ADVANCED METHODS IN BIOENGINEERING LABORATORY

- Introduce professors
- Content & structure
- Safety
- Logistics
Introduce Professors

- Georg Fantner
  - **LJNI** - Laboratory for Bio- and Nano- Instrumentation
  - Lab website, ljni.epfl.ch [georg.fantner@epfl.ch](mailto:georg.fantner@epfl.ch)

- Carlotta Guiducci
  - **CLSE** - Chair on Engineering. Laboratory of Life Sciences Electronics.
  - Lab Website: clse.epfl.ch [carlotta.guiducci@epfl.ch](mailto:carlotta.guiducci@epfl.ch)

- Aleksandra Radenovic
  - **LBEN** - Laboratory of Nanoscale Biology LBEN
  - Lab website: lben.epfl.ch [aleksandra.radnovic@epfl.ch](mailto:aleksandra.radnovic@epfl.ch)
Objectives

1. Learning the quantitative approach in bioengineering
   - How to make use of **quantitative high-sensitive, high resolution technologies** to measure biophysical and biochemical parameters
   - Imaging, trapping and tracking **single biological entities at the nanoscale**
   - Characterizing **molecular binding** by the interpretation of averaged signal on millimeter-square areas
   - Determining sensitivity and selectivity of a **biosensor**
   - Designing and building a **lab-on-a-chip device** (and some of the things you can do with it)
   - How to analyze real life scientific data (get quantitative answers)

2. working in real-life research labs
   - Keeping professional notebook
   - Behavior in a cleanroom

3. Planning, organizing and executing a research project
4. Write a scientific paper in the “Letter to nature” style
CONTENT AND STRUCTURE
Teaching method/Structure of the course

- 4 contact hours per week, 4 credits
- One ex-cathedra introduction session (1 week)
- 3 modules (6 weeks) of pre-prepared exercises
- One “independent” research project based on a real world publication/project (1 week planning + 3 weeks execution)
- Write a paper in “Nature style” about your project (2 weeks)
### Schedule

<table>
<thead>
<tr>
<th>Date</th>
<th>GR1</th>
<th>GR2</th>
<th>GR3</th>
<th>GR4</th>
<th>GR5</th>
<th>GR6</th>
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<td>INTRO</td>
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<td>SPR</td>
<td>AFM</td>
<td>OT</td>
<td>LOAC</td>
<td>SD</td>
<td>BM</td>
<td>HOME: analyse experiments and prepare for experiments on handouts</td>
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Groups

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<tr>
<th>GR1</th>
<th>Chenevas-Paule Clément</th>
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<tr>
<td>GR1</td>
<td>Cuillery Emilie Marie Claudia</td>
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<tr>
<td>GR1</td>
<td>Gasbarri Matteo</td>
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<td>GR2</td>
<td>Lamanuzzi Leonardo</td>
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<td>GR2</td>
<td>Makhlouf Aly Abdelrahman Ismail</td>
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<td>GR3</td>
<td>Mesbah Seyedehgolzar</td>
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<td>Mettraux Nicolas Arthur</td>
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<td>GR4</td>
<td>Nash Pavel Anthony</td>
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<td>Pacifico Giammarco</td>
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<td>GR5</td>
<td>Rudinskiy Mikhail</td>
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<td>GR5</td>
<td>Sprunger Yann Christophe</td>
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<tr>
<td>GR6</td>
<td>Van Roey Pierrick</td>
</tr>
<tr>
<td>GR6</td>
<td>Zemp Dominique Anne</td>
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Teaching method/Structure of the work

- Exercises consist of four hours of **supervised work** in group.

- **Independent work** (with available support from the teacher and assistants during specified office hours):
  - Prepare each exercise prior to the first session on the available handouts and complementary material.
  - Complete of data analysis when requested after the end of the exercise
  - Laboratory Notebook-filling **during** and after (for analysis only, not for rewriting) the practice.

- Independent research project, with support from assistant and teachers.
Teaching material

- The handouts, applets and additional material for respective exercise can be found on moodle (in progress, previously our teaching website http://lben.epfl.ch/teaching)

- Reference books:
  - Intermolecular and Surface Forces, J. Israelachvili, Academic press
  - Surface Plasmon resonance Based Sensors, J.Homola et al., Springer
  - Surface Design: Applications in Bioscience and Nanotechnology, R. Forch, H. Schonherr, A.T. Jenkins, Wiley
Evaluation

- Continuous control:
  - 2/3 Paper written during independent research project
  - 1/3 Evaluation evaluation by the TAs (quiz)
    - compile properly the lab notebook (one for each student)
    - Prepare the exercise in advance by studying the handouts provided on the site
    - You will have to study the handouts (provided on the web site) and prepare for the exercises beforehand. At the beginning of each exercise, a quiz proposed by TA will serve to assess your preparation. If failed, you will get a ZERO POINTS for this part
    - Participate actively and as much as possible autonomously to the exercise
THE SIX LABORATORY PRACTICES
Bioanalytics
- **SURFACE DESIGN** The students will learn some basic techniques of surface design for bioanalytics.
- **SURFACE PLASMON RESONANCE** The students will learn how to plan and interpret surface bio-molecular binding experiments.

