Towards 3-D tracking of colloidal particles in microscopy

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1 Introduction

We investigate a method for 3-D tracking of colloidal particles observed in 2-D bright-field video microscope images. The aim is to estimate diffusion coefficients and pair-wise interaction potential of the particles. These two entities are of fundamental importance in the understanding of colloidal suspensions. Our application lies in the field of pharmacy, where modifications of the active colloidal substances need to be analysed.

Here, our images consists of latex (polysterene) particles performing a Brownian motion in three dimensions. One image in the video sequence is shown in Figure 1. All of the particles are spherical and of equal size, 494 nm in diameter. The apparent differences in size and brightness is due to off-focus placement. Particles in the focal plane are depicted as small, distinct, black spots, while particles above or below the focal plane are either light or dark in the middle. Also, they are larger and more blurred, the further away from the focal plane they are. The resolution in the image is 180 nm per pixel. The time interval between two consecutive images in the video sequence is 20 ms. The movement of our particles in this time is typically only a few pixels, so we need sub-pixel accuracy of our position estimation method.

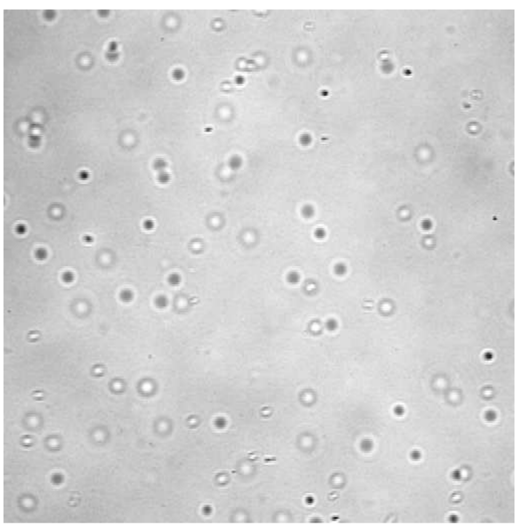

Figure 1: Image from the video sequence.

The idea is to use the out-of-focus optical effect to estimate the depth, or z-coordinate, of the particles. This will be done by using template matching.

Previous efforts in using image processing in colloidal suspension studies have e.g. been made by Crocker and Grier (1996). However, their analyses are restricted to particles at a single focal depth since they have only considered crystalized particles observed with a narrow focal depth of the microscope. The method we are developing is not restricted to these kind of static colloidal systems.

2 Template construction

In addition to the sequence of images like the one above, we also have images where we, for each image, have several particles at a known depth. These images are called z-scans. We have 131 of these, where the difference in depth of the particles present in two consecutive images is 200 nm. These images will now be used to construct templates of what the particles look like at different depths.

We assume that the pixel intensity in a neighbourhood of a particle centre at position $(x_0, y_0, z) \in \mathbb{R}^3$ in one of our images I can be modelled as

$$I(i,j) = \alpha + f_z(d) + \text{ error} \tag{1}$$

where $d = \sqrt{(x_0-i)^2+(y_0-j)^2}$ and $(i,j) \in \mathbb{Z}^2$. The function f_z is called the intensity profile at depth z. The constant α, which corresponds to the background pixel intensity, is generally different for each particle. Since the particles are

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spherical, the assumption (1) of spherical symmetry is reasonable, at least as long as we do not have particles too close to each other. For the z-scans, this is however not the case; the number of particles present in each z-scan is much smaller than in the sequence images.

For each z-scan, we want to estimate the corresponding f_z, using a nonparametric regression smoother, see e.g. Fan and Gijbels (1996). Since the particles centres in the plane are unknown, we have to estimate these as well.

For a given initial position near the true particle centre $(x_0, y_0) \in \mathbb{R}^2$, we use the pair $(\hat{x}, \hat{y}) \in \mathbb{R}^2$ minimizing

$$\mathrm{RSS}(x, y) = \sum_k (\hat{f}_z(d_k) - I(i_k, j_k))^2 \qquad (2)$$

as the estimate of (x_0, y_0). Here $(i_k, j_k) \in \mathbb{Z}^2$ for $k = 1, \ldots, n$, are the pixel positions within distance r_{max} of (x, y) and $d_k = \sqrt{(i_k - x)^2 + (j_k - y)^2}$. $\hat{f}_z$ is the corresponding nonparametric smooth, which is used as the intensity profile estimate for a particle at depth z. To calculate this, we use a locally-weighted linear smoother with a Gaussian kernel. Notice that the estimation of the particle centre in (2) is done at sub-pixel accuracy. Figure 2 shows the

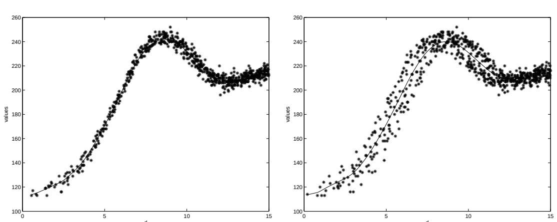

Figure 2: Pixel values vs distance from $(\hat{x}, \hat{y})$ and $(\hat{x} + 0.5,\ \hat{y} + 0.5)$, respectively.

scatter plots $\{d_k, I(i_k, I_k)\}$ for a particle; the left plot is for the position minimizing (2), i.e. the estimated particle centre, and the right is for a position half a pixel away in both x and y from the estimated particle centre. We see that sub-pixel accuracy is achievable.

Using our estimated $\hat{f}_z$ from each z-scan as the truth, we have our templates for the 3-D estimation of the particle centres in a sequence image as the one in Figure 1.

3 Evaluation by estimating the diffusion coefficient

Next, we estimate the diffusion coefficient for the particles in a sequence of images as the one in Figure 1. Since the particles in the images are of known equal size we can calculate the theoretically predicted diffusion coefficient and compare with the estimated. and compare with the estimated. In other words, this sequence is used to evaluate the positioning method.

In Kvarnström (2003), 2-D trajectories from 26 particles in the image sequence, obtained by using the Hough transform, were used to estimate the diffusion coefficient of the system. Our assumption was that the particles were performing Brownian motion with a measurement error on the position estimates. This way, we could also estimated the standard error on the position estimates. The theoretical diffusion coefficient of $0.982\mu\mathrm{m}^2/s$ was within the resulting 95% confidence interval of $0.893 \pm 0.150\mu\mathrm{m}^2/s$.

We plan to generalize this methodology to evaluate the sub-pixel accuracy 3-D positioning method developed here.

4 Practial considerations for automation

After estimating the positions for all the particles in each image of the sequence, we need to link these into trajectories. Here, a number of problem arises. First of all, it should be noted that the images only cover a subset of the total volume in which the particles are confined. Therefore a linking algorithm have to be able to take care of particles going in and out of the image area. Also, when particles get close, they occlude each other, both partially and totally.

At this stage, we have not considered these issues. Instead, we will manually supervise this step of the tracking.

References

J. Crocker and D. Grier. Methods of digital video microscopy for colloidal studies. *Journal of Colloid and Interface Science*, 179:298–310, 1996.

J. Fan and I. Gijbels. *Local Polynomial Modelling and Its Applications.* Chapman and Hall, London, first edition, 1996.

M. Kvarnström. Estimation of the diffusion coefficient in a mixture model. `http://www.math.chalmers.se/~matskv`, 2003. Submitted.