labeling the cell molecules

introduction

- Tracking biomolecules (**proteins, antibodies, amino acids, and peptides**) often requires labeling with a reporter or sensor.
- Observation of molecular processes inside living cells is fundamental to a quantitative understanding of how biological systems function.
- At present, several labeling methods are available for this purpose.

introduction

- In the past decade, rapid developments in fluorescence microscopy, fluorescence correlation spectroscopy, and fluorescent labeling techniques have enabled new experiments to investigate the robustness and stochasticity of diverse molecular mechanisms with high spatiotemporal resolution.
- **Fluorescent label**, also known as a **tag** or **probe**, is a molecule that is attached **chemically** to aid in the labeling and detection of a biomolecule such as a protein, antibody, or amino acid.

introduction

- Generally, fluorescent tagging, or labeling, uses a **reactive derivative** of a fluorescent molecule known as a **fluorophore**.
- The fluorophore **selectively** binds to a specific region or functional group on the target molecule and can be attached chemically or biologically.

introduction

- **Various labeling techniques** such as enzymatic labeling, protein labeling, and genetic labeling are widely utilized.
- Ethidium bromide, fluorescein and green fluorescent protein are **common tags**.
- The most commonly **labelled molecules** are antibodies, proteins, amino acids and peptides which are then used as specific probes for detection of a particular target.

There are currently several labeling methods for tracking biomolecules:

1. Isotope markers

- In this technique, one or more of the atoms of the molecule of interest is substituted for an atom of the same chemical element, but of a different isotope (like a radioactive isotope used in radioactive tracing).
- Because the labeled atom has the same number of protons, it will behave in almost exactly the same way as its unlabeled counterpart and, with few exceptions, will not interfere with the reaction under investigation.
- The difference in the number of neutrons, however, means that it can be detected separately from the other atoms of the same element.

There are currently several labeling methods for tracking biomolecules:

2. Colorimetric biosensors

- Biosensors are attached to a substance of interest. Normally, this substance would not be able to absorb light, but with the attached biosensor, light can be absorbed and emitted on a spectrophotometer.
- Colorimetric assays are normally used to determine how much concentration of one species there is relative to another.

There are currently several labeling methods for tracking biomolecules:

3. Photochromic compounds

- Photochromic compounds have the **ability to switch between a range or variety of colors.**
- Their ability to display different colors lies in how they **absorb light**.
- Different molecule absorbs different wavelengths of light, so that each isomeric species can display a different color based on its absorption.
- These include photoswitchable compounds, which are proteins that can switch from a non-fluorescent state to that of a fluorescent one given a certain environment.

- **Photochromic fluorescent probes can be divided into two classes:**

-Genetically encoded fluorescent proteins.

-Synthetic nanoprobes.

- Fluorescent proteins can easily be integrated with any target protein, and thus eliminate the need for exogenous probes to target to one specific protein.
- Non-genetically encoded fluorescent nanoparticles usually **exhibit higher brightness and increased photostability** as compared to fluorescent proteins.
- Even the brightest photoswitchable fluorescent protein is still much dimmer than some organic small-molecule fluorophores.

