Hypothesis-based particle detection for accurate nanoparticle counting

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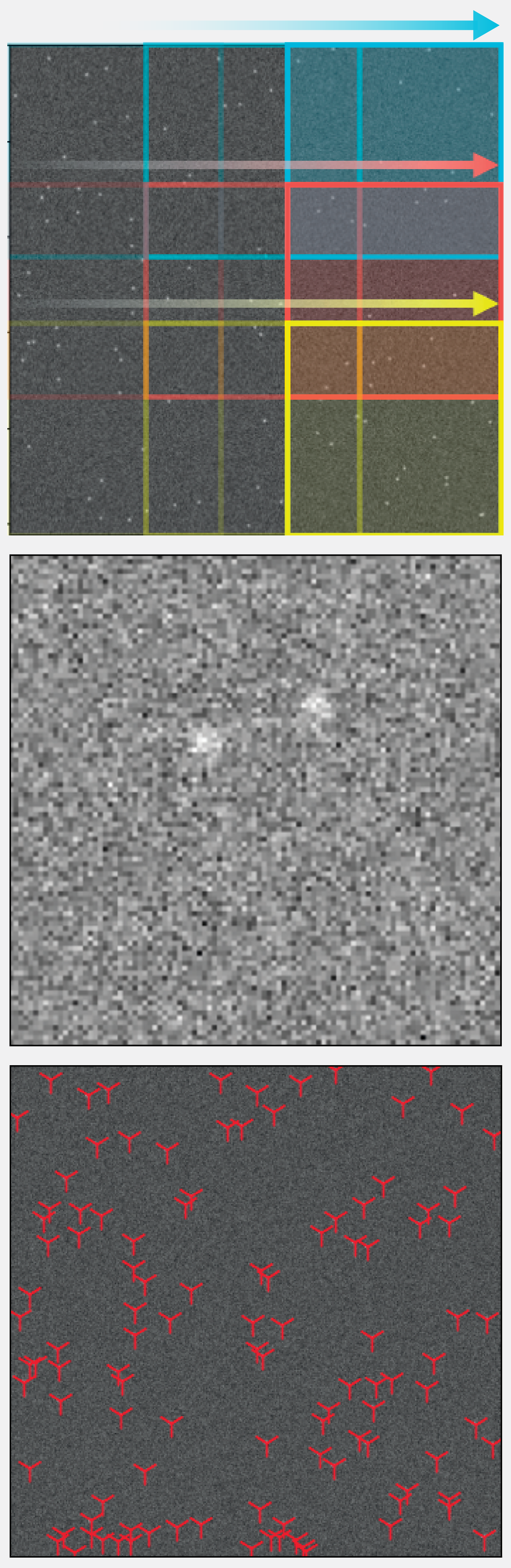
Introduction

- Accurate quantification of individual biomolecules represents a fundamental shift from traditional diagnostics that rely on ensemble measurements of large molecular populations.
- Digital detection enables precise analysis of the concentration of low abundance analytes crucial for applications in early disease diagnostics and personalised healthcare [1,2].
- Nanoparticle imaging based assays are a powerful digital diagnostic tool where enumeration of surface bound particles provides direct, calibration-free quantification of analyte concentration [3].
- Low signal levels e.g. from weak scattering, can lead to significant errors in particle detection, with generally unknown rates of false alarm.
- Probabilitistic methods, incorporating knowledge of noise sources can enable robust detection without arbitrary parameter tuning [4].
- We present a novel maximum likelihood hypothesis based approach for nanoparticle counting.

Algorithm structure



- Image segmentation with a rolling window divides large field of view into small regions containing only a few particles on average.
- Image modelled as a random Poisson process with mean intensity given by:

$$\lambda(\mathbf{r}) = \lambda_{bg} + \sum_{k=1}^{N} \frac{I_k}{2\pi\sigma^2} \exp\left[-\frac{|\mathbf{r}-\mathbf{r}_k|^2}{2\sigma^2}\right]$$

