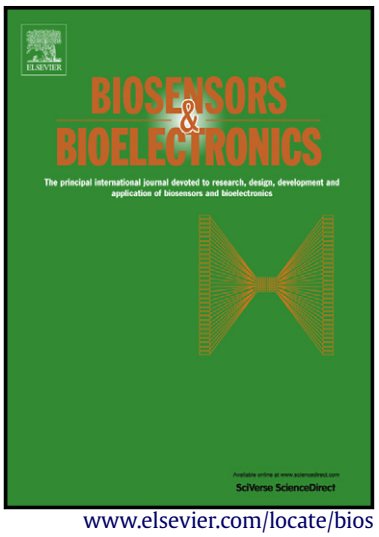

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Bacterial Detection: from Microscope to Smartphone

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Abstract

The ubiquitous nature of bacteria enables them to survive in a wide variety of environments. Hence, the rise of various pathogenic species that are harmful to human health raises the need for the development of accurate sensing systems. Sensing systems are necessary for diagnosis and epidemiological control of pathogenic organism, especially in the food-borne pathogen and sanitary water treatment facility's bacterial populations. Bacterial sensing for the purpose of diagnosis can function in there ways: bacterial morphological visualization, specific detection of bacterial component and whole cell detection. This paper provides an overview of the currently

available bacterial detection systems that ranges from microscopic observation to state-of-the-art smartphone-based detection.

Key words: bacteria, pathogen, biosensor, 16S rRNA

1. Introduction

Bacteria are microorganisms that are a few micrometers in length and take the shapes of spheres, rods, or spirals. Bacteria sense and respond to temperature and pH changes, nutritional starvation, stresses to the outer and inner membranes, new food sources, toxins, and quorum sensing signals (Salis et al., 2009). One of the processes associated with bacterial infection is chemotaxis, which involves the attraction or repulsion of the bacteria from chemical stimulus (Grebe and Stock, 1998). Pathogenic bacteria are harmful species that cause bacterial infections and contagious diseases that result in many serious complications. Common pathogenic bacteria and their effects include *Escherichia coli* and *Salmonella* (food poisoning), *Helicobacter pylori* (gastritis and ulcers), *Neisseria gonorrhoeae* (sexually transmitted disease), *Neisseria meningitidis* (meningitis), *Staphylococcus aureus* (boils, cellulitis, abscesses, wound infections, toxic shock syndrome, pneumonia, and food poisoning), and *Streptococcus* spp. (pneumonia, meningitis, ear infections, and strep throat). Records from the Foodborne Diseases Active Surveillance Network show that between 1996 and 2005, 121,536 cases of laboratory-confirmed bacterial infections were reported in the United States, resulting in 552 deaths, of which 215 were due to *Salmonella* and 168 due to *Listeria* (Behravesh et al., 2011). Epidemic diseases caused by foodborne pathogenic bacteria are major health issues, which necessitate an immediate detection strategy (Zhang et al., 2013). This is because the clinical presentations for most of the pathogenic infections are not specific enough for definitive diagnosis. The main issue in diagnostic is to distinguish between the closely related sub-species and between pathogenic and non-pathogenic strains. Sensing strategies developed should be rapid and involve sensitive procedures for early screening and proper enumeration of the identified pathogenic strains. The rapid and accurate detection of pathogenic bacteria is vital for the administrations of appropriate antibiotic treatment to control the spread of

the disease and to assess the drug resistance information. Detection of pathogenic agents is also a vital step for the identification of infection source anywhere from home, hospital to outdoor settings (Klompas et al., 2009; Allegranzi et al., 2011; Polin et al., 2012).

Fundamental identification of bacteria relies on the morphological features of the cells, which can be visualized via microscopic observations. Other common methods include Gram staining, culturing, and biochemical assays and sequence-based detection. In addition, probes (e.g., aptamers or antibodies) that are specific to surface/flagellin proteins of the bacterial or the bacterial whole cell, are also used to detect the presence of bacteria. These probes are often applied in biosensors for the detection of specific types of bacteria. This review provides an overview of the currently available bacterial detection strategies.

2. Microscopic examination

Microscopes are optical-based instruments that are used to visualize samples that cannot be seen by the naked eye (Figure 1a & b). Bacteria were initially observed by the Dutch microscopist Antonie van Leeuwenhoek in 1683 using a single-lens microscope (Porter, 1976). Direct observation of bacteria under microscope is the most simplest and cheapest way for bacteria identification. Bacteria are identified and classified based on the various shapes, sizes and arrangements (for examples coccus, bacillus, filamentous, in pair or in chain formation). Beside, based on cell wall properties, bacterial can be identified under microscopic observation due to their staining properties, such as Gram positive, negative, or acid-fast staining). The application of labeled tags (fluorophores, quantum dots etc) has also enhanced the precision of the microscopic identification of bacteria. For example, fluorophores tagged to probes specific to a given microorganism can be visualized with a fluorescence microscope. Recently, fluorescence microscope was used to visualize *Pseudomonas aeruginosa* with the aid of fluorescently labeled nucleic acid probe (Wang et al., 2011b). Steingart et al. (2006) reported that use of a fluorescence microscope that enhanced the smear microscopic visualization by 10% over the conventional Ziehl-Neelsen (ZN) staining method for *Mycobacterium tuberculosis* visualization. Another microscope, LED (light-emitting

diodes) microscope, which is characterized by long lifespan and low power consumption, is beneficial in countries with limited resources. LED microscope was used to visualize *M. tuberculosis* (Trusov et al., 2009; Albert et al., 2010). Although not as sensitive as fluorescent microscopes for visualizing *M. tuberculosis*, the LED microscope provides faster reading with exceptional reproducibility and easy to be used (Bonnet et al., 2011).

Another technology that may prove to be useful for laboratories with limited resources is the portable digital microscope known as CellScope, which has the capacity of both bright-field and fluorescence microscopy (Chang et al., 2012). The images obtained are binarized images that have patch sizes that match the size of *M. tuberculosis* bacilli (2–4 μm in length, 0.5 μm in width). This microscope, together with the portable automated low-cost bright-field/fluorescence microscope, may slowly replace the bulky high-cost, conventional laboratory-grade optical microscopes (Schaefer et al., 2012). With the advancements of technology, microscopes with higher resolutions have been introduced, including scanning electron microscopes, transmission electron microscopes, environmental scanning electron microscopes and atomic force microscopes (Jung et al., 2010). Although microscopic observation represents a simple and cheap way for bacterial identification, it cannot be used for differentiate between different bacterial strains. However, this is possible by using sequence specific detection. [Insert Figure 1]

3. Sequence specific bacterial detection

Specific sequences of the bacterial genome are used to classify bacteria into specific genera or species. For example, the sequencing and analysis of 16S ribosomal RNA has lead to the division of prokaryotes into two evolutionary domains, namely the Bacteria and Archaea (Woese et al., 1990). 16S rDNA sequencing became an important tool for the identification of bacterial isolates, especially bacterial species with unusual phenotypic profiles, rare bacteria, slow-growing bacteria, uncultivable bacteria, and culture-negative infections (Woo et al., 2008). 16S rRNA sequences have a region

that is highly conserved across species that can be used for general bacterial detection as well as highly variable specific-specific regions that can be used for species-specific bacterial detection (Woo et al., 2008; Rajendhran and Gunasekaran, 2011) (Figure 2a). Using 16S rDNA sequences, Woo et al. (2008) have identified 215 novel bacterial species, 29 of which belong to novel genera, from human specimens collected between 2001 and 2007. Another species-specific sequence, *IS*6110 (an insertion element that is found in *M. tuberculosis* complex species), has proved to be an important diagnostic marker for the detection of *M. tuberculosis* complex species (Dale Thierry et al., 1990; et al., 1999; Tang et al., 2013). Hence, sequence-based detection of this strain is a more prominent alternative than microscopic-based observation, as it provides species-specific detection. Moreover, the presence of multiple copies of *IS*6110 at different locations in the genome can ensure efficient DNA fingerprinting of the bacteria. To specifically detect these sequences, Polymerase Chain Reaction (PCR) is an assay that allows the amplification of the specific sequences to an amount enough for detection to ensure precise diagnostic. [Insert Figure 2]

3.1. Polymerase Chain Reaction (PCR)

PCR is a molecular amplification process whereby copies of target DNA are amplified through a stepwise process of denaturation, annealing, and extension of the target DNA. Due to its sensitivity, only a few copies of the pathogen genome in the sample are necessary to detect target DNA. Ahmad et al. (2012) used PCR to detect the presence of *Leptospira*; they reported a sensitivity of detection that reached 1.8 pg and 1×10^3 leptospires/ml. Similarly, Tang et al. (2013) reported that use of multiplex PCR-based detection of *M. tuberculosis* achieved sensitivity of 93.1% and specificity of 89.6%, which exceed those of the microscopy/culture method. In both of these PCR assays, a protein-coding gene was used as the target for detection. In contrast, Nithya et al. (unpublished data) successfully demonstrated the feasibility of a non-protein coding RNA (npcRNA) gene as the target for the PCR assay for detection and differentiation of *Salmonella typhi* and *S. paratyphi A*. Sensitivity of 10 pg (10^3 bacteria) was reached for both *S. typhi* and *S paratyphi A*. This result showed that npcRNA gene sequences could be used as the target for PCR-based diagnostic tests. A potpourri of

npcRNAs discovered in a wide array of microorganisms (Chinni et al., 2010; Raabe et al., 2011) also suggests the potentiality of relying on npcRNA as the potential target for PCR-based diagnostic.

