Single Particle Raster Image Analysis

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May 24, 2016

Outline of the talk:

- Aim / Motivation;
- Data collection;
- Method;
- Results on simulated and experimental data;
- Conclusions and future work.

- In many areas the microstructure plays a major role in determining the properties of soft biomaterials.
- Functionalities such as controlled release of drugs and water management in foods depend to a large extent on the mass transport properties.
- Use methods from Image Analysis and Spatial Statistics to detect and map heterogeneity and diffusion properties locally in soft biomaterial structures.

There exists many different methods to tackle the problem. The main ones are:

- Image correlation spectroscopy techniques (TICS, RICS, STICS,...);
- Fluorescence recovery after photobleaching;
- Single particle tracking.

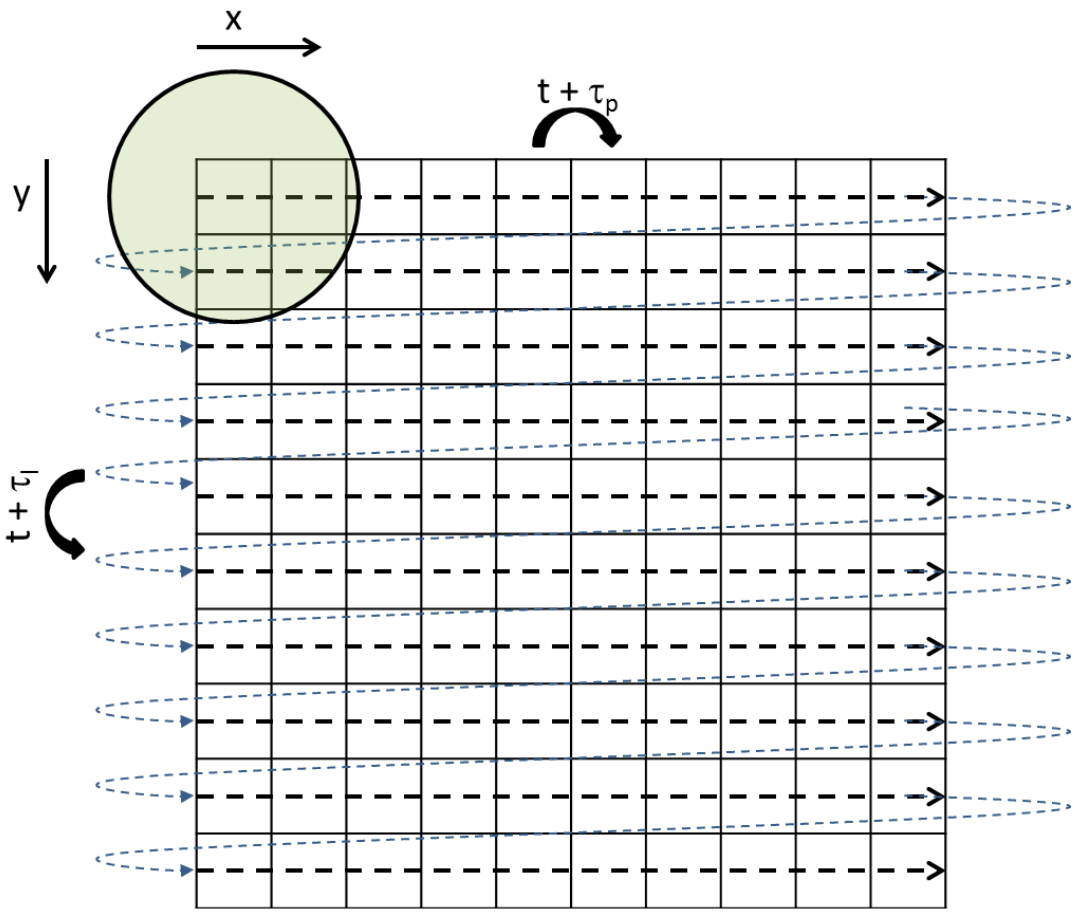

Figure: Movement of the scanning beam according to the raster scan pattern used. The scanning time between adjacent pixels in the x- and y-directions are τ_p and τ_l, respectively, and $\tau_p \ll \tau_l$.

