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Accurate, predictable, repeatable micro-assembly technology for polymer, microfluidic modules

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Abstract

A method for the design, construction, and assembly of modular, polymer-based, microfluidic devices using simple micro-assembly technology was demonstrated to build an integrated fluidic system consisting of vertically stacked modules for carrying out multi-step molecular assays. As an example of the utility of the modular system, point mutation detection using the ligase detection reaction (LDR) following amplification by the polymerase chain reaction (PCR) was carried out. Fluid interconnects and standoffs ensured that temperatures in the vertically stacked reactors were within $\pm 0.2\text{ }^{\circ}\text{C}$ at the center of the temperature zones and $\pm 1.1\text{ }^{\circ}\text{C}$ overall. The vertical spacing between modules was confirmed using finite element models (ANSYS, Inc., Canonsburg, PA) to simulate the steady-state temperature distribution for the assembly. Passive alignment structures, including a hemispherical pin-in-hole, a hemispherical pin-in-slot, and a plate-plate lap joint, were developed using screw theory to enable accurate exactly constrained assembly of the microfluidic reactors, cover sheets, and fluid interconnects to facilitate the modular approach. The mean mismatch between the centers of adjacent through holes was $64 \pm 7.7\text{ }\mu\text{m}$, significantly reducing the dead volume necessary to accommodate manufacturing variation. The microfluidic components were easily assembled by hand and the assembly of several different configurations of microfluidic modules for executing the assay was evaluated. Temperatures were measured in the desired range in each reactor. The biochemical performance was comparable to that obtained with benchtop instruments, but took less than 45 min to execute, half the time.

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Keywords

micro-assembly; precise; repeatable; polymer; modular; microfluidics

1. Introduction

Molecular analysis is one of the fundamental tools enabling a deeper understanding of many fields, particularly molecular biology, medicine, and forensics [1]. Sophisticated assays for a wide variety of applications provide researchers and practitioners with more detailed and timely information to guide diagnostic decision-making and/or threat assessment. Most of these assays require the use of a series of processing steps and multiple benchtop instruments to carry out each step [2]. Although the cost of these tools is decreasing rapidly, market penetration is limited by the size, initial cost, maintenance costs, and the need for trained operators to perform the required assays and interpret the results.

Progress in the development of micro- and nanofabrication technologies and the understanding of the behavior of fluids on the micro- or nano-scale has enabled implementation of fluidic reactions in very small channels [3–5]. It has the potential to replace benchtop protocols for performing complex biological and chemical reactions with low-cost, disposable systems without the need for an expert operator due to the process automation afforded with integrated microfluidic systems [6,7]. As these systems allow inexpensive, high-throughput analyses by reducing the reagent volumes, cutting processing times, and performing multiple assays simultaneously, they should play a major role in a variety of fields that require molecular analyses [1,8,9].

Micro- and nanofluidic systems for complete biochemical analyses can perform most of the typical laboratory tasks done with conventional instrumentation. For genetic analyses, steps including isolation and purification of the target material, amplification, and detection of biomarkers, may be carried out by various combinations of functional components, such as reactors, mixers, valves, sensors and actuators, integrated into microfluidic systems. There are two principal strategies for the integration of a variety of functional components in a microfluidic system: (1) a monolithic approach [10] and (2) a modular approach [11]. In the monolithic approach, all of the functional components, which are needed for an intended analysis, are integrated onto a single substrate. Full monolithic integration is preferred to minimize device size, but the need for relatively complex, often across mixed scales, fabrication techniques is increased by putting all of the components on a single plane [10,12,13]. Maintaining distinct temperature zones is more difficult in planar devices with a trade-off between larger footprints, greater complexity, and biochemical performance [14,15]. Compromises are required in materials selection; for example, poly(methyl methacrylate), PMMA [16], or cyclic olefin copolymer (COC, Topas 6013) [17,18] is better for functionalization in a capture device, but cannot withstand the typical denaturation temperatures used in amplification, such as PCR, due to its low glass transition temperature; polycarbonate [19] or high temperature grades of COC [20,21] because of its higher glass transition temperature can be used for PCR. Applying design changes to obtain better performance or incorporate new technologies is more difficult with monolithic microfluidic

systems. The costs of production may be too high to produce large numbers of disposable microfluidic systems in comparatively small markets.

In the modular approach, each device or component is developed separately and connected by fluidic interconnects to build a fluidic system [22]. Modular micro- and nanofluidic systems provide flexibility in design and fabrication enabling device-specific material selection and the ability to add additional functions, or extra options, to enhance system performance [23,24]. In addition, each device or component, although designed for one application, may be used for other applications. To obtain the advantages of a modular approach, fluidic interconnects must be designed to minimize dead volume, support easy assembly, and withstand operating pressures. It is one of the major challenges facing broader application of the modular approach.

