User Manual for
ImageStudio Software

Software Version: V3.0

Manual Version：A3.0

Revision History

Manual Version	Software Version	Revised Date	Description
A0	V1.0.0	Feb. 2023	• First release
A1	V2.0.1	Mar. 2023	• Support mIF solution
A1.0	V2.0.2	May. 2023	• Fix the problem that the software is installed in the default path during "Online Update", and the custom path is invalid • Fix the abnormality of 'QCPassFlag', 'StitchEval' and 'BitDepth' fields in the IPR file, in the "Image Quality Control" module • Fix the image in tiff format, the problem of reporting an error in QC, in the "Image Quality Control" module • Fix the problem of DAPI opening exception, in the "Tissue Segmentation" module • Update the upload type configuration for "Image Quality Control"module
A2.0	V2.1.x	July. 2023	◆ Added the adaptation of Zeiss multi-channel microscope for staining type with "DAPI&mIF". ◆ Added the stitching the method of adjacent FOVs. ◆ The data after image Quality Control (including success and failure) can be used as the input of the three modules of image stitching, tissue

			segmentation and cell segmentation.
A3.0	V3.0.x	Oct. 2023	◆ Added support for H&E image quality control, stitching, and segmentation. ◆ Added performance monitor for memory usage, disk storage space, CPU usage, and I/O usage.

Note: Please download the latest version of the manual and use it with the software specific for this manual.

User Manual

1 Product Introduction

STOmics ImageStudio is an offline image processing software that is specifically designed to provide multiple functionalities for Stereo-seq Chip images. It includes easy-to-use functional modules for microscope image quality evaluation and manual adjustment (image stitching, image calibration , tissue segmentation and cell segmentation). Image quality evaluation mainly evaluates the clarity and stitching quality of an image by referring to the background tracklines on the Stereo-seq Chip in order to meet requirements for further downstream bioinformatics analysis. When automatic stitching or segmentation algorithm can not satisfy the needs for further downstream analysis, manual adjustment modules can provide manual tools for image processing.

2 Related Softwares

2.1 SAW

SAW:Stereo-seq Analysis Workflow (SAW) software suite is a set of pipelines bundled to process

sequencing raw data of Stereo-seq to biologically informative data. The spatial gene expression matrix generated from SAW is the starting point of the downstream bioinformatic analysis.

2.2 StereoMap

StereoMap is an interactive HD visualization desktop application intended for displaying Stereo-seq analysis results. SAW output files such as gene expression matrix GEF file, image RPI and IPR files, as well as clustering results, can be visualized via StereoMap.

2.3 Version Requirements

ImageStudio	SAW					StereoMap			
	v5.4.x	v5.5.x	v6.0.x	v6.1.x	v7.0.x	v1.0.x	v2.0.x	v2.1.x	v3.0.x
V1.0	☑	☑	☐	☐	☐	☑	☐	☐	☐
V2.0	☐	☐	☑	☐	☐	☐	☑	☐	☐
V2.1	☐	☐	☐	☑	☐	☐	☐	☑	☐
V3.0	☐	☐	☐	☐	☑	☐	☐	☐	☑
Note: The new version is compatible with the functions of the old version.									

3 Installation instructions

3.1 General Information

STOmics ImageStudio software is intended for assessing the quality of microscope images and assisting offline image manual operations, which includes five functional modules: image quality control, image stitching, image calibration, tissue segmentation and cell segmentation. Currently,

ImageStudio can only be installed on the Windows system.

3.1.1 Software logo

3.1.2 Computer system requirements

- Hardware requirement: memory of at least 16G.
- Operating system: Windows 10 - 64 bit
- Connection requirement: Not necessary unless image uploading to a computer cluster or cloud is required.

3.1.3 Software download or update links

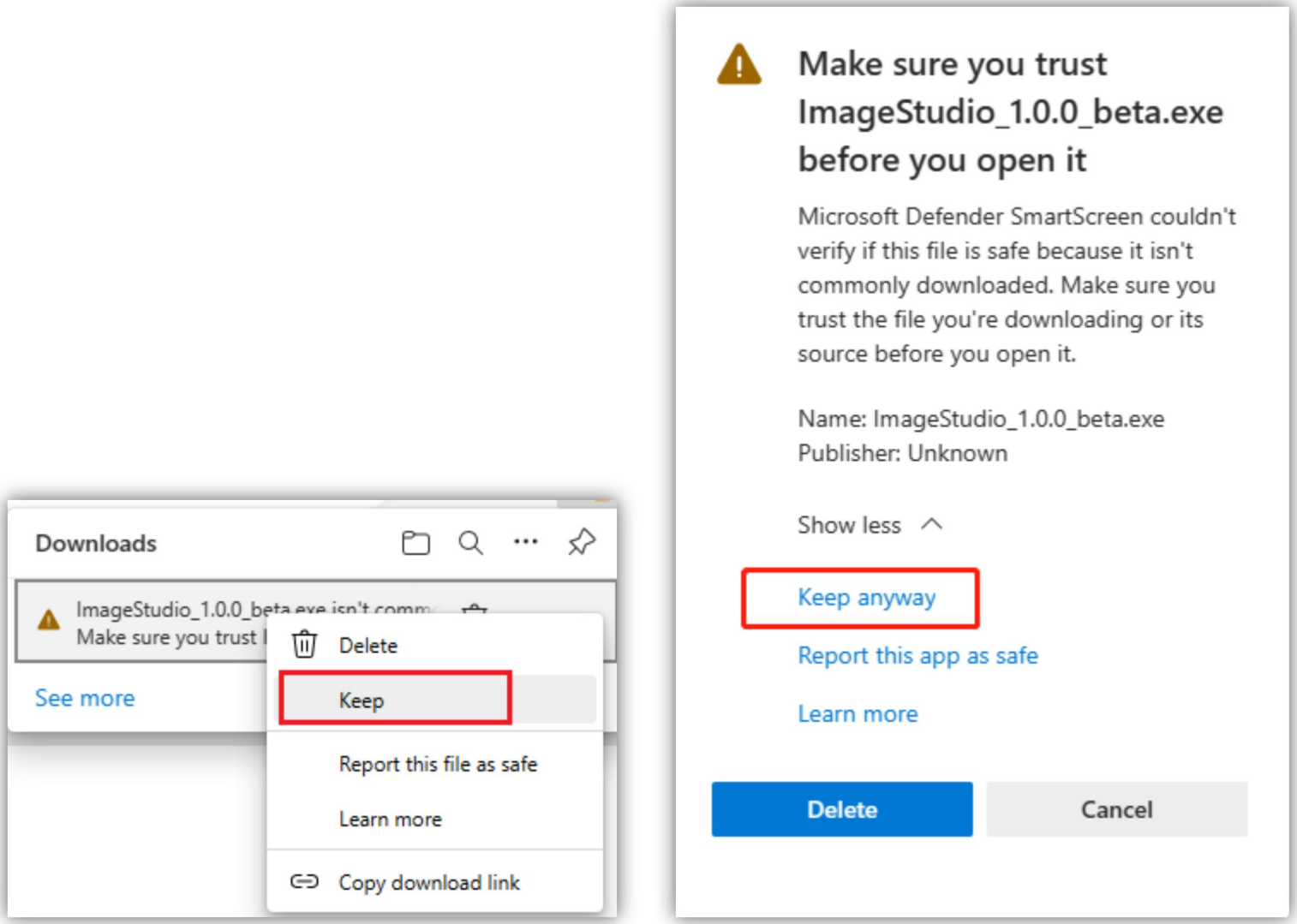
https://www.stomics.tech/BioinfoTools

3.2 Installation Instructions

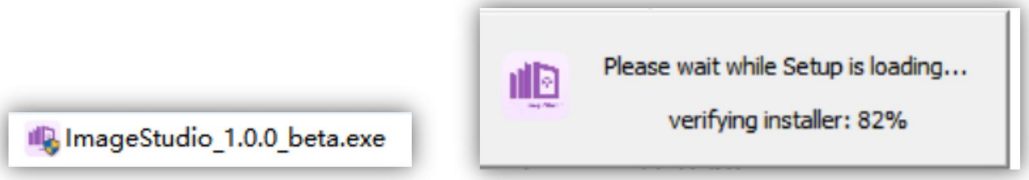
You can install different functional modules based on your requirements. The installation instructions are as follows:

3.2.1 First Installation

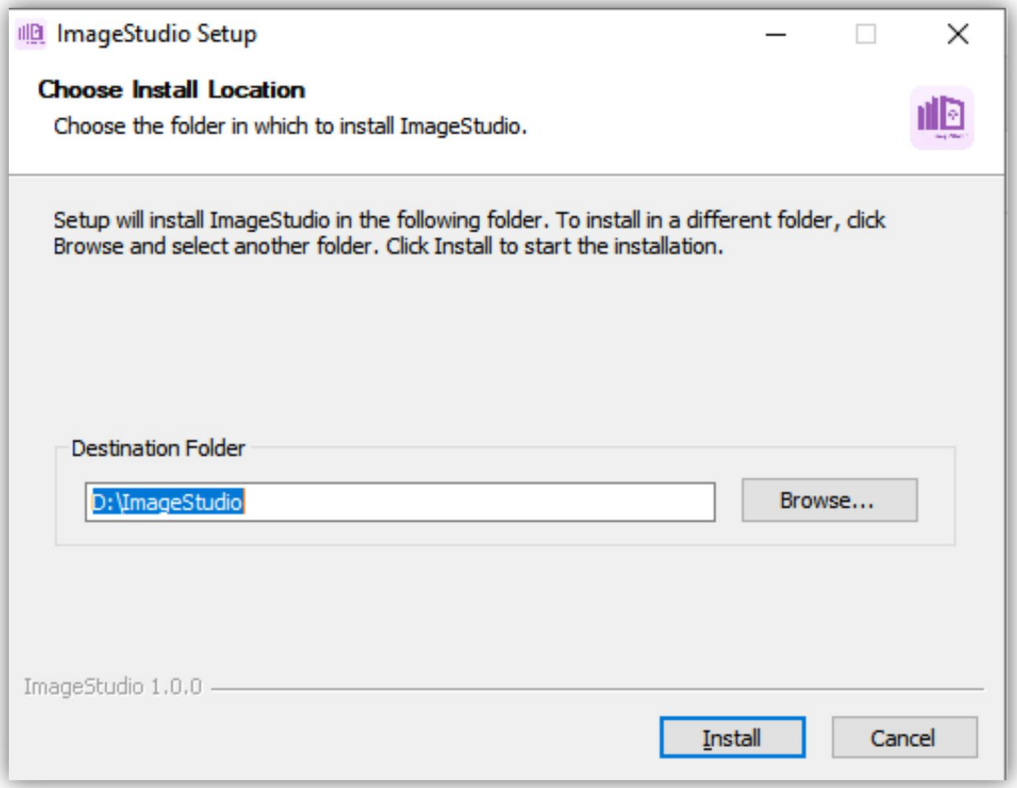
1. Download **'ImageStudio_1.0.0_beta.exe'** installation package from links given in section 1.3. The software installation might get blocked, simply click on the '...' button and select 'Keep' to recognize the software installation package as a trusted file.



2. Double click the downloaded file '**ImageStudio_1.0.0_beta.exe**'



3. Select a destination location. (The system creates the ImageStudio folder on the *D: Drive*.)



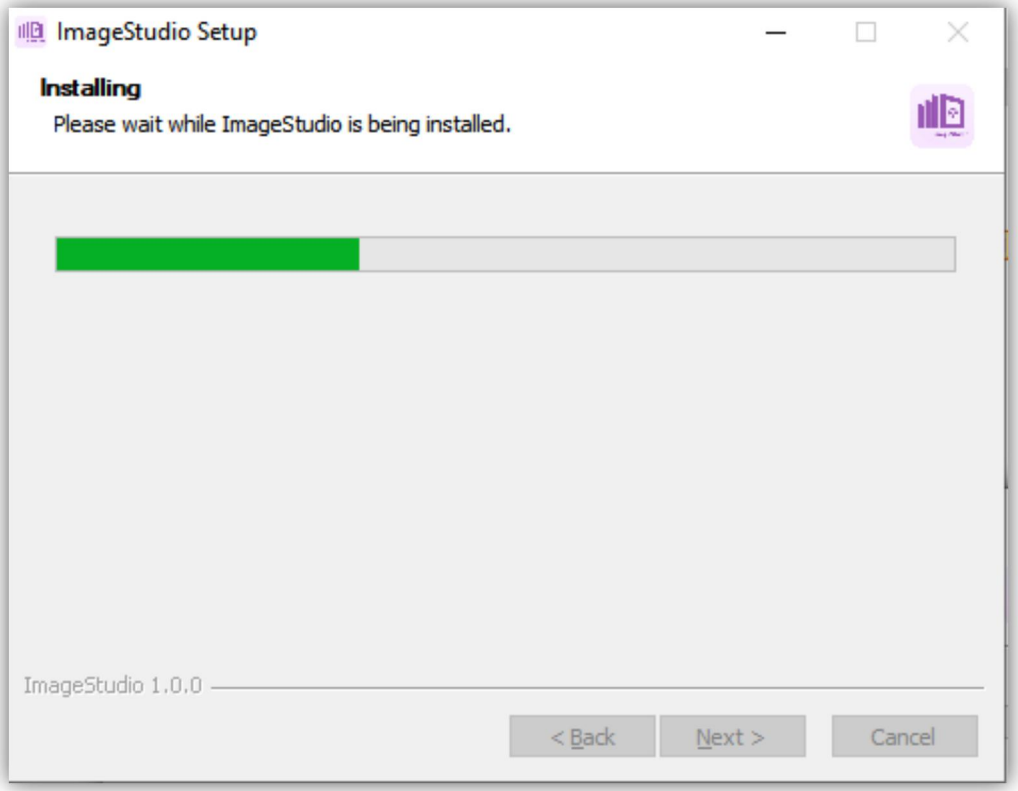
⚠

- Changing the default installation directory is not recommended, If necessary, please do not install into the C:

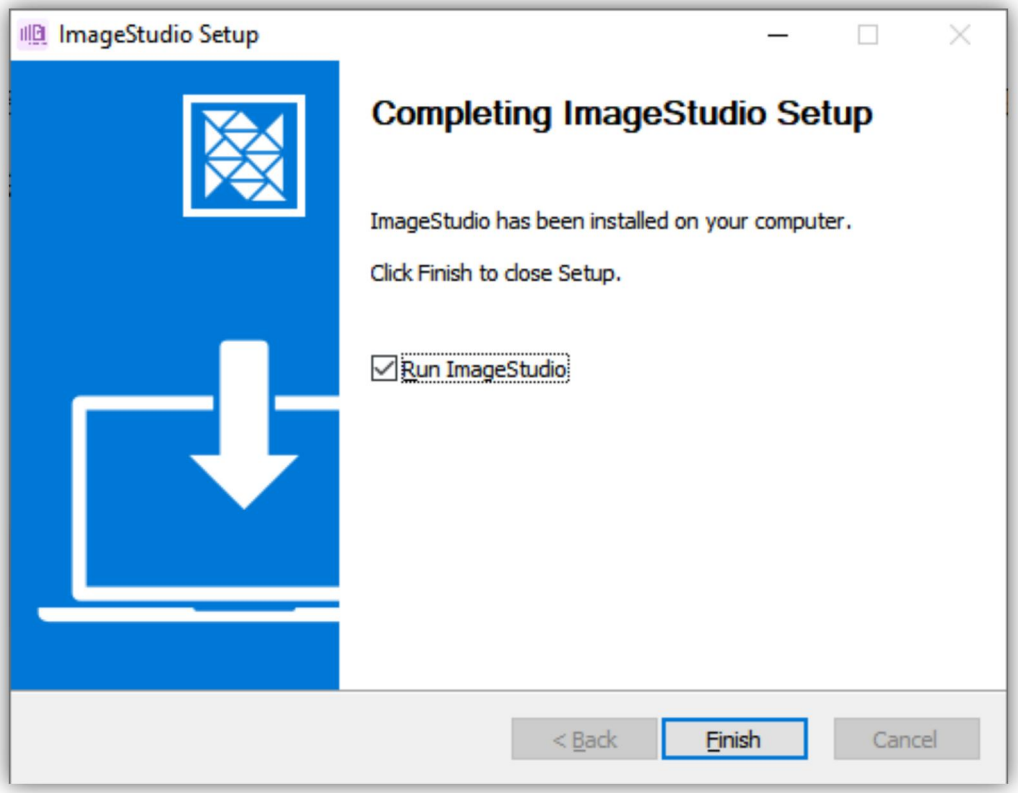
Drive as errors might occur due to lack of administrative permission.

- Please ensure there is enough space on *D: Drive*. It is recommended to have at least 20G storage.

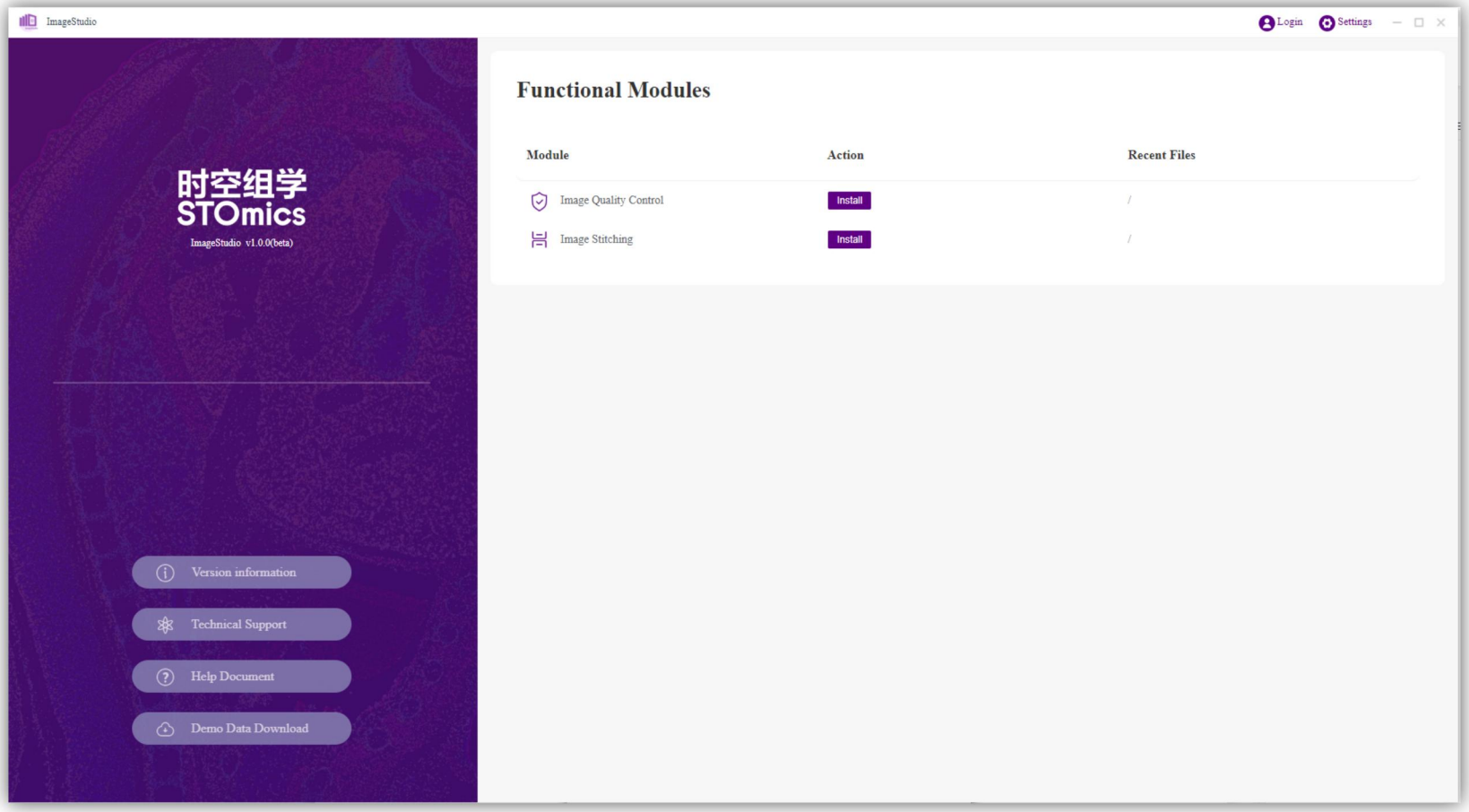
4. Click "Install"



5. Once the installation is completed, select the option ‘Run ImageStudio’ and click ‘Finish’ to launch the program.

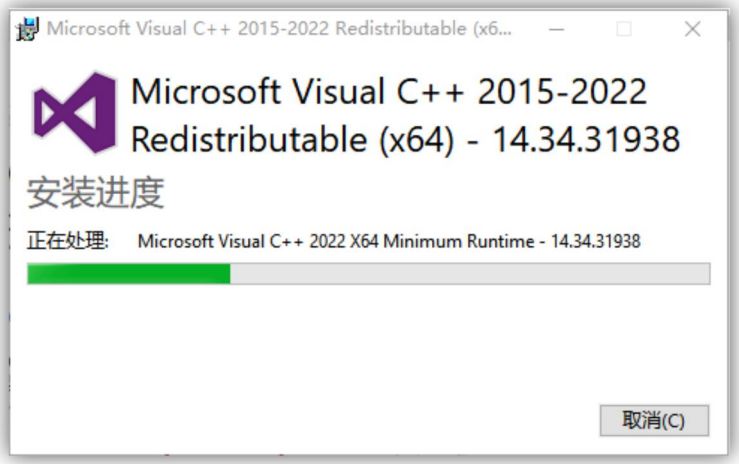


6. After the installation, double-click the shortcut icon on the desktop to launch.

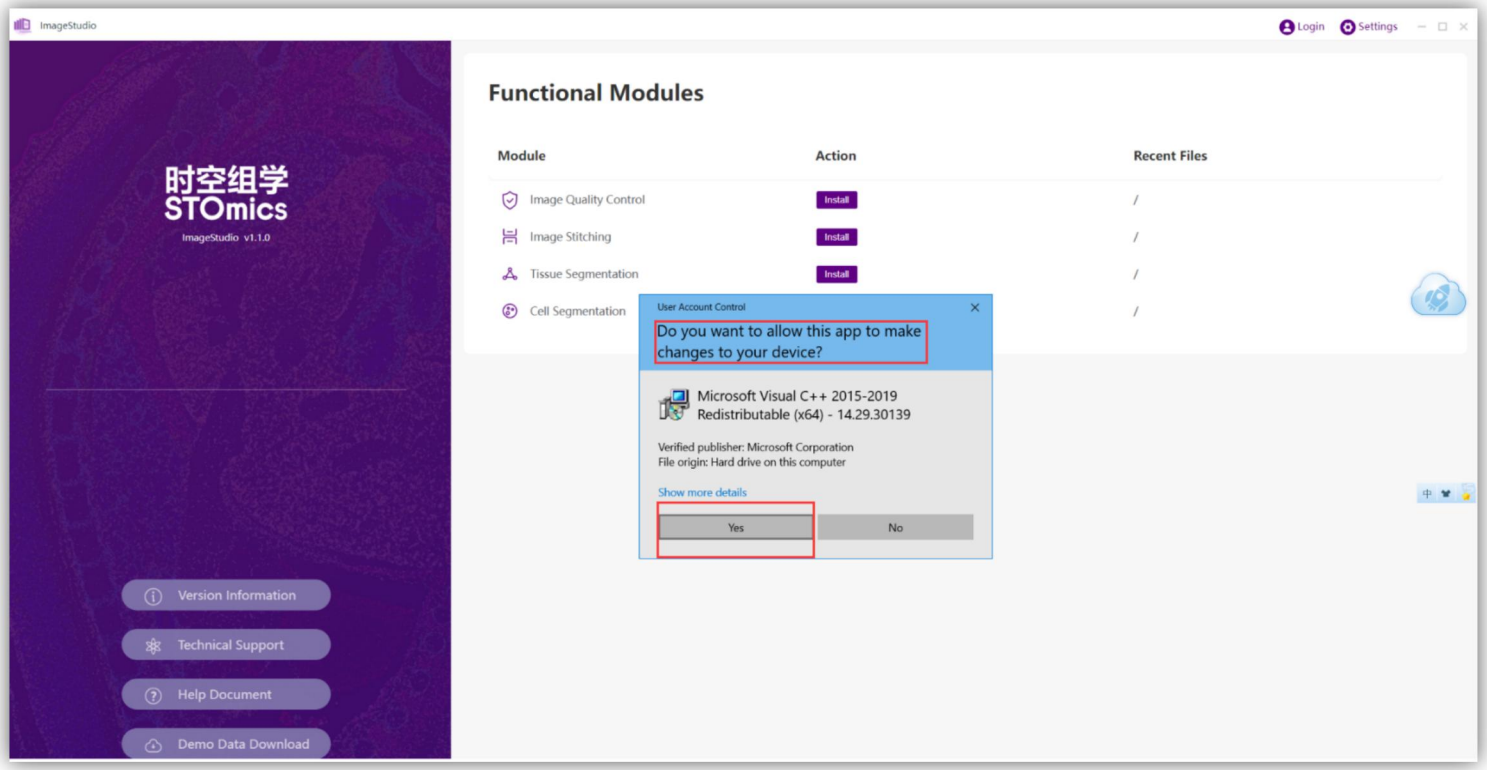


⚠

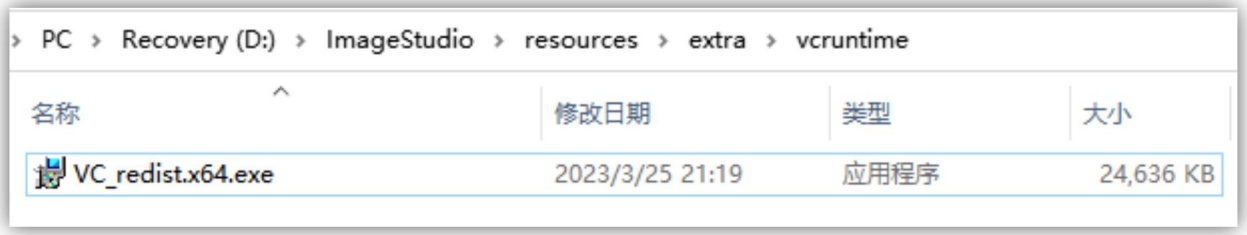
- Because the image quality control module needs the Visual C++ dependent environment of Windows10 operating system, if the environment is not installed on the personal computer, the system will be installed silently.
  - If silent installation failed because of permissions problems, the user needs to manually click on the "install".



- During the silent installation process on some PCS, the following notification will be confirmed by clicking "Yes".

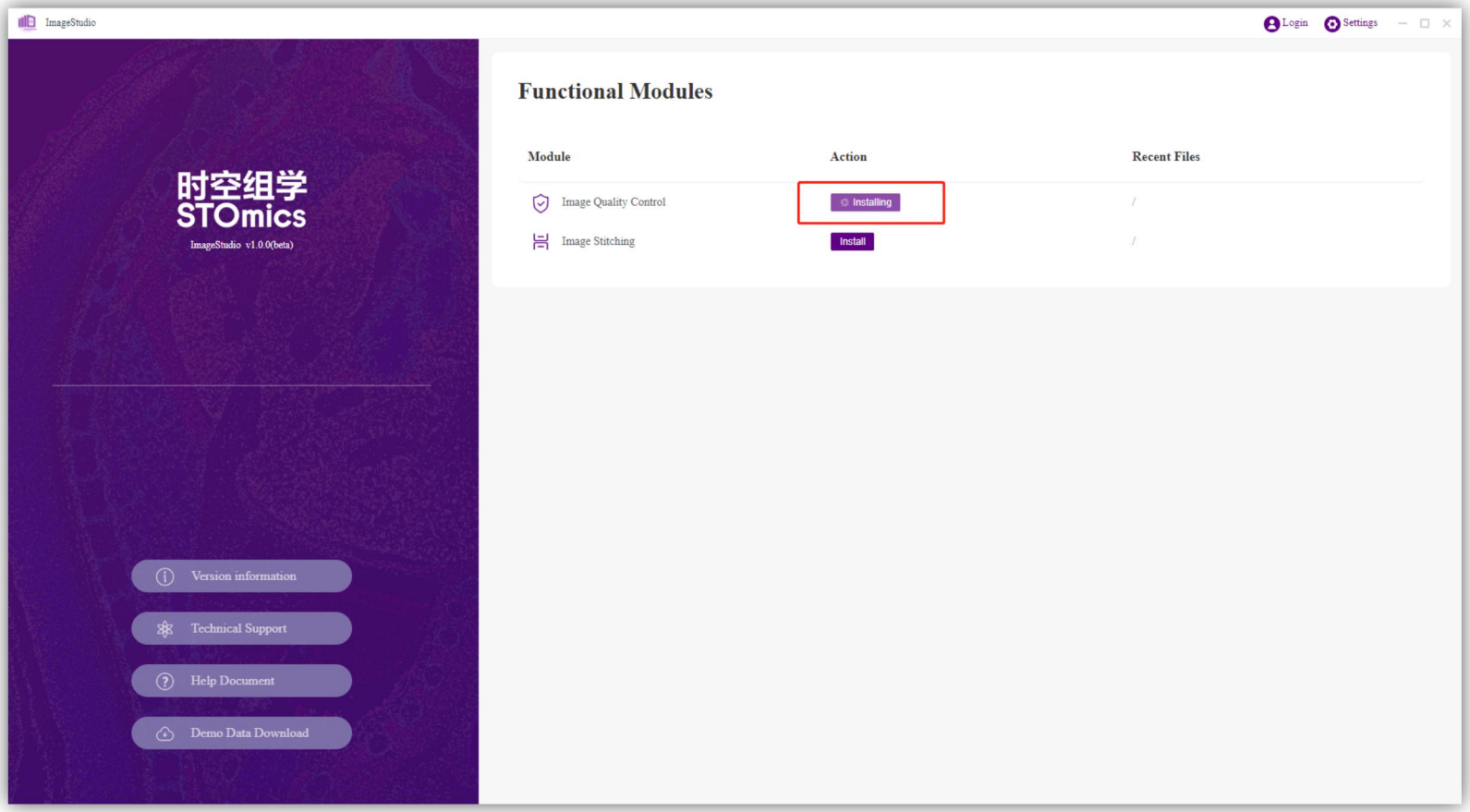


◆ If the user clicks "no" by mistake, you need to manually double-click the vc_redi.x64. exe file in the: *installation directory (for example: D:\ImageStudio) \resources\extra\vcruntime* to install.



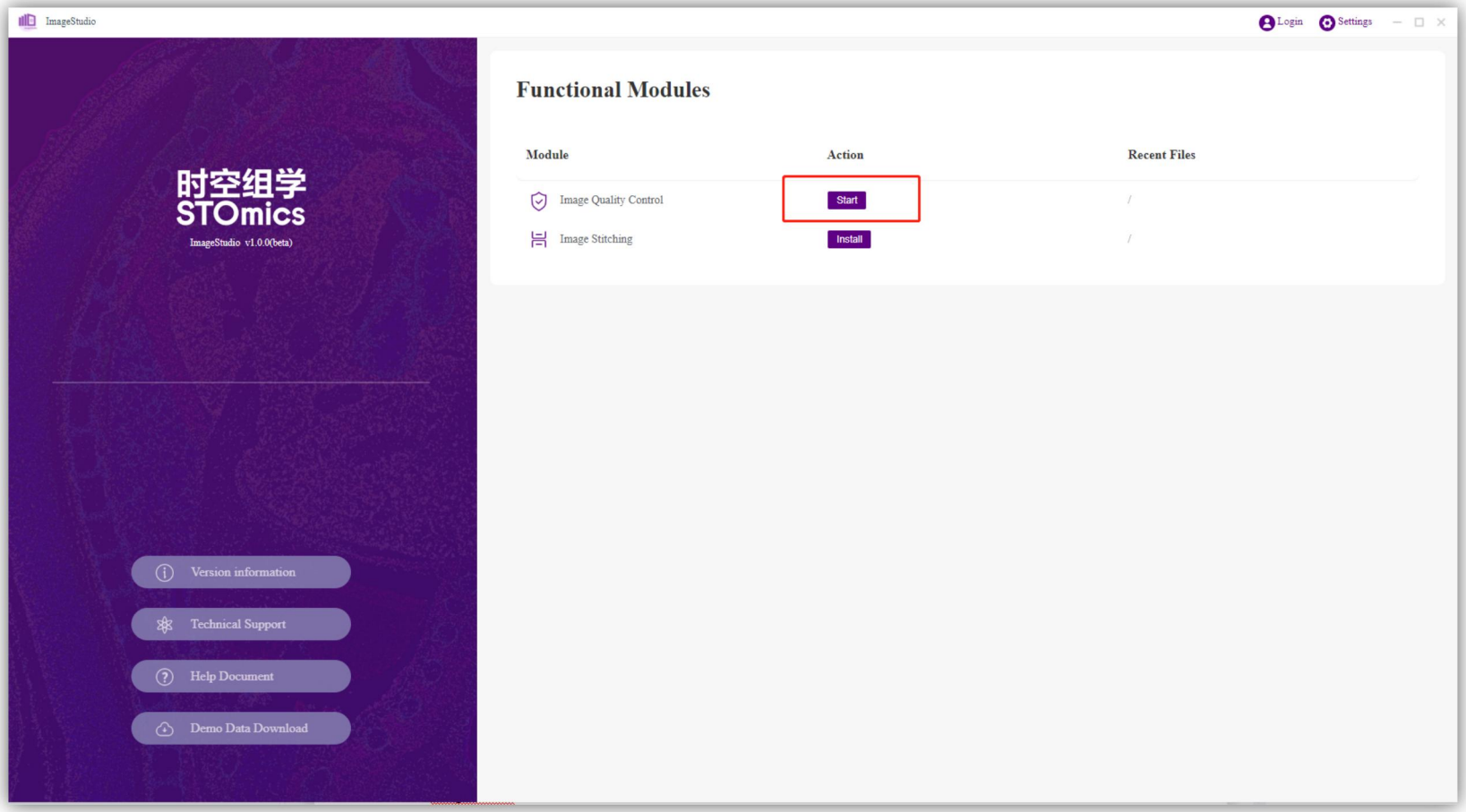
- Please ensure there is enough space on *D: Drive*. It is recommended to have at least 20G storage.

7. Click to install the corresponding functional module. Here, we use ‘Image Quality Control’ as an example:



⚠ Do not install all modules at the same time. Install the next functional module once the previous one has been finished.

8. After the functional module has been installed, click ‘Start’ to access the module’s interface.

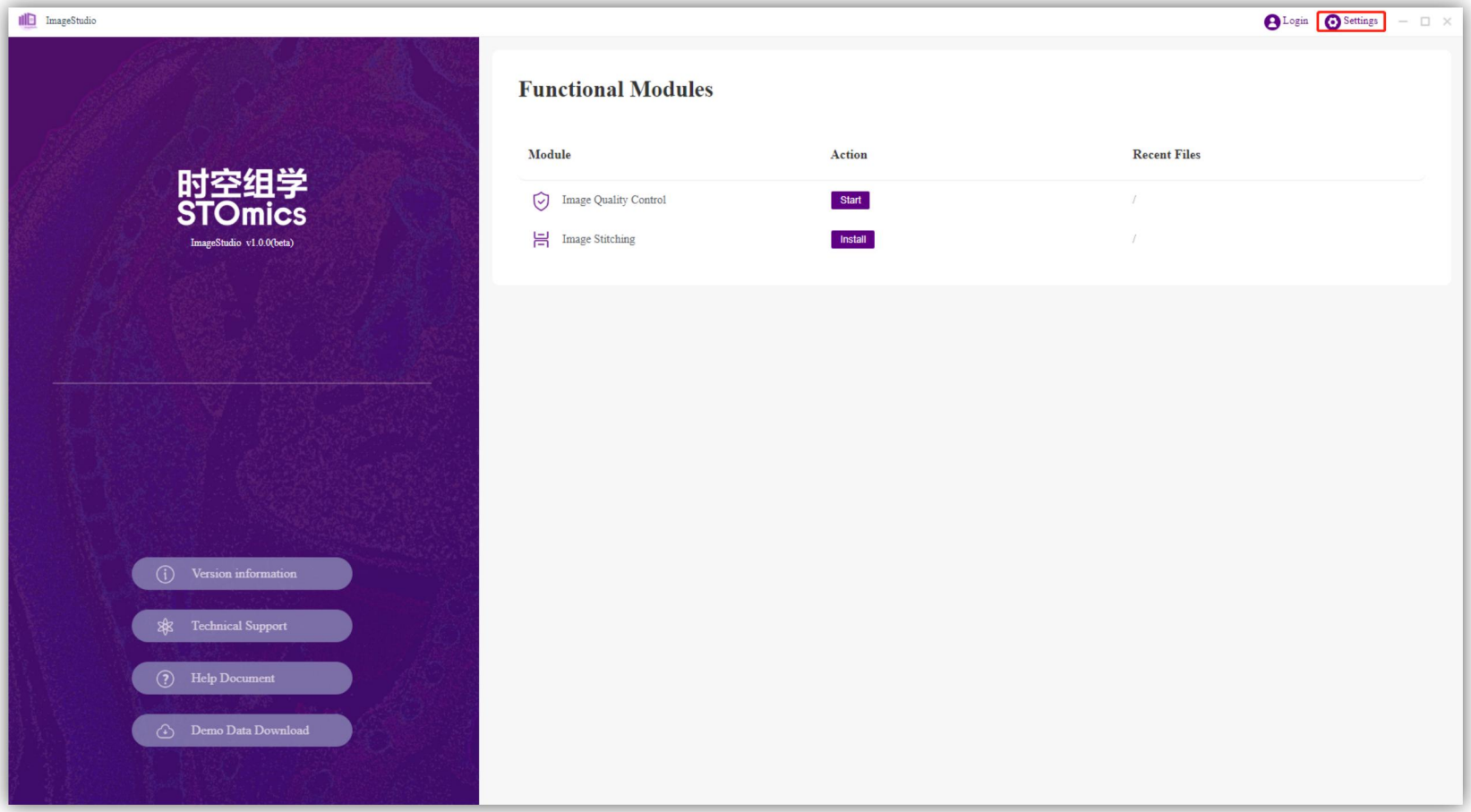


9. Repeat step 7 to install other functional modules.

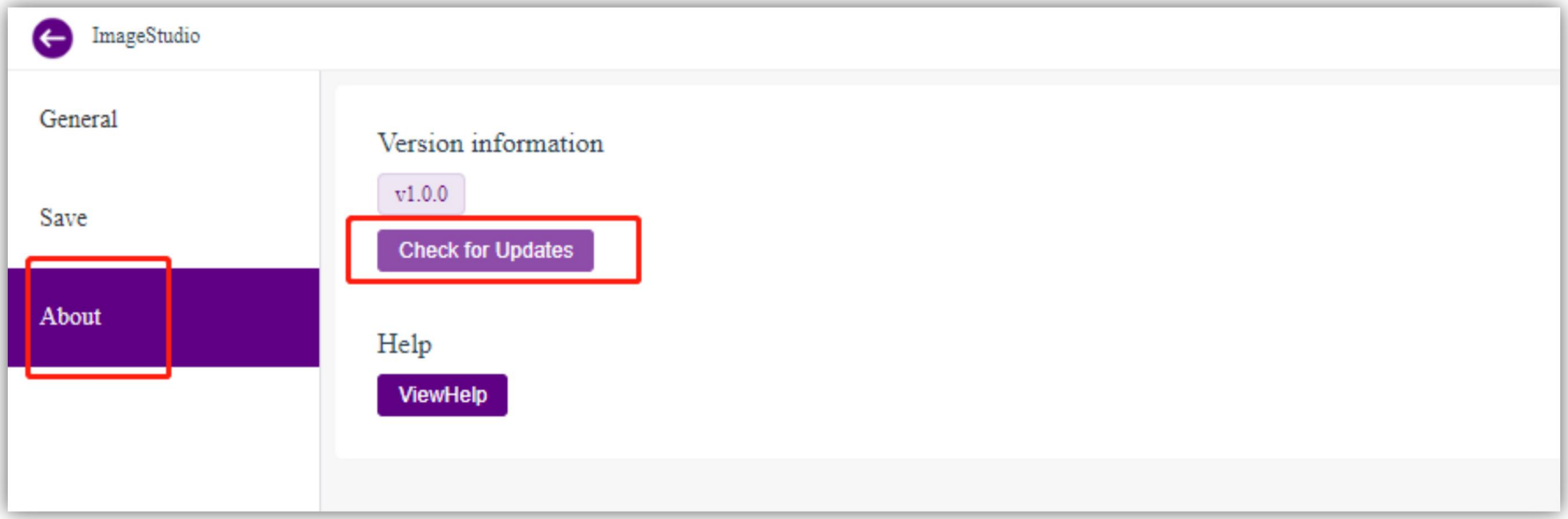
⚠ The installation instructions and screenshots this manual use V1.0.0(beta) as an example. Other versions are the same as V1.0.0(beta).

3.2.2 Software Update

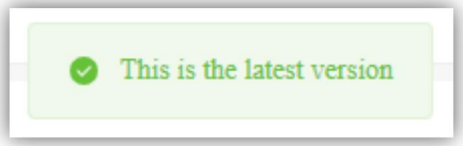
1. Once the installation is completed, open ‘Settings’ .



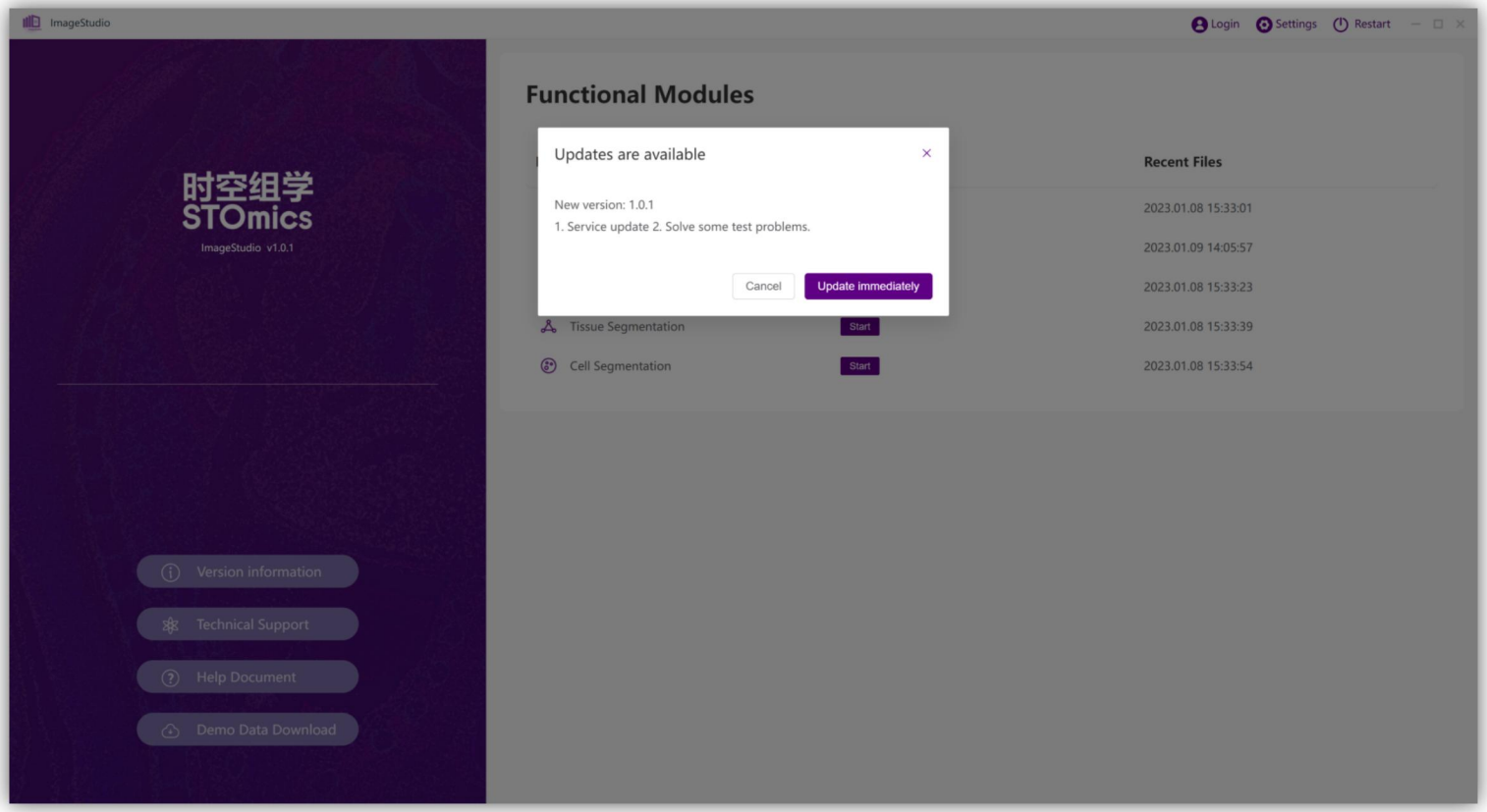
2. Click *About* and check for updates. The system can automatically check for updates with stable internet connection.



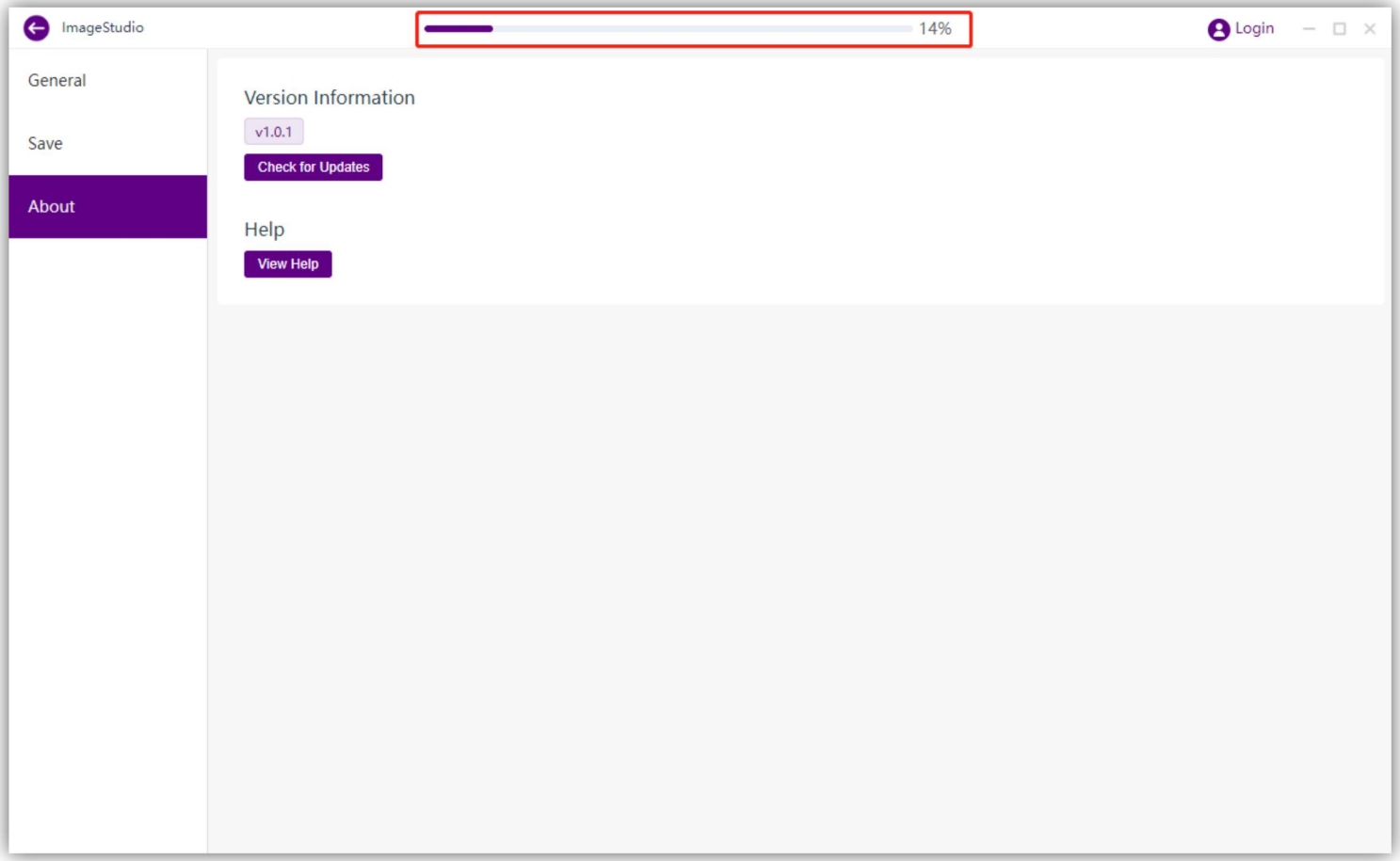
3. If there is no version to be updated, the following information will display.



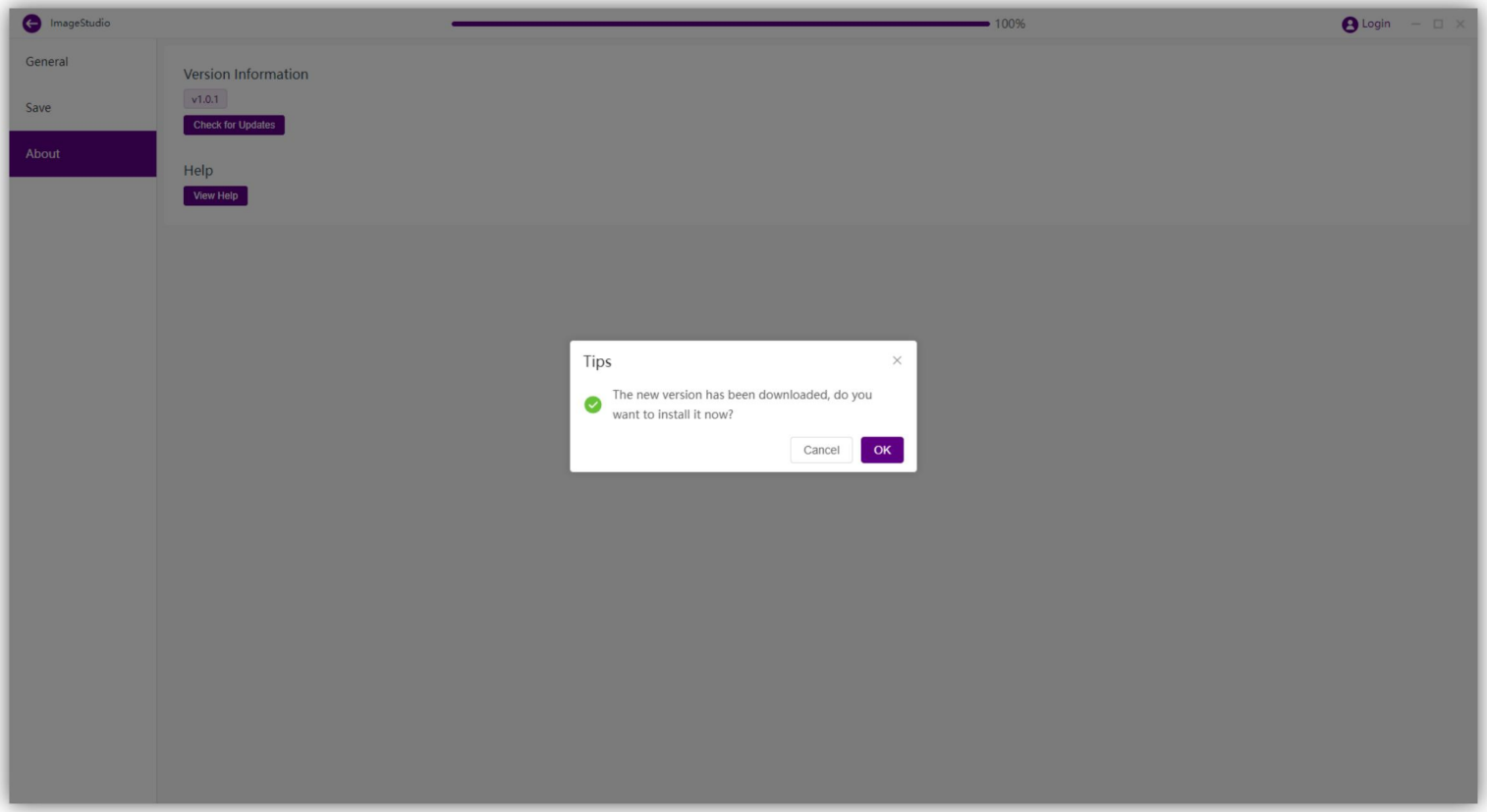
4. If new version is available for update, a pop-up message will display.



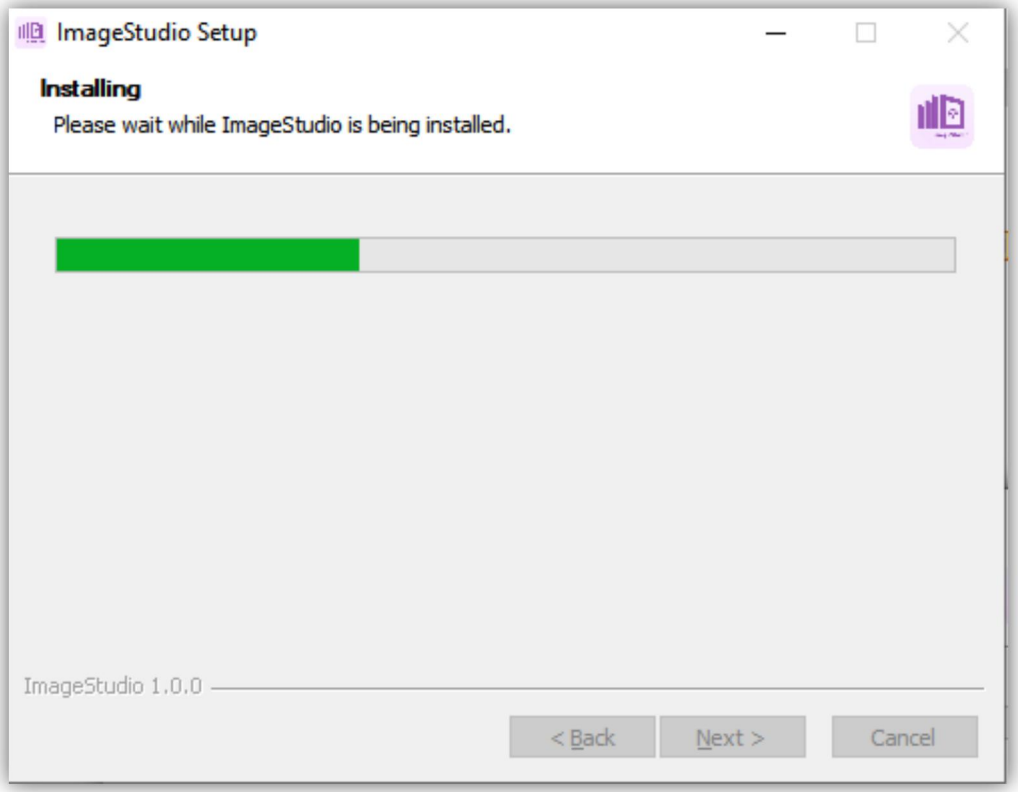
a. Click ‘Update Immediately’ to download the installation package.



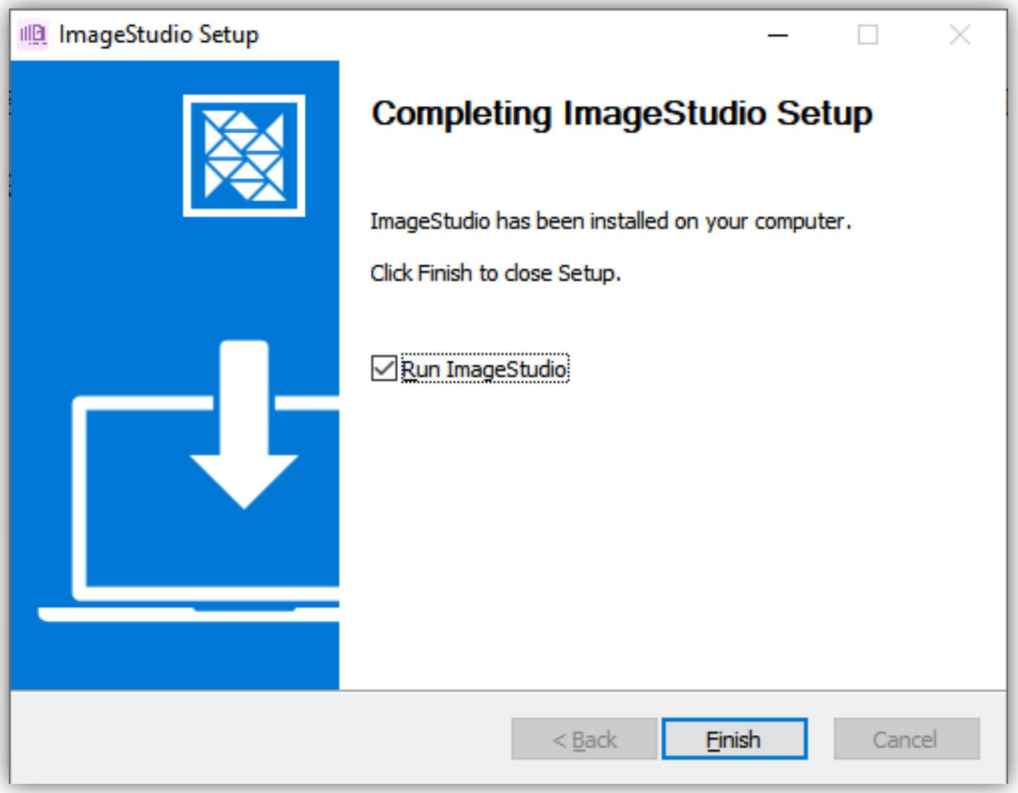
b. Once complete, click ‘OK’ .



c. Enter the Installation interface.



d. Now the update has been completed.

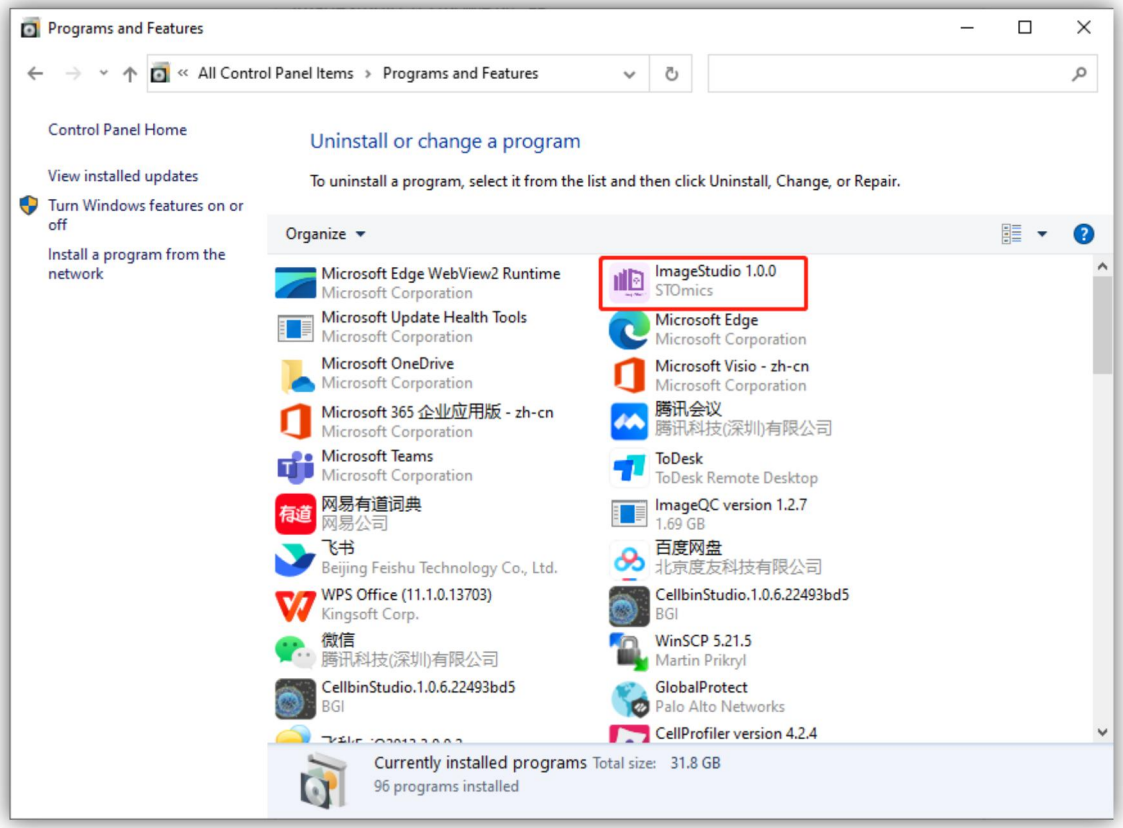


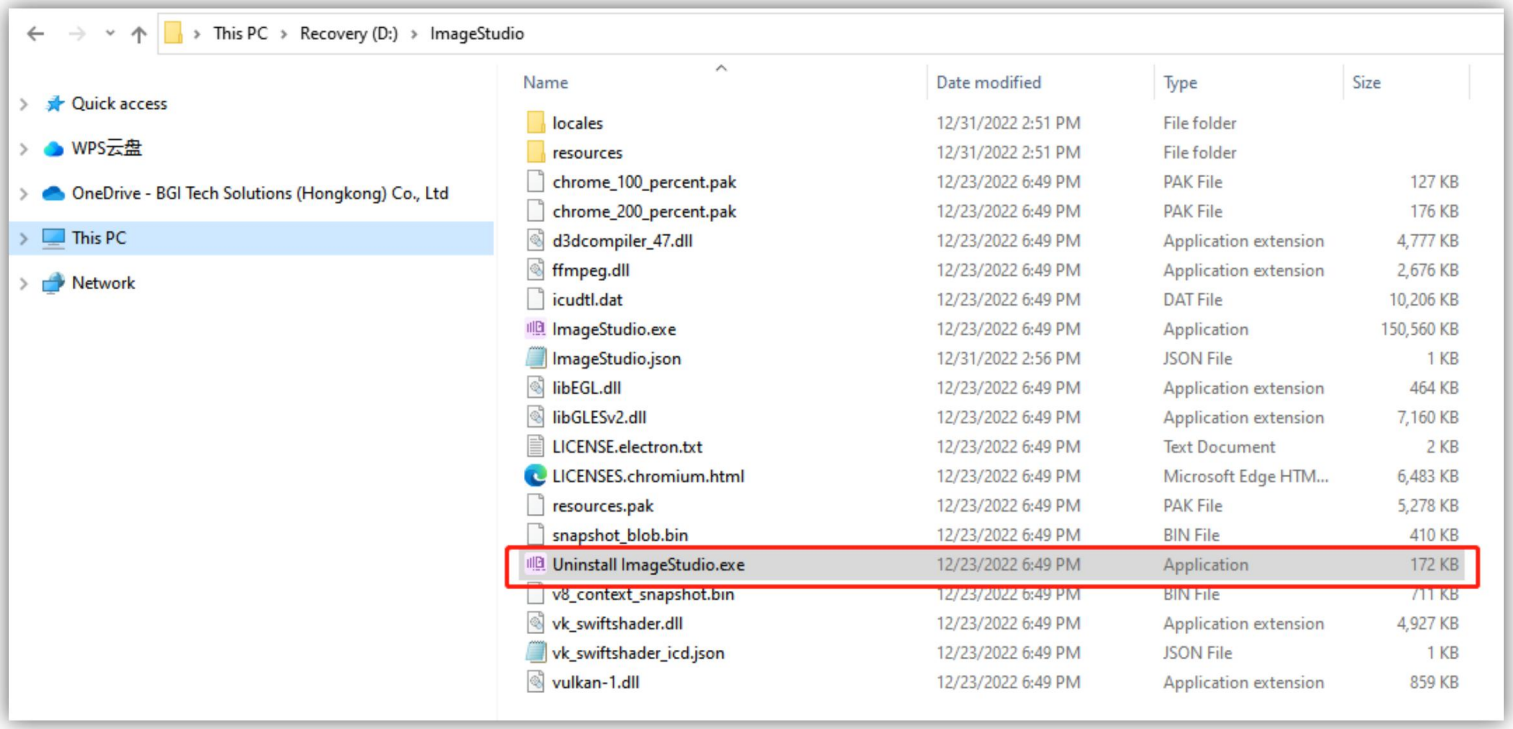
⚠ Make sure to update the software under internet connection. If internet connection is unavailable, contact your local FAS for assistance.

3.2.3 Software Uninstallation

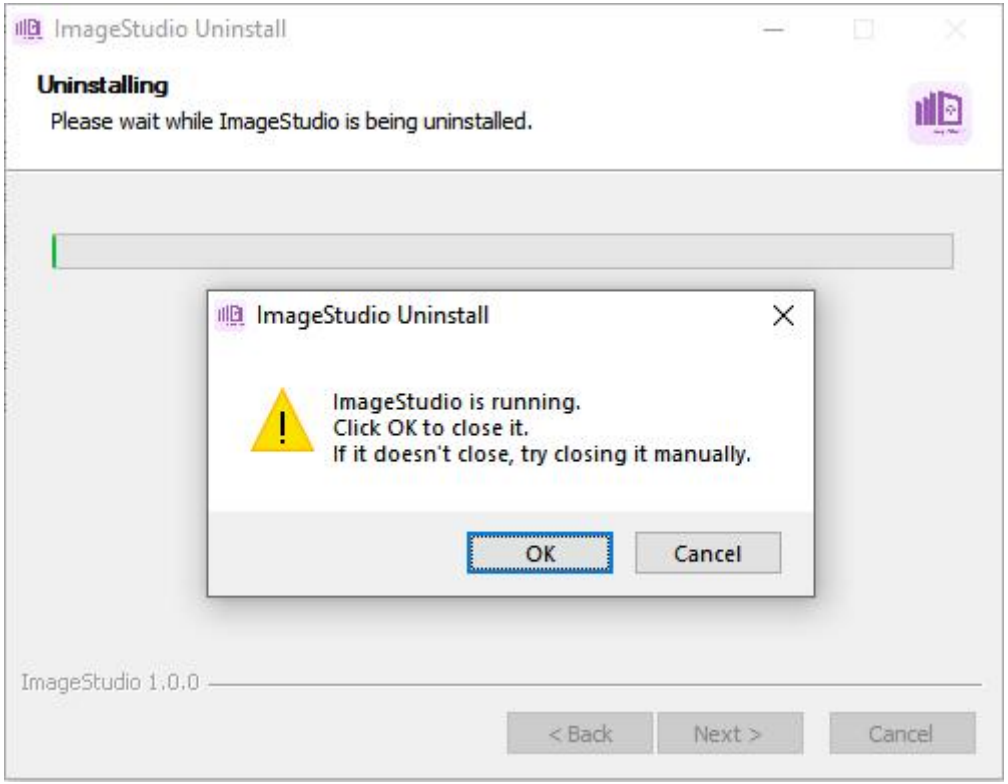
1. Open the system’ s control panel or find ‘**Uninstall ImageStudio.exe**’ under the program installation directory (Default: drive *D:/ImageStudio*). (Users can save the installation package of

the latest version as a backup.)

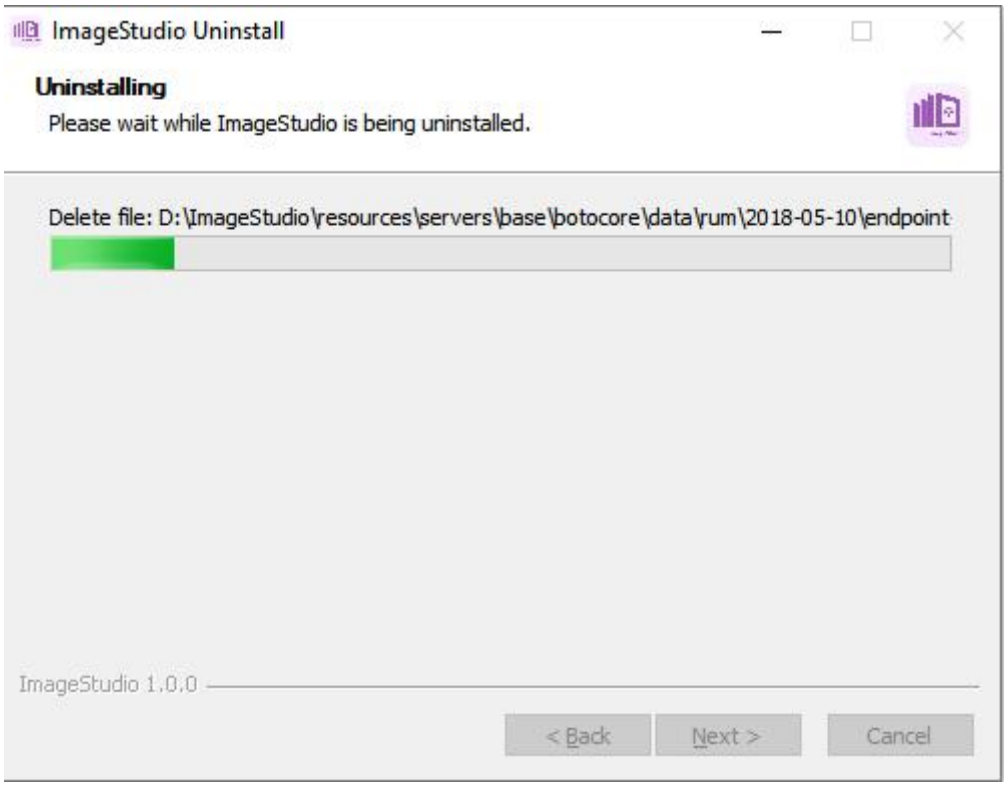




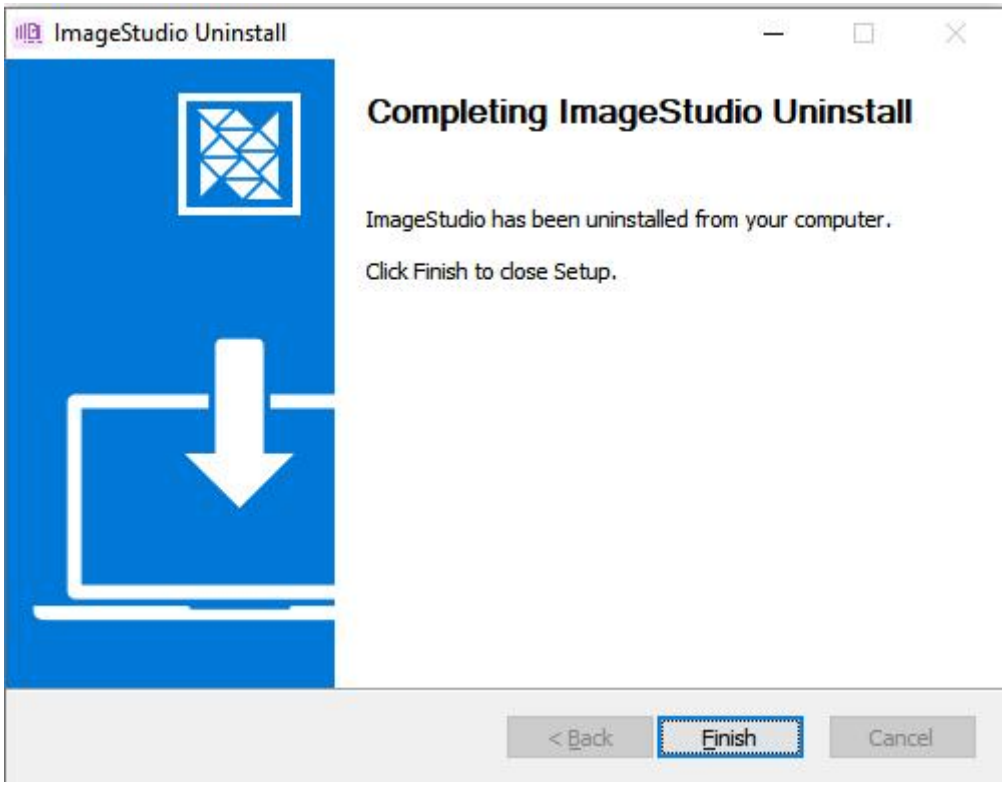
2. If a pop-up prompts that the program is occupied, click "OK" to close the ImageStudio program window.



3. Click ‘Next’ to uninstall.



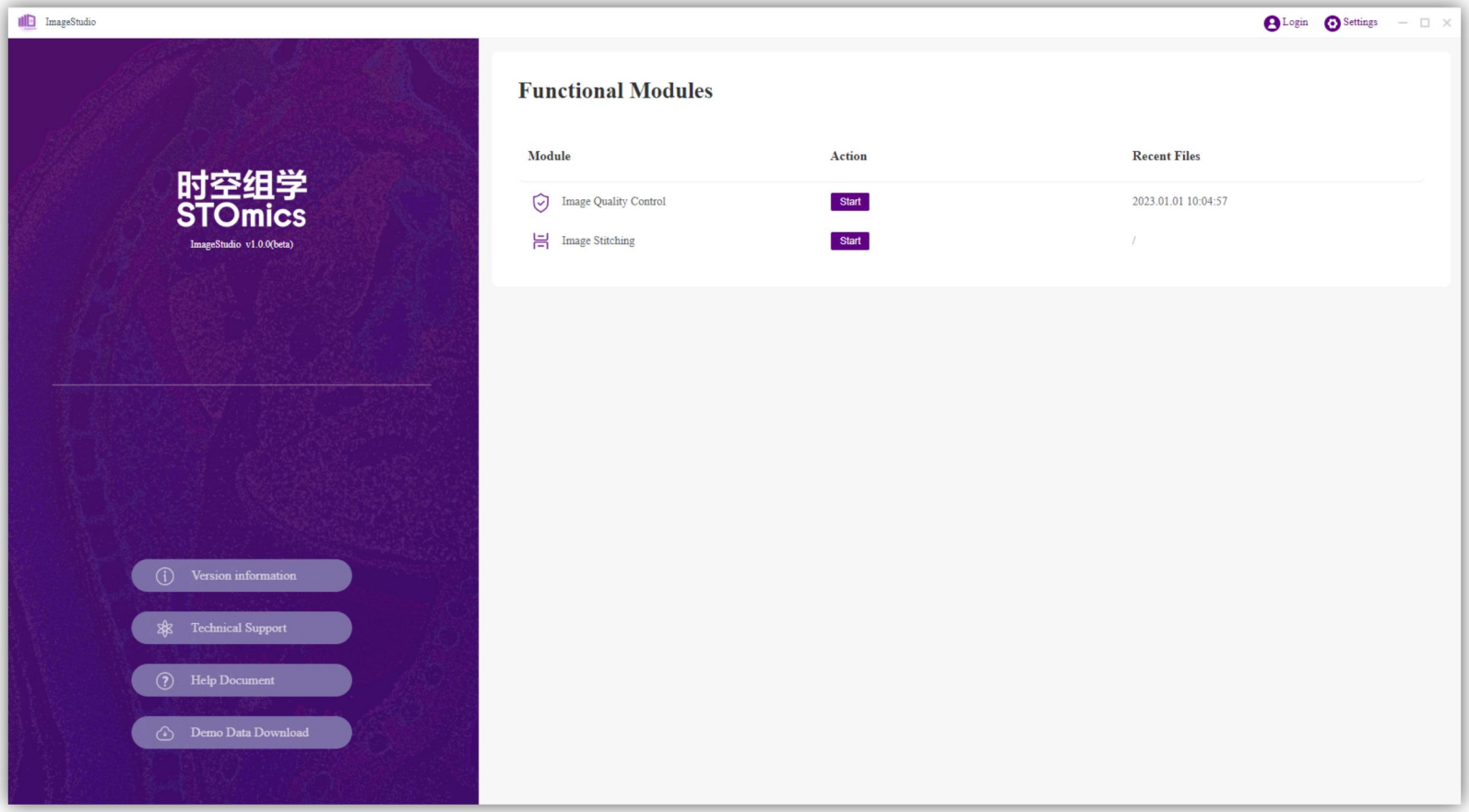
4. Click ‘Next’ to complete the uninstallation process.



4 Operation guide

4.1 Startup Page

Launch ImageStudio after installation. The startup page is shown here.



4.1.1 Left Side Column

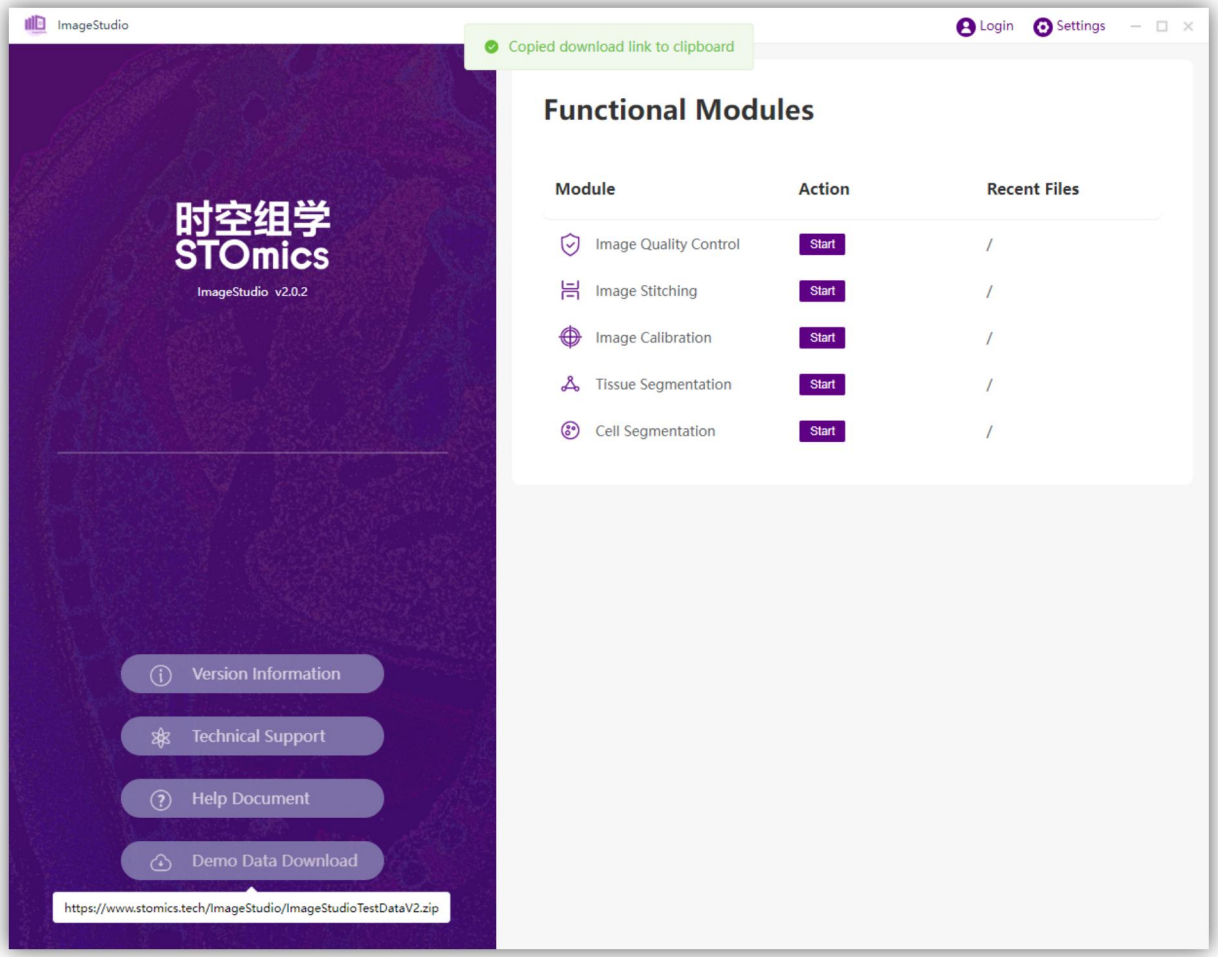
(1) Click *version information* to view the software version information.

(2) Click *technical support* to view technical support.

(3) Click *help document* to view the relevant document information of each module.

(4) Click *demo data download* and select the file path to download the demo input data of each module.

Note: If failed to download data after clicking the Demo Data Download, hover the mouse over the Demo Data Download button, then the system will automatically copy the download link, which can be downloaded in the browser.

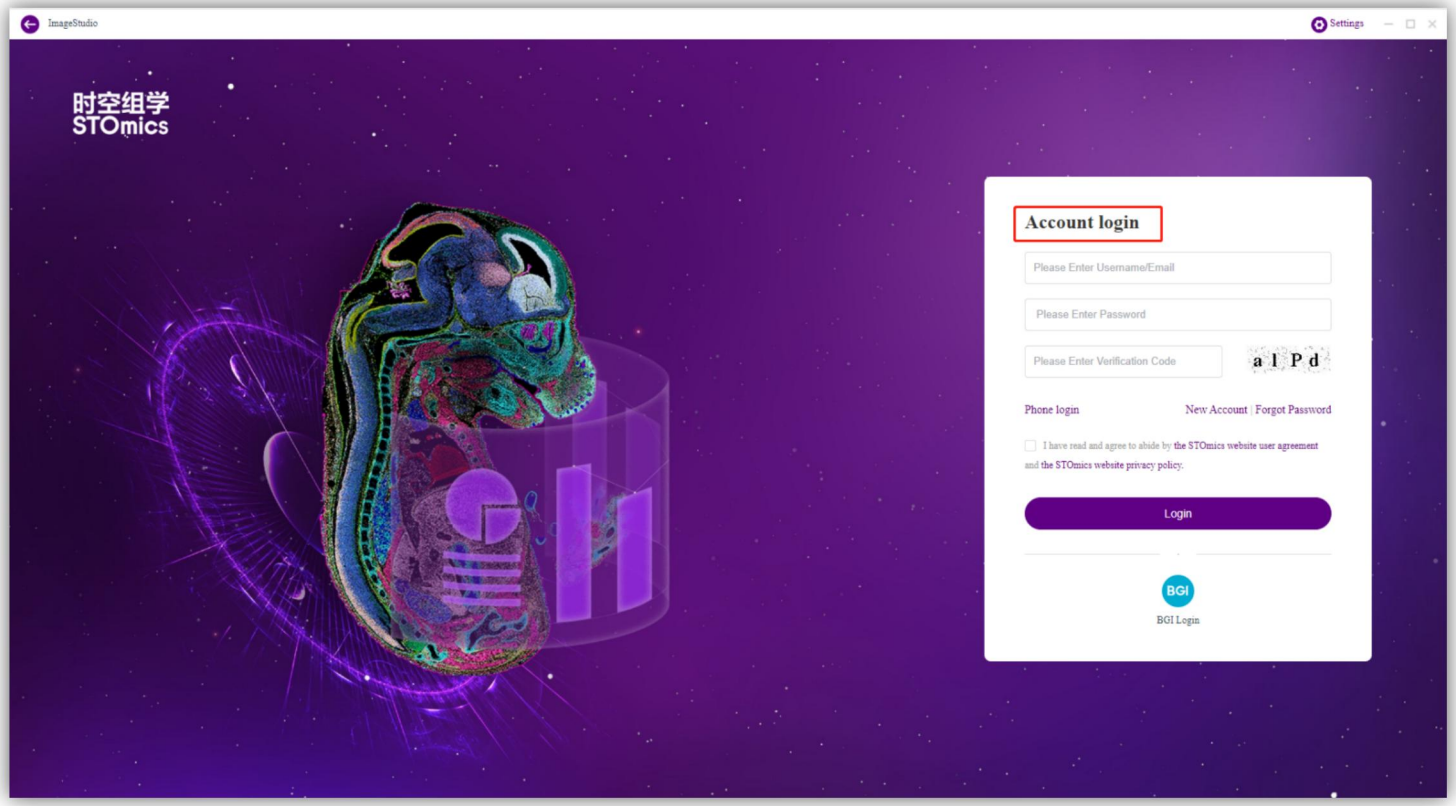


4.1.2 Login (Optional)

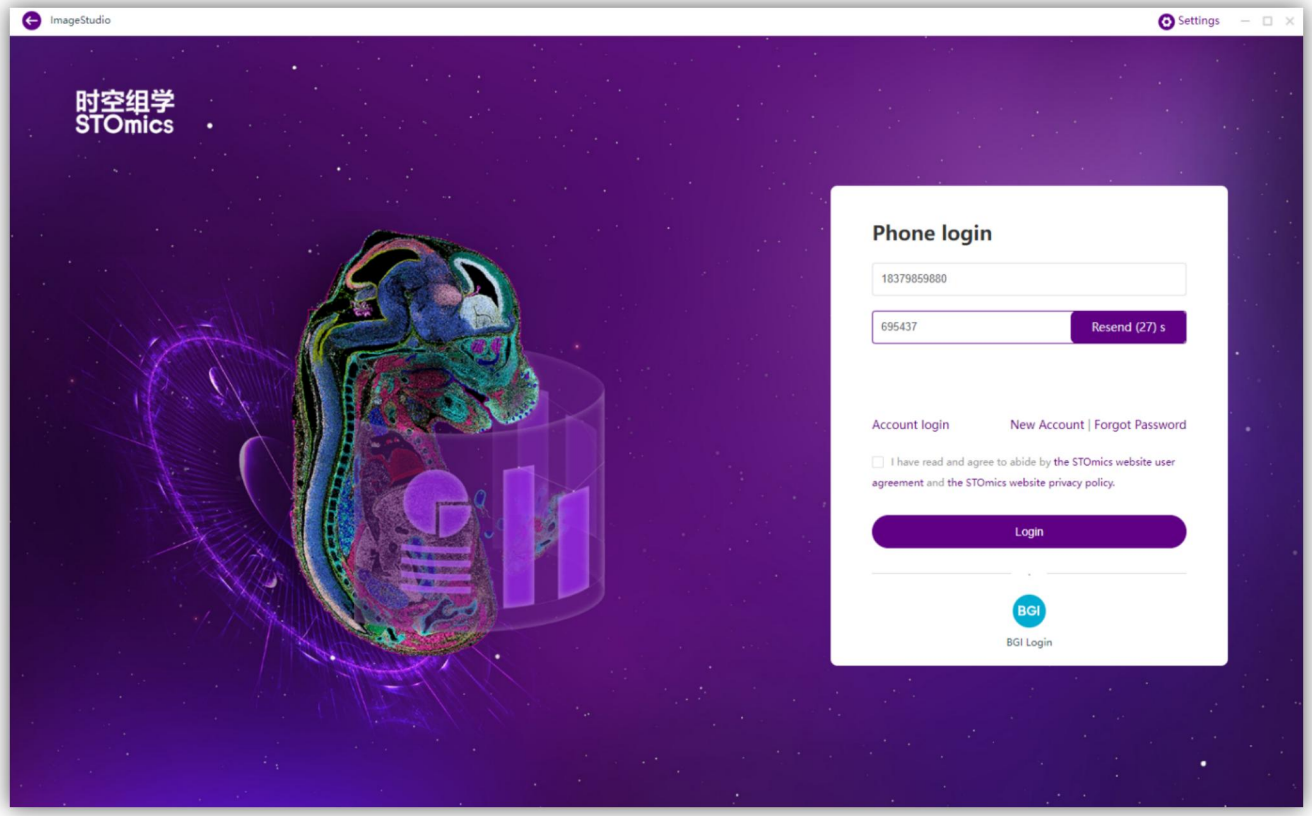
User login is optional which is only required for internal users who have access to STOmics' s online platform (Stereo-seq Analysis Platform, STOmics Cloud). STOmics Cloud registered users can directly

log in to ImageStudio or create a STOmics Cloud account on ImageStudio’ s login page. There are three login methods available (same as STOmics Cloud).

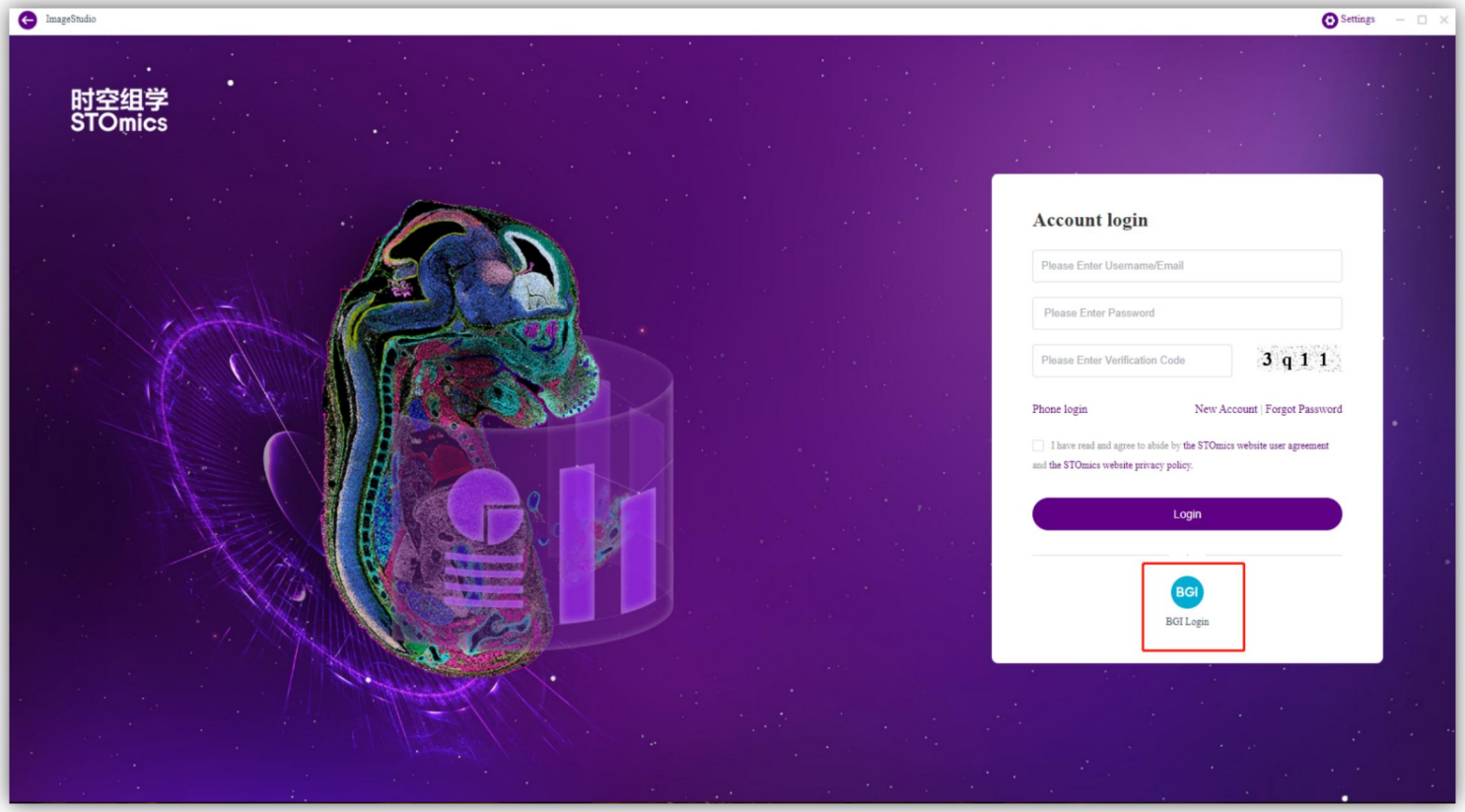
(1)Account login - enter username/email and verification code to log in.

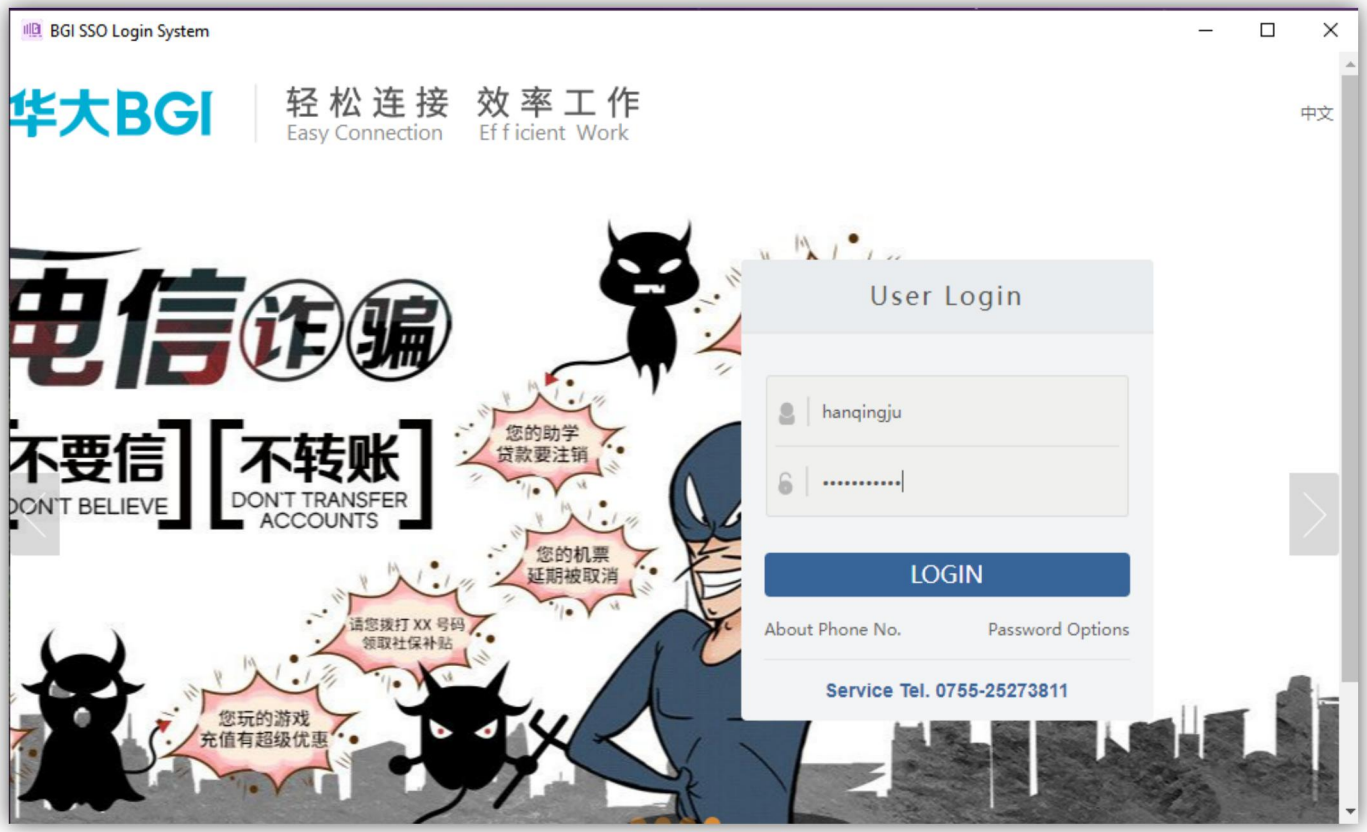


(2)Phone login - enter phone number and verification code to log in.

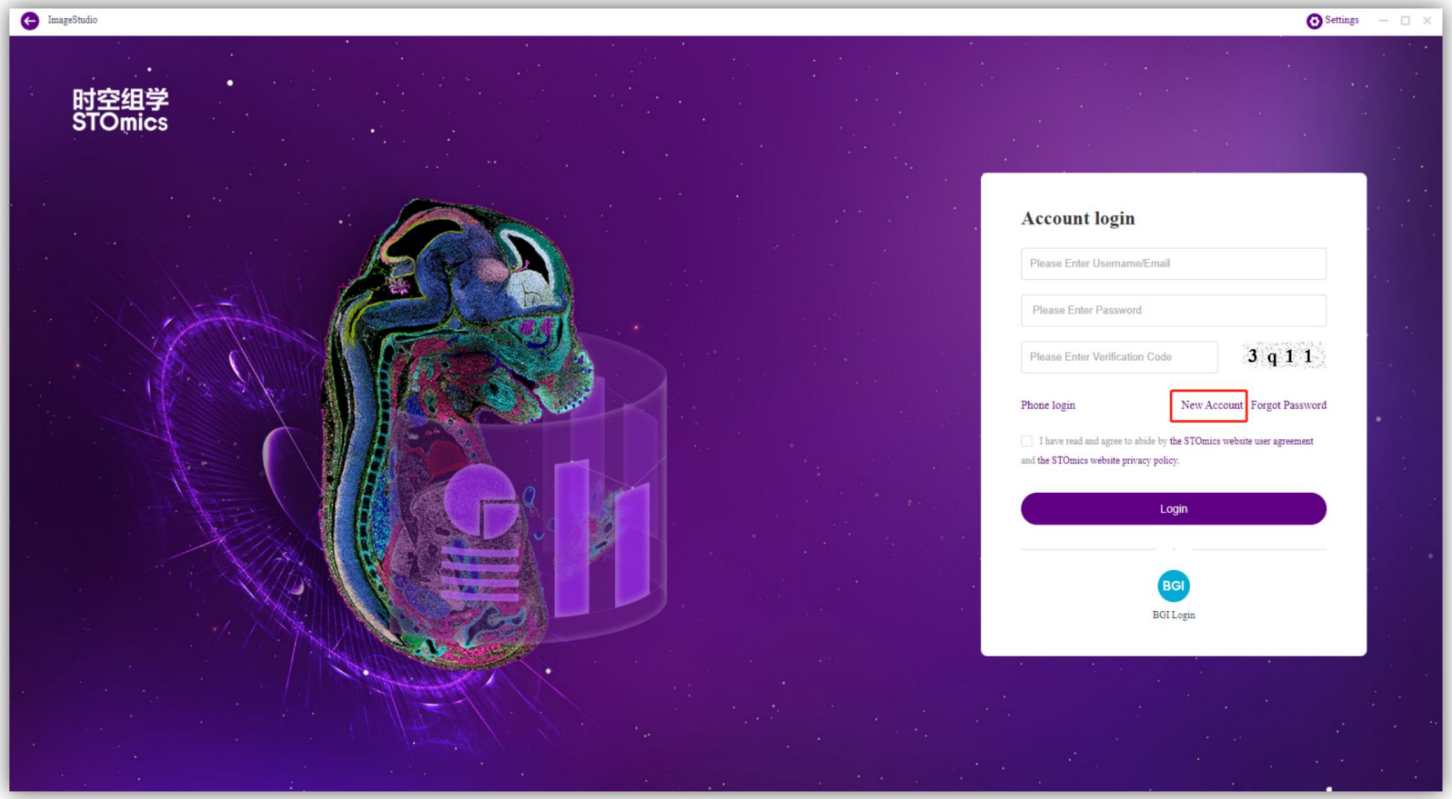


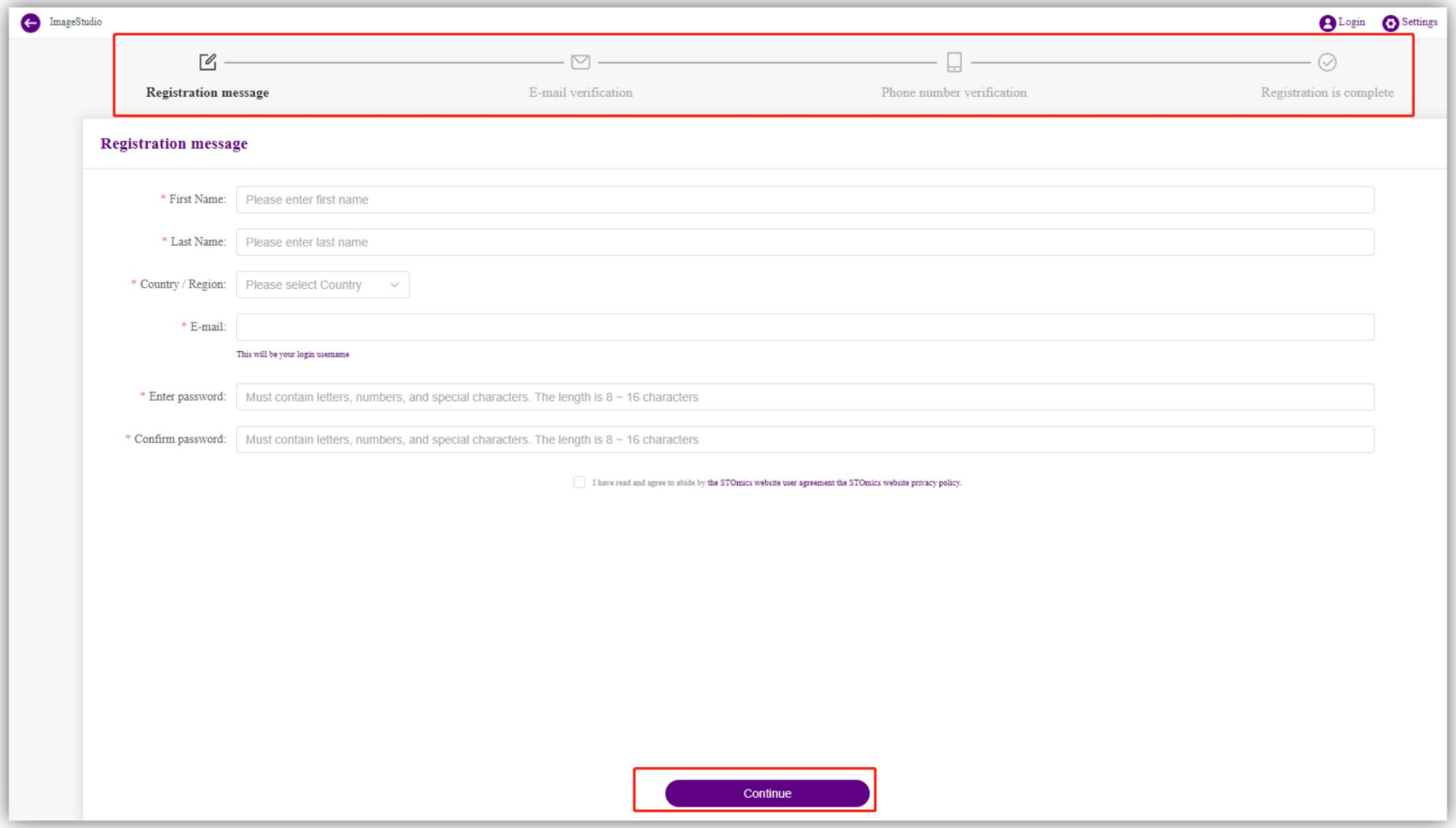
(3) BGI Login – Applicable for internal users only, enter user name and password to log in.


ImageStudio
Settings
时空组学
STOmics
Account login
Please Enter Username/Email
Please Enter Password
Please Enter Verification Code
3 q 1 1
Phone login
New Account | Forgot Password
I have read and agree to abide by the STOmics website user agreement and the STOmics website privacy policy.
Login
BGI
BGI Login

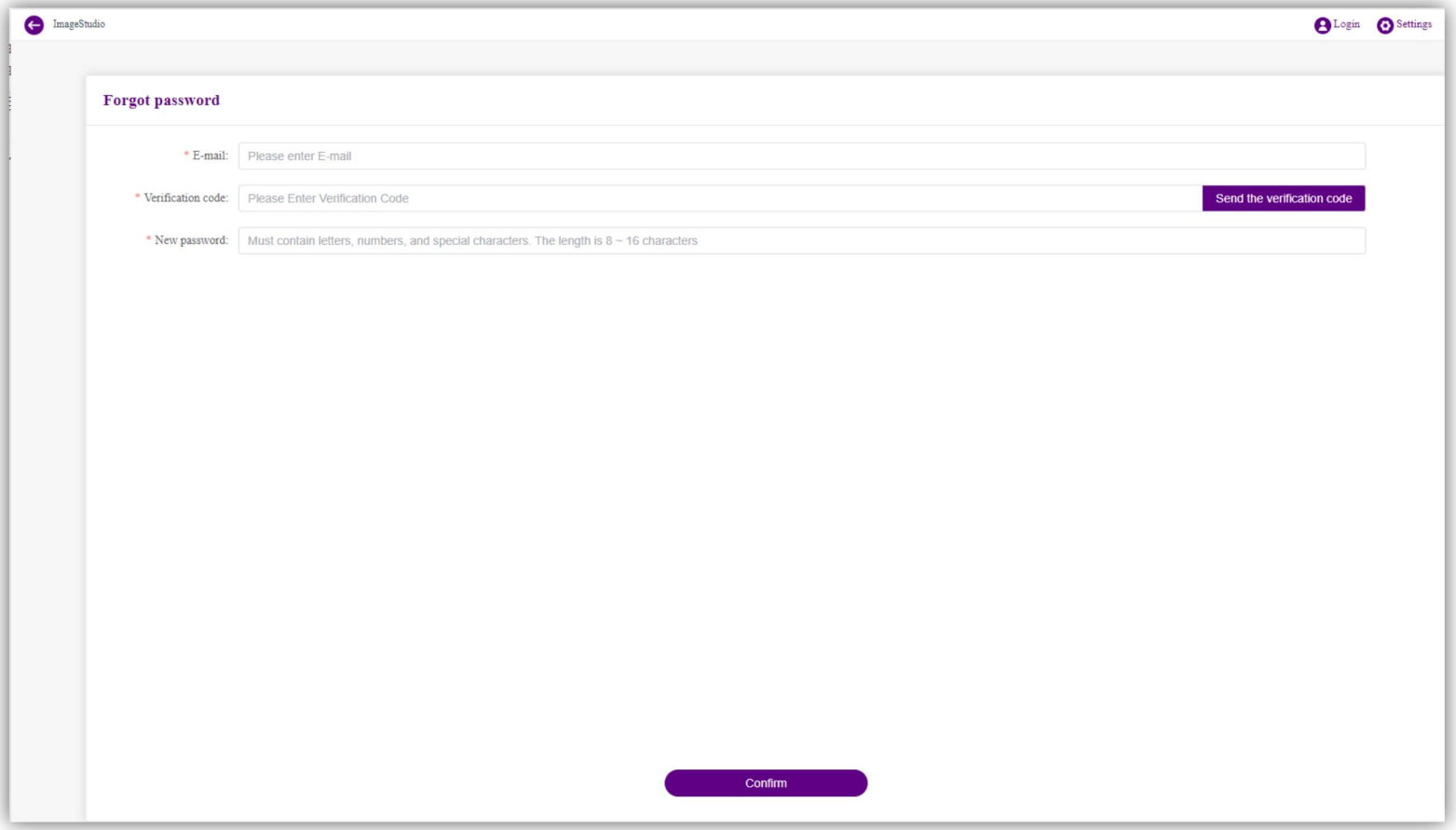


(4) Account registration (same as STOmics Cloud). Complete via four steps: enter registration information, verify email, verify phone number, and confirm registration.


ImageStudio
Settings
时空组学
STOmics
Account login
Please Enter Username/Email
Please Enter Password
Please Enter Verification Code
3 q 1 1
Phone login
New Account
Forgot Password
I have read and agree to abide by the STOmics website user agreement and the STOmics website privacy policy.
Login
BGI
BGI Login



(5)Change password (same as STOmics Cloud).

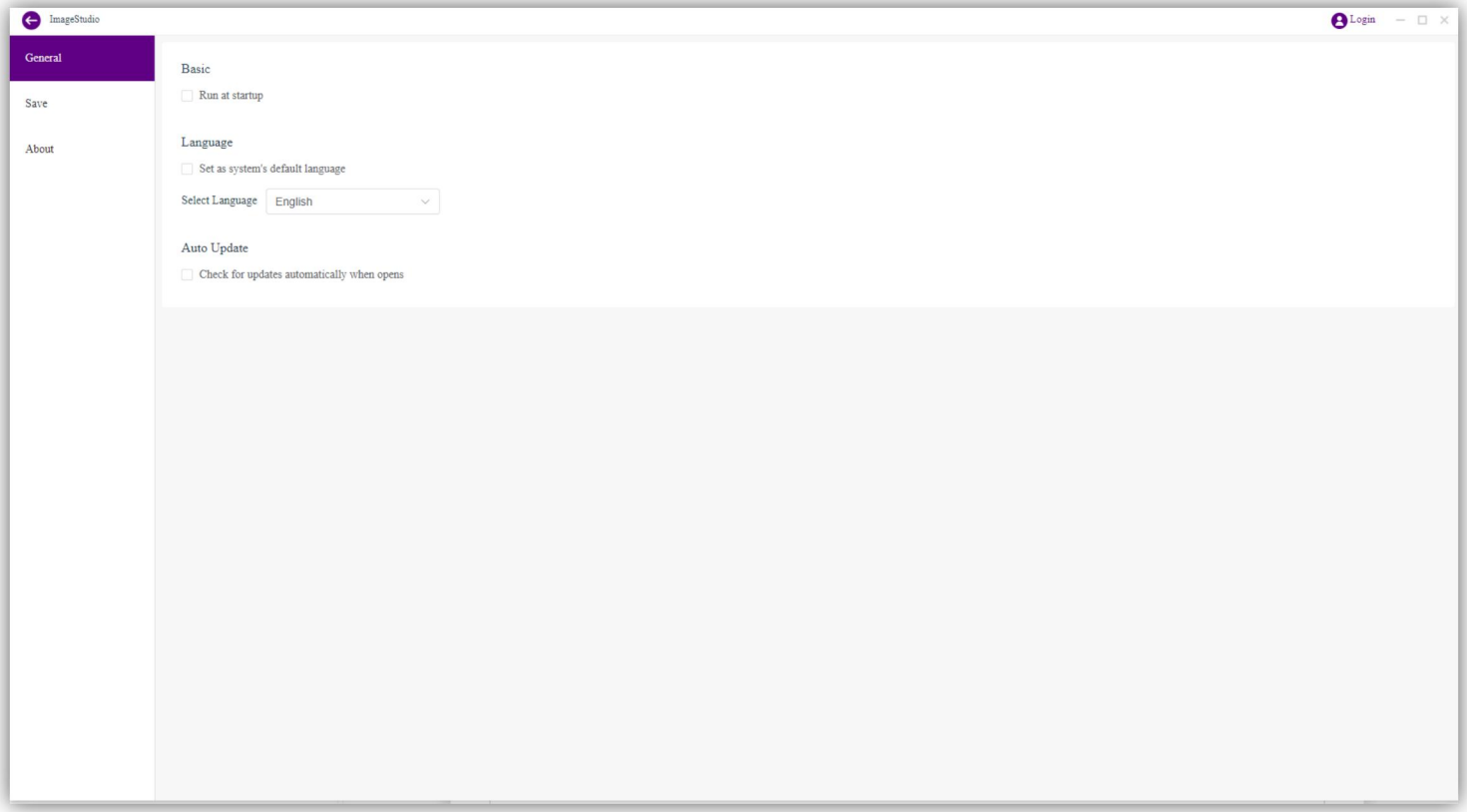


⚠The login system adds the following additional features for BGIers.

- ‘Image Quality Control’ function can verify whether the input SN number is correct or not.

4.1.3 Settings

(1) *General* – Settings for 1) whether to run ImageStudio at startup, 2) language selection and 3) auto update of general functions.

