Measurements Lab

Fundamentals of Instrumentation for Detection of Optical Signals

> Excitation sources Polarization and dispersion Detectors

NC State University

Fluorometer

A standard fluorometer consists of an excitation source, sample compartment, dispersion (monochromator) and detector. The output of the detector is usually connected to a computer, which can record the signal as a function of wavelength when the monochromator is scanned.

Because of the importance of removing all sources of stray light, the source is usually passed through a double monochromator. This can give clean relatively narrow bandwidth excitation source. However, one can replace such a source with a laser diode or laser in some applications. This will be particularly important for timeresolved fluorescence applications.

Fluorometer schematic



Excitation of fluorescence

- Xe arc lamps
- Hg arc lamps
- Laser diodes
- Lasers

Xe arc lamp (cw and flashed)



Spectral corrections to account for discrete lines in the Xe lamp



Hg arc lamp

Mercury arc lamps have much sharper lines that Xe lamps. They are less used for general applications that require Broad band light, but they are much cheaper than lasers And can provide relatively intense light at certain wavelengths.



Laser diodes

Another light source is the laser diode. In contrast to LEDs, laser diodes emit monochromatic radiation. Laser diodes are available with wavelengths ranging from about 355 to 1500 nm. Laser diodes are convenient because the output is easily focused and manipulated. In contrast to arc lamps or incandescent lamps, the output of LEDs and laser diodes can be pulsed or modulated. LEDs can be amplitude modulated up to about 100 MHz, and laser diodes can be modulated to several GHz.

Laser diodes are p-n junctions with a depletion region between the two junctions. When a bias voltage is applied holes and electrons are injected into the depletion region from p- and n-doped ends. Recombination across the band gap results in emission.

Laser diodes currently span wavelengths from 335 – 2000 nm

These commerical laser diodes show the typical size and geometry used in making experimental laser diodes. The diodes can be tuned over a narrow range using temperature.



Lasers in Chemistry

Light Amplification by Stimulated Emission of Radiation I. Requirements for a laser II. Two state, three state, and four state systems. III. Survey of tunable lasers. IV. Applications

Requirements for a laser

- Gain (population inversion)
- Laser cavity (reflecting mirrors)
- Output coupler (semi-reflective mirror)
- Standing waves can be obtained for $n\lambda=2L$
- The bandwidth in the laser cavity can be modulated using birefringent filters, etalons, or grating/prism combinations.

An example: Nd:YAG



A practical laser must have a pump source to create an excited state population. It must have a laser medium that is in an optical resonator. There must be an output coupler, which is a mirror that is only partially reflective to let some of the light out.

Gain can only be achieved with population inversion

- Gain represents the increase in photons emitted from the sample compared to a Boltzmann distribution.
- The Boltzmann distribution will never allow for more population in an excited state than in the ground state.
- Gain represents a non-Boltzmann distribution induced by optical or electrical pumping.

Basic physical justification

The figure shows why a population inversion is needed. It you want to get more photons out than you put in you need many molecules in the excited state to start a cascade of output photons.



A two level system cannot be used to build a laser

- For states 0 and 1 the Einstein B coefficients are equal $(B_{12} = B_{21})$
- High fluence limit results in a ratio of populations determined by microscopic reversibility
- At equilibrium

 $W_{absorption} = W_{emission}$

 Since Bp is the same, in the limit of large r we have that

 $n_1 = n_0$, and there is no population inversion $(n_1 > n_0)$.



A Three Level System: minimum requirement for a laser

- Optical or electrical pumping is used.
- Pump to intermediate state that decays into longlived emissive state.
- Ground state population must be overcome to make a population inversion in the emissive state.
- Example: the ruby laser, Cr:Al₂O₃ in which Cr d-d transitions give a population inversion in a state that emits at 594 nm.

A three-level system provides a route to a population inversion

- We can simplify the rate equations by assuming that
- If $k_{21}n_2 >> n_1B\rho$ then a population build-up in n_1 can lead to an population inversion $n_1 >> n_0$.
- Still has disadvantage that population in n₀ is dominating at equilibrium.



A four-level system produces superior results for laser design

- A four level system provides ease of creation of an inversion since state 3 is essentially unpopulated at equilibrium.
- In order for this scheme to work the rate from 1 to 2 and from 3 back to 0 must be rapid. The population inversion is set up between 2 and 3.



Comparison of thermal and pumped population



The laser cavity



The partial reflector is usually called the output coupler.

