Microscope in Action

European Learning Laboratory for the Life Sciences (ELLS)

Educators PowerPoint:

Basic physics of microscopy

Additional information about fluorescence microscopy

EMBL

Content

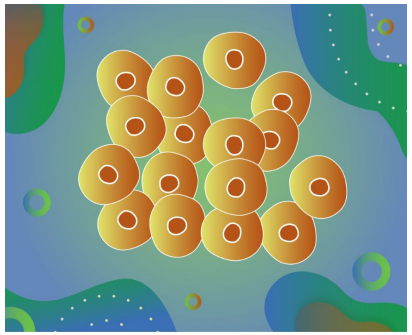
1. The European Molecular Biology Laboratory
2. Fluorescence – Glowing with bright colours
3. The green fluorescent protein (GFP) – a green revolution
4. Introduction to light microscopy
5. Fluorescence microscopy
6. Assembling your fluorescence microscope
7. Advanced fluorescence microscopy – breaking the limit of resolution
8. Applications and outlook

1. The European Molecular Biology Laboratory

European Molecular Biology Laboratory

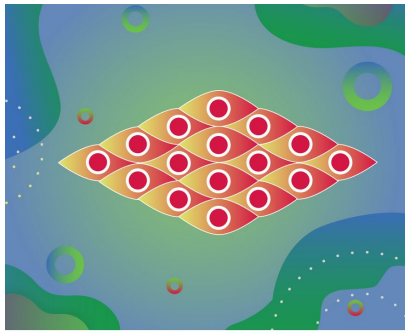
- Offers vital services to scientists in the member states and the world
- Actively engages in technology transfer and industry relations
- Trains scientists, students, and visitors at all levels
- Coordinates and integrates European life science research

Life sciences research by >80 independent groups



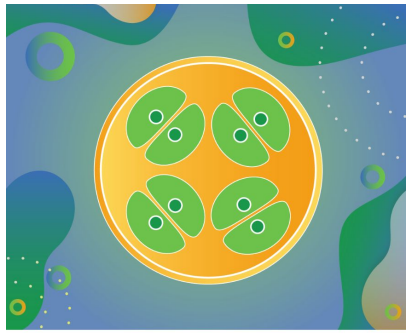
Cells

Understanding how cells work and how they are organised



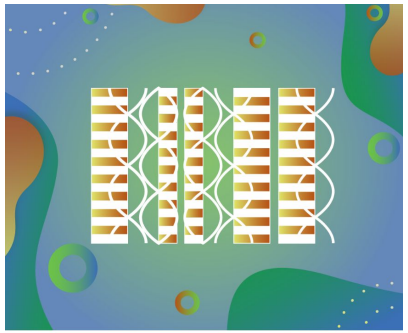
Tissues

How biological tissues develop, work, regenerate, and heal



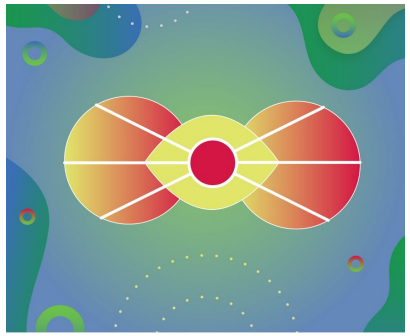
Development

How a single cell becomes a multicellular organism



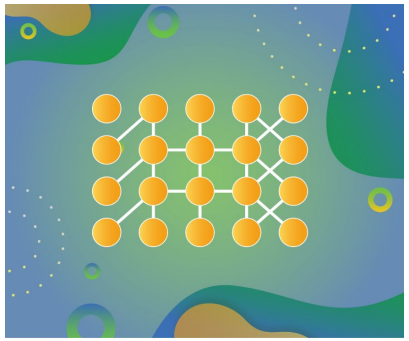
Bioinformatics

Computing to analyse data from a range of biological experiments



Imaging

Cutting-edge technology, zooming into life at the tiniest scales

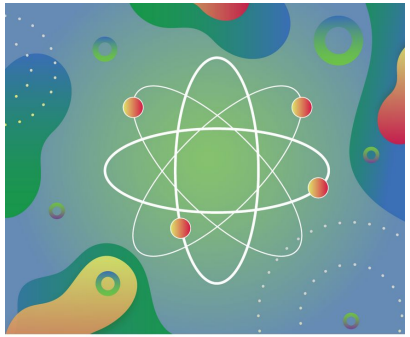


Disease models

Cell and statistical approaches to better understand diseases

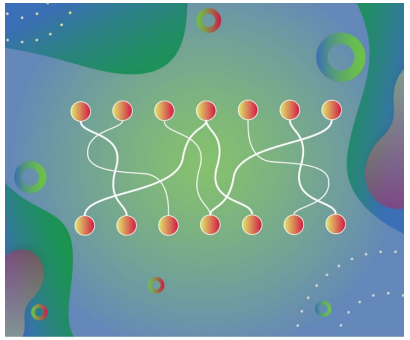
Chemistry

Chemical tools to answer biological questions



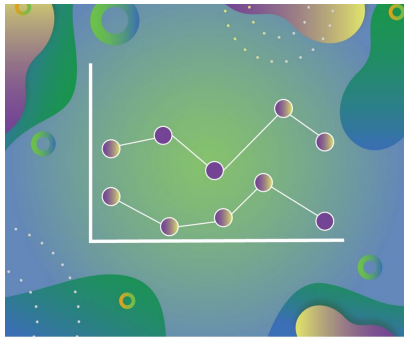
Physics

Enabling technology for a host of experimental methods



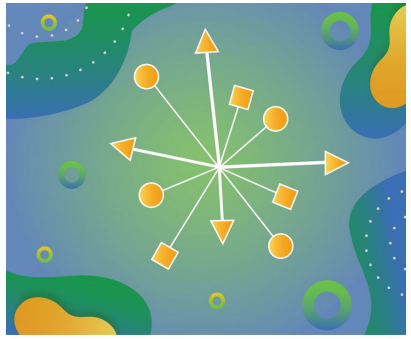
Engineering

Key engineering principles support a range of biological methods



Statistics

Mathematical analysis is crucial to the data-rich science of biology



Multidisciplinary

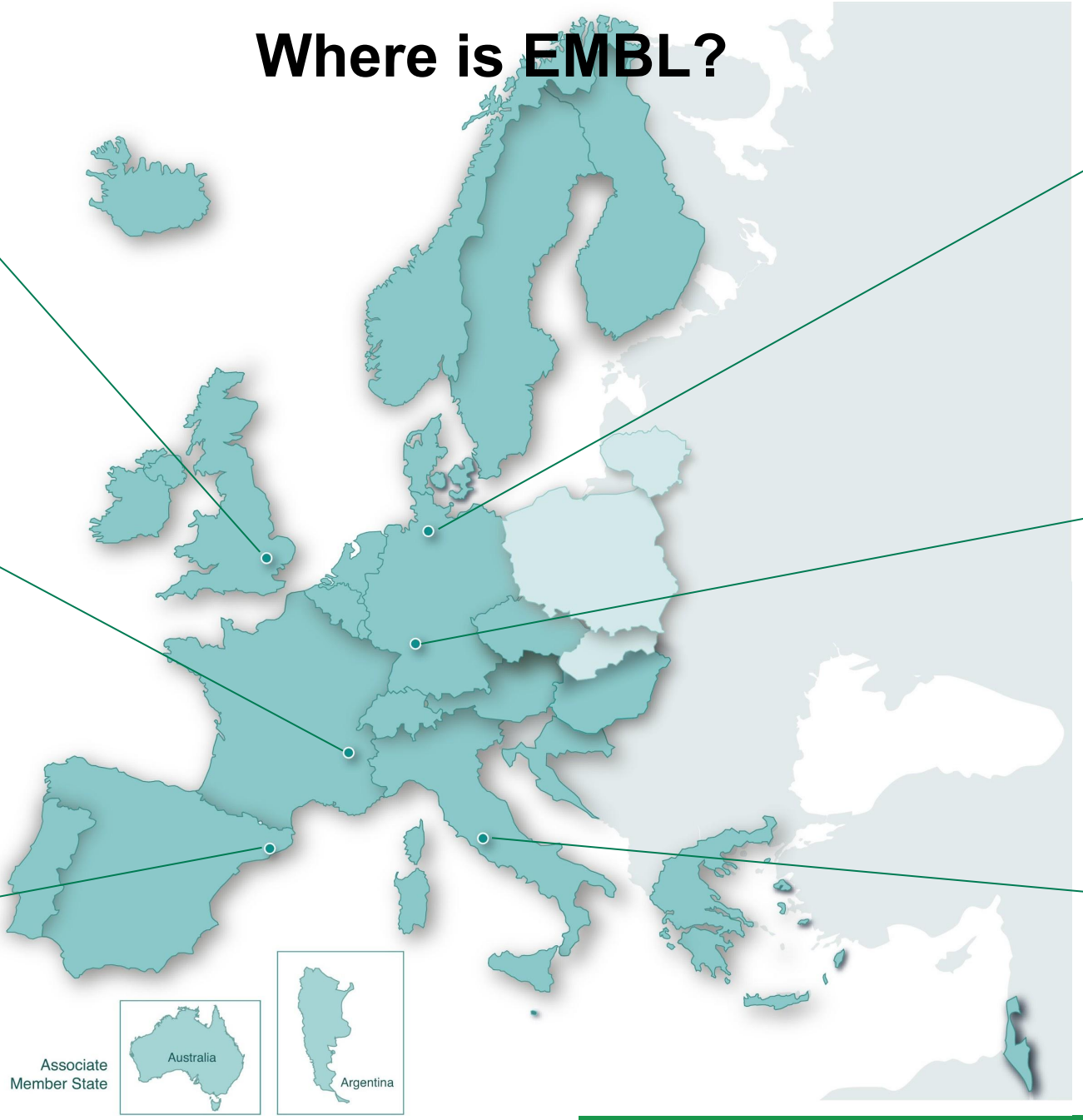
Sharing ideas across disciplines to revolutionise biology

Where is EMBL?

Bioinformatics

Structural Biology

Tissue Biology & Disease Modeling



Structural Biology

Life Sciences

Neurobiology & Epigenetics

ELLS – European Learning Laboratory for the Life Sciences

EMBL

ELLS - EMBLs science education department: bridging science and schools

- Transform new ideas gained from EMBL research & technology into useful knowledge for teachers and schools
- Show how science works

Inform

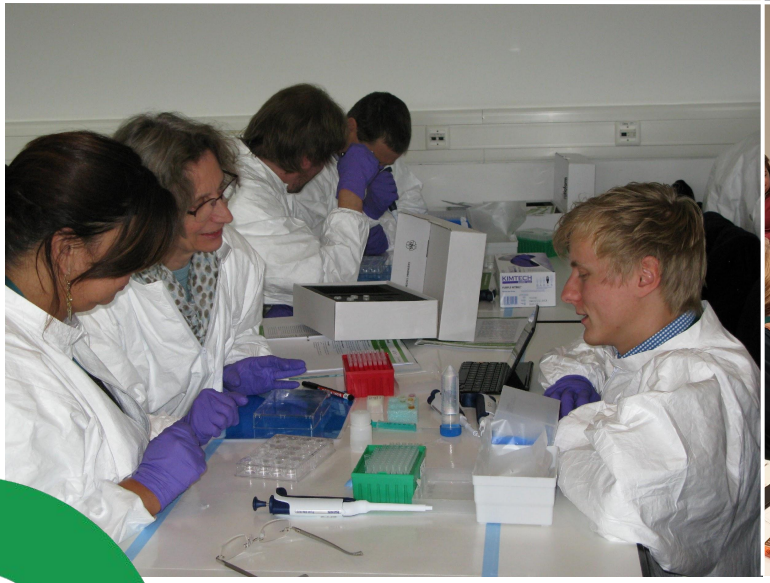
- Make young people and the public curious, well educated and critical consumers of scientific knowledge

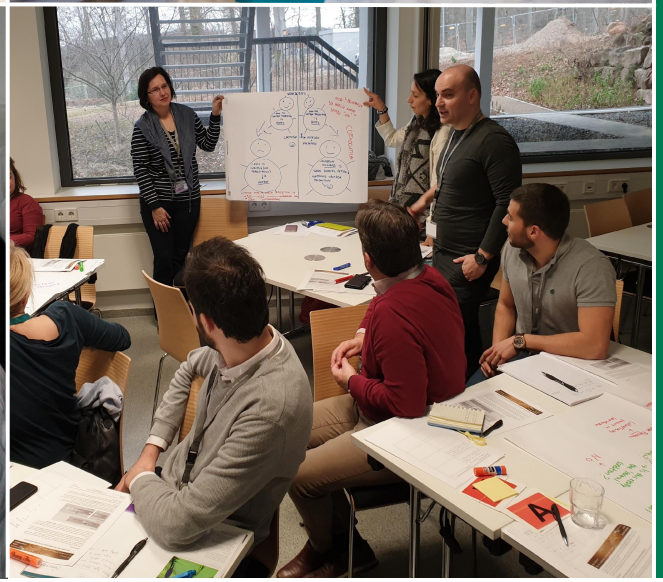
Inspire

- Raise awareness of science careers
- Engage scientists in public engagement activities

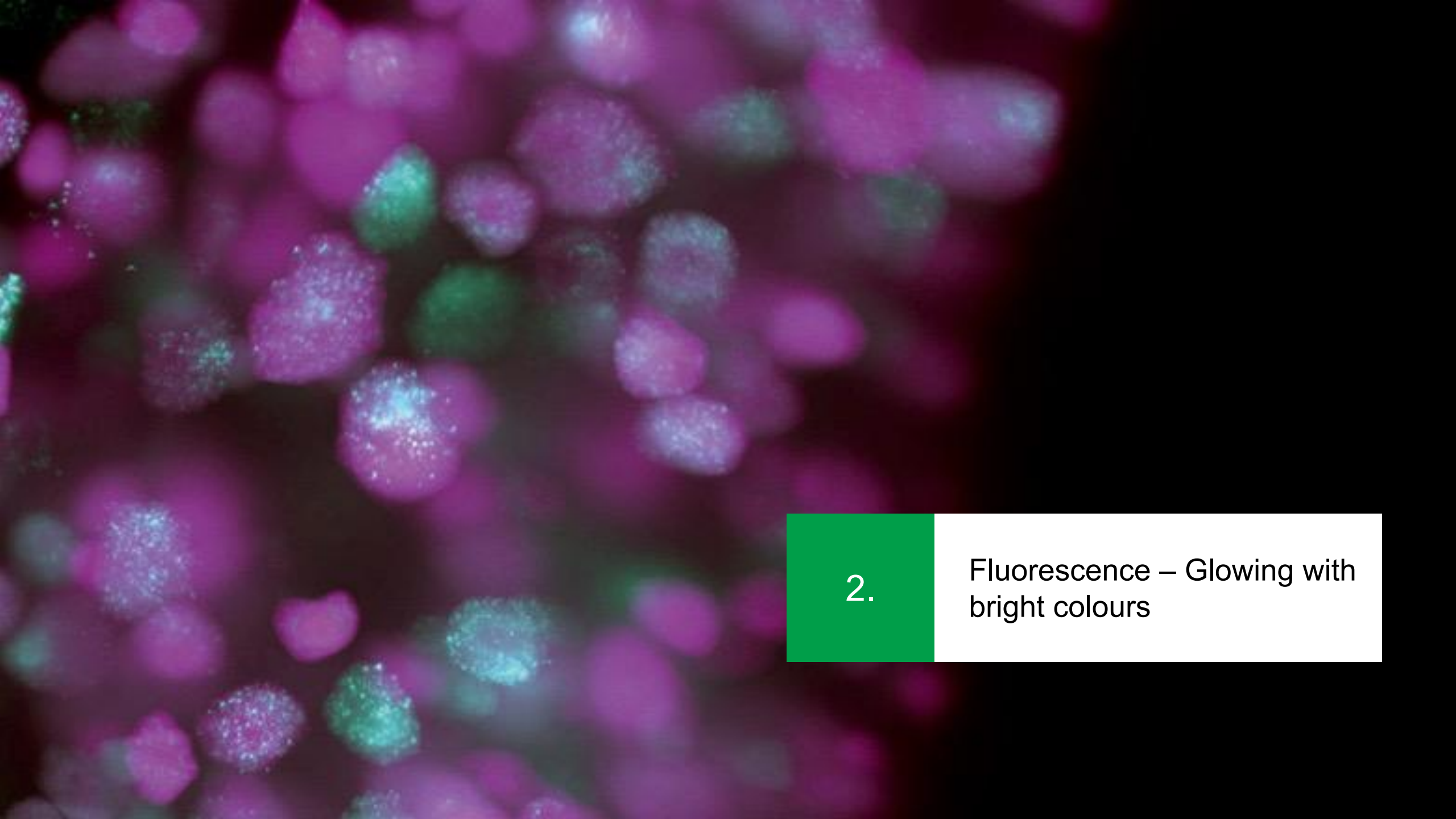
Educate

Teacher training (Europe-wide)





ELLS LearningLABs & online teacher training courses are delivered to secondary school science teachers in Europe on-site, off-site or online.



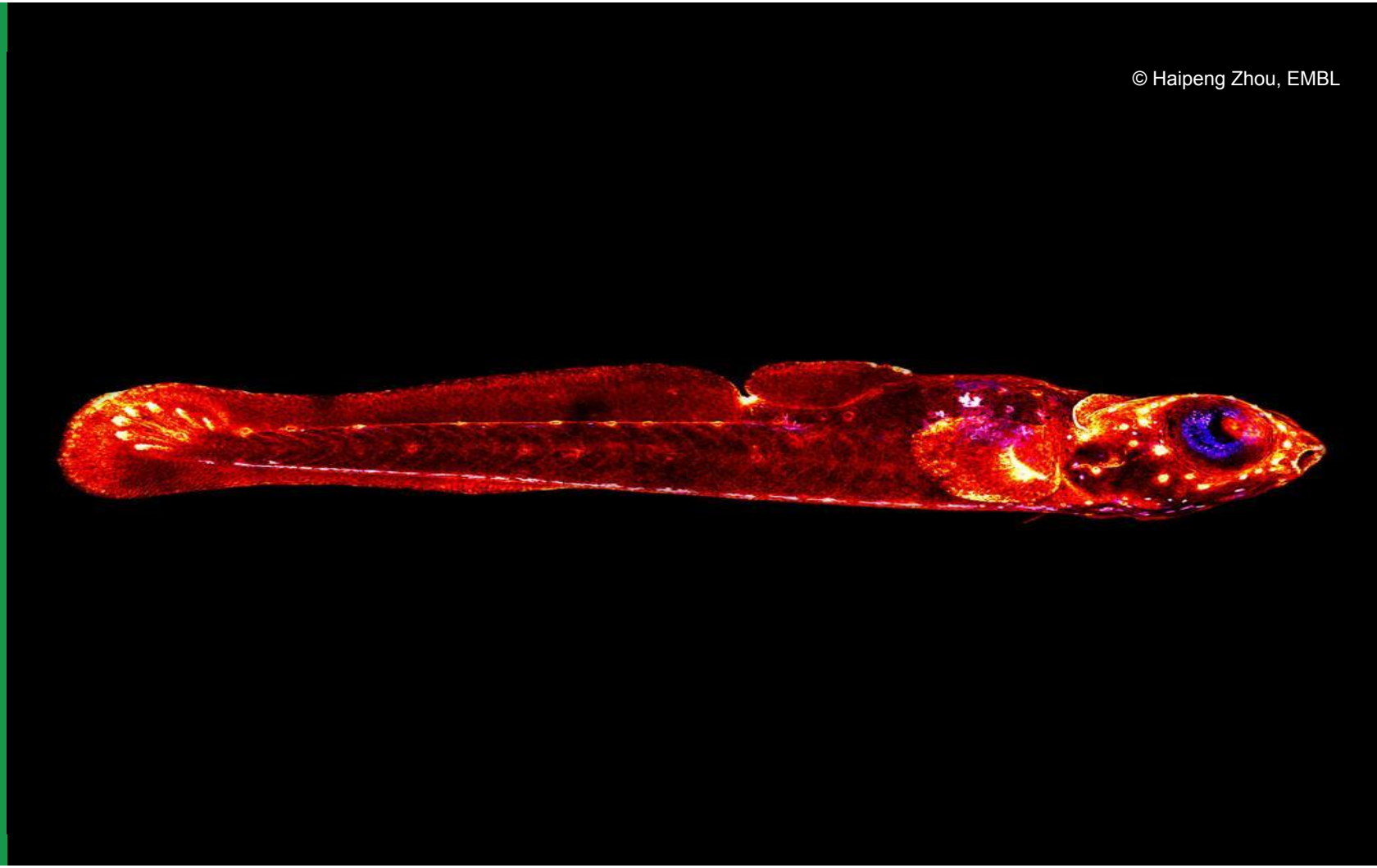
2. Fluorescence – Glowing with bright colours

The principle of fluorescence
Glowing with bright colours

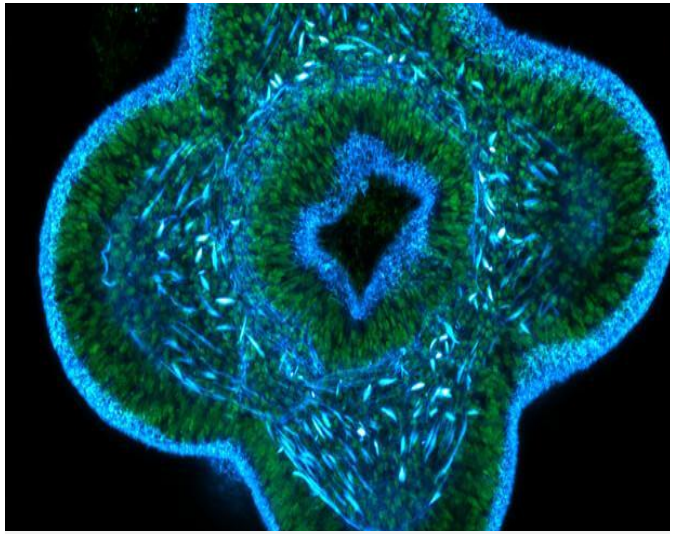
The image shows an upside down *Medaka* larvae expressing a fluorescent protein in cells that also express interleukin-1.

