CHAPTER – 1
INTRODUCTION

1.1 Importance of Detection technologies

Development in the field of Material Science has paved new ways for engineers to solve emerging problems in biology. With the advent of Nanotechnology, Material scientists are continuously establishing arenas for multidisciplinary research by involving all the domains of sciences to address global healthcare issues. Detection of harmful disease causative agents is one of such areas, where material scientists are exploiting novel nanomaterials for fabricating facile, cost effective methods early detection of harmful microorganisms. These methodologies are crucial not only for successful circumvention of a disease but also for elucidating its cause, limiting spread, preventing outbreaks and avoiding wastage of resources in futile treatments [1]. In the current times, testing of samples for disease causative agents are performed in a “health provider centered model” where centralized laboratory setups with high end sophisticated instrumentation facility located either in hospitals or specialized centers perform testing on contaminated samples [2]. The main drawback of this conventional model is large turn over time from sample collection to result communication, mostly when tests are performed in resource limited settings (RLS). Sample collected from rural and remote areas firstly need to be transported to centralized laboratories located away in big cities. At laboratories, samples are processed in batch manner and then results are transmitted to health workers, from where clinical decisions are communicated with effected peoples [3]. This long procedure of confirmatory diagnosis and the fact that test samples that are far from these set ups are less likely to follow up for timely appointments, leads public health worker strenuous to manage a disease outbreaks [4]. Especially, pathogen contamination in water and food needs a continuous on site testing and monitoring to prevent epidemics. Ironically, majority of techniques employed for diagnosing harmful bacterium in water and food samples require culturing of samples which may take time from days to weeks. This delay or late detection contributes to spread of infections since precautions cannot be taken and in many cases poor assessments implies usually worst disease management.
Bacterial illness due to harmful pathogens is an avoidable yet leading cause of deaths and hospitalization across the globe [5]. Pathogens are pervasive in human environment and cause several infectious diseases in humans and animals. Food and water contamination represents a major route of infections by these pathogens. The center for disease control and prevention USA, reports 31 major pathogens are responsible for majority of food and water borne illness [6]. Most of these illnesses are prompted by norovirus, followed by non-typhiodal Salmonella spp., Clostridium perfringens, Campylobacter spp., Toxoplasma gondii, and Listeria monocytogenes. According to UNICEF and WHO, every day 4000 children die due to intake of contaminated drinking water and almost one in ten people fall ill after ingestion of contaminated food [7]. Although, the global incidences of pathogenesis from food and water borne diseases are difficult to estimate, but according to a recent report by WHO in 2016, around 550 million people suffers from diarrheal diseases each year, which includes approximately 50% children under the age of 5 years [8]. With low infectious dose of less than 100 CFU, it remains a continual challenge to tackle outbreaks of water borne diseases. Recently, a project commissioned by British government “Review on antimicrobial resistance” estimates a near future 10 million deaths a year and $100 trillion loss to economy, far more than cancer [9]. Besides, long term exposure to these microorganisms prompts severe chronic conditions such as ocular failure and kidney damage [10].

As discussed earlier, food and water borne diseases are preventable by identifying contaminated food and water at first glance. The most commonly applied tools applied for pathogen detection are polymerase chain reaction, cell culture and immunological approaches [11]. These traditional and modern molecular techniques are well established and are highly sensitive, specific and yields robust results when used in combination. Even though with such interesting qualities, these traditional techniques are labor intensive, expensive and takes long turnover times [12]. This is clearly scant and more efforts are required for development of advanced, facile and cost effective systems for pathogen detection as these will not only conserve precious human lives, but also avert the unnecessary cost associated with medical treatments.

Advances in the area of water diagnosis are currently focused on different areas such as the synthesis of new nanomaterials for transduction and amplification on contaminant detection with biomolecular recognition elements [13], [14]. These sensing
devices based on biomolecular detection techniques have shown capabilities for designing a fast, cost effective and straightforward approach for fabricating sensitive, specific and miniaturized detection systems [15], [16]. These devices equipped with highly efficient nanomaterial based amplifications upon recognition of target bacterium appear to address the challenges of rapid and facile testing of water and food samples.

