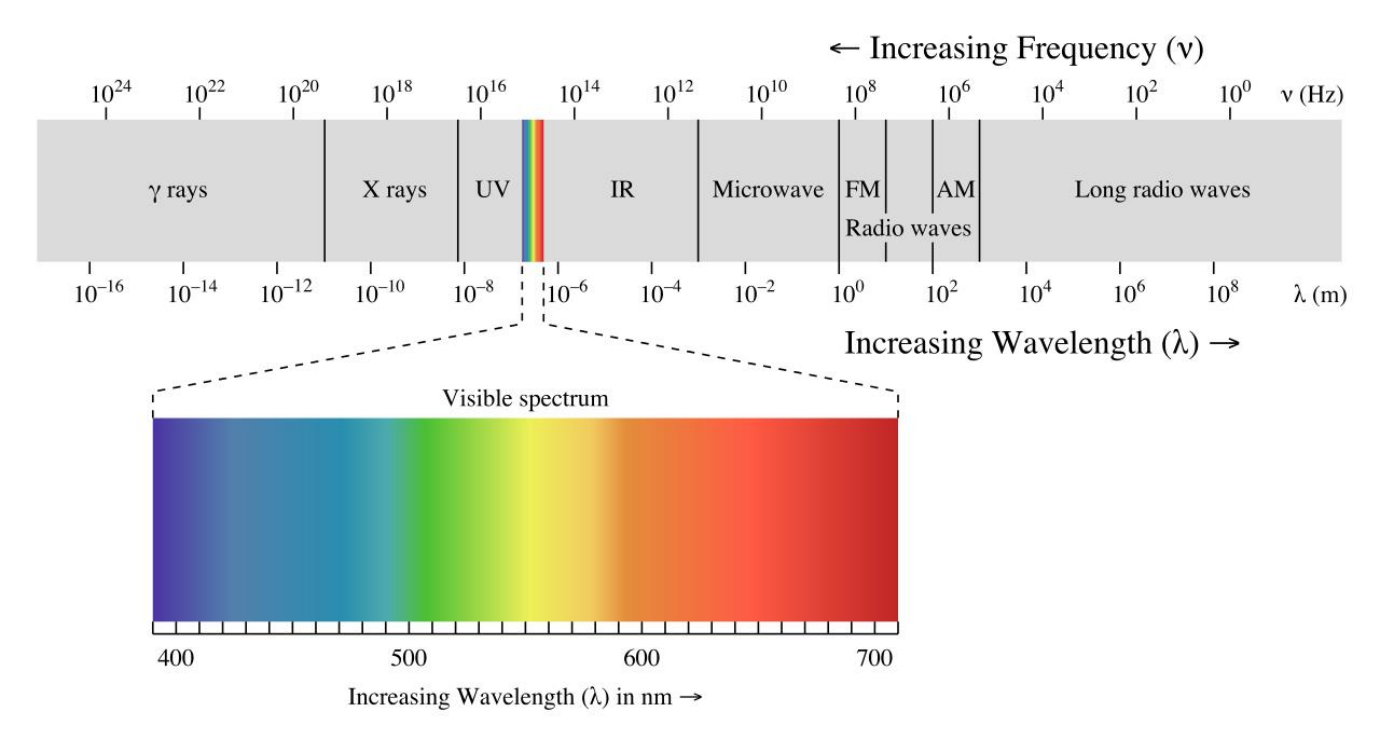
Optical Spectroscopy:
The study absorption and emission of light in nature

Abid Khan (past slides from Virginia Lorenz and Kai Wen Teng)

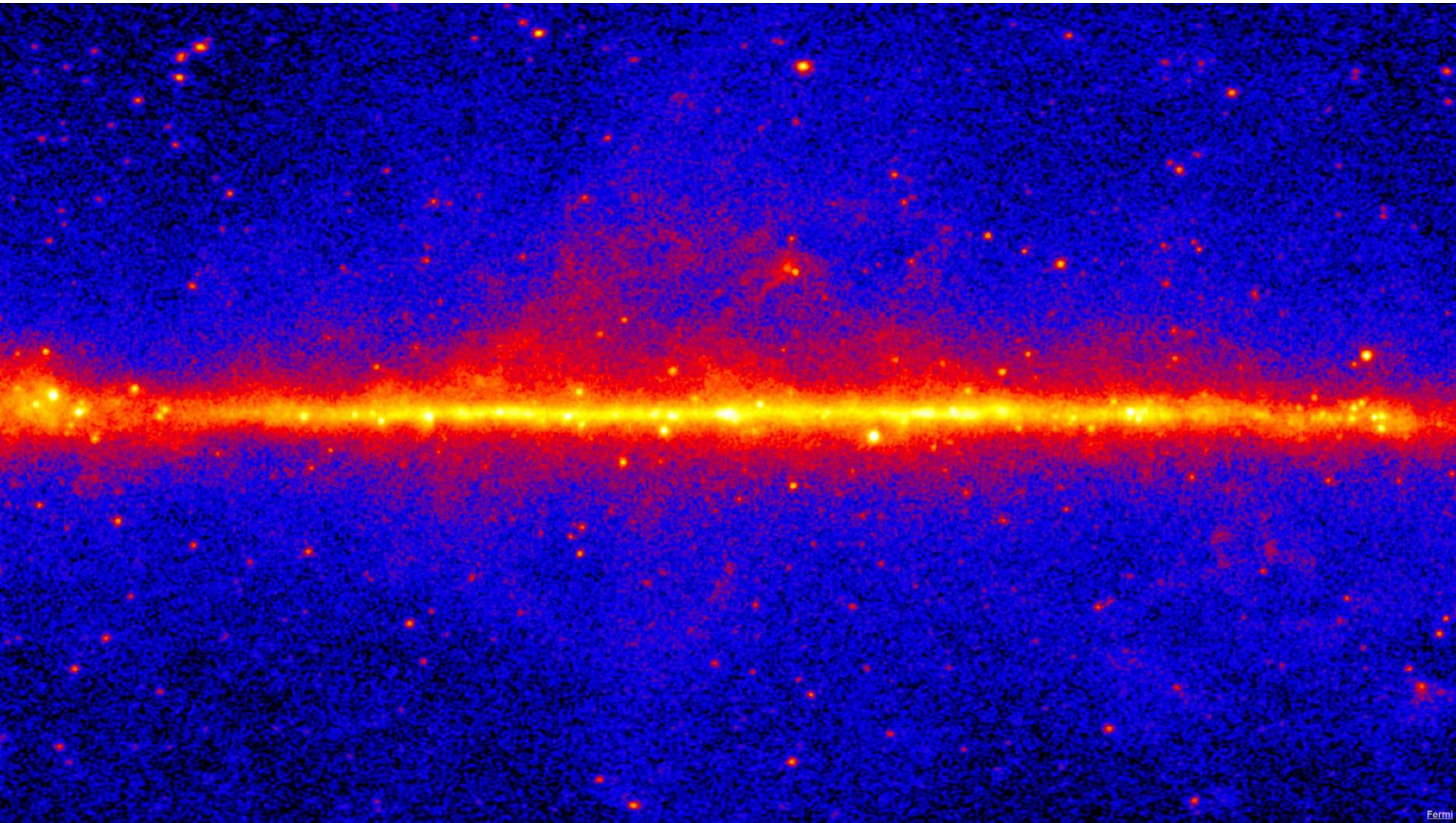
PHYS 403 Spring 2019



Optical Spectroscopy in Astronomy

- By looking through a standard telescope, you are observing the night sky at the visible light spectrum

