Nano-Bio-Analytical Systems for the Detection of Emerging Infectious Diseases

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Abstract

The global damage caused by the spreading microbial infection is evident from the devastating COVID-19 pandemic. The infectious diseases caused by emerging viral and resistant bacterial pathogens have been a worldwide medical threat and economic burden. To combat this threat, a technology able to rapidly identify pathogen infection and determine the pathogen resistance profile is needed. The current methods for emerging infectious disease detection are mainly molecular methods based on polymerase chain reaction (PCR) for the detection of the specific pathogenic gene or resistant gene mutations. While sensitive, it requires prior knowledge of the pathogenic cells, which fail to output a negative result when a new pathogen or new resistant strain occurs and wrongly output a positive result when resistance genes are simply present but are not expressed or are not contributing to resistant phenotypes. Considering the life-threatening condition of an emerging infectious disease and the increasing prevalence of emerging pathogens and bacteria with antibiotic resistance in hospitals, automated and fast diagnostic facilities are required. This chapter summarizes the emerging nano-bio-analytical systems for the rapid detection of pathogen infectious diseases and antibiotic susceptibility testing for antibiotic resistance determination that will empower humans to win the epic war between human wits and microbial genes. In viral infection detection, we discussed nano-bio-analytical systems for the detection of viral infectious diseases including point-of-care immunoassay systems, electrochemical detection systems, and plasmonic-based

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systems. In resistant bacterial infection detection, we reviewed the emerging optical imaging systems for rapid phenotypic antibiotic resistance determination.

Keywords

Emerging infectious diseases · Pathogen detection · Point of care · Biosensor · Optical imaging system

7.1 Introduction

Following the severe acute respiratory syndrome (SARS) in 2003 and the Middle East respiratory syndrome (MERS) in 2012, the COVID-19, which is the third large-scale pandemic caused by coronavirus in the last 20 years, has been declared as a public health emergency of international concern. COVID-19 has a remarkable efficiency in human-to-human transmission, with relatively high morbidity and mortality, especially among the aged and those with underlying comorbidities (Morens and Fauci 2020; Morens et al. 2020). Unlike other conventional diseases, infectious diseases are caused by infectious pathogens, which can impair the normal functioning of the host, and often spread from person to person or sometimes from animals to humans. Researchers have sorted the infectious agents into categories of bacteria, viruses, fungi, parasites, and prions. The incidence of emerging infectious diseases (EIDs) has increased over the past two decades and is likely to increase in the near future (Wilson 1999). Emerging diseases have been classified as newly emerging, re-emerging, "intentionally emerging", and "accidentally emerging" infection diseases (Table 7.1). Despite the differences, these four categories share something in common: newly emerging diseases can persist and then re-emerge through intentional or accidental release (Morens and Fauci 2020; Morens and Fauci 2012; Morens et al. 2008; Morens et al. 2004; Satcher 1995; Excler et al. 2021).

Global health, economic boom, social stability, and security of human society have been threatened by all EIDs, especially caused by viruses and bacteria. Therefore, it is incredibly important to develop precise and timely diagnostic systems to

Table 7.1 Major categories of emerging infectious diseases (Morens and Fauci 2020)

The category of EIDs	Comments
Newly emerging infectious diseases	Diseases first discovered in humans, e.g., HIV/AIDS (1981), Nipah virus (1999), SARS (2003), MERS (2012), COVID-19 (2019)
Re-emerging infectious diseases	Diseases that have historically infected humans but continue to re-emerge in new locations (e.g., West Nile in the United States and Russia in 1999) or in drug-resistant forms (e.g., methicillin-resistant *Staphylococcus aureus*)
Intentionally emerging infectious diseases	Diseases related to intent to harm, including mass bioterrorism
Accidentally emerging infectious diseases	Diseases unintentionally released by humans, e.g., epizootic vaccinia and transmissible vaccine-derived polioviruses

facilitate the recognition and intervention of EIDs for the prevention of infectious diseases into epidemics and to improve public health (Ozer et al. 2019).

Currently, the major strategies for the clinical diagnosis of EIDs are to cultivate pathogens and to identify or detect specific antigens, antibodies, or nucleic acids. The most common and efficient methods for identifying and diagnosing infectious disease pathogens are nucleic acid-based assays (Koo et al. 2018; Sin et al. 2014). Generally, RT-PCR is regarded as the gold standard of viral nucleic acid detection. The highly sensitive PCR-based methods have disadvantages such as being time-consuming, labor-intensive, and expensive. Biosensors facilitate the rapid detection of targeted biomarkers, enabling real-time disease diagnosis (Mujawar et al. 2020). The combination of nanotechnology and biosensors holds the potential to improve the accuracy, speed, and sensitivity of devices detecting bacterial and viral. At the same time, great advances have been made in deeper understanding and comprehension of the genome and proteome of pathogens and their interactions with hosts (Wrapp et al. 2020).

Therefore, this chapter described the nano-bio-analytical systems for emerging infectious diseases from two aspects: (1) viral infection detection system and (2) resistant bacterial infection detection system. In the field of the viral infection detection system, point-of-care immunoassay, electrochemical, and plasmonic-based platforms were introduced with system design and sensing mechanism. As for the resistant bacterial infection detection system, real-time microscopy system, microfluidic imaging system, surface plasmon resonance imaging system, and light scattering imaging system were introduced with system design and their application in rapid bacterial resistance determination.

7.2 Nano-Bio-Analytical Systems for Emerging Viral Infection Detection

The SARS-CoV-2 event is widely and rapidly spreading around the world, and because of the terrifying contagiousness of this virus, the development of point-of-care testing (POCT) diagnosis assays to detect and manage the disease is urgently needed in the afflicted area, even though RT-PCR test has become the gold standard to recognize SARS-CoV-2 disease (Orooji et al. 2020).

