Photochemical Techniques

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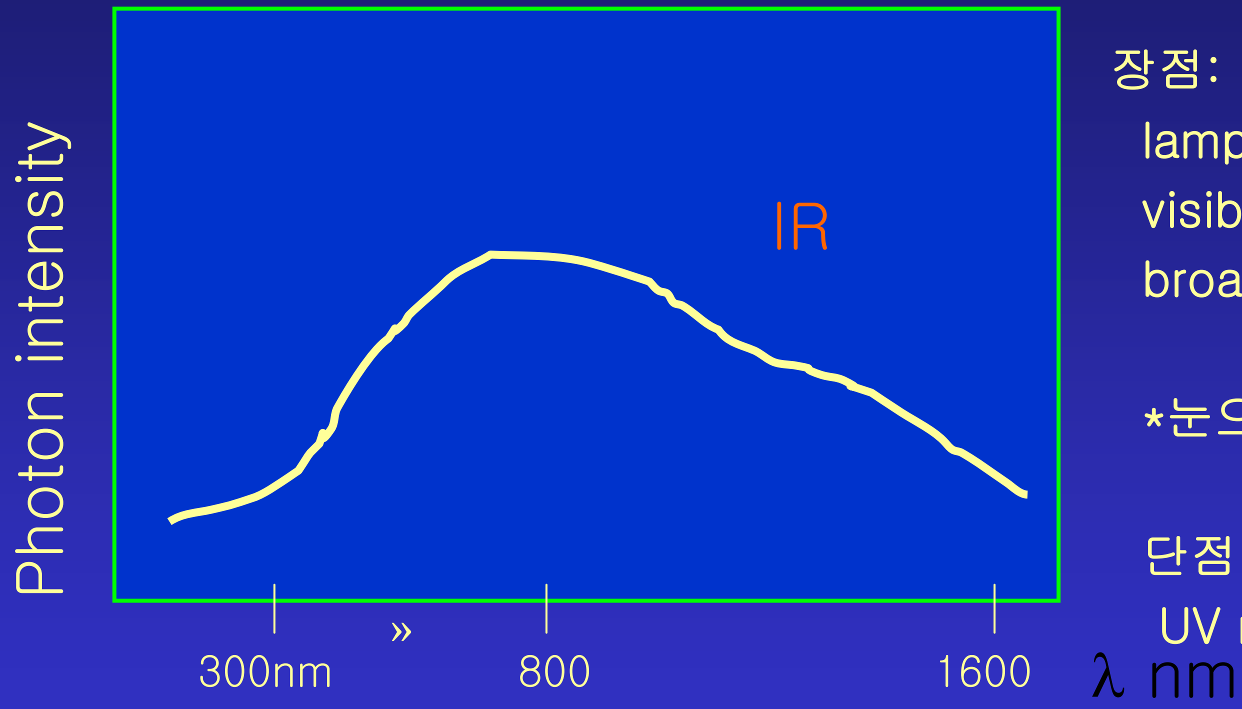
Photochemical Technique: Light source

- Tungsten Lamp



장점:

lamp 값 저렴

visible~IR region까지

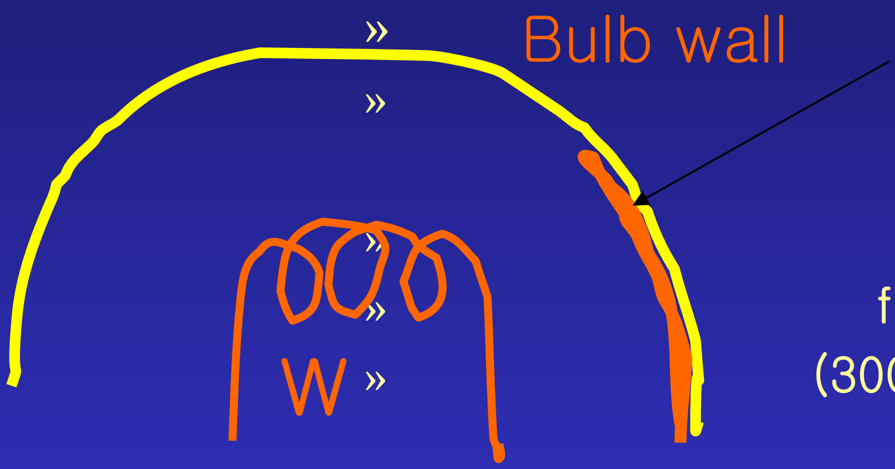
broad continuous spect.

*눈으로 볼 땐 노란색

단점 :

UV region 거의 없다.

- Tungsten-halogen Lamp



표면에

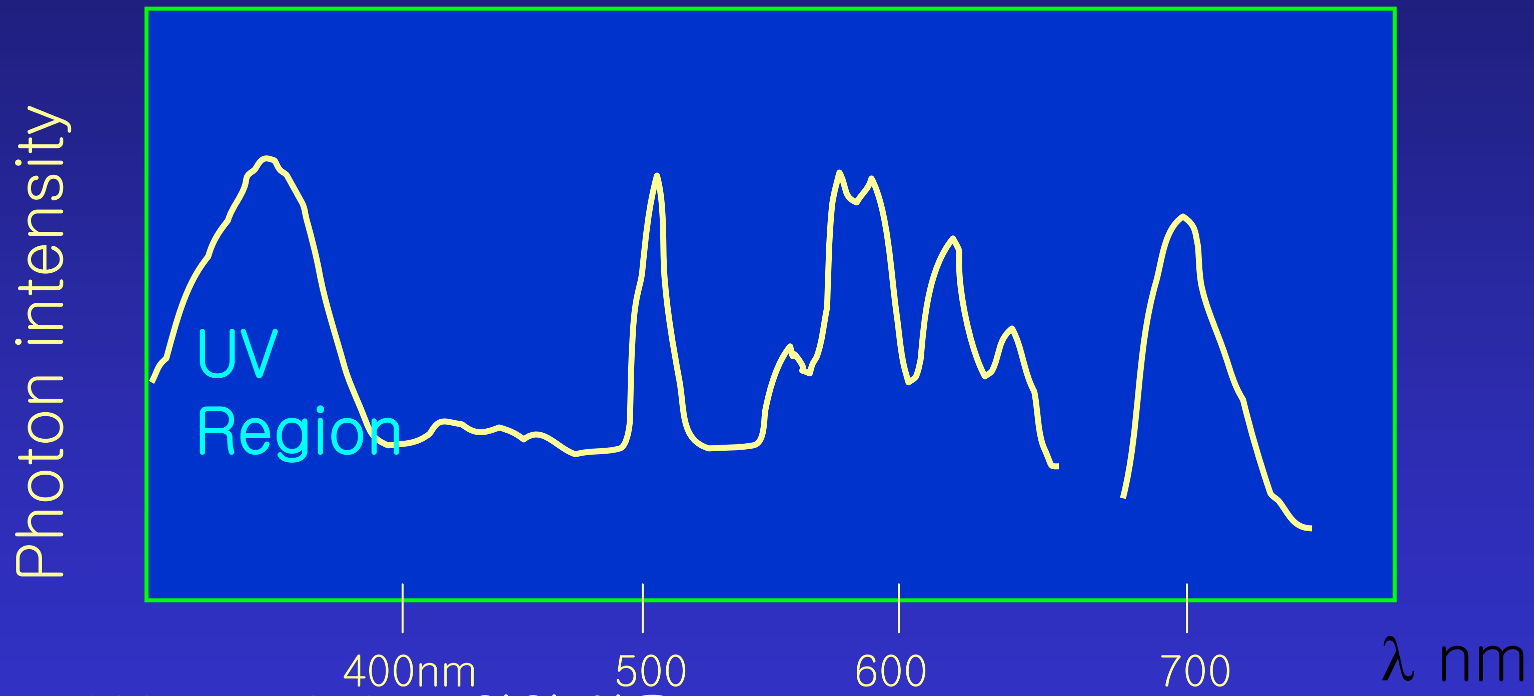
evaporated W + haolgen

Halogen은 W가 가열되면서
filament에 떨어지게 한다.
(3000~3200 K 가능하게 한다.)

- Bulb wall의 온도를250C로 유지시켜야 한다.
- 온도를 높게 해주면 visible 쪽으로 shift

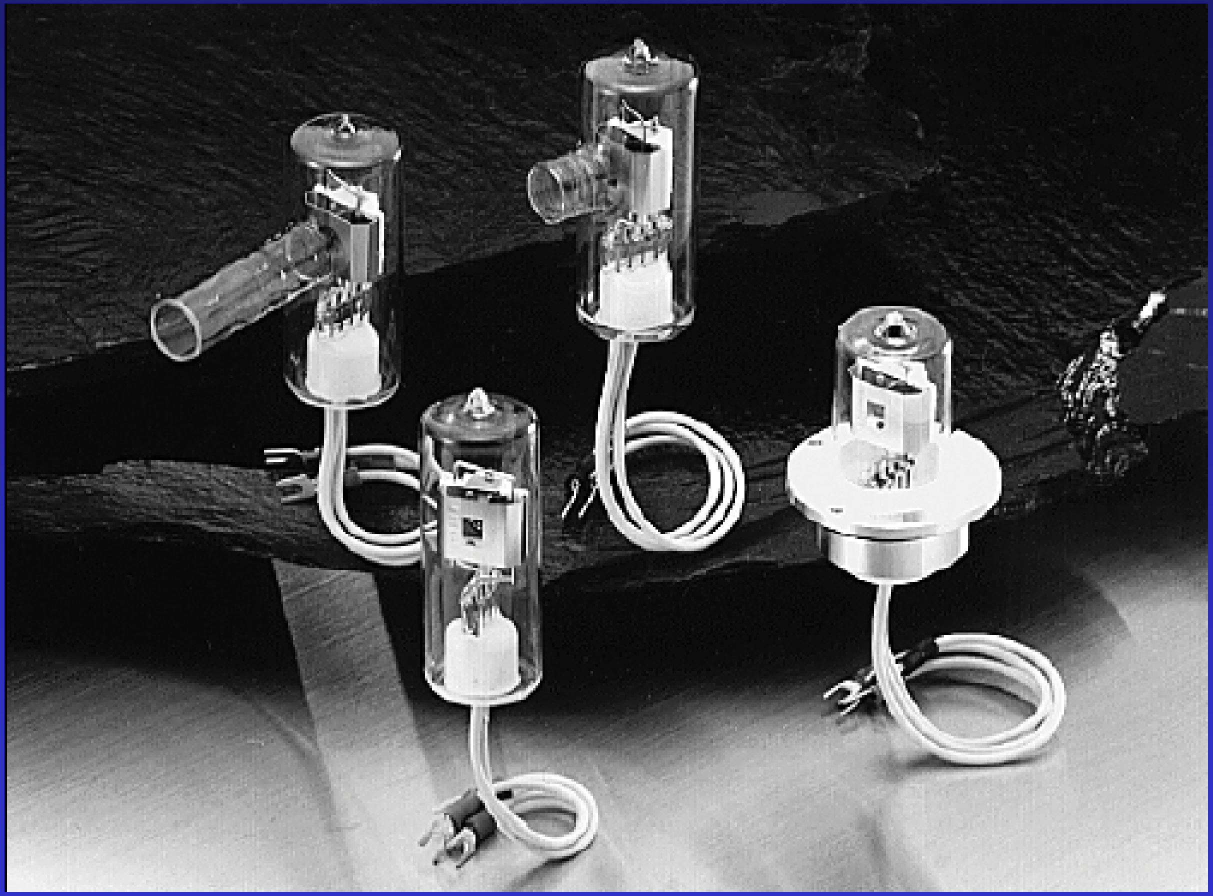
- (태양 표면 온도 6000C => UV 영역의 빛이 많이 나온다.)

- Deuterium Lamps
- UV spectrophotometer의 light source
- 단점 low output

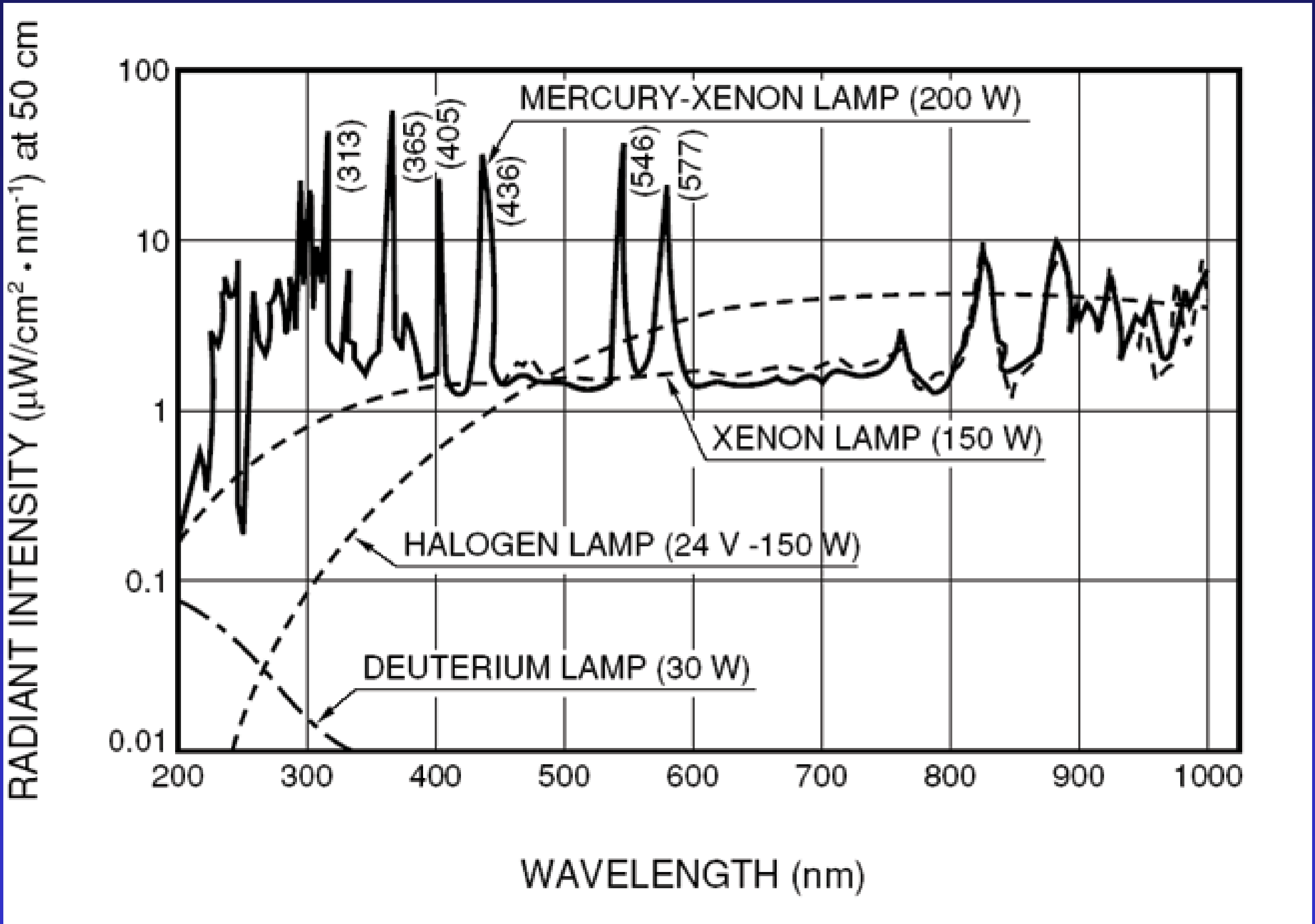


- 180nm ~ 350nm 영역 이용
- 350nm 근처에서 W 에서 D_2로 바꿔준다.

- Deuterium Lamps

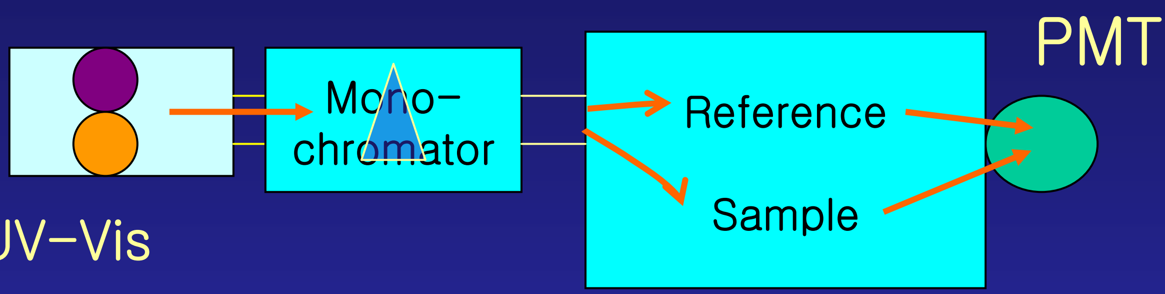


Photochemical Technique: light source spectra



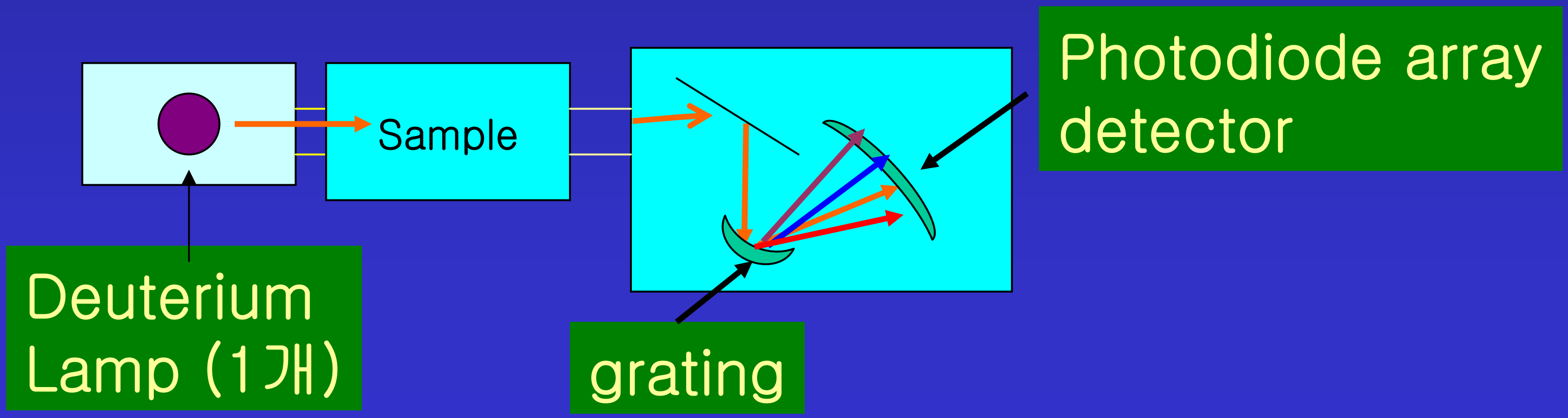
Photochemical Technique: UV-visible spectrophotometer