Example of fluorescent protein EosFP

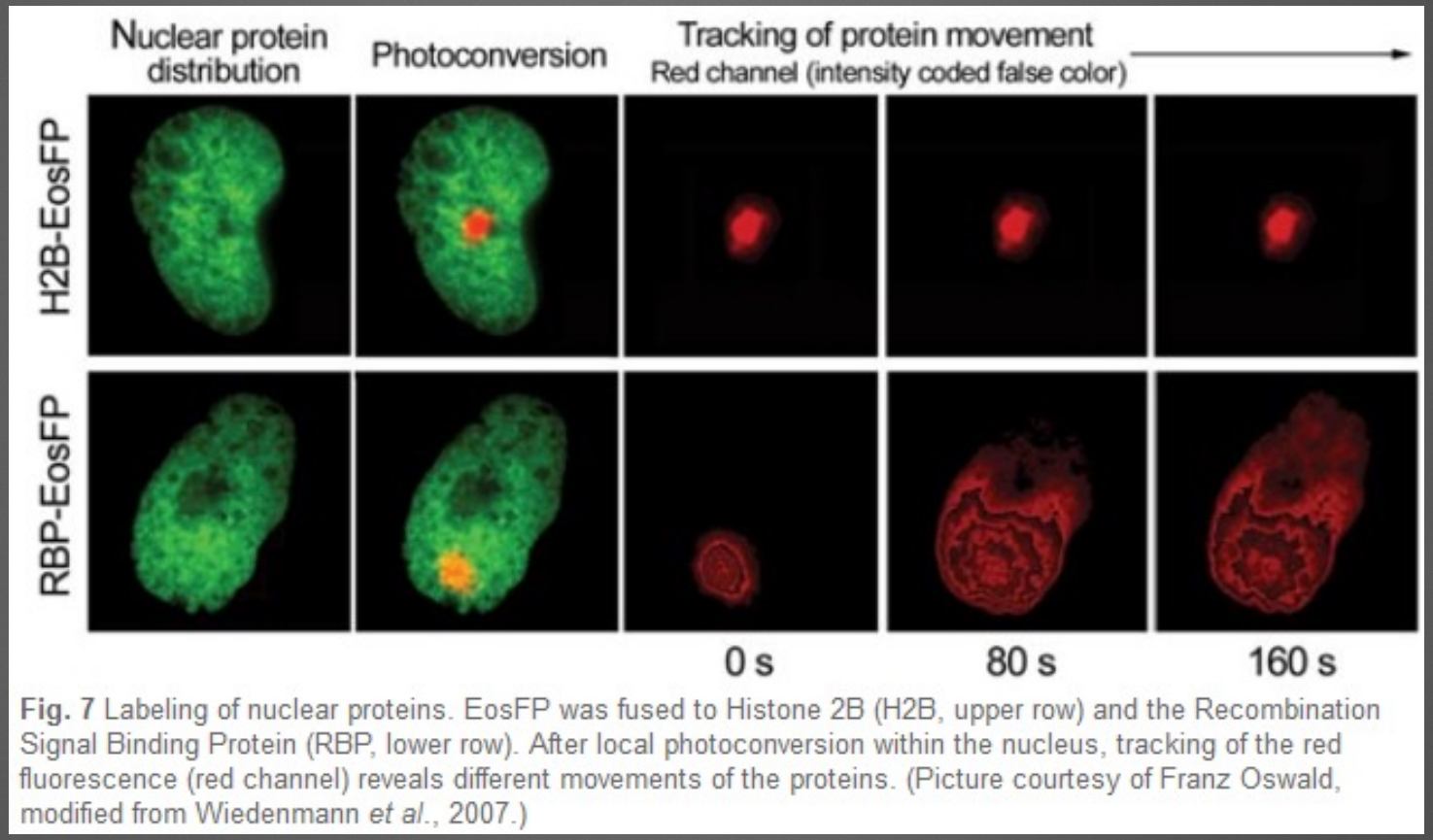


Fig. 7 Labeling of nuclear proteins. EosFP was fused to Histone 2B (H2B, upper row) and the Recombination Signal Binding Protein (RBP, lower row). After local photoconversion within the nucleus, tracking of the red fluorescence (red channel) reveals different movements of the proteins. (Picture courtesy of Franz Oswald, modified from Wiedenmann *et al.*, 2007.)

There are currently several labeling methods for tracking biomolecules:

4. Fluorogen labels

- A fluorogen is ligand (fluorogenic ligand) which is **not itself fluorescent**, but when it is bound by a specific protein or RNA structure becomes fluorescent.
- For instance, **Y-FAST** (**Yellow Fluorescence-Activating and absorption-Shifting Tag**) is an variant of Photoactive Yellow Protein which was engineered to bind chemical **mimics** of the **GFP** tripeptide chromophore (is the part of a molecule responsible for its color).
- Likewise, the Spinach aptamer is an engineered RNA sequence which can bind **GFP** chromophore chemical mimics, thereby conferring conditional and reversible fluorescence on RNA molecules containining the sequence.

