A Prototype of Simple Implantable Optofluidic Device Enabled by 3D Printing Technology

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A Prototype of Simple Implantable Optofluidic Device Enabled by 3D Printing Technology

by

Yu Chang

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Mechanical and Industrial Engineering

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Abstract

A Prototype of Simple Implantable Optofluidic Device Enabled by 3D Printing Technology

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Superhydrophobic surface-based optofluidics have been introduced to biosensors and unconventional optics recently with unique advantages such as dynamic adjustable optical properties, low light loss, and high versatility. Most of these platforms were manufactured by Microelectromechanical systems (MEMS) manufacturing technology and usually need to be further packaged or sealed. Although the MEMS-based technology is prone to make an integrated device containing sensing and electronic components, the technology may constrain the design of the morphology of the featured structures. For example, it was hard to manufacture a three-dimensional, fully enclosed round channel with complex structures along the inner surface. The limitations could prevent the researchers from potentially advanced and innovative designs. Microstereolithography (µSLA) has emerged as a versatile solution primarily due to its comparable resolution and compact manufacturing processes. This technology has been found widespread in applications across various fields, particularly in the realms of biology and chemistry. In this study, we harnessed the power of µSLA to revolutionize the design of superhydrophobic surface-based optofluidic probes. Our approach involved the creation of optofluidic chips with diverse T-shape structured configurations, followed by transmission measurements and ray tracing simulations. After the analysis, we were able to identify the main design factors (solid area fraction at the solid/water/air interface, the cross-section shape, and the effective cladding layer
composition) and determine the optimal dimensions for the curl T-shape design, which were found to be approximately 524 µm in width, around 50 µm in thickness, and 350 µm in length, with longitudinal spaces of 260 µm between them. To validate the feasibility of our optofluidic probes, we conducted experiments in two distinct settings. The first setting mimicked an in vivo scenario, utilizing human plasma as the medium and a thyroid biopsy training phantom as the environment. The second setting, designed to simulate ex vivo conditions, involved the use of fluorescence-stained mouse brain slices. In conclusion, our study preliminarily highlights the potential of µSLA-based optofluidic probes, particularly in animal soft tissue research.
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CHAPTER 1. INTRODUCTION

This chapter provides a concise overview of optofluidic development. We first delve into the basic concept of optofluidic systems, proceeding to explore various sections that introduce typical configurations and their applications. We investigate the materials, setup, and design considerations from current literature. Our focus then shifts to the dedicated section on optofluidic-based in vivo devices, a topic closely aligned with our research. Additionally, we provide a summary that encompasses the findings from reviewed publications, ongoing improvements, and the challenges posed, which highlights potential avenues for further research. Lastly, we unveil the roadmap of this dissertation as a summary of the proposed approach.

1.1 Optofluidic

Optofluidic is usually considered as a merge of optics and microfluidics. The characteristics and advancement of microfluidic technology have spurred the development of optofluidic platforms. The characteristics of microfluidics, similar to many other microdevices, are small volume, precise control, and versatility, which powered much optofluidic research to study the light-matter interactions at the micro and nanoscales or build innovative optical elements to obtain alternative solutions for conventional optics over decades. Additionally, since microfluidics exploits the liquid as a working medium, the characteristics of the fluids, such as interchangeability and mobility, could result in a highly adjustable system for various purposes. Such optofluidic platforms can be applied to fluorescence-based biosensing devices, medical studies, absorption spectroscopies, toxic gases detection, and optics. Compared to the conventional optical elements, which are manufactured in solid with permanently fixed functionalities, the fluid-based light control system could lead to
reconfigurable optical components even in a timely manner. In general, the optofluidic has but is not limited to, the characteristics of fluids, microdevices, and optical components. It can be further integrated with conventional optical and electronic components. For example, optofluidic devices built for sensing deoxyribonucleic acid (DNA) and ammonia have been established. They can be made more compact by integrating a photon detector and other optical components into a portable DNA analyzer.

Nowadays, the overall size of solid-state optical devices has been continually reduced primarily due to the requirements emerging from the market of digital display, which indicates a compact miniaturized system. Similarly, the goal of the optofluidic design is to achieve complete integration of optical functions within a single microchip, eliminating the need for external bulky optics. The major manufacturing technique used for miniaturization is MEMS manufacturing technology. It includes a wide range of sub-techniques, such as lithography, plasma etching, deposition, and sputtering. The microfabrication technique shows advances in broad fields of applications, from power electronics and biosensors to communication engineering. With the technology, both the physical structure and precisions can be down to nano-scale. This approach has the potential to reduce system costs and minimize its physical footprint, thus becoming a great candidate for point-of-care (PoC) diagnosis.

1.2 General setup

A conceptual scheme of the optofluidic system is shown in Figure 1. From the top of the figure, an optofluidic chip is the core of the setup consisting of a complex microfluidic with functionalized structure or channels. The device can fulfill the transportation and accommodation of the target-contained solutions. The second block represents the integrated control unit for fluids, which is responsible for pumping or
replacing the liquid with the monitored flow rate and amount, such as a digital syringe pump. Following that, the optical components represent the light source, such as lasers, to interact with the target molecules. In the very beginning, the excitation source was bulky and expensive, for example, the conventional mercury and deuterium lamp proposed by Jorgenson et al. or the laser with high coherence. The problem was solved by using the Light-emitting diode (LED) technique. Other components can also be incorporated with the chip to achieve a comprehensive setup to guide the light into the channel or post-process the transmitted light signals, such as lenses, fibers, optical filters, and micro-mirrors. The control unit for the system, such as an adjustable optical stage, is to assemble the parts for operation, which can even be automated. While the transmissions within the optofluidic channel can undergo absorption and scattering, the integration of the above sub-systems enables real-time and precise measurements for those changes in the light with a relatively small sampling volume.

![Figure 1](image)

**Figure 1.** The conceptual scheme of the optofluidic system.

1.3 Optofluidics configuration: liquid core and liquid cladding

The design of optofluidic devices can be distinct from one to another. One of the main reasons is multiple material options, including liquids, gases, and conventional
solid-state materials. The requirements for the materials depend on intended applications, for example, heat resistance, transparency, and biocompatibility. One of the optofluidic devices, similar to other light-guiding devices, typically encompasses core and cladding layers. Both layers are designed in the liquid state. A simple conceptual example is depicted in Figure 2. An optional pre-mixer is connected to the device in case the target molecules and their buffer solutions need to be prepared within the system. Fluid 1 can be a light channel with or without target molecules (samples). The Fluid 2 is an optional design used in some research and serves as cladding. According to the constitution shown in the figure, we can (1) interchange the kinds of fluids to acquire different optical properties directly, which can result in dynamic control over these optical components. We can also (2) adjust the properties of the fluids, such as flow rate, temperature, and concentrations, which leads to a refractive index (RI) gradient change of the fluid. Moreover, (3) the deformation in the interface between fluids can be realized through proper fine-tuning of the fluid. Depending on the altered morphology of the fluid interface, various optical elements, including waveguides, lenses, and prisms, can be tailored to specific designs.

![Figure 2](image)

**Figure 2.** The conceptual scheme of the optofluidic channel contains multiple fluids.

The configuration has been applied in the optics field. In 2004, Daniel et al. established a waveguide with liquid core and liquid cladding to manipulate the light beams through the control of continuous laminar flow within the channel.\(^{17}\) Yang et al.
also designed an optofluidic platform to direct the light based on laminar flow control, which demonstrated a dynamic tuning of dielectric properties.\textsuperscript{10} In both investigations, the differences in the flow rates of the fluids can lead to a series of changes at the interfaces, thus realizing dynamic reconfiguration of the devices. The RI gradient can be defined by the diffusion of microfluidic laminar flows. A tunable lens is presented by Chen et al.\textsuperscript{18} In this case, air is used as “fluid 2,” and it flows perpendicular to fluid 1. While a light source is applied perpendicularly to the channel, one can adjust the airflow rate or pressure to deform the interface, thus changing the curvature. In addition, the RI of the liquid can be tuned by modifying its concentration. Furthermore, the multiple fluids configuration can also be applied to measure the RI change.\textsuperscript{19} In the protocol, a pre-mixer is designed to deliver a solution consisting of graphene oxide (target) and ethanol. The solution then flows into the main channel with reference fluid (fluid 2). Here, the main channel is designed in a zigzag geometry for a secondary mix of fluids. While the mixed flows interacted with the applied laser, the correlations between the varied target concentrations and the received transmissions are studied. The configurations can be extended to biosensing and environmental monitoring.

1.4 Optofluidics configuration: liquid core and solid cladding

The optofluidic device with a liquid core and a solid cladding is shown in Figure 3a. Here, the liquid core is not necessarily a specific layer but can be a porous structure\textsuperscript{20} with a similar morphology to multimode optical fibers (Figure 3b). The liquid core is designed to accommodate the solutions containing fluorescent-labeled target molecules while delivering the light of excitation and emission. The design of solid cladding varied in terms of the materials and the geometries. This kind of optofluidic configuration can be found in many fluorescence-based applications. Fluorescence measurements are widely utilized in many applications, which can be divided into
steady-state and time-resolved fluorescence. The former provides information on the emission spectra to identify the interested molecular targets within the measured samples, such as DNA sequencing\textsuperscript{21}, phosphate monitoring for seawater\textsuperscript{22}, water quality analysis\textsuperscript{23}, and monitoring of proteins\textsuperscript{24}. The time-resolved one can provide additional information, such as fluorescence lifetime, but with relatively higher noise. It has been applied in DNA detection\textsuperscript{25}, biological imaging\textsuperscript{26}, reactive sulfur species mapping\textsuperscript{27}, and diagnostic method\textsuperscript{28}. Recently, a fully sealed hydrogel liquid core probe\textsuperscript{29} was developed for fluorescence measurements. The molecular targets within the sampling solutions may gradually enter and diffuse in the core and cladding, leading to a dynamic change in the guided light signal.

Many of the solid substrates of the devices are silicon\textsuperscript{30}, which is a worldwide material option for photonic and micro devices due to its compatibility with most of the micro or nanofabrication processes. It is also prone to integrate with other integrated circuit components to implement multi-tasks. Polydimethylsiloxane (PDMS)\textsuperscript{31} and other polymers have been used for conducting optofluidic devices for decades. They are known for ease of manufacturing, such as nanoimprinting\textsuperscript{32}, and are compatible with soft lithography techniques. It is also a biocompatible material for applications like lab-on-a-chip (LOC) systems for medical diagnostics\textsuperscript{33}. Glass is another option since the previous materials may not have sufficient optical transparency in a wide range of wavelengths. It is also a cost-effective material with good chemical stability to many solvents. Practically, glass-based optofluidic devices can be manufactured via femtosecond laser machining techniques\textsuperscript{34}. However, the RIs of the solid-state materials above are higher than that of the liquid. When the incident light goes from the core layer to the cladding layer with an incident angle that is less than the critical incident angle, according to the mechanism of total internal reflection (TIR), the RI of the core layer...
should be higher than the cladding layer to efficiently guide and confine the light energy in the core of the device.

![Figure 3. Conceptual schemes of the Liquid-core-solid-cladding optofluidic devices. (a) The basic configuration of the device has a liquid core layer (navy color) and a solid cladding layer (grey color). (b) Porous type configuration of the device, whereas the holes can be filled with fluids or air. The designs of the interface between the core and cladding layers (blue color) are shown in (c) a series of air spaces (green color) among the nano and microstructures and (d) an air layer with a thin solid layer.](image)

One of the common solutions is to select the appropriate materials for core or cladding layers to maximize the difference in the RIs.\textsuperscript{35} For example, hydrogels have relatively lower RIs (<1.33). It is also a biocompatible material that has been employed in the development of drug-delivery systems\textsuperscript{36} and tissue engineering platforms.\textsuperscript{37} Silica-aerogels\textsuperscript{38,39} are another nanostructured material offering a RI close to air (~1), compatible with many fluids. Even though the light source with a wavelength shorter than 300 nm is not applicable due to the absorbance of silica. The other solution is to design a complex cladding layer.\textsuperscript{40} Lately, devices have been introduced that use superhydrophobic surfaces to guide light, trapping air within micro and nanostructures.\textsuperscript{41,42} A conceptual scheme of the hydrophobic structures and the air spaces between them is shown in Figure 3c. This, in turn, reduces the contact area
between the solid substrate and liquid core at the interface, which improves the light-guiding performance. In Figure 3d, a thick air layer between the solid layers demonstrates an improvement strategy based on an air layer\textsuperscript{43} rather than porosity or tiny air pockets. This thin-wall optofluidic platform attained a better detection limit compared to a standard microplate reader since it trapped the light within the core or induced lower dispersion.

In general, two significant challenges need to be addressed for the devices based on the conventional nanofabrication method. Firstly, most of them are initially constructed with planar-like micro and nanostructures\textsuperscript{44,45} and are then subjected to packaging processes, such as oxygen plasma bonding. The subsequent bonding or packaging may cause significant light intensity loss in case the sealing quality is low. The quality can be affected by factors such as inappropriate surface roughness for the bonding area, particle contamination, and weak bonding strength. Secondly, altering the morphology of nanostructures is quite challenging with conventional nanofabrication methods, especially in the case of trying to create intricate three-dimensional (3D) structures onto a non-flat substrate. Due to the limitation, the cross-sections of conventional optofluidic channels are typically square or rectangular. Kraiczek et al. report the analysis of the cross-section geometries of channels in terms of the 3D ray simulations.\textsuperscript{46} A square cross-section (for core and cladding layers), as shown in Figure 4, is the second-best design. In contrast, a round cross-section is the top design since the light beams within such devices tend to reflect toward the center of the channel, resulting in a more linear performance for fluorescent applications.
Figure 4. The heterogeneous geometries of the liquid core waveguide's cross-sections are investigated and reported in the reference.\textsuperscript{46}

1.5 Optofluidics configuration: solid core and liquid cladding

Optofluidic platforms with a solid core, usually optical fibers\textsuperscript{47}, and liquid cladding are rapidly developed because of the progress in microfluidics and the mature optical fiber technique. A conceptual scheme is shown in Figure 5a. Optical fibers are utilized in the solid core surrounded by fluids mixed with analytes or dye elements.\textsuperscript{48} Another design with partial liquid cladding is shown in Figure 5b. A portion of the cladding layer is removed and coated with functionalized chemical groups (e.g., amine coupling) to identify the molecular targets.\textsuperscript{49} A small amount of solutions containing analytes is sampled and placed in the surface functionalized area. Afterward, the sensors display a correlation between the output signal and the analyte concentrations within the liquid cladding or partial liquid cladding. The design has advantages, including high sensitivity and fast response. The principle of TIR is easier to fulfill since the RI of the liquid cladding is much lower than the crystal fiber core.
Figure 5. The scheme of the optofluidic device with a solid core and (a) liquid cladding or (b) partial liquid cladding. The purple marks the functionalized surface to capture the molecular targets within the sampling solutions. Note that the cladding area with grey color represents solid cladding.

To date, the design has been applied to various kinds of sensors. Oraie et al.\textsuperscript{50} developed a RI sensor based on an optofluidic channel with multiple fluids. The platform was designed with an optical fiber surrounded by liquid cladding in the center. The fluids of liquid cladding, ethylene glycol, or distilled water are used as reference, while the unknown liquid state material is mixed with reference flow. The resolution is reported as 7*10\textsuperscript{-6} RIU. A temperature sensor\textsuperscript{51}, consisting of a water cladding and a tapered optical fiber in the core, is proposed to implement the measurements in proximity to room temperature (30-50°C), which is a good candidate for chemical reactions or marine environment monitoring. A label-free method\textsuperscript{49} to measure the copper ions, or potentially other heavy metals, within the water is also achieved by partially replacing the fiber's original cladding with sampled liquids. During the experiment, Tran et al. modified the surface of the fiber core by applying chitosan linked with ethylenediaminetetraacetic acid. This modification is particularly effective in capturing copper ions due to its improved chelation properties.

1.6 Optofluidic-based in vivo devices

Optical-based in vivo devices are designed to implement a variety of tasks in in vivo conditions rather than a cell culturing setting, including but not limited to nano-photosynthesis biosystem for generating oxygen\textsuperscript{52}, hollow waveguide for tissue
ablation, arrayed waveguide grating for human retinal imaging, bioabsorbable waveguide for photomedicine, and other stretchable optical biosensors. As described in the previous composite optofluidic configurations, the fluidic part of the devices cannot only deliver the solutions containing nanomaterials, which are potentially photoactivated nanoparticles for intracellular delivery or even tumor destruction, toward the designated locations in the tissue but also be reconfigurable according to the requirements of measurements. The optical units transferring the light from light sources and receiving the optical responses, whether in terms of tomography imaging or light spectrum, can help evaluate the therapeutic methods and understand the specific light-matter interaction during the treatment without conventional bulky instruments. Complementary metal-oxide-semiconductor (CMOS)-compatible process, nanoimprinting techniques, and even laser cutting are widely used in the previous works. Nowadays, these optofluidic systems are usually constructed in a compactly integrated optical probe form and have demonstrated their values in vivo and ex vivo applications such as the neural probe, coherent tomography imaging, intracellular delivery, temperature sensor, and even integrated multifunctional device.

