Biology 542

# Syllabus

# Light Microscopy for Biology & the Biomedical Sciences

Bovine pulmonary artery endothelial cell – Structured Illumination Microscopy – Talley Lambert, Harvard Medical School

# Welcome to Biology 542 - Light Microscopy for Biology & the Biomedical Sciences

Instructors	Professor Nathanaël (Nat) Prunet (he/him) – <u>nprunet@unc.edu</u>	
	Professor Paul Maddox (he/him) – <u>pmaddox@unc.edu</u>	
	Teaching assistant: Grace McLaughlin (she/her) – gamclaug@live.unc.edu	
Class schedule	Tuesday & Thursday, 3:30 – 4:45 pm	
Lecture	Genome Sciences Building 1374	
Lab	Thursday, 3:30 – 4:45 pm, Wilson Hall 130	
Office hours	Nat: Wednesday, 10 am – 12 pm, Genome Sciences Building 1120	
	Grace: Tuesday, 5 – 7 pm, Genome Sciences Building 1152	
Course website	https://uncch.instructure.com/courses/36504	

# Course description

Richard Feynman, a theoretical physicist and Nobel laureate, famously said: "It is very easy to answer many of these fundamental biological questions; you just look at the thing!". As biologists, we have daily reasons to scoff at that statement; however, "looking at the thing" is undeniably a powerful way to try to understand biological phenomena. Over the last two decades, light microscopy has seen an explosion of new techniques and provided us with invaluable tools for biological research. In this class, we will cover light microscopy techniques currently used in research laboratories. Lectures will cover the basics of light microscopy

(image formation, magnification, resolution, contrast), widefield and fluorescence microscopy, optical sectioning (confocal, multi-photon, light-sheet and TIRF) and super-resolution microscopy. Lab sections will include hands-on time on a simple, rail-based microscope; and on research microscopes (brightfield/DIC/epifluorescence, confocal and super-resolution); and quantitative image analysis on ImageJ.



Rail microscope, with all the components of an infinity-corrected compound microscope aligned on a rail

### Learning outcomes

This course is designed to give you, as biologists, an understanding of light microscopy techniques currently used in research laboratories, from basic to cutting-edge: how they work, what they can do, and their limitations. You will gain a theoretical and practical knowledge of light microscopy that will help you understand biological research and provide you with valuable skills that you can use in biological research laboratories.

#### Course materials

Materials that we will discuss in class will be posted on the website.

Textbook: *Fundamentals of Light Microscopy and Electronic Imaging*, Douglas Murphy, Wiley-Liss, 2001 (available on course website).

# Grading

You will be evaluated on multiple, lower-stake lab assignments and exams, which gives you more paths to success. Your final grade will be calculated from the following:

Midterm	25%
Weekly assignments (10)	50%
Independent project	25%

We do not grade on a curve, to foster maximum interaction and collaboration between students. However, we may lower the cutoffs for letter grades to ensure enough students get As and Bs.

# Midterm

- The midterm will focus on material and concepts covered in lectures.
- The midterm will be posted on Gradescope on Friday 09/22 at 12 pm
- You will have 24h to return your exam but a 2h time limit to take it.
- You will have access to your notes during the exam. However, it is important that you study the course materials before the exam.

# Weekly assignments

- You will have 10 assignments.
- Assignments will be posted weekly on Gradescope on Wednesday at 4pm.
- You will have a week to return your assignment.

# Attendance and participation

- We would like you to be intellectually engaged in the learning process.
- We encourage you to attend, and participate in lectures as much as possible. Lectures will be recorded to give you flexibility to study at your own rhythm.
- You are required to attend labs. Labs will not be recorded.



Zeiss LSM 880 confocal microscope

- University Policy: As stated in the University's <u>Class Attendance Policy</u>, no right or privilege exists that permits a student to be absent from any class meetings, except for these University Approved Absences:
  - Authorized University activities: <u>University Approved Absence Office (UAAO)</u> <u>website</u> provides information and <u>FAQs for students</u> and <u>FAQs for faculty</u> related to University Approved Absences
  - 2. Disability/religious observance/pregnancy, as required by law and approved by <u>Accessibility Resources and Service</u> and/or the <u>Equal Opportunity and Compliance</u> <u>Office</u> (EOC)
  - 3. Significant health condition and/or personal/family emergency as approved by the <u>Office of the Dean of Students</u>, <u>Gender Violence Service Coordinators</u>, and/or the <u>Equal Opportunity and Compliance Office</u> (EOC).

