VNIVERSITAT DE VALÈNCIA

Course Guide
33141 Cellular analysis techniques

COURSE DATA

Data Subject	
Code	33141
Name	Cellular analysis techniques
Cycle	Grade
ECTS Credits	4.5
Academic year	2022 - 2023

Study (s)

Degree	Center	Acad. year	Period
1109 - Degree in Biochemistry and Biomedical Sciences	Faculty of Biological Sciences	3	Second term

Subject-matter

Degree	Subject-matter	Character
1109 - Degree in Biochemistry and Biomedical Sciences	10 - Métodos instrumentales	Obligatory

Coordination

Name	Department
RODRIGUEZ FERRON, SACRAMENTO	357 - Cellular Biology, Functional Biology and Physical Anthropol.

SUMMARY

“Methods in Cell Biology" is a compulsory subject taught in the second semester of the third year of the degree in Biochemistry and Biomedical Sciences and corresponds to 4.5 ECTS. This subject is included in the block "Methods in Molecular Biosciences" and has a mixed theoretical-experimental nature. *Methods in Cell Biology* comprise the set of techniques and approaches for the structural-functional analysis of biological material at the microscopic level. Four aspects will be covered: instrumentation, techniques for the preparation of biological material for microscopic observation, techniques developed to obtain information on biological processes (in situ detection) and techniques for manipulating cells and tissues. In the theoretical part, the student will obtain an updated view of the observation techniques of different biological samples at the various levels of resolution offered by the current modern microscopes. In the practical part, students will get hands-on experience in the preparation of samples for their analysis at the microscope level, in the use and understanding of the equipment and in techniques for the in situ detection of molecules and processes at the cellular level.

PREVIOUS KNOWLEDGE

Relationship to other subjects of the same degree

There are no specified enrollment restrictions with other subjects of the curriculum.

Other requirements

COMPETENCES (RD 1393/2007) // LEARNING OUTCOMES (RD 822/2021)

1101 - Degree in Biochemistry and Biomedical Sciences

- Capacidad para trabajar correctamente en los laboratorios de bioquímica, genética, biología molecular y celular incluyendo seguridad, manipulación, eliminación de residuos y registro anotado de actividades.
- Capacidad para utilizar la instrumentación básica en experimentación molecular y celular.
- Tener una visión integrada de las técnicas y métodos utilizados en biociencias moleculares y biomedicina.
- Capacidad para diseñar experimentos y aproximaciones multidisciplinares para la resolución de problemas concretos.
- Capacidad para presentar, discutir y extraer conclusiones de los resultados de los experimentos científicos.

LEARNING OUTCOMES (RD 1393/2007) // NO CONTENT (RD 822/2021)

• Understanding the basis of the main techniques used for the microscopic analysis of biological samples and processes.
• Knowledge about the main applications of microscopic techniques in the field of experimentation in molecular biosciences.
• Understanding the basis of the equipment used in microscopic analysis and sample preparation techniques for observation at different levels of resolution.
• Knowledge about how to handle the equipment used in microscopic analyses.
• Capacity for the resolution of theoretical and practical exercises.
• Knowledge about how to interpret, present, and discuss data and experimental results.
• Acquisition of skills in designing experiments to solve specific problems in the area of the subject.

DESCRIPTION OF CONTENTS

1. INTRODUCTION TO METHODS IN CELL BIOLOGY

Introduction to methodology and samples used in cell biology. Types of samples and their handling. Cell cultures. Preparation of living samples. Imprints and smears. Subcellular fractionation. Introduction to histological technique.

2. LIGHT MICROSCOPY

Conventional light microscopy. The light microscope: theory of image formation. Elements of the microscope. Resolution. Optical aberrations and their correction: types of objectives. Dark field. Light interference. Phase contrast. Interferential phase contrast. Fluorescence. Fluorescence microscope. Confocal scanning microscopy. Microcaptura optical tweezers and laser.

3. HISTOLOGICAL TECHNIQUE

Fixing: Background and fixing utilities. Types of fixation. Chemical binding agents. Immersion and perfusion. Inclusion: A Foundation for inclusion. Means of inclusion. Embedding in paraffin. Freezing. Embedding. Microtomy: Getting sections. Microtomy of paraffin. Freezing microtome. Vibratome. Handling and mounting sections on slides. Staining of biological samples. Dyes. Conventional stains. Staining procedures and sample preparation.

