AN ABSTRACT OF THE THESIS OF

Chuckaphun Aramphongphun for the degree of Master of Science in Industrial Engineering presented on August 31, 2001. Title: Application of Ultrasonic Welding to the Rapid Prototyping of Microfluidic Systems for Biotechnology.

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Abstract approv

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This paper introduces an alternative technique for the development of microfluidic systems for biotechnology based on mechanical machining and ultrasonic welding. Advantages of this approach over existing prototyping approaches involving the rapid development of tooling include: (a) short cycle time, (b) design flexibility, and (c) low cost manufacturing. In addition, the process provides a migration path to high volume production. A limitation of this system is that it cannot practically produce microchannels smaller than about 250 μm (0.010 in). However, for many biological cell-based biosensors, this feature scale seems well suited based on cell viability results. Several issues are discussed relevant to this approach, including bond strength, seal leakage, and sterilization.

Application of Ultrasonic Welding to the Rapid Prototyping of Microfluidic Systems for Biotechnology

by

Chuckaphun Aramphongphun

A Thesis

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented on August 31, 2001
Commencement June 2002

Master of Science thesis of Chuckaphun Aramphongphun presented on August 31, 2001

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ACKNOWLEDGMENTS

I would like to first deeply express my sincere gratitude to my major professors, Drs. Brian Paul and Dean Jensen, for their invaluable guidance, encouragement, and assistance in every aspect during this research.

I would like to thank my committee members, Drs. David Kim and Roy Rathja for their advice and help in reviewing my thesis. Appreciation is extended to Drs. Frank Chaplen and William Warnes, who allowed me to use their laboratories. I also would like to thank Dr. Rosalyn Upson, Mr. Stephen Etringer and Mr. Corwin Willard for their help and very useful advice.

This thesis would not have been complete without the help and support of my host, Dr. Clara Jarman and her family. She was always available for assistance and gave me sincere hospitality and warmth during my stay in Corvallis. In addition, I would like to thank my fellow graduate students, Kannachai Kanlayasiri and Satoko Kimpara, for their friendship and support.

Finally, I am most grateful to my family, who has provided enormous encouragement throughout the progress of this research and my studies at Oregon State University.

TABLE OF CONTENTS

	Page
INTRODUCTION	1
Polymer Microfabrication and Bonding Processes	5
Research Objectives	11
Research Questions	12
MATERIALS AND METHODS	13
Device Design	13
Fabrication of Polymeric Microfluidic Channels	14
Sterilization of Microfluidic Channels	17
Populating Cells in the Chambers	18
Cell Viability Test	19
RESULTS AND DISCUSSIONS	21
Cell Viability	21
Rapid Prototyping	27
High Volume Production	28
Optical Detection and Scratching	29
Bond Strength and Leak Resistance	30
Sterilization	32
CONCLUSIONS	34
RECOMMENDATIONS FOR FUTURE RESEARCH	36
BIBLIOGRAPHY	37

TABLE OF CONTENTS (CONTINUED)

		Page
APPENDICES		39
APPENDIX A:	ULTRASONIC WELDING PROCEDURE	40
APPENDIX B:	PROTOCOL IN PREPARING THE TISSUE CULTURE OF BETTA FISH CHOMATOPHORES	42
APPENDIX C:	SEQUENCE OF STEPS TO CAPTURE AN IMAGE AND MEASURE THE AREA OCCUPIED BY THE COLORED PIGMENT GRANULES	44
APPENDIX D:	CRI OF AUTOCLAVED AND ETHANOL STERILIZD CHANNELS IN WEEKS 4-7	45
APPENDIX E:	CRI OF AUTOCLAVED ULTRASONICALLY WELDED CHANNELS AND MULTIWELL CELL CULTURE DISHES IN WEEKS 4-7	46
APPENDIX F:	MACHINING TIME FOR WHITE PC PARTS WITH ENERGY DIRECTORS AND CHANNELS	47
APPENDIX G:	MACHINING TIME FOR FLAT TRASPARENT PC PARTS	48
APPENDIX H:	PRODUCTION TIME FOR PROTOTYPING ULTRASONICALLY WELDED CHAMBERS	49
APPENDIX I:	AVERAGE BOND STRENGTH (MPa) OF ULTRASONICALLY WELDED CHAMBERS	50
APPENDIX J:	COMPARISON TABLE OF THREE DIFFERENT ARCHITECTURES	51
APPENDIX K:	MERCURY CHAMBER	52
APPENDIX L:	MERCURY DRAWING	53
APPENDIX M:	PROCEDURE FOR FABRICATION OF MERCURY CHAMBER	56
APPENDIX N:	PRODUCTION TIME FOR PROTOTYPING MERCURY CHAMBER	58
APPENDIX O:	GEMINI CHAMBER	59
APPENDIX P:	PROCEDURE FOR FABRICATION OF GEMINI CHAMBER	60
APPENDIX Q:	PRODUCTION TIME FOR PROTOTYPING GEMINI CHAMBER	62
APPENDIX R:	AVERAGE BOND STRENGTH (MPa) OF SOLVENT-WELDED CHAMBER	63
APPENDIX S:	SOLVENT-WELDED SPECIMEN TO TEST BOND STRENGTH	64

LIST OF FIGURES

Figure		Page
1	The dimensions of energy director and welded joint before and after ultrasonic welding	11
2	Designs of microfluidic channels	15
3	Scheme describing the fabrication of enclosed microchannels in PC	16
4	The final dimensions of a microchannel chamber	17
5	Populating colored pigment cell in the channels by using a micropipette	18
6	CRI of autoclaved and ethanol sterilized ultrasonically welded chambers over time	22
7	CRI of autoclaved chambers and multiwell cell culture dishes over time	24
8	Two surface areas where scratching occurs	30
9	A pin alignment fixture with two circular slots	30
10	A bond strength testing of the chamber	31
11	Instrument set up to test the leak resistance of channel seals	32

LISTS OF TABLES

Table		Page
1	CRI of autoclaved and ethanol sterilized chambers in each week	21
2	The results of an equal variance two-sample t-test to find out whether the mean CRI of the channels sterilized by autoclaving differs from that of the channels sterilized by ethanol	23
3	CRI of ultrasonically welded chambers and multiwell cell culture dishes in each week	24
4	The results of an unequal variance two-sample t-test to test whether the mean CRI of the autoclaved ultrasonically welded chambers is less than that of the multiwell cell culture dishes	26

APPLICATION OF ULTRASONIC WELDING TO THE RAPID PROTOTYPING OF MICROFLUIDIC SYSTEMS FOR BIOTECHNOLOGY

INTRODUCTION

In recent years, microfluidic systems have become a vital technology applied in many fields such as biology, chemistry, toxicology, and pharmacology. The function of microfluidic systems is to deliver and manipulate small quantities of liquid or gas samples in microscale (10-500 μm) channels for the purpose of integrating various "on-board" laboratory functions, such as purification, reaction, separation and detection. As a result, these microfluidic systems are sometimes referred to as "lab-on-a-chip" technology with the ability to speed up many laboratory procedures due to the small quantities of fluid being manipulated. The application of microfluidic systems has been categorized into four broad areas: (i) miniaturized analytical systems or microscale total analysis systems (μTAS), (ii) biomedical devices, (iii) tools for chemistry and biochemistry, and (iv) systems for fundamental research (McDonald et al., 2000). This miniaturization offers a wide range of advantages, including reduced cost in manufacturing, decreased consumption of samples and chemicals, shorter analysis times, increased sensitivity, greater separation efficiency, disposability and portability (Duffy et al., 1998; McDonald et al., 2000).

Currently, one of the most promising applications of microfluidics is molecular biology. This field provides many useful discoveries for solving environmental and health problems. The techniques and tools of biotechnology have made it simple to detect toxins in food and diagnose specific diseases. Thus, biotechnology has played an important role in mitigating environmental problems, developing treatments for dangerous diseases (e.g. AIDS, cancers), as well as creating synthesized products such as insulin and hormones (Wright, 2001).

An interesting application of analysis tools for biotechnology is the biosensor. Biosensors are devices that use biological elements to measure analytes of interest. Biosensors promise to improve the detection of substances in a number of fields of endeavor, including toxicology, pharmacology, medical diagnostics, environmental monitoring, and scientific discovery. In toxicology, for example, biosensors could improve the testing of food and water. In pharmacology, biosensors could be used to help in discovering new drugs and in evaluating the efficacy of drug preparations. In the practice of medicine, biosensors already play a substantial role in glucose monitoring; many types of antibody tests currently used in medical diagnostics and forensics exemplify a major sub-category of biosensors.

Some key requirements of cell-based biosensors include: (a) cell viability, (b) optical detection, and (c) autoclaving compatibility. Cell viability deals with the ability of the microfluidic systems to sustain the cell. Optical detection emphasizes the fact that most cell-based biosensors detect activity in cell optically. Autoclaving compatibility emphasizes the need to efficiently sterilize the chamber.

A key technology necessary for enabling biosensors is microfluidics fabrication, which generally consists of a microlamination approach: patterning and bonding. Many microfluidic systems documented in literature (Voldman et al., 1999; Shoji and Esashi, 1993) use silicon or glass microfabrication techniques employing standard consecutive process steps: (i) photolithography to provide an etch mask in photoresist, (ii) wet etching to produce microchannels on the planar substrate, and (iii) bonding of two substrates together. The advantages of this process are high resolution (about 100 nm) and good accuracy (Whitesides and Stroock, 2001). However, as pointed out by Becker and Gartner (2000); Soper et al. (2000); and Folch et al. (1999), this approach has some disadvantages in the prototyping environment:

a) The fabrication process is comparatively long. The procedure consists of many steps: mask making, substrate cleaning, photoresist coating, X-ray or UV exposure, development, and wet etching.

b) Costs of substrate and wet chemistry in the process are relatively expensive. This factor highly affects production cost and disposability of the devices.

c) Properties of substrate material limit optical detection and channel geometry, which is important for many biosensor applications. Silicon is an opaque material; therefore, it cannot be used for detection by optical techniques. Since glass (amorphous SiO_2) is a transparent substrate, optical detection can be performed. However, due to the amorphous property of

glass, only shallow, hemispherical channel cross sections can be obtained from wet etching.

d) Silicon or glass bonding processes require high voltages or temperatures. These requirements increase the cost of the process.

Because of these drawbacks, innovative, polymeric fabrication techniques have been developed and studied to help speed up the development of microfluidic systems. Polymers, compared to silicon and glass, have several outstanding points as follows (Becker and Gartner, 2000):

a) Higher volumes of channels can be created rapidly by processes such as microinjection molding, replica molding, and precision embossing, without the need to perform photolithography on each substrate. Rapid production of tooling for these methods can largely reduce prototyping time.

b) These techniques provide a migration path to high volume production.

c) Polymers are inexpensive. For instance, polymers like polymethylmethacrylate (PMMA) are of the order of \$0.002-0.020 per cm^2, while boro-float glass (e.g., Corning Pyrex) is of the order of \$0.10-0.20 per cm^2.

d) Channels can be formed in a large variety of geometries, particularly vertical sidewalls with a high aspect ratio.

e) Polymer substrates can be bonded at much lower temperatures.

f) Moreover, since many polymers are transparent, optical detection can be compatible with these materials.

