Electronic Supplementary Material (ESI) for Lab on a Chip.

Nitrate measurement in droplet flow: gas-mediated crosstalk and correction

Electronic Supplementary Information

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Supplementary figures

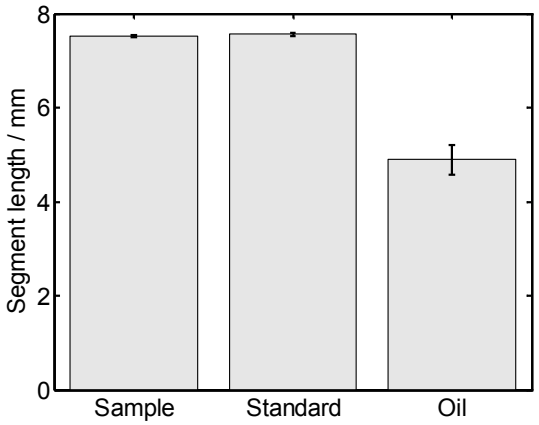

Figure S1: Lengths of sample droplets, standard droplets and separating oil slugs, generated by the setup shown in Fig. 1, flowing through 500 µm ID PTFE tubing at a mean linear flow rate of 2.75 mm/s. In each case n=100 and the error bars indicate the standard deviation of each measurement.

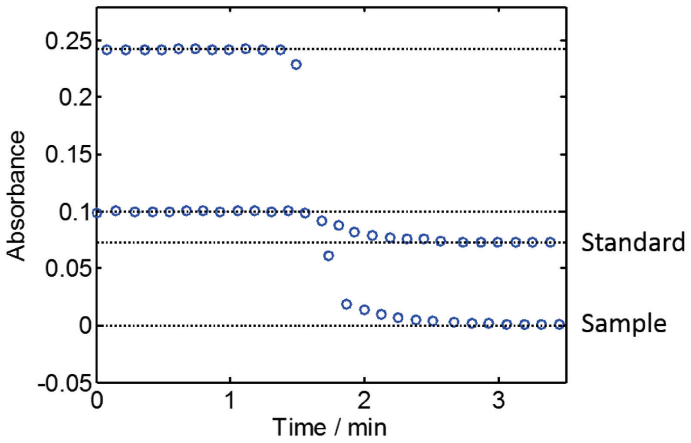

Figure S2: Representative droplet absorbance data showing the absorbance of standard droplets (200 µM NO_3^-) shifting in sympathy with a change in sample (from 600 µM to 0 µM NO_3^-) in the absence of any surfactant.

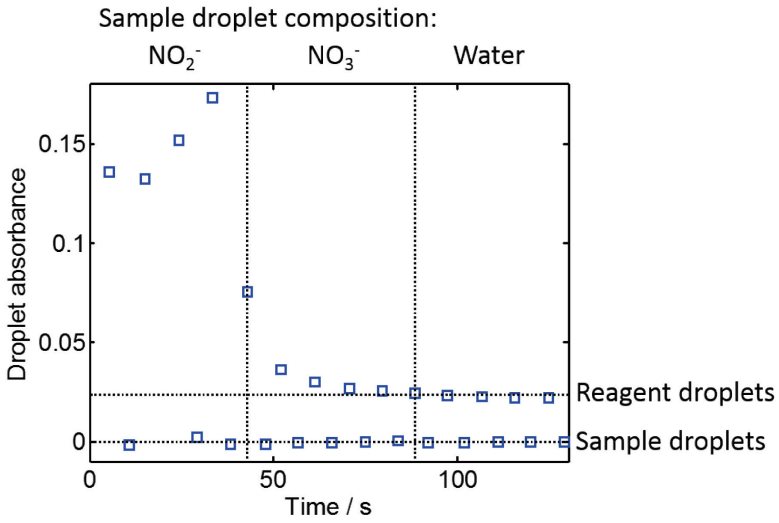

Figure S3: Absorbance of alternating droplets of sample and reagent generated by the manual aspiration method (see Fig. 2 and accompanying text for explanation of the method) using silicone oil as the continuous (non-droplet) phase. The sample composition was changed from nitrite (NO_2^-) to nitrate (NO_3^-) to water. Inter-droplet crosstalk was seen from nitrite droplets only, as previously seen in experiments using perfluorinated oil (Fig.s 2,3).

