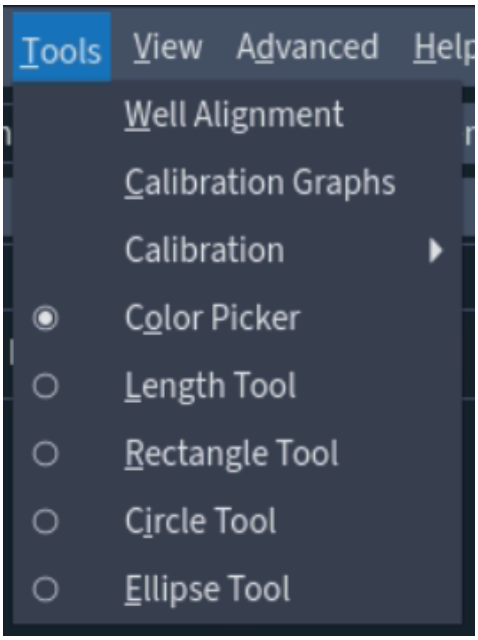
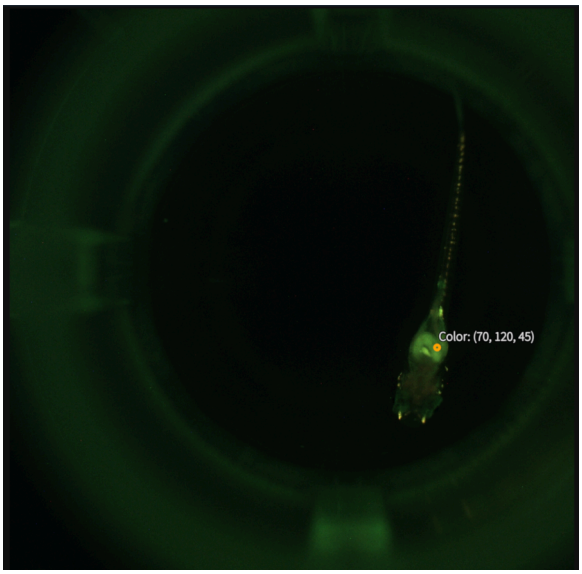
Tools

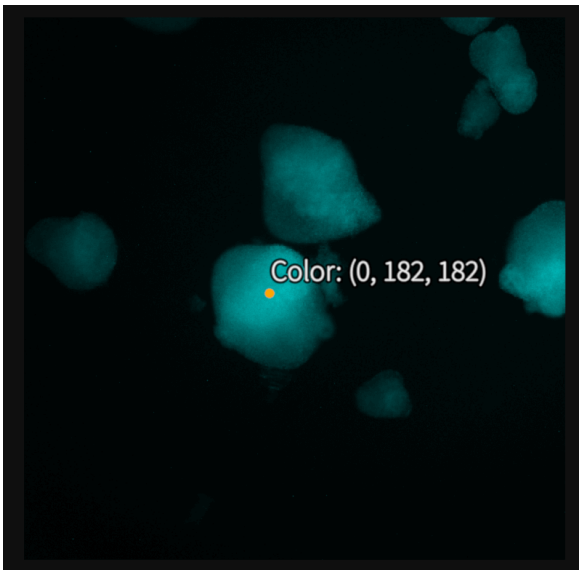
Available both in the **MCAM Acquisition Software** and the **MCAM Viewer Software**.

Color Picker

When the Color Picker tool is selected, clicking on the screen will display the pixel color at this location. Values are displayed as (Red, Green, Blue) integers corresponding to the three color channels of the image.



