

**COURSE DATA****Data Subject**

Code	44697
Name	Image analysis techniques
Cycle	Master's degree
ECTS Credits	2.5
Academic year	2022 - 2023

Study (s)

Degree	Center	Acad. year	Period
2224 - Master's Degree in Research and Development in Biotechnology and Biomedicine	Faculty of Biological Sciences	1	First term

Subject-matter

Degree	Subject-matter	Character
2224 - Master's Degree in Research and Development in Biotechnology and Biomedicine	1 - New technology	Obligatory

Coordination

Name	Department
MORANTE REDOLAT, JOSE MANUEL	357 - Cellular Biology, Functional Biology and Physical Anthropol.

SUMMARY

The subject 'Técnicas de Análisis de Imagen' (hereinafter TAI) is a clear example of what has meant the enormous advancement of microscopy and other image acquisition techniques in the training of future professionals in Biology, Biotechnology and Biomedicine. Currently, there is a large distribution of computer-integrated microscopy equipment and a wide variety of software packages and new algorithms available for digital image analysis. This kind of technology, which has advanced rapidly since the beginning of the 21st century, along with the constant increase in the number of images to be processed, requires the training of specialized researchers who must combine image analysis skills with biological knowledge.



The TAI subject is presented as an introductory course to digital image treatment techniques applied mainly to biological images, that is, to Bioimage Analysis (BIA). This consists in the identification of biological elements of interest in images and in the quantification of a great variety of characteristics to tackle a biological problem in an unbiased way. To do this, different software packages consisting of 'collections' of image processing and analysis algorithms (known as components) are used.

However, BIA does not consist solely in 'learning to use a certain software' since, for each specific problem, it is necessary to assemble a series of components in a specific order, adjusting their functional parameters to the problem to be solved, obtaining a 'workflow'. This workflow takes image files as input and generates numerical values and / or processed images as a result. Furthermore, component assembly can also be done in the form of an executable script. Converting workflows to scripts has two main advantages: 1) It allows the automation of repetitive tasks, and can even develop scripts for high content BIA. 2) The scripts also stand out for their usefulness as documentation, that is, they serve as a record of a workflow, which ensures the reproducibility of a BIA protocol.

Therefore, there is a growing demand for experts in the optimal assembly of image processing algorithms for a practical development of the BIA: bioimaging analysts. This group of professionals join and complement the three classic categories of experts involved in "BioImaging": scientists in the field of biosciences (e.g. biologists), instrumentalists (e.g. microscopists) and developers (e.g. programmers).

In the 2.5 credits, we cover the fundamentals of the BIA beginning with the characteristics and properties of digital images and their specific problems; continuing with the different algorithms and techniques for the construction of workflows and their logic to obtain workflows capable of obtaining significant quantitative data from images. Finally, we acquire the basic knowledge needed for the automation of these workflows as scripts to be able to process large numbers of images.

The subject is conceived as a fundamentally practical package, so each of the theoretical concepts that are introduced during the course, has its immediate practical application in the following sessions.

PREVIOUS KNOWLEDGE

Relationship to other subjects of the same degree

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

Other requirements

There are none

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

2224 - Master's Degree in Research and Development in Biotechnology and Biomedicine

- Students should apply acquired knowledge to solve problems in unfamiliar contexts within their field of study, including multidisciplinary scenarios.



- Students should be able to integrate knowledge and address the complexity of making informed judgments based on incomplete or limited information, including reflections on the social and ethical responsibilities associated with the application of their knowledge and judgments.
- Students should possess and understand foundational knowledge that enables original thinking and research in the field.
- Be able to integrate new technologies in their professional and/or research work.
- Utilizar adecuadamente las herramientas informáticas, métodos estadísticos y de simulación de datos, aplicando los programas informáticos y la estadística a los problemas biomédicos y biotecnológicos.
- Adquirir destrezas en el manejo de las metodologías avanzadas empleadas en las biociencias moleculares y en el registro anotado de actividades.
- Aprendizaje del uso de la instrumentación y equipamientos empleados en los laboratorios de biotecnología y biomedicina.

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

Understand the principles of operation of the digitization of the image and its implications in the processing and obtaining information of biological image.

To provide the student a set of simple tools to get effectively morphometrics measures, in the field of Molecular and cellular biology.

