Perspective

Lighting up Biosensors: Now and the Decade to Come

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Lighting up Biosensors: Now and the Decade to Come

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Abstract

Optical biosensors are defined as portable optical devices that use biorecognition molecules interrogate a sample for the presence of a target. The capabilities of optical biosensors have expanded rapidly with advances in miniature optical components and molecular engineering. Biosensors to meet needs in health, environmental monitoring and food safety have become commercially available, with many more in the pipeline. We review the innovative approaches to overcoming existing hurdles to practical biosensor designs and explore potential areas for future breakthroughs in optical biosensor technology.

Introduction

Introduction

Ten years ago, *Analytical Chemistry* published a Perspective on Optical Biosensors.[1] Such biosensors, defined as optical devices that included biological recognition elements, were just beginning to move out of the lab and to the point of use.[2] The perspective identified emerging technologies, including improved arrays of recognition molecules, miniaturized optics, and automated fluidics as critical elements required to make optical biosensors useful products to improve people's lives. Other critical hurdles discussed included sampling, automated sample processing, systems integration, and expansion into "low cost—high value" applications. In the last decade, progress has been astounding. The worldwide biosensor market is growing at a rate of 9-10% per year and is expected to increase to $27B by 2022 (https://www.psmarketresearch.com/market-analysis/biosensors-market; https://www.marketsandmarkets.com/Market-Reports/biosensors-market-798.html?gclid=EAIaIQobChMIxOr8oJ2H4AIVqKOzCh37VQlHEAAYAiAAEgLROvD_BwE). While electrochemical sensors are predicted to maintain 70% of the market, largely due to glucose monitoring, optical biosensors are the fastest growing segment within this industry, with optical biosensors commercialized for diagnostics, environmental monitoring, biomanufacturing, and food safety. The relatively expensive, special-use optical biosensors of the first decade of this century are being replaced by inexpensive, simple-to-use devices that provide rapid responses for actionable information; however, those available now are just the tip of the possible iceberg. While the configuration of optical biosensors in which the recognition molecules are attached to a surface for target capture and spectral interrogation still predominates, evolution of intracellular markers and small imaging systems is pushing formats where the recognition molecules interact with the sample *in situ* or in solution to generate an optically measurable change. Here, we will present a perspective on the breakthroughs of the last 10 years that are already revolutionizing optical biosensors and explore our crystal ball to anticipate possibilities for the next decade.

Products commercialized in the last decade

For a long time the advantage electrochemical sensors had over optical biosensors was that they were more amenable to developing portable analytical devices as the electronics could easily be miniaturized into a handheld device. The use of the past tense is apt as this is no longer true; most of us now walk around with optical devices on our being every single day. The rise of the mass fabrication of optical devices, such as the CMOS cameras on our mobile phones, has not only revolutionized daily life but also the way we think about optical sensing. Coupled with the incredible advances in CMOS camera-based detectors are the equally amazing advances in lighting and diode-based light sources in particular. The optical sensing devices we have in our smart watches are only a prelude to the variety of wearable optical sensors in the R&D pipeline.

Leading the list of newly developed optical biosensors are those for point-of-care diagnostics, both human and veterinary. This market segment is dominated by the lateral flow assays which have branched way beyond just being pregnancy tests. Lateral flow assays are now available in formats that test several markers simultaneously (usually $\leq$5) and have been sold to detect heart attacks, water pollutants, drugs of abuse and biological warfare agents.[3] Portable, often hand-held, optical readers to use with these tests justify the additional cost and complexity by providing quantitative data and controlling for variation in human vision. Abbott, for example, is selling a variety of tests, including HIV, influenza and cardiometabolic markers, using its Alere series of optical readers (https://www.alere.com/en/home/products-services/solutions.html?gclid=EAIaIQobChMItY722-6c3QIVBI_ICh0yVgbwEAAYAiAAEgI_8fD_BwE). Conducting the biological recognition process on optical waveguides increases sensitivity and quantitative reliability in systems such as those sold by mBio Diagnostics (http://mbiodx.com/). The company patented waveguides that can be manufactured at low cost, used in resource-limited settings, and provide reproducible data. The mBio waveguide system has demonstrated utility for infectious disease diagnosis (including a successful 2700 person trial in Africa), therapeutic monitoring of AIDS patients, veterinary pathologies, and microtoxins in the ocean.

