

# Developing Diagnostic Products Using Polymer Laminate Technology

*Polymer laminate technology can open opportunities for creation of molecular diagnostic devices.*

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In the development of newer molecular and immunodiagnostic products, panels of tests are launched on a single platform. To accomplish this, complex fluid circuits that perform sample preparation and sample distribution interface with other functional elements. When a device incorporates microfabricated components that are injection molded or embossed, changes in the design to optimize performance are carefully considered because of the expense in retooling. To avoid these costs, modifications are made reluctantly, and other work-arounds may be proposed. While such fixes lower the short-term development costs, the end result may be a merely adequate, not superior, product.

Polymer laminate fabrication offers an alternative that permits iterative and empirical testing without the need for tooling. The result is a superior product that is optimized and well characterized.

The strength of polymer laminate technology is in applications for single-use devices where the fluidic component needs to perform multiple tasks. The device takes the complexity of the test out of human hands and puts it into a small fluidic card, hence the term Lab-on-a-Chip.

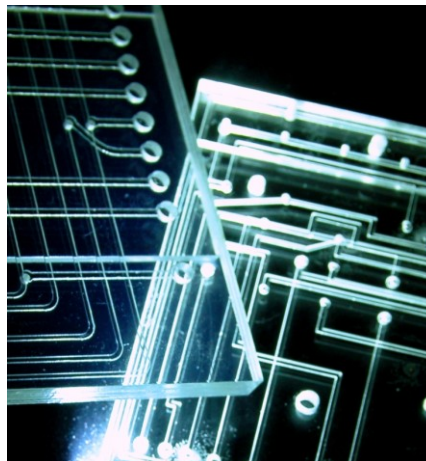
## What Is Polymer Laminate Technology?

Polymer laminate technology is a microfabrication method for rapid prototyping and manufacture of microfluidic devices. The fabrication process for polymer laminate devices involves thin sheets or films of commercially available polymeric materials that are laser cut, then stacked to form fluidic channels and vias. The layers are bonded (laminated) together using pressure-sensitive adhesive, thermal, or diffusion bonding. Channels and other functional structures are precision laser cut in each of the layers including the bonding layers.

## Addressing Manufacturability

Microfluidic devices find use in any application where nano- to microliter volumes of samples or precious reagents are involved, in preference to traditional laboratory disposables

that require tens to hundreds of microliters of fluid. In addition to applications for immuno or molecular diagnostics, there are applications in spectroscopy, environmental analysis, sample preparation, separations, and the automation of biological assays and cell culturing. Microfabrication technologies used in research and development, such as micro injection molding, embossing, and etching in silicon or glass, are expensive to use for commercial product development and risky to implement in molecular and immunodiagnostic product manufacture for a couple of reasons.



*Figure 1. Polymer laminates made using thicker acrylic material perform well as manifolds. In the examples shown here, a five or seven layer laminate was made using batch fabrication. Two different layers of interconnected channels are incorporated into each design the channels are 1 mm wide. The seventh layer in these examples have a step feature. These manifolds were made to interface to a pneumatic ally driven manifold using gaskets.*

Fabrication in glass or embossing in plastic is generally not compatible with high-volume manufacture of a single-use product for the diagnostics market. Micro injection molded components are economical at high volumes (tens of thousands to millions of devices per year, depending on cost per test) because they require a significant investment in tooling. Products

manufactured with such mass-production technologies would be expected to address large markets. Products that address smaller, niche markets justify neither large development nor tooling costs nor the time delays associated with such activities. Interface to the instrument for fluid control and data collection is also not well developed with newer microfabrication technologies except in the case of some larger research platforms that do not address the diagnostics market, and packaging for interconnection between the micro platform of the device and macro world of the controlling instrumentation have not been standardized for commercial products.