In laboratory and microfluidic systems development environments, the need exists to quickly develop and test concept devices in short iterations. Requirements of the prototyping method include the need to rapidly incorporate new design concepts within the prototype (design flexibility). The method must be fast to execute (short cycle time) with low prototyping costs. It would be preferable if the prototyping method could be performed with commonly available materials and equipment without the need for high cost facilities (e.g. photolithographic equipment).

Polymer Microfabrication and Bonding Processes

Numerous micromanufacturing and bonding processes have been applied to produce microfluidic channels in the past. Key techniques are summarized below. These microfabrication techniques can be categorized into two main groups: direct machining and replication methods. Brief details of the processes are described below.

Direct Machining Methods

Mechanical micromachining. By using a micro-cutting tool, a mechanical micromilling process can create channels in PMMA as small as 22 μm and as deep

as 62 μm, with vertical sidewalls and good roughness (65-90 nm) (Friedrich et al., 1995). These structures can be employed as microchannel parts or masters for replication. The advantages of this technique are that a wide range of materials can be machined and the prototyping times are comparatively short because the mask making and photolithography steps are not required. Nevertheless, channel cross-sections with aspect ratios more than 3:1 or channel widths less than 20 μm cannot be created.

Laser ablation. High aspect ratio microchannels and three-dimensional microstructures can be manufactured by this method. This technique applies a UV excimer laser pulse (e.g. ArF, KrF) to fracture molecular bonds of the plastic, and ablated debris is then removed from the region to create the channels (Roberts et al., 1997). Many commercial polymers, including polymethyl methacrylate (PMMA), polycarbonate (PC), polyethylene terephthalate (PET), polystyrene (PS), polytetrafluoroethylene, and nitrocellulose, can be machined by this method. However, laser ablation techniques provide inferior surface smoothness of channels than replication methods such as injection molding and hot embossing (Madou and Florkey, 2000).

Replication Methods

LIGA. LIGA is the German acronym for X-ray lithography, electroforming, and molding. The process consists of 3 steps: (1) Photolithography involves coating of photoresist (e.g. PMMA) or thick negative photoresist (e.g. SU-

8) onto a conductive substrate, X-ray or UV radiation exposure through a mask, and development to create a three-dimensional resist structure; (2) electroforming to deposit the resist structure with a metal and create a metal mold insert; and (3) molding to replicate plastic microstructure in the mold insert. With this technique, it is possible to create microstructures with very high aspect ratios (up to 100:1), submicron feature sizes, and very smooth walls (surface roughness < 50 nm). These microstructures can be fabricated from a variety of materials, including metals (e.g. Au, Ni, Cu), metal alloys (e.g. nickel-iron, nickel-cobalt, nickel-tungsten alloys), thermoplastics (e.g. PMMA), and ceramics (e.g. ZrO_2) (Lowe et al., 1997). The slow growth rate in the electroforming step (10-100 μm/hr) is a limitation of this process.

Injection Molding. A mold insert obtained from either electroforming in LIGA, mechanical micromilling, or laser ablation is required for this process. Typical cycle times for microinjection molding are in the range of 1 to 3 min (Becker and Gartner, 2000). A raw, polymeric material is fed into the cylinder by a heated screw. The molten material is then injected under pressure into a die (a metal block with a cavity and a mold insert) used to form a plastic shape. The mold set is cooled down to room temperature, and the plastic part is ejected from the mold (Soper, 2000).

Hot Embossing. Like injection molding, hot embossing requires a master, which is created from electroforming in LIGA, mechanical micromilling, or laser ablation. The master and polymer substrate, primarily PMMA and PC, are

assembled in an embossing machine and heated slightly above the glass transition temperature (T_g) of the polymeric materials. The master is embossed in the substrate by using pressure. They are then cooled to below T_g under pressure. At room temperature, the master and substrate are taken apart, and result in the desired features in the polymer substrate. The cycle time of the PMMA embossing process is approximately 5 minutes (Becker et al., 1999).

These two replication methods, injection molding and hot embossing, offer potential benefits: good surface finish and capability of high volume production that allows microfluidic channels to become disposable devices.

Soft Lithography. Polydimethylsiloxane (PDMS), an elastomeric polymer, is utilized in this process to replicate a pattern. Desired channels are formed by casting PDMS against a master. A rapid prototyping of microfluidic systems in PDMS was developed by Duffy et al. (1998). The design of a channel network was created by using a Computer Aided Design (CAD) program. This design was printed on a transparency by using a high-resolution printer. Instead of using a chrome mask, this transparency was used as a mask in photolithography to transfer the pattern into a photoresist whose thickness defined the channel depth. After exposure and development, the pattern of photoresist was used as the master to cast replicas in PDMS. This rapid prototyping technique could not create features with widths smaller than 20 μm. The PDMS was cast against the master to form an elastomeric replica containing channel networks, and cured at 65∘C for 1 hour. To make an enclosed channel, the surface of this replica, and that of a flat

piece of PDMS, were oxidized and sealed irreversibly when brought into conformal contact. Oxidized PDMS was also sealed to other materials used in microfluidic systems, such as glass and silicon.

The advantages of this rapid prototyping are that an entire microfluidic channel in PDMS can be fabricated in less than 24 hours, and the sealing of the channels in oxidized PDMS is fast (within a few minutes). Elastomeric PDMS replica could also be used as a mold to replicate microchannels in thermoplastic polymers (Xia and Whitesides, 1998).

Plastic Bonding Methods

Adhesives. Proper adhesives can bond the channel substrate and the cover plate together to generate an enclosed channel. In addition, a 30 μm thick PET sheet coated with a high temperature adhesive is employed to bond. The adhesive layer melts by using a heated roller, and combines the two pieces together. However, the adhesive tends to flow into and obstruct the channel flow (Becker and Gartner, 2000).

Heat and Pressure. In this method, the substrate and cover plate are bonded by clamping together and heated at slightly below T_g for 10 minutes in an oven. Because of the heat and pressure, the cover plate is sealed to the channel substrate. However, when the plastic temperature is close to its T_g, the material could vastly deform and generate bubbles (Soper et al., 2000).

Ultrasonic welding. This process is widespread and suitable for joining macroscale thermoplastics because it is fast and economical. Nevertheless, in microscale systems, no application of ultrasonic welding has been applied to microfluidics up to this time. (Becker and Gartner, 2000).

Ultrasonic welding (USW) is a joining process for thermoplastic parts (e.g. polycarbonate) by applying high frequency vibrations between two parts placed under pressure. The welding unit converts standard 50/60 Hz alternating current (AC) from the main supply into high frequency (20 or 40 kHz) electrical energy, which is then converted by a piezoelectric crystal into mechanical vibration at the same frequency. This vertical mechanical motion is transmitted to a booster, which magnifies the amplitude of the vibrating motion. Ultimately, the vibrational energy is conveyed to the horn, which interfaces with the part. The vibrations produce a sharp rise in temperature at the interface of the parts, due to intermolecular friction. When the temperature is high enough, the plastic between the parts flows. After coalescence of the materials, the material solidifies and forms a welded joint. Because the process operates at a high frequency, above the range of human hearing (> 18 kHz), it is called "**ultrasonic**".

Energy directors are triangular-shaped protrusions of plastic material, which concentrate the ultrasonic energy at the contact spot between two parts. The energy director increases the strength of the welded joint and decreases the welding time due to higher heating rates in the parts. Typical heights for energy directors are about 500 μm (0.020 inch) for crystalline thermoplastics, and about 250 μm (0.010

inch) for amorphous thermoplastics. The size of the energy director must be optimally designed in order to allow the plastic to flow completely throughout the desired area. The rule of thumb to obtain the joint width is to multiply the height of the energy director by eight as shown in Figure 1 (Mainolfi, 1984). This formula was utilized to predict the joint width and prevent the material flow into the channels.

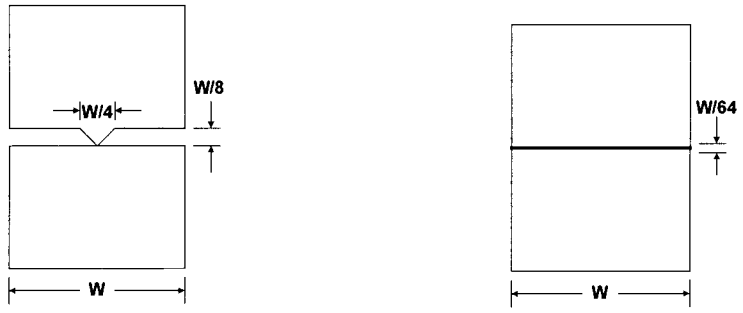


Figure 1. The dimensions of energy director and welded joint before and after ultrasonic welding (Mainolfi, 1984).

Research Objectives

1. Apply the capability of ultrasonic welding to prototype microfluidic systems for biotechnology such as cell-based biosensors.
2. Investigate features of ultrasonically welded devices, such as cell viability, bond strength, hermetical sealing and sterilization compatibility.

Research Questions

1. Can ultrasonic welding (USW) be used for prototyping microfluidic systems for biotechnology? The first issue involves the ability to form the small energy directors without the use of a capital-intensive process such as injection molding. In this research, Computer Numerical Control (CNC) machining is used to produce these features. The second issue is whether 250 μm high energy directors can be formed repeatedly through the use of CNC machining. The third issue involves whether 250 μm high energy directors can be used to form microchannels, which are 500 μm wide by 750 μm deep.
2. Is there a difference of mean cell viability between ultrasonically welded devices and multiwell cell culture dishes?
3. What are the advantages of ultrasonic welding?

MATERIALS AND METHODS

Device Design

The subject device for this research was designed for biosensor applications, which use biological elements to measure analytes of interest. The specific design of the device is a flow chamber with two channels that hold fish chromatophores for biological reagent detection purposes. Chromatophores are pigmented cells that are responsible for the brilliant and changeable colors of fish, amphibians, and reptiles. These cells have been applied in biosensors, because their appearance is changed by many types of biological reagents, such as toxins, drugs, and metabolites.

The size of the channel was initially determined by long-term viability studies, which have shown that a channel size on the order of 500 μm wide and 750 μm deep is most suitable to support the exchange of oxygen and nutrients and to remove cell wastes necessary for cell viability over a long period of time (4-8 weeks). The fabrication of this device consisted of a two-step microlamination approach: (1) patterning, which involved the machining of the material, and (2) bonding, which involved ultrasonic welding.

Polycarbonate (PC), an amorphous thermoplastic, was selected as the material to fabricate microfluidic devices since it is biocompatible, easy to machine, easy to ultrasonically weld, and features exceptionally high impact strength and dimensional stability over a wide temperature range (-40° to 240° F). Components

of the microfluidic chamber contain two machined parts: a flat transparent PC and a white PC with channels and energy directors -- small triangular-shaped protrusions used in ultrasonic welding. Figure 2 shows the design of channels and energy directors that were used to fabricate the microfluidic channels in PC.

