THORLABS

Optical Microscopy Course

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Based on the Course at

UC Berkeley

Instructor Notes

Optical Microscopy Course

Instructor Notes Preface

This kit grew out of our desire to make the course materials and equipment for our Berkeley class easily accessible. We hope these materials make it easier for you to provide hands-on optics and microscopy instruction to your own students. Running any lab class over many years requires a fair bit of organization. In these Instructor Notes, we have collected our hardware setup checklists, notes for new lab instructors (at Berkeley these are typically graduate student instructors), and our weekly notes on things that help make the labs run smoothly. Some comments on the nature of the class:

Course Structure

For course instructors – the faculty in charge of the class – there are a number of decisions which can be made. We designed the class to have 10 labs, suitable for a 10-week academic quarter. For 14-week semester-based systems (as at Berkeley), this allows us to allocate one lab session to a midterm with a practical (hands-on) exam, and to have three lab sessions for student projects at the end of the semester. Each week there is a 3-hour lab and 2 hours of lecture.

The labs are designed to work in sequence as a full set, and we have taught them as such for many years. However, for those interested in exploring other possibilities, there are natural divisions in the labs:

- Labs 1 - 4 involve the basic setup of a microscope system, and some investigation of digital imaging, resolution, some aberrations, and illumination.
- Labs 5 - 8 involve the Abbe theory of image formation, building from Köhler illumination through darkfield imaging to phase contrast, and covering the Modulation Transfer Function.
- Labs 9 - 10 cover fluorescence imaging in a transillumination configuration. Lab 9 covers the basics of fluorescence imaging, while Lab 10 and its associated problem set are focused on spectra (lab) and quantitative filter selection (problem set).

Faculty can use the equipment for a variety of purposes beyond the 10-lab sequence. For those doing so, Lab 5 (Köhler, conjugate planes, and darkfield), Lab 6 (Abbe theory), and Lab 9 (Fluorescence) are particularly conceptually rich. However, we did not design the labs to be stand-alone, and using them in such a fashion will require significant effort/involvement on the part of the faculty.

Lab Preparation

The key to success for the labs (and class) is for the lab instructors to work through the lab on their own well ahead of time (ideally a day or two prior to the student lab session). This prepares the lab instructors to be ready for (and often to anticipate) student difficulties and questions. The labs are reasonable in the time allotted, but there are many potential pitfalls, and it is very difficult to efficiently answer student questions if one has not had one's own hands on the equipment recently. In the case of graduate student instructors (teaching assistants), who may be somewhat unfamiliar with the entirety of the theory, having worked through all the lab steps (including at least some of the data reduction) is especially important.

Target Students

We designed this course for students doing biological microscopy, although the physical concepts involved cover far more. Because such students – even graduate students – often have no upper-division optics (or, for biology students, any upper division physics), we aimed the class at students who have had only a good lower-division physics class including optics and waves (as most lower-division physics

sequences do.) Years of teaching at Berkeley have demonstrated that such students are more than capable of doing excellently with the material. However, review of critical concepts in lecture is very important.

Lectures

We have not tried to provide lecture materials. This is in large part because, while the labs are well setup to run with varying levels of student preparation, the level of the theory portion of the class can vary widely with the students enrolling for the class at a given institution. We have taught this material to many audiences: undergraduates with no upper-division physics experience, upper-division undergraduate students, graduate students, and post-doctoral scholars (sometimes simultaneously) without anyone getting either overwhelmed or bored. However, the nature of the lectures naturally varied. Individual instructors can decide how they wish to handle the lecture component of their class; we do suggest approximately 2 hours of lecture per week.

Reading Quizzes: We find the best way to have students be ready for lab is to give a short reading quiz during lecture (or quickly at the start of lab) covering the theory and details of the upcoming lab. Questions are designed to be quick if the students have read the material.

Exam Practicals

We typically have two exams, both with a written portion and a 10-15 minute (per student) practical examination where students must demonstrate their knowledge of microscopy using the hardware. For the midterm, this usually involves being able to properly set up Köhler illumination and properly adjust cameras and apertures for good resolution, while on the final questions are a bit more creative. The practical exams (especially the midterm, coming early in the class), serve nicely to focus students on really mastering the equipment, instead of just working through lists of steps during a lab. For this reason, we highly recommend having practical portions to the exams, despite the logistical overhead involved. Suggestions for making them successful are included in the Appendices to these Notes.

These Instructor Notes fall into two categories:

1. Lab Instructor Notes. These involve documents summarizing things needed to make each week's lab run smoothly.
2. Appendices with setup and training documents.

We hope these are useful; please suggest any changes for the documents that would be beneficial to others so we can improve them (and especially let us know of any errors). Many thanks!

Neil Switz and Dan Fletcher

Berkeley, CA

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THORLABS

Lab 1 Notes: Introduction to Optical Imaging (I)

Optical Microscopy Course

Lab 1 Instructor Notes:
Introduction to Optical Imaging (I)

Before You Start

It will be very helpful if you have built an entire rig, using the EDU-OMC1(/M) Manual and the construction and alignment videos in the *Videos* tab at www.thorlabs.com/OMC. This will give you a decent sense of what is coming, and allow you to assist students better.

If you do not have time for that, then there is simply no substitute for having run through the entire lab ahead of time – a day or two before – so that you have likely encountered everything the students will.

General Items

Things to cover in the first class / lab section:

- **We spend time in the first class explaining neat stuff they will be doing, in order to build excitement.**
- Basic Administration: Where the course notes can be found online, etc.
    - You should **bring copies of the Lab Notes for the first day (1 copy for each student)**; students will very likely not have them yet.
    - Students will need to choose lab partners, if these are not assigned by the instructor.
    - Remind students that there is a template lab report they can work from (in Appendix A of the Lab Notes). We find this helps enormously in ensuring decent quality in student reports.
    - **Explain the due date, submission location, and naming convention you want the students to use for their lab reports.**
- Cost Warning: We explain to students that the spectrometers cost $3000; the cameras $375 each; lenses are often $100 each, and the test targets we use are $900 each, so it is very important for them to be careful with these. Tell them it is always best to ASK if there is any confusion before doing something.
    - **Emphasize that if critical parts get broken, that may be one less lab group we can have in the course next year. It really matters!**

Lab Preparations

- Check your room lights. Fluorescent ones have good spectral lines, which are important since they match the lines from Hg Arc lamps often used (though increasingly superseded by lasers and LEDs) in microscopy.
- Make sure all cameras are set correctly (see Appendix B: Preparing for the Course).
- In advance, make sure you have:
    - LEDs and USB power supply
        - Our IR LEDs are 940 nm or so. This is still detected well by the mono camera, but far beyond the IR cutoff filter (usually ~ 650 nm) for the color camera.
    - The focal-length-test lenses used in the lab – LA1765 with f = 75 mm, and LA1031 with f = 100 mm.
        - **DO NOT label the focal lengths of these, or tell them to the students!**
    - USB cables for cameras.
    - If you do not have fluorescent room lights, bring a fluorescent light into the classroom for the students to take a spectrum of.

Example Quiz 1 Questions

In our course at UC Berkeley, we give this quiz, trade and grade, then tell students we are not recording the scores this (first!) week.

