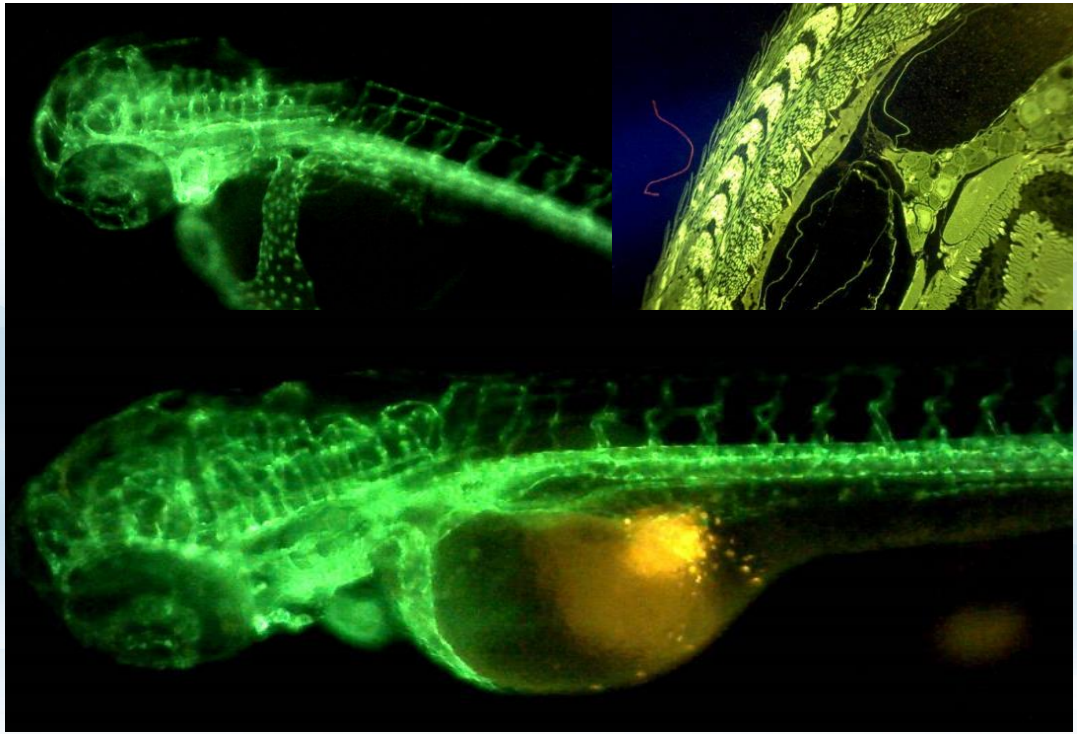
Dino-Lite *knowledge & education*

Fluorescence Microscopes

Dino-Lite Fluorescence models

- Smallest fluorescence microscope in the world
- Revolution to biomedical and educational applications
  - ✓ Flexible
  - ✓ Easy to use
  - ✓ Affordable
- Broad range of fluorescence spectrum
- Software suite included
- Low cost of ownership

Application Images



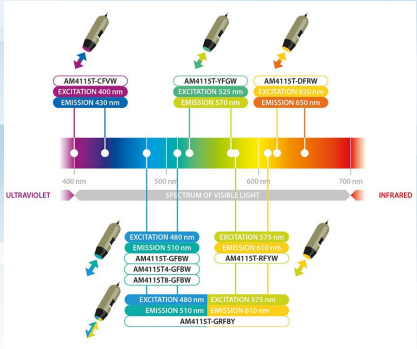
Images of a zebrafish

Made with the AM4115T-GFBW

Fluorophore used: GFP

All Fluorescence models are based on the Dino-Lite Edge AM4115T model.

- USB 2.0 Connection
- 1.3 Megapixel
- Different excitation and emission wavelengths available
- Magnification up to 400x
- DinoCapture 2.0 and DinoXcope software



