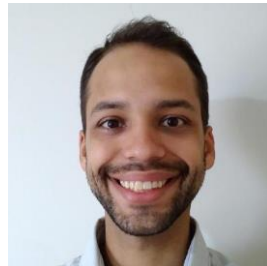


# napari-FLIM-phasor-plotter – a collaborative project

a plugin to generate interactive phasor plots from raw FLIM data

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## Cornelia Wetzker

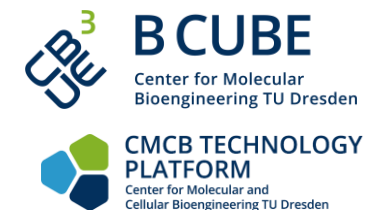
Data steward (B CUBE/ CMCB, TU Dresden and  
NFDI4BIOIMAGE)  
previously Light Microscopy Facility staff member  
(CMCB, TU Dresden)



## Svetlana Iarovenko

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

With materials from Marcelo  
Zoccoler



# Scientists' needs and wishes for FLIM software

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

How can I chose a colorblind-friendly lookup table?

Do I need licensed software to analyse FLIM data?

How to apply downstream workflows?

Are there open source software solutions?

Why is it so time-consuming?

# napari-flim-phasor-plotter



license **BSD-3-Clause** pypi **v0.0.6** python **3.8 | 3.9 | 3.10** tests **passing** codecov **62%**

\* napari hub **napari-flim-phasor-plotter**

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

## Contributors 3



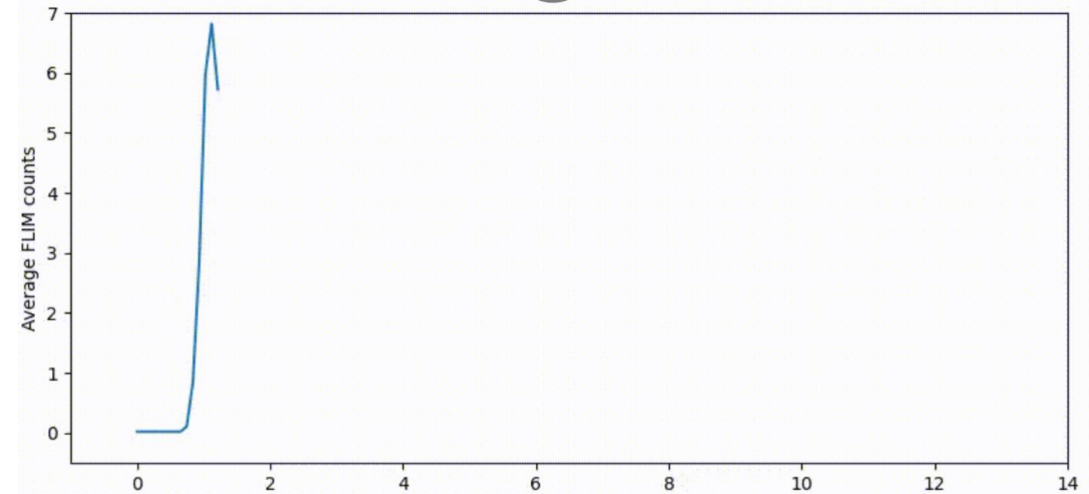
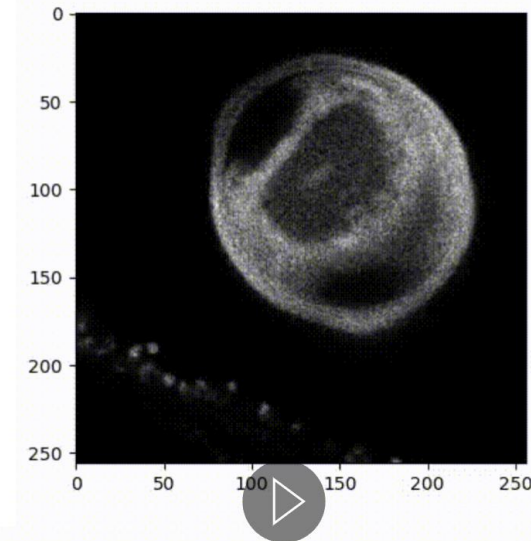
**zoccoler** Marcelo Zoccoler