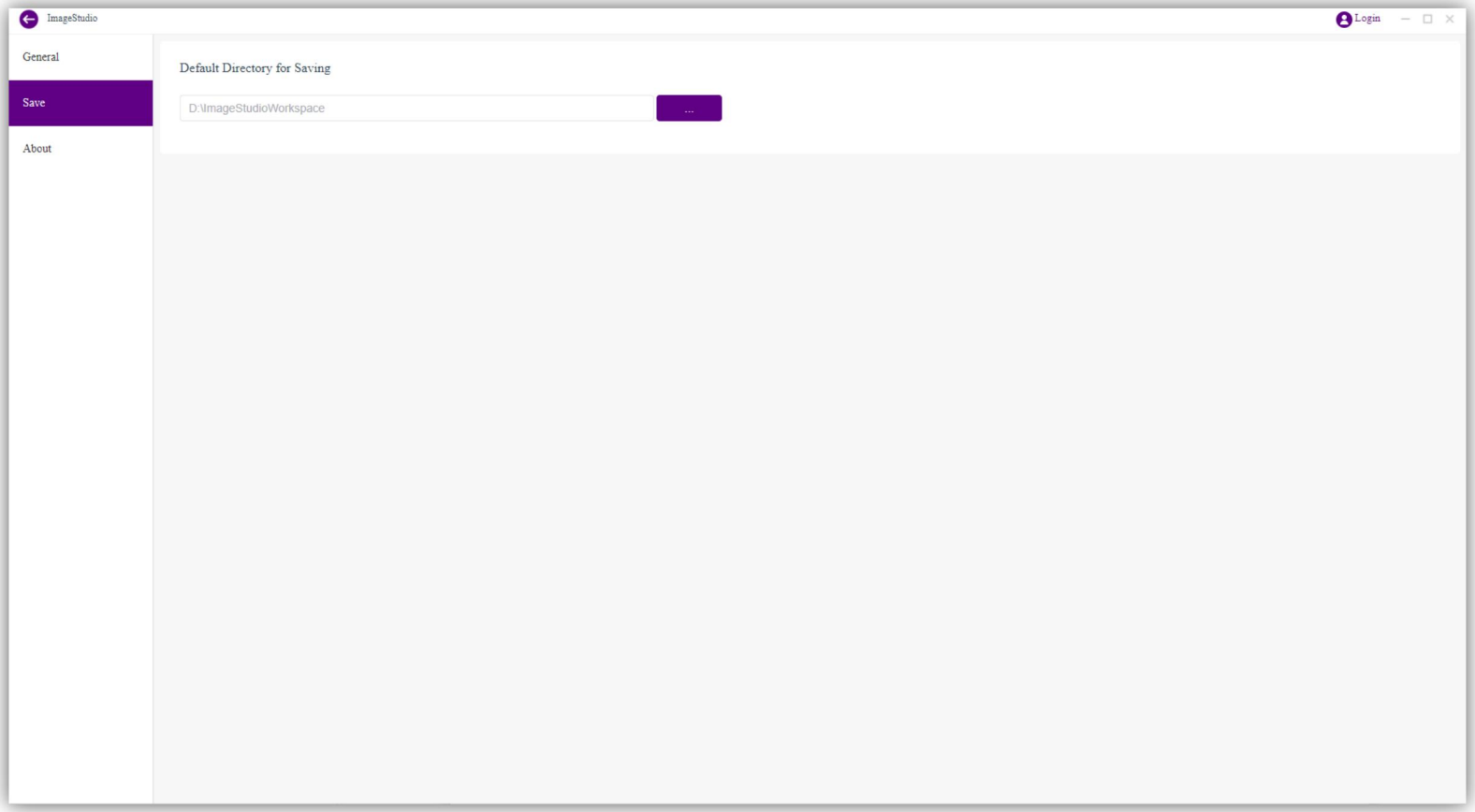


- The default language can be *set to system's default language*. Only two languages are supported

currently: English and Chinese.

- '*Check for updates automatically when opened*' is unselected by default. If necessary, check the box for Auto Update.

(2) Save, set the default directory for saving.



- The output file directory of each module is saved in *D:\ImageStudioWorkspace* on your computer

by default. Click the *three dots* to modify the file path.

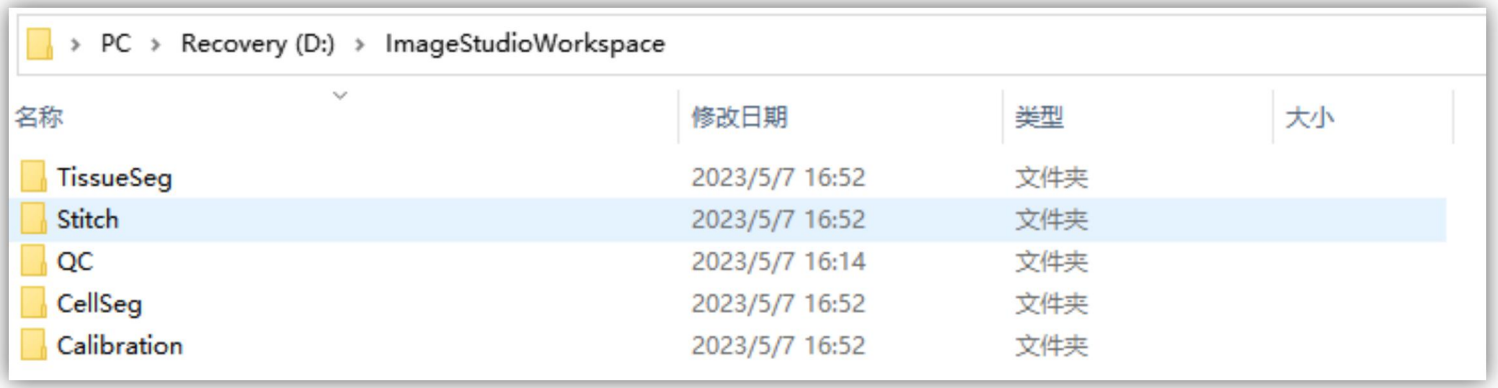
- Different directories are created in *D:\ImageStudioWorkspace* directory depending on the module.

  Image Quality Control: QC

  Image Stitching: Stitch

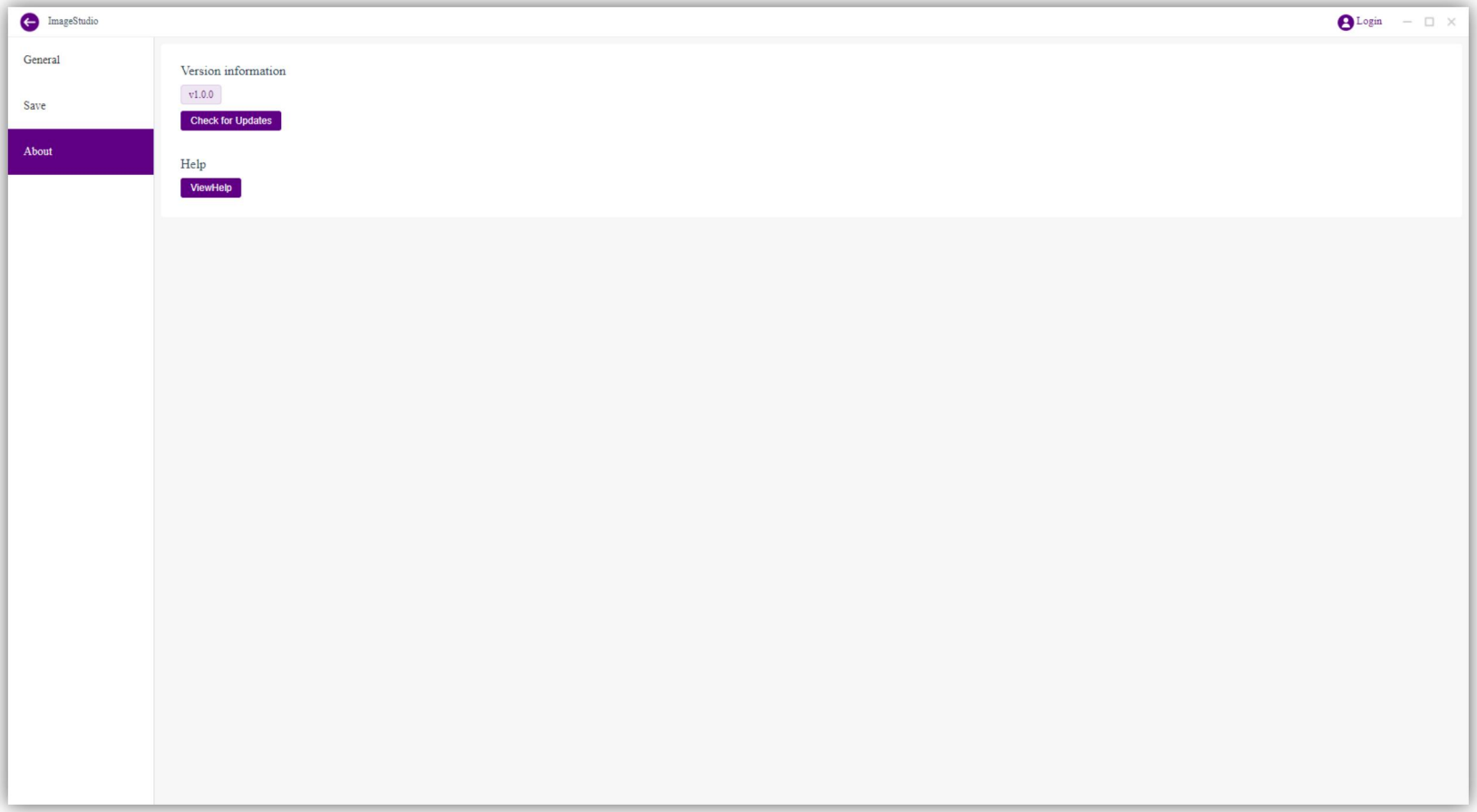
  Tissue Segmentation: TissueSeg

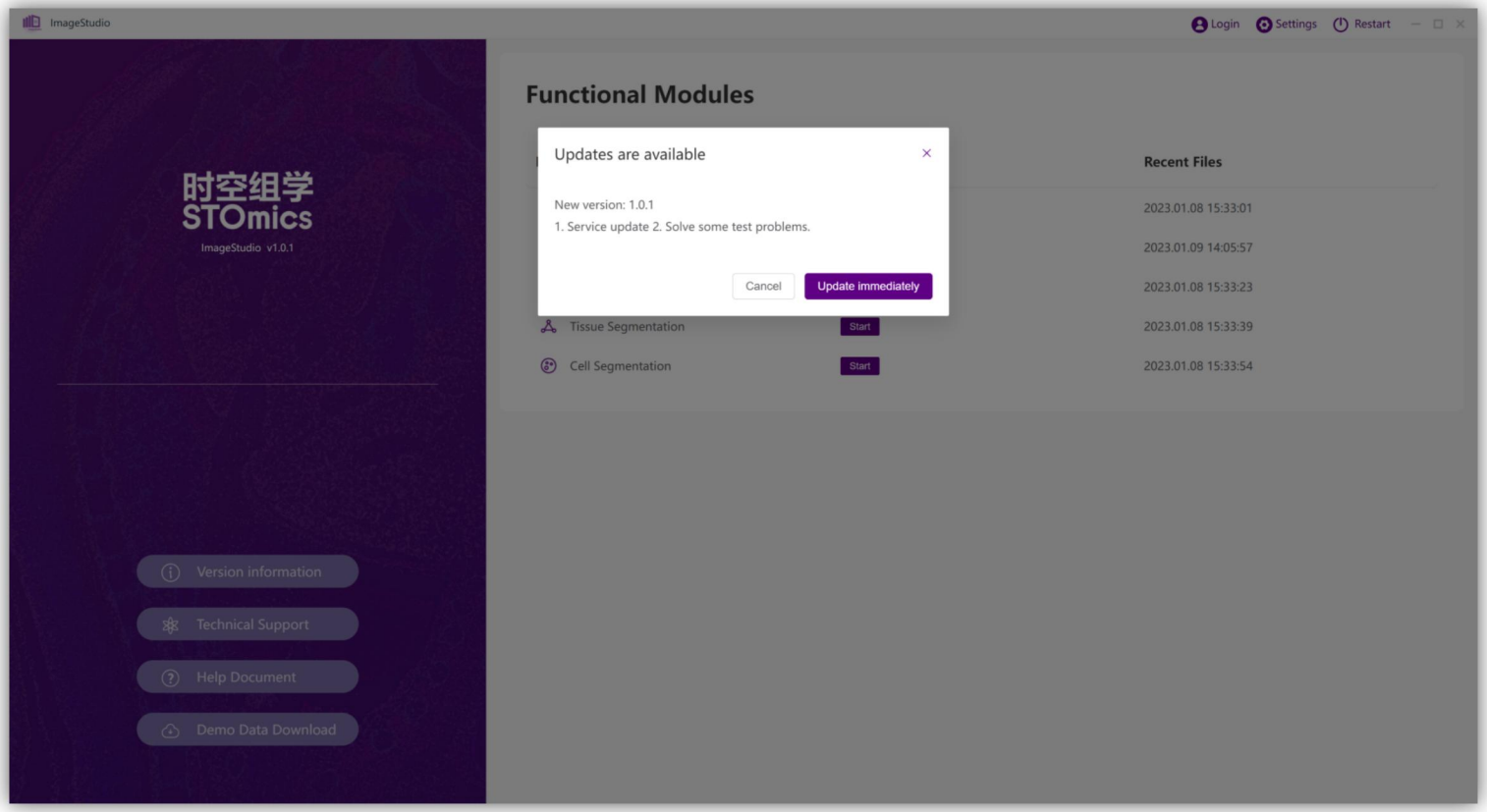
  Cell Segmentation: CellSeg



Note: Each directory will generate a .mst file which users can delete but cannot modify the content within.

(3) *About*, Check for Updates and ViewHelp.


ImageStudio
Login
General
Save
About
Version information
v1.0.0
Check for Updates
Help
ViewHelp



- Click 'Check for updates'. System can check for updates automatically with the internet connection.
- Click 'Update Immediately' to update online.
- Click 'View Help' to view the relevant document information of each module.

4.2 Image Quality Control

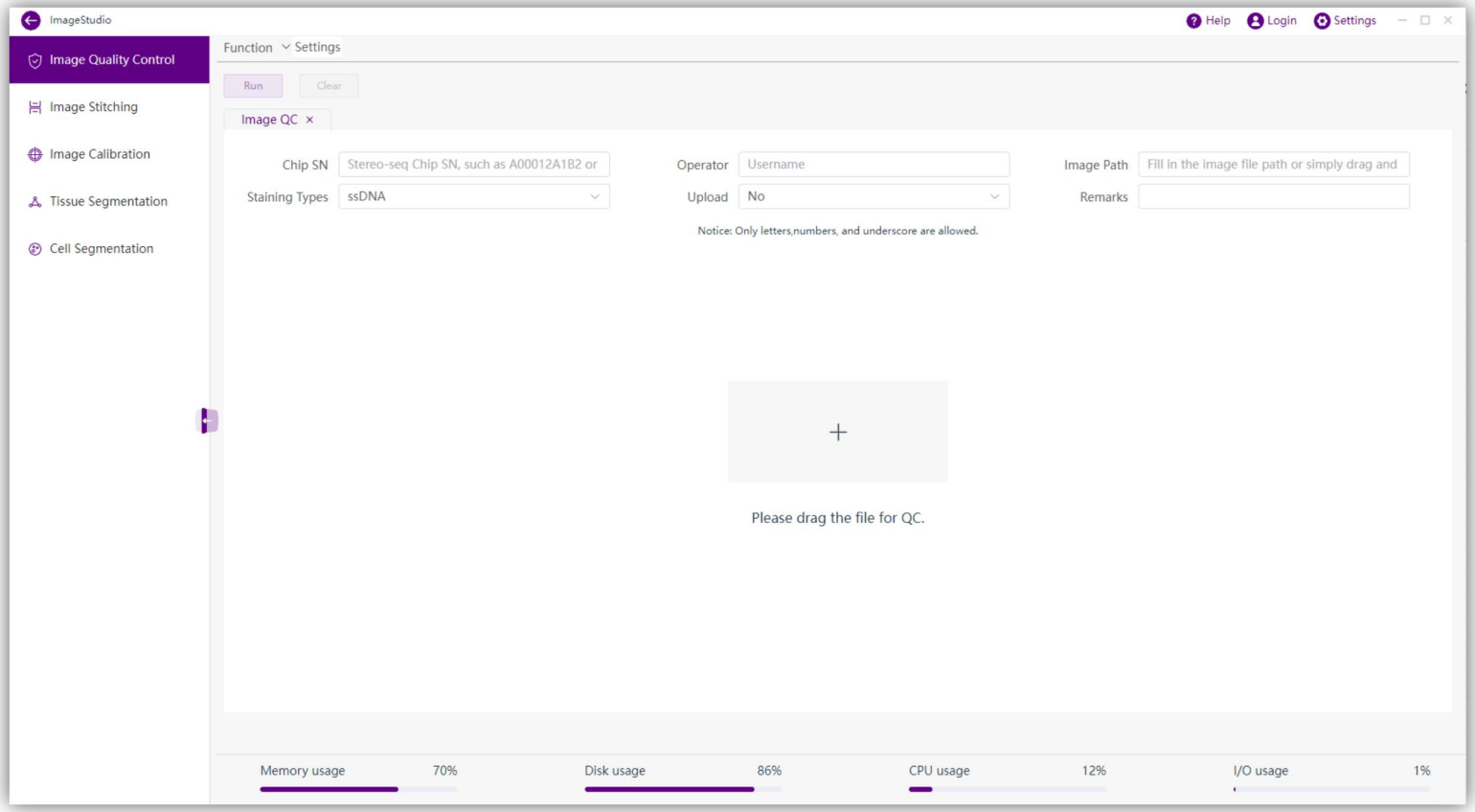
Image Quality Control is intended for assessing the quality of Stereo-seq microscopic images to

ensure further downstream analysis. It includes three functions: Image QC for nuclei (ssDNA or DAPI) stained images or DAPI&mIF , TIFF/PNG Image upload and upload information configuration.

> Note: The saving path of microscope images and the output path of image quality control cannot contain Chinese or special characters.

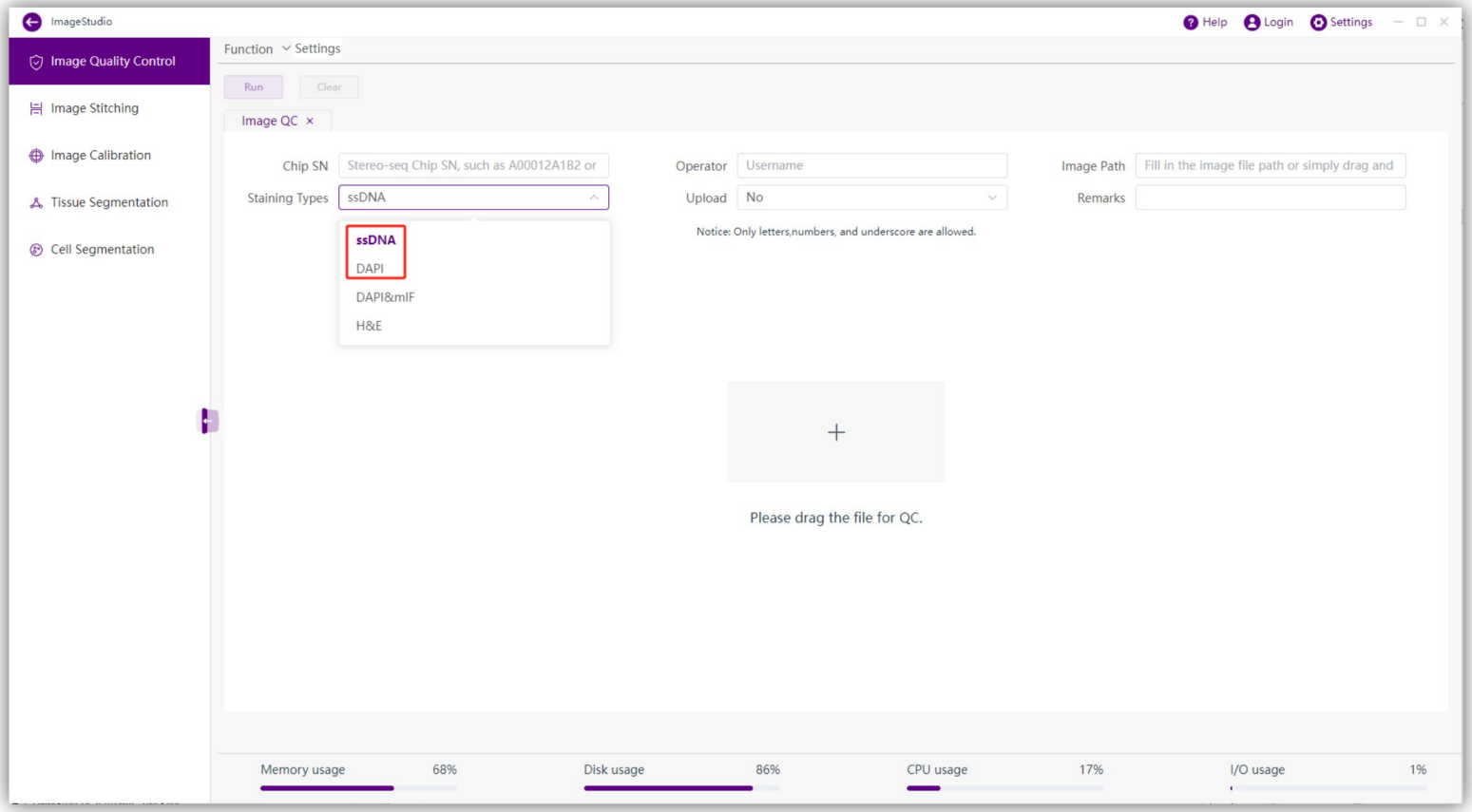
4.2.1 Image QC

The interface of image QC is as follows:

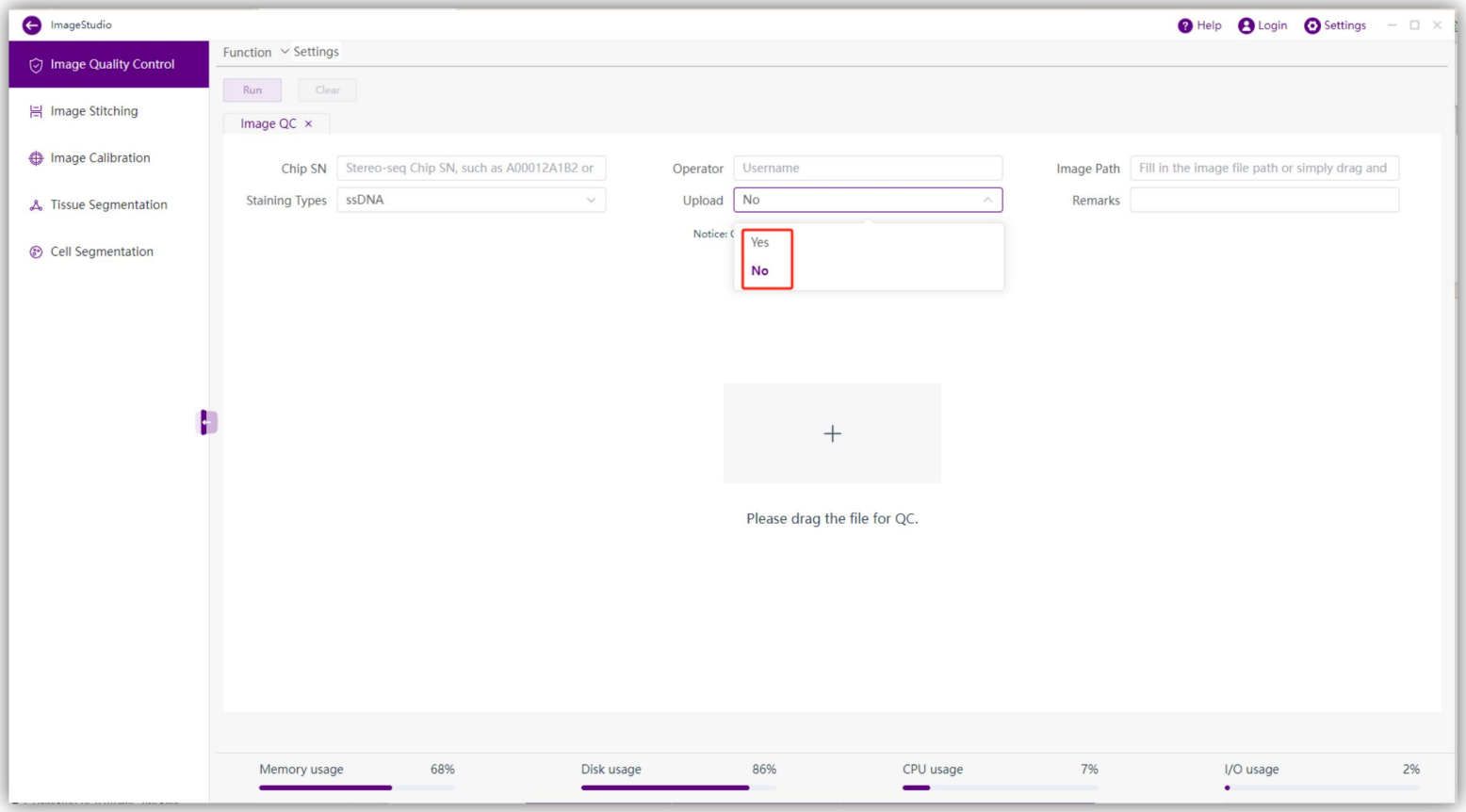


4.2.1.1 Nuclei-stained images with ssDNA or DAPI

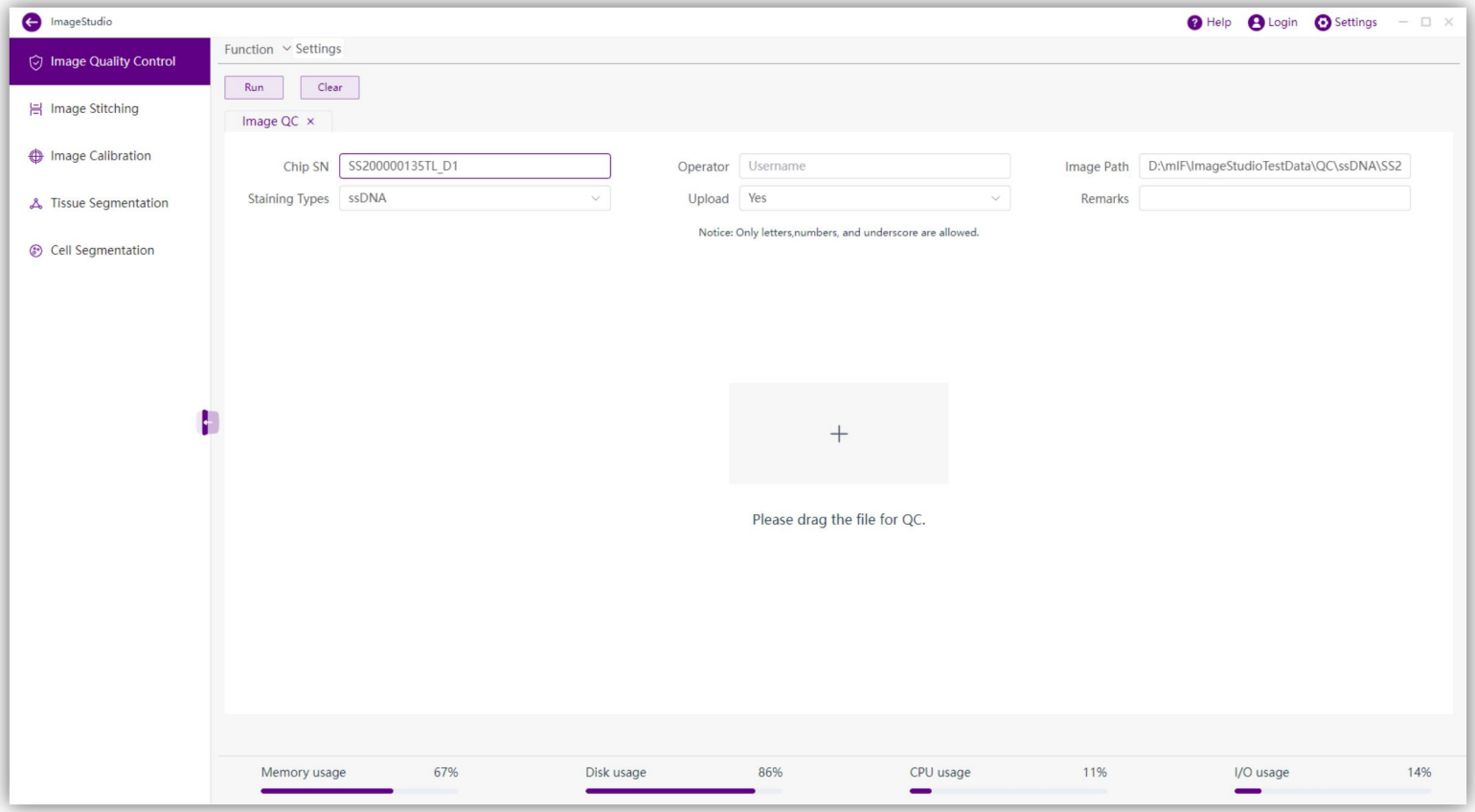
(1)Select the staining type "ssDNA" or "DAPI"



(2) Select "Yes" or "No" for "Upload" to upload the image (after QC) to the selected computer cluster or cloud or not.



(3) Drag and drop image files or folders to the program.



(4) Description of microscopes and data formats that are supported by ImageStudio QC module.Output Directory: *D:\ImageStudioWorkspace\QC*

Microscope	Input file	Output files that are supported by SAW
Zeiss Axio Scan Z1	Raw image file in CZI	• IPR file that stores image

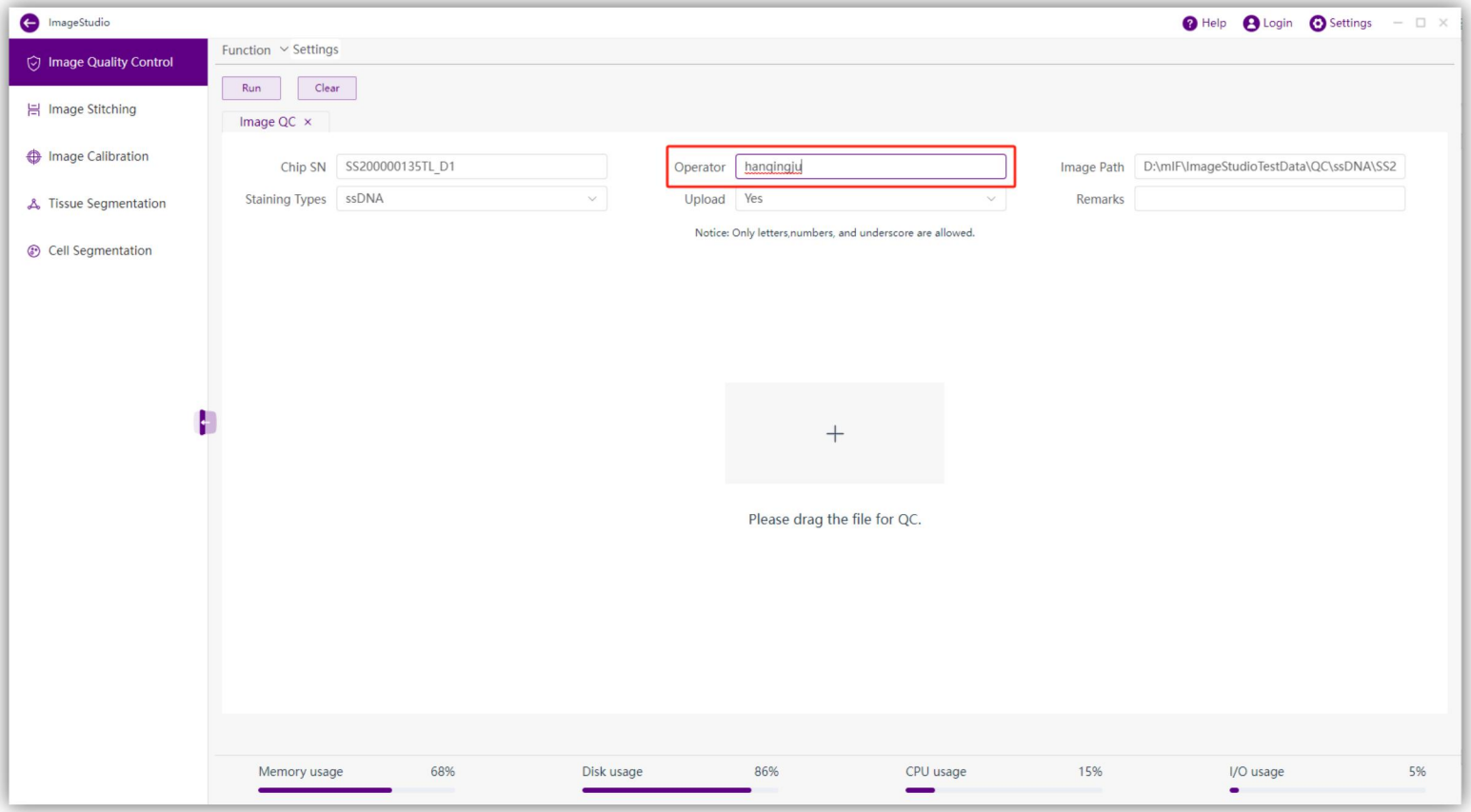
Zeiss Axio Scan 7	format	capturing information and Image QC information • Zipped image files in TAR.GZ format
Leica DM6-M Leica DM6-B	Stitched image in TIFF format Tile images (individual FOV)	
Motic for BGI custom	Raw image file in mdsx format	
Go Optical	Tile images (individual FOV)	
Other microscopes (before API development):	Stitched image in TIFF format	
Other microscopes (after API development):	Tile images (individual FOV) or raw image file	
/	The output	

compressed

file(TAR.GZ) of after

Image QC

(5) Fill in the Stereo-seq Chip Serial Number (SN) and the operator’ s email address.

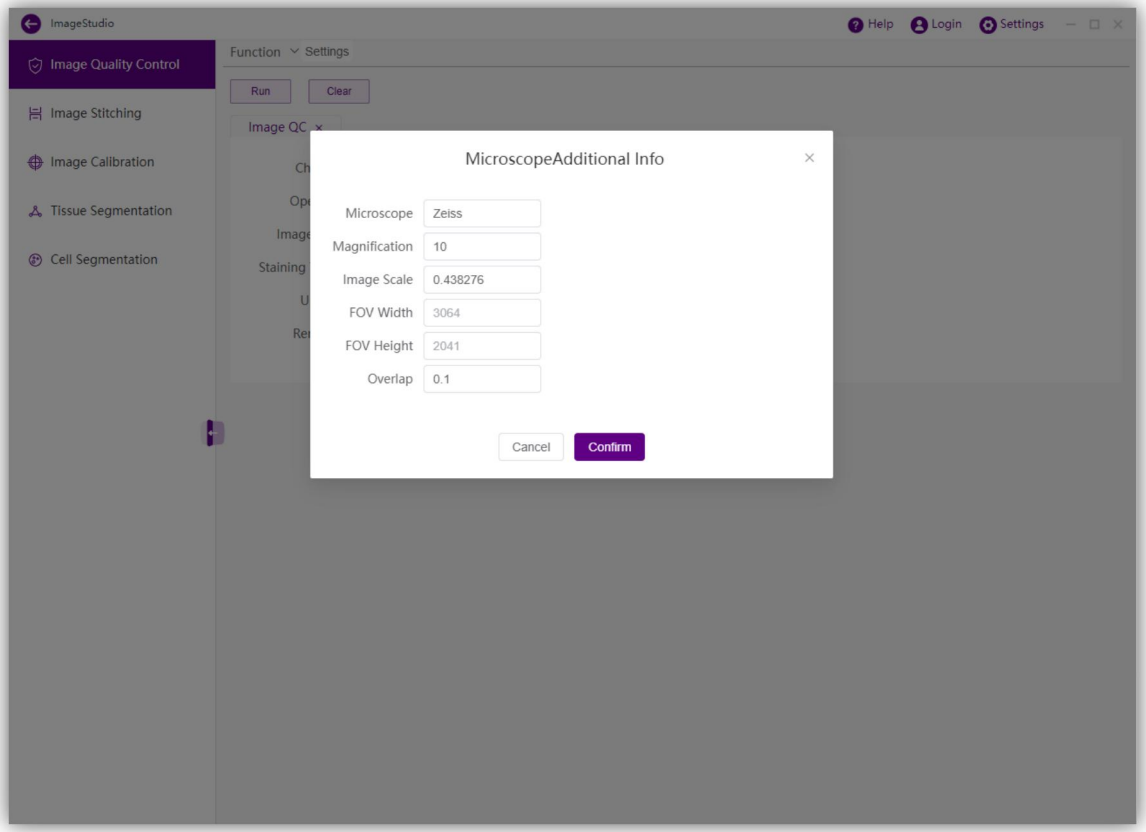


- **Chip SN**: Chip serial number of a Stereo-seq Microscope Assessment Chip T or a Stereo-seq Chip T that is used for imaging. ImageStudio software can only recognize the standard Stereo-seq Chip SN from BGI STOmics.
- **Operator:** Full email address of the operator, or mailbox prefix of a BGI employee.

Note: The Stereo-seq Chip SN and the operator email will be recorded and filled automatically on the next entry. For a new user or a warning raised, please re-type the correct email address and try again.

- **Image Path:** File path for the stitched panoramic image or the file directory path for the tiled FOV images. It can be manually typed in or automatically filled in by the program after dragging and dropping the file/folder into the program.
- **Staining Types:** Options are ssDNA, DAPI, and DAPI & mIF.
- **Remark:** Record additional details in the remark field if needed.

(6) For the stitched panoramic image, users will be requested to fill in the image parameters manually in the pop-up window as shown below. Click 'Confirm' to finish the entry, and click 'Run' to start QC.



⚠ These parameters are not required for tiled FOV images in TIFF/ PNG format.

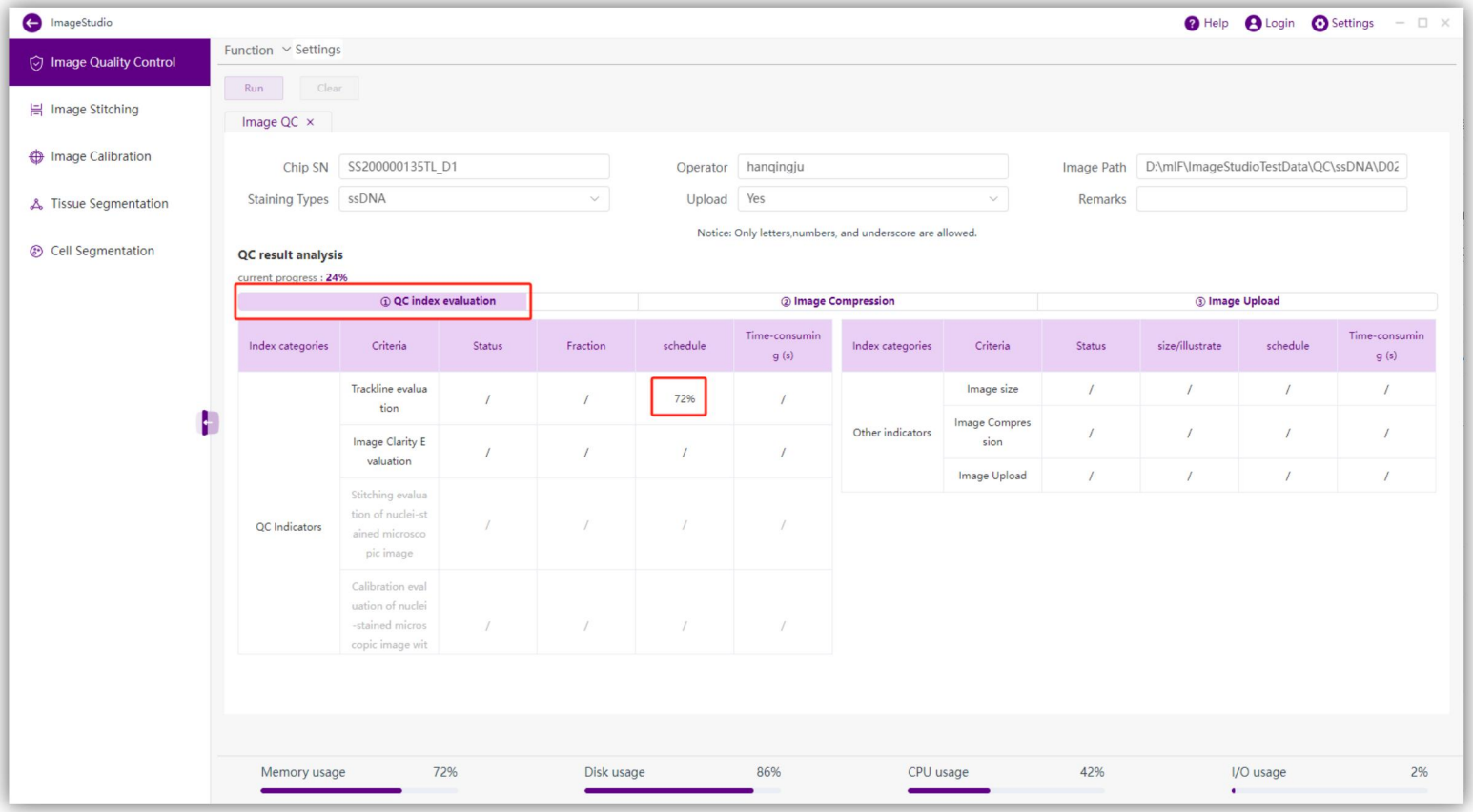
- **Microscope**: Manufacturer of the microscope, such as Zeiss, Leica, Motic, Olympus, etc.
- **Magnification**: Objective lens magnification. For example, if the image was taken by a 10x objective lens, enter 10.
- **Image Scale**: Image Scale can also be defined as 1/resolution in the unit of μm/pixel, with at

least 3 decimal places.

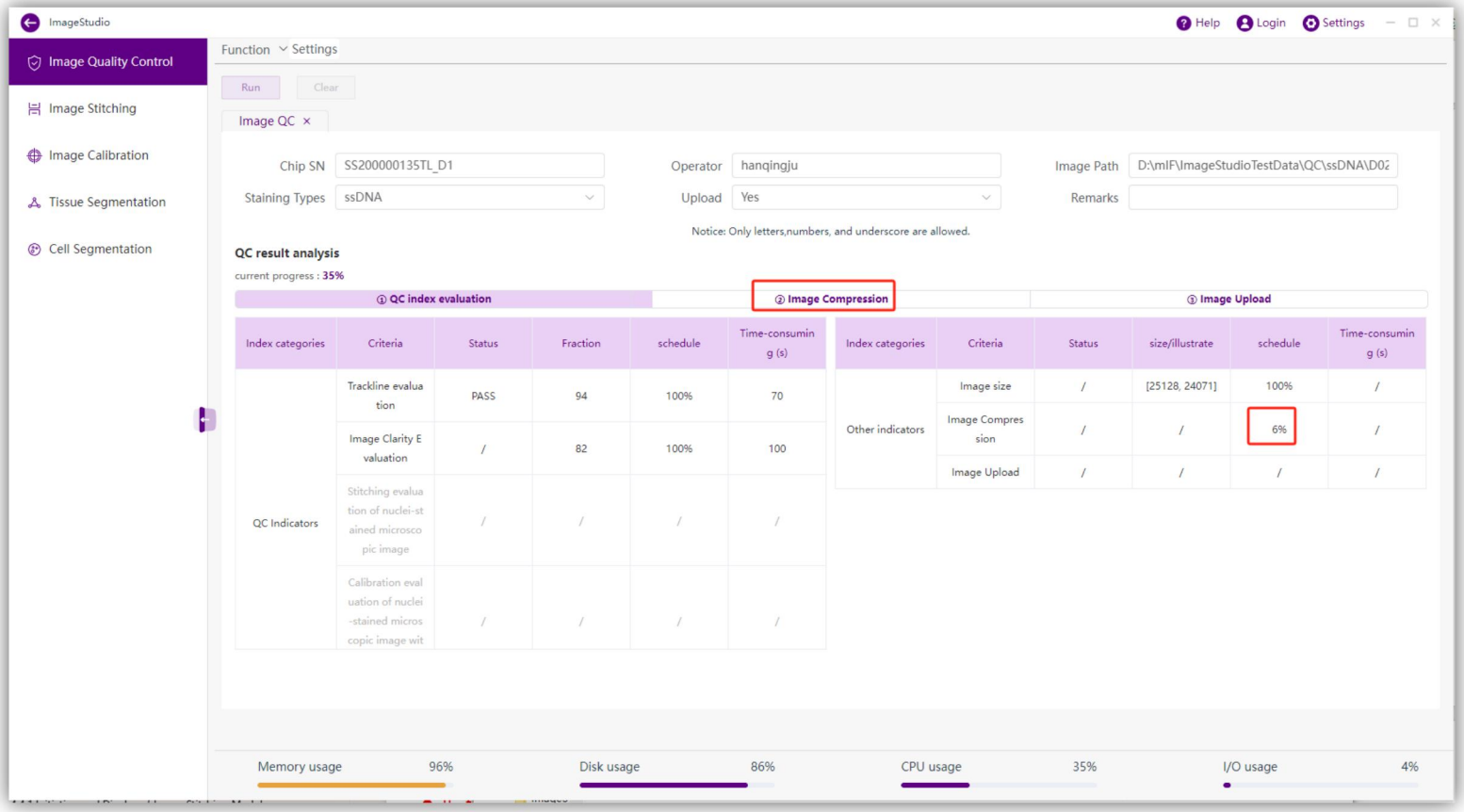
- **FOV width and FOV height**: Width and height of each tiled FOV image or individual FOV (in pixels) .
- **Overlap:** Overlap ratio between two adjacent FOVs (if the overlap ratios at the width and height are different, take the larger one). For example, if the overlap is 10%, enter 0.1.

(7) Click the ‘ Run’ button to start QC. The program will sequentially perform indicator evaluation, image compression, and image uploading (optional). The progress bar will display the current location, and the table will display the progress percentage and time consumed for each step. Please wait patiently for completion.

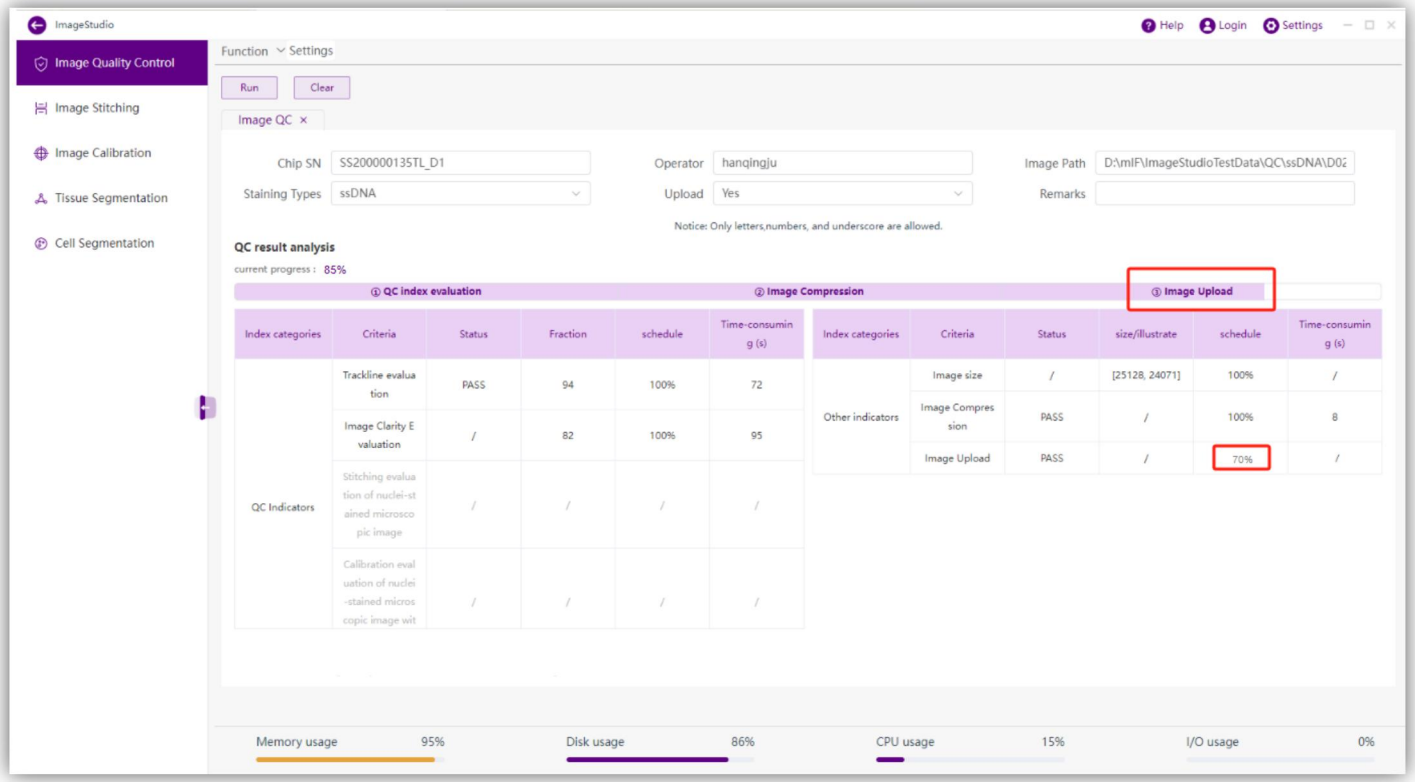
- QC indicator evaluation: display the score of each indicator, pass or fail status, progress percentage, and time consumed. The unapplicable indicators are colored gray.



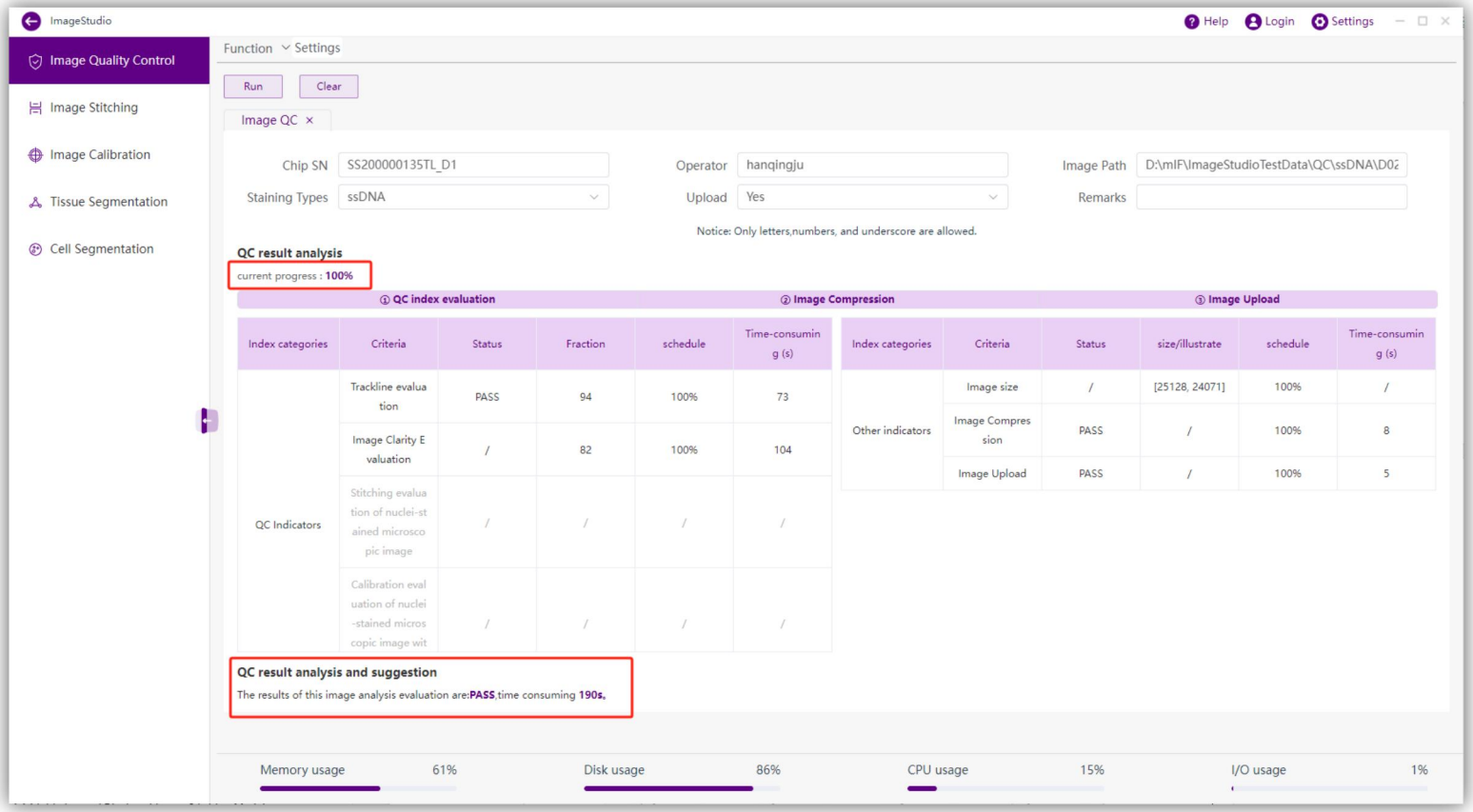
- Image Compression



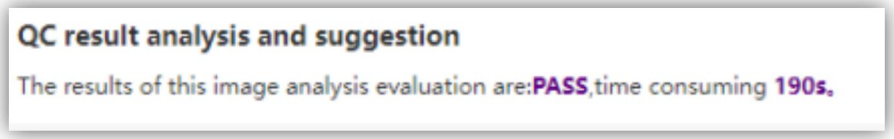
- Image Upload



(8) After QC has been done, the result will be displayed at the bottom. The displayed information includes QC pass or fail, total time consumed, and the recommendations for automation analysis.



- If the QC result is PASS, it indicates that the image can be analyzed by the automation image processing pipeline in SAW.

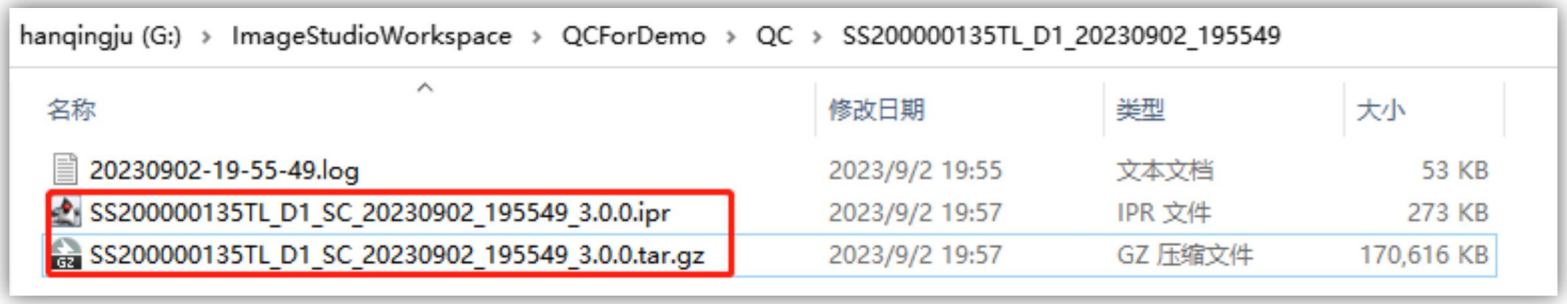


- If the QC result is FAIL, it indicates that the image can not be further analyzed by the automated

image processing pipeline in SAW. The result will also provide suggestions to scan again or perform manual analysis on the input image.

Note: Please make sure all the images were taken following the ***Imaging Guidelines***. If the image QC fails, please confirm the image quality, chip SN, or re-take the image. Contact a STOmics FAS for assistance if necessary.

(9) Once the image passes the evaluation, the program will compress the files to tar.gz and record image evaluation and essential information into .ipr file.



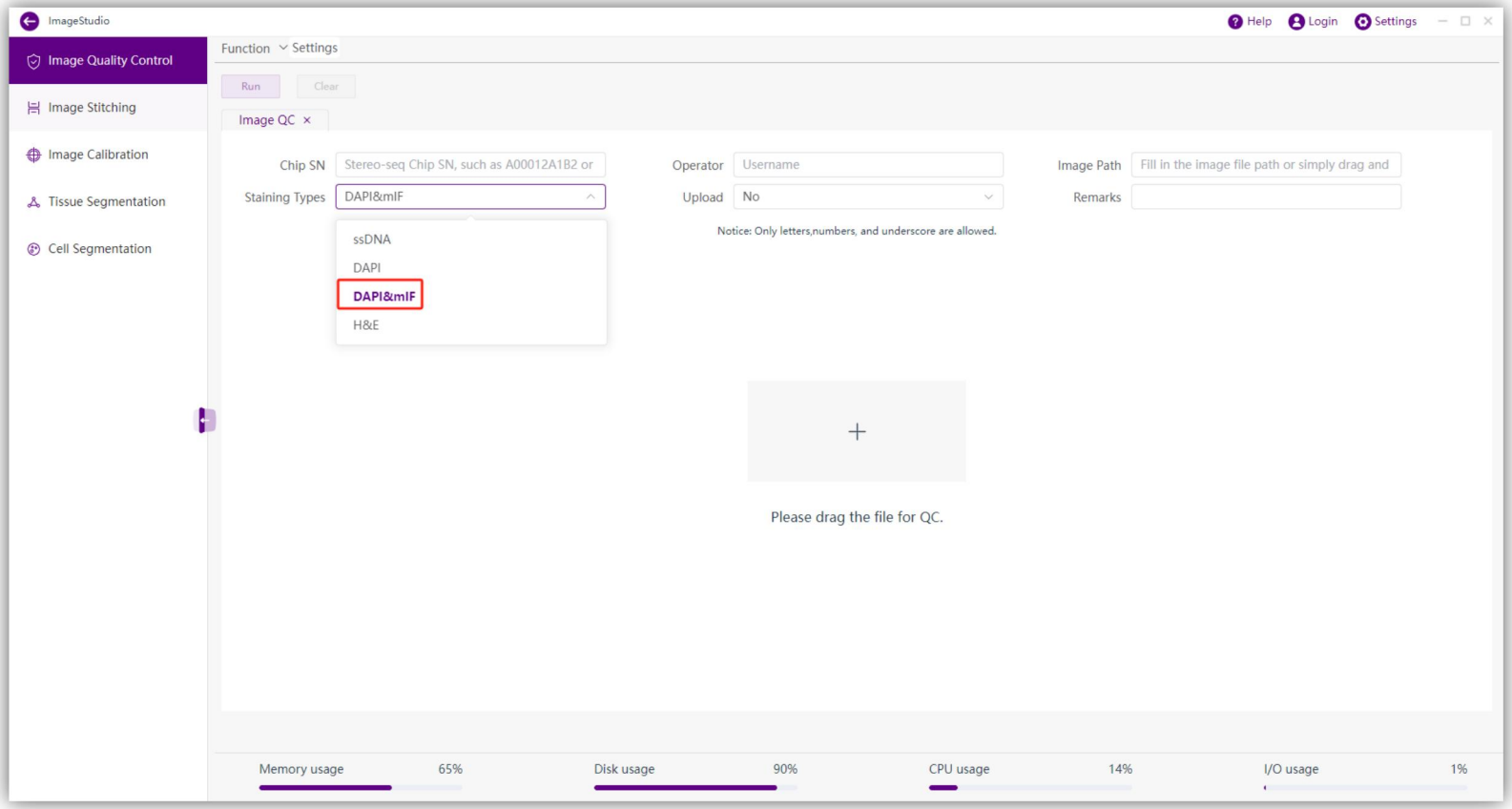
(10) If the auto-uploading function is turned on, the output files (TAR.GZ and IPR) will be automatically uploaded to the selected computer cluster or cloud. Click the "Clear" button to clear

the contents, and then drag another image for a new run.

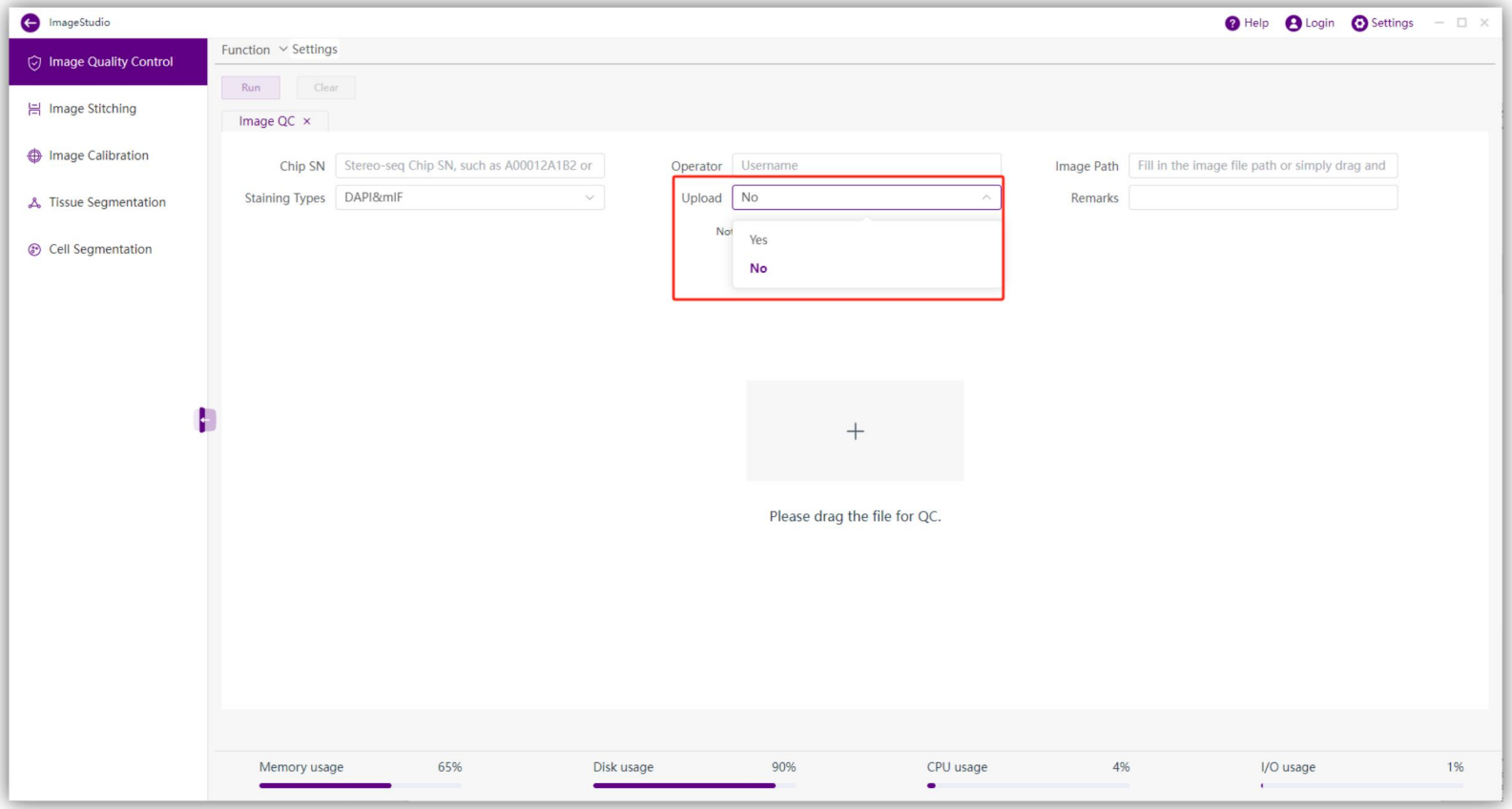
(11) Go to *D:\ImageStudioWorkspace\QC* to find the output files.

4.2.1.2 DAPI&mIF

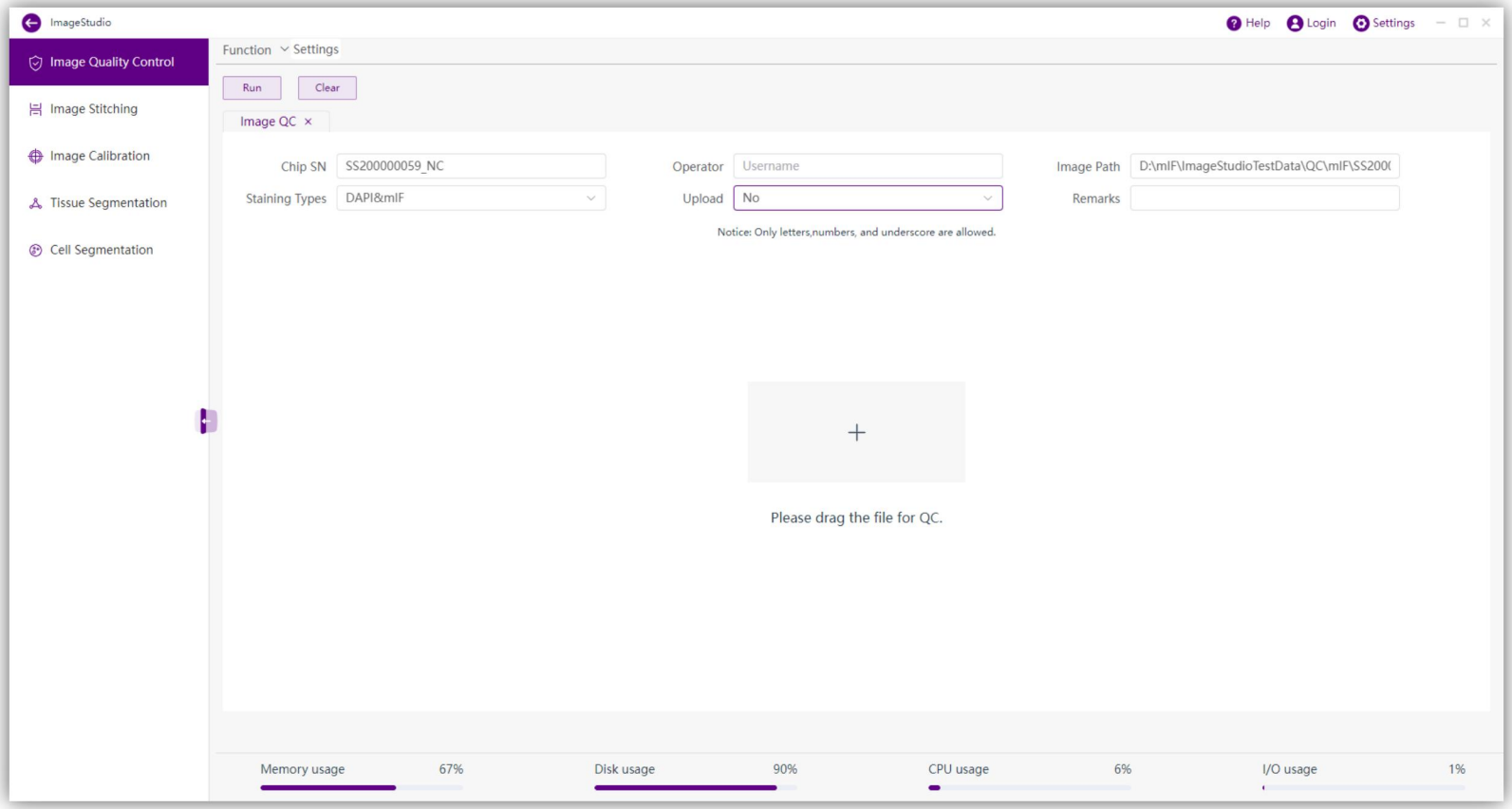
(1)Select the staining type "DAPI & mIF"



(2) Select "Yes" or "No" for "Upload" to upload the image (after QC) to the selected computer cluster or cloud or not.



(3) Drag and drop image files or folders to the program.



(4) Description of microscopes and data formats that are supported by ImageStudio QC module.

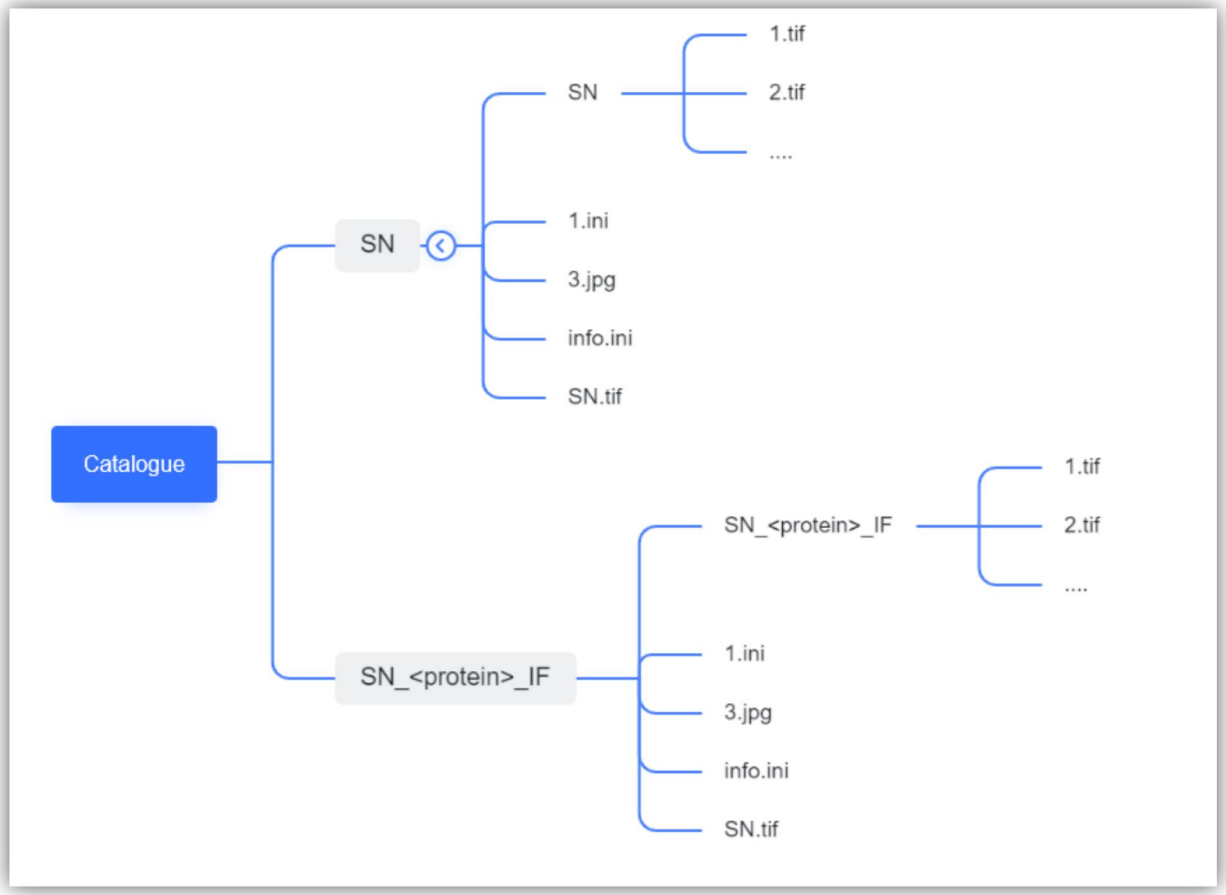
Output Directory: *D:\ImageStudioWorkspace\QC*

Microscope	Input file	Output files that are supported by SAW
Zeiss Axio Scan Z1	Raw image file in CZI format	• IPR file that stores image

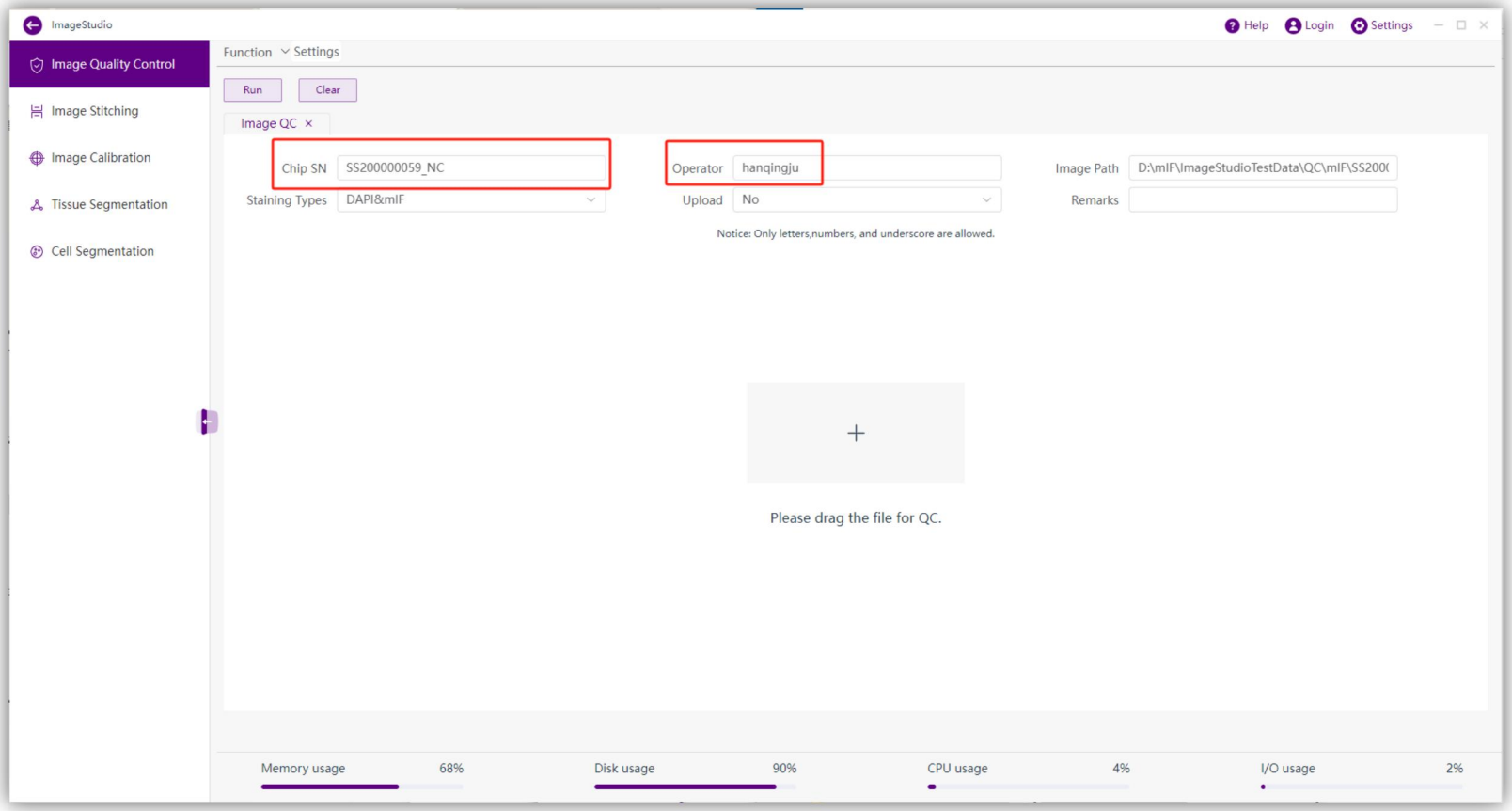
Zeiss Axio Scan 7		capturing information and Image QC information • Zipped image files in TAR.GZ format
Leica DM6-M	Stitched image in TIFF format	
Motic for BGI custom	Raw image file in mdsx format	
Other microscopes (before API development):	Stitched image in TIFF format	
Other microscopes (after API development):	Tile images (individual FOV) or raw image file	
/	The output compressed file(TAR.GZ) after Image QC	

- Input file specification

Type	User naming convention	Example
DAPI	Stereo-seq Chip SN	SS200000655_LB
IF	Stereo-seq Chip SN _Protin_IF	SS200000655_LB_CD31_IF



(5) Fill in the Stereo-seq Chip Serial Number (SN) and the operator’ s email address.



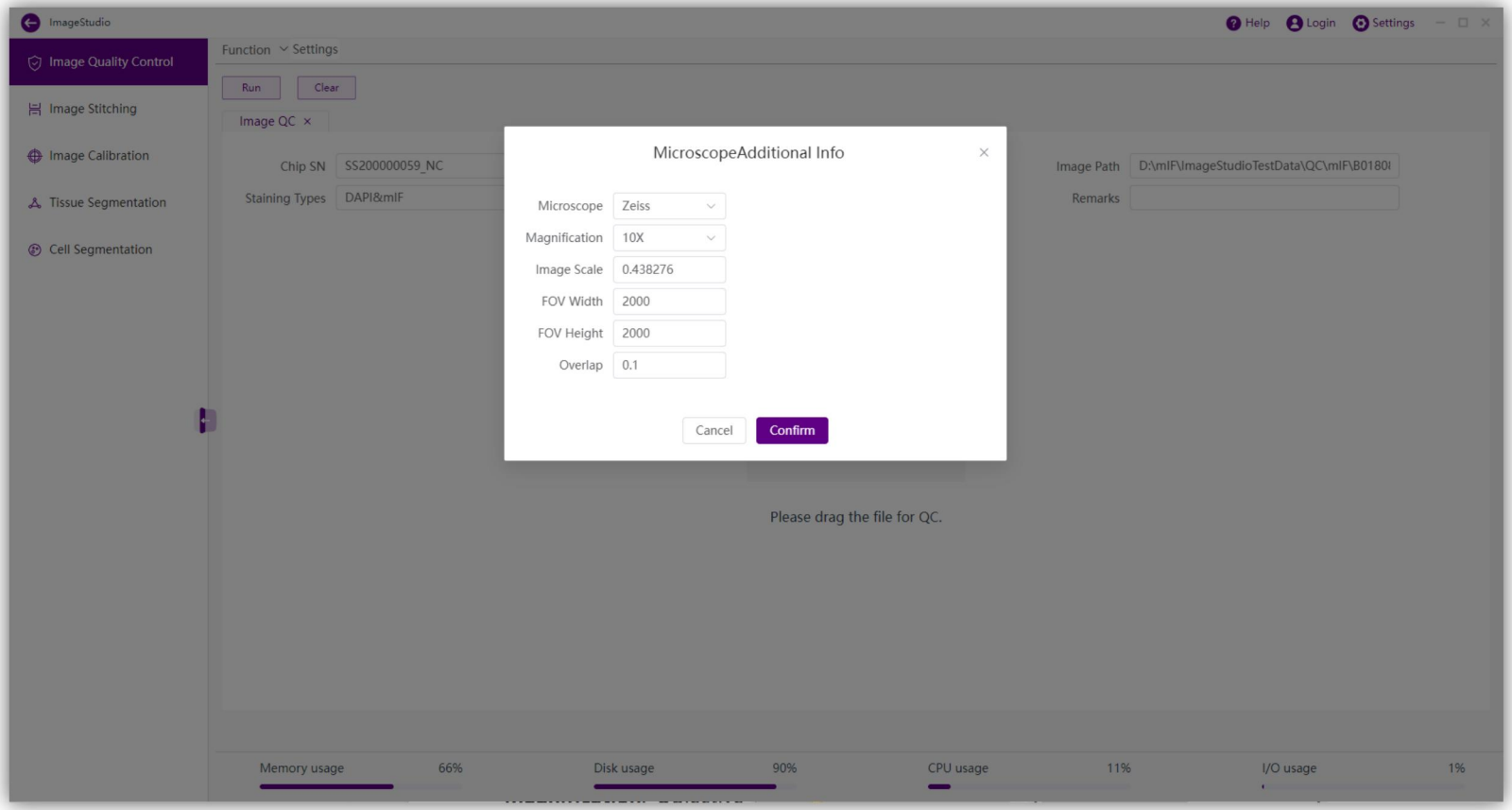
- **Chip SN**: Chip serial number of a Stereo-seq Microscope Assessment Chip T or a Stereo-seq Chip T that is used for imaging. ImageStudio software can only recognize the standard Stereo-seq Chip SN from BGI STOmics.
- **Operator:** Full email address of the operator, or mailbox prefix of a BGI employee.

Note: The Stereo-seq Chip SN and the operator email will be recorded and filled automatically on the next

entry. For a new user or a warning raised, please re-type the correct email address and try again.

- **Image Path:** File path for the stitched panoramic image or the file directory path for the tiled FOV images. It can be manually typed in or automatically filled in by the program after dragging and dropping the file/folder into the program.
- **Staining Types:** Options are ssDNA, DAPI, and DAPI & mIF.
- **Remark:** Record additional details in the remark field if needed.

(6) For the stitched panoramic image, users will be requested to fill in the image parameters manually in the pop-up window as shown below. Click 'Confirm' to finish the entry, and click 'Run' to start QC.



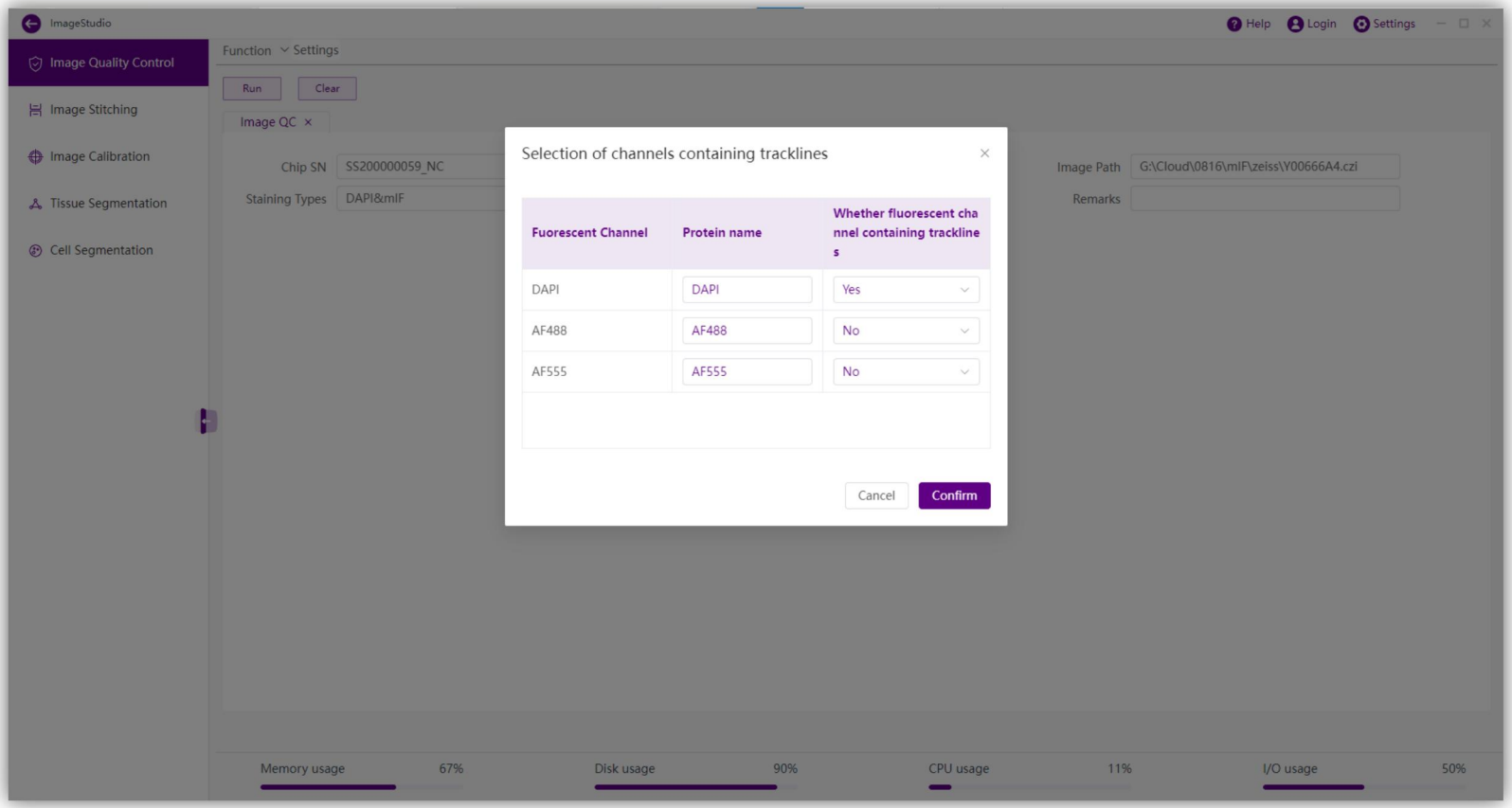
These parameters are not required if exported tile images in TIFF format are used.

- **Microscope**: Manufacturer of the microscope, such as Zeiss, Leica, Motic, Olympus, etc.
- **Magnification**: Objective lens magnification. For example, if the image was taken by a 10x objective lens, enter 10.
- **Image ratioScale**: Image Scale can also be defined as 1/resolution in the unit of μm/pixel, with

at least 3 decimal places.

- **FOV width and FOV height**: Width and height of each tiled FOV image or individual FOV (in pixels)
- **Overlap**: Overlap ratio between two adjacent FOVs (if the overlap ratios at the width and height are different, take the larger one). For example, if the overlap is 10%, enter 0.1.

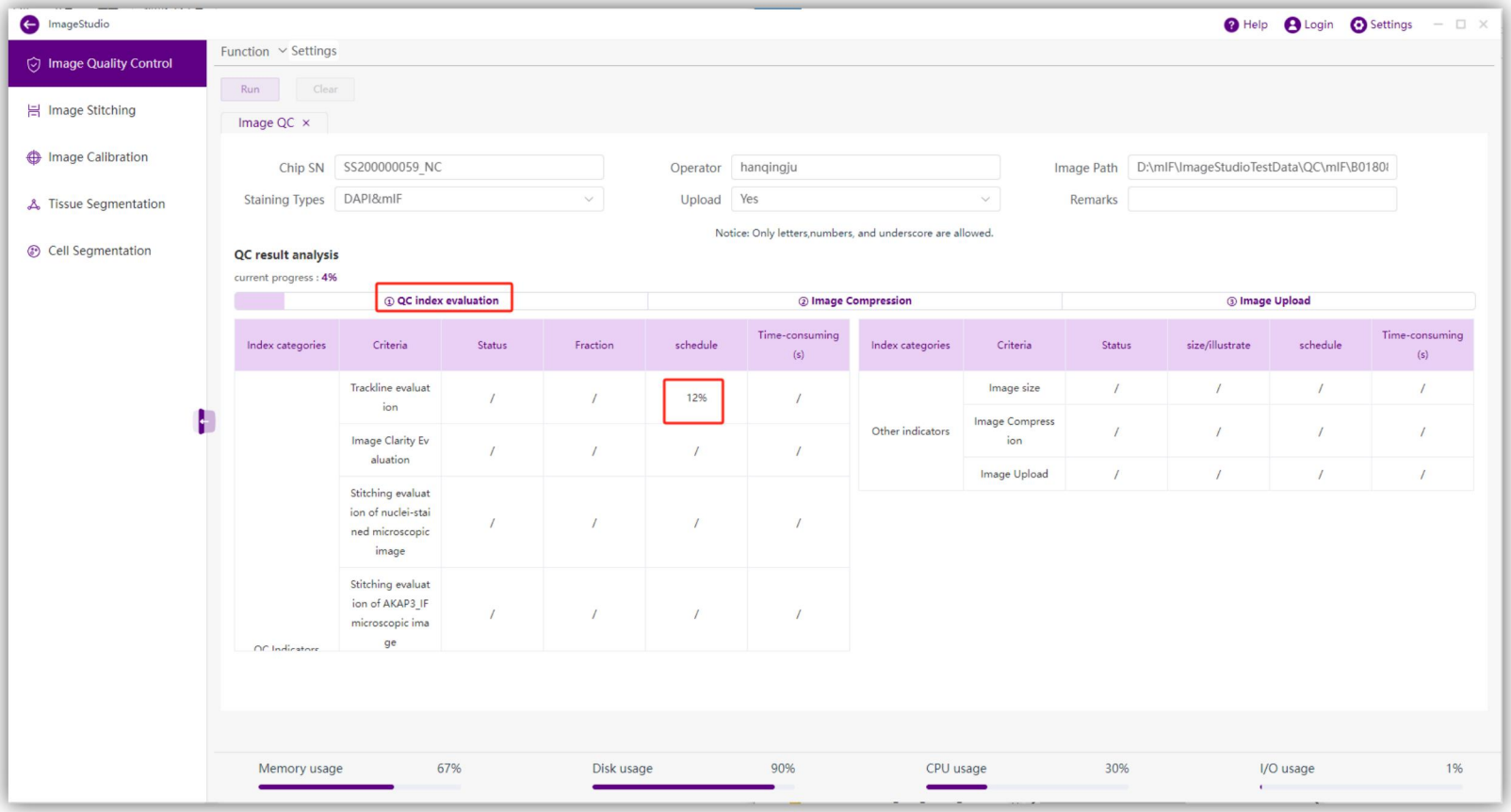
(7) User will be requested to confirm the protein name and trackline channel in the screenshot below and click 'Run' when the multi-channel image is uploaded. By default, the channel name will be used as the protein name.



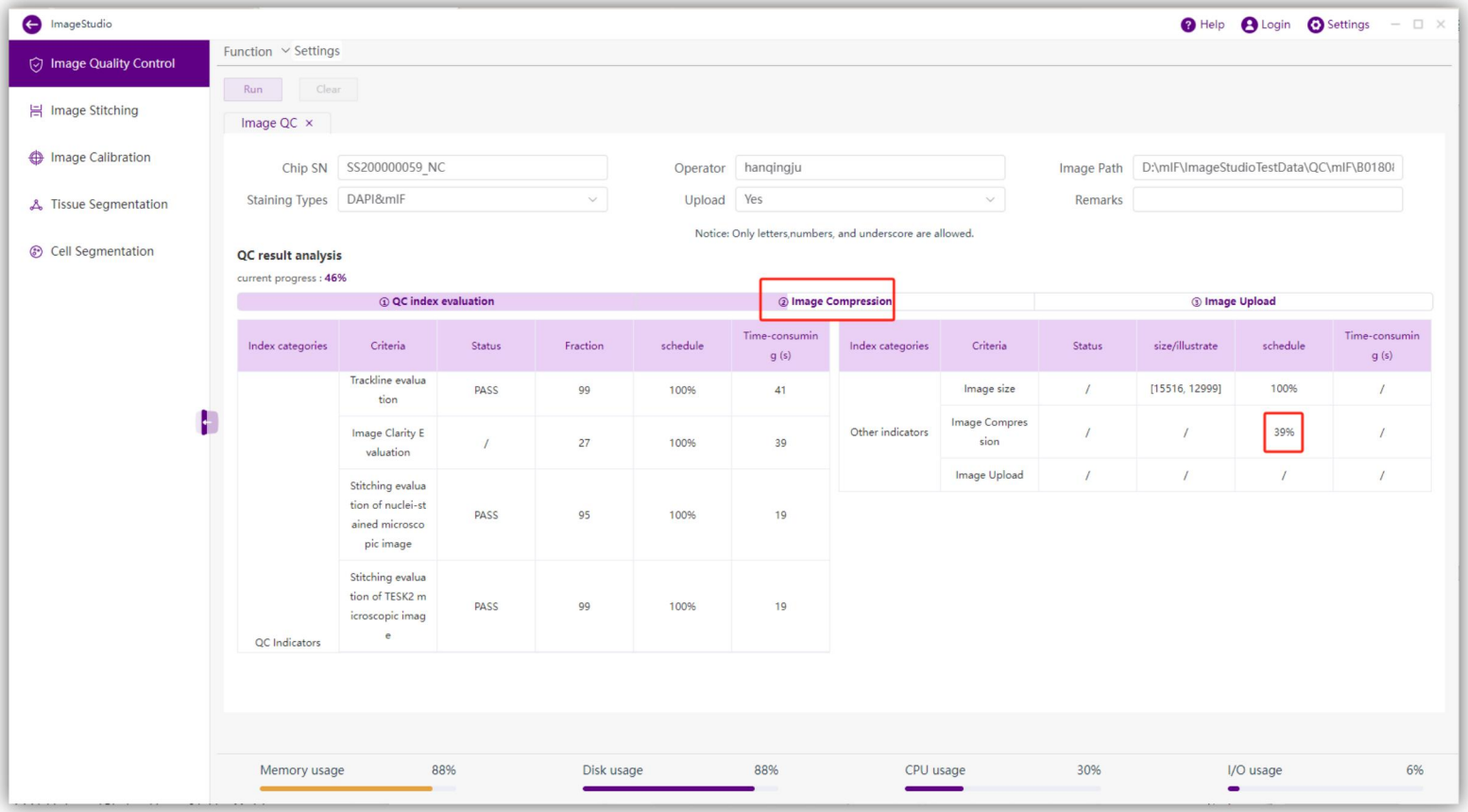
(8) Click the 'Run' button to start QC. The program will sequentially perform indicator evaluation, image compression, and image uploading (optional). The progress bar will display the current location, and the table will display the progress percentage and time consumed for each step. Please wait patiently for completion.

- QC indicator evaluation: display the score of each indicator, pass or fail status, progress percentage,

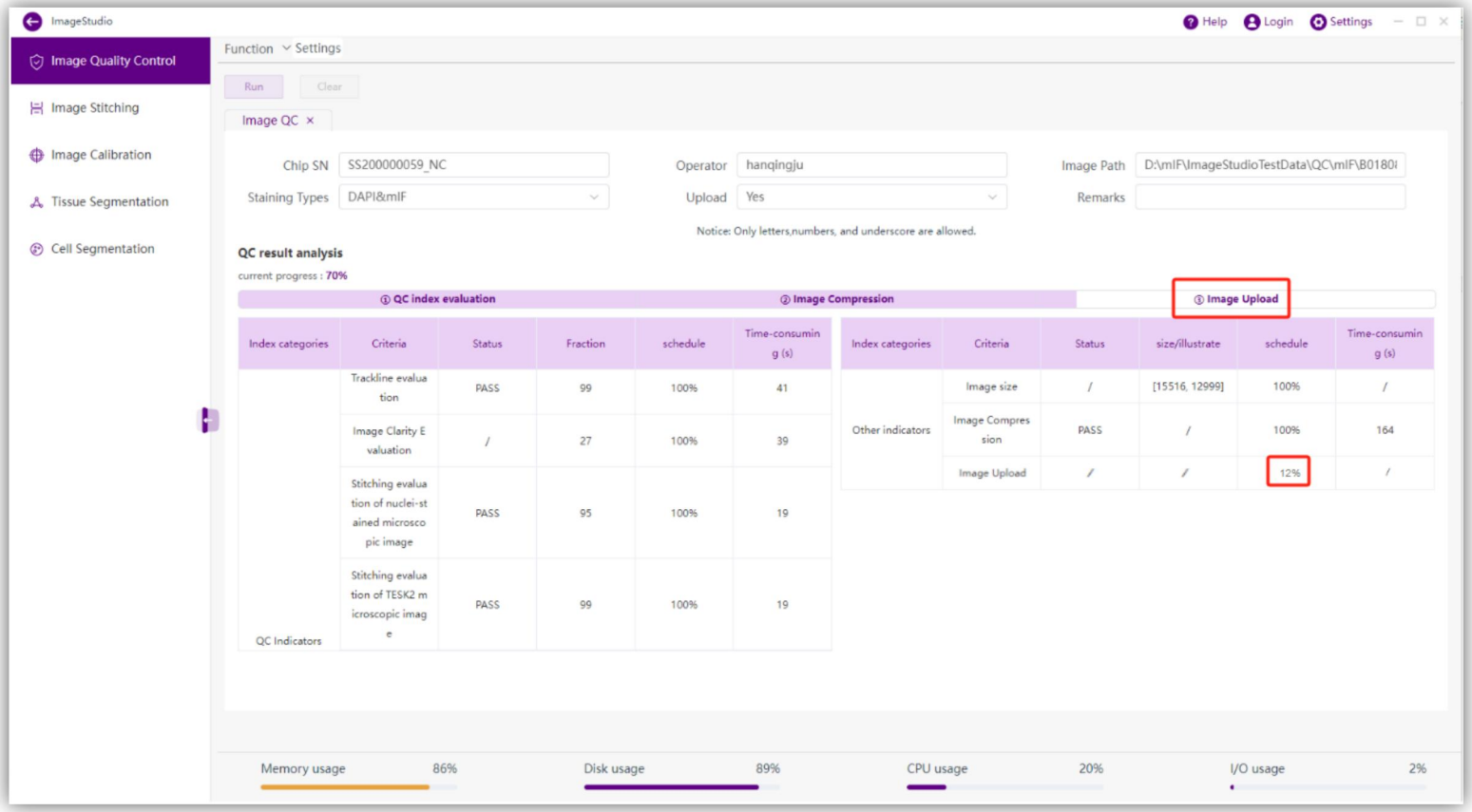
and time consumed. The unapplicable indicators are colored gray. The QC indicators include trackline detection evaluation, stitching evaluation, and DAPI vs mIF calibration evaluation.



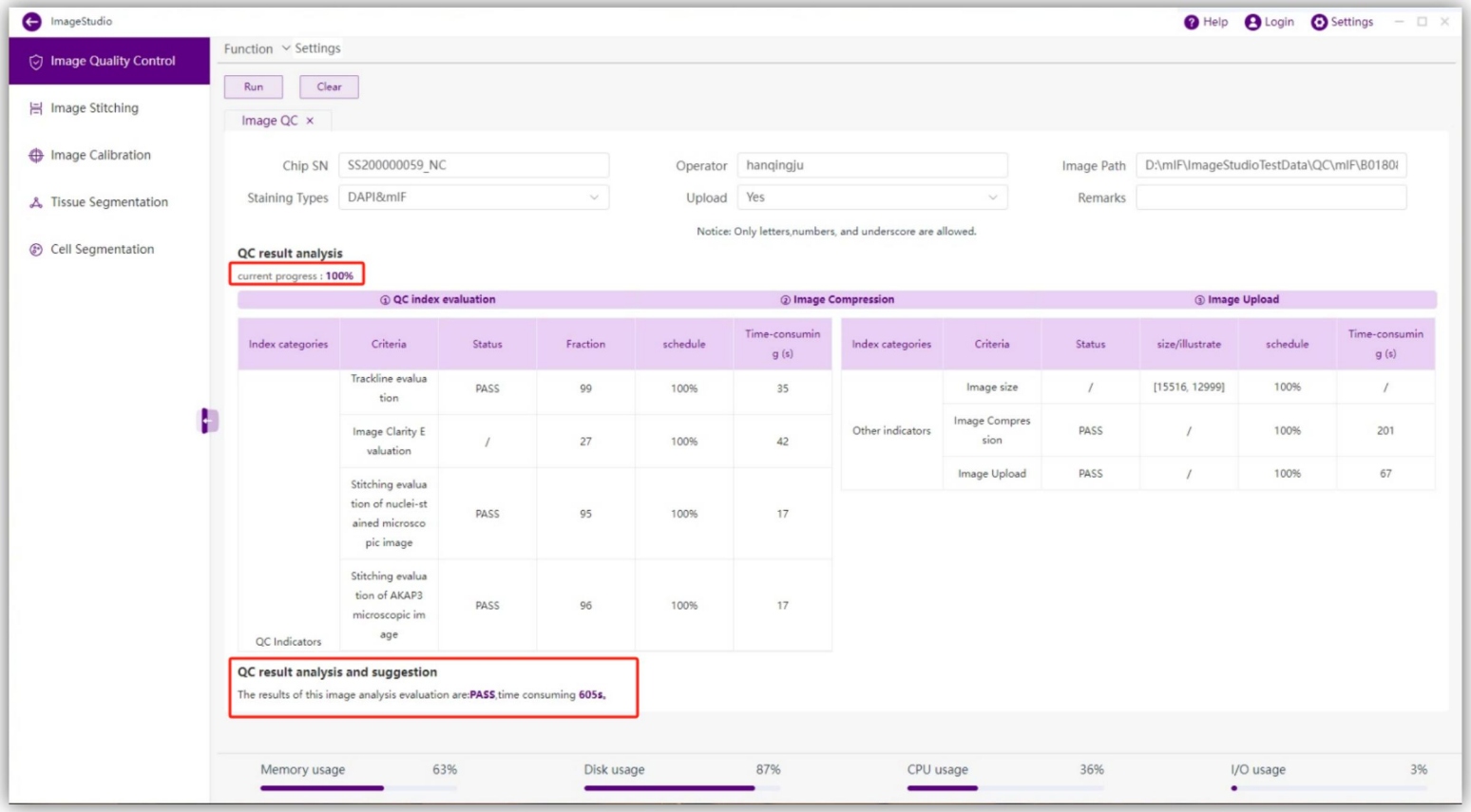
- Image Compression



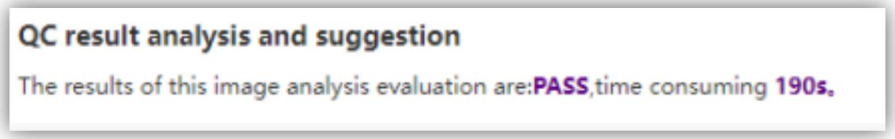
- Image Upload



(9) After QC has been done, the result will be displayed at the bottom. The displayed information includes QC pass or fail, total time consumed, and the recommendations for automation analysis.



- If the QC result is PASS, it indicates that the image can be analyzed by the automation image processing pipeline in SAW.

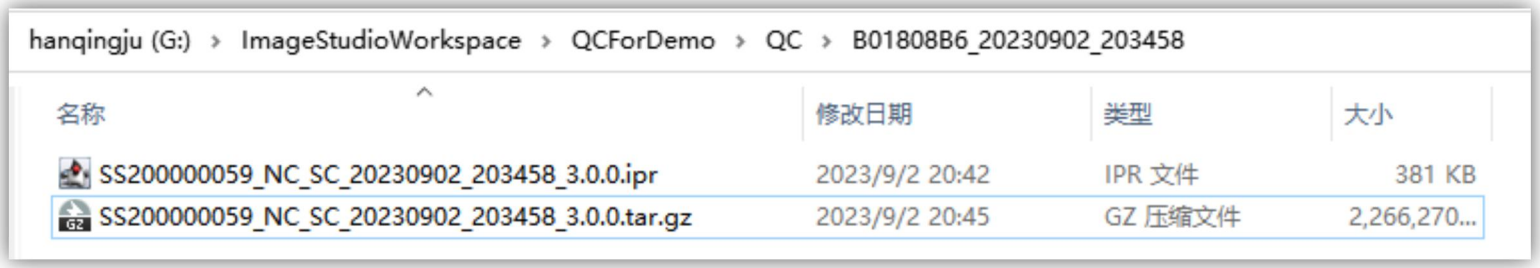


- If the QC result is FAIL, it indicates that the image can not be further analyzed by the automated

image processing pipeline in SAW. The result will also provide suggestions to scan again or perform manual analysis on the input image.

Note: Please make sure all the images were taken following the ***Imaging Guidelines***. If the image QC fails, please confirm the image quality, chip SN, or re-take the image. Contact a STOmics FAS for assistance if necessary.

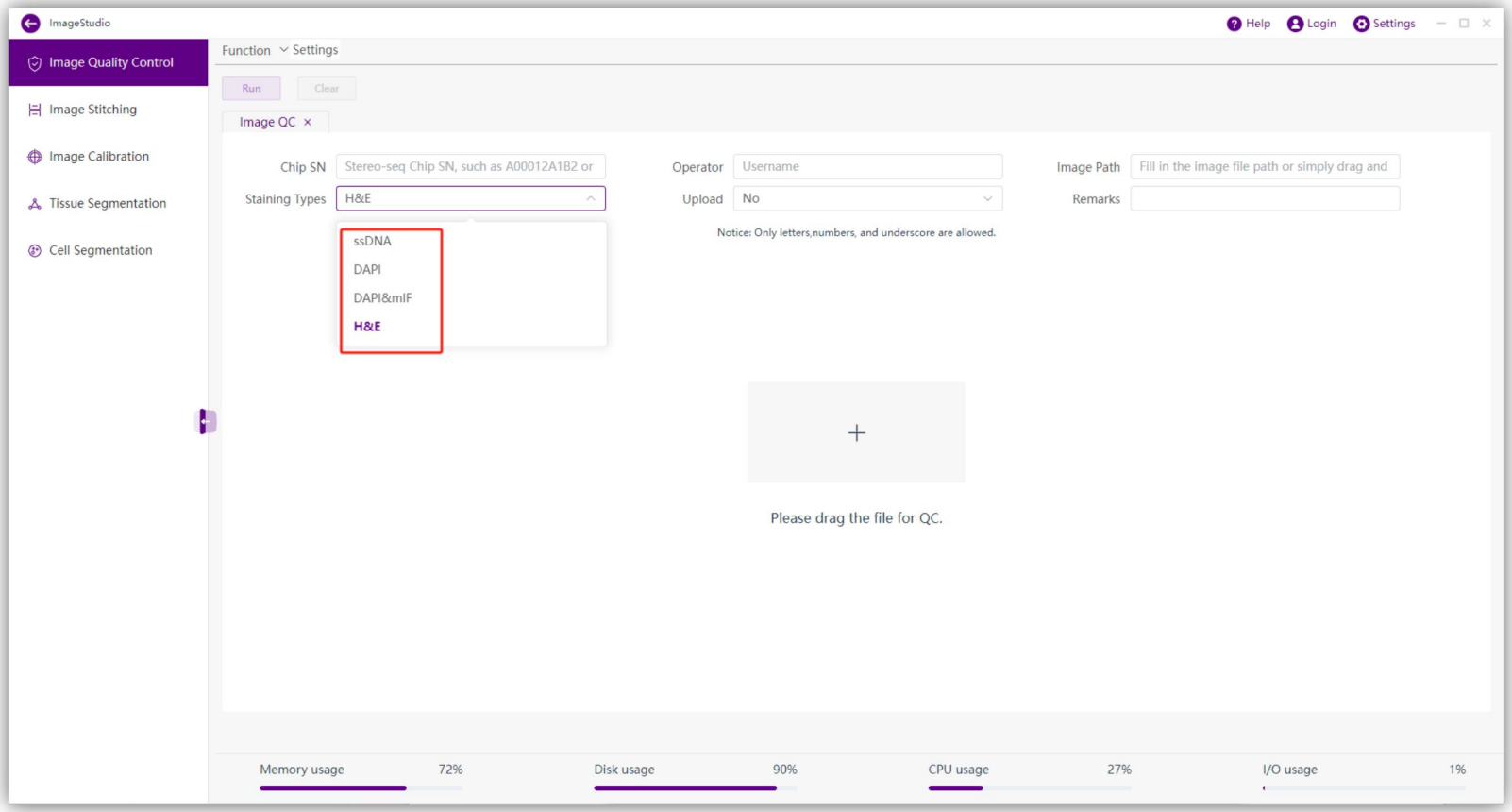
(10) Once the image passes the evaluation, the program will compress the files to tar.gz and record image evaluation and essential information into .ipr file.



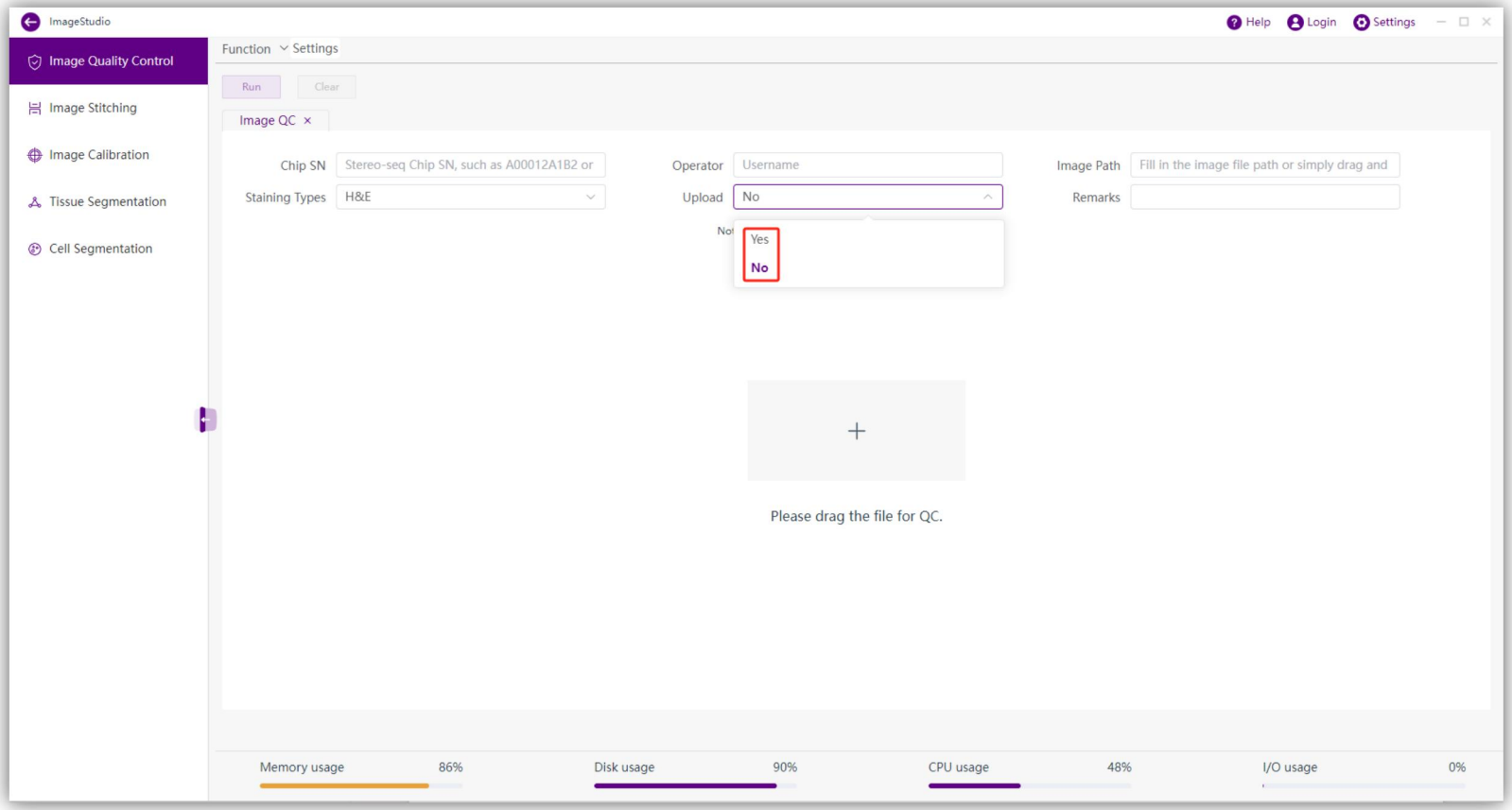
(11) If the auto-uploading function is turned on, the output files (TAR.GZ and IPR) will be automatically uploaded to the selected computer cluster or cloud. Click the "Clear" button to clear the contents, and then drag another image for a new run.

