SECTION 1: Principles of biochemistry, cell biology, physics and microelectronics

Lecture 1.1: Introduction to nanotechnology and nanobiotechnology
(Instructor: **Dr Evangelos Gogolides**)

- Historical introduction to the science of miniaturization. From electron to fluid flow.
- Going up and down the nanoscale.
- Definition of nanotechnology and nanobiotechnologies.
- An analysis of what nanotechnology is and what one expects from it.

Lecture 1.2: Cell biology principles (Instructor: **Dr Dimitris Mastellos**)

- Introduction to the cell and its organization (from prokaryotes to eukaryotic cells, distinct cellular compartments, main organelles and related functions)
- Cell membranes - structure and topography
- Protein biosynthesis and turnover-regulation and degradation
- Cell signaling: relaying the signal from the cell membrane to the nucleus
- The cell cycle - deregulated cell division and cancer
- The immune system-General overview :
  - Innate and acquired immunity, target cells and main immune mediators
  - Antibody structure and function - B and T- cell receptors
  - Monoclonal antibodies - applications
- Towards model biological systems-Divergent signal integration within the cell

Lecture 1.3: Structure of biological macromolecules (Instructor: **Prof. Elias Eliopoulos**)

- Introduction to building blocks of biological macromolecules
- Principles of protein structure
- Principles of DNA structure
- Sequence / Structure relationships. Structural motifs. Examples
- Introduction to in silico prediction of 3D-structure
- Structure / function relationships. Examples

Lecture 1.4: Microelectronic Materials and Device Technology (Instructor: **Dr Spyros Gardelis**)

- Semiconductors (What is a semiconductor? energy gap, conduction and valence band, electrons and holes, p-type, n-type)
- Basic Devices (p-n junction, bipolar transistor, MOS transistor, MOSFET, CMOS technology)
- Optical properties of semiconductors (direct and indirect band gap semiconductors, laser, photodetector)
- Applications of microelectronics in every day life
- Integration - miniaturization revolutionize the way we live (Moore's law)
- Examples of devices used in every day life (logic devices, memories, lasers, displays, detectors, sensors (chemical, gas, bio-sensors)

SECTION 2: Core Nanobiotechnology methods and practices

UNIT 2.1: Micro and Nano-fabrication science and technology

Lecture 2.1.1: Conventional patterning schemes for hard substrates for bioanalytic microdevices (Instructor: **Dr Evangelos Gogolides**)

- The top-down approach in nano fabrication. History of microfluidics, and motivation for going micro and nano for life sciences and chemistry
- Lithography fundamentals: Light Sources, Lithography Systems, Photoresists, Lithographic Processes
- Wet etching and Silicon micromachining
- Plasma etching and its mechanisms. Isotropic and Anisotropic etching
- Deep etching processes for Si bioanalytic microdevices
- Glass micromachining
- Novel Deep etching processes for polymer bioanalytic microdevices

Lecture 2.1.2: Microfabrication technologies for plastic analytical microfluidics (Instructor: **Dr Angeliki Tserepi**)

- Why use plastic substrates?
- Master Fabrication for plastic patterning
- Injection molding
- Hot embossing
- Soft lithography and variations thereof
- Other/ emerging methods
- Surface modification and sealing
- Examples of analytical microfluidic devices / systems

Lecture 2.1.3: Patterning of biomolecules and other biological substances (Instructor: **Dr Panagiotis Argitis**)

- The necessity for the patterning of biomolecules and other biological substances on solid surfaces
- Chemical /physical binding of biomolecules on surfaces
- Patterning methods :
  - microspotting, mechanical methods for delivery and synthesis, dip-pen lithography
  - microcontact printing methods (soft lithography related methods)
  - photochemical modification of SAMs, light guided DNA and protein synthesis
  - photochemical modification of polymeric films, photoresist-based methods
  - other / emerging methodologies
- Potential applications and comparison

UNIT 2.2 : Nanomaterials for bio-applications, Characterization, Imaging

A. DRUG DELIVERY METHODS/ MATERIALS

Lecture 2.2.1: Drug Delivery and Targeting Systems - Focus on Liposomes
(Instructor: **Prof. Sophia G. Antimisiaris**)

Why controlled release? Therapeutic Background and Rationale for Time control and Place control requirements. Liposome Technology. Applications in Therapeutics.

