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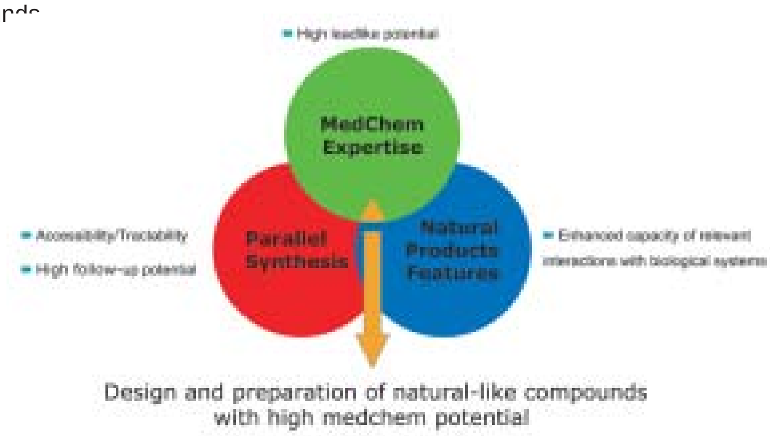

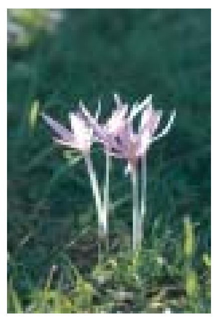

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- Design and Prediction of relevant physicochemical parameters
- High Throughput Chemistry
- High Throughput MS driven analysis and purification
- Experienced samples and data handling

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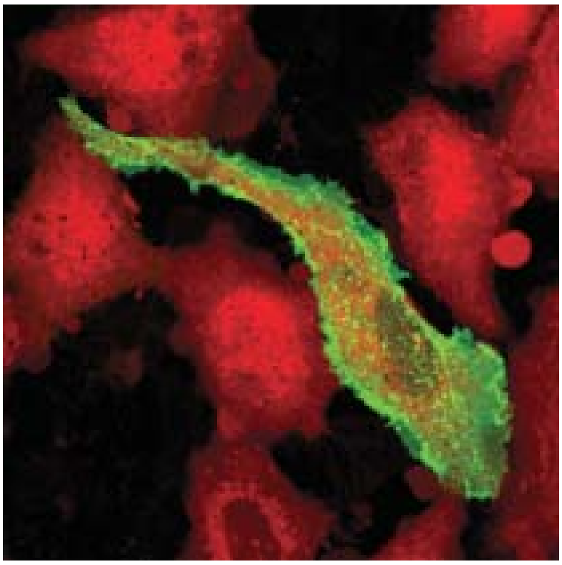
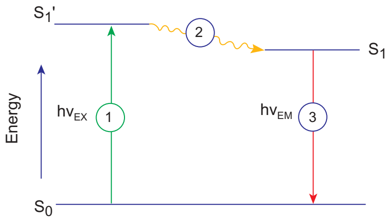


The ABC's of Fluorescence

Fluorescence is the property of some atoms and molecules to absorb light at a particular wavelength and to subsequently emit light at longer wavelength after a brief interval (=fluorescence lifetime). The process of phosphorescence occurs in a manner similar to fluorescence, but with a much longer lifetime. These are part of luminescence processes in which excitation can be induced by other sources, including temperature, friction, chemical reaction, or enzymatic reaction (bioluminescence).

Regarding specifically fluorescence, absorption and emission characteristics depend on molecules and surrounding conditions; it spans from ultra-violet absorption to visible (photoluminescence) and also infrared wavelengths. Absorption and emission spectra can be represented along the continuous spectrum from UV to IR. Convenient parameters are useful to characterize dyes, so let review important ones:
- Maximum emission and absorption wavelengths,
- Light absorption and transfer efficiency (main responsible of brightness).
- Combinaison of dyes for special applications: FRET, Quenching…



*Standard fluorescence model

Theory reminder Molecules absorb photons ('light particles') at specific wavelengths. The energy of light E=hn (where n is the vibration frequency of photons, and h the Planck constant) excite electrons to higher energy state. When electrons return to their original state, energy is converted in photons, usually in the infra red region (heat). In fluorescent compounds however, the global electronic environment of the molecule (and surrounding molecules) makes possible to convert a high quantum of light energy in photons (of lower energy), eliciting a light emission with relatively narrow wavelengths generally in the visible or infra-red region, but at a lower wavelength than the original light. The process occurs on the nanosecond time scale (10^{-9} sec).

Molar Absorption / Extinction Concentration (EC, e [$M^{-1}cm^{-1}$])
Absorption efficiency is appreciated by a coefficient of absorption of light at the maximum absorption wavelength. Higher is EC more energy is caught by the dye (so long the light source power is not limiting).

