THORLABS

Optical Microscopy Course

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Based on the Course at

UC Berkeley

Instructor Notes

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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!

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Berkeley, CA

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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 and aligned 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 the 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 3.45 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 **do not** 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 CS165CU(/M) 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.

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Lab 2 Notes: Introduction to Optical Imaging (II)

Optical Microscopy Course

Lab 2 Instructor Notes:
Introduction to Optical Imaging (II)

General Items

- Optical Rail: **Discuss how to avoid damaging the rail parts – do not crank hard on the setscrews, etc.** Seriously – finger-tight is fine for almost everything; it is easy to generate enormous forces using screws, even by hand, and thus dent the aluminum dovetail. Using a driver gives you even more leverage, allowing for higher torques and potentially easier damage. Be gentle!
- Resolution Targets: **The resolution targets are very expensive, and easy to break.** Before they are taken out, we spend some time at the start of class going over how to handle them (this works best if discussion is right before they are used, rather than at the start of class, since most of the class is spent in camera setup). Things especially useful to mention:
    - **Emphasize again how expensive these are, and how easy to break.**
    - Wear gloves when handling, and ONLY handle from the edges.
    - DO NOT touch the chrome surface or put the chrome surface face down on anything!
    - ONLY put the targets back in their special plastic holder.
    - Removing Target from Box:
        - Place the box flat on table so if something goes wrong, the target cannot fall far.
        - Twisting fingers on the box tabs causes it to pop open without sudden motion
            - Pulling on the tabs causes the box to jerk, and the target to fly out.
    - Putting the Target in the Stage:
        - Loosen the (4) setscrews before putting the target in.
        - Make sure the stage's upright element is tightened down (setscrew on top), with sufficient room for the target (but not too much slop) before inserting the target fully.
        - Once the target is inserted, gently tighten the setscrews to keep it in place.
            - VERY gently; if you do it right, you will be able to take the target in and out later without re-adjusting them.
                - You may want to do only the bottom left and top right screws, so it is easier to undo them later.
            - We tell students to tighten by twisting the hex driver holding the driver by the shaft, not the handle. This reduces the torque they can put on the screws. It is easy for them to overtighten these – make sure they are cautious.
        - Removing the Target from the Sample Stage:
            - While wearing gloves, grab the top at the edges and lift it out.
            - Place it immediately into the plastic holder and snap the holder closed.

Lab Preparations

- Laptops: For focusing cameras at infinity, it can be helpful to have a pair of laptops with USB cables and the ThorCam software installed. That way students can do the focusing near a convenient window (maybe on a different floor of the building) that faces some distant objects.
- Optical Rail: We find it best to set this up prior to the start of class. The optimal configuration is outlined in the EDU-OMC1(/M) Manual and the table in these Instructor Notes (see the Lab 4 Instructor Notes).
- Stages: The stage mounting is more sensitive than one would expect; it is best to do this for all stages ahead of time, and then just give them to the students.
    - Be careful when tightening the translation stage against anything else. The roller bearings in the translation stage can easily dent the ways they travel along if they are torqued hard. To avoid this, be sure to hold the bottom (not the sides) of the translation stage with your fingers when (e.g.) tightening the post against the bottom of the stage.
        - Same thing for tightening the sample stage bracket against the top of the translation stage: hold the top (not the sides) of the translation stage with your fingers when tightening the screws.
    - The markings on the face of the sample stage should face away from the micrometer.
    - When placing the stage on the optical rail, the horizontal part of the stage should be over the rail; the vertical post hangs down over the side of the rail carrier. The micrometer faces away from the camera.
- Construction Notes:
    - It is always a good idea to have the socket head of any setscrew facing OUT of the more expensive part, so it is less likely to get stuck in the expensive part. E.g., we have the socket head sticking OUT of the ND filter wheel and going IN to the ½" post.
    - Also, it is useful to show the students how to use the through-hole inside of the post to get leverage on the post by putting a driver through it to get purchase (apply torque) when trying to screw the post onto something.
        - **REMIND THEM not to torque too hard on things!**
- LEDs: Check that they work, and the USB cable / power supply is available.
- Achromatic Lenses: Either have these installed in the lens focus-tubes before the start of class or be ready to explain to students how to handle them properly.
    - If you are installing them, make sure to install them all facing the same way; this will make your life much easier when troubleshooting different students' setups. Otherwise the correct tube lengths/focus positions will vary since the lenses are quite thick; their principle plane locations vary by several mm depending on orientation.
    - The correct orientation for the tube lens is with the flatter side facing the camera and the more curved side facing away.
        - The reason for this is that aberrations are minimized by spreading the ray bending more effectively across multiple surfaces. This is covered in the reading for Lab 4 (lens orientation, in the Melles Griot/CVI/IDEX optics guide.)
        - See the EDU-OMC1(/M) Manual for assembly details (and proper orientation) for all lenses.

Example Quiz 2 Questions

1. Write down the thin lens equation (for finding where images are formed)
2. If you want to focus your camera at infinity, how far do you place the camera face from the lens?
3. Write down a formula for the magnification of a lens (or pair of lenses – either formula is fine)
4. Write down the definitions for NA, both paraxial and for high NA.
5. Why do you need to be careful not to tighten the setscrews on the rail carriers too much?
6. When handling the resolution test target
    a. Name two things you should always do:
        i. Tell them that 'don't break it', and 'be careful' are true but not what is being asked for.
        ii. Answers include: use gloves, only put it down in its special case, always handle by the edges, etc.
    b. Name two things you should never do:
        i. Answers include: force it into the sample holder, touch the face with your fingers, place the chrome side down on the table, overtighten the setscrews on the stage, etc.

Lab Tasks

Main Things to Emphasize:

- What an image is. E.g., the light from the computer monitor makes no image on a piece of paper held a few centimeters away. A lens collects this light and makes an image at the focal plane.
    - If you can put a piece of paper there and see the object on it, then that is an image plane for the object!
- Basic camera controls; how to set up proper exposure, do a line scan, etc.
- What the depth of field is. This is easy to see when focusing at infinity, since as you start panning the camera to objects closer than "infinity" you will reach a distance close enough that objects begin to be noticeably blurry. That is the hyperfocal distance. Objects ~ 1 km away will be sufficiently far away.
- What resolution really is, especially that there is not any single best definition.
    - Rayleigh Limit: Point out that the amount of "bumpiness" in their line-profile through the resolution bars varies from small to large, and the "cutoff" one assigns to the "resolution limit" is a bit arbitrary. It is helpful for them to understand the Rayleigh limit as more of a rule of thumb.
    - Contrast: Draw their attention to the contrast level – how big the dip is between two resolution target bars – and help them think about how any practical definition of resolution depends also on the contrast one can see. Resolution and contrast are not identical, but are very closely related.

General:

- Stage: If you are having students set this up, see notes on page 2-2.
- Achromats: If you are having students install the achromats, we have them do it by following these steps:
    - Take the retaining ring out of the focus mechanism.
    - Open the lens box.
    - Place the tube down over the lens (so they never touch it at all with their hands).

- Invert the box and tube so the lens falls into the tube.
- Tap the base of the tube against the table to get the lens to settle to the bottom.
- Put in the retaining ring using the spanner wrench.

- Mounting the PCX Lens: See the *PCX Objective Assembly* Section in the EDU-OMC1(/M) Manual
- Connecting Tubes**:** Students often connect tubes, so they have only a ½" long tube on the end, and then screw the 1" focus mechanism into that. Then they cannot adjust the focus over its full 1" range. Wait for them to mess this up, then help them figure it out (otherwise they often cannot).

Focusing Cameras in the Room:

- Write something up on the board (or in marker on a piece of paper taped to a wall) before students try to focus the camera in the room. It may be worth having pages/large writing around the room so that each team can focus on one that is far from their setup.
- Camera Setup:
  - Set Black level set to 0, Frames Per Trigger to 'continuous', and Frames to Average to 1.
  - Leave Frame-Rate Control checkbox un-checked; this will result in the camera running at the highest rate available given the USB bandwidth and the exposure setting.
  - Show the students how to use the exposure time slider (checkbox on camera properties menu).
    - Exposure: In order to be able to focus, students will need to have the exposure time short enough to get a high (ideally > 15fps) frame rate. However, this is short enough that the image will be dim, and they will need to use Gain in order to brighten it.
      - It can be helpful to click the Exposure "Max Slider Range" and set it, e.g., for 100 ms. That way students can adjust the exposure more easily, without lowering the frame rate fps too badly.
      - Gain is given in dB, according to the standard dB = 20 log(multiple) formula, giving a linear gain of ~ 251 at 48 dB.
        - Arguably the formula *should* be 10 Log(multiple), since the cameras measure photons, proportional to power. However, the standard throughout the camera world is to use "20".
  - Make sure they also open a line-profile, so they see how it works and what it looks like during exposure and gain changes.
    - A good way for them to work is to set the exposure at a value (e.g. ~ 20-50 ms) that allows a reasonably high frame rate, then adjust the gain until the image is well-exposed (line profile near the maximum, without ever clipping / hitting the maximum).
  - Have them click the "Fit to Screen" icon on the ThorCam software so they can see the whole image. Best if they make the window large as well – a small image makes it harder to tell if you are in focus.


"Fit to screen" icon.

- Lens Position:
  - Most students seem to focus at objects nearby (e.g. their computer monitors) rather than some far away object. After they do this for a while, make sure they have focused on something far across the room (otherwise they will start far from the correct infinity focus).

        - It can be worth working out the difference on the board: for a f = 150 mm lens, the difference in lens position for an object 2 feet (60 cm) away and 6 m away is 200 mm vs 154 mm from the camera – almost 5 cm (2 inches)!
    - Tips:
        - Tell students which side of camera is down (text on back of camera should be oriented right-side-up); this really helps them understand how to orient the camera when focusing.
            - When focusing, it helps if students have the camera oriented so the image will be right-side-up. Many students will not realize they only need to rotate the camera in their hands in order to have the image right-side up. **This is a teachable moment – wait for them to mess this up, then help them after a while.**
        - Tell students to brace the camera lens tube against something – monitor, rail, whatever. This makes it easier to adjust focus.
- Make sure the students have a 3", ½", and three 1" lens tubes (the 1" tube has the SM1 focus in it) on the camera before going to focus out a window. Otherwise the distances won't work out for focusing at infinity.
- Before going to the window (our labs are usually in basements/windowless areas), we ask students, "Which way will the lens have to move when focusing at a more distant object, like the building across campus?" We do not leave the room until they can all clearly explain this – which saves a lot of confusion and time once we go up to the window/area for focusing the cameras at infinity.
- Also, if available, bring some spare ½" and 1" (12.5 and 25 mm) lens tubes because almost always some group has omitted at least one.

Focusing at Infinity:

- There is a trick with the locking ring on the focus mechanism:
    - Turn the SM1 lens tube ~1/8 turn the wrong way
    - Lock the ring down gently
    - Tighten the ring – as you do this, usually the focus tube also rotates ~1/8 turn back to the position you wanted.
- Cameras:
    - The cameras automatically adjust to maximize the frame rate; however, if you are setting the frame rate manually, be sure it is high enough (5-10 fps) that it is not hard to focus.
    - Note: If you get "Dropped Frames" errors on the computers, use the 'Frame Rate Control' checkbox and reduce the frame rate until "Dropped Frames" is 0%. The checkbox allows you to set the maximum frame rate for the camera and so can help reduce workload on the data transfer and CPU. The frame rate will automatically drop further if required for long exposures; in this case a notification will appear.

Lamp, Stage, and Resolution Target Imaging:

- **Make sure students get the camera at the right height ~9.5 cm** from the upper rail center to the center of camera SM1 lens tube.
- Set up the lamp so the bulb filament is at roughly the same height as the camera.
    - **Remind students the lamp gets hot!**
- Make sure they try to get the alignment right – the camera lens tube should be parallel to the rail.
    - You can explain to them that otherwise the errors will add up – you can have another lens angled the opposite way to compensate, but overall imaging gets worse and worse.

- Plano convex (PCX) lens should be the same height as the camera; show students how to:
    - Put it in a rail-carrier next to the camera lens tube.
    - Adjust height so it matches the camera lens tube by feel, lock it down in the rail carrier.
    - Put on post collar.
    - Move PCX to a rail carrier near the sample holder.
    - Note: The Assembly Video (see the *Videos* tab at www.thorlabs.com/OMC) is quite handy to watch here as part of your preparation.
- Always use the lamp at max intensity (do not install a dimmer or power it from a lower voltage or power supply – as voltage changes, so does power, and thus temperature, and thus the peak of the Planck blackbody distribution from the filament). You want the wavelength to be consistent throughout all your work; if one needs to reduce the intensity of the illumination (on this or a research microscope), one should use the ND filters.
- Camera Setup:
    - Make sure the students have a line-profile open; horizontal line-profile works best (easiest to see how it corresponds to the features on the screen).
    - Be sure students are doing proper exposures – max intensity just below saturating, per the line-profile.
    - (If being manually set) Frame rate should be at least 5-10 fps so it isn't too hard to focus.

Resolution:

- Students have a hard time understanding how to use the resolution target – worth going around and making sure they understand that:
    - Roughly, the resolution will be determined by the 3-bar group that has the smallest spacing they can distinguish by eye.
    - A nice demo is to have them use the line-profile (especially using ImageJ) to see when the "bumps" between the 3-bar sets cease being distinguishable. There is some arbitrariness in where you set your limit on this; usually ~9% is a good rule of thumb as the limit below which it is hard to distinguish the separation of the lines by eye.
- Resolution and Contrast:
    - Having the students see that contrast drops as you go to more closely spaced lines on the resolution target is a good goal; being quantitative about this is unnecessary.
    - Diffuser: without this, the illumination is fairly coherent, and so one sees nasty fringes in the image. We DO NOT want to get into this with students during Lab 2 – we start discussing it in Labs 3, 4. For now, just make sure they install the diffuser, which will make the fringes go away.
        - If they ask, explain that we will return to this in great detail.
        - For student-instructor reference (do not get into with the students themselves): one can think of the fringes as being the result of keeping only the first few terms of the Fourier expansion of the 3-bar set (a square wave) that are cut off by the finite lens NA. Those terms will have spatial frequencies (sizes, fringe spacings) like ~m λ / NA, where m is some odd integer – so larger than λ, especially for lower NAs (e.g. the ~0.08 NA or so of the f = 25 mm PCX lens).
        - **Note: The diffuser results in a lot of background in the images (they look very washed-out). This is OK for this lab.**
    - Exact Contrast Calculation:
        - We purposefully do not get into this too much in Lab 2; the preferred definition is $C = (I_{max} - I_{min}) / (I_{max} + I_{min})$, but **it is better not to overwhelm the**

students with too much information at once.; omitting this definition for now is fine, and perhaps preferable.

- One can improve this by having the students subtract off the background first – easiest to do by:
    - Save the image as a TIFF, then open it in ImageJ.
    - Move the cursor over a fully dark area of the image to see how dark "dark" is.
    - Use Process/Math/Subtract to subtract this value off the whole image.
    - Using $C = (I_{max} - I_{min}) / (I_{max} + I_{min})$ to get a better value for C.
        - Note: This will correct for losses in contrast due to image background, but not due to other factors (e.g. modulation transfer limits, etc.) due to where we suggest measuring the background.