**cwetzker** Conni Wetzker



**sviaro** Svetlana Iarovenko



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

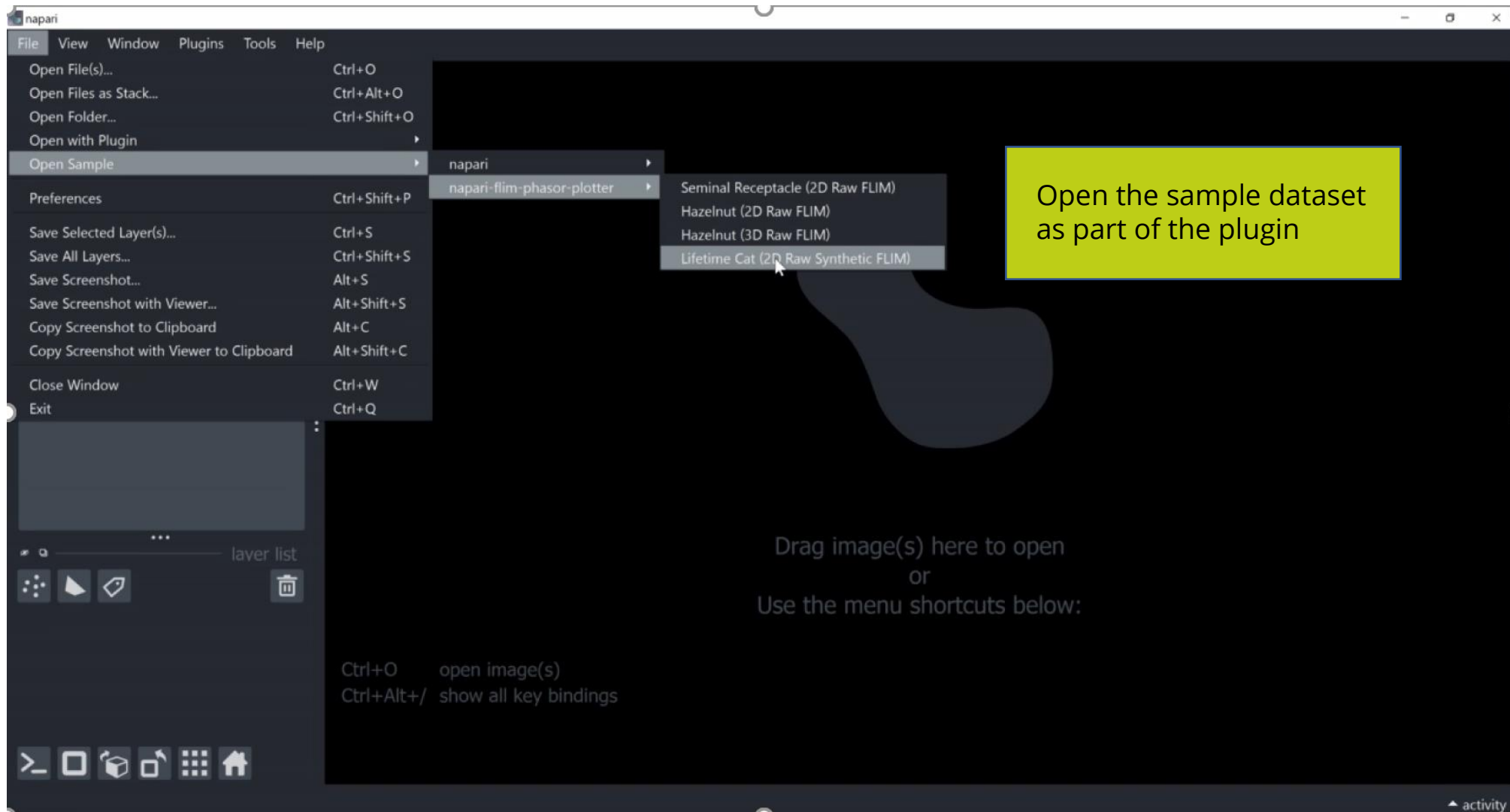


Extendable by various plugins (widgets and user interfaces, readers and writers)

# Usage of synthetic data – the lifetime cat example image



Svetlana Iarovenko



# Usage of synthetic data – the lifetime cat example image



Svetlana Iarovenko

The screenshot shows the napari software interface. The main window displays a white silhouette of a cat sitting on a black background. On the left, the 'layer controls' panel is visible, showing settings for opacity (1.00), contrast limits, auto-contrast (set to 'once'), gamma (1.00), colormap (set to 'gray'), blending (set to 'translucent'), and interpolation (set to 'nearest'). Below this is the 'layer list' panel, which contains two entries for 'lifetime cat syntheti...'. The bottom entry is selected and highlighted with a blue border. A yellow callout box with the text 'Select the image layer containing with lifetime information' points to this selected layer. The bottom status bar shows '0 | 15 | 255' and 'activity'.

# Usage of synthetic data – the lifetime cat example image



Svetlana Iarovenko

The screenshot shows the napari software interface. The 'Plugins' menu is open, and the 'napari-flim-phasor-plotter' plugin is selected. The 'Calculate Phasors' option is highlighted. A yellow callout box on the right side of the interface contains the text: 'Select the calculation of the phasor plot in the plugin'. The main view displays a grayscale image of a cat, and the layer list at the bottom left shows two layers named 'lifetime cat syntheti...'. The status bar at the bottom right indicates '26 | 255' and 'activity'.

# Usage of synthetic data – the lifetime cat example image



Svetlana Iarovenko

The screenshot shows the napari software interface. The central view displays a grayscale image of a cat. On the left, the 'layer controls' panel is visible, showing settings for opacity (1.00), contrast limits, auto-contrast (set to 'once'), gamma (1.00), colormap (set to 'gray'), blending (set to 'translucent'), and interpolation (set to 'nearest'). The 'layer list' at the bottom left shows two layers named 'lifetime cat syntheti...'. On the right, the 'Calculate Phasors (napari-flim-phasor-plotter)' plugin is active, with the following settings: image layer (lifetime cat synthetic image), Laser Frequency (MHz) (40.000), harmonic (1), threshold (10), apply median (checked), and median n (1). A 'Run' button is located below these settings. A yellow text box is overlaid on the right side of the interface, containing the text: 'Define parameters for the calculation of the phasor'.



# Usage of synthetic data – the lifetime cat example image



Svetlana Iarovenko

The screenshot displays the napari software interface. On the left, the 'layer controls' panel shows settings for a layer labeled 'Labelled\_pixels\_from\_...' with an opacity of 0.70 and a blending mode of 'translucent'. The central view shows a grayscale image of a cat with a white hood. On the right, the 'Phasor Plot Widget (napari-flim-phasor-plotter)' is open, showing a plot of S versus G. The plot features a white parabolic curve and several colored data points (red, blue, purple) clustered near the top right of the curve. Below the plot, the 'Plotting' panel shows the 'Labels layer' set to 'Labelled\_pixels\_from\_lifetime cat synthetic image', with axes 'G' and 'S'. A yellow text box is overlaid on the bottom right of the cat image, containing the text: 'Adjust the visualization settings of the phasor'. The bottom status bar of the napari window includes navigation icons and the text: 'use <1> for activate the label eraser, use <2> for activate the paint brush, use <3> for activate the fill bucket, use <4> for pick mode'. The bottom right corner of the napari window shows '26 | 255' and 'activity'.

