Metabolomics Image Analyzer (MIA)

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1 Introduction

Metabolomics or proteomics studies involve investigation of hundreds of different metabolites or proteins present in different types of biological samples. The understanding of these fields has largely originated from the research on cell lines. Frequently the result interpretation of metabolomics studies performed on cell line experiments depends on appropriate normalization of the data to account for variability in cell numbers that may be introduced by several factors including different treatments and different time courses for incubation. Since most metabolite concentrations correlate with cell numbers, normalization methods that do not adequately account for alterations in cell numbers can lead to erroneous conclusions [1].

Many present protocols to evaluate cell population are invasive using a cell counter or flow cytometry which disables time-course study on the cell samples. Non-invasive protocols typically utilize microscope images with florescence to enhance contrast of characteristic objects. Nevertheless, florescent agents may contaminate the cell extracts, leading to measurement inaccuracy at metabolomics and proteomics levels.

Detection of non-confluent adherent cells under bright field microscopy (BFM) without fluorescence has been reported using off-focus imaging [2]. Foreground segmentation of adherent cells under phase contrast microscopy (PCM) for highly confluent cell culture has been reported to calculate cell confluence [3]. Nevertheless detection of individual adherent cells under PCM for highly confluent culture has been challenging. Most cells anchor to a substrate that allows adhesion, while others can also adapt to suspension culture in a free-floating manner. The shape of suspended cells is typically quasi-spherical due to surface tension. Therefore each of them acts as a convex lens and exhibits local intensity maximum about the centroid. The shape of the adherent cells, however, is typically amorphous due to interaction with the container. These cells exhibit low-contrast intensity minimum due to chromatins. The contrast of the cells is so low that advanced techniques need to be developed to capture essential details.

Here we present a software tool, metabolomics image analyzer (MIA), to detect cells under PCM with different confluences. We use a self-adaptive algorithm to determine the parameters used in the tool. The detection of individual cells can be used to calculate characteristics of cell culture including count, confluence, and spatial distribution of cells. The structure of MIA is shown in Figure 1.

