
Object-Oriented Polarization Software (**OOPS**) User Manual

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<https://github.com/Mattheyses-Lab/OOPS/releases>

November 29, 2023

Introduction

Object-Oriented Polarization Software (OOPS) is a GUI-based MATLAB tool designed to facilitate object-based image analysis of fluorescence polarization microscopy data. You can download the latest release [here](#). You will need an installation of MATLAB R2023b¹ to run it.

Contents

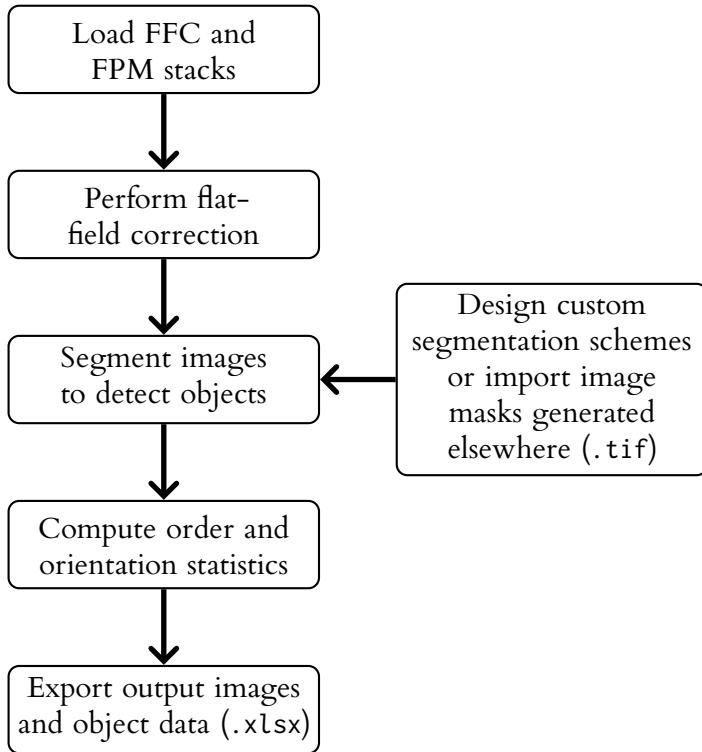
1 Getting Started	3
2 Navigating the Interface	4
2.1 Menubar	4
2.2 Settings Panel	6
2.3 Summary Panel	7
2.4 Selection Listboxes	7
2.5 Threshold Adjustment Panel	8
2.6 Display Area	8
2.7 Log Panel	12
3 Starting a New Project and Importing Data	12
3.1 Create a New Project and Define Groups	12
3.2 Import Image Data	13

¹ <https://www.mathworks.com/downloads/>

4 Processing the Data	13
4.1 Flat-field Correction	13
4.2 Image Segmentation and Object Detection	14
4.3 Computing FPM Statistics	15
5 Mask Adjustment	15
5.1 Image Level Adjustments	15
5.2 Object-Level Adjustments	16
6 Visualizing the Image Data	16
6.1 Average Intensity	16
6.2 Order	17
6.3 Azimuth	18
7 Examining Individual Objects	19
8 Plotting the Object Data	20
8.1 Scatterplot	20
8.2 Scatterplot Matrix	21
8.3 Swarmplot	21
8.4 Polar Histogram	22
9 Object Selection	23
9.1 Select Objects Manually	23
9.2 Select Objects Using Property Filters	24
9.3 Select Objects by Label	24
10 Object Labelling	24
10.1 Create and Apply New Labels Manually	24
10.2 Label Objects Using k-means Clustering	25
10.3 Delete Object Labels	26
10.4 Combine Object Labels	26
11 Designing Custom Segmentation Schemes	26
12 Defining Custom FPM Statistics	27

13 Exporting Data	30
13.1 Image Data	30
13.2 Object Data	31

Quick Guide to Workflow



1 Getting Started

1. Download and install **MATLAB R2023b**. You will also need the following **MATLAB** toolboxes:
 - Curve Fitting Toolbox
 - Image Processing Toolbox
 - Parallel Computing Toolbox
 - Signal Processing Toolbox
 - Statistics and Machine Learning Toolbox
2. Download the latest release of **OOPS** and unzip it. You can find the latest release [here](#).
3. Make sure **OOPS** is discoverable on the **MATLAB PATH**. Open **MATLAB** and click the **HOME** tab in the **MATLAB** toolbar at the top of the editor window. In the **Environment** section, click **[Set Path]**.
4. In the **Set Path** window, click Add with Subfolders.... **MATLAB** will open the file browser.

5. Navigate to the directory where you unzipped the **OOPS** download. Select the unzipped folder and click **[Open]**.
6. In the **Set Path** window, click **[Save]** and then **[Close]**. You are now ready to run **OOPS**.

2 Navigating the Interface

OOPS uses a graphical user interface (GUI) to enable interactive data import, processing, visualization, analysis, and export ([Figure 1](#)). This section defines the different components of the interface and describes how to navigate between **Views**.

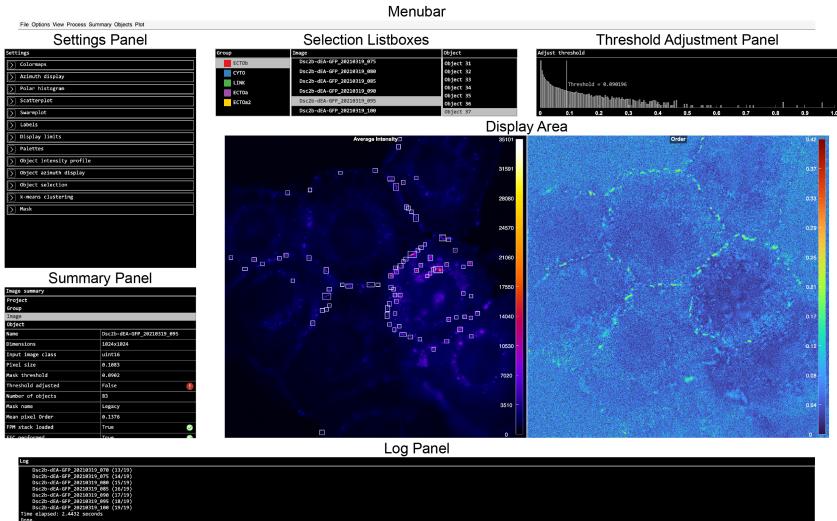


Figure 1: Schematic showing major components of the **OOPS** GUI.

2.1 Menubar

Menus holding controls for data import/export, data processing, data analysis, object selection/labeling, GUI display, etc.

File ...

New project – Start a new project

Load project – Load a previous project from file (.mat)

Save project – Save the current project to file (.mat)

Load FFC stacks – Import FFC stacks for the currently selected group

Load FPM stacks – Import FPM stacks for the currently selected group

Load reference images – Import reference images for the currently selected image(s)

Load mask images – Import mask images for the currently selected image(s)

Export images – Export various types of output images for the currently selected image(s)

Export object data – Export stacked object data tables (.mat,.xlsx) for the entire project

View ...