Polymer laminate technology offers flexibility in style of interconnection, while lowering development costs because it does not require tooling, facilitating iterative, empirical testing of fluid components. With care in the development of a robust and repeatable fabrication process, polymer laminates are readily scalable to support development, clinical trials, through to product launch and volume manufacture. The equipment and processes in lamination are ubiquitous and well established, ensuring a path to volume manufacture without specialized manufacturing equipment. For microfluidic device fabrication, tools and processes familiar to other industries such as printed circuit board manufacture and lamination are used in a new way, with proprietary processes developed to address the unique aspects of microfluidics. For scale-up to volume manufacture, the development costs are reduced and risk is lowered because the path to volume manufacture is well understood. As diagnostic tests shift from single, highly complex tests (that

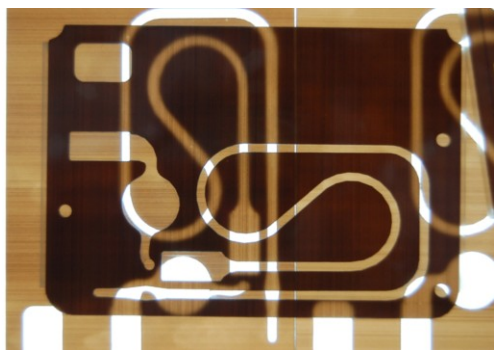


Figure 2. Five layer laminate component for Osmetech's eSensor XT-8, molecular diagnostic test for susceptibility to Warfarin that was recently FDA approved. The laminate functions to bring together both an injection molded component for sample introduction, and array of electroactive sites. The laminate itself functions as a pneumatically-driven recirculating pump.

are performed in traditional diagnostic labs and take two to three days to render results) to near-patient panels that provide results in mere minutes to hours, the need for complex microfluidic components increases. These near-patient tests are designed to be as user friendly as possible, requiring simple addition of sample while the device does the

rest of the work. With remote healthcare on the horizon, such simplicity will be required for personalized medicine or to assist in patient compliance to therapeutic regimens.

### Flexibility in Material and Structures

Polymer laminate fabrication permits the incorporation of complex functionality, such as flexible pneumatic valves, recirculating pumps, and filtration components, while offering a simple interface to optical or electroactive components. A wide choice of materials with essential performance characteristics is available (e.g., polymethylmethacrylate (acrylic; cast or extruded), polyethylene ester terphthalate, polystyrene, polypropylene, fluoropolymers, polyimides, and silicone sheets that are available in medical grade. These materials are all inexpensive commodities, ensuring volume supply of the critical material. Because polymer laminate fabrication processes are run at room temperature, thermally sensitive reagents are readily incorporated within the finished device. A common misconception regarding polymer laminates is that the walls of integrated channels cannot be made smooth or that the adhesive extends into the channel, causing reagents to "hang up" (adsorb) during the course of an assay. However, the high quality of the bonding adhesives provides edges as smooth as the edges of the substrate materials that are cut, with no running or bulging of the adhesive into the channel. With channels wider than 100 micrometers, the edge effects do not have significant effects on device performance. Experience has shown that cell culture, immunoassays, and PCR, as well as washing with 70% alcohol, are all compatible with laminate devices fabricated using select adhesives. In applications where the devices have been used with multiple wash steps, the clearance of the channels was within the expected five volumes calculated from fluid modeling. In single-use applications, carry-over or hold-up volume is easily compensated by providing a small over-volume. Non-specific binding can be a concern with some assays and certain plastics; the choice of buffer, additives, and/or surface modification (e.g., "blocking" exposed surface with BSA) can mitigate the effects of large surface-area-to-volume ratios. Reduced enzyme activity for immunoassays or for PCR can also be compensated by choice of additives to the buffer system.

### Precision Registration of Fine Features

Repeatable performance in microfabricated devices requires fixtures that ensure repeatable alignment of the layers that are stacked and bonded to create the 3-D fluidic network. The alignment tolerances are 50–125  $\mu\text{m}$  with feature sizes in the device between 100–1000  $\mu\text{m}$ . Alignment is achieved using fixtures with alignment pins that mate with features in the device. Stacks of 13 layers have been made with alignment tolerances of 50  $\mu\text{m}$  throughout the stack [reference?]. Typically, alignment requirements can be relaxed by oversizing features at the point where they connect to another layer.

