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Development of a Micro-Blood-Typing System Using Micro-Stereolithography Technology

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ABO typing is the first test done on blood used for transfusion. A person must receive ABO-matched blood, as ABO incompatibility is the major cause of fatal transfusion reactions. Until now, this blood typing has been done manually. There is a need for an automated typing machine that uses a very small volume of blood. In this paper, we present a new micro-blood-typing system with a fully 3-dimensional geometry, which was realized using micro-stereolithography. This system was fabricated using a novel integration process based on a virtual environment, and blood-typing experiments using this system were successfully performed.

1. Introduction

Blood typing is a test to classify blood by determining the absence or presence of antigens on the red blood cells and the presence of antibodies to these antigens in the serum. Generally, in manual blood typing, the volume of blood needed is above 20 μl.[(1)] However, although this volume is small, blood must be drawn repeatedly if a patient's blood is needed for additional testing or close examination. Above all, it is very hard to collect infant's blood.

Therefore, an ABO typing device has been developed to provide efficiency and convenience through automation and minimization of the sample size. However, the commercialized ABO typing machines are very big and expensive. Consequently, these machines can be used only in certain places such as for major medical centers or blood

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banks. They also have limitted use for emergency blood transfusions because the machines take significant amounts of time for blood sedimentation and require the use of a centrifuge. Moreover, there is little difference in specimen volume compared to manual blood typing.[2,3]

For these reasons, we present a new micro-blood-typing system using microstereolithography.[4–12] This system may form the basis of an automated machine, in function and capacity suitable for use in a hospital. In addition, the proposed system is capable of being applied in clinical examinations based on the agglutination reaction, in addition to ABO typing.

2. Theory

2.1 *Design of an ABO typing device*

Generally, the ABO typing process has two steps: cell typing and serum typing. Cell typing (forward typing) is used to detect the presence or absence of A and/or B antigen on an individual's red blood cells. An individual's ABO group is determined by testing the red blood cells with reagent anti-A and anti-B sera. A schematic picture of a cell typing device is shown in Fig. 1. Serum typing (backward typing) is used to detect ABO antibodies in an individual's serum. The patient's serum is mixed with reagent group A cells, B cells and O cells. The outcome of serum typing is compared with the outcome of cell typing to ensure accurate ABO determination.

After ABO, the most important antigen in transfusion practice is D. The D antigen is a member of the Rh system. Detecting the D antigen consists of testing the individual's red blood cells with anti-D. Rh typing is similar to ABO typing. The patient's red blood cells are mixed with serum containing and then observed for agglutination. If this occurs, then the patient has Rh-positive blood. If the blood cells do not stick together, the patient has Rh-negative blood.

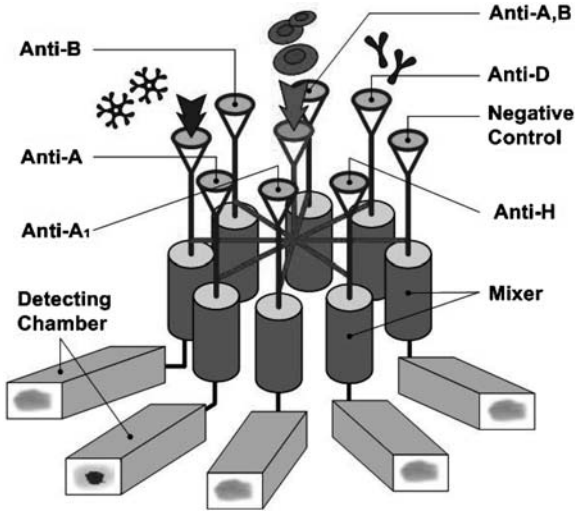


Fig. 1. Schematic diagram of blood-typing system.

The subgroups of A and B are caused by decreased amounts of antigen on the red blood cells. They are inherited conditions. These subgroups cause some clinical troubles in that we often cannot know exactly what the ABO type is in general ABO typing, and it is possible to misjudge because of uncertain reactions. Table 1, shows the interpretation of ABO typing results. Sometimes, ABO incompatibility occurs. In that case, additional blood typing tests must be performed to examine the cause of the incompatibility. It also causes delay in the interpretation of blood typing.

