napari-superres

an open-source implementation of methods for Fluorescence Fluctuation-based Super-Resolution Microscopy (FF-SRM)

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1. Fluorescence nanoscopy

- Fluorescence microscopy is a well-developed imaging modality that is used in life sciences to study tissues, organoids, and cells. The inspection on the microscopic scale is limited by the resolving power of the used optical system, but more **specialized techniques for Super-Resolution Microscopy (SRM) are able to render even nanoscopic details of biological samples.**

- There are **numerous hardware-based methods for SRM**. For example, Structural Illumination, Localization Microscopy, STED, and Airyscan microscopy **require each a different type of complex and expensive hardware (Fig. 1)**. Furthermore, those technologies are often sold as commercial package with proprietary software by microscopes manufacturers.

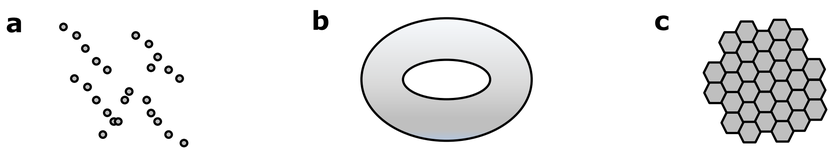

Fig 1. Quiz: identify the hardware-based Super-Resolution technology associated to: a) bee stings b) a honey donut c) a beehive.

2. Access to technology is not obvious

- However, the accessibility to SRM has been historically hindered by the availability of high-tech, usually expensive, microscopy platforms. Unfortunately, **scientific advances of nanoscopy in countries of Africa, Latin America, and Eastern Europe have historically lagged behind developed nations, due to the lack of geographically well-distributed infrastructures.**

- In fact, **the cost of a microscopy platform dedicated to super-resolution can span between 100 k£ and several hundreds of k£,** for very complex hardware. The market price depends on the novelty of the technology, and on factors such as the market request, the possibility that well-funded institute have to bundle multiple purchase orders, the ability of the facility manager to negotiate, and the overall scientific leverage. **Moreover, the cost of maintenance contracts** (~ few 10 k£) **may not be sustainable by small institutions**.

- Unfortunately, **the abundance of big institutes and organised consortia might be a characteristic of few rich countries** where scientific research is well financed. E.g. Janelia HHMI (US), EMBL (multiple sites across Europe), The Francis Crick Institute (UK), Euro-BioImaging network, etc.

2. Fluorescence Fluctuation-based Super-Resolution Microscopy (FF-SRM)

- **What is the FF-SRM?**

The analysis of statistical properties of fluorescence intermittency (FF-SRM) applied on diffraction-limited images can deliver nanoscopic information which is fundamental to study biological systems in unaltered conditions (live nanoscopy). **Fig. 2** shows a time line of FF-SRM methods.

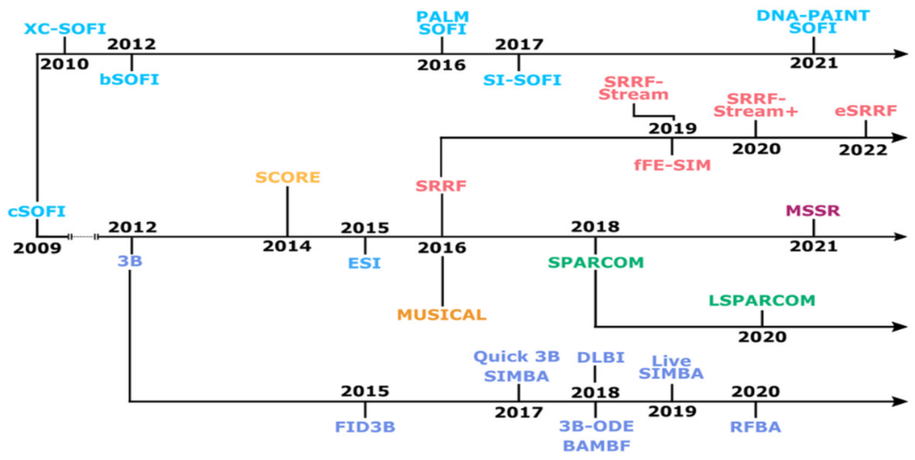

Fig 2. Time line of the development of FF-SRM methods [Alva et al., 2022]