Understand and apply macro programming language instructions and control structures. Create macro instructions to automate image analysis workflows for both individual images and multiple files and/or stacks of images.

Introduce the student to the digital image processing techniques.

To relate the knowledge of the statistic, the microscopic techniques and the instrumental techniques of analysis with the quantitative techniques that are taught in IAT.

Train the students in the use of free distribution programs for Image Analysis.

Understand and apply macro programming language instructions and control structures.

Create macro instructions to automate image analysis workflows for both individual images and multiple files and/or stacks of images.

DESCRIPTION OF CONTENTS



0. Session 1: Presentation of the course: Complete diagram of the workflow in image analysis

Concept and purpose of Bioimage Analysis. Aspects to take into account when measuring. Human perception vs. computational analysis. Current microscopy: volume of images generated. Everyday examples of image analysis. The profession of Bioimage Analyst. Basic scheme of work in Bioimaging Analysis. The workflows. Concrete examples. Image analysis software: ImageJ / Fiji and the importance of Open Source. Fiji: Simple Commands, Integrated Commands, Plugins and Macros. Fiji Updater.

1. Session 2: Properties of the digital image

The digitization process. Sensor types. The numerical nature of the digital image. Color digitization. Light and optical systems. Digital image properties: size and resolution, calibration, bit depth. Color spaces. Image file types. False color palettes. Dimensions of biological images.

2. Session 3: Enhancement and restoration of the digital image

Real image vs. digitized image vs. corrected image. Histogram of a digital image. Perform and restore operations: operations that change the number of pixels, color decomposition, point-to-point operations, operations between neighboring pixels (in the spatial domain), operations in the frequency domain. Examples.

3. Session 4: Segmentation

Segmentation concept. Human brain vs analysis software. Neighborhood criteria. Segmentation techniques: manual segmentation, threshold segmentation, segmentation based on edge detection, region-based segmentation, clustering segmentation, pixel classification segmentation (machine learning).

4. Session 5: Segmentation exercises

5. Session 6: Binary Image

Concept and properties of the binary image. Operations with the binary image: Boolean operations (AND, OR, XOR) concept and applications. Operations with the binary image: Mathematical morphology (Erosion, Dilation, Opening, Closing, Outline, Fill Holes, Skeletonize, Analyze skeleton, Euclidean distance map, Ultimate Erode Points, Watershed, Voronoi, Binary Reconstruction).



6. Session 7: Integration Exercises

Exercises to combine the contents of sessions 1 to 6

7. Session 8: Morphometry

What to measure?: Spatial and spectral information. Measure vs. result. Accuracy vs. precision. Primary and derived form descriptors: Perimeter, Area, Feret Diameters, Surrounding Rectangle, Centroid, Area Fraction, Fit to an Ellipse, Aspect ratio, Solidity, Roundness, Form Factor. Densitometric measurements. Examples.

8. Session 9: Integration exercises

Exercises to combine the contents of sessions 1 to 8

9. Session 10: Automation. Macroinstructions (I)

Macroinstructions: concept and use. The macro language in ImageJ. Built-in Macro Functions. Annotations. Variables: definition and use. Operators. Concatenation. Vectors: definition and use. Control structures (conditionals and loops). Creation and use of dialog boxes.

10. Session 11: Automation. Macroinstructions (II)

Guided exercises to automate workflows using macro instructions.

11. Session 12: Final exercises. Examination approach

Final exercises of integration of the whole subject.
Presentation of the exercises for the final evaluation.

12. Session 13: External speaker visit

Presentation of real practical cases by external speaker.



WORKLOAD

ACTIVITY	Hours	% To be attended
Theory classes	15,00	100
Tutorials	10,00	100
Study and independent work	17,50	0
Preparation of evaluation activities	20,00	0
TOTAL	62,50	

TEACHING METHODOLOGY

The development of the course is structured around three axes: the theory sessions, the practical sessions and the presentation of working models by professionals with experience in the application of digital processing techniques.

The theoretical classes alternate with practical sessions so that first the concepts of the image analysis technique will be explained, and then put into practice.

Practical classes, during tutoring sessions, which will be conducted in small groups, will be developed following two complementary strategies: in some sessions will apply the knowledge gained in previous session theory, by a number of problems-type. In other sessions integrators exercises, which serve to relate the knowledge gained so far will be made. The role of the practical sessions will rest primarily on the student, as it will be he who will have to solve similar problems of greater complexity. Students will be distributed in groups of two, and the teacher will handle guide and help at all times. Once the work is completed, the problems will be corrected and analyzed by the same students in the classroom.

