Hybrid subtractive-additive-welding microfabrication for lab-on-chip applications via single amplified femtosecond laser source

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Abstract. An approach employing ultrafast laser hybrid subtractive-additive microfabrication, which combines ablation, three-dimensional nanolithography, and welding, is proposed for the realization of a lab-on-chip (LOC) device. A single amplified Yb:KGW femtosecond (fs)-pulsed laser source is shown to be suitable for fabricating microgrooves in glass slabs, polymerization of fine-meshes microfilter out of hybrid organic–inorganic photopolymer SZ2080 inside them, and, finally, sealing the whole chip with cover glass into a single monolithic piece. The created microfluidic device proved its particle sorting function by separating 1- and 10-μm polystyrene spheres in an aqueous mixture. All together, this proves that laser microfabrication based on a single amplified fs laser source is a flexible and versatile approach for the hybrid subtractive-additive manufacturing of functional mesoscale multimaterial LOC devices.

Keywords: femtosecond laser three-dimensional (3-D) microfabrication; 3-D printing; nanotechnology; microfluidics; lab-on-chip.

1 Introduction

Since the invention of the laser, it became an irreplaceable tool in advanced material processing.1-4 The recent development of industrial design, high-stability, high-power, and high-repetition rate lasers with tuneable pulse duration capability (from hundreds of fs to tenths of ps) enables performing both additive and subtractive manufacturing using a single laser system. Such systems are capable of multimaterial and mesoscale fabrication varying from nanometer resolution to overall size in range of centimeters.5

Recently, functionality and integrability of lab-on-chip (LOC) devices have been strongly advanced.5,7 One of the current goals in this field is to reduce the price of such chips, making them more affordable for the end-user.8 This requires simplification of technologies used in their production, which can be achieved by combining various material processing methods and fabrication steps into one manufacturing system. Additionally, it might be desired that the created microstructures would perform their tasks in a passive fashion, i.e., without the use of any external energy source.9,10 This paves the way for using such devices in remote locations short of electricity or accessibility to modern equipment.

In this work, we use an amplified femtosecond Yb:KGW laser for both subtractive and additive fabrication techniques in order to build a passive LOC particle separator. First, laser ablation is used to produce microfluidic channels in the glass substrates. Then, three-dimensional laser lithography (3DLL) is employed to integrate microfilters into the channels. Finally, the chip is sealed with a glass cover by laser welding. All the steps are performed using an Yb:KGW amplified fs laser source (Pharos, Light Conversion), which is a fully automated industrial design laser enabling full control (via PC) of output power, pulse duration, and repetition rate. We demonstrate that the integrated filters can sort 1- and 10-μm diameter microparticles in water. Overall, these results demonstrate that multiple technological steps required to produce a functional LOC device can be performed using a single fs laser source in a hybrid subtractive-additive fabrication approach.

2 Materials and Methods

In order to create the required structures, four distinct fabrication steps had to be undertaken: direct channel ablation [Fig. 1(a)], inlet cutting via filament assisted ablation [Fig. 1(b)], filter polymerization [Fig. 1(c)], and welding of upper and lower parts of the structure [Fig. 1(d)]. For the channel formation, a 1-mm-thick borosilicate glass microscope slide was used. The channels were sealed with a 150-μm cover glass slide. For the filter mesh fabrication, a hybrid organic–inorganic photopolymer SZ2080 was chosen as it exhibits high mechanical strength,11 a wide fabrication window12 and, if need arises, could be easily combined with organic13 or inorganic14 additives for increased functionality. It was mixed with 1 wt. % photoinitiator 2-benzyl-2-dimethylamino-1-(4-morpholinophenyl)-butanone-1 (also known as Irgacure 369). One of the advantages of this material is its hard gel-form during fabrication, which results in minimal shrinkage after developing.11 The liquid SZ2080
turns into gel during a prebake step when the solvent is removed from the mixture. The prebake is performed in a “ramp” fashion, with three temperature levels of 40°C, 70°C, and 90°C each lasting 20 min and separated by 5-min temperature increase intervals. Development is performed in 4-methyl-2-pentanone for 1 h. In all these experiments, an Yb:KGW femtosecond laser “Pharos” (Light Conversion Ltd.) was employed, as it offers a broad tuning range ($\lambda$ from 260 nm to 1.6 μm) with single pulse energies up to 1 mJ (f0, single pulse to 200 kHz; P, up to 20 W) which is enough for both additive and subtractive manufacturing.