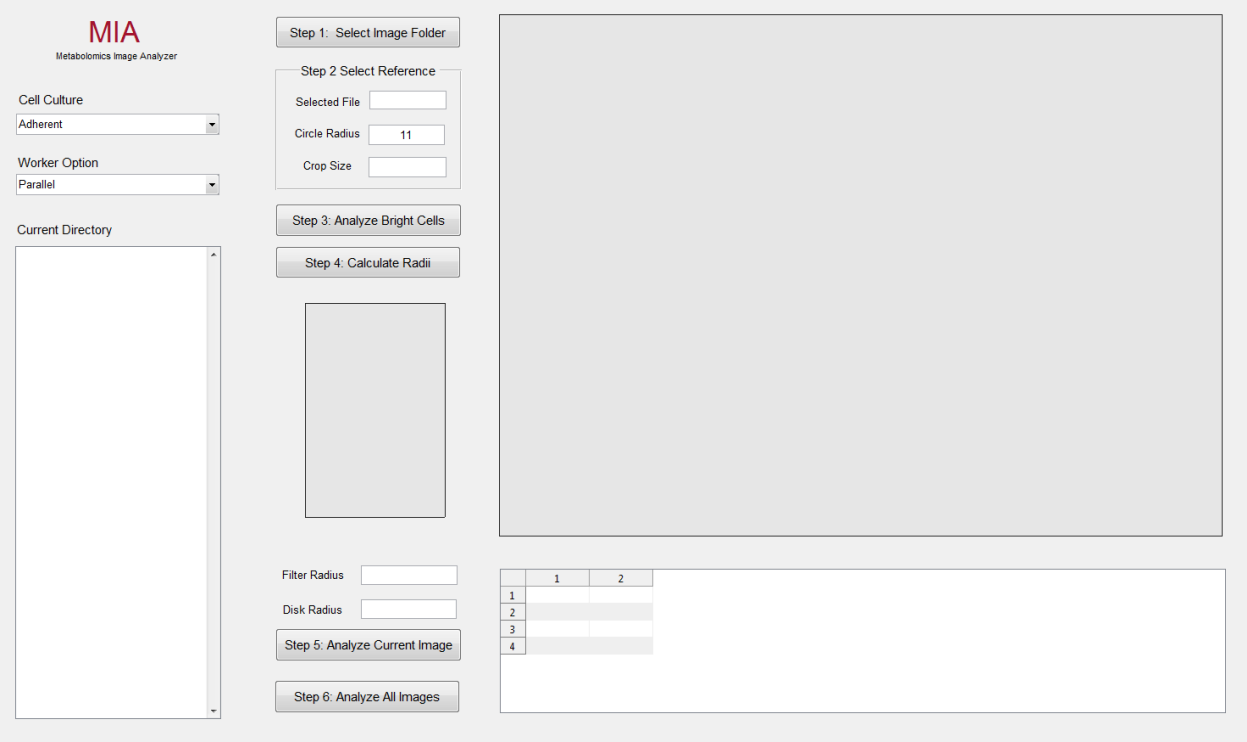

Figure 1 The structure of MIA.

2 Prerequisites

MIA is developed under MATLAB 2016b, and is packaged using MATLAB compiler. Similar to Java application, the software tool requires installation of MATLAB Compiler Runtime (MCR), which is included in the package. Double-clicking "MyAppInstaller_web.exe" will initialize the installation. The installation time is dependent on the internet connection of the users. After installation of MCR is complete, one can use MIA as an executable.

Even if one has MATLAB installed, it is still recommended that one download MCR before using MIA. Different versions of MATLAB have different functions, and sometimes the same function are called by different syntaxes.

3 Applications

As an executable with graphic user interface, MIA is very easy to use. The workflow is illustrated by the following steps. Although buttons need to be clicked, the workflow is automatic as no parameter input is required in addition to image file selection. The software is designed this way so one is aware of what MIA is doing in each step and can make sure everything is done in a satisfactory manner.

1. Cell Culture popup menu allows one to choose cell culture type, and Worker Option popup menu allows the users to decide whether Step 6 is performed in a single worker or in parallel. Normally parallel computing leads to less computation time, and the computation time reduction is more significant when more images are processed.

2. The Current Directory lists all TIFF files in current directory. If the current directory is where the images of interest are stored, then Step 1 can be skipped. Otherwise, click "Step 1 Image Folder" to browse the image folder.

3. In Step 2, one can click on each file in current directory to preview the TIFF image. The highlighted image file will be chosen as the reference image in this step. Reference image is used to preview the processing result and calculate the filter radius and disk radius for all files in the folder, typically from the same sample. Circle Radius has a default value of 11, and can be adjusted to capture the bright cells. Since bright cells take up a small portion of the total count, the choice of this value does not noticeably affect the total count. For large images, one can crop the image with a margin size defined in Crop Size text box. The default value is 10% of the size of the image.

4. (Adherent Cells Only) Step 3 analyzes bright mitotic cells in the image. The analyzed image is shown in the preview window.

5. (Adherent Cells Only) Step 4 calculates filter radius and disk radius for dark cell detection in the reference image, which will be used for other images in the folder to save computation time.

6. (Adherent Cells Only) Step 5 analyzes the dark resting cells in reference image. The analyzed image is shown in the preview windows.

7. Step 6 analyzes all images in the folder.

4 Example

Here an example of using MIA to process two adherent cell images is shown. The example images are also included with the package. We use the default options in the popup menus. In Step 1, we change the image folder to “adherent_test”. The two TIFF files will show in current directory. In Step 2, we click on either image, the preview window on the right of the tool will show the image (Figure 2).