4.2.1.3 H&E

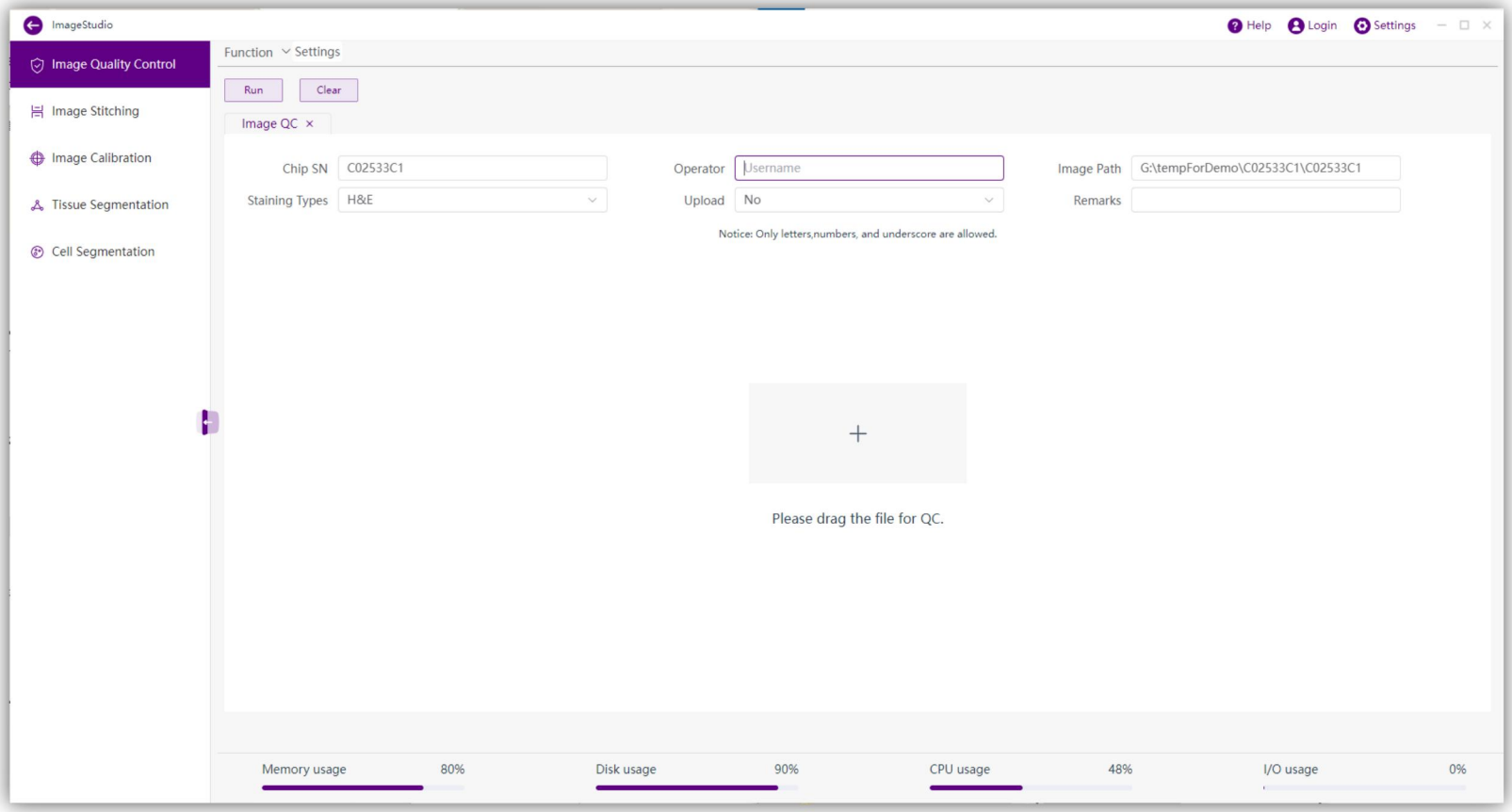
(1) Select the staining type "H&E “



(2) Select "Yes" or "No" for "Upload" to upload the image (after QC) to the selected computer cluster or cloud or not.



(3) Drag and drop image files or folders to the program.



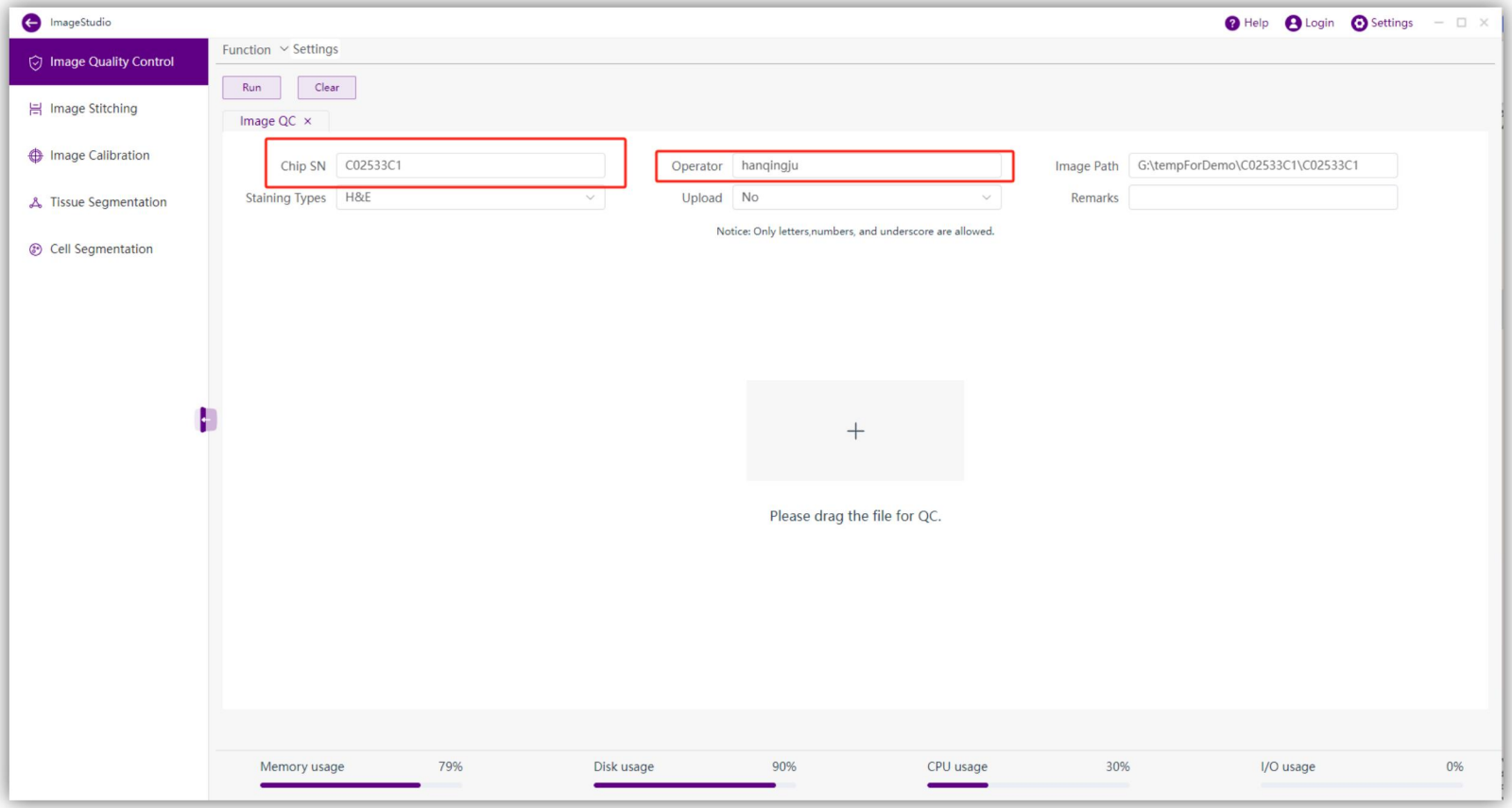
(4) Description of microscopes and data formats that are supported by ImageStudio QC module.

Output Directory: *D:\ImageStudioWorkspace\QC*

Microscope	Input file	Output files that are supported by SAW
Zeiss Axio Scan Z1	Raw image file in CZI	• IPR file that stores image

Zeiss Axio Scan 7	format	capturing information and Image QC information • Zipped image files in TAR.GZ format
Leica DM6-M	Stitched image in TIFF format	
Motic for BGI custom	Raw image file in mdsx format	
Go Optical	Tile images (individual FOV)	
Other microscopes (before API development):	Stitched image in TIFF format	
Other microscopes (after API development):	Tile images (individual FOV) or raw image file	
/	The output compressed file(TAR.GZ) of after	

(5) Fill in the Stereo-seq Chip Serial Number (SN) and the operator’ s email address.



- **Chip SN**: Chip serial number of a Stereo-seq Microscope Assessment Chip T or a Stereo-seq Chip T that is used for imaging. ImageStudio software can only recognize the standard

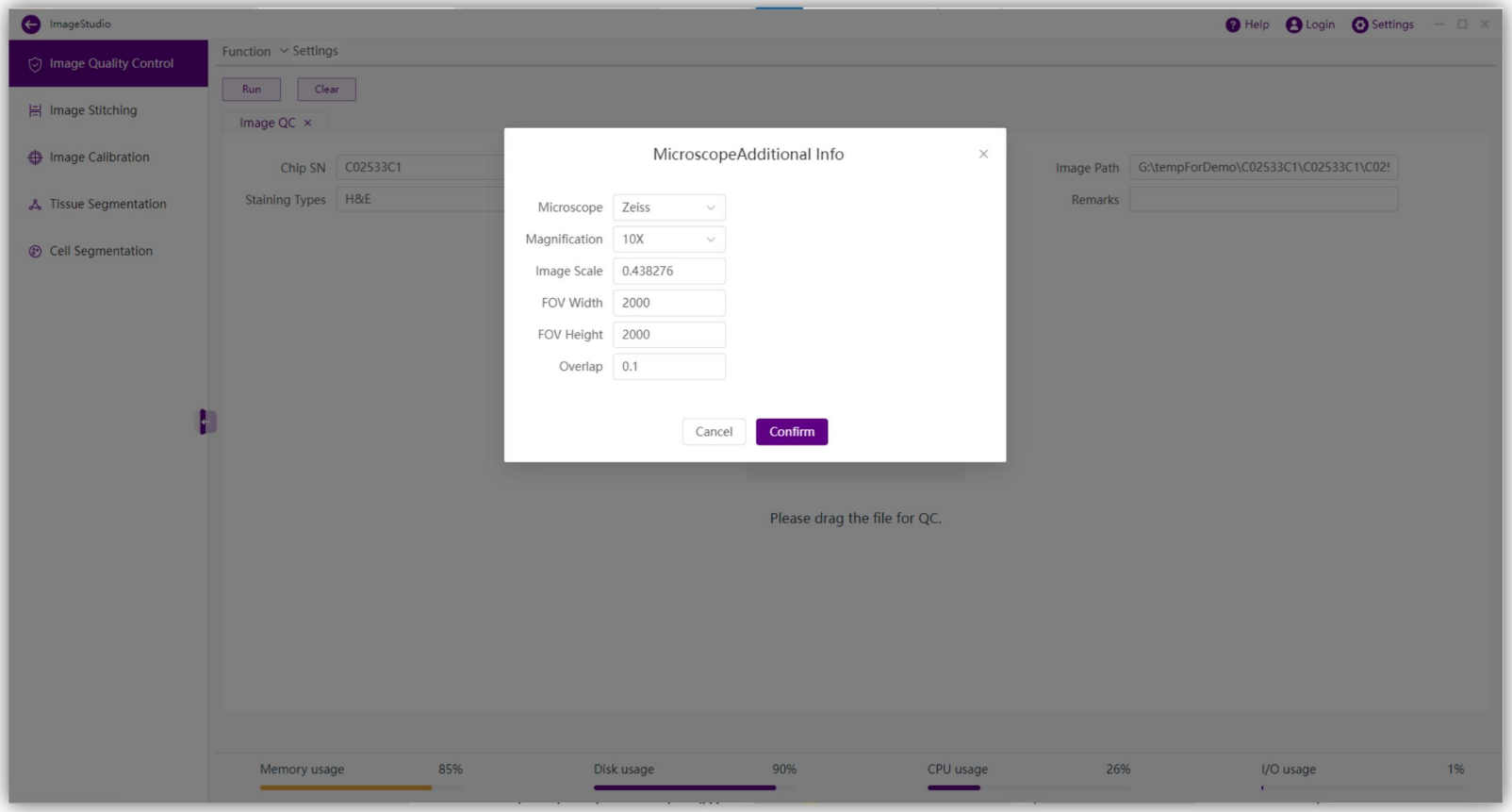
Stereo-seq Chip SN from BGI STOmics.

- **Operator:** Full email address of the operator, or mailbox prefix of a BGI employee.

Note: The Stereo-seq Chip SN and the operator email will be recorded and filled automatically on the next entry. For a new user or a warning raised, please re-type the correct email address and try again.

- **Image Path:** File path for the stitched panoramic image or the file directory path for the tiled FOV images. It can be manually typed in or automatically filled in by the program after dragging and dropping the file/folder into the program.
- **Staining Types:** Options are ssDNA, DAPI, and DAPI & mIF.
- **Remark:** Record additional details in the remark field if needed.

(6) For the stitched panoramic image, users will be requested to fill in the image parameters manually in the pop-up window as shown below. Click 'Confirm' to finish the entry, and click 'Run' to start QC.



⚠These parameters are not required for tiled FOV images in TIFF/ PNG format.

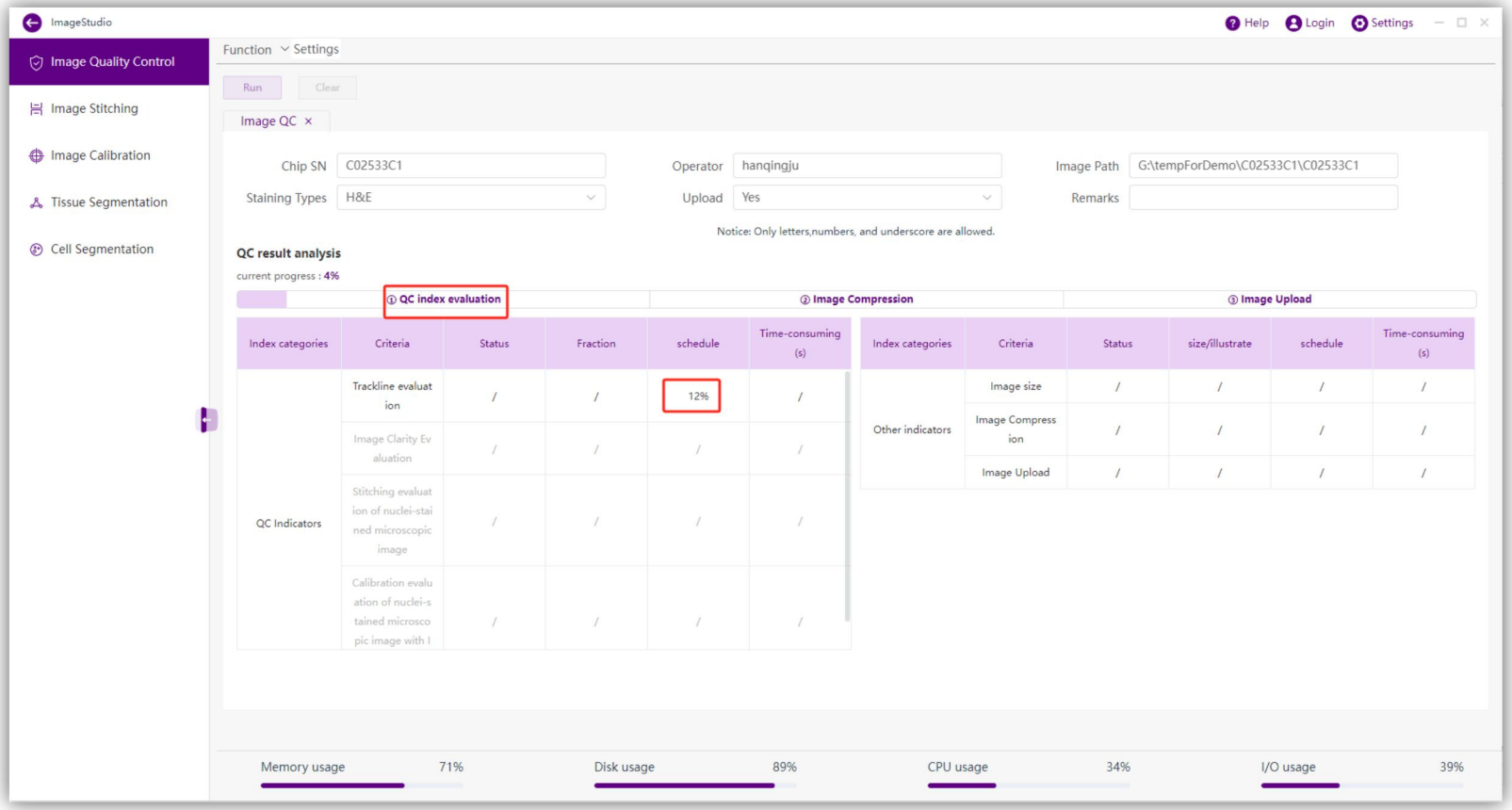
- **Microscope**: Manufacturer of the microscope, such as Zeiss, Leica, Motic, Olympus, etc.
- **Magnification**: Objective lens magnification. For example, if the image was taken by a 10x objective lens, enter 10.
- **Image ratioScale**: Image Scale can also be defined as 1/resolution in the unit of μm/pixel, with

at least 3 decimal places.

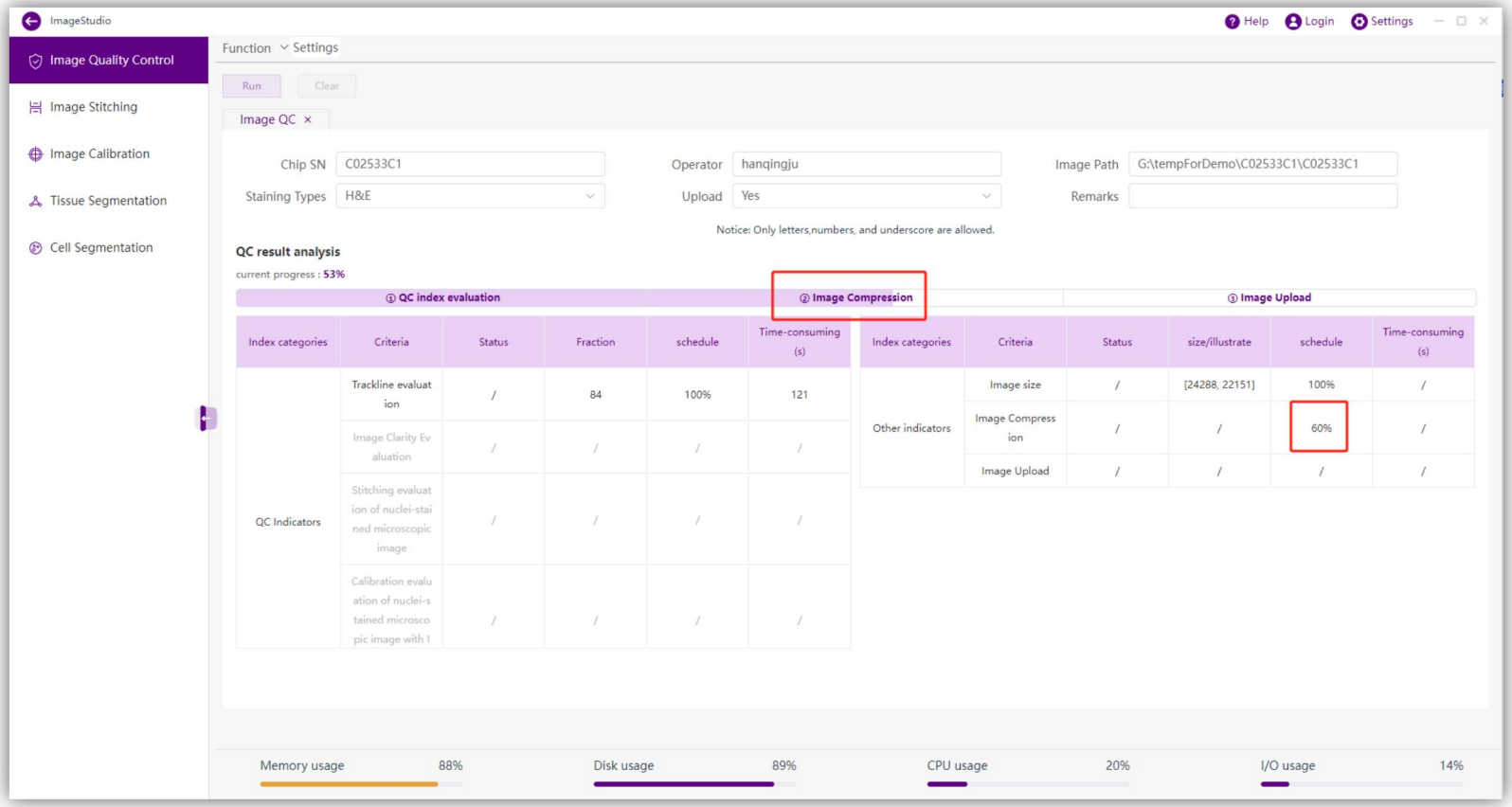
- **FOV width and FOV height**: Width and height of each tiled FOV image or individual FOV (in pixels)
- **Overlap**: Overlap ratio between two adjacent FOVs (if the overlap ratios at the width and height are different, take the larger one). For example, if the overlap is 10%, enter 0.1.

(7) Click the ‘ Run’ button to start QC. The program will sequentially perform indicator evaluation, image compression, and image uploading (optional). The progress bar will display the current location, and the table will display the progress percentage and time consumed for each step. Please wait patiently for completion.

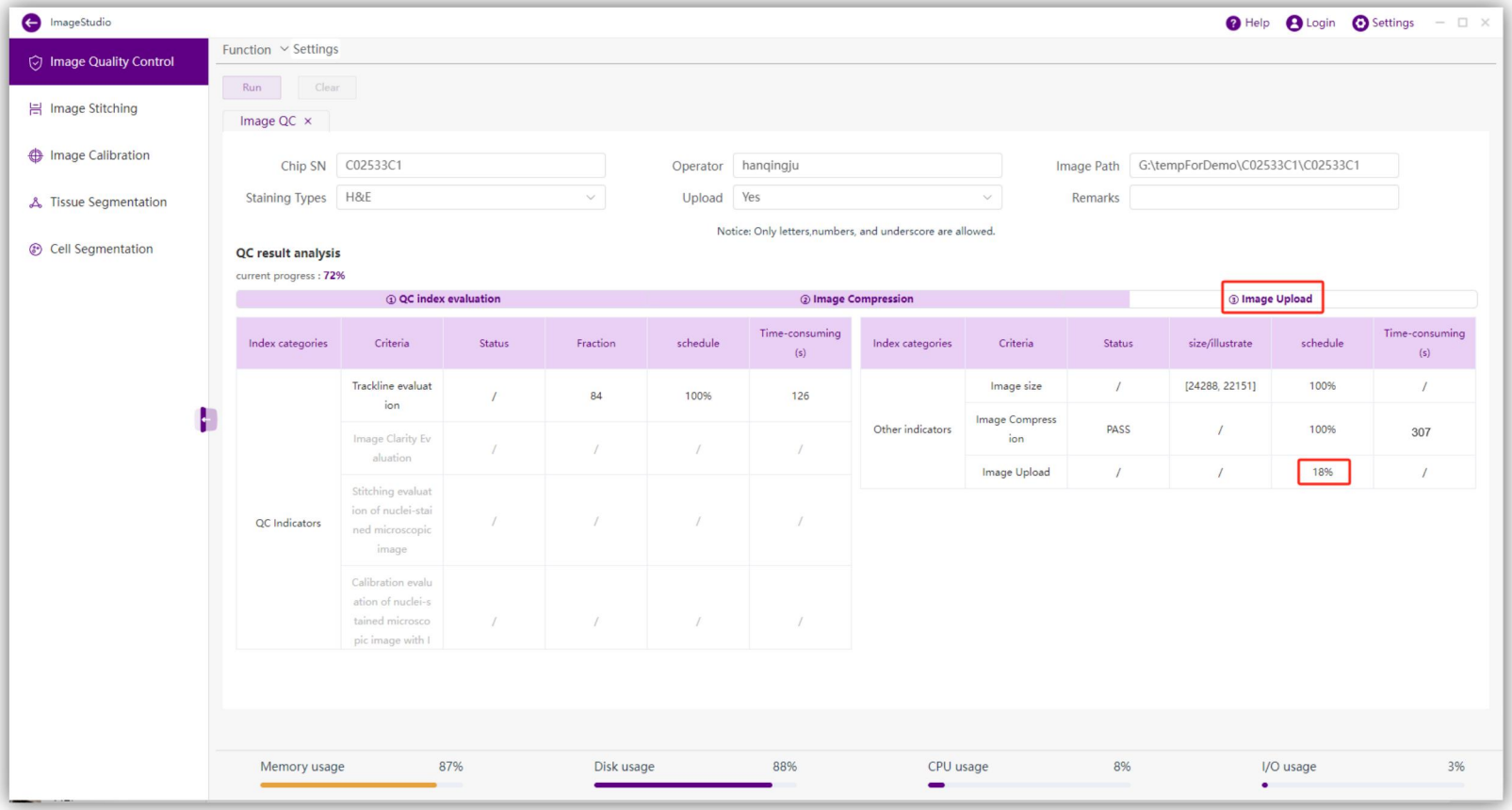
- QC indicator evaluation: display the score of each indicator, pass or fail status, progress percentage, and time consumed. The unapplicable indicators are colored gray.



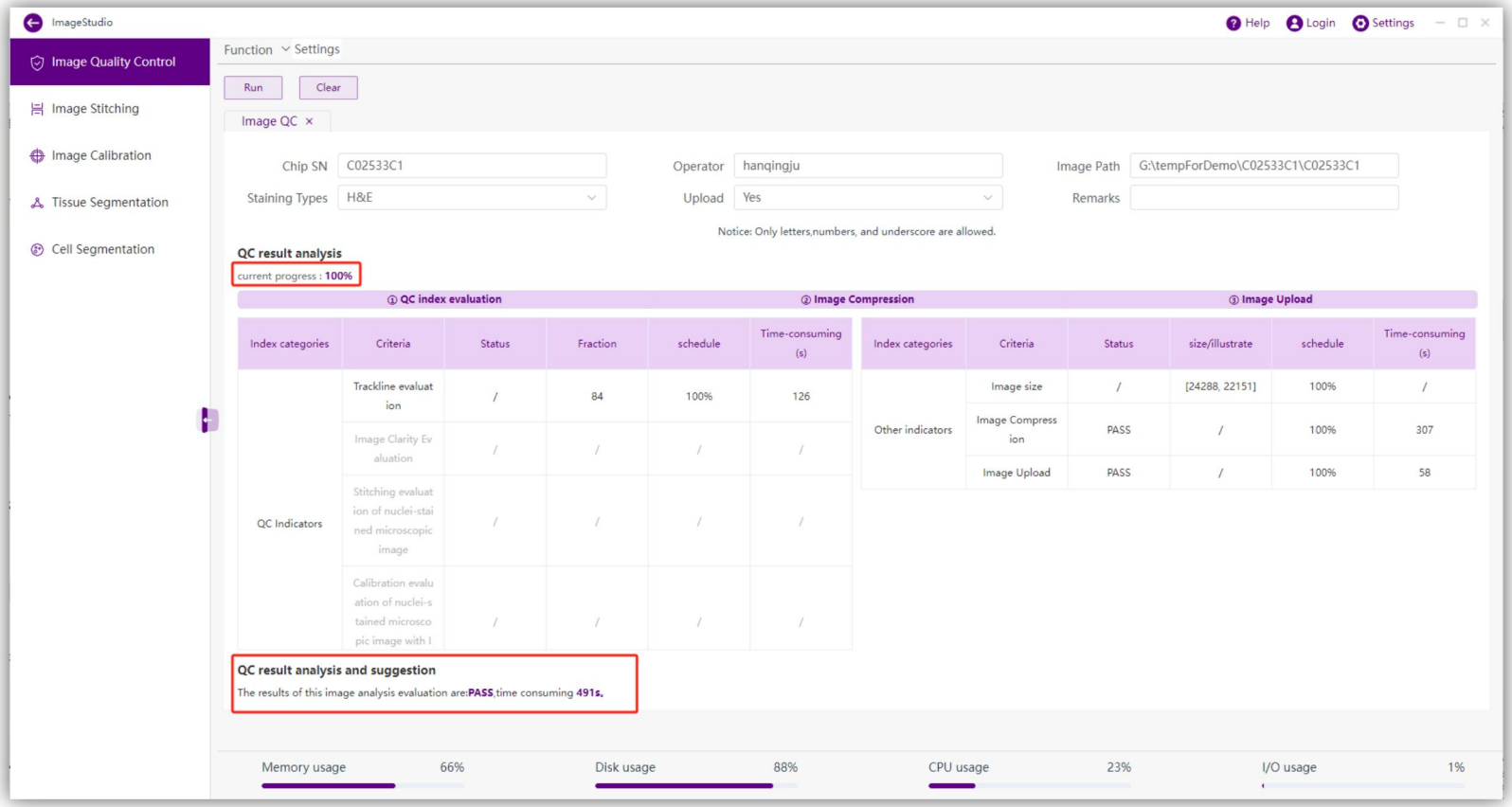
- Image Compression



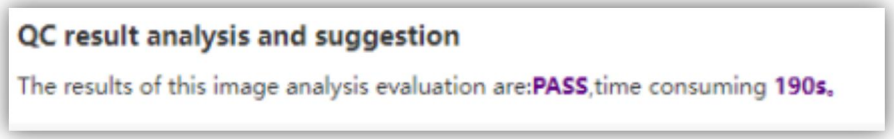
- Image Upload



(8) After QC has been done, the result will be displayed at the bottom. The displayed information includes QC pass or fail, total time consumed, and the recommendations for automation analysis.



- If the QC result is PASS, it indicates that the image can be analyzed by the automation image processing pipeline in SAW.

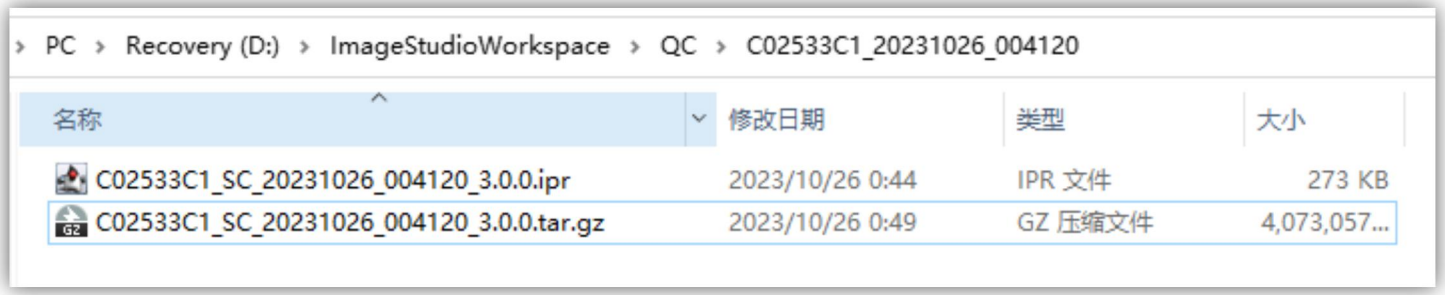


- If the QC result is FAIL, it indicates that the image can not be further analyzed by the automated

image processing pipeline in SAW. The result will also provide suggestions to scan again or perform manual analysis on the input image.

Note: Please make sure all the images were taken following the ***Imaging Guidelines***. If the image QC fails, please confirm the image quality, chip SN, or re-take the image. Contact a STOmics FAS for assistance if necessary.

(9) Once the image passes the evaluation, the program will compress the files to TAR.GZ and record image evaluation and essential information into IPR file.

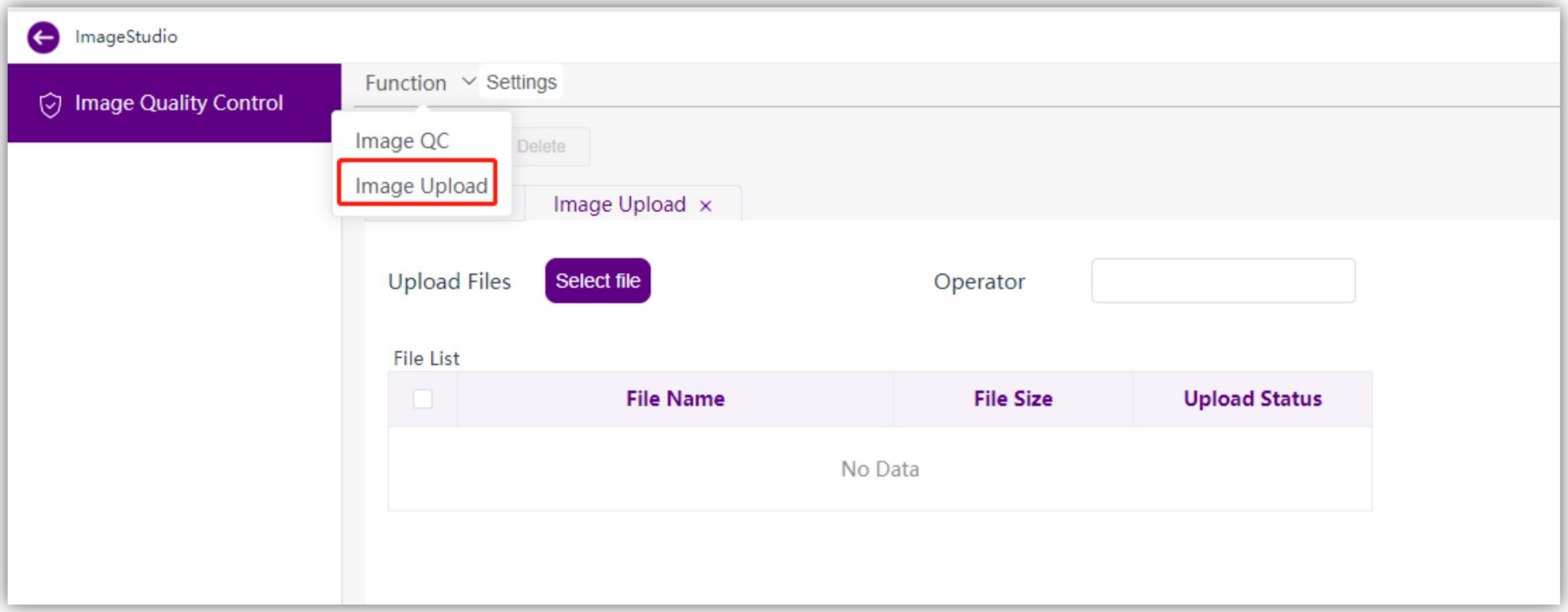


(10) If the auto-uploading function is turned on, the output files (TAR.GZ and IPR) will be automatically uploaded to the selected computer cluster or cloud. Click the "Clear" button to clear

the contents, and then drag another image for a new run.

4.2.2 Image Upload

Stitched images in TIFF formats can be uploaded to a computer cluster or cloud using this function. Upload interface as shown in the screenshot below:

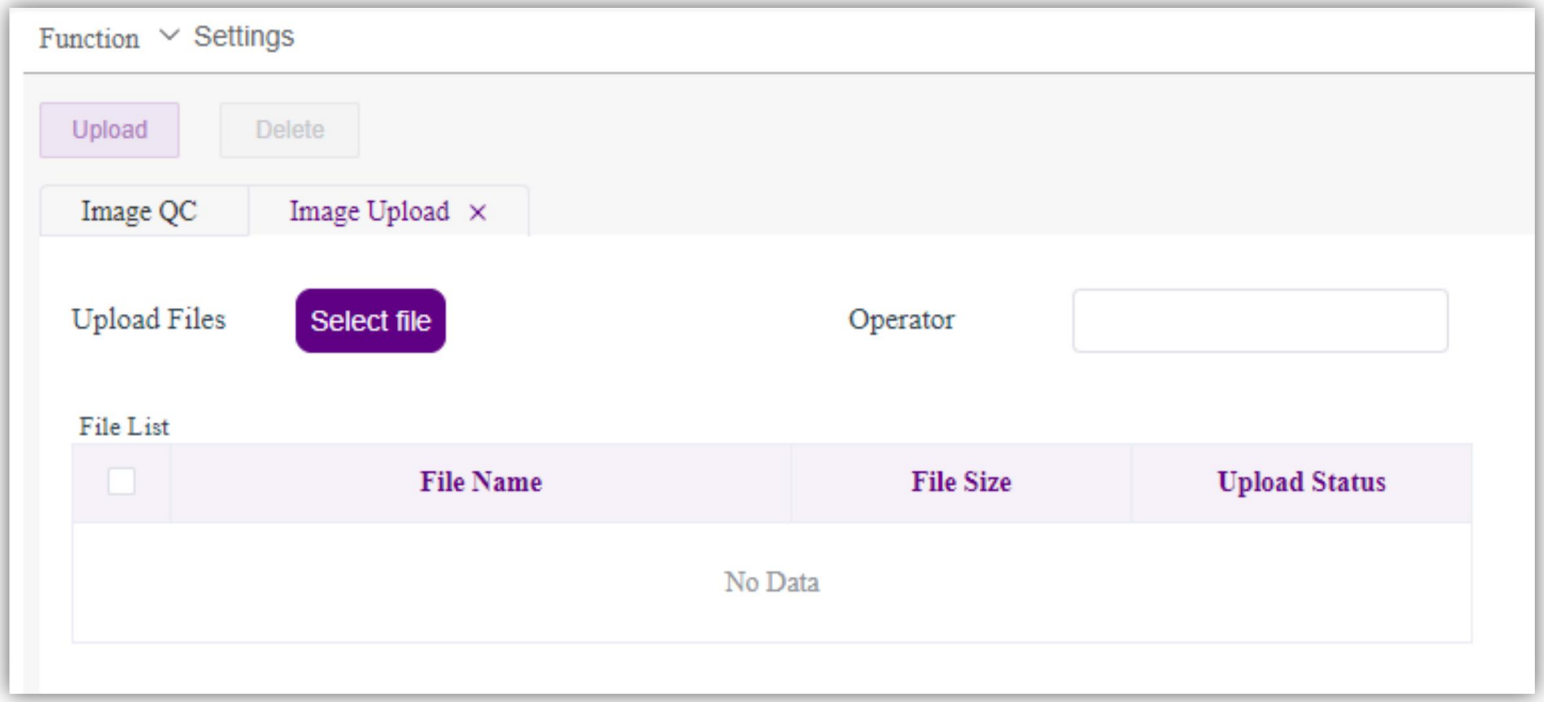


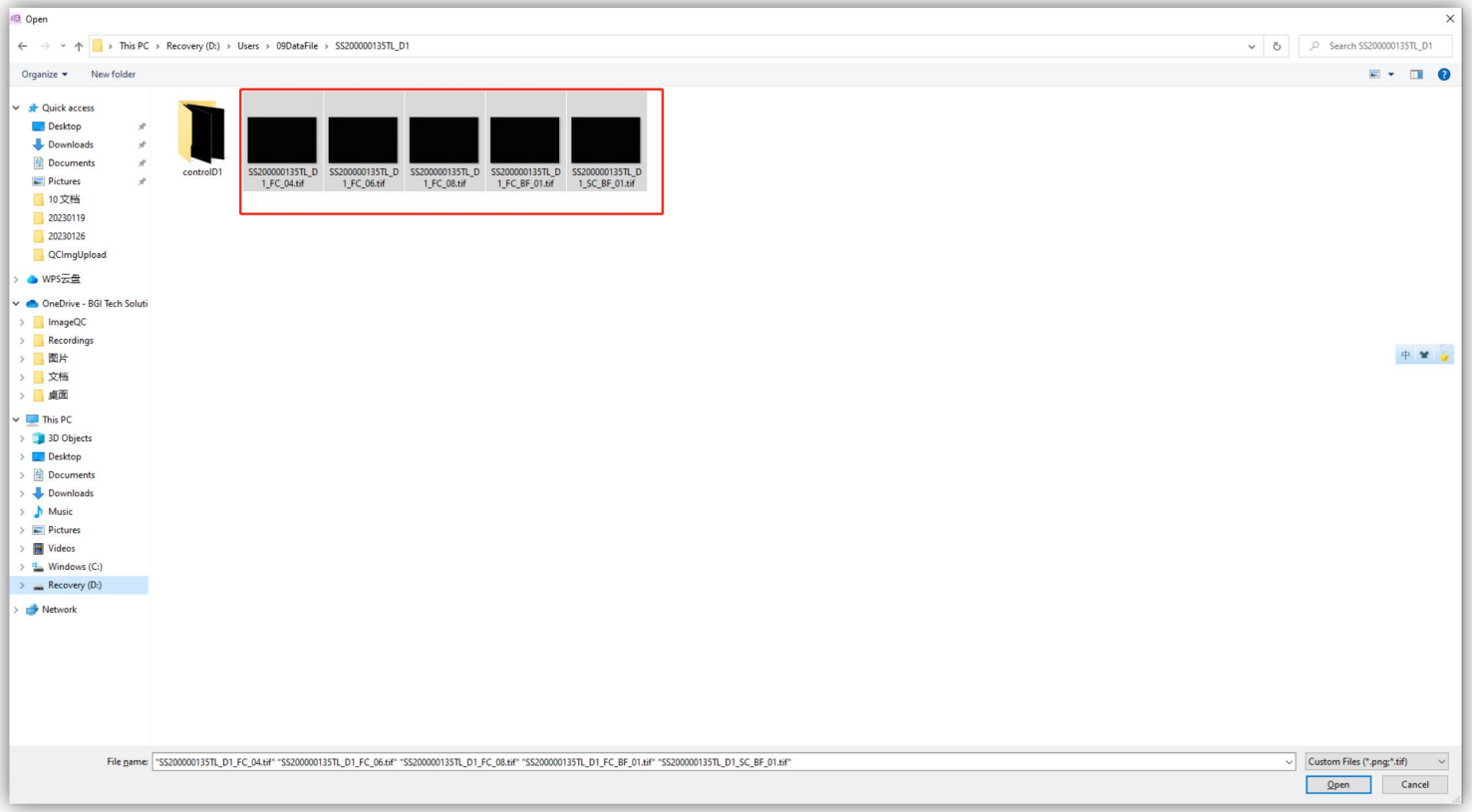
Note: For images being uploaded to the cluster, users need to upload images according to certain naming conventions, otherwise the system will detect naming convention errors.

Image naming convention

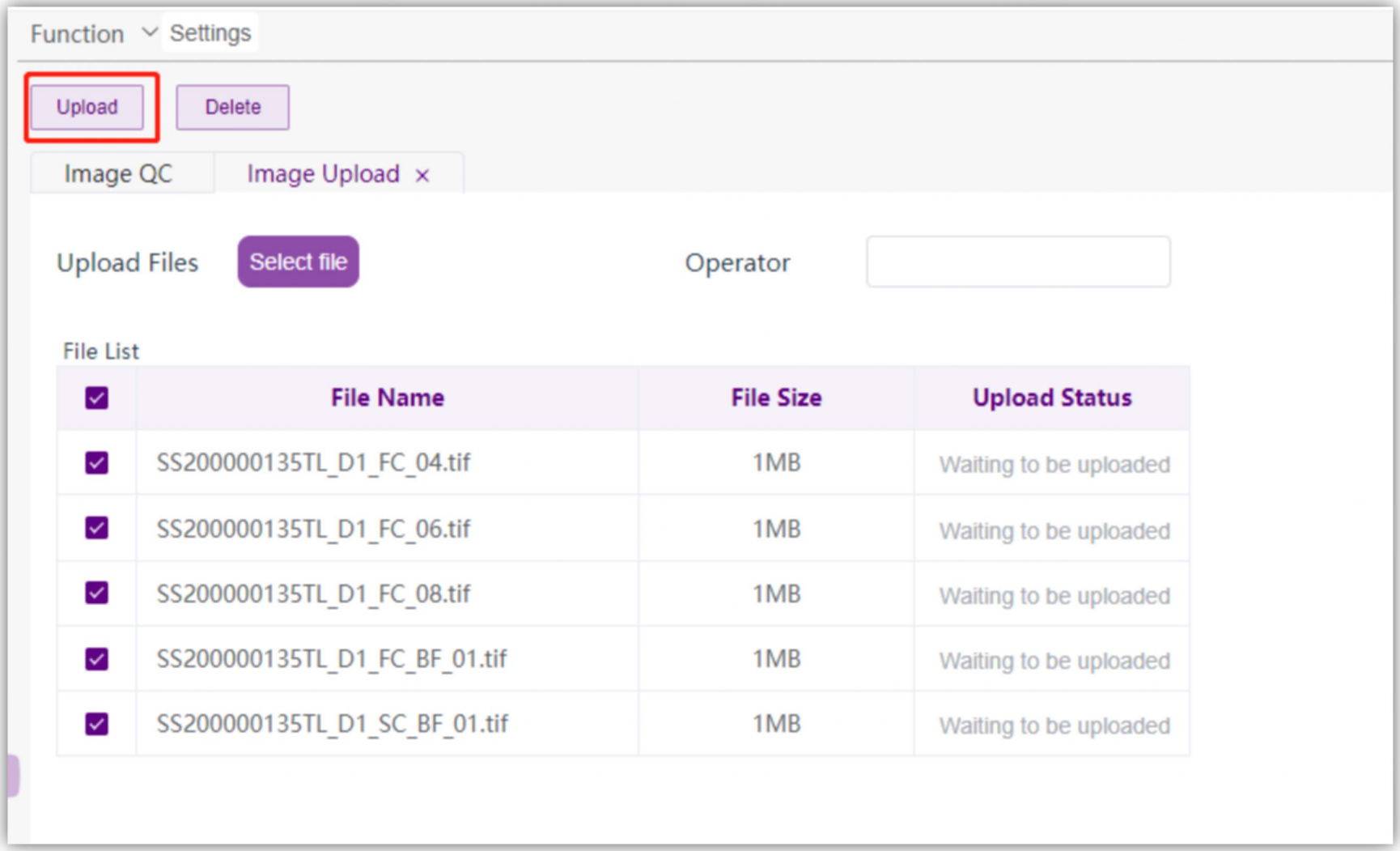
Image Type	Whether chip SN	User naming convention	Example
Permeabilization time optimization images captured using TRITC channel at different time points	No	Stereo-seq Chip SN_FC_permeabilization time point in two digits	SS200000135TL_D1_FC_06
Bright field images	Yes	Stereo-seq Chip SN _FC/SC_BF_numbering in two digits	SS200000135TL_D1_SC_BF_01

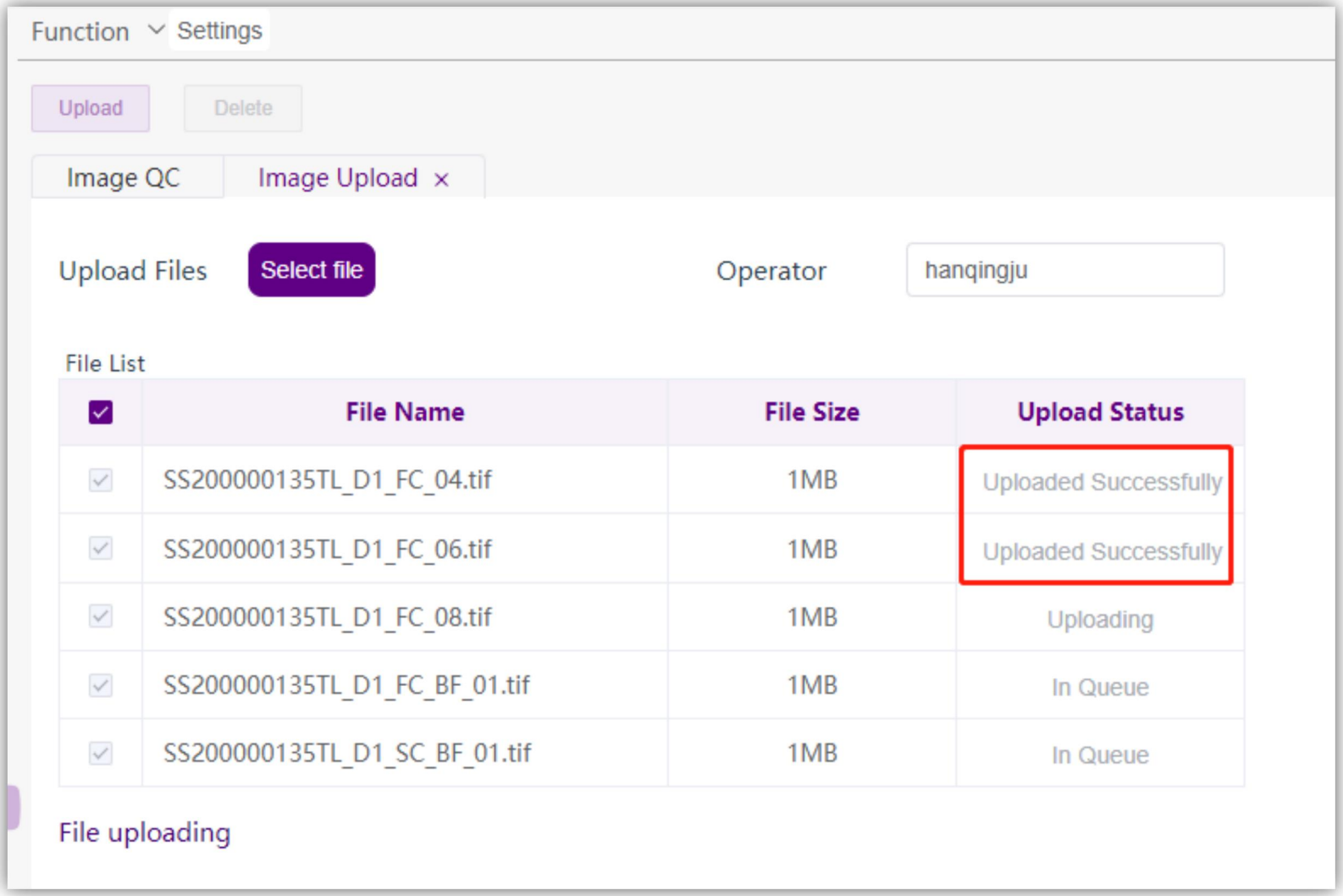
(1) Click 'Select file' then open the file to be uploaded.


Function
Settings
Upload
Delete
Image QC
Image Upload
Upload Files
Select file
Operator
File List
File Name
File Size
Upload Status
No Data

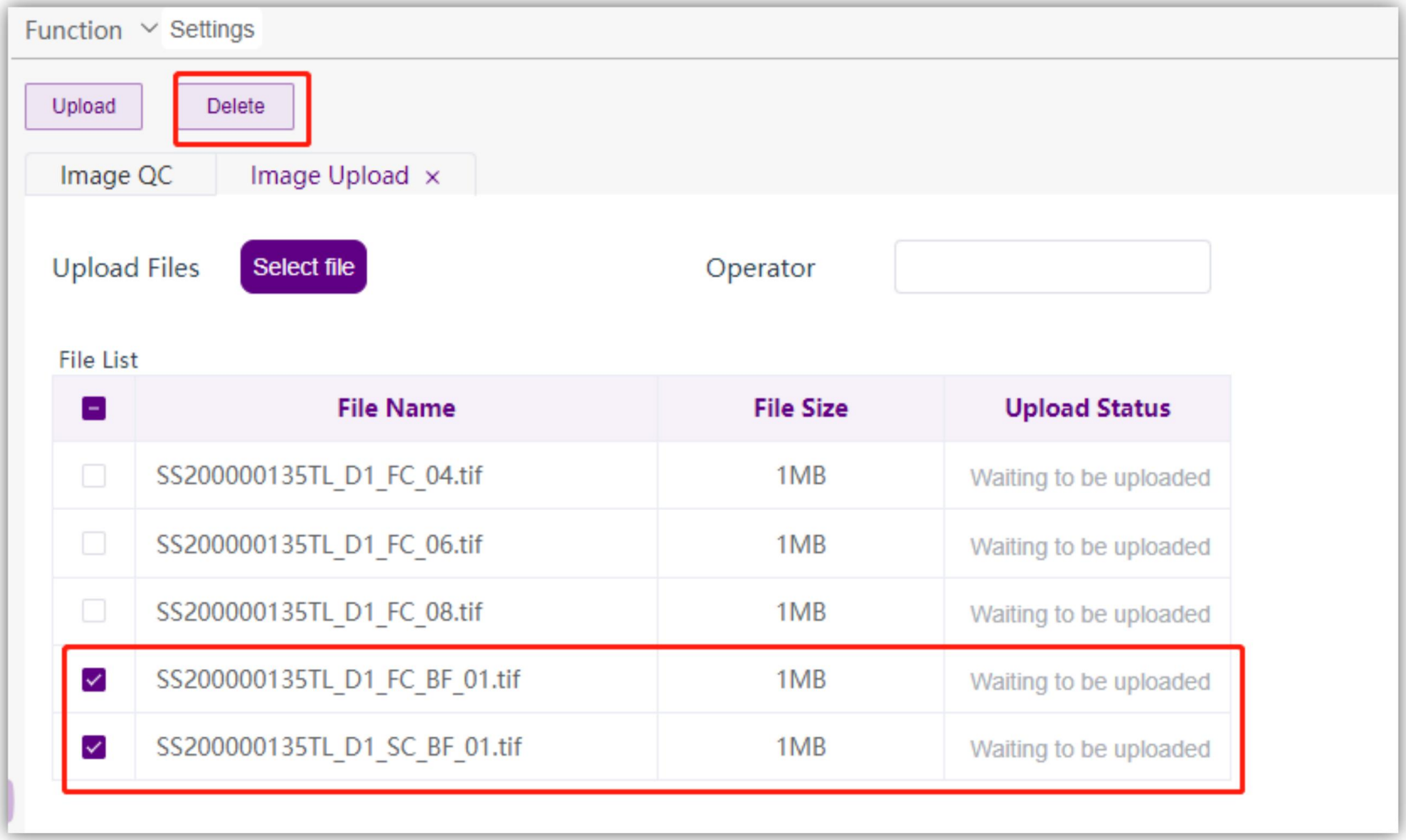


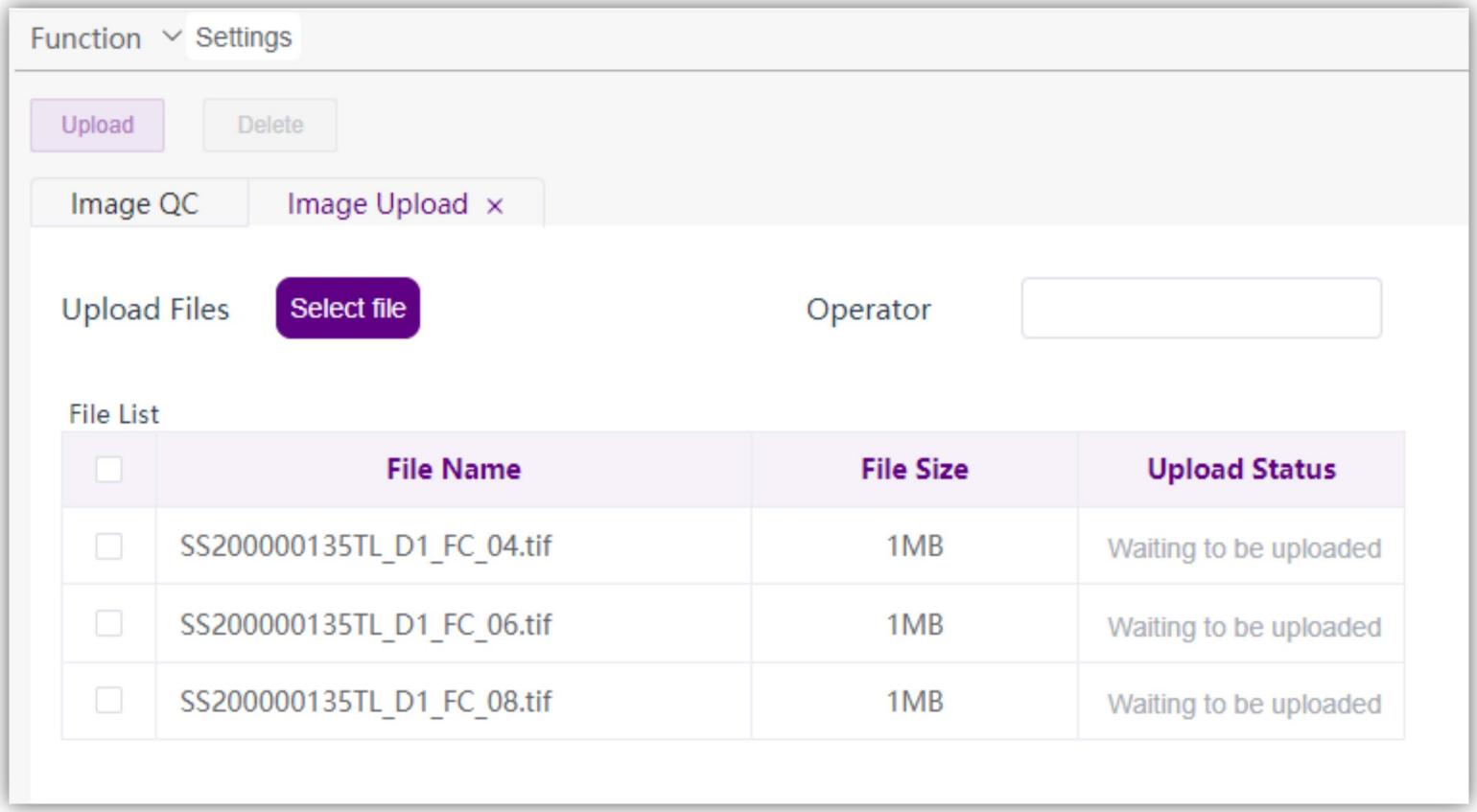
(2)Click 'Upload' and wait for it to complete.


Function
Settings
Upload
Delete
Image QC
Image Upload
Upload Files
Select file
Operator
File List
File Name
File Size
Upload Status
SS200000135TL_D1_FC_04.tif
1MB
Waiting to be uploaded
SS200000135TL_D1_FC_06.tif
1MB
Waiting to be uploaded
SS200000135TL_D1_FC_08.tif
1MB
Waiting to be uploaded
SS200000135TL_D1_FC_BF_01.tif
1MB
Waiting to be uploaded
SS200000135TL_D1_SC_BF_01.tif
1MB
Waiting to be uploaded

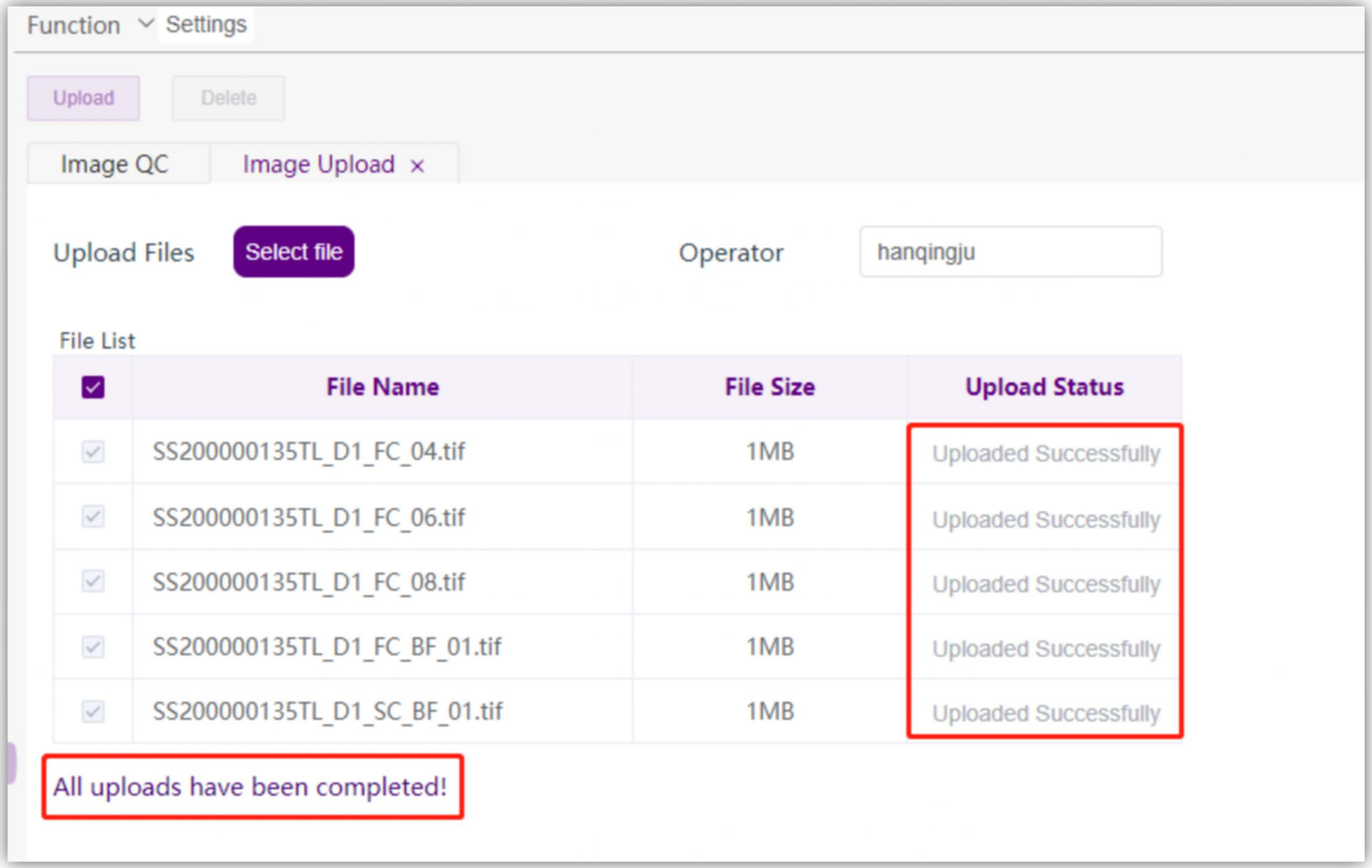


(3)If you need to delete files from the file list waiting to be uploaded, select the file and click 'Delete'.


Function
Settings
Upload
Delete
Image QC
Image Upload
Upload Files
Select file
Operator
File List
File Name
File Size
Upload Status
SS200000135TL_D1_FC_04.tif
1MB
Waiting to be uploaded
SS200000135TL_D1_FC_06.tif
1MB
Waiting to be uploaded
SS200000135TL_D1_FC_08.tif
1MB
Waiting to be uploaded
SS200000135TL_D1_FC_BF_01.tif
1MB
Waiting to be uploaded
SS200000135TL_D1_SC_BF_01.tif
1MB
Waiting to be uploaded

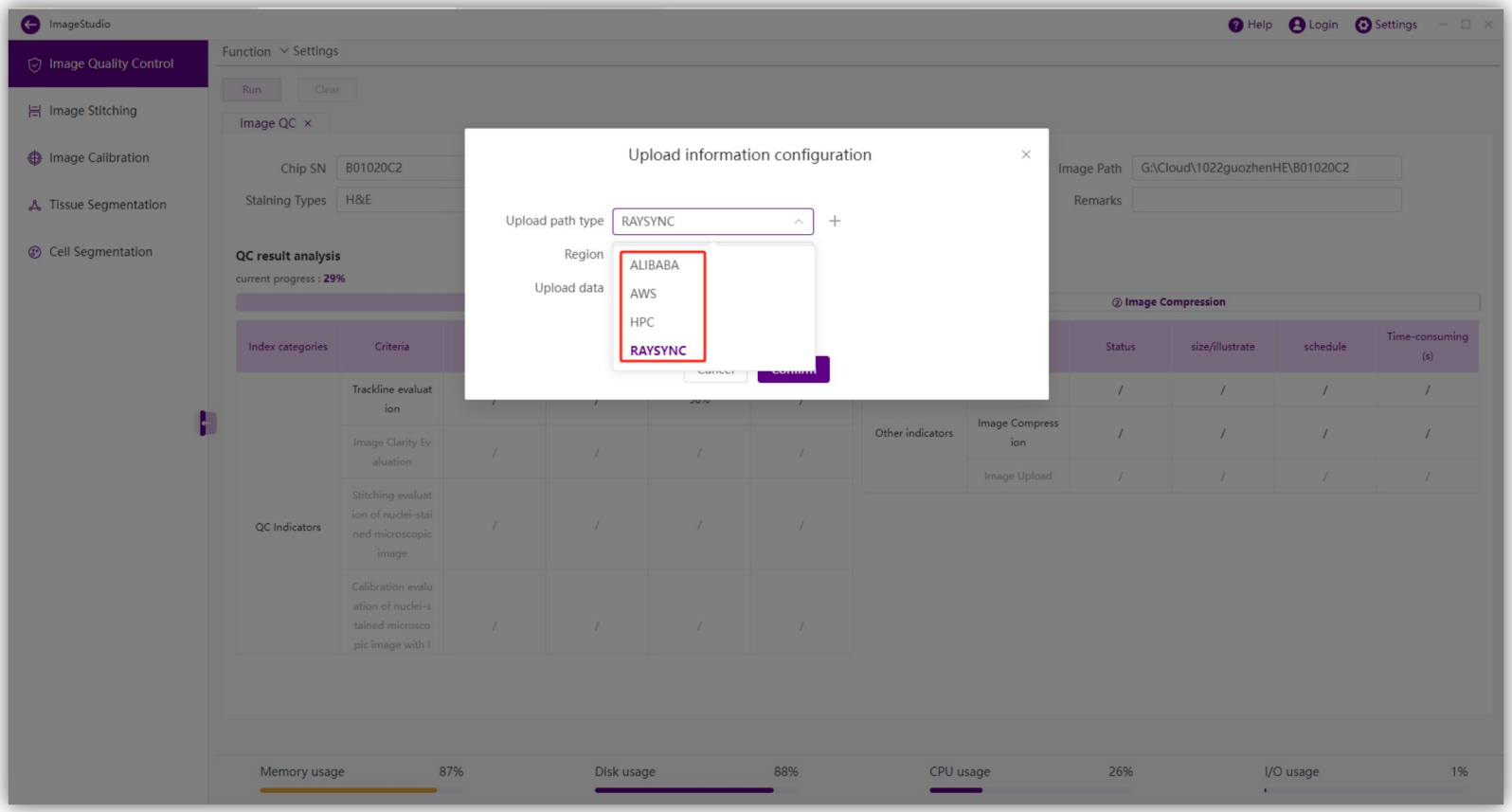


(4)Once all files have been uploaded successfully, the interface display is shown as below:


Function
Settings
Upload
Delete
Image QC
Image Upload ×
Upload Files
Select file
Operator
hanqingju
File List
File Name
File Size
Upload Status
SS200000135TL_D1_FC_04.tif
1MB
Uploaded Successfully
SS200000135TL_D1_FC_06.tif
1MB
Uploaded Successfully
SS200000135TL_D1_FC_08.tif
1MB
Uploaded Successfully
SS200000135TL_D1_FC_BF_01.tif
1MB
Uploaded Successfully
SS200000135TL_D1_SC_BF_01.tif
1MB
Uploaded Successfully
All uploads have been completed!

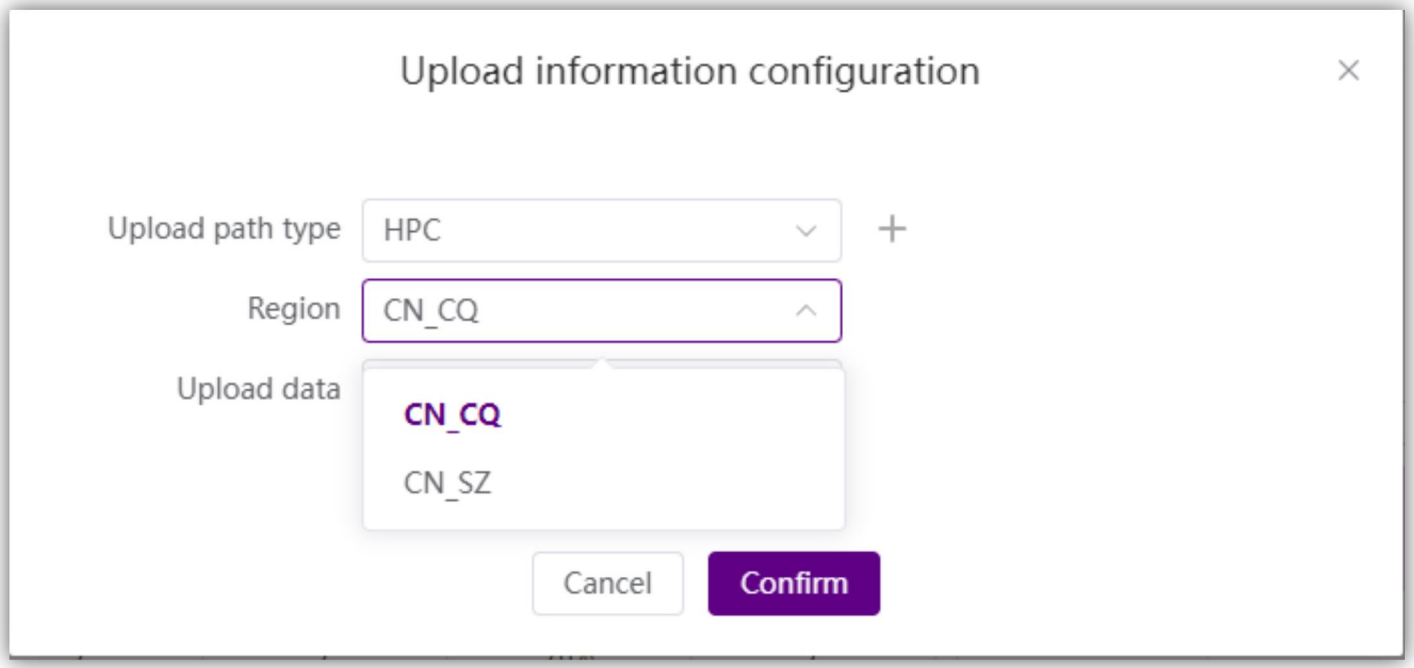
4.2.3 Upload Type Configuration

Set the upload path of the image.



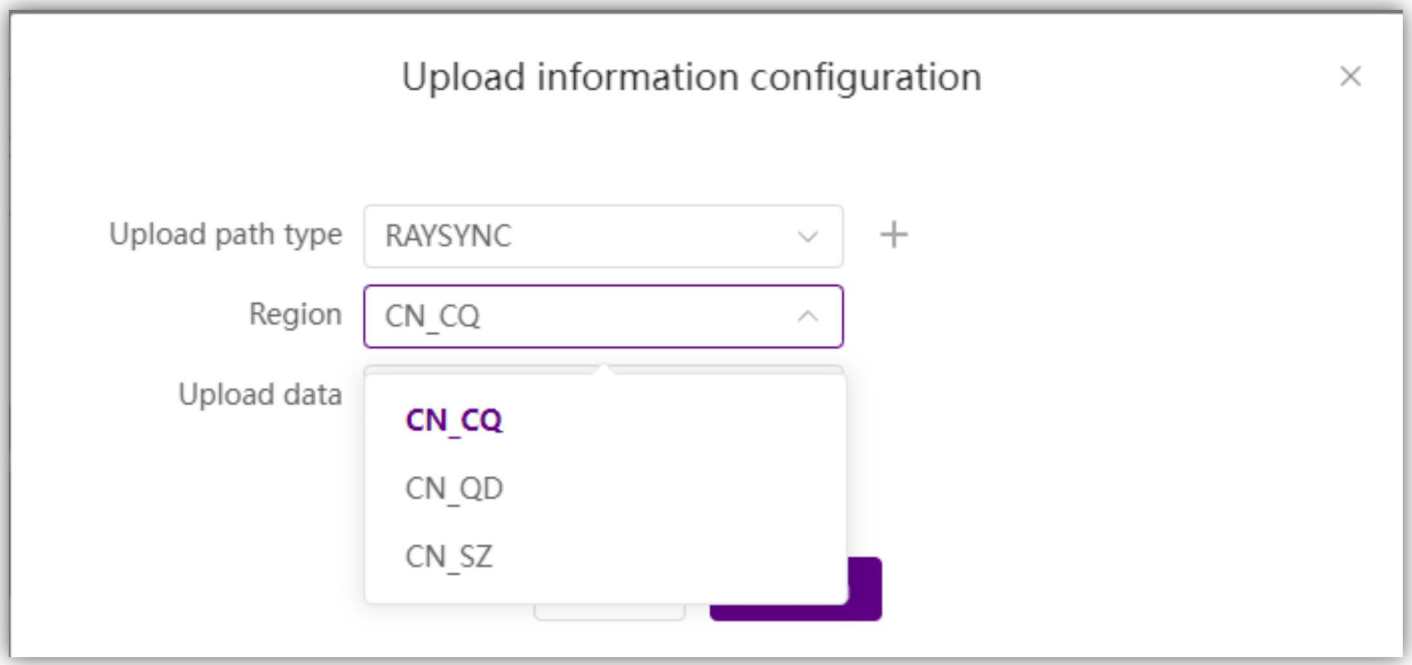
(1) Description of different types of upload paths:

a. HPC is for local upload of Image Quality Control output files to the computer cluster in region. Please set up the network configuration in advance. BGI intranet is required.



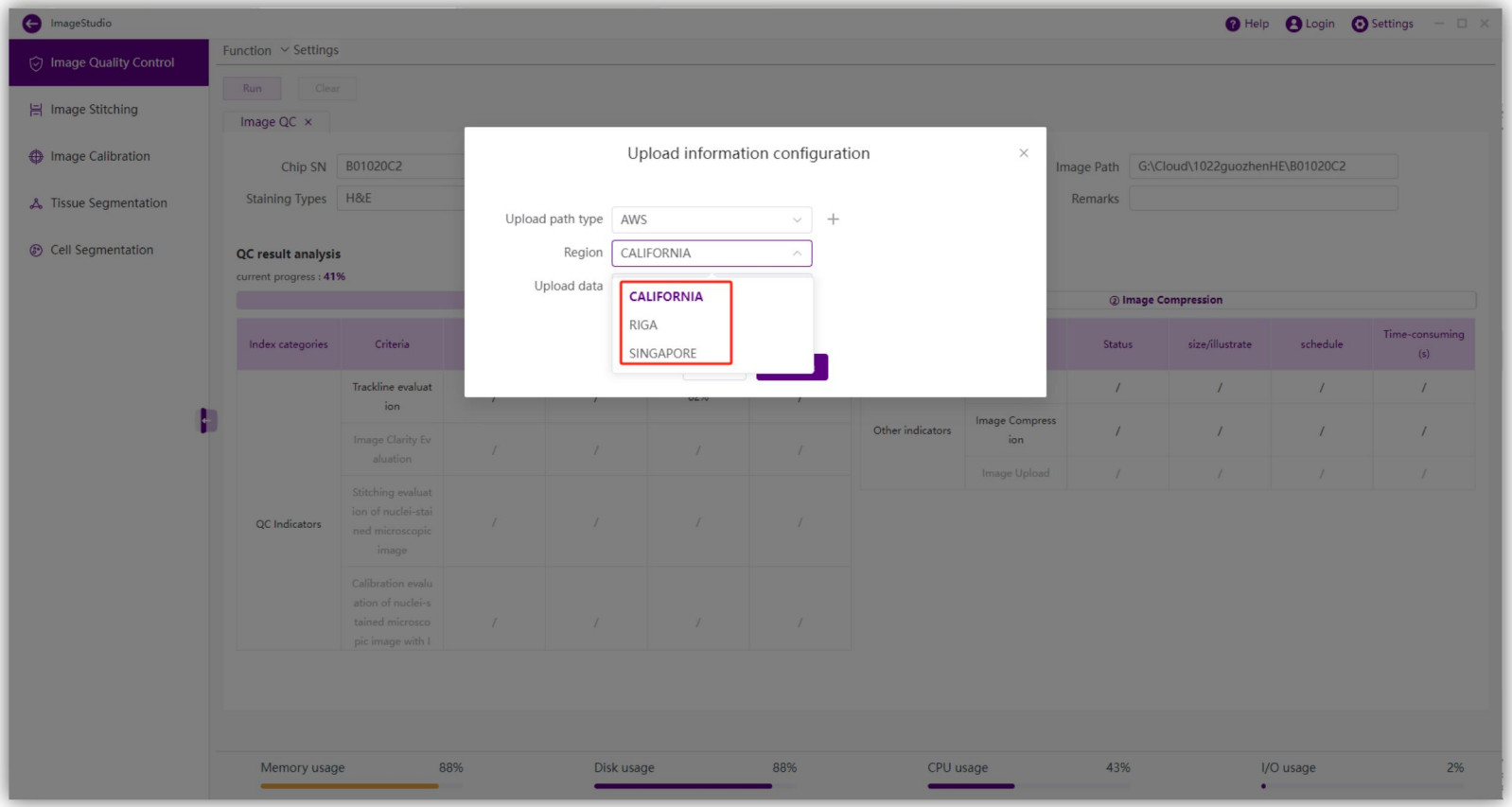
⚠The regions of this upload type are Shenzhen(CN_SZ) or Chongqing(CN_CQ) , and the degault is Shenzhen.

b. RAYSYNC uploads Image Quality Control output files via Raysync software to region’ s computer cluster. It requires only internet without additional network configuration.



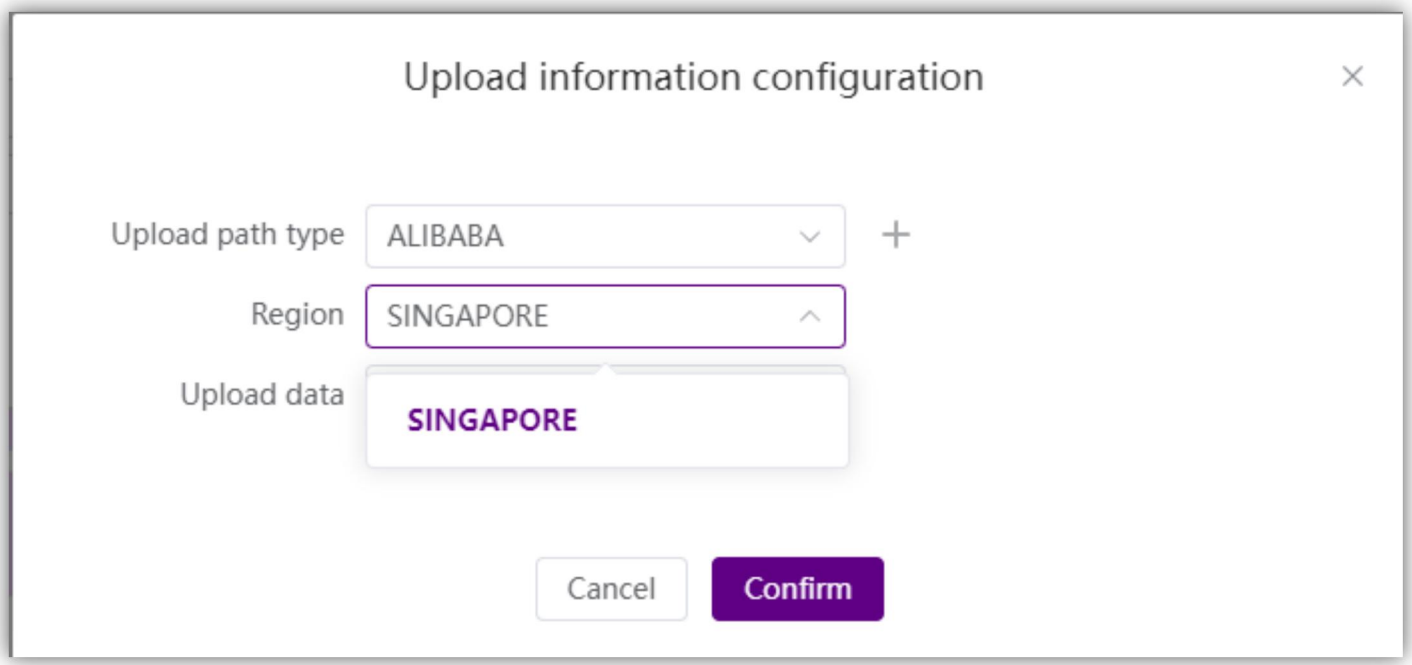
⚠The regions of this upload type are Shenzhen(CN_SZ) , Chongqing(CN_CQ) or Qingdao(CN_QD), and the default is Shenzhen.

c. AWS stands for Amazon Web Service. AWS will upload Image Quality Control output files to the Amazon cloud. Currently, it is only suitable for regions where BGI’ s proprietary STOmics Cloud has been deployed on the AWS cloud. Currently, RIGA, Singapore and California areas are configured in the system, and new regions will be added in the future.



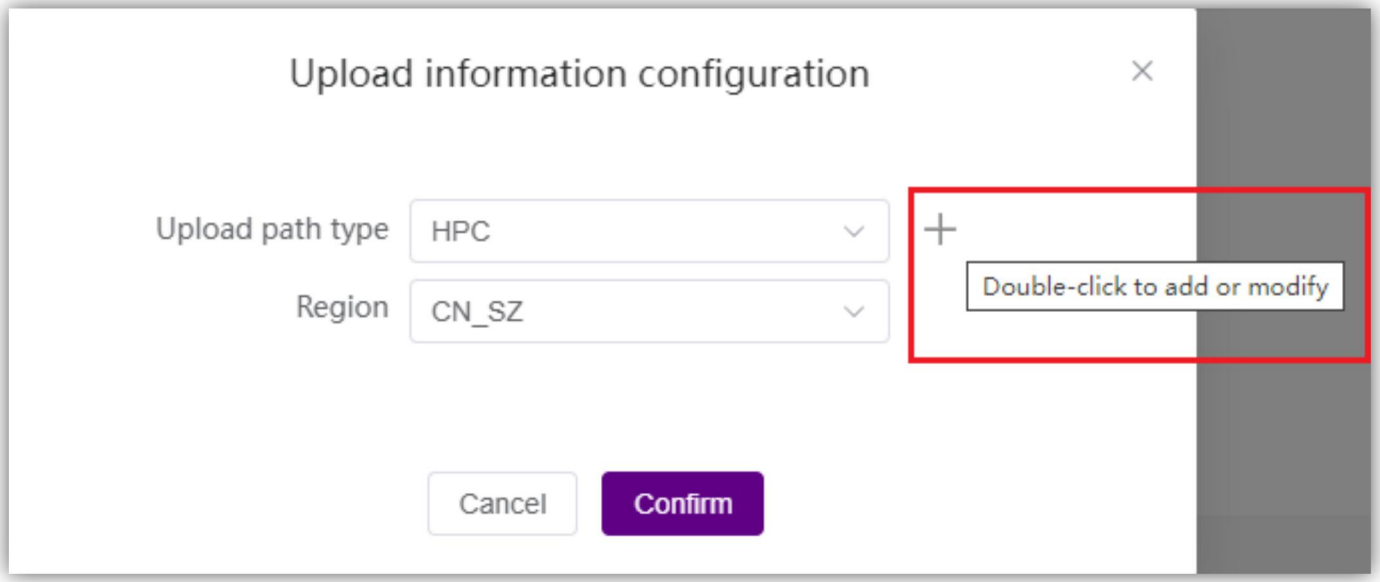
4

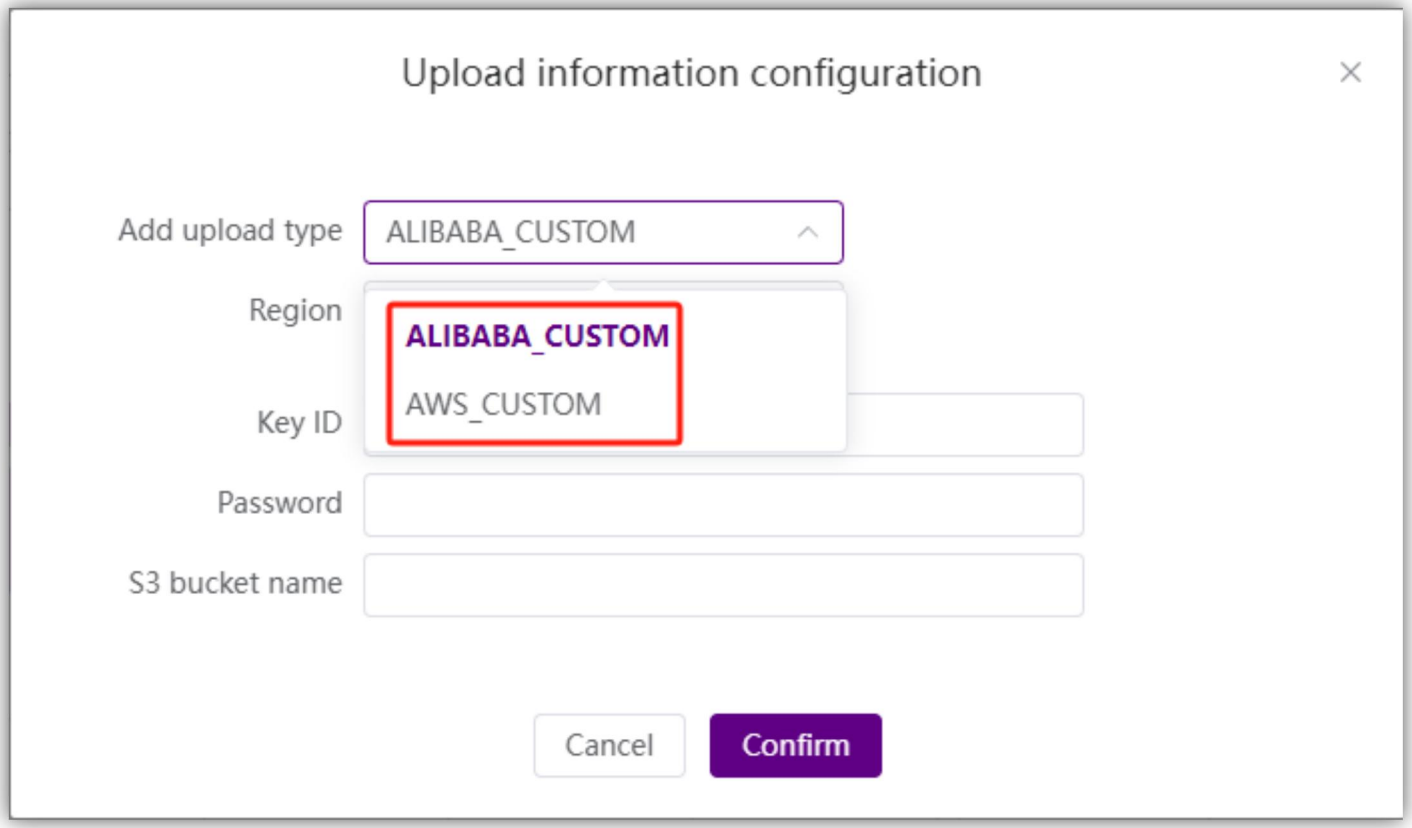
d. Upload to "ALIBABA" will upload Image Quality Control output files (TAR.GZ and IPR) to Ali Cloud. Up to now, Singapore (4-APAC) is the only region that uses Alibaba Cloud for analysis. Please contact headquarters FAS for additional paths or new regions.



(2) Modification of custom upload type:

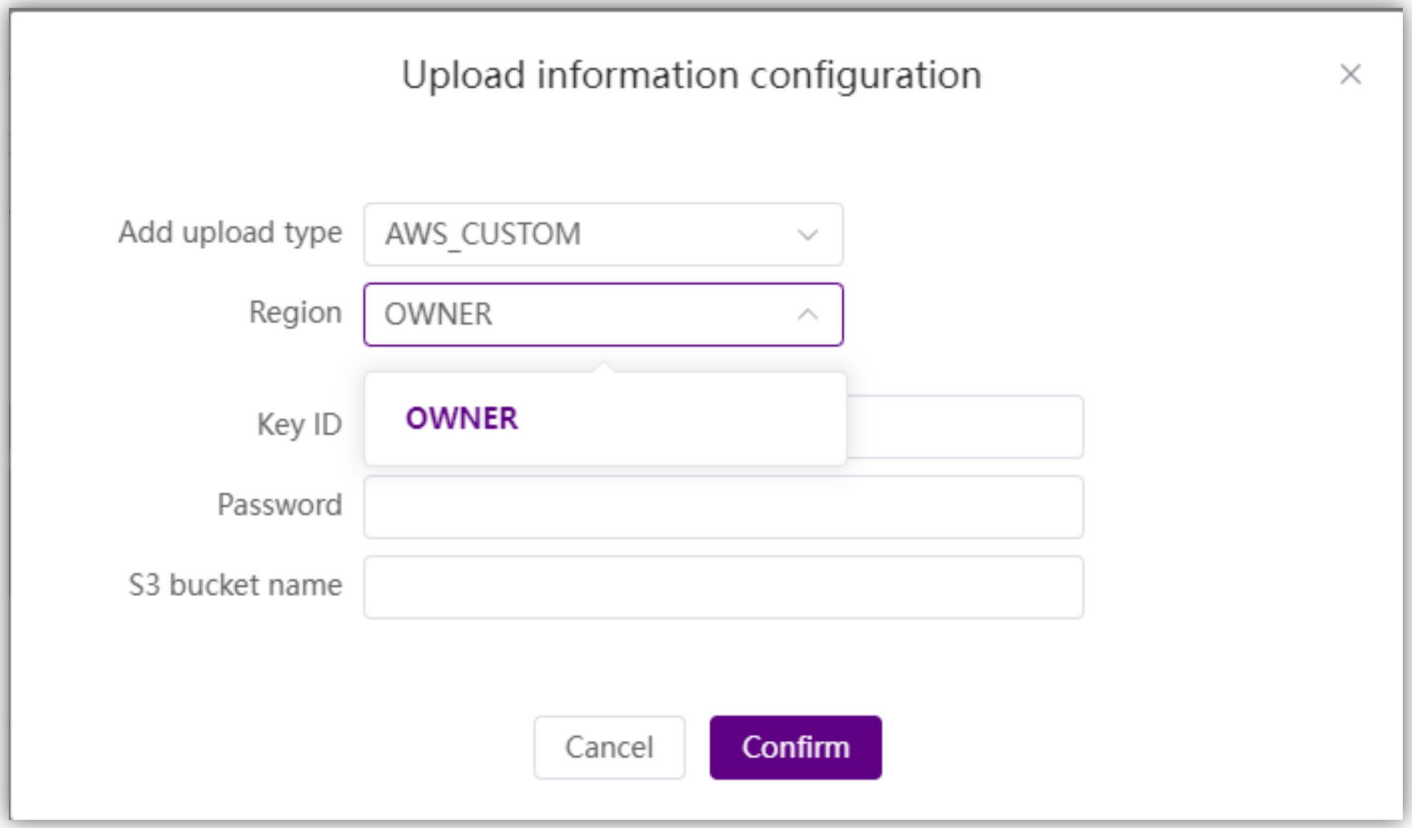
a. Double-click the ‘ +’ sign on the right to modify the custom upload type.


Upload information configuration
Upload path type
HPC
Region
CN_SZ
Double-click to add or modify
Cancel
Confirm



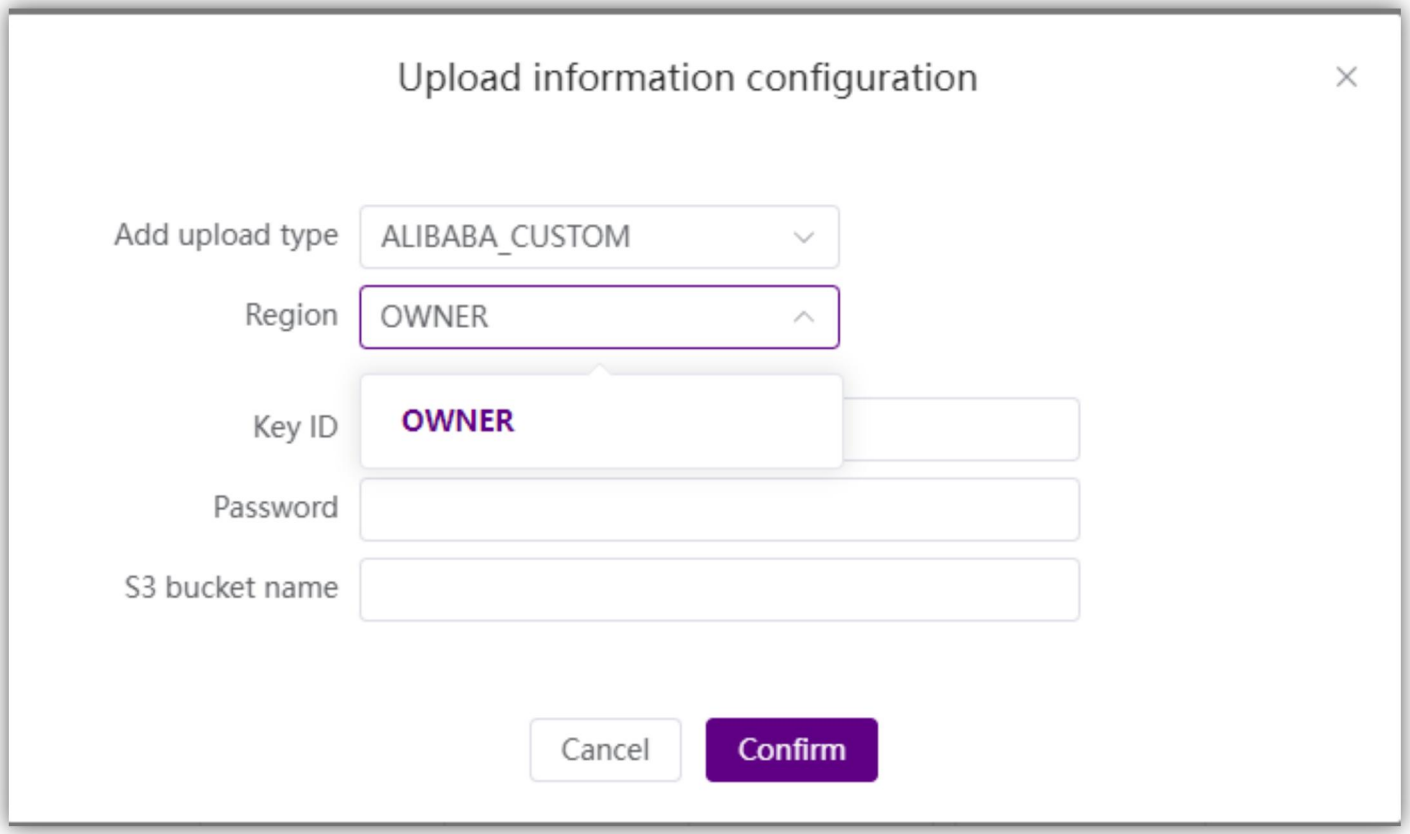
b. Choose 'AWS_CUSTOM' for Amazon cloud upload type.

- If users have purchased the AWS cloud service, select the region of OWNER, configure the key ID, password and S3 bucket path. After the configuration is completed, you can add the upload type options for AWS_CUSTOM under OWNER region.



c. ALIBABA_CUSTOM stands for customized Ali Cloud upload.

- If the user has purchased Alibaba Cloud service, double-click the "+" button to set up a customized upload configuration. Select "ALIBABA_CUSTOM" for "Add upload path type" and "OWNER" for "Region", then fill in the "Key ID", "Password", and "S3 bucket name". Click "Confirm" to finish the setup. The user could find a new "OWNER" region option under the "ALIBABA_CUSTOM" upload type option.



⚠After the modification of the custom upload type, you can select different types to upload data as needed.

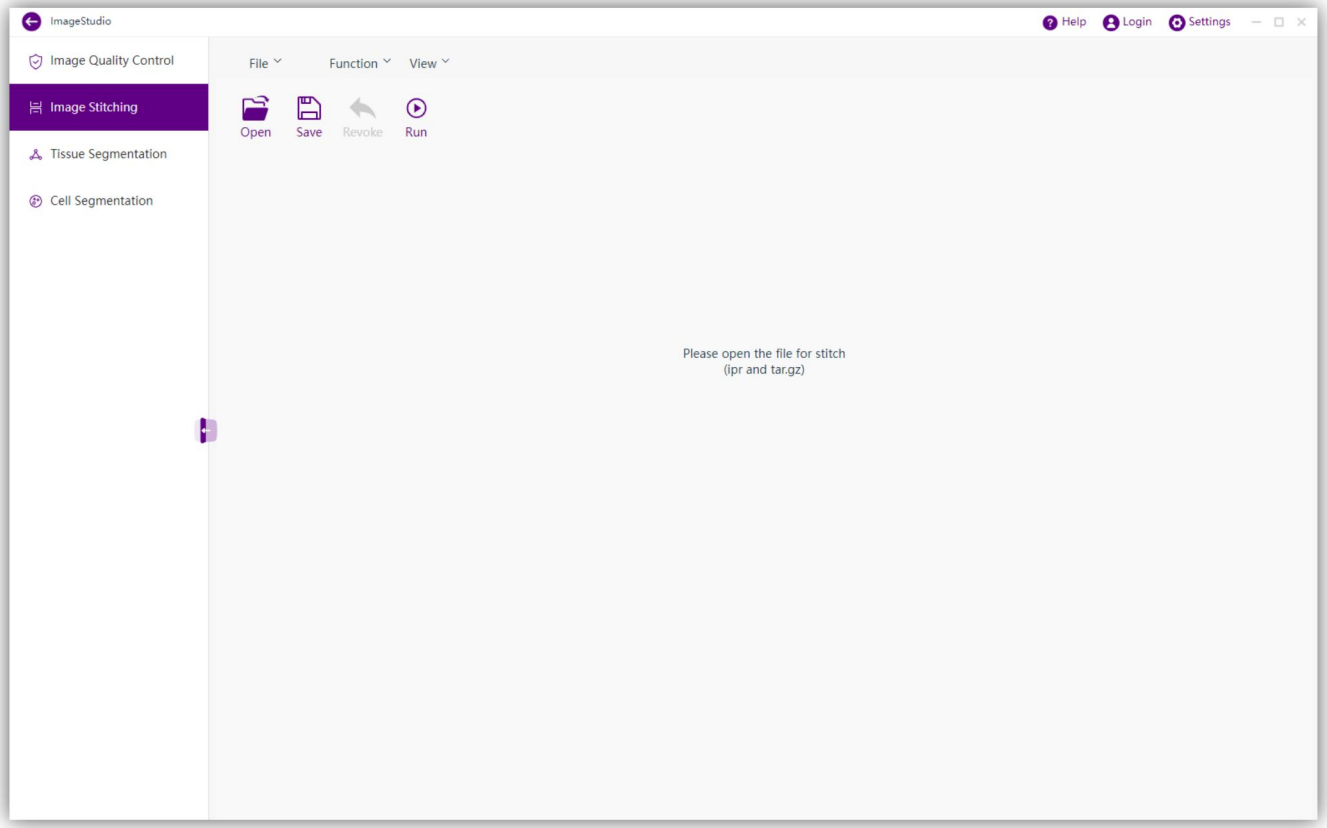
4.3 Image Stitching (tiled FOV image only)

The image stitching module provides manual image stitching tools to adjust microscope stitching before running the standard automatic Stereo-seq Analysis Workflow (SAW) pipeline.

⚠ The prerequisite for running this module is that the tile image file has been generated .

4.3.1 Initiation and Display of Image Stitching Module

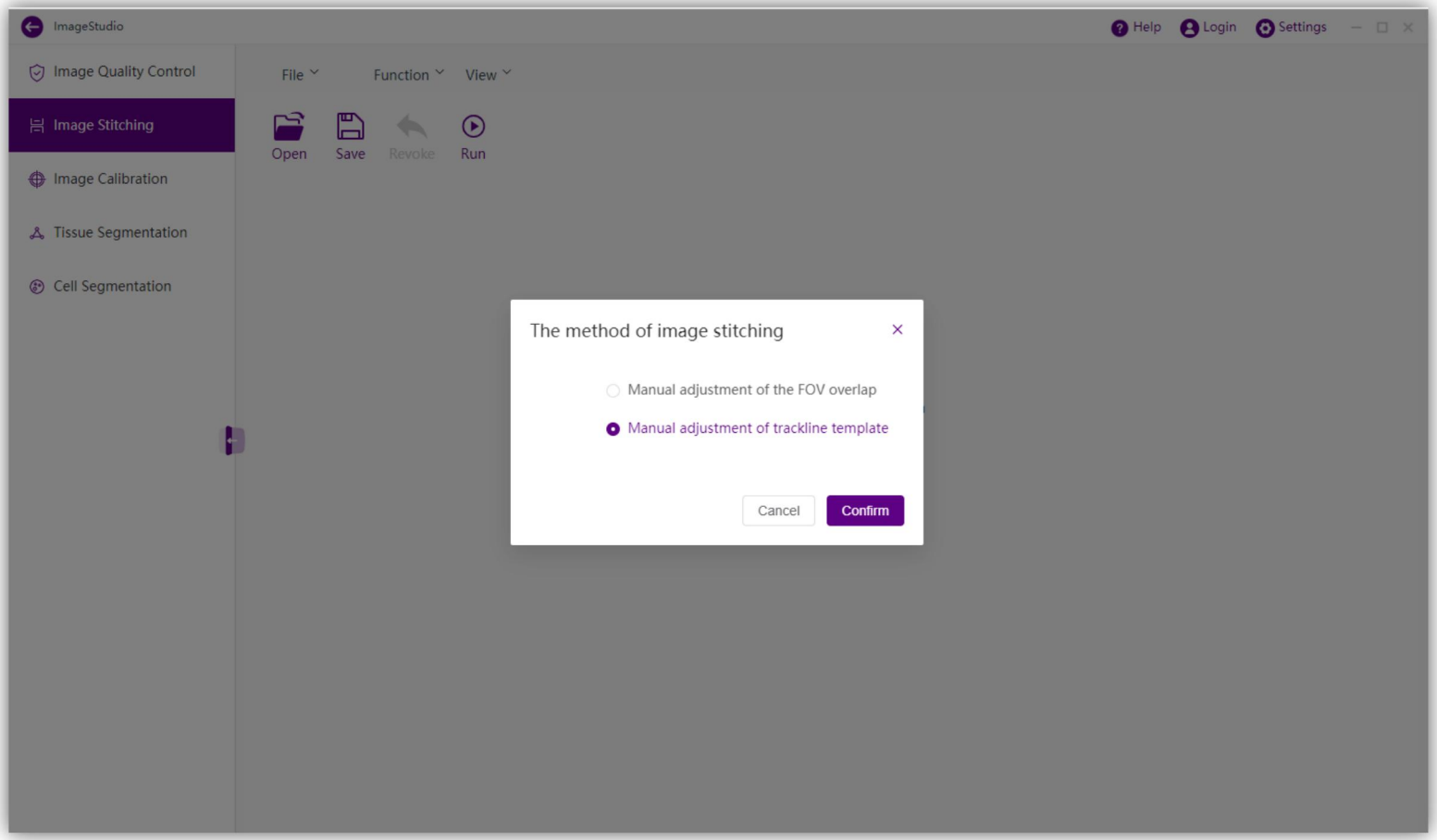
(1)Run the image stitching module.

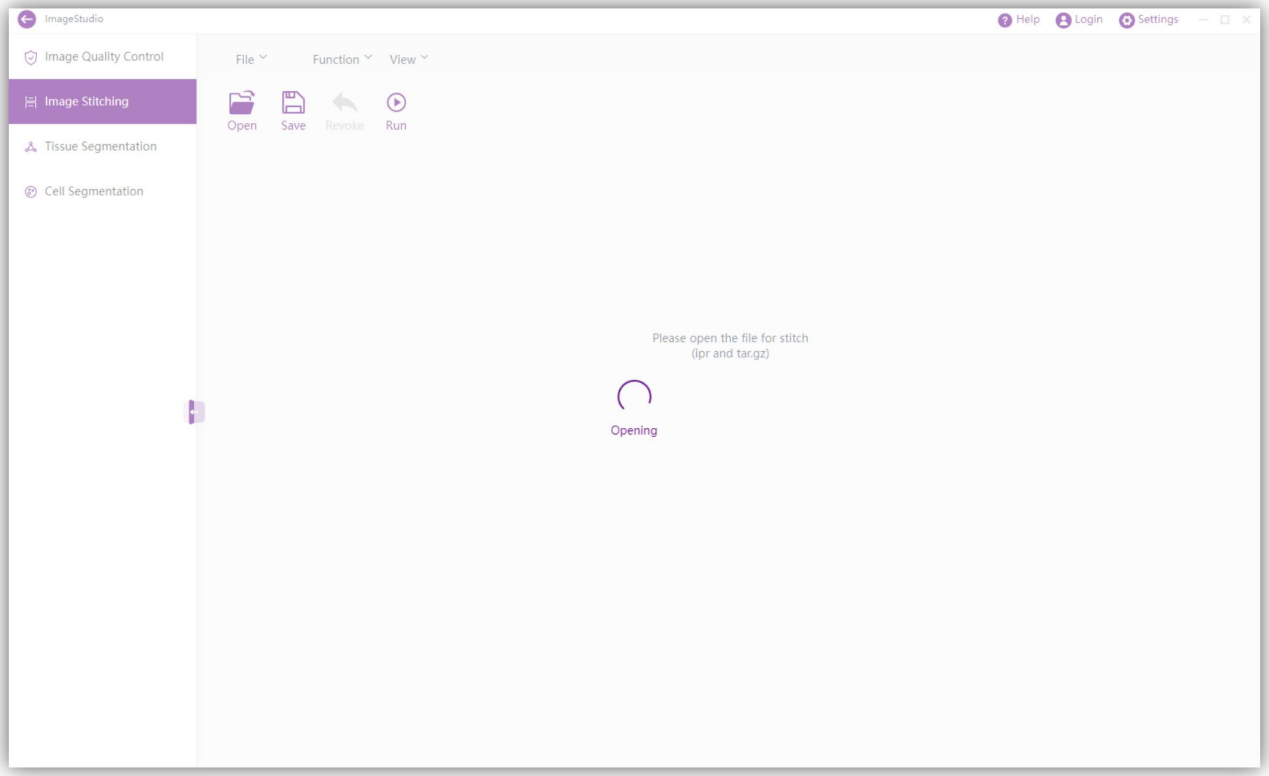


(2)Click 'Open' , select the method of image stitching (the default is the manual adjustment of

trackline template), and then select the the image file that is ready for manual stitching.

Output Directory: *D:\ImageStudioWorkspace\Stitch*

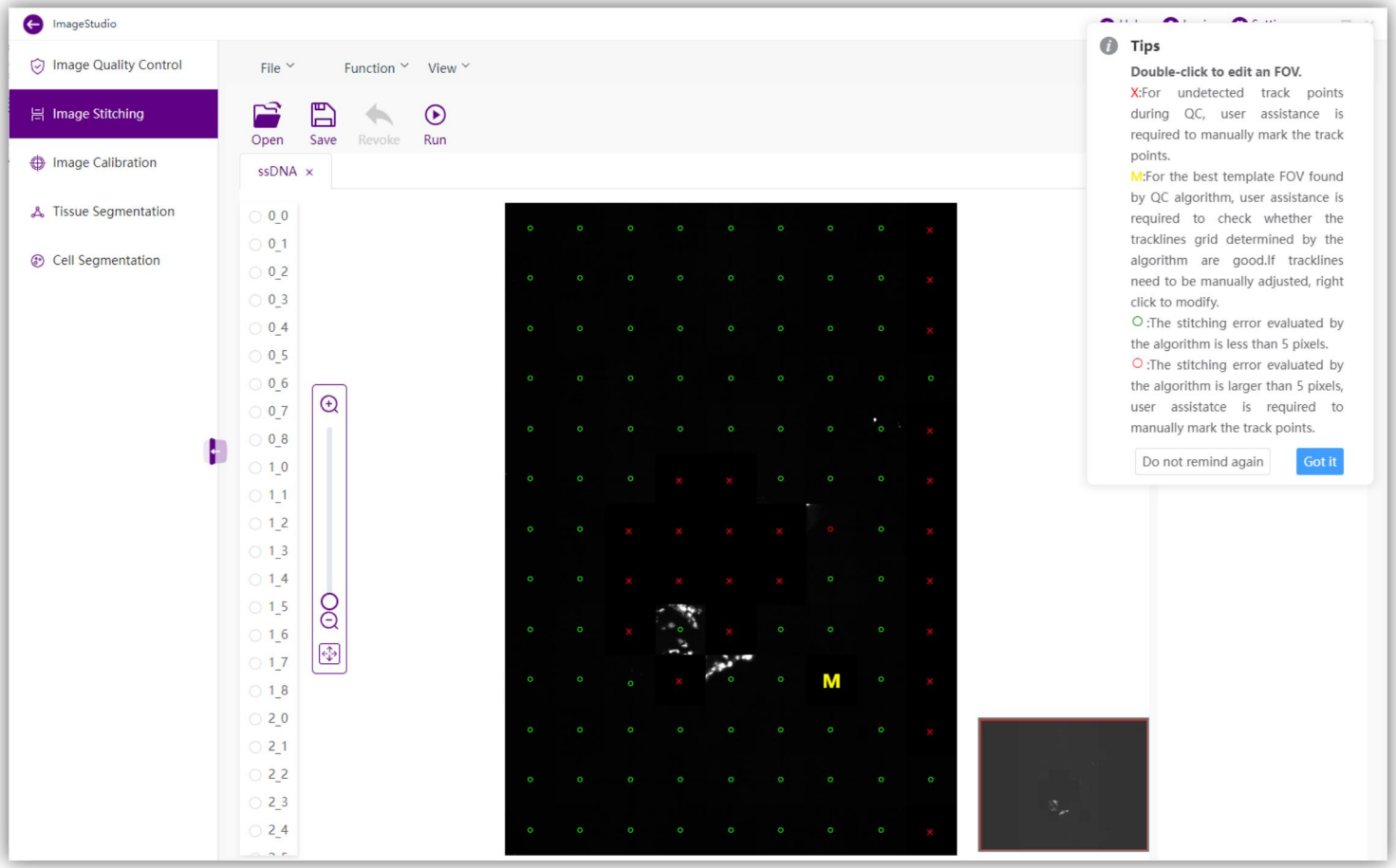




Module	Input File	Output File
Image Stitching	• IPR file and the corresponding TAR.GZ file that have gone through the ImageQC or SAW-register or SAW-rapidRegister processing	• IPR file that records manual stitching information • fov_stitched.tif for stitched image in TIFF format • File of template FOV points

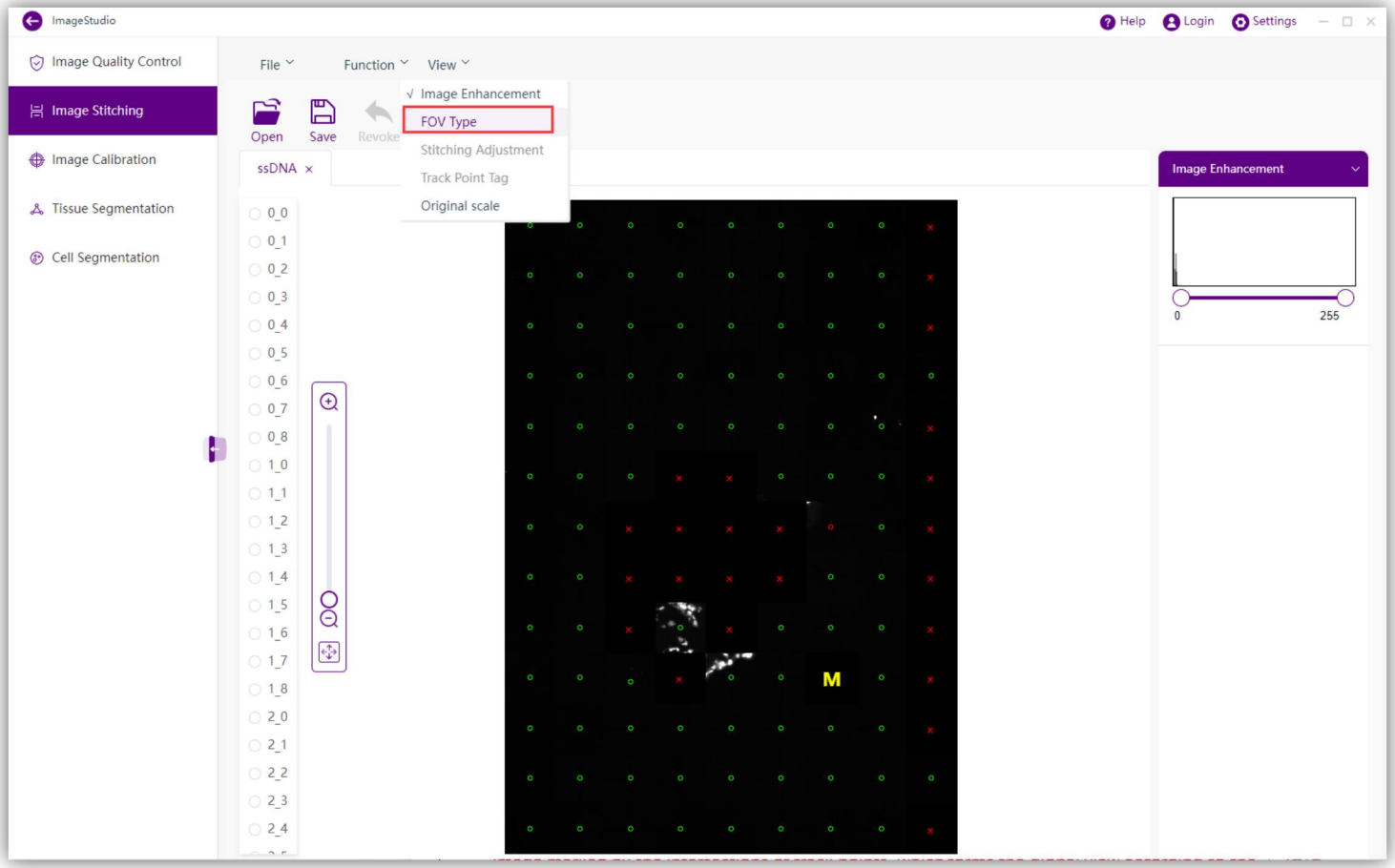
	module within Stereo-seq Analysis Workflow (SAW) pipeline	• fov_stitched_transformed.tif of pre-registered stitched image in TIFF format • File of template FOV points post pre-registration

(3) If you choose "Manual adjustment of trackline template", the program will open the IPR file and TAR.GZ file which record the track point information of each FOV detected by Image Quality Control module. Each square displays the local view of each FOV image marked by the intersections of track points, which forms the local view according to the row and column numbers of all the FOV.