Gamma Rays



Black hole binaries in disks of plasma are sources of gamma rays

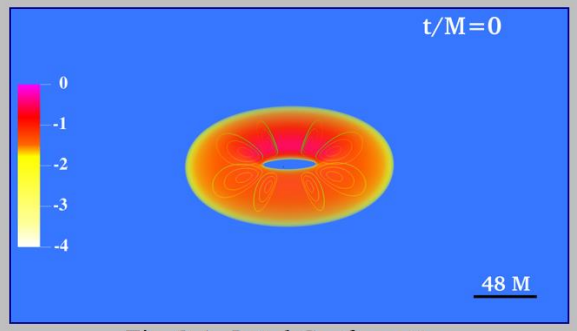


Fig. 2-1: *Inital Configuration*

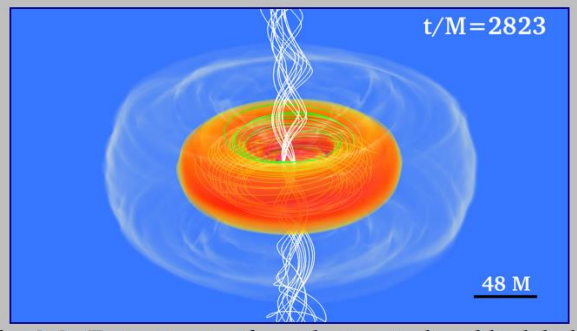


Fig. 2-2: *Twin jets rise from the inspiraling black holes*

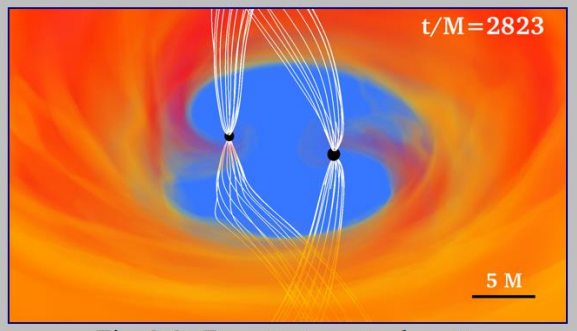


Fig. 2-3: *Zoom in to central cavity*

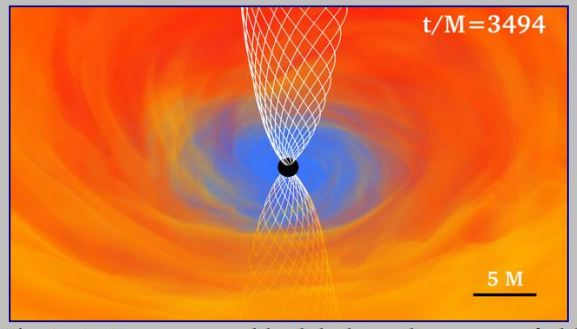


Fig. 2-4: *Post merger black hole and magnetic field*

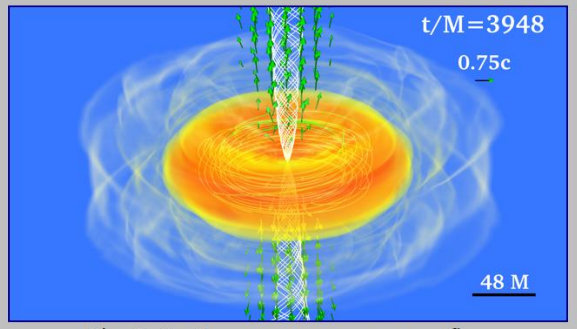
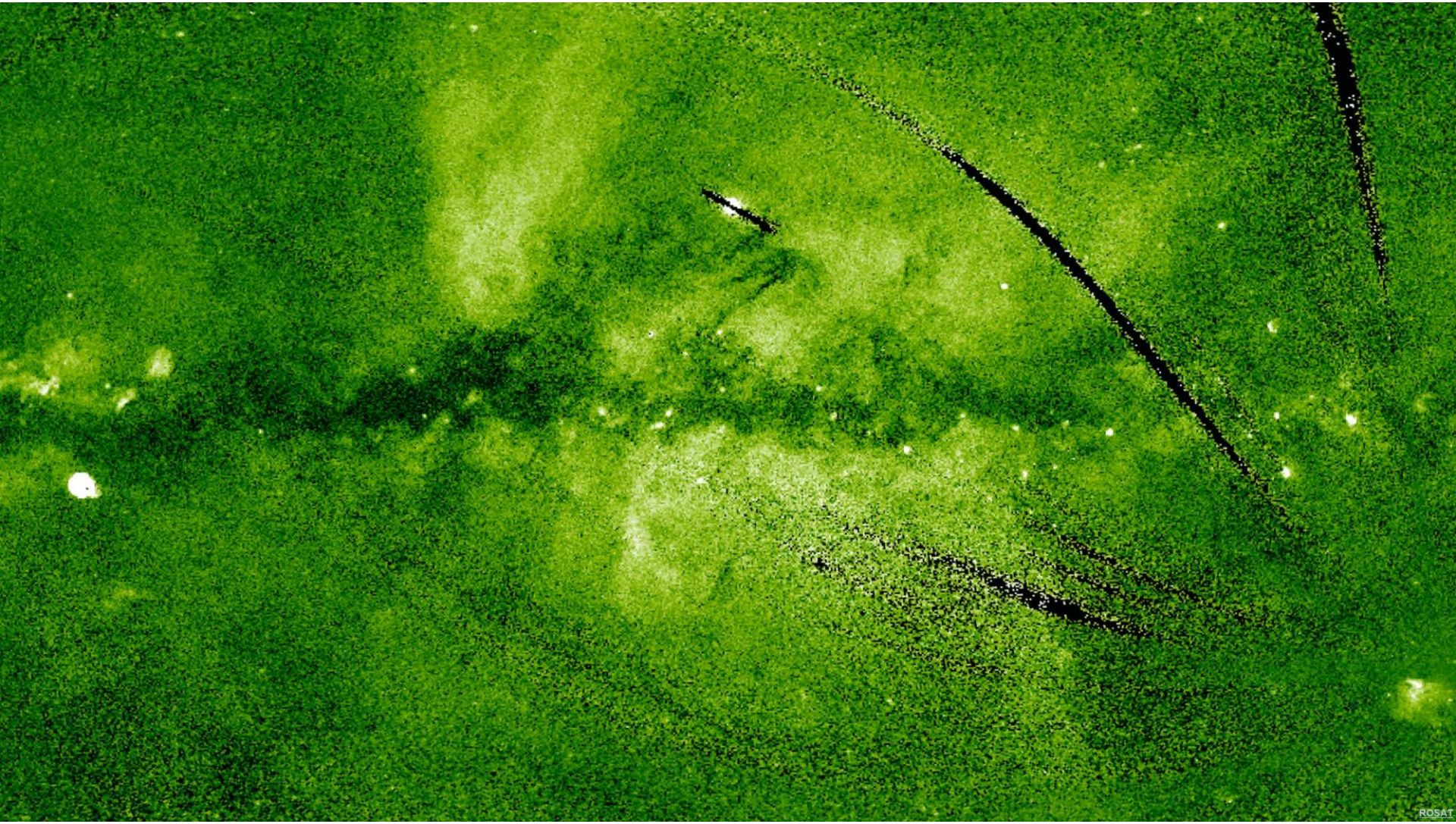


