

April 1, 2005

> April Fool's Day Cancelled Due to Leap Year ☹

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Effect of Concentration

$$I_f = \Phi(I_0 - I)$$

Fluorescence Intensity

Fluorescence Quantum Efficiency

Incident Radiation Intensity

Transmitted Radiation Intensity

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Substitute and Simplify

Beer's Law: $I = I_0 e^{-\epsilon bc}$

Gives: $I_f = \Phi I_0 (1 - e^{-\epsilon bc})$

Expand:

$$I_f = \Phi I_0 \epsilon bc [1 - (\epsilon bc/2!) + ((\epsilon bc)^2/3!) \dots]$$

If ϵbc is small (< 0.05), then:

$$I_f = \Phi I_0 \epsilon bc$$

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Analytical Implications

$$I_f = \Phi I_0 \epsilon bc$$

> At specific λ_{exc} with constant I_0 :

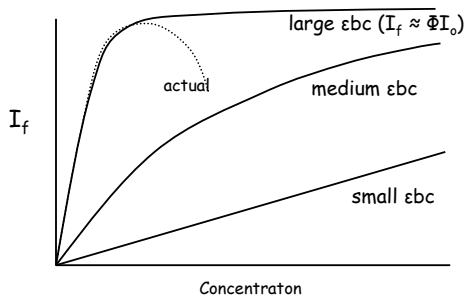
$$I_f = Kc$$

> $I_f \propto I_0$

• So, signal will increase with increased source intensity

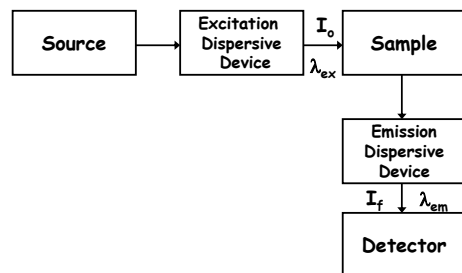
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Quantitative Considerations



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Instrumentation



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Sources

Continuum

- **Xe Arc lamp**
 - Intense UV/Vis source

Line

- **Hg Vapor Lamp**
- **Laser**
 - N₂- or Nd:YAG-pumped tunable dye laser
 - Ar⁺ or Kr⁺ laser

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Dispersive Devices

➤ Excitation

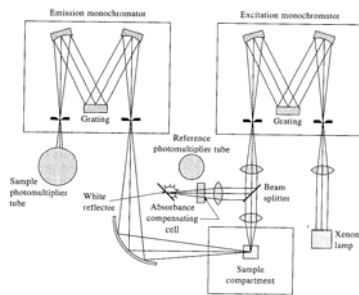
- Only needed for *continuum sources*
- modest resolution, high light throughput

• Emission

- modest resolution
- high stray light rejection (*double-monochromator*)

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Typical Spectrofluorometer



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Applications

➤ Quantitative Analysis

- Need molecule with high fluorescence quantum efficiency *OR* one that can react quantitatively to form a highly fluorescent compound
- Linear relationship with concentration only at low concentrations
- Fluorescence signal directly proportional to *source intensity*:

Laser sources should give best results

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Laser-Excited Fluorescence

Why use Laser Sources?

➤ Saturation Effects

- **I_f indep of I_o**
 - variation of source intensity not observed in signal
 - decreased noise
- **I_f is maximized**
 - increased signal
 - best detection limits (ppt and lower)
- **Extends LDR**
 - by lowering detection limits

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