- Introduce professors
- Content & structure
- Safety
- Logistics

ADVANCED METHODS IN BIOENGINEERING LABORATORY

Introduce Professors

- Georg Fantner
  - **LBNI -** Laboratory for Bio- and Nano- Instrumentation
  - Lab website, lbni.epfl.ch georg.fantner@epfl.ch
- Carlotta Guiducci
  - **CLSE** - Chair on Engineering. Laboratory of Life Sciences Electronics.
  - Lab Website: clse.epfl.ch carlotta.guiducci@epfl.ch
- Aleksandra Radenovic
  - **LBEN -** Laboratory of Nanoscale Biology LBEN
  - Lab website: lben.epfl.ch 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

Schedule ABML 2017-2018								
	GR1	GR2	GR3	GR4	GR5	GR6	DEADLINES	
1st 02/19	INTRO	INTRO	INTRO	INTRO	INTRO	INTRO		HOME: prepare for experiments on handouts
2nd 02/26	SPR	AFM	OT	LOAC	SD	BM		HOME: analyse experiments and prepare for experiments on handouts
3rd 03/05	SPR	AFM	OT	LOAC	SD	BM		HOME: analyse experiments and prepare for experiments on handouts
4th 03/12	SD	SPR	LOAC	OT	BM	AFM		HOME: analyse experiments and prepare for experiments on handouts
5th 03/19	SD	SPR	LOAC	OT	BM	AFM		HOME: analyse experiments and prepare for experiments on handouts
6th 03/26	OT	SD	AFM	BM	SPR	LOAC	Preference of exercise sent by students	HOME: analyse experiments and prepare for experiments on handouts
7th 04/09	OT	SD	AFM	BM	SPR	LOAC	Assignement of projects communicated by teachers	HOME: analyse experiments and prepare plan
8th 04/16	Meeting with teachers and brainstorming on plan of experiments							HOME: work on plan
9th 04/23	EXP							HOME: work on analysing experiments and on paper
10th 04/30	EXP							HOME: work on analysing experiments and on paper
11th 05/07	EXP						hand first version of the paper by Friday	HOME: work on analysing experiments and on paper
12th 05/14	feedback from teacher and assistants on the first version of the paper							HOME: work on finalizing paper
13th 05/28	work with assistants on paper						hand final version of paper by Friday end of the semester	HOME: work on finalizing paper

Groups

GR1	Chenevas-Paule Clément
GR1	Cuillery Emilie Marie Claudia
GR1	Gasbarri Matteo
GR2	Lamanuzzi Leonardo
GR2	Makhlouf Aly Abdelrahman Ismail
GR3	Mesbah Seyedehgolzar
GR3	Mettraux Nicolas Arthur
GR4	Nash Pavel Anthony
GR4	Pacifico Giammarco
GR5	Rudinskiy Mikhail
GR5	Sprunger Yann Christophe
GR6	Van Roey Pierrick
GR6	Zemp Dominique Anne

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
  - "Introduction to Error Analysis: The Study of Uncertainties in Physical Measurements," Taylor, John R., 1997, University Science Books,
  - Optical Trapping Review : K.C. Neuman & S.M. Block, "Optical trapping,"Rev. Sci. Instrum. 75 (2003).
  - Lab on a Chip Technology, Volume 1: Fabrication and Microfluidics, Keith E. Herold and Avraham Rasooly, Caister Academic Press, 2009
  - http://www.afmworkshop.com/atomic-force-microscope-book.php

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

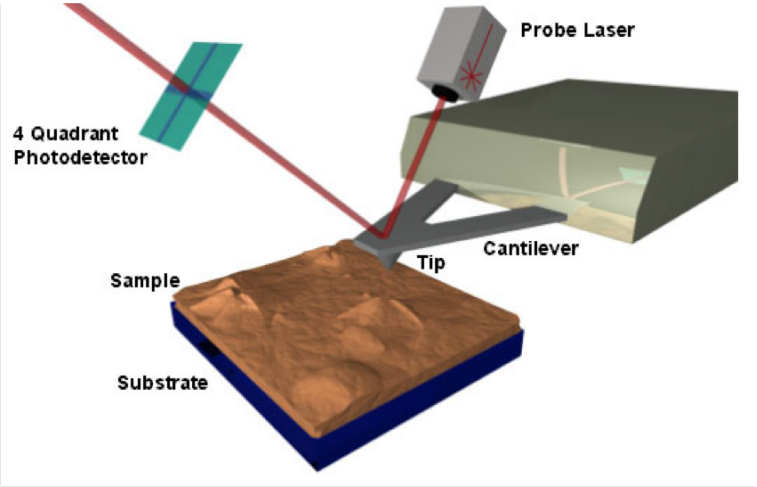
THE SIX LABORATORY PRACTICES

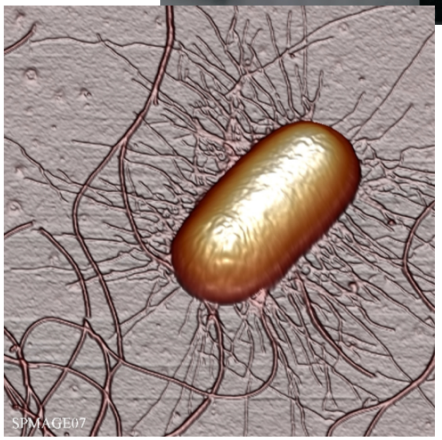
- **Bioanalytics**
  - **SURFACE DESIGN** The students will learn some basic techniques of surface design for bioanalytics.
  - **SURFACE PLASMON RESONANCE** The students will learn how to plan and interpret surface bio-molecular binding experiments.

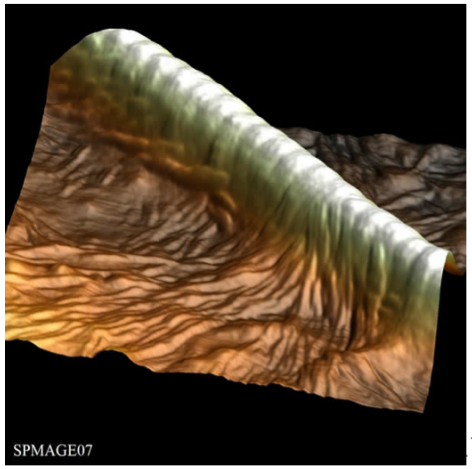
- **Working with single biological entities**
  - **BROWNIAN MOTION** The students will learn how to simulate and analyze Brownian motion of single particles in Matlab, use brightfield and darkfield microscopy. They will be introduced to the image data acquisition, theory and software design for image 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



