

### Microfluidic device fabrication

Many of the fabrication techniques used in microfluidic device technologies were adopted from the semiconductor industry. Most of the recent development work has utilized glass or fused-silica substrates, which exhibit better electrical insulation and optical properties than semiconductors, or polymer materials that reduce costs<sup>48,49</sup>.

Typical glass or fused-silica microfabrication steps include:

- 1 Deposition of a thin chromium or gold film onto the substrate to act as an etch mask.
- 2 Spin-coating of a light-sensitive polymer film (photoresist) onto the metal film.
- 3 Exposure of the photoresist to ultraviolet light through an appropriate mask to create a pattern.
- 4 Development of the photoresist to strip away exposed areas.
- 5 Removal of the metal film in the exposed areas by etching with KI/I<sub>2</sub> (for gold) or K<sub>3</sub>Fe(CN)<sub>6</sub>/NaOH (for chromium).
- 6 Etching of the substrate using a dilute HF/NH<sub>4</sub>F solution.
- 7 Sealing the microchannels by thermally bonding a cover plate of the same material to the patterned substrate<sup>48</sup>.

Microchannel dimensions are typically 20–100 μm wide, and 5–30 μm deep. Thermal bonding of the cover plate to the substrate is carried out by bringing the parts into contact and applying heat and pressure to increase compliance and promote formation of covalent Si–O–Si bonds. The process is sensitive to the presence of particulates on the bonding surfaces, hence most fabrication steps are carried out in a clean room. Access holes for addition of reagents or buffers are fabricated in the cover plate before bonding using techniques such as ultrasonic drilling.

### Microfluidic device properties

The small dimensions of microfabricated devices give rise to unusual fluidic properties. The Reynold's number (Re) is the ratio of inertial forces to viscous forces acting on a fluid<sup>50</sup>. For very small Reynold's numbers (Re ≪ 1 for microfabricated devices), the inertial forces are essentially zero and the fluid dynamics are dominated by viscous drag. Fluid flow in this 'low Reynold's number' or 'Stokes flow' regime is characterized by laminar flow profiles (there is no turbulence in the device), by mixing through lateral diffusion and by the effects of surface tension.

Control of fluid flow in these devices has been carried out using a variety of techniques, including pressure-driven flow, and more recently, electro-osmotic flow. Pressure-driven control of fluid flow in microchip devices is often achieved simply by direct connection of external pressure and/or vacuum sources (e.g. programmable syringe pumps or peristaltic pumps). Microfabricated pumps integrated directly into the device have also been demonstrated. Micropump designs using thermopneumatic,

electrostatic and piezoelectric actuation have all been described in the technical literature<sup>15–17</sup>.

### Electro-osmotic flow

In electro-osmotic flow, the flow velocity of the bulk solution ( $u_{\text{Bulk}}$ ) is proportional to the applied electric field (E) and the electro-osmotic mobility ( $\mu_{\text{EO}}$ ):

$$u_{\text{Bulk}} = \mu_{\text{EO}} E$$

Electro-osmotic mobility is in turn proportional to the electrostatic potential at the hydrodynamic plane of shear along the microchannel wall and depends on both the charge density on the channel wall and the ionic strength of the fluid<sup>51</sup>. Electric fields required to generate fluid velocities of approximately 1 mm s<sup>-1</sup> are typically several hundred volts per centimeter, and are manipulated via electrodes brought into contact with the fluid-filled microchannels. Because electro-osmosis is a surface-driven phenomenon, fluid transport is characterized by 'plug flow' (i.e. the fluid velocity is nearly constant across the width of the channel)<sup>52</sup>. In addition, because the flowstream follows the electric field lines, it is possible to create virtual valves in which fluid flows around corners at intersections of microchannels with essentially no leakage into the other branches of the device<sup>53</sup>. The principal disadvantage of electro-osmotic fluid transport is the tendency for un-wanted separation of components owing to differences in electrophoretic mobility.

### Electrophoretic separation

Charged species present in the bulk fluid undergo electrophoresis as a result of the applied electric field, and move with an electrophoretic velocity of:

$$u_{\text{EP}} = \mu_{\text{EP}} E$$

where the electrophoretic mobility ( $\mu_{\text{EP}}$ ) is dependent on the net charge of the molecule and hydrodynamic radius. The net velocity of the charged analyte ( $u_{\text{Analyte}}$ ) is thus given by:

$$u_{\text{Analyte}} = (u_{\text{EP}} + u_{\text{Bulk}}) = (\mu_{\text{EP}} + \mu_{\text{EO}}) E$$

Molecules having different electrophoretic mobilities will travel through the system at different velocities, and hence a discrete injection of a sample containing a mixture of analytes quickly separates into bands of individual molecular species migrating at different rates. Several unique features of the microchip format enable very rapid, high-resolution electrophoretic separations to be performed in these devices<sup>1,2,48,49,53</sup>. The flat velocity profile that is characteristic of electro-osmotic flow essentially eliminates the band broad-