Interest in the modular approach for the design of microfluidic systems is increasing. An early stacked microfluidic system was reported by Verpoorte *et al.* who demonstrated fluidic manifolds in silicon [25]. Pyrex wafers were used to implement a microfluidic circuit board concept [26]. Building blocks in poly(dimethylsiloxane), PDMS, were introduced by Hofmann and used to demonstrate a sheath flow [27]. A bus modular microfluidic architecture was developed for detection of biochemical species [11] and for cell pre-concentration [28]. The latter consisted of passive fluidic components and active electromechanical control structures. Another modular approach was reported to form customized microfluidic systems [29]. Individual microfluidic components, called microfluidic assembly blocks (MABs), were made from PDMS and aligned and assembled on either a glass cover slide or a PDMS coated glass slide, by tweezers with an optional use of a high magnification stereoscope. A breadboard approach, the Smart Build Plug n Play™ system from Corning, used a fluidic motherboard and different types of functional modules, connected using modified luer-type connectors to build planar and 3-D systems [30,31]. Another breadboard approach employed a fluidic motherboard including thermal reactors for biochemical reactions and two functional modules. The minimal unswept volume, fluidic interconnects between modules were formed using microtubes centered in conical ports hot embossed in the facing surfaces [32]. Another system used tapered microtube interconnects between several functional modules and a fluidic substrate [33].

The combination of multiple components, which can be manufactured in high volume at low cost, is necessary to develop microfluidic systems for various applications. As shown by the development of other industries such as automobiles or computers, modularization may be the most effective way to reduce both time and cost in the development of products. The trend may follow the cost-effective ways of other fields [34].

Polymers have emerged as important materials for use in microfluidic applications due to their many advantages [34,35]. Polymers are inexpensive when compared with silicon or other options [36]. The use of polymers as substrates allows for low cost, batch fabrication by using replication-based methods, such as hot embossing for small lots and injection molding for larger production runs. Different polymers offer a wide range of properties that are unavailable in other materials for biological and chemical applications. PDMS is a key material for easy, rapid, and low cost prototyping of microfluidic devices for research

purposes at the early stages of development, but has several problematic characteristics [37]. Thermoplastic polymers, such as polycarbonate (PC), poly(methylmethacrylate) (PMMA), cyclic olefin copolymer (COC), and polystyrene (PS), have also been used in many microfluidic applications. Compared with PDMS, they are preferred for mass production at low cost and their surface chemistry is more stable [38]. A variety of polymer microfluidic systems were reported in the field, based on PDMS [39,40] and thermoplastic polymers [41–45].

A modular microfluidic architecture and fabrication methods allow reticulation of complex biological assays into distinct functional elements and enable optimization of individual components. A vertically stacked modular microfluidic system made from thermoplastics was developed. Simple assembly techniques using polymer passive alignment structures and fluidic interconnects were designed to facilitate the realization of customized microfluidic systems with a modular architecture. Detection of point mutations in the *K-ras* gene of the human genome with the ligase detection reaction (LDR) preceded by PCR, shown schematically in the Supplemental Information, was selected as a demonstration of the vertically stacked system [46,47]. Several different configurations of microfluidic modules for executing the assay were assembled with two, one comprised of three steps and the other four, characterized in detail biochemically [23,24].

2. Materials and Methods

2.1 Microfluidic Device Design

2.1.1 Module Architecture—The modules integrated different combinations of the following steps in a flow-through format: (1) PCR to amplify target variations; (2) mixing of the PCR products with the purification reagents; (3) purification of the PCR products; (4) mixing of the PCR products and the LDR reagents; and (5) LDR to detect point mutations. Vertical and horizontal spacing of the different steps was determined by the optimal temperature required for each reaction. Schematic polymer chips for the individual reactors are shown in Fig. 1A (three device configurations) and 1B (two device configuration).

Two types of modular interconnects were used in the assembly of the modules: (1) A combination of a microfluidic passage and a precision standoff; and (2) a precision standoff. Microscale passive alignment structures were incorporated to exactly constrain all of the components in the module. They were used to align chips containing functional fluidic structures with cover plates containing thermal management structures and on the microfluidic interconnects between the functional devices.

