Optics and Microscopy by David Wu

1 Introduction

Microscopy is one of the main tools for visualization in the biological sciences. In order to keep a record of what we see, we must transfer an image of an object to film or hard disk - to that end, we interpose and need optics. Thus, we seek a grasp of what our microscopes are doing, and how to get the most out of them. This short write up is meant to accompany power point slides, available in the form of a 'pdf' on the course website. Most of the material was taken and modified from either the Olympus microscopy website, the Newport tutorials, CVI Laser tutorials, Fourier Optics by Goodman, and especially, Optics by Hecht.[1]

Following the power point presentation, we'll start with a few quick pointers on how to clean optics you will encounter in Aph162 lab, a brief review of geometric optics, aberrations upon those optics, Fourier optics, and finally talk about some of the microscopy configurations in the lab, if I can get it done.

2 How to handle and clean optics

We will be dealing with lasers, halogen or mercury lamps, objectives of various sorts, and bare lenses, all, perhaps, with coatings. But we want clean optics because high power lasers or high power arc sources can interact with contaminants on the optics and cause damage to the various coatings on the substrate, or ignite due to the intense heat. Moreover, dust, fingerprints, etc, will inevitably reduce the quality of our images.

That being said, the first rule of cleaning optics is don't clean optics unless one absolutely needs to. Any amount of cleaning will inevitable introduce microscopic scratches in the optic; multiple cleaning rounds will reduce the quality of the optic multiplicitally. But if one needs to, then the first thing to do is to lightly blow off the dust - that way, when lens tissue is applied to the surface, small particles won't be dragged around and scratch the surface. When blowing off dust or lint, one should generally use air sources without propellant, and with low particle number, such as purified nitrogen sources, a bulb blower, or camel hair lint brushes. It is generally discouraged to use office cleaning blowers, such as THF, since they have large diameter particles, and if one is not careful, propellant comes out and ends up covering the optic. Since THF is organic, it just might dissolve any organic coatings. Hence, if one must use office cleaning supplies, then light pressure and keeping the canister upright - so no propellant comes out - will do.

After removing as much lint as possible, there are three main techniques used to clean optics. The first thing to mention is the necessity of wearing gloves while handling bare optics. This is essential for avoiding getting fingerprints on the optics. The first method is drag and drop. A drop of methanol is placed on lens tissue (Kodak - not VWR!!), and the wet portion of the wipe is dragged across the surface, exerting no pressure.

The wipe method is used for harder stains, and involves folding the lens tissue until a single untouched corner is available; the tisse is then handled by a pair of hemostats or tweezers,

[1]For example, see the Olympus microscopy website, http://www.olympusmicro.com, or the wiki-book http://en.wikibooks.org/wiki/Optics, or chapters 5 and 6 in Hecht. The first 100 or so pages in Goodman's Fourier Optics suffices.

solution is applied to the wipe and excess shaken off. The wipe is then applied from the center of the optic outwards. The tissue is then discarded, regardless if another wiping must be used.

The last method is called the immersion technique, and is used for most metallic coatings. After the surface dust is removed, the optics can be immersed in acetone and rinsed in fresh solvent until clean. In general, bare metal coating and filters should not be touched in any way.

In the case of removing immersion oil from our microscope objectives, we fold a lens tissue and using a hemostat or tweezers, apply it to the surface with the oil on it, and let the oil soak into the lens tissue - we do this without moving the tissue at all. Scratching the surface of an objective is bad news. We then proceed by using the wipe method as described above. Again, once we move out of the region of the objective lens, we don't go back!

There are, of course, other more specialized ways of cleaning optics, but these are the methods that are appropriate for our use. So what solvents should we use for which surfaces? For our mirrors (non-metallic) or lenses, we use isopropyl alcohol, methanol or acetone or a combination thereof. Acetone plus alcohol forms an azeotrope, meaning that the mixture will not separate into its constituent components before evaporation; acetone increases the volatility of the solved, thus allowing for faster evaporation and less streaking. It is also more organic than either methanol or isopropyl alcohol. For the filters we have (including dichroics), methanol is generally okay (especially if the optic was made from a reputable company) but often times, we are just not sure (sometimes the coating is organic, meaning we don't use organize, otherwise the coating will be dissolved!). In this case, we need to call the company to ask. And again, for metal-coated optics, we only use the immersion technique, as described above.

