

Chemistry 4631

Instrumental Analysis

Lecture 14



Molecular Luminescence Spectrometry

Emission and Excitation Spectra

- **Excitation spectrum**
 - absorbance spectrum
- **Fluorescence and Phosphorescence**
 - excitation at fixed λ while recording emission intensity as a function of λ

Molecular Luminescence Spectrometry

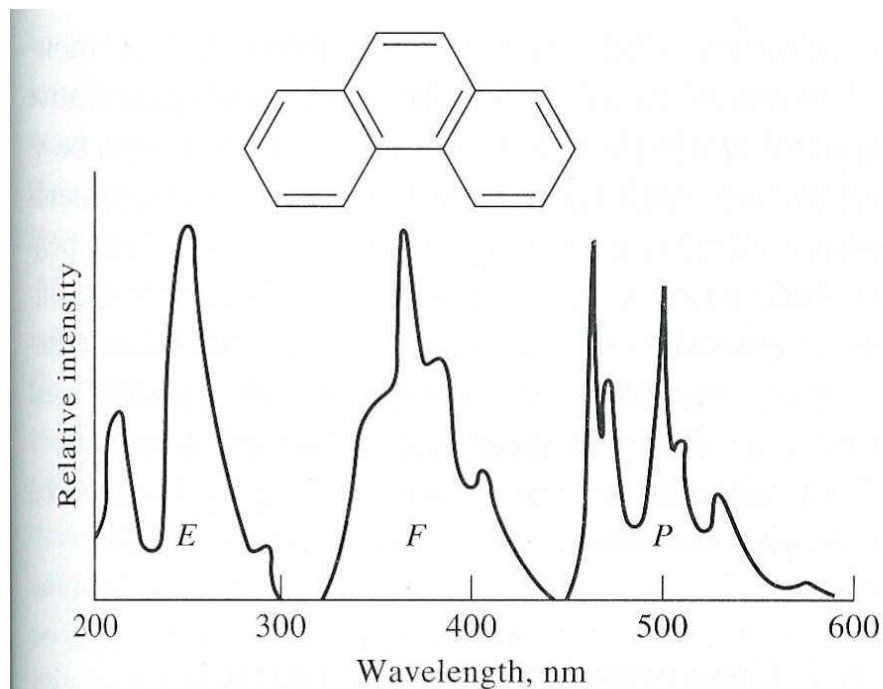


Figure 15-3 Spectra for phenanthrene: *E*, excitation; *F*, fluorescence; *P*, phosphorescence. (From W. R. Seitz, in *Treatise on Analytical Chemistry*, 2nd ed., P. J. Elving, E. J. Meehan, and I. M. Kolthoff, Eds., Part I, Vol. 7, p. 169. New York: Wiley, 1981. Reprinted by permission of John Wiley & Sons, Inc.)

Molecular Luminescence Spectrometry

Instrument

Components are very similar to those for absorbance.

Fluorescence instruments incorporate double-beam optics to compensate for fluctuations in radiant power.

Molecular Luminescence Spectrometry

Instrument

Fluorescence is emitted in all directions but best observed at 90° .
The right-angle geometry minimizes contributions from scattering and the intense source radiation.

Molecular Luminescence Spectrometry

Instrumentation

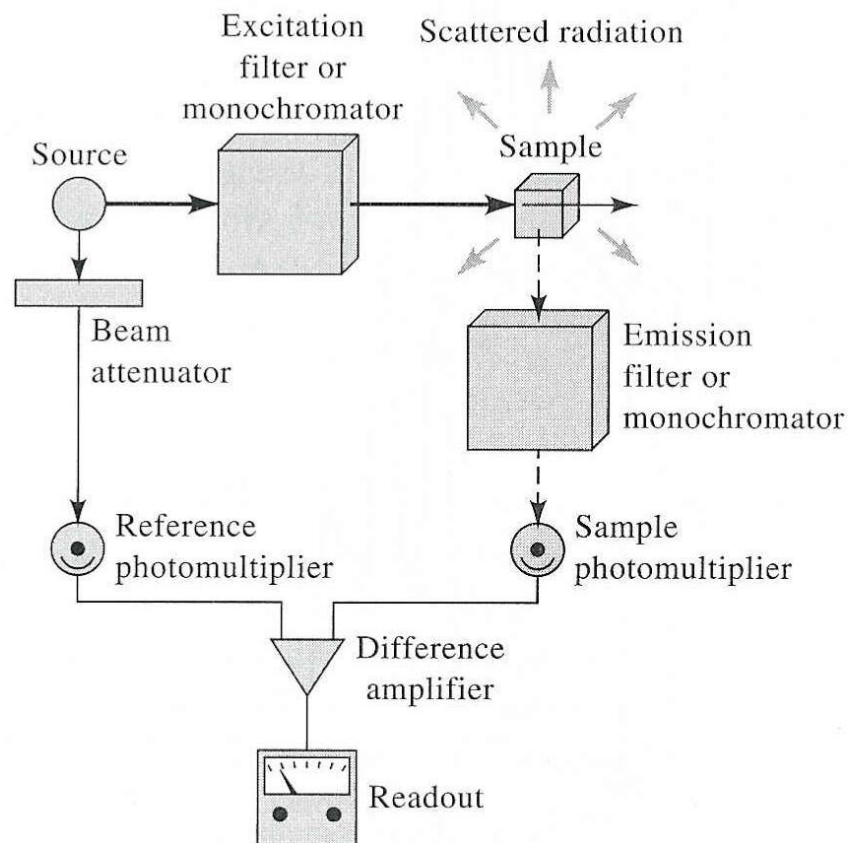


Figure 15-4 Components of a fluorometer or a spectrofluorometer.