Currently, there are over 50 companies working on bacterial diagnostics. Some companies such as Roche Diagnostics, Chiron Corp., Gen-Probe Inc., Bayer Diagnostics, BD (Becton, Dickinson and Co.), Innogenetics, Digene Corp., bioMérieux SA, Qiagen NV and Eurogentec SA, have successfully developed PCR-based platform for bacterial detection. They have generated a fully integrated sensing platforms that can perform self-contained, multiplex and automated detection, which may represent the future direction of bacterial detection. Verigene gram-positive blood culture nucleic acid test is a random access, multiplexed, automated nucleic acid test for the direct detection and identification of genus, species and genetic resistance determinants of gram-positive bacteria (such as *Staphylococcus* spp., *Streptococcus* spp., *Listeria* spp., and *Enterococcus* spp.) from blood culture broth. This technique also reduces the time of detection from several days associated with blood culture to only a few hours of detection (Scott, 2013). On the other hand, iCubate system facilitates a multiplex detection of PCR assay, which is empowered by ARM-PCR (Amplicon Rescued Multiplex PCR) technology for simulatenous detection of multiple targets in a single reaction. Moreover, this system is equipped with a reader, one processor, a computer with preinstalled software and PPI algorithm (http://icubate.com/index.php/technology/). Another state-of-the-art multiplex PCR system is the Filmarray. This system is an easy and fast two stages PCR system, which consists of extraction/nucleic acid purification system and nested multiplex PCR system. The detection is performed by a singleplex second-stage PCR amplification, that amplifies the products from the initial multiplex PCR (http://filmarray.com/the-panels/).

3.2. Real-time PCR-based platform

Typically, PCR-based detection methods require the use of agarose gel electrophoresis and ethidium bromide staining to visualize the amplicon. However, real-time monitoring of pathogen genomic DNA via real-time PCR obviates the need for agarose gel electrophoresis, as monitoring is achieved in real-time with the use of SYBR green or fluorescently labeled probe/primer. Real-time PCR assay was developed for the differentiation of *Schistosoma japonicum* and *S. mekongi* (the causative agent of Human schistosomiasis) by using primer pair specific against the 18S ribosomal RNA gene. The usage of the real-time PCR is better than the use of microscope, which cannot differentiate cercariae and eggs of these 2 species (Kongklieng et al., 2013). In another study, a microfluidic chip was designed that contains both DNA extraction and real-time PCR system. The chip contains a monolithic aluminum oxide membrane (AOM) for DNA extraction. As a proof-of-principle for multiple target detection, methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) genomic DNA (gDNA) used for detection that can be detected up to 300 fg (100-125 copies) (Oblath et al., 2013). Real-time detection of live *Salmonella* sp. cells was also demonstrated via real-time PCR using primers targeting the *invA* gene (González-Escalona et al., 2009).

3.3. Isothermal-PCR based platform

The conventional PCR-based amplification method has evolved into isothermal amplification, which requires a single temperature as opposed to the thermocycling required for PCR. Isothermal amplification techniques include strand displacement amplification (SDA), loop-mediated isothermal amplification (LAMP) (Chang et al., 2012), and nucleic acid sequence-based amplification (NASBA) (Compton, 1991). Isothermal-based amplification is an attempt to eliminate the need for a thermal cycler, which is expensive and bulky. Huang et al. (2013) used the isothermal-based amplification method known as helicase-dependent isothermal amplification (HDA), which consists of a disposable DNA amplification platform, to detect DNA extracted from a stool sample

containing *Clostridium difficile.* This electricity-free system, which operates on a microfluidic chip in a Styrofoam cup (the insulator), was able to maintain its temperature at 65 ± 2 °C while achieving a detection limit of 1.25 x 10^{-2} pg of DNA (Huang et al., 2013). Besides cost saving, this isothermal-based amplification systems can be beneficial for places with very low-resource settings and can be performed faster compared to normal PCR-amplification to incur appropriate treatment after diagnosis.

3.4. Microarray

Although PCR and Real-time PCR enable multiple target detection, the application of these assays for high-throughput analysis can incur expensive costing and is liable to amplification bias, false positivity and inaccuracy in relative abundance quantification (Janse et al., 2012). In this case, one elegant sequence-based detection system that enables high-throughput analysis for a wide panel of bacterial detection while maintaining low cost per sample is microarray. Microarray is carried out on any suitable surface/surfaces of nanosensors. DNA microarray is now widely used in molecular biology for gene profiling, rapid detection of mutations and polymorphisms, gene discovery, and gene expression monitoring. In principle, microarray involves several thousands of specific DNA sequence spotted in picomoles amounts. Among the various high-throughput assays that have been developed, microarray technology has grown most rapidly (Silzel et al., 1998; MacBeath and Schreiber, 2000). The technology originally evolved from Southern hybridization, in which the sequences of DNA to be analyzed are spotted on the surface of a membrane and then probed with known gene sequences. To identify the genes involved in the interferon modulated expression profile, Kulesh et al. (1987) used the DNA array technique by spotting cDNAs on a filter paper, and Schena et al. (1995) miniaturized microarrays for gene expression profiling. Ekins and Chu (1992) described the first immunoanalytical microarray during the same period of time that DNA arrays were being developed. Hybridization of probe and target is usually detected and quantified via fluorophore-labeling of the target (Figure 2b). Perraut et al. (2002) proposed the use of a new generation of scanners for DNA chips that offer high sensitivity, high resolution, high performance, and reduced cost. Using

microarray and comparative genomics, Schoolnik (2002) studied the genetic basis of bacterial pathogenicity; this study involved characterization and quantification of the extent of genetic variability within natural populations at the gene level of resolution. In another study, Mitterer et al. (2004) described a DNA microarray-based PCR approach for the quick detection and identification of bacteria from cervical swab specimens. This method has successfully identified bacteria that were not detected by the conventional method (Mitterer et al., 2004).

Most microarray imaging results are obtained by scanning of laser excitation coupled with photomultiplier tube light detectors. For quantitative imaging, Hamilton et al. (2006) used approaches based on wide field imaging and charge-coupled devices (CCDs). Cell array technology has become an efficient analytical tool, in a scope that resembles DNA microarray biochips. Details of cell array were overviewed by Melamed et al. (2012). Recently, Ballarini et al. (2013) designed the BactoChip microarray for the simultaneous detection and quantification of multiple types of bacteria. This chip enables the differentiation of bacterial species from 21 different genera based on 60-mer probes designed from in silico identified species-specific marker genes. This array has a low limit of detection (0.1%) and can handle species-level relative abundances over a 100-fold dynamic range in complex bacterial communities.

3.5. Neutralizer displacement and micro-nuclear magnetic resonance (μNMR)

Label-free sequence-based detection strategies that do not require fluorophores, SYBR Green and ethidium bromide staining can be cost-saving detection systems without compromising the sensitivity. Some of these strategies rely on the measurement of electrical signal changes or the monitoring of changes in NMR spectra. For example, the neutralizer displacement assay allows detection of biomolecules based on the electrostatic changes of a sensor surface. The specific probe is conjugated to a neutralizer (which consists of a conjugate of a peptide nucleic acid and a cationic amino acid), which results in charge neutralization of the probe. Due to the mismatch in the probe-neutralizer complex, the target specific for the probe will bind more strongly to

displace the neutralizer. The result is a large increase in the electrocatalytic current. Das et al. (2012) used a 20-mer DNA probe to detect bacteria in an unpurified lysate, and the reported detection limit reached 0.15 cfu μL^{-1} within 30 min. This detection limit was even better than that of direct analysis (Figure 3a). [Insert Figure 3]

Other assays involve tracking the changes in NMR spectra that are associated with the relaxation rate of the sample. In one study, magnetic-DNA nanoparticles were used to detect several types of bacteria in a clinical sample. In this system, two probes complementary to the two ends of the target 16S rRNA are immobilized on the surface of the polymeric microsphere. Following total RNA extraction and symmetric reverse transcription-PCR to produce copies of single stranded sense DNA strands, these strands are captured by one of the target probes on the surface of the microsphere. Next, another probe, which is complementary to the other end of the target strand and is immobilized on the magnetic nanoparticles, hybridizes to the overhanging edge of the target DNA. This results in the shortening of the transverse relaxation rate (R2) of a sample, which is detected using a miniaturized μNMR device. This assay has been used for general and specific detection of several clinically relevant bacterial types with sensitivity of detection that reached up to a single cell. Moreover, it offers high sensitivity of detection as the background level is very minimal and has many advantages over PCR/real-time PCR in terms of cost and time (Figure 3b) (Chung et al., 2013).

4. Bacteria cell surface-based detection/whole cell protein-based detection

Apart from the specific sequences that reside in the genome of the bacteria, a number of different bioreceptors that include proteins, polysaccharides, and flagella on the surface of the bacteria also often become the target of detection. Bacteria cell surface proteins are responsible for a host of processes such as adhesion, nutrient acquisition, and signaling. Depending on the method of probe generation, some of these probes can target the individual protein or multiple protein targets on the cell surface (whole cell-based probe generation). Two dominant probes, antibodies and

aptamers, have been the focus of many biosensor development studies (Bai et al., 2012; Liu et al., 2013).

