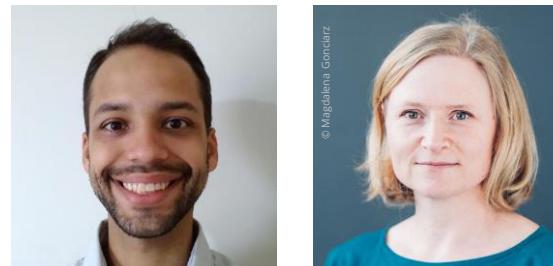


napari-FLIM-phasor-plotter – a **collaborative** project

a plugin to generate interactive phasor plots from raw FLIM data

Marcelo Leomil Zoccoler

Postdoc in Bio-Image Analysis
Development Group (Physics of Life, TU Dresden)



Cornelia Wetzker

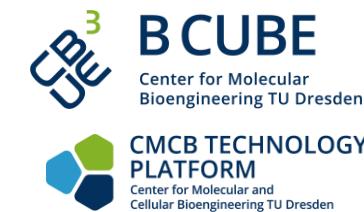
Data steward (B CUBE/ CMCB, TU Dresden and NFDI4BIOIMAGE)
previously Light Microscopy Facility staff member
(CMCB, TU Dresden)



Svetlana Iarovenko

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

With materials from Marcelo Zoccoler



The content of these slides is shared under the terms of the [Creative Commons Attribution License CC-BY 4.0](#) unless stated otherwise.

Scientists' needs and wishes for FLIM software

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

Do I need licensed software to analyse FLIM data?

How can I chose a colorblind-friendly lookup table?

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



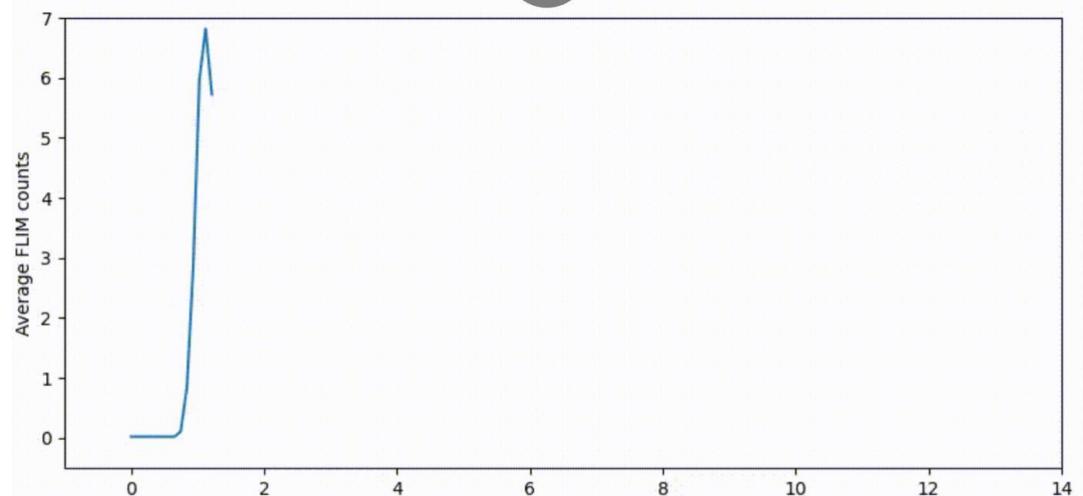
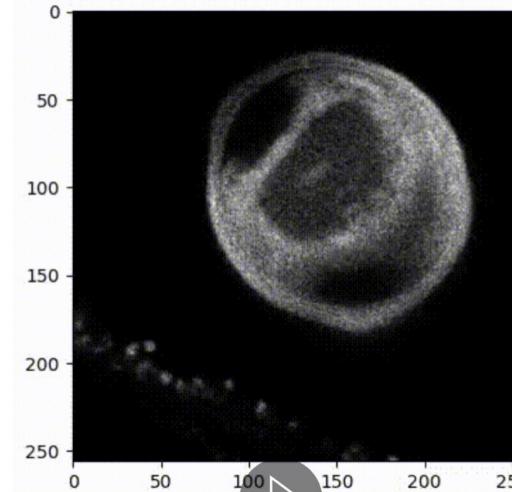
[zoccoler](#) Marcelo Zoccoler



[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.



GerBi FLIM workshop 2024 München – napari-flim-phasor-plugin Conni Wetzker

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

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



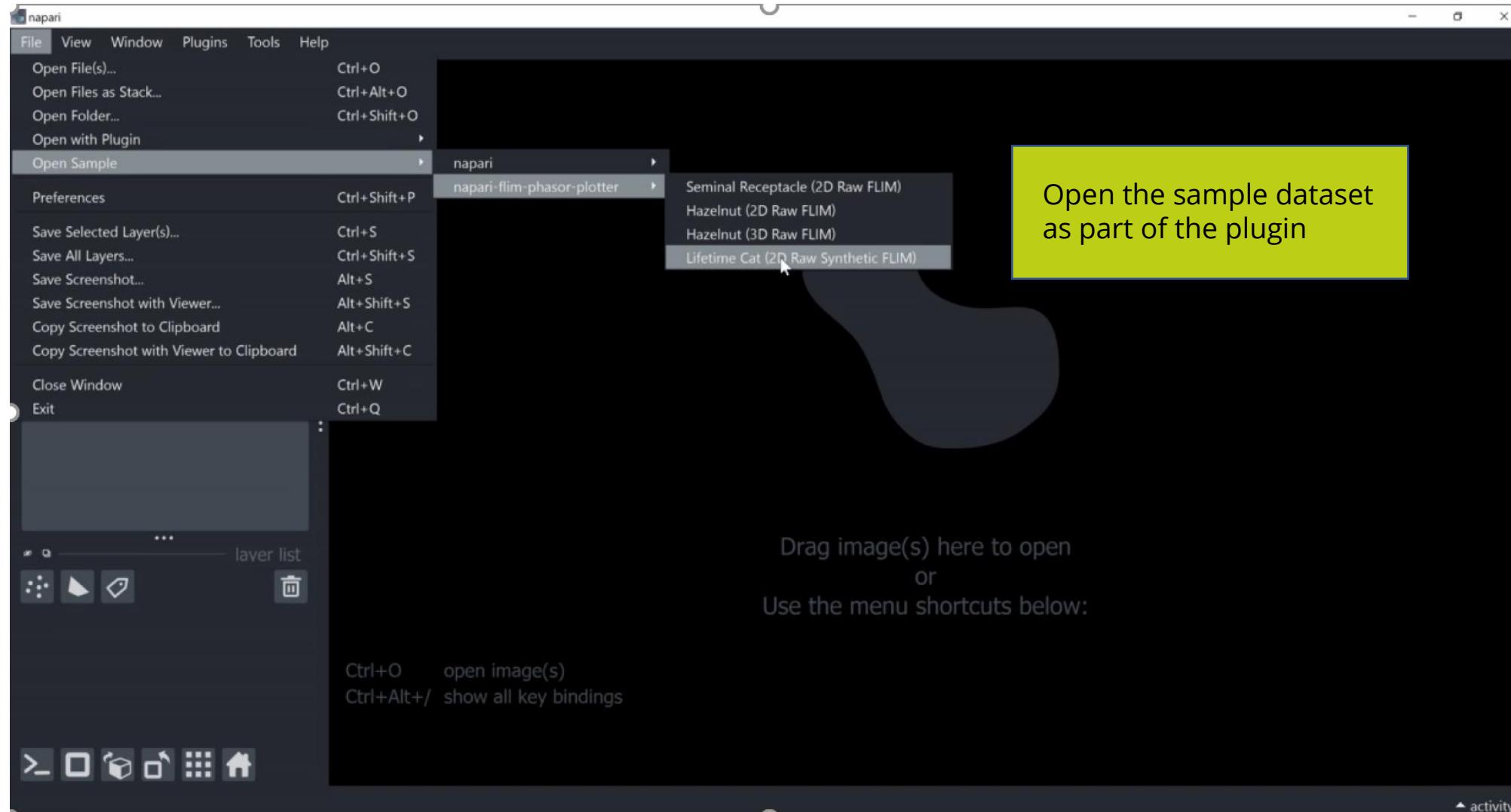
Extendable by various plugins (widgets and user interfaces, readers and writers)