The developed platforms can be categorized by the optical and fluid channels' layout. The most common design includes well-sealed microchannels fabricated in a fluidic chip form, while the optical unit is built into another chip, which bonds with the fluidic one later. For example, the optical part of the probe shown in Figure 6a consists of an electrode and microscale inorganic LEDs (μ-ILED), whereas the fluidic channels are placed onto it. Both parts are positioned on a flexible substrate eventually. These multilayer chips are modulable and can be further packed with other control units such as thermally actuated micropumps, μ-ILED, and wireless modules. Unlike the
multilayer chip configuration, some chips possess similar arrangements in terms of the separated optical and fluid units but with a needle form. As shown in Figure 6b, researchers built a probe consisting of an optical core and liquid channel to deliver the molecular cargo intracellularly to demonstrate the application concept for cancer treatment. One can penetrate and direct to the target soft tissue based on the miniaturized volume. As Moreover, some designs use a coaxial configuration, as shown in Figure 6c, where the optical fiber is encapsulated in the center of a needle. The gap between the fiber and the cover shell serves as a liquid channel where the liquid can be injected from the hole created on the needle shell. Here, the fluid (saline) is selected according to the requirement of RI matching.
Figure 6. (a) Simplified conceptual scheme of multilayer optofluidic chip configuration.\textsuperscript{63} (b) An optofluidic probe in a needle form, consisting of a liquid channel for conveying the molecular cargo and an optical core for light transmitting.\textsuperscript{60} (c) Simplified conceptual scheme of the coaxial arrangement for the optical and fluidic channels.\textsuperscript{59} The dotted circles represent the open holes for liquid injection and optical reading.

The development of PoC technologies has become a powerful candidate for dynamic diagnostics, particularly in resource-constrained areas, since PoC devices are usually known for their timely care practices with user-friendly instructions, portability, and affordability.\textsuperscript{67} Another terminology, LOC, describes the multifunctional platforms to analyze the biotargets such as viruses and bacteria with high sensitivity and rapid detection but small sampling volume.\textsuperscript{68} Not surprisingly, both liquid-core-solid cladding and solid-core-liquid-cladding configurations can be applied in developing miniaturized in vivo optofluidic platforms. These compactly integrated devices are
promising to address a wide range of challenges regarding PoC and LOC applications. Nevertheless, some extensions can be made to promote this rapidly evolving field.

1.7 Societal context and research questions

We introduced the significant implications of optofluidic systems through various systematic configurations and their applications. In the optical domain, conventional solid-state optical elements can be replaced by adaptive optofluidic components. The technique can be utilized in developing imaging technologies or even solar energy fields. For instance, a solar concentrator that is capable of performing dynamic control based on the meniscus interface between the multiple fluids, realizing an electrowetting tracking of solar energy. In the healthcare and biomedical domain, precise fluidic, nanoparticle, and optical manipulations at the microscale enable cell sorting, drug delivery, and photopharmacology functions. Particularly, the modulable in vivo optofluidics can be used to rapidly and timely detect biomarkers and pathogens, image the soft tissue, and assess the drug without having a bulky setup. Last but not least, optofluidic systems also show their capabilities in the domain of environmental monitoring, such as water quality analysis. The combination of microfluidic and optical sensing features enables real-time data collection to address environmental concerns. In summary, the powerful features such as tunability, reconfigurability, and miniaturization lead to a versatile system for multiple purposes.

In the context of ongoing improvements, researchers made many efforts in the aspects of material integration, technology integration, miniaturization, and user-friendly operation. As mentioned in the previous sections, the material requirements varied according to the measuring environment, application, and integrated materials, implying the importance of material integration. While many photonic materials have good optical properties and chemical compatibility, some unique materials, such as
nanostructured silica-aerogels, are used in biomedical applications, and the RI matches with liquids. The functions of optofluidic devices can be expanded by integrating with other sensing technologies, such as fluorescence spectroscopy, surface plasmon resonance (SPR), or localized surface plasmon resonance (LSPR). Recently, a dual-optofluidic-channel sensing platform incorporating the SPR technology was established to analyze multiple liquid analytes simultaneously. The platform consists of an optical fiber in the core along with two fluidic channels, whose channel walls were coated with an Ag layer. The channels were filled with different solutions, resulting in distinct resonance wavelengths under the applied excitation, providing a label-free protocol. Furthermore, the miniaturization of the devices has been accomplished via mature MEMS technology or femtosecond laser machining technique, which paves the way for integration with other on-chip systems. For example, a platform to detect pathogens incorporates continuous flow polymerase chain reaction (PCR), one of the microfluidic-based technologies. An LED, optical filters, and charged-coupled device (CCD) camera were also compactly integrated. This all-in-one device is a user-friendly design that can be used in an on-site detection setting.

Although immense progress has been made in enhancing the capacity of optofluidic devices, some challenges still need to be addressed. First of all, the complexity of manufacturing the devices has gradually increased while the researchers are seeking compact and multifunctional systems. As described, many of the miniaturized fluidics are enabled by the intricate MEMS technology. The technology is composed of a series of manufacturing steps, from substrate preparation, pattern definition, and building structures to device packaging, in a cleanroom environment, which indicates time-consuming and less cost-efficient. In addition, the construction was usually established on a flat substrate, which may confine the potential geometrical
improvements of the devices. The manufactured devices are generally planar-like, limiting the flexibility to alter their morphology. Alternatively, additive manufacturing, also known as the 3D printing technique, has been introduced to the optofluidic field in terms of its rapid prototyping feature.\textsuperscript{77,78} Even though the morphology of the current 3D printed microdevices are relatively simple (e.g., straight and flat microchannels), it is a novel candidate for constructing complex objects in one or a few steps. The second challenge is material compatibility. One of the solutions, as described, is to use some nanostructured materials, such as aerogels. The material aims to match the measuring conditions while retaining mechanical flexibility. The designs of these nanostructures are mostly porous structures. However, its manufacturing technique comes with several solvents and drying procedures and even more complicated fabrication to conquer the low mechanical strength.\textsuperscript{79} The other challenges need to be taken into account while the researchers deal with the previous two: reducing the optical losses and cost. The variables in optical losses are inevitably induced by the inherent absorption of the materials, scattering, and refraction during light manipulations, and the quality of sealing and alignment among various components. The cost of the overall design depends not only on the material selection but on the complexity of device manufacturing. Both of them are essential factors during the research development. In short, optofluidic technology fuses interdisciplinary technology into one spot, which is promising in a wide range of applications. A new strategy is necessary to deal with the challenges in manufacturing complexity, material compatibility, optical losses, and cost.

1.8 summary of the proposed approach

The roadmap of the proposed research is shown in Figure 7. In the first and second phases, we investigate possible strategies to conduct an optofluidic device with reduced manufacturing complexity. One of the solutions is to choose biocompatible liquid
materials to construct the liquid core of the device. The material, simply sugar water, was easy to acquire, and their RIs can be tuned through various sugar concentrations. The solutions were then injected into the rectangular PDMS channel for fluorescent measurements. Theoretically, the RI of the solutions should be compatible with the PDMS cladding, which has a RI of around 1.4, indicating high sugar concentrations are preferred. Another investigated solution is to adopt an efficient molding method along with the femtosecond laser micromachining to manufacture PDMS-based optofluidic platforms. The molds with various geometries were made from computer numerical control (CNC) machining and milling or ceramic 3D printing technology. The channel consists of two pieces, where the inner surfaces could be sent to femtosecond laser treatment for producing periodic hierarchical nanopattern. We obtained a strongly hydrophobic surface by combining the periodic nanostructure and stearic acid. We found that the surfaces' hydrophobicity depends on the pattern's roughness. Thus, one can vary the laser scan rate (interval between pulses) to modify the hydrophobicity. The hydrophobic surfaces are expected to serve as an air-solid mixed cladding to enhance the light-guiding performance. The other solution, which attracts our attention the most, is a one-step 3D printed chip. We can acquire a feasible liquid-core-solid-cladding platform with the methodology while greatly simplifying the manufacturing process. Furthermore, we are able to design and build a 3D and more sophisticated morphology for possible improvements or extension applications in the optofluidic field.
In the third phase, we utilize µSLA\textsuperscript{80}, an innovative technology for fabricating intricate microstructures at a miniature scale.\textsuperscript{81,82,83} This technology is instrumental in creating a fully enclosed optofluidic chip with a complex interface between the core and cladding. By utilizing this method, we are able to produce chips with a circular cross-sectional shape to enhance their performance according to the previous ray tracing analysis.\textsuperscript{46} Furthermore, we introduce a variety of microstructures on the inner surface of the device. These microstructures, coated with polytetrafluoroethylene (PTFE), function as a hydrophobic layer, forming a complex cladding that traps air within the microstructures. We conducted a comprehensive study on factors such as the solid fraction of the cladding layer within an effective range of thickness and the geometries of the chips' cross-sections, all of which influence the chip's ability to guide light. Eventually, we applied the device to quantum dot-labeled clustered regularly interspaced short palindromic repeat (CRISPR) Cas-12a biosensing.
We have developed various microstructured chips based on prior research into microstructure design. As shown in the fourth phase, the “T-shape” configurations are employed as prototypes for 3D-printed optofluidic probes. In our experimental phase, we successfully fabricated an array of micropillars onto each “T-shape” structure with a feature size of 50 microns within a round channel. Importantly, the liquid channel and the optical channel were built as a whole. We explored and varied the T-shape morphology and its dimensions, subjecting them to transmission measurements, fluorescence assessments, and ray tracing simulations during the fifth and sixth phases of our work. To demonstrate the practical utility, we conducted in vivo-like fluorescence measurements in a simulated environment using a training phantom and human plasma, with results aligning with our prior analyses.

Concurrently, in the last phase, we achieved the successful detection of fluorescence signals on stained C57BL/6 mice brain slices, affirming the probe’s ability to observe a stationary fluorescence source. Our preliminary findings underscore the potential of this probe for in vivo or ex vivo fluorescence measurements. We anticipate that ongoing improvements and innovation in this realm will unveil new possibilities, pushing the boundaries of optofluidics and its real-world applications.
CHAPTER 2. OBJECTIVES

2.1 Organizing the designing principles for optofluidic devices

Investigate the methods and principles to develop optofluidic devices. The evaluations for the appropriate materials and the complex light guiding design should be included in the studies. Summarizing and organizing preliminary designing principles as the knowledge base for extension work.

2.2 Developing a 3D optofluidic device

Build 3D optofluidic devices rather than planar-like chips by exploring various manufacturing techniques and applicable materials. In addition, identify the contributions from the established work compared to the conventional methods. More comprehensive design guidelines should be obtained in this phase, and improving plans are expected to be drafted.

2.3 Developing a simple implantable optofluidic device

Build an all-in-one prototype of an easy-to-make, operate, and innovative design of the implantable optofluidic device capable of delivering the liquid as well as the transmissions or fluorescence. The device should be able to apply in a setting where autoclave sterilization is necessary. Evaluations for new materials and the machining methodologies may be included in the new design if necessary. Since this is still a preliminary stage of steady-state fluorescent measurement, studies about the limitations of the proposed work and the improvement plans should be included along with supplementary information.
CHAPTER 3. BACKGROUND

This chapter serves as the foundational framework established through an extensive literature review. First, we introduce the current development of hydrophobic surface manufacturing techniques along with their connections with optofluidics. Subsequently, we briefly overview the simulations, from Snell's law to the commercial analytical method within this field. In the later section, we elaborate on the strategies to build an advanced device with feasible performance while possibly reducing processing complexity: RI tuning and micro-scale 3D printing technology. Simple tests are included while we go through the strategies for preliminary evaluation. Finally, we encapsulate the entire body of literature, offering a comprehensive summary and elucidating the contributions made by our proposed work in addressing the challenges within this field.

3.1 Hydrophobic surface and optofluidics

As introduced, optofluidic platforms are an interdisciplinary field between light-guiding and microfluidic systems. Due to the combination of both, the platforms are eventually re-configurable, thus providing multiple solutions to study light-matter interactions for single or multi-species molecular targets. Designing a complex cladding layer is one of the solutions to improve the light-guiding performance for the optofluidic device built in a liquid core and a solid cladding. Hydrophobic surface-based devices have been recently introduced to trap air within micro and nanostructures. Many methods have been published for building hydrophobic surfaces with micro and nanostructures through sol-gel techniques, imprinting and spraying, femtosecond laser pulses, atmospheric pressure plasma jet, and nanofabrication.

A brief flow of the hydrophobic aerogel fabrication is shown in Figure 8. A
tetraethylorthosilicate (TEOS) based solution was mixed with an acid and base catalyst to transform into a sol and gel sequentially at room temperature. After the gelation, the alcogels were bathed in an aging solution for 24 hours at 50 °C. The material’s densities can be controlled by keeping them in an aging solution for varying periods. A wash step for removing the impurities and a supercritical extraction for removing the solvents were then applied to extract the finished sample. Lastly, the silica aerogel was sent to hexamethyldisilazane (HMDS) vapor treatment to result in a hydrophobic surface. After the manufacturing process consisting of many kinds of solvents, acids, and alkaline substances above, the resultant substrate has a porous structure with an effective RI lower than 1.09, which is an ideal candidate for water-filled cores.

![Diagram of the protocol to build a hydrophobic aerogel substrate](image)

**Figure 8.** The protocol to build a hydrophobic aerogel substrate.95

Imprinting may be a relatively low-cost method. Recently, an optofluidic compound eye with an adjustable view angle (up to 104°) was developed by using a photoresist and PDMS-based imprinting process. The imprinted features are a convex array of microlenses, each with a diameter of 50 μm. A conceptual scheme is shown in
Figure 9. It underwent three pattern define-transfer stages, one for the upper part and two for the lower part, to approach the main body of the device. In detail, the device’s main body is manufacturing approximately 15 steps, along with nine kinds of materials. Compared to the fabrication for the micropillars, it shows that the number of pattern define-transfer cycles will increase along with the complexity of the design morphology. In the experiments, the volumetric change of injecting solutions would induce the deformation of the eye’s profile, thus varying the focal length. Eventually, an optofluidic-based variable optical imaging is realized. In addition, the laser-imprinted nano and microstructures can be used to enhance the hydrophobicity compared to the flat material itself. Such surfaces with antireflection features may be suitable for solar energy applications.

Femtosecond laser treatment is usually used to write arbitrarily shaped patterns for
the microfluidic channels on the glass, PDMS, or aerogel substrate. The desired structures can be obtained by micromachining, namely the ablation or selective etching, or by laser-induced photoreaction, such as local photopolymerization and photoreduction. Through the application of appropriate laser fluence, it is possible to generate periodic structures on a substrate using the laser-induced ablation-resolidification process. For instance, George et al. introduced a localized surface plasmonic-based sensor for detecting Rhodamine 6G molecules by employing a commercial femtosecond laser. This laser was utilized to create grid patterns featuring a size of 20 μm on the polymethylmethacrylate (PMMA), illustrated in Figure 10a. Such a structured hydrophilic surface serves as a template for the PDMS substrate through a soft lithography process involving molding and demolding operations. Subsequently, the patterned PDMS substrate is immersed in an AgNO₃ solution to facilitate the diffusion of Ag ions, followed by the reduction of Ag particles induced by the residual Si-H group on the PDMS. The PDMS surface, structured with metal particle coating, exhibits hydrophobic properties (contact angle~150°). Droplets containing fluorescent analytes were placed on this surface, resulting in Raman signals that were sharper and more pronounced in comparison to the unpatterned PDMS matrix. Alternatively, Li et al. described a surface plasmonic sensor based on a silicon substrate structured with grooves and coated with gold particles using a femtosecond laser-induced photoreduction process by immersing the Si sample in an Au-ion-contained solution. Nowadays, many of the femtosecond laser-involved optofluidic or plasmonic devices are either developed on functionalized hydrophobic/hydrophilic surfaces or are integrated with other fabrication methodologies, as depicted in Figure 10b. In this Figure, the laser is employed to create a microchannel in the previously mentioned hydrophobic aerogel.
Huang et al. use argon atmospheric pressure plasma in a chemical vapor deposition (CVD) process to produce a superhydrophobic surface. During the process, HMDS is utilized as a precursor for polymerization. In the deposited thin film with nanopores structure (surface roughness~190 nm), enormous hydrocarbon compositions were polymerized, leading to a superhydrophobic surface with a contact angle of over 160° that possesses anti-icing potentials. Despite the fact that extensive research indicates that the atmospheric pressure plasma jet has the capability to alter the hydrophobic nature of the sample (e.g., Polystyrene and epoxy resin), giving it a structure resembling nanopores, less is employed in optofluidic device manufacturing.

Superhydrophobic surfaces offer several benefits, including self-cleaning properties and resistance to biofouling. The schematic representation of a hydrophobic surface adorned with nano or microstructures is depicted in Figure 11a. An exemplary optofluidic device, comprising a silicon wafer bearing periodic nanopore patterns sandwiched between PDMS slabs, is presented. The hydrophobic patterns were
manufactured using laser interference lithography and deep reactive ion etching (DRIE), which are conventional cleanroom processes, followed by the application of spin-coated Teflon. In this investigation, a waveguide loss of 4.9 dB/cm at 14° incidences was observed, a figure higher than that of the flat and hydrophilic-based channels. This loss can be further mitigated to 1.8 dB/cm by infusing oil into the porous structure, thereby creating slippery liquid-infused porous surfaces (SLIPSs) as shown in Figure 11b.