1. Which direction should the camera be facing when you take the cap on or off?
   - Facing down, to keep dust/crud from the threads from falling onto the camera face.
2. What is the name of the free image-analysis software should you have installed on your laptop?
   - ImageJ
3. What is the first thing you do before beginning to handle optics?
   - Put on clean gloves.
4. What will you name your lab report?
   - Lab 1, Firstname Lastname
5. This week's lab notes had guides to the features of several software programs you will be using. Name two of them (listing the thing they do is fine – do not need the exact name).
   - ImageJ, Camera, Spectrometer

Lab Tasks

Main Things to Emphasize:

- What the image plane is.
- That cameras only detect light intensity at some spot – all you see is what you would see if you held paper at that spot. A lens is required to put an image on the detector.
- Light has a spectrum; draw the link to colors you can see (and note that some cameras can detect colors you cannot see…one needs to know the details of one's camera!)

General:

- Have students put their names on the desktop folder shortcuts so you know which is which.
- You will need to help them figure out their camera settings.
  - Especially the line-profile window; emphasize this so they start to understand what it is, and what it means to saturate the camera / why that matters (one no longer knows how bright something is if it has hit the maximum level the pixel can handle).
  - Students may loath to expose the camera to room light, being worried it might get damaged. Explain that this is a good concern, but applies only to intensified cameras, ones with electron gain (EM gain), or photomultiplier tubes (PMTs), and not to usual inexpensive CCD or CMOS cameras like these.
- Kimwipes, especially with a ragged edge, work better than some lens paper for imaging on the camera surface.
  - You need to use the (quite directional) LED light to cast a good shadow onto the camera surface; if you illuminate from too many directions (e.g. using diffuse room light) the edge will not be sharp, since the shadows from each illumination angle will fall in different places on the camera surface. There is no need to get into this level of detail unless some group asks (and then respond to them, not everyone). This is only an issue due to the thick (~3 mm+) cover glass on the image sensor.

- Imaging:
  - If your classroom has very diffuse ceiling lighting, provide another source of light for the students to image with the lenses.
    - Exit signs, etc. work well, as do recessed ceiling lights, LED lights, etc. It is important to test this before the class – different lighting will yield different results. The important thing is for the students to have some sharp light/dark feature in their image on which to focus, so they can see it on the table, then put the image onto the camera sensor and see it displayed on their screen. They should see more detail (and less total area of the light fixture) on the camera image than when they look at it on the desk, since the pixels are very close together on the camera (~5.2 μm spacing), a distance hard for your eye to resolve from a foot or more away – resolution of the eye under normal room lighting will be in the ballpark of ~100 μm from 30 cm distance).
  - Emphasize the digital magnification (not optical magnification) that is happening: the 5.2 μm spaced pixels are displayed on much larger pixels (our displays have ~100 μm pixels or so) on the computer monitor, or, if the image is resized, possibly displayed over multiple pixels.
- Lens Focal Length:
  - Write the lens law $\frac{1}{f} = \frac{1}{S_i} + \frac{1}{S_o}$ on the board and mention it before starting this section.
  - Get students to image with the lens directly under a ceiling light. Being off to one side makes everything harder to measure.
  - Smarter groups will image onto the floor, to get a longer (object) distance to the lights.
  - Make sure students understand that the error in their focal length measurement is NOT the difference between what they measure for S_i from the lights and what it would be if S_o were infinity. They should know their uncertainty in distance measurements (say, 10 ± 1 cm for BOTH S_i and S_o) and then figure the actual error accordingly, e.g. by putting worst-case (maximum error, plus or minus) values into the lens equation for S_i and S_o and seeing what the variation in calculated focal length can be. This is especially easy for students to do using a spreadsheet, a skill we emphasize in class.
    - NOTE: we very purposefully **<u>do not</u>** get into derivatives and formal propagation of errors / adding errors in quadrature. The emphasis in this course is on quick/approximate techniques; most students (and most engineers) do not go to calculus first to get a rough idea of the error in this situation; rather, we have them calculate the focal length for all combinations of plus/minus errors on S_i and S_o using Excel. For reference, however, the standard propagation-of-errors way to do it is:
      - Thin lens equation: $\frac{1}{f} = \frac{1}{S_i} + \frac{1}{S_o}$, so $\boldsymbol{f = \frac{S_o S_i}{S_0 + S_i}}$ (and the expected value of f is given by plugging one's measured values into this equation).
      - From this, $\frac{\partial f}{\partial S_o} = \frac{S_i}{S_o+S_i} - \frac{S_o S_i}{(S_o+S_i)^2} = \left[\frac{S_i}{S_o+S_i}\right]^2$ and similarly $\frac{\partial f}{\partial S_i} = \left[\frac{S_o}{S_o+S_i}\right]^2$
      - The error in f due to the uncertainty in $\boldsymbol{S_o}$, $\boldsymbol{\Delta S_o}$ is then $\Delta f \cong \frac{\partial f}{\partial S_o} \Delta \boldsymbol{S_o}$, where $\frac{\partial f}{\partial S_o}$ is evaluated using the measured values of S_i and S_0, and $\boldsymbol{\Delta S_o}$ is the estimated error in that measurement. A similar relation holds for the Δf due to the error ΔS_i.

- These errors are presumed to be statistically independent, and so add in quadrature, giving a final total error $\Delta f \cong \sqrt{\left(\frac{\partial f}{\partial S_o}\,\Delta S_o\right)^2 + \left(\frac{\partial f}{\partial S_i}\,\Delta S_i\right)^2}$

- IR LED Test on Cameras: For more advanced students (or all of them if you have time) have them calculate the ratio of exposure times between the color and mono cameras; usually the color one barely shows anything, while the mono one saturates even at low exposure.
  - On the Thorlabs DCC1645C color camera, the IR LED usually shows up as blue-ish. The reason for this is that the filters over the blue pixels appear to leak more in the IR than the red and green ones. In general, it is not obvious which filters would leak worst in the IR. It is worth explaining (to students who ask) re: Bayer filter arrays, and that each pixel has a separate filter (as opposed to each pixel detecting all three colors). That means that the RGB color values seen for each pixel are not real, but interpolated ("demosaicing" is the term; you can refer students to Wikipedia). This is discussed in more detail in Lab 10 on optical filters.
- Spectra:

  Do the IR LED portion of this even if you do not have a spectrometer – it will help the students understand the IR filters on the color camera (and later, in the illumination path).

  Assuming you use the spectrometer,

  - If you do not have fluorescent room lights, bring a fluorescent light into the classroom for the students to take a spectrum of.
  - Draw links for the students about the following:
    - Fluorescent lights have a spectrum similar to mercury (Hg) arc lamps used for fluorescence microscopy.
    - Depending on your computer monitors (i.e., if they are old fluorescent-backlit ones, not newer LED-backlit ones) when students look at the spectra from the computer screen they should be able to note peaks similar to the room lights. They should be able to infer (help them think about it) that this implies the backlight for the LCD monitor is also a Hg-based fluorescent light.
    - Most white LEDs, e.g. in an LED flashlight, consist of a blue (usually 460 nm) LED exciting fluorescent material coated above it, so you get multiple wavelengths and the output looks "white."
      - You can note the Stokes shift of the fluorescence from excitation peak (usually 460 nm) to the emission peak (spectral bump at longer wavelengths); comment to the students that we will discuss that more later (in Lab 9 of fluorescence).
    - The halogen lamp also looks "white," but help students notice that it has much more red/IR than the LED flashlight.
  - Be sure they save all their spectra for use in the lab report!
    - It is OK for them to simply paste the images into Word, or whatever.
    - Have them save at least one into a spreadsheet (e.g. Excel), so they can plot the data.