Files – Switch to **Files** view

FFC – Switch to **FFC** view

Mask – Switch to **Mask** view

Order – Switch to **Order** view

Azimuth – Switch to **Azimuth** view

Plots – Switch to **Plots** view

Polar Plots – Switch to **Polar Plots** view

Objects – Switch to **Objects** view

Process ...

Flat-field correction – Perform flat-field correction for selected images

Build mask – Segment the selected images using the current segmentation scheme

Compute FPM statistics – Compute built-in and custom FPM statistics for the selected images

All – Perform all processing steps for all images in the project

Summary – Open a new window with a summary table of all the objects in the project

Objects ...

Select by property – Open a separate window to define property filters to select objects

Delete selected objects ...

In project – Delete all selected objects

In group – Delete selected objects in the current group

In image – Delete selected objects in the current image

Clear selection ...

In project – Deselect all objects

In group – Deselect objects in the current group

In image – Deselect objects in the current image

Label with k-means clustering – Perform k-means clustering and relabel objects according to cluster

Show object images by label – Open a separate scrollable window showing all object intensity images grouped by label

Plot ...

Group scatter plot matrix – Select and plot multiple object properties in a grid of scatterplots

Object intensity profile – Plot the object pixel intensity sinusoidal fit plot for the current object in a separate window

Azimuth stick plot – Plot the **Average Intensity** image with overlaid azimuth sticks in a separate window

Images – Choose from various types of output images to plot in a separate window

2.2 Settings Panel

The **Settings Panel** is an accordion-style container with expandable items holding controls for various display/processing options. The list below gives the name of each item and the settings it controls.

1. **Colormaps** – Select the colormaps/lookup-tables(LUTs) used to display each type of image.
2. **Palettes** – Select the color palettes for groups and labels.
3. **Display limits** – Adjust the dynamic display range for different image types.
4. **Labels** – Create, delete, merge, apply, and edit object labels.
5. **Mask** – Change the active segmentation scheme.
6. **Object selection** – Adjust the appearance of object selection boxes.
7. **k-means clustering** – Select options for k-means clustering.
8. **Swarmplot** – Adjust the appearance of the swarmplot shown in the **Plots** view.
9. **Scatterplot** – Adjust the appearance of the scatterplot shown in the **Plots** view.
10. **Polar histogram** – Adjust the appearance of the polar histograms shown in the **Polar Plots** view.
11. **Azimuth display** – Adjust the appearance of the azimuth sticks shown in the **Azimuth** view.

12. **Object azimuth display** - Adjust the appearance of the azimuth sticks shown in the **Objects** view.
13. **Object intensity profile** - Adjust the appearance of the object intensity fit plot shown in the **Objects** view.
14. **GUI** - Adjust the appearance of the OOPS interface.

2.3 Summary Panel

The **Summary Panel** shows a table summarizing different properties and status indicators for different levels of the data hierarchy. There are four levels to choose from:

- **Project**
- **Group**
- **Image**
- **Object**

2.4 Selection Listboxes

1. **Group Listbox** - Shows the list of groups in the project and their colors, with the current group highlighted ([Figure 2](#)).

To switch to another group, select its name in the listbox. To change the name of a group, double-click its name in the list box, type in a new name, then press **Enter**. To delete a group, right-click its name and select **Delete group**. To add a new group, right-click anywhere in the listbox and select **New group**.

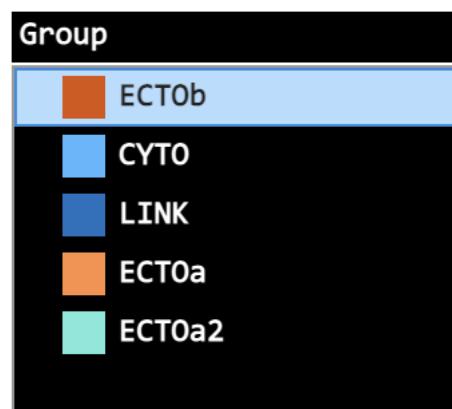


Figure 2: **Group Listbox**

2. **Image Listbox** - Shows the list of images in the current group, with the current image(s) highlighted.

To switch to another image, select its name in the listbox. To select a continuous group of images, click on the name of the first image, then click on the name of another image while holding . To add or remove images from the selection one at a time, click on their names while pressing . To delete the selected image(s), right-click their name(s) and click **Delete selected**. If multiple images are selected, only the first image will be shown in the **Display Area**.

3. **Object Listbox** – Shows the list of objects in the current image, with the current object highlighted.

To switch to another object, select it in the listbox. Only one object can be selected at a time. The object listbox only controls what object is shown in the **Display Area** in the **Objects** view and in the **Summary Panel**. To select objects for deletion/labeling, see [section 9](#).

2.5 Threshold Adjustment Panel

The **Threshold Adjustment Panel** contains an intensity histogram plot with a sliding bar for user adjustment of certain image masks. For details, see [subsection 5.1](#).

2.6 Display Area

Dynamic collection of large and small panels to visualize input images, output images, and plots, organized into different **Views**.

Output plots and images are grouped into distinct **Views** and shown in the **Display Area**. This list below describes the content shown in each **View**. To change **Views**, click **View** followed by the name of the desired **View**.

1. **View > Files** – Eight small panels showing the user-uploaded FPM (top) and FFC stacks (bottom) ([Figure 3](#)).

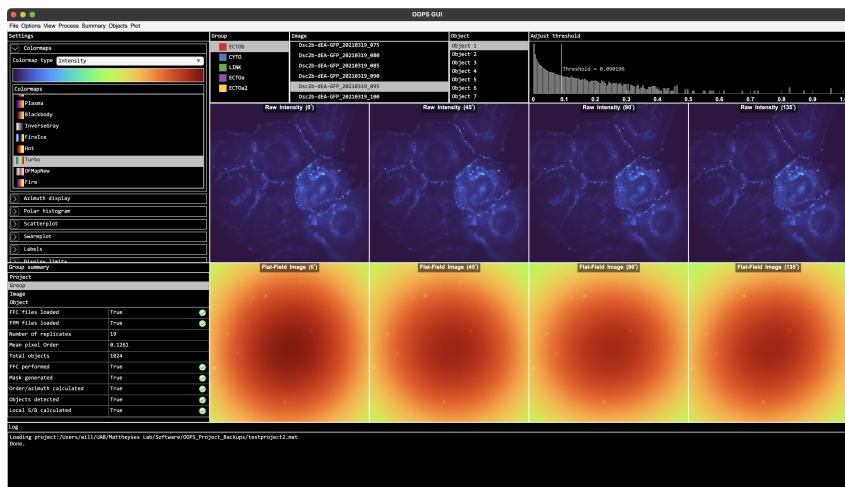


Figure 3: Screenshot showing an example of the **Files** view.

2. **View FFC** – Eight small panels showing the raw FPM stacks (top) and flat-field corrected FPM stacks (bottom) (Figure 4).