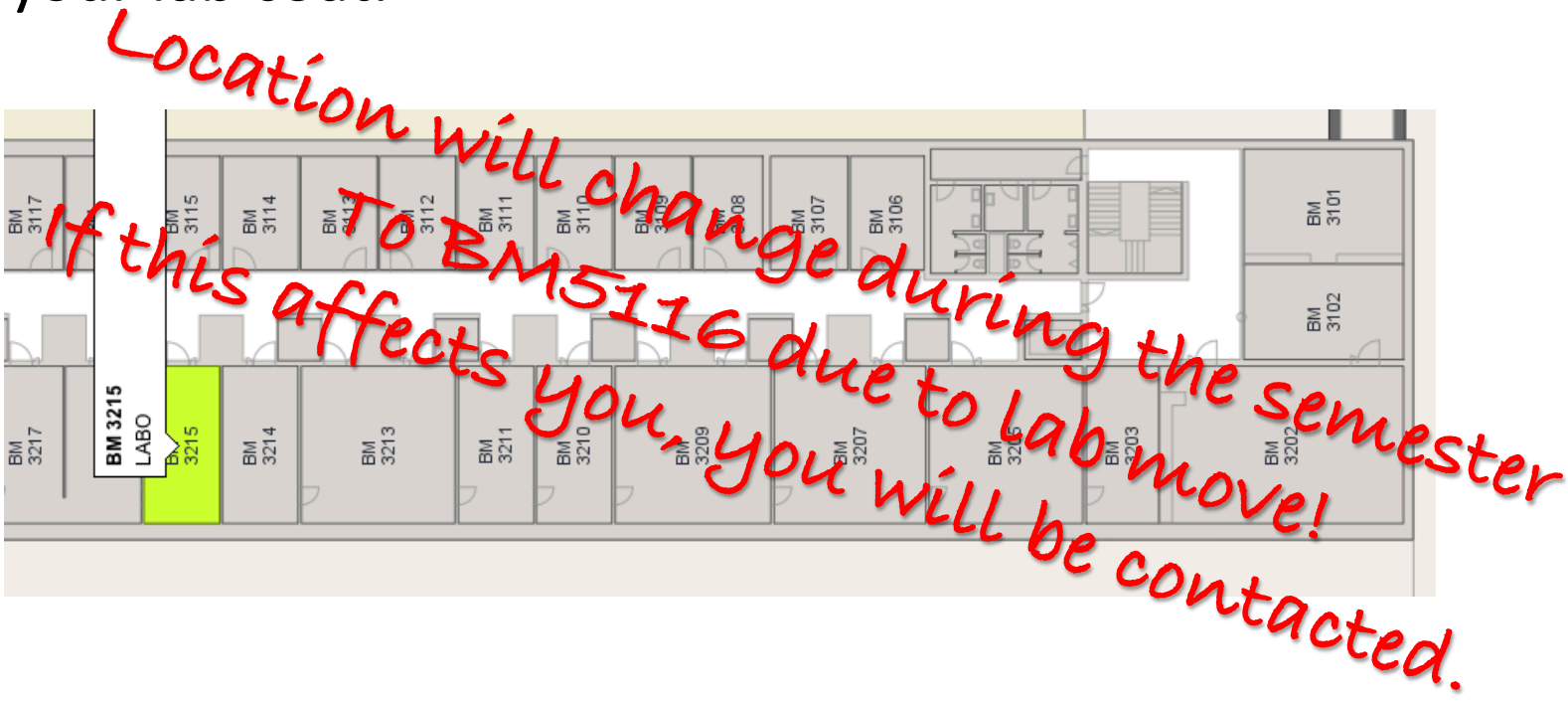




- Things you will learn:
  - Make surface topography images with sub nanometer resolution
  - Nanoscale imaging of E.coli.
  - Extract tendons from rat tails, image microfibrils of collagen and measure the characteristic D-banding structure of collagen fibrils
  - How to process and analyze AFM data

Locations and dress code

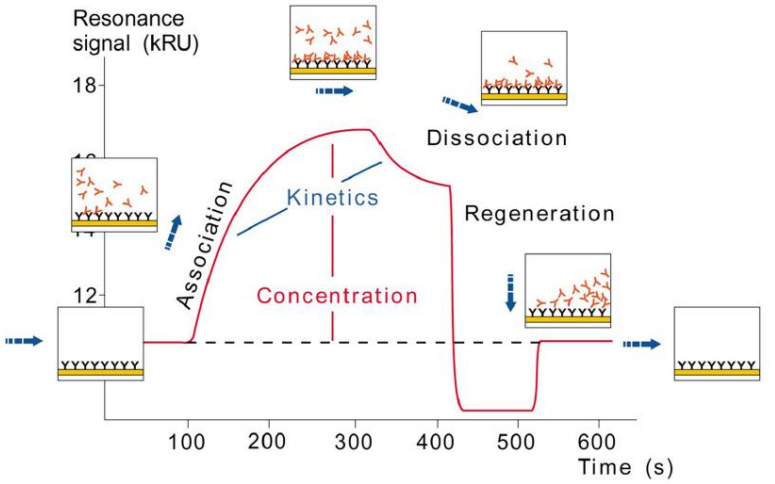
- AFM
  - dress code: wear pants and close toed shoes. Bring your lab coat.



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:

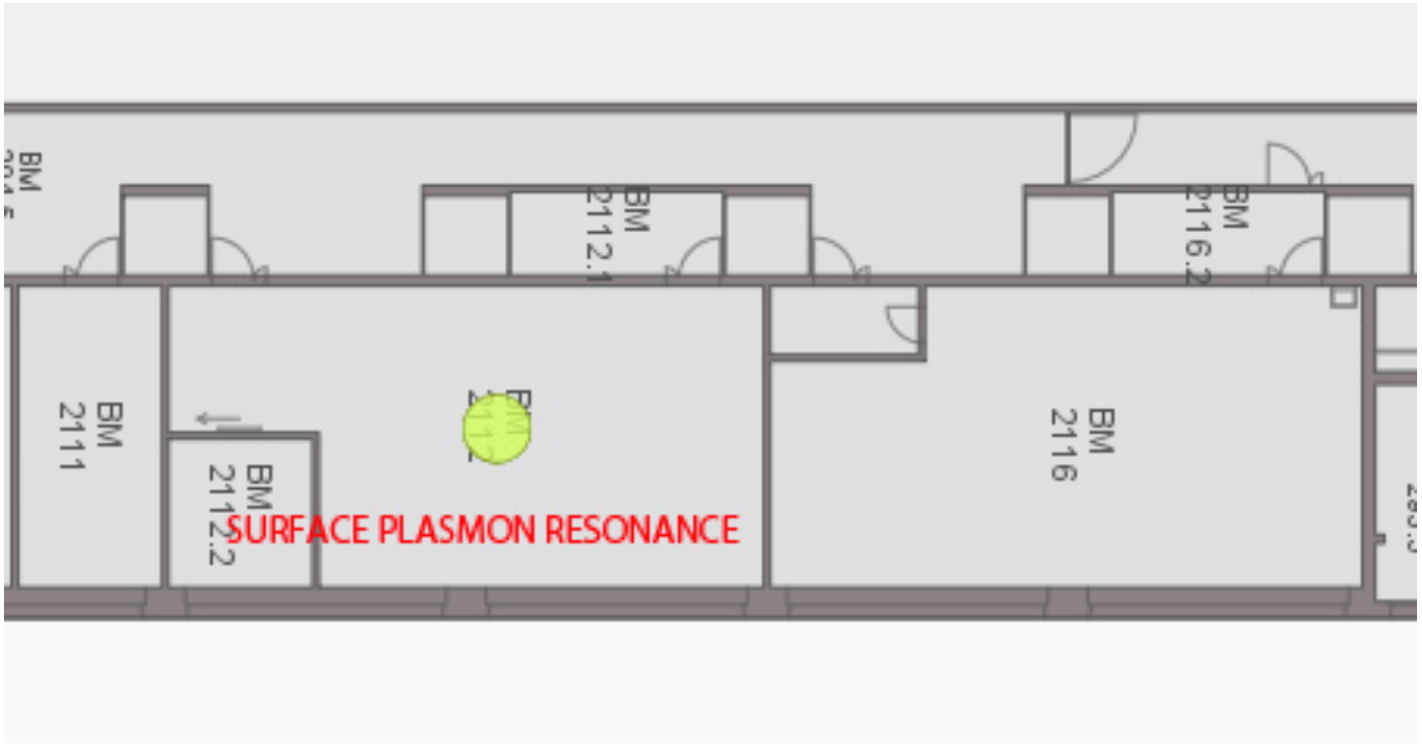
- understanding the importance of real-time measurements of biomolecular binding interactions
- Perform kinetic analysis for ligands immobilized on a sensor chip by amine coupling chemistry
  - Direct coupling
  - Ligand-mediated coupling