2.1.2 Design and Analysis of Passive Alignment Structures—Passive alignment structures were developed to assemble microfluidic components fabricated by polymer microfabrication technologies such as hot embossing and injection molding. Combinations of features can be assessed by screw theory, which can be used to represent and analyze the degree of constraint provided by different assembly features. A well-defined toolkit of features, which can be used directly in engineering design, with the twist matrix for each was presented by Whitney *et al.* [48] and Adams and Whitney [49]. You *et al.* showed the feasibility of using polymer passive alignment structures, comprised of three pairs of

hemisphere-tipped posts mating with v-grooves, to exactly constrain modular devices [50] and the accuracy of that alignment for injection molded parts [51]. Over- and under-constraint can introduce unpredictable dead volumes due to variability in assembly forces and feature dimensions. To demonstrate the flexibility and applicability of the technology, an alternative set of alignment features were selected to exactly constrain the rigid plates used for module fabrication based on their manufacturability. These included a hemispherical pin-in-hole, a hemispherical pin-in-slot, and a plate-plate lap joint, as shown in Fig. 2.

Each feature can be modeled as a twist matrix as shown in the Appendix [49]. A resultant twist matrix (TR) for the assembly, which consisted of the intersection of the feature twist matrices, was used to analyze the relative motion permitted by the alignment features. The resulting null twist matrix indicated that there were no unconstrained degrees of freedom with the selected features, so these parts were unable to move relative to each other. To determine the presence of over-constraint in the design, a constraint analysis was done by evaluating the intersections of all of the wrench matrices for each feature (see Appendix). The resultant wrench matrix (WR) for the assembly considering all of the features simultaneously was null, so there were no residual forces or moments. In addition, the resultant wrenches for all of the different combinations of the features were checked independently, because they may constrain a particular degree of freedom. All of the subsets, each consisting of two or three assembly features, were checked systematically and found to be null also, so there was no over-constraint in the assembly.

The set of constraints can be used to locate a part at any particular desired position with respect to another part. It helped to prevent infinitesimal motions and minimize misalignment between assembled components without any additional instruments or jigs.

2.1.3 Thermal Design—The biological reactions for both amplification and ligation required thermal cycling for the analysis, so thermal management was an important design criterion. During each thermal cycling step the temperatures must match the specified reaction conditions to obtain higher purity and better yields of the amplified or ligated products. Both the continuous flow PCR (CFPCR) and continuous flow LDR (CFLDR) modules needed multiple temperature zones for thermal cycling. In addition, the temperatures in the passive micromixer needed to be balanced between thermal isolation to avoid bubble formation at the mixing junctions and thermal activation to enhance mixing in the diffusion channel. Chen *et al.* showed that in a planar polymer CFPCR, well-defined temperature zones were sufficient to increase amplification yield by over 300% over the same device without thermal management measures taken [14]. For the vertically stacked modular microfluidic architecture including two thermal reactors for the PCR and LDR, heat transfer was considered in both the lateral and vertical directions to minimize thermal crosstalk between components.

Finite element-based thermal simulations using ANSYS (v.11.0, Canonsburg, PA) were employed to ensure uniform temperature distributions in the PCR and LDR reactors, isolation of the mixing junction in order to avoid heat-induced bubble formation in the micromixer, and minimal thermal cross-talk due to the asymmetric thermal zones in the PCR and LDR reactors (see Fig. 3A). A 3D thermal solid element, a ten-node tetrahedron

(SOLID87) [52], was used to mesh the vertically stacked, polymer, microfluidic module, including the PC, copper plates, and thermal insulators. Grid sensitivity was evaluated using three different element sizes to ensure that the simulated results were independent of element size. The number of elements in the coarse mesh was 153,184, the medium mesh had 387,402 elements and the fine mesh 869,472 100 μm elements.

2.2 Fabrication and assembly

2.2.1 Fabrication of polymer parts—All polymer parts, including reactors, cover plates, and interconnects, were molded from clear, PC stock (LEXAN 9034, GE Plastics, Pittsfield, MA) using micro-milled (MMP, KERN Micro- and Feinwerktechnik GmbH & Co KG, Eschenlohe, Germany) brass mold inserts [53]. Depending on the features on each, parts were replicated with either single-sided or double-sided hot embossing using a Jenoptik HEX-02 ((Jena, Germany).

The passive alignment features were applied to the assembly of the components of each microfluidic reactor, aligning reservoirs on each component before bonding. The patterns for the alignment structures were also micro-milled on the brass mold inserts for each part (see Fig. 2). The hemispherical pins were machined using a 794 ± 0.25 μm diameter ball end mill (McMaster-Carr, Elmhurst, IL, USA). The nominal depth was 397 μm on the mold insert. The diameters of both the holes and slots were selected as 770 μm . The height was 450 μm with a 3° draft angle on the brass mold insert.

Continuous, serpentine microfluidic channels used as reactors were fabricated for the required thermal cycling for the PCR and LDR. The reactors were single-sided hot embossed in 500 μm thick PC substrates.

