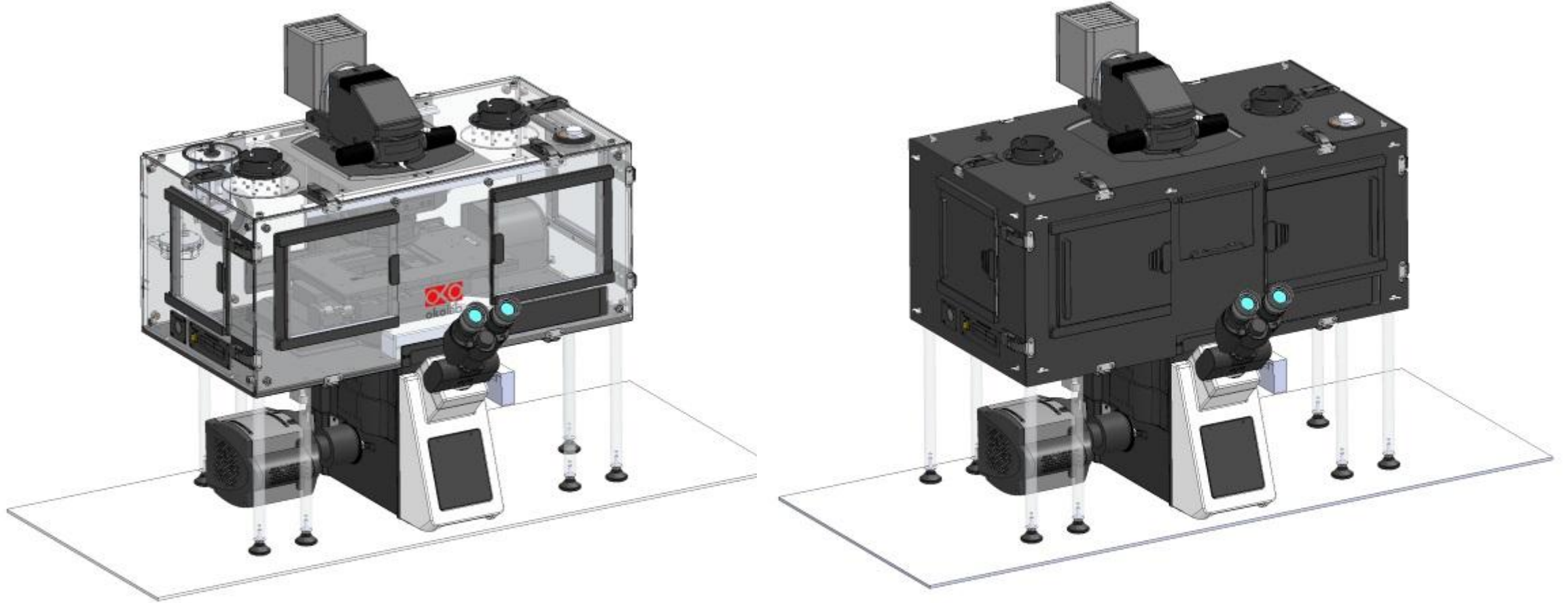
- Inverted microscope
- Imaging chamber for live cells
- Objective