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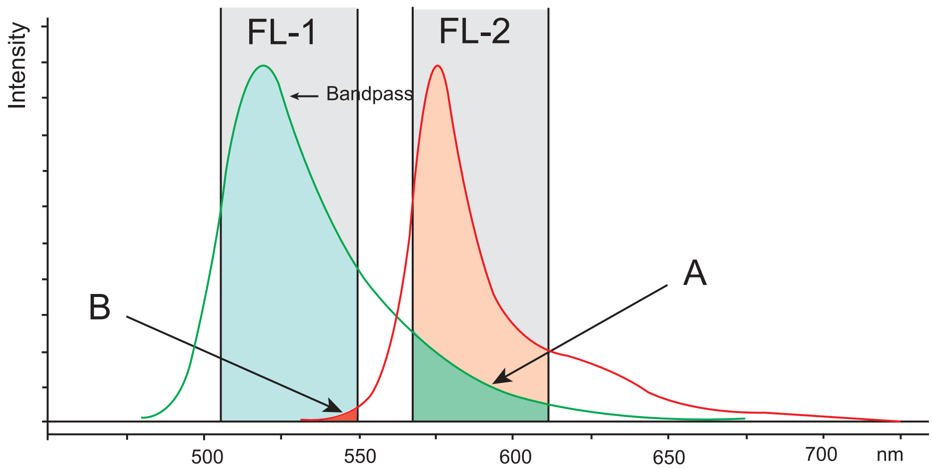
Quantum yield (QY)
QY is the efficiency of energy transfer from light input and light emission, thus the ratio of the number of quanta emitted by the specimen divided by the number of quanta absorbed. Higher is the quantum yield, higher is the fluorescence intensity. Low quantum yield results in low fluorescence that can merely be compensated but a high molar absorption, and eventually if it is possible to increase light output of the source.

Stokes shift / Multiple fluorescent detections
It is the shift of wavelength between the maximum absorption and maximum emission wavelengths. Small stokes shift may affect correct excitation and measurement, especially when multiple fluorescence detection is used.

Wavelength pass (absorption or emission peak width)
A wide excitation wavelength pass may be useful to catch more light if it fits to the excitation source. A wide emission wavelength pass may be useful if a filter/measurement detector is not available to fit the optimum, but usually it is undesired because it will overlap absorption or emission of other dyes. The use of a proper filter, to catch the light in a definite window, improves greatly the possible combinations of fluorescence detections.

Overlapping spectrum / compensation
Measurement of one dye may interfere with the measurement of another one in case of overlap between absorption and emission spectra (insufficient stokes shift, too much large wavelength pass …). I.e., a red-collecting channel (FL2, in the FACScan) used for PhycoErythrin can detect significantly FITC fluorescence (A in the tail of absorption peak), as well in opposite way, PhycoErythrin fluorescence is detected in the green channel/filter (FL-1) (B in the tail of emission peak). In cytometry, such interference can be partially compensated by parameter settings.



Fluorescence lifetime (t).
This is the time between excitation and emission. It ranges from 10^{-9} sec (fluorescence) to hours (bioluminescence glow). Long fluorescence lifetime allows reducing background since autofluorescence ends before light measurement.

Dissociation affinity constant
For dyes that bind ionic species or other ligand, generally modifying their fluorescence characteristics, the dissociation constant is a useful parameter. K_d is defined as the concentration of ligand that dissociates from the dye according to the equilibrium reaction. Kd should fit in the range of Ligand concentration that is studied.

$$\text{Dye + Ligand} \underset{Kd}{\overset{Ka}{\Longleftrightarrow}} \text{[Dye : Ligand complex]}$$

Equilibrium equation $$\frac{Ka}{Kd} = \frac{\text{[Dye : Ligand complex]}}{\text{[Dye] x [Ligand]}}$$

Fluorescence and associated important features

Hydrophobic/hydrophilic sensitivity
Fluorophores are generally rather hydrophobic molecules, and their fluorescence properties are affected by hydrophobic/hydrophilic environment or sample interactions. Fluorescence lifetime and the closely related quantum yield may strongly depend on the molecular environment, like the surrounding solvent, or the local pH. High environment-sensitivity of fluorescence is profitable in membrane indicators dyes. Hydrophobic dyes are useful to enter / cross biological membranes. In most applications where the dye serves simply as a label, fluorochromes that are hydrophilic and pH insensitive are generally sought.

Photostability
Many fluorochromes fade upon continuous illumination, and even degraded. This might be detrimental to their stability during storage or use under day light exposure. Photostability is furthermore crucial during lasting measurements observation. (I.e. brightness may decline during a scanning by confocal microscopy). This is the case with most fluoresceins. FluoProbes provides improved and photostable dyes like FluoProbes dyes. When available, photostable alternatives of each homologue conventional dye are indicated.

pH sensitivity
Fluorescence of several compounds vary with acidic or alkaline conditions. Such pH sensitive fluorophores are therefor used as pH indicators. However, this is usually detrimental for labeling applications and others. FluoProbes provides improved pH stable fluorescent labels, as FluoProbes dyes. Others are indicated as alternative in the description of each homologue conventional dyes.

Thermal sensitivity
Fluorescence properties may be affected by temperature.

Fluorescence of free dye versus conjugated dye
The effect of dye conjugation on fluorescence is important mainly for :

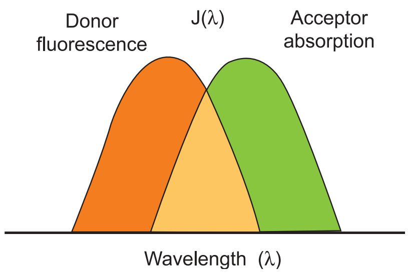
- Dyes for labeling ; It is also wished the free dye be non fluorescent until conjugation gives it highly fluorescent (no background due to unlabeled dye), but it should be also combined with stability of fluorescence in different environment conditions.
- Some reactive dyes for cell tracing and probing (ions, pH, potential...) : fluorescence of the dye should change quantitatively and/or qualitatively (see Ratiometric dyes) after conjugation.
- FRET quenchers : same as labeling dyes, but concerning quenching ability because no fluorescence is expected.