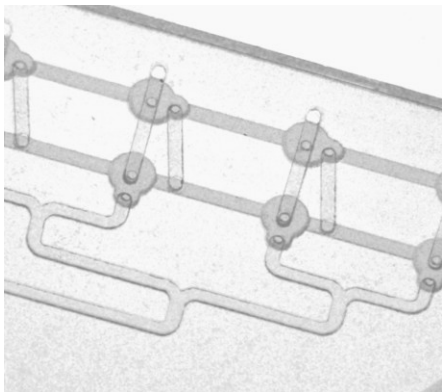
### Reduced Device Footprint and Cost

A unique feature of polymer laminates is the ability to form enclosed three-dimensional structures, such as channels or chambers, and stack them in the vertical dimension. In addition,

the density of features possible with polymer laminates, with edge distances between features as small as 0.5 mm, is much greater than with traditional injection molded channels. Compact devices in small-footprint instruments are compatible with the workflow in a hospital or near-patient setting. A panel can be run on a single small chip rather than having separate chips for each test. The ability to stack channels and functions in the vertical dimension facilitates the integration of all the required steps. To accomplish the same functionality with traditional injection molding would limit the device to three layers, in which functionality can reside on one or both sides of the device. With polymer laminate fabrication, the channels and vias that connect them can be stacked up to 5 layers or more, enabling a compact, low-dead-volume system.

### Rapid Prototyping

Polymer laminate technology is suitable for rapid prototyping and permits empirical testing. It facilitates the implementation of experimental design to understand the critical features that affect device performance. Changes in channel dimension or geometry are simple, or a range of geometries tested in a single run of prototypes. Thus, a range of modifications to the design can be evaluated in parallel. In addition, a single fabrication run can incorporate a variety of different modifications to avoid the need for multiple prototyping runs to test different designs. The fabrication process itself is not a function of the material composition. Hence materials, geometries, and different surfaces are readily explored without changing the process.



*Figure 3. Branched channel structure showing on-board, keyhole-shaped pneumatic valves. Fluidic device used for multiplexed analysis and integrates with an injection molded structure containing the reservoirs.*

### Customization of Diagnostic Platforms

Once a diagnostic platform is developed, it may become possible to apply that platform to a number of different diagnostic tests, almost invariably with some minor modifications to the fluidic design being required. Polymer laminate technology makes this customization possible without a large investment in new tools and fixtures.

### Robust Adhesion

There is a common misconception about the quality and performance of the adhesives used in this platform technology. Device manufacturers sometimes fear that the adhesives between polymer substrates will lack structural integrity and interfere with the device's performance. However, the adhesives used in polymer laminate manufacturing have been shown to be strong and biocompatible; they are already widely used in the medical diagnostics industry. They can be optically clear and provide a permanent bond to the substrate with proper surface treatment and curing. In some cases, the bond can be so strong that the material that brings the two pieces together breaks apart before the adhesive bond fails. One alternative to pressure-sensitive adhesives is the use of a 25  $\mu\text{m}$  polypropylene thermal bond adhesive (melt temperature of 60°C), which bonds materials through Van der Waals interactions with the substrate surface as the polypropylene wets the surface, or direct bonding through surface activation with plasma or ozone followed by pressure and heat, well below the melt temperature of the plastic.

### Interfacing with Traditional Components

Polymer laminates can also play an important role in facilitating the interconnections between other fluidic components, such as injection molded pieces for reservoirs or for sample addition, or detection components such as electrochemical arrays or optical windows such as microscope slides. The outermost layers of the laminate can be constructed with pressure-sensitive adhesives, while the internal fluid channels and through holes have very little or no exposure to adhesive. This allows simple presses and fixtures to be used for assembly of a final functional unit.

Laminates also serve as a 'functional double-sided tape' to mate with injection-molded components or with electroactive or optical components or glass slides. The laminate can incorporate the fluidic circuit, which serves as a platform for the complex steps of mixing, metering and washing, while cost-effectively bringing together the critical interface to the user and the detection platform. Thus, polymer laminate technology is important in facilitating the commercialization of microfluidic technologies.

### Scalability

Further efficiencies are achieved from the scalability of the fabrication process. Because the process is linearly scalable through duplication of processes and equipment at a reasonable cost, increasing manufacture from 1000 to 100,000 per month is achievable in concert with volumes needed to address the market. Higher volume manufacture can be addressed through die cutting of some layers where designs permit. While polymer laminate technology is robust, proven, and inexpensive, it is not a panacea. It is not cost-effective for fabricating millions of multilayer laminates of seven or more layers. Designs suitable for volume manufacture are typically no more than five layers; they can include devices with sets of vertically stacked channels.