Each student will have a personal copy of the work programs to be used in class, and so they can continue to work individually also outside the computer room of the Faculty. During the first sessions we will take special care to train the students in the use and installation of software, to prevent that the use of this material they have an additional cost.

EVALUATION

The evaluation of student learning will take place in 2 different stages:

1. continuous evaluation of the progress and the work developed throughout the course, which will be based largely on questions and problems carried in the practical sessions. The note obtained in this section will constitute 20% of the final grade.
2. The acquired knowledge will also be assessed by examination at the end of the course, which contribute 80% to the final mark.



The exam will consist of a set of biological problems, of which the images will be provided in the corresponding sentences, which the students will solve individually. Each one will provide a reasoned report of the proposed solution to each problem and an executable program on the imageJ platform that will automatically solve it. The technical contributions that the student makes to the resolution of the problem related to the Image Analysis techniques will be valued, as well as the originality and simplicity of the proposed protocol.

REFERENCES

Basic

- Bray M.A. et al (2012). Workflow and Metrics for Image Quality Control in Large-Scale High-Content Screens. *J Biomol Screen.* 17(2):266-274.
- Chaki J. & Dey N. (2020). *A Beginners Guide to Image Shape Feature Extraction Techniques*. Boca Raton: CRC Press.
- Eliceiri K.W. (2012). Biological Imaging Software Tools. *Nat Methods.* 9(7):697-710.
- Ferreira T.A. & Rasband W. (2012). *The ImageJ User Guide Version 1.46r*. <http://imagej.net/docs/guide/user-guide.pdf>
- Guiet R., Burri O. & Seitz A. (2019). Open Source Tools for Biological Image Analysis. In: Rebollo E., Bosch M. (eds) *Computer Optimized Microscopy. Methods in Molecular Biology*, vol 2040. Humana, New York, NY.
- Jost A.P.T & Waters J.C. (2019). Designing a Rigorous Microscopy Experiment: Validating Methods and Avoiding Bias. *J Cell Biol.* 218(5):1452-1466.
- Meijering E., Carpenter A.E., Peng H., Hamprecht F.A. & Olivo-Marin J.C. (2016). Imagining the Future of Bioimage Analysis. *Nat Biotechnol.* 34(12):1250-1255.
- Miura K., Tosi S., Möhl C., Zhang C., Paul-Gilloteaux P., Schulze U., Nørrelykke S.F., Tischer C. & Pengo C. (2016). Bioimage Analysis Tools. In: Miura K. (ed) *Bioimage Data Analysis*. Wiley-VCH, Heidelberg.
- Miura K. et al (2017). Workflows and Components of Bioimage Analysis: The NEUBIAS Concept. Zenodo. doi: 10.5281/zenodo.1042570.
- Myers G. (2012). Why Bioimage Informatics Matters. 9(7):659-660.

Additional

- Pertusa J.F. & Morante-Redolat J.M. (2019). Main Steps in Image Processing and Quantification: The Analysis Workflow. In: Rebollo E., Bosch M. (eds) *Computer Optimized Microscopy. Methods in Molecular Biology*, vol 2040. Humana, New York, NY.
- Rueden C.T., Schindelin J., Hiner M.C., DeZonia B.E., Walter A.E., Arena E.T. & Eliceiri K.W. (2017). ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics.* 18(1):529.
- Russ J.C (2015) *The Image Processing Handbook, Seventh Edition* 6th Edition CRC Press. London.
- Sbalzarini I.F. (2016). Seeing Is Believing: Quantifying Is Convincing: Computational Image Analysis in Biology. In: De Vos W., Munck S., Timmermans JP. (eds) *Focus on Bio-Image Informatics. Advances in Anatomy, Embryology and Cell Biology*, vol 219. Springer, Cham.
- Schindelin J., Arganda-Carreras I., Frise E., Kaynig V., Longair M., Pietzsch T., Preibisch S., Rueden



C., Saalfeld S. & Schmid S. (2012). Fiji: an open-source platform for biological-image analysis. Nat Methods. 9(7):676-682.

Schneider C.A., Rasband W.S., Eliceiri K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 9(7):671-675.

