

The counterbalancing bulk flow can be applied electroosmotically if the electro-osmotic mobility does not differ too much from the electrophoretic mobility of the analyte. If the electro-osmotic mobility is written as $\mu_{EO} = \mu_{EO}^0 \eta(20)/\eta(T)$, then by adjusting the ratio of the lengths of the hot and cold channels, (assuming $f_{EP}(T) = \text{constant}$) focusing can be achieved if $f(\text{cold})/f(\text{hot}) < -\mu_0/\mu_{EO}^0 < f(\text{hot})/f(\text{cold})$, where $f(\text{hot}) > f(\text{cold})$. If $f(\text{hot}) < f(\text{cold})$, then the inequalities have the opposite sign. If x is the fraction of the total channel length that is hot, then focusing will occur if: $x \cdot f(\text{hot})/f(\text{cold}) + (1-x) < -\mu_0/\mu_{EO}^0 < x + (1-x) \cdot f(\text{cold})/f(\text{hot})$, where $f(\text{hot}) > f(\text{cold})$. By adjusting x , it is then possible to tune the range of analyte mobilities that are focused.

It should be noted that this can also be done for microchannels of non-constant cross-section. The final results are essentially unchanged, since in most instances, the dependence on the cross-sectional area of the channel cancels out in the equations. As a result, it is possible to generate the temperature gradient using Joule heating within the microchannel. This would serve to simplify the design and operation of a microfluidic device using this technique even further, since the focusing and the temperature gradient could be produced using the same pair of electrodes as illustrated in FIG. 4(a).

Microchannel 20 shown schematically in FIG. 4(a), has electrodes, 22, 24, and two sections, sections 26, 28, of different cross-sectional area. Section 26 has a cross-sectional area of 27 and section 28 has a cross-sectional area of 29. The electrical resistance per unit length of each section is given by: $R_1 = 1/(\sigma \cdot A_1)$, where σ is the conductivity of the buffer in the microchannel 20. When a current, I , is passed through the microchannel 20, the power per unit length dissipated through Joule heating in each section will be: $P_1 = I^2 \cdot R_1 = I^2/(\sigma \cdot A_1)$. In general, the resulting temperature in section 28 will be higher than that in section 26, as shown in FIG. 4(b): $T_2 > T_1$. The electric field in each section of the microchannel 20 is given by the current multiplied by the resistance per unit length: $E_i = I \cdot R_i = I/(\sigma A_i) = I \cdot \eta(T_i) \cdot f(T_1)/(\sigma_0 \cdot h(20) \cdot A_i)$.

The electrophoretic velocity of an analyte in each section of the channel is: $u_{EP}^i = \mu_0 \cdot f(T_1) \cdot I/(\sigma_0 \cdot f_{EP}(T) \cdot A_1)$. If a bulk flow velocity is applied along the channel, it will not be the same in each section, but will instead be given by $u_B^i = u_B^0/A_i$, where, u_B^0 is a constant. The ratio of the electrophoretic velocity to the bulk velocity is then given by: $u_i^{ratio} = \mu_0 \cdot f(T_i) \cdot I/(\sigma_0 \cdot f_{EP}(T) \cdot u_B^0) = u_0^{ratio} \cdot f(T_i)/f_{EP}(T)$ via adjusting u_B^0 so that $|u_1^{ratio}| > |u_2^{ratio}|$ as shown in FIG. 4(d), which can result in focusing. Because the ratio of the electrophoretic velocity to the bulk velocity does not depend on the cross-sectional areas of the two sections, the same considerations as above apply if bulk flow is applied electroosmotically.

One preferred buffer system is composed of 0.9 mol/L Trizma base and 0.9 mol/L boric acid in water (1.8 M Tris/boric), with an expected pH of about 8.7 (at room temperature). From measurements of the conductivity of the buffer, the function $f(T)$ was determined to vary from 1 at 20° C. to 0.77 at 70° C.

