ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

# MASTER HANDOUT

This document presents some important information of Advanced Bioengeneering Methods Laboratory course. Please print this document and always bring with you during sessions.

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# 1. COURSE OUTLINE

## 1.1 Content

- The teaching proceeds with weekly alternating sessions of laboratory practice and classroom data analysis. Both the laboratory practice and classroom data analysis sessions are supervised work in groups (3 people / group).
- The core of this course consists of 6 exercises that are meant to be performed in either 2 weeks/sessions each for a total of 12 weeks :

  I. LAB-ON-A-CHIP: learn how to design and fabricate miniature chemical and bio-chemical analysis systems, also known as Labon-a-Chip systems, referring to the idea of shrinking a complete chemical analysis laboratory onto a small chip.

  II. BROWNIAN MOTION: learn how to how to simulate and analyze Brownian motion of single particles in Matlab, use brightfield and darkfield microscopy. They will be introduced to the image data acquisition, theory and software design for image filtering and particle tracking in Matlab.

  III. OPTICAL TRAPPING: learn the basics of operating a high-end optical tweezers to record mechanical transitions of single molecules.

  IV. SURFACE DESIGN: learn some basic techniques of surface design for bioanalytics.

  V. SURFACE PLASMON RESONANCE: learn how to plan and interpret surface bio-molecular binding experiments

  **VI. LAST ONE**

- Student can find all the documents necessary for this course on the web site: **……. (Pass: …)**

## 1.2 Evaluation

- **2/3**: Written exam on the 30th of May. The exam will contain the same kind of questions presented you in handouts.
- **2/9**: Evaluation of the quality of the lab notebook. Student must compile properly the lab notebook.
- **1/9**: Evaluation of the student's preparation (before sessions) and participation (during sessions). Student has to study the handouts (provided on the web site) and prepare exercises beforehand,. Quizzes are available online for self-evaluation.

## 1.3 Preparation

- Students are asked to prepare lab sessions before the lab sessions, meaning understanding the theory and protocols, so that practical work can progress smoothly. Quiz for each topic is available on the web site, please do it beforehand for self-evaluation.

- Students have to answer to questions (those one can answer without doing the experiment) so that they can discuss about with TA during sessions. Keep in mind that you will have the same or same kind of questions during the written exam; hence it is very important to really understand questions.

## 1.4 Lab notebook

Keeping a complete and accurate record of experimental methods and data is a vital part of science and engineering. Your laboratory notebook is a permanent record of what you did and what you observed in the laboratory. Learning to keep a good notebook now will establish good habits that will serve you throughout your career.

A lab notebook should contain:

- Record of important procedures for experiments you have developed during your experimentation: everything you do, and why you did it, just like a diary!
- Record of your mistakes and difficulties. You will frequently learn more from these failures!
- Record of the results of experiments that you have performed.
- Enough information so that someone else, with an equivalent technical background to your own, is able to repeat your work, and obtain the same results by using your notebook.

Lab notebook is not a:

- Copy of the handouts.
- Text book explaining the theory of phenomena you study or manual of machines.
- Simple list of results of experiments. Comment and draw conclusions!

What goes into your notebook? :

1) Always use **pen** and write neatly and clearly
2) **Page numbers**: in the upper outside corner of each page. These are important so you can refer back to frequently used tables, procedures, or results. You can also be sure that there are no missing pages (leading to missing steps) if following a past procedure.
3) **Table of contents**: The first few pages should be reserved for this, it allows you to quickly find the information you are looking for and makes the book a useful reference. Later on you will be able to find a particular experiment without having to read every page.
4) **Dates**: Every entry, or at the very least every day that you record data should be dated
5) When you start a **new topic**, start on a right side page (odd), and record its **Title** and **Objectives** at the top of the first page dedicated to this topic.

6) **Procedure** of the experiment**,** all steps you have performed. Record **Reasons** for decisions made during an experiment as well. What we did isn't always good enough, why we did what we did is just as important to record.

7) **Unusual conditions** during an experiment. Something is went **wrong** or was **unexpected**

8) If you make a **Mistake**, don't obliterate it! You may need to read your mistake later; perhaps you were right the first time! Use a single cross out and **Explain** why it was an error.

9) **Data** typed into the computer must be printed and taped into your lab notebook. **Plots** of data made in lab should also be printed and taped in your lab notebook.

| Metric | Requirements | Worth |
|---|---|---|
| Pen | Write in pen, not pencil | 10 % |
| Date | Date **every page at the top** | 10 % |
| Right Side | Begin each experiment on odd page | 10 % |
| Printouts | Attach printouts and plots of data as needed | 10 % |
| Legible | Obvious care taken to make it readable,<br>even if you have bad handwriting | 10 % |
| Mistakes | Mistakes crossed out with one line and explained | 10 % |
| Organized | table of contents<br>title of experiment on 1st page<br>objectives of experiment<br>clear from notebook what you were doing when | 20 % |
| Informative | all required data and information<br>descriptive comments of your observations | 20 % |

# 2. SAFETY

In any laboratory, there is potential for injury if certain common-sense practices are not followed. In AMBL this is minimal, but it's still important to follow a few basic rules. In case of any doubt or question don't hesitate to ask, and if you do some mistake **REPORT IT IMMEDIATELY**: doing mistakes is not an issue, you are here to learn, not reporting them is a big issue, since it can result in unexpected-unexplainable results, and even worst, can be harmful to you or for someone else in the lab. Always ask on how to dispose used stuff.