THORLABS

Lab 3 Notes:
Aberrations and Illumination

Optical Microscopy Course

Lab 3 Instructor Notes:
Aberrations and Illumination

General Items

- This is a short lab; let the students know that that is expected.
- **Be sure you know how to read the stage micrometer – you will have to explain it to the students.**
- **Discuss how to avoid damaging the parts:**
    - Proper handling of optics (wear gloves, do not touch surfaces, etc.)
    - Remind students of how expensive and fragile the resolution target is, and how to mount/handle it properly.
    - Do not torque hard on the screws.
    - Be gentle and careful.
- **Be sure to describe how to handle the iris when assembling the home-built objective (see below), so students do not break them.**
- The quiz question about which surface to clean is actually rather important – it helps to emphasize where the image is in the system, and gets students thinking in terms of image planes even before we really emphasize it.

Lab Preparations

- LEDs: It is important to have LEDs ready (with fresh batteries, if you are using batteries). Ideally a full set for each lab group (though in a pinch they can swap them around), with blue (~405 or 470 nm; yes, we know 405 is really violet), green (~525 nm), red (~630 or 670 nm), and IR (~940 nm works well). See Appendix B, "Preparing for the Course," for details on LED selection.
- Color Filters: If you do not have LEDs, you can do this using the Roscolux color filters. If doing that, then in Part IV, "Aberrations: Color," leave the lamp in its normal position and instruct students so they do not set up the LED portion of the system.
    - The LEDs do work better for different parts of the experiments, having smaller emitting areas (and thus providing more spatially coherent illumination) and narrower emission bandwidths (purer color for the resolution comparison; also, the 470 nm and 930 nm LEDs are separated by enough to give a very convincing demonstration of the resolution change.)
- Remove IR Filters from the Lamps (If they are on; sometimes they are left from previous classes/labs.): If present, they result in much better imaging than with the bare (and high-IR content) lamp light. This lab and the following lab purposely start students with poor imaging and then show how to improve it using methods such as narrowing the spectral bandwidth.

Example Quiz 3 Questions

Note: **We never allow calculators** – students need to learn to handle approximate calculations by hand.

Assume: A PCX lens has f = 25 mm, diameter = 25 mm, red light has λ = 630 nm.

1. Which side of your PCX lens should be facing the sample in order to minimize aberrations in your image?
2. Estimate the NA of your PCX lens in the configuration in which you are using it.
3. Estimate the (Rayleigh) resolution you should achieve with your PCX lens. State the color of light you are assuming.
4. Estimate the magnification of the USAF target onto your camera using the PCX and 200 mm achromat tube lens as you have set up.
5. Later in this lab you will use an objective lens consisting of an achromat with a focal length of 25 mm and a diameter of 12.5 mm; would you expect it to image with better or worse resolution than your PCX lens based on the Rayleigh criterion?
6. **If there is dirt in your image, and no matter which optical elements you move it stays the same place, what item should you clean?**

Lab Tasks

Main Things to Emphasize:

- How to set up proper exposure for your camera – line scan, exposure settings
- How illumination matters – simple things like: Diffuser (coherence) dramatically affects the way the image looks, as well as the resolution. Color (λ) affects resolution and aberrations, brightness determines whether you need a long, noisy camera exposure, etc.
- What resolution is, roughly, and that it depends on contrast too (i.e., how do you even know if you have resolved something if you have no contrast?).

General:

- Coherence: Moving the lamp further away makes the illumination more spatially coherent (effectively the sample is illuminated from a smaller spread of angles). Because the filament has a rectangular cross section, the illumination will be more coherent on one axis than the other, and this can be noticeable when looking at the 3-bar patterns (e.g., the vertical ones may look different than the horizontal ones). Placing an iris in front of the lamp can help fix this; nonetheless, alignment and other effects typically make the horizontal and vertical bars look a bit different.
    - **Try to steer the students to noticing the fringing (diffraction) effects as they move through focus, usually most visible on the smaller sets of bars.**
    - Then mention how a bunch of fringes will make it harder to resolve closely spaced bars.
    - Using LED illumination (with the LED at a distance) can make this effect even more noticeable than using color filters.
    - **Advanced Instructor Note**: The spatial coherence of the source is given by the van Cittert-Zernike theorem (see *Principles of Optics*, 6th ed., by Born & Wolf; p. 520). This is beyond most students in the class; however, the formula for the diameter of the area over which initially incoherent light will be coherent after propagation is d = 0.16 λ/NA, where NA is given by NA ~ r/s, where s is the distance to the light source and r is the

radius of the light source emitting area – i.e., it is the angle subtended by the light source. For comparison, a green (λ = 525 nm) LED with a 100 µm diameter emitting area, placed 500 mm from an illuminated sample, will provide coherence over a diameter d = 840 µm (nearly a millimeter), and covering the entire field of view. Conversely, a lamp filament 2 mm tall will produce only d = 42 µm, and even less if taking the ~5 mm width of the filament into account.

- Chromatic Aberration: The images with just an LED and diffuser are noticeably better than what students get with the lamp; point this out to them!

Preparation:

- LEDs: Make sure you have them and the batteries are fresh (if using batteries) or have a USB port and the extension cable next to the rail (if using the USB-powered LEDs).
- If you want students to gain hands-on experience with the optomechanics, have the home-built objectives disassembled for them to work with.
    - Leave the ½" (12.7 mm) lenses in the barrels to avoid damage. Students only assemble the rest of the objective; we do not have them do that step.

Resolution Targets:

- Remind students to be careful!
    - **Open box only when it is sitting on the table.**
    - **When putting the target away, it goes only in its box (and make sure to snap box shut).**
    - **Handle target only by the edges, while wearing gloves.**
    - **Careful when inserting in the sample holder.**
        - **Do not scrape chrome on any parts.**

Reading the Stage Micrometer:

- This should be explained to the students – gather them around a rig and show them.
    - The major barrel divisions are in 0.5 mm (500 µm) increments
        - On the top and bottom of the barrel there are marks every 1 mm; these are offset such that going from one mark on the bottom of the barrel to one on the top = 0.5 mm (one full turn of the knob).
    - The numbered divisions on the barrel are in 10's of µm.
        - i.e., "24" = 240 µm.
- Add these to get the total: 2 major divisions visible on the main shaft, with the barrel reading "32" means (2*500 + 32 * 10) µm = 1320 µm = 1.32 mm.
- Note: The expected focusing difference between red and blue light is ~500 µm for the BK7 PCX lens – it is not a subtle effect.

Objective Assembly:

- The achromat is quite thick and can bind up in the tube. DO NOT force it by trying to thread a retaining ring onto it or poking it with a driver or other "stick".
        - **We avoid having students struggle with this by pre-assembling the lens into the barrel.**
    - Open the lens box.
    - Place the SM1V10 down onto the lens.
        - NOTE: You want the flattest side of the lens to end up facing the sample. This matches what would be true for a PCX lens and so may seem obvious, but

actually the proper orientation for a multi-element lens depends on its design. In this case, the proper orientation is in fact to have the flattest side (which is the negative element of this doublet) toward the finite conjugate (the sample).

- Invert so lens is in SM1V10 focus mechanism.
- Tap base on desk until lens settles.
  - Only then put in the retaining ring.
- If this does not work, flip it over and dump the lens back into the box (or, if necessary, tap the side of the barrel with a driver until the lens falls out).
- Forcing it will result in a broken $100 lens.
- Warn students: **DO NOT torque the screws down too much** – this can damage parts.
- (Only if having student assemble objective): Especially when doing this, go through an assembly of the objective with them gathered around, emphasizing each step:
  - Socket in screw is facing out of post.
  - Screw engages enough threads on the slip-ring.
  - Using a driver, drive the setscrew far enough down into slip-ring that it cannot scratch lens tube when you tighten the slip ring.
    - The rule of thumb is that you need to engage 3 full threads on a screw to get the full mechanical strength of the connection.
  - Using a driver in the through-hole in the post to get leverage, tighten the slip ring onto the post, but just finger tight to avoid damage.
  - **When tightening the iris onto objective, grab the outer edge of the iris so you are not applying torque to the delicate iris adjustment ring.**
  - **Have iris index marks facing up** (so you can see them later) before tightening down slip-ring (again, not too tight – enough to be secure, but explicitly explain to them they do not need to (and should not) torque really hard).

Objective Focusing:

- The trick to getting the objective properly set up is to:
  - Orient the objective with its lens facing the camera lens.
  - Have the lamp illuminate the back (iris) of the objective.
  - Close the BFP iris all the way down (~1 mm).
  - Adjust the objective lens focus until the iris comes into focus on the camera!
    - Usually there is some dust on the inner edge of the iris which makes it easier to focus on.
    - If the camera is saturating when displaying the iris image, you will be unable to see the edge properly. Be sure to use a line-profile to check for saturation.
- You will later use this same trick for the condenser.
- **DO NOT give this alignment/focusing approach away to the students too soon! This is a great learning moment. Let them (at least try to) figure out how to do it!**

ImageJ:

- Worth (re-)explaining line-profiles to any students that do not seem proficient. Students take a while to get quick about this, and it can help to show them the tricks:
  - Once you make a little line, you can drag it to new areas of the screen (say, a different 3-bar element) and hit Ctrl+K to get a new line-profile. In this way, they can get line-profiles of all 6 elements in a few seconds, instead of many minutes.

Chromatic Aberration:

- Focal Length vs. Wavelength:
  - There are two main points to make for students here:
    - First, the focal length varies a good bit with wavelength. Depending on time, you could work out with students, at the board, what the blur spot size would be for a lens of this NA when shifted 0.5 mm away for best focus; it is huge. Link this to why imaging is poorer in broad-spectrum (white) light.
    - Second, the achromat lens shows vastly smaller focal-length shift with wavelength, which makes sense, given its name. Students can get hung up on the shapes of the f vs. λ curves and miss the big difference in the absolute change occurring for the two different lenses.
  - Separately, the PCX lens shows monotonic increase in f with λ (since n(λ) tends to increase as one goes to shorter wavelength). This is NOT TRUE for the achromat objective. The Wikipedia article on "Achromatic lens" has a nice graph demonstrating this, showing (as the student data will match) a focal shift that is concave up (assuming wavelength on the horizontal axis of their plot). This is a good time to discuss how an achromat is made, and that it will have the "correct" focal length at *two* wavelengths, and, when in between those wavelengths, will tend to deviate less from the design focal length than a PCX might. Going to triplets and more complex lenses (Apochromats) results in correction at multiple wavelengths and decreasing error from desired focal length when between the extreme design wavelengths. A decent high-NA apochromatic objective is $6k. It is worth helping students understand why one pays for the "apochromatic" part.

THORLABS

Lab 4 Notes:
Köhler Illumination

Optical Microscopy Course

Lab 4 Instructor Notes:
Köhler Illumination

General Items

- Teaching Rig (optional):
    - It is easiest to teach the course if you have a separate optical rail set up to allow you to do demos (for instance, we connect a projector to the computer, and can then demonstrate directly in class what the students will see, or did see, and discuss it).
    - If you have sufficient equipment to allow this, then set the teaching rig up **before class** for full Köhler illumination, as in these notes.
        - Then reference students to it as they are working, so they can go up and look at it to see how to construct things.
- Literature references (academic papers) are not compiled as part of these notes; they must be obtained by the course instructor. In general, not having any of the professional references is not a critical hindrance for the students. We have left discussion of them in the notes so the instructor or students can pursue them if they wish (see the Lab 4 Lab Notes recommended reading, and Appendix E of these Instructor Notes).

Lab Preparations

- Irises: It helps if these are calibrated correctly (with student use they often get out of calibration). The setscrews for this seem to be glued in during manufacture (a standard technique), leaving few options. (Gently) turning the iris ring a little too far in either direction can help adjust the zero position, but surely voids any warranty; caveat emptor.
    - Checking if they are off calibration can help you advise students. Obviously, if they are off and this is not taken into consideration, the analysis will come out poorly.

Example Quiz 4 Questions

1. Name two places where, if you place a piece of paper in the optical train, you should be able to see an image of the lamp filament on the paper.
2. Name two places where, if you place a piece of paper in the optical train, you should be able to see an image of the field stop on the paper.
3. What do you adjust to get the field stop focused at the proper place?
4. Write down the full formula for the Rayleigh resolution of a trans-illuminated sample.
5. What does Köhler illumination involve that makes the light more uniform at the sample?

Lab Tasks

Main Things to Emphasize:

- How to set up Köhler illumination.
    - Proper alignment. If a rig is properly aligned, the field of view should be illuminated fairly uniformly (within 15% or so), and that should be obvious from horizontal and vertical line-profiles.
- Resolution scales with NA (per the Rayleigh resolution formula).
- Image brightness scales with NA (let them figure out how; it goes like NA^2 for low NA).

- Practice using the camera controls, histogram window, etc.

General:

- A common alignment difficulty occurs when students try to set things up with any (or all) of the irises closed. Imaging can then be very strange, and when that happens it is easy to forget to check the irises. If a student asks for help, and things look weird on the screen, check the irises first.
- We never need to set the gain > 0 in this lab; it is recommended to leave it at zero.
- **Even if the functions are available, avoid using auto-gain and/or auto-exposure for this lab**. The auto-functions work based on the average of the pixels in the image. If the whole field is black except for one small, bright iris (e.g. when bringing the field stop into focus by adjusting the condenser position) then the auto-gain will badly overexpose the small bright area to bring the total pixel average to a midrange value.

Preparation:

- For roughing out a teaching rig (an example for students to look at and emulate), the distances in the following table are a decent place to start.
    - Note: The Assembly and Köhler illumination videos can be quite helpful here and are worth watching first. See the *Videos* tab at www.thorlabs.com/OMC.

PLEASE DO NOT give this table out to students, who may follow it slavishly. It is much better to set up the rail itself (on the breadboard), and put the sample stage in a consistent position, the camera at the left end, and the lamp at the right end, and let the rest of the optics fall into their natural positions as the rig gets built up.

Component	Distance from Left Edge	Details
-	0 cm	Left side of rail; all distances are figured from this.
Mono Camera Rail Carrier	~12 cm	Exact position is non-critical and will vary depending on where the slip-ring is secured on the lens tube – ideally that is about mid-way on the tube. Important thing is to keep the camera body ~2 cm inside end of rail.
"Dog-Leg" Rail	26.5 cm	**Distance from the left end of the main rail to the left side of the perpendicular dog-leg rail. It is very helpful if this position is the same for all rigs.**
70/30 Beamsplitter Rail Carrier	~33 cm	Will vary a bit depending on whether the 30% side is against the back of the mount or against the SM2 retaining ring (we use the latter configuration, so the retaining ring side of the mount faces the BFP camera). Note: there is a correct orientation that minimizes the secondary (and slightly off-set) reflection of the aperture stop, visible on the BFP camera.
BFP Camera	-	The BFP camera gets mounted near the very end (<1 cm from edge) of the dog-leg rail when using the homebuilt achromat objective. Note: when you install the 10X Phase objective you will have to slightly reposition the BFP camera to best capture the phase ring (and also usually reposition the condenser slightly to bring the field stop into the best focus).
Homemade Objective Rail Carrier	~39 cm	Exact position will vary with device, and where the slip-ring is attached.
Sample Stage	43.5 cm	**It is helpful if this position is the same for all rigs. Measure from the left edge of the rail to the left edge of the sample stage rail carrier.**
Condenser	~53 cm	Exact position will vary with the field stop location and slip-ring positioning.
ND Filter Wheel	~66 cm	Very insensitive to exact positioning.
Flip-mount Filters	~71 cm	Very insensitive to exact positioning.
Field Stop	~79 cm	Exact position will vary with the lamp and collector lens position.
Collector Lens	~82 cm	Exact position will vary with the lamp and condenser lens position.
Lamp Post	~84 cm	Assuming the lamp housing is oriented so the bulb is closest to the camera on the camera side of the lamp post, the exact position not critical. Important thing is to keep the lamp from extending off the edge of the rail. (Corresponds roughly to the right edge of the lamp rail carrier being ~5 cm from the right edge of the rail).