Molecular Luminescence Spectrometry

Instrument

Sources

Need to be more intense than for absorbance - since the magnitude of the output signal is directly proportional to the source radiant power P_0 .

Molecular Luminescence Spectrometry

Instrument

Sources

- **Low pressure Hg vapor lamps with a fused silica window.**

Has excitation lines at 254, 302, 313, 546, 578, 691, and 773 nm.

Lines are isolated with filters.

Molecular Luminescence Spectrometry

Instrument

Sources

- **High pressure xenon arc lamps**
75 to 450 W gives continuum from 300 - 1300 nm approximates that of a blackbody, weaker radiation produced down to 200 nm. Can pulse at constant frequency to get higher peak intensities.

Molecular Luminescence Spectrometry

Instrument

Filter and monochromators same as for absorbance, except must have 2 of them.

First monochromator called the excitation monochromator.

Second monochromator emission monochromator - separates the scattered light from the wanted light.

Molecular Luminescence Spectrometry

Instrument

Cells are glass or silica which is clear on all four sides entrance and exit slits are at a 90° angle.

Cell compartments lined with baffles
Avoid fingerprints - skin oils
fluorescence.

Molecular Luminescence Spectrometry

Instrument

Transducers

The fluorescence signal tends to be low intensity so need a sensitive detector, i.e. PMTs, diode array, charge transfer.

PMT's – most common

Molecular Luminescence Spectrometry

Instrument designs

Because of day-to-day variations in the instrument, it must be calibrated daily.

A standard solution, such as quinine sulfate (10^{-5} M) is usually used.

Excited at 350 nm and emits at 450 nm.

Molecular Luminescence Spectrometry

Instrument designs

Fluorometers

If the instrument uses only filters it is called a fluorometer.

Filter photometers very simple, inexpensive, compact, rugged, easy to use can do quantitative fluorescence analysis, cost \$1000 - 5000.

Molecular Luminescence Spectrometry

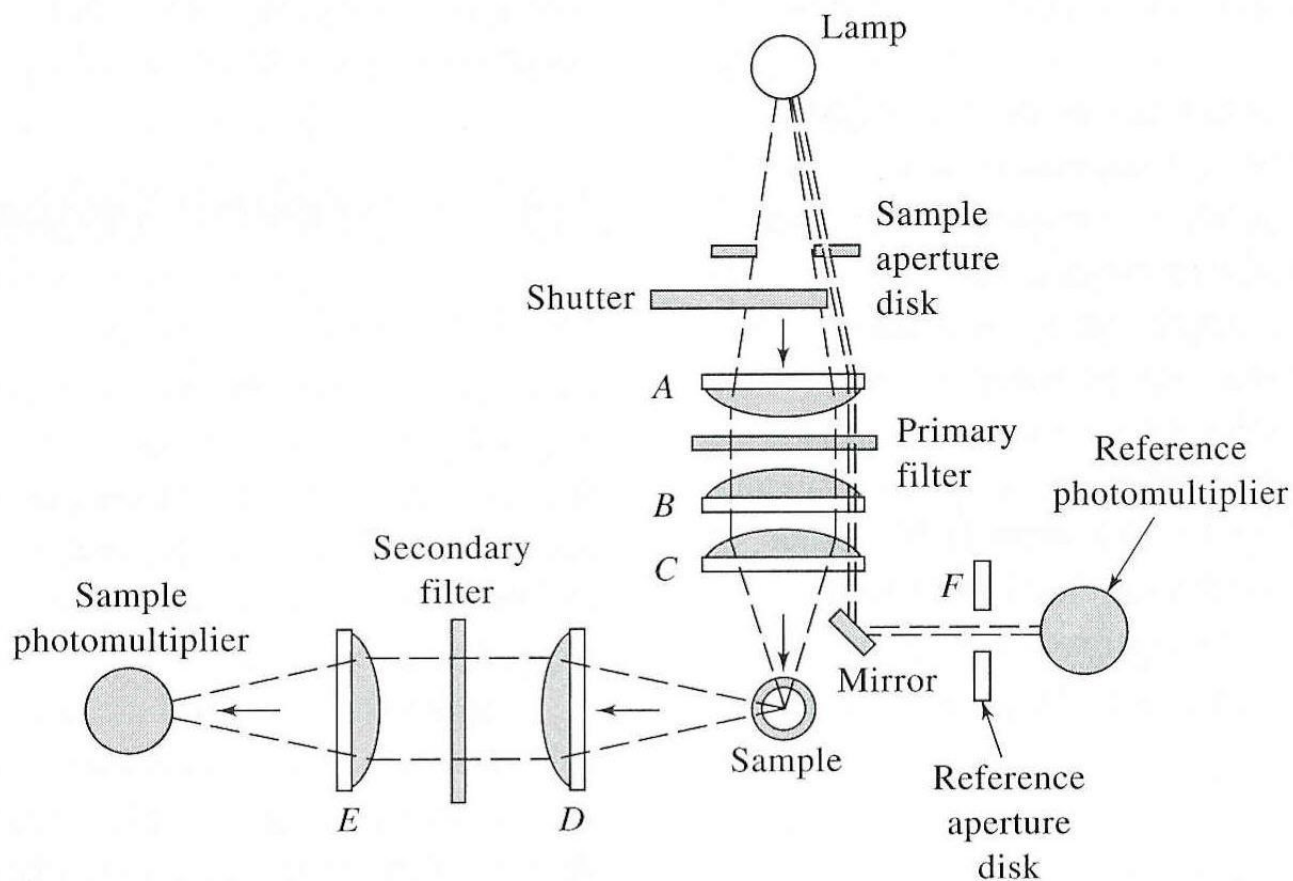


Figure 15-6 A typical fluorometer. (Courtesy of Farrand Optical Co., Inc.)

