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Physics 352 – Winter 2008

6 A FEW CONTEMPORARY TOPICS IN OPTICS

Optics is both very old subject and a very modern one. Imaging the microscopic world, for example, makes use of lenses whose optical principles were unraveled in the 17th century; yet on the list of *Science*'s top ten breakthroughs for 2006, we find clever new techniques for extending the powers of microscopes [*1*]. We'll very briefly survey just a **few** contemporary topics in optics. (A thorough survey could keep us occupied for months!) First, let's remind ourselves of the "diffraction limits" of imaging.

6.1 Diffraction limited imaging

As we saw earlier in the course, the wave nature of light sets restrictions on the spatial resolution of imaging techniques. Light of wavelength λ gathered by an aperture of size D can pinpoint its source only to within an angle of roughly $\theta_{min} = \lambda/D$ – this sets the resolution of telescopes. A lens used for magnifying an image can resolve distinct objects only if they are separated by at least $x_{min} \approx \lambda/2$ – this sets the resolution of microscopes[4]. One of the key challenges of modern optics is to surmount these diffraction limits.

6.2 Optical interferometry

One obvious way to increase the resolution of a telescope is to increase its size (D). As we discussed earlier in the course, one can effectively create a "larger" telescope by carefully combining the signals received by more than one individual telescope, separated by a well-measured "baseline." The effective aperture size of the telescope is now the length of the baseline. This principle of interferometry has been used for radio astronomy for decades. Visible wavelengths are of course vastly shorter than radio waves, and so very precise measurements and exquisite stability of the component telescope locations are required for interferometry to be possible. Nonetheless, this has been achieved in recent years, and promises to be a good way of directly observing the orbits of extrasolar planets.

The Cambridge Optical Aperture Synthesis Telescope, for example, is an optical interferometer than can create baselines of up to 100 meters! (By comparison, the largest telescope on Earth, the Keck telescope in Hawaii, has a diameter of 10 meters, though it is already in the process of being turned into part of a multi-element interferometer.)

[4] More precisely: $x_{min} = \lambda/(2\, n \sin\theta)$, where n is the index of refraction of the medium and θ is the maximal collection angle of the lens. At best, $\sin\theta \approx 0.9$.

6.3 Fluorescence

Fluorescence microscopy is not new, but many contemporary techniques below make use of it, so we'll introduce it. A fluorescent molecule is one that absorbs light of one wavelength and then emits light at a different (longer) wavelength. The excitation and the emission are separated in time, typically by a nanosecond or so. There are a wide variety of fluorescent probes available, in all sorts of colors (including "Oregon Green"!). See Figure 6.2 for an example of a tissue section stained with various fluorescent probes.

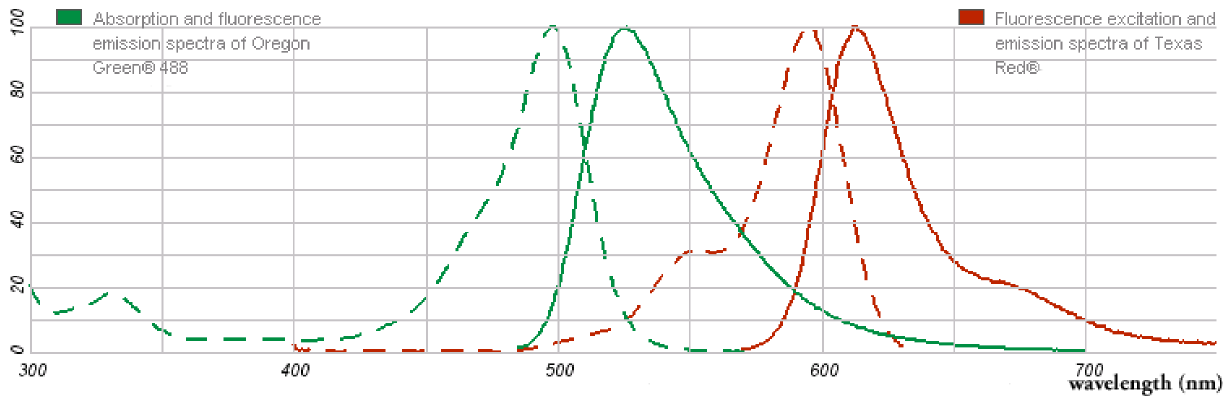


Figure 6.1. Examples of excitation spectra (dashed) and emission spectra (solid) for two commonly used fluorescent molecules, Oregon Green (green, left) and Texas Red (red, right). Note that emission occurs at higher wavelengths (and hence lower frequencies and lower energies) than excitation. From *Molecular Probes / Invitrogen.*