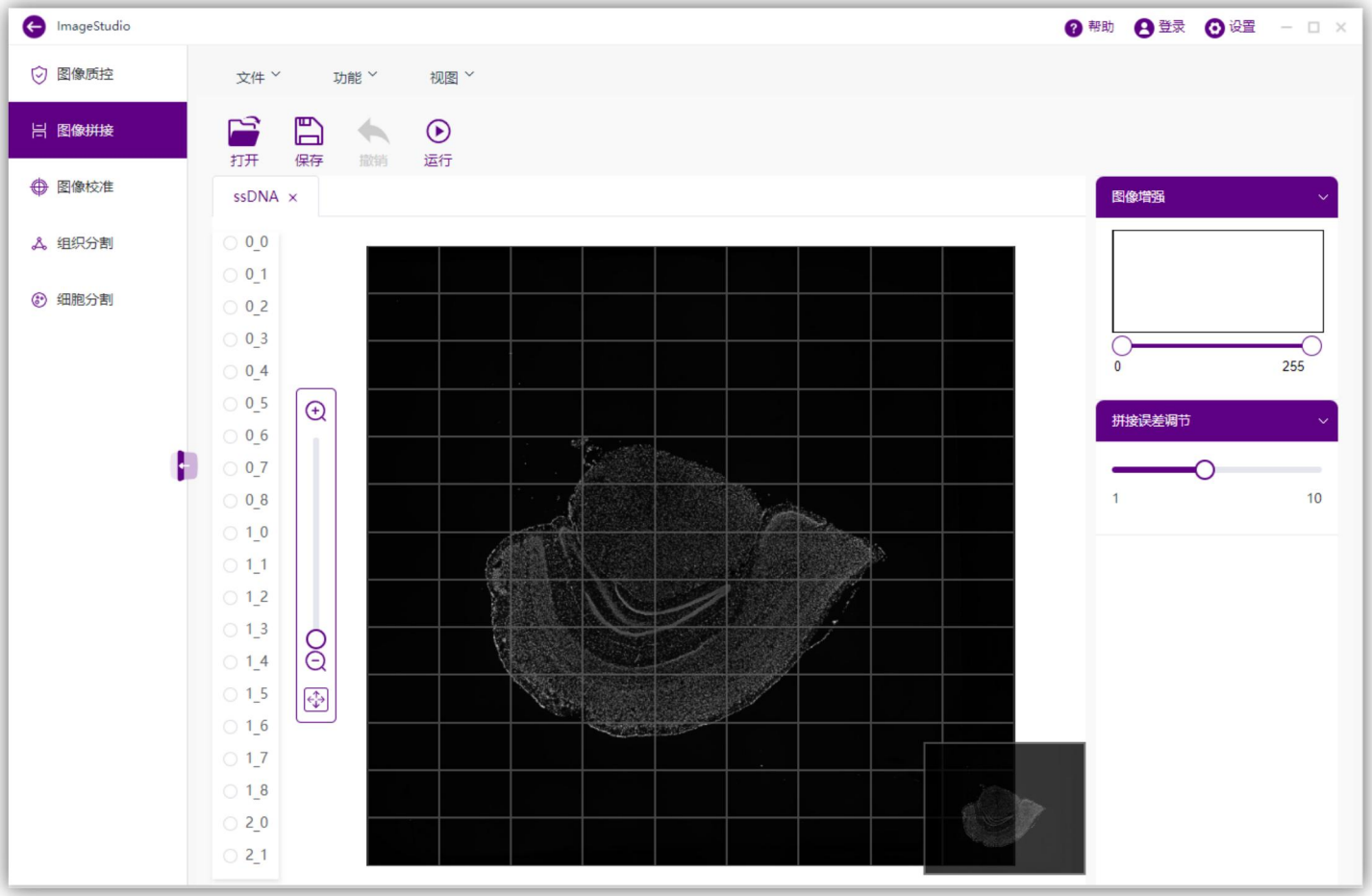
Note: For IF images, the system will generate a global map based on DAPI.

- Click 'Do not remind again', the 'FOV description' banner will not be displayed again until the next start-up.
- Click 'Got it' to close the current banner. Click 'FOV Type' under 'View' to re-open the banner.

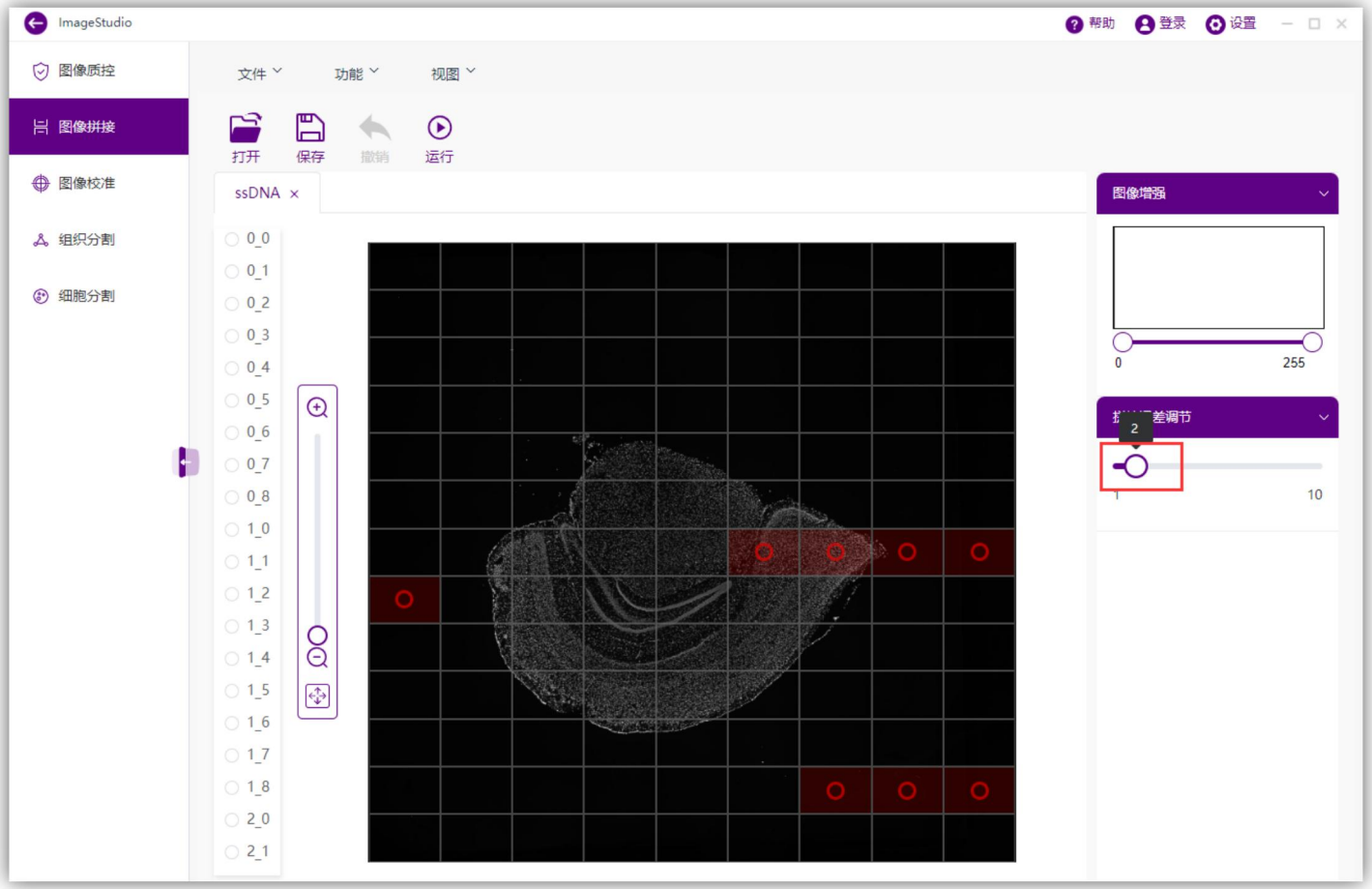


(4) If you choose "Manual adjustment of FOV overlap"

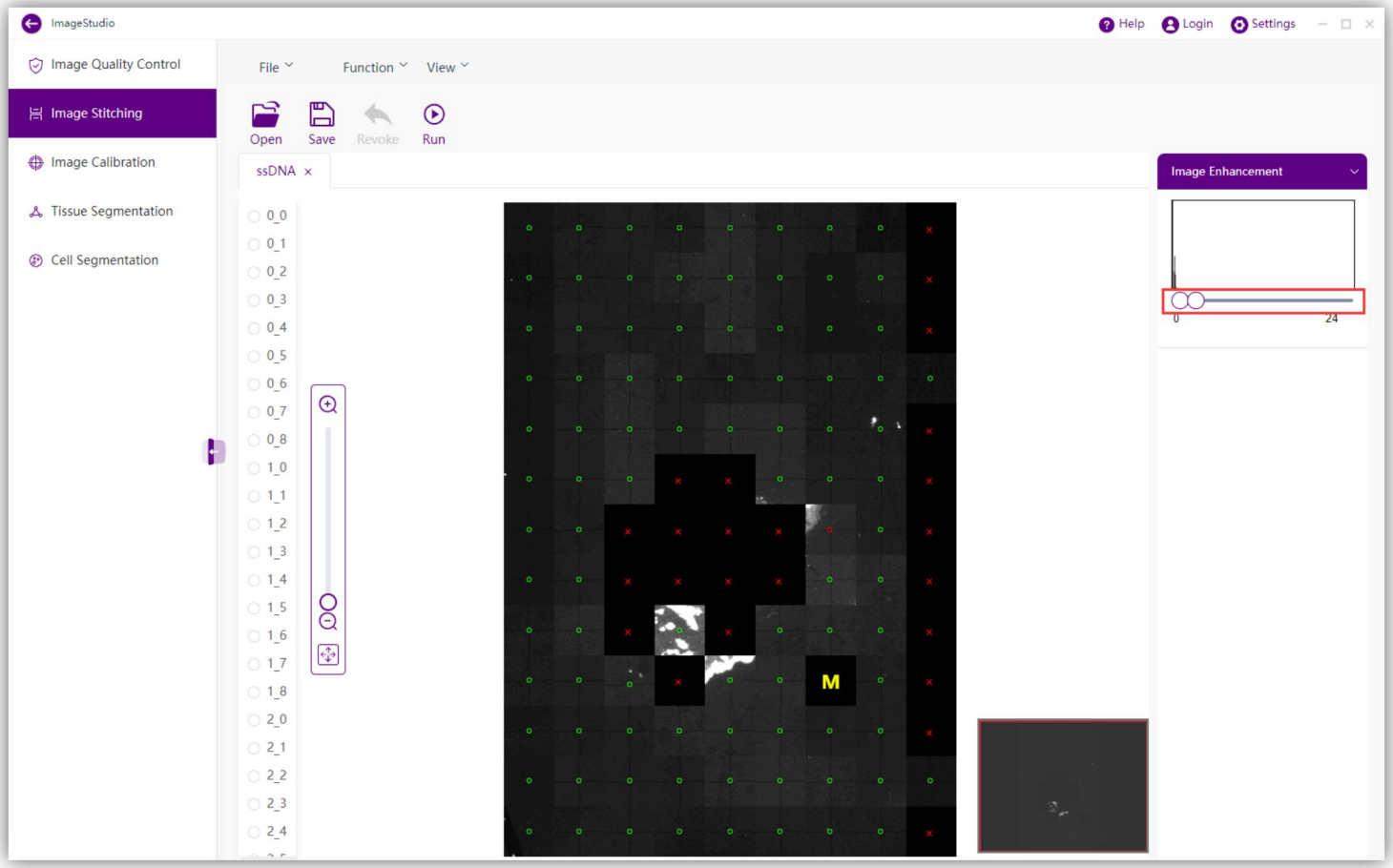
① The program will open the IPR file and TAR.GZ file which record the track point information of each FOV detected by Image Quality Control module. Each square displays the global view of each FOV image marked by the intersections of track points, which form the global view according to the row and column numbers of all the FOV.



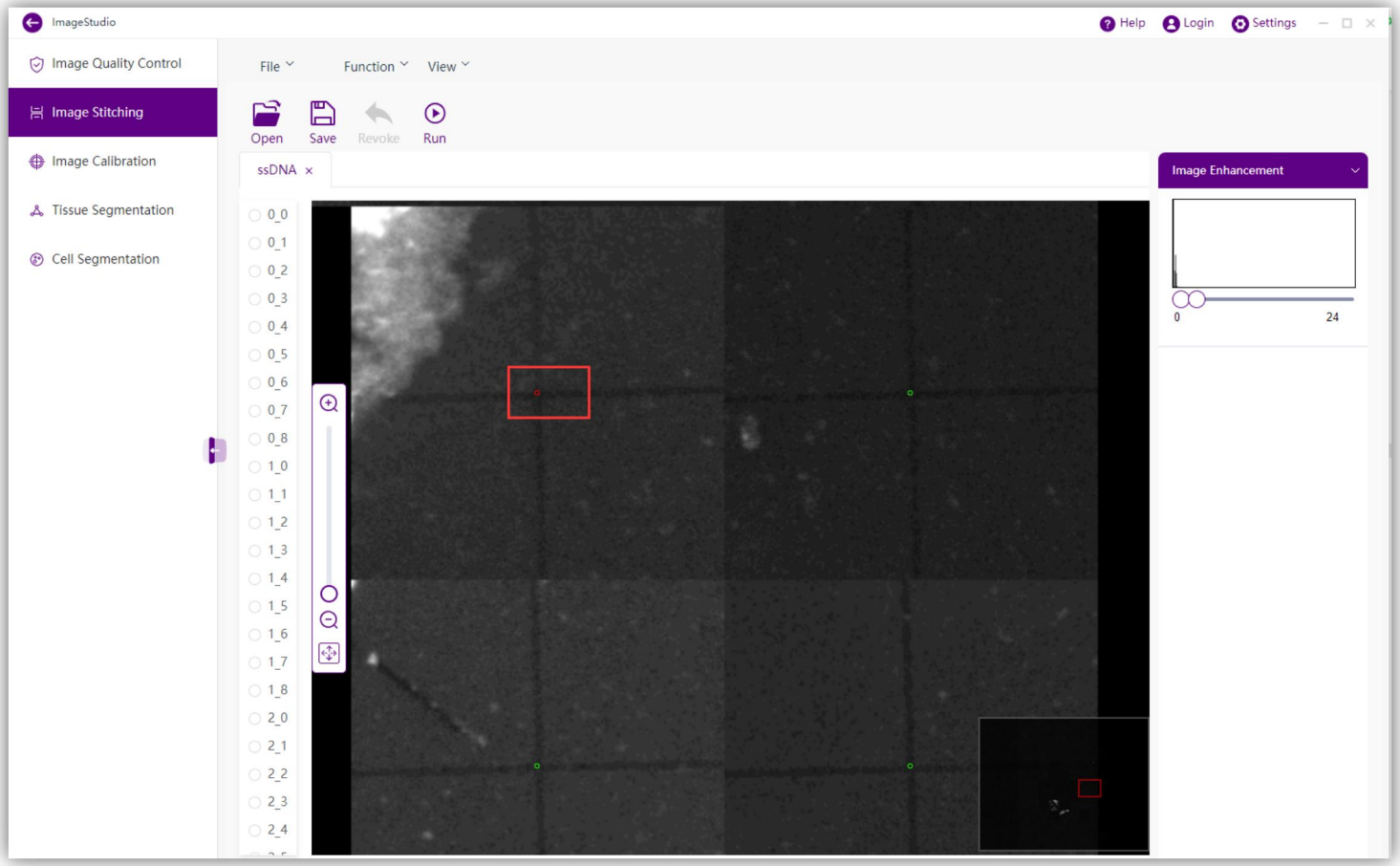
② Drag the slide bar of "Stitching Error Adjustment" to display algorithm detects a FOV greater than this error and marks it in red.The smaller the stitching error set by the user, the more red FOVs in the global large image.



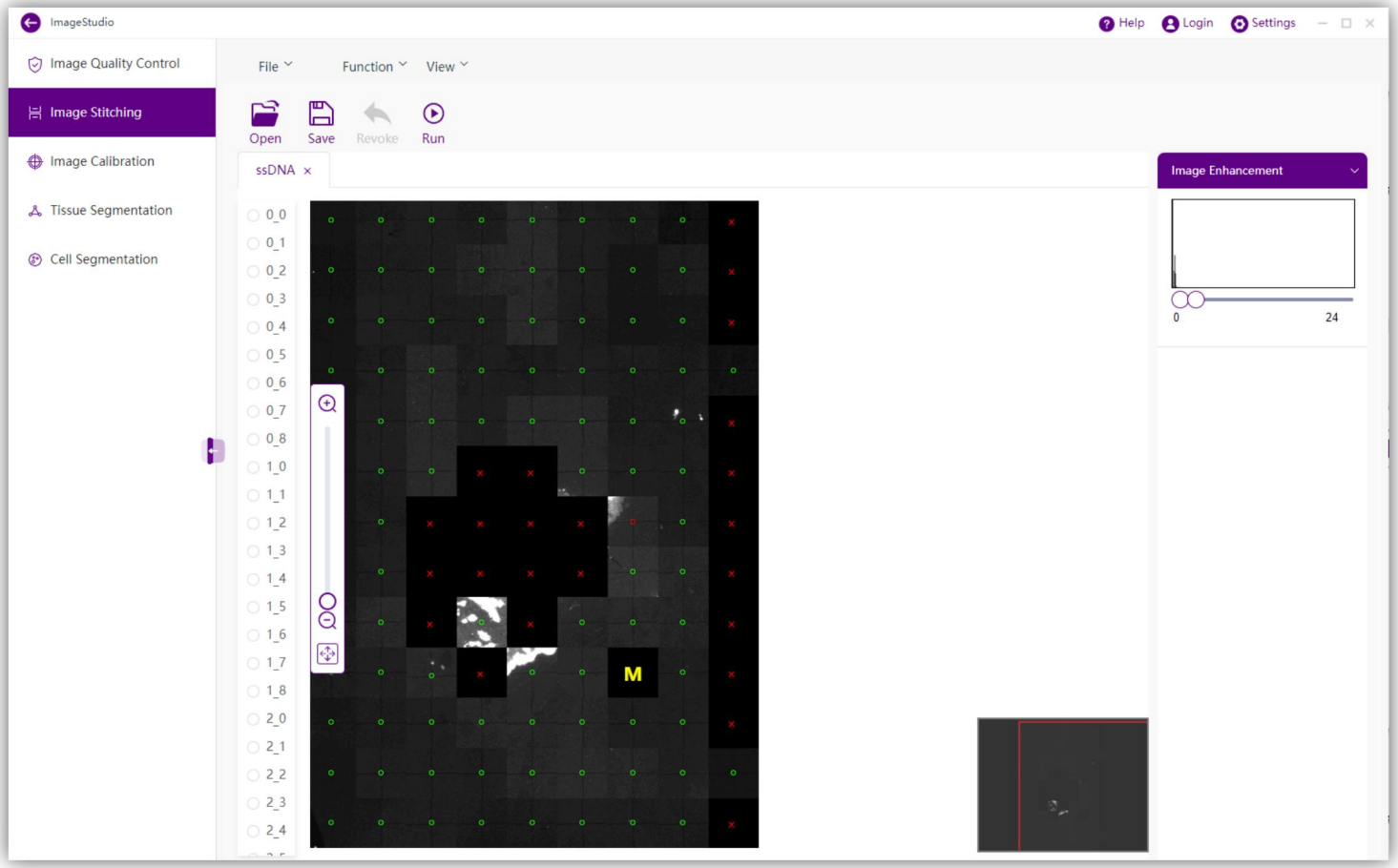
(5) Drag the slide bar of 'Image enhancement' to display the trackline of the image.



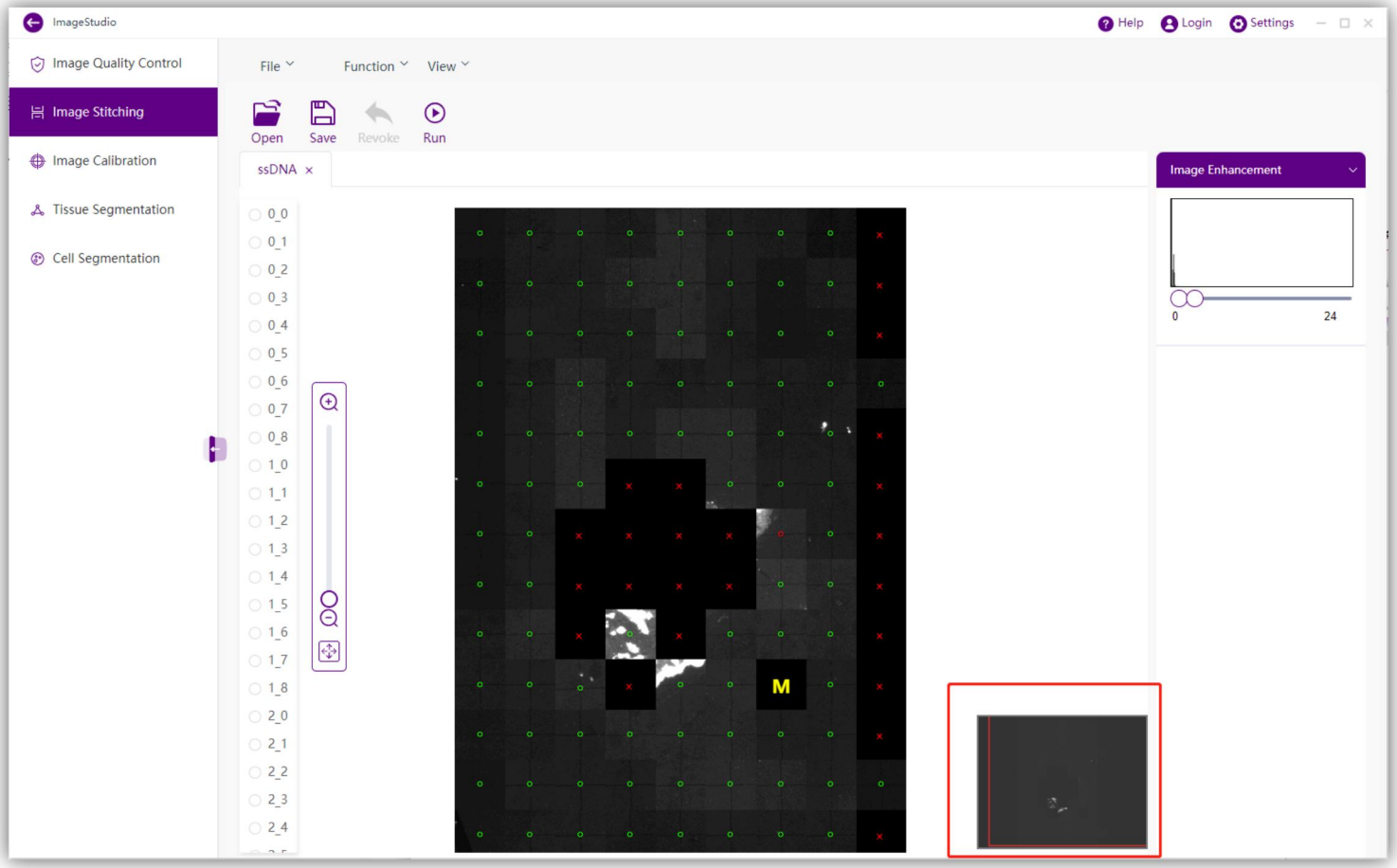
(6) Press and hold Ctrl+ while using the mouse wheel or click on the magnifying glass icon to zoom in or out of the image. Use this function to get a closed up look of the FOV and check whether the trackpoints have been marked correctly.



(7) Left-click mouse to move the positioning of the image.



(8) Left-click within the local view window to move the field of view.



4.3.2 Manual Adjustment of Trackline Template

The FOV marked with an M in the Global view is the template FOV, which is the best FOV evaluated by the trackline QC process. In order to estimate the image stitching performance, ImageStudio deduces

the quality of trackline alignment of the stitched image according to the tracklines identified by the template FOV through a proprietary algorithm which checks whether the tracklines identified by the template FOV (in green) coincide with the tracklines of the bottom image.

Decription of each type of icon:

X:QC undetected track point, which requires user assistance to manually mark the track point.

M:Best template FOV determined via QC, which requires user assistance to confirm the quality of marked tracklines via algorithms.

○: Stitching error evaluated by the algorithm is less than 5 pixels, which requires user assistance to confirm the stitching quality.

○: Stitching error evaluated by the algorithm is greater than 5 pixels, which requires user assistance to manually mark the track points.

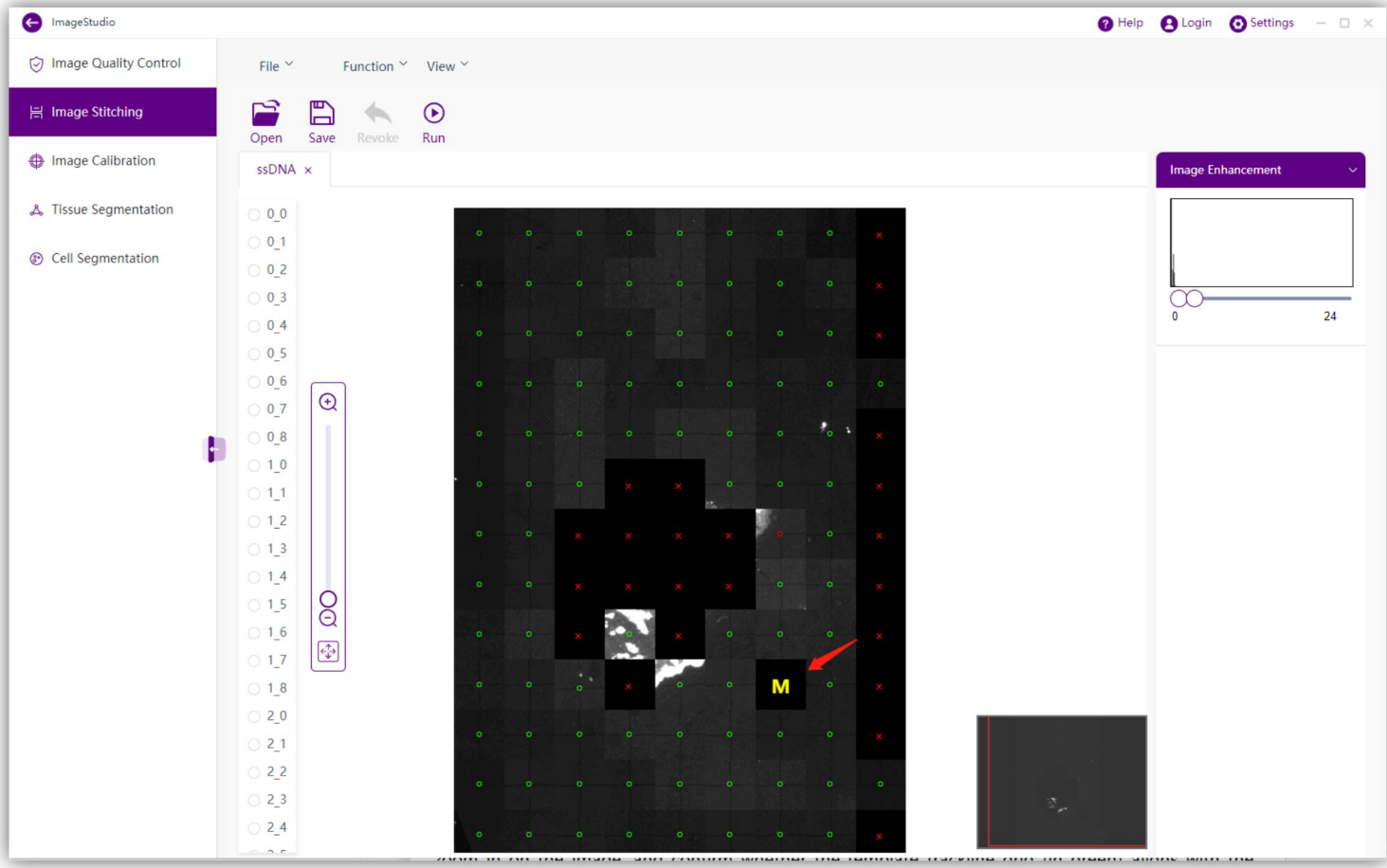
Suggested user actions:

- Check whether the M point mark is correct. If not, manual adjustment of the M point mark is required.
- Check for the FOV marked by red circles, since the stitching error evaluated by the algorithm for this FOV is greater than 5 pixels (required).

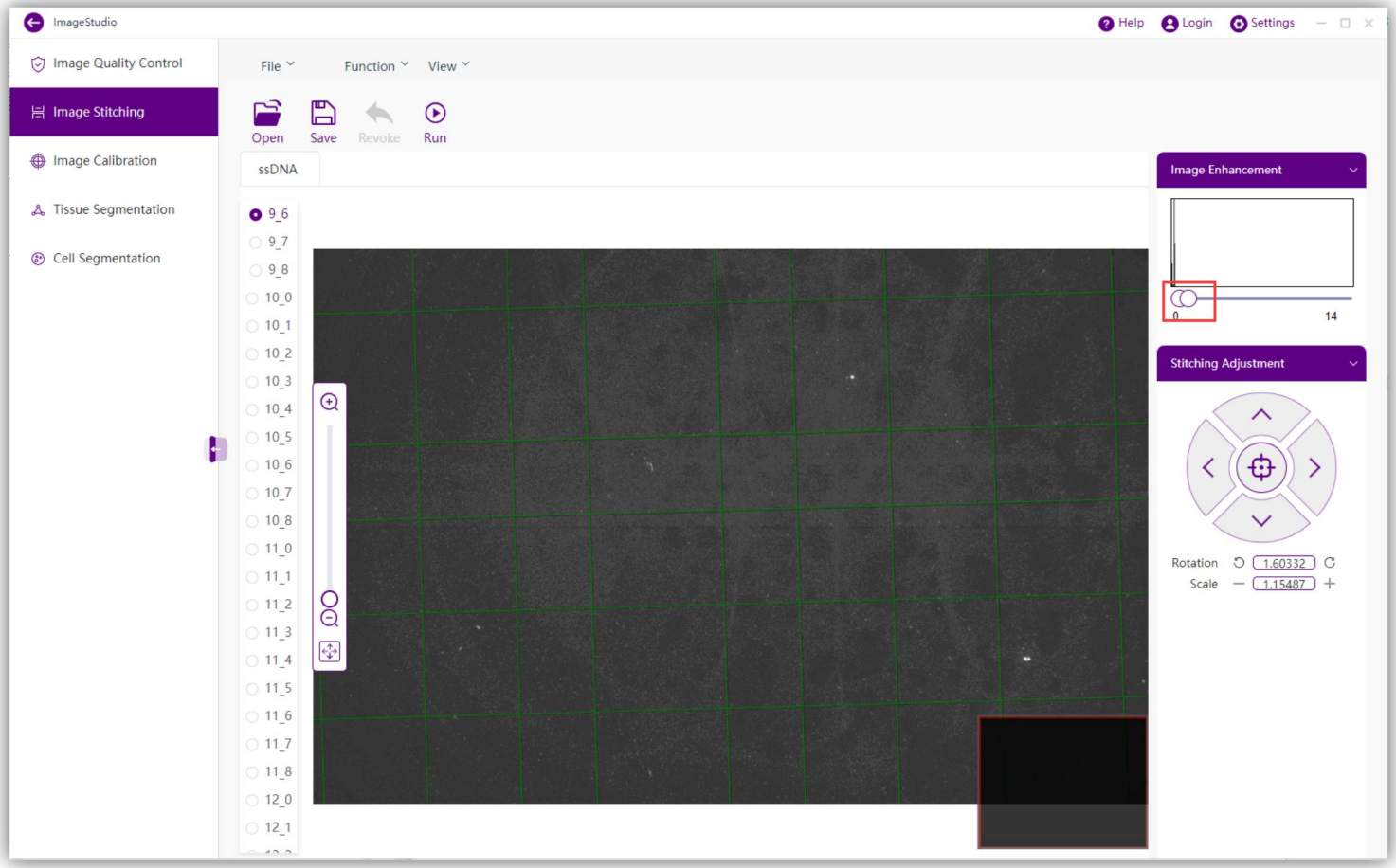
- Check for the FOV marked with a red X (tracklines cannot be found by the alforithm). Users need to manually mark the track points (optional).
- Check and confirm the FOV marked by green circles (optional).

(1) FOV marked with the M point: Mainly adjust the trackline grid and align the algorithm generated trackline grid (in green) with tracklines on the Stereo-seq Chip.

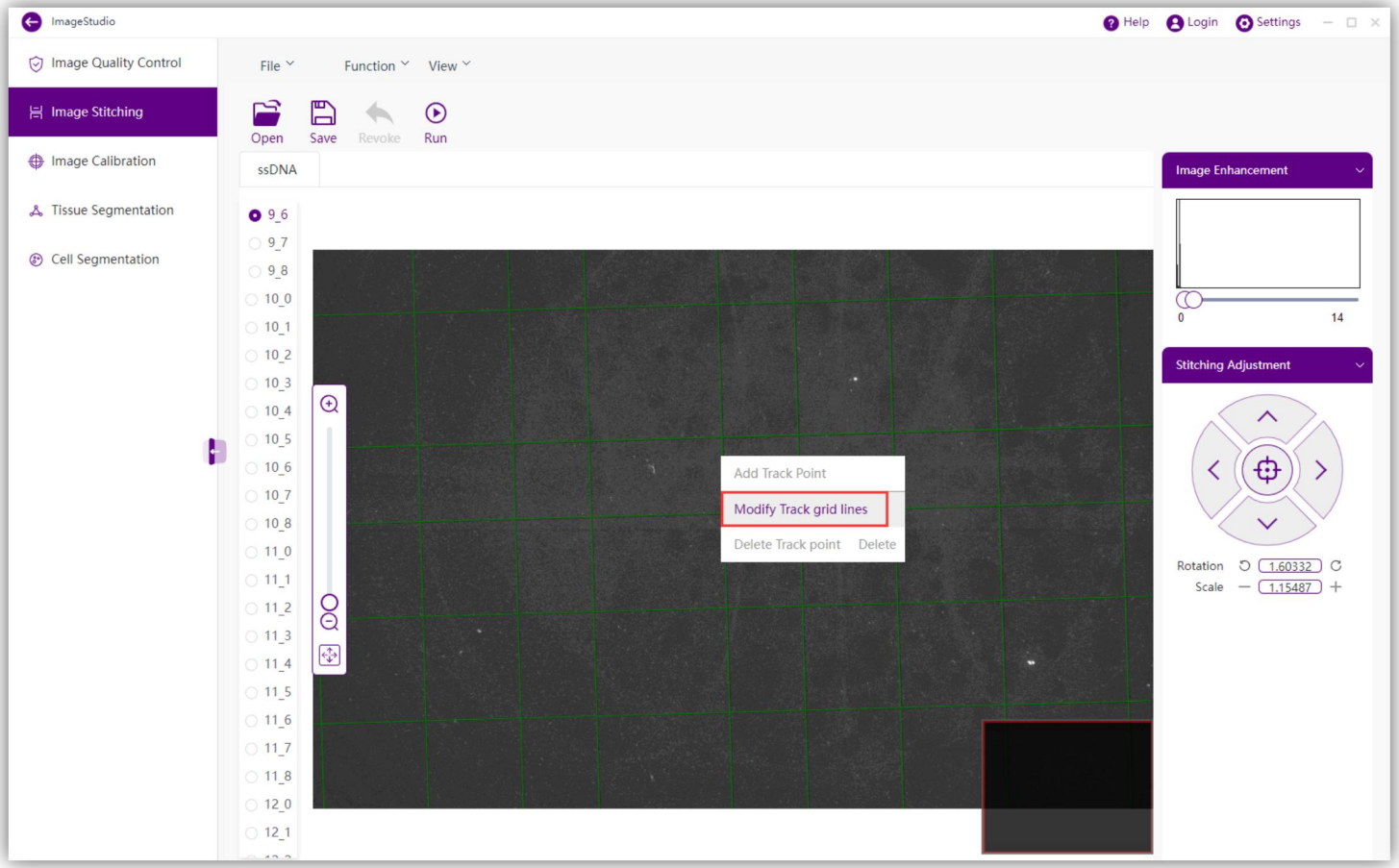
1. Double click to open the FOV marker with an M point or click the list of FOVs on the left to open.



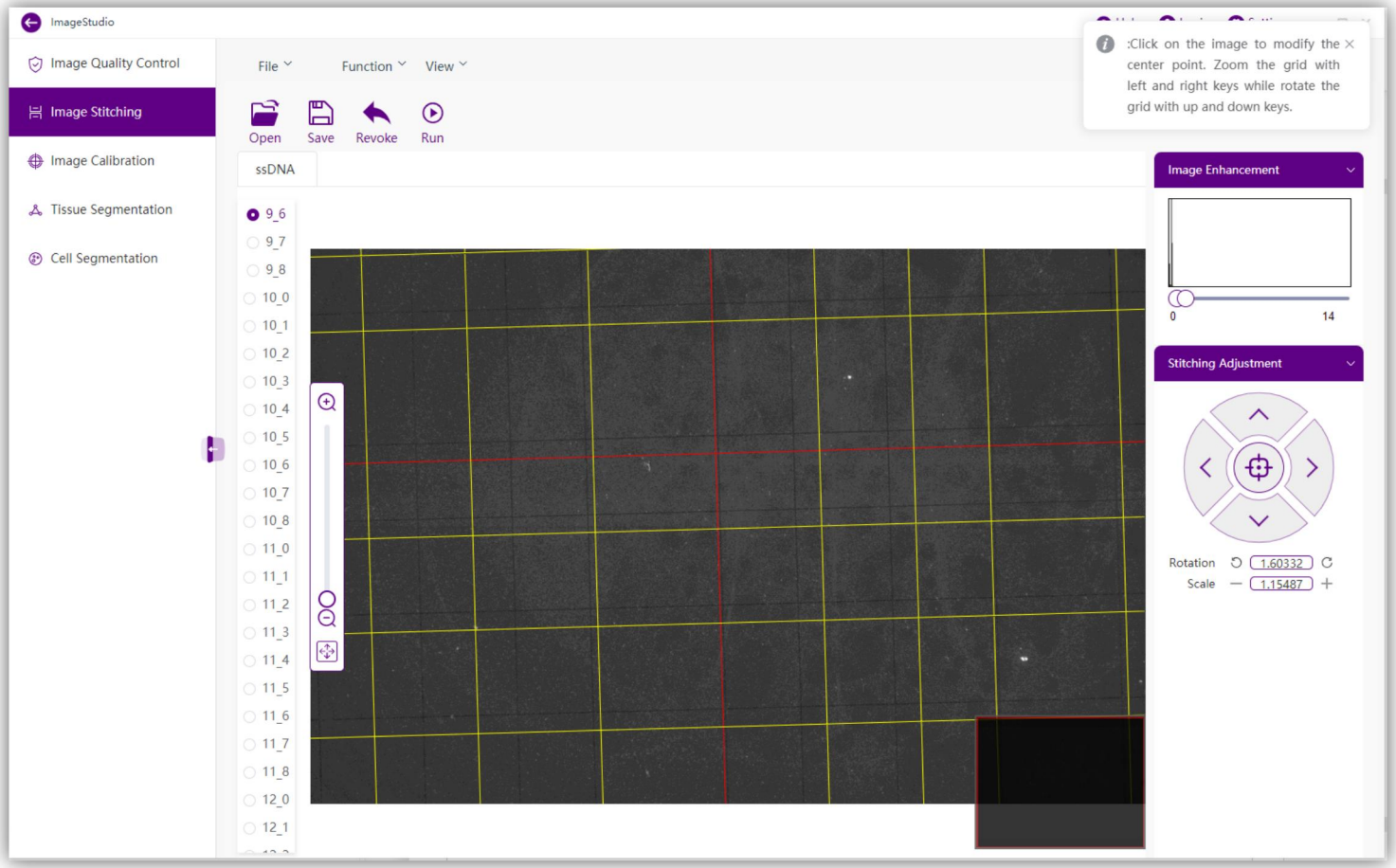
2. Drag the slide bar to enhance the image while pressing and holding Ctrl+ with mouse wheel to zoom in on the image, and confirm whether the template trackline grid (in green) aligns with the tracklines on the chip.



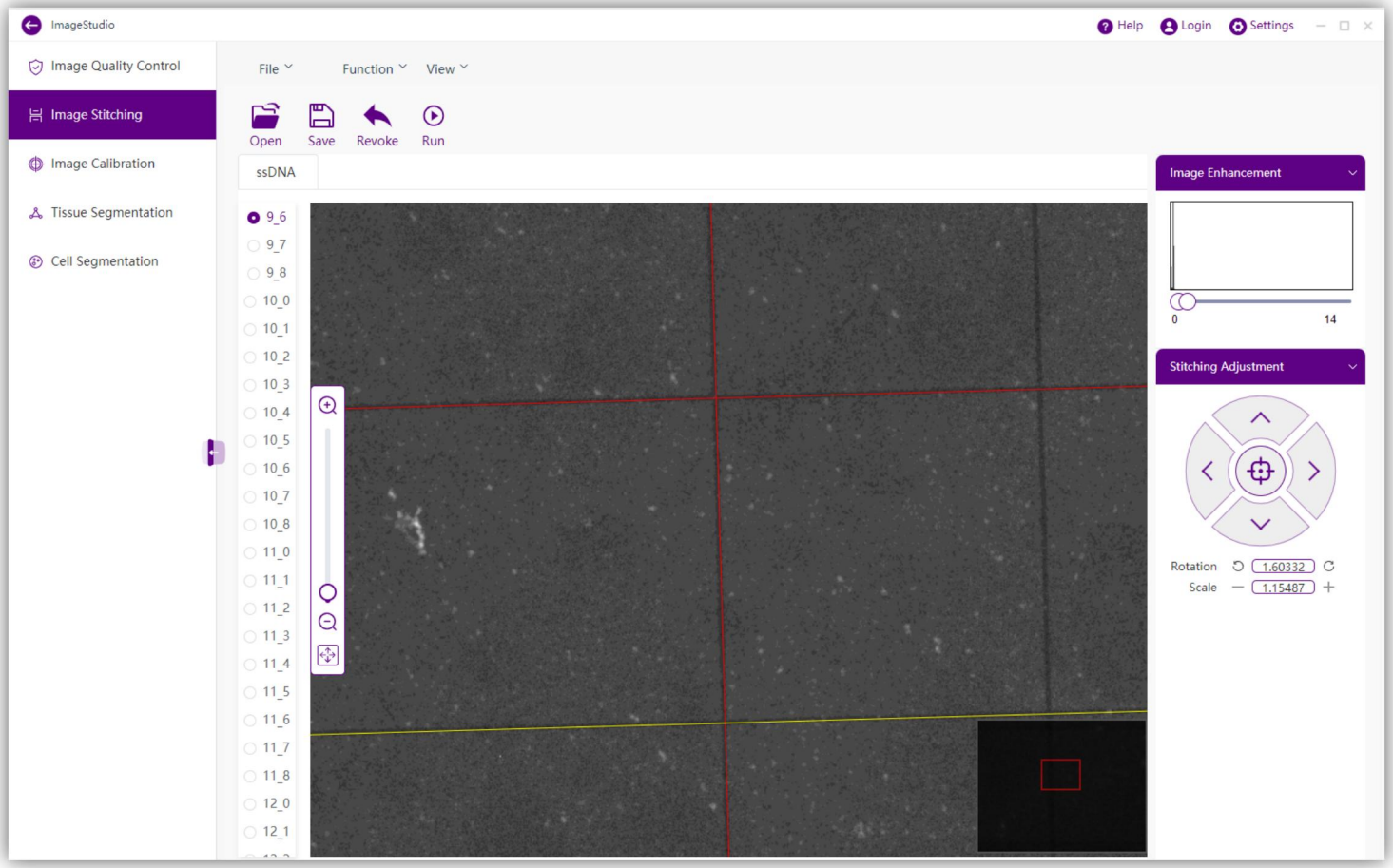
3. If the tracklines are not aligned, right-lick on the image and select 'Modify Trackline Grid'.



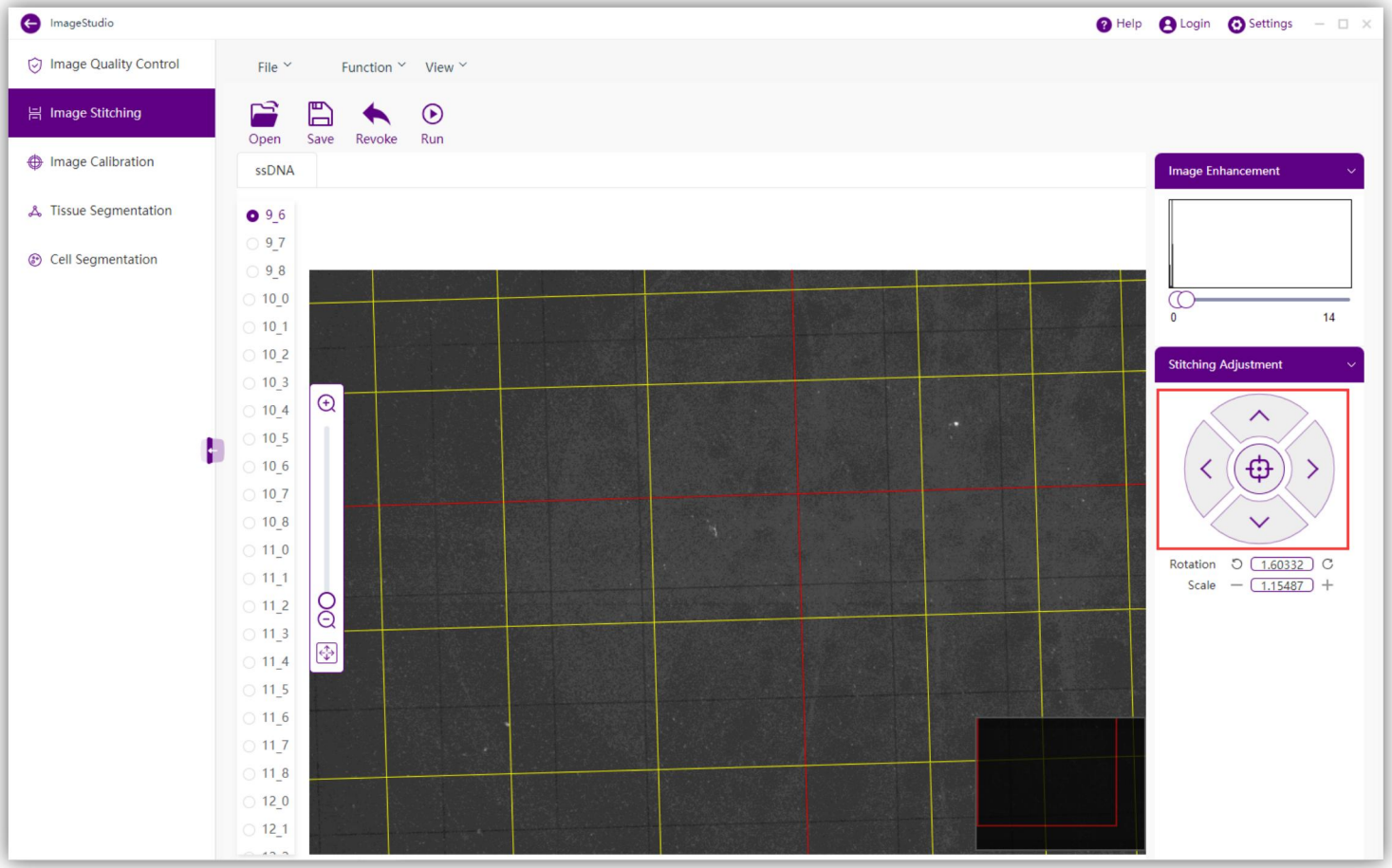
4. Then, red and yellow trackline grids will appear. Trackline grids shown in red represent tracklines that are within focus whereas yellow trackline grids are tracklines that are out of focus. Rotation, sclaing and other manual operations can only be done on the red tracklin grids.



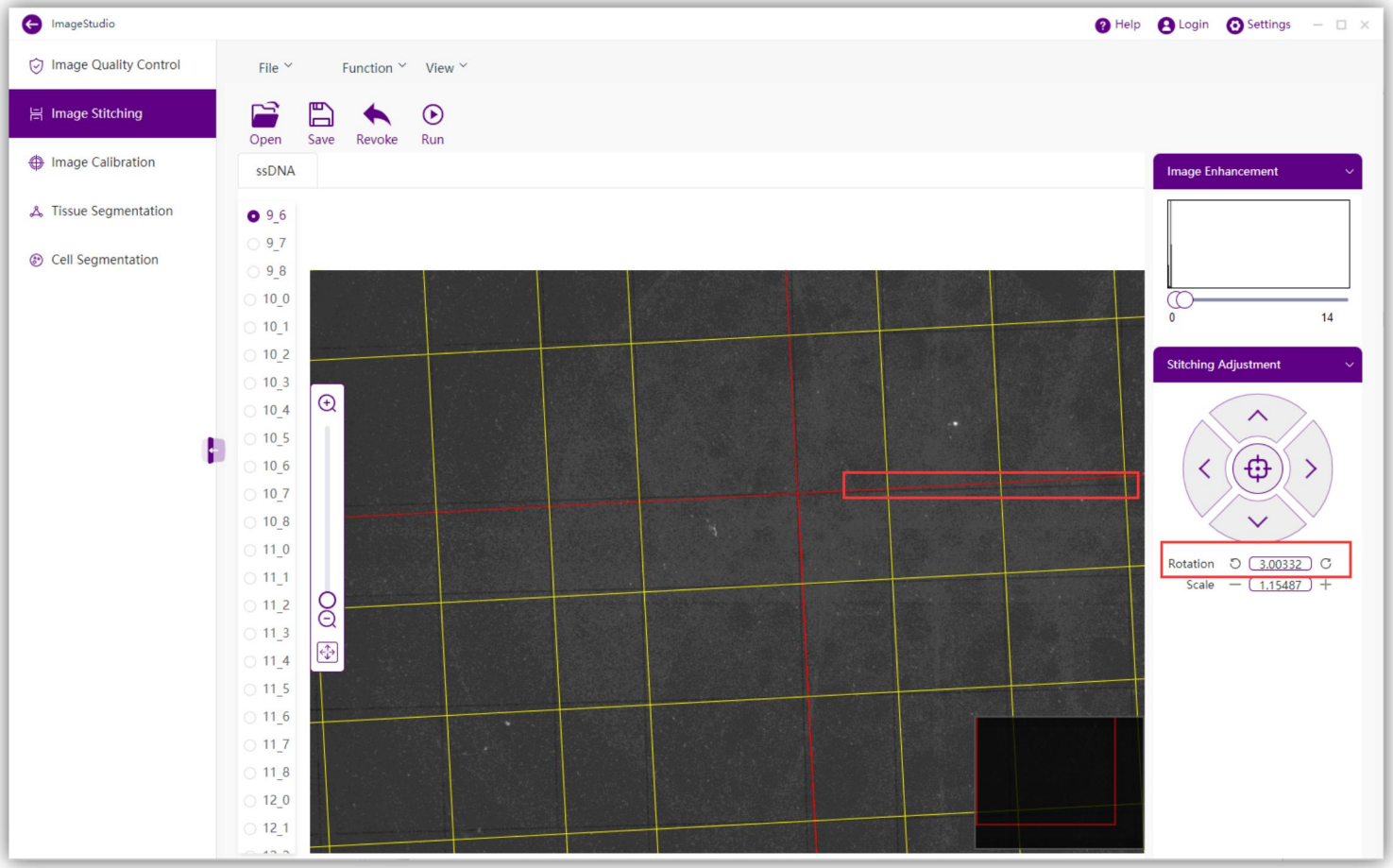
5. Find a clear track point and left-click to move the red trackline grids to the selected track point on the image.



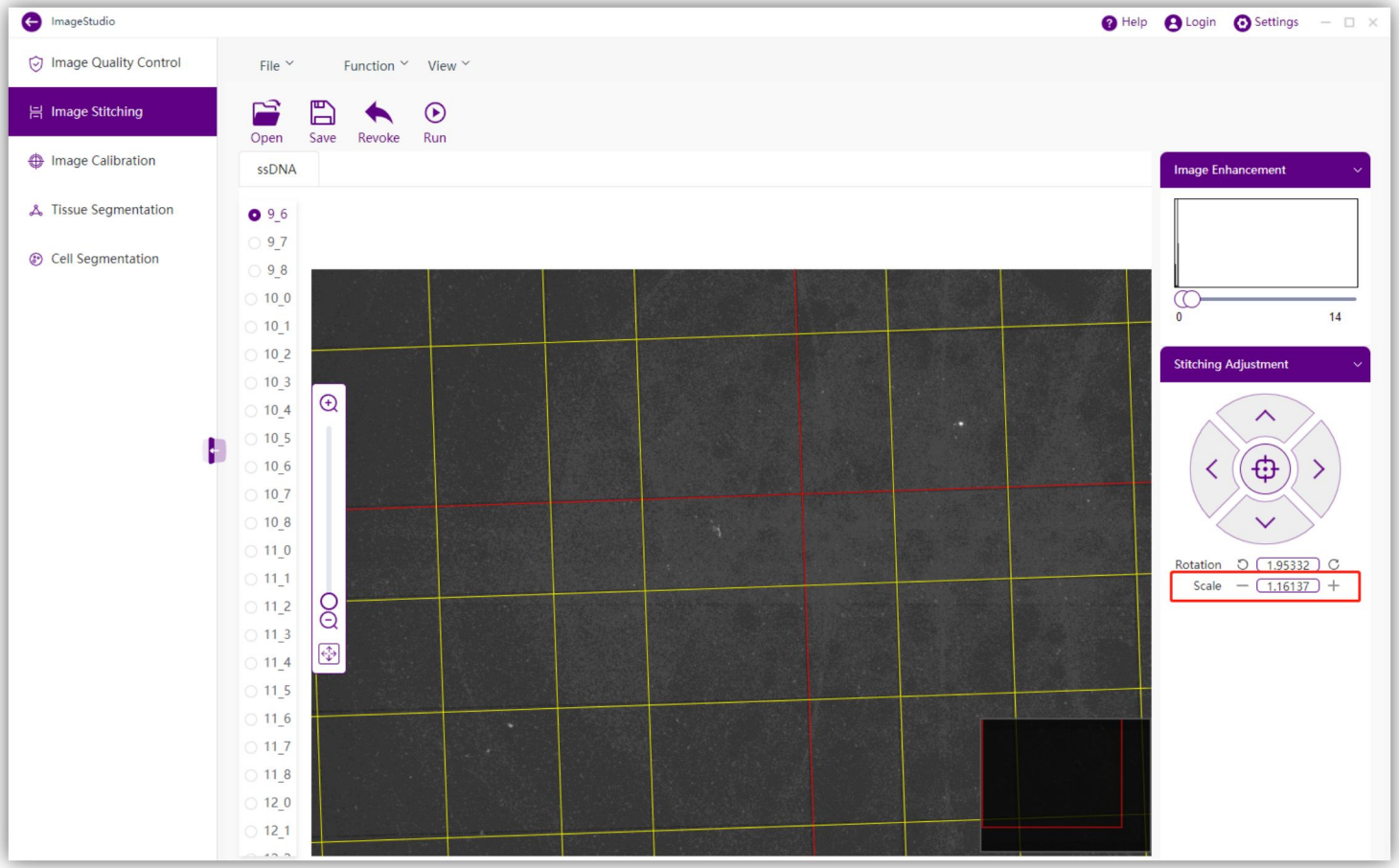
6. Zoom out the image, click the template to adjust the direction, adjust the horizontal and vertical directions, and ensure that the yellow line is aligned with the picture track, and the number of adjustments should not exceed 9 times at most.



7. Left-click on the plus and minus sign of ‘Rotation’ under 'Template Adjustment' to adjust the rotation angle of yellow trackline gird with red trackline grid as the center. Press ’ + ‘ to rotate clockwise and ’ -’ to rotate counter-clockwise.

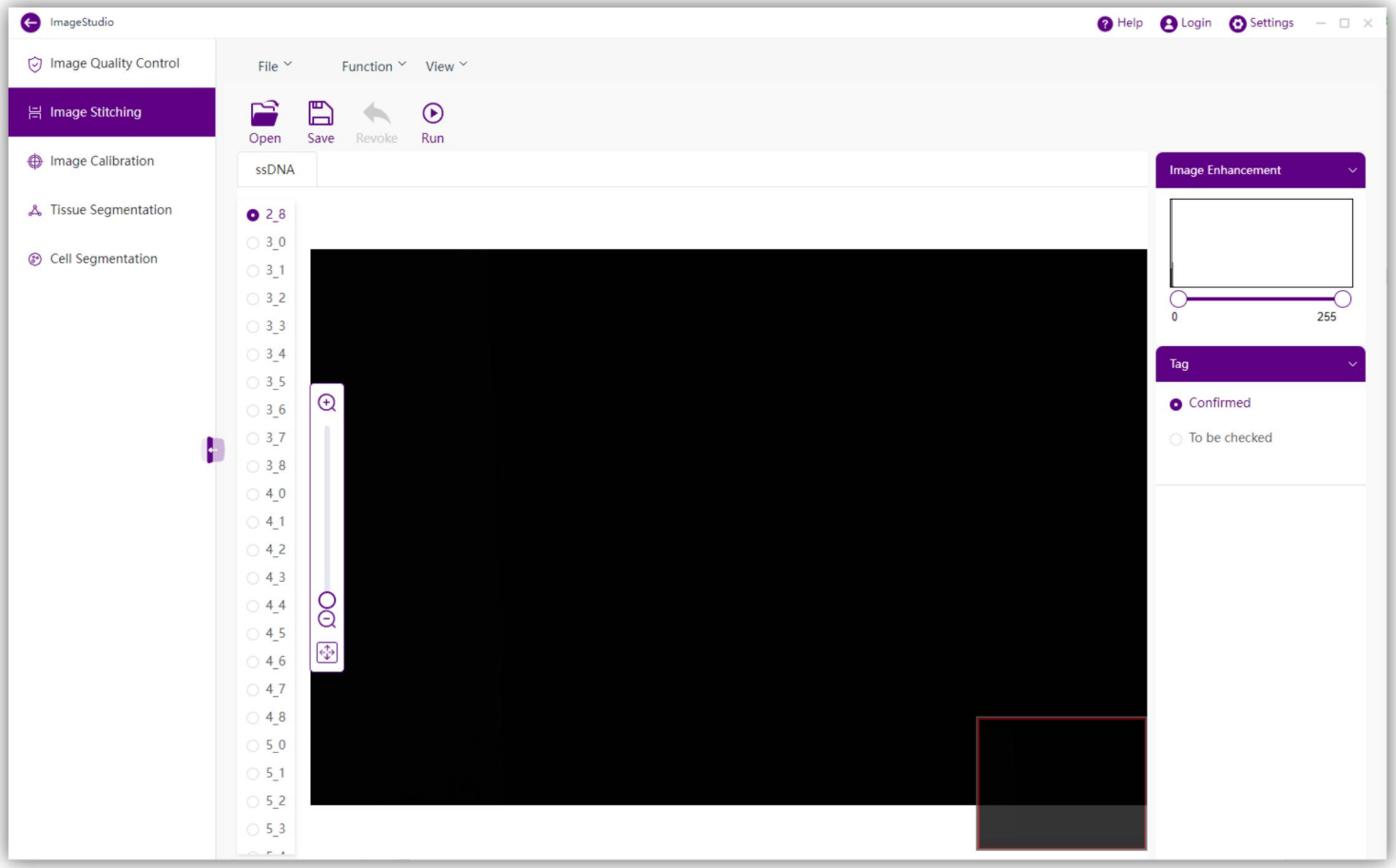


8. Left-click on the plus and minus sign of ‘Scale’ under 'Template Adjustment' to scale up and down the yellow trackline gird with red trackline grid as the center, respectively.

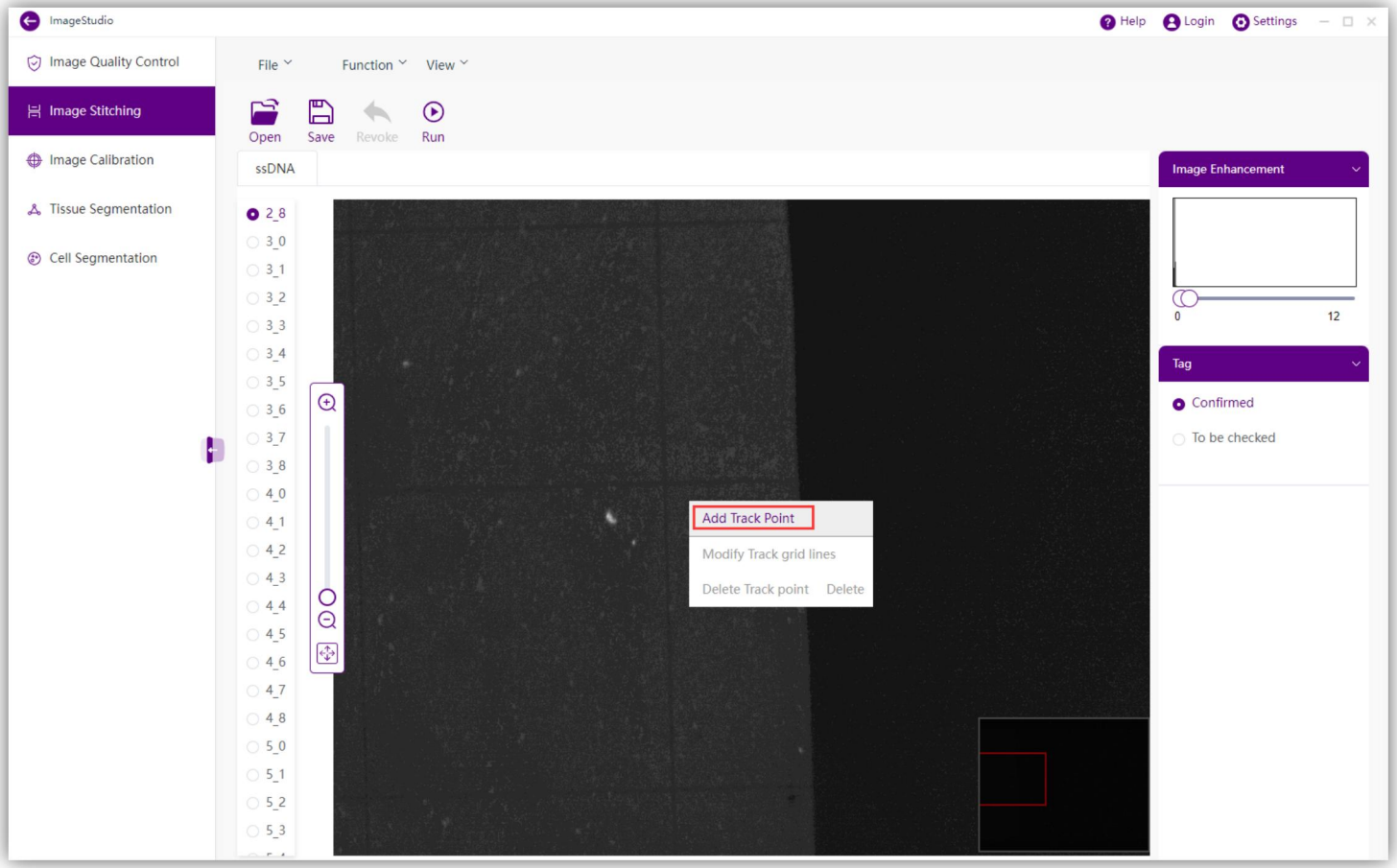


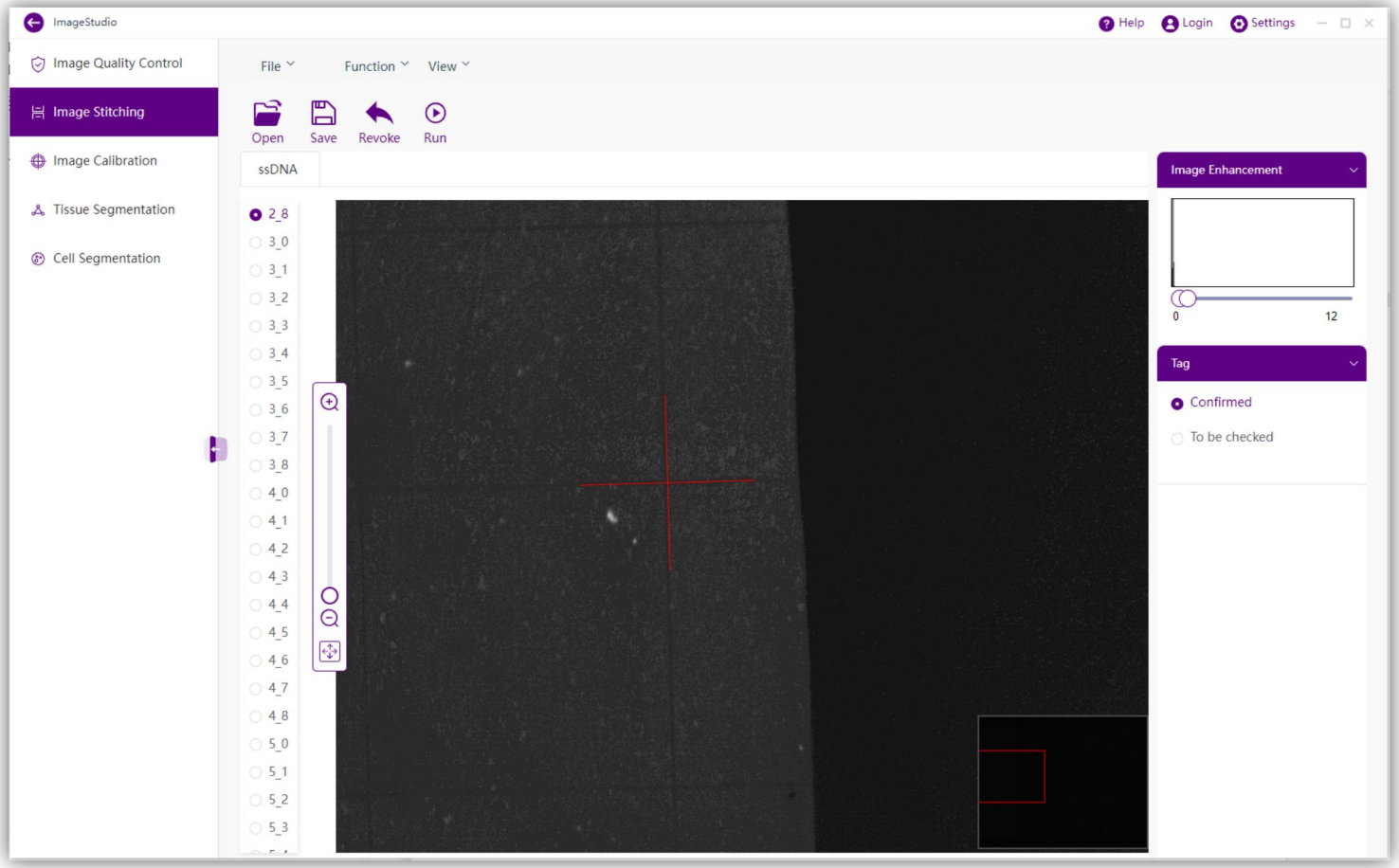
(2)Other FOV (non-template FOV): Mainly adjust the track points marked with red X and ○.

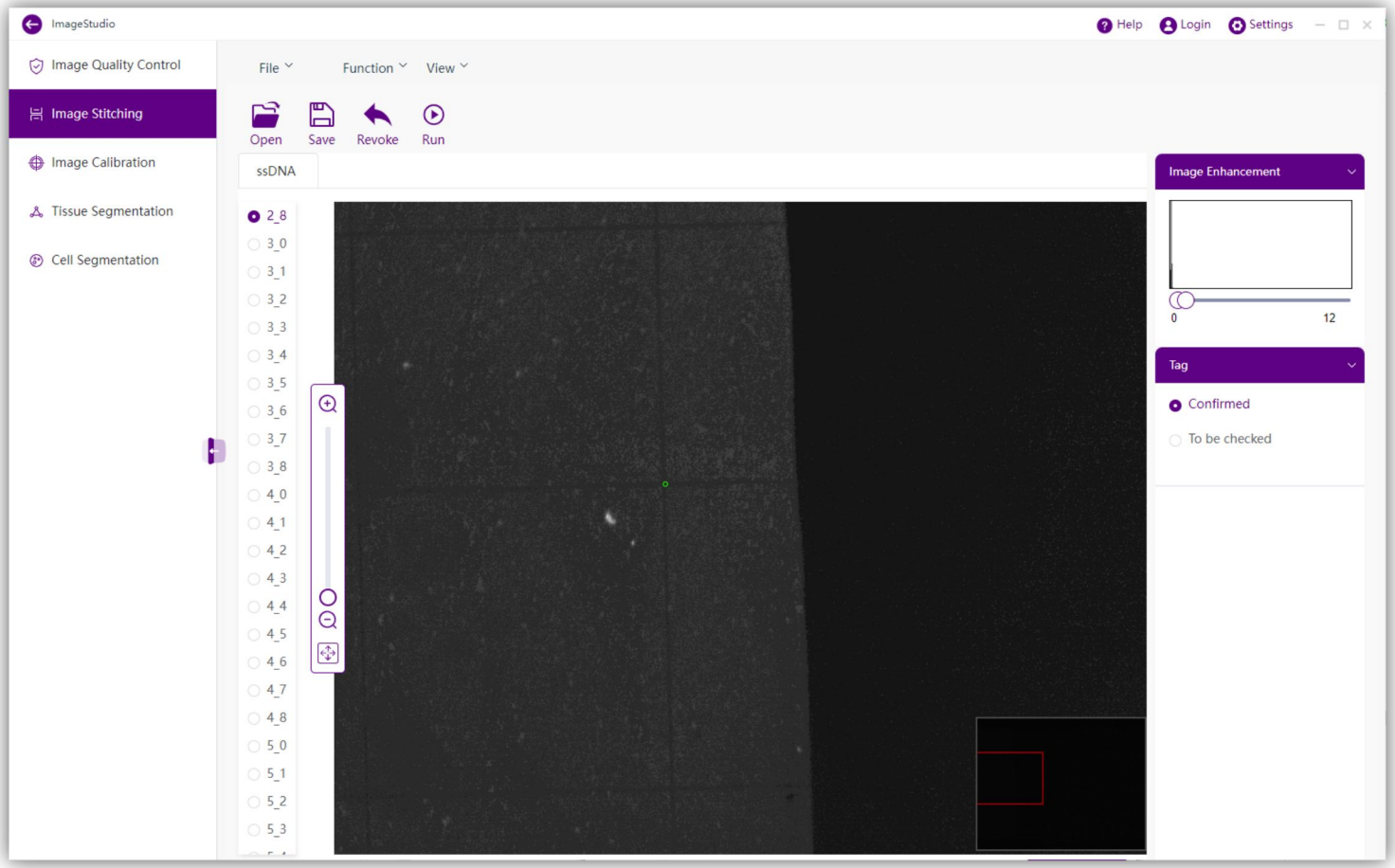
1. Double click on the FOV marked with redred X or ○ to start adjusting the position.



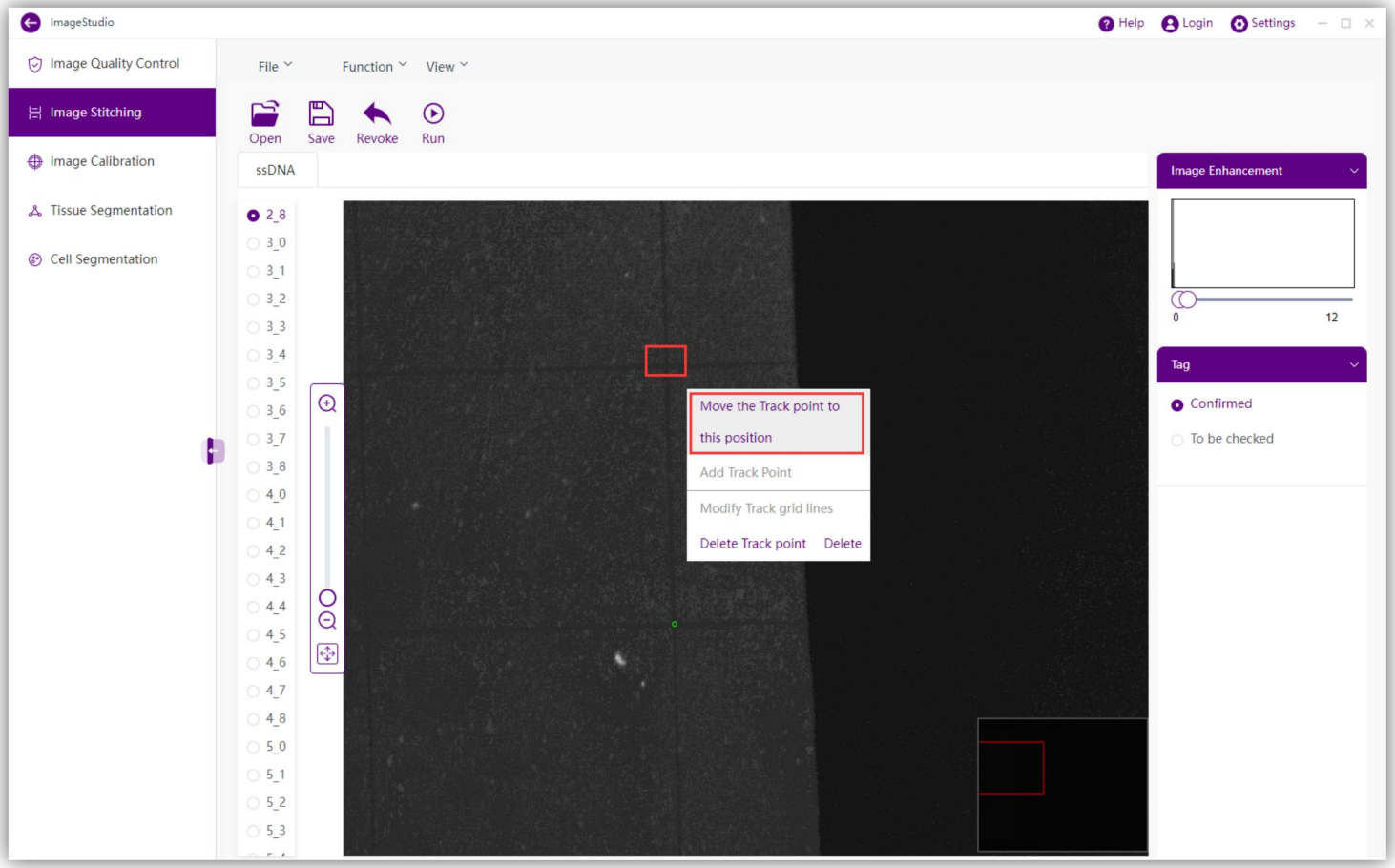
2. Find a clear crossed track point then right-click the intersection point to select ‘Add track point’. A green circle appears on the manually selected track point which can be repositioned to an actual trackline point on the chip. The green circle is shown as a red cross during repositioning to better align with the trackline on the chip.

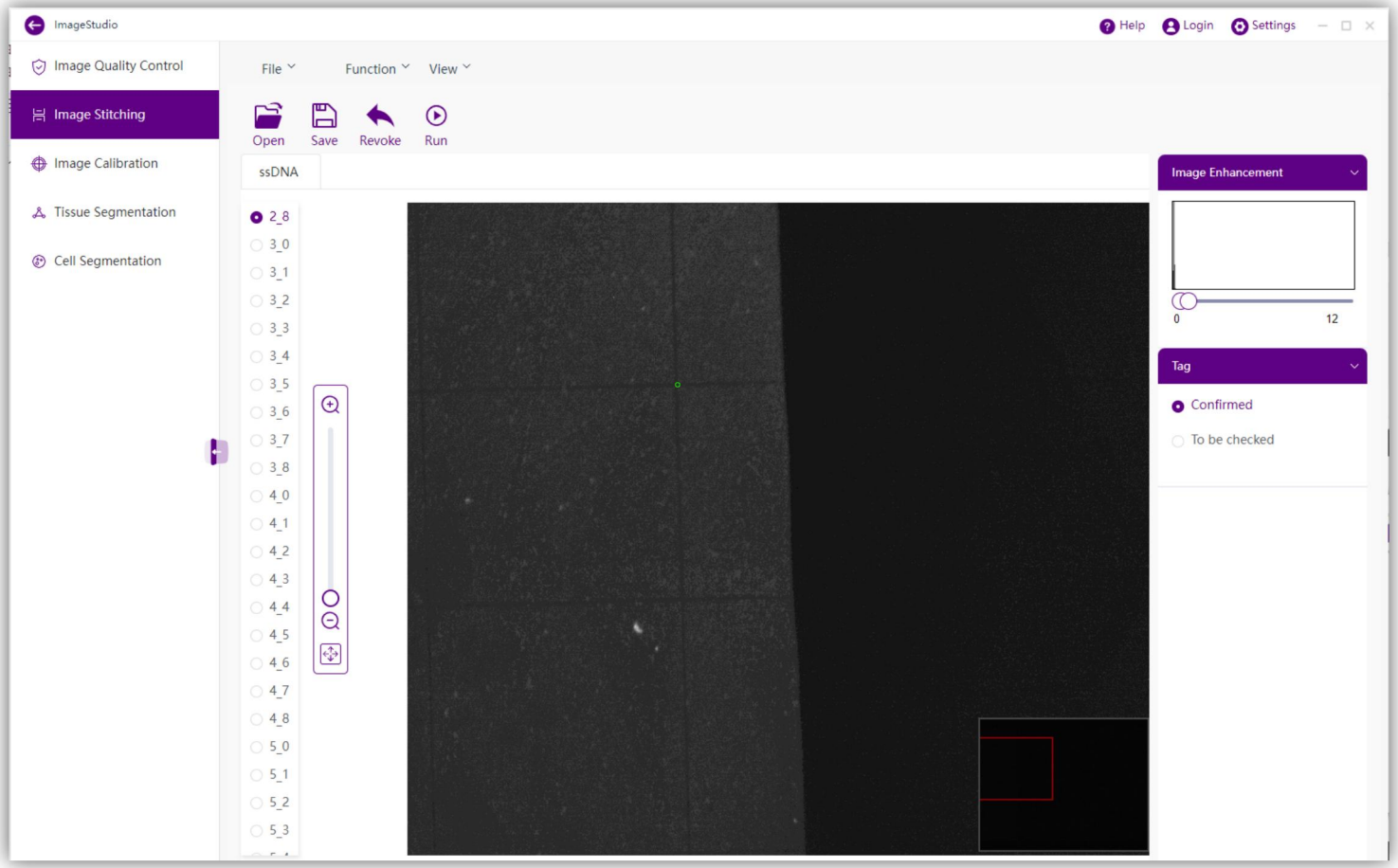

ImageStudio
Help
Login
Settings
Image Quality Control
Image Stitching
Image Calibration
Tissue Segmentation
Cell Segmentation
File
Function
View
Open
Save
Revoke
Run
ssDNA
2_8
3_0
3_1
3_2
3_3
3_4
3_5
3_6
3_7
3_8
4_0
4_1
4_2
4_3
4_4
4_5
4_6
4_7
4_8
5_0
5_1
5_2
5_3
Add Track Point
Modify Track grid lines
Delete Track point
Delete
Image Enhancement
0
12
Tag
Confirmed
To be checked


ImageStudio
Help
Login
Settings
Image Quality Control
Image Stitching
Image Calibration
Tissue Segmentation
Cell Segmentation
File
Function
View
Open
Save
Revoke
Run
ssDNA
2_8
3_0
3_1
3_2
3_3
3_4
3_5
3_6
3_7
3_8
4_0
4_1
4_2
4_3
4_4
4_5
4_6
4_7
4_8
5_0
5_1
5_2
5_3
Image Enhancement
0
12
Tag
Confirmed
To be checked

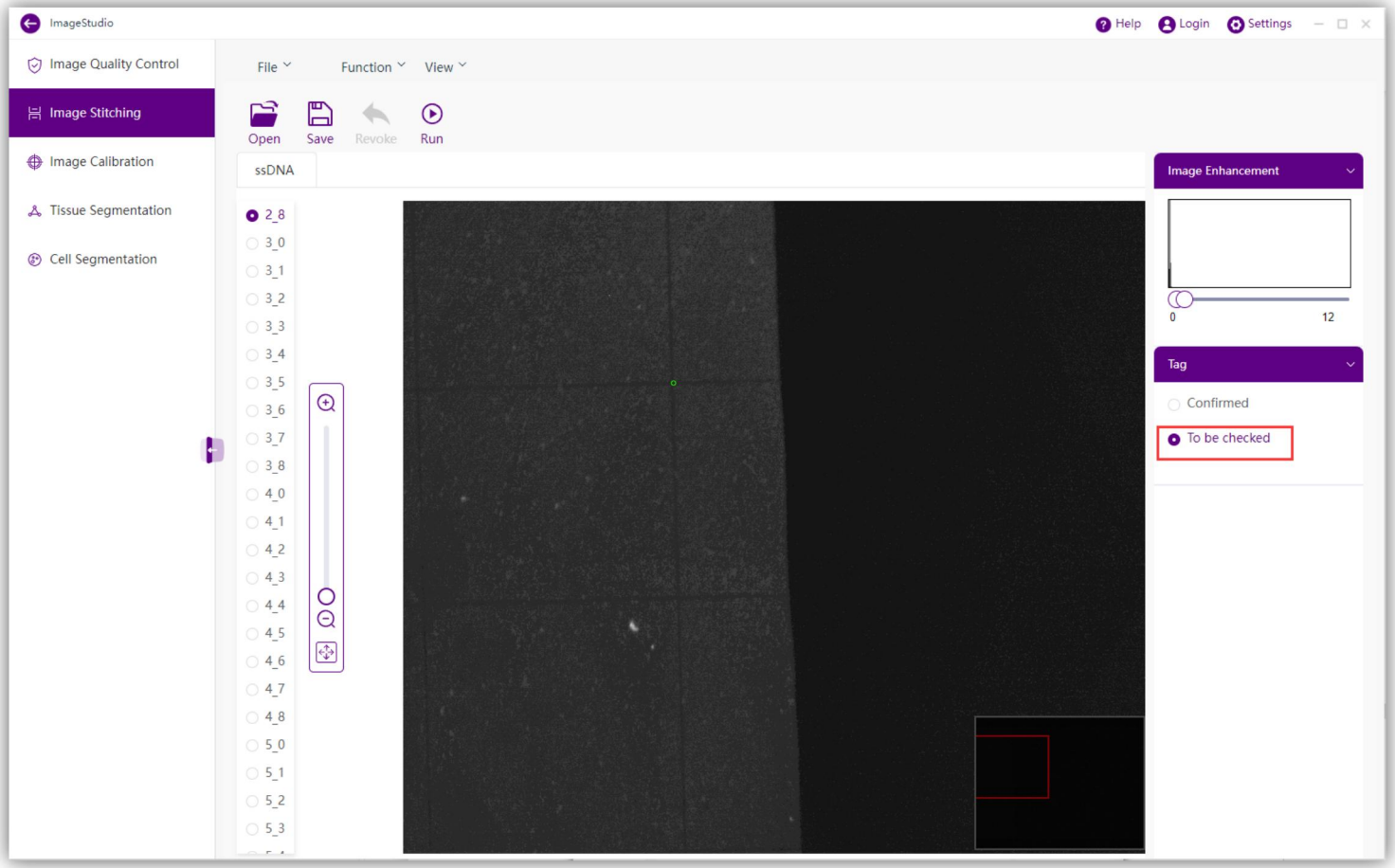


3. If unsatisfied with the repositioning, simply right-click and select 'Move the Track Point to this position' to move the green circle to the point of selection.

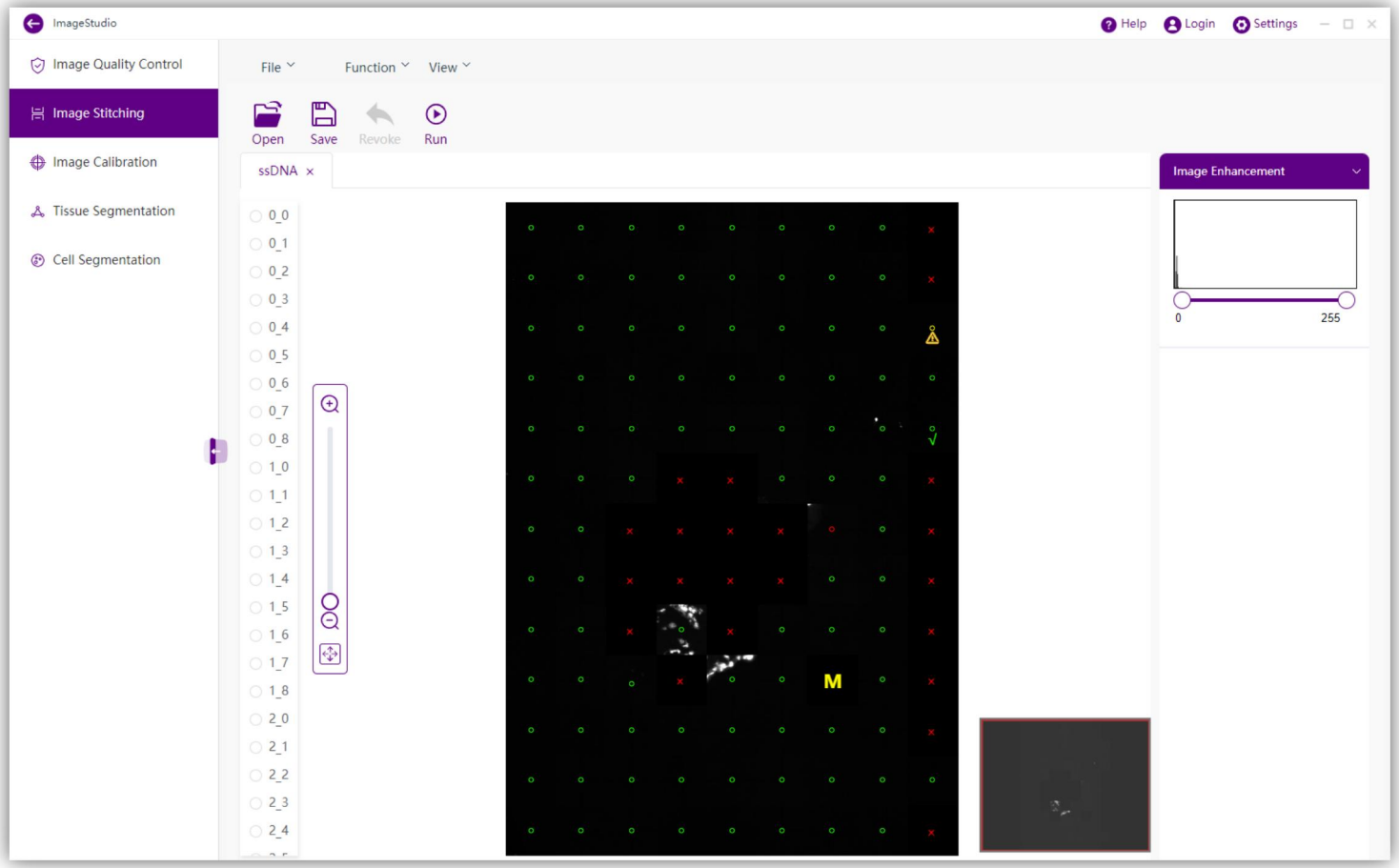

ImageStudio
Help
Login
Settings
Image Quality Control
Image Stitching
Image Calibration
Tissue Segmentation
Cell Segmentation
File
Function
View
Open
Save
Revoke
Run
ssDNA
2_8
3_0
3_1
3_2
3_3
3_4
3_5
3_6
3_7
3_8
4_0
4_1
4_2
4_3
4_4
4_5
4_6
4_7
4_8
5_0
5_1
5_2
5_3
Move the Track point to this position
Add Track Point
Modify Track grid lines
Delete Track point
Delete
Image Enhancement
0
12
Tag
Confirmed
To be checked



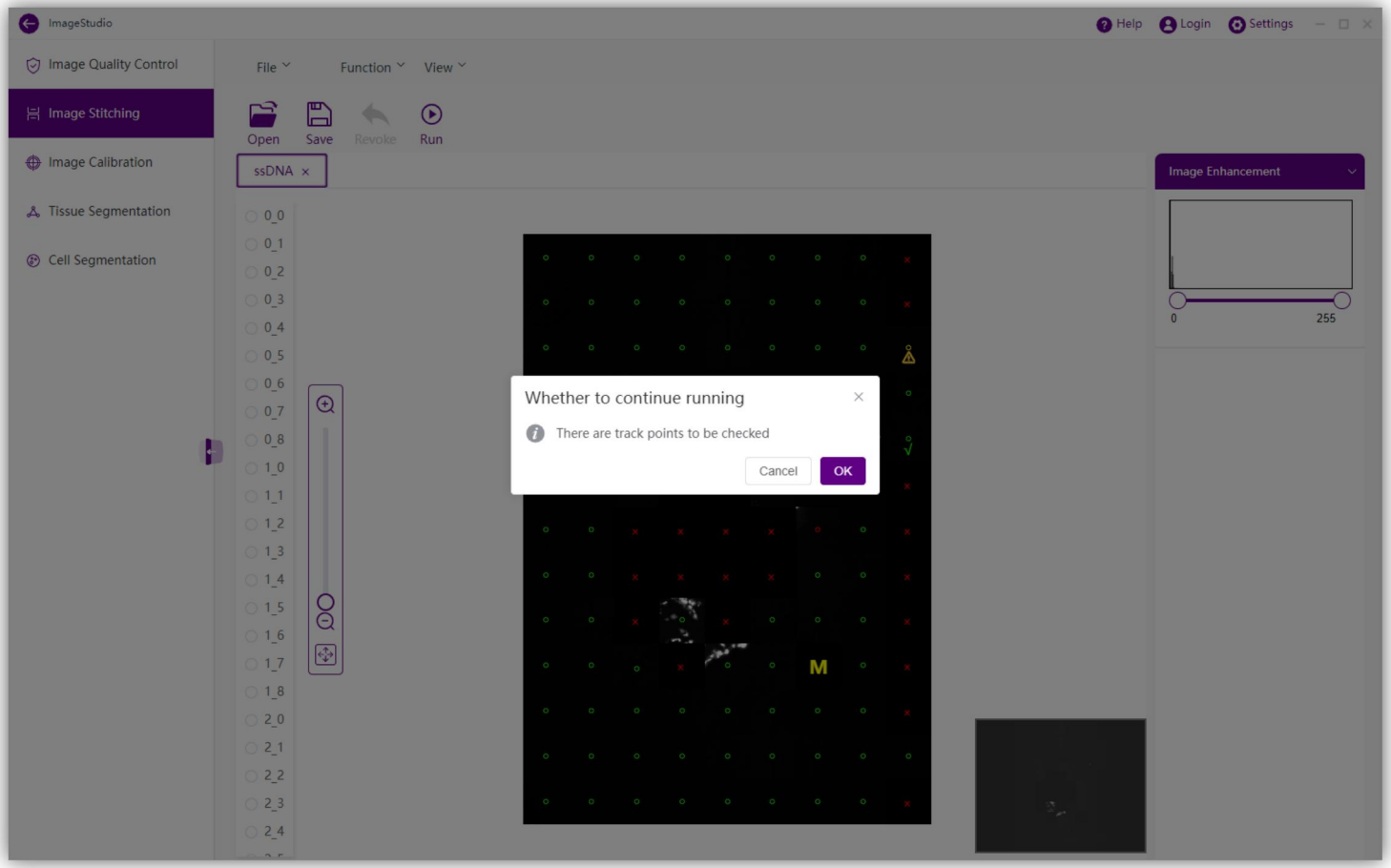
4. Once the modification is done, the default status after modification is set to ‘Confirmed’. If a certain FOV position is still unsured, set the status to Pending'.



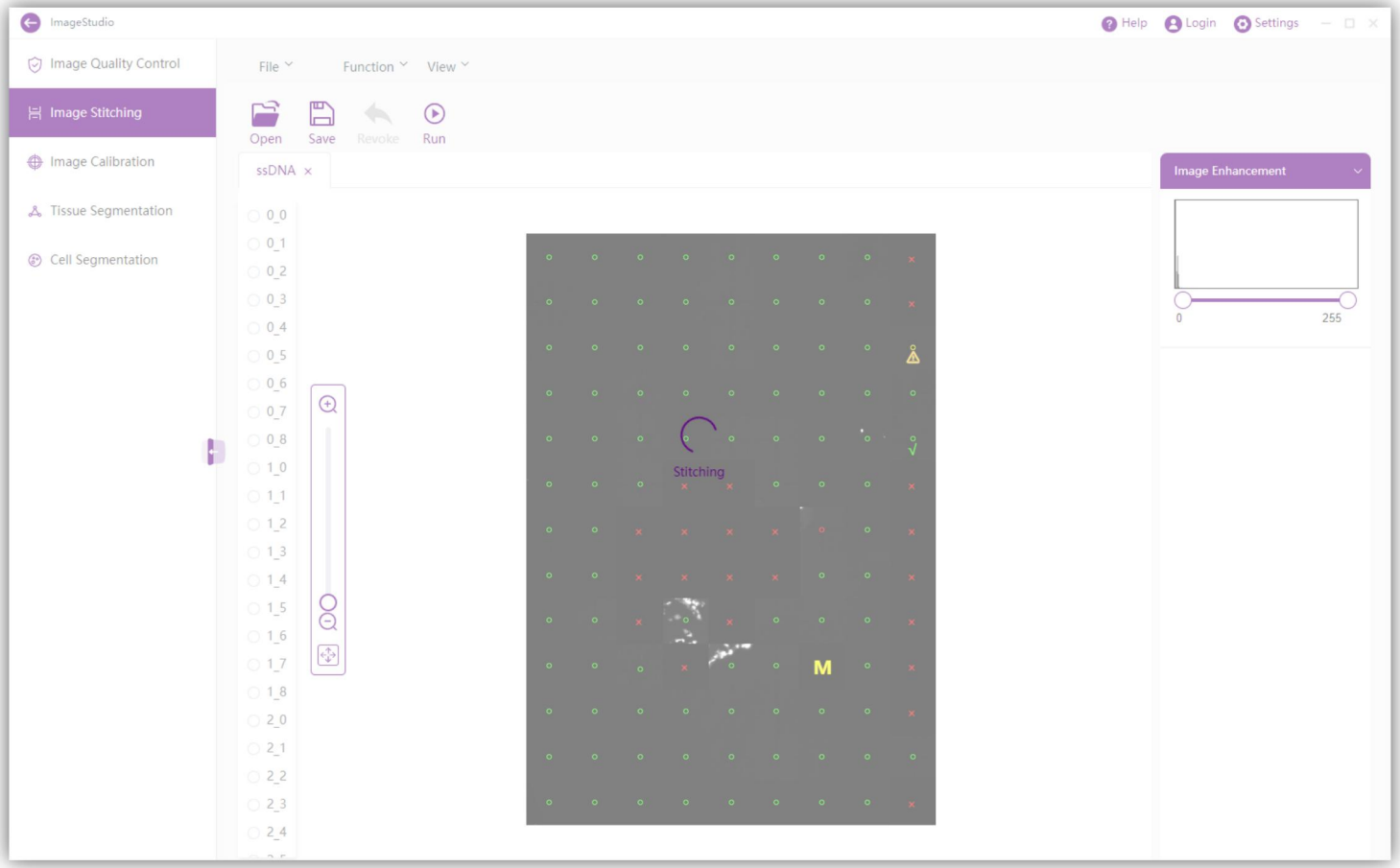
5. After modification, return to the global view. The 'Confirmed' FOVs are indicated by a green circle and √ sign, while the 'Pending' for verification ones are indicated by a yellow exclamation sign.



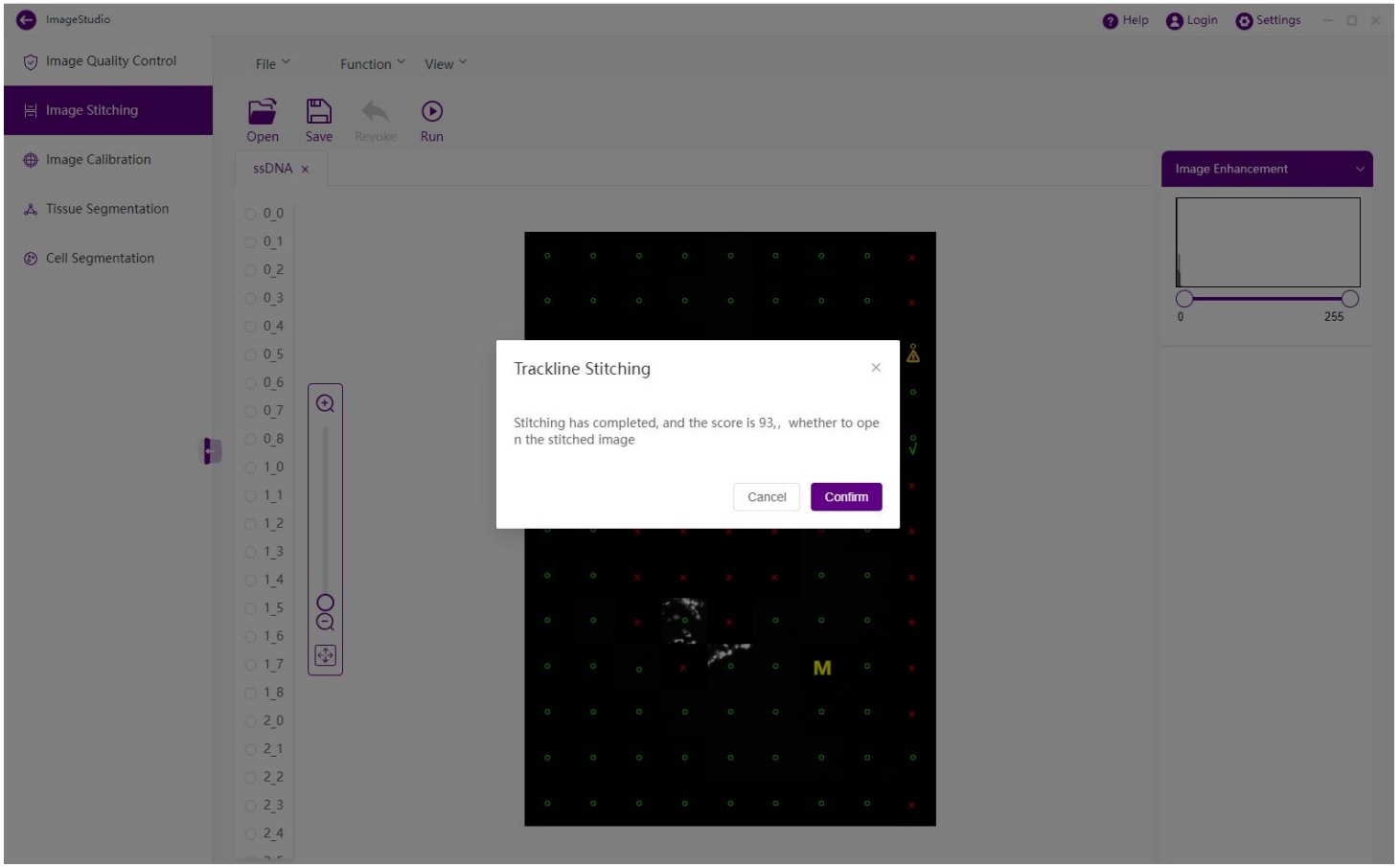
6．Once all the FOVs within the global view have completed modification, click ‘ Run’. If 'Pending' FOV are left to be checked, the system will ask whether to run after checking.



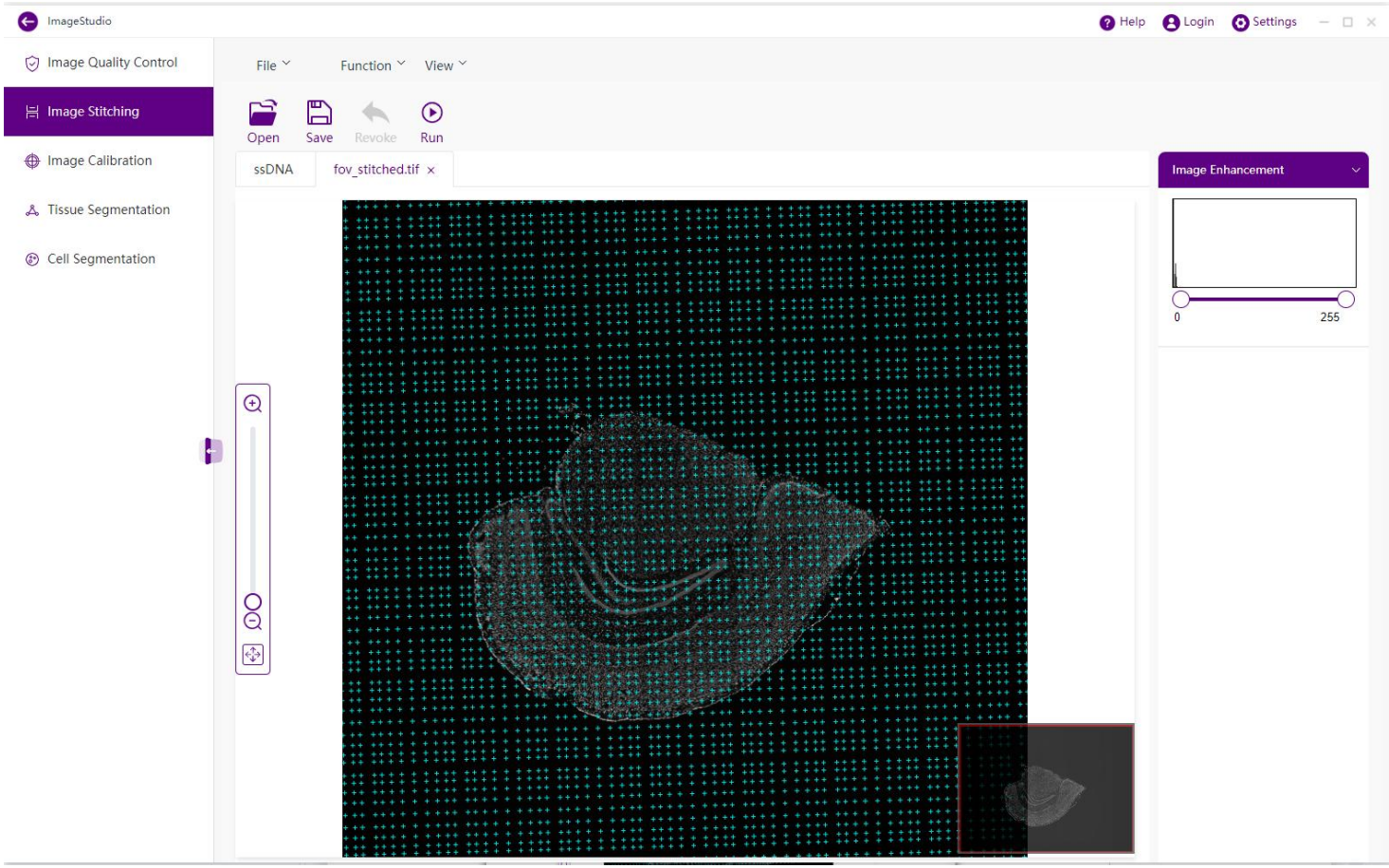
7. If all the FOVs have been confirmed, click ' Run' and the system will initiate re-stitching of the FOV images based on the manually selected track points for each FOV within the global view.



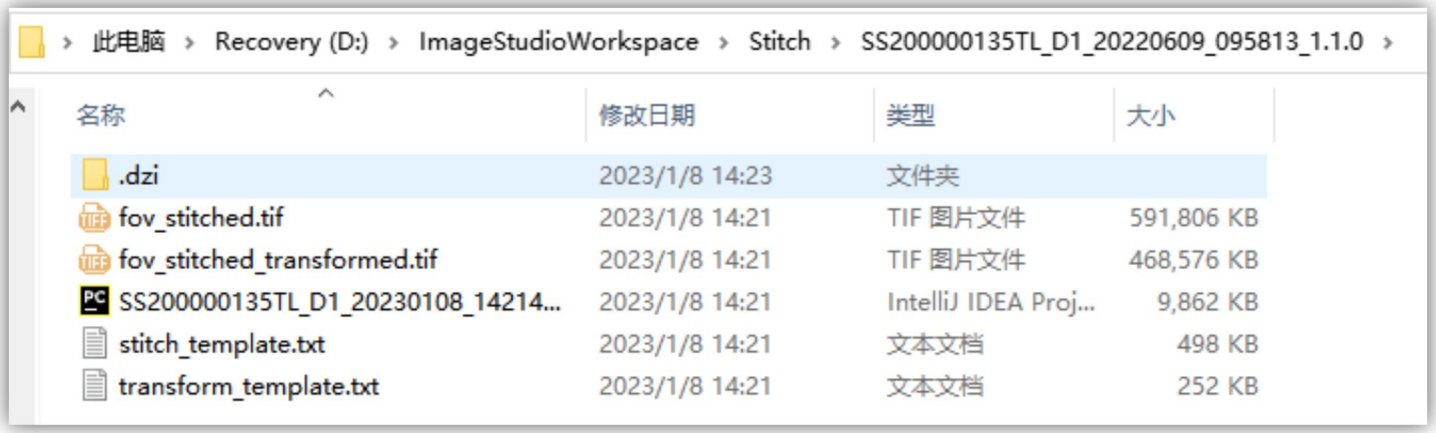
8. Once the stitching process has been completed, a score will be given based on the evaluated re-stitching quality and the system prompts whether to open and check the stitched image.



9. Click 'Confirm' to open the image; The deduced template trackline will be mapped on to the image. Check whether the mapped trackline conincidice with the trackline on the Stereo-seq Chip.



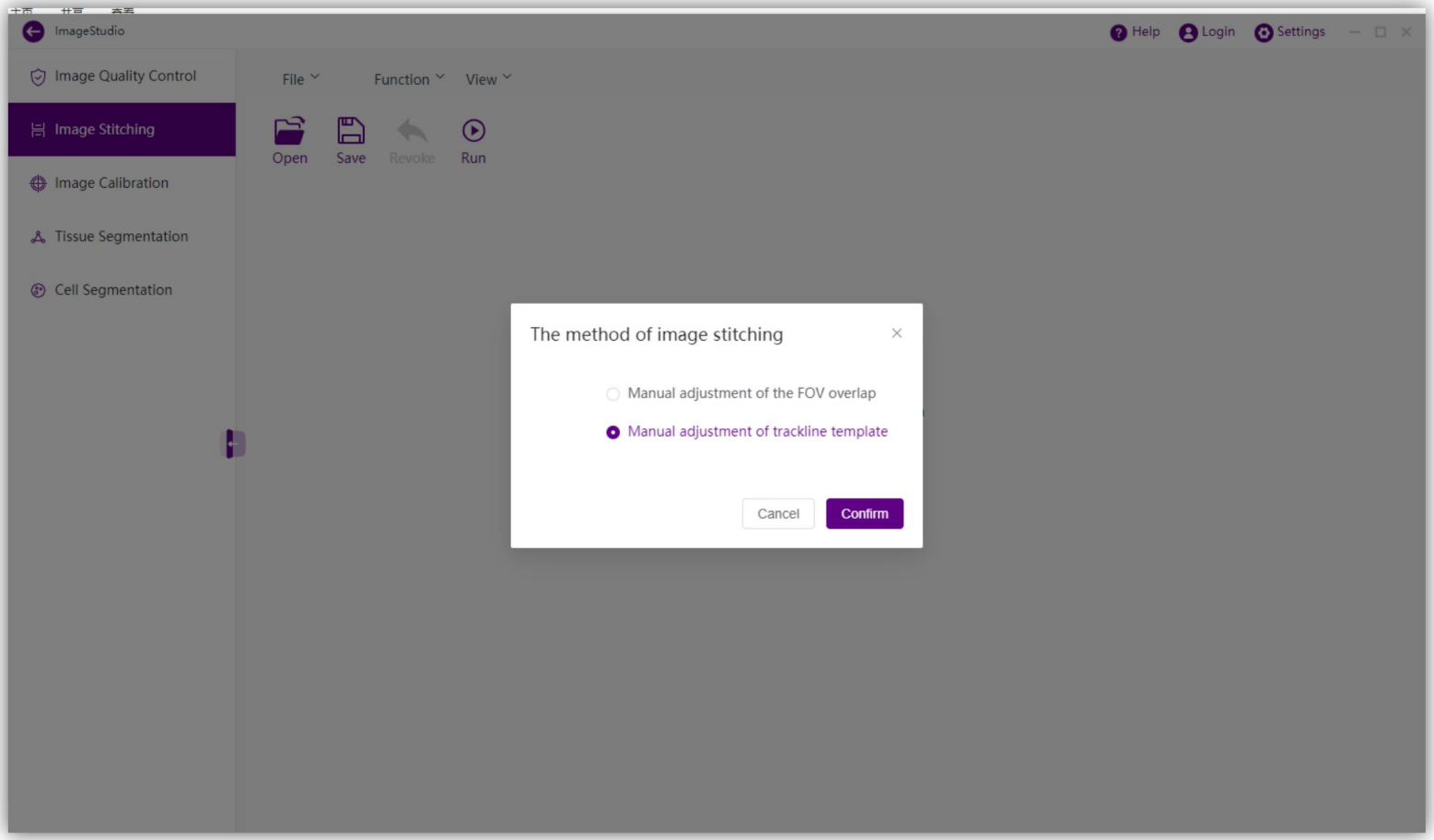
10. Final output files will be saved to the default directory: *D:\ImageStudioWorkspace\Stitch*



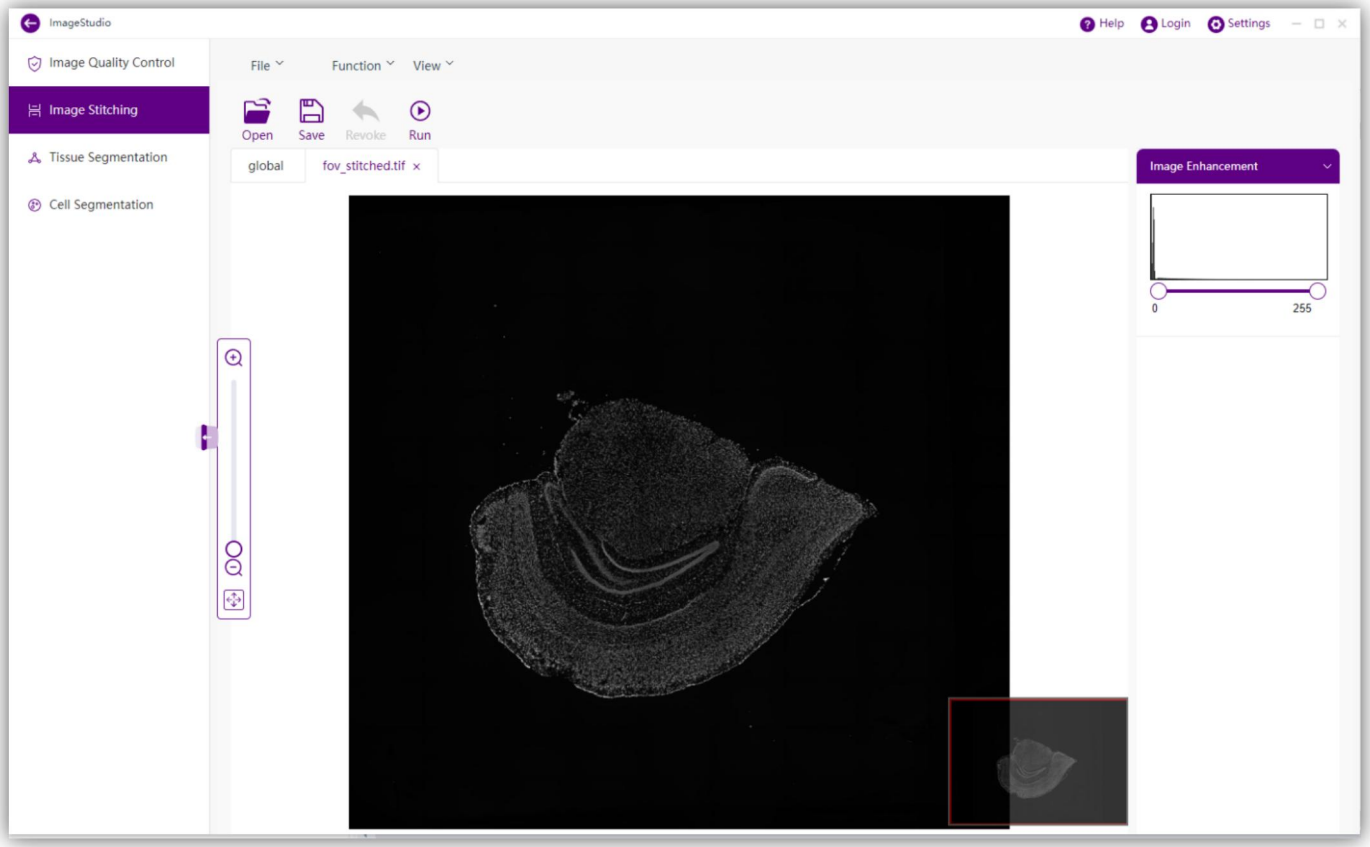
4.3.3 Marking of Template Trackline

The purpose of this function is to assist in verifying that the trackline deduced from the re-stitched image coincidice with the trackline on the Stereo-seq Chip.

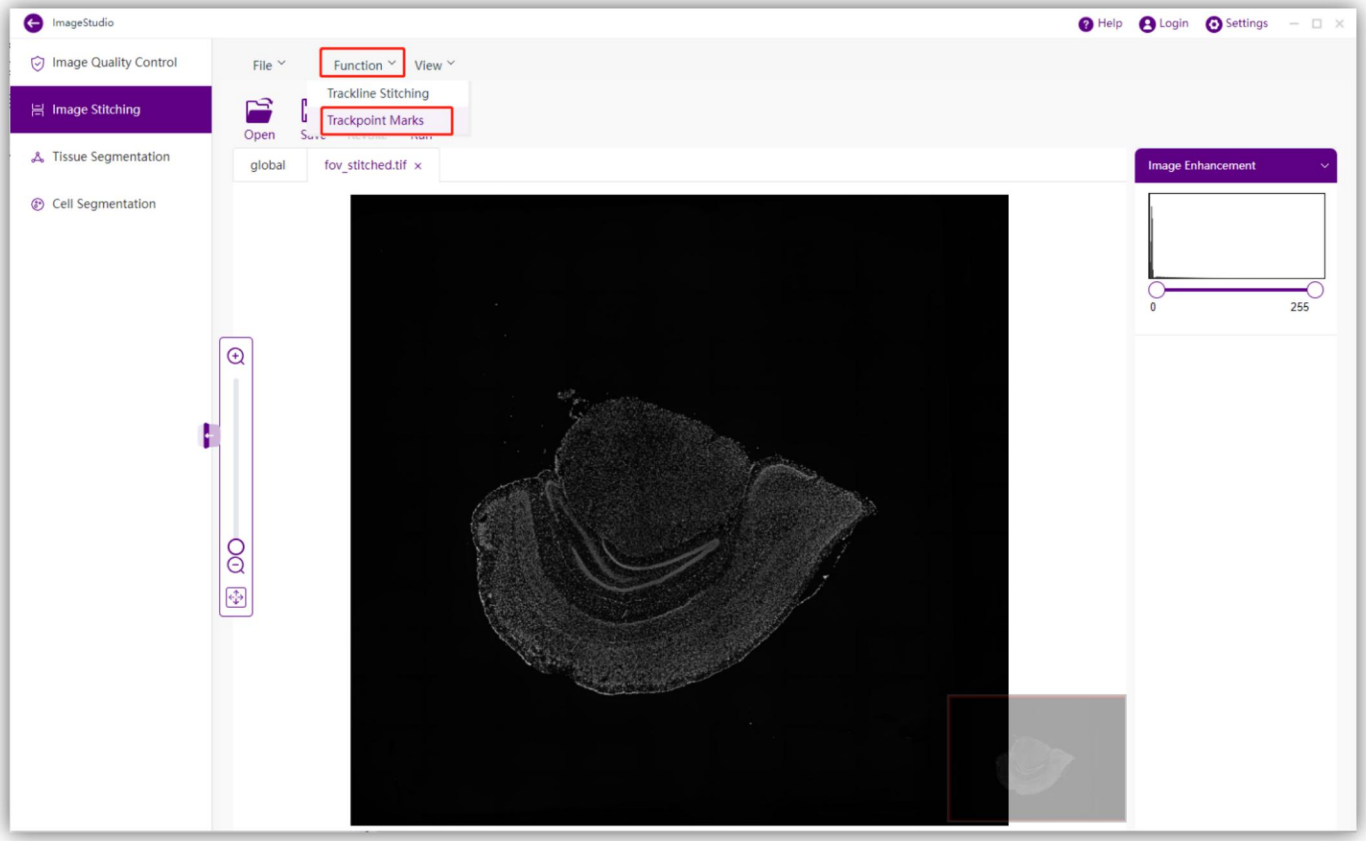
(1)Open file and select either method of image stitching.

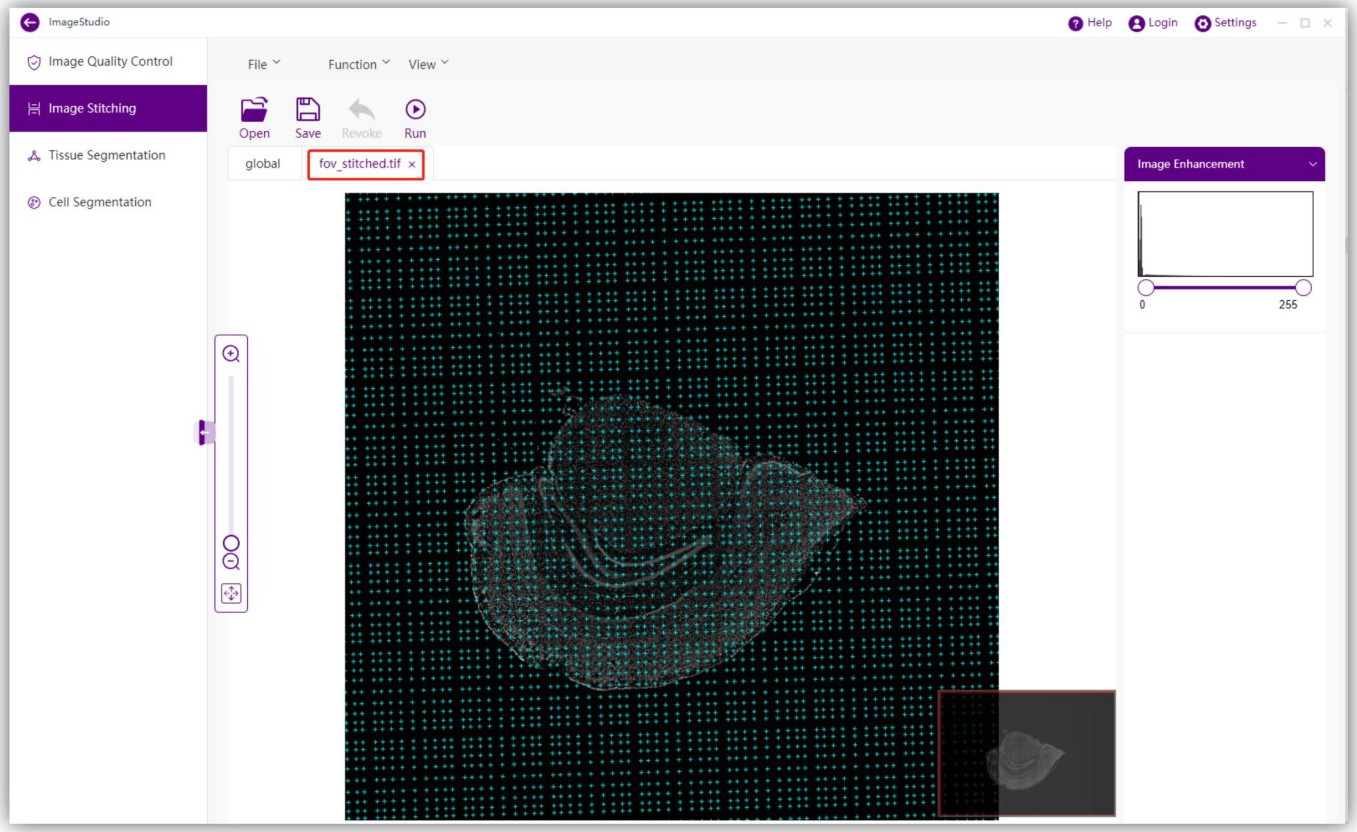


(2) Select fov_stitched.tig (stitched image) or (fov_stitch_transformed.tif (stitched image that has been rotated). Supported formats: TIFF

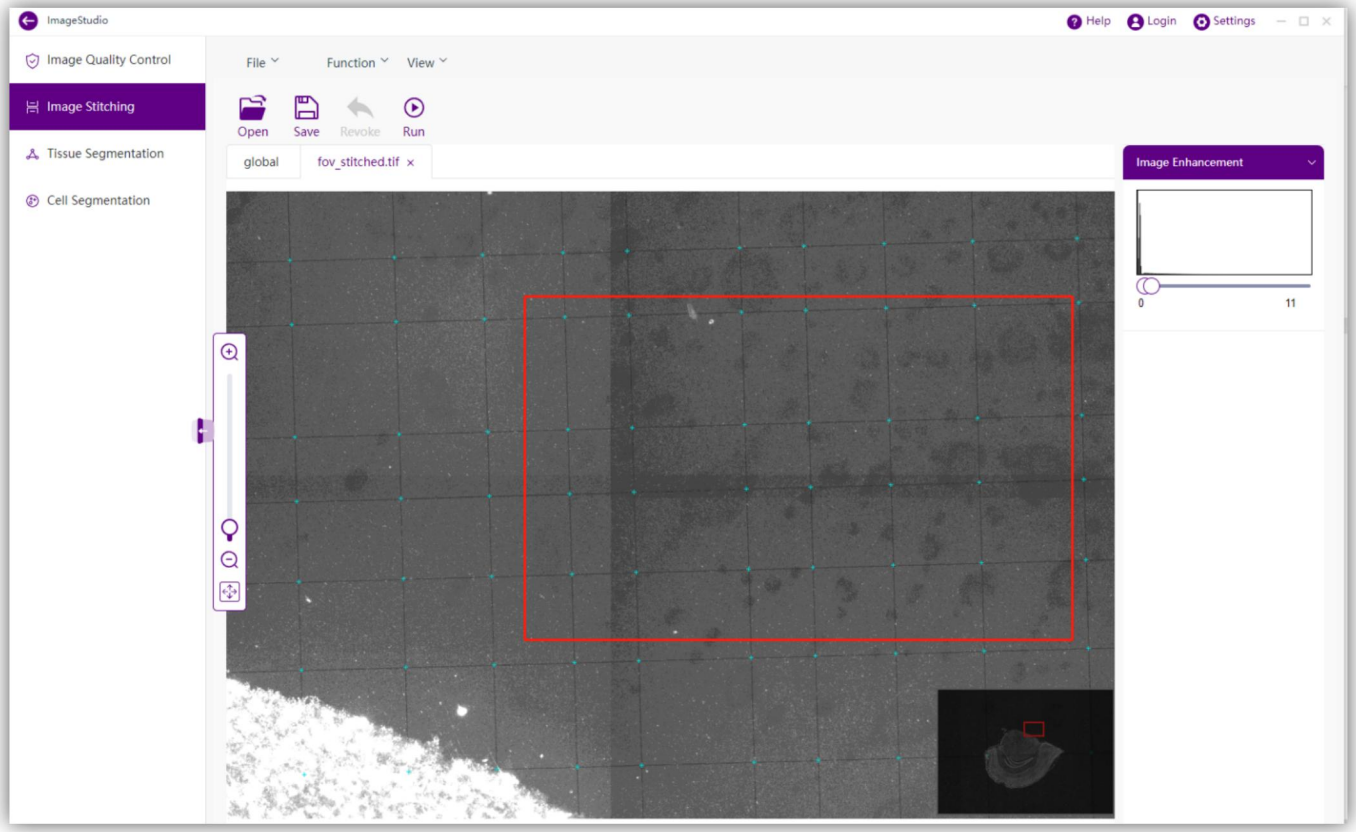


(3) Click ' Track Point Positioning Marks ' under ' Function', select the template file (template.txt), and mapped the template points onto the chip. Select stitch_template.txt as the template file for fov_stitched.tif while for fov_stitch_transformed.tif, select transform_template.txt as the template file.


ImageStudio
Help
Login
Settings
Image Quality Control
Image Stitching
Tissue Segmentation
Cell Segmentation
File
Function
View
Trackline Stitching
Trackpoint Marks
Open
global
fov_stitched.tif
Image Enhancement
0
255



(4) Adjust the image enhancement, and zoom in or out to check whether the track points are aligned with the tracklines on the Stereo-seq Chip.

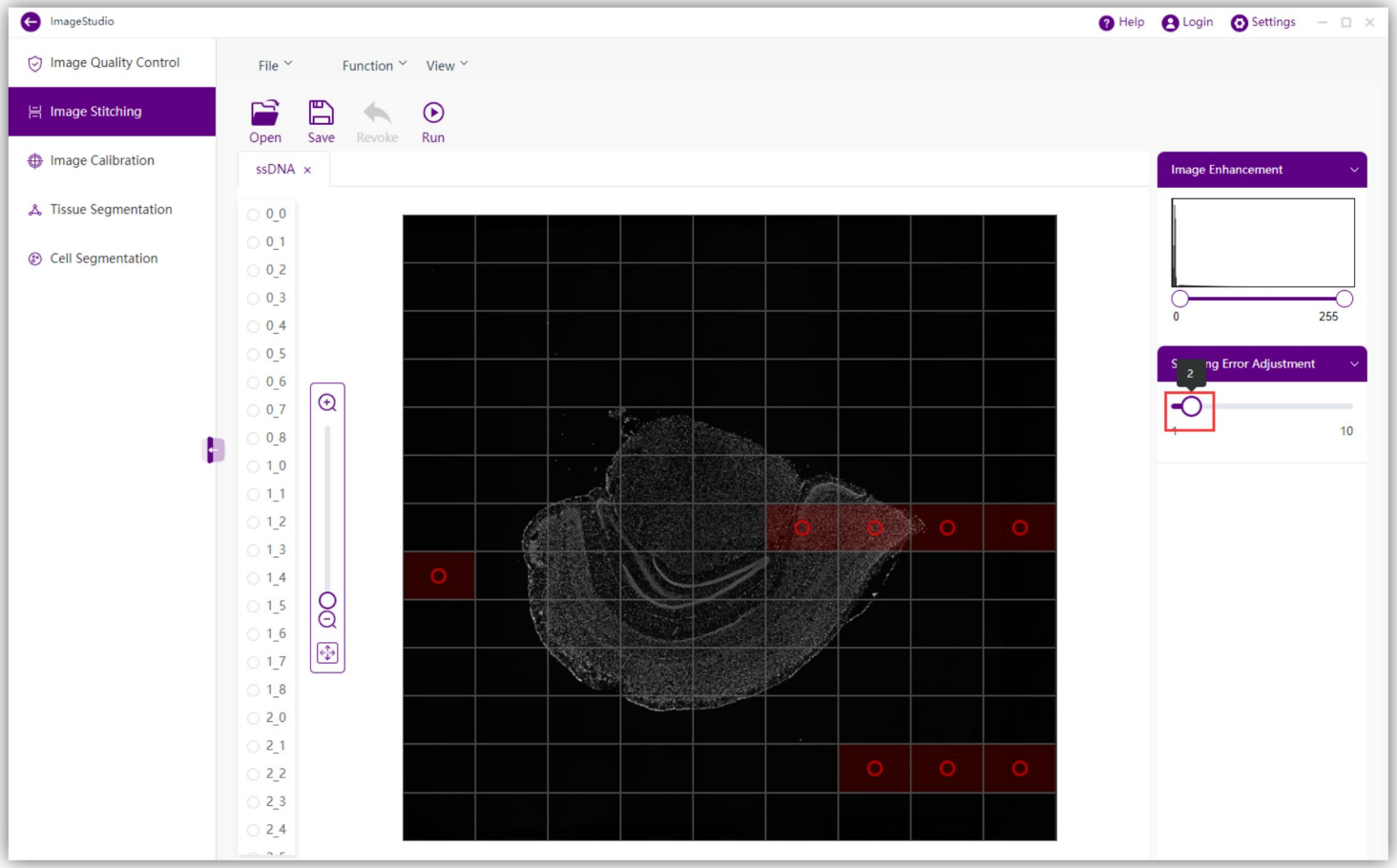


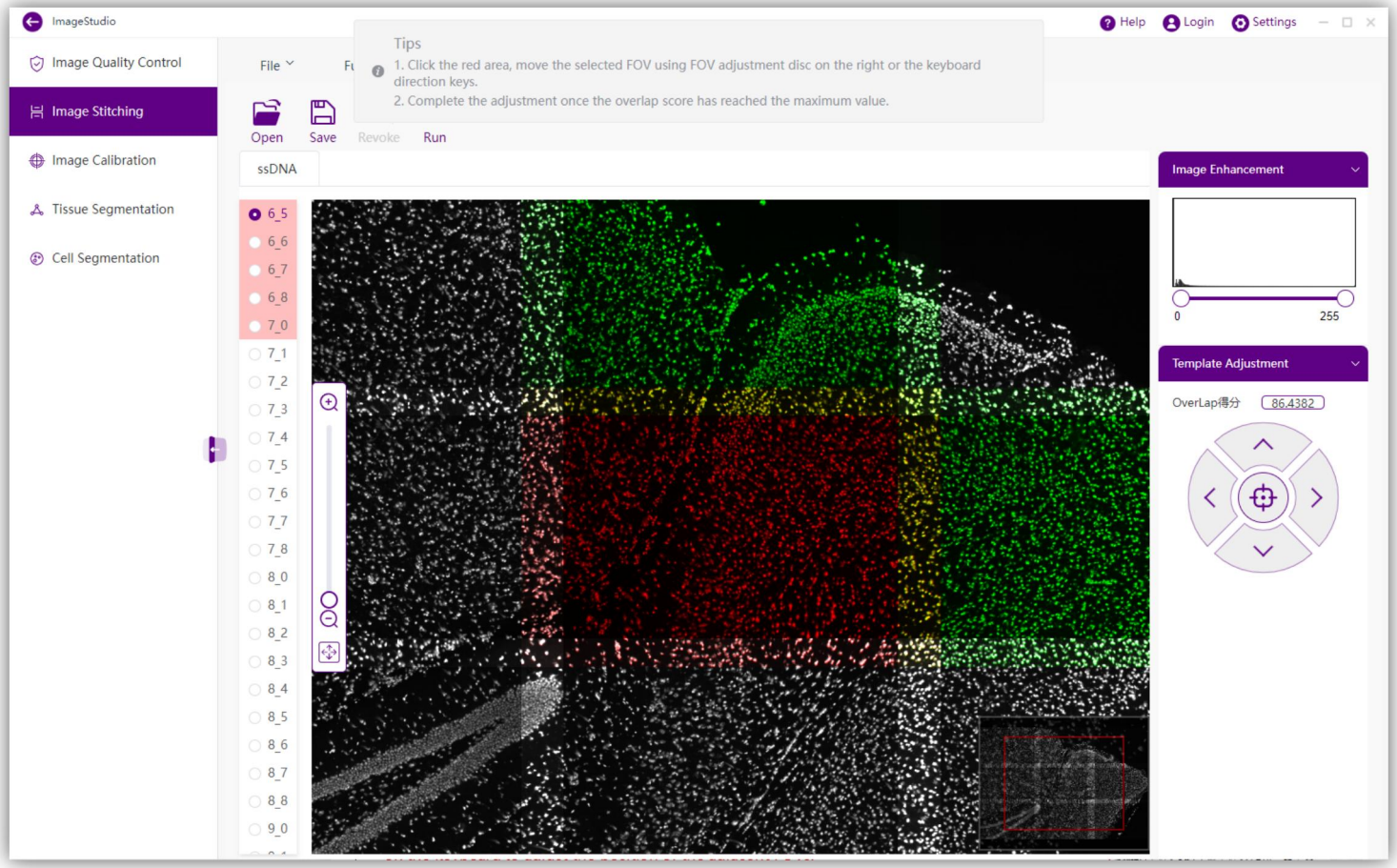
4.4.4 Manual Adjustment of The FOV Overlap

The FOV marked with a red circle in the stitched image is evaluated by the automatic algorithm when the stitching error of the FOV is greater than the set value (the default value is 5 pixels). Users can drag the "Stitching Error Adjustment" slide bar on the right and the number of red circles will

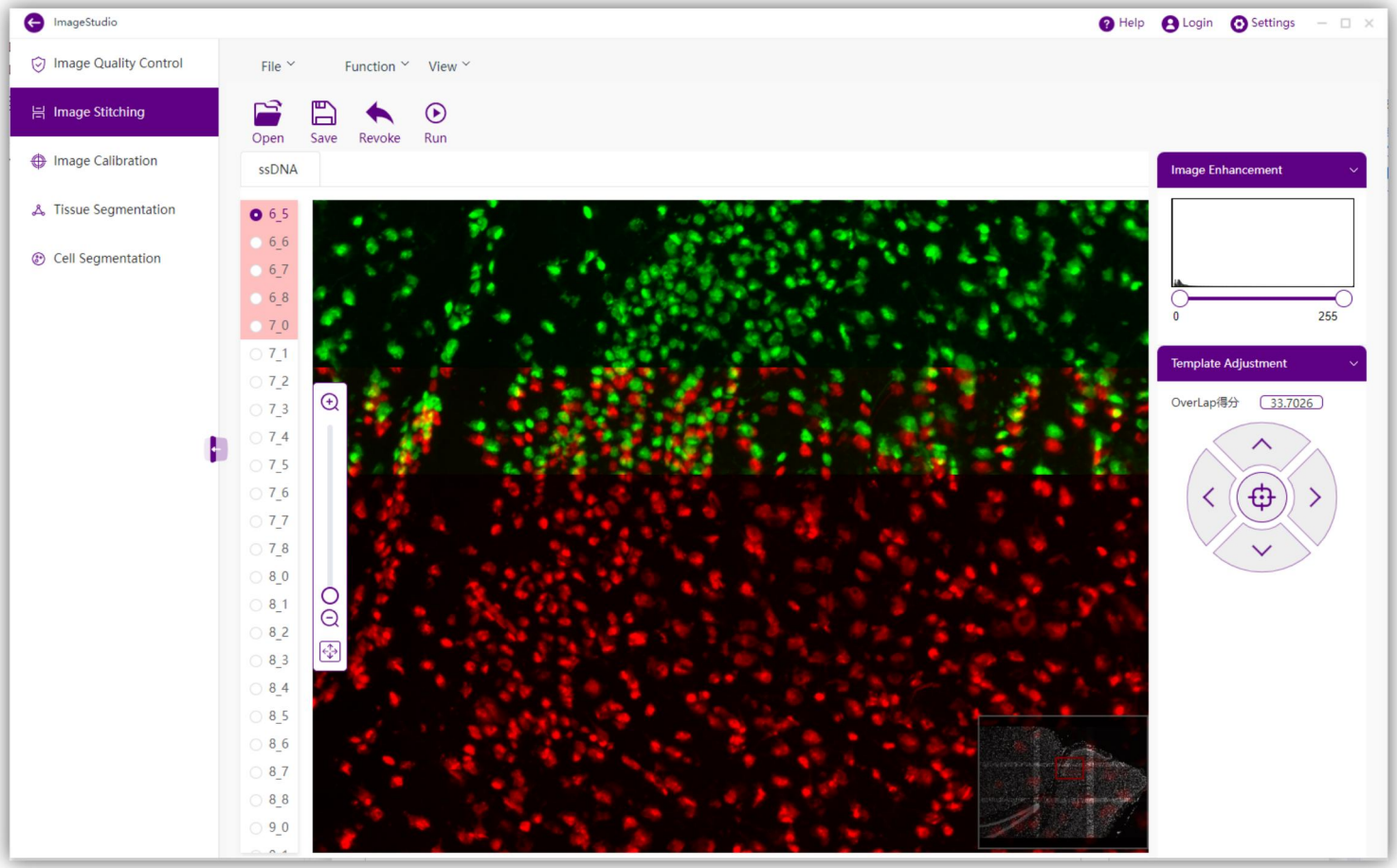
gradually increase as the stitching error decreases.

(1) Double click to open the FOV marked with a red circle or click the list of FOVs on the left.

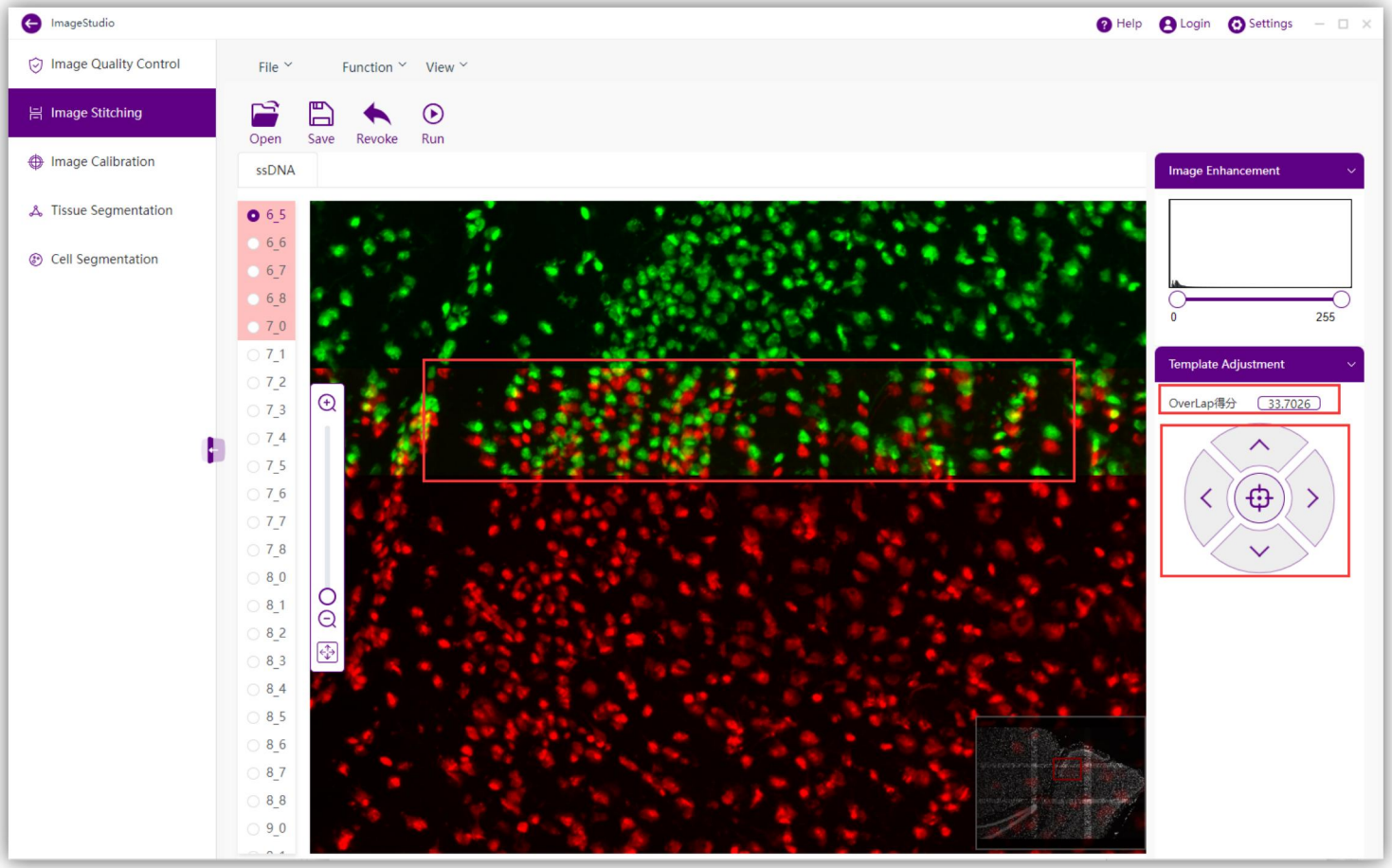


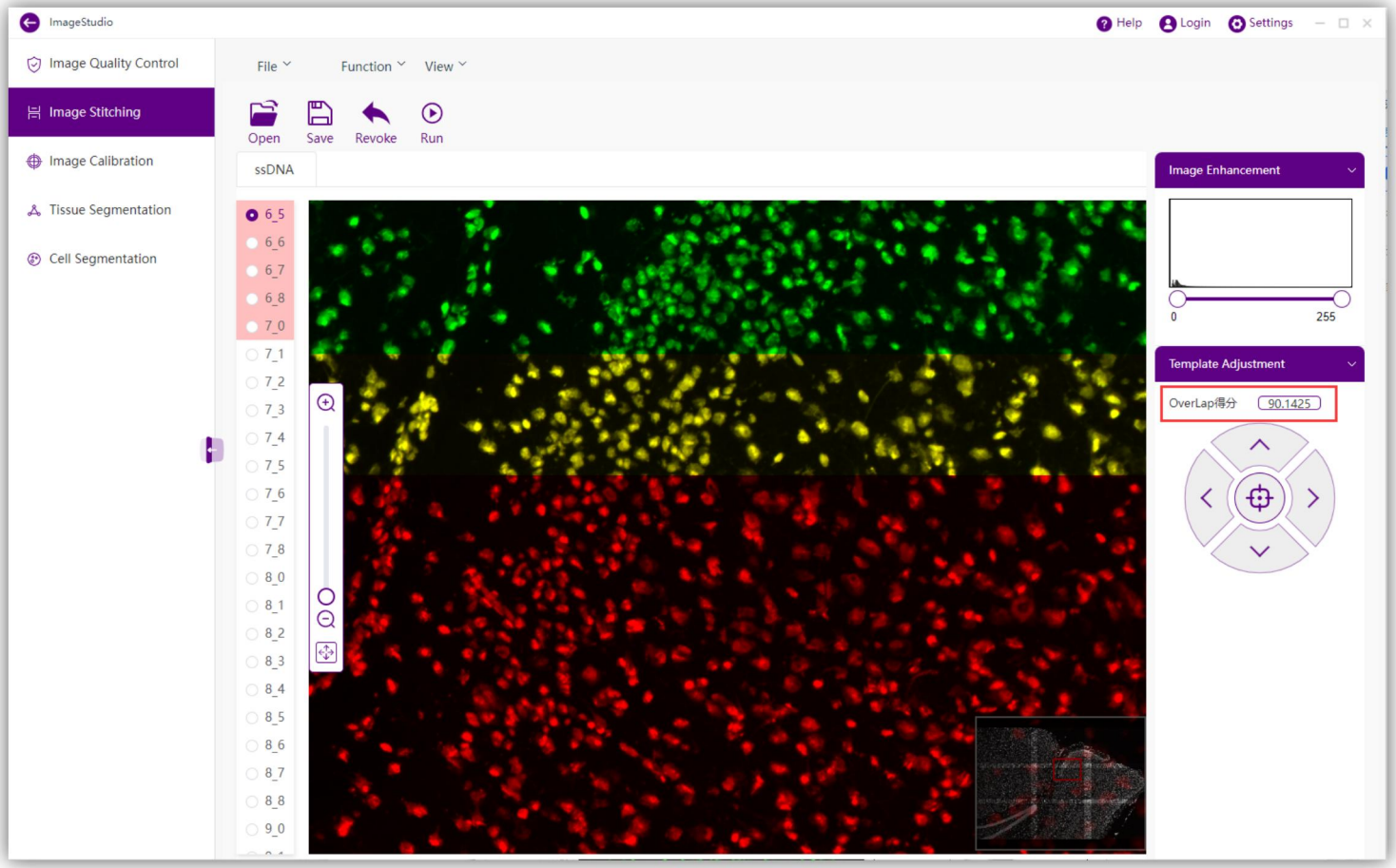


(2) The system generates an image composed of up to 9 FOVs. Drag the slide bar to enhance the image, you can click the "Stitching Adjustment" panel or press 'up and down', 'left and right' keys on the keyboard to adjust the position of the adjacent FOVs.



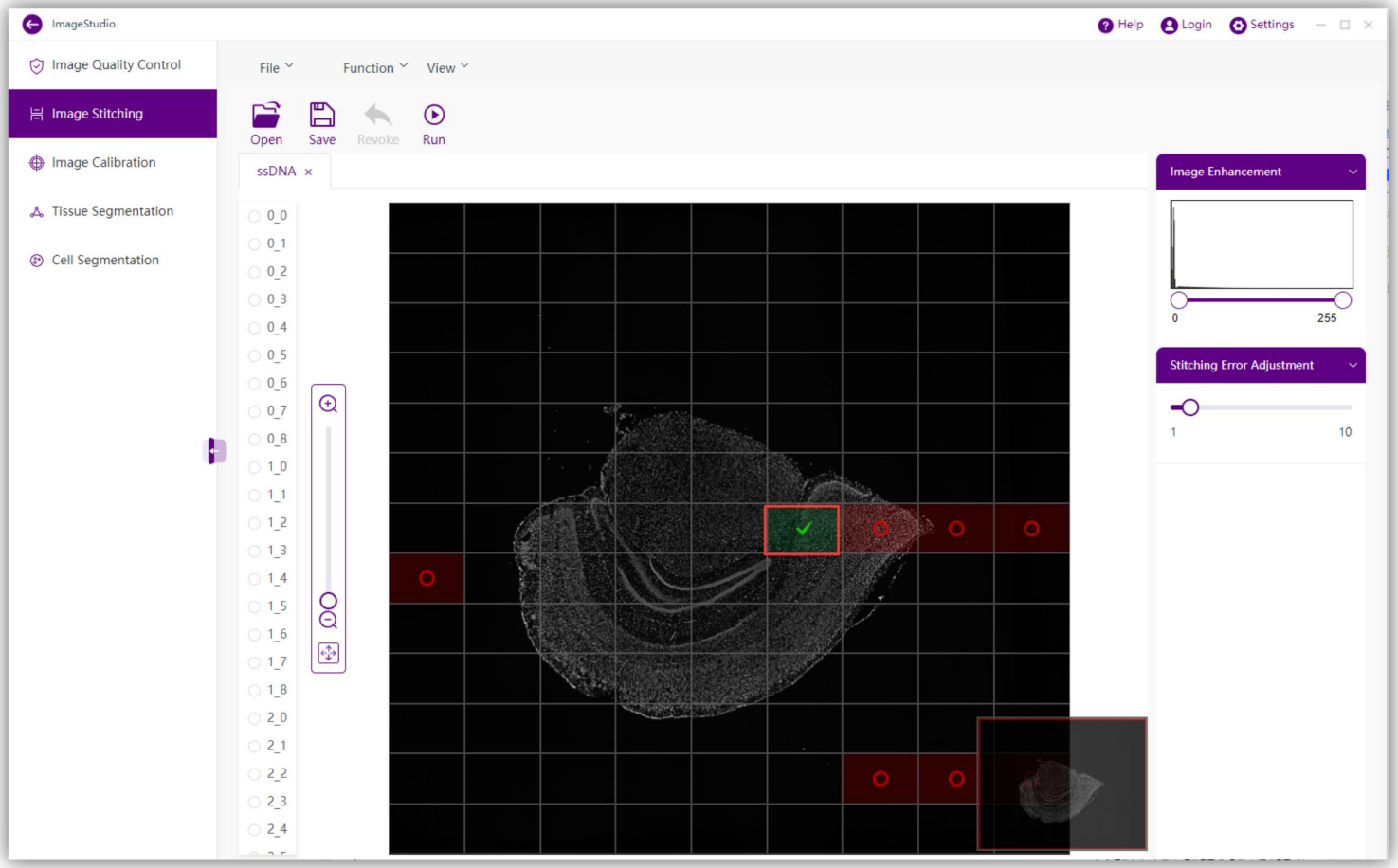
(3) The stitching effect of adjacent FOV images can be judged according to the score of OverLap or the degree the colors overlap with each other (yellow).


ImageStudio
Help
Login
Settings
Image Quality Control
Image Stitching
Image Calibration
Tissue Segmentation
Cell Segmentation
File
Function
View
Open
Save
Revoke
Run
ssDNA
6_5
6_6
6_7
6_8
7_0
7_1
7_2
7_3
7_4
7_5
7_6
7_7
7_8
8_0
8_1
8_2
8_3
8_4
8_5
8_6
8_7
8_8
9_0
Image Enhancement
0
255
Template Adjustment
OverLap得分
33.7026

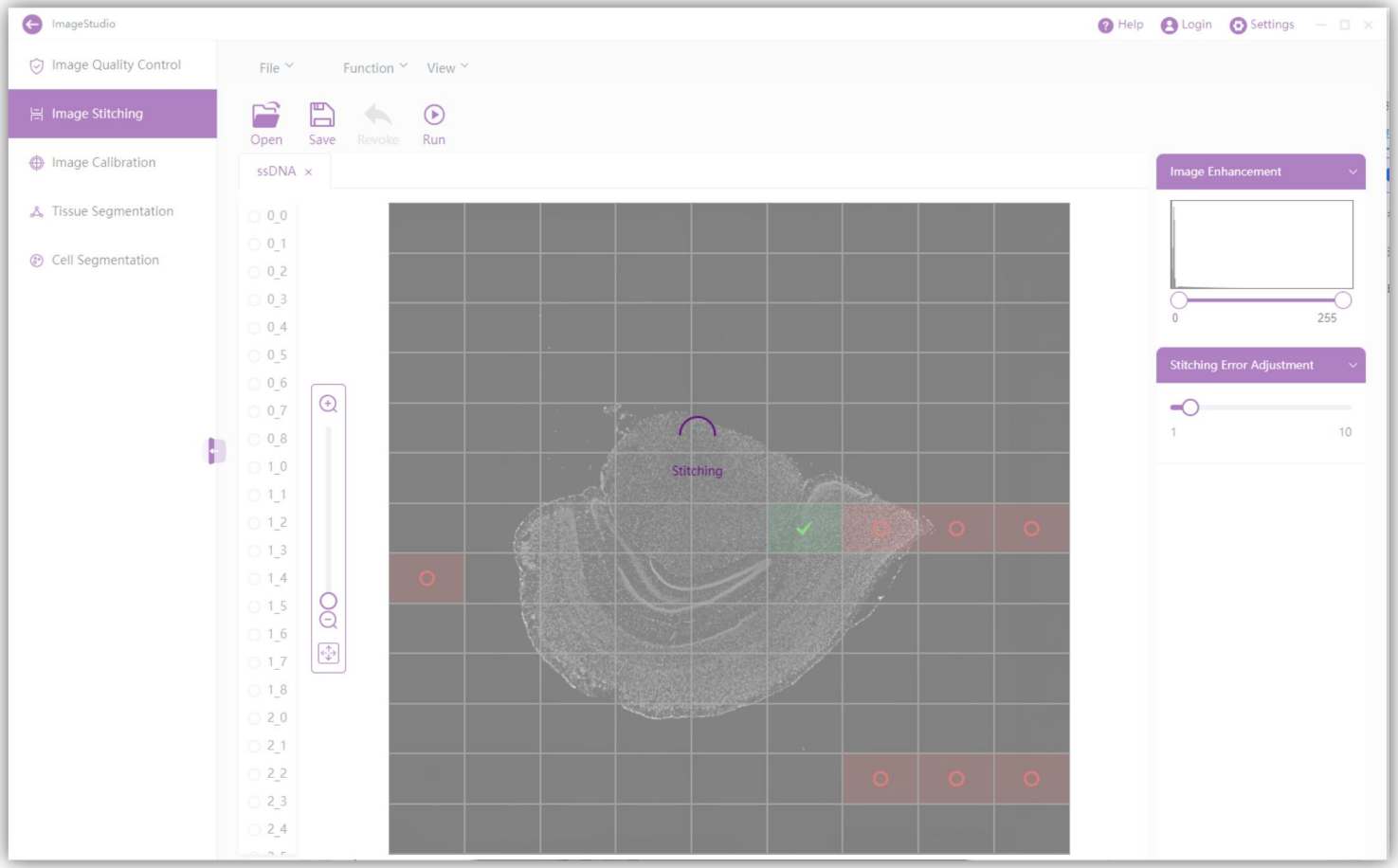


Note:The score range of OverLap is between 0 and 100. The higher the score, the higher the overlap of degree of the adjacent FOVs and the smaller the stitching error.

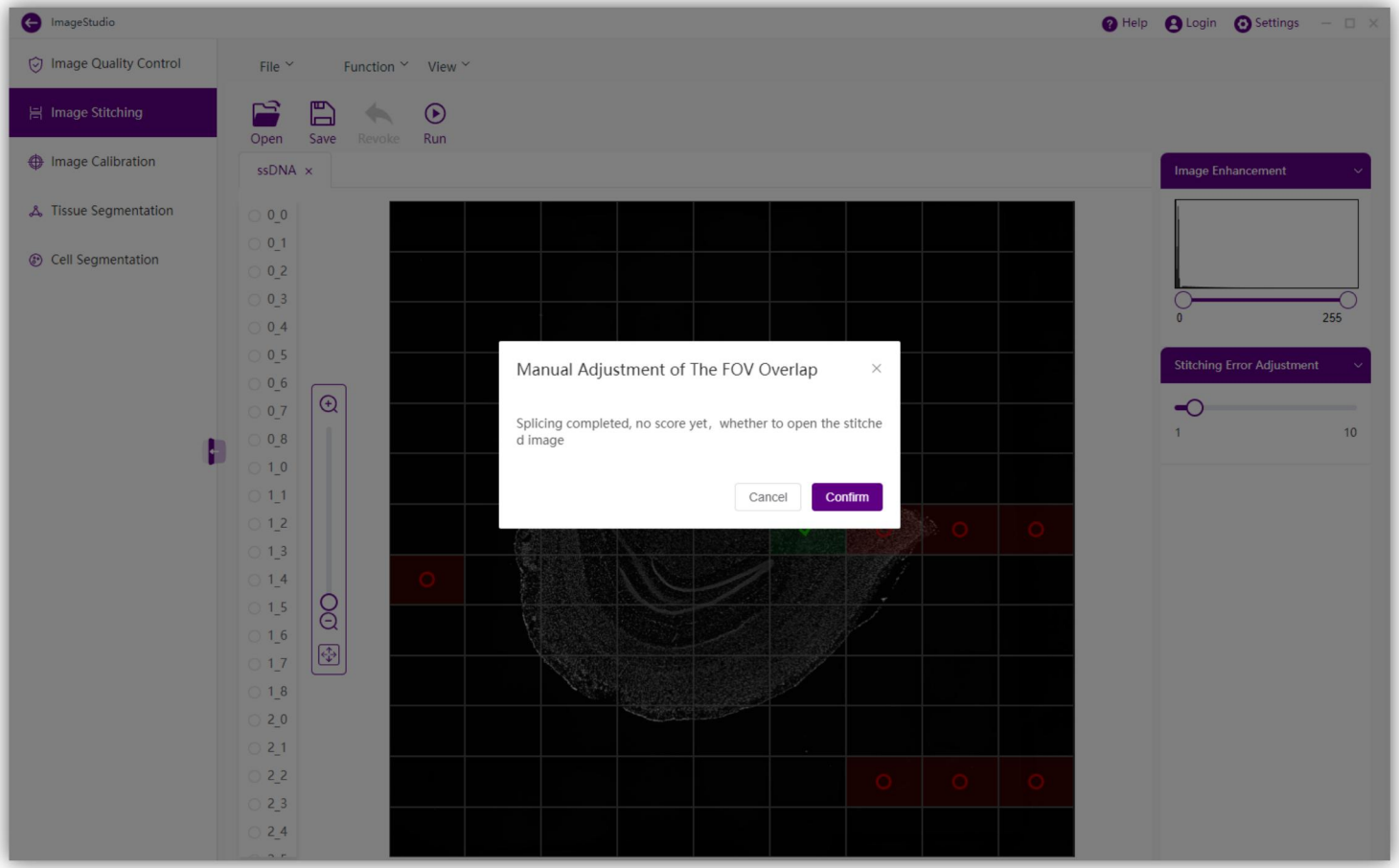
(4) After modification, click "Save"and return to the global view. The modified FOVs are indicated by a green circle and a √ sign.



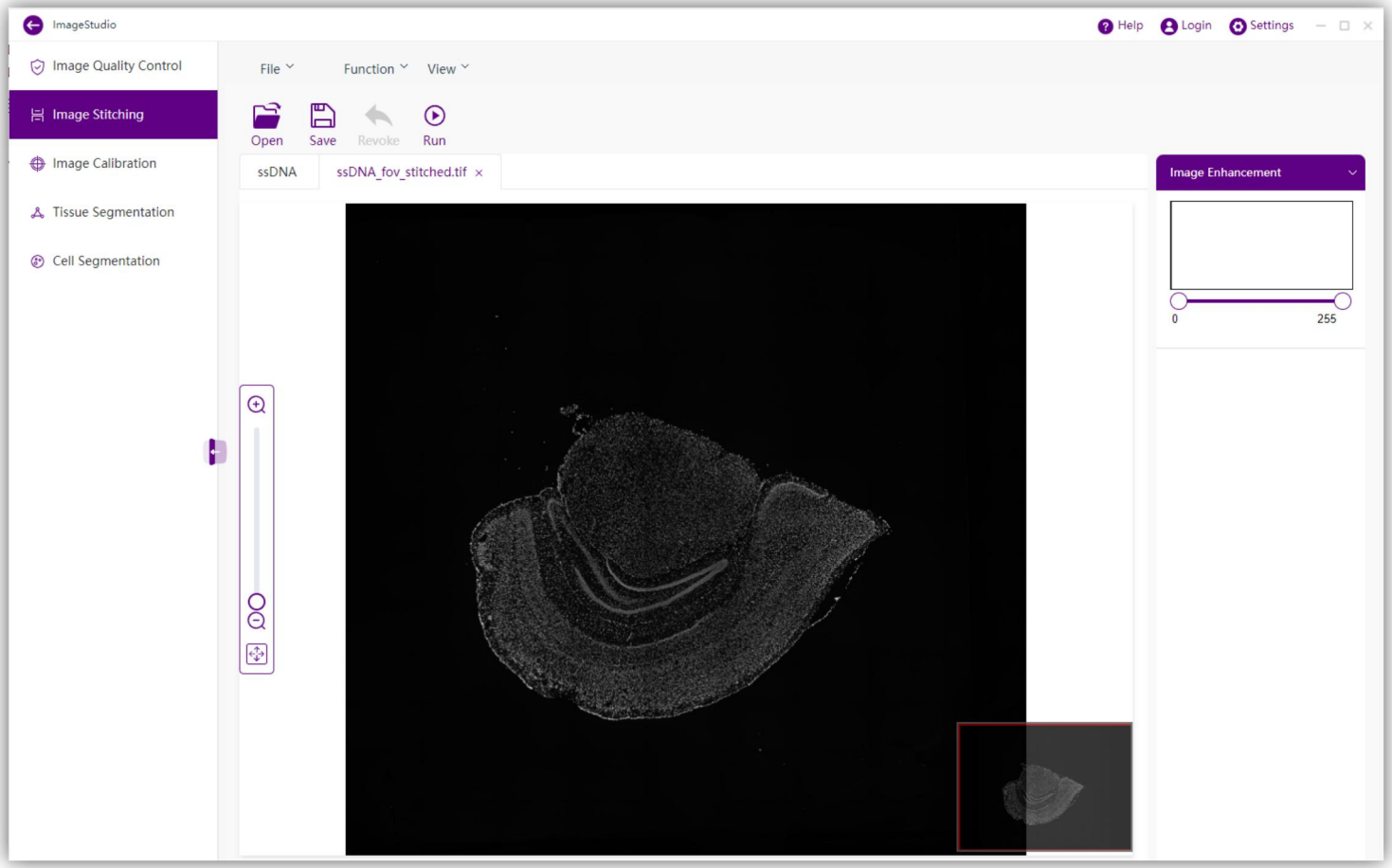
(5) If all the FOVs have been confirmed, click ' Run' and the system will initiate re-stitching of the FOV images based on the manually selected track points of each FOV within the global view.



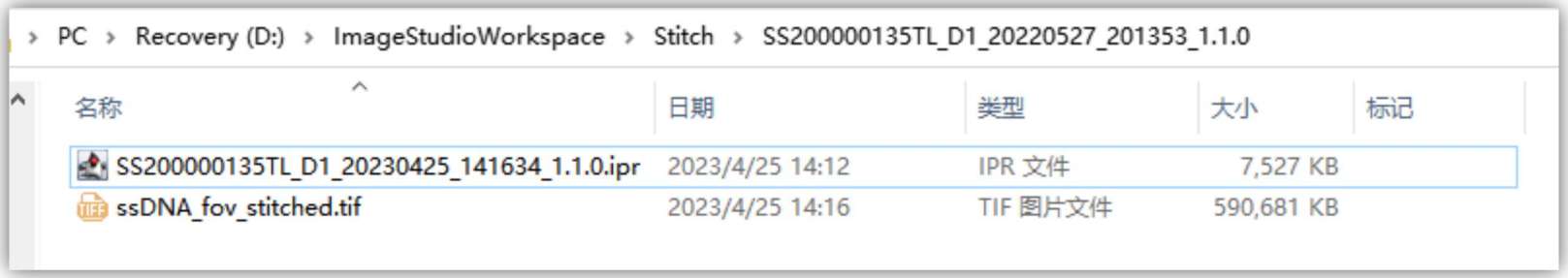
(6) Once the stitching process has been completed, the system prompts whether to open and check the stitched image.



(7) Click 'Confirm' to open the image.



(8) Final output files will be saved to the default directory: D:\ImageStudioWorkspace\Stitch

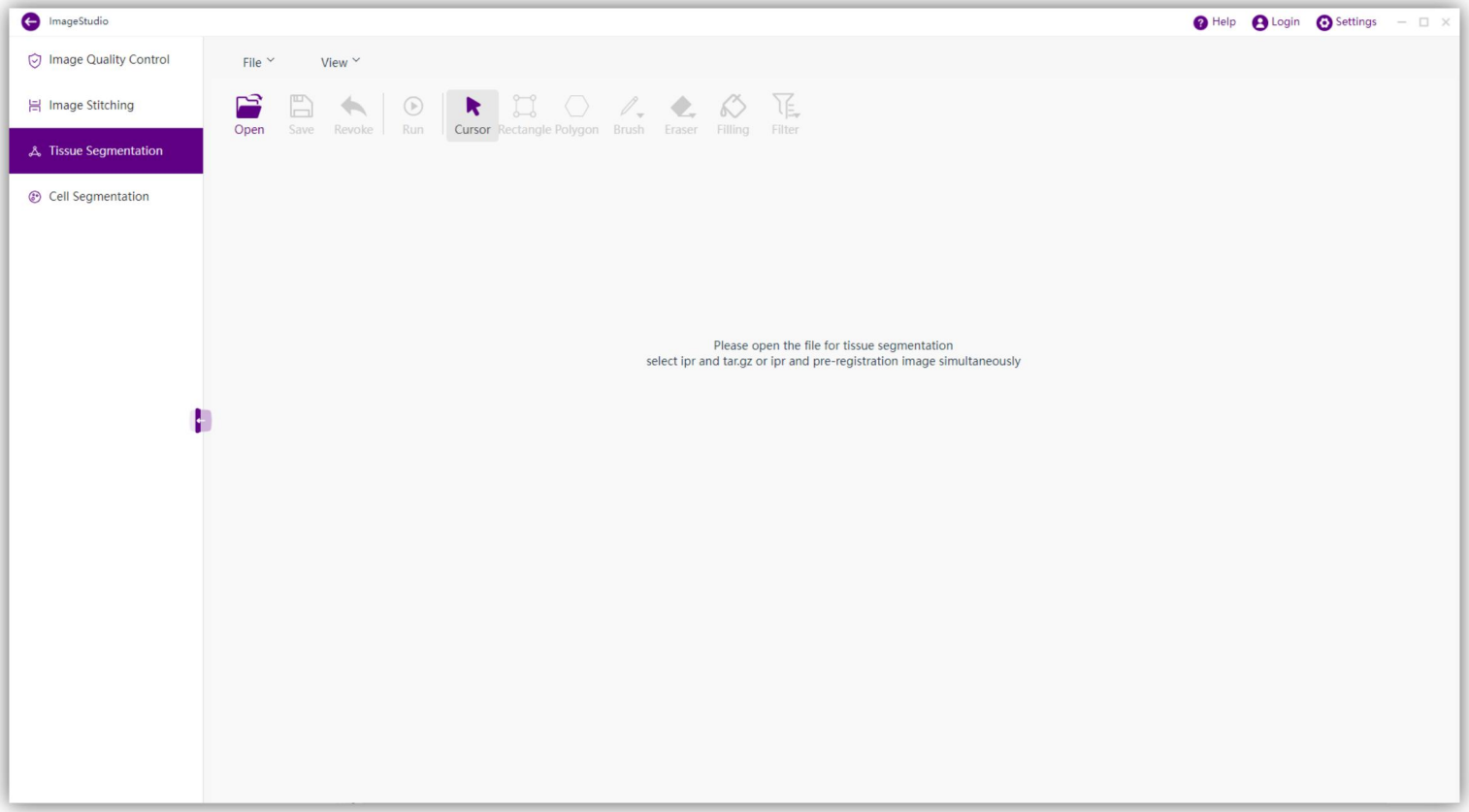


4.4 Tissue Segmentation

Tissue segmentation module is for manually adjusting the segmented tissue mask image.

4.4.1 Basic Operation

(1) Initiate the Tissue Segmentation module.



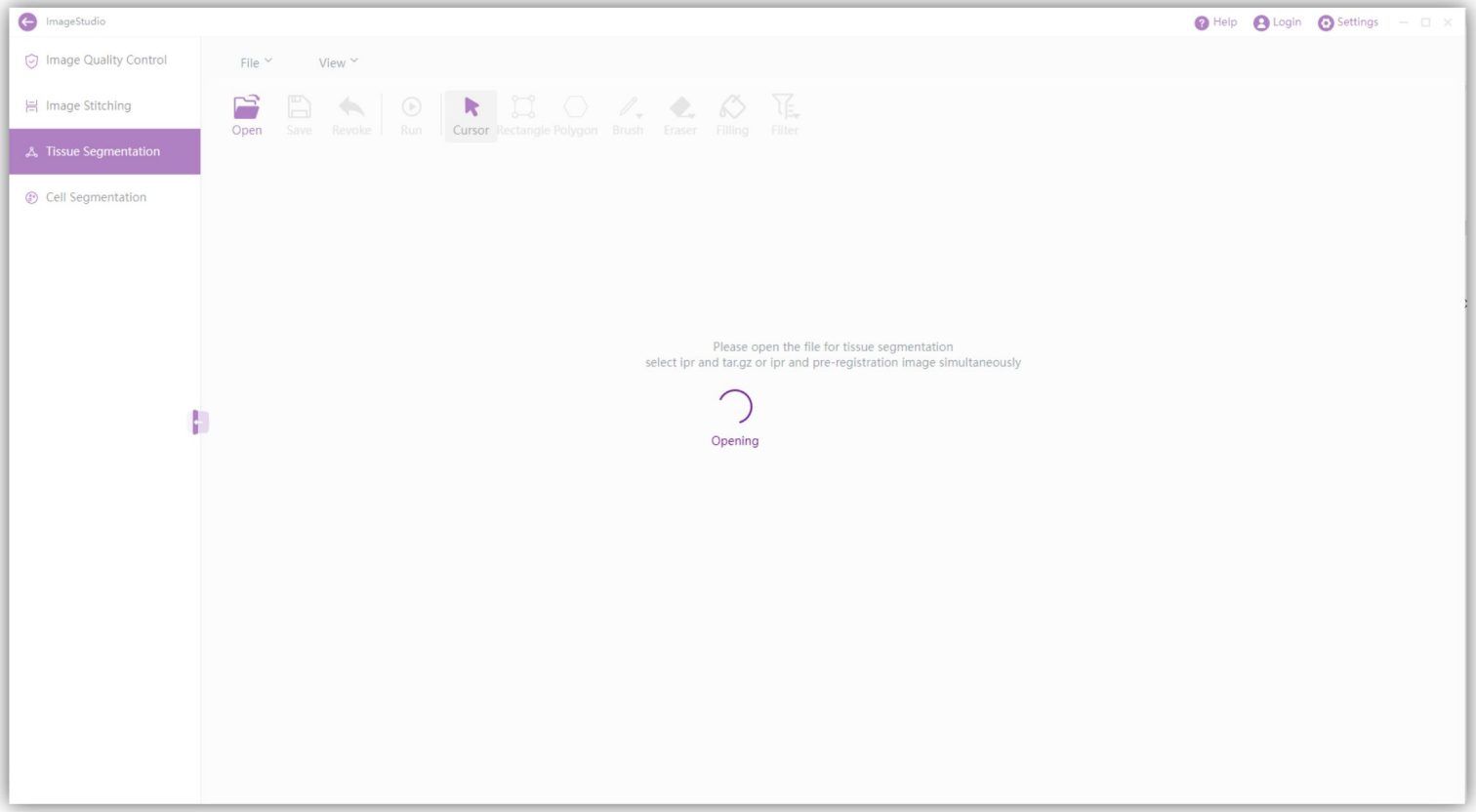
(2) Click 'Open' and select the image file.

Output directory: *D:\ImageStudioWorkspace\TissueSeg*

Module	Input File	Output File
Tissue Segmentation	• IPR file and the corresponding TAR.GZ file that have gone through	• IPR file that records manual processing information

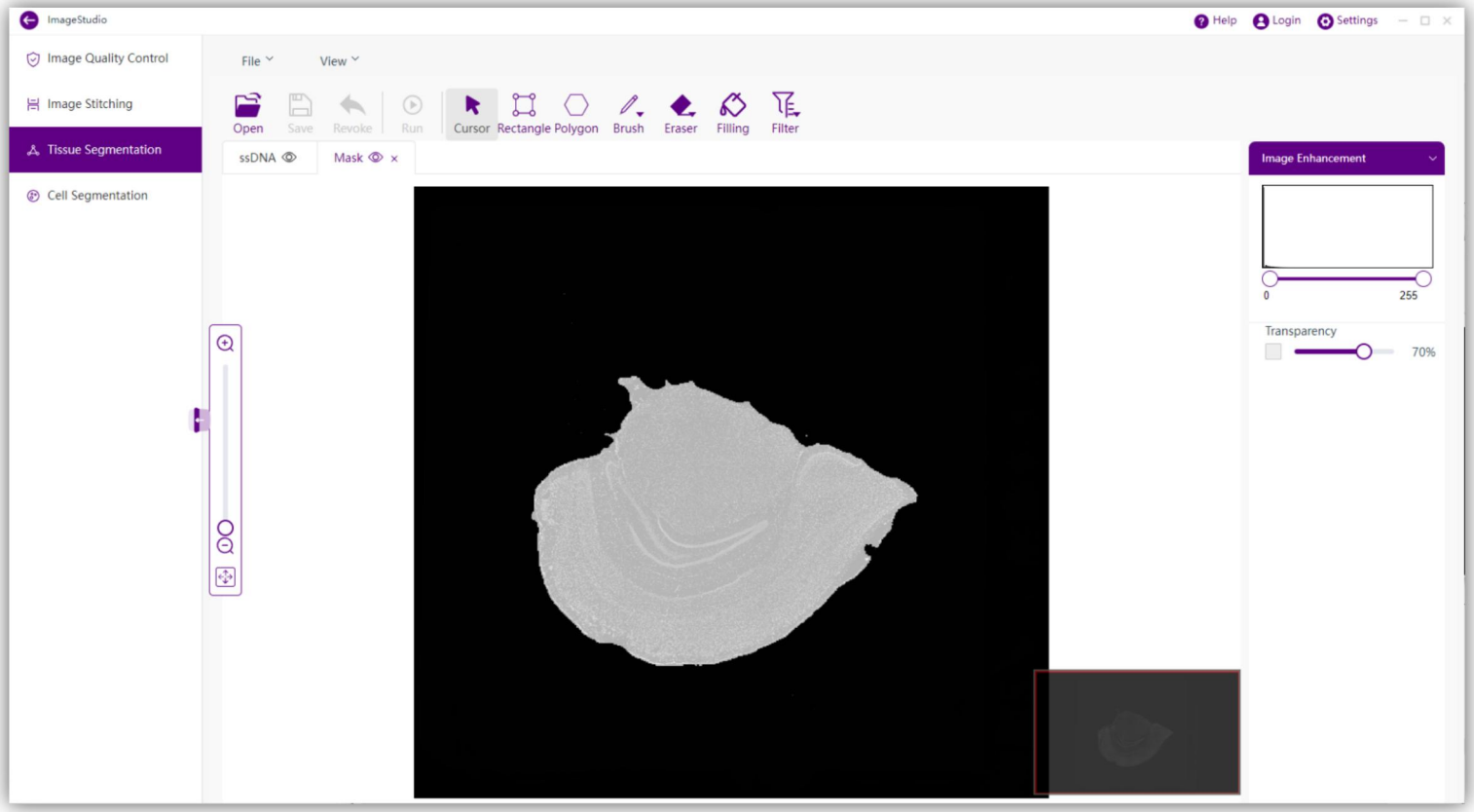
the ImageQC or SAW-register or SAW-rapidRegister processing module within Stereo-seq Analysis Workflow (SAW) pipeline

- IPR and ssDNA Image
- fov_stitched_transformed.tif for pre-registered stitched image in TIFF format
- tissue_mask.tif for tissue mask image



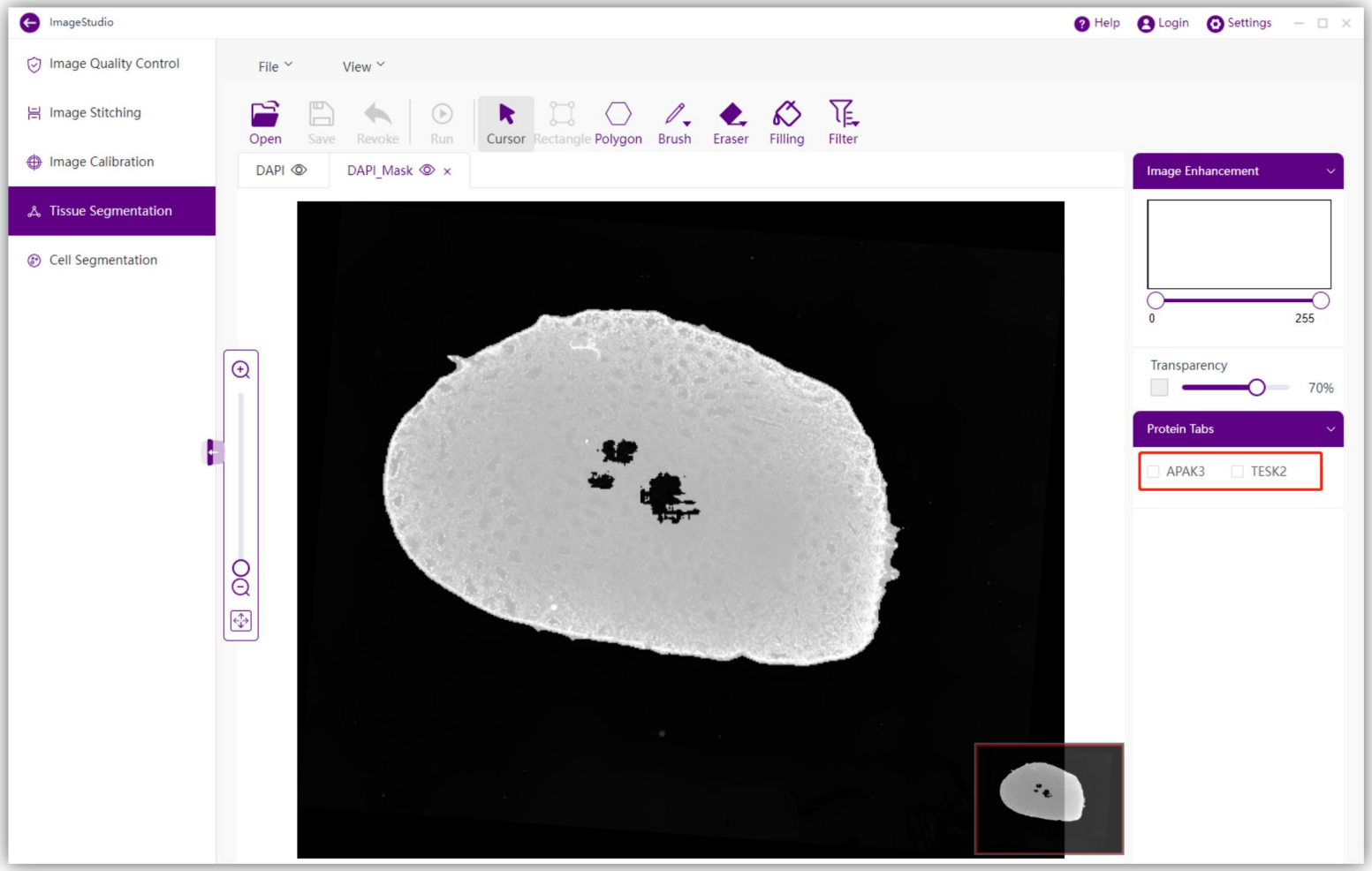
⚠Since generating the image pyramid is time-consuming, please only open one Stereo-seq Chip file at a time. One 1cm by 1cm Chip T will take about 3~5min to open.

(3) If the input is ssDNA or DAPI , two tabs will be opened and display the ssDNA or DAPI image or tissue mask image, Whether to display tissue mask image depends on whether the IPR file by user contains segmented Mask information.

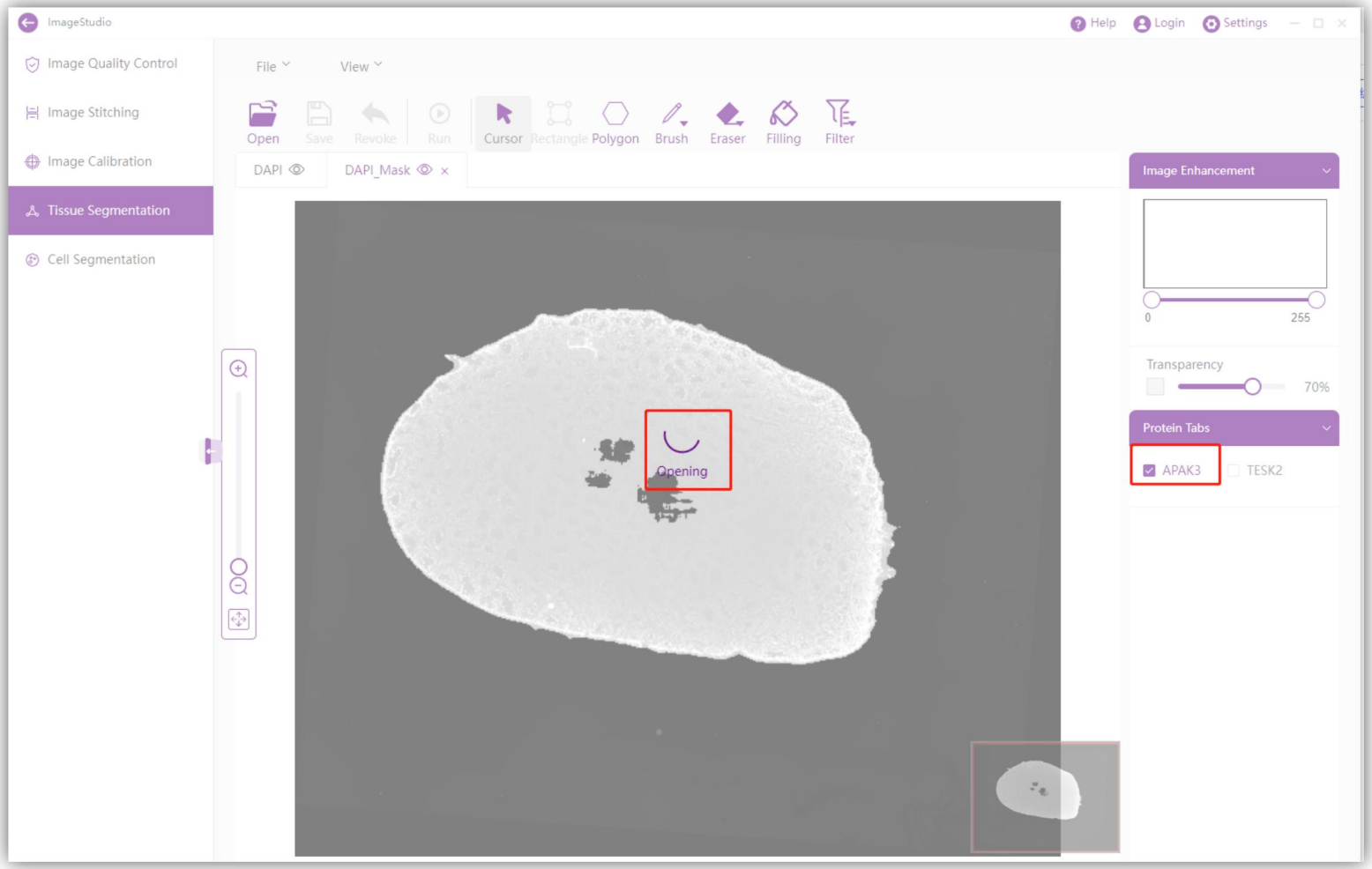


(4) If the input is DAPI with mIF

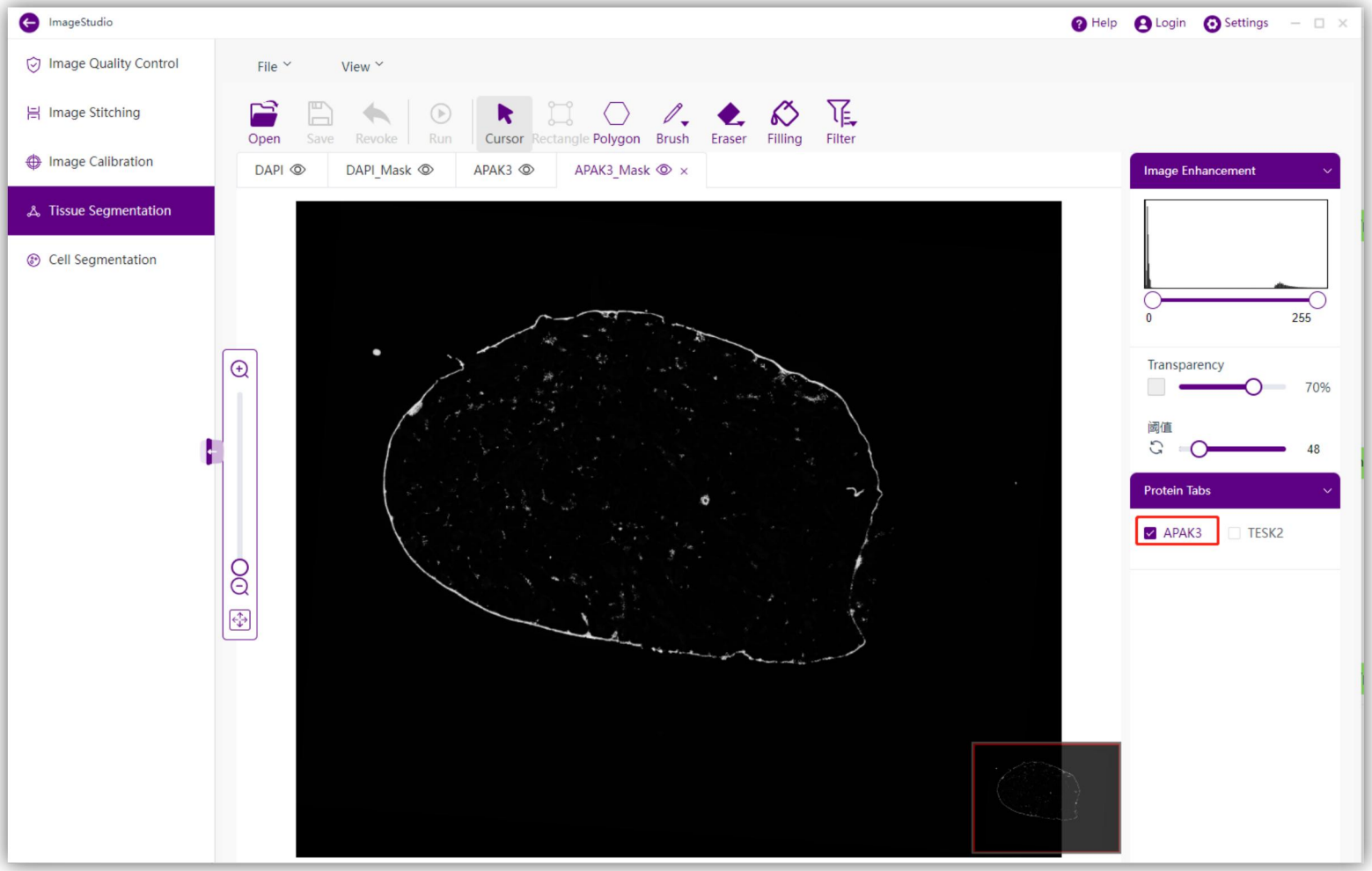
- Two tabs will be opened and display the DAPI image or tissue mask image by default, Whether to display tissue mask image depends on whether the IPR file by user contains segmented Mask information.



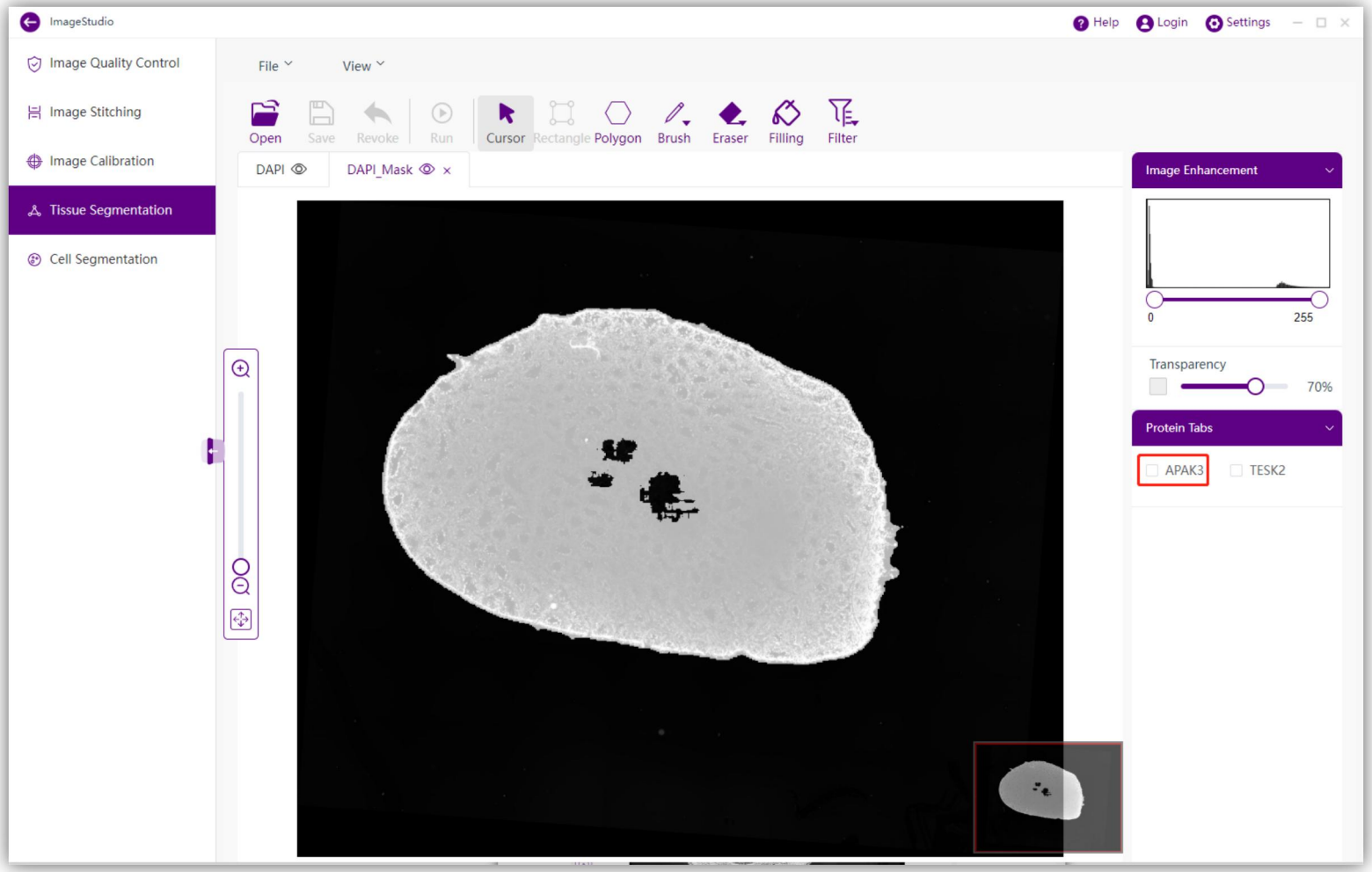
- Users can choose whether to display other proteins by selecting the box under "Protein Tabs"on the right.



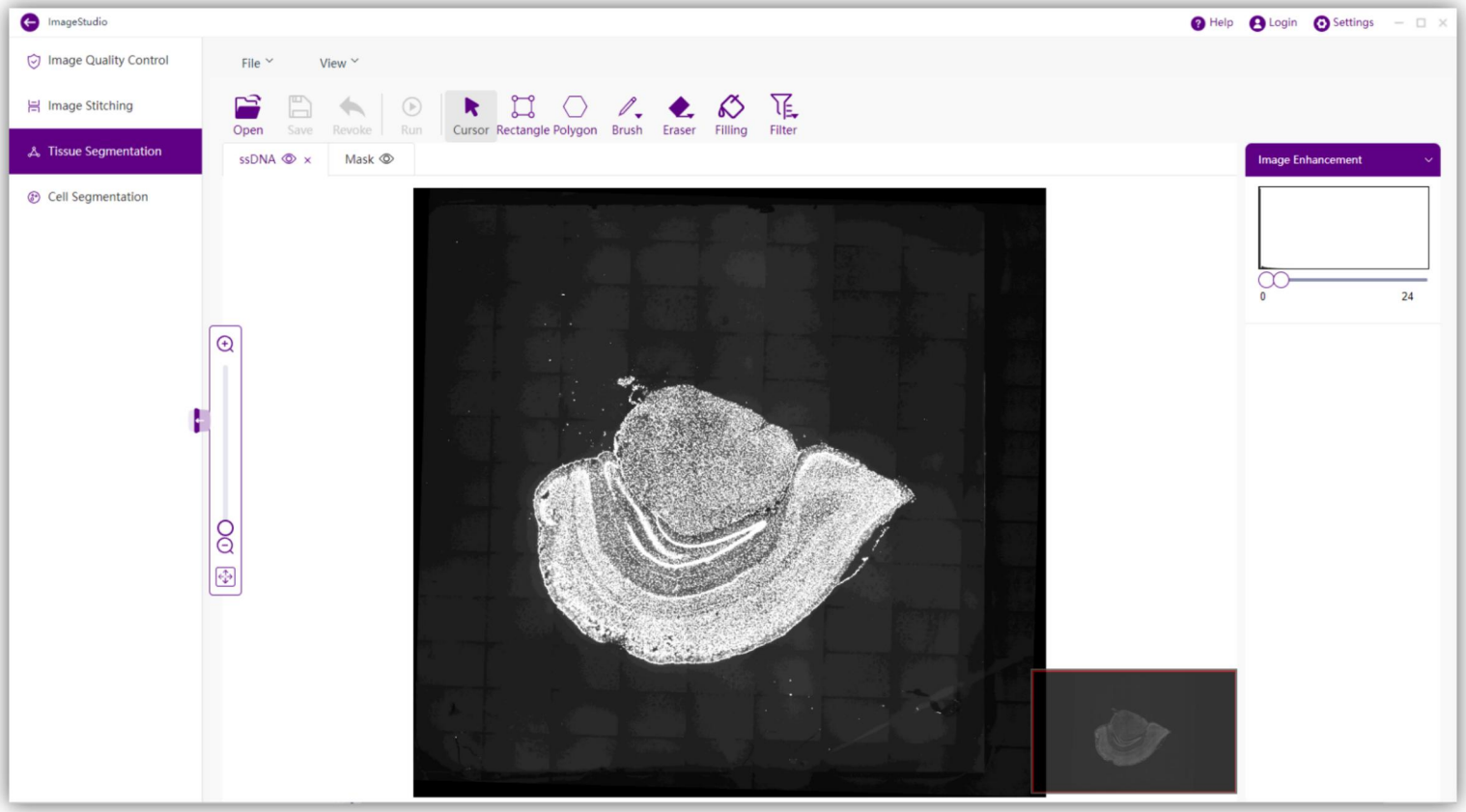
- The display of AKAP3 protein is shown below.



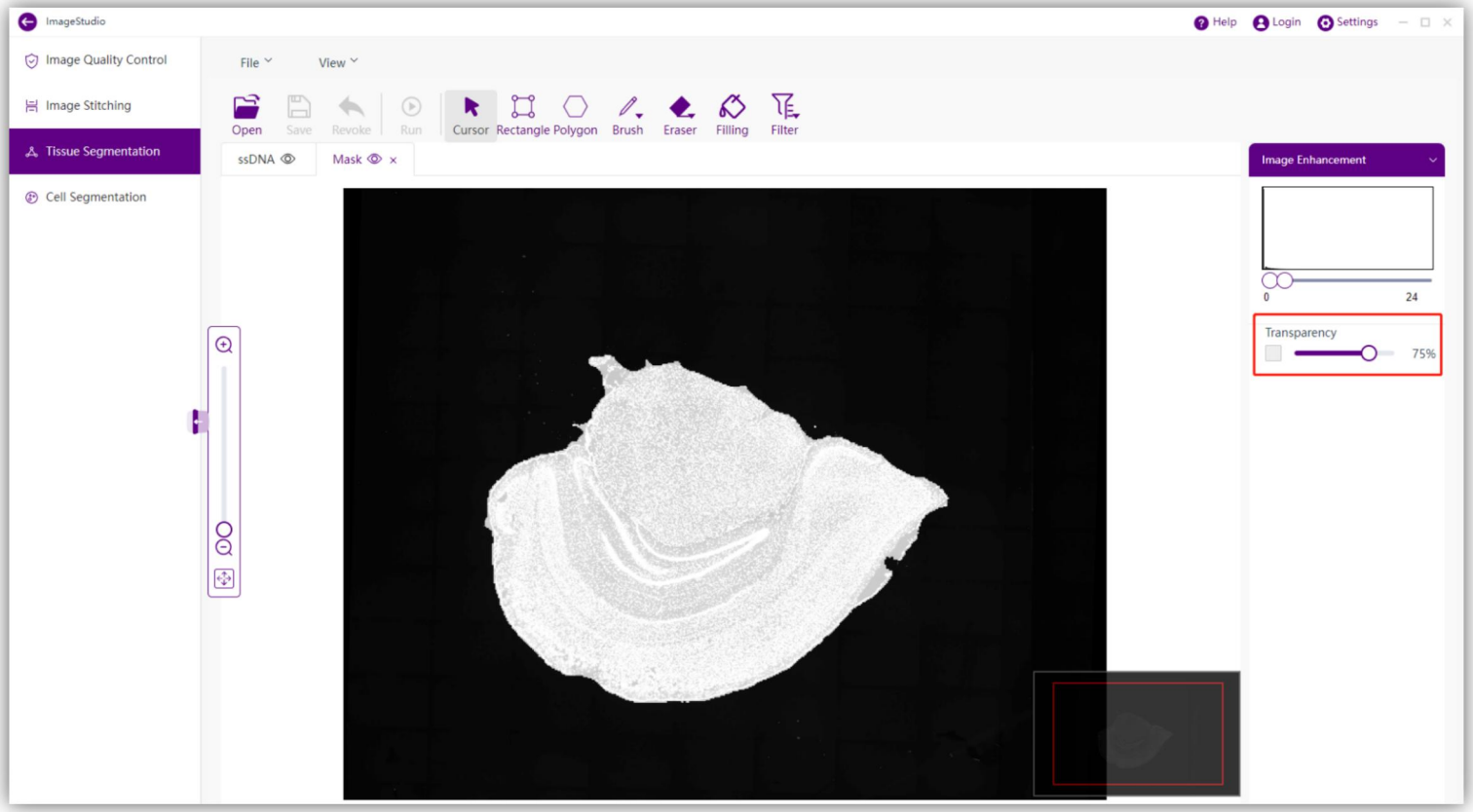
- Users can choose whether to turn off the display of other proteins by deselecting the box under "Protein Tabs"on the right.



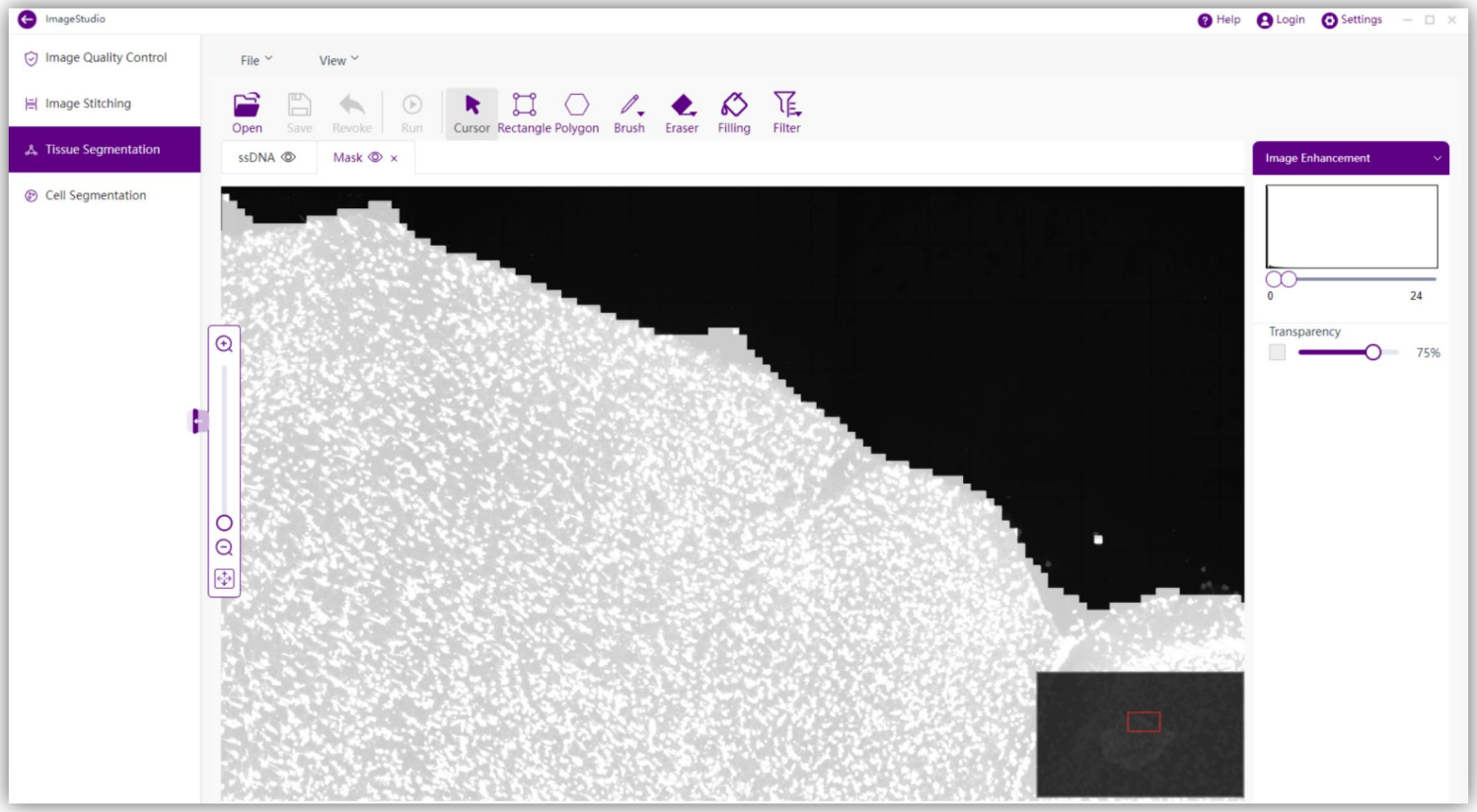
(5) Drag the 'Image Enhancement' slide bar under the stitched image tab to better display the tissue contour and background.



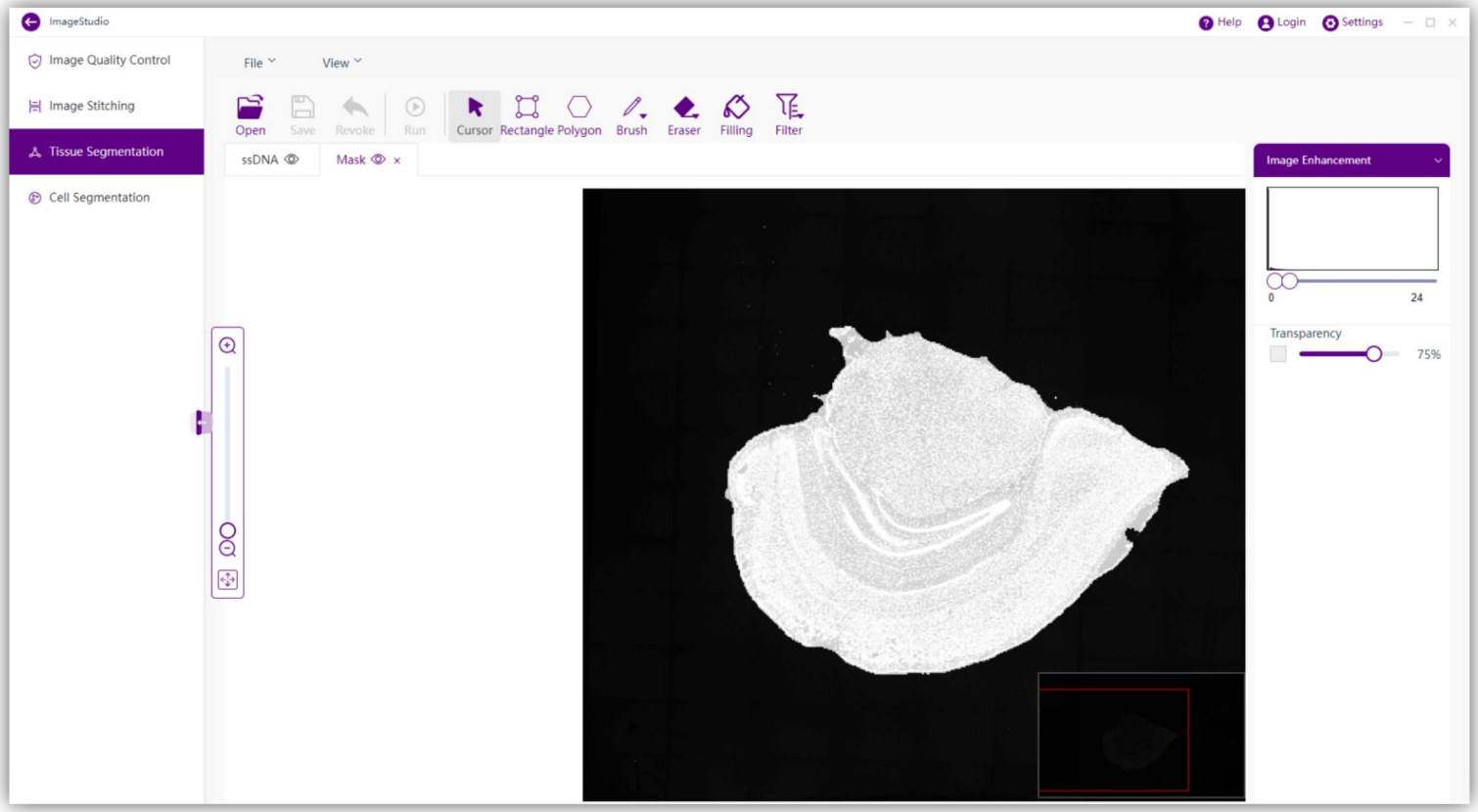
(6) Drag the 'Transparency Adjustment' slide bar under the Mask tab to adjust the transparency of tissue mask image for a clearer view of the segmented tissue contour.



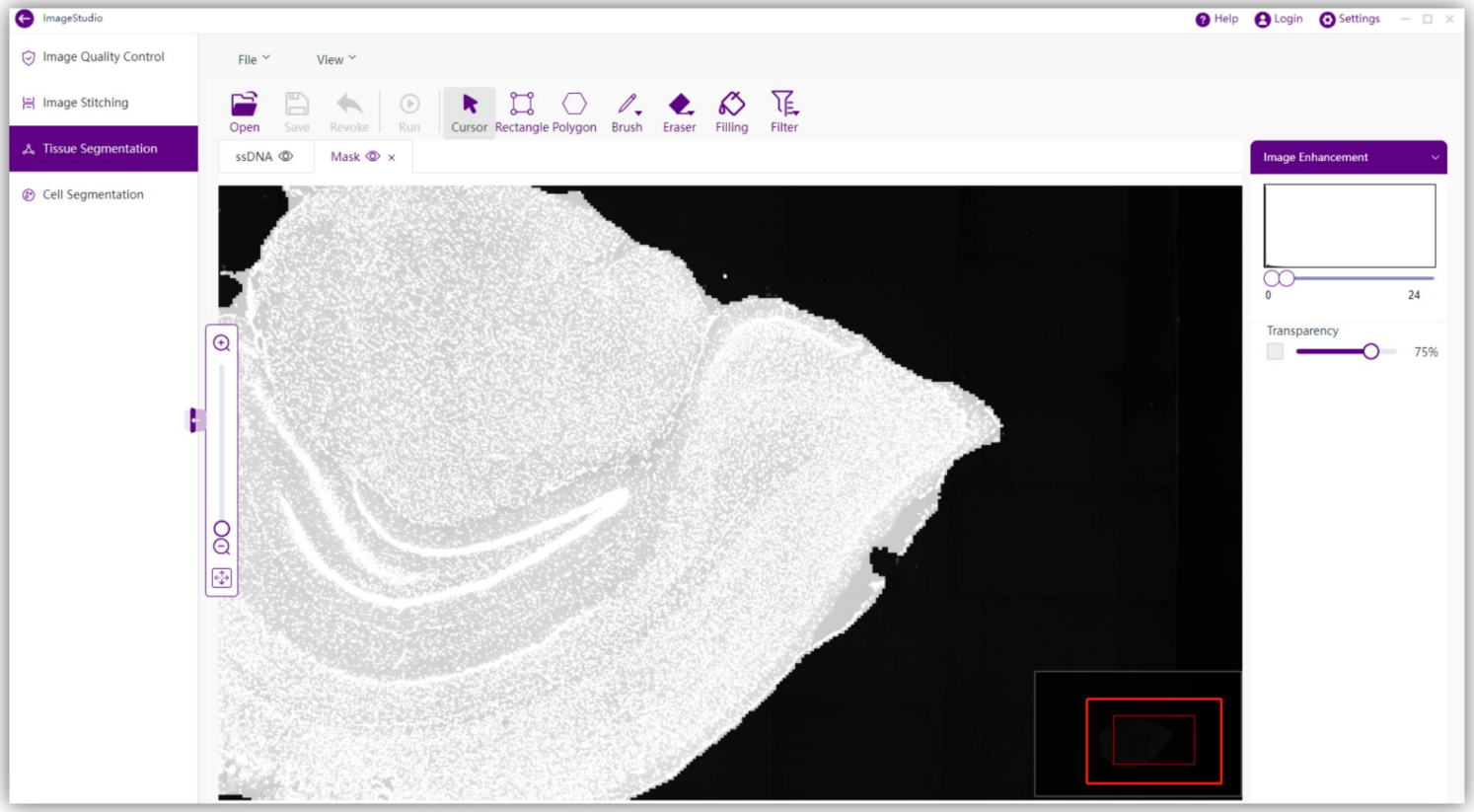
(7) Press and hold Ctrl+ while using the mouse wheel or click on the magnifying glass icon to zoom in or out of the image.



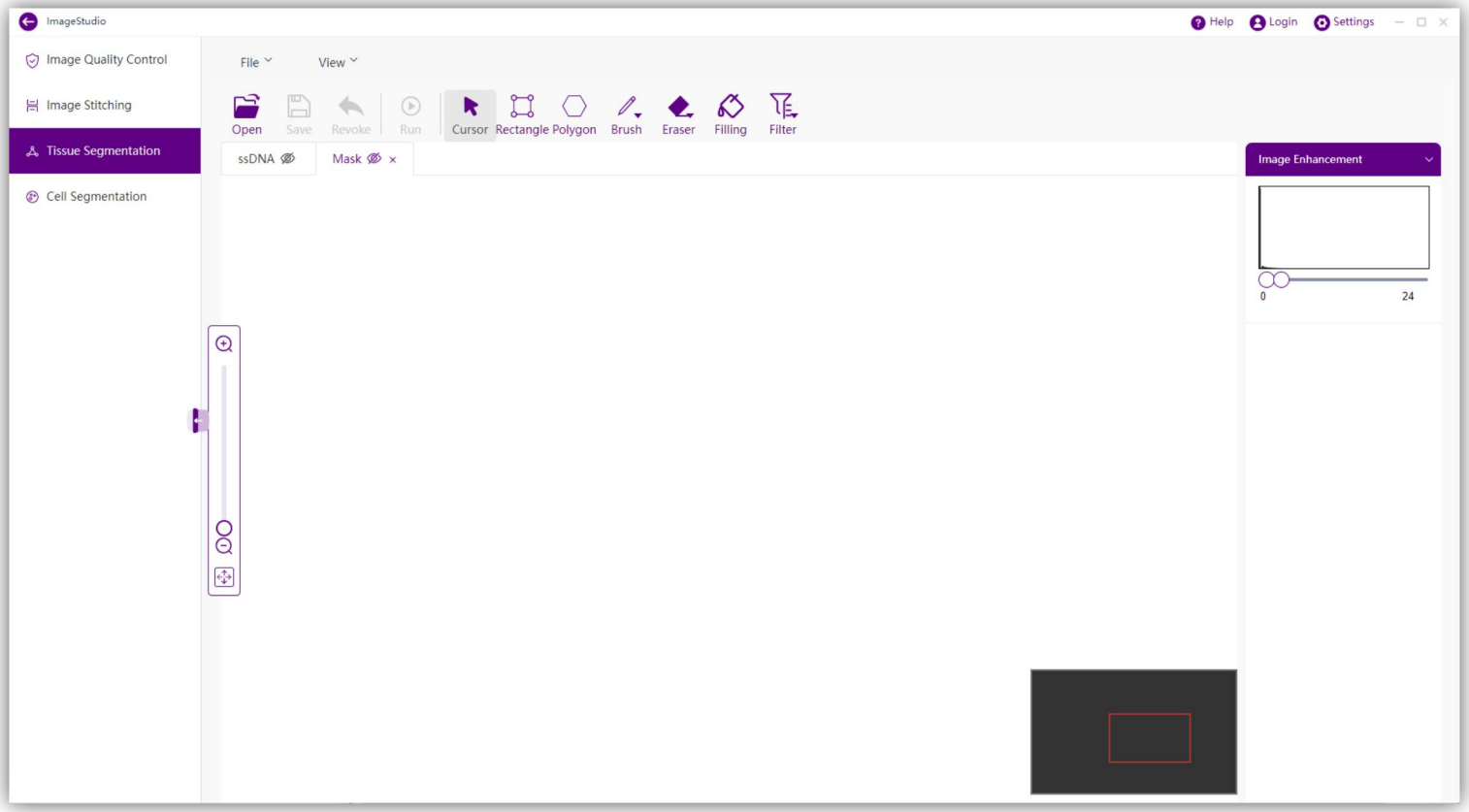
(8) Left click to move around the image.

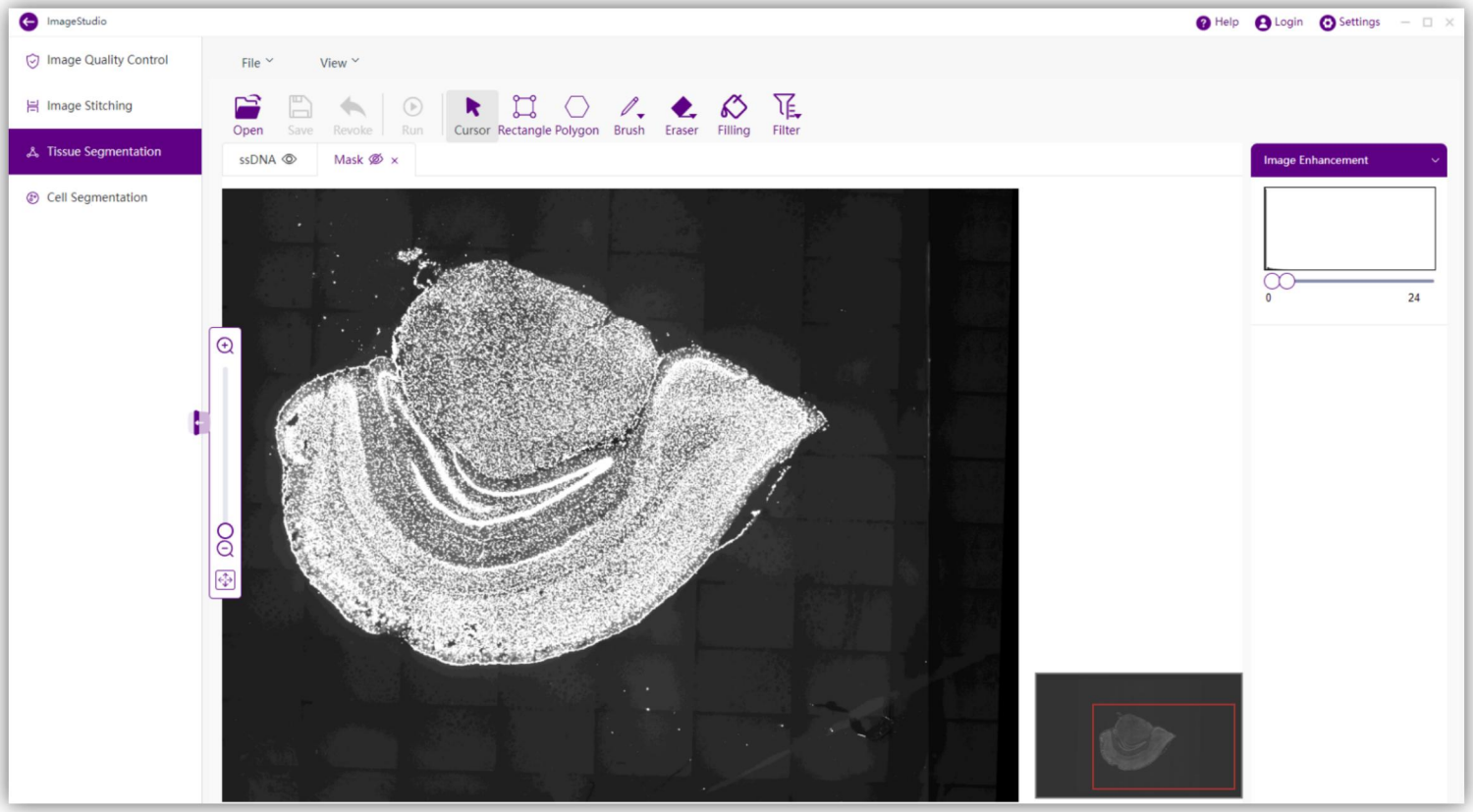


(9) Left-click within the local view window to move the field of view.



(10) Click on the 'eye icon' to hide or display the image under the tab.

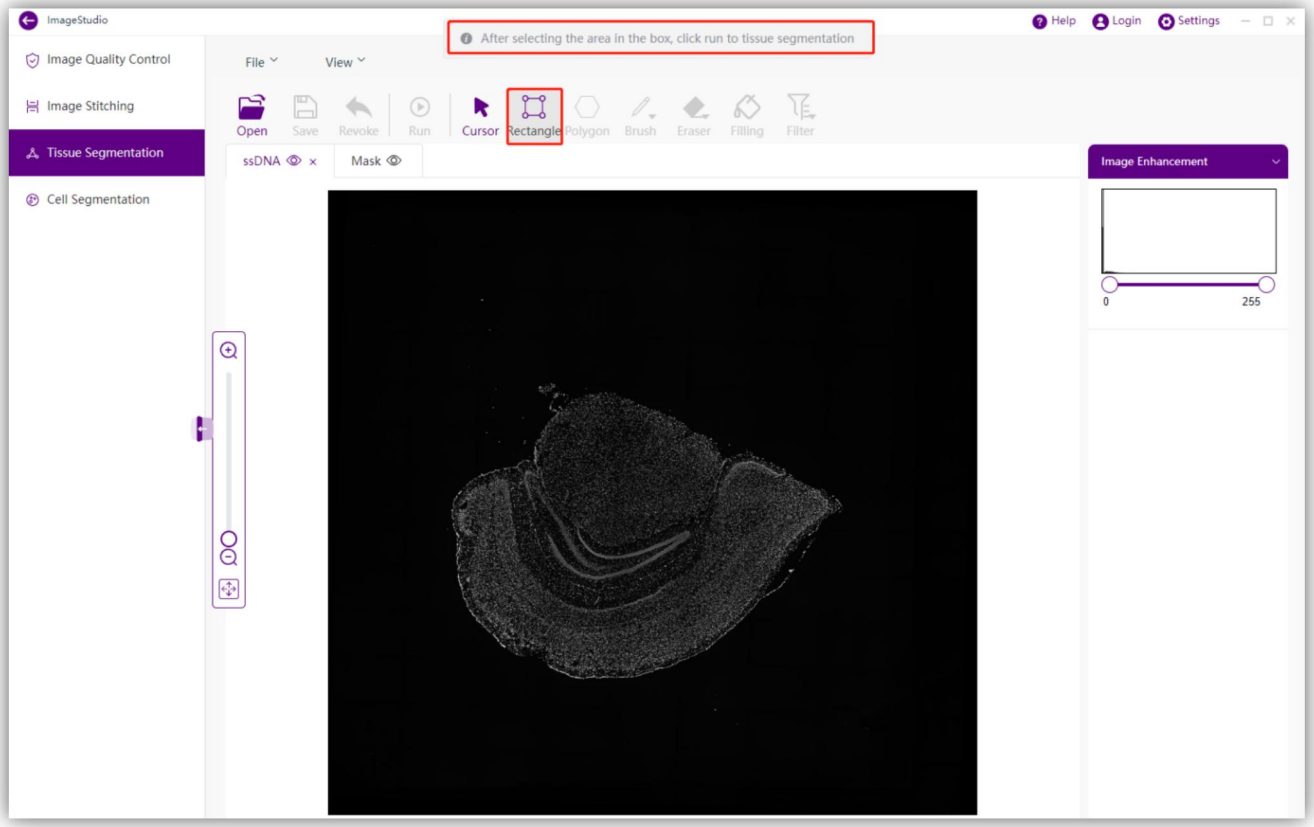

ImageStudio
Help
Login
Settings
Image Quality Control
Image Stitching
Tissue Segmentation
Cell Segmentation
File
View
Open
Save
Revoke
Run
Cursor
Rectangle
Polygon
Brush
Eraser
Filling
Filter
ssDNA
Mask
Image Enhancement
0
24



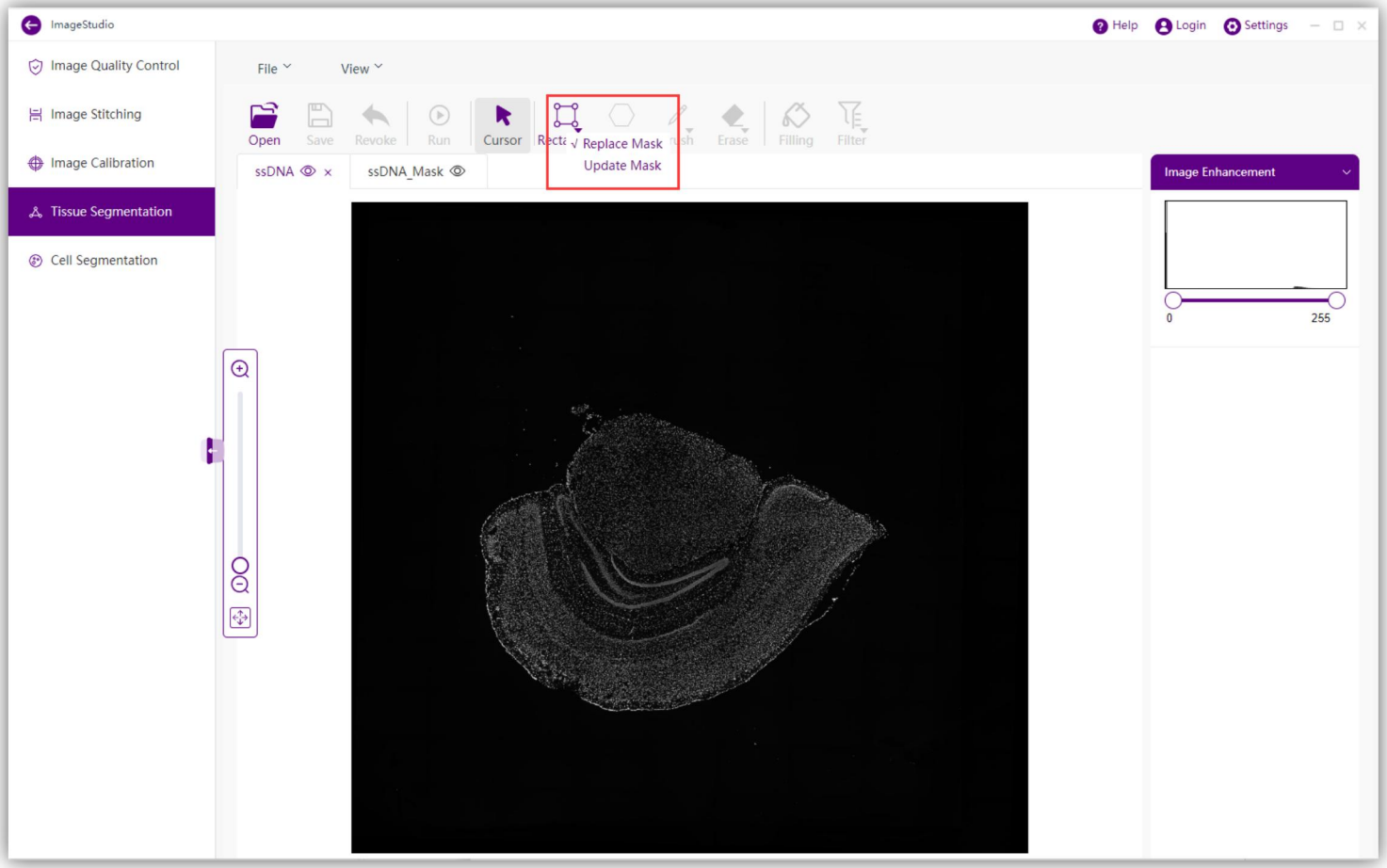
4.4.2 Rectangle

This function mainly operates on the stitched image for extracting algorithm generated tissue mask file according to the box selected ROI via tissue segment algorithm.

