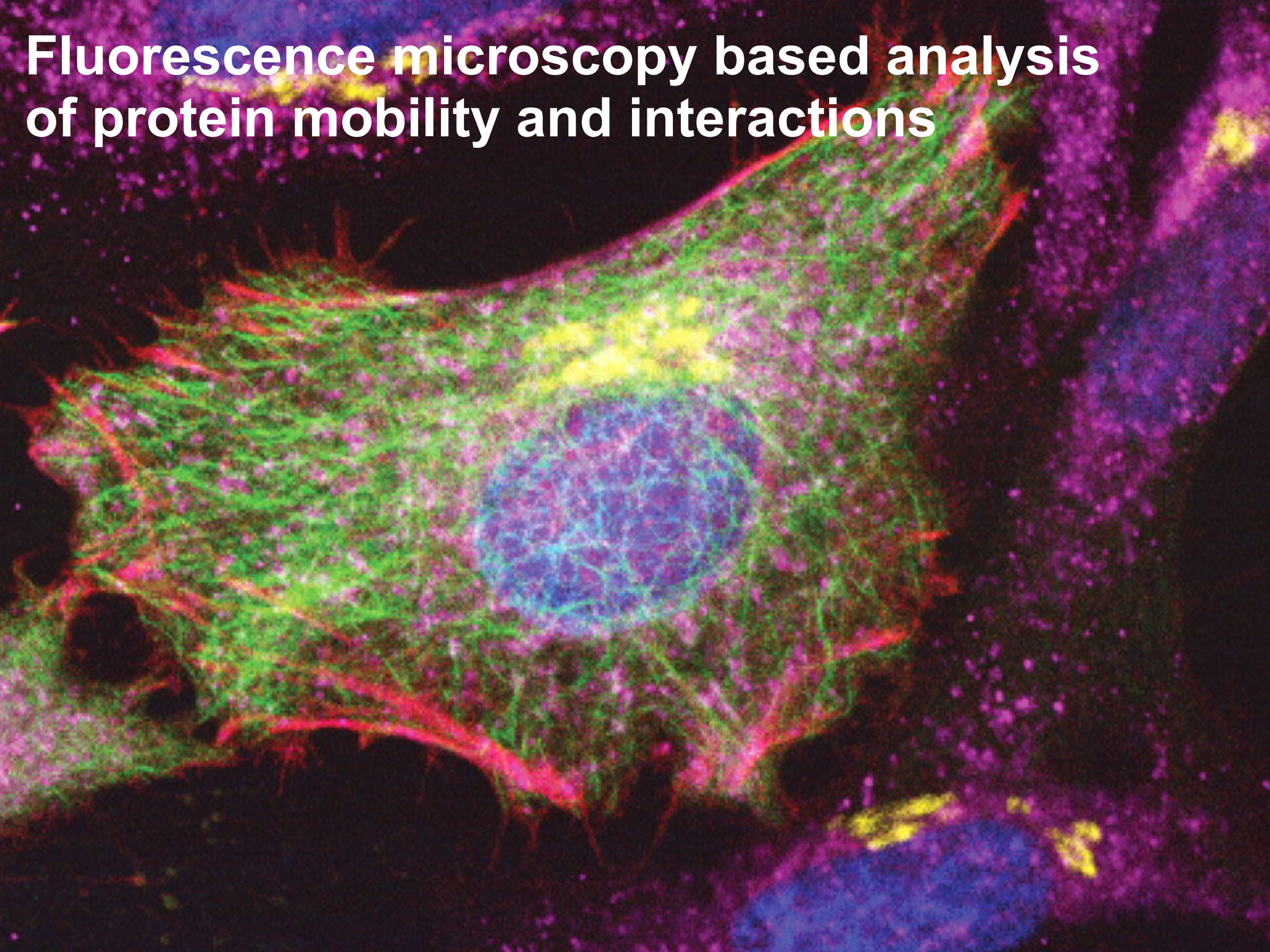
Fluorescence microscopy based analysis of protein mobility and interactions