# Usage of synthetic data – the lifetime cat example image



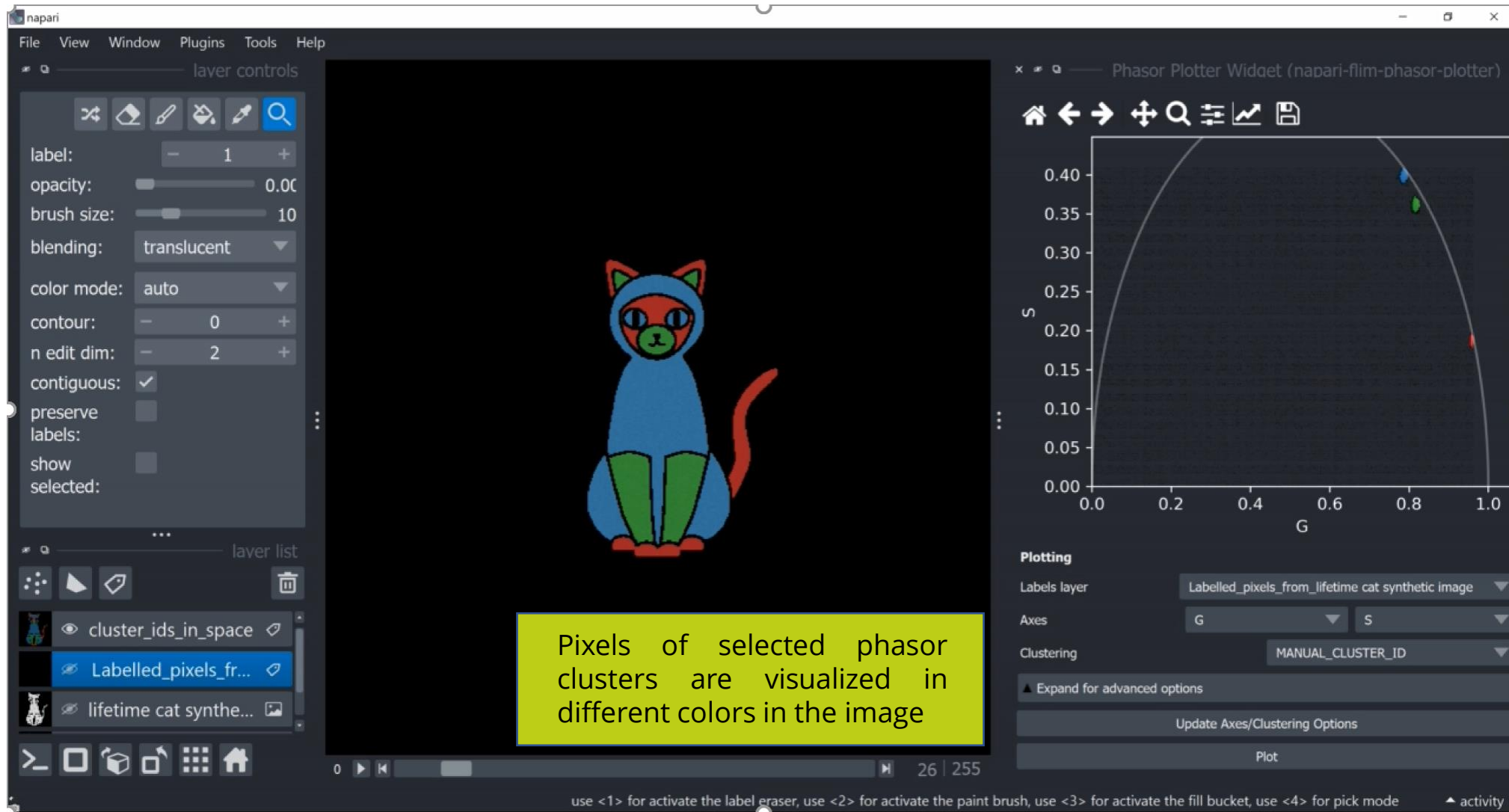
Svetlana Iarovenko

The screenshot displays the napari software interface. On the left, the 'layer controls' panel shows settings for a layer labeled '1', including opacity (0.00), brush size (10), and blending (translucent). The 'layer list' on the bottom left shows three layers: 'cluster\_ids\_in\_space', 'Labelled\_pixels\_fr...', and 'lifetime cat synthe...'. The central view shows a synthetic image of a blue cat with green paws. On the right, the 'Phasor Plot Widget (napari-flim-phasor-plotter)' is open, showing a plot of S versus G. The plot has a white arc and several colored points (blue, green, red). A blue circle highlights a red point on the arc. The plot's axes are labeled G (0.0 to 1.0) and S (0.00 to 0.40). The plot title is 'Phasor Plot Widget (napari-flim-phasor-plotter)' and it shows coordinates x=0.9529, y=0.1664. Below the plot, the 'Plotting' section shows 'Labels layer' set to 'Labelled\_pixels\_from\_lifetime cat synthetic Image', 'Axes' set to G and S, and 'Clustering' set to 'MANUAL\_CLUSTER\_ID'. A yellow text box at the bottom of the plot area contains the text: 'Select clusters, here manually by drawing ROIs in the phasor plot'. The bottom status bar of the napari window shows '26 | 255' and instructions: 'use <1> for activate the label eraser, use <2> for activate the paint brush, use <3> for activate the fill bucket, use <4> for pick mode'.

