napari-FLIM-phasor-plotter – a collaborative project

a plugin to generate interactive phasor plots from raw FLIM data

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With materials from Marcelo Zoccoler

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Student at CMCB TU Dresden,
soon PhD student (IMP Vienna)

Scientists‘ needs and wishes for FLIM software

How can I chose a colorblind-friendly lookup table?

How can I access and export data at all steps of analysis?

How to apply downstream workflows?

Do I need licensed software to analyse FLIM data?

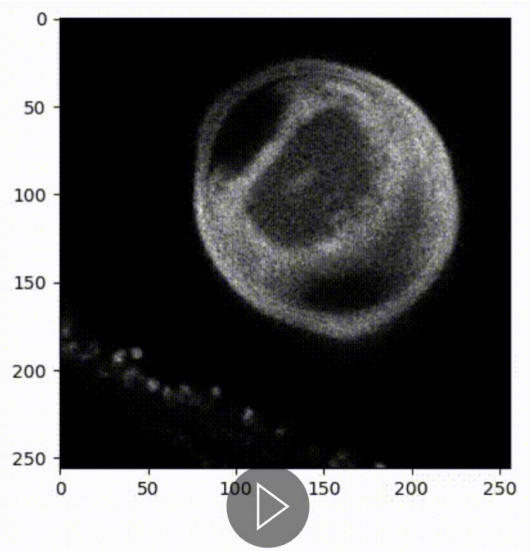
Are there open source software solutions?

Why is it so time-consuming?

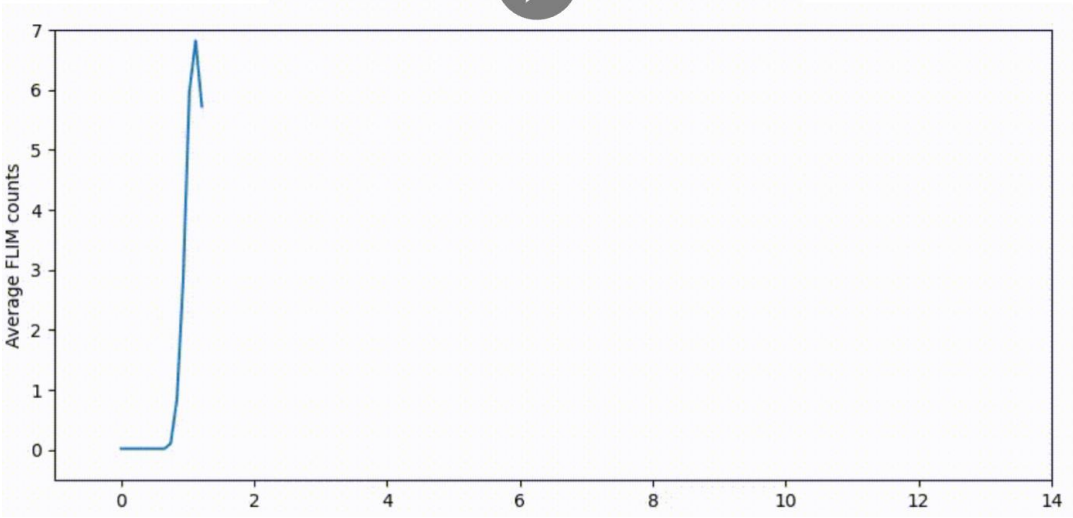
napari-flim-phasor-plotter

https://github.com/zoccoler/napari-flim-phasor-plotter

Contributors 3



Napari-flim-phasor-plotter is a napari plugin to interactively load and show raw fluorescence lifetime imaging microscopy (FLIM) single images and series and generate phasor plots. These are Fourier transforms of the decay data being visualized using the napari-clusters-plotter plotter, adapted to suit the FLIM context. This allows qualitative and quantitative downstream analysis of FLIM images.



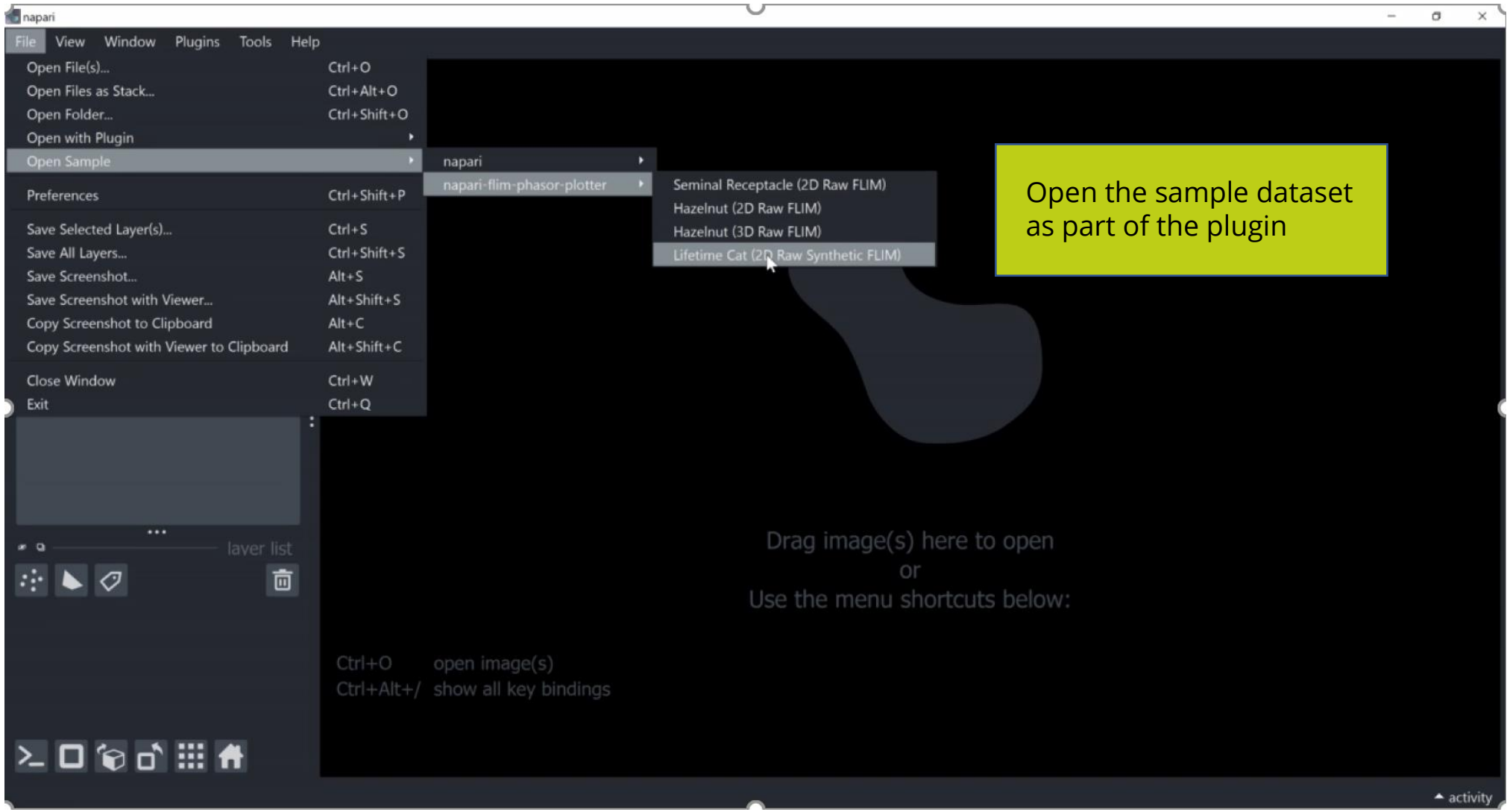
Napari: a fast, interactive viewer for multi-dimensional images in python

Extendable by various plugins (widgets and user interfaces, readers and writers)

Source: napari.org, https://www.napari-hub.org/plugins/napari-assistant, https://biapol.github.io/