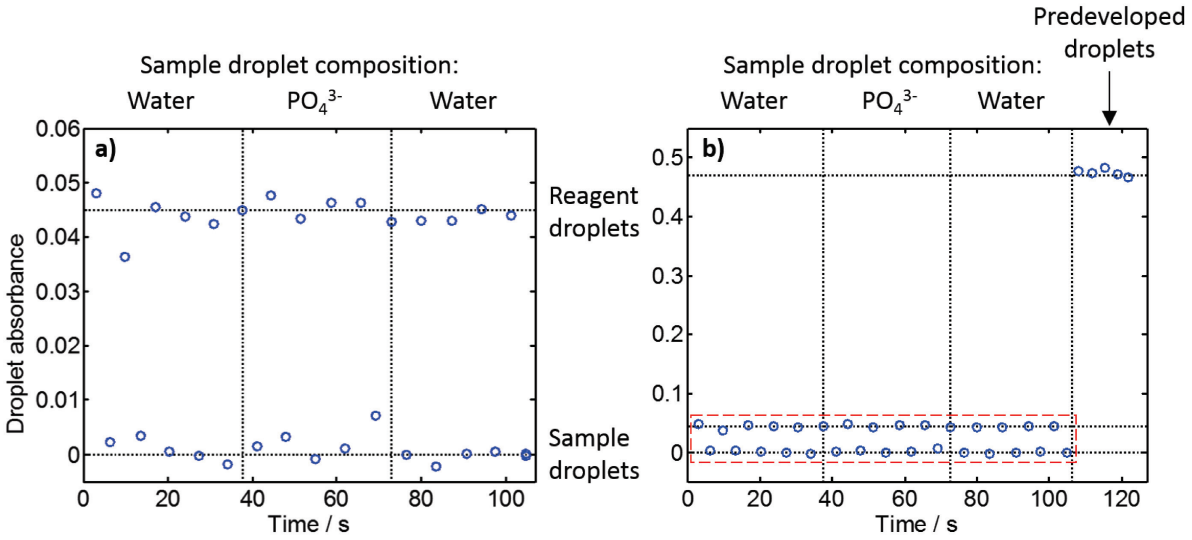

Figure S4: a) Absorbance of alternating droplets of sample and (phosphate-specific) reagent generated by manual aspiration. The sample composition was changed from water (left) to 100 µM phosphate (PO_4^{3-}, centre) to water (right), with no colour development evident in the reagent droplets. b) The same data (highlighted in the red dashed box) shown alongside a series of predeveloped droplets (formed by mixing the phosphate solution with the reagent at a volumetric ratio of 1:1) – showing the colour development that can be attained when phosphate reaches the reagent.

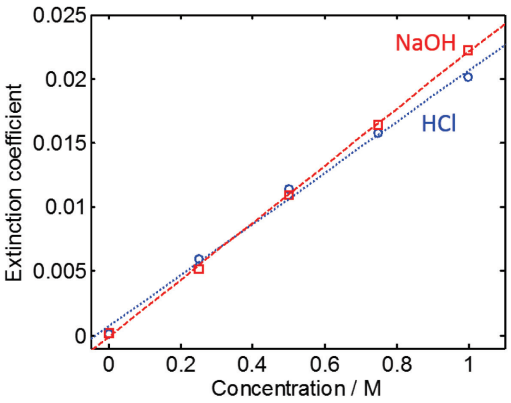

Figure S5: Extinction coefficient of different concentrations of NaOH and HCl flowed through a flow cell, similar to that used in the setup shown in Fig. 1. Both give an increase in extinction coefficient of similar magnitude, consistent with the data shown in Fig. 3. In contrast to Fig. 3, here we label the y-axis as the more formally correct "Extinction coefficient" rather than "Absorbance" as the increase here is solely attributable to the change in refractive index.