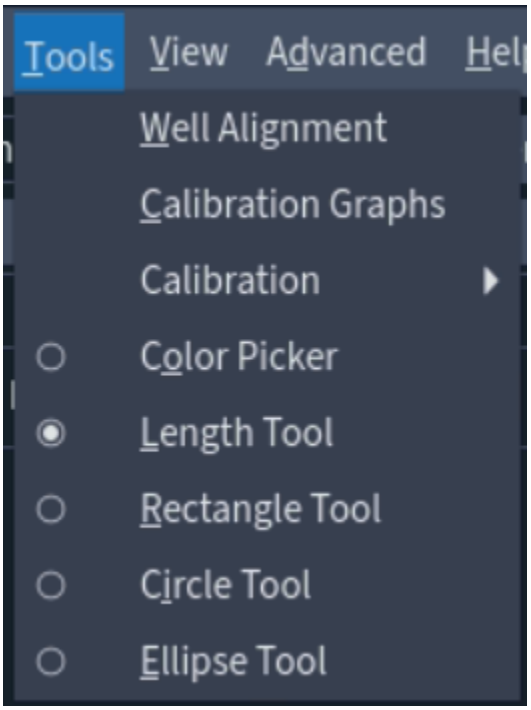


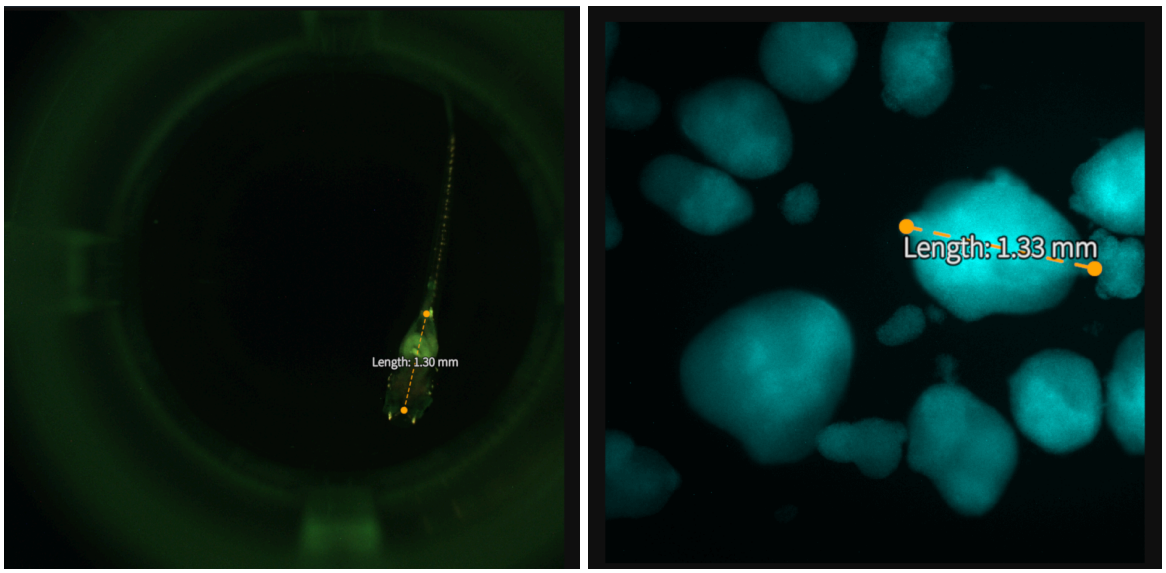


Step	Function
1	**Open the MCAM** software.
2	**Go to Tools > Color Picker**.
3	**Click on a pixel in the image** to get the displayed color at the location.
4	In the MCAM software, **go to View** which will give the user several options to alter the viewing color space. Changing this will alter the pixel values displayed by the color picker.
5	To take another measurement simply **repeat step 3**. To remove the Color Picker **repeat step 2**.

Length Tool

When the Length Tool is selected, straight lines can be measured on the screen. The measurement and unit will be displayed on the screen.

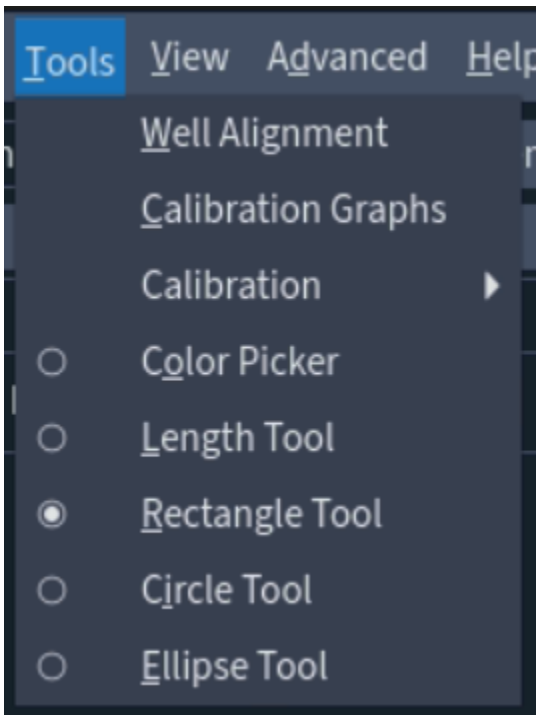


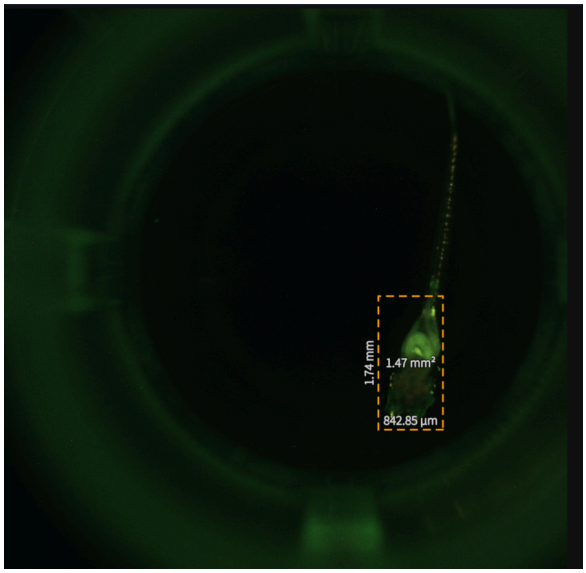


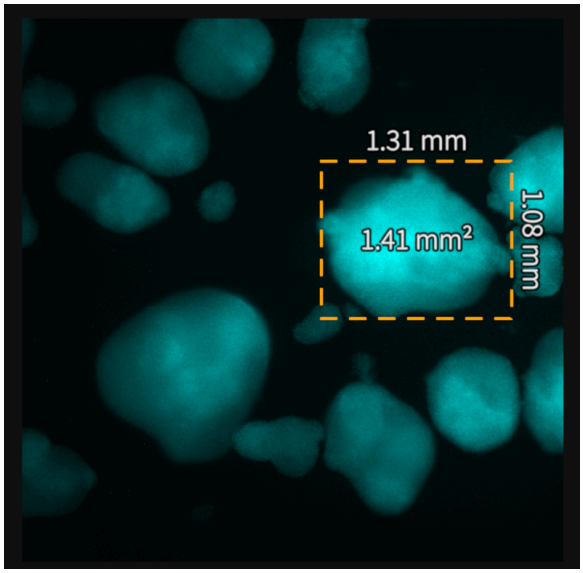
Step	Function
1	Open the **MCAM** software.
2	G**o to Tools > Length Tool**.
3	**Click once with the left mouse button** to define the beginning of the line, **move the cursor** to extend the line and finally **click again with the left mouse button** to define the end of the line.
4	To take another measurement simply **repeat step 3**. To remove the Length Tool **repeat step 2**.