Temperature control



Plexiglas incubation cube with chamber for CO₂ atmosphere



Heated cell imaging chamber, CO2 atmosphere controller



Ordering Info
H301-K-FRAME
H301-KOEHLER-LID
GS35-M

And heating for the objective –



**Metal Foil Blanket
with Velcro Anchor**



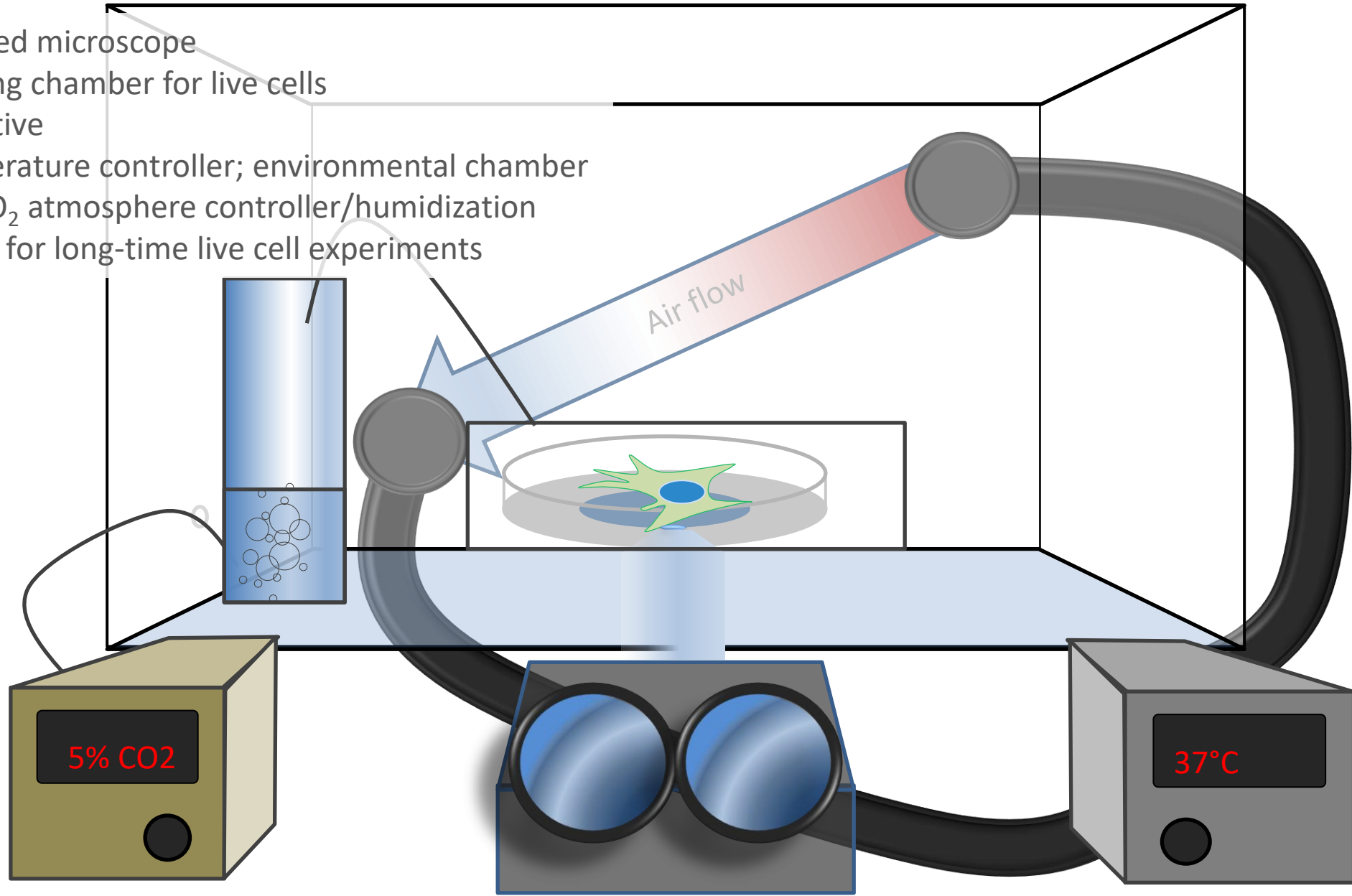
**Copper Tubing
Water Jacket**



**Proportionally-Controlled
Closed Loop Heater**

Live cell imaging – essential equipment

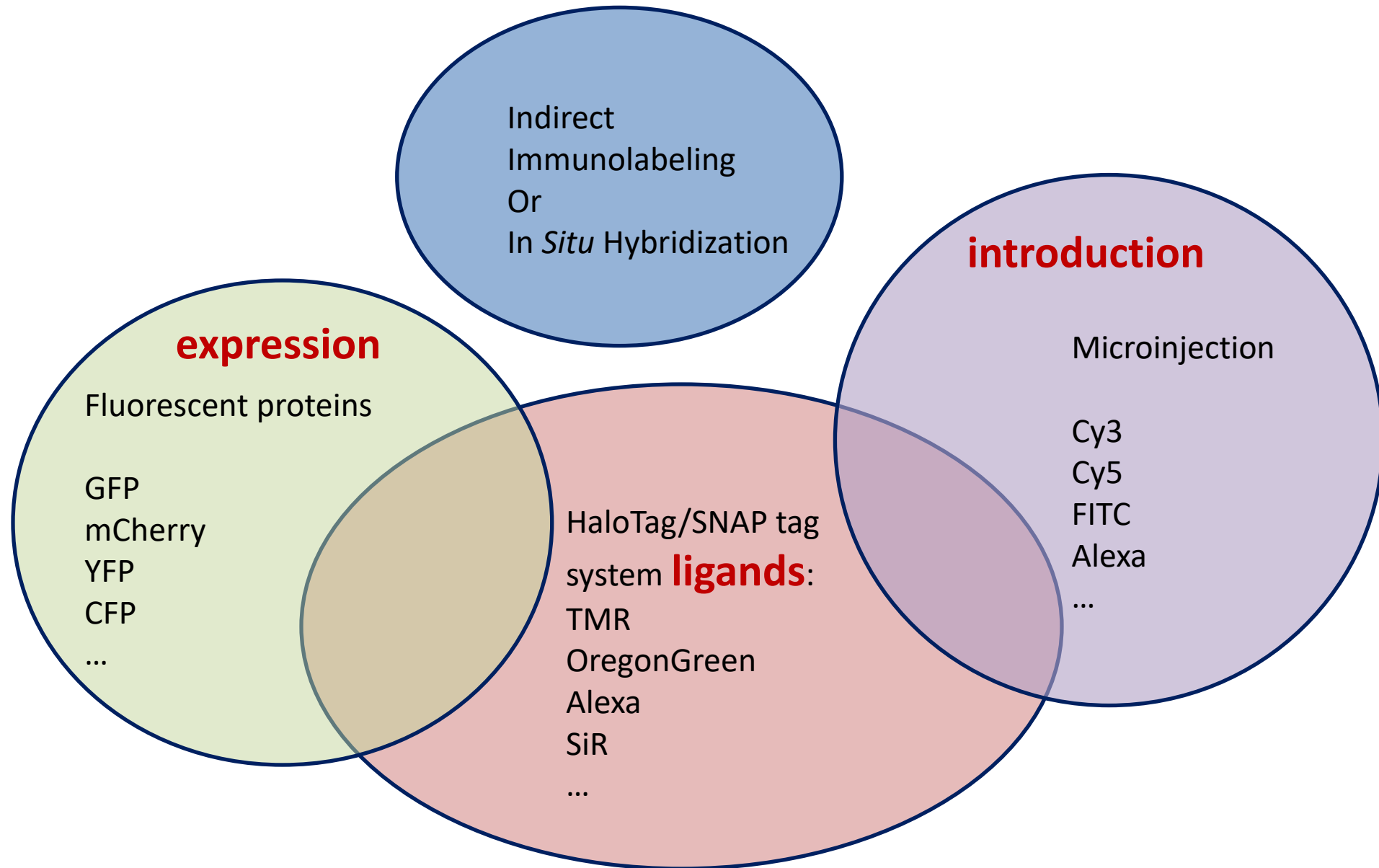
- Inverted microscope
- Imaging chamber for live cells
- Objective
- Temperature controller; environmental chamber
- CO₂ /O₂ atmosphere controller/humidization for long-time live cell experiments



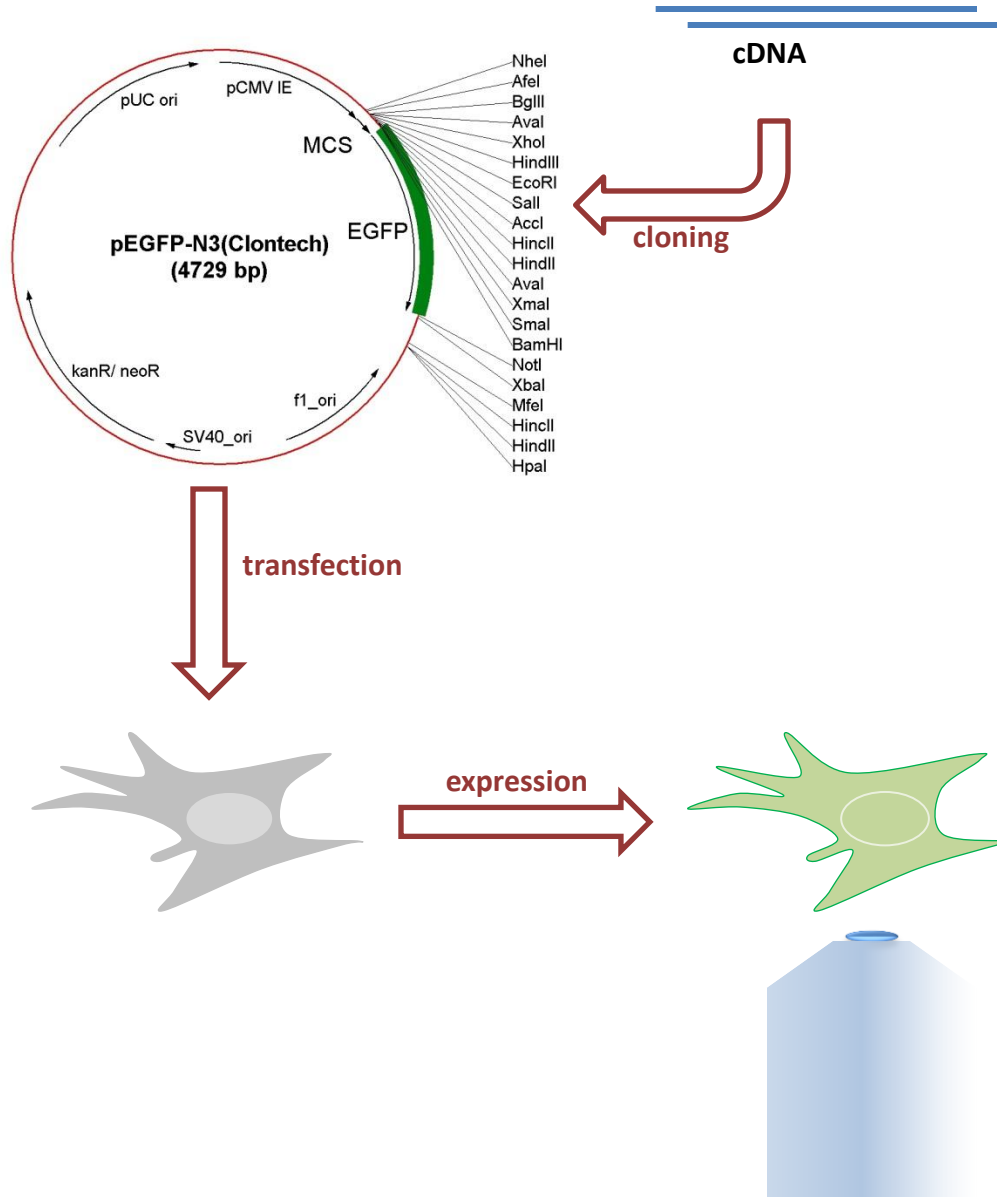
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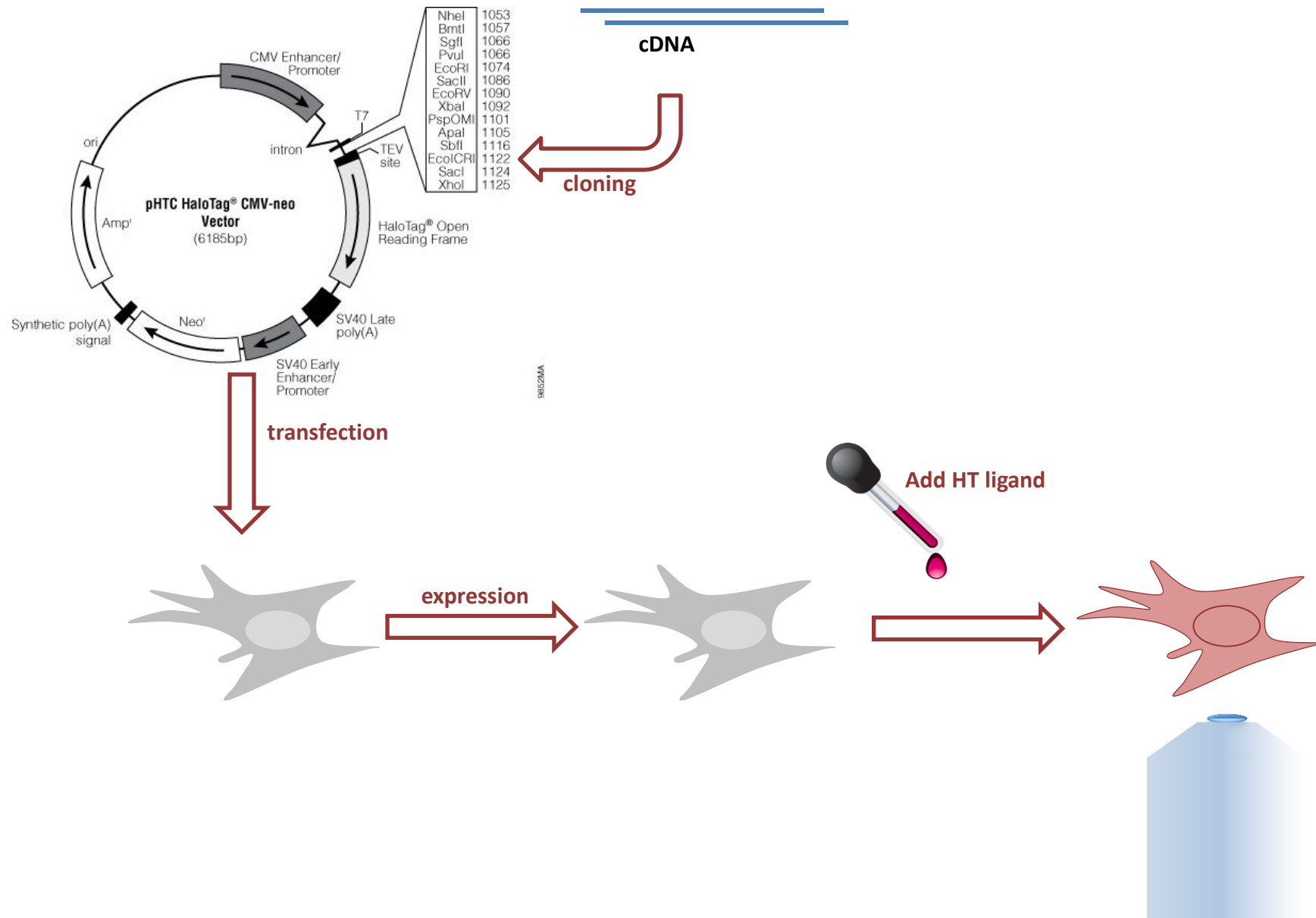
Visualization of cell structures in fluorescence microscopy



