

Fluorescence and Hole Burning

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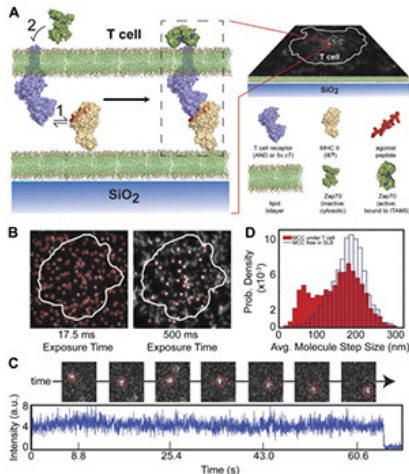
October 2, 2019

Previously on CHM676...

We heard from Shalini Low-Nam about using fluorescence imaging to study cell signal-response at the single-cell, single-molecule level.

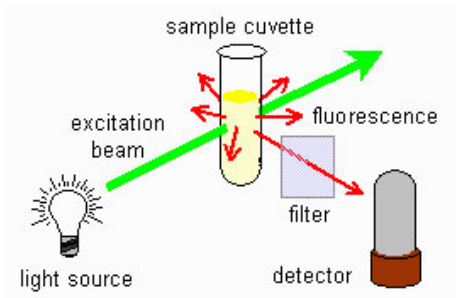
Today: How does fluorescence fit into the response theory framework?

<http://groveslab.cchem.berkeley.edu/science.html>



Fluorescence

- 1 Excite the sample
- 2 Collect radiant light **at 90°**
- 3 Monitor in one of two modes:
 - **Emission:** Spectrum = Light intensity *as a function of* frequency, with a *fixed* excitation wavelength
 - **Fluorescence Excitation:** Spectrum = light intensity at a *fixed* wavelength *as a function of* excitation frequency.



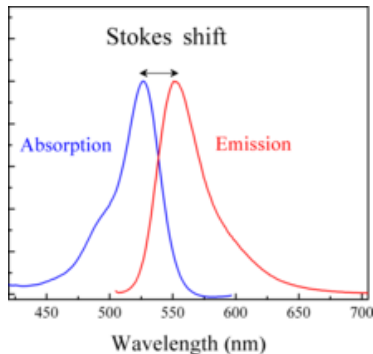
<https://www.chromedia.org/dchro/gfx/ZgbwordJmB.jpeg>

Fluorescence Excitation spectra typically resemble absorption spectra.

Stokes Shift

Experimentally: Absorption and emission peaks are separated by a **Stokes Shift**.

Results from electron/vibration interactions and from

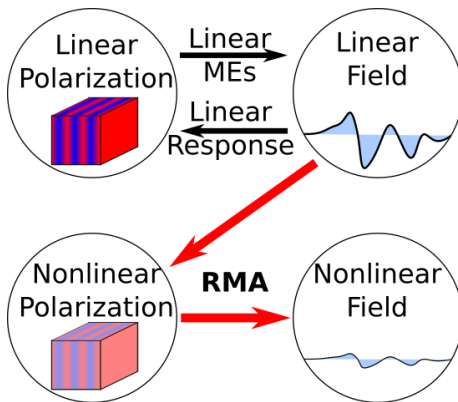


QUANTUM MECHANICS!



Response Theory

Q: So how does fluorescence fit into our response theory framework?

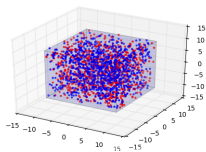


A: It doesn't!

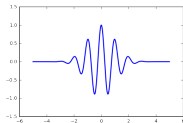
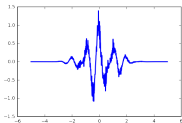
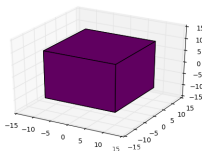
Recall: Response theory works with *macroscopic* fields:

$$\mathbf{E}(\mathbf{x}, t) \equiv \langle \mathbf{e}(\mathbf{x}, t) \rangle_M$$

Microstate



Macrostate



In **incoherent** processes, the average *field* is zero – but the average *intensity* is not!

Fluorescence gives a typical example:

- A *single* dipole emits a *coherent* field with a well-defined phase
- An *ensemble* of dipoles with uncorrelated phases emit an *incoherent field* with a stochastic phase.

Fluorescence: Light after Dephasing

Consider an ensemble of dipoles with the same frequency ω and polarization ϵ but *random phases*:

$$\langle \mathbf{E}(t) \rangle = \frac{1}{2\pi} \epsilon \int_0^{2\pi} \sin(\omega t + \phi) = 0.$$

$$\begin{aligned} \langle I(t) \rangle &= \frac{\epsilon^2}{2\pi} \int_0^{2\pi} \sin^2(\omega t + \phi) \\ &= -\frac{\epsilon^2}{8\pi} \int_0^{2\pi} \left(e^{2i(\omega t + \phi)} - 2 + e^{-2i(\omega t + \phi)} \right) = -\frac{\epsilon^2}{2}. \end{aligned}$$

Although the **average field** is zero, the **average intensity** is not! \Leftarrow **Fluorescence**

Fluorescence corresponds to radiation of light from a completely *dephased* sample – the phase of each microscopic emitter is random!

This process falls **outside** the scope of *our development* of response theory since the average field is zero. (Though it can be related!)