- 기존 UV-visible spectrophotometer



- UV-Vis
  light source

- Diode array type

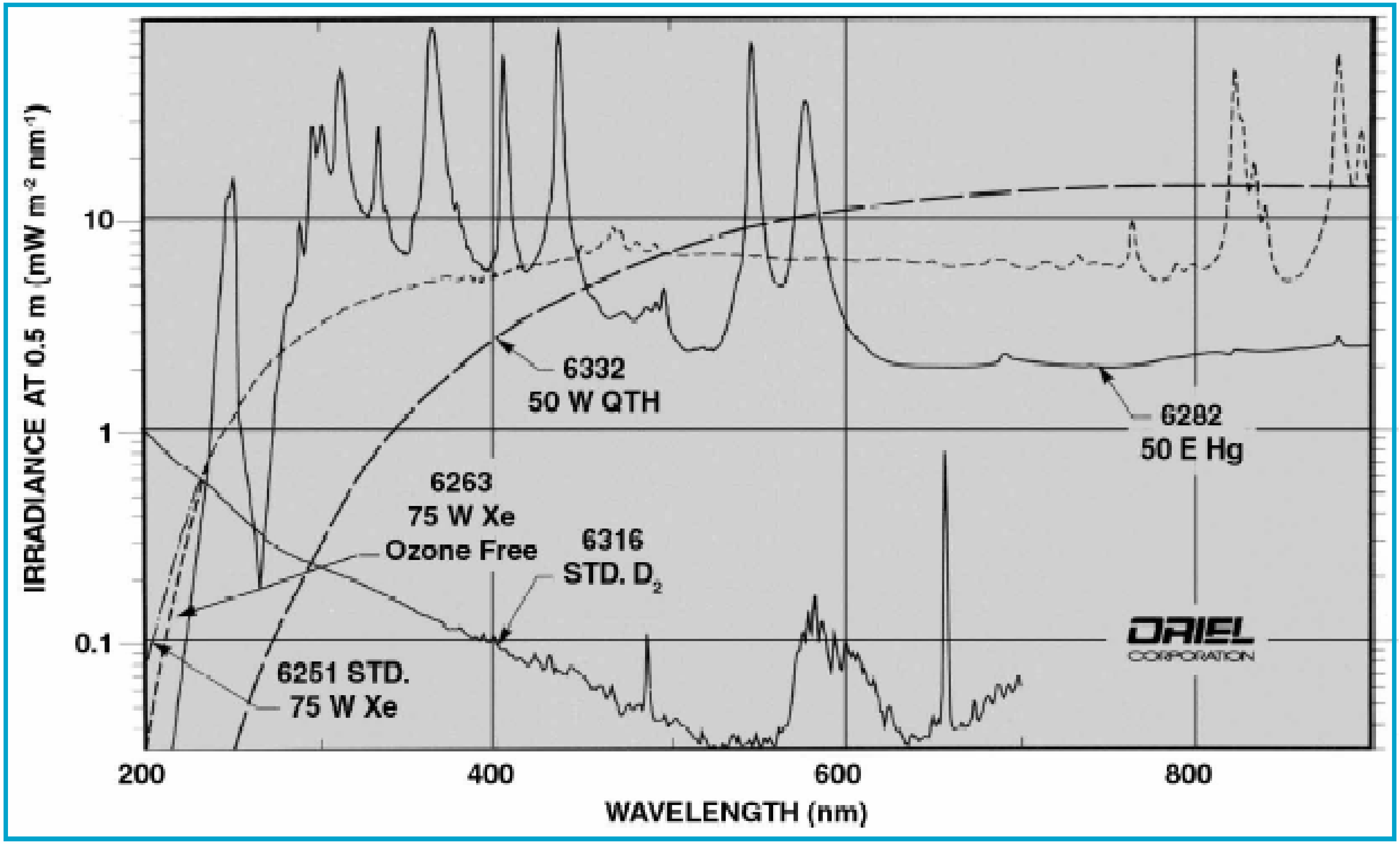


Photochemical Technique: Mercury arc lamps

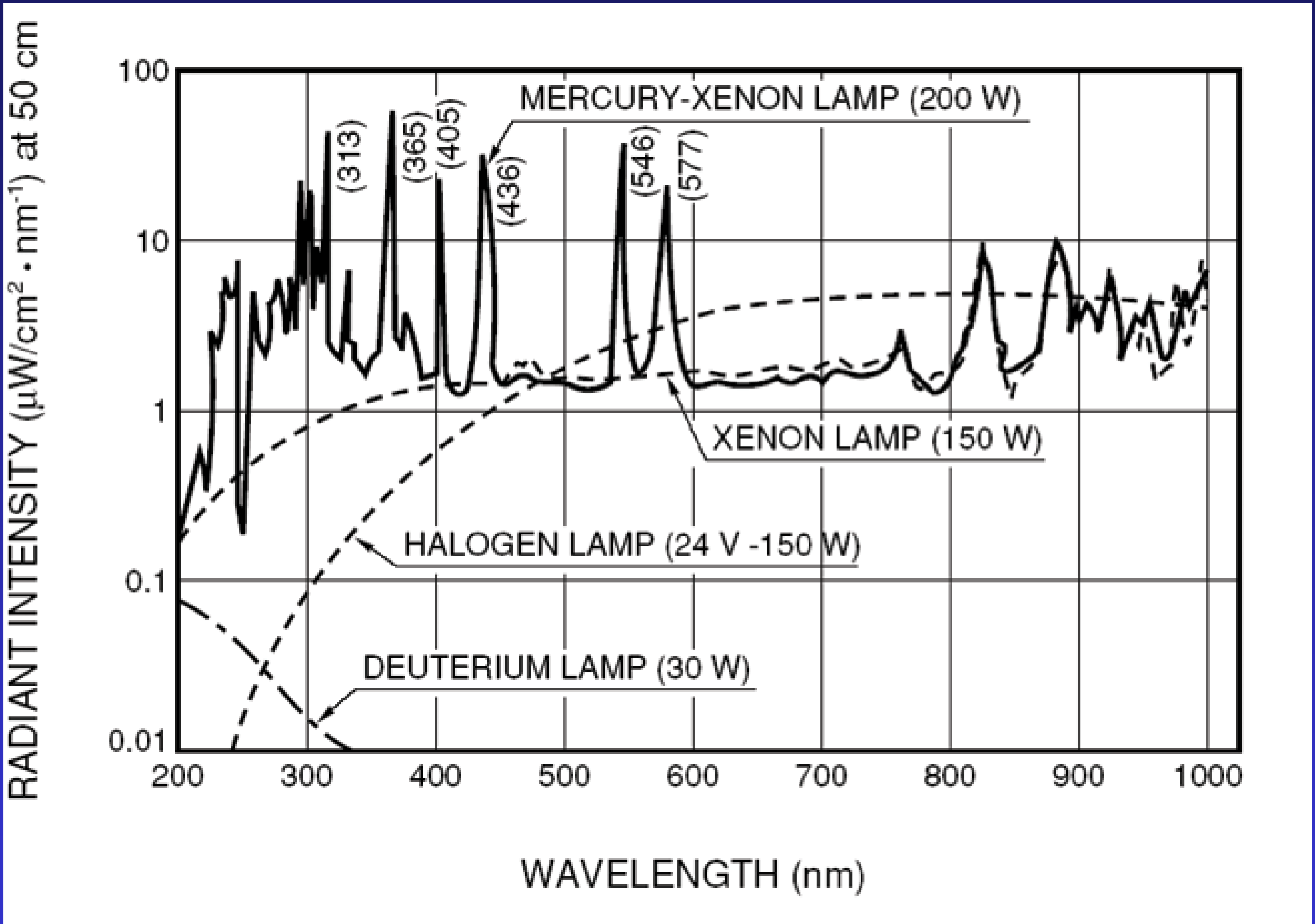
- Electrode 사이에 arc 를 만들어서, mercury를 여기시켜 빛 발생

1. Low pressure : Hg vapor pressure ~ 10^{-3} torr (184.9nm, 253.7nm를 주로 이용)
2. Medium pressure ~ 1–1.3 atm
3. High pressure ~ 100–400 atm

Photochemical Technique: Xe-Mercury arc lamps



Photochemical Technique: light source spectra



Photochemical Technique: Mercury arc lamps

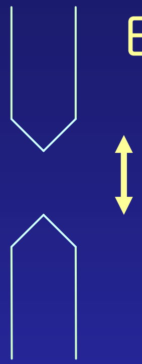
- Low Pressure Lamp – arc가 크다.
  (arc 가 작을 수록 밝기가 밝다.)

파장	248.2 nm	253.7	265.2 ~ 265.5	275.3
상대강도	0.01	100	0.05	0.03
파장	280.4	289.4	296.7	302.2 ~ 302.8
상대 강도	0.02	0.04	0.2	0.06
파장	312.6 ~ 313.2	365.5 ~ 366.3	404.5 ~ 407.8	435.8
상대강도	0.6	0.54	0.39	1.0
파장	546.1	557~579	1014	1128.7
상대강도	0.88	10.1	–	–

- Grating 교정 (low pressure Hg lamp 또는 laser 사용)

Photochemical Technique: Medium pressure lamp

- 유럽에서는 high pressure 라고 함.



Electrode

electrode 사이 거리가 많이 떨어져 있고, 에너지가 많이 들어간다.

거리가 100mm 이상인 것을 medium pressure lamp.

High pressure lamp는 medium prs. Lamp에 비해 압력이 높고 (P ~ 20 atm 이상), 온도 높고 전류 높다.

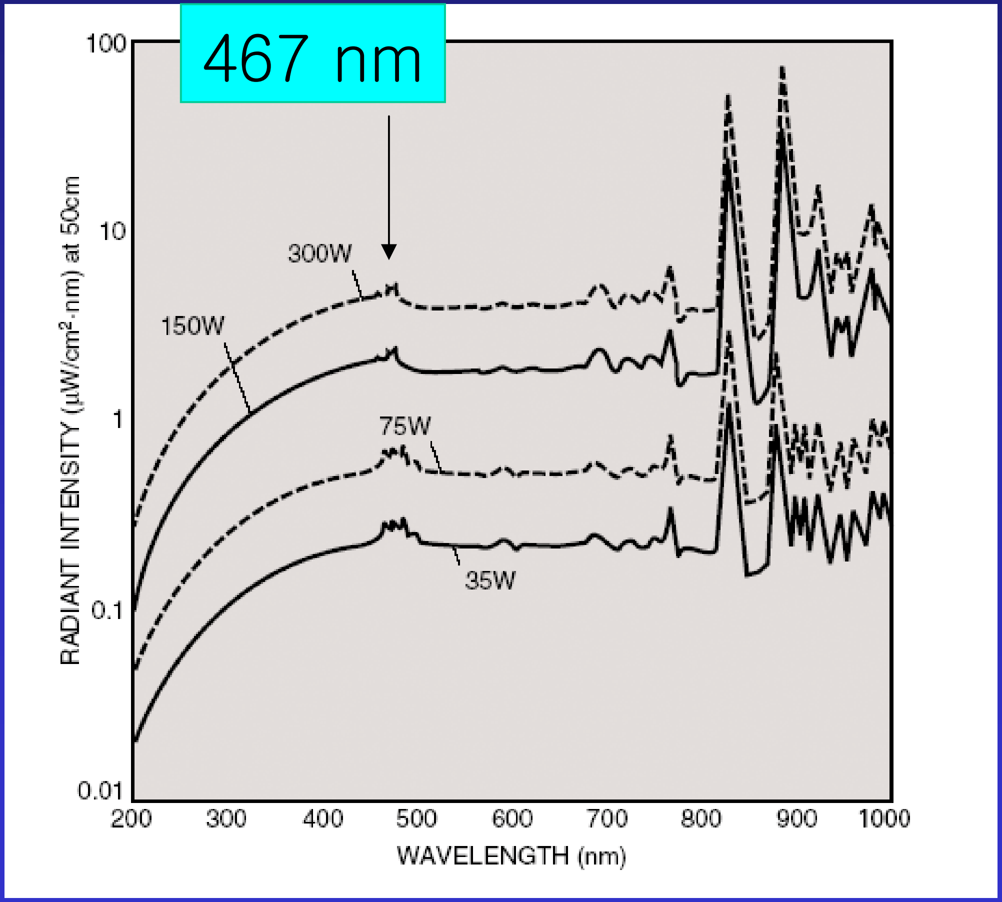
High pressure는 < 5mm : power supply 가 상당히 크다.

(cf; capillary lamp : 10 ~ 30 mm)

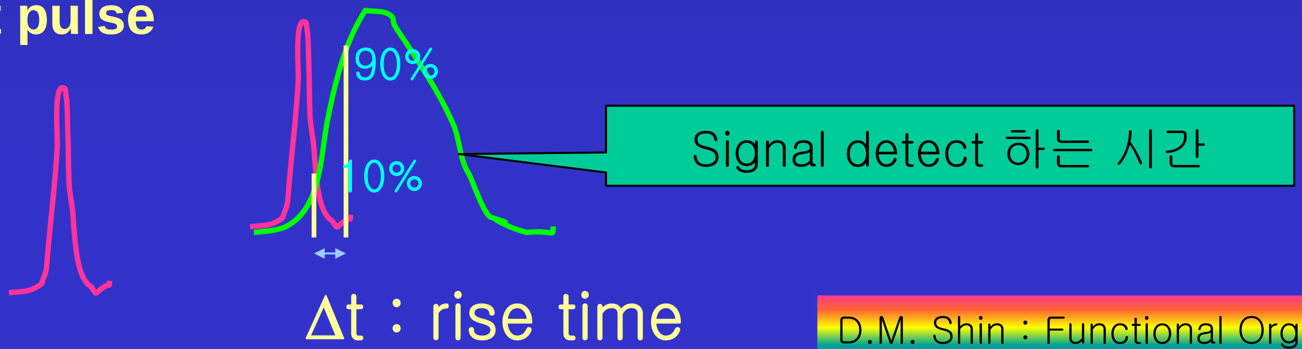
용도: Photochemical reaction에서 UV region의 irradiation이 필요한 경우

Photochemical Technique: Xe-arc lamp

- **Operate at high pressure (20 atm)**
- **Start arc.**
- **Focusing 하면 종이가 탄다. -> ir filter를 사용**

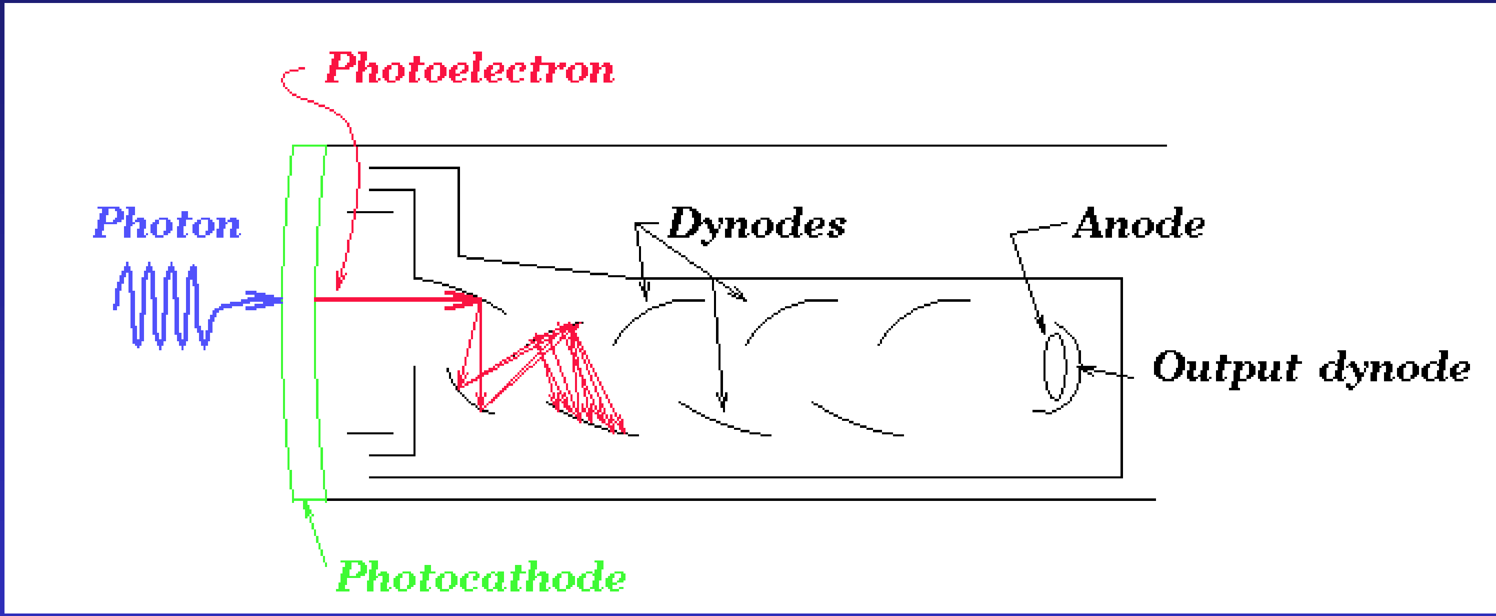


1. **Radiant Power (Flux)   unit: W ; radiation energy (in joule) / time**
2. **Luminous Power (Flux)   unit : lm ; radiant power modified by the response of the human eyes (주로 visible)**
3. **S/N ratio (signal current/noise current, or voltage) 클수록 좋다.**
4. **Noise Equivaent Power (NEP) : noise 와 같은 level의 signal을 만드는데 필요한 radiant power.**
5. **Detectivity   D = 1/ NEP**
6. **Spectral response : plot of the radiant sensitivity for wavelength**
7. **Quantum efficiency : # of electrons / # of photons (1개 photon 이 들어와서 1개의 electron 이 만들어지면 q.e =1)**
8. **Rise time : the time for a detectoer signal to rise from 10 % go 90 % of peak amplitude for illumination with a delta function light pulse**

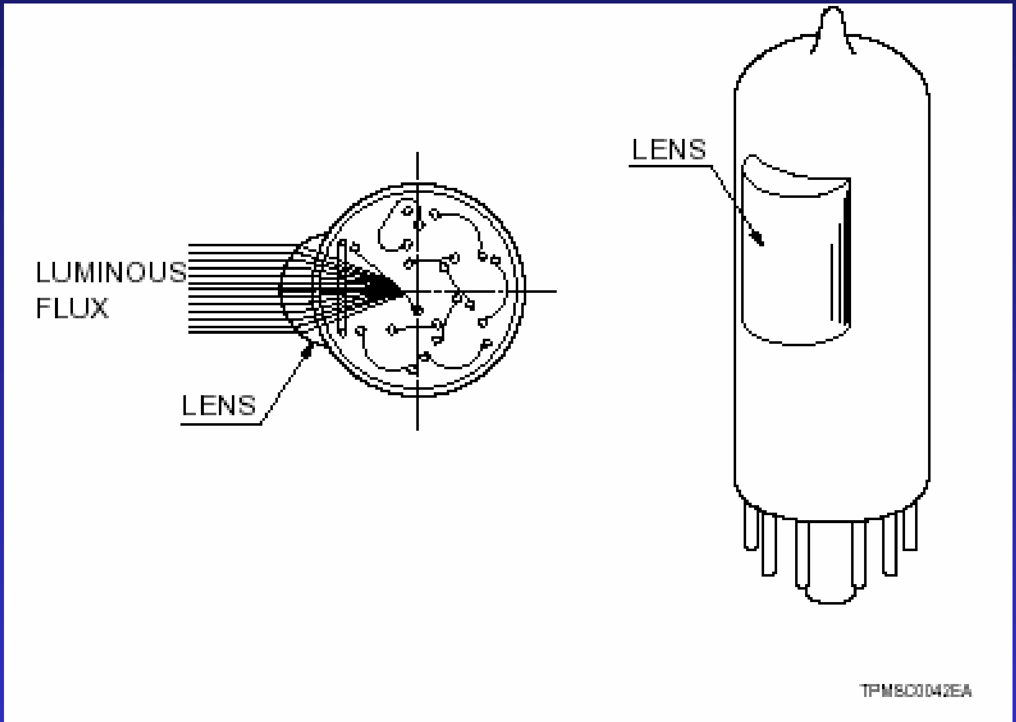


1 photoelectron -> 4 secondary electrons / Dynode

Head-on PMT



Side-on PMT

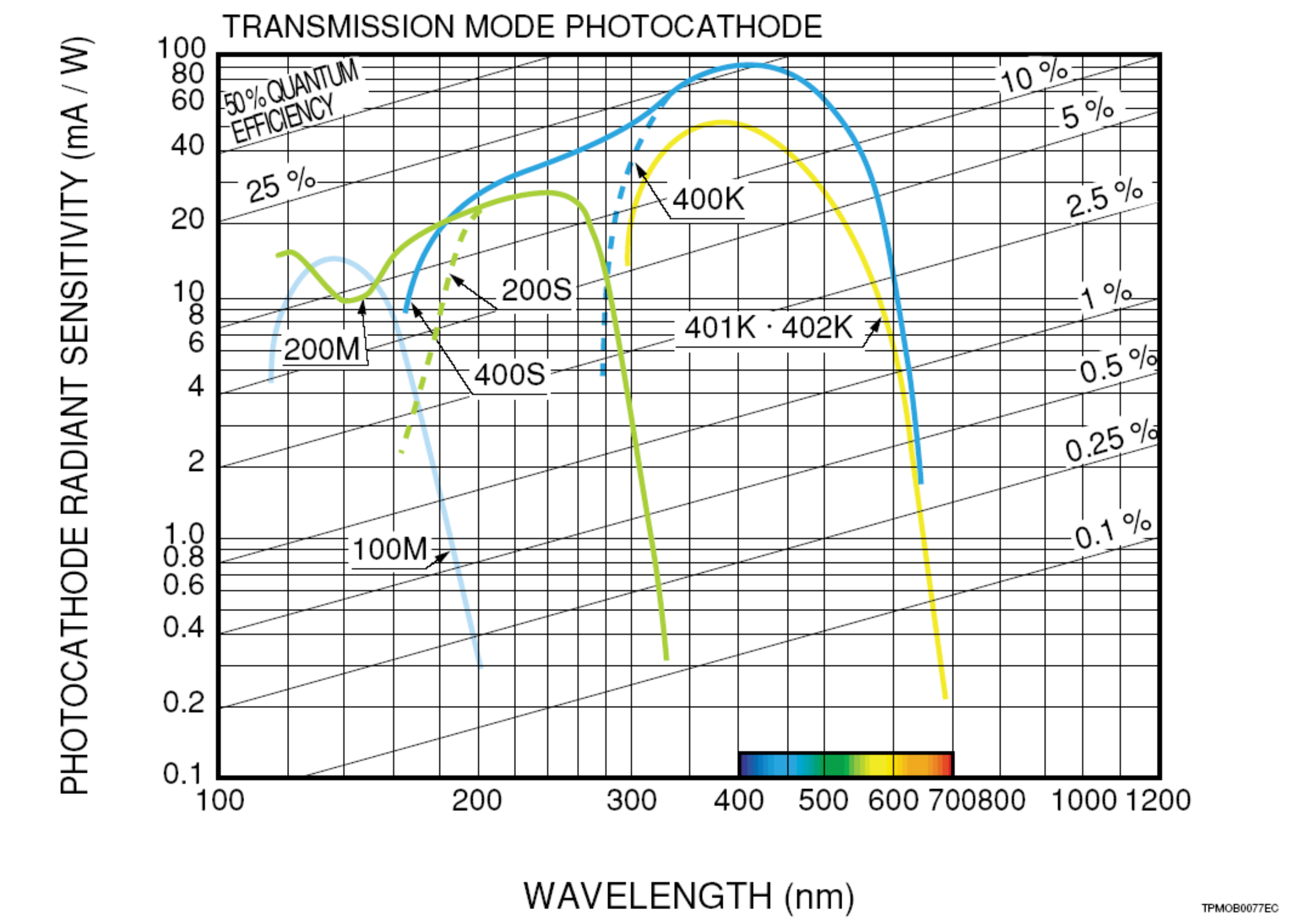


PMT의 **response sesitivity**는 **stage** 개수에 따라 결정

Secondary electron은 **quantum efficiency**에 따라 나오는 양이 다르다.

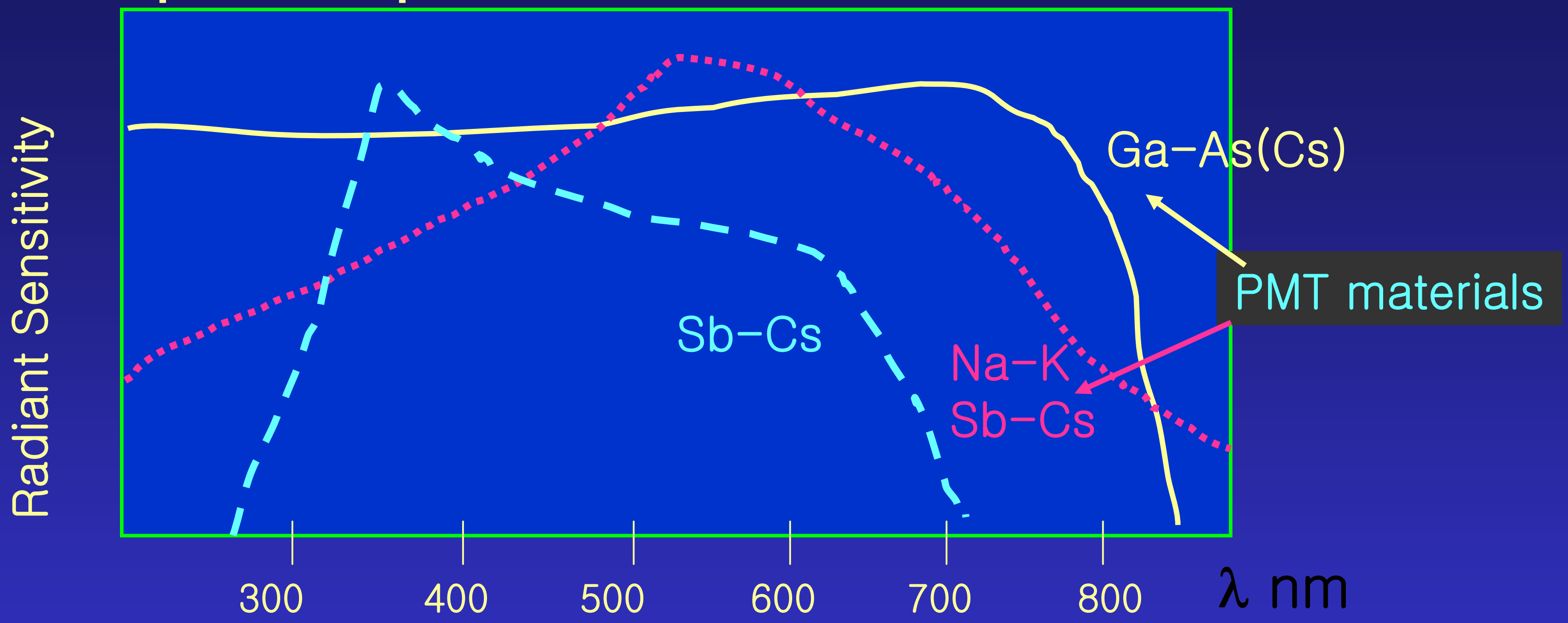
이것이 다른 **cathode** 를 때려서 **electron**이 증가된다. **(10^8** 배 **/14dynode)**

Spectral Response – PMT 종류에 따라 다름.



