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Applications of Quantum Dots in Biology

An Overview

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Summary

This chapter summarizes the properties of fluorescent semiconductor nanocrystals (quantum dots), and their relationship to performance in biological assays. The properties of quantum dots (optical, structural, compositional, etc.) are described. Recent work employing these entities in biological studies (immunofluorescent labeling, imaging, microscopy in vivo applications, encoding) is discussed.

Key Words

Quantum dot; semiconductor nanocrystal; labeling; biological imaging; immunohistochemistry; fluorescence microscopy; multiplexing.

1. Introduction

Highly luminescent, colloidal semiconductor nanocrystals, or quantum dots, have been known since the early 1990s ***(1–3)***; however, not until 1998 were these materials were first utilized as biological probes ***(4,5)***. The emission wavelength of these unique fluorescent probes can be altered with a change in the size of the quantum dot, allowing their emission to be tuned to any wavelength within a range determined by the semiconductor composition. Although there have been a number of reports of biological applications of quantum dots since the pioneering articles, it is clear that the use of these novel probes is still in its infancy. Both protocols for using quantum dots and the methods for preparing these reagents are continually being improved. Because many of the properties of quantum dots differ from those of other fluorescent biological probes, quantum dots can be enabling for a given application. These key properties are discussed in relation to their performance in biological applications.

From: *Methods in Molecular Biology, vol. 303: NanoBiotechnology Protocols*
Edited by: S. J. Rosenthal and D. W. Wright © Humana Press Inc., Totowa, NJ

2. General Properties

2.1. Optical Properties

2.1.1. Absorbance Characteristics

Quantum dots absorb light differently than dye molecules. Fluorescent dyes typically absorb light efficiently in an absorbance band that has a slightly shorter wavelength than the emission (*see* **Fig. 1A**). This can be advantageous for selective excitation of a fluorophore but also requires that each fluorescent dye be excited separately when multiple colors are used together (multiplex). This can decrease throughput and increase instrument cost, particularly when lasers are required for excitation. The absorbance band of a fluorescent dye is usually spectrally close to the light emitted, making efficient collection of the emitted light more difficult owing to scatter, autofluorescence, and the need for precise optical filters.

Quantum dots, by comparison, absorb light at all wavelengths shorter than the emission (**Fig. 1B**). This allows multiple colors of quantum dots to be effectively excited by a single source of light (e.g., lamp, laser, LED) far from the emission of any color. The effective "Stokes shift," or wavelength difference between maximum absorbance and maximum emission (typically ~15–30 nm for organic dyes), can be hundreds of nanometers for a quantum dot.

Not only can quantum dots be excited far from where they emit, but extinction coefficients (i.e., the measure of absorbed light) are much larger than for typical fluorescent dyes and, thus, absorb light much more efficiently (**Fig. 1D**). For example, the extinction coefficients for some common dyes compared to quantum dots are provided in **Table 1**.

In addition, the use of many colors of quantum dots simultaneously (multiplexing) requires only one excitation source to excite all colors efficiently. This can be particularly valuable in multicolor fluorescence microscopy, enabling one to visualize simultaneously many colors of quantum dot-labeled probes.

2.1.2. Emission Characteristics

2.1.2.1. Shape of Emission Spectrum

By their nature, quantum dots exist in polydisperse collections of nanocrystals of slightly different sizes. The emission spectrum of a solution of quantum dots is the sum of the spectra of many individual quantum dots that differ slightly in size. Consequently, the width of the observable emission spectrum depends on the uniformity of the quantum dot size distribution (*see* **Subheading 2.2.**). A sample that has a very uniform quantum dot size distribution will have a narrower composite emission spectrum than a sample that is less uniform. Typically, the size distribution is nearly normally distributed and the emission spectrum nearly Gaussian shaped. This is in contrast to most fluorescent dyes that display

