COURSE (TITLE):

Molecular bases of cell function

IBP module: "Structures and mechanisms controlling the cell function"

LECTURER:

YEAR and SEMESTER: 1st year and 2nd semester

CREDITS (CFU): 3

SECTOR (SDS): BIO13

ACADEMIC YEAR:

ASSESSMENT:

LOCATION: Department of Environmental, Biological and Pharmaceutical Science and

Technologies, Via Vivaldi 43 Caserta

COURSE OBJECTIVES/OUTCOMES:

The aim of this course is to provide the techniques and the fundamental principles of the microscopy for the analysis of the cell structure as well as of specific cell functions/mechanisms. In details, the course includes the study of DNA replication/repair/ recombination mechanisms in eukaryotic cells and the related experimental methodologies; the principles of innate immunity in physiology and pathology, and the cellular and molecular mechanisms underlying it. The purpose of this course is to make students aware of the importance of fundamental theoretical concepts for the analysis of cell structure, concerning the choice of instrumentation, sample preparation, study/characterization of specific cellular mechanisms.

SYLLABUS (overview)

Microscopy: fundamental principles of the microscopic observation; contrast techniques and study of living cells; several fluorescence microscopy techniques for the static and dynamic study of cellular functions; super-resolution and electron microscopy for the study of cell structure. Mechanisms of DNA replication/repair/recombination and their evolution from Bacteria to Eukarya and related analytical methods. Evolution of the immune response to pathogens: host-pathogen interaction, innate immunity, inflammation, physiological defensive mechanisms vs. pathological activity, vaccination and immunotherapy.

SYLLABUS (Detailed description):

- 1. Microscopy for cell studies:
 - Fundamentals of microscopic observation
 - Optical Microscope: components, tools and accessories
 - Contrast techniques and study of living cells:
 - Dark field microscopy
 - Phase contrast microscopy or interferential contrast microscopy (DIC)
- Introduction to fluorescence microscopy
- Immunofluorescence: principles and applications
- Confocal microscopy
- Two-photon microscopy

• "F" techniques and their applications: "Fluorescence Resonance Energy Transfer" (FRET), "Fluorescence Recovery After Photobleaching" (FRAP), "Fluorescence Loss in Photobleaching" (FLIP), "Fluorescence Lifetime Imaging Microscopy" (FLIM), etc. • Super-resolution microscopy

- Principles and applications of electron microscopy
 - Correlative microscopy
- 2. Mechanisms of DNA replication/repair/recombination and related techniques:
 - Analysis of replication fork parameters by DNA fibre track assay.
 - Isolation and identification of proteins on nascent (iPOND methodology).
 - Visualisation of homologous recombination events by sister-chromatid exchange (SCE) assays.
 - Single molecule biophysical assays of DNA enzymes and proteins (total internal reflection microscopy, TIRF; <u>co-</u>localization <u>single molecule spectroscopy</u>, CoSMoS).

3. Innate immunity

- Defensive mechanisms through evolution
- Effector cells of innate immunity
- Soluble effectors of innate immunity (antimicrobial peptides, complement, cytokines)
- The inflammatory response in innate defence
- The pathological deragments of inflammation
- Immunotherapy: regulation of innate/inflammatory responses

TEXTBOOKS:

ADDITIONAL READING: