Molecular Luminescence Spectrometry

Emission and Excitation Spectra

- **Excitation spectrum**
  - absorbance spectrum

- **Fluorescence and Phosphorescence**
  - excitation at fixed $\lambda$ while recording emission intensity as a function of $\lambda$
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Instrument

Components are very similar to those for absorbance.

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

Figure 15-4  Components of a fluorometer or a spectrofluorometer.
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**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 Po.
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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.
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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.
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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)
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**Sources**

Semiconductor LED vs LASER

![Graph showing LED and Laser regions](image-url)
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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
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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
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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.

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- 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.
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!!
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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.
Visible LED

LED to emit visible light, the band gap of the materials that are used must be in the region of visible wavelength = 390 - 770 nm. This coincides with the energy value of 3.18 eV - 1.61 eV which corresponds to the colour spectrum.

<table>
<thead>
<tr>
<th>Colour</th>
<th>Energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Violet</td>
<td>3.17</td>
</tr>
<tr>
<td>Blue</td>
<td>2.73</td>
</tr>
<tr>
<td>Green</td>
<td>2.52</td>
</tr>
<tr>
<td>Yellow</td>
<td>2.15</td>
</tr>
<tr>
<td>Orange</td>
<td>2.08</td>
</tr>
<tr>
<td>Red</td>
<td>1.62</td>
</tr>
</tbody>
</table>

The band gap, $E_g$ that the semiconductor must possess to emit each light.
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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
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Sources

- Blue light-emitting diodes (LEDs)

Important parameter - quantum efficiency (\( \eta \)): 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)
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Sources

• Blue light-emitting diodes (LEDs)

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).
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Total Internal Reflection

Reflection and transmission coefficients for non-normal incidence can be calculated from the Fresnel equations.

Light striking a medium with a lower index of refraction can be totally reflected.

Critical Angle $\theta_c$

$n_1$ high index material

$n_2$ is totally reflected.
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Sources

• Light-emitting diodes (LEDs)
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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.

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

- Lasers tunable dye laser pumped by pulsed N\(_2\) gas or Nd:YAG laser—minimize interferences.
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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.
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**Instrument**

Filter and monochromateters same as for absorbance.

First monochromater called the excitation monochromater.

Second monochromater emission monochromater - separates the scattered light from the wanted light.
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Instrument
Transducers

The fluorescence signal tends to be low in intensity so need a sensitive detector, i.e. PMTs, diode array, charge transfer.
PMT’s – most common
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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.
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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.
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Figure 15-6 A typical fluorometer. (Courtesy of Farrand Optical Co., Inc.)
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Instrument designs

Spectrofluorometers

Produces both excitation and emission spectra. Usually has 2 grating monochromaters, radiation from the 1st monochromater is split, part goes to reference PMT and part goes to sample. The fluorescence coming from the sample goes to the 2nd monochromater and detected by 2nd PMT.

Chem 4631
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.
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.
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>
<thead>
<tr>
<th>Ion</th>
<th>Reagent</th>
<th>Wavelength, nm</th>
<th>LOD, µg/mL</th>
<th>Interferences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al(^{3+})</td>
<td>Alizarin garnet R</td>
<td>470 absorption 500 fluorescence</td>
<td>0.007</td>
<td>Be, Co, Cr, Cu, F(^{-}), NO(_3^-), Ni, PO(_4^{3-}), Th, Zr</td>
</tr>
<tr>
<td>F(^-)</td>
<td>Quenching of Al(^{3+}) complex of alizarin garnet R</td>
<td>470 absorption 500 fluorescence</td>
<td>0.001</td>
<td>Be, Co, Cr, Cu, Fe, Ni, PO(_4^{3-}), Th, Zr</td>
</tr>
<tr>
<td>B(_4)O(_7^{2-})</td>
<td>Benzoin</td>
<td>370 absorption 450 fluorescence</td>
<td>0.04</td>
<td>Be, Sb</td>
</tr>
<tr>
<td>Cd(^{2+})</td>
<td>2-(o-Hydroxyphenyl)-benzoxazole</td>
<td>365 absorption 450 fluorescence</td>
<td>2</td>
<td>NH(_3)</td>
</tr>
<tr>
<td>Li(^+)</td>
<td>8-Hydroxyquinoline</td>
<td>370 absorption 580 fluorescence</td>
<td>0.2</td>
<td>Mg</td>
</tr>
<tr>
<td>Sn(^{4+})</td>
<td>Flavanol</td>
<td>400 absorption 470 fluorescence</td>
<td>0.1</td>
<td>F(^{-}), PO(_4^{3-}), Zr</td>
</tr>
<tr>
<td>Zn(^{2+})</td>
<td>Benzoin</td>
<td>-- absorption Green fluorescence</td>
<td>10</td>
<td>B, Be, Sb, colored ions</td>
</tr>
</tbody>
</table>

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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.
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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.
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Applications

Lifetime Measurements

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

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

Example. Determination of nitrogen monoxide

\[
\text{NO} + \text{O}_3 \rightarrow \text{NO}_2^* + \text{O}_2
\]

\[
\text{NO}_2^* \rightarrow \text{NO}_2 + \text{hv} \ (\lambda = 600-2800 \text{ nm})
\]
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Analysis of Gases

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

Example. Determination of atmospheric sulfur compounds

\[ 4\text{H}_2 + 2\text{SO}_2 \leftrightarrow \text{S}_2^* + 4\text{H}_2\text{O} \]

\[ \text{S}_2^* \rightarrow \text{S}_2 + \text{hv} \ (\lambda = 384 \text{ and } 394 \text{ nm}) \]
Assignment

• HW4 – Due Today
• HW5 Chapter 14: 1, 6, 9
• HW5 - Due 2/20/19
• Read Chapter 15
• HW6 Chapter 15: 1, 2, 4, 9, 13
• HW6 - Due 2/22/19