Diffusing particles

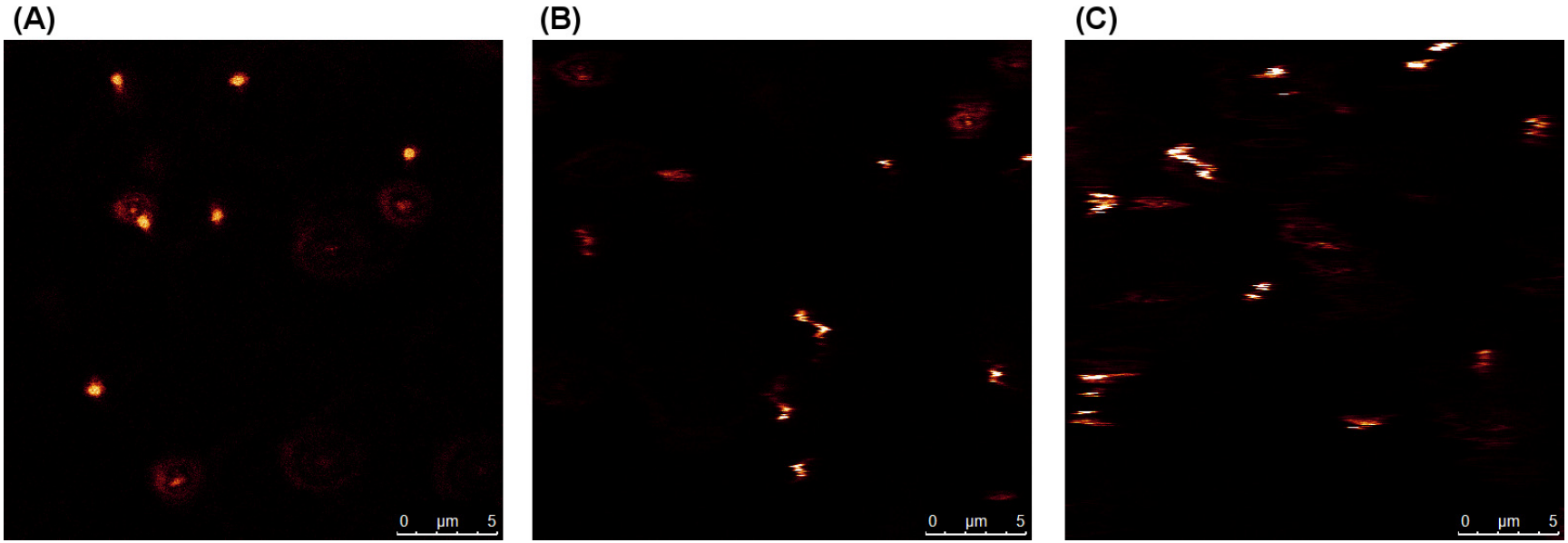

Figure: Effect of varying scan rate visually on experimental data with 175nm beads, scanned at decreasing scan rate: (A) at scan rate 8000Hz; (B) at scan rate 400Hz; (C) at scan rate 100Hz.