3 Geometric Optics

We can think of objects in space as collections of point sources that emanate spherical waves upon illumination of light. A lens, therefore, acts as a collector of light by reshaping those spherical waves and imaging at the focal point of the lens. Since the lense will be inevitably imperfect, and can never collect all the light emitted, the image of the object will be blurred somewhat from the original - that is, the image is diffraction limited. We will return to this point in the Fourier optics section.

But for now, let's ignore any talk of waves or wavelength, and keep only the interposition of refracting objects in our light (ray) path. This limit is the geometric optics limit, and suffices for many practical aspects of optics.

Now, in order to form an image, the refracting surface must be shaped such that all light rays arrive at the focal point at the same time. So, if we consider a point emitter of light rays, and interposed in the path of the rays, an object of different refractive index but spherical in shape, it can be shown that for a certain ray that arrives at the spherical surface at height h, that

$$\frac{n_1}{l_o} + \frac{n_2}{l_i} = \frac{1}{R}\left(\frac{n_2 s_i}{l_i} - \frac{n_1 s_o}{l_o}\right) \tag{1}$$

where the notation can be found in the accompanying power point (slide 6). For small ψ, $cos\psi \approx \psi$ and $sin\psi \approx \psi$ so $l_o \approx s_o$ and $l_i \approx s_i$ and so

$$\frac{n_1}{s_o} + \frac{n_2}{s_i} = \frac{1}{R}\left(n_2 - n_1\right) \tag{2}$$

This is the ‘paraxial’ approximation or 1st order optics or Gaussian optics; ray optics are appropriate when rays are of small deviation from the optical axis. This might also give you an idea of how far off we will be if we deviate from small angles; we will return to this point later.

Now lets consider light approaching a spherical surface and passing back through some material of a certain thickness into the original medium, forming an image point. So beginning with

$$\frac{n_1}{s_o} + \frac{n_2}{s_i} = \frac{1}{R}\left(n_2 - n_1\right) \tag{3}$$

we end up with (slide 7)

$$\frac{n_l}{s_{o1}} + \frac{n_m}{s_{i2}} = \left(\frac{1}{R_1} - \frac{1}{R_2}\right)\left(n_l - n_m\right) + \frac{n_l d}{s_{i1}\left(s_{i1} - d\right)} \tag{4}$$

and we note that as $d \to 0$, and if we set either s_i or s_o to ∞ (where we ignore the secondary subscripts) the conjugate parameter becomes either f_o or f_i, we have

$$\frac{1}{s_o} + \frac{1}{s_i} = \frac{1}{f} \tag{5}$$

that is, the Gaussian lens formula. Note that since $d \to 0$, this is also the thin lens approximation. This equation is central to any starting optics calculation when we set up optics, but in fact we barely even use this in a conventional microscope. Instead, we usually buy infinite conjugate optics, because thinking about optics in Fourier space is much easier. Also, we are usually focusing and collimating rays of light, and so the object is placed at the focus of a positive lens, and so its image is at infinity. Why we do this will be clear in later sections. A few more useful definitions:

Magnification is defined as $-s_i/s_o$. f/# is the ratio of focal length to diameter. We will also see that this ratio will be useful in determining how aberrated our images are. Numerical aperture is the ‘light collecting power’ of a lens, and is given by $NA = nsin\theta_{max}$, where n is the refractive index.

4 Aberrations

But then, why did we choose a spherical surface to make all our calculations (in the geometric sense), anyway? It turns out that it is feasible to make high quality surfaces shaped spherically. According to Hecht, this is because two spherical surfaces of the same radius of curvature will always fit on top of each other. Thus by randomly vibrating the cutting surface, the apposing material will be smoothed out nicely. If the surface were some odd aspherical shape, this clearly would be impossible. In face, apherical lenses are usually made from injection moulding - their properties will be touched upon later - but then their surface quality is at the mercy of the smoothness of the mould.

We will touch upon briefly, the two chief problems that will degrade an image: geometric aberrations and chromatic aberrations. The former is due to the fact that we use spherical surfaces to image our objects, or because our equations are for small angles, and the latter because light bends differently, depending on wavelength.

Spherical Aberration - These artifacts occur when light waves passing through the periphery of a lens are not brought into focus with those passing through the center - they over focus, producing a blurry image (slide 9). Waves passing near the center of the lens are refracted only slightly, whereas waves passing near the periphery are refracted to a greater degree resulting in the production of different focal points along the optical axis.

