Bioinformatics, 32(2), 2016, 318–320
doi: 10.1093/bioinformatics/btv576
Advance Access Publication Date: 6 October 2015
Applications Note

Bioimage informatics

SIMToolbox: a MATLAB toolbox for structured illumination fluorescence microscopy

Pavel Křížek[1], Tomáš Lukeš[2,3], Martin Ovesný[1], Karel Fliegel[2] and Guy M. Hagen[4,*]

[1]First Faculty of Medicine, Charles University in Prague, Prague 12800, Czech Republic, [2]Department of Radioelectronics, Faculty of Electrical Engineering, Czech Technical University in Prague, Prague 16627, Czech Republic, [3]Laboratoire d'Optique Biomédicale, École Polytechnique Fédérale de Lausanne, Lausanne CH-1015, Switzerland and [4]UCCS center for the BioFrontiers Institute, University of Colorado at Colorado Springs, Colorado Springs, CO 80918, USA

* To whom correspondence should be addressed.

Associate Editor: Robert Murphy

Received on July 22, 2015; revised on September 28, 2015; accepted on September 30, 2015

Abstract

Summary: SIMToolbox is an open-source, modular set of functions for MATLAB equipped with a user-friendly graphical interface and designed for processing two-dimensional and three-dimensional data acquired by structured illumination microscopy (SIM). Both optical sectioning and super-resolution applications are supported. The software is also capable of maximum *a posteriori* probability image estimation (MAP-SIM), an alternative method for reconstruction of structured illumination images. MAP-SIM can potentially reduce reconstruction artifacts, which commonly occur due to refractive index mismatch within the sample and to imperfections in the illumination.
Availability and implementation: SIMToolbox, example data and the online documentation are freely accessible at http://mmtg.fel.cvut.cz/SIMToolbox.
Contact: ghagen@uccs.edu
Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

In structured illumination microscopy (SIM), a sequence of images is acquired, each taken with a different position of an illumination pattern in a fluorescence microscope but without a mask in the detection path (Heintzmann, 2006). Subsequent processing of the raw data is required to reconstruct the final image. Current SIM methods include optical sectioning microscopy (OS-SIM) (Křížek *et al.*, 2012; Neil *et al.*, 1997; Wilson, 2011) and super-resolution SIM (SR-SIM) (Gustafsson, 2000; Gustafsson *et al.*, 2008; Heintzmann and Cremer, 1998).

Several different algorithms can be used to process SIM data (Heintzmann, 2006). SIMToolbox offers a set of MATLAB functions for processing both OS-SIM and SR-SIM data according to a variety of methods. SIMToolbox can also apply an alternative method for processing the data, maximum *a posteriori* probability estimation (MAP-SIM) (Lukeš *et al.*, 2014). The tools can be accessed from a user-friendly graphical user interface.

Our goal is to offer a collection of processing methods, which were developed based on extensive testing with real data. The software can be used to process data acquired by commercial SIM systems; however, some of the tools and documentation provided are meant for researchers developing their own systems. In addition to the software, we provide example data, a user's guide and a detailed description of the implemented methods and algorithms. Please refer to the Supplementary Note for further details.

2 Features and methods

2.1 OS-SIM

SIMToolbox can process OS-SIM data using homodyne detection (Křížek *et al.*, 2012; Neil *et al.*, 1997), maximum–minimum projection (Heintzmann, 2006) or several forms of scaled subtraction (Heintzmann, 2006; Křížek *et al.*, 2012). When using scaled subtraction, it is necessary to have knowledge of the illumination pattern positions

in the camera image. We accomplish this by spatial calibration using an alignment pattern (Křížek *et al.*, 2012). Residual stripes are corrected using a method introduced by Cole *et al.* (2001).

2.2 SR-SIM

SIMToolbox can process SR-SIM data using the Heintzmann–Gustafsson method (Gustafsson, 2000; Gustafsson *et al.*, 2008; Heintzmann and Cremer, 1998). Briefly, the image processing steps are as follows:

1. Forward fast Fourier transformation (FFT) of the raw data.
2. Identification of peaks in the frequency spectrum. The software can accomplish this with high accuracy using spatial calibration methods (Křížek *et al.*, 2012) or using spot detection methods (Ovesný *et al.*, 2014).
3. Shifting of spectral components in frequency space.
4. Apodizing the frequency spectrum according to one of several possible functions. SIMToolbox offers several apodizing functions including a standard incoherent model, cosine bell, Butterworth, triangle and application of the Lukosz bound (Righolt *et al.*, 2013).
5. Inverse FFT with an adjustable inverse filter parameter to recover an image with resolution beyond the conventional limit.