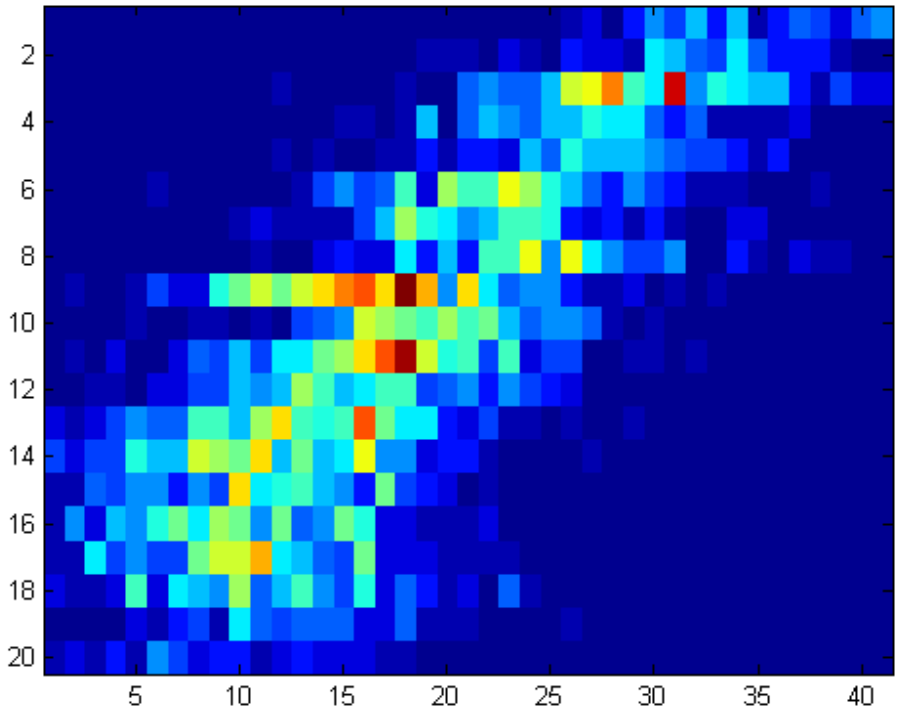

Figure: A simulated raster scan image of a 175nm particle.

Likelihood

The likelihood of photon counts $\{N(x, y_k, t_k)\}$ in the k-th horizontal line of an extracted particle, scanned at time $t_k = t(y_k)$ given the corresponding particle position S_k has the form:

$$L(\{N(x, y_k, t_k)\}|S_k) = \prod_x \frac{E(x, y_k, t_k)^{N(x,y_k,t_k)}}{N(x, y_k, t_k)!} e^{-E(x,y_k,t_k)}.$$

where $E(x, y, t)$ is the expected number of photon scanned at time t in the pixel at position (x, y). $E(x, y, t)$ depends on the Point Spread function and other characteristics of the particles.