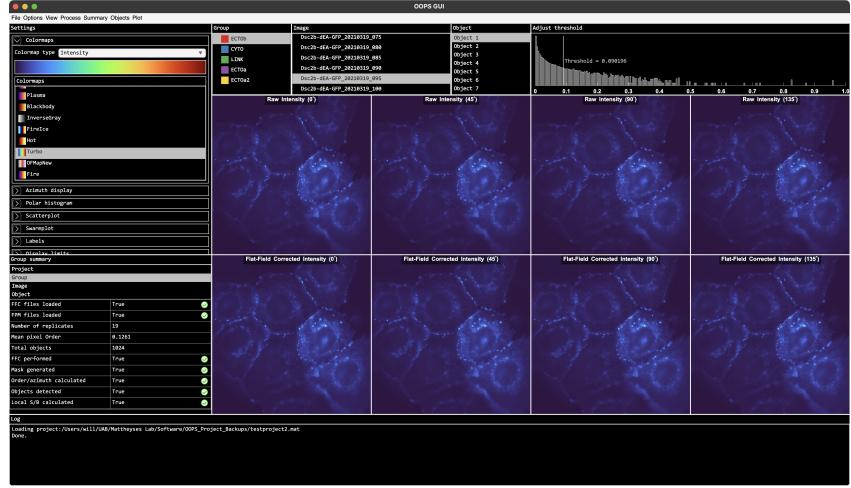


Figure 4: Screenshot showing an example of the **FFC** view.

3. **View Mask** – Two large panels showing the **Average Intensity** (left) and **Mask** images (right) (Figure 5).

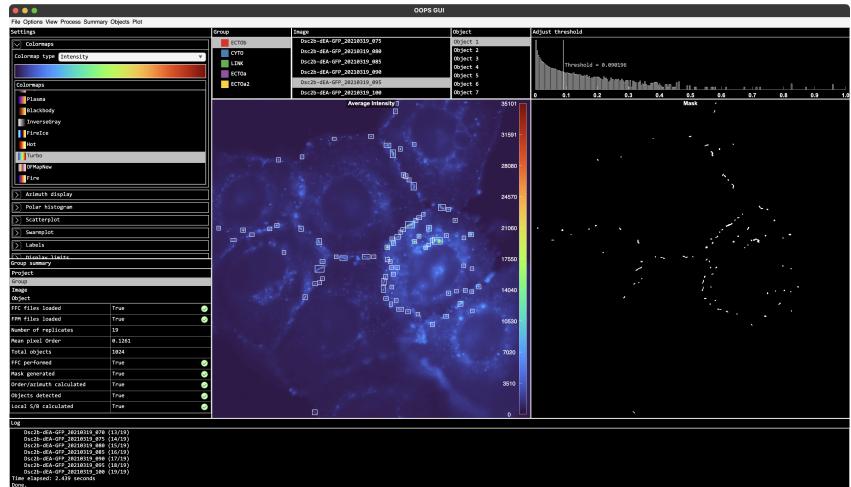


Figure 5: Screenshot showing an example of the **Mask** view.

4. **View Order** – Two large panels showing the **Average Intensity** (left) and **Order** images (right) (Figure 6).

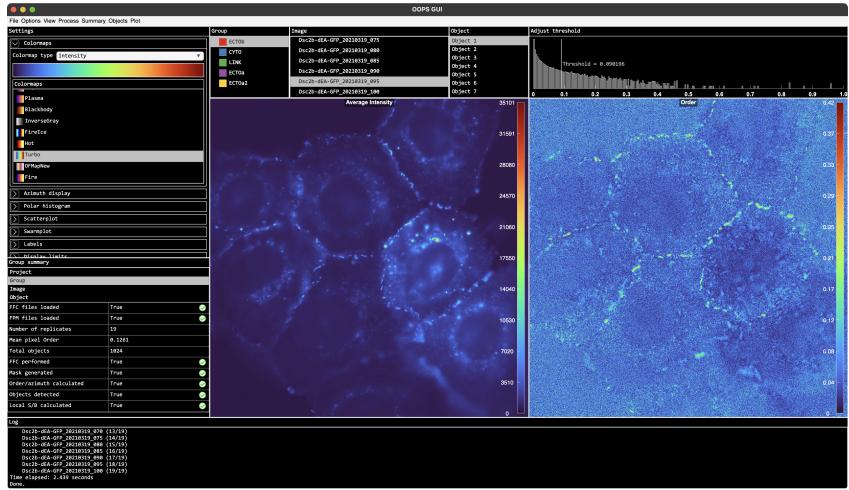


Figure 6: Screenshot showing an example of the **Order** view.

5. **View > Azimuth** – Two large panels showing the **Average Intensity** (left) and **Azimuth** images (right) (Figure 7).

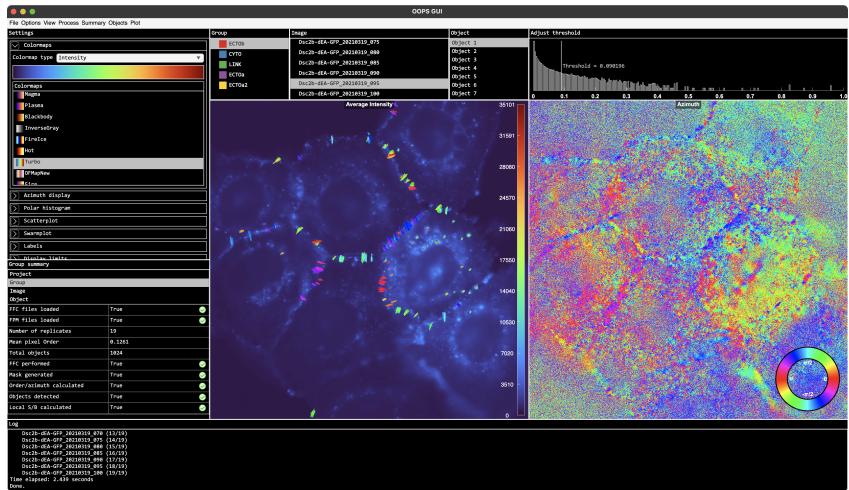


Figure 7: Screenshot showing an example of the **Azimuth** view.

6. **View > Plots** – Two large panels showing the object **Scatterplot** (left) and **Swarmplot** (right) (Figure 8).

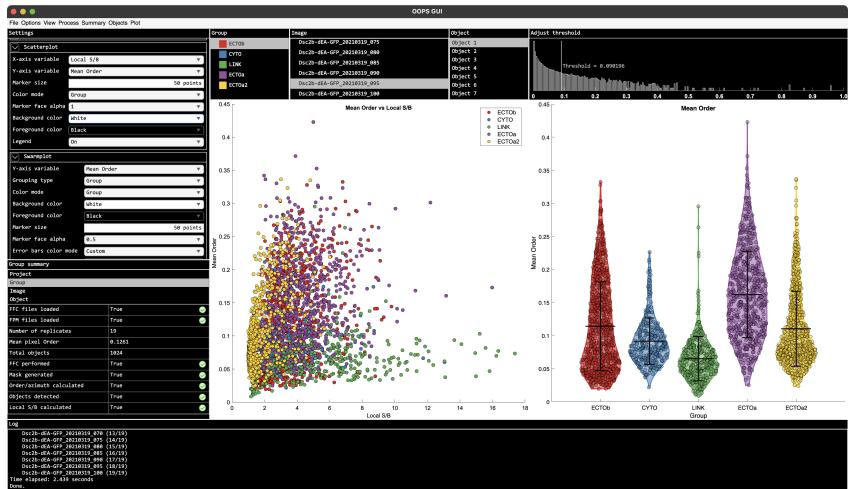


Figure 8: Screenshot showing an example of the **Plots** view.