Molecular Luminescence Spectrometry

Instrument designs

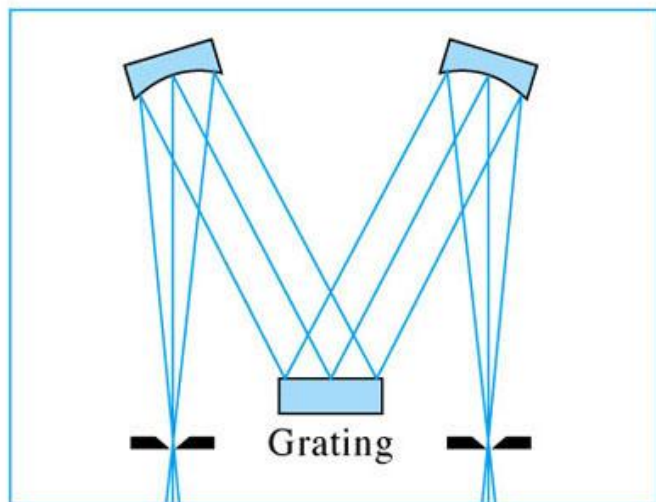
Spectrofluorometers

Produces both excitation and emission spectra.

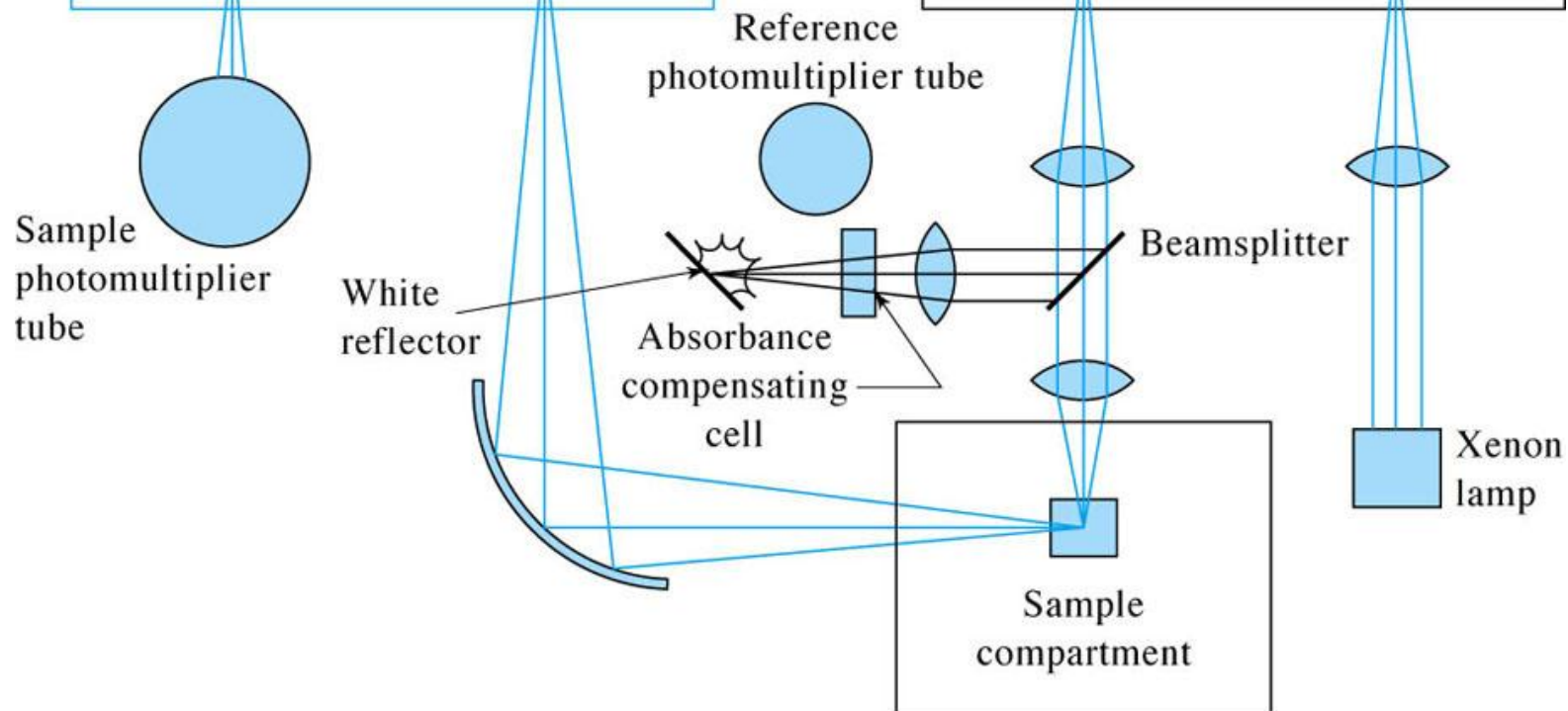
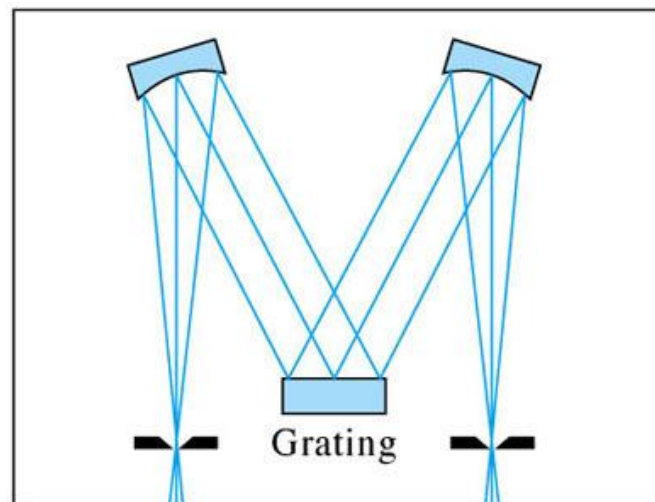
Usually has 2 grating monochromators, radiation from the 1st monochromator is split, part goes to reference PMT and part goes to sample.