7.2.1 Point-of-Care Immunoassay Systems

Immunoassay is a type of bio-analytical method in which the interaction of an analyte (i.e., antigen) and an antibody is the basis to measure a specific analyte (Huang et al. 2020; Byrnes et al. 2020). Immunological assays, particularly the enzyme-linked immunosorbent assay (ELISA) and lateral flow assay, assess viral infection rates or vaccine efficiency by detecting viral antigens or antibodies against viral antigens (Orooji et al. 2020). Immunosensing assays are commonly used to detect various sorts of viruses with high sensitivity, for instance, SARS

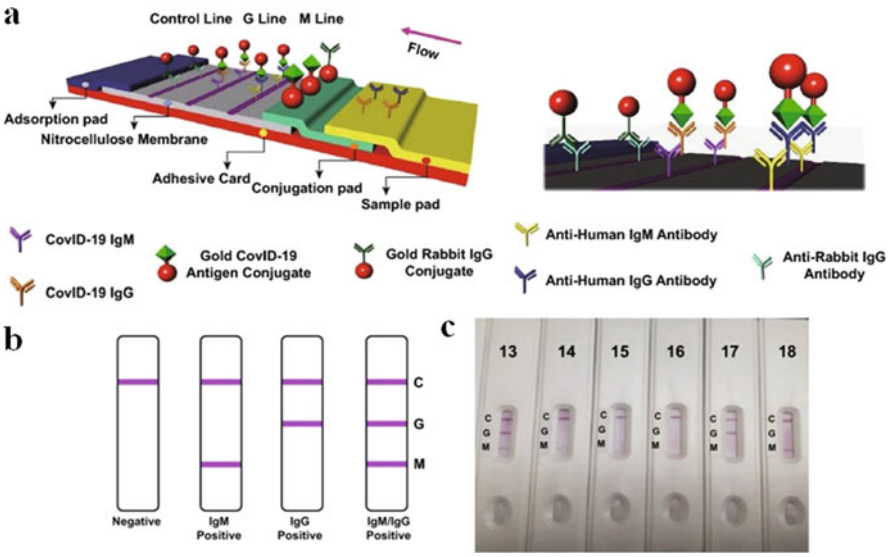

Fig. 7.1 Schematic explanation of rapid SARS-CoV-2 IgM-IgG combined antibody test. (**a**) Schematic diagram of the identification device. (**b**) Interpretation of several experimental results. (**c**) Relates to the control line, G means IgG line, M means IgM line. Reproduced from reference (Orooji et al. 2020)

CoV-2 (Padoan et al. 2020; Chen et al. 2020), HIV (Sevenler et al. 2020; Stalter et al. 2020), HCV (Eshetu et al. 2020; Patel and Sharma 2020), and so on.

ELISA is the most used immunoassay for viral detection in clinical laboratories, for example, ELISA is the gold standard for HIV diagnosis. In the ELISA test, the chromogenic substrate is converted into a colored molecule by using capture antibodies and detection antibodies modified with enzymatic tags. ELISA can test multiple samples and can be automated to increase throughput, but the sensitivity may vary (Carter et al. 2020).

Aiming to detect EIDs, the lateral flow immunoassay (LFIA)-based POCT assay has been developed. Lateral flow tests, also known as lateral flow immunochromatographic assessments, are simple and direct paper-based devices designed to identify the presence of target analytes in a fluid sample without the need for any specific and exorbitant hardware. Figure 7.1 shows a diagram of a quick SARS-CoV-2 IgM-IgG combined antibody test (Orooji et al. 2020).

Yu et al. (Yu et al. 2017) have designed a nanostructured microfluidic immunoassay pathogen detection platform with high sensitivity and selectivity. By utilizing the three-dimensional morphology as well as the unique optical property of the ZnO nanorods, the detection limit of H5N2 AIV can be improved to as low as 3.6×10^3 EID50/mL (EID50, 50% embryo infectious dose), which is approximately 22 times more sensitive compared to conventional ELISA. In Chan's paper, a microfluidic integrated rGO transistor chip was established to detect the gene of H5N1 influenza in a flowing environment (Chan et al. 2017). Lu et al. (Lu et al. 2020) present a novel

digital microfluidic H1N1 virus detection platform using a one-aptamer/two-antibody assay on magnetic beads, and the limit of detection (LOD) is 0.032 hemagglutination units/reaction. This is the first demonstration of a digital microfluidic platform capable of performing the whole diagnostic process for influenza A H1N1 viruses by using electromagnetic force. Iswardy et al. (Iswardy et al. 2017) used a microfluidic dielectrophoresis (DEP) chip with mouse anti-flavivirus monoclonal antibody-coated beads for rapid detection of dengue virus (DENV) in vitro. The platform is capable of accelerating immune response time, with an on-chip assay time of 5 minutes and DENV detection capability down to 10 (Morens and Fauci 2012) PFU/mL.

7.2.2 Electrochemical Detection Systems

Among various approaches used to distinguish viral pathogens, including Zika (Cecchetto et al. 2017), influenza (Hushegyi et al. 2016), HIV (Shafiee et al. 2013), and so on, electrochemical biosensor technology is leading the way in the development of POCT devices owing to the advantages of high selectivity, sensitivity, quick response, and ease of miniaturization (Wang et al. 2021; Huang et al. 2016).

Enzyme-catalyzed reactions between immobilized biomolecules and target analytes generate electrons that affect the electrical properties of the solution, which is what electrochemical biosensors typically rely on to detect. An electrochemical system containing an electrode and a pathogen solution can convert the chemical energy derived from the target pathogen and biorecognition elements to the electrical signals captured by electrodes.

Li et al. (2021) showed that for the detection of HIV p24 antigen, the ZnO-NW-enhanced EIS biosensors proved their high sensitivity with LOD down to 0.4 pg/mL. Li et al. (2013) presented an EV71-specific nanogold-modified working electrode for electrochemical impedance spectroscopy and reached a LOD of 1 copy number/50 μL reaction volume in the detection of EV71, and the interval from sample preparation to detection was 11 min. Navakul et al. (Navakul et al. 2017) present a technique of DENV based on EIS aiming to detect, classify, and screen antibodies (Fig. 7.2). In this research, DENV was used as a component to functionalize a graphene oxide (GO)-polymer surface and make the polymer surface more selective and sensitive to the virus by inducing a self-assembly process. The EIS sensor can detect DENV ranges from 1 to 2×10^3 pfu/mL (LOD down to 0.12 pfu/mL) and classify the virus serotypes accurately.

7.2.3 Plasmonic-Based Systems

Plasmonic-based platforms, which consist of surface plasmon resonance (SPR) and localized surface plasmon resonance (LSPR), have become indispensable tools for