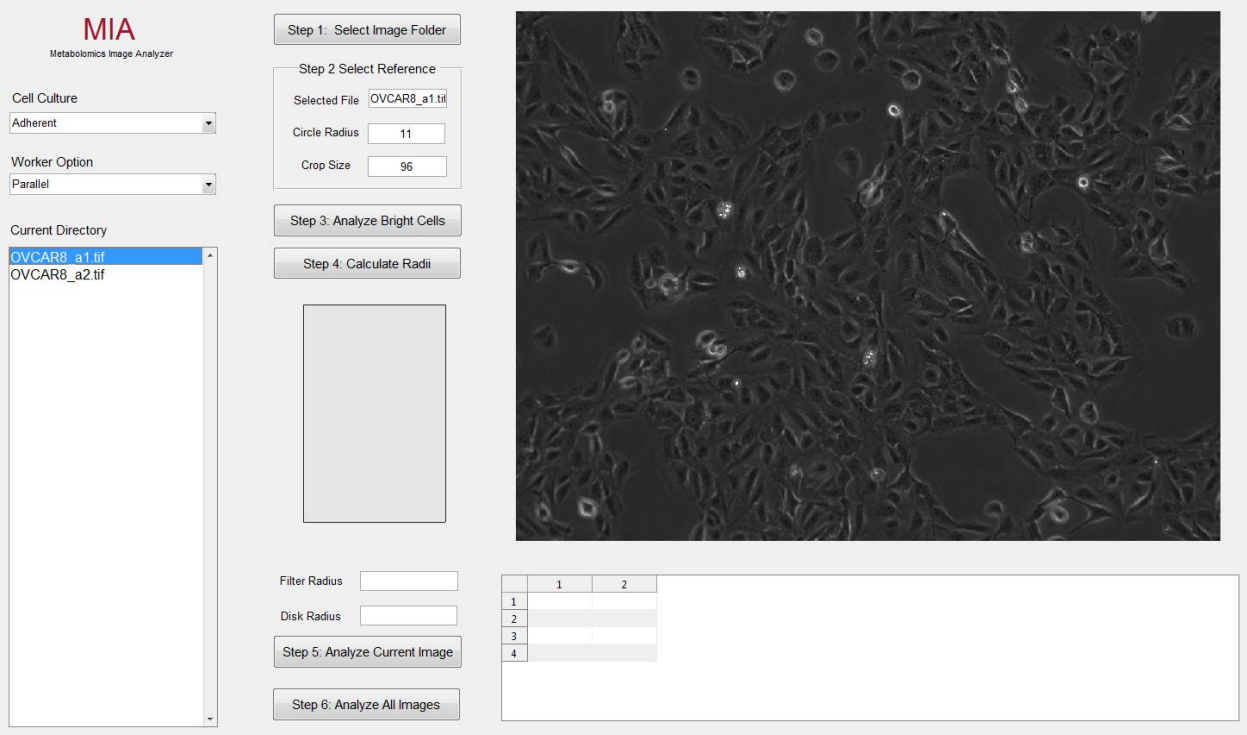

Figure 2 Step 1 and 2.

In Step 3, we analyze the bright mitotic cells in the image. The analysis is dependent on Circle Radius and Crop Size, which are automatically defined in Step 2. However, one can adjust these two values. Here we use 300 as crop size to expedite the process for illustration purposes (Figure 3).