(1)Click 'Rectangle' in the stitched image tab.



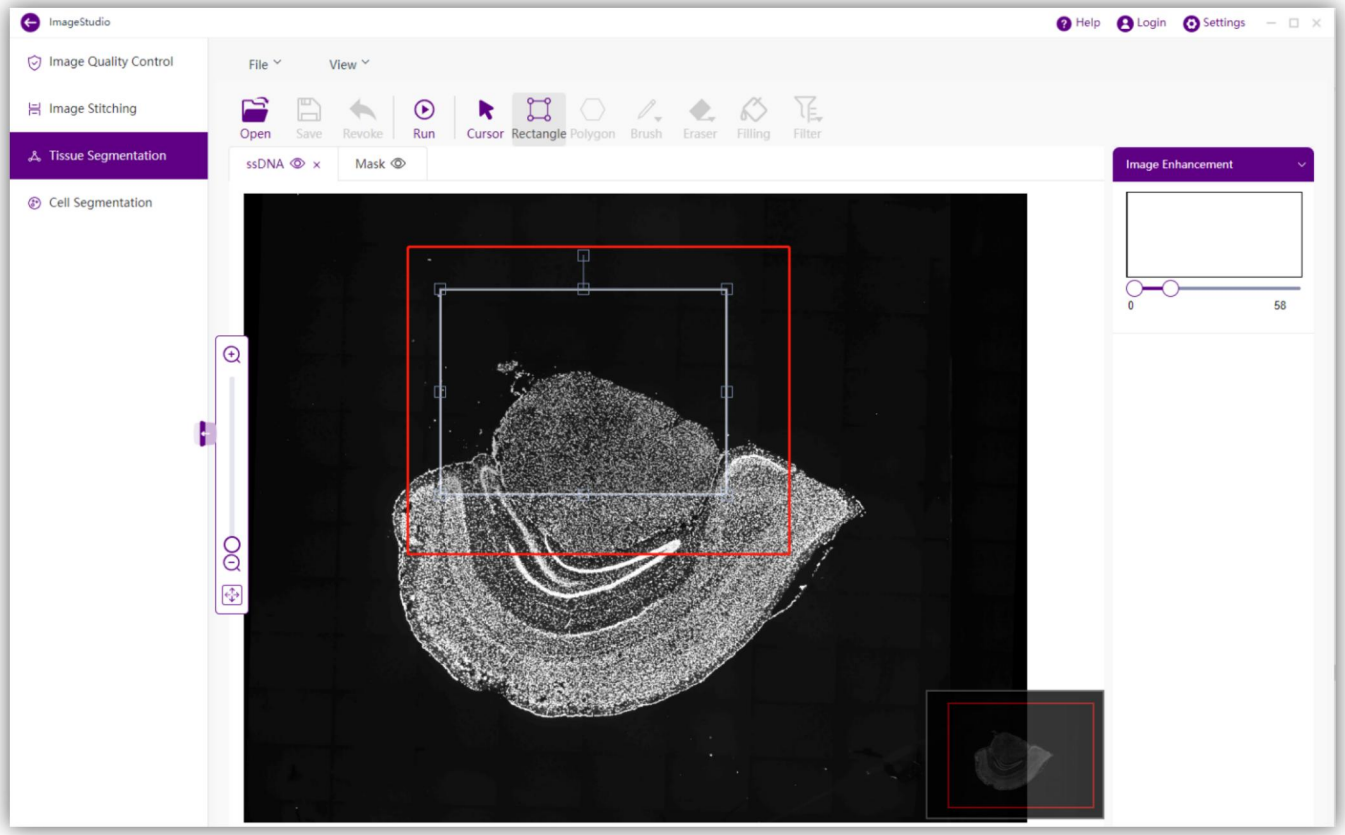
(2) User can choose either "Replace Mask" or "Update Mask", and the default option is to replace Mask.



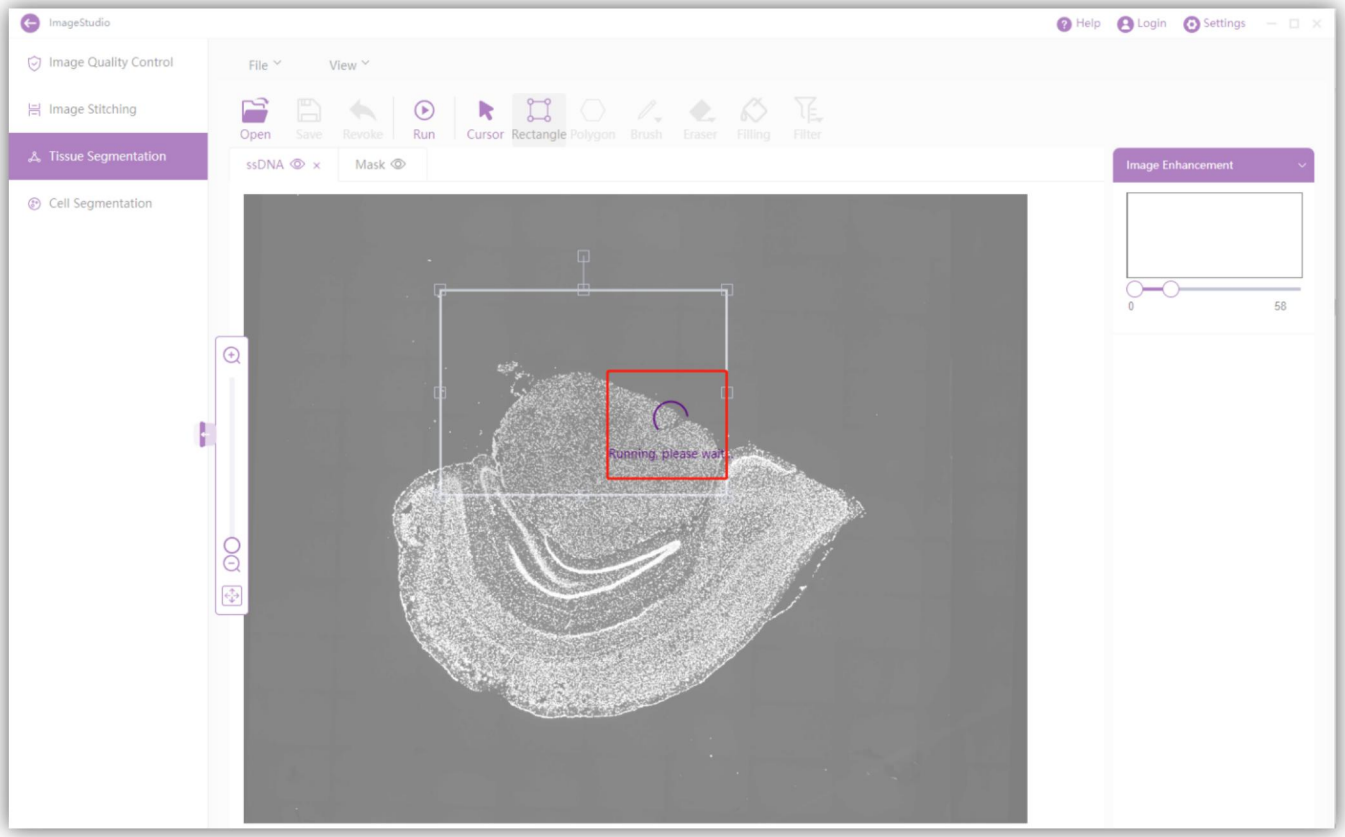
Note:

- "Replace Mask" extracts a new Mask according to the box selected ROI, which replaces the stitched Mask file.
- "Update Mask" updates the corresponding Mask according to the box selected ROI, and the Mask of other areas remain unchanged.

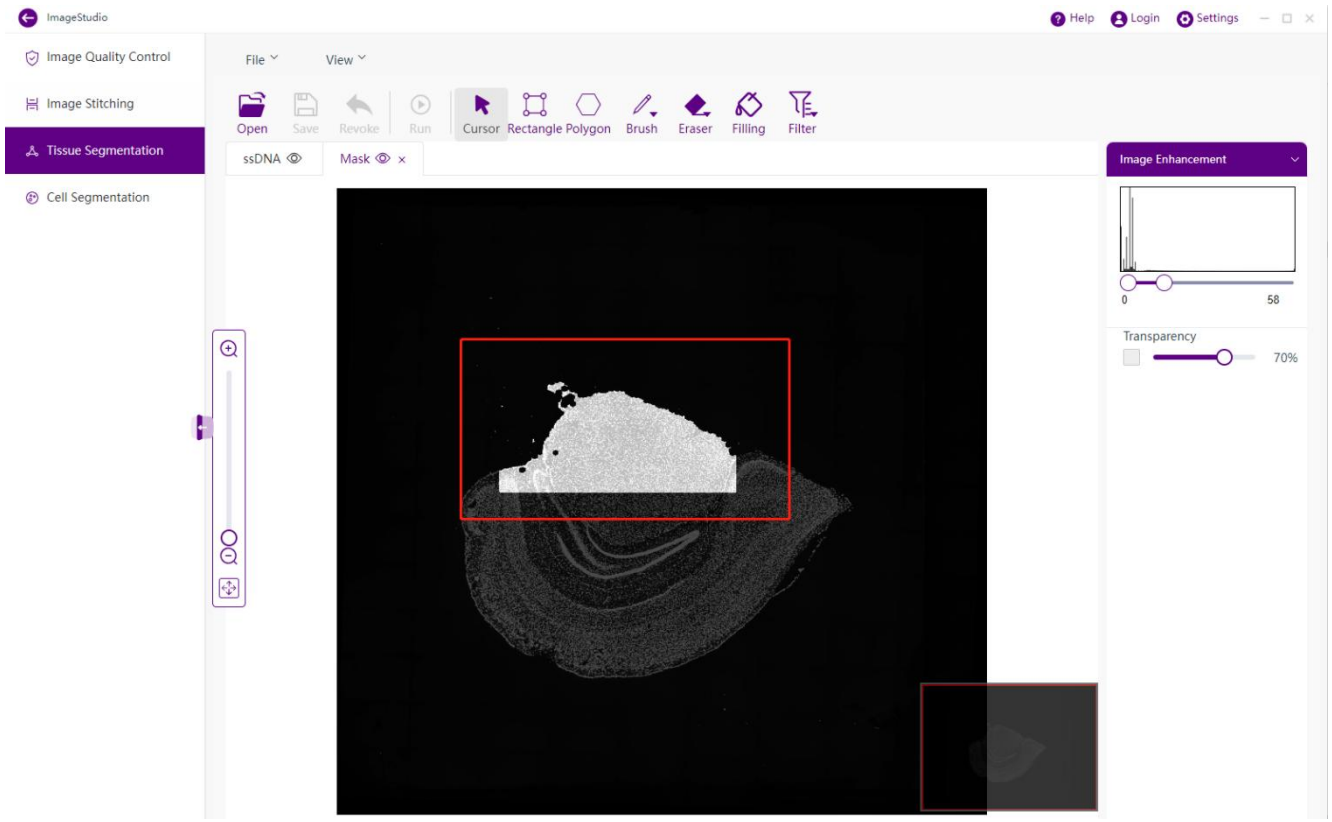
(3) Box selects region of interest using the rectangle.



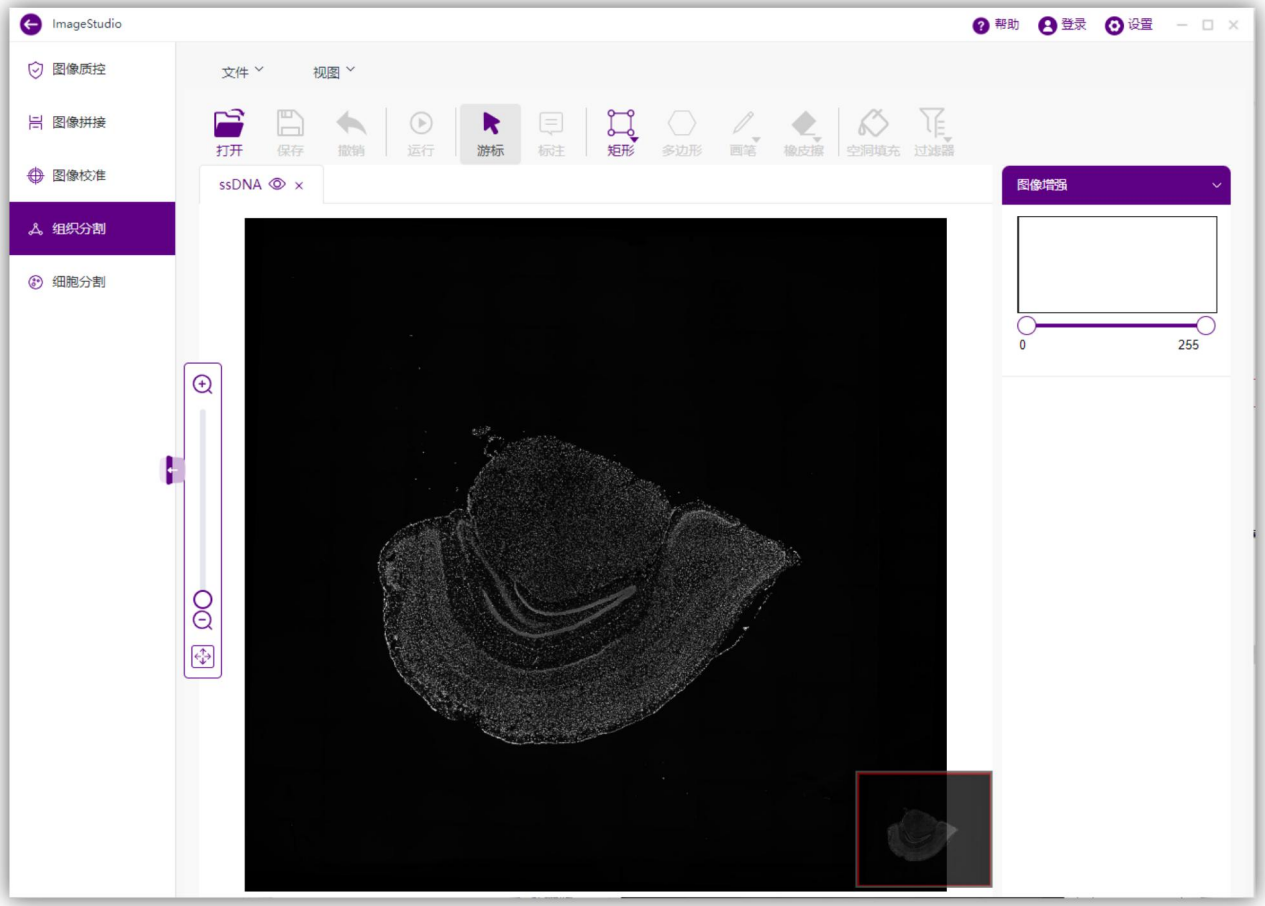
(4) Click 'Run'.

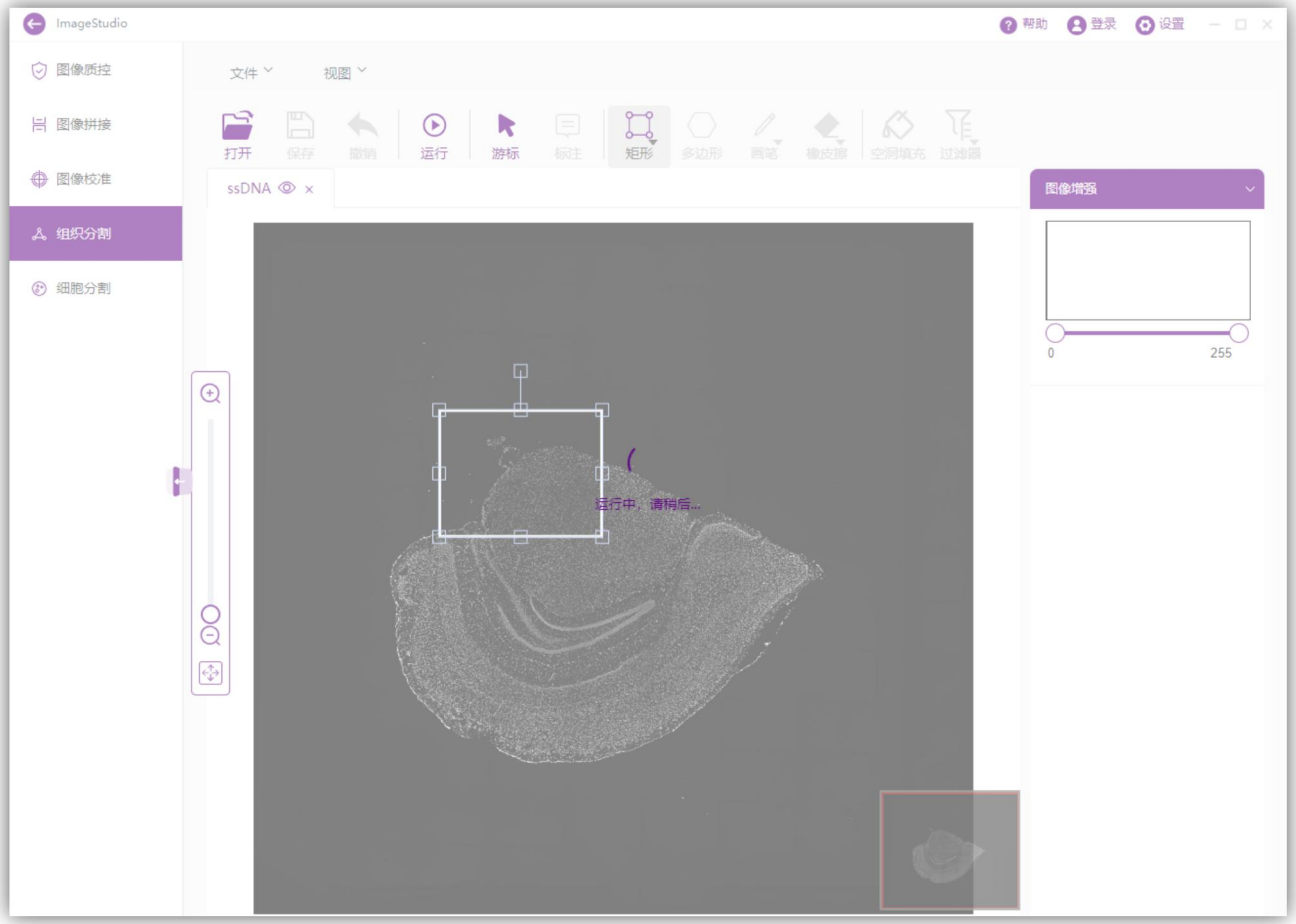


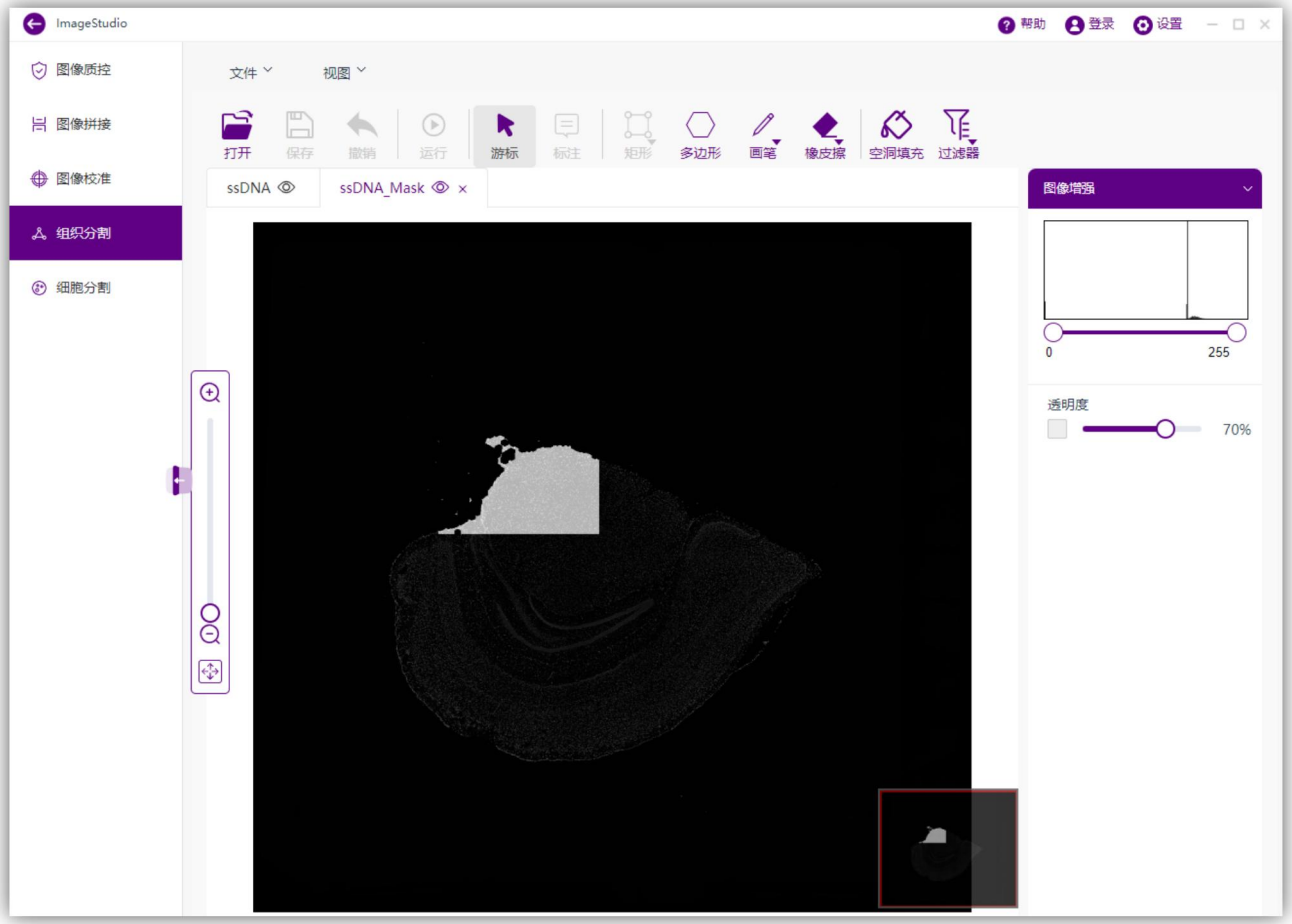
(5) Tissue segmentation algorithm will extract tissue Mask file according to the box selected ROI, write it into the IPR file.

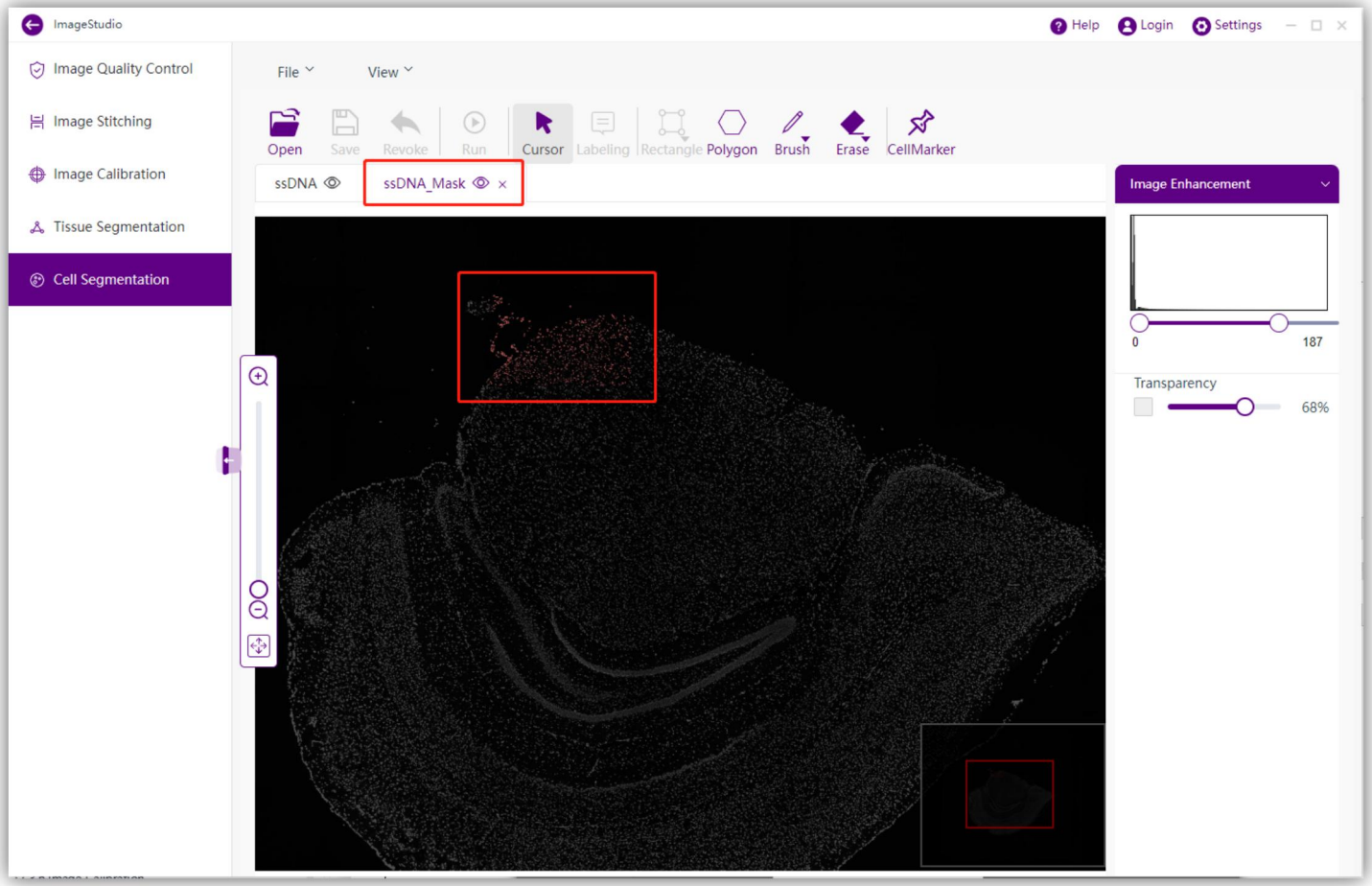


(6) If the input data has not gone through tissue segmentation, a new "Mask" tab will be generated after running tissue segmentation using rectangle tool.


ImageStudio
帮助
登录
设置
图像质控
图像拼接
图像校准
组织分割
细胞分割
文件
视图
打开
保存
撤销
运行
游标
标注
矩形
多边形
画笔
橡皮擦
空洞填充
过滤器
ssDNA
图像增强
0
255


ImageStudio
帮助
登录
设置
图像质控
图像拼接
图像校准
组织分割
细胞分割
文件
视图
打开
保存
撤销
运行
游标
标注
矩形
多边形
画笔
橡皮擦
空洞填充
过滤器
ssDNA
图像增强
0
255
运行中，请稍后...


ImageStudio
帮助
登录
设置
图像质控
图像拼接
图像校准
组织分割
细胞分割
文件
视图
打开
保存
撤销
运行
游标
标注
矩形
多边形
画笔
橡皮擦
空洞填充
过滤器
ssDNA
ssDNA_Mask
图像增强
0
255
透明度
70%

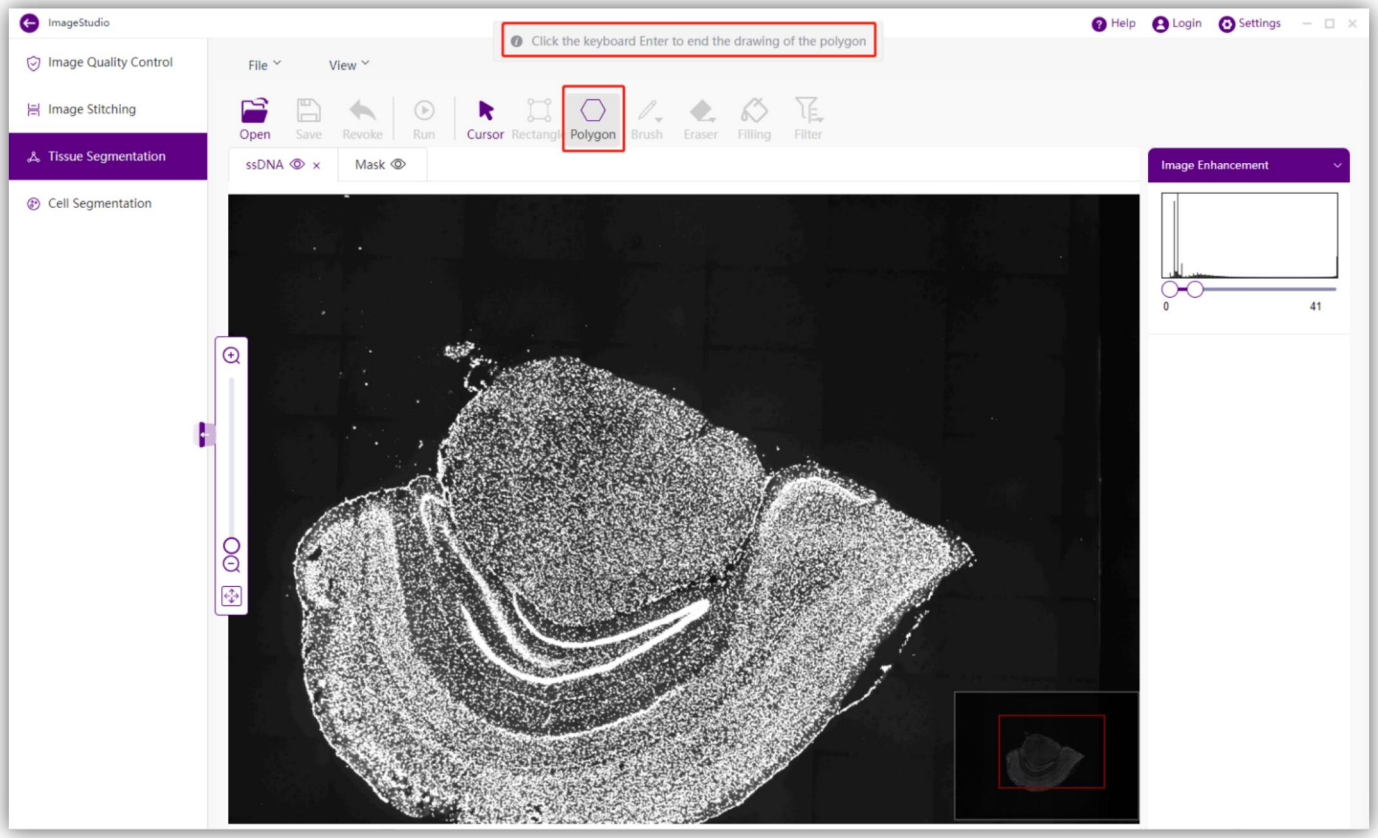


4.4.3 Polygon

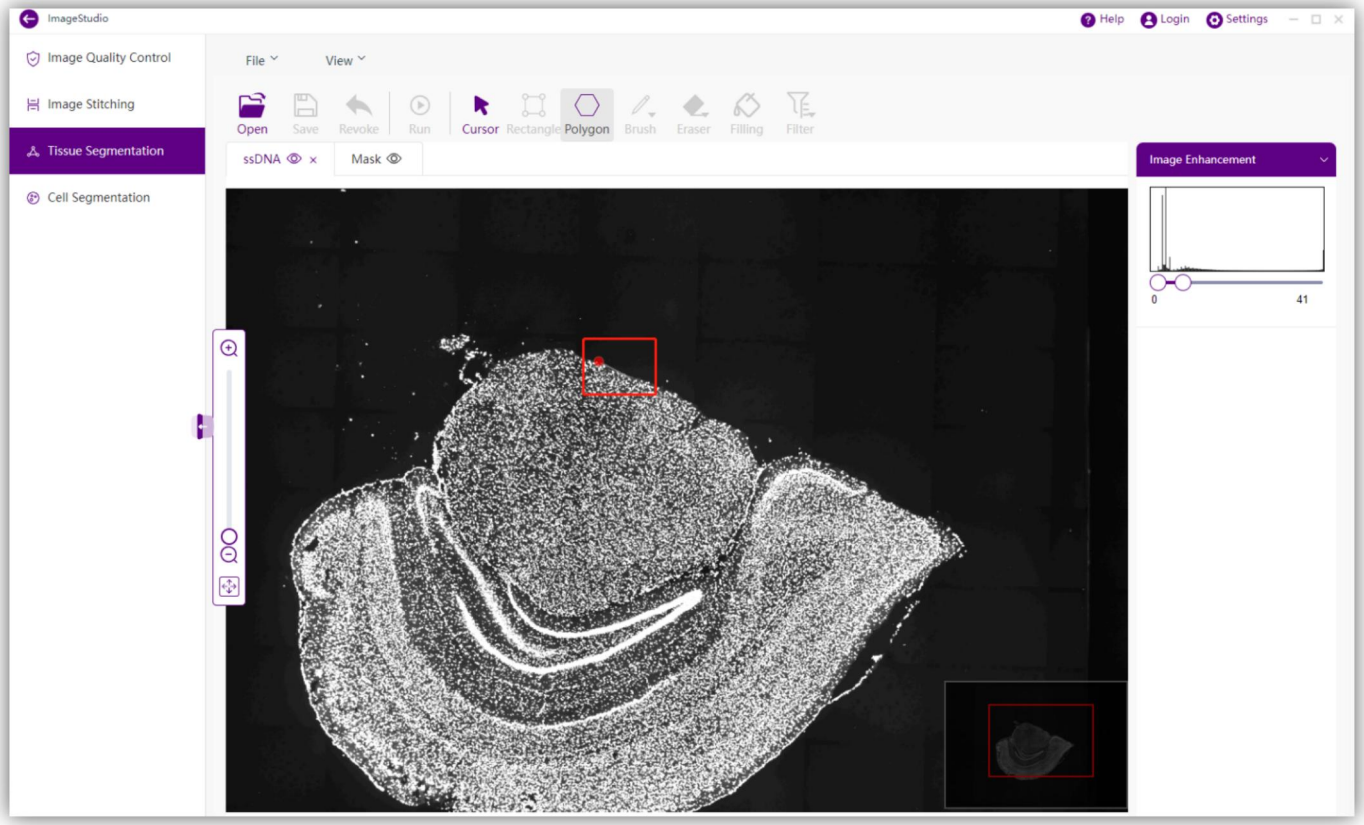
This function mainly operates on the stitched image for modifying algorithm generated tissue Mask file according to the manually drawn tissue contour using polygon selection and overlaying the

stitched tissue Mask file. Multiple ROIs could be drawn using the polygon selection.

(1)Click 'Polygon' in the stitched image tab.

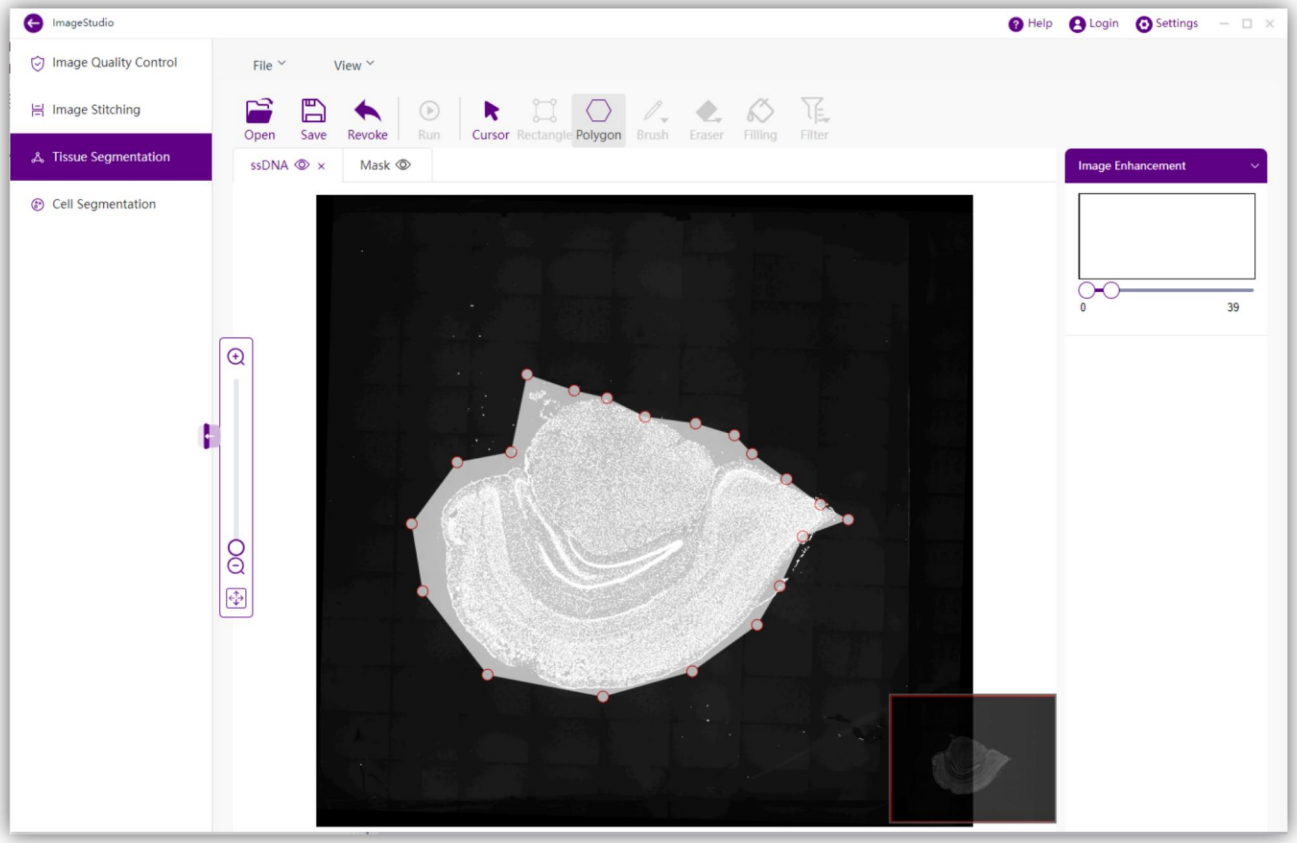


(2)Start with a point, left-click to add points until an enclosed polygon has formed.

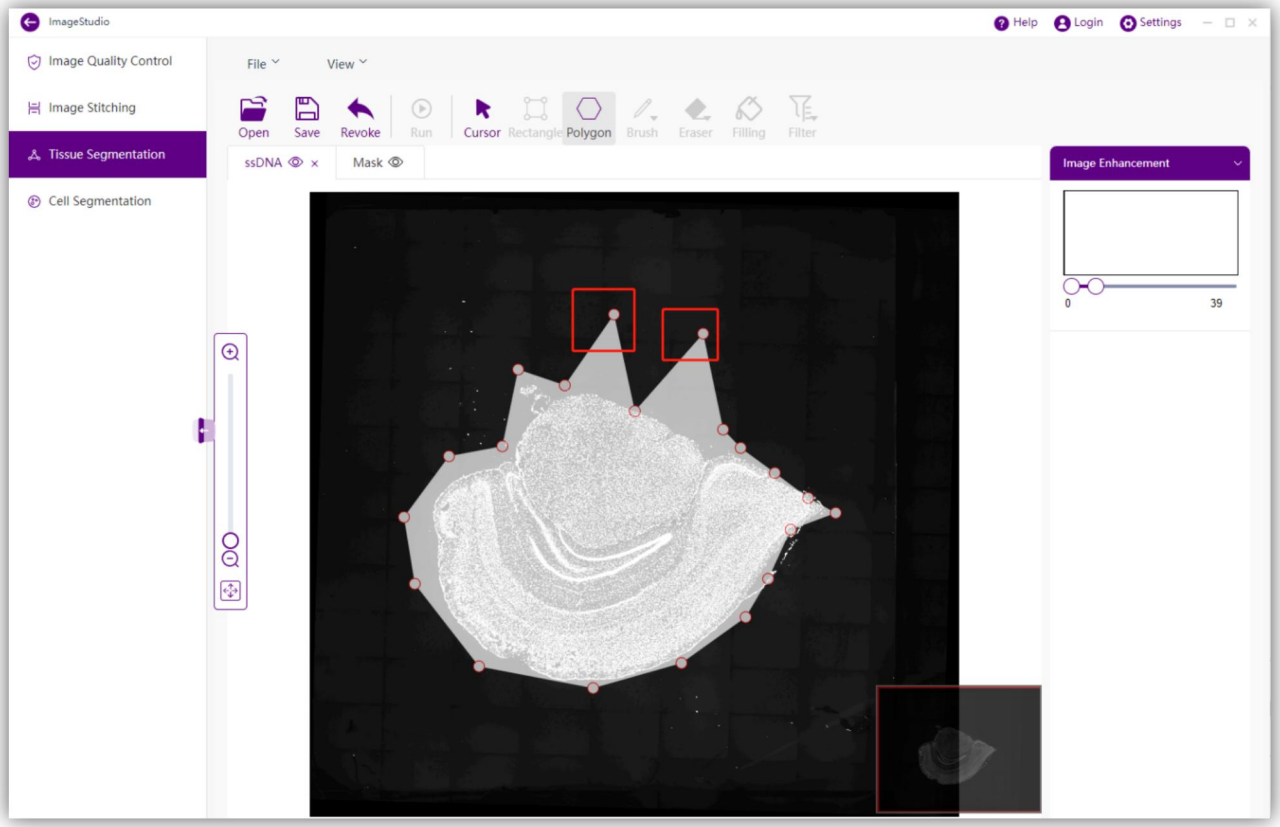


Currently, overlapping of ROIs using polygon selection is not supported.

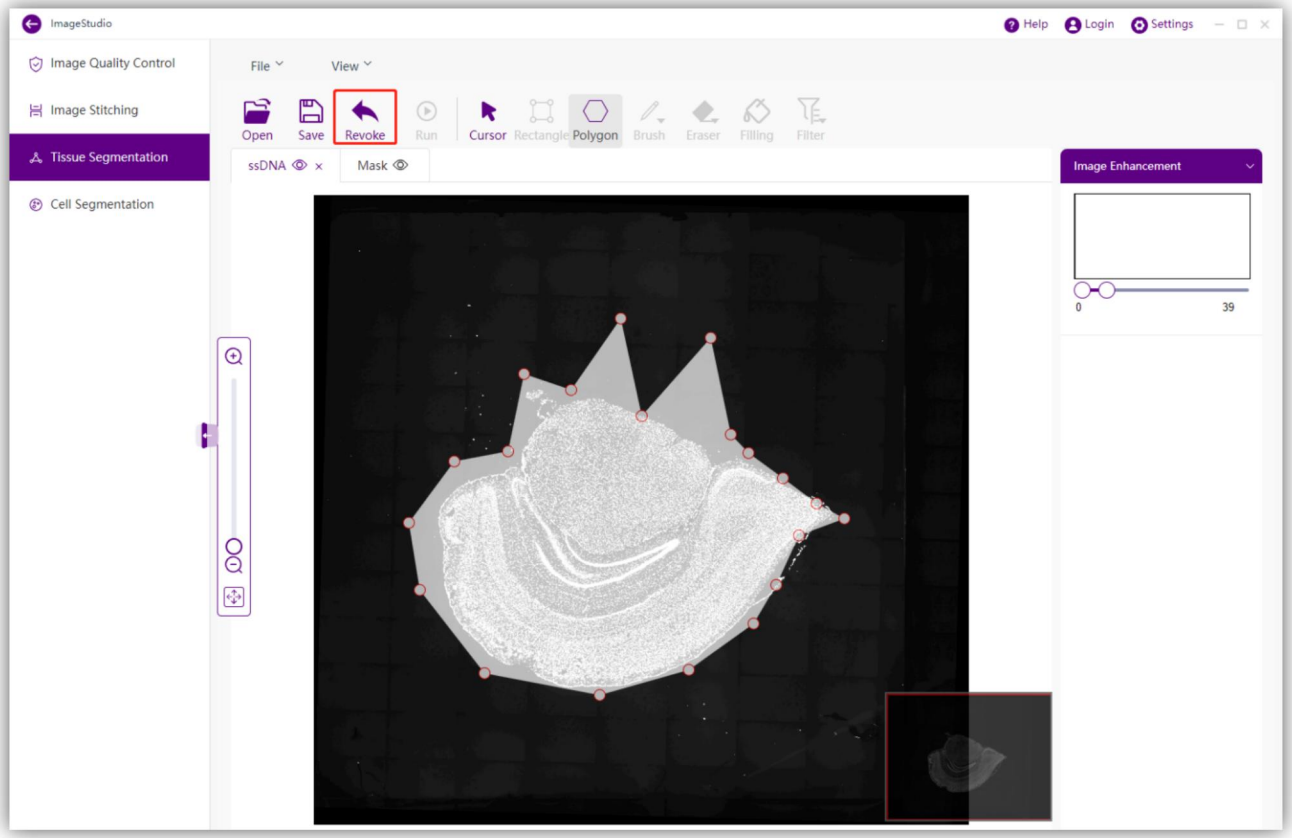
(3) Press and hold Enter on the keyboard to stop adding more points and enter polygon editing mode.

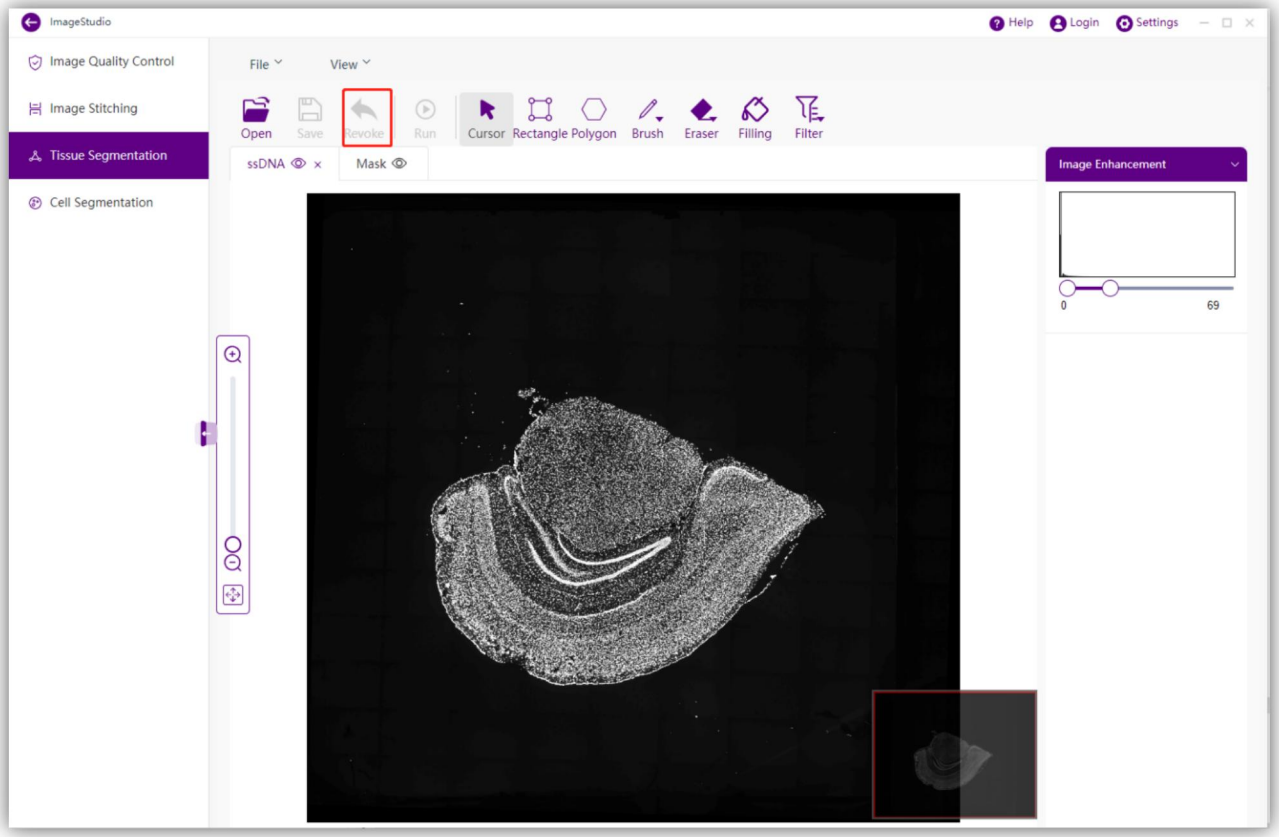


(4) Click on the points on the enclosed polygon trace then left-click and hold to drag and reposition the shape of the polygon.

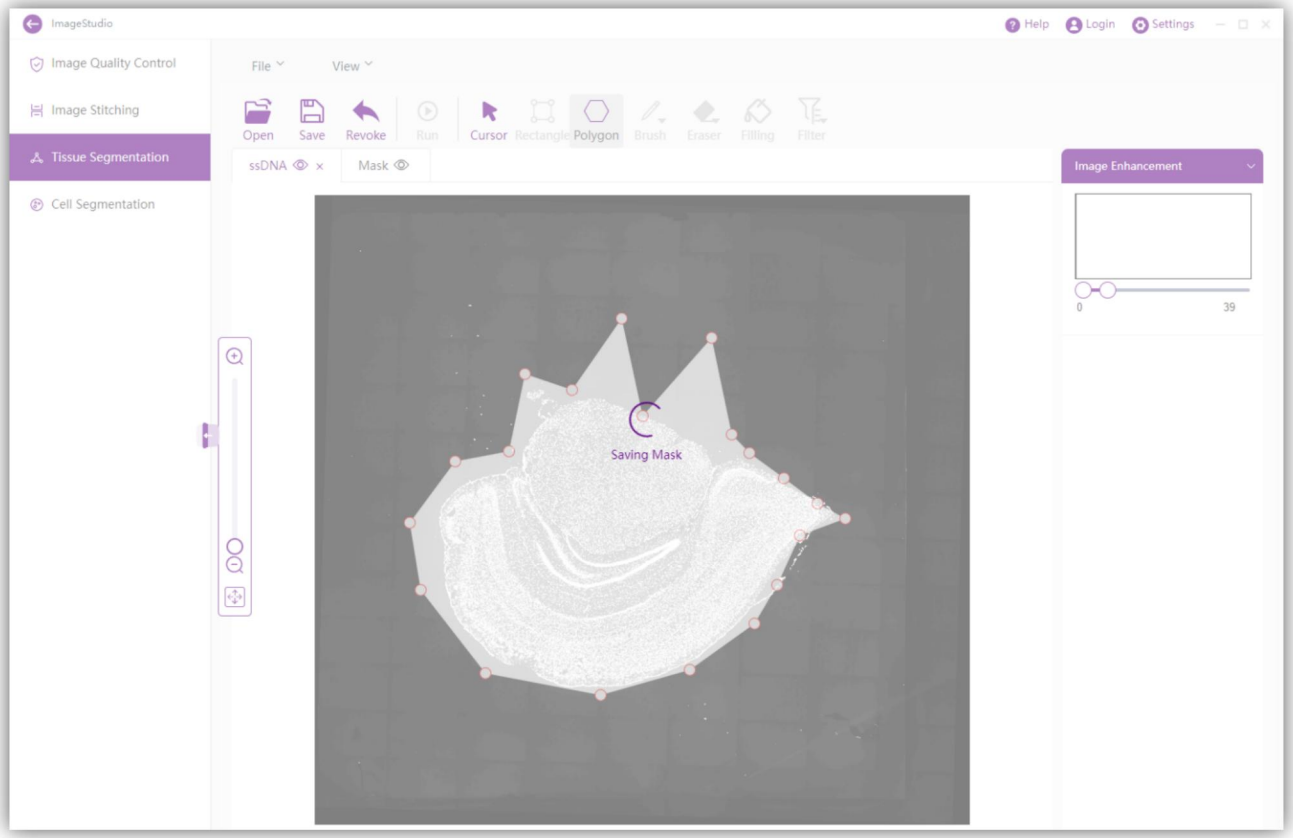


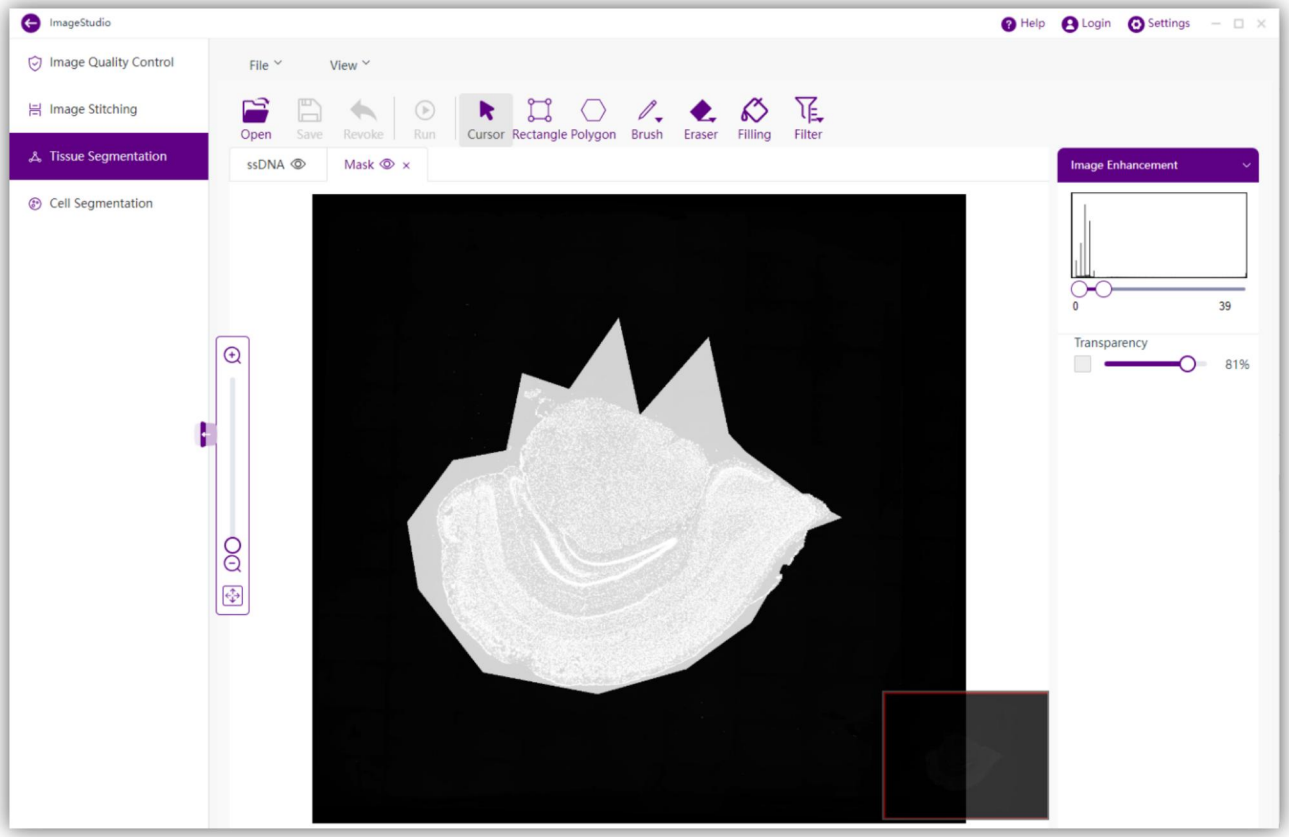
(5)Undo the point editing action by clicking 'Revoke' before saving.


ImageStudio
Help
Login
Settings
Image Quality Control
Image Stitching
Tissue Segmentation
Cell Segmentation
File
View
Open
Save
Revoke
Run
Cursor
Rectangle
Polygon
Brush
Eraser
Filling
Filter
ssDNA
Mask
Image Enhancement
0
39



(6)Click 'Save' after editing to write the new tissue Mask file into the IPR file.


ImageStudio
Help
Login
Settings
Image Quality Control
Image Stitching
Tissue Segmentation
Cell Segmentation
File
View
Open
Save
Revoke
Run
Cursor
Rectangle
Polygon
Brush
Eraser
Filling
Filter
ssDNA
Mask
Image Enhancement
0
39
Saving Mask

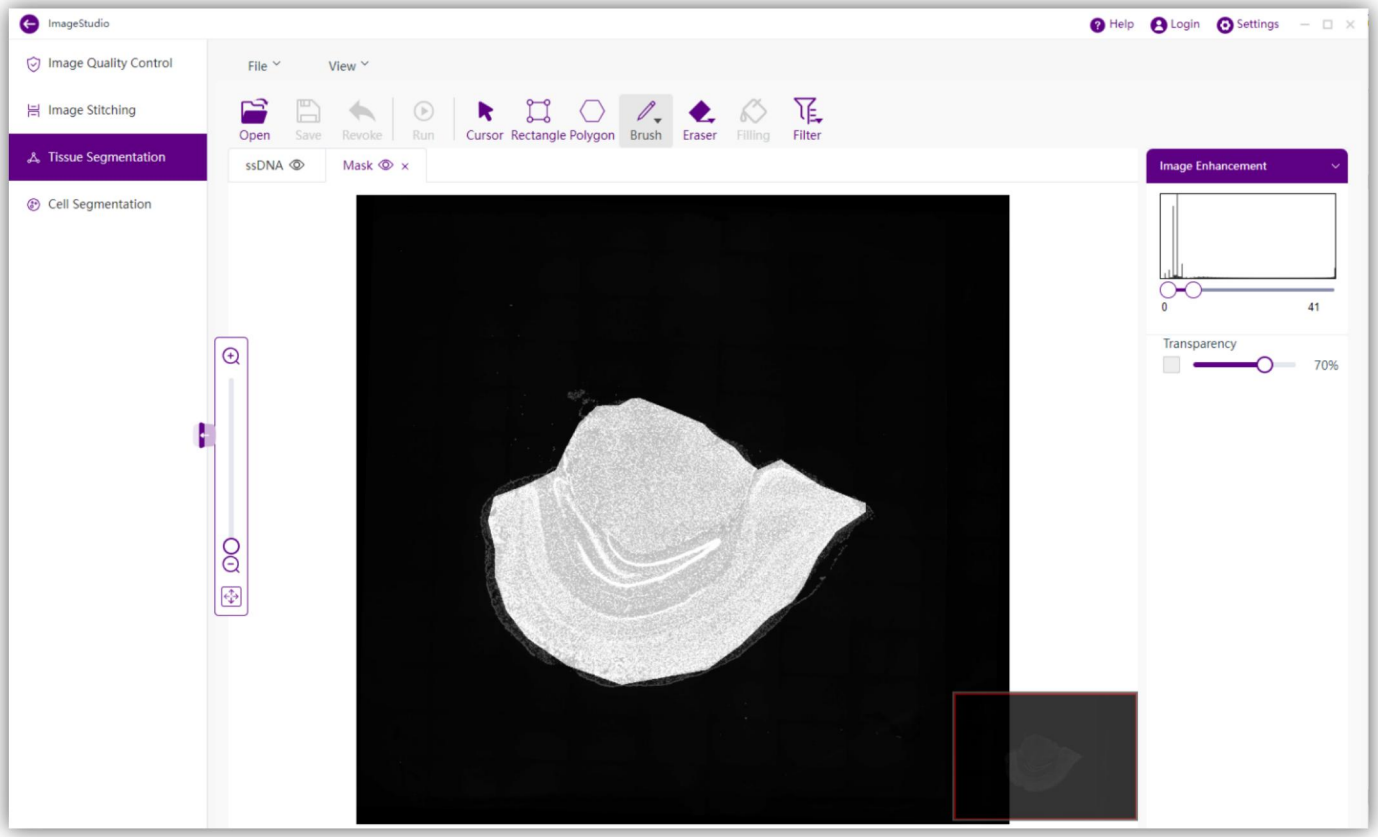


(7) Click 'Cursor' to exit the polygon editing mode.

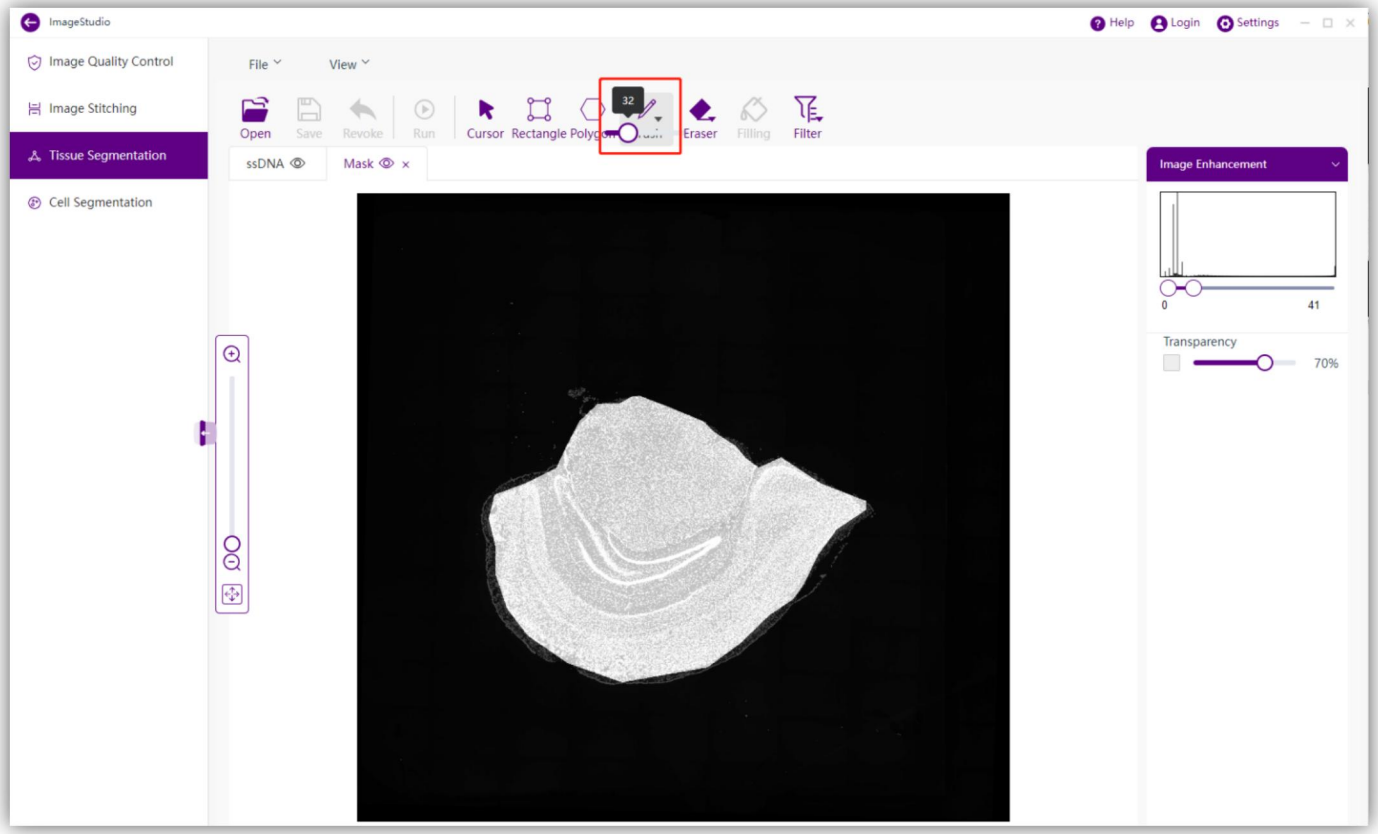
4.4.4 Brush

This function mainly operates on the Mask image for modifying algorithm generated tissue Mask file according to the manually drawn tissue Mask using a brush tool.

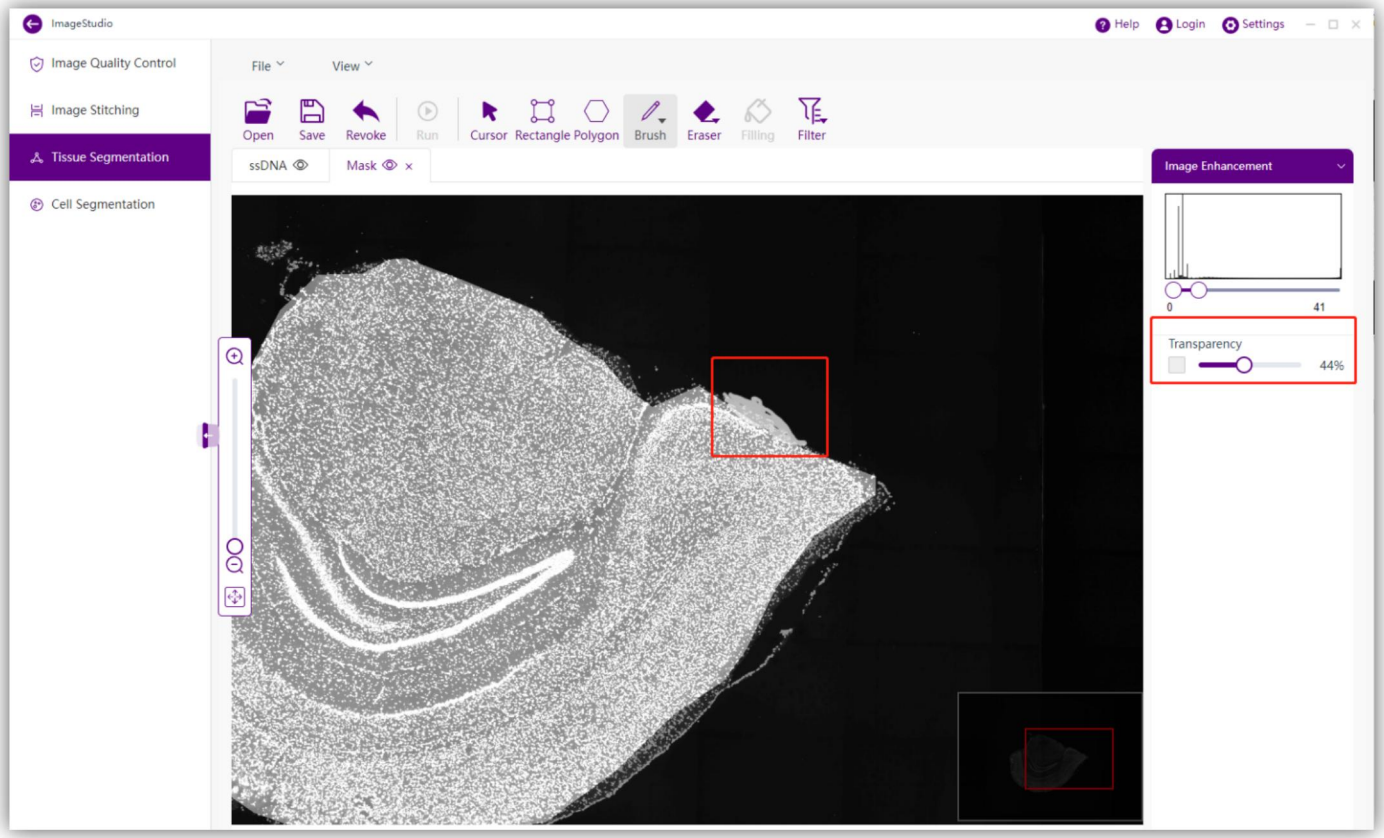
(1)Click 'Brush' in the Mask image tab.



(2)Click on the triangle icon next to 'Brush' to adjust the size of the brush. Range: [0, 1000], unit: pixel.

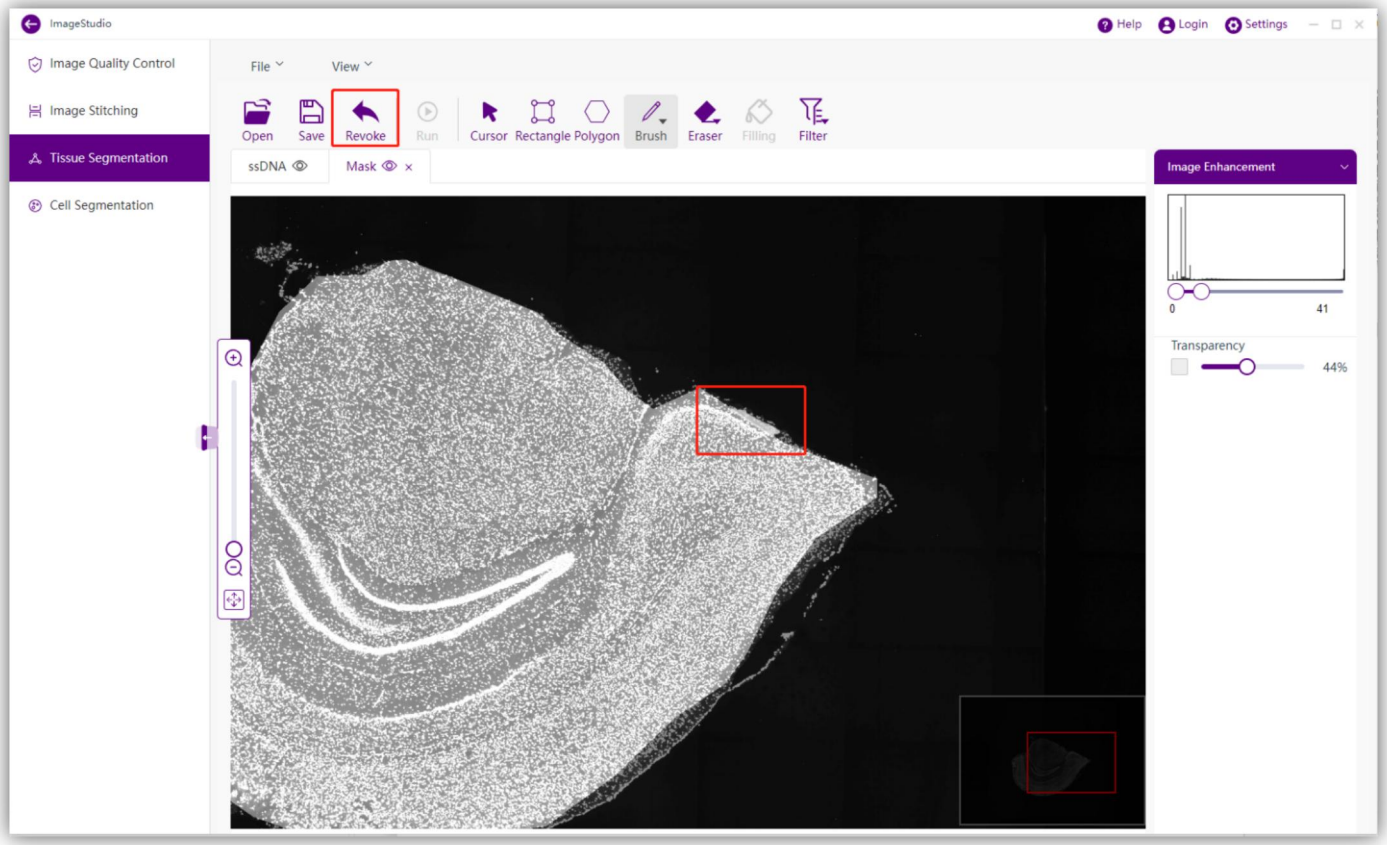


(3) Adjust 'Transparency' to get a clearer view of the tissue contour. Left-click and hold to draw on the tissue Mask outline.

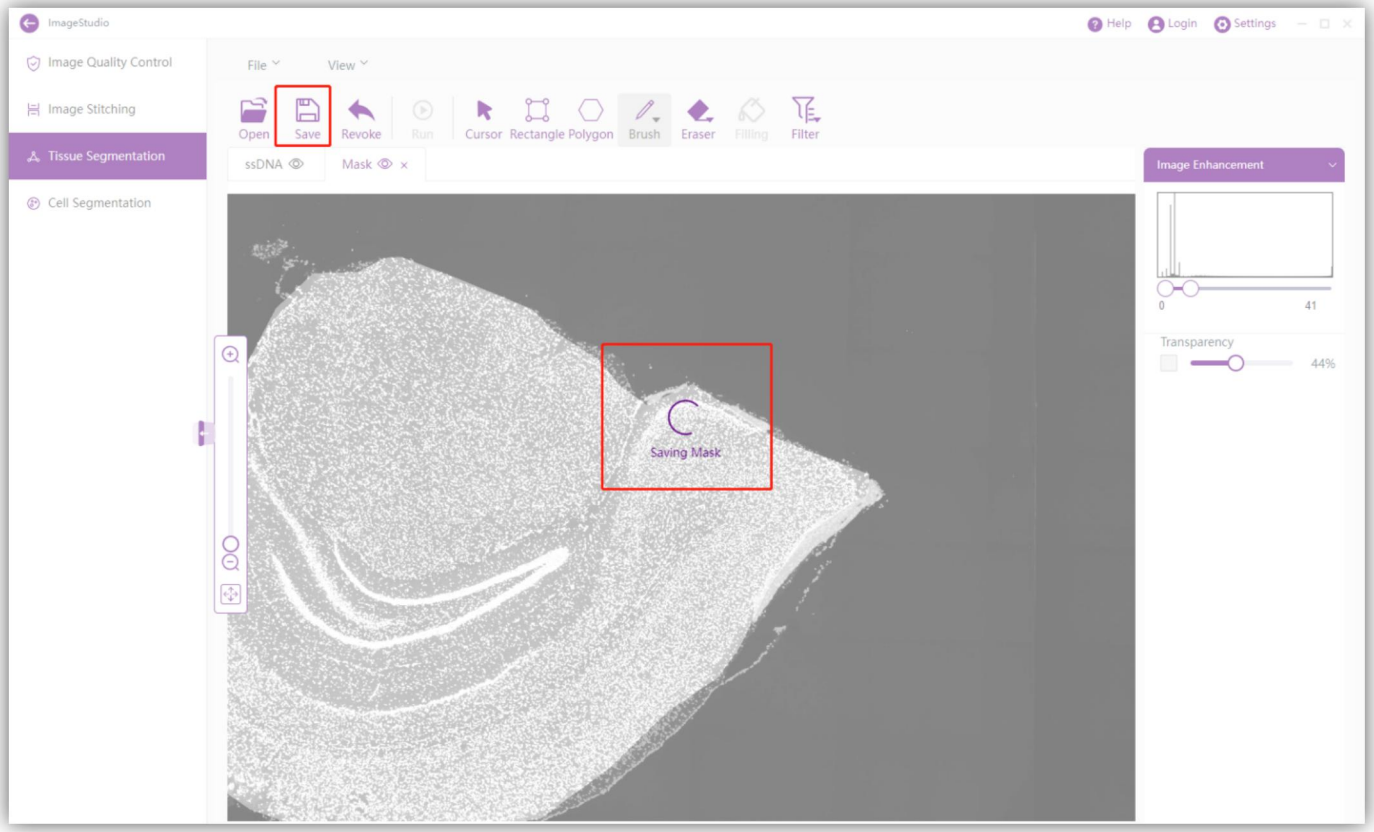


⚠Left-click within the local view window to move the field of view.

(4)If mistakes were made, click 'Revoke' before saving to revoke current action.



(5)After drawing is completed, click 'Save' to update the tissue Mask image and write it into IPR file.

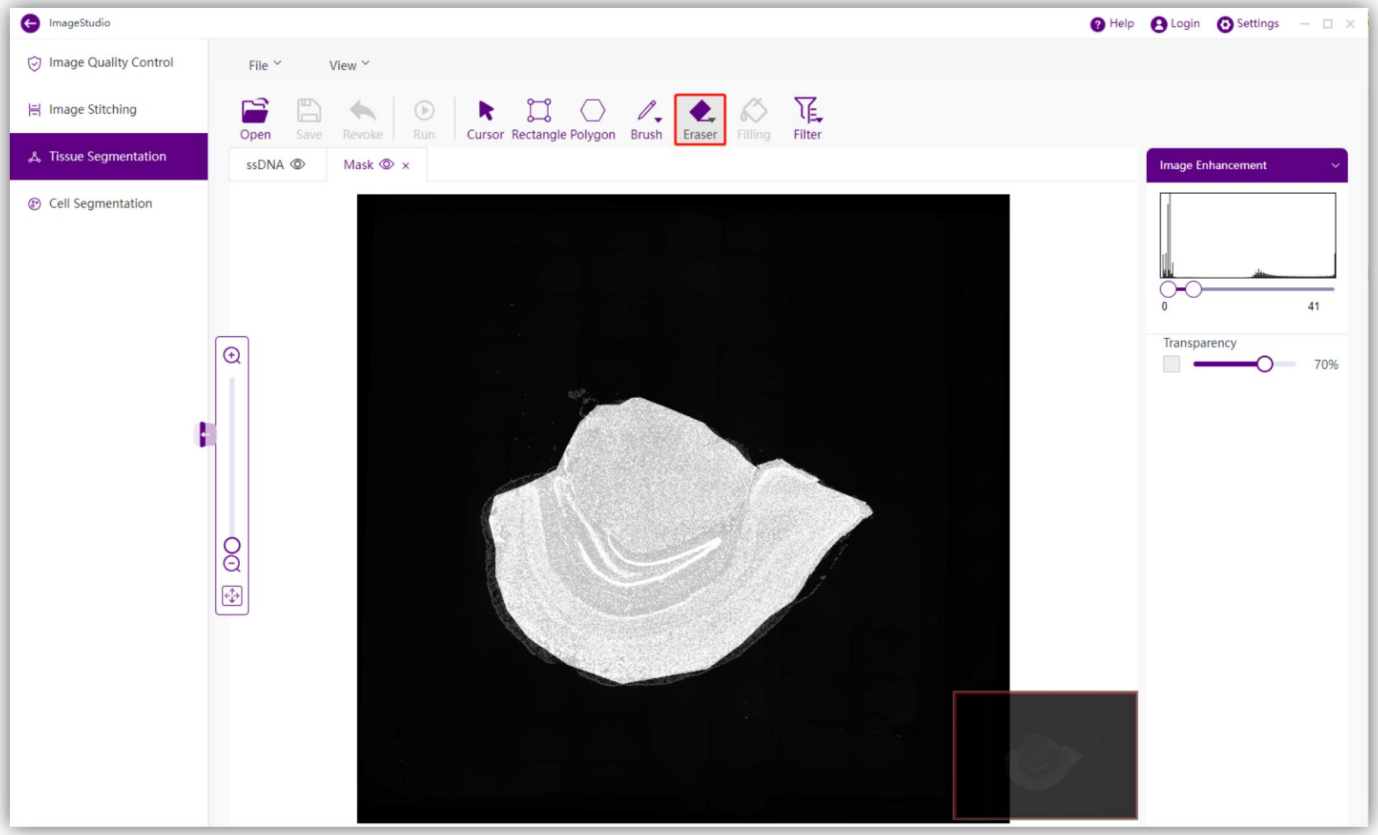


(6) Click 'Cursor' to exit the brush editing mode.

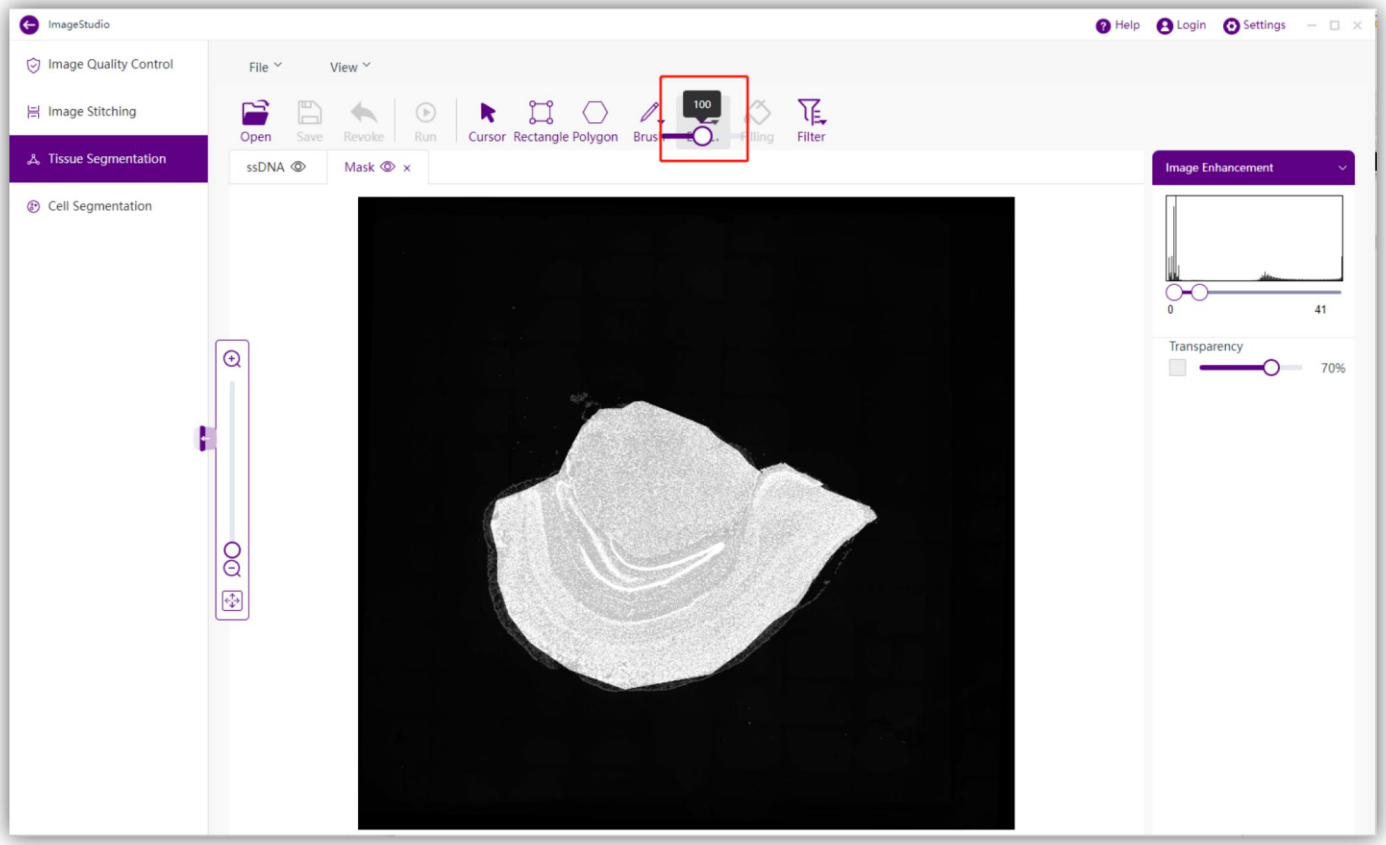
4.4.5 Eraser

This function mainly operates on the Mask image for modifying algorithm generated tissue Mask file with the eraser tool to manually erase unwanted tissue Mask region.

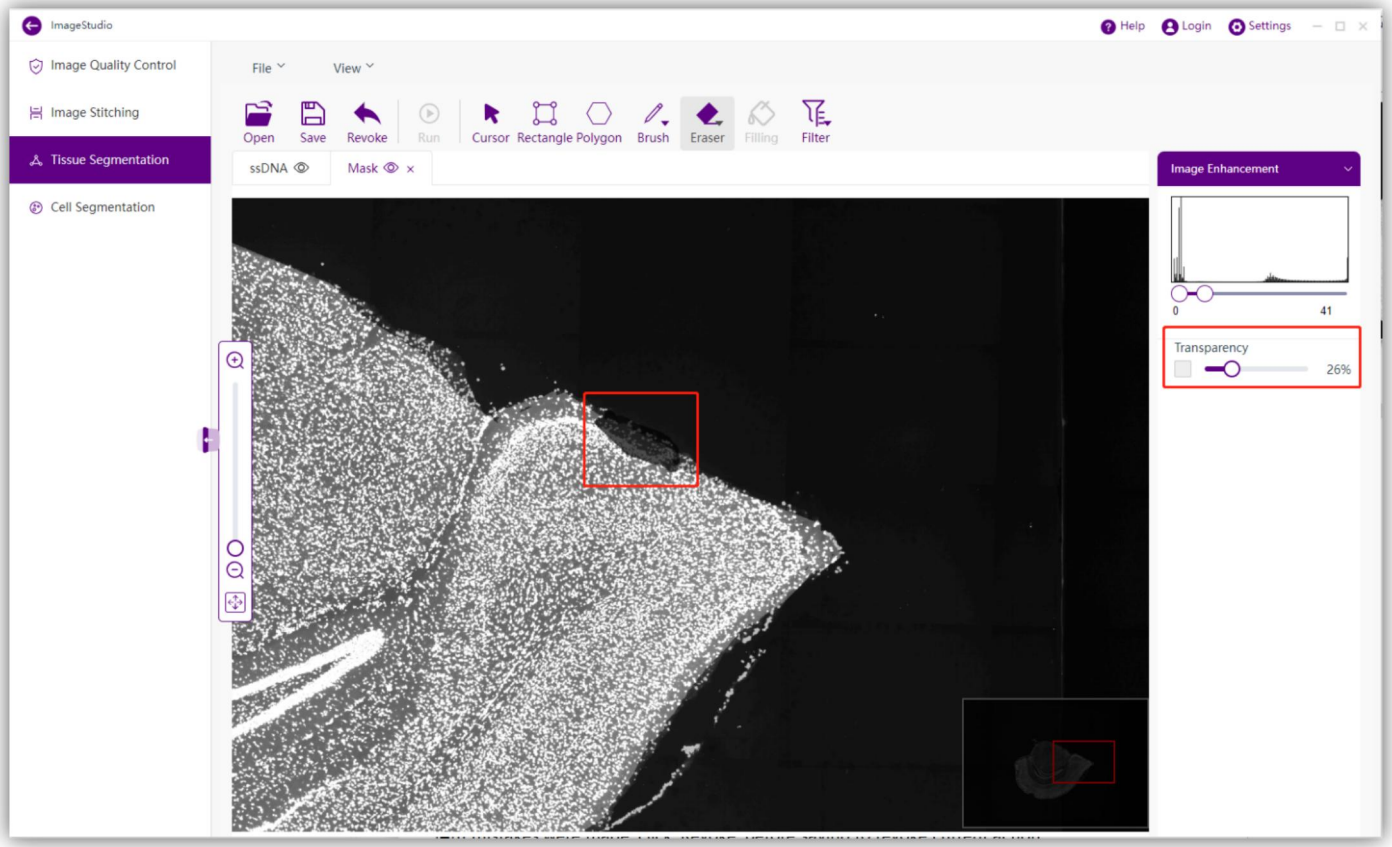
(1)Click 'Eraser' in the Mask image tab.



(2)Click on the triangle icon next to 'Eraser' to adjust the size of the eraser. Range: [0, 200], unit: pixel.

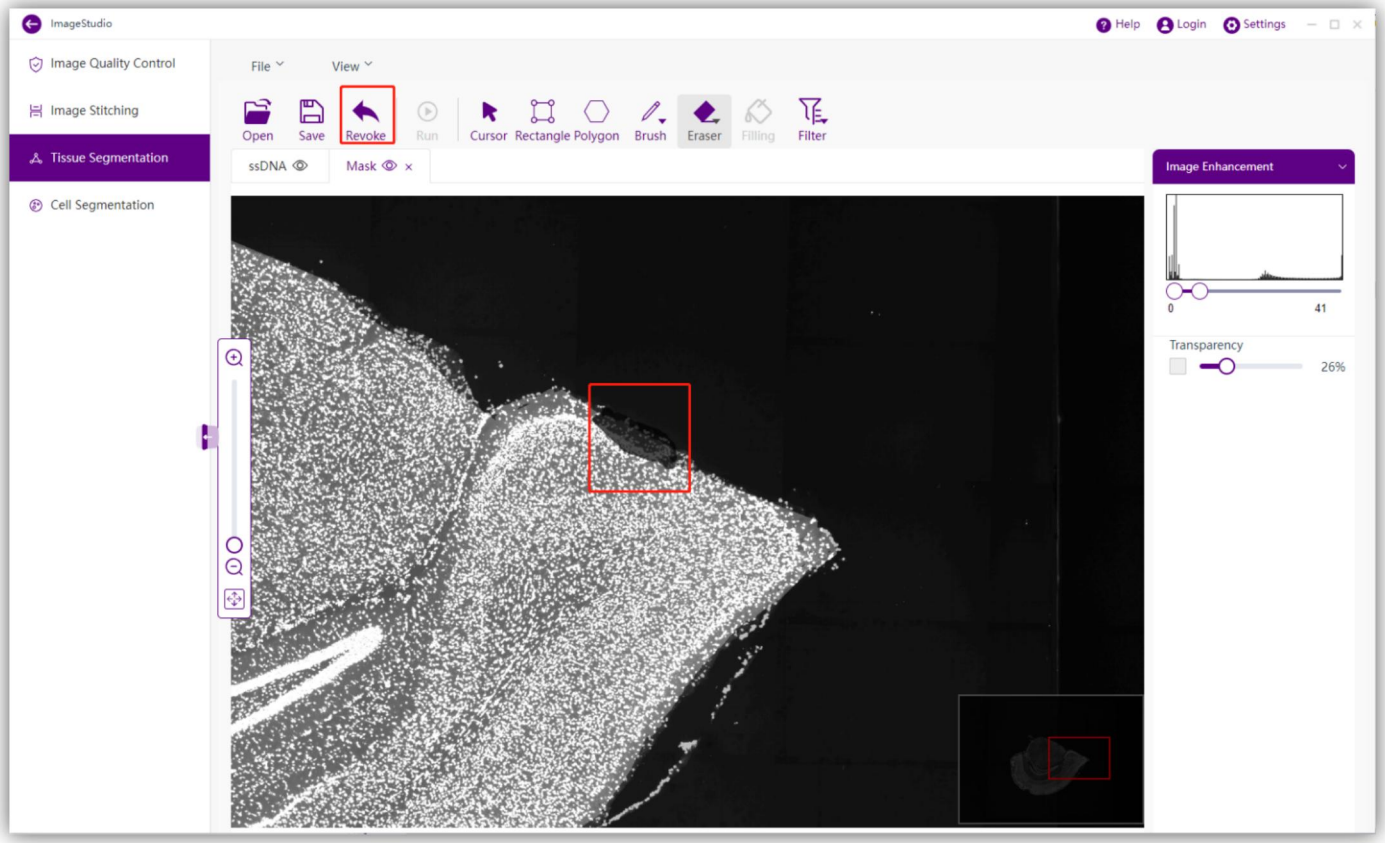


(3) Adjust 'Transparency' to get a clearer view of the tissue contour. Left-click and hold to erase unwanted tissue Mask region.

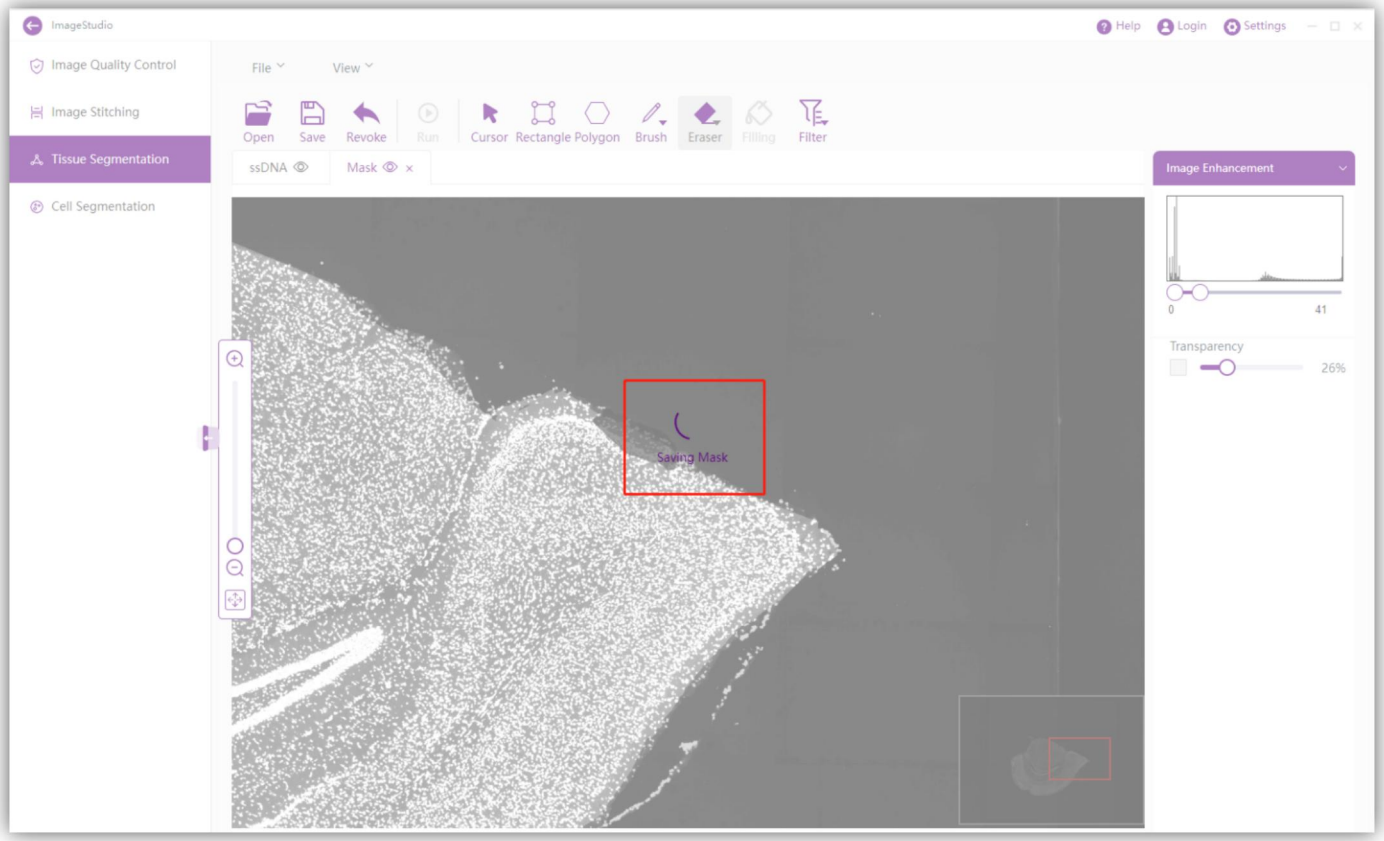


⚠Left-click within the local view window to move the field of view.

(4)If mistakes were made, click 'Revoke' before saving to revoke current action.



(5) After erasing is completed, click 'Save' to update the tissue Mask image and write it into the IPR file.

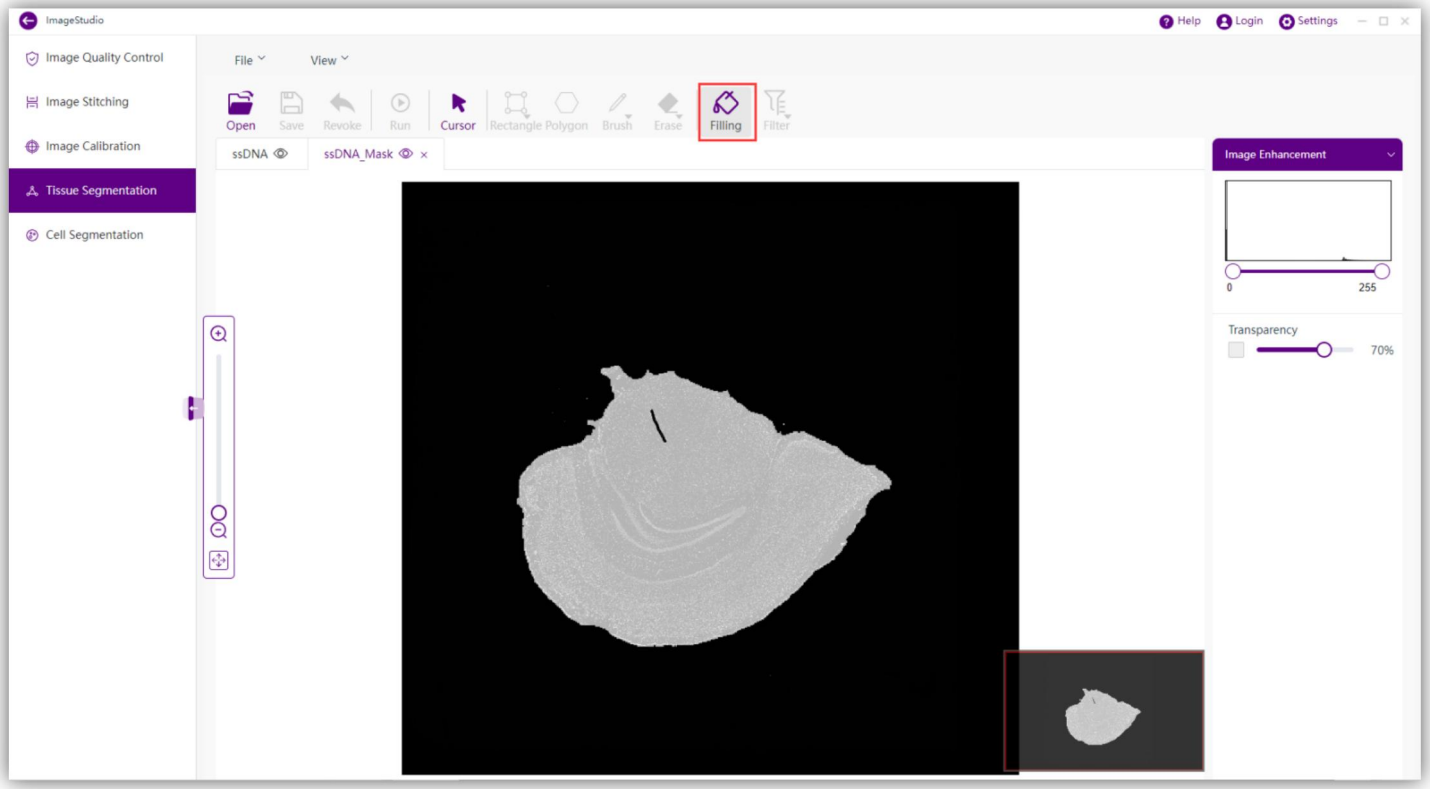


(6)Click 'Cursor' to exit the eraser editing mode.

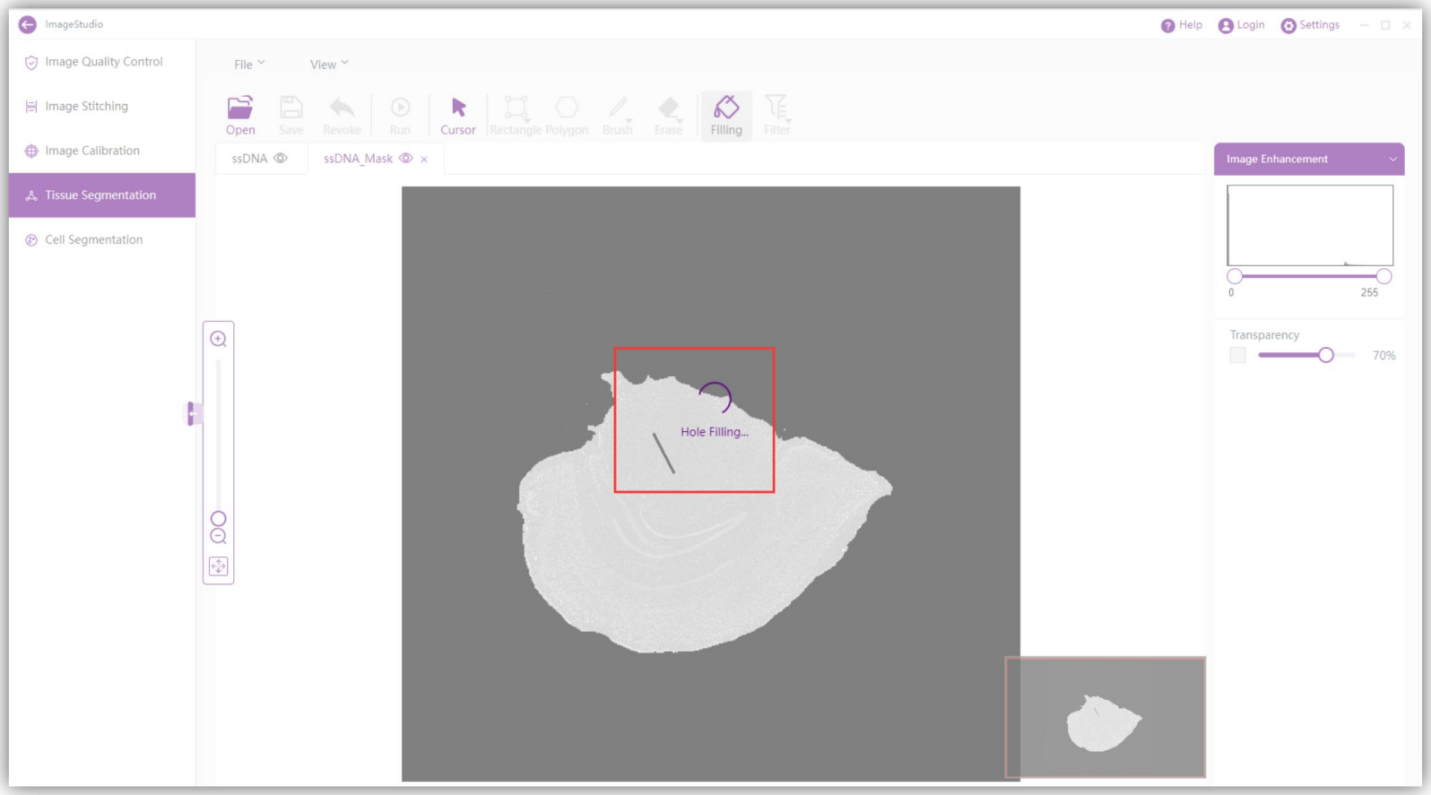
4.4.6 Filling

This function mainly operates on the Mask image for manually filling vacancies in the tissue Mask image.

(1) Click 'Filling' in the Mask image tab.

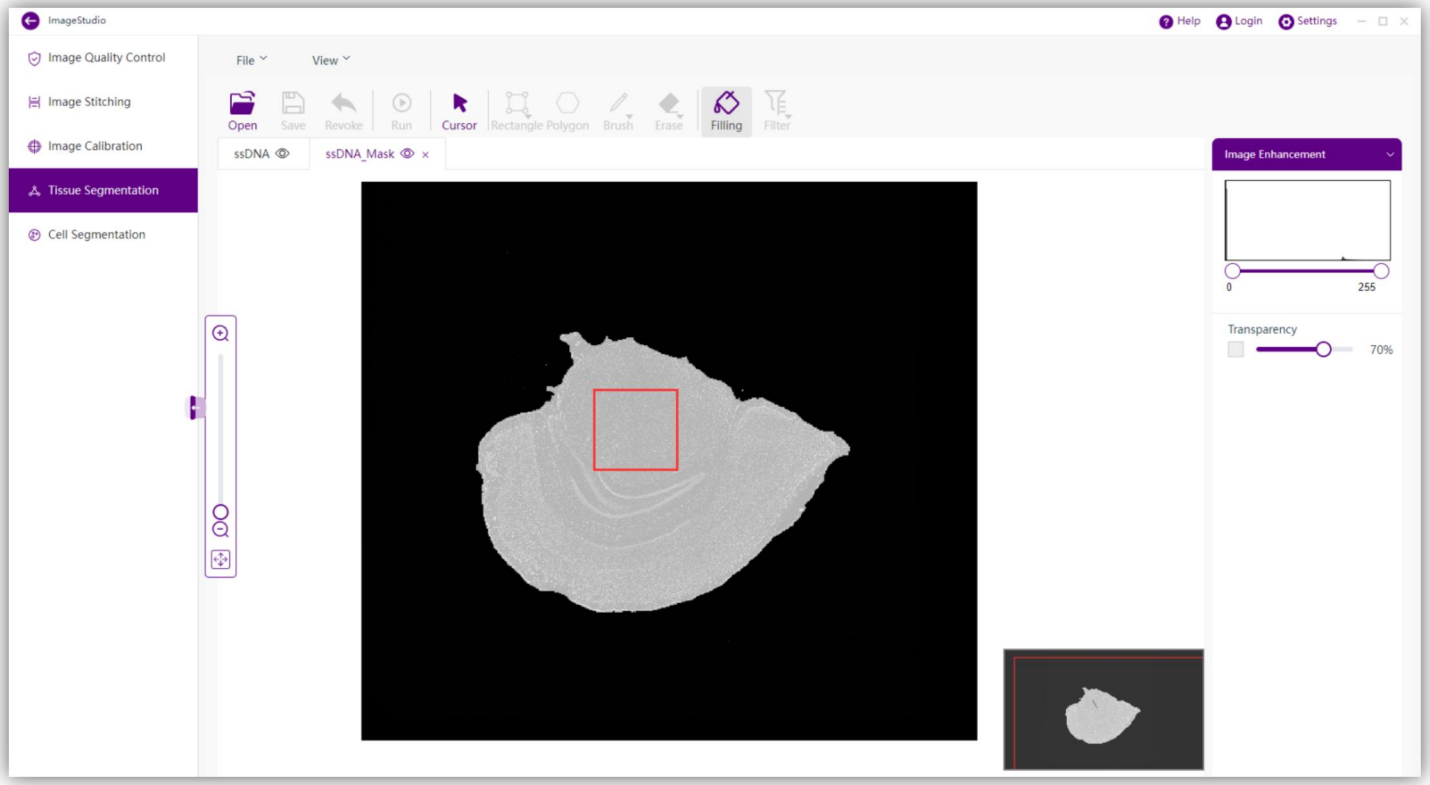


(2) Right-click to vacancies hole that need to be filled in the image, and the algorithm will fill in the vacancies automatically.

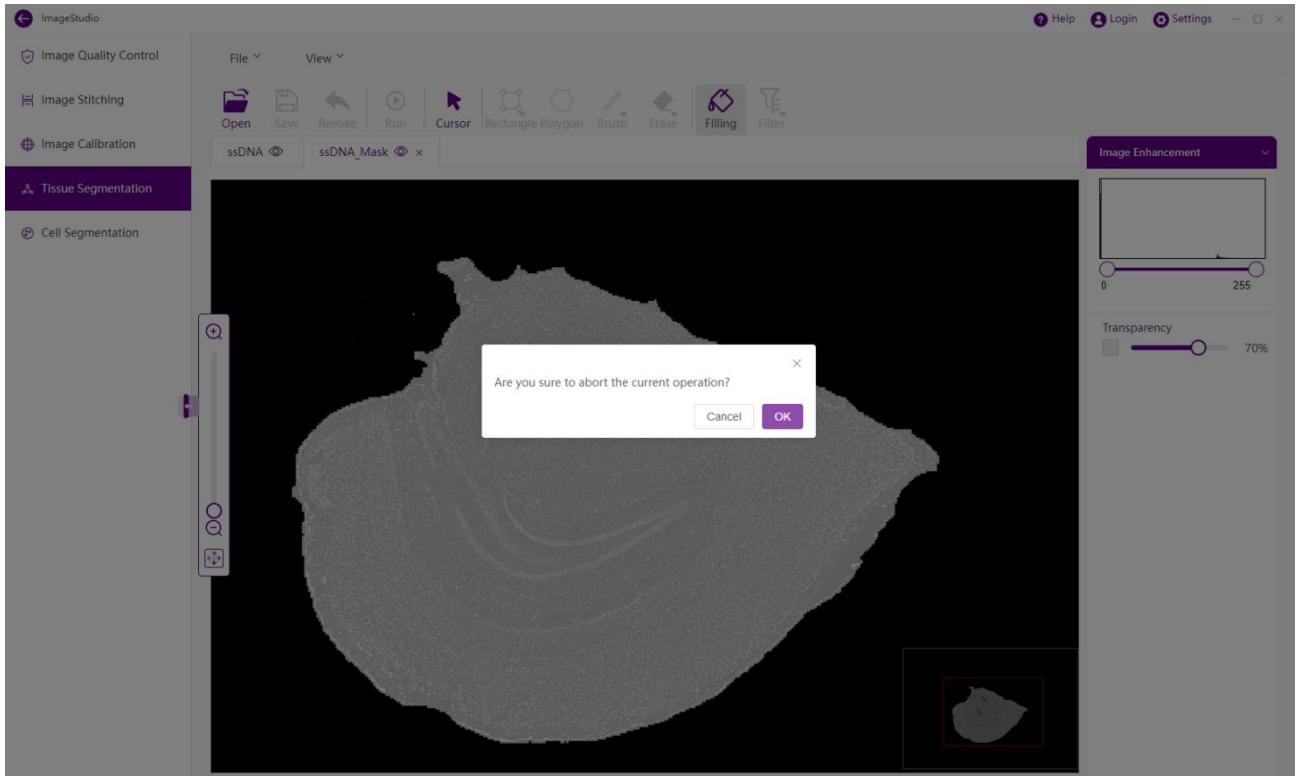


Note: If you need to fill other holes, right-click again to vacancies hole that need to be filled in the image.

(3)Once completed, click 'Save' to update the tissue Mask image and write it into IPR file.



(4)Click 'Cursor' to exit the fillling editing mode.

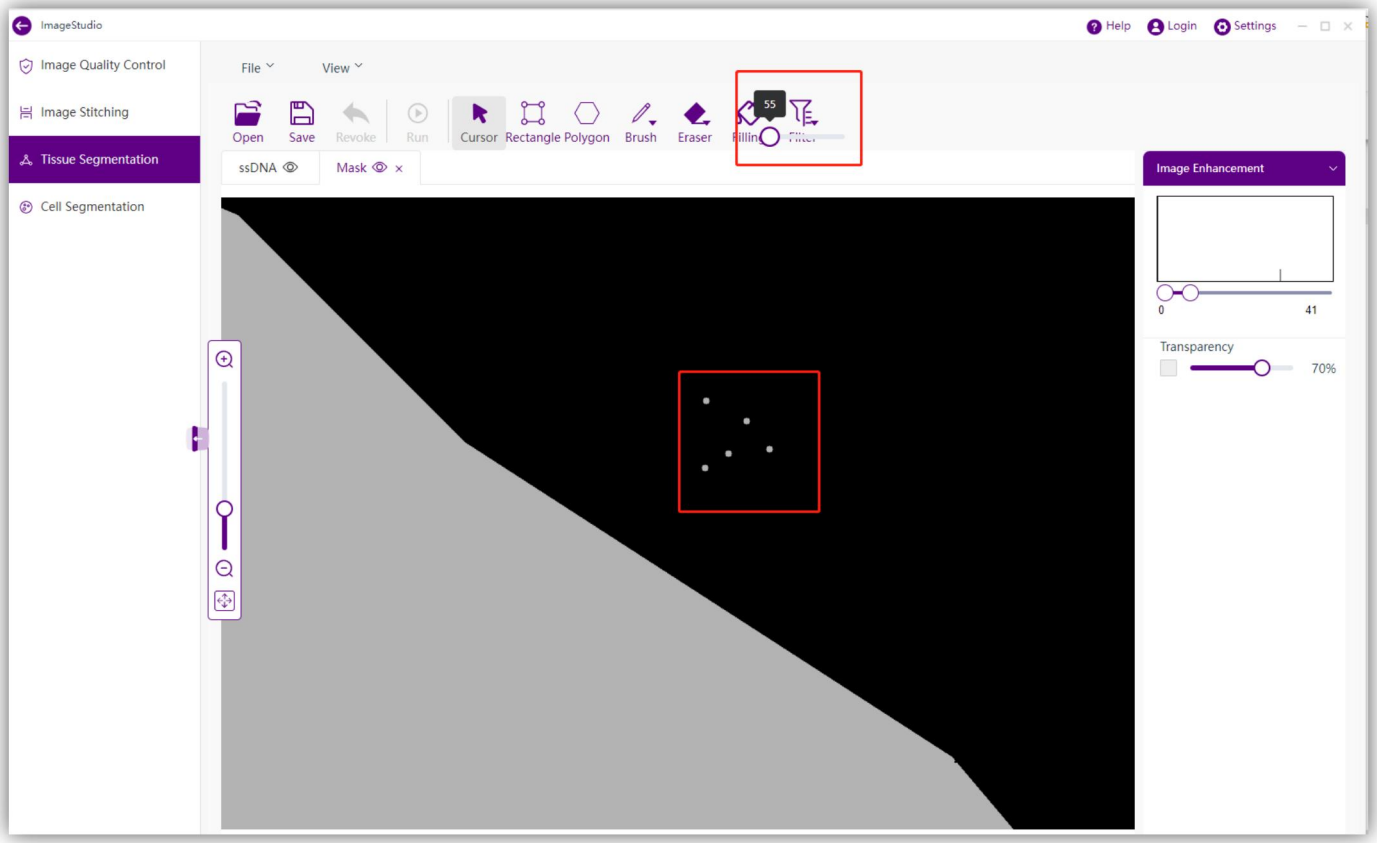


⚠The vacancy is defined as a black region surrounded by a white tissue Mask area.