Usage of synthetic data – the lifetime cat example image

Svetlana Iarovenko



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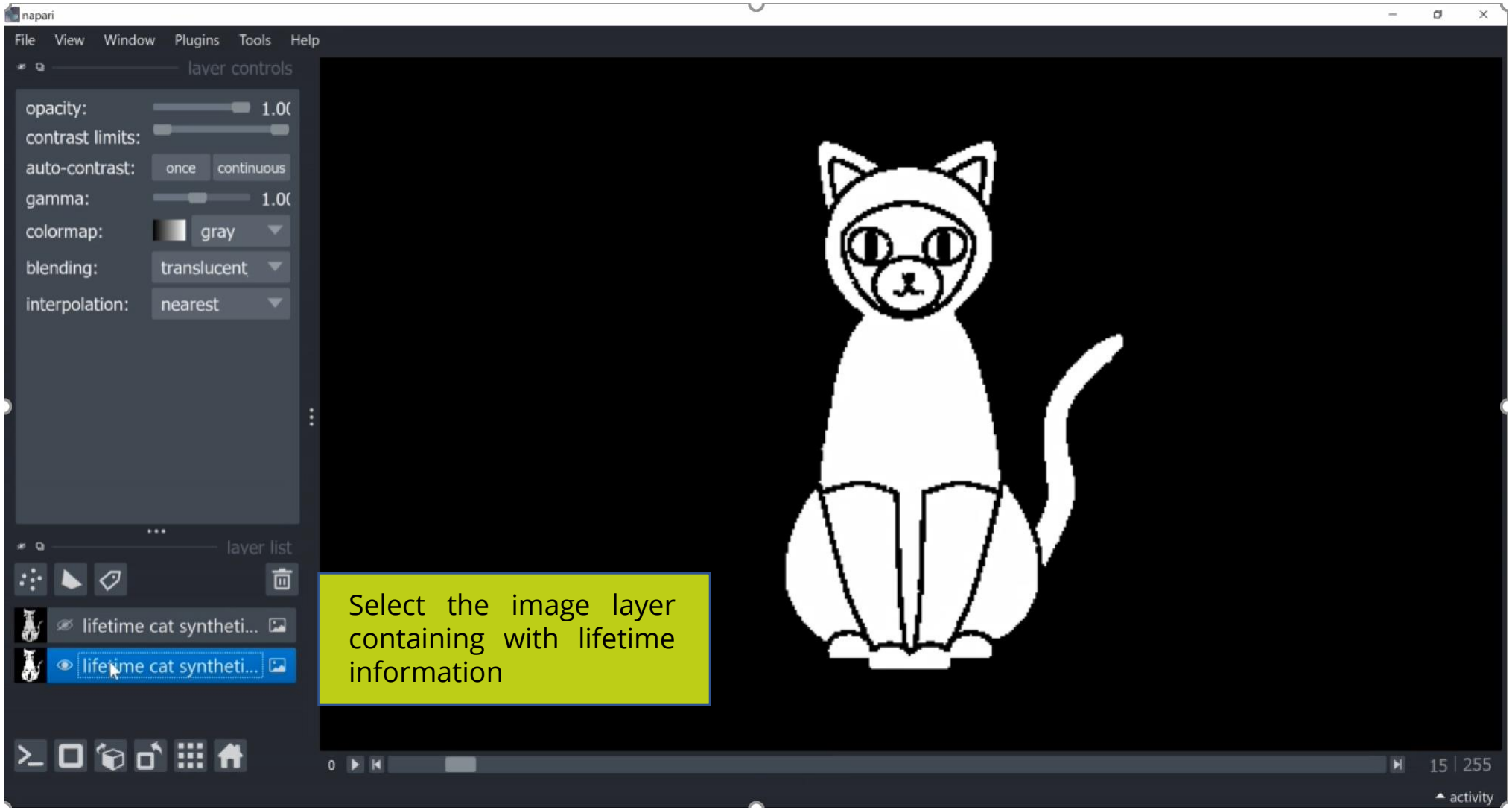

Usage of synthetic data – the lifetime cat example image

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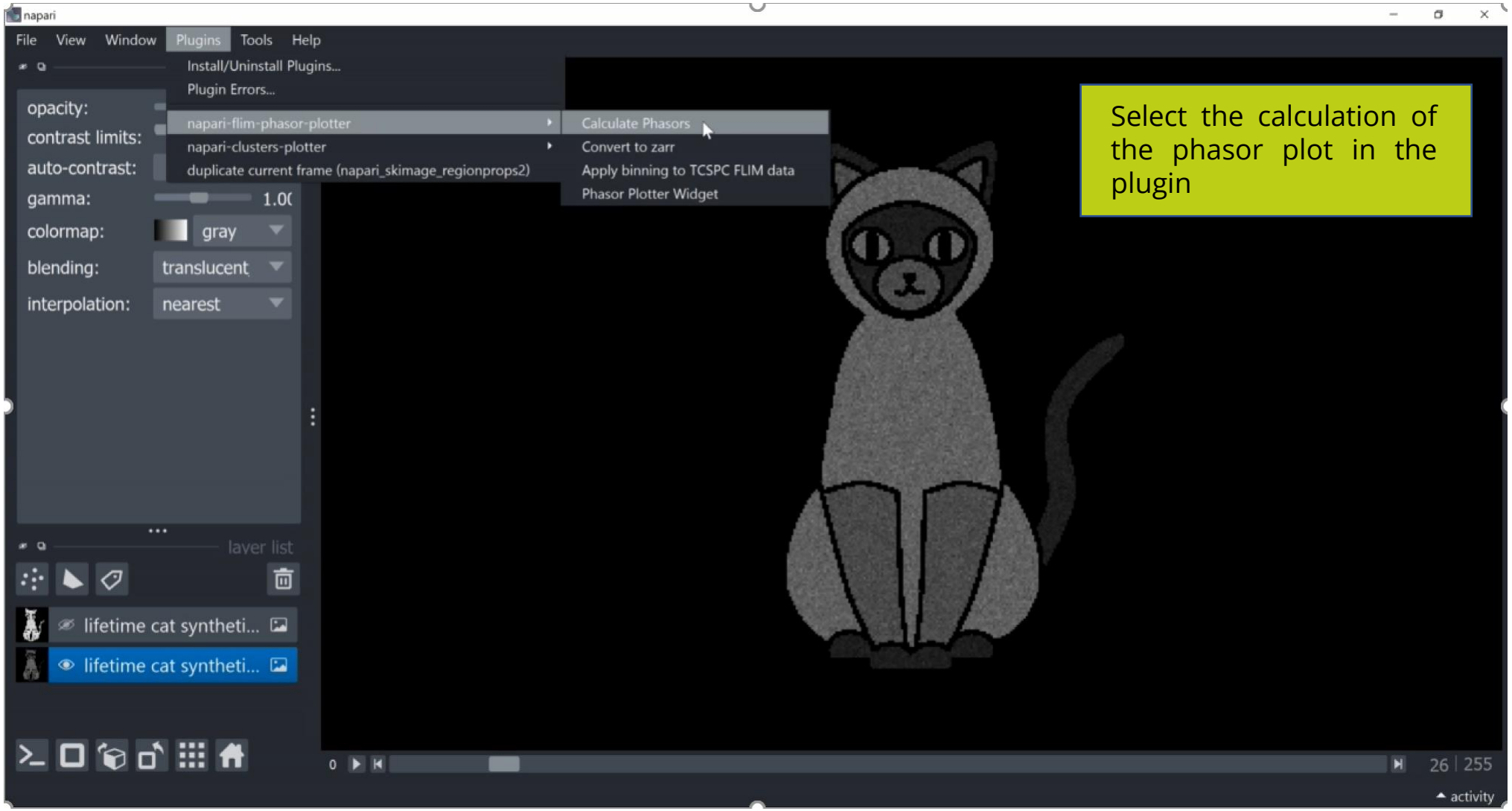
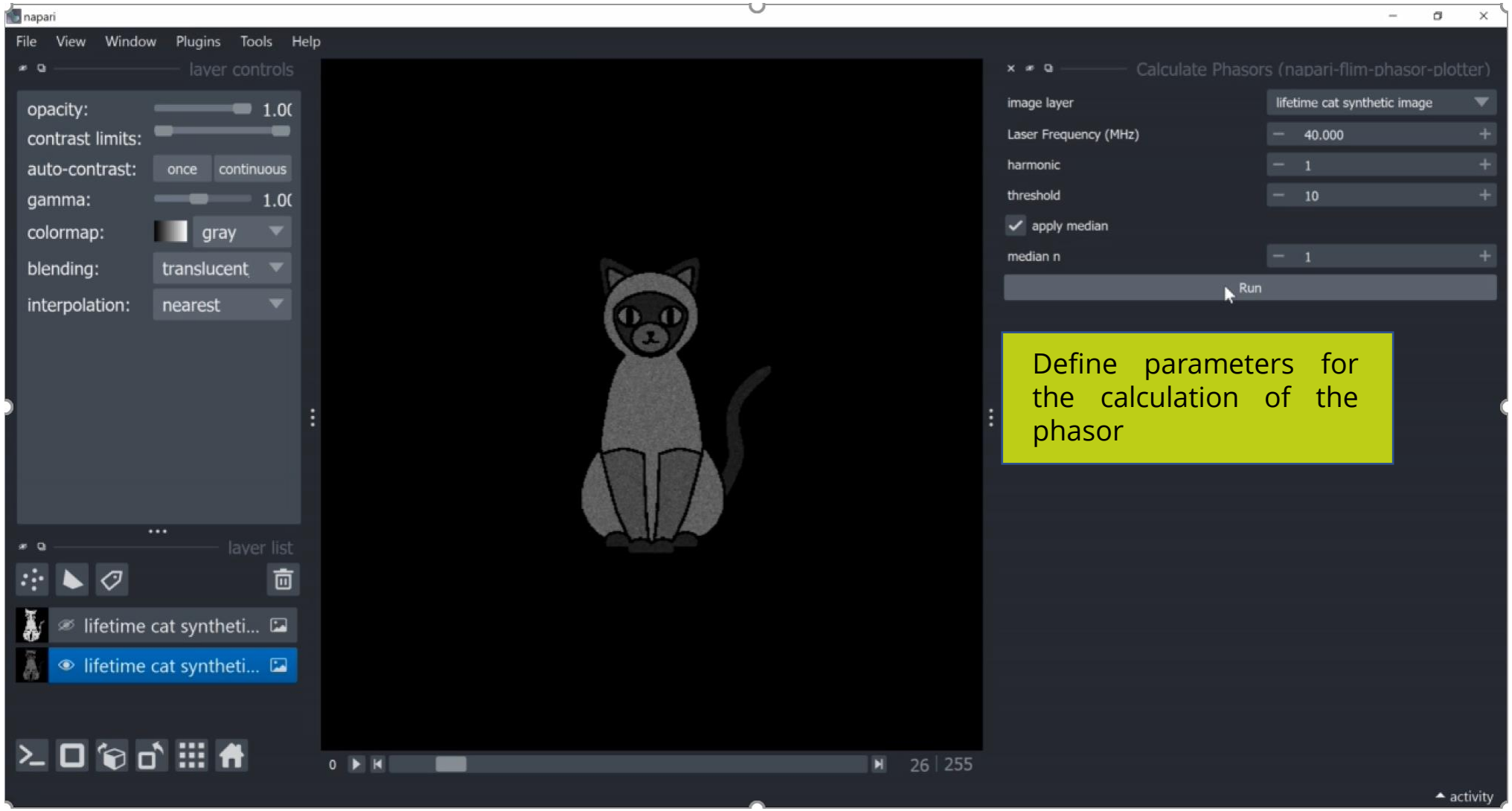