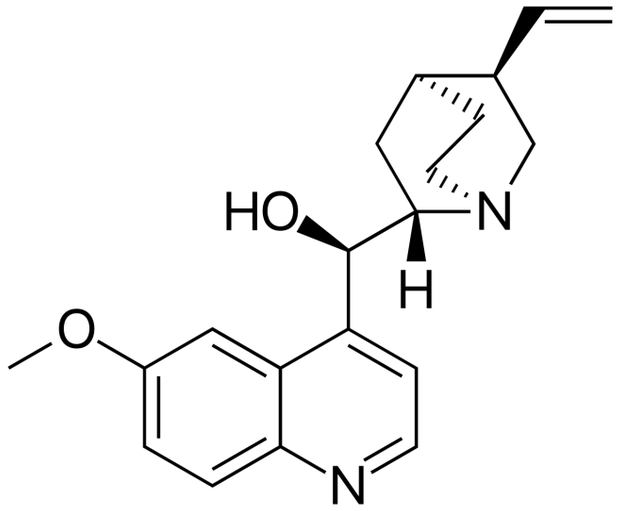


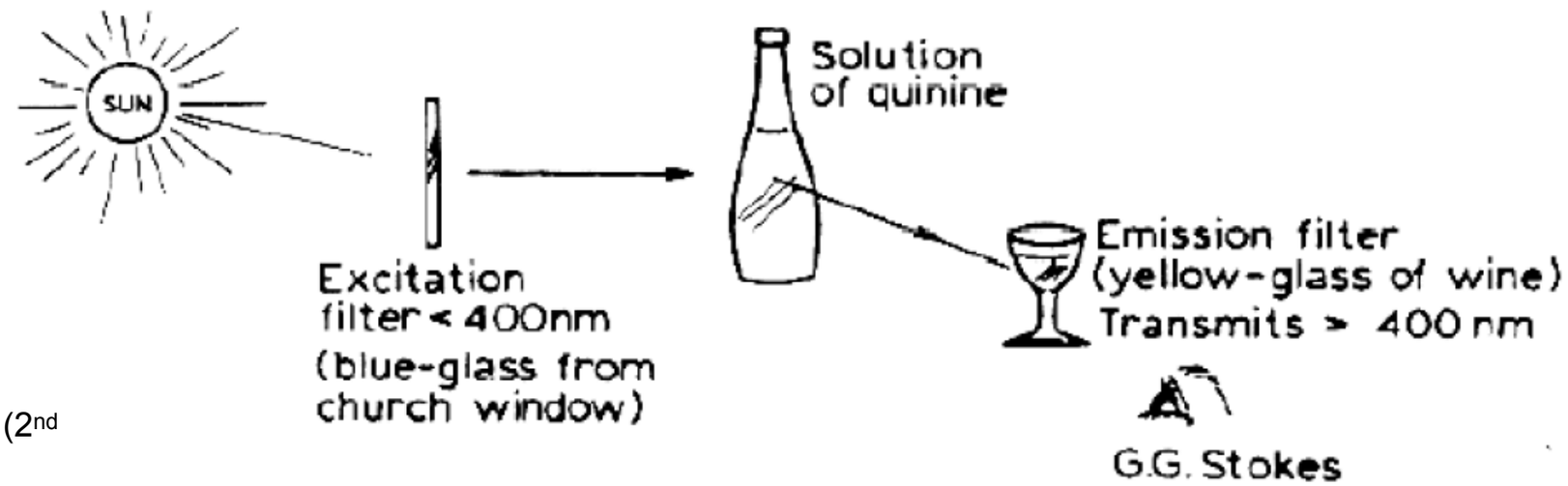
The first observations of fluorescence

– or how drinking helped

UV

blue

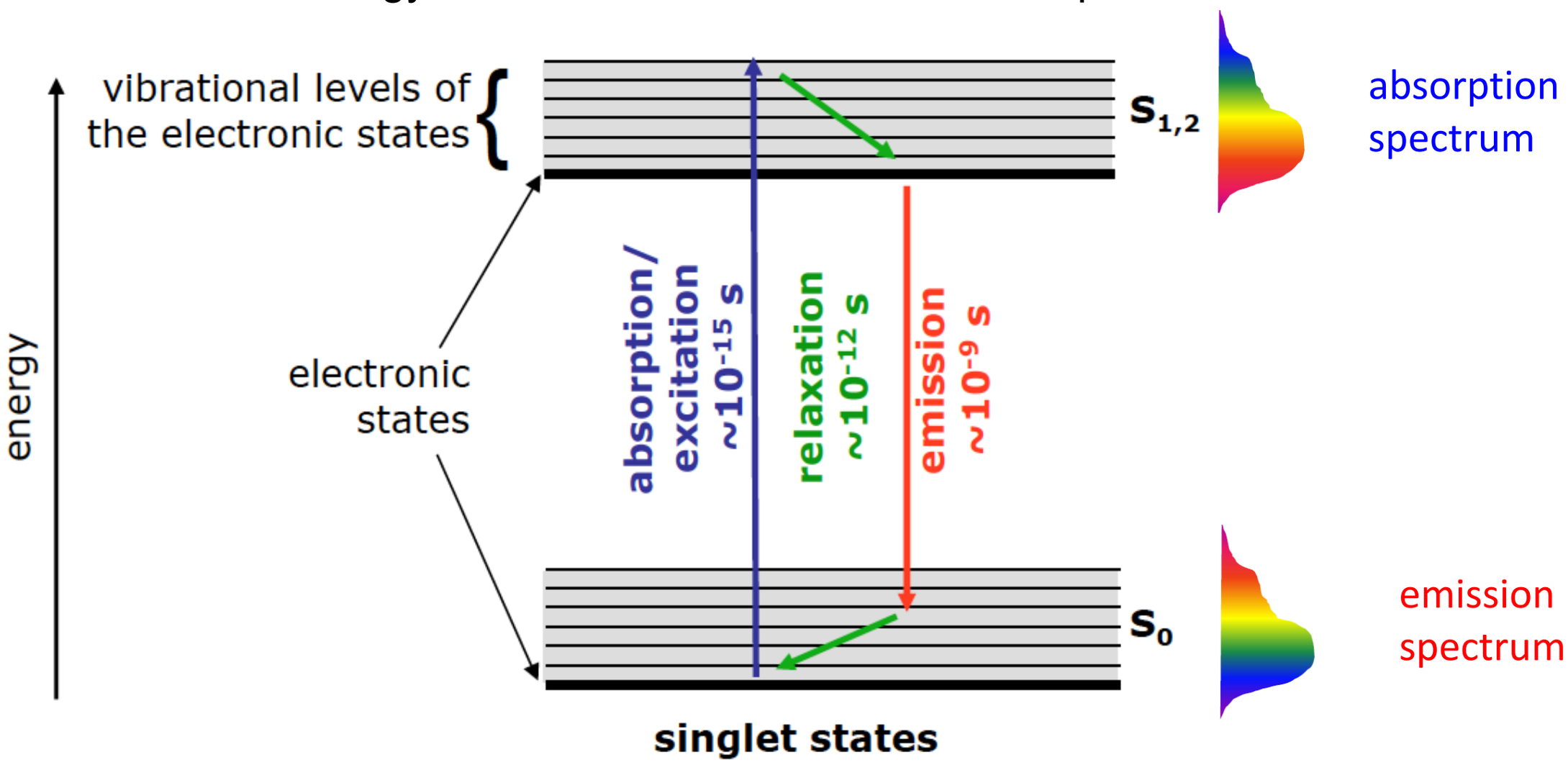




Lakowicz, JR: Principles of Fluorescence Spectroscopy (2nd edition), Kluwer Academic, New York (1999)

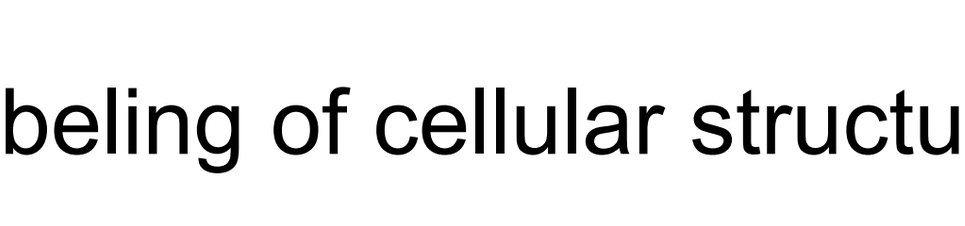
Steps of the fluorescent process

In 1953, Alexander Jablonski described fluorescence for the first time using a diagram of the different energy levels involved in the fluorescent process