Photochemical Technique: Phototube

Spectral response

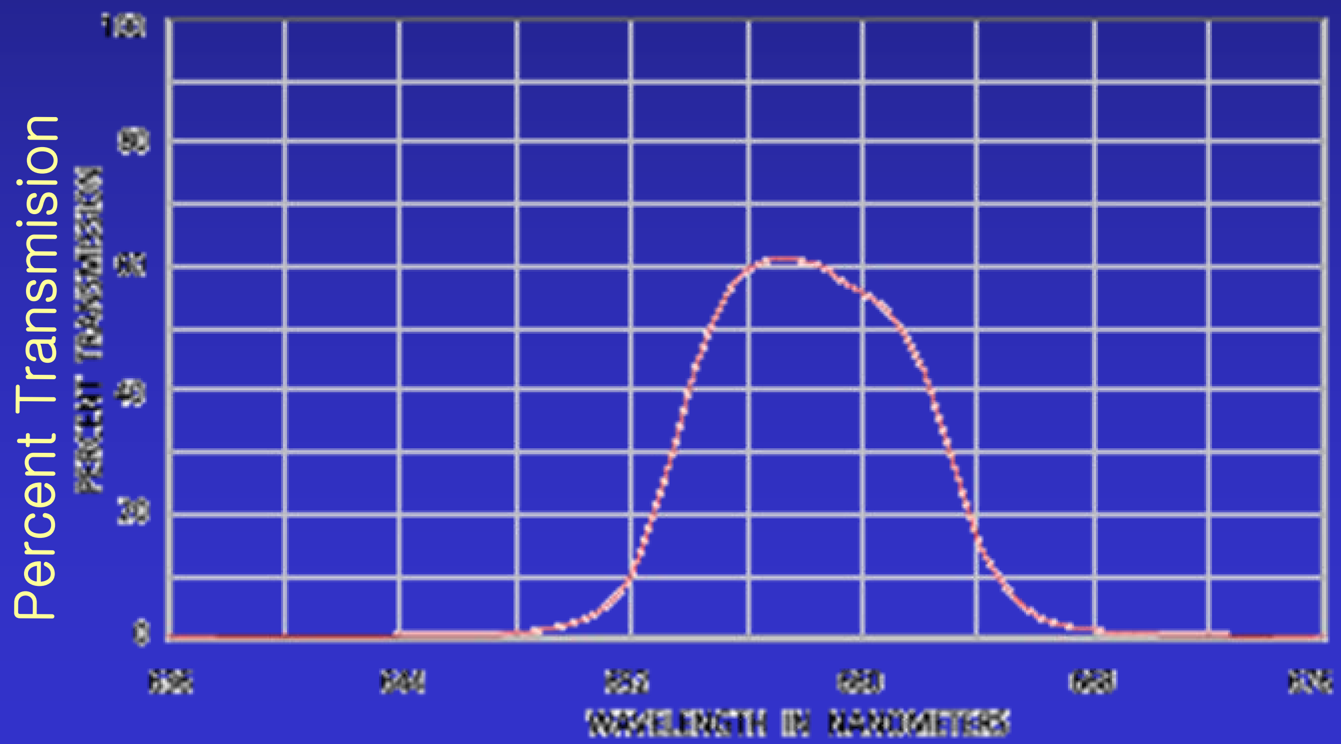


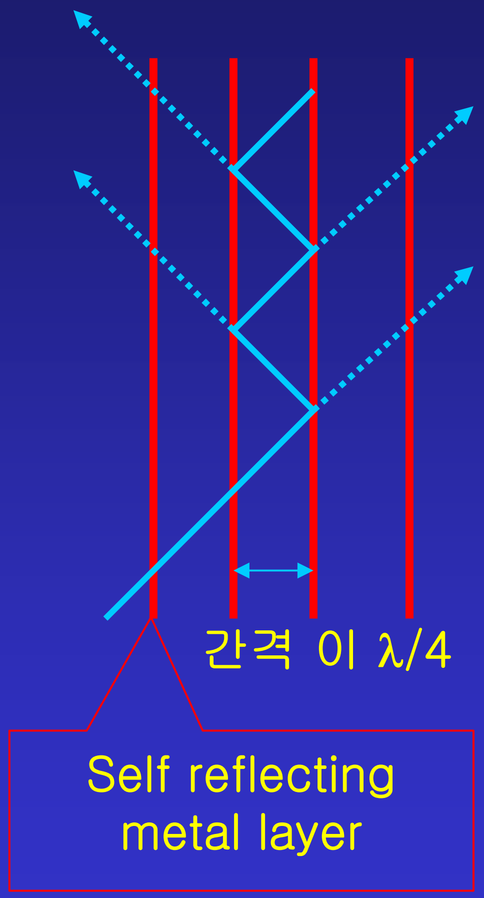
Cooling 을 하면 **spectral response** 가 달라진다 **(dark signal** 줄어듬 **)**
(Uv-visible 영역에서는 증가되고**, IR** 영역에서는 큰 변화 없다**.)**

- **IR sensitive PMT – Cooling** 요함**.**
- **PMT dark signal (**빛 새는 것**,** 주위 온도 높아서**,** 전류가 흘러서**)**

Photochemical Technique: Band path filter

Bandpass Filter : A filter that transmits a continuous range (band) of wavelengths but reflects or absorbs wavelengths above and below this band. Bandpass filters are specifed by the full width at half maximum (FWHM) of their transmission peak: wideband (>60 nm FWHM), medium band (20–60 nm FWHM), and narrowband (<20 nm FWHM). A typical narrowband transmission curve is shown below.





$$2n_e t\cos\theta = m\lambda$$

n_e : spacer refractive index

t : spacer thickness

θ : internal angle of incidence

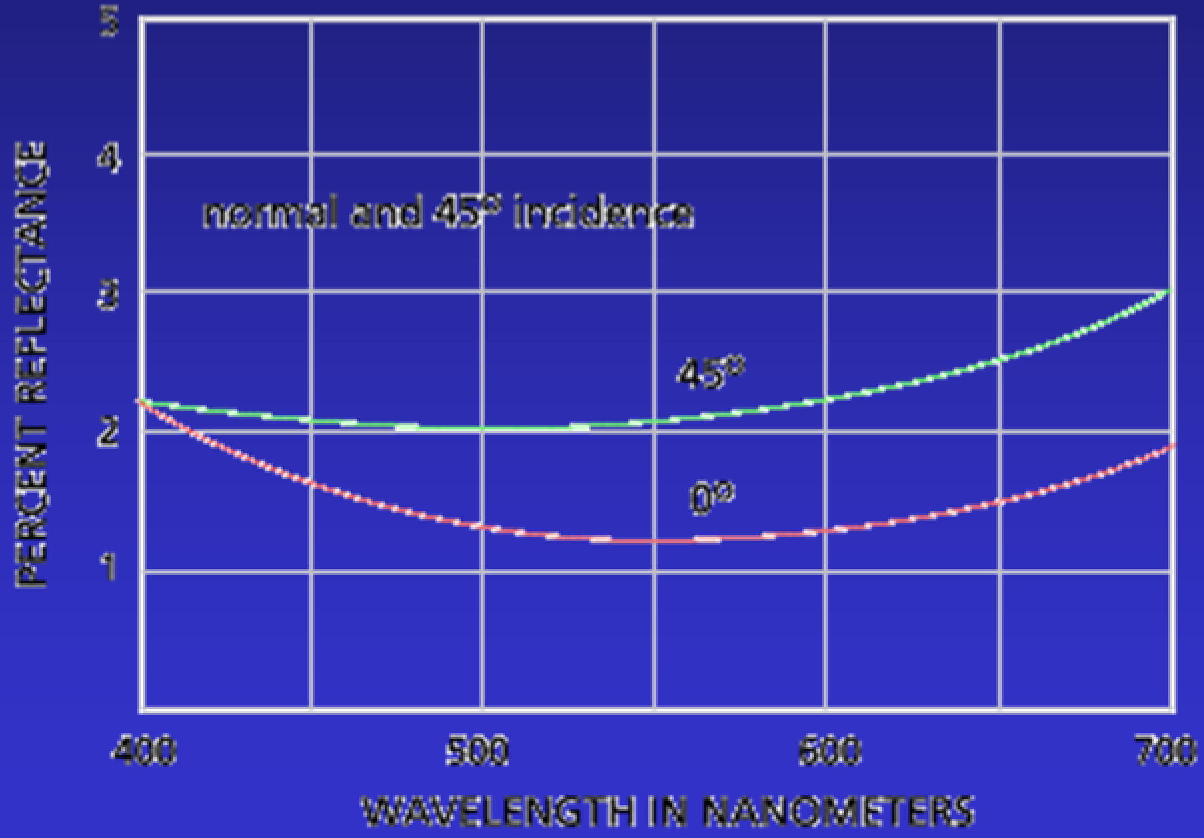
m : 특정파장의배수(자연수)

금속면이 광원 방향으로 향하게 !!

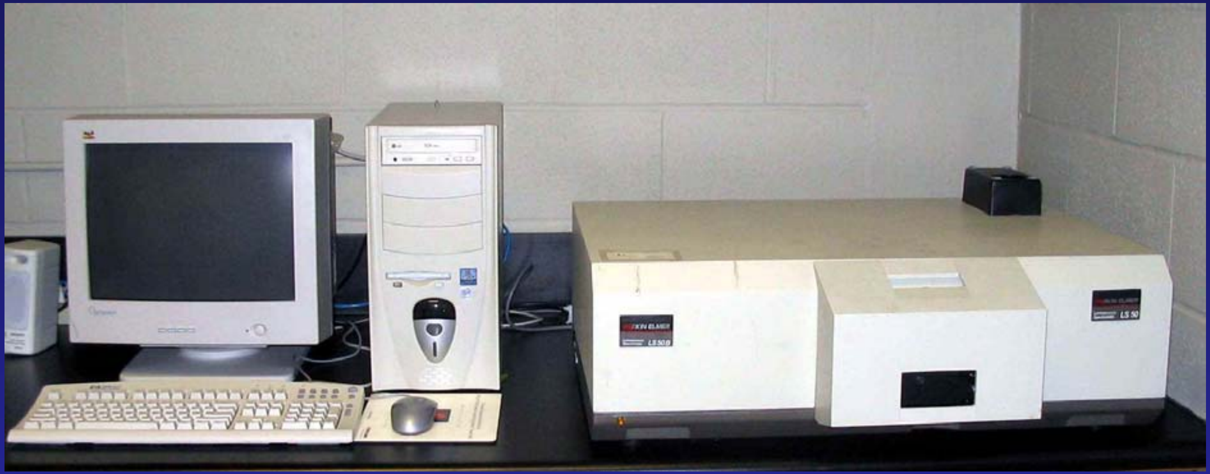
Antireflection Coatings (AR) At a simple interface between two dielectric materials, the amplitude of reflected light is a function of the ratio of the refractive index of the two materials, the polarization of the incident light, and the angle of incidence.

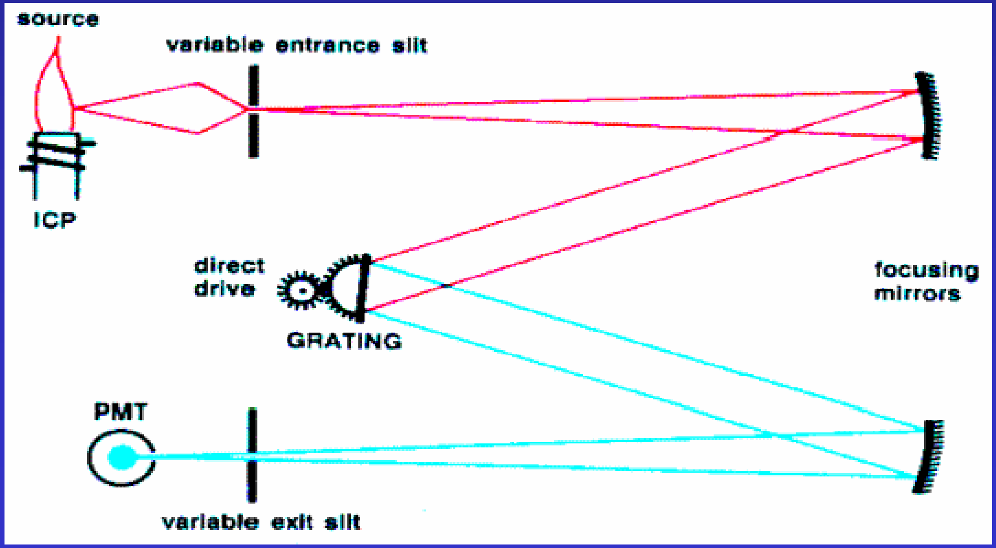
For light normally incident on an air/glass interface with the glass having a refractive index of 1.5, the intensity of the reflected light will be 4% of the incident light. For an optical system containing ten such surfaces, the transmitted beam will lose 1/3 of its intensity from reflection losses alone.

Surface reflection losses on an optical element can be reduced significantly by adding an antireflection (AR) coating. There are several types of antireflection coatings, the broadband single-layer coatings, broadband multilayer dielectric coatings, and V-coatings (narrowband multilayer dielectric coatings).

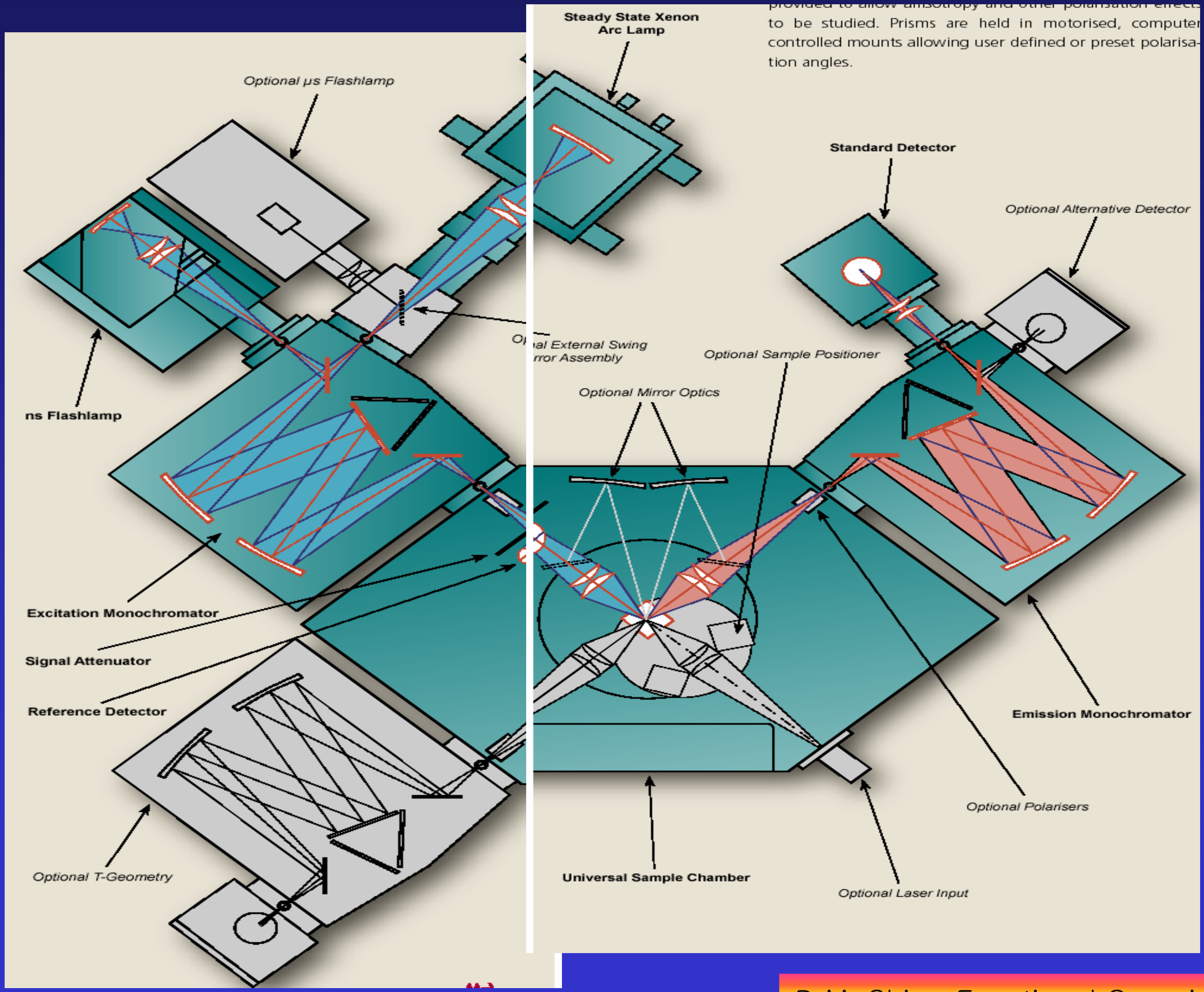
- **Single-layer coatings:** **Magnesium fluoride (MgF_2) is commonly used for single-layer coatings because of its almost ideal refractive index (1.38 at 550 nm) and high durability. At normal incidence, reflections are reduced to less than 2% per surface. A typical reflectance curve is shown below.**
- 