4.1. Antibodies

Antibodies are protein-based probes that recognize their target antigen with high affinity and specificity. They are produced using an immunized animal or via cloning/recombinant DNA technology. As glycoproteins belonging to the immunoglobulin group, antibodies have different isotypes with an amine (NH_2) terminus at antigen binding regions and the carboxyl (COOH) terminus at the stem. There are two different types of antibodies: polyclonal and monoclonal. In a number of studies, polyclonal antibodies were produced to detect *Salmonella* spp. (Kramer and Lim, 2004; Hahm and Bhunia, 2006), *E. coli* (Geng et al., 2006), and *Listeria monocytogenes* (Geng et al., 2004; Gray and Bhunia, 2005). In addition, monoclonal antibodies have been generated against antigens such as the whole cell (Zhao and Liu, 2005), the cell wall (Thirumalapura et al., 2005), and virulent proteins on the cell surface (Hearty et al., 2006). Compared to polyclonal antibodies that recognize multiple epitopes on the target, monoclonal antibodies are more specific, as they recognize only a single epitope on the surface of the antigen. Thus, monoclonal antibodies exhibit less cross-reactivity compared to polyclonal antibodies. Synthetic peptides have become a preferred choice to alleviate problems of cross-reactivity in antibodies. Synthetic peptides are much smaller than the whole protein; typically they contain 10–20 amino acid residues that harbor the epitopes against which antibodies bind. For example, IgY antibodies were isolated against the urease peptide from *H. pylori* (Shin et al., 2004). Antibodies were also produced against a wide array of synthetic peptides from *S. aureus, Streptococcus* spp.*, H. pylori,* and others (Huesca et al., 2000; Shin et al., 2004; Bialek et al., 2006).

The most common method of antibody-based detection is the enzyme linked immunosorbent assay. Other than this assay, several sensing strategies have been generated using antibody; for example, Kleo et al. (2012) developed a two antibody-based biosensor for *F. tularensis*. The first system uses a quartz crystal microbalance (QCM), in which sensor chips were modified by a specific antibody. The QCM technique is combined with a microfluidic flow system that allows online detection of binding

events between antibody and whole bacteria. The second system is based on the aggregation of antibody-functionalized gold nanoparticles and bacteria. Aggregation is detected as the change of particle diameter using dynamic light scattering and the shift of the absorption peak using UV/Vis spectroscopy. A detection limit of 10^3 CFU/ml was acquired (Kleo et al., 2012). The availability of antibody against bacteria can facilitate the development of many other antibody-based detection assays such Western blot and dot-blot for the sensitive detection of bacteria. Moreover, for improved detection limit of bacteria, the available antibodies can also be harnessed in immuno-PCR, as was evident with the development of immuno-PCR for the detection of fish pathogen, *Pasteurella piscicida* (Kakizaki et al., 1996). The analogue of antibody known as aptamer is also gradually gaining attention due to the advantages over antibody.

4.2. Aptamers

The catalytic reactions executed by the group I intron of *Tetrahymena thermophila* and the RNA portion of RNase P demonstrated the ability of the RNA to perform a range of reactions. The functional capabilities of this nucleic acid molecule were further illustrated by the discovery of ribozyme, which led to the concept of RNA world (Cech, 1986; Orgel, 1986). These discoveries showed that RNA molecules are associated with phenotype and genotype and they undergo structural conformations to carry out certain functions and to carry information. To further study the functions of RNA, *in vitro* evolutions were performed in test tubes, which have engendered an in vitro selection system that can generate high affinity aptamers in the early 1990s. This system, named SELEX (Systematic Evolution of Ligands by Exponential Enrichment), was developed using a randomized library of molecules. It involves the selection of molecules from the nucleic acid libraries (ssDNA or RNA) that have high affinity against the target by iterative processes of incubation, separation, and amplification. The high-affinity nucleic acid ligands obtained by this method are called aptamers, and they are initially generated against different targets (T4 DNA polymerase, organic dyes) and to alter the cleavage activity of a ribozyme (Ellington and Szostak, 1990; Robertson and Joyce, 1990; Tuerk and Gold, 1990).

Aptamers are widely used in diagnostic applications for wide range of bacterial targets, due to their advantageous properties, such as low molecular weight, reversible denaturation, and ease of being functionalized. Aptamers can be produced rapidly, and the availability of automated robots to carry out the SELEX process has further sped up the process (Eulberg et al., 2005). The RNA aptamer specific for type IVB pilus, which is the point of entry into human cells for *Salmonella enterica* serovar *typhi*, was generated via SELEX (Pan et al., 2006). This aptamer was found to inhibit the action of this pathogen by reducing cell invasion. Another aptamer was generated against *Campylobacter jejuni*, a causative agent of human campylobacteriosis, which is caused by consumption of undercooked poultry (McMasters and Stratis-Cullum, 2006). *Francisella tularensis*, which is the agent that causes Tularemia, was also targeted for aptamer generation. Vivekananda and Kiel (2006) generated 25 different classes of aptamer specific against *F. tularensis* sub-species *japonica*. In other studies, magnetic beads were used to generate aptamers against Cholera toxin, Staphylococcal enterotoxin B, and anthrax spores (Bruno and Kiel, 1999; 2002).

Hamula et al. (2008) generated a DNA aptamer that can bind the bacterium *Lactobacillus acidophilus*. The resultant aptamer exhibited a very high affinity for the target, with an estimated dissociation constant of 13 ± 3 nM (Hamula et al., 2008). Using cell-based SELEX or complex target SELEX, Cao et al. (2009) isolated a series of DNA aptamers that can bind to *S. aureus*. This type of SELEX can generate a group of aptamers that can bind to various target proteins on the surface of *S. aureus*; in contrast, single target SELEX generates aptamers that are able to recognize a single target. Cao et al. (2009) produced five different classes of aptamers (SA20, SA23, SA31, SA34, and SA43), and they were able to recognize their target in the nanomolar range. The synergistic binding of all aptamer groups to the target can be more prominent compared to that of the individual aptamer in detecting *S. aureus* (Cao et al., 2009). Using a fluorescence assay and electrochemical detection, Kim et al. (2014) studied the binding efficiency of a cocktail of three DNA aptamers generated to detect *E. coli* and compared it with that of a single aptamer. The detection limit of the cocktail was 18 times greater than that of the single aptamer. One of the developed SELEX procedures, called cell-SELEX, has enabled generation of aptamers against the whole

cell as the target (Figure 4). Cell-SELEX creates an aptamer that binds to molecules on the surface of target cells, so specific information about the target is not needed (Sefah et al., 2010). Using cell-SELEX, Labib et al. (2012) generated high-affinity DNA aptamers against live *Salmonella typhimurium* with a counter selection strategy against heat-killed *S. typhimurium* and a mixture of related pathogens, including *S. enteritidis*, *E. coli*, *S. aureus*, *P. aeruginosa*, and *Citrobacter freundii*, to obtain target-specific candidates. A high-affinity DNA aptamer against spores of the nonpathogenic strain *Bacillus anthracis* was generated and applied in an aptamer–magnetic bead–electrochemiluminescence sandwich assay scheme (Bruno and Kiel, 1999). Ozalp et al. (2013) immobilized two different aptamers specific against *E. coli* and *S. typhimurium* on the surface of magnetic beads and quantified the presence of bacteria using real-time PCR. They showed that even in the presence of 100% milk, detection of approximately 100 CFU/mL for both *E. coli* and *S. typhimurium* could be reached within 2 hours in spiked milk samples. Kolesnikov et al. (2012) provides a detailed overview of aptamer-based diagnosis of bacterial infection. Aptamer-based detection is a nucleic acid-based detection that has many advantages based on the features associated with aptamer such as the absence of batch-to-batch variation, reusability due to stability against repetitive denaturation and ease of modification for conjugation on a variety of surface platform (Table 1). [Insert Figure 4; Table 1]

5. Biosensors

A biosensor is a device that combines a biological component, such as an aptamer/antibody, as the detection agent with a detector component that primarily is responsible for displaying the occurrence of biomolecular interactions. For a biosensor to be valuable for both research and commercial applications, it must contain a suitable biological recognition element that exhibits high sensitivity. Moreover, the structures used for construction of biosensors must be compatible for biological molecules such as enzymes, antibodies, antigens, whole cells, and DNA strands. Using glass or SiO_2 materials as the surface, biomolecular interactions are monitored via surface-assisted modifications (McCauley et al., 2003; Kumar et al., 2004; Ho et al., 2006; Wang et al.,

2006; Yang and Zhu, 2007; Gopinath et al. 2010, 2013). Many biosensors have been developed using gold (Au) as the surface; gold can be easily immobilized with biomolecules through thiol linkers (Huber et al., 2004; Gopinath et al., 2008). Checa and Soncini (2011) attempted to develop a selective Au-based sensor that allows species harboring resident Cu-homeostasis systems to eliminate the Au toxic ion without perturbing the acquirement of Cu in Au-rich surroundings. One-dimensional ceramic nanomaterials have been developed, and one-dimensional nanofibers can be fabricated via electrospinning, sol-gel, or the combination of the two (Teo and Ramakrishna, 2006; Ramaseshan et al., 2007). Oxide sensors designed from these nanomaterials (size of 30–50 nm) can be used for biomolecular detection with high sensitivity (Ramaseshan et al., 2007).