Instructors may work with students to meet attendance needs that do not fall within University approved absences. For situations when an absence is not University

approved (e.g., a job interview, illness/ flu or club activity), instructors are encouraged to work directly with students to determine the best approach to missed classes and make-up assessment and assignments.

# Temporary schedule

Date	Weekday	Торіс	Room	Instructor			
Aug 22	Tuesday	Introduction	GSB 1374	Prunet			
Aug 24	Thursday	Reflection, Refraction, Interference	GSB 1374	Maddox			
Aug 29	Tuesday	Introduction: Discussion of life inside a cell	GSB 1374	Maddox			
Aug 31	Thursday	Digital Imaging 1	GSB 1374	Maddox			
	Reading assignment for Sept 10: Fundamentals of light microscopy and electronic imaging						
	pages 1-13 (I	Basic illuminating light paths, conjugate image planes) a	nd pages 15	-50			
	(Geometrical optics and basic imaging light paths)						
Sept 5	Tuesday	Image formation and magnification	GSB 1374	Prunet			
Sept 7	Thursday	Conjugate image planes and Infinity Corrected optics	GSB 1374	Prunet			
Sept 12	Tuesday	Wave optics and phase microscopy	GSB 1374	Maddox			
	Reading assig	gnment for Sept 12: <u>https://www.microscopyu.com/tut</u>	orials/kohle	<u>r</u>			
	https://www	microscopyu.com/microscopybasics/components	1				
Sept 14	Thursday	Polarization and DIC	GSB 1374	Maddox			
	Reading assig	gnment for Sept 17: <i>Fundamentals of light microscopy</i> a	nd electron	ic imaging			
	pages 117-13	33 (Polarized light interaction with matter)					
Sept 19	Tuesday	Introduction to fluorescent probes, their fundamental	GSB 1374	Prunet			
		properties and how they can be used as bio-sensors					
Sept 21	Thursday	Applications of Light microscopy	GSB 1374	Prunet,			
				Maddox			
		Take home exam 1					
Sept 26	Tuesday	Lab 1 - Cell Phone Microscopy		Maddox			
Sept 28	Thursday	Lab 2 - Lenses, Focal Length, and Creating an Image		Maddox			
	Reading assignment for Oct 1: Resolution and point spread function (A beginner's guide to						
	rigor and rep	producibility in fluorescence imaging experiments by Lee	e et al)				
0 et 2	nttps://www	/.molbioicell.org/doi/10.1091/mbc.E17-05-0276		Madday			
Oct 3	Tuesday	Lab 3 - Ray Diagramming and Multi-Lens Systems		Maddox			
Oct 5	Thursday	Lab 4 - Calibrating Wagnification		Maddox			
Oct 10	Tuesday	Canford Lucasing		Maddox			
Oct 12	Tuesday	Confocal Imaging		Maddox			
0017	Tuesday	Lab 6 - Darkneid Microscopy		IVIAUUUX			
Oct 10	Thursday	Widefield vs Confecal comparison, Pesolution and		Maddox			
00119	mursuay	Point spread function: A beginner's guide to rigor and		IVIAUUUX			
		reproducibility in fluorescence imaging experiments					
Oct 24	Tuesday	Getting quantitative information from photon		Maddox			
00027		counting to digital images and digital image processing					
		(259-281)					