Labs 2 through 10 have been omitted from the online version of the Instructor Notes. The full version is included on the USB stick shipped with the EDU-OMC1(/M) kit.

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Appendix A: Overview / Preparation for Lab or Student Instructors

Optical Microscopy Course

Appendix A Instructor Notes:
Overview / Preparation for Lab or Student Instructors

This is mainly a lab course, and its success depends entirely on the knowledge and efforts of the instructor (and especially any student instructors) teaching it. A number of students have said this is one of the best courses they have taken; not all courses generate that kind of feedback, so this can be a fun opportunity for the student instructors – it is certainly more enjoyable to teach students who are engaged and interested.

The tips below are ones we have found to be useful for making the course run smoothly, and also some that make the student instructors more effective in the context of this course. **We strongly encourage both instructors and student instructors to review them prior to the start of the course.**

General Preparation

- Having read the notes well ahead of time, being in lecture, and (perhaps most of all) **working through the labs beforehand** makes a major difference. If you do not understand something fully, chances are a student will not either, and someone will ask you about it. Better to figure it out or ask someone ahead of time than to get confused during the lab section. You need to be up on the Course Notes and Lab Notes so you can answer questions. **It is very bad form to not remember equations the students themselves are being quizzed on**, etc. Worse is to give erroneous answers to questions because you yourself are unprepared.
    - Work through the lab yourself, doing all steps, a day or two before your section.
        - This is Critical: You will be MUCH better able to answer questions if you have done it all yourself, recently (even if you taught it last year… we know!)
        - It really is critical to do all steps: often the devil is in some detail (ImageJ has trouble with a file format, etc.) and it can be very hard (and embarrassing) to have to sort that out during class.
        - Use the exact same equipment the students will use; often results vary significantly if you use a slightly different green filter, or different length focus tube, etc.
        - Make notes as to where there are changes to (or errors in) the lab notes, or where students may have trouble or equipment may be damaged
            - **Address these with students at the start of lab section.**
        - Be ready to step in to help students at points where they may struggle, and to help them use the equipment without damage for those sections where it may be at risk (e.g., changing lenses). You will be ready for this if you have just recently done the lab yourself – you will have struggled at many of the same spots, etc.
- **Please keep a list of items that need fixing (with reference to which lab/step/document the issue comes from) so that you can repair them (or report any typos on the *Feedback* tab at www.thorlabs.com/OMC).**
- When working with students, it is far, far **better to say you are unsure, and then answer later** (by email, or in the next lab section) than to give a wrong or overly confusing answer. There is no

shame in this – we do it regularly, and we designed the class. Students are always able to push your knowledge, and even things you derived yourself may have slipped your mind 5 weeks later. Students appreciate it when you are honest with them, as long as you do come back and answer the question later.

- Make a big effort to **learn the students' names early on**. We make a cheat sheet with the names of each set of lab partners laid out the same way they are in the room, and keep it handy on a counter. Usually takes us a few weeks, but does make a big difference.

Running Lab Section

- We often start with a (short) review: We initially ask for questions, then move on to things that students were frequently confused about on the last lab report. I then wind up with comments (and maybe an example) regarding things that they may not have seen in a while but which are useful for the current lab, and issues they may run into – equipment tricks, changes in the lab steps, typo corrections, etc. **Students do not like it if this part extends past ~15 minutes.**
- **Circulate and check on all the tables/groups regularly.**
    - **This really is critical. Good instructors circulate steadily among the groups; students learn far less if you stand (or sit) waiting for them to come to you with questions.**
    - Some students/groups are less likely to ask for help, but may nonetheless be struggling. The only way to find out is to check in with them periodically
    - Do not just walk by, but spend a minute watching them to see how they are doing; this also gives them the opportunity to notice you have paused and ask you a question without having to go get you or flag you down.
    - ASK a quick "how's it going?" as you make your rounds; it will frequently elicit a question, or else reveal all is not well.
- Be aware **not all students feel comfortable asking questions** – if you pay attention to this you will quickly figure out which groups/people these are. Spending a little extra time to check in with them/engage in a bit of conversation about how it is going can make the difference in their asking a question they need help with.
    - If you find you are always talking with the same one or two lab groups (and this is easy to fall into: as they say, the squeaky wheel gets the grease), stop and readjust your approach.

      Students can often seem to understand more of what you say than they actually do (it is a frequent thing for a student to say 'yes' when you ask if they understand what you are saying, when actually they do not – this seems to be nearly universal; I find myself doing exactly the same thing when overwhelmed technically in a situation where I do not feel comfortable getting into a longer discussion). Making yourself available for a slightly longer period, so the discussion can be slower, avoiding too much jargon or idioms, and circling back to check in again after a short time all seem to help. Asking them a related follow-up question can help you determine if they are understanding things, but be careful not to make people feel as if they are put on the spot.

- Make sure all students are comfortable with the environment.
    - If one table is making raucous or inappropriate jokes, consider steering their discussion back to the lab.
    - Be aware that students take cues about a field from their instructors… Especially for student instructors, remember that you are NOT viewed as their peer, but rather an instructor. Make sure the environment is welcoming to all.
- Try not to explain "too much" – it is easy to want to explain to students the neatest extra bit/extension of some result. Our surveys of the class have consistently revealed that most students are struggling to conceptually master the very basic material in the labs. Be sure to cover that first, and if discussing refinements pay attention and notice when you are hitting the point of diminishing returns – if students lose confidence/get confused again, the additional discussion was not helpful. Less is often more in this context.
- Don't give away questions: the "concept questions" and other lab exercises are designed to lead students to understand things. If you tell them what they will see, etc., they will not struggle with them and thus will not learn the concepts in a way they can reproduce (e.g. on the exam). If students ask about something where the Lab Notes have posed a question, restate the question, and help them think through how to approach it (mention things that might contribute, or ask a leading question about what they might expect based on some prior step leading to this one), but don't give it away until they have really worked on it. We will often give some suggestions or leading questions, then tell them we will come back in a little while. We then come back in ~5 minutes to see if they are still stuck, or to go over what they now understand in order to help emphasize it (if they have it right, we are sure to emphasize that they "got it exactly" for instance, which helps build their confidence in the new knowledge).
- Remember (and emphasize) the main learning goals for the course: PSF, Abbe theory, Resolution, Contrast, Spectra – you can emphasize these as they come up in different contexts, and help the students make connections and to see the unity in the course material. In addition to running the course, you are one of the critical instructional components – at least as much as the course notes, lecture, or the labs themselves.
- Practical Exams: These are tricky – while the exams are not hard, students often feel great pressure, and in every class a couple of students choke completely. The critical trick is to catch it early if someone is panicking and give appropriate guidance (which you can deduct from their points later) so that they get moving and can demonstrate what they do know. The essential element here is helping students demonstrate what they do know – the exam is not about finding out what they do not know; help them over that. Be aware that often the students who choke are the better-prepared ones, and are not completely unprepared but rather wondering about some additional detail… some brief discussion and a suggestion to move on ("don't worry about that," etc.) can get them moving again and let you know that they were considering an unnecessary (at the level of the practical exam) refinement rather than being totally confused.