Structure:

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

Locations and dress code

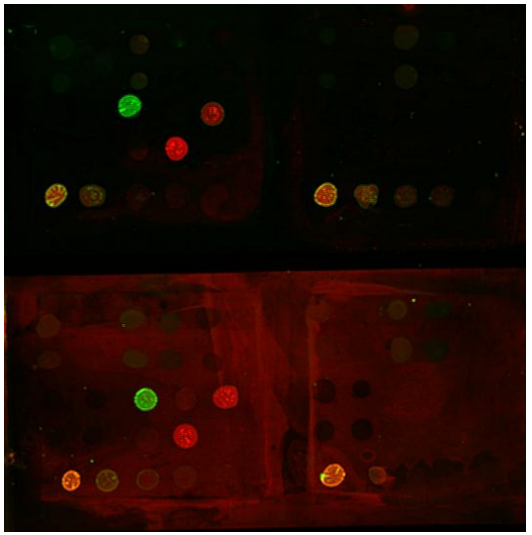
- **SURFACE PLASMON RESONANCE**
  - location: **TP SSV and CLSE BM2112**
  - Meeting in TPSSV for first session and in front of **BM2112 for second session.**
  - dress code: lab coat. Closed shoes. Long pants.



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:

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

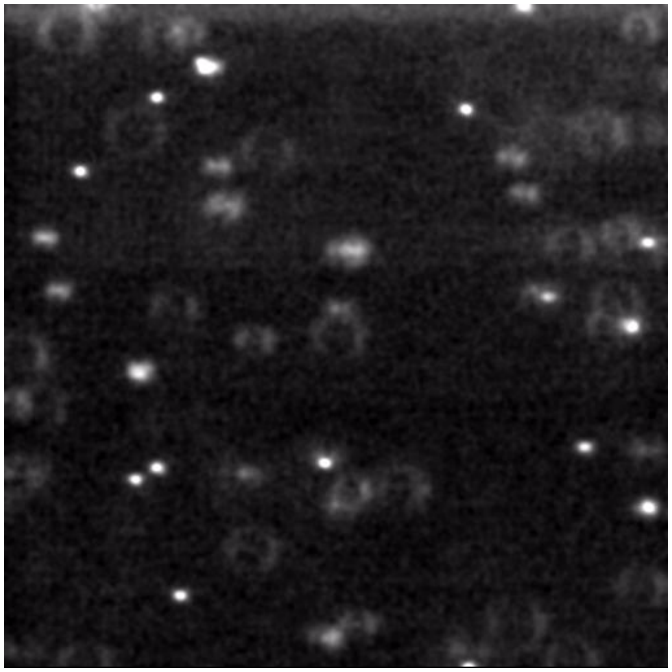
Locations and dress code

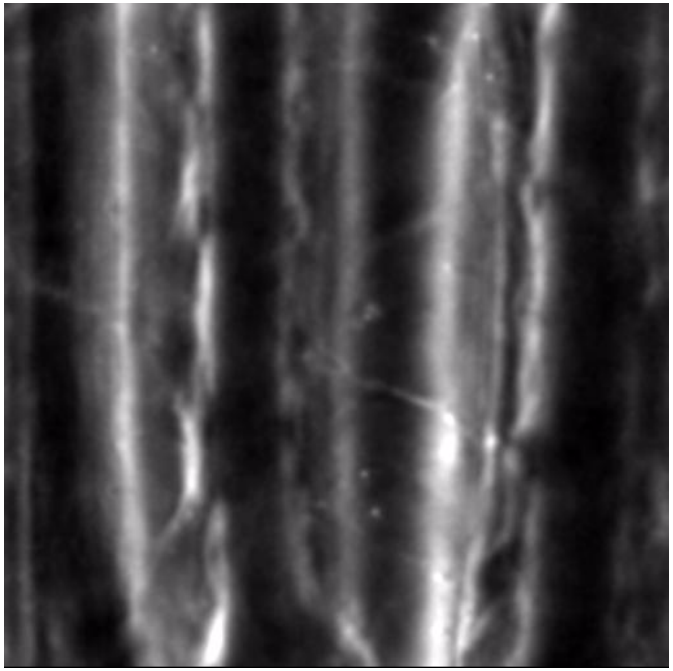
- **SURFACE DESIGN**
  - location: **TP SSV**
  - dress code: lab coat. Closed shoes. Long pants

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.