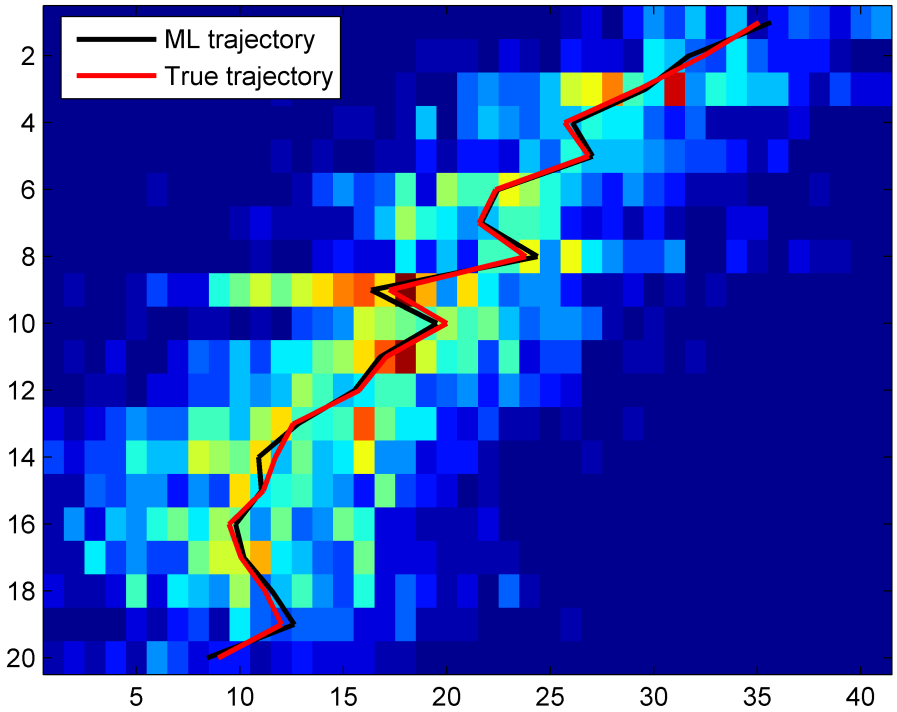

Figure: A simulated raster scan image of a 175nm particle with the true trajectory (red line) and the corresponding maximum likelihood estimate (black line).

Let K be the number of lines a particle has been observed and X_k be the x-position of the particle during the scanning of the k-th line. For diffusing particle it holds:

$$X_k - X_{k-1} \sim N(0, 2D\Delta t),$$

so we can estimate the diffusion coefficient by using the mean square displacement:

$$\tilde{D} = \frac{1}{2\Delta t(K-1)} \sum_{k=2}^{K} (X_k - X_{k-1})^2 \qquad (1)$$

Heterogeneous system

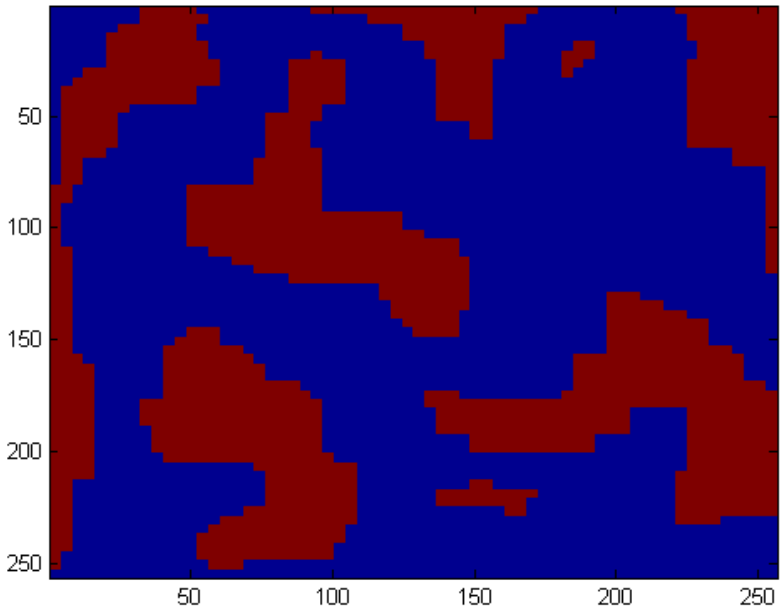

Figure: Simulated structure made of two media with different viscosity.

How to use the information about the dynamics of the particles to obtain information about the structure?

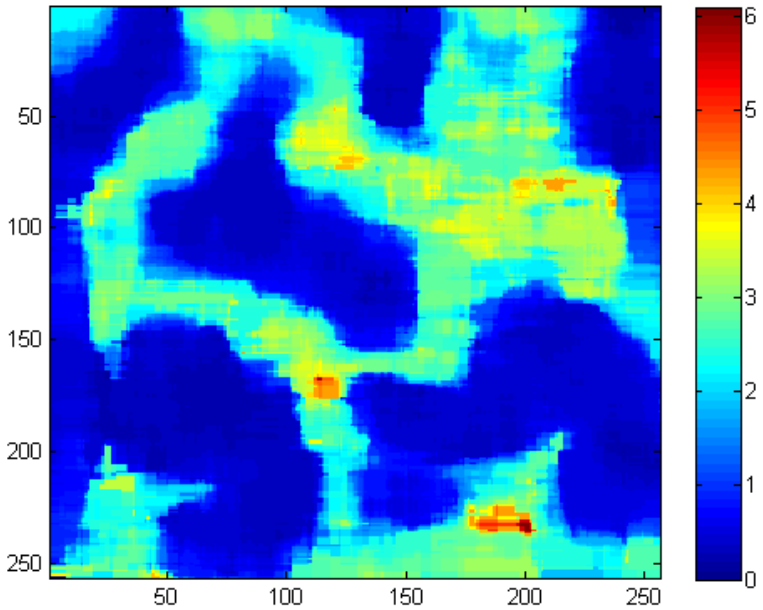

Figure: Diffusion map based on 5000 particles. The color is a representation of the estimated diffusion coefficient in $\mu m^2 s^{-1}$.

