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BrightEyes-MCS: a control software for multichannel scanning microscopy

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Introduction

Fluorescence microscopy is an essential workhorse of biomedical sciences, thanks to its capability to provide specific and quantitative information on the observed specimens. The advent of super-resolution techniques has further improved the quality of the images produced by optical microscopes. Among the numerous super-resolution techniques, image scanning microscopy (ISM) emerged as a robust and reliable technique, being able to provide gentle imaging at a high signal-to-noise ratio (SNR) with excellent optical sectioning (Castello et al., 2019; Perego et al., 2023; Tortarolo et al., 2022). An ISM microscope shares the same architecture as a confocal laser scanning microscope (CLSM) but exploits a pixeled detector. Each detector element acts as a displaced small pinhole and generates a confocal-like image after a full scan of the field of view. As the detector is made by a matrix of pixels, the raw ISM dataset is not a 2D image, it is a 4D dataset, which can be seen in two ways: each single pixel scanned on the sample plane is associated with a micro-image of the detector; vice-versa, for each physical pixel on the detector plane is associated a full-scanned field of view on the sample plane (Müller & Enderlein, 2010; Sheppard, 1988). Using tailored reconstruction algorithms, the images of the raw ISM dataset can be fused to produce a single super-resolution image using all the photons collected by the detector, guaranteeing an excellent SNR (Zunino et al., 2022, 2023, 2024).

The capabilities of ISM can be further extended if coupled with a fast detector, such as an array of single photon avalanche diodes (SPAD) (Buttafava et al., 2020; Slenders, Perego, et al., 2021). This type of detector features the readout of each pixel is independent and async, preserving the temporal information of each photon arrival. In other words, it is possible to tag each photon with its arrival time with respect to the excitation laser, expanding the dataset to a 5D array. This data can be used for estimating the fluorescence lifetime allowing the fluorescent lifetime imaging scanning microscopy (FLISM), a microscopy technique that merges the temporal information with the ISM advantages mentioned above (Rossetta et al., 2022; Tortarolo et al., 2024).

Statement of need

In live-cell microscopy, the demand for high-resolution imaging coexists with the necessity to protect sample integrity. Fast pixel dwell times are instrumental in this pursuit, minimizing sample damage while enhancing image quality. Leveraging the capabilities of the SPAD array detector, which can achieve megahertz photon flux per single pixel, requires an advanced data acquisition and control system based on FPGA technology. Existing open-source microscope control software, such as ImSwitch (Moreno et al., 2021) and μManager (Edelstein et al., 2010), while general-purpose, flexible, and scriptable, are primarily designed for camera-based systems. They are not optimized to handle the precise synchronization and high-performance demands

of ISM instruments, especially for configuration with pixel dwell times as low as 1 µs and photon flux rates in the megahertz range.

In contrast, BrightEyes-MCS (Microscope Control Suite) is an open-source software specifically designed for controlling laser-scanning microscopes equipped with SPAD detectors. It overcomes the limitations of other software by providing real-time synchronization, high-throughput data acquisition, and seamless compatibility with the high-speed scanning demands of ISM systems.

BrightEyes-MCS not only features a user-friendly graphical interface (GUI) for microscope control, but also supports real-time previews and efficient data saving in the HDF5 format, which is ready for internal processing or integration with external analysis tools. Although tailored for ISM, BrightEyes-MCS is versatile and can be adapted for various applications, including fluorescence correlation spectroscopy (FCS), spectroscopy, and other advanced imaging techniques. This flexibility makes it a valuable resource for researchers working with diverse microscopy setups that require precise control and high-performance data acquisition.

BrightEyes is the name of the project founded by the ERC in 2018 (Consolidator Grant, N. 818699). In this context, other open-source tools have been developed, like BrighEyes-TTM, an open-hardware time-tagging module (Rossetta et al., 2022), BrightEyes-ISM (Zunino et al., 2023), a python library for ISM data analysis.