4. CYTO/HISTOCHEMICAL TECHNIQUES

Classical histochemical techniques: Detection histochemistry of lipids, carbohydrates, nucleic acids and metals. Histoenzimology. Histoenzimology Utilities. Histochemical detection of enzyme reporters. Histological technique. Controls. Techniques based on fluorescence cell biology: Marking with lectins and other natural ligands. Detecting molecules by fluorophores. Detection of organelles by fluorophores. Introduction of fluorescent compounds into cells. Fluorescent Detection of receptors, cytoskeletal molecules, second messengers.

5. IMMUNOCYTO/HISTOCHEMICAL TECHNIQUES

Immunocytochemistry: polyclonal and monoclonal antibodies. Antibody preparation. Enzymatic systems and fluorescent detection. Direct methods, indirect and three layers. Histological technique. Controls.

6. IN SITU DETECTION OF CELLULAR PROCESSES

Endocytosis, intracellular trafficking and interaction of proteins: Techniques of analysis of the processes of endocytosis. Introduction of proteins in cells. Techniques to assess movement and interaction of proteins. FRET, FRAP. Assessment analysis of the polarity and adhesion. Aggregation analysis. Analysis of transmigration. Analysis of polarity. Techniques for assessing cellular proliferation. Nucleotide incorporation technique. Immunocytochemical markers of cell cycle. Measures the amount of DNA. Analysis of mitosis. Determination techniques of cell degeneration. Apoptosis and necrosis. Techniques for determination of cytotoxicity and viability. TUNEL technique. Annexin technique.

Immunocytochemical markers of apoptosis. Autoradiographic techniques: radioactive isotopes. Pulse-capture experiments. Autoradiographic techniques. Radioligand receptor and determination. Radioactive techniques for cell proliferation.

7. TECHNIQUES FOR NUCLEIC ACID HYBRIDIZATION

Situ hybridization techniques: Hybridization of nucleic acids. Probes. Hybridization conditions. Detection systems. Isotopic and non isotopic techniques. FISH.

8. ELECTRON MICROSCOPY

The electron microscope: transmission and scanning EM. Elements. Resolution. Sample preparation. Ultrastructure: Fixation. Resin embedding. Ultramicrotomy. Contrast with heavy metals. Critical point. Special techniques: Immuno-histochemistry for EM. Cryo-ultramicrotomy. Freeze-fracture.

9. Program of practical sessions

1. Histological technique: fixation, embedding and microtomy. Preparation of various samples for microscopic observation. Inclusion and freezing. Paraffin microtomy, cryomicrotomy and vibratome sectioning. Handling of sections.
2. Color and fluorescence. Routine histological staining. Staining with fluorochromes.
3. Histochemistry. Techniques for the in situ detection of various molecules. Histoenzimology. Analysis of the expression of reporter genes.
4. Immunocytochemistry. Detection of antigens. Immunocytochemical methods and strategies.
5. In situ detection of cellular processes. Determination of proliferation and degeneration processes.
6. Electron microscopy. Sample preparation. Use of instruments.

WORKLOAD

ACTIVITY	Hours	% To be attended
Laboratory practices	24,00	100
Theory classes	21,00	100
Development of individual work	7,50	0
Study and independent work	25,00	0
Readings supplementary material	5,00	0
Preparing lectures	15,00	0
Preparation of practical classes and problem	15,00	0
TOTAL	**112,50**	

TEACHING METHODOLOGY

The development of the subject is divided into:

Lectures. Presentation and discussion of previously announced selected topics. Teaching and bibliographic resources will be available to students in multimedia. The teacher will present the basics of the subject, devoting more time to highly complex issues, and will guide the students in the integration of the contents with related issues of other subjects.

Laboratory practical classes. A program of laboratory experiences will connect theoretical classes with research approaches in the field. Attendance is compulsory.

Tutorials. There will be two one-hour tutorials to discuss the development and results of the laboratory practical classes and to provide an integrated view of the methologies. During the tutorials, the progress of the course will be discussed. One of the tutorial will be also used to resolve problems related to the topics studied during the classes and the second tutorial will be a research talk.

EVALUATION

The evaluation of the **theoretical contents and lab experience** will be done through a single integrated exam which may include questions of different formats (multiple choice, experimental resolution of issues,...) to assess knowledge and understanding acquired by the student on the subject and his/her ability to use scientific language in an appropriate way. The grade in this exam will represent **70% of the final grade**.

