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Unveiling the limits of precision in iterative MINFLUX

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Abstract

In single-molecule microscopy, a big question is how precisely we can estimate the location of a single molecule. Our research shows that by using iterative localisation microscopy and factoring in the prior information, we can boost precision and reduce the number of photons needed. Leveraging the Van Trees inequality aids in determining the optimal precision achievable. Our approach holds promise for wider application in discerning the optimal precision across diverse imaging scenarios, encompassing various illumination strategies, point spread functions and overarching control methodologies.

KEYWORDS
localisation precision, MINFLUX, SIMFLUX, single-molecule localisation microscopy, super-resolution

1 | INTRODUCTION

In single-molecule localisation microscopy (SMLM), sparsely activating fluorescent emitters sequentially improves resolution beyond the diffraction limit.[1] Modulation-enhanced SMLM (meSMLM) utilises patterned illumination to enhance localisation precision.[2] Methods like SIMFLUX, SIMPLE and repetitive optical selective exposure employ standing-wave intensity patterns, while MINFLUX uses a doughnut-shaped pattern.[3–6]

The localisation precision can be iteratively improved around emitters using prior information in iterative meSMLM (imeSMLM). In an iterative MINFLUX variant,[9] the emitter positions are estimated through triangulation with doughnut-shaped illumination patterns, leading to improved precision. Distributing the limited photon budget over iterations is favoured over increasing signal photons per iteration for better information content.

Characterising (me)SMLM methods' localisation precision involves using the Cramér-Rao lower bound (CRLB).[7] imeSMLM iteratively updates prior information, and additional information can be gained from photoactivation. The CRLB, which requires unbiased estimators, cannot incorporate a prior distribution on estimands.[10]

Kalisvaart et al.[8] propose using the Van Trees inequality (VTI) as a Bayesian alternative to the CRLB due to available prior information.[11–13] The VTI establishes a fundamental limit on imeSMLM precision, especially with standing-wave illumination. Simulation results demonstrate the exponential increase in information content with iteration count in ideal conditions, but nonideal conditions require optimal design considering practical imaging conditions. The VTI serves as a performance metric for in silico control strategy design.

