Super-resolution microscopy system for single-molecule tracking, including hardware and software, designed for bench-top use

Marijonas Tutkus[1,2], Mohammad Nour Alsamsam[1,2], Aurimas Kopustas[1,2], Meda Jureviciute[1], Juste Paksaite[1]

1 Vilnius University, Life Sciences Center, Institute of Biotechnology, Vilnius, Lithuania
2 Center For Physical Sciences And Technology, Vilnius, Lithuania
marijonas.tutkus@gmc.vu.lt, marijonas.tutkus@ftmc.lt

Fluorescence microscopy (FM), which uses molecule-specific fluorescent markers to measure and track the locations, and interactions of labelled biomolecules [1]. FM augmented with approaches such as super resolution microscopy (SRM) based on single-molecule (SM) localisation (SMLM) achieves nanometric resolution in 3D with SM sensitivity (2014 Nobel prize award) [2-4]. SMLM combined with SM tracking (SMT) reveals live-cell dynamics (e.g. diffusion) of individual biomolecules [5]. However, often the lack of user-friendly and inexpensive equipment forms a roadblock for many labs to use SMLM for their research.

In our previously published work on the miEye Bench-top super-resolution microscope system [6], we demonstrated exceptional performance using affordable equipment. This SRM systems is dedicated to SMLM technique, but also can be used to many other imaging applications such as single-molecule FRET. With this system, we achieved a lateral sample drift of approximately 10 nm over 5 minutes, while our autofocusing system effectively controlled Z drift. Additionally, we achieved a ground-truth resolution of approximately 16 nm using DNA PAINT in vitro and less than 30 nm using dSTORM in fixated cells. The miEye system is an open-microscopy project, and we have made all information, including parts list, assembly guide, and software code “microEye” [7] for microscope control, data acquisition, and analysis/visualization, available as open-source [8].

In this presentation, we will unveil the latest updates to our microscope's hardware and software, which includes the installation of a dual-view emission path and 3D localization using astigmatism. We also will present results of extensive testing of various industrial-grade CMOS cameras for SMLM applications and compare them to our reference sCMOS camera performance. We will showcase our miEye system's capabilities through demonstration experiments, such as reliable tracking of HaloJF647-tagged Kinesin molecules in living eukaryotic cells on fluorescently labelled microtubules (while super-resolving and tracking microtubule network dynamics), highlighting the applicability our system. Our presentation will cover advancements in our SRM system and its use in exploring biological systems using SMLM and SMT techniques [9-10].

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