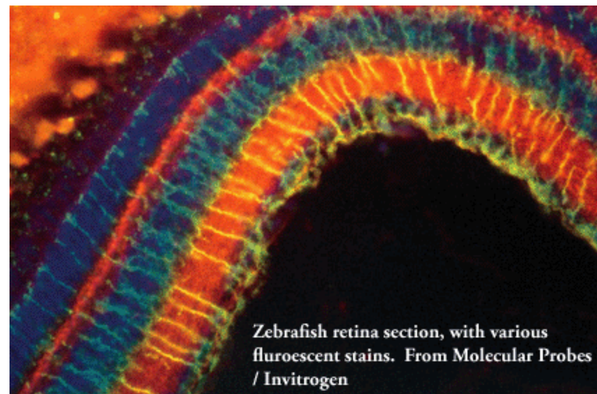


Figure 6.2. A fluorescence image (see image for details).

6.4 Fluorescence resonance energy transfer (FRET)

Fluorescence resonance energy transfer (FRET) provides distance information in the Angstrom to few-nanometer range. Suppose a fluorescent probe (Probe A, e.g. Oregon Green, above) is normally excited at wavelength λ_1 and emits light at λ_2. Suppose a second type of probe (Probe B, e.g. Texas Red, above) is excited at wavelength λ_2 and emits light at λ_3. If Probe A and Probe B are in close proximity to one another, and Probe A is excited, then rather than emit light it

can transfer its energy to Probe B, which then emits light at wavelength λ_3. Probe A is dimmer than it would be if B were not around, and B is brighter than it would be if A were not around. Molecules can be thought of as electromagnetic oscillators, and Probe A resonantly drives the oscillations of B, since A's emission frequency matches the excitation frequency of B. The coupling between the two oscillators depends very strongly on distance, since the molecules' dipole electric fields decay sharply within a few nanometers, and so the fluorophore intensities can be used to measure distance over very short length scales. Probe "A" is called the donor, and "B" the acceptor.

One of the many uses of FRET has been to examine the dynamics of ATP synthase, a "molecular motor" that synthesizes ATP at your cellular membranes by mechanically turning a "shaft" (blue) in the center of "stator" enzymes (orange). In the experiment shown below (Fig. 6.3), one stator has the acceptor fluorophore (red) and the shaft has the donor (green). As the machine rotates, three distinct levels of FRET energy transfer are seen, corresponding to three distinct inter-molecular distances, the magnitudes and time-scales of which describe the molecular motion.