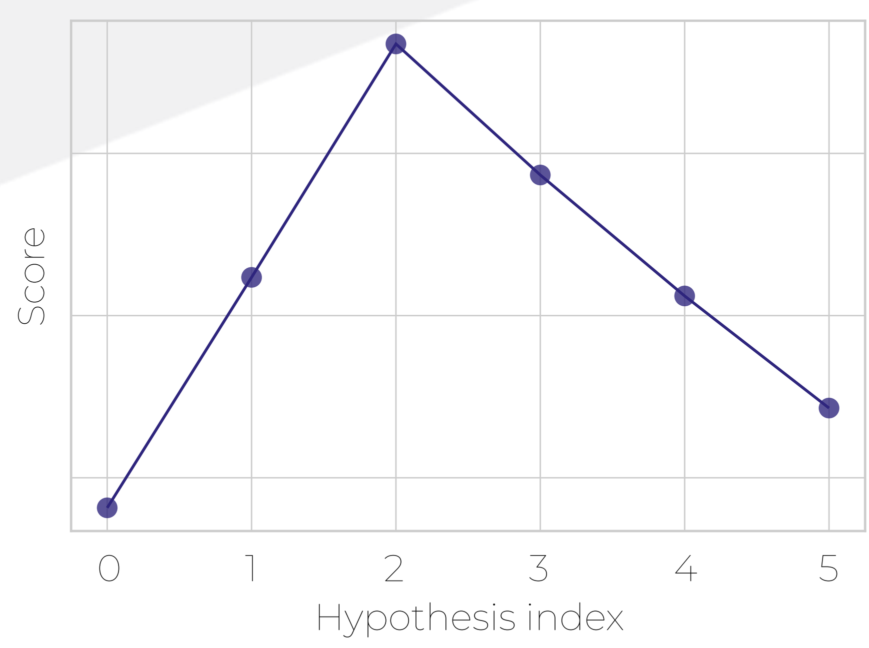
- Model parameters for each image segment found via maximum likelihood estimation (MLE) for hypothesis that 0, 1, 2, ... particles are present.
- Preliminary coarse peak detection used to initalise MLE fitting.
- Out of bounds (oob) penalty applied to log-likelihood function to constrain allowed particle positions.

$$\mathcal{M} = \sum_{i=1}^{M} (v_i \log(\lambda_i) + \log(v_i!)) - \mathcal{P}_{\text{oob}}$$

- Each hypothesis scored using a modified log-likelihood function. The Laplace penalty term, using observed Fisher information matrix, reduces over fitting.

$$\mathcal{S} = \mathcal{M} - \frac{1}{2}\log[\det \mathbb{J}]$$

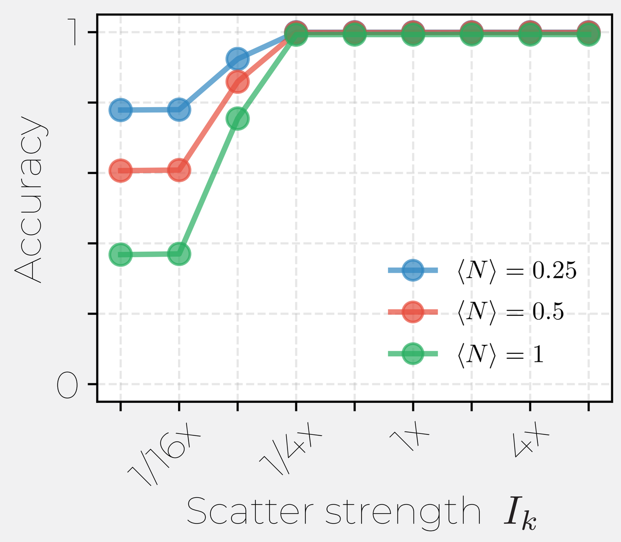
- Hypothesis with the highest score is selected yielding number of particles.
- Algorithm also provides particle properties for later analysis, e.g. classification.