1.2 Modern Detection Techniques

Development in the field of modern nanomaterial based facile detection strategies have started their journey from colorimetric assay developed by Mirkin’s group in 1996 [17][18]. They did so by rationalizing size dependent optical properties of gold nanoparticles for selective detection of analytes through biomolecular recognitions. From then to till date development and advances in the field of microelectronics, biotechnology and nanotechnology have shaped these devices into much handy, low cost and rapid units capable of detecting multiple diseases within minimal time intervals.

Although these detection techniques are relatively new in the family of diagnostic devices yet they represents a facile, time saving and cost effective alternates for qualitative and quantitative detection of analytes by using nanomaterials as amplification component [19]. The recent needs for fast and low cost devices prove themselves as an alternative approach of detection than conventional culturing and biochemical techniques [20]. These detection techniques are essentially consist up of four components. First is biological sample, which includes cell culture, human (urine, blood, saliva, stool etc), animal, environmental and food samples. Second is a a recognition element that binds specifically to its analytes. Some examples of these recognition elements are antibodies, aptamers, molecularly imprinted polymers, drug or any other chemical or biological moiety that possesses specific binding affinities. The third element is the signal amplification unit. Conventionally, this unit comprises of enzymes or other catalytically active molecules, but now-a-days there is a constant effort to replace these with much sensitive nanostructures. Given the nature of amplification technique employed, the readout generated can be optical, electrochemical or mass based measurements. The fourth element is the readout for users. This unit takes amplified signals and displays it to user or physician. Traditionally, this unit was comprised of sophisticated instrument display but now with the advancement in technology low cost substrates like paper,
arduino and smart phones are employed. Figure 1 illustrates basic components of a modern diagnostic device.

![Figure 1](image)

**Figure 1** – Basic elements of a diagnostic device in a core diagram. Inner core comprises of analyte to be detected followed by its recognition element and amplification element to amplify this detection to a visible signal on a paper, smart phone, arduino or any other specialized device.

### 1.3 Classification of detection techniques

These detection techniques can be classified on the basis of their various attributes and the type of transduction and amplification employed. Each type of technique has its advantages and limitations. A number of factors like type of analyte, sample, sensitivity required, cost effectiveness, type of signal readout generated governs the applicability of a particular detection technique [21] [19].

However, the field of diagnostic devices is completely diverse and comprises of many different detection strategies, for the scope of this thesis we have divided these devices into four main classifications based on the detection strategy these devices employs (1) catalytic affinity based detection (2) labeled or label free (3) Type of bio-
recognition element (4) Transduction or amplification of signal. A brief overview of classification is illustrated in Figure 2.

**Figure 2** – Block diagram of classification of detection techniques based on various attributes

### 1.3.1 Recognition element

Detection techniques on the basis of recognition element can be classified mainly as (a) Enzymatic (b) immunosensor (c) DNA sensors.

Enzyme based strategies are based on the catalytic conversion of an organic substrate into a colorimetric or fluorimetric product by specialized enzymes [22]. Enzymes are linked with affinity biomolecules like antibody, antigen or hapten and upon specific recognitions generate and amplify an optical or electrochemical signal. The most commonly used enzymes are horseraddish peroxidase (HRP), alkaline phosphatase and beta-galactosidase [23]. Different types of enzyme based strategies are reported and are vastly used in for biodetection and medical diagnostics. Enzyme Linked immunosorbent assay (ELISA), is one such example widely used in diagnostic laboratories [24], [25]. In this technique, biodetection is performed by coating a specific recognizing probe on a polymeric surface. Followed by addition of sample containing analyte molecules and
sequential washing another enzyme labeled recognizing probe is added, which generates a detection signal.

DNA based assays are based on high affinity between complementary strands of single stranded nucleic acid sequences and specifically designed single stranded DNA molecules known as aptamers for detecting a specific analyte. This type of technique employs either immobilization of ssDNA probes on the surface similar to ELISA or colloidal detection by using nanomaterials [26]. In contrast to enzymatic assays, colloidal assays based on ssDNA aptamers are cost effective, rapid and less perishable.