	Reading assi microscope	gnment: Intro. Video and Digital Cameras, digital images control: A Multi-Mode Digital Imaging Microscope (Meta	, digital image acquisition, Morph and Image J; 236-
	238; 260-26	7; Image J Instructions)	
Oct 26	Thursday	Practical Applications, counting molecules, nanometer localization accuracy in wide-field	Maddox
		Reading assignment: Point Scanning Confocal and Multi-photon Microscopy: Basic concepts and practical aspects (205-231)	
Oct 31	Tuesday	Advanced confocal techniques; <mark>FCS, FLIM,</mark> FRET, FRAP. TIRF, Light Sheet: 1	Maddox
Nov 2	Thursday	Advanced confocal techniques; FCS, FLIM, FRET, FRAP, TIRF, Light Sheet: 2	Maddox
Nov 6	Tuesday	Intro. Super-Resolution Microscopy: <mark>SHREC</mark> , <mark>SIM,</mark> PALM, STORM	Prunet
	Reading assi	gnment for Nov 12: Getting quantitative information fro	m photon counting to
	digital image	es and digital image processing (259-281)	
Nov 8	Thursday	<mark>3-d deconvolution</mark>	Maddox
Nov 13	Tuesday	Examples of Single Molecule Imaging of protein function with TIRF; Examples of FRET BioSensors: phosphorylation, tension	Maddox
Nov 15	Thursday	Cell based screening for identifying functional proteins	Maddox
Nov 20	Tuesday	Real world examples: single molecule imaging for protein function using TIRF; FRET biosensors for measuring phosphorylation, tension, etc	Maddox
Nov 22	Thursday	Future Developments in Light Microscopy	Maddox
Nov 27	Tuesday	Review session; Final Exam start	
Dec	Thursday	Final Exam (Take Home) due (Noon)	

# We are here to help you succeed!

- We are committed to work with you to ensure your success. Our goal as instructors is to help you understand the material and think about how the concepts and methods are applied in the real world.
- Ask questions. Please feel free to interrupt us to ask questions. The spontaneous discussions that sometimes occur as a result are very useful for mastering various topics.
- **Student hours.** Please take advantage of student hours. We also welcome you to email us to ask questions or make an appointment outside of student hours.

• Feedback. We encourage your feedback at any time throughout the quarter about things that are helping you learn, or things that aren't helping. Please communicate with us if there are ways that we can improve the course to better support student learning.

## Syllabus Changes

The instructor reserves the right to make changes to the syllabus including project due dates and test dates. These changes will be announced as early as possible.



Drosophila ring neurons – Airyscan – Nat Prunet

### Honor Code

All students are expected to follow the guidelines of the UNC Honor Code. In particular, students are expected to refrain from "lying, cheating, or stealing" in the academic context. If you are unsure about which actions violate the Honor Code, please see me or consult studentconduct.unc.edu.

# Acceptable Use Policy

By attending the University of North Carolina at Chapel Hill, you agree to abide by the University of North Carolina at Chapel Hill policies related to the acceptable use of IT systems and services. The Acceptable Use Policy (AUP) sets the expectation that you will use the University's technology resources responsibly, consistent with the University's mission. In the context of a class, it's quite likely you will participate in online activities that could include personal information about you or your peers, and the AUP addresses your obligations to protect the privacy of class participants. In addition, the AUP addresses matters of others' intellectual property, including copyright. These are only a couple of typical examples, so you should consult the full Information Technology Acceptable Use Policy, which covers topics related to using digital resources, such as privacy, confidentiality, and intellectual property. Additionally, consult the <u>Safe Computing at UNC</u> website for information about data security policies, updates, and tips on keeping your identity, information, and devices safe.

# Accessibility Resources and Service

<u>Accessibility Resources and Service</u> (ARS – <u>ars@unc.edu</u>) receives requests for accommodations, and through the Student and Applicant Accommodations Policy determines eligibility and identifies reasonable accommodations for students with disabilities and/or chronic medical conditions to mitigate or remove the barriers experienced in accessing University courses, programs and activities.

ARS also offers its Testing Center resources to students and instructors to facilitate the implementation of testing accommodations.

Faculty and instructors with any concerns or questions about accommodations and/or their implementation, are invited to <u>reach out to ARS</u> to discuss.

#### Counseling and Psychological Services

UNC-Chapel Hill is strongly committed to addressing the mental health needs of a diverse student body. The <u>Heels Care Network</u> website is a place to access the many mental health resources at Carolina. CAPS is the primary mental health provider for students, offering timely access to consultation and connection to clinically appropriate services. Go to their website <u>https://caps.unc.edu/</u> or visit their facilities on the third floor of the Campus Health building for an initial evaluation to learn more. Students can also call CAPS 24/7 at 919-966-3658 for immediate assistance.