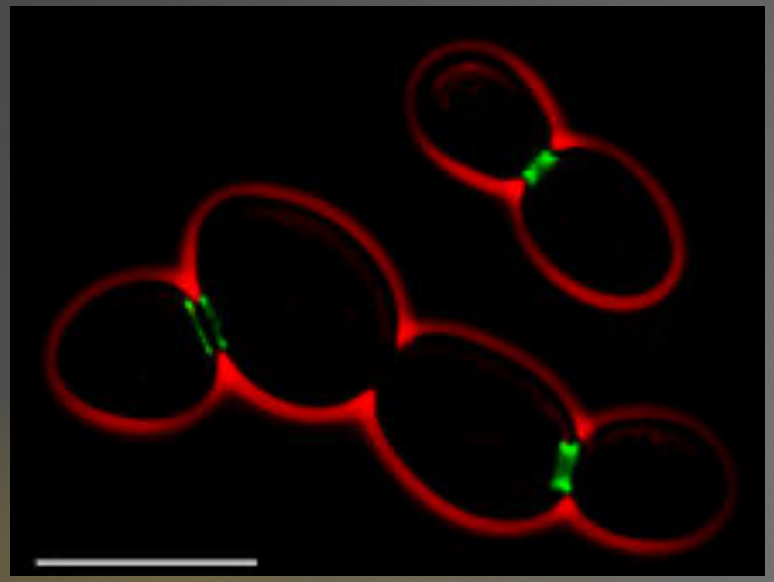
There are currently several labeling methods for tracking biomolecules:

5. Fluorescent labels

- Among the labeling methods, **fluorescent labeling has the upper hand due to:**

- Its non-destructive nature
- The high sensitivity of the fluorescence technique
- Meeting the requirements of small measurement volume and low concentration of the fluorescent material.

- Fluorescent labeling is generally accomplished by using **a reactive derivative** of the fluorophore that **selectively** binds to a functional group contained biomolecule.

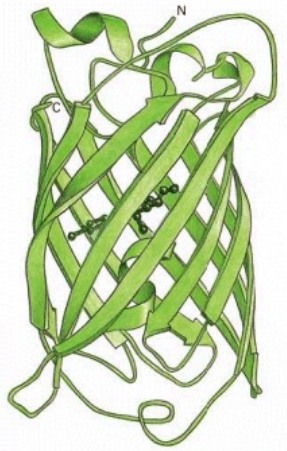


S. cerevisiae septins revealed with fluorescent microscopy utilizing fluorescent labeling

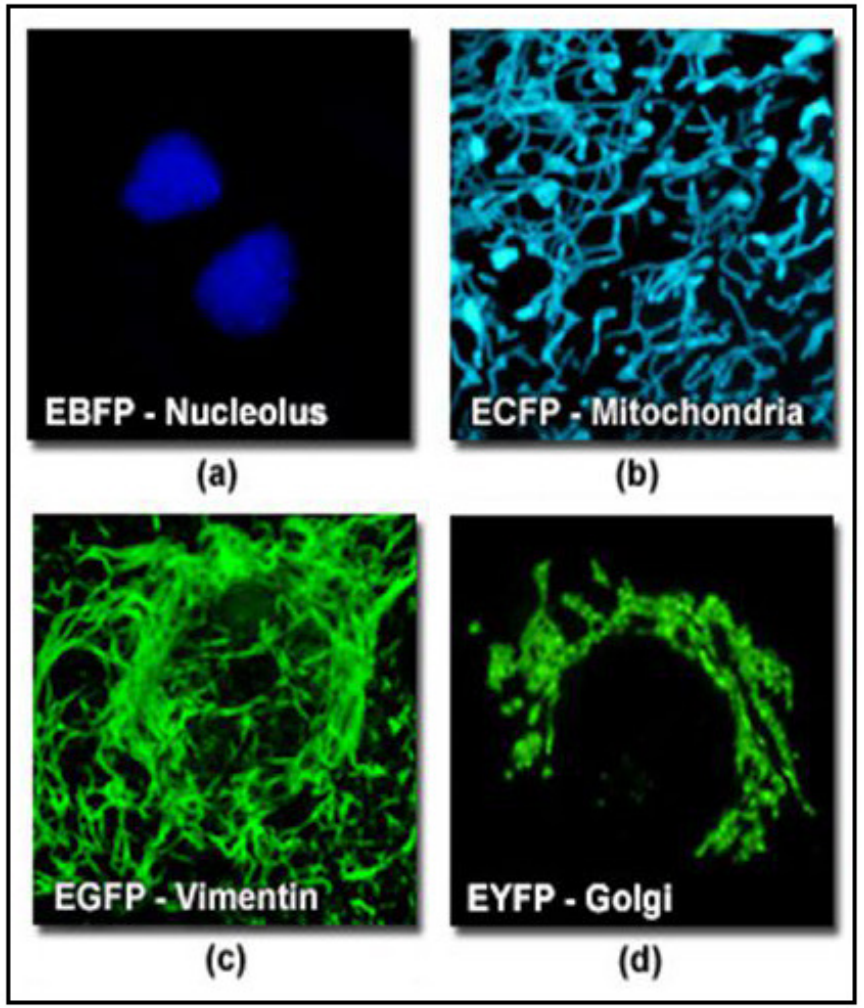
- Fluorescent molecule attachment to the biomolecules can be achieved **chemically** (chemical modification through non-covalent or covalent binding) or **biologically** (genetic incorporation of unnatural amino acids, fusion of biomolecules with a peptide tag having a fluorescent probe, or enzyme catalysis).
- Besides the above-mentioned chemical and biological labeling methods, a new type of labeling is widely employed (i.e., tag-labeling) which can be carried out both chemically and biologically.

