EMITO-METRIX APPLICATION: INSTALLATION TUTORIAL

EMito-Metrix is a high-performance pipeline for analyzing mitochondrial morphology and ultrastructure in multiple tissues from low to high-resolution images acquired with Electron microscopy (EM). The application 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 to determine how any given experimental condition would impact on mitochondrial morphology and ultrastructure

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

This section describes installation instructions for Windows, Linux and MacOS operating systems

- SYSTEM REQUIREMENTS

Windows, Linux and MacOS are supported for running the EMito_Metrix application. Before installing, we recommend to check the following requirements for each software and environment used by the application:

- Anaconda distribution requirements:
 https://docs.anaconda.com/anaconda/install/
- Fiji software requirements:
 https://imagej.net/software/fiji/downloads#system-requirements
- Cellpose software requirements:
 https://github.com/MouseLand/cellpose?tab=readme-ov-file#system-requirements

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EMITO-METRIX INSTALLATION FOR WINDOWS

1- CELLPOSE PYTHON ENVIRONMENT: INSTALLATION AND SETTING

- Anaconda distribution installation

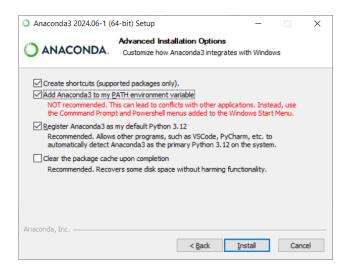
Install an Anaconda distribution for Python 3 using the following URL:

https://www.anaconda.com/download/success

Download the latest version of Anaconda installer for Windows 64bits. After downloading, go to your download folder and double-click the installer to launch Anaconda installation.

It is recommended that you install Anaconda using the *Just Me* option, which will install Anaconda Distribution to just the current user account (doesn't require Windows Administrator privileges).

During installation, add Anaconda to your PATH environment variable, as shown below:



See here for detailed instructions of Anaconda installation and settings

Cellpose installation & setting

Once you installed anaconda environment, you will need to create and set a python virtual environment for Cellpose application (which is essential for the EMito-Metrix application).

Open an anaconda prompt

From the Windows start menu (), type anaconda prompt in the search bar and click on the Anaconda application to start it. This will open a new command prompt window with Anaconda enabled, as shown below:



• Create the Cellpose Python environment

We are going to create a **Python virtual environment (venv) for Cellpose** application.

A virtual environment is simply a Python subdirectory with its own file structure, python packages and settings. This Virtual environment let you have a stable and reproducible Cellpose environment, which is essential for the proper running of the EMito-Metrix application.

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

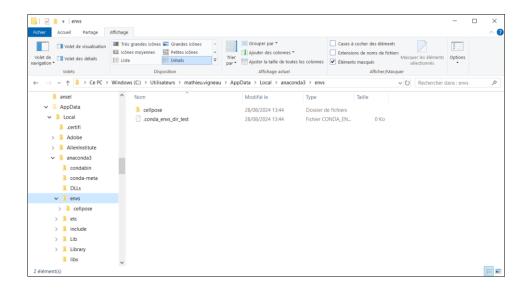
```
conda create --name cellpose python=3.9
```

This command line will create a new python3.9 environment named cellpose.

This will create a new folder, containing your Cellpose python environment. This cellpose folder is found under the following Windows path:

<u>C:\Users\username\AppData\Local\anaconda3\envs\cellpose</u> or

C:\Users\username \AppData\anaconda\envs\cellpose



Cellpose environment activation

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

conda activate cellpose

• Cellpose package installation

Next, you need to install Cellpose application from your Cellpose python venv. In your opened anaconda prompt, type the following python command line, and press Enter:

```
python -m pip install cellpose[gui]
```

This command line will install Cellpose python packages in your python virtual environment, also including the **Cellpose Graphical user Interface**.

• (Optional) Cellpose GPU package installation

If you have a CUDA supported GPU installed on your computer (see here for CUDA GPU compatibility), you can install a GPU version of Cellpose.

Please see this <u>URL</u> for the install instructions of CUDA toolkit and GPU version of Cellpose.

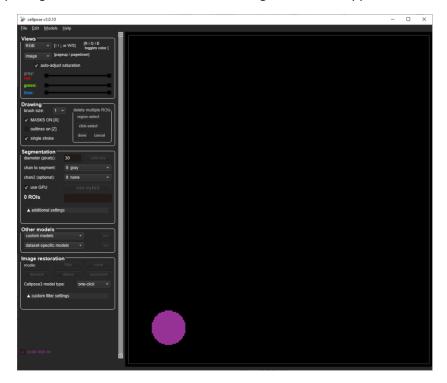
If you need more information about Cellpose detailed requirements, dependencies and installation instructions, see here.

