Microscopic images from a point process perspective

T Honnor, A Johanson, J Brettschneider

Introduction

Point processes are a rich and flexible class of models that can be put to use to answer quantitative questions in science and engineering. Determining the best way to map a real-world application to the models is not always straight forward and the interpretation of any findings needs to be done in light of the subject matter interpretation of the point process model.

We use point processes for to model the location of microtubules in cell biology. Image data is collected using fluorescent microscopic. It captures the locations of certain protein species under different conditions. We have developed distribution-free procedures to quantify the significance of observed differences between the resulting point pattern structures.

The approach is illustrated by experimental data shedding light on the interplay between subcellular structures called microtubules and chemicals involved in mitosis.

Methods: Point Processes Models

$$\chi_2 := \{(\underline{x} = x_1, x_2, \ldots, x_{n(\underline{x})}) : n(\underline{x}) \in \mathbb{N}, x_i \in \mathbb{R}^2 \text{ for } i = 1, 2, \ldots, n\}$$

Model pattern as realisations of a point process:

Random subset $\underline{X}$ on $\mathbb{R}^2$.

For B in Borel σ-algebra $\mathcal{B}(\mathbb{R}^2)$ on $\mathbb{R}^2$: $\underline{X}_B = \underline{X} \cap B$

Counts (random variable): $N(B) = n(\underline{X}_B)$ = number of points of $\underline{X}$ in B

Intensity measure $\mu(B) = \mathbb{E}[N(B)], \quad \forall B \in \mathcal{B}(\mathbb{R}^d).$

Let $\underline{x}$ be a realisation of $\underline{X}$ on the observation window W.

Estimator for the **intensity** of $\underline{X}$: $\hat{\rho} = \frac{n(\underline{x})}{|W|}$

Let $\text{nn}(x_j)$ be the (set of) nearest neighbours of point x_j.

$$\text{nn}(x_j) = \{x_k : k = \text{argmin}_l ||x_l - x_j||\},$$

and $\text{nnd}(x_j)$ its nearest neighbour distance

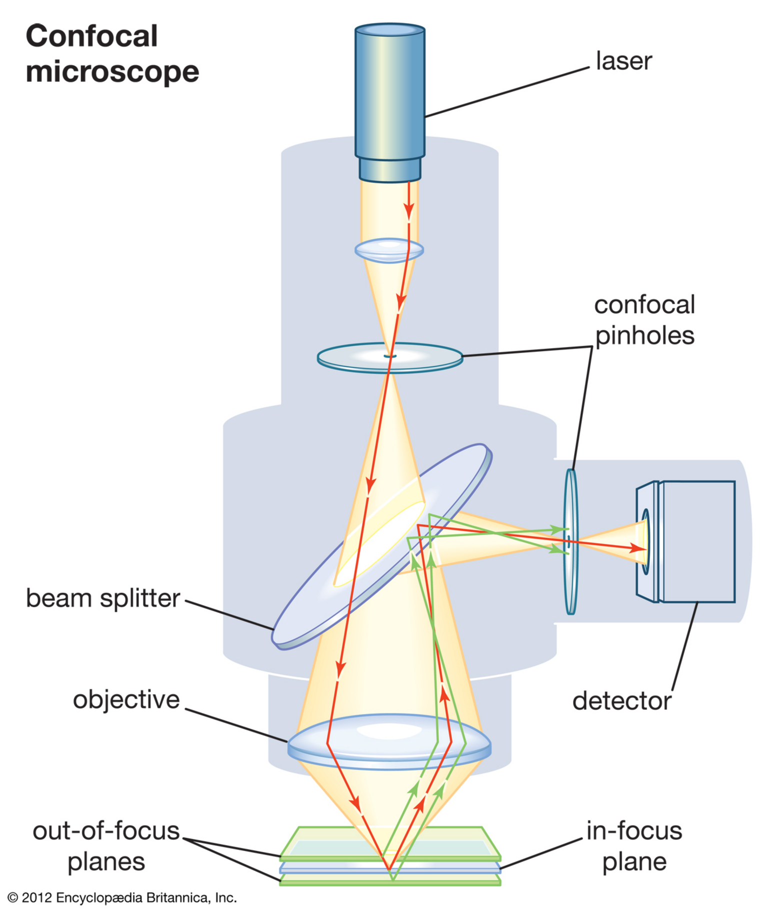
$$\text{nnd}(x_j) = \inf_{x \in \text{nn}(x_j)} \{||x_j - x||\}.$$