Interleukin-1 is involved in sensing damage and signaling this to neighbouring cells. It is expressed in all immune tissues.

The image was taken with a confocal laser-scanning-microscope.



Fluorescence



Absorption and emission of photons almost simultaneously

nanoseconds

Phosphorescence

Absorption and emission of photons are a long time apart

seconds to hours

Bioluminescence

Emission of photons by chemical reaction of luciferin via luciferases (enzymes)

chemical reaction

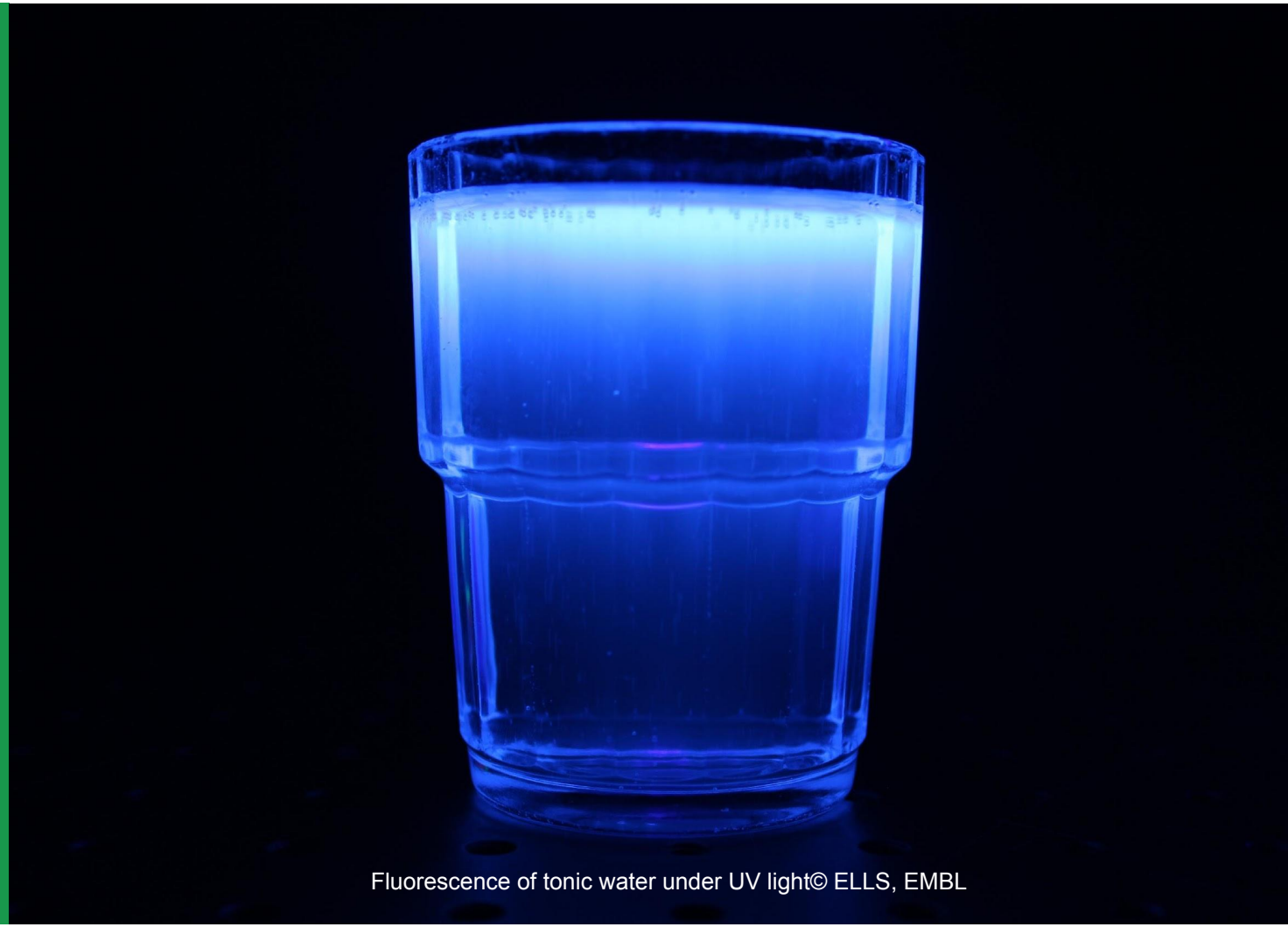
Definition and discovery

*'Luminescence that is caused by the **absorption** of radiation at one wavelength followed by nearly immediate **reradiation** usually at a different wavelength and that ceases almost at once when the incident radiation stops.'*

– Merriam-Webster

This kind of luminescence was first described in the 1560s in regards to an infusion of certain wood types (*Lignum nephriticum*) that exhibited blue light.

It was later discovered in fluorites as well as in quinine solutions (Herschel, 1845) and after a more detailed description in all sorts of matter, e.g. chloroplasts.



Fluorescence of tonic water under UV light© ELLS, EMBL

Description of fluorescence

After reading the publication by Herschel, Sir George Gabriel Stokes researched the phenomenon and coined the process as “fluorescence”, reminiscent of the luminescence fluorite exhibits under certain circumstances.

He also described the fact that the emitted light is of longer wavelength than the excitation light (Stokes’ shift).

Important publication: On the change of refrangibility of light (1853)

“*I was inclined to coin the term ‘fluorescence’…*”

https://en.wikipedia.org/wiki/Sir_George_Stokes,_1st_Baronet#/media/File:Ggstokes.jpg

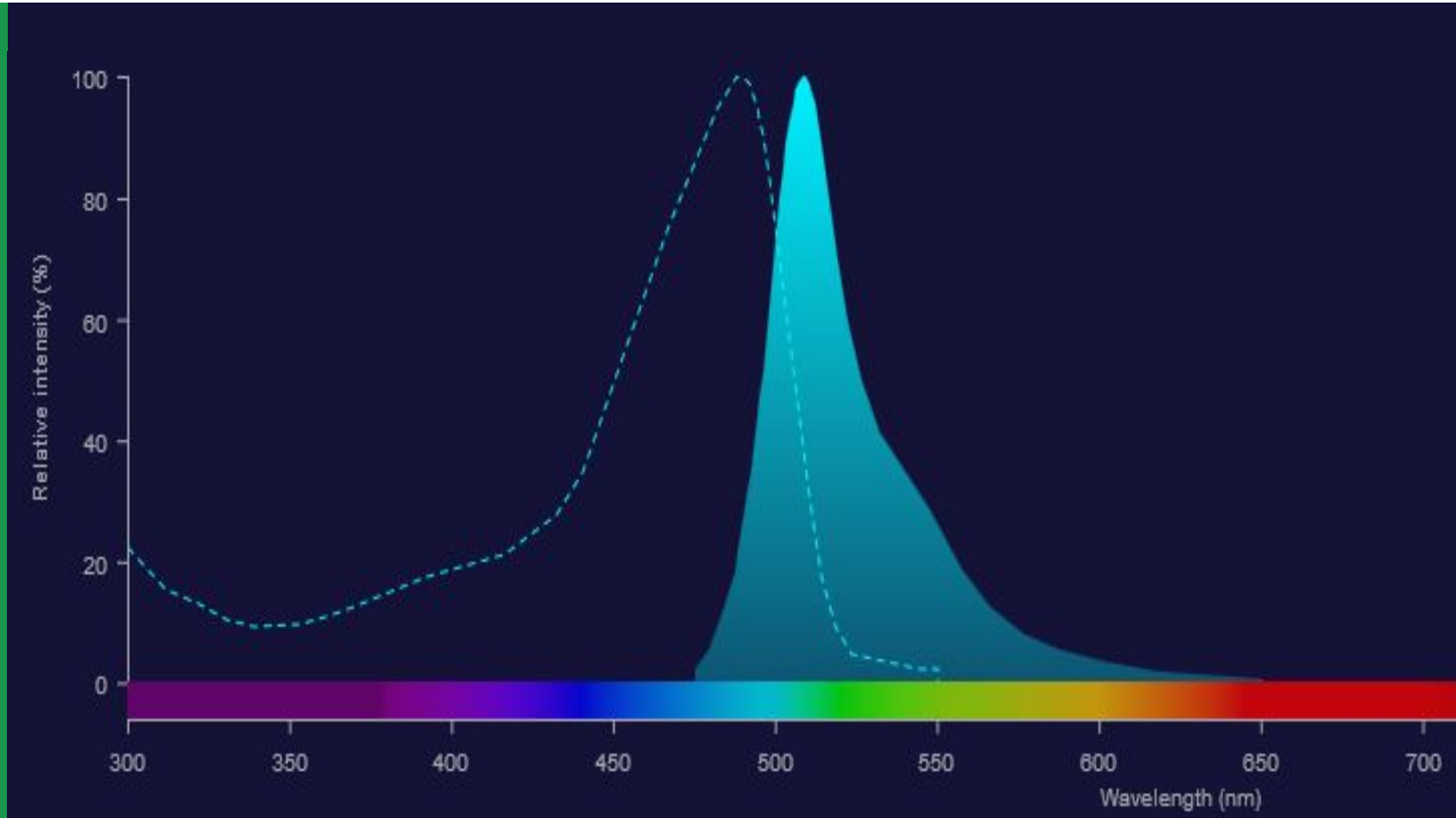
Fluorescence spectrum

The image shows the spectrum of eGFP, one of the modified versions of the original GFP.

The excitation is shown as dotted line.

The area of wavelength in which both excitation and emission are possible is called spectral overlap.

For both excitation and emission exist Maxima with the highest relative intensity



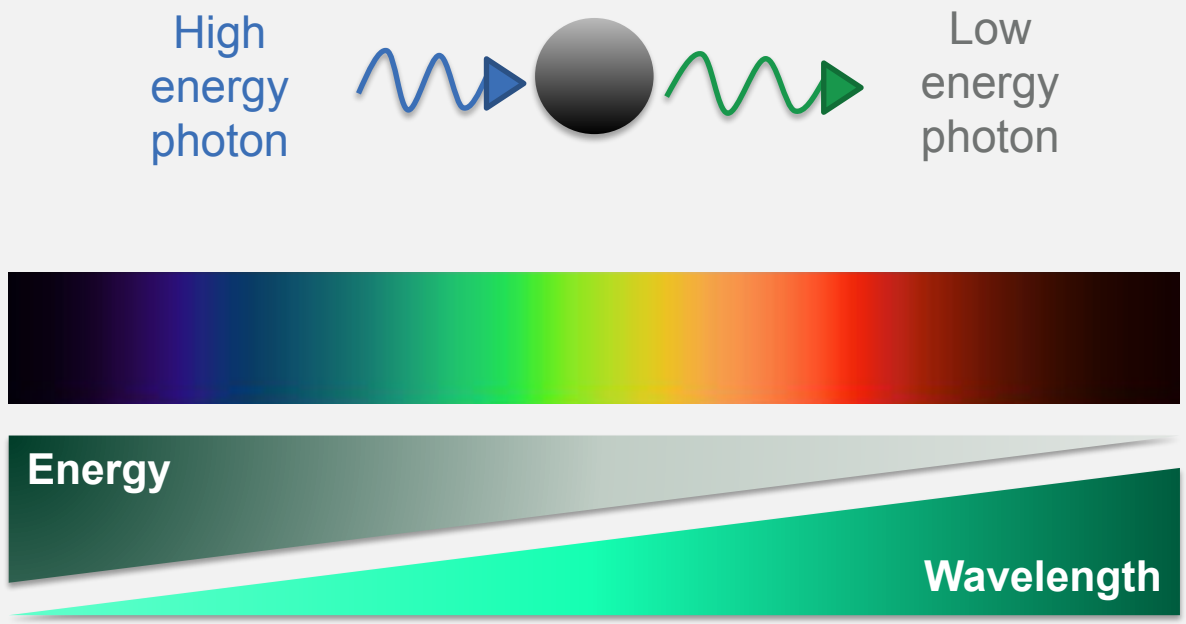
ThermoFisher Scientific Fluorescence Spectra Viewer
https://www.thermofisher.com/de/de/home/life-science/cell-analysis/labeling-chemistry/fluorescence-spectraviewer.html#!/

Absorption, emission and Stokes' shift

An object is excited by high energy photons (low wavelength), raising an electron to a higher energy level.

This energy is released by radiation of lower energy photons and the electron relaxes to its ground state.

Stokes' Shift: The emitted light is always of higher wavelength (less energetic) than the excitation light.

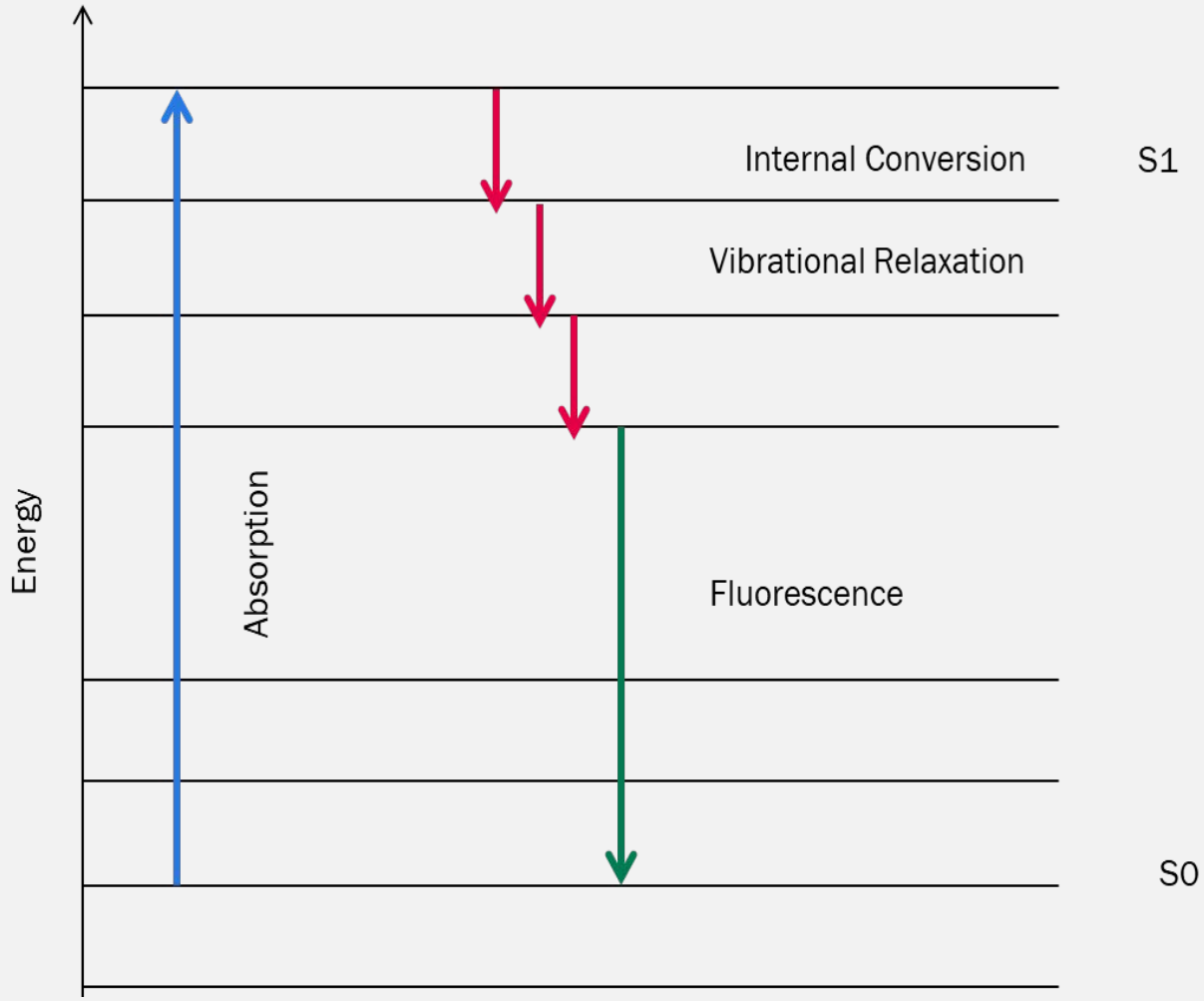


The Jablonski diagram and electron orbitals

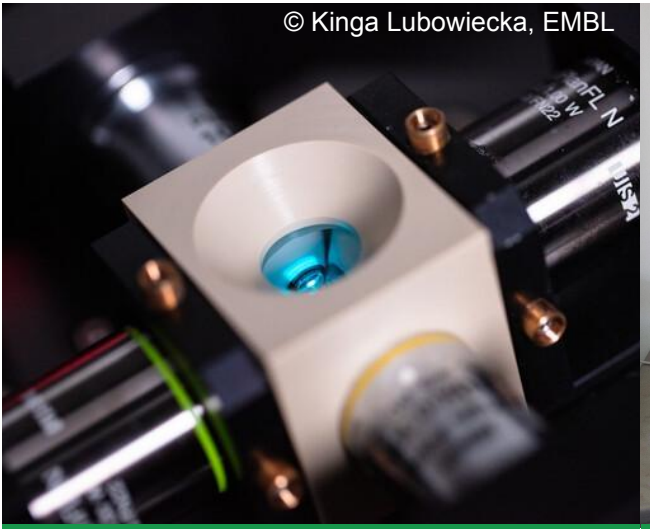
The excited electron is elevated from the S0 to the S1 orbital, a higher energy state. This higher energy is rapidly lost by internal conversion and vibrational relaxation (see next slide).

The low energy state S1 electron emits energy in form of a longer wavelength light and falls back to its original S0 state.

The process of reradiation happens within 10^{-9} – 10^{-7} seconds (range of nanoseconds).

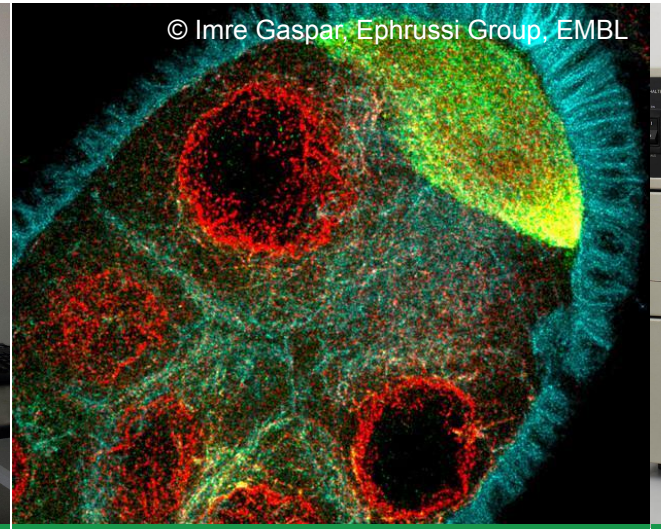


Applications of fluorescence in science



Microscopy

DNA/RNA Sequencing



Hybridization

Cell sorting

Applications of fluorescence in daily life

Bank note security

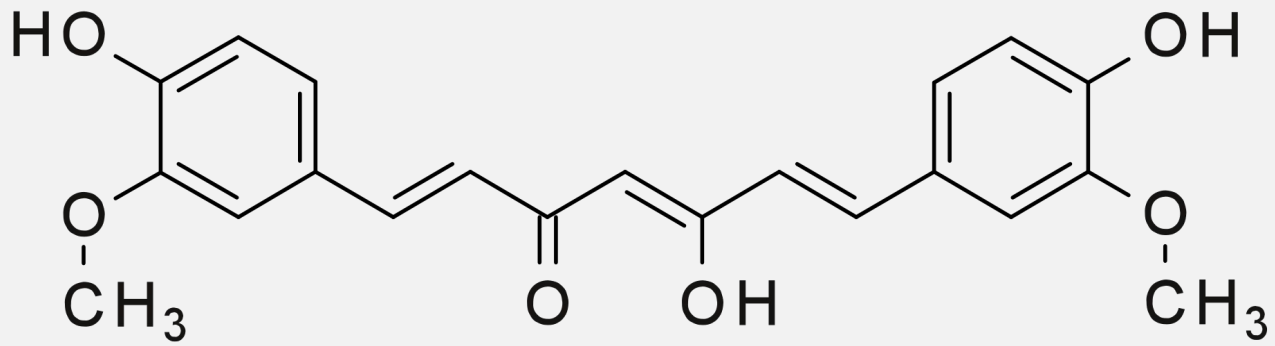
Clothing (High visibility/Black light) and Paint

Highlighter Pen

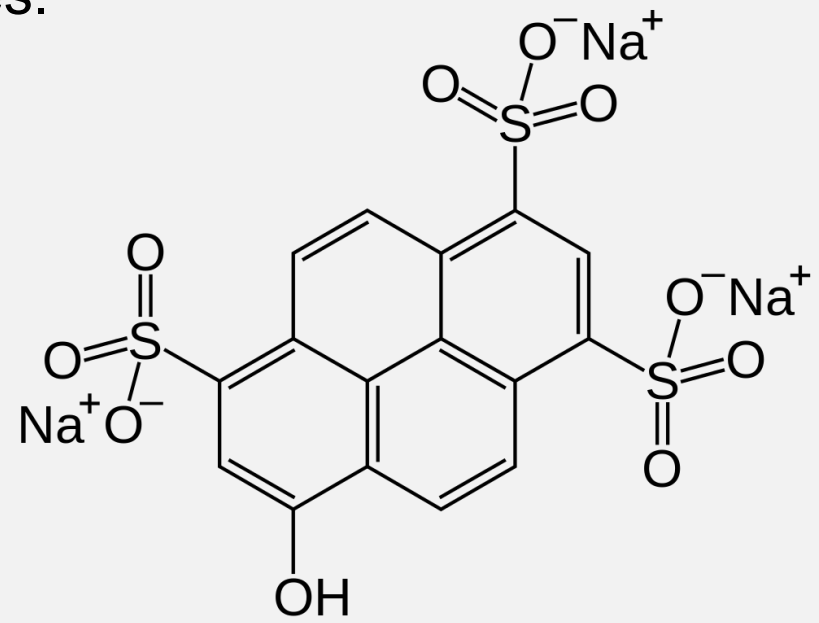
What is a fluorophore?

