Selecting the Best Microfluidics Device Material for Your *In Vitro* Diagnostic

Balancing Performance and Cost with Glass

A Production Mindset

When examining a material for IVD products, one should also consider the production manufacturing scale options for fabrication that will ultimately influence design, cost, and quality.

"In vitro diagnostics" is a term for a broad industry encompassing everything from benchtop or larger bioanalytical instrumentation applications such as protein analysis and next generation sequencing to point of care devices. As if *in vitro* diagnostic (IVD) development wasn't difficult enough just from the biology itself, the instrumentation and platform development add layers of complexity.

Examined here are the advantages and challenges of using a microfluidic flow cell approach for automating and miniaturizing IVDs from the standpoint of selecting the best material for the microfluidic component. The materials' strengths and weaknesses are reviewed in the context of general biological assay applications. In this context, recommendations for applications where glass can aid in achieving the desired IVD performance while still maintaining a favorable production cost are made. General guidelines for choosing materials for microfluidics-based IVDs are also provided.

INTRODUCTION

Microfluidics, or lab-on-a-chip technology, is a powerful tool that sits at the intersection of biotechnology, automation, and functional integration. By using photolithography and other CMOS fabrication techniques to make fluid flow cells, microfluidic devices can shuttle

picoliter, and smaller, volumes of fluids into functional regions, enabling powerful *in vitro* diagnostics (IVD) harnessing everything from digital biology, to next generation sequencing, to organs-on-a-chip, to high-throughput cell-based assays, to high-density multifunctional chips for drug discovery and bioanalysis, for shrinking sample and reagent volumes.

Materials selected for the IVD device often define the patterning and processing tools and techniques applied (rather than the other way around) and ultimately the manufacturing, performance, functionality, and the cost of the device. Typical IVD device materials are primarily glass, silicon, and polymers (plastics and photoresists). However, paper, 3D printed materials, hybrid devices, and other new materials are emerging [1].

Glass, with favorable and well-understood physical, chemical, and thermal properties, is best for applications that require optical transparency, a low fluorescence background and a low channel surface roughness. Silicon is historically favored for the ability to achieve nano- to microscale high-aspect aspect ratio features and multifunctional layering. Polymers are primarily used to control manufacturing costs at the production scale [2]. Paper is emerging as a material for patterned lateral flow assays, but it does face some challenges in implementation, with the main drivers being ease of use and low cost. Additive methods, including the 3D printing of biocompatible and biological materials, have applications in the development of the so called “organ-on-a-chip” and “bioreactor-on-a-chip” technologies [1].

Thanks to advances in laser machining, photolithography, etching automation, wafer bonding, functionalization, room temperature UV-adhesive bonding and other process improvements, glass and glass-hybrid materials are often both the best-performing and most cost-effective consumable material for IVD devices.

MICROFLUIDIC IVD DEVICE DEVELOPMENT DRIVERS AND CHALLENGES

The desire to reduce sample size and reagent consumption, increase speed of analysis, and decrease cost, is pushing IVD device fabrication to new limits while increasing the device functional integration and complexity. Post-fabrication modifications, such as biological recognition molecules, coatings, and other steps, also influence device production workflow and cost.

A microfluidic flow cell approach allows for the automation of the analysis of volumes smaller than a drop of blood. However, the dimensions and complexity of microfluidic flow cells for IVDs present significant challenges to IVD device design, fabrication, and production scale-up.

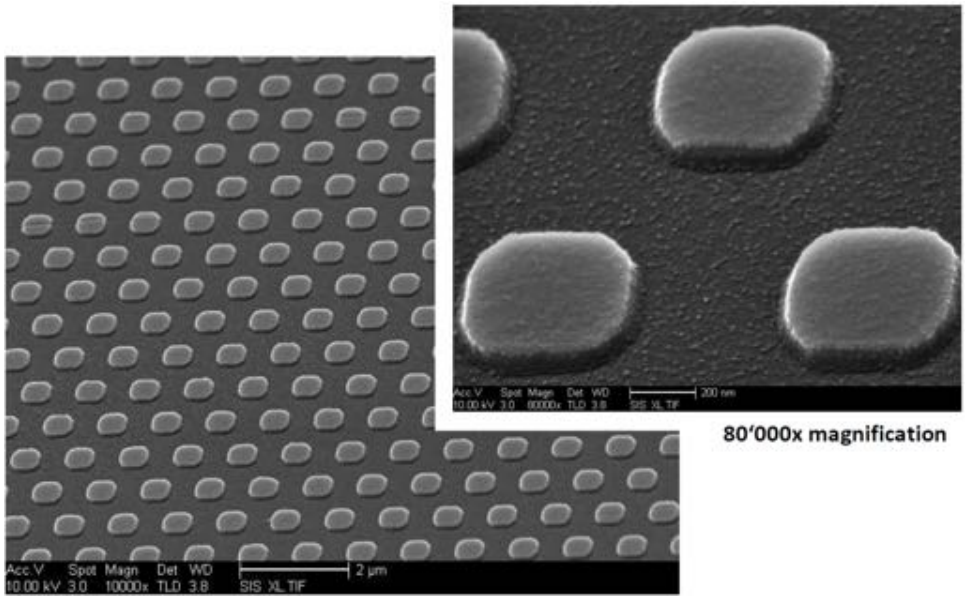
The Impact of Reduced Feature Size

"There's plenty of room at the bottom."

- Richard Feynman, 1959 [2]

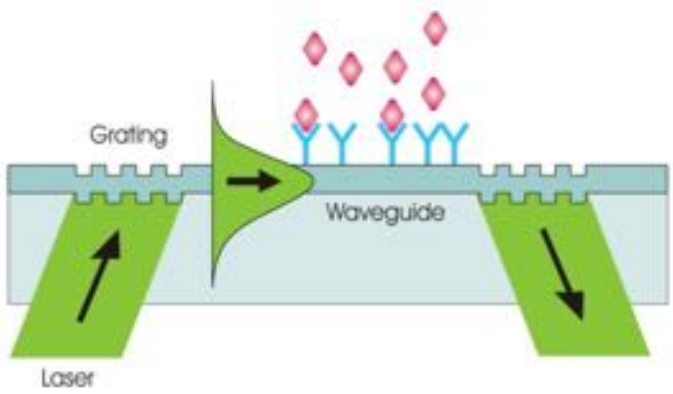
As the dimensions for fabrication technology are pushed lower and lower, what was once considered a surface defect becomes a feature, rather than a flaw. Fluidic devices can take advantage of the ability to use microscale and nanoscale wafer processing techniques to make nanostructures to harness the subsequent effects of these features whose dimensions are on the same order of magnitude as those of biopolymers and cell structures.

Reactive-ion etching (RIE) of glass wafers creates a nano-roughened glass surface that can be exploited to create increased surface area for cell and biomolecular docking stations. For example, an increased surface roughness of nanostructured glass, R_q=150 nm, vs. a smooth glass surface, R_q=1 nm, that cancer cells adhere to more readily, can be exploited to create a glass/polymer hybrid circulating tumor cell microfluidic flow cell [3]. The increased surface roughness can be coupled to a molecular recognition element, such as an aptamer, for increased selectivity [4].

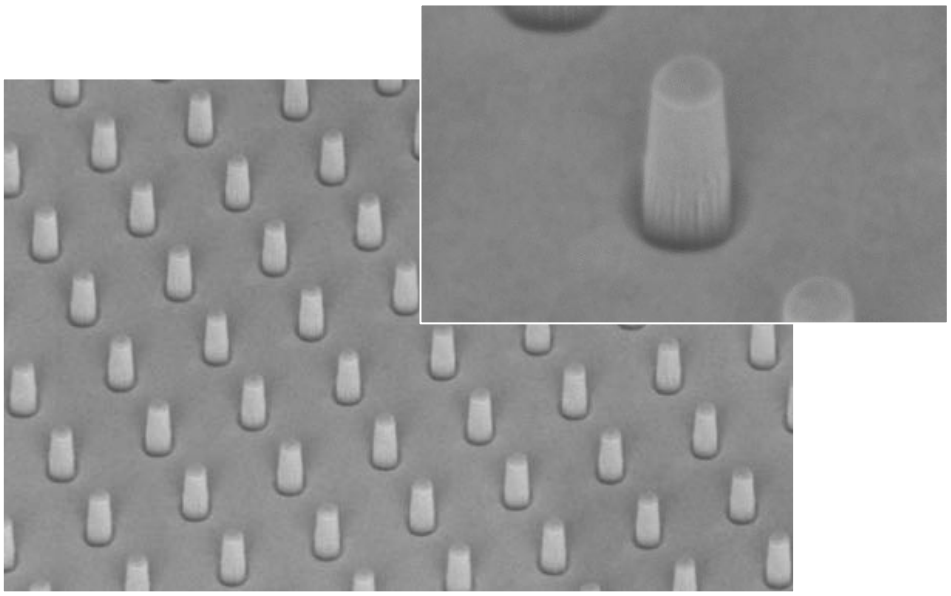


Nanostructures on glass, metallic, and dielectric materials. (Source: IMT)

Nanofluidics employs submicron channels to confine volumes and harness phenomena at the same scale as the biomolecules themselves. Applications are vast [5], including DNA separation, creating artificial pores for cell studies [6], and commercialized platforms for drug discovery, such as the opto-nanofluidics platform for cell sorting created by Berkeley Lights [7]. In its early days, nanofluidics showed up primarily in patents for nucleic acid analysis, with some applications in particle analysis [8].



