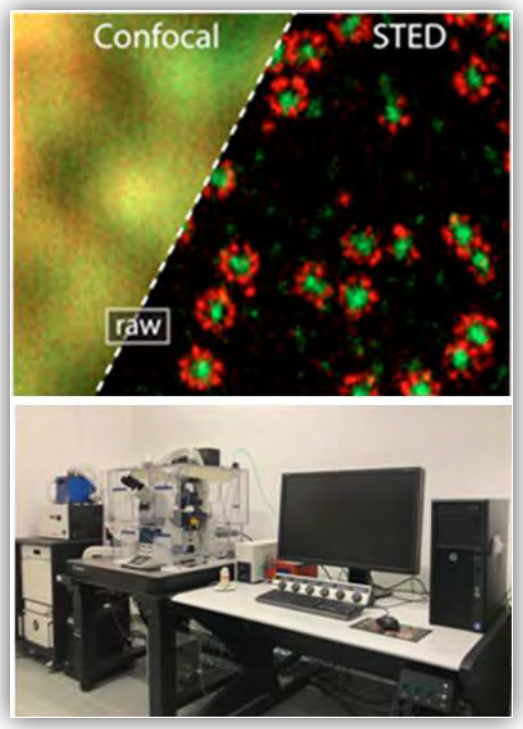
SUMMER SCHOOL LABORATORY ACTIVITIES

ACTIVITIES	Monday 18th	Tuesday 19th	Wednesday 20th	Thursday 21st
1 and 2	**A**	**B**	**C**	**D**
3 and 4	**B**	**C**	**D**	**A**
5 and 6	**C**	**D**	**A**	**B**
7 and 8	**D**	**A**	**B**	**C**

The students are divided into 4 groups (A, B, C, D) of 7-8 students each

Activity – 1 (Prof. Antonio Frigeri)

High resolution microscopy for the analysis of protein channel aggregation into the plasma membrane of cells and tissues

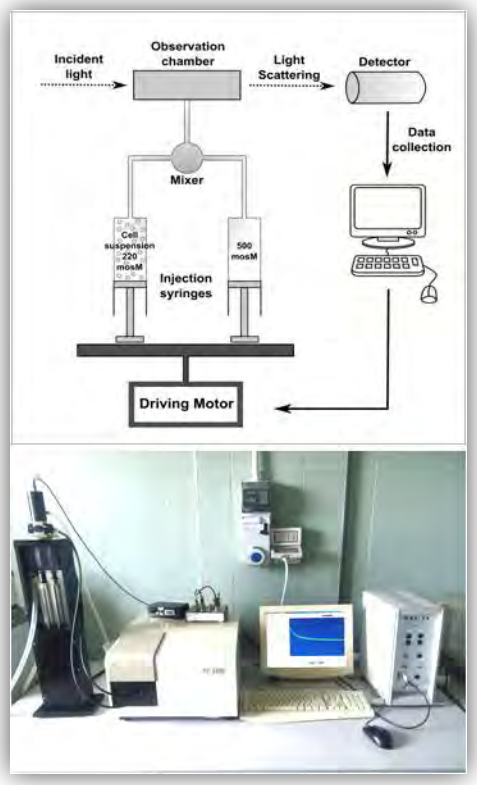


In recent years, a number of "super-resolution" fluorescence microscopy techniques have been invented to overcome the diffraction barrier, including techniques that employ nonlinear effects to sharpen the point-spread function of the microscope, such as stimulated emission depletion (STED). These methods have yielded an order of magnitude improvement in spatial resolution in all three dimensions over conventional light microscopy. The aim of this laboratory experience is designed to give participants the opportunity to evaluate the capabilities of the super-resolution microscopy and in particular with the use of the most recent STED microscope set up (Leica TCS SP8 STED 3x). The participants will familiarize with all components of the instrument and apply different experimental conditions to reach lateral resolution below 50 nm for membrane protein aggregation analysis. Finally, participants will use specific software for imaging and data analysis.