System Architecture

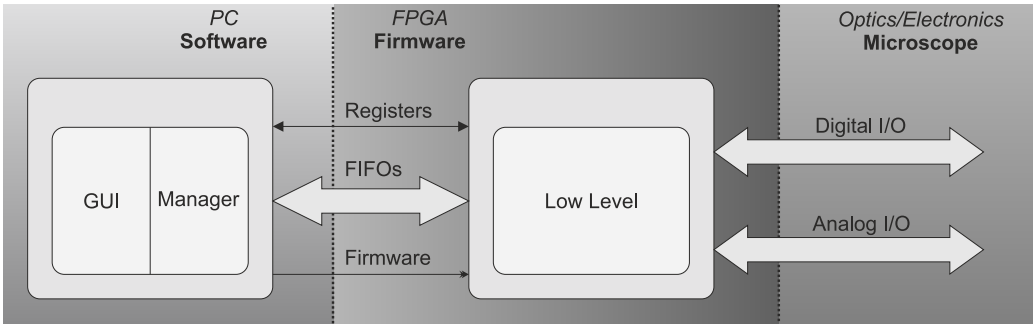

Figure 1: Scheme of BrightEyes-MCS architecture.

The BrightEyes-MCS controls and handles the data stream with the underlying hardware, based on a field-programmable gate array (FPGA) from National Instrument (NI). These boards provide the digital inputs/outputs (I/O), and some models also provide analog I/Os. Figure 1 shows a sketch of the system architecture.

The system architecture features two parts: i) the low-level firmware (BrightEyes-MCSLL) managing the hardware and the electronics, running on the FPGA; ii) the high-level software (BrightEyes-MCS) which includes the GUI and its libraries running on the PC.

The firmware was developed specifically for image scanning microscopy, on NI LabView for FPGA. It is a separate project, free, downloadable but it is not open source. The firmware can be controlled by the high-level software through registers and the data are streamed through dedicated FIFOs. Details are out of the scope of this paper and together with its documentation are available in the BrightEyes-MCSLL repository.

Each time an acquisition starts, BrightEyes-MCS automatically loads the firmware into the FPGA, sets the registers (for example for configuring the number of pixels, and dwell-time), and waiting for the data arriving through FIFO.

BrightEyes-MCS supports up to 25 digital channels (plus 2 extra channels) and up-to 2 analog inputs. It controls the scanning and the positioning with a maximum speed of 1 us for pixel dwell time and it is possible to set a further time-subdivision of the same pixel down to 0.5us per bin.

There is also the possibility of activating the so-called Digital Frequency Domain (DFD) mode (Tortarolo et al., 2024). It is a heterodyne technique that allow to obtain higher time resolution. In this mode, the laser pulsing is driven by the FPGA, and for each detector element it is acquired the histogram of the time-arrival of the photons with respect to the laser pulse (known as Time-Correlated Single Photon Counting, TCSPC), with a bin precision of 0.3 ns.

The software has been tested in a machine equipped with an Intel Xeon CPU (2.2GHz) with 12 cores, and 32GByte of RAM. This system was able to acquire datasets up to 2000x2000x25x81 (x, y, ch,t).

Specifications

The specifications of BrightEyes-MCS depend on the hardware features of the NI FPGA used. To actuate the scanners, analog outputs are needed. Since not all FPGA models provide them, they can be replaced with an external independent board.

Channels	25		
Extra Channels	2		
Analog Inputs Channels	2 (selected out of 8), or not supported with external DAC		
Analog Output Channels	8, or 4 with external DAC		
Pixel Dwell Time	1.0 µs		
Minimum time bin	normal mode	0.5 µs	
	DFD mode	0.2 ns	
Data storage	HDF5, data and metadata		
Data array dimension	repetition, z, y, x, time-bin, detector-channel		
Boards tested	*25 ch.*	▪ NI USB-7856R	
		▪ NI USB-7856R OEM	
		▪ NI PXIe-7856R	
		▪ NI PXIe-7822R	(with external DAC*)
		▪ NI PCIe-7820R	(with external DAC*)

*As external DAC has been tested the commercial evaluation board EVAL-AD5764 from Analog Device.