Gratings on borosilicate glass wafers. (Source: IMT)



Nanopillars in glass to increase surface area for interactions. (Source: IMT)

Though using materials such as polydimethylsiloxane (PDMS) with soft-lithography is often the first and only step of a well-established manufacturing road-map for academic research, glass has many advantages to these materials, largely due to its mechanical, chemical, and optical properties (Table 1) [9, 10]. For example, for nano or microchannels, wall rigidity and stability becomes important, particularly if it is to survive the bonding or sealing process. Additionally, as dimensions shrink to the "extended nanospace," the superior ability of bonded glass to withstand high pressures from fluid flow becomes a significant driver for glass material as the material of choice [11].

Applications for drug discovery, synthesis, DNA sequencing and PCR may require the thermal properties of glass, as well as its stability to chemicals, and negligible auto-fluorescence. Finally, the glass surface is infinitely tunable with silanization reactions, allowing device developers to imbue the surface with specific properties [12].

High aspect ratio channels are often desired for biological applications. For example, high aspect ratio features may be used to confine a single cell or create a specific flow profile. In many cases, silicon is the best material choice for high aspect ratio features, with the processing methods for silicon offering the possibility of arbitrarily high aspect ratios [10].

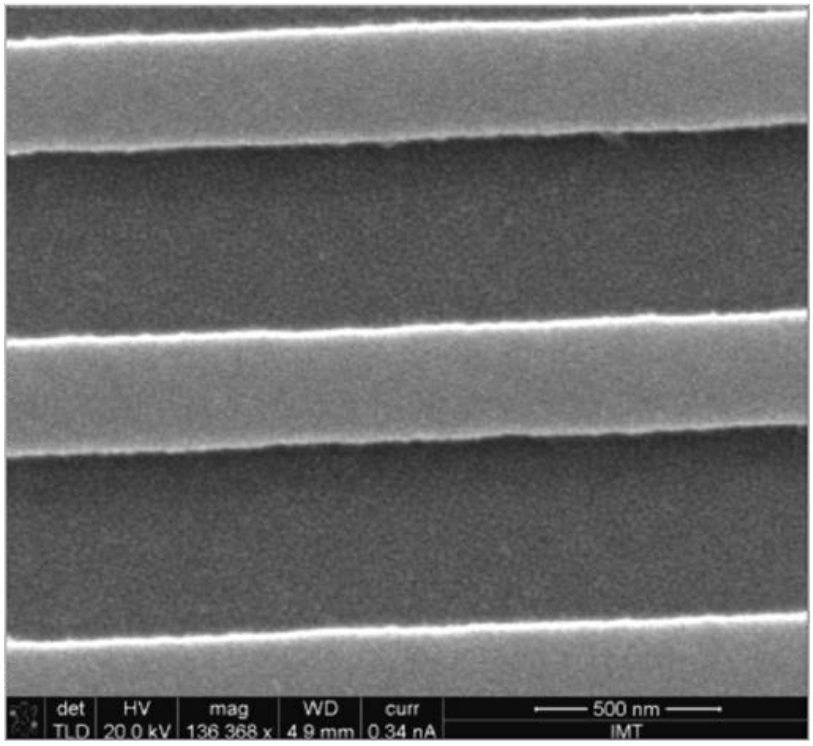
Table 1. Comparison of materials based on mechanical, chemical, and optical properties. [10]

	SILICON/GLASS[a]	ELASTOMERS	THERMOSET	THERMOPLASTICS	HYDROGEL	PAPER
Mechanical Properties						
Young's (tensile) modulus (GPa)	130–180/50-90	~0.0005	2.0–2.7	1.4–4.1	low	0.0003–0.0025
Common Technique for Microfabrication	photolithography	casting	casting, photopolymerization	thermomolding	casting, photopolymerization	photolithography, printing
Smallest Channel Dimension	<100 nm	< 1 μm	<100 nm	~100 nm	~10 μm	~200 μm
Aspect Ratio	limited 3D	3D	arbitrary 3D	3D	3D	2D
Thermostability	very high	medium	high	medium to high	low	medium
Chemical Properties						
Resistance to Oxidizer	excellent	moderate	good	moderate to good[b]	low	low
Solvent Compatibility	very high	low	high	medium to high	low	medium
Hydrophobicity	hydrophilic	hydrophobic	hydrophobic	hydrophobic	hydrophilic	amphiphilic
Surface Charge	very stable	not stable	stable	stable	N/A	N/A
Permeable to Oxygen (Barrer[c])	<0.01	~500	0.03–1	0.05–5	>1	>1
Optical Properties						
Optical Transparency	no/high	high	high	medium to high	low to medium	low
Auto-fluorescence	no/some	some/high	some/high	some/high	?	high

[a]Photosensitive glass is considered as thermoset.
[b]Excellent for Teflon.
[c]1 Barrer = 10^{-10} [cm^3 O^2(STD)] cm cm^{-2} s^{-1} $cmHg^{-1}$

However, when other glass material features (*e.g.* optical smoothness, dielectric constant, metal layer processing step requirements, bonding methods etc.) are also desired, it is possible to use glass as the substrate in some cases where high aspect ratios are required.

Because of the existing methods for fabricating optical gratings and other optical features in glass, fused silica wafer processing offers a path to create microchannels and nanochannels with high aspect ratios. Thanks to a process that combines laser micromachining and chemical etching, aspect ratios as high as 20:1 have been demonstrated by Bellouard, *et al.*, in glass [13].



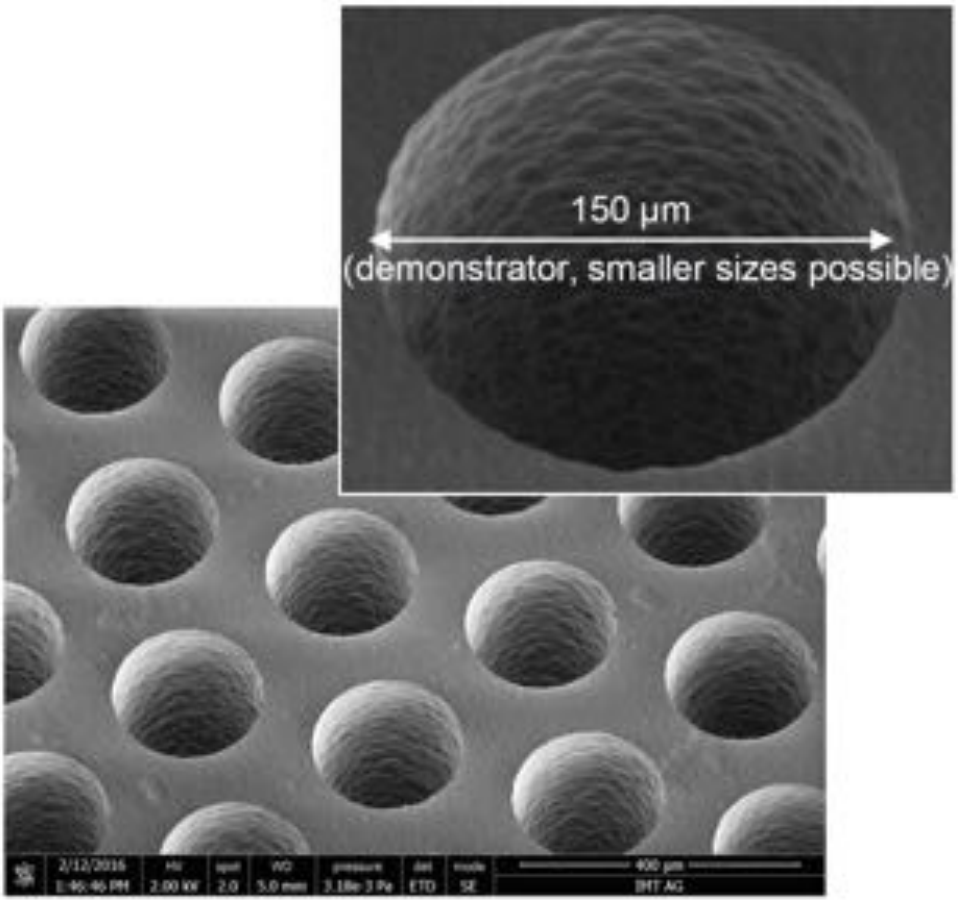
Nanochannels for DNA stretching. (Source: IMT)

One unintended consequence of using microfluidic flow cells is the increased dominance of channel surface properties at micron and submicron dimensions. As volumes decrease, the total surface area-to-volume ratio increases, which can exacerbate problems that may be minor at the test tube or even microtiter plate scale. For example, with decreased volumes being employed to minimize sample and reagent volumes, the impact of the increased surface area-to-volume ratio on non-specific binding can create sizable sample and reagent losses [14].

