CORE LECTURES

Topical Field	Core lectures	Lecturer
MOLECULAR & CELL BIOLOGY	**Molecular biology of the cell I, II**	Gruber, Schütz
BIOPHYSICS (IMAGING & SPECTROSCOPY)	**Biophysics**	Hinterdorfer, Schütz
STRUCTURAL & FUNCIONAL ANALYSIS AND MODELING	**Biological interaction analysis (BIA)**	Gruber, Hinterdorfer, Klar, Müller, Pohl, Romanin, Schütz, Sixt, Heisenberg,

Two core lectures were derived from the standard curriculum of the participating institutions. **Biophysics** was a mandatory core lecture for students with a major in biology, whereas **Molecular biology of the cell** was mandatory for students who majored in physics and chemistry to provide them with the the molecular basis of important cellular functions. This first lecture was mainly taken in the first semester of the student's thesis. The two lectures (30 semester hours, 3 credit points) were held on a weekly basis (2 hours) at JKU, TUW, and IST.

The core lecture **Biological Interaction Analysis (BIA)** (30 semester hours, 3 credit points) was a mandatory course for all students of NanoCell. It was held as one-week block (3 morning lecture hours and 3 afternoon lecure hours per day) in Linz, the first course in October 2014, when most of the students started, and the second course two years later. This lecture course was newly established by several PIs of the consortium. It lead the students to the state-of-the-art on *in vivo* and *in vitro* ensemble and single molecule methodologies specific for detection and characterization of molecular interactions. Furthermore, a deep coverage of high resolution structural methods such as X-ray crystallography, NMR, and cryo-electron microscopy was provided.

LECTURE COURSES

A variety of specialized and mostly newly developed lecture courses were offered within the three topical fields by the PIs dedicated to their particular field of expertise. The students had to collect a minimum total of 3 credit points from these lecture courses that should be tightly connected to the topics of their thesis. The following table surveys the lecture courses that were specially organized within the DK.

Topical Field	Lecture courses	Lecturer
MOLECULAR & CELL BIOLOGY	**Quantitative biology**	Heisenberg, Sixt
	Membrane channels and transporters	Pohl
	Principles in cell and developmental biology	Heisenberg, Sixt
IMAGING & SPECTROSCOPY	**Characterization of bio-nano-structures**	Hinterdorfer
	Optical microscopy on biomolecules	Schütz
	Nano-optics	Klar

	Ultrahigh resolution in optical microscopy	Klar
	Dynamic imaging of cells and tissues	Sixt
	NMR for biomolecular studies	Müller
STRUCTURAL & FUNCTIONAL ANALYSIS AND MODELING	**Theoretical biophysics I, II**	Renger

MOLECULAR & CELL BIOLOGY

Quantitative Biology - Heisenberg, Sixt

This course discusses classical publications on quantitative topics in biology and compares this work with recent developments. The aim of the course is to provide an interdisciplinary analysis of biological questions and discuss the work from a theoretical as well as an experimental angle.

Membrane Channels and Transporters - Pohl

We illuminate protein insertion into the membrane by the translocon, starting with the principles of membrane protein assembly. We subsequently discuss structure function relationships of the protein translocation channel. Taking the bacterial potassium channel KcsA as an example, we unravel the origin of ion channel selectivity. Next, the structural features underlying (i) ion channel gating and (ii) receptor functioning (acetylcholine receptor) are examined. Illuminating the function of water and protein channels completes the picture of physiologically important channels. We then use the chloride proton exchange transporter and the sodium glucose cotransporter to introduce the principles of secondary active transport. The last chapter details the function of pumps (Ca-ATPase; Na/K-ATPase).

Principles in Cell and Developmental Biology - Heisenberg, Sixt

We provide an overview of key principles in cell and developmental biology. This include background on key approaches in these disciplines focusing on biochemical, genetic and embryological methods used to uncover basic processes controlling the function of cells, tissue and developing multicellular organisms. We also provide insight into merging extension of these fields in systems biology and synthetic biology. The general aim of this lecture series is to provide a general overview about the background and recent advances in cell and developmental biology.

IMAGING & SPECTROSCOPY

Characterization of Bio-nano-structures - Hinterdorfer

This course covers the introduction of a broad range of nano-technologies apt for resolving structure and function of bio-molecules. Techniques include atomic force microscopy, fluorescence microscopy,

optical tweezers, force spectroscopy. Applications to proteins, lipids, nucleotides, membranes, and cells are discussed.