## 2.1 Electrical safety

Electrical injuries happen when large amounts of electrical power are dissipated by the body. Most often, this happens in high-current situations (mush more dangerous than the voltage!) and it's a good idea to avoid becoming a current path.

In AMBL, we will work with only low-power electronics, and nothing we do is likely to cause injury. However, some common-sense precautions are in order:

- DO NOT connect supply voltages directly to ground
- DO NOT touch any current-carrying conductor with your bare hands

These simple rules will keep you from injuring yourself and damaging circuit components. Some components will have maximum power ratings that should not be exceeded, so pay attention to these values.

## 2.2 Laser safety

You will use a 300mW NIR diode lasers with $\lambda$=975nm during the Optical tweezers' session. The hazards come from its higher power level, and because it is invisible, making it harder to be aware of its location/direction. The beam will be largely constrained in the apparatus, and you will not need to make adjustments that might put you in the beam path.

Please keep in mind the following things:

- Always know the path of the beam, and keep any body parts or reflective items (rings, watches, etc.) out of the beam path.
- Safety goggles will be available, but not required.
- Always read the pre-labs and know what special precautions you need to take associated with lasers or optics.
- When in doubt about doing something, don't do it before checking with the lab instructor.

## 2.3 Chemical Safety & Biosafety

- DO NOT bring food or drink into the lab. The electronics will appreciate it.
- Always wear lab coat, gloves and goggles while working at the bench or at the chemical hood
- Dispose chemical/biological waste and sharps in proper containers.
- Wash your hands with soap and water after removing gloves and before leaving the lab.
- Please report any spills or injuries to the lab instructor immediately

# 3. BASIC BIOENGINEERING TOOLS

We present you here some basic technique you will use during the practical work. Please read carefully this part beforehand so that sessions can go ahead smoothly.

## 3.1 Pipetting

1) Adjust the volume of fluid dispensed by rotating the thumbwheel until the desired volume
2) Depress the push button to the first stop.
3) Dip the tip under the surface of the liquid in the reservoir to a depth of about 1 cm and slowly release the push button. Withdraw the tip from the liquid touching it against the edge of the reservoir to remove excess liquid.
4) Deliver the liquid by gently depressing the push button to the first stop. After a delay of about one second, continue to depress the push button all the way to the second stop. This action will empty the tip.
5) Release the push button to the ready position.
6) If necessary, change the tip and continue pipetting.

⚠ **NEVER** allow liquid to enter the body of the micropipette
⚠ Change the tip each time you change the solution (for nanoparticle suspensions for instance).

## 3.2 Serial dilution

It is a stepwise dilution of a substance in solution. Usually the dilution factor at each step is constant, resulting in a geometric progression of the concentration in a logarithmic fashion. A X fold dilution can also be written as a 1:X dilution, meaning that you have to pipette 1 volume of the solution to be diluted and mix it with (X - 1) volumes of solvent (buffer); where X is the volume final of the diluted solution for each step. The different parameters necessary to perform the dilutions can be fund by using the dilution equation at each step:

Dilution Equation: $M_1V_1 = M_2V_2$

Where $M_1$ = Molarity of initial solution to be diluted, $V_1$ = volume of initial solution to be diluted, $M_2$ = Molarity of final diluted solution, and $V_2$ = volume of final dilute solution

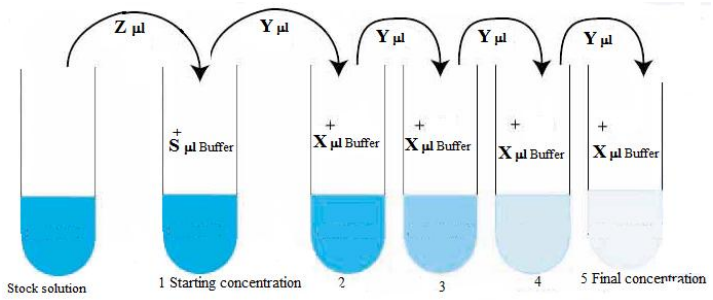


**Figure 1.** Scheme of the serial dilutions procedure.

## 3.3 Microscope

You will use the Olympus inverted system microscope (IX 71 or IX 81) illustrated in Figure 2

