IMPORTANT: This syllabus form should be submitted to OAA (gsbs_academic_affairs@uth.tmc.edu) a week before the start of each semester.

NOTE to STUDENTS: If you need any accommodations related to attending/enrolling in this course, please contact one of the Graduate School's 504 Coordinators, Cheryl Spitzenberger or Natalie Sirisaengtaksin. We ask that you notify GSBS in advance (preferably at least 3 days before the start of the semester) so we can make appropriate arrangements.

Term and Year: **Summer 2025** **Course Number and Course Title:** Advanced Fluorescence Microscopy – Emphasis in live imaging and state-of-the-art technologies **Credit Hours:** 3 Prerequisites (if any): **N/A** Meeting Location: **GSBS Classroom** Building/Room#: **BSRB S3.8371**	**Program Required Course: No** **Approval Code: Yes** **(If yes,** the Course Director or the Course Designee will provide the approval code.) **Audit Permitted: Yes** **Classes Begin:** May 20th,2025 **Classes End:** July 3rd,2025 **Final Exam Week:** July 3rd (Projects Presentation)

Class Meeting Schedule

Day	Time
May (20,22,27,29) June (3,5,10,12,17,24,26,30) July (3rd)	**9am to 11 am**

Course Director Name and Degree: **Adriana Paulucci, Ph.D.** Title: Principal Research Scientist, Director- Genetics Advanced Microscopy Laboratory Department: Genetics Institution: MDACC Email Address: apaulucci@mdanderson.org Contact Number: 713-794 1159 **Course Co-Director/s:** (if any) Name and Degree: **Travis Moore, Ph.D.** Title: Assistant Professor, Director – Center for Advanced Microscopy	**Instructor/s** (Use additional page as needed) 1.Name and Degree: **Adriana Paulucci, Ph.D** Institution: MDACC Email Address: apaulucci@mdanderson.org 2. Name and Degree: **Travis Moore, Ph.D.** Institution: UTH Email Address: travis.i.moore@uth.tmc.edu 3. Name and Degree: **Leoncio Vergara, Ph.D** Institution: Texas A&M Email Address: leovergara@tamu.edu

Department: Department of Integrative Biology and Pharmacology

Institution: UTH

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Contact Number: 713-500-6514

NOTE: Office hours are available by request. Please email me to arrange a time to meet.

Teaching Assistant: (if any)

Name and Email Address: : **Ryan Durham, Ph.D.**
Institution: UTH
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4. Name and Degree: Alloysius Budi Utama, Ph.D.

Institution: Rice University

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5. Name and Degree: Allan Ferreon, Ph.D

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6. Name and Degree: Reid Powell, Ph.D

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Email Address: rpowell@tamu.edu

7. Name and Degree: Anna Karin Gustavsson, Ph.D

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Email Address: ag134@rice.edu

Course Description:

The Advanced Fluorescence Microscopy course will teach basic and advanced principles of fluorescence microscopy with special emphasis in live imaging and state-of-the-art technologies. Basic principles will include basic Fluorescence Spectroscopy and Spectral Analysis and Unmixing, Microscope Architecture and Proper Adjustments, Sample Preparation and Choice of proper fluorophores, endogenous probes and biosensors. It will explain Wide-field versus Optical sectioning microscopy and Confocal Microscopy in depth. The course will take an in depth look into advanced microscopy modalities including super-resolution (SIM, STED, SoRa, STORM) and the latest innovations in confocal super-resolution. It will teach live imaging TIRF (total internal reflection) and TIRF use for single molecule studies. A great emphasis will be given to live imaging to study protein dynamics inside tissues, cells, and small organisms, such as actin dynamics. The Live Imaging Modalities will also include Spinning Disk with SoRa super-resolution, lightsheet, dual camera systems, multiphoton, microfluidics and high-content imaging. Technologies such as Ratiometric Imaging (ex. Calcium, pH, NADH), Ablation (Including DNA ablation), Traction Force Microscopy, FCS (Fluorescence Correlation Spectroscopy), FRAP (Fluorescence Recovery After Photobleaching), FRET (Forster Resonance Energy Transfer) and FLIM (Fluorescence Lifetime Imaging) will also be covered in detail by experts in these fields. The course will also focus on proper image analysis and visualization according to current published microscopy guidelines. The main objective is to teach students how they can combine state-of-the-art technologies and advanced microscopy modalities to excel in their projects. At the end of this course students will be able to generate hypotheses that include state-of-the-art microscopy and, most importantly, they will be able to explain the microscopy and will be able to produce high quality microscopy data that follows the current microscopy guidelines for data quality and reproducibility.