Usage of synthetic data – the lifetime cat example image



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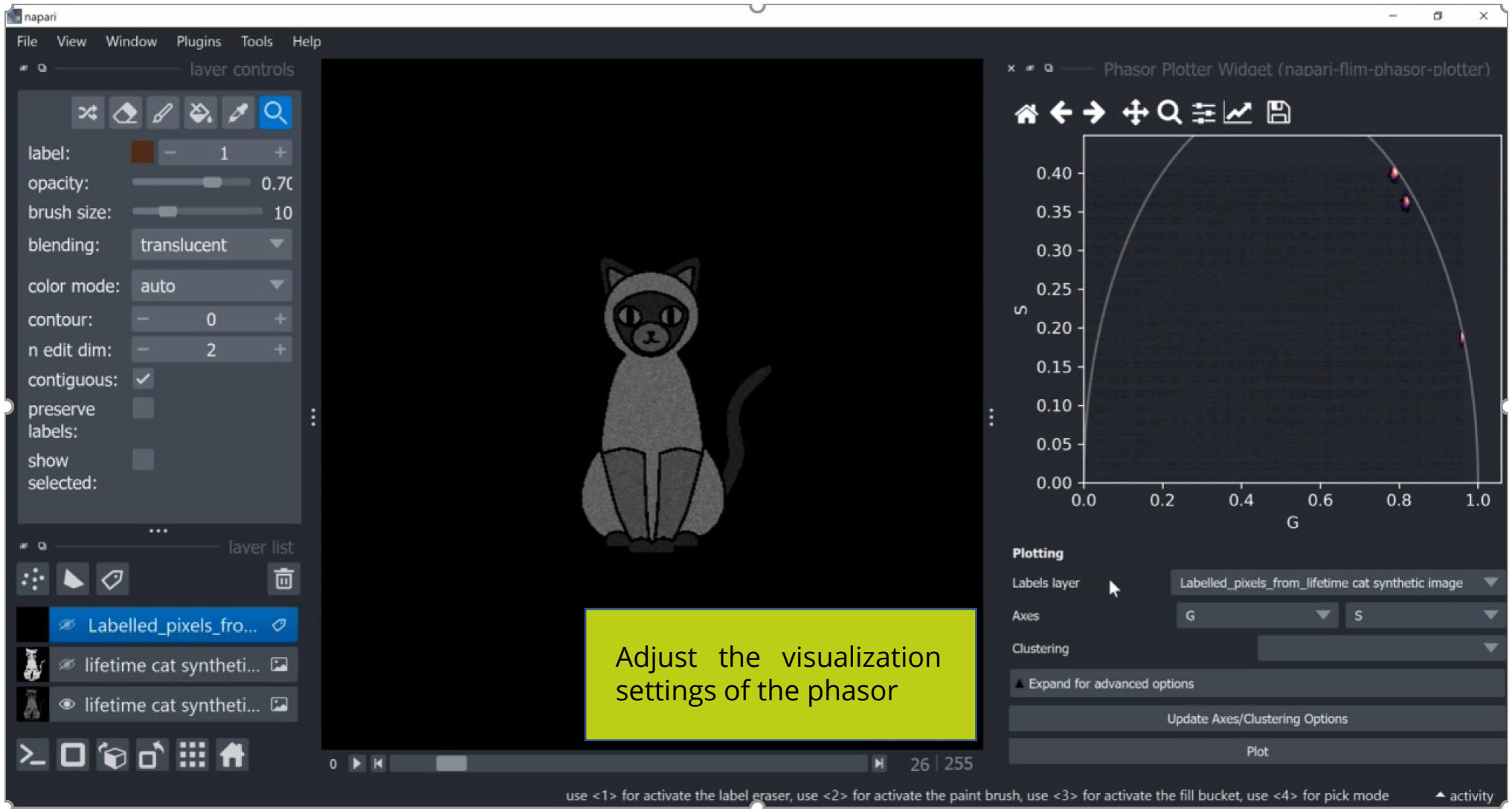

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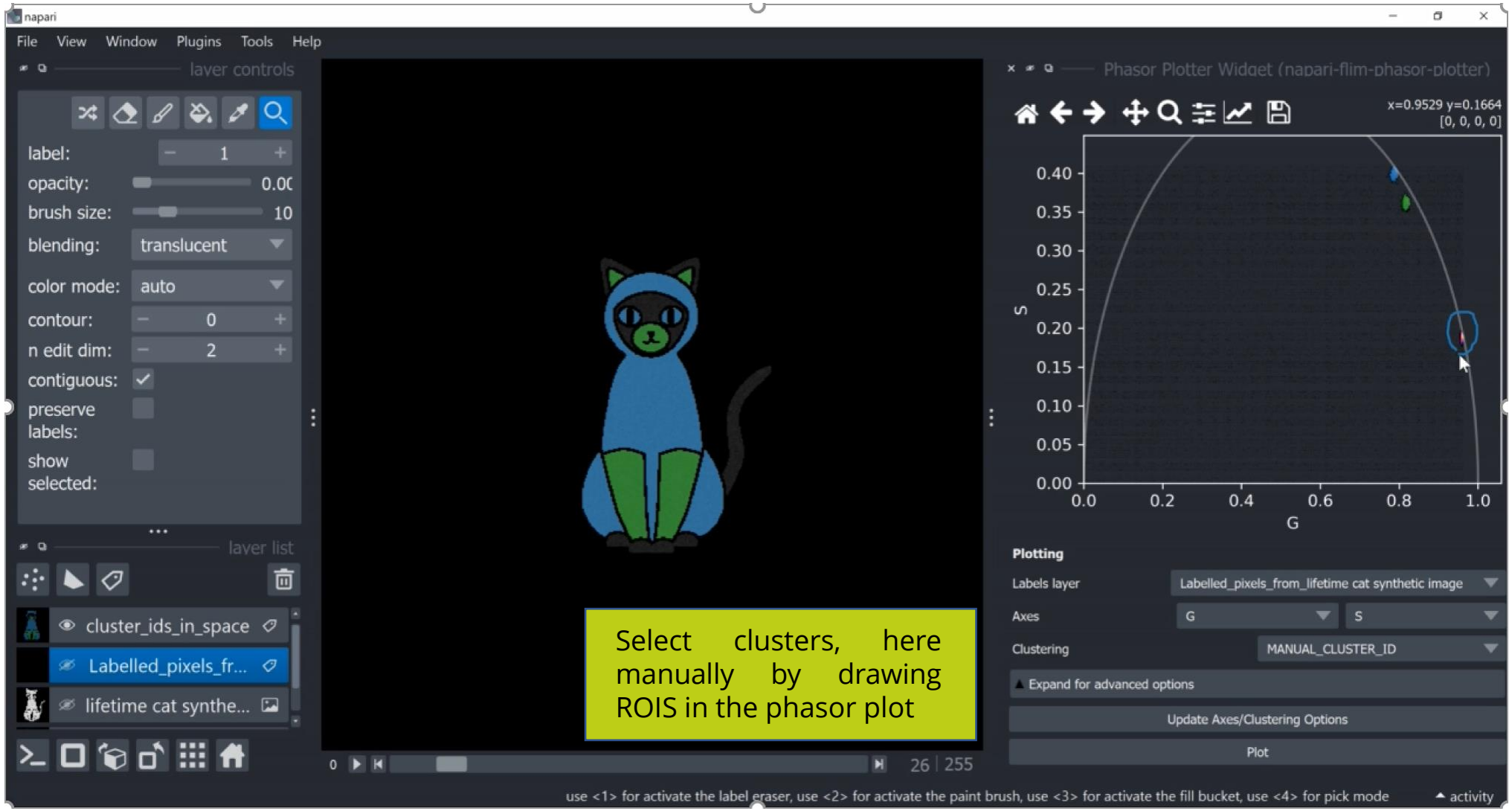