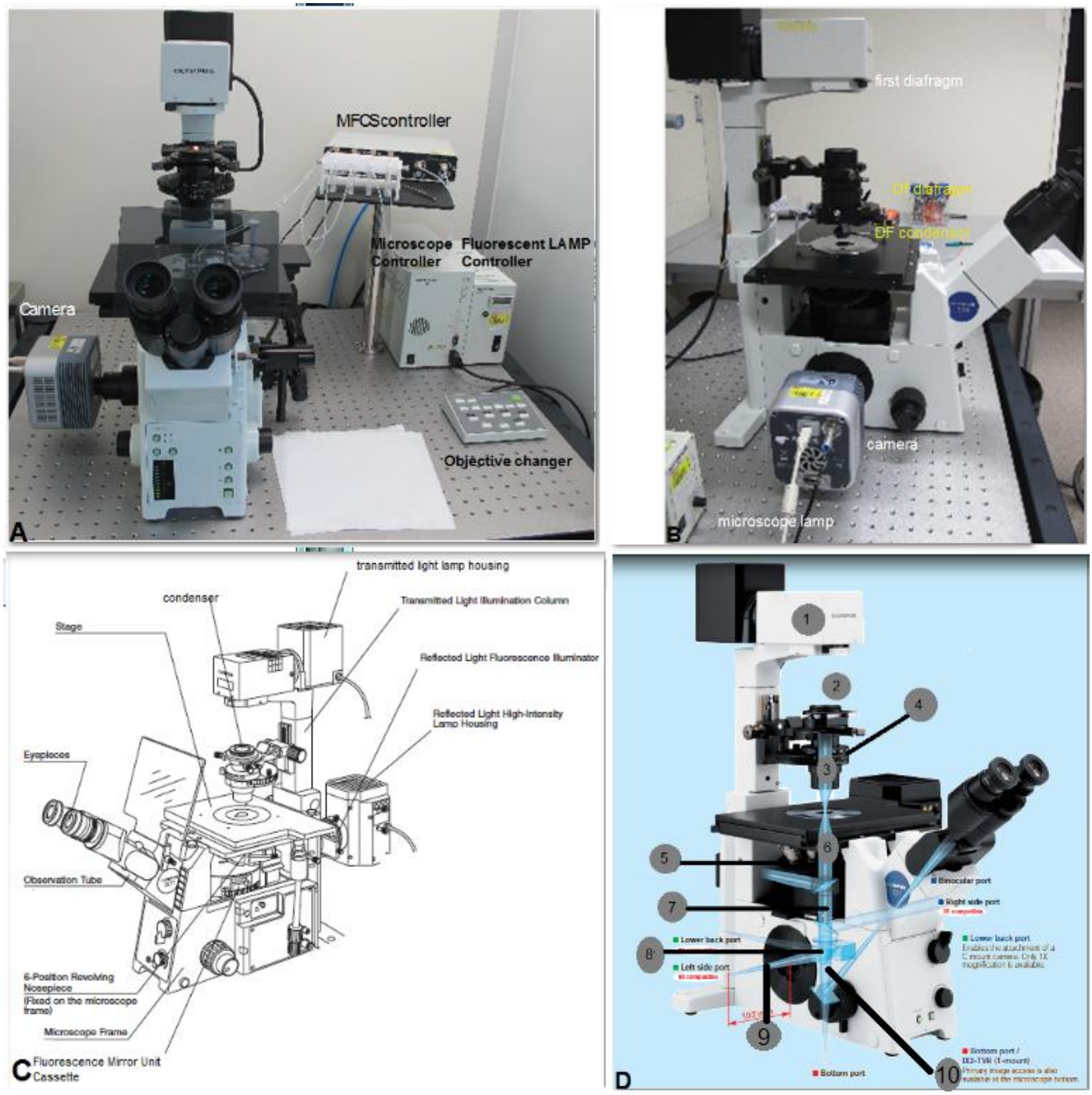


**Figure2. (A)** IX 81 microscope with fluorescent lamp, microscope and lamp controller (used in Lab on chip). **(B)** IX 71 microscope used in Brownian motion Lab. (**C**) Microscope (IX 71 or IX 81) description. (**D**) Microscope illumination path both for IX 71 or IX 81.

Since the two microscopes IX 71 (Fig2.B) and IX 81 (Fig2.A) have almost the same components some of them are presented in Fig2.C. In Fig2.D is presented the optical path of the light starting from the microscope variable intensity halogen light source to the camera for data acquisition or to the eyepiece for observation by naked eye.

① The halogen light source emits light at the desired intensity
② The light from the halogen lamp goes through the condenser.
③ The condenser focuses light from the halogen bulb onto the sample under observation.
④ A rotating turret in the condenser holds several irises and stops that are used for different contrast methods such as bright- and dark-field. (As shown, the condenser iris is selected, as would be the case for normal bright-field illumination.)
⑤ The objective nosepiece rotates to select one of four objective lenses: 5x, 10x, 20x, and 40x. The objectives are sophisticated, compound optical elements.
⑥ Light enters the microscope body through the selected objective.
⑦ The light after passing through the objective is optionally split into two paths at various levels inside the microscope body. This is done by dichroic mirrors.
⑧ A setting wheel selects whether 0 or 100 percent of the light is diverted to the left sideport for the camera or to the eye pieces.
⑨ The goes to a CCD camera mounted with its sensor at the focal plane located just outside the sideport. Images captured by the camera are transferred to the PC via a Firewire connection.
⑩ The light going toward the eyepieces is first deviated by a mirror and then passes through a binocular tube that splits the image headed toward the eyepieces in two for direct observation with both eyes. The eyepieces are 10x, providing overall magnification of 50x, 100x, 200x, or 400x depending on which objective is selected.

***The microscope is a delicate instrument. It is never necessary to force any adjustment!***

- Do not remove any parts from the microscope (except for eyepieces) or attempt to clean the optics.
- If the microscope needs cleaning, ask TAs or Professors for help.
- Do not use canned air on the microscope. It can leave a difficult-to-remove residue.
- To lengthen the lifetime of the halogen lamp, turn off the light source with the HAL on/off switch (Figure 2) when it is not in use.

As mentioned previously, these microscopes are outfitted with four objective lenses: 5x, 10x, 20x, and 40x (for IX 71) or 100X (for IX 81). Microscope images look best when properly illuminated. The procedure developed by August Köhler's is nearly universally used to achieve uniform illumination with little reflection or glare and minimal sample heating. The most challenging technique you will learn is the proper adjustment of the condenser turret and iris diaphragms to achieve good bright-field **Koehler illumination and dark-field illumination**. It is worth learning this technique carefully, as it makes a big difference in the contrast and sharpness of your image. You will also be briefly introduced to fluorescence imaging.