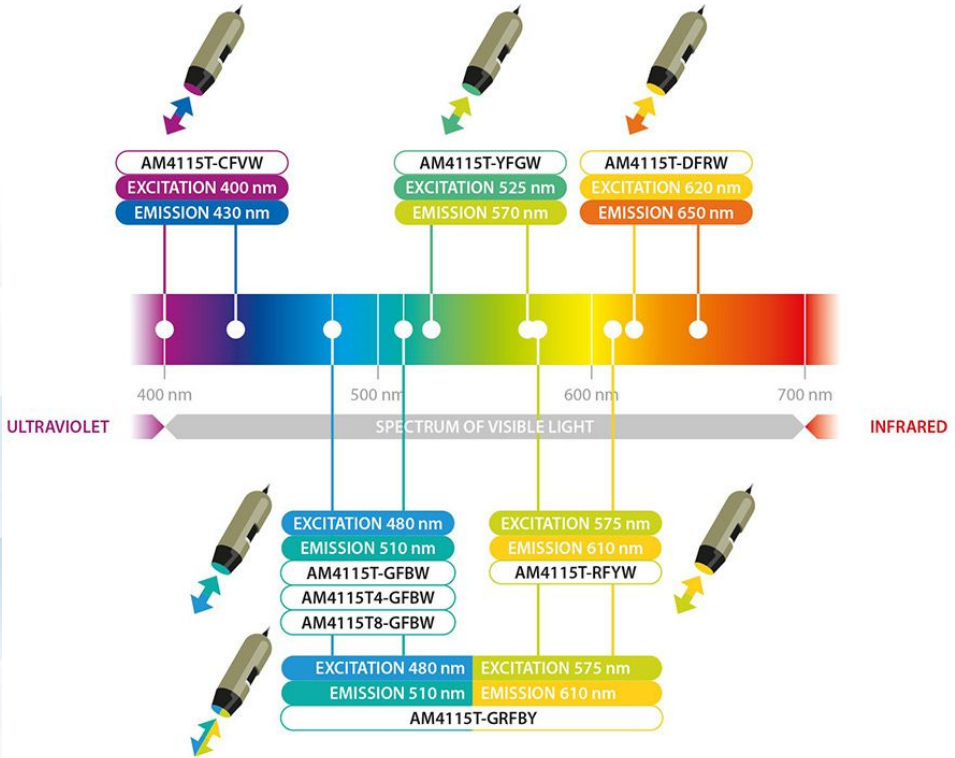
Choosing the right Dino-Lite for fluorescence

1. Know the fluorophore used (the customer must give you this info).
2. Check the excitation and emission spectrum of the fluorophore.
3. Select the right microscope on excitation and emission.

Keep in mind:

- Maximum magnifications are 220x and 470x
- How is the sample mounted? Slide, petri dish, whole animal, etc. Select the right stand.
- Daylight or lamps interfere with the image quality. Preferably a dark environment for capturing images
- Photobleaching: sample will fade over time. The speed of this process depends on the fluorescence sample

Spectrum Overview



Fluorescence Models

Model	Excitation	Emission Filter	Applications
AM4115T-CFVW	400nm	430nm	• Subcutaneous injuries and wounds on living and deceased bodies, bruising, bite marks, blood spatter, fingerprints, and fibers. • Observing DAPI/ Hoechst stained specimens, like nuclei
AM4115T-GFBW	480nm	510nm	• Observe green fluorescence protein (GFP) marked samples or FITC labeled specimen) • Viewing smaller specimens (zebrafish and mice) injected with green or red fluorescent proteins research pertaining to oncology, targeted genes, and viruses. • Analyze the organization, dynamics and function of microtubules in fungal genetics and biology • Commonly used in Drosophila research as marker for certain characteristics.

Fluorescence Models

Model	Excitation	Emission Filter	Applications
AM4115T-YFGW	525nm	570nm	• Observation of orange fluorescence (like Cy3/TRITC) • Visualizing small blood or lymphatic vessels of an organ in micro-angiography analysis • Examining water samples (sea or fresh) • Detect certain cyanobacteria that emit fluorescence
AM4115T-RFYW	575nm	610nm	• Observation of red fluorescence, mainly mCherry/TxRed • Visualizing small blood or lymphatic vessels of an organ in microangiography analysis
AM4115T-DFRW	620nm	650nm	• Cy5 labeled specimen (has to be bright)

Fluorescence Models

Model	Excitation	Emission Filter	Applications
AM4115T-GRFBY	480nm & 575nm	510nm & 610nm	• Observation of dual stained GFP/FITC and mCherry/TxRed samples with one microscope • Localization and comparing
AM4115T4-GFBW	480nm	510nm	• GFP/FITC labeled samples which require 400-470x magnification; detailed structures, individual cell localization. Some bacteria colonies, not individuals

Other combinations available on request.
Please contact us with fluorophore used and what type of sample.

Fluorescence Microscopy

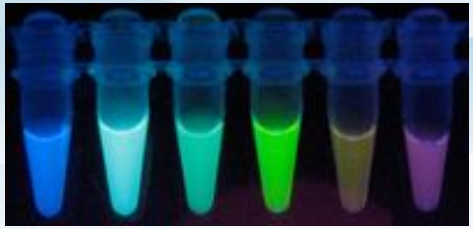
(Additional Information)

What is a fluorescence tag/label/probe/marker?

It is a fluorescent molecule that is chemically or biologically attached to help detecting biomolecules such as a protein, antibody, amino acids, enzymes and peptides. This fluorescent molecule is known as a **Fluorophore (or fluorochrome).**

Fluorophores selectively bind to a specific region of the specimen.