Fig. 2-5: *Quasistationary jet outflow*

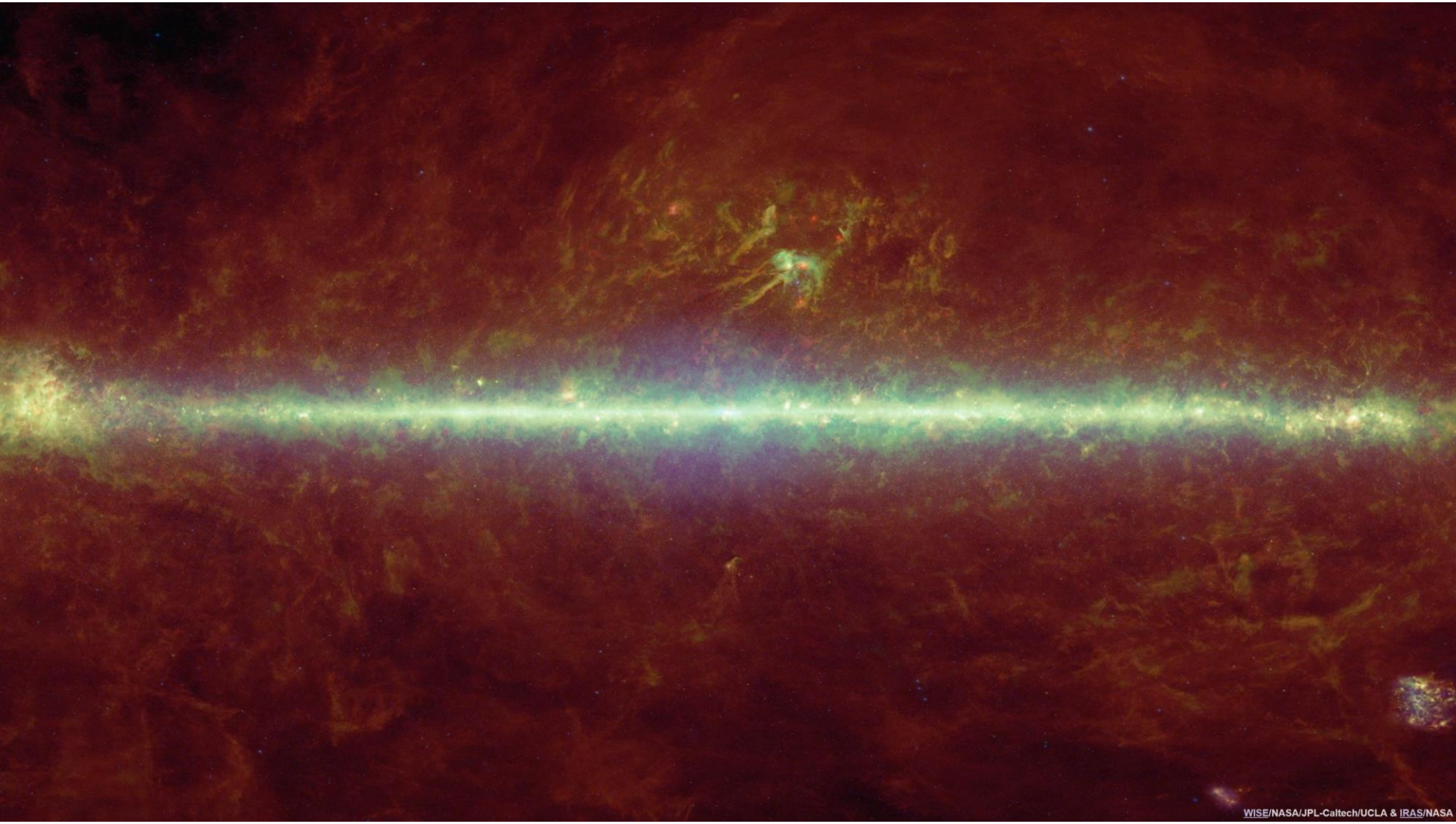
Khan, Paschalidis, Ruiz, Shapiro. 2018

X Rays



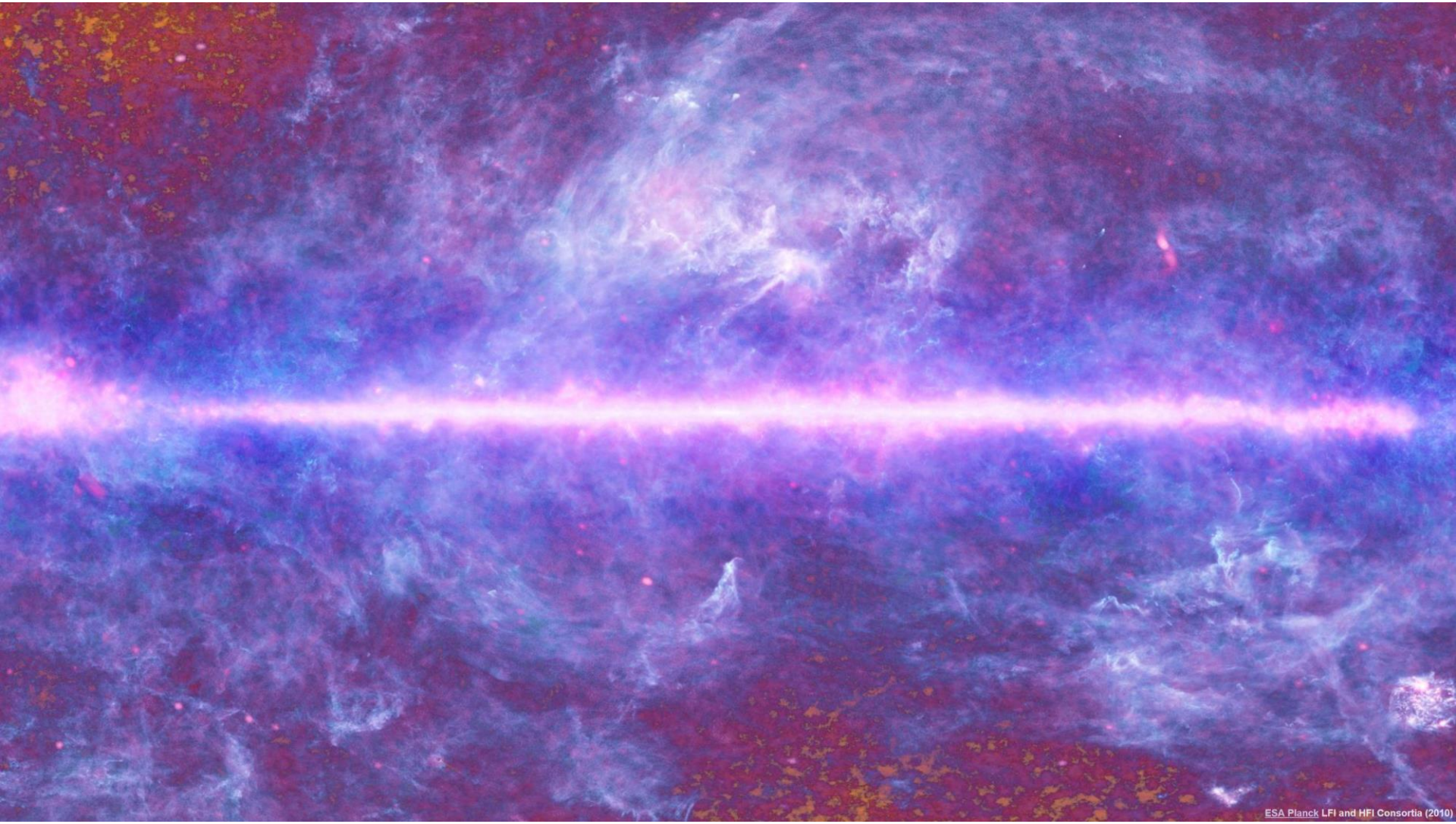
X-ray sources include stars, supernova, gaseous shells ejected during a violent explosion of a dying star, and synchrotron radiation