## 3.4 Koehler illumination

As shown in Figure1 there are two irises in the illumination path of the microscope. The one on top (closest to the light source) is the field iris and the condenser iris is a little farther down. The condenser iris adjustment is located at the edge of the turret disk near the bottom of the condenser. You will adjust both of these irises to achieve the best image.

**It is possible to make the tip of the objective collide with the slide. If you crash an objective into the slide, you will very likely break the slide. This will release the liquid from the slide on to the objective and make it very dirty. Try very hard not to do this.**

The Koehler illumination is set up by following the five easy steps shown in Figure3 and commented below:

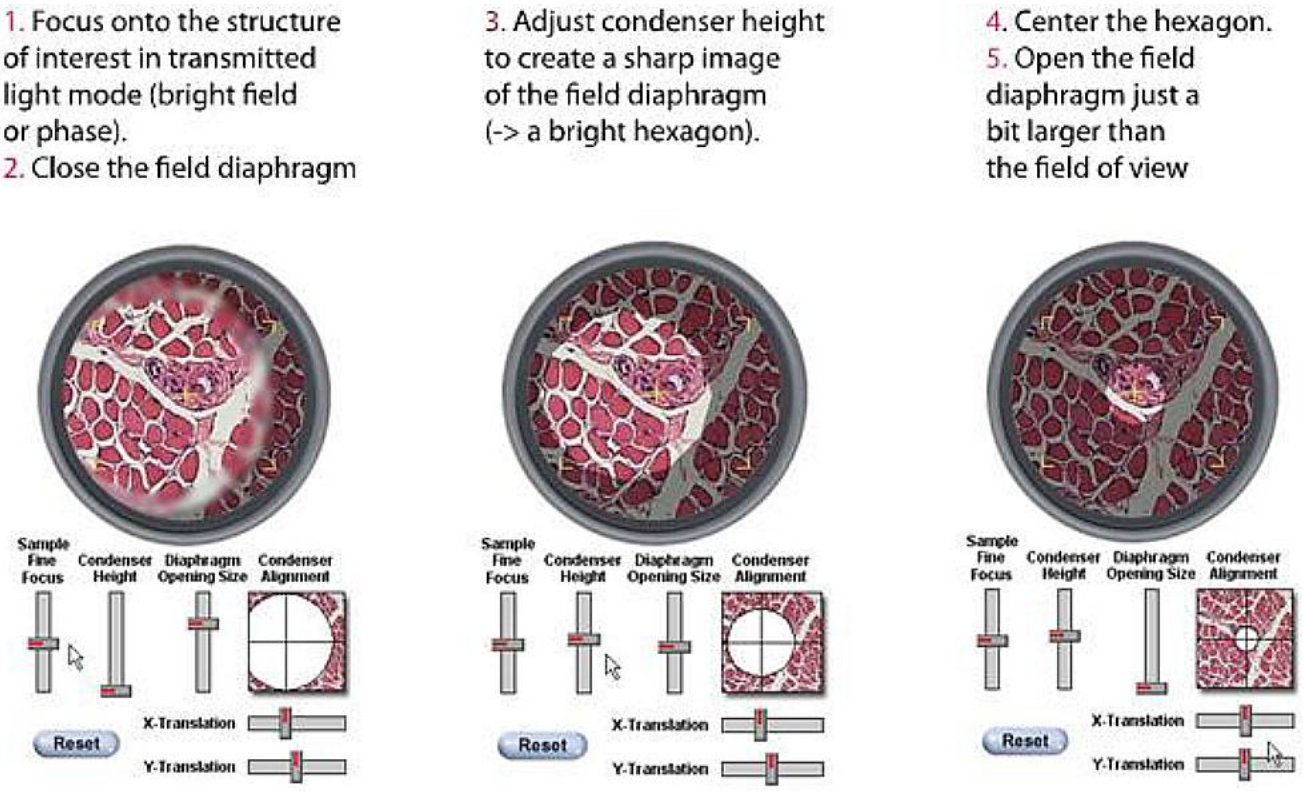


**Figure3**. Five Steps to Align the Light (Köhler-Illumination)

1) Focus on the sample, then adjust the distance between the eyepieces on the binocular tube by rotating them toward or away from each other until the distance between the eyepieces matches the distance between your pupils (when you see images in both eyes).

   The samples are usually difficult to focus on because they have very little contrast. If you have trouble focusing, try starting with the 10x objective. At higher magnification, it is sometimes helpful to focus on the edge of the slide first to get the setting close.

⚠ The eyepieces are designed to be used while wearing eyeglasses. If you do not wear glasses, don't get too close to them.

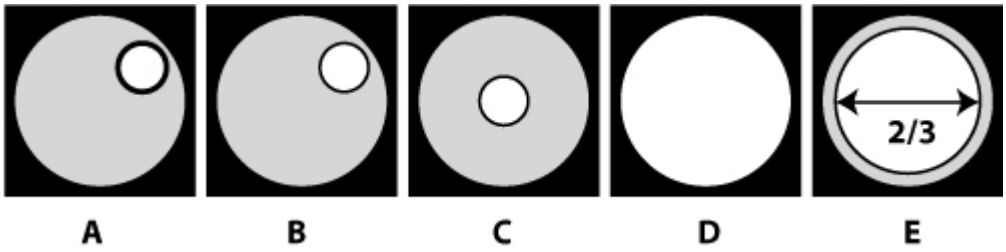


**Figure 4.** Adjusting the irises

2) Close the field iris (diaphragm) until you can see its outline in the eyepieces (Fig.4A). If you do not see the outline in the eyepieces, the condenser may be too high. Move it down using the height adjustment knob (Fig.4B).