This laboratory experience will be held by Prof. Antonio Frigeri, professor of Physiology at the Dept. of Basic Medical Sciences, Neuroscience and Sensory Organs of the University of Bari – Medical School.

Activity – 2 (Prof. Giuseppe Calamita)

Stopped-flow light scattering measurements of membrane osmotic and solute permeability



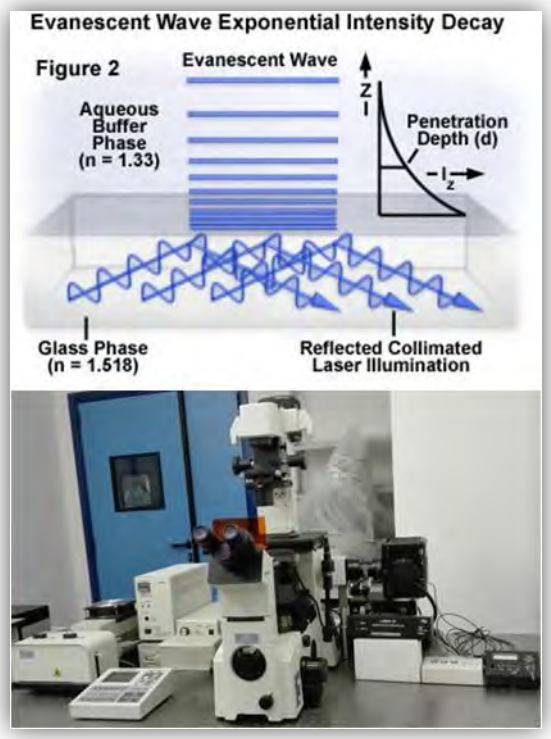
Stopped-flow light scattering (SFLS) is among the most widely used biophysical methodologies to assess the membrane permeability to neutral solutes and water of whole cells, organelles, sealed membrane vesicles as well as artificial liposomes. Membrane solute and water permeability are assessed by measuring the changes in light intensity scattered by the specimen shrinkage or swelling following osmotic or chemical gradients opportunely created across the membrane specimen.

In SFLS technique, small volumes of solutions are used, and the kinetic equations for modeling the solute and water fluxes are equivalent to those used in conventional methods in which concentration and time are measured. SFLS is useful for studying fast molecular transports that have half-lives as short as a few milliseconds.

The SFLS activity will allow the students to use properly the experimental set-up and instrumental device employing isolated whole cells, sealed membrane vesicles and artificial liposomes. SFLS training will be held by Prof. Giuseppe Calamita and Dr. Patrizia Gena, physiologists with major expertise in methodological approaches to measure water and solute permeability in living cells and sealed membrane vesicles.

Activity – 3 (Dr. Maria Grazia Mola)

Total Internal Reflection Microscopy (TIRFM) for water transport measurements across astrocyte plasma membrane



TIRFM is a well-established method to study water and solute permeabilities and cell volume regulation in cells of arbitrary shape and size. Adherent cells labeled with an aqueous-phase dye, are rapidly exposed to an osmotic gradient and the time course of fluorescence signal is detected.

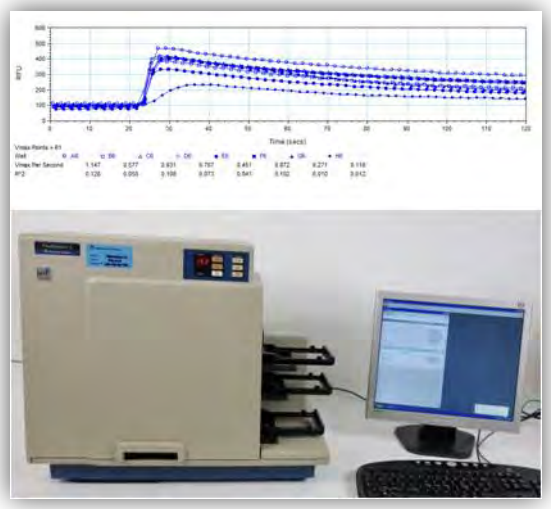
TIRFM uses an evanescent wave instead of direct illumination to selectively excite fluorophores in a very thin layer of cytosol adjacent to the glass-water interface. This allows the observation of membrane-associated processes and reduces dye photobleching.

This activity will allow the students to use properly TIRFM experimental set-up that includes a conventional microscope equipped with a high-numerical-aperture objective, a laser source and a cell perfusion system.

TIRFM training will be held by Dr. Maria Grazia Mola a researcher at the Dept. of Bioscience, Biotechnology and Biopharmaceuticals, University of Bari with the support of Dr. Claudia Palazzo a PhD student with expertise in methodological approaches to measure water permeabilities in living cells.

Activity – 4 (Dr. Maria Grazia Mola)

Use of a semi-automatic platform for a medium throughput screening of compounds able to modulate protein channel function through fluorescence based assays



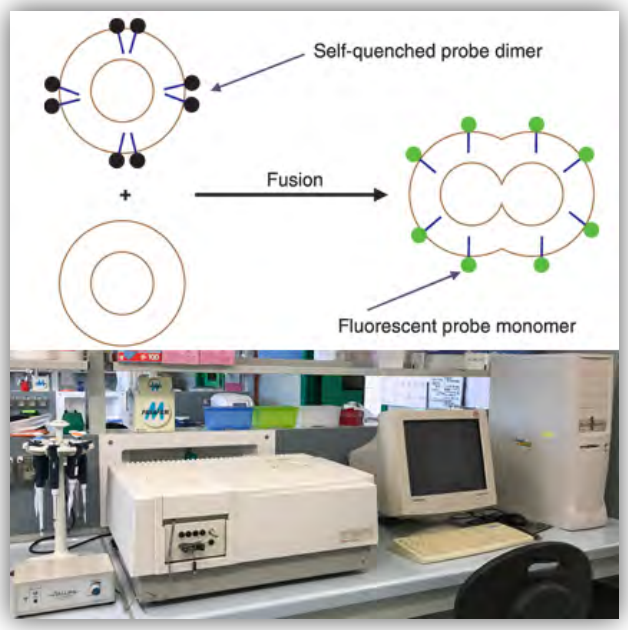
The requirements of a cell-based screening assay include good target sensitivity and specificity, robust readout, day-to-day reproducibility, technical simplicity, suitability for automation, and low cost.

The screening procedure proposed in this activity will be carried out using a benchtop fluorescence plate reader (FlexStation3, Molecular Devices) able to perform functional cellular assays and to detect real time fluorescence kinetic data in the 96-well format. The participants will be introduced to the pratical aspects of a medium throughput screening (MTS) for potential modulators of water channel function in cells labeled with a volume-sensitive fluorescent dye.

MTS training will be held by Dr. Maria Grazia Mola a researcher at the Dept. of Bioscience, Biotechnology and Biopharmaceuticals, University of Bari expert in drug discovery program for modulating membrane protein channel function with the support of Dr. Claudia Palazzo a PhD student with expertise in methodological approaches to measure water permeabilities in living cells.

Activity – 5 (Prof. Giovanna Valenti)

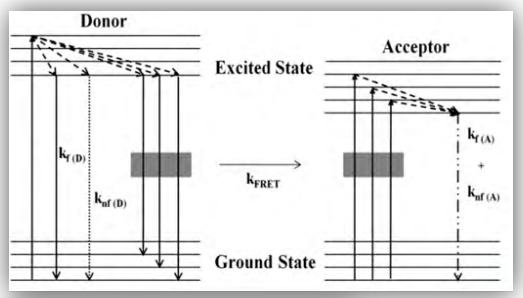
Fluorescence Assay to Monitor Membrane Fusion Kinetics