Height: If the camera lens tube lens center is ~9.5 cm above the center of the top rail, and everything else is set to the same height, you will have enough leeway to adjust the sample as needed.

Collector Lens: Should have flat face toward the nearer object (the lamp) to minimize aberrations.

Condenser:

- Check Assembly: See "Condenser Assembly" Section in the EDU-OMC1(/M) Manual.
- Focusing: Setting the condenser focus so the lens is properly positioned with respect to the aperture stop is done just as with the objective:
    - Place the condenser near the camera lens, with the condenser lens facing the camera.
        - This is also a good time to check that the lens height is correct (center ~9.5 cm above rail.)
    - Set the aperture stop iris to minimum diameter.
    - Illuminate the iris with the lamp.
    - Adjust the condenser lens focus mechanism until the edges of the iris are clearly imaged by the camera.
        - Make sure the camera is not saturating or it will be impossible to properly see the iris edges.
- Initial Placement: Start with the condenser ~50 mm from the sample, since its focal length is 50 mm and the field stop (which it focuses onto the sample) is a comparatively long way ("infinity") from the condenser lens.

Flip Mounts**:**

- Assembling flip mount: See "Flip Mount Assembly" Section in the EDU-OMC1(/M) Manual.
- The Thorlabs convention is that arrows point in the direction light is intended to pass through the filter (in this case, towards the camera).

Köhler Illumination:

- The gist here is to get the lamp filament imaged onto the aperture stop (condenser iris). Since this iris is one focal length from the condenser lens, the filament is thus focused at infinity ("totally out of focus") with respect to the sample, and consequently illumination is more uniform.
    - At high intensity (low ND values for the ND filter wheel) one can see the filament by eye on the closed condenser iris. At lower intensity, the coils of the filament are easily visible on a piece of paper held against the iris.
    - Since the aperture stop iris is one focal length from the condenser lens, and the objective BFP iris is one focal length from the objective, the combination images the aperture stop (and thus the filament) to the objective back focal plane, and it is also easily visible if a piece of paper is held there.
- Place the field stop iris a few centimeters left of the collector lens (i.e., on the condenser side of the collector), to allow for later adjustments to the collector lens position without having to rearrange everything else.
- Adjust the condenser position along the rail until the field stop iris is in focus at the sample.
- **REMEMBER**: You must focus on the sample first, before trying to position the condenser.

Filters:

- The camera is quite sensitive in the IR, and the blackbody spectrum from the lamp is rising rapidly toward the IR (and is much lower toward the blue), so there are many more IR photons. Without the IR filter, a large part of the imaging is effectively being done at long wavelengths. Also, some of the color filters (if used) have some transmission in the IR, so using the IR filter in conjunction with them still helps minimize IR effects.
    - The lamp is bright; screwing the IR filter (Thorlabs FGB37M) directly onto the lamp helps generally reduce glare.
- Green filters are used (instead of, e.g., blue, which would seem better since it provides shorter wavelength and thus better resolution) mainly because the human visual response peaks strongly in the green, and also because thermal light sources typically have blackbody peaks in the IR, and thus the amount of light is also falling off quite strongly as one moves to shorter wavelengths. The combination tends to make blue very dim to the human eye, the historical detector.
- Plots for the Write-Ups:
    - Contrast: This plot trends as expected (it is not, obviously, really the MTF, but it is an approximation of sorts). The results will be very sensitive to background level, etc. – background problems are the usual issue in students' results.
        - **The important thing is for students to see that contrast falls off with resolution, and to get used to thinking of BOTH contrast and resolution as SEPARATELY important (and often related).**
    - Resolution: If well aligned, the plot of resolution vs. NA looks roughly as you would expect – not as good as theory, due to aberrations, but with the correct trend.
    - Brightness: Scales well like NA^2 for small NA. Since the filament image does not fill the condenser aperture well (hence the use of the diffuser), this scaling falls off (instead of increasing beyond a square-law, as it should) at higher objective NAs. Helpful to know in case students pursue this farther than they are asked to in the write-up instructions.
        - Also, the condenser aperture is imaged at M = 0.5 to the objective back focal plane, so the scaling will be off any time the objective aperture diameter > 0.5 * condenser aperture diameter (i.e., whenever the objective NA exceeds the condenser NA).
        - **The important thing here is for students to get used to thinking of NA as affecting BOTH resolution *and* the amount of light collected (image brightness). Again, these are SEPARATELY important.**

THORLABS

Lab 5 Notes:

Köhler, Conjugate Planes, and Darkfield Imaging

Optical Microscopy Course

Lab 5 Instructor Notes:
Köhler, Conjugate Planes, and Darkfield Imaging

General Items

- Spherical Aberration Images: It is very handy to have these as posters in the classroom. (See the *Reference Links* tab at www.thorlabs.com/OMC.)

Lab Preparations

- Irises: It helps if these are calibrated right (with student use they often get out of calibration). The setscrews for this seem to be glued shut, leaving few options. (Gently) turning them a little too far in either direction can help adjust the zero position, but surely voids any warranty; caveat emptor.
    - Checking if they are off calibration can help you advise students. Obviously, if they are off and this is not taken into consideration, the analysis will come out poorly.
- **The room must be dark** – block out any windows, etc., if need be (aluminum foil blocks light exceedingly well, and is easy to cover windows with)
- It is helpful to have a teaching rig already set up, so students can see everything working, and use it to help figure out where their difficulties lie, if they are having any.
- Be sure to have a roll of aluminum foil in class for the parts of the lab that require it.
- Make sure you have LED lamps and/or flashlights ready (with batteries charged) – if the room lights are off it can get difficult if students do not have them.
- It is helpful to have the students read Course Notes Appendix B, on Zernike's Phase Contrast. The use of the zero-order mask matches some of this description exactly.

Example Quiz 5 Questions

1. What is the full Rayleigh resolution formula?
2. What is the formula for Magnification in terms of NA and in terms of focal lengths?
3. Sketch your microscope and indicate the planes conjugate to the lamp filament.
4. What special task might you use a ball-driver for in this lab?
5. Where in the optical path would you put a scratched green filter so it won't make your images look bad?
6. Where is the high resolution image information/light in the objective BFP?
7. Instructor: Draw an Aperture Stop mask (with a hole or opening in it off axis or somewhere) on the board and ask students what shape a mask in the objective BFP would need to be in order to obtain darkfield imaging.

Lab Tasks

Main Things to Emphasize:

- Proper Use of the Camera Controls (exposure, etc.):
    - It can be useful to watch (and have students watch) the ThorCam Quickstart video prior to this lab (See the *Videos* tab at www.thorlabs.com/OMC).

- Students may need help figuring out how to set the frame rate and exposure, since these are interdependent. Make sure they understand that if they set ***longer*** exposure times the frame rate may automatically decrease to accommodate.
    - Draw their attention to the notification "Exposure is limiting frame rate to…" which shows up in the Camera Settings dialogue when the Frame Rate Control box is checked and the exposure is turned up.
- Especially emphasize the use of the line-profile to see if the area of the image of interest is properly exposed (and explain that "proper" exposure is having peak intensities near, but not at or above, 1022 counts, so that one is using the full camera dynamic range without saturating).
    - It helps to explain to students why saturation is bad. We use the bucket-in-the-rain analogy for a pixel with incident photons: once the bucket starts to overflow, you no longer know exactly how much rain has really fallen at that spot.
    - The CS165MU camera is a 10-bit camera, so one might expect that the highest value would be 1023 counts (since one level is assigned to zero; otherwise it would go 1-1024). However, the sensor manufacturer (Sony) uses the terminal bit as an indicator in their camera interface, and consequently the highest reported value is in fact 1022.
- Showing students the camera "Histogram" function (icon to the left of the Line profile icons on the top toolbar) is also helpful – it provides different but related information to the line profiles, and is sometimes easier to watch while tweaking a system. It is also frequently used in the high-end microscope software students may encounter later.

- What the Point-Spread Function ***means***. Students have a hard time grasping this.
    - Help by explaining to them that it is just a way of talking about the 3-D distribution of light at the focus on the system.
        - It is best to explain this while they are racking the focus back and forth looking at the 5 µm spot in the Zoom Window – the Airy rings should be visible as they do this (if not, try turning up the exposure and/or gain further, or opening the BFP iris to 3 mm).
        - Getting students to look at the PSF images (on the web or in poster versions in the classroom) while looking at (and manipulating) what they have on their own screen is best
        - **Make sure they open and close the BFP iris while watching what happens to the spot/ring diameters. This is a key learning moment.**
- Help them (but not before they have struggled sufficiently to be ready to appreciate the information) to draw the connection between blocking part of the BFP and subtracting images.
    - Note: Subtracting images. Obviously, the subtracting-images approach to computationally faking darkfield is sort of rough – optically it is the *fields* that get subtracted, then the resulting image is the magnitude squared of the result, which will have interference terms. But both visually and heuristically "subtracting images" is a good way for students to start thinking about it, especially at an introductory level, and we strongly suggest you leave it at that unless someone asks for additional detail. Remember: it is easier than one might think to overwhelm students with too much information. There are many new concepts in this lab; do not add to them unnecessarily.

General:

- It is best for students to use image zoom (with the mouse-wheel) and Pixel Peek when they have the BFP iris set to 3 mm (and maybe when they are looking at the 7.5 μm diameter spot). Everything gets harder when features get dim and the exposure gets long. Start with something they can see, then let them move to the smaller 5 μm spot after they have the brighter 7.5 μm spot figured out.
- It is impossible to focus once the frame rate drops below ~5 fps, and hard below ~10 fps. It may help to have students set the exposure "Max Slider Range" to ~ 200 ms so they cannot accidentally force the fps too low by using a very long exposure. They can then increase the gain to get a sufficiently bright image to allow for coarse focusing while using a short exposure. Only then should they turn the gain back to zero and increase the exposure (for lower-noise imaging for their final images), which may automatically decrease the frame rate and make focusing in that state difficult.
- Emphasize that students should, before leaving class, do enough of the analysis that they are sure they are able to use their images from BFP = 7.5 mm and BFP = 1 mm to make a darkfield image. Sometimes students leave this part for after the lab, and then realize later that their images were misaligned and they are unable to get a "darkfield" image.
- If/When Students Use the Gain Slider:
    - Explain the difference between Gain (multiplies everything, <u>including</u> readout noise and background, plus adds some extra noise) and exposure (always best if you can get enough light, since it <u>does not multiply/increase the readout noise</u>, and if background is low, only increases signal).
    - Since the maximum gain is 48 dB, ~ 250X, a nice demo can be to have the students turn the gain up to 48 dB, adjust exposure to have a properly exposed (non-saturating) image, and then turn the gain to zero and the exposure up by a factor of 250. The decrease in noise/increase in image quality is quite noticeable.
- The green interference filter lets through much more light than the green glass filter; hence it can be useful to use the interference filter for the long exposure image of the 5 μm pinhole. (Assuming the filter is already installed in the flip-mounts; not worth handling it separately just for this step.)
- The diameters of imaged spots from the 5 μm pinhole come out exactly as expected from the Rayleigh criterion, assuming the imaging has been done well and the BFP diameter ≤ 3.0 mm (note: remember to consider magnification in the calculation). For larger BFP diameters (especially 7.5 mm), the PSF is sufficiently smaller than 5 μm that the image (which is, roughly speaking, a convolution of the PSF with a 5 μm diameter top-hat function) will be "too large" compared to the calculation, and this can confuse students. We suggest not getting into this issue with them, unless they ask – students are already quite challenged by digesting the basics of this lab, and extra material is not necessarily helpful to them.
- Aluminum Foil: It is easiest to get a straight-edge with the foil by tearing off a large piece, then folding it over – the fold will create a nice straight edge to put across the back of the aperture stop, etc.
    - It is actually physically/mechanically easier to put the foil across the BFP first, then adjust the foil on the aperture stop to get darkfield; however, this is conceptually reversed from the way it is explained in the notes, so (to maintain pedagogical consistency) the Lab Notes have the students start by doing it the other way.

- Alignment: A common problem is that students do not have their rig aligned well, so that the image of the aperture stop in the objective BFP is not centered. In that case, if you close down the aperture stop and the BFP iris, and the image of the small aperture stop is offset from the opening in the BFP, no light will get through. The only cure for this is better alignment (getting all the optics aligned parallel to the optic axis).
    - You can try to fix it by adjusting the angle of the condenser – can be done by tapping on the side of the condenser with a ball-driver handle until the light comes through OK / alignment is good.
        - Do not adjust the objective or sample camera, or the images won't all register and students won't be able to do later analysis. If substantial adjustments must be made to alignment, have students take the 7.5 mm and 1.0 mm images again afterwards.
    - The best alignment procedure is to:
        - Move the sample camera rail carrier far enough back on the rail that you can get the condenser and objective in front of the tube lens.
        - Remove the entire condenser rail-carrier, mount it in front of the sample camera lens tube, and slide the condenser lens tube so the edge is flush against the camera lens tube.
            - Adjust the condenser height until both tubes are at the same height (if they are not) and adjust post angles such that the tubes become aligned (being flush with each other will force the tubes into alignment along the rail).
            - Tighten both post-thumbscrews holding the camera and condenser posts into their rail carriers. Mark the heights with a post collar on each post.
        - Move the condenser rail carrier (with condenser in it) back to the original position. You can adjust the condenser position along the rail to get the field stop back into focus (i.e., Köhler).
        - Repeat the alignment steps using the objective: move its rail carrier back to the front of the sample camera lens tube, and with the BFP iris against the lens tube check the height and adjust the objective angle such that it and the camera lens tube are in alignment along the rail.
            - Tighten the post thumbscrews, mark the heights post collars, and move the objective back to its location near the sample stage. Adjust the objective position (and the stage micrometer) to get the sample back into focus.
        - At this point, the sample camera lens tube, objective, and condenser should be well aligned along the rail.
        - Close down the BFP iris and aperture iris alternately, looking on the BFP camera image to see if the irises are concentric (closing down evenly onto the other iris).
            - If not, try very slight taps on the condenser and/or objective to get the irises to close down symmetrically on each other.
            - If that does not work, consider repeating the alignment steps above.
            - Remember: There are no vertical-plane adjustments to the lens mounts, so if the irises are closing down symmetrically on the left/right (horizontal axis) but not the vertical (up/down), and all optics are at the

same height, there is not much you can do. Small misalignment in the vertical direction is generally not a big problem in this lab.

- Note in Advance of Lab 6: The above steps should work for aligning the zero-order mask (small spot on mask screwed into the objective BFP for darkfield imaging) – follow the steps above, then screw in the mask.
  - While watching the image on the BFP camera, open the BFP to 7.5 mm (larger just results in scattered light in the objective barrel) and close down the aperture iris to see that it closes evenly on the zero-order mask spot. Tap the condenser/objective lightly with a ball driver to get the AS and darkfield mask spot to superpose. See the images in the Lab 6 Lab Notes for reference.