5.1. Luminescence-based biosensors

Biosensors with high sensitivity for biomolecular detection often involve detection by luminescence (e.g., fluorescence and chemiluminescence). For example, Meir et al. (2008) have developed a chromatic/fluorescence sensor for rapid and sensitive detection of bacterial proliferation. They constructed agarose-embedded chromatic films that are able to produce color changes and fluorescence transformations in response to bacterial growth. The color changes (blue-red transformation) of the sensing constructs, which consist of glass-supported Langmuir–Schaeffer phospholipid/polydiacetylene films, is due to the interaction of the construct with the amphiphilic compounds secreted by the bacteria. Using an aptamer specific against spores of *B. anthracis* (nonpathogenic Sterne strain), an electro-chemiluminescent-based detection system was developed; it achieved a detection limit of 10^2 to 10^5 spores (Bruno and Yu, 1996). In another fluorescent assay based on FITC-labeled aptamers against *P. aeruginosa*, the limits of detection, quantification, and linearity of *P. aeruginosa* were 5.07, 5.64, and 100 cells/mL, respectively (Kim et al., 2013). The low detection limit shows that the fluorophore-labeled aptamer can detect the presence of *P. aeruginosa*, even at a very low concentration. Duan et al. (2013) recently developed a dual-color flow cytometry

protocol for the simultaneous detection of *Vibrio parahaemolyticus* and *S. typhimurium* using an aptamer conjugated with quantum dots (Duan et al., 2013).

Polymer-assisted sensing is another strategy that is used predominantly for developing high performance sensors with excellent sensitivity, specificity, and less non-fouling (Nagasaki, 2011). Recently, Wan et al. (2013) developed a quaternized magnetic nanoparticle-fluorescent polymer system for detection and identification of bacteria. The system uses three magneto particles conjugated with amine groups, and a fluorescent polymer with a carboxylic side chain acts as the transducer. After attachment of different bacterial species, they could find the replacement of polymer and measured the emitted fluorescence signal (Wan et al., 2013).

5.2. Real-time monitoring of bacteria

Among the biosensors currently available, those that allow real-time monitoring and detection of bacteria are favored, especially in industries involved in the manufacture of human consumable items and in clinical diagnosis in which pathogenic bacteria are involved. Acquisition of detailed information about the presence of bacteria in real time is imperative, as it allows the execution of the steps necessary to eliminate pathogens. Furthermore, biosensors that do not involve the usage of labeled probes are essential, as labeled probes can mask important binding sites and result in a false-negative reaction. Surface-enhanced Raman scattering and disc technology represent important biosensing applications that operate without the requirement for labeled probes.

5.2.1. Surface-enhanced Raman scattering (SERS)

Raman scattering is an inelastic process whereby the frequency of the scattered photon differs from that of the incident photon. Immobilization of biomolecules on the surface of a metal nanoparticle and its subsequent interaction with other biomolecules results in further enhancement of the Raman scattering, producing an enhanced effect termed SERS (Nie and Emory, 1997). Similar to surface plasmon resonance, SERS can

be used to detect biomolecular interactions on the surface of metal nanoparticles via monitoring of the shift in the SERS spectra (Abell et al., 2009). Due to the high sensitivity of this method, SERS is widely used for monitoring biomolecular interactions in real time. Wang et al. (2011a) showed that SERS was useful for multiplex detection of *S. typhimurium* and *S. aureus* in complex food matrices. Antibodies specific against the pathogen, which were immobilized on silica-coated magnetic probes, bound to the protein on the surface of the bacteria and were detected using gold nanoparticles functionalized with a Raman reporter. The detection limit achieved was 10^3 CFU/mL (Figure 5). In another assay, Temur et al. (2012) immobilized a peptide aptamer (peptamer) on the surface of a magnetic gold nanorod particle. Following the interaction of Staphylococcal enterotoxin B with the aptamer, unbound Staphylococcal enterotoxin B was separated from the bound form. Detection was carried out using a sandwich configuration, with the bare gold nanorod particles serving as the SERS probe. The detection limit reached up to 224 aM (Temur et al., 2012). [Insert Figure 5]

5.2.2. Disc technology

Disc technology was initiated by the invention of the digital compact disc (CD) by Klaas Compann in 1969. In 1970, Pete Kramer generated the first glass disc prototype for use in laser diffraction. Varma et al. (2004a,b) described the use of an optical biosensor based on spinning disc interferometry (BioCD) for ultra high-throughput immunological assays. Akin to conventional CD, BioCD operates as an analog sensor, but it is immobilized with biomolecules instead of digital recording. The output is the signal of the biomolecular interaction in lieu of the music/movie (Nolte, 2009). BioCD operates on the principle of micro-diffraction quadrature, which achieves sensitive linear detection of bound molecules. Using spinning disc interferometers, immobilized specific biomolecules can be detected with high speed and high sensitivity. Wang et al. (2008) used a combination of fluorescence and label-free interferometric detection on a BioCD system to detect protein.

BioDVD is another disc technology. BioDVDs have a diameter of 12 cm and a thickness of 0.6 mm, and they contain land and groove widths of 340 nm and a groove

depth of 39 nm. Via radiofrequency magnetron sputtering, an indium tin oxide film (140 nm thickness) is sputtered onto the surface of the substrate. Signal read-out is achieved using an optical disk drive tester with a laser wavelength of 405 nm and a numerical aperture of 0.65. As a proof-of-concept, *E. coli* detection was performed using an optical disk drive tester. A sample containing *E. coli* (OD_{600} of 0.1) was dropped onto the surface of the oxide-coated disk, and two-dimensional imaging of the *E. coli* was acquired; it was similar to the imaging obtained using an optical microscope. This system was beneficial for determining the identification and number of *E. coli* (Figure 6). [Insert Figure 6]

6. Portable biosensor

PCR, real-time PCR, SERS, and disc-based biosensors for bacterial detection require bulky instruments and are less feasible in places where resources are very limited. To meet the need for bacterial detection in low-resource settings, portable devices such as paper-based biosensor and smartphone come into play. These biosensors can allow point-of-care (POC) diagnostic, in which detection can be carried out anywhere to enable prompt treatment. These portable biosensors are in compliance with the World Health Organization (WHO)'s criteria of ASSURED (A-Affordable, S-Sensitive, S-Specific, U-User-friendly, R-Robust and rapid, E-Equipment-free and D-Deliverable to those who need the test) (Rozand, 2014).

6.1. Paper-based biosensor

Made of nitrocellulose material, paper is becoming the frontier for POC diagnostic especially in ares with low resource settings (Li et al., 2012; Byrnes et al., 2013; Yetisen et al., 2013). Advantage of using paper is that paper can be patterned with hydrophobic channels, through which the liquid samples are distributed by capillary action to enable simultaneous multiple assays. Another advantage of using paper is it is cheap, have adsorption and absorption features and can be transported easily due to low weight (Martinez et al., 2010). Govindarajan et al. (2012) have developed a paper-

based assay of low cost for POC extraction of bacterial DNA from raw viscous samples. This 'microfluidic origami' enables bacterial DNA extraction from the bacterial load from as low as 33 CFU mL^{-1}. The reagents are stored dry and can be hydrated with the addition of the sample, water, elution buffer and ethanol on the surface of the paper, which precludes the need for cold-chain transport (Govindarajan et al., 2012). In another assay, paper-based diagnostic was developed for the detection of the sialidase-related disease such as bacterial vaginosis. To actualize this. poly(ethyleneimine) (PEI) microcapsules were prepared and incubated with the negatively charged 5-bromo-4-chloro-3-indolyl-a-d-N-acetylneuraminic acid (BCIN) solution and nitro blue tetrazolium (NBT). Following this, microcapsules were added into paper pulp, which changed the colour of the paper from white to dark purple in the presence of sialidase (Zhang et al., 2013). In a paper-based detection of *Mycobacterium tuberculosis* complex, primer specific against the region of the RNA polymerase beta subunit locus was conjugated to the gold nanoparticle. Using wax, 384 wells were printed on the surface of the paper and were filled with salt $MgCl_2$. The target DNA sequence and the gold nanoparticle-probe were mixed together and added into the wells. The presence of the target DNA (*Mycobacterium tuberculosis* complex) will result in red colour while the absence of the target DNA (non-*Mycobacterium tuberculosis* complex) results in blue colour (Veigas et al., 2012). The paper-based assay was then further integrated with smartphone, which aids in the data transmission to the central laboratory for the quantification of the colorimeteric changes on the surface of the paper.