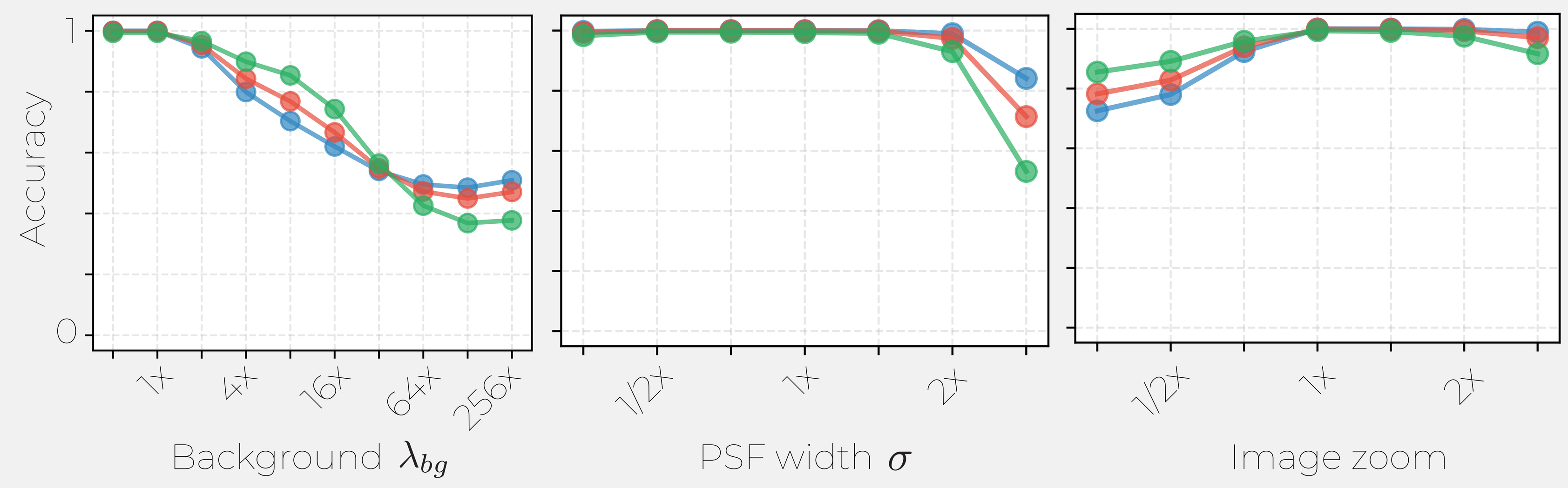


Performance analysis

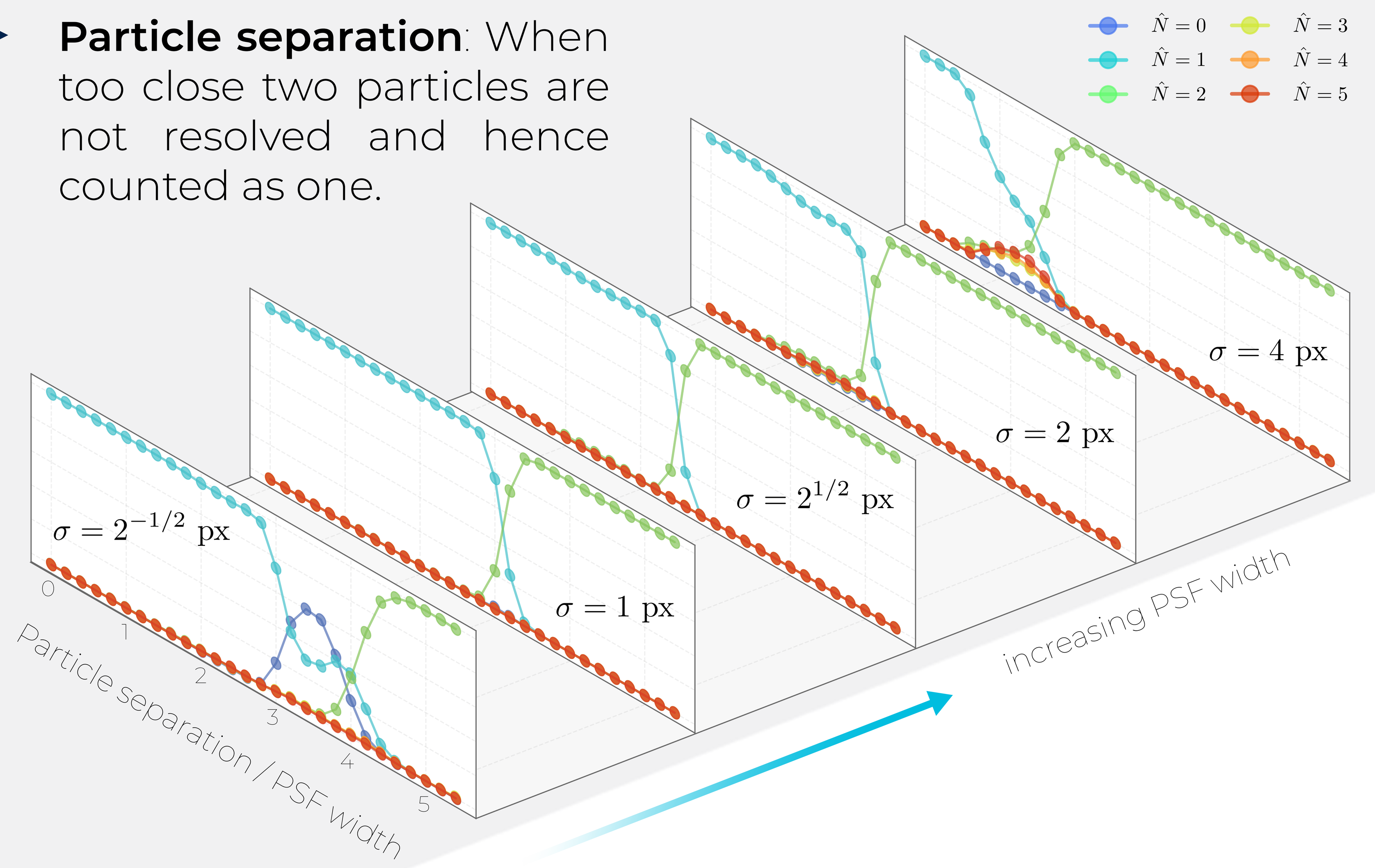
- Noisy simulated data used to assess performance characteristics of particle counting algorithm.
- Confusion tables were calculated and observed accuracies weighted by Poisson distribution to yield expected accuracy.
- **Scattering strength**: Accuracy improves for stronger scatterers due to increasing SNR and SBR.
- **Background intensity**: Reduced SBR degrades expected accuracy.
- **Point spread function**: broadening of PSF reduces intensity on each pixel and hence reduces SNR.
- **Image zoom**: competing effects of reduced background and broader point spread function give optimal range.