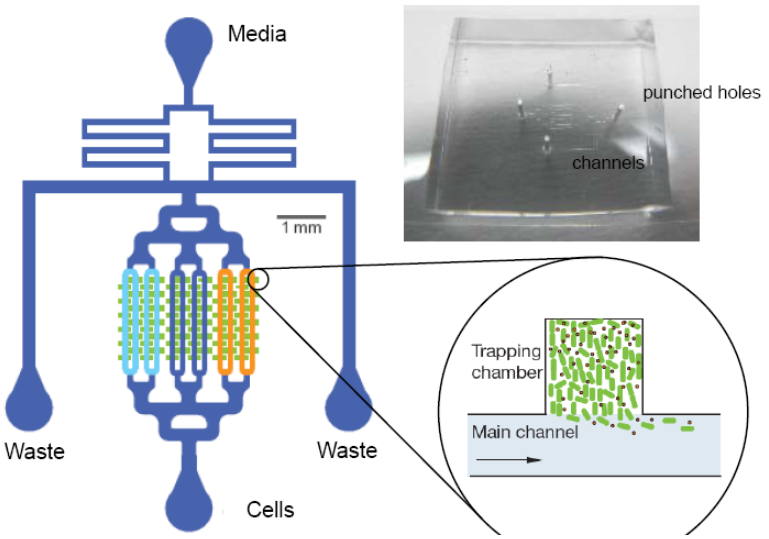
Aleksandra Radenovic

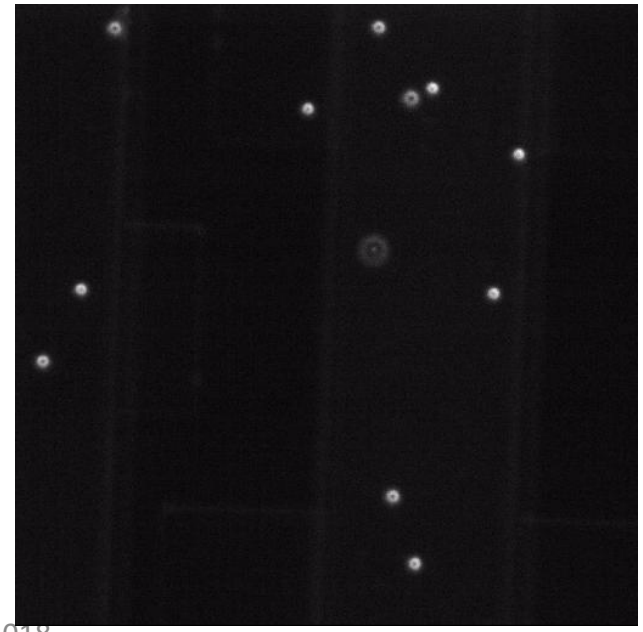
Locations and dress code

- **OPTICAL TRAPPING**
  - location: **MED2 1120**
  - dress code: lab coat, ***IR goggles***

Lab-on-a-chip

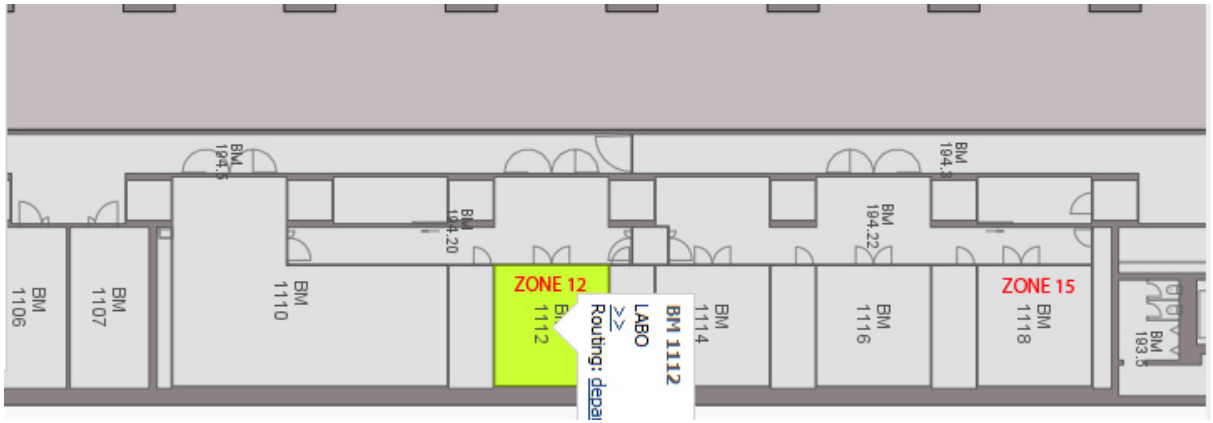
- Lab-on-a-chip (LOC) exercise will introduce students to the fundamental elements of moving fluids in LOC systems such as flows, pressure driven flow, electro-osmotic driven flow, capillary effects, surface forces.