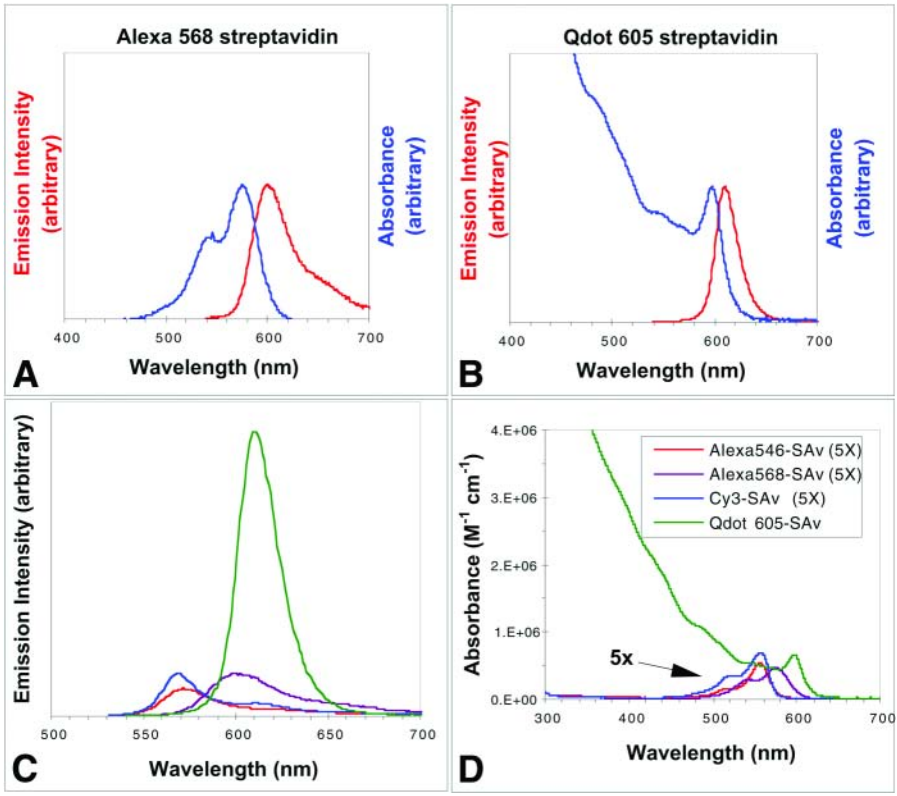


Fig. 1. Comparison of absorbance and emission spectra (normalized) of **(A)** Alexa® 568 streptavidin conjugate and **(B)** Qdot® 605 streptavidin conjugate. Note that the quantum dot conjugate can absorb light efficiently far to the blue of the emission. **(C)** Comparison of emission spectra (nonnormalized) of streptavidin conjugates of Qdot 605 (—), Alexa 546 (—), Alexa 568 (—), and Cy3® (—). The spectra were taken under conditions in which each fluorophore absorbed the same amount of excitation light. The measured quantum yields of the conjugates were 55, 8, 16, and 11%, respectively. **(D)** Comparison of absorbance spectra (nonnormalized, each 1 μ*M* flurophore) of Qdot 605 streptavidin conjugate (—), Cy3 streptavidin conjugate (—), Alexa 546 streptavidin conjugate (—), and Alexa 568 streptavidin conjugate (—). Note that all dye spectra are enhanced fivefold for clarity. Alexa, Cy3, and Qdot are registered trademarks of Molecular Probes, Amersham Biosciences, and Quantum Dot Corporation, respectively.

asymmetric emission spectra that tail (sometimes dramatically) to the red (*see* **Fig. 1C**). Additionally, typical high-quality quantum dot size distributions result in emission spectrum widths (at half maximum) of 20–35 nm, which is noticeably narrower than for comparable dyes. These narrow, symmetric emission

Table 1
Optical Properties of Quantum Dots Compared to Common Dyes[a]

Fluorescent dye	$\lambda_{excitation}$ (nm)	$\lambda_{emission}$ (nm)	ε(mol^{-1}-cm^{-1})
Qdot 525	400	525	280,000
Alexa 488	495	519	78,000
Fluorescein	494	518	79,000
Qdot 565	400	565	960,000
Cy3	550	570	130,000
Alexa 555	555	565	112,000
Qdot 585	400	585	1,840,000
R-Phycoerythrin	565	578	1,960,000
TMR	555	580	90,000
Qdot 605	400	605	2,320,000
Alexa 568	578	603	88,000
Texas Red	595	615	96,000
Qdot 655	400	655	4,720,000
APC	650	660	700,000
Alexa 647	650	668	250,000
Cy5	649	670	200,000
Alexa 647-PE	565	668	1,960,000

[a]The extinction coefficients (ε) are generally much larger for quantum dots than for fluorescent dyes. Furthermore, the excitation wavelength ($\lambda_{excitation}$) can be much farther from the emission ($\lambda_{emission}$).

spectra make possible detection of multiple colors of quantum dots together (multiplexing) with low cross-talk between detection channels.

2.1.2.2. Quantum Yield

Quantum yield is a measure of the "brightness" of a fluorophore and is defined as the ratio of light emitted to light absorbed by a fluorescent material. Some organic dyes have quantum yields approaching 100%, but conjugates (from biological affinity molecules) made from these dyes generally have a significantly lower quantum yield. Quantum dots retain their high quantum yield even after conjugation to biological affinity molecules (**Fig. 1C**).

