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Applications

- Extrinsic Fluorescence – ANS, SYBR Green, etc.
- Intrinsic Fluorescence
- Fluorescence microscopy
- Lateral Movement of Membrane Proteins

Extrinsic Fluorescence

- ANS, SYBR Green, etc.
- ANS which emits only weak fluorescence in polar environment, i.e. in aqueous solution.
- However, in non-polar environment, e.g. when bound to hydrophobic patches on proteins, its fluorescence emission is significantly increased and the spectrum shows a hypsochromic shift; λ_{max} shifts from 475 nm to 450nm.
- It can also be used in competition assays to monitor binding of ligands and prosthetic groups.

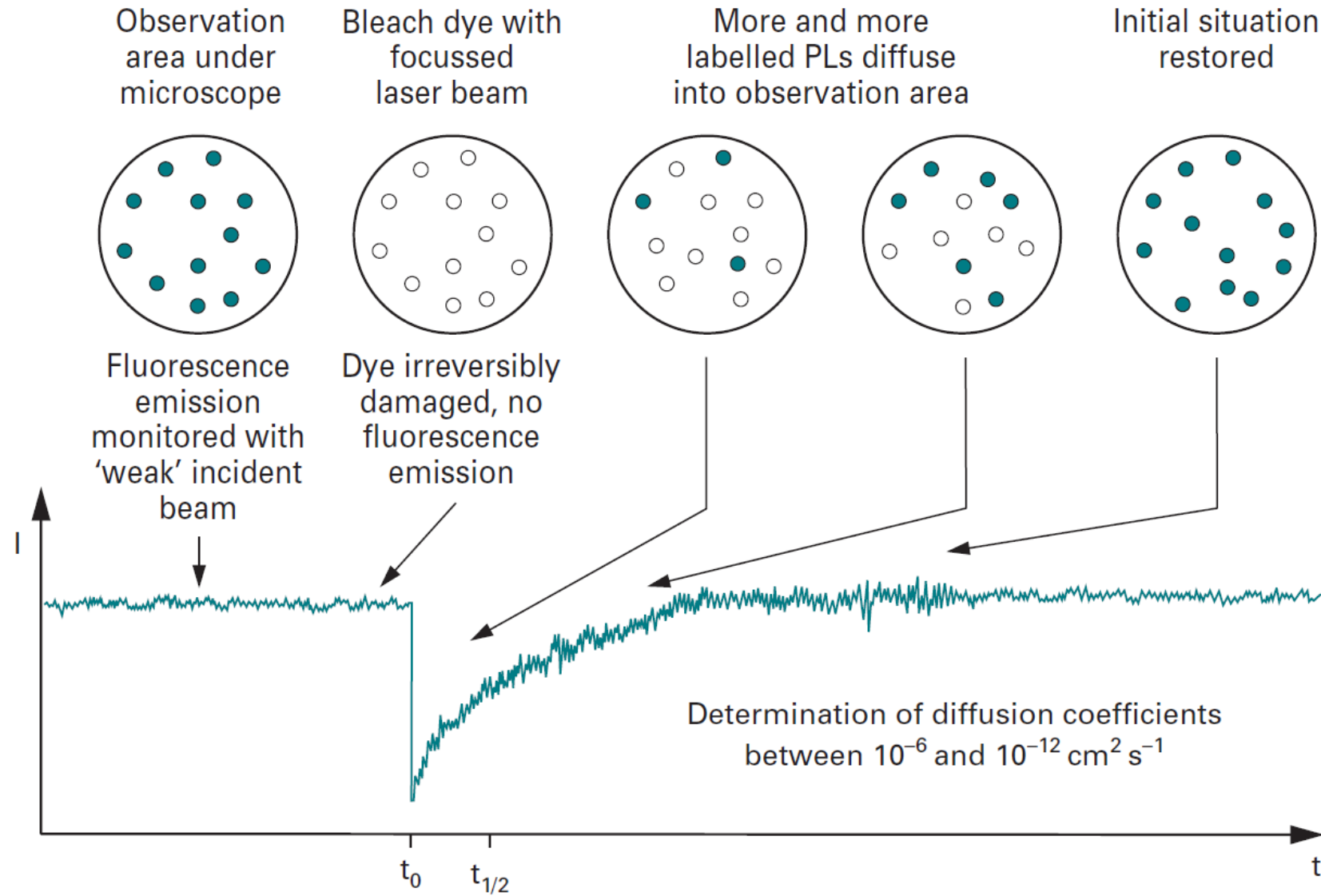
Intrinsic Fluorescence

- Proteins possess three intrinsic fluorophores: tryptophan, tyrosine and phenylalanine.
- As a fluorophore moves into an environment with less polarity, its emission spectrum exhibits a hypsochromic shift (λ_{max} moves to shorter wavelengths) and the intensity at λ_{max} increases.
- Fluorophores in a polar environment show a decrease in quantum yield with increasing temperature.

Fluorescence Resonance Energy Transfer

- Fluorescence microscopy
- The green fluorescent protein (GFP) from the jelly fish *Aequorea victoria* or
- The red fluorescent protein from *Discosoma striata*.

Fluorescence Recovery After Photo Bleaching





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