Chapter 1

INTRODUCTION AND BACKGROUND

This chapter articulates a brief overview of the prevailing scenario at both, local and global levels, in the field of micro fabrication of lenses and imaging through them. It lays the foundation for the research conducted and reported in this thesis. This chapter, starting with a brief background and prevailing situation, furthers by delineating the literature review, research gap, motivation, thesis organization, and concluding remarks.

1.1 A BRIEF BACKGROUND AND PREVAILING SITUATION.

The evolution of technology has brought commendable positive changes in different facets of our life, and the healthcare ecosystem is no exception. Continuously evolving healthcare facilities, such as magnetic resonance imaging (MRI), infusion pumps, and various diagnostic technologies have astonished the medical professionals who practiced a few years ago. Though a significant population is benefitted from such developments, a considerable population still does not have access to pathological and diagnostic facilities. Given the condition that trained manpower and infrastructural facilities are short by 15 to 70%, there is an extensive need to develop a low-cost robust healthcare ecosystem, catering to the needs of the underprivileged.[1] There are five domains identified for the technological transformation of the healthcare ecosystem,

- Electronic health records
- Surgical technologies
- Artificial intelligence and augmented reality
- Telehealth and

- Personalized treatment

To provide healthcare facilities up to remote and resource-limited locations, personalized treatment and a telehealth ecosystem need to be developed. To achieve such goals, it is important to provide low-cost diagnostic equipment and testing facilities, which can be operated by semi-skilled labor.

In the last few years, there have been significant research efforts have been made to develop point-of-care testing devices to be used in resource-limited settings.[2] With the progress and advent of new methods of microfabrication techniques, a variety of diagnostic devices have been developed. Thin-film based technology is one such domain of microfabrication technique, which provides low-cost miniaturization solutions to develop various devices such as capacitors, cells & batteries, electrodes, and many more.[3–5] Thin films are two-dimensional structures placed over a solid support called the substrate. By reducing the thickness of one dimension, a natural occurrence of electronic tunneling through an insulating layer, high resistivity and low-temperature coefficient of resistance, an increase in the critical magnetic field and critical temperature of the superconductor, and optical interference have been observed. Keeping such interesting properties in view, the fabrication of nano/micro structured patterned surfaces has received attention due to their immense potential of applications in Microfluidics[6–9], Smart Adhesives[10,11], Micro and Nano-optics systems[12], Micro-Electro-Mechanical Systems (MEMS)[13], Antifouling/Antireflective Coatings[14,15], Structural Color coatings[16], etc. There are several methods have been reported to generate functional patterned surfaces as Moulding (Embossing) or Nano/Micro Imprinting[17], Laser Ablation and Scanning[18], lithography[19], etc. However conventional techniques of fabricating patterned surfaces require sophisticated instruments and state-of-the-art facilities. Thus, Facile methods of surface patterning have been studied widely in recent years, including self-organization surface techniques such as Dewetting of thin films[20], Wrinkling of surfaces[21], Micro-Origami[22], etc. Figure 1.1 shows various dimensions and applications of Self-organizing patterns in thin films. There is an immense potential for self-organized thin film microstructures, to be utilized as different kinds of sensors.

Figure 1.1 Schematic representation of the different types of facile fabrication methods.

In such a scenario, self-organization techniques have been seen as the future of thin film devices, where different domains of instrumentation and sensing techniques have been explored with significant ease of fabrication. The optical properties of thin film microstructures and the self-organization of thin films in a variety of conditions have drawn attention for their low-cost high-resolution imaging techniques and sensing methods.[23,24] Such microstructures have found promising potential for the development of various optical sensors at a considerably low cost. Therefore, we strive to look into the possibilities of developing a micro-optical setup for high-resolution imaging and optical sensing. In the next section, we will review the self-organization of thin film microstructures and previous reports depicting their potential for the development of point-of-care testing devices.