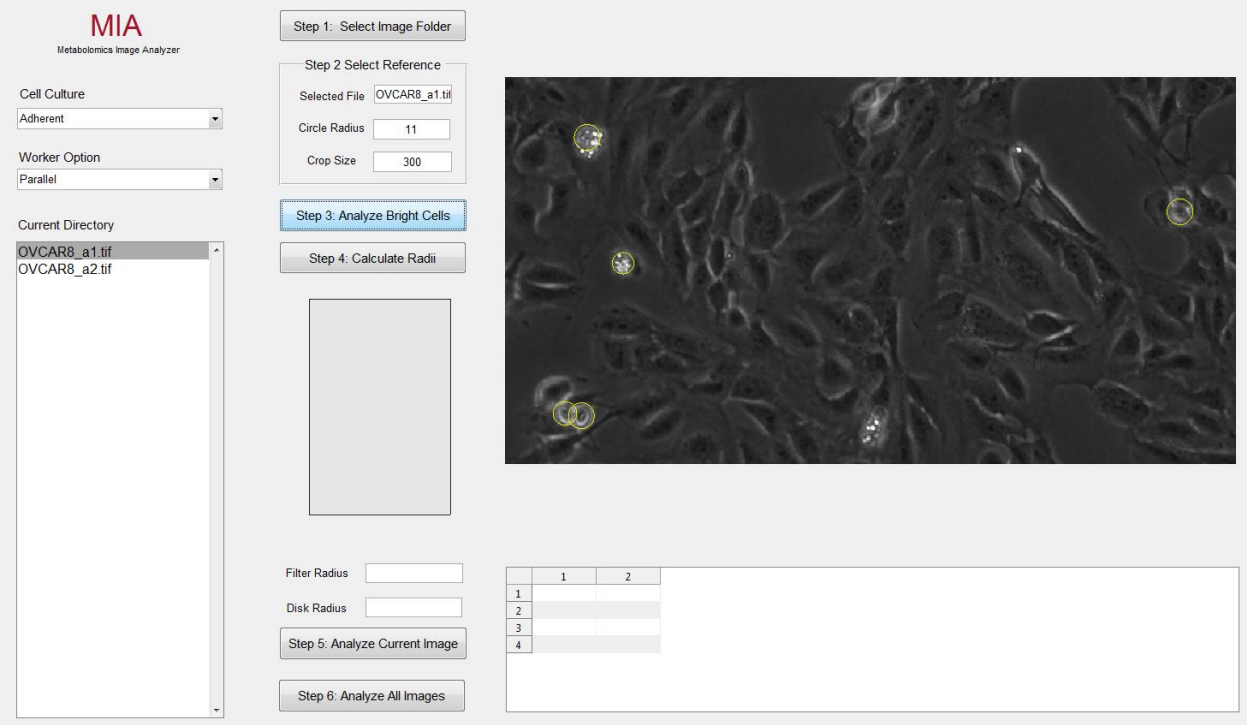

Figure 3 Step 3.

In Step 4, we need to calculate the two parameters from the reference image. This will take some time, but is not always required. One can directly type input values into the two text boxes. The values will be used in Step 5 and 6. Using the radii calculated from Step 4, we are able to get the processed reference image after clicking Step 5. The processed image is shown in the preview window as well. The analysis result is shown in the table below the preview window.

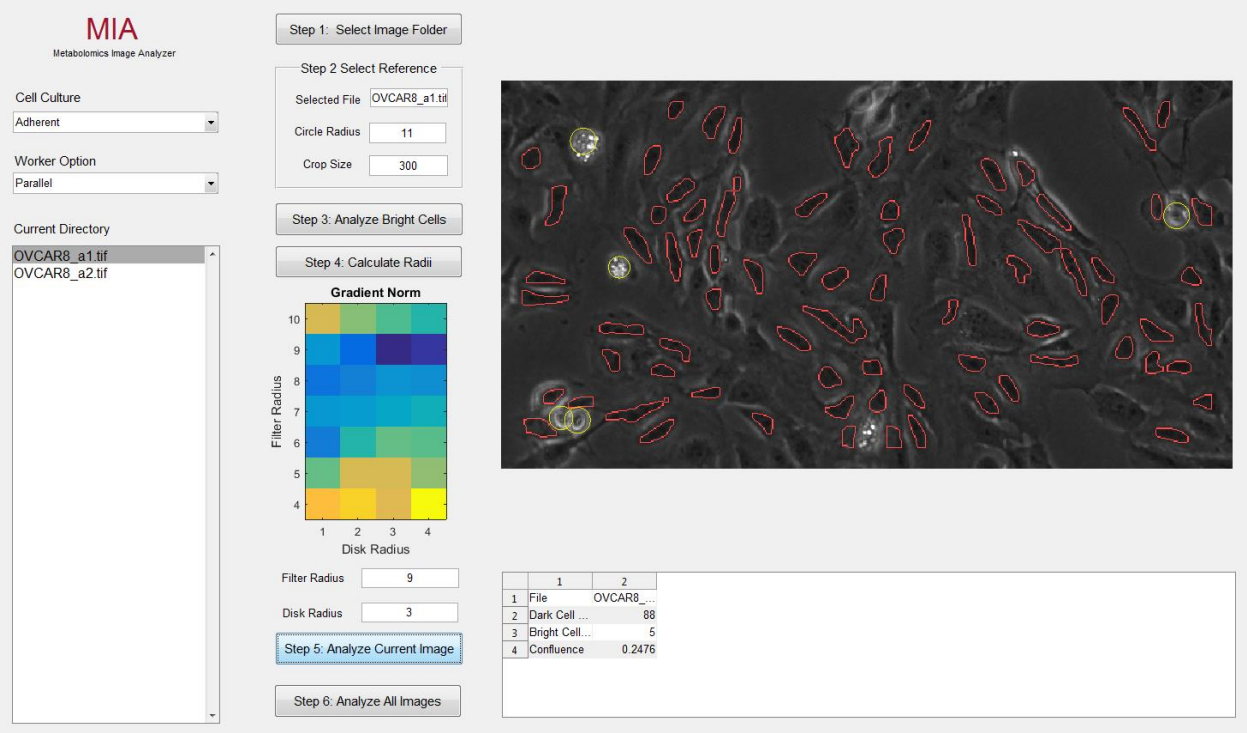

Figure 4 Step 4 and 5.