Gaussian Mixture Models

Let Y_{ij} be the measurement in the pixel (i, j) of the image. The Gaussian Mixture model can be written as:

$$\pi(Y_{ij}|\theta) = \sum_{k=1}^{K} w_{ij}^{k} \pi_k(Y_{ij}|\theta_k) \tag{2}$$

where:

- K is the number of classes;
- w_{ij}^{k} is the prior probability of Y_{ij} belonging to class k;
- $\pi(\cdot|\theta_k)$ is the distribution of class k, i.e. $N(\mu_k, \Sigma_k)$.

Markov Random field Models

By noting that Y_{ij} as the same distribution as $\sum_{k=1}^{K} z_{ij}^k G_{ij}^k$, where

- $G_{ij}^k \sim N(\mu_k, \Sigma_k)$;
- $z_{ij}^k = \mathbb{1}(x_{ij} = k)$;
- $x = \{x_{ij}\}$ has multinomial distribution.

We can let x be a (discrete) Markov random field to introduce spatial dependency.

Use the Expectation Maximization algorithm to estimate the parameters of the model and get

$$P_{ij}^k = \text{posterior probability that} Y_{ij} \text{comes from class k.}$$

Put the pixel (i, j) in class $\hat{k}$ if

$$P_{ij}^{\hat{k}} = max_{k=1,\ldots,K} P_{ij}^k.$$

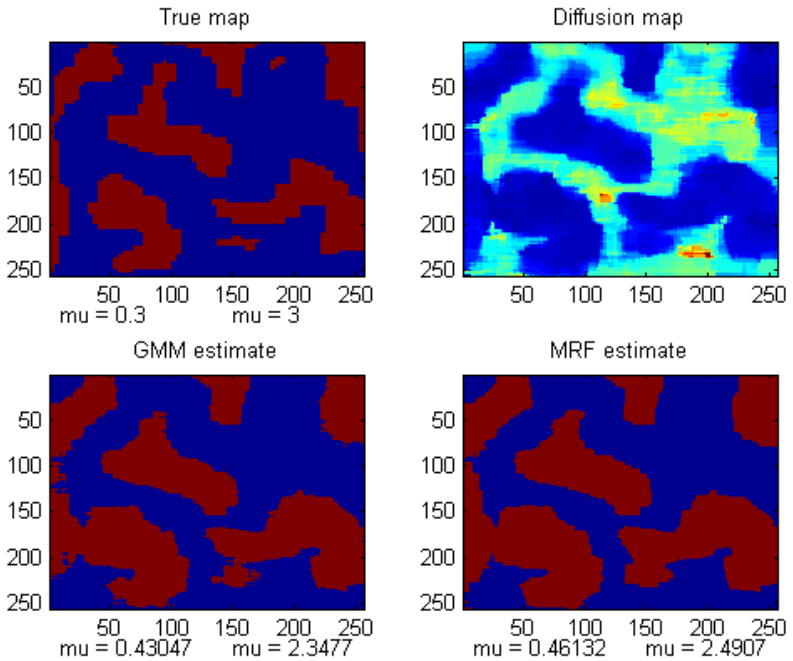

Figure: Top Left: True structure where red is one phase and blue the other; Top Right: Estimated diffusitivity map based on 5000 particles. Bottom Left and Bottom Right: discrimination via two different methods.

- EC-HPC is used to make films that cover pills;
- Understanding of the structure is crucial to control the drug release process.