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

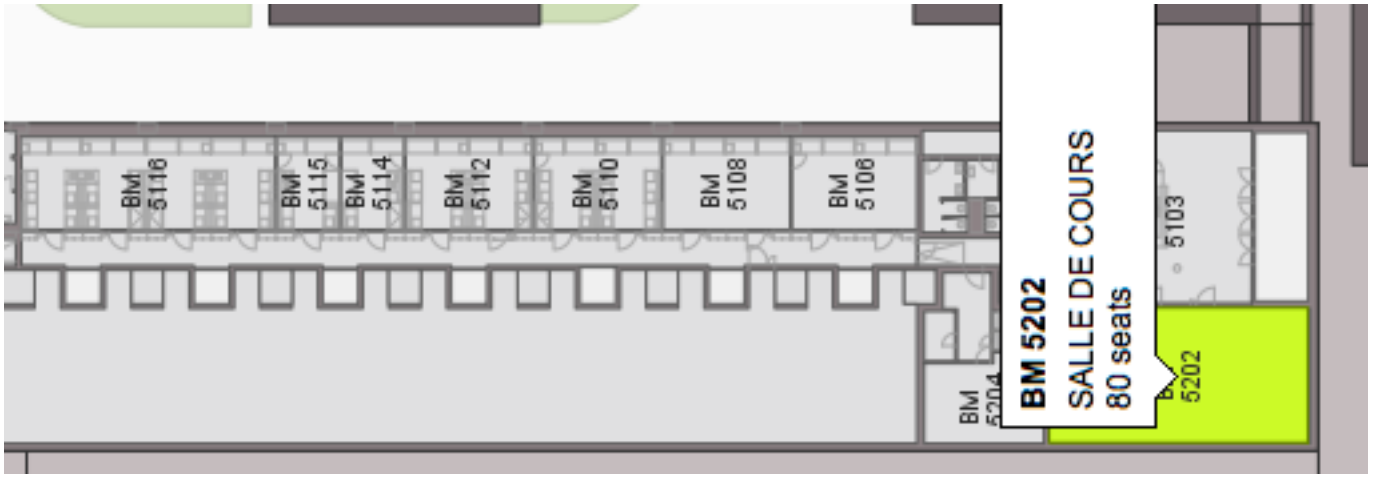


CMI+

LBEN

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

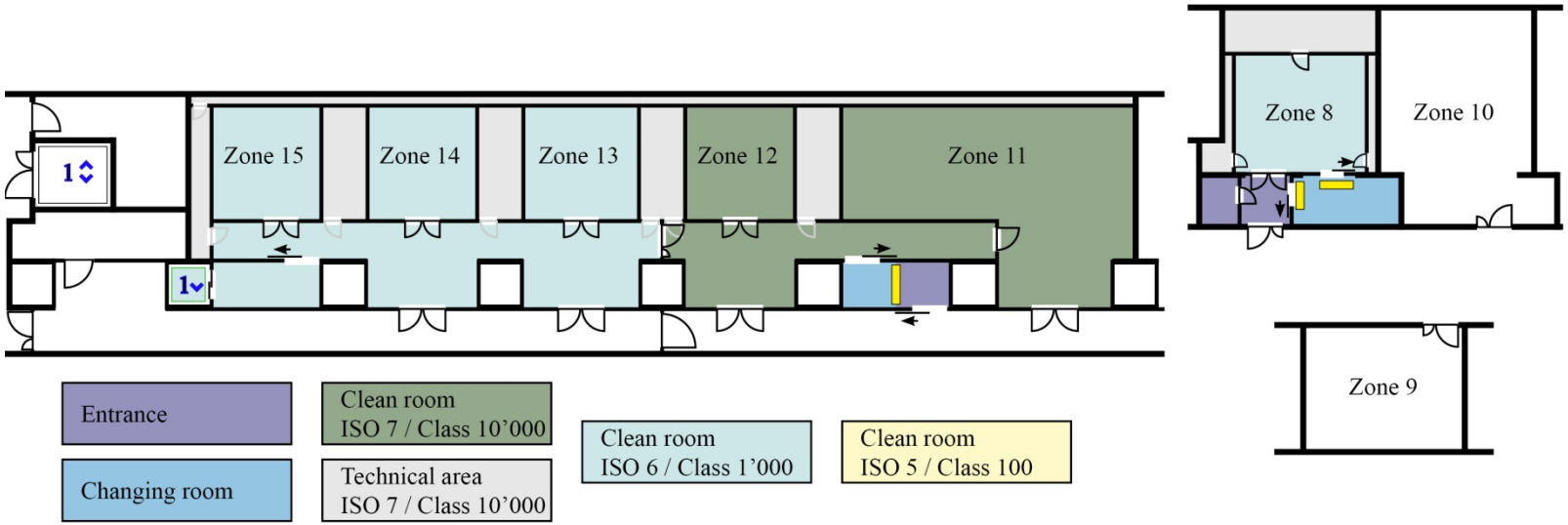
- Though there is minimal wet work in AMBL please do not bring food or drink into the lab. The electronics will appreciate it, and we will also later be handling some bacteria and fluorescent dyes.
- When needed, latex gloves will be provided, as well as proper containers for disposing of chemical/biological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