The average laser power $P$ was measured prior to fabrication and the peak intensity was calculated by employing the following equation:

$$I_p = \frac{2PT}{f\omega^2\pi}. \quad (1)$$

where $f$ is the pulse repetition rate, $\tau$ is the pulse duration, $\omega = 0.61\lambda/NA$ is the waist (radius) of the beam, $M^2$ defines the beam quality (1.2 for “Pharos” laser$^{[15]}$, and $T$ is the transmittance of the objective. Exact parameters describing the fabrication process are listed where it applies.

The structural examination was performed using a scanning electron microscope (SEM) TM-1000 (Hitachi, Ltd.) or an inverted optical microscope setup. Water was used as the liquid medium in all the flow experiments.

3 Results

3.1 Femtosecond Manufacturing of Glass Channels

Glass is a favorable material for a microfluidics as it is mechanically robust, chemically inert, and transparent in all the visible region of the spectrum. The latter property makes it suitable for fluorescence measurements and Raman spectroscopy.$^{[16]}$ Thus, it was chosen as a model material for channel fabrication in this study.

Glass structuring for microfluidic applications can be performed in several different ways. One of them is the combination of standard lithography and chemical etching.$^{[17]}$ when a polymeric mask is created on the glass surface and then etched in hydrofluoric acid (HF). Some of the limitations of this approach are that there is very little control of channel aspect ratio and how steep the channel walls will be. Alternatively, lasers can be used to make strain-induced regions of densified glass where a Lewis-base structure is created via formation of a small-member-structure of tetrahedral rings in silica which are prone to a high contrast etching in HF solution.$^{[18,19]}$ By recording a nanograting pattern inside glass, an additional directionality of HF etching can be obtained.$^{[20]}$ This allows a lot of freedom in designing the microfluidic systems. Etching is a diffusion-limited process and is comparatively slow, which poses constrains in selection of glass and etchant. Fused silica is a strongly preferred host for LOC devices as channel etching in HF is comparatively fast in this material. However, due to the hazardous nature of HF etching, in this work, direct laser ablation was employed for the manufacturing of the channels as it is a well established method of producing microfluidic systems on the surface of the glass substrates.$^{[21]}$

Ablation was performed using the fundamental (1030 nm) laser wavelength, 260-fs pulse duration, 25-kHz repetition rate, and 196-TW/cm² peak intensity at the focus of the f-theta lens with a focal distance of 100 mm. The translation velocity of the sample was 100 mm/s. As the laser spot was around 28 μm in diameter (1/e² level), the 200-μm-wide channels were fabricated by raster scanning the sample along the direction of a channel 11 times. The lateral distance between scans was set to 20 μm. The depth of a channel was controlled by changing the number of scans performed along the same path. It was established that the depth of a channel depended on the number of scanning repetitions almost in a linear fashion: five consecutive scans provided the depth of 40, 10 to 70, 15 to 110, 20 to 150, 25 to 190 μm. The final channels that were used for LOC experiments in this work were 200-μm wide and 100-μm deep (Fig. 2) with surface roughness in the range of tenths of μm. It took just a few minutes to produce channels for one LOC device.
3.2 Inlet Cutting

The next challenge was to cut holes in 1-mm-thick slide glass for the inlets and outlets of the system. This was achieved by water assisted femtosecond filament ablation. By having a ∼1-mm-thick water layer above the glass sample, fs-laser radiation focused into the water initiates the formation of a light filament, which is capable of cutting arbitrary shapes in the millimeters-thick samples. In order to increase the efficiency of this method, before submerging the glass into the water it was washed with soap to make it more hydrophilic. As a result, 1.2-mm-diameter through-holes were successfully cut. Parameters used in this step were similar to the ones applied for direct ablation. More details on this technology can be found elsewhere.22