Photochemical Technique: Fluorescence Spectroscopy

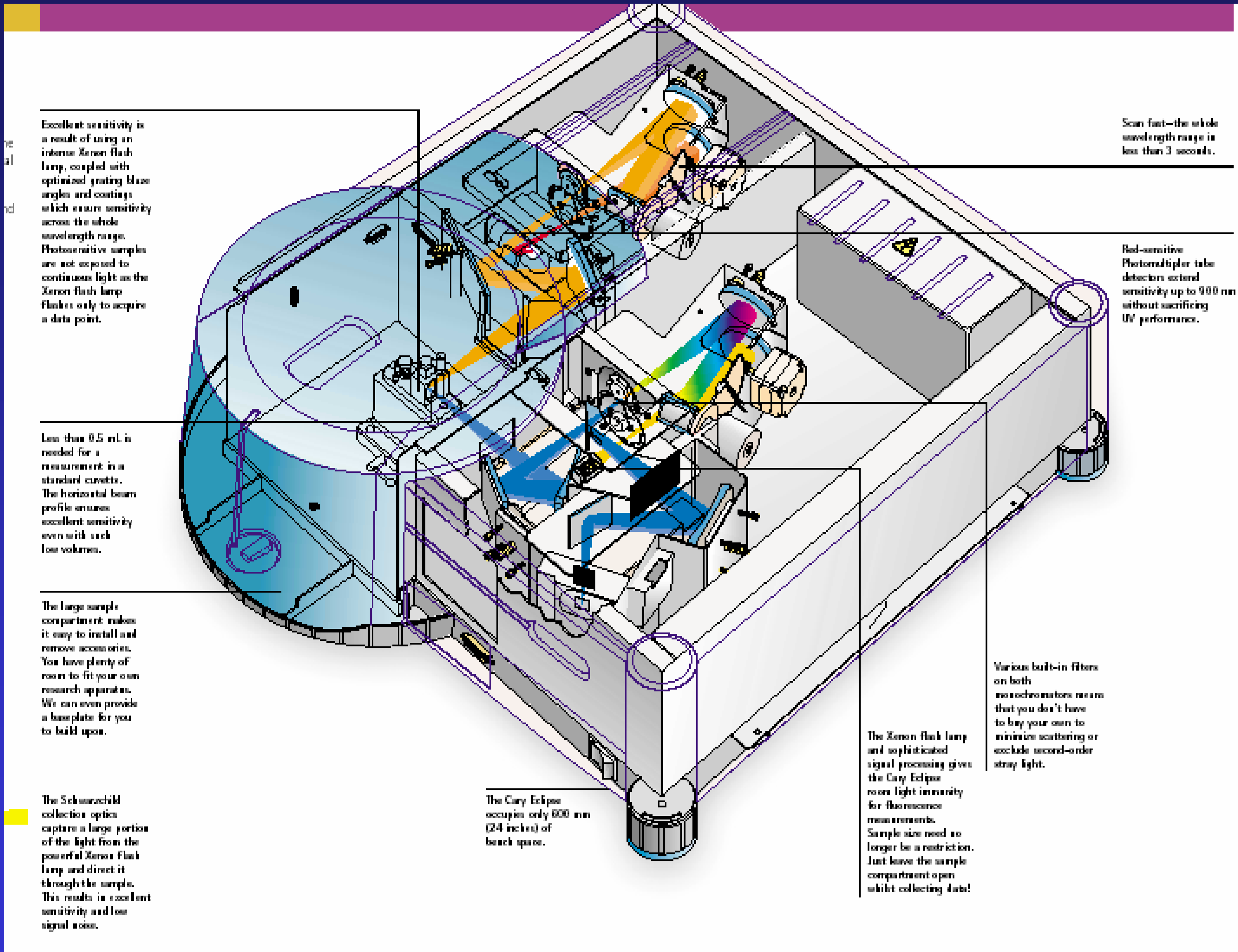




Photochemical Technique: Fluorescence Spectroscopy

- 

Photochemical Technique: Fluorescence Spectroscopy



Photochemical Technique: Fluorescence Spectroscopy

- **Emission Spectrum**

Excitation wavelength 고정

Emission scan

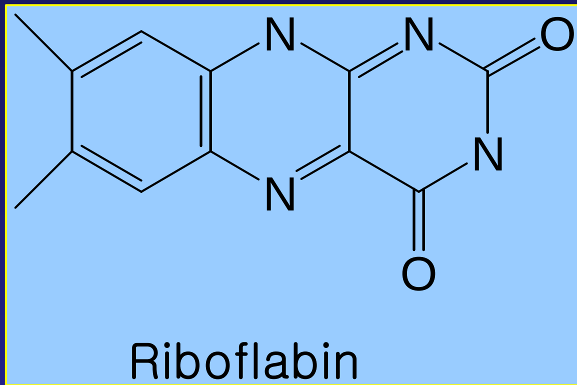
- **Excitation Spectrum**

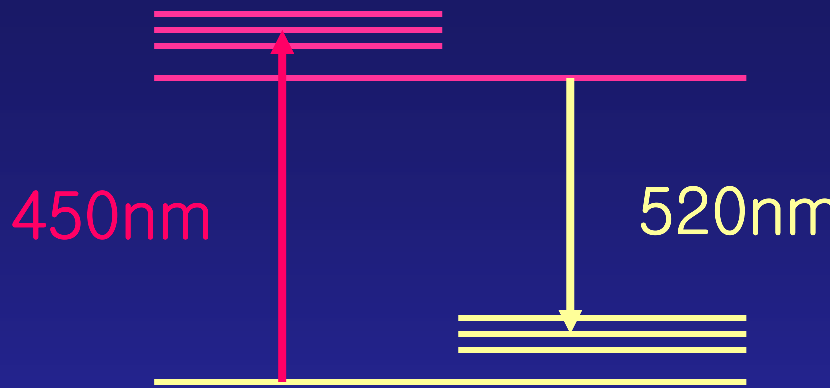
Emission wavelength 고정

Excitation scan

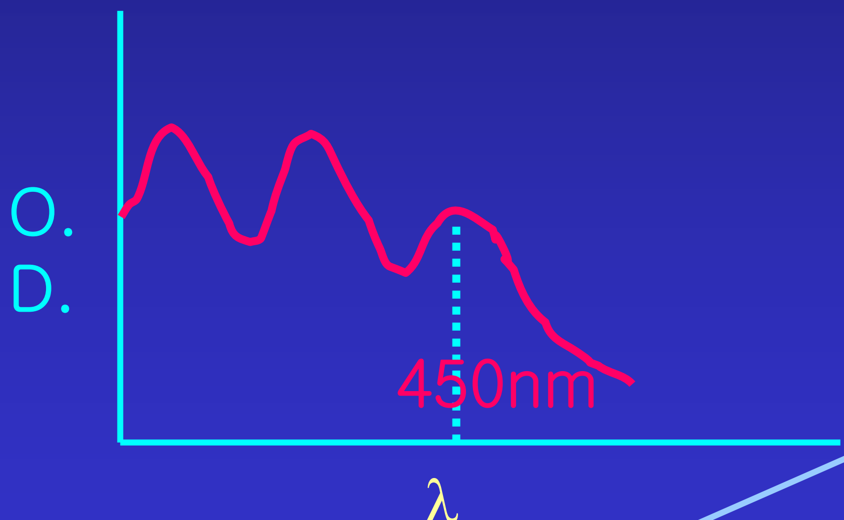
-

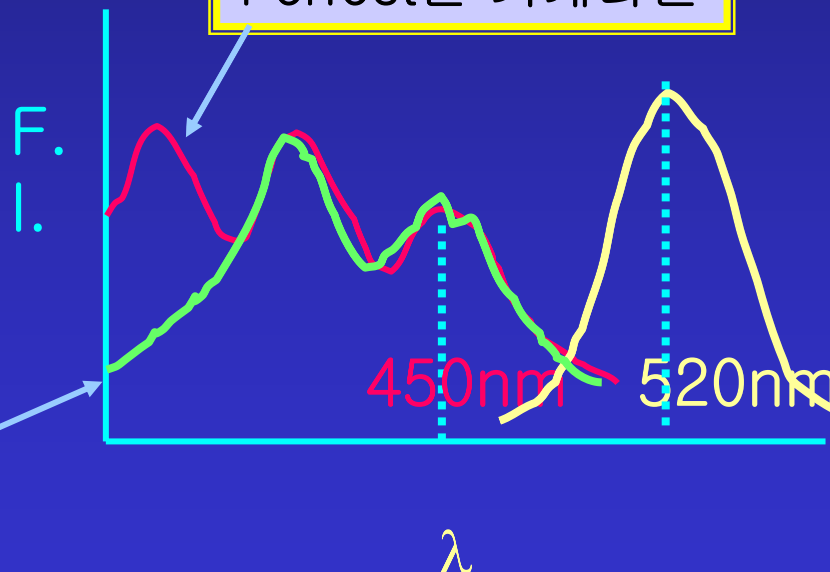
Photochemical Technique: Fluorescence Spectroscopy





Perfect한 기계라면





Correction 필요

– Excitation spectra obtnd at 520nm

$$I_F = I_0(1-10e^{-O.D})\Phi_F$$
$$\cong I_0 \times OD \times \Phi_F$$

(용액이 5% 이내로 충분히 희석된 경우에 아래식 성립)

- **Factors affecting I_F**

1. I_0
2. OD
3. Φ_F
4. PMT spectral response
5. Monochromator spectral response
6. Other optics spectral response

- **Spectral response corrections**

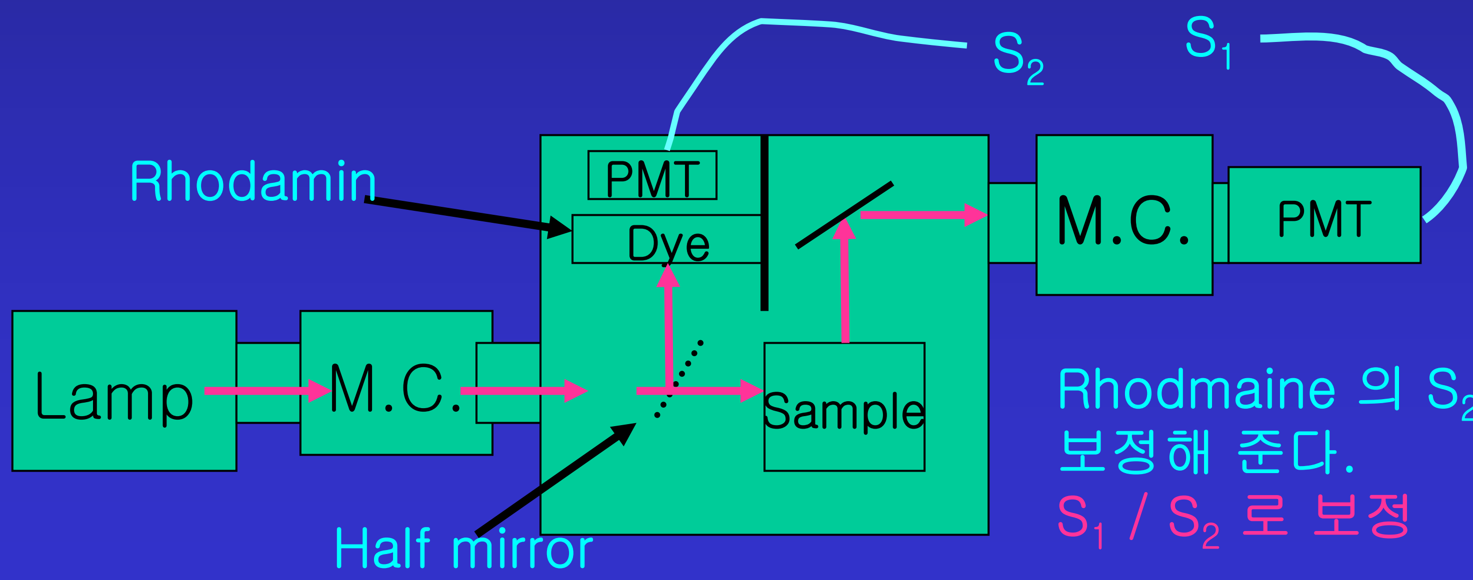
Lamp : Hg-Xe Arc

Fluctuation이 심하다.

I_0 : 시간에 따라 변하면 문제 발생

ex) 200nm에서 **600nm**로 **scan**시 **I_0** 같아야 한다.

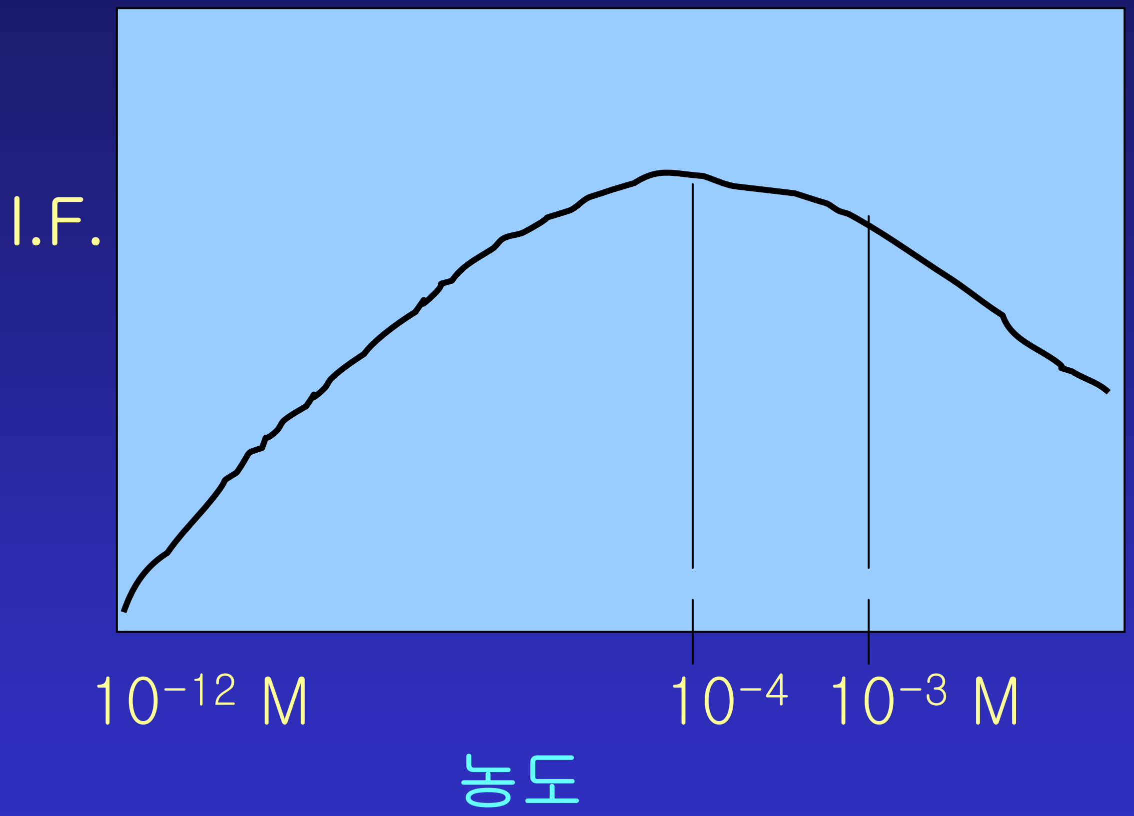
5시간 후에도 **I_0**가 같아야 한다.



Rhodmaine 의 S_2가 S_1을 보정해 준다.

S_1 / S_2 로 보정

- 문제: 다음과 같이 농도가 높을 때 형광이 줄어드는 이유는?



- 문제: 다음과 같이 농도가 높을 때 형광이 줄어드는 이유는?
- **Beers Law:**

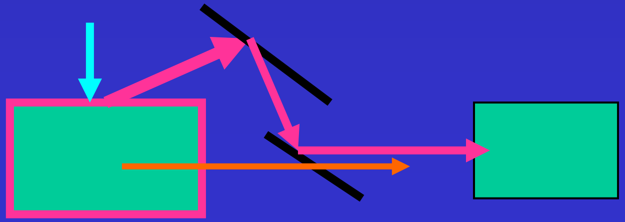
$$\log \frac{P_0}{P} = A = abc$$

- **a : 흡광계수 (extinction coefficient)**
- **b: path length c: concentration**
- 예)

$$A = 0.8, \quad \frac{P_0}{P} = 10^{0.8} \quad \mathrm{T} = \frac{P}{P_0} = 0.16 \quad 16\%\text{통과}$$

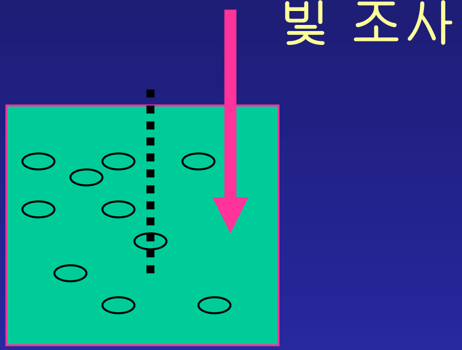
$$A = 2.0, \quad \frac{P_0}{P} = 10^{2} \quad \mathrm{T} = \frac{P}{P_0} = 0.01 \quad 1\%\text{통과}$$

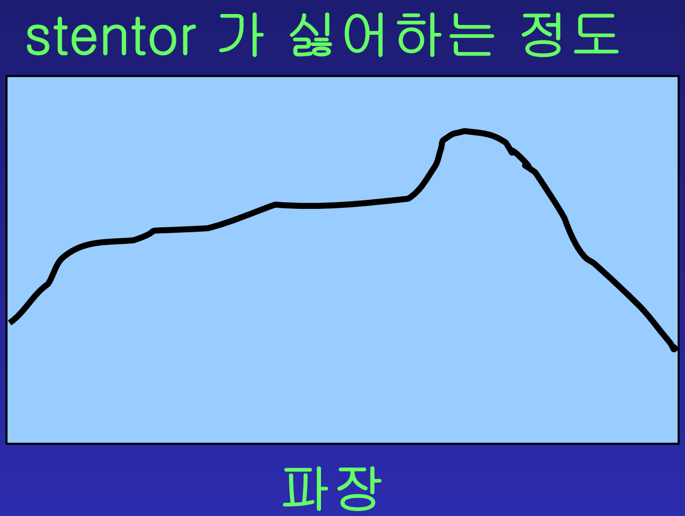
- 표면에서 **(front surface)**에서 빛이 모두 흡수된다.



front surface에서 나오는 빛을 모아서 **detect**한다.

- Action Spectra
- 형광을 내는 물질 또는 생물 소자의 파장에 따른 특성
-



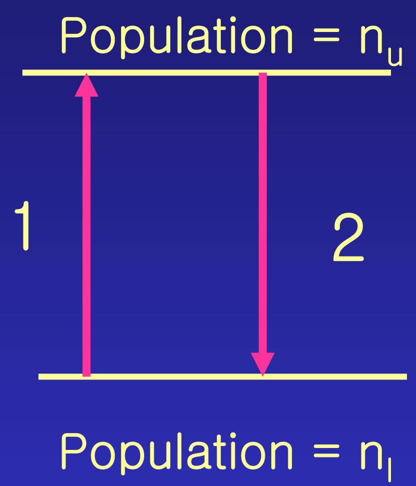


Photochemical Technique: Laser

- **Light Amplication by Stimulated Emission of Radiation**

Stimulated Emission

(일반적인 emission은 spontaneous emission이다)



$$\textit{Rate}\ \text{of absorption} = n_l B_{lu} \rho$$

n_l : # of molecules in the lower state

B_{lu} : Einstein Coefficient

ρ : a given radiation density

$$\textit{Rate}\ \text{of emission} = n_u B_{ul} \rho$$

n_u : # of molecules in the upper state

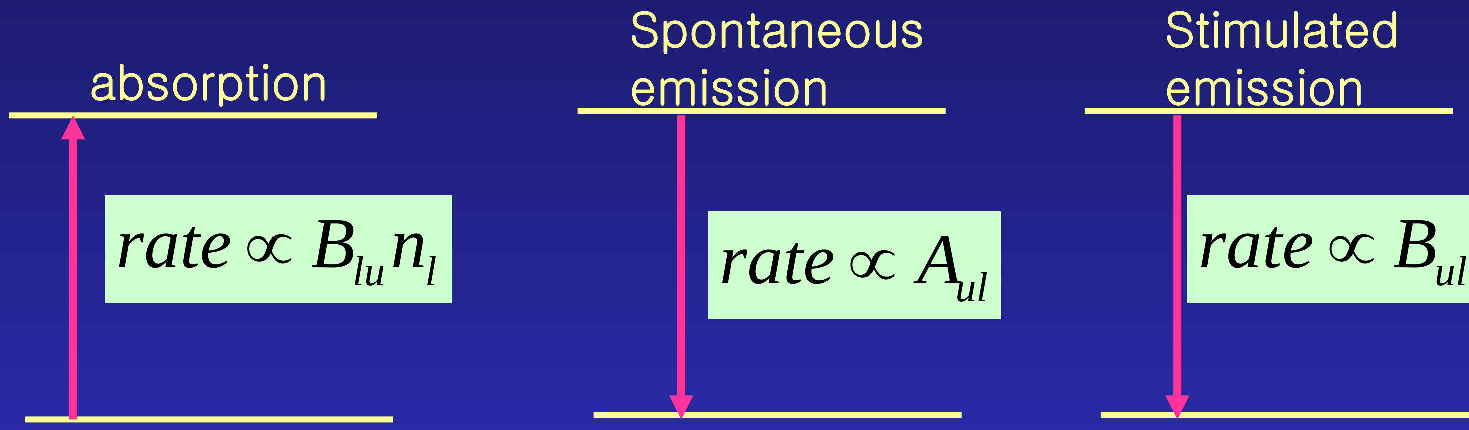
$$B_{ul} = \frac{64\pi^4 \nu^3}{3hc^3} \langle \psi_u | \mu | \psi_l \rangle^2$$

Normally $n_\ell > n_u$

Ref: Handbook of Organic Photochemistry
J. C. Scaiano CRC Press 1989, Vol 1.

Photochemical Technique: Laser

- If 2 process is suppressed,
- In other words: rate of absorption > rate of emission



$$rate \propto B_{lu} n_l$$

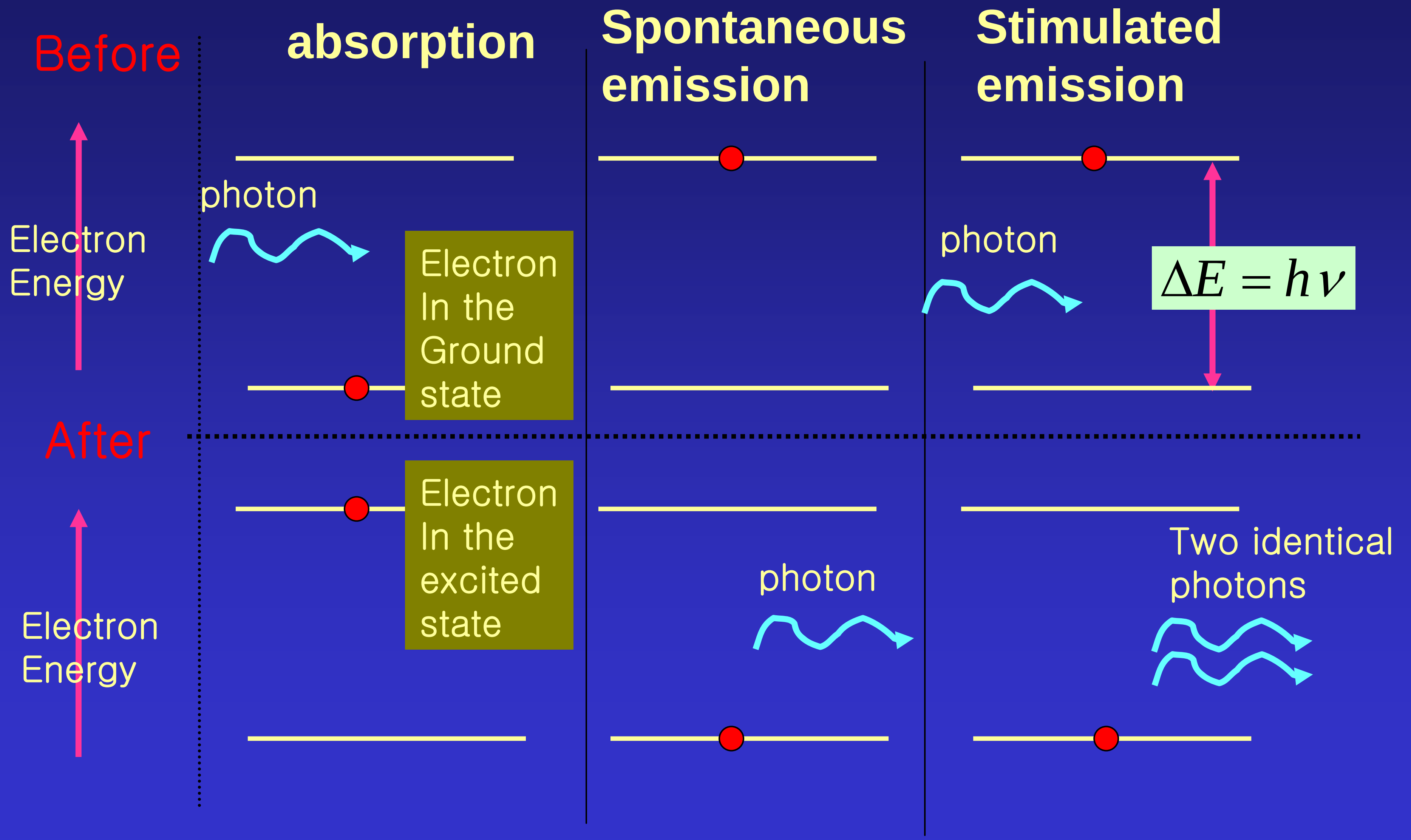
$$rate \propto A_{ul}$$

$$rate \propto B_{ul} n_u$$

Then $n_l < n_u$

Ref: Handbook of Organic Photochemistry
J. C. Scaiano CRC Press 1989, Vol 1.