Optical biosensors are also being used for environmental monitoring.[4] The review by Walper et al.,[3] while focused on the detection of biological threats, provides an excellent comparison of various technologies used for detection in environmental and food samples as well as for clinical diagnostics. For applications of concern to the more general population, cost and ease of use have become recognized as major drivers.

Food testing in particular needs to be fast inexpensive, and capable of analyzing large, heterogeneous samples. The sampling issues have delayed commercialization of rapid tests for bacteria in food. Though biosensors that are sensitive for bacteria have been available for over a decade, testing is still generally done in core laboratories in order increase bacterial concentration through sample preculture. Optical sensors for measuring other aspects of food quality have had more success reaching the market. One of the fastest optical sensors commercially available is a palm-sized infrared scanner (www.Tellspec.com/eng/) that combines spectral analysis, cell phone communications, and real-time cloud-based analysis to evaluate factors such as sugar and acidity in fruit, decay of seafood, or melamine contamination of baby formula. The infrared analysis does not include the use of biorecognition molecules for high specificity analyses, but the potential is clearly there to do so. The market for detecting allergens in foods is also driving a lot of activity at the research and development level. Antibody-based lateral flow devices to detect peanuts and gluten that are designed for home use are available already for online purchase (www.nimasensor.com; www.ezgluten.com); these devices claim ppm sensitivity, but the websites do not provide data on the efficiency of the allergen extraction from food solids into the solution that is actually tested.

Drinking water testing has become a major international application of optical biosensors as contaminants of drinking water, such as lead, arsenic and pesticides, are recognized as major health hazards, and populations around the world are demanding inexpensive methods to insure drinking water safety. Optical microfluidic sensors for detecting pollutants (http://www.optiqua.com/eventlab.html#.XBk3vlxKgVs) and the water-borne parasite *cryptosporidium* (http://www.ntuitive.sg/our-start-ups/water-optics-technology-pte-ltd) are commercially available. In labs across the world, discrete samples are being tested using technologies that combine nanoparticles coated with biomolecules such as oligonucleotide sequences that bind metal ions or antibodies against pesticides or bacteria with colorimetry or fluorescence signals that are amplified by the nanoparticles. These nanoparticle-mediated signals have been generated in small tubes and on paper as inexpensive platforms where semi-quantitative information is sufficient and in microfluidic cartridges with simple, laser-based optical readout systems where quantitation is important.[5] As the cost of producing these tests comes down, more and more of the higher sensitivity, nanoparticle-based optical sensors should become commercially available.[6]

One of the advantages of optical approaches for bioanalysis, compared to electrochemical methods, is they are more compatible with continuous monitoring. Electrodes exposed to complex samples tend to foul over time and decrease in sensitivity for a specific target.[7] With appropriate system materials and geometries, optical sensors can provide continuous analysis with minimum fouling or signal drift, especially if the presence of a target can be spectrally distinguished from the background (as in fluorescence sensors, for example). Furthermore, optical transducers in some cases can have no direct contact with the sample for analysis, as in systems relying on spectral analysis or imaging.

The community developing wearable sensors has taken advantage of noninvasive optical methods for continuous measurement of bodily functions, including pulse, oxygenation, glucose utilization and lactate generation.[8] Optical sensors based on proven semiconductor materials are inherently robust, and light scattering and absorption processes capture clinically relevant information through the skin.[9] Measurement of hemoglobin properties provides data about heart rate and tissue oxygenation over extended periods. Use of soft electronic materials enhances conformal contact with the skin, reducing motion artifacts, and facilitating ultraminiaturization with organic light sources and photodetectors. RF electronics and near field communication technologies bypass the need for batteries and enable data transmission to off-skin receivers such as watches and phones. While these optical wearable sensors are not yet widely adapted to chemical or biochemical sensors, information such as the heart rate obtained from the three diodes on the back of many smart watches, combined with smart algorithms, serves as a surrogate for biochemical information. With consumers becoming comfortable with the use of wearable sensors, wearable sensors that include biological recognition molecules or biochemically reactive species are expected to be quickly accepted. Laboratory systems have already demonstrated the potential for monitoring biochemical targets in perspiration or interstitial fluid. Wearable “sweat sensors”, while not FDA approved for clinical diagnostics, are already being touted in the popular press to protect firefighters and athletes. Hydrogel coatings that recognize glucose to produce an optically measurable signal are nearing commercial feasibility (Figure 1).[10] A wearable sensor for peripheral oxygenation (https://profusa.com/lumee/) measures spectral changes in a hydrogel fiber inserted under the skin; this sensor in advanced development has the potential to drastically improve the quality of life for patients with diabetes or peripheral artery disease.