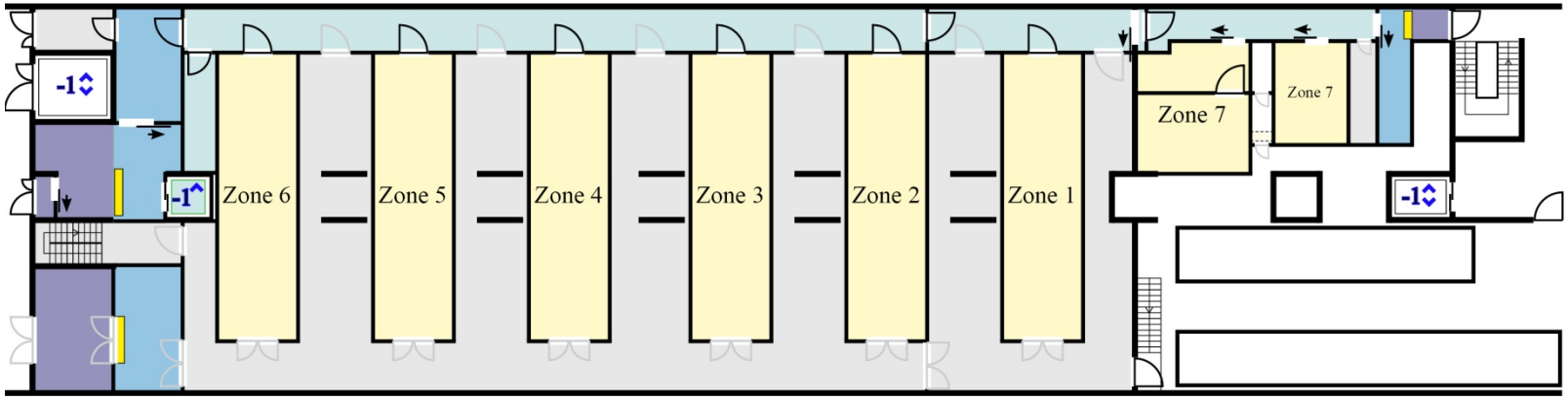
Safety and Behaviour in the cleanroom

Description of the cleanroom

CMI
BM+1

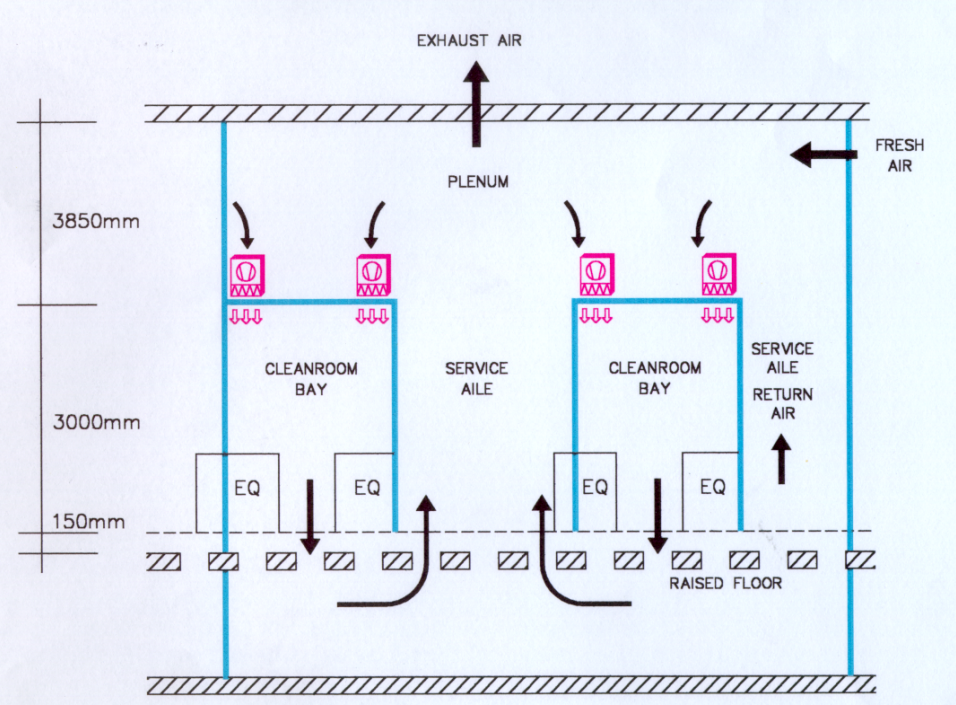


CMI
BM-1



Description of the cleanroom

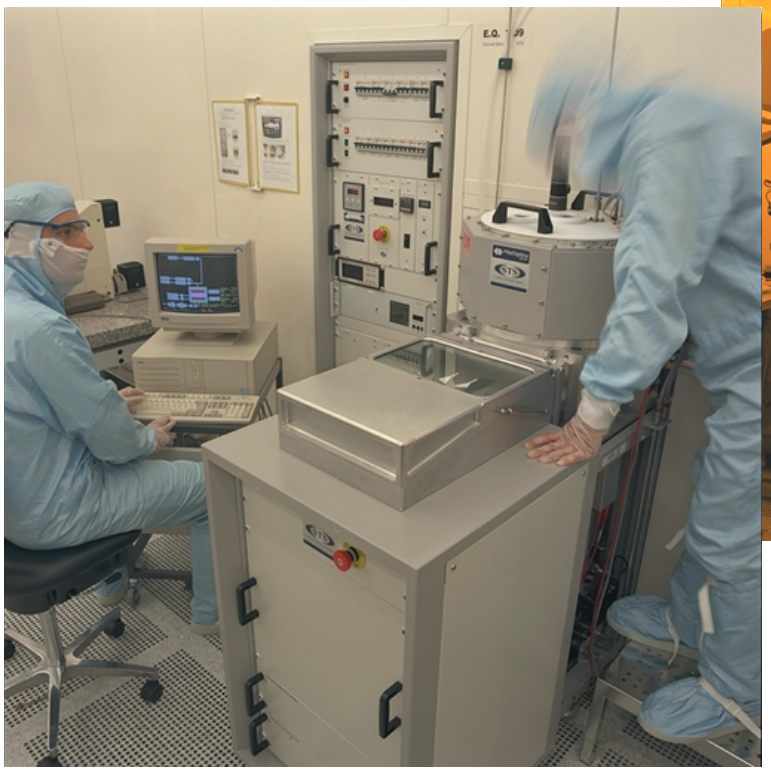
Air filtration and circulation

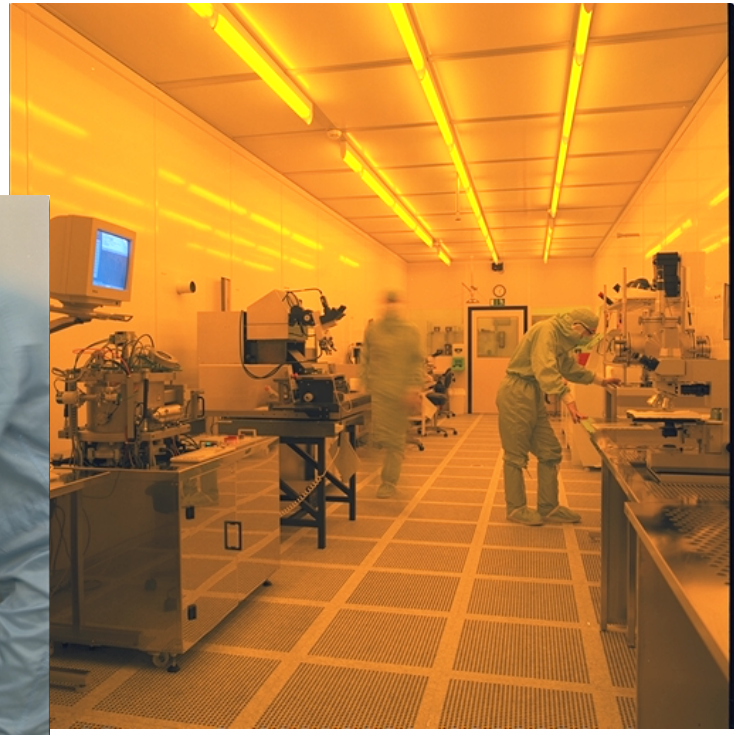


<u>ACTUAL VALUES</u> :
(2/3 of maximum capacity)

- **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

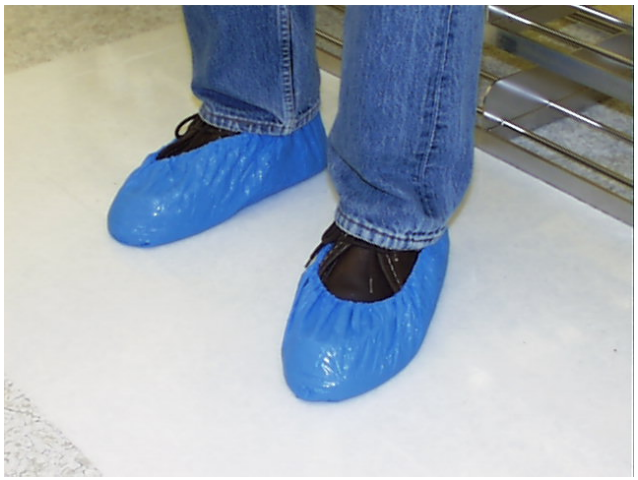




Procedure to access CMI BM+1

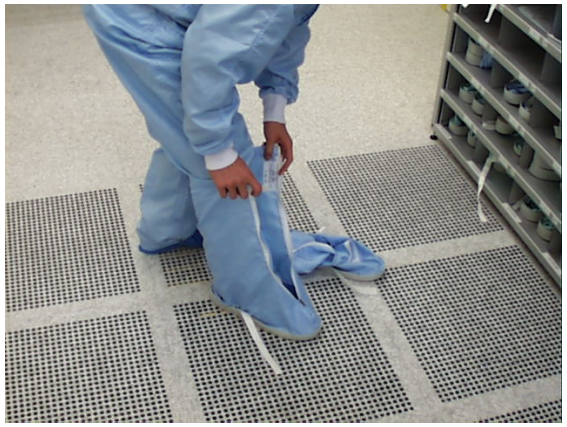
- Quick access is possible to CMI BM+1
  - Contract to sign
  - Short project disscusion
  - Basic instruction are given orally
  - MSDS + SOP required if non standard chemical use
  - Attending the next full cleanroom safety training
- Enter via the “BM+1 SAS”,
  - lockers for personal items
  - cleanroom 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



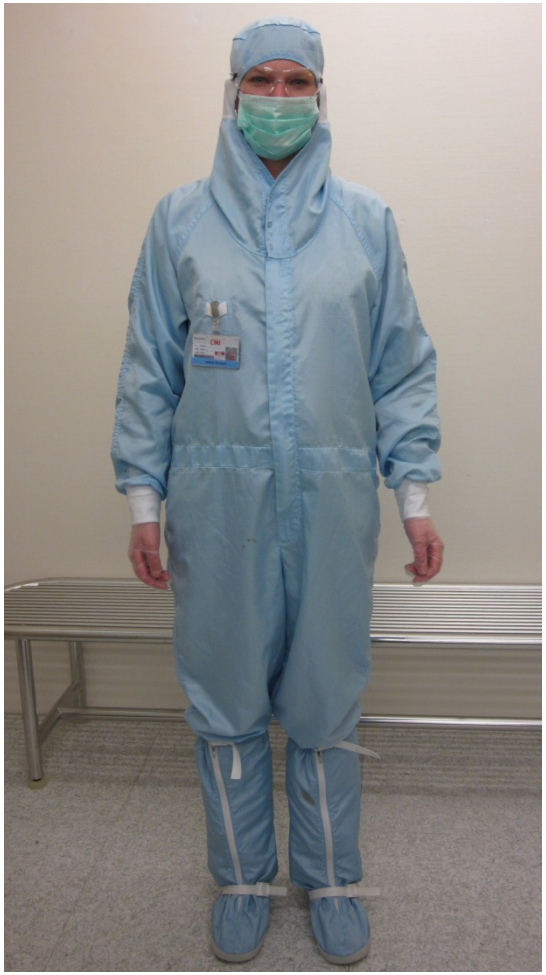
- over shoes
- cleanroom suit
- ...