1.2 LITERATURE REVIEW

In this section, detailed literature on different aspects of the self-organization micropatterning techniques and microstructures reported in this thesis has been reviewed. The various aspects covered have been schematically shown in figure 1.2.

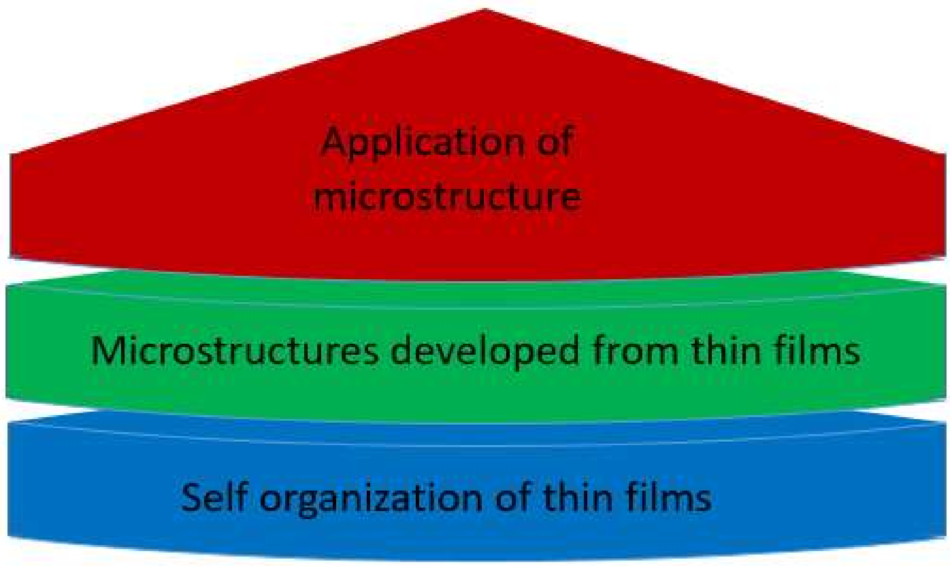

Figure 1.2 Schematic representation of various aspects for which the existing literature review has been given.

1.2.1 Self-organizing patterns

Self-organizing patterns are usually formed due to surface instabilities in the thin film layer or due to the release of stress in the thin films placed over a substrate. There are broadly two types of forces working on a film, namely stabilizing forces and destabilizing forces.[25] These forces can be gravitational, thermocapillary, attractive van der walls, and a host of other factors between substrate film and surrounding.[26-28] By changing the surrounding and surface treatment methods the balance and equilibrium of these forces may result in various micropatterned structures. There are various methods of creating instability or stress in thin films, which will be discussed in the subsequent sections.

1.2.2 Dewetting of thin films

Dewetting is the phenomenon of retraction of a fluid layer when comes in contact with another surface. Instability occurs in the free surface of a thin film and deforms spontaneously, due to the excess molecular interactions.[29-31] Excess molecular interactions (van der walls force for apolar fluids) increase when local film thickness increases.[31] Dewetting is one of the most robust methods of surface patterning, with fair control over the fabricated pattern. A different method of generating thin film instability for dewetting has been reported as thermal, solvent, and surface-modulated dewetting.[32-37] Dewetting has been observed as an undesired phenomenon in paint and stable coating applications. However, better control over the shape and size of the dewetted patterns and their ability to focus light has shown their potential to be used as optical waveguides.[38] Recently demonstrated applications in the fabrication of self-organized microlenses have proved it to be a useful tool in low-cost miniaturized optical applications.[39,40]

It has been demonstrated that thin polymer film gives almost hemispherical droplets after complete dewetting.[41] Certain polymers like polystyrene and PMMA showed the concentration of light and hence magnification.[42] Such droplets provide promising potential in miniaturized optofluidic applications. Since these lenses are having very small focus lengths they have a very large angle of view and hence these microlenses provide a large numerical aperture as numerical aperture (NA) is given as $NA = n\ Sin\ \alpha$ as explained in figure 1.3.