2.1.2.3. Photostability

Fluorescent dyes tend to be organic molecules that are steadily bleached (degraded) by the light used to excite them, progressively emitting less light over time. Although a wide range of photostability is observed in various

fluorescent dye molecules, the stability does not approach that observed in quantum dots (*see* **Fig. 2**). Even under conditions of intense illumination (e.g., in a confocal microscope or flow cytometer), little if any degradation is observed ***(6)***. This property makes quantum dots enabling in applications requiring continuous observation of the probe (cell tracking, some imaging applications, and so on), and potentially more valuable as quantitative reagents.

2.1.2.4. Fluorescence Lifetime

Quantum dots have somewhat longer fluorescence lifetimes than typical organic fluorophores (approx 20–40 vs <5 ns, respectively) ***(7)***. While this lifetime is shorter than "long-lifetime" fluorophores, such as lanthanides (hundreds of microseconds), the difference could be exploited to reduce autofluorescence background in some measurements, such as those made on polymer substrates. A short delay between excitation and collection of the emitted light can nearly eliminate autofluorescence of polymeric substrates (or potentially other media such as blood) and still allow collection of the majority of the quantum dot-emitted light (Quantum Dot Corporation, unpublished data). Additionally, the relatively short lifetime of quantum dots does not significantly reduce emission at high excitation power owing to saturation.

2.2. Physical Properties

2.2.1. Structure

Quantum dot conjugates are complex, multilayered structures, and many process steps are required to produce a useful, biological conjugate (**Fig. 3**). Some terminology that is used in describing quantum dot structures is as follows:

1. Core quantum dot: The central quantum dot nanocrystal, and what determines the optical properties of the final structure. Most preparations produce core quantum dots that are hydrophobic.
2. Core-shell quantum dot: Core nanocrystals that have a crystalline inorganic shell. These materials are bright, stable, and, like cores, are hydrophobic and only soluble in organic solvents.
3. Water-soluble quantum dot: Core-shell quantum dots that are hydrophilic and are soluble in water and biological buffers. Commercially available water-soluble quantum dots have a hydrophilic polymer coating.
4. Quantum dot bioconjugate: Coupling a water-soluble quantum dot to affinity molecules produces a quantum dot bioconjugate.

Unlike samples of dye molecules in which every molecule is identical, each core quantum dot in a sample contains a slightly different number of atoms and thus can be slightly different in some of the properties (*see* **Subheading 2.3.**). Consequently, the methods developed to synthesize quantum dot cores are

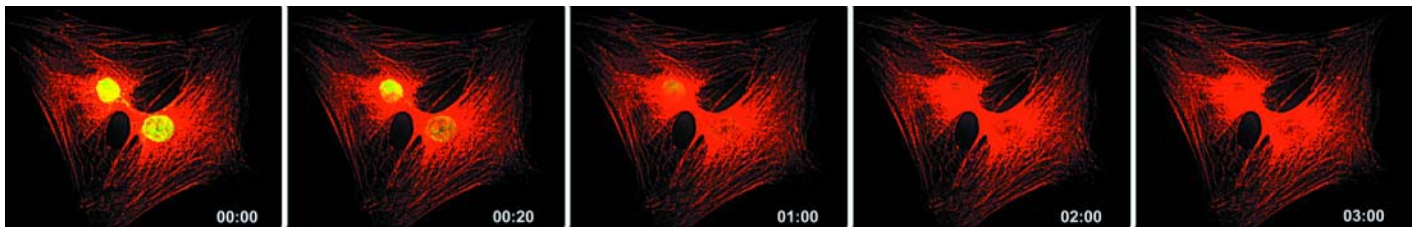


Fig. 2. Comparison of photostability between Qdot® 605 and Alexa Fluor® 488 streptavidin conjugates. Actin filaments in two 3T3 mouse fibroblast cells were labeled with Qdot 605 streptavidin conjugate (red), and the nuclei were stained with Alexa Fluor 488 streptavidin (green). The specimens were continuously illuminated for 3 min with light from a 100-W mercury lamp under a ×100 1.30 oil objective. An excitation filter (excitation: 485 ± 20 nm) was used to excite both Alexa 488 and Qdot 605. Emission filters (emission: 535 ± 10 and em 605 ± 10 nm) on a motorized filter wheel were used to collect Alexa 488 and Qdot 605 signals, respectively. Images were captured with a cooled charge-coupled device camera at 10-s intervals for each color automatically. Images at 0, 20, 60, 120, and 180 s are shown. Whereas Alexa 488 labeling signal faded quickly and became undetectable within 2 min, the Qdot 605 signal showed no obvious change for the entire 3-min illumination period.