Fabrication of Polymeric Microfluidic Channels

Figure 3 shows the process steps involved in the rapid prototyping of microchannels in PC.

Machining of Channels and Energy Directors. In order to create energy directors and channels, computer-aided design (CAD) files were exported to a computer-aided manufacturing (CAM) program used to generate a numerical control (NC) program for manipulating a computer numerical control (CNC) machining center. The machining center was utilized to drill holes for alignment and mill energy directors with the dimensions 500 μm (0.020 in) wide by 250 μm (0.010 in) high by using a 90 degree angle end mill. The machining center also machined vertical sidewall channels with a cross section of 500 μm (0.020 in) wide by 762 μm (0.030 in) deep by using a commercial miniature carbide end mill (500 μm diameter).

Bonding. A machined white PC lamina and a flat transparent PC lamina (for optical detection) were cleaned with 100% ethanol and deionized water, and then dried under a stream of air. The two laminae were assembled in a pin alignment

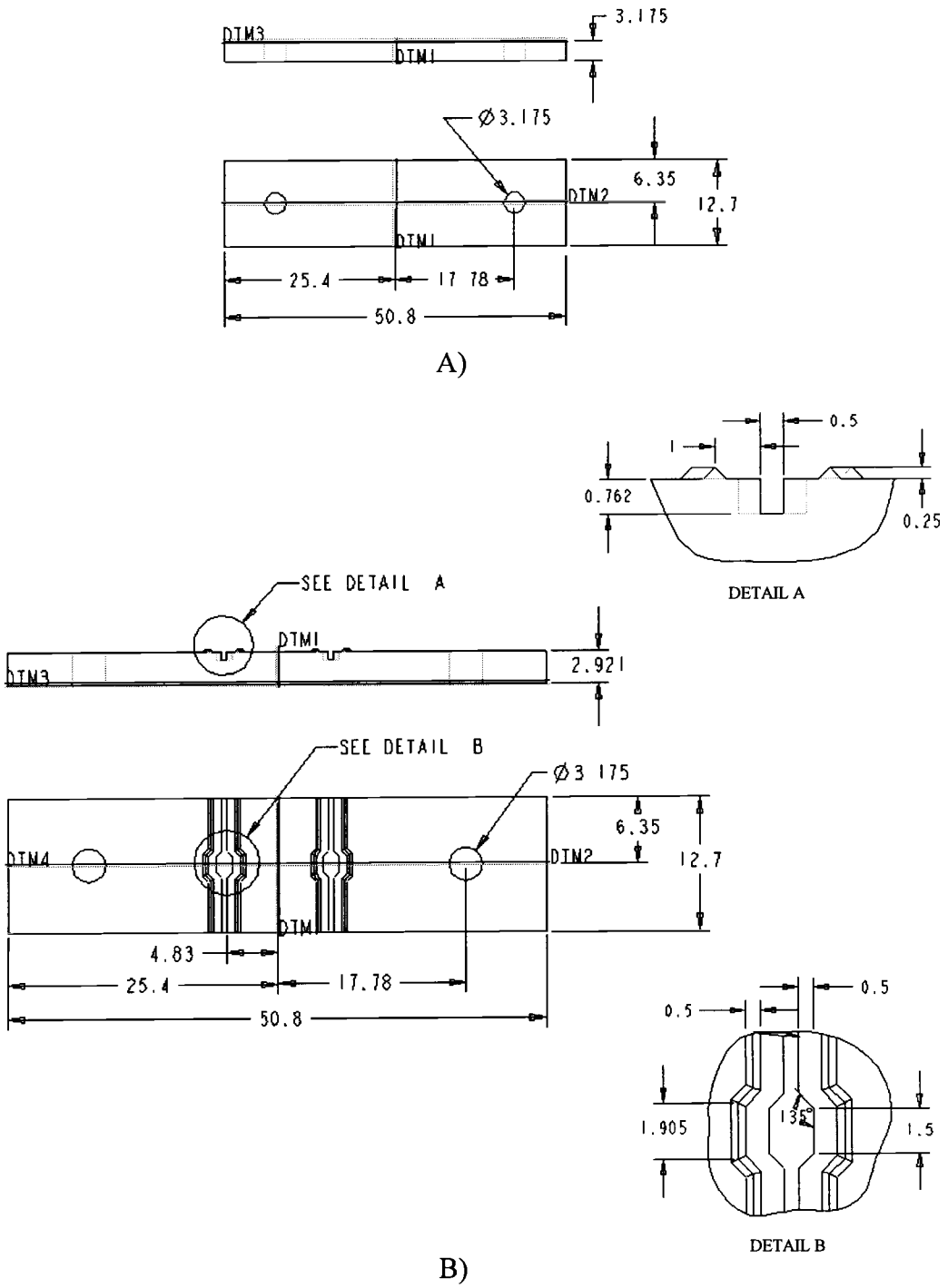
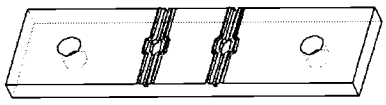


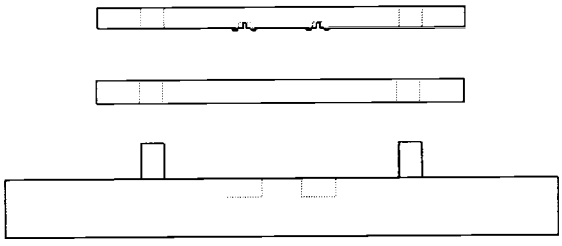
Figure 2. Designs of microfluidic channels (in mm). (A) flat transparent PC. (B) white PC with energy directors and channels. Holes are used for alignment during ultrasonic welding



A white PC with energy directors and channels

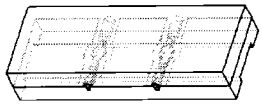
A flat transparent PC

A)



A pin alignment fixture

B)



C)

Figure 3. Scheme describing the fabrication of enclosed microchannels in PC. (A) milling a white PC with energy directors and channels as well as a flat transparent PC. (B) aligning the two parts by using a pin alignment fixture and bonding by an ultrasonic welding process. (C) final shape of the chamber after machining.

fixture as shown in Figure 3 and ultrasonically welded. This bonding process was performed at 38 psi and 150 joules for 100 to 150 milliseconds.

Machining of channels' conduits and final shape chambers. In order to easily load cells and analytes, a chamber requires inlet and outlet conduits at both ends of the channels. A chamber was milled by using a 1.5 mm diameter end mill to a depth of 3.175 mm from both ends. In addition, a manual milling machine was used to cut out two alignment holes and make a slot (6.35 mm wide, 1 mm deep) on the white PC underneath the area of interest to optimize the light intensity. Figure 4 presents a complete chamber of two microchannels.

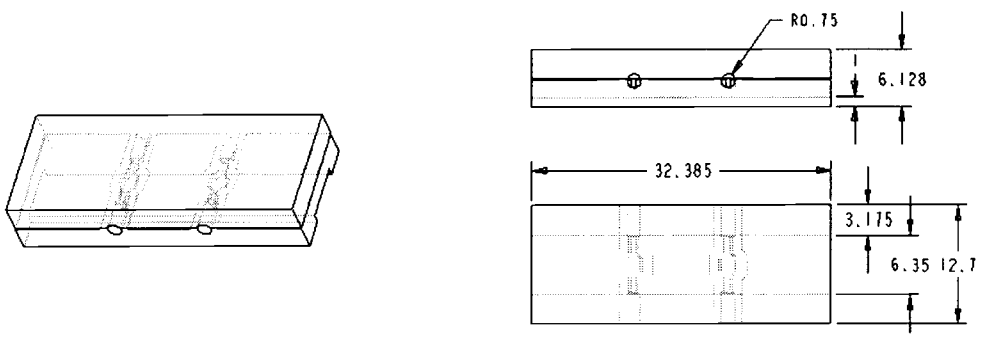


Figure 4. The final dimensions of a microchannel chamber (in mm).

Sterilization of Microfluidic Channels

In biological applications, sterilization is an important step, which is used to inactivate microorganisms (e.g. fungi, bacteria, and viruses) and therefore protect cell cultures from contamination. In this research, the chambers were cleaned for 15 minutes in 100% ethanol. Then they were either sterilized by autoclaving

(steam under pressure at 121°C and under 15 psi for 20 minutes) or by immersing the chambers in 70% ethanol overnight.

Populating Cells in the Chambers

In this research, we used a primary cell culture of colored pigment cells isolated from *Betta Splenders* to populate the channels. In order to introduce cells into the channels, the chambers are immersed in Liebowitz's Media (L-15), insuring that the interior of the chambers were filled with media and did not contain any bubbles. 30 μL of the cell suspension was dispensed into each channel of the chamber by using a P-200 micropipette with a tip as shown in Figure 5.

Figure 5. Populating colored pigment cells in the channels by using a micropipette.

The cells were allowed to settle, undisturbed for 30 minutes, and then fetal bovine serum (FBS) was added to a final concentration of 5%. 10 nM melanocyte

stimulating hormone (MSH) was also added to this media for two reasons. First, MSH causes the chromatophores to maximally disperse, thus providing the greatest possible difference between dispersed and aggregated states for the measurement of cell viability. This was important because of the magnification limitations imposed by the stereomicroscope, and the resultant limitations in sensitivity of detection of the changes when the neurotransmitter norepinephrene (NE) was added. Also, MSH promotes the overall health of the chromatophore culture. On the following day, the L-15 media containing 5% FBS was replaced and 50 μL of media were gently flushed through each channel of the chamber to ensure complete exchange of media.

Cell Viability Test

Viable chromatophores are capable of aggregating their pigment granules when exposed to the NE, while dead chromatophores do not respond to NE. Under normal conditions, the pigment granules within chromatophores are evenly dispersed throughout the cytoplasm. When stimulated to aggregate by NE, the pigment granules migrate to the center of the chromatophore, and the area occupied by the colored pigment granules becomes much smaller. This characteristic of the chromatophores can be used to determine the cell viability.

The relative measure of cell viability, called the Cell Response Index (CRI), was defined by using the following formula:

$$(\%)\ \text{Cell Response Index} = \frac{(\text{Area}_1 - \text{Area}_2)}{\text{Area}_1} \times 100$$

$$= \left[1 - \frac{\text{Area}_2}{\text{Area}_1}\right] \times 100$$

where Area_1 was the pixel area occupied by the colored pigment granules before exposure to NE, and Area_2 was the contracted pixel area occupied by the colored pigment granules after exposure to NE.

To perform the viability experiments, images of cells in each channel and 24 well cell culture dish were recorded once a week with a Leica MZ7 stereomicroscope at 5x magnification and a Spot Insight camera using ImagePro image analysis software. The total areas of pigment granules were measured before and after exposure to 1 μM norepinephrine by using the ImagePro software. The sequence of steps used to obtain the area occupied by the colored pigment granules within the field of view of an image was as follows: (1) images were first segmented in the area of interest to distinguish the pigment granules (colored objects) from the white background, and (2) the total area of the pigment granules was then measured for all colored objects in the segmented image. From this data, we could estimate the percent CRI, which represents the viability of the cells in microfluidic channels.