In Step 6, we process all images in the folder, presumably from the same sample or at least the same cell line. The analysis result is shown in the table below the preview window. Users can click on each column in the table to preview the processed image result. Users can also save one image of interest by clicking on the save button at the top-left corner. The image will automatically be save into the current folder with JPEG format. At any step, users can change the crop size to be able to switch from fast computation to accurate calculation.

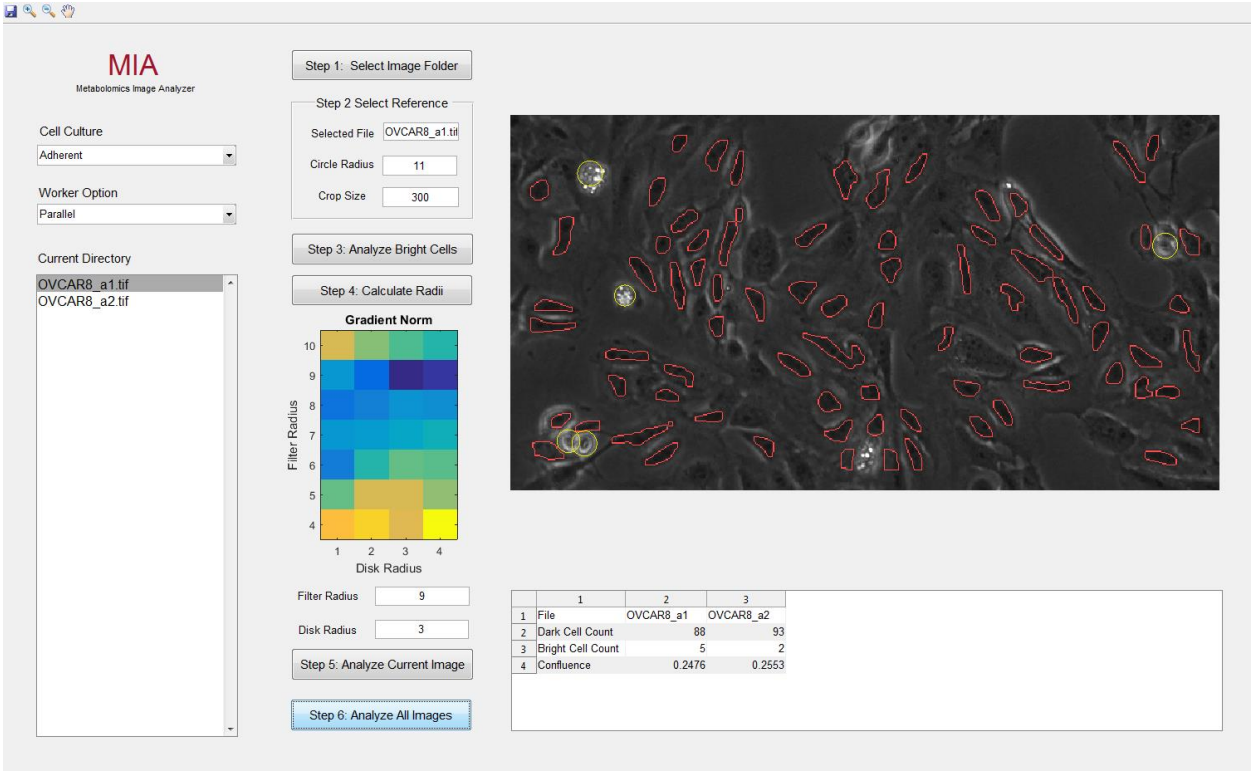

Figure 5 Step 6.

5 References

1. Silva, L.P. et al. Measurement of DNA concentration as a normalization strategy for metabolomic data from adherent cell lines. *Analytical chemistry* **85**, 9536-9542 (2013).
2. Ali, R. et al. Automatic segmentation of adherent biological cell boundaries and nuclei from brightfield microscopy images. *Machine Vision and Applications* **23**, 607-621 (2012).
3. Jaccard, N. et al. Automated method for the rapid and precise estimation of adherent cell culture characteristics from phase contrast microscopy images. *Biotechnology and Bioengineering* **111**, 504-517 (2014).