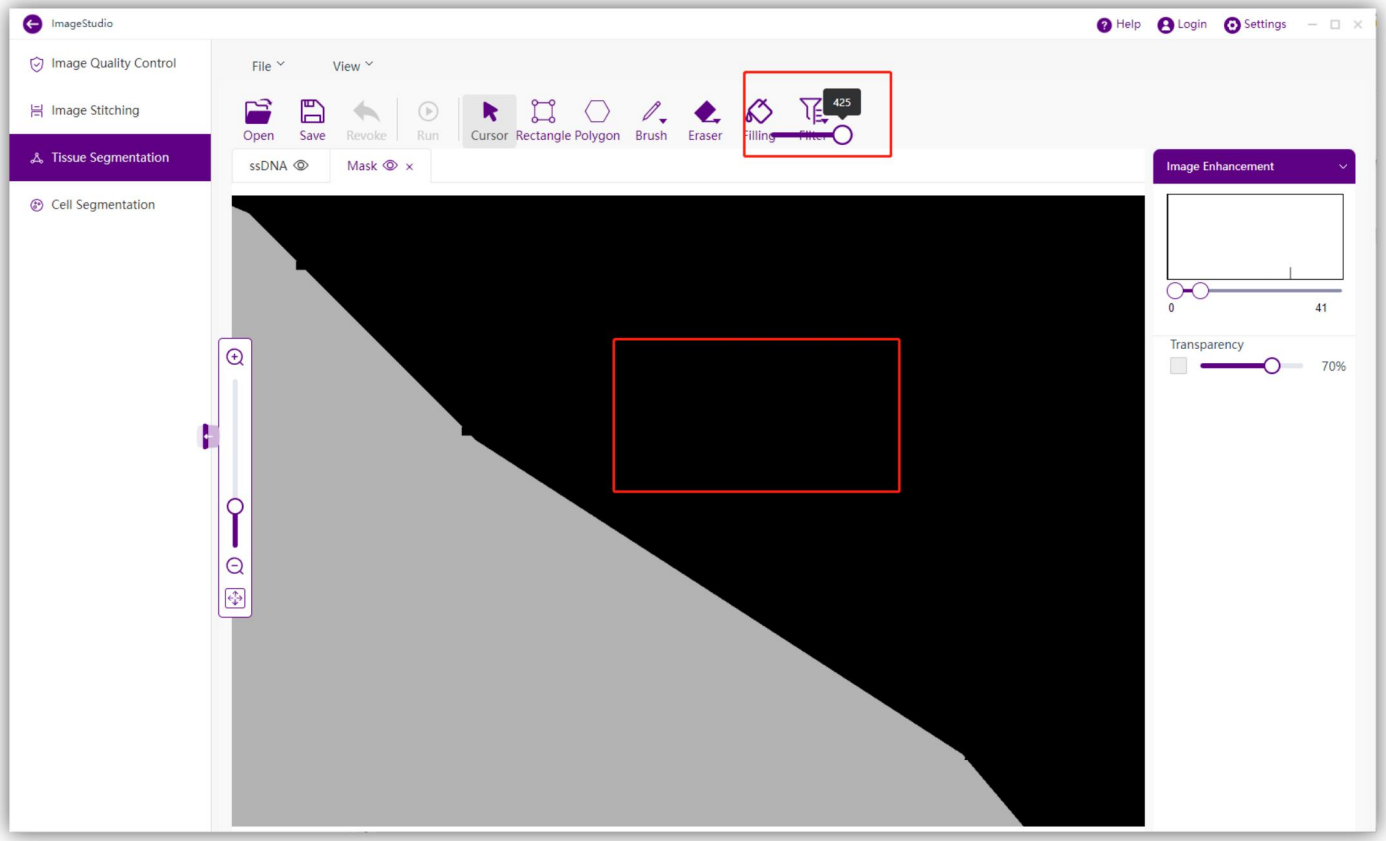
4.4.7 Filter

This function mainly operates on the Mask image for filtering background noise during imaging.

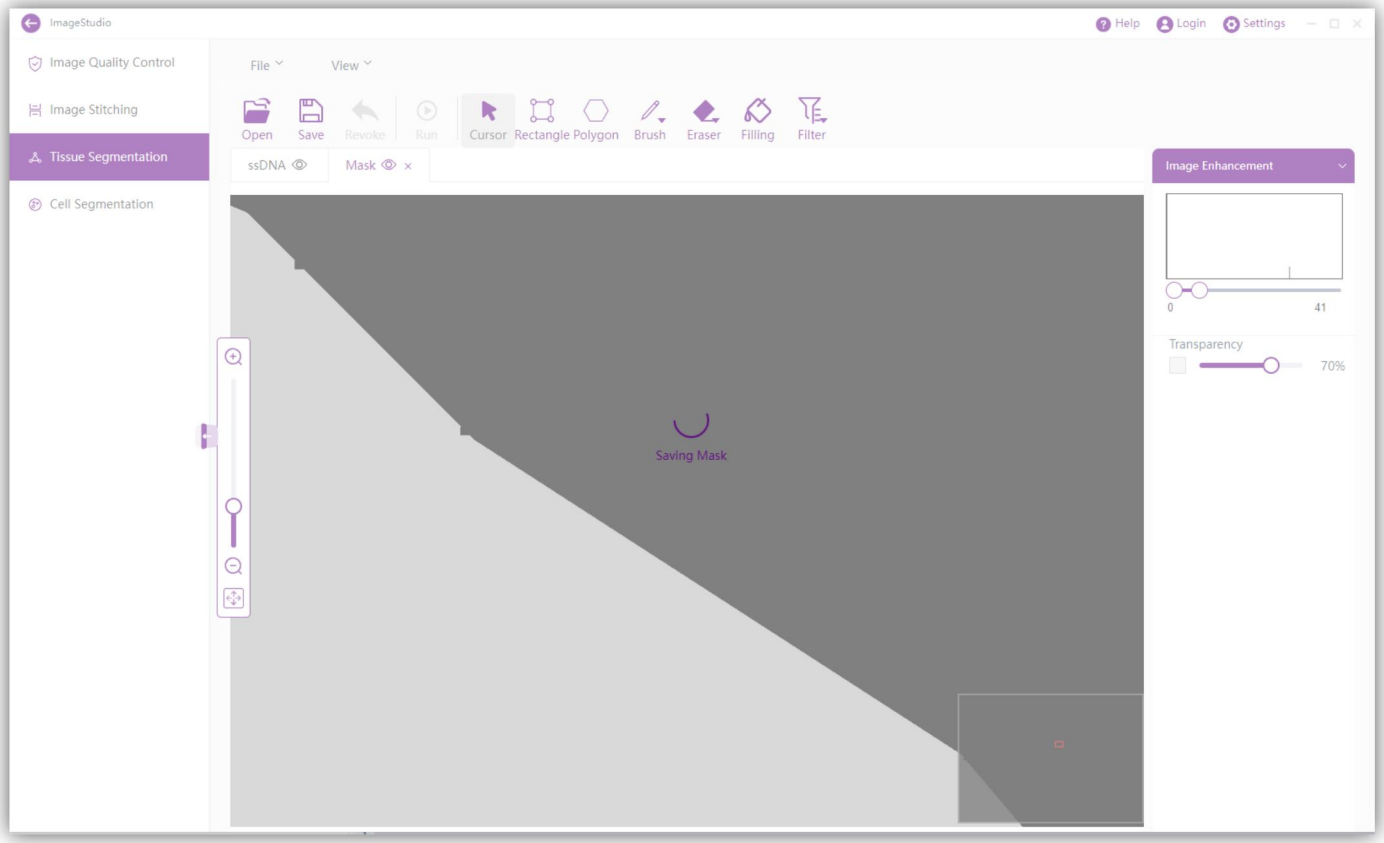
(1)Click 'Filter' and drag the bar to adjust the filtration parameters.



(2)View the filtering results in the Mask image tab.



(3)Click 'Save' to update the tissue Mask image and write it into IPR file.

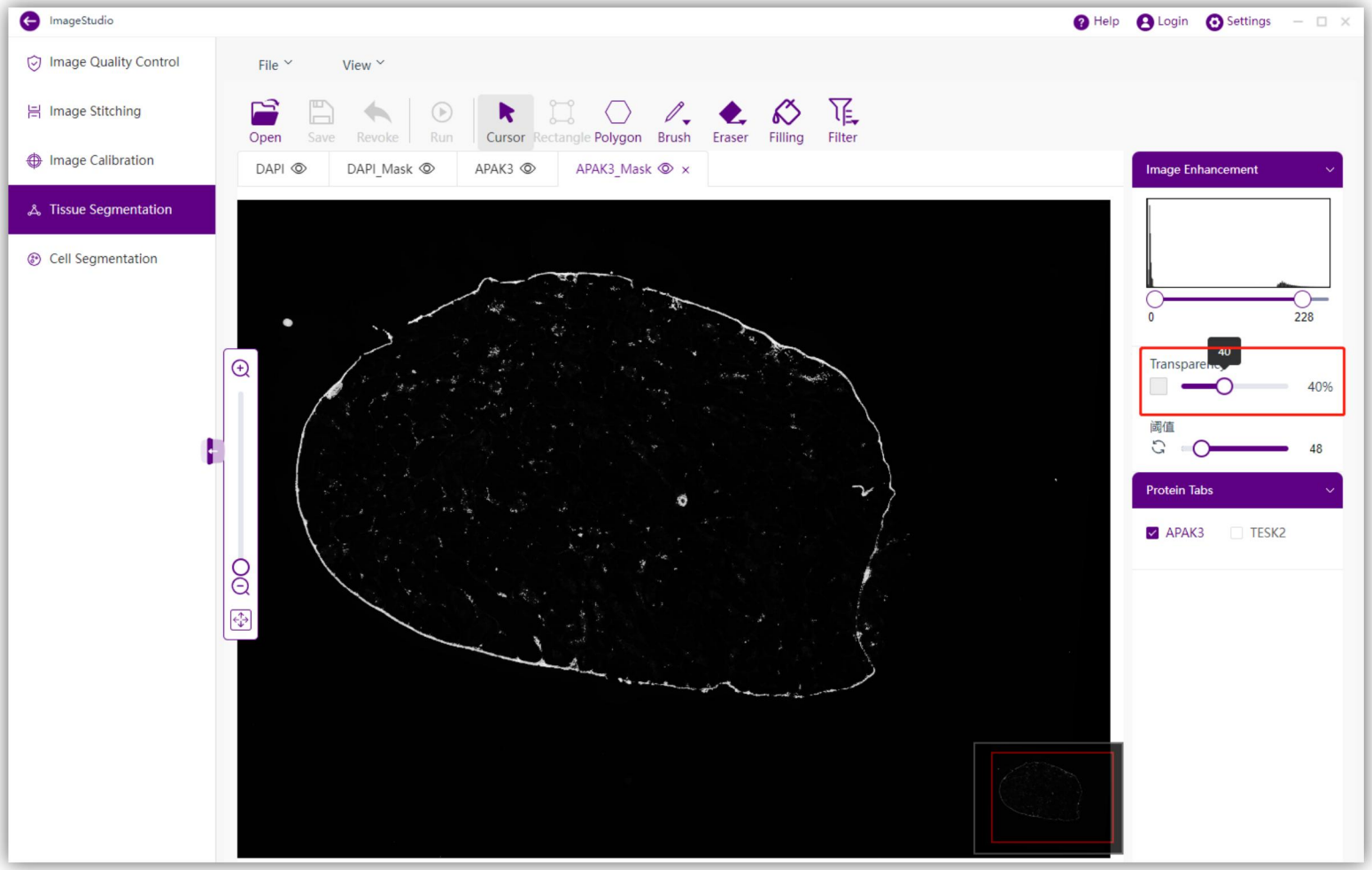


(4) Cursor, brush and eraser tools can be used simultaneously.

4.4.8 Threshold

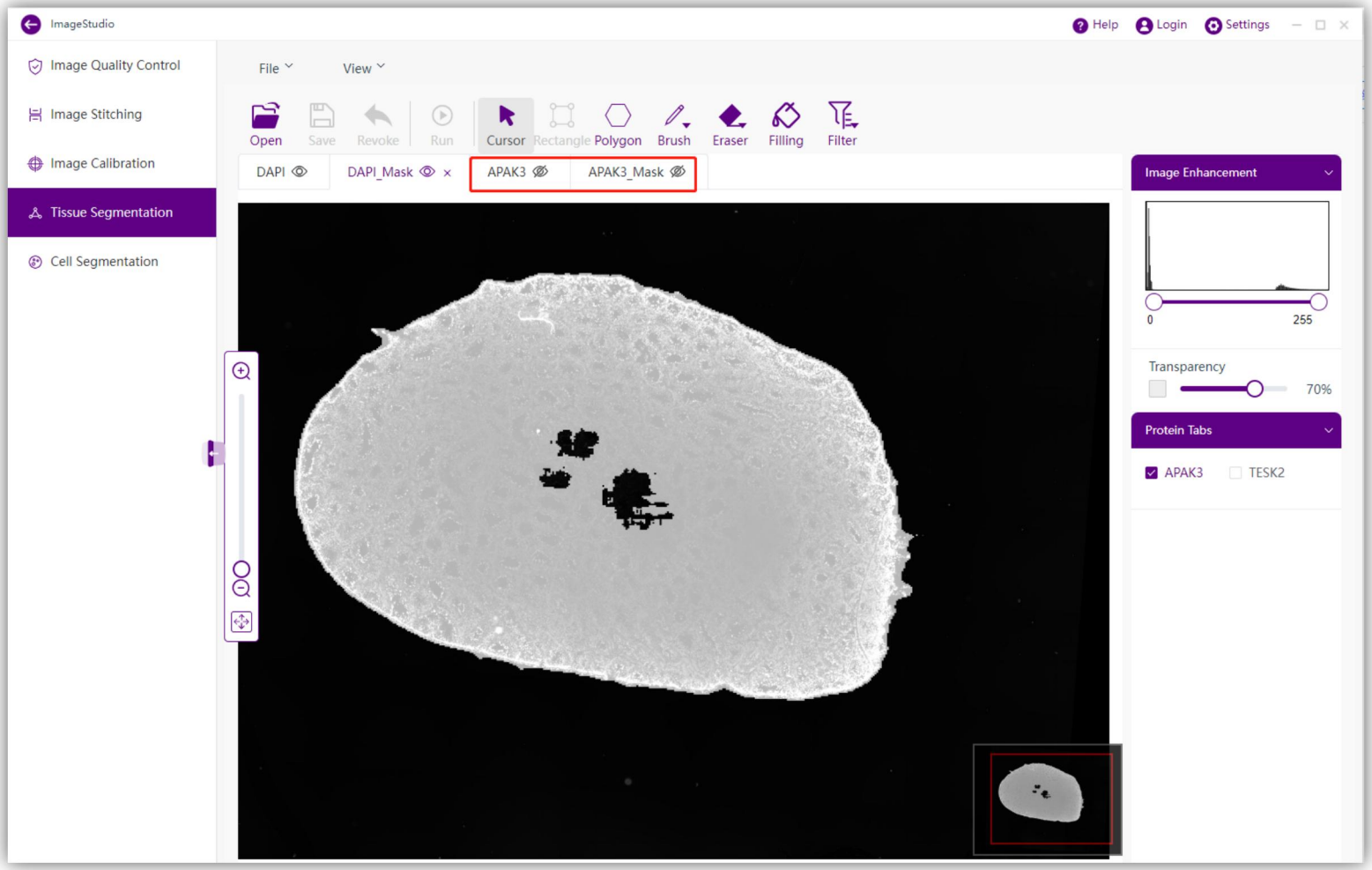
The display of a protein signal intensity can be enhanced or diminished by adjusting the cut-off threshold of the protein mask image.

(1) On the page of a protein mask, adjust the "transparency" to better reflect the overlapping region between the stitched image and the Mask image.



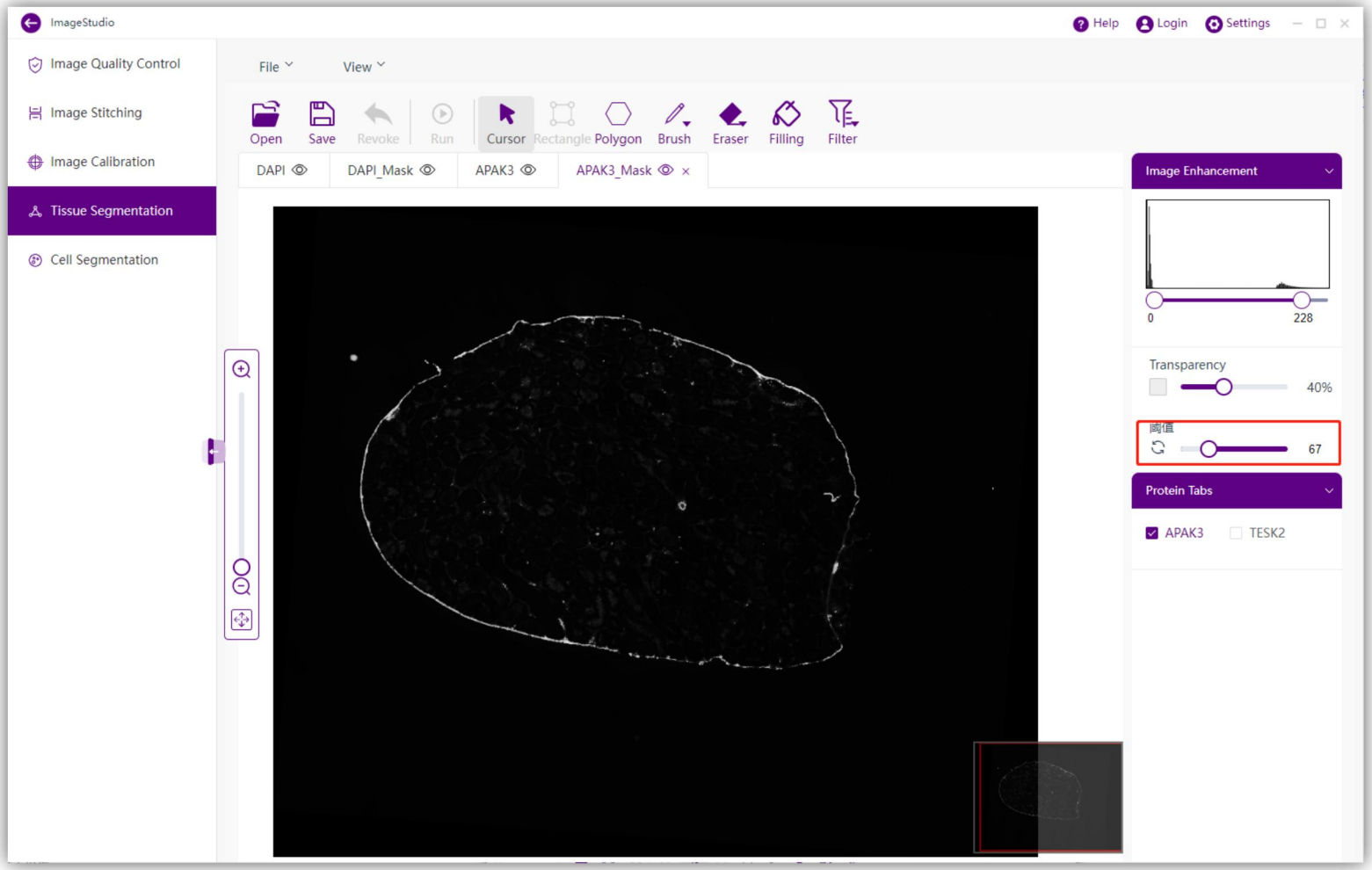
(2) Click on the 'eye icon' to hide or display the other proteins under the tab. Avoid the presence of

other proteins that may cause background noises on the current protein image.



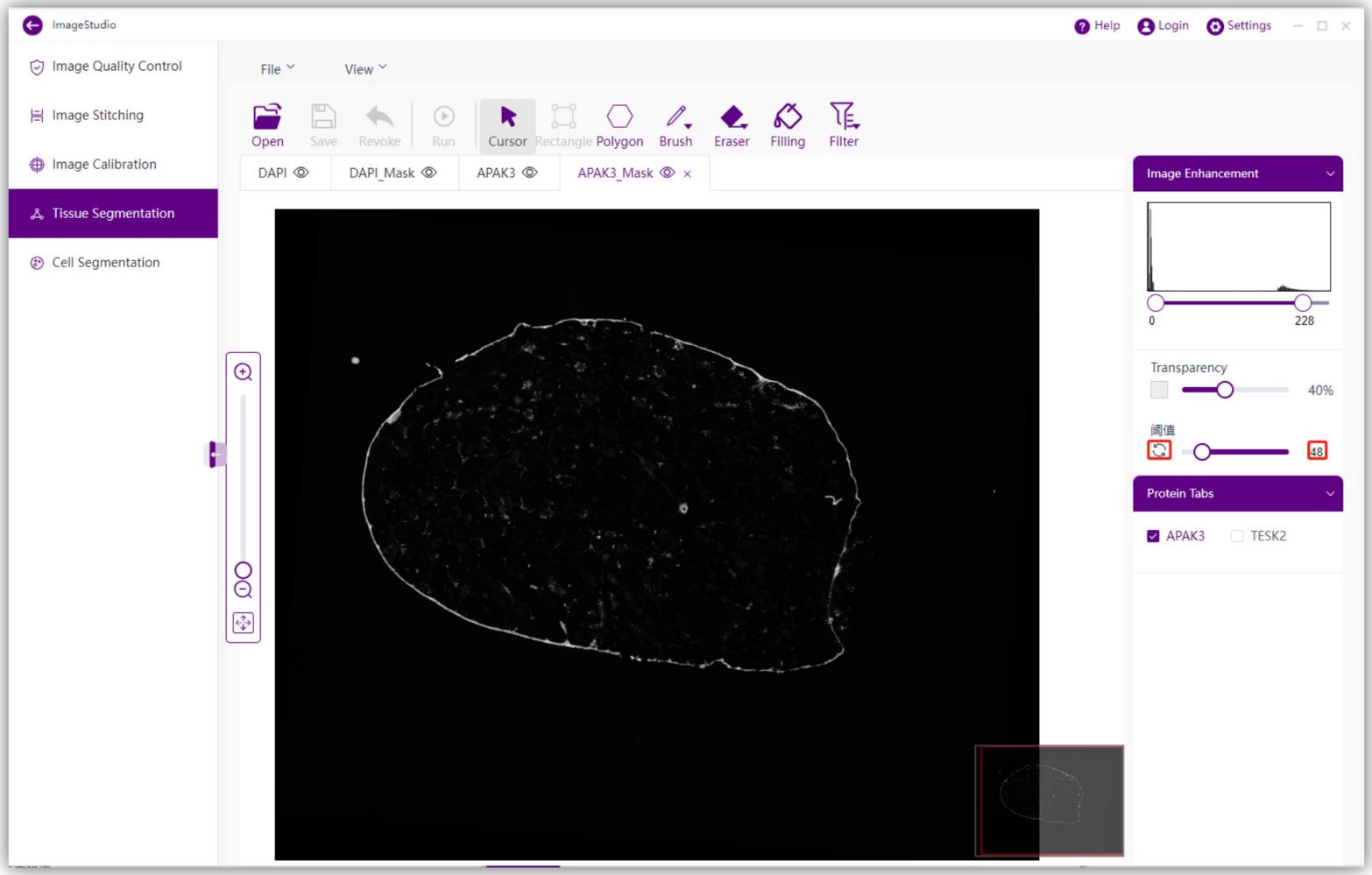
Note: The image layer are displayed from bottom to top in tab's sequential order from left to right, e.g. in this case, DAPI is the bottom layer.

(3) On the Protein Mask page, adjust "Threshold".

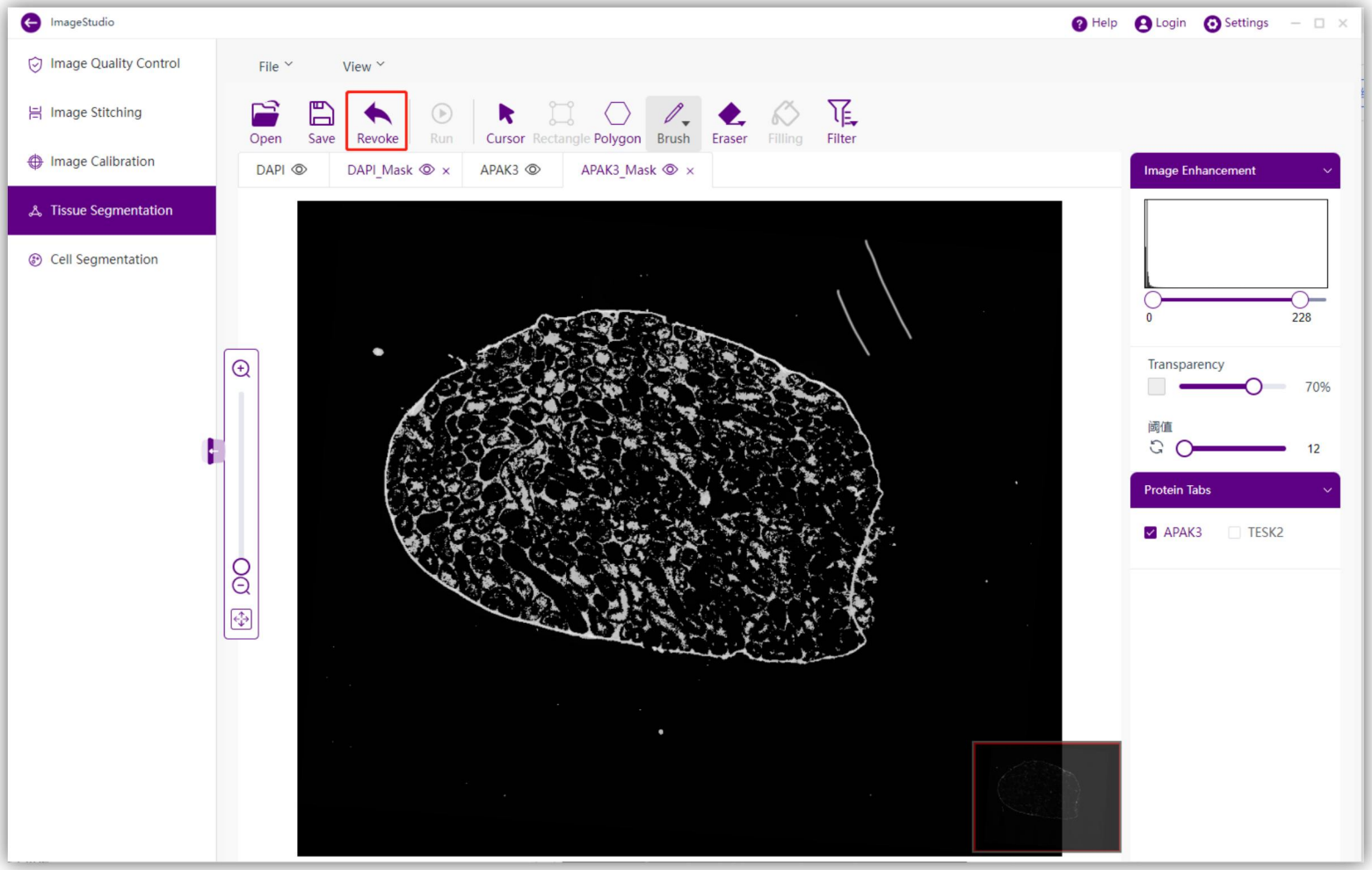


Note:It is recommended to adjust the Mask's threshold value first, and then adjust it with other tools such as brush and eraser

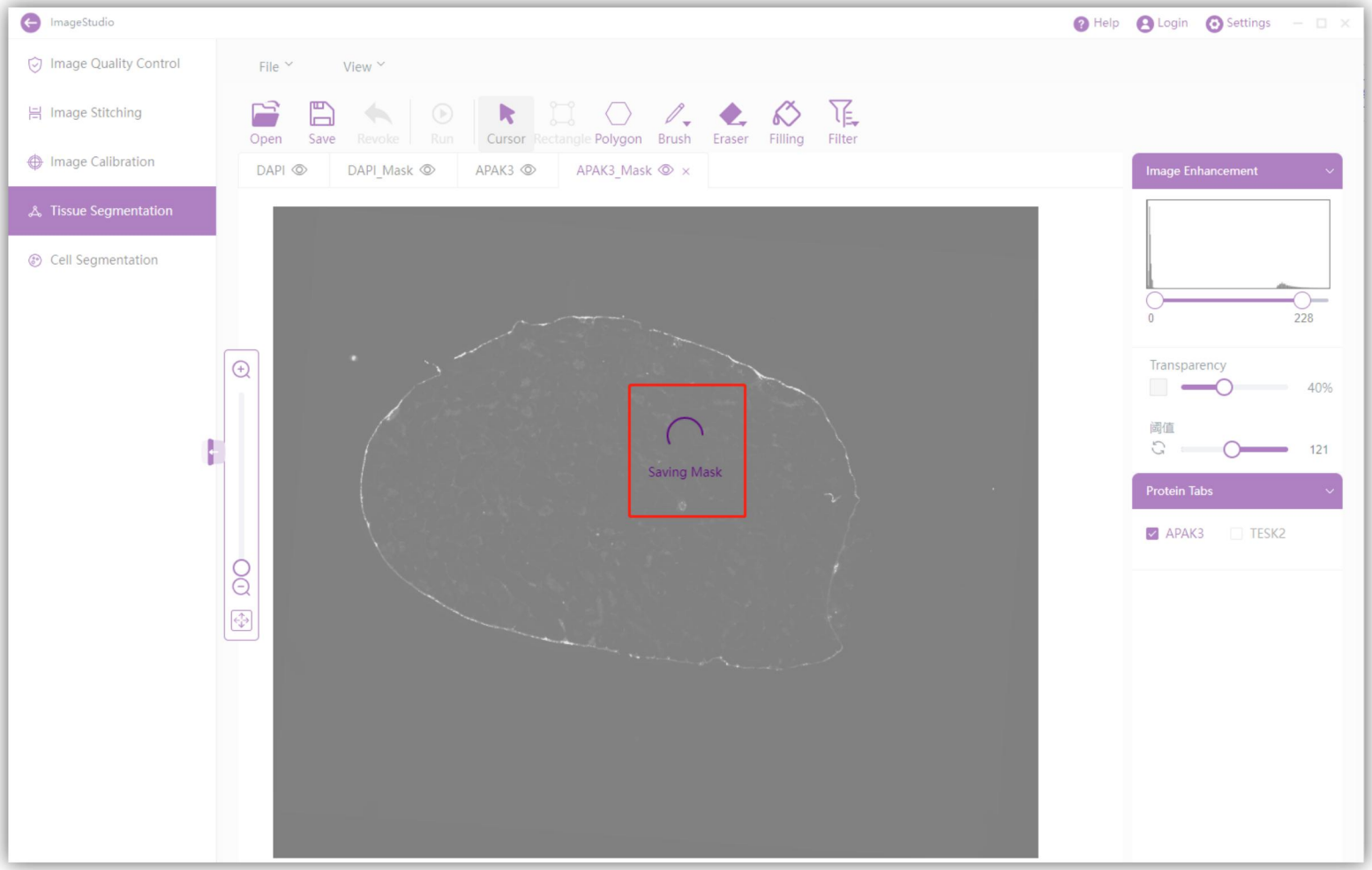
(4)After the adjustment, click reset to restore the default value if necessary.



(5) If mistakes were made, click 'Revoke' before saving to revoke current action.



(6) After all protein adjustment is completed, click "Save" and the updated Mask is written into IPR file.



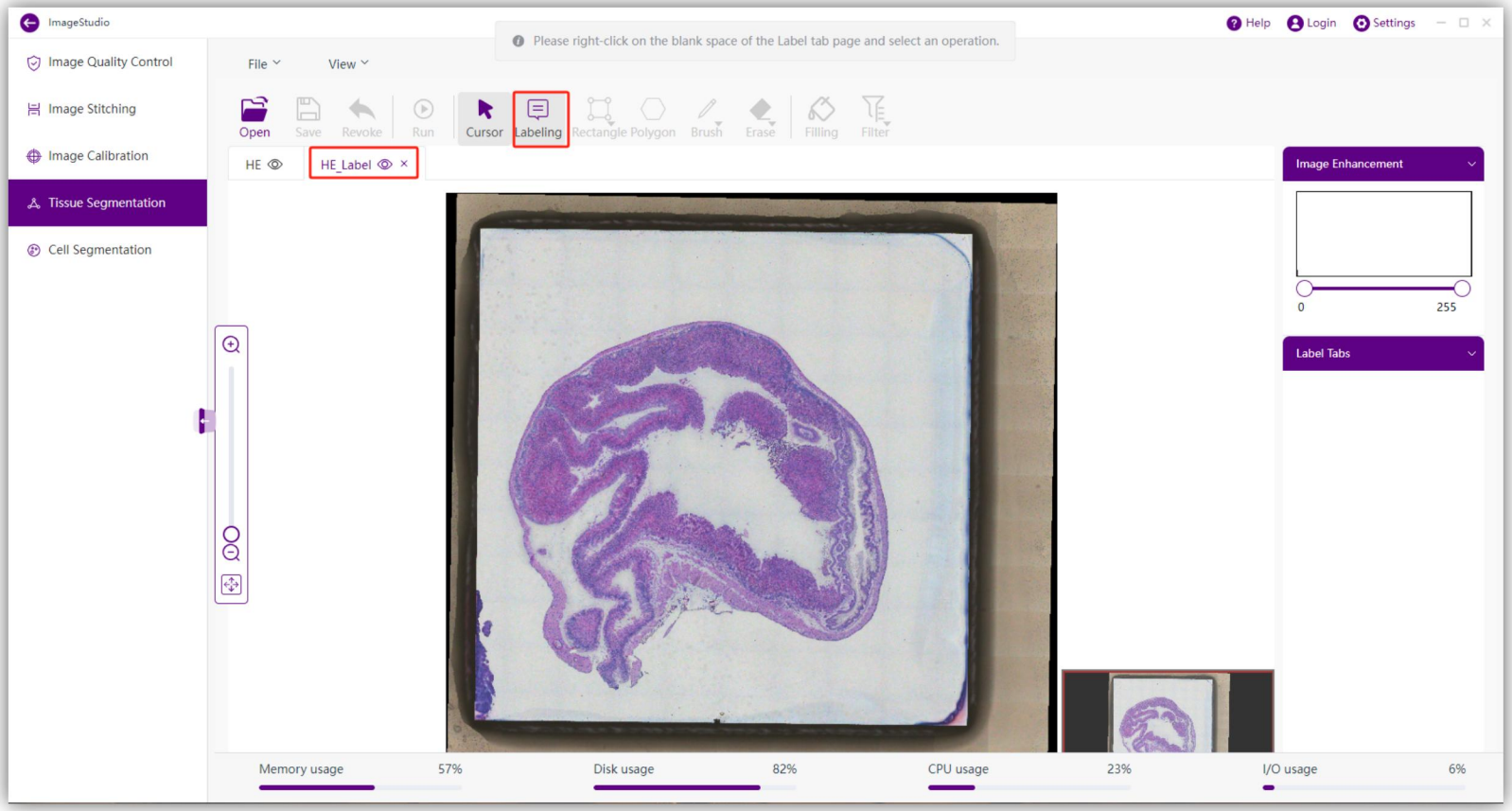
(7) Users can use cursors, brushes, and eraser at the same time.

4.4.9 Labeling

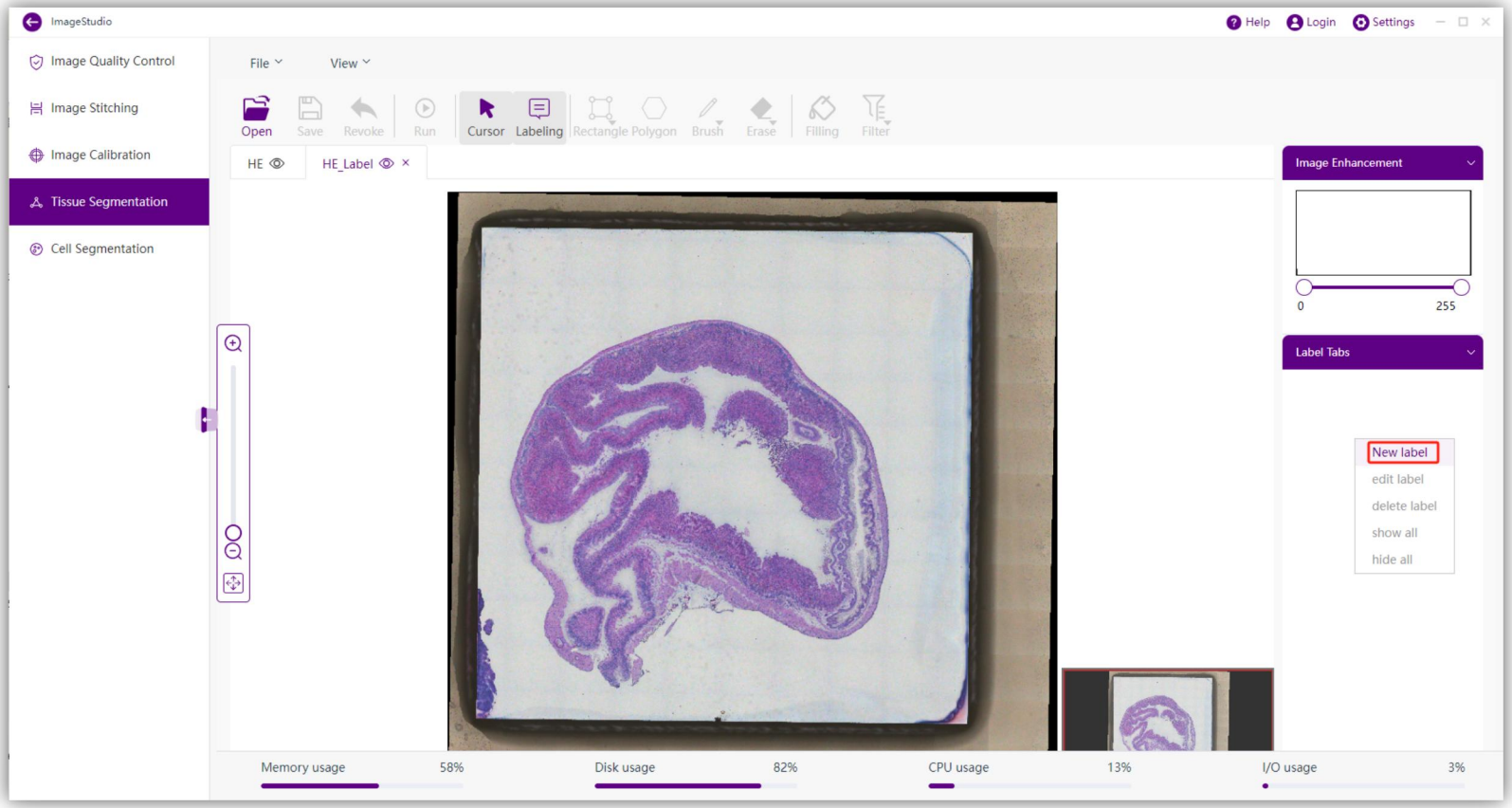
Different from tissue segmentation which marks the region for the entire tissue, the labeling function allows users to mark multiple regions of the tissue which can be used in pathological annotation. Users may use the polygon tool, brush, and/or eraser to create labels for cancer, tumor, normal, or any region of interest. The labels can be defined and edited in HE_Label tab.

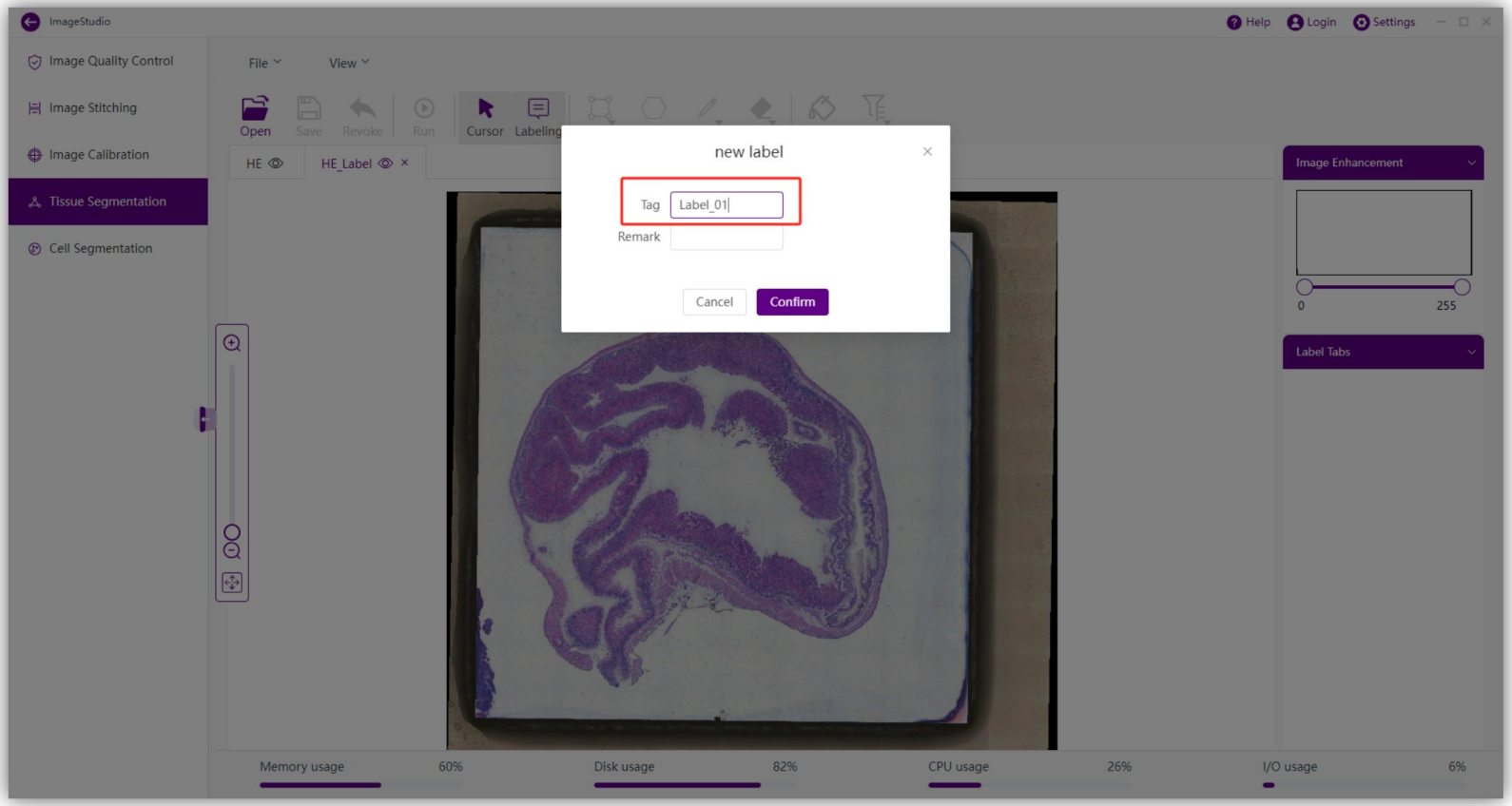
Note:The Labeling function in Version 3.0.x is only available for H&E images.

(1) Click "Labeling" to launch labeling mode. A new "HE_Label" tab will be generated and a new "Label Tabs" will be displayed on the right side.



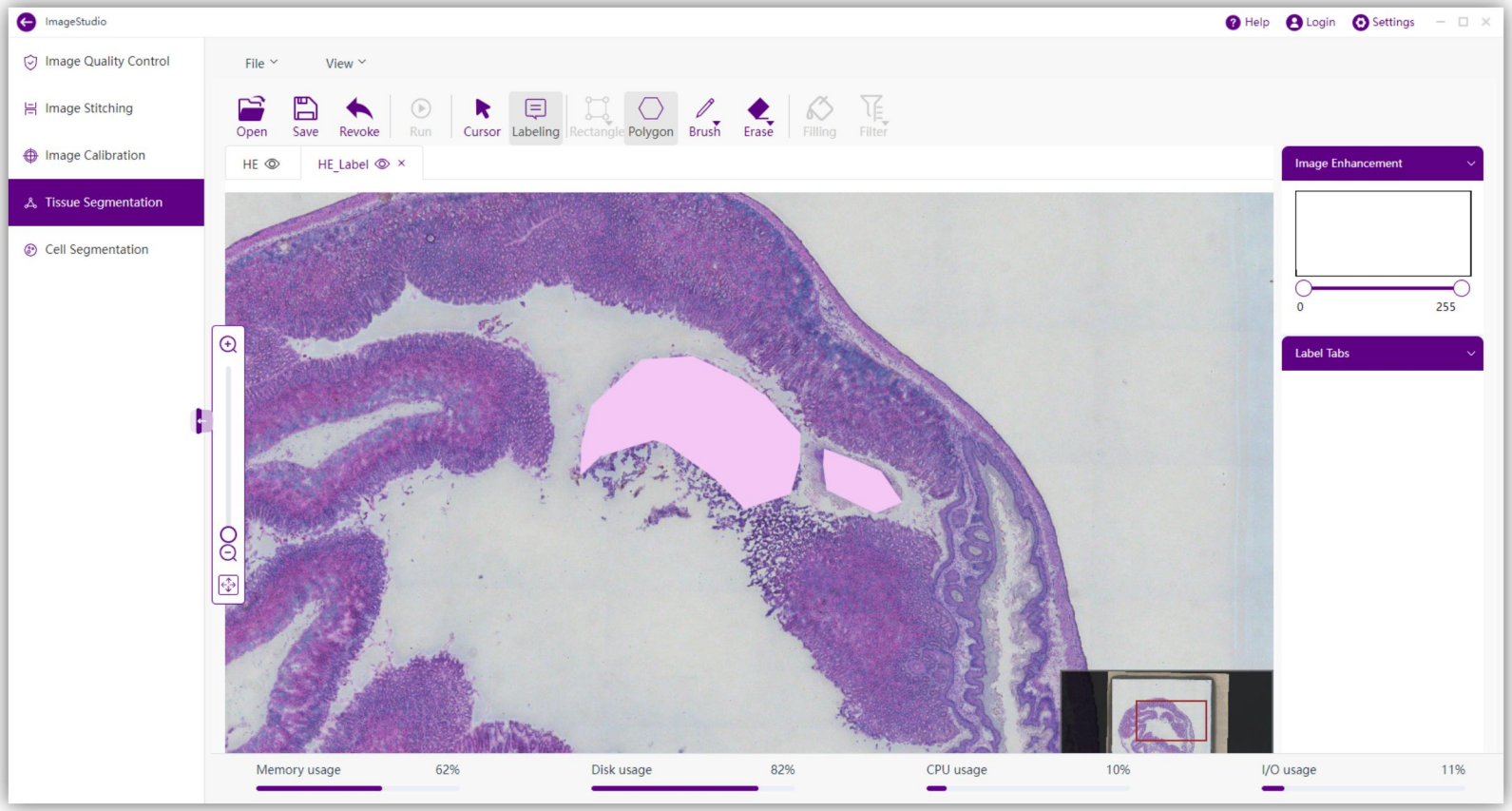
(2) Right-click at the whitespace under "Label Tabs", select "New Label", enter the label name and notes in the pop-up box and click "Confirm" to create a label tag.


ImageStudio
Help
Login
Settings
Image Quality Control
Image Stitching
Image Calibration
Tissue Segmentation
Cell Segmentation
File
View
Open
Save
Revoke
Run
Cursor
Labeling
Rectangle
Polygon
Brush
Erase
Filling
Filter
HE
HE_Label
Image Enhancement
0
255
Label Tabs
New label
edit label
delete label
show all
hide all
Memory usage
58%
Disk usage
82%
CPU usage
13%
I/O usage
3%

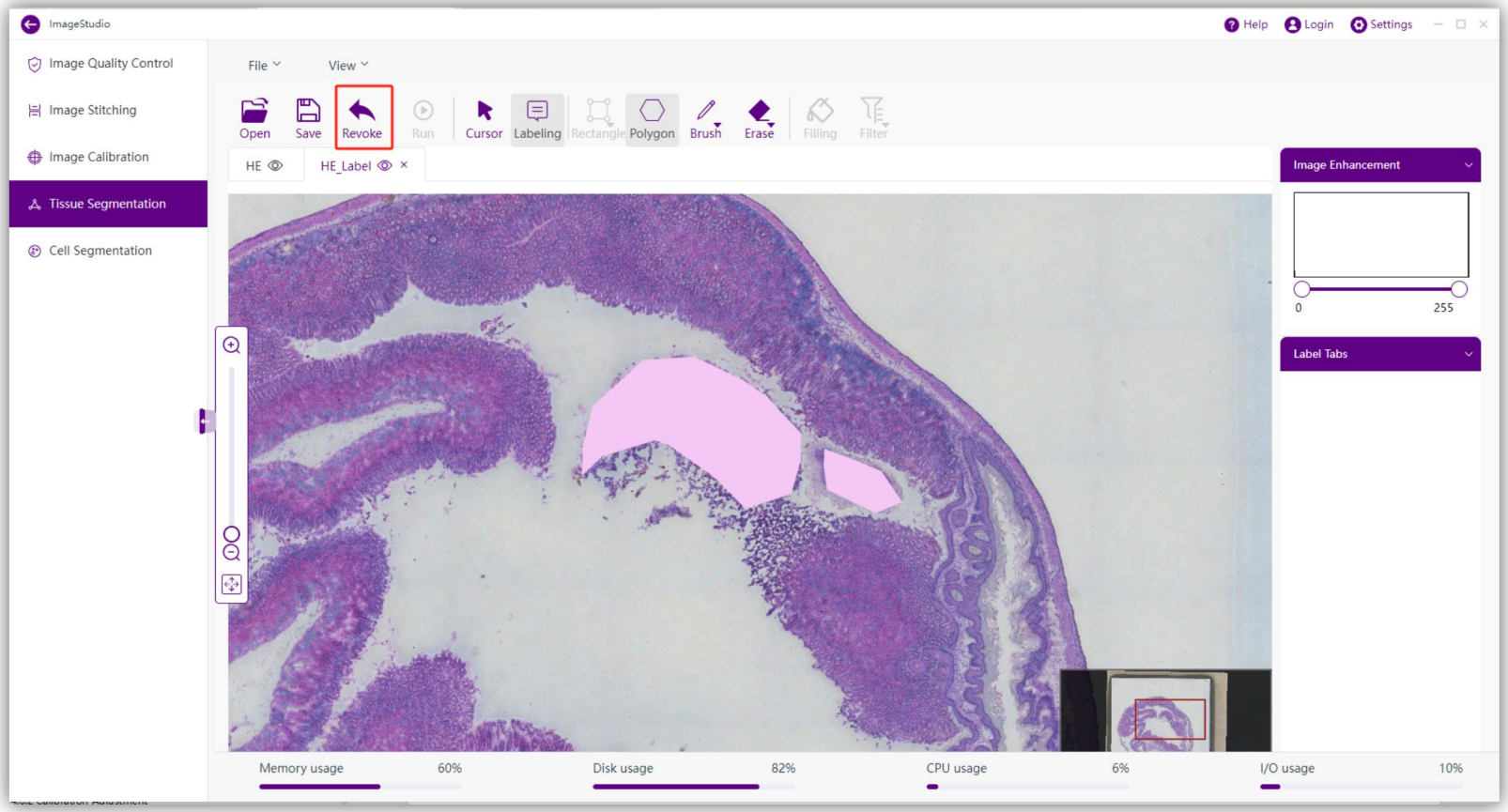


Note: A valid label name can contain only letters, numbers, and underscores.

(3) Users can manually mark tissue areas of interest using polygons, brushes, and erasers.Then, use polygon, brush, and eraser tools to delineate the region belonging to the label that created earlier.

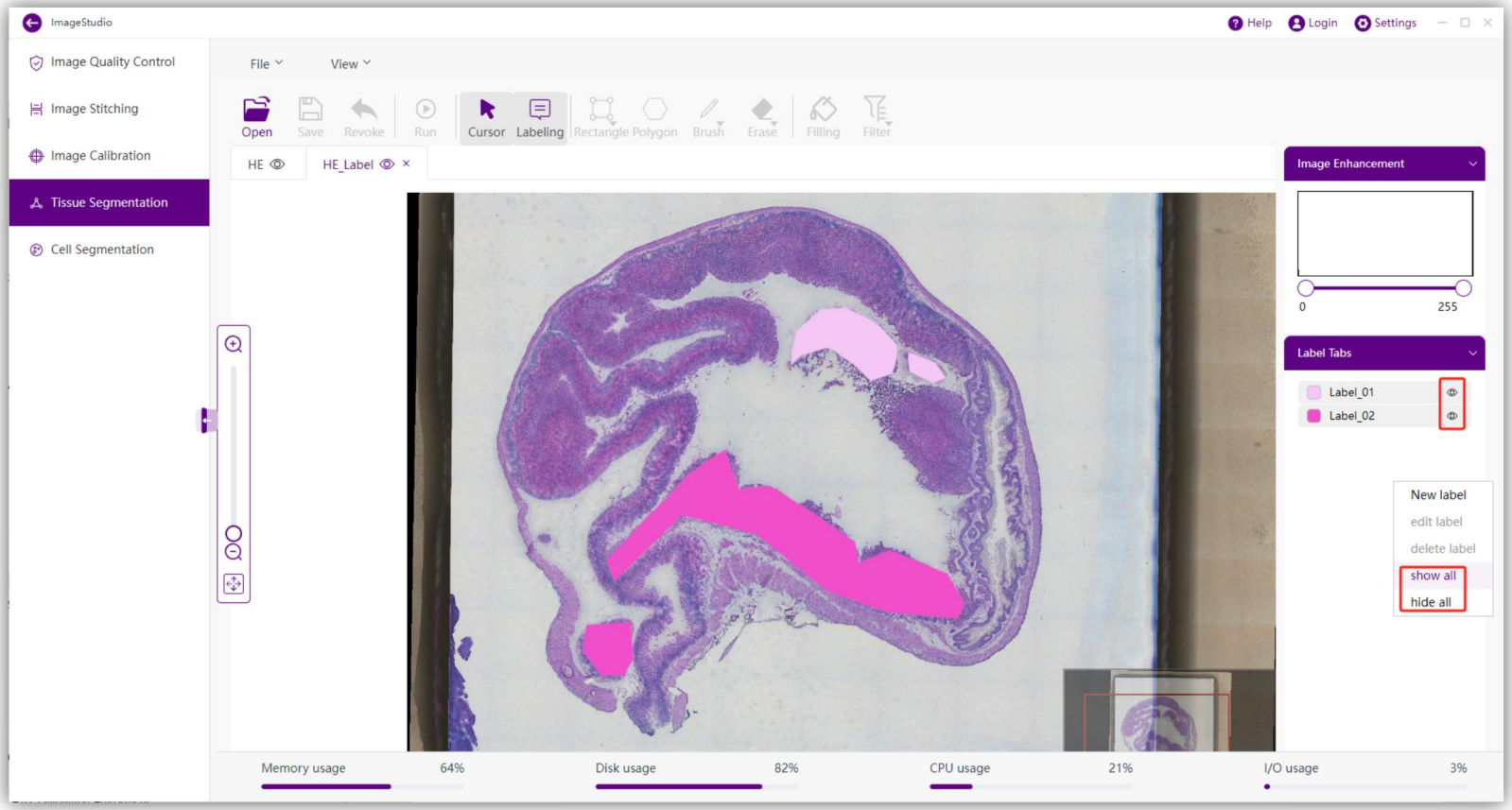


(4) Click "Revoke" to undo the last action. Only valid before saving.

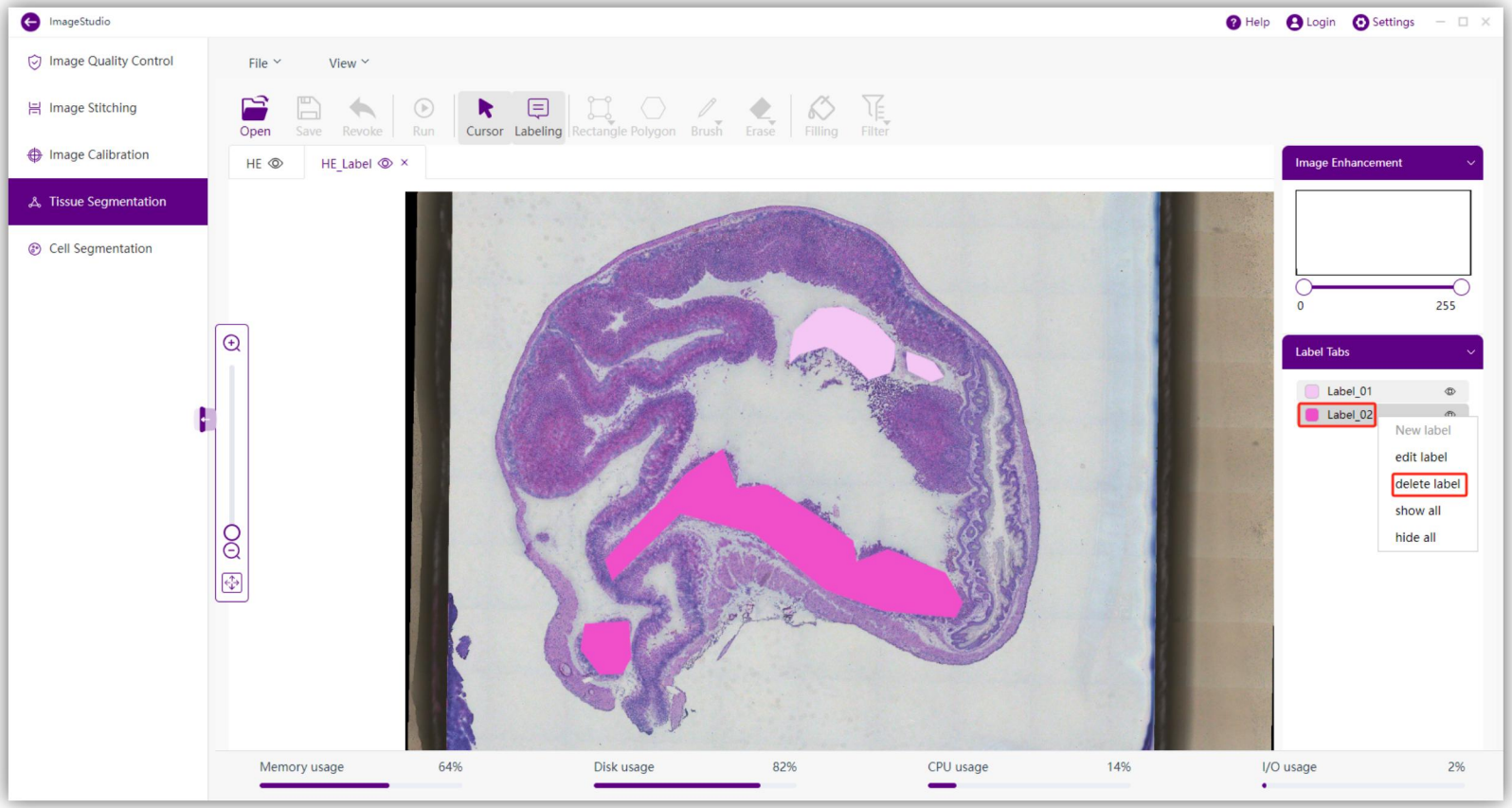


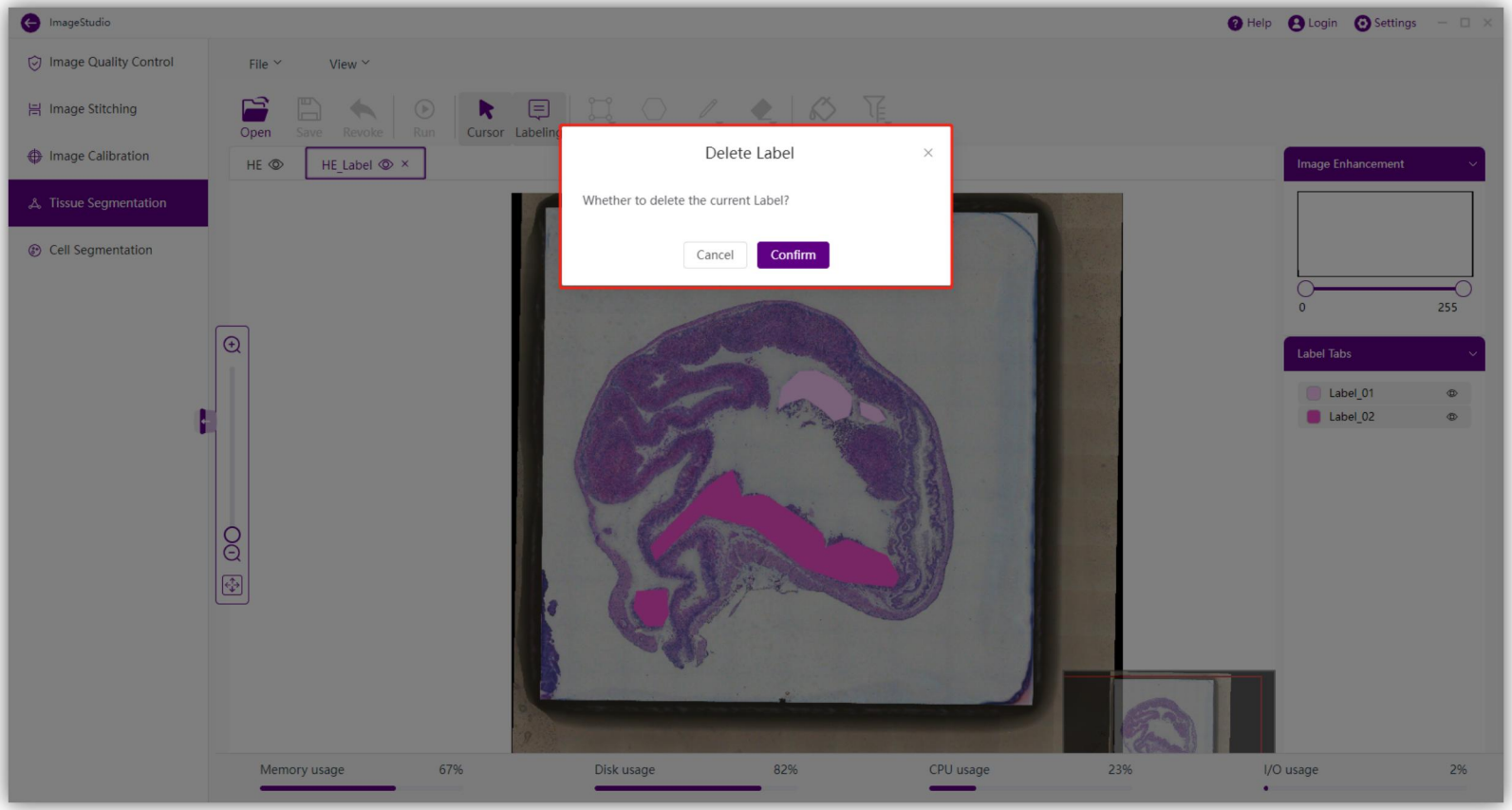
(5) Click "Save" to convert the drawing to the label displayed in the "Label Tabs" section with a program-assigned color, and then write the label mask into the IPR file.

(6) Click the eye icon in front of the label to show or hide a label layer. Right-click at the whitespace and select "show all" or "hide all" to show or hide all labels together.

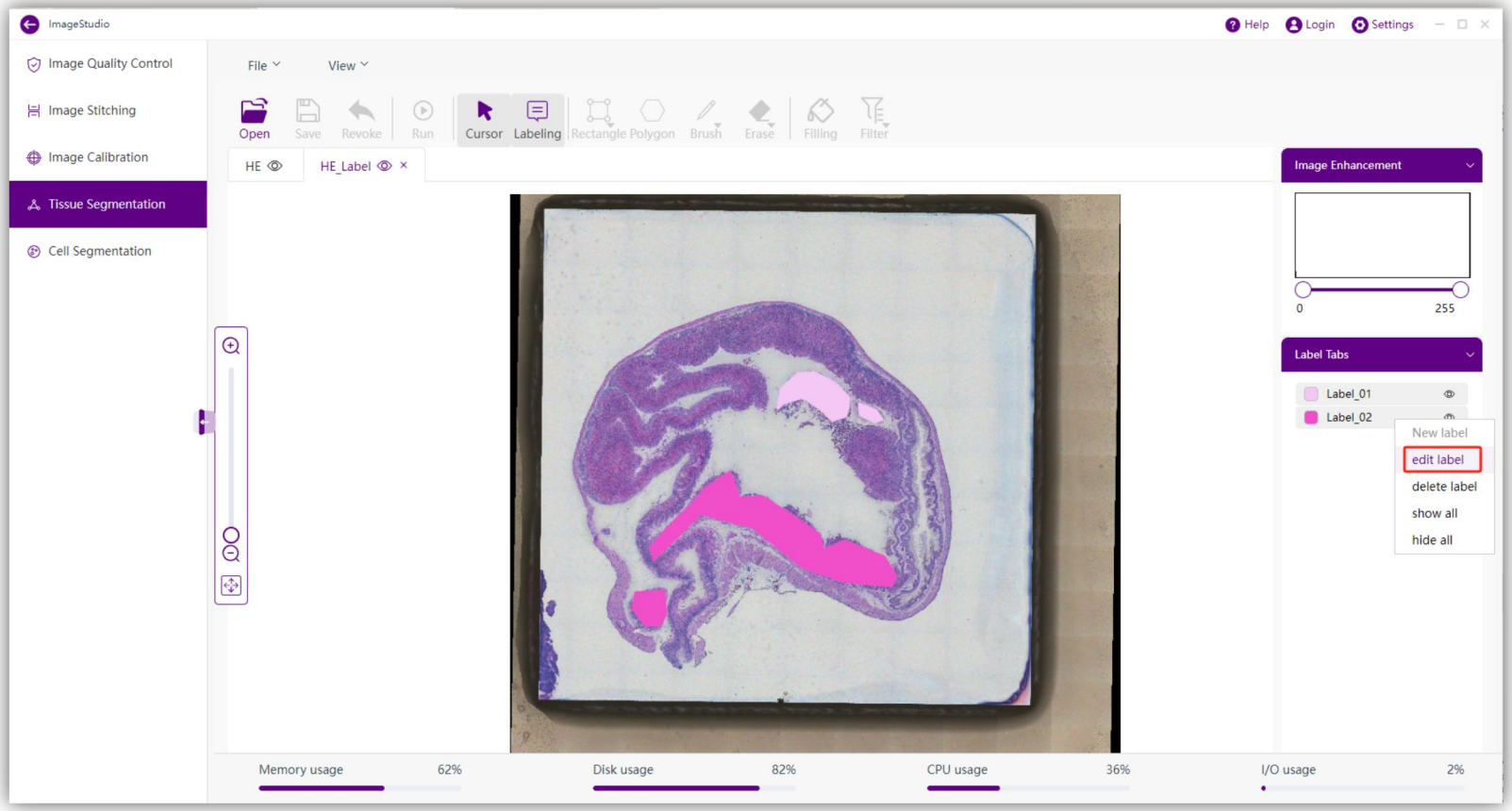


(7) Right-click the label name in "Label Tabs" and select "delete label" to remove a label.

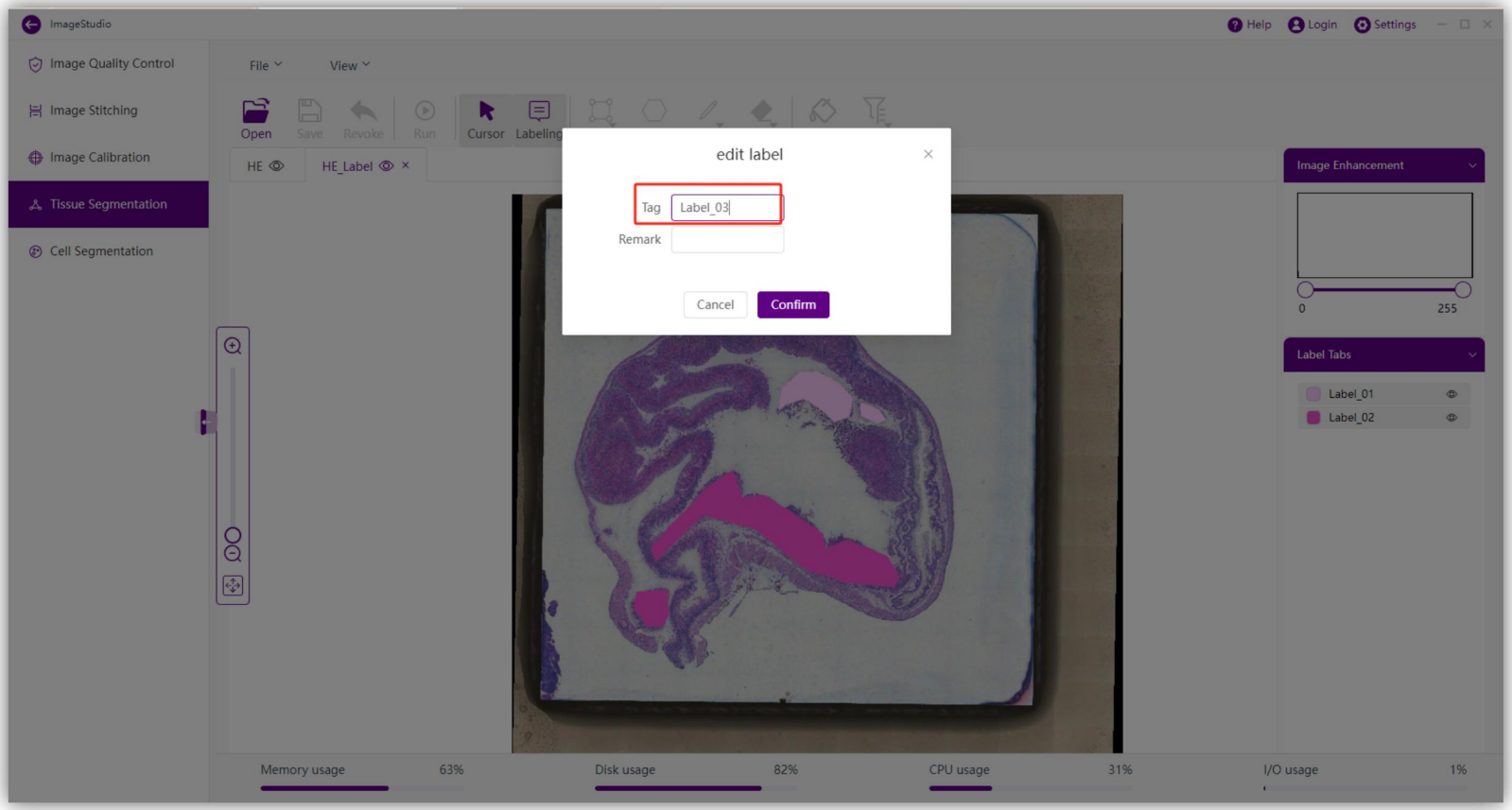

ImageStudio
Help
Login
Settings
Image Quality Control
Image Stitching
Image Calibration
Tissue Segmentation
Cell Segmentation
File
View
Open
Save
Revoke
Run
Cursor
Labeling
Rectangle
Polygon
Brush
Erase
Filling
Filter
HE
HE_Label
Image Enhancement
0
255
Label Tabs
Label_01
Label_02
New label
edit label
delete label
show all
hide all
Memory usage
64%
Disk usage
82%
CPU usage
14%
I/O usage
2%



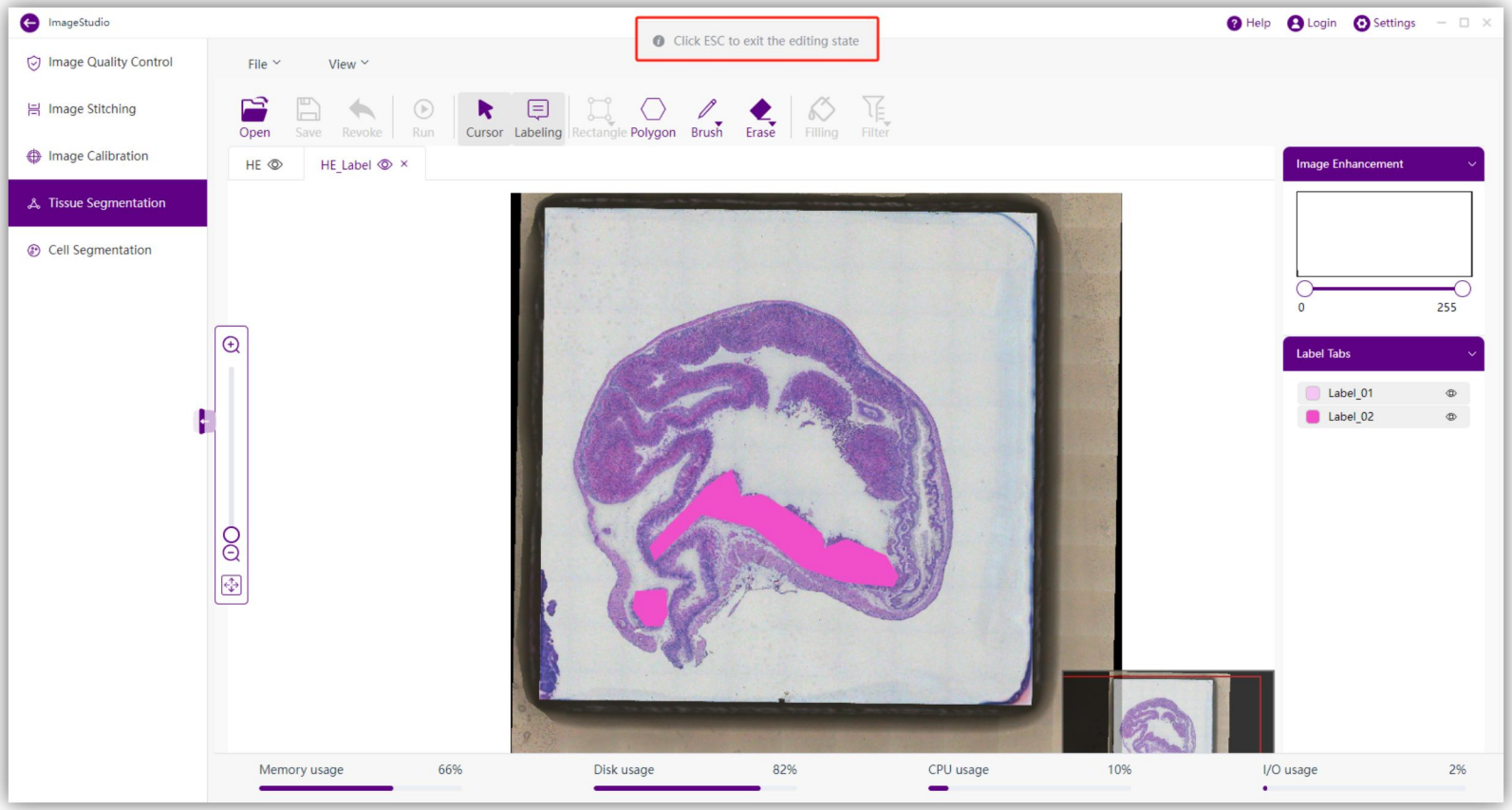
(8) Right-click the label and select "edit label" to start editing the name and the region of this label.



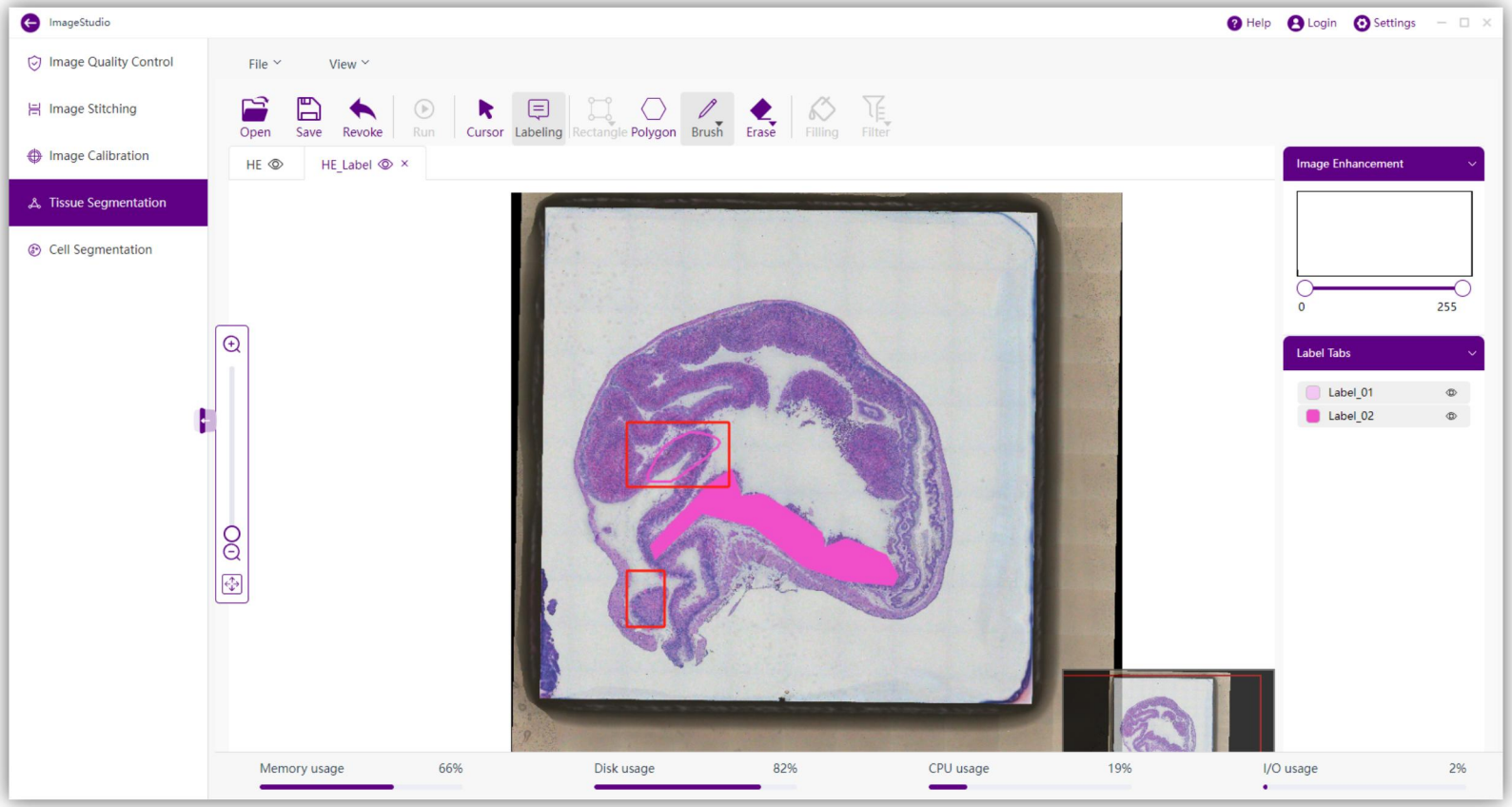
(9) After turning on the label editing mode, the pop-up window allows user to change label name and remark information.



(10) Click "Confirm" to finish editing the label name and remark, and allow you to edit the label region with polygon, brush, and eraser. Press "ESC" to exit editing mode.

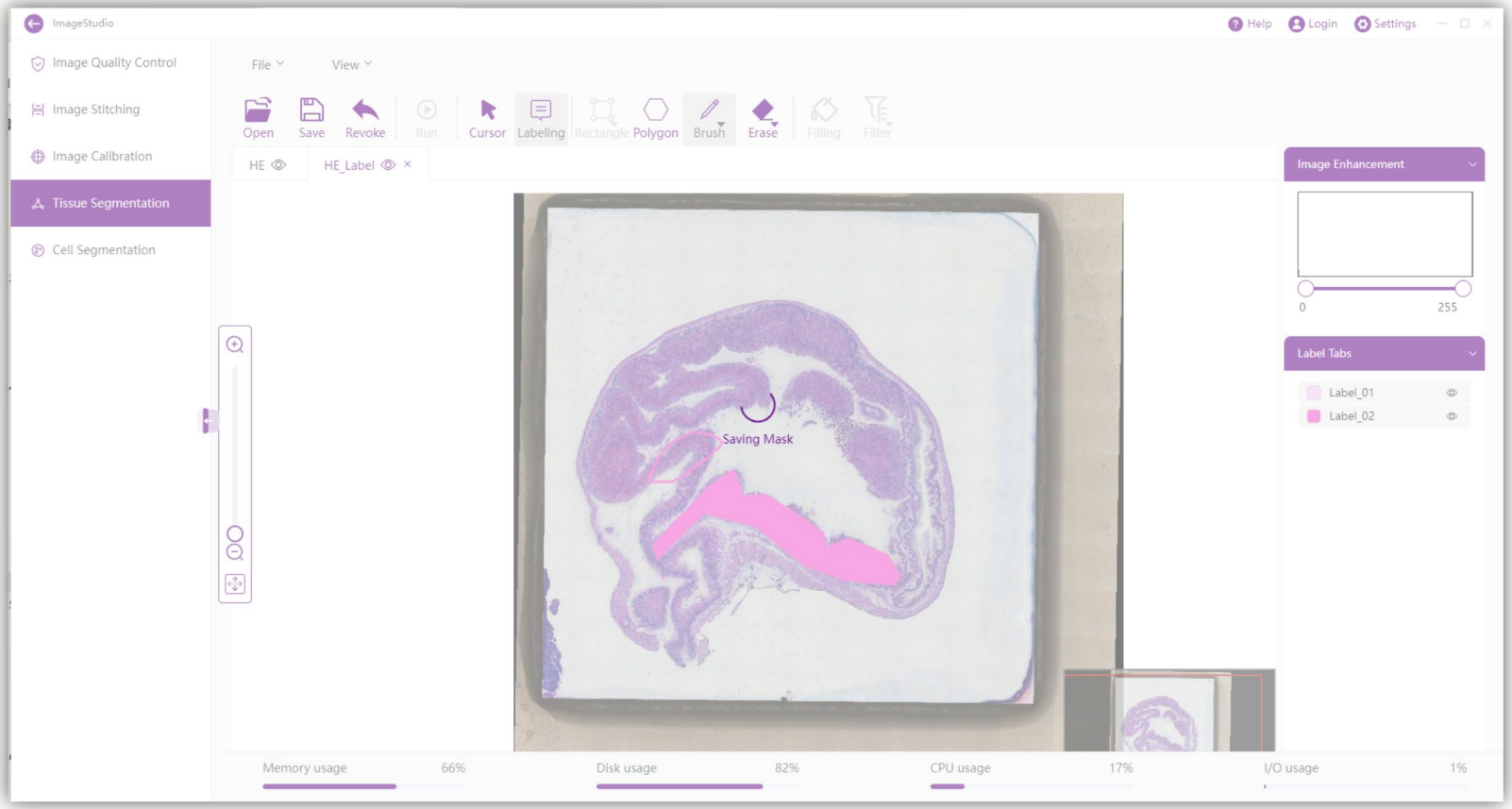


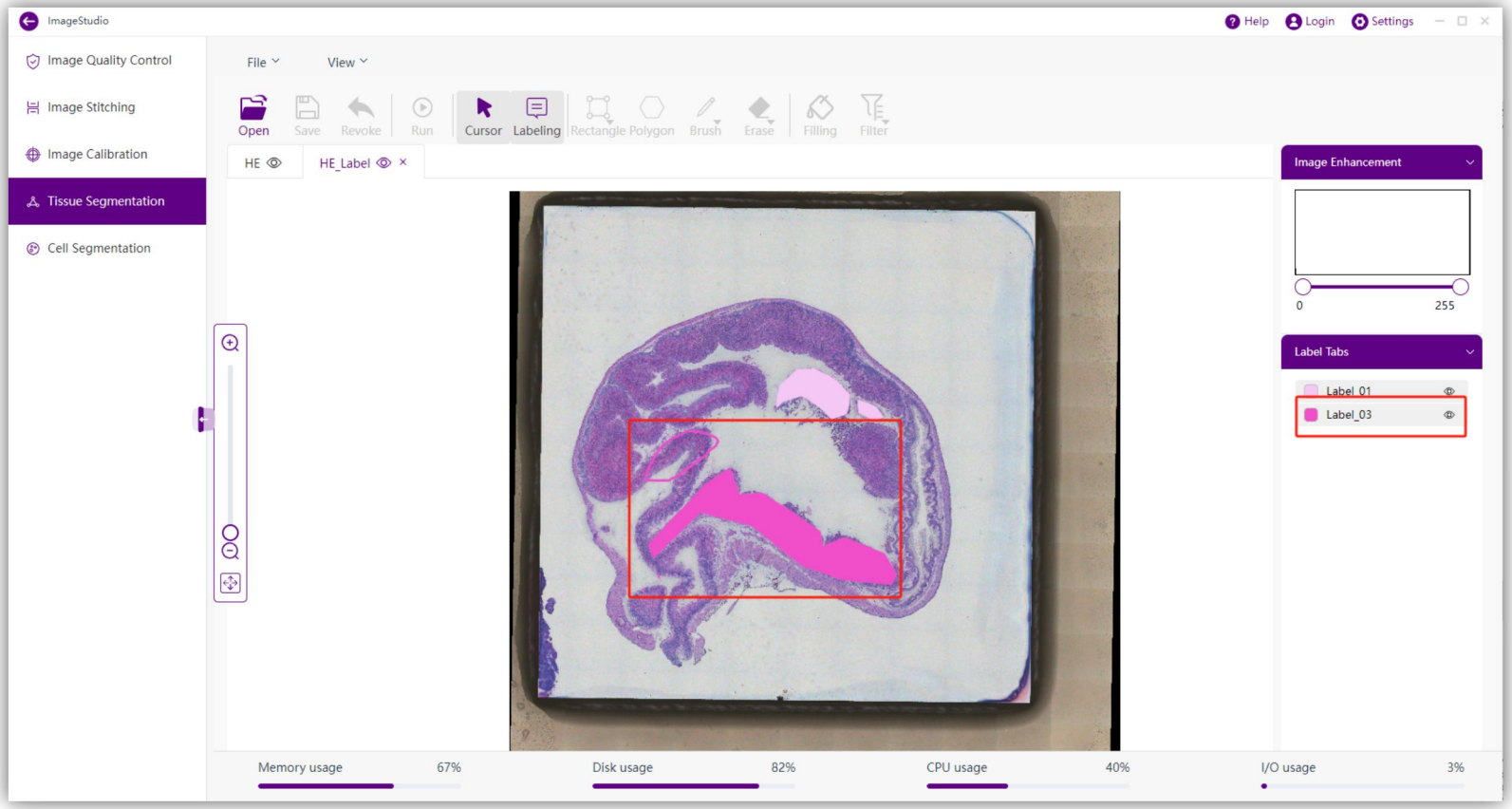
(11) Select polygon or brush tool to manually add regions, and select eraser to remove regions.



Note: Recommend to hide other labels while editing, to avoid any interferences on other labels.

(12) Click "Save" to save edited labels into the IPR file.


ImageStudio
Help
Login
Settings
Image Quality Control
Image Stitching
Image Calibration
Tissue Segmentation
Cell Segmentation
File
View
Open
Save
Revoke
Run
Cursor
Labeling
Rectangle
Polygon
Brush
Erase
Filling
Filter
HE
HE_Label
Saving Mask
Image Enhancement
0
255
Label Tabs
Label_01
Label_02
Memory usage
66%
Disk usage
82%
CPU usage
17%
I/O usage
1%

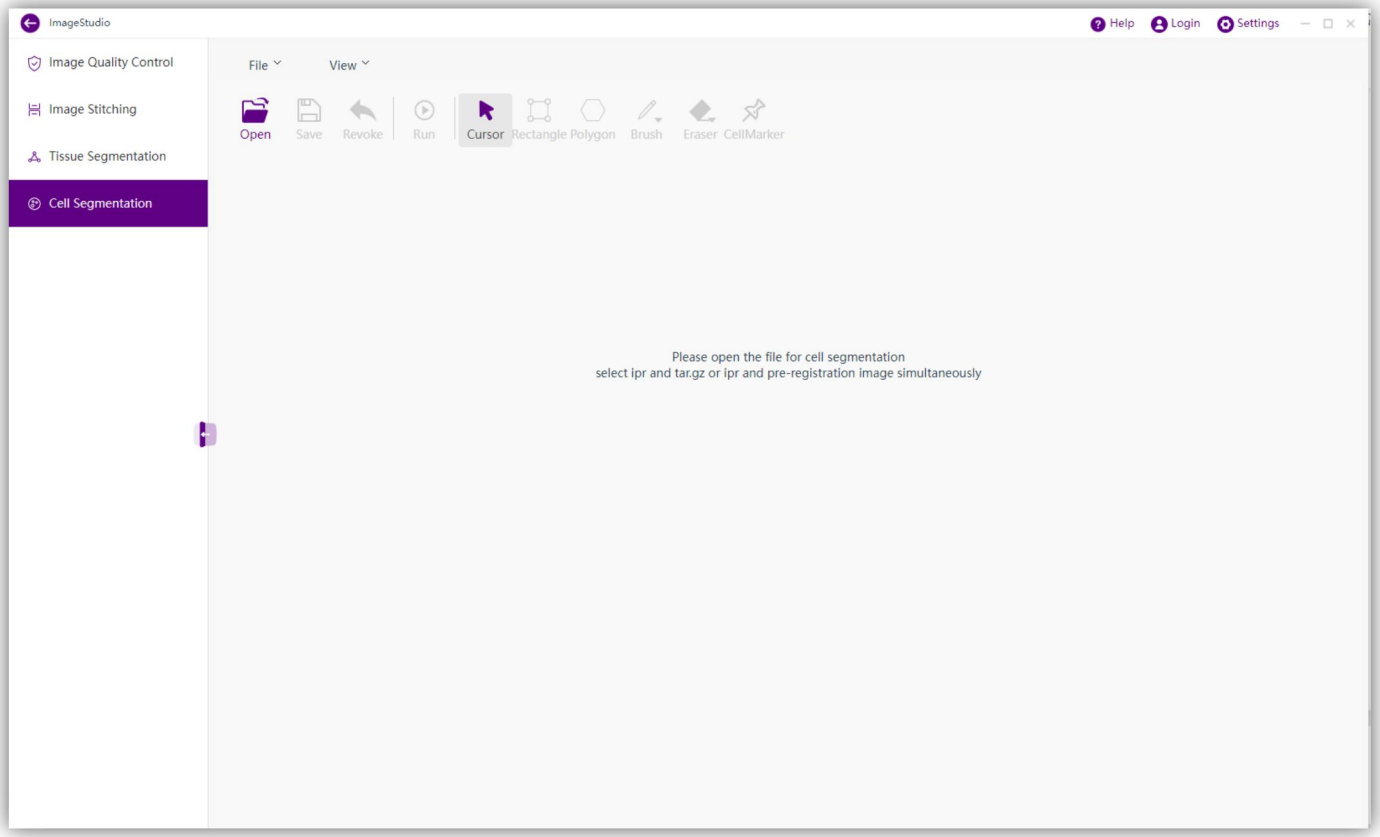


4.5 Cell segmentation

Cell segmentation module is for manually adjusting the segmented cell mask image.

4.5.1 Basic Operation

(6)Initiate the Cell Segmentation module.



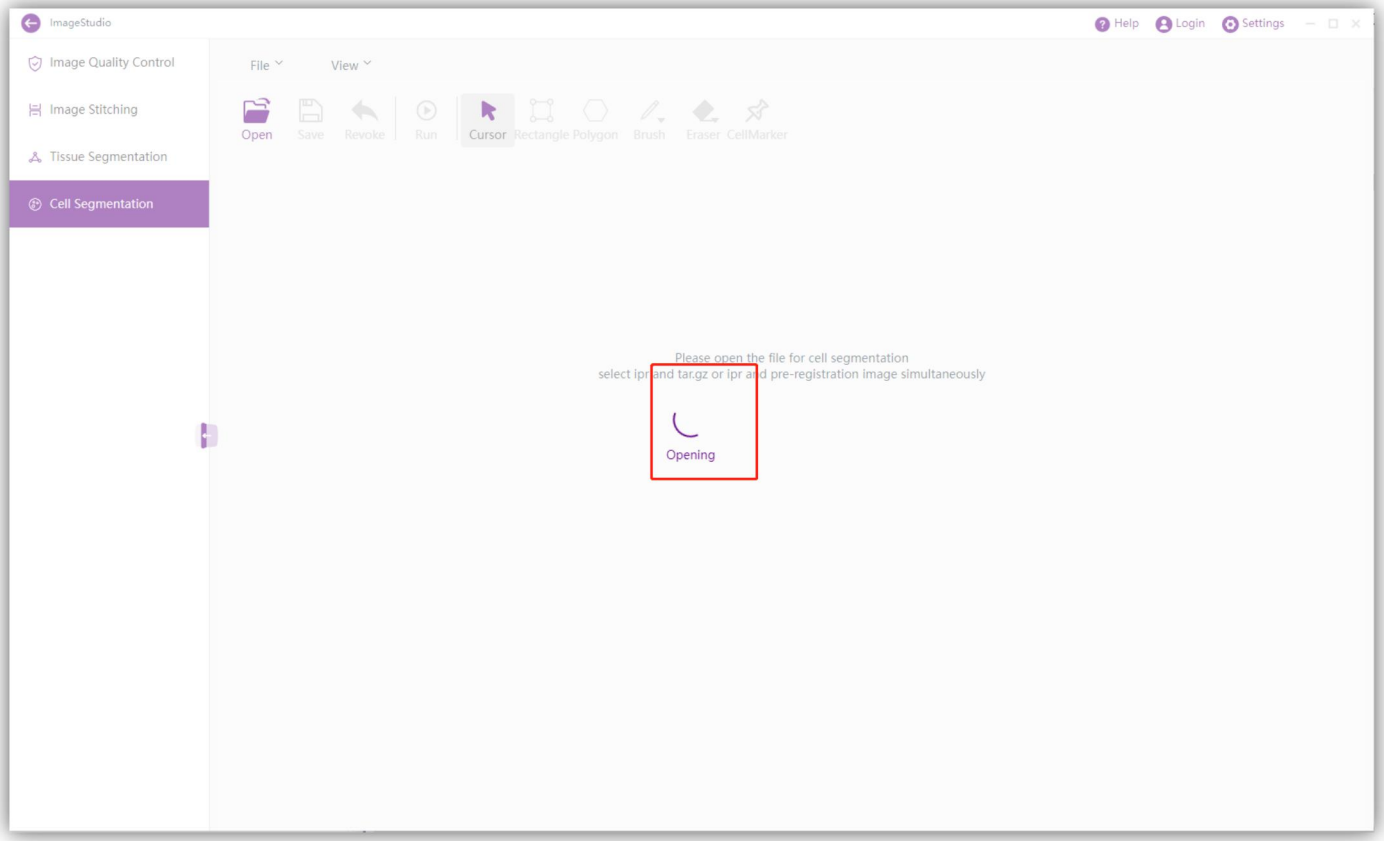
(7) Click 'Open' and select the image file.

Output directory: *D:\ImageStudioWorkspace\CellSeg*

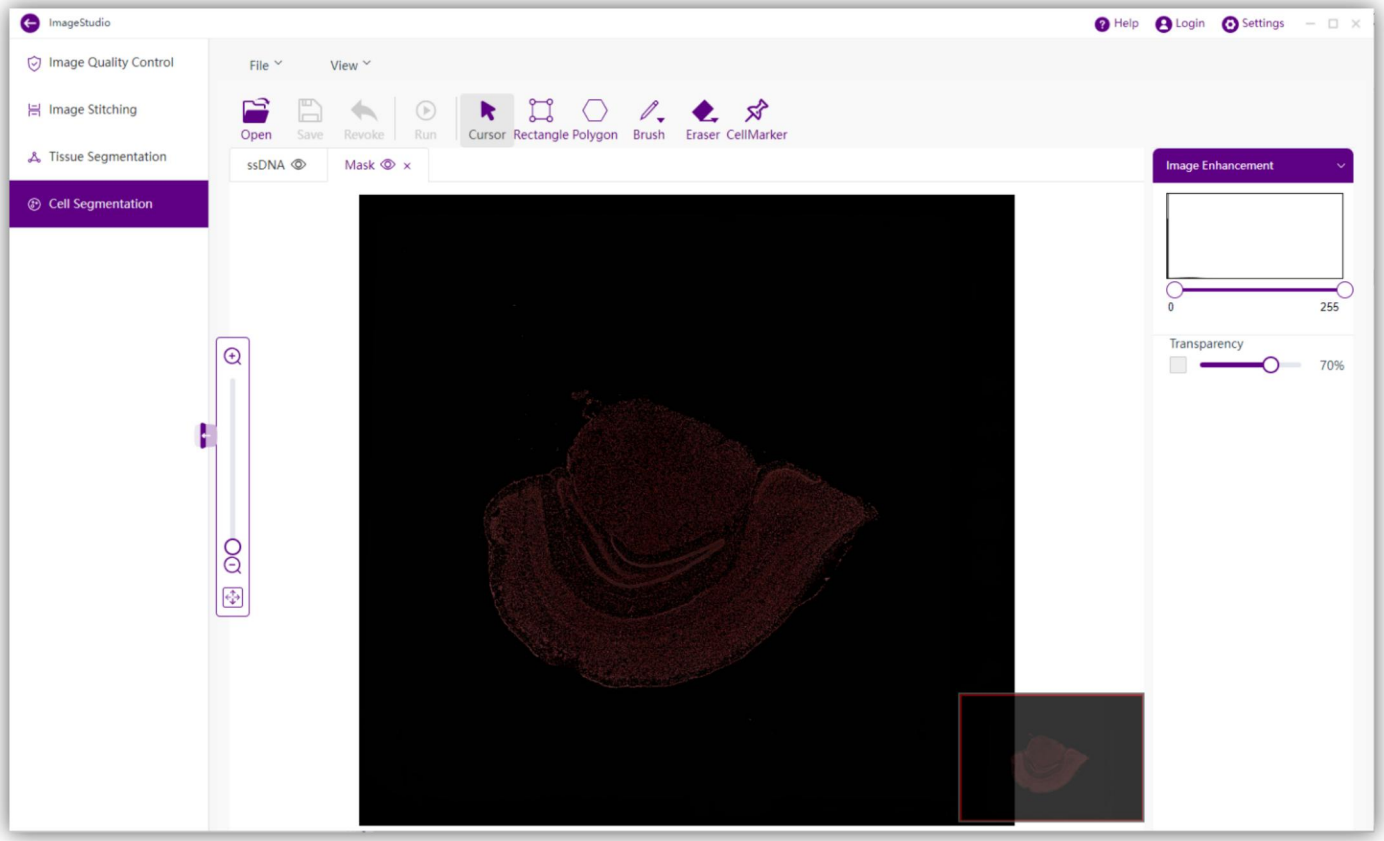
Module	Input File	Output File
Cell Segmentation	• IPR file and the corresponding TAR.GZ file that have gone through	• IPR file that records manual processing information

the ImageQC or SAW-register or SAW-rapidRegister processing module within Stereo-seq Analysis Workflow (SAW) pipeline

- fov_stitched_transformed.tif for pre-registered stitched image in TIFF format
- cell_mask.tif for cell mask image



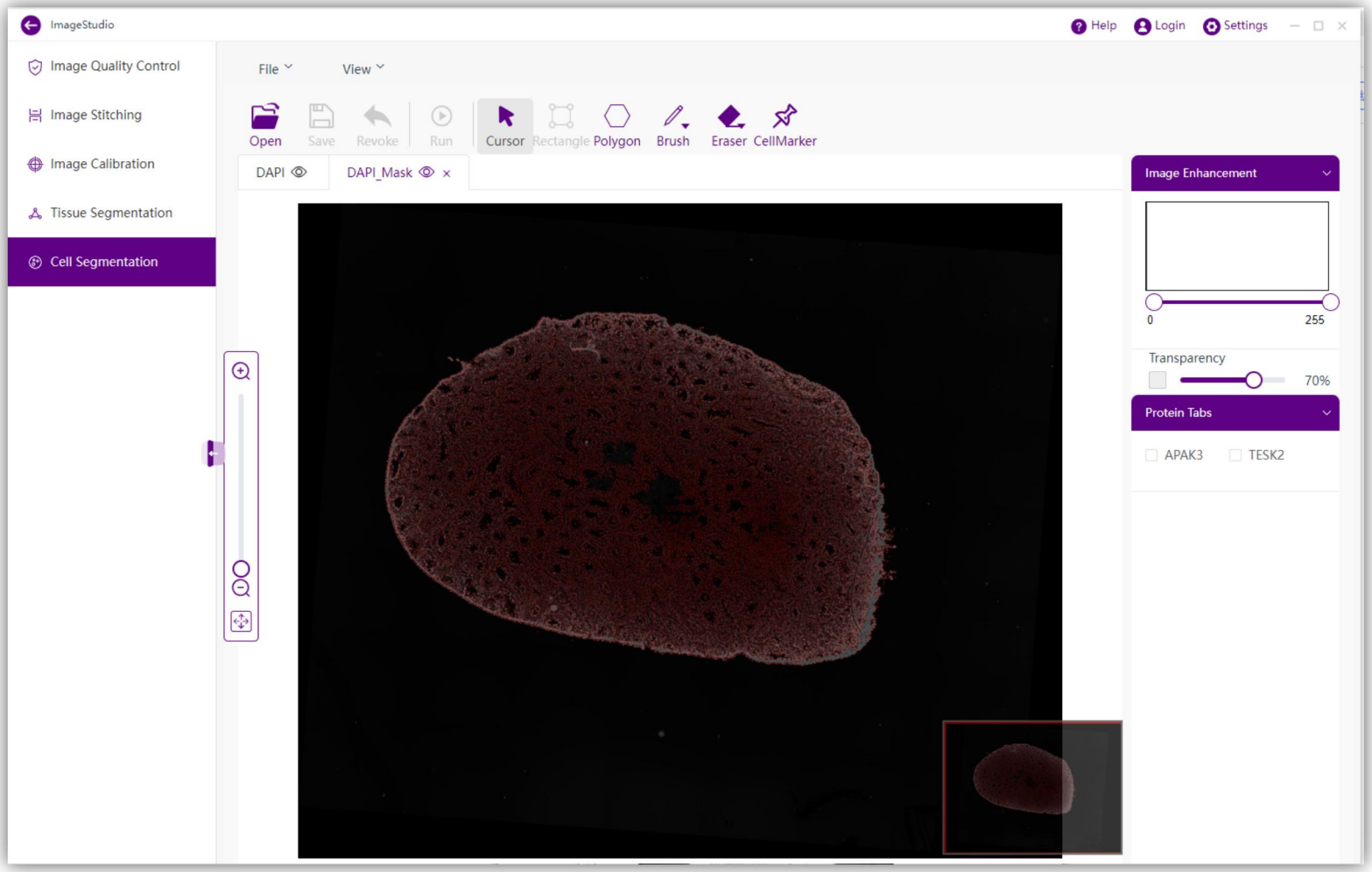
(8)Two tabs will be opened and display the ssDNA image and cell Mask image.



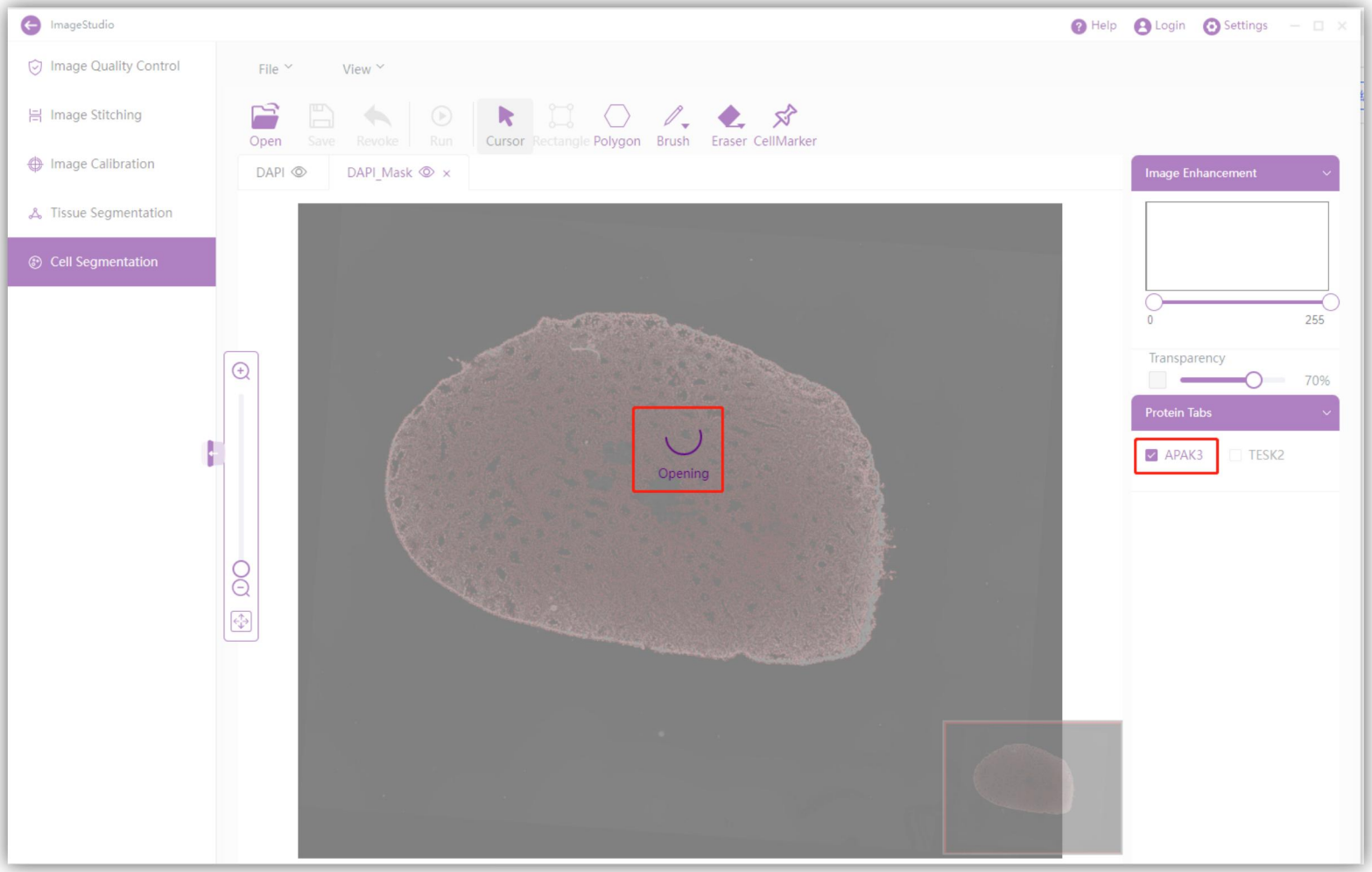
⚠Since generating the image pyramid is time-consuming, please only open one Stereo-seq Chip file at a time. One 1cm by 1cm Chip T will take about 3~5min to open.

(9)If the input is DAP with mIF

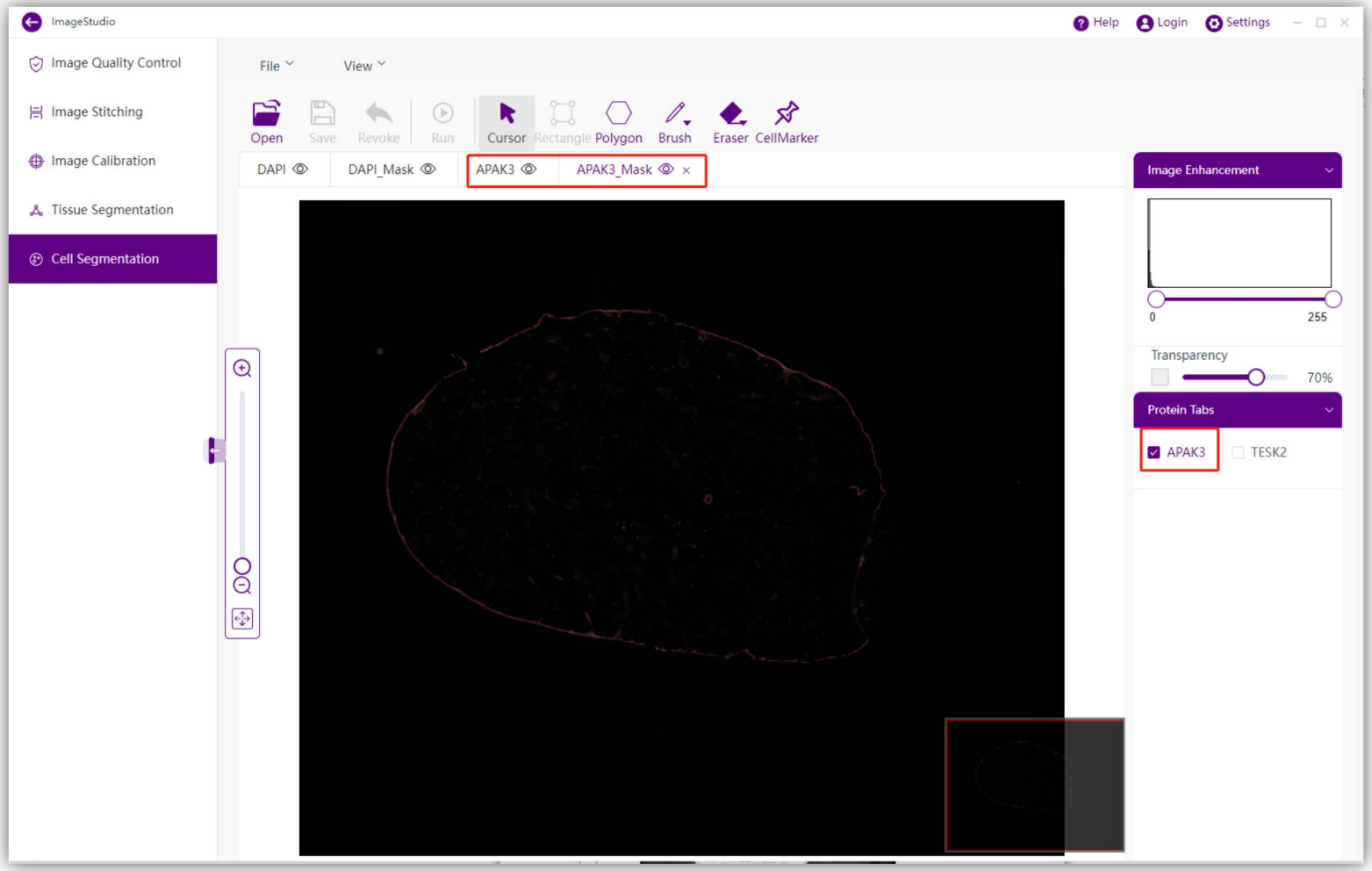
- Two tabs will be opened and display the DAPI image and tissue Mask image by default.