RESULTS AND DISCUSSIONS

Cell Viability

The CRI was used to evaluate the viability of the cells in ultrasonically welded chambers and 24 well cell culture dish. Three experiments were performed in this research. No data was collected in the first experiment due to contamination. The second experiment was recorded for four weeks. To verify data obtained, the experiment was repeated using a primary cell culture from a different fish.

In the second experiment, we observed data for four weeks, from weeks four to seven, to estimate the CRI over time. The chambers were sterilized using two types of sterilization techniques, autoclaving and ethanol. The CRI of the autoclaved and ethanol sterilized chambers over time is shown in Table 1 and Figure 6.

Table 1. CRI of autoclaved and ethanol sterilized chambers in each week.

	Autoclaved Chambers				Ethanol sterilized Chambers			
	n	Mean	95% CI	SD	n	Mean	95% CI	SD
Week 4	4	57.431	± 5.557	5.670	4	51.462	± 4.083	4.166
Week 5	4	55.693	± 5.538	5.651	4	50.434	± 3.732	3.808
Week 6	4	54.739	± 5.045	5.148	4	49.084	± 3.410	3.480
Week 7	4	52.130	± 5.941	6.063	4	47.788	± 2.769	2.825

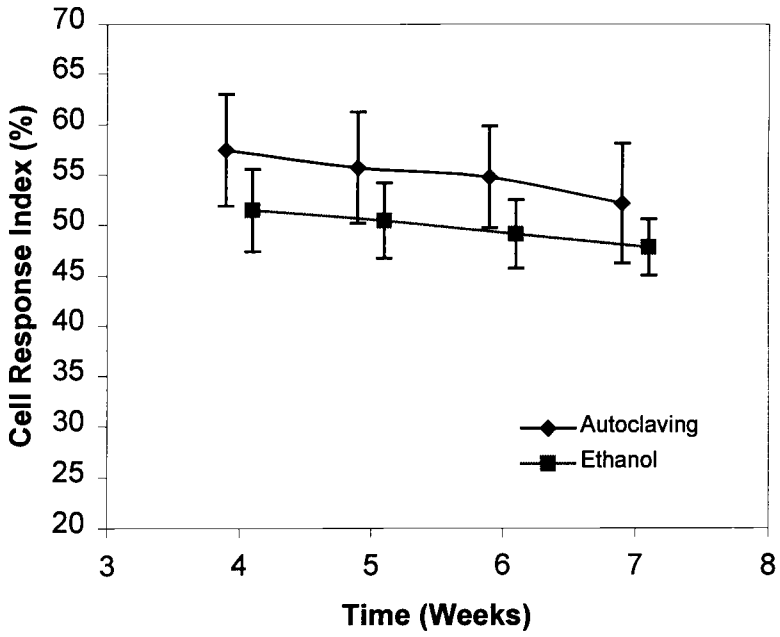


Figure 6. CRI of autoclaved and ethanol sterilized ultrasonically welded chambers over time.

A statistical analysis was employed to find out whether the mean CRI of the channels sterilized by autoclaving differs from that of the channels sterilized by ethanol. In Week 4, an equal variance two-sample t-test was performed comparing the mean CRI of the autoclaved channels (mean = 57.431, SD = 5.670) with that of the ethanol-sterilized channels (mean = 51.462, SD = 4.166). The alpha level was 0.05. This test was not statistically significant (t-stat = 1.697, d.f. = 6, two-sided p-value = 0.141) indicating that the mean CRI of the channels sterilized by autoclaving is not different from that of the channels sterilized by ethanol. A 95% confidence interval for the difference of CRI is from -2.639 to 14.577 %.

The results of the two-sample t-test from weeks five to seven are summarized in Table 2. Overall, it was found that the mean CRI of the channels sterilized by autoclaving is not different from that of the channels sterilized by ethanol.

Table 2. The results of an equal variance two-sample t-test to find out whether the mean CRI of the channels sterilized by autoclaving differs from that of the channels sterilized by ethanol.

	Statistical Results (alpha = 0.05)			
	t-stat	d.f.	p-value	95% CI
Week 4	1.697	6	0.141	(-2.639, 14.577)
Week 5	1.543	6	0.174	(-3.078, 13.595)
Week 6	1.820	6	0.119	(-1.947, 13.259)
Week 7	1.381	6	0.216	(-5.578, 20.040)

The result of the second experiment also showed that the pigment cells lived for ten weeks in the ultrasonically welded channels, while the pigment cells in multiwell cell culture dish were dead after six weeks of the experiment. This was contrary to expectations. As a result, a second experiment was performed to validate these findings. From the result of the statistical analysis in the first investigation, because autoclaving consumes much less time than ethanol sterilization, the autoclaving process was employed to sterilize all chambers for the third experiment.

The third experiment ran for four weeks. The CRI of the autoclaved ultrasonically welded chambers and multiwell cell culture dish was calculated over

time, as displayed in Table 3. Figure 7 shows the results from both experiment 2 and 3.

Table 3. CRI of ultrasonically welded chambers and multiwell cell culture dishes in each week.

	Ultrasonically Welded Chambers				Multiwell Cell Culture Dish			
	n	Mean	95% CI	SD	n	Mean	95% CI	SD
Week 1	7	71.118	± 5.267	7.111	9	86.259	± 1.053	1.612
Week 2	7	67.101	± 4.603	6.214	9	85.861	± 1.273	1.949
Week 3	7	62.666	± 4.904	6.619	9	80.329	± 1.520	2.327
Week 4	7	60.801	± 5.821	7.858	9	77.079	± 1.763	2.699

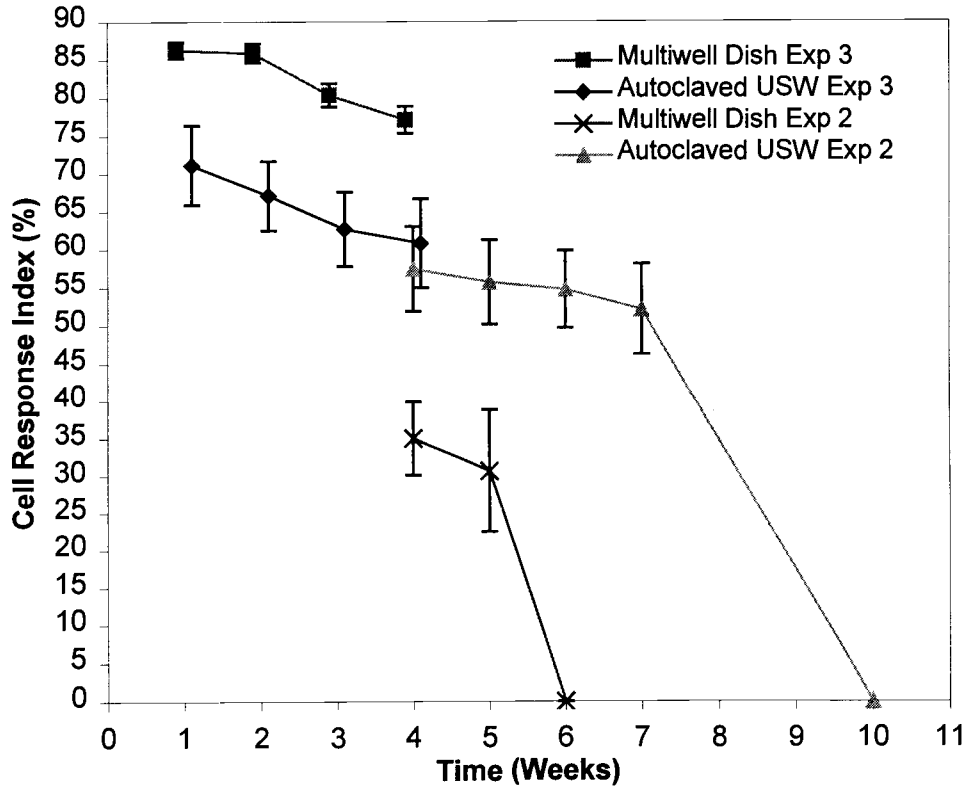


Figure 7. CRI of autoclaved chambers and multiwell cell culture dishes over time.

A statistical analysis was employed to test whether the mean CRI of the autoclaved ultrasonically welded chambers is less than that of the multiwell cell culture dishes. In Week 1, an unequal variance two-sample t-test was performed comparing mean CRI of the autoclaved ultrasonically welded chambers (mean = 71.118, SD = 7.111) with that of the multiwell cell culture dish (mean = 86.259, SD = 1.612). The alpha level was 0.05. This test was found to be statistically significant (t-stat = -5.524, d.f. = 6.482, one-sided p-value = 0.0006) indicating that the mean CRI of the channels sterilized by autoclaving is significantly less than that of the multiwell cell culture dish. A 95% confidence interval for the difference of CRI is from -α to -9.885 %.

In addition, from the result of an unequal variance two-sample t-test in each week from weeks two to four as summarized in Table 4, it was found that the mean CRI of the autoclaved ultrasonically welded channels is significantly less than that of the multiwell cell culture dish.

Furthermore, the data shows that the variability of CRI in each channel was much larger than that in each well of the cell culture dish. This might occur for two reasons: (1) the handling of the cells in loading the chamber may significantly increase the difference in cell density in each channel, which directly affects the variability of CRI (the greater the cell density, the greater the CRI), and (2) because of a white background of the chamber (compared to the transparent background of the multiwell dish), the chamber gave less contrast to capture the

image and measure the total area of the cells. This may also influence the variability in the CRI of the chamber.

The proximity of the CRI of the chamber in Week 4 from both experiments might suggest that the chamber provides more robustness and repeatability, while the multiwell dish was comparatively easy to contaminate and may have caused the discontinuity of the CRI in Week 4. In addition, the variability in the CRI of the multiwell dish in the second experiment was relatively large. This may be from inconsistency in the cell density in each well.

Table 4. The results of an unequal variance two-sample t-test to test whether the mean CRI of the autoclaved ultrasonically welded chambers is less than that of the multiwell cell culture dishes.

	Statistical Results (alpha = 0.05)			
	t-stat	d.f.	p-value	95% CI
Week 1	-5.524	6.482	0.0006	(-∞, -9.885)
Week 2	-7.699	6.923	0.0001	(-∞, -14.136)
Week 3	-6.743	7.159	0.0001	(-∞, -12.717)
Week 4	-5.246	7.107	0.0006	(-∞, -10.413)

From the results of both observations, it was also observed that the CRI of both ultrasonically welded chambers and multiwell cell culture dish decreased over time. As the cell culture ages, some cells die and become unresponsive to the effects of NE, thus over time, the ratio of the area occupied by NE-treated cells ($Area_2$) over that occupied by untreated chromatophores ($Area_1$) becomes greater and, consequently, the CRI ($1-Area_2/Area_1$) becomes smaller.