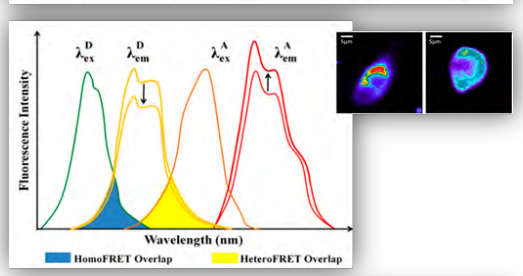


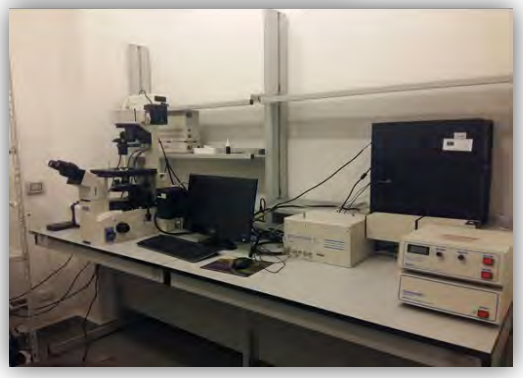
The aim of this training is to monitor, in real-time, the fusion between membranes using a fluorimetric method for assaying membrane fusion exploit processes in a cell-free model system that reflects the final step of exocytosis. Specifically, isolated vesicles are labeled with a lipid-soluble fluorescent probe (octadecylrhodamine R18), at self-quenching concentration and, as a consequence of fusion with unlabelled membrane, lipid probe dilution occurs, leading to an increase in the fluorescent emission signal. The membrane fusion is monitored upon the addition of a cytosolic fraction using a spectrofluorophotometer (RF-5301PC Series) under basal condition and after selective inhibition of ion channel or water channel functionally involved in the fusion process. The student will be introduced to the proper use of the instrument and the use of a fluorescent lipid probe at self-quenching concentration. The training will be held by Professor Giovanna Valenti, Dept. of Bioscience, Biotechnology and Biopharmaceuticals, University of Bari, expert in cell physiology techniques focused on intracellular trafficking pathways with the support of Dr. Mariangela Centrone a PhD student who has a specific expertise in the membrane fusion assay.

Activity – 6 (Dr. Marianna Ranieri)

Detecting cAMP with FRET-based sensors in single living cells







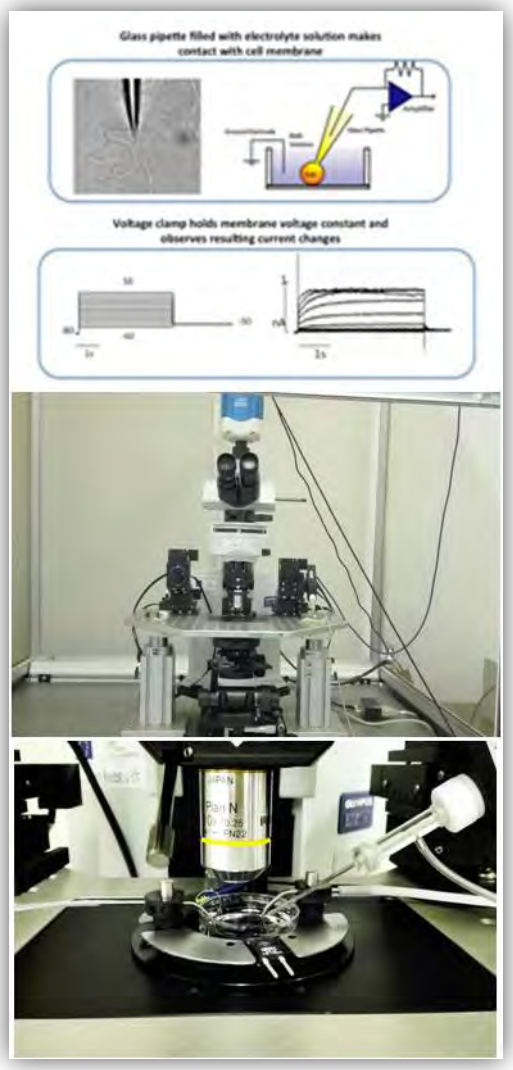
The experience and training will consist in the real-time evaluation of cAMP changes in human embryonic kidney (HEK-293) living cells. The cAMP increases will be monitored in real time, under control condition and after stimulation with forskolin (FK).