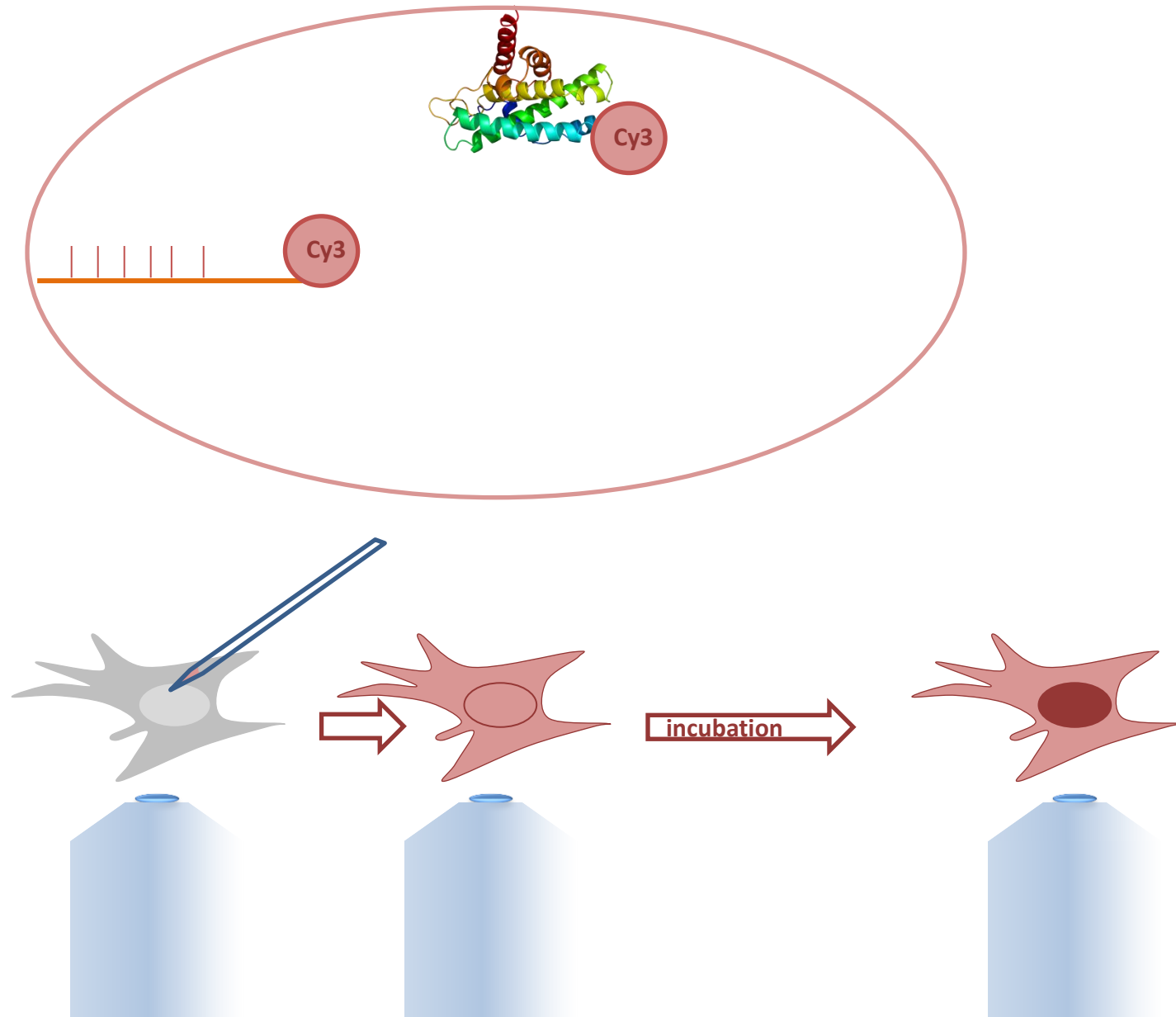
Fluorescent proteins



HaloTag/SNAP tag systems...