- **Three Types of Optical Transitions**

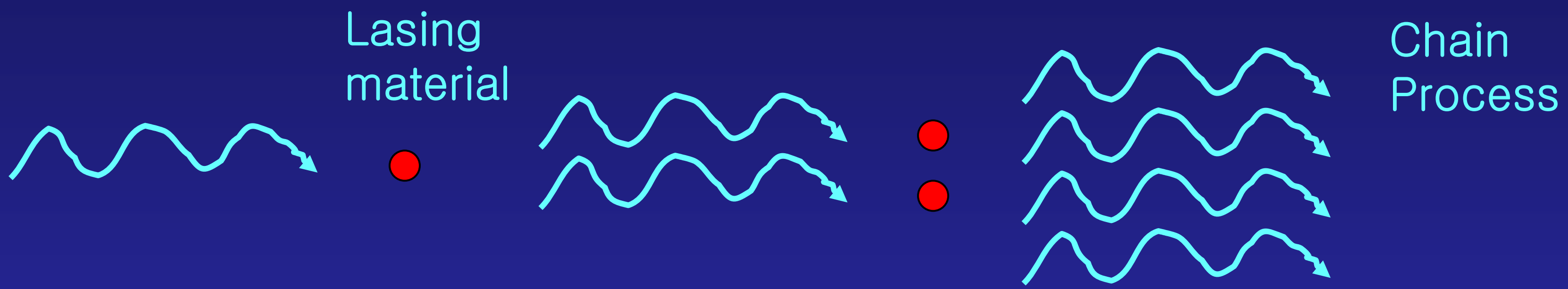


- If $n_u > n_l$, there is possibility of average overall amplification for an array of photons passing through a volume of these particles.

- Spontaneous emission depletes n_u at a rate proportional to A_{ul}, which is coefficient of spontaneos emission.

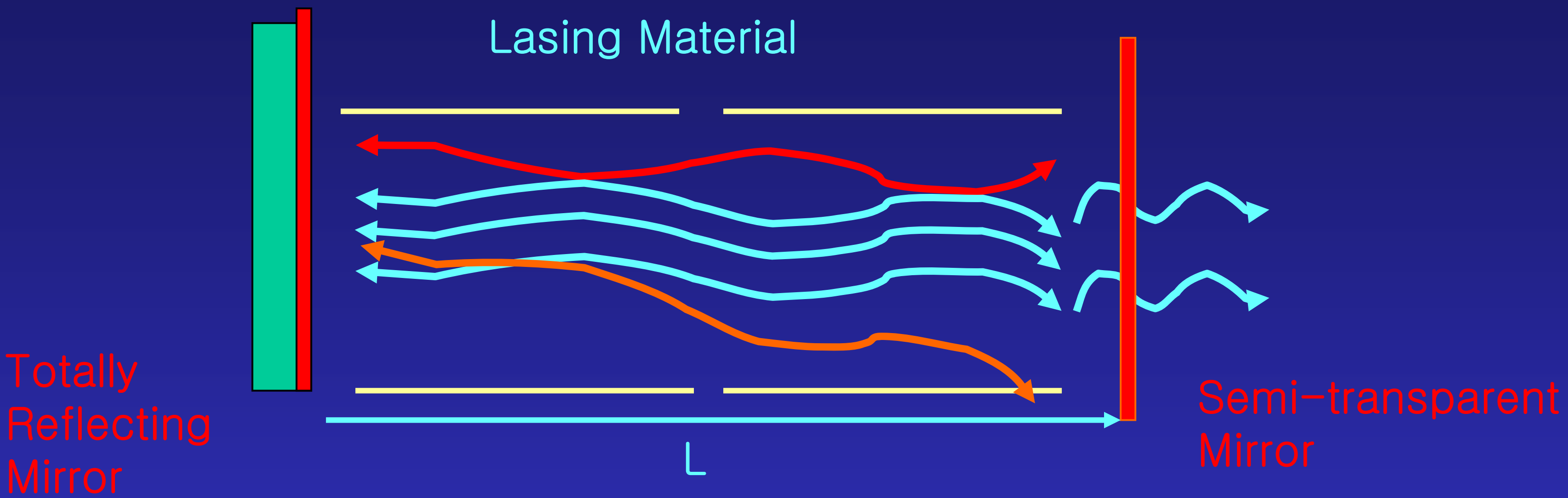
$$A_{ul} = \frac{8\pi h \nu^3}{c^3}$$

- **Schematic Diagram of a Laser**



- 한 순간에 **population inversion**을 없애고 빛을 내 보낸다. **(**약 **1 μsec)**

- **Schematic Diagram of a Laser**



- **Two parallel mirrors are separated by**

$L = n(\frac{1}{2}\lambda)$ 이때 $E_u - E_l = hc/\lambda$를 만족

- **Light reflected from a mirror will be in phase with the indident wave (constructive interference)**

- **Characteristics**

All lasers produce intense beams of light that are monochromatic, coherent, and highly collimated. The wavelength (color) of the laser light is extremely pure (monochromatic) when compared to other sources of light, and all of the photons (energy) that make up the laser beam have a fixed phase relationship (coherence) with one another. This causes the light to form a beam with a very low rate of expansion (highly collimated) that can travel over great distances, or can be focused to a very small spot with a brightness that can approximate that of the sun. Because of these properties, lasers are used in a wide variety of applications in all walks of life

Characteristics

1. **Coherence**

   Phase가 다르면 destructive interaction에 의하여 소멸된다. In-phase만 남는다.

   Phase 뿐만 아니라, mirror와 mirror사이가 standing wave가 되도록 조정되어 있다.

2. **Highly monochromatic**

   한가지 frequency 만 증폭되게 된다.

3. **Accurate parallelism**

   두개 거울에 수직이 되지 않는 빛은 cavity에서 튀어나간다.

   Beam size 작고, E가 크다 (short pulse laser, 10^9 W/cm^2).

4. **Brightness**

   10^{-3} W ~ 10^{12} W power를 갖는다.

Photochemical Technique: Laser 종류

Attribute	HeNe		Ion		Diode		HeCd		DPSS	
	λ (nm)	P (mW)	λ (nm)	P (mW)	λ (nm)	P (mW)	λ (nm)	P (mW)	λ (nm)	P (mW)
Wavelength/Power	543 594 612 633	3 5 7 40	457 488 514 568 647 752	50 150 150 20 100 20	405 408 440 635 640 660 685 780 785 830	1 24 1 3 12 22 15 7 35 40	325 442	55 130	430 442 457 473 532 1064	10 10 400 15 3000 5000
Beam Quality	Excellent		Very Good		Good		Very Good		Very Good	
Cooling	Convection		Forced Air		Convection		Forced Air		Convection/ Forced Air	
Efficiency	Good		Poor		Excellent		Good		Very Good	
Battery Operation	Yes		No		Yes		No		Yes	

Photochemical Technique: Laser – 파장별

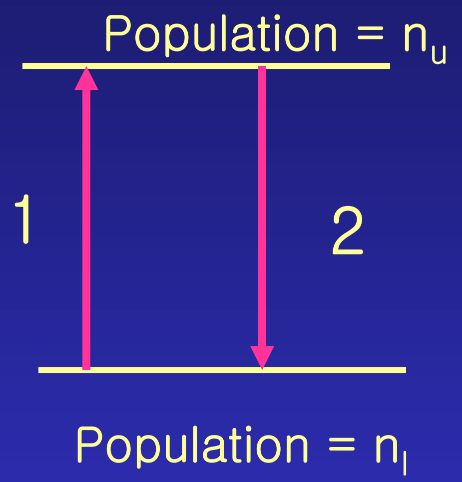
Spectral Region	Available Wavelengths (nm)	Output Power (mW)
Multiline Visible	**457 – 647**	**25 – 400**
Tunable Visible	**454 – 647**	**4 – 195**
Violet	**405, 430, 440, 442, 457**	**1 – 130**
Blue	**457, 473, 488**	**4 – 400**
Green	**514, 532, 543**	**0.2 – 3000**
Yellow	**568, 594**	**0.35 – 20**
Orange	**612**	**0.5 – 2**
Red	**633, 650, 685, 647**	**0.5 – 100**
Near Infrared	**752, 830, 1523**	**0.8 – 20**

Laser와 polpulation inversion 방법

- **Two Level**

$$B_{lu} \approx B_{ul} \qquad \rho : \mathrm{same}$$

$$n_u \approx n_e$$

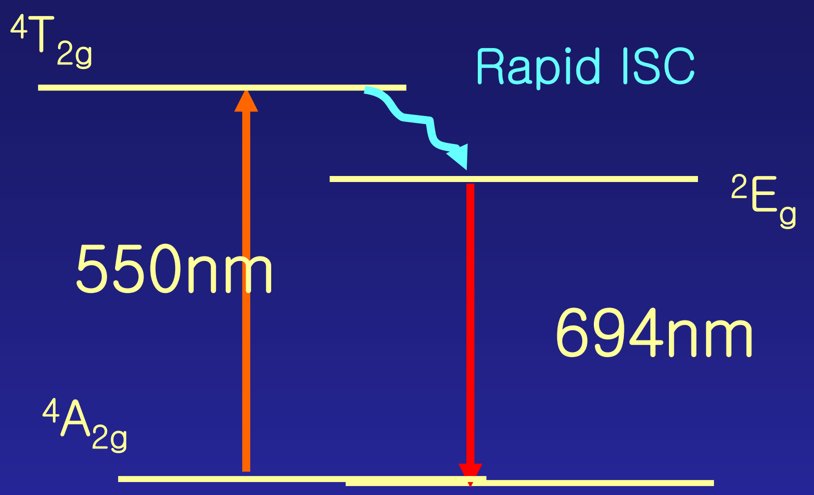


- **따라서 population inversion 이 어렵다.**

-> Three level system으로

Laser와 polpulation inversion 방법

- **Three Level**



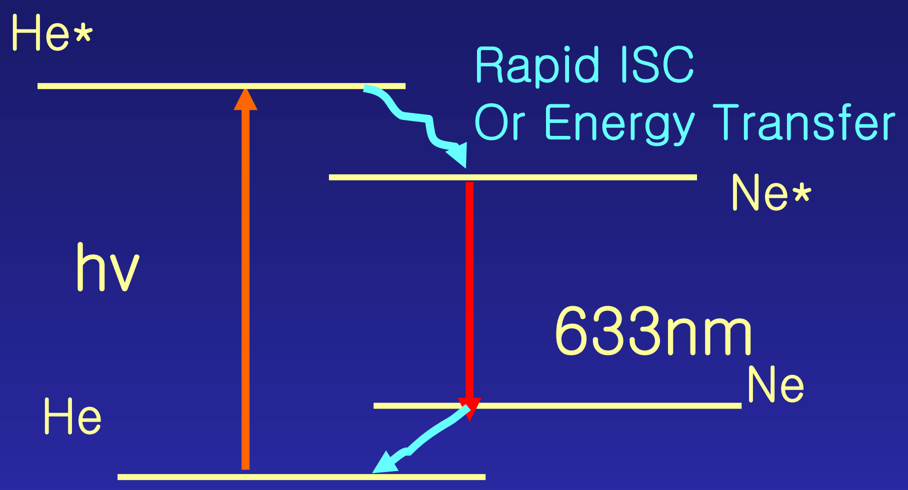
Energy levels for Cr^{3+} ions in Luby Laser.

- **Rapid intersystem crossing populates the ^2E_g state.**

- **The characteristic red laser emission occurs from the ^2E_g state.**
- 문제점**: inefficient more than 50% of Cr ion** 이 ^2E_g**state**로 여기되어야만 **lasing.**

Laser와 polpulation inversion 방법

- **4 Level system**



Energy levels for He-Ne Laser.

- 이런 경우 계속해서 **population inversion**이 가능하므로 **continuous operation**이 가능하다**.**

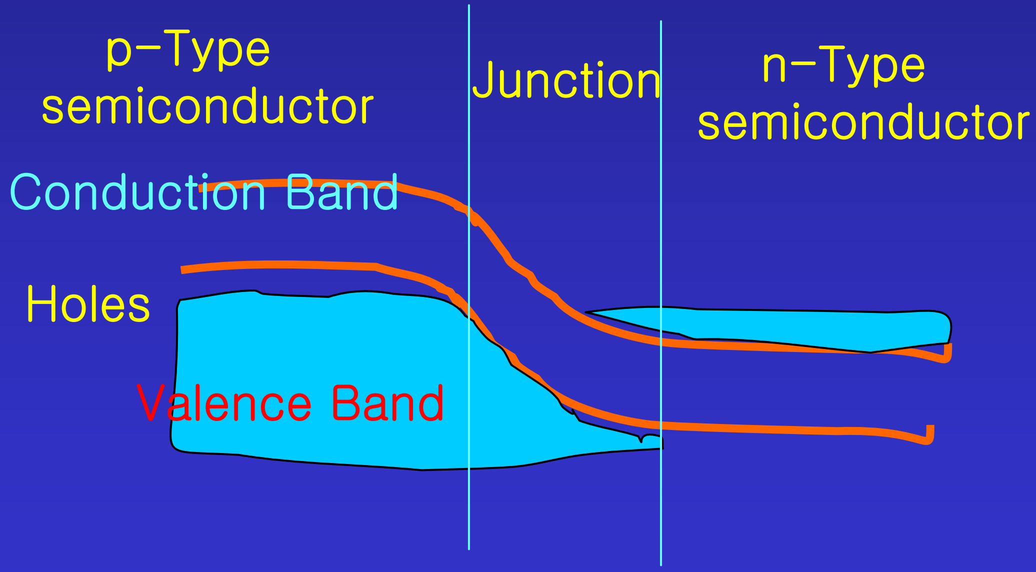
Photochemical Technique: Laser

Laser와 polpulation inversion 방법

Lasing at Simple semiconductor juctions

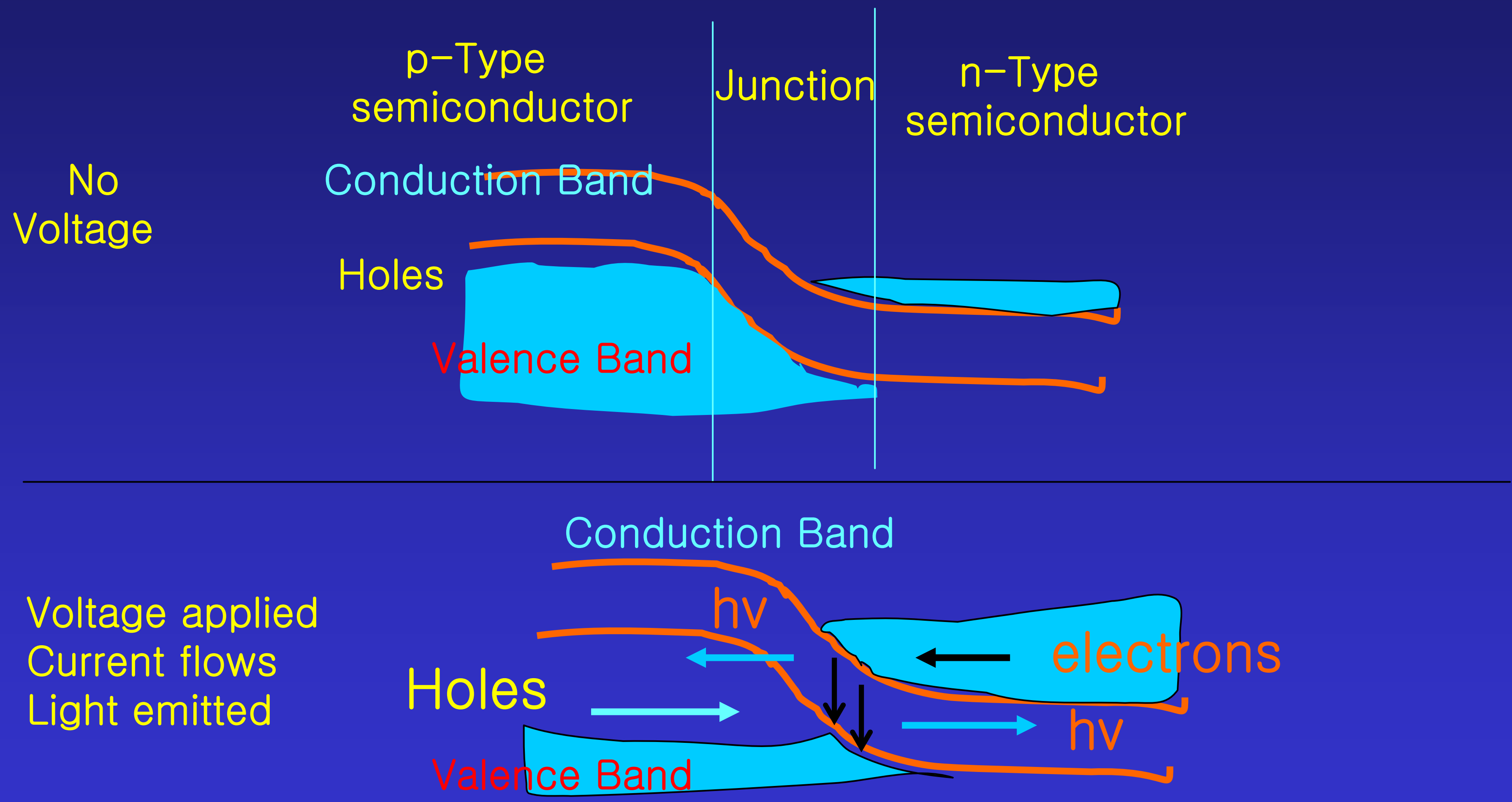
- **No voltage applied**

- **Missing e$^-$, mobile holes** **extra mobile e$^-$,**



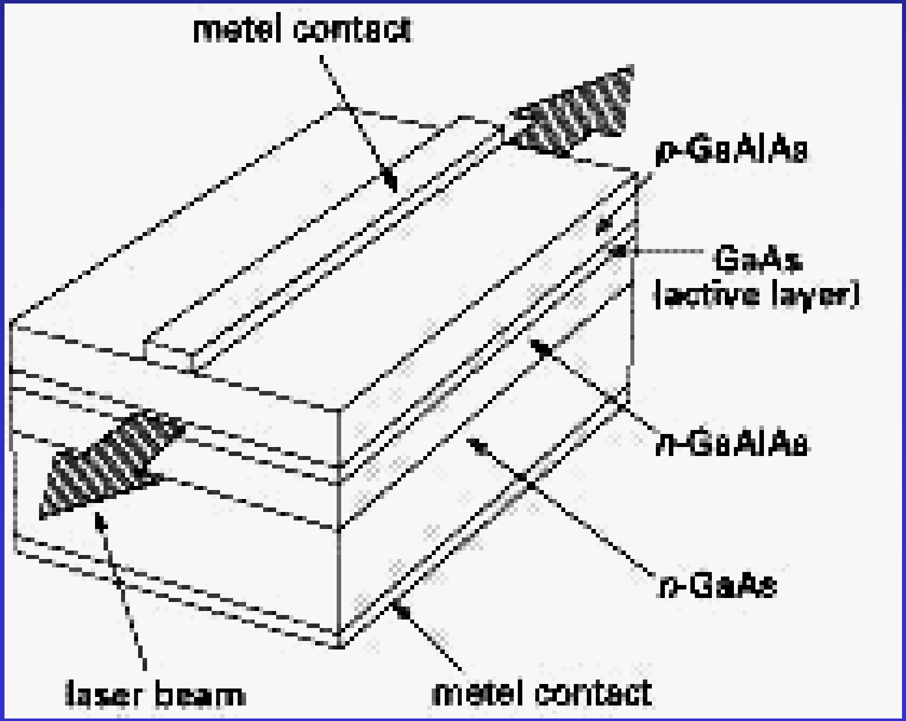
Laser와 polpulation inversion 방법

Lasing at Simple semiconductor juctions



- **When current flows across the junction, holes and electrons recombine: electrons relaxed from the conduction band to valence band. The relaxation energy is released as photons.**

- 여기에 **p-n junction**이 잘 **doping** 되고**,** 높은 전류를 걸면**, population inversion of electrons and holes can be induced in the junction region.** 이것을 **optical cavity**에 걸면 **laser.**

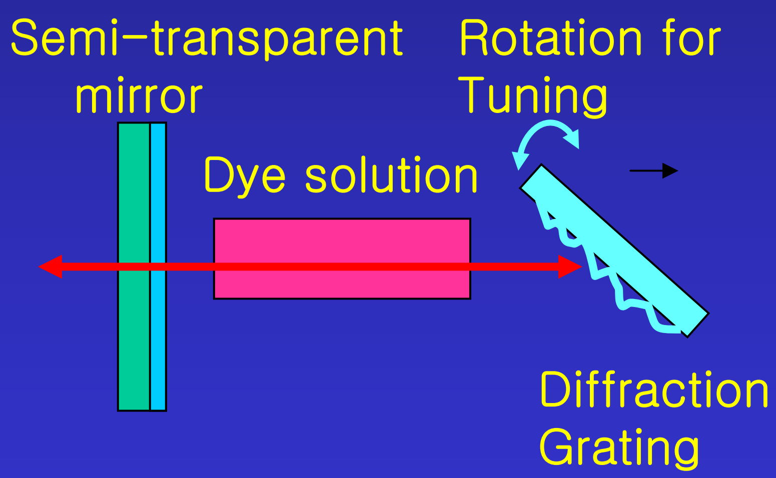


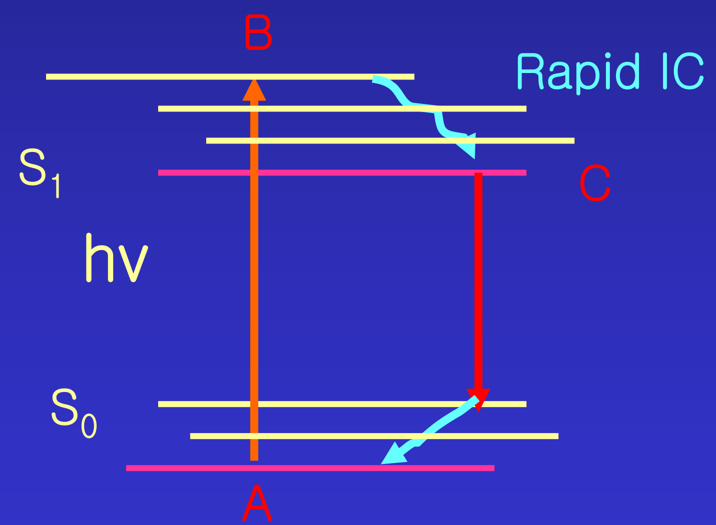
They can be tuned.

Wavelength of their light output can be varied.