6.2 The smartphone

The first smartphone-based detection for the visualization of a single bacterium or virus was demonstrated by, Zhu et al. (2012), who have attached a cost-effective bacterial detection instrument to the cell phone. This first cell phone-based imaging system aims to meet the need for bacterial detection in low-resource settings. The system was applied to *E. coli* as a proof-of-concept. Anti-*E. coli* O157:H7 antibodies were immobilized on the interior surface of a capillary tube. Using a battery powered peristaltic pump or a mechanical spring-based pump, samples containing *E. coli* were pumped through the capillary tube, followed by the addition of biotinylated secondary

anti-*E.coli* antibodies and streptavidin-conjugated quantum dots. Imaging and quantification of the bacteria were executed via fluorescent visualization using an inexpensive microscope (~28 grams) attached to the cell phone. With a detection limit of 5–10 cfu mL^{-1}, this hand-held device is highly specific for detecting *E. coli*, even in samples such as a complex food matrix. In this state-of-the-art smart phone-based direct visualization of the fluorophore-labeled bacteria, the compact and light-weight opto-mechanical device is attached to the camera of the phone. The attached fluorescent imager produces compact laser-diode-based excitation at 450 nm that imparts a high incidence angle (approximately 70°) in the sample plane and contains a long-pass thin-film interference filter, an external low numerical aperture lens, and a translation stage for focus/depth adaptation. This system is able to efficiently filter off the background signal as the scattered light is blocked by the LP filter. It also can capture even a weak signal from single cell bacterium or nanoparticle. The combined weight of the fluorescent imager and the cell phone is approximately 186 g, which enables imaging of bacteria or viruses anywhere in the field (Figure 7) (Wei et al., 2013). [Insert Figure 7]

Park et al. (2013) recently illustrated the applicability of smartphones for detecting bacteria using digital images as a point-of-care diagnostic tool. They used a paper-based microfluidic instrument, which is a portable device that contains channels, that was immobilized with anti-*S. typhimurium* and anti-*E. coli* antibodies conjugated to submicroparticles. The paper-based microfluidics were dipped into samples containing the bacteria, which led to immunoagglutination. This state of immunoagglutination was analyzed via Mie-scattering of the nanoparticles based on the digital images captured by the smartphone. This system is very rapid (1 minute) and also enables single cell bacterial detection. Khatua and Orrit (2013) speculate that cell-phone-based bacterial detection using spectral resolution of fluorescence may eventually allow for the simultaneous detection of different bacteria.

7. Future perspectives

Aberrant or inappropriate macromolecular complexes in the body can cause pathological conditions. Hence, the modulation of such complex interactions by small organic molecules offers possibilities for the treatment of human diseases. These small molecules can modulate the complexes via competition and act as a drug that can block the interactions of whole bacterial cells/target molecules on the host cell. High-throughput screening for these small molecules from a given library can be easily achieved using array systems. This strategy can identify suitable inhibitory molecules from the initial chemical library that have high affinity for the bacteria. Such inhibitory molecules will be highly useful for developing compounds that can be used to treat disease. Another detection system based on measuring the changes in the terminator and anti-terminator operating system in bacteria cells (e.g., in the tryptophan and histidine-utilizing operon systems (Figure 8) may prove to be an effective new approach to study the bacterial regulation. [Insert Figure 8]

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Figure Legends

Figure 1

Microscopic observation of bacteria. (a) Basic set-up of a light microscope and (b) Microscopic image of *bacteria*.

Figure 2

Sequence-based analyses of bacteria: (a) Detection of 16S rRNA on agarose gel-electrophoresis. A cartoon illustration of a ribosome is shown. (b) Fluorescent-based microarray used to detect specific bacterial sequences. The oligonucleotide probes that are immobilized on the surface hybridize with the fluorescently labeled secondary strand. Following washing to remove the unbound strands, detection of the specific bacterial sequences is conducted by fluorescence imaging.

Figure 3

(a) Sequence-based detection by the neutralizer displacement assay. Probe conjugated to neutralizer is immobilized on the sensor surface. The addition of the target displaces the neutralizer, which results in a large change in the electrocatalytic current (Das et al., 2012). (b) Detection of bacteria using μNMR (Chung et al., 2013). Total RNA extraction and symmetric reverse transcription-PCR (RT-PCR) generate single stranded sense DNA strands that are captured by the target probe on the surface of microspheres. The other probe, which is immobilized on magnetic nanoparticles and is complementary to the other region of the target strand, binds to the other end of the target DNA. The transverse relaxation rate (R2) of the sample is thereby shortened, and the R2 is detected by the μNMR device.

Figure 4

Cell-SELEX. Whole cell bacteria are mixed with a randomized pool of DNA or RNA molecules. Counter selection is performed using negative cells. Bound molecules are eluted and amplified for the next round of SELEX. This process is repeated several times prior to cloning and sequence analysis.

Figure 5

Surface-enhanced Raman scattering (SERS)-based detection of bacteria. Specific probes immobilized on the sensor surface interact with the bacteria, thereby shifting the SERS spectra.

Figure 6

Disc technology for detection of bacteria. Bacteria in water interact with the specific probe on the surface of the disc, resulting in a change in the signal read-out.

Figure 7

Detection of bacteria by smartphone. An opto-mechanical device containing a fluorescent imager is attached to the smartphone to capture images of the bacteria to visualize them. (Wei et al., 2013).

Figure 8

Terminator in the bacterial system. Synthesis of full-length transcript through destabilized terminator upon binding of the corresponding protein. In the absence of the protein, the transcription is terminated. This system can be a possible bacterial detection scheme.

Table 1: Aptamer-based biosensor for bacterial detections

Target	Sensor	Sensitivity	Application	Reference
B. anthracis (nonpathogenic Sterne strain)	Electro-chemiluminescent-based detection system	10^2 to 10^5 spores	Detection of *B. antracis*	Bruno and Yu, 1996
Staphylococcus aureus	Flow cytometry	-	Inhibition of *S. aureus* by combined aptamers	Cao et al., 2009
Shigella sonnei	Enzyme-Linked aptamer sedimentation assay	-	*Detection of S. sonnei*	Masoudipour et al., 2011
Escherichia coli	Electrochemical	112 cfu/mL	Screening pathogen	Luo et al., 2012
Salmonella typhimurium	Impedimetric	600 cfu/mL	Viability test	Labib et al., 2012
E. coli & S. typhimurium	Colorimetric	10^5 cfu/mL	Rapid detection of bacteria	Wu et al., 2012
Streptococcus hemolyticus	Fluorospectrophotometer	33 cfu/mL	Detection of *S. hemolyticus*	Le et al., 2012
Staphylococcal enterotoxin B	SERS	224 aM	Detection of *Staphylococcal aureus*	Temur et al., 2012
S. typhimurium	silver enhancement signal amplification technology	7 cfu/mL	Detection of S. typhimurium	Yuan et al., 2013
S. typhimurium	Fluorescence spectrophotometer	25 cfu/mL	Detection of *S. typhimurium*	Duan et al., 2013
Pseudomonas aeruginosa	Fluorescence spectrophotometer	5.07 cells/mL	Detection of *P. aeruginosa* in drinking Water	Kim et al., 2013
Salmonella Paratyphi A	Chemoluminescence	10^3 cfu/mL	Detection of Salmonella	Yang et al., 2013
Staphylococcus aureus	Light-scattering	Single cell	Sensitive detection	Chang et al., 2013
Vibrio parahaemolyticus	Dual-color flow cytometry	-	Detection of *Vibrio parahaemolyticus*	Duan et al., 2013
Eight pathogens	Magnetic nanoparticle-fluorescent polymer system	10^7 cfu/mL	Detection of eight different pathogen samples	Wan et al., 2013

Highlights

- Ubiquitous nature of bacteria enables them to survive in a wide variety of environments and the rise of various pathogenic species.
- Sensing systems are needed to monitor pathogenic and food-borne bacterial populations.
- Bacterial sensors for the purpose of diagnosis can function in two ways: specific detection of a bacterial component and whole cell detection.
- Bacterial detection systems range from microscopic observation to state-of-the-art smartphone-based detection.
- Currently available bacterial detection systems are overviewed.

Figure(s)