# Usage of synthetic data – the lifetime cat example image



Svetlana Iarovenko



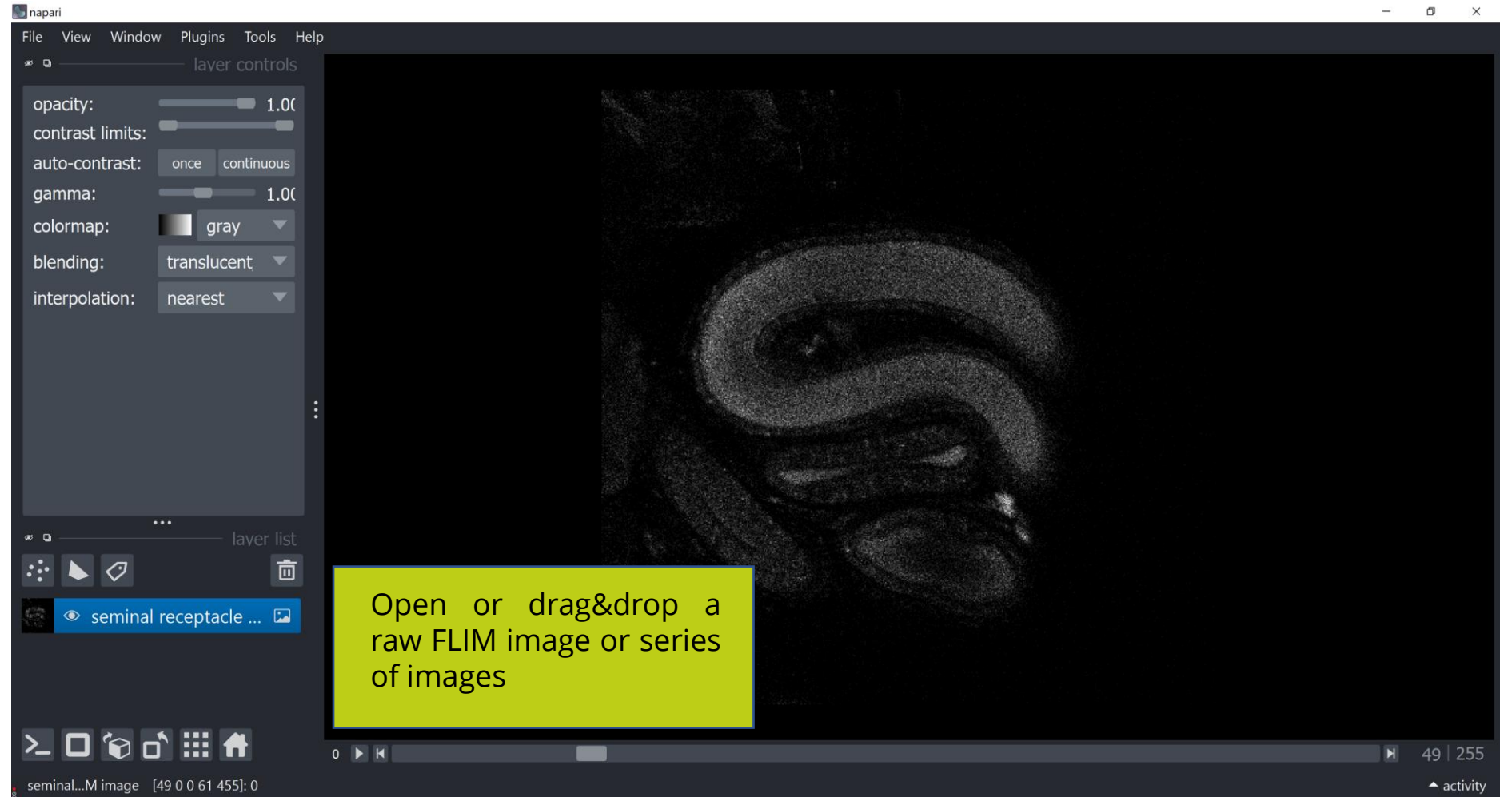
Pixels of selected phasor clusters are visualized in different colors in the image

# Integrating FLIM analysis into a bio-image analysis workflow

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

Example workflow:

- Open a raw FLIM image/ dataset

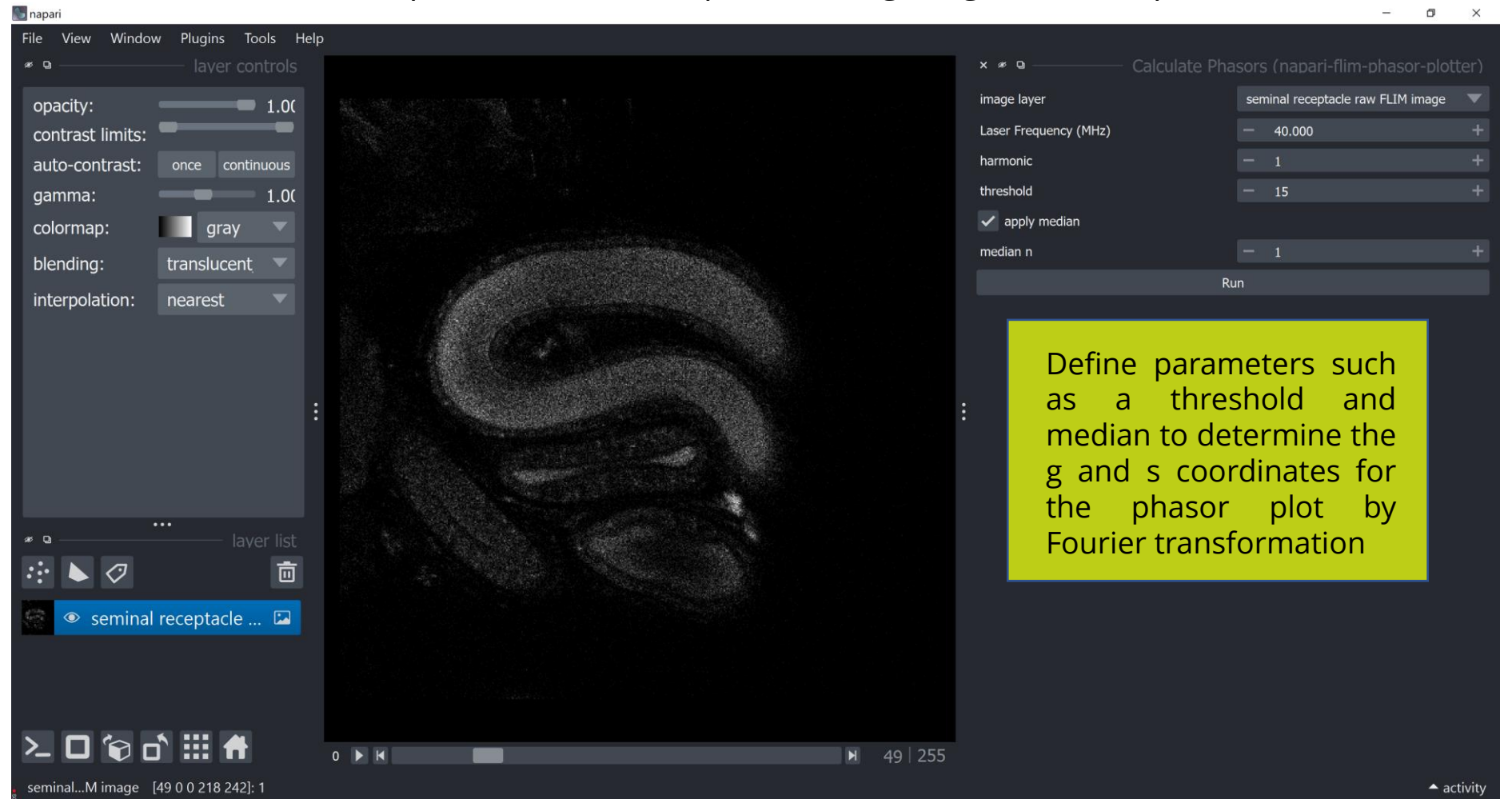


# Integrating FLIM analysis into a bio-image analysis workflow

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

Example workflow:

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



# Integrating FLIM analysis into a bio-image analysis workflow

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

Example workflow:

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

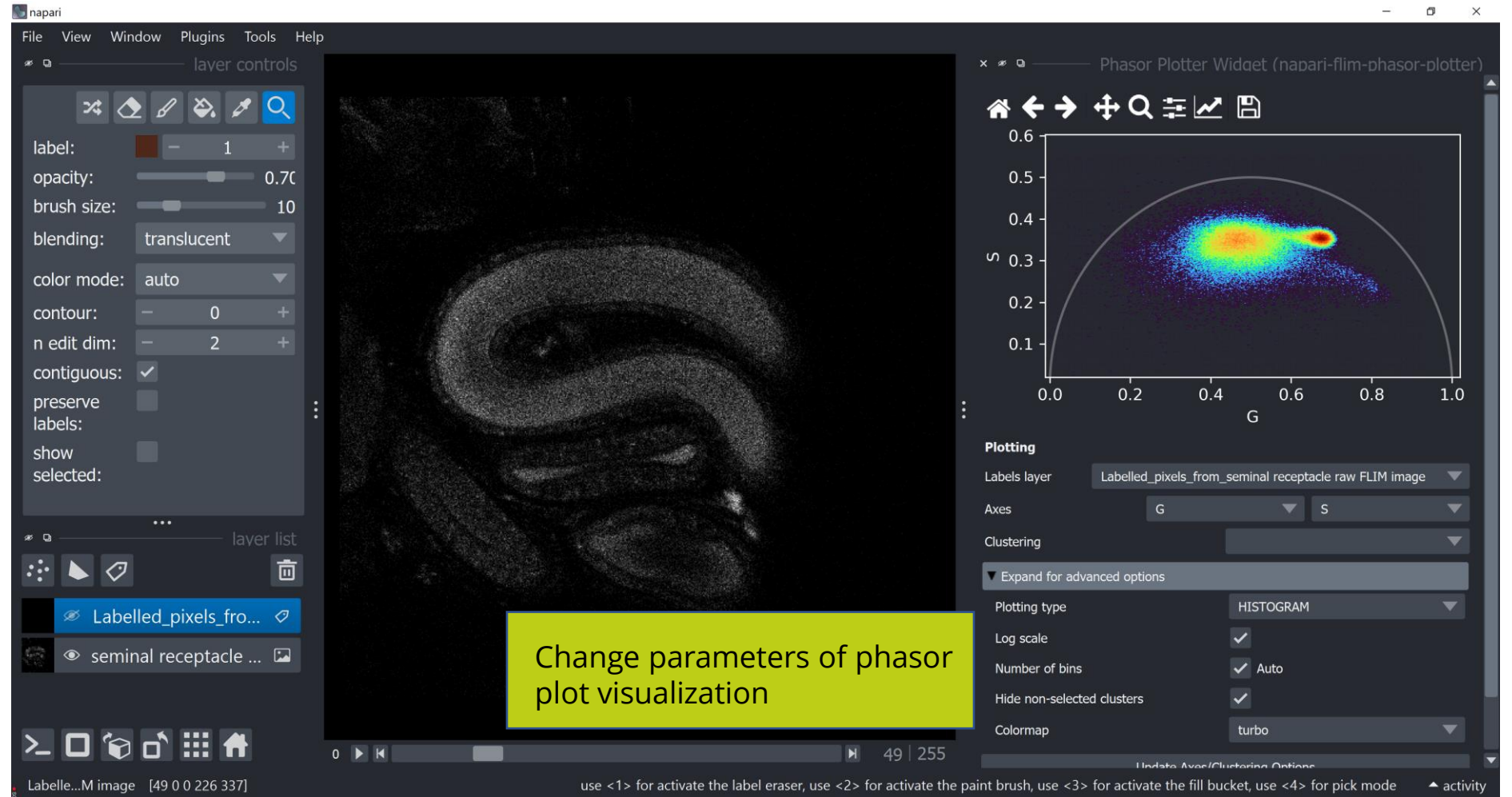


# Integrating FLIM analysis into a bio-image analysis workflow

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

Example workflow:

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



# Integrating FLIM analysis into a bio-image analysis workflow

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

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

The screenshot displays the napari software interface. The central window shows a grayscale FLIM image of a sperm storage organ. On the left, the 'layer controls' panel is visible, showing a 'Labelled\_pixels\_from...' layer with a label of 1, an opacity of 0.70, and a brush size of 10. Below it, the 'layer list' shows the 'Labelled\_pixels\_from...' layer selected. On the right, the 'Clustering (ncp)' plugin is open, showing the 'Labels layer' set to 'Labelled\_pixels\_from\_seminal\_receptacle raw FLIM image'. The 'Measurements' section lists 'G', 'S', and 'frame', with a yellow callout box pointing to 'S' and 'frame' containing the text 'Select the parameters used for clustering'. Below this, the 'Clustering Method' is set to 'HDBSCAN', and the 'Minimum size of clusters' and 'Minimum number of samples' are both set to 40. A yellow callout box points to the 'Update Measurements' button with the text 'Select the clustering method'. At the bottom, the 'Run' button is visible.