Svetlana Iarovenko

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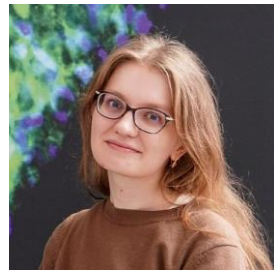

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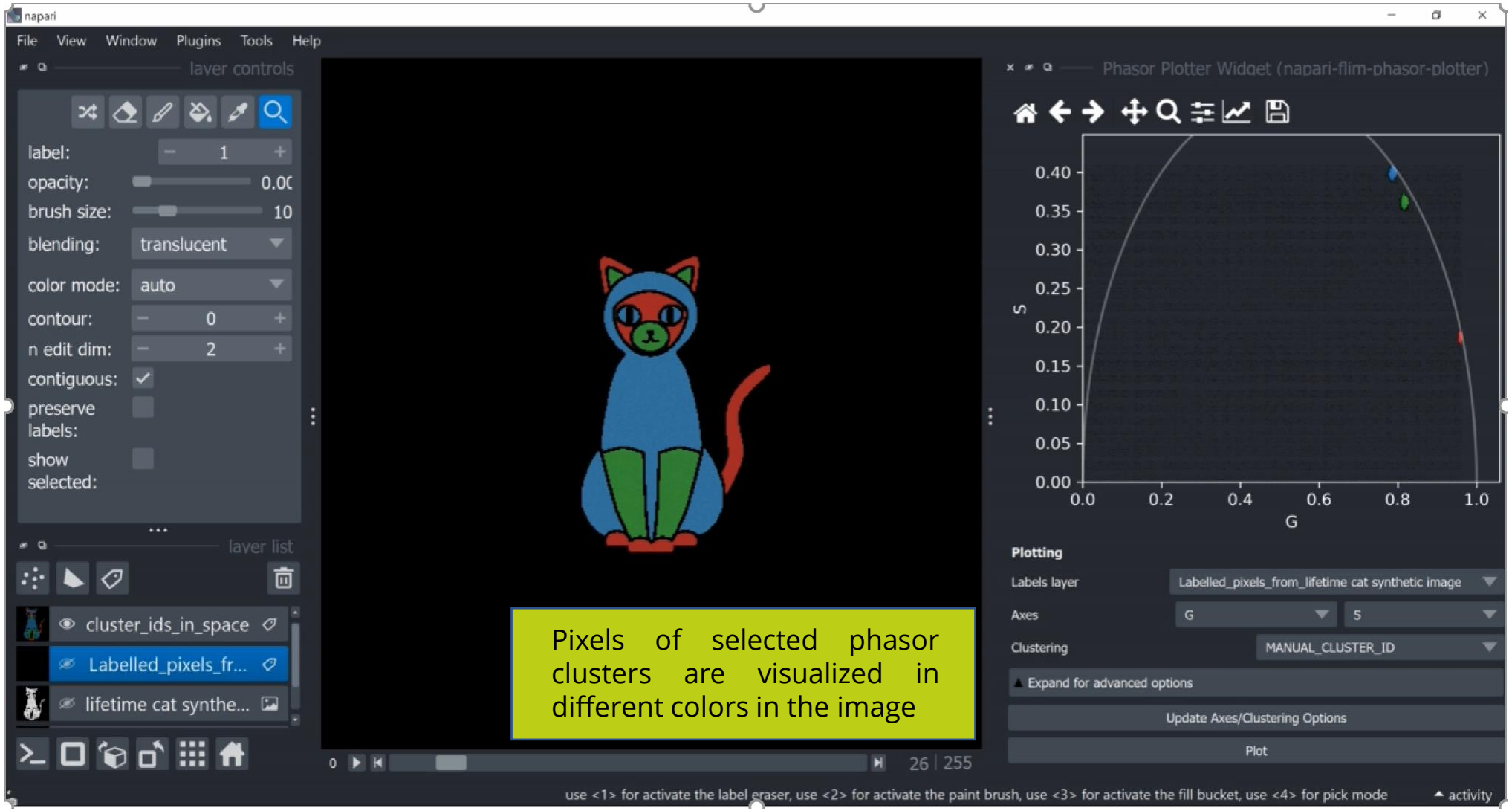

Integrating FLIM analysis into a bio-image analysis workflow

Example workflow:

- Open a raw FLIM image/ dataset

Metabolic FLIM of sperm in a female sperm-storage organ in *Drosophila*

Integrating FLIM analysis into a bio-image analysis workflow

Example workflow:

- Open a raw FLIM image/ dataset
- Generate a Phasor Plot

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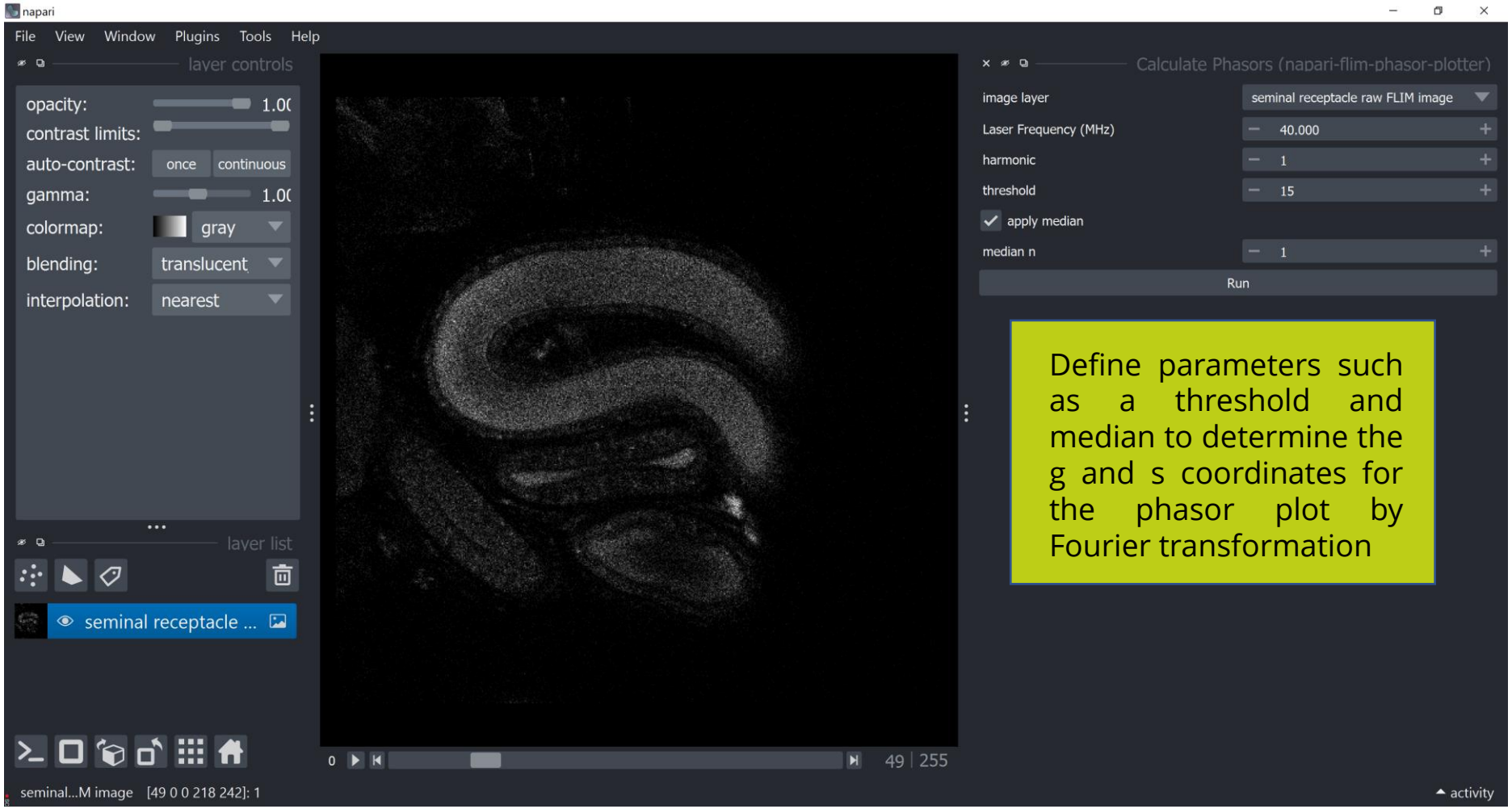