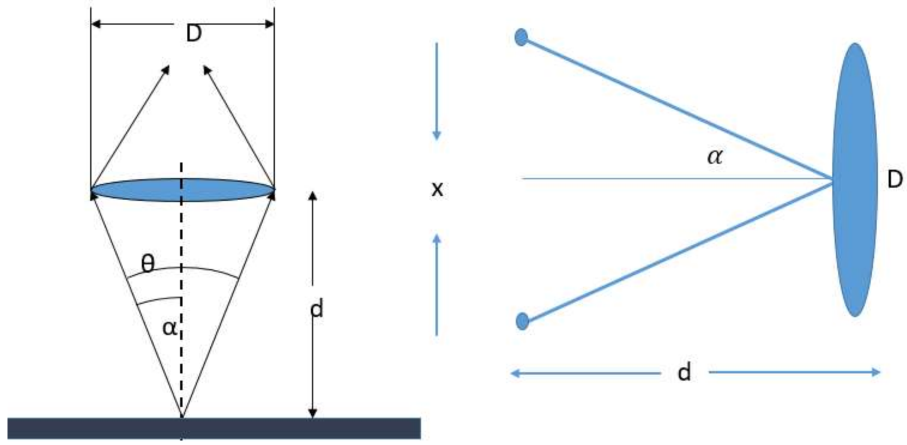

Figure 1.3 Schematic representation of the numerical aperture of a lens. By reducing the distance d, we can increase the numerical aperture using microlenses in a microfluidic setup.

An array or randomly distributed closely spaced microlenses can act as a compound eye, which can cover a large area under observation.[43] Stationary patterns and letters have been demonstrated to be magnified by many of the researchers using microlenses.[44,45] However, observing moving particles of less than 5μm through microlenses is still lagging. Such developments will lead to the detection of Red Blood Cells (RBC), Platelets, bacteria, and other parasites with a very small volume of sample assay.

1.2.3 Wrinkling of Thin Films

Soft materials such as elastomers and polymeric gels can easily undergo large deformation and various morphological stabilities in response to environmental stimuli (e.g., mechanical forces, temperature, humidity, pH value, electric field, and van der Waals interactions).[46-48] The stimulus-sensitive property of soft materials makes them promising candidates for applications as intelligent materials in therapeutics, sensors, microfluidic systems, nanoreactors, and biological scaffolds.[49] Much experimental and theoretical effort has been directed toward exploring the characteristics of surface patterns at the critical state of buckling and the subsequent post-buckling evolution, as well as understanding the underlying physical mechanisms in different types of materials.[50] Morphological instability of a soft material typically exhibits three phenotypes: wrinkling, folding or creasing, and delamination as shown in figure 1.4. Wrinkling refers to periodic or chaotic surface undulations appearing on an originally flat surface. It is often detected during the buckling of thin structures with or without lateral supports.[51] In a two-dimensional (2D) system, for example, a stiff film anchored on a compliant substrate may buckle into sinusoidal waves. Folding refers to a buckling-induced surface structure with localized, deep surface valleys.[52] Folds are often observable, for instance, during the post-buckling evolution of surface wrinkles in a hard layer bonded to a soft substrate or floating on a liquid.