A fluorophore is every compound or molecule that fluoresces.

Fluorophores often contain aromatic rings.



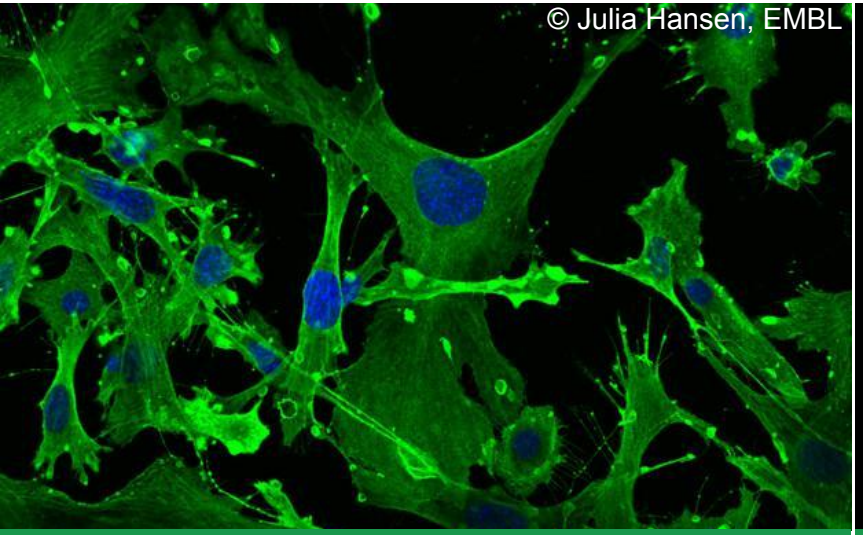
Curcumin emits green light when excited by blue light. It is the primary component of the traditional indian spice turmeric. Depicted is the enolic form.



Pyranine – the chemical compound in yellow highlighter shows green fluorescence.

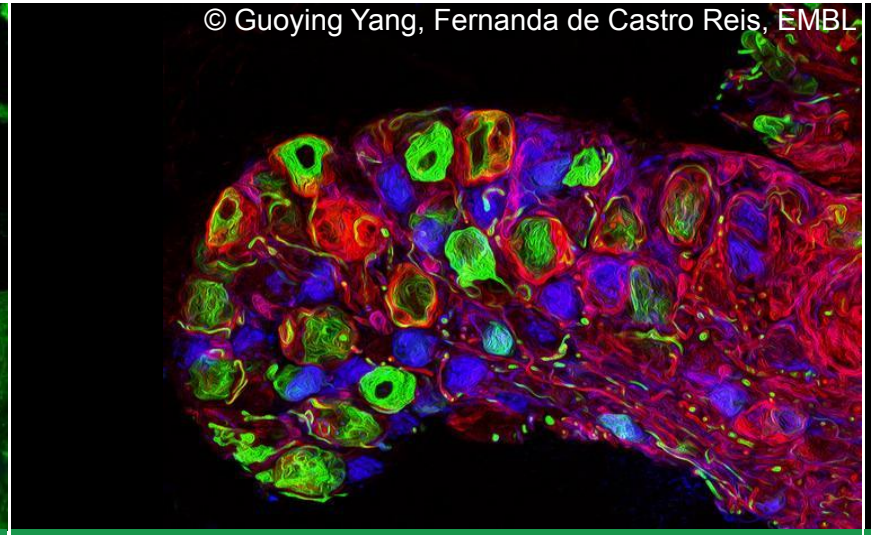
Both Curcumin and Pyranine can be used for staining of biological samples

Fluorophores can be used as...



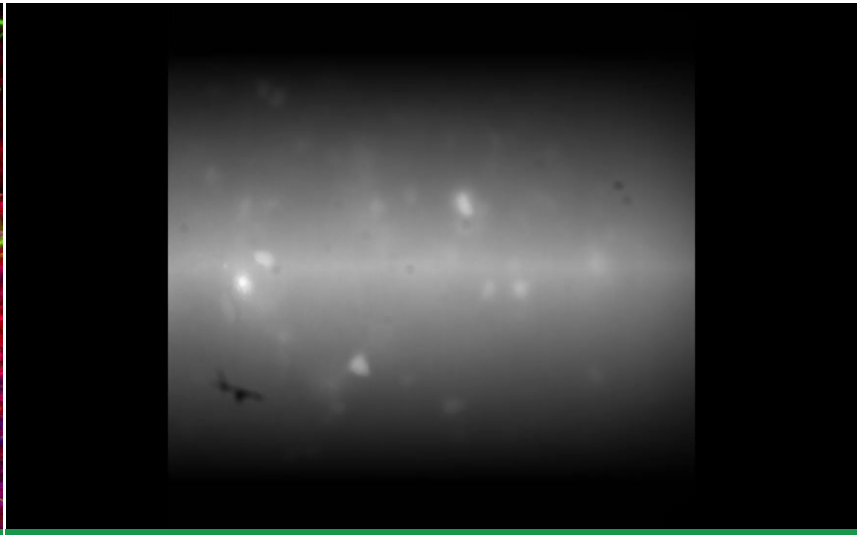
Dyes
Staining of cellular structures

(e.g. Nucleus)



Tags
Tracking movements of molecules

(e.g. a protein moving in a cell, localization to cellular compartments)



Indicators
Signaling the presence of a certain
molecule or ion
(e.g. presence of Calcium ions, pH indicator)

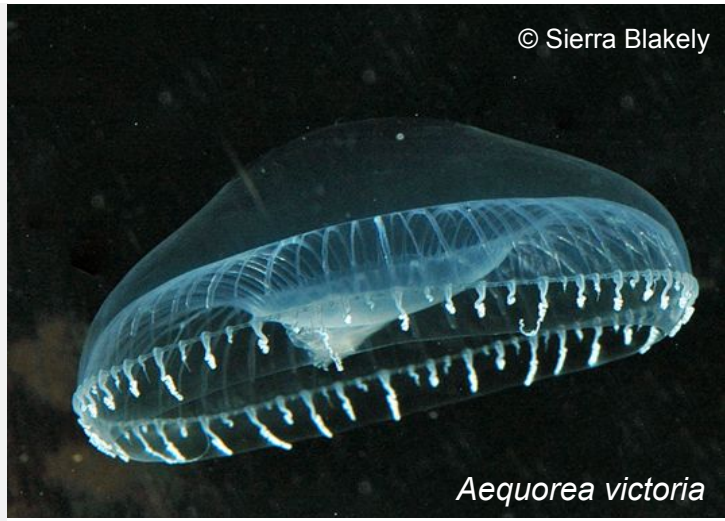
3. The green fluorescent protein (GFP) – A green revolution

The green fluorescent protein (GFP)

Nobel Prize in Chemistry 2008 for the discovery and development of the green fluorescent protein, GFP

- **Osamu Shimomura**
- **Martin Chalfie**
- **Roger Y. Tsien**

- Extracted from jellyfish (*Aequorea victoria*) in 1960s by Shimomura
- Excitation wavelength: 400 nm
- Fused to other proteins it can give information about their distribution in living cells, tissues and organisms.
- About 30 kDa in size
- Revolutionized the life sciences by showing that specific protein tagging with GFP is possible *in vivo* (Chalfie et al.)
- Tsien et al. worked on many alterations to GFP, resulting in a huge variety of fluorescent proteins in the whole visible range



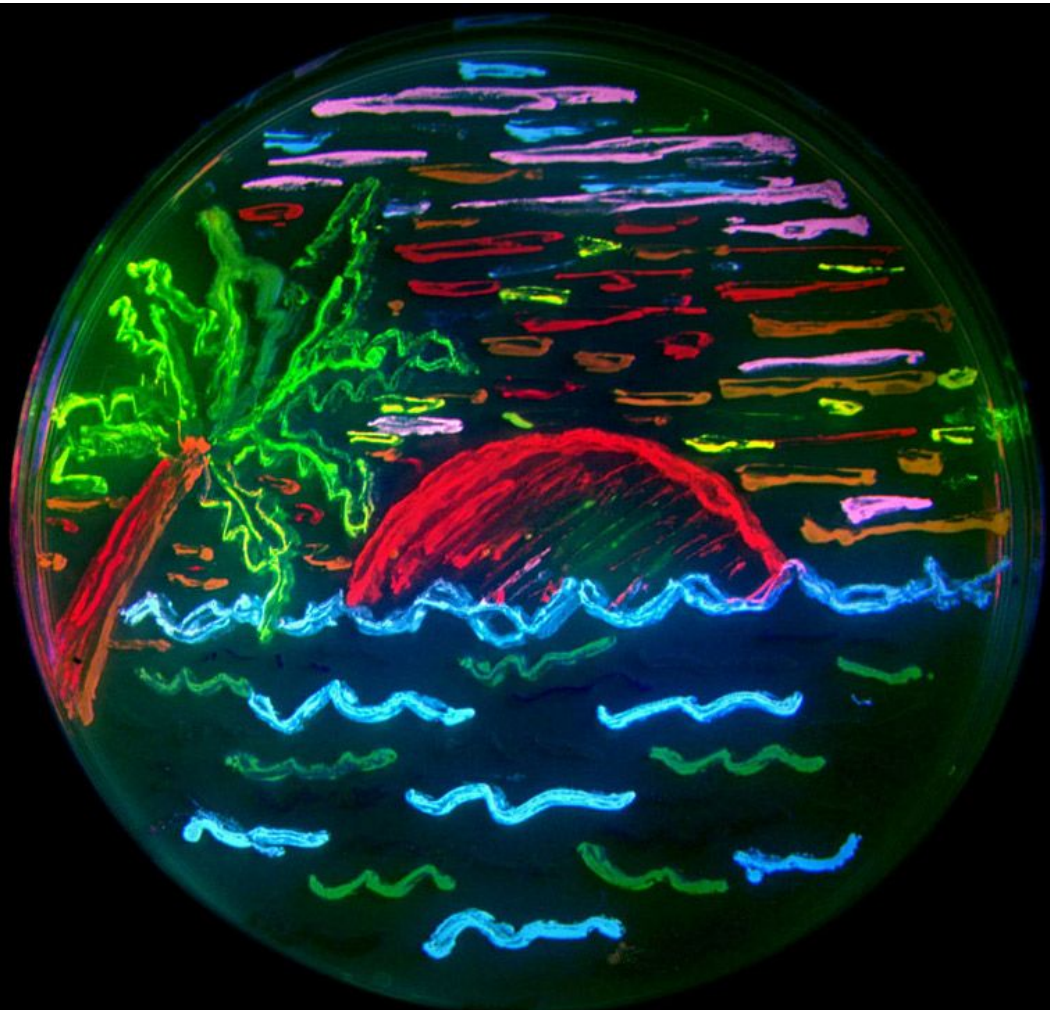
Designing fluorescent proteins - a rainbow of colours

GFP

- Emerald
- Citrine
- mTFP1
- BFP

dsRed

- mOrange
- mApple
- mCherry
- mGrape



Using the green fluorescent protein in research

The gene for GFP (or other fluorescent proteins) can be added to genes of interest via cloning and transformation:

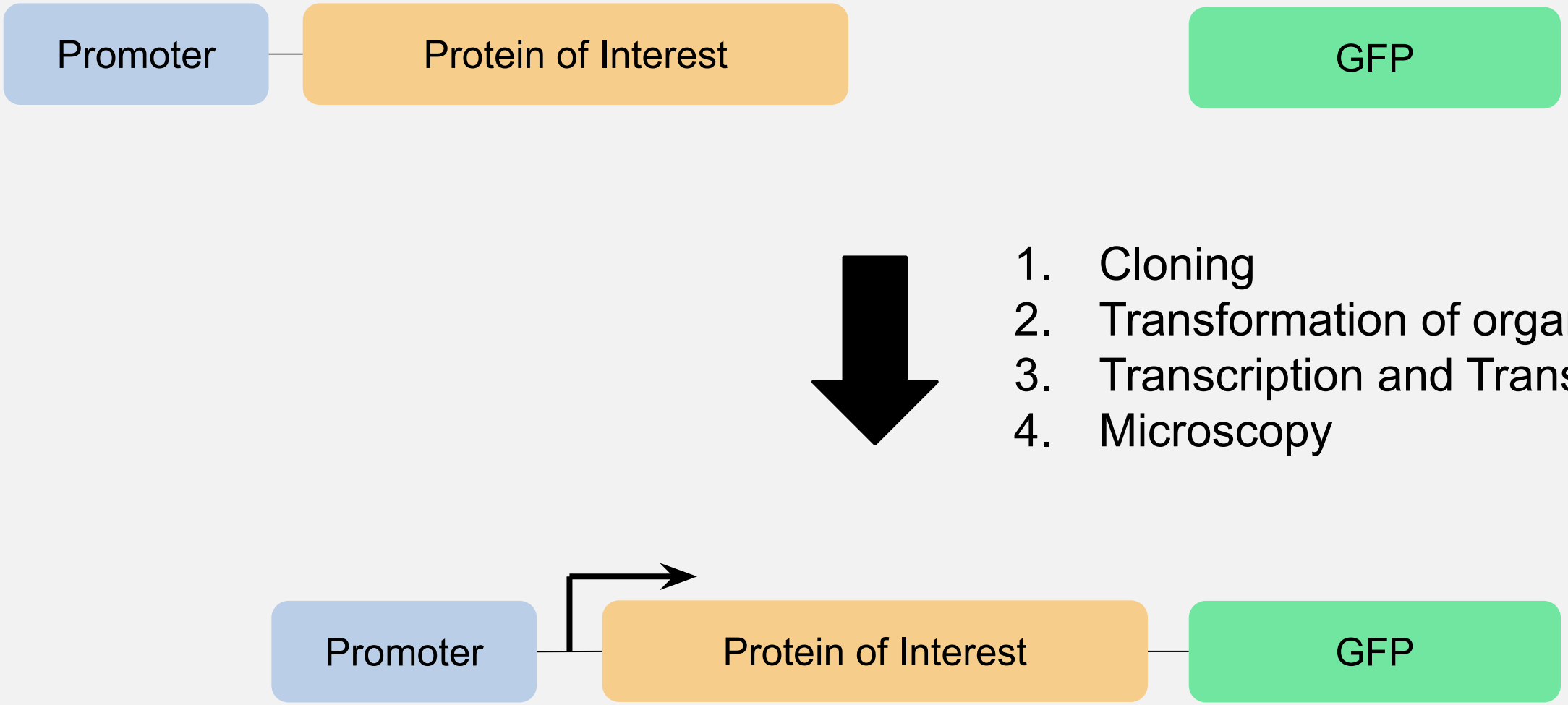
1. The gene for GFP is cloned to the C- or N-terminal end of the sequence of a protein of interest in bacteria.

2. The DNA is then purified and used to transform the respective model organisms. This can be done in many different ways.

3. The newly expressed ‘chimeric protein’ is tested for a change in function due to the addition of GFP.

4. Now, the tagged protein can be localized within the cell by microscopy or used for other studies involving GFP.

GFP expression cassette



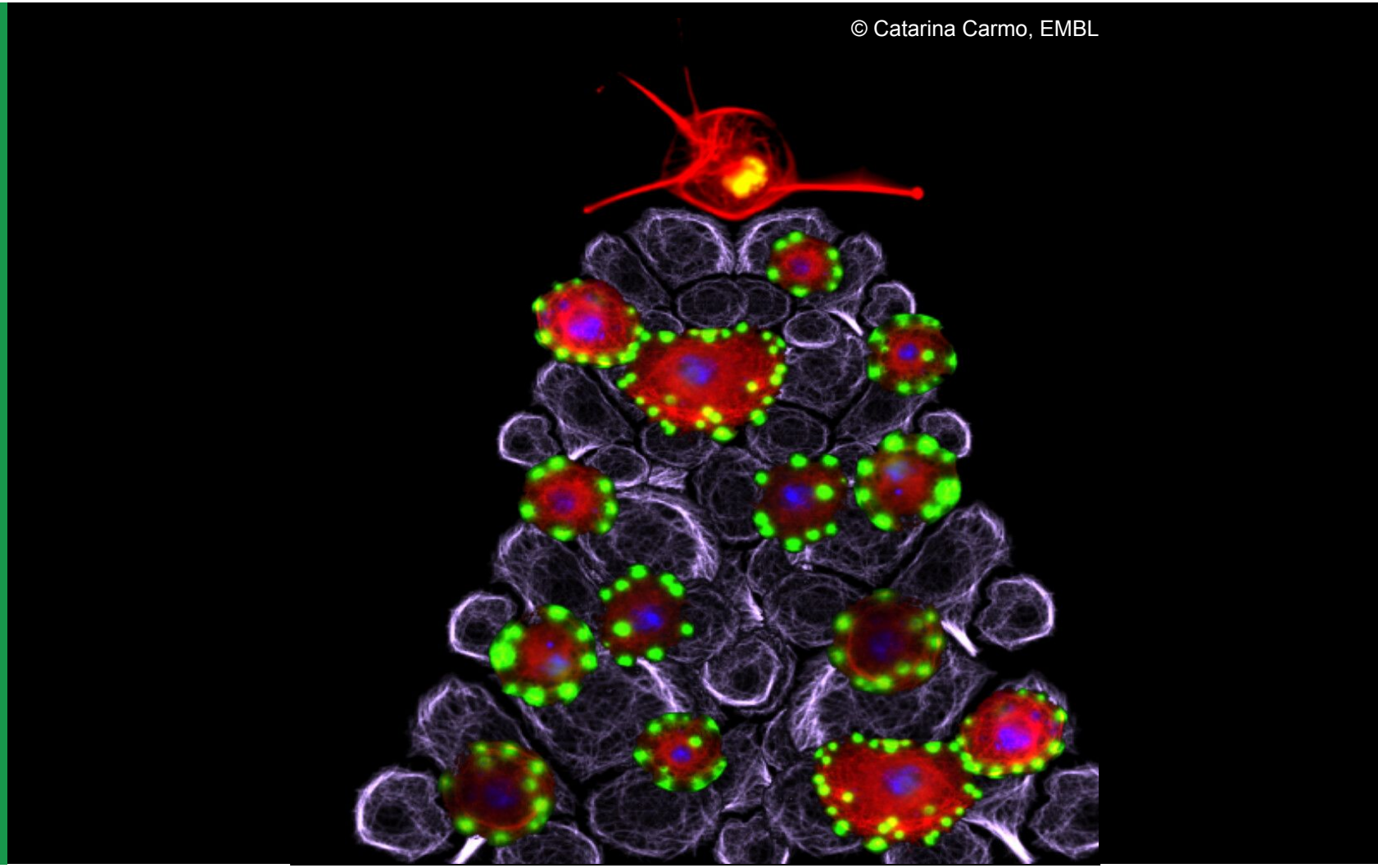
Example for protein tagging with GFP - Drosophila christmas tree

The image shows the cytoskeleton protein tubulin either in grey or in red.

DNA is seen in blue, and in yellow at the top cell.

The green dots are a protein tagged with GFP that was overexpressed, i.e. a large amount of the fusion protein was produced by the cells.

This overexpression resulted in clumping of the cells, leading to this tree-like shape.

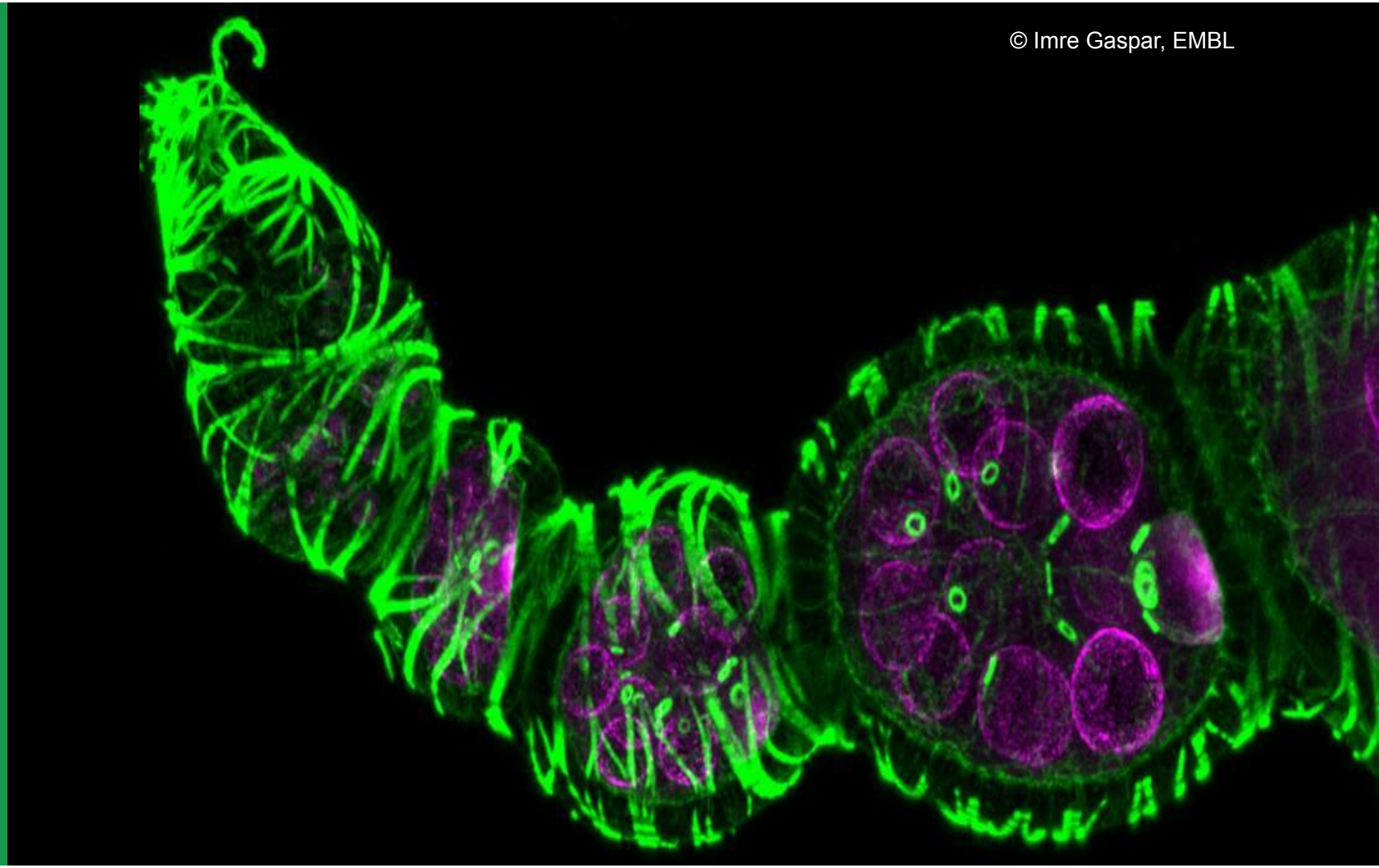


Example for protein tagging with GFP - Drosophila egg chambers

Actin is shown in Green, as ring canals.