Because of the immense body of work describing the modification of glass surfaces, glass as a substrate has by far the most chemically tunable surface. Silanization reactions with glass are well known [12], and it is also possible to create hybrid material devices with glass, including glass structures with polymer surfaces.

The Impact of Enhanced Device Complexity

The complexity required by biological applications can include the integration of optics, optical windows, electrodes, electronics, additional three-dimensional structures such barriers, pores, and similar, not to mention any surface modification requirements for the biological application itself. Glass, Foturan®[d], and fused silica have their strengths depending on application and design parameters. Using a combination of subtractive techniques such as ultra-fast laser fabrication and chemical etching with additive methods such as electroless plating, CVD, and other material deposition techniques, it is possible to create the complex, multifunctional chips (including those with polymer surfaces), in these substrates [15, 6, 5, 8].



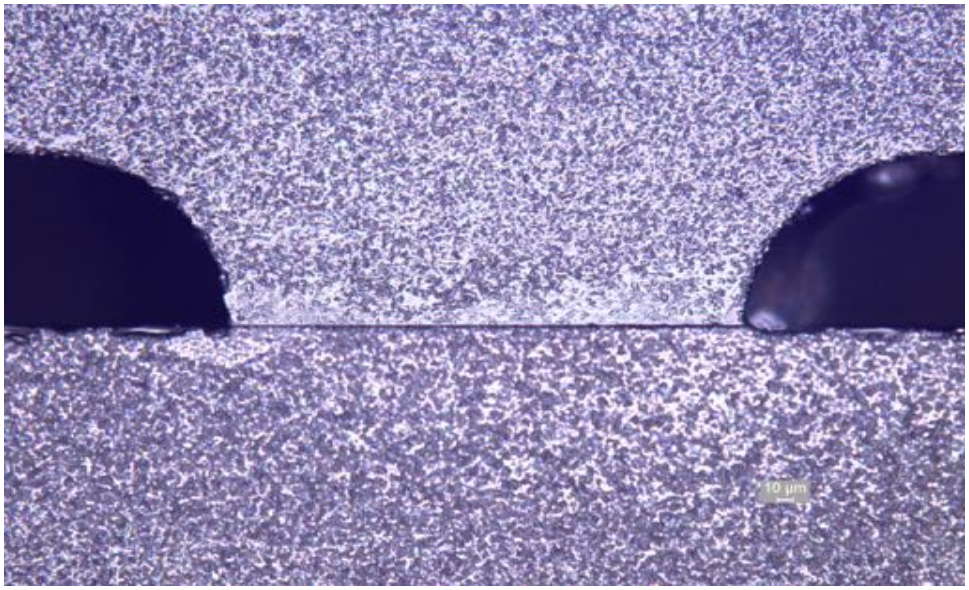
Foturan® holes. (Source: IMT)

The Impact of Biological Complexity

IVD cell culture systems on a chip lack the three-dimensional advantages of an *in vivo* experiment, however, they allow researchers to isolate cell function without interference from the complex environment *in vivo*, reduces animal care expenses, cost, and animal suffering, and allow for optical observations. However, depending on the cells, the media, and the phenomena one desires to measure or observe, the best material, configuration, and post-fabrication processing varies. Even the channel design can affect cell viability as some cells are more susceptible to shear stress than others [16].

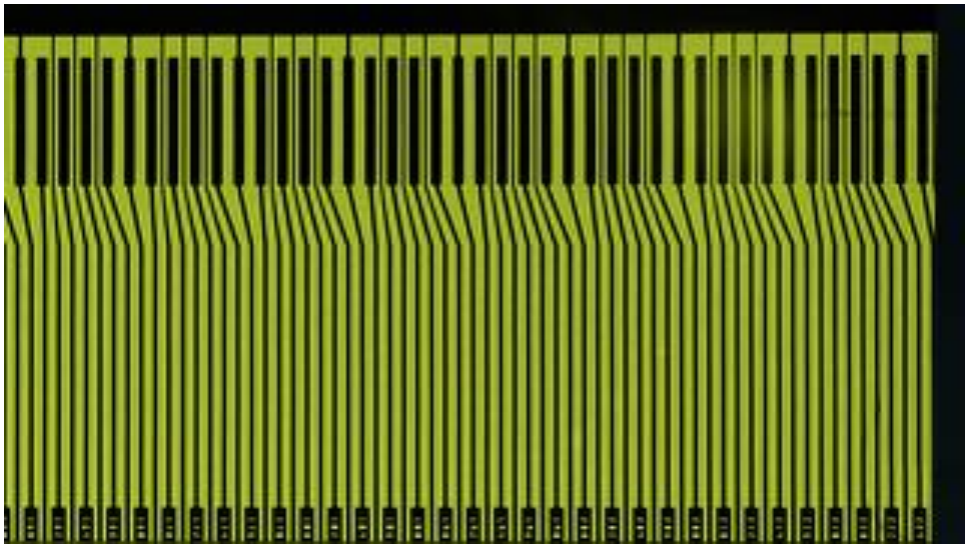
PDMS has often been used because of the favorable gas permeability, allowing for gases to be exchanged between the cells on a chip and the environment surrounding the chip. However, this can also result it unintended migration of gases and ions that can compromise cell health as well. Similarly, patterning to locate cells in one region of a device has many advantages, but cells not meant to be in contact with a surface may eventually lose activity as the surface affects the cell shape and physiology. Depending on the assay, requirements for cell activity may vary from a few hours to a few days. Material interactions with cells may be less important if the interaction occurs after the cell behavior to be measured.

Neuronal cells are often measured using electrodes, making the standardized patterning of electrodes on glass or silicon a reason to choose either material [16]. The ability to use ITO electrodes that are transparent can be an advantage to using glass, as the cells can be monitored using a microscope, even when on an electrode surface.



UV Adhesives room temperature glass:glass bond used to preserve bioactivity of encapsulated biomolecules/cells. A >0.3mm glass wafer with isotropically etched channels bonded with UV-adhesive to a 1mm thick glass wafer. (Source: IMT)

Because of the desire to use optical methods, glass is often the best material. Fortunately, it is possible to use silane chemistry to modify the glass post-bonding for more favorable surface properties. Even better, room temperature bonding techniques using UV adhesives can be employed [17]. UV-adhesives are well known in industry with verified biotoxicity and biocompatibility data and often implemented for biomedical applications.