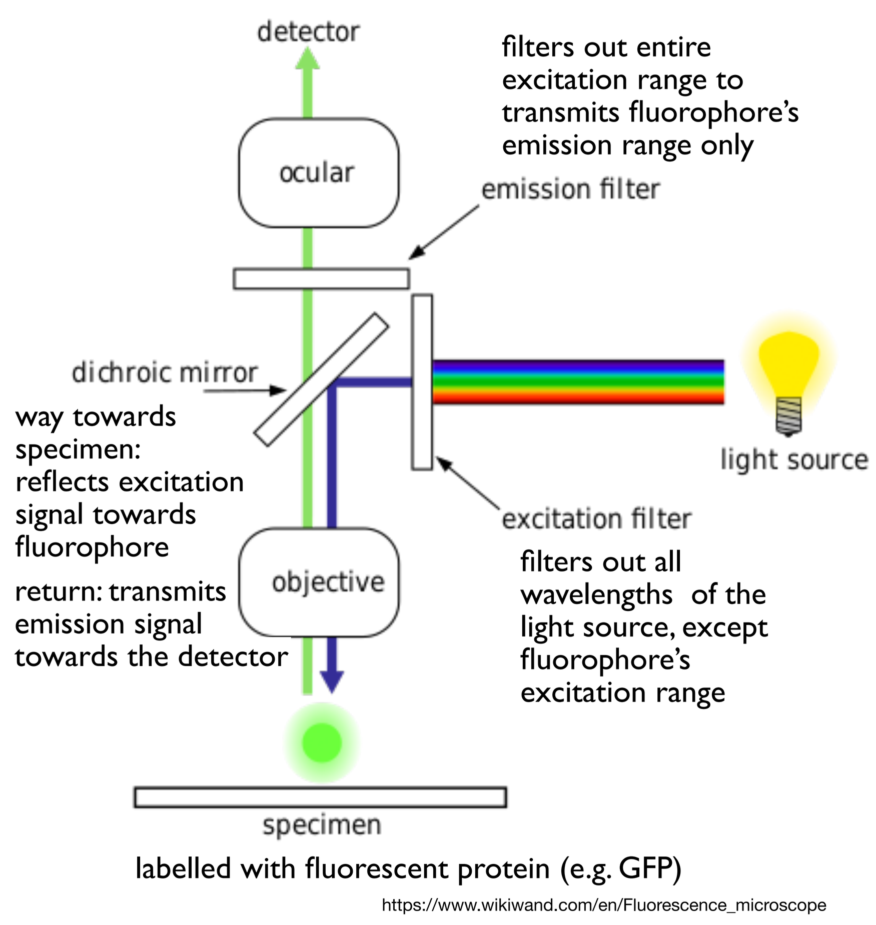
Nearest neighbour function (Diggle 2003):

$$G(r) = \frac{1}{\rho|B|}\mathbb{E}\left[\sum_{x \in \underline{X}_B} 1_{\{\underline{X} \setminus x) \cap b(x,r) \neq 0\}}\right] \qquad \hat{G}(\underline{x}, r) = \frac{1}{n(\underline{x})}\sum_{j=1}^{n(\underline{x})} 1_{\{\text{nnd}(x_j) \leq r\}}$$

for finite B in $\mathbb{R}^2$, and $b(x,r)$ the disc centred at x with radius r.

