

5 Quantum Dots for Microscopy

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I am picking one particular example of an application of nanofabrication, firstly because I learned quite a lot about it a few years ago, when my wife got excited about using quantum dots to stain microbiological specimens, and secondly because it relates to the physics of semiconductors, to the use of lasers, and to the advances in microscopy that have been made possible by sophisticated computer control and analysis of scanning processes. I could have chosen to discuss nanofabrication of semiconductor layers, but that is a much bigger subject, and I only know about a few corners of it.

Telescopes and microscopes were first developed at the beginning of the scientific revolution in the 1600s, and there have been continual developments of both classes of instruments, both using visible light, and using other sorts of radiation of shorter or longer wavelength. In microscopy shorter wavelengths enable smaller length scales to be seen, because the resolution is increased, but shorter wavelengths also do more damage to biological specimens.

Confocal microscopy is a technique that allows high contrast pictures to be obtained by using a point source of light to be focused on the focal plane of the microscope, and then scanned across the entire focal plane, in a way similar to the scanning of the picture onto a traditional television screen. Three-dimensional pictures can be constructed by also scanning the focal plane of the microscope while keeping the illumination focused on the microscope's focal plane. The method was invented more than fifty years ago, but needed modern developments to make it tolerably fast. This allows the image to be recorded near one point at a time, while there is little illumination on the rest of the sample, so keeping background illumination at a low level, and reducing the average exposure of the whole sample.

Many biological samples are rather homogeneous, with refractive index and density that do not differ much from those of water. One way that particular features of such materials can be shown up is by labeling antibodies to particular components of the sample with some brightly colored dye. This is somewhat similar to the use of antibodies in a radioimmune assay, as was discussed in Chapter 3. These *stains* can show up as bright patches of color where they are attached to the appropriate antigen. The effect can be enhanced by using luminescent stains, that can be stimulated to emit a lower frequency radiation when stimulated by higher frequency radiation. This has

the advantage that the higher frequency radiation can be filtered out by a colored filter that is transparent to the frequency emitted by the stain.

Organic dyes are somewhat unstable, and liable to be bleached by the radiation that excites the luminescence, so people, including a group at Bell Labs, tried to develop minute semiconductor crystals, tens of nanometers across, that could be used instead of these organic dyes. Two of these people, Mounji Bawendi and Paul Alivastos, moved to MIT and UC Berkeley respectively, and developed water soluble *quantum dots* of this sort, and these were fabricated commercially by the Quantum Dot Corporation, around 2002. An account of these materials can be found in the article by Watson, Wu and Bruchez in *Biotechniques* **34**, 296 (2003); the title is *Lighting up Cells with Quantum Dots*. Since that time the Quantum Dot Corporation has been swallowed by one or two larger corporate fish.

5.1 Semiconductor nanocrystals as stains

We know that semiconductor crystals are luminescent, since photoexcitation by photons whose energy is greater than the band gap produces electron-hole pairs, which first decay down to the bottom of the conduction band and the top of the valence band respectively, and then, for direct gap semiconductors, recombine, emitting photons whose energy is close to the band gap energy. Light near the top of the visible spectrum, or in the near ultraviolet, will not do structural damage to stable crystalline semiconductors, so will not destroy the characteristic photoluminescence, in the way that an organic dye may be bleached by exposure to such radiation. In a nanocrystal the momentum of the electron hole pairs is not conserved, because they exchange momentum with the boundary of the crystal, so the difference between a direct band gap and an indirect band gap is not important. A very useful feature of quantum dots used for this purpose is that the size of the crystal can be used to tune the frequency range of the photoemission. The smaller the crystal is the larger the band gap is, since confinement within a small volume increases the kinetic energy of the electrons, which scales like the $-2/3$ power of the volume, and similarly increases the energy of the holes. The quantum dots can be made in a range of distinct sizes, each attached to a different antibody, and this allows different proteins to be distinguished by the different colors emitted by the different sized crystals.

It is desirable to use semiconductors that emit light well into the visible range if you actually want to be able to see the image in the microscope. It is

therefore necessary to have a fairly large band gap above about 2.0 eV, which corresponds to a light wavelength of 620 nm. to get these band gaps within the usual zinc blende crystal structure (diamond-like), the II-VI materials are most suitable. The Quantum Dot Corporation's nanocrystals were made of a CdSe core with a ZnS outer shell. The outer shell of semiconductor is, I think, a sort of optical coating to cut down reflection of the emitted light. The outer shell of semiconductor was coated with a transparent polymer which keeps the water out of the crystals, and a layer of the protein streptavidin binds on the inner side to the polymer coat, and on the outer side to suitable antibodies. A 4 nm quantum dot constructed in this way emits green light centered on a 540 nm wavelength, and a 6.5 nm quantum dot emits red light centered at about 650 nm wavelength. Some details and comparisons with other techniques can be read in the paper by X. Michelet *et al.* in *Science*, **307**, 538 (2005).