Infared



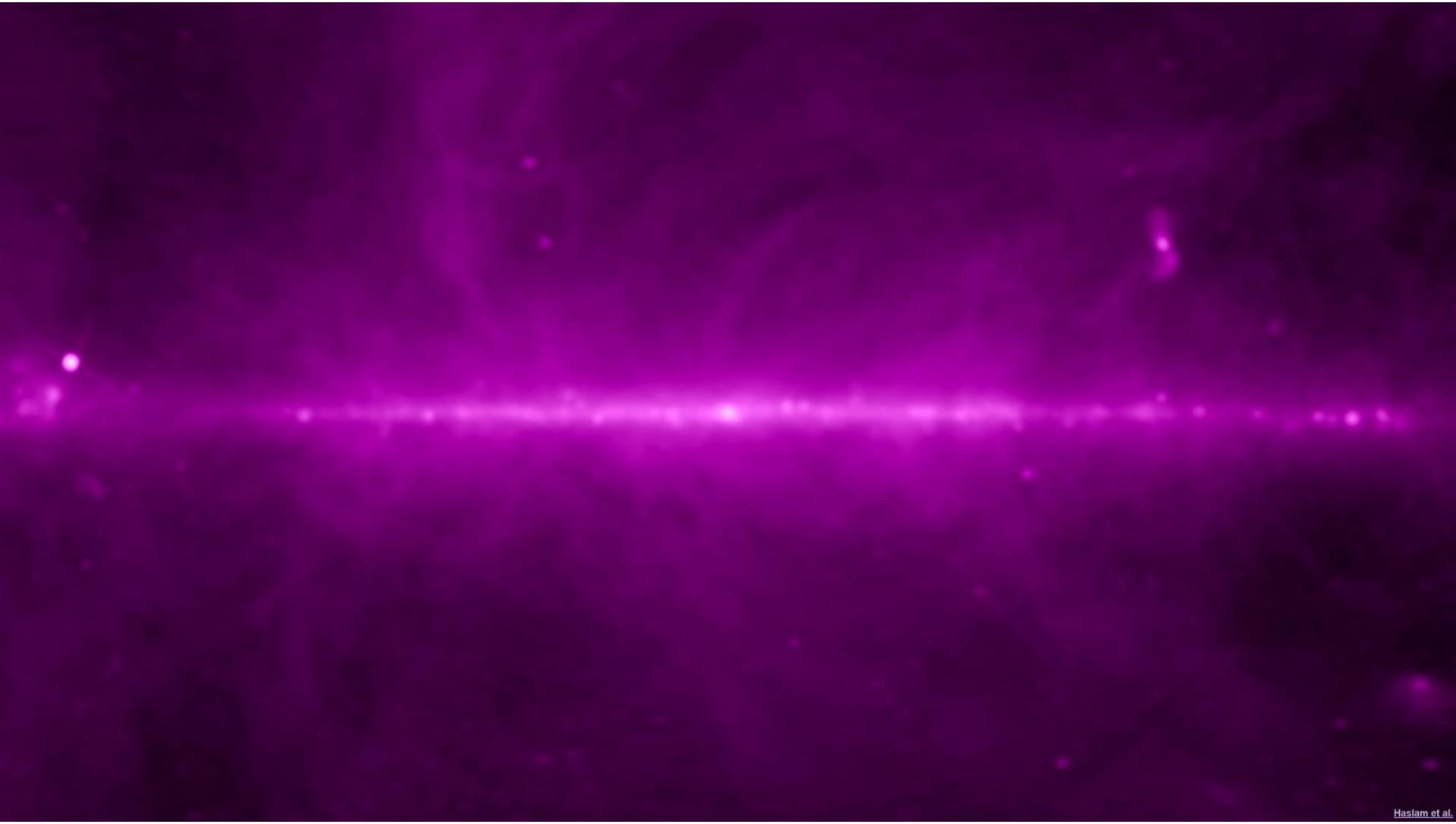
Sourced from stars

Microwave



Cosmic microwave background radiation emitted from the big bang and inflation

Radio



cold intergalactic hydrogen mostly found in the milky way emits radio waves.