Hardware and Electronics

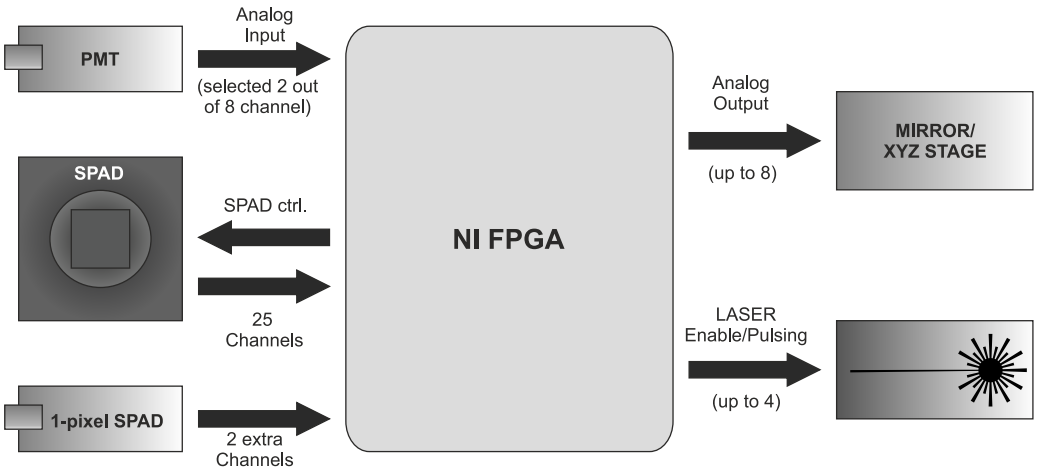

Figure 2: Scheme of the hardware of BrightEyes-MCS system.

This sketch shows the main electronics components controlled by BrightEyes-MCS.

The sample positioning and the scanning are controlled by the analog outputs connected to positioner. They can be either linear galvo mirrors or piezo stages. The analog outputs supported are 8 and the user can select for each channel to be used as X, Y, Z, or a constant voltage. The possibility of setting a constant voltage is useful for other types of elements such as Acousto-optic modulators (AOM).

The support of photomultiplier tubes (PMTs) is given by reading out two analog inputs at the same time. The user can select the two analog inputs out of 8 channels.

In the case of the NI FPGA board used lacking the analog I/O, the analog outputs are provided by an external DAC connected to the digital I/O of the FPGA. Similarly to it, we are planning to support for external ADC for providing the analog inputs.

The BrightEyes-MCS can control up to 4 lasers through 4 digital output lines. They can be switched on and off easily at the start and end of a measurement. In the case of DFD mode – i.e. time-of-arrival / lifetime measurement – the system provides the synchronization clock to the lasers for triggering the pulses.