- "Faking" the PSF using convolution: this is easiest when using a nested set of targets (e.g. the USAF 1951) so both large and small bars are visible at the same time; however, if the students focus their attention on the barely-resolvable features (e.g. the holes in the eight in "128") it also works out fine with the NBS 1963A 5-bar target.
- Blocking the BFP with a ball driver: it is best to use the smallest driver that works; a large driver blocks much more of the low-spatial-frequency light, and worse, its thicker shaft blocks more of the BFP in total, which can make the image look strange.
  - A common student problem is putting the driver into the BFP in such a way that the shaft of the driver blocks many of the diffracted orders from the sample – easy to do if they all fall along a line, e.g. if they are diffracted spots from a Ronchi ruling. Bringing the driver shaft in at a slight angle (i.e., not exactly along a horizontal or vertical axis) resolves this difficulty.
  - Using aluminum foil (e.g. if a sheet of foil is crimped around the post or rail carrier, with just a little molded stalk sticking up into the BFP), especially if the tip is kept no larger than necessary, can be quite helpful when taking images, since it won't move.
  - Consider discussing with students which half of the objective BFP they had to mask and why it was that side. This gets at a subtlety of the Magnification – it is actually negative in this configuration, and hence one ends up blocking the same sides of both apertures.

THORLABS

Lab 6 Notes:
The Abbe Theory of Image Formation (I)

Optical Microscopy Course

Lab 6 Instructor Notes:
The Abbe Theory of Image Formation (I)

General Items

- None.

Lab Preparations

- This is one of the most important and content-filled labs of the entire course. It will be very hard for students to understand what is happening if they are not well prepared. It is worth making this clear to them in class (or by email) ahead of time, underlining the importance of having read the Course Notes, Lab Notes, and having watched the videos by Dr. Peter Evennett ***before*** coming to lab; see the *Videos* tab at www.thorlabs.com/OMC.
- Practice setting up and aligning the BFP camera beforehand so you can help students do so during the lab. The video on setting up the BFP camera may be useful; see the *Videos* tab at www.thorlabs.com/OMC.

Example Quiz 6 Questions

1. How could you illuminate your sample with a plane wave using your system?
2. From the perspective of Abbe theory, what do you get after a plane wave hits a sample?
3. What do plane waves at the sample look like in the BFP of the objective?
4. What do points of light in the BFP of the objective look like at your camera?
5. Why is resolution lower in the image than is actually true in terms of the sample?
6. If you had two sinusoidal samples, one with a spatial frequency of k and the other with a spatial frequency of 2k, which would require a higher numerical aperture to image?
7. Where is the high resolution image information in the objective BFP?
8. Sharp, fine features correspond to what spatial frequencies and scatter light at what angles?
9. A point in the FFP (front focal plane) of a lens corresponds to what kind of wave at the BFP?
10. What is the minimum number of diffracted orders required to form an image?
11. What is the formula for the reflection at an air-glass interface?

Lab Tasks

Main Things to Emphasize:

- **Keep People Moving** – this is a long lab, and if they do not keep a good pace they will not finish on time. Worse, the most important element of the lab is the very last one (by necessity). It is critical to keep students moving so they get to it.
- BFP Camera Lens Orientation: Students struggle with this, because both the image and object distances are finite – neither is infinity. Remind them to try to spread the ray bending across multiple surfaces, and use the example of a point focused to infinity (where having the flat side of the lens facing infinity means no bending at all happens at one of the two

surfaces) to lead them to having the flat side of the lens face the closer conjugate. This is discussed also in the *IDEX/CVI/Melles Griot Fundamental Optics Guide* (Lens Shape and Lens Combinations, ~ p. 1.18; see the *Reference Links* tab at www.thorlabs.com/OMC).

- The "Ghost Image" of the BFP: This is a learning moment; do not give it away too fast; let students figure out where it comes from. Do not get too far into details unless the student in question is very clearly already fine with all the other content. For most students, a complete discussion is too much at this stage.
    - There is a correct and incorrect orientation of the beamsplitter (BS) plate (discussed further, below). One can test this just by measuring the ratio of intensity in the 0th order spot to the ghost image spot, then rotating the BS plate 180° and realigning to get the image on the camera. Then re-measure 0th order spot and ghost image. Clearly one BS plate orientation is better. There is further discussion of this below, but it is too much for most students given the time available and length of this lab.[1]
- Students often ask where the "flaring" comes from when aligning the BFP camera and beamsplitter. It is serious aberration (coma, etc.) due to the light coming through the lens off-axis and not all being bent to focus at the same point (direct them to the *IDEX/CVI/Melles Griot Fundamental Optics Guide*, p. 1.11, Aberrations, and 1.14, Coma; see the *Reference Links* tab at www.thorlabs.com/OMC).
    - How this works can be illustrated holding an LED a good distance (50 cm) from one of the "find the focal length" lenses from Lab 1 and using that lens to focus it to a point on the desk. Then tilt the lens to show the coma / flaring.
- Coherent vs. Incoherent Illumination: Students often ask about this. This is a deep and confusing topic; for this class we urge you not to go farther than something like this:
    - Coherent Illumination: Illumination with the aperture stop closed down, or from any source so far away that the angles of light hitting the sample from it are all close to the same (note that with the aperture stop fully closed, the illumination at the sample is approximately a plane wave, which by definition has no angular spread).
        - Very roughly speaking, any part of this light can interfere with any other part, across the entire illuminated area of the sample.
    - Incoherent Illumination: Illumination with the aperture stop wide open, or from any source so close to the sample so light from it hits the sample at lots of angles. Note that with the aperture stop wide open, there is a converging cone of illumination at the sample with a large NA (defined by the condenser focal length and the aperture stop radius).
        - Very roughly speaking, light diffracted from the sample will only interfere with other diffracted light originating from the same spot on the source (filament). If the sample is illuminated by many parts of the filament then the separate, independent *intensity* images formed due to light from each part of the filament will add up to form the final image (and these separate images will not mutually interfere with each other – just add). This naturally affects the resolution and imaging behavior.

[1] For instructor reference, the best orientation is with the more highly reflective surface facing the incident light -- i.e., with the oxide coating of the EBP2 beamsplitter facing (at 45°) toward the back of the objective and BFP camera. If assembled as per the EDU-OMC1(/M) Manual, Section 5.2.10 "Back Focal Plane (BFP) Beamsplitter Assembly," the coated side will be facing the lip of (and thus will be closest to the edge of) the LMR2(/M) optic mount, and hence the lip side of the LMR2 would properly face the incident light. We will reiterate that having students figure the correct orientation out for themselves -- either experimentally, theoretically, or both -- is an excellent exercise. To assist with this, we provide discussion of the theory later in these Lab 6 Instructor Notes.

- This a deep topic (as most recently indicated by Glauber's 2005 physics Nobel for studies of optical coherence). Coherence is discussed a bit in the Lab 7 Course Notes, but interested students should be referred to other resources.
  - See the discussion of diameter of the coherence area and the van Cittert-Zernike theorem in the Lab 3 Instructor Notes.
  - For those craving deeper discussion, Wolf (of Born & Wolf fame), has a short volume, ***Introduction to the Theory of Coherence and Polarization of Light.***

General:

- The secondary reflection ("ghost") from the beamsplitter: Students have a hard time understanding where this comes from and why it is different for the two orientations. For more advanced students, solving the following can be a fun exercise; however, as with the last lab there are many new concepts in this lab, and students are frequently already overloaded. Save this explanation for those students who are clearly looking and ready for an extra challenge.
- The calculation is a bit complicated for the EBP2 beamsplitter, which has an oxide layer, etc., but the principle is easy to present if one assumes a traditional partially silvered glass plate, which has one (partially silvered) side which reflects 30% of the light hitting it, and one side which (being just an air-glass interface) reflects $[(n_{glass} - n_{air})/ (n_{glass} + n_{air})]^2$. Since $n_{glass} = 1.5$ and $n_{air} = 1$, this gives $(0.5 / 2.5)^2 = 4\%$. Students should know this formula from the "Equations to Memorize" sheet in Appendix A of the Course Notes.
  - Note: Of course 4% is not correct for 45° incidence (further modified by Snell's law as it transmits into the glass). For quantitative predictions, they will want to check the actual Fresnel coefficients (at the right angles, etc.) However, it is better to have students learn to approximate rather than to confuse them with too many issues at once when the results are still very similar.
- There are two possible orientations for the silvered glass plate:
  - Silvered Side toward BFP Camera:
    - 30% reflects off first surface.
    - 70% goes through, and 4% reflects off the back (air-glass) surface. On the way to the BFP camera this hits the silvered surface again, losing 70%, so 70% * 4% * 70% ≈ 2% gets to the camera.
    - The ratio of "ghost" to main spot intensity is thus 2% to 30%, = 1:15.
  - Glass-Side toward BFP Camera:
    - 4% reflects off the first surface.
    - 96% goes through, and 30% of this reflects off the back (silvered) surface. On the way to the camera this hits the glass surface again, losing another 4% (and transmitting 96%), so 96% * 96% * 30% = 28% gets through.
    - The ratio of the "ghost" to main spots will then be 4% to 28%, = 1:7.
  - This is obviously a major difference (factor of ~2) in the relative intensities of the ghost. Note that the position on the camera of the main and "ghost" spots will be reversed as well, based on whether the first or the 2nd reflection is most intense.
    - Note: Having the light hit the highest-reflecting surface first thus results in the smallest relative secondary (ghost) reflection, and in the class geometry will result in a main spot on the right in the image, with a ghost to its left. So the oxide layer (most reflective surface) should face the objective.

Preparation:

- **DEFINITELY PRACTICE** getting the BFP camera (the color camera) aligned *before* class; otherwise it will be very hard to help the students, who will probably struggle with this. The video on setting up the BFP camera may be useful to watch in advance; see the *Videos* tab at www.thorlabs.com/OMC.
- It is helpful to have a teaching rig already set up, so students can see everything working, and use it to help figure out where their difficulties lie, if they are having any.
- Make sure you have LED flashlights ready – if the room lights are off it can get difficult if students do not have them.

THORLABS

Lab 7 Notes:
The Abbe Theory of Image Formation (II)

Optical Microscopy Course

Lab 7 Instructor Notes:
The Abbe Theory of Image Formation (II)

General Items

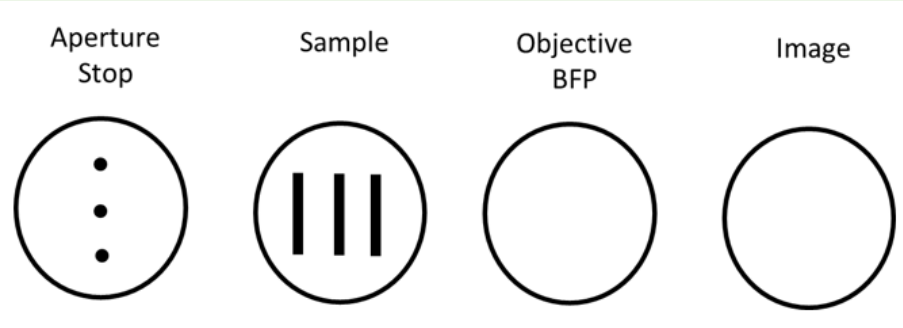
- Course Notes Appendix B, on Zernike's phase contrast method, is extremely helpful for students to review prior to this lab.
- Campbell-Robson Contrast Sensitivity Chart: We have made a poster for the classroom of a variable frequency and contrast sine wave. This allows students to "see" quite literally, something analogous to the "MTF" of their eye and makes for a great lecture discussion.
    - There is a PDF available for download on the *Reference Links* tab at www.thorlabs.com/OMC. You can print this out and hang it in your classroom.
    - The best version we have found online is a pattern is a Log-F Contrast Chart using Imatest, which is a variant of the Campbell-Robson chart. See the *Reference Links* tab at www.thorlabs.com/OMC for links.
    - Students may ask why they perceive contrast to be dropping at *low* spatial frequencies as well as high – after all, the MTF of a normal lens system is highest at low spatial frequencies. The answer for this is related to the biology of the retina, particularly on-retina processing related to lateral inhibition by neurons in the retina. References that offer a good explanation for the low frequency roll-off in the CSF include:
        - *Sensation and Perception*, by Wolfe, Kluender and Levi, ISBN 9781605352114
        - *Foundations of Vision*, by Wandell, ISBN 0878938532 and available free on the web. See the *Reference Links* tab at www.thorlabs.com/OMC for link.

Lab Preparations

- **DEFINITELY PRACTICE this lab ahead of time**; it is heavily dependent on good alignment, and students may need assistance figuring out the many steps to get from an image of a Ronchi ruling to the contrast of the lines.
    - In particular, if the system alignment is off, then the plotted contrasts vs. spatial frequency do not come out looking at all like the Course Notes plots, and students will get confused and frustrated.
        - If time permits – carefully keeping people on track during the lab can help make sure there is extra time at the end – then keeping students in the classroom until they have reduced and plotted at least some of their data can help catch problems before they go home and have no chance to retake images if the analysis is not working out.
- Make sure ImageJ is installed and working on the computers.
    - The Slanted Edge MTF (v.2) plug-in must be installed in ImageJ**.** See the *Reference Links* tab at www.thorlabs.com/OMC for link.
    - It is worth practicing with this to make sure you are familiar with its use before the students start using it.
        - **In particular,** SE MTF **only works if you draw a box around an edge that is dark on the left and light on the right side. The reverse does not work.**

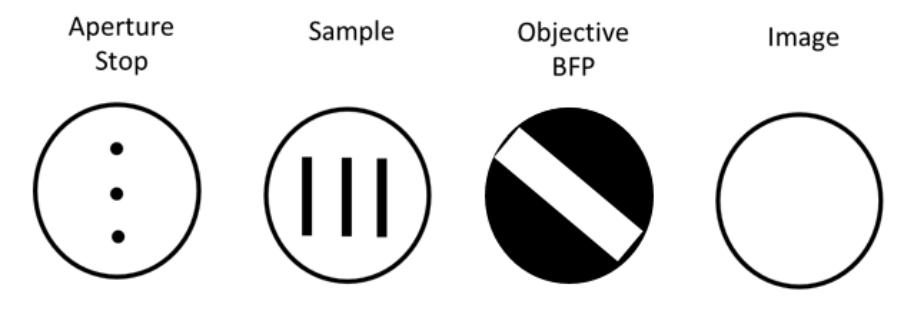
Example Quiz 7 Questions

Question 1: Given the Aperture Stop and Sample, draw the resulting BFP and Image:

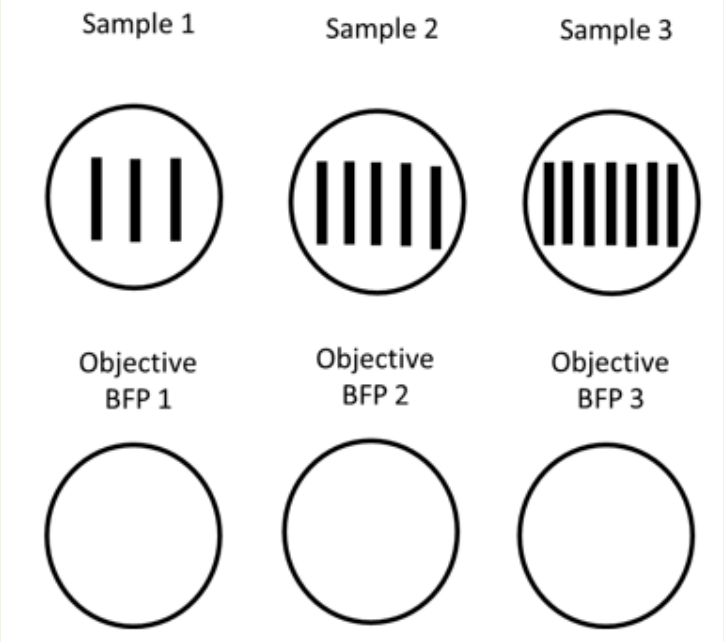


Question 2: Is this coherent or incoherent illumination?