7. **View > Polar Plots** – Two large panels showing polar histograms of object azimuth data for the current image (left) and group (right) (Figure 9).

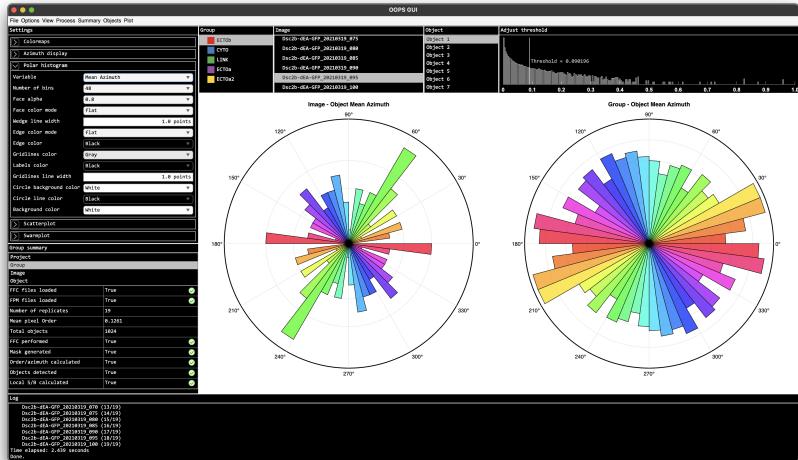


Figure 9: Screenshot showing an example of the **Polar Plots** view.

8. **View > Objects** – Collection of large and small panels showing various object subimages/plots including the normalized average intensity image (top left), mask image with detected midline (top middle), order image (bottom left), azimuth stick overlay (bottom middle), normalized intensity stack (top right), and a plot of object pixel intensities fit to sinusoids (bottom right) (Figure 10).

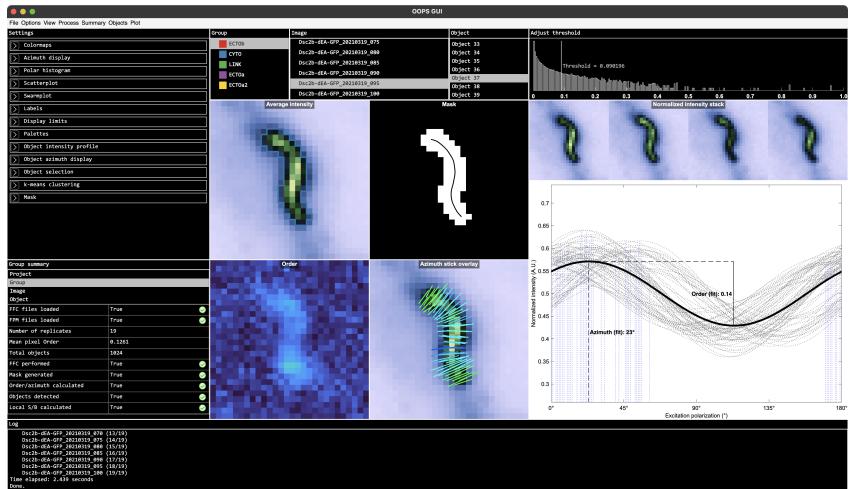


Figure 10: Screenshot showing an example of the **Objects** view.

2.7 Log Panel

The **Log Panel** shows various status updates and warnings during different processing and analysis steps.

3 Starting a New Project and Importing Data

3.1 Create a New Project and Define Groups

1. To launch the software, type **OOPS** into the **Command Window** and press **[Enter]**.
2. Once the window appears, start a new project by selecting **File** → **New project** from the menu bar or press **[⌘]+[N]**.
3. OOPS will then prompt you to specify the name of the project and the number of groups. Once you are finished, click **[Continue]**.

|| Avoid using special characters when naming your project. The number of groups must be ≥ 1 . You can always add or remove group later on.
4. OOPS will then ask you to specify the names of each group. Enter a name for each group and click **[Continue]**.

|| Make sure your group names are unique and again avoid special characters.
5. OOPS will display your groups in the **Group Listbox** at the top of the window (Figure 2). The listbox highlights the currently selected group, which will be the first group by default. You can switch between groups by selecting them in the listbox.

3.2 Import Image Data

1. Select **File > Load FFC stacks**. In the file browser, navigate to the directory containing the FFC stacks for the selected group. Select them all at once and click **Open**.

OOPS will open the images, average them along each excitation polarization, and then normalize the result. The normalized, average FFC stack will then appear in the bottom half of the display area. If you do not select all of the FFC stacks, or if you select the wrong files, just repeat this step and the incorrect files will be replaced.

2. Select **File > Load FPM stacks** from the menu bar. In the file browser, navigate to the directory containing the FPM stacks for the current group. Select them all at once and click **Open**.

OOPS will open the selected files and populate the **Image Listbox** with the names of each FPM stack. The currently selected FPM stack will then appear in the upper half of the display area. Navigate between the images by clicking on their names in the listbox. When the listbox is active, you can also navigate using the up/down arrow keys.

3. Click through each image to make sure they were imported correctly.

If you load additional FPM stacks, they will be added to the end of the list. If you need to delete any images, select them, then right-click in the listbox and click **Delete selected**.

4. Navigate between groups using the **Group Listbox** and repeat steps 1-3 for each group in the project.

4 Processing the Data

Once you have loaded the raw FFC and FPM stacks, there are three base processing steps to perform. To quickly perform all processing steps for all images in the project using default settings, just click **Process > All**.

To run each step individually for the currently selected images, you will select it from the **Process** menu in the menu bar. During each step, OOPS will display a progress bar dialog box indicating the completion status. After each step, the window will update to reflect the results.

4.1 Flat-field Correction

1. Select all of the images for the current group using the **Image Listbox**.

To select all images in the current group, click on first image in the **Image Listbox**, then click on the last image while holding .

2. Select **Process** \gg **Flat-field correction** to perform the flat-field correction for each selected image.

Unless you upload new FFC stacks, you only need to perform this step once for each image.

4.2 Image Segmentation and Object Detection

OOPS offers several methods for image segmentation. Depending on your sample morphology and desired analyses, you can:

- Segment images using one of the built-in schemes ([step 1](#))
 - Design custom segmentation schemes by linking together a set of pre-defined image operations using `CustomMaskMaker.m` ([step 2](#))
 - Upload binary mask images (***.tif**) from file ([step 3](#))
1. To use built-in schemes, expand the **Mask** settings item in the **Settings Panel**, click on the **Mask type** dropdown menu, and select **Default**. Select the desired scheme from the **Mask name** dropdown menu, either **Puncta**, **Adaptive**, or **Filaments**. Proceed to [step 4](#).

The choice of mask will depend on your specific sample morphology. If the fluorescent structures in your image are small, punctate, and well-separated, choose **Puncta** or **Adaptive**. In general, **Puncta** is recommended, but **Adaptive** might provide better results if the structures are very close to one another or if the intensity varies drastically throughout the image. Alternatively, if your objects are large and mostly linear, choose **Filaments**.