Usage of synthetic data – the lifetime cat example image



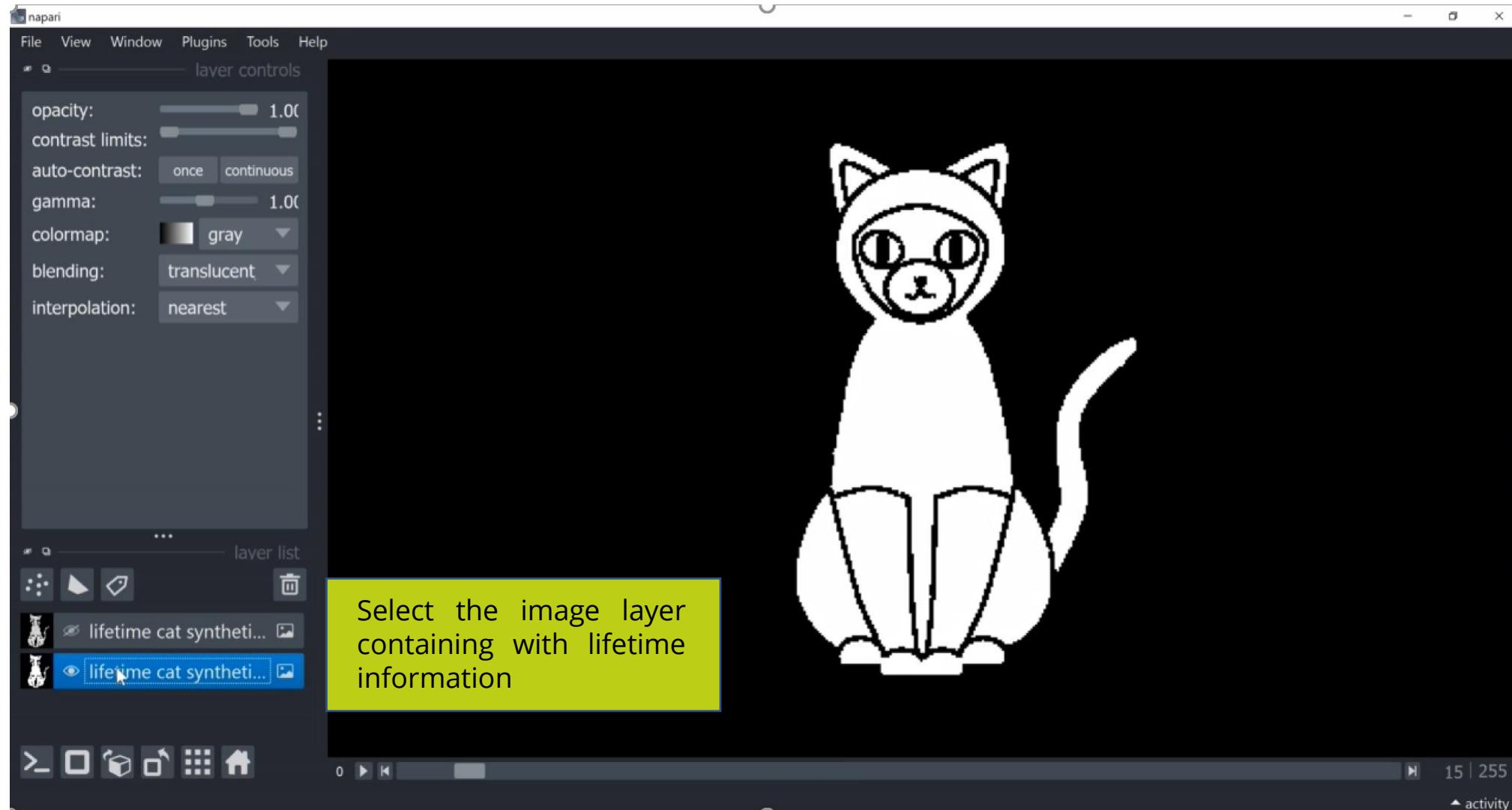
Svetlana Iarovenko



Usage of synthetic data – the lifetime cat example image



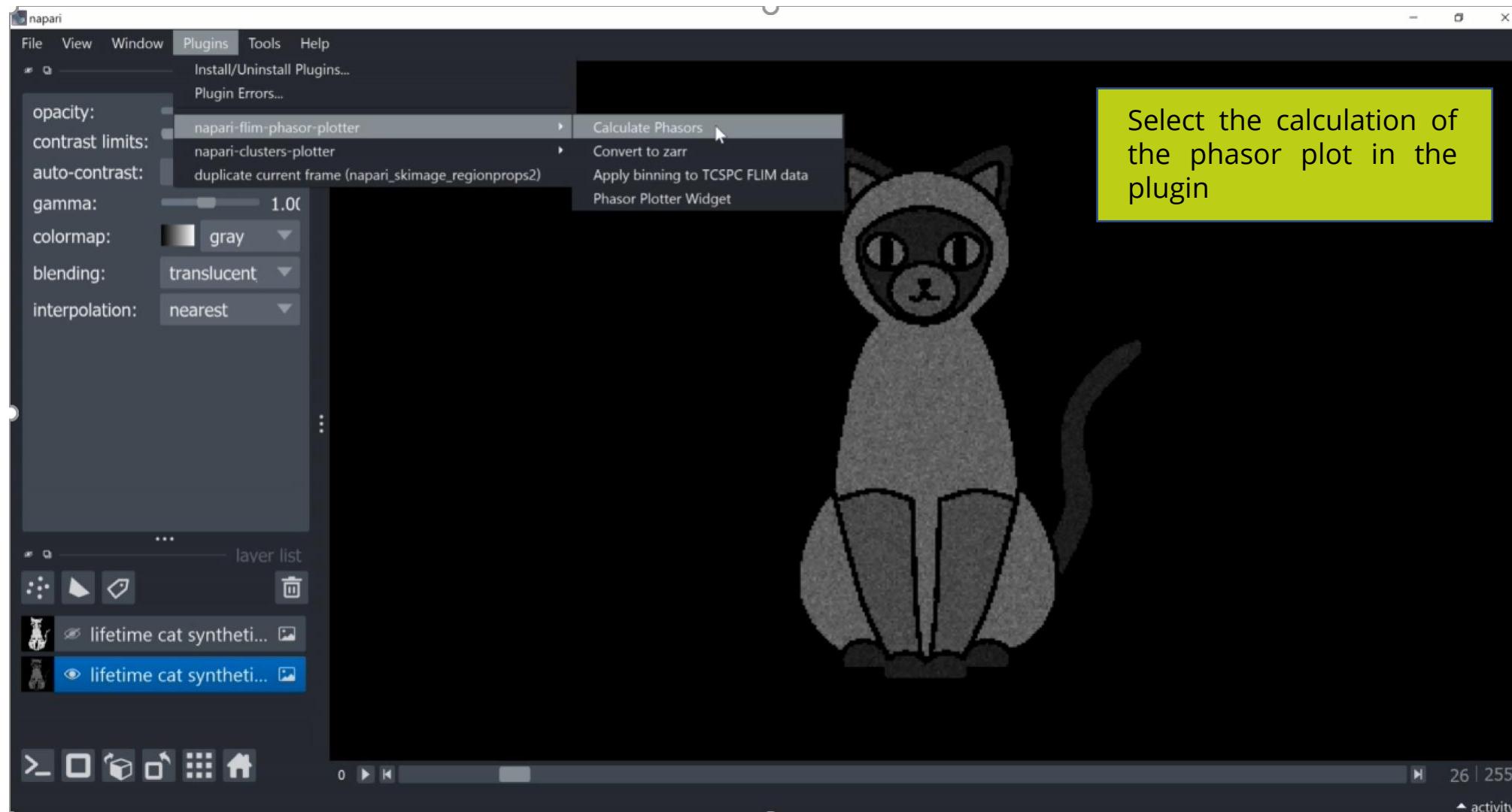
Svetlana Iarovenko



Usage of synthetic data – the lifetime cat example image



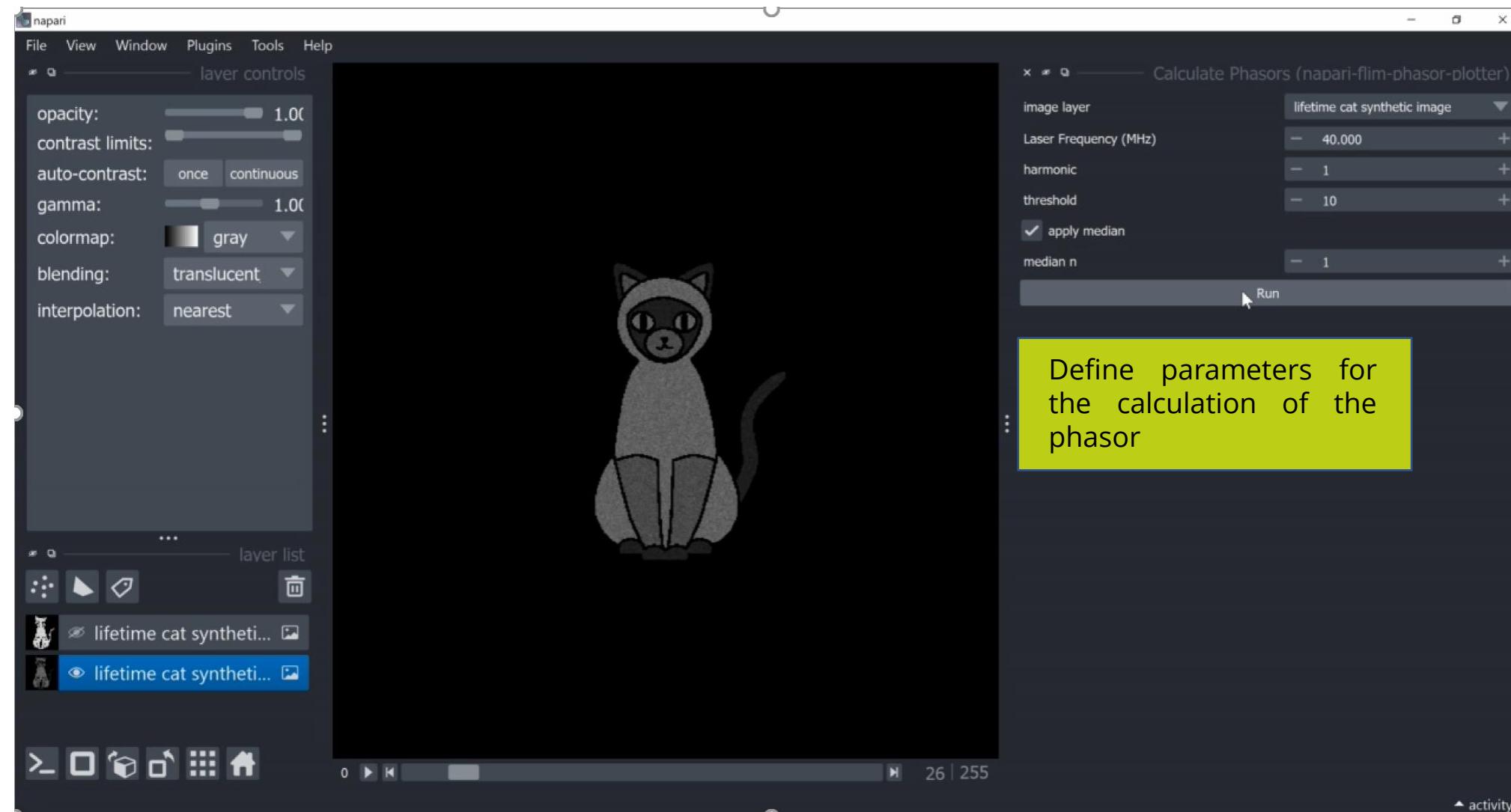
Svetlana Iarovenko



Usage of synthetic data – the lifetime cat example image



Svetlana Iarovenko



The screenshot shows the napari software interface. On the left, the 'layer controls' panel displays settings for opacity (1.00), contrast limits, auto-contrast, gamma (1.00), colormap (gray), blending (translucent), and interpolation (nearest). The 'layer list' panel shows two layers: 'lifetime cat synthetic...' and 'lifetime cat synthetic...'. The main canvas displays a grayscale image of a cat. To the right, the 'Calculate Phasors (napari-flim-phasor-plotter)' panel is open, showing parameters for the 'lifetime cat synthetic image': Laser Frequency (MHz) at 40.000, harmonic at 1, threshold at 10, and a checked 'apply median' option with a median n of 1. A yellow callout box highlights the parameter definitions with the text: 'Define parameters for the calculation of the phasor'.

Usage of synthetic data – the lifetime cat example image



Svetlana Iarovenko

The screenshot shows the napari software interface. On the left, the 'layer controls' panel displays various settings for a layer named 'Labelled_pixels_from_labeled_cat'. The main canvas shows a grayscale image of a Siamese cat with its pixels labeled in red. A yellow callout box with black text reads: 'Adjust the visualization settings of the phasor'. To the right, a separate window titled 'Phasor Plotter Widget (napari-flim-phasor-plotter)' shows a plot of G versus S. The x-axis (G) ranges from 0.0 to 1.0, and the y-axis (S) ranges from 0.00 to 0.40. Several red dots are plotted along a curve. The 'Plotting' section of the widget includes dropdown menus for 'Labels layer' (set to 'Labelled_pixels_from_labeled_cat'), 'Axes' (set to 'G' and 'S'), and 'Clustering'.



Usage of synthetic data – the lifetime cat example image



Svetlana Iarovenko

The screenshot shows the napari software interface. On the left, the main canvas displays a synthetic lifetime cat image with blue and green pixels. A yellow callout box contains the text: "Select clusters, here manually by drawing ROIs in the phasor plot". To the right, a Phasor Plotter Widget is open, showing a plot of G (x-axis) vs S (y-axis). The plot features a unit circle and several data points. One point is highlighted with a blue oval ROI. The widget's control panel includes sections for "Plotting" (Labels layer: "Labelled_pixels_from_lifetime cat synthetic image", Axes: G and S, Clustering: "MANUAL_CLUSTER_ID"), "Advanced Options" (Expand for advanced options, Update Axes/Clustering Options), and a "Plot" button. The bottom status bar provides keyboard shortcuts: <1> for label eraser, <2> for paint brush, <3> for fill bucket, and <4> for pick mode.