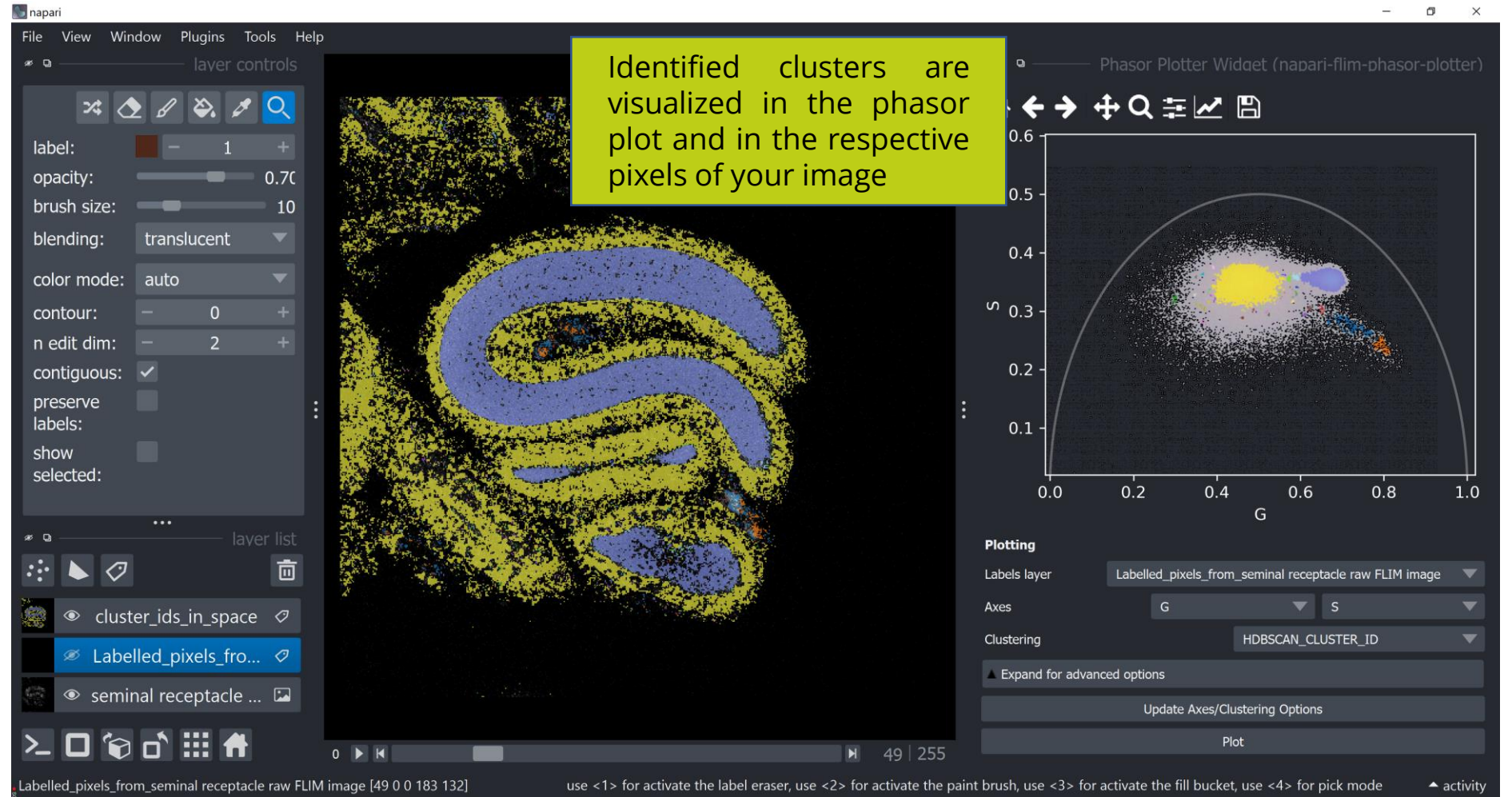


# Integrating FLIM analysis into a bio-image analysis workflow

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

Example workflow:

- Open a raw FLIM image/ dataset
- Generate a Phasor Plot
- Change Phasor Plot display options
- Apply a clustering algorithm to the Phasor Plot (HDBSCAN) using the napari-clusters-plotter