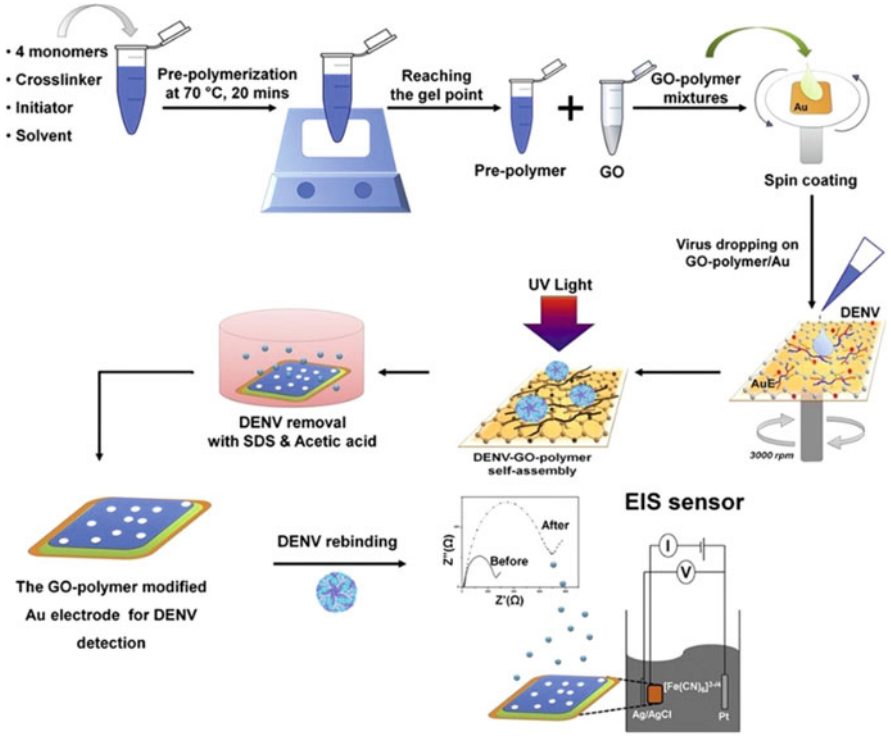

Fig. 7.2 Schematic illustration of the preparation of GO-polymer for DENV detection on a gold electrode. Reproduced from reference (Navakul et al. 2017)

POCT diagnostic applications (Table 7.2) for their advantages of stable, real-time, highly sensitive, and label-free. Plasmonic-based platforms are regarded as a critical candidate for next-generation diagnostics (Li et al. 2019a).

So far, SPR-based sensor has rapidly emerged as the most powerful detection type of optical biosensors. The SPR-based sensor could detect the binding events between the capture probes and analyte molecules by monitoring the refractive index change at the metal-dielectric interface. Inherently as a refractometric device applied in biology, SPR biosensors realize the detection and quantification of analytes by measuring parameters of incident light such as angle, intensity, phase, or wavelength.

As a promising one-step and label-free virus detection method, several SPR-based biosensors have already been adapted into POC devices of EIDs (Li et al. 2019a; Farzin et al. 2020), such as HIV (Kosaka et al. 2017), H1N1 (Su et al. 2012), H7N9 (Chang et al. 2018), etc. Detecting, identifying, and understanding viruses are of great importance for EIDs prevention, diagnosis, and control. When it comes to the detection as well as quantification of the antigens by plasmonic platforms, the work of Kosaka et al. (Kosaka et al. 2017) can detect HIV-1 p24 antigens in human serum less than a week after infection with the LOD of 10^{-17} g/

Table 7.2 Various optical techniques to diagnose different infectious diseases

Detection method	Analyte	LOD	Detection time	Reference
SPR aptasensor	H5N1 AIV	0.128 HAU	1.5 h	Bai et al. (2012)
Intensity-modulated surface plasmon resonance (IM-SPR) biosensor	H7N9	144 copies/mL	<10 min	Chang et al. (2018)
4-MBA/Au SPR chip	Recombinant nucleoprotein of Ebola (EBOV-rNP)	0.5 pg/mL	~ 1 h	Sharma et al. 2020)
Au/DSU/NH2rGO-PAMAM/IgM thin film-integrated SPR sensor	DENV-2 E-proteins	0.08 pM	8 min	Omar (et al. 2020)
GBP-E-SCVme-coated SPR biosensor	SARS coronavirus surface antigen (SCVme)	200 ng/mL	10 min	Park et al. (2009)
LSPR-induced Qdot-MB biosensors	ZIKV RNA	1.7 copies/mL	3 min	Adegoke et al. (2017)
Affinity peptide-guided plasmonic biosensor	Human norovirus	9.9 copies/mL	10 min	Heo et al. (2019)
LSPR-amplified immunofluorescence biosensor	Nonstructural protein 1 (NS1) of the ZIKV	1.28 fg/mL	–	Takemura et al. (2019)
LSPR biosensors based on thermally annealed silver nanostructures	Dengue NS1 antigen	9 nm/(μg/mL)	~30 min	Austin Suthanthiraraj and Sen (2019)

mL, which equals to one virion in 10 mL of plasma. Recent reports demonstrate that the SARS-CoV spike and SARS-CoV-2 spike share the same functional host cell receptor, angiotensin-converting enzyme 2 (ACE2) (Zhou et al. 2020; Wan et al. 2020). Furthermore, Wrapp et al. (Wrapp et al. 2020) provide evidence through SPR both biophysically and structurally to point out the fact that SARS-CoV-2 spike protein has a better affinity than SARS-CoV when bound to ACE2. By integrating IM-SPR biosensor with a newly generated monoclonal antibody, Chang et al. (Chang et al. 2018) developed a simple but reliable platform, being capable of sensitive and quick detection of H7N9 virus with a detection limit at 402 copies/mL in mimic solution, which dwarfs commercial RIDT, homemade target-captured ELISA, and even qRT-PCR. Almost excluding any sample preparation, Huang et al. (Huang et al. 2021) use a spike protein-specific plasmon nanoarray SPR chip to detect SARS-CoV-2 virus particles in one step using a spike protein-specific plasmon nanoarray SPR chip.

Wang et al. (Wang et al. 2010) use high-resolution surface plasmon resonance microscopy (SPRM) and successfully demonstrate label-free imaging, detection, and

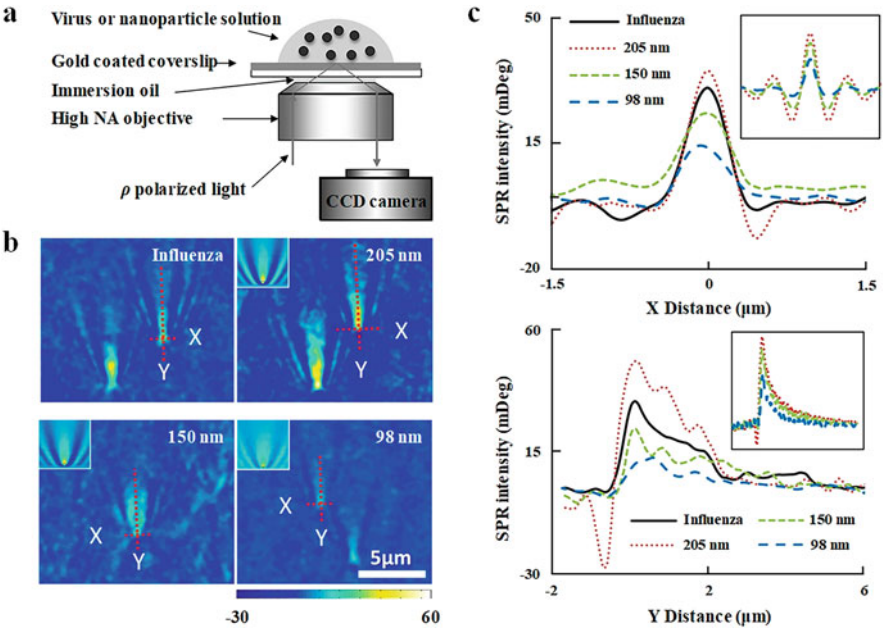

Fig. 7.3 Label-free imaging, detection, and mass measurement of single viral particles by surface plasmon resonance. (**a**) Schematic of the SPRM experiment setup (drawing not to scale). (**b**) SPRM images of the H1N1 influenza A virus and silica nanoparticles of three different sizes in PBS buffer. (**c**) The SPR intensity distributions along X and Y directions for selected particles (respectively, indicated by the dashed lines in b). (Insets) Corresponding profiles from simulated images. Reproduced from reference (Wang et al. 2010)