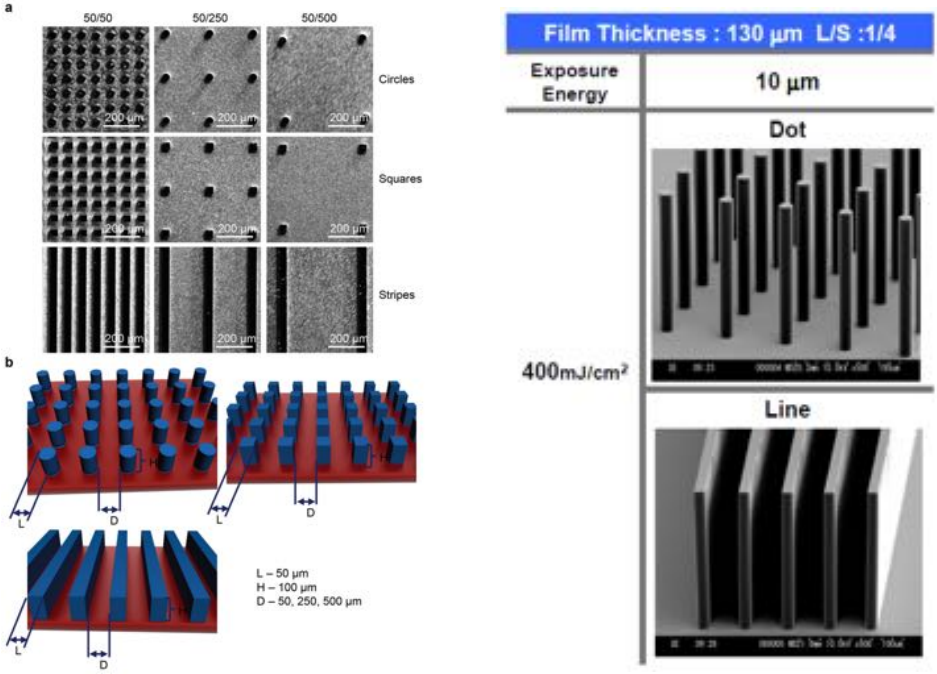


Gold electrode array. (Source: IMT)

Of course, often the best solution is to take advantage of the properties of each material, with optical features like ITO electrodes patterned on glass, polycarbonate as the base for additional sensing features, and PDMS forming the cell culture layer [18]. More and more, it is the hybrid material devices that are able to overcome the challenges of the complexity of using live cells [1].

Fabricating for Cell Adhesion

Cell adhesion is dependent on cell type, length of time on the pattern or surface, cell density, and media type. Micro and nano-patterning a surface modification is often exploited to confine cells to a specific region. For example, Toner's group demonstrated that collagen (cell adhesion) surrounded by albumin (non-adhesive) could create cell and cell-free zones as long as the cell culture medium was free of serum proteins. By exposing the surface to hepatocytes in serum free media, then fibroblasts in serum containing media, a two-cell type pattern of hepatocytes and fibroblasts were defined [19]. Another strategy is to use TMMF, a photostructurable material, as an intermediary layer to create so-called "2.5D" structures for cell capturing and culturing [20].



Modulation of collective cell behaviour by substrates with patterned surfaces for cell culturing. (Source: "Modulation of collective cell behaviour by geometrical constraints," M. Lunova, V. Zablotskii, N. M. Dempsey, T. Devillers, M. Jirsa, E. Syková, Š. Kubinová, O. Lunov and A. Dejneka, Integr. Biol., 2016, 8, 1099. DOI: 10.1039/C6IB00125D **- Published by The Royal Society of Chemistry.)**

Another effective strategy is to use self-assembled monolayers (SAMs) to chemically define surface functionality. Depending on the end functional group, surfaces can be tailored for adhesion, lubrication, wettability, or protein physisorption [12]. Thiol-based SAMS are the most commonly used, reacting with gold [16], [12]. a metal readily patterned on glass. Organosilanes are another powerful chemical tool, as their reaction chemistry with the free hydroxyl groups found on the surface of the glass itself is very well-understood [16]. Common surface properties important for IVDs that can be created by organosilane modification include [12]:

- Increasing hydrophobicity for cell/biomolecule adhesion, or droplet/digital microfluidics;
- Increasing hydrophilicity to prevent biological material adsorption;
- Adding a surface charge for ionic association or other purposes.

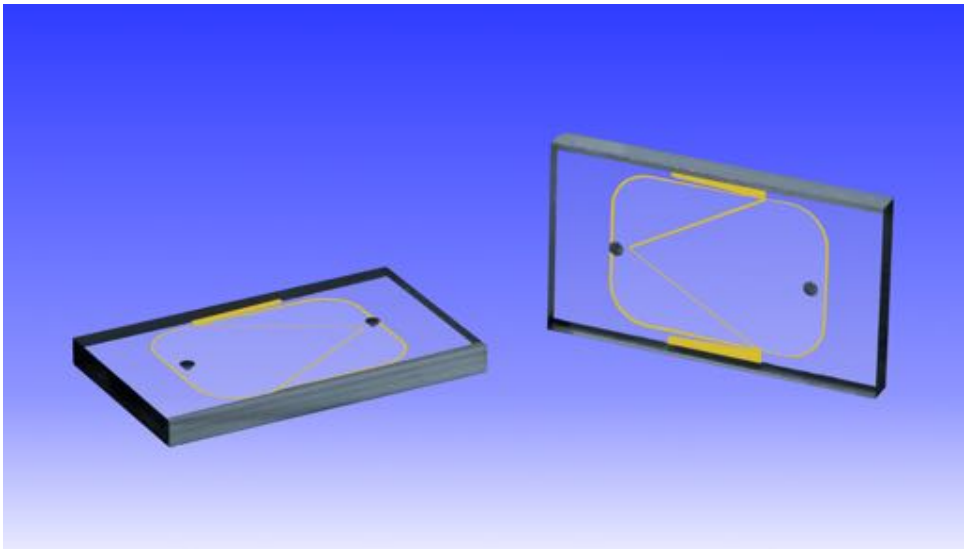
Recently, a burst of studies utilizing cell micro- and nanopatterning techniques revealed that the manipulation of cell geometry, such as confinement to circular or square patterns independently of the cell spread area, affects many crucial cellular processes. To produce uniform substrates for biological applications, deep reactive ion etching is used to pattern silicon wafers so as to produce sets of substrates with surfaces consisting of arrays of silicon micropillars of different geometry and with different values of inter-pillar spacing. The patterned silicon substrates were coated with a parylene, which is a biocompatible, inert and very low permeability material [20], [21].

Alternative scenarios can be envisaged by the structuring of photo-structuring Photoimaginable Bonding Adhesives (PBA) to create complex fluidic channel systems incorporating capture and fusion of cells or droplets directly on a glass wafer by using standard MEMS processes [22]. This method far more economical then typical hybrid approaches as it does not require extra passivation layers due to availability of bio-compatible PBAs. The combination of bio-functionalization and UV-adhesive sealing can lead to a cost-effective and up-scalable production of cell-trapping flow cells [21].

Fabricating for Cell Sorting

Cell sorting moves cells from an entry point where a heterogeneous population begins, to a selection zone where a subpopulation is selected, and finally to a diversion zone where one subpopulation follows a different path than the rest [23]. The need to sort heterogeneous cell populations is expanding to the isolation of rare target cell populations such as the enrichment of circulating tumor cells (CTCs), hematopoietic stem cells (HSCs), and circulating fetal cells (CFCs).

It has wide applications in research, in the health and biopharma industries for diagnostics, theranostics and personalized medicine. The earliest, best known methodology is fluorescence-activated cell sorting (FACS), where a cell is selected based on its fluorescent signal. This technique is associated with high efficiencies and requires a flowing stream and is the technique to which all others are benchmarked.



Chip for dielectrophoretic accumulation of magnetic beads for a virus detection system. (Source: IMT)

There are three types of cell sorting – fluorescence label-based, bead based, and label-free. Within each category, there are a variety of mechanisms for selection: optical forces, electrokinetic, acoustophoresis, magnetic, mechanical, or passive.

Depending on the fluorescence efficiency of the target, wavelength, and limit of detection (LOD), glass or fused silica tends to be the material of choice due to low auto-fluorescence. Even if other materials are required, such as electrodes, glass is still a strong choice. However, for acoustophoresis and magnetic applications, depending on the level of integration required, silicon-metal - glass hybrids may be a better choice because of the CMOS fabrication and integration processes available for the silicon substrate.

Label-free methods are not necessarily free from the optical transparency and low auto-fluorescence requirements that drive the choice of glass. If inherent fluorescence, optical or optoelectronic tweezers are used, then glass, or a glass-hybrid, is often the best material. Label-free electrokinetic methods still benefit from the favorable high dielectric constant of glass, not to mention the ease of depositing and patterning electrode materials on glass.

Due to the ability to create channels with arbitrarily high aspect ratios, and ease of patterning electrode and other materials, silicon, or silicon/glass or silicon hybrid/multilayered materials, is often the best choice for label-free acoustophoresis and magnetophoresis. For passive cell sorting, glass, silicon, polymers can be used depending on feature sizes, type, aspect ratio desired, optical and chemical properties required.

Fabricating for PCR

As with other bioassays, the best material for high-throughput, small volume nucleic acid analysis is largely determined by the detection mechanism and thermal properties of the materials. For optical detection methods, glass is still the material of choice. However, with electronic detection methods proliferating, other materials from silicon to polymers are suitable.

Glass is an attractive substrate for other reasons – fused silica does not have a strong inhibition effect on PCR enzymes [24]. It does not outgas or take on water, nor undesirable ions that can foul an enzyme reaction.