Textbook/Supplemental Reading Materials (if any)

- **Handbook of Biological Confocal Microscopy by James Pawley (consultation only)**
- **Instructors will provide articles and chapters that supplement classes**

Course Objective/s:

Upon successful completion of this course, students will be able to properly choose and apply microscopy modalities and technologies to their current projects. They will present the microscopy related portion of their projects or hypothetical projects and they will have to write and present a short project with aims introducing the proper microscopy modalities and technologies chosen for their projects. During this final presentation they will have to explain how the microscopy modalities and technologies work and how it addresses their biological questions and they will have to show the experimental designs and methods that they will use for analysing their data.

***Specific Learning Objectives*:**

1. Basic and advanced modalities of Fluorescence Microscopy
2. Learn how to combine proper technologies to answer questions that go beyond the optical limit resolution. That means know how and when to use FRET, FRAP, FCS, ABLATION, Photo-activation,Traction Force Microcopy, FLIM, DNA paint, etc.
3. How to plan their microscopy experiment properly from sample preparation to data collection. How to properly store, analyze, write methods, and present microscopy data.
4. How to follow the current microscopy rigor guidelines for data quality and reproducibility.
5. How to excel on their projects by using the right microscopy modality and technology, and how to write properly the microscopy part of their projects.

Student Responsibilities and Expectations: Students enrolled in this course will be expected to perform the following activities each week.

1- Attend and participate in 2 hours lectures every week. At the end of classes there will be either breakouts or round tables that will allow for evaluation of student progress.
2- Attend and participate in 2 hours lab each week.
3- Make proper notes and save data from labs to be analyzed latter.
4- Students are expected to attend the Image Analysis and Breakout classes (considered major part of grading)
5- Prepare for laboratories in advance by meeting their lab-mates and to pose questions to course director and co-director.
6- Prepare for the final presentation. A hypothetical or actual project must be presented that inlude several microscopy modalities and few technologies. Students will present how samples will be prepared, data will be collect and what they expect for results. To achieve this objective students will have to meet outside of class and they are also encouraged to meet Dr. Paulucci and Dr. Moore to help with project development)
7- "Plagiarism and failure to properly cite scientific literature and other sources will not be tolerated and are grounds for dismissal from the course and further GSBS disciplinary action. Cheating or engaging in unethical behavior during examinations (quizzes and final) will be grounds for dismissal from the course without credit and further GSBS disciplinary action"

Grading System: **Letter Grade (A-F)**	
Student Assessment and Grading Criteria : *(May include the following:)*	
Percentage	**Description**
Homework **(20 %)**	Students will have to prepare for laboratory classes together with their labmates. They will also have to meet their groups to discuss their projects and their final presentation. They will be encouraged to bring questions to Dr. Paulucci and Dr. Moore after each classs during round table for discussions.
Imaging Analysis Exercises Quiz **(10%)**	Students will have to analyse their data acquired in the lab with the guidance of intructors. These analyses will count as a test, or quiz.
Presentation **(30 %)**	Students Final Detailed Presentation of 30 minutes per group. They will have to present a hyphtetical project with chosen microscopy modalities and techologies, explain how they work, how they would collect their data and how they would analyse the data. They will have to answer questions from their peers and instructors.
Workshop or Breakout-Session **(10%)**	The breakouts will be interactive and involving image analysis and advanced microscopy data interpretation
Participation and/or Attendance **(30%)**	Students are expected to attend 2 hours lectures (TUE) and 2 hours lab (THU) per week. Participation will be evaluated in each class/lab.