Cover plates sealed the microfluidic channels in the reactors, incorporated thermal management features to decrease lateral heat conduction between temperature zones, and mated with the interconnects on the opposite side. Thermal isolation grooves were patterned on the back-side mold using a 200 μm diameter end mill (Contour Fine Tooling, Marlborough, NH, USA). For the cover plates, 3 mm thick PC was double-sided hot embossed. The fluidic interconnects, with reservoirs and alignment structures, and standoffs, with only alignment structures on each side, were double-sided hot embossed in 4.8 mm (3/16 inches) thick clear PC.

After the embossed parts were diced by a table saw (MicroLux Miniature Table Saw, Micro-Mark, NJ, USA), the back-side of the microfluidic reactor was planarized using a fly cutting machine (Precitech, Keene, NH, USA) to clear the holes and slots of the alignment structures. Through holes in the cover plates and interconnects were made using ArF 193 nm excimer laser ablation (Rapid X 1000, Resonetics, Nashua, NH).

Prior to thermal fusion bonding, parts were cleaned using the following steps. First, they were immersed in a 1% detergent solution in an ultrasonic cleaner (Branson, Danbury, CT, USA) for 1 min. Then they were washed with isopropyl alcohol (IPA) and deionized (DI) water in sequence. Third, they were cured in an oven at 85°C overnight; and then, UV exposure was used to activate the contact surfaces and reduce the glass transition

temperature at the contact surfaces in order to avoid significant deformation of functional structures and enable a bonding temperature below the T_g [54,55].

The reactors were thermal fusion bonded with the cover plates using the guidance of the passive alignment features without any additional tools. They were sandwiched between borosilicate glass plates and placed in a convection oven (VWR 1602, VWR Scientific, Inc., Radnor, PA, USA) at 146 °C for 2 h.

2.2.2 Fabrication of heater modules—Temperature control units were fabricated and installed on the modules. Kapton heaters (HK5569; HK5574, Minco, Inc., Minneapolis, MN) were mounted on 1 mm thick copper sheets with the same cross-sectional areas. Mini-hypodermic, Type T thermocouples (HYP0, Omega Engineering Inc. Stamford, CT) were inserted into end milled microchannels, with dimensions of 0.22 mm wide, 0.22 mm deep and 14 mm long, at the center of each copper sheet. Separate heating units were attached to each temperature zone for both the PCR and the LDR reactors using thermally conductive tape (3M, St. Paul, MN, USA).

The heating units were connected to a Fieldpoint system (National Instruments, Inc., Austin, TX, USA), a real-time controller. LabVIEW (v6, National Instruments, Inc., Austin, TX, USA) with a PID toolkit was used to control and monitor the temperatures in the module for real-time feedback. A PI algorithm was employed for temperature control.

2.2.3 Module Assembly—Circular, adhesive labels (Office Depot, Boca Raton, FL, USA) about 3 mm in diameter were punched out using a standard paper hole punch. These were used as protective masks for the reservoirs on the devices and interconnects. The remaining exposed contact surfaces were coated with SU-8 adhesive in an aerosol form (MicroSpray, MicroChem, Newton, MA). After the components, interconnects, and standoffs were assembled, they were clamped between glass plates and cured at 90 °C for 10 minutes in a convection oven.

2.2.4 Temperature Measurement—Microchannels, 0.22 mm wide, 0.22 mm deep and 14 mm long were fabricated to monitor temperatures at the center of each temperature zone in the microfluidic PCR and LDR reactors. After the reactors were aligned with cover plates, they were thermally bonded. The same method for the module assembly was employed to produce a module for temperature measurement. After the mini-hypodermic, Type T thermocouples were inserted into the microchannels, the heating units were mounted on each temperature zone. Actual temperatures at the center of each temperature zone were measured.

2.2.5 Polymer Micro-Channel Preparation—A solution including bovine serum albumin, BSA (NEB, Ipswich, MA) with 1 $\mu\text{g}/\mu\text{L}$ was injected into the fluidic network for a leakage test of the module. The solution also helped decrease enzyme absorption on the surfaces of the microchannels in the module that could deactivate the enzyme. After each use, the module was completely purged by air and dried in an oven at 45 °C for 1 h.

3. Results and discussion

3.1 Thermal Management

3.1.1 Thermal Simulations—Two thermal reactors were used in the PCR/LDR modules. Thermal system performance was evaluated to estimate the steady-state temperatures in each temperature zone with the spacing between adjacent temperature zones and the height of the air gap between reactors varied. Thermal cross-talk between temperature zones and devices can significantly degrade the performance of each analysis step.

Representative temperature distributions are shown for the PCR (Fig. 3B) and the LDR (Fig. 3C) with an air groove 2 mm deep and 3 mm wide between each temperature zone and 4.5 mm high air pockets between reactors. Most of temperature zones were at the target temperatures, within a variation of ± 1.1 °C except in the transition zones and close to the edges of each temperature zone. These produced the desired temperature distribution and uniformity in each reactor and were used in the modules.