Additionally, we developed a prototype that integrates black silicon nanostructures, similar to the previous sandwiched device, fabricated via DRIE on a flat substrate, serving as a cladding layer\textsuperscript{105}. We achieved a minimal loss of 0.1 dB/cm at a zero-incident angle, where a slight portion of light beams still experience reflection, depending on the concentration level of the incident light beam. The effectiveness of tiny air spaces formed by the superhydrophobic nanostructures demonstrates advancing light-guiding performance. Jonáš et al. conducted a study involving a tapered optical fiber waveguide connected to a droplet situated on a superhydrophobic surface, aimed at assessing the quality factors of individual optical resonances\textsuperscript{106} and a self-supporting optofluidic device based on a superhydrophobic surface\textsuperscript{107}. Both hydrophobic surfaces, featuring nano-patterns, were crafted by applying silica nanoparticles onto chosen substrates through a spin-coating process. This approach offers an alternative means of generating nanostructured surfaces instead of relying on conventional etching techniques.
3.2 Simulation

Snell’s law\(^{108}\) has been applied to many conventional waveguide designs. The law is still applicable for the optofluidic devices. To realize an efficient light guiding function, according to Snell’s law, a medium whose RI\((n_1)\) is higher than another medium whose RI\((n_2)\) is lower, with an incident angle \(\theta\) being less than the critical angle \(\theta_c\), can be totally reflected, namely TIR.

\[
\theta_c = \sin^{-1} \left( \frac{n_2}{n_1} \right)
\]

The incident light with an angle greater than the critical angle would undergo reflection and refraction, and the refraction of the light would lead the incident light to decompose into several split rays. This is so-called the effect of temporal dispersion. The simulations of the devices were primarily aimed at analyzing the split of light rays at distinct interfaces, employing meridional ray approximations as the foundation.\(^{109}\) The division of light rays corresponds to the division of transmission intensity. A two-dimensional model\(^4\) (Figure 12) for the liquid-core-solid-cladding configuration is used to understand and predict the optical loss during propagation. As described, the light beam would be partially reflected, thus leading to multiple splits along with the propagation. In Figure 12, the geometry of the light path can be simplified and expressed into a series of relations:
\[ \frac{h_1}{2} = L_1 + L_2 + L_3 = L_s \tan(\theta_1) + h_w \tan(\theta_2) + L_a \tan(\theta_3) \]

\( n_0, n_1, n_2 \) represented the RI of the environment, core layer, and the cladding layer, respectively. The refractive angles can be derived from Snell's law.

\[ \theta_2 = \sin^{-1}\left( \frac{n_0}{n_2} \times \sin(\theta_1) \right) \]

\[ \theta_3 = \sin^{-1}\left( \frac{n_2}{n_1} \times \sin(\theta_2) \right) = \sin^{-1}\left( \frac{n_2}{n_1} \times \sin\left( \sin^{-1}\left( \frac{n_0}{n_2} \times \sin(\theta_1) \right) \right) \right) \]

\[ \theta_1 = \frac{\pi}{2} - \theta_3 = \frac{\pi}{2} - \sin^{-1}\left( \frac{n_2}{n_1} \times \sin\left( \sin^{-1}\left( \frac{n_0}{n_2} \times \sin(\theta_1) \right) \right) \right) \]

We can rewrite \( \theta_2 \) as

\[ \theta_2 = \sin^{-1}\left( \frac{n_1}{n_2} \times \sin(\theta_1) \right) \]

\[ = \sin^{-1}\left( \frac{n_1}{n_2} \times \sin\left( \frac{\pi}{2} - \sin^{-1}\left( \frac{n_2}{n_1} \times \sin\left( \sin^{-1}\left( \frac{n_0}{n_2} \times \sin(\theta_1) \right) \right) \right) \right) \right) \]

Once we input the known conditions, such as the incident angle, the device dimensions, and the constitution, we can obtain the light ray propagation at the beginning. Subsequently, we can conduct the iterations for every “site” shown in the conceptual model, combined with the random sampling numerical methods for simulations.

**Figure 12.** The conceptual scheme of the two-dimensional light propagation model.

To facilitate this simulation process, a commercial software tool, TracePro® by Lambda Research, has been harnessed for simulating the propagation of light. This software is based on the Monte Carlo model. It has been instrumental in the
development of bioabsorbable waveguides, offering a reliable platform for comprehending the intricacies of light behavior and interaction within the context (e.g., intensity decay along with the propagation length) of the devices. During the process, the starting point, the medium's attenuation characteristic, and the geometry of the design will be taken into account. The expression can also be applied to scenarios such as light refracting at the mismatched boundaries and locally altered optical properties.

A free Monte Carlo model-based software is also accessible (Multi-Scattering). Its validation is accomplished by Jönsson et al. by comparing the experimental and computing results, which is light propagation in a phantom medium.

Briefly speaking, the model starts with the assumption that the photons are recognized as classical particles with no polarization and wave phenomenon. The model is then used to predict random traveling distance between the scattering or absorption events of the photon, which is induced by the various mediums. Based on the prediction, the traveling distance of the photon within the simulated coordinates can be calculated from the probability distribution functions, enabling the simulation of a wide range of scenarios. For example, Hamed et al. introduced a Monte Carlo-based algorithm for simulating the Hermite Gaussian laser beam within a turbid medium, emphasizing the polarization characteristics of the scattered photons.

A multipath light propagation model in 3D space is recognized as a representation of real-world scenarios. For example, the multipath effect has been exemplified by Li et al., showcasing its impact on absorbance performance within high-performance liquid chromatography (HPLC). Moreover, Kraiczek et al., Wei et al., and Wu et al. have illustrated simulations involving various factors like cross-sectional geometry, channel length, and metamaterial properties. These simulations have contributed to determining the optimal parameters for constructing a liquid-core optofluidic system.
3.3 Improving strategy 1: RI tuning

RI tuning is an important strategy to configure both the liquid and solid state materials to match the counterpart of the devices, either the core or cladding layer. The RI describes the extent of light refraction in the material. Typically, the RI of the optofluidic core layer should be higher than the cladding layer to fulfill the TIR. The solid and liquid substances can be tuned by either designing the material's chemical essence or combining them with substances with distinct RI.\textsuperscript{117} In practice, two methods to modify the effective RI for the solid substrate are producing nanostructured material, such as aerogels mentioned in section 1.4, or doping nanoparticles.\textsuperscript{118,119,120,121} For the liquids, RI tuning is essential since the solutions within the optofluidic devices usually accommodate the analytes, which should be taken into account while designing a liquid medium with proper RI. Preparing the liquid material is relatively simple since one can modify its optical properties by mixing the different liquid materials\textsuperscript{17,122} or molecules.\textsuperscript{123,124}

In the initial phases of designing implantable optofluidic devices, the adjustment of refractive indices in solid materials plays a pivotal role. This is particularly crucial because the RI of the 3D printing material, which serves as part of the cladding, tends to be notably higher compared to the majority of fluids. In cases where another solid material is considered for integration with the 3D printing substrate and serves as a cladding part, the material should exhibit strong adhesion to the 3D printed cladding layer and possess a RI that is compatible with both the core layer and the 3D printed substrate. It is equally important to prevent any heat treatment that may lead to deformation or distortion of the 3D-printed structures. Instead of designing the substance itself, there is another way to create a complex structure to fulfill or improve the light-guiding function: to develop a complex cladding layer—for example, multi-
layer materials\textsuperscript{125,126} or structures with various air-solid fractions.\textsuperscript{127,85}

3.4 Improving strategy 2: Micro-scale 3D printing technology

Additive manufacturing, also known as 3D printing technology, has been applied in various fields, including the communication field\textsuperscript{128}, optics and metaphotonics\textsuperscript{129}, pharmaceutics\textsuperscript{130}, and micro-electrochemical energy storage devices\textsuperscript{131}. The technology has the features such as fast and simplified production. Currently, micro- and nano-scale 3D printing methods are capable of building complex geometry with advanced printing resolutions and have emerged as promising alternatives for nanofabrication processes, such as conventional lithography methods, which are usually limited to planar patterning. The published applications, to name a few, include photonics for telecommunications\textsuperscript{132}, microchannels for the optical stage for fluorescence microscope\textsuperscript{133}, biomimetic hydrophobic surface for droplet manipulation\textsuperscript{134}, hydrogel-based direct solar vapor generation to mitigate water scarcity\textsuperscript{135}, and a lens-in-lens probe for in vivo endoscopy\textsuperscript{136}.

Fused Deposition Modeling (FDM) is a 3D printing technique based on material extrusion. Figure 13 illustrates the typical setup, wherein one or two nozzles are used to load the printing material(s). These nozzles are connected to molten chambers that transform thermoplastic polymer filaments, such as Polylactic acid (PLA), polyvinyl alcohol (PVA), and polycaprolactone (PCL), into a liquid state. These materials are widely employed in the development of particle-reinforced composites\textsuperscript{137}, medical devices\textsuperscript{138}, and topological self-interlocking (SIL) structures\textsuperscript{139}. In essence, a SIL structure is engineered to withstand defects within a single element when subjected to concentrated force loads, preventing the failure of the overall assembly\textsuperscript{140}. A FDM printed hollow-core fiber\textsuperscript{141}, consisting of polyethylene terephthalate glycol (PETG), for guiding mid-wave infrared light is also built for potential security and biological
applications. In dual nozzle mode, one can load and print two kinds of material simultaneously, which is a unique feature among the printing technologies. The platform can be heated up and maintain the proper temperature for printing. The nozzle size, whose standard size is 0.4 mm, and the extrusion rate can directly affect the printing resolutions. The printed resolution on the X-Y axis usually comes with a value greater than 150 μm.\textsuperscript{142} The surface roughness of the printed sample can be observed in the inset of Figure 13. A similar extrusion-based process is direct ink writing (DIW), which is distinct from the previous one since it offers broad material options from glass to metal due to highly customizable inks for preparing printing materials\textsuperscript{143}. For instance, the technology has enabled graphene\textsuperscript{144} as well as PCL-bioactive glass composite\textsuperscript{145} scaffolds with feature sizes from 100 μm to 400 μm for electronic or biomedical applications.

**Figure 13.** The commercial FDM printer (FlashForge). The left photo represents a front view, and the right photo represents a top view. The inset shows the FDM-based printed tanks consist of thermal plastic polymer.

Powder-based 3D printing processes can be categorized in terms of the powder material: polymer and metal. Microscale selective laser sintering (μSLS) and multi-jet fusion (MJF) are two of the common polymer powder-based techniques. In Figure 14a, In Figure 13a, the printing cycle is achieved by moving down the printing bed, depositing powder (recoater), and implementing a laser scan on the designated area.
Thus, no supporting structure is needed. For μSLS, the sizes of the powder and the sintering laser spot are the key factors that contributed to the printing resolution. Moreover, it is less anisotropic in terms of mechanical properties than FDM\textsuperscript{146}. In practice, after introducing the Ag nanoparticle as a sintering material and the continuous wave laser as a sintering (melting) light source, a planar chip with 10 µm diameter pillars is proposed for microelectronic use\textsuperscript{147}. Another published work is to build unmanned aerial vehicles\textsuperscript{148}. In Figure 14b, MJF is distinguished by the utilization of two distinct inks, referred to as the fusion ink and the detailing agent. The first treatment is implemented by applying the laser and fusing agent to a specific focusing area of the printing bed, while the fusing agent can raise heat absorption. The second is applying a detailing agent along the edge of the area for cooling and smoothening the "details" of the parts\textsuperscript{149}. MJF is reported to have a faster printing speed compared to the FDM and SLS methods\textsuperscript{150}. Also, MJF is better than SLS in terms of surface roughness\textsuperscript{151}, but its mechanical properties may be lower due to the lower density. Currently, an MJF-printed microchannel device with depth and width of 300 µm and 250 µm is published. It has been evaluated for developing radiotherapy immobilization devices\textsuperscript{152} or bioreactors\textsuperscript{153}. The inset in Figure 14b shows a practical sample printed by a MJF printer.
Figure 14. The conceptual schemes of μSLS and MJF are shown in (a) and (b), respectively. A MJF printed mold consisting of acrylonitrile butadiene styrene (ABS) thermoplastic material is shown in the inset.

Digital light processing (DLP) is a photopolymer-based printing system comprised of the following components: a curing light source, a photosensitive resin, a light control unit, and a post-curing station. These structures are then sliced into numerous pattern layers, typically spaced a few microns apart. The exposure required for patterning is accomplished using digital display techniques, which encompasses a projection system and a digital micromirror device. Due to the free-radical photopolymerization, the liquid state material at the predefined location could be cured based on the spatial control of light exposure. As shown in Figure 15a, the printed
samples were attached on a movable platform and waited for removal. The removed samples will be subjected to the post-curing process, which is implemented by the yellow box shown in Figure 15b, to fully cure the parts. In commercial products, transparent resin is applicable, as shown in Figure 15c. Recently, the feature sizes of DLP-based microfluidics can be improved from 100 µm\textsuperscript{155} to 20 µm\textsuperscript{156}, which is a significant step for bio-related applications (e.g., organ on a chip). A simple comparison of FDM (left) and DLP (right) is depicted in Figure 15d. Two hollow cylinders with concentric hole arrays, where each hole has a diameter of 750 µm and a length of 1 cm, are built. DLP printed sample demonstrates a better printing quality in terms of surface roughness and hole morphology. Additionally, a variant of DLP, lithography-based ceramic manufacturing (LCM)\textsuperscript{157}, is capable of printing dense and precise ceramic materials. The slurry-like photopolymerizable suspensions with ceramic particles are distributed into the printing tank with a similar optical arrangement to DLP, where an ultraviolet (UV) laser is directed and focused at the selected spot at the bottom of the platform. Later, the material is subject to an oven for sintering to obtain a fine and dense ceramic part. A ceramic printed mold with fine surface quality is tested and printed in Figure 15e. It is ideal for dental restorations, such as molar crown production.\textsuperscript{158}
Figure 15. (a) The printing setup with printed green samples on the moving stage. A transparent film is fixed at the bottom of the tank and attached to a light source panel. (b) The DLP printer (Elegoo). (c) The photo shows the samples printed by DLP. (d) The comparison of FDM (left) and DLP (right) samples. The samples were designed as hollow cylinders with concentric hole arrays. Each hole has a diameter of 750 µm and a length of 1 cm. The scale bars denote 1 cm. (e) The photo shows the mold printed by LCM.

Among these photopolymerization-based methods, µSLA, also referred to as projection microstereolithography (PµSL), stands out as one of the notable instances. The control mechanism for the curing light source closely resembles that of DLP\textsuperscript{159,160}. The distinction between DLP and PµSL, as shown in Figure 16, lies in the incorporation of optical elements\textsuperscript{161} (the upper part of the photo). An LED light source operating at 395 nm is conditioned through designed diffusers. A beam-splitting cube is responsible for polarizing the light and incorporates an anti-reflective coating. A pellicle beam splitter is responsible for directing the light to a CCD camera, which is utilized for verifying system focus and monitoring the printing process. An liquid crystal on silicon (LCoS) display\textsuperscript{162}, as a digital mask, is used to define the printing pattern. These
augmentations significantly enhance both the resolution and precision of the printing process. The achieved feature size of a fine thread can be as small as 0.6 μm\textsuperscript{163}, in contrast to the feature size attainable with DLP. The potential applications include microbial bioprinting for biofilms\textsuperscript{164} and micro tweezers for handling biosamples\textsuperscript{165}. The process can also be extended to build the printing with multiple photocurable materials without contaminating the resins by incorporating a glass palette onto a translational platform\textsuperscript{166}, which is competitive with dual nozzle mode FDM.

![Image of µSLA printer setup]

**Figure 16.** The photo of the µSLA printer setup.

An ultra-short laser-based printing technology within photonics and biosciences is two-photon polymerization (2PP).\textsuperscript{167} Likewise, it is a maskless process. The fundamental principle underlying 2PP is a nonlinear optical process\textsuperscript{168} that triggers photo-initiators within the photoresist material. This activation process can result in the creation of structures with feature sizes as miniature as 65 nm\textsuperscript{169} and even less than 50 nm\textsuperscript{170} through the utilization of ultrashort laser pulses. Typically, the local polymerization necessary for this process is initiated and controlled by precisely directed laser beams. To date, micro-resonators and bio-scaffolds can be produced with the technology without a cleanroom environment.\textsuperscript{171} An additional technology relying on ultra-short laser pulses is known as direct laser writing (DLW). The nonlinear
excitation and local polymerization combination enhances the solidification resolution beyond the treated light's diffraction limit. DLW has been suggested for the fabrication of an in vivo microdevice (with a feature size of 11.5 µm) designed to capture circulating tumor cells (CTCs) based on their physical attributes. In a simulated artificial arm vein environment, human prostate cancer cells that were introduced into donor blood were effectively captured at a concentration of 1,000 cells per ml.

An important common fact for the introduced 3D printing technologies is that the attainable feature size is heavily affected by the design complexity, for example, whether it involves straightforward planar pillar arrays or intricate structures embedded within the sidewalls of microchannels. In this research, the adoption of PµSL with a resolution of 20 µm becomes a practical choice due to its relative accessibility compared to other 3D printing technologies. In detail, it has fewer material-related issues (e.g., wastes, clogging, and the number of options) and a higher printing resolution based on the non-planar design than many of the technologies except for 2PP and DLW. Nonetheless, 2PP and DLW comes with a comparatively high expense and limited print volume, whereas PµSL’s resolution can be enhanced through optical arrangement improvements, and its versatility can be broadened by integrating biomass materials. In conclusion, the accessibility is primarily attributed to factors including cost considerations, process complexity, and printing quality.

3.5 The contributions of the proposed work

This dissertation primarily focuses on the comprehensive design and application of optofluidic systems, specifically emphasizing the configuration comprising a liquid core and solid cladding. This particular setup involves a hydrophobic structured interface strategically employed to enhance the optical performance of the device.
Essentially, the complex cladding within the liquid channel, where interaction between light and the sample occurs, has provided a significant scope for improving this configuration.

Our investigation encompasses various techniques concerning hydrophobic surfaces and their associations with optofluidic devices. In general, a hydrophobic surface involves two primary elements: the design of nano or microstructures and the application of coatings to diminish surface energy. Most of these surfaces involving nano and microstructures exhibit planar characteristics, such as the array of pillars, grids, or holes, as introduced in section 3.1. The quality of the resulting structure and the maturity of the process have been validated in numerous published research works. The cost, time, and complexity of manufacturing are closely linked to the desired structures. Introducing specialized patterns, such as convex lenses mentioned in imprinting processes, inevitably increases the cost, time, and process complexity, thereby limiting the flexibility to alter their morphology. Another essential consideration involves enhancing the quality of the device's production to prevent issues like leaks from bonded interfaces, thus ensuring effective manipulation of liquid samples. This underscores the importance of streamlining chip design while maintaining the device’s efficiency.