mass/size measurement of individual virus particles in solution. Based on the Kretschmann configuration, a high numerical aperture objective and an inverted microscope were used in the SPRM (Fig. 7.3a). For the SPRM setting, the sensing area is the whole image area with a size of 0.08×0.06 mm^2. Figure 7.3b is an image showing the discrepancy between silica nanoparticles of three different sizes and the H1N1 virus in PBS buffer, which is obtained by SPRM. For the two viruses studied in this work, the mass and diameter are found to be 6.5 ± 0.8 fg and 218 ± 10 nm for HCMV and 0.80 ± 0.35 fg and 109 ± 13 nm for H1N1 influenza A/PR/8/34, respectively (Wang et al. 2010). The sensing area can detect as small mass as 1 ag of the binding of a single particle, with a corresponding mass detection limit of about 0.2 fg/mm^2 per unit area, while the typical detection limit of the conventional SPR is nearly four orders of magnitude worse than this work (Homola 2008).

When measuring short-range changes in the refractive index owing to a molecular adsorption layer, the response of SPR spectroscopy and LSPR spectroscopy become similar, even though the former is much more sensitive to changes in bulk refractive index (Qiu et al. 2018; Qiu et al. 2019). The enhanced plasmonic field near the nanostructures increases the sensitivity of LSPR sensing systems to local refractive

index changes during molecular binding. Among various biosensing technologies, LSPR biosensing systems are capable of detecting various categories of analytes of clinical interest (Haes et al. 2005).

The LSPR technique may have the potential to substitute to detect SARS-CoV-2 and diagnose COVID-19. The overall performance could be improved by combining the plasma detection and amplification process. Meanwhile, the energy loss of the plasma and the related nanoscale heat generation, also known as the plasmonic photothermal (PPT) effector thermoplasmonics, could bring benefits to a wide range of research and innovation topics. For example, Qiu et al. (Qiu et al. 2020) developed a dual-functional LSPR biosensor using LSPR sensing transduction and PPT effect (Fig. 7.4a), combining both functions on a cost-effective two-dimensional gold nanoislands (AuNI) chip for the detection of SARS-CoV-2 viral nucleic acid. Aiming to improve the stability, sensitivity, and reliability of sensing, plasmonic resonances in LSPR and PPT are excited with two different light sources, i.e., 532 nm laser (normal incident angle) and 580 nm laser (attenuated total reflection mode). The biosensor can sensitively detect the RdRp gene with a LOD value as low as 0.22 pM. Based on previous work, Qiu et al. (Qiu et al. 2021) further expand the use of the photothermal-assisted plasmonic sensing (PTAPS) system and introduce the concept of thermoplasmonic-assisted dual-mode transducing (TP-DMT) (Fig. 7.4b), which allows direct detection of LOD up to 0.1 ± 0.04 pM. Cyclic

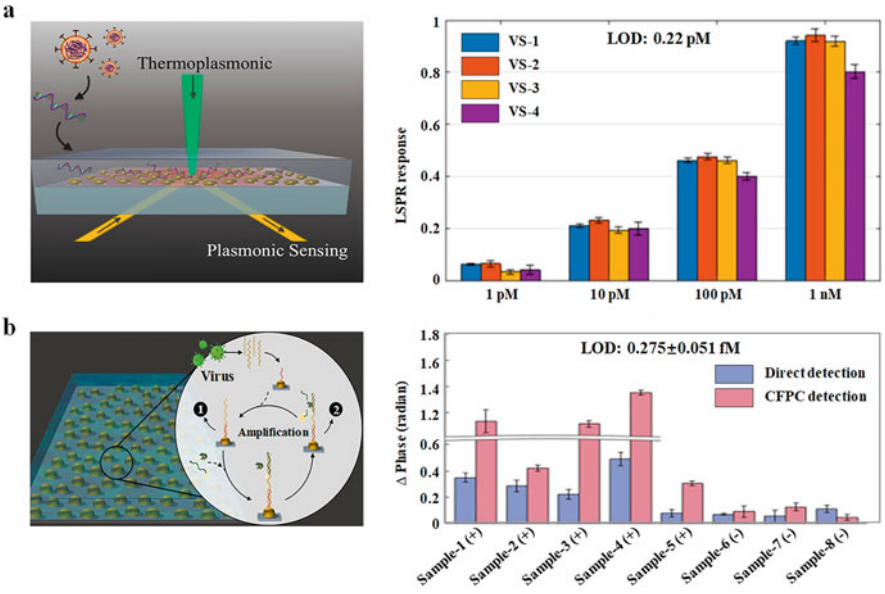

Fig. 7.4 (**a**) Dual-functional plasmonic photothermal biosensors for highly accurate detection of SARS-CoV-2. Reprinted with permission obtained from (Qiu et al. 2020). (**b**) Thermoplasmonic-assisted cyclic cleavage amplification for self-validating plasmonic detection of SARS-CoV-2. Reproduced from Ref. (Qiu et al. 2021)

fluorescence probe cleavage (CFPC) improves the sensitivity of quantitative detection by stimulating transient and cumulative LSPR responses, and the LOD of the second LSPR biosensing can be improved to 0.275 ± 0.051 fM.

7.3 Nano-Bio-Analytical Systems for Emerging Resistant Bacterial Infection Detection

Antibiotic-resistant bacterial pathogens are an emerging health threat, spreading rapidly and widely around the world (CDC 2019; Solomon and Oliver 2014). A critical reason for this worldwide concern is the overuse and misuse of antimicrobials (Laxminarayan et al. 2013; Tacconelli et al. 2018). A technique that could rapidly identify pathogen infection and determine antibiotic resistance profiles is needed to combat this threat. Antibiotic susceptibility testing (AST) is extensively used in the clinic to detect antibiotic susceptibility of isolated bacteria, guide more potent antibiotic treatment regimens, and evaluate therapeutic results. However, aiming to realize AST, the golden standard method often require microorganism culture, separation, and further subculture steps, which depend on overnight cell culture (Jorgensen and Ferraro 2009; Bauer et al. 1966). Therefore, rapid POCT bacterial resistance infection detection methods are necessary to avoid empirical prescription of antimicrobials and improve antibiotic stewardship.

