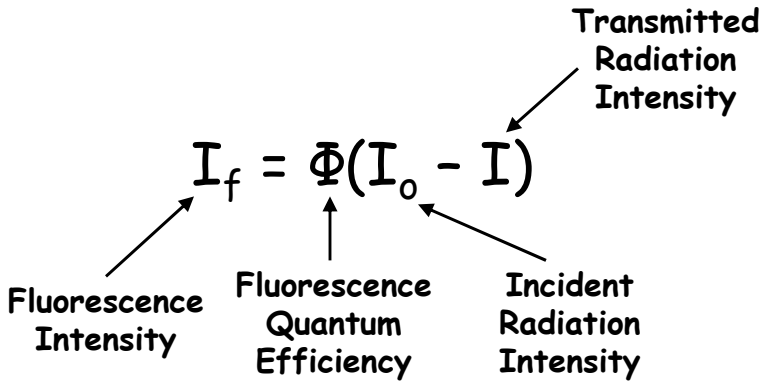
April 1, 2005

- **April Fool's Day Cancelled Due to Leap Year ☹**

Effect of Concentration



Substitute and Simplify

Beer's Law: $I=I_o e^{-\varepsilon bc}$

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

Expand:

$$I_f=\Phi I_o \varepsilon bc[1-(\varepsilon bc/2!)+((\varepsilon bc)^2/3!) \ldots]$$

If εbc is small (<0.05), then:

$$\boxed{I_f=\Phi I_o \varepsilon bc}$$

Analytical Implications

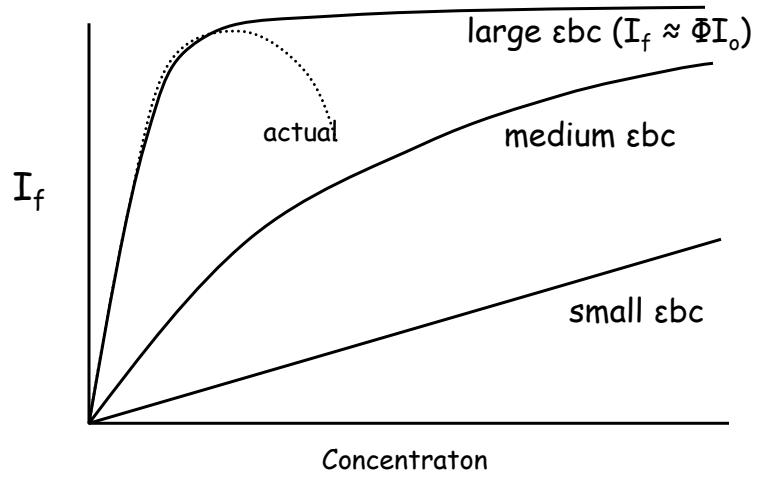
$$I_f=\Phi I_o \varepsilon bc$$

- **At specific λ_{exc} with constant I_o:**

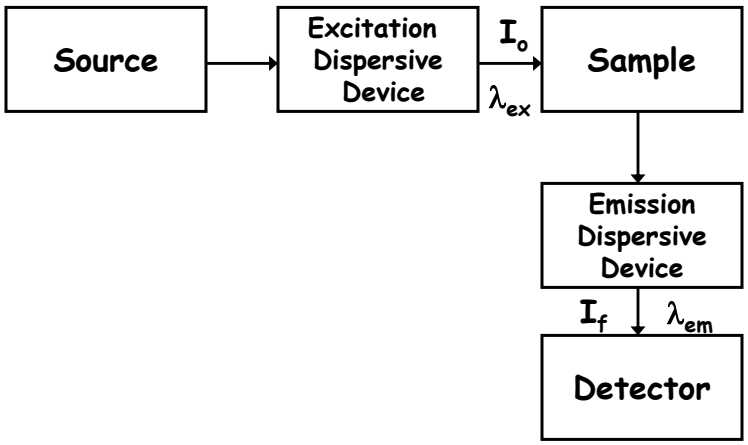
$$I_f=Kc$$

- **$I_f \propto I_o$**
  - **So, signal will increase with increased source intensity**

Quantitative Considerations



Instrumentation



Sources

Continuum

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

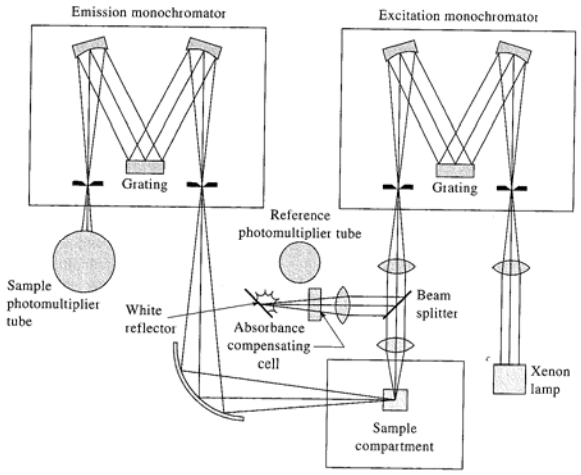
Line

- ***Hg Vapor Lamp***
- ***Laser***
  - N_2- or Nd:YAG-pumped tunable dye laser
  - Ar+ or Kr+ laser

Dispersive Devices

- **Excitation**
  - Only needed for *continuum sources*
  - modest resolution, high light throughput
- **Emission**
  - modest resolution
  - high stray light rejection *(double-monochromator)*

Typical Spectrofluorometer



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

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