Let's begin again with the paraxial equation

$$\frac{n_1}{s_o} + \frac{n_2}{s_i} = \frac{1}{R}(n_2 - n_1) \tag{6}$$

If instead of approximating with 1st order, we try higher order approximations, then we end up with

$$\frac{n_1}{s_o} + \frac{n_2}{s_i} = \frac{1}{R}(n_2 - n_1) + h^2 \left[\frac{n_1}{2s_o} \left(\frac{1}{s_o} + \frac{1}{R} \right)^2 \frac{n_2}{2s_i} \left(\frac{1}{s_i} + \frac{1}{R} \right)^2 \right] \tag{7}$$

We see that as rays go beyond small ψ, the rays over focus. This is called spherical longitudinal abberation. We also see how to solve this problem. If we stop down with an aperture then we get rid of these rays that are out of focus then the image becomes more in focus. The price we pay, however, is that there is less light falling on our focusing screen, which means that we must integrate longer to get the same image brightness. Hence, lenses with a small numerical aperture are referred to as 'slow'. Another thing is to add another spherical surface to the other side of the lens, producing biconcave lense instead of simple plano-convex lenses. This will reduce the spherical aberration somewhat. Another possibility is to use proper aspheric surface, such as in point-and shoot cameras.

Coma refers to the phenomenom when off axis light sources do not come to a common focal point (slide 10). This is easily seen by taking a pair of spectables and letting some sunlight through. If one tilts the glasses enough, the image of the sun will be flared out, like a comet.

Astigmatism (slide 10) refers to when rays that are on different axes focus onto different planes. This depends on the power of the lens, as well as the angle of rays.

Field curvature (slide 11) is conceptually similar to spherical abberations - emitters off the central axis, for a flat object, are focused by the lens onto a concave surface at the image plane. We see that a field when looked through the lens will be slightly curved inward. This means that while the center of an image may be in focus, the edges will not (or vice versa). We also see how this can be corrected, by using a compesating negative lens. In fact, many of the lenses used in this lab are doublets - that is, a negative lens cemented against a positive lens to eliminate field curvature and spherical aberrations at the same time.

The last geometric distortion is called distortion (slide 11). Different areas of the lens have different focal lengths of different magnitude, resulting in so-called pin-cushion or barrell distortion. Unfortunately, how does one know if any of these are present, or is it that the specimen just looks that way? We use standards such as grids with know (or zero) distortion to calibrate our microscope.

Chromatic Aberrations - This type of optical defect is a result of the fact that white light is composed of numerous wavelengths. When white light passes through a convex lens, the component wavelengths are refracted according to their frequency. Blue light is refracted to the greatest extent followed by green and red light, a phenomenon commonly referred to as dispersion. The inability of the lens to bring all of the colors into a common focus results in a slightly different image size and focal point for each predominant wavelength group. Again, by using multiple elements in lenses with different refractive indices, one can correct for differences in color. Note that most so-called 'achromats' are corrected for green light.

5 Fourier Optics

We started off talking about a lens collecting the spherical waves emanating from a point source. In the geometric scheme, if an object is placed at the focal plane, its image is effectively at infinity, because that's how we draw the waves. In the diffraction scheme, the Fourier transform of a point is a plane (in 2-D), so that the wave emitting from the lens will never focus into an image of the point; i.e., the image is at ∞.

But why do we even need to talk about optics beyond the paraxial approximation? It turns out, by thinking about optics as linear systems, we gain a frequency representation of optics, and that provides us with a nice way to characterize optical systems beyond mere rules of thumb, including ways to truly define resolution.

An easy way to picture this is to decompose an object into its various sine and cosine frequency components, with low spatial frequency components near the center and higher frequency components near the edge of the Fourier image. This meshes quite nicely with how we think a lens limits the resolution of an optical system; lower collection angle means lower collection efficiency of diffraction orders, which means we can never reassemble the diffraction orders by refocusing, resulting in blurring of the object in the image. In any case, one can do tricks to filter the image, for example by masking edges of the Fourier transform to create a blurrier image (loss of high frequency components, such as edges) or conversely block the center of the Fourier image to remove the average brightness of an image and help with enhancing contrast, since the average brightness is nothing but the zero-frequency component in Fourier space.