1.3.2 Label or Label Free

The use of labeling by enzymes or particles classifies a detection technique into labeled or non labeled assay. Some of the labels are fluorophores or chromophores, which are binded to a bio-recognition element and produce a signal when illuminated. Moreover, there are other labels also like radiolabels, magnetic particles and metal nanoparticles. The use of labeling marker is also associated with high cost and quenching or decay of fluorescence with time, possible deterioration of bio-molecule, generation of toxic waste (radiolabels).

Label free bioassays detect biomolecule-bioreceptor interactions without exogenous element adhered to molecule [27]. Modern sensitive techniques like SPR (surface Plasmon resonance), where binding of bioreceptor generates a change in optical properties, Quartz crystal microbalance, where binding of bioreceptor introduces a mass change producing a shift in resonance change of quartz crystal microbalance [28]–[30]. Recently, impedance based electrochemical sensing employing changes in resistance upon biomolecule binding are also employed for detection of various analytes [31], [32].

1.3.3 Catalytic detection

Catalytic detection here refers to catalytic activity of enzymes and nanoparticles. Analyte detection can be performed by (1) conversion of analyte into detectable product by enzyme (2) detecting enzyme inhibition and activation by analyte (3) monitoring modification in properties of enzyme or nanoparticles by analyte. These bioassays allow lower limits of detections compared to affinity detection as enzyme or nanoparticle activity amplifies the signal produced by biomolecular interactions [22].
1.4 Readout signal in detection techniques

The readout signal generated upon analyte detection depends upon type the technique employed and varies as a function of sensitivity required, cost and analyte to be detected. For example, electrochemical and optical readout techniques are largely employed for point of care analysis, whereas mass based techniques like mass spectroscopy and quartz microbalance are used for sophisticated laboratory setups. Brief overviews of these techniques are discussed in this section.

**Electrochemical:** Use for electrochemistry for modern biomolecule detection techniques was first described by Clark in 1962 [23]. In this technique change in electrochemical signals upon receptor analyte interactions are detected. These electrochemical signals are detected through electrical transducer, that may be amperometric, potentiometric or impedometric [33], [34]. Many such strategies uses oxido-reductase enzymes to join its substrate causing a redox reaction, measured by an electrochemical transducer.

**Mass based:** Signal generated by this technique is based on the change of mass upon bioreceptor analyte binding. These mass change causes change in resonance of a quartz resonator or sagging of a micro cantilever [35].

**Optical:** Optical techniques uses interactions of light and matter to report the detection of an analyte [15]. The main advantage of optical signal read out is direct visual representation of results without any requirement of sophisticated instrumentation. For example, a colorimetric assay like as of pregnancy test strip can be easily employed for detection of various analytes even at RLS without need of any trained manpower [36].

Optical sensors measures changes in fluorescence, luminescence, absorbance and reflectance upon analyte detection [37]. Various optical bioassays are reported in literature for detection of chemical and biological analytes employing different spectral regions i.e ultraviolet, visible, near infrared spectrum of light. Easy to read signal output is one of the major advantages of optical techniques over other strategies. Optical signal readouts can be generated in both in label and label free bioassays. In labeled bioassay, a receptor element is labeled with enzyme or nanomaterial which generates an optical signal (colorimetric, fluorescence, luminescence) upon addition of appropriate substrate. In label free bioassays, the interaction between analyte and transducer (i.e nanomaterial) is used for signal generation. Among all the optical signal readout principles, the most
common method of detecting and quantifying biological compounds is colorimetric and fluorescence based. Fluorescence based techniques employ molecule that have an electronic structure which allows excitation and emission of light in visible and near infrared spectrum commonly referred as fluorophores. These molecules when conjugated with a specific receptor probe can be used for detection of analyte, through binding events. Fluorescence signals can be easily isolated using optical filters and collected using photodiodes. A part from conventional fluorescence based immunoassays novel Forster resonance energy transfer based assays are also gaining momentum in field of research and analysis [38]. In this thesis, our major goal was aligned to develop novel optical detection techniques for biomolecule detection platforms.