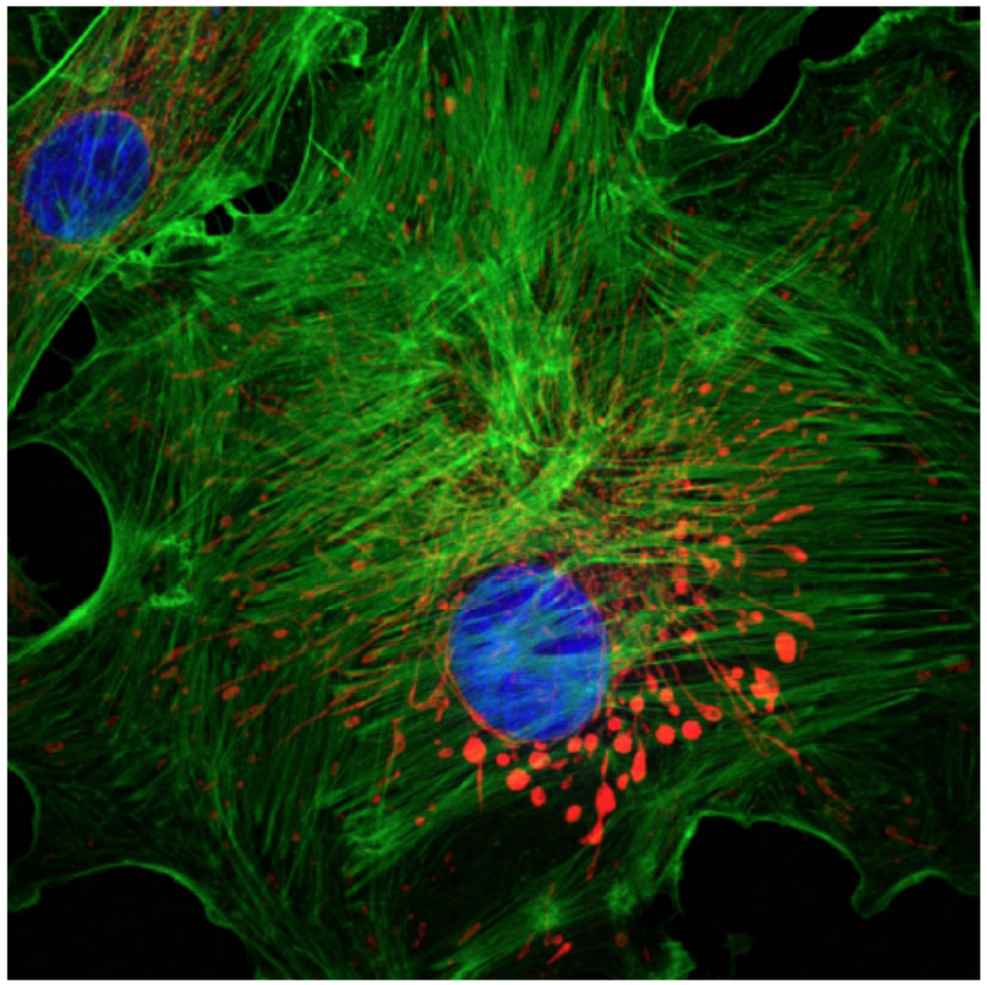


Labeling of cellular structures with different fluorophores

Fluorophores emit across the whole spectrum



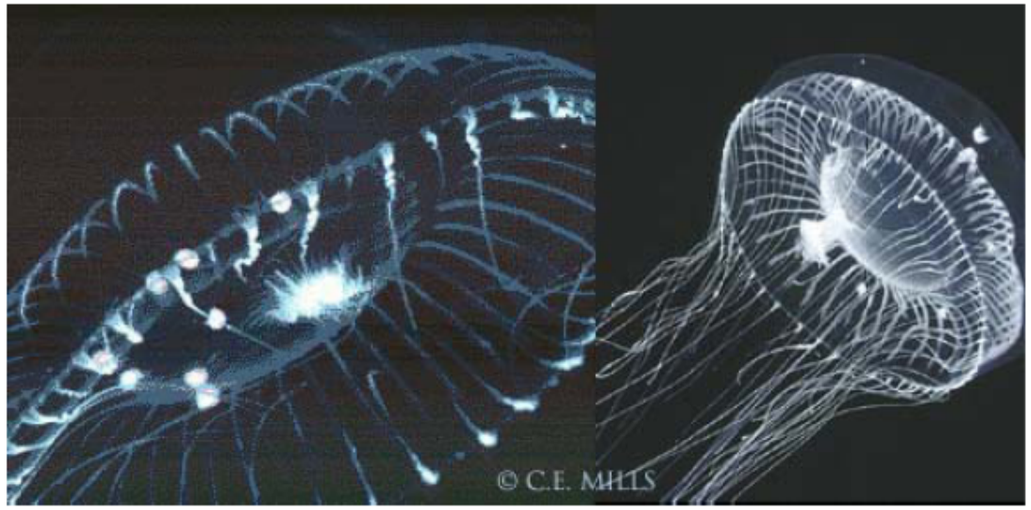
340-400 nm	Near Ultraviolet (UV, invisible)
400-430 nm	Violet
430-500 nm	Blue
500-560 nm	Green
560-620 nm	Yellow to Orange
620-700 nm	Red
>700 nm	Near Infrared (IR, invisible)



Molecular Probes FluoCells

Blue:	DAPI (nucleus)
Red:	Mitotracker Red (mitochondria)
Green:	Alexa488 (actin)

Autofluorescent proteins:
green fluorescent protein GFP



jellyfish *Aequorea victoria* (Cnidaria)
bioluminescent organism

Timeline:

1962: Discovery by Shimomura et al., J. Cell. Comp.Physiol. 59, 223

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1992: Cloning and sequencing of GFP by Prasher, et al., Gene 111, 229

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1995: Expression of fluorescent protein by Chalfie et al., Science 263, 802

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thousands of publications...

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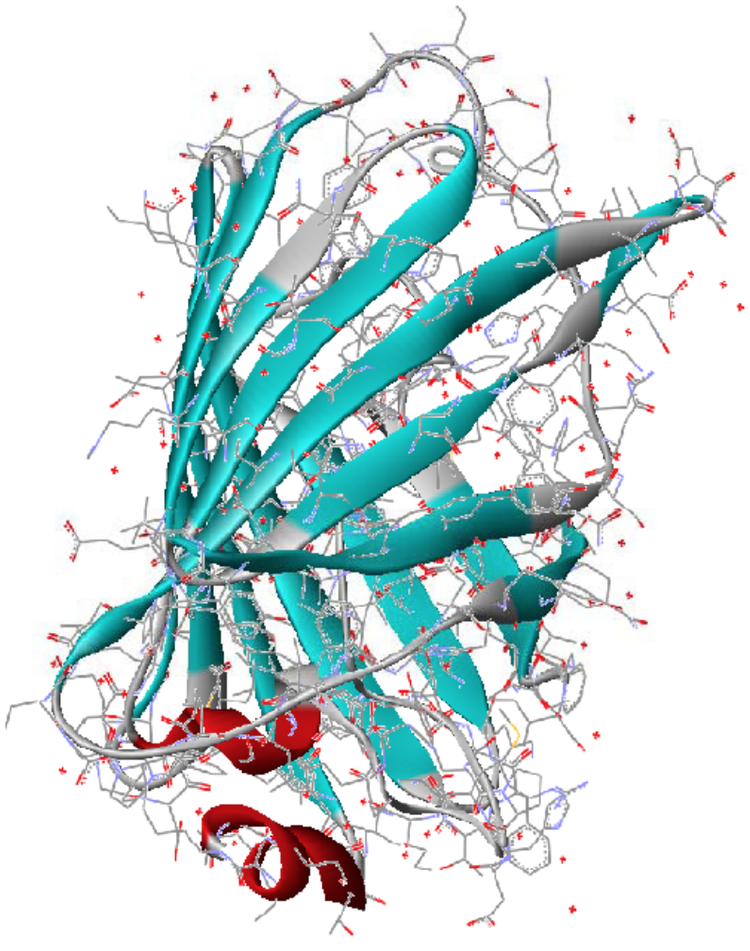
2008: Nobel Prize for Osamu Shimomura, Martin Chalfie, Roger Tsien

|



MW ~ 27 kDa
238 amino acids

Parameters that characterize a fluorophore

- Excitation and emission wavelength (important for spectral separation of scattered excitation light as well as emission between multiple colors)
- Stability toward bleaching
- Extinction Coefficient ε

  ε capacity of light absorption at a distinct wavelength (usually at the absorption maximum)

- Quantum Yield ϕ

  ϕ is a measure of the integrated photon emission over the fluorophore spectral band

$$\phi = \frac{Number\ of\ emitted\ photons}{Number\ of\ absorbed\ photons}$$

- At sub-saturation excitation rates, fluorescence intensity or brightness is proportional to the product of ε and ϕ