The proposed micro-blood-typing system consists of two blood-typing devices: a cell typing device and a serum typing device. A schematic picture of the cell typing device is shown in Fig. 1. In cell typing, an individual's ABO group is determined by testing the red blood cells using anti-A and anti-B sera. First, the patient's cells are inserted into the inlet, and then they are divided into seven equal parts by micro-separation channels. Each separated cell is mixed with anti-A, anti-B, anti-AB and anti-D by a micromixer, and is sent to a detecting chamber. In the detecting chamber, the agglutination reaction is observed by CCD camera.

Additional testing of the red blood cells with anti-A_1 and anti-H sera is carried out to detect weak subgroups of A or B. Since weak A or B subgroups have fewer antigens present on the red blood cells, anti-A or anti-B serum may produce a very weak or negative reaction, while anti- A_1 serum gives a stronger or positive reaction. The negative control test line is used to detect the self-agglutination reaction.

A schematic picture of the serum typing device is showed in Fig. 2. In serum typing, an individual's ABO group is determined by testing the serum using cell-A, cell-B and cell-O. This blood-typing process is similar to the cell typing process. The patient's serum is inserted into the inlet and divided into three equal parts by micro-separation channels. Each separated sample is mixed with cell-A, cell-B and cell-O by a micromixer. Then it is sent to a detecting chamber, where the agglutination reaction is observed by CCD camera.

2.2 *Assembly-free process*

The proposed micro-blood-typing system has a fully 3-dimentional structure, which was realized using micro-stereolithography. It is fabricated with a novel integration process based on a virtual environment.

Table 1
Interpretation of ABO typing results.

Cell typing		Serum typing			ABO blood type
Reaction with patient cell		Reaction with patient serum			
Anti-A	Anti-B	A Cells	B Cells	C Cells	
(–)	(–)	(+)	(+)	(–)	O
(+)	(–)	(–)	(+)	(–)	A
(–)	(+)	(+)	(–)	(–)	B
(+)	(+)	(–)	(–)	(–)	AB

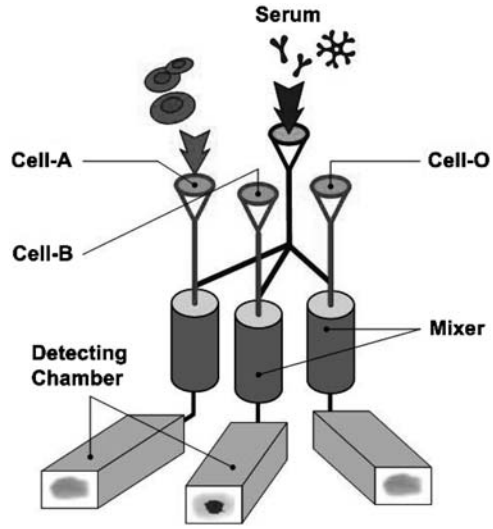


Fig. 2. Schematic diagram of serum typing system.

Micro-stereolithography technology is based on conventional stereolithography, in which a UV laser beam irradiates the open surface of a UV-curable liquid photopolymer, causing it to solidify.[13–15] This technology makes it possible to fabricate complex free-form 3D microstructures in a single process, so that assembly is not required. However, to fabricate the microstructures in a single process, all of the information required for the fabrication of the microfluidic system (laser power, scanning speed of the laser beam, etc.) must be available.

For that reason, we present a novel integration process based on a virtual environment. It provides all the information required to fabricate complex microfluidic systems. This process also makes it possible to easily assemble unit devices in cyber-space and to generate the fabrication details for a micro-blood-typing system.

The proposed process has two main functions. First, it obtains all the details required to fabricate a given system in a single process. Second, various microsystems can easily be created using this process because each unit device can be quickly and efficiently assembled in cyberspace using the assembly-free process. To realize this process, a data structure was designed to provide direct access to the fabrication information for a unit device, as this information is required in the assembly-free process. Virtual assembly and fabrication information extraction algorithms for the integration process were also developed. Figures 3 and 4 show the data structure and a flow chart of the integrated process, respectively.