The gain bandwidth

Although laser light is perhaps the purest form of light, it is not of a single, pure frequency. All lasers produce light over some natural bandwidth or range of frequencies. A laser's bandwidth of operation is determined primarily by the gain medium that the laser is constructed from, and the range of frequencies that a laser may operate over is known as the gain bandwidth. For example, a HeNe gas laser has a gain bandwidth of 1.5 GHz Ti:sapphire has a bandwidth of about 128 THz Exercise: convert these to wavenumbers!

Longitudinal modes

These standing waves form a discrete set of frequencies, known as the longitudinal modes of the cavity. These modes are the only frequencies of light which are self-regenerating and allowed to oscillate by the resonant cavity; all other frequencies of light are suppressed by destructive interference. For a simple plane-mirror cavity, the allowed modes are those for which the separation distance of the mirrors L is an exact multiple of half the wavelength of the light λ , such that $L = N\lambda/2$, when N is an integer known as the mode order.

Longitudinal modes

In practice, the separation distance of the mirrors L is usually much greater than the wavelength of light λ , so the relevant values of N are large (around 10^5 to 10^6). Of more interest is the frequency separation between any two adjacent modes N and N+1; this is given (for an empty linear resonator of length L) by Δv : $\Delta v = \frac{C}{2I}$

where *c* is the speed of light ($\approx 3 \times 10^8 \text{ m} \cdot \text{s}^{-1}$).

Output modes

The figure on the right shows the combination of the gain bandwidth and longitudinal modes. This leads to a discrete set of wavelengths that are output from the laser within the bandwidth.



Common lasers used in laserinduced fluorescence

- Ar and Kr ion lasers
- Nd:YAG and Nd:YLF lasers
- Excimer lasers
- Dye lasers
- Transition metal lasers
- Optical parametric amplification

Ar and Kr ion lasers

- Noble gas ions are created in an electric discharge. The ions have specific electronic transitions.
- Ar ion lines: 514, 501, 488, 476 and 457 nm.
- Kr ion lines: 406, 413 and 752 nm.
- Continuous wave (cw), non-tunable, useful narrow bandwidth source for pumping cw dye lasers.

Argon ion laser

A high discharge current is used to form ions.

Powerful Continuous Wave operation can be achieved by discharge (15-50 Amps!) to maximize the pumping power. Several watts CW output or upto 1 kilowatt in microsecond pulses can be generated.

Pump to 4P states, 35eV above ground state by multiple collisions. Transitions correspond to 4P-4S,

514.5nm and 488nm. Use Brewster window at ends of gas tube to isolate a single polarization with minimum reflection losses.



Nd:YAG and Nd:YLF lasers

- Flash lamp pumped transitions with strong lasing at 1.064 μm. There are narrow transitions due to the fact that f-orbital transitions are buried below 5s and 5p.
- Cavity is Q-switched for 10 ns every 0.1 seconds. Q-switching is an electronic method to create a short pulse (or burst) of laser light.
- Frequency doubling using KDP to achieve 532 nm, the most frequently used wavelength in biophysical studies of biological molecules.

Frequency doubled Nd:YAG



Nd:YAG

- Nd:YAG is the commonly used laser material today.
- Neodymium-doped yttrium aluminium garnet; Nd:Y₃Al₅O₁₂) is a crystal that is used as a lasing medium for solid-state lasers.
- It is particularly useful in nanosecond pulsed lasers.
- The Nd:YAG fundamental occurs at 1064 nm.



Nd:YLF

- Nd:YLF is used in a diode pumped configuration.
- Neodymium yttrium lithium fluoride; $LiY_{1-x}Nd_xF_4$).
- It is particularly useful in as a reliable pump source for Ti:sapphire.
- The Nd:YAG fundamental occurs at 1047 nm.



Dye lasers

- Tunable lasers pumped both cw by Ar and Kr ion and in pulsed laser applications by Nd:YAG.
- Common dyes

Rhodamine λ_{max} =590 nm Stilbene-1 λ_{max} =430 nm Coumarin λ_{max} =430 nm

Dye lasers are messy



Pumped liquid contains dye. Dye degrades over time. Result is a frequent exchange of laser dyes.

Solid state alternative: Ar ion laser exciting a Ti:sapphire rod



d-d transitions can give a useful four level system for tunable lasers

- A titanium sapphire laser Ti:Al₂O₃ is a four level system.
- This is part of a vibronic mechanism which results in a Jahn-Teller distortion of the complex. In this distorted complex e_u is the fourth state.



Ti is d¹ leading to population of a single low energy state in the d-orbitals

Comparison of three and four state systems

- Cr is d³ and this results in a different behavior than for Ti.
- Because each of the t_{2g} levels is occupied by a single electron there is no Jahn-Teller distortion.
- Ruby has the disadvantage as well that other t_{2g} -> e_g transitions can occur absorbing the light.