## Rapid Turnaround

The higher feature density of multiplexed diagnostic devices requires more expensive tooling and long turnaround times— as much as six weeks. With polymer laminates, development time is drastically reduced, with initial prototypes typically available within three to seven days.

## Applications of Polymer Laminate Technology

**Example 1: Electrochemical Detection-Based DNA Microarrays.** Osmetech Molecular Diagnostics has obtained FDA clearance for a DNA microarray device which incorporates polymer laminate technology. The eSensor XT-8 is a DNA microarray device for warfarin sensitivity testing. Warfarin, one of the most commonly prescribed anticoagulants in the United States, exhibits a narrow therapeutic range, a wide inter-individual variation in dosage required to reach optimal therapeutic effect, and severe adverse effects from overdosage due to excessive bleeding. The eSensor XT-8 cartridge device establishes an individual's genotype. It consists of a printed circuit board chip, a cover, a plate, and a microfluidic component composed of a laser-cut multilayer laminate. The microfluidic component includes a diaphragm pump and check valves in line with a serpentine channel that forms the hybridization chamber above the array of electrodes. The PCB chip is prepared for an eSensor assay by depositing DNA capture probes and insulator molecules on the working electrodes. Each specific deposition solution is dispensed on the appropriate electrode using a robotic pipette system. The capture probe and insulator react with the gold surface to form an insulating self-assembled monolayer. After capture-probe dispensing, the PCB chips are washed, dried and assembled with the laminate, plate, and plastic cover into a cartridge to form a microfluidic circulating system that can hold approximately 140  $\mu\text{l}$ .

Osmetech is one of the few companies to provide electrochemically based detection technology for nucleic acid analysis. Conventional microarray devices are expensive and rely on fluorescent detection-based technologies that require bulky optical detectors. Polymer laminate is low in cost and also has a small footprint, which conserves bench space in a typical clinical laboratory. According to the company, previous studies demonstrated the feasibility of a single-use, sample-to-answer eSensor device, and recent developments in microfluidics provide additional tools to perform the required functions. Point-of-care microarray systems using electrochemical detection of nucleic acids can meet critical healthcare needs, including rapid genotyping. 1

**Example 2: Particle Sorter** CFD Research Corp. (CFDRC) conducts high-end R&D for government agencies and other organizations. The company uses computational tools for component and system design, turns them over to be manufactured using polymer laminates and other fabrication technologies, and then performs testing with the completed devices. One of CFDRC's products that incorporates polymer laminates is a dielectrophoresis (DEP) -based particle sorter,

designed to allow intelligent discrimination of target particles from background clutter. The sorter can be used to effect separation based on size (respirability) or particle type (dielectric properties). It can operate in batch, stopped-flow, or continuous-flow modes. Polymer laminate technology is uniquely suited for the DEP sorter because of the two-sided nature of the device, which has a metal layer on the bottom, followed by a polymer laminate layer that contains the fluidic channels, topped with another metal layer, all bonded together with pressure-sensitive adhesives. The device sorts heterogeneous particle populations, such as bacteria mixed with pollen for the purpose of extracting the bacteria. The pollen are separated out on one side under the influence of an electric field, while the bacteria end up on the other side. Microsystems such as these have very small channels that call for microfabrication. The channel in which the sample is introduced is about 100 by 500  $\mu\text{m}$ , which is difficult to manufacture using traditional machining methods. An alternative approach is traditional lithography, but two-sided lithography is not very well established. Substrates such as glass or silicon could be used, but plastics are often more cost-effective for technology demonstration. Another advantage of laminate-based technology is the ability to stack multiple layers and attach them using a pressure-sensitive adhesive. For a silicon or glass substrate, it would be necessary to use anodic or fusion bonding with either high temperature or high voltage or both to attach the top and bottom layers. The problem with these options is that both bonding processes are cumbersome (especially glass-glass bonding) and they are permanent. The adhesive that bonds laminate layers, on the other hand, can be dissolved so that the layers can be reused. Instead of manufacturing 100 devices, you can make 20 and reuse them five times. The reusability combined with lower cost makes plastics more attractive than either silicon or glass at the proof-of-concept stage. CFDRC's next step is to develop an integrated sample preparation cartridge for processing complex liquid samples to test for and identify pathogens. It will combine multiple components on a single chip that would interface with off-chip controllers to carry out multiple steps in an automated fashion. The components are modular by design and may be combined to yield an integrated sample prep unit. For example, the particle sorter may be combined with a device that uses an electric field to extract the intracellular content from the bacteria and have it analyzed by a sensing element. The resulting product would be a handheld sensor for biological analysis.