Each fluorophore has its own spectral **excitation** and **emission** characteristics.



- A Dino-Lite fluorescence microscope is chosen based upon the required excitation and emission spectrum (of the fluorophore)

How does fluorescence microscopy work?

Short version: (long version see page 14)

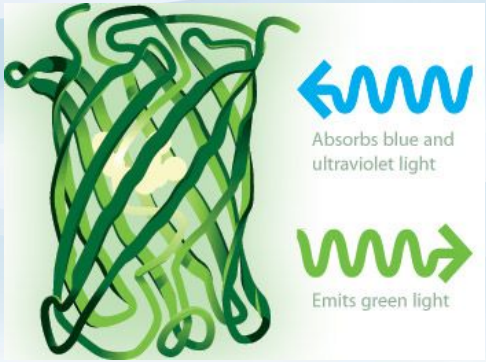
Illuminate (**excite**) the specimen with a specific wavelength, depending on the fluorophore used.

This light is absorbed by the fluorophore. The fluorophore reacts by **emitting** light of a longer wavelength.

Example: GFP Fluorophore.

EX light: 425-490nm (peak at 475nm)

EM light: 490-550nm (peak at 509nm)



Dino-Lite AM4115T-GFBW; EX: 480nm, EM: 510nm and up

Why fluorescence microscopy?

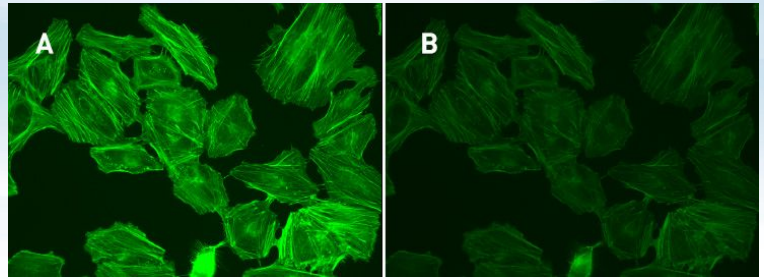
- Specific region is stained; other parts will not appear or limit the visibility
- Higher signal-to-noise ratio than normal bright field microscopy

Other things to consider:

- **Photobleaching:** overtime the fluorophores lose their ability to fluoresce
- **Phototoxicity:** UV/V light can have a toxic reaction to living cells
- Only the stained region is visible, other information of the sample is not visible

A: Original GFP image of cells

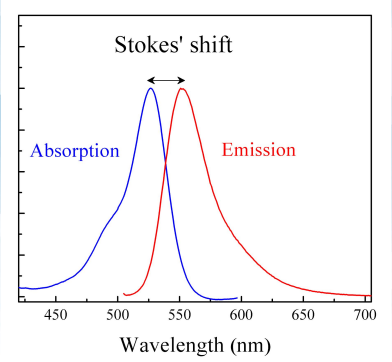
B: Photobleaching in progress, after continuous illuminating the cells.



How Does Fluorescence Microscopy Work? (Long)

Fluorescence is Luminescence emitted by certain compounds (fluorophores) when they absorb excitation light.

- A photon's energy is rapidly absorbed (10-15 sec), and shifts one of the fluorophores electrons from a ground state to an excited state
- The electron then loses some of the gained energy through smaller vibrational states (in the form of heat)
- Electron hangs in the excited state for 10-9 sec and then emits a single lower energy photon (**Stoke's shift**)



Fluorescence Labeling

Fluorescence Molecule labels

Fluorescent molecules will bind to certain structures in cells or other targets due to native structure or connectors

DNA DAPI, Hoechst

Proteins FITC, TRITC, Cy3,5, TxRed, Alexa

Lipid membranes Fluorescein

Immunofluorescence

Fluorescence molecules which are bound to antibodies that attach to specific proteins in the cells. (Alexa 488-Anti Goat)

Fluorescence Proteins

Expression of DNA constructs for the protein of interest fused to a fluorescent protein

Fluorescence Terms

Extinction Coefficient:
Light absorbing capacity of dye. At max absorption this would be 1000 - 10,000 for poor dye and 250,000 for excellent dye.

Quantum Efficiency or Quantum Yield (QY)
Measurement of efficiency of dye.

Bleaching
Fluorophores can only be excited so many times. Oxidation in the excited state leads to bleaching. I.e. FITC may have 30,000 cycles before bleaching.

Saturation
Ground state depletion. More photons do not result in more fluorescence.

Photo Damage / Photo toxicity
Damage caused to cells or tissue due to the energies in fluorescence excitation light
Higher energy light causes more damage. UV is more damaging to cells than IR.