Rectangle Tool

When the Rectangle Tool is selected, rectangles can be drawn across a desired area of the screen. Height, width, area and their corresponding units will be displayed on the screen.





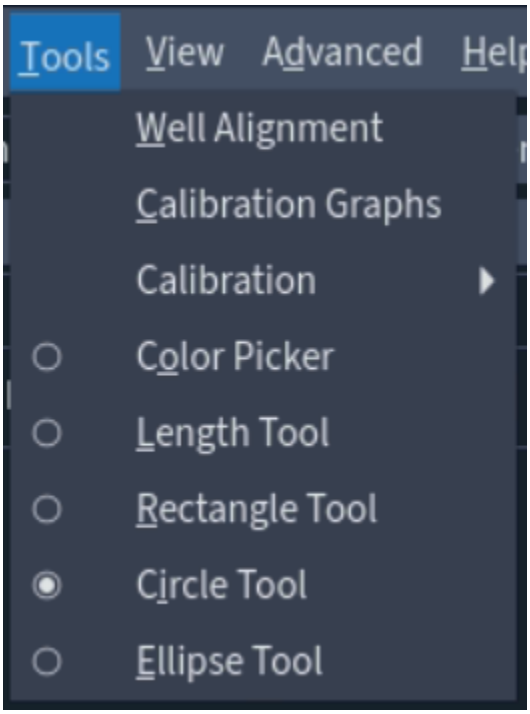


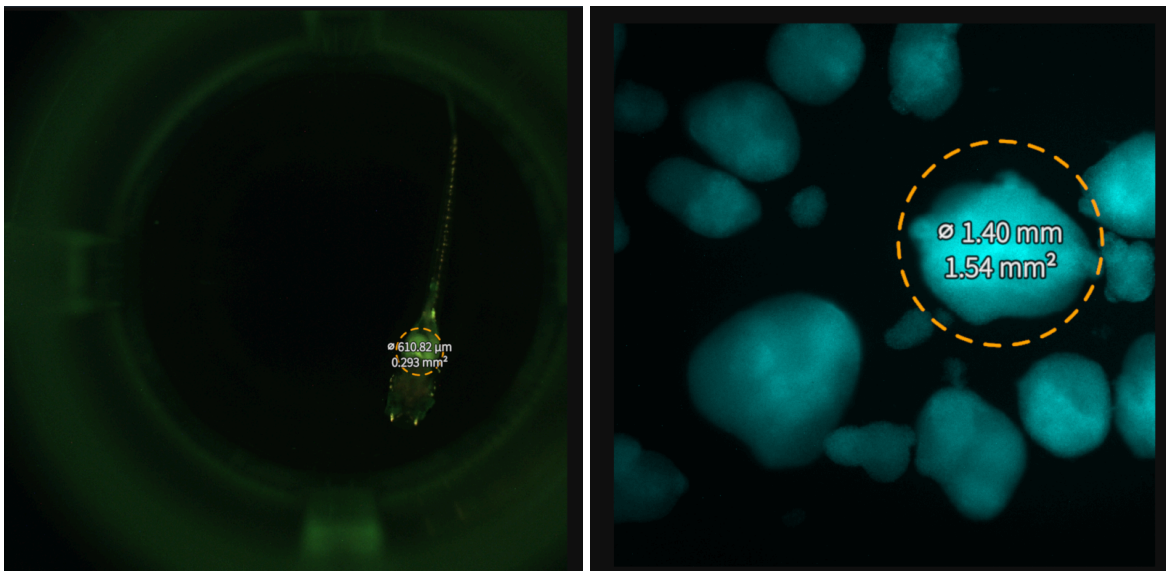
Step	Function
1	**Open the MCAM software.**
2	**Go to Tools > Rectangle Tool**.

3	**Click once with the left mouse button** to define one corner of the rectangle, **move the cursor** to extend the area covered by the rectangle and **click again with the left mouse button** to define the second defining corner of the rectangle.
4	To take another measurement simply **repeat step 3**. To remove the Rectangle Tool **repeat step 2**.

Circle Tool

When the Circle Tool is selected, circles can be drawn across a desired area of the screen. Diameter, area and their corresponding units will be displayed on the screen.