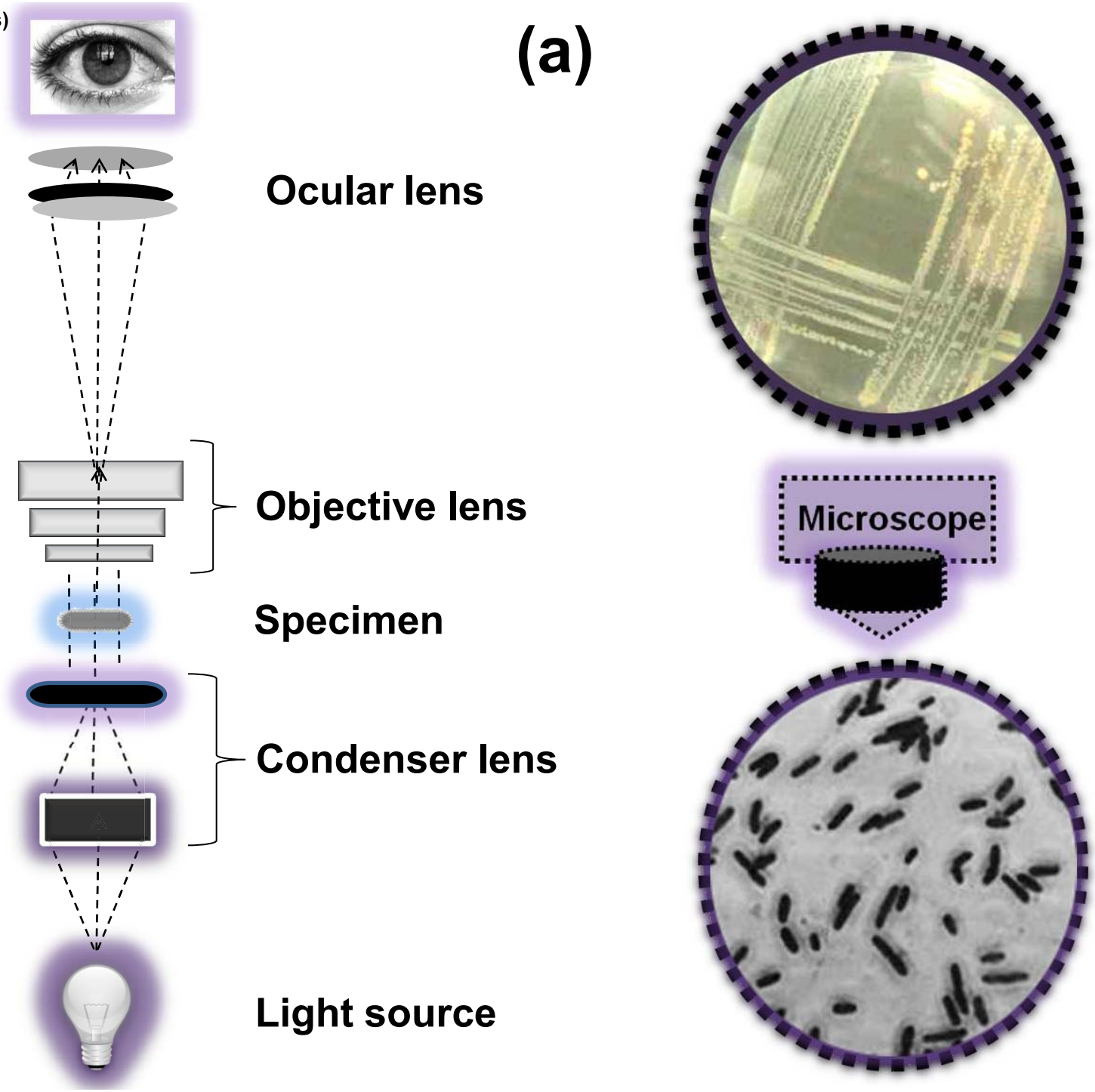

Fig. 1

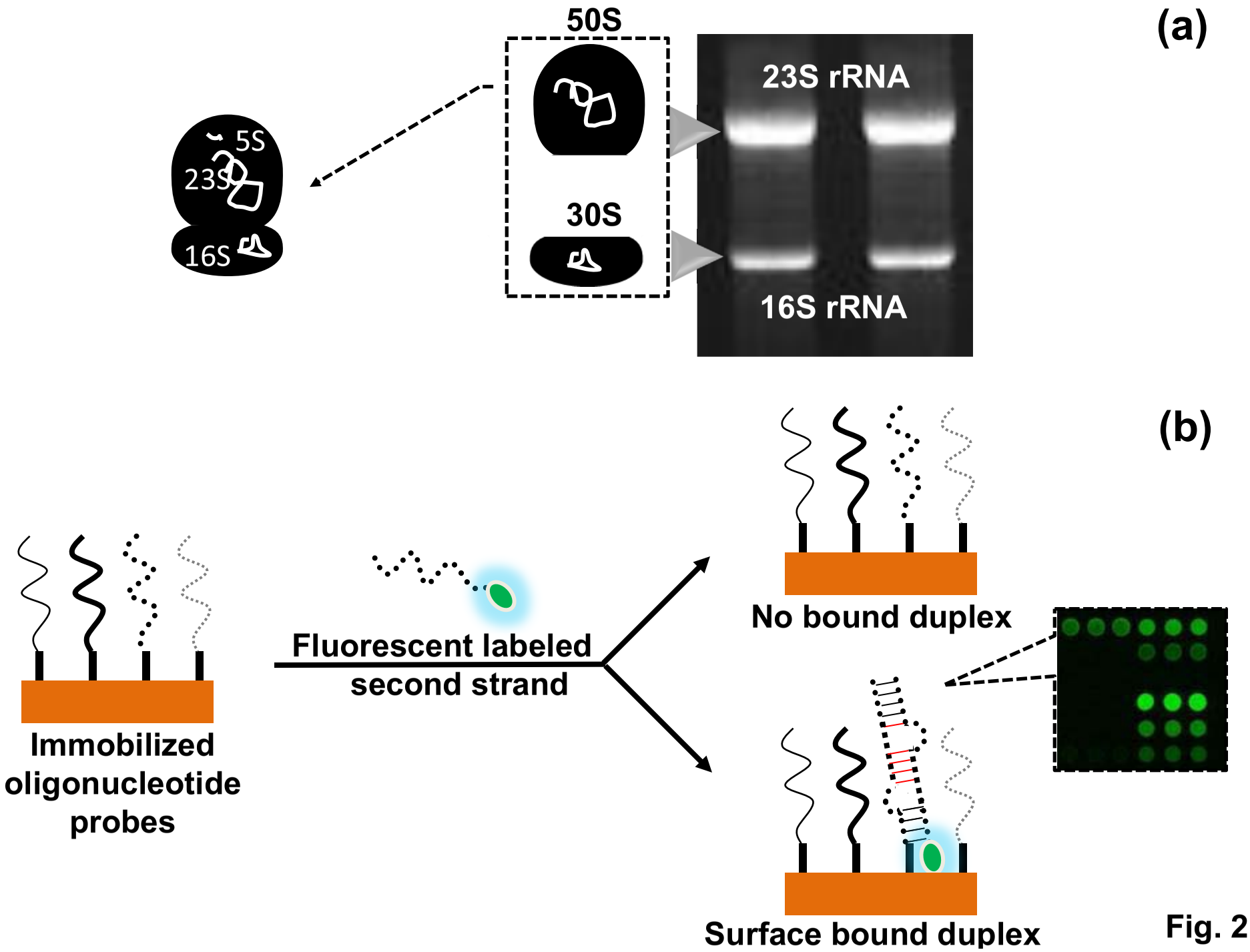

Fig. 2

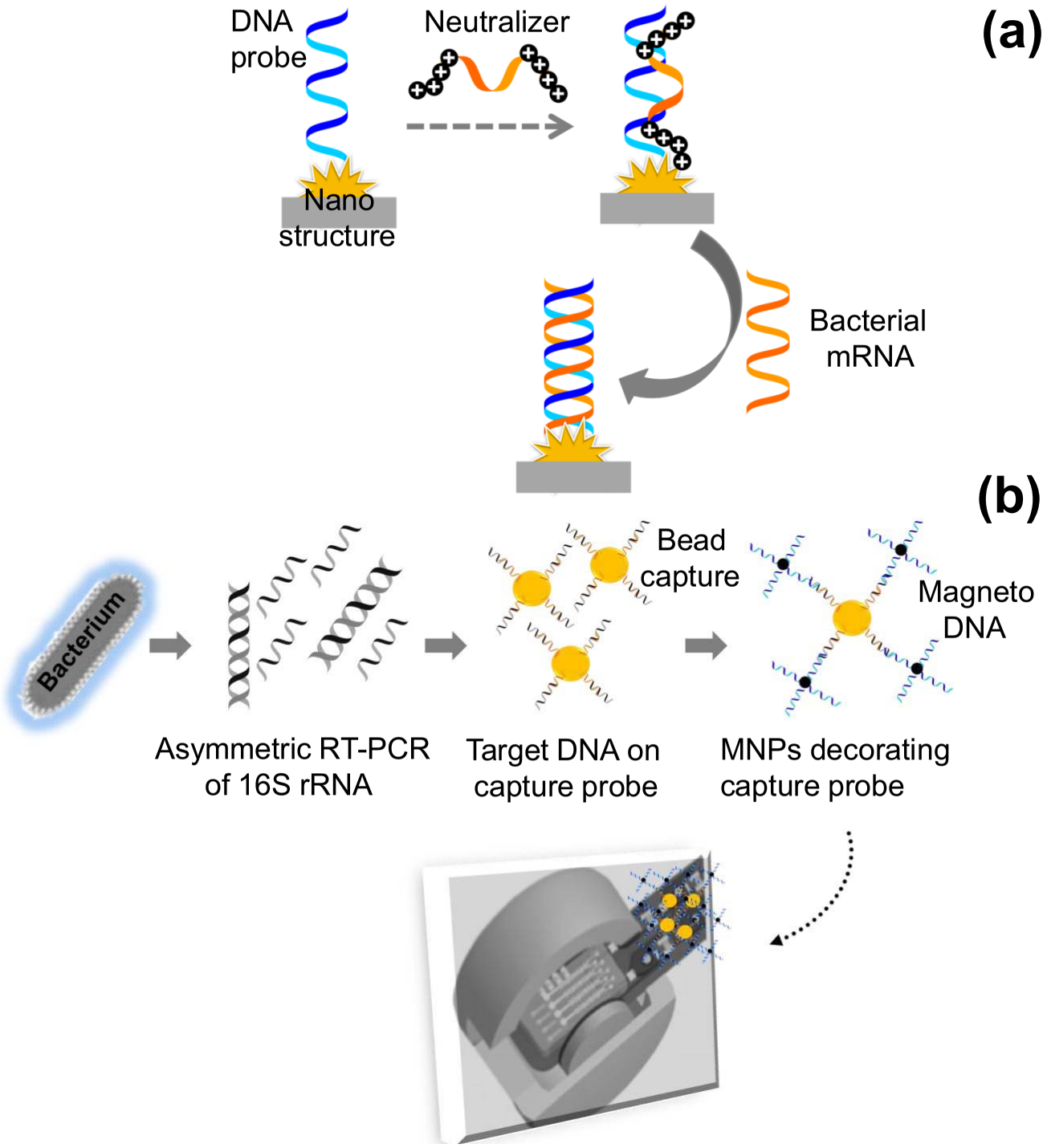

Fig. 3

