

Developments In The Microfabrication Of Biochips Using Laser Micromachining

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Biochips are miniaturised devices designed to carry out a broad range of biotechnological processes and can be divided into two categories. The first are the microarray biochips which are typically two dimensional surfaces containing defined regions of attached biomolecules for undertaking parallel chemical detection measurements on specimens. In these devices chemicals or chemical groups within the specimen react with biomolecules in specific regions within the biochip. The results of these reactions are measured, typically using optical techniques, to quantify the amount of each chemical within the specimen. Microarray biochips are currently being exploited for carrying out many routine biological tests ranging from allergy, infection and drugs of abuse detection, through to complete genome measurements on a single chip. Microfluidic biochips form the second category. These devices are designed to move fluids or particles through networks of channels where they may undergo a range of reactions or measurements. In this way, these biochips can be thought of as miniaturised biotechnology laboratories on a chip.

A conceptual diagram of such a device is shown in figure 1. Here a sample from anyone of a number of sources undergoes a series of preparation, analysis and detection processes within the integrated chip. The output of the device is a combination of analytical data and reaction by-products. The biochip illustrated in figure 1 represents a long term ideal which has yet to be reached. However, in the past decade there has been substantial develop towards this goal from both academia and industry. The drivers in this development are the advantages miniaturisation can offer biotechnological processes. For instance, reactions occur in microfluidic channels of similar dimensions to a human hair and so sample volumes are typically measured in nanolitres which, in turn, leads to reductions in reagent costs. Reactions also complete in less time allowing large numbers of tests to be carried out quickly.

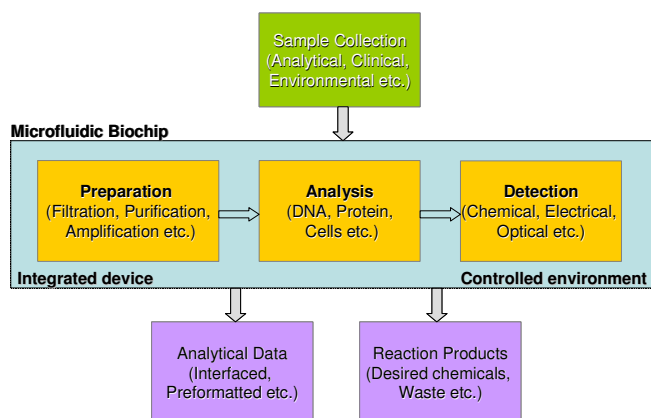


Figure 1. Schematic illustration of an ideal microfluidic biochip

Pharmaceutical industries are looking to biochip technology to enable over 1 million tests per day to be performed in drug discovery programmes. Miniaturisation also has advantages in process control since environmental parameters such as temperature and pressure can easily be controlled within an integrated biochip. Additionally, as a result of the small dimensions involved, parallel processing for multi-analyte investigation is commonplace.

The challenge to the microengineer is to manufacture biochip devices in an accurate, cost-effective and rapid manner. Unlike conventional silicon MEMS, biochip devices often make use of a broad range of materials within a single device. Issues of biocompatibility necessarily take precedence over ease of manufacture. Typical materials for use in biochips include glasses, polymers such as polyimide, polycarbonate, PMMA, epoxies, thin metal films, bulk metals and elastomers. Such a diverse range of materials can be problematic for conventional photolithographic-based microfabrication but is well suited to laser micromachining. While the creation of a complete biochip will usually employ a number of different fabrication processes, described here is the use of excimer laser machining to create key components within biochip devices.

Electrokinetic systems

Electrokinetic processes are becoming increasingly popular for the electric field induced manipulation and interrogation of particles within biochips [1-3]. In such processes the direction and speed of particle movement is a function of the dielectric properties of the particles and its suspending medium as well as the electric field geometry. Particles can be trapped or corralled in defined regions, transported around devices or analysed by using different combinations of static and moving electric fields. One such process is travelling wave dielectrophoresis where particles can be transported and, if desired, fractionated on long arrays of microelectrodes of a width and spacing comparable in size to the particle being transported. The electrode array is energised using quadrature sinusoidal voltages to create a travelling electric field. To allow the fabrication of arbitrarily long arrays energised by a just four electrical contacts a multilayered fabrication process must be used involving bus-bars and electrical via holes. This is conceptually illustrated in figure 2a with an example electrode array fabricated using 248nm excimer laser ablation shown in figure 2b. The electrode array consists of 10µm wide electrodes separated by 10µm gaps fabricated on a glass substrate using thermally evaporated 70nm gold films deposited with a 5nm chromium adhesion layer. Several methods can be used to pattern these electrodes including single pulse demetalisation. However, a key issue in these devices is the smoothness of the electrode edges. Due to the strong chrome adhesion layer and mechanical damage from the shock wave generated, single pulse

demetalisation tends to result in electrodes with edge roughnesses of around 1 μ m. Mechanical damping of the shock wave in the form of a thin polymer film spin coated over the surface of the metal prior to demetalisation can improve the

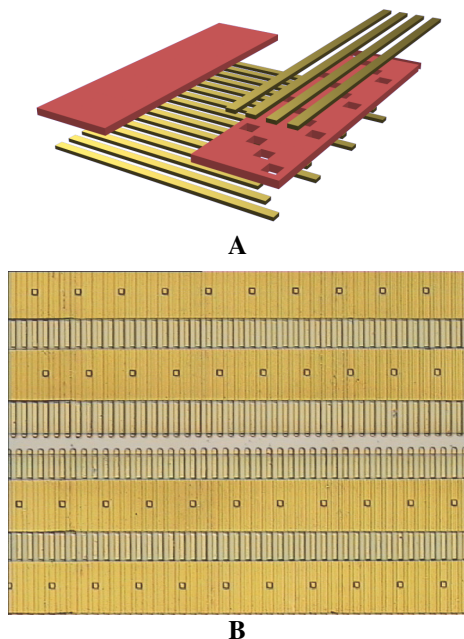


Figure 2. (a) Conceptual illustration of a multilayered travelling wave dielectrophoresis electrode array. (b) Example of multilayered array of 10 μ m electrodes energised by 4 bus-bars.

machining quality. In the case of figure 2b, after deposition of the gold film the substrate was coated with a 2 μ m thick layer of polyimide (DuPont) prior to patterning the fine vertical electrodes. The ablation characteristics of this particular polyimide are shown in figure 3. It can be seen that machining at a fluence of less than 180 mJcm⁻² allows the polyimide to be removed without ablating the gold film. In practice fluences of around 100 mJcm⁻² are used to minimise any thermal transfer to the gold film which can cause localised diffusion between the gold and chrome films which, in turn, can make the film difficult to etch if needed in later processing. The electrodes are formed by first machining the polyimide to reveal the unwanted gold film and then removing the gold with a single high fluence pulse (>200 mJcm⁻²). The electrode patterning is

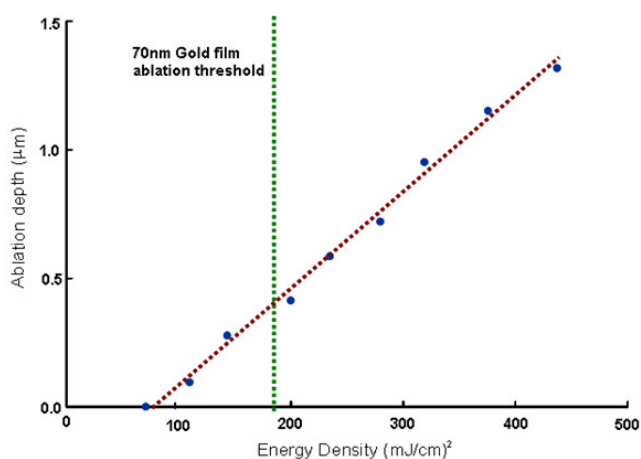


Figure 3. Ablation vs fluence characteristics for polyimide and 70nm gold film.

then completed by chemically removing the remaining polyimide film. An added benefit of this process is that the polyimide coating can also act as a debris shield so preventing ablated metal recasting in the electrodes. Using this patterning technique electrodes edge roughnesses of <100nm can be achieved.

Travelling wave dielectrophoresis electrodes need to produce high field strengths in aqueous mediums with typical energising voltages between 4 and 12V. Therefore, the electrodes need to be capable of carrying a significant electrical current. The reliability of multilayer electrode arrays is largely governed by the current carrying capabilities of the electrical via holes that connect the field producing electrodes to the bus-bars. Via holes are formed by machining a small aperture over every fourth electrodes and then depositing a second chrome gold film followed by subsequent single pulse demetalisation. The electrical connection between the bus-bar and the electrodes is dependent on the quality of the metalisation of the machined aperture side walls. Since physical vapour deposition processes such as thermal evaporation are primarily line of sight coating processes, vertical walls tend to receive poor quality coatings. To overcome this limitation, and improve the reliability of multilayer electrode arrays, the via holes of multilayered electrode arrays need to be contoured to provide a shallow side wall angle. Such a task is difficult to achieve using conventional lithography but simple using laser processing techniques. Figure 4 shows electron micrographs of two example contoured via holes. In each case the vias have been formed in a 2 μ m thick polyimide film spin coated over a set of laser patterned field producing electrodes and subsequently heat polymerized. Contouring is achieved by moving the workpiece between

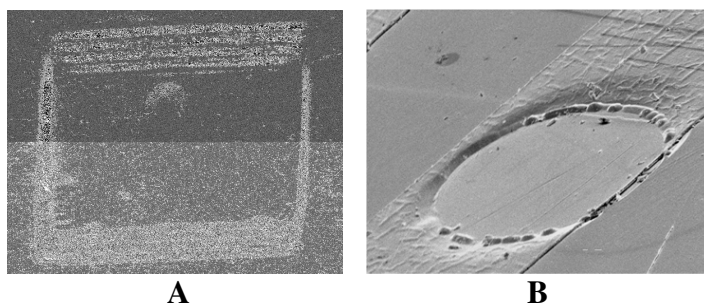


Figure 4. Contoured electrical vias (a) 5 steps, (b) many steps.

consecutive machining pulses or batches of pulses. The wall angle measured as a deviation from the substrate is given by

$$\theta = \tan^{-1}\left(\frac{s}{d}\right)$$

where s is the distance moved by the workpiece between pulses or batches of pulses and d is the ablation depth per pulse or batch of pulses. The horizontal length, l , of the via hole side wall is given by

$$l = \frac{st}{d}$$

where t is the thickness of the polyimide. Figure 4a shows a via hole machined in 5 pulses with a displacement of 400nm between 175 mJcm⁻² to give a wall angle of approximately 45° and wall length of approximately 2 μ m. In this case the individual steps in the wall are clearly visible. Figure 4b shows the effect of reducing the machining fluence and hence increasing the number of steps in the side wall. In this case the steps are no longer visible. However,

even though the workpiece displacement was reduced to 100nm, the wall horizontal length is significantly increased. Figure 4b shows a via hole created using a circular aperture with a linear displacement. The effect of this is an ellipsoidal entrance (upper surface) and circular exit (bottom surface). The image shows good metalisation of the side walls. Also visible is the underlying field producing electrode running perpendicular to the upper bus bar.

Microfluidics

The production of channels for transporting samples and reagents is essential to the operation of microfluidic biochips. Laser micromachining offers a quick, often material independent, means of creating such channels and hence is a valuable tool in the prototyping of biochip devices. Channel system with cross-sectional dimensions typically measured in tens or hundreds on micrometers can be fabricated by simple pattern transfer using serial writing or mask projection techniques into a bulk material. Generally, such techniques produce rectangular cross-section channels which are adequate for most microfluidic applications. However, fluid flow within microchannels is virtually always laminar in nature and so possesses a parabolic velocity profile with zero velocity at the walls of the channel and maximum velocity in the centre of the channel. There exist some applications where the rectangular cross-section of channels provides unacceptably low flow velocities in the corners of the channel which can lead to transport inefficiencies and possibly the loss of valuable reaction products. Serial writing of microfluidic channels using non-rectangular beam profiles allow microchannels with a wide variety of cross-sections to be manufactured. As an example, figure 5 shows an array of 20µm wide microfluidic channels with a semicircular cross section fabricated in 30µm thick dry film polymer laminate. Circular cross-section pipes can be formed by bonding a second, identical, inverted array onto the upper surface of the channels.

Whilst non-rectangular cross-section channels have limited applications in biochips, one area where accurate channel profiling is an advantage is in the manufacture of manifold systems to distribute or combine samples from different parts of the biochip. In designing manifold systems careful consideration has to be taken of the fluid velocities along with forward and back pressures created by the manifold. Often, since mixing is commonly undertaken by diffusion processes in biochips, manifold structures also have to include defined transport sample times. Therefore, the ability to create smooth contoured manifold systems capable of maintaining laminar



Figure 5. An array of 20µm wide semicircular cross-section fluidic channels fabricated in a dry film laminate.