Cr(III) ligand field is nearly O_h so help is required from other metals

- Note that the rate equations for triply degenerate t_{2g} ground state of Cr(III) imply that the rate of absorptive transitions is 2 times higher than for the stimulated emission.
- From this view point we see that ruby is definitely not the ideal material. Alexandrite (CrBe:Al₂O₄) is a four level system and that overcomes these problems.
- Ruby and Alexandrite are transition metal lasers.

Z-shaped laser cavity standard in Ti:sapphire design



A Ti:sapphire tuning curve



Dispersion, polarization and filtering of emitted light

Polarizers



If the second polarizer is rotated through an angle θ the intensity is given by

$$I = I_0 \cos^2 \theta$$

Glan-Thompson Polarizer

Best properties for fluorescence. Birefringent Calcite prisms, i.e. refractive index is different along each optical axis of the crystal. Angle of crystal is cut so that one polarized component undergoes total internal reflection at the interface, and the other continues along its optical path.



The purpose of the second prism is to ensure that the desired beam exits the polarizer in the same direction as the entering beam.

Gratings

Gratings are essential for the resolution of the different frequencies of emitted light. There are both reflection and Transmission grants.



Example: Transmission grating

The diffraction of HeNe laser light through this transmission Grating reveals that various orders of diffraction.



Grating efficiency



Bandpass and cutoff filters

Filters can be used to compenstate for suboptimal performance by the monochromator. In turbid samples the stray light may be greater than the fluorescence and the ability to select certain wavelengths of light for observation can be essential for the success of the experiment.



Detectors

Photomultiplier tubes

Current is proportional to light intensity.

Photon strikes the plate (photocathode) and causes an "electron cascade", which is an amplification through a chain of dynodes.

Photomultiplier tube plates are sensitive only over a narrow range of the spectrum. The next two slides show the combination of window coatings and photocathode materials that lead to specific spectral response.

The dynode chain

When an electron strikes the photocathode it initiates a cascade of electrons which in crease in number for each dynode. The amplification occurs in the dynode chain because a large bias voltage.



Photomultiplier tube windows



Photocathode spectral response



Linearity of detector response

Photodetectors are characterized by a photocurrent response that's linear with incident radiation over a wide range. Any variation in responsivity with incident radiation represents a variation in the linearity of the detector. If we plot the output current of the detector versus the input radiation level, the slope of the line from the lowest level of radiation to the highest level of radiation should not change. Noise in the detector or system will determine the lowest level of incident radiation detectable. The upper limit of this input/output linearity characteristic is established by the maximum current capability that the detector can handle without becoming saturated (no change in output for a change in input).

Sources of noise in photodetectors

Noise can be divided into two categories: externally induced noise, and internally generated noise. External noise includes those disturbances that appear in the system as a result of an action outside the system. Two examples of external noise are hum picked up from 60-hertz power lines and static caused by electrical storms. Internal noise, on the other hand, includes all noise that's generated within the system itself. We now know that every resistor produces a discernible noise voltage and every electronic device (such as vacuum tubes and semiconductor elements) has internal sources of noise. You can think of internal noise as an ever-present limit to the smallest signal that the system can handle.

Voltage fluctuations as intrinsic noise

A record of the output from a random noise generator might look like that shown in the figure. Because of the random nature of the noise, the voltage fluctuates about an average value V_{av} . A simple average of these fluctuations is a meaningless description, since the average of the variations is zero. Rather, it's convenient to use the average of the squares of the deviations about V_{av} . The average is taken over a time interval T that's much longer than the period of the fluctuations in the voltage.



The root-mean-square voltage as a definition of noise

The mean square voltage is:

$$\langle \mathbf{V} - \mathbf{V}_{av} \rangle^2 = \frac{1}{T} \int_0^T (\mathbf{V} - \mathbf{V}_{av}) dt$$

This is a convenient way of defining the fluctuation in a real System. The square root of this voltage is one measure of noise

$$V_{\rm rms} = \sqrt{\langle V - V_{\rm av} \rangle^2}$$

Shot noise

The term "shot noise" is normally associated with vacuum tubes in which the stream of electrons creates a noise due to the random fluctuations in the rate of arrival of electrons at the anode. This noise may be likened to the noise of a hail of shot striking a target. Hence the name shot noise. Shot noise is present in all photon detectors due to the random arrival rate of photons from the source of radiant energy under measurement and background radiation. This shot noise is often called "photon noise." Photon noise is the true ultimate limitation to detector performance. Even if all internal noise sources were eliminated, photon noise would set the ultimate limit for detector performance. Shot noise is proportional to the square root of the total number of counts on the detector. It is proportional to the square of the number of counts.

$$N_{shot} = \sqrt{N}$$

Silicon photodiode

Silicon photodiodes are not sufficiently sensitive to be used in most fluorescence applications where low light levels are common. However, the form the basis for technologies that are widely used such as Si avalanche photodiodes and CCDs.