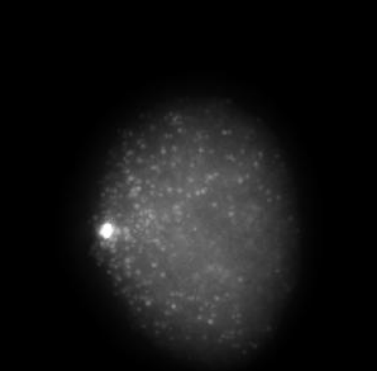
Microinjection



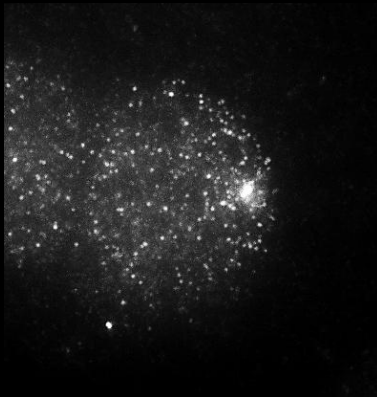
The same reporter, different visualization



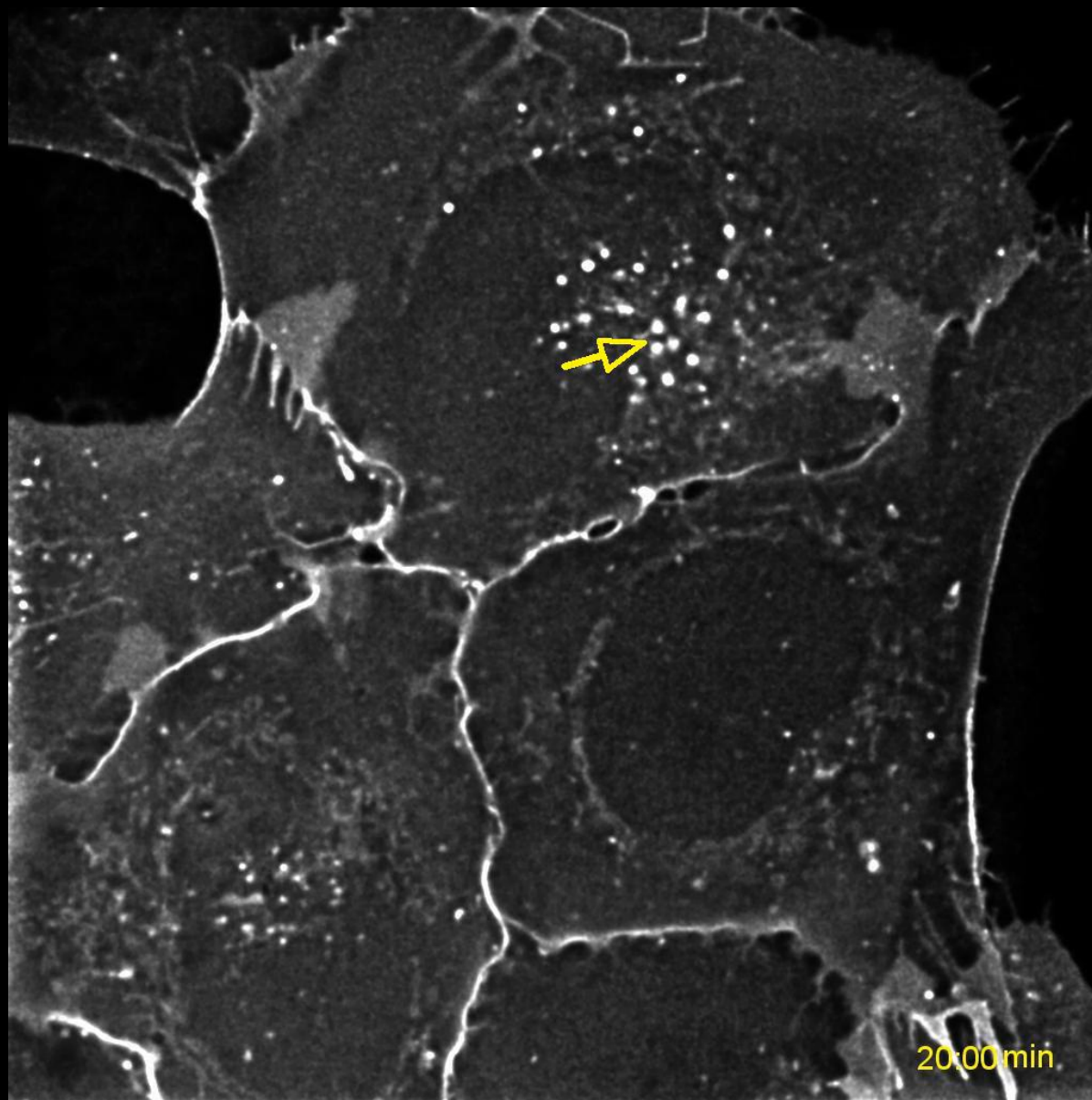
GFP tagged protein of interest



HaloTagged protein of interest + TMR ligand



Microinjected Cy3 tagged protein



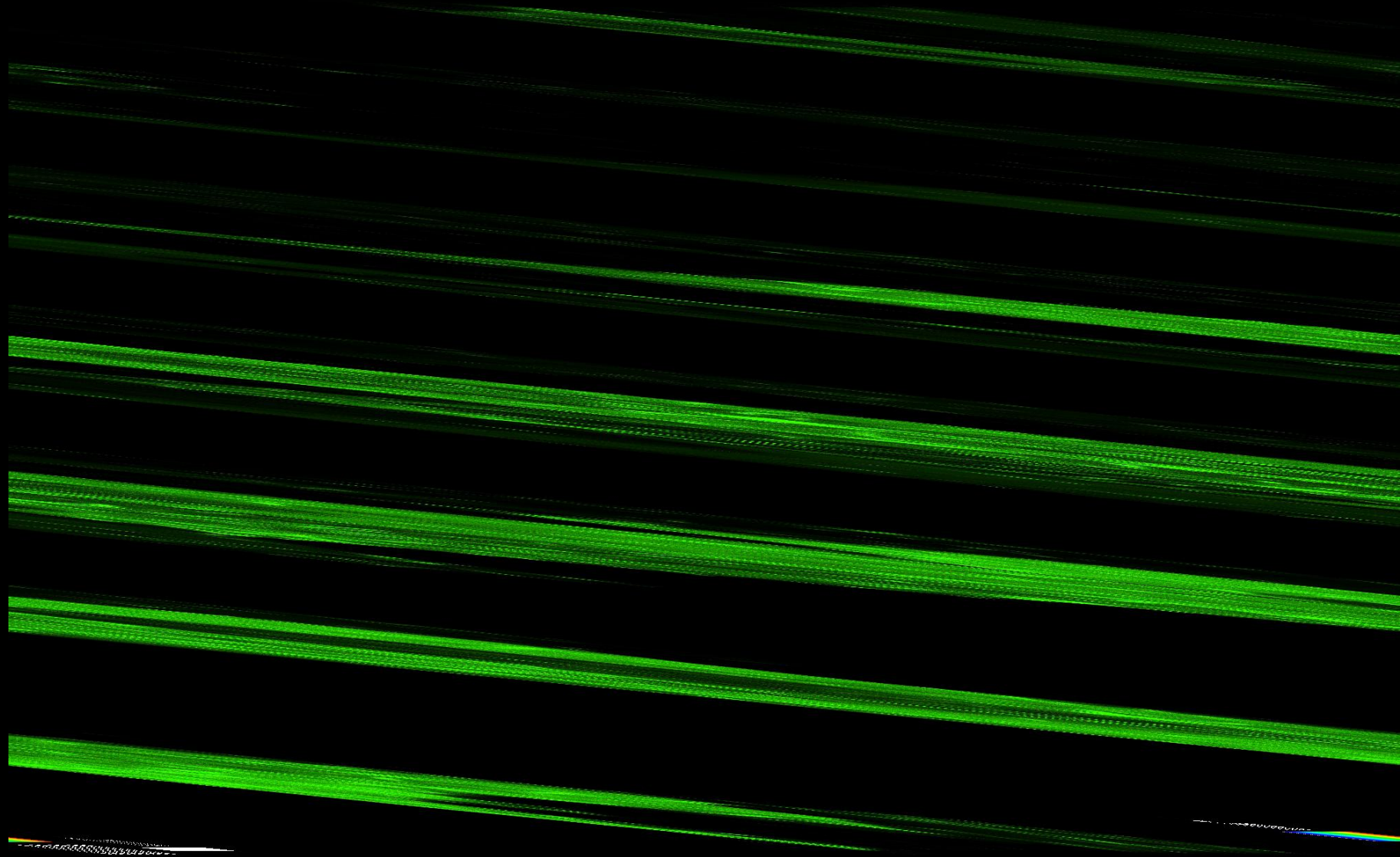
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Which methods use the live cell imaging – the main purpose