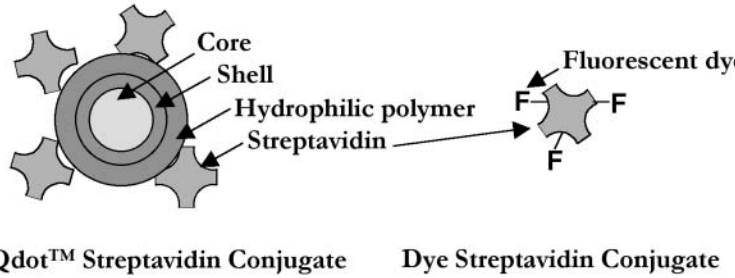


Fig. 3. Schematic of Qdot™ Nanocrystal Probe compared to a typically labeled fluorescent dye protein conjugate (see text for descriptions). Proteins generally carry several fluorescent dye labels (F). By contrast, each quantum dot is conjugated to multiple protein molecules.

continually being optimized to produce more uniform materials ***(8,9)***. This increased uniformity of size and shape produces samples that have narrower (sharper) emission spectra, allowing colors that are closer in wavelength (color) to be used together.

Although these "core" quantum dots determine the optical properties of the conjugate, they are by themselves unsuitable for biological probes owing to their poor stability and quantum yield. In fact, the quantum yield of quantum dot cores has been reported to be very sensitive to the presence of particular ions in solution ***(10)***. Highly luminescent quantum dots are prepared by coating these core quantum dots with another material (in the case of cadmium selenide cores, zinc sulfide or cadmium sulfide is generally used), resulting in "core-shell" quantum dots that are much brighter, and more stable in various chemical environments ***(3,11)***. These core-shell quantum dots are hydrophobic and only organic soluble as prepared.

A number of methods have been reported to convert these hydrophobic "core-shells" into aqueous-soluble, biologically useful versions ***(4,5,12,13)***. Although a comprehensive comparison of these approaches does not exist, there are significant differences in the stability and brightness, and therefore the performance of the resulting aqueous materials. Frequently, investigators do not report quantum yields of the bioconjugates prepared, and often the limit of detection is not reported in a way that allows comparison of performance with that of another method. The stability of the conjugate, a property that is essential for a quantitative reagent, is generally not determined either. For example, some preparations lack stability toward dilution (e.g., losing brightness on dilution in buffer); other methods produce materials that exhibit poor storage stability, or that become less bright in particular chemical environments. High-quality, water-soluble quantum dots do not show significant

change in peak emission wavelength, or quantum yield, as a function of environment or time.

Other than the difference in optical properties just outlined, quantum dots differ from dye conjugates in another important respect. Quantum dots are polyfunctional; there are a number of affinity molecules (proteins, oligonucleotides, small molecules, and so on) per quantum dot. In the case of traditional fluorescent labels, there is generally a one-to-one correspondence of dye to small molecule, and more than one dye molecule per protein or other large molecule (**Fig. 3**).

2.2.2. Size

Water-soluble quantum dot conjugates are in the 10 to 20-nm size range (as measured by transmission electron microscopy, size-exclusion chromatography, and dynamic light scattering), making them similar in size to large proteins (*see* **Fig. 4**). This might preclude them from certain applications, however, their size does not prevent use in the labeling of cell surfaces and tissue sections, or from accessing intracellular targets in fixed and permeablized cells.

2.3. Material

A bulk (i.e., arbitrarily large) piece of semiconductor has a defined emission wavelength. When the size of the semiconductor particle is diminished to the nanometer scale, "quantum confinement" becomes operant, and the emission wavelength becomes dependent on the particular particle size (hence, the term *quantum dot*). Quantum confinement is due to the energy cost of confining the excited state (of an emitting quantum dot) to a smaller volume than it would ideally occupy in the bulk material. Thus, smaller core quantum dots are higher energy and emit "bluer" than larger ones. The useful consequence of this property is that a range of colored fluorescent probes can be generated from a single material simply by preparing different sizes of quantum dots. The range of wavelengths within which a quantum dot can emit is determined by the semiconductor core material.

Cadmium selenide is the material used in virtually all of the quantum dot biological labeling to date, and its emission spectrum conveniently spans the visible light range (~450–660 nm). Materials such as cadmium telluride and indium phosphide potentially allow probes in the far red (up to ~750 nm), and cadmium sulfide and zinc selenide give access to the ultraviolet. Generation of far-red and near-infrared (IR) quantum dot probes will likely be extremely valuable in whole-blood assays in which absorption by hemoglobin limits the detection of shorter-wavelength materials. Deep tissue and in vivo imaging are other areas in which near-IR probes will find use, because scatter by tissue is minimized in this region of the spectrum. A variety of semiconductor materials and the range of emission wavelengths achievable by altering their size are shown in **Fig. 5**.