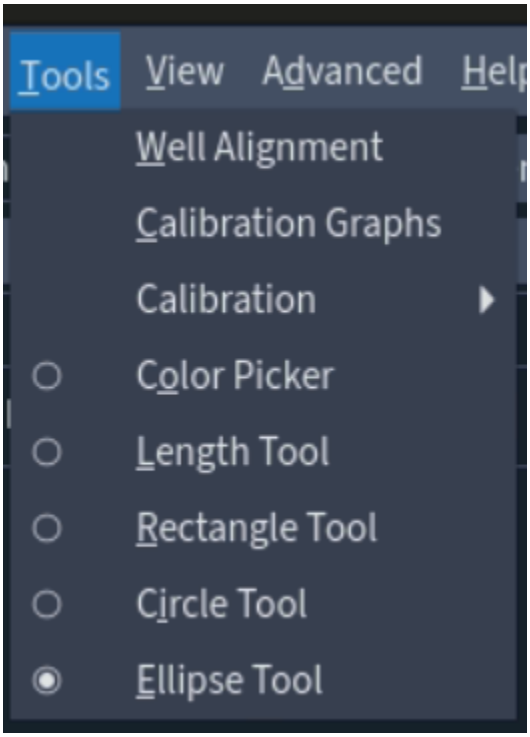


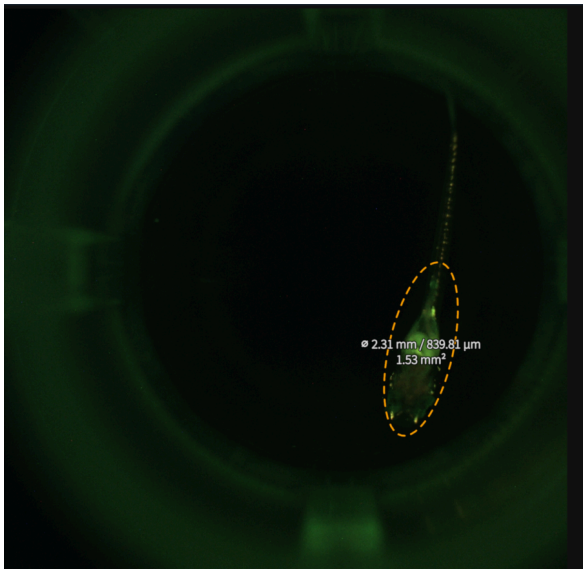


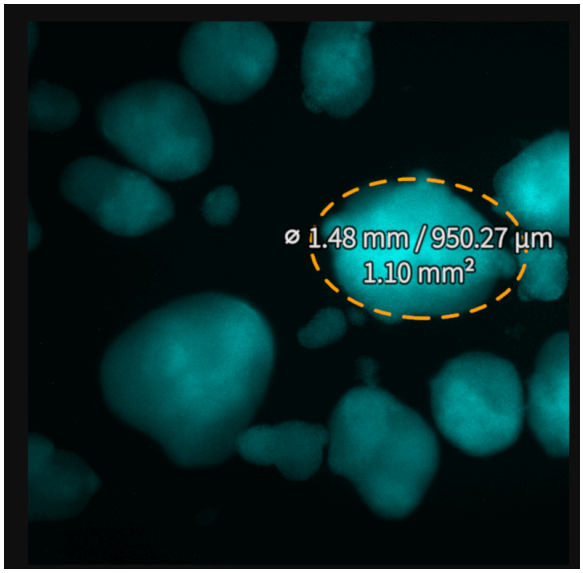
Step	Function
1	**Open the MCAM software.**
2	**Go to Tools > Circle Tool**.
3	**Click once with the left mouse button** to define the center of the circle, **move the cursor** to extend the circle's radius and finally click once with the left mouse button a second time to define the final shape of the circle.
4	To take another measurement simply **repeat step 3**. To remove the Circle Tool **repeat step 2**.

Ellipse Tool

When the Ellipse Tool is selected, ellipses can be drawn across a desired area of the screen. Width, height, area and their corresponding units will be displayed on the screen.





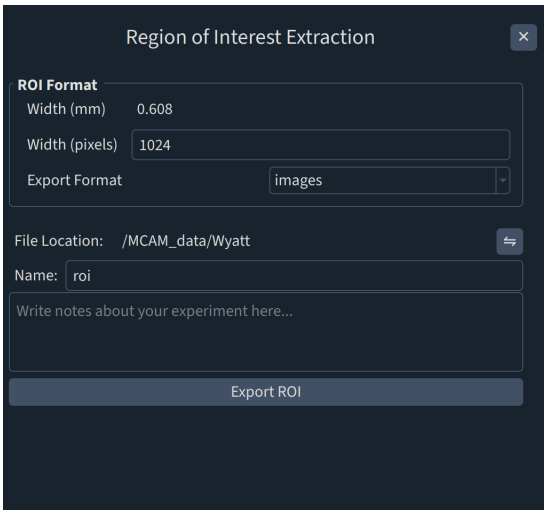


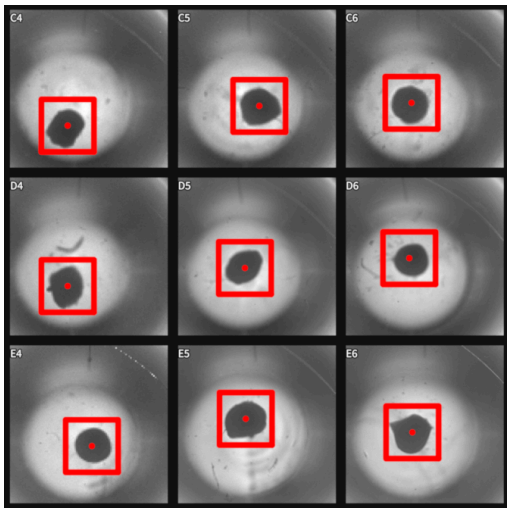
Step	Function
1	**Open the MCAM software.**
2	**Go to Tools > Ellipse Tool**.