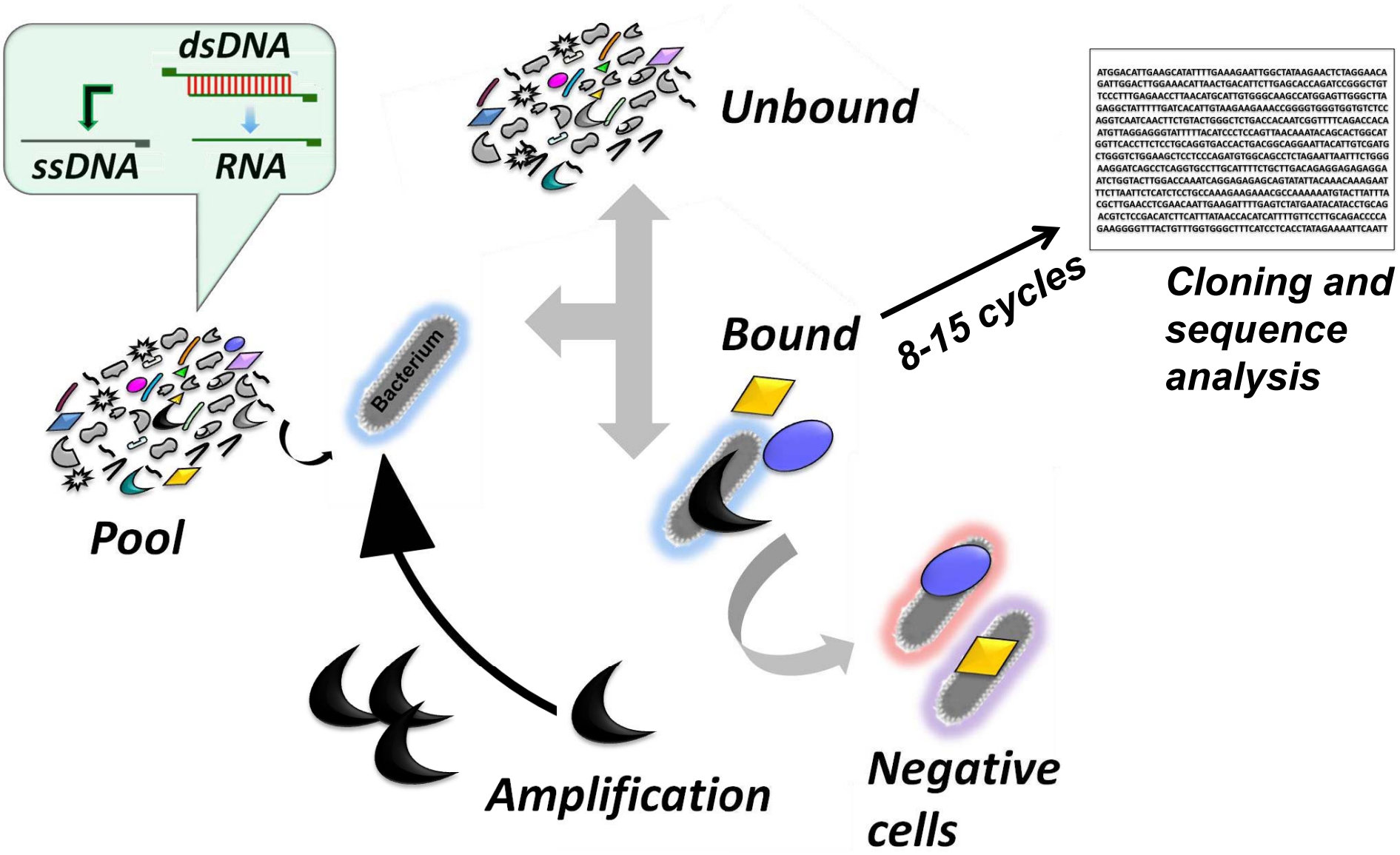

Fig. 4

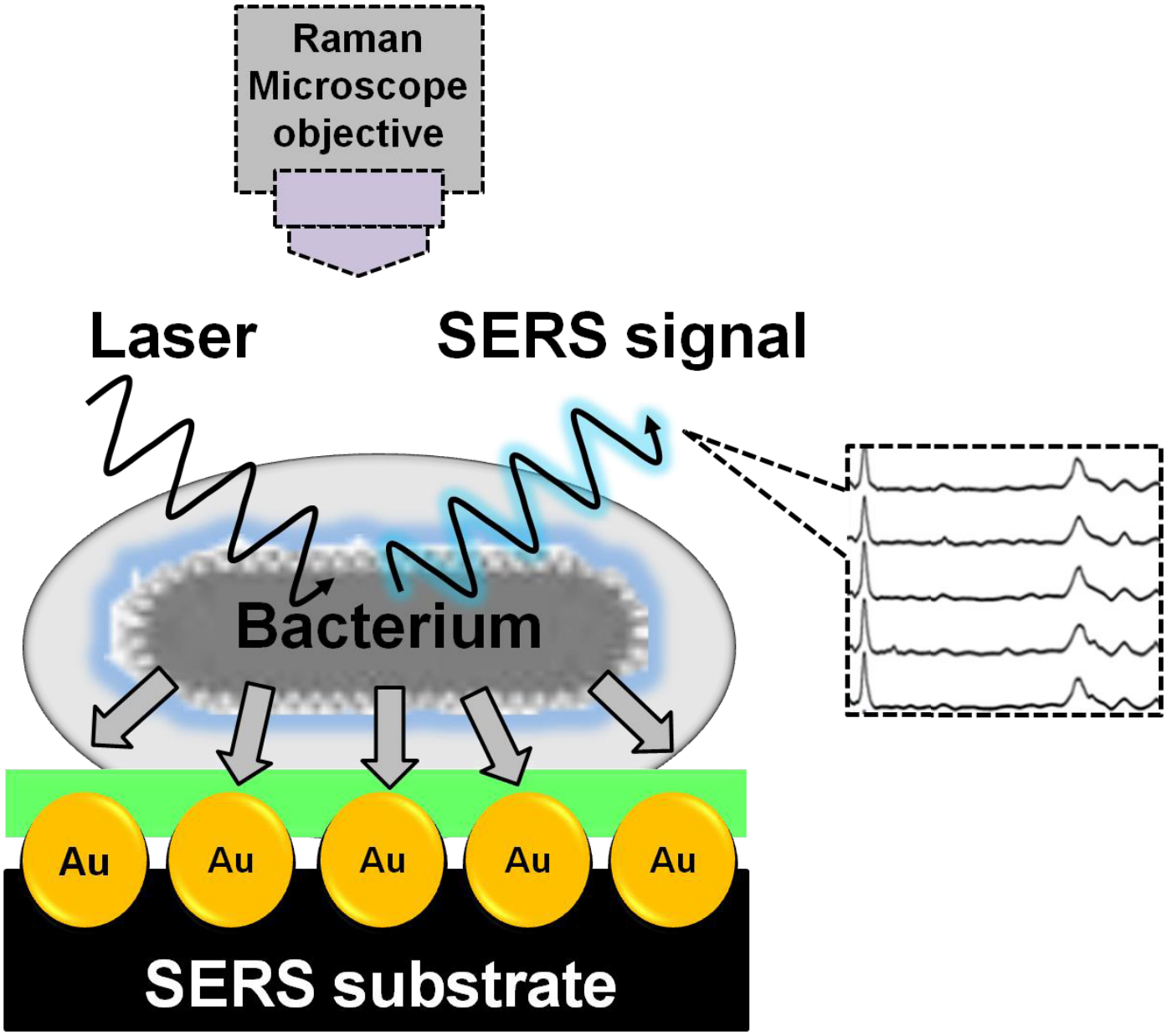

Fig. 5

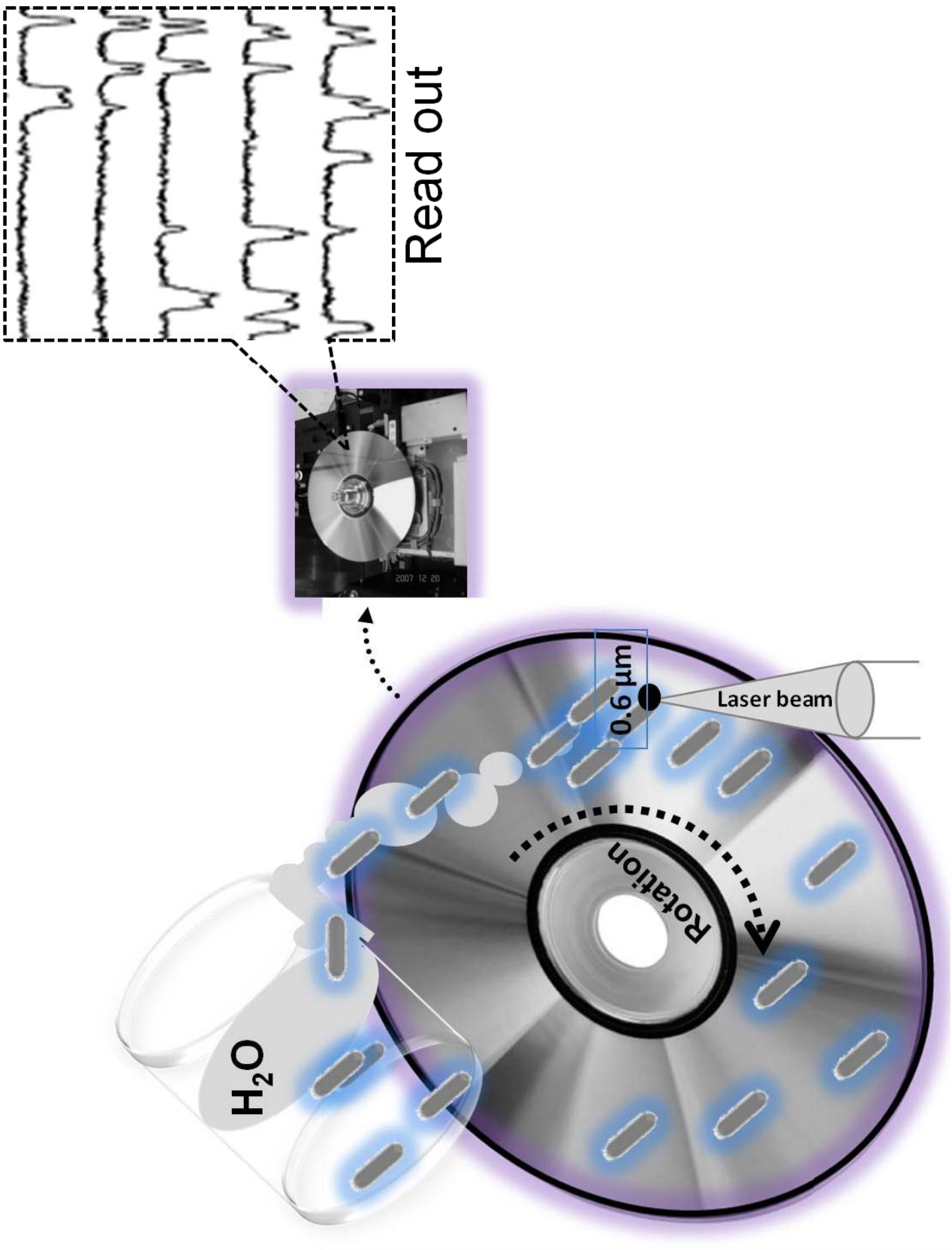

Fig. 6