Dividing Drosophila stem cells (left end of the picture) which result in rapidly dividing cystoblasts. On the right of the image there are 16 daughter cells, one of which acquired the oocyte fate: it is recognizable because it has 4 ring canals. The other 15 cells become nurse cells with over-expanded nuclei (magenta spheres). The ring canals are used for transport of molecules between nurse cells and oocyte.

The nuclei of the nurse cells have a perinuclear protein tagged with GFP (also green).

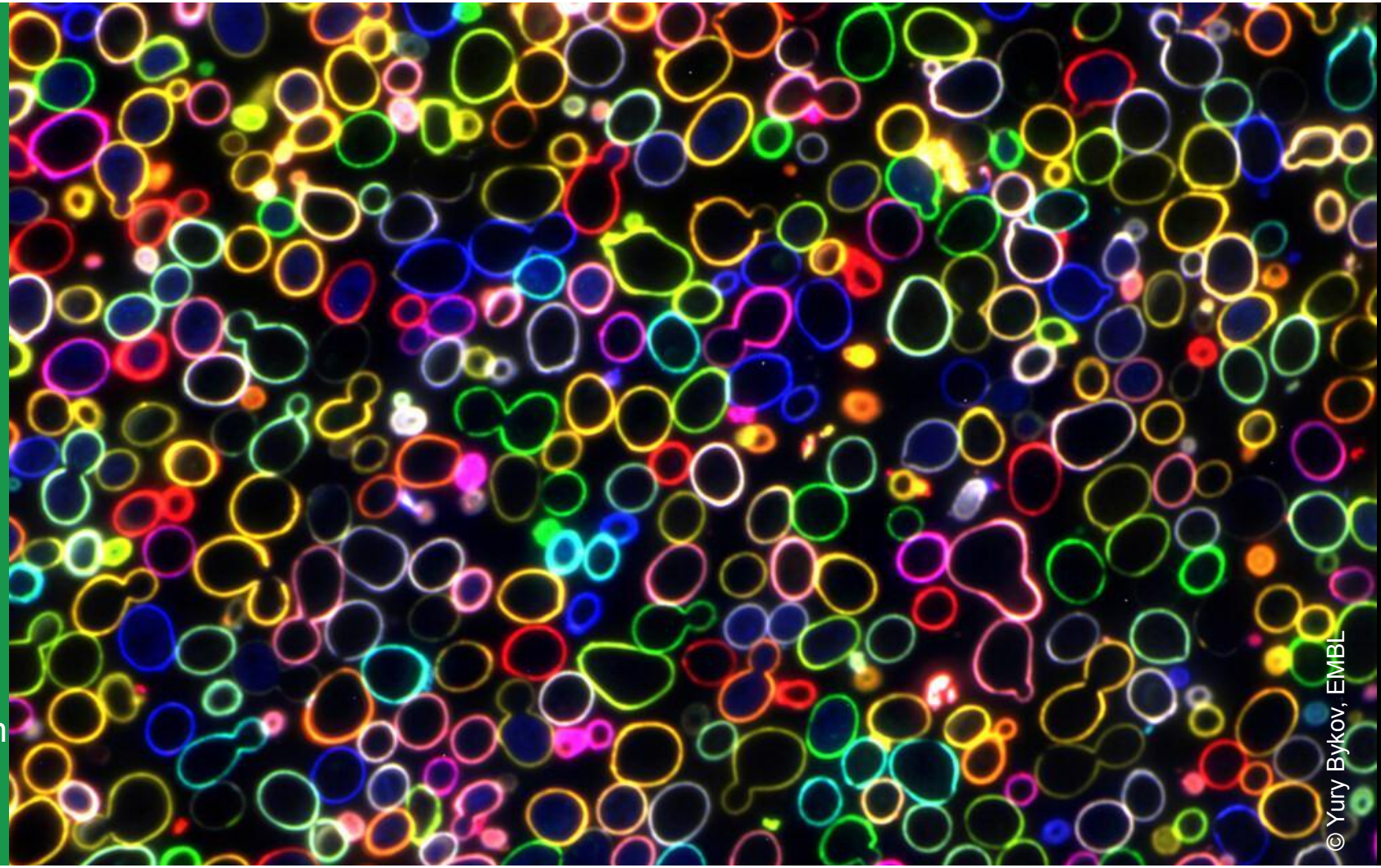


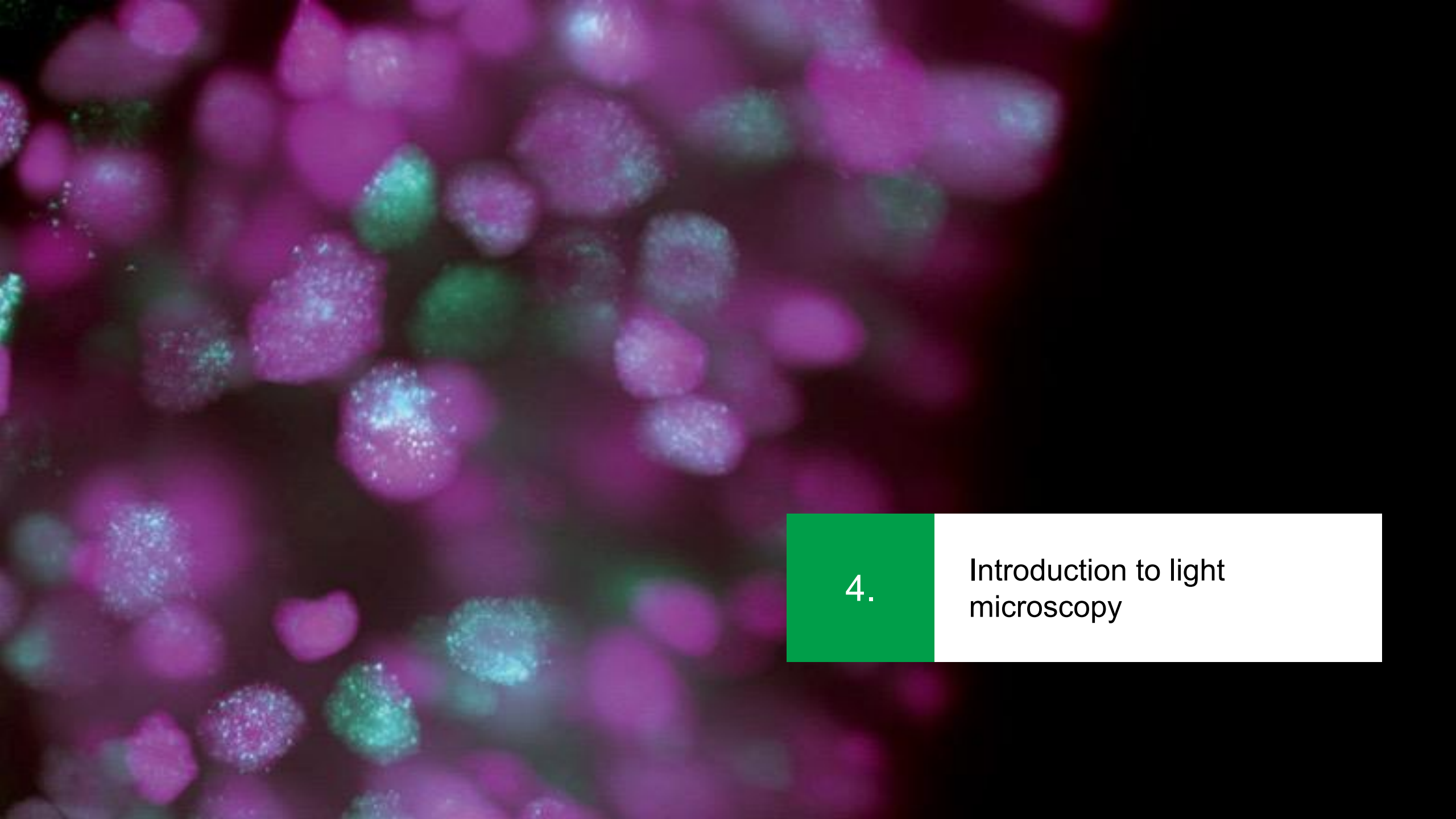
Staining with fluorescent dyes - Yeast barcoding

Saccharomyces cerevisia (Brewer's yeast) is used extensively in science as model organism. Here, yeast cells are labeled with different fluorescent dyes.

Cells from different experiments can be mixed and still be easily distinguished from on another. This makes simultaneous study and comparison possible.

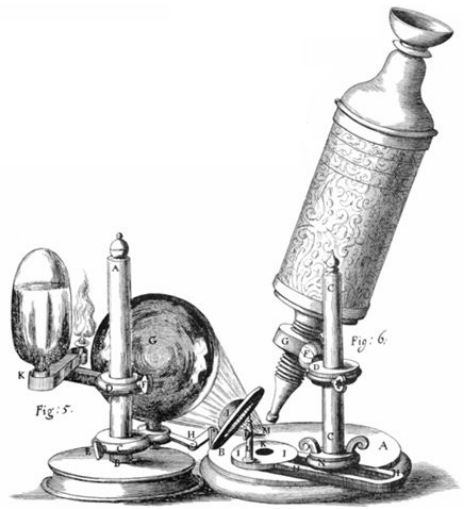
The technique called "barcoding" was established by EMBL alumnus Yury Bykov for use with automated electron microscopy to study small structures within the cells.

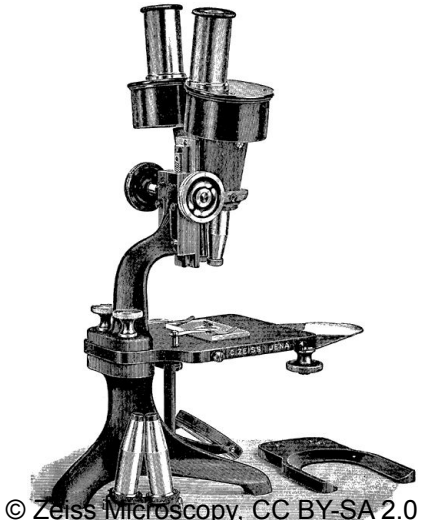


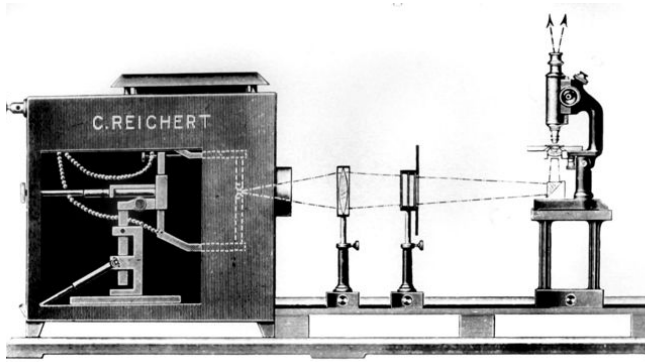


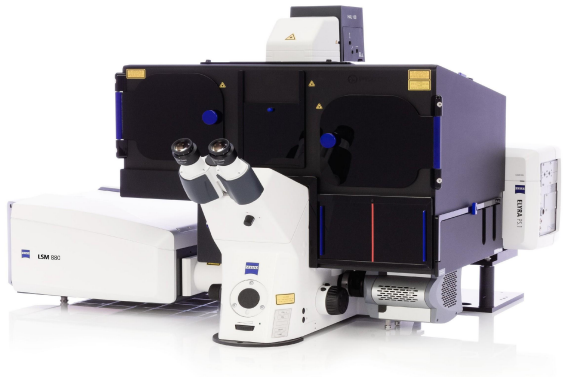
4. Introduction to light microscopy

The evolution of light microscopes



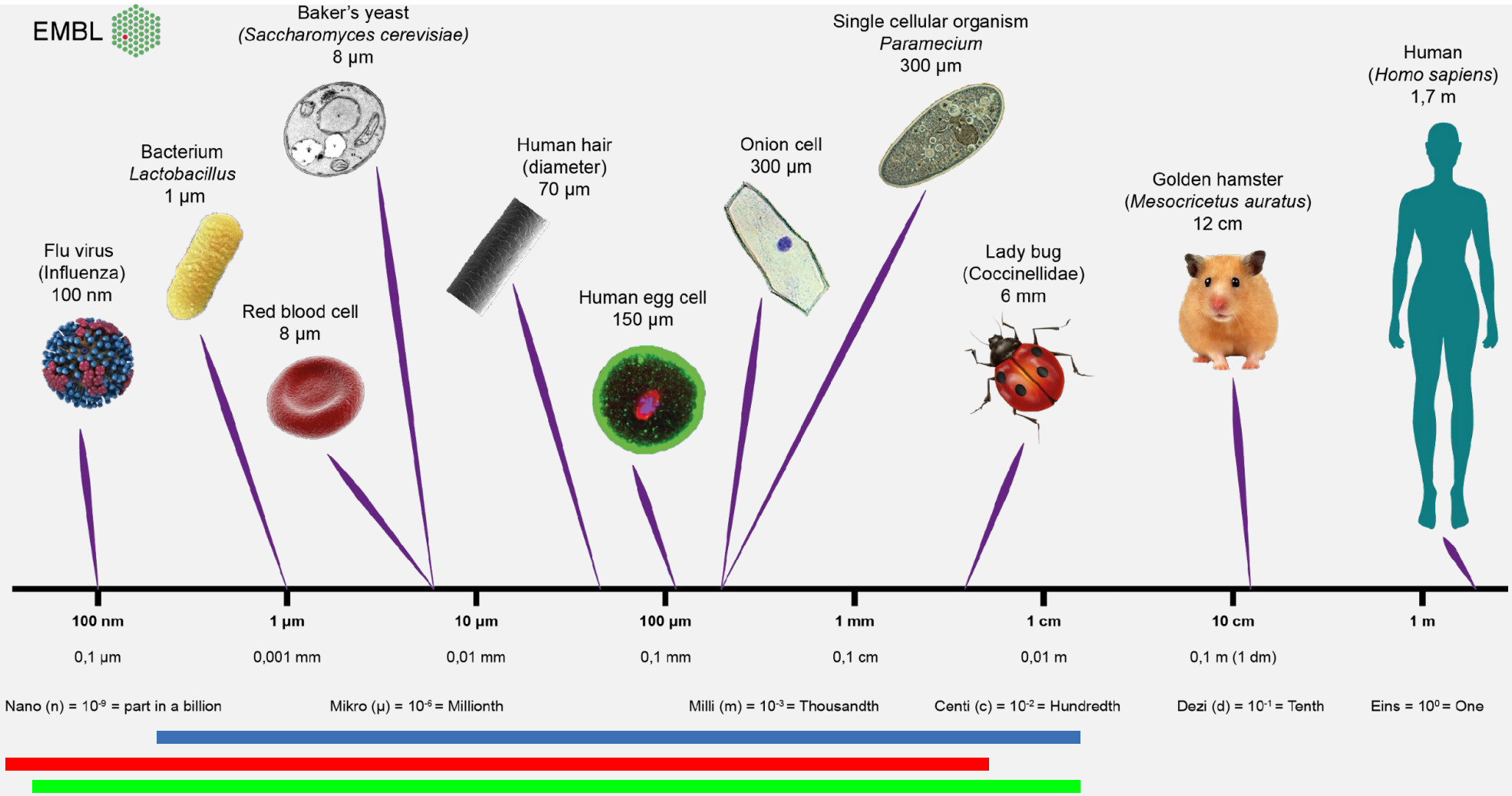






Hooke's compound microscope	Commercial light microscope	First fluorescence microscope developed by Oskar Heimstädt	Super resolution microscope
1665	1896	1911	Today
Resolution 10 µm			Resolution 10 nm

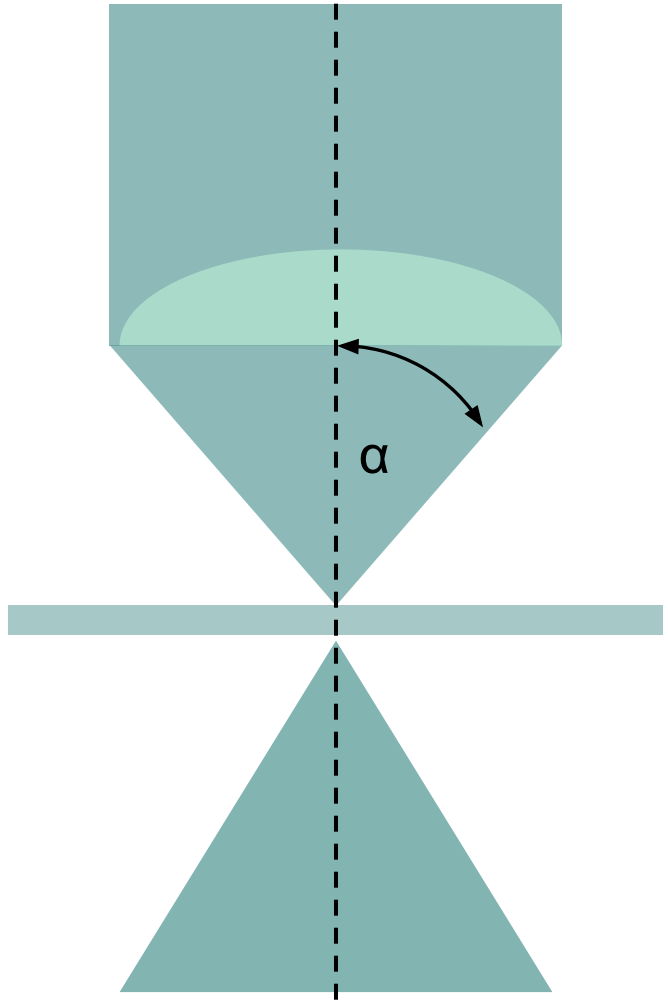
The scale of life



The numerical aperture

The numerical aperture (NA) describes the actual opening width of an objective.
It is described by $NA = \sin\alpha * n$,
where n = index of refraction (1 for air)
and α = ½ opening angle of the objective.

Basically, the NA can be viewed as the light-gathering capacity of an objective.



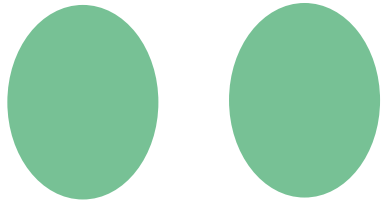
Objective Lense

Object plane

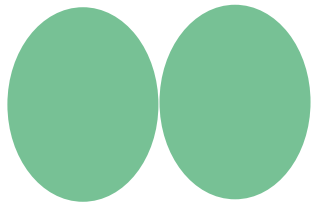
Light microscopy resolution

The resolution of a light microscope is the ability to distinguish between two points at a given distance. It is dependent on the NA of the microscope as well as the wavelength. The calculation for resolution as defined by Abbe is: $d = \frac{\lambda}{2NA}$, where d = resolution and λ = the wavelength.

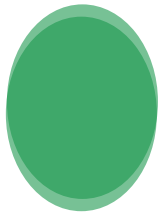
Therefore, the theoretical resolution limit is about 200 nm.