In general, with all bioassays, it is possible for the material to inhibit the enzymes used in the assay.

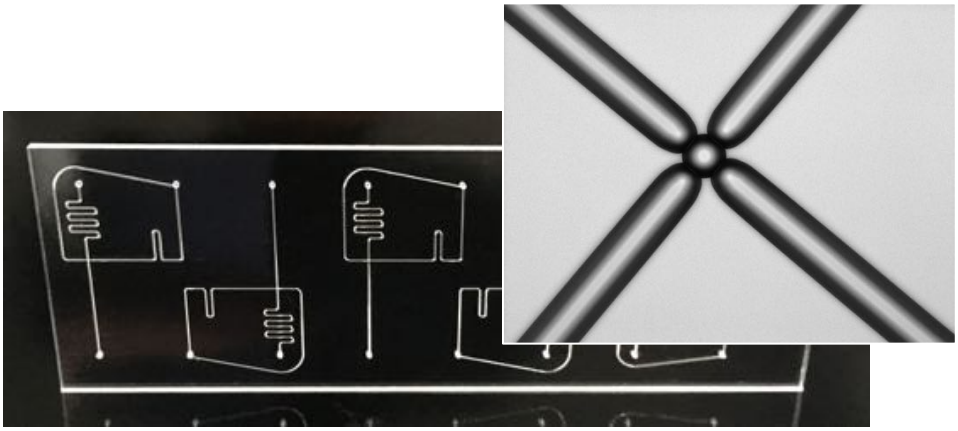
One of the major innovations achieved by the drive to reach the $1000 genome is the recognition of the role of sample preparation including the amplification and quantitation of DNA libraries using digital PCR. Digital PCR (dPCR)has been in existence for since 1992 by P. J. Sykes, *et al* [25], and is a method of digitizing PCR by separating the sample into many partitions to try and get to a single molecule per partition. With smaller partitions, the ability to quantify different sequences whose presence occurs in small percentages (< 1 %) increases. This was originally done using multiplexed capillaries and arrays in 384 well plates. Normal sample volumes for this array approach, however, required relatively large volumes and were dauntingly expensive [26].

Microfluidic devices designed to create droplets from an emulsion in a small volume version of flow injection analysis (droplet microfluidics) or harness electrowetting on an electrode array to create and move droplets (digital microfluidics), have come to dominate the approach to dPCR. Droplet and digital microfluidics has allowed for the confining of single molecules, single cells, and extremely small volumes of many reagents in a wide range of applications.

The ability to create thousands to millions of uniformly sized droplets is key to success for this approach, and this is heavily dependent on the design and manufacture of the digital microfluidic chip. The end result is better precision and selectivity when used for either standalone dPCR or dPCR that is a part of the NGS workflow. These advances have been applied to improve the understanding complex diseases and expression pathways such as cancer, and is now being used in theranostics approaches such as liquid biopsies [27].

Companies such as Advanced Liquid Logic (now part Illumina), Raindance and Quantalife (both now part of BioRad) have perfected creating droplets using the geometry and surface chemistry of the microfluidic channels as they interact with the droplet-enclosed fluid (hydrophilic) and droplet enclosing fluid (hydrophobic).

The droplet microfluidics chip is designed to create droplets by combining an aqueous and hydrophobic liquid using pressure driven flow, hydrophilic and hydrophobic surface chemistry, and channel geometry [28].



Chip for droplet microfluidics. (Source: IMT)

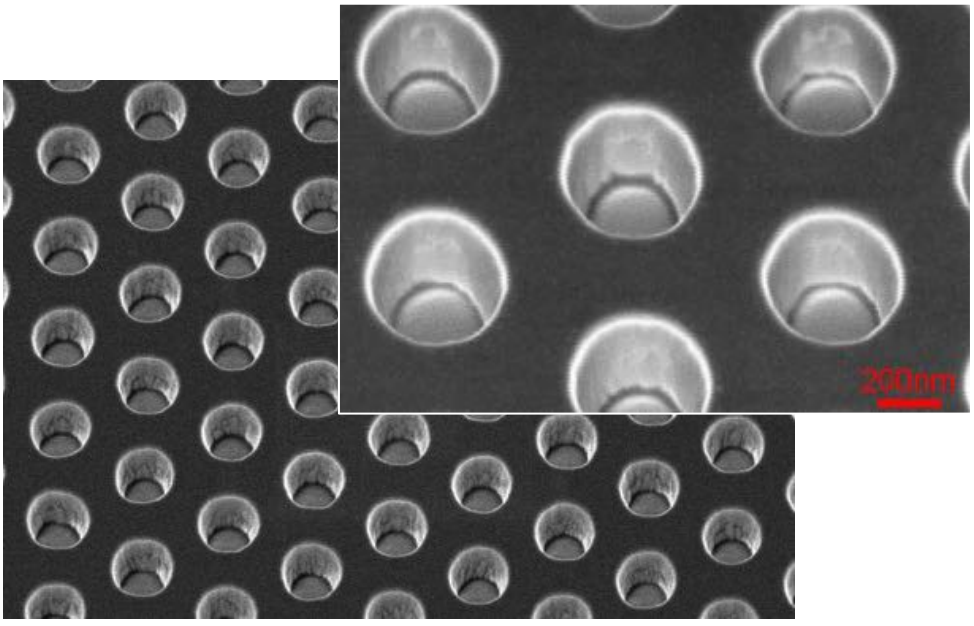
Glass plays a major role in the successful manufacture of pressure-driven droplet microfluidic components since it provides excellent surface properties, *e.g.* low surface roughness, chemical inertness and high accuracy of manufacturing tolerances to ensure reproducible droplet volume. Additionally, because of the well-known surface chemistry, it is relatively easy to pattern hydrophobic regions on hydrophilic glass to create uniform droplet volumes and aid in droplet sorting.

Digital microfluidics uses an array of electrodes and electrowetting to create and manipulate droplets [29]. Electrowetting is the property of an applied voltage to modify the wettability of a surface. When an aqueous solution hits a hydrophobic electrode, surface forces cause the solution to bead up, forming a droplet. Applying an electric field between the droplet and the electrode allows the droplet to spread out. By creating an electrode pattern, it is possible to control droplet size and movement. For this effect, glass is the best material, as the high-density ITO electrodes required are readily patterned on glass.

Fabricating for Next Generation Sequencing

Next Generation Sequencing, so-called "NGS", is the term coined for the massively parallel sequencing resulting in gigabytes of sequence data per day to quickly identify gene sequences. NGS is being harnessed for everything from personalized medicine to rapidly identifying microbial food contaminants [30].

Many technologies use optical methods – and in this use case, glass is offers the best optical properties. NGS sequencers such as those by Illumina and Pacific Biosystems continue to use glass. Pacific Biosystems is pushing the limits of detection for its new single molecule sequencing product, so background fluorescence and scattering are particularly damaging to performance. Emerging next gen sequencing powerhouse, LaserGen, utilizes fluorescence for detection with glass microfluidic flow cells.

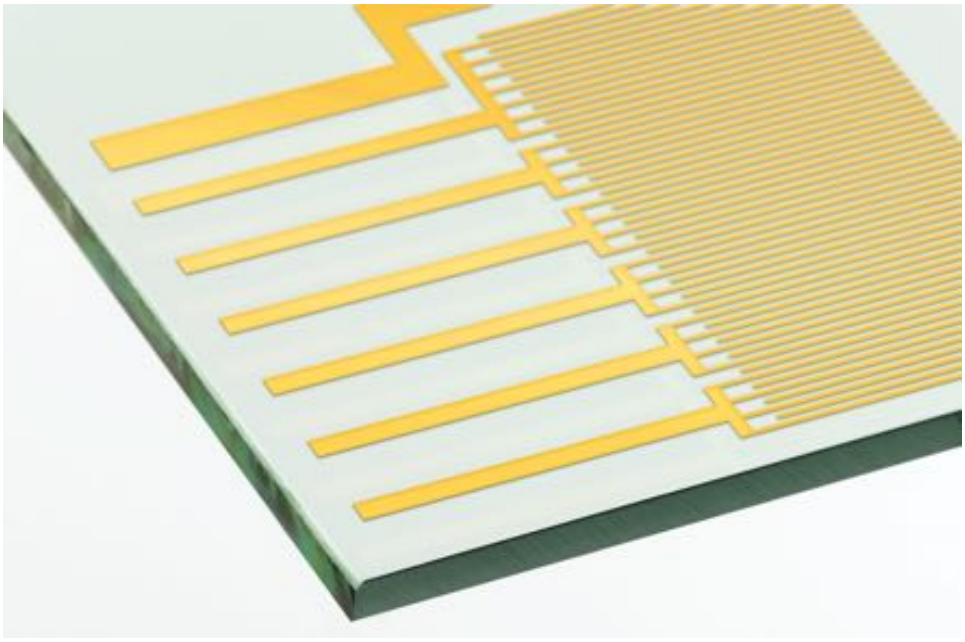


Nanowells in quartz to increase surface area and feature density. (Source: IMT)

Illumina managed to reach the $1,000 genome target by harnessing the power of glass fabrication. By structuring the glass surface, creating patterned flow cells, it was possible for Illumina to increase the density of the sequencing space and reduce image acquisition time since patterned images are easier to overlap rather than unstructured images [31]. Important features to this success are the high accuracy of sub-micron patterning on a wafer level. This enables the extremely dense packaging of the sequencing space within the microfluidic chip, reaching the optical resolution limit of conventional fluorescent imaging systems. Details of the patterned flow cells at Illumina, Inc. can be found on the Illumina website [32].