One primary strategy for constructing an efficient optofluidic device involves RI tuning. Initially, the approach consists of utilizing a liquid core matrix with a comparable RI to the solid state cladding, enabling the function of guiding light. Another strategy pertains to the micro 3D printing method. 3D printing technologies offer a superior option for rapid and straightforward manufacturing. While these technologies have evolved from macro to micro and nanoscale designs, their application in optofluidics requires further exploration. Unlike conventional
nanofabrication methods, micro 3D printing techniques offer the potential to explore advanced morphology for potential extensions. In our research, leveraging micro-scaled 3D printing technology could simplify both the manufacturing process and the integration setup for implantable optofluidic systems. As depicted in Figure 17a, conventional in vivo optofluidic systems have typically been configured using a stack of planar chips or probe-like (or needle-like) designs. For the probe-like designs, one exhibits a concentric arrangement, while the other adopts a parallel configuration. Given the preference for a probe form to facilitate penetration, we have devised several chips in a compact probe form, illustrated in Figure 17b, wherein the optical unit and the liquid channel coexist within a single channel. Analogous to the concentric design, our configuration enhances the area for light-matter interaction (microstructured channel) without the need for an additional optical channel. Furthermore, the transition of the chips from a flat design to a microstructured channel and the seamless integration of additional optical elements (e.g., Fresnel lenses) can be achieved in a single step with optimized space utilization. The overall volume of each design is roughly the same. Although 3D printing techniques have been used to construct microfluidic substrates or platforms, the designs of microstructured channels, especially multiple structures within an enclosed round 3D chip, are still rare.178
Figure 17. A scheme of (a) previously summarized in vivo optofluidic devices (Figure 6) and (b) the proposed compact and streamlining chip designs.

The contributions of the proposed work have been summarized in Figure 18. First of all, we introduced the femtosecond laser treatment to construct a PDMS-based channel. As described, femtosecond laser has been utilized to create functionalized hydrophobic surfaces and write the fluidic channel on a substrate. Here, we further combine the previous two application concepts- construct a channel with a hydrophobic inner surface. Next, we integrated µSLA into the design of microstructured optofluidics,
marking a relatively recent linkage between manufacturing technology and the application of optofluidics. Furthermore, the morphology achieved through one-step printing, wherein intricate microstructures were constructed along with the inner surface of a circular channel, highlights the substantial capacity to generate arbitrary shapes. This capability sets it apart from the majority of planar designs typically found in published works on optofluidics or microfluidics. Simultaneously, concerns such as the packaging quality of optofluidics and potential integration with other optical components are also addressed. Finally, our methodology for developing the implantable prototype indicates the ease of modifying the device's design using the manufacturing technique employed, thus enabling potential expansions for the optofluidic device across various configurations.

Figure 18. The sub-research fields under the optofluidic that have been explored in the dissertation.
CHAPTER 4. APPROACH

In the proposed research, the manufacturing and design of the implantable prototype were inspired by multiple technical paths, as mentioned in the previous chapter. The investigation of the liquid core with a high RI was conducted in the very beginning, followed by the hydrophobic based PDMS channel design and the design of the μSLA-based chip in the end. In the last phase, we built several experimental environments to mimic the real in vivo and ex vivo scenarios. All the materials, methods, and models that were employed to explore the possible solutions are introduced in the section.

4.1 Materials

To design fully enclosed or implantable devices, many materials were collected to address the components like the core, the cladding, and the fluorescence label. Moreover, both solid and liquid materials were evaluated in the case of implantable chip design. The solid core-based design indicates that the channels for the light rays and the applied liquid are isolated, which has been used in much research. In contrast, the liquid core-based design represents the light propagating along the liquid core where the measured sample is carried.

4.1.1 Liquid-core materials

A variety of biocompatible liquid-core materials were chosen for RI adjustment. Deionized (DI) water, phosphate-buffered saline (PBS), and human plasma were selected as solvents. Glucose, fructose (Sigma-Aldrich group), and commercial sugar packs were chosen as solutes. The solutions with high RI based on the above were prepared in distinct concentrations.

4.1.2 Solid-core materials

The solid-core design was evaluated for light transmission in the early stage of implantable device development. Numerous coatings were taken into account in terms
of RI, mechanical stability, and curing method. Parylene-c, and UV curable photosensitive resin (IP-n162, Nanoscribe Inc.) were included. IP-n162 is typically used in negative-tone 2PP. The RI of IP-n162 and parylene-c are 1.62 and 1.64, respectively. They are ideal for producing micro-optics with low shrinkage and a smooth structural surface finish.

4.1.3 Solid-cladding materials

To manufacture a complex cladding layer in either enclosed or implantable cases, PDMS and UV-curable resin were chosen based on their transparency, biocompatibility, and flexibility in machining processes.

4.1.4 Fluorescent molecules

Carboxyl quantum dots (Qdots, Qdot™ 605 ITK™, size: 15-20 nm; Thermo Fisher Scientific), organic laser dyes (Biotium CF® 488 and 405), and Alexa Fluor® 350 and 488 (Thermo Fisher Scientific) were chosen to evaluate the performance of the chip in the fluorescence measurements. It is also a methodology to label the molecular target for recognition.

4.2 Methods

4.2.1 Liquid core design

Liquid core design is different from the other investigations regarding device development. The design is to adjust the RI by increasing the concentrations of sugar water. A refracting index refractometer is applied in the experiments to characterize the liquid-core material (Figure 19). A small amount of liquid-core materials (~60 µL) will be sampled and placed in the right hole to confirm the RI. The machinery methodology and the geometrical design to build structured channels, both enclosed and implantable devices, are introduced in the following section.
4.2.2 Fully enclosed device
4.2.2.1 PDMS based device

As depicted in Figure 20, a variety of molding techniques were employed to fabricate PDMS-based optofluidic platforms. In particular, Figure 20(a) reveals ceramic molds generated through 3D printing, featuring diverse dimensions. Contrarily, Figure 20(b) and Figure 20(c) present the molds crafted through CNC machining and milling. While the dimensions of the molds in Figure 20(a) and Figure 20(b) remain consistent, variations in surface roughness stem from the distinct manufacturing methods employed. In particular, Figure 20(b) has a round cross-section, whereas Figure 20(c) exhibits a square configuration. The mold exemplified in Figure 20(c) was subsequently utilized to produce the PDMS channel, illustrated in Figure 20(d). This channel is composed of two sections, offering the possibility of subjecting the inner surfaces to femtosecond laser treatment. The operational configuration of the channel, which contains an inlet and an outlet for the fluorescence solution, is demonstrated in Figure 20(e). Furthermore, Figure 20(f) through Figure 20(h) display molds generated via the HP MJF process. Compared to the previous molds, a superior control over channel wall thickness could be afforded with these new molds. Here, the channel walls were defined by the dimensions of holes (Figure 20(g)) and gaps (Figure 20(f) and

Figure 19. HI96800 refracting index refractometer from Hanna Instruments
Figure 20(h)) instead of manual cutting. The actual manufacturing process involves the pouring of PDMS into the desired area within the mold. Subsequently, the de-molded PDMS materials are assembled as outlined in the conceptual schematics found in Figure 20(i) and Figure 20(j). The ratio between PDMS and its curing agent is 10:1. In order to solidify the molded components, a baking step lasting for 3 hours at a temperature of 60°C is executed for the molding as mentioned above experiments.
Figure 20. Molds for optofluidic with various lengths and diameters are prepared in a variety of ways. (a)-(c) represents the molds manufactured by ceramic-based 3D printing, CNC machining, and milling, respectively. (d) the partition of the PDMS channel and its mold. (e) the photo of the operated channel. (f) 3D printed mold for the round channel. (g) 3D printed mold for the round channel with the biopsy punch aside to build a hollow core. (h) 3D printed molds for rectangle channels. (i) and (j) conceptually depict the round and rectangle cross-section channels, respectively.

4.2.2.2 Femtosecond laser machining

The femtosecond laser was applied to treat the inner surfaces of the PDMS
rectangle channel, as shown in Figure 21. Practically, the surface ablation that results in the creation of a hierarchically structured nanopattern was conducted after a sequence of ultrashort pulses governed by the laser technique. The surface configuration, characterized by periodic nanostructures, is harnessed in conjunction with stearic acid to produce a remarkably hydrophobic surface.\textsuperscript{179} Since the hydrophobicity of the surfaces depends on the resultant roughness of the pattern, we can vary the laser scan rate (interval between pulses) to obtain various roughness, which could be considered the means of modifying the resultant surface hydrophobicity.

![Laser treatment and SEM photos](image)

**Figure 21.** The conceptual scheme on the left shows the laser treatment applied on the inner surface. The scanning electron microscope (SEM) photos of femtosecond laser-treated surface of PDMS. The nanostructure treated by the laser was zoomed in on the middle figure, and the top view of part of the surface was shown on the right.

4.2.2.3 µSLA and microstructured channel design

The technique of µSLA has enabled the creation of various intricate 3D microstructures due to its superior resolution and user-friendly manufacturing operation. The process starts with multiple digital masks converted from the sliced engineering designs (20 µm per layer), which were sent to a printer (MicroArch\textregistered P140, Boston Micro Fabrication) to print them. To ensure the microstructures’ quality, wash and post-curing stations are employed to eliminate uncured liquid resin and break any remaining unactivated photoinitiators, respectively.

In order to study optimal designs for minimizing optical loss, several distinct microstructure morphologies (Figure 22 (a) to Figure 22 (d)) with a 100 µm feature size
for fully enclosed channels were fabricated. Following the printing, a hydrophobic surface is achieved within the microstructured channel by applying PTFE (Teflon AF 6%, Chemours) through dip-coating and subsequent post-baking at 110°C for 3 hours. This results in a coating thickness of approximately 1.5 µm, measured by the profilometer as illustrated in Figure 23.

In the configuration, the core of the enclosed channel is confined by a complex cladding layer comprised of printed microstructures and air spaces amongst the structures. The presence of air spaces between these microstructures reduces the ratio of liquid-solid contact area or the absorption induced by printing material, thereby increasing the number of light rays reflected into the core. Here, four main microstructures are manufactured and displayed in Figure 22a through Figure 22d. The first two structures, micro-gratings (Figure 22a) and micro-pins (Figure 22b), have been extensively utilized in superhydrophobic-related research.41,94 A "T-shape" structure with a double overhang is showcased in Figure 22c, possessing exceptional super-repellent properties that retain the solution on the T-heads due to minimal contact angles instead of filling the air gaps.180 This is followed by an "umbrella" feature shown in Figure 22d, similar to the T-shape but inclined at 13.31° instead of flat, leveraging hydrophobicity based on inclined structures. The hydrophobicity based on the inclined nanostructures was reported.89

The T-shape structured channel's components, air gaps, liquid core, and solid printing structures are demonstrated in Figure 22e. The effect of PTFE treatment is evident in the contact angle shift, seen in Figure 22f and Figure 22g, going from 58° to 120°. This hydrophobic capability, aided by both the PTFE coating and specialized structure, effectively prevents liquid from entering air gaps beneath the T-heads. T-
shape structures spaced as 2.8 mm and 70 µm are shown in Figure 22 h and Figure 22 e, respectively. Human plasma-based contact angle measurement is shown in Figure 22 i. The light's path at the interface of solid, liquid, and air is presented in Figure 22 (j), revealing partial internal reflection where a portion of the incident light at a 35° angle is reflected into the core layer. The overall proposed optofluidic chip is depicted in Figure 22 (k), featuring enclosed channels where the liquid is introduced and replaced through designated inlet and outlet points. The design is fully enclosed to prevent liquid leaks.

4.2.2.4 Transmission measurements

A lab-built setup is shown in Figure 24. An LED driver (LEDD1B, Thorlabs) is employed to modulate the power of the fiber-coupled LED (M00559094, Thorlabs). The LEDs have peak wavelengths at 405, 488, 595, and 1100 nm. One end of a fiber is connected to the LED, with an integrated UV lens situated at the opposite end. The fiber is fixed and adjusted on a three-axis stage (MBT616D, Thorlabs). The manipulation of the incident light beam's angle is fulfilled by the stage, ultimately stabilizing it at 5° to achieve maximum intensity without saturation. Similarly, another fiber is arranged on the three-axis stage and connected to the spectrometer (FLMS16493, Ocean Insight). Various filters were mounted and placed on a rotation stage between the chip and the fiber, which could be adjusted based on the measured wavelength. The optofluidic chip is positioned on the stage and can be vertically adjusted, facilitating precise alignment with the two fibers.
Figure 22. The SEM of the microstructures are shown, accompanied by their digital photos. (a) Micro-grating with an aspect ratio of 15:1. (b) Micro-pin with an aspect ratio of approximately 10:1. (c) Double overhang T-shape with an aspect ratio (the height to the cap width) of roughly 3:1. (d) Umbrella with an aspect ratio of roughly 3:1. (e) The photo of a T-shape cross-section where the water is filled in the core. The yellow line marked the multiphase interface comprised of liquid, air, and solid substrate. The contact angles before (panel (f)) and after (panel (g)) PTFE treatment were measured on a flat printing sample. (h) The droplet is supported by the structures with a 2.8 mm gap. (i) The human plasma droplet on the structure demonstrates a contact angle 112°. (j) Reflection of a light beam at the interface between solid, water, and air. (k) The graph of a functional platform with integrated light and liquid core paths. Liquid is pumped into the core via embedded micro-tubing. Flow directions within the chip are illustrated as the inset. Chang, 2022, On-Demand Fully Enclosed Superhydrophobic–Optofluidic Devices Enabled by Microstereolithography, 38, 10673. Copyright © 2022, American Chemical Society. Adapted with permission.
Figure 23. The photo of the profilometer monitor, Tencor P2, employed to measure the coating thickness of PTFE.

Figure 24. The setup for transmission and fluorescence measurements.

4.2.2.5 Fluorescence measurements and CRISPR-Cas12a sensing

The previously described lab-built setup is also adopted for conducting fluorescence measurements. Carboxyl Qdots are used in the fluorescence measurements, prepared in various concentrations ranging from 0.1 to 6.25 nM, with each solution having a volume of 211 µl. The Qdots are excited at approximately 405 nm, where the emission is at around 607 nm. Three measurements are taken and averaged for each concentration intensity. Additionally, the lab setup is utilized to demonstrate an application involving the CRISPR system in combination with
associated proteins (Cas). As depicted in Figure 25, the CRISPR-Cas system is triggered in the presence of target DNA. The experimental procedure involves preparing mixtures comprising 1 µl of binding buffer, 9.5 µl of nuclease water, 62.5 nM of single-stranded DNA, and varying concentrations of target DNA. These mixtures, both with and without target DNA, are then incubated for 2 hours at 37 °C. Subsequently, 1.68 nM streptavidin-coated Qdots and anti-fluorescein-coated magnetic beads are sequentially introduced into the mixtures and allowed to incubate for 30 minutes at room temperature. In cases where the CRISPR-Cas system is activated by the presence of target DNA, the DNA probes are degraded within the mixtures. This is in contrast to situations where the DNA target is absent, resulting in the formation of conjugates between the DNA probe, magnetic beads, and Qdots. Following these reactions, those degraded components were separated from the supernatant while the supernatant was removed. The supernatant is then appropriately diluted and subjected to fluorescence analysis using the optofluidic device.

**Figure 25.** The method involves utilizing the CRISPR system in combination with associated proteins to achieve a fluorescence-based detection for nucleic acids.
4.2.3 Implantable device

4.2.3.1 PDMS based device

As shown in Figure 26, a manufacturing approach based on PDMS was employed to evaluate the designs. A funnel-like device is sketched in Figure 26 (a). The two red lines on the PDMS indicate the light transmission channels for excitation and detection made from 3D printing material (IP-n162, Nanoscribe). The diameter of the line is 0.4 mm. The 3D printing material is cured by UV light. In Figure 26 (b), a 3D printed mold in terms of several components was assembled. In particular, the center mushroom-like part was utilized to define the space for the light transmission channel, as shown in Figure 26 (c). Multiple numbers of channel designs were manufactured to produce the PDMS substrate. The de-molded PDMS piece with the preserved channels for further building the light transmission channels is shown in Figure 26 (d). Next, a 3D-printed PVA part was inserted into the PDMS base to confine the 3D printing material in the channel (Figure 26 (e)). The PVA material (Figure 26 (f)) can be removed by rinsing in an ultra-sonic bath for several hours.
Figure 26. (a) The conceptual scheme of preliminary optofluidic design. The red lines denote the channel for light transmission. The center hole represents the channel for liquid injection. The following photos represent the components of the molds and the PDMS piece. (b) 3D printed mold for the proposed PDMS-based device. The blue arrow denotes the gap that will be filled with PDMS. (c) The center part for making the light transmission channels. (d) The de-molded PDMS piece. (e) 3D printed PVA mold combined with PDMS device during the molding process for the transmission channels. (f) 3D printed PVA mold for producing transmission channels.

4.2.3.2 Microstructured channel design

Several configurations of the 3D printing designs were demonstrated in Figure 27. Initially, these designs were intended to be constructed with multiple layers, each composed of different solid materials. In Figure 27. (a), materials 1 and 2 represent photosensitive polymers, IP-n162, and HTL resin (Boston Micro Fabrication), respectively. Within this design, channels marked as A, B, and C were incorporated for the purposes of detecting, transmitting, and transporting liquids, respectively. In detail, A and B were made from the same material, while the remaining material served as solid cladding layers. An air gap was maintained between the two composite layers to prevent the interferences of the transmitted and emitted light rays. The dimensions of the concentric multilayer solid light guiding device were varied (80 µm to 600 µm), as depicted in the lower-right photos. In addition, an alignment was constructed to position
the optical fibers towards designated layers, as shown in Figure 27 (b1). In the experiments, the previous proposed optofluidic device, including the PDMS-based design, should be assembled with the alignment depicted in Figure 27 (b2) and Figure 27 (b3). Arranging the fibers for excitation and detection was involved in this assembly, along with the delivery tube for the solution, along the specified alignment points.