Software Architecture

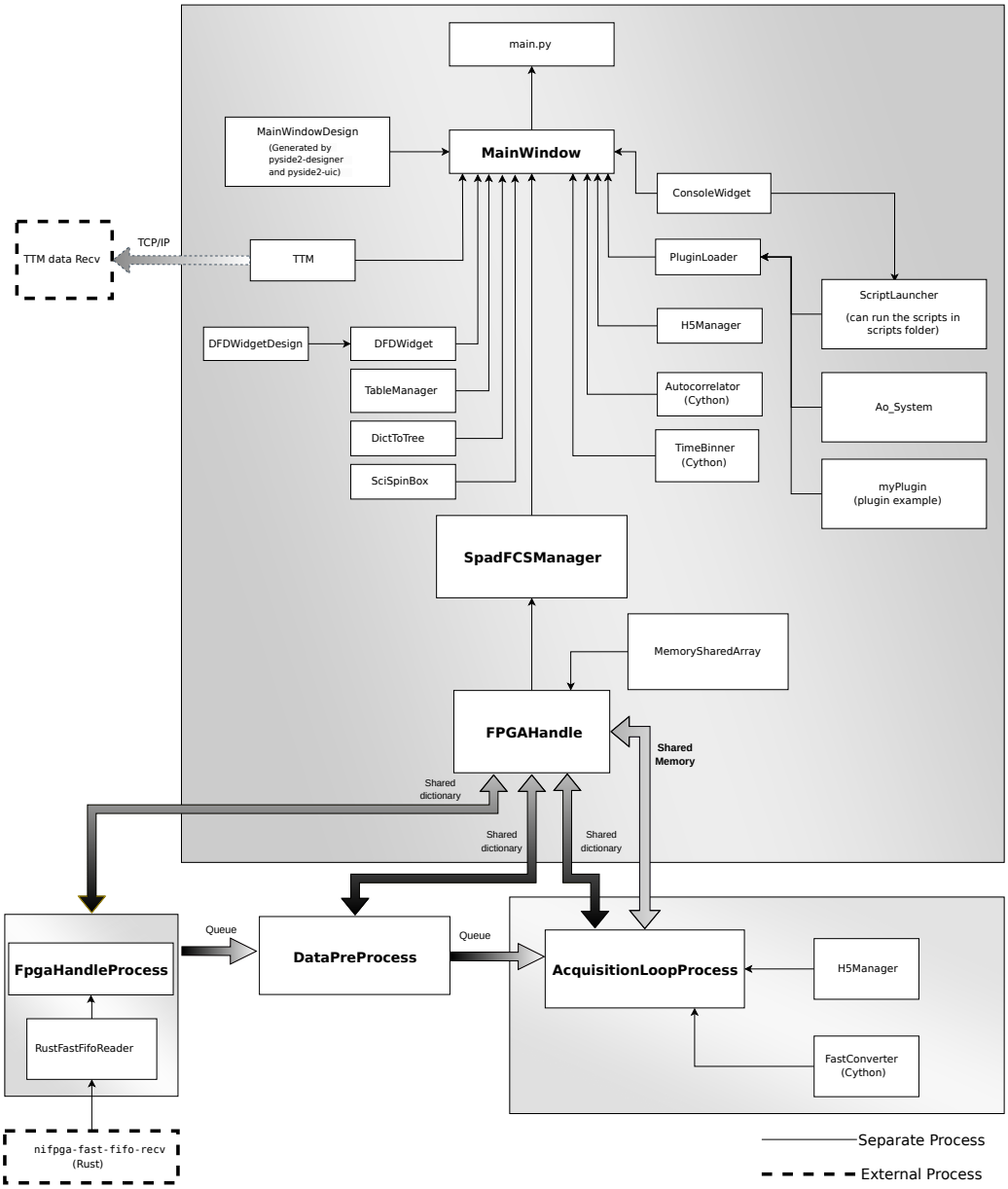

Figure 3: Scheme of the software architecture

The Figure above shows the main parts of the BrightEyes-MCS Python code. The main process runs the GUI (MainWindow). When an acquisition is started the FPGAHandle instantiates the other three parallel processes.

These processes are "infinite loops" (until the event end-of-acquisition), implemented using the multiprocessing library. As they are independent Python instances, the communication between needs "shared" objects such as mp.Dict, mp.Event and MemorySharedArray (which uses mp.Array). Here below a short description of what the three processes:

1. The FpgaHandleProcess uploads and runs the firmware on the FPGA, listen to commands from the Main, and executes them. These commands are mainly related to read/write registers. This process continuously reads data available on the FIFOs from the FPGA,

sending them to the DataPreProcess via a mp.Queue. The communication with the data is given by the nifpga library. The FIFO readout can performed either with nifpga library or with nifpga-fast-fifo-recv, a Python library written in Rust which we developed for reaching higher readout performances.

2. The DataPreProcess is just waiting for data from the input Queue and cumulate them up to a given value and sends them to the AcquisitionLoopProcess with an mp.Queue.
3. The AcquisitionLoopProcess converts the raw data to a numpy array. This is performed by FastConverter, a function developed in Cython due to performance reasons. The converted data are reshaped and stored into a buffer used for the live preview and for saving the data to the HDF5 file h5py library.

The GUI is implemented with PySide2 library. The images and the other plots are drawn by PyQtGraph library. The GUI also provides an integrated Python console (which uses the library QtConsole) which exposes all running objects. This means that on the console, the user can modify parts of the running software, in-live. This allows to run scripts for automatized operation, moreover, it allows to easily run scripts for example a quick data analysis at the end of the measurement.