Usage of synthetic data – the lifetime cat example image



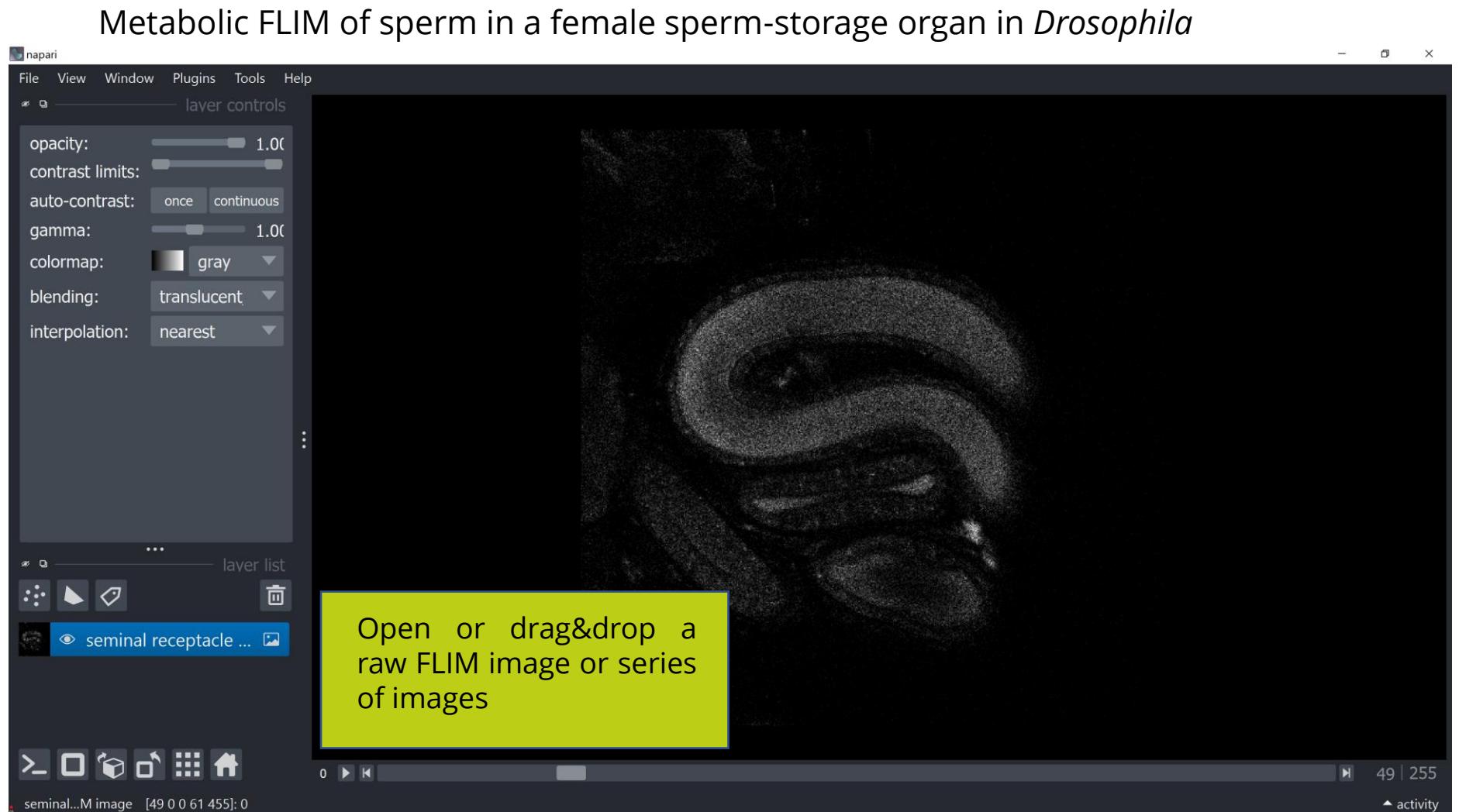
Svetlana Iarovenko

The screenshot shows the napari software interface. On the left, the 'layer controls' panel displays various parameters for a selected layer, including 'label', 'opacity', 'brush size', 'blending', 'color mode', 'contour', 'n edit dim', 'contiguous', 'preserve labels', and 'show selected'. The 'layer list' below shows three layers: 'cluster_ids_in_space', 'Labelled_pixels_fr...', and 'lifetime cat synthetic...'. A yellow callout box highlights the 'Labelled_pixels_fr...' layer. The main canvas displays a stylized cat with blue, red, and green pixels. A text overlay on the canvas reads: 'Pixels of selected phasor clusters are visualized in different colors in the image'. To the right, a 'Phasor Plotter Widget' is open, showing a plot of G versus S. The plot area contains several colored points (blue, green, red) following a circular path. The widget has sections for 'Plotting' (Labels layer: 'Labelled_pixels_from_lifetime cat synthetic image', Axes: G and S, Clustering: 'MANUAL_CLUSTER_ID'), 'Advanced Options' (expandable), and 'Axes/Clustering Options' (Update Axes/Clustering Options). At the bottom, a keyboard shortcut for activating the label eraser, paint brush, fill bucket, and pick mode is listed: 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.

Integrating FLIM analysis into a bio-image analysis workflow

Example workflow:

- Open a raw FLIM image/ dataset

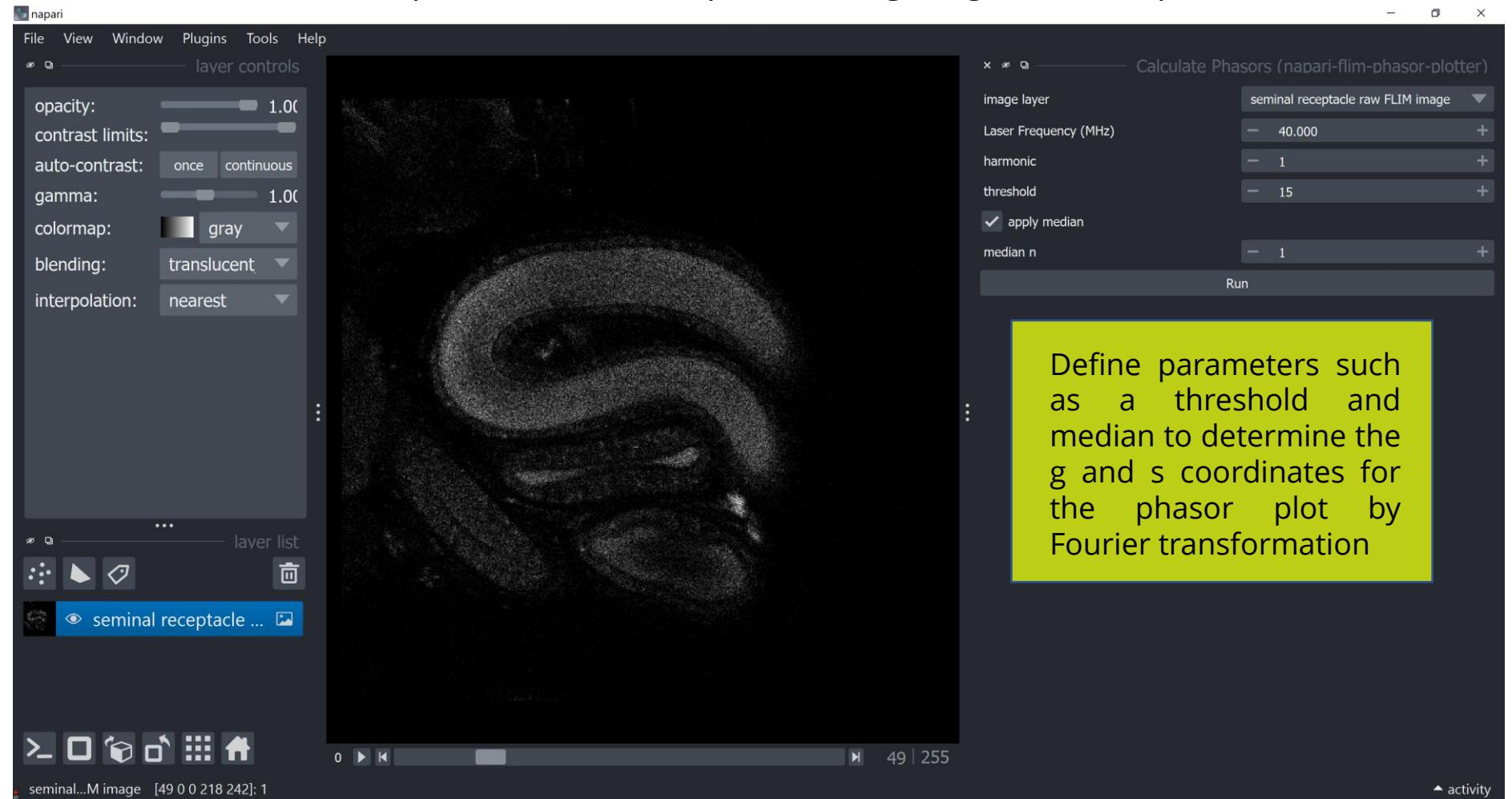


Integrating FLIM analysis into a bio-image analysis workflow

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

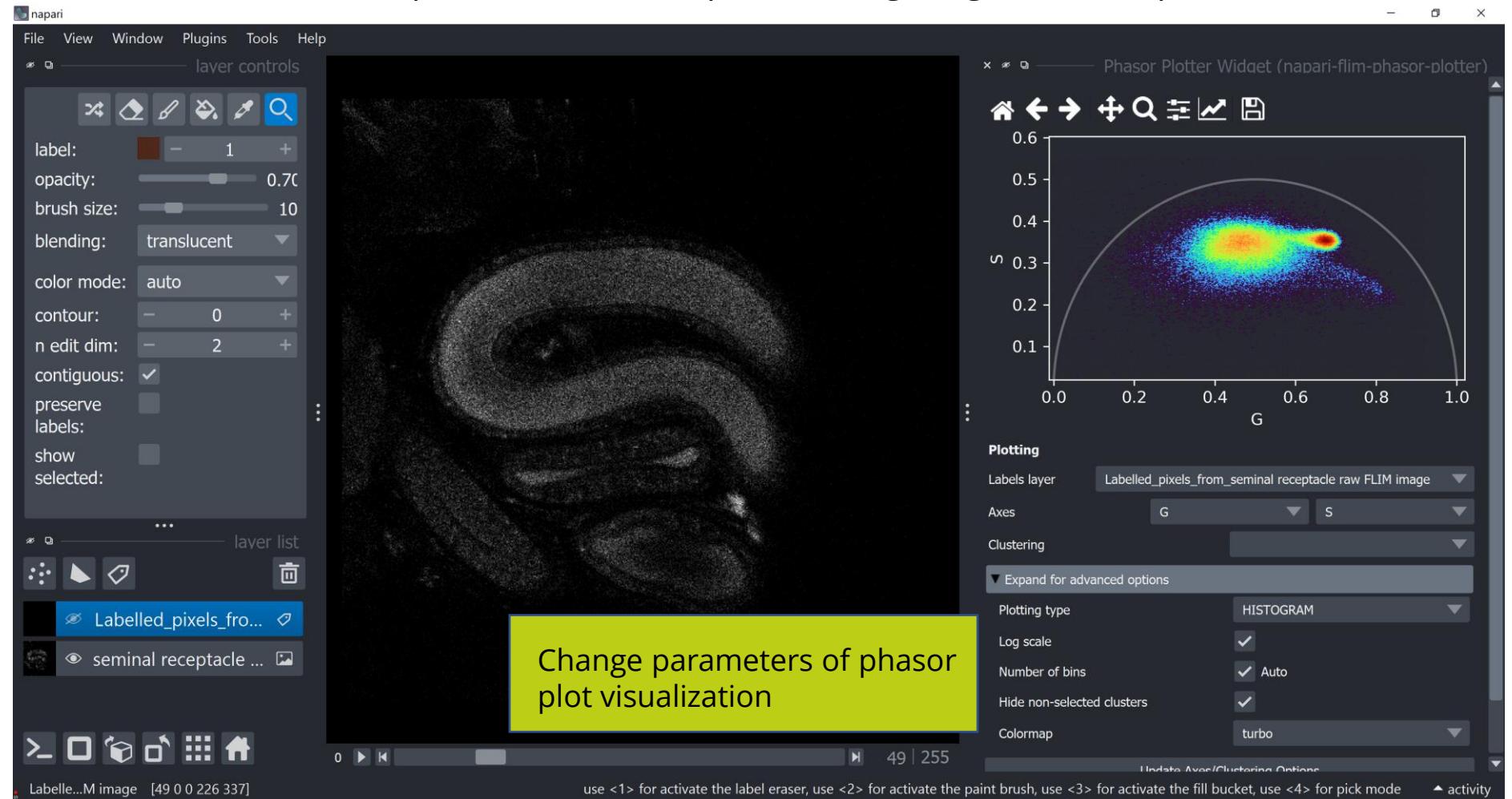


Integrating FLIM analysis into a bio-image analysis workflow

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