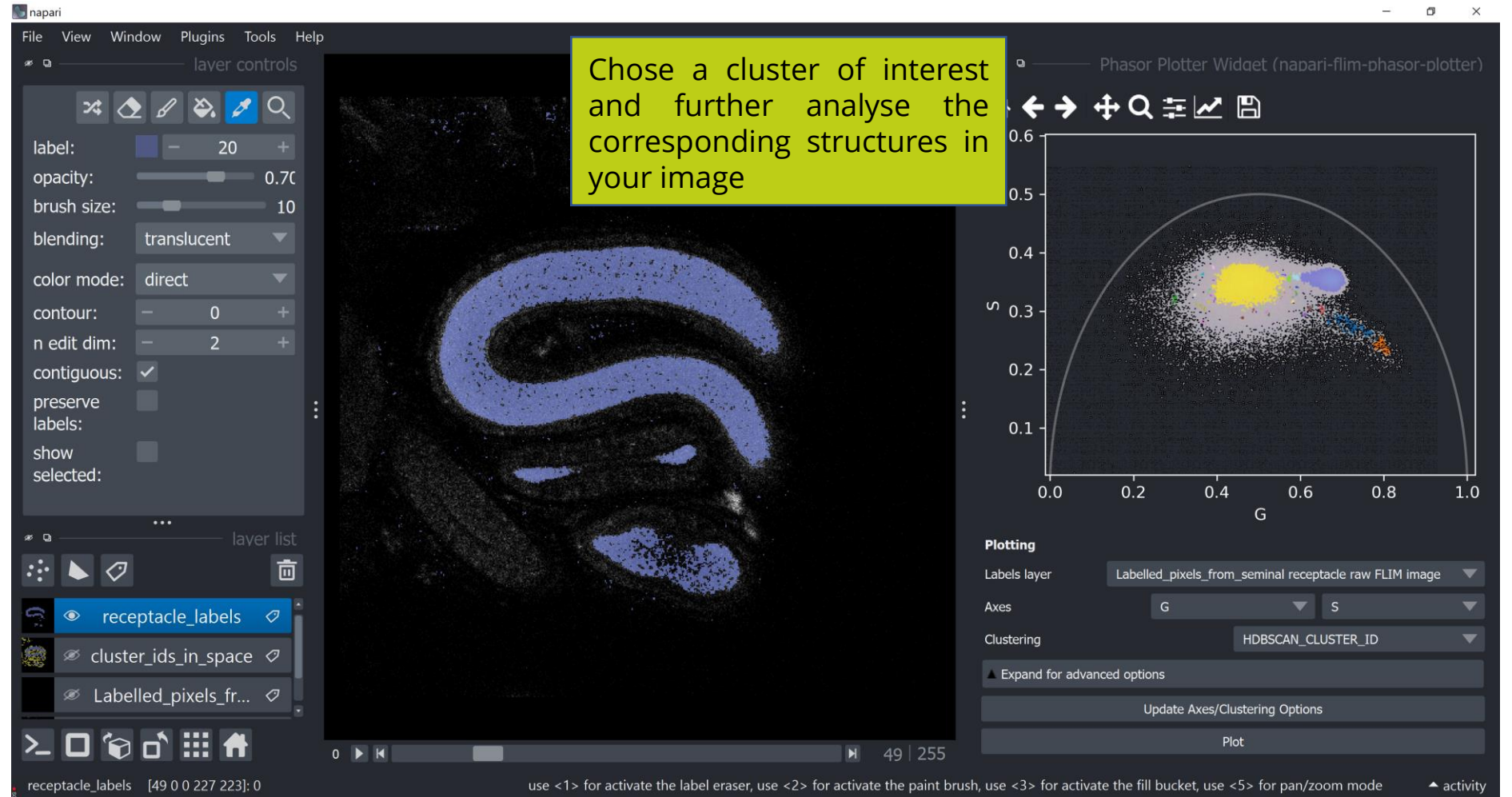


# Integrating FLIM analysis into a bio-image analysis workflow

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

### Example workflow:

- Open a raw FLIM image/ dataset
- Generate a Phasor Plot
- Change Phasor Plot display options
- Apply a clustering algorithm to the Phasor Plot (HDBSCAN) using the napari-clusters-plotter
- Extract cluster/class of interest



# Integrating FLIM analysis into a bio-image analysis workflow

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

Example workflow:

- Open a raw FLIM image/ dataset
- Generate a Phasor Plot
- Change Phasor Plot display options
- Apply a clustering algorithm to the Phasor Plot (HDBSCAN) using the napari-clusters-plotter
- Extract cluster/class of interest
- Post-processing: apply morphological operations

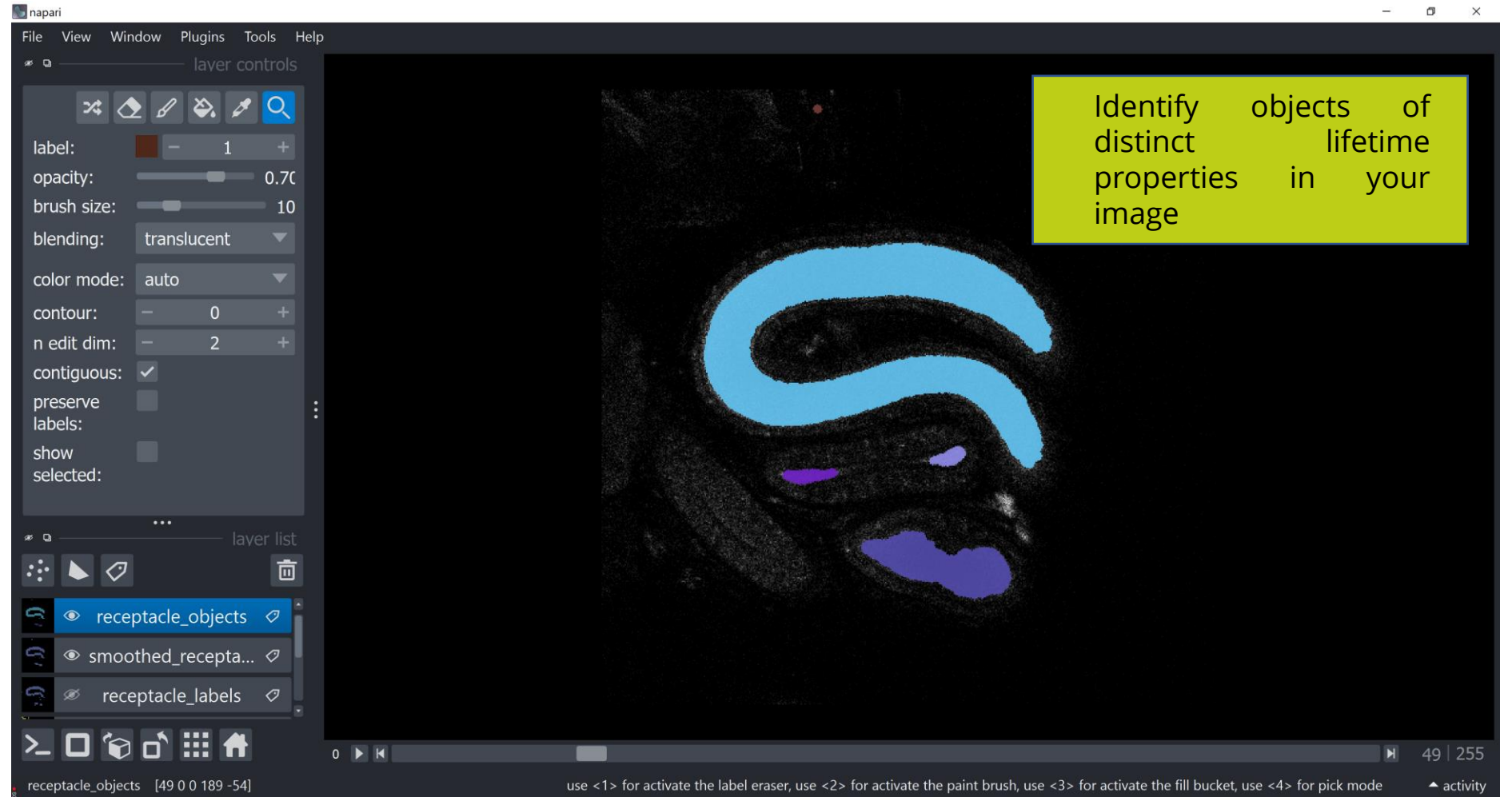


# Integrating FLIM analysis into a bio-image analysis workflow

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

Example workflow:

- Open a raw FLIM image/ dataset
- Generate a Phasor Plot
- Change Phasor Plot display options
- Apply a clustering algorithm to the Phasor Plot (HDBSCAN) using the napari-clusters-plotter
- Extract cluster/class of interest
- Post-processing: apply morphological operations
- Perform instance segmentation



# Integrating FLIM analysis into a bio-image analysis workflow

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

### Example workflow:

- Open a raw FLIM image/ dataset
- Generate a Phasor Plot
- Change Phasor Plot display options
- Apply a clustering algorithm to the Phasor Plot (HDBSCAN) using the napari-clusters-plotter
- Extract cluster/class of interest
- Post-processing: apply morphological operations
- Perform instance segmentation
- Extract Features