Optical Microscopy on Biomolecules - Schütz

The lecture introduces students to fundamentally new microscopy concepts that are currently revolutionizing the life sciences. The course first works out the basics on optical microscopy, including detailed treatments of resolution, image formation, and contrast enhancement. On this basis, newer bioimaging modalities including confocal microscopy, near-field microscopy, total internal reflection microscopy, single molecule microscopy, and the various superresolution microscopy concepts are discussed via their application to biological questions.

Nano-optics - Klar

This lecture introduces a variety of nanoscopic light sources (dye molecules, plasmonic scatteres, quantum dots) and their interactions (Förster resonant energy transfer, surface enhanced Raman, surface enhanced IR spectroscopy etc.). Fields of applications are in bioimaging, biosensing and in optoelectronics.

Ultrahigh resolution in optical microscopy - Klar

This lecture covers modern techniques in superresolving optical microscopy. It starts with an introduction into imaging theory (including vectorial Debeye Theory), near field optical microscopy, optical sectioning using confocal and two photon microscopy, structured illumination microscopy including I5M and 4Pi, local switching techniques such as STED and RESOLFT microscopy, temporal stochastic switching techniques such as PALM and STORM and finally a glimpse into superresolution in optical lithography. Ultrahigh resolution in optical microscopy.

Dynamic Imaging of Cells and Tissues - Sixt

A block seminar introducing the currently available technology to dynamically image at the cell and tissue level. This will be an overview of the available technology and its suitability for specific biological questions rather than an in depth discussion of microscopic techniques. The focus will not be on biophysical methods that are discussed in other courses, but mainly on confocal techniques, multiphoton microscopy, total internal reflection microscopy etc. and their applications in cell and tissue biology.

NMR for Biomolecular Studies - Müller

This course covers the strategies and main experimental techniques for assigning biomolecular NMR spectra and for deriving three-dimensional structures of proteins from multi-dimensional NMR data by computational approaches. The course also highlights aspects of molecular dynamics analysis by NMR and their physicochemical backgrounds. Hands-on demonstrations and home assigments of assignment and structure elucidation software will be included.

STRUCTURAL & FUNCTIONAL ANALYSIS AND MODELING

Theoretical biophysics I+II - Renger

This course covers the introduction of theoretical tools for the modeling of biological macromolecules and their photophysical and chemical reactions. In the first part of the course the physical foundations is laid for the description of the structure, dynamics and optical properties of biological macromolecules in general and proteins in particular Different hierarchies of the modeling and their connections are discussed, ranging from electronic structure calculations via molecular mechanics force field up to continuum electrostatics calculations and non-equilibrium density matrix theory. The main focus of the second part of the course is on particular numerical methods as, e.g., the solution of the Poisson Boltzmann equation in combination with Monte- Carlo approaches for the calculation of protonation probabilities of titratable residues of a protein as a function of ionic strength and pH of the solvent, molecular dynamics and Monte-Carlo methods for the sampling of conformational substates of proteins and quantum chemical methods for the parametrization of the Hamiltonian of biomolecules. In parallel to these lectures, a computer lab course “Modeling of biological Macromolecules” is offered, where the students can apply and deepen their knowledge.

The PhD students also took other specialized courses within their own or an external institution:

Topical Field	Lecture courses	Institution
MOLECULAR & CELL BIOLOGY	**Immunology**	Med.Uni Vienna
	Biomembranes	TUW
	Molecular cell biology II - Advanced	IST
	Biological physics	IST
	Shapes and patterns	IST
	Choosen chapter in biophysics: Mechanisms of membrane transport	JKU
IMAGING & SPECTROSCOPY	**Ultrasensitive techniques of genetics**	JKU
	Ultra-high resolution microscopy	JKU
	Dye chemistry and functional dyes	JKU
	Photochemistry	JKU
	Bio-nano sensor technology	JKU
	Basic microscopy	IST
STRUCTURAL & FUNCIONAL ANALYSIS AND MODELING	**Automating NMR workflows: introduction to python programming**	Uni Gothenburg, Sweden
	Biomolecular NMR	Uni Gothenburg, Sweden
	Excursion polymer chemistry	JKU
	Bioanalytics I, II	JKU

LABORATORY COURSES

In addition, students took practical laboratory courses (3 credit points each; limited to 4 students per course) at the PIs institutions as blocks. The students had to attend two of these practical courses (minimum total of 6 credit points), one in a complementary field to the thesis. One substitution with a practical course that was taken during the visit at the foreign collaborator was permitted. The following table surveyes the practical courses taken be the students.