- Pharmacokinetic / Therapeutic Based considerations for type of control required
- General Methods to control Drug Release Kinetics (time) from Nanosized DDS (Liposomes or PN). Kinetics of drug release. Membrane - Matrix - Swelling controlled systems -Environmentally sensitive hydrogels, etc.
- Liposomes (Technology, Drug Release mechanisms, In vivo fate - Routes of Administration, Targeting)
- Comparison of Lipid-based and Polymer based systems; Special constructions
- Examples of Therapeutic applications (Targeting cancer, Brain targeting possibilities, Development of Drug-eluting Biomaterials)

Lecture 2.2.2: Drug Delivery and Targeting Systems –
Focus on cyclodextrin delivery, studied by NMR and XRD
(Instructors: **Dr Konstantina Yannakopoulou, Dr Irene Mavridis**)

- The basis of cyclodextrins as pharmaceutical excipients
- Inclusion complexes of cyclodextrins with drugs - Pharmaceutical applications
- Basics in NMR spectroscopy and X-ray crystallography as methods to study
- Cyclodextrin inclusion complexes

B. NANOSTRUCTURED MATERIALS FOR BIOAPPLICATIONS

Lecture 2.2.3: Magnetic Nanoparticles for Bioapplications (Instructor: **Dr Ioannis Rabias**)

Experimental techniques for magnetic characterization of ferrofluids and Applications of ferrofluids in medicine

- A study in ferrofluids and their uses in nanotechnology
  - A review on what is a ferrofluid?
  - The origin of their magnetism, their stability as magnetic colloids
  - Synthetic methods and applications
  - Nature, properties and their high technological value
- Synthetic Routes for Hydrosol and Organosol colloidal fluids
  - Differences and similarities in these two approaches
- Applications of ferrofluids in medicine. Two case studies:
  1) Hyperthermia and 2) Contrast Agents for Magnetic Resonance Imaging (MRI)

Two applications of ferrofluids in medicine will be discussed:

- Hyperthermia covers a wide variety of techniques in which elevation of temperature in ferrofluids is achieved using low-frequency electromagnetic radiation. In this way, hyperthermia is a promising approach for cancer therapy, by locally heating a tumor without damaging the healthy tissues in the tumor surrounding
- Magnetic Resonance Imaging (MRI) is one of the most powerful diagnostic techniques in medicine. The use of superparamagnetic contrast agents based on ferrofluids, which enhance image contrast, will be discussed

C. IMAGING OF BIOMATERIALS

Lecture 2.2.4: Scanning Probe Microscopy in Nanobiotechnology (Instructor: **Dr Eleni Makarona**)

- Scanning probe techniques
- Scanning tunneling microscopy
- Atomic force microscopy
- Near-field optical microscopy
- Optical tweezers force spectroscopy

UNIT 2.3 : Molecular and Cellular biology and Applications

A. PROTEOMICS AND ANALYSIS

Lecture 2.3.1: Gel-based protein analysis methods (Instructor: **Dr Antonia Vlahou**)

- Definition and significance of proteomics research
- Introduction to protein separation methodologies: Principle of two-dimensional electrophoresis (2DE) and liquid chromatography
- Steps of analysis by 2DE : isoelectric focusing , vertical polyacrylamide gel electrophoresis, protein spot detection and quantification
- Principles of matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS)
- Identification by peptide mass fingerprinting
- Coupling of 2DE with MALDI
- Protein profiling with MALDI MS analysis
- Applications of 2DE-MALDI MS in biomedical research
- Limitations-Avenues for improvement

Lecture 2.3.2: Non-gel based protein analysis methods (Instructor: **Dr Spiros D. Garbis**)

- Separation of proteins and peptides with high performance liquid chromatography (HPLC)
- Combining two or more chromatographic chemistries for peptide/protein separation (multi dimensional HPLC approaches)
- Introduction of principles of electrospray ionization (ESI)
- Coupling micro- and nano-bore LC with mass spectrometry ionization sources (MALDI; ESI; nano-ESI)
- Introduction to the principles of tandem mass spectrometry using ion-trap, time-of-flight, quadrupolar mass analyzer designs and their variants
- Protein identification by tandem mass spectrometry of their peptide products
- Protein quantification by LC-MS analysis of isotope-labeled peptide products
- Applications of LC-MS to biomedical research
- Limitations-Avenues for improvement

B. ASSAYS AND ARRAYS

Lecture 2.3.3: Binding Assays and Immunosensors (Instructor: **Dr Sotirios Kakabakos**)

- Binding assay principles
- Binder molecules
- Antibodies: structure and production
- Labels and labelling procedures
- Standard solutions for quantities determinations
- Surface immobilization of biomolecules
- Configurations of binding assays
- Signal amplification systems
- What is a (immuno)sensor?
- Main immunosensor configurations
- Principles of optical immunosensors
- Examples of optical immunosensors developed at NCSR “Demokritos”

Lecture 2.3.4: DNA and Protein arrays: fabrication, detection and applications
(Instructor: **Dr Panagiota Petrou**)

