Supplementary Information for

Generation of Linear and Non-linear Concentration Gradients along Microfluidic Channel by Microtunnel Controlled Stepwise Addition of Sample Solution

Cheuk-Wing Li\textsuperscript{1}, Rongsheng Chen\textsuperscript{2} and Mengsu Yang\textsuperscript{1,2,*}

1. Department of Biology and Chemistry, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong SAR, China
2. Key Laboratory of Biochip Research, Shenzhen Research Institute of City University of Hong Kong, Shenzhen, China

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S1. Microfabrication details:

The procedure to prepare the PCB master involved (I) the design of the photomasks by a computer aided design software (CorelDRAW 12.0 Corel Corporation, UK), (II) the transfer of photomask patterns on the positive photoresist coated on the PCB (Kinsten, Chiefskill, Taiwan) by UV irradiation (KVB-30 exposure unit, Chiefskill), (III) the removal of the exposed photoresist by PCB developing reagent (Kinsten, Chiefskill, Taiwan) and (IV) the etching of the unprotected copper layer on the developed PCB by ferric chloride solution. The PCB master featured with main channels and microtunnels were cast with degassed PDMS prepolymer (Sylgard 184, Dow Corning, Midland, MI). Then, the PDMS was cured on the PCB master at 65°C for 2 hours to form negative replicas. With access holes punched, the replicas were oxidized in a plasma cleaner (PDC-3XG, Harrick Scientific, Ossining, NY) and irreversibly sealed against another thin PDMS slab to form a gradient generator. For scanning electron microscope (SEM) imaging, cured PDMS replicas were sliced into cross sections by using a vibrating blade microtome (VT-100S, Leica Microsystems, Deerfield, IL, USA) and were coated with gold by a sputter coater (SCD 050, BAL-TEC, USA). High vacuum mode of a scanning electron microscope (XL30 ESEM FEG/EDAX system, Philips Electron Optics, The Netherlands) was used to obtain the tilted SEM image in Fig S2.
S2. SEM image of microtunnels and main channels:

Fig S2. SEM image of a 45 degree tilted gradient generator featured with main channels, microtunnels and cavity microstructures. The microtunnels were much shallower in height when compared with the main channels. Hence, fluidic resistance of a microtunnel was much higher than in a main channel, a prerequisite for stepwise addition of sample solution. Inset showed the cross section of a microtunnel structure. Scale bar is 5 μm. As a side note, the cavity microstructures could be used to accommodate biological cells or microorganisms for studying cellular behavior under linear and non-linear gradients of a stimulant. However, tests regarding the functions of these cavities were not performed in this study.
**S3. Explanation of identical pressure differential across microtunnels:**

The basic principle to establish a chemical gradient along fluidic flow is by introducing sample solution through a series of microtunnels fabricated alongside the main channels. The control of chemical gradient profile was achieved by varying the length of each microtunnel that would alter the volume of sample solution being dispensed convectively into the buffer stream of main channel.

A sketch comprising upper and lower main channels was built (Fig S3a, sketch) to elucidate this design principle. The two main channels are of five units in length and are interconnected sideways by a series of 11 microtunnels. These microtunnels are assumed to have very high fluidic resistance so that the majority of flow goes along the main channels. Equal pressure (5 units) is applied to both inlets of the main channels to drive fluidic flows from left to right. A plot of pressure versus distance coordinate is built below the sketch, where two dotted lines illustrate the positions corresponding to the first and the last microtunnels. Since the pressure profiles of the upper and lower main channels are identical, their profiles are superimposed in the plot indicating that there is no pressure differential across all microtunnels.

Let us now consider another sketch where the relative positions between two main channels are horizontally displaced in a fashion depicted in the sketch of Fig S3b. While other parameters are unchanged in both sketches, pressure differential is present between two main channels in sketch of Fig S3b. Since the pressure profiles of the two main
channels are identical, the horizontal displacement results in an identical pressure differential across all microtunnels (Fig S3b, plot).
Fig S3. Identical pressure differential across microtunnels is achievable by horizontal displacement between two main channels. The applied pressure (arbitrary unit) is indicated by the number inside the inlet and outlet ports (circular). The length of the main channels is metered by the distance coordinate axis underneath the sketches. “mt” refers to the distance between the first and the last microtunnel. “x” and “y” refers to the distance of microtunnel away from the inlet and outlet ports, respectively. Identical pressure differential also exists in other x and y values as long as the total lengths of both upper and lower main channels are identical and $x_{up} \neq x_{lo}$. 