Suggested Breakdown of Time:

- This assumes a Student Instructor paid for 20 hrs/week.
- **If instructor time is more limited, the most important thing is for them to have worked through the lab ahead of time on their own.**
    - 3 hrs: doing all reading for course for week, working through any parts you do not understand.
    - **5 hrs: going through lab fully, doing parts of write-up (even making the tables, etc.) to make sure you have seen exactly what the students will.**
    - 5 hrs: lab section + office hour + prep time before lab, etc.
    - 1 hr: email support for student questions
    - 2 hrs: attending lecture
    - 3 hrs: grading
    - 1 hr: meet with professor to go over lab for the week, any parts you are unclear on.

  This totals 20 hrs/week; not all weeks will require this – it is usual, for instance, to be able to go through the lab and reading much faster than the students do, and student email support varies a lot by week. Typical weeks will be closer to 15 hours. HOWEVER: **Lack of preparation will severely impact the course: it is critical for the lab instructor to be able to do and understand ALL parts of the lab before the students walk into the room.**

We hope this is useful; please suggest any changes for the documents that would be useful to others so we can improve them (and especially let us know of any errors you catch). Many thanks!

Appendix B:
Preparing for the Course

Optical Microscopy Course

Appendix B Instructor Notes:
Preparing for the Course (Or for a New Class Offering)

This course requires a good bit of setup prior to each offering. The three main tasks are updating the computers (if your campus IT does not do that for you), checking that all parts are on hand, and replacing disposable parts. Completing these tasks is also an excellent opportunity for the instructor (or student instructor) to familiarize themselves with the equipment; we have found that – even though we designed the course ourselves – during the setup process we always re-learn things we did not know we had forgotten.

The following things are important to check each year before starting the course. Rigs can usually be fully set up in under an hour each, if parts have not been allowed to go missing. Computer updates can take substantially longer – often the better part of a day if updating a classroom full of machines.

Setting Up the Rigs

- **Before each semester, we set up and align every rig to be used for the class. This reveals any missing or damaged parts, and assures a degree of skill on the part of any instructors new to the system. It is impossible to overstate how important this step is.**
- If this is the first time this is being done, we suggest watching the videos showing assembly, Köhler illumination setup, and BFP camera alignment (as well as potentially the DCC and OSA Quickstart videos). The EDU-OMC1(/M) Manual can also be helpful. All of these are available under the *Videos* tab at www.thorlabs.com/OMC.

Beyond this point, hardware setup will be very instructor dependent:

- In some years we take everything completely apart, and have students build it all from scratch during the labs. This is a better learning experience; however it is also both time consuming and increases the likelihood of damaging the equipment (especially the AR-coated lenses).
- More typically we only partly disassemble the main components. We:
    - Leave the rail itself set up on the breadboard.
    - Change the focus settings of any lenses with adjustable mounts.
    - Leave most lenses and filters in their tubes.
        - Usually we leave a couple of absorption filters and uncoated PCX lenses (e.g. the f = 35 mm collector lens and f = 25 mm PCX objective) out so students can learn with the less expensive optics. We also show them (or have them remove and look at) a single achromat during the appropriate lab just to see what the thicker, multi-element lens looks like.
    - Before the lab where they are first used, we will typically partially disassemble and often rearrange the lens tubes in some of the components (e.g. the camera lens tubes, or the condenser) so students can rebuild and align them.

Checking Disposable Parts

We find many parts either go missing or require replacement. Check each setup and compare it to the Kit Components list in the EDU-OMC1(/M) Manual. You can choose to rebuy these items from Thorlabs or source your own supplier. Many parts can be found directly on Thorlabs' website, while others may have to be requested from techsupport@thorlabs.com.

- Thorlabs Lens Paper: One pack. We find Thorlabs lens paper really does work best, not for the obvious reason that it is a Thorlabs product, but because it has more structure and is less opaque than many other lens papers, which is useful for the steps where students image it.
- Highlighters: The "fluorescent" yellow and pink highlighters do a nice job of approximating fluorescein and rhodamine, but you must check them – not every "yellow" marker does the job, nor every pink one (the more "fluorescent"-looking ones do best). Test before using them in a class. We have found the "Stabilo® Boss Super Plus #56" works well.
- Microscope Slides: MS10UW2 microscope slides, 1 mm thick, white marking region, pack of 200. Standard slides work fine; we prefer the ones without frosted edges.
- Coverslips: CG00C2 cover glasses, #0 thickness, 22 x 22 mm, pack of 200. Standard coverslips also work fine; we suggest between #0 and #1.5. For the objectives in this kit, the difference in imaging with different coverslips (due to spherical aberration) is not particularly noticeable.
- Clear Nail Polish: No particular brand
- USB Extender Cables: USB-C-72
- Sharpie Marker: We like medium or fine tip.
- Rulers: Flexible and transparent rulers work best.
- Samples (Can Be Obtained from Thorlabs):
    - Diatom slides: "Diatom strew," a random mix, often works fine.
    - Tissue slides: If you wish to go beyond the ones supplied, try various H&E stained tissue / pathology slides.
    - Beads (fixed, also fluorescent)
    - Transparency slides / printouts of Abbe's name and image
- Plastic Sheet Color Filters: Roscolux® or similar filter booklet
- Polarizers
- LED Flashlights
- LEDs: The Thorlabs LEDs provided in the kit are handy since they are pre-wired and powered via USB. If you wish to make more of your own, we tend to use IR, red, green, and blue (around 930 nm, 630 nm, 525 nm, 470 nm, respectively). We use diodes with between 20° and 30° spread in the 5 mm / T1-¾ size. These should be wired with ~12" between the battery-connector and the LED itself, so the battery can sit on the table while the LED is mounted. This matters less if you set it up to run off a 12 V supply (if you do, use a connector that does not allow them to be connected backwards, and also adjust the resistor values to get the appropriate currents per the datasheets). The LED should be in series with an appropriate-value, current-limiting resistor (and

ideally a diode to prevent damage from reverse connections) to a 9 V battery connector. Note: the long leg of the LED goes to the "+" terminal of the battery.

- Tape: Scotch® permanent double-sided tape, and matte Scotch® Magic® adhesive tape are both very helpful.
- Kimwipes®: Lint free; makes these very useful.
- Paper: Standard copier paper – useful for tearing off bits to look at the image at different parts of the system, etc.
- Aluminum Foil: Necessary for some of the labs (e.g. Labs 5 and 6), useful for others. A standard roll is fine.
- Thorlabs 3/16" (M5) Thumbscrews for adjusting the rail carriers.
- Storage Bin to keep parts (rulers, flashlights, etc.) in one place on the student desk or workbench.
- It is also helpful to print (in color) the four pages of camera/spectrometer/ImageJ controls from the end of the Lab 1 Lab Notes (see also the end of the Lab 10 Lab Notes), and tape them to the lab benches near the computer monitors but where they will not be covered by the breadboard.