The fluorescence coming from the sample goes to the 2nd monochromator and detected by 2nd PMT.

Emission monochromator



Excitation monochromator



Molecular Luminescence Spectrometry

Another type of Fluorescence instrument is a Fluorescence Microscope

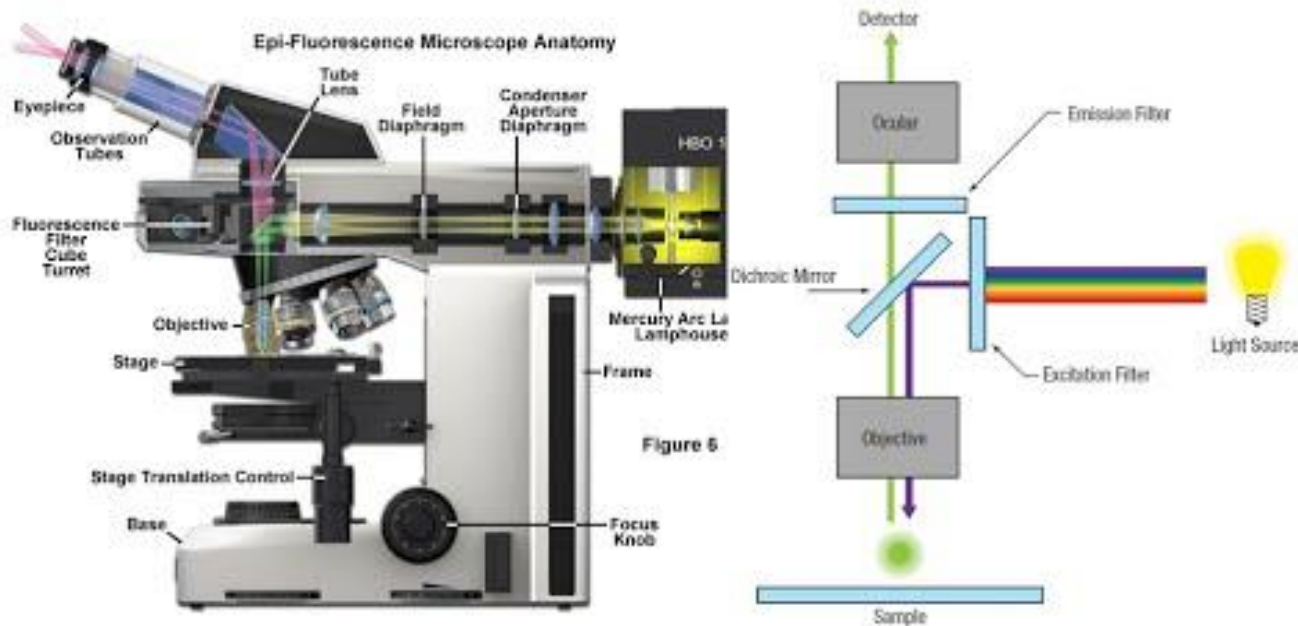
For these microscopes – the source can be LEDs

LED Light Sources: A Major Advance in Fluorescence Microscopy

Benefits of LEDs, include compact size, low power consumption, minimal heat output, high emission stability and extremely long life span.

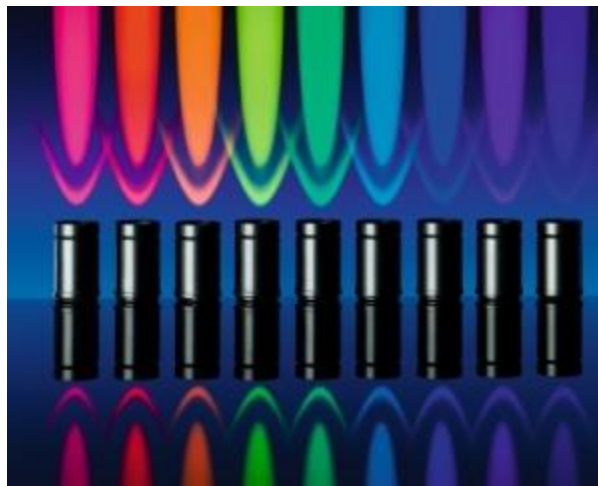
Molecular Luminescence Spectrometry

Fluorescence Microscopy



Molecular Luminescence Spectrometry

Benefits of LEDs, include compact size, low power consumption, minimal heat output, high emission stability and extremely long life span.