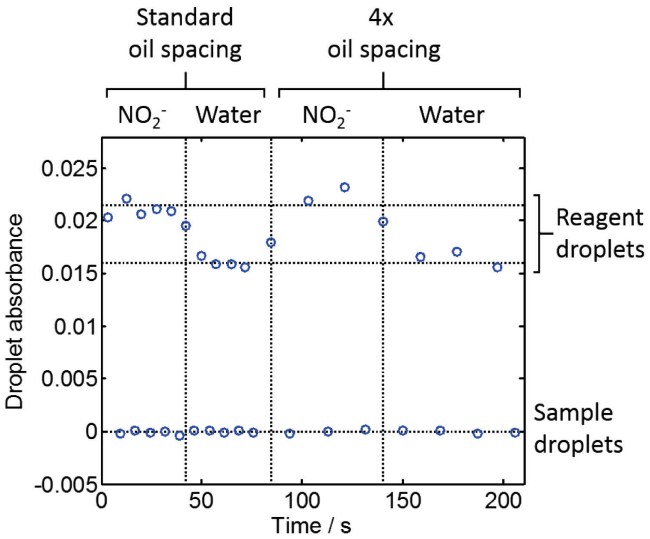

Figure S6: Absorbance of alternating aspirated droplets of sample and reagent generated by manual aspiration. The sample composition was alternated between water and nitrite (NO_2^-) with the spacing between droplets changing from standard (same volume as the droplets) to 4 times greater. The colour development evident in the reagent droplets is the same regardless of oil spacing, consistent with all NO in the oil having been consumed (in the droplets) in each case. As the drops were aspirated and then flowed through the heater and flow cell (as shown in Fig. 2), each set of droplets will have undergone an identical residence time in the heated section.

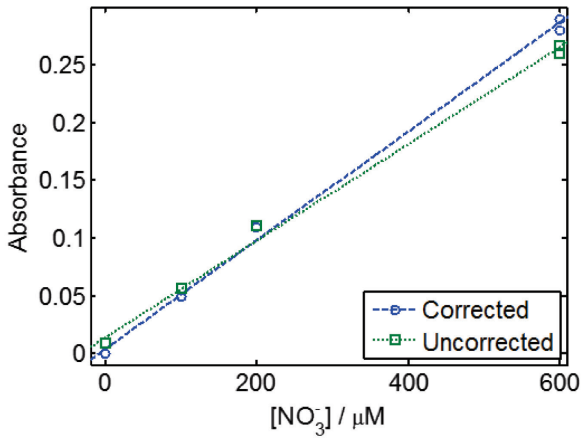

Figure S7: Calibrations for measuring river samples, using uncorrected (green squares) and corrected (blue circles) measurement of 0, 100, 200, and 600 µM NO_3^- standards.

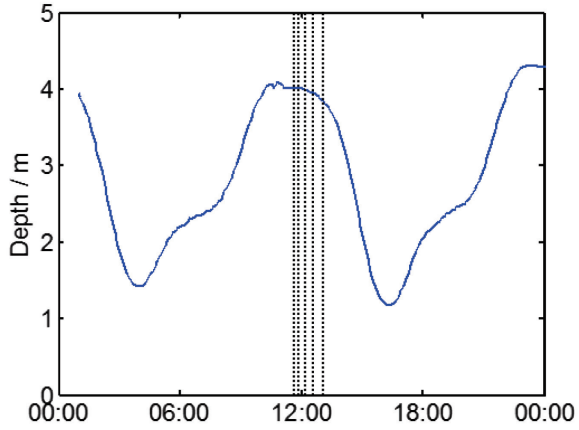

Figure S8: The tide state in Southampton on the day that samples were taken from the River Itchen (7th June, 2017). The vertical dashed lines indicate the time each sample was taken from the sampling positions (shown in Fig. 5 in the main text) in the order: 4, 5, 3, 2, 1. The depth (y-axis) represents the tide height as measured at Southampton docks (50 53.01N, 1 23.66W), data obtained from www.sotonmet.co.uk.

