

# AutoMito Network

This document provides users with step-by-step instructions for extracting mitochondrial network features from autofluorescence imaging data. It can also be used with other types of fluorescence images, such as labeled mitochondrial data. For up-to-date documents and code, please visit

<https://github.com/shannonhandley/AutoMitoNetwork>

## Installation instructions

- Open *MyAppInstaller\_web* and follow prompts to download the application.
- Once completed, a folder labelled *AutoMitoNetwork* will appear in your selected save location.
- Open *application* folder.

📁 > This PC > Windows (C:) > Program Files > AutoMitoNetwork

Name	Date modified	Type	Size
📁 application	29/09/2023 8:52 AM	File folder	
📁 uninstall	29/09/2023 8:52 AM	File folder	
🖼 icon_48	29/09/2023 8:14 AM	PNG File	3 KB

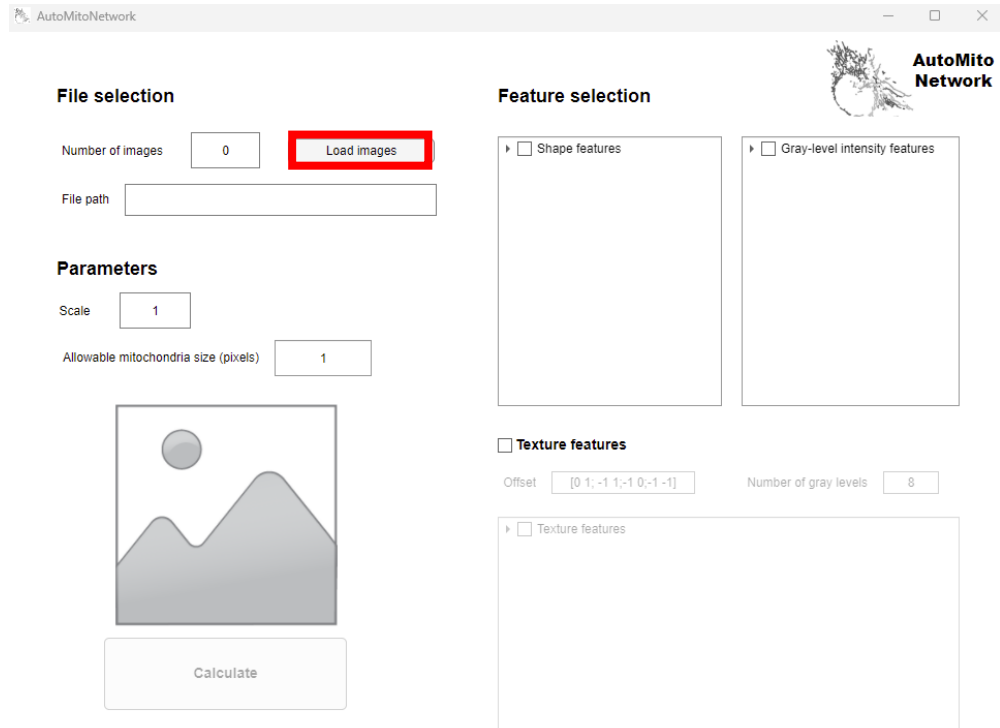
- Select *AutoMitoNetwork* to open application and start analysis.

📁 > This PC > Windows (C:) > Program Files > AutoMitoNetwork > application

Name	Date	Type	Size
🖼 AutoMitoNetwork	29/09/2023 8:18 AM	Application	1,239 KB
🖼 icon	29/09/2023 8:14 AM	ICO File	6 KB
📄 readme	29/09/2023 8:18 AM	Text Document	2 KB
🖼 splash	15/05/2023 9:41 AM	PNG File	234 KB

## Application analysis instructions

1. Press load images to upload image(s) for analysis.  
The number of images selected and the folder path they are in will appear.



The screenshot shows the AutoMitoNetwork application window. The title bar reads "AutoMitoNetwork". The interface is divided into two main sections: "File selection" and "Feature selection".

**File selection:**

- "Number of images" is set to 0.
- The "Load images" button is highlighted with a red rectangle.
- "File path" is an empty text input field.

**Parameters:**

- "Scale" is set to 1.
- "Allowable mitochondria size (pixels)" is set to 1.
- Below the parameters is a placeholder image of a landscape with mountains and a sun.
- A "Calculate" button is located at the bottom of the parameter section.

**Feature selection:**

- There are two large empty boxes for selecting features.
- The first box is labeled "Shape features" with a dropdown arrow.
- The second box is labeled "Gray-level intensity features" with a dropdown arrow.
- Below these boxes is a section for "Texture features" which is currently unchecked.
- Under "Texture features", there is an "Offset" field with the value "[0 1; -1 1; -1 0; -1 -1]" and a "Number of gray levels" field with the value 8.
- At the bottom, there is another unchecked checkbox labeled "Texture features" with a dropdown arrow.

The "AutoMitoNetwork" logo is visible in the top right corner of the application window.

2. Input the following optional parameters:  
Scale (1=default). If scale is known can input value here, otherwise units are in pixels.  
Allowable mitochondria size (pixels) (1=default). This removes detected mitochondrial networks with an area less than the inputted value.

AutoMitoNetwork

File selection

Number of images

2

Load images

File path

C:\test\


Parameters

Scale

1


Allowable mitochondria size (pixels)

1



Calculate

Feature selection



AutoMito Network

Shape features

Gray-level intensity features

Texture features

Offset

[0 1; -1 1; -1 0; -1 -1]

Number of gray levels

8

Texture features

### 3. Select shape and gray-level intensity features.

A checked box in the parent node 'Shape features' or 'Gray-level intensity features' means all features are selected.

A coloured box in these parent nodes mean only certain features were selected.

AutoMitoNetwork

File selection

Number of images

2

Load images

File path

C:\test\


Parameters

Scale

0.1

Allowable mitochondria size (pixels)

100



Calculate

Feature selection

Shape features

Area

Perimeter

Major axis length

Minor axis length

Eccentricity

Orientation

Convex area

Circularity/form factor

Filled area

Euler number

Equivalent diameter

Gray-level intensity features

Mean intensity

Median intensity

Std intensity

Minimum intensity

Maximum intensity

Mode

Skewness

Kurtosis

Texture features

Offset

[0 1; -1 1; -1 0; -1 -1]

Number of gray levels

8

Texture features

- Check texture features if texture features are to be analysed. If checked proceed to the following step otherwise skip to step 8.

AutoMitoNetwork

File selection

Number of images

2

Load images

File path

C:\test\


Parameters

Scale

0.1

Allowable mitochondria size (pixels)

100



Calculate

Feature selection

Shape features

Area

Perimeter

Major axis length

Minor axis length

Eccentricity

Orientation

Convex area

Circularity/form factor

Filled area

Euler number

Equivalent diameter

Gray-level intensity features

Mean intensity

Median intensity

Std intensity

Minimum intensity

Maximum intensity

Mode

Skewness

Kurtosis

Texture features

Offset

[0 1; -1 1; -1 0; -1 -1]

Number of gray levels

8

Texture features

5. Input offset for the gray-level co-occurrence matrices as a matrix. The offset is the distance between the pixel of interest and its neighbour. Each row in the matrix is a two-element vector, [row\_offset, col\_offset], that specifies the relationship, or offset, of a pair of pixels. row\_offset is the number of rows between the pixel-of-interest and its neighbour. col\_offset is the number of columns between the pixel-of-interest and its neighbour. See <https://au.mathworks.com/help/images/ref/graycomatrix.html> for more information.

Default = [0 1; -1 1; -1 0; -1 -1], which is four offsets: one pixel to the right, one pixel up, one pixel 45° to the right and one pixel 45° to the left. The figure below shows how offsets can be defined.

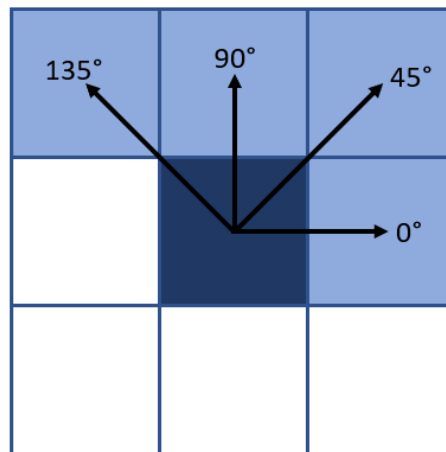


Figure 1. Diagram of offsets used to generate GLCM. In total four offsets (light blue) were taken from the centre pixel (dark blue). The four offsets were one pixels at 0°, 45°, 90° and 135° from the centre pixel.