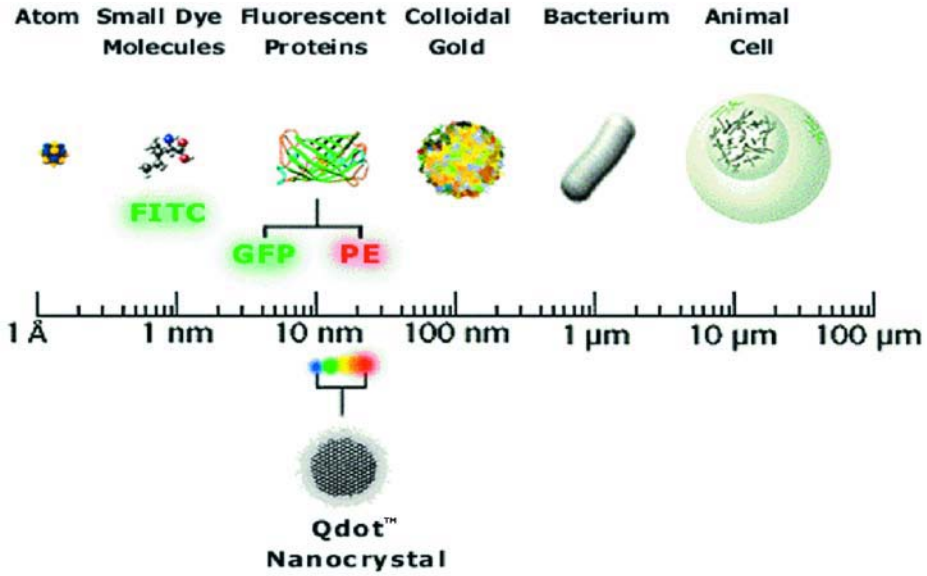


Fig. 4. Physical size of quantum dots compared to related entities.

3. Applications

3.1. Quantum Dots as Labels

For the purpose of this chapter, we define "labels" as single quantum dots conjugated to biological affinity molecules (as differentiated from encoding applications, described in **Subheading 3.2.**). These analogs to traditional fluorescent dye-labeled proteins, antibodies, oligonucleotides, and so on can be used in many biological applications, some of which are unique to quantum dots. Most of the work published on quantum dot labels to date has been "proof-of-concept" work—demonstrating the use of quantum dots in an application, but typically not solving a particular biological problem. Furthermore, the publications have used different or evolving preparations of quantum dots, making results difficult to compare among investigators.

3.1.1. Immunohistochemistry and Other Microscope-Based Techniques

A standard fluorescence microscope is an ideal tool for detection of quantum dot labels. Lamp-based excitation can be applied through a very wide excitation filter for efficient excitation of the broad quantum dot excitation spectrum. Since the emission spectrum is narrow, a narrow emission filter can be used to maximize signal to background. Alternatively, a long-pass emission filter can be used to observe several colors simultaneously. Finally, the excellent photostability provides additional time for focusing and sample inspection without bleaching.

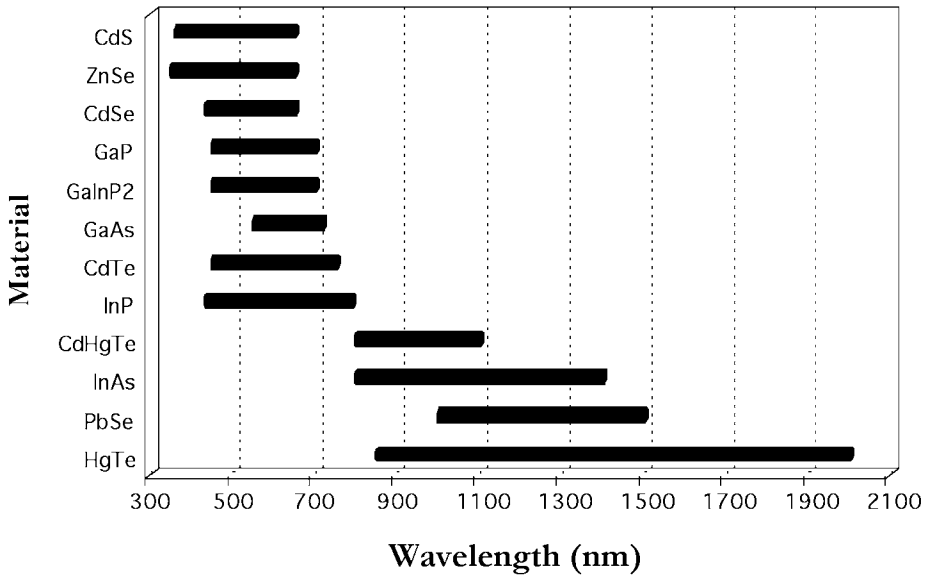


Fig. 5. Wavelength ranges obtainable by varying size of quantum dots made from a number of different semiconductor materials. Each bar approximately represents the range of wavelengths obtained from the smallest (left end) to largest (right end) quantum dot made from the material listed.