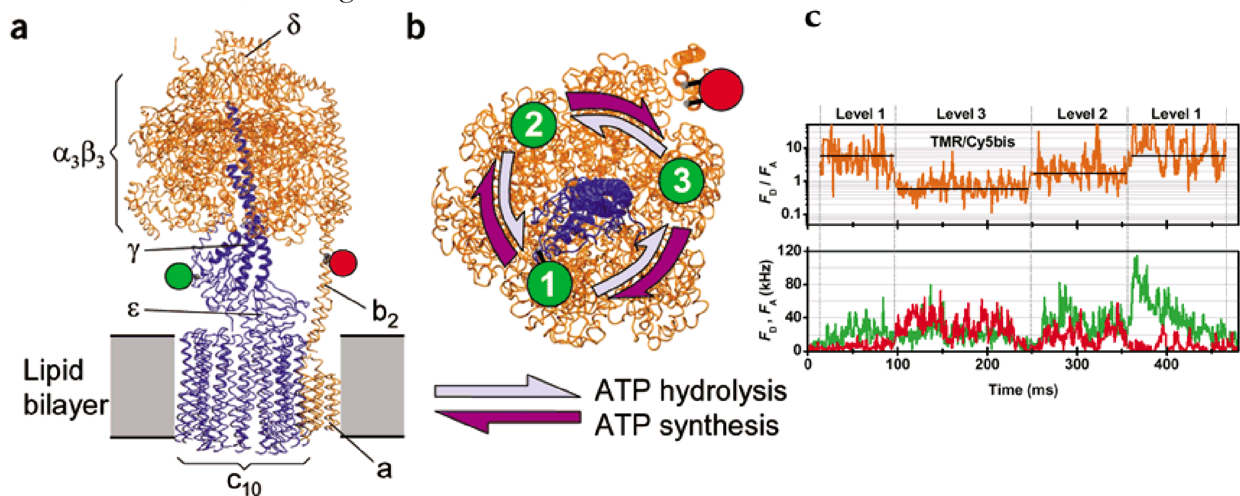


Figure 6.3. Molecular information from FRET (see text for details). From "Proton-powered subunit rotation in single membrane-bound F_0F_1-ATP synthase," M. Diez *et al.*, *Nature Structural & Molecular Biology* **11**, 135 - 141 (2004).

6.5 Total Internal Reflection Fluorescence Microscopy (TIRF)

In class, we discussed total internal reflection, in which light incident above a critical angle from a medium of higher index of refraction to one of lower index is completely reflected. A more complete electromagnetic analysis would show that this is almost, but not quite true. There is no propagating wave transmitted to the second medium, but there is a weak "evanescent wave" with an exponentially decaying amplitude – like that of an overdamped oscillator – that extends into the "forbidden" medium. The characteristic length of this decay is typically around 50-100 nm.

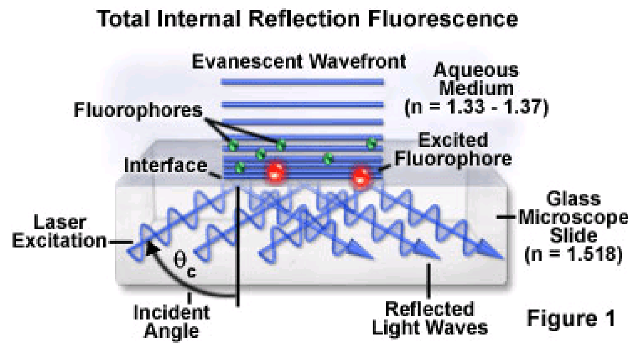


Figure 6.4. From Nikon, http://www.microscopyu.com/articles/fluorescence/tirf/tirfintro.html

Recently, people have made use of this evanescent field for Total Internal Reflection Fluorescence Microscopy (TIRF). Imagine a situation in which one has fluorescent probes throughout an object (e.g. a cell), but one is only interested in a small fraction of probes near the "bottom" of the cell (e.g. adhesion proteins). With standard fluorescence illumination, the signal of interest is "washed out" by the fluorophores in the bulk. Exciting under TIR conditions, however, one excites only those fluorophores within the evanescent field decay length from the surface, cleanly seeing only the molecules located in the region of interest (see Figures 6.4, 6.5).

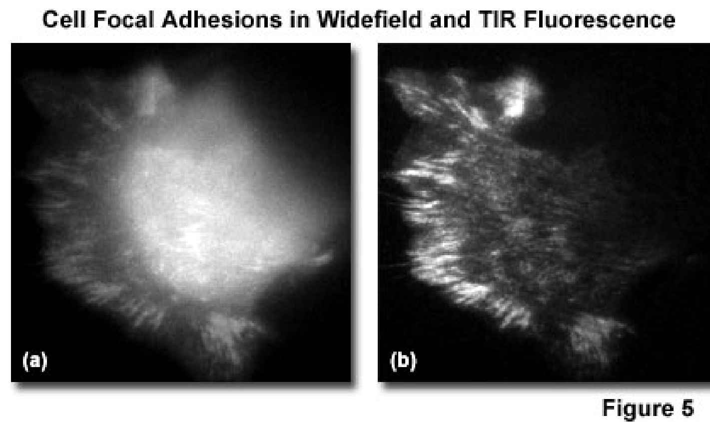


Figure 6.5. From Nikon, *http://www.microscopyu.com/articles/fluorescence/tirf/tirfintro.html*