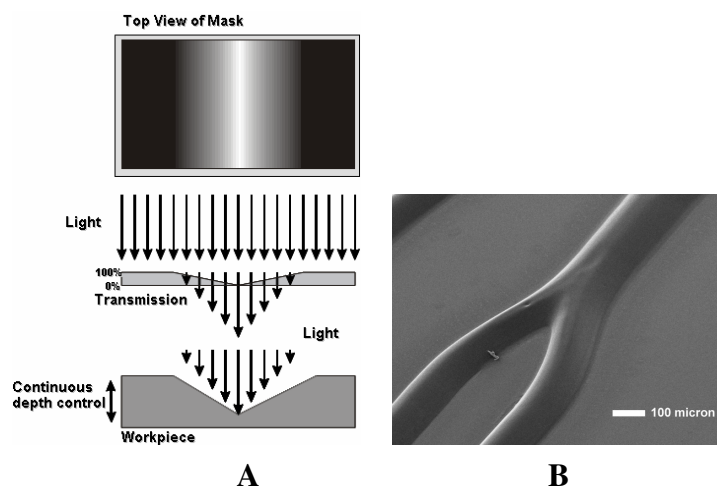


Figure 6. (a) An illustration of the principles of greyscale mask-based ablation. (b) An electron micrograph of a 100µm wide microfluidic manifold moulding tool produced in cured SU-8.

flow without vortex creation due to sharp corners or transitions in channel profile is an advantage.

Whereas multiple mask based ablation has been shown to produce high quality contoured structures such as large area microlenses [4], greyscale mask based ablation allows the production of contoured features in a single exposure process [5,6]. Conventional 'binary' masks consist of regions of zero or 100% transmittance and all exposed areas are illuminated with the same fluence. This results in equal degrees of ablation for all illuminated regions and accurate pattern transfer. Greyscale or halftone masks allow spatially varying transmission on a single mask as illustrated in figure 6a. When used for laser micromachining, greyscale masks can be used to spatially control the workpiece fluence and hence ablation depth, so allowing contoured surfaces to be created in a single exposure. Low-cost greyscale machining can be achieved by creating greyscale masks using conventional binary mask technology and binary dither patterning techniques similar to those used in printing or computer graphics applications. The dithering process considers the mask area as a bitmap which can be divided up into a matrix of $n \times n$ pixel elements. By controlling the distribution of filled pixels in each element the average transmission of the element can be varied. At the same time, if the dimensions of the individual elements are below the maximum resolution of the beam delivery optics, the pixel distribution within each element will not be transferred to the workpiece, rather the ablation depth will be a function of the average transmission of each mask element. Many dithering algorithms are available for creating greyscale images from binary dot patterns. In many cases the principle application of these algorithms is an accurate conversion of the image when perceived with the human eye. Such algorithms are not necessarily the best for laser micromachining. Figure 6b shows a two into one manifold structure machined (248nm, excimer) in a cured layer of the epoxy-based photoresist SU-8 using a greyscale mask created using the clustered dot ordered dither algorithm [7]. The channel structure is shown as a positive, raised, feature allowing it to be used as a mould tool. When embossed into a polymer such as polycarbonate the tool forms a half channel which can be solvent bonded to a matching half channel to produce a full manifold structure.

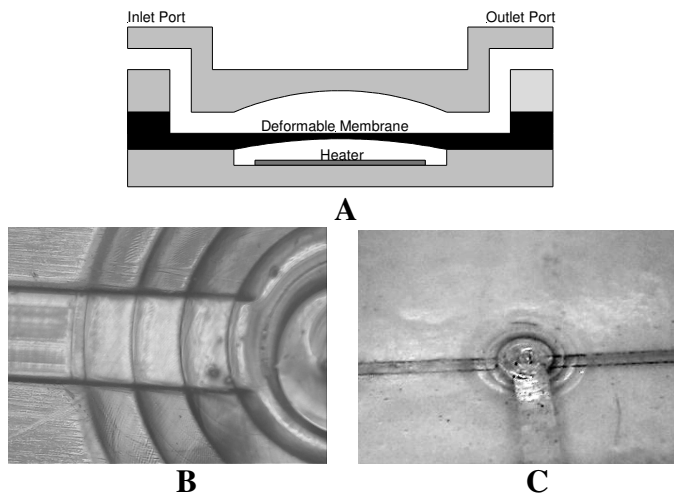


Figure 7. A thermo-pneumatic microfluidic valve (a) conceptual diagram, (b) contoured rubber membrane with integrated channel, (c) complete view of the microvalve