- **Green fluorescent protein** (**GFP**) is a **naturally occurring fluorescent** protein from the jellyfish Aequorea victoria that is widely used to tag proteins of interest.
- **GFP** has been modified by changing the wavelength of light absorbed to include other colors of fluorescence.
- (**YFP**) or **yellow fluorescent protein**, (**BFP**) or **blue fluorescent protein**, and **(CFP)** or **cyan fluorescent protein** are examples of GFP variants.
- These variants are produced by the **genetic engineering of the GFP gene**

Aequorea victoria



Green fluorescent protein (**GFP**)



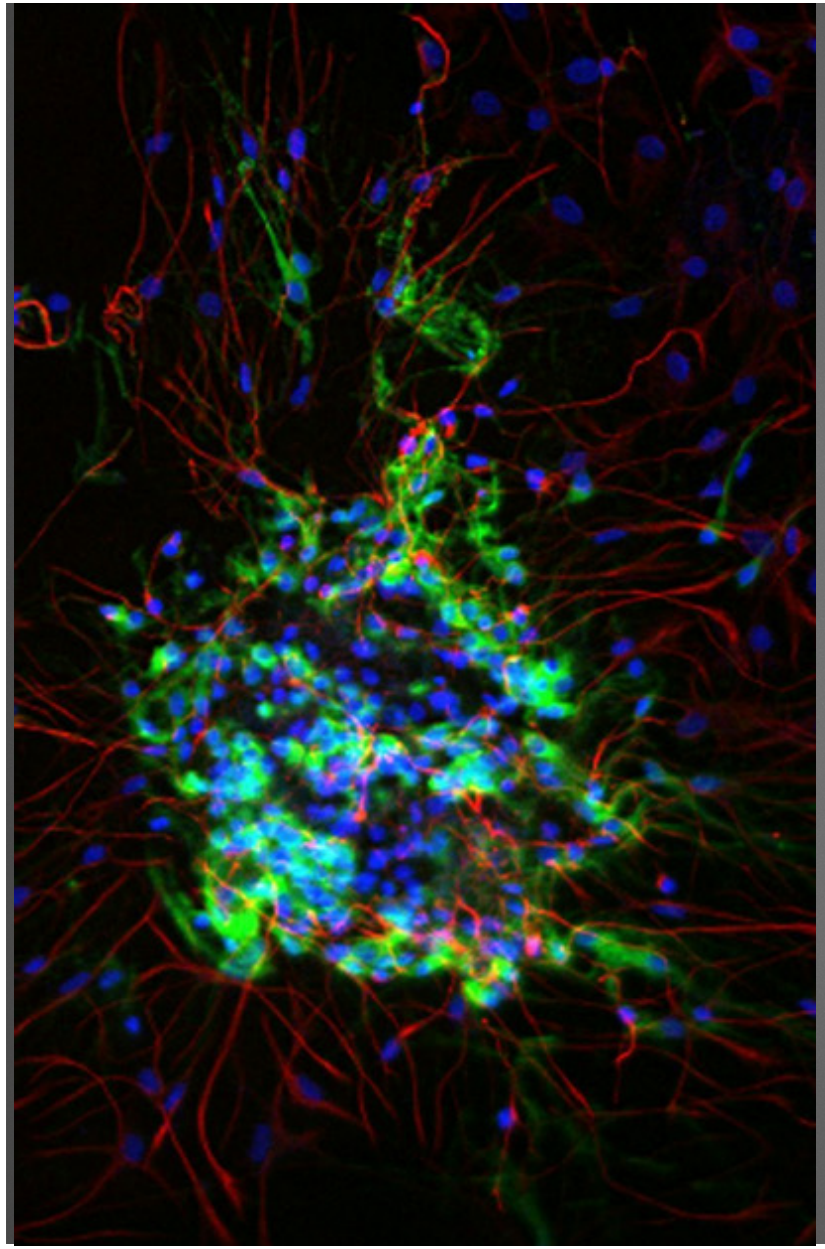
- **TagYFP** is mainly intended for protein labeling. It can also be used for cell and organelle labeling and for tracking the promoter activity.