However, a modified design was introduced in Figure 27. (c) due to certain challenges encountered during the manufacturing and testing phases, which will be discussed in more detail in the subsequent chapter. Unlike the previous funnel-like devices, the channel came with a uniform inner diameter of 4 mm. Three curl-T shape structures were built to confine the fluorescence-contained solution in the core, as indicated in the diagram below. The structures were inspired by the fully enclosed device and could result in a complex cladding layer in terms of the liquid core, 3D printing material, and the air spaces in between. The core has a diameter of 1 mm. The T shape's thickness and width are approximately 150 µm and 600 µm.

Lastly, the structural configuration of the optofluidic chip was designed in Figure 28. There are three distinct sections in the device, marked as A, B, and C. A denotes the liquid inlet, facilitating the replacement of fluids within the device. B represents a hole with a porous bottom to couple with the optical fibers. As illustrated in the inset, the porous film serves the dual purpose of preventing fluid leakage from the channel and regulating a reasonable level of absorbance caused by the printing material. C represents a microstructured channel to carry the solution and the light regardless of the transmission and emission. Throughout the experiments, various parameters were assessed, including the pore size within the porous film, the diameter of the fiber coupling hole, and the length of the microstructured channel. Initially, the
microstructured channel was designed with a length of 1.2 cm to avoid scenarios such as saturated transmission and fluorescence intensities. The ultimate channel length was settled at 8 mm, aligning with the dimensions of the mouse brain between the dorsal and ventral regions, approximately 8 mm. Furthermore, the pore size of the porous film underwent variations, ranging from 50 µm to 300-400 µm holes, while ensuring the prevention of water leakage and the reduction of optical absorption. These meticulously considered parameters were implemented in the prototype for conducting fluorescence measurements.

To some extent, the microstructured channel is similar to the previous design shown in Figure 27 (c). However, the channel could be distinguished by featuring a reduced volume and an array of functionalized structures lining its inner surface. The specific configurations of these microstructures are elucidated in Figure 29. In Figure 29 (a), the adoption of a flat T-shaped design, similar to the enclosed device design, was primarily chosen for its meritorious mechanical stability. These structures are highly tunable, allowing for a reduction in the solid fraction. Notably, adjustments can be made not only in the circumferential direction but also in the longitudinal orientation.
Figure 27. (a) Conceptual scheme of the optofluidic device. Material 1 (purple) indicates the light transmission layer. Material 2 (yellow) indicates a solid cladding layer. In the top-right scheme, the light transmission goes through the inner layer (B) to the bottom end while the detection is received from the outer layer (A). (C) indicates the liquid injection channel, similar to the PDMS-based design. The air gap between the inner and outer layers varied, as the photos showed. (b1) The photos represent the device as a combination of optical fiber, 3D printed fiber alignment, and the 3D printed chip. The photos before and after the assembly are shown in (b2) and (b3). (c) The 3D-printed chip with a core confined by the T-structures.
Figure 28. The images depict the optofluidic chip, highlighting specific features denoted as A, B, and C. A signifies the liquid inlet, B corresponds to the fiber coupling hole, and C represents the microstructured channel. Within the fiber coupling hole, there is a porous film featuring multiple holes with diameters ranging from 300 to 400 µm. During the initial evaluation, the microstructured channel, marked as C, measures 1.2 cm in length, whereas, for the final application, a preferred length of 8 mm is recommended.

The curl T shapes, characterized by head thicknesses of 90 µm and 25 µm, are introduced in Figure 29 (b) and Figure 29 (c), respectively. A recognizable curvature difference is apparent in the insets, compared to Figure 29 (a). There are two distinct morphologies in Figure 29 (d) and Figure 29 (e). The former has been modified from the prior T-shaped design to feature a circular head, while the latter possesses a secondary structural design marked by protrusions. In Figure 29 (e), the thickness of the T-shaped heads gradually tapers from 75 µm at the center to 25 µm at the edges, while each protrusion exhibits a diameter and height of 50 µm. Importantly, all of these microstructures are coated with PTFE to imbue them with hydrophobic properties. In Figure 29 (f), a visualization of the three-phase composition was sketched for the relative positions between the liquid core, solid printed structures, and the air space within the microstructured channel.
Figure 29. Various configurations of optofluidic chips are captured by SEM imaging. The half cross-sections are shown in the insets of the first three figures. (a) The chip with an array of flat T-shape structures. (b) The chip featured curled T-shape structures. (c) The chip with thin and curled T-shape structures. (d) The chip with circular T-shape structures. (e) The chip is characterized by curl T-shaped structures modified with protrusions on the top. (f) The photo refers to the junction where the liquid core within the optofluidic system meets the intricate cladding.

4.2.3.3 Transmission measurements and ray tracing simulation

In Figure 30a, an experimental arrangement was established for conducting transmission measurements. The light source unit consists of an LED driver and a 405 nm LED from Thorlabs. To capture the light signal, a spectrometer is employed. A lab-made transmission setup, where its main body is constructed by stereolithography technology (Elegoo), is positioned between the light source and the spectrometer. The details of the transmission setup is illustrated in Figure 30b. The microstructured chip is positioned within the central aperture of a tank. The tank is printed in the dimensions of 38 mm in length, 38 mm in width, and 13 mm in height. Distilled water is introduced
into the tank through a tube connected to the chip. In the setup, an optical fiber placed between an LED and the fiber coupling port on the chip is included. Another optical fiber is mounted at the base of the setup to capture the transmitted light from the sensing port.

To predict and analyze the transmission performance across various structural designs, the simulations, TracePro® (Lambda research), is applied. A Monte Carlo-based ray tracing methodology is utilized in this software. In the schematic illustration of Figure 30c, the configuration used during the simulation is comprised of:

- The light source is represented by the purple component, positioned within the coupling hole.
- The chip itself is filled with water, represented in blue.
- The receiver is signified by the brown segment, where an illuminance map is generated on its surface.

During the simulation, 1,500 incident rays were applied to a range of chip designs, allowing the calculation and analysis of the differences in absorbed flux across the various maps generated.

![Figure 30.](image)

**Figure 30.** (a) The lab-built transmission setup (b) The lab-printed transmission box is integrated with the optofluidic chip, fibers, and tube. (c) The simulation's arrangement and illuminance map.
4.2.3.4 Fluorescence measurements

Two methods were employed for conducting fluorescence measurements. In Figure 31a, the initial approach is related to the configuration used for transmission measurements. Here, the detection fiber is moved from the bottom of the tank to the fiber coupling hole. This adjustment is to verify a probing protocol in an invasive way. In this arrangement, a solution of distilled water containing Qdots with an excitation wavelength of 405 nm and emission wavelength of 605 nm was introduced into the tank via a connected tube with varying concentrations. The choice of Qdots was made due to their remarkable brightness. In Figure 31b, another distinct setup was devised to demonstrate a more realistic in vivo operation. In this configuration, the tank was substituted with a thyroid biopsy training phantom (EDM Medical Solutions) featuring a center cubic hole of similar dimensions to a mouse brain. Instead of distilled water, the human serum (H4522, MilliporeSigma) is used to better represent physiological conditions. The modifications above and choices in the experimental setup were made to ensure that the fluorescence measurements closely resembled real-life scenarios in in-vivo settings, enhancing the relevance of the acquired data.
Figure 31. (a) Fluorescence measurement platform. (b) The experimental setup for mimicking the fluorescence measurements in an in vivo setting. The real photo shows the cubic hole with dimensions of 8 mm in the center.

4.2.3.5 Fluorescence measurements on the mice brain slice

An alternative application of the proposed chip is depicted in Figure 32a. In this experiment, the chip was placed onto brain slices obtained from mice that had been stained with wheat germ agglutinin (WGA). WGA is a lectin known for its specific binding to glycoproteins, which are commonly distributed across cell membranes. The standard WGA staining procedure was used, as shown in the upper part of Figure 32b, following a previously established protocol\textsuperscript{182} for conducting fluorescence staining. In the staining process, Alexa Fluor\textsuperscript{®} 350 and 488 were used to mark the lyophilized WGA lectin powder. The PBS was used while preparing the dye-contained solutions to obtain 100 $\mu$g/ml concentration. To conserve the structure of the brain tissue, 4\% Paraformaldehyde (Thermo Fisher Scientific) was exploited to treat the frozen slices (C57BL/6, coronal cross-orientation, Zyagen) for a period of 15 minutes in the first place. Subsequently, these brain sections were subjected to a 30-minute, room-temperature incubation with the WGA staining solution in a dark surrounding. Upon completion of the staining process, the fluorescence images, as demonstrated in
the lower part of Figure 32b, were verified through a fluorescence microscope, rendering the stained samples ready for further analysis. The next step involved filling the chip with distilled water, serving as the probing medium, to read the fluorescent signal. As previously described for the measurement setup, both the excitation and detection fibers were positioned within the fiber coupling hole. Two LED light sources were selected to excite the fluorescence within the brain slices, emitting light at wavelengths of 365 nm and 490 nm and connected with the microstructured chip, respectively.

![Diagram of the application](image)

**Figure 32.** (a) A conceptual scheme of the application is illustrated by utilizing fluorescence measurements on stained mice brain tissue. (b) The process of WGA binding to glycoproteins is depicted in the conceptual diagram. The accompanying images are the marked lyophilized WGA lectin powder, with Alexa Fluor® 350 on the left and Alexa Fluor® 488 on the right. The bar represents the scale of 3mm.
CHAPTER 5. RESULTS

In this chapter, we organized the experimental data into three stages: liquid core design based on the simple RI tuning, PDMS-based optofluidic device, and 3D printed chips. For the last two sections, we present enclosed and open-hole (implantable) designs based on each material. The material evaluations, transmission and fluorescence measurements, simulations, and real sample-based fluorescence testing were covered throughout the development. Eventually, we will be able to conclude a design guideline from all the trials and testing results.

5.1 Material experiments: RI tuning

As described, optofluidic is composed of a liquid core and a solid cladding layer. RI tuning for the liquid core design was first implemented. Theoretically, the RI of the liquid core should be higher than the one of the cladding layer, which is assumed to be PDMS (~1.4295). The biocompatible materials were chosen to construct the proposed device, and their RIs were measured, as shown in Table 1. DI water and PBS RIs were roughly the same, whereas human plasma was a little higher. However, the transparency of human plasma was not as good as the previous one, which corresponded to higher optical absorption. Moreover, the RI can be tuned through various sugar mixtures.

The materials were then sent to fluorescent measurements. As shown in Figure 20 (e), a simple PDMS-based device was utilized during the tests. 0.05% organic dye, which possessed an emission spectrum centered at 605nm while excited at 405nm, was injected into the device. The results are shown in Figure 33, which demonstrates that the RIs of sugar mixtures increased along with the concentrations while the fluorescence intensity decayed. This indicated that the RI and light absorption should be considered for the liquid-core design. In addition, the high viscosity under the
concentrated sugar water would increase the difficulties for operation. Such high-concentration solutions can only be applied in an enclosed channel because of the possible cell damage caused by high osmotic pressure while the solutions are injected into the tissue.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Refractive Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI water</td>
<td>1.333</td>
</tr>
<tr>
<td>PBS</td>
<td>1.334</td>
</tr>
<tr>
<td>Human plasma</td>
<td>1.350</td>
</tr>
<tr>
<td>30% human plasma within PBS</td>
<td>1.338</td>
</tr>
<tr>
<td>30% sugar within PBS</td>
<td>1.377</td>
</tr>
<tr>
<td>30 % glucose within PBS</td>
<td>1.383</td>
</tr>
<tr>
<td>30 % fructose within PBS</td>
<td>1.381</td>
</tr>
<tr>
<td>30 % glucose within DI water</td>
<td>1.380</td>
</tr>
<tr>
<td>30 % fructose within DI water</td>
<td>1.380</td>
</tr>
<tr>
<td>30% sugar within DI water</td>
<td>1.387</td>
</tr>
<tr>
<td>50% fructose within PBS</td>
<td>1.419</td>
</tr>
<tr>
<td>80% fructose within PBS</td>
<td>1.500</td>
</tr>
</tbody>
</table>

*Table 1.* Refractive indexes of the materials for liquid-core design. The sugar denotes commercial sugar packets.
Figure 33. The correlation among fluorescent intensity, RI, and sugar concentration.

5.2 PDMS-based fully enclosed device

PDMS-based designs are widely used because of their ease of operation, even though post-processing is usually applied to the devices. Here, the complex cladding design for PDMS-based enclosed devices was studied. A variety of PDMS molds are shown in Figure 20. For an enclosed device, a round cross-section channel is preferred because of its symmetry along the axis of the core layer. However, it was hard to implement femtosecond laser treatment on the curled inner surface. Therefore, several square cross-section channels were made. Various laser scan rates were conducted on the PDMS surfaces, as shown in Figure 34. Periodic grooves were observed by comparing Figure 34 (a) and (b), where the surface treatment was applied to the latter. Figure 34 (c)-(f) represents the structures under various scan rates. The intervals were narrower under the lower scan rates because the lower scan rate led to a shorter spatial interval between pulses. Next, the contact angles under various scan rates of the laser were measured in Figure 35, indicating the lower the scan rate, the better the hydrophobic effect due to varied roughness.
Figure 34. The photos of PDMS surfaces (a) without and (b) with laser treatment. The periodic structures under different laser scan rates are shown in (c) 10 mm/s, (d) 20 mm/s, (e) 30 mm/s, and (f) 50 mm/s, respectively.

Figure 35. The contact angles with various laser scan rates.

PDMS channels with and without hydrophobic cladding layers were also sent for transmission measurements. An ignorable difference was observed in the absorption spectrum of Figure 36. That said, the hydrophobic cladding layer is insufficient to serve
as a complex cladding design. One of the reasons is that the thickness of this layer is far thinner compared to the core size (2 mm by 2 mm). Although a small portion of the incident light rays could be reflected by those tiny air bubbles within such a porous layer, an enormous amount of light would be absorbed by the thick PDMS channel wall (~1.5 mm). It is more like surface roughness than a reasonable cladding design. The other one was the cross-section geometry as mentioned. From the simulation, the absorption performance of the light guiding device with a non-round cross-section was non-linear and would not be as good as a round one.

Figure 36. The absorption spectrum of PDMS-based devices with and without complex (hydrophobic) cladding layer. The insets are the photos of the devices.

5.3 PDMS-based implantable device and preliminary 3D-printed device

PDMS-based implantable devices were designed as a liquid channel surrounded by a solid core and cladding. The materials (Table 2) were evaluated for solid-core design in both PDMS-based (Figure 26) and some of the 3D-printed designs (Figure 27). Theoretically, the material was expected to adhere to the cladding layer well. A high RI compared to the solid cladding is also necessary. In addition, it would be better
if no heating treatment is applied during the manufacturing process to avoid possible twisting and shrinking of the structures, film-like structures in particular. From the table, IP-n162 are suitable for our application because it is not solvent-based, no heat treatment is needed, and the mechanical stability is reasonable.

However, the manufacturing process with IP-n162 was not feasible after the evaluation. First of all, the typical printing volume for 2PP is 1 mm$^3$. In our design, the length of the channel is around 1.5 cm, which indicates a much longer printing time (several days). Second, the channel should be printed on the PDMS substrate directly to acquire consistent dimensions. Since the direct writing operation along the inner channel surfaces was not feasible, it was then replaced by the PDMS-PVA molding method (Figure 26e). Nevertheless, the material failed to be cured and fixed in the designated gaps because of the leaking issue between PDMS and PVA molds. The resin calls for a femtosecond laser as a curing light source, indicating that the lab's UV LED laser source is incompatible with the resin, even though the material is possible to be cured with UV light in general. The resin failed to be cured after 24 hours of exposure to the UV laser, as shown in Figure 37.

Figure 37. (a) IP-n162 resin. (b) IP-n162 resin (yellow) after LED UV light treatment
for 24 hours.