2. To use custom schemes, expand the **Mask** settings item in the **Settings Panel**, click on the **Mask type** dropdown menu, and select **Custom**. Select the desired scheme from the **Mask name** dropdown menu. Proceed to [step 4](#).

For an explanation of how to design custom segmentation schemes, see [section 11](#).

3. To upload masks generated elsewhere, select **File** \gg **Load mask images** \gg **Label 4-connected objects** or **File** \gg **Load mask images** \gg **Label branches**. In the file browser, select a number of mask files matching the number of currently selected images and click **Open**. Proceed to [step 5](#).

Mask files must be 8-bit binary images (***.tif**) with dimensions matching the height and width of your FPM stacks. When you upload masks, OOPS will open the files and label connected components in the image depending on your label type selection. If the objects in your mask are small, punctate, and well-separated, you should select **Label 4-connected objects**. If the objects in your mask represent large, linear structures with multiple connected branches (such as filaments), select **Label branches**.

4. With all images still selected, click **Process** \gg **Build mask** to segment the images using the currently selected scheme.

In addition to segmenting the images, OOPS will label connected components in the mask to construct the objects and then extract a large set of object features. Depending on the segmentation scheme chosen and the size/number of the objects, this is typically the most time-consuming step. However, unless you change segmentation schemes, you will only need to perform this process once. Therefore, if you are experimenting with different masks, it is recommended that you only process a few images at a time until you identify an appropriate scheme.

5. Click through each image to examine the segmentation results.

4.3 Computing FPM Statistics

1. With all images still selected, click **Process > Compute FPM statistics** to compute the pixel-wise FPM output images.

The default FPM statistics calculated by OOPS are **Order** and **Azimuth**. It is possible to define additional custom statistics using the configuration class `customFPMStatistic.m`. To define custom statistics, see [section 12](#).

2. Click through each image to examine the results.

By default, the display will be set to **View > Order**. To view the azimuth images, select **View > Azimuth** from the menu bar.

5 Mask Adjustment

Optionally, masks can be adjusted at the image-level by changing the intensity threshold or at the object-level by selecting and removing undesirable objects. Image-level adjustments will nullify any object-level adjustments, so they should be performed prior to deleting individual objects.

5.1 Image Level Adjustments

1. To view image masks, click **View > Mask**.

The display area will update to show the average intensity image on the left and the mask image on the right.

2. Locate the threshold slider in the upper right corner of the window. To adjust the threshold, click and drag the vertical bar left or right. The mask image will update in real time as you adjust the threshold.

You can only adjust the threshold for the built-in **Puncta** and **Adaptive** schemes, as well as certain custom schemes. The **Puncta** scheme and custom schemes defined with **Otsu** binarization employ a single global threshold; dragging the bar left or right will lower or raise the intensity threshold. Lowering the threshold will include more objects. Conversely, the built-in **Adaptive** scheme or custom schemes using **Adaptive** binarization employ multiple locally defined thresholds; dragging the bar left or right will lower or raise the sensitivity. Increasing the sensitivity will include more objects.

5.2 Object-Level Adjustments

You can perform object-level mask adjustments by selecting one or more objects, and then deleting them.

1. Select the objects you wish to delete.

| There are multiple ways to select objects. For details, see [section 9](#).

2. Click **Objects** > **Delete selected** > **In project** or press **⌘ + D**.

| This will delete all selected objects across the entire project. If you only want to delete selected objects in the current image, click **Objects** > **Delete selected** > **In image**. To delete selected objects in the current group, click **Objects** > **Delete selected** > **In group**.

6 Visualizing the Image Data

There are three main types of pixel-wise output images:

- **Average Intensity**
- **Order**
- **Azimuth**

The **Average Intensity** image can be visualized from several different **Views** and will always be shown on the left-hand side of the **Display Area**. The **Order** and **Azimuth** images have dedicated **Views** and will be shown on the right-hand side of the **Display Area**. The **Azimuth** data can also be shown as sticks overlaid on the **Average Intensity** image.

6.1 Average Intensity

1. To view the **Average Intensity** image, click **View** > **Mask**, **View** > **Order**, or **View** > **Azimuth**.
2. To change the colormap used to display the image, expand the **Colormaps** settings item in the **Settings Panel**, click **Intensity** in the **Colormap type** dropdown menu, and select the desired colormap from the **Colormaps** listbox.
3. To adjust the dynamic display range of the image, expand the **Display limits** settings item and drag the thumbs of the **Average Intensity** slider to change the minimum and maximum display limits.
4. To show or hide the colorbar, click the  icon in the toolbar.
5. To apply the object mask to the displayed image, click the  icon in the toolbar.

6. To overlay the **Average Intensity** image and its **Reference** image, click the  icon in the toolbar.

This will only work if you have uploaded a **Reference** image for the currently selected image. You can adjust the colormap and display limits of the **Reference** image the same way you did for the **Average Intensity** image.

7. To enable pan/zoom behavior, click the  icon in the toolbar.

As you move your cursor through the image, a bar at the bottom of the image will display the (x,y) coordinates and value of the pixel under the cursor. To increase zoom, left-click. To decrease zoom, right-click. To freeze the image at the current position, left-click while holding . To undo the freeze, left-click again while holding . To temporarily switch to the neutral (no zoom) position, double-click. To change the focus of the pan/zoom behavior while it is active, click the  icon in the toolbar of another image. To disable pan/zoom, click the  icon in the toolbar of the image where it is active.

8. To perform a linescan, click the  icon in the toolbar.

OOPS will draw an interactive linear ROI on the image and open a new window to plot the linescan. Click on the endpoints of the line and drag to change its angle or length, or click on the center of the ROI line and drag to translate it. The linescan plot will update in real time as you adjust the line. For the **Average Intensity** image, the plot shows the normalized, integrated pixel values. When finished, click the  icon again or close the linescan window.

9. To export the **Average Intensity** image, click the  icon in the toolbar.

OOPS will prompt you to specify a location and filename for the exported file. This will export the current view of the image at 600 dpi as it appears on your screen, including all annotations (object boxes, colorbars, etc.). If you want to export the actual image data with native resolution, see [subsection 13.1](#).

6.2 Order

1. To view the **Order** image, click .
2. To change the colormap used to display the image, expand the **Colormaps** settings item in the **Settings Panel**, click  in the **Colormap type** dropdown menu, and select the desired colormap from the **Colormaps** listbox.
3. To adjust the dynamic display range of the image, expand the **Display limits** settings item and drag the thumbs of the **Order** slider to change the minimum and maximum display limits.
4. To automatically set the display limits, click the  icon in the toolbar.

When active, the **Order** image will automatically be scaled to the range [0, max], where "max" is the maximum value across all pixels. This behavior will persist until you click the  icon again, even if you switch to another image.

5. To show or hide the colorbar, click the  icon in the toolbar.
6. To apply the object mask to the displayed image, click the  icon in the toolbar.
7. To enable pan/zoom behavior, click the  icon in the toolbar.
8. To perform a linescan, click the  icon in the toolbar.