3.3 Integration of Microfilters

Before sealing the channels, 3DLL was applied to form microfilters inside. The experiments were performed with a 1.4-NA objective using second-laser harmonic (515 nm), 200-kHz repetition rate, 0.525-TW/cm² peak intensity, and 0.5-mm/s sample translation velocity. Because of the versatility of the 3DLL technology,23 filters of basically any shape or orientation could be formed inside the channel at the chosen position (Fig. 3). Thus, the geometry of a filter rotated at 45 deg was selected [Fig. 3(f)], as it could be integrated directly into the channel intersection and conform with the flow-reflective geometry at the channel’s intersection. The microbeads that were intended to be separated were 1 and 10 μm in diameter, thus the chosen pore size was in the middle of this interval—6 μm.

3.4 Sealing Channels via Laser Welding

The laser welding of the channel system and a cover glass was the last step in assembling the microfluidic system.24 One of the main requirements for a successful welding is to make sure that both glass layers would be in direct contact with each other. In order to achieve that, three strategies were proposed and implemented. The first one was to clean the glass surfaces in an ultrasound bath first in acetone, then in isopropanol, and finally in water followed by drying using nitrogen gas and then compressed together. The second approach was to put the glass slides with an isopropanol layer between them in a vacuum chamber with the intent of removing isopropanol and making an optical contact. In the last approach, glass slides were cleaned only by acetone and blown with nitrogen before being compressed together. The second approach did not provide satisfactory results as the glass slides were not entirely in contact to one another. The first and the third approaches showed consistent results in channel sealing. Thus, because of simplicity, the third strategy was chosen.

Welding was performed using 200-kHz repetition rate, 12.5-TW/cm² peak intensity, 10-mm/s translation velocity, and 1030-nm wavelength. At these parameter settings, glass melts at the interface between two glass surfaces and permanently bonds them together. Overall 10 rectangular welds spaced apart by 200 μm were formed to achieve a sufficiently strong system. The assembled chip with metal pipes secured in the inlet and outlets is shown in Fig. 4.

3.5 Testing of the Microsystem

To prove the concept of particle separation using a filter inside such a microfluidic chip, a stream of differently sized beads was mixed in water and pumped into the channel [Fig. 5(a) blue arrow (1)]. The filter allows only smaller particles to pass through [Fig. 5(a) yellow arrow (2)], whereas the bigger ones are carried with the main flow [Fig. 5(a) red arrow (3)]. In this case, a significant amount of small particles was carried together with the main flow (red arrow). To improve separation efficiency, several different channel geometries were fabricated. Figure 5(b) shows a geometry...
with an additional wall marked “U” polymerized in order to increase the pressure for particles entering the filter. The geometry shown in Fig. 5(c) serves a similar purpose and because of simpler fabrication was chosen for further studies. To improve the separation efficiency even more, a three-stage filtering LOC was fabricated [based on the geometry shown in Fig. 5(c)] shown in Fig. 6.

To observe the behavior of the microparticles, an inverted microscope setup was utilized. As was previously mentioned, the glass slide chosen for channel sealing was 150-μm thick, allowing use of a high-magnification objective lens which is capable of imaging single beads of both sizes...
This enables use of the LOC system for in situ observations. Clear redirection of larger particles was observed (Fig. 8). Examination of the liquid after the filtering revealed that bigger beads are absent. Clogging of the channel was happening if bigger particles started to gather in front of the filters. Such conglomeration of the microbeads was broken by a brief reversion of the liquid flow. It was noticed that it is not only effective, but also had no negative impact on the microfiltering action. Therefore, the created microfluidic system performed the planned task and was proven to be mechanically robust.