We will focused on the different compartmentalized measurements of cAMP in the cytoplasm, in the plasma membrane or in the intracellular organelles, using different Epac-based probes. The student will be instructed to the proper use of this instrument composed of monochromator, inverted microscope, cooled enhanced ECCD camera and computer with the software for the acquisition and analysis.

Dr. Marianna Ranieri, a researcher at the Department of Bioscience, Biotechnology and Biopharmaceuticals, at the University of Bari, is a physiologist with expertise in imaging applications both in static and in real-time measurements. She will hold this laboratory experience with the support of Dr. Annarita Di Mise, a Postdoc who has a specific expertise in imaging techniques used for measuring in real time calcium and cAMP levels in single cells.

Activity – 7 (Prof . Monica Carmosino)

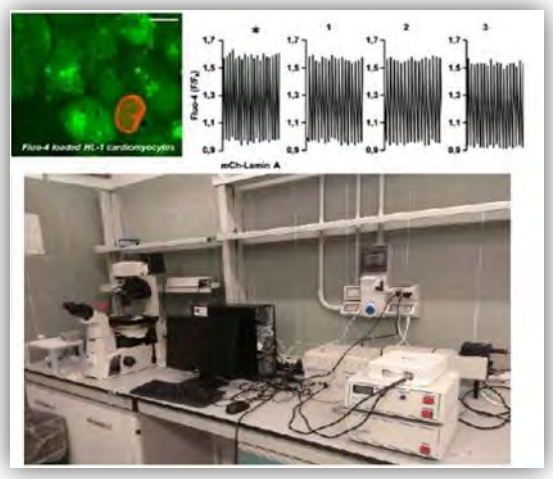
Electrophysiological characterization of KCNH2 potassium voltage-gated channels



Aim of this laboratory experience and training will be the evaluation of the biophysics properties of the potassium voltage-gated channel sub family H member 2 (KCNH2), involved in the repolarization of the cardiac action potential. Both channels activation and the inactivation will be evaluated by voltage-clamp and whole-cell patch clamp experiments in HEK293 cells expressing the fluorescent version of the human KCNH2 channel. The student will be introduced to the proper use of our experimental set-up of voltage clamp to study voltage-gated channels. Stepwise changes in voltage produced by this technique will cause KCNH2 channels to interconvert between different states (activated-inactivated), and these transitions will be monitored as changes in membrane current. This laboratory experience will be held by Monica Carmosino, Associate Professor at the Department of Sciences, University of Basilicata, with the kind collaboration of Dr Roberta De Zio, PhD student at the Department of Bioscience, Biotechnology and Biopharmaceutics, University of Bari.

Activity – 8 (Dr. Andrea Gerbino)

Fluorescence detection and imaging of cytosolic calcium oscillations in cardiomyocytes



Aim of this laboratory experience and training will be the real-time evaluation of cytosolic Ca^{2+} oscillations in cardiac cell models (such as HL-1 cells) loaded with Ca^{2+}-sensitive dyes. The Ca^{2+} oscillation patterns will be monitored under control condition and after stimulation with physiological challenges that mimic either parasympathetic or sympathetic activation.

The student will be introduced to the proper use of our experimental set-up which is composed of a monochromator, an inverted microscope, a cooled CCD camera and a computer with the acquisition software. How to choose the fluorescent dye that best fit the researcher's project will be considered.

This laboratory experience will be held by Dr. Andrea Gerbino a researcher at the Department of Bioscience, Biotechnology and Biopharmaceuticals, University of Bari. Dr. Gerbino is an electrophysiologist with additional expertise in imaging applications used for measuring in real time calcium and cAMP levels in single cells.