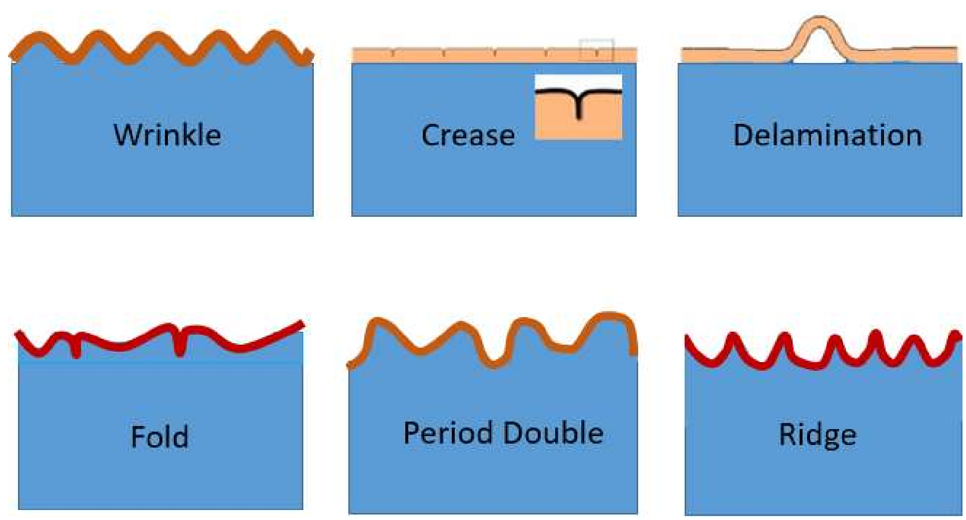

Figure 1.4 Morphological patterns due to instabilities in thin films.[53]

In pursuit of low-cost fabrication techniques, mechanically stimulated surfaces, generating wrinkles have received wide attention among researchers. When stress is increased in a thin film in the form of shear, tension, torsion, or compressive strength, wrinkling occurs to reduce the increased stress. Various regular or irregular wrinkles can be readily observed on elastomer surfaces, which may pose a limit on the performance of materials and is often thought to be a nuisance, that should be avoided. On the other hand, the physics and characteristics of wrinkles have immense potential to create tunable patterns, fabricate functional surfaces, design flexible electronics, and/or measure the mechanical properties of materials.[54] Smart optical surfaces based on wrinkled patterns have attracted attention due to their comparative ease of fabrication and reversible tenability.[55] An important consideration is to align these wrinkles or thin-film microstructures, to get desired properties for targeted applications. Alignment of the thin film instabilities provides ordered microstructures, making them suitable for various applications. Dual-stimuli-responsive wrinkles of polyvinyl alcohol (PVA) on the PDMS surface have been demonstrated for smart windows and rewritable optical displays.[56] However, the tunability with stimulus and alignment of wrinkles remain a challenge for such applications, as they depend upon the topology modulation during the operation. Such problems can be avoided if a gradient topology pattern of wrinkles can be obtained on a single substrate, which provides modulation in light intensity at different points of the substrate. To obtain aligned wrinkles for advanced applications, fracture-induced alignment of wrinkles and circular torsion and mechanical methods have been reported.[57,58] Among various methods to create a wrinkled pattern on a substrate, mechanically stimulated surface patterning methods have proved to be more robust.

Thin layered materials develop surface undulations or wrinkles when they experience small compressive strain. This response is the result of a complex interplay between the deformation of the top layer and its foundation. Indeed, wrinkles release in-plane compression of the film, as bending is energetically less costly than compression, and this is accompanied by bulk deformation of the foundation material. The prediction of the wavelength and the distribution of elastic energy of the wrinkles is therefore non-trivial and depends on the mechanical properties, both of the film and the viscoelastic substrate, as well as on the geometric features of the stress field. However, on further compression, the wrinkles become unstable and new morphological phases emerge depending on the elastic nature of the foundation.[59]

A significant effort has been put to understand the stress-induced wrinkling phenomenon and its application in smart optical surfaces.[60] The capability of wrinkles to interact with the light wave and their intensity modulation, make them a suitable candidate for the smart window, advanced radar, and other applications. In this thesis, we will apply existing knowledge to create wrinkles on a gradient compressive stressed substrate for a novel application of photomicrography.