(often over as much as 50 nm)

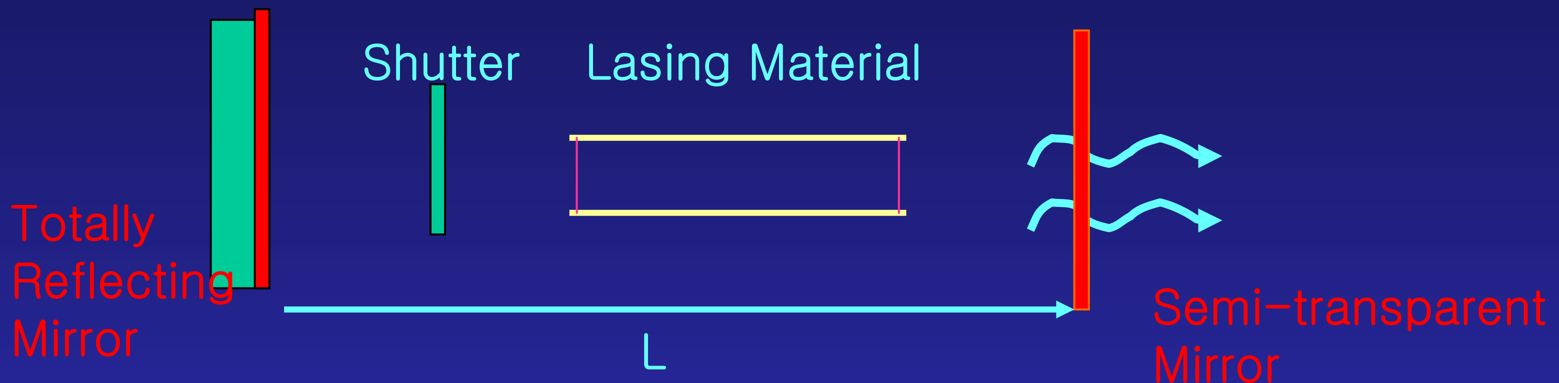
- **Dye laser**는 **2 level system** 같아 보이나 사실상 **4 level system**이다. 따라서 **continuously operate**가능





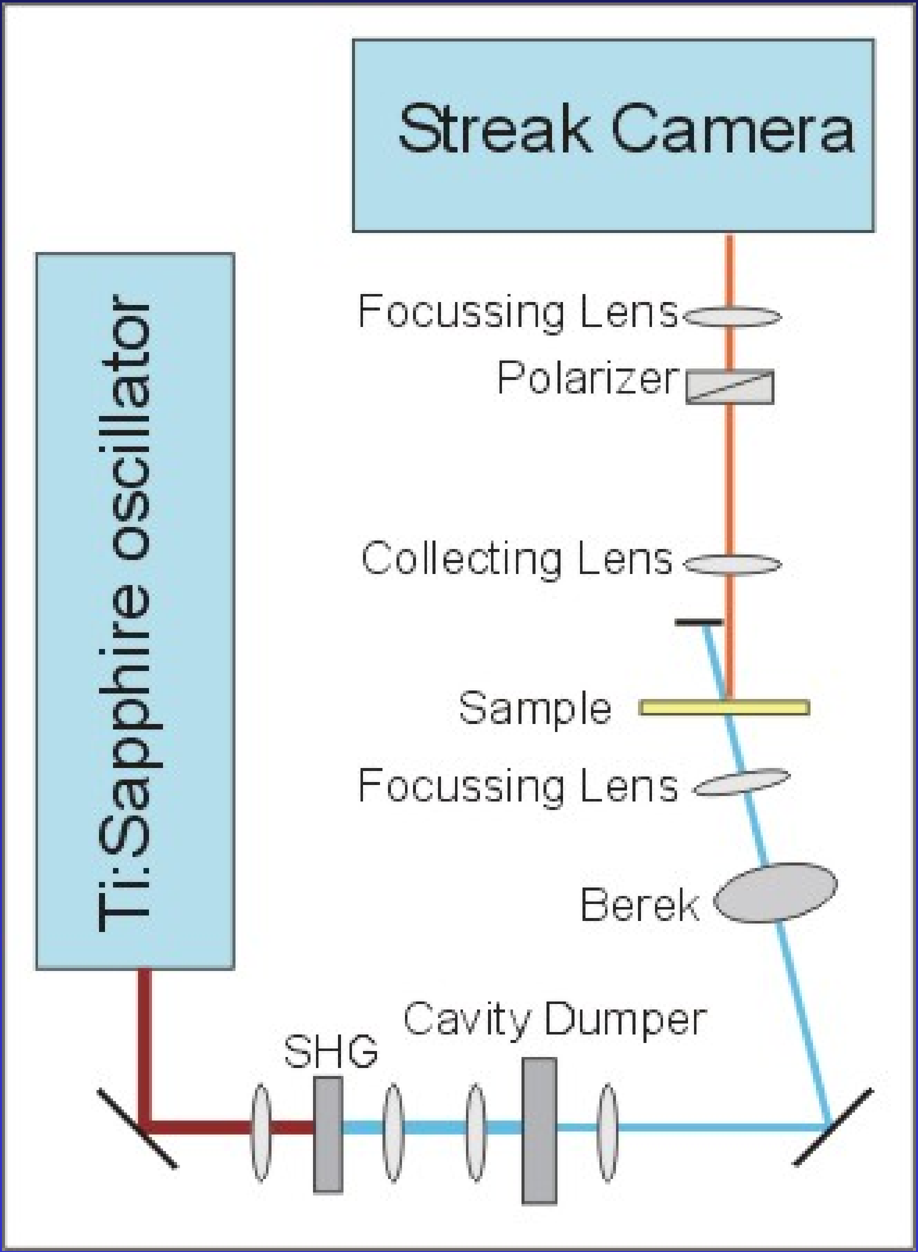
Energy levels for Dye Laser.

• Q-switching Technique



- **Simple laser**는 약 **1 μsec** 정도의 **pulse** 를 갖는다**.** 이유는 **laser emission**이 **population inversion**이 달성되어야만 생기기 때문**.**
- **Shutter opens with extreme rapidity.**
- 처음에는 **shutter closed & radiative absorption (**이 경우 **amplification** 은 거의 없다**.)**
- **Shutter** 갑자기 열면 **amplification**이 갑자기 상승**.**
- 저장되었던 **energy**들이 **giant pulse**로 되어서 **release.**
- **1 J**의 **ruby laser**가 **Q-switch**로 **10ns duration, peak power 100MW**의 **pulse** 발생

형광 lifetime 측정 Set-up의 하나.

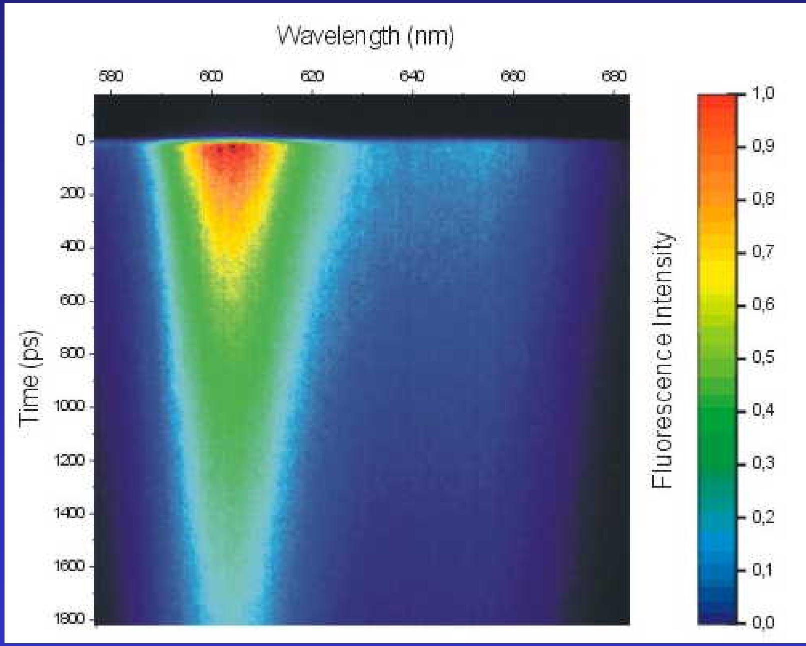


The experimental setup in figure 1 has - besides the streak camera itself - four main elements:

- A BBO-crystal that is used to frequency double (SHG) the light from the Ti:Sapphire oscillator.
- A cavity dumper that reduces the repetition rate from 82 MHz to 4 MHz. This ensures that there will not be an overlap in the fluorescence decay from two consecutive pulses in molecules with a long lifetime.
- A polarizer that is fixed to transmit vertical polarized light only since the sensitivity of the streak camera depends on the polarization.
- A Berek polarization compensator that is used to turn the polarization of the excitation light. It is thereby possible to detect fluorescence parallel, at magic angle (54.7°), and perpendicular relative to the excitation pulse without turning the polarizer in front of the streak camera. This is highly convenient in anisotropy measurements.

The Streak Camera - general facts

The streak camera used in the department uses a Hamamatsu C 4742-95 camera. The streak camera enables detection of the fluorescence as a function of the spectral and the time evolution simultaneously. The fluorescence can be detected in two spectral windows with the width of 50 nm or 100 nm. There are six different time windows to choose from, varying in size from 100 ps up to 2 ns. The time resolution in the shortest window is 1 ps, when the system is well aligned.



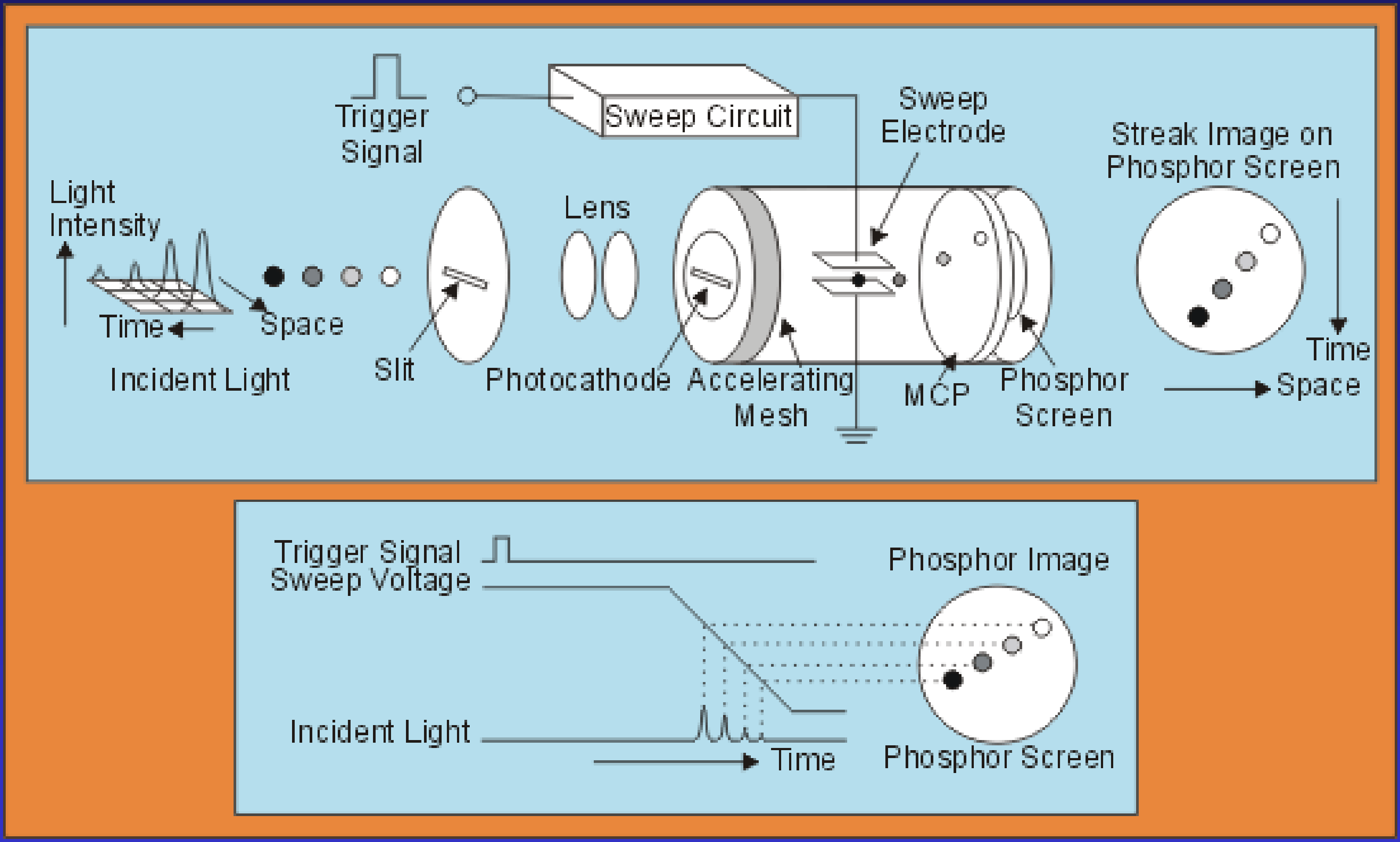
An example of a 3D image obtained with the streak camera. The streak camera measures the fluorescence intensity -illustrated with the colours, increases from black/blue towards red- as a function of both wavelength (horizontally) and time after excitation (vertically).

The principles of the streak camera

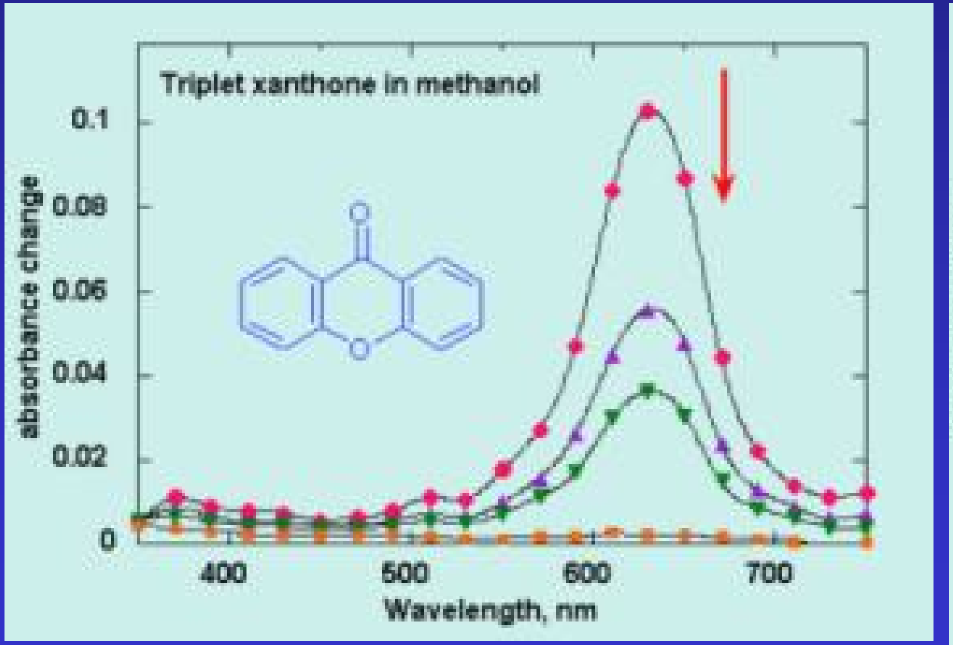
The Ti:sapphire oscillator excites the sample and it emits fluorescence.

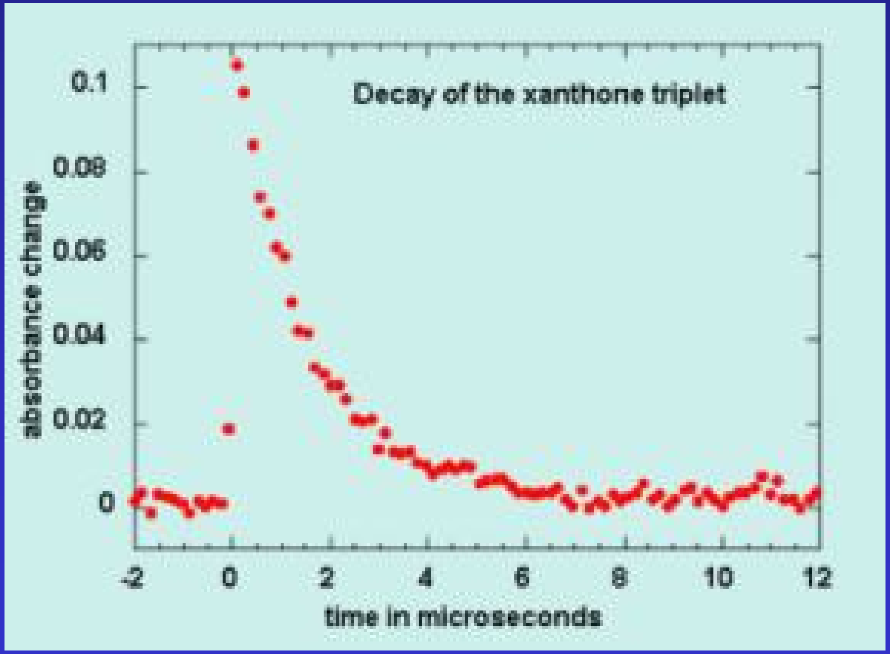
The fluorescence from the sample is distributed in space by a grating giving a 3D picture of the incident light, which is projected on the slit. After passing through two lenses, the light hits a photocathode connected to a mesh plate that accelerates the generated electrons. A sweep circuit - synchronized to the laser pulses - deflects the electrons onto the multi channel plate (MCP) depending on the moment of their emission, thereby introducing the time resolution. Hence the photons emitted directly after excitation experience a higher voltage then the photons emitted at a later time. The signal is then transferred to a phosphor screen and detected by a CCD camera yielding a 3D picture.

The principles of the streak camera



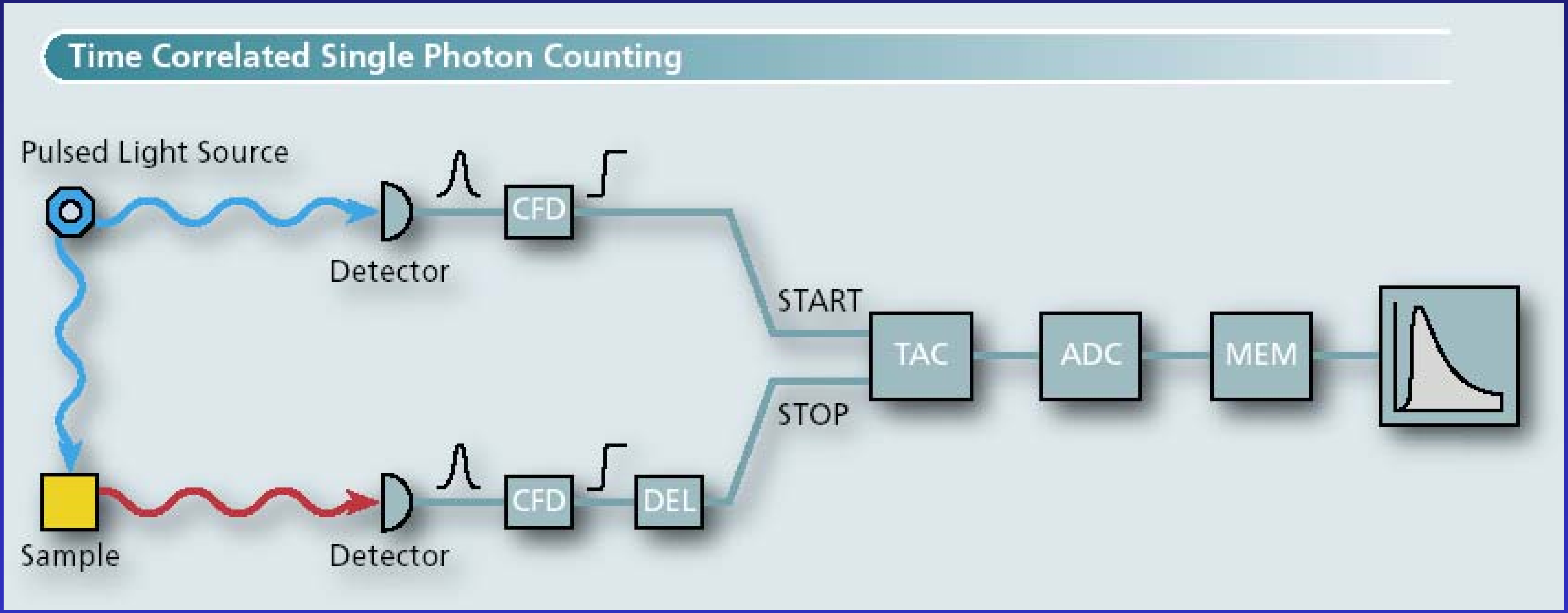
일반적인 **PMT**를 사용하거나 **PDA**를 이용하였을 경우 얻어지는 **spectrum**의 형태

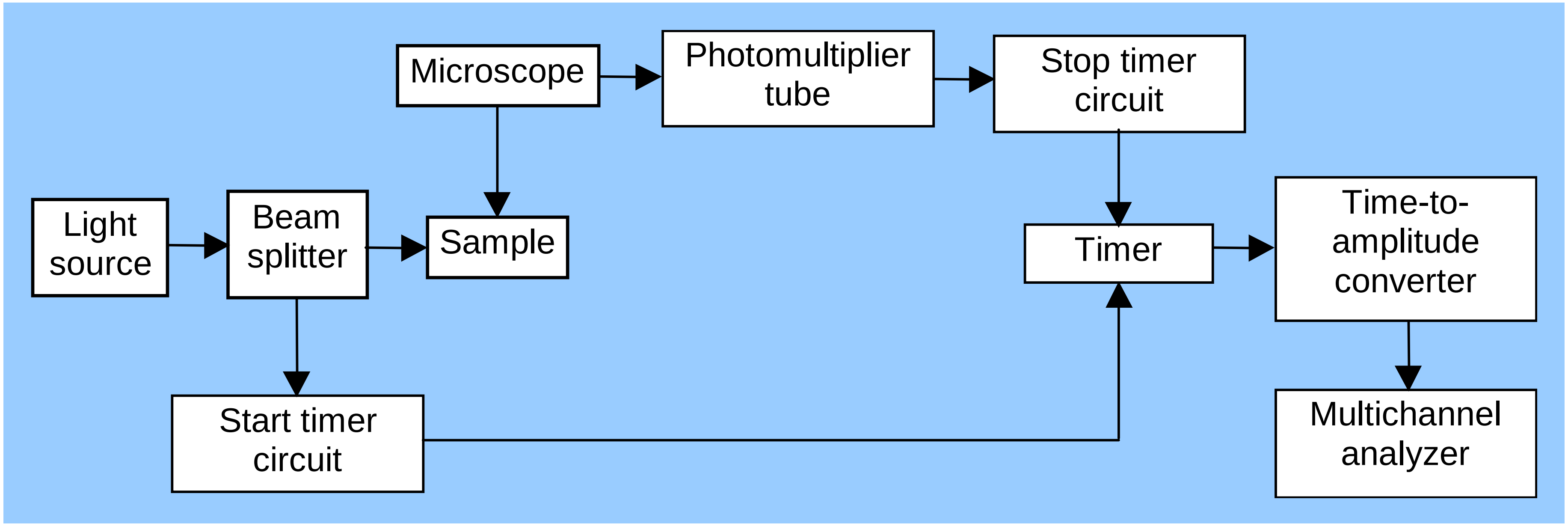




Photochemical Technique: Time Correlated Single Photon Counting

•

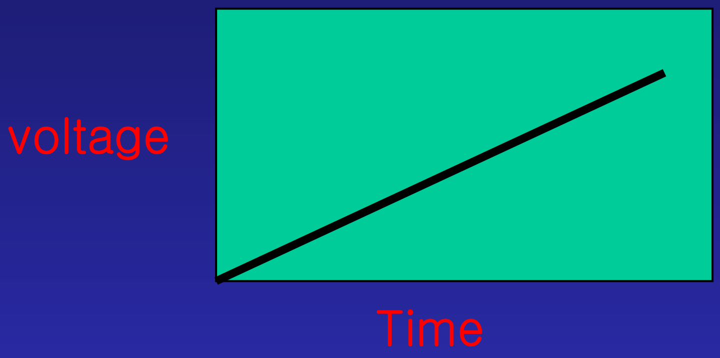




Time-correlated single photon counting system block diagram. When a light source provides an excitation pulse, some of the light is deflected to the start timer circuit while the rest illuminates the sample. A single fluorescent photon is detected by a photomultiplier tube, which generates a pulse that stops the timer. The time difference is then converted to an amplitude. The various amplitudes are recorded in the multichannel analyzer and a profile of different time intervals versus number of photons in that interval are displayed.