⚠ Make sure **not to lower the condenser into the slide**. If you crash into a slide, you will break it, making the objective and the condenser very dirty in the process, and possibly damaging them.

3) Bring the field iris into focus by moving the condenser up or down using the condenser height adjustment knob. **Do not adjust the focus of the objective.** When you have successfully focused the condenser, you will see a regular hexagon of light through the eyepieces. Continue to focus until the perimeter of the hexagon is highly defined

4) Remove one eyepiece. Center the image of the iris using the condenser centering knobs until the image looks like in Figure 4C. If you do not see any light, open the field iris until you can see its edge. Use the condenser centering knobs to move the opening closer to center and then continue closing the field iris. Replace the eyepiece and look at the position of the hexagon. Use the condenser centering knobs to further center it within the field of view.

5) Looking into the eyepieces, open the field iris until its edges are just out of view (Fig. 4D). Use the image intensity switch on the front of the microscope to set a comfortable overall illumination level.

⚠ Never use the field or condenser iris to adjust the brightness.

⚠ Remember to set up Köhler illumination each time you change objectives.

## 3.5 Dark field illumination

Dark field illumination allows for observation of small particles under the microscope that would not normally be visible. This is due to the scattering of light onto the particles. The effect is similar to looking at stars during the daytime versus night.

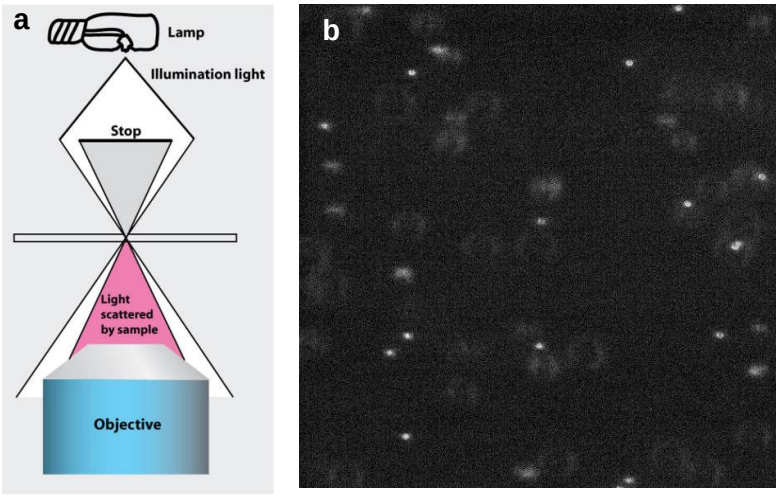


**Figure 5. a)** Principle of dark-field illumination. All the illuminating light is rejected, and light scattered by the sample is collected by the objective **b)** Darkfield image of single 0.94 µm diameter polystyrene spheres.

1) Set the desired objective and establish the Köhler illumination.
2) Rotate the condenser turret disk one position to the right. You should see an upside down number "2" just to the right of the condenser column. Remember that for bright field, the upside down number "2" is to the left of the condenser column; the upside down number "2" is to the right of the condenser column in dark field.
3) To get an idea of how this effects sample illumination, tilt the condenser column back and hold a piece of white paper under the condenser. You should see a disk of light. In this position, an opaque disk blocks the light in the middle of the illumination field.
4) To get an idea of how this effects sample illumination, tilt the condenser column back and hold a piece of white paper under the condenser. You should see a disk of light. In this position, an opaque disk blocks the light in the middle of the illumination field.
5) Open the field iris all the way.
6) Increase the light intensity using the Toggle Switch for Illumination Intensity. You will need to turn the light level up significantly in order to see the small amount of light scattered by the smaller nanoparticles (even though the PS spheres will be easily visible).

## 3.6 Fluorescence imaging

Fluorescence microscopy is the most popular method for studying the dynamic behavior exhibited in live cell imaging. This stems from its ability to isolate individual proteins with a high degree of specificity from non-fluorescing material. The sensitivity is high enough to detect as few as 50 molecules per cubic micrometer. Different molecules can now be stained with different colors, allowing multiple types of molecule to be tracked simultaneously. These factors combine give fluorescence microscopy a clear advantage over other optical imaging techniques, for both in vitro and in vivo imaging.

Fluorescence microscope is a light microscope used to study properties of organic or inorganic substances using the phenomena of fluorescence instead of, or in addition to, reflection and absorption. In most cases, a component of interest in the specimen is specifically labeled with a fluorescent molecule called a fluorophore

(such as GFP, Green Fluorescent Protein). GFP is a fluorescent protein that was first found in the jellyfish Aequorea Victoria. It has the useful property that its formation is not species specific. This means that it can be fused to virtually any target protein by genetically encoding its cDNA as a fusion with the cDNA of the target protein. This can be done in a live cell, and hence the movement of individual cellular components can now be analyzed across time.

