

User-Friendly Deconvolution Techniques for Advanced Microscopy

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Lay Summary

Microscopy datasets are inherently degraded by the fundamental physics of light propagation. Diffraction, scattering, and photon noise combine to blur fine structures, reduce contrast, and obscure quantitative detail. These limitations are particularly acute in live-cell imaging, where excitation of light must be minimised to reduce phototoxicity and bleaching.

Deconvolution computationally restores these distortions by modelling image formation and estimating an improved approximation of the true signal over multiple iterations. The most widely used approach, the Richardson–Lucy (R–L) algorithm, iteratively maximises the likelihood of the true image under Poisson noise. When implemented effectively, R–L improves resolution and signal-to-noise ratio. However, it is computationally and temporally demanding, with performance dependent on accurate PSF characterisation, careful iteration control, and efficient memory use. Large three-dimensional datasets can therefore become prohibitively slow to process, forcing a trade-off between fidelity and practicality.

PETIKIT (MATLAB), CLIJ2 (ImageJ/Fiji), and Napari with PyCUDADeconv (Python) using large red blood cell time-series and 3D RPE1 metaphase stacks. Runtimes were decomposed into pre-processing, deconvolution, and post-processing steps, while accuracy was assessed against a 92-kinetochore ground truth. Napari consistently achieved the shortest runtimes, completing large datasets in under 4 minutes, ~5-fold faster than PETIKIT and >10-fold faster than CLIJ2 (Fig. 2a). Accuracy analysis showed Napari and PETIKIT recovered 98–99% of kinetochores, whereas CLIJ2 plateaued at ~84% (Fig. 3). Pareto analysis confirmed Napari offered the best trade-off between speed and fidelity (Fig. 4). These results demonstrate that implementation, not algorithm alone, determines deconvolution usability. Napari emerges as a robust open-source solution, enabling efficient and reliable integration of deconvolution into routine advanced imaging workflows, making imaging quantitatively more reliable at higher resolutions without damage to the cell.

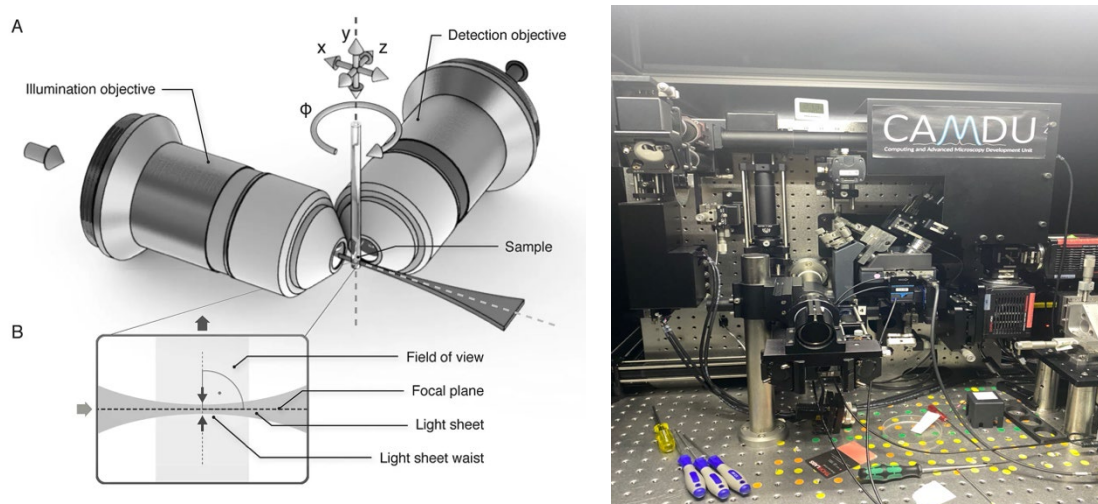


Figure 1. A schematic diagram illustrating the lattice light sheet principle, with structured Bessel beams forming a thin optical sheet for volumetric imaging.

AIM: To amend deconvolution approaches on different software to assess the usability, speed, and accuracy of three different deconvolution approaches for advanced microscopy.

METHODS

Two live samples were analysed: (i) a large RBC time-series representing extended volumetric acquisition, and (ii) a 3D stack of RPE1 cells expressing EGFP–Ndc80 in metaphase. Kinetochores served as ground truth features (~92 per cell) counted manually. Imaging was performed on a 3i Lattice Light Sheet Microscope (Figure 1).

Pipelines:

- PETIKIT (MATLAB)
- CLIJ2 (ImageJ/Fiji)
- Napari + PyCUDADeconv (Python, CUDA kernels)

All employed Richardson–Lucy deconvolution with harmonised PSFs and iteration settings.

Each dataset was processed five times per pipeline. Runtimes were separated into pre-processing, deconvolution, and post-processing. Accuracy was assessed using TrackMate for automatic spot detection compared with the manual count of 92-kinetochore ground truth after 0, 5, 10 and 15 iterations. Non-parametric tests (Kruskal–Wallis with Wilcoxon post hoc) assessed runtime differences.

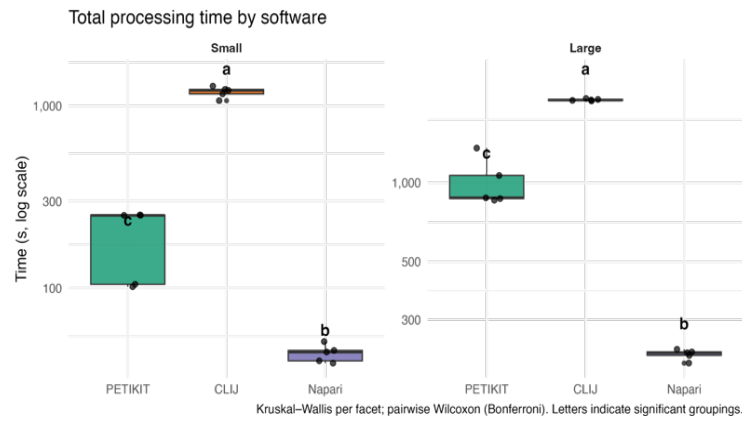


Figure 2a. Total processing times for PETIKIT, CLIJ2, and Napari across large (RBC) and small (metaphase) datasets

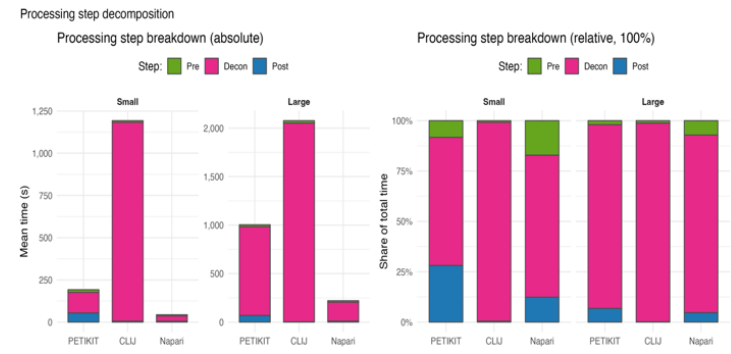


Figure 2b. Step-level runtime distributions (pre-processing, deconvolution, post-processing) for each pipeline.

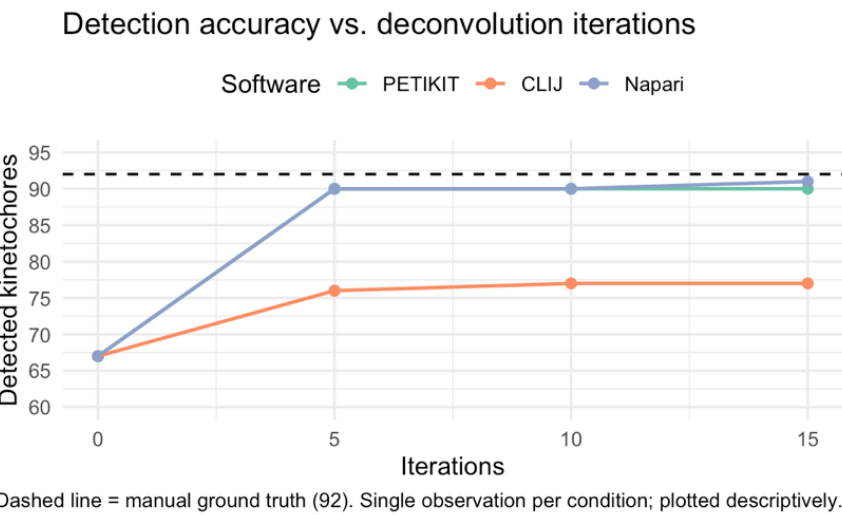


Figure 3. Kinetochores detection counts after 0, 5, 10, and 15 Richardson–Lucy iterations compared with the manual ground truth of 92 spots.

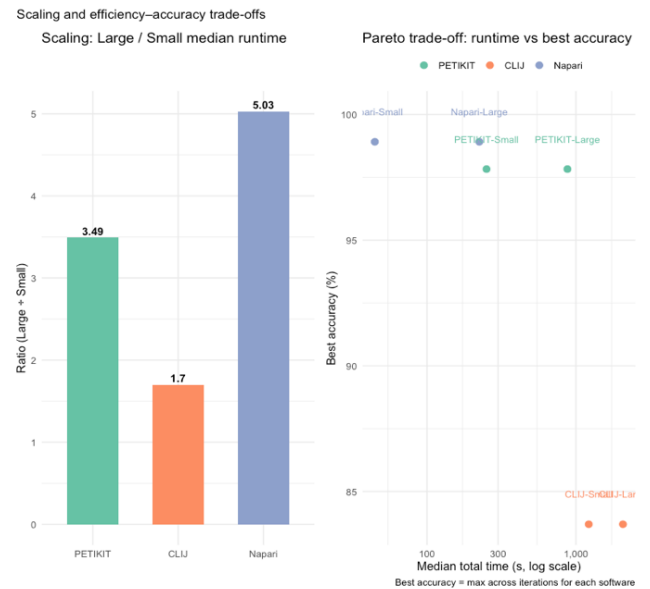


Figure 4 a. ratio of large to small dataset runtimes (median values), showing scaling efficiency. napari scaled most efficiently.
Figure 4 b. pareto trade-off plot comparing runtime (log scale) against best achieved accuracy.

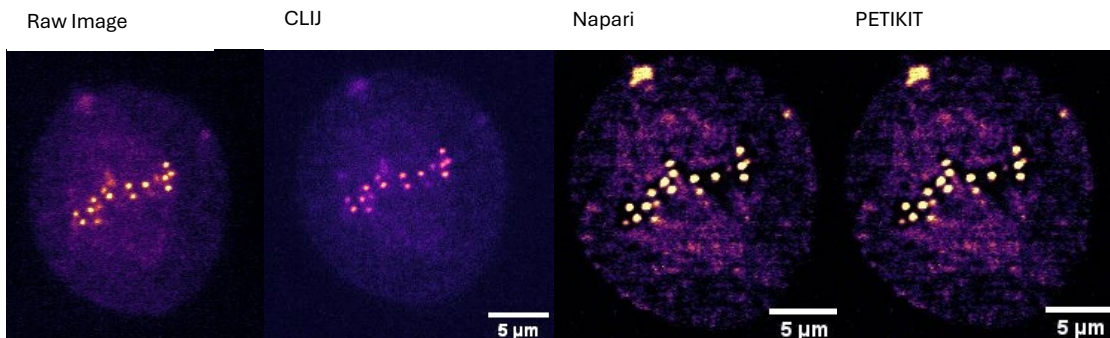


Figure 5. Example of lattice light sheet imaging of RPE1 kinetochores in metaphase before and after (10 iterations) deconvolution with each pipeline.

