# 1 Introduction

Thank you for using **MitoCellPhe**.

# 2 Installation and Setup

## 2.1 MitoCellPhe Skeletonizer

To use MitoCellPhe Skeletonzier, you will need the following software on your PC:

**CellProfiler**: `www.cellprofiler.org/previous-releases`

- In order to generate the best possible skeletons for the mitochondrial anaylsis, we recommend using our skeletonizer pipeline for CellProfiler. The pipeline is currently designed for version 3.1.9.
- Once CellProfiler 3.1.9 is installed, you will need to install the requried modules to run the pipeline.

To see where the required modules should be installed, launch CellProfiler and go to File → Preferences → "CellProfiler plugins directory". The default folder can be used, or another folder can be selected. Whatever folder is chosen, navigate to that folder in File Explorer, creating it if it does not exist. Then, copy `clahe.py`, `coverrectangle.py` and `savecroppedobjectsplusplus.py` to the folder. Close and relaunch CellProfiler, and the modules should be available for use.

With the modules available for use, the MitoCellPhe Skeletonizer pipeline can be loaded into CellProfiler. Go to File → "Open Project" and load one of the `.cpproj` files depending on the type of cells analyzed.

## 2.2 MitoCellPhe Analyzer

To use MitoCellPhe Analyzer, you will need the following software on your PC:

**Fiji**: `www.imagej.net/Fiji/Downloads`

- Fiji can be installed anywhere on your local machine. Please keep the Fiji install folder handy for use with MitoCellPhe, as you must link to this folder when setting up your batch processing. You will need to input the path to the Fiji folder that you extracted in the MitoAnalyzer, so make sure you know where it is.

- Your Fiji install must include `Jython.jar` so it can process the Python script that performs batch processing. To do this, open Fiji, go to Plugins → Macros → Run..., and open any Python (`.py`) file. If Jython is not installed, this will open a prompt that allows you to download and install the correct `.jar` file. Once the `.jar` is installed, you can close Fiji.
- If you do not have a python script you can create one. Open notepad or another text editor. Save an empty file as "test.py", ensuring that the file extension is ".py". You can use this file for the above step and then delete it afterwards.

**Java JDK:** `www.oracle.com/java/technologies/javase/javase-jdk8-downloads.html`

- Download and run the installer for your OS.
- For Windows 10, after completing the install, you will need to edit some environment variables to make sure the software runs (see `https://bit.ly/34D1tDW` for an explanation of how to edit environment variables in Windows 10).
  - Add `JAVA_HOME` to system variables, with the path `C:\Program Files\Java\jdk1.8.0_261` (version number may vary, as long as it starts with "jdk").
  - Edit `PATH` system variable and add to the end: `C:\Program Files\Java\jdk1.8.0_261\bin` (version number may vary).
  - Add `PYJNIUS_JAR` to system variables, with the path (install folder)`\jnius\pyjnius.jar` (replace install folder with where MitoCellPhe is located).

**Apache Maven:** `maven.apache.org/download.cgi`

- Download Apache Maven "Binary Zip archive" from the link above.
- For Windows 10, extract the folder contained within the downloaded zip file to `C:\Program Files\`.
  - Edit `PATH` system variable and add to the end: `C:\Program Files\apache-maven-3.6.3\bin`

After installing the prerequisites, extract the .zip file containing MitoCellPhe analyzer anywhere easy for you to access. Then, click on "MitoCellPhe Analyzer.exe", and the application will open. If the application opens, you should see the application as presented in Figure 1.

## 3 Using MitoCellPhe Skeletonizer

To use the skeletonizer, first decide whether you would like to perform differentiated or undifferentiated skeletonization. Differentiated skeletonization will attempt to segment individual cells in the image and outputs skeletons of each segmented cell. Undifferentiated skeletonization will not segment the cells, the output is a skeleton of the entire input image.

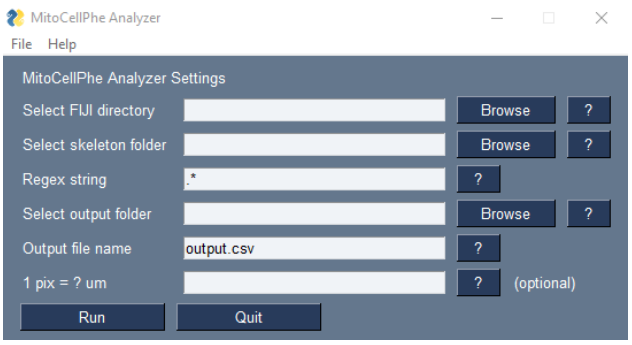


Figure 1: The MitoCellPhe Analyzer window.

First, open CellProfiler 3.1.9 and go to File → "Open Project". Open one of the following two project files: `differentiated_pipeline.cpproj` or `undifferentiated_pipeline.cpproj`. Next, clear the Image file list, then add your stain image files by dragging and dropping them into the Images module. You can also drop in entire folders.

Change any filters and regular expressions in the Image module as needed to filter your input data folders to select only the mitochondria stain images. Once you have the images loaded in the Images module, press the 'Analyze Images' button to generate the skeletons. The skeletons will be saved into the same location that the input files are located, with suffixes attached.

Each skeleton will be a new file with the same file name as the input stain image it corresponds to and "`_cp_skel`" or "`_cp_skel_invert`" is appended to the file name to distinguish the skeletons from the input images. The former label represents skeletons with a black background and a white foreground and the latter is the opposite. If you would like the skeletons to be saved elsewhere, modify the SaveCroppedObjects++ (or SaveImages module for the undifferentiated pipeline) modules' "Output file location" setting.

After the pipeline has finished processing, you should have a set of skeletonized images in the same directory as your original data with "_cp_skel" appended to differentiate them from the input images. Now, you can close CellProfiler and use the MitoCellPhe Analyzer to analyze the skeletons.

## 4 Using MitoCellPhe Analyzer

Opening MitoCellPhe Analyzer should present the window shown in Figure 1 and a blank console. As actions are performed in MitoCellPhe, they are outputted to the console for reference. This is useful in case there are any errors that need to be diagnosed.

To run the analyzer, set the parameters in the Analyzer window then click the 'Run' button. Each parameter has an associated help window with extra information about the parameter accessible through the '?' buttons. Through the File menu in the left-hand corner, you can save and load parameter settings. The parameters are described below.

1. **Select FIJI directory**: Browse to the folder which contains the FIJI ImageJ executable.

2. **Select skeleton folder**: Browse to the root folder which contains all of the skeletons for analysis. If you followed the instructions to skeletonize your images, this is the same folder that your input stain images are located in.

3. **Regex string**: Enter a regular expression pattern to determine which files to select. If you followed the instructions above to skeletonize your images, then the regex string should be: ".*_cp_skel.*" (without quotation marks).

4. **Select output folder**: Browse to the folder that you would like the output file to be saved to. This can be any folder you like or a new folder.

5. **Output file name**: Choose a name for the output file. The output file name should end in ".csv" because the output file is a comma separated values (csv) file.

6. **1 pix = ? um**: Set the pixel to micrometer scale. How many micrometers are expressed in 1 pixel? Enter '1' to keep the output measurements in pixels.

After setting the parameters, click the 'Run' button. If the parameters are set correctly and MitoCellPhe Analyzer and its dependencies are installed correctly, the software will analyze the skeletonized image files and report the results of each image's analysis as a row in the output file. If you would like to save the parameters you entered, go to File → "Save Parameters". To load parameters from a file go to File → "Load Parameters". This makes it easy to repeat the analysis or re-run similar analyses, such as using the same scale or the same data files.

# 5 Troubleshooting