The data structure was constructed to provide direct access to the information needed to fabricate a unit device. Generally, the fabrication details for a unit device consist of its micro-stereolithography production processes. The data include the fabrication information for each layer, since micro-stereolithography technology is a layer-by-layer process. This is accomplished by storing the laser path and layer thickness in the data structure, which is sufficient information to fabricate each layer of the unit device. The path of the

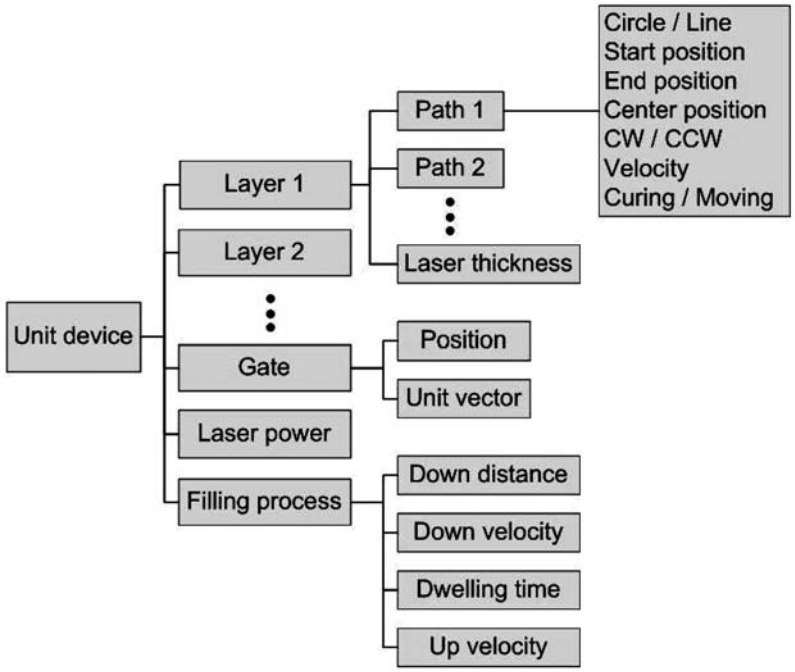


Fig. 3. Data structure to store fabrication information for a microfluidic device.

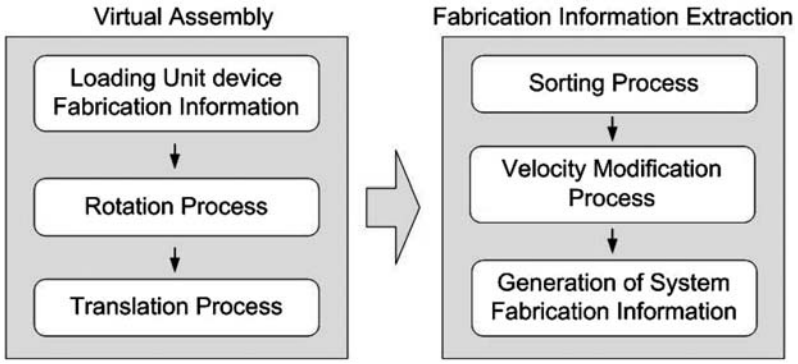


Fig. 4. Flow chart of the integrated process based on a virtual environment.

focused laser beam consists of a number of parameters, including its start, end and center positions, and its velocity, rotation and direction.

The information regarding the position and direction of the exit/entry gate of the unit device, as shown in Fig. 5, is also defined in the data structure. This information is used to virtually assemble each unit device in cyberspace.

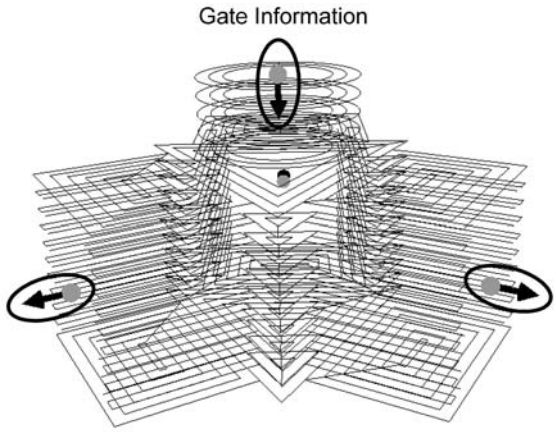


Fig. 5. Example of a microfluidic device that was stored for data structure.

In cyberspace, a unit device is expressed graphically as the line of each path that constitutes the device. Using this graphical expression and the virtual assembly algorithm, a virtually assembled microfluidic system can be expressed in cyberspace as shown in Fig. 6.