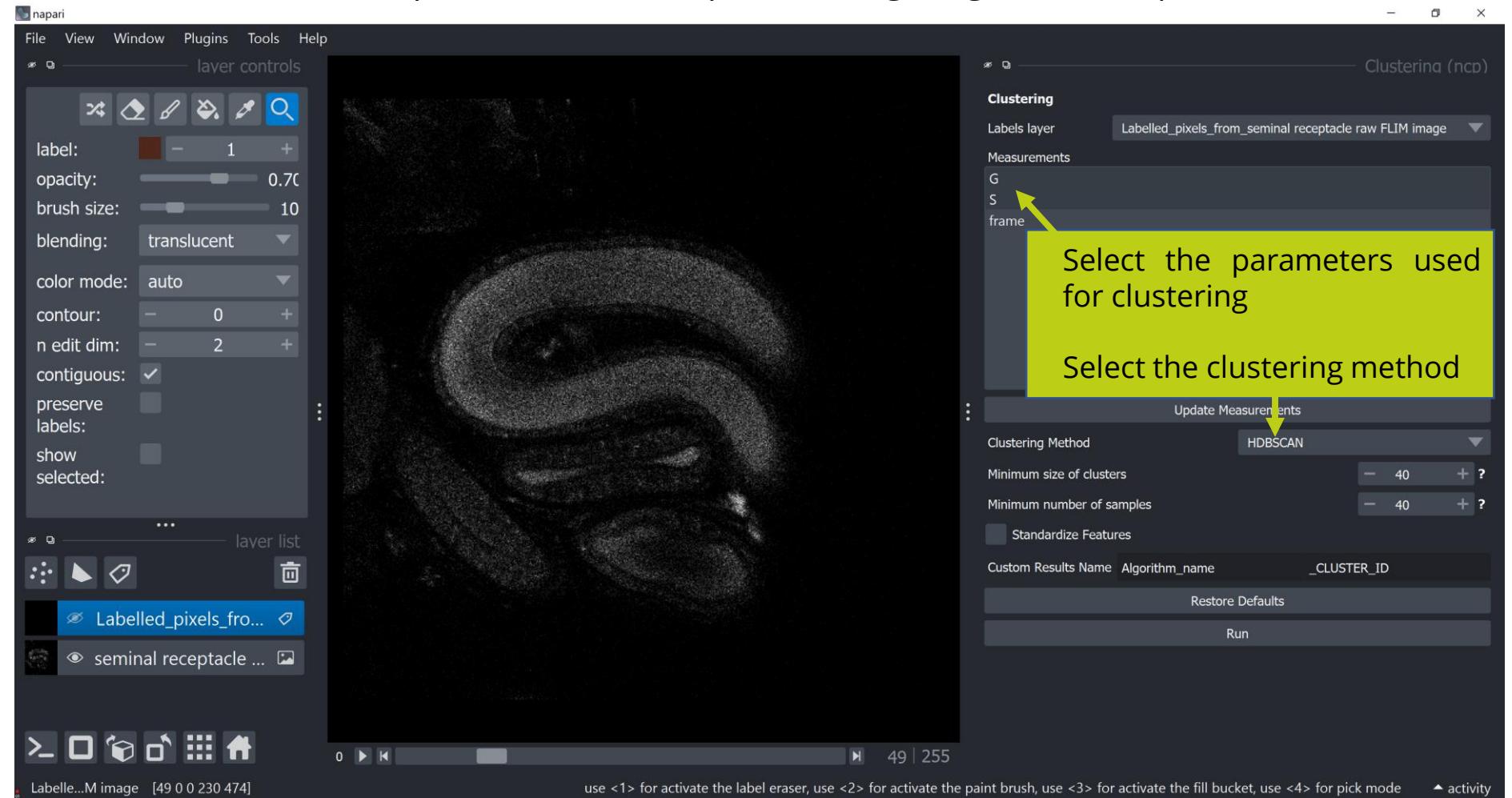


Integrating FLIM analysis into a bio-image analysis workflow

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

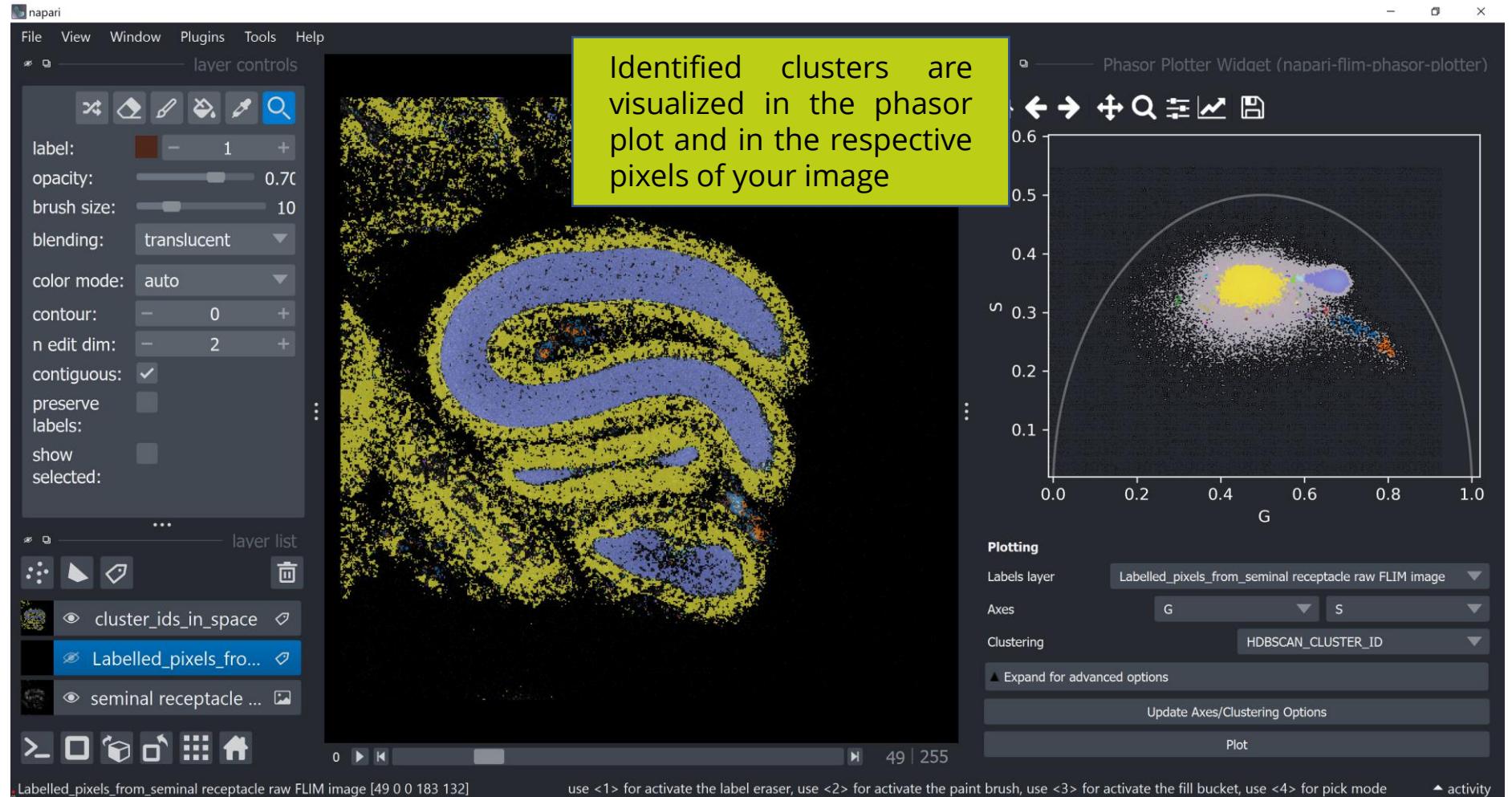


Integrating FLIM analysis into a bio-image analysis workflow

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

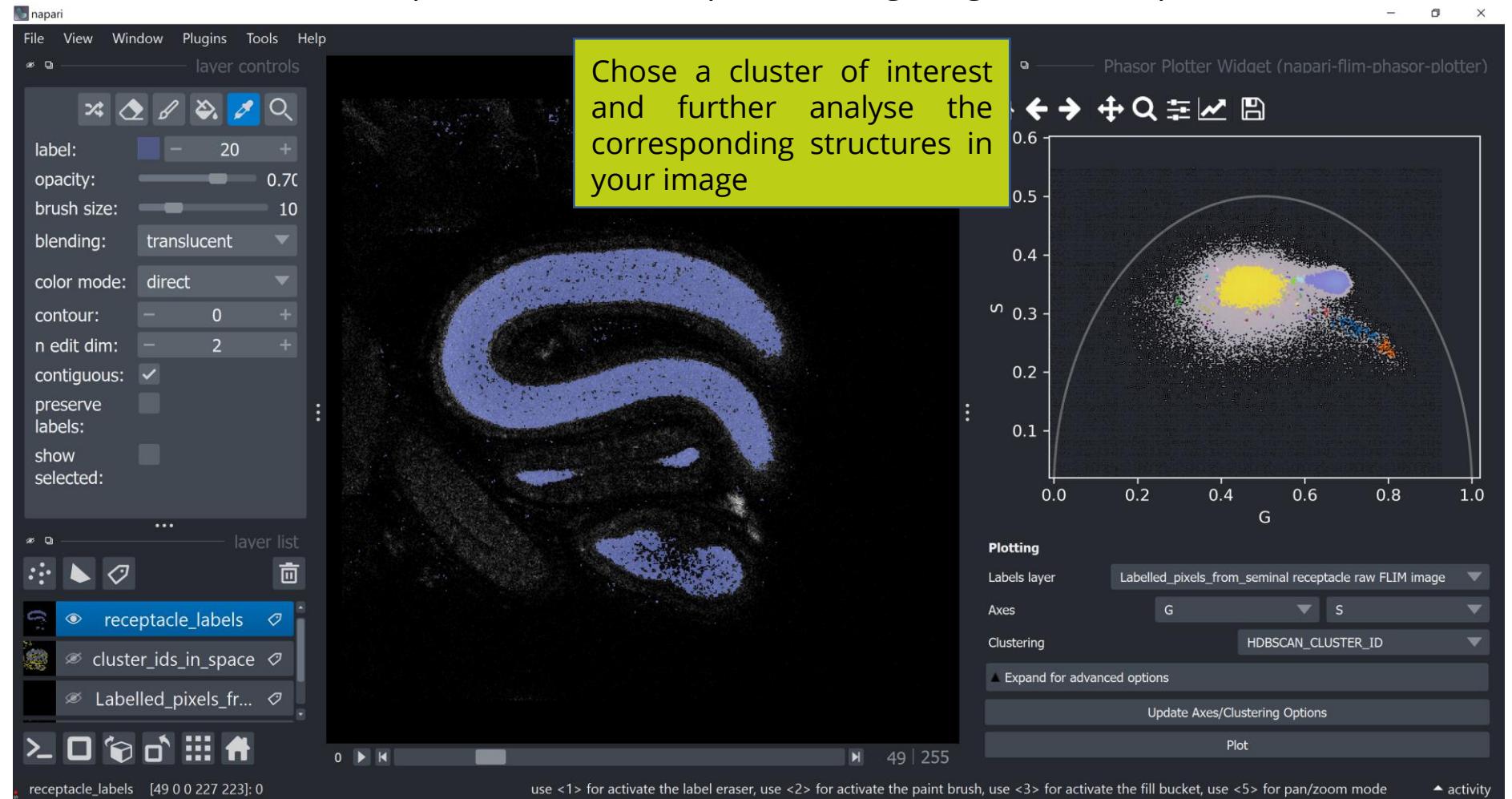