Working with single biological entities
- **BROWNIAN MOTION** The students will learn 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.
- **OPTICAL TRAPPING** In this students will learn the basics of operating a high-end optical tweezers to record mechanical transitions of single molecules.
- **ATOMIC FORCE MICROSCOPY** The students will learn how to use AFM on various biological samples. Learn image processing and how to extract meaningful data at the nanometer scale.

LAB-ON-A-CHIP
- The students will learn how to design and fabricate miniature chemical and bio-chemical analysis systems, also known as Lab- on-a-Chip systems, referring to the idea of shrinking a complete chemical analysis laboratory onto a small chip.
AFM a Versatile Tool for Nanoscale Measurements

- Things you will learn:
  - Make surface topography images with sub nanometer resolution
  - Nanoscale imaging of *E. coli*.
  - Extract tendons from rat tails, image microfibrils of collagen and measure the characteristic D-banding structure of collagen fibrils
  - How to process and analyze AFM data
Locations and dress code

- **AFM**
  - dress code: wear pants and close toed shoes. Bring your lab coat.

If this affects you, you will be contacted.

To BM5116 due to lab move! Location will change during the semester.
**SPR**

**Description:**
The exercise consists in the employment of label-free biosensors for the observation of binding kinetics. Real-time biomolecular bindings will be observed for different molecules.

**Objectives:**
- Understanding the importance of real-time measurements of biomolecular binding interactions
- Perform kinetic analysis for ligands immobilized on a sensor chip by amine coupling chemistry
  - Direct coupling
  - Ligand-mediated coupling

**Structure:**
- 1st week: Introduction to SPR Technology and Surface preparation
- 2nd week: SPR experiment and kinetic analysis

**Diagram:**
- Resonance signal (kRU)
- Kinetics
- Association
- Dissociation
- Regeneration
- Concentration
- Time (s)
Locations and dress code

- **SURFACE PLASMON RESONANCE**
  - location: **TP SSV and CLSE BM2112**
  - Meeting in TPSSV for first session and in front of **BM2112 for second session**.
Surface design

Description:
Modern bioanalytics is based on surface detection of biomolecules. The exercise will explore a surface modification technique commonly employed in biosensor and microbiosensors.

Objectives:
- Learn how to design an experiment of biomolecular detection on arrayed surfaces
- Perform an analysis in terms of hybridization efficiency according to different conditions

Structure:
- 1^{st} week: surface cleaning and deposition of an arrayed pattern of molecular probes (DNA oligonucleotides)
- 2^{nd} week: hybridization with complementary sequence, data acquisition and analysis
Locations and dress code

**SURFACE DESIGN**

- **location:** TP SSV
- **dress code:** lab coat. Closed shoes. Long pants
Brownian motion

- In the first part of this exercise, the students will replicate Perrin's work with modern equipment. Next they will investigate intracellular vesicle transport inside living cells and determine if the vesicle transport is accomplished by Brownian motion or by directed transport.

Aleksandra Radenovic
Locations and dress code

- **BROWNIAN MOTION**
  - location: MED 31521
  - dress code: lab coat
Optical Trapping

- Optical trapping is one of the most successful technology transfers from a physics lab to biology. The goal of this exercise is to provide hands on experience to the bioengineering students of one of the mostly used single molecule technique.
Locations and dress code

- **OPTICAL TRAPPING**
  - location: MED2 1120
  - dress code: lab coat, *IR goggles*
Lab-on-a-chip

- Lab-on-a-chip (LOC) exercise will introduce students to the fundamental elements of moving fluids in LOC systems such as flows, pressure driven flow, electro-osmotic driven flow, capillary effects, surface forces.
Locations and dress code

- **LAB-ON-A-CHIP**
  - **location:**
    - CMI+ zone 12. dress code: wear pants. You will be given instruction on what to dress-LOC
    - LBEN lab BM2124
  - meeting for the first session in front of CMI+ and for the second session in BM5202
Locations and dress code

- **ANALYSIS SESSIONS**
  - Location: BM5202 (for AFM, Brownian motion and Optical trapping exercises)
LAB SAFETY
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.
Electrical Safety

- Electrical injuries happen when large amounts of electrical power are dissipated by the body. Most often, this happens in high-current situations, which is why you always hear that “it’s not the voltage, it’s the current that is dangerous.” Strictly speaking, both are dangerous, 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:
  - don’t connect supply voltages directly to ground
  - don’t 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.

