EMITO-METRIX APPLICATION: QUICK START TUTORIAL

EMito-Metrix is a high-performance pipeline for analyzing mitochondrial morphology and the ultrastructure in multiple tissues from low to high-resolution images acquired with Electron microscopy (EM). Interface allows to compute a set of morphometrics and texture measurements, and provides a list of graphs for optimizing data visualization using dimensionality reduction (UMAP or PCA) and more conventional depiction of data distribution (density curves, histograms, violin plots or star plots). Additionally, a machine learning (ML) module with predictive analytic tools allows determining how any given experimental condition would impact on mitochondrial morphology and ultrastructure

EMito-Metrix plugin was written by Mathieu Vigneau, Emmanuel Doumard and Jean-Philippe Pradère from the RESTORE Institute.

This section describes EMito-Metrix instructions for running and output descriptions

- TABLE OF CONTENTS

•	Warnings, advices & prerequisites	Ì
•	Emito-Metrix running	Ø
•	Emito-Metrix output description	Ð

WARNINGS, ADVICES & PREREQUISITES

1- WARNINGS & ADVICES ABOUT ELECTRON MICROSCOPY IMAGES

- About EM magnification & EM image resolution

In electron microscopy (EM) mitochondrial analysis, panoramic images enable the visualization of the tissue but not the details of each mitochondrion. Larger images are useful for gaining finer mitochondria measurements, such as Cristae's orientation, parallelism, and Cristae's quantity within the mitochondria.

In optical microscopy, the resolution of images are closely related to the magnification used during acquisition. The higher magnification, the greater resolution for morphology and ultrastructure details (see figure below).



Magnification 2k

Magnification 12k

EM image of the same skeletal muscle biopsy of Zebrafish, using either a magnification of 2k (left panel) or 12k (right panel). The higher magnification, the greater resolution of mitochondrion structure.

Before acquiring your EM images, we recommend that you adjust your magnification to the accuracy you would like to obtain for your morphological and ultrastructure mitochondrial measurements.

Modifying magnification during the acquisition will affect the resolution of EM images, i.e. the resolution of mitochondrial features. Therefore, when running EMito-Metrix analysis, this magnification variation will result in variations of some mitochondrial measurements – especially morphological measurements -, as shown in the following figure.



Impact of EM magnification on mitochondria metrics measurements from images of Mouse's skeletal muscles (left panel) and Drosophila's skeletal muscle (right panel)

If you plan to analyze and compare one-by-one several conditions, we highly recommend that you use the same magnification so that you can compare the corresponding morphological measurements

- About the effect of magnification on our trained model accuracy

While using a high magnification may be necessary to obtain high-resolution images of mitochondria, it is worth noting that precision and sensitivity of the model used for mitochondria segmentation is highly dependent on mitochondria's environment, i.e. the type of tissue imaged (see below).



Magnification 1,2k

Magnification 15k

EM image of the same skeletal muscle biopsy of Drosophila, acquired at magnification of 1.2k (left panel) or 15k (right panel). For each case, we have projected mitochondria segmentation on the raw EM image. We observe an erroneous segmentation from the 15k magnification, which is due to the absence of tissue's context

When setting both magnification and tissue's region to acquire, we recommended that you choose an area containing several mitochondria (at least 2 mitochondria) associated with their tissue environment. This should guarantee better mitochondria detection (see below).

We tested if our fine-tuned GM model was working at high magnification. Specie per Specie, we compared mitochondria segmentation from 1,2k to 20k resolution (see figures below). We found that mitochondria segmentation was working very well but with a variable accuracy depending on the specie. Thus, we established that our model works at maximum 15k images for Fly, 12k images for Z-Fish and 20k images for Mouse.



• Impact of resolution on DROSOPHILA mitochondria segmentation

• Impact of resolution on MOUSE mitochondria segmentation



• Impact of resolution on ZEBRAFISH mitochondria segmentation



- About the heterogeneity of mitochondria's size in an EM image

The Cellpose neural network used in our EMitoMetrix application has been separately trained on images of skeletal muscles from different species, resulting in so many different species-specific models. For each model, we annotated mitochondria with the same diameter; however, using Cellpose neural network in an automatic way needs a user-defined mitochondria diameter (in pixel) as an input for segmentation.

User defines the size value as the average diameter of mitochondria from a single image, or from an image-by-image basis. Changing the diameter will change the results of the algorithm outputs (i.e. the objects segmented). When the diameter is set smaller than the true size, then the neural network model may over-split mitochondria. Similarly, if the diameter is set too big, then the neural network model may over-merge mitochondria.

User can define a single diameter per image. Therefore, if the tissue's region imaged contains mitochondria with strong size heterogeneity (i.e. large and small mitochondria in the same image), the neural network model may detect mitochondria with a poor accuracy (for example an over-splitting of large mitochondria and/or an over-merging of small mitochondria, as shown below).



Low user-defined diameter

High user-defined diameter

Projection of EM image of skeletal muscle biopsy of Mouse with mitochondria segmentation map (color-coded)*. We used either a low value (left panel) or a high value as a user-defined diameter for mitochondria detection

*Images from CBM (CSIC-UAM), UAM University - Laura Formentini.

For better precision and sensitivity of detection, we recommend choosing a tissue's region to acquire with a good homogeneity of mitochondria's size.

- About the Generalist & Specialist trained models used for mitochondria detection

As previously explained, each species-specific model has been trained using images of the same tissue - skeletal muscle - from a single specie (i.e. ZebraFish, Mouse, Fly or Human). This implied that dataset used for each model training had a high homogeneity of tissue's environment. On this opposite, we used images of skeletal muscle from several species (i.e. ZebraFish, Mouse, Fly and Human) to train our Generalist model, that is dataset with a very high heterogeneity of tissue's environment. Because of this heterogeneity, and depending on the tissue and/or specie you use, you may have a better mitochondria detection using the Generalist model than the Species-specific models (see below).



Projection of EM image of skeletal muscle biopsy (left panels) and liver biopsy (right panels) of Mice with mitochondria segmentation map (color-coded)*. For each tissue, we used either Generalist or Mice-specific model as an input for Cellpose mitochondria detection. The number of detection is indicated for each condition

* Images from CBM (CSIC-UAM), UAM University - Laura Formentini

For better precision and sensitivity of detection, we recommend testing both Generalist and Specific models on your dataset, before starting EMitoMetrix application

We do not recommend using Cellpose trained models (cyto2, nucleus, cyto), as these models have not been trained on EM images.

2- APPLICATION EXPECTATIONS

Expected input files & folders

EMito-Metrix application allows: 1- analyzing mitochondrial morphology and ultrastructure in EM images **from multiple experimental conditions**; 2- **comparing these conditions one-by-one** in a single (and same) analysis.

Before running such analyze, we recommend that you organize your experimental conditions and input files (i.e. raw EM images) as shown below:



 Save all input files and conditions to analyze and compare in a unique parent folder (mystudy_INPUT)

No preferences regarding the location of mystudy_INPUT folder

- *mystudy_INPUT* must contain as many folders as conditions to compared/analyze *Example:* "control_condition vs test_condition
- *mystudy_INPUT* must contain nothing but the condition folders to compared/analyze. No other files or folders saved in mystudy_INPUT folder
- Each condition folder must contain all input (raw) EM images to analyze. Condition folders <u>must contain nothing but the input (raw) images</u>
- Folders name and input files name must respect the following rules: no special characters: []!#\$%&'()/*,:;<=>?@^`{[]~] no spaces, no accents no dot (except the one for file extension)
- We recommend the following file formats for the analysis: tif images, tiff images, bmp images

!!Even if accepted, we do not recommend using jpg, jpeg and png file formats, because of their poor resolution!!

To exclude conditions and/or images from the analysis, insert the character # at the beginning of the folder's/image's name you need to exclude (see example below).

<mark> </mark> 🕑 📕 =	Gérer	Condition_Control		-	\Box ×	
Fichier Accueil Partage Affich	age Outils d'image				~ ?	
\leftarrow \rightarrow \checkmark \uparrow \blacksquare « EMito-Metrix_	DATA > mystudy_INPU	T > Condition_Control	~ Ŭ	Rechercher dans : Con	dition_C 🔎	
pycache	Nom ^	Date	Туре	Taille	Mots clés	
> 📜 Adobe	🜌 #2m-02	29/06/2022 16:08	Fichier TIF	8 639 Ko		
> 📜 Dell	🜌 #2m-03	29/06/2022 16:08	Fichier TIF	8 639 Ko		— Excluded image
✓	🌌 2m-01	29/06/2022 16:07	Fichier TIF	8 639 Ko		Ũ
✓	🌌 2m-04	29/06/2022 16:08	Fichier TIF	8 639 Ko		
#Condition_Test3	🌌 2m-05	29/06/2022 16:09	Fichier TIF	8 639 Ko		
Condition_Control	🌌 2m-06	29/06/2022 16:09	Fichier TIF	8 639 Ko		
Condition Test1	🌌 2m-07	29/06/2022 16:11	Fichier TIF	8 639 Ko		
Condition Test2	🌌 2m-08	29/06/2022 16:12	Fichier TIF	8 639 Ko		
	🌌 2m-09	29/06/2022 16:13	Fichier TIF	8 639 Ko		
mystudy_OUTPOT	🧟 2m-10	29/06/2022 16:14	Fichier TIF	8 639 Ko	~	
30 élément(s)						
uded condition/folder						

- Expected output folders

Create an output folder (i.e. *mystudy_OUTPUT*) dedicated to output folders and files generated by the EMito-Metrix application. **You must organize** *mystudy_OUTPUT* as below:

- mystudy_OUTPUT must be empty when performing the application for the first time
- mystudy_OUTPUT name must respect the following rules: no special characters: []!#\$%&'()/*,:;<=>?@^`{|}~] no spaces, no accents
- While running, the application will create output folders and files in *mystudy_OUTPUT* folder. *mystudy_OUTPUT* <u>must contain nothing but outputs</u>.

Do not modify output folders name and images name, which may abort the application running

Mitochondria segmentation settings

Before running EMito-Metrix application, you must estimate **mitochondria** size as well as **trained model** to use for mitochondria segmentation.

To set these parameters, **we recommend using Cellpose graphical user interface**, following these instructions:

• Starting Cellpose application

Open an anaconda prompt

From Windows start menu, type anaconda prompt in the search bar, or open a Terminal window from Linux

To activate your Cellpose python environment, type the following python command line in your opened anaconda prompt, and press Enter:

conda activate cellpose

To start Cellpose graphical user interface, type the following python command lines from your Cellpose python environment and press Enter:

cellpose

• <u>Setting mitochondria segmentation</u>

In Cellpose window, drag and drop your input image to analyze. Here is an example of the Cellpose graphical interface and its functionalities:



Using Cellpose application, estimate the following segmentation settings:

- **Mitochondria size (in pixels):** defined as the average diameter of mitochondria to detect in the image (see <u>here</u> for more details about diameter definition)

Use the purple circle (at the bottom of the view) as a scale disk of the user-defined diameter value

- Trained model to use for automatic segmentation (see here for more details)

You can choose either a Cellpose trained model (cyto2, nucleus, cyto) or one of our custom Generalist or Species-specific EM models.

Once you have set both diameter and trained model, click on the *run button* to check the mitochondria segmentation. Adjust diameter accordingly if the segmentation is not working.

Depending on mitochondria size heterogeneity of your dataset, you may need to set your diameter value by image, by condition (single value for all images of the condition) or by study (single value for all conditions)

For more details about Cellpose GUI instructions, check out this documentation

EMITO-METRIX RUNNING

1- HOW TO LAUNCH EMITO-METRIX APPLICATION?

EMito-Metrix setting and running are performed using Fiji application (see *EMitoMetrix_Installation* tutorial for installation instructions).

- In your Fiji application directory, double-click the ImageJ-win64.exe file to start Fiji software
- Once the Fiji application has started, go to *EMito_Metrix* menu from Fiji *Plugins* menu, and start the application selecting *EMito_Metrix_Fiji*, as shown below :



2- SETTING EMITO-METRIX ANALYSIS

- Checking EMito-Metrix application & environment

Once you have launched *EMito-Metrix* application, a window will ask you to confirm that the following application & environment settings are fulfilled:



• <u>Software configuration</u> (see installation guide for detailed instructions)

- Python environment

Python3 environment installed, using Python-like distribution (Anaconda for example)

- Python dependencies

Python packages installed for data display and data prediction modules

- Cellpose Python environment

Python virtual environment installed and set for Cellpose application. Cellpose GUI installed

- Fiji installation and setting

Fiji software distribution installed. Fiji plugin updating and setting ok

- Cellpose Fiji wrapper

Cellpose Fiji-wrapper set, using Cellpose python virtual environment

- Emitometrix Python environment

Python virtual environment installed and set for data display and data prediction

• Input files & folders (see here for detailed instruction)

- Folder tree

Valid input folders containing condition folders and raw input EM images

- Folder name

Valid input folders name and condition folders name

- File name

Valid input files name

- **Output folders** (see <u>here</u> for detailed instruction)
 - Folder location

Valid output folder containing nothing but the output files

- Segmentation parameters (see here for detailed instruction)
 - Mitochondria diameter

Estimate the average mitochondria size (in pixels) for each image/condition to process

- Trained model to use

Define trained model to use for mitochondria detection

If not verified, the application execution will abort and invited you to check each application and environment settings

- Setting Python virtual environments

If the EMito-Metrix application installation and setting is correct, the next window will invited you to set these following python parameters:

Plugin configuration X		
Select folder with your CELLPOSE VIRTUAL ENVIRONMENT CELLPOSE folder Browse		
Did you install Cellpose with GPU computing ?		
GPU computing ? No		
EMITOMETRIX folder Browse		
Choose your computer environment		
Environment: Windows 💌		
(See Help Button for plugin instructions and installation)		
OK Cancel Help		

- Cellpose folder: specify parent folder containing your Cellpose virtual environment

from Windows: C:/Users/username/AppData/Local/anaconda3/envs/cellpose/ from Linux: /home/username/anaconda3/envs/cellpose/ from MacOS: /opt/anaconda3/envs/cellpose/

🛃 Plugin configuratio	n.		×			
Select folder with y	our CELLPOSE VIP	TUAL ENVIRONM	ENT			
CELLPOSE folder	[Browse			
Did you install Ge	lipose with GPU co	mputing ?				
GPU computing ?	No •					
Select folder with		🛃 Select a Folde	-			×
EMITOMETRIX folder		Rechercher dans :	conda-envis		- 600	
Choose your con Environment (See Help Button	ipuler environment Windows 🗨 for plugin instructio	Documents A Buresu Documents	emitometria			
		GEPC	Nom du dossier : Type de fichier :	D: Proiponde en sipelpose Tous les fichiers	e	Select Acruater

- GPU computing: specify if you have install a GPU version of Cellpose
- EMitometrix folder: specify parent folder containing your Emitometrix virtual environment from Windows: C:/Users/username/AppData/Local/anaconda3/envs/emitometrix/ from Linux: /home/username/anaconda3/envs/emitometrix / from MacOS: /opt/anaconda3/envs/emitometrix /
- Environment: specify your computer environment used for EMito-Metrix running Compatible with Windows, MacOS and Linux environments

The application will check the validity of your specified Cellpose and Emitometrix virtual environments (see installation instruction guide for Python virtual environment setting).

If virtual environment folders are not valid, the application will abort and invite you to check it

- Setting the analysis: workflow steps, input & output folders

If virtual environments are correct, you will have to define the following analysis settings:

image analysis : General parameters X	
1- Enter the EXPERIMENTAL NAME : mystudy	
2- Select WORKFLOW STEP to perform	
Mitochondria Segmentation No 💌	
Morphology & Ultrastructure Analysis No 💌	
Morphometrics Display & Visualization No 💌	
Data Computation & Prediction Yes 💌	
3- Select INPUT folder containing raw images to process	
INPUT directory D:/Pro/Datatest/MyStudy_INPUT/ Browse	
4- Select OUPUT folder containing output files	
OUTPUT directory D:/Pro/Datatest/MyStudy_OUTPUT/ Browse	
(See Help Button for plugin instructions and installation)	
OK Cancel Help	

- Experimental / protocol name

String of characters

• Select Workflow step to perform

- Mitochondria Segmentation

Image preprocessing and mitochondria segmentation

- Morphology & Ultrastructure Analysis

Quality control of segmentation & Morphometrics measurement of segmented mitochondria

- Morphometrics Display

Data visualization of morphometrics, using graphs & distributions

- Data computation & prediction

Predictive analytic tools that determine the impact of one condition on morphometrics

The application checks the following conditions: 1- even if not selected, the "mitochondria segmentation" option is run when the application is unable to find mitochondria segmentation maps in the output folder; 2- even if not selected by user, the application will automatically run "morphology & ultrastructure analysis" option when unable to find mitochondria metrics files in the output folder.

The option to run "morphometrics display" and "data prediction" without selecting "Mitochondria segmentation" and "morphology & ultrastructure" steps allows running separate condition comparisons, without restarting mitochondria detection and morphometrics measurements (less time consuming).

Input & output folders

- **INPUT Directory**: specify root directory containing condition folders and input files *Example: C:\Users\username\Documents\EMito-Metrix_DATA\mystudy_INPUT*
- **OUTPUT Directory**: specify root directory used to save output files, images and measurements *Example: C:\Users\username\Documents\EMito-Metrix_DATA\mystudy_OUTPUT*



The application will check the validity of INPUT and OUTPUT folders and files (see here for detailed instruction)

If INPUT and/or OUTPUT folders and files are not valid, the application execution will abort and invite you to check INPUT and OUTPUT folders/files.

- Setting the analysis: workflow parameters for each step

Once INPUT and OUTPUT folders are valid, define settings for each workflow step previously selected, as shown below:

🛃 Image analysis : Parameters - part 1 🛛 🕹 🗙
1- GENERAL PARAMETERS Unit of length for output pixel Normalization method Standard score 💌
2- MITOCHONDRIA SEGMENTATION Filter images for optimized segmentation : Yes v Mitochondria size : Same size v
3- MORPHOLOGICAL MEASUREMENTS MitoMetrics to use for display and prediction default 💌
4- Data DISPLAY & COMPUTATION Graphic to display default 💌
5- PREDICTION ANALYSIS Prediction model to use default
(See Help Button for plugin instructions and installation) OK Cancel Help

• General parameters

- Unit of length: unit used for morphometrics measurements

pixel or calibrated

If you select *calibrated*, you will have to define *pixel width*, *pixel height* and *unit of length*.

Image analysis : Pixel dimensions X					
Places fill out the following parameters:					
Unit of Length					
Pixel Width	0				
Pixel Height	0				
	OK Can	cel			

- **Normalization method**: Raw EM images are normalized on amplitude to attenuate global gray level variations observed between images, which may be related to variations in the amount of staining agent.

<u>min-max scaling</u>: consists in rescaling the range of features to scale the range in [0, 1]. This method is preferred when your data does not follow a normal distribution. Does not handle outliers well.

<u>standard score</u>: values (x) are centered on mean (mean(x)) with a unit standard deviation (sd(x)). This method is preferred when your data follows a normal distribution. Handles outliers well.



• Mitochondria segmentation

Filtering images for optimized segmentation: when EM images are acquired with high resolution and/or high magnification, mitochondria detection can be improved using Gaussian filtering

Yes or No (default value)

Gaussian filtering applied for segmentation only, not for mitometrics analysis

Mitochondria size: decide if mitochondria size varies across conditions or/and images or is the same for all conditions and images

same size (default value) or variable size (across conditions and/or images)

If you select variable size: select the level of mitochondria size variability

"Depending on condition" or "Depending on images"



Morphological measurements

Morphological and texture measurements used for data display and prediction analysis

default (all metrics) or custom (user-defined metrics)

• Data display & computation

Graphs and data distributions used for morphometrics visualization

default (all data distributions) or custom (user-defined distributions)

Prediction analysis

Machine learning models used for data prediction analysis

default (all models) or custom (user-defined models)

- Setting the analysis: mitochondria segmentation parameters

Depending on the options set in the previous section (see <u>here</u>), you will have to define the following mitochondria segmentation settings:



- Trained model used for mitochondria segmentation (see here for detailed instruction):

Cellpose models (cyto, cyto2 or nucleus), EMito-Metrix trained models (Specialist or Generalist models) or custom models (your own trained/fine-tuned model)

- If custom model: specify your own custom trained/fine-tuned model

Select your trained/fine-tuned model file with a dialog box

- **Mitochondria diameter** (in pixels): average size of mitochondria to detect in the image (see <u>here</u> for detailed instruction)

Same size: the user-defined value must be set only once.

Variable size, depending on condition: the user-defined value must be set for each condition

Variable size, depending on image: the user-defined value must be set for each image

- Setting the analysis: morphometrics measurements selection

Next, you will have to select morphological and texture measurements to use for data display and prediction analysis.

Mitochondria Me	trix Analysis				×
Select AT LEAST	r 3 mitochondria metrics t	o compute			
1- GENERAL P	ARAMETERS :				
Condition Name	Mito ID	Nito CentroidX 🔽 Mito C	SentroidY	Condition	n & mitochondrial labels
2- MORPHOLO	GICAL MEASUREMENTS				
Mito Area	Mito Perimeter	AreaPerimeter Rat	io 🔽 Mito Circularity	Mito Roundness	Mitochondrial morpholo
Mito Solidity	Mito AR	Mito Feret Diamete	r 🔽 Mito FeretX	Mito FeretY	
3- TEXTURE ME	EASUREMENTS :				
Mito MeanInt	🔽 Mito MedianInt	Mito TotalInt	Intensity SD	Intensity SD percent	● Mitochondrial tex
3- TEXTURE ME	EASUREMENTS - CORRE	CTED :			
Mito MeanInt COF	RR 🔽 Mito Mediai	nInt CORR 🔽 Mito 1	Fotalint CORR	Intensity SD CORR	✓ Intensity SD percent CORR
3- CRISTA MEA	SUREMENTS :				
Skewness	✓ Kurtosis	I ⊂ CristaOrienta	ition Major 🔽 Crista	Orientation Minor 🔽 CristaOrie	entation Angle 🔽 CristaOrientation Area
(See Help Butto	on for plugin instructions a	nd installation)		1	
			Mitoch	nondrial Cristae	OK Cancel

General parameters

Condition & images name, mitochondria labels and spatial position (Centroid X&Y)

Morphological measurement

Mitochondria shape measurements

METRIC NAME	DEFINITION	Illustration
Mito_Area	Area of the mitochondria	STD
Mito_Perimeter	The length of the outside boundary of the mitochondria.	QTD
AreaPerimeter_Ratio	Ratio between Perimeter and Area	QID
Mito_CentroidX and Mito_CentroidY	X and Y coordinates of the center point of the mitochondria.	ETO
Mito_Circularity	Shape of the mitochondria defined as (4 π *area/perimeter^2). A value of 1.0 indicates a perfect circle. As the value approaches 0.0, it indicates an increasingly elongated shape	STD

Mito_Roundness	Shape of the mitochondria defined as (4*area/(π*major_axis^2)), or the inverse of the aspect ratio.	
Mito_Solidity	Ratio between area and convex area	STO
Mito_AR	Aspect Ratio : Ratio between major axis and minor axis	
Mito_Feret_Diameter	The longest distance between any two points along the mitochondria boundary, also known as maximum caliper	Est Ample
Mito_FeretX and Mito_FeretY	Starting coordinates of the Feret's diameter	

• <u>Texture measurements</u>

Ultrastructure measurements, based on distribution of normalized gray values in mitochondria

METRIC NAME	DEFINITION	Illustration
Mito_MeanInt	Average intensity calculated from mitochondria's normalized gray values	
Mito_MedianInt	Median intensity calculated from mitochondria's normalized gray values.	
Mito_TotalInt	Sum of the mitochondria's normalized gray values	
Intensity_SD	Standard deviation of the mitochondria's normalized gray values used to generate the mean intensity. Measure of crista's density within the mitochondria	
Intensity_SD_percent	Ratio between Intensity_SD and MeanInt. Measure of crista's density within the mitochondria	Mean Intensity

• <u>Texture measurements – corrected</u>

Ultrastructure measurements, based on distribution of corrected gray values calculated in mitochondria.

Corrected gray values are calculated from normalized gray values, by filtering noise with a high frequency filter (Fast Fourier Transformation).

METRIC NAME	DEFINITION	Illustration
Mito_MeanInt_CORR	Average intensity calculated from mitochondria's gray values after High frequency filtering (FFT noise correction)	
Mito_MedianInt_CORR	Median intensity calculated from mitochondria's gray values after High frequency filtering (FFT noise correction)	
Mito_TotalInt_CORR	Sum of the mitochondria's gray values, after High frequency filtering (FFT noise correction)	
Intensity_SD_CORR	Standard deviation of the mitochondria's gray values used to generate the mean, after High frequency filtering (FFT noise correction). Measure of crista's density within the mitochondria	
Intensity_SD_percent_CORR	Ratio between Intensity_SD_CORR and MeanInt_CORR. Measure of crista's density within the mitochondria	Mean intensity

• Crista measurements

Measurements of cristae's organization and orientation

METRIC NAME	DEFINITION	Illustration
Skewness	Third order moment about the mean. Measure of the asymmetry of the mitochondria's normalized gray values about the mean intensity	Mean Modal Median
Kurtosis	The fourth order moment about the mean. Measure of the "tailedness" of the mitochondria's normalized gray values about the mean intensity.	

CristaOrientation_Major	Primary axis of the best fitting ellipse calculated from the frequency spectrum of the mitochondria's normalized gray values. Measure of the Crista's orientation, alignment and number within the mitochondria.	
CristaOrientation_Minor	Secondary axis of the best fitting ellipse calculated from the frequency spectrum of the mitochondria's normalized gray values. Measure of the Crista's orientation, alignment and number within the mitochondria.	
CristaOrientation_Angle	Angle (between the primary axis and a line parallel to the x-axis of the image) of the best fitting ellipse calculated from the frequency spectrum of the mitochondria's normalized gray values. Measure of the Crista's orientation, alignment and number within the mitochondria.	
CristaOrientation_Area	Area of the best fitting ellipse calculated from the frequency spectrum of the mitochondria's normalized gray values. Measure of the Crista's orientation, alignment and number within the mitochondria.	

- Setting the analysis: graphs and data distribution selection

Then, select graphs and data distributions used for morphological and texture visualization:

	🛃 Mito Metrics Data o	computation		Data distribution type									
	Select at least 1 g	raph or data distri	bution to display	~									
1	I▼ PCA		✓ violin	🔽 density	🔽 histogram	🔽 radar	🔽 spatial cluster	ring					
2	C Don't save outp Don't display ou (See Help Button	out graphic utput graphic for plugin instruct	tions and installation	Output set	ttings								
							OK Canc	el					

• PCA distribution

The principal component analysis (PCA) is a linear dimensionality reduction technique that consists in transforming data onto a new coordinate system such that dimensions are orthogonal and capture the most variation in the data. We keep only the first two dimensions to be plotted.



PCA distribution of mitochondria (one point per mitochondria), according to the magnitude used for EM acquisitions.

UMAP distribution

The Uniform Manifold Approximation for Projection (UMAP) is a non-linear dimensionality technique based on Rimannian manifold. It connects each point to its nearest neighbors in high dimension, and project the manifold in low dimension to be plotted. Its properties ensure that closest on the projection are close in high dimension, and points that are far from each other on the projection are far from each other in high dimension.



UMAP projection of mitochondria (one point per mitochondria), according to the magnitude used for EM acquisitions.

• Violin distribution

A violin plot is used to easily compare distributions between conditions. Each condition is plotted on a separate axis, but they are aligned with each other for comparison. The density is smoothed by a kernel density estimator.



Violin plots of mitochondria measurements (one plot per measurement), according to the magnitude used for EM acquisitions.

• Density distribution

A density plot is used to represent the distribution of each condition, superimposed on each other. The line represent the kernel density estimator smoothed distribution of density.



Density distribution of mitochondria measurements (one distribution per measurement), according to the magnitude used for EM acquisitions.

• Histogram distribution

Similar to density plots, histograms are used to represent the density of the distribution of each condition, superimposed to each other. It depicts the distribution with more precision, as it is not smoothed by a kernel density estimator.



Histogram distribution of mitochondria measurements (one distribution per measurement), according to the magnitude used for EM acquisitions.

• Radar plot distribution

A radar plot (or radar chart) represent all the features of each condition on a single radial plot for comparison. To compare between features, two scaler have been used: a MinMax Scaler, rescaling all values between 0 and 1 for all features, and a Standard Scaler, substracting the mean value of each feature and dividing by its variance in order to have a pseudo-gaussian distribution centered on 0 with unit variance.



Radar plot distribution of mitochondria measurements according to the magnitude used for EM acquisitions.

• Spatial clustering distribution

The spatial clustering plot simply represent the physical position of the centroid of each mitochondria on a two-dimensional plot. A density-based clustering algorithm, HDBSCAN, is used to automatically capture groups of mitochondria, and each of them are averaged for each variable to be compiled in an output csv file.

!! Spatial clustering will increase calculation time !!



Pre-processed TEM image



(X,Y) projection of Mitochondria's centroid

• Others data computation settings

- Don't save output graphic

Graphs and data distributions are displayed during the analysis, but <u>not saved</u> in the output folder

- Don't display output graphic

Data graphs and distributions are saved in the output folder, but <u>not displayed</u> during the analysis

	Mito Metrice	s Data computation	D	Data distribution type							
	Select at le	ast 1 graph or data distr									
1	I▼ PCA	UMAP	🔽 violin	🔽 density	🔽 histogram	🔽 radar	🔽 spatial clust	ering			
2	C Don't sav	ve output graphic play output graphic Button for plugin instruc	tions and installatio	Output set	ttings						
					-		OK Car	icel			

We developed a machine learning (ML) module with predictive analytic tools in order to determine how any given experimental condition would influence mitochondrial morphology and ultrastructure. In the next window, you have to select at least on machine learning model to use for the predictive analysis.

Data computation and prediction X											
Select at least 1 model to use for prediction											
R RF XGB MLP											
Print out the explanation for each model											
🗖 Don't save output graphic											
🔲 Don't display output graphic											
(See Help Button for plugin instructions and installation)											
OK Cancel Help											

- Confusion Matrix (LR, RF, XGB, MLP)
 - A confusion matrix presents the predictive performances of a model (see below). The cell (i,j) represents the proportion of mitochondria of true class j that have been predicted of class i by the model. The diagonal (from top-left to bottom-right) represents the correct predictions. Train and test performances have been separated for overfitting assessment, and models performances in term of precision and f1-score are displayed above each matrix.



Four models are proposed for the computing of confusion matrix:

<u>Linear Regression model (LR)</u>: tree-based algorithms <u>Random Forrest model (RF)</u>: tree-based algorithms <u>XGBoost model (XGB)</u>: tree-based algorithms <u>MultiLayer Perceptron model (MLP)</u>: neural network

• Summary plots (explanation option):

A summary plot represents the explanation of a model, for a given class (see below). The feature are displayed from the most important (at the top) to the least important (at the bottom). Only the 14 most important features are displayed, and if there are more, they are summed together on a 15th axis. On each axis, each dot represent a mitochondria, its position on the x-axis represent the contribution of the associated feature to the prediction, and its color represent its relative feature value (red represents high values, while blue represents low values). For binary prediction, a single summary plot is displayed, because the summary plot for the second class is the exact opposite of the first one.



SHAP values generated with Linear Regression algorithms (LR), for the magnitude_04k condition

To compute summary plots, activate print out the explanation function

• Others data computation settings

- Don't save output graphic

Graphs and data distributions are displayed during the analysis, but <u>not saved</u> in the output folder

- Don't display output graphic

Data graphs and distributions are saved in the output folder, but not displayed during the analysis

- Setting live image display

A batch mode is proposed, that allows masking image display during application execution.

Macro Execution : Parameters	×
Live image displaying during macro execution ? No 🗸	
Warning : displaying images will slow down macro execution	ı
OK Cance	I

Selecting live image displaying (Yes settings) may slow down the application execution

- Setting image size reduction of input EM images

Before running mitochondria segmentation and morphological analysis, you can crop your input EM images for each condition separately:

- If it is necessary, click on Yes. Then, select an area of interest to keep in your image using the rectangle tool from Fiji application. Once the region of interest is selected, click ok. The application will crop all images of the condition, in the same way.



- If it is not necessary, click on No. The application will continue without cropping images.

- Live quality control of Mitochondria segmentation

Once mitochondria detection is done, validate or invalidate mitochondria segmentation for each input EM image of each condition, as shown below:



- <u>Click on No button</u> if you consider that mitochondria detection is not good.

- <u>Click on Yes button</u> if you consider that mitochondria detection is good.

Once segmentation validation is done, the application will continue using all images and all mitochondria but the invalid ones

- Selecting conditions to use for data display & prediction analysis

Select conditions to use for data visualization, and those for data prediction. You must select at least one condition for data visualization, and two conditions for data prediction (see below)

Aito Metrics Data computation	×
Coloct conditions to use for data display and	
Select conditions to use for data display and	computation
Condition Control Condition Test1 C	ondition Test2
(See Help Button for plugin instructions and	nstallation)
	K Cancel
	Gancer

EMITO-METRIX OUTPUT DESCRIPTION

In this section, we describe folders, images, measurement files and data distributions saved in the output folders.

📙 🖂 📜 🗢 🛛 MyStudy_OUTPUT					- 0	×
Fichier Accueil Partage Affichage	2					~ 🕐
\leftarrow \rightarrow \checkmark \uparrow \blacksquare \rightarrow Ce PC \rightarrow DATA (D:) > Pro > Datatest > MyStudy_OUTPUT >			~ Ū	Rechercher dans : MyStudy_O	<i>م</i>
📜 autres 🔨	Nom	Modifié le	Туре	Taille		
CN_CNRS_IE_2023	Data_visualization	04/07/2024 14:35	Dossier de fichiers			
📕 Pro	Data_visualizationspatial_clustering	04/07/2024 11:35	Dossier de fichiers			
BDD_Median	Image_output	04/07/2024 11:35	Dossier de fichiers			
📜 conda-envs	Log_files	04/07/2024 13:13	Dossier de fichiers			
📜 Datatest	Measurements	04/07/2024 11:38	Dossier de fichiers			
MyStudy_INPUT	Measurements_ALL	04/07/2024 11:43	Dossier de fichiers			
MyStudy_OUTPUT	Measurements_TMP	04/07/2024 14:34	Dossier de fichiers			
Data visualization	Prediction_Analysis	04/07/2024 14:31	Dossier de fichiers			
Data_visualizationspatial	ROI_mito	04/07/2024 11:38	Dossier de fichiers			
Image_output						
Log_files						
Measurements						
Measurements_ALL						
Measurements_TMP						
Prediction_Analysis						
ROI_mito						
📜 Fiji.app 🗸 🗸						
9 élément(s)						=

- Image_output folder
- processed_images: normalized & cropped EM images
- cellpose_mask: label maps containing segmented mitochondria

One value (i.e. one region of interest ROI) per mitochondria detected



Pre-processed TEM image

Label map of mitochondria

- control_quality_mask: projection of mitochondria segmentation and normalized EM image

Used for the quality control of mitochondria detection



Pre-processed TEM image

Mitochondria label projection

• <u>ROI_mito folder</u>

Label regions of mitochondria detected with Cellpose or custom models. These regions of interest (ROI) files are opened and displayed using the *Fiji-ROI manager tool*

Drag and drop your EM image in Fiji, then open the ROI file using Analyze/Tools/ROI manager



• Measurements folder

Morphometrics files containing morphological and texture measurements (see <u>here</u> for a detailed description of mitochondrial measurements).

One text file per image and one line per mitochondria detected

																			-		
_ 1	🦲 2m_	01_MITO_measurem	ents - Bloc-notes														-	o ×			
- 1	Eichier	Edition Format	Affichage Aide																		
	xperi	Iment Name Con	dition Name	Imag	e Name	Mito ID Mito A	rea	Mito Per	imeter AreaPer	meter F	latio Mito M	eanInt Mito Me	anInt CORR	Mito Med	LanInt	Mito Medi	anInt COF	RR IA	× .		
	qdf	02M_wt_1	2m_01	1	6981	312.5341	22.3368	-0.06107	-0.0040	26	-0.05716	-0.0008295	-426.3378	-28.1025		0.4677 0	.2227 -7	765.900:		-	
	qdf	02M_wt_1	2m_01	2	11893	511.73 23.240	8 -0.7016	-0.09933	-0.6979	-0.0971	-8344.3512	-1181.3179	0.3563 0.2324	-50.7793		-234.0036	16	517.133(\sim	
- 1	df	02M_wt_1	2m_01	3	1953	173.2376	11.2735	-0.603	-0.02239	-0.5685	-0.02142	-1177.6578	-43.7275	0.3939 (9.2237	-65.3282	-9	999.008			and mitachandrie
	df	02M_wt_1	2m_01	4	8872	380.9188	23.2911	-0.6594	-0.007736	-0.654	-0.006852	-5850.3188	-68.6349	0.3697 (9.2366	-56.071 -	3058.3494	1 E			one milochondria
- 1	df	02M_wt_1	2m_01	5	6543	323.0193	20.2558	-1.0429	-0.0587 -1.0027	-0.0553	-6823.4232	-384.0978	0.4204 0.2438	-40.3126		-415.3535	19	948.2381			
- 1	df	02M_wt_1	2m_01	6	13190	471.9554	27.9476	-0.1724	0.003827	-0.1623	0.005157	-2273.4178	26.0162 0.3545	0.2401	205.67	85 6	272.5357				
	ldf	02M_wt_1	2m_01	7	6897	324.2914	21.2679	-0.9806	-0.004952	-0.9355	-0.00003239	-6763.2874	-34.1567	0.4517 (9.2379	-46.0667	-4	1802.93			
- 1	df	02M_wt_1	2m_01	8	3935	301.5462	13.0494	-0.9806	-0.1174 -0.9683	-0.1118	-3858.7965	-461.8261	0.4034 0.2352	-41.1351		-200.4353	18	389.486			
	df	02M_wt_1	2m_01	9	10724	449.0021	23.8841	-0.7	-0.003162	-0.6819	-0.0005848	-7506.3373	-33.9143	0.4055 (9.2327	-57.9361	-7	7358.50			
- 1	df	02M_wt_1	2m_01	10	1243	131.7817	9.4323	-0.3205	-0.01397	-0.2963	-0.01684	-398.434	-17.3625	0.3838 (9.2186	-119.733	-1	1565.07			
- 1	df	02M_wt_1	2m_01	11	7742	341.9899	22.6381	-0.6879	-0.00009083	-0.6623	0.006115	-5325.4526	-0.7032 0.4424	0.2322	-64.311	5 -	255669.15	53 :			
- 1	df	02M_wt_1	2m_01	12	4785	285.9066	16.7362	-0.5823	-0.00126	-0.5725	0.001737	-2786.36	-6.0285 0.342	0.2184	-58.735	5 -	17333.961	17 :			
	df	02M_wt_1	2m_01	13	6753	326.0488	20.7116	-0.7786	-0.006959	-0.767	-0.007595	-5257.9681	-46.9932	0.3685 (9.2252	-47.3298	3	3235.77			
	df	02M_wt_1	2m_01	14	5039	265.7645	18.9604	-0.8067	-0.005083	-0.7627	0.001284	-4064.8878	-25.6117	0.4275 (0.2349	-52.9912	-4	4621.784			
- 1	df	02M_wt_1	2m_01	15	2114	205.5807	10.2831	-0.6716	-0.01036	-0.6721	-0.0101 -1419.	8414 -21.907	0.4052 0.2348	-60.3255		-2265.706	5 17	710.144			
	df	02M_wt_1	2m_01	16	3139	223.6224	14.0371	-0.9024	-0.02236	-0.8751	-0.01503	-2832.6219	-70.1731	0.3918 (9.2382	-43.4211	-1	1065.541			
	df	02M_wt_1	2m_01	17	4437	255.5219	17.3645	-0.6175	-0.0005558	-0.5811	0.004858	-2739.6386	-2.4662 0.4268	0.2275	-69.129	-40938.62	29 11	197.298:			
- 1	df	02M_wt_1	2m_01	18	11897	442.4579	26.8884	0.09387	-0.003251	0.0957	-0.002608	1116.7628	-38.679 0.3676	0.2327	391.633	2 -	7158.3372	2 !			
- 1	df	02M_wt_1	2m_01	19	6770	323.7645	20.9103	-1.1791	-0.1385 -1.1894	-0.1363	-7982.3739	-937.4166	0.4402 0.245	-37.3383		-176.9488	19	994.550		_	
- 1	df	02M wt 1	2m_01	20	8434	379.6051	22.2178	-0.3993	-0.002337	-0.4238	0.0008001	-3368.075	-19.7122	0.4542 (9.2292	-113.7369	-9	9807.77		_	
- 1	df	02M_wt_1	2m_01	21	3704	228.5513	16.2064	-0.456	-0.01277	-0.4712	-0.01008	-1689.1481	-47.2848	0.384 (0.2261	-84.2047	-1	1771.27			⁻ one image
- 1	df	02M_wt_1	2m_01	22	1172	130.7107	8.9664	-1.2159	-0.0624 -1.2189	-0.0564	l5 -1425.	0604 -73.129	0.3298	0.2292	27.124	9 -	367.3492				
- 1	df	02M_wt_1	2m_01	23	2338	190.4508	12.2761	-1.3732	-0.01275	-1.389	-0.01201	-3210.5897	-29.8055	0.4 0	9.2418	-29.1302	-1	1896.98:			
- 1	df	02M_wt_1	2m_01	24	6700	330.7767	20.2554	-1.4013	-0.03214	-1.3864	-0.02637	-9388.4246	-215.3667	0.3967 0	9.249	-28.3122	-7	774.7424			
- 1	df	02M_wt_1	2m_01	25	6107	332.6346	18.3595	-1.4623	-0.002317	-1.4329	0.001523	-8930.0728	-14.1503	0.4513 (9.2474	-30.86 -	10678.822	29 :			
- 1	df	02M_wt_1	2m_01	26	6036	322.6518	18.7075	-1.3528	-0.003638	-1.361	0.001198	-8165.2238	-21.9569	0.3919 0	9.2537	-28.972 -	6974.2511	L :			
- 1	df	02M_wt_1	2m_01	27	11179	415.4874	26.9058	-1.2457	-0.05706	-1.2542	-0.05687	-13925.1558	-637.9256	0.4331 (9.249	-34.7685	-4	436.353			
- 1	44	0.0M U+ 1	2- 01	28	2010	240 865 15 768	\$ 1 4457	0 00635	1 1005	0 0015	5606	0116 04 773	0 000 N	0 2583	34 004	5	1107 0030	· · · · ·	1		
_													1.400.014				1177.0	,			
- L													£n 153, Col 1	9 I	00% W	indows (CRLF)	011-8		al		

• Measurements_ALL folder

Contains average morphological and texture measurements for each image (see <u>here</u> for a detailed description of mitochondrial measurements)

One text file per condition, and one line per image

Mouse_M	VITO_measure	ments_Al	LL_IMAGES	- Bloc-note:	s																-		×	1			
Eichier Edit	tion Format	Affichag	e <u>A</u> ide																								
Experimen	nt Name Co	onditio	on Name	Image N	Name Mitc	o TotalNumber	- Mi	ito Densi	ty	Mito To	talArea	Percent	Mito Ar	rea	Mito Pe	rimeter	AreaPer	imeter /	MeanRatio	м	Mito M	eanInt	P ~				
All Speci	ies Mo	ouse	J1_10	123	0.00002935	8.5191	2902.1707	18	9.213	2	13.0212	0.5582	0.5237	0.9027	9174.15	27	0.8676	0.8107	0.9626	1.2549	64.183	7 0.188	76				
All Speci	ies Mo	ouse	J1_5	121	0.00002888	6.8468	2371.0413	18	1.211	12.003	-0.5273	-0.5466	0.6121	-41.397	1	0.832	0.741	0.9548	1.4048	63.244	4 0.1728	0.162	2	— ••			
All Speci	ies Mo	ouse	J1_6	92	0.00002196	6.0687	2764.0435	18	9.691	1	12.7155	-0.7303	-0.7494	0.413	-7.1104	0.8436	0.7418	0.9596	1.3954	66.425	4 0.226	0.245	9		\mathbf{i}		
All Speci	ies Mo	ouse	J1_9	42	0.00001002	2.2307	2225.4524	17	6.274	7	11.6957	-0.5326	-0.5363	0.3706	12.996	0.8386	0.7566	0.9553	1.3776	60.934	5 0.0560	6 0.090	4		-	one ima	ige
All Speci	ies Mo	ouse	J2_1	21	5.0117E-6	0.9491	1893.8095	16	6.132	3	10.7911	0.04659	0.03406	0.5675	-15.249	0.8212	0.7453	0.9454	1.3875	58.247	6 0.1123	-0.01	7				0
All Speci	ies Mo	ouse	J2_2	111	0.00002649	7.3777	2785.0541	19	5.514	12.9017	0.2285	0.1738	0.8069	-407.53	94	0.8346	0.7508	0.9561	1.3674	67.621	4 0.3319	0.232	3				
All Speci	ies Mo	ouse	J2_3	95	0.00002267	6.9063	3046.2 20	34.2157		13.472	-0.0076	984	-0.0804	18	0.8465	-35.141	0.8373	0.7656	0.9577	1.3394	70.222	4 0.364	6€				
All Speci	ies Mo	ouse	J2_9	57	0.0000136	5.0208	3690.8772	22	7.653	5	14.0806	6 0.08414	0.06278	8 0.6056	357.500	13	0.7994	0.7559	0.9448	1.3587	77.176	1 0.194	€				
All Speci	ies Mo	ouse	mouse11	32angle@	01_2kfield10	29	6.9209E-6	1.	0533	1521.96	55	152.191	1	9.1802	0.02955	-0.0441	1	1.1193	831.658	8 0.7699	0.6449	0.933	4 1				
All Speci	ies Mo	ouse	mouse11	32angle@	01_2kfield11	26	6.2049E-6	0.	9577	1543.5	144.630	92	9.5556	0.06391	-0.0083	81	1.0581	1.3663	0.8332	0.6887	0.9541	1.506	9 5				
All Speci	ies Mo	ouse	mouse11	32angle@	01_2kfield12	73	0.00001742	22.	8405	1630.45	21	153.129	4	9.9779	0.00194	-0.0477	7	0.9183	178.158	39	0.8232	0.727	2 €				
All Speci	ies Mo	ouse	mouse11	32angle6	01_2kfield13	38	9.0688E-6	1.	1727	1293.10	53	137.390	9	8.7544	-0.0756	9	-0.149	1.0604	-342.14	185	0.8046	0.673	4 €				
All Speci	ies Mo	ouse	mouse11	32angle6	01_2kfield14	51	0.00001217	71.	8412	1512.78	43	148.378	9.2566	0.1714	0.1244	0.9839	-1135.9	978	0.7995	0.6692	0.943	1.589	6 5				
All Speci	ies Mo	ouse	mouse11	32angle0	01_2kfield15	35	8.3528E-6	1.	1192	1339.88	57	138.768	9.1706	-0.0491	5	-0.1063	1.1036	7295.44	119	0.8359	0.7073	0.953	2 1				
All Speci	ies Mo	ouse	mouse11	32angle@	01_2kfield1	32	7.6369E-6	0.	8865	1160.84	38	134.858	6	8.2363	-0.0072	16	-0.0305	6	0.9063	468.87	25	0.776	6€				
All Speci	ies Mo	ouse	mouse11	32angle6	01_2kfield2	75	0.0000179	2.	6887	1502.14	67	146.763	1	9.5058	-0.0702	2	-0.0766	1	0.9289	-221.2	951	0.818	9€				
All Speci	ies Mo	ouse	mouse11	32angle6	01_2kfield3	28	6.6822E-6	1.	0674	1597.32	14	169.819	5	8.9594	0.9793	0.868	1.2972	-79.062	2 0.7008	0.5198	0.9204	2.145	2 E				
All Speci	ies Mo	ouse	mouse11	32angle@	01_2kfield4	35	8.3528E-6	1.	3519	1618.51	43	160.766	7	9.2195	0.7726	0.6382	1.2646	-60.314	11	0.7626	0.5966	0.938	3 1				
All Speci	ies Mo	ouse	mouse11	32angle6	01_2kfield5	17	4.0571E-6	0.	5798	1429.11	76	139.229	9.4562	0.03175	-0.0372	16	1.0439	1610.7	316	0.8559	0.7718	0.951	7 1				
All Spect	ies Mo	ouse	mouse11	32angle6	01_2kfield6	34	8.1142E-6	1.	2171	1499.97	06	149.024	4	9.6237	0.07248	0.02272	1.025	-255.72	235	0.821	0.6957	0.951	B 1		-		1111
All Speci	ies Mo	ouse	mouse11	.32angle@	01_2kfield7	34	8.1142E-6	1.	0877	1340.5	139.294	14	8.9897	-0.0002	524	-0.0511	2	0.9842	195.629	94	0.8127	0.694	76			 one co 	nditior
All Speci	ies Mo	ouse	mouse11	32angle6	01_2kfield8	45	0.00001074	4 1.	9299	1797.02	22	161.714	5	10.5184	-0.0125	i5	-0.0453	4	0.9869	-21.35	66	0.828	4 E				
All Speci	ies Mo	ouse	mouse11	32angle@	01_2kfield9	35	8.3528E-6	1.	2329	1476.08	57	138.349	6	9.1963	-0.0637	8	-0.0995	5	1.0407	15998.	0319	0.840	96				
<																L	n 1, Col 1		100% V	Windows (C	RLF) U	TF-8	>				

• <u>Measurements_TMP folder</u>

Temporary folder used during application execution

• Data_visualization folder

Folder containing graphs and distributions for data visualization

density.png: density distribution of mitometrics for each condition
histogram.png: histogram distribution of mitometrics for each condition
MinMaxScaler_radar_plot.png: radar plot of MinMax rescaled mitometrics for each condition
StandardScaler_radar_plot.png: radar plot of Standard rescaled mitometrics for each condition
PCA_Condition_Name.png: PCA distribution of mitochondria according to the conditions
UMAP_Condition_Name.png: UMAP projection of mitochondria according to the conditions
violin.png: violin distribution of mitometrics for each condition

• Data_visualizationspatial_clustering folder

Folder containing spatial clustering of mitochondria detected for each image (*spatial_clustering* file), as well as density graph (*density* file) and mean morphometrics (*clusters* file) computed for each cluster

• <u>Prediction_analysis folder</u>

Folder containing SHAP values (*beeswarm* file) and confusion matrix (*confusion_matrix* file) for each machine learning algorithms used.

• Log_files folder

Folder containing application settings (Users_general_parameters.txt) and segmentation settings (Users_Mitochondria_size.txt).