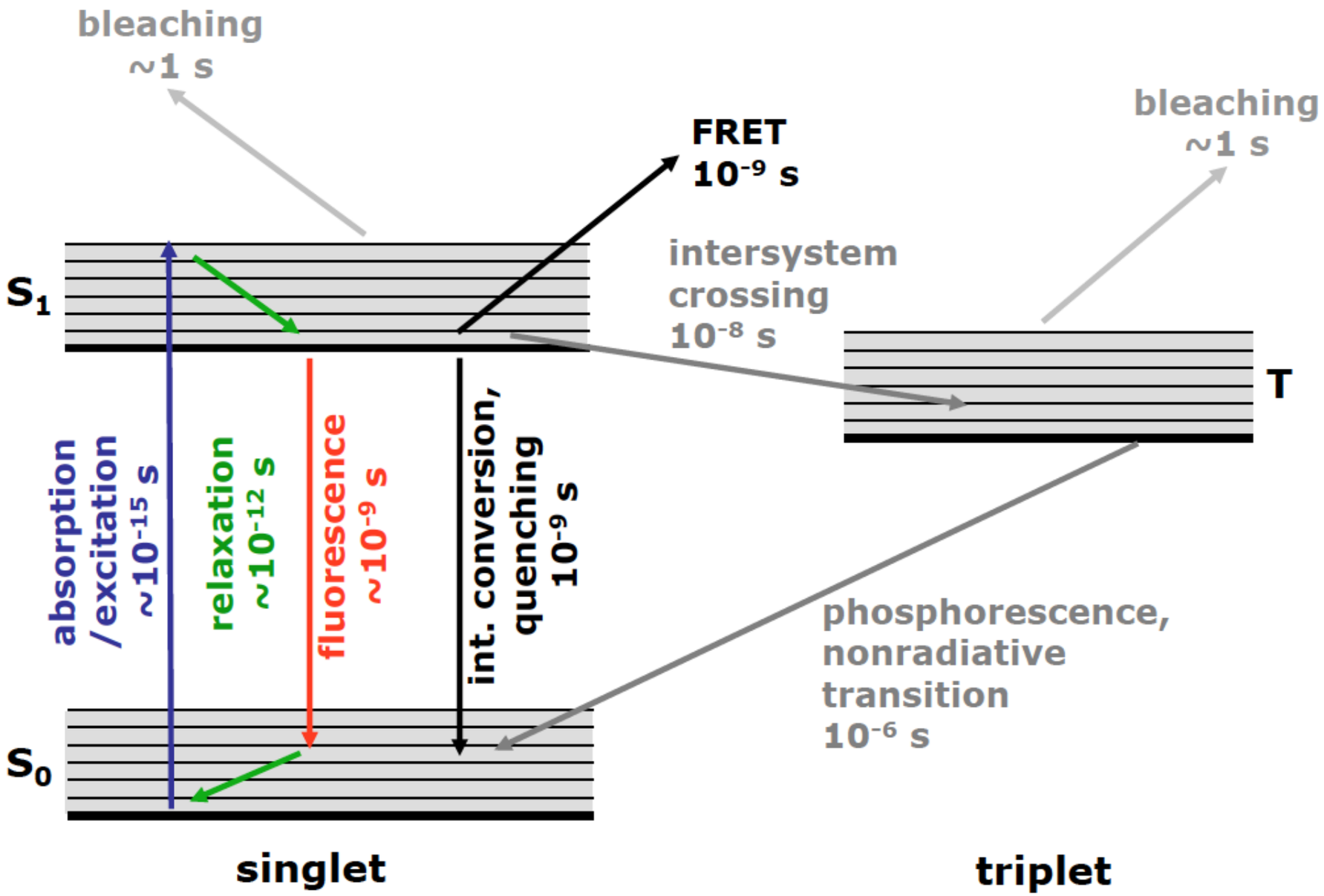
$$I = const \cdot \varepsilon \cdot \phi$$

Autofluorescent proteins

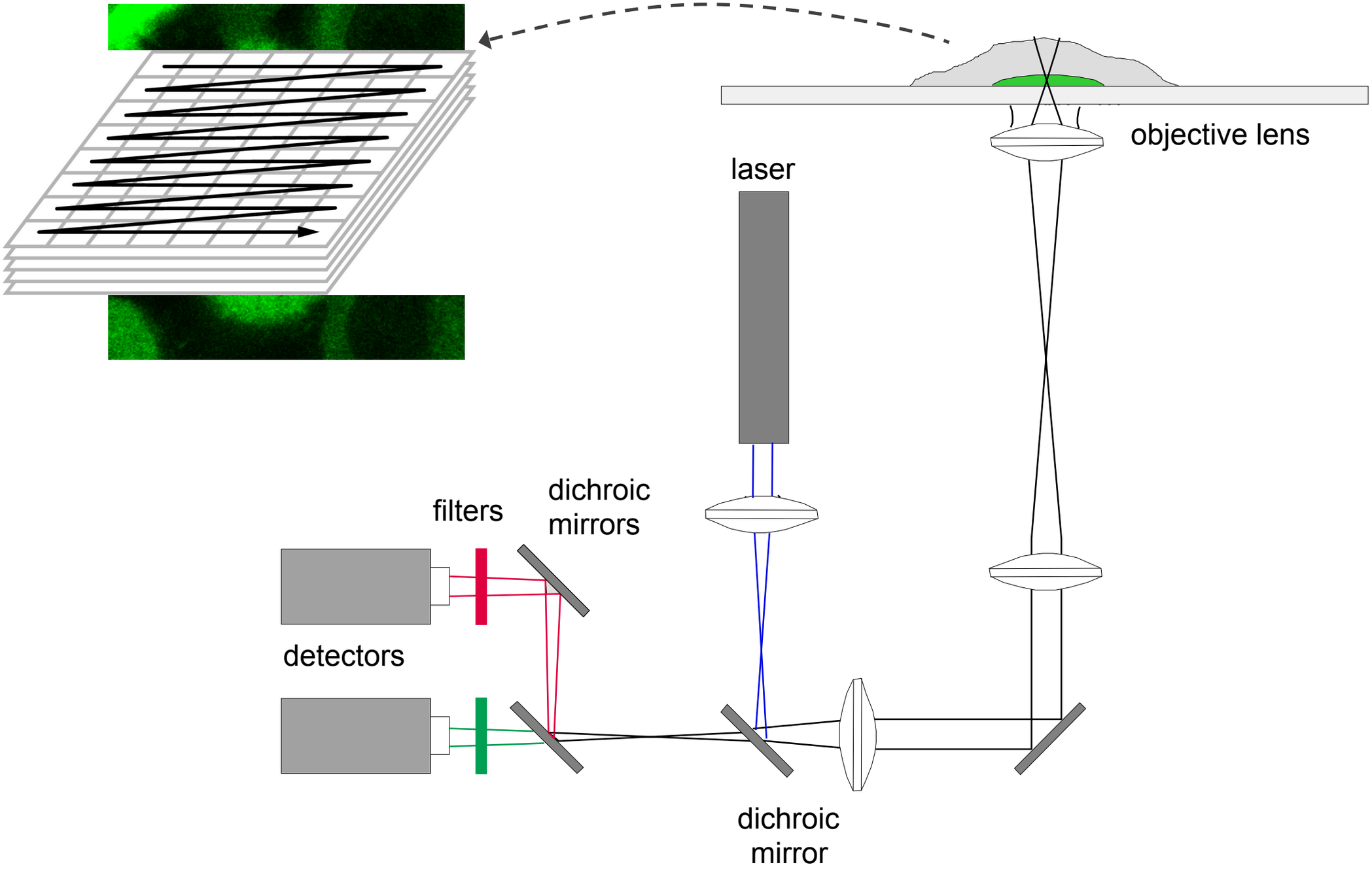
http://www.microscopyu.com/articles/livecellimaging/fpintro.html