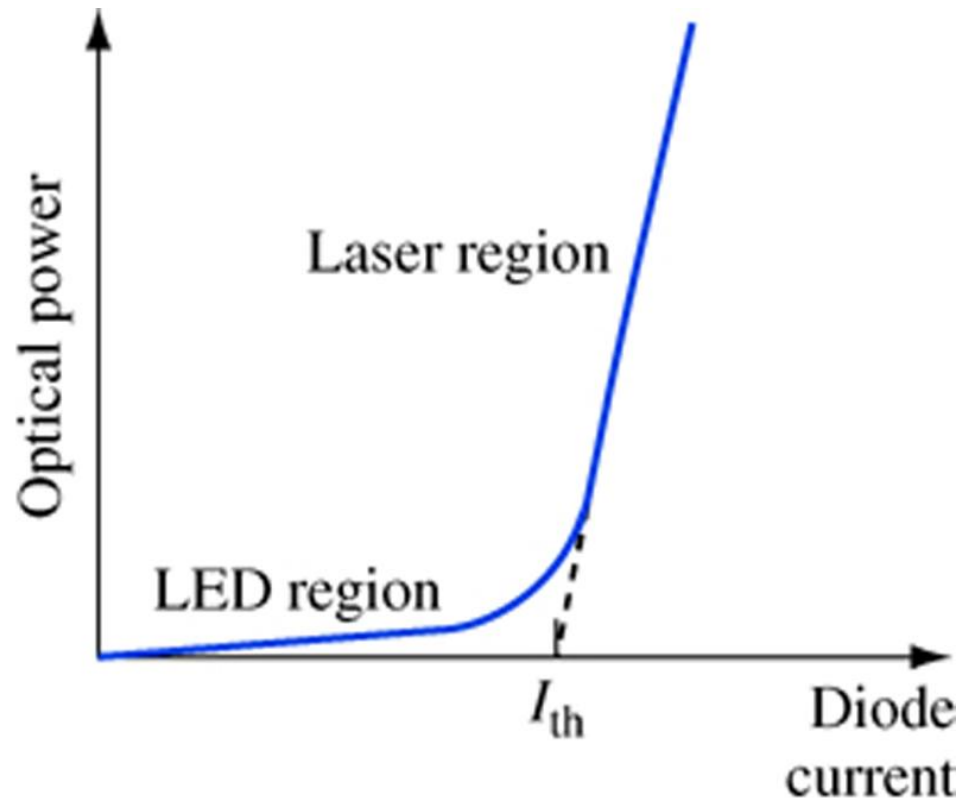


Ten different LED modules which can be easily exchanged are currently available from UV to dark red.

Molecular Luminescence Spectrometry

Sources

Semiconductor LED vs LASER



Molecular Luminescence Spectrometry

Advantages of Light Emitting Diodes (LEDs)

Longevity:

The light emitting element in a diode is a small conductor chip rather than a filament which greatly extends the diode's life in comparison to an incandescent bulb (10 000 hours life time compared to ~1000 hours for incandescence light bulb)

Efficiency: (Presently High 25--30 Lumens/Watt)

Diodes emit almost no heat and run at very low amperes

Lower energy consumption

Smaller size

Red 10x Better than (filtered) incandescent

White 2x better than incandescent

Potential efficiency 150+ Lumens/Watt (2x better than fluorescent)

Greater Light Intensity:

Since each diode emits its own light

Molecular Luminescence Spectrometry

Advantages of Light Emitting Diodes (LEDs)

Cost:

Coming down

Robustness:

Solid state component, not as fragile as incandescence light bulb

No catastrophic failures

Environmentally friendly:

Minimal disposal required

No mercury

Molecular Luminescence Spectrometry

General Structure

A simple LED is a pn junction on a suitable substrate.

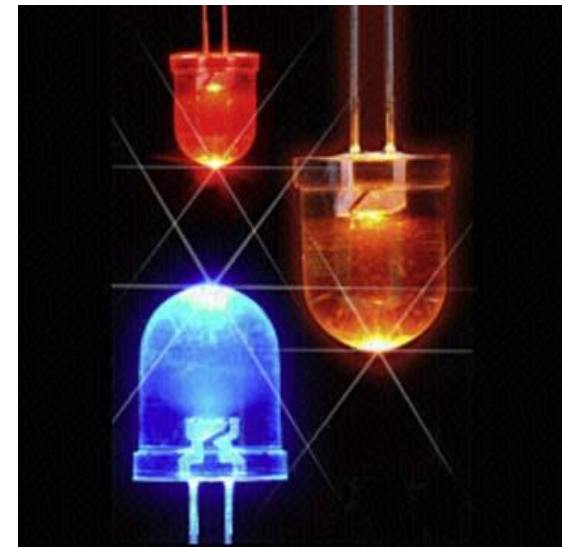
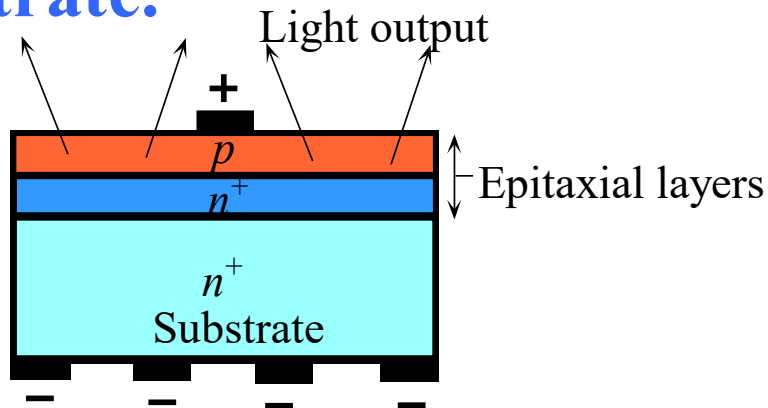


