

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 ( $\mu\text{s}$  - ps)

- temporal discrimination between scatter and fluorescence
- measure fluorescence lifetimes

> **Spatial Resolution**

- focus laser to  $\mu\text{m}$  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  $\epsilon$ ,  $\Phi \approx 0.2 - 1$ , 10-100 ns fluor. lifetime
- **Example** (using  $\text{N}_2$ -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** =  $s_A$

$$S/N = A/s_A = \frac{|s_T \cdot \text{Log } T|}{0.434 s_T}$$

$$= \frac{|(0.001) \text{Log } (0.997)|}{(0.434)(0.001)}$$

$$= (1.3 \times 10^{-6}) / (4.34 \times 10^{-4})$$

$$= \underline{0.003} (\ll 3)$$

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Qualitative Analysis

> As in UV/Vis absorption, **broad emission bands** provide limited selectivity:

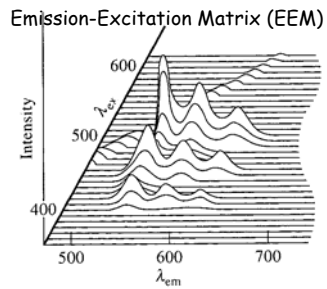
- multicomponent analysis requires separation
- excellent detection method for HPLC of compounds that fluoresce (or are tagged with fluorescent labels)

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## More Qual Analysis

### ➤ What about extra dimension of excitation?

- emission spectrum changes with changing excitation wavelength

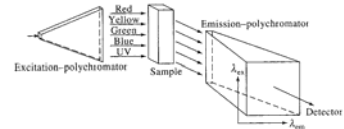


## Measuring an EEM

### The Hard Way

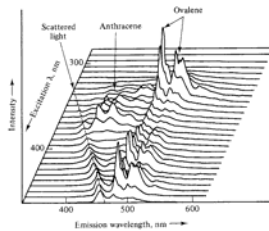
- Acquire emission spectra as  $\lambda_{ex}$  is sequentially changed

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



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## 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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