For the preparation of the report of **practical activities**, students will complete three written exercises related to the laboratory sessions. The grading will be based on the quality of these exercises, along with the personal work of the student during the laboratory sessions, and the mark will represent **30% of the final grade**.

To pass the course it will be essential to attend practical classes and to obtain a minimum score of 5 out of 10 in the exam.

The resolution of online tests related to the theoretical classes will be used to increase the final score up to 5%. The purpose of these test is to promote the revision of theoretical contents.

REFERENCES

Basic

- La mayor parte de los textos de Histología y de Biología Celular incluyen un capítulo metodológico que, aunque muy básico, puede servir al estudiante para obtener una primera visión global de las técnicas de preparación de muestras y de observación.

- Manual de la asignatura generado por los profesores responsables.
- Montuega Badía, L (2014) Técnicas en histología y biología celular. 2ª ed. Elsevier España.

Additional

- También existen páginas web en las que se puede obtener información muy interesante sobre microscopía y técnicas de preparación de muestras (imágenes, protocolos, etc). Éstos son sólo algunos ejemplos:

  http://members.pgonline.com/~bryand/ (tinción de tejidos)
  http://www.bris.ac.uk/Depts/PathAndMicro/CPL/emtechs.htm (microscopía electrónica)
  http://www.medinfo.ufl.edu/dental/denhisto/stains.html#AA14 (tinción de tejidos)
  http://131.229.114.77/Histology (microscopía/microtecnia)
  http://www.bris.ac.uk/Depts/PathAndMicro/CPL/histmeth.htm (tinción de tejidos)
  http://www.cellsalive.com/enhance0.htm (microscopía)
  http://micro.magnet.fsu/primer/index.html (microscopía óptica)
- Para profundizar en cada uno de los apartados del programa podrán utilizarse los libros y publicaciones especializadas listados a continuación.
  Clave de localización: (CI), Biblioteca Ciencias.(M), Biblioteca Medicina. (DEP): Departamento Biología Celular

  - Bancroft, J.D. and Hand, N.M. (1987) Enzyme histochemistry. Oxford University Press, Oxford (CI)
  - Bozzola J.J. y Russell L.D. (1992) Electron microscopy: principles and techniques for biologists. Jones and Bartlett (eds.), Boston. (CI)
  - Burck, H.C. (1969). Técnica histológica. Ed. Paz Montalbo. Madrid. (M)
  - Cuello (1985). Immunohistochemistry. John Wiley & Sons, New York. (DEP)
  - Durfort M. et al (1991) Técnicas de inmunocitoquímica en microscopía electrónica. Publicaciones de la Universidad de Barcelona. (CI)
  - Durfort, M. et al. (1990). La fixació (cinta de video). Publicaciones de la Universidad de Barcelona. (CI)
  - Durfort, M. et al. (1990). La tinció (cinta de video). Publicaciones de la Universidad de Barcelona. (CI)
  - Durfort, M. et al. (1990). La microtomia (cinta de video). Publicaciones de la Universidad de Barcelona. (CI)
  - García del Moral, R. (1993) Laboratorio de Anatomía Patológica. Interamericana. Madrid. (DEP)
- - Lacey, A.J. (1989) Light microscopy in biology: a practical approach. IRL Press, Oxford. (CI)
  - Locquin, M. (1985) Manual de microscopía. Labor, Barcelona. (CI)
  - McManus, J.F.A. and Mowry, R.W. (1968) Técnica histológica. Ed. Atika, Madrid. (CI)
  - Mercer, E.H., Birbeck, M.S.C. (1974). Manual de microscopía electrónica para biólogos. Ed Blume, Madrid. (CI)
  - Peinado M.A. y cols. (1996) Avances en inmunocitoquímica y técnicas relacionadas. Servicio de Publicaciones de la Universidad de Jaén. (CI)
  - Renau, J. y Megías, L. (1998) Manual de Técnicas de Microscopía Electrónica (M.E.T.). Aplicaciones Biológicas. Ed. Universidad de Granada. (DEP)
  - Sauret, M. (1984) Microscòpia. Publicacions i Edicions de la Universitat de Barcelona. (DEP)

- Sampedro, A. et al (1995) Técnicas de fluorescencia en microscopía y citometría. Servicio de Publicaciones de la Universidad de Oviedo. (M)