7. Select texture features to be generated.

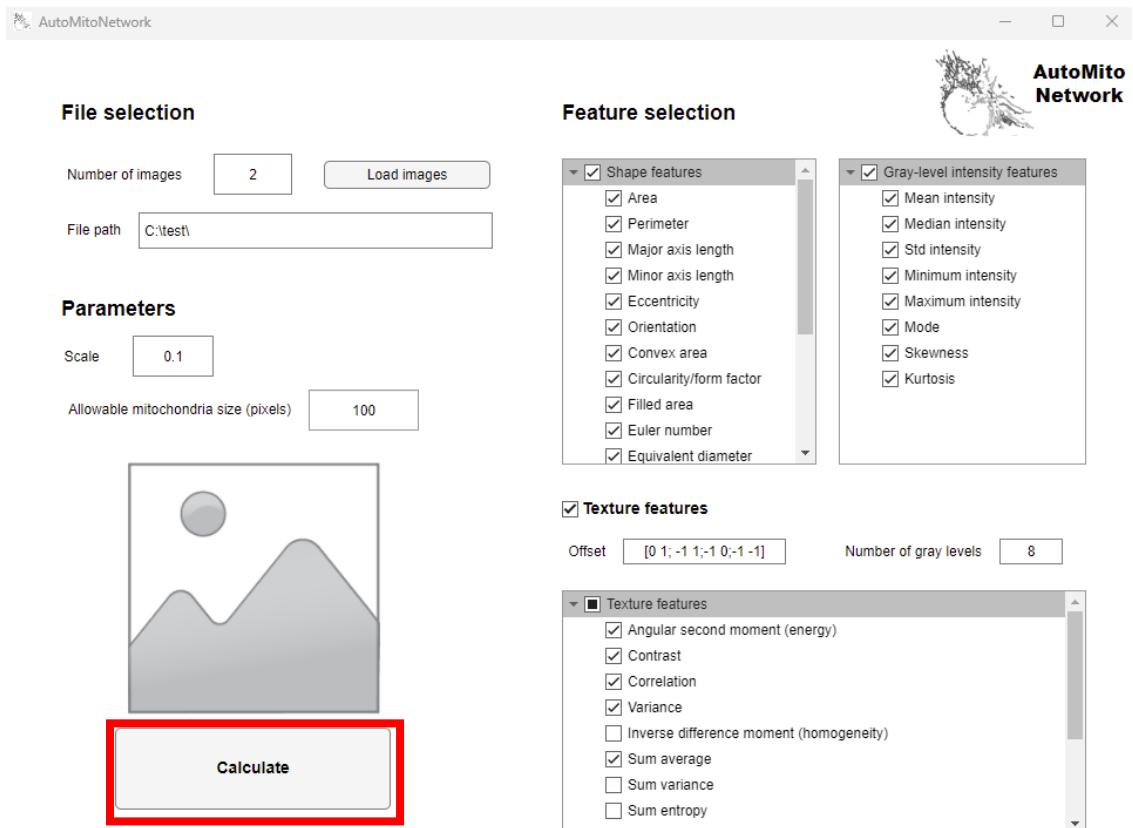
The screenshot displays the AutoMitoNetwork software interface. The window is titled "AutoMitoNetwork" and features a logo in the top right corner. The interface is divided into several sections:

- File selection:** Includes a "Number of images" input field set to 2, a "Load images" button, and a "File path" input field containing "C:\test\".
- Parameters:** Includes a "Scale" input field set to 0.1 and an "Allowable mitochondria size (pixels)" input field set to 100.
- Feature selection:** This section is highlighted with a red border and contains three sub-sections:
  - Shape features:** A list of features with checkboxes, all of which are checked: Area, Perimeter, Major axis length, Minor axis length, Eccentricity, Orientation, Convex area, Circularity/form factor, Filled area, Euler number, and Equivalent diameter.
  - Gray-level intensity features:** A list of features with checkboxes, all of which are checked: Mean intensity, Median intensity, Std intensity, Minimum intensity, Maximum intensity, Mode, Skewness, and Kurtosis.
  - Texture features:** A list of features with checkboxes, all of which are checked: Angular second moment (energy), Contrast, Correlation, Variance, Inverse difference moment (homogeneity), Sum average, Sum variance, and Sum entropy.

Below the "Parameters" section, there is a preview image of a landscape with a mountain and a sun, and a "Calculate" button.

8. Click calculate to perform analysis and generation of features.
- A .xls file is generated and saved in the folder path containing the features for each detected mitochondria network per image. The name of the file is called *filename\_features\_per\_mitonetwork.xls*
  - A .png file is generated and saved in the folder path containing an image of the detected mitochondrial networks. The name of the file is called *filename\_mitomap.png*





- The figure panel in the app will show the final image that was analysed, showing the detected mitochondrial networks and that the program has finished calculations.