Topical Field	Laboratory courses	Lecturer
MOLECULAR & CELL BIOLOGY	**Molecular biology I+II**	Romanin
	Cell culture	Romanin
	Overexpression, purification and functional testing of reconstituted membrane channels / Protein-expression and finction	Pohl
IMAGING & SPECTROSCOPY	**Atomic force microscopy methods**	Hinterdorfer
	Superresolution microscopy with single molecules	Schütz
	TIRF & FRAP microscopy	Sixt
	STED microscopy	Klar
	FRET microscopy	Romanin
	Microscopy on biomolecules	Pohl
	LRET microscopy	Pohl
	Fluorescence correlation spectroscopy	Pohl
STRUCTURAL & FUNCIONAL ANALYSIS AND MODELING	**Biological interaction analysis (BIA)**	Gruber et al.
	Modeling of biological macromolecules I+II	Kraus, Renger
	Single channel recording	Pohl
	Patch-Clamp	Romanin
	Practical NMR	Müller
	Micropatterning of proteins in the live cell plasma membrane	Schütz
	Introduction to Matlab	Bollenbach (ext.)
	Introduction to programming with Python	Bollenbach (ext.)

MOLECULAR & CELL BIOLOGY

Molecular biology I and II – Romanin

In these courses, the graduate students get familiar with basic protocols in Molecular Biology ranging from plasmid preparation to amplification and purification. At an advanced level, mutations are introduced into constructs covering single point, deletion, insertion mutations.

Cell culture – Romanin

Here the students get acquainted with all the basic principles for handling cell cultures in the lab together with various techniques for cell transfection.

Overexpression, purification & functional testing of reconstituted membrane channels / protein-expression and function - Pohl

This laboratory course introduces the students to the protocol of functional reconstitution of membrane proteins. It includes all of the steps that are required to obtain lipid bilayers with reconstituted protein water channels (aquaporins). The students start with the transfection of E.coli cells (or yeast) and finish by measuring the shrinkage of proteoliposomes with stopped flow.

IMAGING & SPECTROSCOPY

Atomic force microscopy methods - Hinterdorfer

This lab course introduces a whole spectrum of atomic force microscopy (AFM) methodologies applied to biological samples, starting from the most common contact topography imaging mode, to intermittent contact mode, single molecule force spectroscopy, recognition imaging, Kelvin probe force microscopy, scanning microwave microscopy, high speed bio-AFM, and combined AFM/fluorescence microscopy Imaging.

Superresolution microscopy with single molecules - Schütz

In the last years, microscopy concepts have been developed which allow for optical resolution below the classical diffraction limit. This practical course introduces one of these methods, single molecule switching microscopy (acronyms are PALM, STORM, dSTORM, fPALM): single molecules are stochastically switched on and off, and the positions of all detected molecules are determined to an accuracy of ~20nm by mathematical fitting. If requested students can bring and characterize their own samples; alternatively, fluorescently labeled cells are used as examples.

TIRF & FRAP microscopy - Sixt

This practical course on total internal reflection microscopy and its applications in visualizing molecular processes close to the plasma membrane of cells. The focus will be on cytoskeletal dynamics. The program will include an introduction into the theory and the application of TIRF. The rest of the tutorial will be spent on sample preparation and actual demonstration of an experiment with possibility for hands on time at the microscope.

STED microscopy - Klar

In the lab course, students are specifically trained to be aware of the shape of the point spread function (PDF) of a light microscope and several methods to reshape the PSF, such as stimulated emission depletion or reversible switching of fluorophores. The benefit from this awareness lies not only in understanding super-resolution but also confocal and two photon microscopic and fluorescence correlation techniques.

FRET microscopy - Romanin

Measurements of protein-protein interactions in living cells is conducted by confocal FRET microscopy. Besides protocols for cell transfection with fluorescent-labeled proteins, theoretical background is provided in the context with analysis of FRET images.

Microscopy on biomolecules - Pohl

After a short introduction into the principles of fluorescence microsopy. They subsequently carry outsimple measurements on proteins and lipids.