Computers

One computer per team is necessary, and updating them before class starts will prevent many problems.

Details are laid out in Appendix C on Computer Setup (in these Notes); however, some general comments:

- Do a full sets of updates, virus scans, and clearing off left-over student materials from the previous class.
    - Worth checking that all keyboards and mice are working
- Check the Following:
    - That all cameras and camera software are working
    - That all spectrometers and spectrometer software are working
    - That on each system one can save a camera image and open it in ImageJ
- Set Camera Software Starting Values: It can be useful to set all systems roughly as follows:
    - Camera Tab:
        - Pixel clock at a reasonable working rate (e.g. 23 MHz)
            - At whatever setting you use, the words "Dropped Frames 0.0%" should appear under the Pixel clock slider, and the camera image should respond to light.
        - Frame rate: Check "Hold" and drag slider to middle position
        - Exposure Time (ms): Check "Hold" and drag slider to middle position
    - Image Tab:
        - Uncheck "Auto" and "Gain Boost" under "Gain"

        - Set gain to 0. (It should then read "1x" under the word "Gain.")
    - Uncheck "Black Level".
    - Set "Software Gamma" to be 1.0 (its minimum value).
    - For the color camera,
        - "Auto White Balance" should be unchecked.
        - All color gains should be 0 (should say "1x" under Red, Green, Blue).
- Image Enhancement Tab:
    - Enhancement to 0 (minimum).
- Color saturation (in color camera) to 1.

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Appendix C: Computer Setup

Optical Microscopy Course

Appendix C Instructor Notes:
Computer Setup

The following instructions are intended for initial setup of computers for a class (often a big task if one is setting up many machines). They can also be used for (important!) updating of machines prior to the start of a new academic year or semester/quarter.

Hardware

- PCs require at least 4 USB ports, at least two of which are capable of full (480Mbit/s) USB2.0 data rates. The advent of USB 3.0 means that hubs may suffice given a single USB 3.0 port.
- Large, color screens are necessary.
- 6-foot (2 m) USB cables for the cameras are important for convenience and preventing students from damaging the camera connections by yanking on the connected cables.

Software

Our experience is that all computers (even brand new) will need updating; this is best done with hardwired internet connections.

- Windows Updates:
    - Do ALL Windows updates (this requires multiple restarts, etc.; run "check for updates" again after the last restart to make sure they are truly all finished.)
        - Make the passwords for all computers the same (and write them down!)
            - Passwords:
                - Admin account:
                    - Admin Password: _____
                - Student Account:
                    - Student Password: _____
- Software Installation: We usually copy a folder with the install files for everything onto each desktop, then do the installations, then delete the desktop folder.
    - We use Firefox, with:
        - Adblock Plus
        - Noscript
        - Set as default browser
        - Check updates on it after installation to make sure it is most recent version
    - Antivirus, install if your university has it free, or turn on "Windows Defender"
    - MS Office (be sure it has registered; often new machines must go through several steps when the programs are first run, and you do not want to run into this during class.)
    - Adobe Reader:
        - DO NOT install any "freebies," like Chrome extension, Intel "Truekey," or browser search bars, etc.
    - ThorCam Camera Software:
        - See the *Software* tab at www.thorlabs.com/OMC for the link to download it.
            - During installation, be sure to check the box to install the USB drivers!

        - After installation, verify that it recognizes cameras (attach a camera using a USB cable and test that it is imaging.)
    - Java: Install, or update to the most recent version.
    - ImageJ:
        - See the *Reference Links* tab at www.thorlabs.com/OMC for a link to download it.
        - We tend to install Java separately, then setup ImageJ after a system restart.
        - Usually all you have to do is put the ImageJ folder into the "program folders" folder, then put a shortcut to the imagej.exe executable onto the desktop and toolbar.
    - Download and install the "SE MTF 2X-Nyquist" plug-in.
        - SE MTF stands for "Slanted Edge Modulation Transfer Function," and it is free online.
        - Requires putting the .jar file into the "Plugins" folder (typically C:/Program Files/ImageJ/Plugins)
        - After installation, run ImageJ and
            - Go to menu bar, and verify that under "Plugins" you see "SEMTF 2xNyquist".
            - Go to menu bar, and under "Help" click on "Update ImageJ" and update to most recent version.
            - Clear default pixel scale: after installing, open ImageJ, open some image (by dragging and dropping on the ImageJ bar), go to Analyze/Set Scale, and in the dialogue box check "Global" and then "Click to Remove Scale."
                - If you do not do this, students will not see the scale in pixels, and will not learn as quickly how to work with that information (and will in fact be confused by the default scaling).
    - Spectrometer Software:
        - **DO NOT plug in the USB spectrometer before installing the software!**
        - Install Thorlabs OSA software if using Thorlabs CCS200 spectrometers.
            - See the *Software* tab at www.thorlabs.com/OMC for link to download it.
        - After installing and restarting the computer, verify that the software works by plugging in one of the USB spectrometers and seeing that you get a spectrum (e.g. from the room lights).
        - Put a software shortcut on the desktop and toolbar.
- Delete all games/students apps/etc. (students tend to find ways to install these).
- Delete the folder you had all the setup files in.
- Empty trash.
- Shut down and restart one last time to make sure all OK; run "check for updates" one last time.

Final Test

- Before class begins, test system(s) by using the ThorCam software to capture and save an image as a TIFF, then open it in ImageJ to see that all systems function properly.
    - Note: It is worth doing this for each setup, i.e. for each computer and for each pair of cameras. That prevents unexpected problems on the first day.
- Also worth testing USB spectrometer(s) for proper functioning (software and hardware.)

Desktop Setup

The computer desktop should be set up as follows:

- Screensaver should be turned off.
    - Set desktop background to a dark neutral (solid dark blue works nicely).
- System Power Settings should be set so computer does not go to sleep in less than 4 hours.
- Desktop:
    - Remove all icons except for the following:
        - Shortcuts:
            - Upper left: (one vertical row of three icons)
                - C: drive
                - Computer
                - Trash
            - Center of screen (two rows of 3 icons):
                - ThorCam Software (for cameras)
                - Thorlabs OSA Software (for spectrometer)
                - ImageJ
                - MS Excel
                - MS Word
                - Folder for each lab section that will use this computer
- Toolbar:
    - Set to autohide.
    - Put icons for ThorCam, Thorlabs OSA, ImageJ, Excel, Word, Firefox onto toolbar. Remove all others.
- Delete (on desktop and C: drive) folders with course info/images/etc. from previous year.
- Note: Leave any course-specific teaching files from previous year on desktop (usually TIFF and PowerPoint images of solid colors, grids of lines – will be instructor-dependent).

Placement

- Put one computer on each desk.
- Set up all monitors.
- Connect all cables/plugs:
    - Keyboards – be sure to route USB cables appropriately, so cables are not all tangled.
    - Mouse – be sure to route USB cables appropriately, so cables are not all tangled.
    - Power cords, including QTH10 lamp power cord, which we tape down on desk.
    - Two USB extender cables, one for each camera – route to desk and tape down with lab tape.
    - Have a spare mini-USB cable for spectrometer, and micro-USB cable for LEDs.
    - (If using): plug in USB hubs to external power and to computer.

