Supplementary Information

Method for Analyte Identification Using Isotachophoresis and a Fluorescent Carrier Ampholytes Assay

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We here provide details of the image processing steps we implemented for obtaining the signal $I(x)$ from the acquired raw images. We also present an example where 2-nitrophenol and 2,4,6-trichlorophenol are simultaneously detected using the fluorescent carrier ampholytes (FCA) assay, and provide a figure summarizing the procedure for analyte identification using FCA.

Background and flatfield correction of raw data

We here describe in the detail the image analysis we perform on the raw images in order to obtain the signal $I(x)$ used in our identification procedure.

- Throughout the experiment, the location of the microscope stage is fixed. We begin acquiring images shortly after initiating the electric field, and continuously acquire images until all fluorescent peaks have passed through the field of view.

- All images acquired before the first fluorescent zone enters the field of view can be regarded as background. We average those images, resulting in a single background image which we denote here as $BG(x, y)$. An example image is shown in Figure S1(a). The background image is acquired with the illumination on, but with no fluorescent species in the liquid-filled channel.

- At the end of each data set acquired at a given location, we fill the channel with a high concentration ($10\mu M$) of fluorescent dye and acquire 100 images. We arithmetically average these 100 images, resulting in a single flatfield image which we denote here as $FF(x, y)$. An example image is shown in Figure S1(b). In this image, it is clear that the illumination intensity decreases toward the right hand side of the field of view.

- Figure S1(c) shows a typical raw data image acquired in the experiment. We denote this image as I_{RAW}. By subtracting the background image from the raw image ($I_{RAW}(x, y) - BG(x, y)$) we obtain a background corrected image, shown in figure S1(d). Applying the same process to the flatfield image ($FF(x, y) - BG(x, y)$) yields a background corrected flatfield image (not shown here).

- We divide the background corrected image by a background corrected flat field image to compensate for non-uniform illumination,

  $$I(x, y) = \frac{I_{RAW}(x, y) - BG(x, y)}{FF(x, y) - BG(x, y)},$$

  and obtain the flatfield corrected data image shown in figure 1S(e).

- Finally, we integrate $I(x, y)$ along the width of the channel, w, to obtain a one-dimensional function describing the variation of area-averaged intensity along the axial coordinate of the channel,

  $$I(x) = \int_0^w I(x, y)dy .$$

Figure 1S(f) presents the area averaged intensity of the raw data image, the background corrected image, and the flatfield corrected image. To better see the effect of flatfield correct, figure 1S(g) present a detailed view of the right-most section of the signal where the illumination intensity is lower.

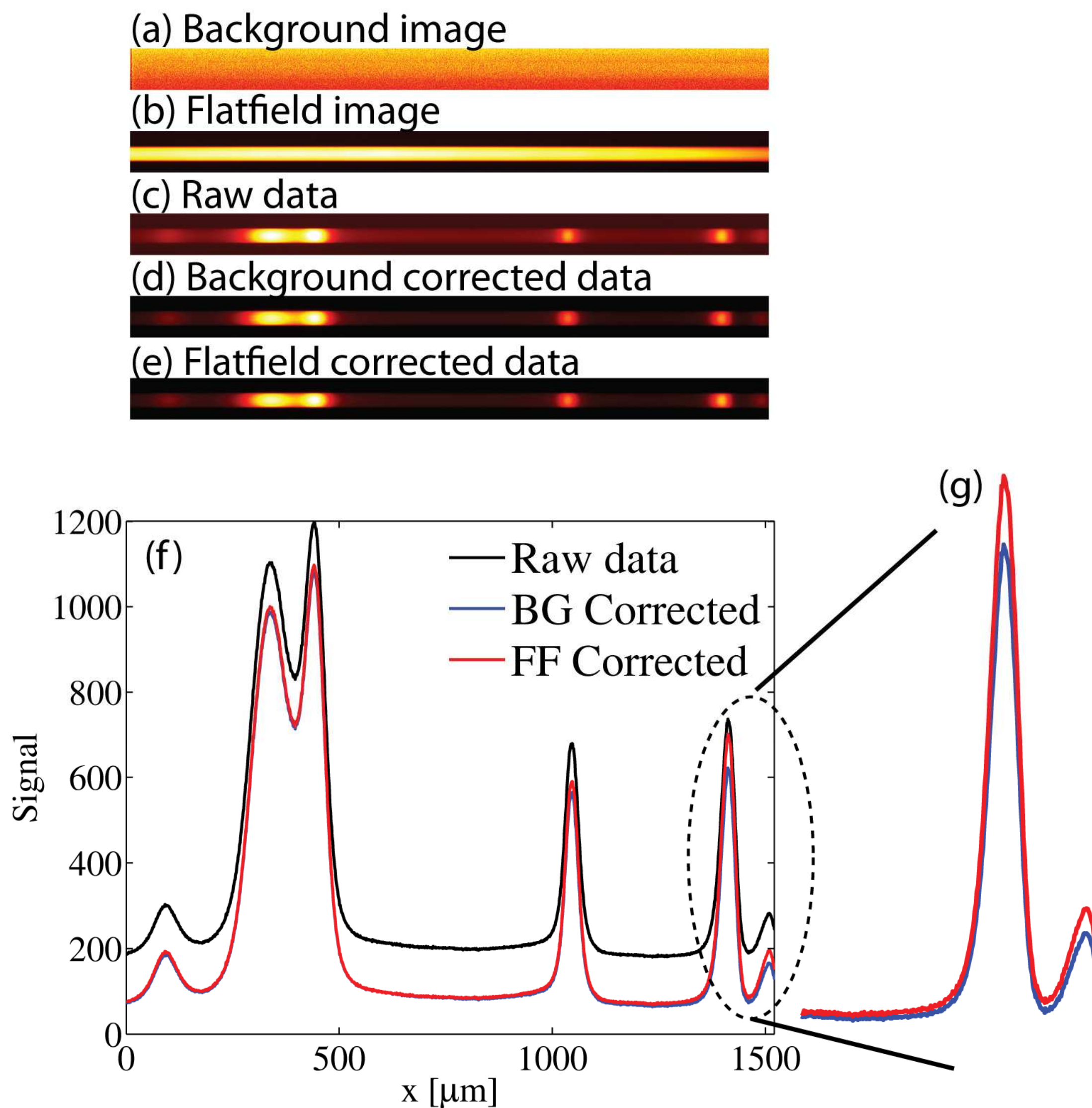

Figure 1S. Example of image processing performed on acquired data. (a) Background image obtain from averaging of images acquired before fluorophores reach the field of view. (b) Flatfield image obtained after the experiment, by imaging a channel filled with uniform fluorescent dye. (c) Raw data image showing several fluorescent peaks. (b) Background corrected image obtained by subtracting the background image from the raw data image. (d) Flatfield corrected imaged obtained by dividing the background corrected data by the background corrected flatfield image. This step results in an increase in intensity of the right-most peak which was located in a region of lower illumination intensity.

Multiplexed Detection of 2-nitrophenol and 2,4,6-trichlorophenol

Below we present typical data showing multiplexed (simultaneous) detection of 2-nitrophenol and 2,4,6-trichlorophenol in a single ITP and fluorescent carrier ampholyte experiment.