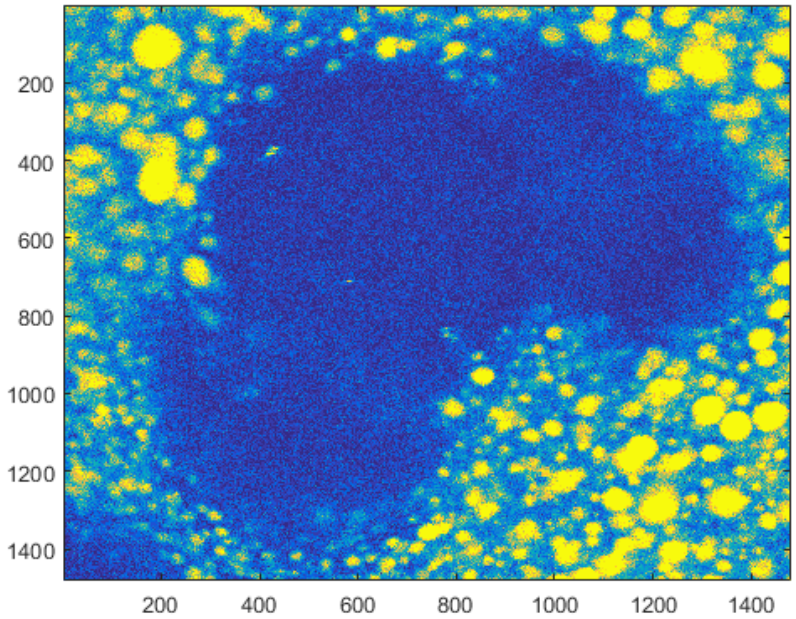

Figure: EC-HPC structure; EC is fluorescently labelled, HPC is not.

Not have enough data to perform a formal classification $\rightarrow$ Gaussian kernel smoothing.

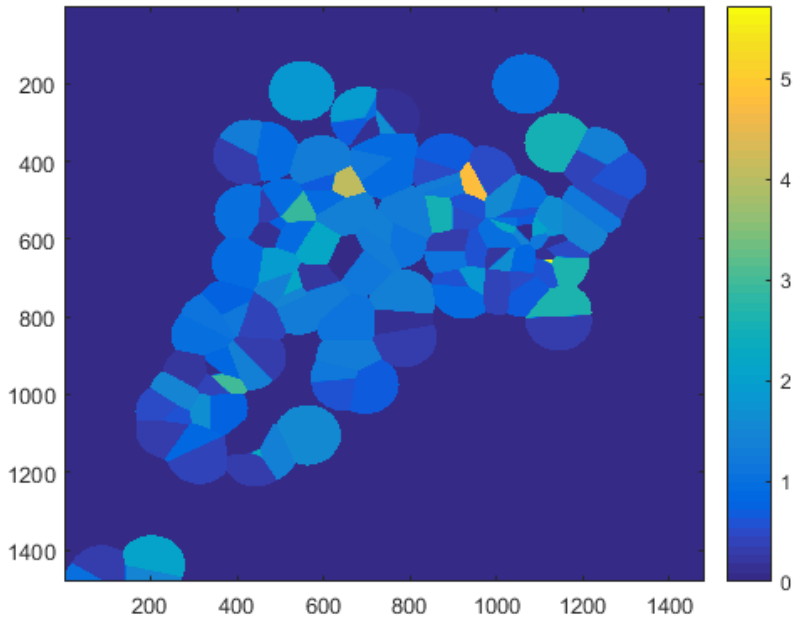

Figure: Estimated diffusitivity map based on 134 particles.

Conclusions and Future work

- The method seems promising to analyse hetereogeneity in soft biomaterials;
- One could theoretically create a high resolution diffusitivity map with the resolution the bead size;
- More data need to be gathered to get better results in the experimental case;
- Analyse more complex cases including binding and flow.

Thank you for your attention!