Laboratory biosensors reported in the last decade

Biosensors with exciting applications have been reported that incorporate revolutionary approaches to paper-based microfluidics and cell phone analyses and/or evolutionary improvements in high sensitivity

nanoparticle assays or optical systems such as Raman or surface plasmon resonance that can minimize the use of labels. The underlying drive for these development initiatives is to produce analytical devices that can provide actionable information inexpensively and simply for use by an appropriate consumer. Point-of-care diagnostics and point-of-use environmental and food analytics for the developing world have received a lot of emphasis, but companies commercializing such systems are frequently even more eager to use them to solve problems in the established markets of US, Europe and Asia.

Going beyond straight-path lateral flow immunoassays, paper microfluidics employ separate arms to sequentially control flow to different regions of the paper for more complex reaction control. The Yager group at University of Washington and the Whitesides group at Harvard spearheaded the use of shaped paper elements to mimic microfluidic systems; Yager cut the paper into appropriate shapes while Whitesides used wax to define the microchannel boundaries.[11,12] These paper tests work well in the lab, but issues such as evaporation, paper quality/reproducibility, and reagent stability continue to challenge large scale manufacture and commercialization.[13]

A variety of nanoparticles have been fabricated and incorporated into lateral flow assays and paper microfluidics, as well as tests in microfluidic cartridges. Building on earlier experience with gold nanoparticles and quantum dots, the literature is full of complex particles with novel combinations of particle materials and biorecognition molecules that generate signals clearly distinguishable from background, i.e. Förster resonance energy transfer, extended lifetime fluorescence or upconverting luminescence. Carefully shaped gold nanoparticles and zinc oxide nanostructures decorated with biorecognition molecules focus light at higher intensity at the particle surface and have enabled highly sensitive surface plasmon resonance and surface enhanced resonance Raman measurements.[14]

Meanwhile, the use of cell phone cameras as biosensor readout devices has exploded. Digital holography demonstrated the capacity to encode 3D information in intensity readings; Ozcan and colleagues used a convolutional neural network approach to reconstruct in-focus images from randomly defocused back-propagated holograms.[15] These computational advances not only provided a method to distinguish objects at lower resolution than the pixel density on the cell phone, but also led to lens-free technology that kept the devices small and less expensive than previous systems . The Ozcan lab also commercialized the first cell phone attachments to read out lateral flow assays (Figure 2),[16,17] and applications such as testing for drugs of abuse in saliva and infectious diseases in clinical fluids are in the early stages of commercialization,[18] with several cell phone readers already on the market.

Multiple papers describe the devolution of the color signals to distinguish bioassay results, but cell phone platforms are evolving mainly in terms of pixel count, acquisition frame rate and dynamic range, so that applications based on color resolution may not be the most promising. The ability to record movies has made cell phones effective devices for recording microfluidic analyses in real time; advances in microfluidic sample processing and analysis are frequently geared now for cell phone readouts. The lack of access to underlying cell phone software, the rapid changes in cell phone capabilities, and the wide availability of 3D printing have encouraged developers to make use-specific attachments for any cell phone or to replicate the simple optical and data transmission capability within the analytical device, relying on the cell phone merely to relay the data to the user. The rapid proliferation of miniature microscopes for cell analysis and point-of-care diagnostics exemplify this approach.[19]

Advanced technologies with application to optical biosensors

New materials and advanced engineering strategies are producing prototype technologies that were hard to imagine in 20th century science fiction novels. The recently reported handheld confocal microscope[20] is very reminiscent of the Star Trek Tricorder (Figure 3) and only needs complementary,

nontoxic dyes and recognition molecules to extend its function to biosensing in living animals. Microflow cytometers[21] are under development that include imaging as well as fluorescence spectroscopy for identification of cells, including marine algae. Particularly intriguing to think about in terms of eventual application for optical biosensors include molecular imaging, rapid sequencing, organic electronics, designer recognition molecules and molecular machines.