Luminescence: Emission of light from any substance

- **Fluorescence**: transition from excited state to ground state is **fast (~ns – ms range)**
- **Phosphorescence**: transition from excited state to ground state is **slow (~s – ks range)**

Perrin-Jablonski energy diagram

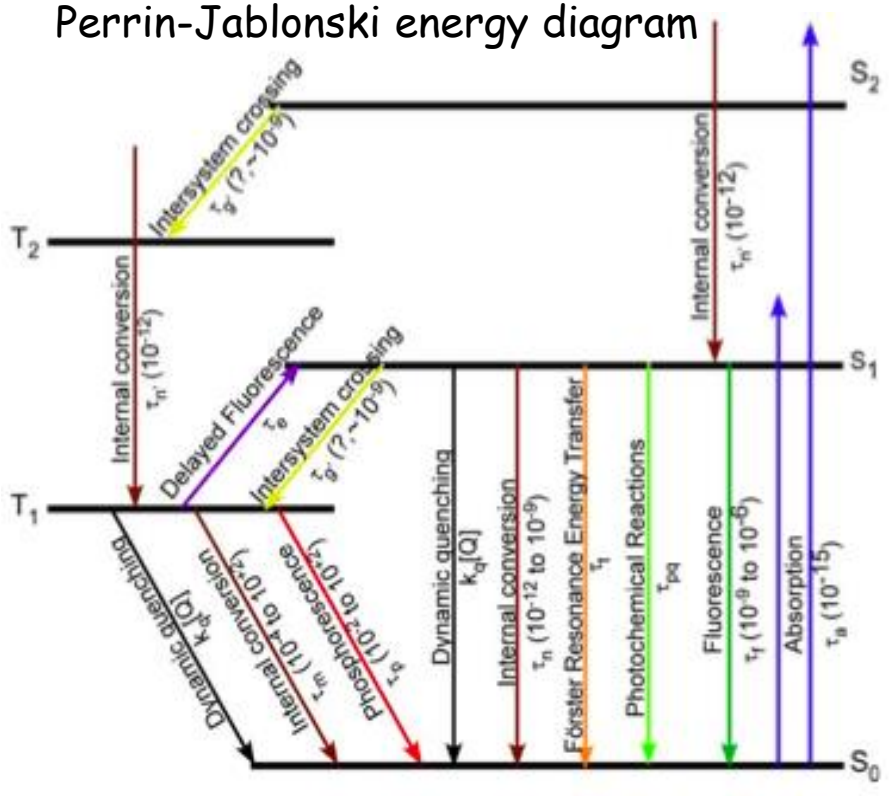


Diagram by Ulai Noomnarm in Photosynth Res. 2009 Aug-Sep;101(2-3):181-94.

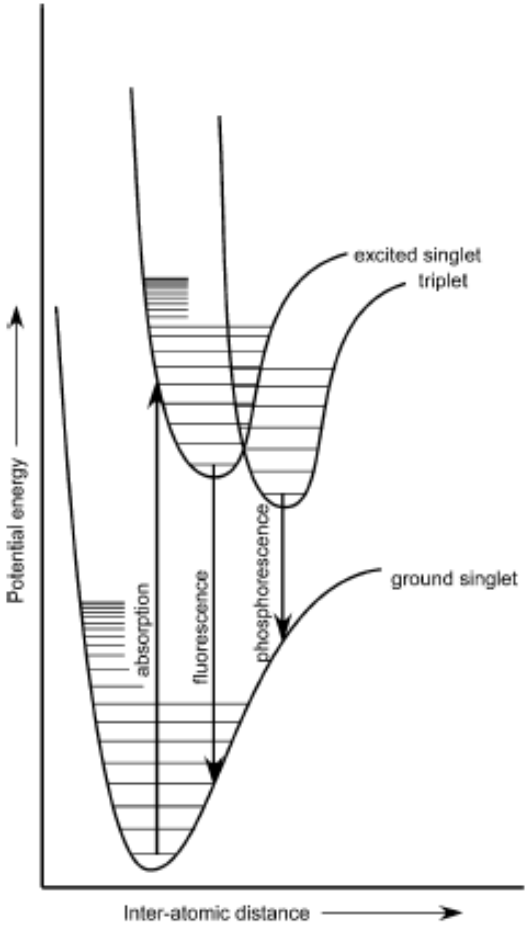
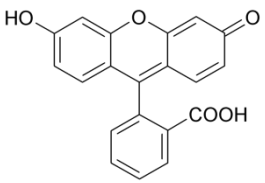


Diagram by Robert Clegg in Photosynth Res. 2009 Aug-Sep;101(2-3):181-94.

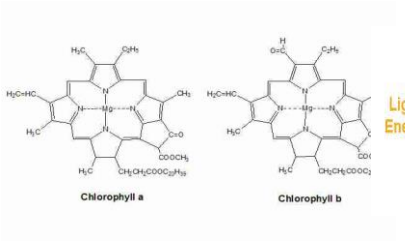
Types of Fluorescent Molecules

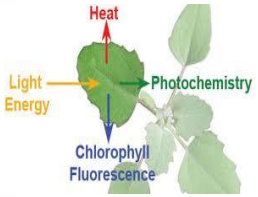
Synthetic Organic:

Fluorescein



Naturally Occuring:

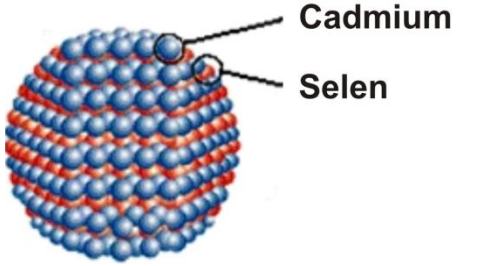


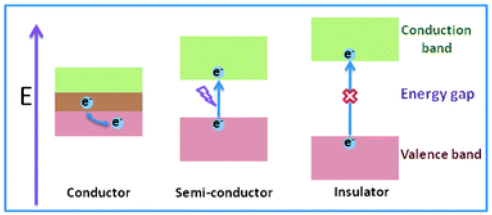


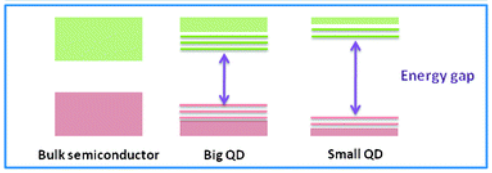
Fluorescent Proteins:

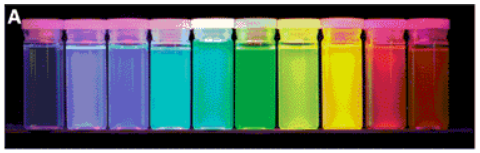
Green Fluorescent Protein

Semiconductor Nanocrystal:









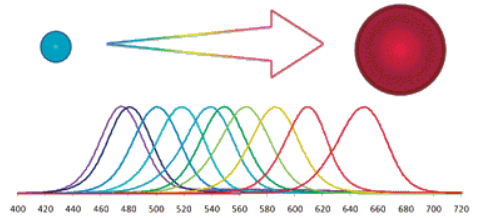
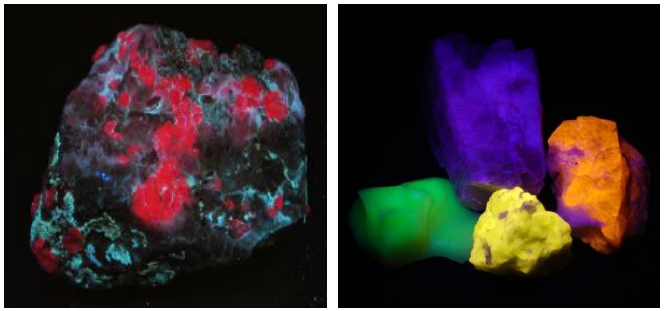


