The fairSIM project: structured illumination microscopes and reconstruction for video-rate imaging

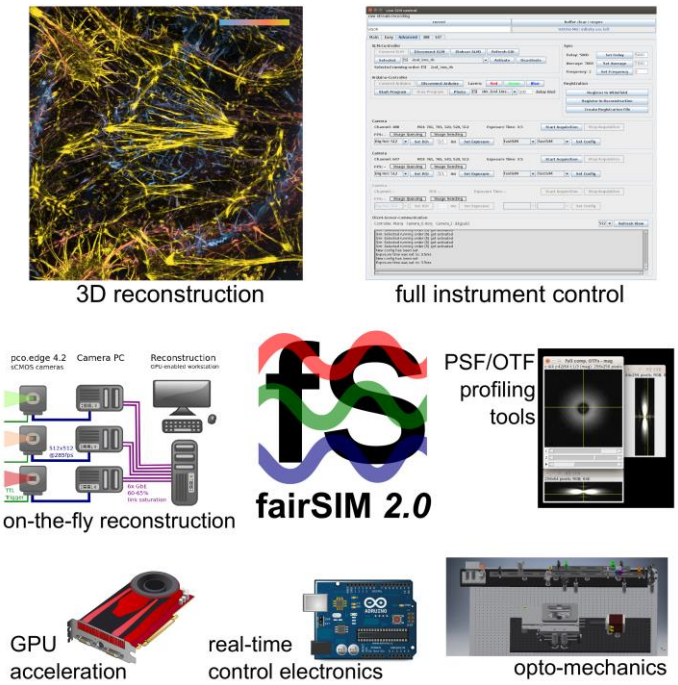
Marcel Müller[1], Andreas Markwirth[2], Alice Sandmeyer[2], Robin Van den Eynde[1], Mario Lachetta[2], Viola Mönkemöller[1], Wolfgang Hübner[2], Rainer Heintzmann[4,5], Mick Phillips[3], Ian Dobbie[3], Lothar Schermelleh[3], Peter Dedecker[1], Thomas Huser[2]

[1]Laboratory for NanoBiology, Department of Chemistry, KU Leuven, Leuven, Belgium.
[2]Biomolecular Photonics, Faculty of Physics, Bielefeld University, Bielefeld, Germany
[3]Micron Advanced Bioimaging Unit, Department of Biochemistry, University of Oxford, Oxford, UK.
[4]Institute of Physical Chemistry and Abbe Center of Photonics, Friedrich-Schiller-University, Jena, Germany
[5]Leibniz Institute of Photonic Technology, Jena, Germany

Email: muellerphysics@gmail.com

Super-resolution structured illumination microscopy (SIM) allows for sub-diffraction imaging with low phototoxicity and high frame rates, making it a popular technique for live cell imaging. Current-generation instruments provide video-rate imaging speeds, and thus enable the observation of even fast dynamical processes with sub-diffraction resolution.

In 2016, we released a free and open-source implementation of the SIM reconstruction algorithm [1]. Since then, the fairSIM project has been greatly extended in various ways: The reconstruction algorithm itself gained new filter options [2], the 3D data processing [3] implementation is well on its way, and microscope profiling tools such as optical transfer function generation have been added. We also pushed for the development of bespoke SIM microscopes, such as a multi-color version of the SLM-based fastSIM [4] system, a cost-effective DMD-based SIM and a modular, customizable and easily replicable SIM illumination system.



Various new features added to the fairSIM project since its initial release in 2016.

All these microscope systems can now be linked to an on-the-fly reconstruction feature, which merges data acquisition and post-processing, and allows the user to obtain an immediate super-resolution views of their samples. This is achieved by tight integration of the data acquisition process with a GPU-accelerated implementation of the reconstruction process. On our fastest data acquisition systems (based on [4]), we can achieve 25fps simultaneously on 3 color channels, and provide reconstructed super-resolution images within half a second after data acquisition.

Here, we present an overview of the fairSIM project, its applications, features and capabilities, but also its current limitations and ongoing development. We hope to connect groups in need of bespoke, high speed SIM imaging with those developing these methods and algorithms.

[1] Marcel Müller, Viola Mönkemöller, Simon Hennig, Wolfgang Hübner, and Thomas Huser. *Open-source image reconstruction of super-resolution structured illumination microscopy data in ImageJ*. Nature Communications, 7, 2016

[2] Victor Perez, Bo-Jui Chang, and Ernst Stelzer. *Optimal 2D-SIM reconstruction by two filtering steps with Richardson-Lucy deconvolution.* Scientific reports 6, 37149, 2016.

[3] Mats Gustafsson, Lin Shao, Peter Carlton, Rachel Wang, Inna Golubovskaya, Zacheus Cande, David Agard, and John Sedat. *Three-dimensional resolution doubling in wide-field fluorescence microscopy by structured illumination*. Biophysical journal, 94(12):4957–4970, 2008.

[4] Hui-Wen Lu-Walther, Martin Kielhorn, Ronny Förster, Aurélie Jost, Kai Wicker, and Rainer Heintzmann. *fastSIM: a practical implementation of fast structured illumination microscopy*. Methods and Applications in Fluorescence, 3(1):014001, 2015.