3. Fabrication

Micropipes, micromixers and micro-pipe connectors were developed as shown in Figs. 7(a), 7(b) and 7(c). Figure 7(d) shows an example of connecting the various pipes with the pipeconnector. Then, the fabrication information for each unit device was stored in the constructed data structure. Using the stored fabrication information of unit devices and the assembly-free process, a prototype of a micro-blood-typing system was developed. Figure 8 shows the first prototype of a micro-blood-typing system composed of 5 micromixers, 5 microchambers, 6 inlets, 5 outlets and 11 micro-pipe connectors. The fabrication time was 13 h. The photopolymer used in this fabrication was SL 5180 (3D Systems, USA).

The size of the fabricated system was 12 mm×12 mm×10 mm. It had five blood typing test lines. The calculated inner volume of each micromixer was approximately 4 μl, and red blood cells diluted by 10 or 15% were used for cell typing.

Thus, theoretically, a sufficient volume of blood was 0.2 – 0.3 μl for the detection of one blood type. Such a volume is too small to detect blood type in commercialized automated devices currently in use. Our system used a barrier-embedded Kenic micromixer (BEKM)[16] to react blood with reagent, and this can be done in a single process.

Moreover, in manual serum typing, at least 40 μl of undiluted serum is required to observe the agglutination reaction. The agglutination reaction does not always occur using only 40 μl undiluted serum. Thus, by detecting blood type using such a small volume of blood, the micro-blood-typing system has great advantages when infant's blood must be drawn or when a patient's blood must be drawn repeatedly for additional blood-typing tests.

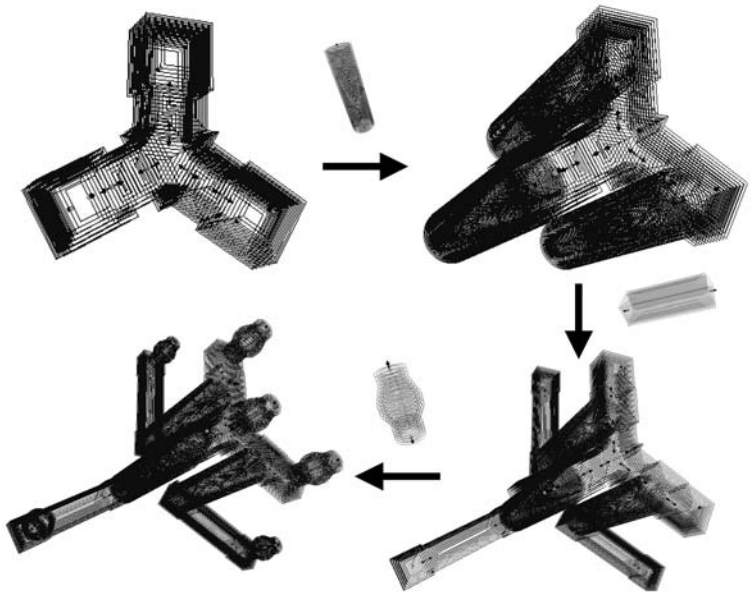


Fig. 6. Virtual assembly process using virtual assembly software.