Based on the thermal simulations, the height of the fluidic interconnects needed to be >4 mm. The fluidic interconnects included hemispherical pins that mated with the hole and slot features on the microfluidic devices for assembly. The final thickness fabricated using double-sided embossing was approximately 4.5 mm.

3.1.2 Temperature Measurements—Temperatures at the center of each temperature zone were measured using needle-type thermocouples. The three temperature zones for the PCRs were within a variation of ± 0.2 °C of the target temperatures at the center of each temperature zone. Initially, the temperature in the 64 °C ligation zone for the LDR device slightly overshoot the target temperature. The denaturation temperature set point was decreased from 95 °C to 90 °C, and the final ligation temperature was within ± 0.2 °C of the 64 °C target value.

3.2 Module Architecture and Assembly

Two modules biochemical performance were tested. The first was comprised of three devices configured in a flow-through format: a microfluidic PCR reactor, a passive micromixer, and a microfluidic LDR reactor (see Fig. 4A and 4B). The second module with essentially the same architecture as the first added a purification step before the addition of the LDR reagents to eliminate unwanted by-products of the amplification. The performance of both modules is presented in Han, *et al* [24]. A third module (Fig. 4D and 4E), incorporated only the passive micromixer and the microfluidic LDR, enabling on-chip ligase detection reaction for point mutation identification if purified PCR amplicons are available was assembled, but not tested biochemically.

Images of the individual device chips for each module are shown in Fig. 4A (3 chip module) and Fig. 4D (2 chip module). Assembled polymer modules for tested configurations are shown in Fig. 4B, with two sets of interconnects used (labeled with numbers 4 and 5 in the figure). The two device module only required a single set of interconnects and the assembled components are presented in Fig. 4E.

Cross-sectional views of the three- and two-device configurations are given in Fig. 4C and 4F, respectively. In Fig. 4C, two alignment structures, outlined in boxes with blue dashed lines, are shown on the top and bottom of the reactor, aligning the reactor with the cover plate and the fluidic interconnect. The laser-drilled fluid port from the reactor through the fluid interconnect is also shown enclosed in the box with yellow dashed lines for its boundary. Fig. 4F shows the laser drilled hole in the two-device module.

3.3 Micro-assembly Accuracy

The passive alignment structures were used to assemble the microfluidic reactors and cover plates. The microfluidic reactors included the microchannel network for nucleic acid processing. The cover plate was needed to seal the microchannel network. It contained a fluid port and grooves for thermal isolation. The accuracy provided by the passive alignment structures used for micro-assembly is shown in Fig. 5. After hot embossing, the microfluidic reactors and cover plates were aligned for thermal bonding using only the passive alignment structures. The location of each hemispherical pin was checked to ensure that it matched with a hole or a slot (see Fig. 5B & 5C). If it matched, the laser-drilled hole in the cover plate was aligned well with the outlet of the reactor (see Fig. 5D & 5E). The distance from the center of the laser drilled hole on the cover plate to the center of the outlet of the reactor was measured to evaluate the alignment accuracy and quantify the mismatch in the assembled parts after thermal fusion bonding (Fig. 5F). Three sets, each including three assembled parts, were characterized. The mean mismatch was $64 \pm 7.7 \mu\text{m}$ that resulted from tooling errors, variation due to molding of the thermoplastics, and variation in the position of the passive alignment structures on the brass mold inserts (see Fig. 2B). The accuracy of the alignment structures enabled the use of smaller, laser drilled holes with $150 \mu\text{m}$ diameter coupled with reactor inlets or outlets of $300 \mu\text{m}$ diameter, reducing the dead volume at each fluidic interconnect and the footprint of the components in the module.

3.4 Module Performance

The assembly features aligned a laser drilled hole in the assembled microfluidic device with a laser drilled hole in the microfluidic interconnects. After adhesive bonding, the aligned holes formed fluidic passages between modules. No leakage was found in any of the fluidic passages when fluorescent dye was pumped through the modules.

The biochemical performance of the system is reported in detail in Han, et al. [24]. A brief discussion of the benchtop experiments used to design the flow-through modules and representative gel electrophoresis and image quantitative analysis results are shown in the Fig. S2 of the Supplemental Information.

4. Conclusions

Interconnects and alignment structures were developed as tools to accurately, repeatably assemble thermoplastic devices into modular architectures and generate fluidic systems to carry out multi-step molecular assays. The modular system proved advantageous for the detection of mutations of the *K-ras* gene. The LDR reaction was used to identify the

mutations in concentrations up to 1:200 mutated to wild type ratios in half the time needed with conventional, benchtop instruments.