• (Optional) Cellpose package checking

Once you installed the Cellpose package, we recommend verifying the validity of the installation. From your Cellpose python environment, type the following python command lines and press Enter:

cellpose

If the package installation is correct, the following window will appear:



If you have issues with installation, see the cellpose docs for more details.

• Cellpose environment deactivation

Once the Cellpose installation is correct, **deactivate your Cellpose python environment** typing in your opened anaconda prompt the following python command line:

conda deactivate

2- FIJI INSTALLATION AND SETTING

- Fiji software installation

Install Fiji software distribution using this following URL:

https://imagej.net/software/fiji/downloads

Download the latest version of Fiji for Windows 64-bit or Windows-32-bits using the *imagej.net* (USA) link. After downloading, go to your download folder and double-click the Fiji-zip file to uncompress it.

It is highly recommended that you store your Fiji app directory in your user space (C:\Users\username\MyDocuments\FijiApp) rather than in the Program Files windows-system directory (write permission issues in these system-wide folders could prevent Fiji updating)

See here for detailed installation instructions and system requirements of Fiji application

- FIJI plugin updating & setting

EMito-Metrix plugin relies on the following Fiji plugins, which are not automatically installed during the Fiji software installation:

- Bio-Formats (https://imagej.net/formats/bio-formats)

The Bio-Formats Importer is a plugin for reading data into Fiji. It can open many dozens of proprietary life sciences formats

- ImageScience (https://imagej.net/libs/imagescience)

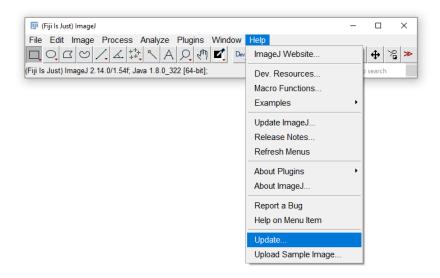
ImageScience is a library of Java classes to facilitate scientific image computing.

- PTBIOP (https://wiki-biop.epfl.ch/en/ipa/fiji/update-site)

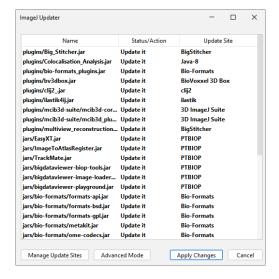
The EPFL - PTBIOP (BioImaging & Optics Platform) is a Fiji package including plugins, macros, ActionBars and Wrappers for external software calls (Cellpose, Elastix, Ilastix...)

- IBMP CNRS

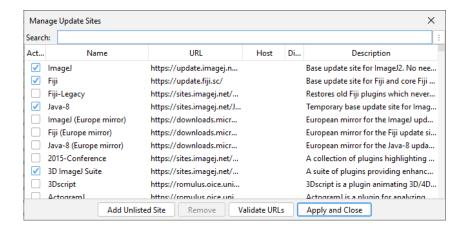
- In your Fiji App directory, double-click the ImageJ-win64.exe file to the start Fiji software
- Once the software is opened, select update from the Fiji Help menu (as shown below) in order to update Fiji plugins



• In the ImageJ Updater window, select the Manage update sites button (see below).



• The *Manage Update Sites* window appears, which contains a list of Fiji plugins you can upload and install in your Fiji application (plugins already installed are checked).



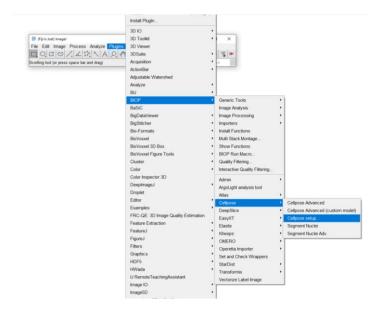
From this Manage Update Sites window, check the following plugins to install it:

- Bio-Formats
- IBMP-CNRS
- ImageScience
- PTBIOP
- Once these plugins are checked, click on Apply and Close button from the Manage Update Sites window. Then, click on Apply Changes button from the ImageJ Updater window
- Once the plugin are installed, restart Fiji application

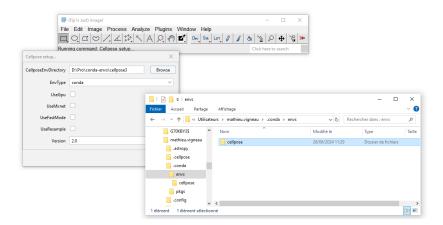
- Cellpose wrapper settings

Cellpose wrapper is a command from PTBIOP plugin that enables performing Cellpose object segmentation through Fiji application, using a python virtual environment with Cellpose package installed (see here to create Cellpose virtual environment).

• In your Fiji App, select *BIOP* from the Fiji *Plugins* menu. Then, select *Cellpose setup* in the *Cellpose* menu, as shown below:



- The Cellpose setup window appears which allows setting Cellpose wrapper. From this window, modify the following settings:
 - CellposeEnvDirectory: select your own Cellpose Python environment folder



- EnvType: python package chosen for your Cellpose environment

Choose **conda** if you have installed Anaconda distribution for Python

- UseGpu: allows using Cellpose with GPU computing (faster than CPU computing)

Activate the checkbox if you have installed a Cellpose GPU version

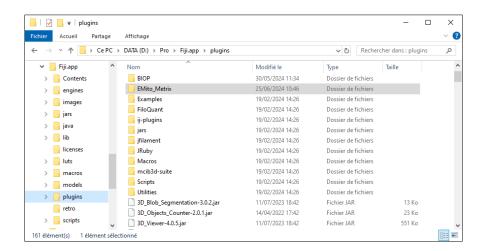
- Version: version of Cellpose launch from the Fiji wrapper

Choose 2.0

Then press OK

• EMito Metrix folder creation

In your Fiji plugins folder (yourFijiFolfer\plugins\), create a new folder named **EMito_Metrix**



• EMito Metrix file upload

Download and save the EMito_Metrix files in your *EMito_Metrix Fiji folder*, using the following GitHub repository:

https://github.com/Restore-Lab/EMito-Metrix

You must upload these following files from the GitHub repository (!! Keep the same file names !!):

- EMito Metrix Fiji .ijm (Fiji plugin file)
- dataComputation.py (python file for data display)
- mlComputation.py (python file for data computation and prediction)
- requirements.txt (python package dependencies)
- GeneralistModel_GM_EM.zip (Cellpose GM model)

Once copied, unzip the file in the EMito_Metrix folder and delete the zip archive

SpecialistModel_SM_FLY_EM.zip (Cellpose SM model for Fly)

Once copied, unzip the file in the EMito_Metrix folder and delete the zip archive

SpecialistModel_SM_HUMAN_EM.zip (Cellpose SM model for Human)

Once copied, unzip the file in the EMito_Metrix folder and delete the zip archive

- SpecialistModel_SM_MOUSE_EM.zip (Cellpose SM model for Mouse)

Once copied, unzip the file in the EMito_Metrix folder and delete the zip archive

- SpecialistModel_SM_ZEBRAFISH_EM.zip (Cellpose SM model for Zebrafish)

Once copied, unzip the file in the EMito_Metrix folder and delete the zip archive

3- EMITOMETRIX PYTHON ENVIRONMENT: INSTALLATION & SETTING

• Create the Emitometrix Python environment

We are going to create a **Python virtual environment (venv) for data computation plugins**.

A virtual environment is simply a Python subdirectory with its own file structure, python packages and settings. This Virtual environment let you have a stable and reproducible environment for data visualization and computation plugins, which are essential for the proper running of the EMito-Metrix application.

To create the emitometrix venv, type the following python command line in your anaconda prompt, and press Enter:

conda create --name emitometrix python=3.11

This command line will create a new python3.11 environment named emitometrix.

This will create a new folder containing your Emito-Metrix python environment. This folder is found under the following Windows path:

C:\Users\username\AppData\Local\anaconda3\envs\emitometrix or

C:\Users\username \AppData\anaconda\envs\emitometrix

• Emitometrix environment activation

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

conda activate emitometrix

• Python dependencies installation

EMito-Metrix plugin relies on the following Python packages, which are not automatically installed during emitometrix python environment installation:

- cloudpickle==3.0.0	- matplotlib==3.9.0	- pyparsing==3.1.2	- six==1.16.0
- contourpy==1.2.1	- numba==0.60.0	- python-dateutil==2.9.0.post0	- slicer==0.0.8
- cycler==0.12.1	- numpy==1.26.4	- pytz==2024.1	- threadpoolctl==3.5.0
- fonttools==4.53.0	- packaging==24.1	- scikit-learn==1.5.1	- tqdm==4.66.4
- joblib==1.4.2	- pandas==2.2.2	- scipy==1.14.0	- tzdata==2024.1
- kiwisolver==1.4.5	- pillow==10.4.0	- seaborn==0.13.2	- umap-learn==0.5.6
- Ilvmlite==0.43.0	- pynndescent==0.5.13	- shap==0.46.0	- xgboost==2.1.0

The list of these packages is included in the *requirements.txt* file that is found under your *EMito_Metrix Fiji* folder

example of EMitometrix folder: C:\Users\username\Documents\Fiji.app\plugins\EMito_Metrix

To install python dependencies in your emitometrix environment, type the following python command line from your opened anaconda prompt, and press Enter:

pip install -r yourFijiFolfer\plugins\EMito_Metrix\requirements.txt

• Cellpose environment deactivation

Once the Cellpose installation is correct, **deactivate your Emitometrix python environment** typing in your opened anaconda prompt the following python command line:

conda deactivate

Once you correctly installed these software and environments, the EMito-Metrix application is ready for running. See the *EMitoMetrix tutorial* for running instructions.

EMITO-METRIX INSTALLATION FOR MACOS

1- CELLPOSE PYTHON ENVIRONMENT: INSTALLATION AND SETTING

- Anaconda distribution installation

Install an Anaconda distribution for Python 3 using the following URL:

https://www.anaconda.com/download/success

Download the 64-bits graphical installer (.pkg) for your system. After downloading, go to your download folder and double-click the installer to launch Anaconda installation.

Double-click the downloaded file and click Continue to start the installation. Answer the prompts on the Introduction, Read Me, and License screens.

Anaconda recommends that you choose Install for all users of this computer (as shown below). As of version 2024.02-1, the default location of the installer is /opt/anaconda3*. To install elsewhere, select Install on a specific disk....



* This folder will be used for the following steps

See here for detailed instructions of Anaconda installation and settings

Cellpose installation & setting

Once you installed anaconda environment, you will need to create and set a python virtual environment for Cellpose application (which is essential for the EMito-Metrix application).

Open an anaconda prompt

From the Finder menu, type *Terminal* in the search bar and click on the *Terminal* icon to start it. This will open a new command prompt window with Anaconda enabled.

• Create the Cellpose Python environment

We are going to create a Python virtual environment (venv) for Cellpose application.

A virtual environment is simply a Python subdirectory with its own file structure, python packages and settings. This Virtual environment let you have a stable and reproducible Cellpose environment, which is essential for the proper running of the EMito-Metrix application.

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

conda create --name cellpose python=3.9

This command line will create a new python3.9 environment named cellpose.

This will create a new folder, containing your Cellpose python environment. This cellpose folder is found under the following Windows path*:

/opt/anaconda3/envs/cellpose

*If opt folder is hidden, open Finder and select Macintosh HD on the left side. Press Command + Shift + .

• Cellpose environment activation

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

conda activate cellpose

• <u>Cellpose package installation</u>

Next, you need to install Cellpose application from your Cellpose python venv. In your opened anaconda prompt, type the following python command line, and press Enter:

python -m pip install cellpose[gui]

This command line will install Cellpose python packages in your python virtual environment, also including the **Cellpose Graphical user Interface**.

• (Optional) Cellpose GPU package installation

If you have a CUDA supported GPU installed on your computer (see here for CUDA GPU compatibility), you can install a GPU version of Cellpose.

Please see this $\underline{\sf URL}$ for the install instructions of CUDA toolkit and GPU version of Cellpose.

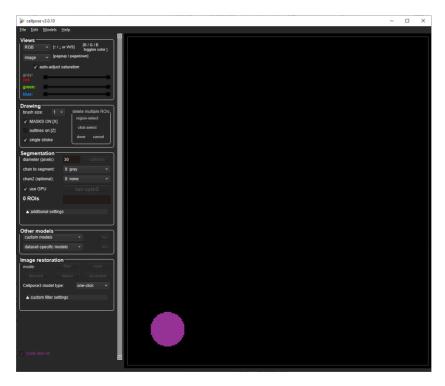
If you need more information about Cellpose detailed requirements, dependencies and installation instructions, see $\underline{\text{here}}$.

• (Optional) Cellpose package checking

Once you installed the Cellpose package, we recommend verifying the validity of the installation. From your Cellpose python environment, type the following python command lines and press Enter:

cellpose

If the package installation is correct, the following window will appear:



If you have issues with installation, see the cellpose docs for more details.

• Cellpose environment deactivation

Once the Cellpose installation is correct, **deactivate your Cellpose python environment** typing in your opened anaconda prompt the following python command line:

conda deactivate

2- FIJI INSTALLATION AND SETTING

- Fiji software installation

Install Fiji software distribution using this following URL:

https://imagej.net/software/fiji/downloads

Download the latest version of Fiji for macOS (x86_64) using the *imagej.net* (USA) link. After downloading, go to your download folder and double-click the Fiji-zip file to uncompress it. Once the application is installed, drag and drop your Fiji.app into the application folder.

See here for detailed installation instructions and system requirements of Fiji application

FIJI plugin updating & setting

EMito-Metrix plugin relies on the following Fiji plugins, which are not automatically installed during the Fiji software installation:

- Bio-Formats (https://sites.imagej.net/Bio-Formats/)

The Bio-Formats Importer is a plugin for reading data into Fiji. It can open many dozens of proprietary life sciences formats

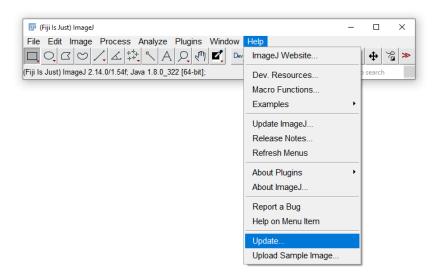
- ImageScience (https://sites.imagej.net/ImageScience/)

ImageScience is a library of Java classes to facilitate scientific image computing.

