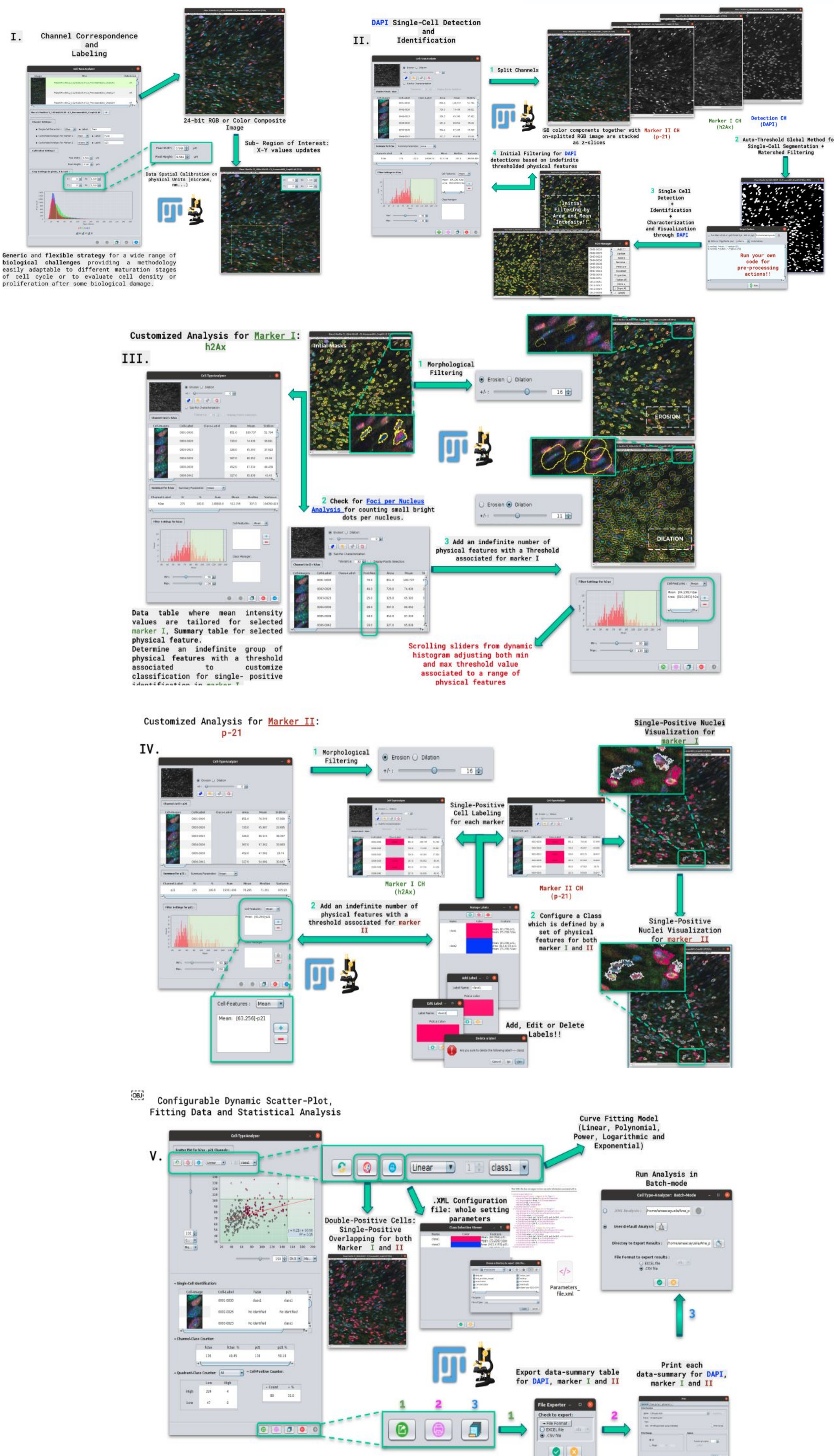


### Abstract

Fluorescence microscopy techniques have experienced a substantial increase for visualization and analysis of many cell biological processes in life science. Here we describe a fully automated and versatile tool called CellTypeAnalyzer to avoid the time-consuming and biased manual counting of cell nuclei. It consists in an open source plugin for either Fiji or ImageJ for visualizing, detecting, filtering, delineating, quantifying, characterizing and generating customized classifications for specific cell types over 2D RGB fluorescence images. The workflow implemented involves: (I) data spatial calibration on physical units (microns, nm...) and sub-region drawing; (II) auto-threshold global methods and watershed algorithms to isolate single cells from background; (III) data tables for single cell characterization and statistical summary for selected physical metric; (IV) initial filtering to select cells based on physical features with a threshold range associated; (V) delineated cell visualization; (VI) morphological filtering for single cell outlines: erosion and dilation; (VII) foci per nucleus analysis for counting small bright dots per single cell; (VIII) configuration of customized classes for specific cell types based on physical features with a threshold value associated; (IX) single-positive cells identification; (X) double-positive cells identification (single-positives which overlaps);

(XI) Configurable scatter-plot to plot any physical feature as a function of another and applying curve models that best fit data. Our software provides an intuitive, modular and flexible strategy to perform analysis through a wizard-like GUI in which the user is guided to each step of the customized analysis. This procedure may be applied in batch mode to multiple microscopy files (requiring less than 5 seconds per image) through loading an editable .xml Configuration file generated automatically which contains the whole setting up parameters for pre- and post-processing actions. An additional GUI for code custom is provided to run pre-processing image actions both by writing in any of ImageJ's supported languages their own code and by browsing for macros (.txt or .ijm) or script files (.js, .bash or .py). In short, together these features make CellTypeAnalyzer well-suited to any researcher without any computational or programming proficiency as well as to deal with challenging situations as an uneven background or experiments where the low number of fluorescently labeled objects determines a variable signal to noise ratio of the image.