Define parameters such as a threshold and median to determine the g and s coordinates for the phasor plot by Fourier transformation

Integrating FLIM analysis into a bio-image analysis workflow

Example workflow:

- Open a raw FLIM image/ dataset
- Generate a Phasor Plot
- Change Phasor Plot display options

Metabolic FLIM of sperm in a female sperm-storage organ in *Drosophila*

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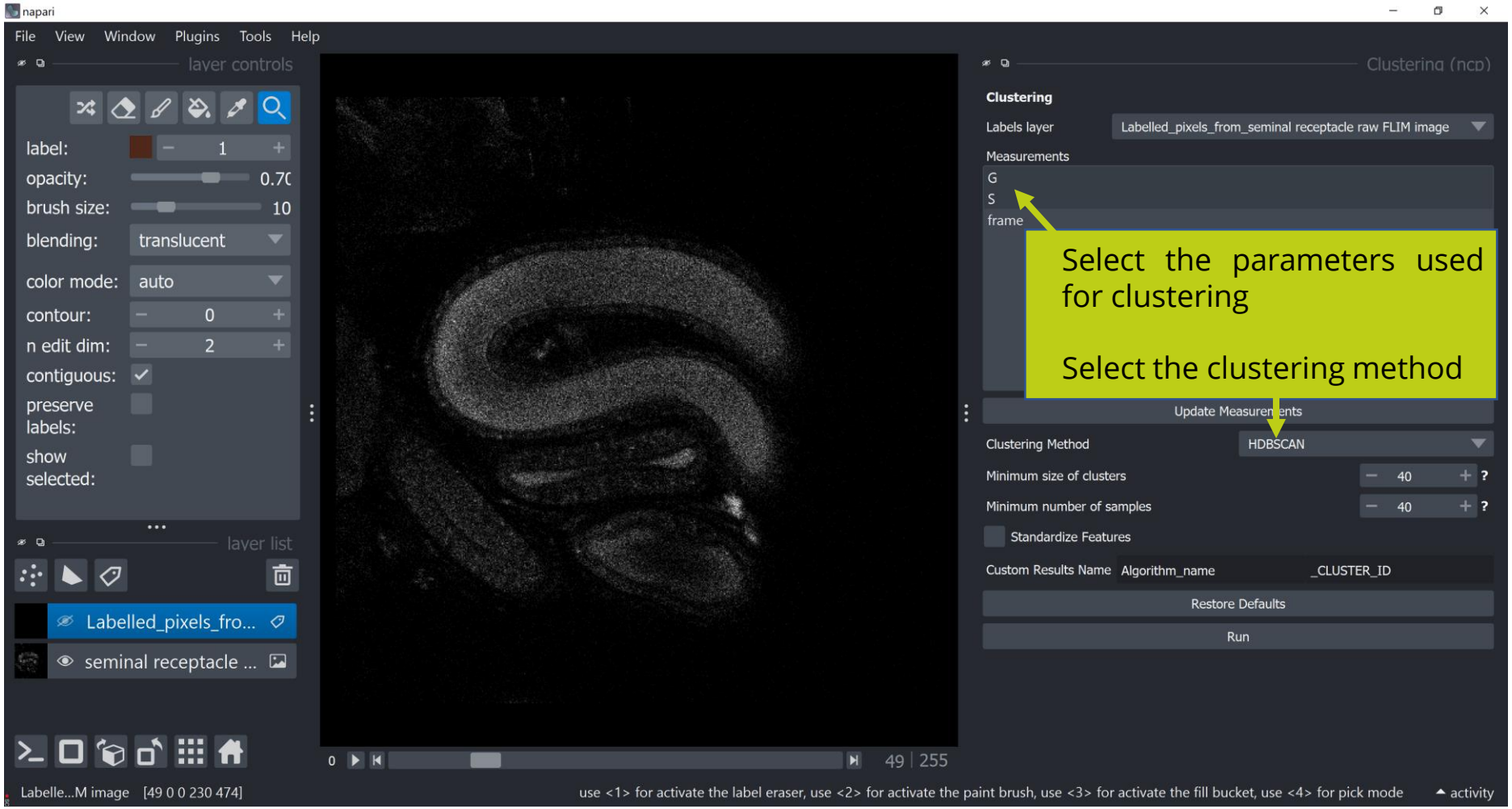
Integrating FLIM analysis into a bio-image analysis workflow

Example workflow:

- Open a raw FLIM image/ dataset
- Generate a Phasor Plot
- Change Phasor Plot display options
- Identify and select reproducibly clusters in your dataset

Metabolic FLIM of sperm in a female sperm-storage organ in *Drosophila*



Integrating FLIM analysis into a bio-image analysis workflow

Example workflow:

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- Change Phasor Plot display options
- Apply a clustering algorithm to the Phasor Plot (HDBSCAN) using the napari-clusters-plotter

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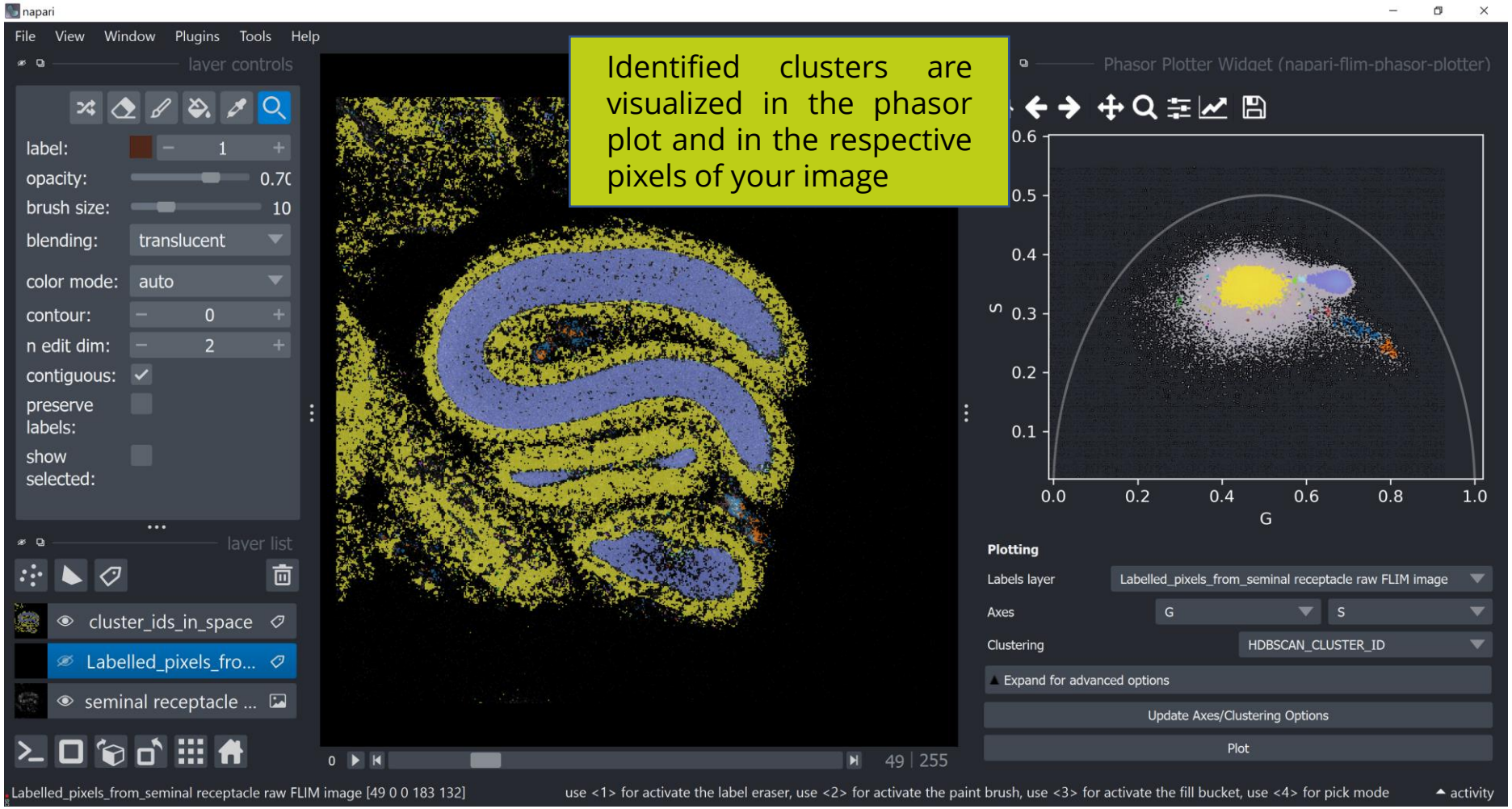

