Single particle raster image diffusion analysis

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Introduction

Raster Image Correlation Spectroscopy (RICS) analyses images collected via a raster scanning pattern to determine the dynamics of a system, e.g. the dynamics of particles. The standard RICS approach developed by Digman et al. (2005) can detect dynamics on a wide time range (microseconds to seconds). Traditionally, RICS data have been analyzed using correlation techniques to estimate the diffusion coefficient. We have developed a new technique based on a Single particle tracking (SPT) algorithm which allows us to obtain Maximum Likelihood (ML) estimates for the diffusion coefficient, brightness, and Point Spread Function (PSF) parameters.

Materials and Methods

One of the major benefits of RICS is that it can be performed with commercial laser scanning microscopes. The acquisition of images is done in the following way: we start scanning the first pixel in the first line, then we proceed with the second pixel in the first line after time τ_p and so on until the entire line has been scanned. Now, at time τ_l, we start scanning the next line and repeat this process until the whole image has been acquired. The raster scanning method is shown in Figure 1. This scanning pattern accesses information at different time scales, as we rapidly scan pixels inside a line while the time between lines is typically two order of magnitude longer.

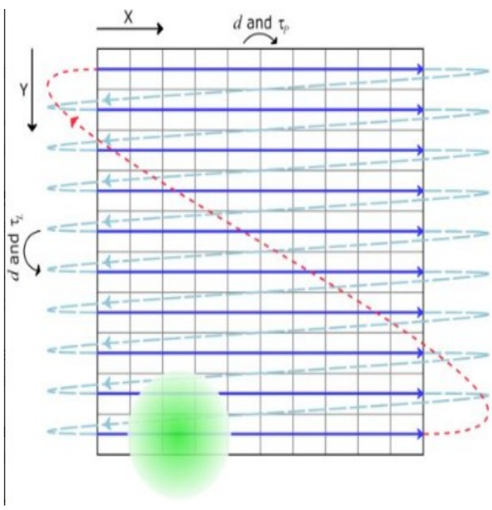

Figure 1. Raster Scan of pixels

The SPT algorithm is applied to a stack of images taken as described above. In particular SPT is divided into two steps: finding, separating and extracting the particles, and analyzing them by the ML method. The detection of particles is based on finding local maxima of photon counts over a threshold. The results of the extraction are shown in Figure 2.

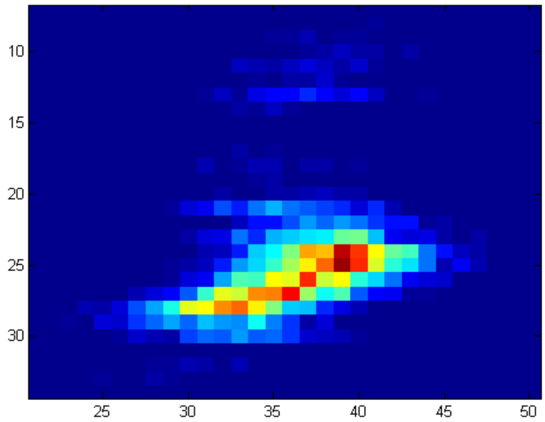

Figure 2. 175nm Particle extracted from an image acquired with RICS; The particle is diffusing with diffusion coefficient D = 3 $\mu m^2 s^{-1}$.

The likelihood of the data (photon counts in all pixels) given a fixed set of parameters (brightness of the particle, position of the particle at a certain time, PSF parameters) simply depends on how we scan the pixels. In particular photon counts in all pixels follow a Poisson distribution with a mean which depends on the brightness and a triple integral of the PSF. We maximise the likelihood through optimization algorithm to obtain a MLE of the position of the particle at times when we scan a line. Finally, since our goal is to have an estimate of the motion of the particle, we can use the mean square displacement obtained from the estimated positions to restore the diffusion coefficient.

Results and Discussion

We compare the results of our method against standard RICS estimates of the diffusion coefficient on a set of simulated data. In particular instead of looking at the real diffusion coefficient, it is more natural to look at the real mean square displacement for our method. We selected approximately 400 particles of diameter 50nm in a stack of images. The results are shown in the table below, where: D is the diffusion coefficient; DT is the true mean square displacement; D SPT is our estimate of the mean square displacement; lower and upper bounds are the confidence interval bounds for SPT estimate; finally D RICS is the standard RICS estimate for D. We used median over all particles.

D	Number of particles	DT	D SPT	Lower Bound	Upper Bound	D RICS
0.5	482	0.460	0.451	0.399	0.491	0.479
1	439	0.939	0.953	0.898	1.010	0.928
2	401	1.759	1.895	1.748	2.019	1.523
4	357	3.462	3.708	3.441	3.887	2.921
8	393	6.778	7.510	6.918	8.023	9.256
16	573	11.858	13.493	12.544	14.217	22.917
32	324	24.630	24.090	19.660	29.200	118.190

The results seem reasonable: the error is of the same order for our method and RICS. Moreover we take advantage of having a large number of particles and build confidence intervals for D; To be able to obtain confidence intervals in the standard RICS, we would need more data, i.e. more recording time. Working with single particles allows us to have heterogeneity, both with particles having different diffusion coefficient and with a region where the diffusion coefficient varies over the region. We plan to improve our method by adding a MLE of the brightness and PSF parameters so that we do not need to estimate them separately from non-moving particles (like in standard RICS). This further improvement may lead to a method which is more independent of initial guesses for parameters than standard RICS, which need a phase of “calibration” on non-moving particles. We are currently also evaluating the SPT method for real data obtained with a confocal laser scanner.

Conclusion

Our method seems worth to be studied and used to better understand dynamics of particles and heterogeneity in materials. It can be implemented without expensive requirements and used in parallel with standard RICS to confirm or improve results. Finally, our method seems more flexible, as we don't need to assume anything about the movement of particles, while RICS analysis needs to be adjusted if we consider any other type of movement (e.g. diffusion and binding).

References

Digman MA, Brown CM, Sengupta P, Wiseman PW, Horwitz AR, Gratton E. 2005a. Measuring fast dynamics in solutions and cells with a laser scanning microscope. Biophys J 89:1317–1327.