OOPS will draw an interactive linear ROI on the image and open a new window to plot the linescan. Click on the endpoints of the line and drag to change its angle or length, or click on the center of the ROI line and drag to translate it. The linescan plot will update in real time as you adjust the line. For the **Order** image, the plot shows the average pixel values. If the object mask is applied, the average will only consider pixels within the mask. When finished, click the  icon again or close the linescan window.

9. To show the **Order-Intensity** overlay, click the  icon in the toolbar of the **Order** image.

This will overlay the **Order** and **Average Intensity** images, with the latter acting as an opacity mask. Adjust the **Order** display limits by dragging the thumbs of the **Order** slider. Adjust the brightness of the image by dragging the thumbs of the **Average Intensity** slider.

10. To export the **Order** image, click the  icon in the toolbar.

OOPS will prompt you to specify a location and filename for the exported file. This will export the current view of the image at 600 dpi as it appears on your screen, including all annotations (object boxes, colorbars, etc.). If you want to export the actual image data with native resolution, see [subsection 13.1](#).

6.3 Azimuth

1. To view the **Azimuth** image, click .
2. To change the colormap used to display the image, expand the **Colormaps** settings item in the **Settings Panel**, click **Azimuth** in the **Colormap type** dropdown menu, and select the desired colormap from the **Colormaps** listbox.
3. To show or hide the colorbar, click the  icon in the toolbar.
4. To apply the object mask to the displayed image, click the  icon in the toolbar.
5. To enable pan/zoom behavior, click the  icon in the toolbar.

6. To show the **Azimuth-Intensity** overlay, click the  icon in the toolbar of the **Azimuth** image.

This will overlay the **Azimuth** and **Average Intensity** images, with the latter acting as an opacity mask. Adjust the brightness of the image by dragging the thumbs of the **Average Intensity** slider.

7. To show the **Azimuth-Order-Intensity** HSV overlay, click the  icon in the toolbar of the **Azimuth** image.

This will display an HSV image where the **Azimuth**, **Order**, and **Average Intensity** are used to set the hue, saturation, and value (brightness), respectively. Adjust the brightness of the image by dragging the thumbs of the **Average Intensity** slider. Adjust the saturation of the image by dragging the thumbs of the **Order** slider.

8. To export the **Azimuth** image, click the  icon in the toolbar.

OOPS will prompt you to specify a location and filename for the exported file. This will export the current view of the image at 600 dpi as it appears on your screen, including all annotations (object boxes, colorbars, etc.). If you want to export the actual image data with native resolution, see [subsection 13.1](#).

9. To adjust the appearance of the **Azimuth** stick overlay, expand the **Azimuth display settings** item in the **Settings panel**.

The **Azimuth** sticks are shown on the **Average Intensity** image and will update as you change the appearance options. To export the stick overlay image, click the  icon in the **Average Intensity** image toolbar.

7 Examining Individual Objects

1. To quickly examine individual objects, switch to the **Objects** view ([Figure 10](#)) by clicking  **View > Objects**.

2. Navigate between the objects in the current image by selecting them in the **Object Listbox**.

If the **Object Listbox** is active, it will be highlighted and you can quickly switch between objects using the up/down arrow keys.

3. To view a more detailed list of properties for the current object, click **Object** in the listbox at the top of the **Summary Panel**.

You can also view this table from any of the other **Views**.

4. To change the appearance of the object **Azimuth** sticks, expand the **Object azimuth display** item in the **Settings Panel**.

5. To adjust the display limits of the object **Order** image or change the visibility of the colorbar, follow the same steps described in [subsection 6.2](#).

If you click the  icon, the pixel values will automatically scale to the maximum value across the whole **Order** image, not just the object subimage.

6. To change the appearance of the plot of object pixel intensities fit to sinusoids, use the **Object intensity profile** section in the **Settings Panel**.

8 Plotting the Object Data

8.1 Scatterplot

Use the **Scatterplot** when you want to examine the relationship between two different object properties.

1. To view the **Scatterplot**, click  **Plots**.

The **Scatterplot** will be shown in the left-hand panel in the **Display Area**.

2. To change the appearance of the **Scatterplot**, use the **Scatterplot** section in the **Settings Panel**.

You can adjust various aspects of the plot appearance including marker color, marker size, marker transparency, plot background color, plot foreground color, etc. The list below only covers a subset of appearance options.

3. To change the data plotted in the graph, select the desired variables from the **X-axis variable** and **Y-axis variable** dropdown menus.

4. To color plot markers based on the group each object belongs to, open the **Color mode** dropdown menu and select .

The colors of each group are shown in the **Group Listbox**. You can modify the group color palette using the **Palettes** section of the **Settings Panel**.

5. To color plot markers based on the label of each object, open the **Color mode** dropdown menu and select .

The colors of each group are shown in the **Label Listbox** in the **Labels** section of the **Settings Panel**. You can modify the label color palette using the **Palettes** section of the **Settings Panel**.

6. To copy the plot to the clipboard as a vector graphic, right-click on the plot and select .

You can easily paste the copied plot into a presentation, document, or external editing software, where you can further adjust the appearance of the plot.

8.2 Scatterplot Matrix

Use the **Scatterplot Matrix** when you want to examine the relationships between several variables at once.

1. To plot the **Scatterplot Matrix**, click **Plot > Group scatterplot matrix** in the **Menubar**.

| OOPS will open a new window for you to specify plot options.

2. In the pop-up window, select the variables you wish to compare in the **Variables** checkbox.

| You must select at least two variables.

3. Specify how the plot markers will be colored using the **Color mode** dropdown menu.

| If you select **Group**, individual markers will be colored according to the group each object belongs to. If you select **Label**, markers will be colored by the label of each object. You can modify group and label colors using the **Palettes** section of the **Settings Panel**.

4. Specify the display type of the diagonal plots in the matrix using the **Diagonal display** dropdown menu.

| If you select **Variable names**, diagonal plots will display the name of each variable. If you select **Grouped histograms** or **Grouped histogram outlines**, diagonal plots will show a series of overlapping histograms for each variable, colored based on the **Color mode** you selected. You can modify group and label colors using the **Palettes** section of the **Settings Panel**.

8.3 Swarmplot

Use the **Swarmplot** when you want to compare the distributions of a particular object property between different groups and/or labels.

1. To view the **Swarmplot**, click **View > Plots**.

| The **Swarmplot** will be shown in the right-hand panel in the **Display Area**.

2. To change the appearance of the **Swarmplot**, use the **Swarmplot** section in the **Settings Panel**.

| You can adjust various aspects of the plot appearance including marker color, marker size, marker transparency, violin color, violin visibility, plot background color, plot foreground color, etc. The list below only covers a subset of appearance options.

3. To change the data plotted in the graph, select the desired variable from the **Y-axis variable** dropdown menu.

4. To compare distributions between groups, open the **Grouping type** dropdown menu and select **Group**.
 - || All of the objects in a single group will be plotted together.

5. To compare distributions between labels in a single group, open the **Grouping type** dropdown menu and select **Label**.
 - || All of the objects in the current group will be grouped according to their labels before plotting.