Ratiometric dyes
The fluorescent properties may be quite identical or largely modified depending on environment conditions (pH, hydrophobicity…), or binding of ligands (ions). The shift of absorption and/or emission allows ratiometric measurements : both forms are detected at different wavelengths, so their ratio improve greatly the accuracy of detection. Ratiometric dyes are a privileged tools for the detection of ions (indicator dyes).

Fluorescence & other special types

Quenching

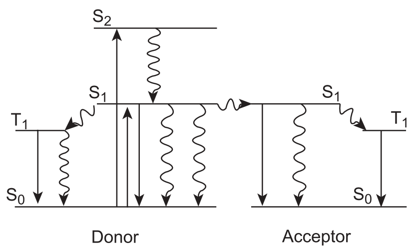
Fluorophores molecules in close contact may inhibit reciprocally their fluorescence properties **(self-quenching).** This is big importance for labeling experiments (degree of conjugation should be optimized), as well for any detection where a high concentration of dye is reached (high density of antigen on membranes, or concentrated in organelles for example). This is a common limitation with PhycoErythrin dye for example. Quenching is taken to good account in some techniques and applications. One most remarkable application is the **FRET** technique (see below)



Overlapping spectra of Donor emission and Acceptor absorption.

FRET

Fluorescence Resonance Energy Transfer (FRET) occurs with some fluorophores when absorption spectrum from an Acceptor molecules (A) overlaps the emission spectrum from a Donor (D), and molecules are in vicinity typically at 30 to 60 Angstroms. In this situation, a fluorophore 'acceptor' is excited, not by light, but by direct transfer of the excited state energy from an initially excited donor. Return to the ground energy state releases photons = fluorescence. For non-fluorescent acceptors, FRET results in a decrease of donor fluorescence quenching. This involves an intermediate energy state (S1) as depicted in figure below.



***FRET interest** relies on lower backgrounds, extended stokes shift of the pair A+D, and the fact FRET occurs at distances in the range of molecules. Important applications include A-D conjugates or A: D complexes that can be used as FRET sensitive probes for detection of agents dis- or as-sociating A : D complexes, detection of molecular interactions, or detection of enzymes that cleaves A-D bonds. If a dark quencher is used as the acceptor molecule, multiple FRET probes can be used, each labeled by a single fluorophore, making these probes suitable to multiplex assays. FRET building blocks (section II). FRET nucleic acid detection (section IV) . FRET proteolyses detection (section V).
*FRET is responsible of **self-quenching** of many fluorophores when present in high concentration (in solution, or on target membranes).

Phosphorescence – Delayed time fluorescence

Phosphorescence is a special way of fluorescence emission that occurs with a delayed time. The return from excited energy state to ground energy state occurs through an intermediate state (T1 in figure above).

Collisionnal Quenching

Collisions between an excited-state fluorophore and other molecules make the return to ground state without emission of photon. Such quenching occurs primarily with molecular oxygen and electron scavengers (Cu^{2+}, Mn^{2+}, halide, Nitrite…) present in the range of millimolar or higher concentrations, or some other compounds.

Chemi- & Bio- Luminescence

Luminescence is close to fluorescence, but is generated by chemical or enzymatic reaction with kinetics spanning from milliseconds-seconds (chemiluminescence 'flash') to minute or hours (bioluminescence «glow»).

Flash (msec-sec) advantages

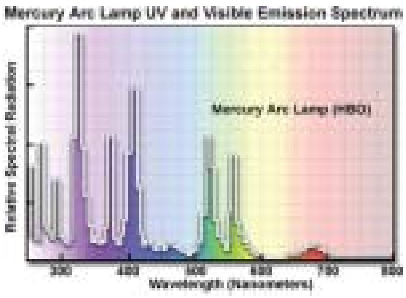
- Rapid time measurement / HTS
- Highest signal to noise ratio
- Low temperature dependency
- Wide dynamic range
- Linear response

Drawbacks are :

- Timing to injection/read
- Mixing reagent is crucial
- Photon counting more difficult
- Signal variations.

Appendix

Fluorescence



Much of the intensity of the mercury burner is expended in the near ultraviolet, with peaks of intensity at 313, 334, 365, 406, 435, 546, and 578 nanometers.

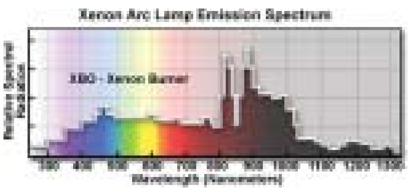
Glow (min-hr) advantages

- Easy to use (Substrate addition non-critical))
- Signal reading can be adjusted for different levels
- High signal levels
- Sequential reading of samples
- Easy to engineer

Drawbacks are high temperature dependency, amplification of background with reading time; interference of additives on signal, long time for stable signal.