CLASS SCHEDULE

Date	Duration (Hour(s) taught by lecturer)	Lecture Topic	Lecturer/s
May 20th	1hour	Principles of Light Propagation and Fluorescence. Sample Preparation.	Adriana Paulucci
May 20th	1hour	Cameras and Image digitalization. Introduction to Resolution and Image Analysis	Leoncio Vergara
May 22nd (Lab)	1 hour	Setting up a microscope for widefield and brightfield. System Maintenance and Optical Aberrations	Adriana Paulucci
May 22nd (Lab)	1hour	Imaging Fluorescent Samples. Proper Adjustments, choices of objectives and Corrections	Leoncio Vergara
May 27th	45 min	Optical Sectioning and Confocal Microscopy, Spatial Array Detectors for Confocal Super-Resolution	Budi Utama
May 27th	45 min	Colocalization, Autofluorescence. Crosstalk, Unmixing and Introduction to FRET	Adriana Paulucci
May 27th	30 min	Discussion and question- round table Preparation for Lab	Adriana and Budi
May 29th (Lab)	1 hour	Confocal Imaging proper adjustments	Budi Utama
May 29th (Lab)	1 hour	Spectral Unmixing and FRET with confocal	Adriana Paulucci
June 3rd	45 min	Introduction to Live imaging (including FRAP ABLATION and Ratiometric Imaging)	Adriana Paulucci
June 3rd	45 min	Live imaging with dual camera systems, TIRF and Traction Force Microscopy and Speckle Microscopy	Travis Moore
June 3rd	30 min	Discussion and questions- round table Preparation for Lab	Adriana P. and Travis M.
June 5th (Lab)	50min	FRAP and Ablation	Adriana Paulucci
June 5th (Lab)	50min	TIRF and dual camera system to study actin cytoskeleton dynamics	Travis Moore
June 10th	1 hour	Super-Resolution STED, STORM, MINFLUX and SIM	Travis Moore
June 10th	1 hour	Breakout- Live Image Analysis- Students will present data analysis from their previous lab	Travis M. and Adriana P.
June 12th (Lab)	2 hours	Super-Resolution Lab (SIM, STORM AND STED)	Travis M and Adriana P.
June 17th	1 hour	Multiphoton, FLIM and Clearing Methods	Leoncio Vergara

June 17th (lab)	1 hour	FLIM laboratory	Leoncio Vergara
Jun 24th	1 hour	FIJI Image Analysis, quantification with Rigor, Planning proper Acquisition Matters	Leoncio Vergara
Jun 24th	1 hour	Breakout- FIJI Image Analysis Students will analyze images from previous lab	Leoncio Vergara
Jun 26th	1 hour	High Content Imaging and Analysis	Reid Powell
Jun 26th	1 hour	Breakout- image analysis using open-source software	Reid Powell
Jun 30th	1 hour	Single-molecule tracking and super-resolution imaging in 3D using light sheet illumination and microfluidics	Anna Karin Gustavsson
Jun 30th	1 hour	Fluorescence Correlation Spectroscopy and Single Molecule Studies	Allan Ferreon
July 3rd	2 hours	Students Projects Presentations	Adriana P. and Travis M.

NOTE: There will be a total of 13 hours dedicated to lectures, 8 hours dedicated to laboratory classes, 3 hours dedicated to breakout involving image analysis and 2 hours for students final presentations, totalizing 26 hours of in- class activities. We expect students to dedicate at least 20 hours outside of class to read articles and materials, prepare projects and presentation and discuss and prepare for the labs. Students will be encouraged to meet with course director (Dr. Paulucci) and co-director (Dr. Moore) to discuss their final projects plan and presentation in advance. They are expected to save some time for this outside of class activity.

Our instructors: All main instructors for this course have many years of experience teaching graduation school with many years of experience in microscopy and managing microscopy laboratories.

AP/jal