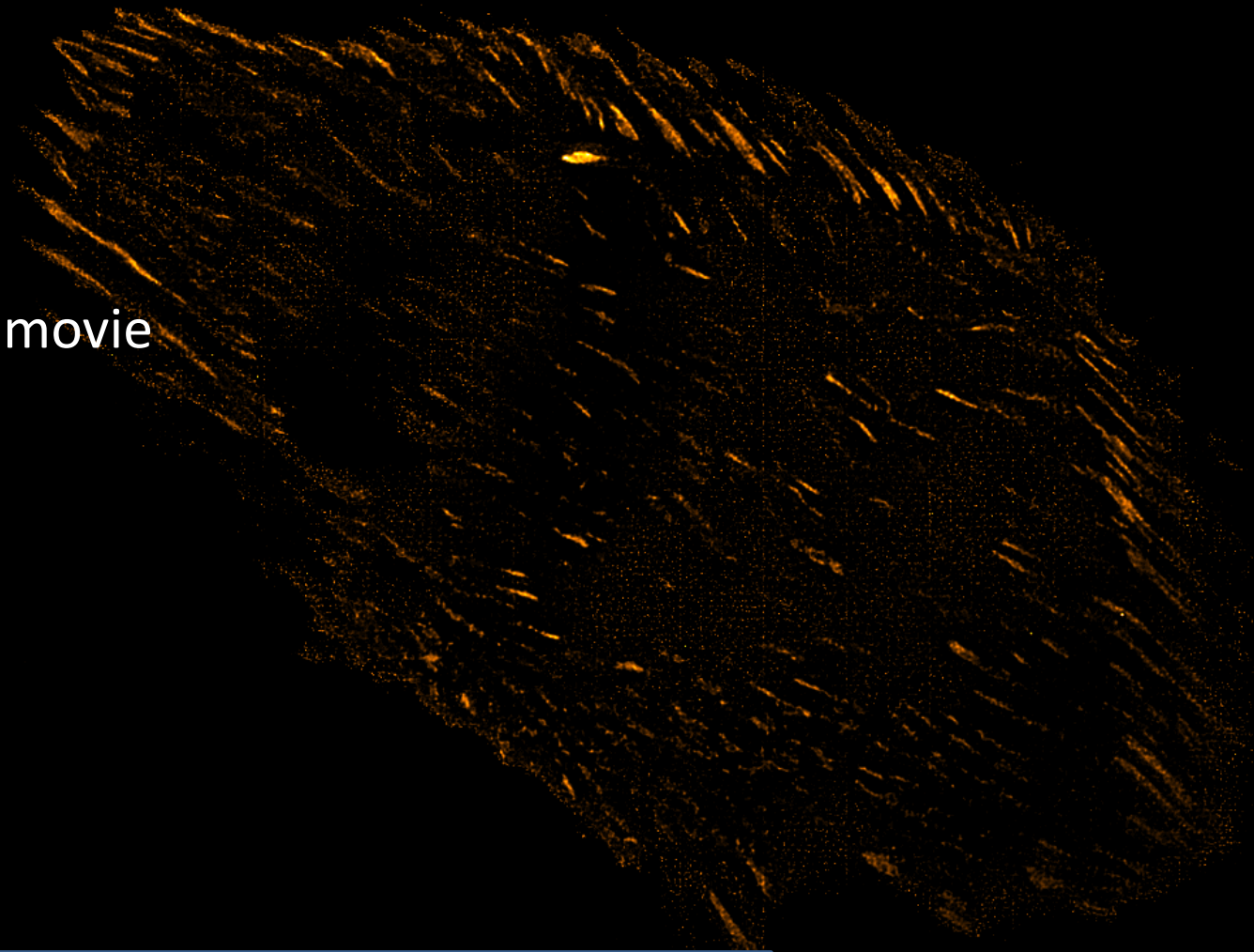
- **Time lapse movie** – monitoring of development, motility, morphology...
- **Photo-kinetic: FRAP/iFRAP/FLIP/Photoactivation..** – monitoring protein and vesicle trafficking, kinetics..
- **TIRF**– observing processes close to the cell membrane
- **FRET-FLIM** – quantifying protein-protein interactions

Time-lapse experiment



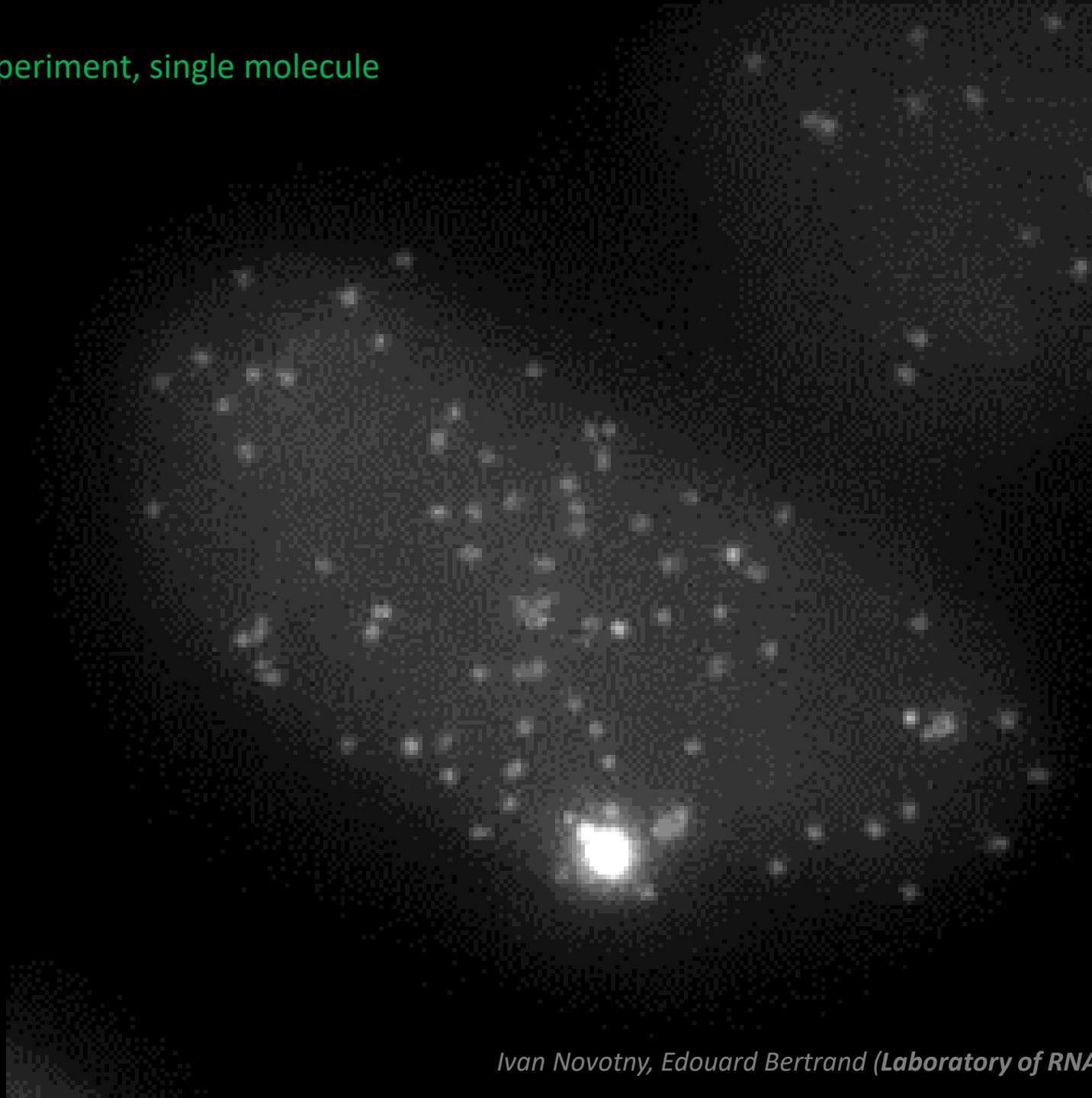
Anastasiya Klebanovych, Pavel Draber (*Laboratory of Biology of Cytoskeleton, IMG CAS*)