LRET microscopy - Pohl

After a short introduction into the principles of lifetime measurements, the students monitor the lifetime of terbium luminescence using a small lanthanide binding peptide in solution. They subsequently carry out distance measurements between the lanthanide binding tag and a fluorescent label which are both introduced into the water soluble motor protein SecA.

Fluorescence correlation spectroscopy (FCS) - Pohl

This practical course starts with measurements of dye diffusion in solution, i.e. with the recordings of fluorescence fluctuations which lead to simple autocorrelation functions. To introduce the students to experiments with cross correlation, we will use lipid vesicles. Dissolving the vesicles leads to a loss of cross correlation between the water soluble dye in the vesicle interior and the lipid anchored dye. The last part of the course is devoted to the diffusion of genetically labeled membrane proteins in live cells.

STRUCTURAL & FUNCTIONAL ANALYSIS AND MODELING

Biological interaction analysis (BIA) – Gruber *et al.*

This laboratory course is newly established by several PIs of the consortium. It leads the students to the state-of-the-art on *in vivo* and *in vitro* ensemble and single molecule methodologies specific for detection and characterization of molecular interactions and high resolution structural methods.

Modeling of biological macromolecules I+II - Kraus, Renger

This computer lab course is planned in parallel to the lecture course “Theoretical Biophysics I, II” (Renger). The students get the chance to practice their new knowledge by solving problems of macromolecular modeling with the aid of computer simulations. In part I, on the one hand they learn to use commercial modeling tools like CHARMM (Chemistry at HARvard Macromolecular Mechanics) in order to build a macromolecule and to perform normal mode analyses and molecular dynamics simulations and APBS (Adaptive Poisson Boltzmann Solver) for all-atom electrostatic calculations. On the other hand the students write their own programs to analyze the output of these calculations to obtain quantities like FRET (Förster resonance energy transfer) efficiencies that can be compared with experiments. In part II of this lab course, the students learn how to implement efficient

Poisson solvers using multigrid techniques. At the end, a small computer program is developed for a coupled molecular dynamics simulation using efficient numerical methods for the solution of ordinary differential equations.

Single channel recording - Pohl

This laboratory course introduces the students to the protocol off setting up single channel recording experiments. It includes all of the steps that are required to obtain such data and provides the students with the theoretical and practical framework for data analysis.

Patch-Clamp - Romanin

The intention of this course is to get familiar with the various configurations of the patch-clamp technique covering both single channel and whole cell recordings from both native as well as transfected cells. Theoretical background is further conveyed to allow for analysis of electrophysiological recordings.

Micropatterning of proteins in the live cell plasma membrane - Schütz

The Schütz group has recently established protein micropatterning in the live cell plasma membrane as a tool to study interactions of membrane proteins (see also thesis project 2 of Gerhard Schütz). In this practical course, the technique is introduced to the students. In brief, soft lithography is used to generate antibody patterns on glass surfaces, onto which cells are grown. The target proteins are reassembled along the micropatterns. Fluorescence microscopy is used as readout. If requested, students can characterize their own samples; alternatively, standardized samples will be available.

LABORATORY ROTATIONS

Students spent at least 2 weeks each in 2 other laboratories within the DK consortium (2 credit points per stay, minimum total of 4 credit points) to learn the techniques and the scientific cultures of these laboratories. Particular attention was given to hands-on experience for their own rearch topics in collaboration with the hosting lab.

Besides frequent stays of the students in other labs of the graduate scholls, the following official lab rotation courses were organized and integrated into the curriculum:

- “LAPAP development methods”, **Sixt**
- “Receptor recycling and cell sorting”, **Sixt, Heisenberg**
- “Stim/Orai coupling and interaction”, **Gruber, Romanin**
- “FRET and LRET modelling and computation”, **Renger, Kraus**
- “Mechanism and forces in protein translocation”, **Pohl, Hinterdorfer**
- “Photochemistry and NMR”, **Knör, Müller**
- “Optical nanoscopy and nanostructural characterisation”, **Schütz, Klar**

SOFT SKILLS

The following soft skill courses were offered within the DK and attended by the students:

- Scientific presentation and conduct
- Workshop on communication skills for scientists
- Workshop on visual communication of science
- Patent law
- Voice and Presence
- Stage coaching
- Scientific writing in technical and natural sciences
- Gender studies and diversity
- German as foreign language
- Presentation skills