Numerous emerging AST methods generally can be divided into two classes: genotypic and phenotypic approaches (Bauer et al. 2014; Khan et al. 2019), which recognize antibiotic resistance genes and directly analyze the phenotypic features, respectively. The genotypic approaches, which detect antibiotic resistance genes (Khan et al. 2019; Fluit et al. 2001; Frye et al. 2010; Park et al. 2011; Athamanolap et al. 2017), are highly desirable for the AST owing to the outstanding sensitivity (Machowski and Kana 2017). But the methods are still challenged for requiring enough prior knowledge of the bacteria; furthermore, the methods may obtain false-negative results when new resistant strains come into sight, and false-positive results when the existing resistance genes are not expressed or do not promote a resistant phenotype. Aiming to directly monitor bacterial cell growth or reproduction, phenotypic AST technologies generally focus on phenotypic characteristics (e.g., number, morphology, size, and length) (Syal et al. 2016; van den Broek et al. 2008; Cermak et al. 2016; Pancholi et al. 2018; Lissandrello et al. 2014; Pantel et al. 2018; Choi et al. 2014a; Longo et al. 2013; Besant et al. 2015; Schoepp et al. 2017). Optical inspection detection techniques, including real-time microscopy, microfluidic imaging, surface plasmon resonance imaging, and light scattering imaging, have been leading the way in rapid phenotypic AST.

7.3.1 Real-Time Microscopy System

Optical microscopy systems are the leading method used in rapid phenotypic AST detection, which can image the phenotypic characteristics (e.g., number,

morphology, size, and length) for direct bacterial cell growth or reproduction measurements (Syal et al. 2016; van den Broek et al. 2008; Cermak et al. 2016; Pancholi et al. 2018; Lissandrello et al. 2014; Pantel et al. 2018; Choi et al. 2014a; Longo et al. 2013; Besant et al. 2015). Multiplexed automated digital microscopy (Metzger et al. 2014; Chantell 2015) was established to achieve rapid identification and drug-resistance analysis of clinical specimens, which isolates bacterial cells from coexisting or interfering species within the clinical specimens (e.g., urine or blood) utilizing gel channels, attaches purified bacterial cells to the sensing surface based on electrokinetic loading, and identifies bacterial cells with fluorescence imaging within 1 h (Chantell 2015). In order to enable quick AST in individual cells morphological analysis (Choi et al. 2014b), an emerging imaging tool uses bright-field microscopy and an automated image-processing and analysis algorithm to facilitate accelerated AST to ascertain antibiotic-induced morphological changes in single bacterial cells. oCelloScope (Fredborg et al. 2015; Fredborg et al. 2013), another optical imaging technique, images the growth of bacterial cell populations in liquid samples and quantifies the changes in the area occupied by the growing cells for rapid AST. The Accelerate Pheno™ system uses microscopic morphogenetic cellular technology to analyze individual cells and colonies and measure growth, providing AST results of bloodstream infections within 7 h (Pancholi et al. 2018; Pantel et al. 2018; Marschal et al. 2017; Burnham et al. 2014; Schneider et al. 2019; Ehren et al. 2019).

Electrical impedance, which detects the current response of a sample under applied potential, is highly desirable for the label-free biosensing platform. The technology can be employed in various applications, including tissue, protein, and cell studies (Ng et al. 2010; Shamsipur et al. 2018; Nwankire et al. 2015; Lei 2014). As an intrinsic characteristic, the sensitive current response of the target bacterial cells to an applied electric field is connected with subtle differences and variations of parameters such as the shape and size of the target bacterial cell, the internal structure of the bacteria, and the dielectric constant and conductivity of the diversified bacterial components. This enables the impedance analysis for studying bacterial external stimulus-response determining the bacterial viability, studying the principles of antibiotic action, and bacterial identification and bacterial isolation (Mannoor et al. 2010; Yang and Bashir 2008; Xu et al. 2017; David et al. 2012). Based on the intrinsic electrical properties of single bacterial cells, Zhang et al. (2021a) reported label-free electro-optical impedance microscopy (EIM) by imaging the cell responses to low-frequency potential modulations to quantify single bacteria impedance. *E. coli* O157:H7 is immobilized on the surface of an indium tin oxide (ITO) electrode, and the impedance response of potentially modulated bacteria is imaged to complete the detection. EIM can map bacterial viability at subcellular resolution by impedance response, which was used to monitor the impedance changes under two distinctive types of drug for comprehensive bacterial viability detection and antibiotic mechanisms study. Figure 7.5a shows the schematic illumination of the EIM system with an inverted microscope. The ITO electrode surface is modified with antibodies for both *E. coli* potential modulation and immobilization. Figure 7.5b is the representative impedance responses of the single bacterial cells

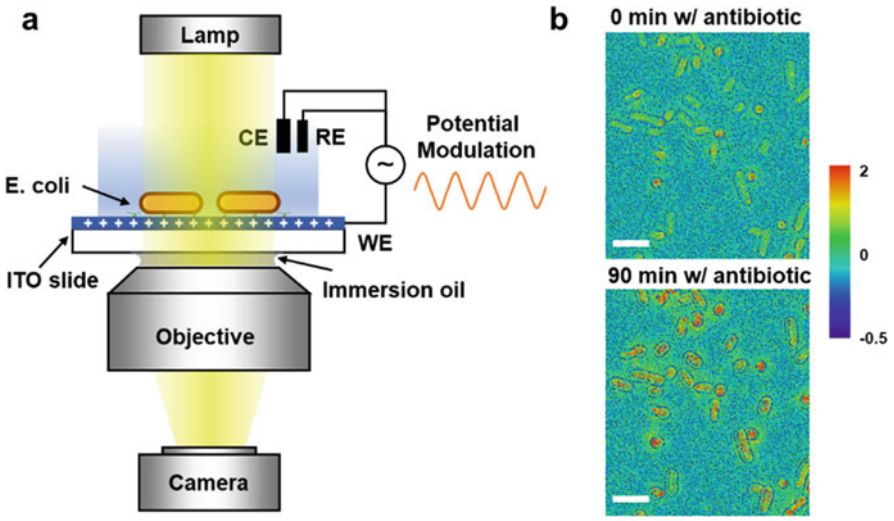

Fig. 7.5 (**a**) Structure diagram of the impedance imaging setup with a standard three-electrode. (**b**) The representative impedance results of the bacterial cells under 90-min antibiotic treatment. Reprinted with permission from reference (Zhang et al. 2021a). Copyright 2021 American Chemical Society

with subcellular resolution under antibiotic treatment, which revealed the heterogeneity response of single bacterial cells. The simple imaging approach can be used to identify resistant bacteria associated with the infectious disease, delivering accurate results more rapidly at a lower cost.