Fig. 6.44: A schematic illustration of one possible LED device structure. First n^+ is epitaxially grown on a substrate. A thin p layer is then epitaxially grown on the first layer.

Molecular Luminescence Spectrometry

- LEDs are semiconductor p-n junctions that under forward bias conditions can emit radiation by electroluminescence in the UV, visible or infrared regions of the electromagnetic spectrum.
- When pn junction is forward biased, large number of carriers are injected across the junctions. These carriers recombine and emit light.
- The quanta of light energy released is approximately proportional to the band gap of the semiconductor.
- The emitted photons must escape without being reabsorbed, so the p-side has to be narrow.

Molecular Luminescence Spectrometry

A typical LED needs a p-n junction

There are a lot of electrons and holes at the junction due to excitations

Electrons from n need to be injected to p to promote recombination

Junction is biased to produce even more e-h and to inject electrons from n to p for recombination to happen

Recombination produces light!!



Molecular Luminescence Spectrometry

Efficient LED

- ❑ Need a **p-n junction** (preferably the same semiconductor material only different dopants).
- ❑ **Recombination must occur** → Radiative transmission to give out the correct color.
- ❑ Color of LED → $hc/\lambda = E_c - E_v = E_g$
→ so choose material with the right E_g
- ❑ **Direct band gap** semiconductors to allow efficient recombination.
- ❑ All photons created must be able to leave the semiconductor.
- ❑ Little or **no reabsorption** of photons.

Molecular Luminescence Spectrometry

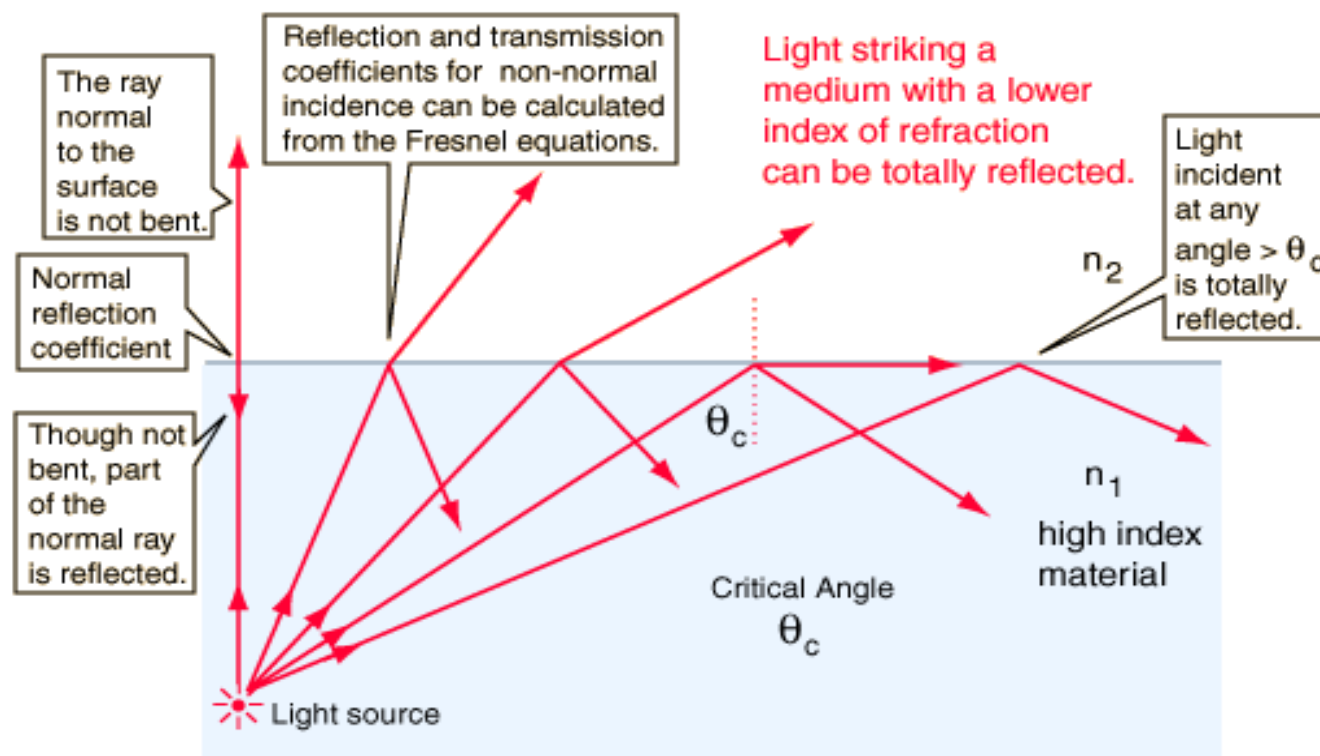
Sources

The quantum efficiency

- Internal quantum efficiency of some LEDs approaches 100% but the *external efficiencies* are much lower. This is due to reabsorption and TIR (Total internal reflectance).