All components were produced by single- or double-sided hot embossing of PC substrates. Components were assembled using passive alignment structures and thermally bonded with a cover plate. Microfluidic interconnects and spacers were developed for stacking components in the vertical direction. Polymer, passive alignment structures enabled accurate relative alignment of through holes of $64 \pm 7.7 \mu\text{m}$, reducing the potential dead volume required. This micro-assembly technology is suitable for the interconnection of microfluidic devices for sequential modular realizations of biochemical assays requiring multiple processing steps.

Each of the biochemical reactions required for mutation detection with LDR were reticulated into separate steps that could be executed in sequence to facilitate realization of a stacked, modular microfluidic system through simple assembly. To enable the assay in the modular format, continuous-flow microfluidic components were developed; batch process components may also be used with the modular architecture, however. Each reactor module can perform its primary function, such as PCR, mixing, or LDR, without assembly as standalone units or they can be combined to perform multi-step assays.

As there is no single modular design that is applicable for all assay conditions, the system can be adapted for different assays by modifying a few components to cover different processing conditions, instead of changing the overall design of a module or system. This is a major advantage in the use of the modular microfluidic architecture for clinical diagnostics, which in many cases require biochemical processing using different steps. Although a system was demonstrated for processing one sample in this study, many samples may be handled in parallel by incorporating arrays of similar reactors, such as the micro-titer plate formatted reactors of Park *et al.* [56] and Chen *et al.* [15] using similar assembly technology.

By using the established replication technologies for thermoplastic polymers, such as injection molding, low cost, disposable detection tools for known disease-causing mutations can be produced. Other PCR-based detection assays in diagnostic applications may also be realized in a similar format. In addition, easy assembly with acceptable accuracy under microscale conditions can allow three-dimensional fluidic operations as well as large-scale fluidic integration in a mass production environment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix: Analysis of passive constraints using screw theory

Two types of screws are used: the instantaneous rotational and translational motions of a rigid body are expressed as twists; and the force and torque exerted on a rigid body are represented by a wrench acting along an about an axis. Twist matrices (T) and wrench (W) matrices can be obtained for the combined action of several alignment features on an assembly.

Each alignment feature can be modeled as a twist matrix (T), as shown in Eqs. 1–3 for the three used in the module:

$$T_{Hemipin-Hole} = \begin{bmatrix} w_x & 0 & 0 & 0 & 0 & -d_y w_x \\ 0 & w_y & 0 & 0 & 0 & d_x w_y \\ 0 & 0 & w_z & d_y w_z & -d_x w_z & 0 \\ 0 & 0 & 0 & 0 & 0 & v_z \end{bmatrix} \quad (1)$$

$$T_{Hemipin-Slot} = \begin{bmatrix} w_x & 0 & 0 & 0 & 0 & -d_y w_x \\ 0 & w_y & 0 & 0 & 0 & d_x w_y \\ 0 & 0 & w_z & d_y w_z & -d_x w_z & 0 \\ 0 & 0 & 0 & 0 & v_y & 0 \\ 0 & 0 & 0 & 0 & 0 & v_z \end{bmatrix} \quad (2)$$

$$T_{plate-plate} = \begin{bmatrix} 0 & 0 & 0 & v_x & 0 & 0 \\ 0 & 0 & 0 & 0 & v_y & 0 \\ 0 & 0 & w_z & 0 & 0 & 0 \end{bmatrix} \quad (3)$$

Substituting the parameter values used in the module, Using that the X and Y components of vectors: d_1 , d_2 , and d_3 are $L_1 = L_2 = L_3 = 7$ mm and $L_4 = 8$ mm, obtained by the dimensions of microfluidic components. $w_x = w_y = w_z = 1$ and $v_x = v_y = v_z = 1$ were assumed. The twist matrices for each feature can be obtained, as shown in Eqs. (4)–(6):

$$T_{F1-Hemipin-Hole} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & -7 \\ 0 & 1 & 0 & 0 & 0 & 8 \\ 0 & 0 & 1 & 7 & -8 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix} \quad (4)$$

$$T_{F2-Hemipin-Slot} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & -21 \\ 0 & 1 & 0 & 0 & 0 & 8 \\ 0 & 0 & 1 & 21 & -8 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix} \quad (5)$$

$$T_{F3-Plate-Plate} = \begin{bmatrix} 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \end{bmatrix} \quad (6)$$

The wrench matrices, which describe the resultant force and moment acting on each feature, can be found by taking the reciprocals of twist matrices because the vector dot product of the twist and the wrench equals zero. They are shown in Eqs. (7)–(9):

$$W_{F1-Hemipin-Hole} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & -7 \\ 0 & 1 & 0 & 0 & 0 & 8 \end{bmatrix} \quad (7)$$

$$W_{F2-Hemipin-Slot} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & -21 \\ 0 & 0 & 0 & 0 & 0 & 8 \end{bmatrix} \quad (8)$$

$$W_{F3-Plate-Plate} = \begin{bmatrix} 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \end{bmatrix} \quad (9)$$