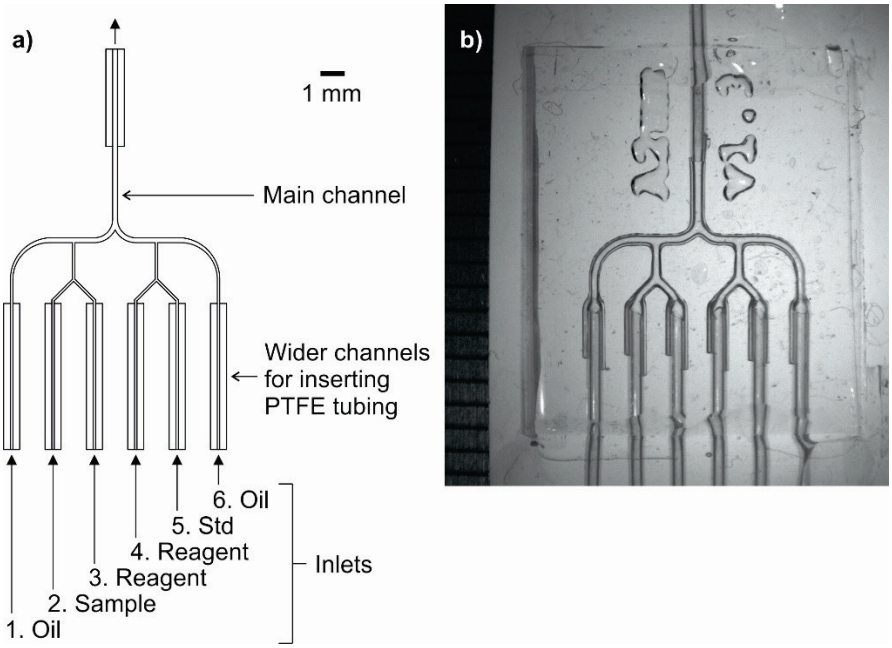

Figure S9: a) Channel design of the microfluidic chip. b) Image of a fabricated chip with PTFE tubing inserted and fixed. A ruler with millimetre divisions is shown on the left edge of the image.

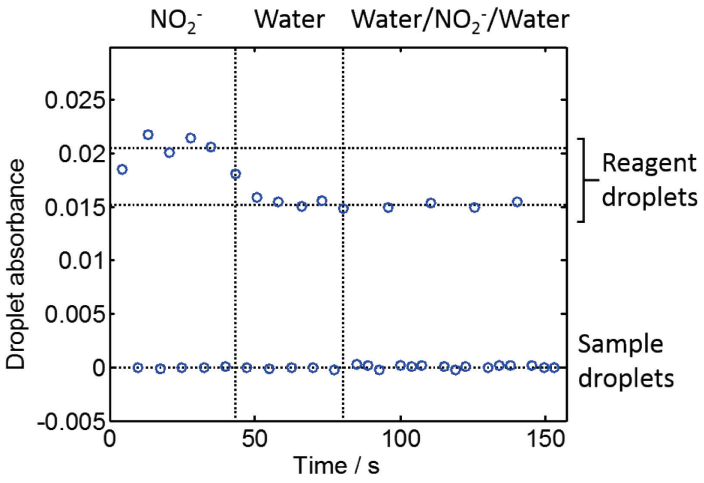

Figure S10: Absorbance of alternating sample and reagent droplets generated by manual aspiration. The sample composition was changed from nitrite (NO_2^-, left) to water (centre) to a three-droplet sequence composed of water-nitrite-water. Colour development is seen where the sample droplets are nitrite alone, but no colour is seen when the sample is water or the water-nitrite-water sequence - indicating that the nitrite only migrated as far as the neighbouring droplets.