As the $1000 genome gives way to the $100 genome, the desire to minimize costs by reducing the volumes of expensive reagents drives the LOD requirement further down. This increases the need for the low auto-fluorescence of the microfluidic flow cell material. Illumina's approach relies also on massively parallel analysis, which requires dense feature patterning on glass chips. QIAGEN's hydrodynamic approach and Bio-Rad's digital droplet microfluidics design can allow for other materials for the droplet generation that is part of the secret to their sequencing speed [30].

Another cost-reducing approach is to eliminate the label altogether. Companies like Oxford NanoPore and Genia-Roche use a label-free pore-based approach that requires the integration of high aspect ratio nanopores with a direct electronic detection method for sequencing, driving the use of silicon as the microfluidic chip material rather than glass [30]. These new methods relying on CMOS chips for direct electronic detection have also been embraced by Ion Torrent-Thermo Fisher Scientific.



Impedance electrodes on glass. (Source: IMT)

While label-free assays may seem to eliminate the need for the optical properties of glass, glass still offers some advantages. For silicon and glass, the submicron control of the channel etch depth that is possible can result in improved signal-to-noise for impedance detection. The ability to readily incorporate ITO electrodes allows for the integration of digital microfluidic sample preparation, helping to reduce precious sample and expensive reagent consumption.

Fabricating for Protein-Protein interactions

There are a variety of receptor-mediated assays that can be automated with microfluidics, but by far and away the most common is the immunoassay. In research, soft-lithography with PDMS is the most popular fabrication method and material for microfluidic immunoassays with cost being the primary driver. A few on-chip immunoassays are made in glass as its low to no auto-fluorescence and strength under pressure has advantages for nanofluidic approaches [33], and the ready ability to pattern ITO electrodes on its surface makes glass one of the best materials for digital microfluidics approaches for IVD immunoassays [34]. However, commercial microfluidic platforms rely primarily on polymers, with a combination of

lower cost per chip and material familiarity (i.e. similar to the microtiter plate) motivating polymers' wide adoption for immunoassay applications [11].

Fabricating for Organ-on-a-Chip applications

With the goal of accelerating drug discovery by providing responses that mimic *in vivo* responses, organ-on-a-chip applications rely more heavily on hybrid materials that can include a planar support for a three-dimensional scaffolding of an entirely different composition [35]. Factors such as the fabrication complexity created by hybrid material insertion, in situ synthesis, or layering with the impact on cell viability based on gas permeability and protecting cells from inorganic and organic poisons are factors. A wide selection of materials, including silicon, glass, PDMS and polymers, have been combined with hydrogels, biopolymers and additive manufactured scaffolding using proteins and the organ cells themselves.

Additive Manufacturing

3D printed Microfluidics for Biological Applications

There are a variety of 3D printing techniques for biological applications, with particular promise in the area of organs-on-a-chip. Printing methodologies include stereolithographic, digital micromirror device-based projection printing two-photon polymerization, FDM, Inkjet and bio printing. Each has its own material of choice, with most being photocurable resins or polymers. FDM uses thermoplastics such as those most often used for microtiter plates: ABS, polycarbonate, as well as polyphenylsulfone, and elastomers. Bioprinting has demonstrated success with hydrogels, viscous materials, photocurable resins, and cells and the biopolymers themselves. Bioprinting is perhaps the most promising printing approach with visions of building organs and scaffolding directly [36].

3D Printing of Glass

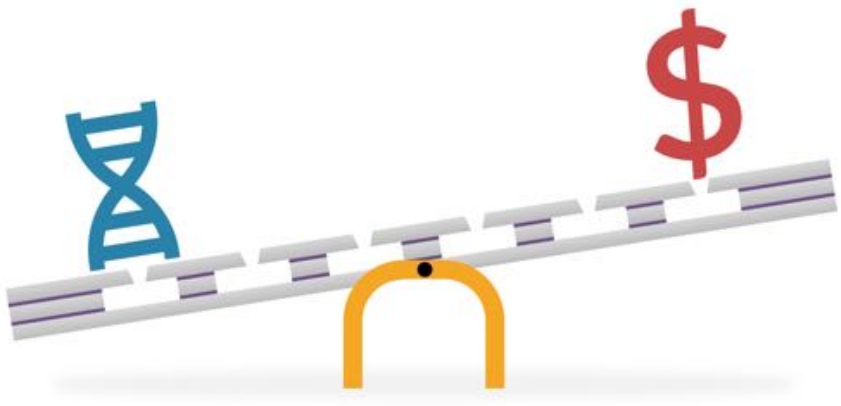
Those interested in creating three-dimensional optical quality materials, however, have proven that something as hard as glass can be printed. Recent demonstrations of transparent fused silica [37] and glass point to a clear future for glass additive printing [38], with interesting possibilities for integrated optics in the future.

Summary

The emergence of new biological assays such as those containing cells and the novel implementation of biopolymers increases the likelihood of chip material interfering with the biological activity or desired effect of the IVD. The viability and functionality of biological material, from the single cell level to the bioreactor and organ level, places new burdens on device materials and fabrication requirements. Additionally, the reduced sample size increases impact of losses from surface interactions.

OVERCOMING OBSTACLES FOR IVD DEVICES

Choosing the right material for a microfluidic flow cell for IVD devices can be the first step in guaranteeing IVD performance within budget and schedule.



The balancing act of IVD performance and cost depends on choosing the best materials with ultimate unit cost in mind.

Each material has its strengths. Silicon is most useful for the arbitrary aspect ratios that can be etched using anisotropic chemical etch or reactive ion etch. However, with the advent of advanced laser microfabrication, glass is becoming more and more feasible.

Both glass and silicon are easy to modify with additive methods such as standard processes from the MEMS and the optical industry e.g. chemical vapor deposition in order to add other materials, metals and dielectric coatings, for additional functionality such as electrodes or waveguides. The patterning of Indium Tin Oxide (ITO), the best material for electrowetting and spectroelectrochemical applications for digital microfluidics, on glass is robust and well understood [39].

Silicon oxide layers can be created on silicon, introducing regions that have some glass like properties. The ability to create three dimensional structures, for example to create a scaffold for cells, is attribute of silicon that makes it a strong candidate for organ-on-a-chip applications. However, soft lithography materials such as PDMS, and 3D printed materials such as hydrogels, biopolymers, and cells, are even more attractive because of their biocompatibility and likelihood of maintaining cell viability.

Hybrid materials – glass on silicon, silicon on glass, and plastic on glass, can also be constructed to enhance surface properties that are more favorable to the health and functionality of the bioassay and its biological components. Post-fabrication modification using silanization chemistry to create hydrophilic, hydrophobic, or chemically reactive surfaces is best understood for glass.