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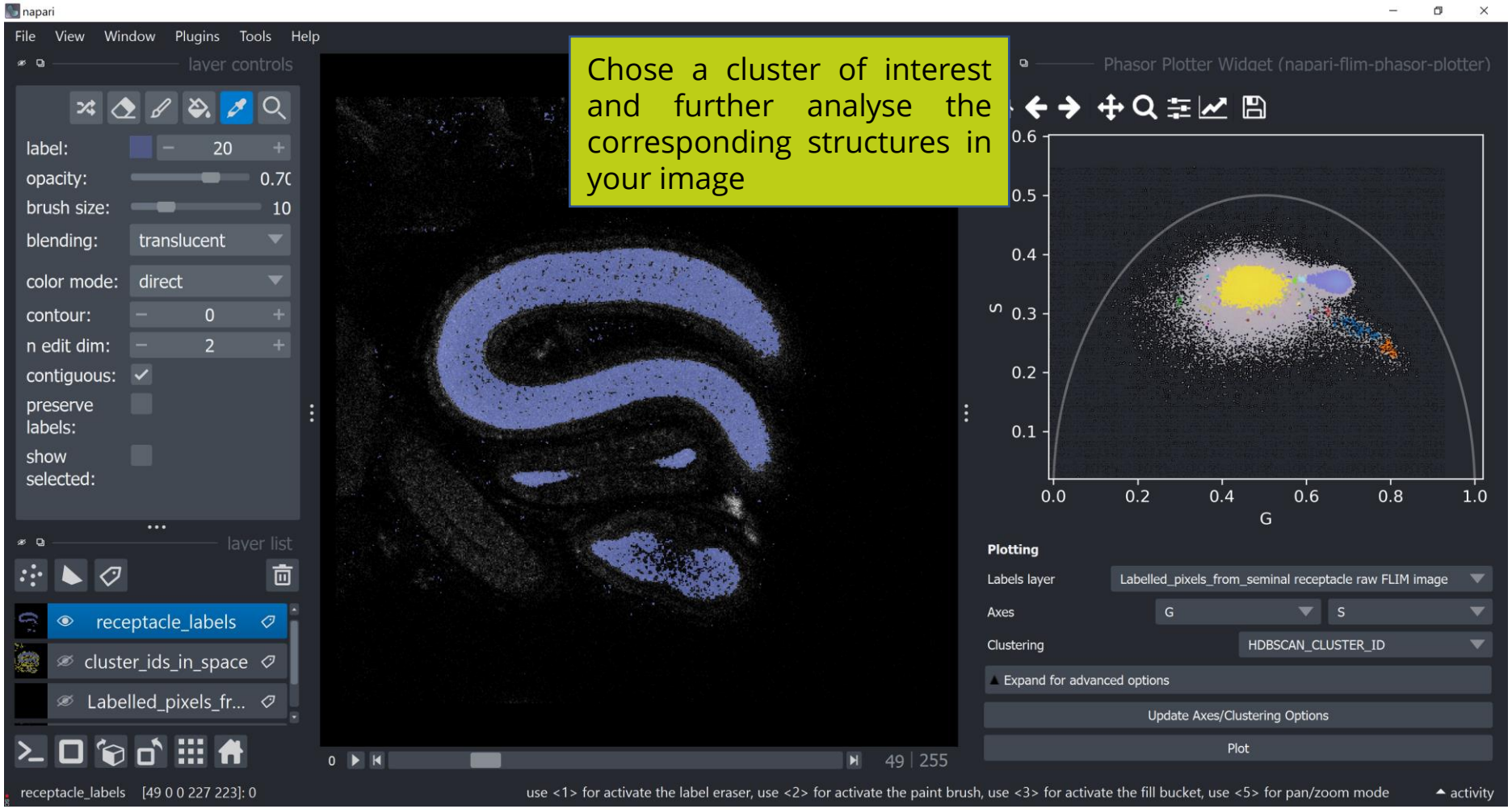

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- Post-processing: apply morphological operations

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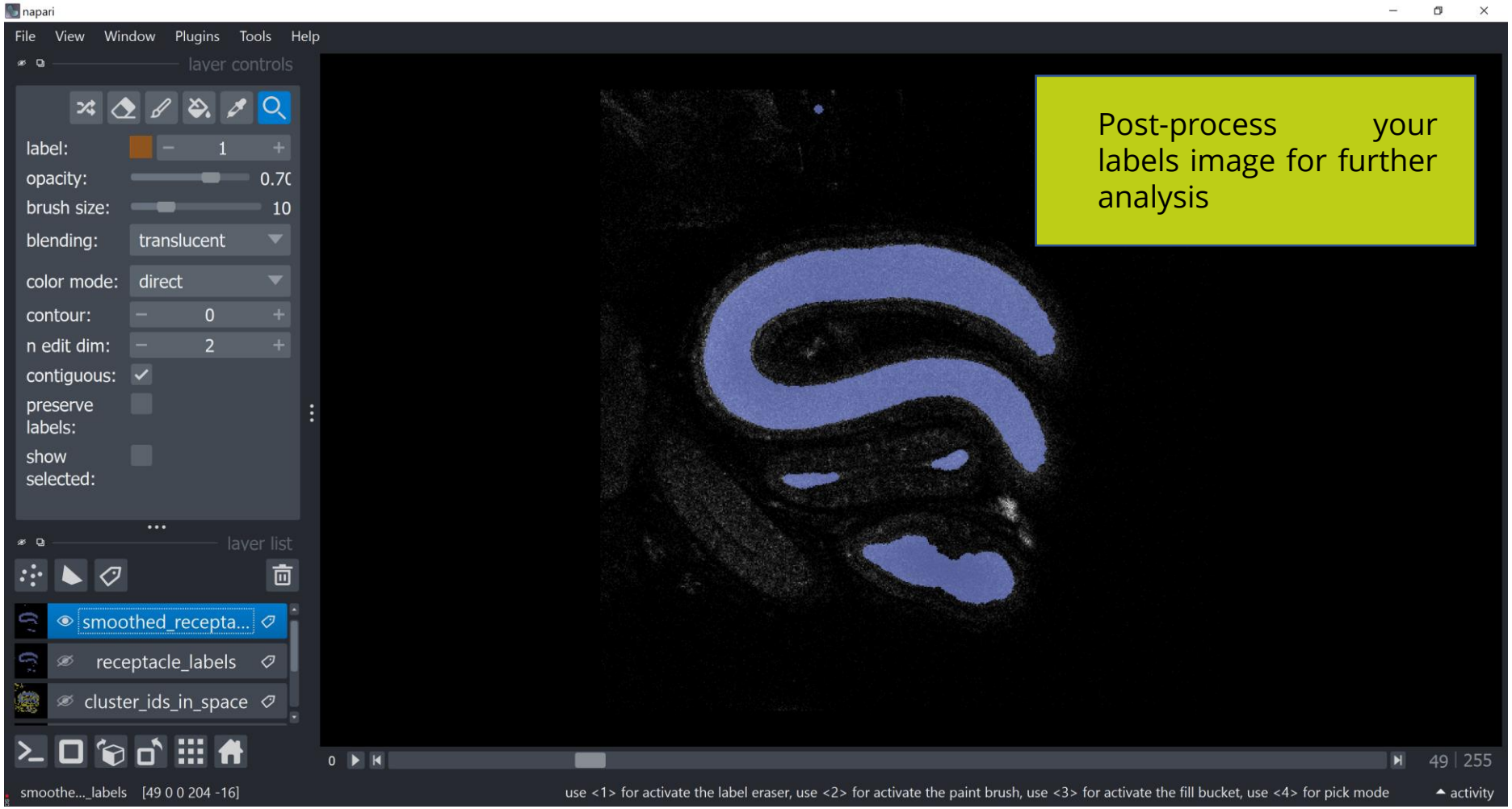