Question 3: Given the Aperture Stop and Sample, and a mask across the BFP that allows only the diagonal strip shown through, draw the resulting Image:



Question 4: For this set of samples, draw what you get in the BFP for each assuming coherent illumination:



Question 5: Roughly sketch, for a system with 0.5 NA and $\lambda = 0.5$ μm

a. The coherent MTF of the system. Label both axes clearly and with appropriate numerical values.

b. The (fully) incoherent MTF for the system. Label both axes clearly and with appropriate numerical values.

Lab Tasks

Main Things to Emphasize:

- Succinctly summarizing how spatial frequency of the sample corresponds to location of diffracted spots in the BFP, and linking the number of diffracted spots that can be captured by the objective aperture (i.e. get through the BFP iris) to the resolution of the system.
- The shape of the MTF. Many students struggle to explain the distinction between the coherent and incoherent MTF – both when one expects to get which shape (a simpler question) and also why they are different shapes (a more complex question). It is definitely worth going over this early (in class, but also at the beginning of lab), so students are as prepared as possible for what they will be doing during the lab.

General:

- Step 2: Making AS and BFP concentric: the easiest way to check this is to get things aligned well (in Köhler, FS in center of mono camera image – do not forget to click "fit to screen" so you are looking at the entire camera), then:
    - Close down AS so it is between 1/3 and 1/2 its normal diameter. Watch this on the color camera (which should not be saturating!)
    - Close down the objective BFP until the image of the edges of the BFP start to cut into the image on the color camera.

        - It can help to do this while imaging the grid target or Ronchi ruling, since those result in a lot of secondary AS images that allow one to see the BFP edges even before they get to the (central, or 0^{th} order) AS image.
            - What matters is that the left and right edges start to get cut off (occluded) symmetrically / at the same time. In this setup you have no way to adjust things if the top and bottom are not symmetrical, so do not worry about that.
    - If the BFP starts to cut into one edge of the AS before the other, check the condenser and objective alignment – tap one or the other, open and close the BFP to check, then repeat until the BFP iris closes symmetrically on the AS.
        - If this really is not working, pull the objective rail carrier off (with the post still mounted), slide it flush against the camera to force alignment, put it back and do the same with the condenser. Then go back to checking BFP and AS alignment by opening and closing, while watching the color camera image.
- Steps 9 and 10: Keeping the camera settings the same, and then getting a "Reference Black" image, allows later subtracting the same background off all the maxima and minima measured from the Ronchi images. Without this, the calculated contrast will come out poorly. Be sure to check that students are doing these steps.
- Steps 14 - 20: Plotting values: it is very much worth having students do this, or at least some of this, during lab, to see that their data is decent (and that they can figure out what to do).
    - Be aware (but do not tell students why!) that, if perfectly measured with these systems the coherent CTF (roughly, the MTF) will not match the Course Notes figure (for the MTF in coherent illumination) due to the finite aperture stop diameter.

THORLABS

Lab 8 Notes:
Contrast Methods and Abbe Theory

Optical Microscopy Course

Lab 8 Instructor Notes:
Contrast Methods and Abbe Theory

General Items

- **DEFINITELY PRACTICE** this lab; it is heavily dependent on good alignment, and students may need assistance figuring out how to achieve it. The basic approach is laid out in the Lab 5 Instructor Notes, which details the process for aligning the objective and condenser by sliding each one up against the sample camera lens tube.
    - **That system provides a process for students to use when trying to get their alignment right.** It is important in this lab that the aperture stop can be closed down symmetrically to the edge of the objective phase ring as part of setting up phase contrast.
- **Keep students moving** – particularly with the diatoms, they can get bogged down. They will need a full hour at least to do the phase contrast; make sure they move from the diatoms to phase with adequate time remaining.
- Students struggle with both:
    - Understanding Phase Contrast: Be prepared for many questions.
        - Phase shift during diffraction: it is best during lab to simply state, without explanation, that the diffracted light undergoes a 90° phase shift, while the undiffracted light (which goes through the center of the BFP) has no phase shift. Showing why this phase shift happens is best left for class lecture. Students have plenty to digest in the lab just understanding what happens to the diffracted and undiffracted beams: where they are in the BFP, why the phase plate matters, and how the additional phase shift results in increased contrast.
            - For reference, a quick way to explain the 90° phase shift is as the natural effect of making small changes in the argument of a cosine. As a plane wave travels through any uniform medium, is accumulates a phase shift ϕ; if that medium is *very slightly* non-uniform (say, the index of refraction varies) then the accumulated phase will be $\phi + \delta(x,y)$, with $\delta(x,y) << 1$. The wave amplitude would then be, say, $\cos(\phi+\delta)$. Of course, the variation in cosine with a small change in argument is $\frac{d}{d\phi}\cos$ times the change in argument, and the derivative of the cosine is the sine, giving the 90° shift. In a similar argument, the Taylor series expansion for the cosine is
$$\cos(\phi + \delta(x,y)) \cong \cos(\phi) - \delta(x,y)\ \sin(\phi) + \cdots$$
so the sample looks like a flat piece of glass (the $\cos(\phi)$ term, which is the same for all points (x,y)) with an additional term proportional to the phase variation $\delta(x,y)$ and with $\sin(\phi)$ again giving the 90° phase shift. (δ^2 and higher-order terms can be ignored for small variations because $\delta << 1$.)
    - MTF vs. Spatial Frequency for Different Condenser NAs:
        - Students struggle to explain precisely why the aperture is best at 70% (which is, of course, only a starting point, providing good contrast at low to moderate spatial frequencies at the cost of some resolution), and how it relates back to the coherent and incoherent MTF.
        - Helping students to understand the plot (from the Lab 7 Course Notes) of MTF vs. spatial frequency for different condenser NAs is important – it can help to have this as a PowerPoint slide, or at least to be ready to draw it on the board during lab discussion.
        - The effect can be subtle, and diatoms are not always the best sample to illustrate the effect on contrast of changing the condenser aperture stop. It depends enormously on the luck of which diatoms appear on a given set of slides.

- It is good for students to look enough, and think about it enough, that they start to get the point. **It is <u>not</u> necessary for them to find perfect examples** of improved contrast/imaging based on condenser NA (aperture stop setting.)

Lab Preparations

- Though clear nail polish included in the kit works OK, it is cytotoxic, and so it can help to have something else to seal the coverslips for the cheek epithelial cells. Other possibilities (not included in the kit) include:
    - Melted beeswax (e.g. in a water bath on a hotplate), which can be swabbed or brushed onto the edge of the coverslip using a cotton swab ("Q-tip").
    - VALAP, a melted mix of 1:1:1 Vaseline, lanolin, and paraffin. Worth doing an internet search if you are not already familiar – there are tricks to making and using it.
        - For both wax and VALAP, be careful of unattended combustible materials on a hot plate! Also, place these in a hood so that compounds do not volatilize and then condense back out on the optics in the room.
    - A nontoxic sealant called Covergrip, from the company Biotium. Advantage: no hotplate; disadvantage: more expensive. See the *Reference Links* tab at www.thorlabs.com/OMC for link.
- If you are making your own bead slides, it can be helpful to draw an "X" or other mark on the slide with a marker near where the beads are placed (we use a fine-tip Sharpie marker; caution – can dissolve in ethanol, which we sometimes use in our bead preps.) This provides a sharp edge to focus on, which is especially useful for small beads, which can otherwise be very hard to find in terms of proper focus.
- Students will need to dispose of their glass slides (with cheek cells) after the lab. Have an appropriate glass disposal box available for this.

Example Quiz 8 Questions

1. (Instructor: draw on the board a shape for an aperture stop – e.g. a half-blocked circle, making a "D"). Then ask students to draw what would be seen at the objective BFP.
    a. Most will miss that the shape gets inverted, but the main point for this lab is that they understand that a "D" shape will end up illuminated in the BFP.
2. Where in the BFP is the light related to fine/small features in the sample?
3. What sort of cells will you use to make a sample today?
4. Where is the phase plate in a phase contrast microscope?
5. Give a formula for contrast (any one of the formulae is fine).

Lab Tasks

Main Things to Emphasize:

- What contrast *is*. Students may gloss over the early steps where they compute the contrast, but understanding that is helpful – particularly that high background decreases overall contrast, and that for small objects it can be very hard to see them against a large background (e.g. in brightfield), while darkfield produces high contrast.
- The utility of being able to see transparent objects – staining usually kills the cell; darkfield, phase (and DIC, etc.) allow observation of living cells. If there is a projector in the room, it can be fun

to show some videos, e.g. of fish keratocytes, macrophages or neutrophils moving. YouTube and other venues will have many such videos, most in phase contrast.
    - Look for the halos around the edges of the cells; pointing that out to students as the signature of phase contrast is also worthwhile. Note: the Nikon phase objectives used in this lab are apodized, reducing the halo effect. From a pedagogical perspective, this is unfortunate but it does provide a chance to mention apodization, though it is probably best only to mention it as a trick for reducing the halos.
    - One video to look for is the movie by David Rogers from Vanderbilt of a neutrophil chasing Staphylococcus aureus. See the *Reference Links* tab at www.thorlabs.com/OMC for link.
- Aperture Stop (AS): Adjusting the aperture stop during brightfield can improve contrast, and it should be natural to reach for and adjust the AS for best imaging. Conversely, the AS should be left alone during phase or darkfield imaging.
- Phase vs. Darkfield: It can be helpful to have students shift from phase contrast to brightfield and back (by adjusting the aperture stop diameter) while observing the cheek epithelial cells.
    - In particular, for transparent objects when one focuses in brightfield the focus is never correct – the generation of contrast is obtained by slight defocus above or below the true focal plane (defocus introduces some phase effects in an otherwise zero-contrast image). This effect can be seen by setting up phase contrast, getting in best focus, then opening the AS so that one is in brightfield. There will be very little contrast, but slight defocusing of the sample will bring out some contrast. When phase contrast is restored (by adjusting the AS), the sample will turn out not be in best focus.
    - Having students compare the difference between darkfield and phase contrast for a couple of samples is also helpful – phase is typically rather expensive, while darkfield (or oblique) illumination are often extremely cheap to set up. Understanding (visually) what you get for the extra money is a nice exercise.
        - Point out that phase provides contrast (roughly) proportional to the sample "thickness" (really, optical path length difference), while darkfield tends to simply pick up edges (which have a lot of high spatial-frequency content, and thus a lot of diffracted light coming through outside the blocked center of the BFP.)

General:

- Change in resolution with condenser aperture:
    - This is not a small effect – students should see internal structure for some diatoms become completely invisible as the AS is closed down, often only halfway or less, only to reappear as it is opened again. The big round diatoms with fine internal structure seem to work well, but details will depend on the luck of the individual diatom strew slide.
    - Since the filament image fills the whole aperture horizontally but not vertically, it may be possible to see (or students may notice) that resolution effects from changing the AS (mainly at larger AS settings) may affect the vertical more than the horizontal resolution.
        - Diatoms, if they have the right structure, can make this fairly clear. However, the right diatoms can be hard to find. To avoid that potential difficulty, one can use the Thorlabs Resolution Target, imaging the 228 lp/mm element. By closing the BFP to ~3.5 - 4.0 mm and the AS all the way down, as the AS is slowly opened some vertical bars will become resolvable while the corresponding horizontal bars do not.
- Depth of Focus: Using the homebuilt achromat objective the results will match fairly well between the DoF as checked by eye and the DoF ~ λ/NA^2 value, assuming $\lambda \sim 525$ nm (appropriate for the IR and green filters in the light path). With the phase objective (NA 0.25 instead of the ≤0.15 NA of the homebuilt objective) it is hard to make a small enough stage translation to be sure the numbers match up.

Preparation:

- Be sure to have the materials for making cheek epithelial cell slides:
  - Slides
  - Sharpie markers
  - Coverslips
  - Sealant (clear nail polish)
  - Something to scrape cells of cheek with – 1 mL pipette tips work fine, as would plastic coffee stirrers, etc.
- **Try it in advance to make sure you can do it yourself!**

Addendum: Home-Made Central Phase Contrast

Inoué's recapitulation of Zernike on the method of central dark ground, and leading from there to phase contrast, is so elegant that we initially followed it in the development of our phase contrast lab (Lab 8). Doing so requires a ¼ λ (quarter wave) plate with diameter ~ 1 mm in the middle of a 25 mm glass plate; such things are not commercially available at any reasonable cost. Almost entirely for that reason, we shifted the phase contrast lab to use a commercial objective when we began working with Thorlabs to make the course available as a kit.

However, should you be inclined to make your own phase masks, it is not hard to do it and allows for doing central (as opposed to annular) phase contrast, which makes a cleaner connection to the central darkfield students have already done. Consequently, we include instructions for making them, and the original lab instructions for using them:

Making Phase Plates:

The basic method is this: moving a coverslip through a candle flame (really, a bit above it where the soot is forming) allows formation of a thin layer on glass which both absorbs and provides a phase delay (e.g. the index for soot from a stearin (a type of wax) candle is n = 2.32 compared to air). Conveniently, the absorption of the soot and the phase shift are both related (by the thickness of the soot layer), and this has been measured and tabulated (c.f. Pluta refrence, below – this method was actually used to make commercial microscopes several decades ago!). Simply light the candle, pass a coverslip through it a time or two, measure the change in transmission through the soot, repeat until the transmission matches the thickness of soot which provides ¼ λ retardation. Then using a razor blade, scrape off all the soot except for a 1.0 to 1.5 mm dot in the center (sufficient to cover the image of the Aperture Stop iris when that iris is fully closed), and mount the coverslip in a short SM1 lens tube so it can be screwed onto the back of the homebuilt objective.

1. Materials:
    a. Some clean 25 mm circular coverslips
    b. A stearin candle (can be ordered from Swedish goods stores like IKEA)
    c. Razor blades
    d. SM1L03 or SM1L05 lens tubes to mount the coverslips in later
2. Preparation:
    a. Put a coverslip in an SM1 lens tube, screw the tube onto something, then put a Sharpie marker against the center of the coverslip and rotate the SM1 lens tube so that you get a perfectly centered dot to use as a template for where to scrape off the soot on the other coverslips.
    b. Set up a microscope rig; after setting up proper Köhler, remove the sample. You will use this to test the transmission of the coverslips.
        i. Hold the clean coverslip in the sample area of the microscope rig and optimize the camera so a clear area of the coverslip is close to, but not quite saturating (1000 counts or so).
        ii. Turn off the room lights – you need very low background, since the correct transmission of the soot is ~ 10% (i.e., 100 counts if 1000 is what you get in a clear area of the glass), so very low background helps.
    c. Light the candle so it warms up.
3. Wave a clean coverslip through the candle, above the main flame where the flame is tapering into soot.
    a. It helps to keep the coverslip normal to the flame axis, and to move your hand at a constant velocity and slowly enough that the wind from your motion does not perturb the candle flame.
    b. Hold the now-sooty coverslip in the sample area of the microscope rig and see what the transmittance is. It should be ~ 10% (say, 100 counts if the original was 1000.)
        i. If it is too low, get a new coverslip.
        ii. If it is too high, wave the coverslip just above the flame again, and re-measure.

iii. If it is just right, move to the next step.

c. Optional:
    i. Per Pluta (see reference below), moistening the soot with a drop of "absolute alcohol" (basically anhydrous alcohol, in this case 99%+ ethanol) toughens the soot layer and (after the alcohol has evaporated) makes the soot layer more robust and easier to process. This is an easy step to add if you happen have reagent-grade ethanol handy, as many biology labs do.
d. Hold the coverslip on a Kimwipe (lint free paper) and scrape the soot off with a razorblade in a circular motion.
    i. Compare to your template; if it looks good, go to the next step.
e. Use a tightly folded Kimwipe to wipe the coverslip down everywhere but the central dark spot (which must remain perfect), to get all other soot off.
f. Put the coverslip in an SM1 lens tube and screw in a retaining ring.