Wu et al. ***(6)*** successfully used quantum dots conjugated to immunoglobulin G (IgG) and streptavidin to label the breast cancer marker Her2 on the surface of cancer cells, stain actin and microtubule fibers in the cytoplasm, and detect nuclear antigens. Labeling was shown to be specific for intended targets, brighter, and significantly more photostable than comparable organic dyes. Using quantum dots of different colors conjugated to IgG and streptavidin, the investigators detected two cellular targets with one excitation wavelength. Although the number of simultaneously observable targets is limited in this study, the number will increase as the number of available quantum dot colors coupled to different affinity molecules increases.

Pathak et al. ***(14)*** used quantum dots coupled to oligonucleotides in *in situ* hybridization. They successfully detected hybridization to the Y chromosome of fixed human sperm cells, although no comparison was made to fluorescent dye fluorescent *in situ* hybridization.

Quantum dots have been shown to be enabling in the area of multiphoton microscopy ***(15)***. Quantum dot probes were reported to have the largest two-photon cross-sections (a measure of the ability to absorb light at twice the normal excitation wavelength) of any probe—close to the theoretical maximum

value. The cross-sections are 2 to 3 orders of magnitude larger than conventional fluorescent probes now in use. With the use of two-photon imaging, quantum dots were intravenously injected into mice and used to dynamically visualize capillaries hundreds of microns deep through scattering media (skin and adipose tissue).

3.1.2. Live Cell Labeling

Quantum dots have been used to label live cells. Jaiswal et al. ***(16)*** demonstrated that a number of cell lines endocytosed quantum dots over a 2 to 3 h period, and the quantum dots became localized in endosomes. These labeled cells were shown to be stable for as long as 12 d in culture. The investigators also labeled live cells by membrane biotinylation, followed by incubation with quantum dot–avidin conjugate, although this method also resulted in quantum dot endocytosis in the cell lines studied. They used the labeling procedure to study the effect of starvation on aggregation of developing *Dictyostelium discoideum* cells that were starved for various durations. Cells starved for different durations were labeled with different colored quantum dots, mixed, and the labeled cells were imaged for 2-s intervals every 2 min for 8 h. It was concluded that the cells' propensity to aggregate is an "on-off" phenomenon, not a continuous function of the degree of starvation. More generally, the work represents the use of quantum dot labels to solve a new biological problem not addressable by conventional fluorescent labeling.

Dubertret et al. ***(17)*** has reported the preparation of quantum dots functionalized with polyethylene glycol (PEG) to study development in *Xenopus* embryos. The quantum dots were microinjected into individual cells of the growing embryo, and because the fluorescence was confined to the progeny of the injected cells, this allowed the embryonic development to be studied for many individual cells. It was found that the quantum dots were stable and had little toxicity.

Quantum dots have also been used to measure cell motility by imaging of phagokinetic tracks ***(18)***. It was demonstrated that cells were capable of engulfing nanocrystals, through an undefined mechanism, as they travel, leaving behind a history of their migratory track. Future research will explore the use of the multiple emission colors of quantum dots to monitor cell motility and migration and simultaneously track specific proteins tagged with complementary fluorescent probes.

3.1.3. In Vivo Applications

Several reports have appeared utilizing quantum dots in vivo. This work is typically accomplished with fluorescent polymers, such as rhodamine green

dextran, or with fluorescent proteins, such as green fluorescent protein. The lack of photostability and brightness of these reagents limits their utility in longer-duration imaging experiments.

Akerman et al. ***(19)*** conducted specific targeting of quantum dot–peptide bioconjugates in mice. Peptides that specifically target lung blood vessel endothelial cells, tumor cell blood vessels, and tumor cell lymphatic vessels were conjugated to quantum dots and intravenously injected into mice. Specific targeting to the lung and tumor vasculature was observed with the appropriate conjugates, and no acute toxicity was observed after 24 h of circulation. The investigators also observed that the quantum dots accumulated in the liver and spleen in addition to the targeted tissues, unless the quantum dot was coconjugated with PEG. While the quantum dot conjugates were specific for the tumor targets, they did not accumulate in the tumor cells, instead remaining in the blood vessel endothelia. The investigators speculated as to the possible causes: the size of the quantum dots, the stability of the mercaptoacetic acid–stabilized quantum dot conjugates used, or slow endocytosis into tumor cells.