1.2.4 Self-organized Thermal Remoulding of Polymer Pellets

Thermal remolding of polymer microstructures has been utilized for creating smooth surfaces. Specially, when the size of the polymer droplet is less than the characteristic length of the molten polymer, then interestingly semispherical structures are obtained.[61] The structure of such semispheres can be controlled using the molecular weight of the polymer, temperature, and size of the pellet. This method of polymer reorganization has been utilized to develop self-healing processable structures.[62,63] Interestingly the polymer pellets of large molecular weight have inherent free volume space occupied between the molecular network.[64] This free space decreases the transparency of the polymer films and structures. If this free space of the polymer can be tuned in a certain process, it can be used for optical lensing applications and can be used with a simple camera to enhance its magnification and resolution.[65]

Already available literature depicts the use of polymer macro lenses in photomicrography, where micron size object was imaged through simple photography devices such as cellphone cameras.[66] For many years, the biological and medical sciences have relied heavily on microscopy to observe and analyze morphological features of specimens and quantitative tools for recording specific optical features. Cellphone-based photomicrography has enjoyed a recent escalation in popularity, due to the introduction of new transparency films and other low-cost accessories such as lenses and optical diffusers.[67,68] In this respect, transmitted light microscopy has proven useful in countless investigations into the mysteries of life. More recently, when microscopy has been employed in the analysis of biological assays and lab-on-chip instruments, cellphone-based photomicrography has placed unprecedented opportunities to record and analyze optical data. The potential of cell phones, being used as a tool for micrography and analysis of images, has developed a significant interest in rapid and low-cost imaging techniques.

Many diseases like Tuberculosis, cell disease, malaria, etc depends on microscopy for their diagnosis. Such, diseases cause more causalities in resource-limited places, where diagnostic health infrastructure is not available suitably. In the last few years, the user base of smart cellphones with better camera and analytical application tools have increased exponentially. These cell phone cameras are the most potential tool for micrography and analysis of biological assays. Various attempts have been made to increase the resolution capacity of the cellphone camera using attachments and transmission mode microscopy arrangements.

One of the important issues in cellphone-based microscopy is the illumination of the sample. Usually, Transmission light microscopy is used in cellphone-based microscopy.[69] Transmitted light microscopy is a mature discipline in which a wide spectrum of illumination techniques are available for contrast enhancement. However, the external source of illumination utilized in cellphone-based microscopy makes it bulky, and difficult to handle in a relocating and remote setup. In contrast, reflected light microscopy just emerging as an important tool for investigating surface features in the materials sciences.[69] However, a cellphone backlight can be used as an illumination source for samples imaged by a cellphone in reflection mode microscopy. A schematic diagram representing the two imaging modes is shown in figure 1.5.

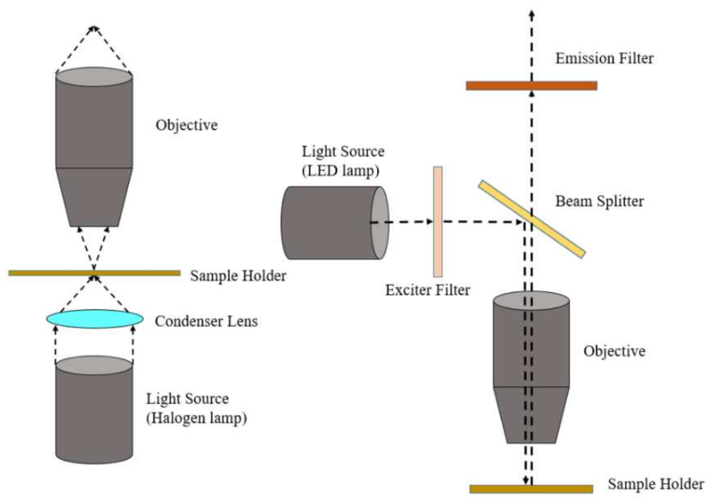

Figure 1.5 Two different modes of imaging in microscopy.