3	**Start by creating a circle** by clicking once with the left mouse button to define the center of the circle. **Move the cursor** to extend the circle's radius and finally **click once with the left mouse button** a second time to define the final shape of the circle.
4	Once the desired circle is created, **click once with the left mouse button** to shape the ellipse based on this circle. **Move the cursor** to adjust the ellipse shape/direction and **click once with the left mouse button** to define the final ellipse shape.
5	To take another measurement simply **repeat steps 3 and 4**. To remove the Ellipse Tool **repeat step 2**.

Region of Interest (ROI) Extraction Tool

The Region of Interest (ROI) Extraction Tool allows users to select the regions they are interested in, with an output folder of tiff or video files containing only the regions users selected.





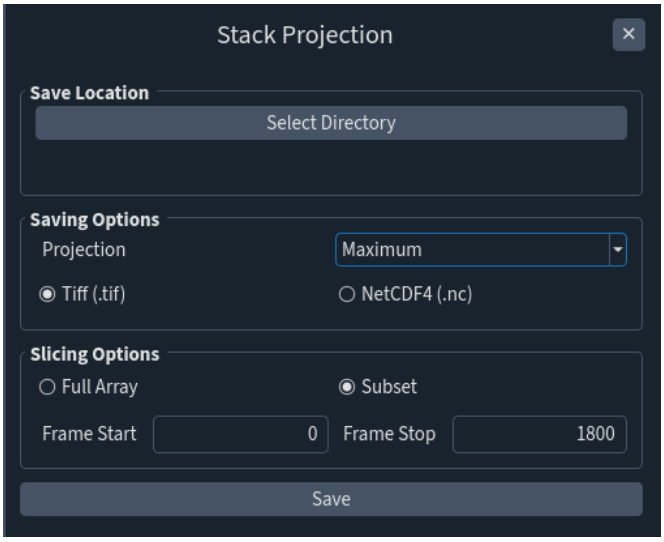
Step	Function
1	**Open an acquired dataset** in the MCAM™ Viewer software.
2	**Navigate to Tools > Regions of Interest.** Red boxes with a dot in the center will appear on the images, and a panel will open on the right.
3	To select the regions of interest: **Click the red box perimeter and drag** to adjust it to the intended size. **Click the red dot and drag** to move the red box to the regions of interest. Users can move the red box individually in each well but the size of the box is the same for all wells.
4	Determine the export format by **selecting "Images"** for image files or **"mp4"** for video files.
5	(Optional) **Select a location** for the new results folder containing tiff or mp4 files of the selected region of interest. By default, the location of the new folder is in the same folder where the folder containing original images/videos is located.
6	(Optional) Enter a name for the new output folder. By default, the new folder is named "roi_(timestamp)".

Updated as of 2026/07/27

7	**Click "Export ROI"** to export tiffs/mp4s of the ROIs selected.

Stack Projection

Projections are a way to combine many z frames into one. It selects pixels of a desired intensity from every slice throughout the volume to construct a 2D image. The MCAM Stack Projection feature allows the user to select if it is constructed based on the following intensity options: maximum, minimum, mean, sum and standard deviation.



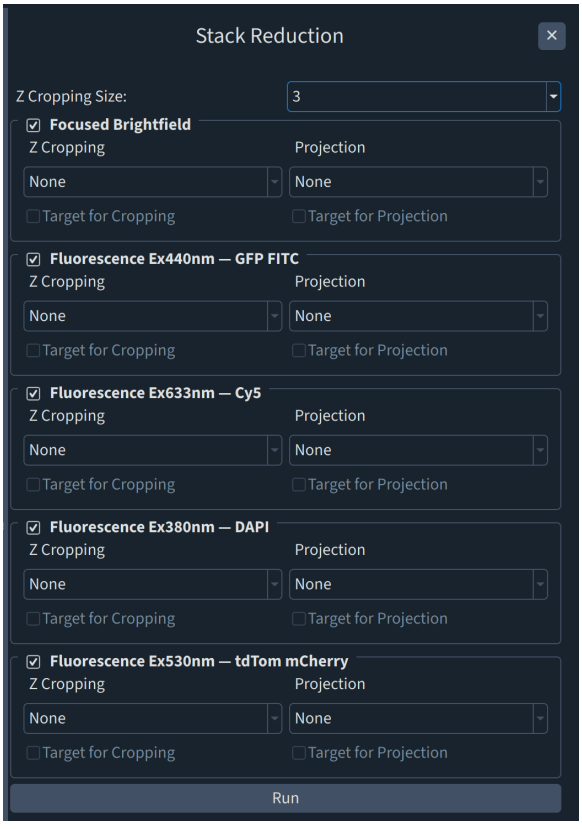
Step	Function
1	**Open the MCAM Viewer** and **click the Open Dataset button**. **Click into the stack dataset of interest**, then **click Open** in the popup to open the stack dataset in MCAM Viewer.
2	**Go to Tools > Stack Projection** to open the Stack Projection Panel.
3	Under Location **click on Select Directory** to choose the file location where the Stack Projection will be saved. As a default it will open up the file location of the stack dataset chosen in step 1.
4	Within Saving Options, **select the intended projection method and choose the save format** (either TIFF or NetCDF4).
5	Within Slicing Options **choose whether the Stack Projection** will be taken from all the frames **(Full Array)** or from a specific subset (Subset). If Subset is

	selected, define it by typing the Frame Start and Frame Stop accordingly.
6	**Click Save** to combine the stacks into the desired Stack Projection and save it.