1.5 Nanotechnology and its implications in detection strategies

In modern biomolecular detection techniques, the demand for cost effective, rapid and sensitive devices is growing and nanotechnology has encouraged researchers and engineers for developing such devices [39] [40]. Nanomaterials are defined as a class of materials having one of their dimension between 0-100 nm [41]. This small size results in increased surface to volume ratio thus increased catalytic efficiency and better optical and magnetic properties. The advancements in electron and probe microscopy have improved our understanding about their properties and nature, making it possible to exploit them in all fields of scientific domain. A few examples of their applicability includes catalysis, nanoelectronics, nanomedicine, energy harvesting, nanofluidics and diagnostic applications [42]. Nano-structured materials are revolutionizing these fields especially of biomolecule detection much effectively by replacing many drawbacks associated with previously reported strategies. For example, fluorescent nanoparticles have much higher life times as compared to conventional fluorophores, catalytic activity of nanoparticles is much higher than conventional counterparts, heat dissipation and magnetic properties are far better than bulk materials etc. These unique properties of nano sized materials are attributed to confinement of electrons within nanostructures. Such confinements are not possible in bulk materials due to overlapping of bands. These confinements of electrons are possible in either one or all of the three dimensions. On basis of these confinements nanosized structures are classified as 0-D, 1-D and 2-D nanomaterials [43]. Quantum dots are excellent examples of 0-D nanomaterials whereas carbon nanotubes and thin films of MoS\textsubscript{2} represent 1-D and 2-D nanomaterials respectively.
The unique properties resulting from such confinements are very sensitive to biological and chemical species in immediate environment and nanomaterials can be used to generate an output signal to detect binding events between two molecular moieties. For example, cystiene capped gold nanoparticles aggregates selectively in the presence of only mercury ions changing color from red to blue, and thus are used for detecting mercury contaminations [44]. In this regard capping or functionalization of nanoparticles plays a pivotal role for designing detection strategies. In above mentioned report, cystiene capping provides selectivity to the bioassay towards mercury, on the other hand if polyethylenimine capped silver nanoparticles are used copper ions can be detected selectively instead of mercury [45]. Therefore in order to translate or produce signal upon detection of analyte, it is necessary to functionalize nanomaterials with different biological and chemical entities. These species may be peptides, nucleic acid, antibodies or some specific chemical entities like drug and molecular imprinted polymers. This functionalization serves dual purpose of providing specificity to nanomaterial for binding a particular analyte and simultaneously increasing its stability [46].

For applications in biomolecule detection, nanomaterials can act as either label or support. As labels they are connected to specific bio-receptor molecule. They function to detect a binding event by giving a particular output such as fluorescence, electrochemical or change in absorption. Noble metal nanoparticles are often used as labels as they can be easily detected through optical or electrical changes [47]. In addition long range surface plasmon resonance of metal nanoparticles assists in ease of probing by absorption spectroscopy. Semiconducting nanomaterials (quantum dots) are also widely used for fluorescence based bioassays [48]. Another example is use of carbon nanotubes and magnetic nanoparticles as a support during biomarker detection. The binding of an analyte onto nanotube changes its electrical properties. Magnetic nanoparticles are also widely used in pre-enrichment steps to remove analyte from complex matrix by using magnets. When nanostructures perform as a support it is used to immobilize receptor and so becomes the surface on which biochemical recognition event occur.

Modern biomedical trends are largely focused towards designing detection strategies for point of care devices [49]. However a substantial development is needed for making these devices a reality. Our growing understanding of nano-materials and their advantages are potential key for realizing these POC devices in reality [50]. Miniaturizing
diagnostic tools, improving detection speeds, portability, reducing amounts of reagent and use of smaller sample sizes can be achieved by using nanomaterials. In addition nanotechnology is amenable to multiplexing, meaning that detection of multiple analytes on a single chip is also feasible prospect for future.

1.6 Microfluidic for diagnostic devices

The essential deception in harnessing these POC devices is to extend the immunoassays on cheap and affordable chips [51]. Microfluidic technologies was developed 40 years back by researchers at Stanford University for gas chromatograph and IBM for inkjet nozzles applications [52]. From then, many different devices ranging from single components such as flow sensors and valves, to complex systems for chemical and biological analyses are developed. Majority of research in microfluidics is focused towards Lab on chip devices particularly for POC diagnostics.