Properties of receptacle objects

	label	area	index
1	1	41.0	1
2	2	23236.0	2
3	3	320.0	3
4	4	431.0	4
5	5	3963.0	5

Extract features of your identified objects

# Integrating FLIM analysis into a bio-image analysis workflow

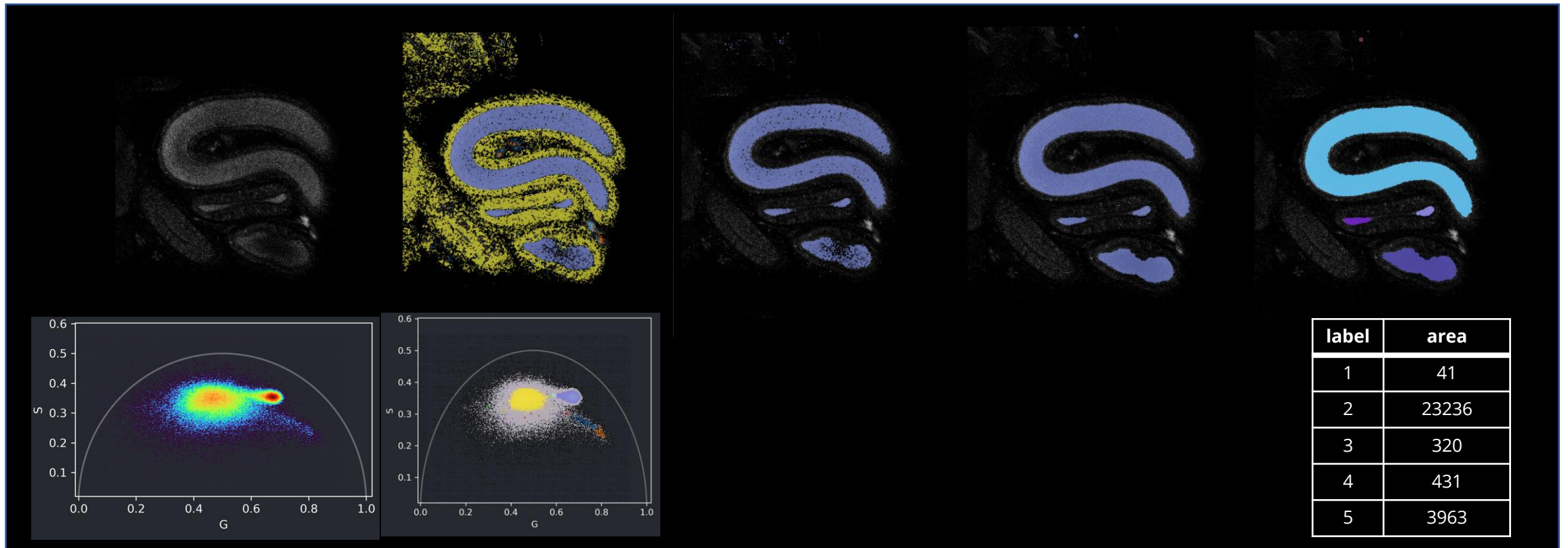
Raw data import & phasor analysis

Cluster analysis

Selection of individual cluster

Processing of selected cluster

Instance segmentation & quantification



# 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

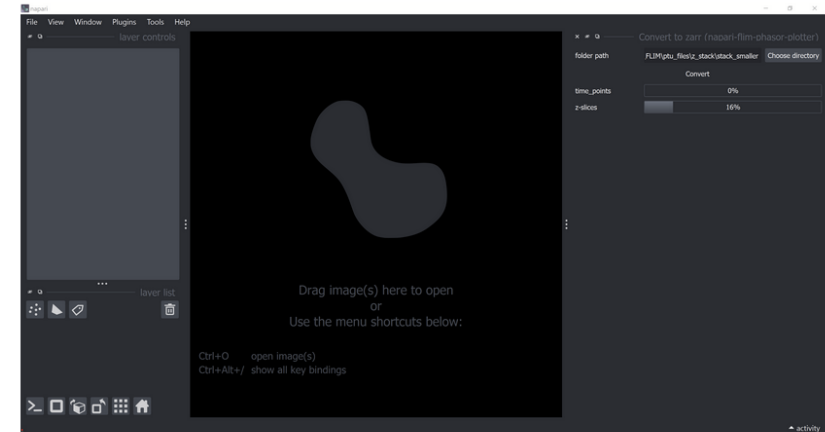


- The plugin can convert raw FLIM files of a folder to .zarr

## 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` followed 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

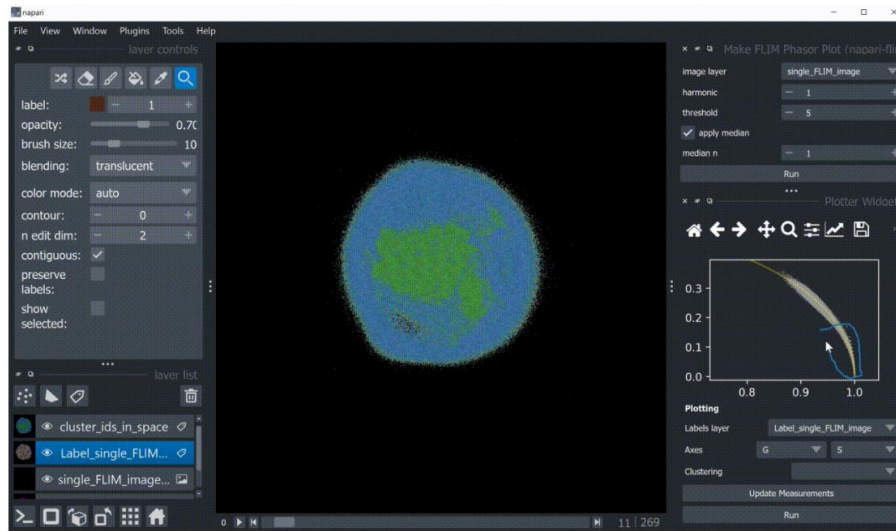
## Including documentation



### 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-clusters-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

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)



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CMCB, TU Dresden