- What is a DNA array?
- The DNA molecule: structure and biological significance
- DNA analysis by conventional methods (Southern Blotting)
- DNA arrays versus DNA chip
- Fabrication of DNA arrays and chips

- Target DNA amplification and labelling by PCR
- Hybridisation and detection formats
- Main applications of DNA arrays
- Limitations of DNA arrays
- Perspectives
- What is a protein array?
- Protein structure & sources
- Formats and surfaces for protein arraysImmobilization of proteins onto solid surfaces
- Fabrication of protein arrays. Detection schemes for protein chips. Antibody arrays: specificity and cross-reactivity Areas of application
- Challenges and bottlenecks
- Perspectives

SECTION 3: Towards Integrated Nanobiotechnology systems

Lecture 3.1: **Principles of Integrated Biosensing Devices**
(Instructor: **Dr Konstantinos Misiakos**)

- ISFETs
- Impedance Spectroscopy Devices
- SAW Devices
- Enzymatic Detection
- SPR Resonance
- Interferometric Devices

HANDS ON EXPERIENCE - LABORATORIES

SESSION 1
Choose 3 out of 4 laboratories

Laboratory 2.1.1 +2.1.2: Fabrication of microfluidic devices on plastic substrates by soft lithography and deep polymer plasma etching
(Instructors: **Dr Angeliki Tserepi**, **Dr Evangelos Gogolides**, IMEL)

Aim of the lab:

- Familiarize attendants with conventional photolithography
- Familiarize attendees with replica molding / soft lithography
- Demonstrate the fabrication and sealing of a PDMS microfluidic device
- Familiarize attendees with the ICP plasma reactor, its operation and diagnostics, as a tool for microfluidics and surface modification
- Perform plasma etching of PMMA microchannels and observe results

Content:

- Fabricate mold on SU8
- Fabricate microfluidic channels by PDMS replica molding on SU8 mold
- Device Sealing
- Demonstration of the plasma reactor (ICP) and diagnostics
- Patterning of microchannels on PMMA using the ICP
- Sealing of microfluidic channels and measurement of flow properties of the microfluidic

Laboratory 2.1.3: SPM Techniques for molecular devices
(Instructors: **Dr Eleni Makarona**, **Dr Dimitrios Velessiotis**, IMEL)

Aim of the lab:

- Familiarize attendants with SPM Techniques (AFM, STM) for molecular systems characterization
- Discuss how the molecular properties can be connected to the device behavior and design

Content:

- Demonstration of AFM, STM measurements
- Analysis of experimental data and extraction of information for the demonstrated systems

Laboratory 2.3.4: Fabrication of protein microarrays using lithography
(Instructor: **Dr Antonis Douvas**, IMEL)

- Photoresist processing fundamentals : processing of chemically amplified photoresists
- Lift-off process : use of lift off process for the patterning of SAMs or proteins, and (optional)
- Patterning of different types of proteins on the same substrate by successive lithographic steps under biocompatible conditions

Laboratory 2.3.5: Fluorescence detection of protein arrays
(Instructor: **Dr Panagiota Petrou**, IRRP)

Aim of the lab:

- Familiarize the attendees with optical detection of protein arrays
- Demonstration of the epifluorescence microscope
- Image processing for quantitative fluorescence measurements

Content:

- Immunoreaction of protein in spots created by photolithography with fluorescently labeled molecules
- Observation of protein spots with the epifluorescence microscope
- Capture of images of the arrays
- Image processing to receive quantitative results

Laboratory 3.1: Operation of a lab-on-a-chip optical device using model assays and real time measurements (Instructor: **Dr Konstantinos. Misiakos**, IMEL)

- Integrated biochip wafer alignment on wafer prober
- Protein/DNA spotting on integrated waveguides
- Washing and microfluidic device application on biochip
- Protein/DNA real time assays through photocurrent monitoring

SESSION 2
Choose 3 out of 6 laboratories

Laboratory 2.3.1: Protein separation by two-dimensional electrophoresis
(Instructor: **Dr Antonia Vlahou**, Bioacademy)

Aim of the lab:

- Familiarize attendees with the isoelectric focusing technology and vertical polyacrylamide gel electrophoretic systems
- Familiarize attendees with automated technologies for spot picking and processing for peptide mass fingerpriniting
- Demonstrate the processing of a cell extract by the 2DE methodology

Content:

- Processing of cell extract on IPG strips for first dimensional separation
- Application of IPG strips on PAGE gels
- Gel staining, scanning and processing for spot excision
- Tryptic digestion of excised spots

Laboratory 2.3.2: Mass spectrometry
(Instructor: **Dr Spiros D. Garbis**, Bioacademy)

Aim of the lab:

- Familiarize attendees with mass spectrometry technologies (MALDI; ESI QqTOF; ESI QIT)
- Demonstrate tryptic peptide analysis by MALDI-MS; LC-ESI-QqTOF MS; LC-ESI-QIT)
- Familiarize attendees with database searches for protein identification
- Familiarize attendees with the custom preparation of capillary LC columns to be used for the LC-ESI-MS based methods

Content:

- Tuning and calibration of mass spectrometers (MALDI; ESI QqTOF; ESI QIT)
- Application of tryptic peptide extracts and proteins and matrix on MALDI targets
- MALDI MS analysis of peptide and protein samples
- Sample preparation for LC–ESI-MS
- LC-ESI-MS-MS analysis of peptide extracts
- Database search methods

Laboratory 2.3.3: Fabrication of protein microarrays using nanoplotter
(Instructor: **Dr George Tsangaris**, Bioacademy)

- Microspoting methods for the fabrication of microarrays : presentation of nanoplotter and familiarization with its use

Laboratory 2.3.6: Bioinformatics basic theory & laboratory
(Instructor: **Dr Sophia Kossida**, Bioacademy)

Aim of the lab:

- Familiarize attendants with biological databases
- Familiarize attendees with software for genome analysis
- Familiarize attendees with software for proteome analysis

Content:

- Introduction into Bioinformatics
- Query and retrieve info from biological databases
- Analyze genes/ genomes with software packages
- Analyze peptides/ proteins with software packages

Lecture 2.3.7: Structural Bioinformatics: Molecular Simulations and Visualization
(Instructor: **Dr Georgios Spyrou**, Bioacademy)

- Applied Bioinformatics in BioNanoTechnology
- Bioinformatics tools for Molecular Simulation and Visualization

Laboratory 2.3.8: State of the art fluoresence imaging & confocal microscopy of biological samples (Instructor: **Dr Stamatis Pagakis**, Bioacademy)

Aim of the Lab:

- Familiarize students with a fluorescence microscope
- Demonstrate the design differences which characterize a confocal microscope
- Demonstrate Image processing and 3D visualisation software

Content:

- Introduction to Fluoresence imaging and 3D image visualization using confocal microscopy
- Two microscope workstations (one confocal) will be used
- Observation of fluorescent proteins (e.g YFP, GFP) and other fluorophores common in fluorescence microscopy
- Hands-on experience and data acquisition on a digital camera fluorescence microscope
- Demonstration of 3D data acquisition using a confocal microscope
- Imaging methods for image deconvonlution, 3D reconstruction and data analysis will be demonstrated on computer workstations with specialised software packages

SESSION 3

Choose 2 out of 3 laboratories

Laboratory 2.2.1: Drug inclusion in cyclodextrins: monitoring in situ by NMR spectroscopy X-ray diffraction characterisation of drug inclusion and 3-D visualisation (Instructors: **Dr Konstantina Yannakopoulou**, **Dr Irene M. Mavridis**, IPC)

- Determination of the formation of an inclusion complex of β-cyclodextrin and the drug piroxicam in situ by NMR spectroscopy
- Determination of the formation of the same inclusion complex (β-CD/piroxicam) in the crystalline state by powder X-ray diffraction
- Molecular docking of the drug inside the cyclodextrin cavity

Laboratory 2.2.2: Intracellular visualisation of Porphyrin-Cyclodextrin conjugates as PDT agents/chemotherapeutic drug carriers by confocal microscopy (Instructor: **Dr Th. Theodosiou**, IPC)

- Cell loading with Porphyrin-cyclodextrin conjugates, with/without a FITC-labelled bioactive compound nested in the cyclodextrin cavity.
- Why conjugate cyclodextrins with porphyrins? A combined photodynamic therapy – chemotherapy paradigm.
- Confocal microscopy on cells incubated with porphyrin-cyclodextrin conjugates:
  - Empty-pocket cyclodextrins and cellular organelle probes to visualise subcellular localisation of the conjugate (Red fluorescence = porphyrin, green fluorescence = probe)
  - Cyclodextrins loaded with a FITC-labelled bioactive molecule (red fluorescence = porphyrin, green fluorescence = FITC)

Laboratory 3.2: Demonstration of a capillary fluoroimmunosensor (Instructor: **Dr Sotirios Kakabakos**, IRRP)

Aim of the lab:

- Familiarize attendees with optical immunosensors
- Demonstration of multi-band capillary fluoroimmunosensor
- Real-time monitoring of the immunoreaction

Content:

- Creation of distinct reaction bands in a single capillary
- Performance of immunoreaction in the capillary
- Demonstration of fluorescent bands detection using the prototype optoelectronic set-up in real time or after assay completion
- Data processing and interpretation of the results