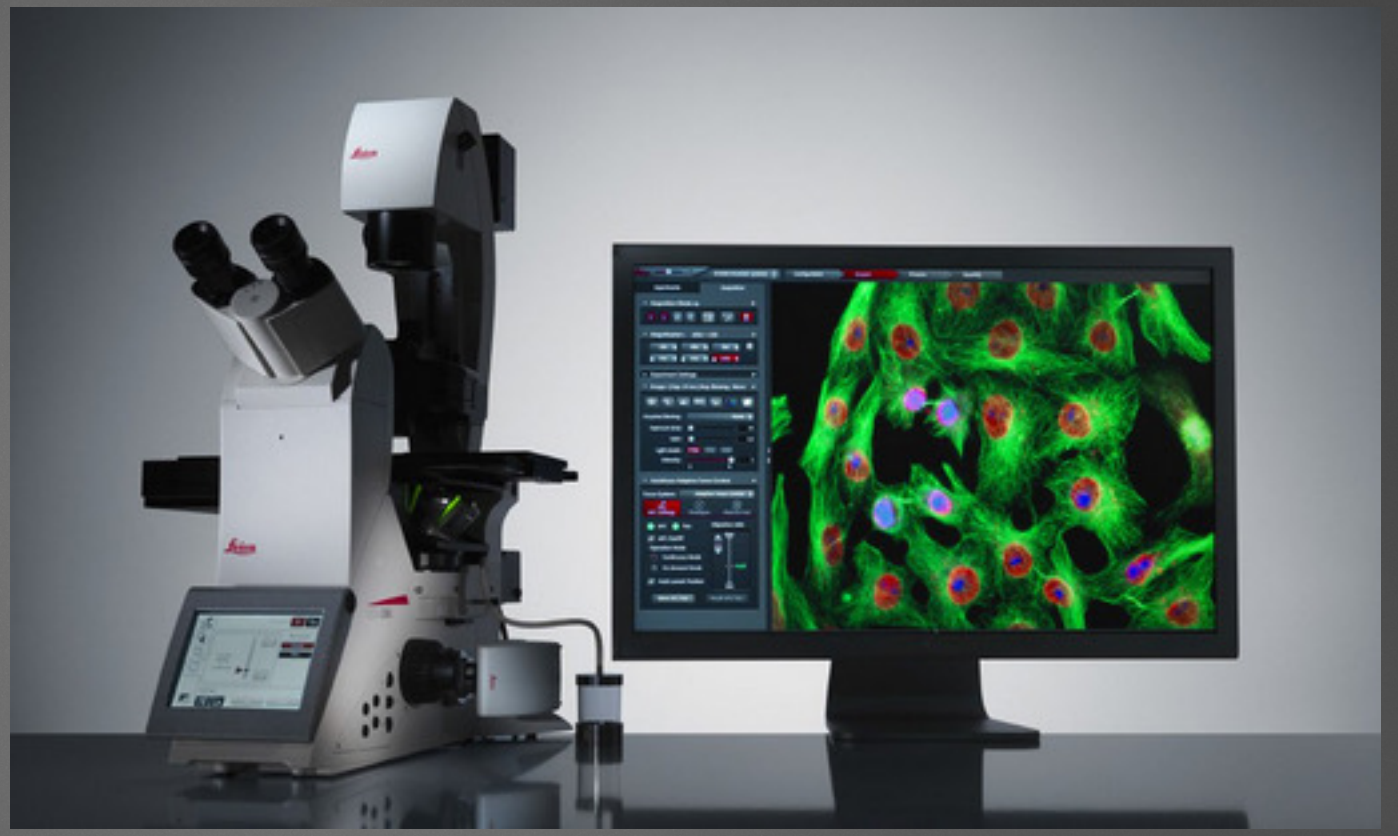
- **TagBFP** is recommended for protein labeling, acidic organelle labeling, FRET (Fluorescence Resonance Energy Transfer) applications.