Choice of light region / wavelength

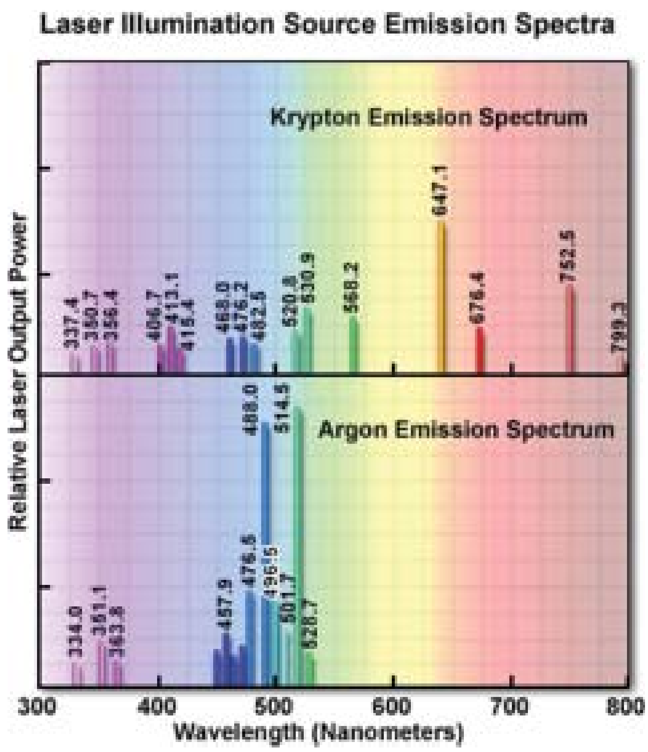
The light region (and suitable dye) is chosen depending on available instrument (light source and detector), but also on the sample type (no light absorption). The near-IR is the region of the spectrum from about 700 nm to 2500 nm (2.5 µm). Light in this region penetrates much more deeply into many samples (tissues, gels, animals, etc.) with minimal scattering or absorption. This allows samples to be imaged at much greater depths than with colors outside this spectrum. Additionally, the autofluorescent background is significantly reduced in this region of the spectrum because biological samples, glass slides, etc. fluoresce only weakly or not at all in the near-IR.



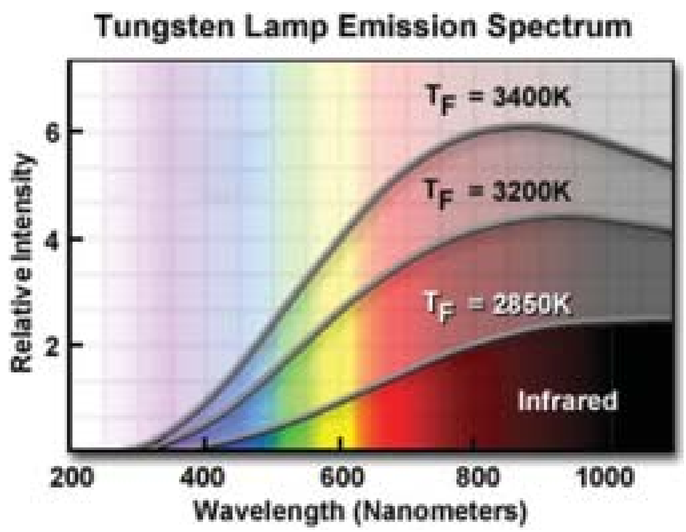
The xenon burners have much more even intensity across the visible spectrum than do the mercury burners , and they expend a large proportion of their intensity in the intra-red (risk of heat).

Light Sources

In order to generate enough excitation light intensity to furnish secondary fluorescence emission capable of detection, powerful light sources are needed. These are usually mercury (ranging in wattage from 50 watts to 200 watts) or xenon arc (burner) lamps (75 watts to 150 watts), which produce high-intensity illumination powerful enough to image faintly visible fluorescence specimens, argon-ion laser with powerful emission capability at 488 and 514 nanometers. Laser sources, despite the high cost, have become especially useful in laser scanning confocal microscopy.



Argon-ion laser with powerful emission capability at 488 and 514 nanometers. Laser sources, despite the high cost, have become especially useful in laser scanning confocal microscopy.



Tungsten-halogen bulbs are used, especially for blue or green excitation with brightly emitting specimens. Their output is relatively even across the visible spectrum.

