Laying Technological Groundwork for Templated Assembly by Selective Removal (TASR) at Biological Length Scales

by

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Abstract

This work presents the size-selective sorting of biological cells using the assembly process known as Templated Assembly by Selective Removal (TASR). This research has demonstrated experimentally, for the first time, the selective self-assembly of mammalian as well as non-mammalian single cells into patterned hemispherical sites on rigid assembly templates using TASR. TASR-based size-selective assembly of biological systems represents a potentially valuable tool for biological applications from cell sorting and isolation of single cells to templating for artificial tissue generation. Not only is the assembly itself demonstrated to work effectively, but it is also demonstrated that this assembly technique can be made low cost and manufacturable by the use of polymer templates replicated from rigid master templates. Furthermore, the assembly process has been proven to be successful with both isotropic (spherical) and anisotropic (non-spherical) assembly components, which enables it to be applied to a wide range of biological specimens. Finally, an integrated theoretical model framework is also presented, which combines all of the key factors that play a role in determining assembly efficiency to produce guidelines for the design of the assembly system, so as to meet the constraints for successful assembly.

In TASR, the system’s free energy is minimized when components assemble in holes in the template surface that match the components’ shapes and sizes. A combination of chemical and mechanical effects selectively removes all poorly matched components. Clonal isolate (SF9) cells derived from the \textit{Spodoptera frugiperda} (Fall Armyworm) IPLB-SF21-AE cells (with a mean diameter of 15 microns) and mouse-derived non-small lung cancer cells (with a mean diameter of 20 microns) were successfully assembled using TASR onto patterned silicon templates in separate sets of experiments. The assembly sites comprised holes with hemispherical profiles etched in a silicon substrate using plasma etching to match the nearly spherical shapes of the cells used. A high assembly yield of a 100% and high cell density were observed for cell placement into sites with uniform diameters for both the cell lines. In addition, size selectivity was established for assembly into sets of heterogeneously patterned hemispherical sites with different diameters. High cell viability of nearly 99% was recorded using multiple testing procedures, confirming predictions from theory that the assembly environment is favorable for the growth and survival of both mammalian as well as non-mammalian cells. These results confirmed model predictions that assembly would be successful with the materials studied here under the experimentally-used assembly parameters. The full set of models and experiments addressed the effects of compliance of the assembly components and templates, components and assembly sites shapes, and the chemical and mechanical properties of the assembly environment. Together, the results of these experiments and models demonstrate that TASR is indeed a very versatile tool for structuring biological systems.

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Figure 7.1: Optical micrographs of portions of the assembly template before assembly with TASR, showing two sets of anisotropic assembly components, one in (A) – (B) matching and the other in (C) – (D) not-matching in shape to the assembly sites, lying on the template. Both components have a starting diameter of 26 μm. While the components in (A) – (B) have straight sidewalls and have a length of 50 μm, those in (C) – (D) have tapered sidewalls and have a length of 150 μm. The SFL technique used for manufacturing these components faces limitations in fabricating components with straight sidewalls for components beyond a length of 100 μm, hence the taper for components with larger lengths is observed. Components with different shapes as shown here are used to assess the shape selectivity of the TASR technique.

Figure 7.2: Optical micrographs showing portions of assembly template after assembly of cylindrical components with TASR. Only the components matching in shape and size to the assembly site are retained in the sites out of the two sets of components used. Most of the components are observed to occupy the edges of
the sites due to maximization of contact area with the site at the edges. It is to be noted that since the matched components are shorter in length than the assembly sites, only diameter matching is demonstrated here. Length matching is not demonstrated due to limitations of the component fabrication technique.
Nomenclature

- $R_c$ — Radius of assembly component
- $R_t$ — Radius of assembly site on template
- $R_{eq}$ — Equivalent radius of curvature of system
- $E_c$ — Young’s modulus of component material
- $E_t$ — Young’s modulus of template material
- $E'$ — Equivalent Young’s modulus of system
- $v_c$ — Poisson’s ratio of component material
- $v_t$ — Poisson’s ratio of template material
- $Y_c$ — Yield strength of component material
- $Y_t$ — Yield strength of template material
- $P$ — Contact load
- $A$ — Contact area
- $a$ — Radius of circular contact region
- $C_r$ — Roughness of assembling surfaces
- $\gamma$ — Interfacial energy for assembly fluid and self-assembled monolayer
- $\rho$ — Density of assembly fluid medium
- $\mu$ — Viscosity of assembly fluid medium
- $E$ — Young’s modulus of assembly fluid medium
- $v$ — Poisson’s ratio of assembly fluid medium
- $U$ — Flow velocity
- $I$ — Intensity of acoustic excitation
- $f$ — Frequency of acoustic excitation
- $d$ — Adhesive distance
- $\omega$ — Interference value
- $\omega_c$ — Critical interference value
- $\gamma'$ — Deformation parameter for assessing substrate deformability
- $K$ — Hardness coefficient
1.1 Self-assembly at intermediate length scales – Concept and Methods

The term ‘self-assembly’ is used to describe processes in which a disordered system of pre-existing components transform into an organized structure without external direction. As mentioned by [1], self-assembling processes encompass multiple disciplines and occur across a range of scales. Self assembly techniques offer a powerful tool set for combining small scale building blocks into more complex systems across a broad range of length scales and under the guidance of a broad range of driving forces, such as in [1-3] among others. While a variety of self assembly techniques have been devised at several different scales, starting from the molecular scale where chemistry and biology assist the self-assembly process, to the meso-scale (from 100 microns-millimeters) where several conventional mechanical forces have been used to assemble components into diverse architectures, significant challenges still remain for assembly in the intermediate scale. Self assembly at the intermediate scale becomes much more challenging because it lacks the enabling advantages enjoyed at the molecular and millimeter scales. At the molecular scale, chemistry is a powerful tool to guide self-assembly. At the millimeter scale, conventional microfabrication techniques excel at creating well-defined architectures with a precise level of control that can promote precise assembly. Control of assembly is more difficult at the intermediate scale, where chemistry alone is not dominant and the level of control over
geometry is reduced. However, even though there are challenges to be faced in achieving precise, well-controlled self-assembly at intermediate length scales, there is a strong motivation to structure systems at these length scales since these are very well matched to length scales for numerous important applications involving optical systems and biological-based systems, amongst other fields.

Past research has successfully demonstrated a range of intermediate-scale assembly capabilities that employ a diverse set of assembly mechanisms. Some common elements that have contributed to these assembly processes include forces to promote adhesion such as chemical forces, electrostatic forces, and capillary forces; another common element is the geometric patterning of the surface on which assembly is to occur in order to guide the final assembled geometry. To some extent, scaling can favor assembly at the intermediate scale; many of the forces that drive assembly processes are relatively weak at the macroscopic scale, but become significant at the intermediate scale of interest. In one example, Aizenberg et al. [4] demonstrated the assembly of charged colloids onto patterned templates with the help of electrostatic and capillary forces. In that work, electrostatic forces introduced at binding sites were used to drive selective assembly of microspheres in a liquid, while capillary forces were used to precisely position the microspheres at the centers of the binding sites during drying of the liquid. In other work, Xia et al. [5] and Cui et al. [6] used capillary forces to drag and pack microspheres into holes predefined on the surface of a template, controlling the number of microspheres packed in each hole by the relative sizes of the microspheres and the holes. Chen et al. [7] demonstrated the assembly of colloids onto patterned templates, using electrostatic and secondary interactions to drive selective organization of microspheres onto regions of a substrate which were patterned with polyelectrolyte multilayer films. Using fluidic and gravitational forces in concert with mechanical selectivity to achieve self-assembly, Smith et al. [8] demonstrated the assembly of light emitting diodes into a
silicon substrate. More recently, self-assembly of objects has also been achieved by tethering them to DNA and then folding these long, single-stranded DNA molecules into DNA ‘origami’ [9] structures with the help of short oligonucleotides. DNA-mediated self-assembly is used to control the positioning of a variety of objects, such as cells, viruses, nanotubes [10], short DNA molecules [11], proteins [12] and gold nanoparticles [13, 14].

Selective self-assembly is a subset of these processes in which a heterogeneous set of structures is correctly sorted into a corresponding set of heterogeneous assembly sites. The present work addresses the theoretical modeling and experimental assessment of one type of selective assembly: Templated Assembly by Selective Removal (TASR) ([15-18]). Some selective assembly techniques obtain their selectivity by the presence or absence of various interactions, such as those mediated by selective chemical binding as in [19]. In contrast, TASR is one of a second set of approaches in which the selectivity is enabled by quantified, controllable variations in the strength of a given component-substrate interaction, such as in [20] and [3]. In essence, this second group demonstrates “analog” selectivity, as compared with the essentially “digital” selectivity of on/off interactions. The analog approach requires quantitative models and system engineering, but it also offers the potential to achieve fine degrees of selectivity within a single assembly process.

TASR relies on a fundamentally shape and size selective mechanism for assembling components at the micro and nano scales. TASR’s capabilities augment the types of functionality previously reported in the literature. For example, references [5, 6], and [21] demonstrated assembly of colloids into holes such that the results depend in part on the relationship between the size of the holes and size of the components. TASR offers an additional degree of selectivity, in that both components that are too large for the holes and components that are too small for the holes are excluded. It enables simultaneous assembly of diverse components into complex and precise systems with potential applications including optically-based chemical and biological
sensors, optical meta-materials, and the creation of systems of biological cells. Because TASR may be used as either a permanent or a reversible assembly process, it has potential not only for construction of final systems but also for applications such as shape and size selective chromatography of synthesis products or biological species.

A schematic of TASR is shown in Figure 1.1. In TASR, the system’s free energy is minimized when objects (micro components, cells, etc.) assemble in holes in the template surface that match the objects’ dimensions. A combination of chemical and mechanical effects selectively removes all poorly-matched objects from the holes. The assembly selectivity (that is, whether adhesive or removal effects are dominant) depends in part on the degree to which the component to be assembled matches the shape and dimensions of the surface topography at that location. The

![Figure 1.1: Schematic illustration of the experimental set-up for TASR, including the assembly fluid and the 1.7 MHz ultrasonic transducer that applies mechanical forces for the selective removal system. A magnified view of the assembly template immersed in the fluid medium illustrates that only the components that match the assembly sites accurately in size are retained, while the ones that do not match the assembly sites are removed by ultrasonic fluid forces.](image-url)
surface topography is designed such that holes at various locations in the substrate surface match the shapes of the components that are intended to assemble there.
1.2 Application to biological systems

Self-assembly also provides for an important tool for structuring biological systems, since selective assembly at the intermediate scales is well matched to the biological length scales. Precise placement of cells and other biological specimens is enabling or even crucial for biological applications from tissue generation to the study of single cell behavior. To be able to perform biological assays for studying the intra-cellular workings of a single cell successfully in micro TAS systems and other microfluidic platforms, techniques for the controlled trapping of cells in a flow are needed. Such techniques can enable the execution of a given set of chemical, biological and/or microfluidic unit operations at the single-cell level in a controlled microenvironment. A high density arrangement of the trapped specimens is desirable in such systems to increase the number of tests that can be carried out. An effective self-assembly technique such as TASR that offers precision and selectivity simultaneously can help achieve exactly that. Some of the conventional techniques used for cell manipulation are discussed below first, followed by the advantages offered by TASR over these techniques for achieving the same target.

1.2.1 Conventional cell manipulation techniques

Some of the known methods in the literature for trapping or positioning of cells are based on techniques that use optical tweezers [22], dielectrophoretic trapping [23], or electrophoretic microwells [24], surface chemistry [25], mechanical trapping [26], microfluidic trapping [27] and methods utilizing ultrasonic fields [28-30]. These techniques have certain drawbacks, such as a complicated set up (e.g. optical-based methods), limited selectivity (e.g. microfluidic and mechanical trapping techniques), or risk of cell damage (e.g. dielectrophoresis or electrophoretic microwells).

Among other methods, ultrasonic separators have been widely used to study the separation
of microorganisms and animal cells [31-33] in an effort to overcome these drawbacks. More recently, “acoustic tweezers” that utilize standing surface acoustic waves have also been used to manipulate and pattern cells and microparticles, as demonstrated in [34]. The frequency used in most of these separators is between 1 and 3 MHz, as frequencies below this range more readily cause fully-developed cavitation, which can cause cell disruption. The existing methods based on ultrasonic separation have established that live cells are able to handle the mechanical and thermal stresses that are generated in such systems due to the standing waves with no recorded change in cell behavior [35, 36]. Such systems are able to separate out single cells from suspension and trap them in a flow. However, none of the previous work in this field has yet demonstrated high density, size-selective placement combined with rapid, highly-parallel operation for the sorting of cells and other biological species using ultrasonic excitation.

1.2.2 TASR for Biological Systems

TASR offers such a self-assembly based technique for manipulating many single cells and other biological specimens in parallel. TASR can trap, position, and sort cells based on size selection with the aid of ultrasonic fluid removal forces that displace incorrectly-sorted cells. The chip-based platform for cell capture presented in the current work combines several features that are not simultaneously possible with existing cell capture technologies, such as size recognition, the precise placement of a single cell inside a matching assembly site, high density, flexible trapping, parallelization, short capture times, reproducible results from experiment to experiment, high sensitivity, and ease of integration with further analysis steps. Because of its rapid, highly-parallel operation and its high selectivity, TASR offers a unique and valuable tool for several important biological applications.

Size-selective cell placement and sorting using TASR can have potential applications in
widespread areas of medical research and diagnostics, such as biological sensing, sorting of tumor cells from healthy cells, distinguishing between different varieties of leukocytes, characterizing cell size just before cell division, cellular stimulation and lysis to extract intracellular proteins for downstream analysis, capturing intracellular signalling dynamics of protein pathways and so on. Tumor cells may be distinguished from healthy cells by differences in their size, structure, function, and growth rate, and certain types of tumor cells will be more readily separated using the TASR process. The identification and sorting of large tumor cells (e.g. soft part sarcoma) using the TASR technique may potentially be easier, as these cells are much larger than normal, easily identifiable cells, such as mesothelial cells. Similarly, tumor cells that are much smaller than normal mesothelial cells (such as some breast cancers and small cell lymphomas) may also be isolated using TASR-based size-selectivity. The isolation of medium-sized tumor cells (e.g. many carcinomas) is the most difficult to accomplish based on sorting by size alone. In such cases, the demonstrated size-selectivity of the TASR process may be combined with shape-selective sorting since these medium-sized tumor cells, having a rounded or spherical appearance in most cases, may differ markedly in shape from normal healthy cells. Since TASR-based sorting and structuring as demonstrated here relies on size selectivity, and changes in cell size mark important changes in the life cycle of a cell as well as changes in the motility of the cell, TASR may also be a valuable tool in assessing the relation between the cell size and changes in cell physiology, and for synchronizing cells based on the cell size. These types of manipulations may be done using chip-scale sorting of macroscale cell populations, as is demonstrated in the present work, or by integrating the sorting functionality into an LOC platform with a smaller population of cells. For example, the cells or other biological species may be contained in the chambers of a microfluidic device that also includes the desired on-chip analytical functionalities. An external ultrasonic transducer may be used to apply excitations to the chip and sort the cells or other species. After
the initial sorting, the chip may be removed from the excitation, and the sorted cells may be assessed using the on-chip analytical functionalities. This approach offers a cost advantage because the ultrasonic actuators are isolated from contamination and may therefore be used repeatedly.
1.3 Previous work on TASR

The TASR approach has been demonstrated experimentally and modeled theoretically for the self-assembly of rigid materials such as silica on silicon template surfaces in previous work [15, 16]. The experiments and model have also been extended to encompass deformable systems, and TASR has been proven to be an effective technique for the self-assembly of polymers such as polystyrene on patterned silicon substrates in previous work [17, 37]. Some details from previous work are discussed below briefly.

Jung et al. [15] did a proof-of-concept demonstration of TASR and established for the first time that the technique can work effectively; in particular, this work demonstrated TASR’s effectiveness for assembly at the micron scale. The experimental concept was extended and developed further by Eid et al. [16], who extended the idea of selective self-assembly to simultaneous and selective self-assembly of different sized micro and nano components onto specific locations on an assembly template. A theoretical model for rigid system assembly using TASR was first proposed by Jung et al. in [15], initially verified in [15], and refined and verified in [16]. The foundations of this model were built on the basic concept of TASR which is stated as follows. In order to achieve self-assembly, components which are to be assembled, as well as the template onto which they are to be assembled, are placed in a fluid environment. The use of fluid media is a natural choice, since many micro-components are manufactured and kept in the form of dispersions in fluid media. In addition, the interaction between the components and substrate in the fluid medium may be tailored to promote the assembly process by changing the fluid medium. There are several means by which the surfaces of interest can be made to adhere to each other in a medium such as oil, for instance. By creation of hydrophilic surfaces on both the component to be assembled as well as the template onto which it is assembled, the two surfaces can be made to adhere to each other in the oily, non-polar medium. Another way of driving adhesion between mating surfaces for the purpose of self-assembly can be by the creation of hydrophobic
surfaces in a polar fluid medium such as water. This concept of creating hydrophobic surfaces is used in many of the demonstrations of TASR in order to achieve selective self-assembly by shape and size matching. Although other forms of component-substrate attraction exist (such as gravitational, electrostatic, or magnetic interactions), hydrophobic interactions are well-suited for use with the TASR mechanism because it is a surface force, so that the strength of the component-substrate interaction scales linearly with the area of contact between the component and the substrate, as discussed below.

In this implementation of TASR, hydrophobic surfaces adhere in water-based fluid media, and the strength of these adhesive forces between surfaces is dependent on the contact area and hence the degree of shape matching between the component and the substrate. At the same time, the assembly fluid is excited by an ultrasonic excitation at a megahertz-range frequency in order to drive TASR’s selective removal process and also to ensure the circulation of components, both of which are discussed in greater detail below. The mechanical forces that are applied to the components as a result of this ultrasonic excitation act so as to remove the components from the surface, though they are not always strong enough to fully remove the components. While the adhesive retention forces dominate in sites where the shape of the component is well-matched with the topography on the template (so that the component stays in the site), the mechanical removal forces emerge stronger in poorly matched sites (so that the component is removed from the site). The mechanism by which these poorly-matched components are removed from the sites is best described as roll-off, in which the poorly matched

![Figure 1.2: Schematic illustration of removal of components from incorrectly matched sites on the template surface by roll-off (Figure adapted from [15])](image-url)
components are rolled out of the assembly sites [15]. The net result is that holes that are well-matched to a type of component that is present in the fluid medium are filled with those components, whereas holes that are poorly-matched to the components remain empty at the end of the TASR process.

Prior modeling [15, 16], determined that rigid assembly components are rolled out of poorly-matched assembly sites by a combination of fluid forces. This removal occurred when the mechanical moments that promote removal that are generated as a result of these removal forces exceeded the mechanical moments that promote component retention that are generated as a result of the retention forces due to adhesion.

It was then concluded that the variation in assembly yield (quantified in terms of the number of holes on the patterned template surface that were filled by the assembly process out of the total number of holes of that size on the template) was related to the progressive increase/decrease in the ratio of the mechanical moments that promoted component retention to the mechanical moments that promoted component removal. Yield approached 0% when the removal moment due to fluid forces exceeded retention moment due to adhesion forces and approached 100% when retention moments exceeded removal moments. Further details of the concept of TASR as well as the theoretical model for TASR proposed and verified in previous work [15-17] are described at length in Chapter 2, Section 2.1.1.

Assembly using TASR was then extended to the assembly of more deformable materials such as polymers. The successful, TASR-based, shape- and size-selective assembly of deformable micro-components on rigid substrates was demonstrated for the first time in [17, 37]. Taking into account the possibility of change in shapes and increased contact area between the component and the substrate as a result of deformation, a theoretical model [17] based on the Hertzian Contact Theory was created to predict the conditions under which TASR-based assembly of deformable components on a rigid substrate will be successful. This model, which complemented the original TASR model [15, 16], was used to identify the mechanical nature of contact at the interface of the assembly component and the
template substrate. More details on the model are described in Section 2.1.2 of this thesis. The success of this model was then demonstrated by comparison of the model predictions with experimental results for a component material (polystyrene) for which the material properties lie near the boundary of how much deformability can be tolerated by the TASR process.

These experimental results were obtained by examining the assembly of polystyrene microspheres on a patterned silica template. Rigid silica components were also assembled under identical conditions in order to compare the results for the assembly of rigid and deformable materials. Patterned silica templates were created which showed excellent conformation to the ideal hemispherical profiles desired for assembly of microspheres into the matched sites. The results for assembly of deformable polystyrene microspheres on these patterned substrates exhibited both a good degree of assembly selectivity of given deformable components into different-sized holes, and very high assembly yields of up to 100% for deformable components in well-matched holes. These results showed agreement with the predictions of the theoretical model for assembly of deformable materials using TASR.

The experimental results were also examined in the context of the original TASR theoretical model [15, 16]. It was found that the original model can be extended to describe the behavior of deformable materials under many (though not all) experimental circumstances despite the variation in contact area which occurs as result of the deformation.

The final model created in previous work to describe assembly in TASR is used to predict the circumstances under which initial placement of components into assembly sites will take place in the TASR system. This theory was proposed by Jung et al., and complete details of the theoretical framework are described in [18]. The theory describes the conditions for ensuring component circulation inside the assembly fluid medium and hence the initial component assembly in TASR. It states that mean fluid flows which drive ‘in-plane’ motion of assembly components at the solid-fluid
interface in TASR give rise to a drag force on the component and make them enter the holes; more
details are described in Section 2.1.3. This is the primary method for the components entering the holes
rather than a vertical descent driven by gravity, for instance.

Together, these models comprise a strengthened theoretical foundation that was used to
successfully demonstrate and analyze experimentally the selective assembly using TASR in rigid as
well as deformable systems. As these models are also highly relevant for the present research, each of
these theoretical frameworks is addressed and described individually in detail in Chapter 2.

Based on this theoretical and experimental framework demonstrated in previous work, this
research seeks to extend the applicability of TASR to a broader range of materials, geometries,
sizes and applications, encompassing biological systems. The motivation to extend TASR to
biological systems has been highlighted already in Section 1.2, where several advantages offered
by TASR over conventional techniques used at these length scales are identified. The main goals
of the current work and key guidelines to be implemented in the TASR process are presented in
the following sections.
1.4 Goals of thesis

This work seeks to provide a platform to structure and self-assemble biological systems using TASR. All of the previous work on self-assembly discussed previously in Section 1.1 and 1.2 have successfully demonstrated different subsets of the attributes required for practical implementation of assembly techniques, such as precision, selectivity, scalability, speed, materials compatibility, etc. To achieve the overall goals of this thesis and create a new capability for selective assembly and sorting of biological systems using TASR requires that a number of subsidiary goals also be met. To this end, the current work seeks to use modeling and experiments to demonstrate how the above requirements can be met simultaneously, and to extend TASR into new materials systems and new geometries. Some specific goals of the present work are listed below.

The first goal is to demonstrate the effectiveness of the TASR approach for size-selectively, simultaneously sorting and assembling biological cells. In particular, the goals of this research include the selective assembly of mammalian and non-mammalian single cells into patterned hemispherical sites on rigid assembly templates (master templates). As highlighted previously in Section 1.2.2, TASR-based size-selective assembly of biological systems represents a potentially valuable tool for biological applications ranging from cell sorting and isolation of single cells to templating for artificial tissue generation. The second goal of this research is to demonstrate that the TASR assembly technique may be made low cost and manufacturable by the use of techniques that allow replication of rigid master templates in low-cost materials. The third goal of this research is to create and demonstrate the capability to assemble components with non-spherical, anisotropic geometries to complement the existing ability to work with spherical, isotropic components. The final goal is to create an integrated theoretical model framework. This framework will combine all of the key factors that play a role in determining assembly efficiency to create guidelines for the design of the assembly system, so as to meet the constraints for successful assembly.
1.5 Thesis outline

The thesis is divided into eight chapters. Chapter 1 provides an introduction to the basic concept and purpose of Templated Assembly by Selective Removal. Until this point, the main principle of TASR has been introduced, wherein competition between adhesive retention forces and mechanical removal forces drives the selectivity of the self assembly. A review of the techniques that have been used in the past for self assembly has been presented, and it has been shown that there is much scope for improvement in the existing techniques to enable higher selectivity and enhanced capabilities, particularly at biological length scales. Previous experimental and theoretical work done using TASR for assembly of both rigid as well as deformable components on rigid patterned templates has also been briefly described [15-17], which serves as the starting point for the extension of this work to biological domains. The need for an integrated theoretical TASR model based on the previous partial models is introduced; this integrated model should address the issues of designing the assembling system and choosing optimal assembly parameters so as to achieve successful assembly, whilst allowing the flexibility to work with a wide range of materials. The assembly system recommended by the integrated model should satisfy all the constraints imposed by the previous theoretical propositions. The main goals of the thesis are then described.

Chapter 2 introduces the integrated theoretical framework for designing and modeling of any TASR system. The previous TASR models for characterizing selective removal, assessing mechanical deformations, and modeling initial component assembly in TASR for the self-assembly of both rigid and deformable structures are first reviewed. Then, based on the possible cases for assembly, as dictated by the mechanical properties of the assembling system, parameter maps for TASR are created in which favorable regions of operation for assembly are identified, given system constraints and properties. Relevant to the current experimental thrust, the case of assembly of biological cells on rigid
substrates is also analyzed, and model results are tabulated. Two biological cell lines of interest for experimental purposes are then identified based on model predictions and system constraints. Model predictions for assembly on deformable templates are also identified to be of particular interest in order to enable the use of low-cost, manufacturable, bio-compatible deformable substrates for biologically-based applications. All of this theory is presented for modeling the assembly of isotropic, spherical-shaped assembly components. A separate mathematical model for the assembly of anisotropic components, (in particular, cylindrical components) is also proposed to provide a framework for testing the effectiveness of TASR with shapes other than spheres.

Chapter 3 lays out the procedures to be followed for template fabrication, which is the first step in experimental demonstration of TASR. Assembly templates must be patterned to demonstrate shape and size selective assembly of components inside them. The fabrication process of the silicon-based templates for self-assembly of all cases of interest here is then described step-by-step, including rigid templates for trapping nearly spherical biological cells, rigid templates for capturing components with anisotropic shapes and finally, the rigid templates which serve as master templates for creating replica templates in more deformable polymer materials.

Chapter 4 describes the experimental set-up used in TASR and provides insights into the parameters that need to be tuned in order to achieve the highest possible efficiency in the assembly process. Template and component preparation techniques and the general experimental protocol for assembly are also discussed here. Specific details of assembly particular to each case are discussed separately in their corresponding chapters.

Chapter 5 describes the experimental work carried out in order to demonstrate and characterize the self-assembly of biological cells on patterned silicon substrates. Clonal isolate (SF9) cells derived from the Spodoptera frugiperda (Fall Armyworm) IPLB-SF21-AE cells (with a mean diameter of 15 microns) and mouse-derived non-small lung cancer cells (with a mean diameter of 20 microns) were
successfully assembled using TASR onto patterned silicon templates in separate sets of experiments. Results for the size-selective assembly of SF9 cells into 12, 15 and 22 micron diameter sites on patterned silicon substrates are first presented and show high assembly yield, selectivity and density. The dependence of assembly results on assembly parameters is presented, and cell volume analysis is carried out to establish size selectivity of assembly. The assembly demonstration is then extended to mammalian cells and the structuring of mouse-derived non-small lung cancer cells using TASR is shown.

Chapter 6 targets the demonstration of assembly using TASR on deformable substrates. Starting with the method for template replication using polymer materials, assembly of deformable components on these replicated templates is then elaborated upon. The results for assembly on deformable replicated substrates are also compared with the assembly on rigid master templates in the context of previous work, and insights into the effects of substrate deformability upon experimental success are derived.