TIRF experiment, 12h movie



HCX PL APO 100x/1.46 OIL CORR CS; FWD 0.09; CG 0.1-0.22
Leica DFC350FX R2 – monochromatic CCD camera; 6,4 μm pixel

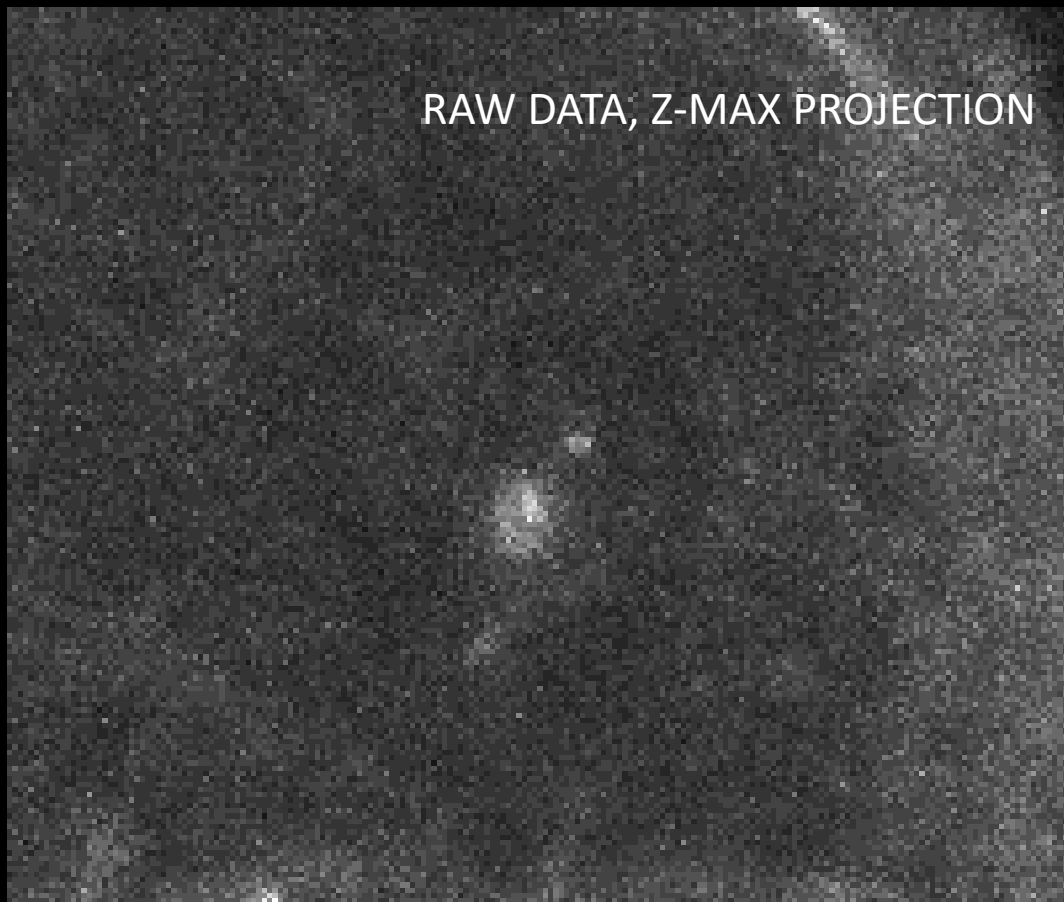
Fast time-lapse experiment, single molecule



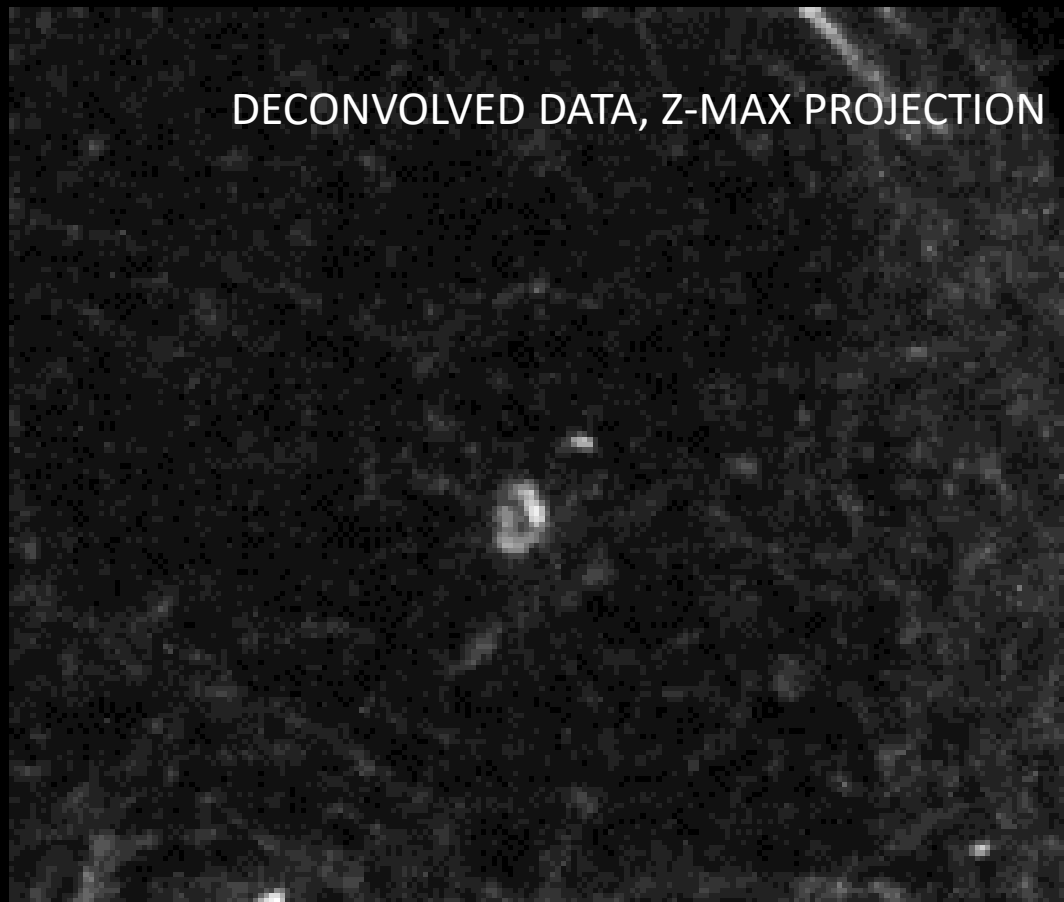
Ivan Novotny, Edouard Bertrand (Laboratory of RNA Biogenesis, IGMM CNRS)