Microfluidics technology is characterized by engineering and manipulating fluid at micro levels of length scale. Microfluidics has shown a great potential towards improving diagnostics and biology research. Conceptually the idea of microfluidics was first developed by semiconductor industry and later expanded by microelectromechanical systems field. Microfluidics has developed largely as clones of semiconductor microelectronics industry. Unlike semiconductor industry where complex silicon structures are predominant, microfluidics device materials have undergone rapid transitions. Microfluidic system uses cheaper and more accessible materials like paper, glass as well as polymers for devices [53]. Silicon and glass are perhaps largely displaced by plastics in these devices. Much research has been done on polymer named poly-di-methylsiloxane (PDMS). PDMS is optically transparent and soft elastomer, thus good choice for optical immunodiagnostic POC devices. Moreover, paper being cost-effective and readily available is also used for developing microfluidic devices. Figure 3 depicts an illustration of PDMS, paper and glass based microfluidic chips for diagnostic devices.
From theoretical point of view the major difference micro and bulk flow is low Reynold number i.e. Laminar flow, surface and interfacial tension and capillary forces. Microfluidics technologies could be easily applied to immunodiagnostics to streamline complex assay protocols. The advantages of microfluidic systems such as low sample requirements with a précised liquid controlling, ability to automate and integrate various steps and cheap fabrication (in bulk quantities only) when coupled with specific and sensitive immunoassay results in a platform which complies with WHO ASSURED criteria [54].

However with such a great number of advantages being offered by microfluidic immunosensors still there is a great lack in delivery of such systems. The field is still at an early age of development with high fabrication and manufacturing costs. Along with these drawbacks we are still seeing many commercial players in market like conbas LIAT, FilmArray, Achira Labs and many more. Microfluidic based diagnostic products constitute a $ 1B+ industry from the whole $3.8B+ microfluidic device segment [55]. Moreover, commonly used dip stick assays and glucose monitoring systems are also good
example of commercialized paper based devices. But still a lot of research is needed in designing robust and ease immunosensing platform and to integrate it with microfluidic devices along with a major addressing to fabrication part [56].

1.7 Smart phone based detection systems

With the ever increasing technological advancements diagnostic paradigm is also shifting towards point of care systems. Smartphone is becoming a universal possession in both high and low income economies [57]. In many areas across the globe where sophisticated devices are not available or deployed, smart phones can be used for providing access to medical diagnostics. The emerging arena of mobile health uses mobile communication and devices for providing healthcare services in remote areas [58]. Over 94% of world population uses mobile phones with 70% of smart phone users from developing countries. The enormous growth along with increased features and reduced prices has vastly expanded the market size of smartphones. Smartphones are equipped with a rich set of sensors and computer like programs and have phenomenal capacity to command and control medical devices [59]. The inbuilt powerful processors and memory helps in analysis and storage of diagnostic results. Moreover, smartphones are also equipped with data transmission capacity through GSM and USB allowing communication between a remote test area and centralized laboratory for consulting professionals. Such type of interfacing is high appreciated in developing countries, where smartphone based diagnostics provides a cost effective alternative to sophisticated instrumentation. In recent years various devices have been developed using smartphone to specifically address the diagnostic challenges of RLS [60], [61]. For example, smart phone based a plug and play type blood pressure monitoring device is already available in market instead of a conventional stethoscope and sphygmomanometer. Many other devices with such applications like heart rate monitoring, calorie counter are available in market for personalized health care. Smart phones are equipped with several in built sensing systems the major sensing operations used in point of care devices are limited to camera using image processing algorithms [62]. However, a variety of sensors could be integrated as accessories to smart phone to increase the scope and accuracy required for diagnostics. Figure 4 illustrates various examples of smart phone based diagnostic devices.
Figure 4 – Adaptation of many commercially available smart phone based diagnostic devices for representation of application of smart phone in medical purposes.