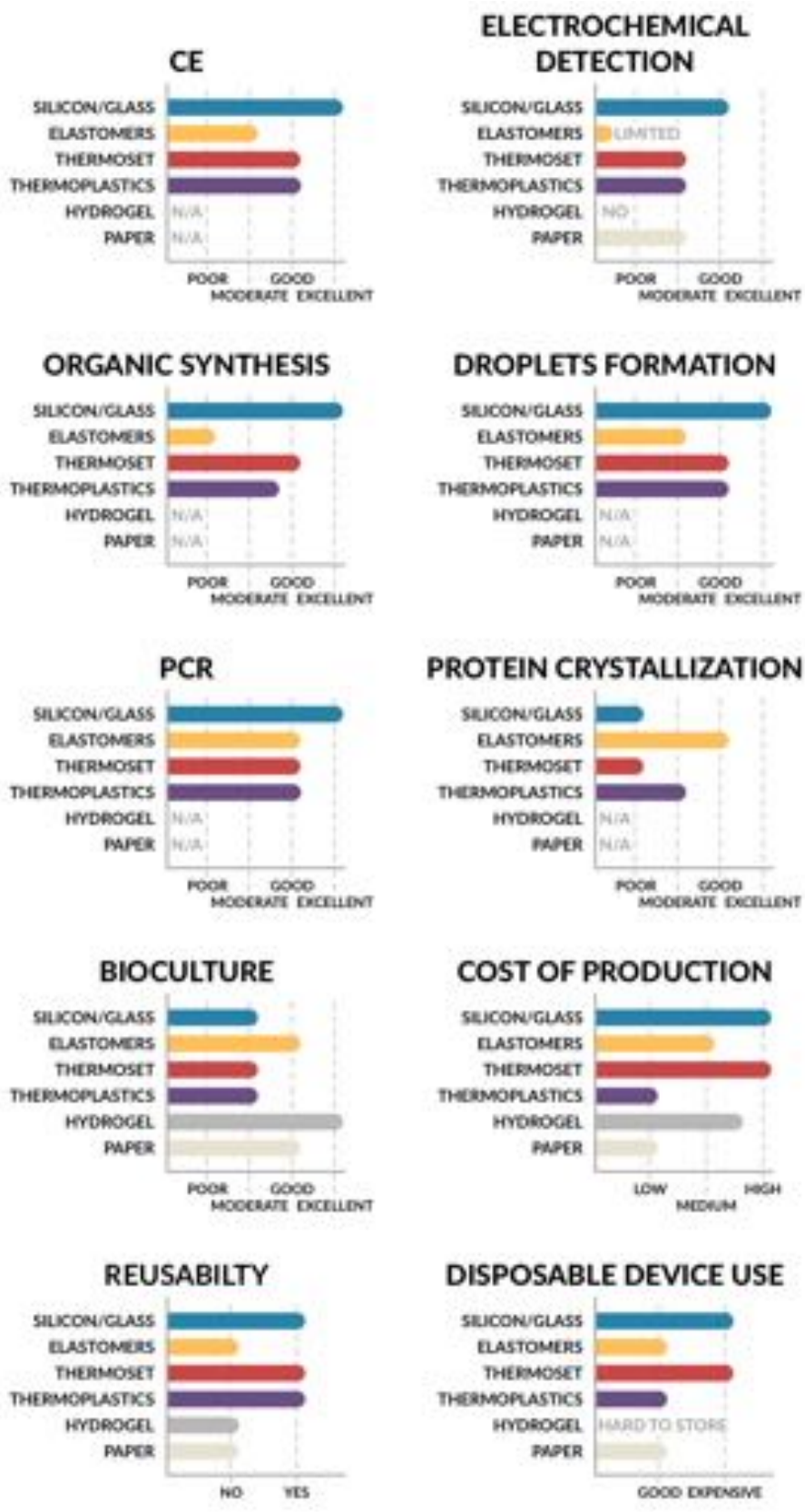
Nonetheless, polymers have their sweet spots. Many applications, such as immunoassay, have well understood methodologies for dealing with polymer surfaces, and the cost for millions of disposables is hard to beat using glass or silicon. Additionally, the cyclic polyolefins can give a “good enough” optical transparency and auto-fluorescence for many applications. The cost of consumable reagents is also driving IVD developers to explore label-free approaches, reducing the need for optical transparence and low auto-fluorescence in those cases.

For applications such as PCR, the physical properties of glass (thermal expansion coefficient and low auto-fluorescence) are hard to beat, though silicon-glass devices and other hybrid devices, are very commonly employed. Where electrokinetic forces are required, glass is often the best choice due to its high impedance and the ability to control its surface charge.

Addressing Biological and Engineering Complexity

The wealth of data on surface modifications in glass, and the current capabilities of glass surface modification with a variety of materials, coupled to its mechanical, thermal and optical properties, makes glass a material equal to the biological complexity and engineering complexity of many IVD applications. Because of the ability to bond glass using thermally and UV-A-cured adhesives, it is possible to perform room-temperature bonding processes, allowing for bio-molecule encapsulation prior to bonding. Automation of UV-adhesive bond equipment simplifies and reduces costs for manufacture. Finally, glass can be sterilized by a variety of methods, a critical part of IVD manufacturing.

Silane chemistry, room temperature bonding techniques, and coating methods also offer methods for patterning and processing glass for cell viability at the prototyping and manufacturing scale. The ability to incorporate optical components such as ITO and multidimensional features in addition to controlling surface properties offers some advantages in using glass for droplet microfluidics IVD applications.

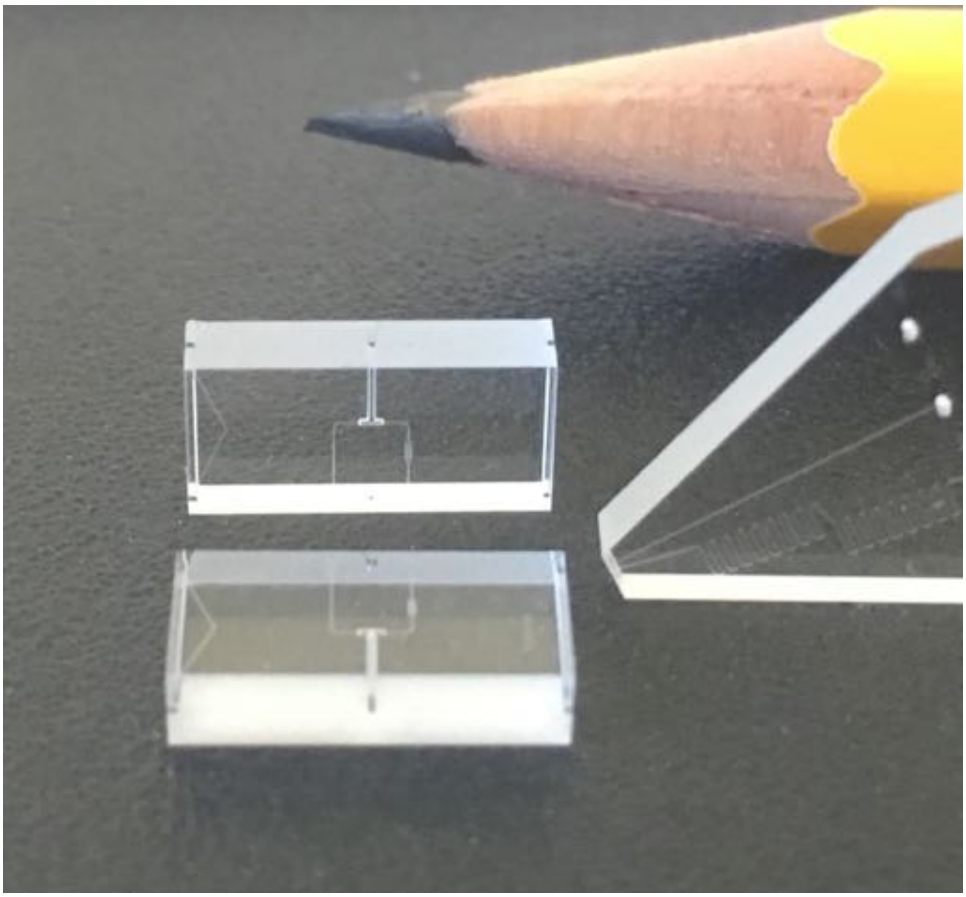


Application-based selection guide for IVD materials. [10]

Economies of Scale

When examining a material, one should also consider the production scale options for fabrication that will ultimately influence design, cost, and quality.