The last decade has fostered incredible developments in microscopic imaging methods that view samples with sub-micron resolution, in some cases even while a cell or organism is alive. This family of super-resolution microscopes, can be subdivided into those that break the Abbe diffraction limit using hardware solutions such as stimulated emission depletion microscopy (STED) and the single molecule localization microscopy (SMLM) methods that exploit the photophysical blinking properties of fluorophores such as photoactivated localization microscopy where the fluorescent species is a protein and (direct) stochastic optical reconstruction microscopy ((d)STORM) where the fluorescent species are organic fluorophores. The focus in the super resolution field has thus far mostly been on imaging of biological processes at the single molecule level where a unique molecular understanding of biological mechanisms can be achieved with the ability to localized the position of molecules with precision as low as 20 nm.[22]

The localization precisions as low as 20 nm achievable with SMLM methods are smaller than the expected space occupied by single biorecognition molecules. This invokes the notion of using SMLM for developing optical biosensors that monitor many single molecule interactions in a massively parallel wide-field format (Figure 4).[23] There are a few initial forays in using these methods for biosensing,[24] but at this stage the methods are not compatible with molecular counting, where calibration would not be required, because switching the fluorophores into the blinking mode required for SMLM is not currently quantitative. As such, the early forays into single molecule microscopy methods by biosensor researchers have mostly used these methods for characterizing how biointerfaces are working at a single molecule level.[25] The knowledge obtained using these sophisticated microscopies will suggest much simpler sensor technologies to assess specific molecular functions.

Further into the future will be sensors that build on high resolution approaches to image in three dimensions. Structural illumination, 3D SMLM, light sheet and even lattice light sheet microscopes have demonstrated the capability of 3D imaging and high resolution through large slices of tissue. Such methods could take us towards a new realm in sensing where ultrasensitive devices can measure concentrations directly *in vivo*. While these microscopes are currently very complex and expensive, the information they provide is likely to generate new targets for biosensor applications and possibly optoelectronic concepts appropriate for miniaturization.

The imaging of single molecules can, however, be achieve with much simpler optics. Notable here is darkfield microscopy where the scattering of light from surface bound plasmonic nanoparticles is sensitive to even a single protein binding to the nanoparticle. Initially, biomolecular binding events were monitored with imaging spectrometers which made the acquisition of the data the limiting factor but in more recent times imaging processing methods and the use of low cost CMOS cameras has meant data from thousands of nanoparticles can be obtained in less than a second.[25] Couple fast data processing with the mobile phone-based dark field microscopes, and it is easy to envisage portable optical biosensors that provide single molecule information. The notion of single molecule biosensors that count individual biomolecular binding events is not science fiction. Nanowell arrays have been combined with digital microfluidics to produce highly sensitive methods for detecting single molecules.[26,27] The success of the single-molecule optical array (SiMoA) technology is exactly that, a quantitative biosensor based on measuring many single molecule interactions (https://www.quanterix.com/products-

technology). Single-molecule sensitivity is achieved by capturing single proteins on antibody modified beads, binding additional immunoreagents that can generate fluorescence, capturing these constructs in an array of wells where each well can only accommodate a single bead and then developing the fluorescence. In this way fluorescence is only observed if the immunosandwich is formed as a result of the analyte being captured. As the number of beads is well in excess of the number of analyte molecules, Poisson sampling statistics makes it highly unlikely that the signal in a given well comes from more than one analyte molecule. In this way the number of fluorescent wells gives quantitative information on the amount of analyte. This method reveals incredible performance in terms of detection limits and sensitivity, and conversion of the more complex commercial devices to a simplified biosensor system is not a long leap.

One of the key features used in the SiMoA technologies to get single molecule resolution is the confinement of the optical signal to a well with femtolitre volume. Confining the volume from which the optical information is derived is used in many of the highly successful advances in optical sensing whether it be in optical fiber waveguides,[28] zero-mode waveguides (Figure 5),[29-31] or plasmonic nanoparticles.[32] In essence the small volumes are used to spatially separate the single molecules. Perhaps the only exception to confining the volume for single molecule sensing is in single molecule localization microscopy where temporal, rather than spatial separation, is used to separate the signals from individual molecules.