FAST RESONANT SCANNER IMAGING, Z-STACK TIME LAPSE MOVIE

RAW DATA, Z-MAX PROJECTION



DECONVOLVED DATA, Z-MAX PROJECTION



HC PL APO 63x/1.40 OIL CS2; FWD 0.14; CG 0.17

Resonant scanner, supersensitive hybrid detectors (HyD), photon counting mode

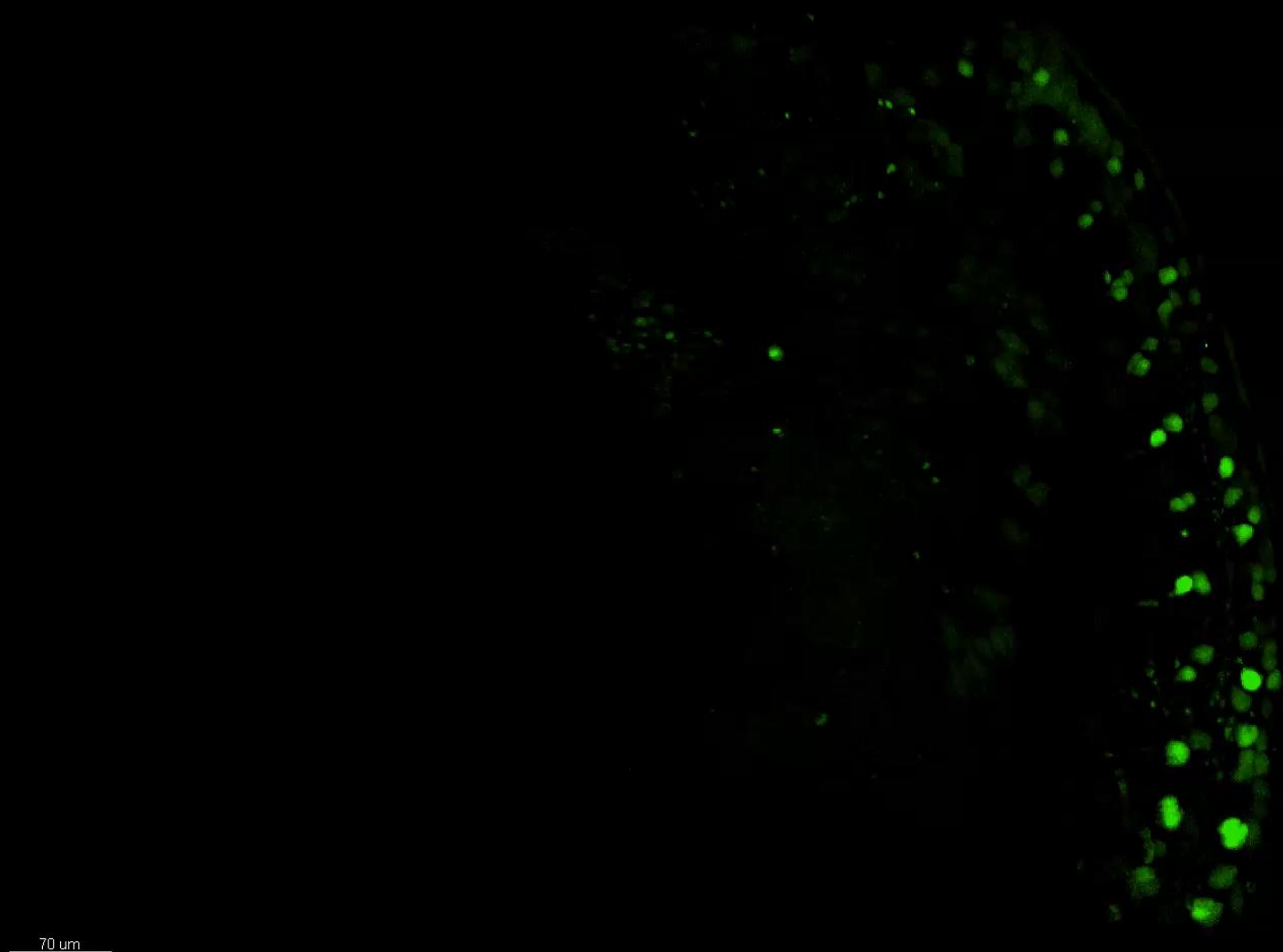
LAS X, lightning

Vladka Sladkova / Ivan Novotny / Honza Valecka

Photokinetic experiment - FRAP



Klara Klimesova, David Stanek (Laboratory of RNA Biology, IMG CAS)



70 μm

0:00:00.000

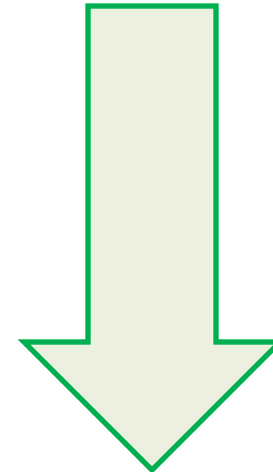
Live cell imaging

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Improve the quality of the imaging

- Minimize the unwanted photo-bleaching
- Minimize the illumination light intensity
- Improve the signal, minimize background
- Protect the cells – choose longer wavelength

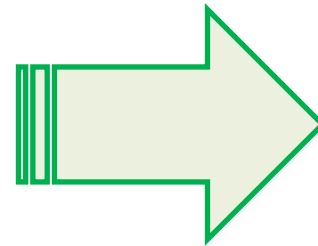
Linked conditions



Improve the quality of the imaging

SENSITIVE/FAST CAMERA

- Lower background, higher signal
- Minimize illumination intensities
- Minimize exposure time

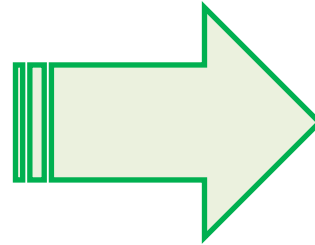


- Lower photo-bleaching
- longer cell viability
- prolong the experiment time

Improve the quality of the imaging

USE APPROPRIATE IMAGING MEDIA

- Lower background, higher signal
- Minimize illumination intensities
- Minimize exposure time