Moreover, using NE as a reagent to test cell viability of the fish chromatophores offers an advantage. Following treatment with NE, the media was changed and the chromatophores recovered and became evenly dispersed again. This treatment did not harm the cells, so the culture could be repeatedly tested over eight weeks for the viability test.

Rapid Prototyping

In this research, it is found that milling and USW processes are suitable for rapid prototyping. By applying milling to form channels and USW to seal the channels, we could rapidly prototype an entirely new microfluidic chamber with two channels within 12 hours. Each chamber takes approximately 20 minutes for fabrication. The CAD program and the CNC machining center made the modification of channel's design simple and quick. It was possible to redesign for minor changes, such as changing the layout of energy directors, within 3 hours. In addition, the width of the channels could be reproduced within 10% variation (average width 541.7 μm in range from 500 μm to 554 μm). It was also found that the USW process to be robust as changes to process parameters (e.g. pressure, weld time) were not required to join energy directors in different patterns of length and layout without significantly changing the quality of weld joint. This is significant for decreasing prototyping times, as it does not require process redesign for each product design iteration.

On the other hand, milling has a scale limitation for microchannel dimensions. In general, due to a constraint of spindle speed (7500 rpm) of the CNC machining

center used in this experiment, the smallest dimension, which could be machined, was 550 μm (0.022 inch). However, it is expected that CNC machining centers with higher spindle speeds could machine parts in a scale size down to 250 μm (0.010 inch), which is on the order for the practical limits for drilling via holes in the printed circuit board industry.

High Volume Production

One requirement of biotechnology-based microfluidic packages is that they must be disposable and inexpensive. To meet this requirement, the prototyping architecture must be compatible with high-volume production processes. The use of USW techniques makes this method particularly compatible with high volume manufacturing. Instead of using a milling process, injection molding of the plastic can be applied to decrease production time. Injection molding can form the parts within seconds while milling takes about 30 minutes to machine. Additionally, there are some other advantages of molding over milling such as better surface finish and better shape resolution. Injection molding also has some disadvantages. Creating a die (a required metal block with a cavity used to form a plastic shape) consumes time (e.g. days) and is expensive. However, these drawbacks can be negligible when compared to the high production rate. Since USW is a very fast and low-cost process, it is appropriate to use it to bond a microfluidic device in both mass production and rapid prototyping systems.

Optical Detection and Scratching

For optical detection purposes, the transparent PC was selected to be the upper part of the chamber and the white PC became the lower part of the chamber (and contrasting background for the cell pictures). Comparing a clear and white colored background, we found that the white colored background provided a better image because it gave more light reflection and assisted in making cells distinguishable.

After bonding by USW, it was discovered that some scratching occurred on the contact surface between the horn and the white PC (A) as well as the contact surface between the transparent PC and the pin alignment fixture (B) (as shown in Figure 8). These scratches were caused by the vibration between the surfaces during USW. Therefore, the scratches appeared on both top and bottom surfaces of the assembled part. These scratches on the top surface of the transparent PC were found to affect the quality of cell images during image capture. This problem was eliminated by avoiding contact between the clear PC and the fixture at the area of interest. For this reason, we made two circular slots (4.76 mm diameter 2.54 mm deep) on the fixture at the area of interest, at the central section of the channels, as displayed in Figure 9. This eliminated any problems in optical detection.

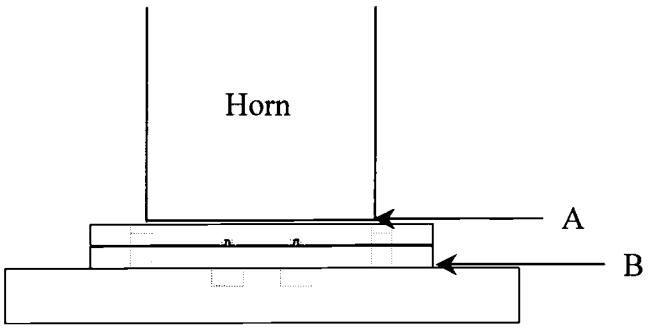


Figure 8. Two surface areas where scratching occurs: (A) the contact surface between a horn and a white PC and (B) the contact surface between a transparent PC and a pin alignment fixture.

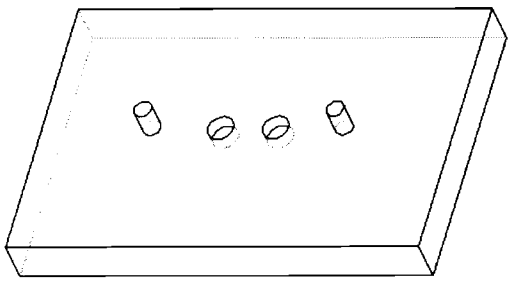
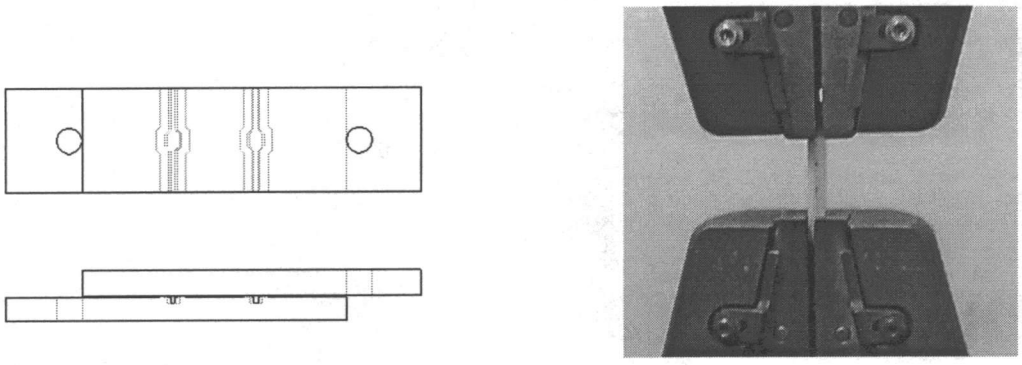


Figure 9. A pin alignment fixture with two circular slots.

Bond Strength and Leak Resistance

Bond strength of the ultrasonically welded part is a factor that helps determine the durability of the chamber. In this research, we used shear strength testing to represent the bond strength of the welded joint. To firmly hold both ends of the part by grippers, the chamber was machined and installed in an INSTRON 4505 testing machine to perform the test as shown in Figure 10.



A) B)

Figure 10. A bond strength testing of the chamber: (A) Specimen of an ultrasonically welded chamber used for bond strength testing and (B) Specimen installed in the tensile tester by gripping at both ends.

The result from the experiments showed that the average bond strength of the chamber sealed by USW was 21.16 MPa, ranging from 20.42 to 21.96 MPa (while the bulk strength of the PC is 56-75 MPa). This strength is high enough that the weld was not broken by hand force.

In addition to the strength of the welded joints, another important factor tested was leakage of the seals. This problem might highly influence the result of the cell response to NE. To perform a test for a leak proof seal, food dye was carried through microporous wicks (Polyacetal plastic resin, 1.0 mm OD, 1.0 cm long) into the channels as shown in Figure 11 and used to indicate any leakage in the channels. Because of the color of the food dye (red 40), if there was a leak inside the channel, it was readily detected by eye. Experimental results showed that USW could be applied to hermetically seal the PC pieces efficiently.

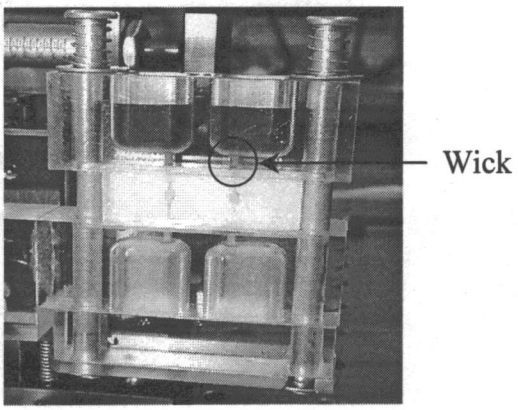


Figure 11. Instrument set up to test the leak resistance of channel seals.

Sterilization

The initial testing of the sterilized, ultrasonically welded device resulted in bacterial and fungal contamination after about 7 days. This was determined to be the result of inadequate cleaning of the components prior to welding. Close attention to cleanliness ended the contamination problem. Autoclaving (steam under pressure) is a comparatively simple, effective, rapid, and inexpensive method and is usually selected as the first choice for sterilization. It is superior in that it effectively kills ethanol-resistant fungal and bacterial spores that sometimes can contaminate a culture. After autoclaving, the chamber did not show any changes in appearance, dimensions or clarity (for the transparent PC). Hence, autoclaving is the sterilization technique of choice for the ultrasonically welded PC chamber

In addition, another method used for industrial-scale sterilization was tested on the PC chamber. Due to the penetrative capability of gamma rays, gamma irradiation is a very effective process. Disposable plastic components (e.g. tissue

culture flasks and dishes, plastic pipettes, syringes, and pipette tips) with individual sealed packages are commonly sterilized in this fashion. After gamma irradiation (cobalt-60, Dose 3 MRad, exposure time 33 hours), it was found that the PC chamber turned a dark brownish-yellow, creating a problem for viewing the chromatophores. Thus, gamma irradiation is not compatible with the PC chamber.

CONCLUSIONS

This research presents an alternative technique for prototyping microfluidic channels (> 250 μm wide) based on mechanical machining of PC and ultrasonic welding. The entire process, including design and manufacturing, takes less than 12 hours. This paper documents the fabrication of a fish chromatophore-based biosensor using this method.

The results have shown that the Cell Response Index, representing viability of the pigment cells in the devices, is less than that of the multiwell cell culture dishes. Several reasons have been suggested; however, this CRI of the ultrasonically welded chamber is high enough to allow the biosensor to perform adequately. Moreover, the microfluidic devices offer benefits over multiwell cell culture dishes, including the use of less sample material, and a portability that allows in-situ and real-time analysis. Also, wicks or tubing inserted in the ports drilled on either side allow for the introduction of a sample in a controlled manner. The experiments have also shown that it is possible to allow pigment cells to live in the channels for ten weeks.

Advantages of the USW method over existing methods for microfluidics device development include: (a) short cycle time, (b) design flexibility, and (c) a commercialization path to high volume production. Other features of the ultrasonically welded chambers, such as high bond strength, efficient hermetic

sealing, and autoclaving compatibility are also suitable for fabrication of microfluidic systems for biotechnology.

A limitation of this manufacturing process is that it cannot practically produce microchannels smaller than about 250 μm (0.010 in). However, for this fish chromatophore-based biosensor, this length scale was well suited, as shown in the cell viability results.

RECOMMENDATIONS FOR FUTURE RESEARCH

There are two interesting points that should be mentioned for future research. First, injection molding can be applied to form PC parts for the patterning step. This process offers advantages over mechanical milling, such as shorter cycle time and better surface finish. It also reduces process steps (e.g. drilling of the channel ports) and, thus, eliminates plastic debris from drilling that may obstruct the channel flow. In addition, automation can be utilized for these techniques, such as injection molding and ultrasonic welding for high volume production of microfluidic chambers.