Molecular Luminescence Spectrometry

Total Internal Reflection



Molecular Luminescence Spectrometry

LEDs

Important parameter -quantum efficiency (η): a number of photons generated per electron-hole pairs

Factors which determine quantum efficiency

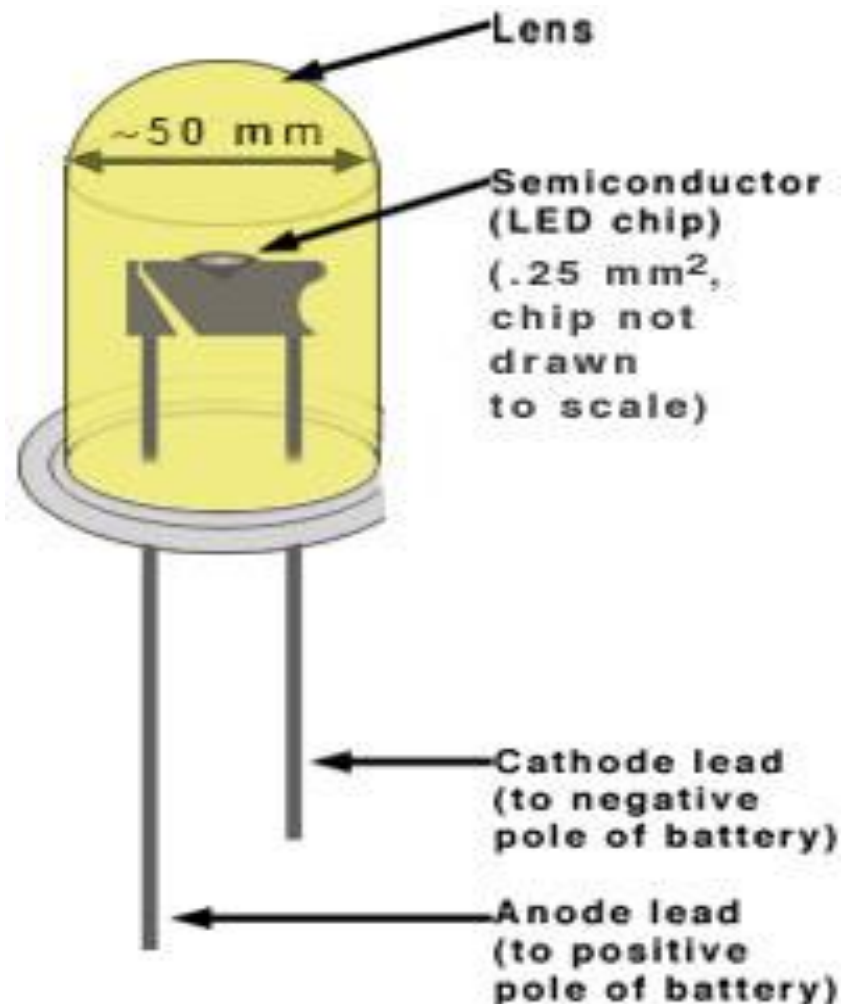
Efficiency of radiative recombination

Internal losses (due to recombination in the depletion region)

Molecular Luminescence Spectrometry

Sources

- Light-emitting diodes (LEDs)



Molecular Luminescence Spectrometry

The nitrides and blue LED

- **Difficulties:**
 - to find suitable substrates for the nitrides
 - to get p-type nitrides
- But with constant R&D work, better materials are produced
- GaN, InGaN, AlGaN → high efficiency LEDs emitting blue/green part of the spectrum.
- First blue LED 1994 Shuji & Nakamura (10 000 hours lifetime)
- SiC can also be used as blue LED - SiC on GaN substrate

Molecular Luminescence Spectrometry

LEDs as Sources

- **Blue light-emitting diodes (LEDs)**

Emit at 450-475 nm

- Use a pn junction under forward bias to produce radiant energy
- The diodes are made from gallium nitride ($\lambda = 465$ nm) or indium gallium nitride ($\lambda = 450$ nm)

Molecular Luminescence Spectrometry

Other Instruments that use Fluorescence:

**Capillary Electrophoresis and
Fluorescence Detectors for
Chromatography**

Sources

- **Lasers tunable dye laser pumped by pulsed N₂ gas or Nd:YAG laser—minimize interferences.**

Molecular Luminescence Spectrometry

Instrument

- Lasers

Advantages:

For microbore chromatography or CE which use only mL or less of sample.

In remote sensing where the collimated nature of the laser beam is needed.

To minimize the effects of fluorescing interferences by using highly monochromatic excitation.

Molecular Luminescence Spectrometry

Applications

Fluorescence and phosphorescence methods more sensitive than absorbance methods since Intensity is measured independently of the source, P_0 .

However precision and accuracy are 2-5 times less than for absorbance methods.

Molecular Luminescence Spectrometry

Applications

Determination of organic species

Used for enzymes, coenzymes, medical agents, plant products, steroids, vitamins, food products and more.

Widely used technique for a vast range of organics – because of structures.