4. You are done. Test the phase plate in your rig to see if it works for phase contrast.

Phase Contrast Lab Steps:

1. Get a decent phase sample (e.g. human cheek epithelial cells, as described in the Lab 8 Lab Notes).
2. Set up your rig for good imaging, in Köhler illumination, etc.
3. Screw your phase plate onto the back of your objective.
4. Open the BFP iris to several millimeters (say, 5.0 mm).
    a. The reason for only opening the objective BFP to 5 mm is to reduce aberrations in the objective; the better the imaging system the harder it is to see clear samples at the plane of best focus. Aberrations introduce phase shifts which can actually make the sample more visible if it is clear.
5. Close the aperture stop iris down to its minimum.
    a. Check in the BFP camera to see that the AS is closing down so that only the area covered by the soot is illuminated. If not, and there is filament visible around the edge of the soot spot, fix your alignment.
        i. If the AS and spot do not overlap perfectly, especially vertically (for which there are no mechanical adjustments), try rotating the phase mask SM1 lens tube; the black spot may not be centered in the mask tube.
        ii. If you cannot center the soot spot, make another phase plate.
6. Once you close down the AS so that only the soot is illuminated, you should be in phase contrast. Open the Aperture Stop iris to get to brightfield; close it to go back to phase.

References:

- "Zernike on phase contrast", from Inoué 1st ed. ISBN 0-306-42120-8, pp. 119-122.
- The article by Zernike (Appendix K in Strong; c.f. References in Appendix E) is excellent, and available as a cheap Dover paperback, or free online from the Open Library (See the *Reference Links* tab at www.thorlabs.com/OMC for link)
- Pluta, Maksymilian; "Stray-light problem in phase contrast microscopy or toward highly sensitive phase contrast devices: a review", Optical Engineering 32(12), 3199—3214 (December 1993). [N.b.: Pluta does not appear to list the wavelength he uses, but it is surely in the visible, and the masks work fine in our experience.]

THORLABS

Lab 9 Notes:
Fluorescence Microscopy

Optical Microscopy Course

Lab 9 Instructor Notes:
Fluorescence Microscopy

General Items

- This lab is harder without spectrometers, but not every student group necessarily has to have their own. We recommend that even if you do not have sufficient spectrometers for each team, you have a station or two where each team can go to do some of the spectrometer portions for themselves.
    - Otherwise, you can work around the absence of spectrometers by adjusting your use of the notes. To this end, lab steps that require a spectrometer are listed below, with suggestions for alternatives. Note that some steps have parts that do not require a spectrometer, but give the same information by eye – certainly include those parts.
- It is important to have the room lights off (and the room fairly dark) during all fluorescence imaging steps. If you have not already arranged for the room to be fully dark, do so prior to this lab.

Lab Preparations

- Practice this lab in advance. Main things to check for:
    - **Can the room be sufficiently darkened?** If not, cover the windows, etc. (Aluminum foil blocks light extremely well and is easy to put up).
        - Note: The room does not need to be blacked out with students in it –light from monitors or other dim additional light is fine. Our experience is that the combination of monitors and rig lamps provides sufficient light for people to see much of what they are doing; the provided clip-on LED lamps and flashlights provide for any additional lighting needs.
        - Remember: It helps to have the monitors facing *away* from the mono cameras.
            - Slight tilting of the monitors can help a lot
            - Many monitors have brightness settings, which allow one to reduce the backlight intensity. This can be helpful.
            - Students sometimes mistakenly leave their beamsplitters in, and these can then be in a position to reflect monitor light directly into the sample camera. Keep an eye out for this problem.
    - Make sure there are some lights. **LED flashlights should be charged/have fresh batteries.**
    - **Pink highlighter: Does it work?** Some do, some do not, but in general, the "fluorescent pink" variety seem to work well, and the more "flat pink" or "regular red-ish" variety do not. The manufacturers do not specify them for this application, and the markers do dry out over the years, so you need to check a new one *before* lab. Give yourself time to find a different highlighter, just in case.
- Have Kimwipes available, and tape (we like Scotch "magic" tape). If you do not have Kimwipes, Thorlabs lens paper also works well (not true of all lens paper – you want paper with fibers and space between the fibers; some other lens paper is fairly continuous).
- **Mount the filters (if this has not already been done).** We do not have students do this, to prevent damage to (fingerprints on) the expensive (and delicate) filters.
    - There is a proper orientation for the filters: the Thorlabs labeling convention is that the arrow faces in the direction the light is intended to go through them, so in this case the arrows on the excitation filter should face away from the lamp, and on the emission filter, away from the sample (which happens to also be away from the lamp in this case).
    - **You really want to have the arrows labeled on the filter mounts (on the TRF90 flip-mount and SM1L05 tube).**
        - We use a half-inch (12.5 mm) SM1L05 lens tube to mount the emission filter because it gives a little extra distance before fingers can hit the filter.

- Keep the lens caps on! We store the filters between classes by screwing all the emission filters together and putting a lens cap on one end (and all into a cabinet, so dust does not get on the other end). We also put a lens cap on the excitation filter in the TRF90 flip-mount and in between classes, flip the green filter in front of the other side, excluding dust.

- Get out the pink plastic slides, and make sure that when presented to students they are not in a box labeled "fluorescent slides" or anything like that. **It is hard to appreciate how difficult it is for students – even though they have just read the Course Notes, and the Lab Notes are entitled, "Fluorescence" – to figure out what is happening. It is a definite teaching moment. *Don't give it away.***

Example Quiz 9 Questions

1. Approximately what wavelength is red light? Green light?
2. What is the formula for the Collection Efficiency of a lens?
    a. You can decide whether to ask for the low- or high-NA version of the formula.
3. The Stokes shift results in what difference between the emitted fluorescence and the excitation light?
4. What is the purpose of the emission filter?
5. When a fluorescent molecule is destroyed by light, it is called _____?

Lab Tasks

Main Things to Emphasize:

- Students really struggle with Step 13 – which is good; it is a fantastic learning moment. If they ask for help, do not give it away, but lead them toward understanding. For instance, we work with the lab groups individually, and often ask them, "what wavelength is hitting the slide?" They always get this. Then we ask what color they see, and what wavelength that is. They can do that. Then we ask where that came from, given that none of that wavelength was hitting the slide. Where *did* it come from?
    - Once they figure out that the light is new, being somehow generated by the slide, then they have it. We often take this moment to point out that this is a fundamentally quantum-mechanical process. The absorption of one color and emission of another is not a classical phenomenon; they are seeing quantum mechanics at work.
- Certain filter combinations do not result in an image. Some students must repeat the process several times before they figure out that they must always keep in mind *what wavelengths will make it through to the camera.*
- Re: Step 20, another good filter example is to have students do the following. Only suggest this after students have completed other steps, and if they seem to be understanding well.
    1. Flip in the excitation filter.
    2. Adjust your mono camera so the image is not saturating (you do not need a sample in).
    3. Hold the emission filter at the sample plane so the light hits it straight on. What do you see on the camera?
    4. Tilt the emission filter 20˚ or 30˚. What do you see on the camera?
    5. Flip the excitation filter back out.

General:

- Steps 5, 8, 12, 20, etc.: We have students hold the fiber while taking spectra, to keep things moving and to keep the focus on the rough result rather than trying to get things exact. However, one can mount the fiber as shown below, and take many of the spectra at once with a consistent experimental geometry. If you do this, spectra to take are:
    - Lamp, with and without the IR filter
    - Lamp with IR filter and VG9 green filter
    - Lamp with IR filter and 520-40 interference filter
    - Lamp light (with IR but no green or interference filters) that has gone through the pink plastic slide

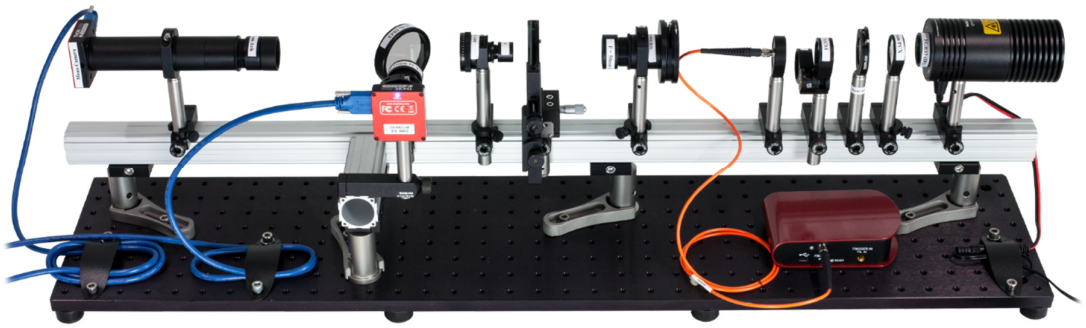


Figure 1: Fiber mounting geometry.

- Step 32: The Carolina Biosciences H&E stained tissue sample "Human prostate gland – young, sec. 31-6542 (H8725)" has a good bit of interesting structure; liver samples often seem to be less ideal.
    - If you want, you can have students read up (Wikipedia is sufficient for this purpose) on the H&E stain (best done while they are viewing the tissue samples – it is important not to overwhelm them with such reading in advance of the lab, but it can be interesting for them if they are looking at light and dark features in a tissue sample and want to try to identify what they are). For instance, the (blue) hematoxylin primarily binds to DNA and RNA, in the cell nuclei, whereas eosin mainly binds to protein, in the cytoplasm. Students may be able to identify some of this in the tissue sample images.

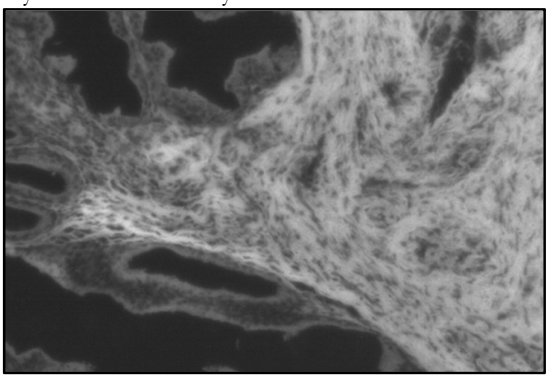


Figure 2: H&E stained human prostate (young) imaged in fluorescence using the kit rig. Note dark staining of nuclei by hematoxylin, and bright (eosin-stained) cytoplasm. An example of chemical-specific contrast!

- Students who image with both long exposure (can be nearly 1000 ms) and no (or small) gain should notice a difference (especially with the bead sample) from an image with maximum gain. It can be worth discussing the difference with them or covering it in class – why longer exposure gives you more signal for a given amount of noise, and thus higher SNR (signal-to-noise ratio), while using gain multiplies both signal and noise and thus does not improve SNR; although use of the gain does allow making optimal use of the camera dynamic range for a given exposure.

- We have found that it is counterproductive to get too far into the details of signal, background, and noise from different sources (dark signal, readout, etc.) – the conceptual content of the basic fluorescence imaging is more than enough for students to digest in one lab.

Without a Spectrometer:

Options:

- We use Thorlabs CCS200(/M) spectrometer, which is quite versatile. If you do not have those,
  - Companies like Vernier and Ocean Optics make educational spectrometers. These will work adequately for many, if not all, of the steps below, and they may already exist somewhere on your campus.
  - Often a campus lab will have a USB spectrometer that can be borrowed – even if there is only one, used by the Lab Instructor for demo purposes, live spectra demos can be very pedagogically effective – more so than just having students look at the printed spectra.
  - Note: Using most USB spectrometers, spectra can be taken live while students watch on a projector. This is very effective for class demos and is a possible approach to a lab with insufficient spectrometers for each team to have one.

Lab Steps that use a spectrometer include:

- Alternative: Provide spectra to the students:
  - Step 5: Incandescent lamp spectrum.
    - The QTH10(/M) lamp has color temperature of 2800 K; if you use a theoretical Planck spectrum as a substitute, remember the filter will remove much of the IR.
      - The FGB37M filter transmission data is available on www.thorlabs.com.
  - Step 8: Lamp with interference filter spectrum.
    - Interference filter is Thorlabs FBH520-40 (500 – 540 nm interference bandpass filter); spectrum available on the product website.
  - Step 12: Light coming from illuminated area of pink plastic slide. (See next section.)
  - Step 25: Lamp spectrum with VG9 green glass filter in place. (See next section.)
    - VG9 transmission data is available on the Thorlabs website.
- Step 13: **DO NOT omit this step; it is the main conceptual content of the lab.**
  - Step 13 can be done by providing students with spectra for the previous steps, or simply having them identify spectra by eye in the earlier steps – the wavelengths are well separated in general.
- Step 20:
  - Step 20 has parts that do not require a spectrometer but give the same information by eye – certainly include those parts, and comment on the meaning/implications of the blue shift as one rotates the filter so the light is going though it at an angle.
  - The emission filter is a Thorlabs FELH0550 (a 550 nm long-pass interference filter); transmission spectrum available on the Thorlabs website.
- Write-up Steps 1 and 2: Instructor will have to decide what to do, depending on how they have handled the lack of spectrometers for each team.

Reference Spectra (If You Don't Have a Spectrometer):

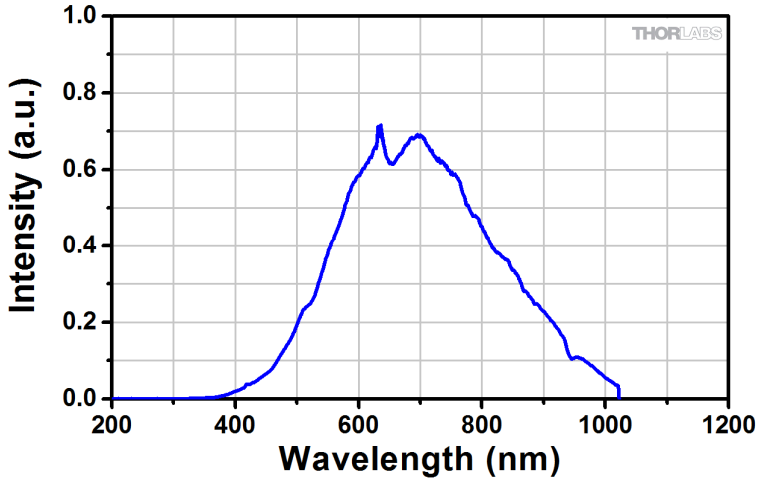


Figure 3: Spectrum (uncalibrated) of light from the QTH10 lamp, with only the collector lens inserted. Integration time = 0.2 ms.

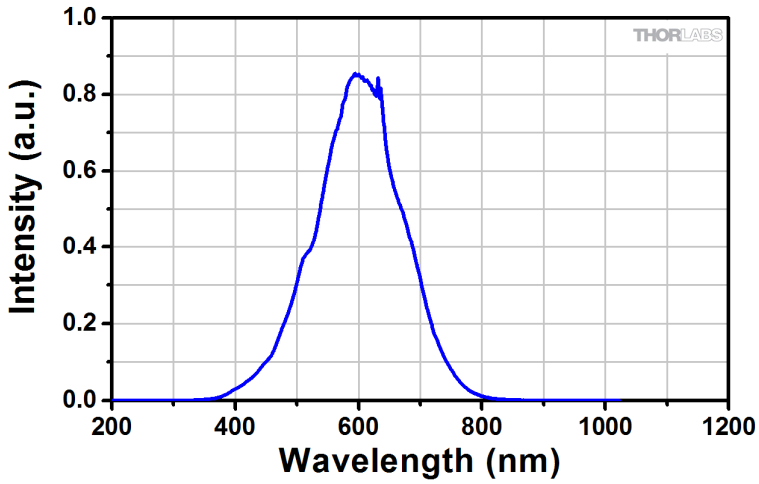


Figure 4: Spectrum (uncalibrated) of light from the QTH10 lamp, with only the KG5 filter and collector lens inserted. Integration time = 0.2 ms.