Image from Zrazhevskiy et al. 2010

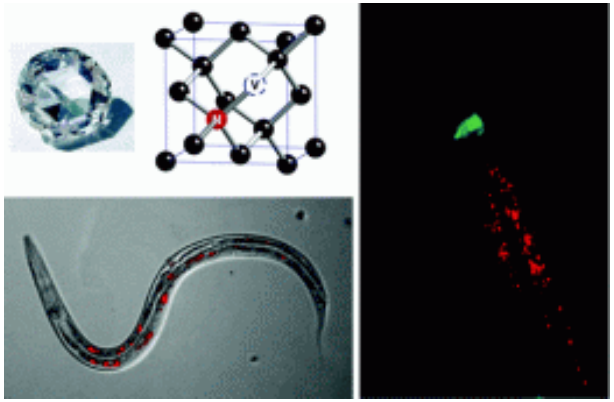
Crystals:



Ruby and assorted minerals

From mineralman.net

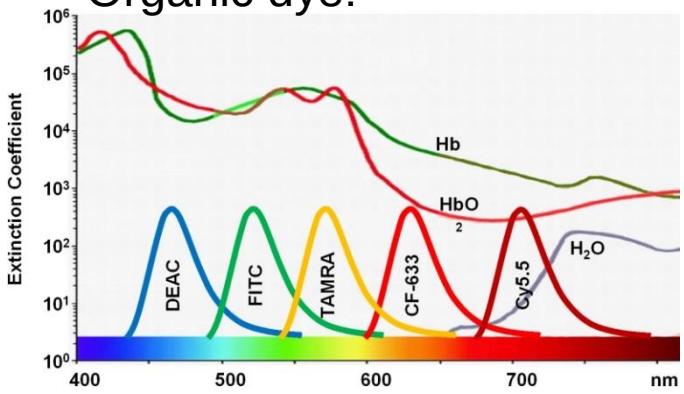
Fluorescent Nanodiamonds



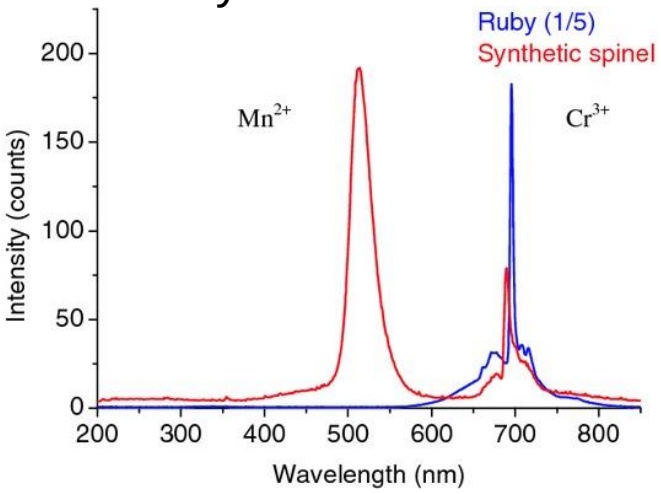
Nano Lett., 2010, 10 (9), pp 3692-3699. DOI: 10.1021/nl1021909

Fluorescence (S_1-S_0)

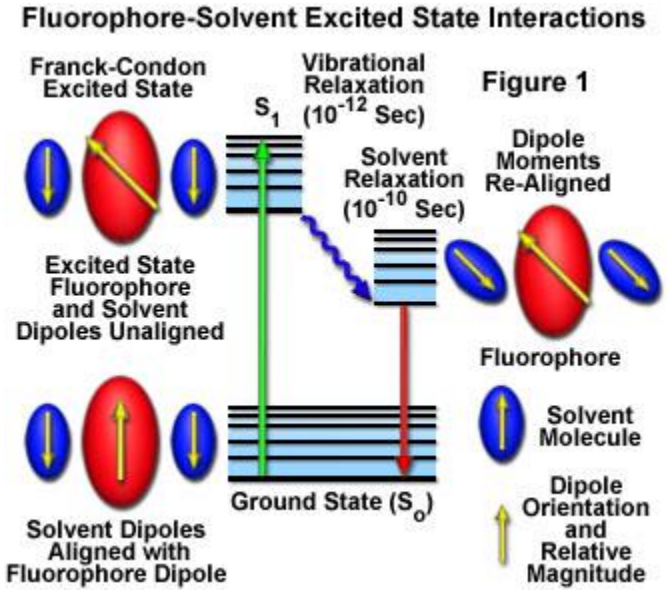
Organic dye:



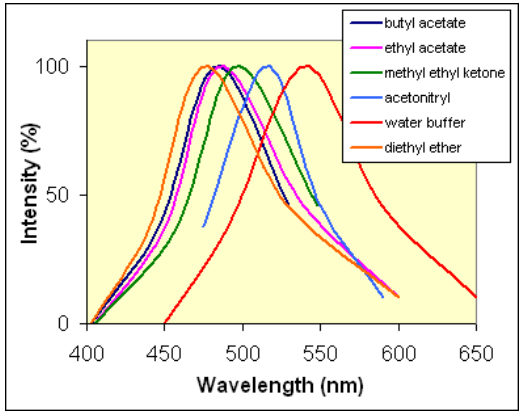
Ruby:



Solvent Effect:



http://micro.magnet.fsu.edu

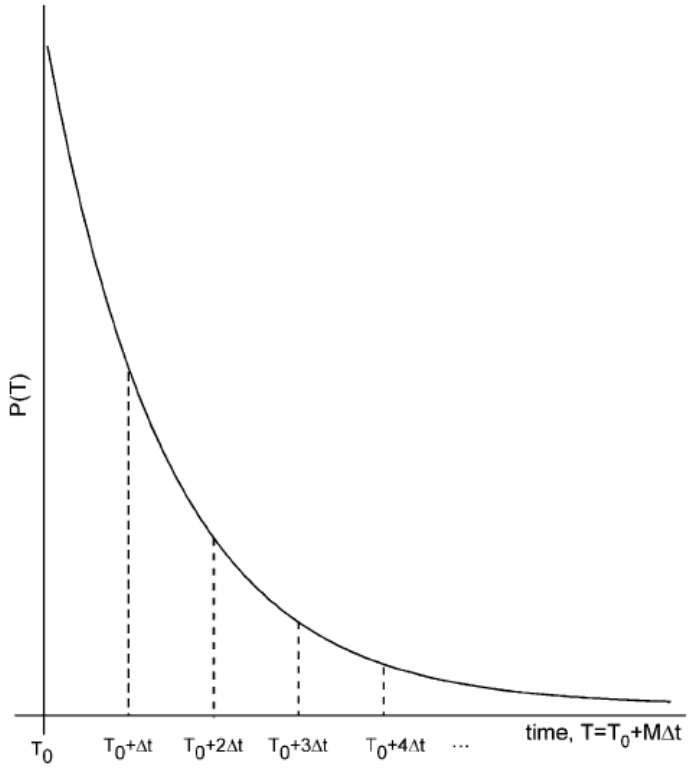


http://www.bio.davidson.edu

Time-Dependent Fluorescence: Fluorescence Lifetime

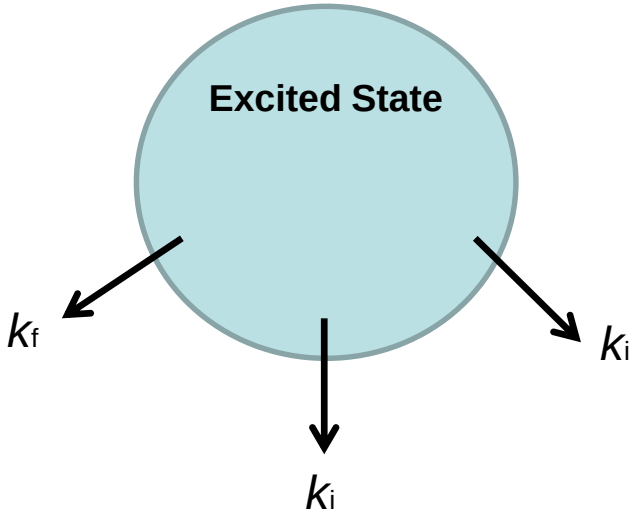
Fluorescence Lifetime: The average amount of time a molecule stays in excited state

Probability of being in the excited state



k_f = rate constant for leaving excited state while emitting a photon

k_i = rate constant for leaving excited state through other means (ie. Dynamic quenching, Energy Transfer, etc)



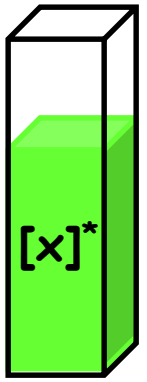
Fluorescence Lifetime: $\tau = \sum_i \frac{1}{k_i}$

Lifetime is sensitive to other decaying pathways present!

Diagram by Robert Clegg in Photosynth Res. 2009 Aug-Sep;101(2-3):181-94.

Measuring the Depletion of the excited state

$$\left[\#x^*\right] = \left[\#x_o^*\right] e^{-(k_F + k_t)t}$$

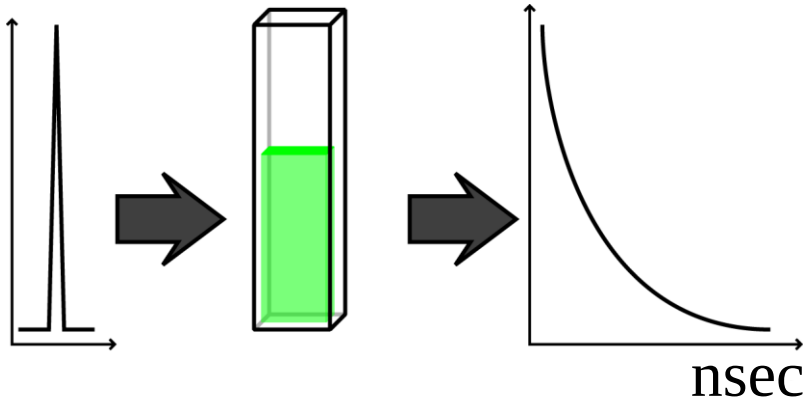


$\left[\#x^*\right](k_F)$ = Intensity that you measure

K_F is rate constant of fluorescence

Intensity measured is proportional to the # of molecules in the excited state!

Measuring Lifetime: Time Domain



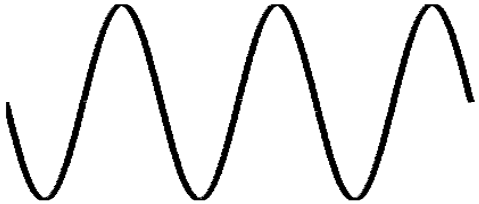
What do you need?

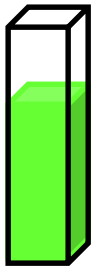
-Collect signal fast enough

-Fitting

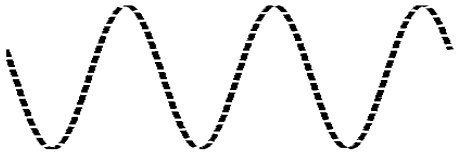
Measuring Lifetime: Frequency Domain

$$E(t) = E_o + E_\omega \cos\left(\omega_E t + \varphi_E\right)$$





$$F(t) = F_o + F_\omega \cos\left(\omega_E t + \varphi_E - \varphi\right)$$



$$\tan\left(\varphi\right) = \omega_E \tau_\varphi$$

$$M = \frac{F_\omega / F_o}{E_\omega / E_o} = \frac{1}{\sqrt{1 + \left(\omega \tau_{Mod}\right)^2}}$$

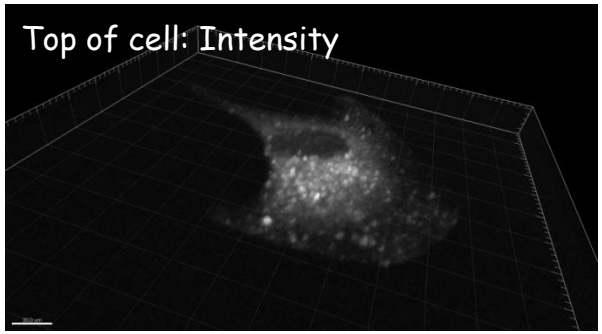
What do you need?