**Example 3: Rapid Prototyping for Fluidic Device** Blood Cell Storage Inc. has submitted a new fluidic device for patent review and is in the process of prototyping using polymer laminate technology. Once the prototypes have been proven and the design finalized, the product will scale up to high-volume production. The company plans to outsource this phase. Because polymer laminate technology is flexible, the turnaround on the prototypes is very fast. The design can be altered and a new prototype batch of 25 parts delivered within two or three days. Laminated construction is particularly suited to the design because it provides the ability to combine layers of dissimilar materials, such as plastic and glass, which is critical to the device. The company can submit various designs and test them much more quickly

than using a most other fabrication techniques. Polymer laminate technology also provides very consistent results. The firm can place an order for 100 parts with delivery in less than two weeks. In the past Blood Cell Storage Inc. built prototypes in house, but lacked the capability to achieve the high-quality bond between the layers. Outsourcing the construction of prototypes saves the firm time and is cost effective because the source is geared up to laminate on a larger scale and therefore can provide an ample supply of matching parts quickly.

**Example 4: Fluidic Card in Nanosatellite for Biological Experiments in Space.** NASA's Gene-Sat 1 Technology Demonstration Mission is a fully automated, miniature space flight system that provides life support for small living things and conducts biological examinations to look for genetic changes in bacteria during spaceflight. Putting novel cellular genetics investigative tools into small spacecraft gives scientists the ability to study and understand the effects of space environments on living things. The Gene-Sat 1 experimental payload was designed to perform assays of biological specimens in an automated fashion. One of its subsystems is a fluidic card that features one inlet fluidic channel and one outlet fluidic channel that feeds 10 sample wells at the same time. The device was constructed using layers of close-tolerance cast acrylic bonded with an acrylic-based pressure sensitive adhesive. The inlet and outlet to each well have a  $0.45\ \mu\text{m}$  porosity nylon membrane which serves two functions: to keep the suspended cell culture from moving between wells, and to provide even flow of the nutrient solution across all 10 cell culture chambers by having

equal membrane area at the inlet and outlet of each culture chamber. The top-most layer of the device consists of a gas-permeable membrane, also bonded using a pressure-sensitive adhesive. The technology demonstration satellite was launched in December 2006 containing 10 experimental



Figure 4. Top view of the cell culture card that flew on NASA's GeneSat experimental satellite to demonstrate autonomous cell culture in near earth orbit. The fluidic card is constructed from layers of acrylic bonded with an optically clear bottom and a gas permeable top. A single inlet distributes nutrients evenly across all ten sample chambers. The system flew in December 2006 and successfully telemetered data on cell growth to earth.

samples of *E. coli* that were genetically modified to produce a green fluorescent protein during growth. Once the satellite was in orbit, autonomous incubation of the *E. coli* cultures was initiated by an increase in temperature and the infusion of a nutrient solution to each of the membrane-isolated cell culture wells built into the laminated microfluidic cell culture card. The bacterial growth was monitored for 96 hours by miniaturized optical scattering and fluorescence detection through the optically transparent polymer walls. Each of the 10 bacterial cell cultures grew and successfully expressed the fluorescence signature. Data from the fluorescent *E. coli* were telemetered back to earth for analysis. Upon reviewing the mission science data, the researchers found it successful. The organisms grew and glowed, and the sensors all worked. The ability to incorporate the porous membranes proved to be critical to the success of the device's performance for this mission. The laminate construction proved to be robust and to meet the rigorous demands of the launch environment.

### New Products to Market

Polymer laminate technology complements, but does not replace, injection-molding. It adds value to the manufacture at volumes exceeding a million devices per year and facilitates the development of devices that might not otherwise reach the market due to tooling costs. It lowers the barrier to entry for device manufacturers with groundbreaking ideas for diagnostic tools, as well as others who want to address smaller, niche markets. By enabling a shorter development time and easing the process of doing multiple iterations inexpensively, polymer laminate technology speeds time to market and makes the microfabrication of devices with complex features affordable.

### References

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