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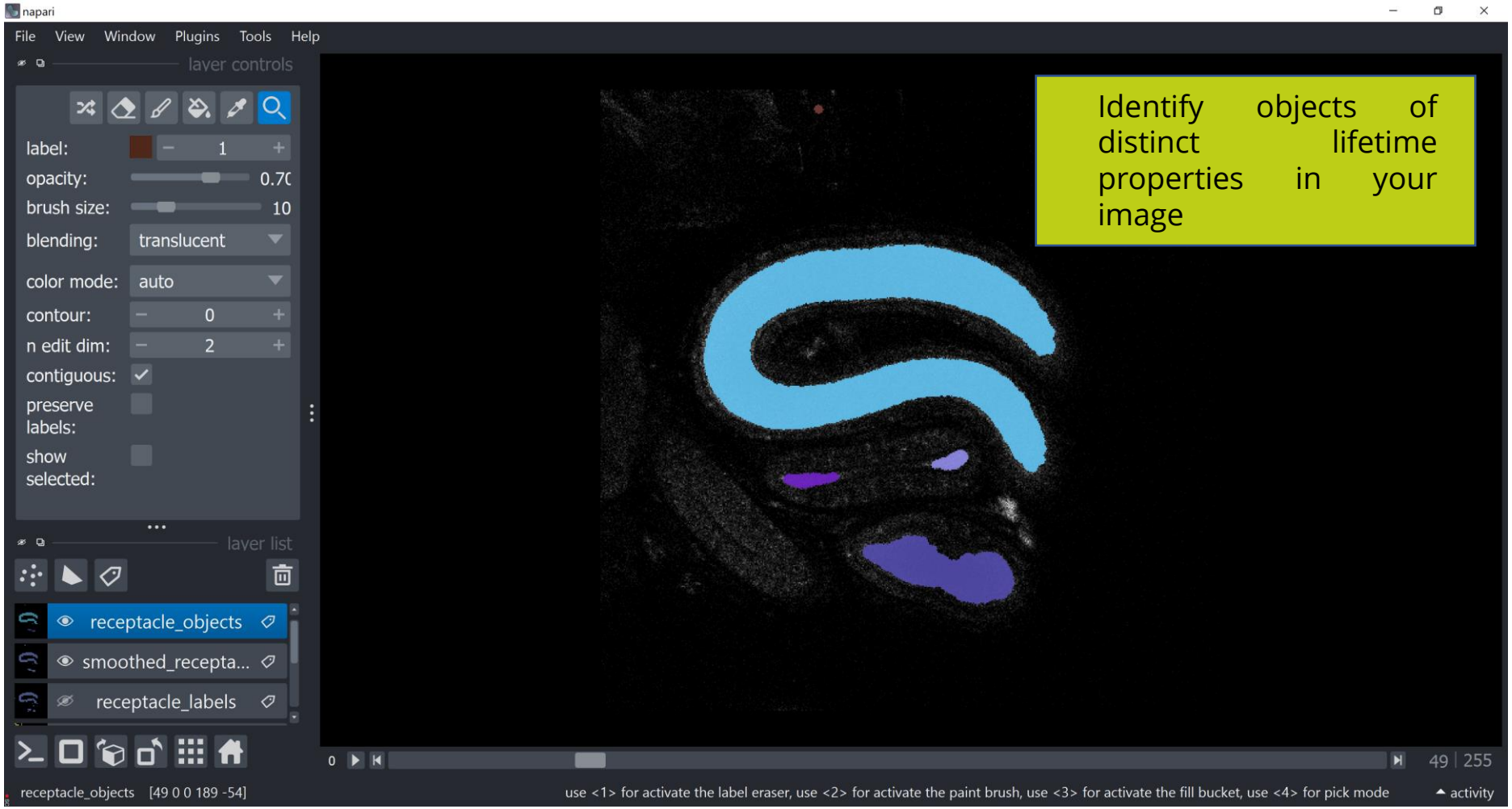

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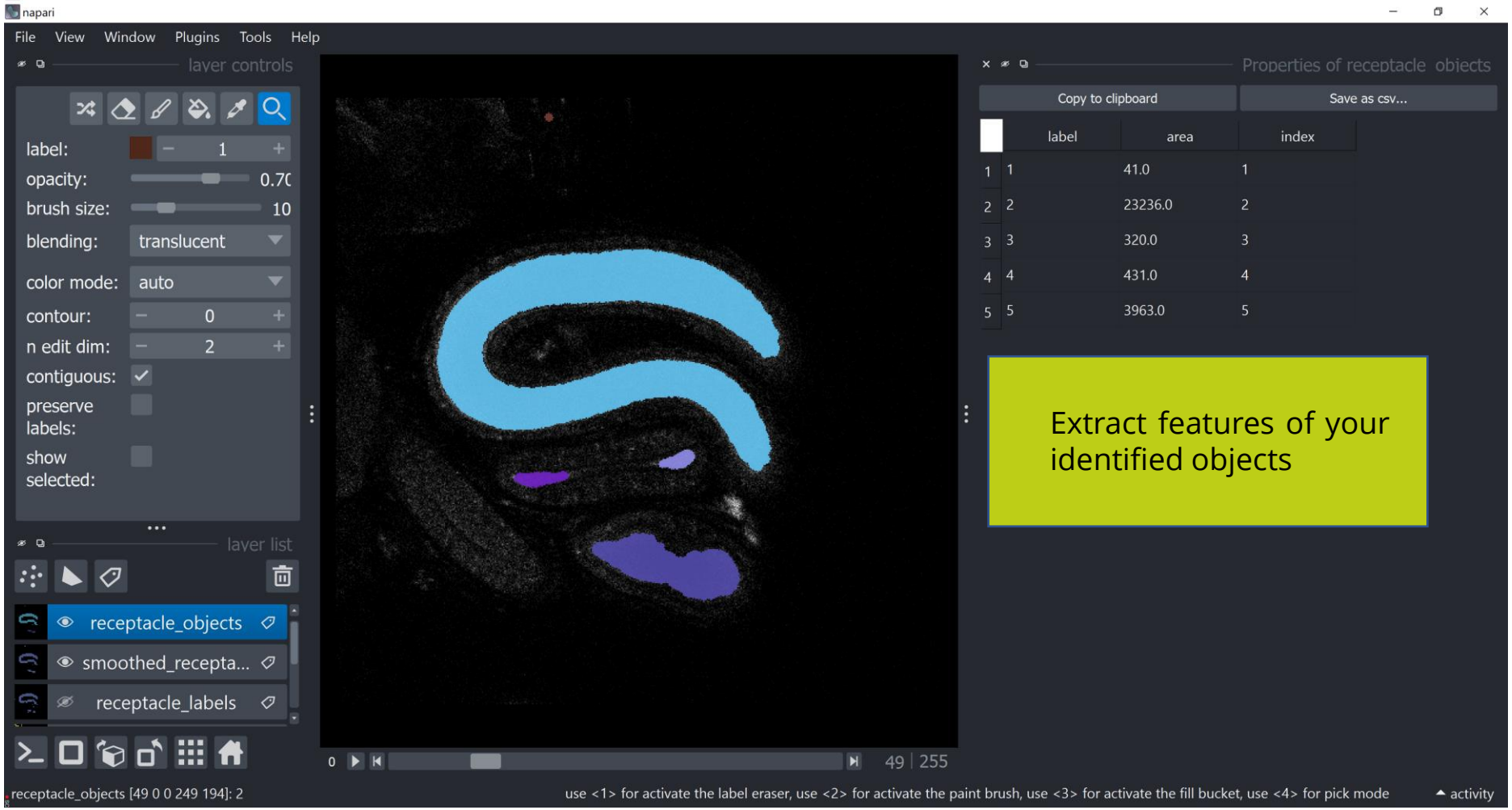