The motion analysis can be done by intersecting all of the twist matrices. The resultant twist matrix (TR) for the assembly, which consists of the intersection of the twist matrices, can be obtained:

$$TR_{Hemipin-Slots-Plate} = [] \quad (10)$$

This null twist matrix indicates that there are no unconstrained degrees of freedom of motion. Therefore, these parts are unable to move relative to each other. The constraint analysis can be done by intersecting all of the wrench matrices. The resultant wrench matrix (WR) for the assembly using the same method is

$$WR_{Hemipin-Slots-Plate} = [] \quad (11)$$

There is no force or moment that is provided by all four features. However, all resultant wrenches should be checked because the different combinations of features can constrain a particular degree of freedom. Therefore, all subsets consisting of two or three assembly features were systemically checked as follows:

$$WR_{subset1} = \cap(W_{F0}, W_{F1}) \quad (12)$$

$$WR_{subset2} = \cap(WR_{subset1}, W_{F3}) \quad (13)$$

$$WR_{subset3} = \cap(WR_{subset2}, W_{F4}) \quad (14)$$

All wrench subsets were checked by Eqs. (12)–(14) with Eqs. (7)–(9). As all of the wrench spaces were null, there was no over-constraint in the assembly. From both the motion analysis and the constraint analysis, the assembly was exactly constrained. This design can be used to locate a part at any particular desired position with respect to another part. It helped to prevent infinitesimal motions and minimize the misalignment between assembled components.

Biographies

Tae Yoon Lee is an assistant professor in the Department of Technology Education and the Department of Biomedical Engineering, Chungnam National University, Republic of Korea. He obtained his BE degree (Mechanical Engineering) from Kyunghee University, Republic of Korea in 2002 and a Ph.D. degree (Mechanical Engineering) from Louisiana State University – Baton Rouge, USA in 2009. His main research interests are concerned with biosensors and microfluidic devices to solve major technical problems for diagnosis of human diseases.

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Sunggook Park is currently the L.R. Daniel Associate Professor of Mechanical Engineering at Louisiana State University. He received his B.S. and M.S. in 1996 and 1998, respectively, from the Department of Chemical Engineering, Yonsei University, Seoul, Korea. He earned his Ph.D. in Physics from the Technical University, Chemnitz, Germany in 2002, with a dissertation on the electronic properties of surfaces and interfaces of organic semiconductors. He moved to the Laboratory for Micro- and Nanofabrication at the Paul Scherrer Institute as a postdoctoral researcher from 2002–2004 where he gained expertise in nanomolding and nanofabrication. His current research interests at LSU focus on fundamentals of the nanomolding process such as thermal and mechanical behavior of polymers, applications of molded structures in BioMEMS/NEMS, and 3-D patterning. He was a recipient of the NSF Young Faculty Development CAREER award in 2007. He is currently a co-PI for the NIH P41 Center for BioModular Multi-Scale Systems for Precision Medicine and is leading the LSU effort for that center.

Steven A. Soper received his Ph.D. from the University of Kansas in 1989 in Bioanalytical Chemistry. He then did a post-doctoral fellowship at Los Alamos National Laboratory, where he worked on single-molecule detection approaches for DNA sequencing. He is currently a Foundation Distinguished Professor at the University of Kansas in Mechanical Engineering and Chemistry and Director of the NIH-sponsored Center for BioModular Multiscale Systems for Precision Medicine. His work is focused on developing polymer-based BioMEMS and BioNEMS for cellular and molecular processing for in vitro diagnostics; single-molecule DNA/RNA sequencing technologies; and surface modification of polymer micro- and nanochannels. He is the founder and currently Chief Scientific Officer at BioFluidica, Inc., which is developing microfluidic platforms for the isolation of circulating tumor cells and Digital Nanogenetics, which is developing modular fluidic systems for disease detection and diagnosis.

Michael C. Murphy, an Emeritus Professor of Mechanical Engineering at Louisiana State University in Baton Rouge, received the BS degree in Mechanical Engineering from Cornell University in 1977 and an MS in Aeronautics from Caltech in 1978. Between 1978–1985 he was with the Missile Systems Group of Hughes Aircraft Company in Canoga Park, CA as a member of the technical staff and staff doctoral fellow. He received his PhD from MIT in Mechanical Engineering in 1990 and spent the next two years as an instructor for design there, before joining the Department of Mechanical Engineering at Louisiana State University in Baton Rouge in 1992. He spent 1992–1993 as a visiting scientist at the Institut für Mikrostrukturtechnik at Forschungszentrum Karlsruhe. At LSU, he helped establish the microsystems research facilities and program. In 1995, he was awarded an NSF CAREER proposal for work in medical applications of microsystems and was awarded a Roy O. Martin Lumber Co. Professorship of Mechanical Engineering from 2007–2008 and 2010–2016. His research interests continue to be focused on biomedical applications of microsystems and modular microsystems, and he is the leader of the system integration effort for the NIH P41 Center for BioModular Multi-Scale Systems for Precision Medicine.