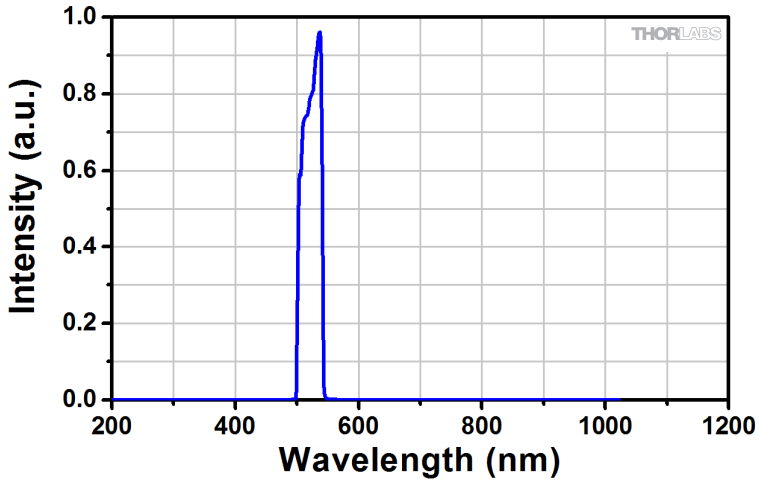


Figure 5: Spectrum (uncalibrated) of light from the QTH10 lamp, with the KG5 filter, collector lens, and 520-40 excitation filter inserted.

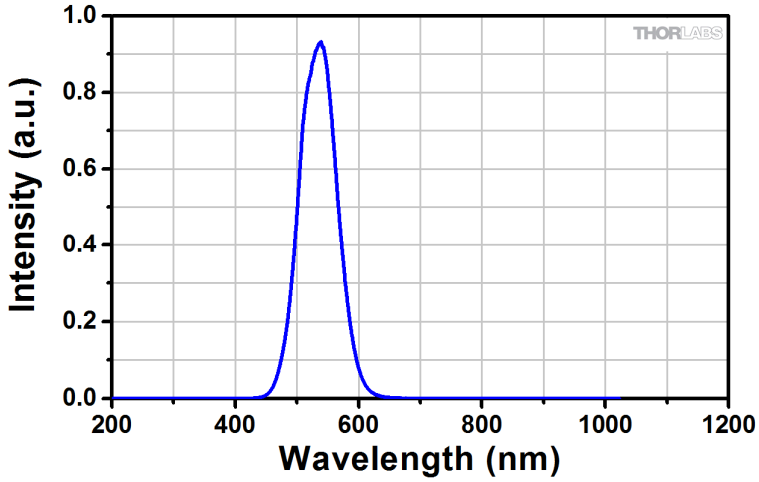


Figure 6: Spectrum of light from the QTH10 lamp, with the KG5 filter, collector lens, and FVG9 filter inserted. Integration time = 1.4 ms.

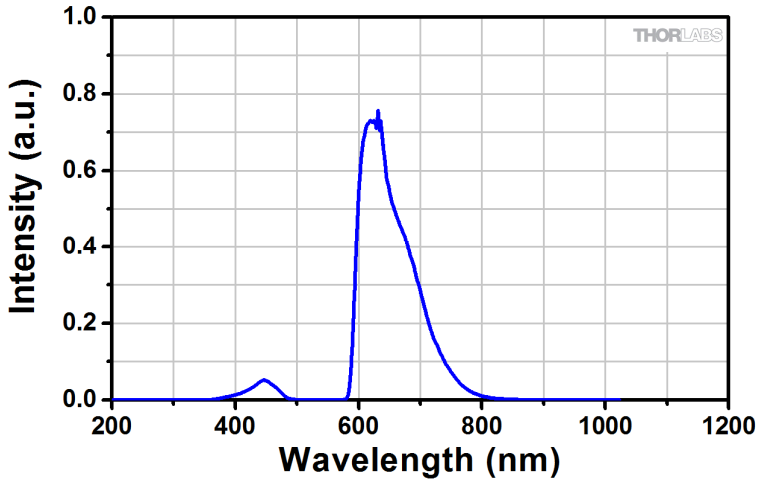


Figure 7: Spectrum of light from the QTH10 lamp, with the KG5 filter and collector lens, with light going *through* the red/pink Thorlabs plastic slide. Integration time = 0.2 ms.

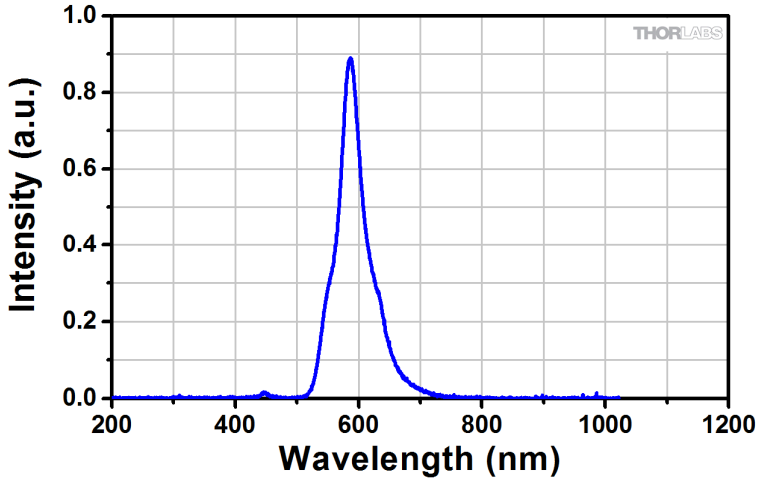


Figure 8: Spectrum of light coming from the pink/red plastic Thorlabs slide, with fiber held against same side as illumination hits slide. Illumination is from QTH10 with collector lens and 520-40 excitation filter in place, as in Fig. 5. Integration time = 200 ms.

THORLABS

Lab 10 Notes:
Spectra and Filters

Optical Microscopy Course

Lab 10 Instructor Notes:
Spectra and Filters

General Items

- This lab has several optional parts, and in addition there is an extensive homework assignment. As an instructor, you should assess your class and decide (in advance of the lab) how much of the optional work to assign, and how much to shorten the lab report for this week. **We strongly suggest minimizing the other tasks to make time for the filter problem set.**
- Filter Selection Problem Set: A major part of Lab 10 is having students use Excel to do quantitative fluorescence filter selection. The Excel file is available on the *Reference Links* tab at www.thorlabs.com/OMC; the first tab has the assignment, and the second tab has an example for students to follow. We highly recommend assigning this exercise; this is sufficiently important that we have written a separate section to help support it (at the end of this lab's Instructor Notes).
    - You can omit this assignment without harm to the rest of the class, e.g. if time is short or students are overburdened with other coursework. What makes sense depends on where this class is placed in your curriculum, how much preparation the students have had, etc. However, we strongly prefer to assign the exercise.
    - Quantitative filter selection is a very useful job skill, and this practice is an excellent engineering (and science) exercise. We explain this directly to the students.
- Excel: Some instructors will prefer to have students use MATLAB, Python, etc., to do this lab. Making such a change will require prior adjustments to the lab notes.
    - Note: We use a spreadsheet (Excel) for two reasons: first, while faculty tend to assume all students are proficient with spreadsheets, this has not been our experience, and the practice is thus helpful for some. Second, in industry – where 85%+ of all students end up working – everyone (engineers, salespeople, the CEO) has Excel, while MATLAB and other expensive packages are not available to everyone, especially to newly hired junior engineers. This fact seems often overlooked from within university training programs, where student editions of the software can be deceptively inexpensive. As a result, being proficient with Excel (in particular; other spreadsheets are less widely adopted) is a remarkably important job skill.
- Office Hours: We have found that a student instructor office hour is helpful during the week of Lab 10, focused on helping students use the software. Typically, our student instructors do not have office hours other weeks, since all of the work is in the lab. The week of Lab 10, especially if one assigns the filter selection problem set, is an exception. An office hour is decidedly helpful for students struggling with Excel, or quantitative filter selection using Excel in particular.
- Etalon Fringe Spacing (Steps 61-65): We make this optional depending on the class. For more advanced students it is an excellent exercise (and they can work out the math on their own – actually quite tractable – or look it up in a book such as Hecht's Optics). For students who do not understand the theory, or struggle with Excel, finding the thickness of the plastic sheet is very difficult – in that case, we suggest allowing them to skip this exercise.
- Relative Quantum Efficiency: We make this an optional part of the lab and place it at the end. Instructors may want to decide ahead of time whether to ask students to do this portion. It is here (and with the Etalon Fringe Spacing) that the bulk of the in-class Excel problems arise for students, when they do.
    - The Relative Intensity measurement is a nice demonstration of both how to (roughly) calibrate an instrument and also the power of the spectrometer software. Given that the steps are laid out, it is more time consuming than actually difficult.
    - The Relative Quantum Efficiency will be harder for students but is an excellent exercise.
        - If you want, you can discuss why the QE is low in the blue (photons are absorbed at the silicon surface, so photoelectrons are not captured well by the charge separation region, which is deeper in the bulk below the electrode) and red (absorption falls off as the photon energy drops close to the bandgap, such that there are fewer electronic states that can be excited and result in

absorption, dropping of course to zero for photon wavelengths corresponding to energies below the semiconductor bandgap). Poorer absorption translates directly to lower photoelectron generation, especially in the charge separation region defined by the doping and electrode(s).

Lab Preparations

- The lab uses Thorlabs spectrometers, with the Thorlabs OSA software. If a different software package or spectrometer is used, prior adjustments should be made in the lab notes.
- **It is extremely helpful for the instructor (or teaching assistant) to familiarize themselves with the Thorlabs (or other) spectrometer before the lab.** Being able to help students make the most of the equipment goes a long way toward decreasing any frustration on their part. The Thorlabs OSA software, in particular, is quite powerful, and it is possible to display calculations and other spectral features in real time, but only if one has practiced ahead of time.
    - The OSA Quickstart video can be useful here, for both students and instructors. (See the *Videos* tab at www.thorlabs.com/OMC).
- A box of fresh microscope slides is necessary for the interference lab steps.
- If the classroom lights are not already fluorescent, then having a fluorescent bulb/lamp available for students to take spectra from is important.

Example Quiz 10 Questions

1. What two spectra do you need to measure to calculate the transmission of an object?
    a. Extra credit for listing Dark spectrum as well (which would make *three* spectra).
2. Roughly what features do you expect to see in the spectrum of a fluorescent lightbulb?
3. What part of an optical fiber should never be touched?
4. What is an etalon (or a Fabry-Perot interferometer)?
5. (Optional) what is the name for a hot object producing a spectrum that perfectly matches theory?

Lab Tasks

- **Warning**: The spectrometer "Auto Setup" feature (available from the Thorlabs OSA software / Sweep tab, but only when sweep is Stopped) can be frustrating for students if they click it when no light is going into the spectrometer (e.g. if the fiber cap is still on, etc.). In that case, the process takes extremely long, and one cannot stop it except by ending the software task from the computer task manager.
    - In our experience, it is far better to have students set the integration time manually, adjusting it until the trace is nearly full scale (and right-clicking on the trace to rescale as necessary). This prevents lengthy autosetups in the case of no light, and also helps the students gain a better intuitive understanding of what the integration time is actually doing.
- Part VI, Steps 54 and 58: Interference effects. Interestingly the interference fringes in the transmission spectrum mostly go away if one holds the slides near the light source, instead of near the fiber. It can be tempting to assume this is due to a larger angular spectrum of light incident on the slides (etalon), but the angular spectrum of light getting through the slides and into the fiber is the same no matter where one holds the slides (assuming it is on the fiber side of the collector lens). The effect is more likely due to the fact that the spacing between the slides varies with position on the slide; thus for a fixed cone angle of light transiting the slides, the illuminated area of the slide – and thus the variation of slide spacings sampled – gets larger as

one moves the slide farther from the fiber face. This variation in the slide spacing causes the fringes to average out.

Main Things to Emphasize:

- That fluorescence imaging depends on the combination of excitation and emission filters blocking all of the illumination (excitation) light.
- The blue-shift in interference filter transmission with increasing angle of incidence.
- "Etalon effect" – the danger of unintentional interference fringes arising from pairs of flat surfaces in an optical system, and that a slight tilt of a flat optic can help avoid this. Note: DO NOT offset the angle of interference filters, since they depend on normal incidence to work properly and their performance degrades (and transmission band blue-shifts) as the angle of incidence is varied.
    - Note: The exception, of course, is if they were designed for a different angle of incidence – dichroics, for instance, are designed for 45° incidence. However, note that the performance of dichroics is far worse than for filters designed for 0° incidence… often a dichroic can barely block at the 1% level, whereas a 0° filter can block at OD 6 to OD 8 (i.e., up to 1 part in 10^8) for modern filters.
- General Lab Technique:
    - Importance of subtracting off baseline from a measurement (e.g. the dark signal in this lab). **Many students have not had that much actual measurement experience, and this is an important element to underscore for them.**
    - That one should check the calibration of one's instruments, and some (very rough) coverage of techniques for doing that when using a spectrometer.
- (Optional): Discuss/point out the quantum efficiency of the detector – particularly, that where it is low one expects the data to be more noisy (since signal is low – we sometimes mention Poisson statistics and $\sqrt{n}$, depending on the class) and noise is also at least partly independent of signal (due to dark signal), so S/N gets bad for multiple reasons as signal drops.

General:

- Students often get confused about the terms "red-shift" and "blue-shift." It is worth explaining that this is shorthand for "shifted to a longer or shorter wavelength," and some examples do not hurt either.
- Students also sometimes struggle with what happens when light goes through more than one absorbing element; e.g. two 50% transmission windows (Question 6 in the lab notes). It can be worth going over this during lecture prior to lab. While it may seem obvious, it is often not for students. Moreover, students have difficulty separating out what it means to have transmission *at different wavelengths*; students who understand the 50% transmission question may then still struggle with figuring out the transmission through multiple elements at different wavelengths (except in the case where one of the transmissions is zero). Expect to assist people with this / keep an eye out for people confused by it.
- If you have students do the Optional RQE portion of the lab, it is helpful to do it yourself in Excel before the Lab period, since questions usually arise.
- Going over the following before class can help make things go more smoothly:
    - Absolute cell references (e.g., using A4 to lock a reference to cell A4 when copying a formula down a column) and the use of the F4 key to toggle the absolute reference quickly/easily.
    - The "max" function
    - How to enter π in Excel using "pi( )"

References:

Absolute spectral power measurements are actually very difficult and depend critically on getting many factors right. Hence, this lab focuses only on *relative* measurements. Furthermore, tungsten-filament lamps are not exact blackbodies. This is mentioned in the notes, but should references be desired (e.g. for a student project), then the following may be of use:

Edmonds, Stephan-Boltzmann Law in the Laboratory. Am J Phys 36, 845 (1968); DOI: 10.1119/1.1975165

- A starting point (for Stefan-Boltzman, not so much the Planck distribution)

PASCO Model TD-8555 Stefan-Boltzman Lamp Instruction Sheet

- Available online – has nice table of resistivity of tungsten vs. temperature.