Integrating FLIM analysis into a bio-image analysis workflow

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

Example workflow:

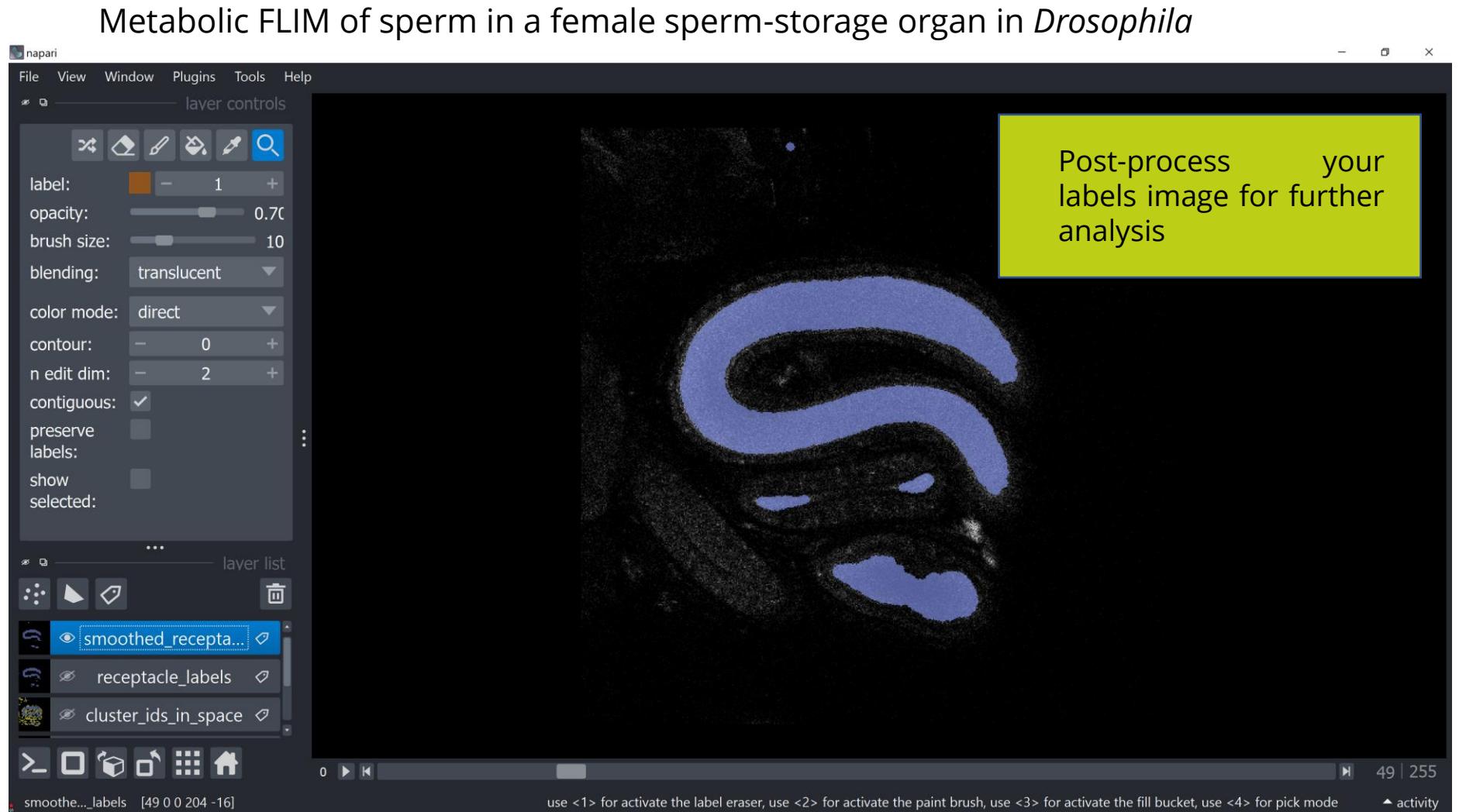
- Open a raw FLIM image/ dataset
- Generate a Phasor Plot
- Change Phasor Plot display options
- Apply a clustering algorithm to the Phasor Plot (HDBSCAN) using the napari-clusters-plotter
- Extract cluster/class of interest