Derivation of crosstalk correction equation

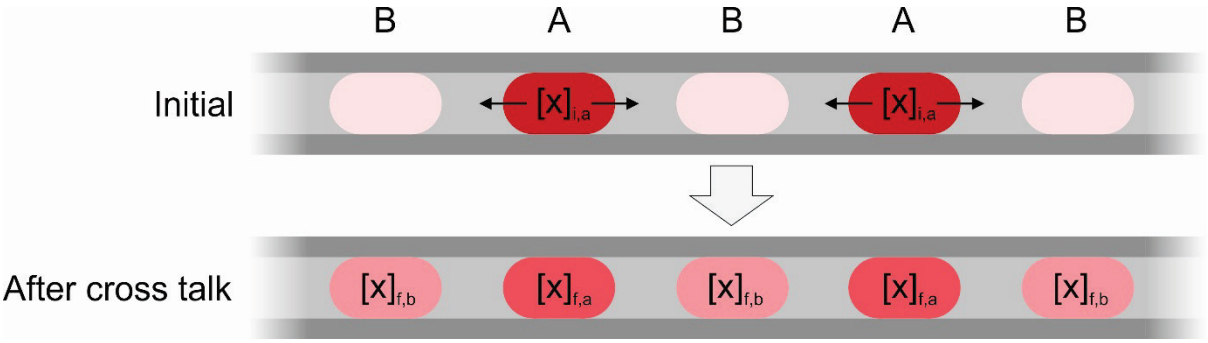

Figure S11: Cartoon illustrating migration of an analyte, x, in a sequence of alternating droplets, A and B. We only consider the analyte initially present in population A (top), a proportion of which subsequently migrates into population B (bottom).

Consider a stream of alternating droplets (A and B). We are concerned with the migration of an analyte "x" from droplet A to the neighbouring droplets, B. The analyte is at an initial concentration of $[x]_{i,a}$ in droplet A and gives final concentrations of $[x]_{f,a}$ in droplet A and $[x]_{f,b}$ in droplet B.

We assume that: a) The concentration remaining in the oil is negligible. b) Analyte cannot travel further than the immediately neighbouring droplet.

These assumptions are reasonable as: a) The diffusion constant of diatomic gases within fluorous oil is high[1, 2] and thus it is likely any nitric oxide that enters the oil will diffuse to a droplet and be captured within it by chemical reaction (as illustrated in Fig. 4a) before measurement. b) In the aspiration experiments (e.g. Fig.s 2 & 3 in the main text) it was generally observed that reagent droplets were only affected by crosstalk from neighbouring sample droplets. This is most effectively shown in the experiment shown in Fig. S10, where blank droplets were introduced in between nitrite and reagent droplets and stopped crosstalk by capturing any migrating species.

First we define the "cross talk parameter", α, which specifies the ratio of x that has left the droplet to the quantity of x that remains following crosstalk:

$$\alpha = \frac{[x]_{f,b}}{[x]_{f,a}} \qquad [1]$$

This parameter can be experimentally determined as described in the main text, and in our experimental setup typically had a value of $0.1 \leq \alpha \leq 0.2$.