Smartphone technology offers great opportunities for improving public health as introducing medical applications in non-medical devices, with which people from non-technical backgrounds also are already familiar with the barriers to access sophisticated medical laboratories for millions of people can be eliminated

1.8 Indian scenario

India is the largest democracy in world with and a country surging ahead in global scenario with its robust economic growth. India has added 250 million people in over 25 years to 2016 and proportion of people living in poverty line fell by half [63]. Along with increase in prosperity, disease burden is continuously raising threat with lifestyle diseases accounted for half of deaths in 2016 [64]. The healthcare industry that has grown to $81.3 billion in 2013 is expected to grow to 17% by 2020 [65]. As this happens, providing healthcare facilities to rural areas is a big challenge. Healthcare system in India is dealing with a plethora of problems. The government spending as a proportion of GDP and per capita health expenditures are among lowest on world, US spend 125 times more and china 5.6 times more [66]. Existing infrastructure of India is not enough to cater emerging disease outbreaks. Moreover, 75% of the qualified doctors practice in urban areas, 23% in semi urban and 2% in rural, whereas 67% of population lives in rural areas [67]. Further, a vast majority of population in north and north east regions lives in hilly terrain and
some territory in remote islands making healthcare reach impossible to these distant areas.

With these challenges Indian government has decided to increase its healthcare spending from 1.4% to 2.5% of GDP by the end of 2017 and also permitting 100% FDI in healthcare sector [68]. Currently 75% of India’s medical devices are imported with 30% from US alone. The healthcare challenges of developing countries are different from that of developed nations. In this case, imports from developed countries lead to a mismatch in design of technologies in implementation according to realities of healthcare infrastructure in developing nations. In conclusion, there is a high need of developing indigenous technologies to met healthcare challenges of India. In a contribution to this the thesis aims at developing new platforms that assist biomedical engineers and public health workers to detect and diagnose bioanalytes in efficient way possible.

1.9 Challenges to Currently available technologies

Developing economies like India and Africa accounts for 90% of global disease burden [69]. These nations lack essential sophisticated devices for timely interventions and approximately all of these devices are designed for high income countries and are inappropriately costly. The poor infrastructure lacking stable electricity sources and clean water, the devices readily malfunction, and thus are not properly exploited reducing their intended benefits [70]. To maximize the healthcare benefits these devices needs to be appropriate for context of environmental conditions of developing world [71] [72]. Advances in field of developing these technologies are potentially constructive towards stream lining healthcare thus significantly improving standard of living [73]. However, to meet growing needs of detection technologies engineers needs to consider the drawbacks of already developed techniques. Some key limitations of developed technologies are explained in this section.

(1) Time and cost effectiveness: Longer time in analysis is detrimental in several ways. This leads to need of advance stage of diagnostics, poor survival, greater disease and treatment related morbidity. In case of infectious diseases detection of pathogens and infections become more important as it may be adversely lead to disease outbreaks and anti microbial resistance as discussed earlier. Culturing is most commonly used technique for detection of pathogenic microorganisms. Culturing is although a cost
effective method but often takes days or even weeks to culture a bacteria. Further biochemical investigation is associated with high costs. Cost effectiveness is an important criterion for any bioassay as people residing in remote and low income nations where disease outbreaks are more prevalent. Modern molecular and chromatographic techniques come with significant expenses and thus cannot be used in RLS. These techniques also require trained manpower and are also not field deployable.

(2) Sensitivity: Although a number of low cost techniques are developed but suffers drawback of poor sensitivity. Low infectious dose of some pathogenic microorganisms hinders there field deploy ability. Moreover, sensitive detection of many disease biomarkers is required for accurate diagnosis.

(3) Interference: A number of interferences exist in samples and thus highly discriminative sensing is required. For example, modern detection techniques like PCR are highly sensitive technique is prone to interferences. Internal peroxidase activity of samples contaminated with pathogens also represents a key drawback of ELISA based systems that uses a peroxidase enzymes for signal generation.

Keeping in view the importance of developing novel detection platforms, our study was aimed at the development of bio-assays for specific, sensitive and rapid detection of analytes namely pathogens and proteins through a synergistic combination of biology and material engineering. Nanotechnology has received a wide interest and our belief is in using this for improving global health and prosperity. For this, following objectives were designed:

1.10 Aims and Objectives

(1) Development of nanoparticle based transduction and amplification mechanism for affinity based point of care devices.

(2) To study diagnostic efficacy of developed platform towards target analytes.

(3) Development of low cost cartridge or fluid cell for platform.

(4) Enabling the platform in device format based on smartphone/arduino.
1.11 References