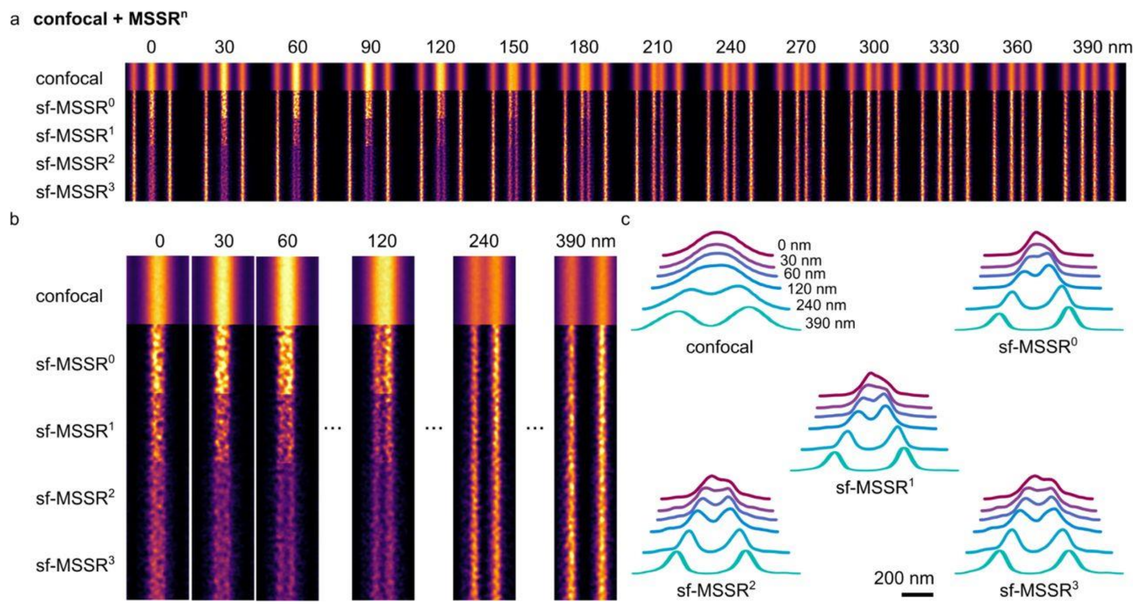

Fig 3. Example of FF-SRM: Mean Shift Super-Resolution (MSSR) [Torres et al., 2021]
a) Super-resolved confocal images of ArgoLight test slide using MSSR on a single frame (**sf-MSSR^n**). **b)** sf-MSSR^0 can resolve the line pair separated by 120 nm, halving the resolution limit for confocal. **c)** Intensity peak separation for different line pairs of the test slide.

3. Superres plugin

- Aim

Existing implementations of FF-SRM are spread over a variety of software GUI (open-source and not) or scripts, and may lack documentation.
This project aims to unify the FF-SRM within the "napari-superres" plugin, including at least six FF-SRM approaches: SOFI, 3B, ESI, MUSICAL, SRRF, and MSSR.
Those **methods** have been **extensively used for live cell imaging at the nanoscales** (reviewed in [Alva et al., 2022]).

- Currently **implemented algorithms** in napari-superres: **ESI**, **SRRF**, **MSSR**.

- In particular, **MSSR has been used to improve our knowledge on structure and function of many biological components** such as viral proteins, microtubule organizing center, etc. (**Fig. 4**).

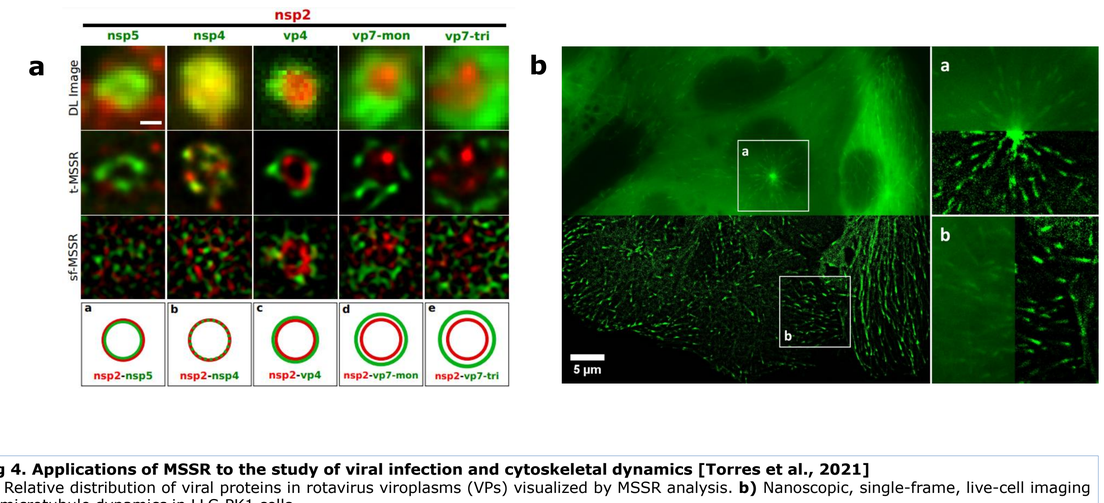

Fig 4. Applications of MSSR to the study of viral infection and cytoskeletal dynamics [Torres et al., 2021]
a) Relative distribution of viral proteins in rotavirus viroplasms (VPs) visualized by MSSR analysis. **b)** Nanoscopic, single-frame, live-cell imaging of microtubule dynamics in LLC-PK1 cells.

4. Conclusions

- We developed a **plugin for super-resolution microscopy that uses diffraction-limited images** and doesn't require any specialized and expensive hardware.

- This allows the execution of some of **most common algorithms for FF-SRM**.

- As an open-source implementation, **napari-superres will remove some economical inequalities by granting global access to nanoscopic imaging**, especially across countries lacking specialized hardware for SRM imaging.

5. Future developments

- **Implement** new FF-SRM methods such as **pySOFI**, **3B**, and **MUSICAL**.

- **Promote computational Fluorescence nanoscopy** among target countries **where technology** for hardware-based super-resolution **is still not available**.

- **Develop a FF-SRM challenge** and use napari-superres as benchmarking tool.

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