Protein (Acronym)	Excitation Max. (nm)	Emission Max (nm)	Extinction Coefficient	Quantum Yield	*in vivo Structure*	Brightness (% EGFP)
GFP (wt)	395/475	509	21000	0.77	Monomer*	48
EGFP	484	507	56000	0.60	Monomer*	100
TurboGFP	482	502	70000	0.53	Dimer	102
EBFP2	383	448	32000	0.56	Monomer*	53
mTagBFP	399	456	52000	0.63	Monomer	98
ECFP	439	476	32500	0.40	Monomer*	39
TagCFP	458	480	37000	0.57	Monomer	63
EYFP	514	527	83400	0.61	Monomer*	151
Venus	515	528	92200	0.57	Monomer*	156
TagYFP	508	524	64000	0.60	Monomer	118
dTomato	554	581	69000	0.69	Dimer	142
TagRFP	555	584	100000	0.48	Monomer	142
mRuby	558	605	112000	0.35	Monomer	117
mStrawberry	574	596	90000	0.29	Monomer	78
mRFP1	584	607	50000	0.25	Monomer	37
mCherry	587	610	72000	0.22	Monomer	47
mRaspberry	598	625	86000	0.15	Monomer	38
dKeima-Tandem	440	620	28800	0.24	Monomer	21
HcRed-Tandem	590	637	160000	0.04	Monomer	19
mPlum	590	649	41000	0.10	Monomer	12

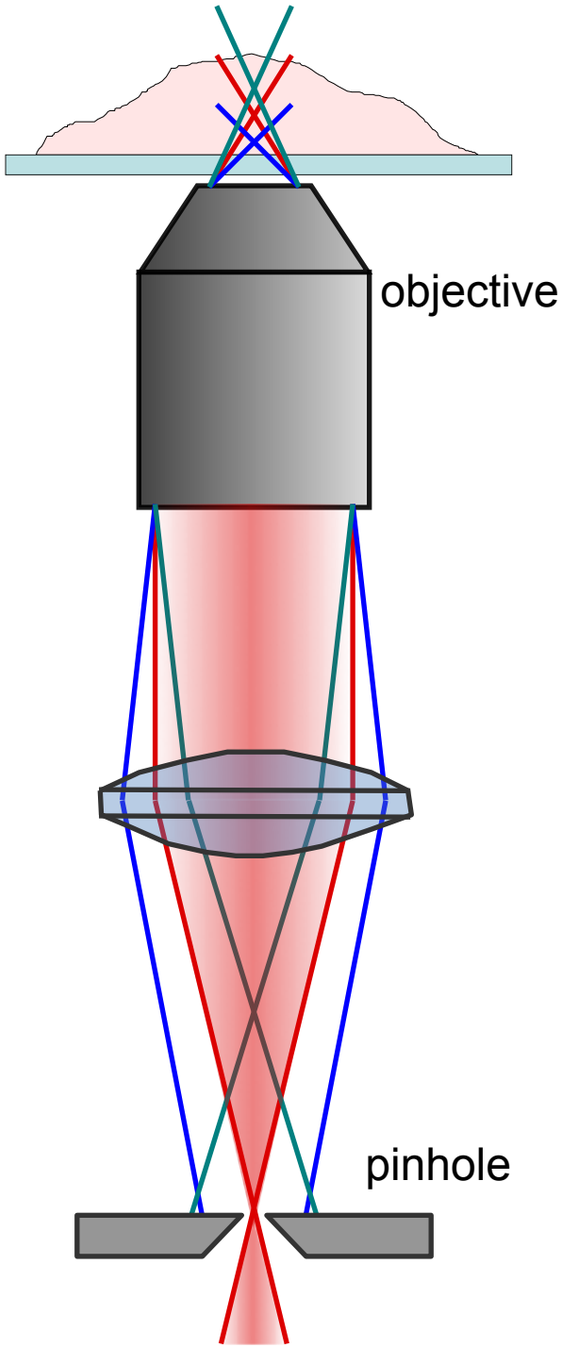
Light and dark – energy states of the fluorescence system