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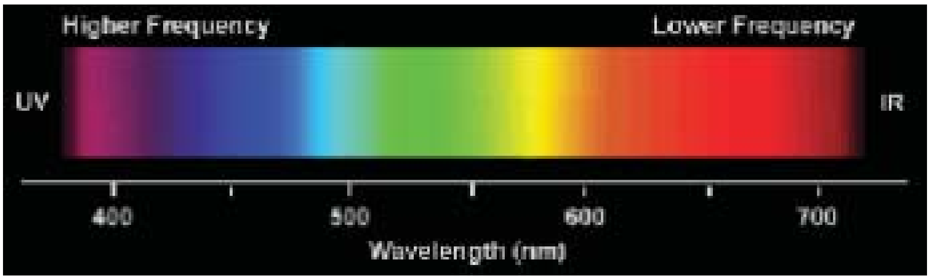
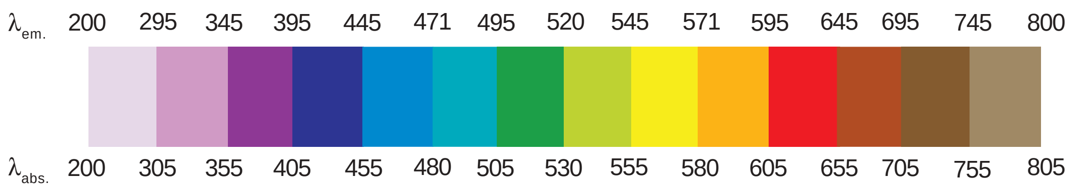
Fluorescence excitation and emission are detected by an instrument called spectrofluorimeter. First, the dye is strongly illuminated by a color of light that is found to cause some fluorescence. Then the spectrum of fluorescence is obtained by scanning with the analysing spectrometer using this fixed illumination color. The analyser is then fixed at the brightest emission color, and a spectrum of the excitation is obtained by scanning with the illuminating spectrometer and measuring the variation in emission intensity at this fixed wavelength.
The quantitative description of these color spectra is made by the wavelength of light. The different colors of the visible spectrum can be separated by approximated wavelength values as shown below :

Visible Spectrum



Violet and indigo are contained between	400 to 450 nm
Blue and Aqua	450 to 500 nm
Green	500 to 570 nm
Yellow and orange	610 to ~750 nm
Red	570 to 610 nm

The visible spectrum is then contained between the UltraViolet, under 320 nm and the Infra-Red, from 750 nm to 2500 nm. Fluorescence spans then from ultra-violet to infra-red wavelenghts. Direct parameters are thus light absorption and (even better) light emission wavelength. Most interesting dyes emit in the visible, so we adopt a color code for emission maximum as follows :



Wavelength (nm)

Fluorescence Microscope

The combination of the important parameters explained before gives a ratio of emitted fluorescence intensity in a typical application of approximatly 10^{-5}. But, current techniques, such as fluorescence in situ hybridization might have ratios as low as 10^{-9} to 10^{-10}.
Then, it is necessary for the fluorescence microscope to attenuate the excitation light without reducing the fluorescence signal, to obtain fluorescent image with correct contrast. Optical filters are dedicated to this operation but also the general configuration of the microscope will greatly contribute to the filtering process.

Basic Requirements of Fluorescence Microscope Optics

Nearly all fluorescence microscopes use the objective lens to perform two functions :

1 - Focus the illumination (excitation) light on the sample.
In order to excite fluorescent species in a sample, the optics of a fluorescent microscope must focus the illumination (excitation) light on the sample to a greater extent than is achieved using the simple condenser lens system found in the illumination light path of a conventional microscope.

2 - Collect the emitted fluorescence.
This type of excitation-emission configuration, in which both the excitation and emission light travel through the objective, is called epifluorescence. The key to the optics in an epifluorescence microscope is the separation of the illumination (excitation) light from the fluorescence emission emanating from the sample. In order to obtain either an image of the emission without excessive background illumination, or a measurement of the fluorescence emission without background "noise", the optical elements used to separate these two light components must be very efficient.

The Dichroic Mirror

In a fluorescence microscope, a **dichroic mirror** is used to separate the excitation and emission light paths. Within the objective, the excitation emission share the same optics.

In a fluorescence microscope, the dichroic mirror separates the light paths.
The **excitation** light reflects off the surface of the dichroic mirror into the objective.
The fluorescence **emission** passes through the dichroic to the eyepiece or detection system.
The dichroic mirror's special reflective properties allows it to separate the two light paths. Each dichroic mirror has a set wavelength value — called the **transition wavelength value** — which is the wavelength of 50% transmission. The mirror reflects wavelengths of light below the transition wavelength value and transmits wavelengths above this value. This property accounts for the name given to this mirror (dichroic, two color). Ideally, the wavelength of the dichroic mirror is chosen to be between the wavelengths used for excitation and emission.
The dichroic mirror is a key element of the fluorescence microscope, but it is not able to perform all of the required optical functions on its own. Typically, about 90% of the light at wavelengths below the transition wavelength value are reflected and about 90% of the light at wavelengths above this value are transmitted by the dichroic mirror. When the excitation light illuminates the sample, a small amount of excitation light is reflected off the optical elements within the objective and some excitation light is scattered back into the objective by the sample. Some of this "excitation" light is transmitted through the dichroic mirror along with the longer wavelength light emitted by the sample. This "contaminating" light would otherwise reach the detection system if it were not for another wavelength selective element in the fluorescence microscope : an emission filter.

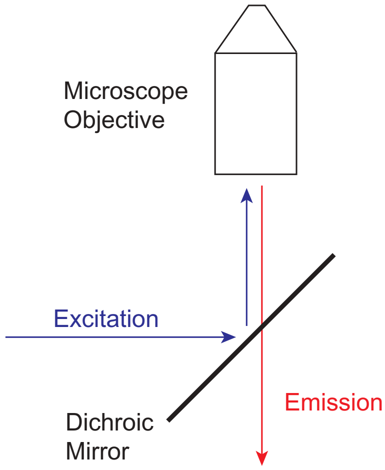


Figure 1 : Dichroic mirror separates excitation and emission light paths.
Note : This diagram shows the dichroic mirror's position in an inverted fluorescence microscope : below the sample. In this type of microscope, the sample is illuminated and imaged from below the stage.