Second, one of outstanding points of thermoplastics is recyclability. Thermoplastics can be recycled to form the components of microfluidic devices for many times. This helps reduce the cost of the material, and decreases the environmental impact of disposal. Further research on the effects of reused thermoplastics with respect to the optimal parameters for ultrasonic welding could be performed.

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APPENDICES

APPENDIX A: ULTRASONIC WELDING PROCEDURE

Make sure to prepare your parts prior to ultrasonic welding. Any debris or liquid still on the surface of the parts may affect the welding process. When welding the chambers, make sure that the parts have been cleaned thoroughly with ethanol, rinsed with deionized water and dried with an air steam. Make sure that the channels, the energy directors, and the chambers have been eliminated debris by using a razor or an Exacto knife. Also to ensure that the chambers remain very clean, be sure to wear gloves while handling the parts.

Step 1 Turn on the air supply to the machine.
The lever is located directly behind the ultrasonic welding machine. Pressurizing the ultrasonic welding system can be achieved by raising the lever counter clockwise so that it is parallel to the copper airflow tubing. The pressure gauge directly below the lever should read between 85 and 100 psi.

Step 2 Power up the machine.
There are two power switches that must be turned on in particular order. The first switch is located on the upper back of the machine. The switch should be in the up position and a green light should appear directly above the switch. The second switch is located on the back upper left corner of the ultrasonic weld controller located directly to the left of the ultrasonic welder. Allow the machine to power up for at least 15 minutes prior to use.

Step 3 Changing the settings.
The ultrasonic welding control system has a set of buttons, each with their own LED. Note that different dimensions of energy director and types of thermoplastic will have different settings. The optimal settings for the chambers are as follows:

Set a pressure of the ultrasonic welding machine at 38-40 psi for the devices.

"ENERGY" LED is on in the "SETTINGS" portion of the control system

"WELD ENERGY"	150 J
"+ LIMIT TIME"	150 mSec
"- LIMIT TIME"	100 mSec

To change one or more of the fields, first press the "ENERGY" button in the settings portion of the control system. The display should read "READY ENERGY MODE X1". Then press the button immediately to the right of the field you wish to change in the control portion of the control system. A red LED should light up indicating that the field can now be changed.

Step 4 Placement of parts.
First, place a transparent PC part of the chamber in the pin alignment fixture, pressing around the part to make sure it is resting firmly on the horizontal work surface. Next, place the white PC part with channels and energy directors bottom

up in the fixture. Once again press around the outside edges to make sure that the energy directors and transparent PC parts are touching.

Step 5 Welding the parts.
There are two red operating buttons to either side of the work surface. Both must be pressed simultaneously for the welding horn to lower. This is to prevent the operator from touching the horn during welding. Continue pressing the buttons until the process has completed. You will know when the parts have been welded because there will be a high pitch noise accompanied by the sound of rushing air during the welding process. When the sounds stop, the process has completed. The buttons can then be released and the horn will retract automatically. This step takes a few seconds to bond the two parts.

Step 6 Removing the parts.
Remove the parts carefully, griping the outside bottom edges and raising the assembly out of the fixture. Note that the part may become stuck on the fixture if one side of the assembly is raised at a faster rate than the other.

Step 7 Shutting down.
To shut down the machine, first switch both of the power switches to the off position. All LED's and lights should no longer shine. Then turn off the air supply by returning the lever perpendicular to the copper airflow tubing.

APPENDIX B: PROTOCOL IN PREPARING THE TISSUE CULTURE OF BETTA FISH CHOMATOPHORES

MATERIALS:

Sterile

100 mm petri dish
Large blunt forceps
Dissecting scissors and fine forceps
~5 50-ml plastic centrifuge tubes
~5 15-ml plastic centrifuge tubes
Transfer pipettes
24 well Costar tissue culture dishes
10 ml syringe
0.2 μm acrodisc syringe filter

Non-Sterile

4 liter plastic beaker containing ice water
10-ml glass beaker with small magnetic stir bar

SOLUTIONS: (Tip: Pipette needed volumes into separate tubes to avoid repeated entry into stock solutions.)

PBS

128 mM NaCl
2.7 mM KCl
10 mM Na_2HPO_4
1.46 mM KH_2PO_4
5.6 mM glucose
Antibiotic/antimycotic pH 7.3

Skinning Solution

1 mM NaEDTA in PBS pH 7.3

Digestion Solution (made fresh)

Collagenase Type I: 220 U/mg (5 mg/ml)
Hyalouronidase: 348 U/mg (0.5 mg/ml)
Make up to a final volume of 7 ml in PBS (use 35 mg collaginase I and 3.5 mg hyalouronidase)
Filter sterilize with a 0.2 μm acrodisc filter immediately before use

L-15 Media

Purchased as pre-made solution without red indicator dye from Gibco-BRL
20 mM HEPES (made from 1 M Gibco-BRL Tisue Culture Grade
Antibiotic/antimycotic (penicillin, streptomycin and Fungizone from Gibco-BRL 15240-062, diluted 1:100)

Fetal Bovine Serum

From Chaplen lab, Hyclone

PROTOCOL (Perform all appropriate steps using sterile technique in the tissue culture hood.)

1. Meanwhile, prepare the digestion solution by weighing out the enzymes and transferring to a 10-ml beaker containing PBS and a magnetic stir bar. Cover and mix on a stir plate.
2. Place fish in a 4 liter anesthetic ice bath for 15 minutes to kill.
3. Place fish in 100 mm sterile petri dish and flood with PBS (about 10 ml).
4. Using surgical scissors and fine forceps, clip the desired fins off the fish and transfer to sterile 50 ml centrifuge tube containing 10 ml of skinning solution.
5. Place the tube on an orbital shaker at 80 - 100 rpm for 20 minutes. During this time, change the skinning solution at least 6 times. First, place on shaker for 5 min while cleaning up. Then perform the next 3 changes manually by rotating and rocking back and forth for 30 sec per change. Then the tube is placed back on the rocker for another 5 min, and the final two changes performed manually.
6. After the last skinning solution change, remove as much skinning solution as possible. Filter sterilize the digestion solution directly into the tube containing the fin pieces. Return the tube to the orbital shaker.
7. After 15-20 minutes, carefully remove only the digestion solution using a 10 ml pipette and place into a 15-ml plastic tube. Avoid sucking up any bits of fin. Spin the 15-ml tube in the Eppindorf table-top centrifuge at 700 rpm and 22°C for 2 min. Transfer the digestion solution (supernatant) back to the 50-ml tube containing the fin pieces, trying not to disturb the cell pellet. Return the 50 ml tube containing the fins and digestion solution to the orbital shaker.

Note: the first cell harvest (pellet) contains mostly epithelial cells and is typically discarded. See below for washing and plating subsequent harvests.

8. Continue harvesting the digestion solution as described in step 7 at about 15 minute intervals. To recover cells from the pellet, add 7 ml of L-15 (no FBS), mix thoroughly, and centrifuge for 2 minutes. Remove the L-15 and resuspend the cells using a 5 ml pipette in 1 to 1.5 ml of fresh L-15, depending on the pellet size. The volume of media used here depends on the size of the pellet and the desired density of the cultures.
9. In order to place cells in the chamber wells, first immerse the chambers in L-15 media, insuring that the interior of the chambers are filled with media and do not contain any bubbles. Using a P-200 micropipettor with tip, dispense about 30 μL of cell suspension in each well of the chamber. Allow the cells to settle undisturbed for about 30 minutes, then add fetal bovine serum (FBS) to a final 5% concentration.
10. The following day, change the media using L-15 containing 5% FBS. No further media changes are needed for at least a week.

APPENDIX C: SEQUENCE OF STEPS TO CAPTURE AN IMAGE AND MEASURE THE AREA OCCUPIED BY THE COLORED PIGMENT GRANULES

Capturing the image

Turn on a CCD camera and a light source (high level) of a stereomicroscope. Acquire image data from a camera.

- Click **Acquire, Select Scanner**, and **Select** [Sources: SPOT Insight Camera]
- Click a **Scanner Icon** to launch the SPOT Camera window.
- Choose **Live** to open a Live Image Window and click **Restart** to display an image.
- Adjust focus of the object and move the object to find out an area of interest.
- Use **Controls** function to adjust brightness and Gamma of the image.
- Click **Snap** to capture an image.

Save the image

- Use **Save** command in **File Menu** or click **Save icon** to save the image in the desired folder.

Processing the image

- Load an image from the folder by click at **Open icon** or choose **open command** from **File Menu**.
- Select the area of interest [801x801 pixels] by using **Rectangular AOI**.
- To make a new image of that area of interest, click **Copy** and **Paste New** from **Edit Menu**.
- Choose **Filters** from **Process Menu** to obtain a clearer image.
- In **Enhancement** option, choose **Sharpen** [Passes: 2, Strength: 7] and click **Apply**.

Measuring the area of pigment cells

- Click **Count/Size** command in **Measure Menu** to estimate area of pigment cells.
- Choose **Manual** option to select colors of the pigment cell.
- In **Histogram Based**, adjust the intensity of the colors from 0 to 255 (Red, Green, and Blue) to count the areas of actual pigment cell.
- Click **Apply Mask** [the image changes from red and white to black and white pictures, white area is pigment cell while black area is background].
- Close a segmentation window.
- Choose **Automatic Bright Objects** and **Count.**
- The software measures the area of bright object representing the area of pigment cell and show the measurement in **Statistics** in **View Menu.**

APPENDIX D: CRI OF AUTOCLAVED AND ETHANOL STERILIZD CHANNELS IN WEEKS 4-7

Channel No.	Week 4		Week 5		Week 6		Week 7	
	Autoclaving	Ethanol	Autoclaving	Ethanol	Autoclaving	Ethanol	Autoclaving	Ethanol
1	57.168	51.159	54.089	50.355	53.291	49.844	52.948	48.672
2	49.505	50.633	48.437	48.987	48.628	46.223	48.117	48.571
3	61.629	46.996	60.939	46.712	60.917	46.573	60.375	43.702
4	61.423	57.061	59.305	55.683	56.122	53.695	47.079	50.208
Sample Size	4	4	4	4	4	4	4	4
Mean	57.431	51.462	55.693	50.434	54.739	49.084	52.130	47.788
SD	5.670	4.166	5.651	3.808	5.148	3.480	6.063	2.825
95% CI	5.557	4.083	5.538	3.732	5.045	3.410	5.941	2.769

SD: Standard deviation, CI: Confidence interval
Autoclaving: Autoclaved chamber
Ethanol: Ethanol sterilized chamber