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Appendix D: Exam Practical Suggestions, Tips, and Templates

Optical Microscopy Course

Appendix D has been omitted from the online version of the Instructor Notes. The full version is included on the USB stick shipped with the EDU-OMC1(/M) kit.

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Appendix E: References

Optical Microscopy Course

Appendix E Instructor Notes: References

This document lists some of the material we have found useful over the years in both industry and academia. This course is specifically intended ***not*** to require a substantial optics background, nor to involve a lot of math, but that is not necessarily true of the books and documents listed below; they are for background reading or future reference. We have tried to give enough information that you can steer yourself to the ones you may find useful.

Optics is one of those fields where wide reading pays off nicely. Many of the concepts are not that hard, and familiarity with how various different effects manifest themselves can make problems melt away. A great way to start is to read the easy parts of the application notes, skipping all the equations (or at least all the hard ones) and just start getting a feel for things. This sort of information will provide the bulk of what you really need to know to build even fairly sophisticated optical systems, and the class will emphasize those equations you will actually need (which are surprisingly few, for such a complex and beautiful subject).

Note to Students: If it were us starting in this class, we had read the following, roughly in this order:

1. Whatever is assigned, especially the Notes documents, which are targeted at the labs you will be doing.

2. Start poking around in the tutorials on the microscopy tutorial websites. These vary in organization from year to year; choose the one that works best for you, or skim all of them for which has the best material regarding your given question.

3. Skim through the Fundamentals of Optics section of the CVI / Melles-Griot Fundamental Optics Guide. Skip anything hard or boring, and read only the interesting bits, focusing on the text explanations of things.

4. Look at various books from the list below. Definitely look in the library to see if you like them before buying any – technical books can be quite pricey.

5. Look at academic papers relevant to your interests.

Technical Notes

This is listed first, before textbooks, for a reason: some of the most helpful material is not actually in books, but rather in the White Papers, Application Notes, etc., put out by various optical equipment companies… after all, they make money if you know how to use their stuff, and thus buy it, so they (sometimes) put a LOT of effort into training materials. We highly recommend starting to familiarize yourself with these resources – it is what practicing engineers (and scientists trying to build experimental rigs) use most. Often, there is no other source for the information – textbooks sometimes lag years behind the industry, or do not cover critical practical details.

All links can be found at www.thorlabs.com/OMC. Links change frequently, so it is hard to keep them properly updated. If one is broken, just search the web for the appropriate terms and it is likely it will turn up elsewhere on the manufacturer's site. Please also report broken links on the *Feedback* tab at www.thorlabs.com/OMC.

Websites

1. Microscopy Websites:
   There are now a number of these, from Zeiss, Leica, Olympus, Nikon. Many of them got their start based off of Michael Davidson's beautiful Molecular Expressions / Florida State University wesbite on microscopy, but all now feature additional material (which varies from site to site, so it can be worth looking at all of them on a given subject). These sites are an excellent place to go learn the basics of some technique quickly, and to generally learn more about microscopy – they may be the only resource set up just to get you up to speed on (mostly biological) microscopy. Very useful, but there are occasional inaccuracies, so check another reference to be sure (if it is really important). The sites vary in organization year to year – look for the one that works best for you. Places to start include:

   - *Molecular Expressions™ Optical Microscopy Primer* – Original FSU site
   - *Microscopy U* – Nikon Version
   - *Microscopy Resource Center* – Olympus version
   - *Education in Microscopy and Digital Imaging* – Zeiss version
   - *Learn & Share: Microscopy Basics* – Leica version
   - *BioDIP Teaching Material* – German BioDIP collection of resources

   For links, please see the *Reference Links* tab at www.thorlabs.com/OMC

2. Fundamentals of Optics section of the IDEX (formerly CVI, formerly Melles Griot) Optics Guide. See the *Reference Links* tab at www.thorlabs.com/OMC for the link.

   Many practicing engineers and grad students doing serious laser work will tell you this is the best source of optics information out there. One advanced text (by Phil Hobbs) even references it as being great. The "Fundamental Optics" section is very well done, as are the others. It's worth reading through the first 36 pages, skimming the math and any confusing parts. Then return to it when you have questions, and/or when you know more… the more you know, the more impressive it is.

   If you use (or plan to use) lasers – which are not part of this class, of course – also definitely look at their Gaussian Beam Optics Guide (most lasers have Gaussian beam profiles). The PDF of the original "Melles Griot Fundamental Optics" version (58 pages long) is easiest to read. Try searching for that online. The same holds true for the "Melles Griot Gaussian Beam Optics" version (14 pages long).

3. Light Collection and Systems Throughput white paper by Oriel/Newport. See the *Reference Links* tab at www.thorlabs.com/OMC for the link.

   Very well done (though sadly the web version was not well adapted from the (nice) original print/PDF version), and introduces and gives examples of how to use some crucial concepts in optics. Worth looking at, and though not critical to the course, it would not hurt to skim it. As well done as the Melles Griot Fundamental Optics Guide, but a little more specialized, so worth looking at after reading the IDEX/CVI/Melles Griot guide.

4. Handbook of Optical Filters for Fluorescence Microscopy by Chroma Corp. See the *Reference Links* tab at www.thorlabs.com/OMC for the link.

   Well done, and easy to read. Chroma has a remarkable range of interference filters for niche fluorescence applications, and long experience in the field.

5. Optics of the human eye**.** See the *Reference Links* tab at www.thorlabs.com/OMC for the links.

   Excellent material on the optics of the human eye, including measured (using adaptive optics) PSFs for different pupil diameters. More interesting as background, since most microscopy these days is done via camera; still, the eye information is fascinating (and relevant to normal vision).

Books

There is no one perfect book on introductory optics, let alone microscopy. Here are some of the standard ones; we suggest picking and choosing parts from different ones as they suit your needs.

* = might be useful for you in this class. The others are for future reference.

1. *Optics, 5th ed., Hecht. ISBN 0133977226

   This is the standard introductory optics text, and is not bad. Unfortunately, it does not really focus on microscopy, and is so broad in its coverage that it covers most things (other than basic imaging) somewhat shallowly. Its best points are the pictures, often quite illustrative, and the fact that it does touch on so many bits of optics that at least you will have had some contact with many of the most important things. Useful as a reference – worth working through the parts the instructor suggests (and any more you feel like), but probably not the one you will go to often for deep coverage of microscopy. Many people start by looking up a topic here, then move on to more advanced books.

2. * Digital Microscopy, 4th ed., Sluder and Wolf, Eds., ISBN: 0124077617.

   A very useful book, and a good example of a "current methods" series – it is a compendium of useful chapters by various experts, put out to help their colleagues and students (this one is done by the famous Marine Biological Laboratories at Woods Hole). The chapter on "Proper alignment of the microscope" is particularly useful (note: we liked the one by Ernst Keller, of Zeiss, in the 3rd edition; we are not familiar with the change in the newer 4th ed.). The chapter authors are eminent. Relatively little math, lots of useful hints, practical tips, and good explanations. Hint: when you need to learn some other technique, look for books like this, i.e., practical series put out by relevant labs – they will help get you up to speed fast.