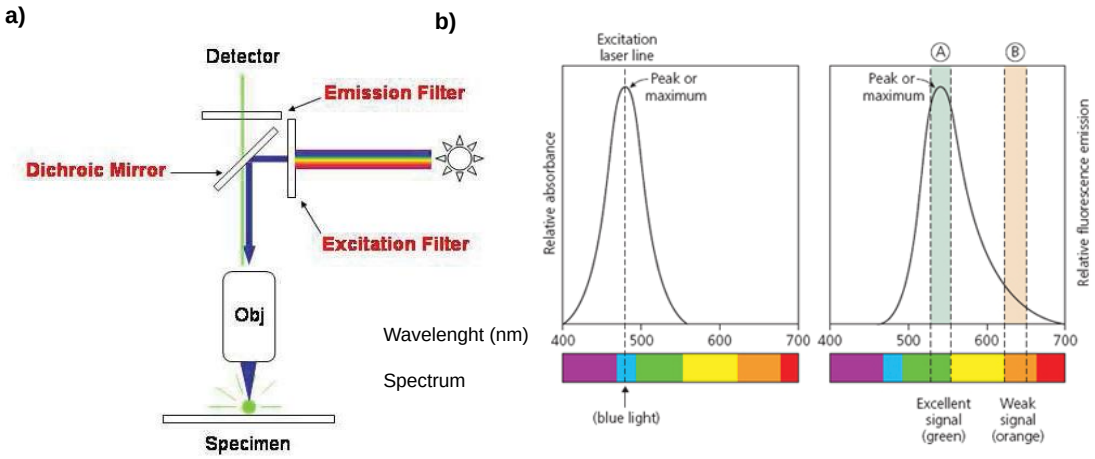


**Figure6. a)** Fluorescence imaging principle (Wikipedia:Fluorescence_microscopy) **b)** Excitation and emission spectra of the dyes used in this practical FITC very close the GFP excitation and emission spectra

There is no requirement to fix and permeabilize the cells first. The discovery of GFP has made the imaging of real-time dynamic processes commonplace, and caused a revolution in optical imaging. The GFP revolution goes even further with the development of different colored GFP isoforms, such as yellow GFP and cyan GFP. This allows multiple proteins to be viewed simultaneously in a cell.

The specimen is illuminated with light of a specific wavelength (or wavelengths) which is absorbed by the fluorophores, causing them to emit longer wavelengths of light (of a different color than the absorbed light). The illumination light is separated from the much weaker emitted fluorescence through the use of a dichroic mirror. Typical components of a fluorescence microscope are the light source (Xenon or Mercury arc-discharge lamp), the excitation filter, the dichroic mirror (or dichromatic beam splitter), and the emission filter. The filters and the dichroic are chosen to match the spectral excitation and emission characteristics of the fluorophore used to label the specimen.

## 3.7 Filters

Filters you will use for Lab on chip session are called FITC (Figure 7) according to the traditional fluorochromes that were earlier commonly used for green and red fluorescence. In the figure, the blue (1) curve shows the excitation i.e. the wavelengths that illuminate the sample. The red (2) curve shows the emission i.e. the wavelengths that are shown to the viewer.

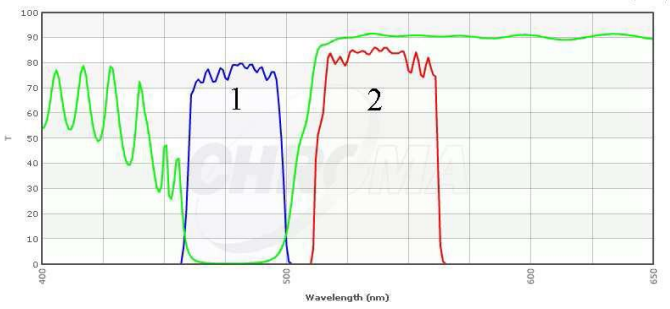


**Figure7.** FITC filter spectrum. 1 = excitation band, 2 = emission band

# 4. VOCABULARY GLOSSARY

- **Adsorption:** Adsorption is the adhesion of atoms, ions, biomolecules or molecules of gas, liquid, or dissolved solids to a surface. This process creates a film of the adsorbateon the surface of the adsorbent[1]. For biosensor, unspecific adsorption must be ideally completely avoided to make the result reliable enough.
- **Affinity:** In chemical physics, affinity is the electronic property by which dissimilar chemical species are capable of forming chemical compounds[2]. This definition might be widened for macromolecules such as proteins or DNA. Affinity might be influenced by non-covalent intermolecular interactions between the two molecules such as hydrogen bonding, electrostatic interactions, hydrophobic and Van der Waals forces[3]. High affinity is usually characterized by a low dissociation constant.
- **Analyte**: A substance or chemical constituent that is undergoing analysis[4].
- **Assay:** Procedure in molecular biology for testing or measuring the activity of a drug or biochemical in an organism or organic sample. A quantitative assay may also measure the amount of a substance in a sample[5].
- **Autocorrelation**: is a mathematical tool for analyzing the relationship between a variable and its pas or future value. It is useful to identify repeating patterns such as periodic pattern in noisy signals. It consists of computing the cross-correlation with itself. A random variable is characterized by a single peak at middle as autocorrelation function. Brownian motion or white noises are characterized by a single peak as well since they are random phenomenon.
- **Avidity effect:** Effect of the overall strength of binding of an antigen with many antigenic determinants and multivalent antibodies. It is influenced by both the valence of the antibody and the valence of the antigen and is more than the sum of the individual affinities[6].
- **Binocular**: An optical device, such as a pair of field glasses or opera glasses, designed for simultaneous use by both eyes and consisting of two small telescopes joined with a single focusing device[7].
- **Bioconjugation:** Method for the mild and site-specific derivatization of proteins, DNA, RNA, and carbohydrates developed for applications such as ligand discovery, disease diagnosis, and high-throughput screening[8].
- **Biosensor**: Small device which utilizes biological reactions for detecting target analytes. Such device intimately couples a biological recognition element (interacting with the Target analyte) with a Physical transducer that translates the biorecognition event into a useful electrical signal[9].
- **Bright field illumination:** The method of lighting the specimen with a solid cone of rays in microscopy. There are two types: the transmitted bright field illumination performed by a substage condenser and the reflected bright field illumination performed by a vertical illuminator[10].
- **Bulk:** The core of the substance or the solution.
- **CAD**: Computer aided design[11]
- **Calibration**: The setting or correcting of a measuring device or base level, usually by adjusting it to match or conform to a dependably known and unvarying measure[12].
- **Calibration curve**: The graphic or mathematic relationship between the readings obtained in an analytic process and the quantity of analyte in a calibration. The relationship is often established as a straight line rather than a curve[13].
- **Cavitation:** The sudden formation and collapse of low-pressure bubbles in liquids by means of mechanical forces or sonication, such as those resulting from rotation of a marine propeller[14].