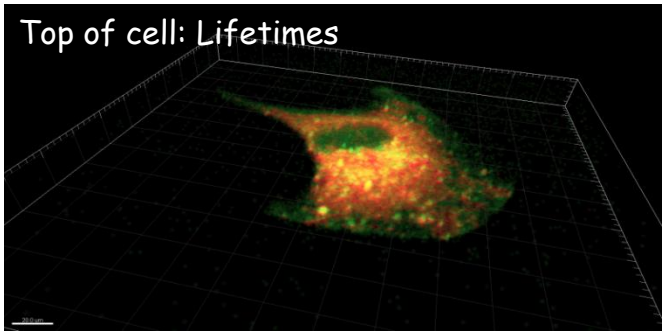
-Intensity modulators

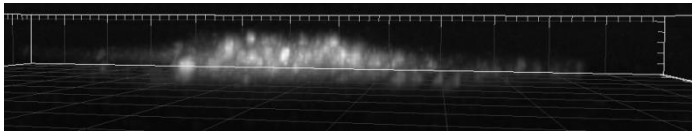
-Synchronization

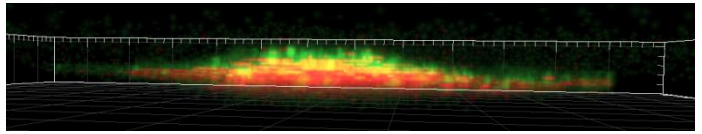
Applications of Fluorescence in Biology

Fluorescence Lifetime Imaging on Live Cells

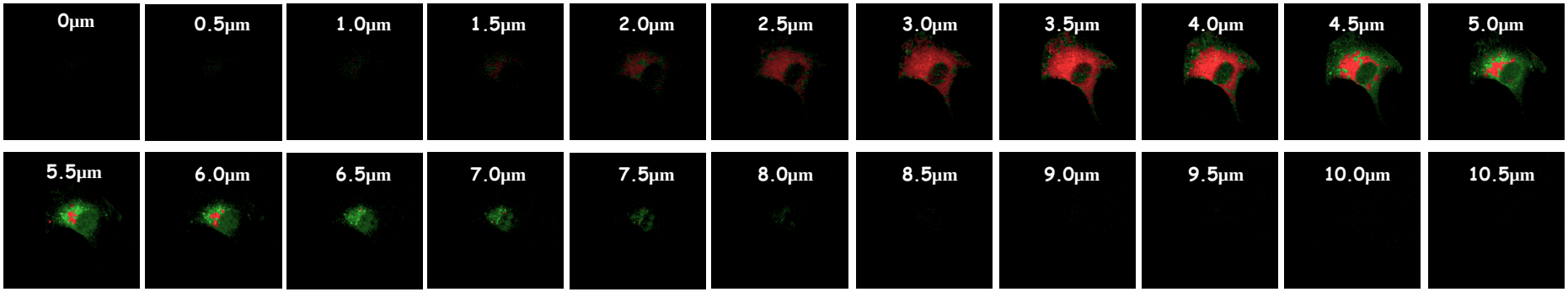


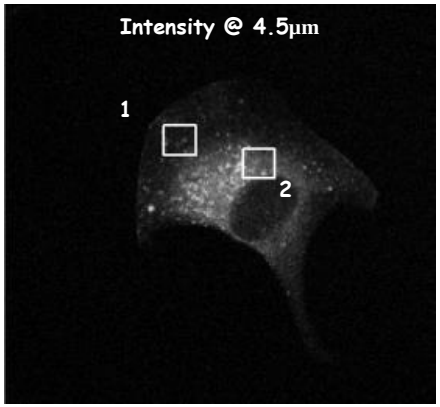


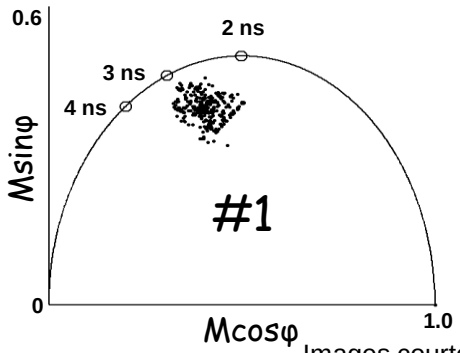


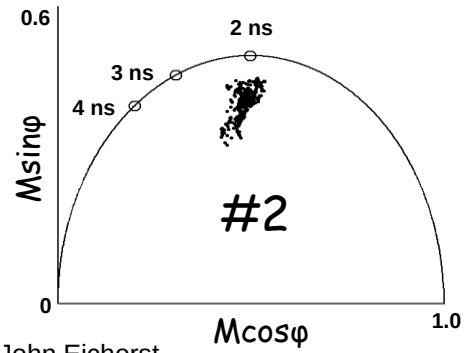


Optical Sections – Rendered by Lifetime



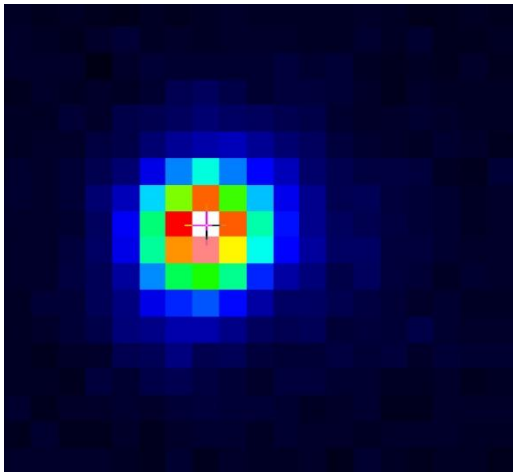


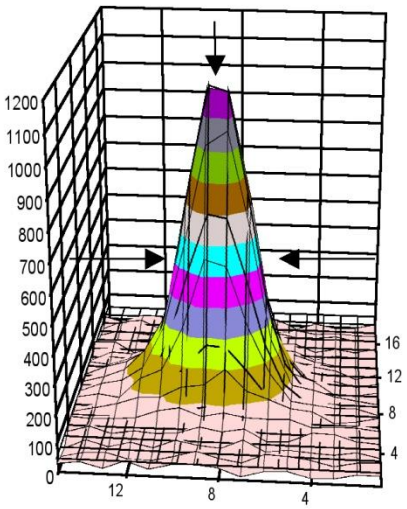


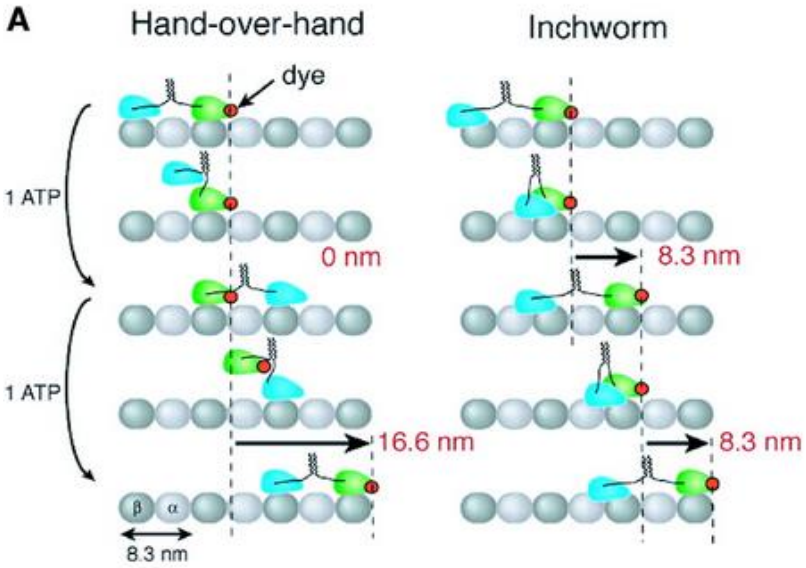


Images courtesy of John Eichorst

Single Molecule Fluorescence Imaging (myosin)







$$\sigma_{\mu_i} = \sqrt{\left(\frac{s_i^2}{N} + \frac{a^2/12}{N} + \frac{8\pi s_i^4 b^2}{a^2 N^2}\right.}$$

Center of the distribution can be determined in ~1.5 nm accuracy if #N is more than 10^4