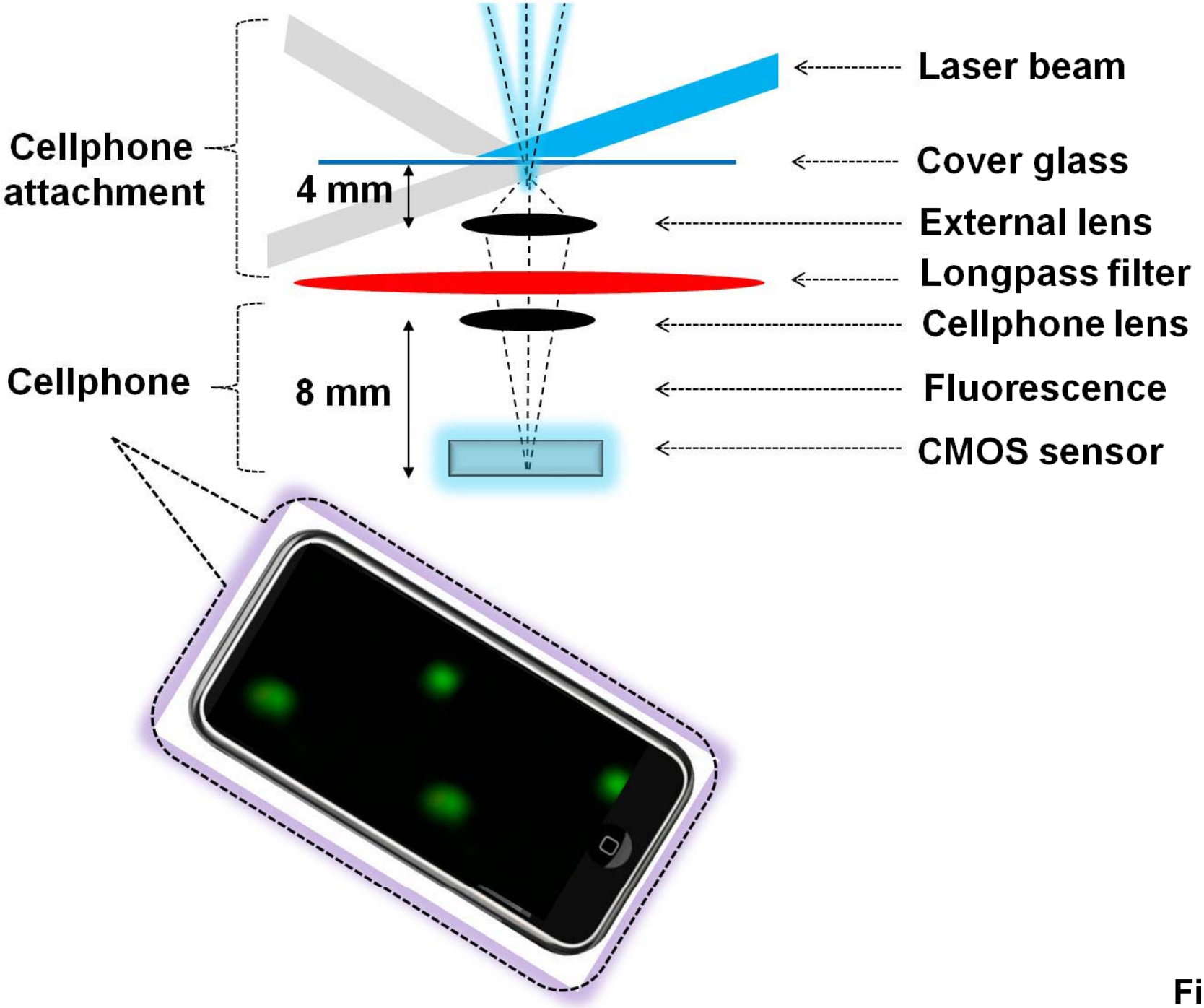

Fig. 7

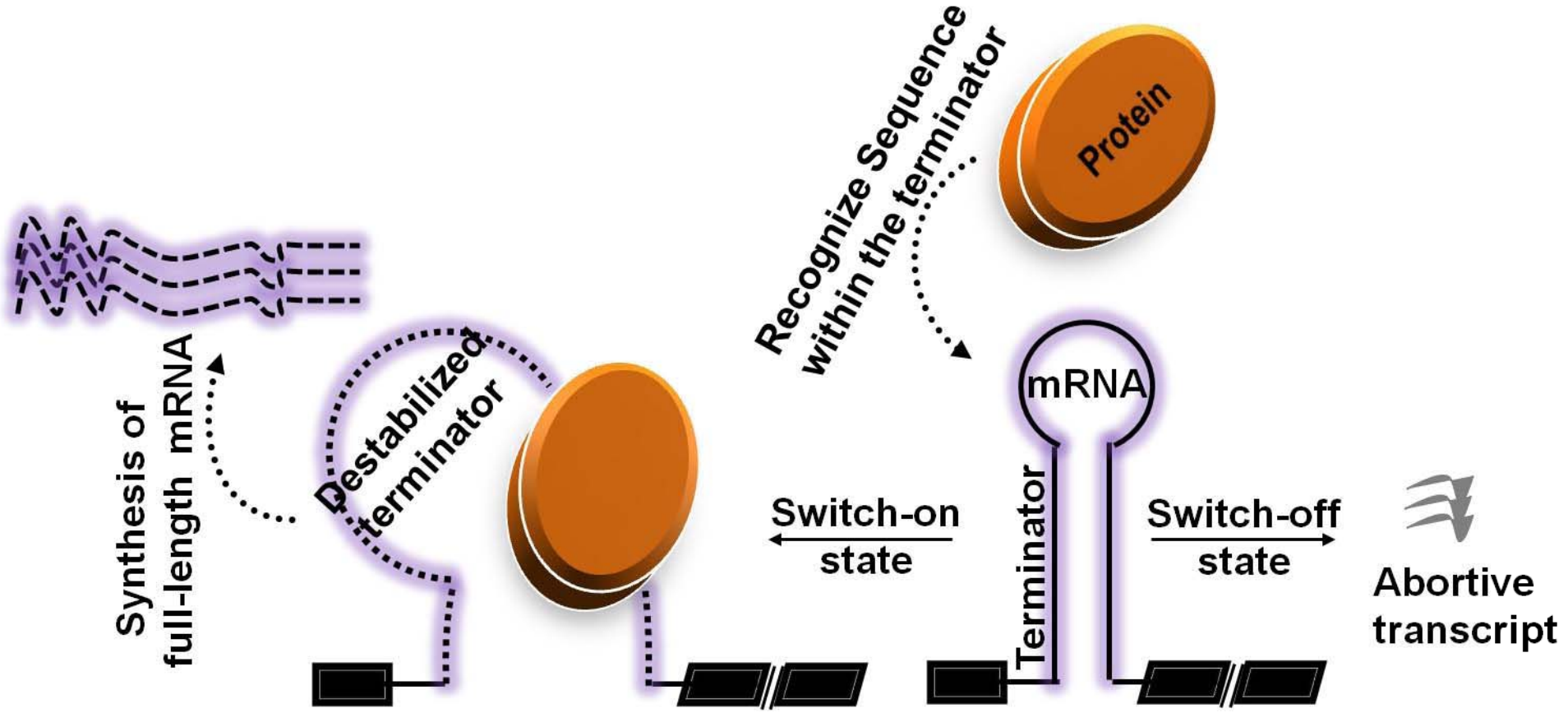

Fig. 8

Figure(s)

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