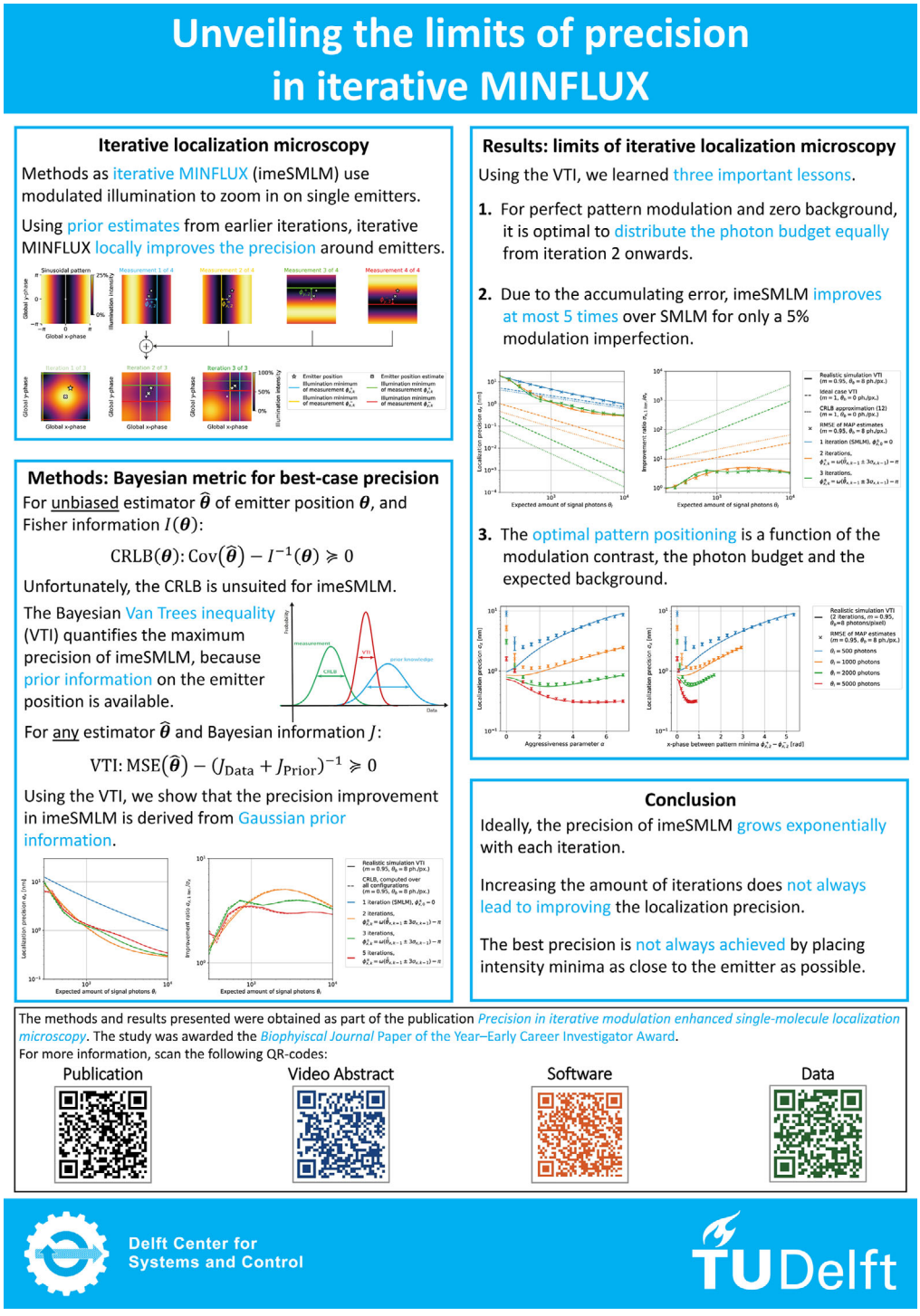

FIGURE 1 Poster summary of Kalisvaart et al.[8] (Introduction) In imeSMLM techniques like iterative MINFLUX, patterned illumination zooms in on individual molecules. Our research highlights the intrinsic limits and trade-offs in localization precision within imeSMLM. (Methods) Traditionally, the Cramér-Rao Lower Bound (CRLB) has set the standard for estimating the highest achievable precision in localization microscopy. However, its application has been limited due to its dependence on unbiased estimators when incorporating prior information from previous experiments. Our study reinterprets imeSMLM from a Bayesian perspective, utilizing the Van Trees Inequality (VTI) to address the CRLB's limitations. The VTI integrates uncertainty from prior information and current measurements, providing a lower bound on the precision achievable with imeSMLM methods. (Results) We found that, in ideal conditions with no background noise and perfect modulation, the information content of signal photons grows exponentially with each iteration. However, this exponential growth diminishes with the presence of background noise, imperfect modulation, or mechanical resolution limits of the illumination positioning system. For instance, with 8 background photons per pixel and 95% modulation contrast, imeSMLM with two iterations achieves, at most, a 5-fold improvement over conventional SMLM. (Conclusion) The VTI proves to be a crucial tool for evaluating illumination control performance, making it the preferred method for optimizing imeSMLM design and implementation.

Practical Considerations

Building upon the groundwork laid by Ober et al.,[7] our research addresses the fundamental question in single-molecule localisation microscopy – the precision in determining the position of a single molecule. Kalisvaart et al.[8] focus on iterative localisation microscopy, exemplified by techniques such as MINFLUX and iterative MINFLUX.[9] Our findings show that the precision enhancement achieved through iterative localisation microscopy stems from the incorporation of prior information, thereby reducing the required number of photons for accurate localisation. In this context, the Bayesian Van Trees inequality emerges as a valuable metric to quantify the optimal localisation precision attainable through iterative localisation microscopy under various practical scenarios.

2 | RESULTS

The VTI was simulated to assess the performance of imeSMLM under 95% modulation contrast. We show that calculating the VTI using unbiased Gaussian prior information is approximately equivalent to calculating the CRLB over all iterations, thereby indicating that the precision improvement in imeSMLM is derived from Gaussian prior information. By using two iterations in total, imeSMLM reaches at most a fivefold improvement over SMLM at 8 background photons per pixel. This shows that the exponential localisation improvement as a function of the iteration count cannot be achieved in realistic experimental conditions, as it breaks down for slight imperfections in the modulation contrast (Figure 1).

The optimal choice of the illumination pattern positions depends on the expected background photon count, the modulation contrast, and the expected signal photon count. To show this, we quantify the distance between the pattern minimum and the estimated emitter position as a function of the aggressiveness parameter α. We show that the optimal aggressiveness parameter α decreases for a decreasing signal photon count. For 95% modulation and 8 background photons per pixel, $\alpha = 2.5$ is optimal at a signal photon budget of 2000 photons, while $\alpha = 5.5$ is optimal at a signal photon budget of 5000 photons. That is, as the signal-to-background ratio increases, the optimal distance between the pattern minimum and the emitter increases as well.

3 | CONCLUSIONS

The proposed framework provides novel insights that challenge existing paradigms in the field of iterative modulation-enhanced SMLM.[14] We outline three significant examples:

1. Our research challenges the conventional belief that the smallest step size guarantees the best performance in iterative localisation microscopy. We establish that the optimal step size is contingent upon factors such as modulation contrast, molecule intensity and background fluorescence. Furthermore, our work reveals that the relationship between localisation precision and step size is nuanced, showing the limited conditions under which the assertions made by Balzarotti et al.[14] hold.

2. Earlier claims suggest exponential localisation improvement under zero background and perfect modulation contrast. Our study demonstrates that such enhancements break down in most experiments, particularly when confronted with slightly imperfect modulation contrast ($m < 1$). This finding nuances assertions made by Gwosch et al.[9]

3. We establish the optimality of isotopically distributing the single-molecule's photon budget across iterations in iterative localisation microscopy, contributing a nuanced understanding of the imeSMLM field.

These insights hold particular significance for (iterative) MINFLUX users, given the diverse expectations associated with these methods. For instance, the application of MINFLUX to DNA-PAINT necessitates optimising the step size due to elevated background levels. In addition, we expect that extending the analysis of (iterative) MINFLUX methodologies using the VTI, for example, to detection and single particle tracking, will reveal new insights. We anticipate that our framework and findings will swiftly be embraced by both method developers and users, fostering nuanced and optimised applications of (iterative) MINFLUX methodologies.[15,16]

DATA AND SOFTWARE

The data and software can be found at https://github.com/qnano/iterative-localization and https://data.4tu.nl/articles/_/19786735.

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