Object A and B (e.g. two Prokaryotes) can clearly be distinguished from another (distance > 200 nm)



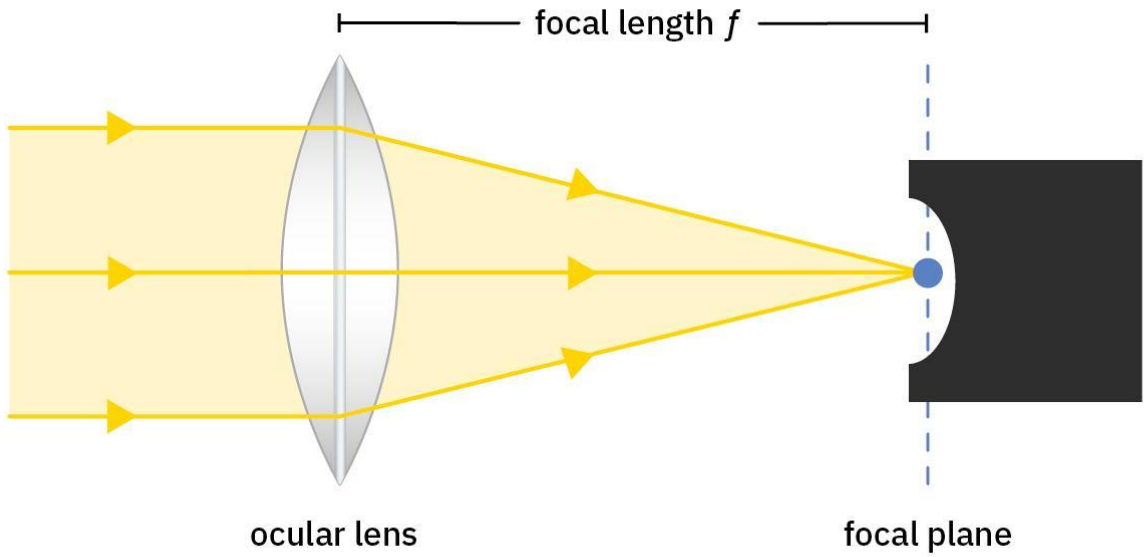
Object A and B nearly overlap and can barely be distinguished from another completely (distance ≈ 200 nm)



Object A and B overlap completely and cannot be distinguished at all (distance < 200 nm)

Focal length

Focal Length: The distance between the centre of the lens and the point where parallel light waves converge to a single point. It should be the distance between the eyepiece/camera and the ocular lens



5. fluorescence microscopy

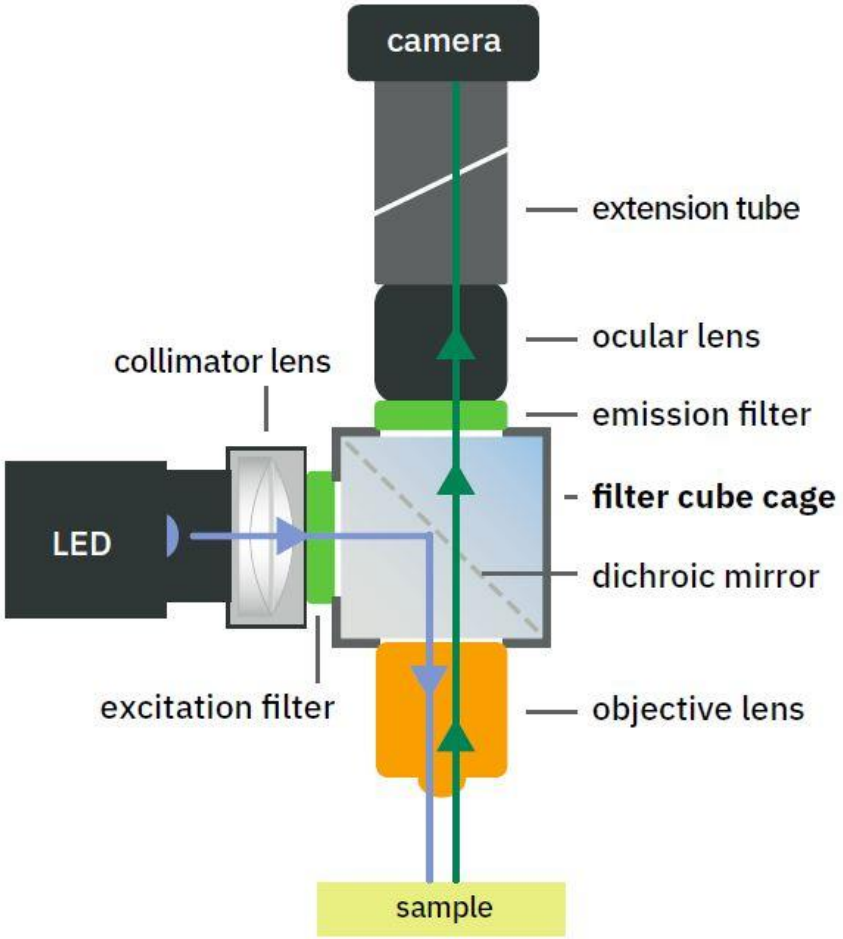
Fluorescence microscopy – short history

- While the first UV-microscope was developed as early as 1904 at Zeiss Optical Works, the first fluorescence microscopes were sold in 1911 and developed by Oskar Heimstädt
- Over the years, many developments were made to improve the microscope as well as the samples to observe
- 1933, the first fluorochromes (fluorescent dyes) were used in cell biology (Taking pictures took about 20 minutes!)
- Changes in Illumination source, development of filters, many different staining techniques and dichroic mirrors led to the rise of fluorescence microscopy as an important research tool
- Advances in computer capabilities and cameras made more advanced microscopy methods possible, with far better lateral and axial resolution

How does a typical fluorescence microscope work?

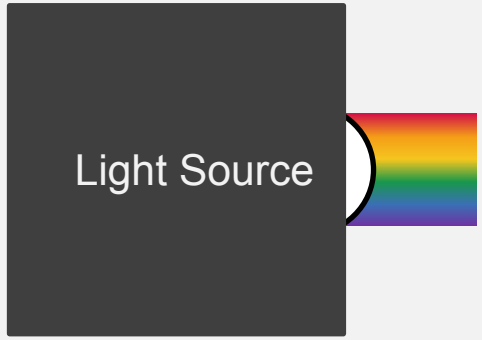
The 3 key components of a fluorescence microscope:

1. Light source
2. Dichroic mirror
3. Cage cube with emission and excitation filters



The path of light – Part 1

Light is emitted by the light source in a directional manner (in combination with a collimator lens). It can either be white light of all colours or of a specific wavelength (as is the case with our Microscope in Action resource).

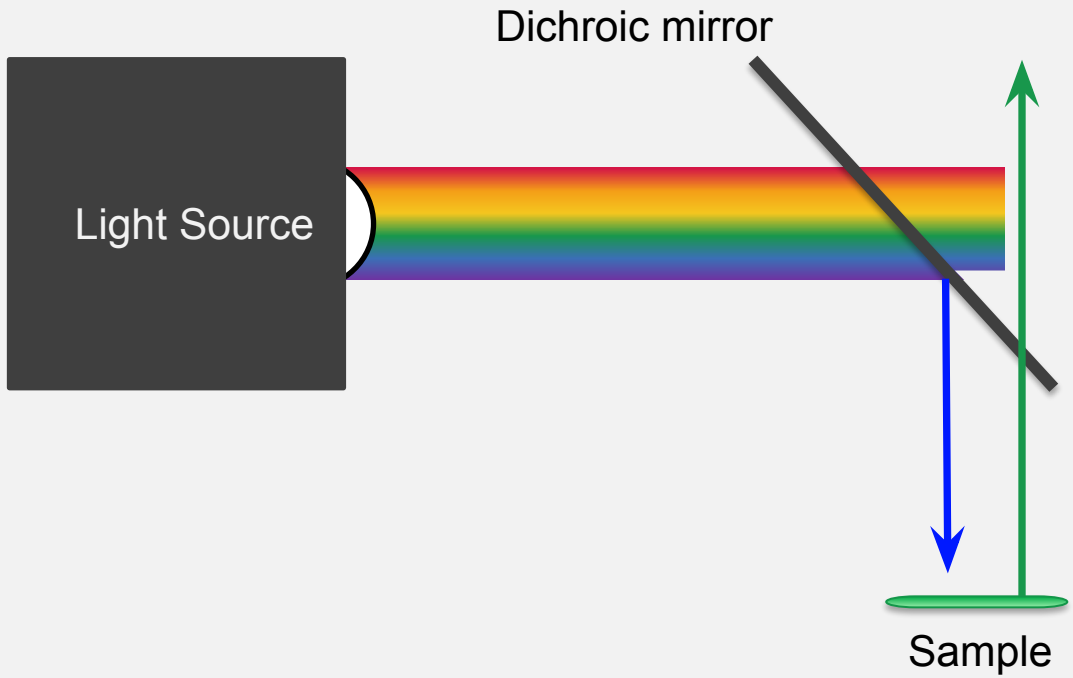


The **Light Source** is either a white light or a LED with specific wavelength, as is the case with your fluorescence microscope kit.

Collimation: The process of altering the direction of light rays in such a way that they start from a point source (the LED) and then, following the collimation lens, proceed in a parallel manner.

The path of light – Part 2

The light hits the coated dichroic mirror. With the correct orientation at 45°, this reflects light of a certain wavelength onto the sample.

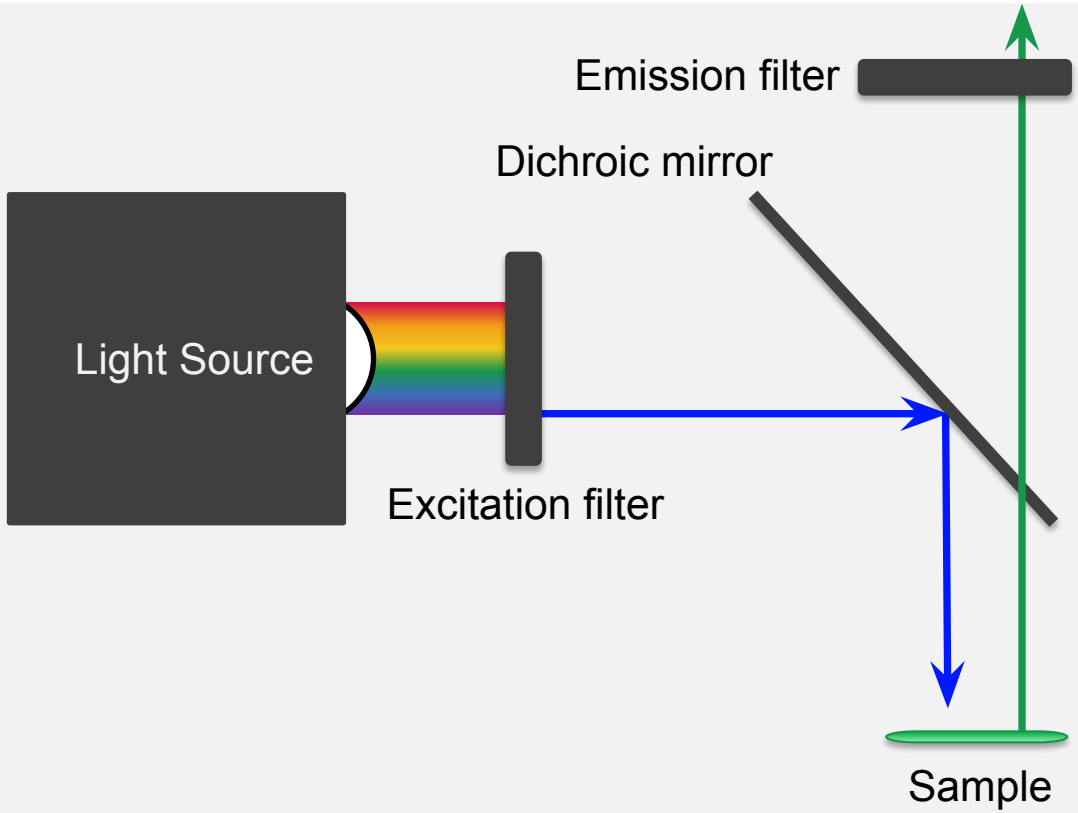


Dichroic Mirror: This is a coated piece of glass that reflects light of a specific wavelength and allows light of other wavelengths to pass through. The dichroic mirror reflects the excitation light onto the sample and lets the emission light pass to the ocular lens. Many different mirrors are commercially available and they can be tailored to the exact need dependent on the fluorophores.

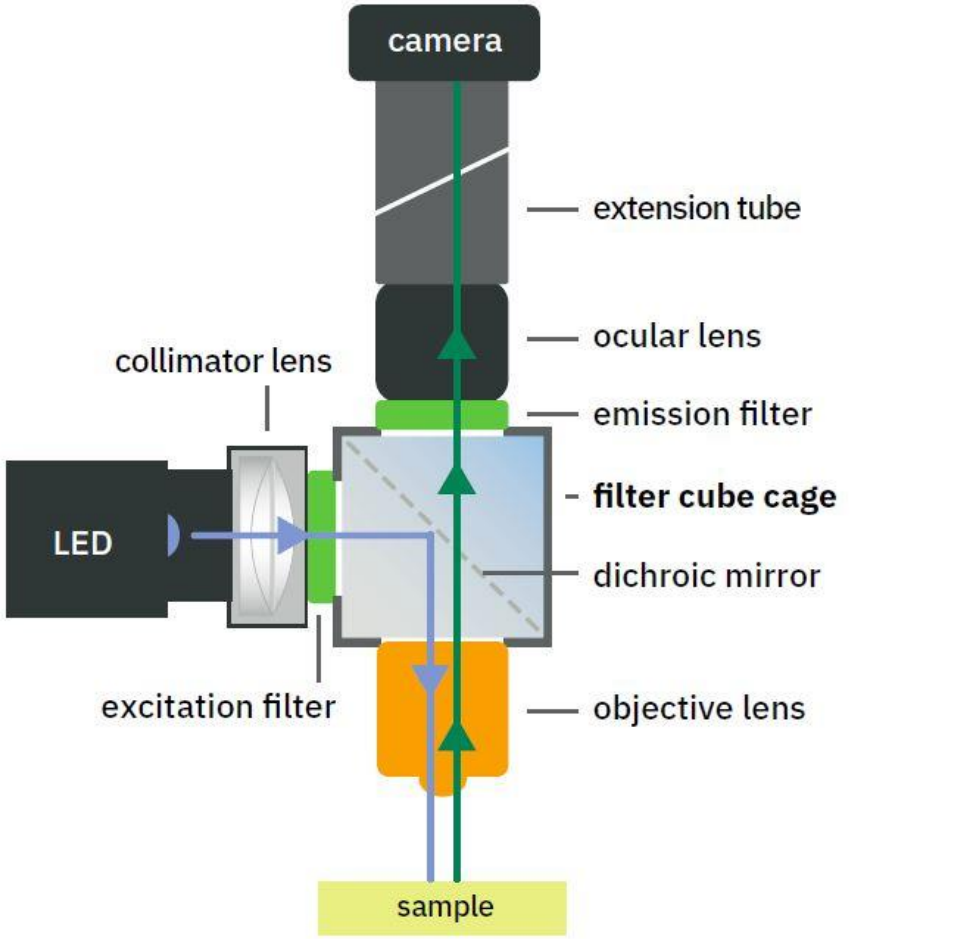
The excitation and emission filters

To select a certain wavelength more specifically and increase signal to noise ratio, two light filters are implemented: The excitation filter is only passable for blue light, while the emission filter only lets green light shine through to the ocular lens.

Both filters work for very specific wavelengths and have a sensitive coating.



The complete fluorescence microscope



Advantages of fluorescence microscopy

- Fluorescence microscopy increases specificity of what can be observed in a sample
- Since UV light can be used for excitation, the theoretical limit of resolution can be overcome
- The signal-to-noise ratio of fluorescence microscopy is high, resulting in a clear image
- *In vivo* and *in vitro* imaging of multiple proteins (Co-localization) is possible

Epi-fluorescence microscope

- The principle of an epi-fluorescence microscope is that both the illuminated and emitted light travels through the same objective lens.
- “Epi” is a Greek prefix and stands for “same”.
- The light of a specific wavelength travels through the objective lens and illuminates the sample. Then, when excitation happens, the light emitted from the sample travels back through the same objective to the eyepiece/camera.

Confocal laser scanning microscope (cLSM)

- CLSM is a widespread microscopy technique that developed from the epifluorescence microscope
- Got increasingly important with better computers
- The sample is scanned by focused lasers of specific wavelengths
- The emitted light is detected and the computer generates a complete image

cLSM versus epi-fluorescence

cLSM

Scanning of multiple channels possible

 Co-localization

Better z-resolution

Specific techniques like FRET, FLIP, FLAM…

 Protein interaction studies

Less overall photobleaching

 If you want to perform specific localisation studies or need high resolution in a thicker sample, cLSM is probably the best choice

Epi-fluorescence microscope

The fluorescence is detected from the same side as the excitation

Faster and easier setup

General observation and imaging is faster

 If you want a standard image of a thin sample, the epi-fluorescence microscope is probably the best choice

More cLSM techniques

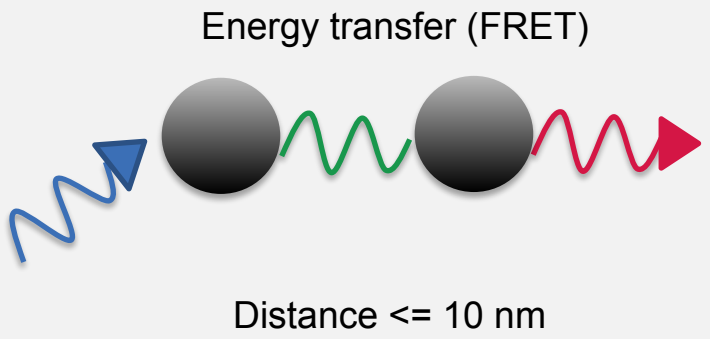
Spinning Disk cLSM: Instead of rastering the sample in straight lines, the light passes a spinning disk. This technique increases imaging speed with some trade-off in light intensity and z-plane resolution

Two-Photon Imaging: Instead of exciting the sample with a high-energy laser of low wavelength, a higher wavelength with stronger light intensity is used.
If two photons of this higher wavelength hit the sample at the same time in the same place, it can be excited by these. This results in lower photobleaching of fluorophores.

Förster Resonance Energy Transfer (FRET)

FRET describes the transfer of energy from one fluorescent molecule to another when they are in close proximity (<= 10 nm)

One fluorophore is excited and transfers its energy to the second fluorophore, resulting in a colour shift and emission of longer wavelength light.



It allows for protein co-localization and interaction studies and can be applied in various ways.

One disadvantage is the difficult setup requiring distinct and non-overlapping fluorescence spectra as well as suitable controls

Fluorescence lifetime imaging (FLIM)

FLIM applies the principle of FRET (donor and acceptor fluorophores) to measure the fluorescence lifetime of the donor molecule.

If donor and acceptor are in close proximity to another (e.g. because of a possible interaction), the fluorescence lifetime of the donor decreases.

To measure the fluorescence lifetime, extremely fast laser pulses are used to excite the donor fluorophore.

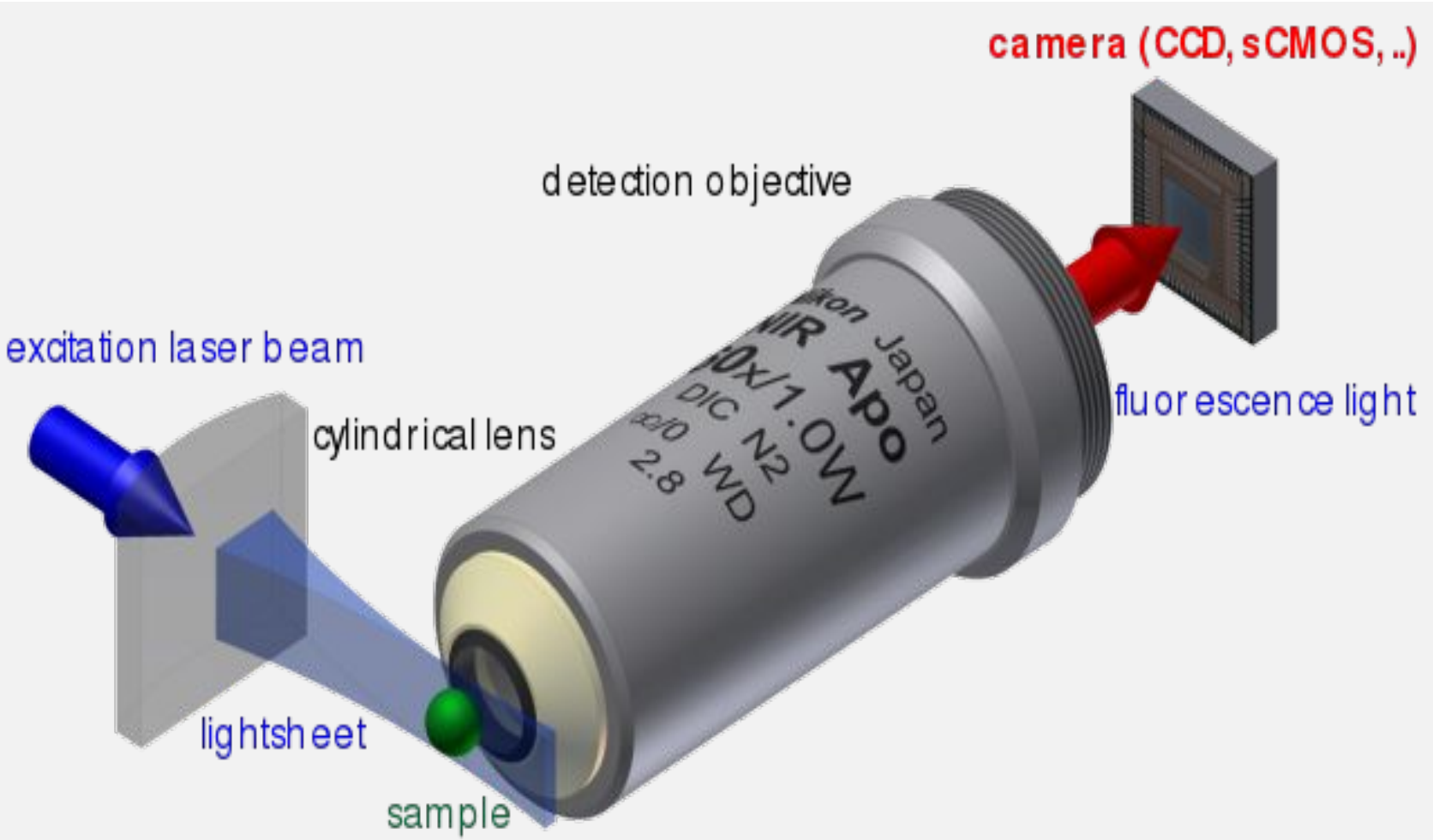
The Fluorescence lifetime describes the timespan an excited donor remains in the excited state.

Light-sheet microscopy

Instead of illuminating the whole sample at once (or at least all z-layers), light-sheet microscopy illuminates only one sample plane at once.