Chapter 7 focuses on anisotropic component assembly. The methods used to fabricate cylindrical micro-components are discussed, and assembly of those components into matching assembly sites is described. While a high local assembly yield and shape matching is demonstrated, the effects on the experiments of challenges faced in the assembly of the components are also discussed.

Finally, Chapter 8 presents the conclusions and scope for future work. A brief summary of the goals achieved by the current work and recommendations for future work are presented.
Chapter 2

Predictive Integrated Model for TASR

2.1 Prior modeling work on predicting assembly success with TASR

Based on previous work [15-18] on modeling self-assembled systems using TASR, it was determined that several key factors come into play in determining whether the technique will be successful with a given set of parameters. In general, the success of assembly with TASR depends on three main criteria:

(A) Selective removal of components from incorrectly matched sites and retention in sites well matched in shape and size – whether the retention or the removal effects dominate

(B) Degree of mechanical deformation in the system – assessing whether or not the mechanical deformations cause a permanent change in the shape and size of the components or template

(C) Initial component assembly – whether or not the circulation is at an appropriate level such that the assembly components are able to enter the assembly sites in the first place

Each of these criteria is discussed in detail in the following section. Together, these individual models are combined into the Integrated TASR Model and presented in Section 2.2, which allows the user to design the system by selecting suitable properties and parameters for successful assembly of the desired system. Some of the relevant parameters in a typical TASR system which help evaluate the above three criteria and are used to generate the parameter map for TASR are classified into the categories below. (The complete nomenclature defining all of the symbols used in the present work is listed before Chapter 1.)
• **Geometry of system:** This includes key dimensions of the assembly components, which for example is the radius of curvature for a system comprising spherical components. The relevant parameters for the complete assembly system include the radius of curvature of the component $R_c$ and the radius of curvature of the assembly site on the template $R_t$.

• **Mechanical properties of the assembling materials:** These include the Young’s modulus of the component material $E_c$, the Young’s modulus of the template material $E_t$, the Poisson’s ratio of the component material $v_c$, and the Poisson’s ratio of the template material $v_t$.

• **Load limits of system:** These include the yield strength of the component material $Y_c$ and the yield strength of the template material $Y_t$.

• **Roughness of the assembling surfaces:** This takes into account the combined (effective) roughness of the component-substrate assembly interface and is quantified by a parameter called the roughness coefficient $C_r$.

• **Chemistry at the interface:** The chemistry at the component-substrate assembly interface is characterized by the interfacial energy $\gamma$ of the interface.

• **Assembly fluid medium:** The mechanical properties of the assembly fluid medium such as the fluid density $\rho$, fluid viscosity $\mu$, the Young’s modulus of the fluid medium $E$, and the Poisson’s ratio of the fluid medium $v$ also need to be taken into account.

• **Acoustic excitation properties:** The intensity of acoustic excitation $I$ and the frequency of excitation $f$ are other important factors.

• **Adhesive distance:** This is the effective distance $d_o$ over which the retention forces can act on the assembly component lying inside an assembly site; in other words, it is the distance over which the retention forces are effective in component adhesion. This is not a fundamental property, and
instead quantifies an approximation that is made for the purposes of obtaining rapid model results to guide the system design, as described further in Section 2.1.1.2.

It is noted that while additional factors may come into play in a given situation, these can in general be combined into the above criteria for self-assembly and can be absorbed into the integrated TASR modeling framework that is proposed in Section 2.2.
2.1.1 Model for Selective Removal

2.1.1.1 Concept of Selective Removal

A brief introduction to the concept of TASR and the previous work done in this domain were discussed in Section 1.3 in Chapter 1. This section describes in detail some of the key ideas and theoretical formulations from the original TASR model. Although this model, as well as the theoretical models discussed in sections 2.1.2 and 2.1.3 were proposed and verified in previous work [15, 17, 18], these models will be of relevance in later sections for the purpose of creating an integrated TASR model and comparing the results presented in current work with those in the previous work.

As discussed before, the key elements in TASR are the components and the patterned substrate onto which they are to be assembled. Self-assembly is achieved in part by setting up a system in which adhesion forces act between the interacting surfaces of the component and the substrate. The strength of this adhesion is controlled by the interfacial energy and by the degree of shape and size matching of the components to features on the template surface. It is the adhesion forces that are responsible for retention of components on the substrate in sites where the components are well matched to the template topography in the presence of removal forces, the origin of which will be discussed in greater detail in following sections.

Adhesion in several TASR systems, as also in previous work on TASR [15], is achieved by working with hydrophobic surfaces in a fluid that contains water. For this purpose, the components and the substrate are coated with a self-assembled monolayer (SAM) that makes the surface hydrophobic (if not hydrophobic naturally, as certain polymeric materials such as polystyrene and PDMS are). Silane-based SAMs, such as Octadecyltrichlorosilane (CH₃(CH₂)₁₇SiCl₃), or OTS for short, is one such adhesion promoter that has been used in previous work [15-17] in order to make the surface of a
material such as silica hydrophobic. The organic tails of the OTS increase the interfacial energy with water, thus generating the hydrophobic effect and promoting component-substrate adhesion.

These hydrophobically-coated components and the coated template are then placed in the assembly mixture, which was a solvent/water mixture in previous work. The assembly mixture contains a certain fraction of water for promoting adhesion of hydrophobic surfaces, and regulating this fraction of water can affect assembly results by affecting the degree of adhesion between interacting surfaces. The assembly mixture also contains an appropriate solvent; the relative amounts of solvent and water control the interfacial energy of the system (described in Section 2.1.1.2). The interfacial energy relates the contact area between two materials to the total free surface energy. Self-assembly, including TASR, works by minimizing the free energy of the system. It is energetically favorable for hydrophobic surfaces to stay in contact with each other in a water based environment, as this lowers the free energy of the system as compared with the case in which the surfaces are apart. Choosing the right solvent and using an appropriate fraction of this solvent in the assembly mixture is critical for achieving the desired interfacial energy in the system and correspondingly, high assembly yield results.

Whereas tailoring the chemistry adjusts the strength of the adhesion per unit area, shape matching increases the area of contact and hence the total strength of the component/substrate adhesion. Shape matching between the components and the features on the substrate then leads to adhesion in the correct sites upon interaction of hydrophobic surfaces in the assembly mixture. The adhesion achieved as a result generates both adhesive forces and adhesive moments that counteract (partially or completely, depending on the circumstances) any forces or moments that may act to remove the components from binding sites. These adhesive moments, which will be referred to here as retention moments, are responsible for binding the components most strongly to well-matched sites. Retention moments are related to the change in contact area (described in greater detail in Section 2.1.1.2) between the component and the substrate in transition from one location to another and to the
interfacial energy of the system. Retention moments are thus a measure of the resistance felt in moving the component from one location, where it has a given contact area with the substrate, to another location, where the contact area and the total interfacial energy are different.

While the initial assembly of components onto substrate is also an important step in deciding the success of assembly, it is the removal process in TASR that drives the assembly process’ ultimate selectivity. The initial component assembly is discussed in greater detail in Section 2.1.3. Here we focus on the selective removal process and the theoretical framework to describe it.

Removal moments are generated in the system by fluidic forces produced by high frequency acoustic excitation of the assembly mixture. A megasonic flow field is generated by the acoustic transducer (which operates at a frequency of 1.7 MHz). Apart from inducing the circulation of components in the flow field, the excitation also produces different kinds of forces – oscillatory as well as non-oscillatory – that act on the component. A subset of these forces (and the mechanical moments that they can produce) tends to remove the components from the substrate by rolling the component out of the site on the template using a mechanism called “roll-off” [15, 16], as shown previously in Figure 1.2. Depending on whether the moments that promote retention are stronger than the moments that promote removal, or vice versa, the components will be either retained or removed. Therefore, by controlling the magnitudes of these removal and retention forces and moments, selective assembly can be achieved.

The original TASR model [15, 16] stated that the retention of the component in a site takes place when the retention moments generated as result of the forces that promote component adhesion to the patterned surface exceed the removal moments which try to remove the component from the site; this promotes the retention of components in well-matched sites. On the other hand, removal from a site takes place when moments that promote component removal (which are generated as a result of the ultrasonically induced fluid forces) exceed the moments that promote component adhesion; this
promotes the removal of components from poorly-matched sites. Prior modeling described how assembly yield varies with the ratio of retention moments to removal moments. It was established theoretically and experimentally by both Jung et al. [15] and Eid et al. [16] that as this ratio increased from a value below about one to above about one, progressively higher assembly yield was recorded. When the retention moments were less than the removal moments, the assembly yield was low, approaching zero (no assembled components) in value. When the retention moments became approximately equal to the removal moments (at a ratio of approximately one), the yield increased sharply. For further increases in this ratio, the retention moments dominated over the removal moments and assembly yields as high as nearly 100% were recorded experimentally.

2.1.1.2 Details of Selective Removal

Following the analysis of Eid et al. and Jung et al. [15, 16], mathematical approximations of retention and removal forces and moments acting on the system can be made.

The interfacial energy between a solid and a liquid surface is defined by the Young's equation which states:

$$\gamma_s = \gamma + \gamma_L \cos \theta$$

(2.1)

where $\gamma_s$ is the energy of formation of the solid surface, $\gamma_L$ is the surface tension of the liquid, $\gamma$ is the interfacial energy between the solid and liquid interface, and $\theta$ is the contact angle between the liquid-solid interface. Measurement of interfacial energy is carried out by measuring the contact angle $\theta$ using goniometry once we know the values of $\gamma_s$ and $\gamma_L$. It is to be noted here that actual contact angle measurements for measurement of the interfacial energy were not carried out here. Instead, literature values [38-40] were used to quantify the interfacial energy of solvent-water mixtures as a function of the fraction of water in the assembly mixture, to obtain trends and values similar to those in previous work [15-17]. Figure 2.1 shows the graphical plot for variation in the interfacial energy of an ethanol
water-mixture with the substrate surface for variation in the fractions of water (by weight) in assembly mixture. Values of interfacial energy of the solvent-water mixtures at concentrations of water other than those plotted in the figure can also be calculated from the plot by means of interpolation. It is also to be noted that the values of interfacial energy plotted here might have slight error due to possible error in measurement of contact angle which, depending on whether is advancing or receding might be measured to have slightly different values. Since these values of interfacial energy of the solvent mixture with the hydrophobic template surface are different for different solvents, as mentioned before, choosing the right solvent with an appropriate fraction of water in assembly mixture is crucial in obtaining a value of the interfacial energy of the system at which best assembly results will be obtained. Therefore, the right solvent must be chosen for assembly using TASR depending on the components to be assembled. **Contact area calculations** were done in order to calculate the retention moments and to study the effect of fractional contact area on assembly results. This section describes

![Graphical plot showing variation in interfacial energy of assembly fluid mixture comprising ethanol and water with the hydrophobic template surface vs. the variation in fraction of water in the assembly mixture. As the fraction of water in mixture increases, the interfacial energy of the system increases. Literature values [38-40] were used to quantify the interfacial energy.]

**Figure 2.1:**

Water concentration (by weight) in ethanol-water mixture

Interfacial energy of mixture, $y$ (J/m$^2$)

Water concentration (by weight) in ethanol-water mixture
succinctly the outline of the approach used in previous work [15-17] to calculate numerically the nominal fractional contact area between the spherical component and the hemispherical hole. Only spherical components and axisymmetric holes are considered in this section. Section 2.3 describes details on contact area calculations and modeling for non-spherical components. Nominal contact area between component and substrate does not take into account the surface roughness effects. Rather, it is the area over which the nominally smooth surfaces of the component and template are separated by no more than a specified distance $d$ when the component is located at a given position on the substrate’s surface [16]. This ‘adhesive distance’, $d$ represents the range of action of adhesive surface forces and is taken to be 1.5 nm based on literature values for the solvent-water mixtures used here [15]. This adhesive distance model is used because the hydrophobic force is predominantly entropic and is not well-described by van der Waals interactions. The hydrophobic interaction can be approximately modeled as having a characteristic decay length that determines its range, taken to be 1.5 nm above in agreement with typical literature values [38]. The contact curve between the spherical component and the hole (binding site) on the template is then defined by identifying the points on the sphere that are within this adhesive distance of the binding site surface; points on the sphere at a distance from the hole outside of this range are assumed not to be in contact. It should be noted that we have assumed in the mathematical description provided here that the sphere’s diameter is smaller than that of hole, which is a valid assumption for the cases that will be discussed using this approach in Chapters 5, 6 and 7. However, it has also previously been shown using conceptually similar arguments that the low contact area between a larger sphere and the smaller hole on top of which it rests also results in the selective removal of spheres that are too large for a given site [16].

Although the basic principle used is simple, contact area calculations become complicated because the shape of the holes obtained on the template surface after the fabrication process (discussed in detail in Section 3.3) is not exactly hemispherical due to factors such as finite lithographic feature size, resist
delamination etc., so that the surface diameter is larger than it would be for a hemispherical hole of the same depth. It is therefore necessary to calculate the contact area numerically [16]. While a few relevant details are discussed here below, complete details on contact area analysis are found in the previous work [15-17]. Atomic Force Microscopy is used to obtain the hole shape profiles, which are determined to be approximately axisymmetric. A commercial surface analysis software package (SPIP by Image Metrology) is then used to fit an 8th order curve of the form y=f(x) to the cross-sectional AFM profile, where y is vertical distance from the bottom of the hole and x is the radial distance from the centerline. The sphere is now assumed to roll along the hole from the lowermost point in the cross-section to any point on the hole surface along the sidewall. Sphere rolling is assumed to take place in the same plane as the cross-section of the hole. The contact curve between the sphere and the hole is defined with the help of contact angles (farthest angular extent of the points on the hole that are within adhesive distance \( d_a \) of the sphere). For each value of azimuthal angle \( \theta \) which is the angle between the plane of the cross-section of hole and the plane passing through the sphere center and the examined point on the sphere surface, the value of these contact angles is calculated and then spherical integration is performed numerically to calculate the contact area \( A_n \).

In order to calculate the real contact area, the surface roughness effects have to be taken into account. This surface roughness might originate from either the non-uniformity of the surface's hydrophobic self-assembled monolayer coating or from the fabrication process itself, which involves some steps that can potentially induce surface roughness. The real contact area (taking into account the roughness) is a fraction of the nominal contact area calculated numerically following the approach highlighted above because roughness may bring some points on the surface out of the contact distance. Therefore, a roughness factor \( C_r \) was defined in order to assess how much of the nominal contact area between the interacting surfaces is in contact in reality. Step-by-step calculation of this roughness factor is described in detail in previous work [15, 16]. In order to describe the surfaces of both the
components and the substrate, two parameters are used to describe each surface: the mean surface roughness (rms) value, $\sigma$, and the peak height, $h$. The roughness factor $C_r$, as in previous work [15, 16, 18], is then calculated using a Gaussian probability distribution function [41] that calculates the fraction of points that are at a height greater than or equal to a certain level from the reference plane of the rough surface. Using the value of the roughness factor for the particular combination of component and template material, we can arrive at the value of the real contact area between interacting surfaces once we know the value of the nominal contact area using the following relation:

$$A' = C_r A_n$$  \hspace{1cm} (2.2)

where $A'$ is the value of the real fractional contact area, $C_r$ is the roughness factor and $A_n$ is the nominal fractional contact area.

The retention force is directly proportional to the differential change in the free energy that takes place upon displacement of the sphere from its original location to a location where it has different contact with the liquid. The change in this free energy in turn is directly related to the magnitude of lost contact area between sphere and hole. Incorporating also the effects of surface roughness, the magnitude of the retaining force which opposes removal of component from substrate is then given by the expression:

$$F_{\text{retention}} = -\gamma \left( C_r \frac{dA_n}{dz} + A_n \frac{dC_r}{dz} \right)$$  \hspace{1cm} (2.3)

where $dz$ is the differential change in distance during the translation of the sphere from one location to the other and $\gamma$ is the interfacial energy between the component and substrate interface. The values of both the roughness factor, $C_r$, and nominal fractional contact area, $A_n$, can in principle change when a component moves to another location [15, 16], and this is taken into account by the expression for the retention force. The retention moment, on the other hand, is calculated using the value $d\Phi$, which is
the differential change in the rolling angle via rotation of the sphere. The roughness of the surface, characterized by the roughness factor, $C_r$, is assumed to be constant and thus any change in its value with the change in the value of $d\Phi$ is ignored. Retention moment, $M_{\text{retention}}$ is then calculated using the following equation:

$$M_{\text{retention}} = -\gamma C_r \frac{dA_n}{d\phi} \quad (2.4)$$

The value of the right hand side in the above equation can then be calculated by making use of the differential chain rule and knowing that

$$ds = R_c d\phi, \quad (2.5)$$

where $R_c$ is the radius of the assembly component and $ds$ is the differential distance covered by the sphere during rolling by an angle $d\Phi$. Since $ds$ can be further expressed in terms of the differential increments along the x and y axis, $dx$ and $dy$, and their values, along with the value of $dA_n$, are calculated in the form of finite differences from the contact area algorithm as described in previous work [15-17], the net value of the retention moment can be calculated numerically, using the expression:

$$M_{\text{retention}} = \gamma R_c C_r \left( -\frac{dA_n}{\sqrt{dx^2 + dy^2}} \right) \quad (2.6)$$

The calculation of the net removal moments involves analytical expressions for several fluidic forces obtained from literature [42] for cases which resemble the situation under consideration.

As mentioned in previous work [15, 16], there are two types of fluid forces in this system. The primary forces, which make the dominant contribution to the removal moment, are due to unsteady low Reynold’s number flow past a sphere, whereas the secondary forces are acoustic in nature. Solving the simplified Navier-Stokes equation that describes the system [43], as shown in Equation 2.7, the
total hydrodynamic drag acting on the sphere is expressed as a result of three primary forces: the added mass force, the viscous drag force and the Basset force [44].

\[ \frac{\partial u}{\partial t} = -\nabla P + \mu \nabla^2 u \quad (2.7) \]

The added mass force is a result of the relative acceleration of the spherical component with respect to the fluid in the vicinity. The viscous drag force, equivalent to the Stokes drag, results from the shear stresses generated as a result of the velocity gradients between the surface of the sphere and the bulk fluid medium. The Basset force is a result of the varying-thickness boundary layer developed around the sphere due to change in its relative velocity with respect to the medium and accounts for deviations of the flow pattern from steady state. Lift and buoyant forces are negligible for the case under consideration here as was also observed in [15, 16], which considered on similar situations.

Along with the primary forces that are generated due to the flow past a sphere, secondary forces are also generated from the megasonic flow field that is created within the assembly fluid by the megasonic transducer. The acoustic streaming drag is one such force that results from the loss of acoustic momentum due to attenuation of sound waves in the viscous fluid medium [45]. Another effect of the sound field is the generation of the radiation pressure force which is exerted on the components due to scattering of the acoustic waves by them [46].

It was demonstrated after detailed calculations in previous work [15, 16] that the net removal moment, \( M_{\text{removal}} \), could be estimated effectively by the expression:

\[ M_{\text{removal}} = M_{\text{added mass}} + M_{\text{radiation}} + \sqrt{\left( M_{\text{viscous}} + M_{\text{Basset}} \cos 45^\circ \right)^2 + \left( M_{\text{added mass}} + M_{\text{Basset}} \sin 45^\circ \right)^2} \quad (2.8) \]

where moments due to the Basset force, \( M_{\text{Basset}} \), the radiation force, \( M_{\text{radiation}} \), the viscous drag force, \( M_{\text{viscous}} \), and the added mass force, \( M_{\text{added mass}} \), were each calculated separately using analytical
expressions [42-48] to arrive at the final moment value. The expressions for each of these moments is listed in equations (2.9)-(2.12) below [16]

\[ M_{\text{added mass}} = \left( \frac{4}{3} \pi^2 f R^4 \sqrt{\frac{I \rho}{c}} \right) \sin \theta \]  

(2.9)

\[ M_{\text{viscous}} = \left( 6\pi \mu R^2 \sqrt{\frac{I}{\rho c}} \right) \sin \theta \]  

(2.10)

\[ M_{\text{Basset}} = \left( \frac{72\pi^3 \mu f}{c} \right) R^3 \sin \theta \]  

(2.11)

\[ M_{\text{radiation}} = 64\rho \left( \frac{2\pi f}{c} \right)^4 R^7 U^2 \sin \theta, \]  

(2.12)

where \( f \) is the ultrasonic wave frequency, equal to 1.7 MHz in our case. The parameter \( c \) is the velocity of the waves created due to ultrasonic vibrations (that is, the speed of sound in the medium) and is related to the material properties of the elastic medium in which it is travelling, such as the Young’s modulus \( E \), Poisson’s ratio \( \nu \) and the medium density \( \rho \), by the following relation [16]:

\[ c = \sqrt{\frac{E}{3\rho(1-2\nu)}}. \]  

(2.13)

\( U \) is the wave velocity amplitude given by [42]

\[ U = \sqrt{\frac{I}{\rho c}}, \]  

(2.14)

and \( I \) is the intensity of the incident acoustic wave at the given location, which is attenuated in the direction of travel and is defined by the relation [49]

\[ I(\alpha) = I_0 e^{-\alpha \alpha}. \]  

(2.15)
$I(a)$ is the intensity at a given location $a$ in the direction of travel, $I_0$ is the intensity at source position $a = 0$, and $\alpha$ is the attenuation coefficient of the travel medium.

### 2.1.2 Model for assessing mechanical deformations in system

#### 2.1.2.1 Concept of assessing mechanical deformations

As discussed previously, TASR is essentially a competition between adhesive retention effects and fluidic removal effects in order to achieve selective self-assembly. Because both the retention and the removal moments increase with component size, TASR can be effective across scales. The component to be assembled is retained therefore in a well-matched site where the geometry of the component to be assembled matches the topography of the template. On the other hand, ultrasonically-generated removal forces will remove it from a site where it is not well-matched. Thus, the shape and size selectivity principle of TASR fundamentally depends on the component’s shape. Since deformable structures can change their shape and size under the application of a load, the component/template contact area may depend on the degree of deformation. Therefore systems incorporating deformable components cannot be described completely by theoretical frameworks such as the original TASR model [15, 16] that do not take into account the deformation of the components and/or substrate. To accommodate the possibility of deformation, a second model describing the effects of deformability on TASR-based self-assembly was proposed in [37]. This model relies on the elastic-plastic contact theory of deformation to determine when the original TASR model is or is not valid. This model complements the original TASR model for rigid structures that was formulated by Jung et al. [15] and Eid et al. [16]. Details of the model are described in section 2.1.2.2, while the basic idea behind the model is discussed here.
The extension to TASR theory proposed here takes into account the mechanical properties of the components and substrate and rests on a simple energy argument. Self-assembly in general and TASR in particular depend on the tendency of systems to minimize their free energy. If the deformations of components and substrate are fully elastic, any reduction in system free energy due to the increase in contact area upon deformation will equal the increase in system free energy due to the storage of elastic energy in the deformed structures. Therefore, for purely elastic deformations, the original TASR model should still apply. In contrast, if the deformation enters the plastic regime, some of the energy will be dissipated, and the TASR model should no longer apply completely. Therefore, the ability to assemble deformable systems (with deformable components and/or substrate templates) comes down to the question of at what point the deformations enter the plastic regime. This in turn depends on a) the mechanical properties of the component and substrate materials, b) the magnitude of the force that holds the component on the substrate, and c) whether the component, the substrate, or both are deformable, as described above.

Although the same concept (the significance of the onset of plastic deformation) applies independent of whether it is the substrate, the components, or both that are deformable, the details of when plastic deformation sets in depend on which elements are deformable, as that dictates the boundary conditions posed on the system. The case of deformable spherical components assembling on an essentially rigid substrate will be considered first, followed by discussion on cases of rigid components indenting deformable substrates. Finally, the case in which deformable spherical components assemble on deformable substrates with comparable mechanical elastic behavior is presented.

*Hertzian elastic contact theory* [50], combined with prior analytical and numerical assessments of the onset of plastic deformation [51-54], is used to assess whether the component deformation is purely elastic or includes a plastic component. The theory that is employed here to describe
deformation is strictly applicable either to two deformable spheres in elastic contact, a deformable sphere in elastic contact with a rigid flat, or to a deformable sphere in elastic contact with a rigid sphere. It may also be approximately applied to the present situation of a deformable sphere inside a hole with a local (but not quite constant) radius of curvature. For assessing the applicability of the TASR process to a given materials system, the parameter of interest is the value of the critical interference \( \omega_c \) that marks the transition from the purely elastic to the elastic-plastic deformation regime. Interference \( \omega \) is a measure of the sphere’s deformation and is equal to the difference between the sphere’s radius and the distance from the center of the deformed sphere to the surface that it contacts. In other words, it is the amount by which the sphere would have had to penetrate into the second surface in order to approach it that closely in the absence of deformation. For values of the interference below the critical interference, the deformation is purely elastic and the original TASR theory is predicted to apply. For values of the interference above this value, the TASR theory is no longer entirely applicable.

### 2.1.2.2 Details of assessing mechanical deformations

The Hertzian closed-form expressions for the mechanics of two deformable spheres in purely elastic contact [50-54] can be used to determine the interference for a single elastic sphere in contact with a flat substrate (or indeed for a sphere in contact with a substrate with a given radius of curvature). Figure 2.2 (A) depicts schematically such a case of a deformable sphere pressed onto a rigid flat. On pressing a deformable sphere of radius \( R \) onto a rigid flat by application of a contact load \( P \), a circular contact between the sphere and the flat is formed which is described by a contact radius, \( a \). An interference depth \( \omega \) characterizes the deformation of the sphere due to the contact load applied by the rigid surface.

The interference \( \omega \) is given in the elastic regime by
where the equivalent radius $R_{eq}$ is given in terms of the component radius $R_c$ and the template’s local radius of curvature $R_t$ by

$$\frac{1}{R_{eq}} = \frac{1}{R_c} + \frac{1}{R_t},$$

(2.17)

and the combined modulus $E'$ is given in terms of the respective Young’s moduli $E_c$ and $E_t$ and Poisson’s ratios $\nu_c$ and $\nu_t$ of the components and template by

$$\frac{1}{E'} = \frac{(1-\nu_c^2)}{E_c} + \frac{(1-\nu_t^2)}{E_t}.$$

(2.18)

The term $p_{\text{max}}$ is the maximum value of the contact pressure at the component/template contact. The contact pressure arises from the net force that presses the component into the substrate template. This force is almost entirely chemical in origin and is determined from the original TASR models [15, 16]. If the contact between component and substrate template is approximated as a contact

![Figure 2.2: Schematic diagram showing the different cases possible with systems comprising deformable materials.](image)

(A) Illustrates the case of a deformable assembly component of radius $R$ contacting a relatively rigid substrate material due to the application of contact load $P$. An interference $\omega$ is obtained upon the contact, with a circular contact region of radius $a$. The deformable sphere is free to expand radially on the rigid substrate. (B) Shows the reverse case of a rigid sphere indenting a deformable substrate material.
between two spherical surfaces, then the pressure may be taken to vary spatially as $\sqrt{1-\frac{r^2}{a^2}}$, where $r$ is the radial distance out from the central contact point and $a$ is the overall radius of the contact area. Within this approximation, the maximum value of the contact pressure $p_{max}$ is simply related to the average contact pressure $p_{avg}$ as

$$p_{max} = \frac{3}{2} p_{avg} = \frac{3}{2} \frac{P}{A}, \quad (2.19)$$

where the average pressure is the ratio of net force to contact area. According to Hertzian theory [50], the contact area between two elastic solids with spherical contact surfaces is given by

$$A = \pi a^2 = \pi \left( \frac{3R_{eq} P}{4E'} \right)^{2/3}. \quad (2.20)$$

Combining these results yields the value of the interference for the physical situation; this value is valid until the onset of plastic deformation but becomes invalid beyond it. The interference $\omega$ is then compared with the critical interference value $\omega_c$ that marks the onset of plastic deformation. The critical interference has been calculated previously [51] to be

$$\omega_c = \left( \frac{\pi KH}{2E'} \right) ^2 R_{eq}, \quad (2.21)$$

where $H$ is the hardness of the component and is related to its yield strength $Y$ as

$$H = 2.8Y. \quad (2.22)$$

In Eq. 2.26, $K$ refers to the hardness coefficient of the spherical component. The value of $K$ was found in [55] by modeling based on finite element results. Their resulting values depend on the Poisson’s ratio of the deformable component material and are given by

$$K = 0.454 + 0.41\nu_c \quad (2.23)$$
Using these results, the ratio \( \omega/\omega_c \) of the interference to the critical interference may be calculated for various component and substrate materials, and for various geometries (radii of curvature of spherical components and the template holes in which they assemble), the expression for which is given below:

\[
\frac{\omega}{\omega_c} = \left( \frac{p_{\text{max}}}{KH} \right)^2
\]  

(2.24)

If \( \omega/\omega_c \) is less than one, the original TASR model is predicted to be applicable, and the assembly is expected to be successful. If the calculated ratio exceeds one, then the TASR model will have begun to become invalid (though the discrepancy between the model and reality may be small for very small amounts of plastic deformation). In addition, for ratios above one, it is known that the interference exceeds the critical interference, but not by how much, because this interference calculation is only strictly valid in the purely elastic regime.