Stack Reduction

The Stack Reduction Tool allows users to reduce the file size of stack datasets. This is achieved by 4 options:

1. Z cropping only where Z stacks are reduced to a smaller stack size with the middle Z stack being the best-in-focus plane.
2. Projection only where Z stacks are reduced to 1 plane using different Z reduction methods.
3. Z cropping+Projection, where Z-stacks are reduced followed by Projection.
4. Channel Extraction, where a subset of channels are saved without reduction through the Z dimension.



Step	Function

1	**Open an acquired dataset** in the MCAM™ Viewer software.
2	**Navigate to Tools > Stack Reduction.** A panel will open on the right.
3	Option 1: Z Cropping only **Select the Z cropping size** from the dropdown menu. For example, if you select "3", your Tiff stacks will be reduced to 3 Z-stacks, with the middle plane the best-in-focus plane. **Go to the channel(s) of interest, and select the intended Z cropping method** from the Z cropping dropdown menu. This Z cropping method will be applied to all other channel(s) automatically. **Deselect the channel(s)** that are not needed. Only the channels that are selected will be kept through this reduction. Optional: **Check the box for "Target for Cropping"** if you want the best-in-focus planes of all channels for the selected z cropping to be dependent on this target channel. If no "Target for Cropping" is checked, best-in-focus planes will be selected on a per channel basis for z cropping. **Click "Run".** Option 2: Projection only **Select the channel(s) of interest, and select the intended Projection method** from the Projection dropdown menu for respective channel(s). **Deselect the channel(s)** that are not needed. Only the channels that are selected will be kept through this reduction. Optional: **Check the box for "Target for Projection"** if you want the best-in-focus planes of all channels for the selected projection to be dependent on this target channel. If no "Target for Projection" is checked, best-in-focus planes will be selected on a per channel basis for projection. **Click "Run".** Option 3: Z Cropping+Projection **Follow the steps from Option 1 followed by Option 2.** Option 4: Channel Extraction **Deselect the channel(s)** that are not needed. Only the channels that are selected will be kept. **Leave both Z Cropping and Projection as None** in their respective dropdown menus. This will leave the z dimension of the selected channels unchanged.
4	The results are saved in a folder named "projection_results_timestamp" in the provided dataset's folder with the metadata and the Tiff files containing the processed images.

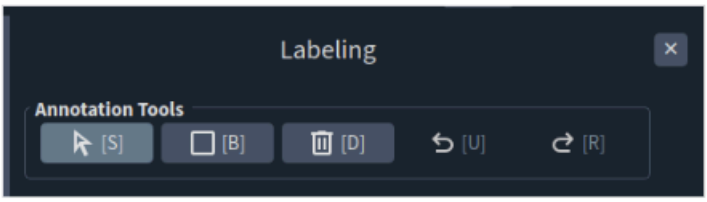
Select the GFP Channel Corresponding to the Brightfield Best Focus Slice

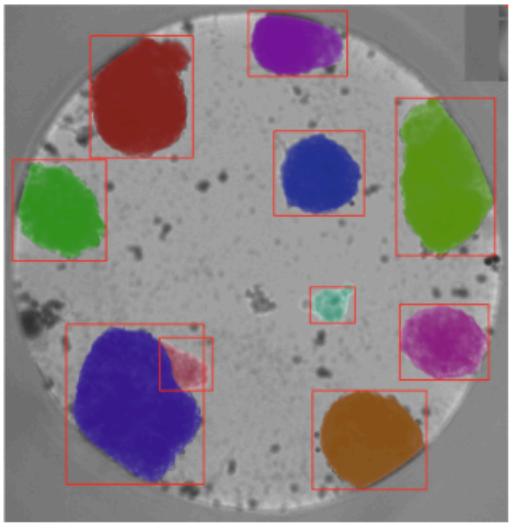
Using the Stack Reduction Tool users can select the best focus brightfield channel and corresponding slices of other color channels. In this example, we select the best focus slice of the brightfield channel and corresponding slice for the green GFP channel. Selecting the best focus slice using the laplacian projection from each channel separately can result in slices representing different planes of the sample.

Step	Function
1	**Open an acquired dataset** in the MCAM™ Viewer software.
2	**Navigate to Tools > Stack Reduction.** A panel will open on the right.
3	On the brightfield channel, **select the Laplacian focus selection method**.
4	**Select the "Target for Cropping" option** checkbox for the brightfield channel. This means that all channels will align around the best-in-focus plane identified in the brightfield channel.
5	As the "Target for Cropping" was set for the brightfield channel, the GFP channel will align to the same Z-plane that was selected as the best focus from the brightfield.
6	The results are saved in a folder named “projection_results_timestamp” in the provided dataset’s folder with the metadata and the Tiff files containing the processed images.