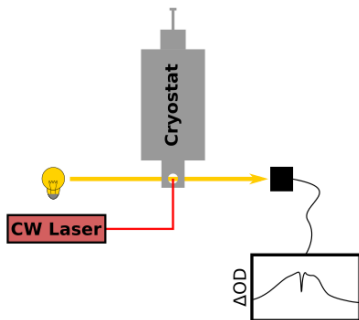
Even though the mean *field* vanishes, the mean *intensity* – and hence the radiant energy – is nonzero.

The **Stokes shift** between absorption and emission spectra results from the quantum population weighting of vibrational energy states.

Hole Burning Spectroscopy

Hole Burning Experiment

- 1 Sample temperature $\rightarrow \sim 5$ K.
- 2 Measure absorption spectrum
- 3 Fry it with a narrow-band laser
- 4 Measure the spectrum again
- 5 Signal = Pre-burn absorption - post-burn absorption



https://www.janis.com/Products/productsoverview/SuperTranContinuousFlowCryostatSystems/ST-100_OpticalCryostat.aspx

Low-Temperature Absorption Spectra

Large low-temperature absorption spectra are determined by two factors:

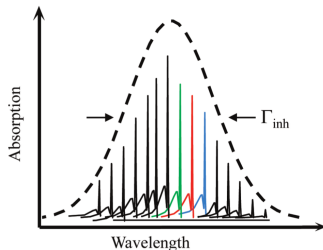
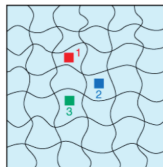
- **Inhomogeneous broadening:**

The distribution of *electronic transition frequencies* (site energies) associated with *different sites* in the material

- **Homogeneous broadening:**

The finite width of the absorption spectrum *at each site*

The bulk absorption spectrum is a **convolution** of the *single-site spectrum* and the *probability density*



Chem. Rev. 2011, 111, 4546-4598

Convolutions

A **convolution** is a mathematical operation that combines two functions:

$$f * g(x) = \int dx' f(x')g(x - x')$$

Key Concept: $f * g$ looks like a *weighted average* of *shifted* copies of $g(x)$, where

- The integral dx' runs over all possible *shifts*
- The function $f(x')$ sets the *weight* at each shift.

The Convolution Theorem

A side note: **Fourier Transforms** are still magical:

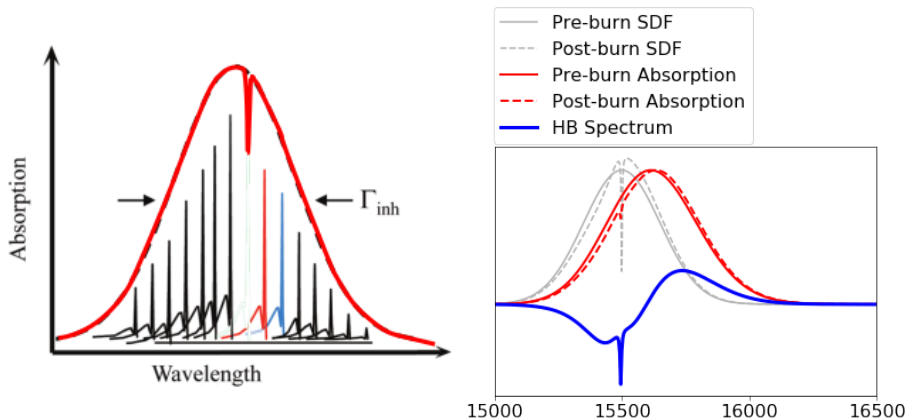
$$f * g(x) = \text{IFT} \left\{ \tilde{f} \cdot \tilde{g} \right\}.$$

Convolutions become products in the Fourier domain!

Examples: <https://github.com/mreppert/CHM676/blob/master/FourierTransforms.ipynb>

Back to Hole Burning

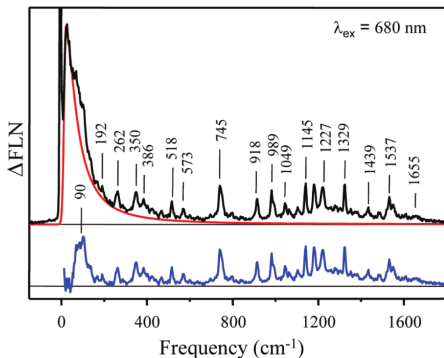
Narrow-band excitation creates a “hole” in the site-energy density function



What does Hole Burning tell you?

- ① Inhomogeneous vs. Homogeneous broadening \Rightarrow Material homogeneity
- ② Line width \Rightarrow Excited state lifetimes
- ③ Satellite holes \Rightarrow Excitonic interactions
- ④ Single-site line shapes \Rightarrow Electronic/vibrational interactions

Hole Burning + Fluorescence $\Rightarrow \Delta \text{FLN}$



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Take-Home Points

Two types of **broadening** in disordered systems:

- **Homogeneous broadening** affects all sites the same \Rightarrow Single-site spectrum
- **Inhomogeneous broadening** affects each pigment differently \Rightarrow Site-energy distribution

Low-temperature spectra are a **convolution** of the single-site spectrum and the site-energy distribution.

The **convolution** $f * g$ is an f -weighted average of all possible *shifted* copies of g .

Fourier transforms convert convolutions to products:

$$\widetilde{f * g} = \tilde{f} \cdot \tilde{g}$$

Take-Home Points

Hole Burning monitors changes to low-temperature absorption due to narrow-band excitation.

The narrow-band laser **selectively excites** resonant pigments and “burns a hole” in the spectrum.

Hole burning can

- Separate homogeneous and inhomogeneous broadening effects
- Provide single-site spectra and site-energy distributions
- Give details on electronic/vibrational interactions
- Uncover excitonic interactions