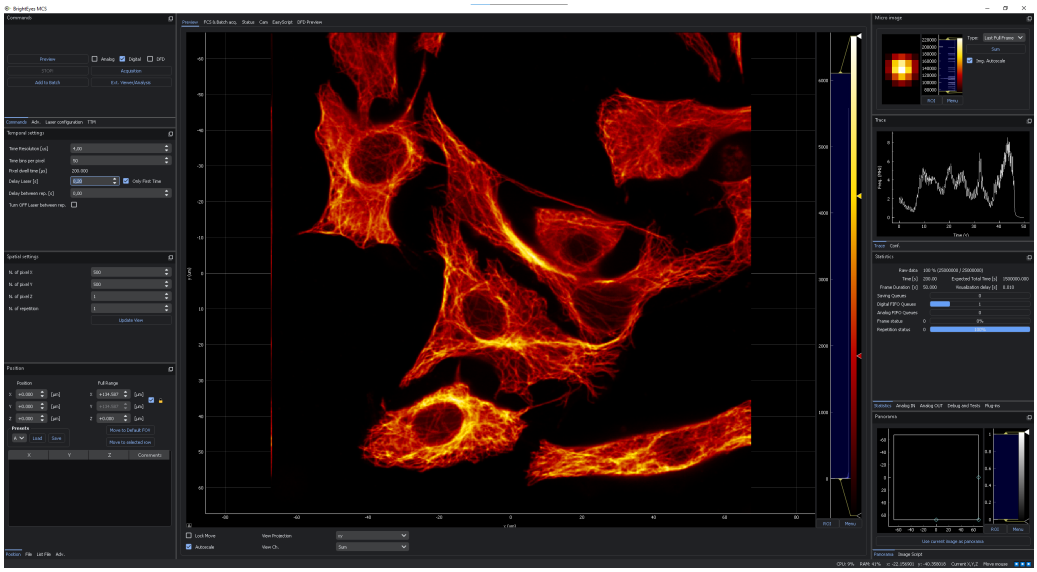

Figure 4: Screenshot of BrightEyes-MCS GUI

Conclusion

The BrightEyes-MCS is a new open-source tool for controlling image scanning microscopes. It provides a real-time preview and supports up to 25 channels. It is designed for image-scanning microscopy with a SPAD array but allows easily to be used on different scenarios. For example, it can be used on a single-detector confocal microscope equipped with a single-pixel SPAD or PMT. It can be used also outside the context of scanning microscopy as for example it is possible to use it in the context of Fluorescence correlation spectroscopy (FCS) (Slenders, Castello, et al., 2021). Furthermore, the software can support single-molecule localization microscopy (SMLM) with non-conventional scanning patterns, such as MINFLUX and ISMFLUX (Slenders & Vicidomini, 2023). Data acquired by BrightEyes-MCS are stored in HDF5 format, which can be easily opened with popular data analysis frameworks like Python notebooks or MATLAB. Additionally, a Python library called BrightEyes-ISM (Zunino et al., 2023) has been developed, enabling users to open these files and apply enhancement techniques such as APR (Castello et al., 2019) and Focus-ISM (Tortarolo et al., 2022). For those using Napari, a plugin named

Napari-ISM is available, allowing the same enhancements to be performed within its graphical user interface.

As Python open-source tool can be easily adapted to other systems. For example, BrightEyes-MCS has been integrated with BrightEyes-TTM, an open-source time-tagging module that allows to time-tag single photons with a resolution of about 30ps (Rossetta et al., 2022). It can be controlled by BrightEyes-MCS: every time an acquisition is starting the TTM remotely starts acquiring data in another machine. Moreover, BrightEyes-MCS can be controlled by external tools via HTTP REST APIs, which allow access to the GUI parameters, main commands (such as start acquisition, preview, and stop), and the preview image. This facilitates integration into a larger control framework, such as ImSwitch (Moreno et al., 2021) or Arkitekt (Roos, 2023).

In conclusion, BrightEyes-MCS presents a promising open-source solution for controlling image-scanning microscopes. It features real-time preview and multi-channel support, and offers great versatility for various microscopy setups. We envision that our open-source and free tool will be widely adopted by the scientific community, contributing to the dissemination of open science culture in microscopy.

Disclosures & Acknowledgements

G.V. has a personal financial interest (co-founder) in Genoa Instruments, Italy. The remaining authors declare no competing interests. We acknowledge our former colleagues, Marco Castello and Simonluca Piazza, now founder and respectively CTO and CEO of Genoa Instruments, for their important contributions to the firmware in the early stages of the project. BrightEyes-MCS is designed to be an open-source software for research purposes and does not reflect the performance of the commercial products offered by Genoa Instruments.

References

The repository of BrightEyes-MCS is https://github.com/VicidominiLab/BrightEyes-MCS.

Buttafava, M., Villa, F., Castello, M., Tortarolo, G., Conca, E., Sanzaro, M., Piazza, S., Bianchini, P., Diaspro, A., Zappa, F., Vicidomini, G., & Tosi, A. (2020). SPAD-based asynchronous-readout array detectors for image-scanning microscopy. *Optica*, *7*, 755. https://doi.org/10.1364/optica.391726

Castello, M., Tortarolo, G., Buttafava, M., Deguchi, T., Villa, F., Koho, S., Pesce, L., Oneto, M., Pelicci, S., Lanzanó, L., Bianchini, P., Sheppard, C. J. R., Diaspro, A., Tosi, A., & Vicidomini, G. (2019). A robust and versatile platform for image scanning microscopy enabling super-resolution FLIM. *Nature Methods*, *16*, 175–178. https://doi.org/10.1038/s41592-018-0291-9