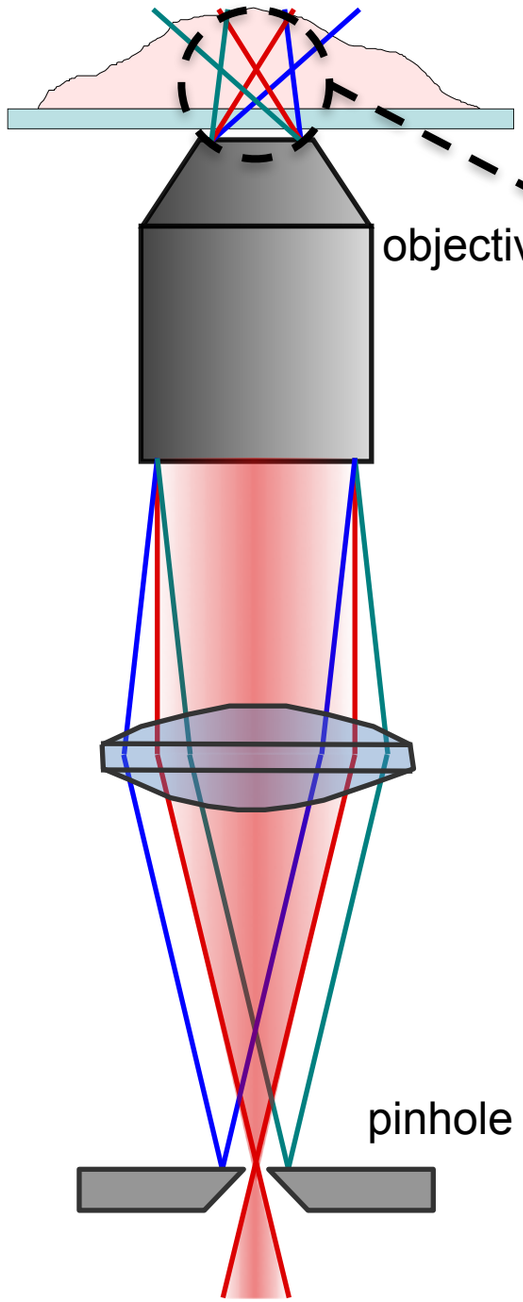


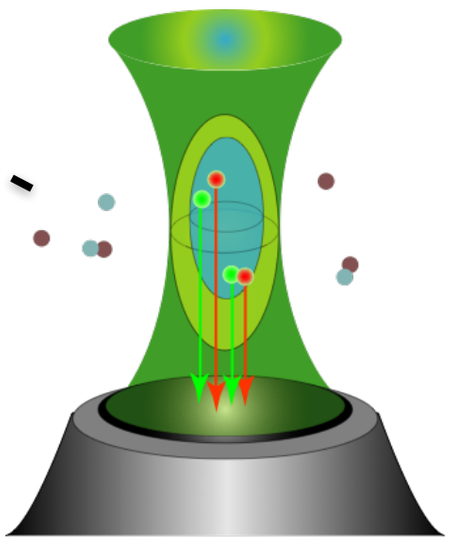
Fluorescence laser scanning microscopy



The confocal principle





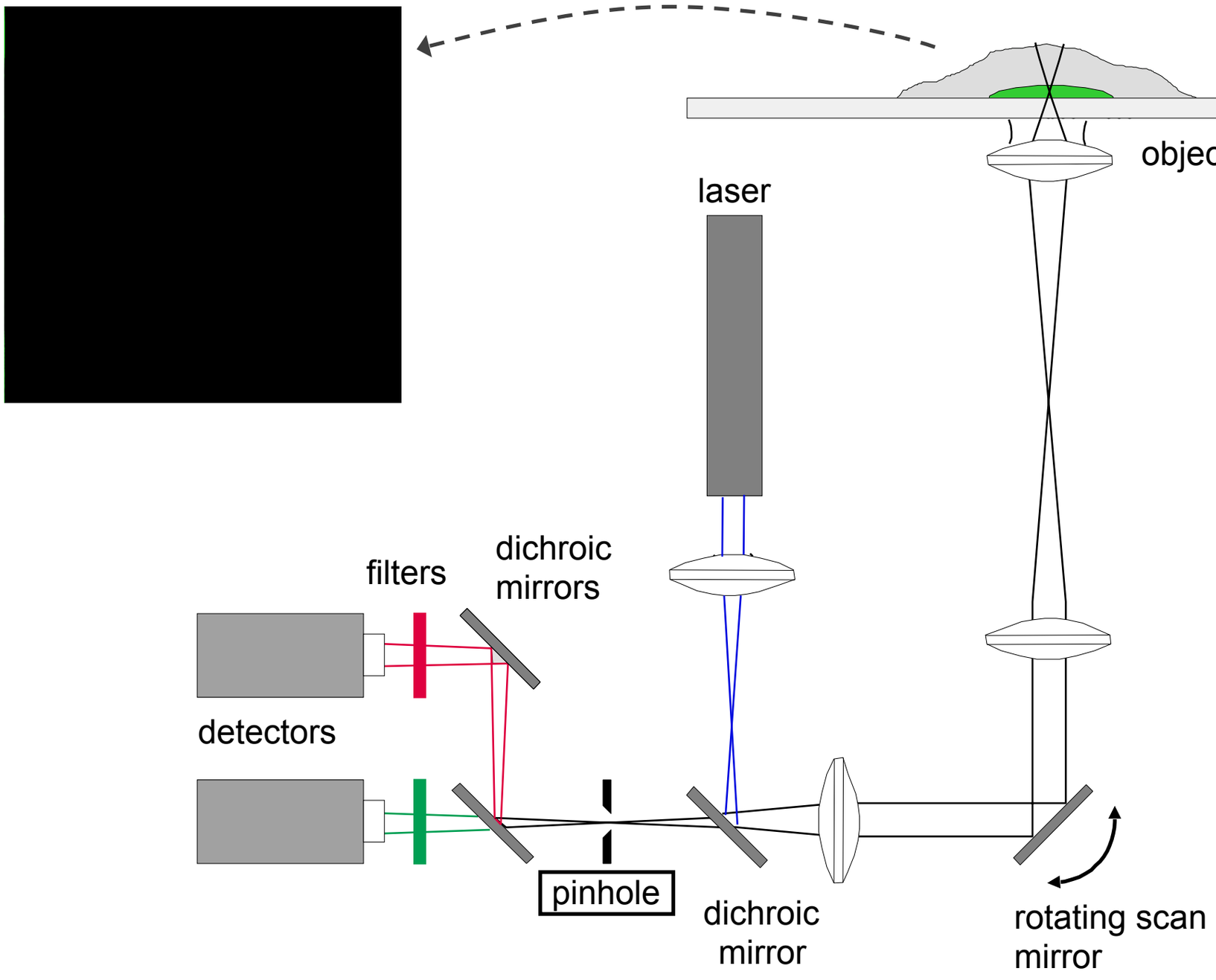


excitation volume

200-300 nm (x,y)
600-700 nm (z)

(< 10^{-15} l)

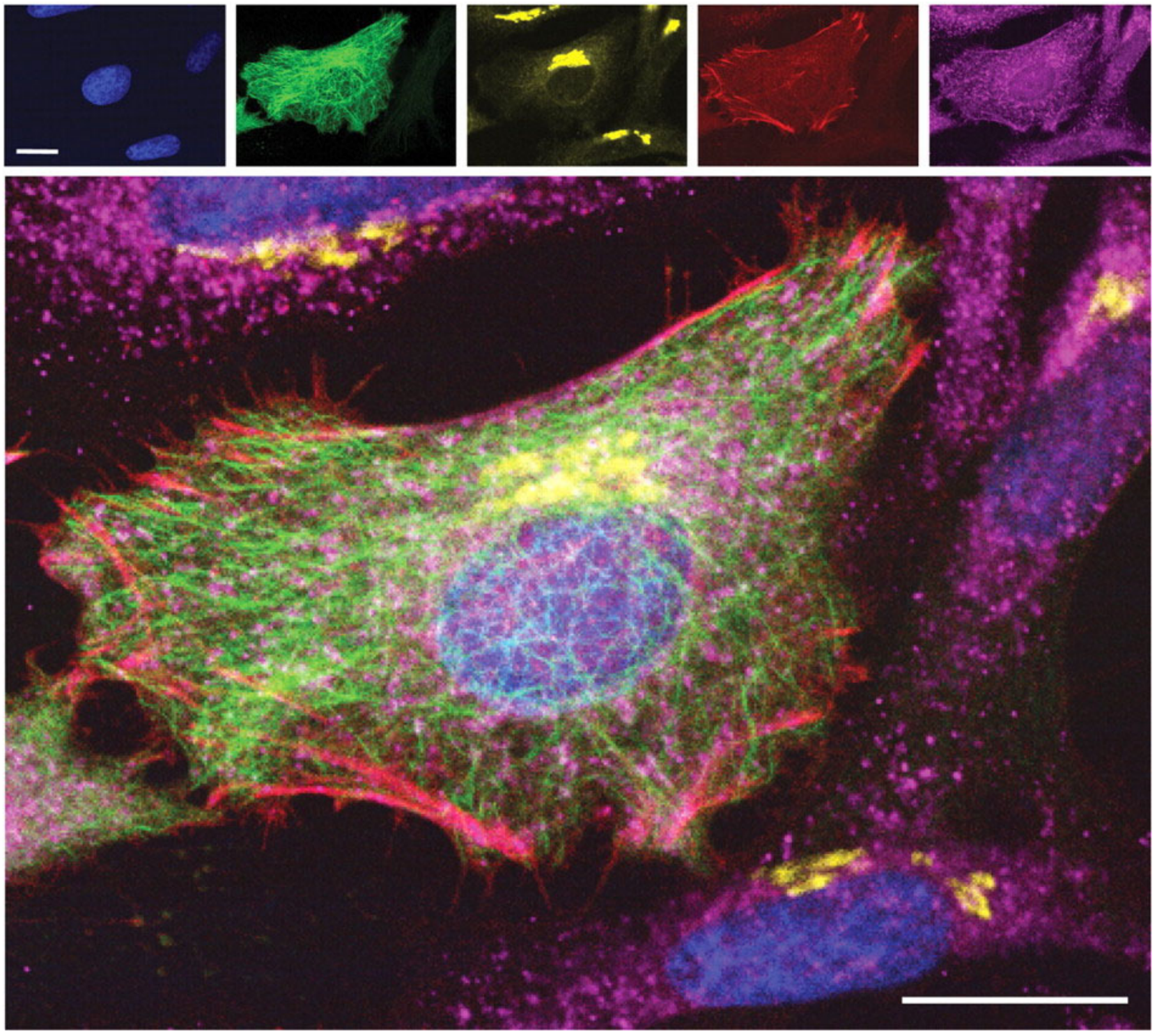
Improved resolution in fluorescence confocal laser scanning microscopy



- confocal/observation volume confined in 3D
- image formation in the confocal laser scanning microscope by raster-scanning and synchronized detection
- flexible targeting of every point in the sample