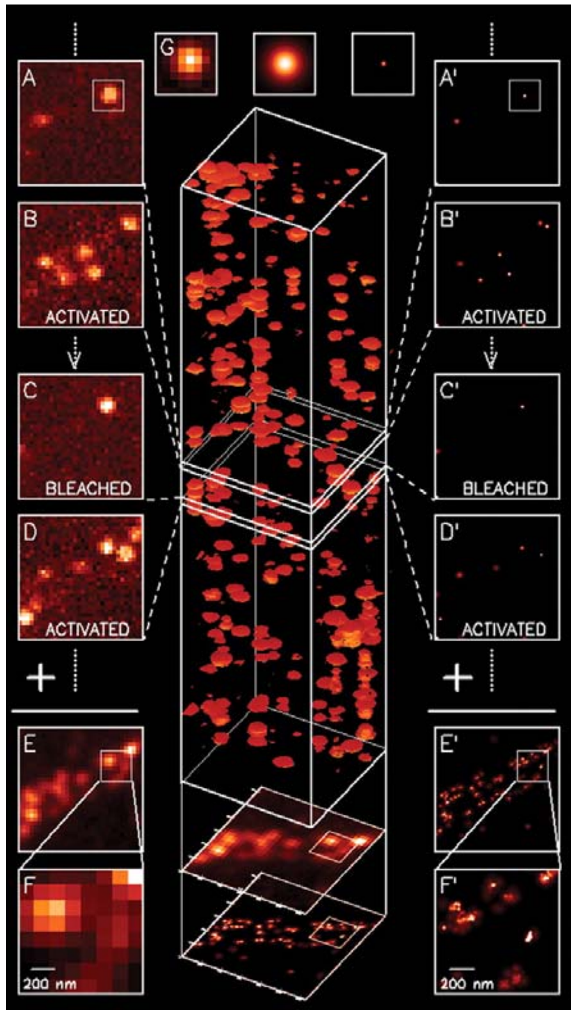
6.6 Single-particle tracking

The diffraction limit states the smallest separation, $x_{\text{min}} \approx \lambda/2$ that can be resolved for two objects. If their separation is smaller, they appear indistinguishable from one object.

Suppose, however, that one knows definitively that only has <u>one</u> object in the area of interest. This object appears as a blur of size x_{min}. Its <u>center</u> can be pinpointed to a much finer precision than x_{min}, and this concept is the basis of single-particle tracking. This is especially relevant to various single-molecule microscopies (see PALM, below) and to **OPTICAL TRAPPING** (which we briefly discussed last term).

6.7 Photoactivated Localization Microscopy (PALM)

As noted above, advances in "breaking" the diffraction limit ranked among *Science's* Top Ten Breakthroughs for 2006 [*1*]. One of the notable techniques cited was Photoactivated Localization Microscopy (PALM) [*2*]. The basic principle involves exciting very few fluorescent probes with a very brief laser pulse. Using single-particle tracking, the positions of these fluorophores are determined. Then the fluorophores are turned "off." (Fluorescent molecules often turn themselves off for a variety of reasons; some can be triggered to go "dark" with laser excitation at certain wavelengths). The process is then repeated, with a (random) other set of fluorophores. After several cycles, a "map" of fluorophore locations is built up.



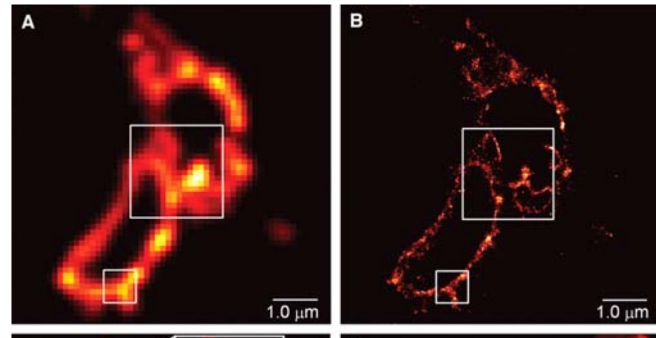


Figure 6.6 PALM
(*left*) PALM, schematic.
(*above*) Images of a cell with an organelle membrane marker. A – TIRF, B – PALM.
From [*2*]

6.8 Optical Traps

We've already discussed optical traps briefly in class, and we'll discuss them more as Phys. 352 progresses, but I can't resist writing a little bit here.

