

MBF_FeatureExtraction (v0.13) -- Provisional Manual (2011.01.17)

PRE-ANALYSIS

File naming. Ensure that the filenames of image data to analyze correspond to the nine-digit naming convention used by the Opera QEHS, as follows: *001002000*, where "001" describes row position as row 1 ("A"), and "002" describes column position as column 2. Image types currently supported include *.flex*, *.tif*, *.jpg* and *.pict*. The filenames must exactly correspond to the corresponding nine-digit *wellindex* entries in the accompanying platemap file (see below).

Plate map reference. In *PlatemapTemplate.xlsm*, specify any of *Cells*, *Dye*, or *Treatment01-Treatment03* by entering appropriate values in the tabs of the same name. Entered values will automatically appear in the table shown in the tab named "platemap", else default values are used. Do not use spaces or special characters in value entries. Ensure that macros are allowed to run, and generate a platemap by clicking *Export platemap* button in the "platemap" tab; this will save a delimited *platemap.txt* in the directory selected from the browser window. *platemap.txt* must be saved in the same directory as the images to be analyzed. (Optional: manually prepare a reference plate map file by filling in the appropriate rows for each image file to be analyzed, with the following columns in order: WellIndex, Cells, Dye, Row, Column, Treatment_Sum, Treatment01, Treatment02, Treatment03; save as a tab-delimited text file.)

ANALYSIS

Mode 1. Open file *v0.13.script* in Acapella. Select image data to be analyzed. Adjust threshold parameters under *Nuclei Detection*, *Cytoplasm Detection*, *Spot Detection* and *Other Detection* as appropriate. Note that, except for nuclei detection, all other detection options are turned off by default. Under *Analysis options*, image display in the Acapella Player may be toggled on and off, field of view to analyze may be specified (default = 0, all fields analyzed), and object masks to restrict analysis may be selected. Hover the cursor over the parameter names for more information on adjustments. Run script.

The Data Explorer displays image and object data for the selected field(s), named *RGB_#* and *obj_#* respectively, where # indicates field number. Data from all fields is appended to both *all_cells* object list and *All_Data* table. Adjust segmentation parameters as required and save parameters for future reference.

Mode 2. Select "Mode 2" under *General* input parameters, and de-select any other mode if needed. From *Features*, select all desired feature suites for calculation. To keep object-level data, select "Write data to file" under *Output* parameters. If desired, input target directory (by default, data will be written to a new "output" folder in the same directory as the image data being analyzed). Data will be saved as *output.txt* by default; to save the data according to experimental treatment as defined in the reference plate map, select "Save data by treatment" and specify the treatment value to be used from the "Filename treatment" menu. Hover the cursor over the parameter names for more information on each

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calculation. Save parameters for future reference. Run script. After analysis, launch MBF_Classify from Matlab to continue with classification (see MBF_classify_manual.pdf).

Note: object-level data in *output.txt* is saved in a directory, created alongside the image data, named by the date of analysis as *YYYY.MM.DD_output*. If analysis continues beyond 11:59PM, then data calculated after 12:00AM will be saved in a new directory tagged by the new calendar date of analysis. Multiple files can be loaded without additional appending into MBF_Classify below, but will need to be present in the same output directory prior to classification.

Mode 3. After running MBF_Classify, load the segmentation parameters used in either Mode 1 or 2 above, and select "Mode 3" under *General* input parameters, de-selecting any other mode if needed. To save image data with classification results, select "Write data to file" from *Output* parameters. By default, a directory named *Image Gallery* will be written to the output directory created during Mode 2; sub-folders according to the number of control groups used during classification will be created here. To adjust the maximum number of objects per field to be saved per classification category, enter the desired value under "Number of object images to output (Mode 3)". To keep whole field views of classified data, select the appropriate option from the "Output field image (Mode 3)" menu. Save parameters for future reference. Run script. At the prompt, browse for the results file, generated by MBF_Classify, that contains coordinate information for analyzed objects. The coordinate file will be re-written as a text file the first time that Mode 3 is run, and future analyses in this Mode will complete in less time.