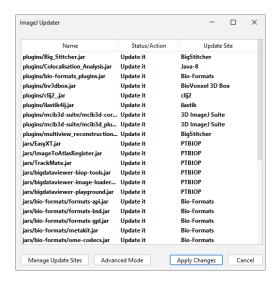
- PTBIOP (https://wiki-biop.epfl.ch/en/ipa/fiji/update-site) (https://biop.epfl.ch/Fiji-update-site) (https://biop.epfl.ch/Fiji-update-site) (https://biop.epfl.ch/Fiji-update-site) (https://biop.epfl.ch/Fiji-update)

The EPFL - PTBIOP (BioImaging & Optics Platform) is a Fiji package including plugins, macros, ActionBars and Wrappers for external software calls (Cellpose, Elastix, Ilastix...)

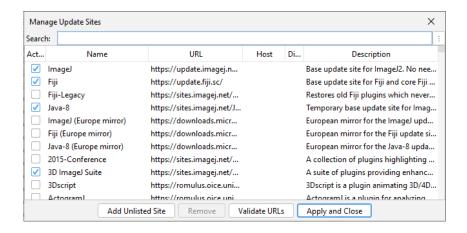
- IBMP CNRS (https://sites.imagej.net/Mutterer/)
- 3D ImageJ Suite (https://sites.imagej.net/Tboudier/)
- In your Fiji App directory, right-click on the Fiji.app file and select open to the start Fiji software.
- Once the software is opened, select *update* from the *Fiji Help menu* (as shown below) in order to update Fiji plugins



• In the ImageJ Updater window, select the Manage update sites button (see below).



• The *Manage Update Sites* window appears, which contains a list of Fiji plugins you can upload and install in your Fiji application (plugins already installed are checked).



From this Manage Update Sites window, check the following plugins to install it:

- Bio-Formats
- IBMP-CNRS
- ImageScience
- PTBIOP

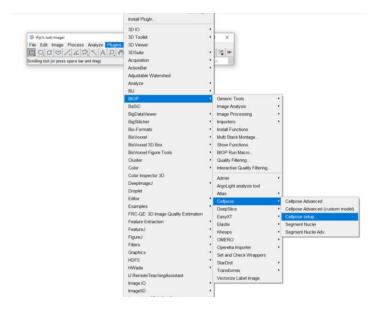
If one or more plugins are not available in the *Manage Update Sites* window, you can add the sites manually using the *Add Unlisted Site* button. For each unlisted plugin, specify the corresponding URL from this <u>list</u>. Once all plugins are manually specified, check it.

- Once these plugins are checked, click on Apply and Close button from the Manage Update Sites window. Then, click on Apply Changes button from the ImageJ Updater window
- Once the plugin are installed, restart Fiji application

Cellpose wrapper settings

Cellpose wrapper is a command from PTBIOP plugin that enables performing Cellpose object segmentation through Fiji application, using a python virtual environment with Cellpose package installed (see here to create Cellpose virtual environment).

• In your Fiji App, select *BIOP* from the Fiji *Plugins* menu. Then, select *Cellpose setup* in the *Cellpose* menu, as shown below:



- The *Cellpose setup* window appears which allows setting Cellpose wrapper. From this window, modify the following settings:
 - <u>CellposeEnvDirectory</u>: select your own Cellpose Python environment folder /opt/anaconda3/envs/cellpose
 - <u>EnvType</u>: python package chosen for your Cellpose environment

 Choose conda if you have installed Anaconda distribution for Python
 - <u>UseGpu</u>: allows using Cellpose with GPU computing (faster than CPU computing)

 Activate the checkbox if you have installed a Cellpose GPU version
 - <u>Version</u>: version of Cellpose launch from the Fiji wrapper *Choose 2.0*
- Then press OK

• EMito Metrix folder creation

In your *Application* folder, right-click on the *Fiji.app* icon and select *Show Package Contents*. In your Fiji plugins folder (*yourFijiFolfer*\plugins\), create a new folder named **EMito_Metrix**

• EMito_Metrix file upload

Download and save the EMito_Metrix files in your *EMito_Metrix Fiji folder*, using the following GitHub repository:

https://github.com/Restore-Lab/EMito-Metrix

You must upload these following files from the GitHub repository (!! Keep the same file names !!) :

- EMito_Metrix_Fiji_.ijm (Fiji plugin file)
- dataComputation.py (python file for data display)
- mlComputation.py (python file for data computation and prediction)
- requirements.txt (python package dependencies)
- GeneralistModel_GM_EM.zip (Cellpose GM model)

Once copied, unzip the file in the EMito_Metrix folder and <u>delete the zip archive</u>