6. To compare distributions between labels and groups, open the **Grouping type** dropdown menu and select **Both**.
 - || All of the objects across all groups will be grouped according to their labels before plotting. If you have many groups and/or labels, the grouping type could produce a large number of individual plots.

7. To color plot markers based on the group each object belongs to, open the **Color mode** dropdown menu and select **Group**.
 - || The colors of each group are shown in the **Group Listbox**. You can modify the group color palette using the **Palettes** section of the **Settings Panel**.

8. To color plot markers based on the label of each object, open the **Color mode** dropdown menu and select **Label**.
 - || The colors of each group are shown in the **Label Listbox** in the **Labels** section of the **Settings Panel**. You can modify the label color palette using the **Palettes** section of the **Settings Panel**.

9. To copy the plot to the clipboard as a vector graphic, right-click on the plot and select **Copy as vector graphic**.
 - || You can easily paste the copied plot into a presentation, document, or external editing software, where you can further adjust the appearance of the plot.

8.4 Polar Histogram

Use the **Polar Histogram** when you want to visualize the distribution of object **Azimuth** data within an image or group.

1. To view the **Polar Histogram**, click **View > Polar Plots**.
 - || The **Image Polar Histogram** will be shown in the left-hand panel in the **Display Area**. The **Group Polar Histogram** will be shown in the right-hand panel in the **Display Area**.

2. To change the appearance of the **Polar Histogram**, use the **Polar histogram** section in the **Settings Panel**.

You can adjust various aspects of the plot appearance including edge color, number of bins, transparency, plot background color, plot foreground color, grid line colors, etc. The steps below only cover how to adjust the **Variable** and **Number of bins**.

3. To change the data plotted in the graph, select the desired variable from the **Variable** dropdown menu.
4. To change the number of bins the data are grouped into, select the desired number of bins from the **Number of bins** dropdown menu.

9 Object Selection

There are a few different ways to select objects:

- Select objects manually in each image ([subsection 9.1](#))
- Select objects by defining property-based selection criteria ([subsection 9.2](#))
- Select objects with specific labels ([subsection 9.3](#))

9.1 Select Objects Manually

1. To select objects manually, begin by clicking **View** **Mask** in the **Menubar**.
2. To show object selection boxes/boundaries, click the icon in the toolbar of the **Average Intensity** image.
3. To select a single object at a time, click on its enclosing selection box.

Continue clicking objects to add them to the selection. To change the appearance of the selection boxes, use the **Object selection** section in the **Settings Panel**.

4. To select multiple objects at once, enable the lasso tool by clicking the icon in the toolbar of the **Average Intensity** image. Click and drag your cursor on the image to select all of the objects in a specified area.
5. To deselect objects, either click their selection boxes, use the lasso tool, or click **Objects** **Clear selection** **In image** to deselect all objects in the current image.

To deselect all objects in the current group, click **Objects** **Clear selection** **In group**. To deselect all objects across all groups, click **Objects** **Clear selection** **In project**.

9.2 Select Objects Using Property Filters

1. To select objects with a specific set of properties, click .

■ OOPS will open the **Define property filters** window.

2. In the **Define property filters** window, select a property and relationship from the dropdown menus and enter a value in the editfield.

3. To add another property filter, click and repeat step 2.

4. To delete an existing filter, click .

■ There must be at least one filter. To exit without selecting objects, click or close the window.

5. When you are finished, click .

■ The **Log Window** will update to indicate how many objects were selected.

9.3 Select Objects by Label

1. To select objects by label, expand the **Labels** section of the **Settings Panel**.

2. Select the label(s) of the objects you want to select in the **Object labels** listbox.

■ To select a continuous group of labels, click the name of the first label, then click the name of another label while holding . To select multiple labels one at a time, click on their names while holding .

3. Right-click on one of the selected labels and select .

■ All objects with the selected label(s) will be added to the selection. Any previously selected objects will remain selected.

10 Object Labelling

10.1 Create and Apply New Labels Manually

1. To create a new object label manually, expand the **Labels** section of the **Settings Panel**.

2. Right-click one of the labels in the **Object labels** listbox and select from the context menu.

■ OOPS will create a new label with a default name.

3. To change the name of the label, double-click its name in the **Object labels** listbox, type in a new name, and press .

4. To apply the new label to the currently selected objects, right-click the name of the label and click **Apply label to selected objects**.

The existing label(s) of the selected objects will be overwritten with the new label.

10.2 Label Objects Using k-means Clustering

1. To label objects using k-means clustering, expand the k-means clustering section of the **Settings Panel**.
2. In the **Variables** listbox, select the object variables you want to use for clustering.
3. To specify how the value of **k** is set, use the **k selection mode** dropdown menu and choose either **Auto** or **Manual**.
4. If the **k selection mode** is set to **Manual**, specify **k** in the editfield.
5. If the **k selection mode** is set to **Auto**, specify the evaluation criterion in the **Criterion** dropdown menu.

The choice of criterion will affect the resulting value of **k**. For **CalinskiHarabasz** see [here](#). For **DaviesBouldin** see [here](#). For **silhouette**, see [here](#).

6. Choose the desired distance metric from the **Distance metric** dropdown menu.
For a description of different distance metrics, see [here](#).

7. Specify whether or not the data should be normalized prior to clustering using the **Normalization** dropdown menu.

It is generally recommended that you should normalize the data using the **zscore** method prior to clustering, especially if your chosen variables differ significantly in their absolute magnitudes.

8. Specify whether or not to display evaluation plots for the clustering solution using the **Display evaluation** dropdown menu.

Even if you select **No**, OOPS will display a stacked bar graph showing the breakdown of clusters within each group.

9. To run k-means clustering with the selected settings, select **Objects** **> Label objects with k-means clustering** from the **Menubar**.

OOPS will create new labels for each cluster and automatically assign them to the objects. **This will replace any existing labels you have created previously.**

10.3 Delete Object Labels

1. Select the label(s) you wish to delete using the **Object labels** listbox in the **Labels** section of the **Settings Panel**.
2. To delete the label(s) and their associated objects, right-click one of the selected labels and click **Delete label(s) and objects**.
 - | There must be at least one object label.
3. To delete the label(s) without deleting their associated objects, right-click one of the selected labels and click **Delete label(s)**.
 - | Any objects with the deleted label(s) will be relabelled with the **Default** label.

10.4 Combine Object Labels

1. Select the labels you wish to merge using the **Object labels** listbox in the **Labels** section of the **Settings Panel**.
2. To merge the selected labels, right-click one of the selected labels and select **Merge selected labels** from the context menu.
 - | Merging requires at least two selected labels. The name of the resulting label will depend on which label you clicked to open the context menu.

11 Designing Custom Segmentation Schemes

Using `CustomMaskMaker.m`, you can define custom segmentation schemes by linking together a set of predefined image operations. For each operation, you will need to specify the operation Type, the operation Name, the operation Target, and various parameters specific to the operation. This can be done independently or from within OOPS. The steps below describe how to design schemes from within OOPS.