1.2.5 Optical spectroscopic techniques in a microfluidic setup

In the addition to the microfabrication of patterns using self-organization of thin films, Microfluidic technology has seen a vehemence surge in the application of point-of-care detection techniques. Optical spectroscopic techniques such as SERS platforms have been developed several years ago, however, their incorporation in the microfluidic setup has been done a few years ago and showed extraordinary results.[70,71] The integration of SERS substrates in a microfluidic setup has been done by directly impregnating the substrate or by developing the SERS substrate in the microfluidic channel.[72,73] A third approach has been explored as the continuous flow of analyte and plasmonic nanoparticles and mixing them to generate SERS enhancement.[74,75] However, due to the low dimension of microfluidic substrates, Reynold's number of flowing streams become very less, and mixing of two streams become very difficult. For the mixing of two streams in a microfluidic channel, active and passive mixers are applied.[76-78] Active mixers are external modulations through mechanical acoustic or electrical stimulation. Whereas, passive micromixers are inbuilt structures in the microchannel that enhances mixing. Active micromixers may provide better mixing, but are not suitable for point-of-care transportable devices. A microfluidic device with passive micromixers giving enhanced mixing will bring analyte and plasmonic nanoparticles in close proximity. It is evident that analyte and plasmonic nanoparticles in such microfluidic devices can generate SERS enhancement in a controlled and reproducible manner, without any external modulation of flow streams. Such devices can be used as microfluidic SERS devices, without any extensive and rigorous fabrication process.

1.3 RESEARCH GAP AND MOTIVATION

Based on the above discussion, it was found that self-organized microlenses have the potential to be used in high-resolution imaging. The high-resolution imaging in a microfluidic setup can bring enormous opportunities for the development of a point-of-care testing device. A merger of microfluidics and optics (optofluidic devices) can bring a solution for the estimation of suspended particles of cellular size. However, there are rarely any reports available of an optofluidic device giving high-resolution imaging of moving particles. Thus, a complete optofluidic device setup, for the estimation of suspended particles in a solution is needed. Also, a microfluidic device, giving enhanced mixing using passive micromixers, with a robust operation method is still needed. Such a device will be used to bring analyte and plasmonic nanoparticles in close vicinity to generate SERS enhancement. The research gap that has been identified during the literature review has been schematically shown in Figure 1.6. Mainly four areas have been identified: -

- Microlens Imaging in the Microfluidic Channel: - There have been many methods reported in the literature for microlens fabrication and imaging through them. However, the images obtained through such microlenses are still patterns and superimposed images. A microfluidic setup embedded with microlenses to observe moving particles can be used for the estimation of bio-cells with a suitable image processing technique.
- Development of Low-Cost Polymer Macro lenses: - Increasing the userbase of smartphones with better imaging capabilities has posed a promising opportunity for the optical detection of micron-sized subjects using handheld cellphones. If such setups can obviate the use of the standard microscope, it will be a breakthrough in point-of-care testing using optical sensing methods.
- Wrinkled Pattern for Diffused Illumination: - A gradient wrinkled pattern giving diffused light of different intensities at different points will help in sample illumination. Such substrate can be helpful in photomicrography setups.
- Continuous Flow Microfluidic SERS device: - Comparatively independent of the previous three problems, a micropatterned microfluidic substrate that brings analyte and plasmonic nanoparticles into proximity is likely to be a promising candidate for point-of-care testing using handheld Raman spectrophotometer. A microfluidic device with passive micromixers to be effective for SERS enhancement has to be developed.