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Highlights

- Applicable to the interconnection of microfluidic devices for sequential modular realizations of biochemical assays requiring multiple processing steps in micro total analysis systems (μ TAS) format.
- Precise, repeatable micro-assembly of molded polymer devices and modules is necessary to lower the cost of biomedical analyses for broader clinical application.
- The mean mismatch between the centers of adjacent through holes was $64 \pm 7.7 \mu\text{m}$.
- Precision assembly leads to predictable, minimal dead volumes between modules.
- While demonstrated for a vertically-stacked single flow path system, it may be applied to highly parallel, micro-titer plate formatted systems in the same manner.

Figure 1.

A single flow path was designed and fabricated to realize the sequential, continuous-flow PCR and LDR reactions modular format. (A) Layout of the first microfluidic module (three-device configurations) in a stacked format including: (1) PCR microfluidic reactor, (2) Passive micromixer, and (3) LDR microfluidic reactor, (4) Microfluidic interconnect (5) Spacer. Reagents are S1: PCR cocktail; S2: two short allele-specific synthetic primers with a reaction buffer; and S3: *Taq* DNA ligase with a reaction buffer; (B) Layout of a two-device microfluidic module for the LDR reaction with two devices: (2) Passive micromixer, and (3) LDR reactor, mated using the (4) Microfluidic interconnect, and (5) Spacer.

Figure 2.

Assembly features for micro-assembly: (A) Schematic of the combination of hemispherical pin in slot, hemispherical pin in hole and plate-plate lap joint. Final dimensions (in mm) are: Module Length = $L = 56$; Module Width = $W = 28$, $L_1 = L_2 = L_3 = 7$; $L_4 = 8$; and (B) Pictures of micromilled brass mold inserts showing the patterns for the alignment features.

Figure 4.

(A) Assembled and thermally bonded microfluidic devices for three-device configurations (PCR/LDR), each of which consisted of a microfluidic reactor and a cover. The devices are: (1) a microfluidic PCR reactor, (2) a passive micromixer, and (3) a microfluidic LDR reactor (shown inverted so that the microfluidic structures are visible. The microfluidic reactor faces down in the final assembly in Fig. 4B to accommodate mounting the heaters). Each pair of devices is assembled with (4) a microfluidic interconnect and spacer between them; (B) Fully assembled microfluidic module in three-device configuration for mutation detection. Two 4.5 mm thick microfluidic interconnects (4) and spacers (5) were used to provide precise and consistent assembly of the different devices. The white dashed line shows the cutting plane through the microfluidic interconnect for the cross-section in Fig. 4C; (C) Cross-section of an assembled module for the three-device configuration, showing the laser drilled fluidic passage (yellow box) and the passive micro-assembly features (blue boxes) used for aligning the reactors and covers with each other and the devices and microfluidic interconnects; (D) Assembled and thermally bonded microfluidic devices for a two-device configuration (Mixer/LDR). The devices are: (2) a passive micromixer, and (3) a microfluidic LDR reactor (the LDR reactor is shown inverted with the microfluidic channels visible. In the assembly in Fig. 4E the microfluidic reactor is facing down to enable the mounting of the heaters); (E) Fully assembled two-device microfluidic module for mutation detection. One 4.5 mm thick microfluidic interconnect (4) and spacer (5) were used to provide precise and consistent assembly of the two devices. The white dashed line is the cutting plane through the microfluidic interconnect shown in the cross-section in Fig. 4F; (F) Cross-sections of an assembled two-device module, with the reactor and fluidic interconnect on the left and the fluid interconnect and the cover plate on the right. The laser drilled fluidic passages (yellow box) and components are shown.

Figure 5.

Images of the assembled and thermally bonded alignment features used to make each microfluidic device, which consisted of a microfluidic reactor and a cover sheet: (A) The hemispherical pin in slot; (B) The hemispherical pin in hole, and plate-plate lap joint; (C) The outlet of the assembled microfluidic reactor and hole in the cover plate; (D) Enlarged image of the microfluidic passage in the device; and (E) Plot of the measured mismatch of the assembled parts showing the x- and y- offset of the center of the reservoir on the microfluidic interconnect from the center of the exit hole on the assembled device.