Now consider the diffraction of light by an aperture in an opaque screen. We must satisfy the wave equation with appropriate boundary conditions. This results in certain equations which we can approximate by noting that the distance from an aperture to observation is many wavelengths.

Now, consider a light wave given by

$$u(r,t) = A(r)e^{i\phi(r)}e^{-i2\pi vt} \tag{8}$$

and that satisfies the wave equation

$$\nabla^2 u - \frac{n^2}{c^2}\frac{\partial^2 u}{\partial t^2} = 0 \tag{9}$$

Thus we are dealing with the Helmholtz equation

$$\left(\nabla^2 u + k^2\right) A(r)e^{i\phi(r)} = \left(\nabla^2 u + k^2\right) U = 0, \tag{10}$$

$k = 2\pi/\lambda$. Appropriate (Kirkhoff) boundary conditions specify that

$$U = \frac{1}{i\lambda}\int\int_{\Sigma} U\frac{e^{ikr_{01}}}{r_{01}}cos\theta ds. \tag{11}$$

See slide 14 for notation difficulties. This is the Huygens-Fresnel approximation. If we further approximate r_{01} as

$$r_{01} \to z\left[1+\frac{1}{2}\left(\frac{x-\xi}{z}\right)^2+\frac{1}{2}\left(\frac{y-\eta}{z}\right)^2\right] \tag{12}$$

i.e. the first two terms of a binomial expansion, then

$$U = \frac{e^{ikz}}{i\lambda z}e^{\frac{ik}{2z}\left(x^2+y^2\right)}\int\int_{-\infty}^{\infty} Ue^{\frac{-i2\pi}{\lambda z}(x\xi+y\eta)}e^{\frac{ik}{2z}\left(\xi^2+\eta^2\right)}d\xi d\eta \tag{13}$$

This is the Fresnel approximation (the binomial expansion), or the near field approximation. Two terms in the expansion are kept because we don't want the exponential in the numerator to blow up. Now if we say that $z >> \frac{k\left(\xi^2+\eta^2\right)}{2}$ then

$$U = \frac{e^{ikz}}{i\lambda z}e^{\frac{ik}{2z}\left(x^2+y^2\right)}\int\int_{-\infty}^{\infty} Ue^{\frac{-i2\pi}{\lambda z}(x\xi+y\eta)}d\xi d\eta. \tag{14}$$

This is the Fraunhofer approximation (far field) (slide 15). We note that this has the form of a Fourier transform. However, for the Frauhofer condition listed above, we see that for visible wavelengths, z has the distance of many, many meters. However, we can bring the Fraunhofer condition closer by interposing a lens between the spherical wave, the aperture and the far field. The lens thus focuses the Frauhofer diffraction pattern at its focal plane, producing a Fourier transform of the diffraction pattern. Thus we see that, according to the Huygens-Fresnel model, image formation by a lens is a double diffraction process - light diffracts off an object, and a lens computes the Fraunhofer diffraction pattern of the previous diffraction, forming an image. Abbe was the first to discover this.

5.1 Consequences

Some useful parameters that arise out of frequency analysis are such things as the point spread function and optical transfer function. While not going into specifics, we talk about it in general.

First off, on resolution. If we compute the Fraunhofer diffraction pattern for a circular aperture, we get an Airy pattern. If turns out that the width of the central peak is $1.22\lambda z/w$, w is the radius of the aperture, then this quantity limits resolution. Another way to think about this is that a lens, due to its finite width, has an aperture. We see from this equation for resolution that the resolution is dependent on spatial frequencies and wavelength. Heuristically, light diffracts off objects, and the smaller the object, the larger the diffraction angle; however, the lens has a limited collection angle, which in turn determines which frequencies are imaged, and hence, the collection angle (numerical aperture) and wavelength, fundamentally determining the resolution.

That being said, we can define a quantity called the point spread function (PSF) that characterizes the response of an optical system to a delta function, or point object. Naturally, an optical system takes the point object and spreads it out (due to the finite bandwidth), hence the moniker. So if an object, then, can be thought of as a set of point object diffracting light, then each point will be consequently spread out at the focal point of the imaging lens, and the image will be a blurry representation of the object. The image, then, is a convolution of the object and the PSF of the system.

On the other hand, in frequency space, the PSF becomes the OTF, or optical transfer function - this describes the spatial frequency bandwidth which the optical system transmits - this is an nice way to characterize an optical system.