This reduces overall phototoxicity (photobleaching and photodamage) and increases spatio-temporal resolution.

It is often used for investigating developmental processes like embryo development or lateral root initiation.

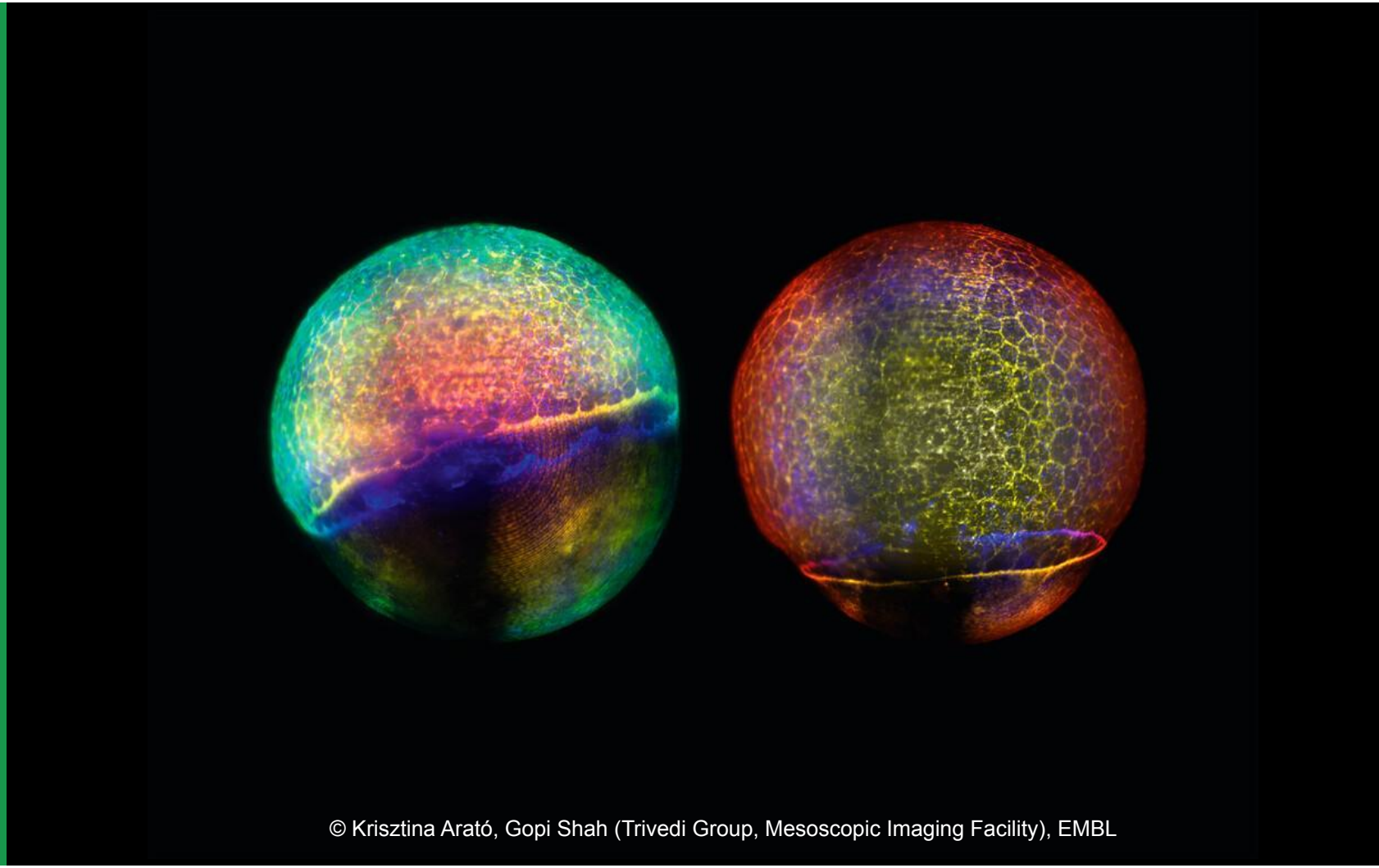


Developing zebrafish embryos

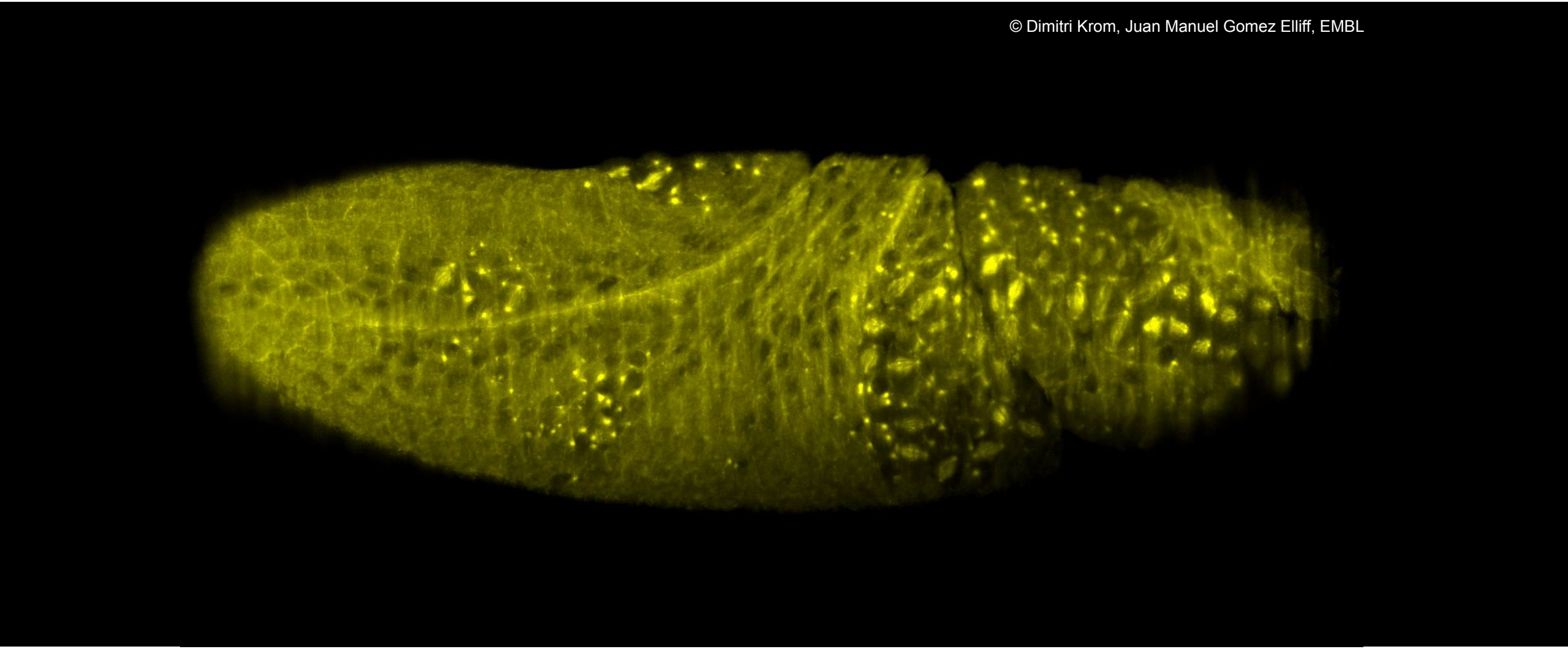
Light-sheet microscopy image of developing zebrafish embryos.

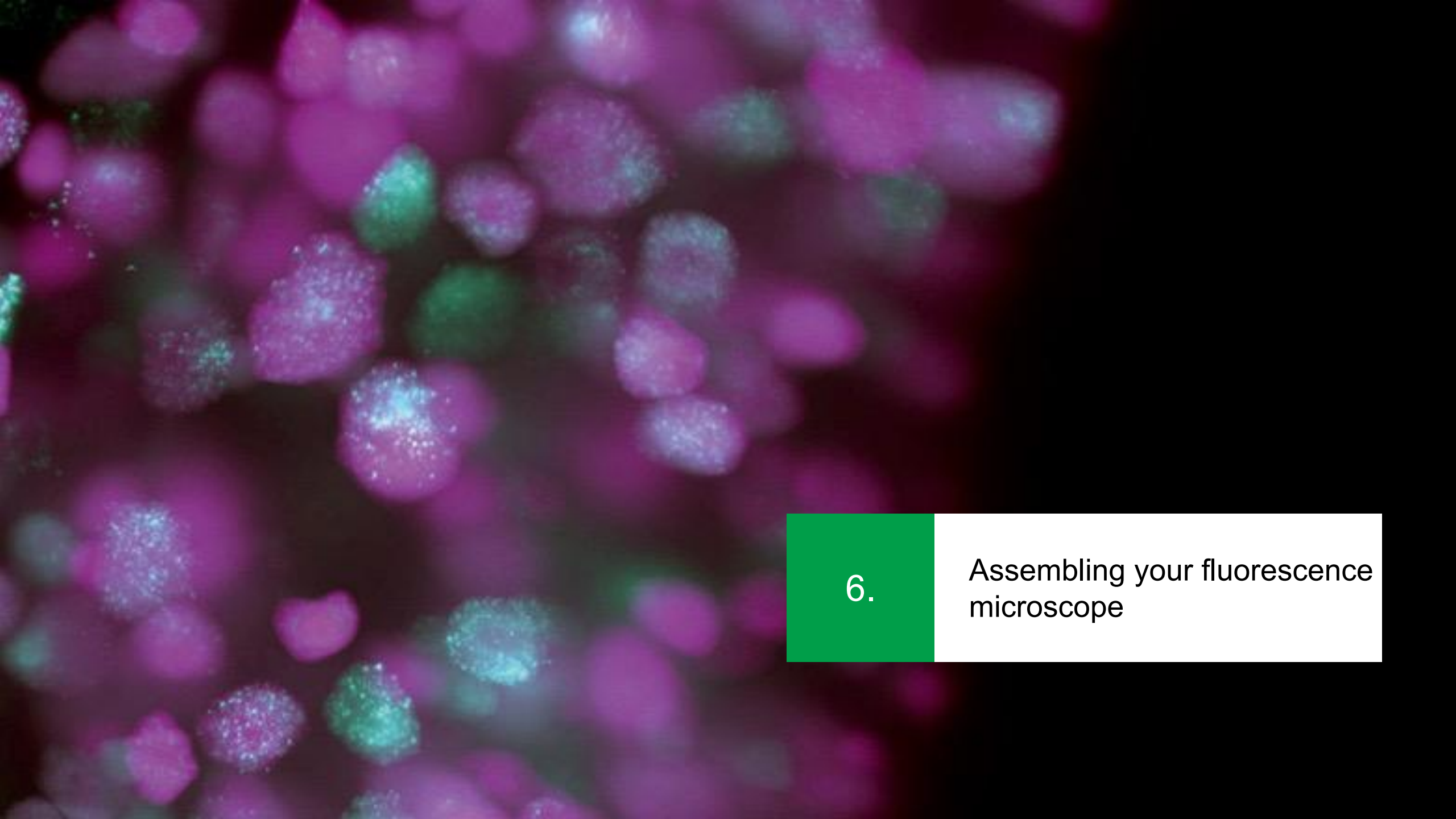
It shows a protein ring around the embryo that is necessary to undergo gastrulation.

Actin was labelled with GFP, but colour code in this picture shows sample depth.



Lateral view of drosophila embryo



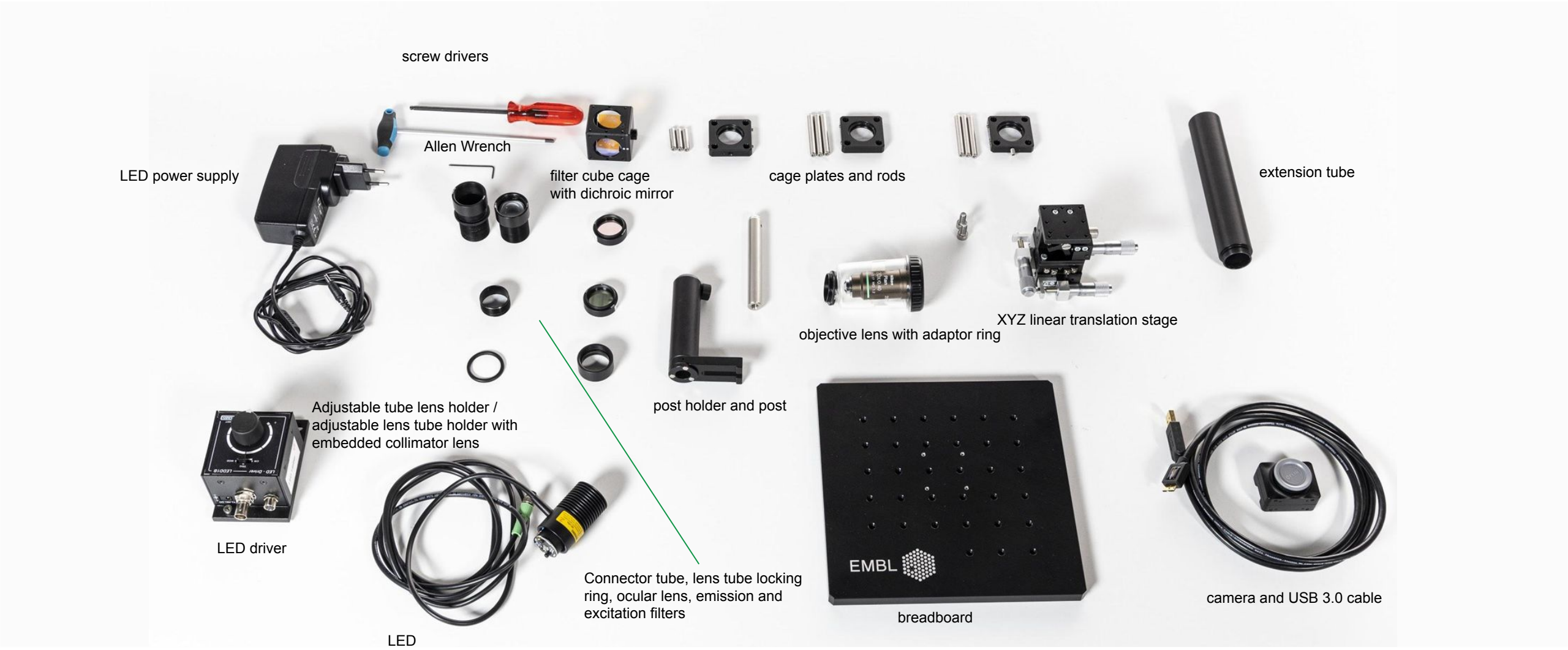


6. Assembling your fluorescence microscope

Safety information

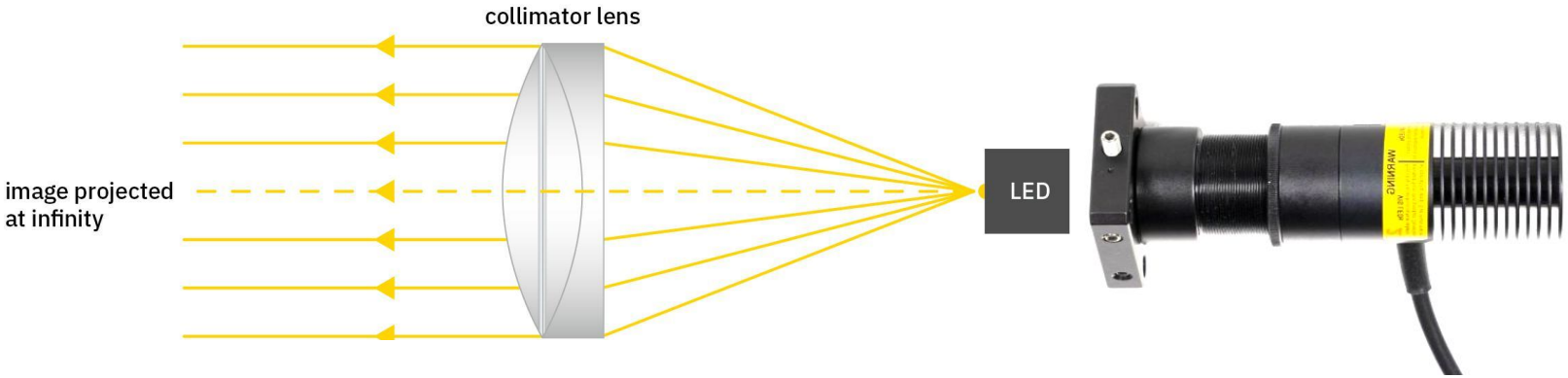
1. **Do not look into the light.** The LED is very bright and might damage your eyes. Turn it off when it's not needed.
2. Do not eat or drink during the assembly.
3. Wear **powder-free gloves** while working with the optical components. Your skin might leave smudges, which are difficult to remove and will limit the functioning of the microscope.
4. To avoid harming yourself or the microscope, make sure that the components or other materials cannot fall off the table while you are working.
5. Handle all components with care. While setting up the microscope, carry heavy components with both hands.
6. **Do not drop or bump the objective lens** against anything, and in particular not against the XYZ stage during assembly and focusing. The objective lens is very sensitive.
7. Unless otherwise specified, the screws of the optical components should only be finger tight.

The fluorescence microscope components



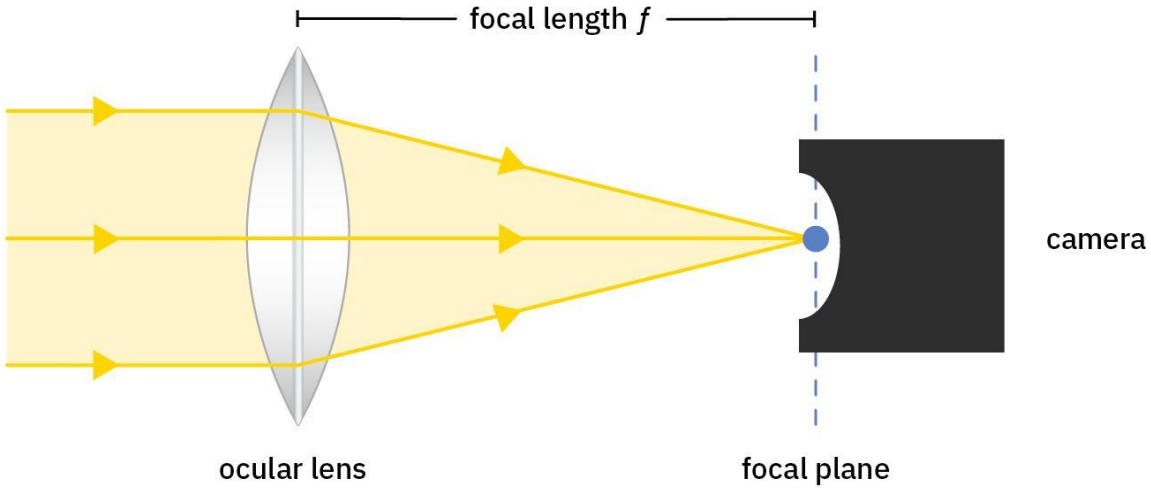
Illumination subsystem (LED)

- **Collimation:** The process of altering the direction of light rays in such a way that they start from a point source (the LED) and then, following the collimation lens, proceed in a parallel manner.



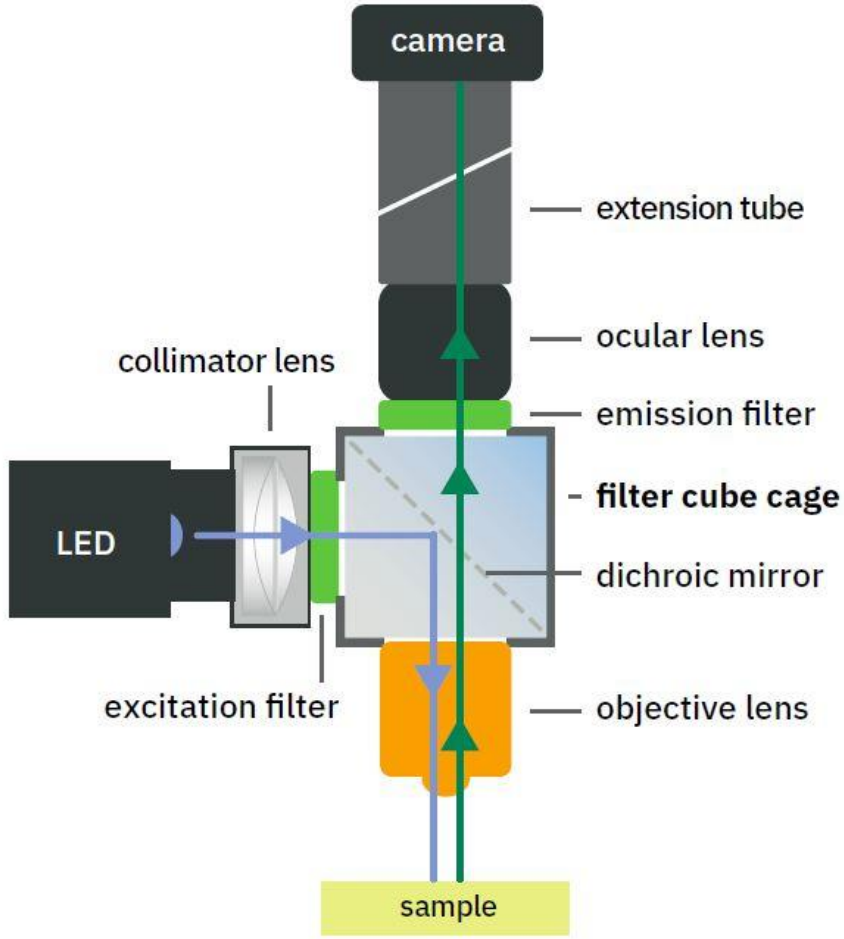
Imaging subsystem (camera)

- **Focal length:** The distance between the centre of the lens and the point where parallel light waves converge to a single point.