The case of rigid/deformable microspherical components assembling on deformable substrates is also of interest to us as mentioned in Section 2.1.2.1 since the possibility of creating deformable, replicable templates from rigid template masters would reduce the total fabrication cost involved and holds potential for simple, low-cost, mass production of templates. Therefore, analyzing these cases theoretically is of significance. This section also highlights the approach for assessing deformations in deformable substrates when indented by microspheres made of a relatively rigid material. Figure 2.2 (B) depicts schematically such a case of a rigid microsphere indenting a flat deformable substrate.

As stated before, a deformable sphere contacting a rigid substrate is different from a rigid sphere contacting a deformable substrate because different constraint conditions lead to different behavior. Therefore, in order to address the case of the indentation of a deformable substrate by a rigid microsphere, a different parameter must be used to decide the nature of the contact between the two mating surfaces. Although the constraint conditions are different, the underlying concept of the transition from the elastic to the elastic-plastic regime remains the same.
Based on the case of Brinnel indentation of an elastic-plastic half-space considered by Mesarovic et al. [53], this parameter of interest describing the nature of deformation in the substrate was found to be $\gamma'$, defined as:

$$\gamma' = \frac{E'a}{R_{eq} Y}$$  \hspace{1cm} (2.25)$$

which is basically the ratio of the indentation pressure ($E'a/R_{eq}$) and the initial yield strength $Y$, of the half-space, or in this case the assembly substrate. Here $R_{eq}$, $E'$ and $a$ are the same parameters as used and described previously in equations (2.17), (2.18) and (2.20) respectively. From finite element predictions [53] of average indentation pressure, the completely elastic Hertzian regime extends for values of $\gamma'$ less than 2.5. Above this value, plastic deformation in the substrate begins.

The Hertzian theory extension used so far for describing the contact between a deformable solid and a relatively rigid solid originates from the theory of contact between two deformable solids with similar mechanical behavior. By combining these concepts of Hertzian theory with the criterion for onset of plastic deformation in a deformable substrate when indented by a rigid material, the theoretical model can then be extended to assess the deformations during the assembly of a deformable microspherical component on an elastic-perfectly plastic, deformable substrate. This is done by first investigating the possibility of the onset of plastic deformation in the deformable substrate material itself [37]. The case of a microsphere made of a relatively rigid material indenting the deformable substrate is considered and the possibility of incurring plastic deformation in deformable substrates is assessed. If the substrate does not deform plastically on contact with a more rigid material, then it may safely be assumed to have elastic behavior on contact with materials that are more deformable than it is under otherwise similar circumstances. The focus of the analysis then shifts to the possibility of deformation in the deformable sphere rather than in the less deformable substrate. Using the same model as described previously for the contact of a deformable sphere on a relatively rigid flat [17], it is
then possible to assess plastic deformation in more deformable spheres on the flat, relatively less
deformable substrate material, which is then essentially treated as rigid compared to the component
material. This model produces a conservative estimate of when the assembly process will be successful
for a given set of deformable materials, a given component/template geometry, and given experimental
parameters. For circumstances in which the model predicts that assembly will be successful, assembly
is expected to be successful. For circumstances in which this model predicts the onset of plastic
deformation, plastic deformation may actually occur, or the system may only be near the onset of
plastic deformation. This uncertainty arises from the assumption that the deformation is being driven
by a rigid component or a rigid template, as described above, which overpredicts the deformation as
compared with a system in which both components and template are deformable. This nonetheless
serves as a useful design tool; when the model predicts success, the design is expected to be effective.
2.1.3 Model for Initial Component Assembly

This model is used to predict the circumstances under which initial placement of components into assembly sites will take place in the TASR system. This theory was proposed by Jung et al., and complete details of the theoretical framework are described in [18]. An outline and some key results are discussed here as these results will be used to combine the model for initial assembly with the previous two models for selective removal (discussed in Section 2.1.1) and the model for assessing mechanical deformations (discussed in Section 2.1.2) together into an integrated model for TASR (to be discussed in Section 2.2). As discussed in detail in [15, 16], there are two main types of flows that come into play in the TASR process – the primary oscillatory flows and the secondary mean flows (also referred to as the acoustic streaming flows). While the oscillatory flows play a major role in driving the selective removal process, it is the secondary mean flows that are the dominant flows for ensuring component circulation inside the assembly fluid medium and hence the initial component assembly in TASR. It is these mean flows that drive ‘in-plane’ motion of assembly components at the solid-fluid interface in TASR and give rise to a drag force on the components, which makes them enter the holes. This is the primary method for the components entering the holes rather than a vertical descent driven by gravity since the in-plane flows contributing to the net mean flows dominate the out-of-plane mean flow component of the same. The drag torque on the components, $T_{\text{drag}}$, under the assumptions of a laminar flow and no boundary layer effects, is estimated by the following relation:

$$T_{\text{drag}} = 6\pi \mu R_c^2 U,$$  \hspace{1cm} (2.26)

where $\mu$ is the viscosity of the assembly fluid medium, $R_c$ is the spherical component radius, and $U$ is the velocity of the acoustic streaming flow. The assumption of no boundary layer effects for the cases discussed here is confirmed from [15], as at the frequency of excitation used here, the boundary layer thickness is assumed to be around 300 nm, which is a small fraction of the diameter of the
microspheres used in current work, and can be accounted for by an error bar on the number for fluid velocity. It is also to be noted that the flow velocity is also closely dependent on the voltage driving the acoustic transducer. The relation for this dependence is given below and is used to calculate the flow velocity $U$ at any given voltage $V$ in the system, once we measure the flow velocity $U_0$ at a reference voltage level $V_0$, as shown below:

$$U = U_0 \frac{V^2}{V_0^2} \quad (2.27)$$

While the drag torque acts to drive the component into the hole, the change in contact area during the process of the component rolling from the flat portion of the template into the hemispherical hole results in a change in the free energy of the system, and hence there exists a net impedance torque that acts to prevent the entry of the component inside the hole. This impedance torque $T_{imp}$ is dependent on the change in contact area as the component tries to enter a hole and is given by the relation:

$$T_{imp} = -\gamma C_r \frac{dA_n}{d\theta} \quad (2.28)$$

where $\gamma$ is the interfacial energy between the surfaces and the assembly fluid, $A_n$ is the nominal contact area between the component and the template, $\theta$ is the rotation angle of the microsphere as it enters the hole and finally $C_r$ is the roughness coefficient that adjusts the value of the nominal contact area to account for the surface roughness effects, assumed to be a constant value in this analysis as previously mentioned. As shown in [18], the maximum change in the contact area, which would also result in the largest possible impedance torque (which would be a value of interest in the following sections), is given by the expression:

$$\frac{dA_n}{d\theta} = -2\sqrt{2dR_c}^{3/2} \quad (2.29)$$
It is to be noted that the analysis for the calculation of the impedance torque above assumes ideal hemispherical hole profiles and does not use actual hole geometries, which, given the hole shapes recorded in the current work and their proximity to ideal hemispherical profiles, is a reasonable assumption. Details of this derivation are not shown here as they are not relevant to the current work, but the key result obtained from the above relation is that the change in contact area as the sphere rolls into the hole depends primarily on the sphere radius $R_c$ and the distance over which the adhesion forces are effective, $d$. Using the above relations, one can then derive a ratio for the drag torque to the impedance torque as follows:

$$\frac{T_{\text{drag}}}{T_{\text{imp}}} = \frac{3\pi \mu \gamma C_r}{\gamma C_r} \sqrt{\frac{R_c}{2d}}$$

(2.30)

This ratio tells us about the conditions under which the circulation effects in the fluid medium are strong enough to enable successful initial component assembly (by overcoming impedance effects), such that if the mechanical deformations are also within the safe limits for assembly, then the selective removal process can drive shape and selective assembly in TASR.
2.2 Integrated TASR Model

Combining all of the ideas from the theoretical models created previously to describe individual aspects of the TASR process, an integrated TASR model is presented here. This model states that in order to achieve successful assembly using TASR, wherein the components that are well-matched in shape and size to the assembly site are retained whereas the components that are not well-matched are excluded from the sites, with a high assembly yield, the following criteria need to be met.

First of all, selective removal of incorrectly matched components from assembly sites and retention inside well-matched sites needs to be ensured. As discussed in Section 2.1.1, the key parameter that determines the success of the selective removal process is the ratio of the torque that promotes component retention inside the assembly site, the *retention torque*, to the torque that promotes the component removal, or the *removal torque*. For the selective removal to take place, this ratio must exceed a value of 1 [15]. This is represented below mathematically:

\[
\frac{T_{ret}}{T_{rem}} > 1 \quad (2.31)
\]

While an exact relation for \( T_{ret} \) as derived in previous work [15, 16] is used to calculate the retention moment numerically, an estimate for \( T_{rem} \) is made using the equation below in terms of relevant variable system parameters

\[
T_{rem} = \sqrt{\frac{72\pi^3 \mu l f}{c} R_c^3} \quad (2.32)
\]

Next, the deformations in the system need to remain within a critical limiting value, in order to ensure that there is no permanent change in the shape and size of the system under the action of mechanical forces that are a part of the system. As discussed in Section 2.1.2, the main concept behind ensuring this is that the deformation in the system is in the purely elastic regime and does not reach the
limit where plastic deformation begins. Also as discussed previously, the definition and actual value of the parameter that is used to characterize the deformation in the system depend on the nature of the system itself, i.e., whether the component or the substrate or both are deformable. In the first case where the component is deformable while the substrate can be treated to be relatively rigid in comparison, the value of the interference \( \omega \) must remain below a critical interference limit \( \omega_c \) in order to ensure successful assembly using TASR [17]. This is also represented below mathematically as:

\[
\frac{\omega}{\omega_c} < 1 \quad (2.33)
\]

This ratio value can be tuned by choosing the relevant system parameters which \( \omega \) and \( \omega_c \) depend on (using equations (2.16)-(2.24)), such as \( P, E', v_c, Y_c, R_{eq} \) etc.

In the second case, on the other hand, in which the substrate material is more deformable while the component material can be treated to be relatively rigid in comparison, the value of the interference ratio parameter \( y' \) must remain below a critical limit number in order to ensure completely elastic deformation and hence successful assembly using TASR [37]. Based on conclusions made in Section 2.1.2, this is represented by the equation below as:

\[
y' < 2.5 \quad (2.34)
\]

In terms of relevant system parameters which one might want to tune depending on the system selected for assembly and using equation 2.25, this is translated as:

\[
\frac{E' a}{R_{eq} Y_i} < 2.5 \quad (2.35)
\]

Finally, as discussed in Section 2.1.3, one also needs to ensure that initial component assembly takes place in the TASR system as that plays a key role in the success of the subsequent steps of assembly. It was shown that in order to achieve this target, the torque that drives the components inside the
assembly sites, the drag torque, must exceed the torque that opposes component entry into the holes, i.e. the impedance torque. This is represented below mathematically:

\[
\frac{T_{\text{drag}}}{T_{\text{imp}}} > 1 \quad (2.36)
\]

By using the respective relations for \(T_{\text{drag}}\) and \(T_{\text{imp}}\) as derived in previous work [18] and also discussed here in Section 2.1.3 earlier (equation 2.30), in terms of relevant variable system parameters, this equation can be simplified to:

\[
\frac{3\pi \mu U}{\gamma C_r} \frac{R_c}{\sqrt{2d}} > 1 \quad (2.37)
\]

Thus, if all of the conditions above are met successfully in the system, when actual system parameters values are used in the above described relations, then the integrated TASR model predicts the assembly in the system to be successful. The following sections show the effects of varying the various system parameters on the criteria determining assembly success, using the integrated TASR model.
2.2.1 Varying intensities, interfacial energies and size scales

As discussed in [18], the value of the intensity of acoustic excitation is a major parameter in deciding the success of the assembly, as it decides the power input to the system and the forces that drive the assembly. The intensity of excitation needs to be above a certain threshold to ensure initial component assembly, i.e. the minimum intensity needs to be such that the drag torque, which acts to drive the component inside the hole, outweighs the impedance torque, which acts to prevent component entry inside the hole. Using previously derived relations in equations (2.36) – (2.37) and the fact that the intensity of excitation is directly proportional to the streaming flow velocity $U$ [18], this requirement can be expressed mathematically as below:

$$I > I_{\text{min}} = \frac{\rho c f y C a}{3 \pi \mu} \sqrt{\frac{2d_{a}}{R_{c}}}$$  \hspace{1cm} (2.38)

where the value of $a$ typically scales as the size of the component $R_{c}$, as discussed in [16]. If the mean values for the parameters $a$, $\rho$, $f$, $d_{a}$, $\mu$, and $C$, are used and these are assumed to be fixed for a given system for assembly, as is usually the case, then these constants are set to fixed values in equation (2.38) above and the variation of the required acoustic intensity with the variation in interfacial energy and the size scale of the system can be studied, which are usually the more variable factors of interest when designing a TASR based system.

While initial component assembly is to be ensured by keeping the intensity level above the minimum level specified in equation (2.38) above, at the same time, the intensity of excitation also needs to be below a certain threshold to ensure selective removal i.e. the maximum intensity needs to be such that the retention torques dominate the removal torques in a correctly matched location. Using previously derived relations [15, 16] for $T_{\text{ret}}$ and $T_{\text{rem}}$ and imposing the criterion mentioned in equation (2.31), this requirement can be expressed mathematically as below:
As before, the value for the parameters $c$, $\mu$ and $f$ are assumed to be fixed for a given system for assembly and are thus set to constant values in equation (2.39) above for that particular system, and the variation of the acoustic intensity with the variation in interfacial energy and the size scale of the system can be then studied, once the value for the retention moment $T_{ret}$ has been calculated numerically.

Thus, as seen above, the ratio of maximum intensity in the system to the minimum intensity needs to be tuned to achieve the optimal level. This can be done by adjusting the operational parameters of interest in the system. The equations (2.38) and (2.39) are then combined to give the criterion on the intensity of the system as follows:

$$I_{\text{min}} < I < I_{\text{max}}$$  \hspace{1cm} (2.40)

where the value of the minimum intensity is calculated using the relation (2.38) and the value of the maximum intensity is calculated using the relation (2.39). One can now select the value of the intensity, size and the interfacial energy of the system which satisfies the above equation (2.40) to ensure that both the criteria for initial component assembly as well as the criterion for selective removal is met, while keeping other parameters in equations (2.38) and (2.39) at a fixed level, depending on the system chosen. The criterion for mechanical deformations is then added to this combined criterion in Section 2.2.2 and 2.2.3, depending on the boundary conditions of the system used.

For instance, with all else held constant, the interfacial energy of the system can be tuned (by changing the fraction of water in the assembly fluid medium, for instance) to change the minimum required intensity value. For any given value of interfacial energy, there is a size range for the system in which the maximum intensity $I_{\text{max}}$ exceeds the minimum intensity $I_{\text{min}}$ and the assembly is
successful, as shown in Figure 2.3 (A), for the case of assembly of polystyrene microspheres onto silica templates in an ethanol based assembly fluid medium, where the value of interfacial energy is assumed to be constant at $\gamma = 1\text{mJ/m}^2$. If possible, one can design the system such that the size of the system falls within this favorable range for assembly. Similarly, this can be done for any other level of interfacial energy, and by plotting curves as shown in Figure 2.3(B), one can extract the size ranges for successful assembly under various conditions. Once the possible or tolerable range for variation in interfacial energy is known, given the constraints of the system, it is then possible to plot a graph showing interfacial energy vs. size of system, from which it is possible to identify a favorable block for assembly using TASR, as shown in Figure 2.3 (C). Then one can operate anywhere within this block and still achieve successful assembly, provided the conditions on mechanical deformations are also met, as discussed in Section 2.2.2 and Section 2.2.3.
Figure 2.3: (A) Plot showing trends for variation of the maximum intensity and the minimum intensity with the size of the assembly system, in terms of the component radius, \( R_c \) for the value of interfacial energy \( \gamma = 1 \text{ mJ/m}^2 \). The hatched region in the graph shows the portion where the maximum intensity exceeds the minimum intensity and represents the desired region for operation with TASR. The desired size range of the system for operation is also extracted from the boundaries of this favorable shaded region and puts a limit on how big the assembly components can be for successful assembly. Plots such as these can be used to identify the trends for variation for different values of interfacial energy \( \gamma \) as shown in (B); actual values will depend on the values of the constant parameters in the system used. (C) shows the plot for interfacial energy vs. the size ranges for the system extracted from plots as shown in (A) and (B) and identifies the favorable operating range for assembly with TASR.
2.2.2 Varying mechanical properties

As discussed previously in Section 2.1.2, mechanical deformations in systems comprising deformable materials also need to be assessed to test the success of assembly using TASR [17]. The criteria imposed upon deformations in the system so as to be within the limit for successful assembly have been presented already in equations (2.34) and (2.36), for different boundary conditions of the system. As seen from these criteria, some of the material properties that are necessary input values in the model for describing deformations in the system include Poisson’s ratio, Young’s modulus, yield strength. The above properties for some of the common materials considered for the purposes of our modeling and experiments are listed in Table 2.1 below.

Table 2.1: Mechanical properties for commonly used deformable materials in the TASR system

<table>
<thead>
<tr>
<th>Material</th>
<th>Young’s modulus, $E$ (GPa)</th>
<th>Poisson’s Ratio, $\nu$</th>
<th>Yield Strength, $Y$ (GPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polystyrene</td>
<td>2.55</td>
<td>0.38</td>
<td>0.012</td>
</tr>
<tr>
<td>PMMA</td>
<td>2.8</td>
<td>0.38</td>
<td>0.05</td>
</tr>
<tr>
<td>PTFE</td>
<td>0.5</td>
<td>0.31</td>
<td>0.015</td>
</tr>
<tr>
<td>Polypropylene</td>
<td>1.5-2</td>
<td>0.35</td>
<td>0.05</td>
</tr>
<tr>
<td>Melamine</td>
<td>11</td>
<td>0.31</td>
<td>0.085</td>
</tr>
<tr>
<td>PDMS</td>
<td>0.0005</td>
<td>0.4</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Once the size of the system is decided based on the criteria described in Section 2.2.1 such that the level of acoustic intensity in the system is at an appropriate value, one can choose the mechanical properties of the system such that the deformation criteria for successful assembly are met. The three main mechanical properties of relevance here, for both the component and the template material, are the Young’s modulus, $E$, the Poisson’s ratio, $\nu$ and the yield strength, $Y$. For a certain known size of the
system, the value of the contact load \( P \) acting on the system (which is the same as the retention force in the system \( F_{\text{ret}} \)), can be calculated as shown previously in equation (2.3) and discussed extensively in previous work [15-17]. Once the value of the contact load and the size of the system are provided as inputs, 3-dimensional plots can be created for the values of \( E, \nu \) and \( Y \), such that knowing the preferred range for any one of these properties based on choice of material, it is possible to see whether the other properties lie in the favorable range or not.

Figure 2.4(A) shows such a 3D plot for the case of a deformable component assembling on a rigid substrate, with a value of contact load \( P \) of \( 1.68 \times 10^{-8} \) N and the size of the system \( R_{\text{eq}} = 1\mu m \). Trends obtained from equation (2.34) that describe this case for assembly are used to generate the 3D plot and subsequent 2D sections of the 3D plot, shown in Figures 2.4 (B) – (D). Here, the values of the component Poisson ratio \( \nu_c \) are set within the range from 0.3 – 0.4 (which is confirmed from Table 2.1 to be the range for most common deformable materials of interest), and the corresponding trends for the component Young’s modulus, \( E_c \), and component yield strength, \( Y_c \), are calculated and plotted. The area beneath this 3D curve represents the favorable operating range for assembly, based on criterion for limiting mechanical deformation in equation (2.34). Plots such as these, created for a particular set of materials and sizes chosen can be used to assess the nature of deformation in the system.

This 3D plot can also be divided into three types of 2D section plots which are easier to read and interpret, as shown in Figures 2.4 (B) – (D). Figure 2.4(B) shows the values of equivalent Young’s modulus plotted vs. Poisson’s ratio for a typical value of the yield strength used in the TASR system. Similarly, Figure 2.4(C) shows the values of yield strength plotted vs. Poisson’s ratio for a typical value of the equivalent Young’s modulus in the TASR system. And finally, Figure 2.4(D) shows the values of equivalent Young’s modulus plotted vs. yield strength for a typical value of the Poisson’s ratio in the TASR system. In all of these plots, the shaded areas under the curves represent the regions where one would choose to operate for successful assembly, given the value of the fixed parameters.
selected. It is again noted that as before, the actual values on the y-axis in all of these 2D plots are not shown since they will depend on the values of the other variables in the assembly system treated as constants in this plot, which vary from system to system. If different values of the fixed parameters are used, similar favorable regions can be identified for that particular system using the same governing relations as those used for the plots described here.

**Figure 2.4:** (A) 3D plot showing trends for variation of equivalent young’s modulus vs. component yield strength and component Poisson’s ratio, for the case of a deformable component assembling on a rigid substrate, with a value of contact load $P$ of 1.68 E-8 N and the size of the system $R_{eq} = 1\mu m$. The area beneath this 3D curve represents the favorable operating range for assembly, based on criterion for limiting mechanical deformation. (B) - (D) show 2D sections of the 3D plot in (A), where trends for variation of two parameters are studied, by keeping the third fixed. In each of these plots, the shaded region represents the region for successful assembly, also based on criterion for limiting mechanical deformation.
2.2.3 Varying boundary conditions in the assembly system

2.2.3.1 Deformable Component on a Rigid Substrate

As seen previously in Section 2.2.1, it is possible to choose the intensity of acoustic excitation such that both the conditions for selective removal as well as initial component assembly are met, for successful assembly. It was also discussed in Section 2.2.2 how the mechanical properties of the component and template material can be chosen such that the deformations in the system lie in the purely elastic regime, though the actual parameter characterizing the system deformation depends on the boundary conditions of the mechanical system, as mentioned earlier in Section 2.1.2.

Here we collapse all of these criteria onto a single plot which can be used to determine whether the system falls within the predicted range for successful assembly or not. First we consider the case where the component material is deformable while the template material is relatively rigid. Then the deformation parameter, as discussed in Section 2.1.2, is the interference ratio \( \omega/\omega_c \). Figure 2.5 shows a plot for the interference ratio vs. the size of the system, expressed in terms of component radius \( R_c \) (flat template surface considered here for simplicity, note that this can easily be expressed in terms of an equivalent radius instead, using equation (2.17)). Figures 2.5(A) shows this trend for one particular value of the yield strength for the deformable component material, fixed at \( Y_c = 0.01 \) GPa. The other key mechanical properties of the system – the Poisson’s ratio for the component and the Young’s modulus for the component are kept fixed at mean values of 0.35 and 2.5 GPa respectively in Figure 2.5(A). The trend shows that the larger the component radius, the lower is the interference ratio for a fixed yield strength of the component material. The value of this interference ratio falls below one for component sizes lying to the right of the green dotted line. For successful assembly, it is predicted that the interference ratio value should be below one [17], as discussed previously in Section 2.1.2. This favorable region for operation of TASR in terms of component sizes is marked with the help of an
arrow next to the green dotted line, pointing towards the right. Also plotted on the y-axis is the ratio for the maximum intensity (remaining below which ensures that retention torque dominates removal torque for well-matched components during selective removal) to the minimum intensity (remaining above which ensures that the drag torque dominates the impedance torque during the initial assembly process). For successful assembly, it is predicted that the intensity should be above the minimum intensity value and below the maximum intensity [18], as also discussed in Section 2.2.1 previously. This favorable region for operation of TASR between the maximum and the minimum intensities is marked as the blue hatched area in the Figure 2.5(A). Thus, the region in the intensity/component radius space that meets all of the assembly constraints described here is the overlapping region between the blue hatched region and the area lying to the right of the dotted green line. This favorable region meeting all of the assembly constraints is indicated by a red hatching on the plot shown in Figure 2.5(A). These types of results can be used to design the system for successful assembly, by using the relevant values for fixed parameters in the system.

Similarly, another plot as shown in Figure 2.5(B) is used to identify favorable regions for assembly for a system in which the value of the component Young’s modulus and component Poisson’s ratio are kept fixed at 1 GPa and 0.35 respectively, and the value of the yield strength of the deformable component is now varied. It is observed that the lower the yield strength, the higher the interference ratio for a given size of the system, which also makes sense physically since a lower yield strength implies larger possibility of plastic deformation in the system. As before, the region of overlap between the region meeting constraints on intensity (blue hatched region) and the region satisfying deformability constraints (portion of plot where interference ratio is below a value of one) is the region identified as the favorable regime for operation with TASR. The same analysis can be now carried out for studying trends obtained upon variation of the equivalent modulus of the system, now keeping the yield strength and Poisson’s ratio for the component material fixed instead. Figure 2.5 (C) shows the
trends for variation in the value of the component Young's modulus, keeping the value of the
cOMPONENT yield strength fixed at 0.01 GPa and the component Poisson's ratio fixed at 0.35. Following
the same guidelines as before, favorable regions for operation with TASR can be identified.
Figure 2.5: (A) Plots showing interference ratio vs. the size of the system, expressed in terms of component radius $R_c$. Trends are shown for a value of yield strength $= 0.01$ GPa for the deformable component material. The Poisson’s ratio for the component material and the component modulus are also kept fixed at mean values of 0.35 GPa and 2.5 GPa respectively. Also plotted on the y-axis is the ratio for the maximum to the minimum intensity. The down pointing arrow shows interference values that meet the assembly constraint on limiting deformation in the system. For this value of yield strength, component sizes indicated by the right pointing arrow lie in the favorable range for assembly by meeting the deformability constraints while the blue hatched area shows regions that meet intensity constraints. Any overlapping regions between these two, such as the red hatched region in this particular figure, meet both constraints. (B) shows similar plots but with varying values of the yield strength for the deformable component, where value of the equivalent modulus and component Poisson’s ratio are kept fixed at 1 GPa and 0.35 respectively. Note that for the two higher values of yield strength, the requirement that the interference ratio have a value below one does not restrict the component size in the range shown. (C) shows plots with varying values of Young’s modulus for the component material, where values of component yield strength and component Poisson’s ratio are kept fixed at 0.01 GPa and 0.35 respectively. For a modulus of 1 GPa, the component sizes need to be above 1 μm radius, for 2GPa, they need to be above 2 μm radius while for the value of 5 GPa radius, there is no region of overlap in the current plot. However, this can be fixed by tuning the constant parameters in the current analysis.
2.2.3.2 Rigid Component on a Deformable Substrate

The case of rigid or deformable micro-spherical components assembling on deformable substrates is also of interest to us as mentioned in Section 2.1.2 since the possibility of creating deformable, replicable templates from rigid template masters would reduce the total fabrication cost and holds potential for simple, low-cost mass production of templates. Therefore, analyzing these cases theoretically is of significance. This section highlights the approach for assessing deformations in deformable substrates when indented by microspheres made of a relatively rigid material.