### 4 Discussion

One of the main reasons that laser material processing became so popular in the industry is the fact that basically any material can be processed using high-intensity laser radiation. The delivered energy must be absorbed by the material in order for the modifications (melting, ablation, evaporation, ionisation, and optical defect formation) to be created. For example, excimer lasers can be used for additive polymer processing, whereas CO₂ lasers are mostly applied for cutting. However, in the case of ultrafast laser sources, because of the high intensity (TW/cm² and more) of the light source induced nonlinear light-matter interactions, basically all materials can be processed with high precision in both subtractive and additive fashion. For instance, in the case of presented results, the intensity needed for polymerization was around 0.525 TW/cm² (additive process), while it was 196 TW/cm² during ablation (subtractive fabrication). Furthermore, tuning of the pulse repetition rate allows switching between light-matter regimes. Minimal thermal effects are achieved while operating at low repetition rates (kHz, so-called “cold processing”), whereas severe heating (several thousand degrees Celsius) is achieved with higher (hundreds kHz) pulse repetition rate. It is important as the best results during ablation are achieved in the former regime, while it is crucial to operate in the latter during laser welding. It is worth noting that the effects of parasitic light (e.g., pre/postpulses) are not taken into account when addressing the ablation/welding phenomena, since the laser used provides contrast values of 1:200 and 1:1000 for pre- and postpulses, respectively. These contrast ratios translate to fluence (from parasitic light) as ∼11.5 and ∼2 mJ/cm², which are a few orders of magnitude lower than the damage threshold of glass.

One of the most delicate procedures to accomplish in LOC fabrication is cover sealing. It has been demonstrated that fs-laser welding can reach ∼30 Mpa tensile and up to twice higher shear strength. Such mechanical sealing strength is higher than a typical plastic lamination used in Si-based microfluidic chips and allows handling fast flows. It is critical to have a separation gap comparable...
to the axial extent of the focal spot of the laser beam. This places a requirement of 1- to 2-μm distance between the channel substrate and cover. Strength of the cover welding is controlled by increasing the surface area of the joint region.

Both additive and subtractive fs laser fabrication methods have some distinct advantages and drawbacks. The subtractive manufacturing offers a way to rapidly produce various structures that are formed on the surface of the sample, such as channels or hydroactive surfaces. Yet it lacks the possibility to easily produce true 3-D structures. On the other hand, additive manufacturing realized with an ultrafast laser is superb in creating and integrating true 3-D structures on a wide array of substrates and structures. What is more, it allows multimaterial fabrication during formation of a single structure thus enabling four-dimensional printing. However, as it is based on a point-by-point structuring, it lacks the throughput to fabricate the whole LOC system fast enough to be commercially viable. Merging these two approaches in one hybrid manufacturing system is a promising prospect, as it reduces the number of required setups (and light sources) to a single one while combining the advantages and eliminating drawbacks of both of these methods.

Alternatives for producing a fully functioning microfluidic system with either only subtractive or additive manufacturing are also possible, yet more complicated. Combining standard 3-D printing with 3DLL seems to be one of the possibilities. In that case, the throughput sufficient for LOC fabrication is guaranteed by the 3-D printer, whereas 3DLL is used for structure integration and ensures nanolevel precision. In a subtractive case, some promising results were achieved in selective glass or crystal (sapphire) etching. Volume-embedded structures were produced this way, hinting at emerging true 3-D capabilities of this approach.

5 Conclusions

In this work, hybrid subtractive-additive-welding fs-laser fabrication techniques were employed to produce a mechanically robust functional microfluidic system capable of sorting different sized (1 and 10 μm) microparticles. This was enabled by utilizing a widely tunable (in power $P$ and repetition rate $f$) Yb:KGW amplified fs-laser system. The presented results clearly show that a combination of subtractive and additive laser fabrication technologies can be used for creating complex functional LOC systems. The demonstrated all-in-glass manufacturing of LOCs using tailored fs-laser structuring is advantageous compared to an all-in-plastic platform due to enhanced chemical resistance, while the multistep filtering is promising for various cell and bacteria sorting tasks.

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