- SpecialistModel_SM_FLY_EM.zip (Cellpose SM model for Fly)

Once copied, unzip the file in the EMito_Metrix folder and delete the zip archive

- SpecialistModel_SM_HUMAN_EM.zip (Cellpose SM model for Human)

Once copied, unzip the file in the EMito_Metrix folder and delete the zip archive

- SpecialistModel_SM_MOUSE_EM.zip (Cellpose SM model for Mouse)

Once copied, unzip the file in the EMito_Metrix folder and delete the zip archive

- SpecialistModel_SM_ZEBRAFISH_EM.zip (Cellpose SM model for Zebrafish)

Once copied, unzip the file in the EMito_Metrix folder and <u>delete the zip archive</u>

• Create the Emitometrix Python environment

We are going to create a **Python virtual environment (venv) for data computation** plugins.

A virtual environment is simply a Python subdirectory with its own file structure, python packages and settings. This Virtual environment let you have a stable and reproducible environment for data visualization and computation plugins, which are essential for the proper running of the EMito-Metrix application.

To create the emitometrix venv, type the following python command line in your anaconda prompt, and press Enter:

conda create --name emitometrix python=3.11

This command line will create a new python3.11 environment named emitometrix.

This will create a new folder containing your Emito-Metrix python environment. This folder is found under the following Windows path:

/opt/anaconda3/envs/emitometrix

• Emitometrix environment activation

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

conda activate emitometrix

• Python dependencies installation

EMito-Metrix plugin relies on the following Python packages, which are not automatically installed during emitometrix python environment installation:

- cloudpickle==3.0.0	- matplotlib==3.9.0	- pyparsing==3.1.2	- six==1.16.0
- contourpy==1.2.1	- numba==0.60.0	- python-dateutil==2.9.0.post0	- slicer==0.0.8
- cycler==0.12.1	- numpy==1.26.4	- pytz==2024.1	- threadpoolctl==3.5.0
- fonttools==4.53.0	- packaging==24.1	- scikit-learn==1.5.1	- tqdm==4.66.4
- joblib==1.4.2	- pandas==2.2.2	- scipy==1.14.0	- tzdata==2024.1
- kiwisolver==1.4.5	- pillow==10.4.0	- seaborn==0.13.2	- umap-learn==0.5.6
- Ilvmlite==0.43.0	- pynndescent==0.5.13	- shap==0.46.0	- xgboost==2.1.0

The list of these packages is included in the *requirements.txt* file that is found under your *EMito_Metrix* folder

example of EMitometrix folder: /Application/Fiji.app/plugins/EMito_Metrix

To install python dependencies in your emitometrix environment, type the following python command line from your opened anaconda prompt, and press Enter:

pip install -r yourFijiFolfer/plugins/EMito_Metrix/requirements.txt

• Cellpose environment deactivation

Once the Cellpose installation is correct, **deactivate your Emitometrix python environment** typing in your opened anaconda prompt the following python command line:

conda deactivate

Once you correctly installed these software and environments, the EMito-Metrix application is ready for running. See the *EMitoMetrix tutorial* for running instructions.

EMITO-METRIX INSTALLATION FOR LINUX

1- CELLPOSE PYTHON ENVIRONMENT: INSTALLATION AND SETTING

- Anaconda distribution installation

Install an Anaconda distribution for Python 3 using the following URL:

https://www.anaconda.com/download/success

Download the latest version of Anaconda installer, depending on your Linus architecture.

See $\underline{\text{here}}$ for detailed instructions of Anaconda installation and settings for Linux operating system

- Cellpose installation & setting

Once you installed anaconda environment, you will need to create and set a python virtual environment for Cellpose application (which is essential for the EMito-Metrix application).

• Open an anaconda prompt

Open a terminal window. This will open a new command prompt window with Anaconda enabled (as shown below).



• Create the Cellpose Python environment

We are going to create a **Python virtual environment (venv) for Cellpose** application.

A virtual environment is simply a Python subdirectory with its own file structure, python packages and settings. This Virtual environment let you have a stable and reproducible Cellpose environment, which is essential for the proper running of the EMito-Metrix application.

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

```
conda create --name cellpose python=3.9
```

This command line will create a new python3.9 environment named cellpose.

This will create a new folder, containing your Cellpose python environment. This cellpose folder is found under the following Linux path:

/home/username/anaconda3/envs/cellpose

• Cellpose environment activation

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

conda activate cellpose

• <u>Cellpose package installation</u>

Next, you need to install Cellpose application from your Cellpose python venv. In your opened anaconda prompt, type the following python command line, and press Enter:

python -m pip install cellpose[gui]

This command line will install Cellpose python packages in your python virtual environment, also including the **Cellpose Graphical user Interface**.