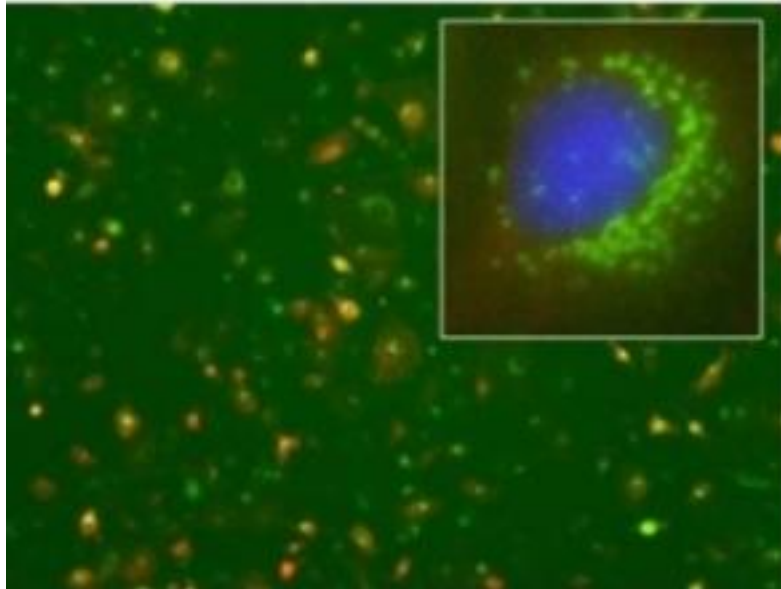


- Lower photo-bleaching
- longer cell viability
- prolong the experiment time

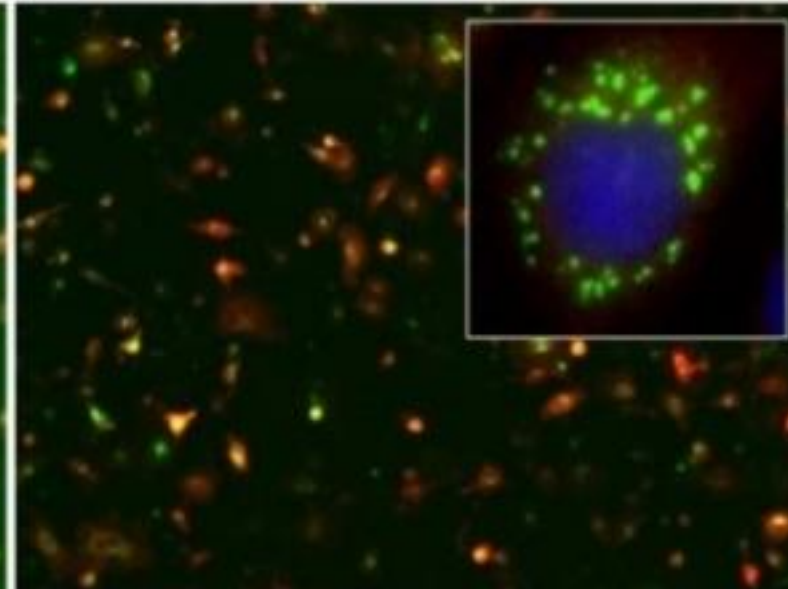
USE APPROPRIATE IMAGING MEDIA



DMEM



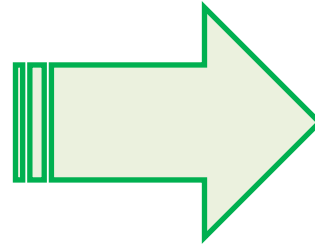
FluoroBrite™ DMEM



Improve the quality of the imaging

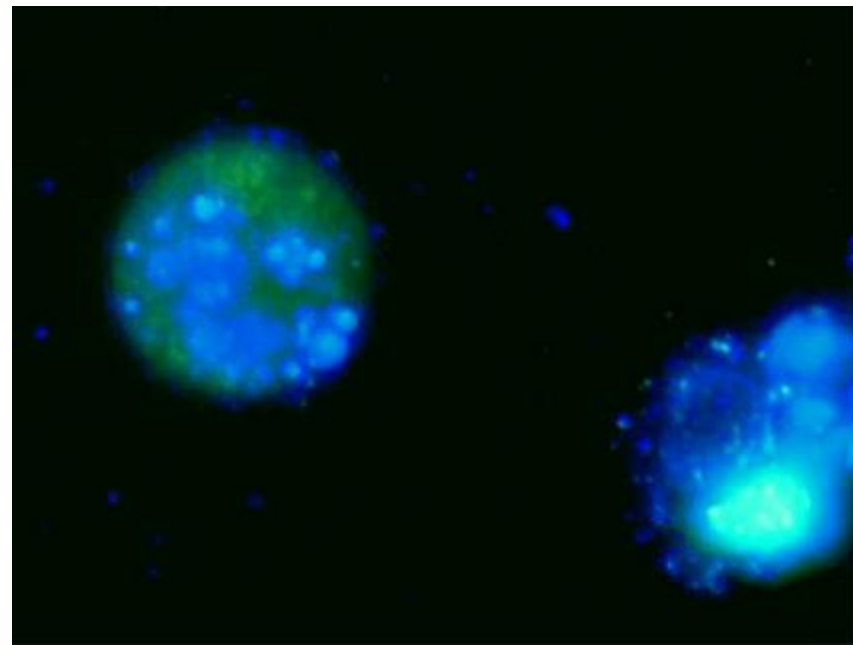
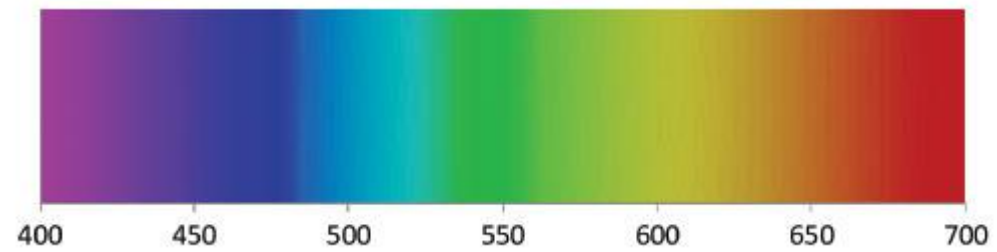
USE ILLUMINATION LIGHT WITH LONGER WAVELENGTH

- Lower photo-toxicity
- Depth of light penetration
- 2 photons?



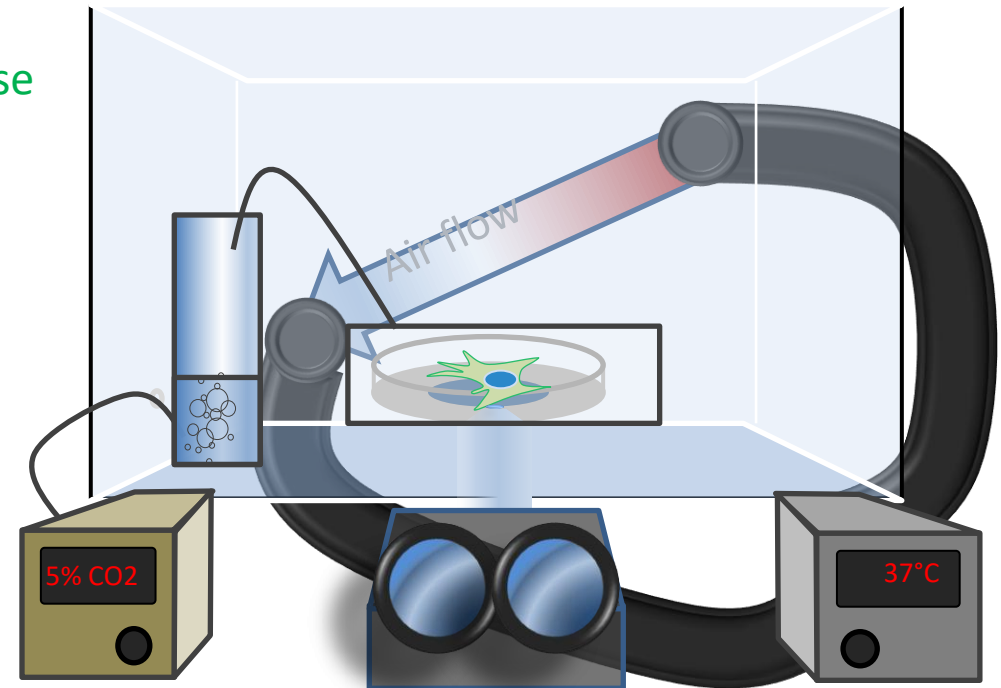
- longer cell viability
- prolong the experiment time
- Bigger objects / thicker tissues

USE ILLUMINATION LIGHT WITH LONGER WAVELENGTH



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Thank you for the attention!