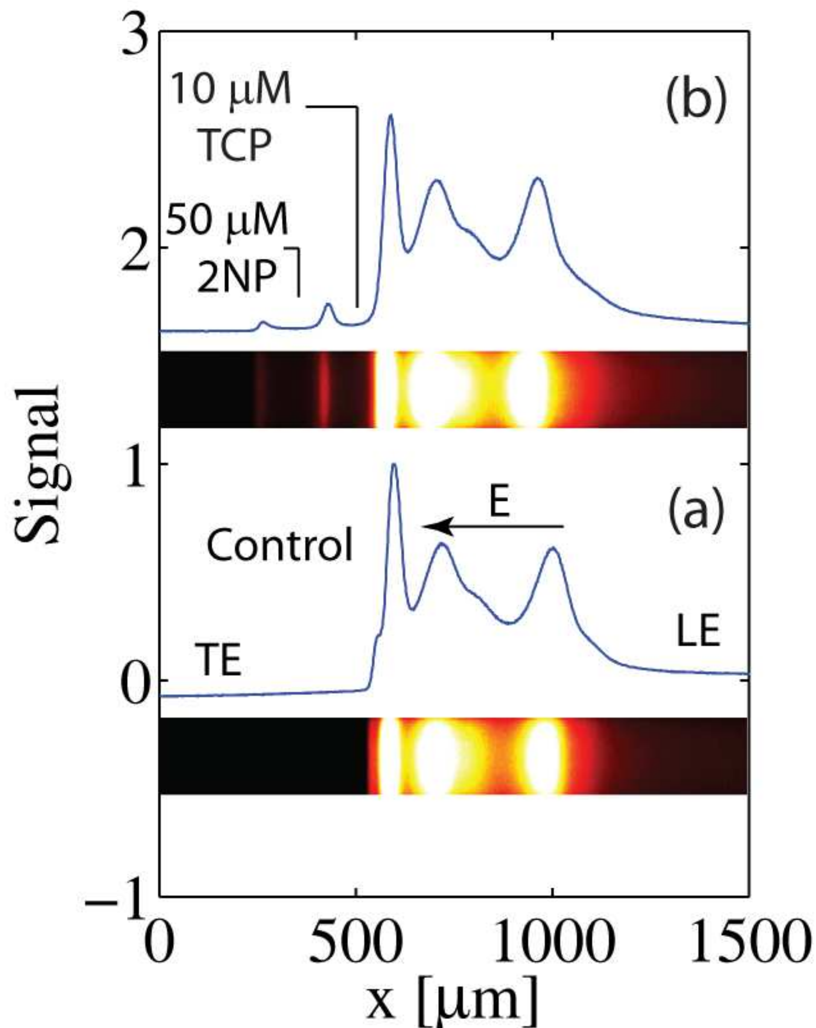

Figure S2. Detection of 2-nitrophenol (2NP) and 2,4,6-trichlorophenol (TCP) in tap water **(a)** The control (no analytes) shows several peaks; the gaps between these likely correspond to impurities in the tap water. **(b)** 50 μM of NP and 10 μM of TCP are here mixed in the TE reservoir. Each displaces a subset of fluorescent CAs resulting in new gaps in the signal. LE is 10 mM Lactic acid, TE is 10 mM tricine and counterion is 20 mM bis-tris. 1 μM of ZOOM 3-10 labeled with Alexa Fluor 647 we mixed in the TE. We here used 1100 V applied voltage with 2 ms exposure.

Summary of analyte identification procedure
The figure below summarizes our analyte identification procedure.

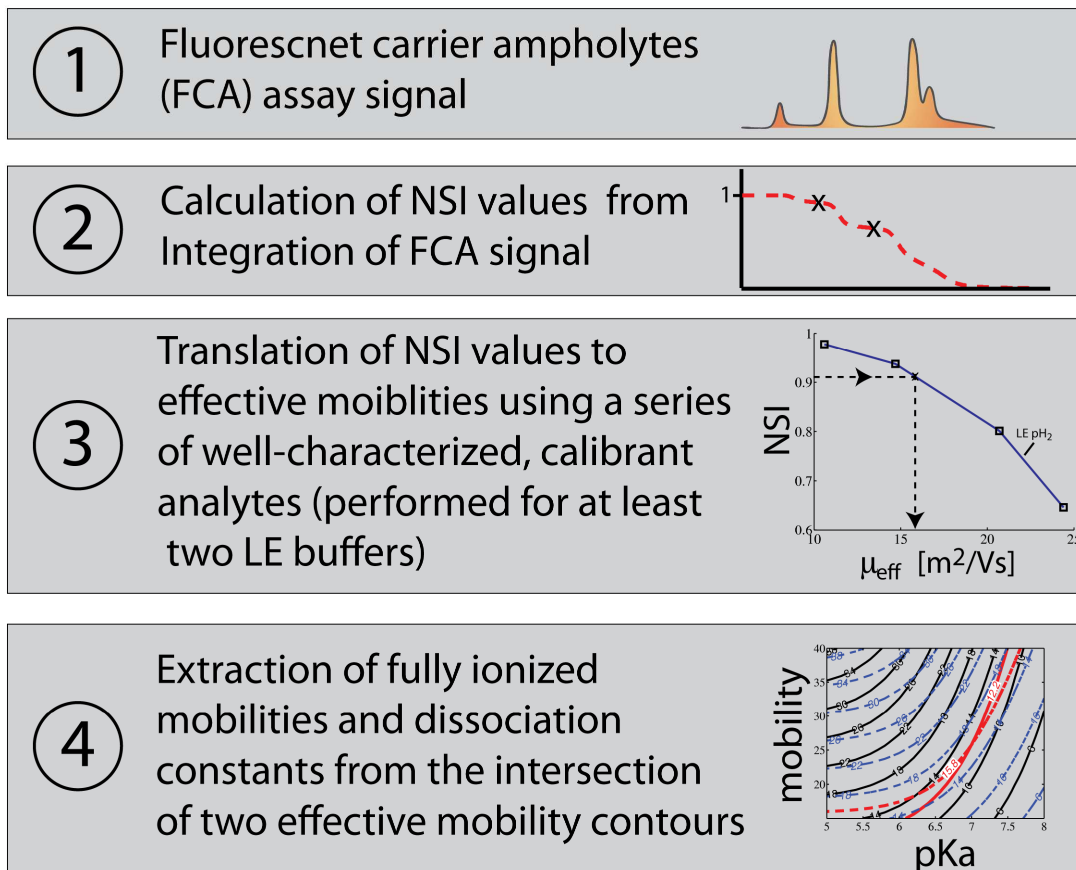

Figure S3. Summary of the analyte identification procedure using the fluorescent carrier ampholytes assay