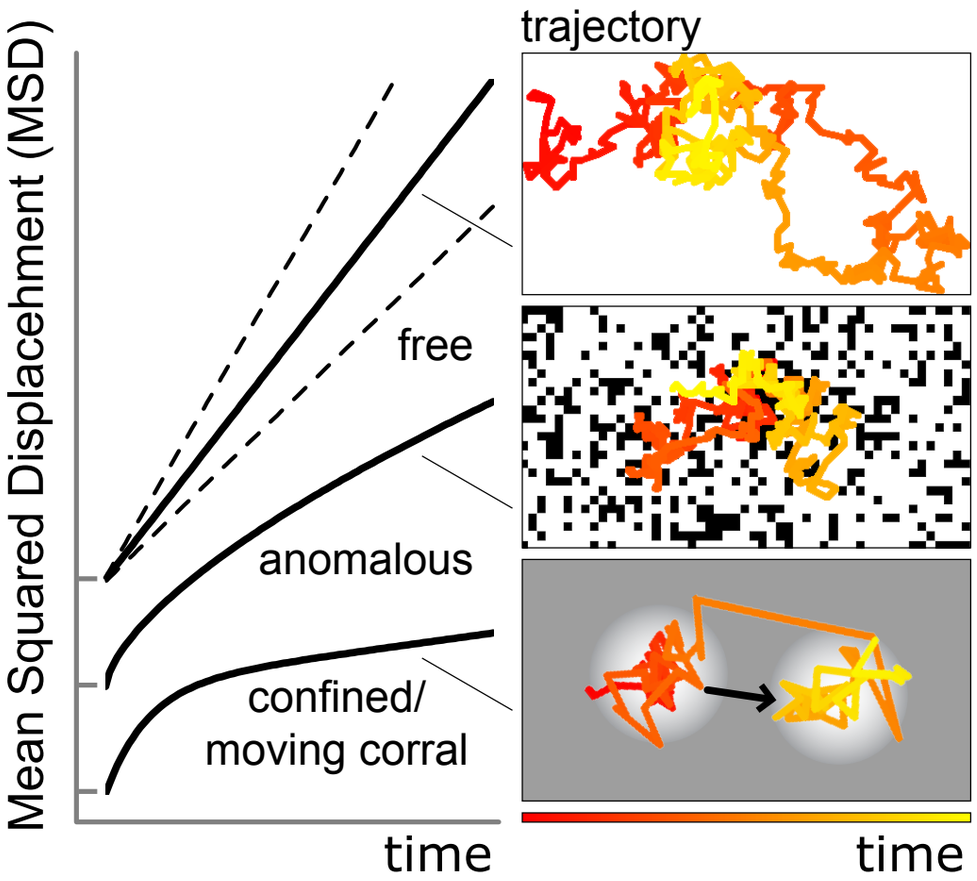
Multi-color fluorescence microscopy

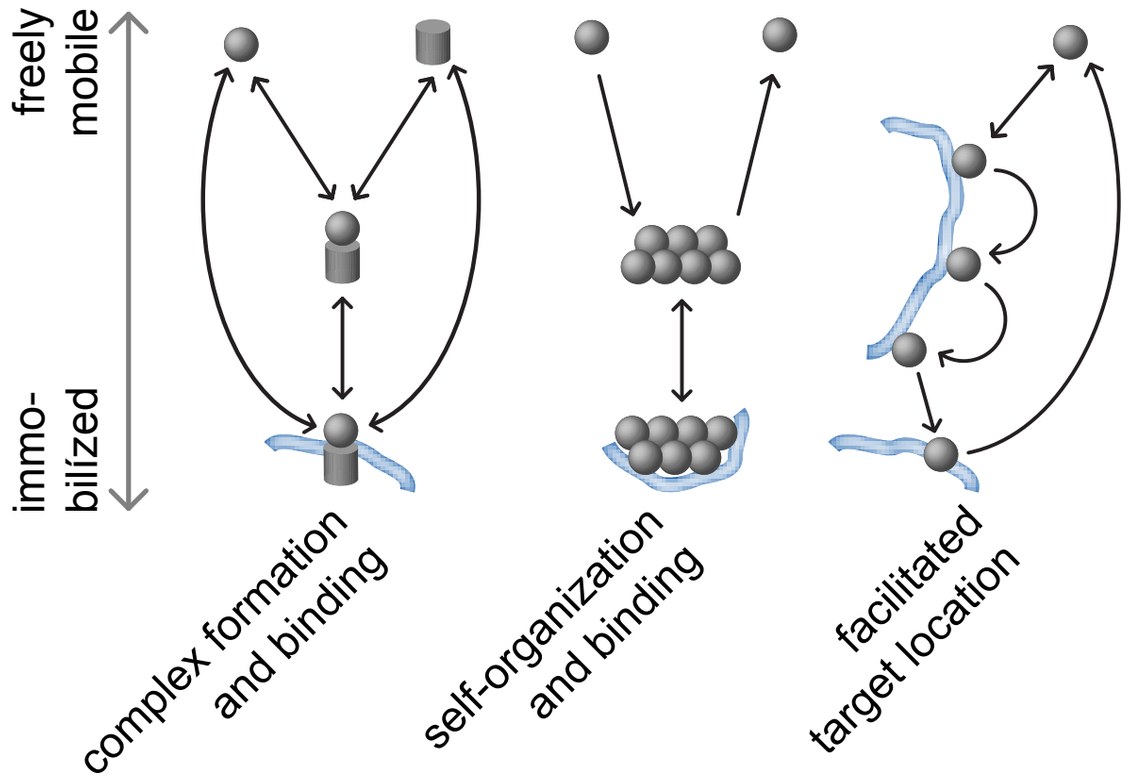
Target:	DNA	α-tubulin	giantin	β-actin	Cytochrome c
Structure:	nuclei	microtubules	golgi	stress fibers	mitochondria



Protein mobility and interactions in the cell

$$MSD = 6\,D\,t^{\alpha}$$





Dependence of diffusion coefficient D and molecular mass *M*

protein: $D \propto M^{-\frac{1}{3}}$ DNA: $D \propto M^{-\frac{1}{2}}$

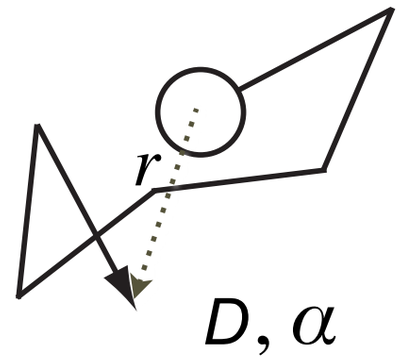
double mass *M* => 0.8 fold lower *D* double mass *M* => 0.7 fold lower *D*

Wachsmuth, M., Caudron-Herger, M. and Rippe, K. (2008). *Biochim. Biophys. Acta* **1783**, 2061-2079.