• (Optional) Cellpose GPU package installation

If you have a CUDA supported GPU installed on your computer (see here for CUDA GPU compatibility), you can install a GPU version of Cellpose.

Please see this <u>URL</u> for the install instructions of CUDA toolkit and GPU version of Cellpose.

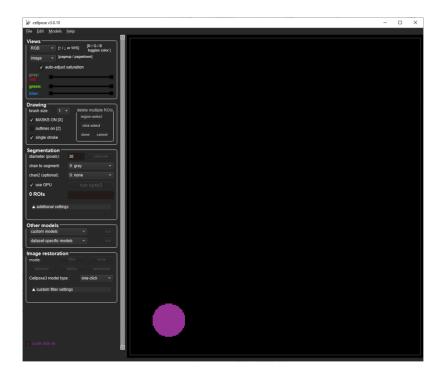
If you need more information about Cellpose detailed requirements, dependencies and installation instructions, see $\underline{\text{here}}$.

• (Optional) Cellpose package checking

Once you installed the Cellpose package, we recommend verifying the validity of the installation. From your Cellpose python environment, type the following python command lines and press Enter:

cellpose

If the package installation is correct, the following window will appear:



If you have issues with installation, see the cellpose docs for more details.

• <u>Cellpose environment deactivation</u>

Once the Cellpose installation is correct, **deactivate your Cellpose python environment** typing in your opened anaconda prompt the following python command line:

conda deactivate

2- FIJI INSTALLATION AND SETTING

- Fiji software installation

Install Fiji software distribution using this following URL:

https://imagej.net/software/fiji/downloads

Download the latest version of Fiji for Linux 64-bit using the *imagej.net (USA)* link. After downloading, go to your download folder and double-click the *Fiji-zip* file to uncompress it.

See here for detailed installation instructions and system requirements of Fiji application

FIJI plugin updating & setting

EMito-Metrix plugin relies on the following Fiji plugins, which are not automatically installed during the Fiji software installation:

- Bio-Formats (https://imagej.net/formats/bio-formats)

The Bio-Formats Importer is a plugin for reading data into Fiji. It can open many dozens of proprietary life sciences formats

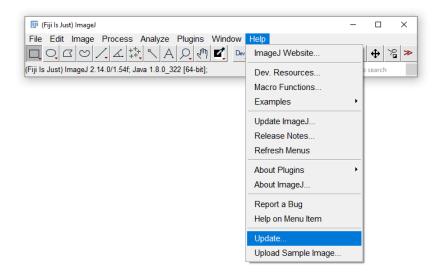
ImageScience (https://imagej.net/libs/imagescience)

ImageScience is a library of Java classes to facilitate scientific image computing.

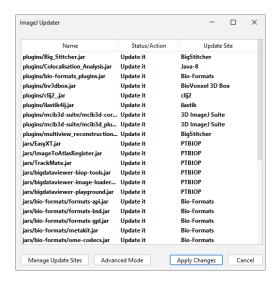
- PTBIOP (https://wiki-biop.epfl.ch/en/ipa/fiji/update-site)

The EPFL - PTBIOP (BioImaging & Optics Platform) is a Fiji package including plugins, macros, ActionBars and Wrappers for external software calls (Cellpose, Elastix, Ilastix...)

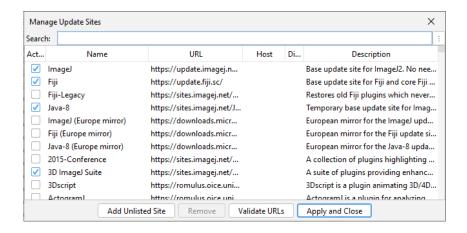
- IBMP CNRS
- In your Fiji App directory, double-click the *ImageJ-win64.exe* file to the start Fiji software
- Once the software is opened, select update from the Fiji Help menu (as shown below) in order to update Fiji plugins



• In the ImageJ Updater window, select the Manage update sites button (see below).



• The *Manage Update Sites* window appears, which contains a list of Fiji plugins you can upload and install in your Fiji application (plugins already installed are checked).



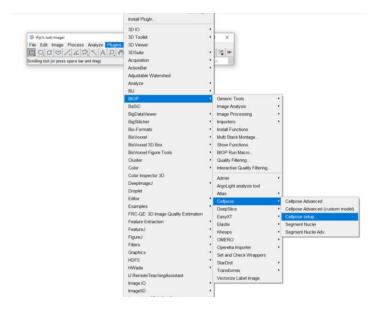
From this Manage Update Sites window, check the following plugins to install it:

- Bio-Formats
- IBMP-CNRS
- ImageScience
- PTBIOP
- Once these plugins are checked, click on Apply and Close button from the Manage Update Sites window. Then, click on Apply Changes button from the ImageJ Updater window
- Once the plugin are installed, restart Fiji application

Cellpose wrapper settings

Cellpose wrapper is a command from PTBIOP plugin that enables performing Cellpose object segmentation through Fiji application, using a python virtual environment with Cellpose package installed (see here to create Cellpose virtual environment).