Identification of targets based on oligonucleotide sequences has long been a biosensor focus. Products range from complex arrays of complementary oligonucleotides on waveguides (e.g. http://www.affymetrix.com/technology/mip_technology.affx) to simple affinity-based biosensors for detecting single nucleotide polymorphisms. In the meantime, the technology for direct sequencing of oligonucleotides has advanced with Illumina systems now capable of sequencing the genome of an organism for $100 (https://www.illumina.com/documents/products/techspotlights/techspotlight_sequencing.pdf) and portable, non-optical devices from Oxford Nanopore sequencing DNA in classroom lab courses (https://nanoporetech.com/products). Portable optical biosensors that can both extract and sequence DNA or RNA cannot be far behind. In fact Bayley and co-workers have extended the nanopore technology from an electrical measurement to an optical measurement by measuring changes in calcium fluxes, using classical calcium fluorophores, through the nanopores as single molecules translocate through the nanopores.[33] The importance of this advance is that single molecule information can still be derived from many more nanopores in an array than can be achieved with resistance measurements and hence the amount of information that can be obtained is dramatically increased.

Many of the advances in imaging and highly parallel molecular analyses have been enabled by the development of LEDs and CMOS cameras; these two types of optical devices have certainly enabled the development of portable optical biosensors. The next round of miniaturization will be enabled by organic electronics. Light sources and detectors based on flexible thin films have come a long way in the last decade, primarily driven by the consumer display market. Critical challenges related to stability and power are being addressed. It has been difficult for biosensor developers to gain access to commercially proven materials because of the huge market opportunity in areas such as large screen displays, but that limitation is disappearing as the technology for making organic photodiode detectors and organic light emitting diodes is disseminated into a broader range of target applications. The marriage between organic electronics and wearable sensors, in particular, offers new opportunities for optical biosensors for real-time health and environmental monitoring.

The last decade has also seen an explosion in the type of molecules developed for molecular recognition. Selection methods for screening binding molecules, such as aptamers, peptides, and antibodies, from large libraries based on target recognition have become commonplace. In addition, the wider exploration of molecularly imprinted polymers has expanded understanding of how side chains on polymers impact target binding. Combining these knowledge bases, a more limited group of researchers has begun to explore the creation of recognition molecules by design. The general approach is to anticipate what molecular properties, especially in terms of flexible side chains, enhance target binding and then to modify a sub-optimum binding molecule to enhance binding or to modify an entire library prior to selection in order to fish out a better recognition molecule. Much of the background work to identify propitious alterations in the recognition molecule can be performed *in silico*. We predict that within the next decade, recognition molecules will be designed with the properties of affinity, avidity, specificity and solubility predetermined *in silico* prior to fabrication. This capability will overcome current limitations in sensitivity and specificity due to suboptimal recognition molecules and expand the applications of optical biosensors

Once the recognition molecules are designed, they can also be adapted to the particular optical biosensor application of interest. Already, recognition molecules have been cloned onto scaffolds that control valency, include groups such as His tags or biotin for subsequent purification or integration with detection schemes, or integrate regions such as hairpin structures that generate conformational responses to binding. Molecular machinery can be assembled that integrates recognition molecules, self-propagating amplification schemes and signaling capabilities to produce exquisitely sensitive optical biosensor strategies. The integration of all these steps into a single event, especially if it can be done in solution after adding an unprocessed sample, offers the lure of incorporating the reagents and strategy into a very simple optical device for a sensitive, specific, easy-to-use biosensor. We predict that the lessons learned from these current molecular design initiatives will produce totally *in silico* design systems for producing recognition molecules with optimum “parts” to produce the needed avidity, specificity, signal amplification, and hooks for purification, labeling and immobilization into sensors.

Optical Biosensors in the Next Decade

Before the turn of the century, the general consensus was that biosensors needed to use electrochemical technologies if portability was required. The communications industry has turned this conclusion into nonsense. Advances in optical materials and components, as well as miniaturization of electronics, creation of molecules *de novo*, and breakthroughs in nanotechnology, have opened new opportunities for optical biosensors. Point-of-care diagnostics developed for low resource environments will gain widespread acceptance for doctor’s office and home use, and these optical biosensors will become a major factor in reducing health care costs for everyone. Simple imaging devices will measure specific functions in living tissues to monitor regenerative medicine, cancer therapies, and physical rehabilitation. The availability of high resolution, handheld imaging systems will trigger the development of new biocompatible recognition molecules and nontoxic dyes to help identify specific cells or molecules in living systems. Wearable optical sensors will rapidly adapt to new applications for health monitoring, and similar devices will become networked[34] for monitoring geographically distributed factors such as epidemics, environmental pollution, animal health, and even crop productivity. Continuous monitoring devices, possibly in a more robust format, will be integrated into urban infrastructure to monitor delivery of safe drinking water (Figure 6) and food supplies.