	Baseline
σ	2 px
λ_{bg}	2,000
I_k	20,000
Size	100 x 100 px





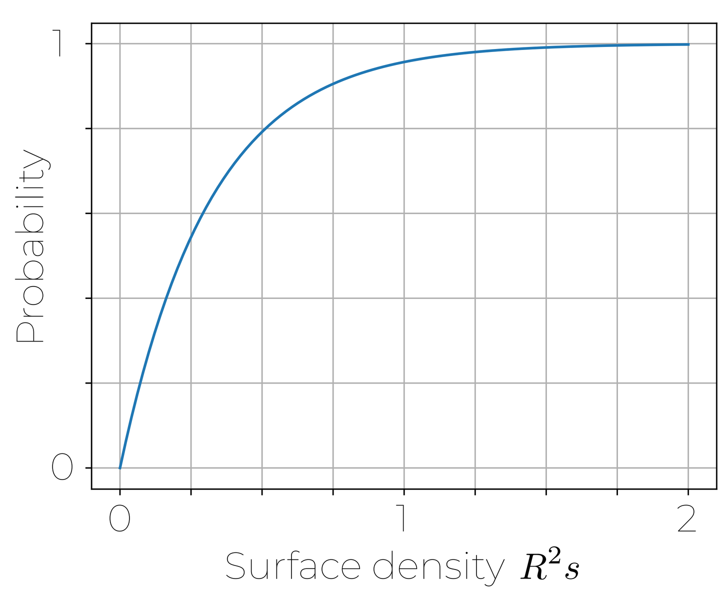
- **Particle separation**: When too close two particles are not resolved and hence counted as one.



- Probability of classifying two particles as a single particle:

$$p_{2\to1} \approx 1 - \exp\left[-\pi R^2 s\right]$$

- Higher order classification errors approximately given by powers of $p_{2\to1}$.



Conclusions

- We have presented a hypothesis-based particle detection algorithm for use in particle counting based nanoparticle imaging assays.
- Performance remains reliable for a diverse range of experimental conditions.
- Arbitrary parameter tuning or large training datasets are not required.

References

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[2] D. C. Duffy, Lab Chip 23, 818–847 (2023).
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[4] C. S. Smith, S. Stallinga, et al., MBoC 26, 4057–4062 (2015).

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