### Title IX and Related Resources

Any student who is impacted by discrimination, harassment, interpersonal (relationship) violence, sexual violence, sexual exploitation, or stalking is encouraged to seek resources on campus or in the community. Reports can be made online to the EOC at <a href="https://eoc.unc.edu/report-an-incident/">https://eoc.unc.edu/report-an-incident/</a> or by contacting the University's Title IX Coordinator (Elizabeth Hall, <a href="https://eoc.unc.edu/report-an-incident/">titleixcoordinator@unc.edu</a>) or the Report and Response Coordinators in the Equal Opportunity and Compliance Office (<a href="reportandresponse@unc.edu">reportandresponse@unc.edu</a>). Confidential resources include Counseling and Psychological Services and the Gender Violence Services Coordinators (<a href="gysc@unc.edu">gysc@unc.edu</a>). Additional resources are available at <a href="mailto:safe.unc.edu">safe.unc.edu</a>.

# Policy on Non-Discrimination

The University is committed to providing an inclusive and welcoming environment for all members of our community and to ensuring that educational and employment decisions are based on individuals' abilities and qualifications. Consistent with this principle and applicable laws, the University's <u>Policy Statement on Non-Discrimination</u> offers access to its educational programs and activities as well as employment terms and conditions without respect to race, color, gender, national origin, age, religion, genetic information, disability, veteran's status, sexual orientation, gender identity or gender expression. Such a policy ensures that only relevant factors are considered, and that equitable and consistent standards of conduct and performance are applied.

If you are experiencing harassment or discrimination, you can seek assistance and file a report through the Report and Response Coordinators (email <u>reportandresponse@unc.edu</u> or see additional contact info at <u>safe.unc.edu</u>) or the Equal Opportunity and Compliance Office at <u>https://eoc.unc.edu/report-an-incident/</u>.

#### Undergraduate Testing Center

The College of Arts and Sciences provides a secure, proctored environment in which exams can be taken. The center works with instructors to proctor exams for their undergraduate students who are not registered with ARS and who do not need testing accommodations as provided by ARS. In other words, the Center provides a proctored testing environment for students who are unable to take an exam at the normally scheduled time (with pre-arrangement by your instructor). For more information, visit <u>http://testingcenter.web.unc.edu/</u>.

## Learning Center

Want to get the most out of this course or others this semester? Visit UNC's Learning Center at <u>http://learningcenter.unc.edu</u> to make an appointment or register for an event. Their free, popular programs will help you optimize your academic performance. Try academic coaching, peer tutoring, STEM support, ADHD/LD services, workshops and study camps, or review tips and tools available on the website.

### Writing Center

For free feedback on any course writing projects, check out UNC's Writing Center. Writing Center coaches can assist with any writing project, including multimedia projects and application essays, at any stage of the writing process. You don't even need a draft to come visit. To schedule a 45-minute appointment, review quick tips, or request written feedback online, visit http://writingcenter.unc.edu.

#### Guidelines for Generative AI

Generative AI is extremely useful; however, it has the following limitations:

- How output is arrived at is not clear as the internal processes used to produce a particular output within the generative AI cannot be determined.
- The output is based on existing data (often scraped from online sources) and may reflect biases that should be acknowledged; it may also be inaccurate or entirely fabricated, even if it appears reliable or factual.
- Al evokes a range of intellectual property concerns; sourcing and ownership of information is unclear, and the status of Al output raises numerous questions—e.g., is output equivalent to a published resource? What citational responsibilities are in place for various Al interactions?

The following sections provide the philosophy and specific guidelines for using these tools and features (increasingly, generative AI capabilities will be integrated with everyday applications). Unless we provide other guidelines for an assignment or exam, you should follow these guidelines.

Use of generative AI in your coursework is based on the following principles:

1. Al should help you think. Not think for you.

Use these tools to give you ideas, perform research (in compliance with point 2 below), and analyze problems. Do not use them to do your work for you, e.g., do not enter an assignment question into ChatGPT and copy & paste the response as your answer.

2. Engage with AI Responsibly and Ethically: Engage with AI technologies responsibly, critically evaluating AI-generated outputs and considering potential biases, limitations, and ethical implications in your analysis and discussions. Utilize AI technologies ethically, respecting privacy, confidentiality, and intellectual property rights. Ensure that the data used for AI applications is obtained and shared responsibly and in compliance with relevant regulations.