While exciting new breakthroughs in science and engineering drive the miniaturization of optical technologies and creation of biosensors to address needs of the earth’s population for health, food production, a clean environment, and personal safety, the biosensor community must also consider the

impact of the information on the users. Is the data reliable and appropriate for the decisions that will be based on that data? Will the impact on the individual or community be appropriate? Are the ethical issues embedded in the biosensor design clearly understood, e.g. is the sensor appropriate for use by all of the intended genders, cultures, and socioeconomic groups? Scientists and engineers developing new biosensors have amazing tools and ideas at their disposal right now; we need to be very thoughtful in how we create new systems to sustain life on the planet.

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Figures

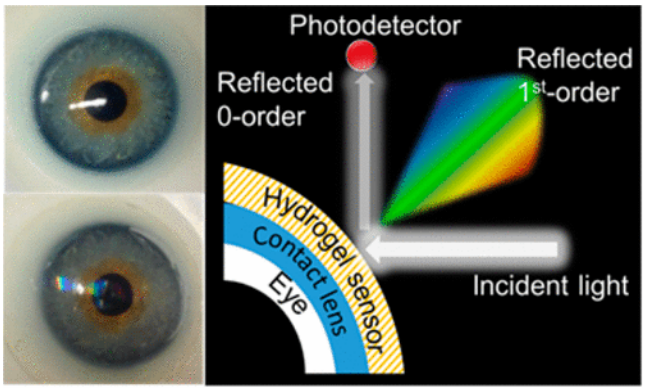


Figure 1. Contact lens coated with a hydrogel containing phenylboronic acid is used to measure glucose concentration in tears. [Reproduced with permission from ref. 10. Copyright (2018) American Chemical Society.]

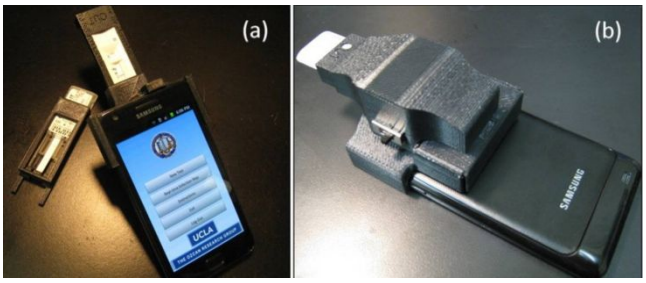


Figure 2. Attachment to cell phone used to read results from lateral flow assays. [Reproduced with permission from ref. 16. Copyright (2012) Royal Society of Chemistry.]

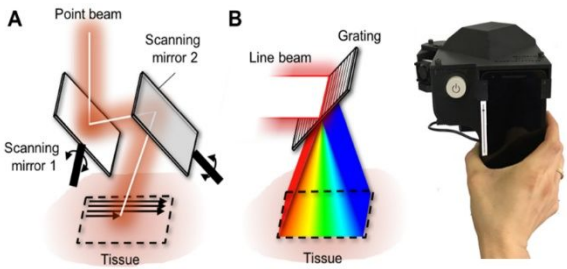


Figure 3. Handheld confocal microscope for skin analysis. [Adapted with permission from ref. 20, Copyright (2018) Sage Publishing].