<table>
<thead>
<tr>
<th>Material</th>
<th>Refractive index</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parylene-c&lt;sup&gt;184&lt;/sup&gt;</td>
<td>1.66</td>
<td>High transmittance in the visible range</td>
<td>• CVD process was needed&lt;br&gt;• Brittle&lt;br&gt; • A hot bake with 130°C above was needed&lt;br&gt; • Local aggregation of the particles&lt;br&gt; • Strong UV absorption&lt;br&gt; • Precursor materials were needed&lt;br&gt; • A 100°C bake for 24h was needed</td>
</tr>
<tr>
<td>ZnO-poly(methyl methacrylate)&lt;sup&gt;119&lt;/sup&gt;</td>
<td>1.507</td>
<td>High transmittance in the visible range (92%)</td>
<td></td>
</tr>
<tr>
<td>ZrO&lt;sub&gt;2&lt;/sub&gt;-PDMS&lt;sup&gt;120&lt;/sup&gt;</td>
<td>1.65</td>
<td>• Particles distributed evenly via Si-based ligand molecule&lt;br&gt; • High transmittance in visible range (93.3%)</td>
<td></td>
</tr>
<tr>
<td>Functionalized Si-silicone hybrid films&lt;sup&gt;121&lt;/sup&gt;</td>
<td>1.727</td>
<td>High transmittance in visible range (&gt;90%)</td>
<td>A hot bake with 150°C was needed</td>
</tr>
<tr>
<td>TiO&lt;sub&gt;2&lt;/sub&gt;-hybrid polymer&lt;sup&gt;118&lt;/sup&gt;</td>
<td>1.936</td>
<td>• Room temperature&lt;br&gt; • Dry approach</td>
<td>• Precursor materials were needed&lt;br&gt; • Atomized spray plasma deposition (ASPD) was needed.</td>
</tr>
<tr>
<td>Episulfide-thiol polymer&lt;sup&gt;185&lt;/sup&gt;</td>
<td>1.707</td>
<td>High transmittance in the visible range with thermal stability</td>
<td>• The temperature for nanoimprinting was 160°C&lt;br&gt; • The RI tuning was based on surface structures, which is not applicable in our case.</td>
</tr>
<tr>
<td>NTT AT’s custom resin&lt;sup&gt;186&lt;/sup&gt;</td>
<td>1.7</td>
<td>• UV curable&lt;br&gt; • High transmittance in visible range</td>
<td>• Solvent-based</td>
</tr>
<tr>
<td>IP-n162&lt;sup&gt;187&lt;/sup&gt;</td>
<td>1.62</td>
<td>• UV curable&lt;br&gt; • Low shrinkage and a smooth structural surface finish</td>
<td>• High viscosity&lt;br&gt; • High curing</td>
</tr>
</tbody>
</table>

| Table 2. The comparison of solid-core materials |
3D-printed implantable devices were designed in a similar way as PDMS-based devices preliminarily, except for the wire-like solid channel. The channel had a liquid channel surrounded by a concentric core and cladding layers (Figure 27a). The cleaved optical fibers, alignment element, and concentric multilayer chips with varied dimensions were assembled in Figure 27b1 to Figure 27b3. The assembly of the setup used in the fluorescence measurements is sketched in Figure 38. No signal was detected under the high concentrations (>50% volumetric concentrations) of laser dyes. This results from high absorption and difficult alignment due to the solid channel design, even though the thickness of the solid substrate is much thinner than the PDMS-based design. Based on the analysis above, a different strategy was necessary. The investigations of the round channels with complex cladding layers in a reasonable thickness range, enabled by μSLA, were manufactured since no additional molding, assembling, or even surface machining steps were needed. They will be discussed in the following sections.

**Figure 38.** The fluorescence setup is shown in the photo, which includes the concentric 3D printing chip, alignment, and optical fibers. The 3D printed tank was filled with laser dyes with high concentration.
5.4 Fully enclosed device: simulations and transmission measurements

To ensure the consistency of the chips' performance (Figure 22a to Figure 22d), we conducted transmission experiments using lasers of varying wavelengths, as illustrated in Figure 39. The transmission intensities for all the measurements in the group of specific wavelengths were normalized by:

\[
\frac{\text{Transmission peak intensity of the microstructured optofluidic chip}}{\text{Maximum transmission peak intensity of the group}}
\]

Among the results, the “T-shape” chip consistently exhibited the highest level of transmission. Comparatively, the flat sample, which has no microstructures, displayed approximately 20% lower transmission than the “T-shape” chip. Following this, the “umbrella” chip, “micro-gratings” chip, and “micro-pins” chip exhibited decreasing transmission levels sequentially. Furthermore, the diverse excitation sources highlighted that our platform obviated the necessity for optical realignment. This versatility was demonstrated by the seamless interchange of the coupling fiber from LED excitation to an alternate source, as dictated by specific application requirements.
Two approaches were adopted to estimate the performance of the chips. One is the calculation of the solid contact ratio at the interface between the liquid core and the complex cladding layer for each device, whereas the other is the calculation of the solid fraction within the intricate cladding designs. The former is simply dividing the liquid-solid contact area by the whole area of the liquid core at the complex interface. The ratios, from high to low, are 100% (Flat sample), 96.8% (“T shape” sample), 52.8% (Micro-gratings sample), 36% (Micro-pins), and 34.1% (“umbrella” sample). The “umbrella” structured sample is predicted to be the best design because of its low solid
contact ratio. The latter was computed according to the ray tracing analysis,\textsuperscript{46} which illustrated that it is essential for the cladding layer thickness to remain below 15\% of the core diameter to ensure that light rays predominantly travel within the core. Given a core diameter of 6 mm, the calculated necessary cladding layer thickness is approximately 900 µm or more minor in theory. For various structured chips, the solid fractions pertaining to each cladding layer are as follows: 86.4\%, 42\%, 41.2\%, 36.9\%, and 29.6\%, for "umbrella" shape chip, micro-pins chip, flat chip, "T-shape" chip, and micro-gratings chip, respectively. Also, note that Teflon, being significantly thinner than the printing material, is ignored during these calculations. In the case of the flat chip, the 400 µm channel wall is considered as the solid substrate since there are no microstructures within the required 900 µm cladding thickness. Theoretically, a lower RI of the cladding is necessary for TIR, which could be achieved by reducing the solid fraction in the cladding layer. It is the primary strategy to reduce optical losses for the developed chips. Consequently, the micro-gratings chip should yield the highest transmission. The second best will be the "T-shape" chip, followed by the flat chip. The orders of the performance from both experiments and predictions are collected in Table 3.

<table>
<thead>
<tr>
<th>Experimental data</th>
<th>Solid contact ratio</th>
<th>Solid fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>T shape</td>
<td>Umbrella (34.1%)</td>
<td>Micro-gratings (29.6%)</td>
</tr>
<tr>
<td>Flat</td>
<td>Micro-pins (36%)</td>
<td>T shape (36.9%)</td>
</tr>
<tr>
<td>Umbrella</td>
<td>Micro-gratings (52.8%)</td>
<td>Flat (41.2%)</td>
</tr>
<tr>
<td>Micro-gratings</td>
<td>T shape (96.8%)</td>
<td>Micro-pins (42%)</td>
</tr>
<tr>
<td>Micro-pins</td>
<td>Flat (100%)</td>
<td>Umbrella (86.4%)</td>
</tr>
</tbody>
</table>

\textbf{Table 3.} The comparison of the orders from transmission experiments, solid contact ratio, and solid fraction.

For the results in Figure 39, the “T-shape” chip consistently displays the highest transmission. The flat chip has almost the second-best performance. Conversely, the
micro-gratings chip and micro-pins chip possess notably lower levels of measured transmission. The disparity between the predictions and the practice is a result of deformation (collapsing issues) experienced by some of the microstructures following the application of the PTFE coating. Even though we have tried with a even lower baking temperature, the micro-gratings, which could be considered as an array of thin film, and micro-pins, which have a high aspect ratio, will twist and collapse eventually and invalidate the “air” gaps at the interface. In contrast, the chips with “T-shape” and “umbrella” display significantly greater mechanical stability, which leads to better light-guiding performance by effectively confining the solutions within the core. Interestingly, the samples' performances in the experimental results align with our initial theoretical anticipation based on solid fraction calculation, except for the two twisted samples.

Additionally, we conducted tests on light transmission as a function of incident angle for all the optofluidic chips. A 488 nm LED laser was used in the experiments. The results are shown in Figure 40. In general, the transmission intensity would decay along with the increased incident angle among all designed chips. Nevertheless, the T-shape chip has the highest intensity while the micro-pins chip has the lowest, revealing that the “T-shape” structural design consistently performs more efficiently than other channels when the incident angle is within the range of up to 15°. Ideally, the light rays should be received under zero incidence with such a straight channel. In practice, the light at zero incidences represents less reflection at the interfaces between the core and cladding or the air space and channel wall. A portion of light beams will still be reflected due to the wide beam angle of the LED laser, as shown in Figure 41.
Figure 40. The transmission of various microstructured chips with varied incident angles.

Figure 41. Conceptual scheme of the scenario under the zero incidence angle.

Additional investigations were conducted to comprehensively understand the factors of the design of the cladding layer, which plays a vital role in the light-guiding capabilities of the chips. Here, the thickness of the structures, the refraction, and the scattering of the light beams are taken into account. Since the flat chip has the second-best performance with such a simple design (no microstructures), it was chosen to assess the transmission intensity with varied wall thicknesses within a range of incidences. In Figure 42a, the chip with 400 µm channel wall, which is 200 µm thinner than the one with 600 µm, shows a significant increment in light transmission. At an incidence of 0°, the transmission intensity of the 400 µm chip was approximately four times higher than that of the 600 µm, primarily due to the light-absorbing properties of the solid reduced fraction within the cladding layer, as denoted in Figure 42b. That said,
the strategy previously discussed to reduce the optical loss could be achieved by simply decreasing the wall thickness in the case, even in the absence of microstructure-aided cladding design. However, the flat sample could be inefficient in supporting the mechanical stresses while the channel wall is further thinner, as marked in Figure 42 (green circle). Thus, further, incorporating “T-shape” structures is required to miniaturize and improve the optofluidic system because we can minimize the thickness of the T structures. At the same time, the channel wall remains the same, which could efficiently provide mechanical stability.

![Graph and diagrams](image)

**Figure 42.** (a) The results of transmissions based on flat chips with varied wall thickness and incidences. (b) The conceptual scheme of the flat chips with 600 µm and 400 µm wall thickness. The range marked as “cladding layer” represents the theoretically required thickness. The yellow section represents the printing material, whereas the blue denotes the liquid core.

We further study the optical mechanism in the “T shape” structured channel. In Figure 43, a major absorption is induced by the thick solid substrate for the traveling light ray indicated by the blue arrow, and a portion of the light rays, depicted by the
purple arrow, is totally reflected at the interface between liquid and air. Importantly, another portion of the light, represented by the red arrow, is partially absorbed and subsequently reflected because of the complex cladding: the thin solid substrate of the “T shape” cap and the large air spaces under the structures. Recalled the experiments on a flat substrate with T microstructure, even when T-shape structures are widely spaced as 2.8 mm, as seen in Figure 22 h, where it was 70 µm in Figure 22 e, the air gaps remain intact due to the influence of surface tension in the vertical direction over the overhangs. The droplet of human plasma was also applied in the contact angle measurements to verify the consistency of such hydrophobicity, as shown in Figure 22 (i). The air gaps play a pivotal role in the cladding layer's design during light guidance and could be considered an “air mirror,” especially when T-cap thickness is minimized, signifying that most of the light intensity would be reflected due to the presence of the thick air cladding. As a result, an ideal optofluidic configuration based on superhydrophobicity is demonstrated, wherein the cap thickness is minimized, and the air cladding thickness is increased.

**Figure 43.** The cross-section scheme of the channel depicts the light absorption and reflection at the interface of the T microstructured sample. Chang, 2022, On-Demand Fully Enclosed Superhydrophobic–Optofluidic Devices Enabled by Microstereolithography, 38, 10675. Copyright © 2022, American Chemical Society. Adapted with permission.
The next factor to be investigated was how the light guiding can be affected by the liquid core's cross-sectional shape, as it can lead to alterations in the light refraction at the complex interface. During the propagation of light, the light rays will gradually turn into miscellaneous refracted beams due to the refractions at the interface of air/solid/liquid, and this multipath phenomenon would result in nonlinear intensity. Ideally, a light guiding channel composed of a core and cladding layers, both with round shapes, can provide superior confinement for the refracted light rays along the center axis, thereby preserving more of the linear light intensity. In Figure 45a, a variety of “T-shape” samples, which have different smoothness of the round cross-section result from the varied dimensions and number of structures at the interface between the cladding layer and core layer, were manufactured. In addition, these structures exhibit varying capabilities in preventing water ingress into the spaces beneath the T head. As illustrated in Figure 44a, the intrinsic contact angle (known as an inherent characteristic at the liquid-substrate interface) of the T shape is approximately 30 degrees, and the cap width (labeled green) is recognized as the liquid suspension line. As demonstrated in Figure 44b, the T shape, featuring an overhang structure, can further suspend the liquid through the surfaces labeled green and orange, achieving the necessary angle down to 0 degrees. Literature\textsuperscript{180} indicates that once the liquid comes into contact with and wets the top surface of the structure, it follows a path along the overhangs. Upon reaching the tip of the overhangs, the motion suspends, and surface tension starts to exert an upward force. The T shape, designed with a double overhang structure (Figure 44c), enables a comparable contact angle for liquid suspension as the T shape depicted in Figure 44b. Furthermore, the air space beneath the T head poses a formidable barrier to liquid infiltration. In general, each device features a nearly identical core layer volume and microstructure height. The solid substrate fraction of the T-1 sample,
measuring 37.5%, is similar to T-3 (38.4%) and T-4 (40%). On the other hand, the solid fraction of the T-2 sample, measuring 28.5%, is comparable with T-4, which is 30.8%.

**Figure 44.** The conceptual scheme shows the liquid suspending mechanisms in terms of the intrinsic contact angles for the designed topologies. (a) T shape. (30°) (b) T shape with overhang structure. (0°) (c) T shape with double overhang structure. (0°)

Among all structured samples, T-1 exhibits the best performance in terms of the transmissions, as shown in Figure 45b. At a 0° incident angle, the light transmission is improved by at least 3.5 times compared to the other designs. This is attributed to the fact that the cross-section of T-1 closely resembles a round shape, and the width of T-
1’s cap is wider than that of the others, except for the sample of T-2. A wider T-cap results in the creation of more “air-mirrors” within the device. Consequently, more light beams can either be entirely or partially reflected by the air cladding or mixed cladding layer composed of air and thin solid material. Conversely, despite having a greater width, the cross-sectional geometry of T-2’s core is less round than that of T-1, leading to fewer light beams being reflected back into the core layer. T-3, T-4, and T-5 also exhibit higher losses because of reduced partial reflection, resulting from their narrower cap widths. Moreover, the overhang structures within the samples of T-3 to T-5 are longer than those in the other designs, excluding the difference in structure lengths when compared to the designs of T-1 and T-2. This increased length may contribute to greater intensity loss. While the unique structure provides additional support for preventing the liquid from air spaces, it must maintain a reasonable thickness to prevent an excessive increase in the solid fraction of the effective cladding layer. After all, T-5 demonstrates the lowest transmission intensity, caused by the previously mentioned facts, such as less round geometry of the cross-section, coupled with the longer overhang length of the structures within the effective cladding layer. These combined modifications to the microstructure design result in a higher optical loss attributable to the solid material and the narrower width of the T-caps.
Figure 45. (a) The engineering drafts of the designed “T shape” samples. The diagram placed in the center of each figure represents the morphology of a single structure used in the sample. (b) Transmissions of the five “T-shape” samples were collected within a range of incident angles. Chang, 2022, On-Demand Fully Enclosed Superhydrophobic–Optofluidic Devices Enabled by Microstereolithography, 38, 10675. Copyright © 2022, American Chemical Society. Adapted with permission.

The factors discussed above can be applied not only to predict the designed optofluidic chips’ performances but also to serve as design guidelines. Nonetheless, the computations may be more complicated when it comes to a more advanced design of the microstructured system. Herein, the simulations of the chips were conducted to aid the development of devices. We simulated the five structured devices and compared them with the experimental data. The arrangement of the light source, receiving spot, and chips are presented in Figure 46a. During the simulations, 1500 light rays were
assumed. The receiver is assumed to be a perfect absorber. The numbers of received light rays of the chips of flat, “T shape,” micro-pins, micro-gratings, and “umbrella” structures are 84, 66, 26, 62, and 69, respectively. Except for the flat device, which has received a relatively higher number of rays, the “T shape” and “umbrella devices have comparable numbers. The micro-gratings device received slightly fewer rays than the previous while the micro-pins device had the lowest. However, the average intensities of the absorbed light on the receiver’s surface showed distinguishable differences in the order of devices’ performances. This is because a portion of the light rays went through more refraction and absorption events at the complex interface than the others, leading to a weaker intensity of the light. Therefore, the average illuminance intensity was adopted in the following comparison. In the results, the received intensity of the “T shape” chip is the highest, depicted in Figure 46c, followed by the Flat chip (Figure 46b), Micro-gratings chip (Figure 46e), “Umbrella” chip (Figure 46f), and Micro-pins chip (Figure 46d). The performance order based on the simulations is very similar to the experimental results.
Figure 46. Transmission simulations of the fully enclosed chips with various microstructures. (a) The conceptual scheme of the arrangement. The dimensions of the cores of each design are the same, with the assumed volume of 211 µL. The medium is assumed to be water. The illuminance maps of the (b) Flat (c) T-shape (d) Micro-pins (e) Micro-gratings (f) Umbrella structured chips. In the map, each grid represents 1 mm.

The simulations of the flat samples with distinct channel thicknesses are established in Figure 47. The arrangement of the setup is based on Figure 46a. In Figure 47a, the thinnest sample has the best performance in terms of the receiving intensity as well as the rays. The receiving intensity of the flat sample with a 1.2 mm thick wall is in close proximity to the sample with 1.8 mm, as shown in Figure 47b and Figure 47c. This is because a thinner wall could lead to an increment in partial reflection or reduction in absorption by the solid substrate.
Figure 47. Transmission simulations of the flat chips with varied channel wall thickness. The high to low thicknesses are (a) 0.6 mm, (b) 1.2 mm, and (c) 1.8 mm.

Finally, the simulations for the “T shape” samples are illustrated in Figure 48. Figure 48a is the simulation setup. According to the average receiving intensities, the T-1 device (Figure 48a) shows the highest level, which agrees with the experimental results. Although fewer light rays were received in T-1’s case, they were reflected to the center under fewer events of refractions while traveling along the channel. T-3 device performance (Figure 48d) is found to be close to the T-1 device, which is the second best among the devices. Following that, T-2 (Figure 48c) and T-4 (Figure 48e) devices are comparable with each other, which received lower light intensities than the previous. Still, the intensity of the T-5 device (Figure 48f) is significantly lower among the designs, which stands in the opposite condition as T-1. In T-5’s case, a larger number of received light rays is obtained but with weaker intensities from those rays, resulting from secondary or more refraction in the propagation. In general, the performance sequence we acquired from the simulations, which is T-1, T-3, T-2, T-4, and T-5, are approximately matches with the practical data.