Interesting websites

Fluorescence microscopy:

- https://en.wikipedia.org/wiki/Fluorescence_microscope
- https://www.microscopyu.com/techniques/fluorescence/introduction-to-fluorescence-microscopy
- http://www.olympusmicro.com/primer/lightandcolor/fluorointroduction.html
- https://www.nobelprize.org/nobel_prizes/chemistry/laureates/2008/illpres.html

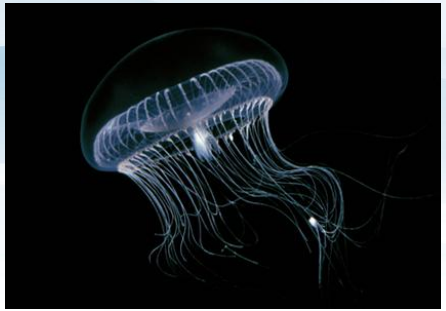
Fluorophores:

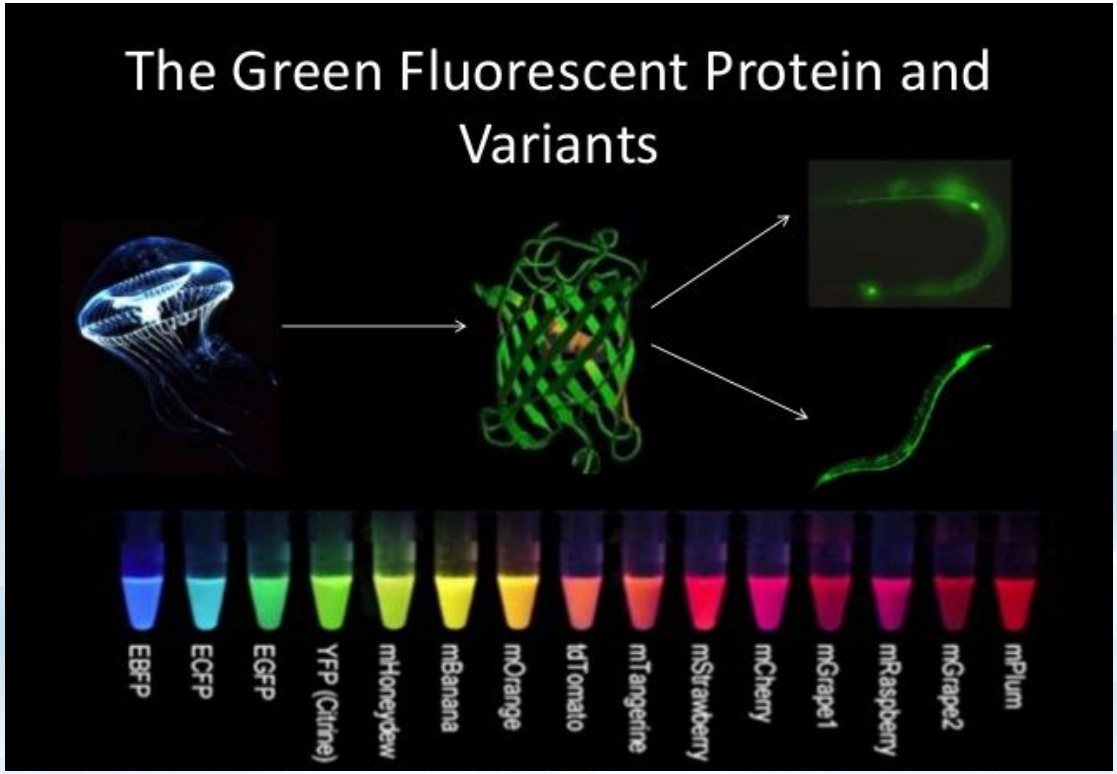
- http://www.fluorophores.tugraz.at/substance/
- https://www.chroma.com/spectra-viewer

History of Fluorescence Microscopy

It was first discovered in materials like Quinine, Fluorspar and other minerals (natural or “auto-fluorescence”).

- Green Fluorescence Protein (GFP) was isolated from jellyfish species in the 1960s but not used as a **marker for gene expression until 1992**.
- Fluorescence proteins have also been found in coral species.
- It is visible in plant material (pollen grains, leaves)
- DAPI, FITC, TRITC, Alexa Fluors, Cy Dyes and can act as stains or be tagged to cellular structures.
- Through molecular biology proteins can be used to study gene expression in non-fluorescence organisms.





The Royal Swedish Academy of Sciences has decided to award the **Nobel Prize** in Chemistry for **2008** jointly to Martin Chalfie, Osamu Shimomura and Roger Y. Tsien, "**for the discovery and development of the green fluorescent protein, gfp".**

Original reference courtesy of Dino-Lite Europe