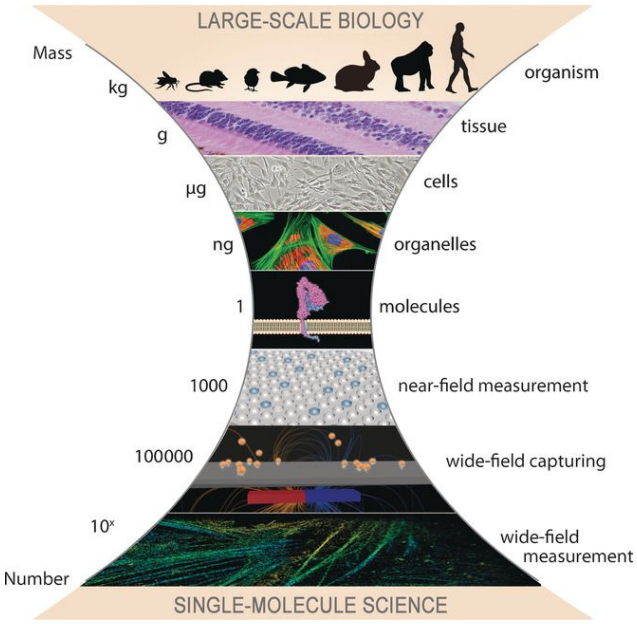


Figure 4. Historically, advances in measurement science have produced methods that are more and more sensitive such that smaller sample sizes and lower concentrations of analyte can be measured and more and more sophisticated scientific questions can be asked. This reduction in amount of sample interrogated has reached its inevitable conclusion of single molecule detection. Measurement science has now entered a phase where more and more single molecule measurements can be performed in parallel to accomplish meaningful quantitative analysis. These highly parallel analyses are achieved either with many simultaneous near-field measurements, by capturing the single molecules in bulk solution before capturing the analytes in small measurement volumes, or by measuring many single molecules directly on a surface in a wide field format. [Reproduced with permission from ref. 23. Copyright (2016) John Wiley and Sons.]

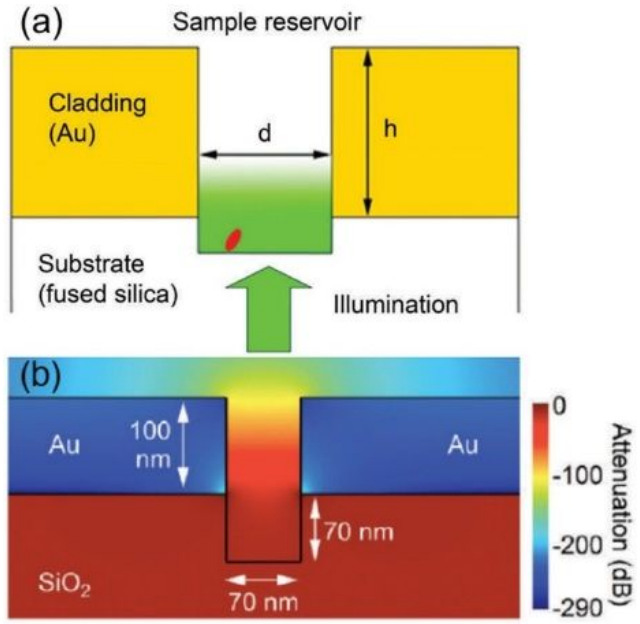


Figure 5. Zero mode waveguides provide one approach for confining single molecules in an optically addressable location. [Reproduced with permission from ref. 29. Copyright (2012) Sage Publishing.]

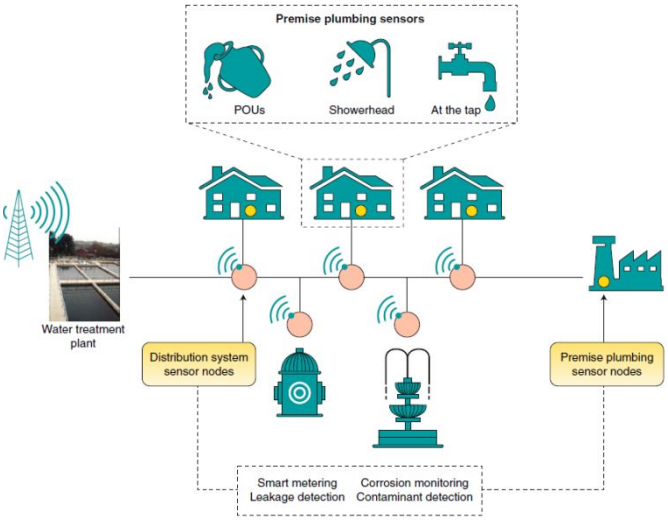
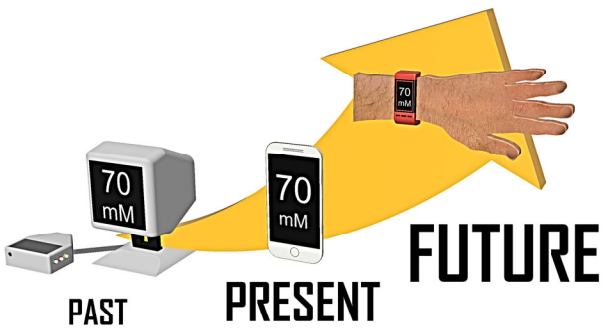


Figure 6. Potential positions where optical sensors and biosensors could be integrated into infrastructure for maintaining water quality. [Reproduce with permission d from ref. 6. Copyright (2018) Springer Nature.]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