Last but not least, the level of the intensities in the simulations can only be
considered as a reference since the number of incident beams is based on our assumption as well as all the material properties, such as the absorption coefficient. Also, experimental investigations to confirm if the air/liquid/solid interface is well-defined are necessary since the simulations of the liquid supporting are not included. The strategy to increase the area of the liquid-air interface within the complex cladding design is an effective way to increase the total reflections as long as the “air gaps” among the structures are in reasonable dimensions for holding the liquids.
Figure 48. The transmission simulations of a series of T shape chips. (a) The diagram of the setup. The morphologies and their illuminance maps are demonstrated in (b) T-1, (c) T-2, (d) T-3, (e) T-4, and (f) T-5.
5.5 Fully enclosed device: fluorescence measurements and CRISPR-Cas12a sensing

Figure 49 illustrates the concept of applying the optofluidic platforms in the Carboxyl Qdots-based fluorescence sensing protocol. The fluorescence concentrations were adjusted by diluting with DI water and prepared across a range of 0.1 to 6.25 nM. An excitation light source, a 405 nm LED laser, was used in the setup, as shown in Figure 24, with an 18° incidence. Each measurement consisted of three scans, with an integration time of 100 ms. The uncorrected fluorescence spectra of different optofluidic chips for Qdots at a concentration of 1.6 nM are depicted in Figure 49a, and these results align with the trends observed in the transmission tests. Particularly, the "T-shape" device exhibits a peak intensity approximately twice as high as that of the flat device. This enhancement is made possible by the TIR occurring at the air-cladding interface and partial reflection within the air-thin solid mixed cladding. Figure 49b displays the integrated fluorescence intensity across various Qdots concentrations, where the "T-shape" device consistently delivers superior performance, with the measured intensity linearly increasing alongside the concentration.

Figure 49. (a) The uncorrected fluorescence emission spectra with an input of 1.6 nM Qdots measured across different optofluidic chips. The inset displays a photograph of the fluorescent solution inside the flat chip, where the green arrow marks the light propagation direction. (b) Cumulative fluorescence signals were detected within the optofluidic chips as the quantum dot concentrations were raised from 0 to 6.3 nM. Chang, 2022, On-Demand Fully Enclosed Superhydrophobic–Optofluidic Devices
Finally, we demonstrate the compatibility of the optofluidic device with a CRISPR-Cas12a system for detecting target DNA. The single-stranded DNA probes undergo denaturation in the presence of the target DNA by the CRISPR complex, resulting in the detachment of Qdots in the solution, as we mentioned and depicted in Figure 25. Conversely, the single-stranded DNA probes remain intact in the absence of the target DNA. The magnetic beads are adopted to capture the Qdots in the solution, which would be further extracted from the supernatant. The supernatant containing CRISPR-activated Qdots is subsequently quantitatively assessed by our optofluidics system. The Qdot concentration is maintained at 1.68 nM while the DNA target concentration is varied. As presented in Figure 50, the integrated fluorescence intensity exhibits a linear increase corresponding to the rise in DNA target concentration from 0.1 to 1 nM. Conversely, the sample lacking the target, which is labeled as NTC, yields a lower signal than the sample of 0.1 nM target DNA strands.

The fluorescence measurements within the solution (liquid core) containing the CRISPR cleavage products are efficiently achieved by the fully-enclosed optofluidic system, which is different from solid core waveguides. This holds great promise for applications in optogenetic therapy, enabling the localized delivery of reagents or even within specific tissues to address a range of conditions, including cancer, sepsis, and neurology related diseases. Employing high-resolution stereolithography, we demonstrate the possible fabrication of smaller “T-shape” structures for microsurgery while maintaining effective light transmission. Additionally, we presented the "T-shape" structures' ability to prevent the wetting of human plasma in Figure 22i, a significant advantage in mitigating biofouling concerns for in vivo applications.
Looking ahead, we envision the 3-D printing and integration of micropumps, microvalves, and even microlight sources with our optofluidics platforms as a unified entity for tasks such as sample loading, sensing, and treatment.197,198

**Figure 50.** The fluorescence intensities were measured for samples with DNA target concentrations of 0.1, 1, and 10 nM. The negative control, lacking the target, is denoted as NTC.

### 5.6 Implantable device: simulations and transmission measurements

In the development of the implantable optofluidic device, our objective was to achieve further miniaturizing of the overall design when compared to the previous enclosed device. We conducted an evaluation of the printing quality during the fabrication of the micro-structures, as illustrated in Figure 29. According to the observation, the printing orientation had an impact on the resulting printing quality, contingent on the specific morphologies involved. In Figure 29e, it can be observed that the protrusions, measuring 50µm in diameter and 50µm in height on the T-structures, exhibited slight distortion due to the influence of gravity. This effect was verified through two distinct printing orientations, as demonstrated in Figure 51. Figure 51a was printed with the micro-structure heads oriented perpendicularly to the printing direction, whereas Figure 51b was printed with the two directions aligned in parallel.
Nevertheless, this distortion is deemed acceptable, given that the dimensional variations among the various designs are more significant, and these differences predominantly impact the performance of light guiding. Furthermore, the solid fraction within the complex claddings remains nearly consistent when comparing the original design to the printed outcome. Therefore, the slight distortion should have a minimal impact on the overall performance.

Figure 51. The photos show the microstructures are printed in the directions of (a) vertical and (b) horizontal.

The micro-structured optofluidic chips underwent a comprehensive assessment, encompassing ray tracing simulations, transmission evaluations, and fluorescence measurements. Ray tracing simulations were employed to gain insights into and predict these chips' transmission performance. In Figure 52a to Figure 52i, multiple illuminance maps were generated to represent the absorbed flux of the receiver based on the simulation arrangement illustrated in Figure 30c. Initially, we conducted simulations for a flat channel devoid of microstructures (Figure 52a), which exhibited the lowest incidence of rays as well as the received flux intensity on the absorber surface. Maintaining a fixed channel length, we proceeded to vary the dimensions of the T structures, with two of them featuring slightly distinct morphologies, as elaborated upon later. The dimensions of the “T shape” structures used in the simulations are shown in Table 4. A notable improvement in transmission performance
(Figure 52b) was observed when employing curl T-structures with a width of 654 µm (Figure 53a), the widest among the designs, in contrast to the flat channel. Subsequently, we achieved an even higher number of incident rays and average receiving intensity (Figure 52c) with a 131 µm width for the curl T head, as presented in Figure 53b, while keeping other dimensions consistent with the previous case. This increase can be attributed to reduced absorption, resulting from a decreased solid contact area between the liquid core and the T-structures while the liquid is well confined within the core. However, practical limitations were encountered when using narrower heads, as they were unable to adequately support and confine the liquid core, causing it to seep into the gaps between the structures. Consequently, the receiving intensity and the number of incident rays in Figure 52d fell between the widest and narrowest curl T cases, owing to a moderate curl T width of 393 µm (Figure 53c). In contrast, Figure 52e and Figure 52f exhibited less noticeable differences in receiving rays and intensities compared to the widest case, as the structures in Figure 52e and Figure 52f were thicker and wider, respectively, resulting in increased absorption due to a higher solid fraction within the complex cladding. Drawing from our experience, thinner T heads had a tendency to reflect more light beams into the core, akin to an “air mirror.” In Figure 52g, the incident rays increased to 305, so as the receiving intensity, after we reduced the length of the structures from 350 to 150 µm (Figure 53d), corresponding to a 40% reduction in the solid contact area compared to the dimensions used in Figure 52d.

Furthermore, Figure 52h and Figure 52i introduced dissimilar morphologies, as depicted in Figure 29a and Figure 29d, respectively, to elucidate the impact of cross-section shape. Theoretically, a round core was expected to better confine light beams along the center axis due to its symmetry. This was confirmed as the rays in Figure 52h
were fewer than in Figure 52f, despite the latter having a wider T, representing a 22% larger solid contact area. Also, the receiving intensity of Figure 52f is higher than that of Figure 52h. In the last, Figure 52i exhibited the lowest incidence of rays among the micro-structured channels. On the one hand, this particular configuration exhibited a lower solid contact area (14% less) and a total solid fraction (34% less) compared to the curl T design used in Figure 52b. On the other hand, the configuration illustrated in Figure 52f exhibited a relatively minor 20% decrease in solid fraction when juxtaposed with the arrangement showcased in Figure 52b. Nevertheless, the intensities of Figure 52f and Figure 52i are close. Consequently, the subsequent experiments would be based on the curl T morphology.
Figure 52. Ray tracing simulations were utilized to collect illuminance maps showcasing absorbed flux in global coordinates for various dimensions of “T-shape” structures. These dimensions are summarized in Table 4. The simulations encompassed a range of configurations, as follows: (a) Flat channel devoid of microstructures, (b) T structure with a wide profile, (c) T structure with a narrow profile, (d) T structure featuring a width of 393 µm, (e) Thick T structure, (f) T structure with a width of 524 µm, (g) T structure with a reduced length, (h) Flat T structure, (i) Circular T structure.
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Table 4. “T-shape” microstructures’ dimensions used in the simulations (Unit:µm)

![Table 4](image)

Figure 53. The images depict the T-structures that were printed according to the dimensions employed in the simulation. These dimensions encompassed the following variations: (a) T structure with a wide profile, (b) T structure with a narrow profile, (c) T structure featuring a width of 393 µm, and (d) T structures with varying lengths and shapes.

Upon analyzing the simulations, it became evident that the ideal curl T shape design should possess the characteristics of being slim, short in the longitudinal direction, and wide around the circumference in terms of maintaining an intact liquid
core and minimizing the optical loss. Subsequently, we proceeded to fabricate the initial seven curl T designs, referred to as T-1 through T-7, where the dimensions are presented in Table 5. We conducted transmission measurements using a setup shown in Figure 30b, which is similar to the simulation setup. In Figure 54a, it was observed that T-1, designed as a flat chip, exhibited the lowest intensity, consistent with the simulation results. Particularly, T-2 and T-3 shared similar dimensions, except for the width of the T head. Surprisingly, T-3, despite having a wider T head at 654 µm, exhibited higher transmission than T-2. This disparity was attributed to the fact that, in the case of T-2, the liquid tended to seep into the gaps between the structures. During the printing process, we determined that a width of 524 µm was optimal for maintaining a circular cross-section shape, as shown in Figure 55a, a dimension we employed in subsequent designs. With a fixed width, we manufactured curl T structures with a thin head labeled T-4, an elongated length named T-5, and a shorter length marked T-6. Most of these microstructured chips displayed considerably higher intensities than T-1 (the flat chip), with the exception of T-4. The issue with T-4 was that its T-head had not been entirely printed. Initially, we aimed to maximize the printing capability by producing T structures with a thickness of 10 µm, closely approaching the printer's optical resolution. However, we realized that the incompletely printed T-4 had a much narrower head width, insufficient to support the liquid core (Figure 55b). Consequently, its cross-section shape deviated from circular, making it less likely to confine light beams along the center axis. Additionally, the air fraction was significantly reduced, leading to diminished internal reflection. Surprisingly, T-6 exhibited slightly better transmission than T-5 due to its reduced length, which equated to a 50% reduction in solid contact area. Theoretically, T-6, with its shorter length and slightly narrower width, was expected to deliver optimal performance. However, experimental results
indicated no significant difference between T-6 and T-3, with T-7 emerging as the best design in the group. After further investigation, it was discovered that the liquid tended to flow into the gaps in the longitudinal (axial) direction, as illustrated in Figure 55c, resulting in a non-straight cross-sectional shape (longitudinal direction), as shown in Figure 55d. Consequently, light beams would be refracted and scattered more diversely compared to the well-confined liquid core case, as shown in Figure 55e and Figure 55f.

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Table 5. For the transmission measurements, the sizes of curl T-shape structures listed were used. (Unit: µm)
Figure 54. The plots display the results of transmission measurements for a total of 10 different designs, and the corresponding dimensions are documented in Table 5. (a) The initial set comprises seven designs featuring a 350 µm spacing. The intensities have been normalized by dividing each intensity value by that of T-7. (b) The second set consists of four designs with varying spacings. Similar to the first set, the intensities have been normalized for comparison, with each intensity value divided by that of T-8. Note that each measurement was conducted and subsequently averaged every three times. Additionally, the experiments for set (a) and set (b) were carried out at different times.
Figure 55. (a) The photos show the scenarios of the droplets standing on the T structures while varying head widths. (b) Incomplete printing of T-4 chip. (c) The solutions failed to be confined in the liquid core. (d) The illustrative representation of the longitudinal cross-sectional design. (e) Well confined liquid core. (f) The illustrative scheme of the cross-section in the well-confined case. In (d) and (f), the red arrow denotes the light rays that are being refracted/reflected.
Furthermore, since T-7 possessed a moderate structural length, it effectively mitigated the adverse effects arising from the irregular longitudinal cross-section compared to T-6 while maintaining a reasonable length to avoid excessive increases in the solid contact area, as observed in T-5. Subsequently, our focus shifted to investigating the spacing between structures. In Figure 54b, T-8, T-9, and T-10 were manufactured based on the design principles of T-7, with modifications made to the spacing and the thickness of the T head. Notably, T-8 and T-10 exhibited stronger intensities than T-9 when a suitable spacing of 260 µm was employed. Additionally, T-10 was printed with a reduced head thickness of 25 µm in an attempt to enhance the number of reflected rays to the core; however, this adjustment did not yield significant improvements. It may indicate that a deduction of the head thickness by 65 µm does not constitute a substantial change in reduced material absorption when compared to prior dimensional adjustments.

5.7 Implantable device: fluorescence measurements

We also conducted fluorescence measurements on the initial seven chips. For this, we employed a lab-printed transmission setup with a bit of modification, wherein we switched the detection fiber from the bottom of the platform to the fiber-coupling hole of the chip, as illustrated in Figure 31a. Our measurements involved assessing the mixtures of Qdots and DI water across various concentrations, as depicted in Figure 56a. It's worth noting that distinguishing differences in intensities between concentrations of 2.5 nM and 5 nM proved to be challenging across all the designs. Furthermore, Only some of the designs exhibited a more substantial positive correlation when the concentrations exceeded 5 nM, as presented in Figure 56b. Among the designs, T-7 exhibited exceptional performance in terms of the correlation coefficient. T-1, T-3, and T-4 also demonstrated commendably high correlation coefficients, with
T-3 ranking as the second-best device. In general, T-7 outperformed the other designs due to its appropriate circumferential and longitudinal cross-section shapes, whereas T-3 featured a round circumferential cross-section, aligning with the observations made during the transmission measurements. The results for T-1 and T-4, representing the samples of flat and incomplete structures, respectively, indicated stable but relatively lower positive correlations. One minor test based on the T-7, the best among the initial chips, was conducted to verify the cross section geometry. Moreover, one minor test based on the T-7, the best among the initial chips, was conducted to re-verify the effect of cross-section geometry. We simply printed the microstructures in the same height, cap thickness, and head width but with different morphologies: a curl T and a circular T structure. The results are shown in Figure 56c, which agrees with our evaluations in the simulations regarding the adverse of its hexagonal cross-section.

To build the conditions resembling in vivo measurements, we created a chamber using a thyroid biopsy training phantom, as illustrated in Figure 31b. In this setup, we substituted the DI water with human plasma, and the dimensions of the cubic cavity were determined based on the average size of a mouse brain. In the results, the chips exhibited relatively lower intensities and displayed weaker positive correlations, as depicted in Figure 56d. The correlation coefficients have been compiled in Figure 56e. It's worth noting that compared to when the chips were securely positioned within the lab-made tank, which has a support structure, the performance observed in the phantom chamber experiments is relatively difficult to identify the order in terms of the slopes and their intensities. Again, this setup aimed to mimic real-world operational scenarios, indicating the chip is free to move and may incur more deviations in the measurements. However, in Figure 57, which are the spectra under the highest fluorescent concentration, we can still conclude that T-7 is the best based on the peak intensities.
among the designs, followed by T-3, aligning with our earlier findings. In terms of peak intensities, T-2 sits the third position, followed by T-1, T-5, and T-4. This order of performance slightly differs from the previous one. Additionally, T-4 exhibited a notably weaker positive correlation in this context. Other than that, a comparison based on the circular and curl T structures, was also investigated in Figure 56f, which demonstrated a similar result to Figure 56c. This reaffirms that the curl T-shaped structures can serve as a promising foundation for designing related applications.

Notably, T-8, T-9, and T-10 were not subjected to the fluorescence experiments since they are improved chips based on all the measurements of the first seven chips. In practice, the order for the first seven chips' performance observed in the results of the transmission and fluorescence measurements (both the lab-printed tank and the training phantom) are similar to each other. Consequently, T-8 to T-10 chips were derived from the previous results for improvements. Since those experiments show similar trends, it could be extrapolated that the improved chips should possess better light-guiding performance based on the transmission tests.
Figure 56. (a) The fluorescence was measured on mixtures containing Qdots and DI water at varying concentrations (2.5 nM to 20 nM) while the chip was placed in a lab-printed tank. (b) The plot showed the chips with a relatively positive correlation, calculated from 5 nM to 20 nM. (c) The fluorescence intensities of T-7 samples, the best design among the initial set, with curl and circular morphologies. The following experiments were implemented while the chip was situated within a thyroid biopsy training phantom. (d) The fluorescence intensity was sampled in human plasma at various Qdots concentrations (from 2.5 nM to 20 nM). (e) The plot showed the chips that have a relatively positive correlation, calculated in a range from 5 nM to 20 nM. (f) The fluorescence intensities of T-7 samples, the best design among the initial set, with curl and circular morphologies. The measurements were conducted three times for each sample at every concentration, and the results were averaged.
5.8 Implantable device: fluorescence measurements on the mice brain slice

In the final phase of our study, we introduced a straightforward application concept aimed at measuring stained mouse brain tissue, as illustrated in Figure 32a. This setup differed from our previous fluorescence measurement arrangement, where the micro-structured chip was no longer inserted in an environment comprising a training phantom and human plasma. Instead, it was positioned on top of a sliced sample (non-contact) obtained from the C57BL/6 mice brain. This configuration ensured that the fluorescence signal emitted from the brain tissue at the bottom of the probe rather than from fluorescent molecules suspended in solutions. Based on the transmission and fluorescence measurements analysis, we concluded that T-7 represented a suitable design. However, there was room for improvement by optimizing the longitudinal and circumferential spacing, as exemplified by designs such as T-8 and T-10.