Aleksandra Radenovic
Chemical Safety & Biosafety

- Though there is minimal wet work in AMBL please do not bring food or drink into the lab. The electronics will appreciate it, and we will also later be handling some bacteria and fluorescent dyes.
- When needed, latex gloves will be provided, as well as proper containers for disposing of chemical/biological waste and sharps. Please make sure to 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.

Aleksandra Radenovic
Safety and Behaviour in the cleanroom
Description of the cleanroom
Description of the cleanroom

Air filtration and circulation

**ACTUAL VALUES**: (2/3 of maximum capacity)

- **FRESH AIR**
  - 38’000 m$^3$/h
  - filter efficiency: 99.97% for particles size 0.1-0.3 µm

- **EXHAUST**
  - 36’000 m$^3$/h

- **FFU**
  - 167 units
  - 0.7 m$^2$ active area
  - total: 189’000 m$^3$/h
  - filter efficiency: 99.999% for particles size 0.1-0.3 µm
Description of the cleanroom
**Procedure to access CMI BM+1**

- Quick access is possible to CMI BM+1
  - Contract to sign
  - Short project discussion
  - Basic instruction are given orally
  - MSDS + SOP required if non standard chemical use
  - Attending the next full cleanroom safety training

- Enter via the “BM+1 SAS”,
  - lockers for personal items
  - cleanroom paper use only

- Transfer of materials and decontamination via “BM+1 SAS”
  - even small items must be decontaminated
  - no material enter without authorisation from CMI staff
Dressing CMI BM-1

- over shoes
- cleanroom suit
- ...

ABML 2017/2018
Dressing CMI BM-1

- over shoes
- cleanroom suit
- cleanroom boots
- face mask
- vinyl gloves
- safety googles
- CAMIPRO card
Dressing CMI BM-1 and BM+1

CMI BM -1

CMI BM +1
Dressing CMI BM-1 and BM+1

Get out the same way you get in
General behaviour in the cleanroom

- NEVER WORK ALONE IN A ZONE
- NO MORE THAN 6 PEOPLE IN A ZONE
- WALK NORMALLY, DON’T RUN
- DO NOT SHAKE HANDS
- DO NOT WORK IN THE CLEANROOM IF YOU HAVE A COLD
- IN CASE OF EVACUATION ALARM, FOLLOW THE SAFETY RULES
- ONLY STAFF FIX THE MACHINES
General cleanroom rules

• lint free cleanroom paper only
• cleanroom notebooks available through CMi ordering system
• pens are available in each zone
• photocopier can be used to transfer notes in and out of the cleanroom
• PC access to public folders in each zone

prohibited:
• normal paper
• pencils & normal pens
Safety Rules

- Never Work Alone
- Only One Emergency N°:
  - tél. 115
- Report any safety problems you encounter
- Wear protective glasses or Medical glasses all the time
Alarms & evacuation

- Double Tone Horn
- Flashing Red Light

⇒ Evacuate immediately with cleanroom dressing

meeting point: BM 1.125 (Ph. Flückiger office) wait there to be accounted for

remark: red alarm can be activated by the push-buttons
Alarms & evacuation

- Yellow alarm is for the staff only
  - Eau DI / DI water
  - Neutralisation
  - ......
Alarms & evacuation
**Working Safely with Chemicals (1)**

[1] **NO NEW CHEMICALS without formal permission through SOP process**
- from Jean-Marie Voirol (CMi safety Manager)

[2] **INFORM yourself**
- Read and understand Material Safety Data Sheets of all chemicals you intend to use, before using them
- MSDS are available in the “SAS du Personnel”
Working Safely with Chemicals (2)

[3] NEVER MIX chemicals (even with water)
- Baths are always prepared by the CMi staff
- Disposal of chemicals after use only in labelled containers

[4] NEVER STORE chemicals on working place

[5] RINSE and DRY working place after each use
- before going to another equipment

[6] Use LABELLED CONTAINERS and tools for your processes

[7] If you have to leave, always label a chemical process in progress with name, date, your expected time of return, where you can be reached, the chemicals involved

[8] Never assume that colourless droplets are just water
Laser safety

- 300 mW NIR diode lasers with $\lambda=975\text{nm}$ (optical traps) The hazards of this Class IIIb laser 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. Safety goggles will be available, but not required.

- In general, other important things to keep in mind:

- Always know the path of the beam, and keep any body parts or reflective items (rings, watches, etc.) out of the beam path.
- 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.

Aleksandra Radenovic