Labeling Tool

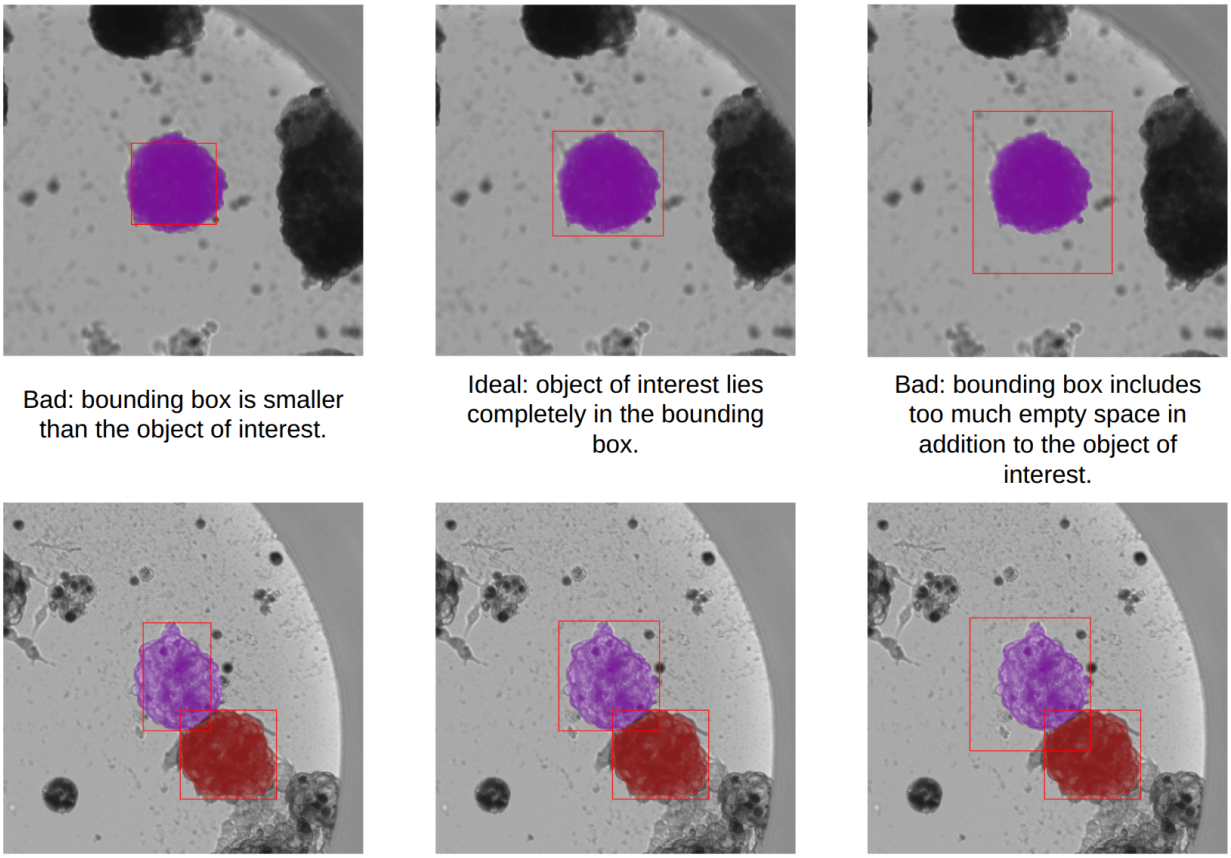
The Labeling Tool allows users to draw bounding boxes for objects of interest (for example, cells or spheroids/organoids). These bounding boxes can then be saved and fed into Ramona's machine learning model training to optimize object detection and segmentation tools for users. This tool is used to label two dimensional images. Three dimensional datasets should be projected to one two dimensional slice to ensure images are in focus before using this tool.





Step	Function
1	**Open an acquired dataset** in the MCAM™ Viewer software.
2	**Navigate to Tools > Labeling.** A panel will open on the right.
3	**Click on [B]** under Annotation Tools. **Navigate to an object of interest**, **then click, hold, and drag**. You will see a box with an orange-dotted line (i.e. bounding box) appear with a colored mask overlay dynamically covering the object while you adjust the bounding box. Make sure your object of interest is completely inside the bounding box before releasing the hold, and that the mask covers the object of interest (see figure below on how to correctly draw a bounding box).
4	(Optional) To delete a bounding box, **click on [D]** under Annotation Tools. **Move your cursor** to the bounding box to be deleted. The bounding box changes from red to yellow. The bounding box is deleted after clicking on it.
5	**Repeat step 3** until all objects of interest are inside their respective bounding boxes. **Click on [S]** to pan around the screen when needed.

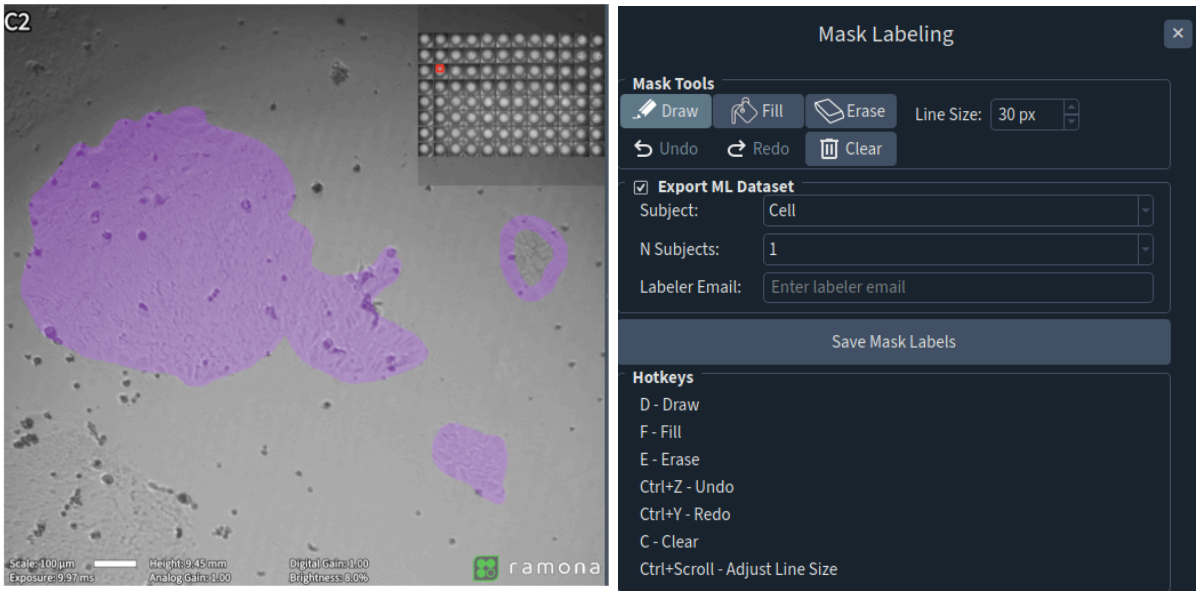
6	**Click “Save Changes”** to save the bounding boxes.
7	The results are saved in a folder named “segmentation_analysis_results” in the provided dataset’s folder with the metadata for the bounding boxes and a png file showing masks on the objects within bounding boxes.



