# Through a Cracked Lens: Alternate Views of Light Microscopy Part 3: Illuminating Illumination

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Did I ever tell you about my first optical engineering project? It was a video projector which used many innovative ideas, including one of the first liquid crystal video displays. It was designed to be the brightest projector ever made and was used in a helmet mounted display prototype. My first job out of college was to gather the parts, put it together, and measure its characteristics. To make a long story short, I had to turn the room lights out to see the image! What made a 20,000-footlambert design into a 20-footlambert display? Illumination! I learned a lot about illumination in those few short weeks, and everything I learned is directly applicable to microscopes. In fact the whole science of illumination was developed by microscopists. Electron microscopes deal with exactly the same concepts.

One of the reasons that illumination is hard to write (and read) about is that all the good words have been redefined to have a specific scientific meaning. So I cannot use perfectly good English words like "brightness," "intensity," or even "quantity" without either carefully defining them first (they don't always mean what you think) and putting everybody to sleep or offending the experts who will complain about my sloppy use of scientific terms. I will try to be precise without being soporific.

Brightness is an easy concept. Imagine that you have two light sources--a two-meter long fluorescent bulb and a tiny Christmas tree bulb. If they both emitted the same amount of light you could read a newspaper just as well with either source. On the other hand it would not hurt your eyes to look at the fluorescent lamp, but it would hurt then to look at the Christmas tree bulb. This is because the light source is more concentrated for the smaller bulb. We would say that the smaller bulb is brighter, even though it gives off the same amount of light. So brightness depends on both the amount of light and the size of the source.

Brightness also depends on one more thing. Light from a bulb usually goes in all directions. Now imagine two small bulbs, both emitting the same amount of light and both the same size. One bulb, however, is throwing its light in all directions, whereas the other bulb has its light concentrated so that all of it hits your face. The second would obviously be brighter than the first. So brightness depends on both physical extent (area) of the source and angular extent (solid angle) of the beam. A laser beam that puts out only milliwatts is brighter than a light bulb that puts out thousands of watts of light. This is because all the light falls within a very small solid angle.

If you put your eye in the beam from a light bulb, the light entering the eye is spread out over the image of the bulb on the retina. If you put your eye in the laser beam (don't try this), all the light entering the eye would be focused down to a tiny spot, which is both why lasers are so useful and why they are so dangerous. If the brightness of the bulb and the laser were the same the bulb would do the same damage as the laser, but such brightness is nearly impossible to achieve with a thermal source of light.

An interesting optics fact is that an optical system doesn't change the brightness of an object. Ignoring for the moment light lost through absorption and surface reflections, the brightness of any image is the same as for the original object. If you make the object larger, you also reduce the angle of illumination in exactly the right proportion to keep the brightness constant. Of course there are losses of light in most optical systems, but the principle is very useful.

When you are trying to get light to go through an optical system it is the brightness than counts. A 100 watt frosted light bulb is not much use in microscopy as you would have to throw away 99.99999% of the light by the time it hits the sample. It gives off a lot of light, but it isn't very bright. A 15 watt bulb with a bare filament is much brighter and is often the best choice. What about a 100 watt bare filament bulb? It turns out that the filaments of the two bulbs are the same brightness, but the filament of the 100 watt bulb has more area. The limit of filament brightness is the rate of evaporation of the tungsten, so if the 15 watt bulb works, the 100 watt bulb would not work any better.

In an optimized microscope illumination system the image of the filament should just fill the entrance pupil of the objective both in area and numerical aperture. This is called Köhler illumination. A larger filament than is necessary to fill the objective lens would only add more scattered light to the system.

The three types of light sources for microscopes are tungsten filament lamps, short arc lamps, and lasers. These are listed in order of brightness and cost. In most uses the tungsten bulbs are great. In cases where more light is needed, for example in projection microscopes, arc lamps are used. Lasers are used mainly in confocal scanning microscopes, which have the additional problem of short dwell time at each picture element. A scan can be completed faster with the laser than with a conventional lamp.

So why was my projector so dim? The light source was a custom-made arc lamp that had plenty of light, but the arc was far too large so its brightness was not what was predicted. The projector was a failure, but as a learning experience it was a big success! ■

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