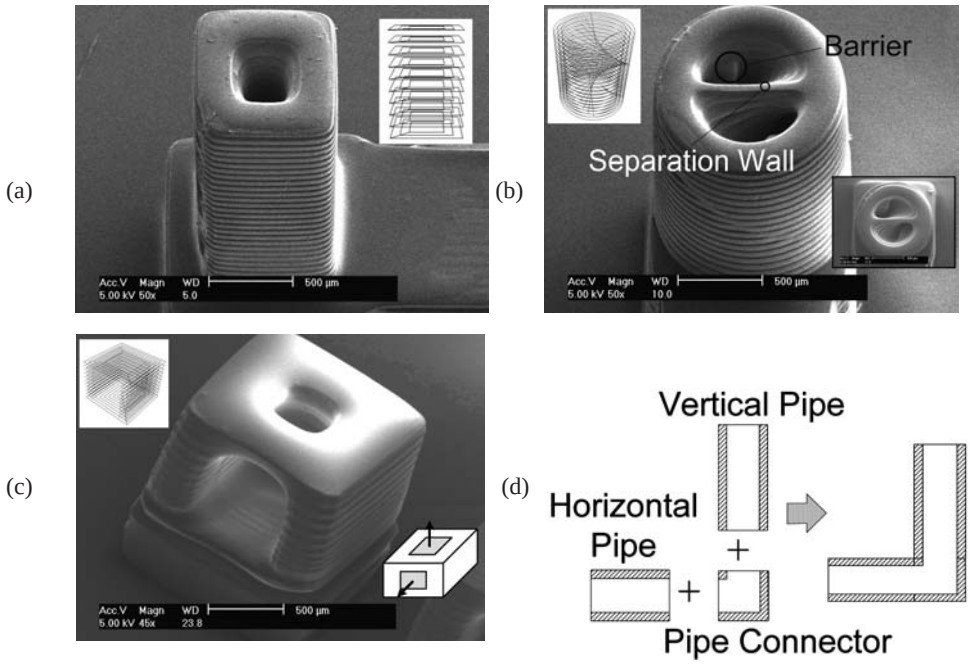


Fig. 7. Developed microfluidic unit device: (a) micropipe, (b) micromixer, (c) micro-pipe connector and (d) an example of pipe connection using the micro-pipe connector.

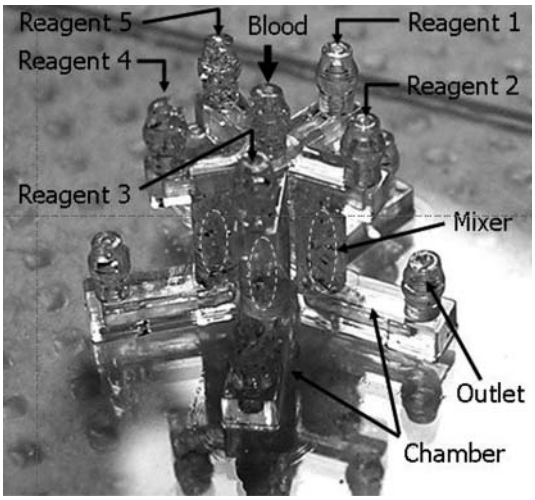


Fig. 8. Fabricated micro-blood-typing system using the integrated process.

4. Results

In this study, experiments were performed using red blood cells and reagents to verify the performance of the micro-blood-typing system we developed. Figure 9 shows the experimental setup for blood typing. Blood (20% diluted red blood cells) and reagents (20% diluted anti-A and anti-B, Ortho-Clinical Diagnostics Inc., USA) were injected into the system using a syringe pump.

Figure 10 shows results of an ABO typing test. In this test, red blood cell-A/B and anti-A/anti-B reagents were injected into the micro blood-typing system at the same time. As shown in Fig. 10(a), the agglutination reaction was observed in the chamber where red blood cell-A was mixed with anti-A reagent. Figure 10(b) shows that agglutination was not observed in the chamber where red blood cell-A was mixed with anti-B reagent. In the same manner, Fig. 10(c) shows that the agglutination reaction was observed in the chamber where red blood cell-B was mixed with anti-B reagent, and Fig. 10(d) shows that agglutination was not observed in the chamber where red blood cell-B was mixed with anti-A reagent.

The microfluidic system was fabricated without the micromixer in order to compare the results in the presence and absence of the micromixer. A hollow pipe with the same dimensions and no twisted separation walls was substituted for the micromixer, and the same blood-typing experiment was performed.

Figure 11(a) shows a complete view of the fabricated microfluidic system without micromixer, and Fig. 11(b) shows the result of the ABO blood-typing test. As shown in Fig. 11(b), agglutination was not observed in the chamber even though red blood cell-A was mixed with anti-A regent, which can be observed when using BEKM.

From this experiment, the agglutination reaction was shown to occur in our system when using BEKM. The performance of our system was confirmed, even when very small volumes of blood (below 1 μl) and reagent were used in ABO typing, whereas agglutination did not occur in the manual test using this small volume of blood.