7.3.2 Microfluidic Imaging System

Microfluidics has revolutionized single-cell manipulation and analysis methods with reduced sample/reagent volumes and the associated costs for sensitivity and POCT. It has been found that the combination of imaging-based tools and microfluidics allows for rapid AST (Park et al. 2011; Choi et al. 2013; Baltekin et al. 2017; Li et al. 2019b). Bacterial cells were sequentially captured in microfluidic chambers (Kim et al. 2015), microchannels (Lu et al. 2013), or droplets (Chen et al. 2010; Boedicker et al. 2008) and imaged to detect changes in the cell number (Metzger et al. 2014; Mohan et al. 2015), size (Choi et al. 2014b), morphology (Quach et al. 2016), and viability (Boedicker et al. 2008) in the presence of antibiotics to perform AST. The Astrego Captiver system is composed of 2000 single bacteria-sized channels, and the longitudinal growth of a single bacterial cell was monitored with a time-lapse phase contrast microscopy for bacterial susceptibility determination within an hour (Baltekin et al. 2017). The droplet-based platform dropFAST (Kaushik et al. 2017) allows single-cell encapsulation, incubation, and drug resistance in less than 60 min. Co-encapsulation of bacterial cells with an active probe (e.g., Alamar blue) has been

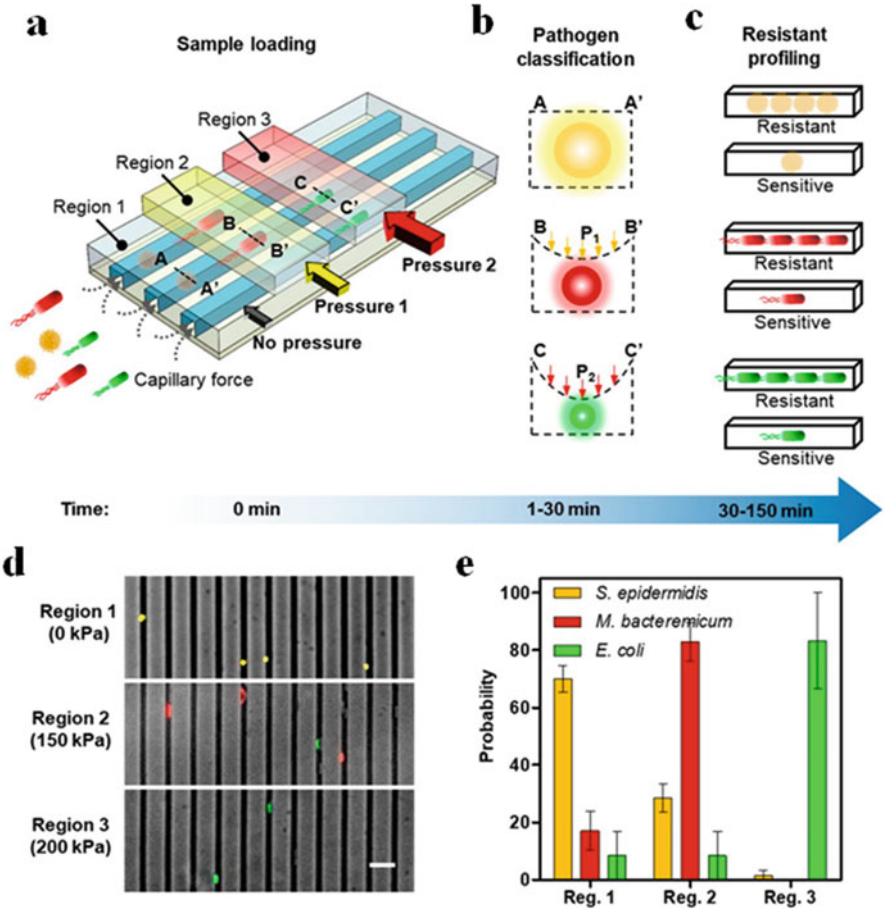

Fig. 7.6 (**a**) Microfluidic device adapted for AST that can be used to classify pathogens at the level of individual bacterial cells. (**b**) Cross-sectional profile of the channel at different air pressures. (**c**) Antimicrobial susceptibility was identified by tracking variances of the bacteria phenotypic growth in the presence of antibiotics. (**d**) Microfluidic separation of three bacterial species (*S. epidermidis*, *M. bacteremicum*, and *E. coli*) by the tunable microfluidic device. (Scale bar, 10 μm). (**e**) Distributions of the bacteria in regions with 0, 150, and 200 kPa applied pressure in the microchannels. Data represent mean ± SEM ($n = 3$). Reproduced from reference (Lu et al. 2013)

used to distinguish AST bacterial subpopulations in not more than 4 hours (Lyu et al. 2018). In another droplet-based method, bacterial cells are captured with antibody-conjugated beads, Hoechst staining is followed by microscopic analysis of cell division and morphology, and drug resistance is determined within 120 min (Sabhachandani et al. 2017).

For direct AST on clinical samples, an adaptable microfluidic system was reported to process clinical specimens (including urine and blood) without any preliminary treatment (Li et al. 2019b). By applying different pressures, the main

pathogens can be captured and classified depending on the shape and size of the polymicrobial sample. A clinical sample was flowed along the microfluidic channel. Once a bacterial cell is noticed with a high-resolution optical microscope, it applies pressure to the channels to trap the cell and then monitors cellular length in the presence of an antibiotic over time. Figure 7.6a shows the adaptive microfluidic device that can be used to classify pathogens at the single bacterial cell level, while Fig. 7.6b shows cross-sectional profiles of the channels at different air pressures. Bacteria remain under different areas of the channel and are sorted according to the applied pressure, which dynamically changes the height of the channel. Antimicrobial susceptibility was identified by tracking variances of the bacterial phenotypic growth in the presence of antibiotics (Fig. 7.6c). Figure 7.6d shows the isolation of three bacteria utilizing a microfluidic device. *S. epidermidis*, *M. bacteremicum*, and *E. coli* were fluorescently stained, mixed, and loaded into the microfluidic system to detect the effectiveness of pathogen isolation ($n = 3$). Figure 7.6e was used to test the effectiveness of bacterial identification by plotting the distribution of bacteria in areas of the microchannel where 0, 150, and 200 kPa pressure was applied.