3. *Video Microscopy, 2nd ed., Inoué and Spring. ISBN 0306455315

   A true "classic" – dense but not ridiculously so. A bit much to just read straight through, but very worth going to for specific questions. Inoué tends to be pretty complete, his technical statements are reliably correct even when simplified (actually a very hard thing to do; Chapter 5: Microscope Image Formation is especially good), and he mentions related considerations, not just the one technique you were looking for. Practical and useful; very little math. In terms of coverage and difficulty, this book falls midway between Sluder & Wolf and Born & Wolf. Note: the first edition

is also worth looking at sometimes – particularly, it has some great information that was dropped from the 2nd edition for space reasons.

4. *Fundamentals of Light Microscopy, Murphy. ISBN 047125391X

   An easy-to-read introduction. Some coverage is too shallow to be very helpful (e.g. on aliasing), but it does give you a place to start when you have no idea what something is. Also, it is easy to read over coffee/beverage of your choice, and that sort of reading can fill you with helpful information that makes you a better experimentalist/engineer.

5. Introduction to Fourier Optics, 3rd ed., Goodman. ISBN 0974707724

   A classic, and a pedagogical gem (i.e., it is quite accessible, and – in the 1st edition – quite short!). The math may be a bit steep if you have never seen a Fourier transform, but it is actually not as bad as it looks – the genius of Goodman is that he picks examples where the math works nicely and the physics becomes clear. Quite readable, and the technique of Fourier transforms in optics is very powerful, so it is worth the effort. Unfortunately, the book does not focus at all on microscopy, so you will have to translate the concepts to that realm yourself (not too hard). A good way to start, that is in keeping with the course material, is with the following sections (in the 3rd edition): 3.10 (angular spectrum of plane waves), 5.1 - 5.3 (coherent optical systems), 6.1 - 6.5 (frequency analysis of optical imaging systems), plus whatever review stuff you might want from Chapter 2 (section 2.1.5 on the Fourier-Bessel transform is quite useful). Note: in the 4th edition, a new chapter 5 on computational work was added. If you use this, the references above become sections 6.1 - 6.3 and sections 7.1 - 7.5.

   Aside: If you are approaching Fourier optics for the first time, an excellent review of the relevant math is in James, particularly the first three chapters, particularly chapters 1 – 3.2:

   Student's Guide to Fourier Transforms, 3rd ed., James. ISBN 0521176832

   This is perhaps the best of the Cambridge "Student's Guide" series, by the way. A more in-depth (and complete) textbook on the relevant math is the classic by Bracewell, The Fourier Transform and Its Applications… but we start students with James' book.

6. Principles of Optics, 7th ed., Born & Wolf. ISBN 0521642221

   THE "classic" optics book, which is to say, almost impenetrable for beginning students. It covers everything, but assumes a very high level of mathematical sophistication, is dense, and is referenced more often than read. But it is virtually the final word on most things in classical optics, and a pleasant (if slow) read once you have sufficient mathematical sophistication. The Born in the title is Max Born, of Quantum Mechanics fame. Sections of particular interest for this class include section 1.5 on reflection and refraction, section 10.4.2 on the van Cittert-Zernike theorem, and section 10.6.2 on the influence of the condenser on microscope resolution. (References are to the 7th ed.)

7. Modern Optical Engineering, 4th ed., Smith. ISBN 0071476873

   This is the classic engineering text for optics, the one (other than Born & Wolf) referred to by those who make their livings in the field of optical design. I mention it as a reference more than a suggested text for this class, but it does cover, and in far more depth, the issues and techniques involved in designing a good optical system. If you *really* want to know how to design good eyepieces, this is your book. Easier to read than that makes it sound, but the book is not really chatty – pretty focused on the basics of any given technique, and works almost entirely from a ray-optics standpoint. Much less math than Born & Wolf or Goodman – mostly algebra, not calculus. In a nice deviation from the norm for technical books, it is well-bound and on good-quality acid-free paper (earlier editions at least).

8. Principles of Fluorescence Spectroscopy, 3rd ed., Lakowicz. ISBN 0387312781

   THE reference for this sort of information. Covers many (but not all) fluorescence techniques, though mostly spectroscopic ones rather than imaging ones (at least in earlier editions of the book). Very complete, quite technical, a fair amount of math, but not nearly as many integrals as in Born & Wolf, or even Goodman.

9. Fundamentals of Photonics, 2nd ed., Saleh and Teich. ISBN 0471358320

   Mostly aimed at non-imaging (often laser) applications, this book has nice (and relatively easy to read, with very good figures) sections on ray, wave, and Fourier optics, along with information on detectors, laser beam optics, etc. A good first place to look regarding some of those more advanced topics, especially to get the idea with as little math as possible. Large and expensive – get it from the library first, then buy it if you need it.

10. Introduction to Optical Microscopy, Mertz. ISBN 0981519487

    Not an "introductory" book – do not look at this unless you are comfortable with Goodman and can read parts of Born & Wolf without too much difficulty. However, this is the only book to really cover the detailed optics involved in microscopy in a single place with a logically coherent structure, and it does it very well. Quite mathematical, and not a great reference if you just want to look "one thing" up, but an excellent introduction to coherence issues in imaging and other advanced topics from the standpoint of microscopy. Note: soon to be out in a 2nd edition from Cambridge University Press; look for this one.

Papers

* Indicates a level appropriate for a student first approaching the subject.

** Indicates related to a good student project (for semester-long classes with a couple of weeks for student projects at the end).

Historical:

* Volkmann, H., "Ernst Abbe and his work," Applied Optics 5 (11), 1720 (1966).

General Microscopy:

* Davidson, M.W. and Abramowitz, M., "Optical microscopy," Encyclopedia of imaging science and technology 2 (1106-1141), 120 (2002).

- Available free online – just search using Google Scholar; see the *Reference Links* tab at www.thorlabs.com/OMC for the link. A nice paper with much of the material from the introductory coverage on the Molecular Expressions microscopy website. Excellent level for student reading; great figures.

* Keller, H.E., "Proper alignment of the microscope," Methods in cell biology 72, 45 (2003).

- Keller is/was a high-level engineer/scientist with Zeiss.

* Evennett, P., "Köhler illumination: a simple interpretation," Proc. R. Microsc. Soc. 28, 10 (1994).

- The same Evennett who made the excellent Abbe theory videos.

Rheinberg Illumination:

** Abramowitz, M., "Rheinberg Illumination," American Laboratory 15 (4), 38 (1983).

- Nice introductory discussion; good starting point for student projects.

** Rheinberg, J., "On an addition to the methods of microscopical research by a new way of optically producing colour contrast between an object and its background, or between definite parts of the object itself," Journal of the Royal Microscopical Society VII, 373, (1896).

- Available free from Google Books – one can download the PDF of the proceedings and find it – worth searching for. Appropriate for student reading.

Gratings, Talbot Effect:

* Talbot, H.F., "Facts relating to optical science, No. I," The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science, 4:20, 112 (1834), DOI: 10.1080/14786443408648274

- Note: The date is sufficiently old that this should be available free (though link is not to free version) – worth checking online / Google Books. Appropriate level for introductory student reading; relatively little math.