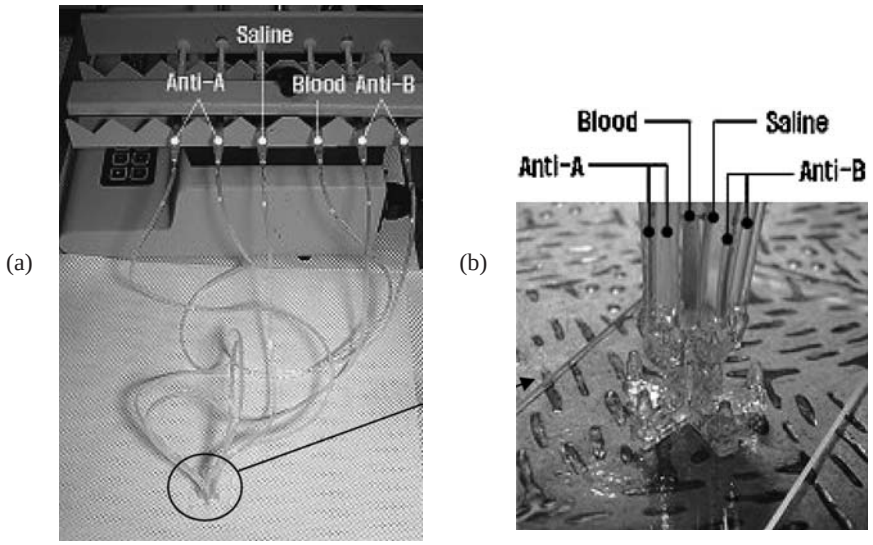


Fig. 9. Experimental setup: (a) whole view and (b) detailed view of a micro blood typing system.

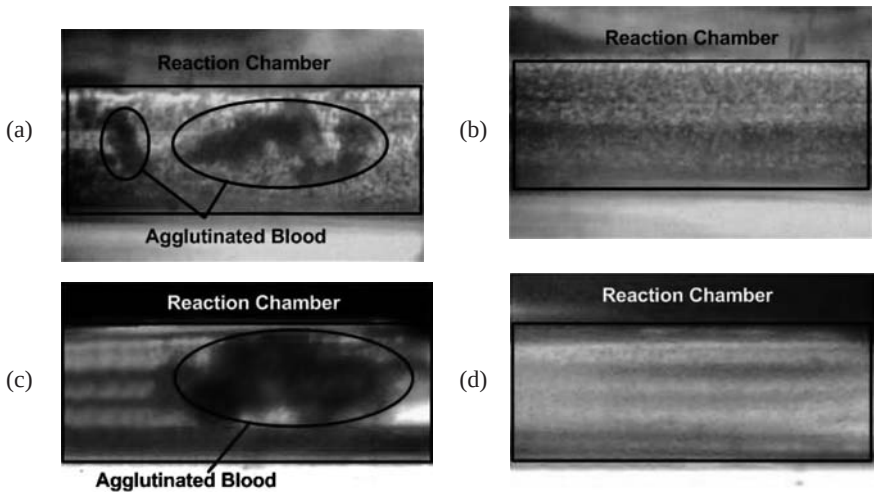


Fig. 10. Result of ABO typing test: (a) agglutinated blood (RBC-A and Anti-A), (b) unagglutinated blood (RBC- and Anti-B), (c) agglutinated blood (RBC-B and Anti-B) and (d) unagglutinated blood (RBC-B and Anti-A).

5. Conclusions

We designed a new micro blood-typing system, which was realized using micro-stereolithography. The blood typing experiments clearly show that the micro-blood-typing system was able to detect ABO blood types well. The micromixer enabled the observation

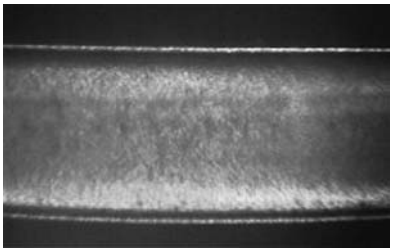
(a)  (b) 

Fig. 11. Fabricated system without micro mixer: (a) whole view and (b) result of ABO typing test (RBC-A and Anti-A).

of agglutination using a small volume of blood (below 1 μl) in ABO typing. Using such a small volume of blood has two major advantages: it is easier to gather blood from infants, and it enables repeated testing after only one blood-gathering step.

In addition, the micro blood-typing system was fabricated using a novel integration process based on a virtual environment. This technology enables the fabrication of a complex micro blood-typing system in a single process, without the need for assembly. The integrated process has a database for each microfluidic device. To realize the proposed process, virtual assembly algorithms and a fabrication information extraction algorithm were developed.

Finally, a prototype of the micro-blood-typing system was fabricated using the integrated process, and blood-typing experiments using this system were successfully performed.

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