3.1.4. Small-Molecule Conjugates

A limitation of traditional small-molecule fluorescent dyes is in the labeling of other small molecules, drugs, transporters, and small-molecule probes to cell-surface receptors. Conjugates of dyes to these small molecules often lack sensitivity or specificity in the detection of the desired targets. Conjugates of small molecules to quantum dots produce conjugates with much greater light output per binding event, owing to the increased absorbance and emission of the quantum dot. Furthermore, there is the possibility of improved avidity compared to a dye conjugate, owing to the combined effect of many molecules of the binding ligand on the surface of the quantum dot. Rosenthal et al. ***(20)*** applied this concept to the study of the neurotransmitter serotonin. They coupled approx 160 serotonin molecules/quantum dot via a short linker and characterized these probes by their interaction with serotonin transporters, electrophysiology measurements, as well as fluorescence imaging. While the results for these initial conjugates show somewhat lower selectivity than high-affinity antagonists, they do show utility in the imaging of membrane proteins in living cells.

3.1.5. Microplate-Based Assays

Assays in microtiter plates are analogous to high-throughput screening. The properties of quantum dots allow a lower limit of detection than other fluorescent dyes, as well as assay simplification compared to enzymatic methods of plate-based detection when used in multiplex format. While many solution-phase fluorescent microplate assays exist, immunosorbant assays, in which the analyte is only present bound to the surface of the plate, are typically accomplished

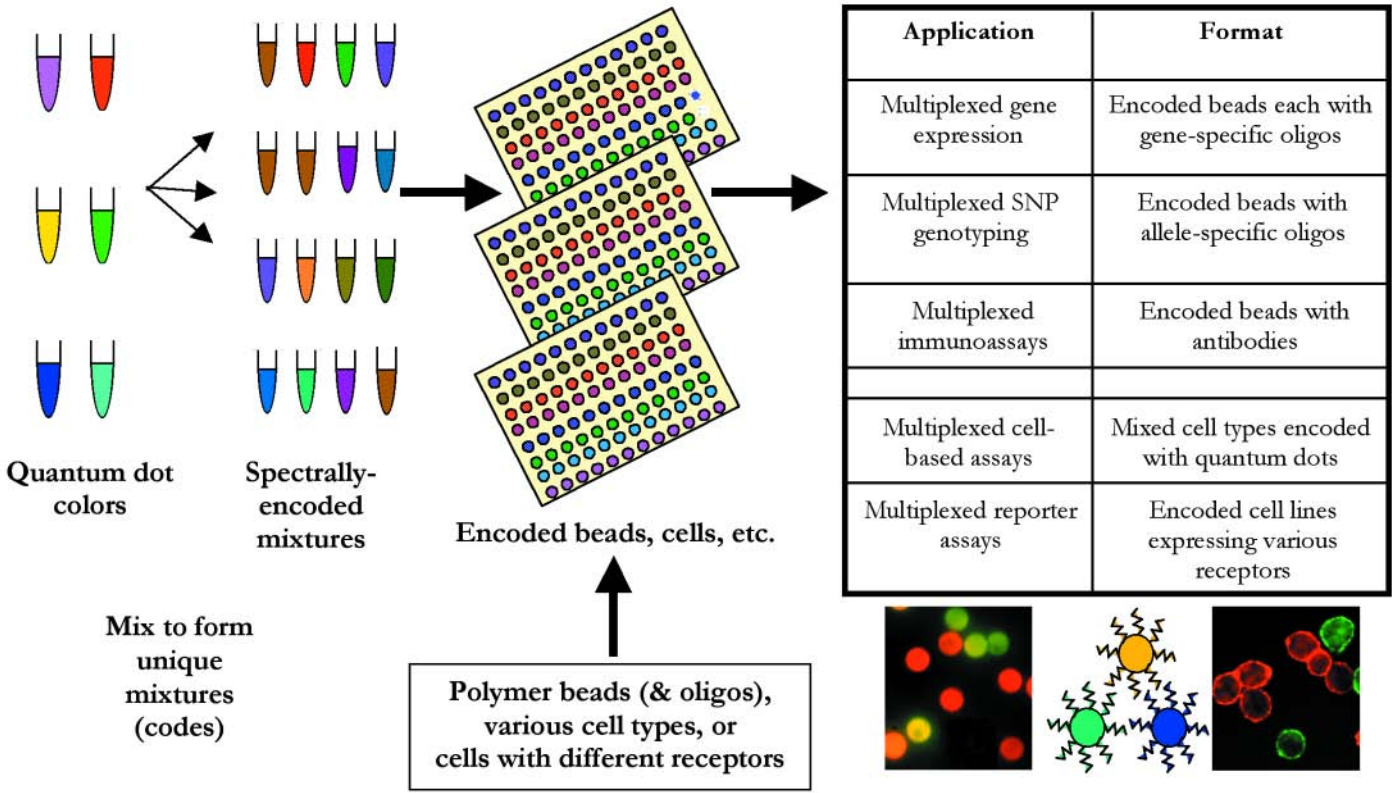
with enzymatic amplification (enzyme-linked immunosorbent assay technique). We have shown that the limit of detection of 605 nm of streptavidin conjugate is at least an order of magnitude lower than phycoerythrin-streptavidin conjugate when used in a microplate reader using 250 nm of excitation for the quantum dot (Quantum Dot Corporation, unpublished data). The use of direct fluorescent detection (as opposed to enzymatic amplification) also allows multiplexed detection without sequential wash and amplification steps. Traditional fluorophores do not give adequate signal to allow their use in these assays.