Integrating FLIM analysis into a bio-image analysis workflow

Example workflow:

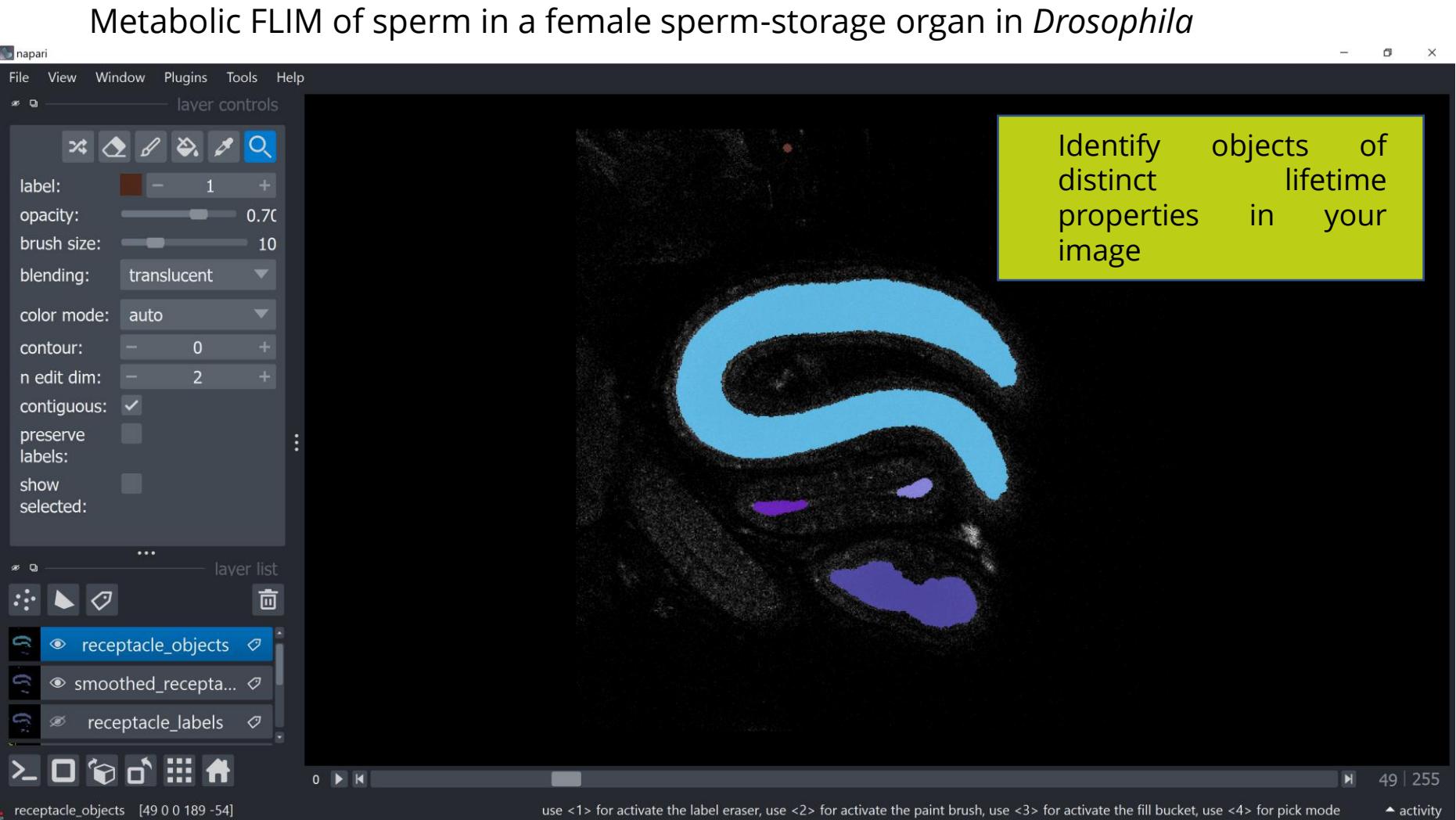
- Open a raw FLIM image/ dataset
- Generate a Phasor Plot
- Change Phasor Plot display options
- Apply a clustering algorithm to the Phasor Plot (HDBSCAN) using the napari-clusters-plotter
- Extract cluster/class of interest
- Post-processing: apply morphological operations