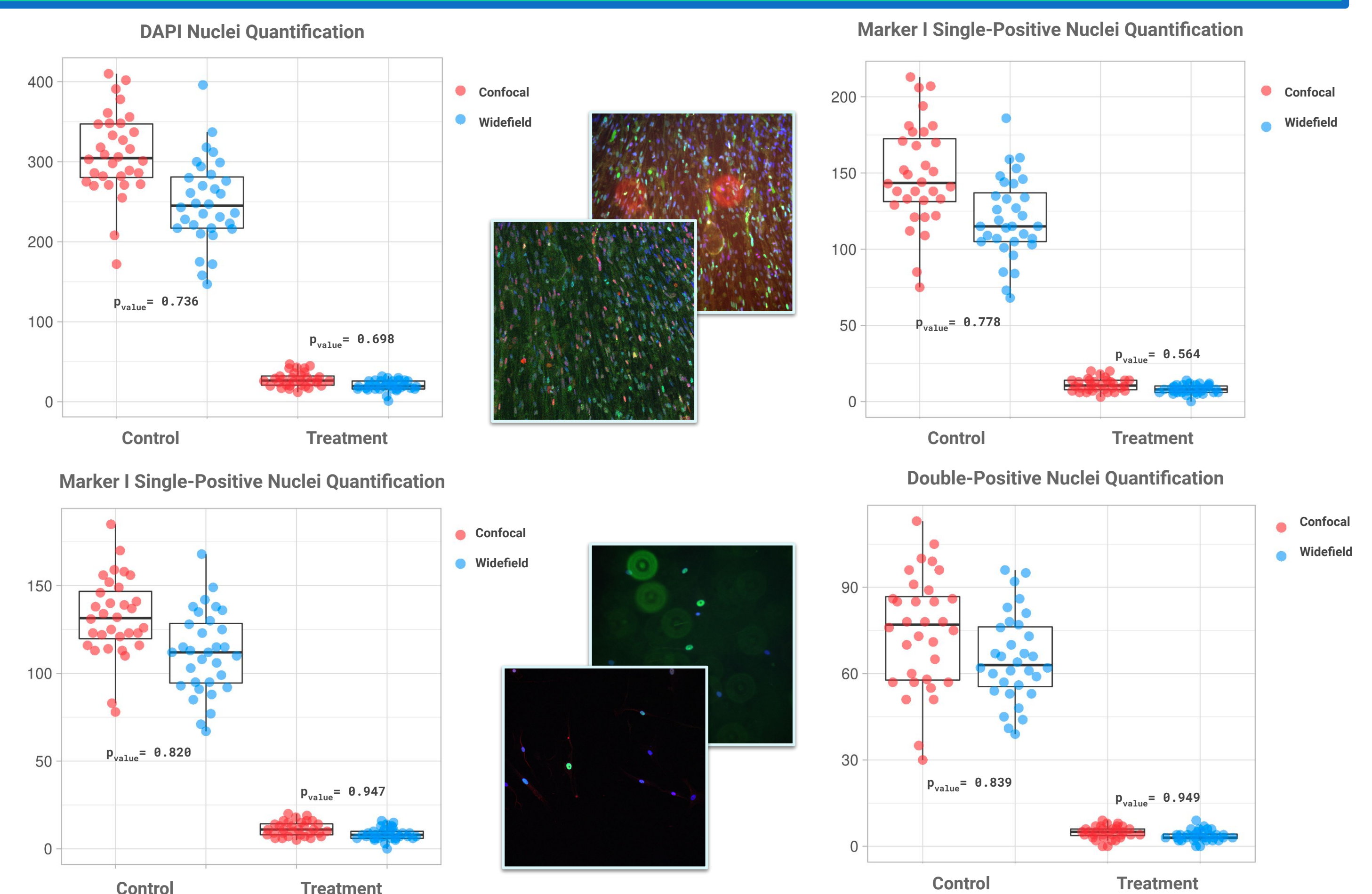
### Overview of Procedure



### Features of CellTypeAnalyzer

- **Supported Image File Formats.** Deal with any file format or multidimensional data supported by Bio-Formats library. Capable of extracting automatically each ".lif" serie as a TIFF file keeping original pixel values and spatial calibration.
- **Inputs.** Images must be from microscope 3-channel acquisition in RGB form with same channel dimension and magnification: 24-bit RGB or Color Composite.
- **Installing CellTypeAnalyzer.** It is packaged as JAR file that may be installed either by placing it into Fiji or ImageJ's plugins subfolder or by following "Plugins" → "Install Plugin" bar options.
- **Scriptable Pre-Processing Actions.** Browse for either macros (.txt or .ijm) or scripts files (.js, .bash or .py). Write and run your own code in any of ImageJ's supported languages without saving, copying it to the clipboard and paste on script editor.
- **Data Spatial Calibration on Physical Units.** Physical metrics may be based on physical units (µm, nm...) and not in pixels units.
- **Draw a Sub-Region of Interest.** Manually draw a closed area using any shape available under ImageJ's ROI tools. Then X-Y values will be updated to reflect the current boundaries.
- **Instant Visualization.** A Z-stack will be generated in which each RGB color component together with non-split RGB will be stacked as z-slices to get more efficient control of performance and to visually confirm each processing step instantaneously.
- **Interactive Navigation through Wizard-Like GUI.** It was designed to interactively guide user through modules enabling navigate back and forth to tune parameters, re-calibrate settings and review unsuitable filters, detections or outputs.