Integrating FLIM analysis into a bio-image analysis workflow

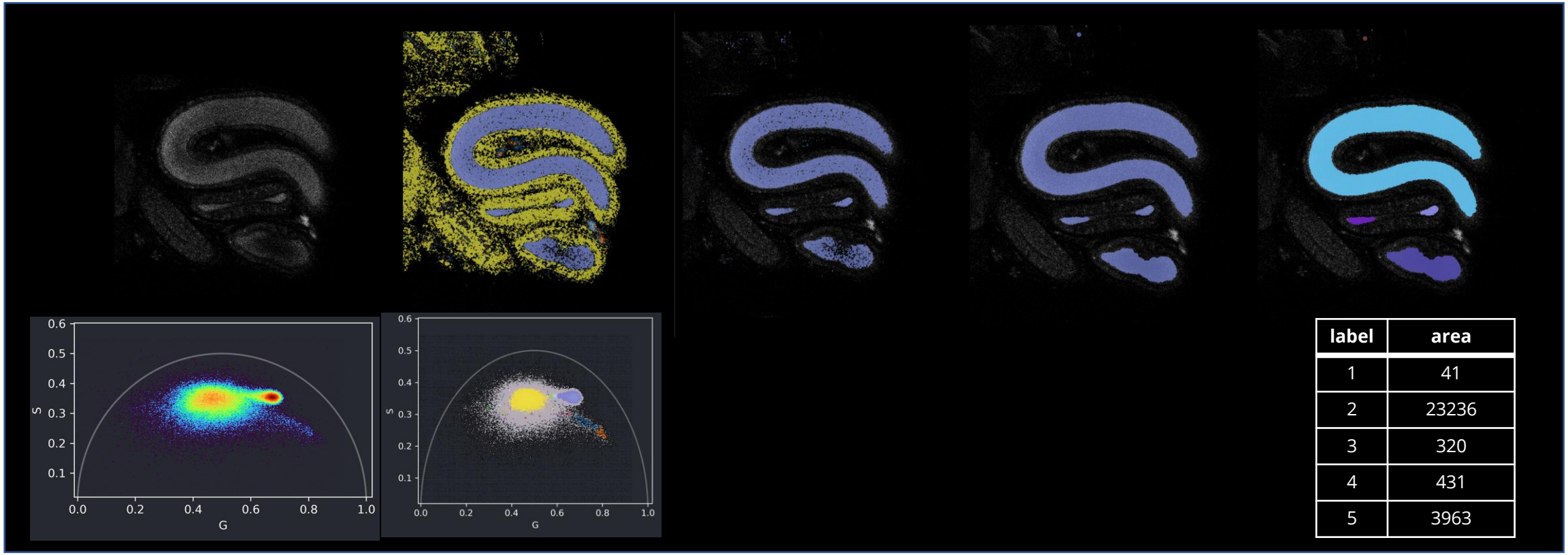
Raw data import & phasor analysis

Cluster analysis

Selection of individual cluster

Processing of selected cluster

Instance segmentation & quantification



label	area
1	41
2	23236
3	320
4	431
5	3963

Input data

- The plugin can import FLIM data of formats
  - .sdt
  - .ptu
  - .tif
  - .zarr

- Of the data shapes:
  - 2D, 3D up to 3D multichannel timelapse FLIM data

- Multidimensional .ptu data folders named _t001_z001 etc.

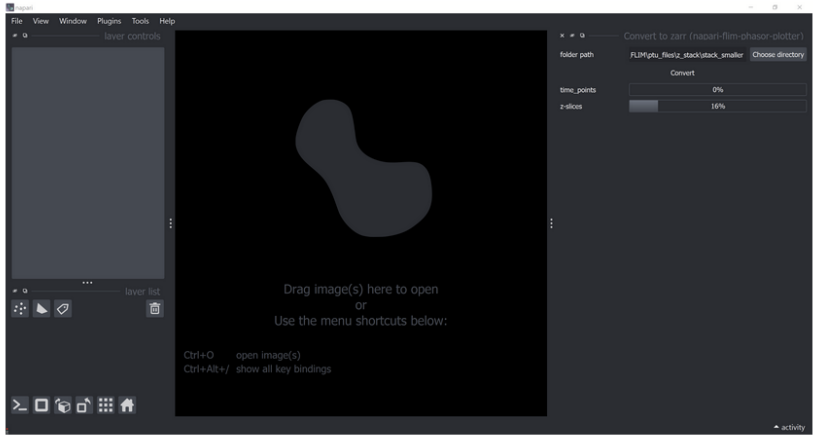
Data conversion to .zarr

- The plugin can convert raw FLIM files of a folder to .zarr

Data Conversion

If a collection of raw (uncompressed) images are larger than 4GB, we recommend converting them to `.zarr`. This can be done via `Plugins > napari-flim-phasor-plotter > Convert to zarr`.

Warning: In the current version, lazy loading with `.zarr` is available, but processing may still load all data into memory, so keep track of your memory usage.



If you have multiple slices or time-points as separated files, you can choose a folder containing the files. In order for the plugin to properly build a stack, the file names must contain some indication about which slice or time-point they represent, i.e., **each file name should contain a `_t` and/or `_z` followerd by a number**.

Here are a few example templates:

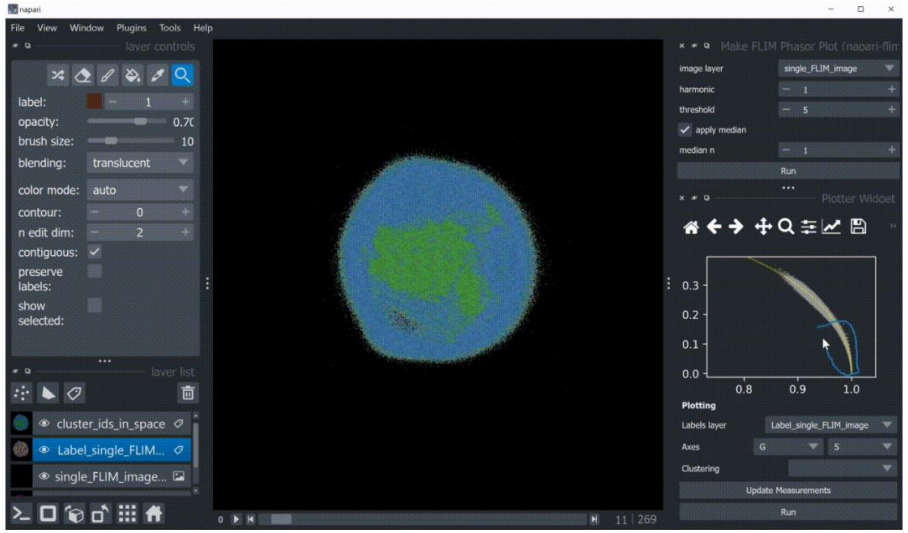
- timelapse:
  - `image_t001.ptu`
  - `image_t002.ptu`
- z-stack:
  - `image_z01.sdt`
  - `image_z02.sdt`
- 3D timelapse:
  - `image_t001_z001.tif`
  - `image_t001_z002.tif`
  - ...
  - `image_t002_z001.tif`

Napari-flim-phasor-plotter – code available on GitHub Including documentation

Usage

Open a FLIM image to visualize it both as a 'FLIM image series' being a sequence of intensity images each corresponding to an individual time point of the FLIM 'micro-time', plus as a timely summed up image. Scrolling through the FLIM time series provides a first glimpse of lifetimes across image regions.

Call the plugin from the menu `Plugins > FLIM phasor plotter > Make FLIM Phasor Plot` to generate a phasor plot by pixel-wise Fourier transformation of the decay data. Hereby, select the FLIM image to be used, specify the laser pulse frequency if not read properly from metadata. Define an intensity threshold to exclude pixels of low photon counts, optionally a median filter, and a harmonic for optimal visualization. `Run` creates the phasor plot and an additional labels layer in the layer list. Below is a demonstration:



Change the color-code of the phasor plot to a density plot of various 'Colormaps' from the pulldown `Expand for advanced options` and select `HISTOGRAM`. Manually encircle a region of interest in the phasor plot to highlight the corresponding pixels in the newly created image layer. Hold 'Shift' to select and visualize several clusters to investigate image regions of similar FLIM patterns.

Installation

You can install `napari-flim-phasor-plotter` via pip. Follow these steps from a terminal.

We recommend using `mamba-forge` whenever possible. Click here to choose the right download option for your OS.
If you use `mamba-forge`, replace the `conda` term whenever you see it below with `mamba`.

Create a conda environment:

```
conda create -n napari-flim-phasor-env python=3.9
```

Activate the environment:

```
conda activate napari-flim-phasor-env
```

Then install `napari` and `napari-clusturs-plotter` (plus git if on Windows):

```
conda install -c conda-forge napari==0.4.17 napari-clusters-plotter git pyqt
```

*Optional: we **strongly** recommend having the `devbio-napari` plugin bundle also installed for post-processing. This can be done with:*

```
conda install -c conda-forge devbio-napari
```

Finally install `napari-flim-phasor-plotter` plugin with:

```
pip install napari-flim-phasor-plotter
```

Alternatively, clone this repository and install the latest plugin development version with:

```
pip install git+https://github.com/zoccoler/napari-flim-phasor-plotter.git
```

In the next few days: install the development version (issue on sdt import)

Take-home message of napari-flim-phasor-plotter

- It is a plugin for napari (a python-based image visualization tool that allows usage of many powerful image processing and analysis plugins)
- open-source and allows contributions from community
- Allows import of FLIM data of .sdt, . ptu, .tif and .zarr format and up to 5D FLIM data (xyzct)
- Allows conversion of FLIM data to .zarr format
- performs phasor analysis of FLIM raw data
- Can implement cluster analysis of phasor plots using napari-clusters-plotter
- Allows further downstream bio-image analysis of available napari plugins
- Provides example datasets to test
- Contributions warmly welcome!

Thanks to

Marcelo Leomil Zoccoler
(maintainer of the napari-flim-phasor-plotter, Bio-Image Analysis Group, PoL TU Dresden)

Svetlana Iarovenko
(soon @ IMP Vienna)

Robert Haase
(ScaDS.AI Leipzig)

Bio-Image Analysis Group, PoL
TU Dresden

Light Microscopy Facility,
CMCB, TU Dresden

Test datasets:

.sdt format - https://zenodo.org/record/7542467
(https://doi.org/10.1038/s41598-019-56067-w)
.ptu format - https://zenodo.org/record/7656540
(DOI: 10.5281/zenodo.7656540)