1. In OOPS, navigate to an image for which you want to define a custom scheme and select it in the **Image Listbox**.
 - | Make sure you have only selected one image and that it has already been flat-field corrected. The image you choose will be used as an example when designing the scheme, but you can apply the scheme to any of the other images once you are done.
2. With the desired image selected, click **File > New segmentation scheme** and OOPS will open `CustomMaskMaker.m`.
3. Select the operation Type using the Operation Type listbox in the upper right of the window.

- When you change the operation Type, the Operation Name listbox will populate with the available operations for that Type.
- 4. Select the specific operation you wish to perform by selecting it in the Operation Name listbox in the middle right of the window.
- When you change the operation Name, the Operation Parameters panel will populate with the available parameters for that operation.
- 5. Select the Target for the operation using the Target dropdown menu.
- The Target specifies which image you wish to perform the operation on. The existing lists of images and operations are shown in panels on the left side of the window.
- 6. Specify any operation-specific parameters shown in the Operation Parameters panel.
- 7. To add your new operation to the scheme, click the `Add to scheme` button.
- 8. Repeat step 3-step 7 to add more operations to the scheme.
- To delete an operation, select it in the Operations panel in the lower left of the window then click `Edit > Delete operation` or press `⌘ + D`. To edit an operation, select it, make the desired changes, then click the `Edit operation` button.
- 9. Once you are finished, close the window to return to OOPS.
- The final output of your scheme represents your mask and must be a binary image. If you use the Otsu or Adaptive thresholding operations to binarize your image, you will be able to further adjust the threshold of each image in OOPS.
- 10. To make the new scheme active and apply it to your other images, see step 2 of subsection 4.2.

12 Defining Custom FPM Statistics

The built-in order statistic, `Order`, is defined based on a specific imaging setup. If your microscope configuration requires computing the order statistic in a different way, you can do so by defining a custom statistic using the configuration class `CustomFPMStatistic.m`. Alternatively, you can use this method to perform custom preprocessing of the image data before the statistic is calculated.

This method should only be used to define custom order statistics. The way the orientation (`Azimuth`) statistic is calculated is virtually identical across many different imaging setups. Note that any custom preprocessing steps defined using `CustomFPMStatistic.m` will only apply to the specific statistic. If you want to change how data are processed prior to computing the

built-in statistics or how the **Azimuth** is calculated, you will need to edit the `FindFPMStatistics` method of the `OOPSImage.m` class.

For each statistic you will define, you will specify four properties:

- **StatisticName** – Name of the statistic used internally by the software (no spaces or special characters)
- **StatisticDisplayName** – Name of the statistic used in image titles, plot labels, tables, etc.
- **StatisticFun** – Handle to the function which calculates your statistic.

Your function must accept a single flat-field corrected FPM stack (`mxnx4 double`) and return the pixel-wise calculation of your statistic (`mxn double`).

- **StatisticRange** – The expected output range of the statistic, used for displaying the image.

Values in the output image returned by your function will be clipped to the range specified by **StatisticRange**.

Use the steps below to create your own custom statistic. In this example, order is calculated in the same way as OOPS, but the image data are denoised with a simple non-local means filter prior to doing the calculations.

1. Create a new script and type the following into the **Editor** window.

```
function out = findDenoisedOrder(in)

% denoise each input frame with
% a non-local means filter
for i = 1:4
    in(:,:,i) = imnlmfilt(in(:,:,i));
end

% compute the order
A = in(:,:,1) - in(:,:,3);
B = in(:,:,2) - in(:,:,4);
C = sum(in,3)./2;
out = hypot(A,B)./C;

end
```

Listing 1: `findDenoisedOrder.m`

2. Save the script as `findDenoisedOrder.m`.

It does not matter where you save the file, just make sure it is accessible on the **MATLAB PATH**.

3. Define the **StatisticName**.

```
>> StatisticName = 'dnOrder';
```

4. Define the **StatisticDisplayName**.

```
>> StatisticDisplayName = 'Denoised Order';
```

5. Define the **StatisticFun**.

```
>> StatisticFun = @findDenoisedOrder;
```

6. Define the **StatisticRange**.

```
>> StatisticRange = [0,1];
```

7. Create a new **CustomFPMStatistic** with the properties you just defined.

```
>> dnOrder = CustomFPMStatistic(...  
StatisticName,...  
StatisticDisplayName,...  
StatisticFun,...  
StatisticRange);
```

8. Navigate to the following directory, where **userPath** is the path to the folder containing **OOPS.m**.

userPath/assets/custom_statistics

Save the **CustomFPMStatistic** you created.

```
>> save('dnOrder.mat',dnOrder);
```

The next time you run **OOPS**, the software will integrate the new statistic into the GUI and add dynamic properties to the data classes. The statistic you defined will be computed automatically whenever you click **Process > Compute FPM Statistics**. A new **View** will be created with the same name as the **StatisticDisplayName** you defined. To view it, click **View > Denoised Order**. In the **Plots** view, you can view the values of the statistic for each object in the **Swarmplot** or **Scatterplot** by selecting the variable **Mean Denoised Order**. When you export object data tables, a new column will be added to the end of the table containing the computed values of the custom statistic for each object.

13 Exporting Data

13.1 Image Data

There are four main output images categories:

- **Intensity**
- **Order**
- **Azimuth**
- **Mask**

Images in each category can be exported with several options:

- **color mode** – color representation mode of each pixel
 - **grayscale** – each pixel is represented by a single value with no color information
 - **RGB** – each pixel is represented by a red, green, and blue value determined by the associated colormap selected in the software and whether or not the exported image is an overlay
- **bit depth** – number of bits used to define each pixel
 - **8-bit**
 - **16-bit**
 - **32-bit**
- **format** – format of the exported image file
 - **PNG**
 - **TIFF**
- **scaling** – how the pixel values are scaled in the exported image (**none**, **auto**, **user**)
 - **none** – output pixel values are unscaled
 - **auto** – output pixel values are scaled to the range $[0, \max]$, where **max** is the maximum value across all pixels in the image
 - **user** – output pixel values are scaled to the range $[\min, \max]$, where **min** and **max** are determined by the display limits set by the user in the **Display limits** section of the **Settings Panel**

Not all options are available for all image categories. For all **RGB** images, the chosen **scaling** will be applied before the image is colorized. For the **Order-Intensity**, **Azimuth-Intensity**, and **Azimuth-Order-Intensity** **HSV** overlay images, scaling only affects the **Order** and **Intensity** components. Overlay images can only be exported as **RGB** images.

1. To export image data for the currently selected image(s), click **File** ➤ **Export images**.

OOPS will open the **Export Images** window, which contains a checkbox tree listing the different output images.

2. Select the image types you wish to export using the **Image types** checkbox tree in the **Export Images** window, then click **Continue**.

OOPS will open the file browser. Navigate to the directory where you wish to save the images, then click **Open**. OOPS will export the selected image types for each of the images currently selected in the **Image Listbox**.

13.2 Object Data

Once you have finished processing and analyzing the data, you can export object data tables either as a MAT-file (.mat) or an Excel spreadsheet (.xlsx).

1. To export object data tables, click **File** ➤ **Export object data**.
2. In the file dialog, navigate to the directory in which you wish to save the data. Enter a file name and use the dropdown menu to choose the file format (*.mat or *.xlsx). Click **Save**.