**MinaAnalyst User Manual**

**Installation**

MinaAnalyst is an application for the analyses of raw images from chromatin tracing, multiplexed error-robust fluorescence *in situ* hybridization (MERFISH), and multiplexed imaging of nucleome architectures (MINA). To install the application:

1. Download and install MATLAB version R2018a or a more recent version following the manufacturer’s instructions.
2. Download the MinaAnalyst package from <https://campuspress.yale.edu/wanglab/mina-analyst/>.
3. Double-click the “MinaAnalyst.mlapp” file in the package to open the graphical user interface (GUI) of the application.

**Inputs**

Required and optional input parameters in the application GUI include:

* Experiment file path (required): folder path of the imaging data on local computer. Please refer to the example data for the default naming conventions of image files.
* Total number of fields of view (required): number of imaged areas in the dataset.
* MERFISH codebook path (optional): folder path for the MERFISH codebook. This input is only required for MERFISH and MINA analyses. When required, the folder should contain a file named “CodeBook.mat”, a file named “AllCodes.mat”, and a sub-folder named “CodeMatrices” containing files named “CodeMatrix1.mat”, “CodeMatrix2.mat”,…, “CodeMatrix140.mat”. These files and sub-folder comprise the codebook necessary for MERFISH analysis, and are generated by the ProbeDealer software during MERFISH probe design. An empty filed indicates that the MinaAnalyst folder contains these files and sub-folder.
* Include lamina association analysis (optional): Select this option for MINA analysis when the dataset includes nuclear (DAPI) imaging.
* Include nucleolar association analysis (optional): Select this option for MINA analysis when the dataset includes nucleolar imaging.

Additional default parameters and detailed annotations of the parameters are listed in the “parameters\_default.m” file in the application package. Users may modify these parameters using the MATLAB script editor or a text editor. Please note the edits in the MinaAnalyst GUI overwrite the default parameters in the “parameters\_default.m” file.

**Performing analyses**

To perform chromatin tracing analysis alone, click the “Run chromatin tracing analysis” button. To perform MERFISH analysis alone, click the “Run MERFISH analysis” button. To perform full MINA analysis (including chromatin tracing analysis, MERFISH analysis, and additional lamina and/or nucleolar analyses if selected), click the “Run MINA analysis” button. Analysis progress is indicated in the GUI panel.

**Outputs**

The chromatin tracing analysis generates an output file named “ChrList.mat” that can be accessed through MATLAB. The file contains a structure array named “Chr”, each element of which corresponds to a chromatin trace. The fields of the structure include:

* x: x coordinates (unit: µm) of each chromatin locus in the trace.
* y: y coordinates (unit: µm) of each chromatin locus in the trace.
* z: z coordinates (unit: µm) of each chromatin locus in the trace.
* r: an array of 1 or 0 values indicating whether each chromatin locus is present (1) or missing (0) in the trace. When a chromatin locus is missing, the corresponding x, y, and z coordinates are set to “NaN” (a “not a number” space holder in MATLAB).
* FOV: serial number of the imaging field of view where this chromatin trace is located.

The MERFISH analysis generates an output file named “SingleCellAnalysisResults.mat” which contains two structure arrays “CellList” and “MolList”. The “CellList” elements correspond to individual cells and contain the following fields:

* CellID: serial number of the cell.
* FOV: serial number of the imaging field of view where the cell is located.
* PixelList: pixel indices (linear indices in MATLAB) of the cell area in the field of view.
* Center: center position of the cell in the field of view (unit: pixels).
* RNACopyNumber: an array of RNA copy numbers for the probed transcript species in the cell.
* TotalRNACopyNumber: total detected RNA copy number in the cell.
* OnEdge: a 1 or 0 value indicating whether the cell is overlapping the edge of the field of view (1) or not (0).

The “MolList” elements correspond to individual RNA molecules, and contain the following fields:

* FiledOfView: serial number of the imaging field of view where the molecule is located.
* MoleculeX: x coordinate of the molecule (unit: pixels).
* MoleculeY: y coordinate of the molecule (unit: pixels).
* GeneID: ID number of the transcript species in the MERFISH codebook.
* CellID: serial number of the cell that contains this molecule, which corresponds to the “CellID” field of the “CellList” structure.
* CellCenterX: x coordinate of the center of the cell in the field of view (unit: pixels).
* CellCenterY: y coordinate of the center of the cell in the field of view (unit: pixels).

The MINA analysis automatically performs both the chromatin tracing and MERFISH analyses, and generates all the output files above. In addition, the chromatin traces are matched to individual cells, and the following new fields are added to the “Chr” structure:

* CellID: serial number of the cell that contains this chromatin trace, which corresponds to the “CellID” of the “CellList” structure. If the matching fails (e.g. due to sample drift, chromatin traces on the edges of an imaging area may be out of the field when imaging cell boundaries), the “CellID” value of the chromatin trace is set to “NaN”.
* MeanXWGA: x coordinate of the center of the chromatin trace in the field of view using the coordinate system of the MERFISH analysis (unit: pixels). The drift correction procedure in the chromatin tracing analysis converts all chromatin locus coordinates to the coordinate system of the Hyb0 imaging round. The MERFISH analysis procedure converts all RNA molecule coordinates to the coordinate system of the cell boundary imaging round. This field (and similarly the next one) records the chromatin trace center position in the latter coordinate system.
* MeanYWGA: y coordinate of the center of the chromatin trace in the field of view using the coordinate system of the MERFISH analysis (unit: pixels).

If the “Include lamina association analysis” option is selected, during the MINA analysis a field named “MinDisToLamina” is added to the “Chr” structure to record the distance from each chromatin locus to the nuclear lamina (unit: µm). If the “Include nucleolar association analysis” option is selected, a field named “MinDisToNucleolar” is added to the “Chr” structure to recode the minimum distance from each chromatin locus to nucleoli (unit: µm). When a chromatin locus is missing in the chromatin trace, the corresponding “MinDisToLamina” and/or “MinDisToNucleolar” value is set to “NaN”.