- **TagCFP** can be detected in cells without adding cofactors or substrates, which makes it ideal for use in live cell assays.

-It can be used for multiplex application to detect two or more events in the same cell or cell population.

-It is ideal for monitoring gene expression and protein localization





Fluorescence Microscopy

- **Synthetic fluorescent probes** can also be used as fluorescent labels.
- **Advantages of these labels include:**

- A smaller size with more variety in color.

- They can be used to tag proteins of interest **more selectively** by various methods.

- Despite their wide array of excitation and emission wavelengths as well as better stability, synthetic probes tend to be **toxic to the cell** and so are not generally used in cell imaging studies.

Fluorescent labeling

- **fluorescent labeling is** one of the most widely used methods for labeling and tracking biomolecules.
- Several techniques of fluorescent labeling can be utilized depending on the nature of the target.
- **In order to select a good fluorescent probe and labeling strategy, there are a few requirements that should be considered:**

1. The fluorescent probes should be small in size and chemically stable, with minimal interference on the folding and biological functions of the target protein.
2. The labeling reaction should be highly efficient and adaptable to the target molecule, preferably establishing a covalent linkage between the synthetic probe and a specific residue in the target molecule.

The most commonly used Techniques of fluorescent labeling

1. Enzymatic labeling

- In enzymatic labeling, a DNA construct is first formed, using a gene and the DNA of a fluorescent protein.
- After transcription, a hybrid RNA + fluorescent is formed.
- The object of interest is attached to an enzyme that can recognize this hybrid DNA.

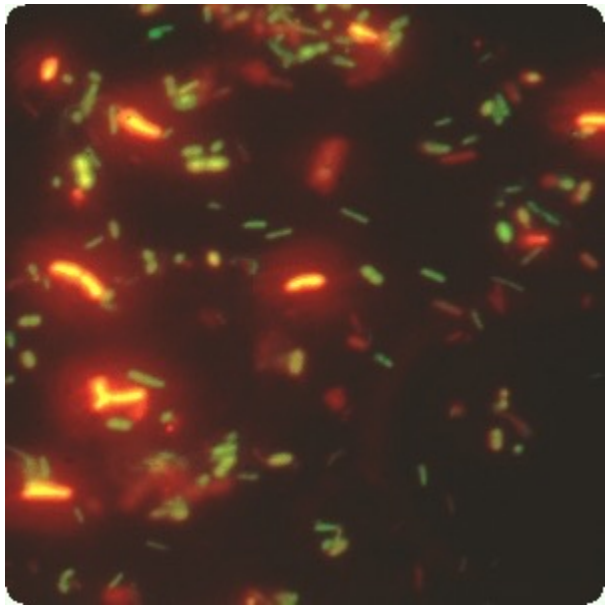
2. Protein labeling

- Protein labeling uses a short tag to minimize disruption of protein folding and function.
- Transition metals are used to link specific residues in the tags to site-specific targets such as the N-termini, C-termini, or internal sites within the protein.

