

April 4, 2005

➤ Today's Office Hour: only until 1:50 pm

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Still More "Why Lasers?"

➤ Temporal Resolution

use *pulsed* lasers (μs - ps)

- temporal discrimination between scatter and fluorescence
- measure fluorescence lifetimes

➤ Spatial Resolution

- focus laser to μm spot size
- efficient measurements with microsamples
- spatial mapping of sample surfaces

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Ultra-Trace Analysis via LEMFS

Determination of Polycyclic Aromatic Hydrocarbons (PAHs)

- **PAH's**: large ϵ , $\Phi \approx 0.2 - 1$, 10-100 ns fluor. lifetime
- **Example** (using N_2 -pumped dye laser, time-resolved):

Detection Limits (PAHs in water):

- ✓ Benzene - 19 ppb (10^{-7} M)
 - ✓ Naphthalene - 1.3 ppt (10^{-11} M)
 - ✓ Anthracene - 8.9 ppt (10^{-11} M)
 - ✓ Pyrene - 0.5 ppt (10^{-12} M)
- Limited by background fluorescence*

- 6 decades LDR

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Emission Versus Absorbance?

- Let's assume a measurement uncertainty of **0.001**

For an emission measurement:

Noise = 0.001

Blank = 0.000

Signal at detection limit = $3N = \underline{0.003}$

Is this signal detectable via absorbance measurement?

For an absorbance measurement:

$s_T = 0.001$

Blank: Transmittance = 1.000

Comparable Signal = $1.000 - 0.003 = \underline{0.997}$

Is this above
the detection
limit?

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Is it Detectable?

Signal = Absorbance (= 0.0013)

Noise = s_A

$$\begin{aligned} S/N &= A/s_A = \frac{|s_T \text{Log } T|}{0.434 s_T} \\ &= \frac{|(0.001)\text{Log}(0.997)|}{(0.434)(0.001)} \\ &= (1.3 \times 10^{-6})/(4.34 \times 10^{-4}) \\ &= \underline{\underline{0.003}} (\ll 3) \end{aligned}$$

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Qualitative Analysis

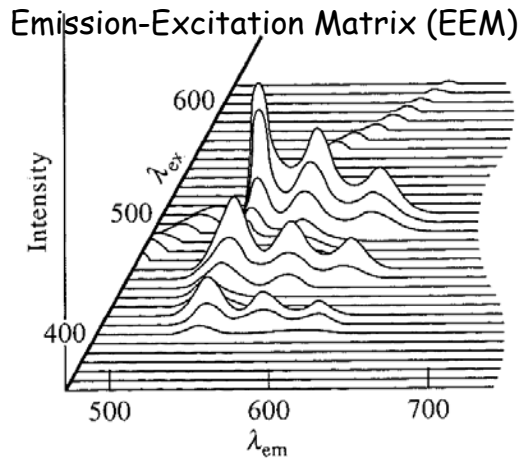
- As in UV/Vis absorption, ***broad emission bands*** provide limited selectivity:
 - multicomponent analysis requires separation
 - excellent detection method for HPLC of compounds that fluoresce (or are tagged with fluorescent labels)

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More Qual Analysis

➤ *What about extra dimension of excitation?*

- *emission spectrum changes with changing excitation wavelength*

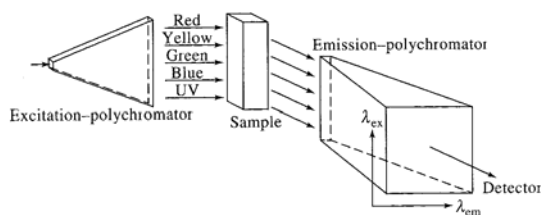


Measuring an EEM

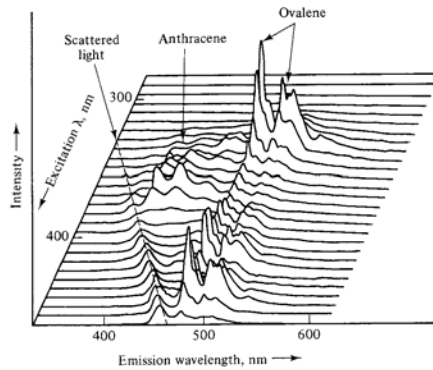
The Hard Way

- Acquire emission spectra as λ_{ex} is sequentially changed

Or: can be done simultaneously using a **Video Fluorometer**



Multi-component EEM



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Summary

- Not universally applicable
- Better detection limits than UV/Vis absorbance
 - nature of emission versus absorbance measurement
 - signal dependence on source intensity
- Limited qualitative analysis capability

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