- **CCD (Charged Coupled Device) camera**: Digital Camera where CCDs are used as sensors for images recording. The CCD captures light and converts it to digital data that is recorded by the camera[15].
- **Chaotropic salt:** Salt having the ability to destabilize hydrogen bonding and hydrophobic interactions[16].
- **Condenser**: One of the main components of many transmitted light compound microscopes. A condenser is a lens that serves to concentrate light from the illumination source that is in turn focused through the object and magnified by the objective lens[17].
- **Cross correlation**: Mathematical tool for analyzing the relationship between a variable and its past or future. It determines the similarity of two functions or variable sets as a function of a time-lag.
- **Cutoff**: In some cases, only one side of the range is usually of interest. Therefore, such targets are often given with only one limit of the reference range given, and, strictly, such values are rather cut-off values[18].
- **Cutoff frequency**: Also called corner or break frequency. It is the boundary in a frequency domain system at which energy flowing through the system begins to be reduced rather than passing through[19] i.e frequency at the corner level. In electronic filters for instance, it is the boundary frequency of passband and stopband.
- **DAQ card**: Device designed to acquire data from a detector or sensor and to store it on a computer for future analysis[20].
- **Dark field illumination**: Specialized illumination technique that capitalizes on oblique illumination to enhance contrast in specimens that are not imaged well under normal brightfield illumination conditions[21].
- **Detergents:** Amphipathic, surface active, molecules with polar and nonpolar domains. They bind strongly to hydrophobic molecules or molecular domains to confer water solubility[22] and are used to purify, isolate or solubilize membrane proteins while preserving the protein's biological activity; they are also used to selectively prepare culture media by inhibiting certain bacteria growth, and to isolate, purify, crystallize or renature proteins.
- **Dielectric**: A dielectric material is a substance that is a poor conductor of electricity, but an efficient supporter of electrostatic fields[23].
- **Evanescent wave:** A wave on the other side of a surface from a total internal reflection and that decays quickly and exponentially with distance[24].
- **Fluorescence**: The emission of electromagnetic radiation, especially of visible light, stimulated in a substance by the absorption of incident radiation and persisting only as long as the stimulating radiation is continued[25].
- **Functionalisation:** The addition of functional groups onto the surface of a material by chemical synthesis methods[26].
- **Gaussian blurring filter**: Filter which blur or smooth an image by a Gaussian function. It is a widely used effect in graphics software, typically to reduce image noise and reduce detail in image processing[27]. The blurring degree is tuned by the variable $\sigma$.
- **GUI**: Graphical user interface
- **Hallmark:** A mark indicating quality or excellence
- **Heterobifunctional reagent:** A reagent that has a reactive group at each end of the molecule that can bind a different molecule[28].
- **Hybridization:** The act or process of mating organisms of different varieties or species to create a hybrid.

But also defined as the process of forming a double stranded nucleic acid from joining two complementary strands of DNA (or RNA) [29].