As stated before, a deformable sphere contacting a rigid substrate is different from a rigid sphere contacting a deformable substrate because different constraint conditions lead to different behavior. Therefore, in order to address the case of the indentation of a deformable substrate by a rigid microsphere, a different parameter must be used to decide the nature of the contact between the two mating surfaces. Although the constraint conditions are different, the underlying concept of the transition from the elastic to the elastic-plastic regime remains the same. As discussed in section 2.1.2, the parameter of interest describing the nature of deformation in the substrate, $\gamma'$, should not exceed a limiting value of 2.5 for the deformation in the substrate to be completely elastic. Above this value, plastic deformation in the substrate begins.

Figure 2.6 shows plots for gamma ratio $\gamma'$ vs. the size of the system, expressed in terms of component radius $R_c$. As before in Section 2.2.3.1, Figure 2.6(A) shows these trends for different values of Young’s modulus for the relatively rigid component material. The other key mechanical properties of the system – the Poisson’s ratio and the yield strength for the substrate material – are kept fixed at mean values of 0.4 and 0.007 GPa respectively in Figure 2.6(A), chosen to reflect a deformable substrate material such as PDMS, which is typically used for template replication. The trends show that higher the modulus, the higher is the value of $\gamma'$ for the same size of the system. For
successful assembly, it is predicted that the interference ratio value should be below 2.5, as discussed previously in Section 2.1.2. This favorable region for operation of TASR is indicated by the down pointing arrow in the figure, to identify the values of deformation lying in the safe regime. Also plotted on the y-axis is the ratio for the maximum intensity (remaining below which ensures that retention torque dominates removal torque for selective removal) to the minimum intensity (remaining above which ensures that the drag torque dominates the impedance torque). For successful assembly, it is predicted that the intensity ratio should lie between the maximum and the minimum intensity values [18], as also discussed in Section 2.2.1 previously. This favorable region for operation of TASR is marked as the blue hatched area in the Figure 2.6(A). Thus, the region on the graph that meets all of the assembly constraints described here is the overlapping region between blue hatched region and the region that meets deformability constraints, and can be used to design the system for successful assembly, by using the relevant values for fixed parameters in the system.

Similarly, another plot as shown in Figure 2.6(B) is used to identify favorable regions for assembly where the value of the Young’s modulus and Poisson’s ratio are kept fixed at 0.0005 GPa and 0.35 GPa respectively, and the value of the yield strength of the deformable component is varied. It is observed that the lower the yield strength, the higher the value of $\gamma'$ for a given size of the system, which again makes sense physically since a lower yield strength implies larger possibility of plastic deformation in the deformable substrate material. Again, as before the region of overlap between regions satisfying individual constraints on intensity and deformability are the regions identified as the favorable regime for operation with TASR.

Using plots as shown in Figures 2.5-2.6 and the guidelines provided in Section 2.2, a complete picture of the TASR framework, in terms of all major factors that play a role in determining assembly success can be derived, and the assembly system can be designed appropriately to meet the required target.
Figure 2.6: (A) Plots showing gamma ratio $\gamma'$ vs. the size of the system, expressed in terms of component radius $R_c$. Trends are shown for different values of Young’s modulus for the deformable substrate material. The Poisson’s ratio and the yield strength for the substrate material are kept fixed at mean values of 0.4 GPa and 0.007 GPa respectively. Also plotted on the y-axis is the ratio for the maximum to the minimum intensity. The down pointing arrow indicates the threshold for the limiting deformation in the system while the blue shaded area shows regions that meet intensity constraints. (B) shows similar plots but with varying values of the yield strength for the deformable substrate, where value of the Young’s modulus and Poisson’s ratio are kept fixed at 0.0005 GPa and 0.4 GPa respectively. As the deformation ratio is below the critical value for all of the cases shown here, substrate deformability does not limit the component radii that may be successfully assembled under these circumstances.
2.2.4 Modeling systems chosen for assembly

Using the guidelines provided in the above sections, the systems for the set of assembly experiments considered here were modeled. The first set of experiments dealt with the assembly of mammalian and non-mammalian cells on the patterned silicon surfaces. This set of experiments falls into the category of the systems where the assembly component is deformable while the substrate is a rigid material, and is hence described by the approach outlined in Section 2.2.3.1, where Hertzian contact theory is used to approximate the measure of deformation in the system and decide the nature of contact. These mechanical considerations are combined with choosing the optimal level of intensity provided to the system to ensure that both the selective removal model and the initial component assembly model predict successful assembly. In the experiments considered here, the cells considered were nearly spherical, so it was a fairly good approximation to model both the mammalian and non-mammalian cells used in current work as spheres with mean values of mechanical properties being a Poisson’s ratio, $v \sim 0.45$ and a Young’s modulus $E \sim 1 \text{ KPa}$ [56, 57]. For a single cell with mean diameter of 15 $\mu$m on a silicon surface (as was the case for the non-mammalian cell line of SF9 cells used in current work), the value of the interference ratio $\omega/\omega_c$ was then calculated to be $6.47 \times 10^{-06}$, using equation 2.24. Similarly, for a single cell with mean diameter of 20 $\mu$m on a silicon surface (as was the case for the mammalian cell line of mouse-derived non-small lung cancer cells used in current work), the value of the interference ratio, $\omega/\omega_c$ was calculated to be $4.4 \times 10^{-06}$. Both of these values are well below the critical limit of 1 where some portion of the deformation becomes permanent and the shape of the deformable components undergoes some change. Thus, the deformations in the system chosen were predicted to be well below the critical limit given by the TASR model for assessing deformations. Then, choosing the right value of intensity and interfacial energy in the system, while also ensuring that biological constraints to the system, such as cell viability, are met, one can optimize the assembly
parameters to ensure successful assembly, as predicted by the integrated TASR framework. The second set of experiments considered here concerned the assembly of deformable polystyrene microspheres that were 2 μm in diameter on deformable template surfaces in PDMS. The material properties and system geometry used in this case correspond closely to those used to produce Figures 2.5 and 2.6, in which successful assembly was predicted. In this case, both the template and the substrate material were deformable. The first step was therefore to assess which of the two materials was more deformable than the other such that the less deformable material could then be treated as relatively rigid compared to the more deformable material. Then a theoretical framework which was well fitted to either one of the two frameworks described in Sections 2.2.3.1 or 2.2.3.2 was extended as described in 2.1.2.2 to describe the system. In this particular case for the contact between a PS microsphere and a PDMS substrate, it was clear that the PDMS substrate was much more deformable compared to the PS sphere based on a simple comparison of the mechanical properties of the two materials, recorded earlier in Table 2.1. Both the Young’s modulus and the yield strength are lower for PDMS than PS, on account of which PS was treated as a relatively rigid material compared to PDMS, and the theoretical framework described in Section 2.2.3.2 was used to assess the deformation degree in this particular system. The value of the parameter $\gamma'$, which shows the extent of deformation in the deformable PDMS substrate was then calculated to be 0.0188 using equation 2.25, which is well below the critical value of 2.5, indicating that this system should also assemble successfully if the right levels of intensity and interfacial energies are chosen for the system, the combination of which are determined using the framework described previously in Section 2.2.1.

Finally, the last set of experiments considered here was the assembly of cylindrical micro-components made of TMPTA on templated silicon surfaces. This set, similar to the first set, was again in the category of the systems where the assembly component is deformable while the substrate is a rigid material, and hence could be described using the approach outlined in Section 2.2.3.1, though the
framework would need to be extended to cylindrical components in this case. The exact values for the
deformation ratios are not recorded here, since the models in the current work are not developed fully
to describe the case for cylindrical components, even though the guidelines for doing so are laid out
subsequently in Section 2.3.

It is noted that while most of the systems considered here experimentally meet the criteria for
deformations below critical value in the integrated TASR model and successful assembly is predicted
as far as the deformations in the systems are concerned, there is a range of values for intensities and
interfacial energies in which only marginal success may be achieved, as can be observed by creating
the relevant parameter plots for the particular system like those shown in Figures 2.5 and 2.6, while at
an optimal value the assembly success is the highest. This is also confirmed later experimentally in
Chapters 5, 6 and 7 where it is recorded that there at the optimal level for these two parameters, the
assembly success is the highest, quantified in terms of ‘assembly yield’.
2.3 Model for cylindrical component assembly

Until now, the entire theoretical framework covered has been discussed only in the context of spherical micro- and nano- components. It is, however, crucial to have the ability to extend the integrated TASR model to anisotropic assembly components as well, such as cylinders. Cylinders in particular also form an important case for analysis in the context of self-assembly not only due to the wide range of applications possible with cylindrically-shaped components, but also because it is quite convenient to

![Diagram of cylindrical contact](image)

**Figure 2.7:** The schematic diagram showing contact between two cylinders, one with radius $R_1$ and the other with radius $R_2$, where the length of the cylinder with radius $R_1$ is $l$, as shown in (A). This case of contact between two cylinders can be extended to the case for assembly of micro-cylindrical components in TASR, where the radius $R_2$ can be taken to be infinity for the case where the component of radius $R_1$ contacts flat template region, and the radius $R_2$ can be taken as a negative number with the value equal to radius of assembly site when the component lies inside a hemi-cylindrical assembly site. As shown in (B), the contact area is of width $b$ and length $l$, equal to the length of the assembly component (cylinder with radius $R_1$).
create assembly sites that are cylindrically-shaped using commercial micro-processing techniques. While the key concepts used to model the assembly of cylindrical components remain essentially the same as those used in the integrated TASR model for spherical components described earlier in Section 2.2, some quantitative details on how to calculate the relevant parameters defining the constraints may vary. For example, because the sphere has a uniform radius of curvature in all directions while a cylinder has two different radii of curvature along two mutually perpendicular axes, the geometry of the problem becomes different when analyzing the mechanics for the interaction between a cylindrical component lying inside a hemi-cylindrical assembly site on the template (or on a plane as in the case of the flat portions of the assembly template). While the case of contact between a sphere and flat (or between a spherical component and a hollow hemispherical assembly site) involves point contact between the sphere and the template, the case of contact between two externally touching cylinders, or one cylinder inside another or of a cylinder and a plane is that of line contact, as shown in Figure 2.7(A). The same equations as described earlier in Section 2.1.2 for calculating Equivalent Radius $R_{eq}$ and Combined Modulus $E'$ for spherical components hold for the case of cylindrical components also, though. For the case of a contact of a micro-cylindrical component with a plane, we set the diameter of the flat template portion $R_t$ in the equation 2.17 to infinity and for the case of concave/internal surface we set its diameter as a negative value, equal to the mean diameter of the hemi-cylindrical assembly site, as done also for spherical components and hemispherical assembly sites. However, while the region of contact between a spherical component and a flat surface is a circle of radius $a$ (expression derived earlier in equation 2.20), the contact area for the case of cylindrical components is a rectangle with length $l$ equal to the length of the cylindrical component and width $b$. Extending the Hertzian contact theory [50] to this case, the width $b$ for the contact area region, as also shown in Figure 2.7(B) is then calculated to be as follows:
\[ b = \left( \frac{4PR_{eq}}{\pi dE'} \right)^{1/2} \]  

(2.41)

where as before in Section 2.1.2, \( P \) is the contact load. The maximum pressure \( P_{\text{max}} \) which is also called the Hertz (compressive) stress is the maximum value of the contact pressure at the component/template contact and is given in this case for cylindrical systems by the expression:

\[ P_{\text{max}} = \frac{2P}{\pi bl} \]  

(2.42)

and the mean pressure over the contact area is given as:

\[ P_{\text{avg}} = \left( \frac{\pi}{4} \right) P_{\text{max}} \]  

(2.43)

Building upon these values of the contact pressure and the known critical load limits of the system for entering the plastic regime, these expressions can then be used to find the deformations as are a part of the system and assess whether the assembly is successful or not, similar to the theory presented for systems comprising spherical assembly components and matching hemi-spherical assembly sites.

Once the contact area is calculated by multiplying the width \( b \) of the contact region with the length \( l \), the change in contact area as the cylindrical component rolls inside the hemi-cylindrical site, is calculated using a similar procedure as is followed for the case of a spherical component rolling inside assembly hole (using guidelines discussed previously in Section 2.1.1.2, also discussed in detail in [15, 18]. Knowing the values of the interfacial energy \( \gamma \) and the value of the roughness coefficient \( C_r \) for the surface, the retention moment is then calculated knowing the value \( d\Phi \), which is the differential change in the rolling angle via rotation of the sphere, as calculated previously in equation (2.4) for the case of spherical components. This value of retention moment can then be compared with the value of removal moment, also found as discussed previously in detail in Section 2.1.1.2. Comparison between the two values then determines the selectivity of removal in the TASR assembly process and governs the assembly yield. It is thus noted that only the geometry of the system changes with the change in the
component shape, for the purposes of contact area calculation and the rest of the analysis for the system follows the same path as that described in detail in previous sections for spherical assembly components.
2.4 Summary

In summary, Chapter 2 provides a general theoretical framework to model any system used in TASR. This theoretical framework combines the main ideas from three models proposed in earlier work on TASR and offers an integrated model to approach any system, whether rigid or deformable. As discussed in this chapter, the three fundamental concepts behind modeling a TASR-based system include the modeling of selective removal [15, 16], modeling of mechanical deformations in the system [17], and lastly, the modeling of initial component assembly [18]. The first model in this list predicts that the TASR process is successful if the torques that promote component retention in a well-matched location exceed the torques that promote component removal, while the reverse is true if the component lies in an assembly site location where it is not well-matched to the assembly site. In particular, the ratio of the retention torque to the removal torque must exceed a value of one for the assembly yield to be high. This is the main metric characterizing the success of the TASR process, along with the degree of selectivity achieved. This model, while originally created to describe rigid systems only, is also applicable to deformable systems, provided that the second model predicts successful assembly. The second model describes deformable systems and by assessing the mechanical deformations in the system, predicts that the assembly is successful provided that the deformations remain below a critical limit. This critical limit is variable depending on which portion of the assembly system – the component, the template or both are deformable, since this governs the boundary conditions imposed on the mechanical system. The main idea in this part of the model is that since TASR depends on shape and size matching, if the deformations are in the purely elastic regime, there is no permanent change in the shape of the system and the assembly is predicted to be successful. On the other hand, plastic deformation leads to dissipation of energy in the system and change in shape, so is not desirable and might affect the assembly adversely, depending on the degree of deformation and
the material used. Lastly, the third model discussed here deals with the initial component assembly in the assembly fluid medium in TASR. This model states that in order for the selective removal forces to come into play inside the assembly site, the fluid forces must be strong enough to drive the component inside the assembly site in the first place. This is determined by the competition between the in-plane fluid torques that try to roll the component inside the hole vs. the impedance torques due to change in contact area that prevent the entry of the component inside the hole. In order for the initial component assembly to be successful, the former must be able to dominate the latter.

The integrated TASR model then combines all of these ideas into a single framework. This model uses the three criteria for successful assembly and studies variations in the criterion parameters with variation in system properties, such as the interfacial energy of the system, the size scale of the system, mechanical properties of the component and template material, and intensity of acoustic excitation, among others. By studying these variations graphically, the integrated model identifies favorable regions for operation of TASR, where all three models predict successful assembly. It is possible to then design one’s system to fall within this favored region for assembly and tune experimental parameters to the optimal level so as to be able to achieve the desired goals using TASR. The chapter covers all of these aspects and also elaborates on the model for anisotropic shapes, since the models discussed above were developed in the context of isotropic shapes, and it is equally crucial to have the ability to model shapes other than spheres for a wide variety of applications.
Chapter 3

Template Fabrication

3.1 Concept of Fabrication

As discussed in previous chapters, TASR is a form of assembly based on shape and size recognition at the micro- and nano-scales. The main idea behind the technique is that assembly components that match in their shape and size to the shape and size of the assembly sites on the template are trapped inside those sites if other conditions are favorable for assembly. Thus, in order to assemble spherical micro-components using TASR, one would need to create hemispherical holes of matching shape and size on the template. Similarly, in order to trap cylindrical components, one would need to create hemi-cylindrical pits that match the components in shape and size. In this fashion, one can go about creating matching assembly pits on the template depending on the structure and the dimensions of the components to be retained inside those pits. This is the guiding concept behind the fabrication of the assembly templates for TASR.

The templates for most of the experiments presented here comprise silicon dies, since it serves as a suitable and compatible substrate material for the purposes of TASR, and also since commercial micro-fabrication technology is very well advanced for structuring the desired features onto a material that is silicon-based. Fabrication of the patterned silicon templates follows an approach that is conceptually similar to the ones described in [15, 16], but the actual fabrication procedure varies according to the components to be assembled onto the fabricated template. The main idea is that pattern transfer and development produce openings in a resist layer. The underlying layer, which might be silicon or silicon dioxide, is then etched isotropically to produce, for instance, the near hemispherical holes required for the assembly of microspheres.
In this work, for creating assembly sites with relatively smooth sidewalls, the silicon templates are coated with a layer of silicon dioxide into which the template holes (that is, the assembly sites) are etched using an isotropic wet etch (buffered oxide etch, or BOE). Because a well-controlled wet etch is available for oxide, this produces smooth sidewalls with reasonable edge control, ideal for assembling small polymer- or silica-based spherical micro-components, which also possess very smooth surfaces. For creating assembly sites with much larger depths, it is less convenient to wet etch an oxide layer since the etch is very slow and also requires large thickness layers of high quality oxide to be grown or deposited in which the features can be etched. An alternative that avoids the need for depositing thick oxide layers would be to use a wet isotropic etch directly in silicon, such as HNA (hydrofluoric, nitric, acetic) etch. However, wet isotropic etches in silicon are less well-controlled than those in oxide. To

![Diagram](image)

**Figure 3.1**: Schematic illustration of sequence for fabrication of assembly template used in TASR. (A) Small patterns are exposed in resist coating the substrate (typically silicon or silicon dioxide) using lithography techniques. (B) Isotropic etching from the exposed spot produces the desired hemispherical sites for spherical component matching. (C) Mask layer is stripped and, if necessary for the particular assembly situation, template may be subsequently functionalized with a self-assembled monolayer (SAM) to make it hydrophobic.
ensure good control and reproducibility, deeper assembly sites were in some cases created using dry plasma etches instead to form approximately hemispherical profiles for assembly sites. Dry etches were used in particular to create sites for assembling biological cells, which are fairly large in size.

A schematic of the general fabrication process is depicted in Figure 3.1 and illustrates the main concept behind template fabrication used in TASR. In this work, there are three main types of self-assembly that have been demonstrated, and each assembly system, with its own specific set of sizes and materials, requires the creation of its own specialized set of templates. The first type of template is for the assembly and sorting of biological cells (SF9 cells and mouse lung cancer cells). The second set of templates is for the assembly of components with anisotropic shapes, such as micro-cylinders. Fabrication of templates for these two purposes is described in Sections 3.3.1 and 3.3.2, while the assembly experiments using those templates are covered in Chapters 5 and 7 respectively. The last set of templates is created for demonstrations of TASR-based assembly on deformable substrates, unlike the relatively rigid silicon-based templates that have been used in all of the work on TASR so far. To create deformable templates, master templates are first created from a relatively rigid material such as silicon or silicon dioxide, and then replicas from a relatively softer material can be manufactured from those rigid master templates. This chapter includes the fabrication of the master templates for demonstration of assembly on soft substrates, described in Section 3.3.3, while the full details of how template replicas are fabricated from these masters and of the corresponding assembly demonstrations are described in Chapter 6. The details for each of these individual template fabrication processes starting with the template layout are described in the sections that follow.
3.2 Template Layouts

As highlighted in the previous sections, assembly sites are created on the template to retain components of matching shapes and sizes using TASR. The template layout is created to meet the specific requirements of the system to be assembled, including the shapes and sizes of the sites, total number of sites, spacing between sites and the density of assembly sites. Each of the three cases under consideration, namely assembly of spherical (isotropic) biological cells, assembly of anisotropic (non-spherical) components, and assembly of spheres on deformable substrates, had its own individual template layout, the details of which are discussed below.

3.2.1 Cell Template Layout

For the assembly of isotropic components, the template is patterned with near hemispherical holes that match the sizes of the components to be assembled. In this set of experiments, the assembly components were biological cells that are spherical and come in a range of diameters (from 12 to 25 μm). The experiments were conducted with two different cell lines, namely SF9 insect cells and mouse-derived non-small lung cancer cells, with the cancer cells as prepared in [58]. In order to establish the size selectivity of cell assembly using TASR, each template was patterned to contain multiple arrays of nearly-hemispherical holes of two different diameters (with the two sizes chosen depending on the cell line used). Thus, each hole on the template was designed to have a single cell trapped inside it. The spacing between adjacent assembly sites was also varied from array to array in order to study its effect on assembly yield obtained using TASR.

Figure 3.2 shows a CAD layout of the chip designed for cell assembly using TASR with grids of holes arranged in different 2-D patterns. The chip is designed with the different types of assembly sites appearing in multiple locations on the chip surface. This helps prevent any variations in assembly conditions across the beaker from
Figure 3.2: (A) CAD layout of the assembly chip designed for cell assembly using TASR with grids of holes arranged in different 2-D patterns. The chip is designed to be a square die with an edge of 8 mm, contains a total of 6000 assembly sites, with 24 arrays of assembly sites and other patterns, and has spacing between consecutive sites varied from 4 μm to 40 μm. The two different colors on the template correspond to two different masks, shown individually in (B) and (C) which are together used to define patterns for hemispherical holes of different sizes on the template for selective cell-trapping. (D) shows a zoomed-in portion of mask in (B) featuring a grid of 1 μm diameter circles, which are starting spots for assembly sites, seen more clearly in (E).

being mistaken for variations in assembly effectiveness across different types of assembly sites. The layout was designed to contain a total of 6000 assembly sites on any single template chip. This high number was chosen to allow demonstration of the capability for high assembly yield and high assembly density using TASR. In this case, the starting spots on the mask pattern from which the assembly sites were created were circles of 1 μm diameter. This feature size was chosen to minimize the size of the resist openings without dropping below the resolution
limits of the lithography system (in this case a contact mask aligner). As also discussed in extensive detail in [15, 17], for the purposes of assembling isotropic components, the larger the starting spot size, the larger is the deviation from the desired ideal hemispherical profile (as confirmed visually in Figure 3.8 later from the actual hole profiles). Hence the smallest possible spot size given the system constraints is used here. Finally, the spacing between the assembly sites is varied between 4 and 40 μm to study the effect of site spacing on assembly. Each die is designed to be a square with 8 mm edge length. This is the maximum allowable dimension for the TASR assembly template given the constraints posed by the experimental set-up used in this work. If the chip size were increased still further, the acoustic flow field would be adversely affected as the template edges will block the circulation of the assembly fluid medium and create undesirable flow patterns in the system [15, 16]. While a smaller chip size does not adversely affect the circulation, the maximum allowed dimension is chosen here in order to maximize number of assembled cells in a given experimental run.

The same template layout was used for both cell lines since the same 2-D pattern and starting spot size was desired. However, the detailed fabrication processes and the final sizes of the assembly sites fabricated using this mask layout were different by design. The different template geometries accommodate the difference in the mean sizes and size ranges for the two cell lines.

3.2.2 Cylinder Template Layout

For the assembly of cylindrical micro-components, the template is patterned with near hemicylindrical holes that match the sizes of the components to be assembled. In this set of experiments, the assembly components were cylindrical and were created in two different nominal diameters (8 μm and 26 μm) and three different lengths (20 μm, 50 μm and 150 μm). In practice, some of the components were not perfect cylinders, as is discussed further in Section 7.2, but the
template was designed for ideal cylinders. In order to establish the size selectivity of the anisotropic component assembly using TASR, each template was patterned to contain multiple arrays of hemi-cylindrical holes of these two diameters (8 μm and 26 μm) to demonstrate diameter matching. The starting feature sizes were lines that were 1 μm in width (for similar reasons as discussed in Section 3.2.1) and of varying lengths (80 μm, 100 μm and 120 μm), so as to assess the effect of length of the assembly sites on the assembly yield.

Unlike the cell assembly template described in Section 3.2.1, in which each hole was designed to hold only a single cell, here, while each hole on the template was designed to hold a single component inside it diameter-wise, it could hold multiple components placed adjacent to each other length-wise, depending on the ratio of the component and the site lengths. The spacing between adjacent assembly sites varied from array to array, with edge to edge spacings ranging from 50 to 300 μm. Figure 3.3 shows a CAD layout of the micro-cylinder assembly chip with grids of assembly sites arranged in different 2-D patterns. As before, the patterns of assembly sites are

Figure 3.3 (A) CAD layout of the assembly chip designed for assembly of cylindrical-shaped micro-components using TASR, with grids of holes arranged in different 2-D patterns. The chip is designed to be a square die with an edge of 8 mm, contains a total of 912 assembly sites and has spacing between consecutive sites varied from 50 μm to 300 μm. The two different colors on the template correspond to two different masks, which are together used to define patterns for hemi-cylindrical holes of different sizes on the template to demonstrate selective assembly, a zoomed in portion of which is shown in (B). (C) shows a further zoomed section of one of the masks, where starting spots for individual assembly sites are seen.
repeated across the die surface to help prevent the spatial variations in the beaker from being confused with the variation of yield across assembly sites. This chip was designed to contain a total of 912 assembly sites on any single template, and the die size was again an 8 mm square.

3.2.3 Master Template Layout for Deformable Replica Templates

In this set of experiments, the assembly of isotropic, polymer-based micro-components on deformable substrates was to be demonstrated. In order to compare the results obtained from these experiments with those from previous work [17] describing the assembly of polymer-based micro-components onto rigid substrates using TASR, the same template layout as used in [17] was also used here. The basic idea behind the design of this template die is discussed here briefly; more details on layout can be found in [17]. It is to be noted Amelia Servi, who was a collaborator on the current work as a part of her undergraduate research project, designed the layout and fabricated the templates used for the set of experiments discussed here.

In this set of experiments, the assembly components were spherical and were 2 μm in diameter. Thus the template was patterned with near hemispherical holes that match the sizes of the components to be assembled. Since the component and hence feature sizes were small in these experiments, it was necessary to have small starting spot sizes so as to have good control over hole dimensions while maintaining close proximity to the desired hemispherical profile. To achieve small starting spot sizes, the features were created using electron beam (e-beam) lithography in this case instead of contact lithography as used in the previous sections. The template die layout had to be created accordingly to fit the requirements of the lithography system.

The CAD layout for the single die pattern used for our templates is shown in Figure 3.4. The e-beam tool exposes spots in the resist according to the CAD pattern. There are 15 arrays of spots in the pattern, including arrays with resist exposure sizes of 45, 50, 55, 60, 65, 70, 100, 200 and 500 nm. The
Spot sizes were varied to study the effect of the starting spot size (and hence hole size, shape, and degree of the hole’s approximation to a hemisphere) on the assembly yield. Each die was created to be a square with 5mm edge length.