Goldman et al. ***(21)*** have developed a series of assays for infectious diseases and explosives using quantum dot conjugates. Systematic efforts have resulted in a well-characterized system of producing conjugates as well as measurement of their performance in assays. Reports by these investigators have shown the current limit of detection for cholera and staphylococcal toxins to be 60 and 15 ng/mL, respectively.

3.2. Encoding

Using single colors to "color-code," or identify, objects; only a relatively small number of objects (probably less than 20) can be uniquely identified. However, using combinations of several colors can produce many distinguishable spectral codes. Quantum dots have several practical advantages when used to produce spectral codes. They have narrow, symmetrical emission spectra, are very photostable; and many colors can be excited by a single wavelength of light. The result is that quantum dot spectral codes can be used effectively for multiplexed assays. Because they are much smaller than objects that scientists would like to define uniquely (cells, latex beads for immuno- or other assays), quantum dots can be combined in colors and ratios to encode these objects by providing a unique spectral "fingerprint" (**Fig. 6**). The encoded entities can be conveniently decoded using imaging methods or flow-based methods to determine their characteristic fluorescence spectra. This concept applied to fluorescent dye–encoded polymer beads has been developed into a commercial system ***(22)***. However, this requires the use of multiple lasers for excitation and limits the number of codes practically attainable by such a system. Using quantum dots for polymer bead encoding has significant advantages in single excitation, such as more closely packed colors and a greater number of colors overall, thus making access to higher numbers of codes more likely ***(23)***. A recent report ***(24)*** describes the use of quantum dot–encoded beads to determine 10 cytochrome P450 genotypes on 94 patient samples. The results show that the call accuracy was higher than with gel-based sequencing.

Living cells can also be encoded using multiple colors of quantum dots together to create codes. A method for encoding cells that is based on the intracellular delivery of quantum dots into live cells was developed ***(25)***. The



Application	Format
Multiplexed gene expression	Encoded beads each with gene-specific oligos
Multiplexed SNP genotyping	Encoded beads with allele-specific oligos
Multiplexed immunoassays	Encoded beads with antibodies
Multiplexed cell-based assays	Mixed cell types encoded with quantum dots
Multiplexed reporter assays	Encoded cell lines expressing various receptors

Fig. 6. Concept of encoding using quantum dots. Quantum dot colors can be mixed to produce spectral codes. These mixtures can be combined with polymer beads to produce encoded beads that can be subsequently coupled to distinct oligonucleotides or other affinity molecules. Alternatively, the quantum dot spectral codes can be used to label cells to differentiate cell lines, or cell lines bearing different receptors. SNP, single nucleotide polymorphism.

quantum dots are nontoxic, photostable, and can be imaged using conventional fluorescence microscopy or analyzed using flow cytometric systems. Unique fluorescent codes for a variety of mammalian cell types were generated, and the potential to create >100 codes was demonstrated. The quantum dot cell codes are relatively inert and do not impact most types of cell-based assays including immunostaining, competition binding, reporter gene, receptor internalization, and intracellular calcium release. A multiplexed calcium assay for G protein-coupled receptors using quantum dots was also demonstrated. The ability to spectrally encode individual cells with unique fluorescent bar codes should open new opportunities in multiplexed assay development and greatly facilitate the study of cell/cell interactions and other complex phenotypes in mixed cell populations.

4. Future Perspectives

Given the unique set of properties that quantum dots offer—that they have demonstrated superior utility in some existing applications and show enabling performance in others—it is likely that new, enabling biological applications will be discovered and developed. The photostability may bring unprecedented means of sample archival to existing applications, as well as continuous imaging for very long durations. The brightness and stability may allow levels of detection previously unachievable and make single-molecule detection more approachable to biological applications. The use of intrinsic properties such as fluorescence resonance energy transfer (FRET) and fluorescence lifetime has been virtually unaddressed, let alone developed. Using quantum dots to encode has the potential to revolutionize high-throughput biology, but little more than simple demonstrations have been made to date. Although detection of quantum dots is possible and easy on conventional instrumentation, the development of quantum dot-specific instrumentation (that takes advantage of unique quantum dot properties) will lead to improved sensitivity, multiplexing, and throughput. Possibilities are DNA microarray detection, flow cytometry, and instrumentation to decode quantum dot-encoded objects. Although quantum dots may not provide advantages in every application, it seems likely that they will become a dominant fluorescent reporter in biology over the next several years.

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