RESULTS & DISCUSSION

Despite employing the same Richardson–Lucy algorithm, the three pipelines produced markedly different outcomes, underscoring the importance of implementation in deconvolution. Napari consistently delivered the shortest runtimes, completing large volumetric datasets in under four minutes and small stacks in less than one minute (Fig. 2a). PETIKIT required approximately 15–20 minutes for the same volumes, while CLIJ2 exceeded 30 minutes. Step-level analysis confirmed that deconvolution dominated total runtime across all platforms (Fig. 2b). The magnitude of these differences highlights how memory handling, GPU integration, and iteration management, rather than the core algorithm, dictate practical usability.

Accuracy analysis against the 92-kinetochore ground truth mirrored these performance trends. Napari and PETIKIT converged rapidly toward near-complete recovery, achieving 98–99% of foci within 5–10 iterations (Fig. 3). In contrast, CLIJ2 plateaued at ~84% recovery despite additional iterations, reflecting limited optimisation of PSF handling and iteration control. Napari's ability to reach near-maximal accuracy within minimal runtimes is particularly significant for time-resolved experiments, where excessive iterations both prolong processing and risk amplifying background noise.

Pareto analysis placed Napari in the most favourable position, combining high accuracy with minimal runtime (Fig. 4b). PETIKIT achieved equivalent fidelity but at disproportionate computational cost, while CLIJ2 underperformed on both axes. Scalability testing further demonstrated Napari's efficiency across dataset sizes (Fig. 4a), confirming its suitability for demanding applications such as lattice light-sheet microscopy and extended live-cell imaging.

Qualitative inspection reinforced these findings. Reconstructions from Napari and PETIKIT displayed sharp kinetochore foci with reduced background, whereas CLIJ2 outputs remained blurred and less biologically informative (Fig. 5). The unexpectedly poor performance of CLIJ2, despite its prominence in the ImageJ ecosystem, illustrates that accessibility and popularity do not guarantee accuracy or efficiency.

Ultimately, Napari's efficiency demonstrates its suitability as a reliable and accessible tool for microscopy workflows especially on the LLSM. Its performance indicates that open-source solutions can equal or surpass commercial deconvolution techniques when appropriately engineered. Future research should focus on further optimisation of Napari, including automated PSF integration, adaptive iteration control, and improved memory management, to broaden accessibility and approach real-time performance. Subsequently, deconvolution could shift from being a computational bottleneck to a routine component of advanced microscopy



WHAT I LEARNED

Experimentally, I developed proficiency on two state-of-the-art platforms: the 3i Lattice Light Sheet Microscope and the Olympus FV3000 confocal system. These systems require a deep understanding of illumination strategies, photophysics, and acquisition optimisation. Working with them gave me first-hand experience of how experimental design and hardware configuration influence the quality and downstream interpretability of results.

Computationally, I advanced my skills by writing code across three distinct environments such as MATLAB, ImageJ/Fiji macro language, and Python, often working in parallel. This demanded adaptability and an ability to translate algorithmic concepts between languages. I also adopted professional software engineering practices, including version control and collaborative code management through GitHub, enabling reproducibility and transparency.

I learned to critically evaluate how algorithm implementation, GPU memory allocation, and data handling strategies affect both performance and biological validity.

Reflection

This project has strongly shaped my academic trajectory; Consequential to this studentship opportunity, I secured admission to the MRes programme within the same department at the University of Warwick, where advanced microscopy and computational image analysis techniques form the methodological core of my project, undertaken with the ultimate intention of enhancing my foundational knowledge and progressing onto a PhD programme.

As my first experience within a professional research environment, participation in such a supportive team provided a formative introduction to the ethos, practices, and collaborative dynamics of cutting-edge research, further reinforcing my motivation to contribute to the field.