**Figure 3.4:** CAD layout of mask pattern used for fabrication of templated surface by electron-beam lithography. The numbers below each of the 15 grids shows the starting spot size used for that grid of spots on the e-beam tool. Starting spot size is varied to study the effect of varying hole shapes on the assembly using TASR (Image courtesy of Amelia Servi)

Where X is the diameter of the holes in the array which is to the left of the array labeled 'mix'
3.3 Template Fabrication

3.3.1 Cell Template Fabrication

The template fabrication procedure involves the creation of patterned nearly-hemispherical sites on a template into which nearly-spherical SF9 cells will assemble based on size matching. The templates for the experiments with biological cells comprise silicon dies into which the template holes (that is, the assembly sites) are etched. Fabrication of the patterned silicon template follows the approach outlined below in detail. The process outline followed below is also depicted schematically in Figure 3.5.

1. The fabrication procedure for the templates starts with a blank 6” silicon wafer. HMDS (hexamethyldisilazane) is first deposited onto the wafer, which is then prebaked for 5 min at 220°C and thereafter cooled for 5 min.

2. Standard positive thin photoresist (OCG 825-20CS) is spun onto the silicon wafer using a spin program involving dispensing the resist at 500 rpm for 8 seconds, spreading at 750 rpm for 8 seconds, and finally ramping the speed up to 5000 rpm for 30 seconds, resulting in a resist layer approximately 1.3 μm thick.

3. Prebake is carried out at 90°C for about 30 minutes to improve resist adhesion to the substrate, avoid mask contamination and prevent erosion during development.

4. In order to pattern hemispherical holes in the template, contact lithography is used to expose small spots in the thin resist layer. The CAD layout of a single die pattern used for these templates has been discussed previously in detail in Section 3.2.1 and shown graphically in Figure 3.2. Exposure of the first pattern on the wafer is carried out (Electronic Visions Model EV620 Mask Aligner) in ‘hard contact’ mode. The process is done in four intervals of 5 seconds each with a cooling time of 10 seconds between each exposure interval to reduce resist heating.
5. Pattern development is then done in OCG 934 1:1 developer for about a minute to produce the corresponding openings in the resist layer.

6. A post-bake is then carried out on the developed wafer for 30 minutes at 120°C to promote hardening of the resist.

7. After post-bake, a nearly-isotropic etch using SF$_6$ gas is carried out in a deep reactive ion etching (DRIE) tool (ST Systems Multiplex ICP) to produce a first set of holes in silicon in the exposed regions. The etch is done for 15 minutes to achieve a depth of 6 µm for the first set of holes for assembly. To lowest order, the isotropic etch produces quasi-hemispherical holes with hole radius approximately equal to the etch depth.

8. In order to remove the resist over the first patterned layer, the wafer is subjected to a piranha clean comprising a solution that is 3 parts sulfuric acid and 1 part hydrogen peroxide.

9. The first pattern is then protected by covering it with an oxide layer of approximate thickness 0.5 µm, deposited (on Novellus Concept One tool) using a plasma-enhanced chemical vapor deposition

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Figure 3.5: Fabrication process flow showing steps involved in creating template for demonstrating cell assembly. Two cycles involving lithography and isotropic etching are carried out to create holes of two different sizes for demonstration of size-selective assembly.
system (PECVD). The tool contains a multiple-station continuous processing sequence, which
cycles each wafer through five separate but identical deposition stations, producing an averaging
effect, which results in enhanced film uniformity, reduced incidence of "pin holes" and improved
process repeatability.

10. A second cycle of lithography is done by spinning thin resist over the oxide, and then repeating the
exposure and pattern development cycle.

11. A Buffered Oxide Etch (BOE) is carried out to open the oxide in the developed regions comprising
the second pattern. The BOE, which consists of a 7:1 solution of 40% NH₄F: 49% HF, is carried
out for 10 minutes with degas applied at the highest power for 5 out of every 30 seconds. The
wafer is subsequently rinsed with water and dried with nitrogen.

12. A second etching cycle is then done to etch the silicon beneath the open regions in oxide. This etch
is done for 28 minutes to achieve a depth of 11 μm for the second set of holes. This produces a 2-D
pattern comprising arrays of holes of two different sizes for the assembly of SF9 cells. Holes of
more than one size are used to establish the simultaneous size selectivity of the assembly process.

13. The wafer is again subjected to a piranha clean to strip the resist.

14. A BOE is now done to remove all of the oxide from the wafer. The BOE, which consists of a 7:1
solution of 40% NH₄F: 49% HF as before, is carried out for 20 minutes with degas applied at the
highest power for 5 out of every 30 seconds.

15. Thin resist is then again deposited onto the wafer to uniformly protect all the features before die-
saw.

16. The wafer is then die-sawed (Disco abrasive system Model DAD-2H/6T) to produce 8 mm x 8mm
assembly templates, each containing several arrays of quasi-hemispherical holes, which were two
of the three sizes - 6, 7.5 and 11 microns deep, and are designed to trap spherical SF9 cells of
matching sizes inside them.
The template was finally cleaned using piranha, followed by rinses in water and then 70% ethanol to disinfect the surface for cell attachment and assembly.

Figure 3.6 (A) and 3.6 (B) show optical micrographs of arrays of holes of different sizes on a template produced using the fabrication procedure outlined here. Scanning electron microscopy (SEM) was used to determine the as-fabricated profiles of the assembly sites. Figure 3.6 (C) shows an SEM image of a 2-D pattern of 15 μm diameter holes that spells out “MIT”. Top view and a cross-sectional image of one of the 15 micron diameter holes are shown in Figure 3.6 (D) and 3.6 (E) respectively.

To lowest order, the isotropic etch produces hemispherical holes with hole radius approximately equal to the etch depth. Careful attention to resist adhesion and etch procedures, along with small starting openings in the resist, enable a good approximation to the ideal hemispherical shape. The finite initial spot size results in etched holes that deviate slightly from the ideal hemispherical shape; the larger the initial spot size, the larger the deviation. Although the fabrication process for templates outlined here requires significant time investment as well as precision, polymer replication techniques are expected to offer a more rapid, less expensive means of creating and mass producing assembly templates in the future.
Figure 3.6: (A) Optical micrograph of a portion of the assembly template for trapping nearly spherical SE9 cells after fabrication, showing grids with holes of different sizes and spacing. (B) Optical micrograph showing another portion of the template with different 2-D patterns (C) An SEM image (top view) of a 2-D pattern spelling out “MIT” on the template. Each circle in the 2-D pattern is a quasi-hemispherical hole that is 15 microns in diameter and is etched using SF₆ plasma etching. (D) An SEM image (top view) of one of the holes forming the 2-D pattern in (C). (E) Cross-sectional view of the hole shown in (D)
3.3.2 Cylinder Template Fabrication

The template fabrication procedure involves the creation of patterned hemi-cylindrical sites on a template into which nearly cylindrical components will assemble based on size matching. The templates for all of the experiments presented here comprise silicon dies into which the template holes (that is, the assembly sites) are etched. Fabrication of the patterned silicon template follows the approach outlined below in detail.

1. The fabrication procedure for the templates starts with a blank 6” silicon wafer.
2. 10 μm of silicon dioxide is deposited (on Novellus Concept One tool) onto the wafer using a plasma-enhanced chemical vapor deposition system (PECVD).
3. HMDS (hexamethyldisilazane) is first deposited onto the wafer, which is then prebaked for 5 min at 220°C and thereafter cooled for 5 min.
4. Standard positive thin photoresist (OCG 825-20CS) is spun onto the silicon wafer using a spin program involving dispensing the resist at 500 rpm for 8 seconds, spreading at 750 rpm for 8 seconds and finally ramping the speed upto 5000 rpm for 30 seconds resulting in a resist layer approximately 1.3 μm thick.
5. Prebake is carried out at 90°C for about 30 minutes to improve resist adhesion to the substrate, avoid mask contamination and prevent erosion during development.
6. In order to pattern hemi-cylindrical holes in the template, contact lithography is used to expose narrow lines in the thin resist layer, with feature (widths) sizes of 1 μm. The CAD layout of a single die pattern used for these templates has been discussed previously in detail in section 3.2.2 and shown graphically in Figure 3.3. Exposure of the first pattern on the wafer is carried out (Electronic Visions Model EV620 Mask Aligner) in ‘hard contact’ mode. The process is done in four intervals of 5 seconds each with a cooling time of 10 seconds between each exposure interval to reduce resist heating.
7. Pattern development is then done in OCG 934 1:1 developer for about a minute to produce the corresponding openings in the resist layer.

8. A post-bake is then carried out on the developed wafer for 30 minutes at 120°C to promote hardening of the resist.

9. After post-bake, a Buffered Oxide Etch (BOE) is then carried out to open the oxide in the developed regions comprising the first pattern. The BOE, which consists of a 7:1 solution of 40% NH₄F: 49% HF, is carried out for 20 minutes with degas applied at the highest power for 5 out of every 30 seconds. The etch is done to achieve a depth of 4 μm and produce the first set of holes for assembly. The wafer is subsequently rinsed with water and dried with nitrogen. To lowest order, the isotropic etch produces quasi-hemicylindrical holes with hole radius approximately equal to the etch depth.

10. In order to remove the resist over the first patterned layer, the wafer is subjected to a piranha clean comprising a solution that is 3 parts sulfuric acid and 1 part hydrogen peroxide.

11. An RCA clean comprising is carried before the subsequent step involving nitride deposition.

12. The first pattern is then protected by covering it with a nitride layer of approximate thickness 0.5 μm, deposited (on Novellus Concept One tool) using a plasma-enhanced chemical vapor deposition system (PECVD).

13. A second cycle of lithography is then done by spinning thin resist over the nitride, and then repeating the exposure and pattern development cycle.

14. The nitride is first etched in the developed regions using plasma etching in a deep reactive ion etching (DRIE) tool (ST Systems Multiplex ICP).

15. A second Buffered Oxide Etch (BOE) is then carried out to open the oxide in the developed regions comprising the second pattern. The BOE, which consists of a 7:1 solution of 40% NH₄F: 49% HF, is carried out for 40 minutes with degas applied at the highest power for 5 out of every 30
seconds, to achieve a depth of 8 μm for the second set of holes for assembly. The wafer is subsequently rinsed with water and dried with nitrogen. This produces a 2-D pattern comprising arrays of holes of two different sizes for the assembly of micro-cylinders. Holes of more than one size are used to establish the simultaneous size selectivity of the assembly process.

16. The wafer is again subjected to a piranha clean to strip the resist.

17. An etch is now done to remove all of the nitride from the wafer, using DRIE as above.

18. Thin resist is then again deposited onto the wafer to uniformly protect all the features before die-saw.

19. The wafer is then die-sawed (Disco abrasive system Model DAD-2H/6T) to produce 8 mm x 8 mm assembly templates, each containing several arrays of 4 and 8 micron deep quasi-hemicylindrical holes, which are designed to trap cylindrical components of matching sizes inside them.

20. The template was finally cleaned using piranha, followed by rinses in water and then ethanol.

The template is subsequently functionalized with a SAM to make it hydrophobic and promote attachment to the hydrophobic micro-cylindrical components. The detailed procedure to do so is discussed in Section 4.2.

Figures 3.7 (A) and (B) show optical micrographs of arrays of holes of different sizes on a template produced using the fabrication procedure outlined here. Scanning electron microscopy (SEM) was used to determine the as-fabricated profiles of the assembly sites. Figure 3.7 (C) shows an SEM image of a 2-D pattern of 26 μm diameter holes that spells out “MIT”. A 3-dimensional view of one of the holes in the 2-D pattern displayed in (C) can be seen in (D), while top-view and cross-sectional view of the same hole are visible in (E) and (F). Smooth sidewalls of the assembly sites are visible from these SEM images and provide for an excellent starting point for assembly using TASR.
Figure 3.7: (A) Optical micrographs of a portion of the template showing assembly sites of different sizes and in different spacings fabricated for demonstration of assembly of cylindrical-shaped micro-components inside them. (B) 2-D patterns created for demonstration of selective assembly on the template (C) SEM image of a 2-D pattern that spells out ‘MIT’ comprising cylindrical-shaped assembly sites (D) 3-D view of one of the assembly sites (E) Top view of an assembly site (F) Cross-sectional view of an assembly site similar to the one shown in (E), showing smooth sidewalls created using isotropic etching with BOE.
3.3.3 Master Template Fabrication

The templates for this set of experiments comprise silicon dies coated with a layer of silicon dioxide into which the template holes (that is, the assembly sites) are etched. It is to be noted that the templates used for the experiments discussed here have been fabricated by Amelia Servi, who was a collaborator on the current work as a part of her undergraduate research project. It should also be noted that the procedure for fabrication of these master templates has already been discussed extensively in detail in [37] and is being reviewed here since the same set of templates is used in current work for creating deformable replica templates, as described in greater detail later in Chapter 6. Fabrication of the patterned silicon template follows an approach that is nearly identical to the ones described in [15, 16].

An oxidized silicon wafer is coated with polymethylmethacrylate (PMMA). In order to pattern hemispherical holes in the template, e-beam lithography is first used to expose various arrays of small spots in the PMMA resist layer with spot sizes ranging from 45 nm to 500 nm. Pattern development produces corresponding openings in the resist layer. The underlying oxide is then etched isotropically with buffered oxide etch (BOE) to produce the near hemispherical isotropic holes required for the assembly of microspheres. A schematic of the fabrication process to be carried out is as depicted in Figure 3.1 earlier, and the details are described below.

1. The fabrication procedure for the templates starts with a blank 6" silicon wafer.
2. An RCA clean is carried out in preparation for the growth of thermal oxide on the wafer.
3. An oxide layer of approximate thickness 1.8 microns is deposited onto the silicon wafer using a recipe which is carried out in 3 iterations, of 200 minutes each at 1050°C.
4. The oxidized silicon wafer is then covered with PMMA, which acts as a positive resist for e-beam lithography, which allows for extremely high resolution (nanoscale) patterns to be defined. HMDS (hexamethyldisilazane) is deposited onto the wafer, which is then prebaked for 5 min at 220°C and thereafter cooled for 5 min. Subsequently, 950K PMMA (C10 Microchem) and chlorobenzene
(ACS, 99.5% Alfa Aesar) are mixed in a ratio of 6:19 by volume to achieve a final PMMA concentration of 2.4% in the mixture. This mixture is spun onto the wafer using a spin program of 500 rpm for 5 seconds and 3000 rpm for 90 seconds resulting in a resist layer 140 nm thick.

5. The coated wafer is then post-baked for 15 minutes at 220°C.

6. In order to pattern hemispherical holes in the template, electron beam (e-beam) lithography is used to expose small spots in the PMMA resist layer, with spot sizes ranging from 45 nm to 500 nm. The CAD layout for the single die pattern used for the templates is shown in Figure 3.4. The e-beam tool exposes spots in the resist according to the CAD pattern and defined beam dose. There are 15 arrays of spots in the pattern, including arrays with resist exposure sizes of 45, 50, 55, 60, 65, 70, 100, 200 and 500 nm. This pattern, as created by Amelia Servi, is written with a beam of aperture 30 μm, a power of 10 keV, a step size of 10 nm and a dose of 120 μAs/cm².

7. After the writing process is complete, the resist is developed in a solution of 1:2 MIBK/IPA at 25°C for 90 seconds to form the desired openings in the resist pattern.

8. This is followed by methanol, isopropanol, and water rinses and then by a Buffered Oxide Etch (BOE) to produce isotropic holes of the required dimension. The BOE etch, which consists of a 7:1 solution of 40% NH₄F: 49% HF, is carried out for 11 minutes with degas applied at the highest power for 5 out of every 30 seconds.

9. The template is subsequently rinsed with water and dried with nitrogen. The resulting pattern on the substrate is 1 micron deep, quasi-hemispherical holes.

10. In order to remove the resist over the patterned oxide layer, the wafer is subjected to a piranha clean comprising a solution that is 3 parts sulfuric acid and 1 part hydrogen peroxide.

11. Finally, the resist is stripped and the wafer is diesawed (Disco abrasive system Model DAD-2H/6T) to produce 5 mm x 5 mm assembly templates, each containing several arrays of 1 micron.
deep quasi-hemispherical holes that match (to varying degrees) the 2 micron diameter polystyrene microspheres to be assembled into them.

Atomic force microscopy (AFM) is used to determine the as-fabricated profiles of the assembly sites. A tapping mode atomic force microscope (AFM D3000 by Veeco Instruments) is used for this purpose, and the profiling is done using a silicon nitride cantilever probe that scans the surface of the template (in the x and y directions). To lowest order, the isotropic etch produces hemispherical holes with hole radius approximately equal to the etch depth. Careful attention to resist adhesion and etch procedures, along with small starting openings in the resist, enable an excellent approximation to the ideal hemispherical shape. The finite initial spot size results in etched holes that deviate slightly from the

![AFM Images](image)

**Figure 3.8:** Atomic Force Microscopy (AFM) images of holes with starting spot sizes (from (a)-(d)) of 50, 100, 200, and 500 nm. Smaller starting hole sizes correspond to better shape matching with spherical components and therefore higher anticipated assembly yield (Image courtesy of Amelia Servi)
ideal hemispherical shape; the larger the initial spot size, the larger the deviation. Therefore, the holes
with a smaller initial spot size are predicted to be a better fit for the spherical components, while the
holes with a larger initial spot size are predicted to be a worse fit. Figure 3.8 shows AFM images of
four quasi-hemispherical holes, each etched from a different size starting hole in the resist.
3.4 Summary

This chapter summarizes the procedures to be followed in order to fabricate templates for assembly using TASR. The main idea behind the TASR technique is that assembly components that match in their shape and size to the shape and size of the assembly sites on the template are trapped inside those sites if other conditions are favorable for assembly. Thus, for instance, in order to assemble spherical micro-components using TASR, one would need to create hemispherical holes of matching shape and size on the template. Similarly, in order to trap cylindrical components, one would need to create hemi-cylindrical pits that match the components in shape and size. In this fashion, one can go about creating matching assembly pits on the template depending on the structure and the dimensions of the components to be retained inside those pits.

Creation of the assembly template starts with the creation of the die layout for an assembly template. The template layout is created to meet the specific requirements of the system to be assembled, including the shapes and sizes of the sites, total number of sites, spacing between sites and the density of assembly sites. In this chapter, procedures for fabricating templates for three individual cases for assembly are described in detail. These three cases under consideration include the assembly of spherical (isotropic) biological cells, assembly of anisotropic (non-spherical) components, and assembly of spheres on deformable substrates. Each of these cases taken up here for experimental study has its own individual template layout and its own particular set of steps to be followed for template fabrication, which are outlined in this chapter. The templates for most of the experiments presented here comprise silicon dies, since it serves as a suitable and compatible substrate material for the purposes of TASR and fabrication technology for silicon substrates is well established. The process for creating deformable replica templates from these silicon master templates is described later in Chapter 7. The main idea in silicon-based template fabrication is that pattern transfer and development produce openings in a resist layer. The underlying layer, which might be silicon or silicon dioxide, is
then etched isotropically to produce, for instance, the near hemispherical holes required for the assembly of microspheres. Similarly, for the creation of hemi-cylindrical assembly sites, designed to trap micro-cylindrical assembly components inside them, a corresponding isotropic etch is carried out in the underlying substrate to the desired depth. While wet etches such as the Buffered-Oxide-Etch give relatively smoother sidewalls and profiles for the assembly sites, they typically are more time consuming. Dry etches like plasma etches, on the other hand, take lesser time but do not give the same level of smoothness as the wet etches. Ultimately, the etching technique and fabrication steps to be followed depends on the materials and the system chosen for assembly with TASR. While creation of silicon-based assembly templates is generally expensive and time-consuming, rapid replication techniques to create replicas of these silicon master substrates are utilized to make the fabrication technique much more scalable, low-cost and manufacturable.
Chapter 4

Experimental set-up

4.1 General assembly protocol

The assembly set-up for TASR is described in detail in this chapter. Experimental protocols include the creation and (when necessary) functionalization of the templates on which assembly is to be carried out, the functionalization of the components that are to be assembled (when necessary), and the actual assembly experiments themselves. Template fabrication has already been described in detail in Chapter 3 for each of the sets of experiments considered here, namely the assembly of biological cells on rigid substrates, the assembly of deformable, polymer-based microspheres on deformable substrates replicated from master silicon templates (as described later in Section 6.2) and finally, the assembly of cylindrically-shaped micro-components on rigid substrates. While experimental details for each of these experiments are addressed individually in Chapters 5, 6 and 7 respectively, the general experimental procedures and experimental set-up common to all of these experiments is described here.

In all of the cases, the template created is a die with a patterned upper surface onto which the assembly components can assemble. Lithography and isotropic etching techniques are used to pattern the template surface with holes that match in shape and size to the components that are intended to assemble inside them. The template is then functionalized (when necessary) in order to make it hydrophobic, so as to promote adhesion in a water-based assembly fluid medium. In this work, template functionalization is done when the template is made from silicon or silicon dioxide-coated silicon and the assembly experiments are carried out in a water-based environment. Since the silicon or
silicon dioxide-coated template is hydrophilic due to the presence of either a native oxide or a deposited/grown oxide, its surface is made hydrophobic by surface functionalization to promote adhesion between the template and the naturally hydrophobic/chemically functionalized hydrophobic assembly components in a water based assembly fluid medium. Component functionalization depends on the assembly case under consideration. In all of the cases considered in the present work, component functionalization is not required. In the first set of experiments considered in the present work for the assembly of cells on silicon templates, no functionalization was necessary for either the cells or the templates since cells naturally tend to adhere to most surfaces. In the second set of experiments concerning assembly of deformable polystyrene spheres onto deformable PDMS substrates, again no functionalization was necessary for either the components or the PDMS templates since both are naturally hydrophobic polymer materials. Finally, for the set of experiments on assembly of cylindrical-shaped TMPTA micro-components on patterned silicon templates, while surface functionalization was not necessary for the naturally hydrophobic TMPTA components, the silicon template surface had to be functionalized in order to make it hydrophobic. Although hydrophobic interactions are not the only possible candidate for providing the necessary surface forces, they are used in the non-biological experiments described here for their convenience and effectiveness. The assembly components are prepared for assembly by suspending them in an appropriate liquid medium and treating them to separate any clusters of components into well-dispersed, individual components. The prepared components and assembly template are then placed in the assembly beaker in the assembly fluid mixture which is subjected to acoustic excitation at a megahertz-scale frequency. The excitation of the fluid mixture promotes selective removal of components from incorrectly matched sites on the template along with ensuring circulation of components in the fluid. The level of excitation is controlled to ensure retention in well-matched sites and removal from incorrectly matched sites. In addition, the number of components in the fluidic medium is controlled so as so to ensure
enough hits between the component and the template, thereby enabling rapid, widespread assembly of
the components in matching sites. The relevant procedures are described in detail in the following
sections.
4.2 Experimental set-up

The main elements in the TASR experiments are the template and the components. As discussed previously, the template is a die with a patterned upper surface, which serves as a platform for the components to assemble on. After template patterning, functionalization (when necessary) of the template and the components surfaces is done by coating them with self-assembled monolayers (SAMs) which render their surfaces hydrophobic, to promote adhesion in a water-based assembly fluid environment. Details on how template and component surface functionalization is done are discussed in Section 4.3. Thereafter, the components are prepared for assembly by thorough dispersion of assembly components inside an appropriate fluid medium, the details of which are also described later in Section 4.3.2.

To perform the experiments, the template and components are placed in a small beaker called the assembly beaker containing the assembly mixture. The assembly mixture is selected to work well with the components being assembled, and it varies from cell culture medium for the purposes of assembling biological cells, to water-solvent mixtures for assembling polymer-based micro-components. A large water-filled beaker located beneath the assembly beaker contains a high frequency acoustic transducer. Electric power is supplied to the transducer to produce acoustic waves which hit the assembly beaker at normal incidence, creating a flow field in the assembly mixture. The flow circulates the components, and the flow field and fluid forces drive the assembly process, acting as the removal agent as explained in Section 2.1.1. A schematic illustration of the experiment was shown in Figure 1.1 earlier. More experimental details are described in the section that follows.
4.2.1 Experimental details

This section describes the experimental details that were common to all of the experiments described in the current work. A large (1325 cc) beaker was filled with water, and a 1.7 MHz frequency acoustic transducer (MMDIT-1.7, by Advanced Sonics) was placed at the bottom of the beaker. The height of water above transducer was kept fixed at about 4 cm. The input voltage (and thus power) to the transducer was controlled by a variable voltage transformer (L10C, by The Super Electronic Company). The input voltage can be varied from 0 V to 130 V, with a corresponding transducer electrical input power varying from 0 W to 36 W. The high transducer frequency ensures that operation is well below the power threshold for fully-developed cavitation. A second, smaller beaker (the assembly beaker) was suspended above the transducer and immersed about 0.75 cm into the water in the large beaker. About 1.25 mL of the assembly fluid medium (fluid medium varying depending on the set of experiments to be carried out) was poured into the assembly beaker. Figure 4.1 shows photographs of the assembly set-up used. The template was placed in the beaker, face-up. Since TASR works well in an oversupply of components [17], a sufficient volume of the dispersed component mixture (also particular to the set of the experiments and materials, as it is governed by the number of sites on the assembly template) was added to the assembly beaker using a pipette. The small beaker was capped, power to the transducer was turned on, and the experiment was allowed to run undisturbed for the experimental duration of 3-5 minutes, the duration varying depending on the set of experiments being carried out. At the end of the experiment, the template was taken out of the assembly mixture, placed on a flat surface and allowed to air-dry. Power to the transducer was shut off after removing the template from the assembly beaker to avoid any sudden changes in ultrasonic flow field that might influence the assembly results [16]. The assembled template was then examined under an optical microscope. After the results were documented, the components were recovered (by detaching cells off the template with Trypsin in case of biological cells and using 60 seconds of
sonication in pure ethanol for polymer-based assembly components), and the template was reused in an effort to ensure geometrical consistency between runs. Experiments were conducted under a variety of conditions, including using a variety of materials as assembly components, different transducer voltages, different component densities and different volume fractions of water in the assembly mixture (when applicable).
Figure 4.1: Photographs of the assembly set-up used for TASR experiments. (A) An overview of the experimental apparatus used. (B) Optical microscope used for imaging the assembly template. (C) A magnified view of a portion of the set-up in (A). A megahertz frequency acoustic transducer is kept at the bottom of a large beaker which is filled with water. A smaller assembly beaker is suspended above the transducer in the larger beaker into which the patterned assembly template is immersed face-up.
4.3 Template and component preparation

As discussed earlier, functionalization (when necessary) of the template and the components’ surfaces is done by coating with self-assembled monolayers (SAMs), which render their surfaces hydrophobic to promote adhesion in a water-based assembly fluid environment. Thereafter, the components need to be prepared for assembly by thorough dispersion of assembly components in the fluid medium to prevent aggregation of components. Protocols to be followed for template and component preparation are discussed below.

4.3.1 Template functionalization

In this work, template functionalization is done when the template is made from silicon or silicon dioxide-coated silicon and the assembly experiments are carried out in a water-based environment. Since the silicon or silicon dioxide-coated template is hydrophilic due to the presence of either a native oxide or a deposited/grown oxide, a self-assembled monolayer (SAM) is grown on its surface to render it hydrophobic and to promote adhesion between the template and the naturally hydrophobic polymer-based components or SAM-coated silica components.

