RNAscope™ HiPlex Image Registration Software v2.1.0 Release Notes

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Brief Description and Version of the Software

RNAscope HiPlex Image Registration Software is designed to register and merge images acquired by performing RNAscope HiPlex Assay.

The currently released version (version 2.1) provides whole slide image file conversion and whole slide image processing functions.

The previously released version (version 2.0) provides improved functions regarding image display, which include automatic pseudo-color assignment, contrast adjustment, thresholding, and image zoom-in / zoom-out capabilities. This version also provides additional functions of autofluorescence background removal for images acquired with Formalin-Fixed Paraffin-Embedded (FFPE) samples or other high-background samples, as well as the option to save images in omero.tif format.

Minimum Systems Requirements

Processor: Intel Core i5
RAM: 16 GB
Windows versions: Windows 10 or 11
Mac versions: MacOS 11, 12, or 13

New Features in Version 2.1

1. Whole slide image file conversion. This feature requires "bftools," which will need to be downloaded separately. It supports conversion of files in czi, lif, and nd2 formats into ome.tiff format
2. Whole slide image processing. This feature supports files in ome.tiff, ome.btf, and qptiff formats. It includes the following sub-features
    a. Whole slide image registration
    b. Whole slide image background subtraction with a blank image
    c. Display of resulting image in QuPath. This sub-feature requires QuPath to be installed
3. Improved registration algorithm. It provides greater robustness for sub-optimally acquired DAPI images

Issues Fixed in Version 2.0.1
1. The potential issue of warping of large images after image registration has now been fixed
2. The image display efficiency when the image is zoomed out has now been improved
3. The previously missing information on "Save Omero Tif" has now been added to page 9 in the user manual

Issues Fixed in Version 2.0

1. The software now shows the main window at the maximum size by default right after launching
2. The software now has a larger image display area; the actual size of the image display area depends on the size of the screen

3. The software now allows the use of mouse wheel to scroll the image list panel of the software
4. The software now allows the use of mouse left key [hold and drag at the right edge of the panel (to the left of the vertical scroll bar)] to resize the image list panel to show long file names; long file names, if not shown in full, can also be revealed by hovering the mouse pointer on top of it (a tooltip will appear to show full file name; Windows version only); the image list panel does not resize itself anymore; manual resizing of the image list panel will also resize the image display area accordingly
5. The software now hides image display adjustment controls if the image is not checked and displayed
6. The software now shows a warning dialog window when the users load files that are not in TIF format
7. The software now shows a warning dialog window when the users load images with inconsistent dimensions
8. The software now shows a warning dialog window when the users try to load new images using the "Open" option while there are currently loaded images, to confirm the replacement of images

New Features in Version 2.0 and above

1. Automatic pseudo-color assignment upon image loading
2. Image zoom-in / zoom-out functions
   a. Mouse wheel control of zoom over the image
   b. Image "Reset Zoom / Position" button
   c. Current zoom indicator / entry box
3. Image display adjustment
   a. Spin box controls for Brightness
   b. Spin box and slider controls for Contrast: Min, Contrast: Max, and Threshold
4. Image preprocessing functions
   a. Remove background without a blank image
   b. Remove background with a blank image
5. Option to save multiple images as a single file in omero.tif format

Known Issues and Limitations in Version 2.0 and above

1. Some anti-virus applications may show a warning during the installation of the software (for example, Symantec anti-virus application may indicate that there is a risk of "SONAR.heuristic.159"). This is a false positive detection and can be ignored. We may work with anti-virus application vendors to remove this false positive detection in the future.
2. The "Remove background without a blank image" function may, in some cases, cause false positives and false negatives in the detection of probe dots. For example, it may have difficulties distinguishing large clusters of dots from autofluorescence background, as the clusters may resemble the pattern of autofluorescence in certain tissue types. In addition, dot-like or filamentous autofluorescence patterns may be accidentally recognized as probe dots. Therefore, caution must be exercised when processing images with this function.
3. Similarly, the "Remove background with a blank image" function (and background subtraction in Whole Slide Image Processing function) may also, in some cases, cause false positives and false

negatives in the detection of probe dots. Caution must be exercised when using this function, especially when adjusting the parameters. We recommend starting with the default parameter values and referring to the user manual as a guide [see Section "Remove background (optional)" in Chapter 4]. In addition, non-probe objects, if only seen in the probe images, may still remain after applying this function.