7.3.3 Surface Plasmon Resonance Imaging (SPRi) System

Surface plasmon resonance (SPR) is an outstanding sensing technique that has many advantages for molecule detection, including in situ, real-time, and fast response. SPR can detect weak refractive index changes on the surface of the sensing chip with high sensitivity. SPR imaging (SPRi) technique can realize real-time imaging and visualization of the local refractive index change near or on the sensing surface, providing spatial information for local mass density sensing. Chiang et al. (Chiang et al. 2009) tested the antimicrobial susceptibility of susceptible and resistant bacteria within a single channel adhered to a gold chip. This is the first report of an antimicrobial test using the SPR system to detect resistant or susceptible strains. To improve the throughput, Ozkaya et al. (Ozkaya et al. 2019) constructed a SPR system with multichannel microfluidic for the multiple detections of methicillin-susceptible *S. aureus*, methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus*, and vancomycin-susceptible *Enterococcus*.

For more sensitive AST detection, Syal et al. (Syal et al. 2016; Syal et al. 2015) introduced the plasmonic imaging and tracking (PIT) technique to monitor and analyze the multidimensional movement of individual bacterial cells, which are closely related to metabolic viability. The PIT equipment is based on an inverted optical microscope, where light from a luminescence diode is directed onto the sensor chip made of gold-coated glass film with immobilized bacterial cells (Fig. 7.7a). Figure 7.7b shows a few snapshots of the plasmonic image, which reveal large fluctuations in the image contrast of a live bacterial cell (*Escherichia coli* O157:H7). The image contrast changes are due to the bacterial cell movement normal to the sensor surface (Z-direction), due to the bacterial metabolism of live cells (Fig. 7.7c), while the dead cell showed very minimal motion (Fig. 7.7d) (Yang et al. 2015; Shan et al. 2014). PIT has been reported to detect the multidimensional

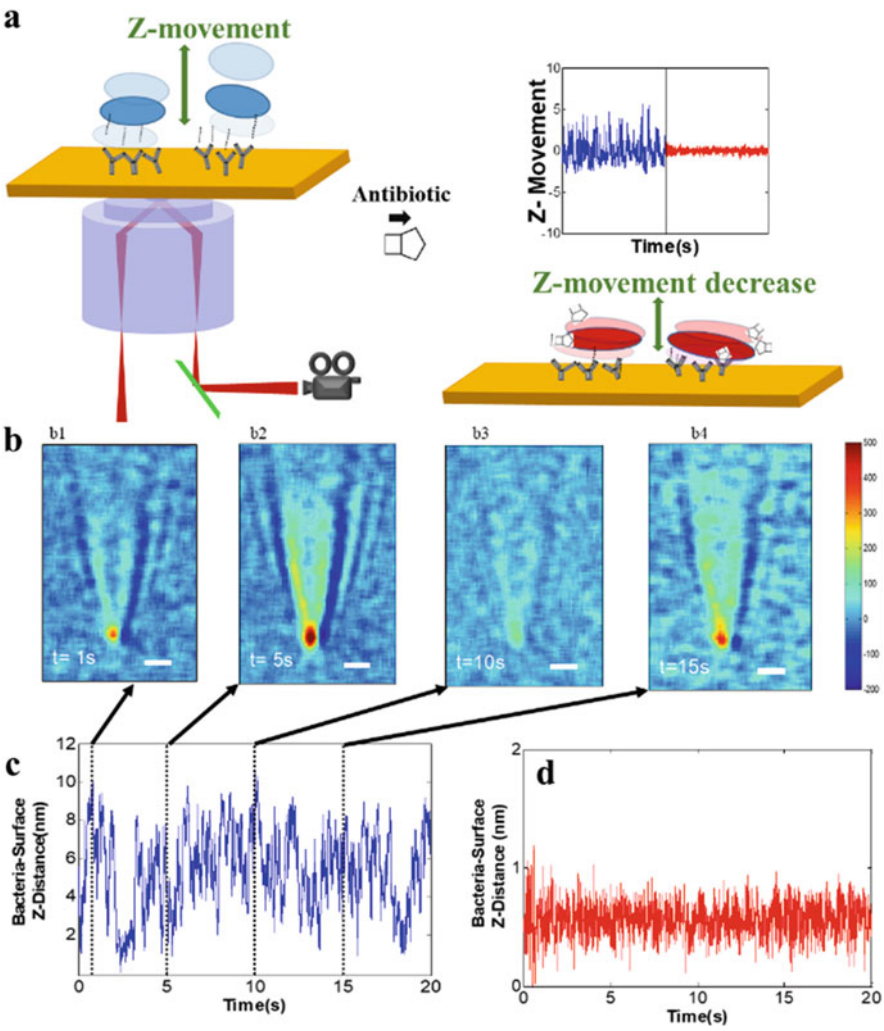

Fig. 7.7 (**a**) Schematic of the plasmonic imaging and tracking of bacterial cell metabolic activity-related 3D movement (referred to here as 3D movement) with nanometer resolution. (**b**) Snapshots of bacteria z-micro-motion. (**c**) z-distance between bacterium and plasmon surface vs. time of an alive (~6 nm). (**d**) Z-displacement plot of a dead bacterial cell showing average motion ~0.50 nm. Reprinted with permission from reference (Syal et al. 2016). Copyright 2019 American Chemical Society

motility of individual bacterial cells and subcellular organelles at spatial resolution <5 nm and temporal resolution <1 millisecond (ms). This detection technique allows rapid monitoring of the multidimensional motility of multiple bacterial cells simultaneously, enabling high-throughput quantitative analysis of single-cell ASTs. The work also demonstrates the feasibility of this technique for the detection of

clinical samples. In addition, PIT has the potential to be used to discriminate and identify bacterial cells in the complex matrix of urine, serum, and other body fluid samples. This will facilitate the real application of PIT and its development into a practical solution for testing real patient samples.

7.3.4 Light Scattering Imaging System

As a label-free and noninvasive analytical tool, light scattering can be widely used to detect and classify small particle analytes such as bacteria and cells in real time (Broeren et al. 2011; Fouchet et al. 1993; Steen 1990). A laser beam is normally used to irradiate a liquid sample for both turbidity and scattered intensity measurement. Generally speaking, measuring light scattered by bacterial cells is the principle of most light scattering-based bacterial growth detection protocols (e.g., BacterioScan (Roberts et al. 2017)). The relative growth of bacteria in culture solutions is commonly characterized by the results of optical density at 600 nm. The principle of this technique is because different concentrations of bacteria scatter different amounts of light randomly in solution. However, this indirect assessment of bacterial growth and cell concentration is often a complex pre-process (requiring inoculation of pure samples and suspension of cells to the appropriate density level) (Sutton 2011). With recent advances in optical detection technology, other light scattering methods have been newly developed to improve detection sensitivity (Roberts et al. 2017; Hayden et al. 2016; Boland et al. 2019). As an important optical detection system in recent years, flow cytometry systems (such as the FDA-approved UF 1000i) integrate light scattering and fluorescence technologies for rapid screening of bacterial cells in urine (Broeren et al. 2011).