Dressing CMI BM-1



- over shoes
- cleanroom suit
- cleanroom boots
- face mask
- vinyl gloves
- safety googles
- CAMIPRO card

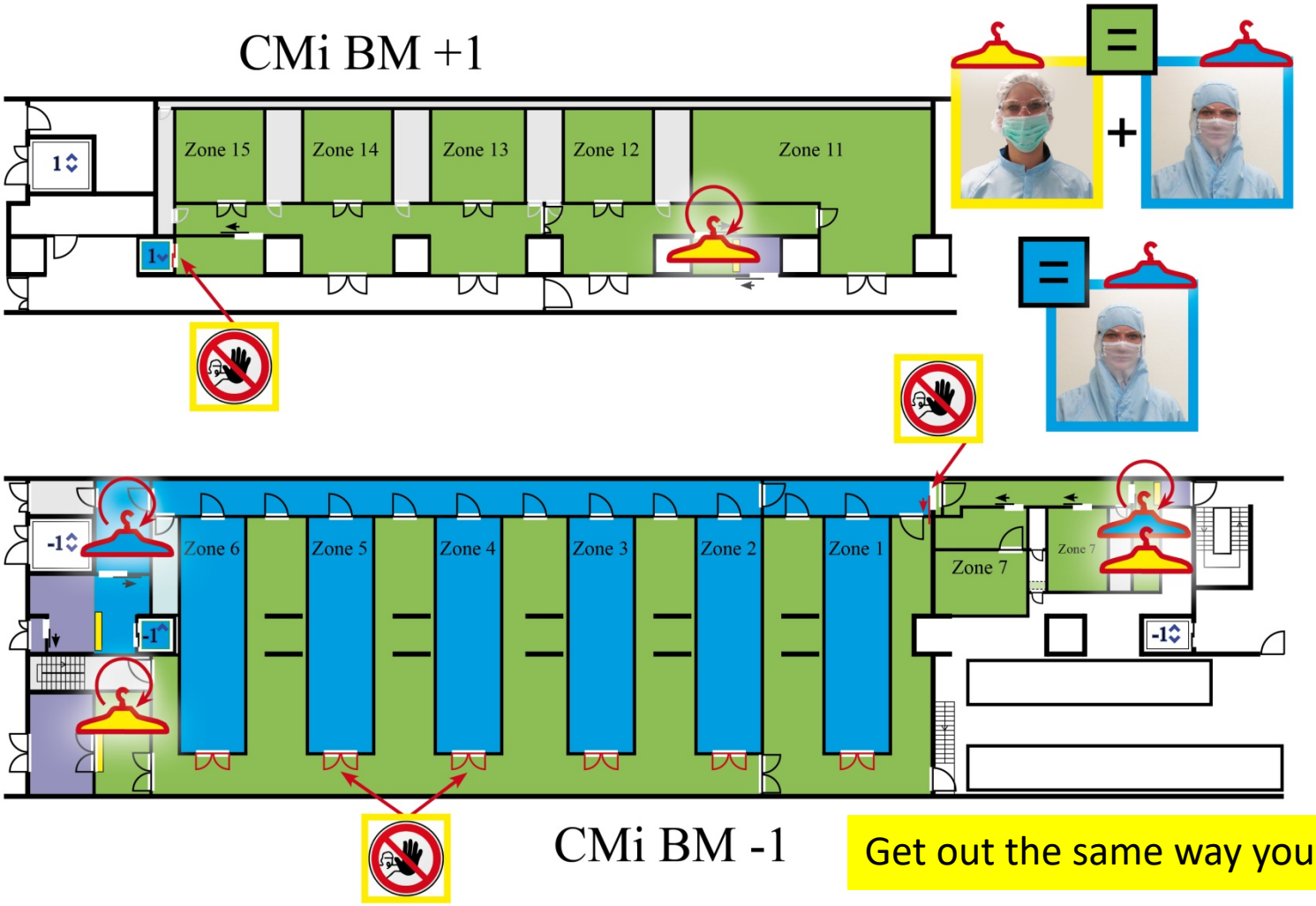
Dressing CMI BM-1 and BM+1



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

prohibited:

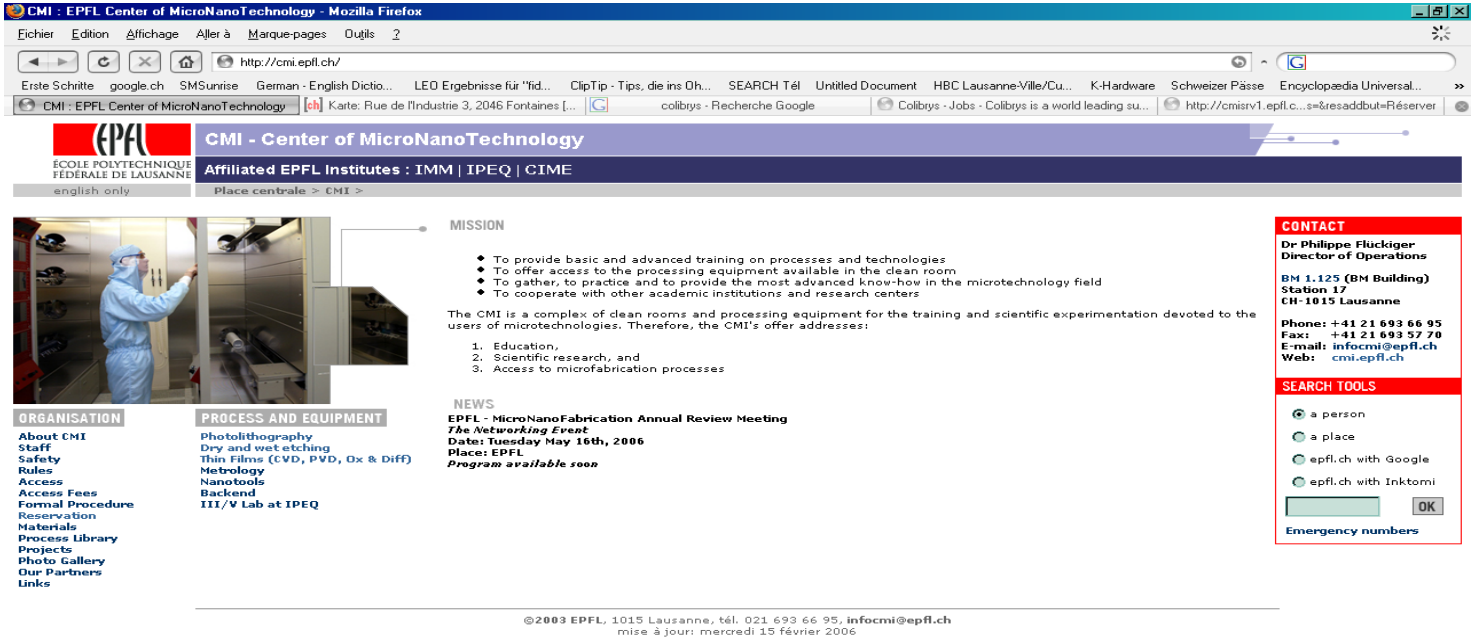
- normal paper
- pencils & normal pens

- 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

CMI Website

http://**cmi.epfl.ch/**

- Reservations
- Orders
- Safety and information

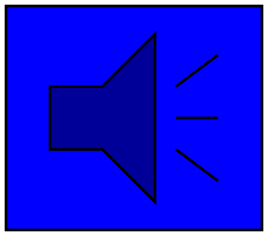


Safety Rules

- Never Work Alone
- Only One Emergency N° :