Filter cage cube

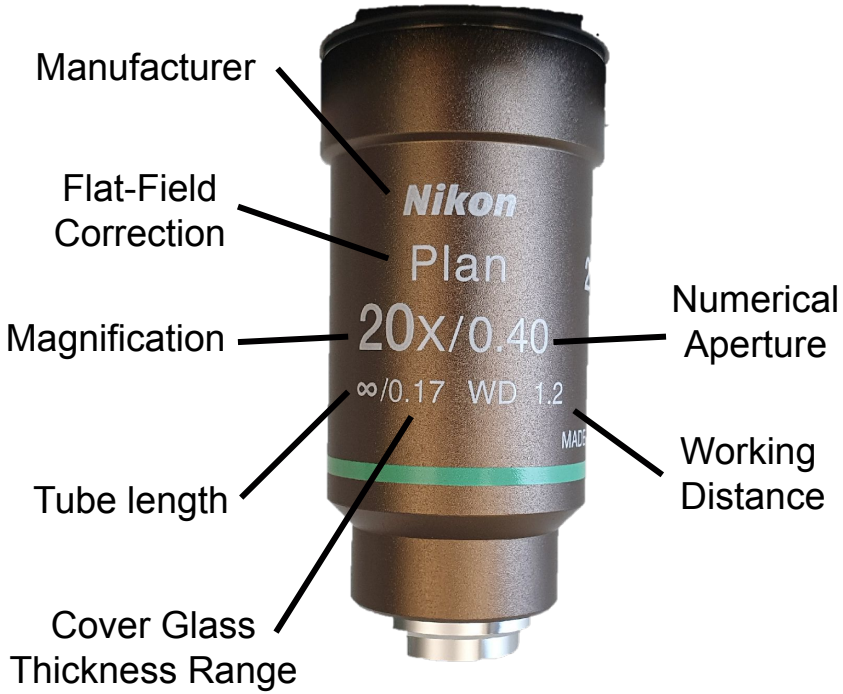
- The filter cage cube is the central piece of the microscope.
- The Dichroic mirror is positioned inside the cube.
- It includes excitation and emission filters.



Objective lens

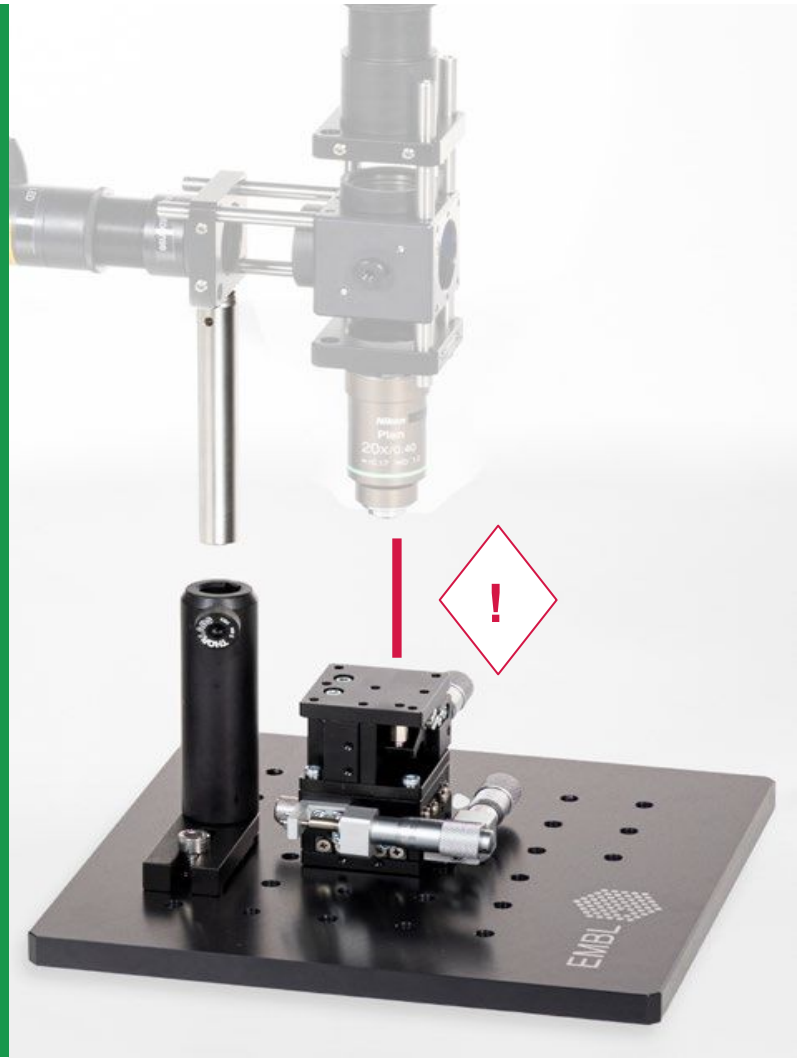
- The objective is the microscope component that is closest to the sample (the distance between the end of the objective and the surface of the sample is called Working Distance). Together with the ocular lens, it increases the overall magnification.

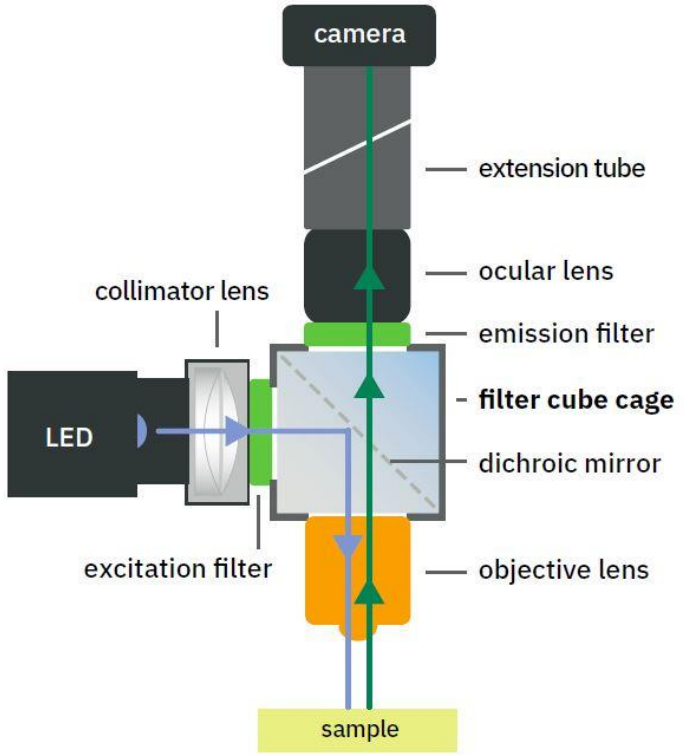
Total magnification = Objective magnification X ocular lens magnification



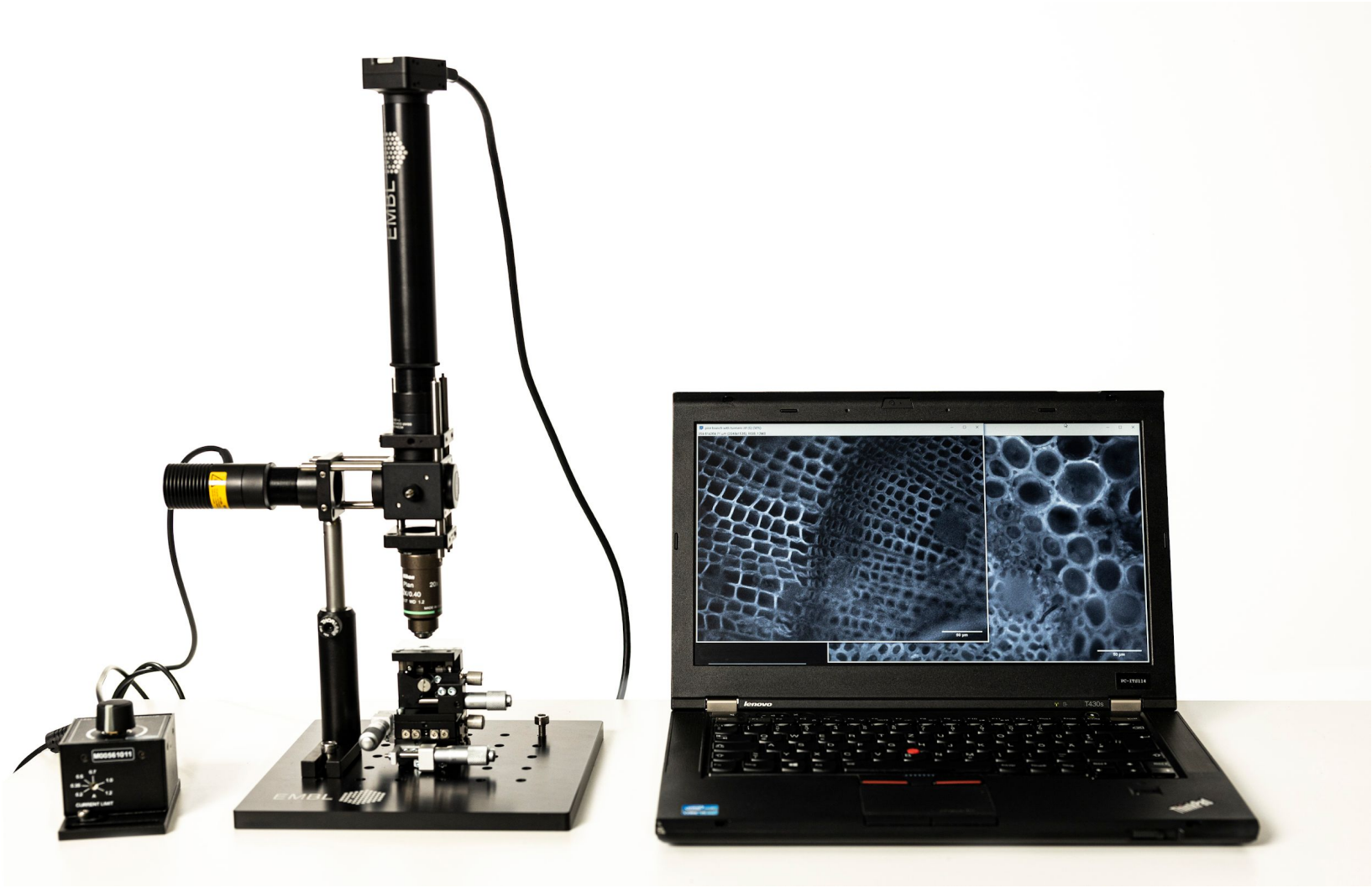
Mounting the microscope on the breadboard

- Do not drop or bump the objective lens against anything, and in particular not against the XYZ stage during assembly and focusing.
The objective lens is very sensitive.

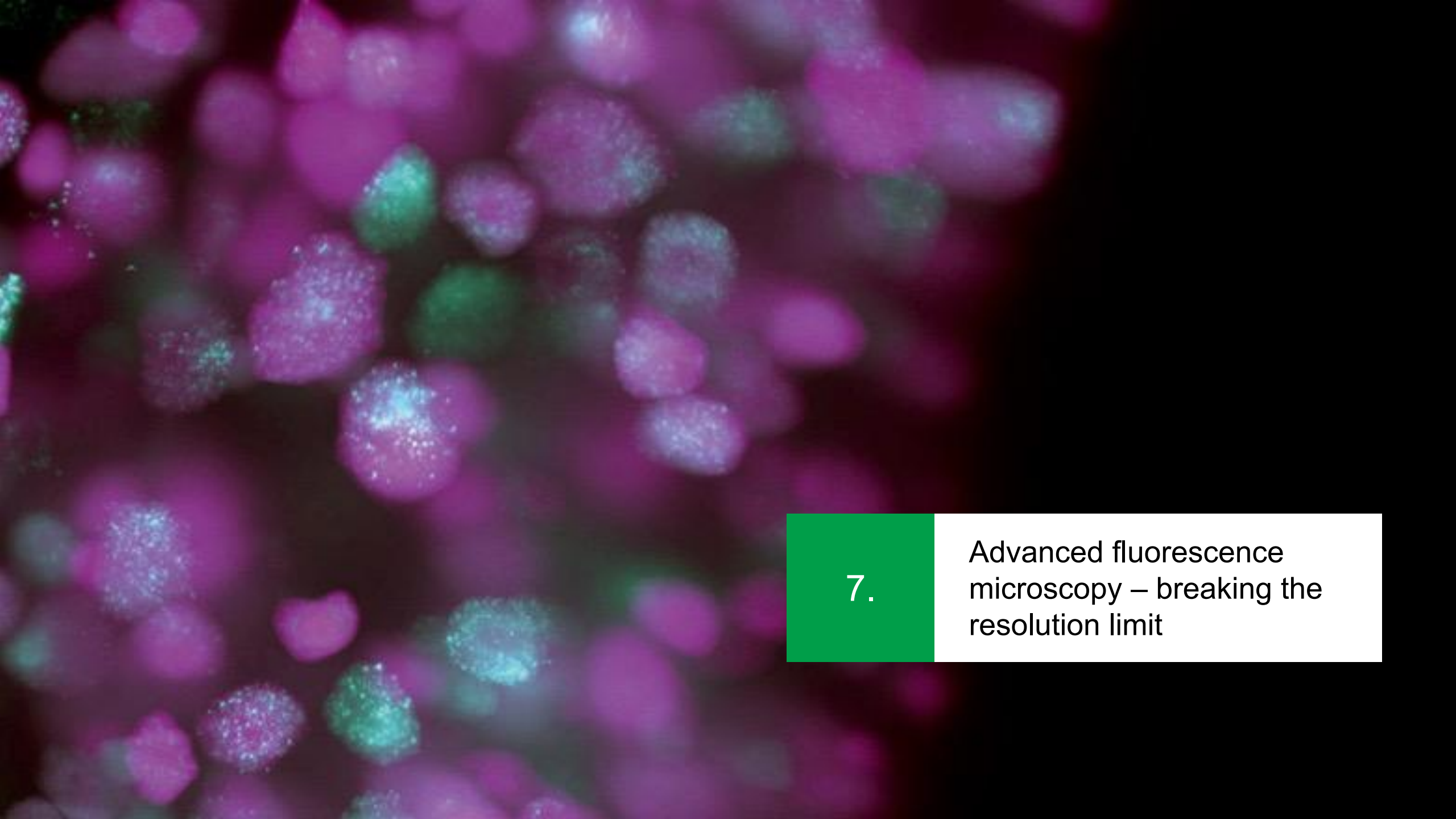




Using the microscope for imaging



Laptop not included in the resource



7. Advanced fluorescence microscopy – breaking the resolution limit

Limits of conventional microscopy

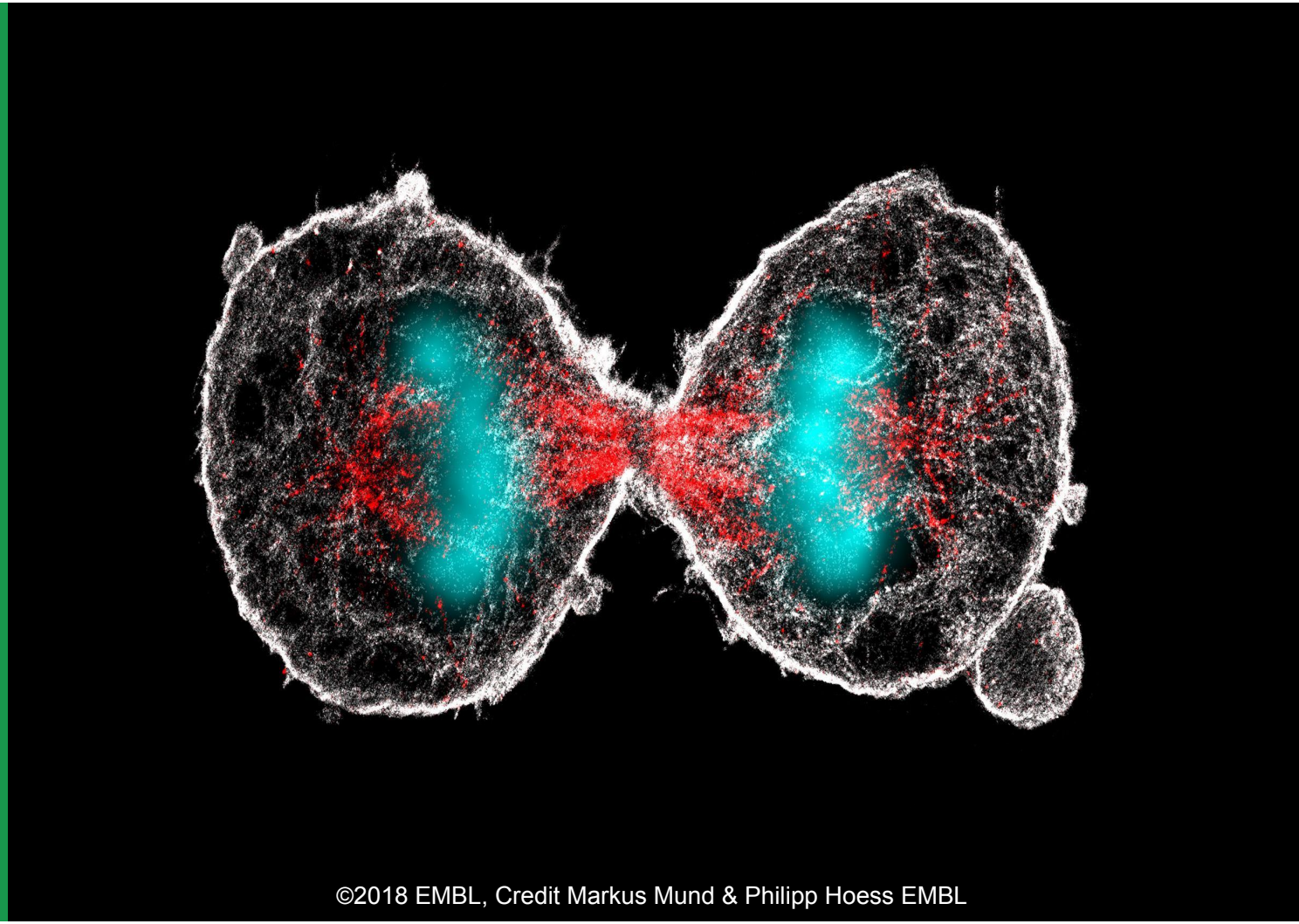
Conventional light microscopy has a resolution limit of about 200 nm (Abbes limit).

Fluorescence Microscopy can surpass this resolution limit under some circumstances (e.g. better z-plane resolution).

In the last decades, huge advances have been made to further surpass the resolution limit of light microscopy:

- STORM
- PALM
- STED
- Resolution limit of up to 10-20 nm

The picture shows the final stage in the duplication of a cell. The chromosomes are visible in cyan and cytoskeleton elements can be seen in red.

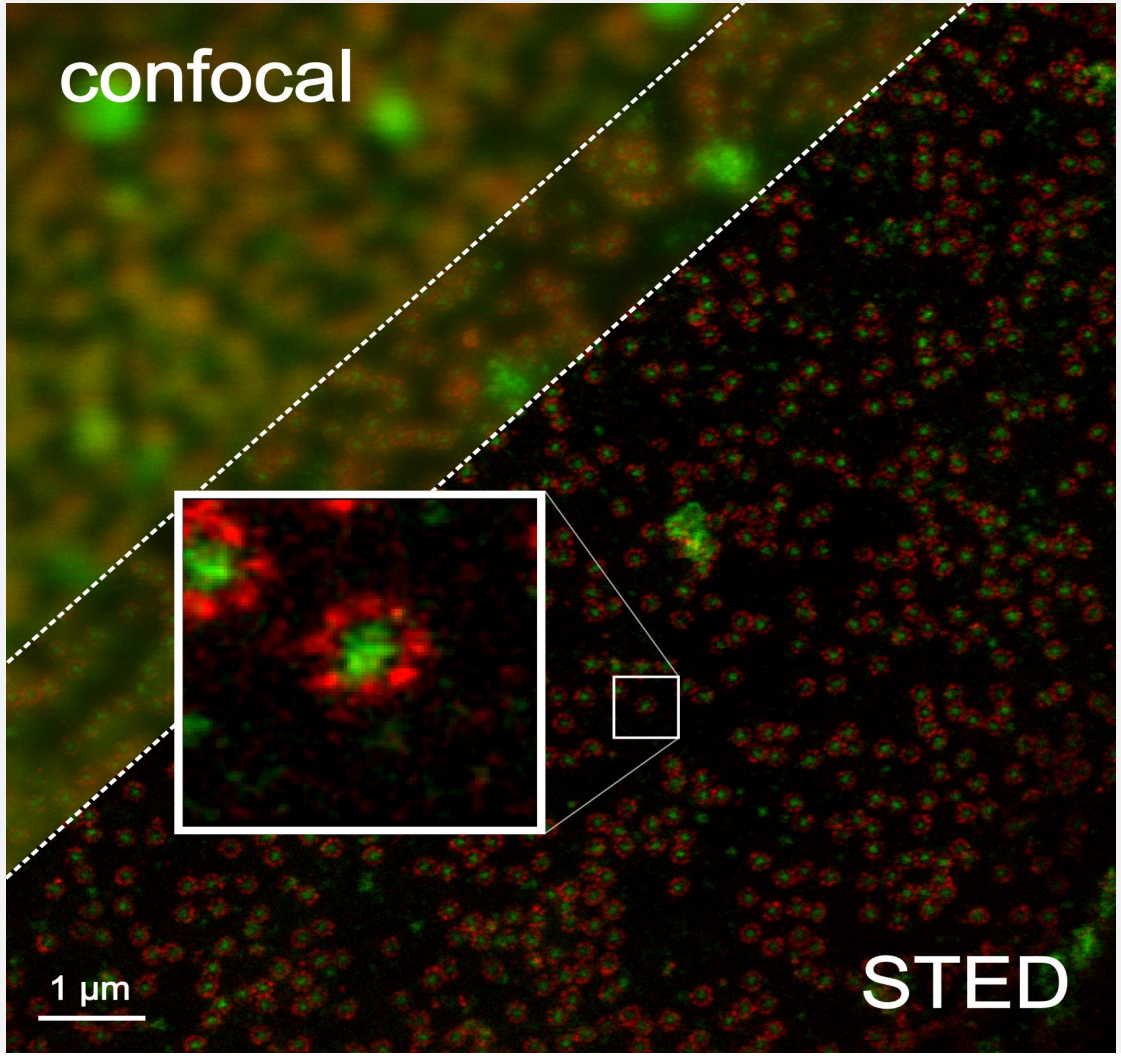


Advancing microscopy – temporal and spatial resolution

- Improving resolution by circumventing Abbe‘s limit

 Super resolution microscopy

- In the picture: The nuclear pore complex (labeled with fluorescent proteins) under confocal and STED microscopy.
STED yields much higher resolution.