• In your Fiji App, select *BIOP* from the Fiji *Plugins* menu. Then, select *Cellpose setup* in the *Cellpose* menu, as shown below:



- The *Cellpose setup* window appears which allows setting Cellpose wrapper. From this window, modify the following settings:
 - <u>CellposeEnvDirectory</u>: select your own Cellpose Python environment folder /home/username/anaconda3/envs/cellpose/
 - EnvType: python package chosen for your Cellpose environment

Choose **conda** if you have installed Anaconda distribution for Python

- <u>UseGpu</u>: allows using Cellpose with GPU computing (faster than CPU computing)

 Activate the checkbox if you have installed a Cellpose GPU version
- <u>Version</u>: version of Cellpose launch from the Fiji wrapper *Choose 2.0*
- Then press OK

• EMito Metrix folder creation

In your Fiji plugins folder (yourFijiFolfer\plugins\), create a new folder named **EMito_Metrix**

EMito_Metrix file upload

Download and save the EMito_Metrix files in your *EMito_Metrix Fiji folder*, using the following GitHub repository:

https://github.com/Restore-Lab/EMito-Metrix

You must upload these following files from the GitHub repository (!! Keep the same file names !!) :

- EMito_Metrix_Fiji_.ijm (Fiji plugin file)
- dataComputation.py (python file for data display)
- mlComputation.py (python file for data computation and prediction)
- requirements.txt (python package dependencies)
- GeneralistModel_GM_EM.zip (Cellpose GM model)

Once copied, unzip the file in the EMito_Metrix folder and delete the zip archive

SpecialistModel_SM_FLY_EM.zip (Cellpose SM model for Fly)

Once copied, unzip the file in the EMito_Metrix folder and delete the zip archive

- SpecialistModel_SM_HUMAN_EM.zip (Cellpose SM model for Human)

Once copied, unzip the file in the EMito_Metrix folder and delete the zip archive

- SpecialistModel_SM_MOUSE_EM.zip (Cellpose SM model for Mouse)

Once copied, unzip the file in the EMito_Metrix folder and delete the zip archive

- SpecialistModel SM ZEBRAFISH EM.zip (Cellpose SM model for Zebrafish)

Once copied, unzip the file in the EMito_Metrix folder and delete the zip archive

Create the Emitometrix Python environment

We are going to create a **Python virtual environment (venv) for data computation** plugins.

A virtual environment is simply a Python subdirectory with its own file structure, python packages and settings. This Virtual environment let you have a stable and reproducible environment for data visualization and computation plugins, which are essential for the proper running of the EMito-Metrix application.

To create the emitometrix venv, type the following python command line in your anaconda prompt, and press Enter:

conda create --name emitometrix python=3.11

This command line will create a new python3.11 environment named emitometrix.

This will create a new folder containing your Emito-Metrix python environment. This folder is found under the following Linux path:

/home/username/anaconda3/envs/emitometrix/

• Emitometrix environment activation

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

conda activate emitometrix

• Python dependencies installation

EMito-Metrix plugin relies on the following Python packages, which are not automatically installed during emitometrix python environment installation:

- cloudpickle==3.0.0	- matplotlib==3.9.0	- pyparsing==3.1.2	- six==1.16.0
- contourpy==1.2.1	- numba==0.60.0	- python-dateutil==2.9.0.post0	- slicer==0.0.8
- cycler==0.12.1	- numpy==1.26.4	- pytz==2024.1	- threadpoolctl==3.5.0
- fonttools==4.53.0	- packaging==24.1	- scikit-learn==1.5.1	- tqdm==4.66.4
- joblib==1.4.2	- pandas==2.2.2	- scipy==1.14.0	- tzdata==2024.1
- kiwisolver==1.4.5	- pillow==10.4.0	- seaborn==0.13.2	- umap-learn==0.5.6
- Ilvmlite==0.43.0	- pynndescent==0.5.13	- shap==0.46.0	- xgboost==2.1.0

The list of these packages is included in the *requirements.txt* file that is found under your *EMito_Metrix Fiji* folder

example of EMitometrix folder: /home/username/Fiji.app/plugins/EMito_Metrix

To install python dependencies in your emitometrix environment, type the following python command line from your opened anaconda prompt, and press Enter:

pip install -r yourFijiFolfer\plugins\EMito_Metrix\requirements.txt

• Cellpose environment deactivation

Once the Cellpose installation is correct, **deactivate your Emitometrix python environment** typing in your opened anaconda prompt the following python command line:

conda deactivate

Once you correctly installed these software and environments, the EMito-Metrix application is ready for running. See the *EMitoMetrix tutorial* for running instructions.