The ability of lasers to machine a broad range of materials can be exploited in the creation of fluid control components such as microvalves and pumps. Figure 7 shows a thermo-pneumatically activated microvalve that relies on the ability to accurately machine a thin rubber film. As can be seen from figure 7a, the valve has an inlet and outlet connected by a microfluidic channel with side walls and floor fabricated from a flexible, easily deformable, material such as rubber. Beneath the floor of the channel is a fluid filled chamber which can be heated causing it to expand. By grading the thickness of the fluidic channel floor it is possible to control its deformation as the heated fluid expands. Pressure from the lower chamber causes the rubber to deform into the channel until it completely blocks the fluidic channel and prevents fluid flow. To assist in providing a strong seal, the ceiling of the fluidic channel can also be contoured to match the deformation of the channel floor. The use of flexible, deformable, materials make this form of microvalve especially suited to use with particle suspensions such as biological cell cultures. In the example microvalve shown in figures 7b and 7c the contouring of the channel has been achieved by machining concentric circles increasing the depth of machining with decreasing circle radius. The channel was constructed from a 100 μm thick rubber film formulated to combine strength and flexibility. Machining used a serial write process using an excimer laser with a wavelength of 193nm. In this case a 200 μm aperture was projected through a 10x demagnification lens to provide a

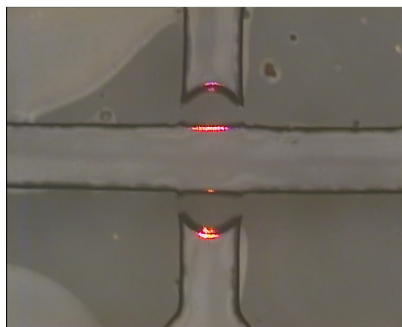


Figure 8. Optical cell counter fabricated in polydimethylsiloxane from a dry film laminate micromould. The walls of the horizontal fluidic channel and vertical hollow light guides have been trimmed using excimer laser micromachining.

20 μm beam at the workpiece. A beam fluence of 1 Jcm⁻² was used with the ablation depth controlled by varying the speed of workpiece motion and hence the number of pulses incident per unit area. Figure 7b shows a close-up image of the fluidic channel machined in the top side of the rubber with the contoured floor machined in the bottom side of the rubber. Figure 7c shows the complete device. The fluidic channel of the valve extends horizontally across the image with the lower, fluid filled, chamber extending vertically down the image. Microelectrode-based heaters are positioned outside the image to allow the pneumatic fluid to be heated expand without heating the contents of the channel.

Optical detection

Increasingly, biochip devices are being developed which incorporate a range of active measurement components which can be used to trigger subsequent sample processing events. In many cases these devices employ optical interrogation processes and hence there is a requirement for the fabrication of wave and light guide structures along with microlenses. An example of such a device is a simple cell counter where cells pass through a light beam causing absorption and scattering and so reducing the measured intensity of the beam. Tracking temporal variations in the beam intensity allows cells to be rapidly counted within a biochip. Figure 8 shows a simple example of excimer laser ablation being employed in the prototyping of lightguides to couple light from an embedded laser diode into a focussed point within a microfluidic channel and to couple transmitted light from the channel to an embedded photodiode. In this case the light guide is a hollow, air filled, structure, fabricated by casting polydimethylsiloxane against a photoresist micromould, where light reflects off the walls of the guide. To focus the light to a point within the channel, the ends of the guides need to be shaped to produce a cylindrical lens. In this case the lens structures have been laser micromachined as an inverse structure in the photoresist mould by serially writing a series of arc using an excimer laser at 248nm with a 200 μm circular apertured beam projected through a 10x demagnification. Additionally, the side wall of the channel has been trimmed to produce a flat surface perpendicular to the guide direction. In this example the micromould was originally produced lithographically using multiple layers of a 30 μm thick dry film laminate resist. Also visible in figure 8 is the scattered light from the laser machined walls of the lightguide and fluidic channel. It should be noted that the light enters from the top of the image and is focussed to a vertical line in the lower quarter of the fluidic channel. This is confirmed by the broad scattering on the upper edge of the channel compared to the fine scatter point on the lower edge of the channel. The advantage of laser micromachining in producing such structures is the ability to modify the mould for subsequent evolutions of the design. For instance, remachining the radius of the light guide lens may produce a more desirable beam geometry through the fluidic channel. Such flexibility is difficult to achieve with lithographic-based fabrication processes.

Conclusions

Biochip devices employ a wide range of materials, many of which are incompatible with conventional lithographic processes. The accuracy, flexibility and often material independence of laser micromachining make it an attractive microfabrication process for the prototyping and production of laboratory-on-a-chip and

biochip devices. Additionally, laser processing has the ability to produce structures which would be difficult or impossible to create cost effectively using lithographic processes. Advances in production scale laser machining and emerging laser processing techniques such as two photon polymerisation also allow laser processing to be strong competition to lithographic mass production.

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