APPENDIX E: CRI OF AUTOCLAVED ULTRASONICALLY WELDED CHANNELS AND MULTIWELL CELL CULTURE DISHES IN WEEKS 4-7

Part No.	Week 1		Week 2		Week 3		Week 4	
	USW	Multiwells	USW	Multiwells	USW	Multiwells	USW	Multiwells
1	68.810	86.902	67.091	82.925	59.194	80.447	57.892	73.454
2	72.199	83.404	65.358	83.962	64.603	79.097	67.867	76.365
3	62.605	86.697	59.074	83.864	57.031	78.532	55.953	75.811
4	63.399	86.324	60.385	87.806	54.239	81.267	52.277	75.437
5	80.249	87.997	74.939	86.361	73.742	80.860	71.153	77.788
6	80.117	85.349	74.605	87.632	67.318	78.880	67.583	76.398
7	70.448	84.461	68.256	84.968	62.534	77.295	52.881	75.685
8	N/A	86.765	N/A	87.776	N/A	85.357	N/A	81.010
9	N/A	88.428	N/A	87.456	N/A	81.226	N/A	81.767
Sample Size	7	9	7	9	7	9	7	9
Mean	71.118	86.259	67.101	85.861	62.666	80.329	60.801	77.079
SD	7.111	1.612	6.214	1.949	6.619	2.327	7.858	2.699
95% CI	5.267	1.053	4.603	1.273	4.904	1.520	5.821	1.763

N/A: Not applicable
USW: Ultrasonically welded chamber
Multiwells: 24 well cell culture dish

APPENDIX F: MACHINING TIME FOR WHITE PC PARTS WITH ENERGY DIRECTORS AND CHANNELS

Operations	Tools	Time	
Cleaning the fixture		1 min	
Deburring the edge of a plastic part			30 sec
Taping and attaching a workpiece on the fixture		1 min	10 sec
Surface Machining	1) 0.50 in dia end mill	1 min	30 sec
	2) 0.25 in dia end mill		15 sec
Drilling holes	no.10 (0.193 in) twist drill		35 sec
Screws insert		3 min	30 sec
Peripheral cutting	0.188 in dia end mill	2 min	25 sec
Energy director machining	90 degree angle end mill	3 min	20 sec
Channels machining	0.020 in dia end mill	14 min	30 sec
Finishing	0.040 in dia end mill	3 min	45 sec
Screws removal		2 min	30 sec
Taking parts from the fixture		1 min	

Total time : **36 min** for 1 set (8 pieces)

Each piece takes : **4 min : 30 sec**

APPENDIX G: MACHINING TIME FOR FLAT TRANSPARENT PC PARTS

Operations	Tools	Time
Cleaning the fixture		1 min
Deburring the edges of a workpiece		1 min
Taping and attaching a workpiece on the fixture		2 min 15 sec
Drilling holes	no.10 (0.193 in dia) twist drill	1 min
Bolts and nuts insert		3 min 35 sec
Peripheral cutting	0.188 in dia end mill	3 min 40 sec
Taking parts from the fixture		5 min 15 sec

Total time : **17 min : 45 sec** for 1 set (16 pieces)

Each piece takes : **1 min : 7 sec**

APPENDIX H: PRODUCTION TIME FOR PROTOTYPING ULTRASONICALLY WELDED CHAMBERS

Process	Time	
Machining a flat transparent PC	1 min	7 sec
Machining a white PC with channels and energy directors	4 min	30 sec
Cleaning two PC parts prior to ultrasonic welding	2 min	
Ultrasonic welding	2 min	
Machining of channels' conduits and final shape chamber	9 min	
Cleaning the complete chamber	1 min	

Total production time (Operator time): **19 min : 37 sec**

APPENDIX I: AVERAGE BOND STRENGTH (MPa) OF ULTRASONICALLY WELDED CHAMBER

Part No.	Maximum Load (N)	Area (mm^2)	Bond Strength (MPa)
1	963.1	46.8	20.58
2	955.6	46.8	20.42
3	1085.0	49.4	21.96
4	1012.0	46.8	21.62
5	1019.0	46.8	21.77
6	964.1	46.8	20.60
		Average :	**21.16**

APPENDIX J: COMPARISON TABLE OF THREE DIFFERENT ARCHITECTURES

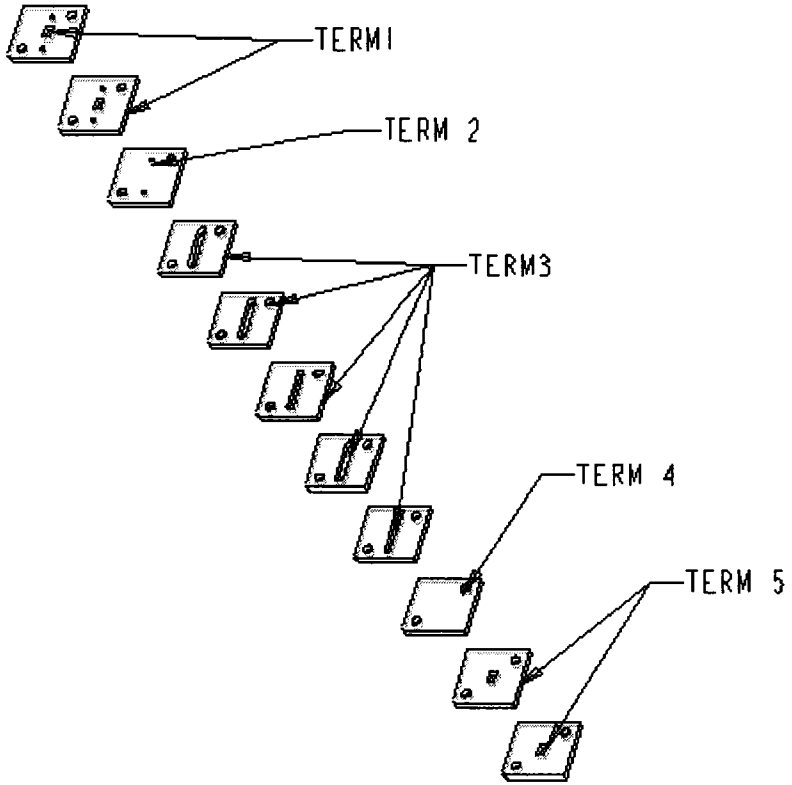
Criteria	Architectures		
	Mercury	Gemini	
		Gemini 1, 2, 3	Gemini 3 USW
Patterning Method	Laser Machining	Laser Machining	Mechanical Milling
Bonding Method	Pressure Sensitive Adhesive	Solvent Welding	Ultrasonic Welding
Design Flexibility	4-6 hr	8-12 hr	6-12 hr
Production Time	2 hr *	3 hr 25 min **	20 min *
Material	Polyester Film	Thick PC	Thick PC
Material Stiffness	No	Yes	Yes
No. of Lamina	11	3	2
Bond Strength	N/A	> 3.18 MPa	21.16 MPa
Sterilization	Gamma Irradiation***	Ethanol	Autoclaving
CRI (at week 4)	N/A	63.7 %	60.8 %

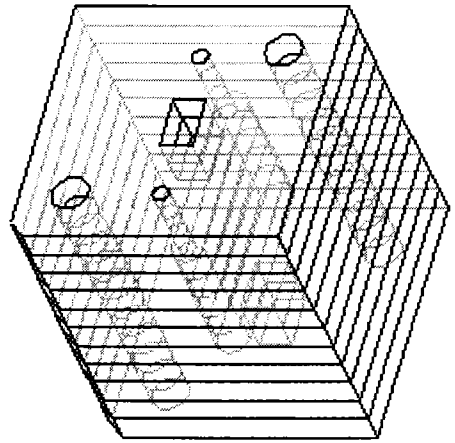
* Operator time

** Total production time: 3 hr 25 min, Operator time: 25 min

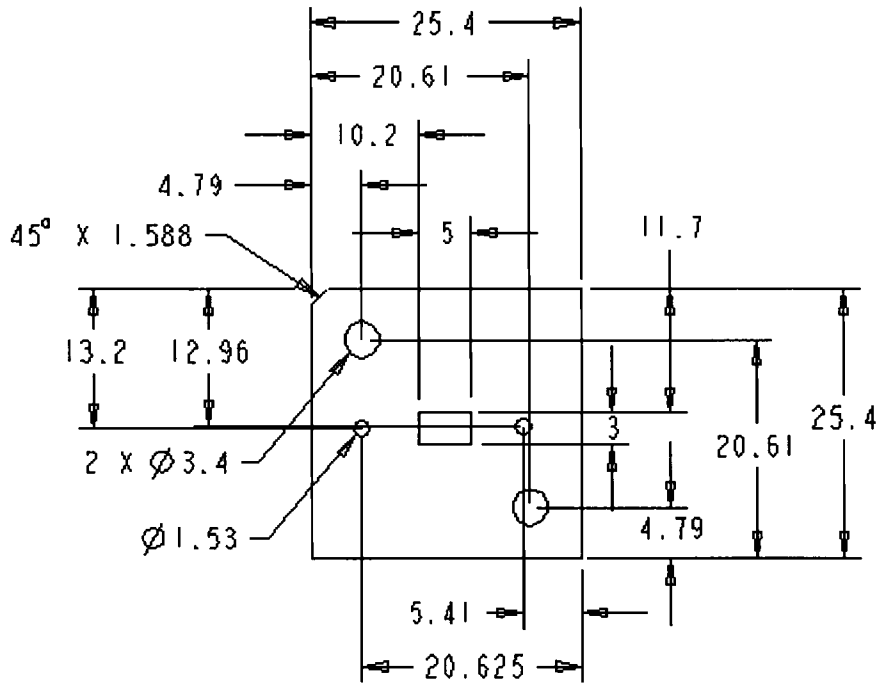
*** 3 MRad, Exposure Time 33 hr

APPENDIX K: MERCURY CHAMBER

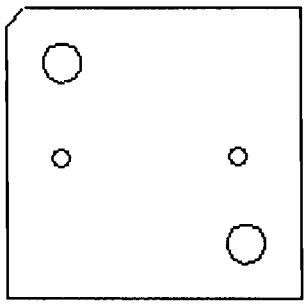




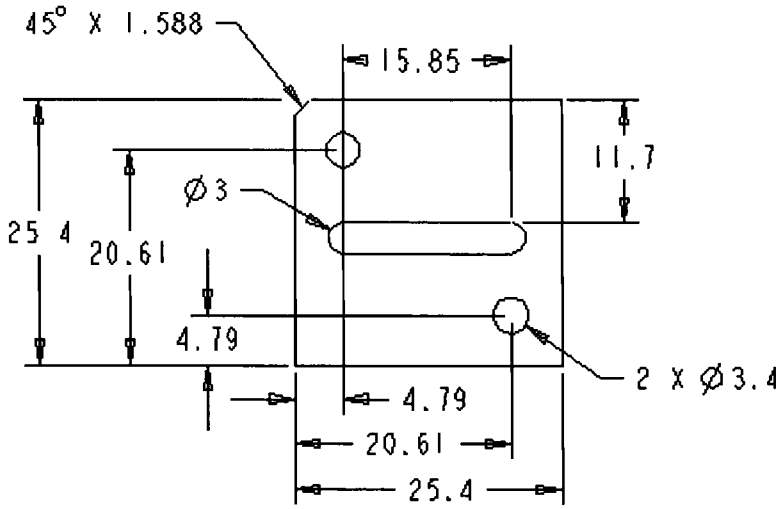
APPENDIX L: MERCURY DRAWING (A) TERM 1 (B) TERM 2 (C) TERM 3 (D) TERM 4 (E) TERM 5



