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:** **GS04 1273: 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 (TBA)**	**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)**	**9:00 am to 11:00 am**

Course Director Name and Degree: **Adriana Paulucci, PhD** 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:** Name and Degree: **Travis Moore, PhD** Title: Assistant Professor, Director – Center for Advanced Microscopy	**Instructors** 1. **Adriana Paulucci, PhD** Institution: MDACC Email Address: apaulucci@mdanderson.org 2. **Travis Moore, PhD** Institution: UTHH Email Address: travis.i.moore@uth.tmc.edu 3. **Leoncio Vergara, PhD** Institution: Texas A&M Email Address: leovergara@tamu.edu 4. **Alloysius Budi Utama, PhD** Institution: Rice University Email Address: budiutama@rice.edu

Department: Department of Integrative Biology and Pharmacology

Institution: **UTHealth Houston**

Email Address: travis.i.moore@uth.tmc.edu

Contact Number: 713-500-6514

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

Teaching Assistant:

Name and Email Address: : **Ryan Durham, Ph.D.**
Institution: UTH
Email Address: Ryan.Durham@uth.tmc.edu

5. **Reid Powell, PhD**

Institution: Texa A&M
Email Address: rpowell@tamu.edu

6. **Anna Karin Gustavsson, PhD**

Institution: Rice University
Email Address: ag134@rice.edu

Course Description:

The Advanced Fluorescence Microscopy course provides a comprehensive understanding of both fundamental and advanced principles of fluorescence microscopy, with a strong emphasis on live imaging and cutting-edge technologies. Students will explore basic concepts, including fluorescence spectroscopy, spectral analysis and unmixing, microscope architecture and adjustments, sample preparation, and the selection of appropriate fluorophores, endogenous probes, and biosensors. The course will cover wide-field versus optical sectioning microscopy, with an in-depth examination of confocal microscopy. Advanced microscopy modalities will include super-resolution techniques (SIM, STED, SoRa, STORM) and the latest innovations in confocal super-resolution microscopy. The course will introduce Total Internal Reflection Fluorescence (TIRF) microscopy, with applications in single-molecule studies and live imaging, with a particular focus on protein dynamics in tissues, cells, and small organisms, such as actin cytoskeleton remodeling. Live imaging techniques will include spinning disk microscopy with SoRa super-resolution, light-sheet microscopy, dual-camera systems, multiphoton imaging and high-content imaging. Students will also gain hands-on experience with advanced technologies such as ratiometric imaging (e.g., calcium, pH, NADH), ablation techniques, fluorescence recovery after photobleaching (FRAP), Förster resonance energy transfer (FRET), and fluorescence lifetime imaging microscopy (FLIM)—all taught by experts in their respective fields. Beyond imaging, the course will emphasize proper image analysis and visualization in alignment with current microscopy guidelines to ensure data quality and reproducibility. By the end of the course, students will be equipped to integrate state-of-the-art microscopy techniques into their research, generate hypothesis leveraging these technologies, and produce high-quality microscopy data that adhere to rigor and reproducibility standards.

Textbook/Supplemental Reading Materials

- 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 select and apply appropriate microscopy modalities and technologies to their research projects. They will develop and present a microscopy-focused component of their actual or hypothetical projects, demonstrating their ability to integrate advanced imaging techniques into experimental design. As part of their final assessment, students will prepare a concise project proposal with specific aims, detailing the microscopy approaches chosen and their relevance to addressing key biological questions. In their final presentation, students will explain the principles behind the selected microscopy techniques, justify their application to their research, and outline the experimental design, data acquisition methods, and analysis strategies necessary for producing high-quality, reproducible imaging data.

***Specific Learning Objectives*:**

1. Understand the principles and applications of basic and advanced fluorescence microscopy techniques.
2. Identify and apply appropriate advanced imaging technologies to address research questions beyond the optical resolution limit.
3. Design and execute microscopy experiments, including sample preparation, data acquisition, analysis, and presentation.
4. Follow microscopy rigor and reproducibility guidelines to ensure data quality and reliability.
5. Optimize microscopy use in research projects and effectively communicate methodologies in scientific writing and presentations.

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

1. Student Responsibilities and Expectations: Students enrolled in this course will be expected to perform the following activities each week.
2. 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.
3. Attend and participate in 2 hours lab each week.
4. Make proper notes and save data from labs to be analyzed latter.
5. Students are expected to attend the Image Analysis and Breakout classes (considered major part of grading)
6. Prepare for laboratories in advance by meeting their lab-mates and to pose questions to course director and co-director.
7. 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)

"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 analyze 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 - Summer 2025

Date	Duration	Lecture Topic	Lecturer(s)
May 20th	1 hour	Principles of Light Propagation and Fluorescence. Sample Preparation	Adriana Paulucci
May 20th	1 hour	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
	1 hour	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
	45 min	Colocalization, Autofluorescence. Crosstalk, Unmixing and Introduction to FRET	Adriana Paulucci
	30 min	Discussion and question- round table Preparation for Lab	Adriana Paulucci and Budi Utama
May 29th (Lab)	1 hour	Confocal Imaging proper adjustments	Budi Utama and Ryan Durham
	1 hour	Spectral Unmixing and FRET with confocal FRET: Acceptor photobleaching Unimixing: crosstalk	Adriana Paulucci and Travis Moore
June 3rd	45 min	Introduction to Live imaging (including FRAP ABLATION and Ratiometric Imaging)	Adriana Paulucci
	45 min	Live imaging with dual camera systems, TIRF and Traction Force Microscopy and Speckle Microscopy	Travis Moore
	30 min	Discussion and questions- round table Preparation for Lab	Adriana Paulucci and Travis Moore
June 5th (Lab)	50 min	FRAP and Ablation	Adriana Paulucci
	50 min	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
	1 hour	Single-molecule tracking and super-resolution imaging in 3D using light sheet illumination and microfluidics	Anna Karin Gustavsson
June 12th (Lab)	2 hours	Super-Resolution Lab (SIM, STORM & STED)	Adriana Paulucci and Travis Moore
June 17th	1 hour	Multiphoton and FLIM	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	Leoncio Vergara

		Students will analyze images from previous lab	
Jun 26th	1 hour	High Content Imaging and Analysis	Reid Powell
Jun 26th	1 hour	**Breakout:** image analysis using open-source	Reid Powell
Jun 30th	1 hour	**Breakout:** Live Image Analysis	Travis Moore and Adriana Paulucci
Jun 30th	1 hour	**Breakout:** Students present data analysis from labs	
July 3rd	2 hours	Students Projects Presentations	Adriana Paulucci and Travis Moore

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