Excitation and Emission Filters

Two filters are used along with the dichroic mirror :
Excitation filter — In order to select the excitation wavelength, an excitation filter is placed in the excitation path just prior to the dichroic mirror.
Emission filter — In order to more specifically select the emission wavelength of the light emitted from the sample and to remove traces of excitation light, an emission filter is placed beneath the dichroic mirror. In this position, the filter functions to both select the emission wavelength and to eliminate any trace of the wavelengths used for excitation.
These filters are usually a special type of filter referred to as an interference filter, because of the way in which it blocks the out of band transmission. Interference filters exhibit an extremely low transmission outside of their characteristic bandpass. Thus, they are very efficient in selecting the desired excitation and emission wavelengths.

The Filter Cube

The dichroic mirror is mounted on an optical block commonly referred to as a **filter cube**. The excitation and emission filters are usually affixed to the filter cube.
This cube provides a convenient means to change the dichroic mirror without direct handling of either the mirror or filters. Figure 2 shows the light path through the filter cube in a fluorescent microscope. The narrow red line emanating from the objective to the filter cube represents the scattered and reflected emission light that must be removed by these optical elements.

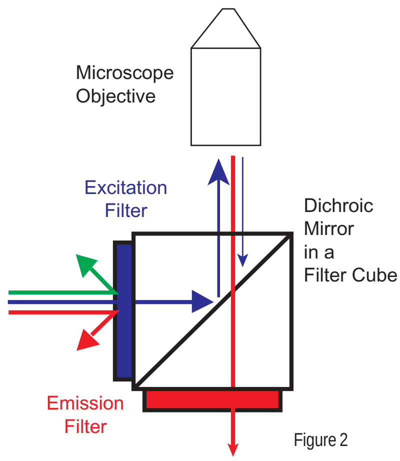
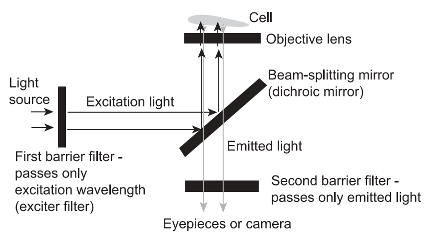
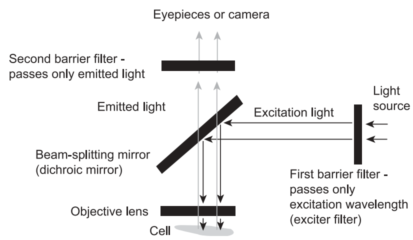


Figure 2

Most microscopes are designed using epi-illumination. In epi-illumination excitation, light goes through the objective lens and illuminates the object. Light emitted from the specimen is collected by the same objective lens.



Sometimes the fluorescent molecule itself is a direct stain or probe for specific structures. In other situations the fluorescent dye is bound to another non-fluorescent probe that recognizes specific structures. For example, the fluorescence molecule, rhodamine may be conjugated to phalloidin, which binds the filamentous actin. One important method to identify specific proteins is to couple fluorescent dyes to antibodies that bind very specifically to macromolecules in the cell.



Size Exclusion Chromatography - Modern SEC material

From the first TSKgel SW column, followed by the TSKgel SWxl, development went to even smaller particle size. The latest TSKgel Super SW generation, consequentially increasing resolution for peptide and protein analysis.

100.000 TP/M for SEC with the TSK-GEL Super-SW column from Tosoh Bioscience.

Compared to the popular TSKgel SWxl, the 4µm particle size silica material of the TSKgel Super sw Shows a 30% improvement in sample resolution, as demonstrated by the separation of four standard proteins on a TSKgel G2000SWxl and on a SuperSW2000 column, shows in fig.1.

As smaller particles show better diffusion parameters, linear flow rates can be increased without loosing resolution.

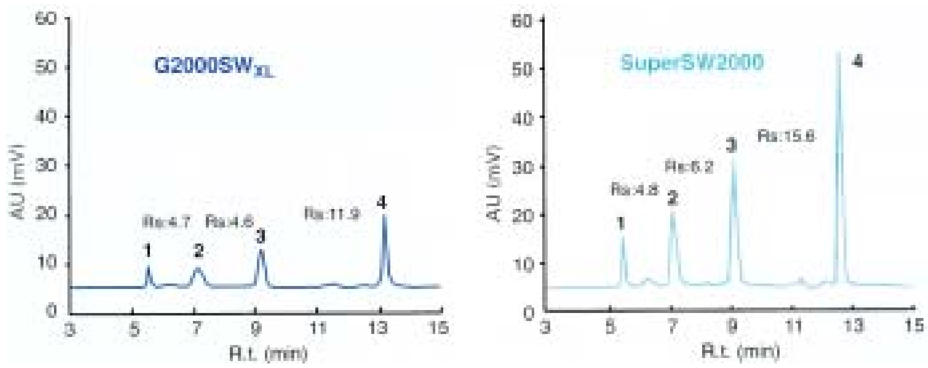


Figure 1 : Comparison of Efficiency : SuperSW2000 versus G2000SWXL
Column : TSKgel G2000SWXL, 7.8 mm ID x 30 cm L ; TSKgel SuperSW2000, 4.6 mm ID x 30 cm L
Sample : 1) Thyroglobulin, 0.2 mg/ml
2) Albumin, 1.0 mg/ml
3) Ribonuclease A, 1.0 mg/ml
4) p-aminobenzoic acid; 0.01 mg/ml
Inj. Vol. : 5 µl
Eluent : 0.1 M Na-phospahte buffer + 0.1 M Na_2SO_4 + 0.05% NaN_3 (pH 6.7)
Flow rate : TSKgel G2000SWXL, 1.0 ml/min ; TSKgel SuperSW2000, 0.35 ml/min
Temperature : 25°C
Detection : UV @ 280 nm