Edelstein, A., Amodaj, N., Hoover, K., Vale, R., & Stuurman, N. (2010). Computer control of microscopes using μManager. *Current Protocols in Molecular Biology*, *92*. https://doi.org/10.1002/0471142727.mb1420s92

Moreno, X., Al-Kadhimi, S., Alvelid, J., Bodén, A., & Testa, I. (2021). ImSwitch: Generalizing microscope control in python. *Journal of Open Source Software*, *6*, 3394. https://doi.org/10.21105/joss.03394

Müller, C. B., & Enderlein, J. (2010). Image scanning microscopy. *Physical Review Letters*, *104*. https://doi.org/10.1103/PhysRevLett.104.198101

Perego, E., Zappone, S., Castagnetti, F., Mariani, D., Vitiello, E., Rupert, J., Zacco, E., Tartaglia, G. G., Bozzoni, I., Slenders, E., & Vicidomini, G. (2023). Single-photon microscopy to study biomolecular condensates. *Nature Communications*, *14*, 8224. https://doi.org/10.1038/s41467-023-43969-7

Roos, J. (2023). *Arkitekt : An open-source framework for modern bioimage workflows* [PhD thesis, Université de Bordeaux]. https://theses.hal.science/tel-04341599

Rossetta, A., Slenders, E., Donato, M., Zappone, S., Fersini, F., Bruno, M., Diotalevi, F., Lanzanò, L., Koho, S., Tortarolo, G., Barberis, A., Crepaldi, M., Perego, E., & Vicidomini, G. (2022). The BrightEyes-TTM as an open-source time-tagging module for democratising single-photon microscopy. *Nature Communications*, *13*, 7406. https://doi.org/10.1038/s41467-022-35064-0

Sheppard, C. (1988). Super-resolution in confocal imaging. *Optik*, *80*, 53–54.

Slenders, E., Castello, M., Buttafava, M., Villa, F., Tosi, A., Lanzanò, L., Koho, S. V., & Vicidomini, G. (2021). Confocal-based fluorescence fluctuation spectroscopy with a SPAD array detector. *Light: Science & Applications*, *10*. https://doi.org/10.1038/s41377-021-00475-z

Slenders, E., Perego, E., Buttafava, M., Tortarolo, G., Conca, E., Zappone, S., Pierzynska-Mach, A., Villa, F., Petrini, E. M., Barberis, A., Tosi, A., & Vicidomini, G. (2021). Cooled SPAD array detector for low light-dose fluorescence laser scanning microscopy. *Biophysical Reports*, *1*, 100025. https://doi.org/10.1016/j.bpr.2021.100025

Slenders, E., & Vicidomini, G. (2023). ISM-FLUX: MINFLUX with an array detector. *Physical Review Research*, *5*, 023033. https://doi.org/10.1103/PhysRevResearch.5.023033

Tortarolo, G., Zunino, A., Fersini, F., Castello, M., Piazza, S., Sheppard, C. J. R., Bianchini, P., Diaspro, A., Koho, S., & Vicidomini, G. (2022). Focus image scanning microscopy for sharp and gentle super-resolved microscopy. *Nature Communications*, *13*. https://doi.org/10.1038/s41467-022-35333-y

Tortarolo, G., Zunino, A., Piazza, S., Donato, M., Zappone, S., Pierzyńska-Mach, A., Castello, M., & Vicidomini, G. (2024). Compact and effective photon-resolved image scanning microscope. *Advanced Photonics*, *6*. https://doi.org/10.1117/1.ap.6.1.016003

Zunino, A., Castello, M., & Vicidomini, G. (2022). Reconstructing the image scanning microscopy dataset: An inverse problem. *Journal of Optics*, *39*, 064004. https://doi.org/10.1088/1361-6420/accdc5

Zunino, A., Garrè, G., Perego, E., Zappone, S., Donato, M., & Vicidomini, G. (2024). *Structured detection for simultaneous super-resolution and optical sectioning in laser scanning microscopy*. https://doi.org/10.48550/arXiv.2406.12542

Zunino, A., Slenders, E., Fersini, F., Bucci, A., Donato, M., & Vicidomini, G. (2023). Open-source tools enable accessible and advanced image scanning microscopy data analysis. *Nature Photonics*, *17*, 457–458. https://doi.org/10.1038/s41566-023-01216-x