- **Run CellTypeAnalyzer in Batch-mode.** Most suitable user-defined parameters may be applied to large sets of images from source folder, loading .XML Configuration file containing the whole processing actions which may be saved from single-image analysis.
- **Customize your own Analysis for marker I and II.** Generate user-defined classes by adding indefinite thresholded physical features for each marker hence customizing your own analysis.
- **Double-Positive Labelling and Identification based on user-defined classes.** A single cell may be double-positive when accomplishes conditions imposed by class for both maker. Thus it is a single-positive cell whose labeling for each marker overlaps.
- **User Configurable Outputs.** User is enabled to configure dynamically scatter-plots to plot any marker physical feature as a function of another applying the curve fitting model that best fit data.

### Testing and Validation



The performance was validated taking into account nuclei quantification from **2 Fluorescence Imaging Techniques (Confocal vs. Widefield)** through **2 conditions: Control (High Cell Density) and Treatment (Poor Cell Density)** to determine whether two conditions are different.

Since  $p_{value} > \alpha (0.05)$  among 2 imaging techniques, this implies that there is no conclusive evidence for an effect within each condition. This supports CellTypeAnalyzer as reliable and robust tool either quantifying **mono- and multi-layered** cell culture.

### References

1. Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., ... Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods*, 9(7), 676–682. doi:10.1038/nmeth.2019