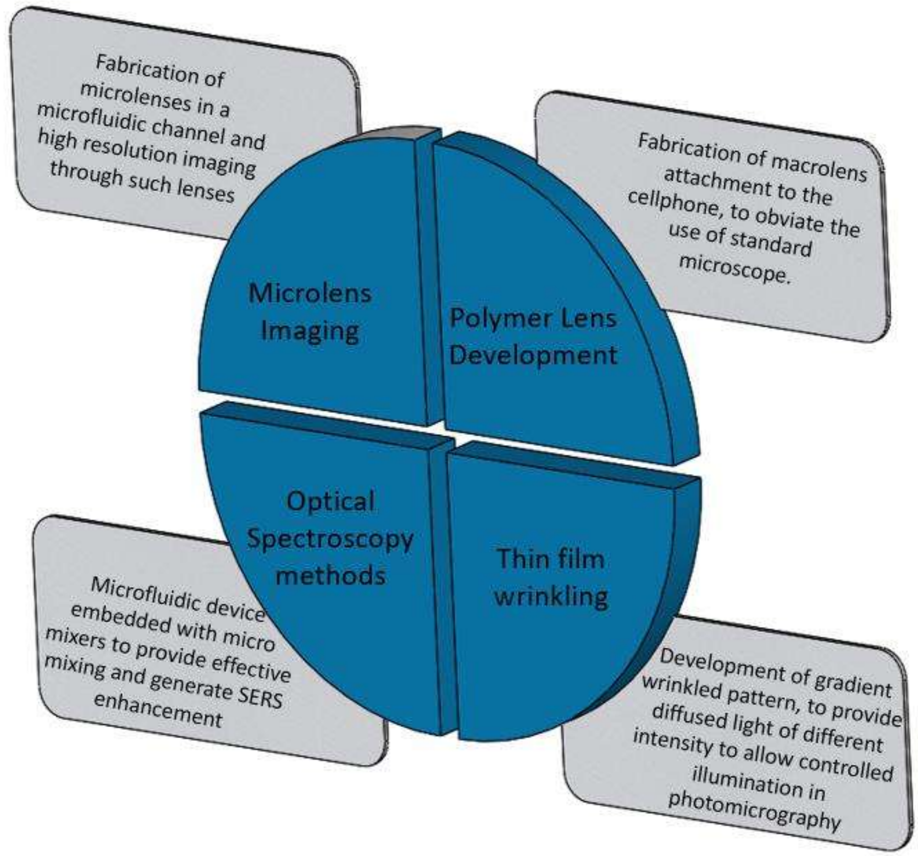

Figure 1.6 Schematic representation of different sections identified during literature review as research gap to be tackled.

Motivated by the gaps in the changing paradigm of the healthcare ecosystem and point-of-care testing systems, in this thesis, attempts have been made to advance/develop methods and techniques in the field of microlens imaging techniques and estimation of cells in a given sample. An approach to developing a complete device ready to be used in the on-field locations is adopted. Also, a microfluidic device utilizing the optical spectroscopy technique was developed, to detect biomarkers without additional sample processing in a reproducible and repeatable manner.

1.4 THESIS ORGANIZATION

The thesis has been segregated into six different chapters, starting with a brief introduction, literature review, and research gap in the first chapter, the subsequent chapters to follow primarily discuss: -

- **Chapter 2: -** Microlens Fabrication and Imaging Methodology, and estimation of the number of cells in a given sample.
- **Chapter 3: -** Development of polymer macro lens to be used as lens attachment, and demonstration of magnification and enhanced resolution of the cellphone camera.
- **Chapter 4: -** development of gradient wrinkled pattern for generation of different intensities of diffused light from a single substrate.
- **Chapter 5: -** Development of micropatterned microfluidic substrate for SERS enhancement without additional sample processing.

Finally, the last chapter concludes the work along with a brief overview for further research and future scope.

1.5 CONCLUSION

This chapter introduces the problem along with an in-depth literature review of various aspects touched through the work reported in this thesis, followed by the research gap and motivation. At the end of this chapter, a brief sequential organization of the various topics covered has been delineated. The next chapter would be discussing self-organization dewetting, microlens formation, and imaging in a microfluidic setup. The development made in the next chapter is the basis for the subsequent two chapters.

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