Poor-Scientists' DIC Imaging:

** Axelrod, D., "Zero-cost modification of bright field microscopes for imaging phase gradient on cells – schlieren optics," Cell Biophysics 3 (2), 167, (1981). DOI: 10.1007/BF02788132

- Nice introductory discussion; good starting point for student projects.

Yi, R., Chu, K., and Mertz, J., "Graded-field microscopy with white light," Optics Express 14 (12), 5191, (2006). DOI: 10.1364/OE.14.005191

- Much more math (level at Goodman or above); complete discussion and description.

Super-Resolution:

Betzig E. et al., "Imaging Intracellular Fluorescent Proteins at Nanometer Resolution," Science 313 (5793), 1642 (2006). DOI:10.1126/science.1127344.

- The Nobel-winning paper on fluorescence microscopy with ~3 nm resolution. Great images, but focus is less on optical technique. Quite readable for most senior-level undergraduates.

Hess, S. T., Giriajan, T. P., and Mason, M. D., "Ultra-high resolution imaging by Fluorescence Photoactivation Localization Microscopy," Biophysical Journal 91 (11), 4258 (2006). DOI:10.1529/biophysj.106.091116

- The team that just missed the Nobel… but did amazing work. This paper discusses the optics in somewhat more detail. Also student-accessible, though a true research paper, not just a quick description.

Gustafsson, M.G.L., "Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy," Journal of Microscopy 198 (2), 82 (2000). DOI:10.1046/j.1365-2818.2000.00710.x

- An excellent paper for a student who understands basic Fourier optics and is looking for a (very cool) advanced application. Also notable since E. Betzig, the Nobel laureate for super-resolution (PALM) has supposedly commented that the Nobel committee should have recognized Gustafsson.

Computational Illumination Techniques:

** Mehta, S.B. and Sheppard, C.J., "Quantitative phase-gradient imaging at high resolution with asymmetric illumination-based differential phase contrast," Optics Letters 34 (13), 1924 (2009).

- This is not necessarily an easy paper to read, but the underlying technique makes for a potentially very nice student project: one takes two images, performs a simple computation with them (difference of the two images divided by the sum – equation 1 in the paper) and obtains interference contrast very similar to the (much more expensive and difficult to set up) DIC

imaging technique based on polarization and shear using a Wollaston prism. This is a nice introduction to the possibilities inherent in computational imaging approaches based on varying illumination; ptychography is a follow-up computational imaging topic – but not suitable for a short project.

Zheng, G., Horstmeyer, R., and Yang, C., "Wide-field, high-resolution Fourier ptychographic microscopy," Nature Photonics 7 (9), 739 (2013).

- An amazing application of Fourier optics, and a nice theoretical project for a student already familiar with Fourier optics (and programming). Interesting even if not a project; NOT good for an experimental student project – implementation is almost surely beyond the scope of a 2-3 week project for students at this level. More appropriate for elements of a theory course building off the material in this class.

Neil, M.A., Juškaitis, R. and Wilson, T., "Method of obtaining optical sectioning by using structured light in a conventional microscope," Optics Letters 22 (24) 1905 (1997).

- NOT a good experimental student project, despite the fact that the images look amazing, but a very cool application (and now a commercial product). Could be a nice (but advanced) theoretical project – just explaining why the technique works, on basic grounds (at the level of this class, skipping full mathematical treatment) would be worthwhile for a student. Warning: at least one of the integrations in the paper is extremely difficult, despite the result just being stated.

Other Illumination Techniques:

** Webb, K.F., "Condenser-free contrast methods for transmitted-light microscopy," Journal of microscopy 257 (1), 8 (2015).

- Possible starting point for a student project. Nice images. Not sure anyone actually implements illumination this way for standard microscopy, but Fourier ptychography and related computational imaging techniques do use related illumination approaches.

** Hinsch, J., "A new way of producing mixed brightfield/darkfield illumination," Microscope 43 (4), 175 (1995).

- Nice introductory discussion; good starting point for student projects. Hinsch worked for Leica, so the paper (which has nice figures) may be available from them.

RICM: Reflection Interference Contrast Microscopy:

* Curtis, A.S.G., "The mechanism of adhesion of cells to glass: a study by interference reflection microscopy," The Journal of Cell Biology 20 (2), 199 (1964).

- The original paper on the subject; quite readable, and a starting point for a possible student project, though it would be a more complicated project than others.

Details of Objectives:

* Abramowitz, M., Spring, K.R., Keller, H.E. and Davidson, M.W., "Basic principles of microscope objectives," Biotechniques 33 (4), 772 (2002).

- Quite accessible; good first paper for students to read on the subject. Same people who did the Molecular Expressions™ microscopy website.

Juškaitis, R., "Characterizing high numerical aperture microscope objective lenses," Optical imaging and microscopy, 21 (2003).

- Excellent, high-level coverage and experimental measurements of objectives. Highly recommended, though not as a starting point for students. Very interesting background on the shape of the first principle plane of an objective, as well as the phase problems in the last 0.1 of the NA of most high-NA objectives.

Absorption Cross-Section:

* Singh, K., Sandhu, G.K., Kaur, G., and Lark, B.S., "Molar extinction coefficients in aqueous solutions of some amino acids," Journal of Radioanalytical and Nuclear Chemistry 253 (3), 369 (2002).

- Derivation of relationship between the cross-section and the molar extinction coefficient. Used in Labs 9, 10; however, deriving this from basic principles can also be an excellent student exercise.

Phase Contrast:

Otaki, T., "Artifact halo reduction in phase contrast microscopy using apodization," Optical Review 7 (2), 119 (2000). DOI: 10.1007/s10043-000-0119-5

- A simple – but not mathematically complete – discussion of apodized phase contrast. This could be a starting point for student investigation as part of a project, but should only be so for a student with prior understanding of Fourier transforms (and, ideally, optics) – a real understanding of what's being done will require more analysis on the part of the student than is included in this paper. The type of apodization discussed matches the Nikon objectives included in this kit.

** Pluta, M., "Stray-light problem in phase contrast microscopy or toward highly sensitive phase contrast devices: a review," Optical Engineering 32 (12), 3199 (1993).

- Excellent starting point for student projects, particularly a phase mask for central phase contrast (akin to central darkfield using the zero-order mask). The critical material is stated in Appendix C of the Course Notes, "Additional Projects," but the full paper is here. Note: Pluta literally wrote the (multivolume) book on microscope design.

* Zernike, F., "The Wave Theory of Microscopic Image Formation," Concepts of Classical Optics, Appendix K (1958).

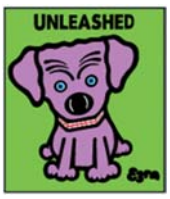
- Now available free online (see the *Reference Links* tab at www.thorlabs.com/OMC for link) and also reprinted by Dover, ISBN 0486432629. Zernike's exposition is extremely readable and (hardly surprisingly) very well done.

* Inoué, S., Video Microscopy, 1st edition, 119-122. ISBN 0-306-42120-8

- An excellent (and excellently phrased) discussion of this same material, based on Zernike's discussion in Appendix K of Strong.

Pluta, M., Advanced Light Microscopy, vol. 1. ISBN 0444989390

- Now dated, but perhaps the most complete discussion of design and details of microscopes. Included for completeness; probably not the best reference for introductory students, but a remarkable work that it is good to be aware of.



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