Light focused to a small, diffraction-limited spot attracts objects with a dielectric constant greater than that of the surrounding medium. There are two ways of thinking about the reason for

this. One you'll encounter in an electromagnetism course: dielectric objects lower their energy in regions where the electric field gradient is strong. Another is that if we were to examine the refraction of light by the object using Snell's Law, and think about the momentum carried by the light, we would deduce that there is a net "inward" force [3]. In any case, the key point is that an intense laser spot acts as a "trap" for particles. These particles can be micron-scale colloids, living cells, cold atoms, etc.

Optical traps have revolutionized biophysics, atomic physics, and other fields. One of the reasons is that the traps act not only as "handles" by which one can manipulate objects, but also as "springs" with which one can measure forces. One can conduct experiments, for example, in which molecular motors "walk" along individual DNA molecules, transcribing RNA, and measure the force exerted by these machines as they perform their biochemical tasks.

In my lab, I make use of "holographic" optical traps [4,5], which are exceptionally versatile. The basic idea is illustrated in Figure 6.7b. A "diffractive element" sculpts the laser wavefronts to be whatever we choose – for example the phase profile that several beams would have, if several beams were incident. What is this diffractive element? A "spatial light modulator" (SLM) – a computer-controlled liquid crystal panel. Each pixel of the SLM shifts the phase of the incident light by some user-determined amount between 0 and 2π. Figure 6.8 shows what the setup looks like.

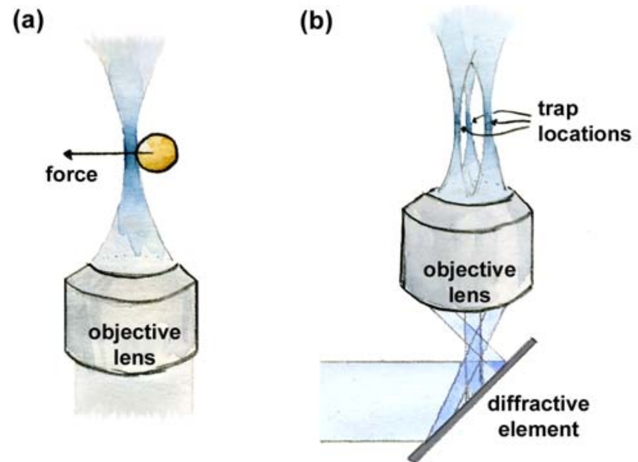
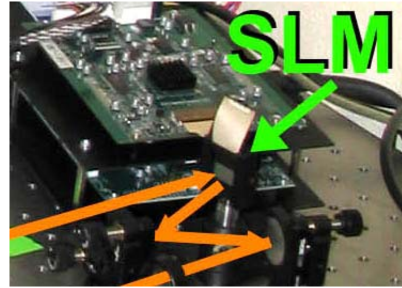
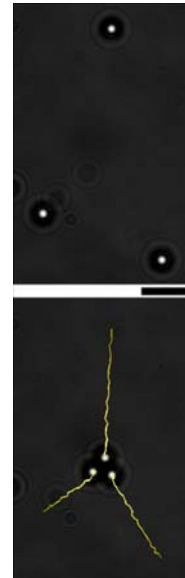


Figure 6.7. Optical traps. (a) A single optical trap. (b) A holographic optical trap

Let's think about this more concretely. A lens shifts the phase of incident light by some amount at each "piece" of the lens – the thick parts shift it more, and the thin parts shift it less. Suppose we replaced the lens by a magical device that shifts the phase at each position by the same amount that the lens would. It would act just like the lens. This, in fact, is what the SLM does. Therefore, we can create optical elements at will.