The light scattering method can further measure the angular variation in the intensity of the light, with this variation being proportional to the number, size, and even shape of the particles, allowing detection limits up to 2 orders of magnitude lower compared to standard methods. Based on the above approach, Zhang et al. developed a rapid AST in the single-cell scattering intensity tracking for direct AST on clinical urine specimens within 60–90 min with a large-volume solution scattering imaging (LVSi) (Mo et al. 2019) by monitoring and tracking individual cell division (Zhang et al. 2020) and by object scattering intensity detection (Zhang et al. 2021b). Traditional optical microscopy can image bacterial cells but requires immobilization of the bacteria on a surface. These limitations, combined with the small field of view of high-resolution optical microscopy, necessitate bacterial enrichment in low-concentration samples. LVSi overcomes this difficulty by illuminating and imaging a large volume, such that the presence of a few bacterial cells in a clinical sample can be tracked continuously, which can image and count low bacterial concentration urine samples, e.g., 10^4 cells/mL for rapid AST (Mo et al. 2019). To accurately track and quantify single division events of bacterial cells in complex clinical samples, a forward scattering optical imaging configuration (Fig. 7.8a) was introduced with a single-cell division tracking imaging processing algorithm. Figure 7.8b shows the representative example of two division events for two cells

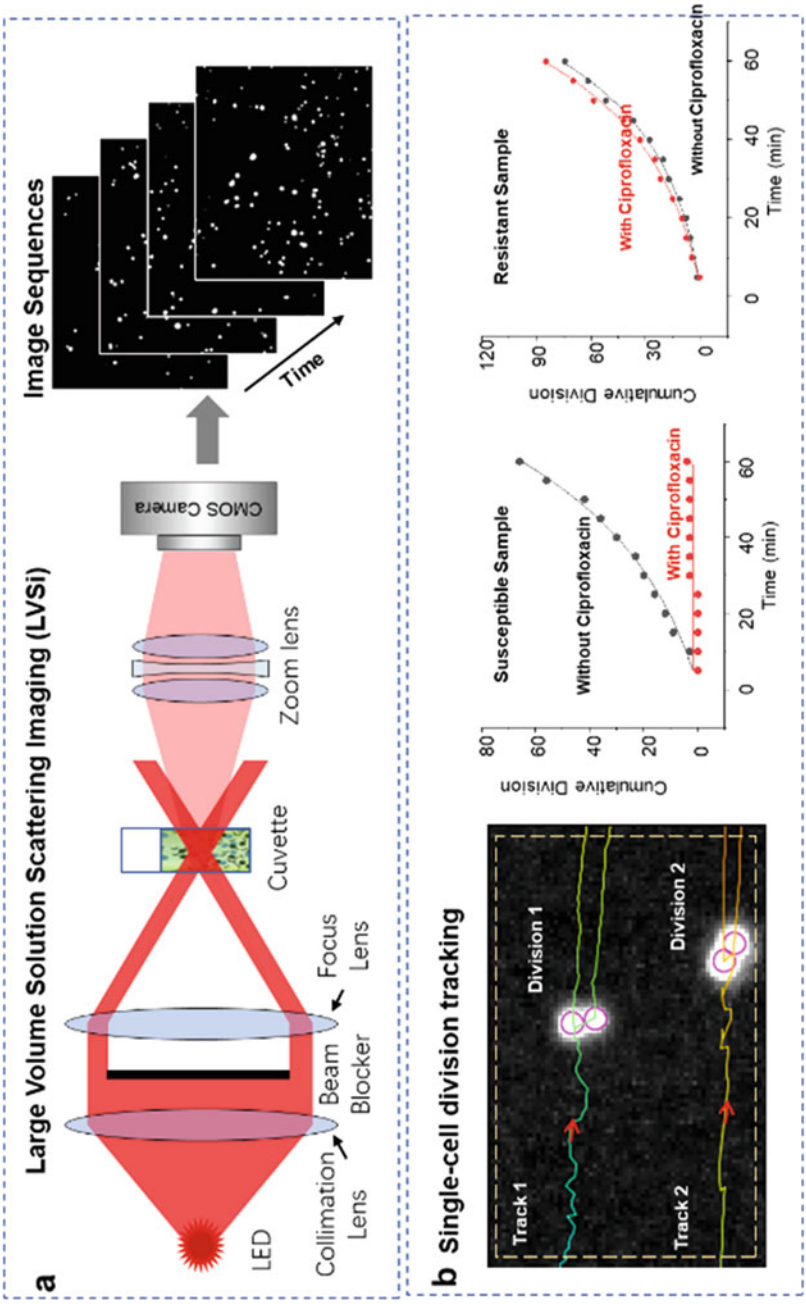

Fig. 7.8 (**a**) Schematic illustration of the LVSi setup which could detect single bacterial cells in urine. (**b**) Demonstration of two-split event detection with processing algorithm and two representative growth curves of susceptible and resistant samples detected by the LVSi-AST tool. Reproduced from reference (Zhang et al. 2020)

tracked using the imaging processing algorithm and two representative growth curves of susceptible and resistant samples detected by the LVSi-AST method. The LVSi-AST method has excellent performance, achieving rapid detection of bacterial infections in 60 clinical samples within 1 h. The digital ASTs of these 30 positive samples were in 100% complete agreement with clinical culture results and field agar coating validation results. This technology will pave the way for more accurate antibiotic prescriptions and prompts proper treatment of the patient within a single clinic visit.

7.4 Conclusion and Future Direction

Current manual and automated techniques for emerging disease detection have become vital tools in today's clinical microbiology labs. In the near future, burgeoning and future innovative technologies, for instance, plasmonic-based biosensing systems, microfluidic-based systems, and light scattering imaging systems, will lead the way in the development of clinical tools for rapid infectious disease diagnosis. These tools have dramatically reduced the time to detect infectious diseases and allow for POCT diagnoses to be made on an outpatient basis. Such rapid and real-time detection tools will not only help save patients' lives but also enable physicians to implement accurate treatments at disease onset, potentially preventing the spreading of EIDs, slowing the evolution of antibiotic resistance, and improving antibiotic stewardship. In summary, given the ever-increasing spread of EIDs, there is an urgent need to develop innovative technologies for the rapid detection of infections, both for real samples collected directly from the patient and for slow-growing or non-cultivable microorganisms.

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