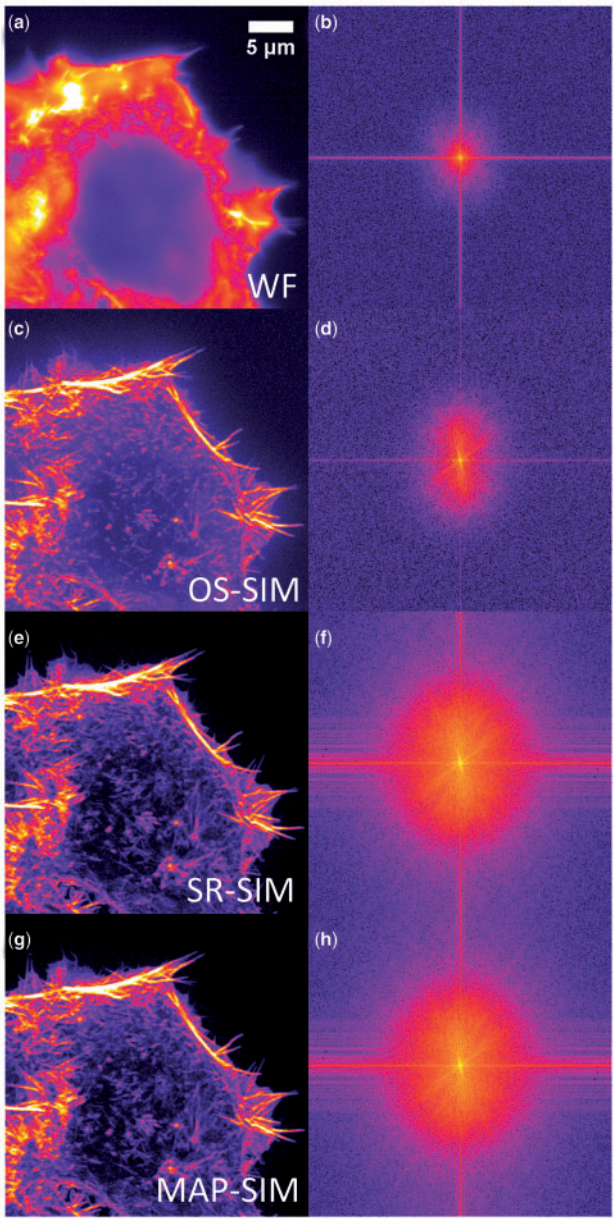

Fig. 1. Widefield (WF) image and OS-SIM, SR-SIM and MAP-SIM reconstructions of images of a HEP-G2 cell in which the actin cytoskeleton has been labeled with Atto565-phalloidin. (**a**) WF. (**b**) FFT of WF. (**c**) OS-SIM, homodyne method. (**d**) FFT of OS-SIM image. (**e**) SR-SIM processing. (**f**) FFT of SR-SIM. (**g**) MAP-SIM. (**h**) FFT of MAP-SIM. Images are maximum intensity projections of 31 axial sections and have been individually brightness adjusted for presentation

2.3 MAP-SIM

An alternative method for processing SR-SIM data is the MAP-SIM method introduced by our group (Lukeš *et al.*, 2014a, b). Here, image details beyond the diffraction limit are estimated by maximum *a posteriori* restoration methods, and optically sectioned, conventional resolution image information is processed by homodyne detection. The two components are then combined by spectral merging in the frequency domain. The result is then apodized, and an inverse FFT is performed to recover the final image.

Our experience has been that MAP-SIM can, in some cases, produce super-resolution images with fewer artifacts than the Heintzmann–Gustafsson method. Such artifacts seem to be primarily caused by problems with the sample, especially refractive index mismatch between the mounting medium and immersion fluid. Reconstruction artifacts can also be caused by high noise levels, by poor SIM pattern contrast or other imperfections in the illumination or by the use of inappropriate parameters in the reconstruction process. For example, we have found that the choice of apodizing and inverse filter parameters (SR-SIM steps 4 and 5 above) have a large impact on the results.

Figure 1 shows a comparison of some of the various methods supported by SIMToolbox, acquired using the methods introduced by our group (Křížek *et al.*, 2012). Please see the Supplementary Note for more details about this SIM experiment.

3 Summary

SIMToolbox is open-source software for processing SIM data. This allows researchers building their own systems to process their data using a unique set of tools and concepts. The software also offers features not currently present in commercially available software, making it a useful choice for reconstructing super-resolution images acquired with commercial equipment.

Funding

This work was supported by the UCCS center for the BioFrontiers Institute and by the CTU (SGS14/148/OHK3/2T/13). T.L. acknowledges a SCIEX fellowship (project code 13.183).

Conflict of Interest: none declared.

References

Cole,M.J. *et al.* (2001) Time-domain whole-field fluorescence lifetime imaging with optical sectioning. *J. Microsc.*, **203**, 246–257.

Gustafsson,M.G.L. (2000) Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. *J. Microsc.*, **198**, 82–87.

Gustafsson,M.G.L. *et al.* (2008) Three-dimensional resolution doubling in widefield fluorescence microscopy by structured illumination. *Biophys. J.*, **94**, 4957–4970.

Heintzmann,R. (2006) Structured illumination methods. In: Pawley,J.B. (ed.) *Handbook of Biological Confocal Microscopy*. Springer, New York, pp. 265–279.

Heintzmann,R. and Cremer,C. (1998) Laterally modulated excitation microscopy: improvement of resolution by using a diffraction grating. *Proc. SPIE*, **3568**, 185–196.

Křížek,P. *et al*. (2012) Flexible structured illumination microscope with a programmable illumination array. *Opt. Express*, **20**, 24585–24599.

Lukeš,T. *et al*. (2014a) Comparison of image reconstruction methods for structured illumination microscopy. In: Popp,J. *et al*. (eds.) *SPIE Photonics Europe*. International Society for Optics and Photonics, Bellingham, WA, p. 91293J.

Lukeš,T. *et al*. (2014b) Three-dimensional super-resolution structured illumination microscopy with maximum a posteriori probability image estimation. *Opt. Express*, **22**, 29805–29817.

Neil,M.A.A. *et al*. (1997) Method of obtaining optical sectioning by using structured light in a conventional microscope. *Opt. Lett.*, **22**, 1905–1907.

Ovesný,M. *et al*. (2014) ThunderSTORM: a comprehensive ImageJ plug-in for PALM and STORM data analysis and super-resolution imaging. *Bioinformatics*, **30**, 2389–2390.

Righolt,C.H. *et al*. (2013) Image filtering in structured illumination microscopy using the Lukosz bound. *Opt. Express*, **21**, 24431–24451.

Wilson,T. (2011) Optical sectioning in fluorescence microscopy. *J. Microsc.*, **242**, 111–116.