For these experiments, SAM coating was done using toluene-based octadecyltrichlorosilane (OTS), the effects of which on interfacial energy in solvent-based systems as used here for some experiments are described at length in [15, 16]. The template was cleaned using a piranha clean, followed by rinses in water and then ethanol, and finally air drying to prevent polymerization of the SAM. During the same time, 6 drops of OTS were added to 75 mL of Toluene, and the resulting mixture was set aside for about 45 minutes in a covered tube. The solution was poured into a flask, and the template was immersed in it face-up. The flask was covered with a lid and placed in an ultrasonic bath (3510R-DTH Branson, manufactured by UL Transonics Corp.) for 30 minutes in order to prevent coagulation of the coating at certain spots on the template and to ensure a more uniform
coating quality. Thereafter, the template was cleaned by spraying it thoroughly with dichloromethane, followed by acetone, to remove any remaining traces of the precursor (toluene-OTS) mixture. The functionality of the coating was then checked by pouring a small drop of distilled water on the template and visually inspecting the contact angle to ensure that coating was successful. A contact angle of greater than 90° between water and the coated surface shows that the surface is hydrophobic, while an angle less than 90° shows that it is hydrophilic.

### 4.3.2 Component preparation

Most polymer-based micro-components are naturally hydrophobic and do not need to be coated with a self-assembled monolayer (SAM). For polymer-based components used in some experiments here, as discussed in Chapters 6 and 7, the components were pipetted into water-ethanol assembly fluid mixtures with varying water fractions (4%, 8% and 20% water). The resulting mixtures were placed in capped microcentrifuge tubes and shaken on a Vortex mixing tool (Vortex-2 Genie, by Scientific Industries) for a few minutes to disperse the components. The polystyrene component dispersions were then shaken in the ultrasonic bath for about 5 minutes to break up any agglomerates of particles. The prepared particles were used immediately to prevent re-agglomeration due to settling.

For the experiments with biological cells, an appropriate volume of cells was pipetted into the fresh, pre-warmed (to room temperature) cell culture medium. The cells were then stained for better visualization against assembly templates following protocols discussed in greater detail in Chapter 5. The resulting mixture comprising stained cells was then placed in sterilized, capped microcentrifuge tubes and shaken on an orbital shaker (VSOS-4P, PRO Scientific) for a few minutes to disperse the cell pellet. Just before dropping a suspension of cells into the assembly beaker, the assembly mixture containing cells was pipetted a few times to ensure maximal dispersion since cells tend to aggregate.
and settle down quickly within a few seconds. Pipetting was done very gently to ensure no mechanical damage to the cells from pipetting.
4.4 Summary

In summary, the experimental protocols to be followed for assembly using TASR are described in this chapter. The main elements in assembly using the TASR technique are the assembly template and the assembly components. Lithography and isotropic etching techniques, as described earlier in Chapter 3, are used to pattern the template surface with holes that match in shape and size to the components to be assembled inside. The template is then functionalized (when necessary) in order to make it hydrophobic, so as to promote adhesion in a water-based assembly fluid medium. The assembly components are prepared for assembly by suspending them in an appropriate assembly fluid medium and treating them to separate any clusters of components into well-dispersed, individual components. The prepared components and assembly template are then placed in the assembly beaker in the assembly fluid mixture, which is subjected to acoustic excitation at a megahertz-scale frequency. This high transducer frequency ensures that operation is well below the power threshold for the onset of fully-developed cavitation. The excitation of the fluid mixture promotes selective removal of components from incorrectly matched sites on the template along with ensuring circulation of components in the fluid, effects of which are described in detail previously in Chapter 2. The level of excitation is controlled to ensure retention in well-matched sites and removal from incorrectly matched sites. By also controlling the number of components in the fluidic medium so as to ensure enough hits between the component and the template, the components are assembled in matching sites. Some of the parameters which are varied during experiments to study the effect of the assembly conditions include different transducer voltages, different component densities and different volume fractions of water in the assembly mixture (when applicable).
Chapter 5

Self-Assembly of biological cells

5.1 Introduction

This chapter describes the experimental procedures and analysis for the selective self-assembly and sorting of biological cells using TASR. The motivation to demonstrate this experimentally has been stressed in detail already in Section 1.4, where several potential applications of structuring cells based on shape and size recognition have been highlighted. As also discussed previously in section 2.2.2, the integrated TASR model, which incorporates the modeling for deformable systems, predicts successful assembly of most biological cells that possess mechanical properties in the mentioned range, and have the desired attributes for assembly with TASR. These attributes, which served as the criterion for selection of biological cells used in the experiments here, once the theoretical model constraints are ensured to have been met as discussed in Section 2.2.4, are now described below.

One of these preferred attributes for successful assembly within the experimental framework used in the current work is for the biological specimen to be nearly spherical in shape. Spherical biological cells are preferred since the fabrication procedures for creating matching hemispherical trapping sites on the assembly templates have been well explored and implemented, and also since the integrated TASR model is well equipped to model spherical components in a broad range of sizes and a vast variety of materials. While the basic modeling approach for assembly of anisotropic components is covered in Section 2.3 and the assembly demonstration of polymer-based components with anisotropic shapes using TASR is described in Chapter 7, assembly of biological specimens with anisotropic shapes is not discussed here, as that is beyond the scope of the present work.
Other preferred attributes in the biological specimens are robustness to shear forces (as are a part of the TASR system). The biological specimen needs to have the ability to withstand the mechanical stresses in the assembly fluid medium created by the ultrasonic excitation in order to prevent cell damage due to shear failure or cell rupture. While most cells fall into this category and should be well-equipped to handle the mechanical forces in the current set-up, some other cell lines which are more fragile, such as human embryonic stem cells or red blood cells (RBC) infected with Thalassemia, for instance, might not be able to withstand the forces in the TASR system due to lack of the mechanical capabilities desired.

The final criterion is temperature. It is possible to design a TASR system in which the assembly bath is maintained at a desired temperature that is different from the temperature in the ambient to accommodate the needs of the specific biological cells in question. However, the TASR system used in the experiments described in this thesis is simpler, and has the assembly fluid maintained close to room temperature. Therefore, for simplicity, it is desired that the biological specimens should have the ability to survive within their respective culture media at the ambient temperature in the TASR system for the duration of the assembly experiments and analysis, which is typically on the order of 30-40 minutes.

Based on the criteria laid out above, two cell lines were chosen for analysis in the current work. First, the assembly of a non-mammalian cell line is described in the subsequent sections, where experiments are carried out with **SF9 cells**, a robust clonal isolate cell line derived from ovarian tissue of the fall army worm, *Spodoptera frugiperda*. These cells are nearly spherical in shape and come in a range of sizes, varying from 10-30 μm in diameter. SF9 cells represent an important group for assembly demonstration using TASR as these cells are useful in the production of vaccines for influenza. Upon infection with baculovirus, these cells grow in size and express recombinant proteins which are thereafter used in vaccine generation. SF9 cells with a mean diameter of 15 microns were
successfully assembled here using TASR onto a patterned silicon template. Thereafter, the extension to mammalian cells is covered in Sections 5.12 and 5.13, where size-selective sorting of *mouse-derived non-small lung cancer cells* using TASR is described. While the assembly of non-mammalian cells is a useful tool to have, it is also crucial to have the ability to achieve successful assembly of mammalian cells, as the scope of applications is much broadened by extending the capabilities of TASR into this regime. The mouse-derived cancer cells used in the current work closely replicate human lung cancer cells in their behavior, and thus represent an important cell type to work with and manipulate using TASR. It is to be noted, however, that while experiments with two particular cell lines are described here, the scope of the technique is in no way limited to these two alone, and can be extended to any cell line that meets all of the requirements posed by the TASR system as mentioned above. Based on the results on assembly of anisotropic components as described in Section 7.3, it is also expected that non-spherical cells have the potential to be assembled using the TASR process.

The assembly sites for both of these sets of experiments comprise holes with nearly hemispherical profiles etched in a silicon substrate using plasma etching. The experimental protocols include the creation of the templates on which assembly is to be carried out, the preparation and staining of the cells that are to be assembled, and the actual assembly experiments themselves. The procedure for fabrication of the specific set of templates for these experiments has already been discussed extensively in Section 3.3.1. The relevant procedures for the remaining steps in the assembly process are described below in detail.
5.2 Cell Culture and Preparation

The SF9 insect cells for TASR experiments were provided by the Sasisekharan Group in the Department of Biological Engineering at MIT. Suspension cultures of SF9 cells (Invitrogen, Carlsbad, CA) were cultured in BD Baculogold Max-XP SFM media (BD Biosciences, San Jose, CA) at 28°C in a shaker incubator. The cells were passaged to a density of about 6 million cells/ml. An optical micrograph of a suspension of SF9 cells dropped onto the silicon template surface without subjecting them to the TASR process is seen in Figure 5.1(A).

In order to enhance visibility on the shiny silicon template surface background, the cells were stained with methylene blue (BioChemika, 03978) and were then resuspended in fresh, pre-warmed culture medium at 37°C. Optical micrographs of cells stained with methylene blue and dropped onto the silicon template surface without subjecting them to the TASR process are seen in Figure 5.1(B), 5.1(C) and 5.1(D). Upon staining, the SF9 cells are seen clearly in a contrasting blue hue against the white assembly sites, on the dark silicon template backdrop. It is clearly noted that there is no visible organization in the cell placement without the TASR process, which is as expected. It can also be confirmed from these optical micrographs that that the SF9 cells come in a range of sizes and are nearly spherical in shape, which is desired for the current experimental set-up, as discussed previously.

SF9 cells, like most other cell lines, tend to aggregate in the culture media. To prepare the cells for the assembly process, it is ensured that any aggregates of cells as dispersed in the culture media are broken apart and that the cells are well dispersed in the assembly media beforehand. This is done since the assembly of a single cell inside any particular assembly site is desired and the acoustic forces at the MHz frequency range are not strong enough to achieve perfect dispersion of cells inside the assembly fluid medium. In fact, any large aggregates of cells might lead to the distortion of the circulation pattern inside the fluid medium and make the process inefficient. In order to overcome that potential obstacle in the assembly process, the cells are carefully pipetted
several times gently, so as to break any cell clusters, while also ensuring that no mechanical cell
damage occurs from rigorous pipetting.

**Figure 5.1:** (A) Optical micrograph of a suspension of SF9 cells dropped onto the silicon assembly template
for TASR before staining, without subjecting the cells to the assembly process. (B)-(D) Optical micrographs of
SF9 cells stained with methylene blue dye and dropped onto the template, also without the TASR assembly
process. Cells are clearly visible in blue against the assembly sites in white, on the black silicon template
background. (B) Cells lying randomly outside and around a grid of assembly sites 12 and 22 μm in diameter
(C) Cells without any organization visible around a pattern formed with assembly sites that spells “MIT” and
(D) Cells lying outside a grid of assembly sites which are 12 μm in diameter and are spaced 60 μm apart.
5.3 Cell Placement and Sorting

The experimental procedure for the assembly of SF9 cells follows the same general outline as covered in Chapter 4; details are discussed here in context of the particular system for assembly.

A large (1325 cc) beaker was filled with water, and a 1.7 MHz frequency acoustic transducer (MMDIT-1.7, Advanced Sonics) was placed at the bottom of the beaker. The height of water above the transducer was kept fixed at about 4 cm. The input voltage (and thus power) to the transducer was controlled by a variable voltage transformer (L10C, The Super Electronic Company). The input voltage can be varied from 0 V to 130 V, with a corresponding transducer electrical input power varying from 0 W to 36 W. Input voltages for the experiments reported here ranged from 15V to 60 V. The high transducer frequency ensures that operation is well below the threshold for fully-developed cavitation.

A second, smaller beaker (the assembly beaker) was suspended above the transducer and immersed about 0.75 cm into the water in the large beaker. About 1.25 mL of the pre-warmed cell culture medium was pipetted into the assembly beaker. The patterned silicon template was placed in the beaker, face-up. A sufficient volume of the stained SF9 cell solution (between 100-200 μl, at a cell density of 6 million cells/ml) was added to the assembly beaker using a pipette. This volume of solution contains a large oversupply of cells, with more than 100 times as many cells as there are assembly sites on the template. The number of components contained in this volume of the solution was chosen to provide an optimum quantity of cells needed for the TASR process, given the number of available sites. It is not sufficient to provide as many cells as there are holes; it has been experimentally observed that the assembly yield does not achieve its maximum value even for ratios of cells to assembly sites as high as 50. On the other hand, for cell to site ratios above 1000 or so, the cells are not dispersed efficiently by the acoustic set-up, and they tend to aggregate together, giving rise to defects and lowering the assembly output. Therefore, an optimum cell density, with ratios of cells to sites in the range of 100-500 was maintained for efficient operation of the TASR technique.
The small beaker was capped with a Parafilm® cover, power to the transducer was turned on, and the experiment was allowed to run undisturbed for 3 minutes. At the end of the experiment, the template was taken out of the assembly mixture and placed on a flat surface. Any remnants of the assembly mixture liquid on the template surface were allowed to air-dry, although for a short time only (on the order of a minute). The assembly template was then examined under an optical microscope to see which assembly sites were occupied after the TASR process. After the results were documented, cells were removed from the assembly sites on the template by trypsinization of the template surface. This was done by placing the template at the bottom of the assembly beaker, adding a small volume (1.25 ml) of trypsin EDTA (25300-054, Invitrogen) to the beaker and allowing the template (with cells on it) to sit immersed for about 5 minutes. The contents of the beaker were then pipetted a few times gently to ensure complete cell detachment from the silicon surface. The template was then reused in an effort to ensure geometrical consistency between runs as well as to minimize the need to fabricate additional templates. The experiments were conducted under different transducer voltages to study the effect of excitation level on the cell assembly yield; the effects observed are discussed in Section 5.7. Experiments were also carried out to test the viability of the SF9 cells subjected to ultrasonic excitation under the conditions experienced in the TASR set-up, using a trypan blue exclusion test. Procedures and results for the viability tests are recorded in Section 5.12. The results of assembly are presented and discussed in the sections that follow.
5.4 Fluorescence Staining

To study the sizes of cells assembled onto patterned templates using TASR, it was necessary to visualize the 3-D geometry of the single cells, as trapped inside assembly sites. In order to achieve this target by using confocal microscopy, the cells were stained with CellTracker™ Red CMTPX (Molecular Probes, C34552) obtained from Invitrogen (with absorption maxima of 577 nm and fluorescence emission maxima of 602 nm). The CellTracker™ dye reagent passes freely through cell membranes, but once inside the cell, is transformed into cell-impermeant reaction products. Excess unconjugated reagent passively diffuses to the extracellular medium.

To prepare the dye working solution, CellTracker™ dye was warmed to room temperature and dissolved in anhydrous dimethylsulfoxide (DMSO anhydrous, Biotium, 90082) to a final concentration of 10 mM. The stock solution was diluted to a final concentration of 10 μM in culture medium. The dye concentration was kept low to maintain normal cellular physiology. The dye solution was then warmed in a water bath to 37°C. SF9 cells were then harvested by centrifugation (Microfuge 18 Centrifuge, Beckman Coulter), and the supernatant was aspirated by pipetting. The cells were then resuspended in pre-warmed CellTracker™ dye solution. The cells were incubated in suspension on an orbital shaker (VSOS-4P, PRO Scientific) for 20 minutes to allow uniform staining of cells, and then centrifuged again. The dye working solution was then replaced with fresh, pre-warmed medium and incubated for another 30 minutes at 37°C. Images of cells stained fluorescently with CellTracker™ dye and imaged using confocal microscopy are shown in subsequent Sections 5.5 and 5.6, in context of the cell assembly analysis.
5.5 Imaging Set-up and Cell Volume Calculation

A laser-scanning confocal fluorescence microscope (PerkinElmer UltraVIEW RS System with Zeiss 200M Microscope) equipped with an X-Cite 120 fluorescence illumination system, Yokogawa CSU-21 Scanhead and a Melles-Griot Argon/Krypton 643 series laser was used. Different microscope objectives were used to image the assembly chip, including one air 20X (0.5 NA, Zeiss), one air 40X (0.75NA, Zeiss) and one oil immersion 100X (1.45NA, Zeiss). All experiments were carried out at room temperature. Images were captured with a Hamamatsu Orca ER cooled CCD camera. Sequential confocal images spaced 0.1 μm apart, as shown in Figure 5.2, were captured across the entire depth of a cell assembled inside a hole. Image processing was subsequently done to reconstruct individual cell slices into a three-dimensional rendering using the open source software ImageJ (http://rsbweb.nih.gov/ij/). This approach provides a precise assessment of cell volume and hence of initial cell diameters before assembly; this enables an assessment of the degree to which the cells match the assembly sites. After the cells are assembled on the template, they undergo a certain amount of mechanical compression during the procedure of imaging with the 100X oil immersion lens. By extracting mean cell diameter from the total volume and the known spherical shape of SF9 cells, accurate dimensions are obtained independent of any mechanical deformations. The cell volume was calculated using the stack of images captured using confocal laser scanning microscopy. A 2-D surface plot for each of these slices was then used to visualize the intensity distribution. Identifying the cell boundary, the area of the cell slice was calculated using the software package. The total volume of each cell measured while assembled inside a site was then calculated by multiplying the area of each slice by the spacing between consecutive slices and summing the individual volumes of the slices.
Figure 5.2: Confocal image surface plots, (A)-(I), of a large and a small SF9 cell assembled inside well-matched assembly sites, 22 and 12 μm in diameter respectively, at varying depths from cell surface. In the images shown here, the scans start from the topmost surface of the smaller cell when the smaller cell is out of focus but some portion of the larger cell is in focus, both being at the same height from the silicon template surface for one particular scan. The scans then progress along the z-axis towards the bottom surface of the small cell when the smaller cell is again out of focus, but the larger cell still remains in focus. Area of a single cell at one depth slice is calculated using Image J software from each scan which is further used to calculate the volume of each slice. Adding up the volumes of all such slices spaced 0.1 microns apart going from top to bottom of the cell surface, the total volume of each cell assembled inside the hole is calculated. The scale bars on each image represent 2μm in length.
5.6 Results

SF9 cells were successfully and selectively assembled into matched sites in the patterned templates using TASR. After assembly, the assembly yield was quantified by calculating the ratio of the number

![Optical micrographs of SF9 cells stained with methylene blue and structured into different 2-D patterns on a silicon template using TASR. A single cell is trapped inside each quasi-hemispherical site that comprises the patterns shown above. Figure 5.3(A) demonstrates high yield of the selective sorting process, with a 100% assembly yield in the placement of cells into a periodic arrangement of holes 15 μm in diameter, as well as a high assembly density of up to 30 sites/mm. Figure 5.3(B) highlights the capability to organize cells into any random 2-D pattern, which in this particular case comprises holes 15 μm in diameter. Figure 5.3(C) demonstrates selective placement of cells into a grid with alternating holes of 12 and 22 μm diameters. The apparent size of the sites is slightly reduced by the overhang at the wafer surface. Precise positioning and manipulation of cells is seen using the TASR technique.](image-url)
of holes of each size that were filled with components to the total number of holes of that size. Figure 5.3(A) and 5.3(B) demonstrate a uniformly high rate of successful assembly for placement of cells (stained with methylene blue) into sites with a uniform diameter of 15 microns arranged both in periodic arrays and in non-periodic 2-D patterns, respectively [59]. The assembly in this case was carried out at a transducer voltage of 55 V. Figure 5.3(C) shows an optical micrograph of SF9 cells assembled into alternating patterned hemispherical sites, 12 and 22 microns in diameter, and highlights the size selectivity of the assembly process. It can also be observed from the optical micrographs that the assembly sites in each of these cases were spaced at different intervals in different grids, and that a high assembly yield is obtained in each case, irrespective of the spacing between assembly sites. Therefore, TASR’s ability to sort out and assemble cells precisely based on size matching and with a high assembly yield does not depend on inter-site spacing in the experimentally-examined range from 4 μm to 40 μm. This versatility can be used not only to capture a very large number of cells in a small active region so as to get the largest density possible, when the spacing between consecutive sites is small, but also for the isolation of certain kinds of cells from other types, when the spacing is enlarged. The ability to regulate the number of cells to be sampled or manipulated at any given time or in any given space can potentially be of use in applications such as medical diagnostics and tissue generation techniques. High assembly density of up to 900 cells/mm² was obtained here using TASR. Confocal images confirming cell assembly results are shown in Figure 5.4. Figures 5.4 (A) and 5.4 (C) show images of SF9 cells labeled fluorescently with CellTracker™, dropped onto the silicon surface without assembly and visualized using confocal microscopy. On the other hand, images of cells labeled with CellTracker™ and observed after subjecting them to the TASR process are shown in Figure 5.4 (B) and (D). In order to study the response of cells and the assembly throughput of TASR at different levels of excitation, the assembly results were documented for a range of transducer voltages. The repeatability of assembly results was also measured by performing nominally identical experiments
(transducer voltage 55V, 100μl stained SF9 cell solution, cell density of 6 million cells/ml) multiple times. Even when different assembly templates were used for each experiment, variation in yield for those assembly conditions was less than 2%.

**Figure 5.4:** Confocal microscopic images of SF9 cells labeled with fluorescent Cell tracker red CMPTX, viewed at 20X magnification in (A) and (B) and at 100X magnification in (C) and (D). Image (A) shows a sample of cells on the silicon surface before assembly with TASR, while image (B) shows single cells, each 15 μm in diameter, trapped in individual sites on the template surface after going through the TASR process. Similarly, when viewed through oil, at a higher magnification, image (C) shows the cells occupying random positions on the template near, in and outside the assembly sites (faint rim of an assembly site on the silicon surface visible in the center of image (C)) before assembly, while image (D) shows a zoomed in view of a single cell trapped inside an assembly site after being subjected to TASR.
5.7 Effect of Voltage Variation

The voltage on the transducer was varied from 15V-60V, and its effect on assembly yield was studied. The cell assembly yield of cells in small holes and cells in large holes was observed to rise steadily with an increase in voltage, as seen in Figure 5.5, with no changes in cell morphology and viability in the voltage range mentioned above. Confirmation of size matching between cells and holes is shown later, in Figure 5.7.

The trend follows similar trends of voltage versus assembly yield as discussed in some of the earlier work on TASR [15-17]. For very low input voltages, there is not enough circulation of the assembly fluid medium, and the cells are placed at random positions on the template surface that are not well-matched in shape and size. As observed in Figure 5.5, these defects, or components that are placed in incorrect positions on the template, are observed to peak at around 20V. At voltages less than 20V, the components mostly either stay settled on the surface of the template or drift slowly in the fluid medium. With a slight increase in voltage, there is a narrow intermediate phase around 20V in which the components are agitated enough to move around a bit but not enough to enable selective sorting. This increases the formation of random defects on the surface while leaving the assembly yield nearly constant or slightly increased. Upon increasing the voltage further, the additional power supplied to the system allows the cells to detach from poorly-matched sites and eventually become trapped in well-matched sites. In well-matched sites, the retention effects arising from high contact area between the contacting surfaces of cells and template and the minimization of the surface free energy [15, 16] dominate over the ultrasonic fluidic removal effects, ensuring that the cells stay inside the well-matched locations. The assembly yield thus starts to increase, since a larger number of cells find the appropriate locations and are retained inside them as the voltage increases. As is discussed in [15, 16], the yield is expected to increase to a peak value and decrease thereafter, since at very high voltages, the removal effects become stronger than the retention effects. Since a peak assembly yield
of about 100% is observed for an operating voltage between 55-60V with SF9 cells, it is unnecessary to go beyond this upper limit to achieve high assembly throughput with TASR for sorting and assembly applications. It should be noted, however, that for voltages exceeding the range described above, changes in cell viability are recorded. Therefore, an upper limit to the voltage is imposed in this case.

![Graph showing fractional assembly yield versus voltage on assembly transducer.](image)

**Figure 5.5:** Fractional assembly yield versus voltage on assembly transducer for the cases of small cells (~12μm diameter) inside 12μm diameter assembly sites and large cells (~22μm diameter) inside 22μm diameter assembly sites on the template. The assembly yield rises with increase in voltage, and the defects (fraction of cells outside intended assembly sites) steadily decrease from their peak levels at 20V.
5.8 Power Input Characterization

Based on the calorimetric measurements made in previous work [15] on TASS, the output power coming directly from the transducer at the operating voltage for assembly of SF9 cells was characterized. For a transducer voltage of 45V, the net power transmitted to the system through the solid-liquid interfaces of the water (contained in the large assembly beaker) and the assembly beaker, and subsequently the interface of the assembly beaker and the assembly fluid (cell culture medium), after taking all losses into account was estimated to be about 0.15 W. For an assembly fluid volume of 1.25 ml in the small assembly beaker, the cells were thus subjected to an input power of 0.11 W ml⁻¹ in the current setup. Power scales with the square of transducer voltage, so in all cases here, the power is much less than the power used in [36] where the effects of ultrasonic excitation on SF9 cell growth, viability, protein expression etc. are investigated and it was concluded that within the given power range, the ultrasonic exposure does not affect the SF9 cell viability and behavior. The effects of acoustic excitation on both mammalian as well as non-mammalian cell cultures have also been studied extensively in [60-62], and in all these cases, no significant loss of cell viability has been observed within power ranges of 0.1 W ml⁻¹ to 0.5 W ml⁻¹. Though cell viability is confirmed experimentally as well in the present work, results of which are presented in Section 5.13, it can also be reasonably deduced from comparison with prior work that the power input in the TASS system should not have any negative effects on the cell behavior. It may also be predicted from this data that if a similar TASS setup is used for the assembly of mammalian cells, then the power input to the system should be favorable for the growth and survival of the cells assembled and sorted using acoustic excitation. Since the transmission of acoustic energy into the system depends on the reflectivity at the solid and liquid interfaces, which in turn depends on the wave velocities of the two media at the given interface [15], the use of a different substrate material (such as polymer replicas of the current substrates) may alter
the net power transmitted to the system slightly, while remaining within the range that is favorable for cell viability.
5.9 Temperature Measurements

The absorption of the acoustic energy in the assembly fluid can cause the temperature of the assembly fluid to rise. Sudden temperature changes in the system or prolonged exposure to extreme temperatures may cause irreversible harm to cells. Therefore, the temperature of the culture medium subjected to high frequency ultrasonic (megasonic) excitation was recorded during experiments to confirm that the assembly liquid stayed within a temperature range that is favorable for the survival of the cells. The temperature increase was recorded as a function of both the driving voltage and time. A temperature-time plot of the assembly fluid medium at different voltages is shown in Figure 5.6. The curves illustrate the range of temperatures that the SF9 cells are subjected to during the course of the assembly process. It is noted from these curves that only a slight increase in temperature, a maximum of about 0.4°C, is observed at any given voltage level during the experimental duration of 3 minutes. The temperature measurements also indicate that the thermal environment in the TASR set-up is at a level where no negative effects are expected on cell behavior, since a temperature range between 22°C

![Temperature-time plot](image)

**Figure 5.6:** Temperature of the assembly fluid medium versus the assembly time at select voltage values on the transducer within the operating range of 15-60V. Only a slight increase in the temperature is observed during the TASR experimental duration of 3 minutes, at any voltage level observed here. Lines are guides to the eye.
and 30°C is favorable for insect cell growth [63, 64]. The viability tests further verify that the acoustic intensities used in the set-up give no indication of being harmful to the SF9 cells. It can also be observed from the temperature measurements that the given set-up is favorable for the assembly of most mammalian cells. Even though the culture and handling temperature for mammalian cells (typically around 37°C) generally needs to be higher than that required for SF9 cells, the concept is consistent with what is presented and discussed in the current work. As long as the assembly fluid medium for the sorting of mammalian cells in the TASR set-up starts out at a higher temperature suitable for mammalian cells, the subsequent increase in the temperature introduced by the experimental conditions can be easily tolerated by mammalian cells [65, 66]).
5.10 Cell Volume Analysis

As discussed in detail in previous sections, confocal laser scanning microscopy and the open source Java-based image analysis software package ImageJ (NIH) were used together to visualize the 3-D geometry of cells assembled on the template (Figure 5.3) and to characterize the selectivity of the process based on the computation of the volumes of cells inside assembly sites. Regions for analysis were chosen at random from the data taken with a transducer voltage of 60 V, and all of the cells within the randomly-selected regions were included in the following analysis.