Initially, we conducted a straightforward experiment to test the new configuration of the system by placing a 40 µl droplet of 1 µM Qdots solution on a glass slide. The aim was to determine if we could discern any distinctions between the emission intensity peak and the background. We opted to use the T-10 chip due to its superior
transmission capabilities when compared to the T-7 chip. Nonetheless, the results, as depicted in Figure 58, revealed no substantial differences in fluorescence intensities. There are several reasons for this outcome. Firstly, the quantity of fluorescent molecules present was significantly lower than in previous setups, whether it was the lab-printed transmission tank arrangement or the training phantom configuration. Secondly, the pathway for detecting emissions, as previously mentioned, was considerably longer in this case compared to the previous scenarios, where emissions were randomly distributed within the environment or channel.

To deal with the adjustments regarding the measuring setup, we developed two alternative chips. One is the Curl T-Protrusions structured device, abbreviated as Curl T-P, which was derived from T-10. The second one is labeled as T-Curl T (thin-curl T structured device), which has only one difference in the channel length compared to the T-10 chip. The Curl T-P featured a reduced T head thickness and included arrays of 50 µm protrusions on the top surface, as the microstructures shown in Figure 29e. Additionally, the two chips have a modified channel length of approximately 8 mm in total, aligned with the distance spanning from the dorsal to ventral regions. The results of the two modified chips, as illustrated in Figure 58, demonstrated enormous improvements (20% - 27%).
Figure 58. The Qdots-based fluorescence measurements with the T-10 chip, T-Curl T chip, and Curl T-P chip. Note that the latter two possess a modified channel length of 8 mm. Each data was sampled three times and then averaged.

In the next stage, we conducted experiments using Alexa Fluor™ 488 and Alexa Fluor™ 350. It is observed that the intensity difference was more readily noticeable with the latter. Alexa Fluor™ 350 exhibited distinct wavelength peaks for both excitation and emission, with a separation of around 90 nm, whereas Alexa Fluor™ 488 had only a 30 nm separation. Consequently, we opted to utilize Alexa Fluor™ 350 for subsequent investigations. In Figure 59a, the intensity difference between control and experimental samples with original channel length, which are non-stained and stained slices, when using the chips was merely 6% for T-Curl T and 2% for Curl T-P, respectively. Again, this lower intensity difference was attributed to the increased transmission distance of the fluorescence and the relatively weaker fluorescence signal. It's worth noting that the fluorescence dyes used in this context were not as bright as Qdots. Next, we implemented the measurements with previous length-modified chips in Figure 59b. In Figure 59b, Curl T-P exhibited a higher intensity difference of 13%, whereas T-Curl T maintained performance levels similar to those of the original length.
Subsequently, we made alterations to the pore size within the porous film situated between the fiber-coupling hole and the micro-structured channel, increasing it from 100 µm to 300 µm. This adjustment yielded an approximately 7% improvement in the results (Figure 59c). The Curl T-P chip exhibited enhanced performance following these optimization measures. In Figure 59d, we utilized the Curl T-P chip to measure various dye concentrations. Compared to the unstained sample, the 20 µg/ml exhibited approximately 1% difference in intensity, whereas the 50 µg/ml expressed an around 4% difference. In the highest concentration case, fluorescence with roughly 20% higher intensity is observed.
Figure 59. The fluorescence sensing experiments using Alexa Fluor™ 350 with the T-Curl T (thin-curl T structures) and Curl T-P (curl T-protrusions structures) chips under the following conditions: (a) Original channel length, which is ~1.2 cm. (b) Shortened channel length, which is ~0.8 cm. (c) An increased pore diameter. Each measurement denoted by names ending with "B" is to signify background intensity. Each measurement was held three times and then averaged. To facilitate comparison, we normalized the intensities by dividing each value by its respective background intensity. (d) The Curl T-P chip with 300 mm holes in the fiber coupling hole is used to measure the fluorescence in various dye concentrations. The “0” denotes the sample with no stained treatment.
CHAPTER 6. CONCLUSIONS AND RECOMMENDATIONS

The conclusions have been structured in alignment with the objectives outlined in Chapter 2, which delineate the three primary stages of this dissertation: formulating the design strategy, constructing an enclosed 3D structured device, and progressing the device toward an innovative and compact implantable prototype. The research questions concerning the complexities in manufacturing, material compatibility, optical losses, and cost are further addressed in subsequent sections. As an initial investigation into implantable optofluidic applications, forthcoming strategies have been formulated based on the discoveries and additional information gathered.

6.1 Summary of designing principles

The design of the proposed optofluidic devices can be categorized into three sections based on their configuration: a liquid core featuring a compatible RI, a complex cladding structure, and an integrated component. The effectiveness of the liquid core design is significantly reliant on the inherent optical properties (e.g., scattering and absorption) of the materials used, while the design of the cladding structure can be influenced not only by the selected material but also by the functionalized structures employed. Theoretically, a favorable cladding design holds the potential for increased compatibility with a diverse array of liquid materials compared to scenarios focused solely on the liquid core design. Traditional optical components, such as fibers, can be seamlessly integrated with the chip without necessitating additional manufacturing steps. The development of an implantable device can be delineated into two stages: the creation of an enclosed device housing the desired light-guiding microstructure and a subsequent advanced extension that expands upon the device's characteristics, integrating additional components and implantable features. The procedure to build a microstructured optofluidic device is listed below.
1. Choose the core diameter for the transmission and liquid sample accommodation. The cladding layer can be calculated on such dimension by exploiting the relation elucidated in the results: core diameter multiplied by 0.15. This is useful for retaining sufficient cladding thickness without overestimating its dimension, leading to a bulk volume.

2. Engineer the curl T structures. Secondary microstructures can also be built for a more advanced performance. The circumferential and axial cross-sections need to be close to round and straight, respectively. The thickness, width, and length of the microstructures should be minimized while the liquid is well confined in the core without falling into the gaps.

3. Confirm the other specification requirements. For example, we modified the length of the channel according to the mice's brain size.

4. Confirm if the implantable device needs to integrate with multiple functional parts, the syringe for liquid injection, the fiber for coupling with a light source and spectrometer, or even microlens. If so, arrange the integration part with the microstructured channel.

5. Optimize the printing parameters based on the designed sample to refine its quality and feature size, which contains the printing orientation, exposure parameters, and photopolymer dwell time.

6. Apply the PTFE treatment for hydrophobicity to define the liquid core better and reduce contamination.

7. Test the printed chips with transmission measurement and fluorescence measurement. The environmental setup of the fluorescence measurement should mimic the scenario in the desired application (in vivo or ex vivo).

In short, our efforts culminate in a set of optimized design guidelines for
superhydrophobic cladding in optofluidic platforms, providing a framework for developing such devices.

6.2 A 3D structured optofluidic device

In the pursuit of enclosed and implantable devices, our initial approach involved PDMS-based designs. These encompassed a sugar water-based liquid core channel, a femtosecond laser-enabled hydrophobic surface-based PDMS device, and an assembly PDMS device constructed using 3D molds. Compared to the previous methods, the µSLA displayed its capability to reduce the manufacturing complexity of the devices through single-step printing. While 2PP shows promise as a high-resolution option for miniaturized devices, it remains a costly and time-consuming methodology. That said, µSLA presents a more viable and cost-effective alternative. Additionally, it highlighted the capacity to create unconventional 3D microstructured morphologies within these devices. As various geometrical designs were introduced, the performance, as observed through fluorescence measurements, exhibited gradual enhancements, indicating the potential for further reducing the optical loss and even extensions to other applications are achievable through this adaptable technology.

Various geometries for superhydrophobic-based enclosed and implantable optofluidic devices were fabricated and assessed through measurements of transmission and fluorescence. Initially, the investigation focused on optimizing the fully enclosed optofluidic devices by reducing the solid contact ratio, as demonstrated in the femtosecond laser-treated channel. However, the porous surface structures constructed by the laser ablation performed minimal influences in the light guiding. Therefore, we analyze both the solid contact ratio and solid fraction of each structured cladding layer, particularly in the thickness theoretically calculated. The effect of the smoothness of the liquid core's cross-section is also studied. Among the different designs, the T-shape
optofluidic chip exhibited the most favorable performance across all tested excitation wavelengths, aligning with our theoretical model. Also, the distinct excitations used in the setup indicate the platform can be easily modified depending on the fluorescent molecules. In detail, the optimized T structure should feature a round cross-section of the core, a thin but wide T-cap, and a short overhang length. The developed optofluidic system featuring optimized T shape structures was employed to quantify a Qdot-based CRISPR-Cas12 assay, thereby introducing an innovative technology suitable for advancing the next generation of CRISPR therapeutics-related applications. Moreover, the “T” microstructures were applied as the default design for the following in vivo application.

6.3 A prototype of implantable optofluidic device

In summary, we developed an implantable prototype after practical printing evaluation, ray tracing simulation, and previously mentioned optical measurements. We referred to the default T-shape designs obtained from the investigation of the enclosed chip. Several variant T shape micro-structures were produced for the printing evaluation in terms of the resolution and the printing orientation. The printed curl T chip with a 25 µm feature size is achieved, and even the secondary structures, a 50 µm protrusions array, are built on each T head successfully. The ray tracing simulation was applied before the experiments to understand and anticipate the priority of proposed morphologies and various dimensions. The results indicated the dimensions in the circumferential direction (structure’s width), the radial direction (structure’s head thickness), the longitudinal direction (structure’s length and the spaces between them), and the cross-section shape would affect the light guiding due to printing material absorption, light refraction and reflection in the interfaces between the solid structure, liquid core, and air space. The radial thickness of the structure should be efficiently thin,
where the circumferential and longitudinal dimensions should be reasonable to lower the solid contact area while the liquid core is well confined like a cylinder, which could raise the light rays collecting efficiency along the center axis. This was verified while we navigated the curl T series in terms of the transmission and fluorescence measurements. Among the initial seven chips, T-7 outperforms the others due to its moderate structural length, as observed in both transmission and fluorescence measurements conducted on the lab-printed tank and the training phantom. This observation underscores its consistency in the performance order of the chips. Consequently, T-8, T-9, and T-10 chips represent advancements derived from evaluating the initial seven chips. The contribution from the cross-section shape in the axial direction was verified by the chips from T-8 and T-10, which are found to have an improved transmission performance.

Furthermore, the fluorescence measurement with the training phantom and human plasma that filled in a chamber similar to a mouse brain volume showed a similar result as previously, which implies the prototype could be potentially used as an implantable probe for mice brains. We also demonstrate a non-implanted protocol by utilizing the chip to measure the fluorescence of a real sample- a mouse's brain slice. Here, the fluorescence is weaker since the number of fluorescent molecules is restricted on the cut, where they are freely moving in the solutions, and the inherent fluorescence is not as bright as Qdots. Collectively, we came up with a modified device, which aligned with the distance from the dorsal to ventral in mice brains, based on the summarized design procedure and the developed optofluidic chip. Eventually, with a dye treatment of 100 µg/ml concentration, an increment of approximately 20 % in intensity was observed for the stained slice. The capability of the micro-structured chips was verified in terms of various measuring environments and discrete protocols. Predictably, the
measuring data would be affected by fluorescent quenching, positioning error (manual operation), and even the fluorescent molecule distribution, which may consequently lower the value of the system. Therefore, µSLA is a crucial enabler in progressing the design of miniaturized optofluidic devices. Our preliminary findings underscore the potential of this probe for in vivo or ex vivo fluorescence measurements. We anticipate that ongoing improvements and innovation in this realm will unveil new possibilities, pushing the boundaries of optofluidics and its real-world applications.

6.4 Recommendation: improvement of coupling efficiency and exploration of hybridized technology

Throughout the dissertation, a significant portion of our focus was directed toward examining the design of the microstructured channel, notwithstanding the potential for the chip's integration with additional functional components as a cohesive unit. Addressing uncertainties in in vivo applications necessitates prioritizing the enhancement of coupling efficiency between the optical elements and the constructed device. To address this concern, two potential extensions can be explored in the future: a hybridized printing process and integration with the lens.

A hybridized printing process denotes a two-stage printing approach. As mentioned, 2PP is deemed unsuitable for our specific case due to potential time and cost constraints. However, it holds promise for potential integration with other photopolymer-based technologies, enhancing device capabilities without significantly escalating process complexity. Grabulosa et al. employed a fusion of one-photon polymerization and 2PP to construct a waveguide, thereby reducing manufacturing duration and enhancing optical confinement.²⁰⁰ Within this framework, one-photon polymerization was induced through blanket irradiation using a UV lamp across the entire sample. Crucial and supportive structures can be printed using integrated
polymerization mechanisms based on specific requirements, such as smoothness, allowing local control. Moreover, materials like glass, crystals, and others can be integrated into this process. This flexibility enables the engineering of the microdevice in one or multiple materials. For our case, 2PP can be utilized to refine secondary microstructures, while the remainder of the channel can be completed using a quicker yet moderate resolution process. Additionally, specific structures, like the film within the fiber coupling hole designed to prevent water leakage, can be printed using glass or other transparent materials.

Another extension is to construct a lens-coupled chip, which is inspired by the 3D printing-based micro lens-in-lens device and contact lens. Both established studies have demonstrated the application of 3D printing technology to manufacture optical elements akin to traditional ones. Moreover, this methodology offers increased flexibility in geometric design to accommodate diverse research requirements. The Fresnel lens, recognized for its distinct structure comprising multiple faceted concentric rings, finds wide application in various optical settings, particularly in lens volume and thickness reduction. An alternative design for integrating such a lens is depicted in Figure 60a. The left side illustrates a transmission mode, where the fiber is positioned in the coupling hole, and the detection fiber is situated at the chip's base. On the right side, the image represents the fluorescence detection mode, where both fibers are placed within the fiber coupling hole. Our testing primarily focused on assessing the design's printability. Before a finished printing sample, a detailed investigation of the evaluation of multiple-material-based printing is necessary. In Figure 60b, simulations were conducted for Fresnel lenses with varying geometries and thicknesses. From our analysis, it became evident that the thinnest lens did not yield the highest flux among the group, highlighting the critical role played by the concentric ring structures.
In summary, both of these extensions have the potential to be integrated to create an advanced optofluidic device. The design guidelines can serve as a valuable resource for reconfiguring not only the initial design but also these extensions, offering an enhancement strategy for future in vivo applications.

**Figure 60.** (a) The drafts of the lens-coupled chips. The left represents a transmission mode, whereas the right represents the fluorescence detection mode. The inset shows the fresnel lens printed in the fiber coupling hole with a minimum thickness in the center of 25 µm. (b) Simulations of the various fresnel lenses. The geometries of the fresnel lenses are displayed in the second row.
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GLOSSARY

3D Three-dimensional
CCD Charged-coupled device
CMOS Complementary metal-oxide-semiconductor
CNC Computer Numerical Control
CRISPR Clustered regularly interspaced short palindromic repeat
CTCs Circulating tumor cells
CVD Chemical vapor deposition
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<th>Acronym</th>
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<tr>
<td>DI</td>
<td>Deionized</td>
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<td>DIW</td>
<td>Direct ink writing</td>
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<td>DLP</td>
<td>Digital light processing</td>
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<td>DLW</td>
<td>Direct laser writing</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>DRIE</td>
<td>Deep reactive ion etching</td>
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<td>FDM</td>
<td>Fused deposition modeling</td>
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<td>HMDS</td>
<td>Hexamethyldisilazane</td>
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<td>HPLC</td>
<td>High-performance liquid chromatography</td>
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<td>LCM</td>
<td>Lithography-based ceramic manufacturing</td>
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<td>LCoS</td>
<td>Liquid crystal on silicon</td>
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<td>LED</td>
<td>Light-emitting diode</td>
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<td>LSPR</td>
<td>Localized surface plasmon resonance</td>
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<td>LOC</td>
<td>Lap on a chip</td>
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<td>MEMS</td>
<td>Microelectromechanical systems</td>
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<td>µ-ILED</td>
<td>Microscale inorganic LED</td>
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<td>µSLA</td>
<td>Microstereolithography</td>
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<td>µSLS</td>
<td>Microscale selective laser sintering</td>
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<td>MJF</td>
<td>Multi-jet fusion</td>
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<td>PBS</td>
<td>Phosphate-buffered saline</td>
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<td>Polycaprolactone</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>PDMS</td>
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<td>PETG</td>
<td>Polyethylene terephthalate glycol</td>
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<td>PMMA</td>
<td>Polymethylmethacrylate</td>
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<td>PTFE</td>
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<td>PVA</td>
<td>polyvinyl alcohol</td>
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<td>PLA</td>
<td>Polylactic acid</td>
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<td>PµSL</td>
<td>Projection microstereolithography</td>
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<td>PoC</td>
<td>Point of care</td>
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<td>Qdots</td>
<td>Quantum dots</td>
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<td>RI</td>
<td>Refractive index</td>
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<td>SEM</td>
<td>Scanning electron microscope</td>
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<td>Abbreviation</td>
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<tr>
<td>SIL</td>
<td>Self interlocking</td>
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<td>SLIPSs</td>
<td>Slippery liquid-infused porous surfaces</td>
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<td>SPR</td>
<td>Surface plasmon resonance</td>
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<td>TEOS</td>
<td>Tetraethylorthosilicate</td>
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<td>TIR</td>
<td>Total internal reflection</td>
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<td>WGA</td>
<td>Wheat germ agglutinin</td>
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