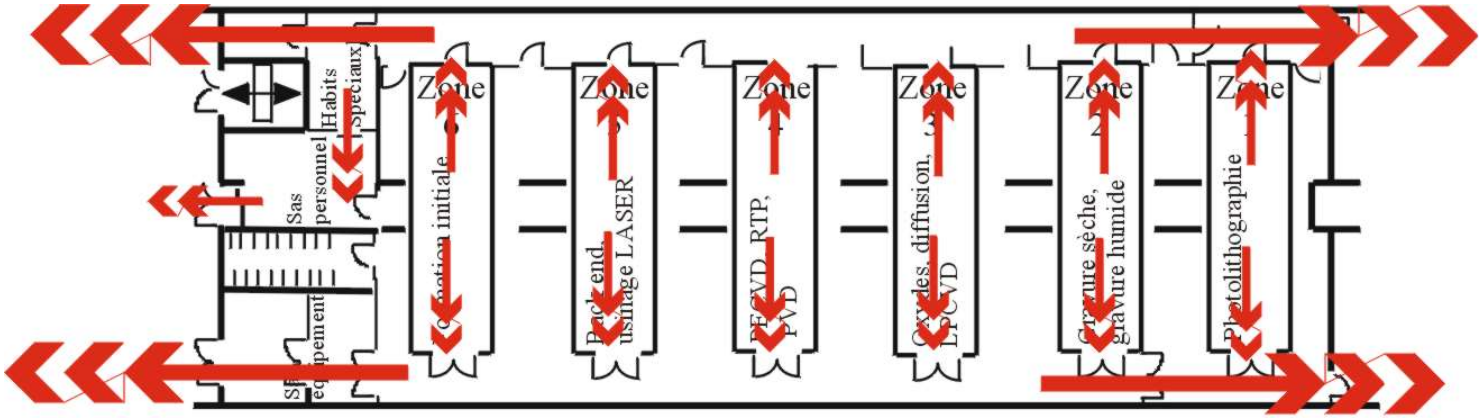
- Report any safety problems you encounter
- Wear protective glasses or Medical glasses all the time

Alarms & evacuation



- Double Tone Horn
- Flashing **Red** Light

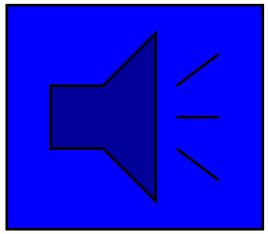
⇒ **Evacuate immediately with cleanroom dressing**



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

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

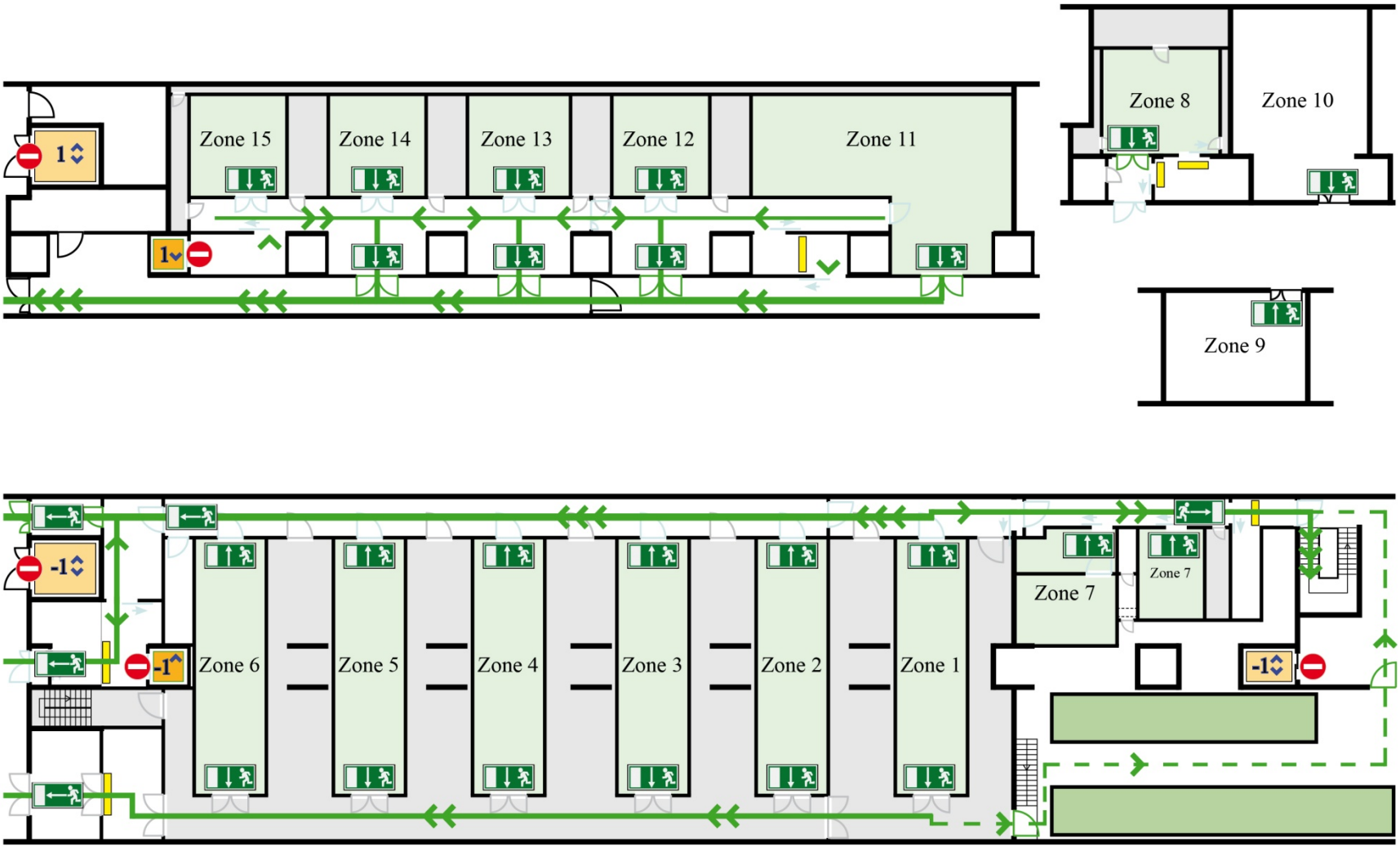
Alarms & evacuation



Single Tone Horn
Flashing Yellow light

- 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 λ=975nm (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