One convenient phase pattern to create is a phase that varies <u>linearly</u> with position along the SLM face. What does this remind you of?...





Bar = 10μm

Figure 6.8. *Left:* The holographic optical trap setup in the Parthasarathy Lab. *Upper right:* The spatial light modulator. Orange lines indicate the path of laser light. *Lower right (two images):* Three initially separated 3-μm-diameter silica microspheres (upper image), are moved into contact (lower image) by the application of a series of phase masks. Colored lines show the particle trajectories.

.... It's just what a mirror, or prism, would do. Building combinations of these phase mask allows the manipulation of particles, as illustrated for example in Figure 6.8.

A lot of the work in my lab involves inventing and implementing "unusual" optical traps – things like **line traps**, for instance, in which light is focused to a line in the focal plane (Figure 6.9). These line traps are useful for measuring energies and forces between colloidal particles, as we'll probably see later this year. Though line traps are less "obvious" in their construction than "point traps," the principles behind them are no different than those we have been exploring. In fact, our lab's phase pattern calculation scheme simply conceptually "builds up" a desired focal pattern using superpositions of the phase profiles of simple mirrors and spherical lenses! Much of the line trap work in my group is done by an undergrad, Gregory Tietjen, who was in Physics 352 last year. If you ever doubt that the optics we're learning in class can be very directly applied to "cutting edge" research, ask Greg!

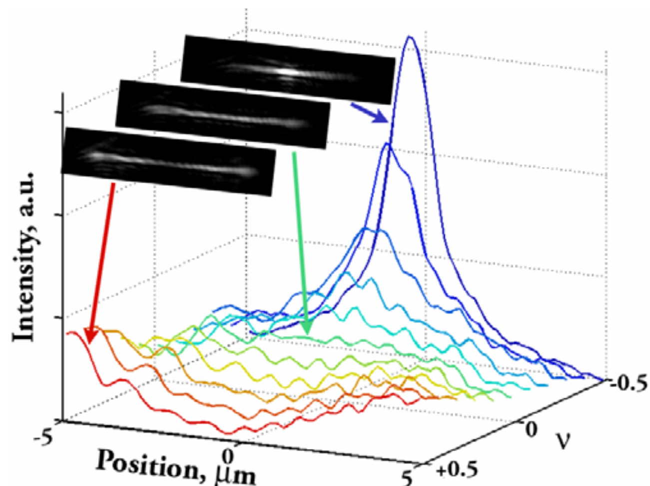


Figure 6.9. A class of "tunable" optical line traps, invented in the Parthasarathy Lab. Particular phase patterns are devised that will cause the incident light to focus to some desired intensity profile. The plot shows measured intensity profiles, together with images of some of the lines.

6.9 Other topics

There are a vast number of topics and techniques that we won't discuss. A few of these include: **Atom optics** – exploring and manipulating the wave-nature of matter, especially at ultra-low temperatures. **Multiphoton microscopy**, in which multiple photons combine to give precise position information. **Near-field microscopy**, in which an optical probe very close ($< \lambda$) to a surface can yield structural information. **Electron microscopy** (not new, but very important) in which electron beams are focused and detected to provide images.

6.10 References for Chapter 6

[1] *Science* 22 December 2006, **Vol. 9**, 1848-1855.
(*http://www.sciencemag.org/cgi/content/full/314/5807/1850a*)

[2] E. Betzig *et al.*, "Imaging Intracellular Fluorescent Proteins at Nanometer Resolution", *Science* 15 September 2006, **Vol. 313**, 1642 – 1645.
(*http://www.sciencemag.org/cgi/content/full/313/5793/1642*)

[3] Ashkin, A. Forces of a single-beam gradient laser trap on a dielectric sphere in the ray-optics regime. *Biophys. J.* **61**, 569-582 (1992).

[4] Grier, D. G. A Revolution in Optical Manipulation. *Nature* **424**, 810-816 (2003).

[5] Curtis, J. E. , Koss, B. A., and Grier, D. G. Dynamic holographic optical tweezers. *Opt. Comm.* **207**, 169-175 (2002).