Methods: Technology & Data



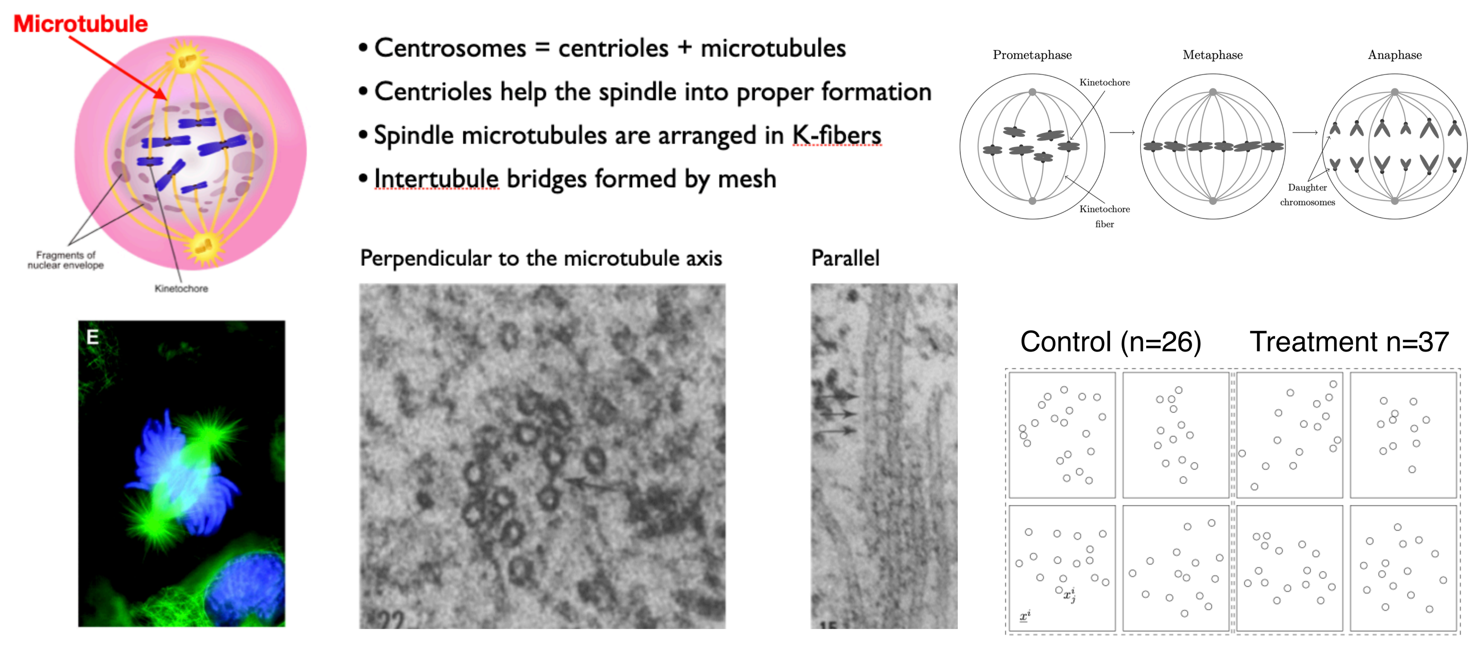


Confocal microscope:
Geometric optics with pinhole in optically conjugate plane in front of the detector to eliminate out-of-focus signal. Long exposure required, but better focus. Scanning arrange-ment to build up image of larger region.