Joule heating may be used to generate the temperature gradient in the microchannel device of FIG. 4(a). The following is a non-limiting example demonstrating Joule heating of a microchannel of the type shown in FIG. 4(a).

The microchannel used for this demonstration was similar to the one shown schematically in FIG. 4(a). The width, i.e., cross sectional area 29, of the narrow channel, i.e., section 28, was about 70 μm , and the width of the wide section, i.e., section 26, was of the cross sectional area 26 was about 350 μm . The length of the tapered portion of the channel was

about 500 μm . The depth of all portions of the channel was about 30 μm . The total length of the microchannel was about 2 cm, with the length of the section 28 divided by the total length, $x \approx 0.8$. Access to each end of the microchannel was provided by a 3 mm hole through the lid piece of the microchannel.

An 8 $\mu\text{mol/L}$ solution of carboxyfluorescein in the 1.8 M Tris/boric buffer was prepared. The analyte to be concentrated was the carboxyfluorescein. Detection of the analyte was performed using a fluorescence microscope and CCD cameras. Simultaneous color and grayscale images were obtained.

To demonstrate gradient focusing using Joule heating, the microchannel was filled with the carboxyfluorescein solution and 1900 V was applied along its length, with the positive voltage V_2 applied to the narrow end via electrode 22, and the wide end held at ground at electrode 24.

After 6 min., the carboxyfluorescein was highly concentrated at the junction between sections 26 and 28 of the microchannel 20. The concentration factor achieved by using this example was typically about 100-fold per minute.

Referring now to FIG. 5, in order to have better control of the temperature gradient, experiments were done using three temperature zones, two cold zones provided by cooling copper blocks 53a, 53b covering much of the ends of the microchannel 50, and one hot zone provided by heated copper block 52. The microchannel 50 was made out of thin (125 μm) sheets of poly(carbonate) substrate 51, which were pressed onto the copper blocks 52, 53a, 53b. Thermal contact between the poly(carbonate) and the copper blocks was insured using a thermally conductive adhesive 56. The copper blocks 52, 53a, 53b were arranged so that there was a 1 mm gap 58 between the heated copper block 52 and the cooling copper block 53a and a 2 mm gap 59 between heated copper block 52 and the cooling copper block 53b.

Microchannel 50 also includes electrodes 55, 54, buffer reservoirs 57, and a narrow hot zone 50a near the middle of the microchannel 50. The heated copper block 52 was heated using a small high-power resistor embedded into the copper and its temperature was regulated using a PID temperature controller (Omega Engineering Inc, Stamford, Conn.). To regulate the temperature of the cold zones, 1/4 inch diameter holes were drilled through the cooling copper blocks 53a, 53b and cold water from a thermostatted bath (Neslab, Portsmouth, N.H.) was passed through them.

Thin polycarbonate microchannel chips, i.e. substrate 51 was attached to the copper blocks 52, 53a, 53b using thermally conductive adhesive 56 in the form of transfer tape (3M). The substrate 51 was pressed against the copper blocks 52, 53a, 53b from above with 3 mm thick PDMS (Sylgard 184, Dow Corning, Midland, Mich.) gaskets 60 and a 2 mm thick acrylic (Acrylite OP-4, Cyro Industries, Mt. Arlington, N.J.) top plate 61, which was secured to the outer copper blocks using nylon screws (not shown).

During temperature gradient focusing, a voltage potential is applied to electrode 55 and electrode 54 is set to ground to allow microchannel 50 to provide focusing and separation of different types of analytes: small dye molecules, amino acids, proteins, DNA, colloidal particles, and cells.

The microchannel 50 may be formed by imprinting with a micro machined silicon template and then sealed with a similar material according to the method disclosed in Ross, D.; Gaitan, M.; Locascio, L. E., *Analytical Chemistry* 2001, 73, 4117-23, herein incorporated by reference.

The copper block arrangement was also used to determine the degree of focusing that could ultimately be reached with temperature gradient focusing. Beginning with a 8 nM