#### 3. You are 100% responsible for your final product.

You are the user. If the AI makes a mistake, and you use it, it's your mistake. If you don't know whether a statement about any item in the output is true, then your responsibility is to research it. If you cannot verify it as factual, you should delete it. You hold full responsibility for AI-generated content as if you had produced the materials yourself. This means ideas must be attributed, facts are true, and sources must be verified.

- 4. The use of Al must be open and documented. The use of any Al in the creation of your work must be declared in your submission and explained. Details on how to source your Al usage are explained below.
- 5. These guidelines are in effect unless we give you specific guidelines for an assignment or exam. It is your responsibility to ensure you are following the correct guidelines.
- 6. Data that are confidential or personal should not be entered into generative AI tools. Putting confidential or personal data (e.g., your One Card details) into these tools exposes you and others to the loss of important information. Therefore, do not do so.

The following sections provide the philosophy and specific guidelines for using these tools and features (increasingly, generative AI capabilities will be integrated with everyday applications). Unless we provide other guidelines for an assignment or exam, you should follow these guidelines.

#### Guideline Specifics

#### Not following these guidelines may be a reportable violation to the UNC Honor Court.

- Writing and Presentation: In principle, you may submit material that contains Algenerated content, or is based on or derived from it, if this use is properly documented. This may include drafting an outline, preparing individual sections, combining elements, removing redundant parts, and compiling and annotating references. Your documentation must make the process transparent – the submission itself must meet the relevant standards of attribution and validation.
- Multimedia Assignments: In principle, you may submit material that contains Algenerated content, or is based on or derived from it, if this use is properly documented. This may include the generation of images, audio, music, video, etc. Your documentation must make the process transparent – the submission itself must meet the relevant standards of attribution and validation.
- Mathematical and Statistical Analysis, Data Analysis, Data Interpretation, Coding of Data, generalizing data to a problem set or any other forms of quantification of language or concepts, etc.: Generative AI can be used for these purposes; however, the output must be verified via your own mathematical calculations and proof of work provided in your assignment.
- Readings and Discussions: Generative AI can be used to analyze readings. However, you must also do the readings. Generative AI analysis is not a substitute for reading the

works themselves. Similarly, participating in online discussions of readings requires that you provide your own contributions. Unless I specifically allow it, do not generate responses to readings using AI.

- Research: If you use AI to support your research, you must account for and document your use. Possibilities include topic brainstorming, search assistance, source evaluation, and summaries and source documentation. Track your use of AI throughout these stages, and then document this assistance as you submit the project. Any material generated through AI in your projects should also be documented in your citations.
- Simulations: In principle, you may use AI tools for advice or brainstorming. It should not, however, be used to find cheats or other unfair advantages. If a report is part of the assignment, your documentation of how you used AI in completing the simulation must make the process transparent.
- Group Work: Group work guidelines are based on the type of assignment above (e.g., a group written assignment will use the guidelines for written assignments).
- In-Class Activities: Instructions on the appropriate use of AI for in-class activities will be provided by me.
- Written & Oral Exams: Unless I explicitly grant permission, the utilization of AI tools is prohibited and could potentially constitute a reportable violation to the UNC Honor Court. If the use of AI tools is explicitly permitted, you are required to adhere to the guidelines concerning AI citation, verification, and clarity as outlined below.

#### Sourcing Use of Al

- Accuracy: Generative AI may invent both facts and sources for those facts. Verification is your responsibility, whether the source of the error is you or the AI makes no difference. You need to check the facts, the quotes, the arguments, and the logic, and document what you did to validate your material.
- Attribution: All ideas that are not originally your own have a source and that source must be attributed. Please be aware that generative AI tends to invent sources. You have a two-fold obligation with respect to attribution:
  - (1) If a source is identified, find and attribute the original source of the idea, identify the location of the text within the source, and provide a working link to the location (if the source is available online). If you are not able to locate the source, delete that content.
  - (2) Document the process by explaining how you used generative AI in a work statement that will accompany your submission of major projects in the class. As you submit a project, develop, and include an appropriate version of the below statements:

"I attest that this project did not use AI at any stage in its development or in the creation of any of its components."

"I attest that this project made use of AI in the following ways:" You must then use the following form to document your usage (note that such attribution is not a valid source for facts, only for the output itself).