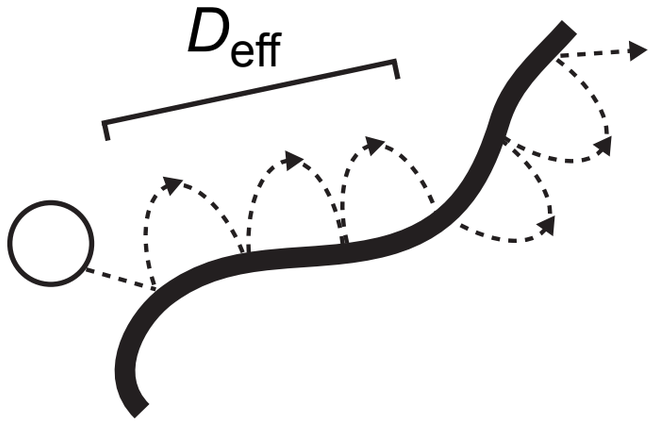
Determining diffusion coefficient D, kinetic binding rates k_{on} and k_{off}, and the apparent equilibrium constant K_{eq}^*

diffusion without binding,

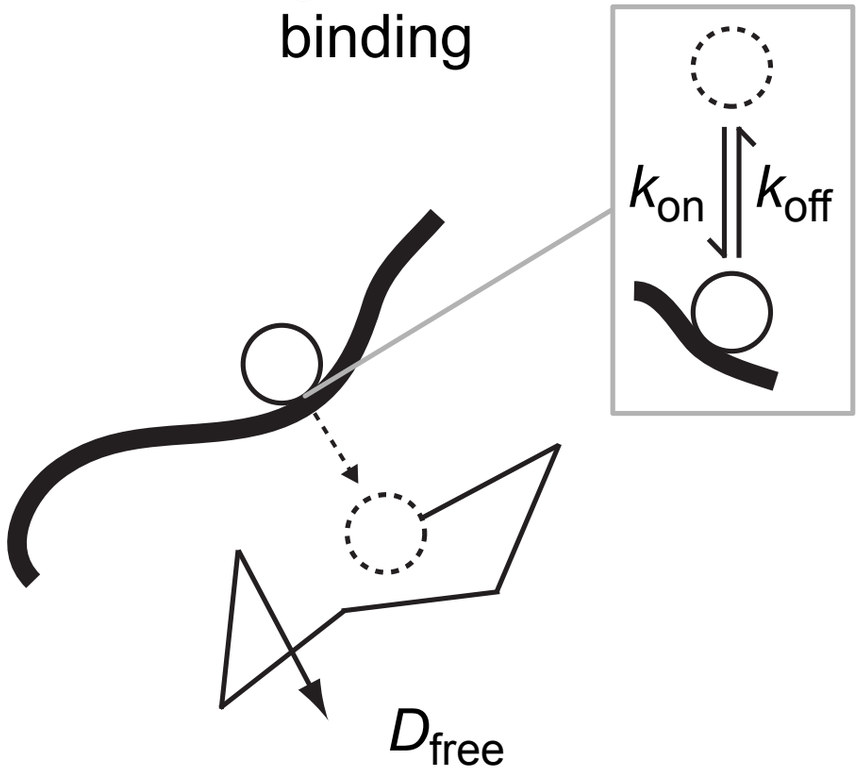
α = 1 for free diffusion



transient chromatin binding



strong chromatin binding



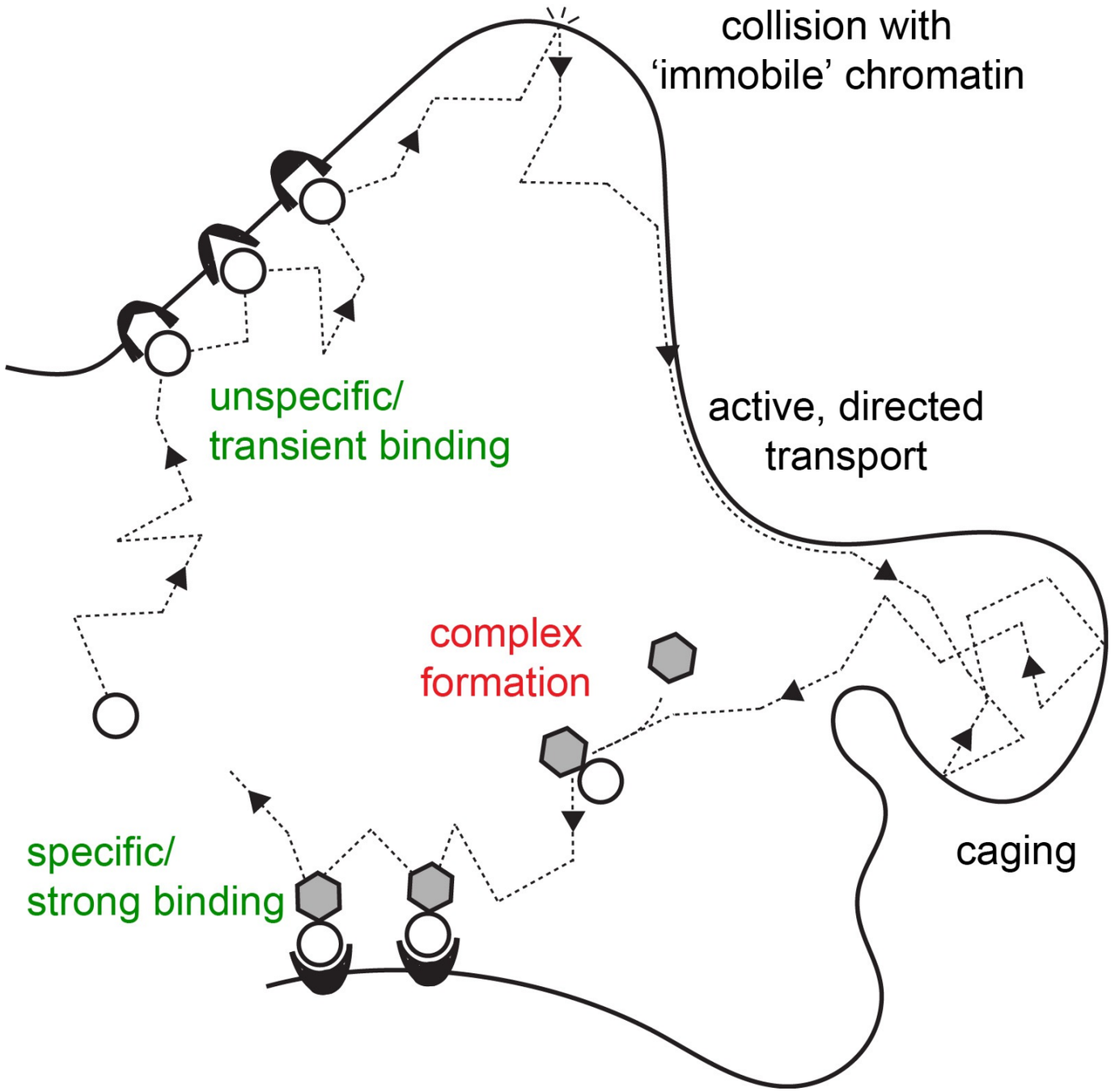
$$\left\langle r^2 \right\rangle = 6 \cdot D \cdot t^{\alpha}$$

$$D_{\text{eff}} = \frac{D}{1 + K_{\text{eq}}^*}$$

$$K_{\text{eq}}^* = \frac{k_{\text{on}}^*}{k_{\text{off}}} = \frac{k_{\text{on}} \cdot [S]_{\text{eq}}}{k_{\text{off}}}$$

Erdel, Müller-Ott, Baum, Wachsmuth & Rippe (2011) *Chromosome Res* **19**, 99–115.

Different microscopic phenomena influence protein mobility and interactions in living cells



Erdel F & Müller-Ott K et al., *Chrom Res* (2010)