A selection of 2-D surface plots that were obtained for a single cell assembled inside a hole and used to calculate the cell's volume are shown already in Figure 5.2. It should be noted that in the process of cell volume calculation, the edge detection of each image slice introduces an error of about ± 0.25 μm in the cell radius measurement. This reflects the fact that in the imaging process, the cell membrane is not marked precisely by a well-defined, sharp boundary. The intensity variations used to distinguish the fluorescently-labeled inner cell region from the dark outer template region are thus spread over a small but finite length.

The calculated volume is representative of the cell volume. By equating this cell volume to the volume of an ideal sphere, the diameter of the ideal sphere that corresponds to that cell size was obtained. The diameters of ideal spheres corresponding to cells of the calculated volumes were then compared with the diameters of the assembly sites on the template, which were measured using an SEM. Figure 5.7 plots a histogram of the number of cells versus the cell diameter in μm, and shows the distribution of sizes in sample cell populations obtained both for 22 cells after TASR assembly and for 50 cells without TASR assembly. The cell diameter plotted here is diameter calculated using the approach highlighted above. It can observed from this plot that only the cells that match the hole diameters created on the template, i.e. 12, 15 and 22 μm ± 0.5 μm are captured on the assembly template using TASR [59]. This spread is commensurate with the uncertainty in cell size from the
confocal microscopy data. All the other cell sizes that do not match the sizes of the assembly sites are eliminated by TASR's selective removal process. The number of cells that has been measured is relatively small, since the 3-D reconstruction technique and volume analysis that enable precise diameter measurements are not suited for the rapid production of large amounts of data. The number of cells observed at each size is therefore not statistically significant, and further information cannot be extracted from the peak heights in this experiment. However, the exclusion of cells that are not commensurate with the assembly sites confirms that the assembly is very highly selective and enables precise positioning of cells inside pre-defined, patterned locations on the chip surface.

![Image: Histogram showing the number of cells observed in each size range plotted versus the corresponding cell diameter calculated using the 3-D reconstruction of single SF9 cells using confocal laser scanning microscopy. Cells characterized with and without TASR assembly are plotted for comparison. Only the cells corresponding to the matching hole diameters on the template were captured by the TASR process. This shows the size selectivity of the TASR assembly and sorting process.]

**Figure 5.7:** Histogram showing the number of cells observed in each size range plotted versus the corresponding cell diameter calculated using the 3-D reconstruction of single SF9 cells using confocal laser scanning microscopy. Cells characterized with and without TASR assembly are plotted for comparison. Only the cells corresponding to the matching hole diameters on the template were captured by the TASR process. This shows the size selectivity of the TASR assembly and sorting process.
5.11 Extension to Mammalian Cells

In an effort to broaden the scope of TASR to applications involving mammalian cells, experiments were also conducted with *mouse-derived non-small lung cancer cells*. These cells were procured from Prof. Sangeeta Bhatia’s group at MIT. The cells were cultured as described in [58].

In order to demonstrate the self-assembly of these cells onto patterned silicon substrates, the same guidelines as those described in Section 5.3 for assembly of SF9 cells were followed. Figure 5.8 (A) shows an optical micrograph of the mouse lung cancer cells dropped onto the template, before

![Figure 5.8: Optical micrographs of mouse-derived lung cancer cells, dropped onto the patterned silicon template without subjecting them to the TASR process and in (A) without staining while in (B)-(D) with the methylene blue stain to offer better contrast inside assembly sites. The cells lie randomly outside and around assembly sites 15 \( \mu m \) in diameter in (C) and outside and around sites which are 25 \( \mu m \) in diameter in (D). Even after cell preparation, clusters of cells are observed in these micrographs since the cells tend to aggregate in the absence of circulation and acoustic excitation forces as exist in the TASR set-up, and do not assemble inside the sites in this case.](image-url)
subjecting them to the TASR process. In order to enhance the visibility of the cells against similarly shaded assembly sites, the cells were stained with methylene blue, as done previously for the SF9 cells. Figure 5.8 (B), (C) and (D) show portions of the assembly templates with a suspension of stained cancer cells dropped onto these, without subjecting them to the assembly process. The cells are seen to be placed without any organized pattern against grids of holes that contain assembly sites 15 µm in diameter in Figure 5.8 (C) and 25 µm in diameter in Figure 5.8 (D), respectively, but are seen in clear contrast against the white assembly sites. The cells are then prepared for the assembly process, using the same procedure as highlighted in Section 5.2, in order to ensure uniform cell dispersion in the assembly fluid medium. The TASR experiments are then carried out at several voltage levels between 40V – 60V and results are recorded. Figures 5.9 (A) and 5.9 (B) show demonstration of complete assembly achieved at optimum parameters with a voltage level of 55V, and highlight the size selectivity as well as high assembly yield for cell placement achieved using this technique.

**Figure 5.9:** Optical micrograph of mouse lung cancer cells self-assembled using TASR into assembly sites that are (A) 15 µm in diameter and (B) 25 µm in diameter, at a voltage level of 55V. A high assembly yield with low defect density and high selectivity is observed in the cell placement using TASR.
5.12 Cell Viability Tests

This section describes the viability tests done for both SF9 cells and mouse lung cancer cells. While a trypan blue staining test was carried out for SF9 cells, a Propidium Iodide (PI) test (more commonly used technique for testing mammalian cell viability) was done for confirming cell viability of the mouse cancer cells. The dye exclusion tests are used to determine the number of viable cells present in a cell suspension. It is based on the principle that live cells possess intact cell membranes that exclude certain dyes, such as trypan blue, Eosin, or propidium, whereas dead cells do not. In the trypan blue staining test, for instance, a cell suspension is mixed with the trypan blue dye and then visually examined to determine whether cells take up or exclude dye. In the protocol presented here, a viable cell will have a clear cytoplasm whereas a nonviable cell will have a blue cytoplasm. A count of the blue stained cells then gives a measure of the cell viability after the TASR experiments. Similarly, in the PI staining procedure, the PI dye does not cross the plasma membrane (PM) of cells that are viable or in the early stages of apoptosis because they maintain PM integrity. In contrast those cells in the late stages of apoptosis or are already dead and have lost PM integrity are permeable to PI and are labeled fluorescently by the dye. The use of two different viability tests provides secondary confirmation of the results.

First, the cellular morphology after experiments was observed in an inverted light microscope. There was no noticeable change in the cell shape after experiments for both the mammalian and the non-mammalian cell lines. Cell viability before and after experiments was then determined using the trypan blue dye exclusion method for SF9 cells. In order to do this, a trypan blue solution (0.4% wt/vol, 15250-061, Invitrogen) was mixed with an equal volume of cell suspension and maintained at room temperature for 3 min. The suspension was loaded on a haemocytometer (Hausser Scientific, 1483), and stained cells were scored as nonviable. Only a 1.2% net change in viability was recorded after the cells were subjected to megasonic excitation in the TASR environment, at the optimal
assembly voltage of 60V, which is also the peak voltage level in the range of voltages used for experiments here (15-60V). The results confirm the predictions from theory that the temperature, power, excitation level and time span of excitation imposed on the cells during TASR do not lead to any significant change in cellular viability or morphology for the SF9 cell line.

Cell viability tests were then carried out for the mouse lung cancer cells using PI staining following standard protocols. Figure 5.10 (A), (B) and (C) show control images of the cells stained with PI and captured using fluorescence microscopy, to assess viability before subjecting the cells to the TASR process. Figures 5.11 (A), (B) and (C) show the images of cells after subjecting them to the assembly process.

**Figure 5.10:** Fluorescent microscopic images of samples of mouse-derived lung cancer cells stained with PI for viability testing, before subjecting cells to the TASR assembly process, as seen in (A) – (C). Dead cells are visible in fluorescent green hue against the silicon template background, whereas the cells not stained are scored as viable. This set of images is used as the control for the images captured after experiments to comparing cell viability.

**Figure 5.11:** Fluorescent microscopic images of samples of mouse-derived lung cancer cells stained with PI for viability testing, after subjecting cells to the TASR assembly process, as seen in (A) – (C). As in Figure 5.10 (above), dead cells are visible in fluorescent green hue against the silicon template background, whereas the cells not stained are scored as viable. Images show that cell viability does not change significantly after the assembly process and a high viability of nearly 99% is recorded even after the TASR experiments.
TASR experiments. Less than 1.1% change in viability is observed after conducting experiments, indicating that the TASR process is not harming cell viability. It is to be noted that the images in Figure 5.11 are captured right after the assembly process, with a short time lapse of less than half an hour between the removal of cells from the assembly set up and imaging under the microscope. Thus, any potential long term effects of subjecting the cells to the TASR process, for example any changes in viability that would only manifest over the course of a day, are not studied here experimentally.
5.13 Summary

To summarize, size selective assembly of mammalian as well as non-mammalian cells was demonstrated experimentally in this chapter. Clonal isolate (SF9) cells derived from the Spodoptera frugiperda (Fall Armyworm) IPLB-SF21-AE cells (with a mean diameter of 15 microns) and mouse-derived non-small lung cancer cells (with a mean diameter of 20 microns) were successfully assembled using TASR onto patterned silicon templates in separate sets of experiments. The assembly sites comprised holes with hemispherical profiles etched in a silicon substrate using plasma etching to match the nearly spherical shapes of the cells used, details of which have been covered already in Chapter 3. A high assembly yield of a 100% and high cell density were observed for cell placement into sites with uniform diameters for both the cell lines. In addition, size selectivity was established for assembly into sets of heterogeneously patterned hemispherical sites with different diameters. The assembly parameters including density of cells in assembly fluid medium and the input power provided to the system were varied to study the effect of these on the success of assembly. Size selectivity of assembly was also confirmed using confocal laser scanning microscopy where sequential confocal images spaced 0.1 µm apart were captured across the entire depth of a cell assembled inside a hole. Image processing was subsequently done to reconstruct individual cell slices into a three-dimensional rendering and reconstruct cell volume to identify mean cell diameter for the cells retained after assembly. It was concluded from this analysis that only the cells that match the hole diameters created on the template, i.e. 12, 15 and 22 µm ± 0.5 µm are captured on the assembly template using TASR. High cell viability of nearly 99% was recorded using multiple testing procedures, confirming predictions from theory that the assembly environment is favorable for the growth and survival of both mammalian as well as non-mammalian cells. These results confirmed model predictions that assembly would be successful with the materials studied here under the experimentally-used assembly parameters.
Chapter 6

Assembly into Deformable Templates

6.1 Motivation for assembly on deformable substrates

Fabricating assembly templates for the TASR process using silicon-based micro-fabrication techniques as described earlier in Chapter 3 is a very expensive and time-consuming task. The method employed for manufacturing templates needs to be made easier, faster, low-cost, manufacturable and repeatable. Therefore, there is a strong motivation to make use of polymer replication techniques to manufacture the TASR templates. Polymer replication techniques can achieve high throughput in the fabrication of micro-devices by replicating micro-fabricated master structures. As more and more commercial applications for polymer based microsystems come into existence, polymer micro-fabrication methods are becoming increasingly well developed and important. The commonly used polymer micro-fabrication technologies are roughly divided into two categories - the direct techniques, in which each single device is manufactured separately, for example using optical lithography, and replication techniques, in which a master structure is replicated into the polymer material. The latter is preferable since it greatly lowers cost while enabling rapid, repeatable fabrication results. Among other deformable materials employed for the purposes of polymer-based micro-fabrication, elastomer materials such as PDMS are of particular interest to us for incorporation in TASR systems since they are cheap, commonly available, non-toxic and bio-compatible. As described in extensive detail in Chapter 5, TASR holds the capability to sort and structure biological cells based on shape and size selectivity. Having biocompatible assembly substrate surfaces for TASR is favorable for tissue scaffolds, cell sorting and cell growth monitoring chip-based systems etc. Such systems are useful in
applications such as biochemical assays, cell counting and sorting, cell growth, detection of biological species, genomics, etc, as already discussed in Section 1.2.2. Some other advantages offered by elastomer materials like PDMS are that they can easily be molded and cured to replicate patterns at the sub-micron levels, are optically transparent, are naturally hydrophobic and so eliminate the need for surface functionalization in water-based assembly environments and finally, are electrically insulating, which assist with the potential creation of embedded circuits for electrical and electronic applications. Thus, replica templates were created in PDMS using the rigid silicon master templates by following the guidelines discussed in the following section and then self-assembly on those deformable replica templates was demonstrated.
6.2 Fabrication of deformable template replicas

Electron beam lithography is an effective means of patterning assembly templates, but it is not economical for manufacturing templates on a large scale. As discussed in the previous section, one potential approach to minimizing the cost of assembly template fabrication is to create polymer replicas of master template patterns using the techniques of soft lithography.

During the course of this research, deformable replicas were created from silica template masters by soft lithography for two main purposes: to demonstrate the technology for low-cost template replication, and to assess the effects of substrate deformability on the TASR process. The process sequence for fabrication of these deformable templates was devised by Amelia Servi, a collaborator in the TASR project at MIT. The process was discussed in previous work [37], but it is documented here briefly for completeness. Polydimethylsiloxane (PDMS) was used to create polymer replicas from the silica templates. PDMS is a commonly available polymer that can be poured in the liquid state on top of a master pattern and subsequently cured. Step-by-step details of the process sequence used are listed below. A schematic description of the two-sequence process is also shown clearly in Figure 6.1. As described previously in Section 3.3.3, the master template fabrication procedure involves the creation of patterned hemispherical sites on a master template into which spherical micro-components may assemble based on shape and size matching, or which may instead be used for the creation of pattern replicas. Fabrication of the patterned silica template follows the approach discussed in detail in Section 3.3.3. Starting spot sizes ranging from 45-500 nm were created on the master template using e-beam lithography; the underlying oxide was then etched isotropically through the resist openings to create nearly hemispherical holes on the template. Once the master silica templates were fabricated, deformable replicas were created from these in PDMS.
Figure 6.1: Schematic diagram showing the procedure for fabrication of the deformable template replica using a low-cost, two-step process. In the first step, liquid pre-polymer is poured onto the patterned assembly template, cured and peeled off to create an inverse pattern of the original. In the second step, the same procedure is repeated with the mold made from the first step, which is then cured and peeled to create a deformable replica of the original template. Finally, the replica is bonded onto a glass slide to create the TASR template for selective self-assembly.

In order to do this, the silica templates were silanized by placing them in a vacuum chamber along with three drops of HMDS on a glass slide for one hour. Silanization of the silica surface was done in order to make the surface of the silica hydrophobic and to reduce the adhesion strength between the silica surface and the PDMS. This step was necessary in order to facilitate the easy separation of the finished device from the silica during the last step in the fabrication process. While the silanization was in progress, the PDMS mix was created. In order to do this, a 10:1 mixture by weight of PDMS pre-polymer with its curing agent (Sylgard 184 elastomer from Dow Corning) was measured out into a container. Since the mixture was observed to be highly viscous, an extra quantity of the mixture (at least 4g more than needed) was created in order to overcome the difficulty in removal of all of the mixture from bottom of the container. The contents of the container were mixed thoroughly with a stick and degassed in vacuum for 10-15 minutes in order to bring the air bubbles generated in the mixture onto the surface, where they were then removed with the help of a nitrogen gun. Since several molds were created in a single batch, PDMS for multiple rounds was mixed at once, and these were used within an hour of the mixing process. In order to constrain the flow of the liquid
PDMS mixture and mold it in the desired shape, a 75x25mm slide box was created. Four glass slides were placed along an adhesive tape in alternating orientation at an angle of 90 degrees from each other. The sides were subsequently folded inwards so that the adhesive was inside the box which was done to assist the peeling off of the mold out of the slide box. The four-slide rectangle was then placed around another slide in order to form the bottom of the box, and adhesive tape was used to seal off the edges. The patterned silica wafer(s), created following the process sequence described in detail in Section 3.3.3, were then put into this slide box. For smaller wafers, a 25x25mm slide box was created instead of the 75x25mm box by standing all slides vertically while still using a slide as the bottom. Upside down wafer shards were then used to provide a larger area at the same height as the silicon chip of interest. After the slide box was created and the wafer placed into it, the PDMS mixture was poured onto the wafer in this box using an approximate amount of 2.5 grams of mixture per 25x25mm area of the wafer. The PDMS layer on top of the template was baked thereafter in the box at 130 degrees C for 20 minutes in order to cure the polymer and promote cross-linking between its molecules. The mold was then peeled out of the box, which was later discarded. Following this process sequence, PDMS molds of the patterned silica wafers were created. In order to get exact replicas of the original silica wafer, the same process was then repeated; the difference in the second run was that the PDMS mold created in the first step was now used as the master for the second molding sequence instead of the silica master as used before.

In order to make molds of the molds, the same directions were followed except for a few changes which are as follows. Since the PDMS molds obtained at the end of the first sequence were naturally hydrophobic, these were not silanized. The same type of slide box was used for the second sequence as that used in the first. However, since the PDMS mold weighed less than the silica wafer, during the second sequence some of the liquid PDMS tended to get under the PDMS master and push it upwards (unlike the case of the silica master, which owing to its greater weight stayed put). In order
to overcome this problem, the first mold was made thicker than usual, using a mixture mass of 3.5gm per 25x25mm area of master instead of the 2.5gm as mentioned previously. In addition, the PDMS master became attached to the bottom slide with PDMS, and the slide was left a permanent part of the PDMS master. In order to ensure smooth removal of the PDMS mold layer from the PDMS master layer once the process was complete, before pouring liquid PDMS onto the PDMS master, adhesive tape was applied onto the flat parts created by the silicon shards used during the original molding. This was observed to be useful for separating the two layers of PDMS from each other upon completion of the process. After baking the mold, the glass sides were taken off of the box, and a razor blade was used to cut the PDMS layers apart along the plane of the adhesive tape. Great caution was exercised in removing the molds very gently, without sliding the razor blade over the active part of the template (which contained the grids of matching sites for components for self-assembly using TASR), since if all of the area surrounding the active area was detached, the layers would peel apart easily. (It would also be possible to promote separation of the layers for example by coating the inverse master with a thin layer of sputtered gold. However, this did not prove to be necessary in the present work.)

Apart from the few differences mentioned here, the rest of the steps and process parameters followed in order to create PDMS molds from the PDMS master were essentially the same as those followed in order to create the PDMS mold off the silica master. At the end of the two sequences discussed above, PDMS replicas of the original patterned silica master had been created. Figure 6.2 shows optical micrographs of the PDMS replicas made from the silica templates in order to demonstrate the effectiveness of this technique for creating deformable patterned surfaces for TASR. The resulting patterns on the substrate are comprised of 1 micron deep, quasi-hemispherical holes that match (to varying degrees) the 2 micron diameter polystyrene microspheres to be assembled into them. The finite initial spot size results in etched holes that deviate slightly from the ideal hemispherical shape; the larger the initial spot size, the larger the deviation, if the manual replication process is well
controlled and the deformable PDMS templates accurately replicate the master templates. This can be confirmed from Figure 6.3, which shows AFM profiles of well-formed quasi-hemispherical holes each

Figure 6.2: Optical micrographs of the deformable template replicas showing the different 2-D patterns transferred onto them from the rigid silica master templates. (A) Layout of a PDMS chip showing several grids containing holes with different starting spot sizes (on master silica template). (B) Closer view of some grids on the chip. (C) A grid of assembly sites made from the same starting spot size on the master template. The holes are all uniform in shape and size and replicate the master template pattern very well, when all the influential parameters in the manual replication process are well controlled.

Figure 6.3: Atomic Force Microscopy (AFM) images of holes created in the deformable PDMS replica templates from master silica templates, with starting spot sizes (from (a)-(d)) of 45, 50, 55 and 70 nm. Smaller starting hole sizes correspond to better shape matching with spherical components and therefore higher anticipated assembly yield.
etched from a different size starting hole in the resist. These profiles were obtained from templates in which all of factors involved in the manual replication process were carefully controlled to obtain the desired hole shapes, replicating accurately the hole profiles on the master templates.

It is to be noted, however, that since there are several steps involved in the replication process which are done manually in the initial demonstrations presented here, the hole profiles obtained for replicated templates in some cases were not found to be exactly identical to those on the master templates, though this was the case only for a few templates (as seen for holes in Figure 6.4). As a result, some holes were either not well-formed (hole profiles were not hemispherical), as shown in

![Figure 6.4](image-url)

**Figure 6.4:** Atomic Force Microscopy (AFM) images of holes created in the deformable PDMS replica templates from master silica templates, which are not well-formed in the replication process. Since there are several steps involved in the replication process which are done manually in the work presented here and require very precise and repeatable control of replication environment, the hole profiles obtained for replicated templates in some cases were not obtained to be exactly identical to those on the master templates, such as the ones shown above in (A) – (D). Some of the defects observed were attributed to missing material on the hole sidewalls as in (A) and (C), or due to extra bumps inside as in (B), or even due to possible defects in the master templates, as in (D), in which case the hole in the master template did not etch completely.
Figures 6.4 (A), (B) and 6.4 (D), or the resulting holes formed on the replica templates were larger in size than the holes on the master templates due to improperly formed hole edges, even if the actual hole shape itself was good, as seen in Figure 6.4 (C). This might be attributed to a larger force than required being exerted on the replica during the step where the PDMS mold is peeled off from either the master template or from its own copy; another cause of flaws in some cases is the trapping of air bubbles inside the liquid PDMS pre-polymer leading to edge defects. For the long term, the repeatability of replication can be ensured by a precise control of the replication conditions every time, for example by making the system automated or by using other methods to assist detachment of the mold from master, for example, by coating the inverse master with a thin layer of sputtered gold, as also mentioned previously. For the present experiments, the fact that some of the hole profiles obtained on the template were not well-matched for assembly of the assembly components used in the present work was useful to assess the selectivity of the assembly set-up in the removal of assembly components from these holes, which were not well-matched.
6.3 Assembly on deformable substrates

The basic set-up and protocols for the experiments described here were the same as those discussed in Section 4.1. Input voltages for the experiments reported here ranged from 20 V to 60 V. Deep-blue dyed polystyrene microspheres, with a diameter of 2.004 (± 0.08) μm, were purchased dispersed in water from Phosphorex, Inc. (catalog no. 1002KB). The color was chosen to offer high contrast to the transparent PDMS polymer surface for improved visibility during optical imaging.

Polystyrene is naturally hydrophobic, and hence the assembly components did not need to be coated with a SAM. Similarly, since PDMS is also naturally hydrophobic, it was also directly used as a TASR template without the need to functionalize it with a hydrophobic SAM. The assembly fluid mixture was prepared by pipetting the polystyrene microspheres into various ethanol–water mixtures (4%, 8%, and 20% water). The resulting mixture was placed in capped microcentrifuge tubes and shaken on a Vortex mixing tool (Vortex-2 Genie, by Scientific Industries) for a few minutes to disperse the components. The prepared particles were used immediately to prevent re-agglomeration due to settling. About 2 mL of the assembly fluid medium was pipetted into the assembly beaker. The patterned PDMS template was placed in the beaker, face-up. A sufficient volume of the polystyrene component solution (between 300-400 μL, at a density of 2.3 x 10^9 components per mL) was added to the assembly beaker using a pipette. This volume of solution contains a large oversupply of components, with more than 10^5 times as many microspheres as there are assembly sites on the template. The density of components contained in this volume of the solution was chosen to provide an optimum quantity of components needed for the TASR process, for reasons similar to those described earlier in detail in Section 5.3. The small beaker was capped with a Parafilm® cover, power to the transducer was turned on, and the experiment was allowed to run undisturbed for 5 minutes. At the end of the experiment, the template was taken out of the assembly mixture and placed on a flat surface. Any remnants of the assembly mixture liquid on the template surface were allowed to air-dry, although
for a short time only (on the order of a minute). The assembly template was then examined under an inverted optical microscope to see which assembly sites were occupied after the TASR process. After the results were documented, components were removed from the assembly sites on the template. This was done by placing the template at the bottom of the assembly beaker, adding a small volume (4 ml) of ethanol to the beaker and placing the system in an ultrasonic bath for about 2 minutes. The template was then reused in an effort to ensure geometrical consistency between runs as well as to minimize the need to fabricate additional templates. The experiments were conducted under different transducer voltage levels (between 20 – 60 V) to study the effect of excitation level on the assembly. The assembly yield, defined as the number of assembly sites that were occupied with matching spheres after assembly as a fraction of the total number of assembly sites on the template, was recorded for each of these voltage levels. Experiments were then also conducted under similar conditions for the self-assembly of polystyrene microspheres on the master silica template, using procedures described in detail in previous work [17, 37]. Results obtained from these two sets of experiments were compared to assess the effect of the deformability of the template surface on assembly yield in TASR. The results are presented and discussed in the following section.
6.4 Results and Analysis

The deformable polystyrene spheres were successfully and selectively assembled into matched sites in the patterned replicated templates using TASR. After assembly, the assembly yield was quantified by calculating the ratio of the number of holes of each size that were filled with components to the total number of holes of that size. Figure 6.5 shows optical micrographs highlighting the progressive stages of assembly. At low values of excitation from the transducer (up to 20V), very low assembly yield is observed and most of the assembly sites are empty (Figure 6.5(A)). This observation is explained by the fact that at low voltages, the drag torque is less than the impedance torque, so that impedance effects dominate and the acoustic excitation is not able to drive the components into the assembly sites in the first place. As the voltage is increased, the assembly yield starts increasing, also

![Figure 6.5](image)

**Figure 6.5:** Optical micrographs showing progressive stages of assembly in the TASR process upon increasing voltage on the acoustic transducer. (A) shows a portion of the TASR template at 20V, most of the holes are observed to be empty. (B) shows the assembly template region upon increasing the voltage to 30V. More and more holes start filling up on increasing voltage, the holes that have a black circle in the center are filled with microspheres whereas the ones with a white circle in the center are empty. (C) shows the same portion of the template after subjecting it to the optimum assembly voltage of 50V. All of the holes are filled with microspheres that match them in shape and size, demonstrating successful assembly.
seen in Figure 6.5(B), since more and more components are now able to get into the assembly sites and stay there if they match the topography of the site. The ability of the components to stay trapped within the site is described by a comparison of the retention torque to the removal torque [15, 16], as discussed in chapter 2. At a peak value of excitation voltage of about 50 V, the assembly yield climbs up to a high value of nearly 100%, as observed in Figure 6.5(C). At this stage, the retention effects dominate the removal effects. With a sufficient increase in voltage beyond this point, the removal effects dominate and the yield starts decreasing. The assembly in this case was carried out in 8% water - 92% ethanol mixture. The pattern on the master silicon template, from which the deformable replicas are fabricated, is made using e-beam lithography by exposing various arrays of small spots in the PMMA resist layer, with spot sizes ranging from 45 to 500 nm. The template array shown in Figure 6.5 is entirely comprised of holes that were etched to a 1 μm depth from 50 nm openings in the resist on the master silicon template, so that the deviation from the ideal hemispherical shape is quite small, and the holes are all well-matched in shape and size to the polystyrene spheres, as seen from the AFM profiles of one of the holes shown earlier in Figure 6.3 (B). Although several different water concentrations (4%, 8%, 20%) in the assembly fluid were used for assembly at various times, the results presented here focus on the 8% water data. A detailed discussion of the effects of varying fluid composition in TASR may be found in [15-17]. Higher assembly yields are obtained for holes with smaller starting spot sizes since they match better with the component in terms of shape and size as compared with the holes created from larger starting spot sizes. The repeatability of assembly results was measured by performing nominally identical experiments (50 V and 8% water fraction with polystyrene components) multiple times. Even when different assembly templates were used for each experiment, variation in yield for those assembly conditions was less than 7%.