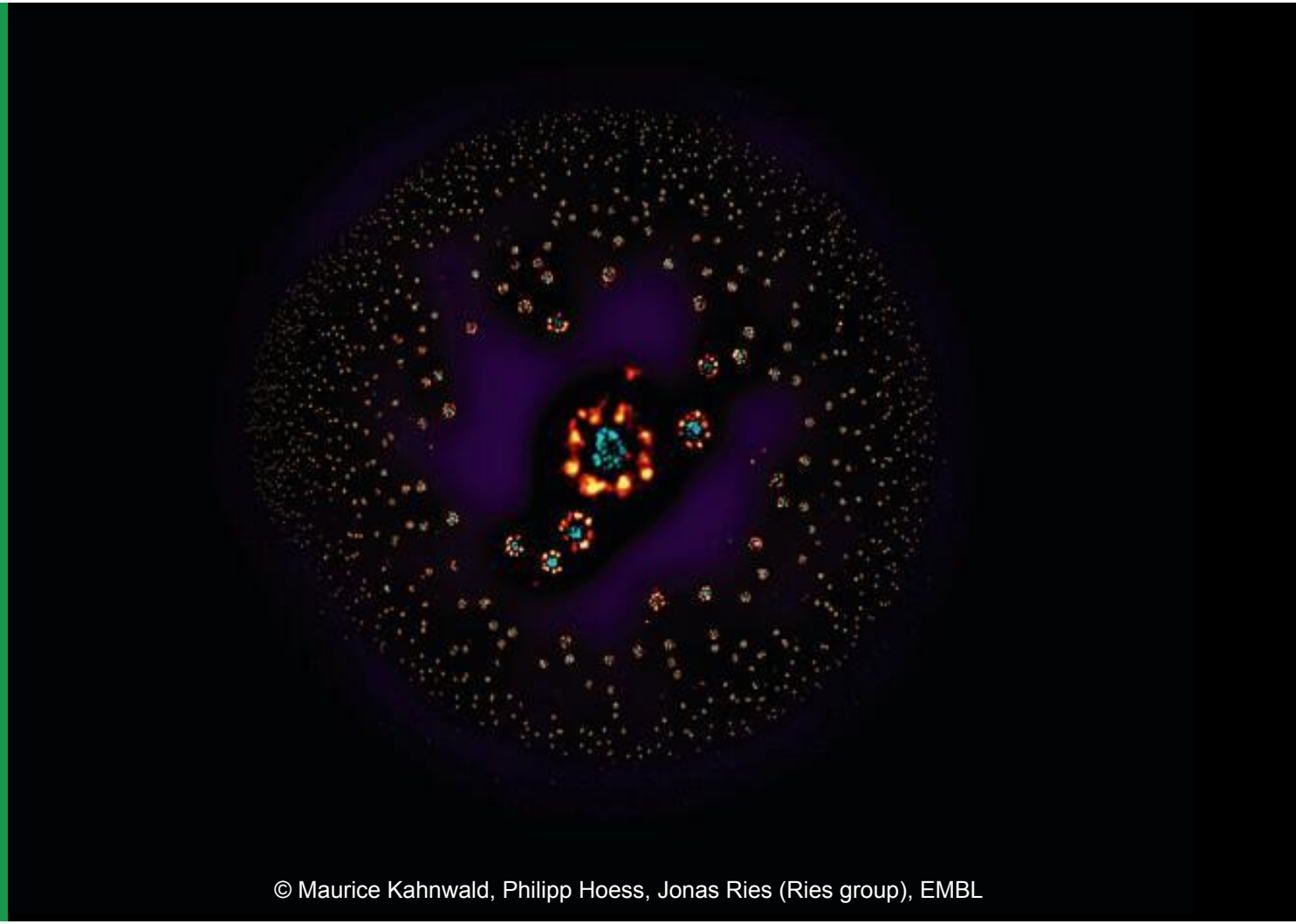
Super resolution microscopy

Image of the Nuclear Pore Complex (NPC) (diameter ca. 100 nm).
The NPC is a channel between cytoplasm and nucleoplasm.

It is one of the largest protein complexes in the human cell.

Red: Protein Nup-96, Nuclear pore marker WGA (cyan).

This image was artistically rendered using a fish-eye view.



STORM - Stochastic Optical Reconstruction Microscopy

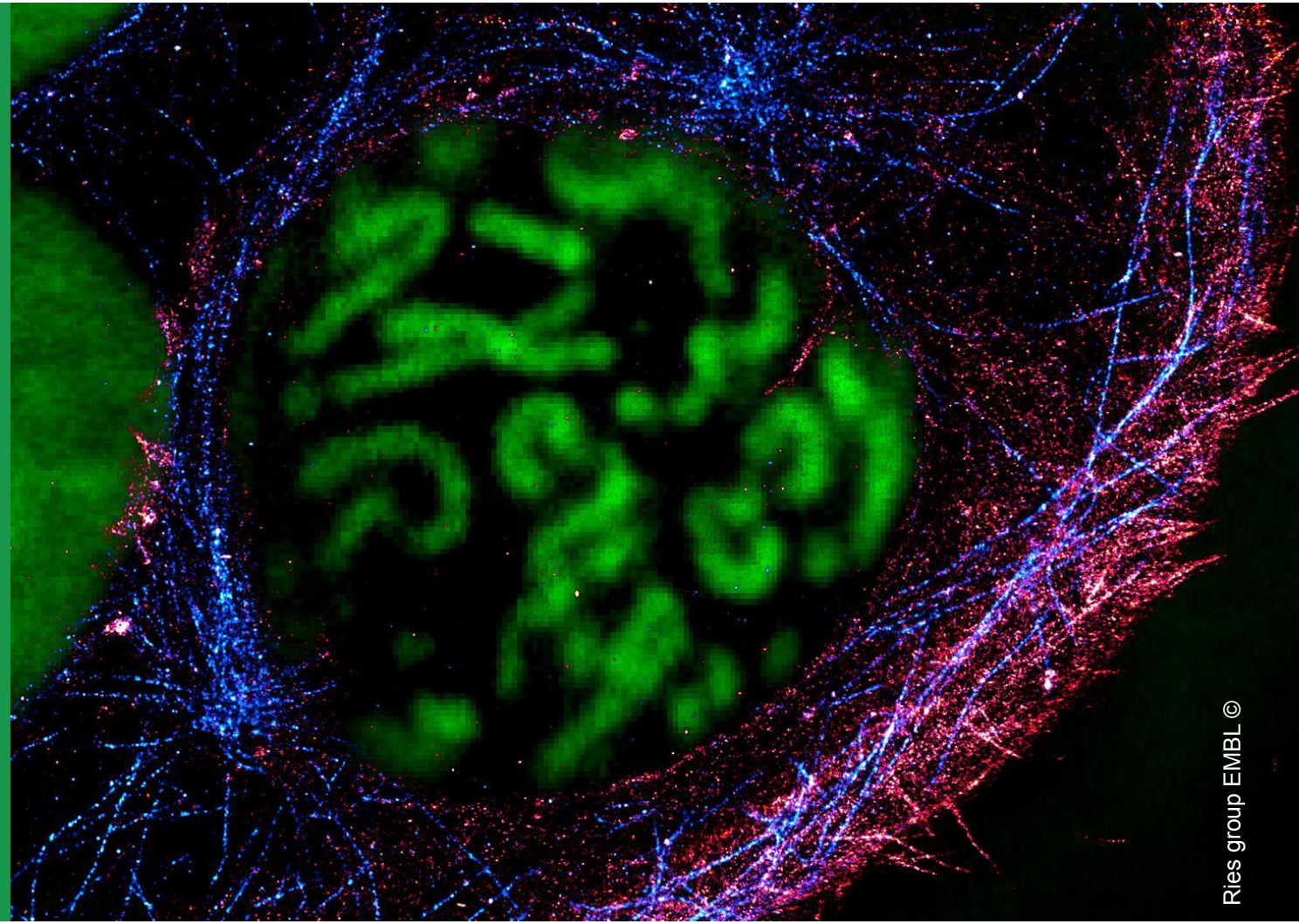
STORM allows the detection of single molecules

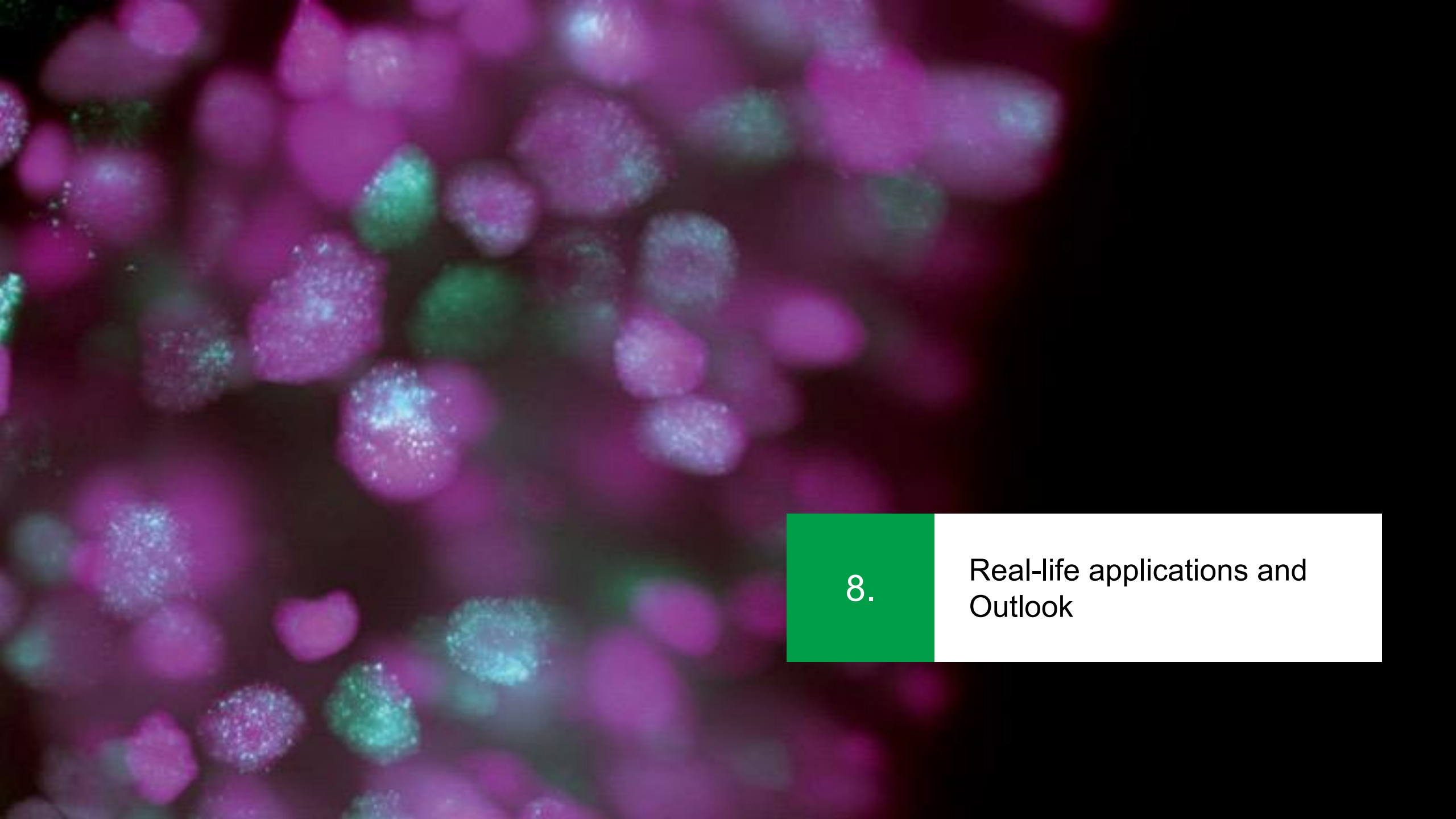
Resolution limit is about 20 nm

Different fractions of the fluorophores are excited during multiple imaging cycles

A complete image is reconstructed by the data gathered during these cycles

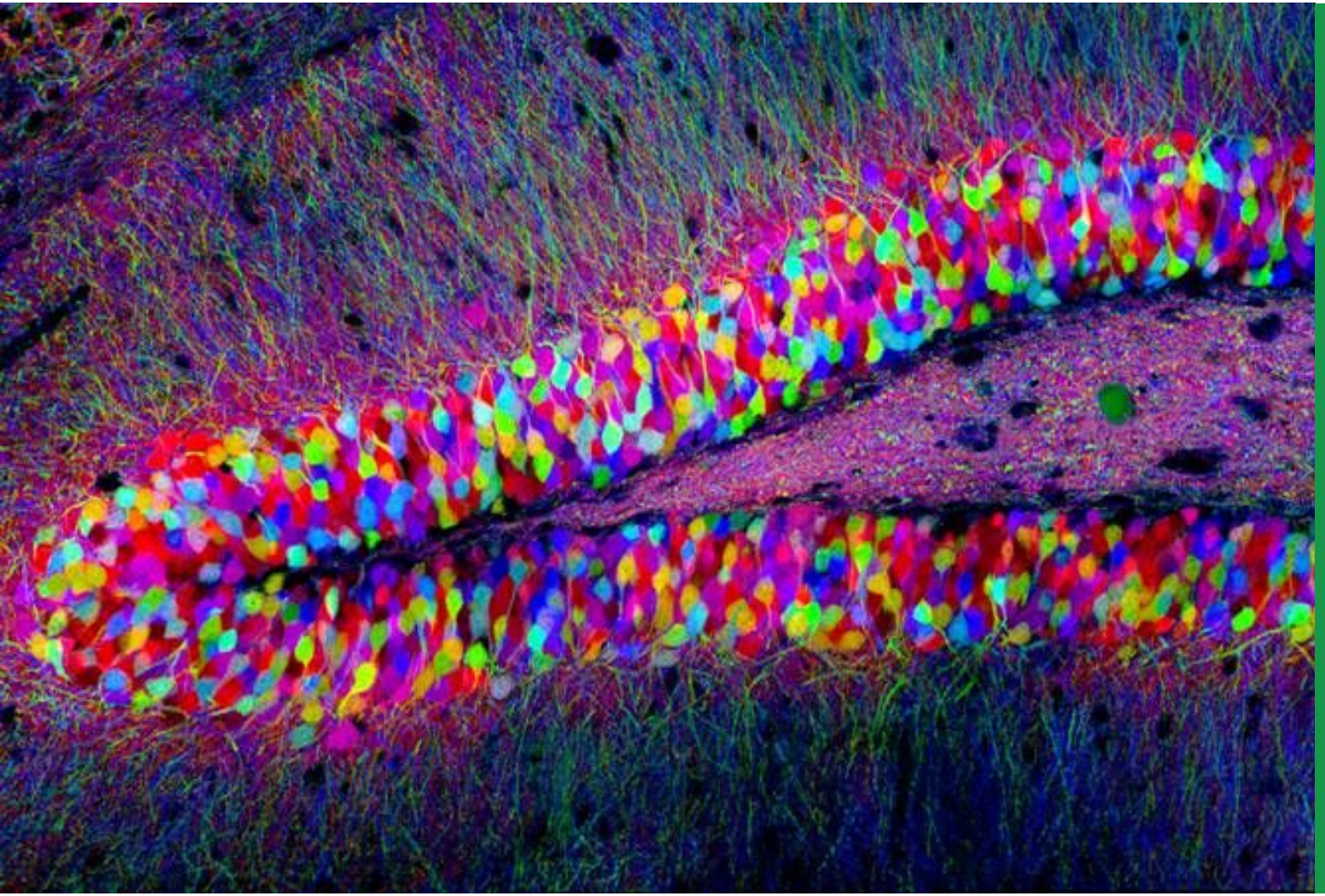
The image shows a U2OS cell (human bone marrow) in prophase. Actin is coloured in red, microtubules in blue and chromosomes in green.





8. Real-life applications and Outlook

The Brainbow



- **Brainbow** = Application for cell sorting and cell identification using different fluorophores

- It can also be applicated in other tissues (such as intestines) and many different species

- Tamily A. Weissman, Y. Albert Pan: '*Brainbow: New Resources and Emerging Biological Applications for Multicolor Genetic Labeling and Analysis*', Genetics, 2015

Outlook

- Ever increasing relevance of fluorescence in life sciences.
- Increasing relevance of fluorescence in diagnostics, for example:
  - Fluorescence for surgery https://www.ted.com/talks/quyen_nguyen_color_coded_surgery
  - Zheng et al.: '*Fluorescence-guided surgery in cancer treatment: current status and future perspectives*', Ann. Transl. Med., 2019
- Constant improvement of imaging techniques and resources, e.g. in electron microscopy and the development of new fluorophores.
- New Imaging Center at the campus EMBL Heidelberg to provide these resources and the necessary expertise internationally.

Further reading - fluorescence

- Malte Rentz: '*Fluorescence microscopy – A historical and technical perspective*', Journal of Quantitative Cell Science, 2013
- Further Reading on FISH: https://www.nature.com/scitable/topicpage/fluorescence-in-situ-hybridization-fish-327/
- Katherine M. McKinnon: '*Flow Cytrometry: An Overview*.', Curr. Protoc. Immunol., 2018
- Sir Gabriel Stokes: '*On the change of refrangibility of light – No. II*', The Royal Society Publishing, 1853 (not open access)
- Next Gen Sequencing: Illumina https://www.youtube.com/watch?v=fCd6B5HRaZ8

Further reading - GFP

- Osamu Shimomura: A story about the discovery of GFP
  https://www.nobelprize.org/prizes/chemistry/2008/shimomura/lecture/

- Martin Chalfie: '*GFP – lighting up life*', PNAS, 2009 – A story about implementation of GFP in organisms and how discovery in life sciences is build on the discovery of others
  https://www.pnas.org/content/106/25/10073

- A video about GFP, its discovery and applications:
  https://www.youtube.com/watch?time_continue=86&v=Z4vJ8rQCNgg&feature=emb_logo

Further reading – light microscopy

- History of Light Microscopy from Airy discs to Rayleigh Criterion: https://www.leica-microsystems.com/science-lab/microscope-resolution-concepts-factors-and-calculation/

- The anatomy of a microscope including the path of light: https://www.olympus-lifescience.com/en/microscope-resource/primer/anatomy/components/

- Timeline of light microscopy: http://light.ece.illinois.edu/ECE564/_OK_Lectures/00_Timeline_milestones.pdf

- Two videos about light microscopy: https://www.youtube.com/watch?v=tVcEEw6qbBQ https://www.youtube.com/watch?v=-b3Eejf4rDQ

Further reading – fluorescence microscopy

- Malte Rentz: '*Fluorescence microscopy – A historical and technical perspective*', Journal of Quantitative Cell Science, 2013
- H.C. Ishikawa-Ankerhold, R. Ankerhold, G.P.C. Drummen: '*Advanced Fluorescence Microscopy Techniques—FRAP, FLIP, FLAP, FRET and FLIM*.' Molecules 2012
- Y. Sako , A. Sekihata, Y. Yanagisawa, M. Yamamoto, Y. Shimada, K. Ozaki, A. Kusumi: '*Comparison of two-photon excitation laser scanning microscopy with UV-confocal laser scanning microscopy in three-dimensional calcium imaging using the fluorescence indicator Indo-1*' , J. Microscop., 1995
- James Jonkman, Claire M. Brown: '*Any Way You Slice It—A Comparison of Confocal Microscopy Techniques*', J. Biomol. Tech., 2015
- Rory M. Power, Jan Huisken: '*A guide to light-sheet fluorescence microscopy for multiscale imaging*', Nature, 2017 (not open acces)
- Peter O. Bayguinov, Dennis M. Oakley, Chien-Cheng Shih, Daniel J. Geanon, Matthew S. Joens, James A. J. Fitzpatrick: '*Modern Laser Scanning Microscopy*', Current Protocols in Cytometry, 2018

Further reading – advanced fluorescence microscopy

- Emily J. Guggenheim, Abdullah Khan, Jeremy Pike, Lynne Chang, Iseult Lynch, Joshua Z. Rappoport: *'Comparison of Confocal and Super-Resolution Reflectance Imaging of Metal Oxide Nanoparticles*', PLOS ONE, 2016

- J. Vangindertael, R. Camacho, W. Sempels, H. Mizuno, P. Dedecker and K. P. F. Janssen.: '*An introduction to optical super-resolution microscopy for the adventurous biologist*', Methods and Applications in Fluorescence Vol. 6, Nr. 2, 2018

- Michael J. Rust, Mark Bates, Xiaowei Zhuang: '*Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM)*', Nature Methods 3, 2006

- Anna Szymborska, Alex de Marco, Nathalie Daigle, Volker C. Cordes, John A. G. Briggs, Jan Ellenberg: '*Nuclear Pore Scaffold Structure Analyzed by Super-Resolution Microscopy and Particle Averaging*', Science Vol. 341, 2013