The most commonly used Techniques of fluorescent labeling

3. Genetic labeling

- Fluorescence in situ hybridization **(FISH)** is an example of a genetic labeling technique that utilizes **probes** that are specific for **chromosomal sites** along the length of a chromosome, also known as **chromosome painting.**
- Multiple fluorescent dyes that each have a distinct excitation and emission wavelength are bound to a probe which is then hybridized to chromosomes.
- A fluorescence microscope can detect the dyes present and send it to a computer that can reveal the karyotype of a cell.
- This technique allows abnormalities such as deletions and duplications to be revealed.

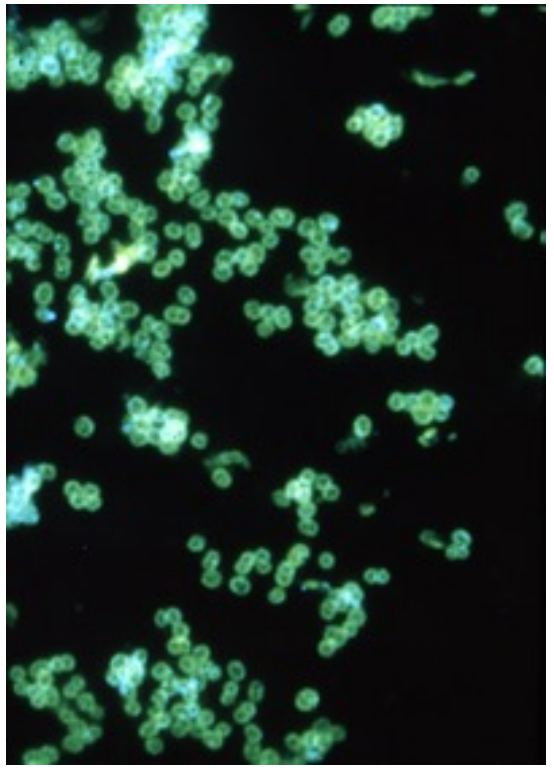


FISH image of bifidobacteria

The most commonly used Techniques of fluorescent labeling

4. Chemical labeling

- Chemical labeling or the use of chemical tags utilizes the interaction between a small molecule and a specific genetic amino acid sequence.
- Chemical labeling is sometimes used as an alternative for GFP. Synthetic proteins that function as fluorescent probes are smaller than GFP's, and therefore can function as probes in a wider variety of situations.
- Moreover, they offer a wider range of colors and photochemical properties.



In a direct fluorescent antibody test, antibodies have been chemically linked to a fluorescent dye

- **chemical labeling** is of great interest as it permits novel types of experiments by targeting chemicals with a wider range of functionality.
- From the chemical labeling point of view, covalent attachment of the chemical probes with reactive moieties and specific amino acids has the advantage of greater irreversibility compared to the non-covalent binding.
- As a result of their nature and strategy, the chemical labeling methods are very **robust**, **easy to handle** and **provide maximum efficiency** with a wide range of fluorophores that can be coupled covalently to the target molecule.
- Chemical labeling methods are **more suitable** for in vitro studies rather than in vivo.

Advantages of fluorescent labeling

- Although fluorescent dyes may not have the same sensitivity that radioactive probes did, they are **able to show real-time activity of molecules in action**.
- Moreover, radiation and appropriate handling is no longer a concern.
- With the development of fluorescent tagging, fluorescent microscopy has **allowed the visualization of specific proteins** in both fixed and live cell images.
- **Localization of specific proteins** has led to important concepts in cellular biology such as the functions of distinct groups of proteins in cellular membranes and organelles.
- In live cell imaging, fluorescent **tags enable movements of proteins** and their **interactions** to be monitored..

Advantages of fluorescent labeling

- Latest advances in methods involving fluorescent tags have led to the **visualization of mRNA** and its **localization** within various organisms.
- Live cell imaging of RNA can be achieved by **introducing synthesized RNA** that is chemically coupled with a fluorescent tag into living cells by **microinjection**.