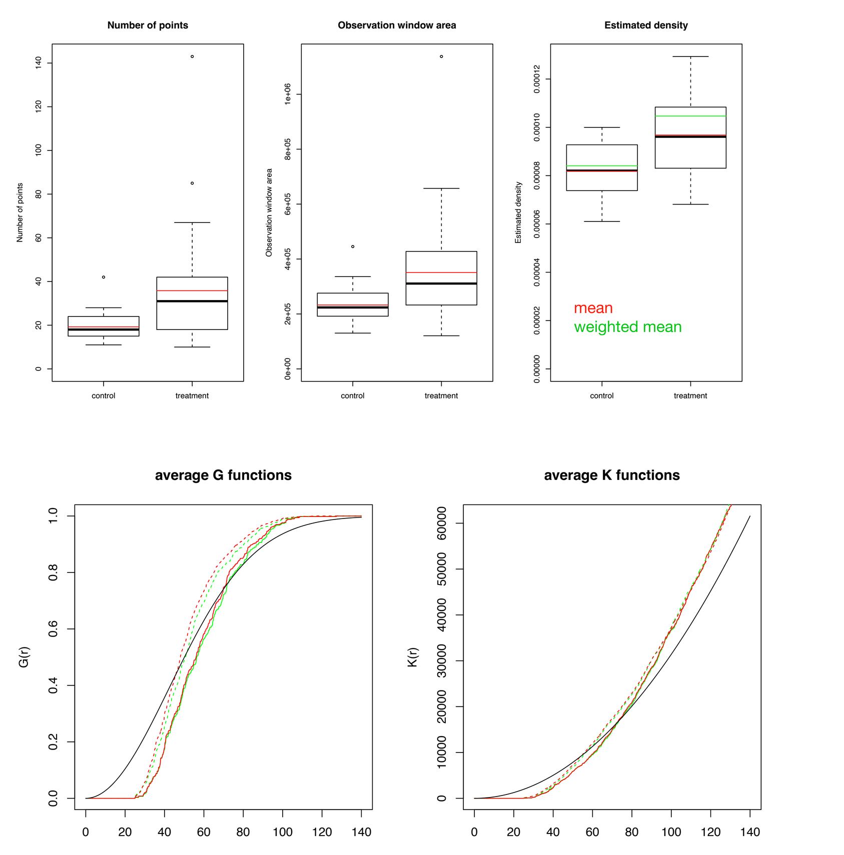
Fluorescent microscope:
High intensity light source excites fluorescent species tagged onto sample which emits specific wavelength

Experimental case study

Stephen Royle's Lab (Centre for Mechanochemical Cell Biology, WMS):
Role of TACC3 protein for the structure of microtubules within K-fibres and mesh?
Experiment: Overexpression of TACC3 through treatment versus control.
Microscopic images collected in planes perpendicular to the fibre axes.



Exploratory analysis and nonparametric tests



Result confirmed by formal testing.
Procedure:

- Based on permutation tests (nonparametric)
- Need exchangeability under the Null under suitable set of operations
- Statistics under permutations are identically distributed
- p-values are uniformly distributed (test e.g. with KS)
- Exact or approximate (subset of operations)
- Still significant after Bonferroni adj. for multiple testing

δ_N	0.0005	δ_{nnd}	0.0057	δ_K	0.1092	δ_{EFT}	0.0011
δ_W	0.0018	$\delta_{\text{nnd},\omega}$	0.0005	$\delta_{G,1}$	0.0061	$\delta_{EFT,\omega}$	0.0005
δ_ρ	0.0001	δ_{msd}	0.0019	$\delta_{G,1,\omega}$	0.0005		
$\delta_{\rho,\omega}$	0.0002	$\delta_{\text{msd},\omega}$	0.0005	$\delta_{G,\infty}$	0.0087		
				$\delta_{G,\infty,\omega}$	0.0013		

Operations $\Gamma = \{\gamma_0, \gamma_1, \ldots, \gamma_m\}$, where $\gamma_0 = \text{Id}$. (random subset)

p-value for two-sided test of H_0 using statistic t :

$$p = \frac{1}{m+1}\sum_{\gamma \in \Gamma} 1_{\{|t(\gamma x)| \geq |t(\gamma_0 x)|\}}$$

Conclusions

Point process models in conjunction with nonparametric test statistics are suitable methods for microscopic image data:

- can deliver results were experiment not interpretable by just eyeballing
- computationally feasible

Experimental case study: the collection of test statistics provides evidence that

- Nearby microtubules are bound together (in K-fibers by a mesh-like structure)
- TACC3 over expression (treatment) is associated with an impact on the mesh

This means, the hypothesised treatment effect could be confirmed

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