To be able to correct for crosstalk we must relate how the final droplet concentrations relate to the initial concentration. Assuming no analyte is lost through the tubing and the concentration left in oil is negligible,

$$[x]_{i,a} = [x]_{f,a} + [x]_{f,b}$$

which rearranges to:

$$[x]_{f,a} = [x]_{i,a} - [x]_{f,b} \qquad [2]$$

$$[x]_{f,b} = [x]_{i,a} - [x]_{f,a} \qquad [3]$$

Putting [2] into [1] gives:

$$\alpha = \frac{[x]_{f,b}}{[x]_{i,a} - [x]_{f,b}}$$

which rearranges to:

$$[x]_{f,b} = \frac{\alpha[x]_{i,a}}{1+\alpha} \qquad [4]$$

Likewise, putting [3] into [1] and rearranging gives:

$$[x]_{f,a} = \frac{[x]_{i,a}}{1+\alpha} \qquad [5]$$

Equations [4] and [5] allow us to relate the starting concentration of a droplet (i.e. the concentration we would expect in the absence of crosstalk) to the concentration found in each droplet population after crosstalk.

We now consider a more general experimental setup (such as that shown in Fig. 1a) where both droplets have an initial concentration of analyte and where the final analyte concentrations are measured by colorimetry (though we note that the final derived equation should apply to other methods where the measured property is proportional to analyte concentration). In colorimetric assays we measure the absorption of a coloured product produced from an analyte-specific reaction using the Beer-Lambert law:

$$A = \varepsilon l[product] \qquad [6]$$

where ε is the extinction coefficient of the product and l is the optical path length. In reactions such as the Griess reaction the reaction kinetics are pseudo first-order when the reagent is in excess. At a given reaction time the concentration of reaction product is proportional to the starting concentration of the analyte.[3-5] Therefore we can rewrite [6] in terms of the analyte:

$$A = \varepsilon lk[x] = k'[x] \qquad [7]$$

where k is a constant and $k' = \varepsilon lk$. If we measure the contents of droplets A and B after crosstalk has occurred, each will be a sum of absorption resulting from analyte that was retained from the initial concentration, and analyte that has travelled from neighbouring droplets. So using equations [4], [5] and [7] we can write the absorbance, A_a, of droplet A and the absorbance, A_b, of droplet b as:

$$A_a = k'\left(\frac{[x]_{i,a}}{1+\alpha} + \frac{\alpha[x]_{i,b}}{1+\alpha}\right) \qquad [8]$$

$$A_b = k'\left(\frac{[x]_{i,b}}{1+\alpha} + \frac{\alpha[x]_{i,a}}{1+\alpha}\right) \qquad [9]$$

[8] rearranges to:

$$[x]_{i,a} = (1+\alpha)\left(\frac{A_a}{k'} - \frac{\alpha[x]_{i,b}}{1+\alpha}\right)$$

If we insert into [9] and rearrange, we obtain:

$$k'[x]_{i,b} = \left(\frac{1+\alpha}{1-\alpha^2}\right)(A_b - \alpha A_a)$$

Considering [7], the expression on the left here constitutes the absorbance measurement we would expect from the initial concentration (i.e. if there had been no cross talk), hence this equation allows us to correct for crosstalk:

$$A_{b,corrected} = \left(\frac{1+\alpha}{1-\alpha^2}\right)(A_b - \alpha A_a)$$

and similarly:

$$A_{a,corrected} = \left(\frac{1+\alpha}{1-\alpha^2}\right)(A_a - \alpha A_b)$$

1. J. E. Kreutz, A. Shukhaev, W. B. Du, S. Druskin, O. Daugulis and R. F. Ismagilov, *Journal of the American Chemical Society*, 2010, **132**, 3128-3132.
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4. G. S. Clinton-Bailey, M. M. Grand, A. D. Beaton, A. M. Nightingale, D. R. Owsianka, G. J. Slavikt, D. P. Connelly, C. L. Cardwell and M. C. Mowlem, *Environmental Science & Technology*, 2017, **51**, 9989-9995.
5. M. Yucel, A. D. Beaton, M. Dengler, M. C. Mowlem, F. Sohl and S. Sommer, *Plos One*, 2015, **10**, e0132785.