- **Time to amplitude converter (TAC)**

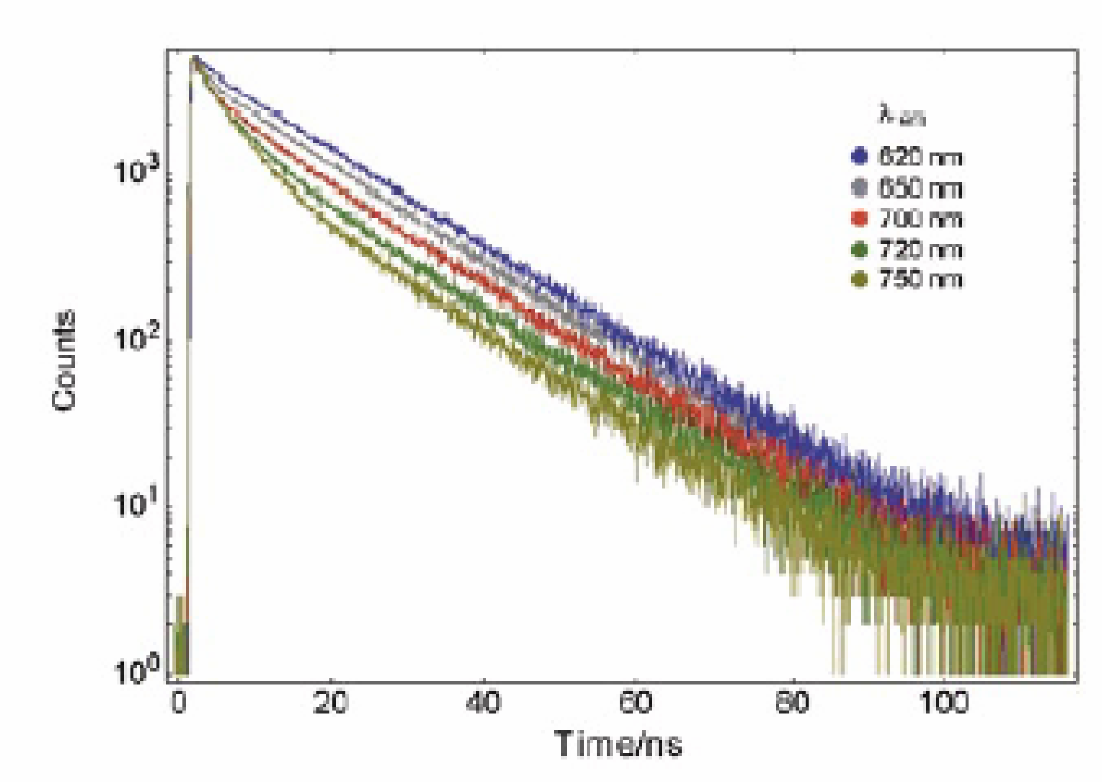
Start signal이 **TAC on** 시키면 **voltage** 증가하기 시작한다.



Stop signal 이 도착하면 **TAC off** 이 때 **voltage**를 읽으면 이것이 시간으로 전환된다.

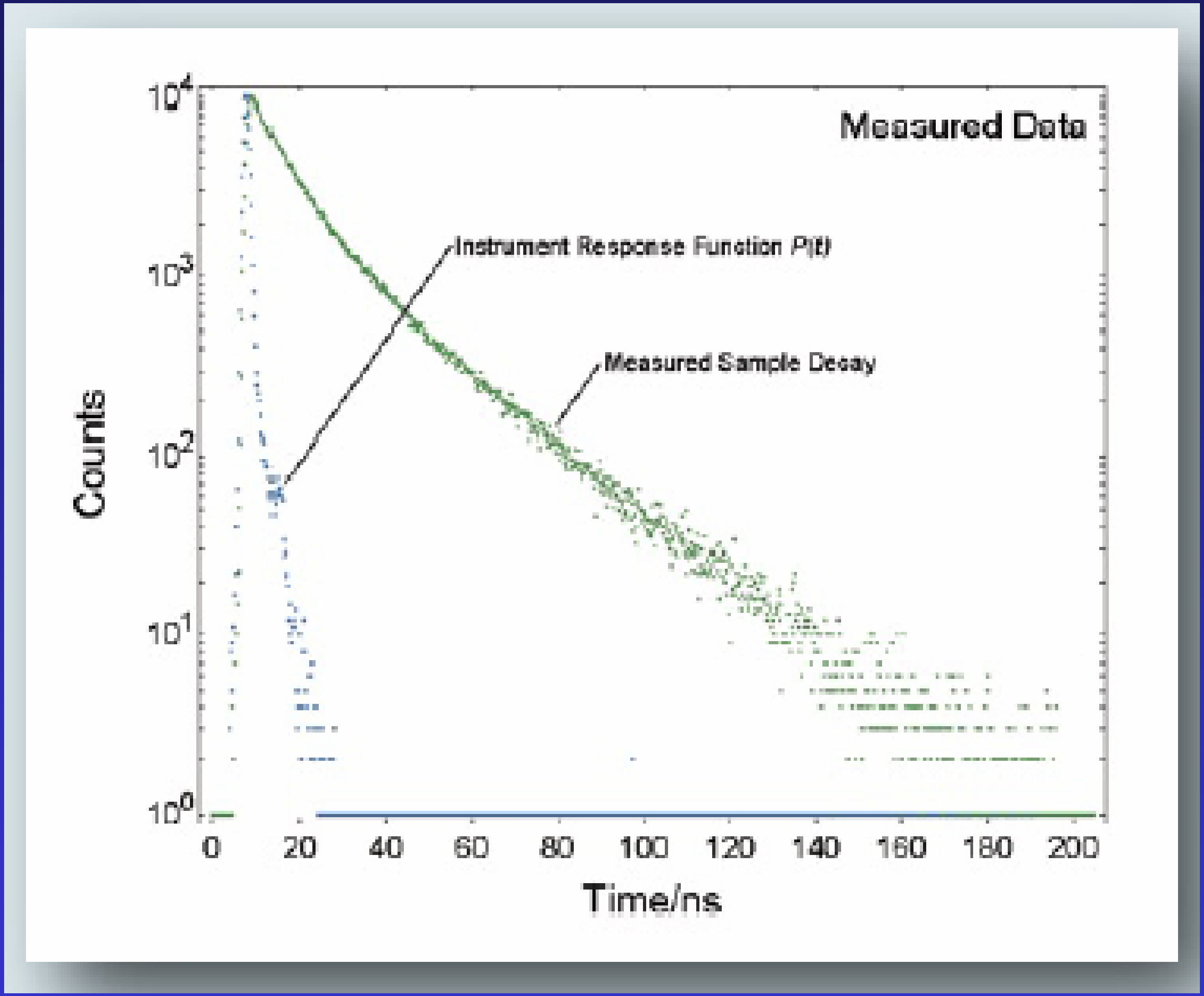
Short lifetime의 경우 **short pulse laser**를 사용한다. **(**예**: argon-ion laser) – single photon**만 측정하기 때문에 **laser**의 **power** 는 중요하지 않다.

Photochemical Technique: SPC



Sample: Hematoporphyrin IX in water at pH 7.2.
Measurement Conditions: Picosecond Diode Laser, rep.rate = 1MHz, MCP-PMT Detector, λ_{ex} = 400nm
Emission changes from single lifetime of 14.8ns at 620nm to a triple exponential decay of 0.8ns, 4.4ns and 14.8ns at 750nm

Photochemical Technique: SPC



Photochemical Technique: SPC

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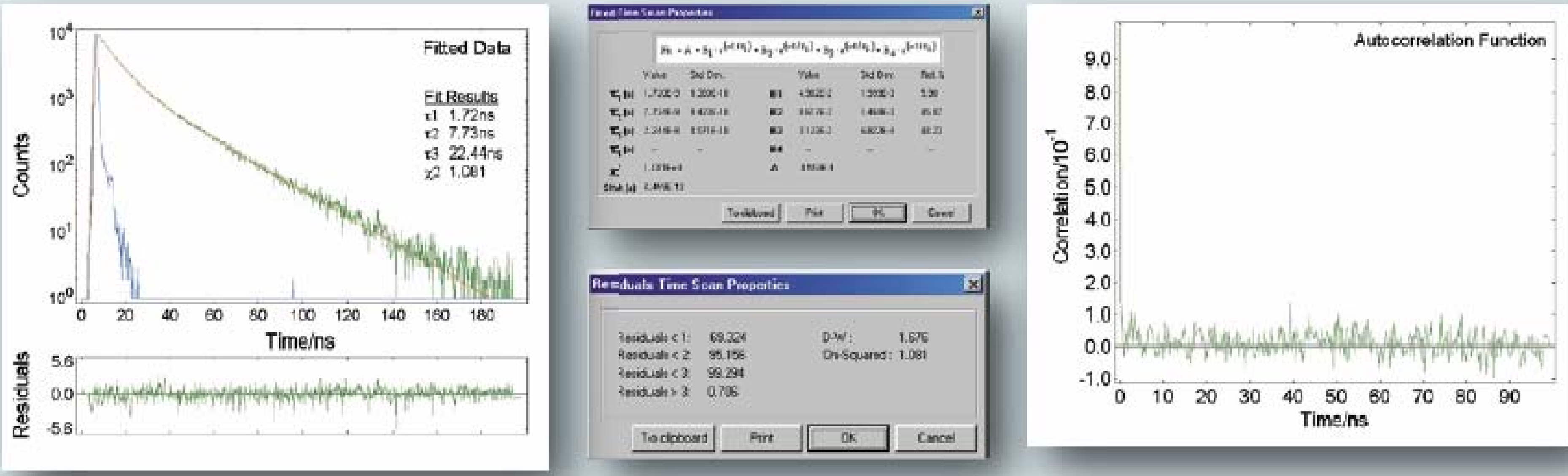
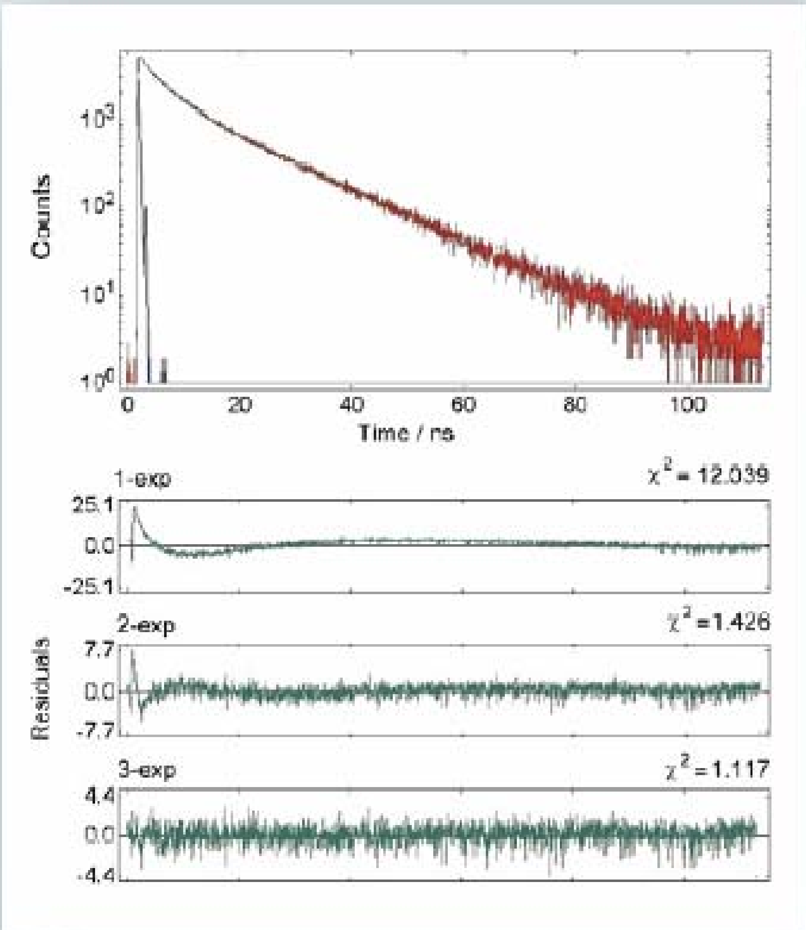


The correct fit is found for three exponential decays with a χ^2 value of 1.081, a clean autocorrelation function, more than 99% of all data points falling within 3 standard deviations and a Durban Watson parameter of 1.676.

Photochemical Technique: SPC

•



Sample: Hematoporphyrin IX in a phosphate buffer (pH 7.2)

Measurement Conditions: picosecond diode laser, λ_{ex} = 398nm, rep. rate = 1MHz, MCP-PMT detector, λ_{em} = 720 nm

Fit Result: (relative fluorescence contribution in brackets)

τ_1 = 14.80 ± 0.06 ns (69.69%)

τ_2 = 4.62 ± 0.09 ns (27.11%)

τ_3 = 0.81 ± 0.05 ns (3.20%)

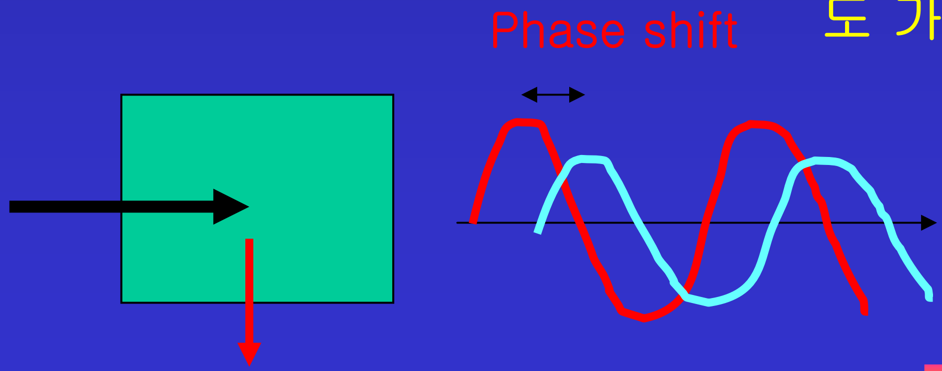
• Time Domain vs Frequency Domain

TCSPC

- Excitation by pulse
- Directly measures entire decay behavior
- Improve readings by increasing measurement time

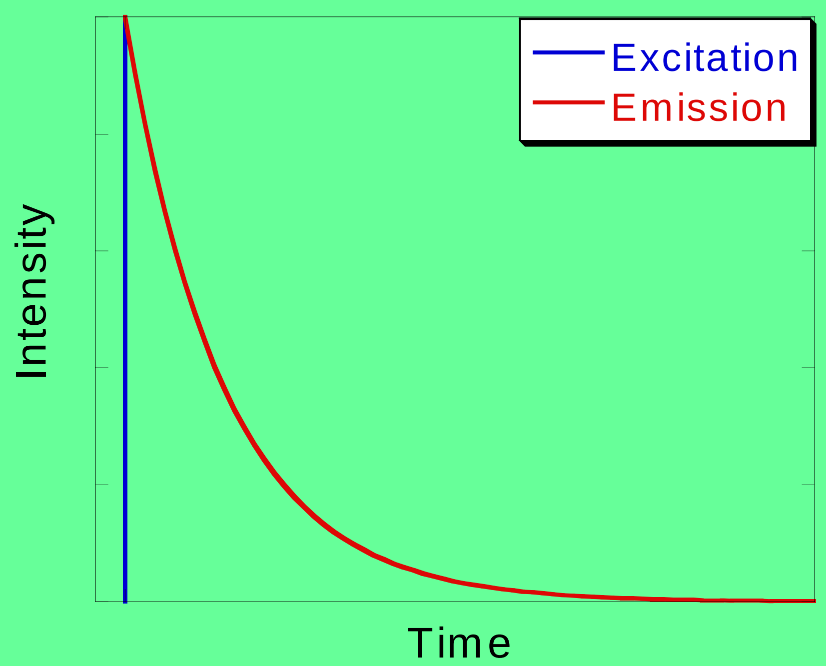
Phase-Modulation

- Intensity modulated excitation
- Phase angle and modulation give two independent measures of lifetime
- Improve readings by measuring more frequencies (10 psec까지도 가능)