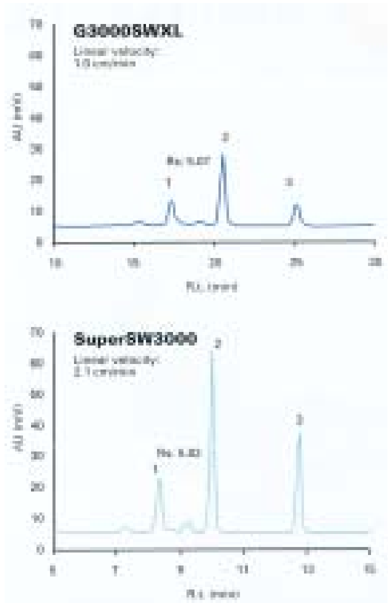


Figure 2 : Comparison of Analysis : SuperSW3000 vs. G3000SWXL
Comparison of Analysis Time : SuperSW3000 vs. G3000SWxl
Column : TSKgel SuperSW3000, 4.6 mm ID x 30 cm L
TSKgel G3000SWxl 7.8 mm ID x 30 cm L
Sample : 1) Bovine serum albumin (BSA) 1 mg/ml
2) Myoglobin (Myo) 1 mg/ml
3) p-Aminobenzoic acid 0.04 mg/ml
Inj. Vol. : 5 µl
Eluent : 0.1 M Na-phospahte buffer + 0.1 M Na2SO4 + 0.05% NaN3 (pH 6.7)
Temperature : 25°C
Detection : UV @ 280 nm

In figure 2 analysis conditions are choosen such, that for the separation of three stnadard proteins, resolution is equivalent on both columns. Thus, linear velocity of the eluent could be increased on the Super SW3000, resulting in nearly 50% shorter analysis time.

By reduing the internal column diameter from 7.8 to 4.6 mm, volumetric flow rates are reduced by the three fold, thud the analyst enjoys the advantages of less solvent consumption. Researcher with limited sample quantities will also like the small sample volume required and the increased sensitivty by 300% (fig.3).

With the introduction of a new TSKgel SuperSW material with small particle size, available in narrow bore or capillary columns, SEC is driven to a modern, high performance and highly sensitive analysis method. Scientist used to work with classical/standard TSKgel SW material now can easily modernize their applications.

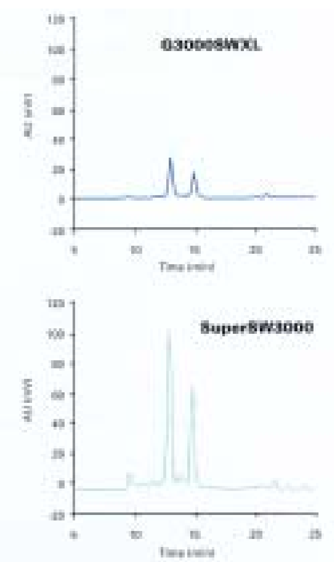


Figure 3 : Comparison of Sensitivity for the Separation of IgG from Albumin in Mouse Ascites fluid : SuperSW3000 vs. G3000SWXL
Comparison of sensitivity for the separation of IgG from albumin in mouse ascites fluid : TSKgel SuperSW3000 vs. TSKgel G3000SWxl
Column : TSKgel SuperSW3000, 4.6 mm ID x 30 cm L
TSKgel G3000SWxl 7.8 mm ID x 30 cm L
Sample : Mouse ascites fluid
Inj. Vol. : 5 µl
Eluent : 0.05 M Na-phospahte buffer + 0.1 M Na2SO4 (pH 6.7)
Flow rate : 0.58 ml/min for G3000SWxl ; 0.2 ml/min for SuperSW3000
Temperature : 25°C
Detection : UV @ 280 nm

Useful formula and datas for Chemicals, DNA, Proteins

Conversion of weight / mole of proteins
Moles = grams of proteins / MW of protein
i.e. 1µg of a 150 000 MW protein is 6.7pmol

Conversion of weight / mole of nucleic acids
MW (average DNA base) = 330
MW (averageRNA base) = 340
1µg (100bp dsDNA) = 1.5pmol
1kb of DNA encodes 333 amino-acids (x average MW : 110) = MW : 36 630

Spectrometric absorbance
The concentration of molecules can be determined by spectrometry according Beer's law.
*Nucleic acids :
50mg/ml (dsDNA) = 1 A_{260}
33mg/ml (dsDNA) = 1 A_{260}
40mg/ml (dsDNA) = 1 A_{260}
*Proteins : A_{280nm} is widely used, because UV absorbance of aromatic amino-acids.
Concentration of protein (M^{-1}) = A280 unit of protein (AU / cm) / EC ($M^{-1}cm^{-1}$)