How to draw bounding boxes correctly around objects of interest (legends in the middle apply to figures above and below).

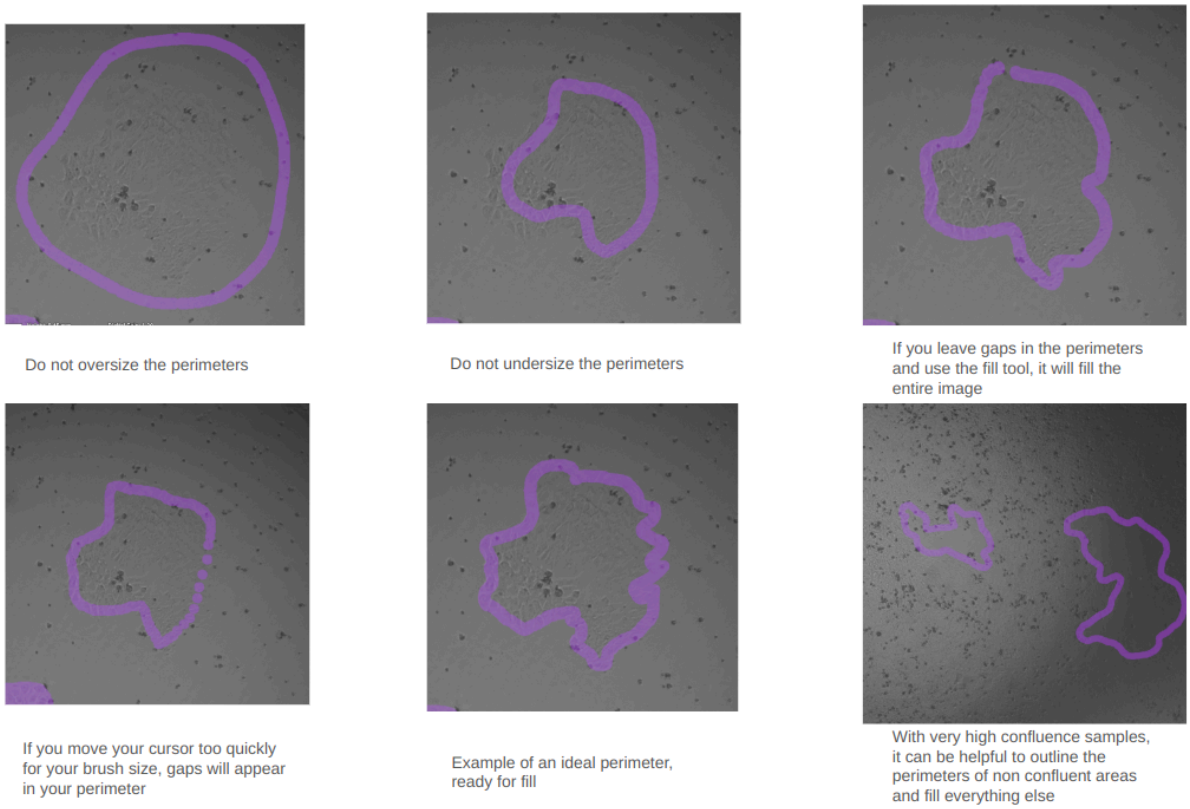
Binary Mask Labeling Tool

The Mask Labeling Tool allows users to manually draw binary masks. These masks can then be saved and fed into Ramona's machine learning model training to optimize the segmentation and confluence tools for users.



Step	Function
1	**Open an acquired dataset** in the MCAM™ Viewer software.
2	**Navigate to Tools > Mask Labeling.** A panel will open on the right.
3	**Click "Draw"** under Mask Tools. This is your paintbrush. Navigate to an object of interest, then **click, hold, and drag your cursor around its boundaries.** Your goal is to paint the perimeter of the object of interest.
4	**Click "Fill"** to use the fill tool, and fill the object. Note that the boundaries of the sample image also count as perimeter to the fill tool, filling a sample will not overflow into other images.
5	(Optional) To undo a change to the annotations, **Click Undo**. To redo a change after an undo, **Click Redo**.
6	**Repeat step 3 and 4** until all objects of interest are masked. **Click the Middle Mouse Button** to pan around the screen when needed.
7	**Click "Save Mask Labels"** to save the masks.

8	The results are saved in a folder named “mask_labeling_analysis_results” in the provided dataset’s folder with the metadata for the masks.



How to draw mask perimeters correctly around objects of interest.