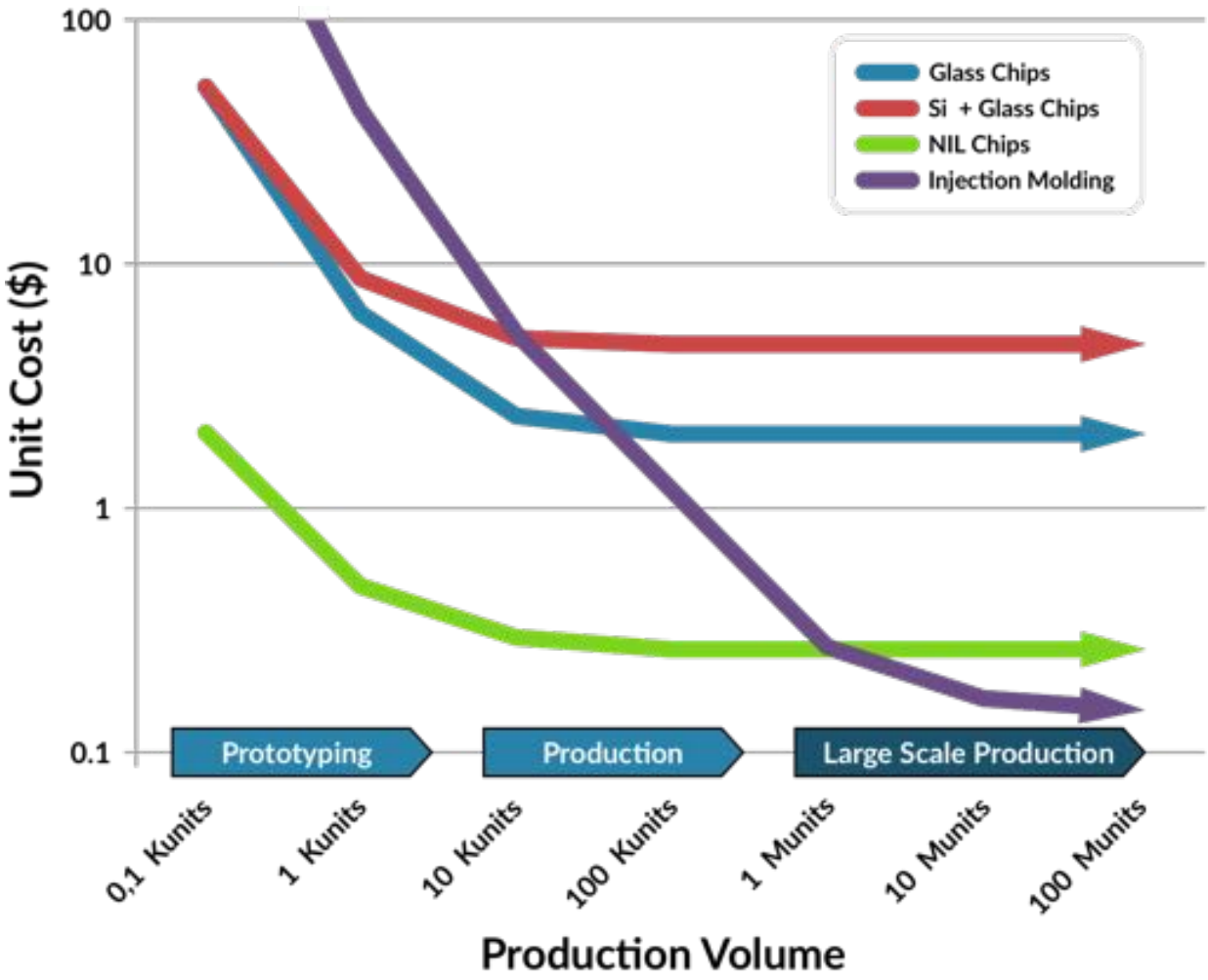
For glass and silicon, one should consider the wafer size manufacturing capabilities of most foundries. Design should be optimized for the use of wafer real estate AND the functionality of the microfluidic flow cell itself in the IVD. Additionally, one must consider factors such as the types of negative manufacturing steps (removing material) and additive manufacturing steps (depositing material), the adhesion layer requirements for the underlying material, and the final removal of each component from the wafer for individual packaging.



Laser cut microfluidics component with side connections. (Source: IMT)

Are you making one device, one thousand, or one million? At the proof-of-concept or prototyping stage, injection molding and other polymer manufacturing methods is too expensive, because you need to make the $10,000 - $100,000+ mold first. PDMS or glass may be your best bet. At the production scale, glass is also a strong choice. Move to the 1 million mark, if you don't need glass for its other properties, injection molded polymers become your material of choice [40], [41].

Production volume can determine which material reduces unit cost
[Source: Yole Développement: Microfluidic Applications – 2015]



The Impact of Material Choice on Manufacturing Processes

Deciding what material to use is driven mainly by the application. However, it is wise to consider the manufacturing process it will drive. When choosing between glass and a polymer, one is also often choosing between glass wafer processing and injection molding. Table 2 below summarizes the differences between each process.

Table 2. Comparison of glass and injection molding process on device properties.

PROPERTY	GLASS	INJECTION MOLDING
Mechanical Stability	Strong	Weak
Sterilization Possible	Easy	Difficult
Outgassing	None	Yes
Reproducibility	High, MEMS accuracy	High, but eventual erosion of the injection molding matrices
Flatness	Excellent	Injection molded parts are always warped
Water Absorption	None	Degree is dependent on material, but always a factor
Stability of Production Process	High, MEMS accuracy	Dependent on injections machine parameters (modern machines have 1,000 configurable parameters)
Influence of Environmental Conditions (at use, at storage)	Negligible provided good packaging	High (temperature, humidity)
Specification of Material	Well documented and proven in use	Only medical grade granulate has exact specifications, variability from batch to batch is possible
Scratch-Resistance	High	Low
UV-Stability	High	Low

Microfluidics Inside

One important perspective to keep in mind – the IVD product will not be the IVD device. It is unlikely a person or machine will ever see the microfluidic chip inside. Most IVDs are a product, and what is seen is the package, not the device. Thus, there is a final element of to consider – the interface between the microfluidic device, which is a component, and its packaging. The interface between device and its package is decided on a company-by-company and product-by-product basis. Here pick-and-place technologies and other integration processes can facilitate scalable production of complete flow cells, including packaging and sterilization.

N.B. The microstructures are inside, the packaging is what the world sees.
Genechip Image **by: Ricardipus**

IMT, along with other companies engaged in manufacturing microfluidic devices and products from across the supply chain are trying to facilitate the microfluidics market growth by addressing standardization issues. The aim of this standardization process is to hasten the adoption of microfluidics solutions by simplifying the integration of microfluidic components and systems and pushing towards lower costs, shorter time-to-market, and reusability in multiple applications. Our approach is to define industry-wide supported guidelines and standards that will enable reliable microfluidic interconnections and affordable integration. Adoption of these guidelines is supported by the development of application-specific verification tests to guarantee usability.

The discussion, which started a few years ago about the microfluidic chip connections, has now expanded towards microfluidic building block dimensions, along with verification testing. The goal is to define objective testing standards for quality control and instrument/component comparisons. As a first step, an ISO IWA was set in place and a first standardization meeting was held at the facilities of NIST [42], [43], [44].

CONCLUSIONS

IVD developers are constantly balancing the requirements of biology with those of engineering the device. When trying to automate, miniaturize, and manufacture an assay either for biological targets and/or with biological components, the assay performance cannot be degraded by the automation or manufacturing approach. While microfluidics solves many problems of automation, the most basic step, choosing the material(s) of the microfluidics flow cell employed, can make or break the IVD, especially when it comes time for production scale manufacture. Understanding the trade-offs of different materials at each stage of IVD development, and its impact on design and manufacture, helps keep these requirements in balance, and the ultimate application successful.

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About IMT

Our Expertise. The capabilities of sub-micron structuring within IMT is well established and documented from our classical business, i.e. optical components. This technology is based on internal IMT-developed processes, *i.e.* not standard stepper photolithography, and we have demonstrated high quality, flexible production of such structures on an industrial scale (not compared to the levels of a MEMS foundry), but with the advantage of utilizing non-MEMS materials, such as glass, gold, and silanes.

With this expertise, we offer an alternative path for our customers. The typical path companies choose to take, due to lack of a one-stop-shopping solution in the market, is to define and utilize a rather intricate and complex in-sourcing production of flow-cell sub-components and doing the functionalization and sealing of the flow cells in house. The latter is chosen mainly out of two reasons:

- The companies prefer not to give the specific binding chemistry to vendors in fear of losing competitive advantage
- No vendors exist with the required capabilities

IMT AG's glass wafer processing capabilities allow an automated production-scale manufacture of complex, multilayer microfluidic flow cells for life sciences applications including protein, DNA and cell handling and analysis to deliver cost-effective precision glass consumables.

IMT AG offers a unique advantage for companies developing life science products, because we offer a complete in-house production workflow at IMT AG. This liberates IVD companies from the cost and complexity of developing their production internally.

IMT AG's Vision. We provide complete flow cells which are customizable to our customer-specified dimensions and layout. We manufacture the specified bio-functionalized pattern of micrometer or sub-micrometer feature sizes in sealed flow cells. The combination of our applied anti-fouling coatings with intelligent patterning of functional structures in the flow channels result in bioactive microfluidic flow cells customized for each application. The final assembly of functionalized wafers uses UV-adhesive room-temperature bonding to retain bioactivity.

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