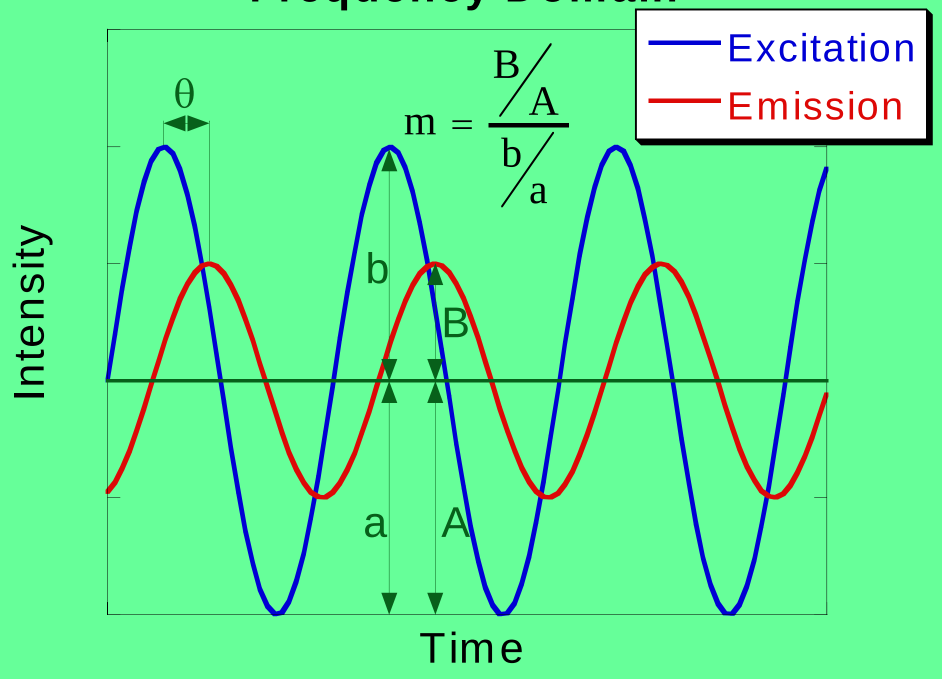
Measuring Fluorescence Lifetime

Time Domain



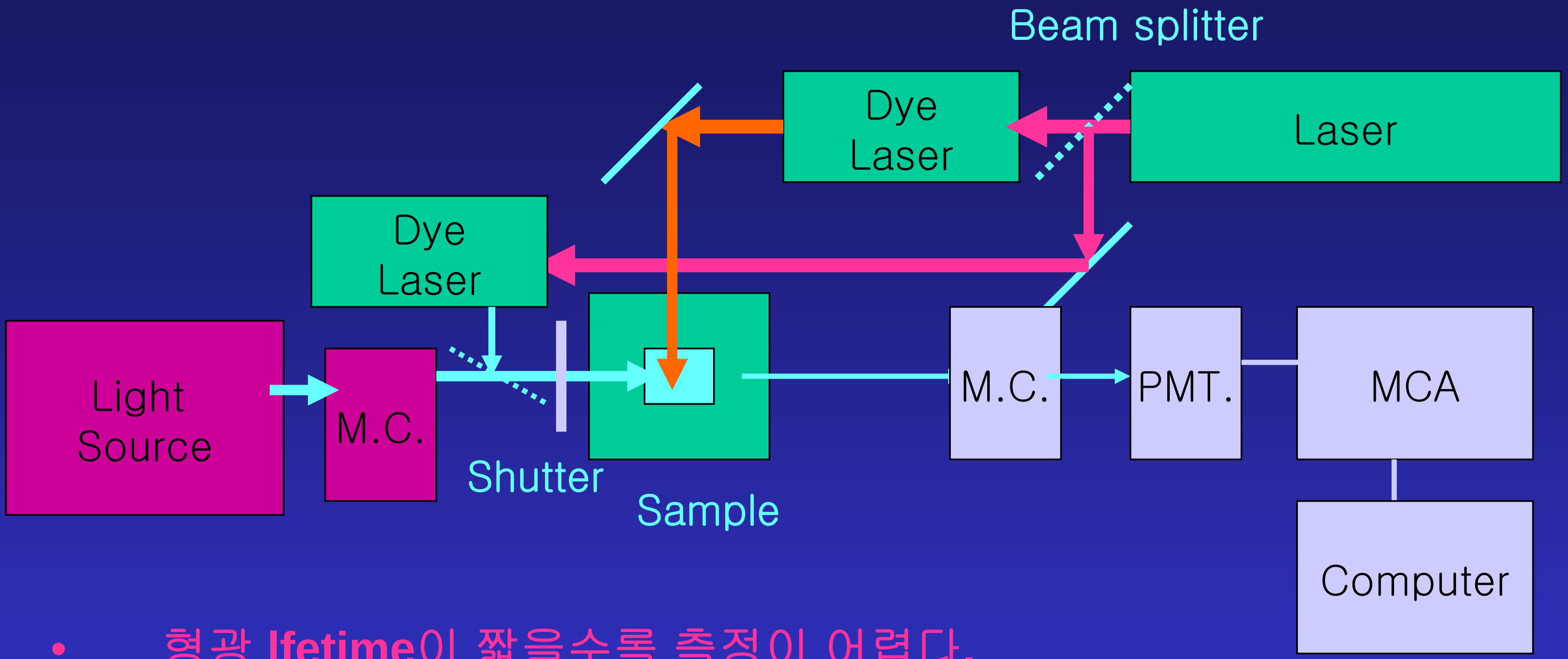
$$I = I_0 e^{-t/\tau}$$

Frequency Domain



$$\tan\theta = \omega\tau$$

$$m = (1 + \omega^2\tau^2)^{-1/2}$$



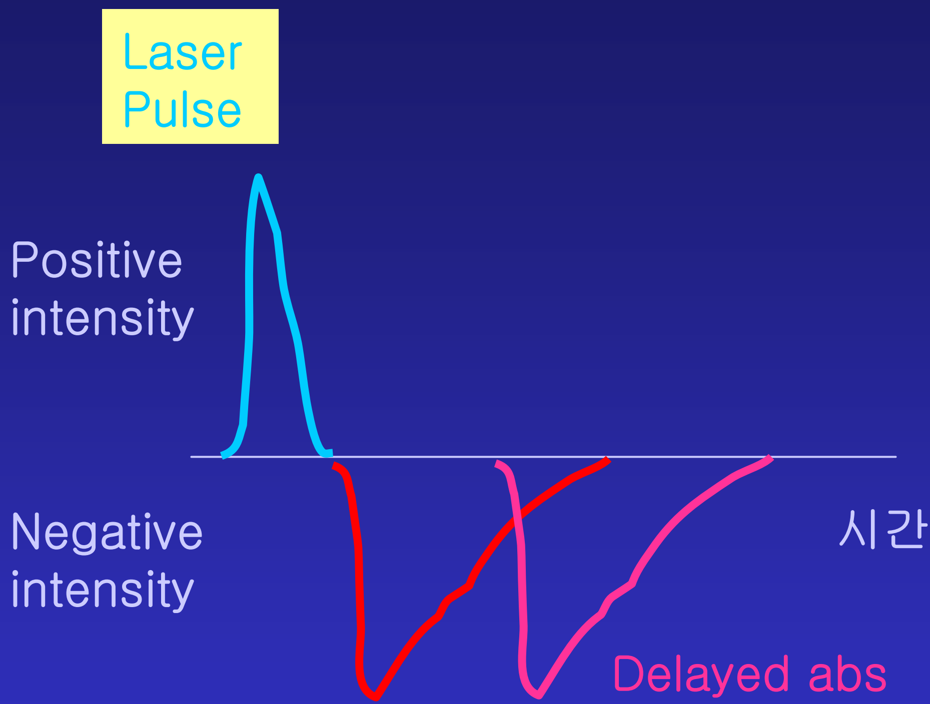
- 형광 **lfetime**이 짧을수록 측정이 어렵다.

$$\log \frac{I_{no-pump}}{I_{pump}} = A = -\sigma_{exct} N_{exct} l$$

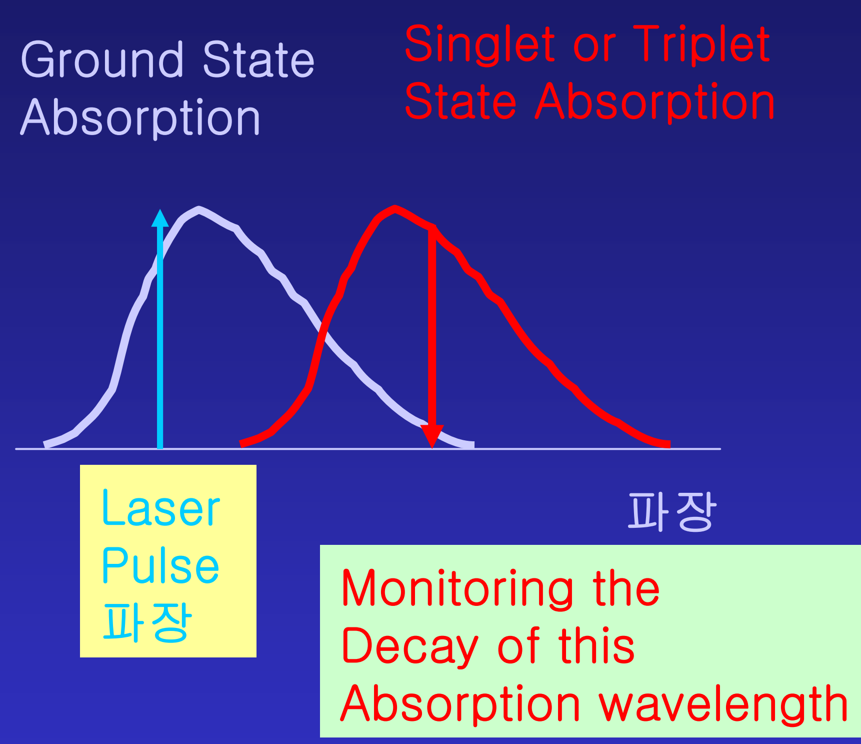
http://www.chem.rochester.edu/~stc/home.htm

Photochemical Technique: transient lifetime

한 파장에서 시간에 대한 광흡수 변화



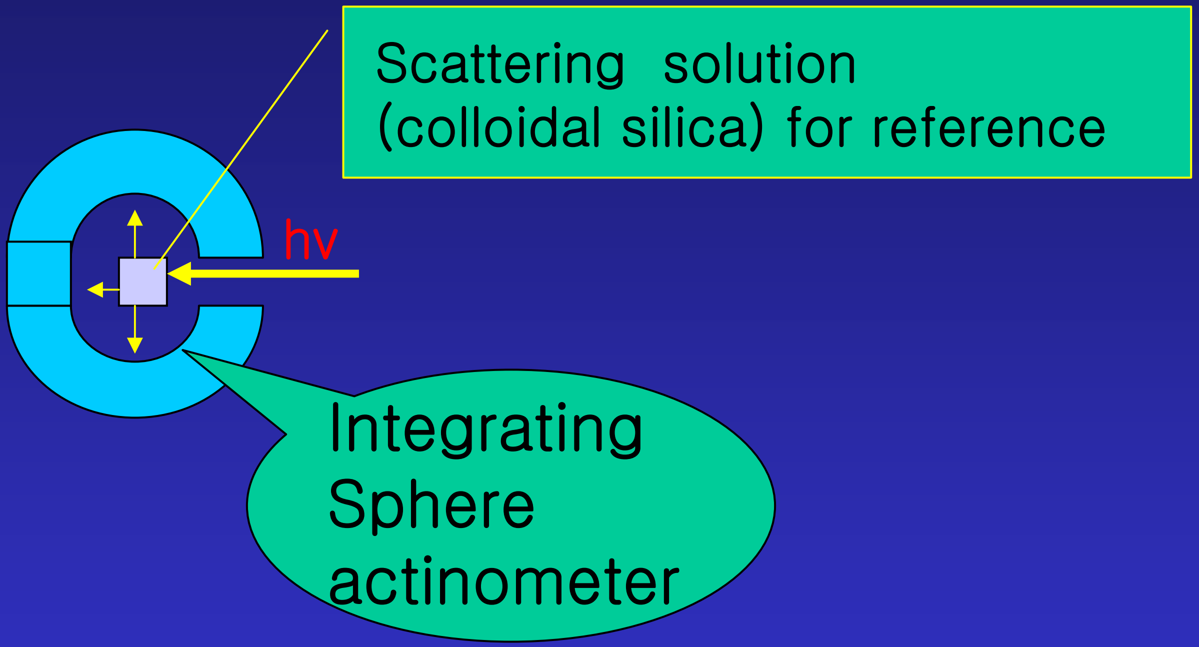
광흡수 측정의 적정 파장



http://www.chem.rochester.edu/~stc/home.htm

1. **Inner filter effect : self absorption**에 의해서 빛의 흡수가 안 되는 경우 **(**농도가 높은 경우**) ;** 표면에서 거의 다 흡수
2. **Wavelength effects on Φ: excitation, emission wavelength** 선택 파장이 **2**배 되는 지점에서도 높은 빛 **intensity** 감지**.**
3. **Refractive index correction;** 차이 나는 곳에서 굴절 및 반사
4. **Polarization effects: grating, light source, condensed phase**
5. **Temperature effects: light source**에서 나온 에너지에 의한 **local heating -> ir filter** 사용
6. **Impurity : (~ppm) impurity**의 양이 적어도 형광 **Φ**의 상대적인 양이 매우 크면 더 크게 보일 수 있다**.** 형광 **quenching effects** 등도 문제**.**
7. **Photochemical stability :** 광 반응성이 좋은 경우 시간 따라 형광의 크기가 달라진다**.**
8. **Raman scattering:** 작은 입자 **(**유기 용매에 들어 있는 물**,** 나노 **crystal)** 들에 의한 **scattering**과 형광과의 구분이 안될 수 있다**.** 특히 형광의 양이 작은 경우에 **.**

- Fluorescence Quantum Yield Measurement
- 1) Primary Method

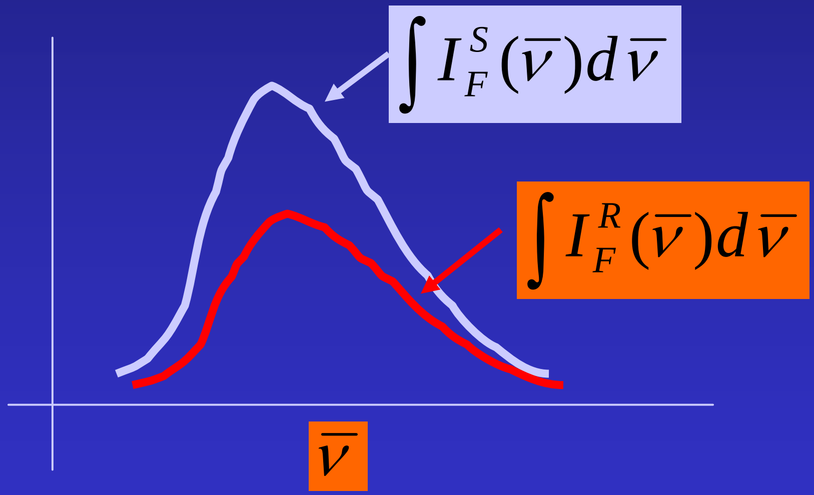


- **Cell**에서 나오는 광의 절대적인 양을 **intergrating sphere**를 이용하여 측정한다.

- **Secondary Method**

$$\frac{\Phi_F^S}{\Phi_F^R} = \frac{\int I_F^S(\bar{\nu})d\bar{\nu}}{\int I_F^R(\bar{\nu})d\bar{\nu}} \times \frac{OD_\lambda^R}{OD_\lambda^S} \times \frac{\eta_R^2}{\eta_S^2}$$

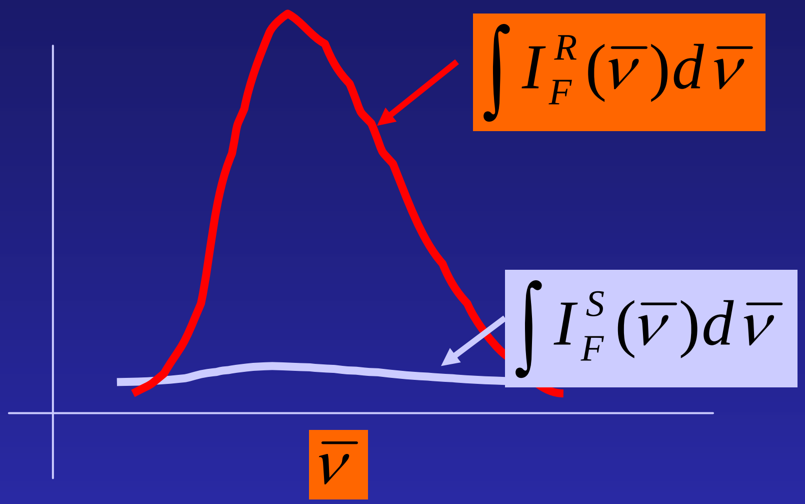
- **η: refractive index (경계면 반사 및 cell 안에서 internal reflection 문제)**



- 고려해야 할 점들
- **Sample**과 **reference**의 파장 영역이 맞지 않는 경우는 가장 가까운 것을 찾아서 **reference**로 사용한다**.** 같은 용매를 사용한 경우가 가장 좋고**,** 형광의 양도 비슷하면 더욱 좋고**,** 흡수도 등 모든 조건이 유사한 **reference**를 고른다**.**
- 흡수도가 다른 농도에서 실험하여 흡수도 와 형광의 양이 직선적으로 변하는가를 확인한다.
- **Reference** 들을 **cross-calibration** 하여 이들이 맞는지 확인하여야 한다**. (reference**의 **quantum yields**가 틀린 경우가 종종 있다**.)**

- 실험 방법
- **1.** 흡수**spectrum**을 얻고 **excitation** 파장에서의 흡수도 기록**.**
- **2. 10mm cuvette**을 이용하여 완전히 **corrected**된 **spectrum**을 얻은 후 면적 적분**.** 둘 모두 같은 용매 사용
- **3. 5**개의 다른 농도에서 실험하여 직선 관계 확인 **(**충분히 낮은 농도에서는 한 농도에서 실험으로 대체**)**
- **4. Reference calibration**한 후 비례 관계로 계산**.**

- 형광의 차이가 아주 많이 나는 경우



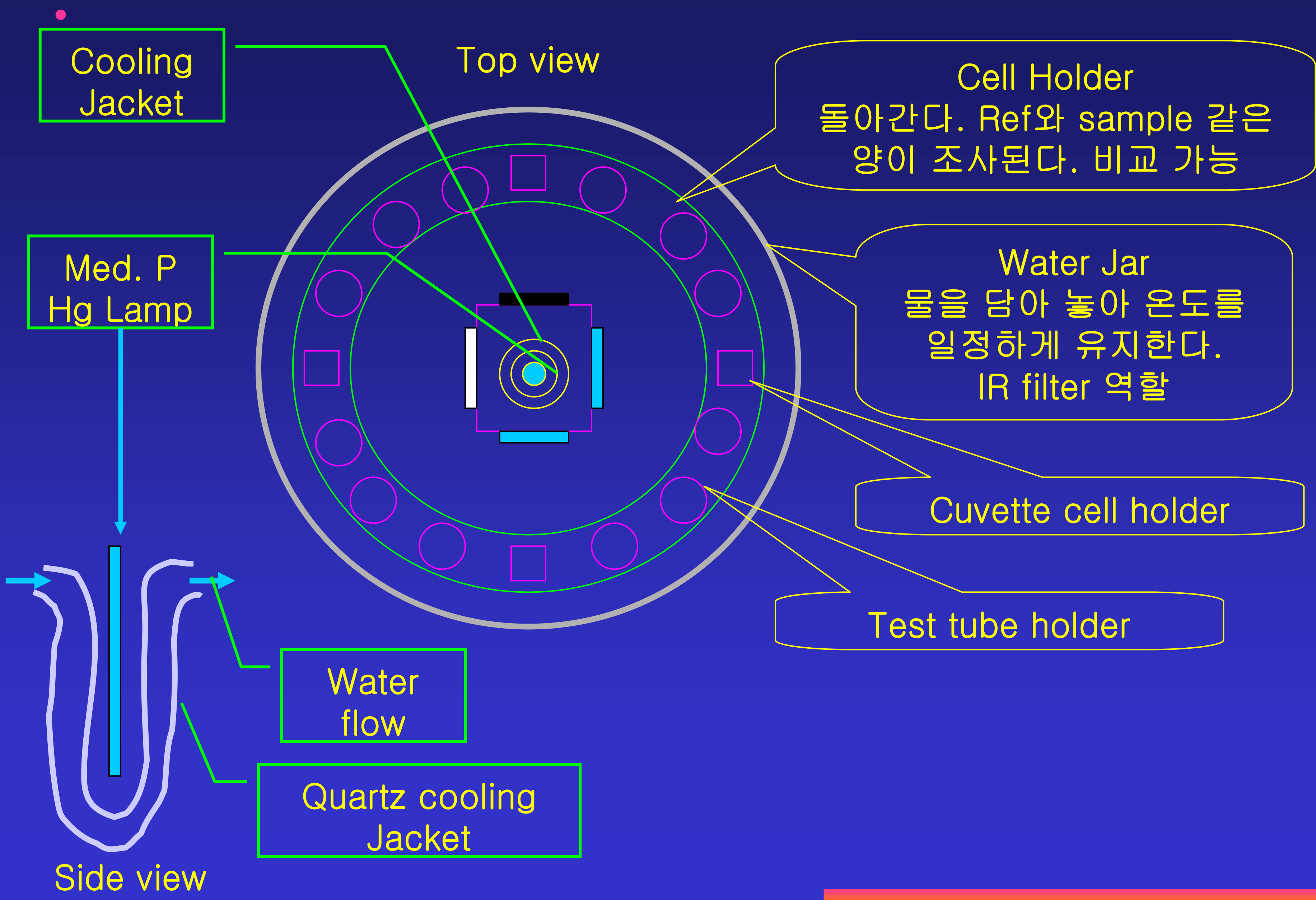
- **1. Reference O.D.** 를 낮춘다.
- **2. Quencher**를 사용하여 **reference**의 형광 양을 줄인다. **(**제일 나중에 사용하는 방법**) :**

$\frac{I_0}{I}, \frac{\tau_0}{\tau}$ 로부터 reference의 Φ_F를 보정한다.

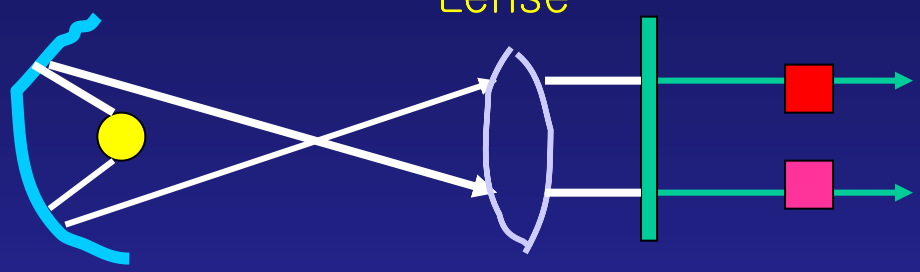
A Table of Standard Materials and Their Literature Quantum Yield Values

Compound	**Solvent**	**Literature Quantum yield**	**Emission range / nm**	**Reference**
Cresyl violet	Methanol	0.54	600-650	*J. Phys. Chem.*, 1979, **83**, 696
Rhodamine 101	Ethanol + 0.01% HCl	1.00	600-650	*J. Phys. Chem.*, 1980, **84**, 1871
Quinine sulfate	0.1M H_2SO_4	0.54	400-600	*J. Phys. Chem.*, 1961, **65**, 229
Fluorescein	0.1M NaOH	0.79	500-600	*J. Am. Chem. Soc.*, 1945, 1099
Norharmane	0.1M H_2SO_4	0.58	400-550	*J. Lumin.*, 1992, **51**, 269-74
Harmane	0.1M H_2SO_4	0.83	400-550	*J. Lumin.*, 1992, **51**, 269-74
Harmine	0.1M H_2SO_4	0.45	400-550	*J. Lumin.*, 1992, **51**, 269-74
2-methylharmane	0.1M H_2SO_4	0.45	400-550	*J. Lumin.*, 1992, **51**, 269-74
Chlorophyll A	Ether	0.32	600-750	*Trans. Faraday Soc.*, 1957, **53**, 646-55
Zinc phthalocyanine	1% pyridine in toluene	0.30	660-750	*J. Chem. Phys.*, 1971, **55**, 4131
Benzene	Cyclohexane	0.05	270-300	*J. Phys. Chem.*, 1968, **72**, 325
Tryptophan	Water, pH 7.2, 25C	0.14	300-380	*J. Phys. Chem.*, 1970, **74**, 4480
2-Aminopyridine	0.1M H_2SO_4	0.60	315-480	*J. Phys. Chem.*, 1968, **72**, 2680
Anthracene	Ethanol	0.27	360-480	*J. Phys. Chem.*, 1961, **65**, 229
9,10-diphenyl anthracene	Cyclohexane	0.90	400-500	*J. Phys. Chem.*, 1983, **87**, 83

Photochemical Technique: Quantum Yields for Reactions



- 간편한 장치



- **Lamp** 자체의 **fluctuation** 때문에 **reference cell** 과 **sample cell**을 같이 설치하여 측정

- **Chemical filter**도 **dye**를 이용하여 사용**,** 많은 경우 **band-path filter** 나 특정 파장 이상을 통과하는 **filter**를 섞어서 사용

- **Potassium Ferrioxalate**
- **Spectral range : 250 ~ 509 nm**
- $2[Fe(C_2O_4)_3]^{3-} \xrightarrow{hv} 2[Fe(C_2O_4)_3]^{2-} + (C_2O_4)^{2-} + 2CO_2$
- **Φ= 1.1 ~ 1.25**
- **436 nm 254nm**
- **1,10-phenanthroline complex**를 만들어서 **510 nm** 에서 **abs**를 측정하여 빛의 강도를 알 수 있다.

- **Ranecke's Salts**
- **Spectral range : 316~600 nm**
- $Cr(NH_3)_2(NCS)_4^{-} \xrightarrow{hv} Cr(NH_3)_2(NCS)_3(H_2O) + NCS^{-}$
- **Φ= 0.276 ~ 0.388**
- **600 nm 360nm**
- Cr^{3+} 와 NCS^{-} **complex**를 만들어 **450 nm** 에서 흡수도 측정