Ikonen, Spectral irradiance model for tungsten halogen lamps in 340-850 nm wavelength range. App Opt v 49, n 5, pp. 880-886 (2010); DOI: 10.1364/AO.49.000880

- Lots of details… more than students will generally need.

If a student wants to dig into the literature on blackbody references, there are a pair of papers from the National Bureau of Standards (now NIST); this gives both a sense of what is involved in work at such a national lab, and (since it is a national lab) are free online. They are:

De Vos, J.C., 1954. A new determination of the emissivity of tungsten ribbon. Physica, 20(7-12), pp.690-714.

Stair, R., Johnston, R.G. and Halbach, E.W., 1960. Standard of Spectral Radiance for the Region of 0.25 to 2.6 Microns. J. Res. Natl. Bur. Stand, 64(4), pp.291-296.

Lab 10 Filter Selection Problem Set

General:

- This can be a fun exercise for students – the better students love to see if they can beat the recommended standard filter set, and who can get the best possible result.
- Doing this exercise can help solidify filter selection for students, which is handy since we always ask them to choose filters on the final exam (we give a bunch of plots for filters, as well as the dye spectra, camera QE, and illumination spectrum, and have them choose the appropriate filters from the ones we give plots for).
- Students can struggle with spreadsheets/Excel. This is half the point of the exercise: being prepared to help them can make their experience better and everyone happier.
    - Consider an extra Office Hour(s) dedicated to Excel.
    - If your campus IT department offers such a thing, we have found that a one-hour "introduction to Excel" workshop for students can make things much easier.

Main Things to Emphasize:

- Students can lose track of the point of doing all the sums. It helps to cover (in class) what is really happening: pick a single illumination wavelength and follow the calculation (actually plug in the numbers based on the spectra): how much light will you get *from* the fluorescent molecules?
    - Illumination photons * ex. filter transmission * molecular absorption = total excitation.

  In general, students will be OK with this, then just point out that the *total* light one will get from the molecules will be that same calculation done at every wavelength and added up.
  Similarly, do it for the light you will detect: it will be some number proportional to how many photons got absorbed (i.e., total excitations) times
    - Emission spectrum * em. filter transmission * camera quantum efficiency (sensitivity)

  Again, students should be OK with this, and again, it's worth doing it by plugging in the actual numbers at some given wavelength (different than the illumination wavelength – pick something near the emission peak). It is worth emphasizing that these are all *relative* numbers, but they allow a nice relative comparison. If one makes the area under the emission spectrum = 1, then it becomes the probability an emitted photon will have a given wavelength, and the numbers become absolute, not relative. Often this is unnecessary since all you usually want to know is how to choose the best filters, or how much better one set is than another. And again, adding the values up for every different wavelength gives the total detected light.
    - It is also worth pointing out that total brightness should scale with (total excitations * total detections per excitation).
- Depending on your class, you may or may not choose to have students do the "filter leakage/background" part of the problem set – it is of secondary importance to the basic idea of maximizing the brightness one gets, and can be left out if that makes sense for your class.
- Students struggle with the use of log plots to look at the filter leakage. Many students do not really understand logs, and log plots; gaining experience with them is yet another reason for them to do this problem set. It is useful to point out (in class) that the number of illumination photons is about a million times the number of emitted fluorescence photons, so blocking must be very good. And on a linear scale, it is hard to see 1%, let alone 1 part in a million. Hence the use of

log scales. Also, this is a moment to go over again with students that what matters is the *product* of the excitation * emission filter transmission (particularly in the region where their transmission bands might overlap).

- This is also a moment to comment on the problem that can occur if a filter is tilted so that the transmission band gets blue-shifted.

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. It is 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 ThorCam and OSA Quickstart videos). All of these are available under the *Videos* tab at www.thorlabs.com/OMC. The EDU-OMC1(/M) Manual can also be helpful.

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.
    - NOTE: student changes of cameras, etc. will rapidly result in changes; these are decent starting points when trying to sort out imaging issues that may arise during a lab.
- Camera General Settings Tab:
        - Exposure Time (ms): 100 ms
        - Frame rate control: Uncheck (unless you see dropped frames). Automatically maximizes frame-rate.
        - Gain: "0"
        - Black Level: "0"
    - Camera ROI and Binning Tab:

        - Click on “Full Frame” which results in a 1440 x 1080 resolution.
    - (For color camera): Camera Color Tab:
        - Color Image Type: sRGB
        - Continuous Auto White balance: Uncheck
        - Click “Clear Gains” on the Camera Settings dialogue, ‘Color’ tab. This sets all color gains to 1 (Red, Green, Blue)

THORLABS

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. Having two separate USB 3.0 ports for the cameras will work best (allowing the highest frame rate on both).
- Large, color screens are necessary.

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(/M) spectrometer.
            - 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(/M) 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.
        - Warning: USB hubs can reduce data transfer speeds. We NEVER connect cameras through a USB hub. However, low-bandwidth items – the keyboard, mouse, LED light, and the individual LEDs – can all be productively run via a single hub.

THORLABS

Appendix D: Exam Practical Suggestions, Tips, and Templates

Optical Microscopy Course

Appendix D Instructor Notes:
Exam Practical Suggestions, Tips, and Templates

Practical exams are extremely useful for incentivizing ***both*** students in a lab team to learn all parts of the equipment (otherwise there can be a tendency for one student to always handle alignment and the other to always handle the computer). It also serves, during the middle of the course, to motivate students to truly learn how to quickly set up Köhler illumination (we tell them well in advance that this will be part of the midterm exam).

We typically break the midterm into two sections: a one-hour written section and an approximately 20-minute practical section (per student.) We allot 30 minutes for each set of students, allowing time to get the previous students out of the room, set all the rigs back to the same state, and bring the new students in.

The most critical aspects of the midterm practical are:

- Tasks should be simple enough that they can be completed without ambiguity in 3 - 5 minutes.
- The placement of any given item should not depend on any other item.
    - E.g., if the collector needs to be moved, one should be able to position it properly without having to move the field stop or lamp.
- Be sure to give hints in a timely manner if a person is stuck.
    - We tell students they can ask for a hint, at the cost of some points. If we see they are truly stuck, yet refusing to ask for a hint, we will eventually press them to accept one (though they always have the right to refuse).
- Students panic. Reassure them that the tasks can definitely be completed in 5 minutes if they think it through and know the material – there are no trick questions, nor is any massive (re)alignment expected.

General Exam Organization (for a class of 2 sections of 12 students, with 2 hours total for the exam):

- The entire class meets in the classroom.
- Half the class then goes to a waiting area, while the remaining half are given the exam and have one hour to complete it.
- Half of the students in the waiting area (25% of the class) continue to wait, while the other 25% come to the lab room to do the practical.
    - This results in one student per rig.
- After 25 min., those taking a practical go back to the waiting area and the remaining students in the waiting area come to take the practical.
    - It is worth having the students returning to the waiting area not pass the students going to the practical room, if at all possible.
- After the second set of students have completed the practical, all students in the waiting area go to the classroom to take the written midterm, and the students who had been taking the written portion go to the waiting area and rotate to the practical as above.

Organization of the Practical

Setup:

- It is important to go through all the rigs which will be used for the practical and make sure that they are all set up identically. This usually means essentially rebuilding the rigs, since students often have not tightened mounts down fully, etc.
    - **This usually takes ~4 hours or so (for 6 rigs), sometime after the previous weekly lab and before the exam.**
    - Depending on the timing of the exam, time may also need to be allocated to return the rigs to their appropriate state for the upcoming weekly lab.
- It is also critical to make sure that all students experience the same problem during the practical. So any changes (misalignments, changes in exposure time, etc.) in the rigs need to be done the same way on each one.
    - **This requires having a list of the changes to be made in each system (e.g. exposure setting, distance condenser is moved from the sample, number of micrometer turns of defocus on the stage, etc.) that is used to misalign each system in identical fashion before each new student uses it for their practical.**
- Typically, each set of students has 20 minutes to do the practical portion. It usually takes an additional 5 minutes just to get students in the door and the old set of students out, and perhaps another 5 minutes to set the systems back up identically before the new team comes in, for 30 minutes total.
    - We usually tell students that they have 20 min., and to give them 15 min., 10 min., 5 min., and "2 min. left" warnings.
    - **Students <u>always</u> panic during the practical** (even, and sometimes especially, the good students.) As a result, tasks to be done during the practical should never take the instructor longer than 5 min.; things that take the instructor 5 min. typically take students in the practical a minimum of 10 to 15 min.

Grading

- Grading is very important. It is critical to catch students who are stuck at some step and to give them an appropriate hint to get them going again while they still have time to demonstrate what they do know.
    - **We are careful to rotate past each table every minute or two and if we see someone who has been stuck for couple of minutes, we let them know that we could give a hint but would have to dock points for that. If they do not want the hint, but are still stuck a few minutes later, we may press them to accept the hint so that they can get moving (and we note any hints on the grading sheet).**
    - Past experience shows that a full 1/3 to 1/2 the students will need some minor hint, and that without hints people who do actually know many of the things being tested will appear as if they know none of them.
    - There is no time to make detailed notes about grading during the practical. **Having grading sheets is critical: one for each student with the steps listed and a checkbox for when they have done each.**
        - We have usually broken these into 5 – 7 steps for the students to do (see example at the end of this document); any more steps is too many for the students to accomplish in the allotted time.

- **It really is important to give students enough hints** (if they need them) that they can have some success on the practical. **Most students are not used to practical exams**, and the point is to incentivize learning the material, not to negatively impact morale if they get flustered and struggle.

Choice of Tasks for the Practical

- Experience has shown that students simply do not have time to do significant realignments or rebuilding of the rigs during the practical. Furthermore, instructors will not have time to set the rigs back up between practical groups if they require a significant realignment.
- As result, simple tasks are best: moving a lens back and forth, getting image at a specific location, doing several steps appropriately in sequence, etc.
- Again, an important rule of thumb is that if the instructor cannot do the practical from scratch in less than 5 min. (really, in less than 3 min. after they already know what to do) then the practical is too long.
- Having too many steps which depend on each other can result in significant chaos.
    - It is better to have steps that are effectively independent. E.g., do not have both the field stop and the condenser as things which the students could move – that results in an infinity of possibilities. Rather, have the field stop fixed and allow the students to move the condenser, or vice versa.

Example

➔ This is only one of multiple practical exams we have done. Others have involved having students set up the tube lens and objective so they can image, etc. We usually have the midterm practical on Köhler and the final practical on something else.

Note on the tasks described below: The point of this practical was to have students demonstrate their knowledge of Köhler illumination, proper camera exposure, and the basics of dark field.

- The initial set up has the:
    - Sample out of focus
    - Condenser at the wrong distance from the sample
    - Collector moved so filament is not imaged at the condenser
    - Camera exposure improperly set
    - Objective back focal plane aperture too small for good imaging
    - Green filter out of the optical path
- **Important**: <u>The field stop and objective remain fixed</u>. This is critical, for otherwise there would be too many possibilities for placement of the condenser and collector. Whatever the practical questions, the student should experience no ambiguity about what needs to be done (assuming they understand the system.) For instance, with the field stop and objective fixed, there is only one
    - proper location for the sample
    - proper location for the condenser
    - proper location for the collector
- The proper approach to this problem (reflected in the example grading sheet below) is:
    - Köhler:
        - Focus the sample on the camera by moving the stage
        - Focus the field stop on the camera (and the sample) by moving the condenser

        - Focus the filament on the aperture stop by moving the collector

    - Imaging:
        - Adjust apertures, camera exposure, and insert green filter (to reduce chromatic aberration) to get best imaging of the USAF target. Points off for failing to do any of those things.
            - Note: Sometimes students use a histogram or line profile to adjust exposure, but then close the window before you come by to grade. If the exposure looks okay and there is no line profile open, ask how they know whether the exposure is set properly to find out if they did it correctly.
    - Darkfield:
        - For this step the instructor opens the aperture stop wide and covers the middle of the aperture with tinfoil, and the student has to produce a darkfield image after being told not to touch the condenser in any way.
        - This can be done simply by closing down the objective BFP iris
        - Some students asked for tinfoil; you can decide whether to give it to them or not.
        - Points are also given for setting exposure decently.
            - Note that smaller features diffract more and are therefore brighter; students may optimize for different Elements or Groups in the USAF target, so do not necessarily take points off if the smallest groups are overexposed.

Setup Prior to the Midterm

- **Rework all student setups so the mounts are tight, properly aligned, etc.**
- Misalign all setups identically (as per checklist on page D-6)
- Put page on table for student to put name on (to prevent any mistakes in grade assignment)
- We use red lab tape to indicate which components students can adjust. For this exam, we put red tape on the:
    - Stage
    - Condenser
    - Collector
    - Filter Flip-Mounts
    - ND Wheel
    - Apertures (BFP, Aperture Stop, Field Stop)
- Put a bit of paper on each breadboard for students to use to look at light in BFP etc.
- Prepare aluminum foil strips for darkfield exercise.
- Computers:
    - Turned on
    - Camera on, window open, set for 1:1
        - Gain = 2 dB
        - Frame Rate ~ 14 fps
        - Exposure ~ 30 ms
            - Set exposure "Max Slider Range" to 300ms
    - No line profile window open.

Practical Exam Grading Sheet

Section II: Practical

Student Name: _____

Grader: _____

Köhler: **Instruction ("Set up Köhler illumination.")**

(XX pts each; 4 questions)

_____ Focused on sample

_____ Focused field stop on sample

_____ Focused filament in aperture stop

_____ Used correct sequence (sample focus 1st, field stop 2nd, filament 3rd.)

Imaging: **Instruction: "Obtain the best image of the target."**

(XX pts each; 2 questions)

_____ Opened aperture stop and objective BFP pretty wide before imaging.

_____ Used green filter

_____ Adjusted camera Gain = 0; ND filters and Exposure to give good exposure/ dyn. range.

Darkfield: **Instruction: "Obtain a good darkfield image; do not touch aperture stop."**

(XX pts each; 2 questions)

_____ Obtained darkfield image without touching condenser or aperture stop.

_____ Adjusted ND filters, exposure, gain for good image/full use of dynamic range.

Practical Exam

Between-Student Setup Sheet

Announcement to all students at the start:

- **Students should write their name, large, on the piece of paper on the table.**
- **Students can:**
  - **Move ONLY the items with red tape on them.**
  - **Open/close any apertures, switch in or out any filters.**
- **DO NOT move things without red tape or twist optical mounts.**
- **Students have 20 minutes from the start of timing.**
- **There will be 15, 10, 5, 2 minute warnings.**

Setup of Rigs for Consistent Misalignment (Reset Prior to Each New Student):

- **USAF 1951 target small lines visible in center of field**
- **Move stage toward objective, to 2nd-to-last tick mark on micrometer**
- **Set ND wheel to ND 0 (open position)**
- **Collector : 2" (50 mm) from lamp**
- **Condenser: 3" (75 mm) from sample**
- **Objective BFP iris closed all the way, then opened to 2.0 mm**
- **Aperture stop, field stop all the way open**
- **Green filter flipped out of beam path**
- **Computer:**
  - **Camera on, window open, set 1:1**
    - **Gain = 2 dB**
    - **Frame rate control checkbox enabled**
    - **Frame rate = 14 fps**
    - **Exposure ~ 30 ms**
    - **Exposure "Max Slider Range" = 300ms**
  - **No line profile open**

Small piece of paper on breadboard (for looking at illumination at different planes)

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'd 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 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 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 review by the famous Tony Siegman 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 (only 14 pages) is packed with useful information.

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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