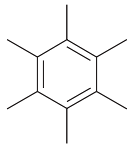
*Protein	MW	pI	EC(280 nm, 1 mg/ml)
Alkaline Phosphatase (calf int.)	140 000	5.7	1.0
Avidin (egg white)	67 000	10	1.54
Biotin	244	3.5	A_{250}=0.111
BSA (bov.ser.albumine)	69 000	4.9	0.67
HRP (horse radish peroxidase)	40 000	7.2	1.34
IgG (hu)	153 000	6.1-8.5	1.36
IgM (hu)	900 000	-	1.45
Protein A	42 000	4.5	0.95
Trypsin (bovine)	23 800	10.5	1.55

Acids and Bases

Compound	Formula	MW (g/mol)	Density (g/ml)	% by weight	Molarity of concentrate
Acetic acid	CH_3COOH	60.1	1.05	99.5%	17.4 M
Ammonium hydroxide	NH_4OH	35.0	0.90	28%	14.8M
Formic acid	HCOOH	46.0	1.20	90%	23.4M
Hydrochloric acid	HCl	36.5	1.18	36ù	11.6m
Nitric acid	HNO_3	63.0	1.42	71%	16.0M
Phosphoric acid	H_3PO_4	98.0	1.70	85%	18.1M
Potassium Hydroxide	KOH	56.1	1.52	90%	13.5M
Sodium Hydroxide	NaOH	40.0	1.53	50%	19.1M
Sulfuric acid	H_2SO_4	98.1	1.84	96ù	18.0m

Temperature / Pressures / Metrics
Celsius °C = 5/9 (°F – 32) °F = 9/5 °C + 32
1 bar = 1 atm = 14.5 psi = 0.1 Mpa = 1KiloPascal = 1.019716 Kg/cm^2
1 Joule (J) = 0.2388459 Calorie = 1 Watt second= 6.24145e^{+18} ElectronVolt
1 meter (m) = 1000mm = 10^6µm = 109 nm = 10^{10} Angstrom = 0.0006213712 mile = 3.28084 feet
rpm = 1000 (RCF x1.12 x r)1/2 rpm: rotation per minute; RCF(g): relative centrifugal force
RCF = 1.12 x r (rpm / 1000)2 r: radius of a rotor in millimeter
1Kg = 10^9 mg = 6.0225e^{+26} atomic mass unit = 9.806652 Newton = 1.215278 pound
1 mol = 6.23 10^{-23} atoms

Chemical groups

Alcohol	R-OH
Aldehyde	R-CO-H
Alkene	>C=C<
Alkyne	-C≡C-
Amide	-CO-NH2
Amine I / II / III	-NH2 / -NH- / -N=
Aromatic ring	
Carbonyl	-C=O
Carboxylic avid	-CO-OH
Disulfide	-S-S-
Ester	-CO-O-
Ether	-O-
Ketone	-CO-
Nitrile	-C≡N
Nitro	-NO2
Thioester	R-CO-S-
Thiol	R-SH
Sulfonate	-SO3Na

Purity

ACS
Materials conforming with the specifications and procedures outlined in American Chemical society specifications

USP Grade
Materials conforming with the specifications and procedures outlined in the United States Pharmacopeia

High Purity grade
Materials of superior quality where there are no publishing standard

UltraPure grade
Ultimate purity : Material with a purity level exceeding the various monograph grades

Oxidant free
Oxidative activity has not be detected. Products are thus devoid of oxidant compounds generated by conventionnal manufacturing procedures or by ageing during storage (i.e; in several detergents), leading to improved results in critical applications (i.e. in proteomics)

Trace Grade
devoid of certains impurities.

DNase/RNase free
DNase and RNase activity has not been detected. Products are thus devoid of nucleases generated by conventionnal manufacturing procedures, leading to improved results in critical applications (i.e. in genomics).

FluoGrade
Quality suitable and tested for Fluorescence detection applications.

Reagent Grade
High Purity materials which suits most standard labs applications.

Analytical Grade
Designates reagents suitable for use in analytical procedures.

Biotech grade
Highest quality suitable and tested for biotech applications: Materials equivalent to Ultra Pure, but particularly suitable for use in Molecular biology applications. Tested for specific contaminants such as nucleases and bacteria where appropriate.

MolecularBiology grade
Highest quality suitable and tested for molecular biology. Products are generally also DNase/ RNase grade

Proteomics grade: Products are Protease free, and eventually Nuclease
Materials conforming to the requirements of protein research which are tested to be nuclease, DNase and/or Protease free where applicable. Appropriate for use in Proteomics research applications.

Certified/certifiable grade
Materials, typically dyes and stains, that meet the requirements of the biological stain commission. Certified reagents have been tested and validated by biological stain commission.

AffiPure
antibodies purified by specific affinity chromatography. Polyclonal antibodies are thus devoid of non-target specific binding, leading to lower background and higher avidity.

Environment friendly
Products are devoid of compounds or packaging that are usually included in equivallent products although inauspicious for the environment or the user: their benefits include better safety, lower toxicity, easier storage or disposal.