These results are consistent with the prediction made based on the theoretical model presented in Section 2.1.2, that TASR can be effective with deformable templates. The interference ratio was
predicted to remain much below the critical value for the onset of plastic deformation for assembly into holes of the sizes considered here, and TASR’s selective removal process succeeded in selectively removing poorly-matched components as predicted.

In order to examine the effect of shape and size of the assembly sites on the assembly process, the assembly yield was recorded as a function of the starting spot size on the template. The yield for polystyrene microspheres on deformable PDMS replica templates in this work was then compared to that of polystyrene microspheres on the rigid silica master templates in the previous work [17]. The plots are shown in Figure 6.6. It was observed that the trends for the yield for the two assemblies were similar, when holes on the master and replica templates were identical in shape and size and the replica template was well-formed, under precise control of replication conditions. On both the relatively rigid

Figure 6.6: Plot of measured fractional assembly yield versus the nominal size of the starting holes made in resist on the master silica template, from which the deformable PDMS replica template is created, comparing the results for the assembly of 2 μm diameter polystyrene microspheres on the replica PDMS template to that of polystyrene microspheres on the master silica template.
silica master template as well as the deformable replica template, the yield was observed to be high for small spot sizes and was found to decrease with increasing spot size, with the highest yield of nearly 100% recorded for spot sizes in the range of 45-50 nm. Thus it was established that as long as the templates were replicated precisely from the master templates and the assembly conditions were well optimized for assembly using TASR, the material used as the substrate did not substantially influence the assembly results. This was an important conclusion for the purposes of extending the applicability of TASR to situations involving the use of deformable template materials.
6.5 Summary

In summary, this chapter describes the procedure for carrying out self-assembly using TASR on templates that are made from a deformable material, unlike previous work which has focused on assembly using templates made from relatively rigid materials like silicon or silicon dioxide. One of the main steps in this demonstration is the process of fabricating deformable replica templates using rigid master templates. The procedure to fabricate the master silica template is described previously in Chapter 3. This involves e-beam lithography and isotropic etching to obtain hemispherical assembly sites on the template, all of which are 1 μm deep but have different hole shapes due to different sizes of the resist opening through which the isotropic etching was carried out. This chapter first describes the procedure to create a deformable replica off of this master template utilizing soft lithography, by making use of commonly available PDMS. The assembly of 2 μm diameter polystyrene microspheres into the patterned assembly sites on the PDMS templates using TASR is then described. The effect of voltage variation on the assembly yield is studied. A high assembly yield of 100% is observed for the holes that are well matched in shape and size to the polystyrene microspheres, at a voltage of 50V. The data for assembly of 2 μm diameter polystyrene microspheres on deformable PDMS replica templates is then compared with the assembly of the polystyrene microspheres on the relatively rigid silica master templates, details of which are described already in previous work [17, 37], in order to assess the effects of template deformability on the TASR process. It is found that the results for assembly onto both template materials are similar, and that template deformability does not substantially influence the success of assembly for this set of material combinations, provided that the assembly sites created in the deformable template closely replicate the assembly sites on the master template in their shape and size. This is an important conclusion for extending the scope of TASR based assembly to low-cost, replicated, polymer-based templated surfaces. The theoretical model predictions for the
assembly of deformable components on deformable templates, described earlier in Section 2.2.4, are also validated using experimental results.
Chapter 7

Anisotropic Component Assembly

7.1 Motivation for working with anisotropic shapes

The work presented so far involves the assembly of only isotropic, spherical components using TASR. However, some applications of interest for TASR involve the assembly of anisotropic components. Successful assembly of shapes other than spheres can open up new possibilities for applications of this technique. In the context of biological systems, the capability to assemble non-spherical shapes can vastly increase the range of biological specimens that TASR can work with and structure, e.g. E. Coli, Salmonella typhimurium bacteria, etc. Since TASR is well matched to MEMS length scales going from a few hundred nanometers to tens of microns, the capability to assemble anisotropic shapes such as cylindrical micro-components also offers a great tool for structuring MEMS. Some potential applications of MEMS-based systems that possess the flexibility to assemble components of different shapes include integrating more expensive, valuable structures into relatively low-cost systems, creating optical meta-materials, or shape and size-selective chromatography. Self-assembled systems comprising cylindrical micro-components, for instance, can be used to create chemical or biological sensors, since the larger surface area of separately fabricated and functionalized cylindrical components can offer the capability to sense a large number of different target species simultaneously.

As a first step towards these types of applications, this chapter describes research focused on the assembly of cylindrically-shaped anisotropic assembly components in order to extend the applicability of the process to new geometries, applications and processes.

Introducing a new shape into the process introduces complexities not only in the experiments but also in the theoretical analysis. One of the potential challenges that could be faced experimentally in
the assembly of components having anisotropic shapes might be controlling the orientation of the component during its approach to the assembly site in order to make sure that it always lands in the assembly site in the proper orientation to match the geometry of the assembly site. The analysis of forces and moments used in the original TASR model to describe the competition of retention vs. the removal will also change accordingly, as discussed previously in Section 2.3. In the theoretical analysis of deformable systems comprised of anisotropic components, the Hertzian contact theory must also be extended to incorporate different radii of curvature of the component in different directions, as is the case for anisotropic components.

Here, cylindrical assembly components with mean diameter of 26 microns and mean length of 50 microns were successfully assembled on a patterned silica template, extending the applicability of the TASR technique to non-spherical shapes that do not have predominantly flat sides. The assembly protocols and procedures for these components are documented below.
7.2 Fabrication of cylindrical components and limitations

The cylindrical components for demonstration of anisotropic component assembly using TASR were fabricated using the *Stop flow Lithography* (SFL) technique [67, 68], in collaboration with Prof. Patrick Doyle, Department of Chemical Engineering at MIT. The components were prepared using a combination of 95% TMPTA and 5% photo-initiator, and were naturally hydrophobic. The main idea used in the component fabrication process is described below. Extensive details can be found in [67, 68]. The SFL technique uses compressed air driven flows to synthesize polymer particles. A flowing stream of oligomer in PDMS microchannels is stopped, and an array of particles is polymerized into it using UV light. The formed particles are then flushed out at high flow rates, and the cycle of stop-polymerize-flow is repeated.

While the SFL technique used to fabricate anisotropic components in the current work is a flexible technique in general, there are some limits to the fabrication process, which need to be kept in mind for the purposes of assembly using TASR. Because particles are synthesized in flow and exposed to finite pulses of UV light, they are smeared when high oligomer flow rates are used. In order to achieve a given particle resolution, there is therefore a limit to the maximum flow rate that can be used. This imposes restrictions on the particle throughput that can be achieved, a problem that is accentuated as particle size is decreased [68]. The typical yield of the stop-flow lithography technique for fabricating components in the size ranges used here is thus between 5000-15000 components/cycle. While it is possible to create more components in a subsequent stop-polymerize-flow cycle, the components created in different batches are not identical. TASR works best in an oversupply of components, so in order to achieve high yield using TASR using the set-up as described in the current work, typically an oversupply of $10^3 - 10^4$ times as many components as the number of sites is needed in each experimental run. (The same components can, however, be re-used.) This poses a logistical limitation on the assembly yield in TASR that can be achieved using components made from this
Another limitation of the fabrication technique is that components that are too small in size are created in a very non-uniform size distribution due to challenges with the resolution at small scales. For example, in the present work, two sets of cylindrical assembly sites were created on the templates which were 8 μm and 26 μm in diameter. These two different sizes for assembly sites were chosen to demonstrate size matching using TASR. While the stop-flow-lithography technique was able to successfully create components that were 26 μm in diameter and 50 μm in length with a tight tolerance on the diameter distribution of ± 0.5 μm, the technique failed in manufacturing the 8 μm diameter components as repeatable cylinders and with a size tolerance that was acceptable for the purposes of TASR.

In order to examine the effects of length of the assembly component on assembly effectiveness, components were also fabricated in different lengths using the stop-flow technique. In the present work, the 26 μm diameter cylindrical components were fabricated in two different lengths – 50 μm and 150 μm. It was observed that while the 50 μm length components had nearly perfectly straight edges, the ones with an intended length of 150 μm diameter had significantly tapered sidewalls, again due to the challenges faced in the fabrication technique. It is difficult to fabricate components precisely with lengths in excess of 100 μm using the technique. Finally, only certain specific materials can be used to create components using this technique.
7.3 Assembly of cylindrical components

The general assembly set-up for TASR is described in detail in Chapter 4. The particular details for the assembly of anisotropic components used in current work are discussed here. As discussed previously, the basic experimental protocols for TASR include the creation and functionalization of the templates on which assembly is to be carried out, the functionalization of the components that are to be assembled (when necessary), and the actual assembly experiments themselves. In this case, for the assembly of anisotropic components, the template created was a silicon die with a patterned layer of oxide on top onto which the components can assemble. Contact lithography and isotropic etching were used to pattern the silicon dioxide layer with hemi-cylindrical holes, using an isotropic BOE etch from a thin line opening in the resist, as already described in extensive detail in Section 3.3.2. These holes serve as matching sites for the assembly of polymer micro-cylindrical components which are 26 μm in diameter. The assembly template was then functionalized in order to make it hydrophobic, using the exact same procedure as outlined in section 4.2.1. The micro-cylinders were prepared for assembly by suspending them in an appropriate solvent and treating them to separate any clusters of components into individual micro-cylinders. The solvent used here for component suspension was a mixture of 8% water and 92% ethanol, by volume. The prepared components and assembly template were then placed in the assembly beaker in the assembly fluid medium, which was subjected to acoustic excitation at a megahertz-scale frequency of 1.7 MHz. The assembly fluid medium used here was identical to the solvent used for component suspension (i.e. a mixture of 8% water and 92% ethanol, by volume). The excitation of the fluid mixture promotes selective removal of components from incorrectly matched sites on the template along with ensuring circulation of components in the fluid. After letting the system run undisturbed for 5 minutes, the template was taken out of the assembly beaker and allowed
to air-dry. It was then observed with the help of an optical microscope. Results are presented and discussed in the following section.
7.4 Results and Analysis

In this chapter, results of the experimental work are presented, followed by discussions and analysis. The assembly results demonstrate shape selective assembly with a high local assembly yield for matching components. The two sets of 26 µm diameter, deformable Trimethylolpropane Triacrylate (TMPTA) microcylinders were assembled using TASR into 26 µm diameter sites in the patterned silica templates using the experimental protocol discussed above briefly in Section 7.3 and in detail in Chapter 4. As with other experiments using TASR, assembly was carried out in a range of voltage levels from 40-60V. Optimum assembly in this case was obtained at a transducer voltage of 45V, and these results are presented here.

Figure 7.1 shows optical micrographs of the two sets of TMPTA assembly components used for testing shape and size selectivity of assembly. While both sets of components have the same starting diameter of 26 µm, they have different shapes due to differences in length. The 50 µm long, straight components shown in Figure 7.1(A)-(B) and the 150 µm long, tapered components shown in Figure 7.1(C)-(D) reflect the SFL technique’s limitations in fabricating perfectly straight sidewalls for components of larger lengths. The fact that the two sets of components have different shapes is, however, useful for establishing the capabilities of the TASR technique.

Figure 7.2 shows optical micrographs of the components that match the geometry of the assembly sites (26 microns in diameter, 10 microns in depth and 120-170 microns in length) self-assembled into those sites. It may be clearly observed from these optical micrographs that only the components that are well matched in shape (and size, in the context of uniform diameter matching) are retained inside the assembly sites. Thus, in this case, only the 26 µm diameter components with the straight sidewalls (which are 50 µm in length) are retained inside the assembly sites, which also have straight sidewalls, while the ones with the significantly tapered edges (which are 150 µm in length) are excluded by TASR’s selective removal process. It can be noted that only diameter matching of components and
sites is demonstrated in the current work since the matched components shown in Figure 7.2 are all shorter in length than the assembly sites, due to challenges with the component fabrication for components of very small or very large lengths. It is also apparent in Figure 7.2 that the most of the components occupy the edges of the sites. This is due to the fact that contact area is maximized when

![Figure 7.1](image_url)

**Figure 7.1:** Optical micrographs of portions of the assembly template before assembly with TASR, showing two sets of anisotropic assembly components, one in (A) – (B) matching and the other in (C) – (D) not-matching in shape to the assembly sites, lying on the template. Both components have a starting diameter of 26 μm. While the components in (A) – (B) have straight sidewalls and have a length of 50 μm, those in (C) – (D) have tapered sidewalls and have a length of 150 μm. The SFL technique used for manufacturing these components faces limitations in fabricating components with straight sidewalls for components beyond a length of 100 μm, hence the taper for components with larger lengths is observed. Components with different shapes as shown here are used to assess the shape selectivity of the TASR technique.
the cylinders (with their rounded end caps) are positioned at the ends of the assembly sites, as compared with the case in which both ends of the cylinder are out of contact with the site when a component lies somewhere in the middle, away from the rounded site ends. Figures 7.2 (A)-(D) show small but magnified portions of the template with matching components retained inside sites in which individual components are seen clearly in focus. Figure 7.2 (E), on the other hand, illustrates a larger area featuring an array of assembly sites almost all filled after assembly except for a single empty site. As in previous experiments with TASR, the assembly yield was quantified by calculating the ratio of the cylinders (with their rounded end caps) are positioned at the ends of the assembly sites, as compared with the case in which both ends of the cylinder are out of contact with the site when a component lies somewhere in the middle, away from the rounded site ends. Figures 7.2 (A)-(D) show small but magnified portions of the template with matching components retained inside sites in which individual components are seen clearly in focus. Figure 7.2 (E), on the other hand, illustrates a larger area featuring an array of assembly sites almost all filled after assembly except for a single empty site. As in previous experiments with TASR, the assembly yield was quantified by calculating the ratio of

Figure 7.2: Optical micrographs showing portions of assembly template after assembly of cylindrical components with TASR. Only the components matching in shape and size to the assembly site are retained in the sites out of the two sets of components used. Most of the components are observed to occupy the edges of the sites due to maximization of contact area with the site at the edges. It is to be noted that since the matched components are shorter in length than the assembly sites, only diameter matching is demonstrated here. Length matching is not demonstrated due to limitations of the component fabrication technique.
the number of holes of each size that are filled with components to the total number of holes of that size. The experiments recorded here correspond to a local assembly yield of 96%; this local high yield is consistent with the fact that the holes are extremely well-matched in shape and diameter to the TMPTA microcylinders with a diameter of 26 μm and length of 50 μm. It is thus noted that despite the limitations of the component fabrication technique resulting in a low component density (as also mentioned in Section 7.4), a high local assembly yield is observed, indicating that the TASR process is working efficiently in a local region where a sufficient number of components is available for assembly. This also implies that global assembly yield may be improved by using larger number of components for assembly, or by moving the assembly template within the assembly fluid during the assembly process to ensure that all parts of the template are exposed to the high component density portions of the assembly bath.
7.5 Summary

In summary, successful assembly of anisotropic, cylindrical-shaped components out of a mixed group of matched and unmatched components using TASR was presented in this Chapter. This is demonstrated in order to extend the applicability of the process to new geometries, applications and processes, for example the ability to encompass cylindrical biological specimens for assembly or for the creation of self-assembled chemical or biological sensing platforms. Here, cylindrical components were fabricated using Stop flow Lithography (SFL) technique [67, 68]. The SFL technique faces limitations in fabricating perfectly straight sidewalls for components of larger lengths. Two sets of TMPTA assembly components were manufactured for testing shape and size selectivity of assembly. While both the sets of components possessed the same starting diameter of 26 μm, they had different shapes due to differences in length (50 μm for one set vs. 150 μm for the second set). Hemi-cylindrical assembly sites were created in the silica layer patterned on silicon assembly templates using isotropic wet etching techniques and were measured to be 26 microns in diameter, 10 microns in depth and 120-170 microns in length. The silica template was functionalized with a self-assembled monolayer to make the surface hydrophobic and to promote adhesion in a water-based assembly fluid medium. The components were naturally hydrophobic and so did not require any surface functionalization for adhesion to the hydrophobic template surface. The system was then tuned for assembly by subjecting it to optimal assembly parameters. Simultaneous assembly of the two sets of components described above was carried out. Only the components that were well matched in shape (and size, in the context of diameter matching) were found to be retained inside the assembly sites. In other words, the components with a length of 50 μm were retained inside the sites after assembly while the components with lengths of 150 μm were removed by the TASR process. High local assembly yield was recorded using the assembly process. Components were observed to occupy the length-wise ends of the sites to maximize contact area, in line with the model predictions on selective removal theory [15]. The global
assembly yield was moderate due to the low component density in the system, as the SFL technique has a limited component throughput. However, the approach holds potential for increasing yield by increasing the component density or engineering better circulation of the existing components among the assembly sites.
Chapter 8

Conclusions and Future Work

8.1 Summary of present work

In this thesis, the successful, TASR-based, shape- and size-selective assembly of biological cells, for both mammalian as well as non-mammalian cell lines on rigid substrates was demonstrated for the first time, and the groundwork was laid out for working with systems at the biological length scales.

Previous work [15, 16, 37] using TASR focused on the assembly of rigid and polymer-based, deformable microspherical components on rigid template surfaces. The assembly of biological specimens, which represent an extreme case for deformable materials using TASR, which works on shape and size selectivity, was identified as a challenging task due to the difficulties encountered in precise placement and sorting of these using conventional biological handling techniques.

A theoretical model was first proposed in order to predict the conditions under which TASR-based assembly of deformable components, such as biological cells, on both rigid as well as deformable substrates will be successful. This model, which combines all of the previous TASR models, was used to design the system for assembly and identify operating ranges for key parameters within which the system should fall for meeting successful assembly requirements. The success of this model was then demonstrated by comparison of the model predictions with experimental results for several materials, some of which were clearly well within the safe regime for tolerable deformation such as biological cells, to materials such as polystyrene, which lie near the boundary of how much deformability can be tolerated by the TASR process.
Based on model predictions, the assembly of biological cells on patterned silicon template was considered experimentally. Patterned silicon templates were created which showed reasonable conformation to the ideal hemispherical profiles desired for assembly of nearly spherical mammalian and non-mammalian cells into the matched sites. The results for assembly of both non-mammalian (SF9 cells) and mammalian (mouse-derived non-small lung cancer cells) cells on these patterned substrates exhibited both a good degree of assembly selectivity of cells in a range of sizes into their corresponding size-matched holes, and high assembly yields of up to 100% for cells trapped in well-matched holes. These results showed agreement with the predictions of the theoretical model for assembly of deformable biological materials using TASR.

A detailed analysis of the effect of varying the experimental parameters on the assembly yield was used to determine the ideal conditions that needed to be maintained in order to achieve high assembly results. The trends in variation of assembly yield on varying these controllable parameters helped elucidate the TASR process for self-assembly of biological systems in greater depth. Cell viability tests were carried out after the assembly process and high cell viability of up to 99% was observed for both the cell lines, showing that the TASR system provides a favorable environment for the growth and survival of both mammalian and non-mammalian cell lines and confirming theoretical predictions.

Based on model predictions, assembly using TASR on deformable templates was also considered experimentally. This was done in an effort to make the TASR technique low-cost and manufacturable, since conventional template manufacturing techniques in silicon were identified to be time-consuming and expensive. Patterned PDMS templates were created by replication from rigid silicon master templates which showed good conformation to the ideal hemispherical profiles desired for assembly of microspherical components into the matched sites. Assembly of deformable polystyrene components was carried out on deformable substrates, in order to demonstrate that deformable materials can be
assembled successful on these replicated substrates, which provide a bio-compatible platform for structuring biological systems. The results for assembly of deformable components on these patterned substrates exhibited both a good degree of assembly selectivity of given components into different-sized holes, and high assembly yields of up to 100%. These assembly results were also compared with the results for assembly of deformable components on rigid master templates, assembled under identical conditions, in order to assess the effect of deformability of the substrate. These results showed agreement with the predictions of the theoretical model for assembly on deformable substrate materials using TASR.

Finally, in order to assess the effects of shapes of the assembly components on the assembly process, the assembly of anisotropic components was also considered experimentally. Successful assembly of anisotropic, cylindrical-shaped components using TASR was presented. Two sets of TMPTA assembly components were used for testing shape and size selectivity of assembly, both of which possessed the same starting diameter but different shapes. Only the components that were well matched in shape (and size, to the extent of diameter matching) were found to be retained inside the assembly sites with a high local assembly yield, while the components with not well matched were removed by the TASR process. These results also showed good agreement with the predictions of the theoretical model on assembly of anisotropic shapes using TASR.

Therefore, a strengthened theoretical foundation was used to successfully demonstrate and analyze experimentally, the selective assembly using TASR for biological systems. Further verification of the groundwork for biological-based TASR systems presented here is possible by extension of experimental work to encompass a broader range of biological specimens.
8.2 Considerations and Future Work

Some of the important aspects of the TASR process and details to be paid particular attention to in the experimental process are recorded here, which would serve to provide guidelines for future work using this method.

The first of a few key points which need to be kept in mind when using this technique, is that since TASR works well in an oversupply of assembly components, much larger number of assembly components than the number of assembly sites are required, as was also the case in the present experiments, since otherwise there is not enough sampling of the sites by the components for assembly. It is to be noted, however, that having too many components in the system is also undesired since this leads to an agglomeration of components in the system and poor circulation in the assembly fluid medium. While the appropriate number of components depends on the number of sites on the assembly template and assembly conditions, typically an oversupply comprising $10^3$-$10^4$ as many components as assembly sites is desired.

It is also crucial to ensure adequate circulation of components inside the assembly fluid medium. While the exact level of circulation desired in the assembly fluid medium depends on the particular details of the experimental set-up for the system chosen, it is possible to improve the circulation in general by moving the chip inside the assembly set-up to locations of higher circulation. This is in a contrast to the approach adopted in the present work, where effort was made to keep the assembly chip stationary at a fixed location inside the assembly beaker, which was consistently the center of the beaker for all of the experiments recorded here.

It is to be noted that these limitations on circulation and oversupply of components in TASR poses some restrictions on the applicability of this technique to applications where an oversupply of the component to be filtered out is not available. For example, for isolation of cancer cells in a biological based system using TASR, if the particular cancer cells to be sorted out selectively from a
sample are very few in number, then the TASR process might not be able to filter all of those out efficiently. It is thus noted clearly that the TASR process is more useful for isolating certain types of components from a large population of components than for filtering out selectively a unique component type amongst many types.

Another aspect of the assembly process to be kept in mind is that since there are numerous parameters which can be tuned in the system so as to regulate the assembly yield, as also discussed previously in the theoretical framework in Chapter 2, consistency in the experimental set-up is a critical criterion to be monitored and regulated. An important point to be noted in the present work is that not all of the template fabrication techniques give the same results in terms of template profiles and surface roughness. For instance, in the present work, wet etches were observed to provide much smoother side-walls for assembly sites than dry etches creating similar dimensions and profiles, which is an important issue to be kept in mind depending on the system requirements for assembly.

Also, as pointed out by the theoretical model results for assembling deformable structures, previously in Chapter 2, it is desired to operate with assembly components that are above a certain component size for successful assembly, where the limit of the component size depends on the particular set of materials in question chosen for assembly. If one chooses to operate with components that are below this critical size level, then success of assembly may be limited by the onset of permanent deformations in the system. The theoretical models, on the other hand, also predict that there is an upper limit to the component size one can go to since for assembly components that are too large, the constraints on the optimal intensity for excitation are not met. Since the previous two constraints on the size of the system mentioned drive the system in different directions, it is important to be careful that the range of sizes chosen for assembly space is not eliminated between the two constraints posed.
Another observation from the theoretical framework proposed here is that as one approaches a larger component size for assembly, a very narrow range of intensity is shortlisted that meets both the criteria on intensity, discussed earlier in Section 2.2. This has also been confirmed in the data obtained in previous work [15, 16], where it was shown that while just after or before a certain level of intensity, the circulation level is still adequate for initial assembly, it is in these same regions also the intensity level is too high for achieving a high assembly yield. While this was observed experimentally in previous work, the integrated theoretical framework proposed in the current work shows clearly why that is the case.

Another feature of interest in the current work was that the selective sorting of biological specimens was achieved without any biological or chemical functionalization of the assembly templates and the specimens themselves, and adhesion between the two was achieved solely on the basis of the natural interaction between the cells and the silicon substrate in the present work. In the future, however, depending on the application targeted, one might need to manipulate the strength of the chemical or biological interaction between mating surfaces in order to produce the desired effect.

Some of the other features of the experiments presented here which can be improved upon in future work are also highlighted here. While high viability of both the cell lines used for experiments here was demonstrated for short durations of time after the experimental procedure, long term viability of the cells over a few days after subjecting them to the TASR process was not studied. While this might provide a useful tool for applications requiring sorting of biological specimens followed by immediate analysis and documentation of results, for application requiring prolonged studies on the cell behavior, carrying out long term viability tests of cells after TASR is highly recommended. In such a case the cells should be cultured and passaged after subjecting them to the TASR process wherein their growth rates should be compared to cell lines not subjected to the TASR process in order to fully study the effects, if any, of TASR system on the cell line chosen.
Since the TASR process has been demonstrated to work with a diverse range of materials, sizes and even shapes, in the current work, the TASR process has been shown to be fully equipped and capable to achieve functionalities for a wide variety of applications. For instance, in the simplest application of the TASR technique building upon the assembly of deformable spherical micro-components shown here, if the components are now functionalized so as to detect a certain set of target species in a given sample, one can potentially utilize such a set-up to create chemically or biologically based sensors.

Similarly, building upon the concept of biological cell assembly with high yield and density demonstrated in the present work, if the chosen cells are assembled in the desired spacing and pattern on a deformable, bio-compatible substrate material, permitting the growth and passage of cells, it is possible to create tissue scaffolds using this technique for a host of further applications.

Another feature of the experiments with cells using TASR is that the cells need to be maintained in a certain temperature range and at a required level in order to sustain viability. It is possible to achieve the desired temperature in such biological systems by placing the assembly beaker (containing cells) in a controlled-temperature water bath, which can not only prevent cooling off to ambient temperatures which is undesirable for cells, but may also prevent temperature rise due to the acoustic heating of the fluid. It is also possible to obtain more insights into the biological mechanisms of assembly during the TASR process by trying to capture some of these interactions into the currently proposed theoretical framework.

While size-selective cancer cell sorting was shown in the present work, it is also very helpful to extend the application of the current method to the sorting of cancerous and non-cancerous cells, capitalizing upon the fact that the two typically differ in size significantly. This would then provide for a very fast and efficient method to selectively sort out tumor cells from an infected sample, provided the constraints posed by the TASR technique itself are met, and long-term viability of cells after
subjecting them to the TASR process is established. An important feature of assembly for biological system using TASR is that once the biological specimens or cells have been sorted selectively by the TASR template, it is possible to recover these cells easily, as described earlier in Chapter 5, for subsequent analysis. Thus, the assembly template, as used in the present work, holds the potential to be integrated into more useful lab-on-a-chip platforms where desired subsequent functionalities are combined into the platform such that the entire sorting and analysis sequence can be carried out in a single run through, thereby vastly increasing the scope of applicability of this tool to biological systems.
References