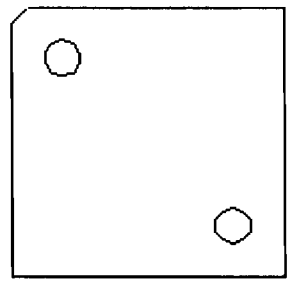
(A) Term 1



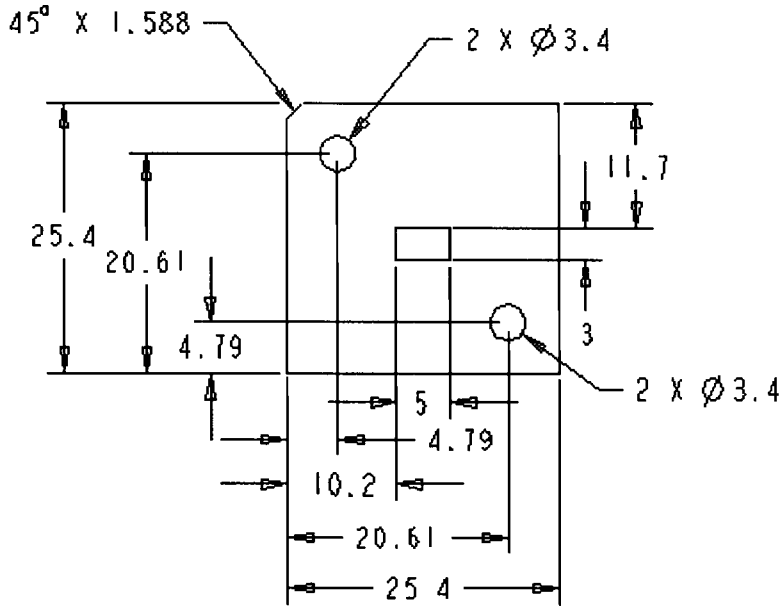
(B) Term 2



(C) Term 3



(D) Term 4



E) Term 5

APPENDIX M: PROCEDURE FOR FABRICATION OF MERCURY CHAMBER

Patterning: Laser machining
Bonding: Pressure Sensitive Adhesive (PSA)

Materials:

1. 3 mil (76.2 μm) thick Melinex 453
2. Double-coated polyester tape, FT 8311
3. Acetone
4. Ethanol
5. De-ionized water
6. Scotch tape

Equipment:

1. ESI 4420 UV laser machine with computer hardware and software
2. Fume hood with water tap, air tap, and sink
3. Pin alignment fixture for laminate
4. Scissors, tweezers, and powderless latex gloves
5. Plastic containers
6. Compression set – requirements include: nut to torque on, clamp stand to secure the compression set while applying torque
7. Torque wrench with an adaptor to a socket, and a socket for the nut on the vise

Procedure:

1. Be certain that the laser is running at 266 nm wavelength.

2. Warm up the laser. Current is 20.5 Amps at 4.5 kHz.

3. Cut the Melinex 453 material from its spool. There should be 6 squares, 1.5 in wide

4. Fasten one Melinex square to the laser platform with scotch tape. Open the SmartCam software file named Term_1. Set the profile speed to 4.5 mm/s, rep rate to 4500 Hz, bite size to 1 μm, and number of passes to 3-4. Then cut the square.

5. Repeat step 4 once for Term_2, twice for Term_3, and once for both Term_4 and Term_5. Be especially careful not to mar the surfaces of Term_2 and Term_4, these are the windows through which the cells are observed.

6. Cut the FT 8311 from its spool into 5 squares, 1.5 in wide.

7. Fasten one FT 8311 square to the laser platform, tacky side up, and open the SmartCam file, Term_5. Use all of the same settings as for the Melinex, except that the number of passes is 25-30.

8. Repeat step 7 three times for Term_3, and once for Term_1. There are no other laminae to cut.

9. While processing the FT 8311, transport the Melinex laminae to the fume hood in plastic containers. Wear a pair of latex gloves.

10. Do an AED wash for each Melinex lamina, and for the alignment fixture, holding the Melinex with tweezers to minimize contamination. The AED wash is as follows: rinse the lamina in the bowl of tap water, squirt acetone, ethanol, and finally with DI water on all sides of the laminae. Dry completely with the air tap.

11. Transport the Melinex laminae in their containers to the fume hood. Cleanliness is the top priority. Lay out the alignment fixture there, as well as the FT 8311 laminae as they are completed.

12. Still using the tweezers, set lamina 11 (Melinex Term_5) in the alignment fixture, then close it to secure the lamina. Set lamina 10 (FT 8311 Term_5) inside with tacky side down. Press the fixture closed firmly with your hands to set the adhesive, and pull the fixture apart again. Then peel the wax paper from the back of lamina 10, set lamina 9 (Melinex Term_4) in the fixture, and press it. Finally, put the fixture in the compression set and torque with the torque wrench to 175 lb-in for 10 seconds.

13. Lay in lamina 8 (FT 8311 Term_3), tacky side down, and hand press it. Then peel off its back, set lamina 7 (Melinex Term_3) in face down, and put the fixture in the compression set. Torque to 175 lb-in.

14. Repeat step 13 for laminae 6 (FT 8311 Term_3) and 5 (Melinex Term_3), then laminae 4 (FT 8311 Term_3) and 3 (Melinex Term_2), and finally laminae 2 (FT 8311 Term_1) and 1 (Melinex Term_1).

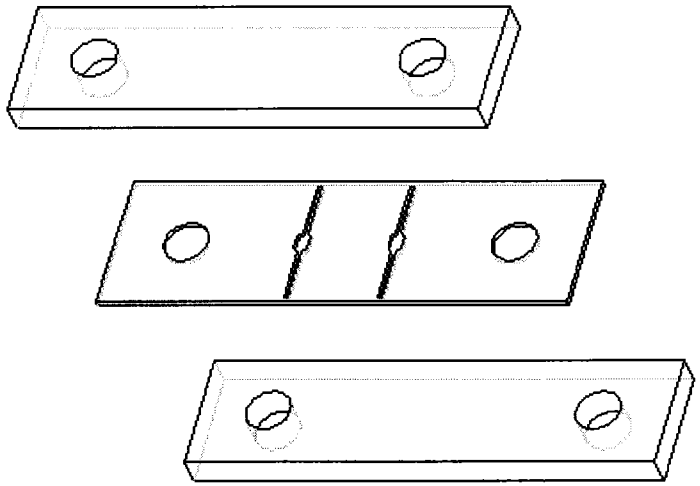
15. Remove the laminate from the alignment fixture and seal it in a zip-loc bag. It is finished.

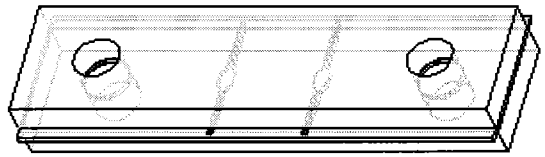
APPENDIX N: PRODUCTION TIME FOR PROTOTYPING MERCURY CHAMBER

Process	Time
Cutting six Melinex laminae by laser (1 min each)	**6 min**
Cutting five FT 8311 Polyester PSA laminae (20 min each)	**100 min**
Cleaning six Melinex laminae	**6 min**
Assembling and bonding	**8 min**

Total production time: **120 min** for a chamber

APPENDIX O: GEMINI CHAMBER





APPENDIX P: PROCEDURE FOR FABRICATION OF GEMINI CHAMBER

Patterning: Laser machining
Bonding: Solvent welding

Materials:

1. (4) 1/8 inch thick Polycarbonate exterior lamina slab
2. (2) 0.030 inch thick Polycarbonate chamber lamina sheet
3. Bottle of solvent with mouth large enough to dip a chamber lamina in the solvent
4. Solvent ingredient: 40% Toluene, 40% Methanol (CH_3OH) and 20% Dichloromethane (CH_2Cl_2)
5. Ethanol
6. Some paper towels and kimwipes

Equipment:

1. Clamp stand
2. Compression set
3. Pin alignment fixture for two chambers in parallel
4. Torque wrench with socket and adaptor for compression set

Procedure:

1. Lay out alignment fixture and Polycarbonate pieces on a clean paper in a fume hood to avoid contamination.

2. Clean Polycarbonate laminae slabs and sheets with Ethanol.

3. Set two 1/8-inch slabs into the fixture.

4. Dip the 0.030-inch sheet in the solvent for five seconds. Optimal conditions would be under a fume hood.

5. Pull out the sheet, shake once to remove the solvent, dry it by an air stream and set into the fixture.

6. Quickly set the other slabs into the fixture, assembly the top of the fixture, and put the fixture in the compression set, which is set in the clamp stand.

7. Using the torque wrench, tighten the compression set down to 120 lb-in.

8. Remove the compression set with the fixture from the clamp stand and keep it in a laminar fume hood for at least six hours.

9. Remove the fixture from the compression set, pull the top from the fixture, and extract the complete laminates. If the laminates stick to the fixture, use a flat-head screwdriver, placed to the outside of the alignment pins, to pry up on the laminates. In doing this, be certain not to mar the viewing window of the laminates or the area of the fixture that contacts them.

10. Keep the laminates in a laminar fume hood for a day to strengthen the bond.

11. Mill off the protruding PC sheet, drill channel ports, then mill the laminate down to the inside edge of the alignment hole, and make a slot underneath the chamber to optimize the light intensity.

APPENDIX Q: PRODUCTION TIME FOR PROTOTYPING GEMINI CHAMBER

Process	Time		
Machining four flat PC parts	4 min	30 sec	
Cutting two PC sheets with channels by laser	30 min		
Finishing PC parts	3 min		
Cleaning the PC parts prior to solvent welding	3 min		
Solvent welding	360 min		
Machining of channels' conduits and final shape chamber	9 min		
Cleaning the complete chamber	1 min		
Total production time:	**410 min:**	**30 sec**	for 1 set (2 chambers)
Each chamber takes:	**205 min:**	**15 sec**	
or	**3 hr: 25 min:**	**15 sec**	

APPENDIX R: AVERAGE BOND STRENGTH (MPa) OF SOLVENT-WELDED CHAMBER

Part No.	Maximum Load (N)	Area (mm)	Bond Strength (MPa)
#1	1233 *	394.6	> 3.12
#2	1301 *	394.6	> 3.30
#3	1261 *	394.6	> 3.20
#4	1235 *	394.6	> 3.13
#5	1254 *	394.6	> 3.18
		Average	**> 3.18**

* All specimen broke at a gripping point.

APPENDIX S: SOLVENT-WELDED SPECIMEN TO TEST BOND STRENGTH