Inverted operational amplifiers

Silicon photodiodes usually operate in a current mode. Light strikes the diode and generates a current. Gain can achieved using a operational amplifier (op-amp).

The circuit for the inverting op-amp configuration is shown. This circuit has the output 180 degrees out of phase with the input. The positive input is grounded.

The input to the op-amp itself draws no current and this means that the current flowing R2 in the resistors R1 and R2 is the same. Using ohms law R1

 $V_{out}/R2 = -V_{in}/R1$. Hence the voltage gain of the circuit is:

Gain = R2/R1



Current to voltage converter characteristic of op amps

Given the gain quation, you might think that it would be Possible to achieve a large gain by making the ratio of The two resistors R2/R1 very large. However, there are Intrinsic limitations. The resistor R1 determines the initial Voltage since $V = I \times R$. The current flowing in from the Photodiode is usually quite smal and thus R1 must be large Enough to achieve an initial voltage in millivolt range at least. Secondly, the time response of the circuit is limited by the RC time constant. If the second resistor is very large then The circuit will be very slow to respond. This is a severe Limitation for time-resolved applications. Photodiodes op-amp Circuits can have time resolution of ca. 10 nanoseconds, but Seldom much better than that for common laboratory experiments.

RC time constant limits gain

Even a bare wire has some resistance and some capacitance. In an op-amp circuit there is a significant resistance since this is the essence of gain. While the capacitance is kept to a minimum, it cannot be zero. So-called stray capacitance is at least a few picoFarads and that is enough to put severe limitations on the time response of an amplifier circuit. RC time constant comes from the equation for charging of a capacitor. $I = C \frac{dV_{out}}{dt} = \frac{V_{in} - V_{out}}{R}$

The solution has an exponential Form with a time constant of RC.

$$V_{\rm out} = \Delta V (1 - e^{-t/RC})$$



Silicon avalanche photodiode

An avalanche photodiode (APD) is a highly sensitive semiconductor electronic device that exploits the photoelectric effect to convert light to electricity. APDs are photodetectors that provide a built-in first stage of gain through avalanche multiplication. From a functional standpoint, they can be regarded as the semiconductor analog to photomultipliers. By applying a high reverse bias voltage (typically 100-200 V in silicon), APDs show an internal current gain effect (around 100) due to impact ionization (avalanche effect). However, some silicon APDs employ alternative doping and beveling techniques compared to traditional APDs that allow greater voltage to be applied (> 1500 V) before breakdown is reached and hence a greater operating gain (> 1000). In general, the higher the reverse voltage the higher the gain. An avalanche photodiode is a silicon-based semiconductor containing a pn junction consisting of a positively doped **p** region and a negatively doped **n** region sandwiching an area of neutral charge termed the depletion region. These diodes provide gain by the generation of electron-hole pairs from an energetic electron that Avalanche Photodiode creates an "avalanche" of N-Contact electrons in the substrate. athode Depletion Region Incident photons pass through P-Contact Incident Holes the **n** and **p** layers before Anode otons Tr-Region entering the depletion region where they excite free electrons Electrons SiO2 Layer and holes, which then migrate to 24 the cathode and anode, respectively. When a semiconductor diode has a N-Layer P-Layer Figure 1 reverse bias (voltage) applied and the crystal junction between the p and n layers is illuminated, then a current will flow in proportion to the number of photons

Si APD vs. PMT

The PMT and the Si APD photodiode both employ a photosensitive surface that captures incident photons and generates electronic charges that are sensed and amplified. PMTs respond when photons impinge on a photocathode and liberate electrons that are accelerated toward an electron multiplier composed of a series of curved plates, known as dynodes. The Si APD has similar concept of operation. Neither detector has spatial resolution.



Quantum efficiencies of APDs and PMTs



CCD cameras

Modern CCD cameras are quite sensitive and can detect wavelengths over a range of the spectrum. Thus, one does not need to scan wavelength in order to obtain a fluorescence spectrum. The emitted light is dispersed across the CCD instead.





CCD principle of operation

In an imaging CCD for, there is a photoactive region (epitaxial Si), and a transmission region made out of a shift register shown in the Series of images on the right. An image is projected through a lens onto the capacitor array (the photoactive region), causing each capacitor to accumulate an electric charge proportional to the light intensity at that location. Once the array has been exposed to the image, a control circuit causes each capacitor to shift the charge and finally to dump it to an amplifer, which converts it to a voltage for storage.