- **Hybrislip: F**lexible, thin covers that minimize fluid friction and facilitate uniform reagent distribution[30] used to create chambers between the microscope slide and the cover slide as well as to hermetically seal them together.
- **Immobilization:** Deposition of a substance on a surface in order to fix it position.
- **Interactant:** Any of the elements involved in an interaction[31].
- **Interface:** A surface forming a common boundary between adjacent regions, bodies, substances, or phases.
- **Ionic strength:** A measure of the average electrostatic interactions among ions in an electrolyte; it is equal to one-half the sum of the terms obtained by multiplying the molality of each ion by its valence squared[32].
- **Iris diaphragm** : A microscope piece that provides continuously variable apertures for applications including setting the numerical aperture of a lens, controlling or measuring the intensity of a diffuse beam, or approximating beam diameters[33].
- **Isoelectric point:** The pH wherein a zwitterion or amphoteric molecule has no net electric charge since it has equal number of cations and anions as well as the net charge of its bound ions is also zero[32]
- **Isotropy**: Uniformity in all orientations. An isotropic property is a property regardless of direction.
- **Laminar flow**: Occurs when a fluid flows in parallel layers, with no disruption between the layers[34]. There are no cross currents perpendicular to the direction of flow, nor eddies or swirls of fluids[35]. In fluid dynamics, laminar flow is characterized by high momentum diffusion and low momentum convection, this property allow fluid separation widely used in microfluidics.
- **Ligand:** A substance (an atom or molecule or radical or ion) that forms a complex around a central atom[36]
- **L E D**: Light emitting diode.
- **Measurement range**: Value of the measurand over which the sensor is intended to measure, specified by upper and lower limits[8]
- **Mediating layer**: A molecular layer with Barrier function (passivation of the underlying substrate) or/and active function (providing attachment sites for further chemical attachment and bioconjugation) [8].
- **Milliq:** Millipore Corporation's trademark, created to describe 'ultrapure' water of "Type 1", as well as the devices used to produce it[37].
- **Monochromatic light**: Light composed of radiation of only one wavelength.
- **Monoclonal antibody**: An antibody produced by a single clone of cells (single clone of hybridoma cells) and therefore a single pure homogeneous type of antibody[38].
- **PBS buffer**: Tampon Phosphate Saline is a buffer solution commonly used in biological research. It is a water-based salt solution containing sodium chloride, sodium phosphate, and potassium chloride and potassium phosphate. The buffer helps to maintain a constant pH. The osmolarity and ion concentrations of the solution usually match those of the human body[39].
- **Photodiode**: A photo sensitive semiconductor device through which current can pass freely in only one direction[40].
- **Polarizability**: The measure of the change in a molecule's electron distribution in response to changing

electric interactions with solvents or ionic reagents. It is a property of matter.

- **Polarizabilities**: Determine the dynamical response of a bound system to external fields, and provide insight into a molecule's internal structure[41]. In optical trapping, the specimen must be polarizable (dielectric such as cellular or bacterial membrane or polystyrene beads…) so that the gradient force, one of the two main trapping forces is generated.
- **Probe:** A substance, such as DNA, that is radioactively labeled or otherwise marked and used to detect or identify another substance in a sample[8].
- **Rack**: A frame for holding vials.
- **Reagent**: Substance or compound that is added to a system in order to bring about a chemical reaction or is added to see if a reaction occurs[32].
- **Reflectance:** A measure of the ability of a surface to reflect light or other electromagnetic radiation, equal to the ratio of the reflected flux to the incident flux[32].
- **Refractive index**: The ratio of the speed of light in a vacuum to the speed of light in a medium under consideration or a measure of the extent to which a substance slows down light waves passing through it. (5)
- **Repeatability**: Variability of the measurements obtained in the same conditions of measurement over a short period of time[8].
- **Reproducibility**: Variability of the measurement system caused by differences in the conditions of measurement[8].
- **Resolution**: Minimal change of measurand value necessary to produce a detectable change at the output. [8].
- **Sampling rate**: The number of samples per unit of time taken from a continuous signal to make a discrete signal. Sampling is an essential step of the data acquisition and must take into account the Nyquist–Shannon sampling theorem so that the data is completely recoverable[42].
- **Scattering of light**: Light scattering can be thought of as the deflection of a ray from a straight path, for example by irregularities in the propagation medium, particles, or in the interface between two media of different refractive index. Most objects that one sees are visible due to light scattering from their surfaces[43].
- **Selectivity**: Extent to which a method can determine particular analytes in mixtures or matrices without interferences from other components[8].
- **Sensitivity**: Ratio of the change in output to the change in the value of the measurand[8].
- **Sensorgram:** A graph of responses versus time in surface plasmon resonance studies[44.]
- **Silanization:** Treatment of a hydrophilic surface with a reactive silane in order to render it more hydrophobic by chemically converting the SiOH moieties of a stationary form to the ester form[45, 8].
- **Sonication:** The act of applying sound waves to agitate particles in a sample or to disrupt particles in a solution[46].
- **Specificity**: "ultimate of selectivity". No component other than the analyte contributes to the result[32].
- **Stiffnes**s: Stiffness is the resistance of an elastic body to deformation by an applied force when a set of loading points and boundary conditions are prescribed on the elastic body (analogous of Hookean spring coefficient if 1DOF). It is an extensive material property[47]. The trap stiffness of an optical trapping gives the "trapping strength" of the device, and is highly related to the resulting force given by both gradient and scattering force.

- **Substrate:** The surface or medium on which an organism grows or is attached. But also The substance acted upon by an enzyme.
- **Substrate chip**: Base chip made with a certain material on which chemical modification are brought for specific applications. Silicon is the most widely used substrate for chips.
- **Surface activation**: Surfaces modification by attachment or adsorption of functional groups to tailor surface properties for specific applications[48].
- **Surface passivation**: Process of making a material "passive", usually by the deposition of a layer of oxide that adheres to the metal surface[49]. For sensors, passivation consists of making a thin layer of inert molecule on the sensor surface, to protect this latter from unspecific adsorption and so allow a better selectivity.
- **Target:** Refers to the material: DNA or RNA that one exposes to the probes on a microarray so that hybridization can be measured subsequently but also molecule that may interact with a drug or drug candidate[50].
- **Transmitted light Microscopy**: The general term used for any type of microscopy where the light is transmitted from a source on the opposite side of the specimen from the objective. The light is passed through a condenser to focus it on the specimen to get very high illumination. After the light passes through the specimen, the image of the specimen goes through the objective lens and to the oculars where the enlarged image is viewed[51].
- **Turbulent flow**: Flow regime characterized by chaotic and stochastic property changes. This includes low momentum diffusion, high momentum convection, and rapid variation of pressure and velocity in space and time[52].

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