Molecular Luminescence Spectrometry

Applications

Lifetime Measurements

To study luminescence decay rates need mode-lock lasers to produce pulses of radiation with widths of 70-100 ps for excitation and fast-rise time PMTs for detection.

Molecular Luminescence Spectrometry

Applications

Lifetime Measurements

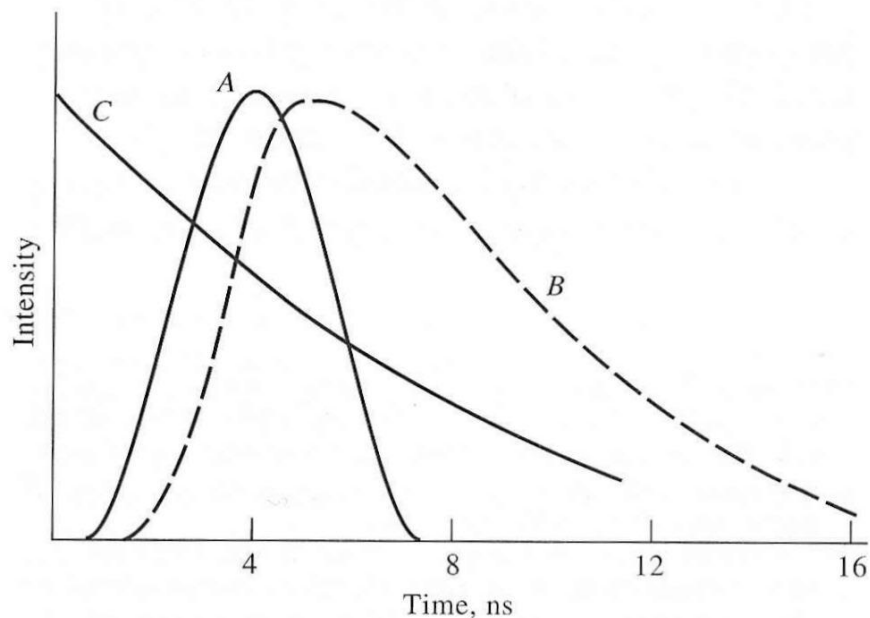


Figure 15-10 Fluorescence lifetime profiles: *A*, excitation pulse; *B*, measured decay curve; *C*, corrected decay curve.

Molecular Luminescence Spectrometry

Analysis of Gases

Used for determining atmospheric pollutants, i.e. ozone, nitrogen oxides, sulfurs.

Example. Determination of nitrogen monoxide

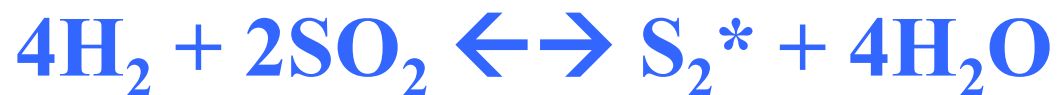


Molecular Luminescence Spectrometry

Analysis of Gases

Used for determining atmospheric pollutants, i.e. ozone, nitrogen oxides, sulfurs.

Example. Determination of atmospheric sulfur compounds



Assignment

- Read Chapter 15
- Homework 5 Chapter 15: 1, 2, 4, 5, 9, 13
- HW5 Chapter 15 due 2-20
- Exam II – March 4th PPTs 9-14

Molecular Luminescence Spectrometry

Chemiluminescence

When a chemical reaction yields an electronically excited species that emits light on its returns to ground state or transfer energy to another species.

Highly sensitive.



Molecular Luminescence Spectrometry

Chemiluminescence

$$I_{\text{CL}} = \phi_{\text{CL}} \frac{dC}{dt} = \phi_{\text{EX}} \phi_{\text{EM}} \frac{dC}{dt}$$

I_{CL} – radiant intensity (photons emitted/sec)

dC/dt – rate of chemical reaction

ϕ_{EX} – excitation of quantum yield (excited states per molecules reacted)

ϕ_{EM} – emission quantum yield (photons per excited state)

ϕ_{CL} – chemiluminescence quantum yield (photons emitted per molecule reacted)

ϕ_{CL} – needs to be 0.01-0.2

Molecular Luminescence Spectrometry

Applications

Determination of Inorganic Species

Non-transition metal ions form fluorescing chelates over transition metals because transition metals tend to be paramagnetic and deactivation is more likely by internal conversion.

Molecular Luminescence Spectrometry

Fluorometric Reagents

TABLE 15-2 Selected Fluorometric Methods for Inorganic Species

Ion	Reagent	Wavelength, nm		LOD, $\mu\text{g/mL}$	Interferences
		Absorption	Fluorescence		
Al^{3+}	Alizarin garnet R	470	500	0.007	Be, Co, Cr, Cu, F^- , NO_3^- , Ni, PO_4^{3-} , Th, Zr
F^-	Quenching of Al^{3+} complex of alizarin garnet R	470	500	0.001	Be, Co, Cr, Cu, Fe, Ni, PO_4^{3-} , Th, Zr
$\text{B}_4\text{O}_7^{2-}$	Benzoin	370	450	0.04	Be, Sb
Cd^{2+}	2-(<i>o</i> -Hydroxyphenyl)-benzoxazole	365	Blue	2	NH_3
Li^+	8-Hydroxyquinoline	370	580	0.2	Mg
Sn^{4+}	Flavanol	400	470	0.1	F^- , PO_4^{3-} , Zr
Zn^{2+}	Benzoin	—	Green	10	B, Be, Sb, colored ions

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