Notes on data analysis for microfluidics laboratory

4 December 2006

Device dimensions

The devices used were of the following two designs:



Device (a) has a $200\pm2 \mu m$ -wide, 30 mm-long diffusion channel. The inlet and outlet channels are each $500\pm2 \mu m$ wide and may each be considered to have effective lengths of 20 mm. Device (b) has a $300\pm2 \mu m$ -wide, 30 mm-long diffusion channel. The inlet and outlet manifolds are each composed of five parallel $500\pm2 \mu m$ -wide channels that may each be considered to have a length of 20 mm. The closely spaced triangular markers are 500 μm apart and the numbered markers are 2 mm apart. The marker closest to the Y-junction lies 500 μm from the Y-junction.

The thickness of the SU-8 layer used as the mold for most devices was $55\pm5 \ \mu m$. Iris and Frances used a device whose mold relief was $40\pm5 \ \mu m$.

Relating pixel intensity, dye concentration and location

If we assume that the intensity, I, of blue light recorded in the image decreases linearly with increasing concentration, C, of red dye in the channel, we have

$$I = I_0 - kC \tag{Eqn 1}$$

where I_0 is the intensity recorded for clear water and k is a constant for the apparatus. For the channel shown in this document, the model presented in Marty Schmidt's November 17 lecture predicts that the concentration will vary with cross-channel position x according to

$$C(x) = \frac{C_0}{2} \left[1 + erf\left(\frac{x}{2\sqrt{Dt}}\right) \right]$$
(Eqn 2)

where C_0 is the concentration of red dye in the red inlet, *t* is the length of time for which fluid at the location under consideration has been in the channel, and *D* is the diffusivity of the dye in water. x = 0 at roughly the center of the channel.

Processing data from the images

First establish how many meters a pixel represents in your images. You have probably captured an image of two of the distance markers; the pitch of the more closely spaced set of markers is 500 µm:



You will probably find that one pixel corresponds to $\sim 1.6 \ \mu m$ on the images.

To analyse your data we suggest using Matlab. The following command loads data from a bitmap file in your working directory to a matrix called image:

image = imread('image.011.000000.bmp','BMP');

To display the image on the screen type: imshow(image)



image(:,:,3) contains the blue intensities, which are of the most interest to us. An arbitrarily chosen row of blue pixels from the image will, on its own, typically give a noisy trace: the command plot(image(200,:,3)) would yield:



We can get a smoother trace by averaging pixels length-wise along the channel:

AveragedBlueIntensity = mean(image(:,:,3));
plot(AveragedBlueIntensity)



One approach to extracting the diffusivity would be to identify the channel walls in the trace, discard data from outside the channel, and fit an error function to the intensity profile within the channel. If taking this approach, it is important to be confident that both the maximum gradient *and* the amplitude of the function are fitted appropriately. Alternatively, you might consider simply drawing a tangent to the intensity profile at its center, finding its points of intersection with appropriate lines of maximum and minimum intensity for the profile, and using the cross-channel distance between the points of intersection to deduce the diffusivity of the dye. The difficulty here lies in visualizing the maximum

gradient of the trace and in choosing the maximum and minimum intensities appropriately. The estimate of diffusivity depends upon the square of the characteristic distance:



A third possible approach is to take the spatial derivative of the intensity profile — which we expect to be Gaussian in shape — and to measure the width of the peak. This method does not rely upon manual skill in interpreting the graph. Neither should the absolute amplitude of the trace influence the diffusivity extracted in this way.

Successful numerical differentiation relies on having a smooth enough intensity trace to begin with. The following command smoothes the trace across the width of the channel by applying a moving average filter with a width of 10 pixels:

SmoothedBlueIntensity = filter(0.1.*ones(1,10), 1, AveragedBlueIntensity);

These commands evaluate and plot the gradient of the smoothed intensity-position trace:

dI_by_dx = SmoothedBlueIntensity(2:640)-SmoothedBlueIntensity(1:639);
plot(-1.*dI by dx)



We now need to discard data from outside the edges of the channel, and normalize the intensity– position gradient. Having normalized the gradient, we can automatically find a characteristic width wof the peak and calculate an estimate of diffusivity:



NormalizedGradient = dI_by_dx(330:420)./min(dI_by_dx(330:420));
plot(NormalizedGradient,'k.-');

% finds the indices of values in NormalizedGradient that are greater than 0.5 CentralPeak = find(NormalizedGradient>0.5);

% finds distance w in pixels
w = max(size(CentralPeak))/2;

From equations 1 and 2,

$$\frac{\partial I}{\partial x} = -k \frac{C_0}{2\sqrt{\pi Dt}} \exp\left(\frac{-x^2}{4Dt}\right)$$
(Eqn 3)

and therefore where $\partial I/\partial x$ falls to half its peak value (i.e. where $x = \pm w$),

$$D = w^2 / [(4 \log_e 2) t]$$
(Eqn 4)

In the lab you measured the peak velocity, V, of polystyrene microbeads in the channel. Reconcile the measured peak velocity with that predicted by the model presented in the 17 November lecture. Where d is the distance from the channel entrance to the position where the image was obtained, t is given by d/V.

If you have images from multiple, known, locations along the channel you might repeat the above procedure for each image and obtain several estimates of the dye's diffusivity. Alternatively, if your data show good enough agreement, you could plot w^2 against *t*, and fit a straight line to those data; our model suggests that the gradient of that line would equal (4 log_e2)*D*. A non-zero *t*-axis intercept would account for channel entrance effects. If your w^2-t results happen to show a clear but *non*-linear trend, can you suggest why?