Integrating FLIM analysis into a bio-image analysis workflow

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

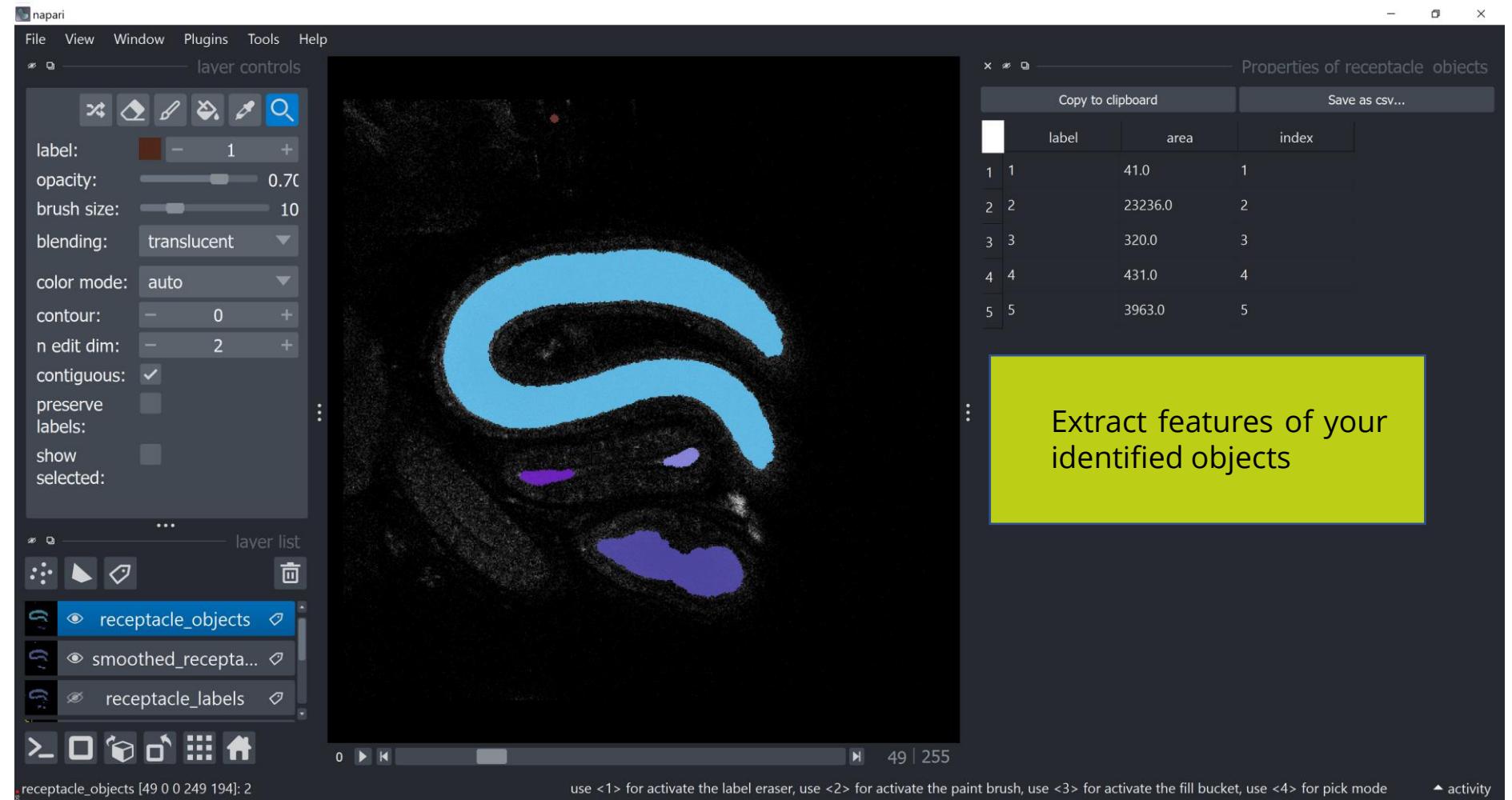


Integrating FLIM analysis into a bio-image analysis workflow

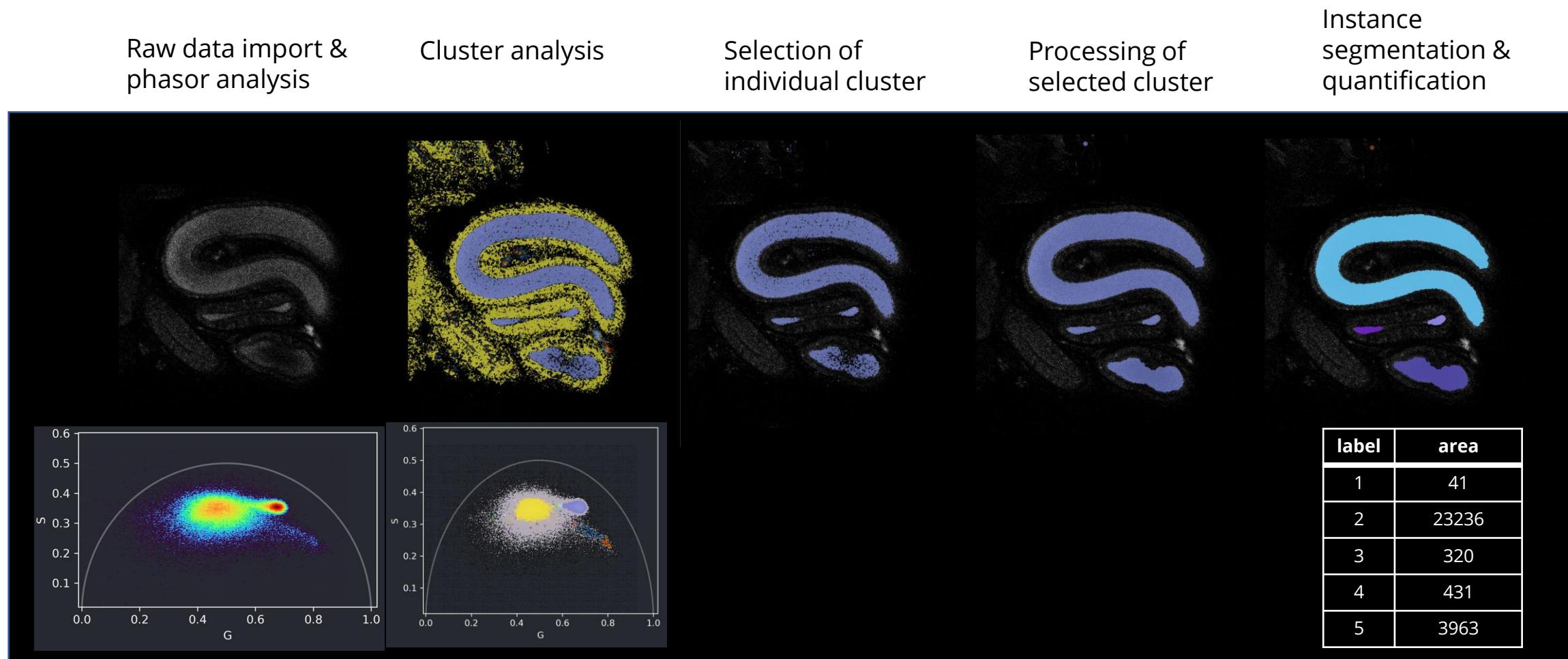
Metabolic FLIM of sperm in a female sperm-storage organ in *Drosophila*

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



Integrating FLIM analysis into a bio-image analysis workflow



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.





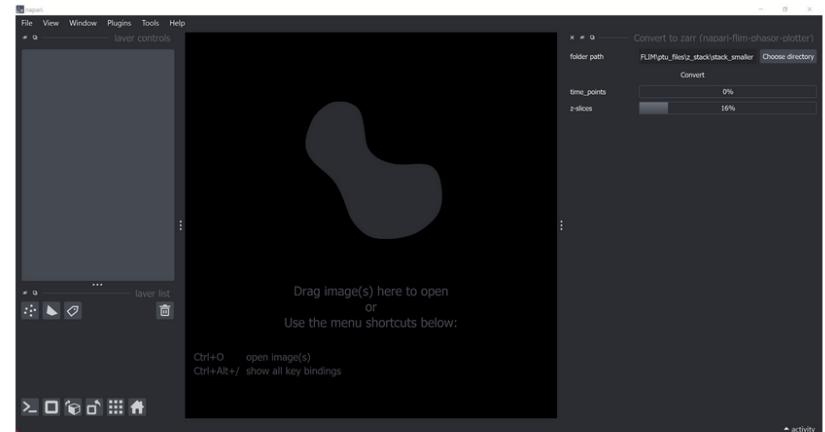
Data conversion to .zarr

- 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`

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

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

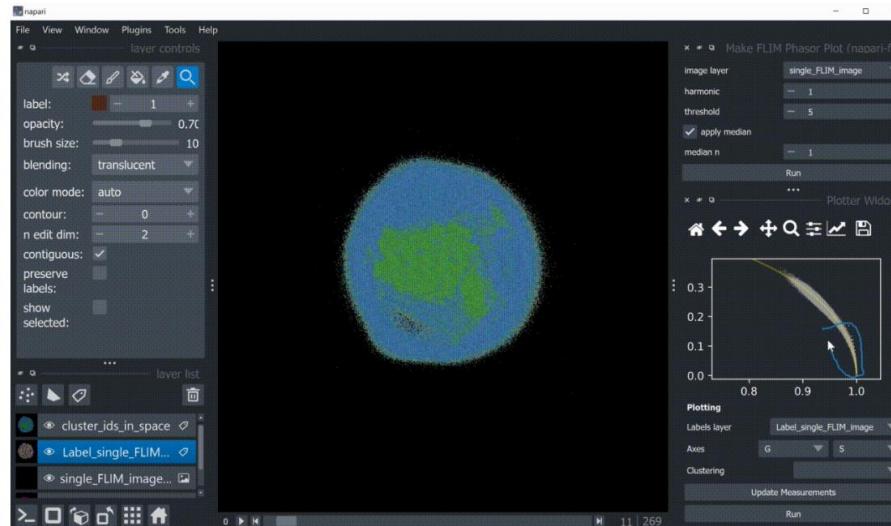
Including documentation



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 larovenko
(soon @ IMP Vienna)



Robert Haase
(ScaDS.AI Leipzig)

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)



Bio-Image Analysis Group, PoL
TU Dresden



Light Microscopy Facility,
CMCB, TU Dresden