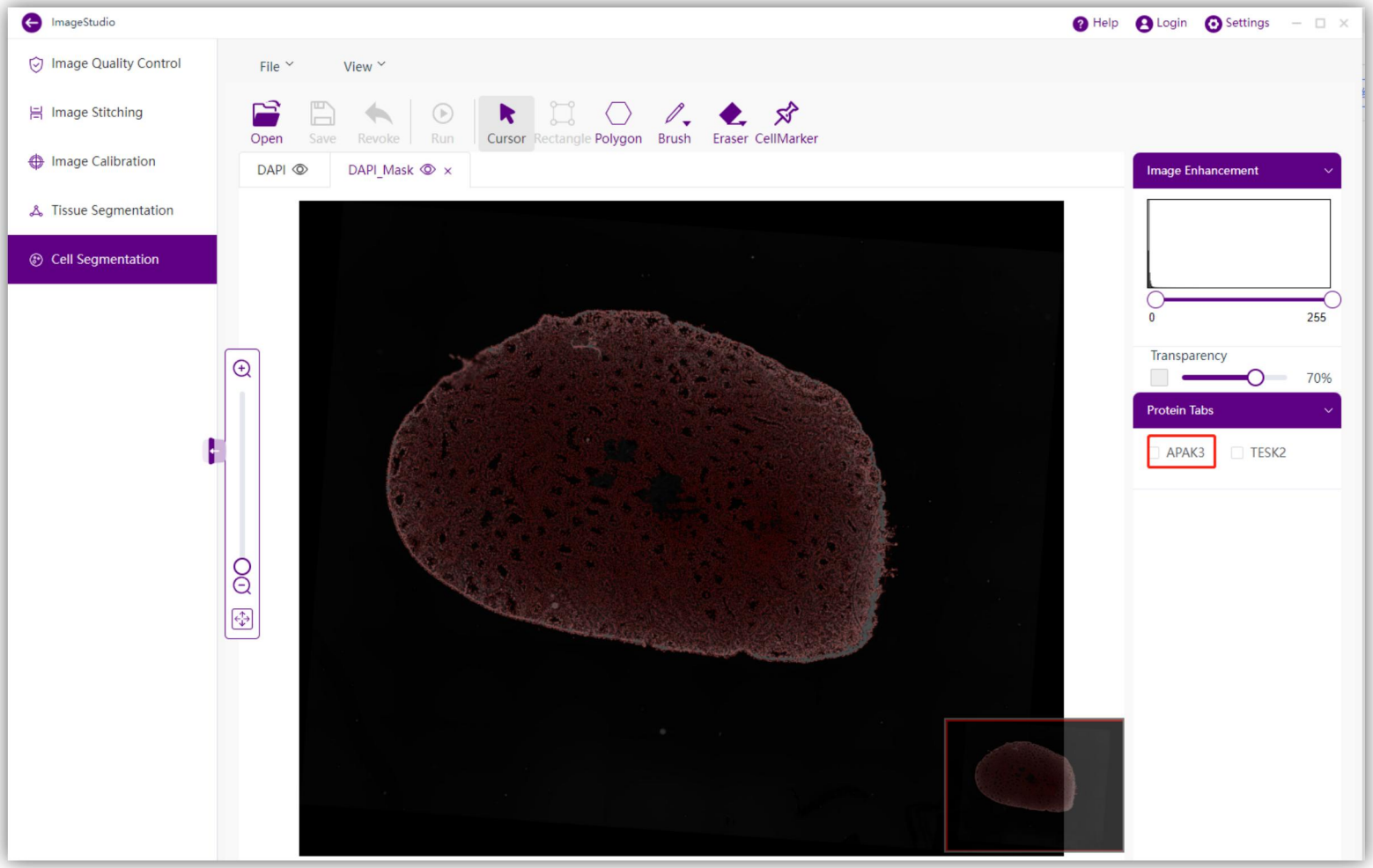
- Users can choose whether to display other proteins by selecting the box under "Protein Tabs"on the right.



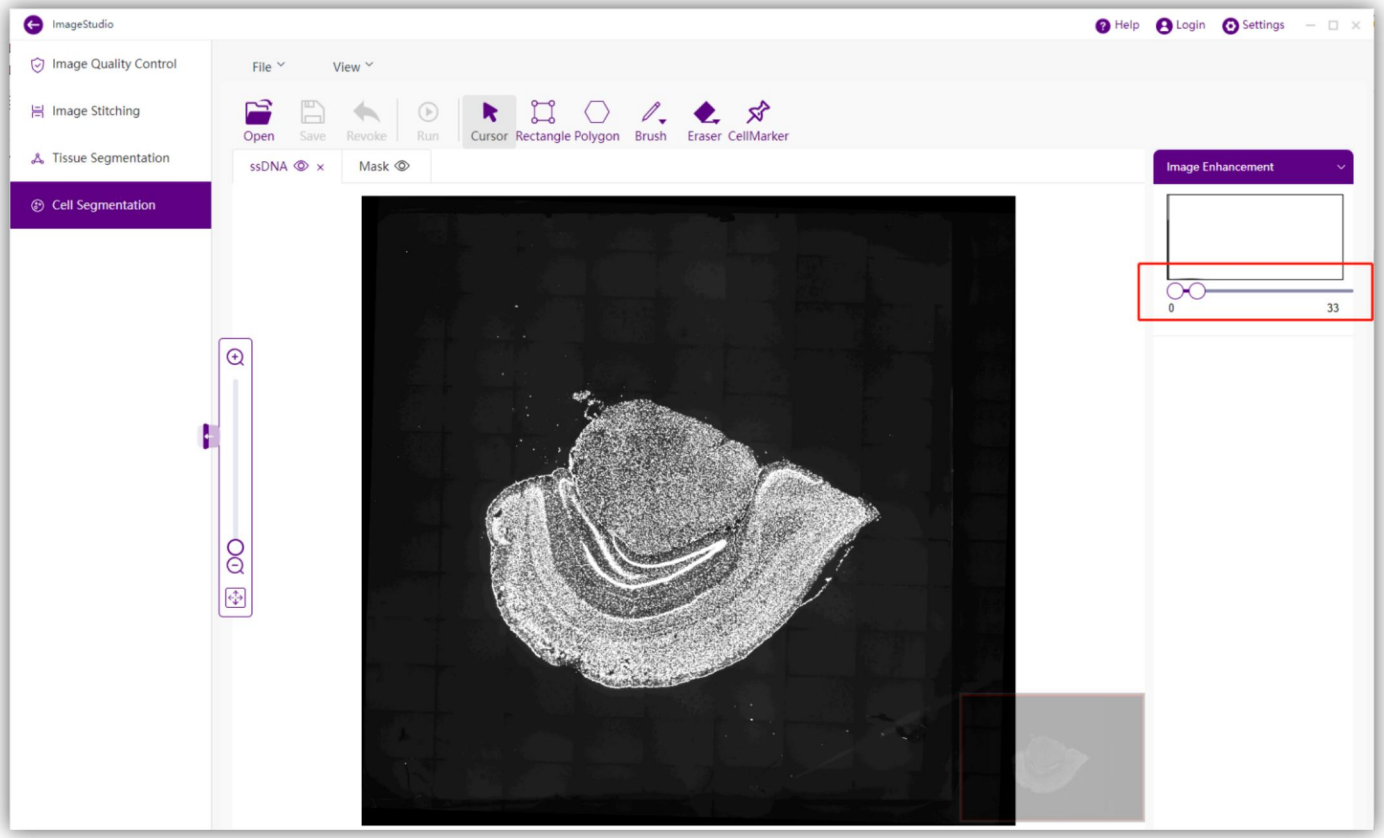
- The display of AKAP3 protein is shown below.



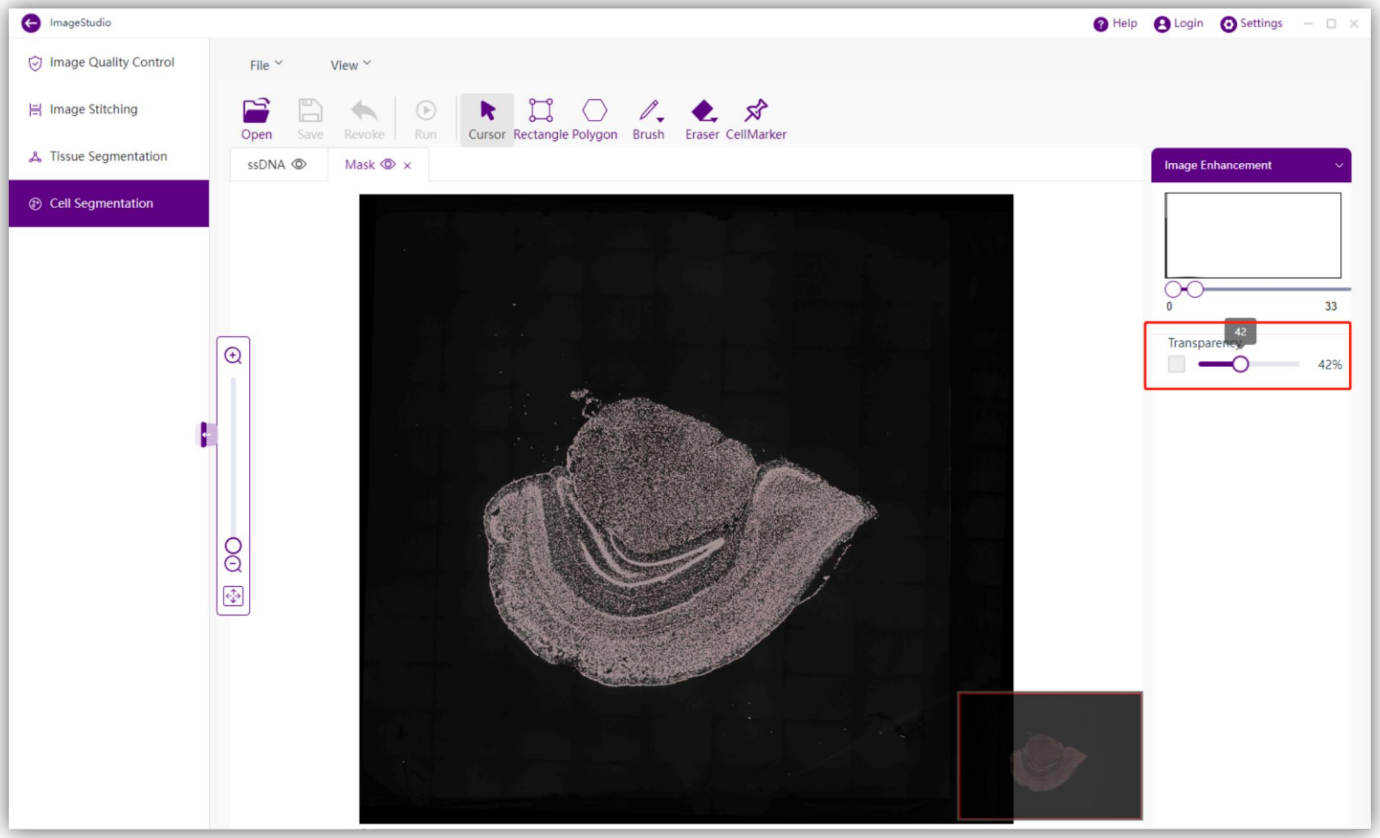
- Users can choose whether to turn off the display of other proteins by deselecting the box under "Protein Tabs"on the right.



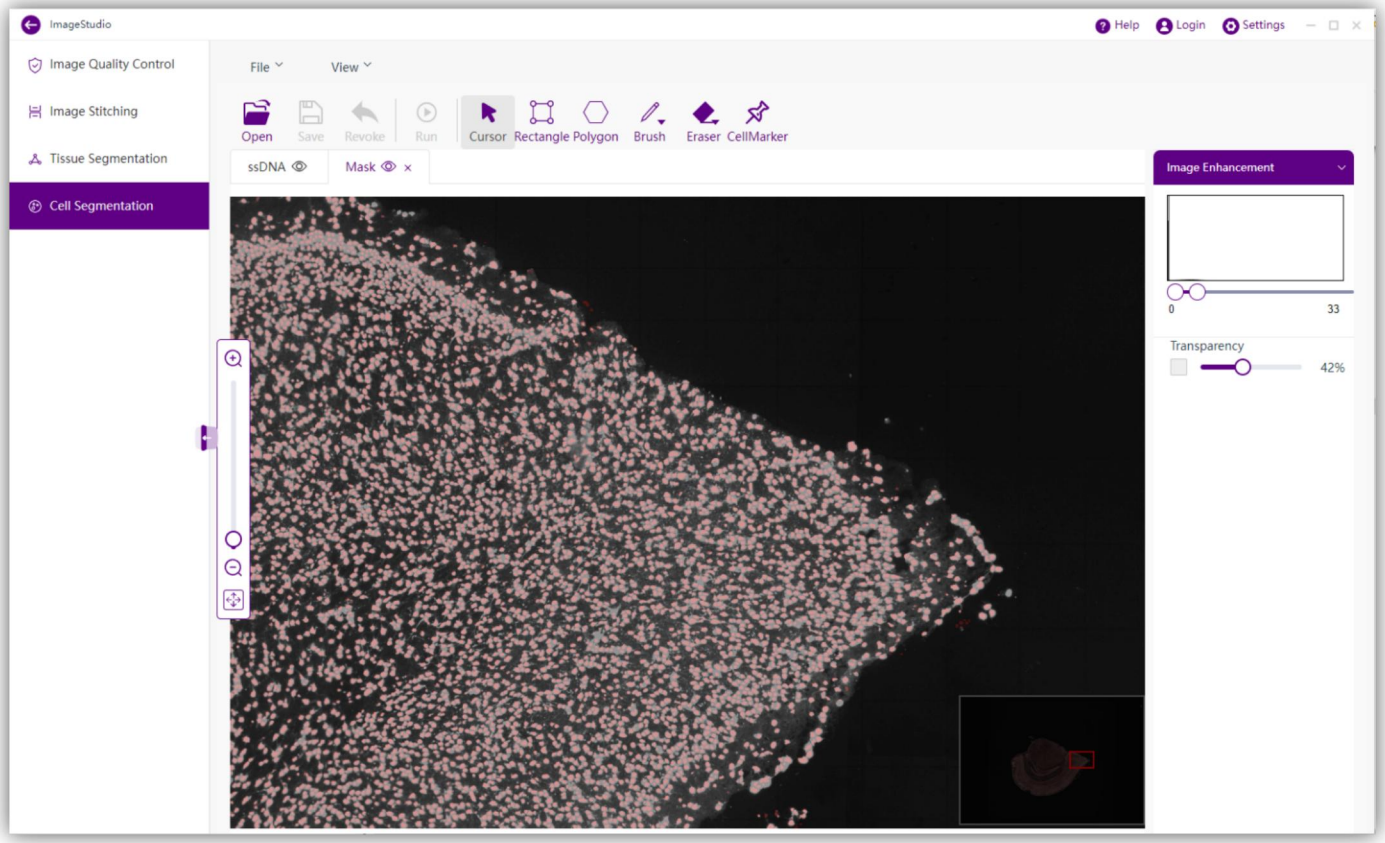
(10) Drag the 'Image Enhancement' slide bar under the stitched image tab to better display the tissue contour and background.



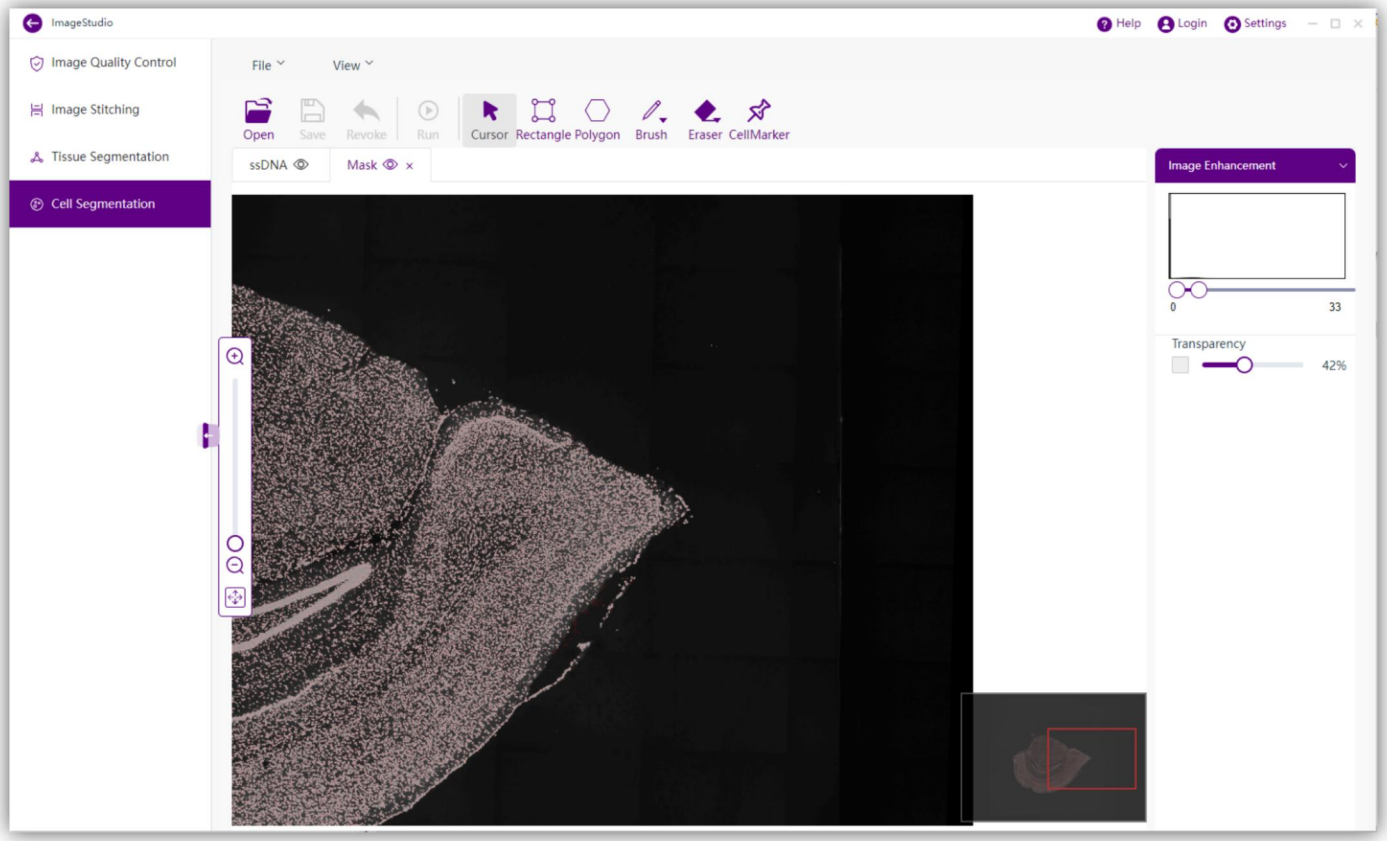
(11) Drag the 'Transparency Adjustment' slide bar under the Mask tab to adjust the transparency of cell mask image for a clearer view of the segmented cellular outline.



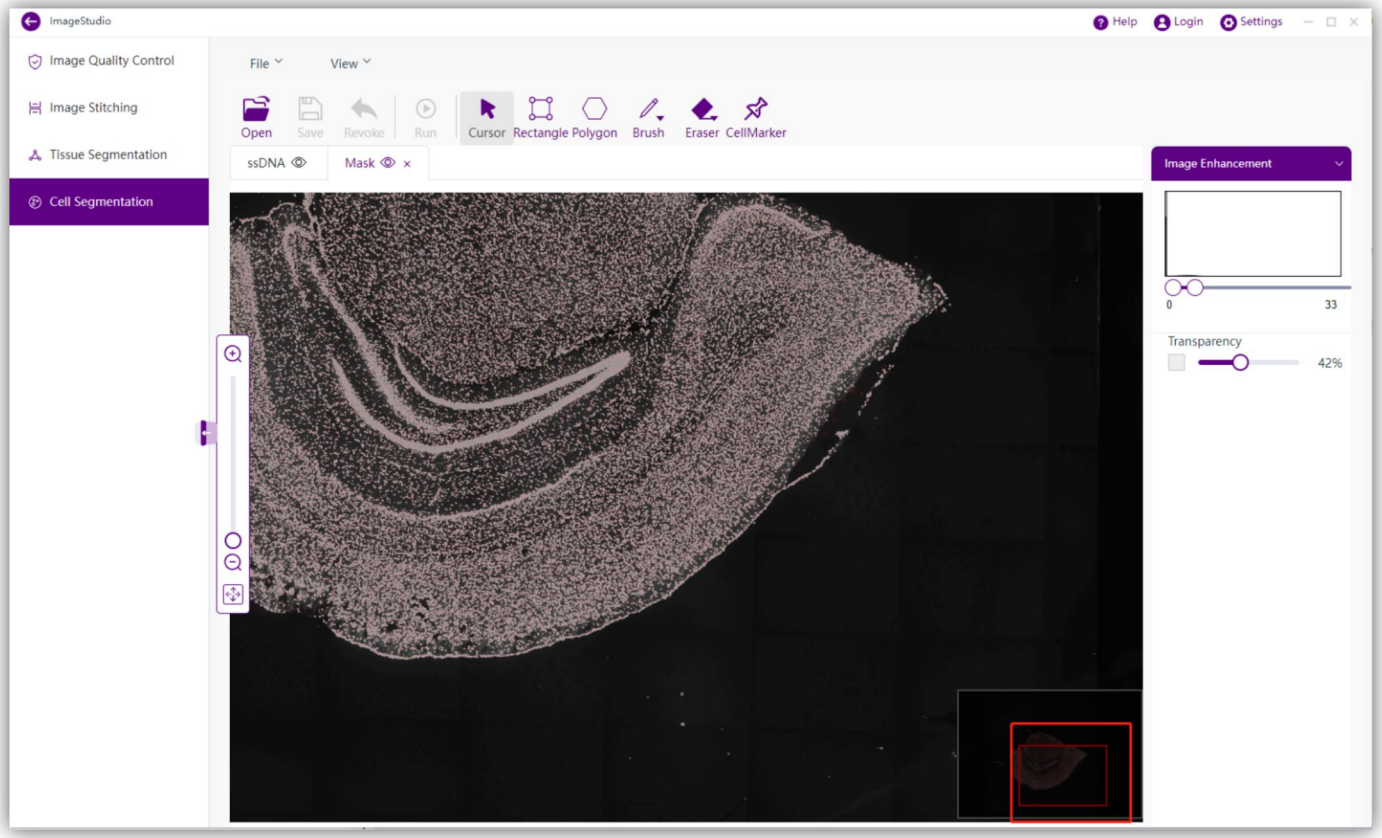
(12) Press and hold Ctrl+ while using the mouse wheel or click on the magnifying glass icon to zoom in or out of the image.



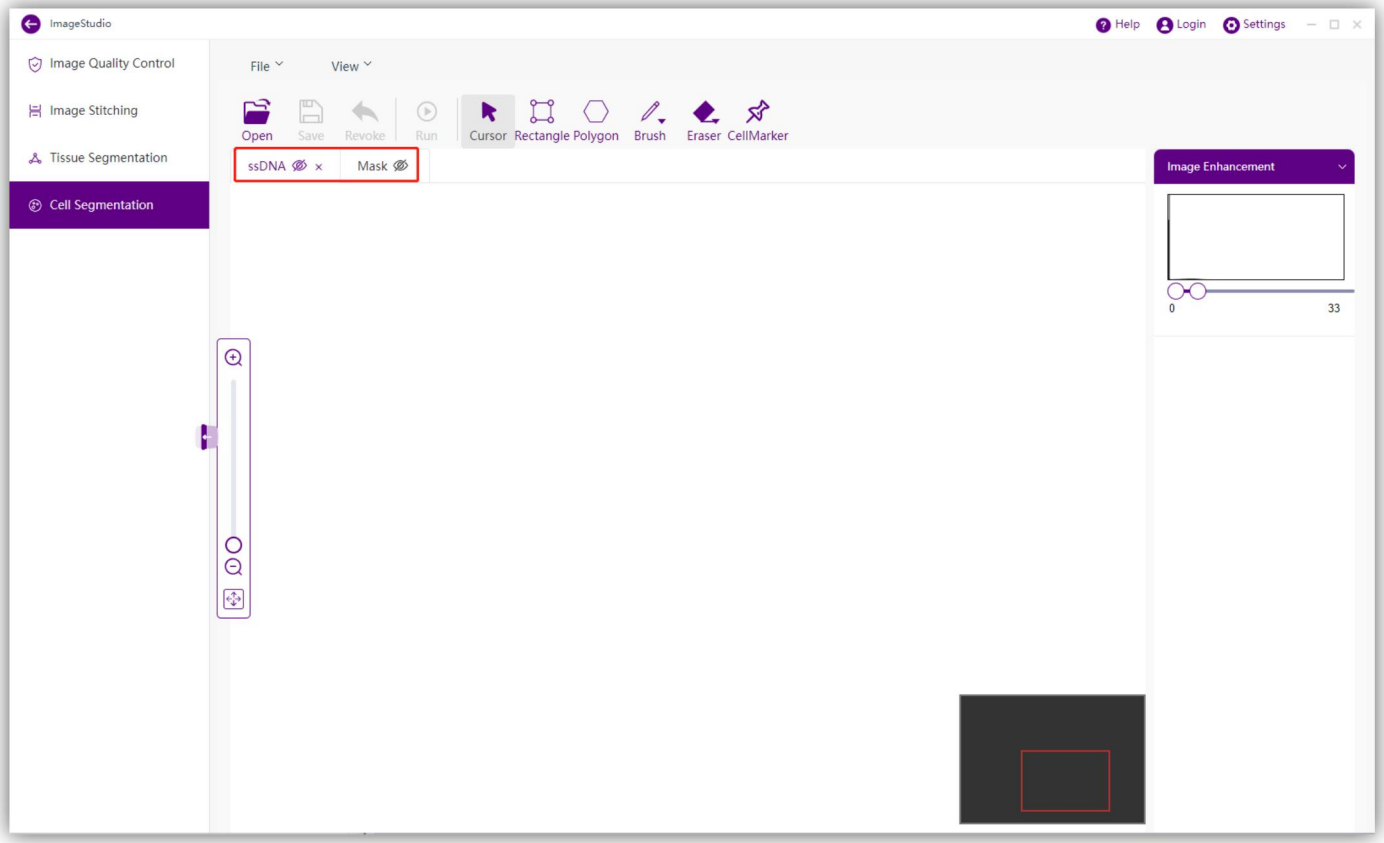
(13) Left click to move around the image.

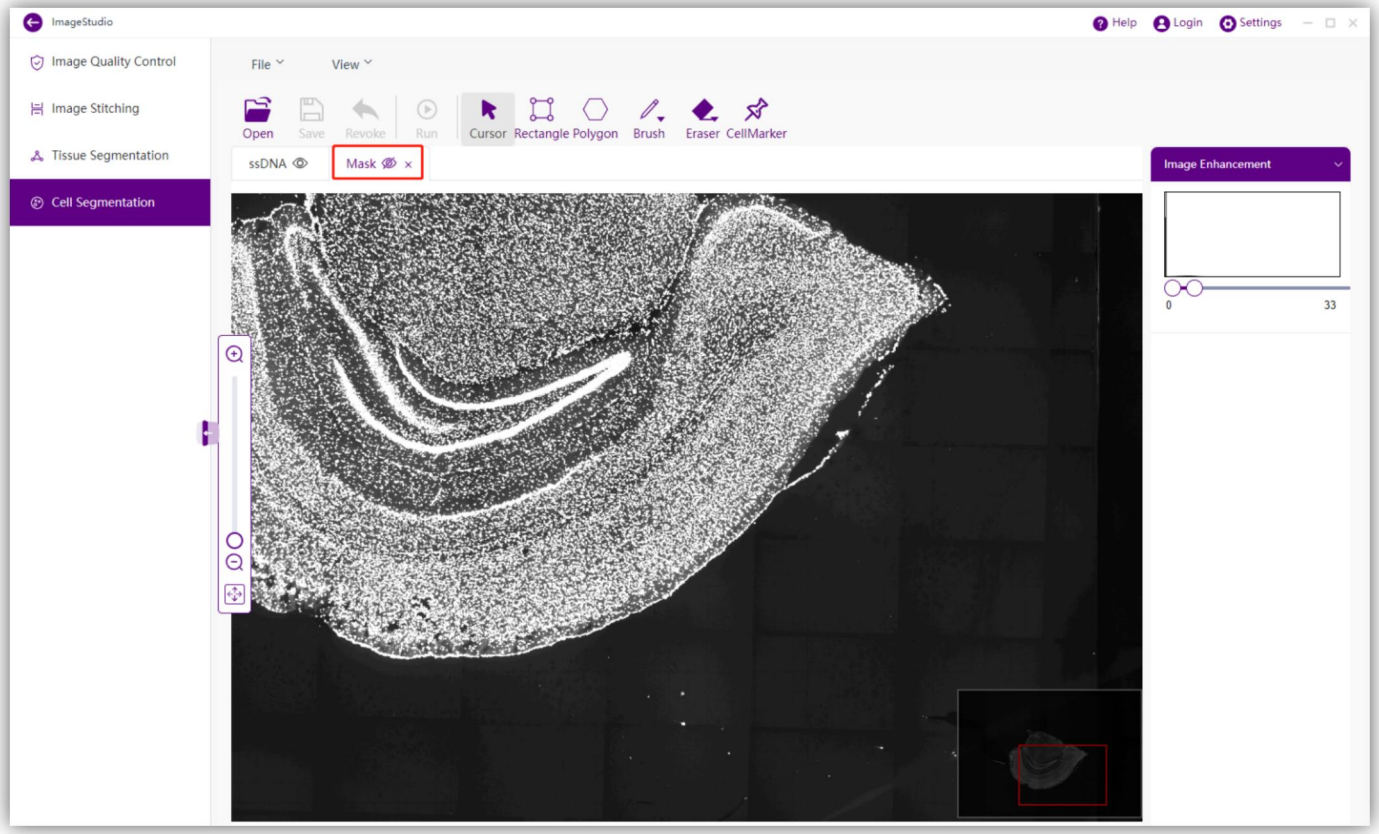


(14) Left-click within the local view window to move the field of view.



(15) Click on the 'eye icon' to hide or display the image under the tab.

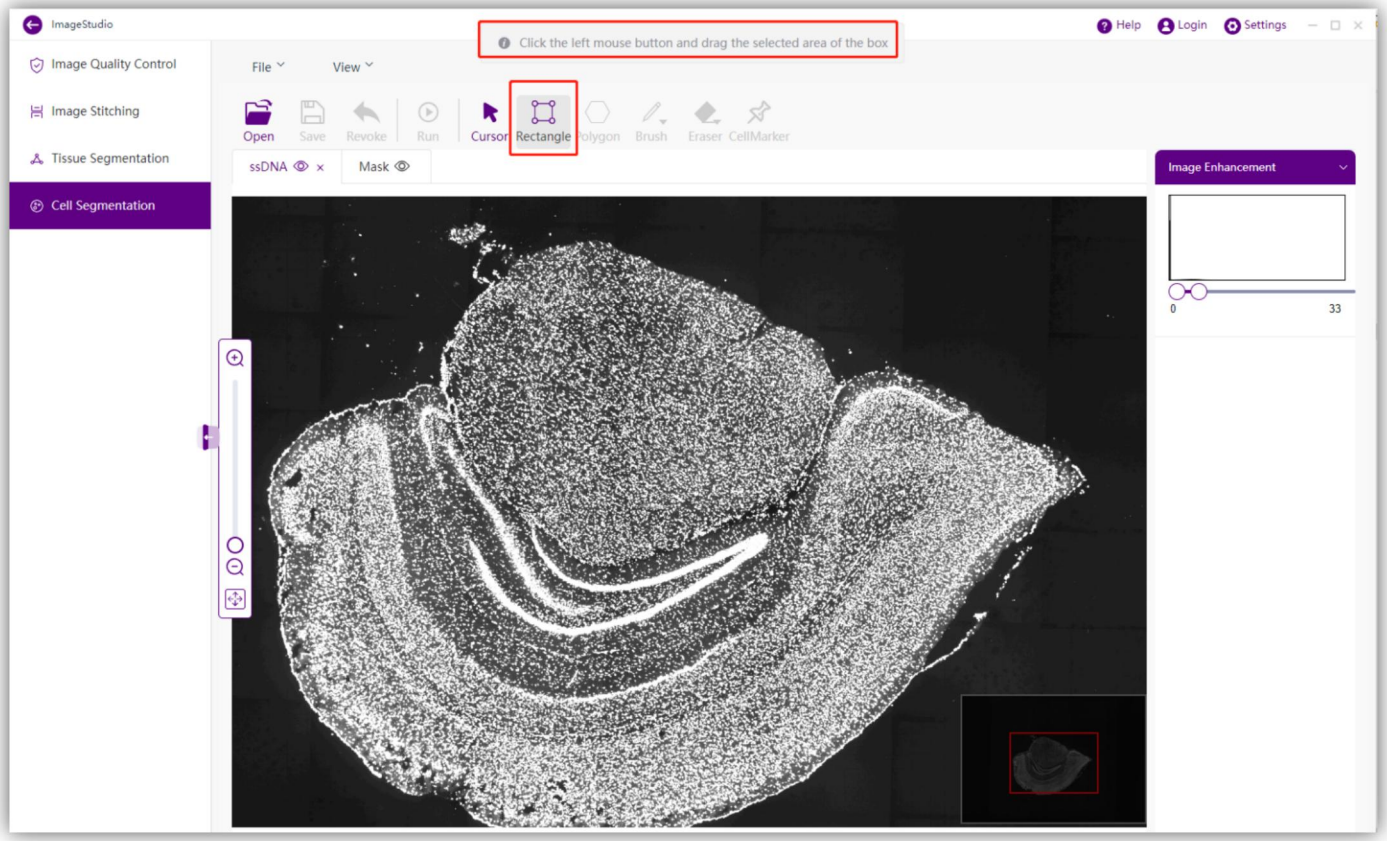

ImageStudio
Help
Login
Settings
Image Quality Control
Image Stitching
Tissue Segmentation
Cell Segmentation
File
View
Open
Save
Revoke
Run
Cursor
Rectangle
Polygon
Brush
Eraser
CellMarker
ssDNA
Mask
Image Enhancement
0
33



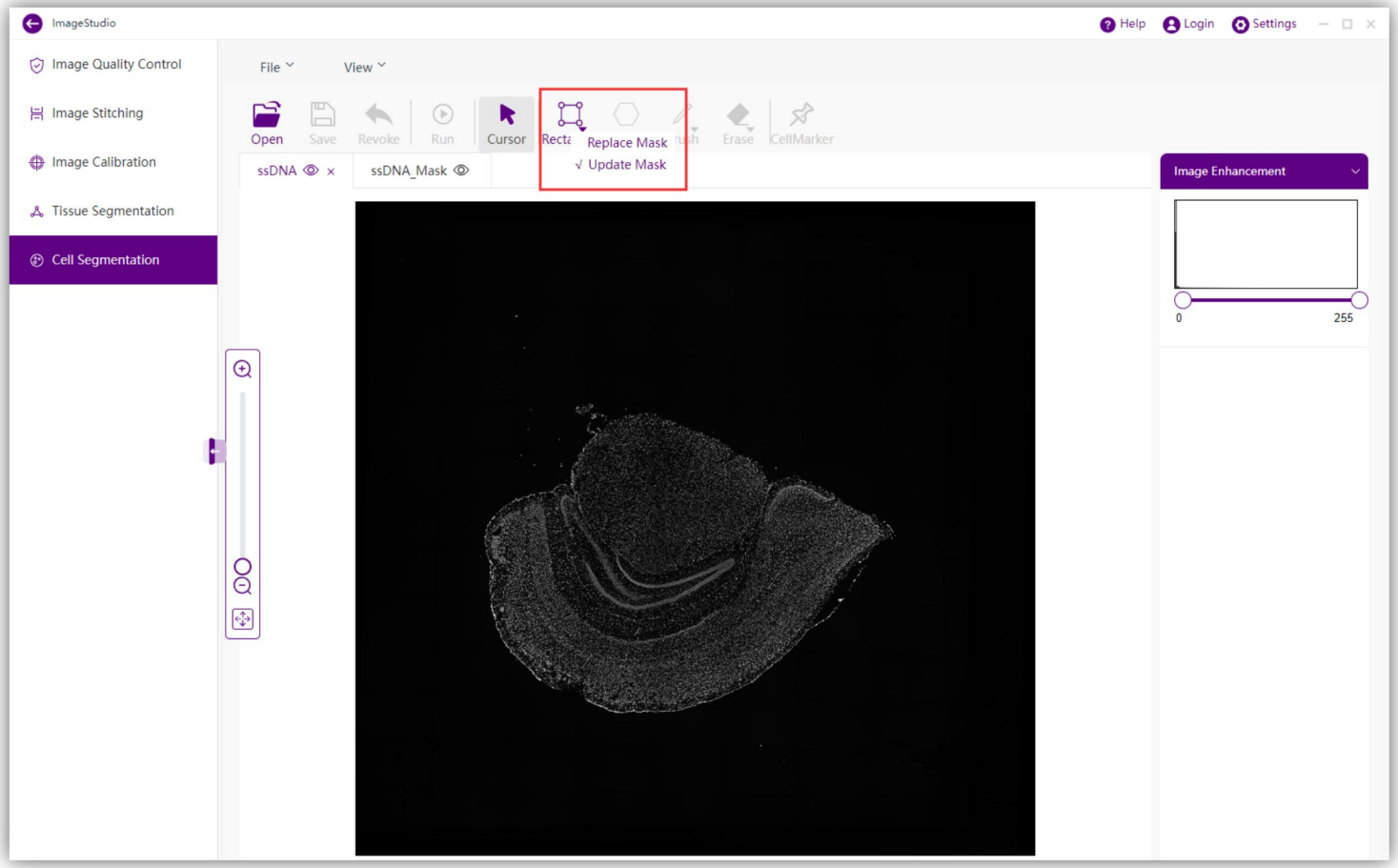
4.5.2 Rectangle

This function mainly operates on the stitched image for extracting algorithm generated cell Mask file according to the box selected ROI via cell segment algorithm.

(1)Click 'Rectangle' in the stitched image tab.



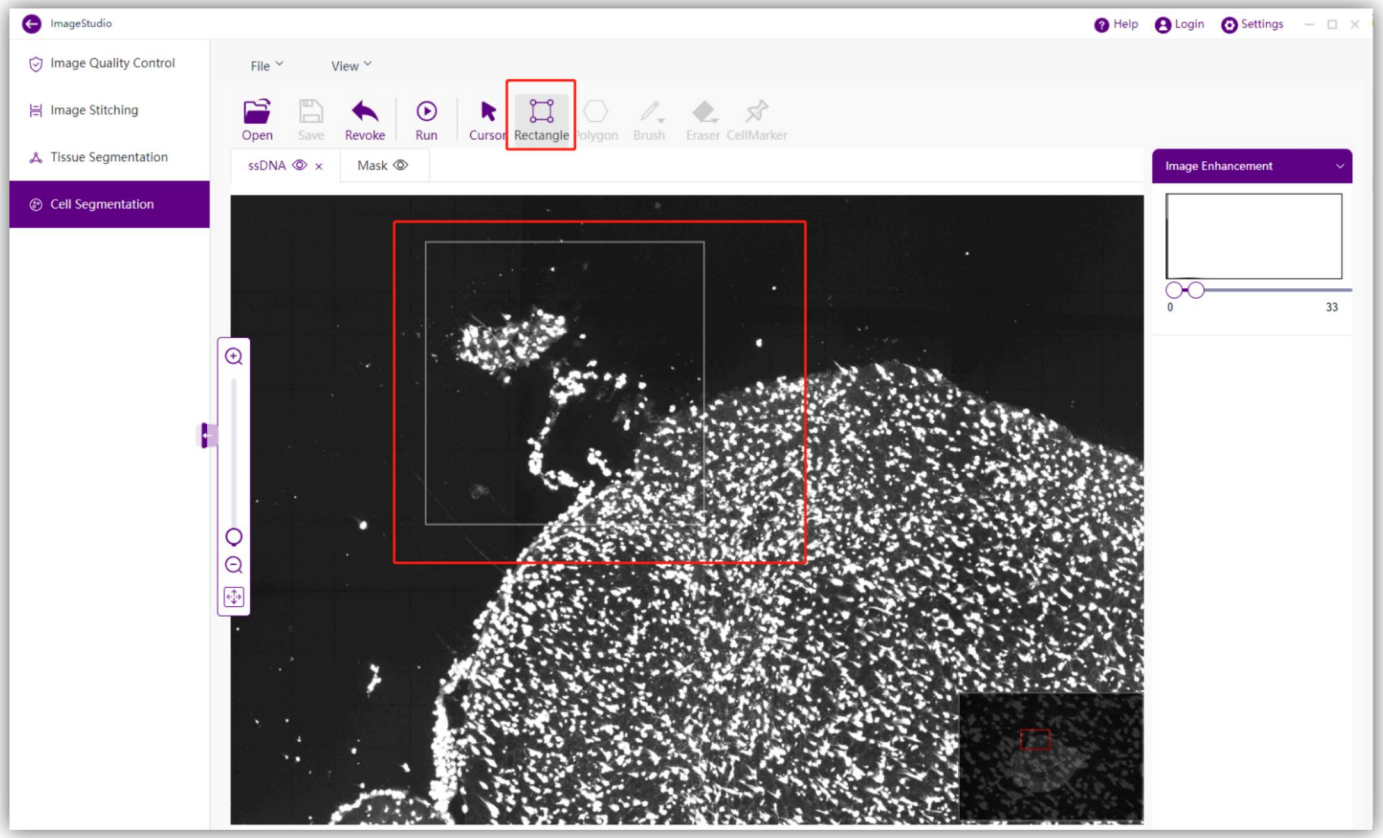
(2) User can choose either "Replace Mask" or "Update Mask", and the default option is to update mask.



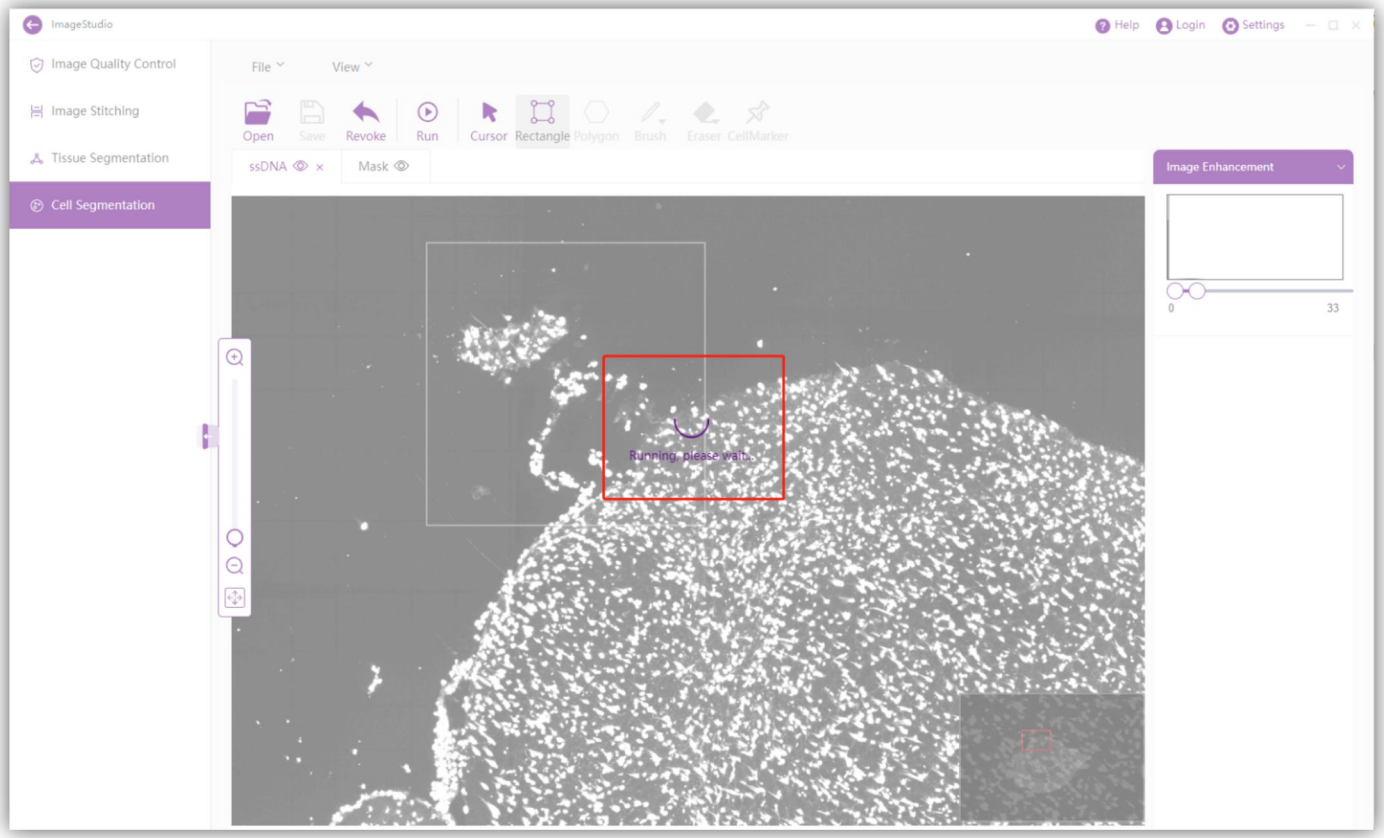
Note:

- "Replace Mask" extracts a new Mask according to the box selected ROI, which replaces the stitched Mask file.
- "Update Mask" updates the corresponding Mask according to the box selected ROI, and the Mask of other areas remain unchanged.

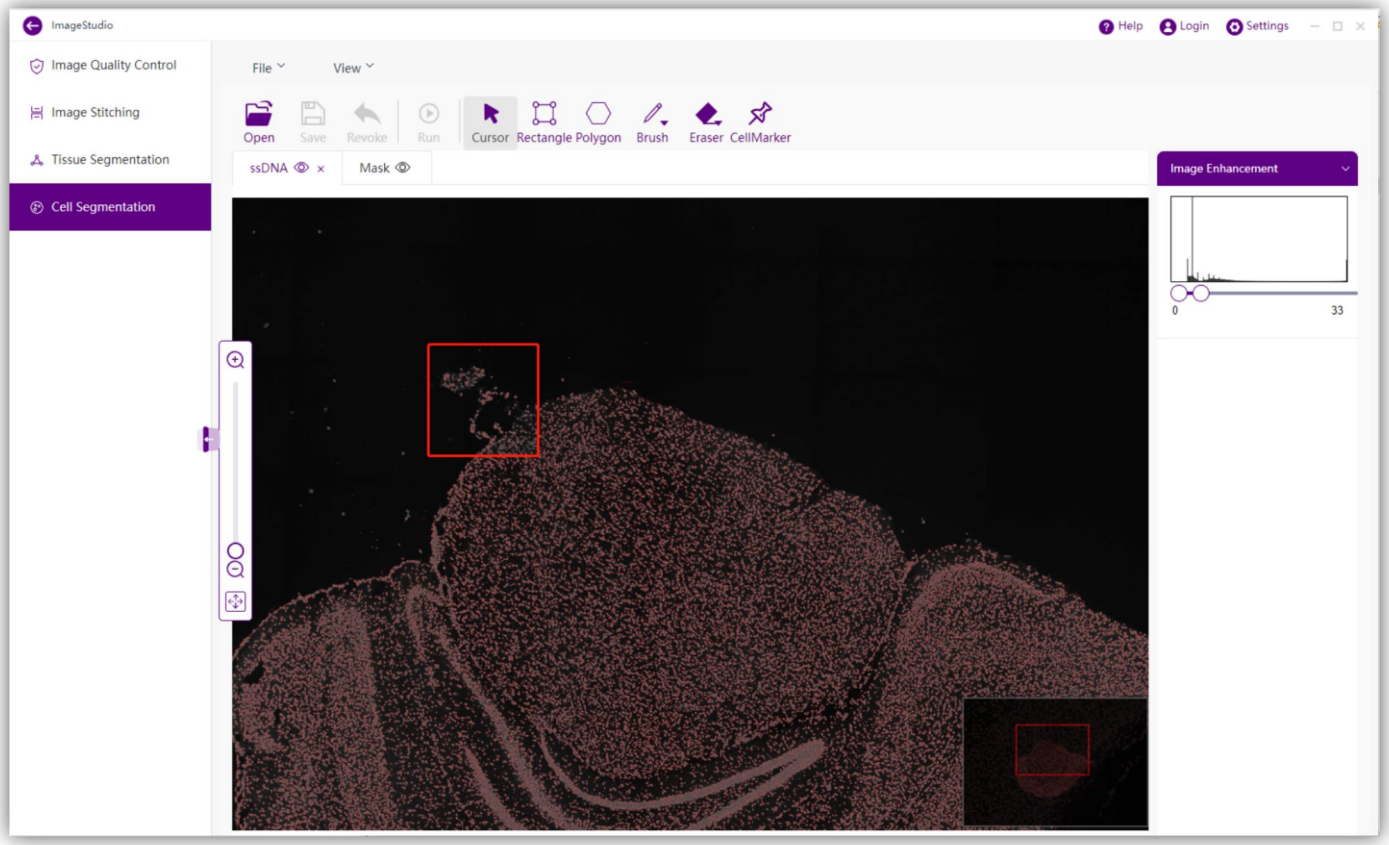
(3) Box selects region of interest using the rectangle.



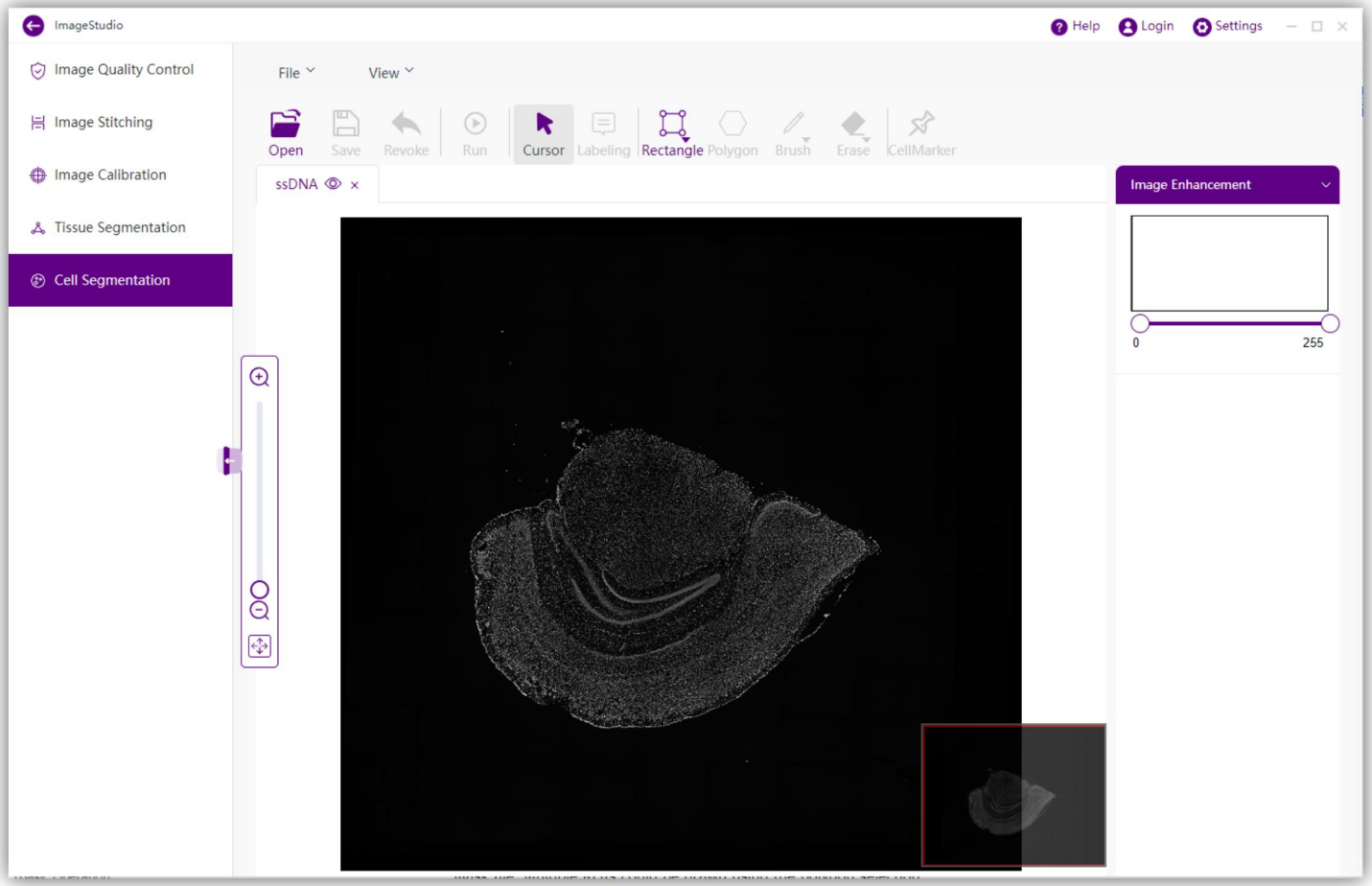
(4) Click 'Run'.

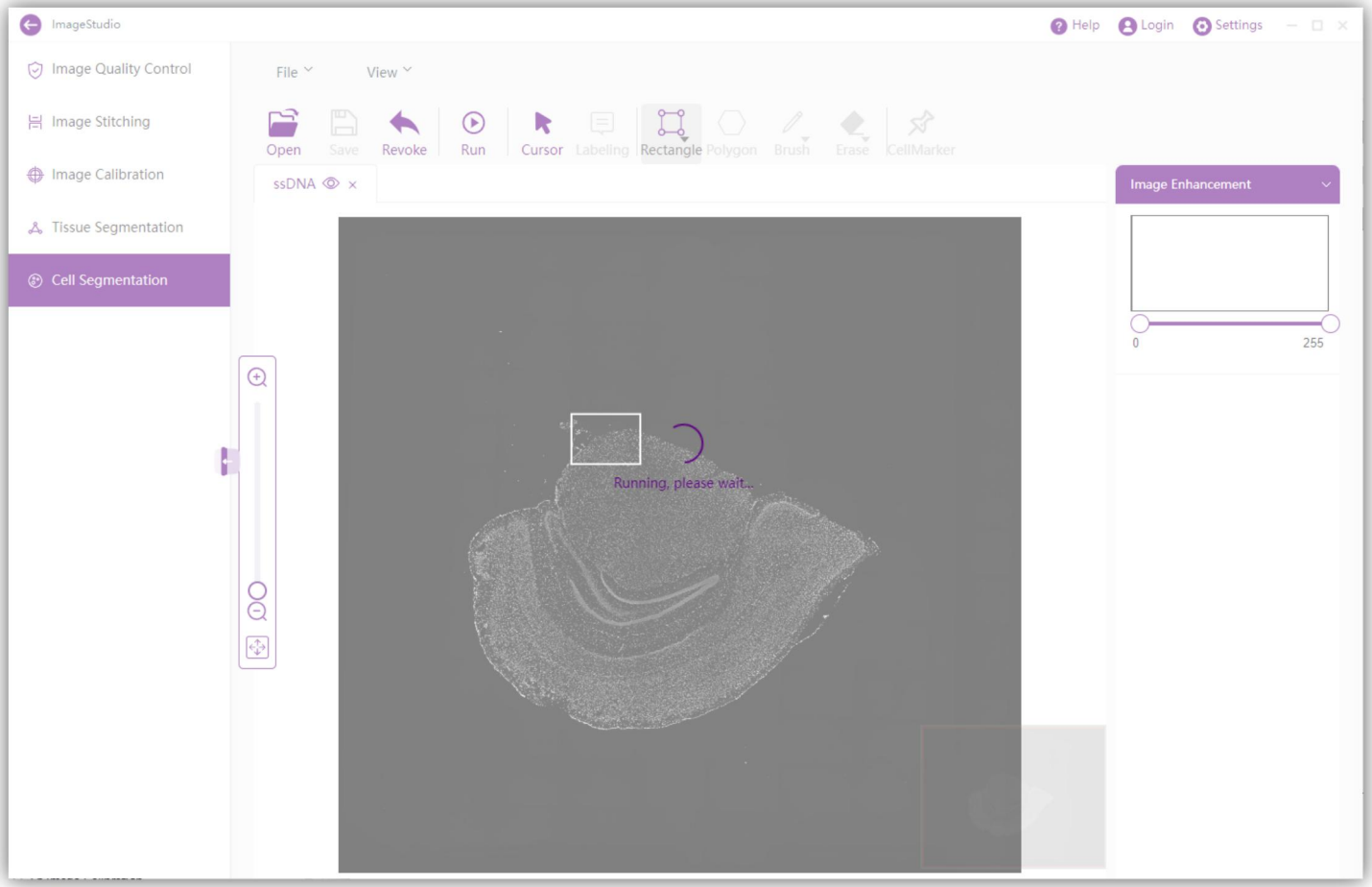


(5) Cell segmentation algorithm will extract cell Mask file according to the box selected ROI, overlay the stitched Mask file, and write it into the IPR file.



(7) If the input data has not gone through tissue segmentation, a new "Mask" tab will be generated after running tissue segmentation using rectangle tool.


ImageStudio
Help
Login
Settings
Image Quality Control
Image Stitching
Image Calibration
Tissue Segmentation
Cell Segmentation
File
View
Open
Save
Revoke
Run
Cursor
Labeling
Rectangle
Polygon
Brush
Erase
CellMarker
ssDNA
Image Enhancement
0
255

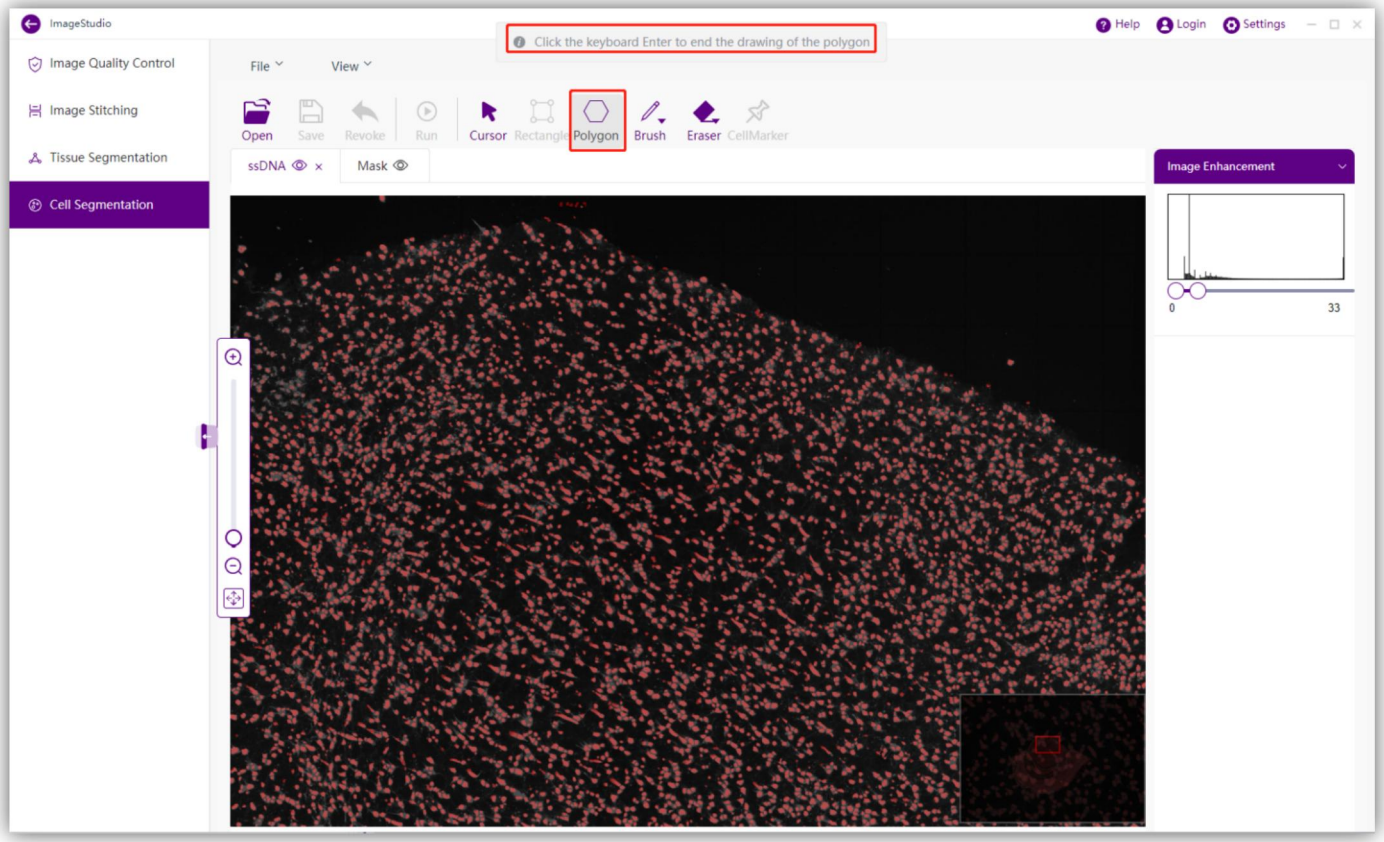


4.5.3 Polygon

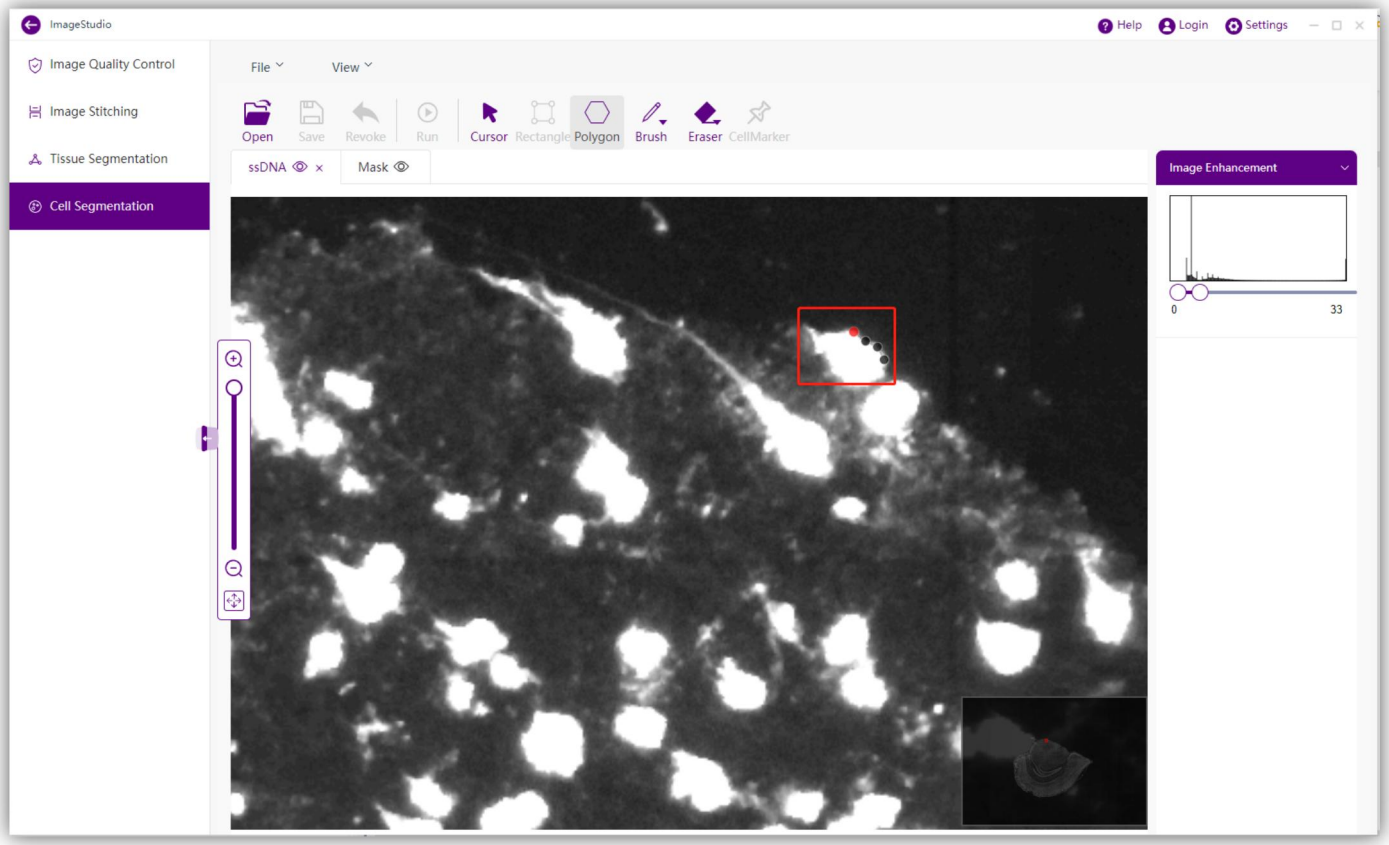
This function mainly operates on the ssDNA image and Mask image for modifying algorithm generated cell Mask file according to the manually drawn cell contour using polygon selection and

overlayinging the stitched cell Mask file. Multiple ROIs could be drawn using the polygon selection.

(1) Click 'Polygon' in the ssDNA image or Mask image tab.

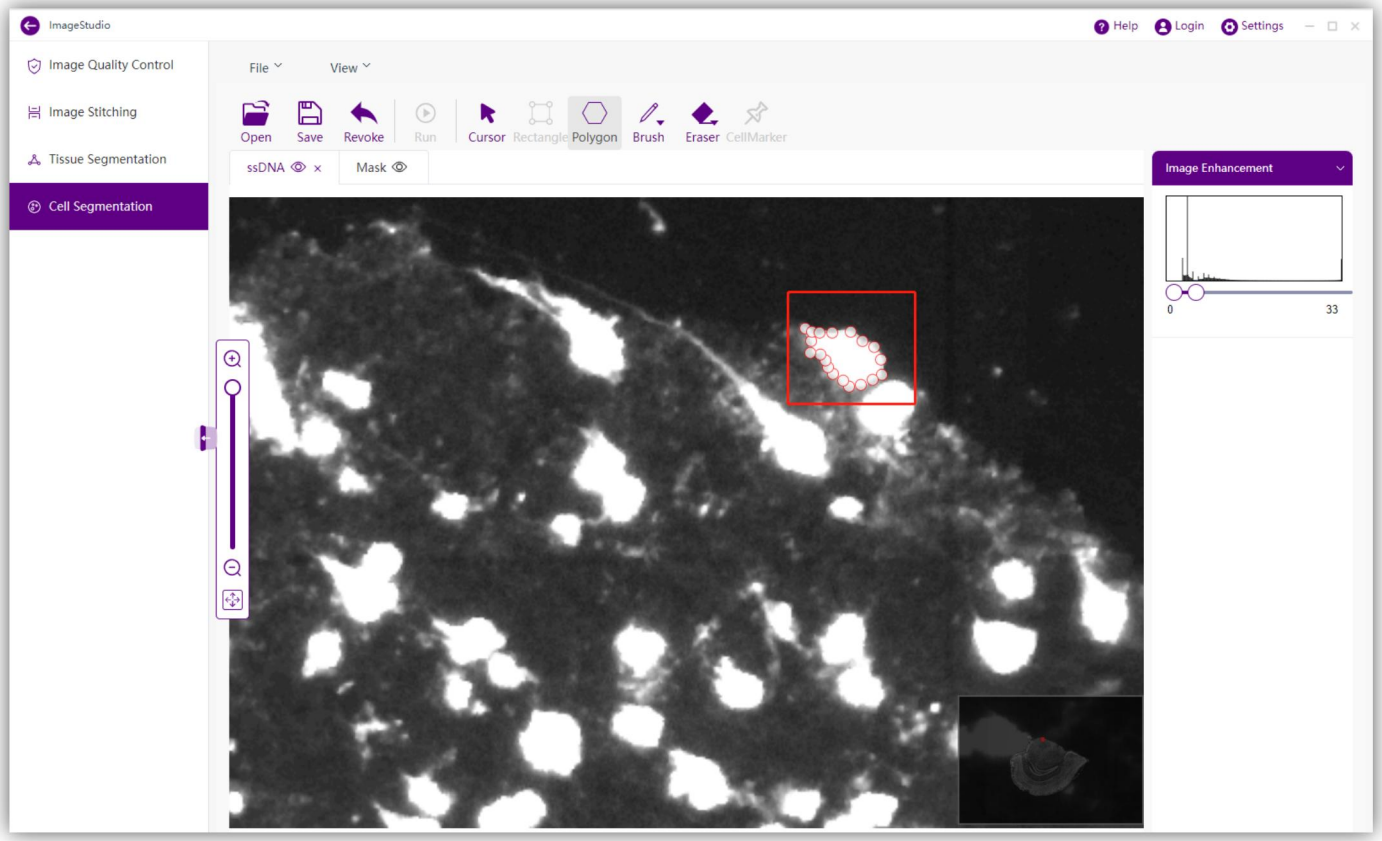


(2) Find the cell that needs to be modified, start with a point, then left-click to add points until an enclosed polygon has formed.

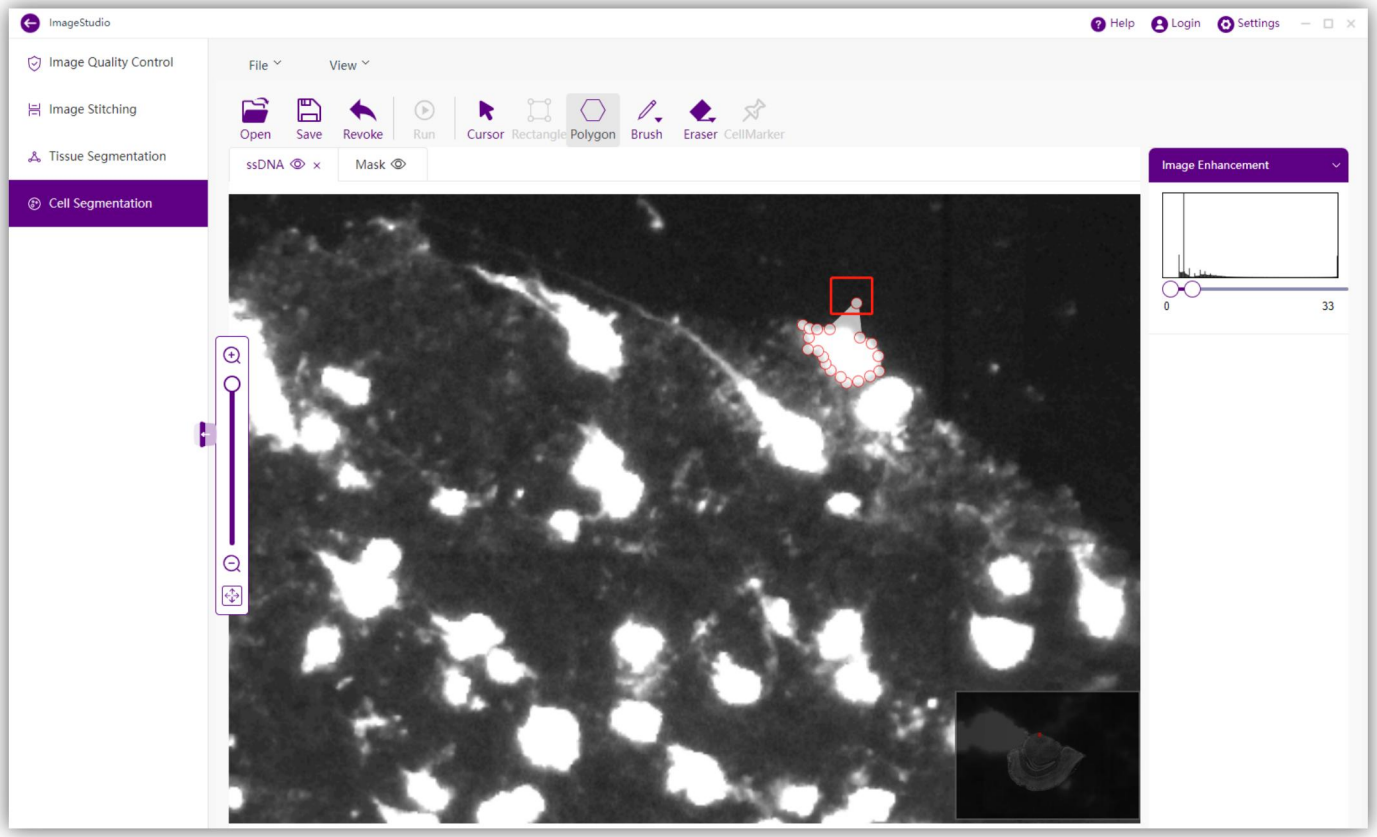


Currently, overlapping of ROIs using polygon selection is not supported.

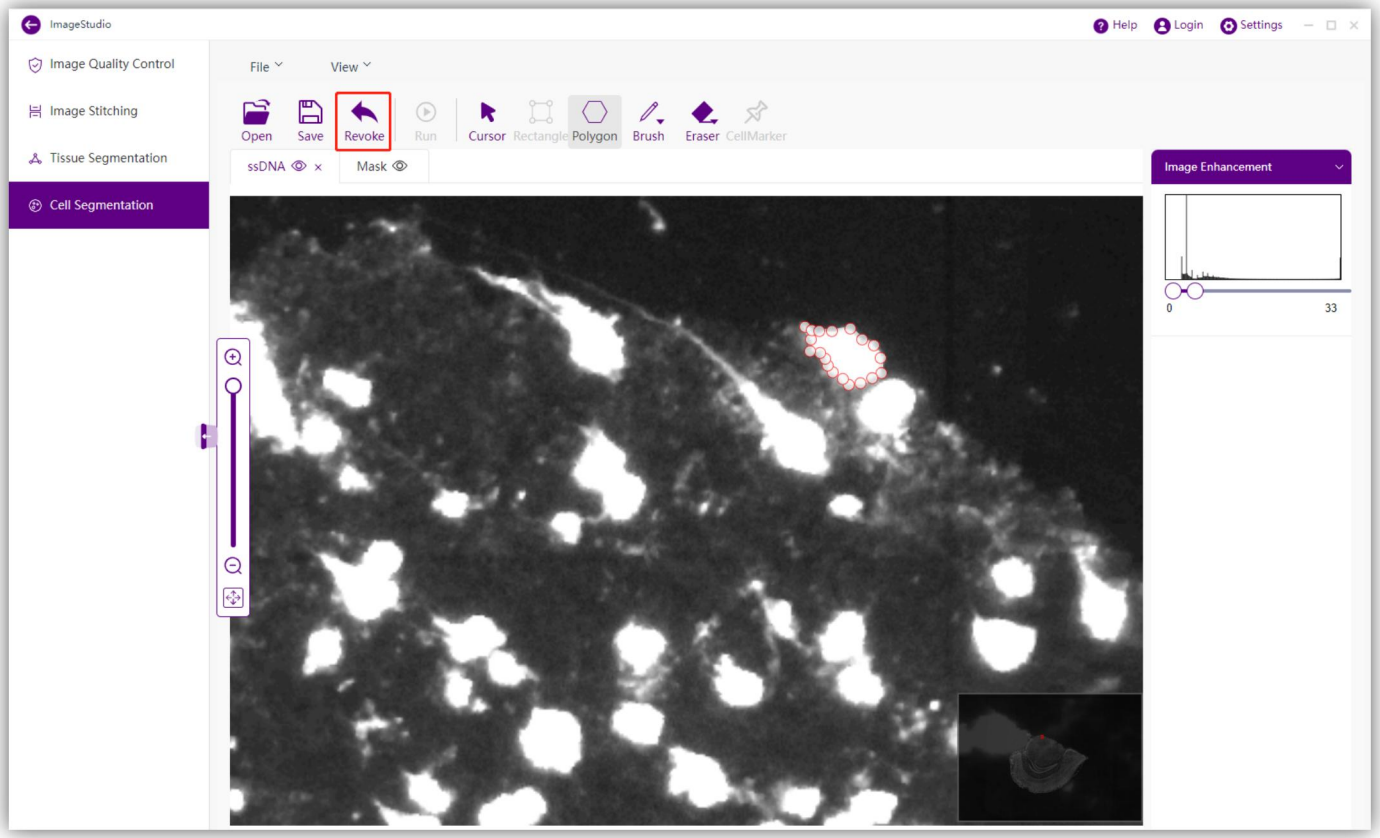
(3) Press and hold Enter on the keyboard to stop adding more points and enter polygon editing mode.

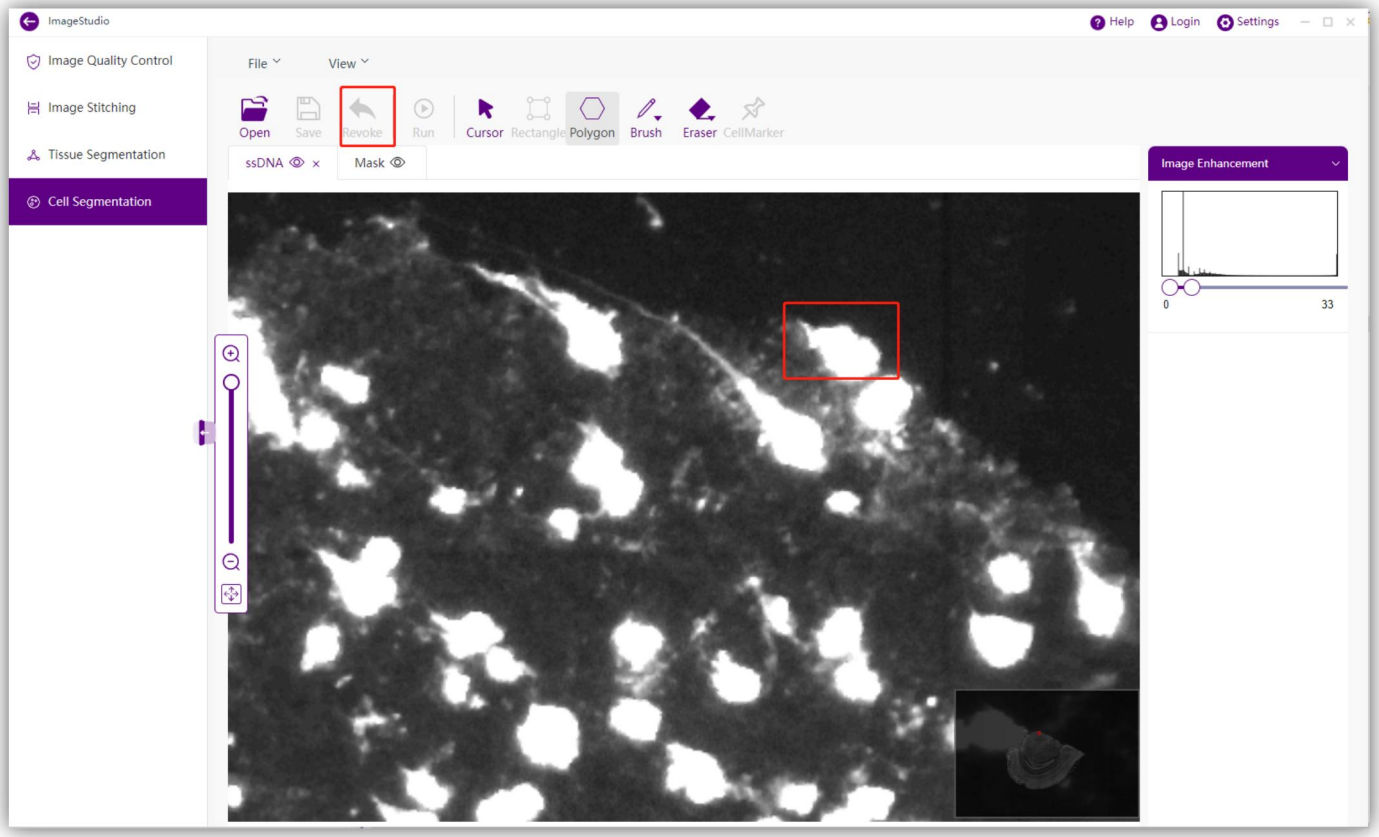


(4) Click on the points on the enclosed polygon trace then left-click and hold to drag and reposition the shape of the polygon.

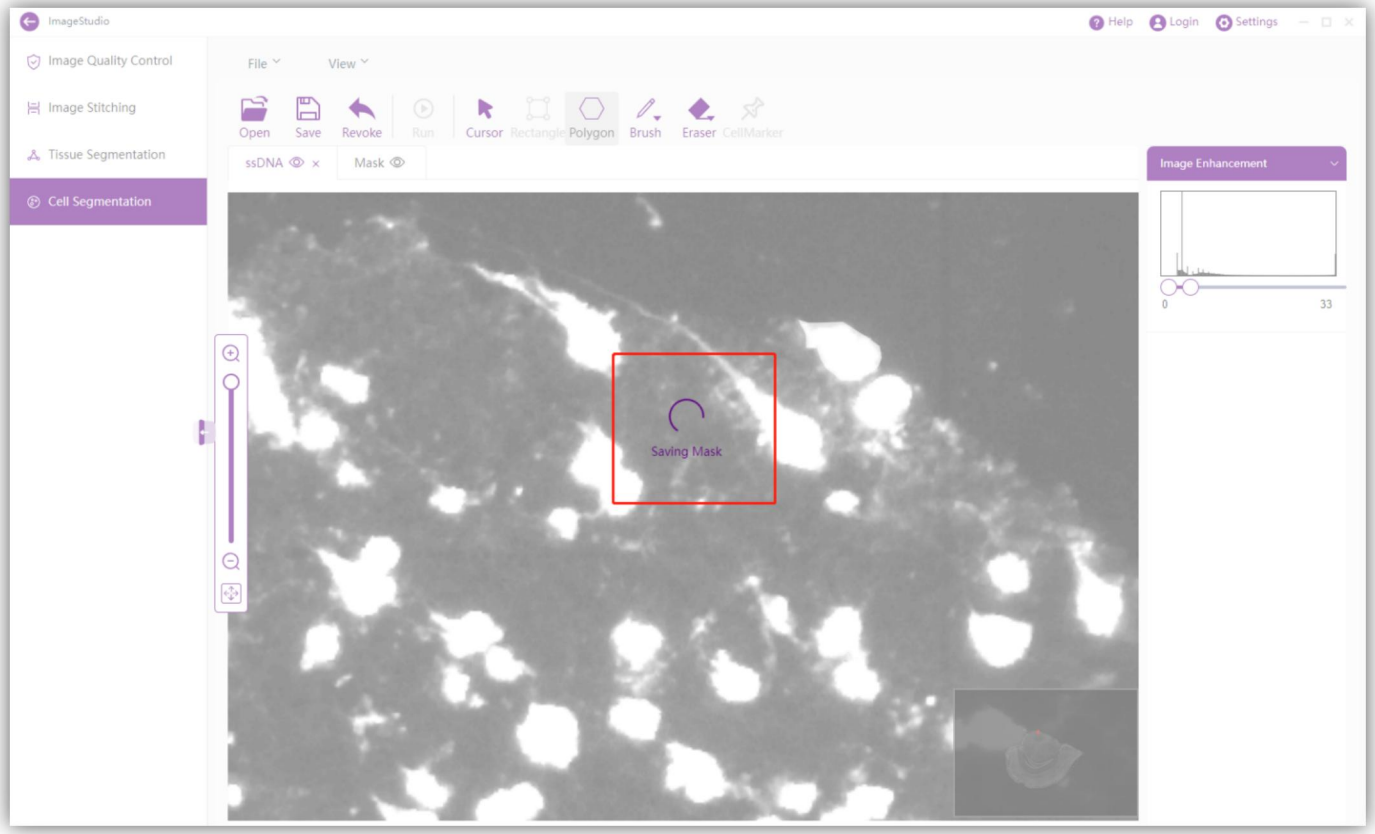


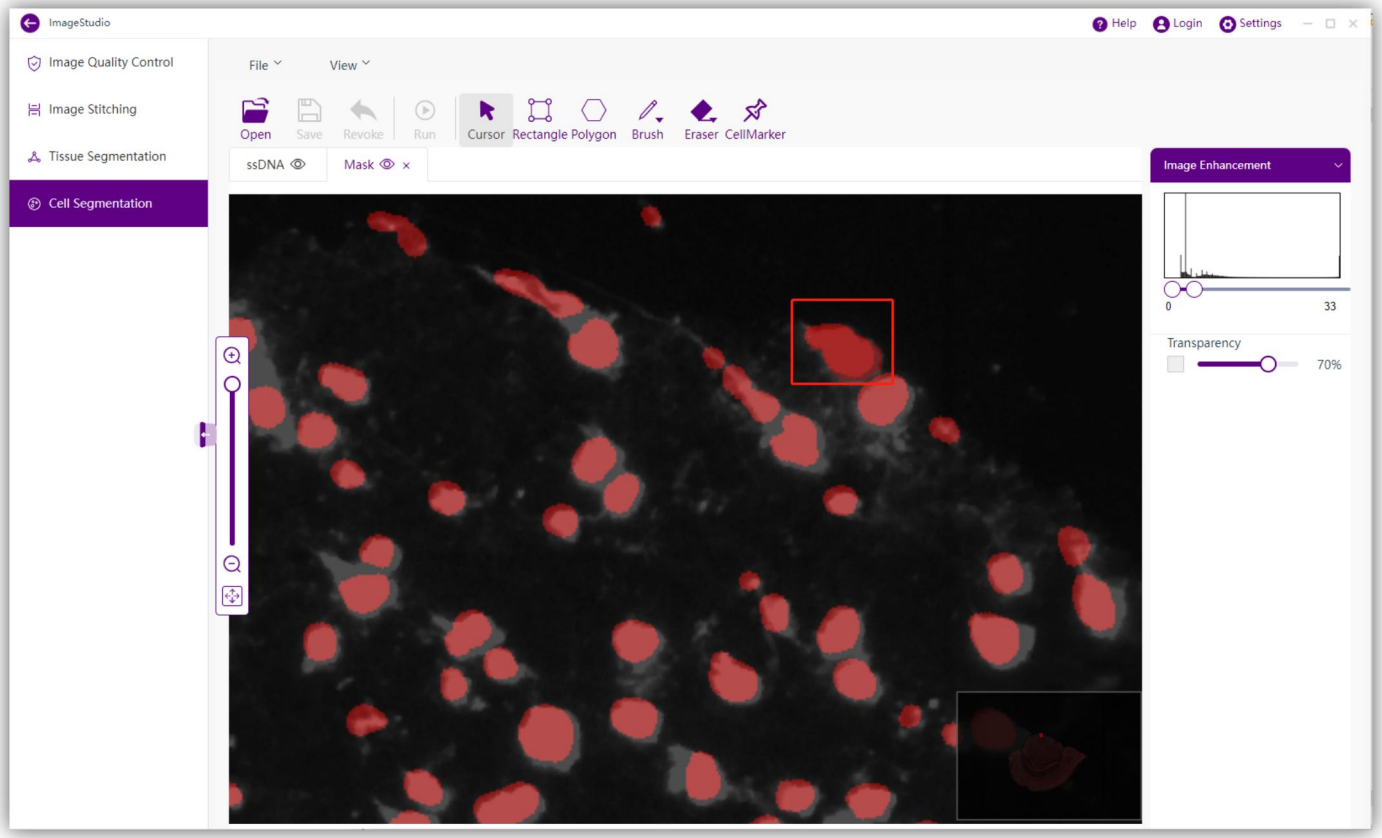
(5)Undo the point editing action by clicking 'Revoke' before saving.


ImageStudio
Help
Login
Settings
Image Quality Control
Image Stitching
Tissue Segmentation
Cell Segmentation
File
View
Open
Save
Revoke
Run
Cursor
Rectangle
Polygon
Brush
Eraser
CellMarker
ssDNA
Mask
Image Enhancement
0
33



(6)Click 'Save' after editing to write the new cell Mask file into the IPR file.


ImageStudio
Help
Login
Settings
Image Quality Control
Image Stitching
Tissue Segmentation
Cell Segmentation
File
View
Open
Save
Revoke
Run
Cursor
Rectangle
Polygon
Brush
Eraser
CellMarker
ssDNA
Mask
Image Enhancement
0
33
Saving Mask

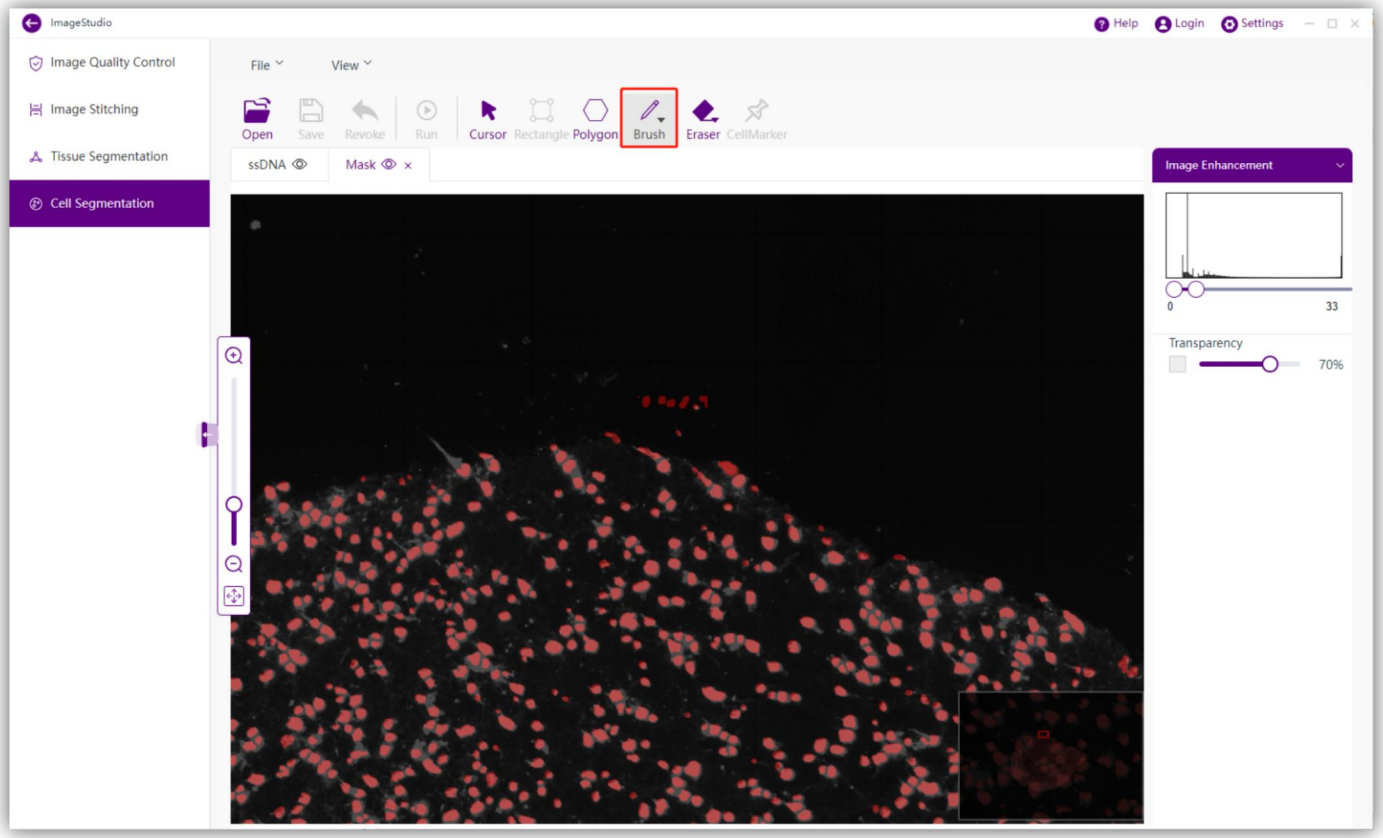


(7) Click 'Cursor' to exit the polygon editing mode.

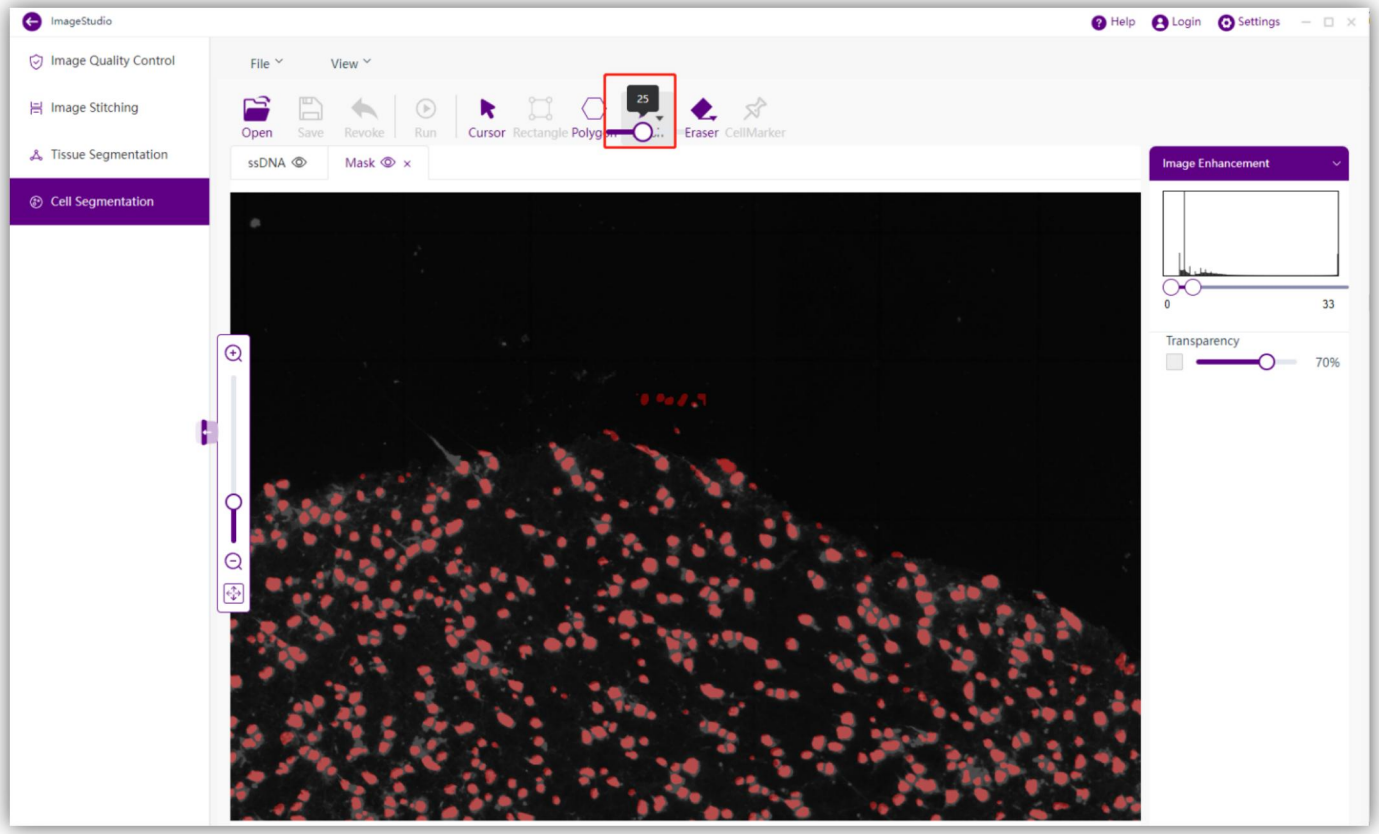
4.5.4 Brush

This function mainly operates on the Mask image for modifying algorithm generated cell Mask file according to the manually drawn cellMask using a brush tool.

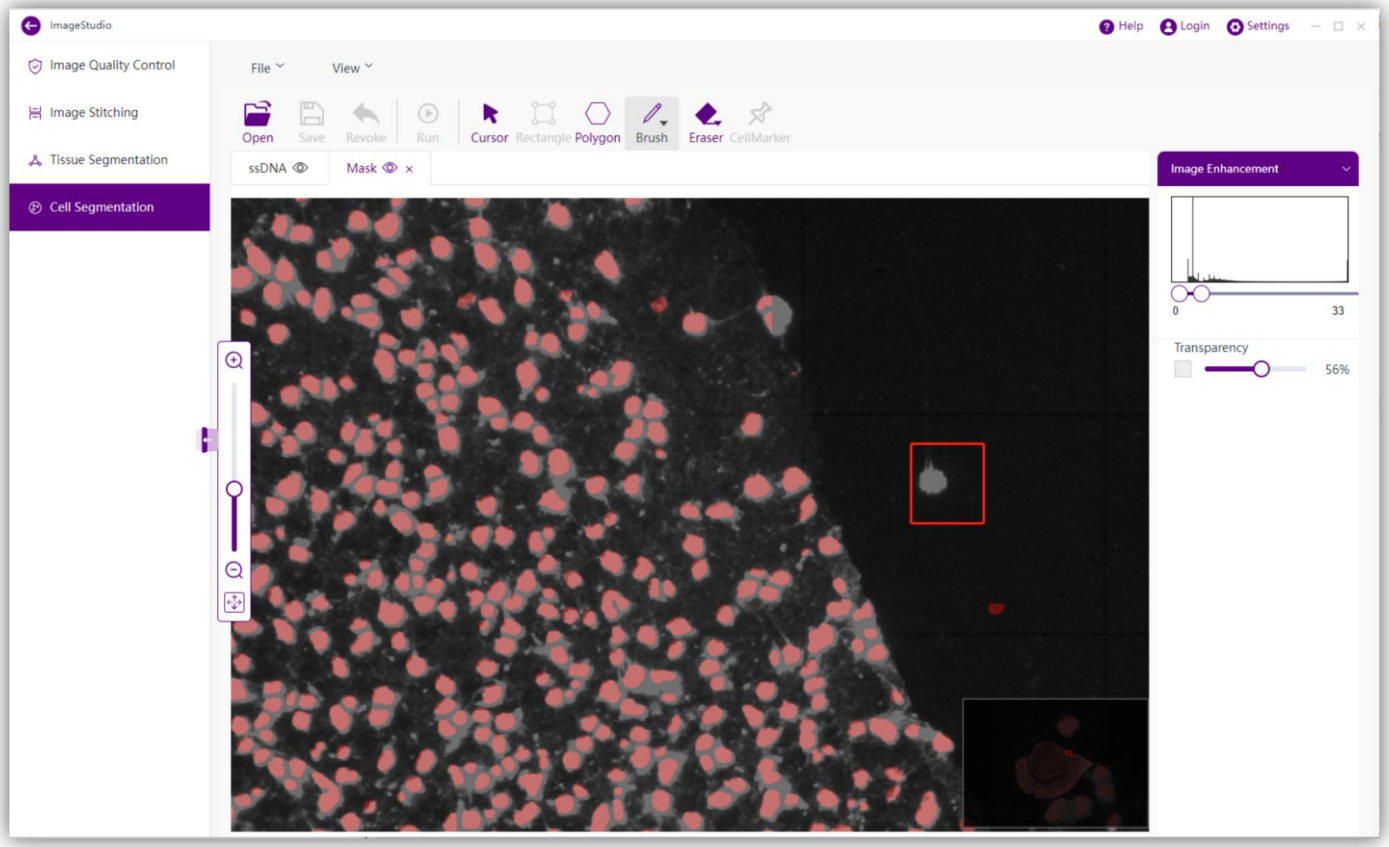
(1)Click 'Brush' in the Mask image tab.



(2)Click on the triangle icon next to 'Brush' to adjust the size of the brush. Range: [0, 50], unit: pixel.

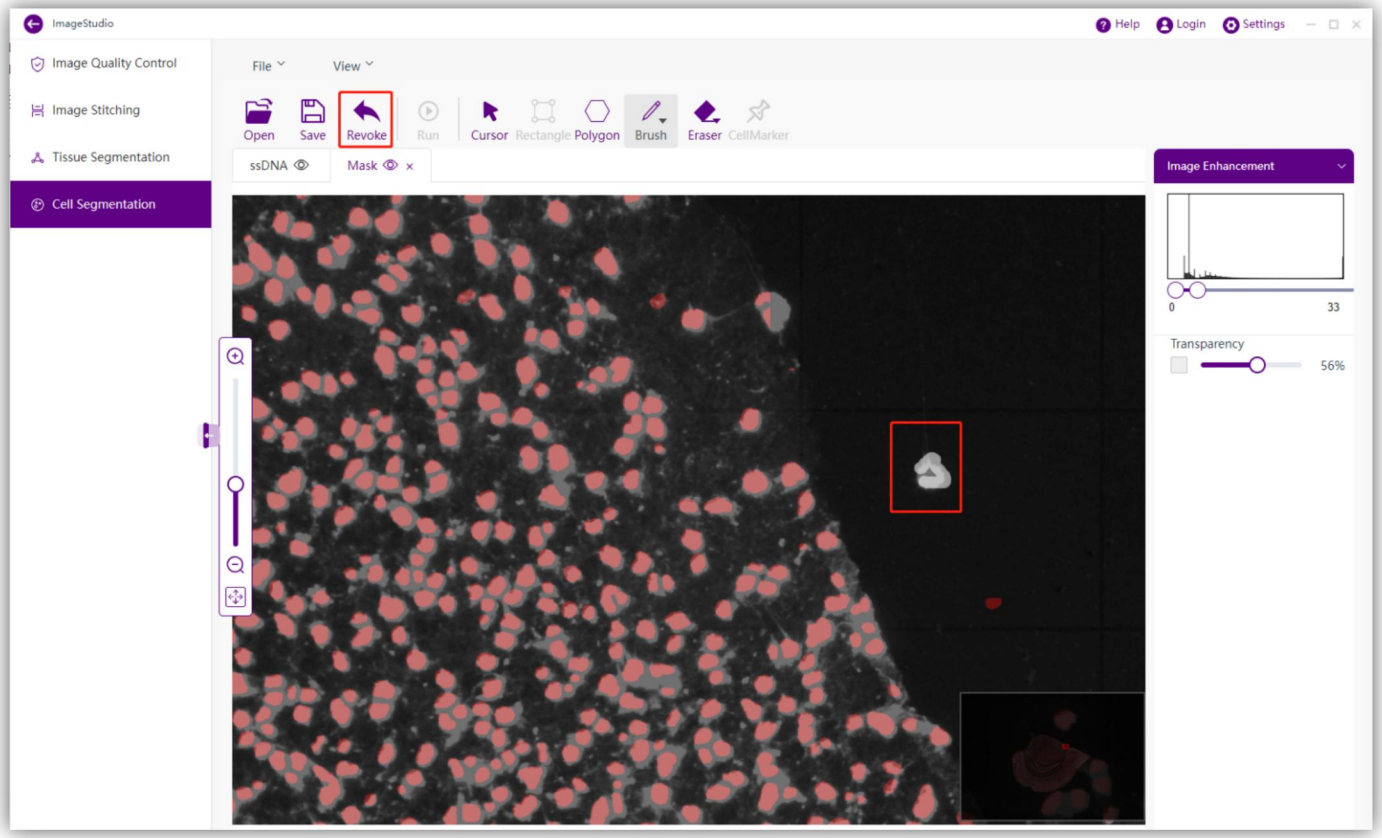


(3) Adjust 'Transparency' to get a clearer view of the cell contour. Left-click and hold to draw on the cell Mask outline.

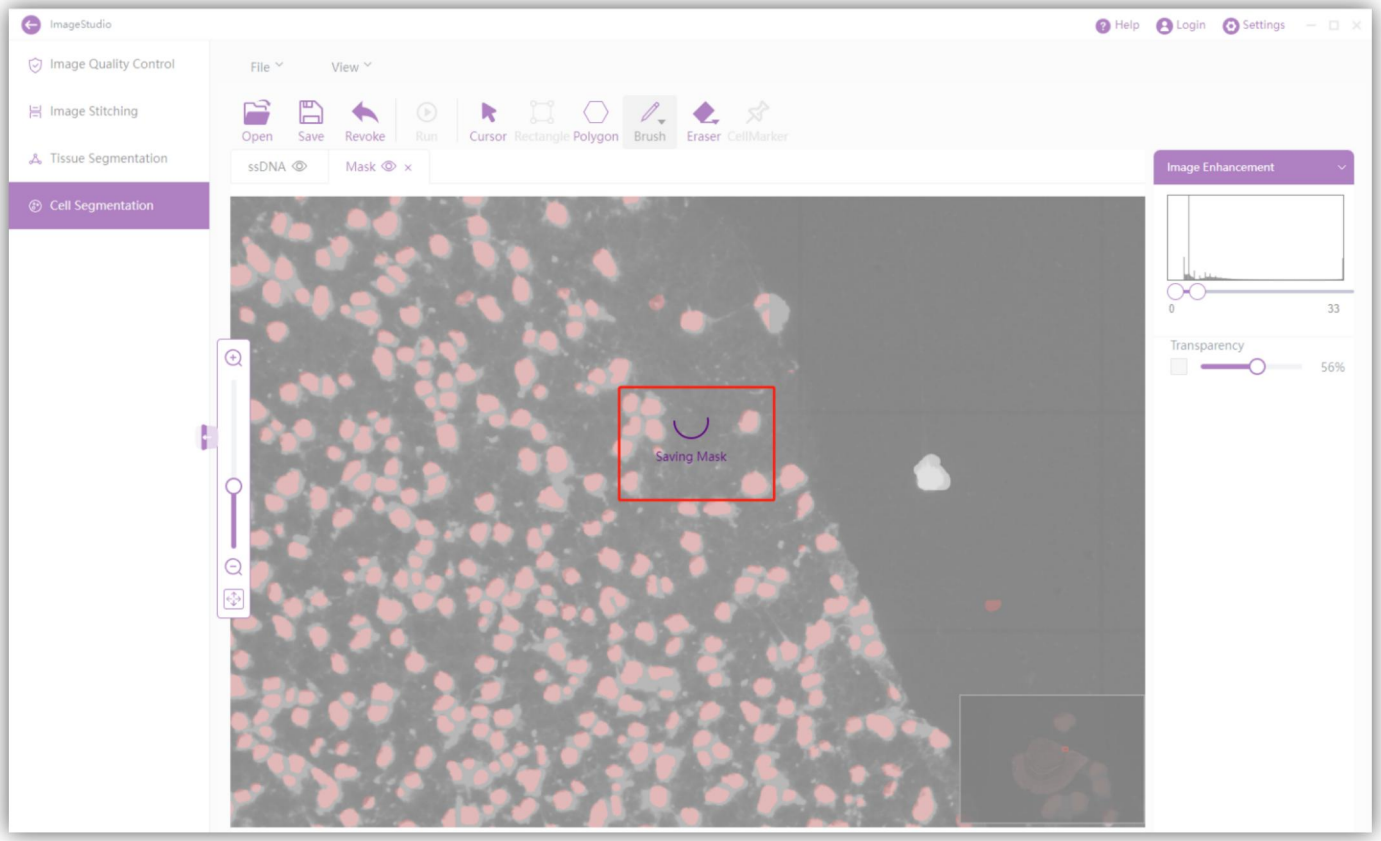


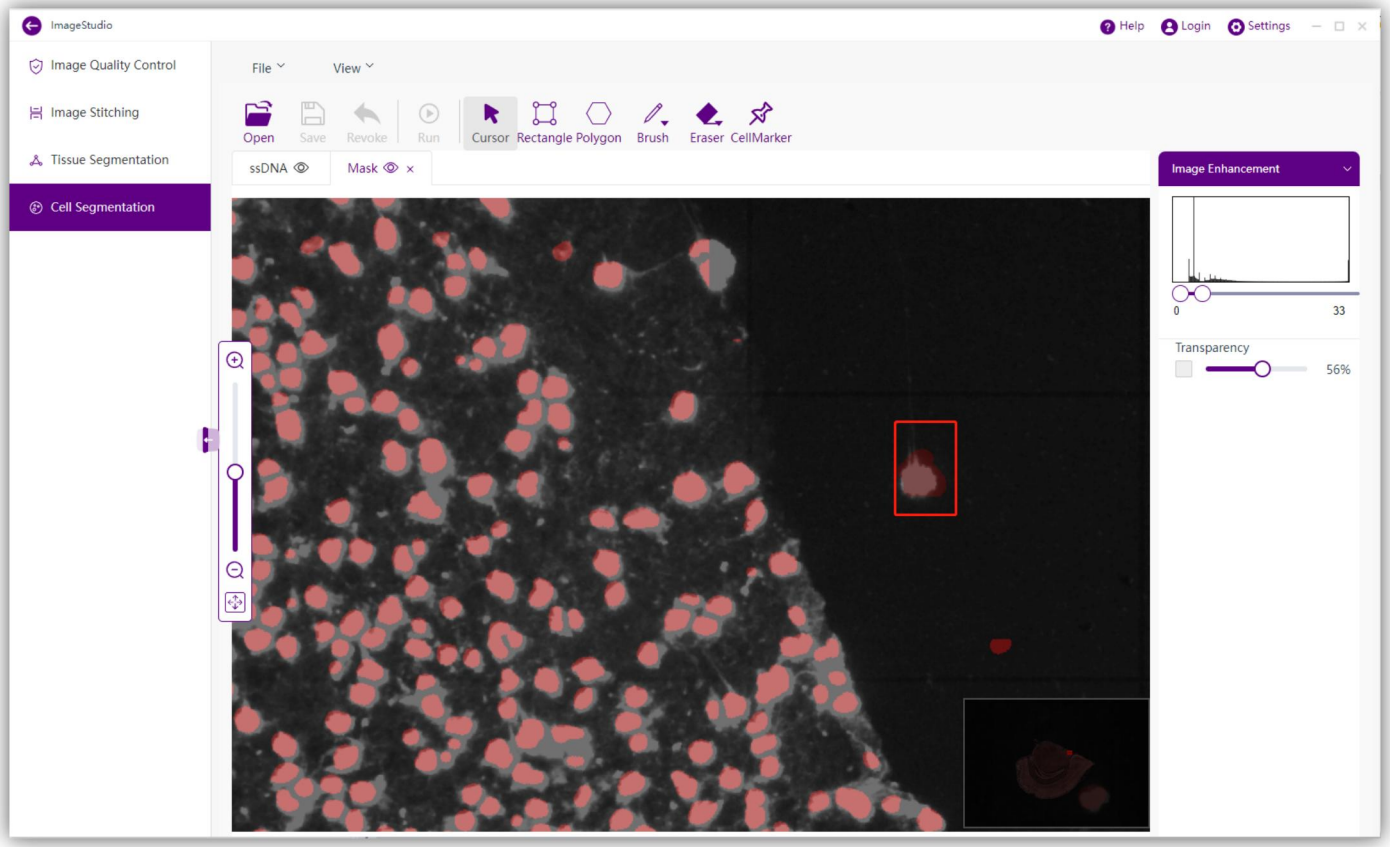
⚠Left-click within the local view window to move the field of view.

(4)If mistakes were made, click 'Revoke' before saving to revoke current action.



(5)After drawing is completed, click 'Save' to update the cell Mask image and write it into IPR file.


ImageStudio
Help
Login
Settings
Image Quality Control
Image Stitching
Tissue Segmentation
Cell Segmentation
File
View
Open
Save
Revoke
Run
Cursor
Rectangle
Polygon
Brush
Eraser
CellMarker
ssDNA
Mask
Saving Mask
Image Enhancement
0
33
Transparency
56%



Note: Updated cell mask will shown in red.

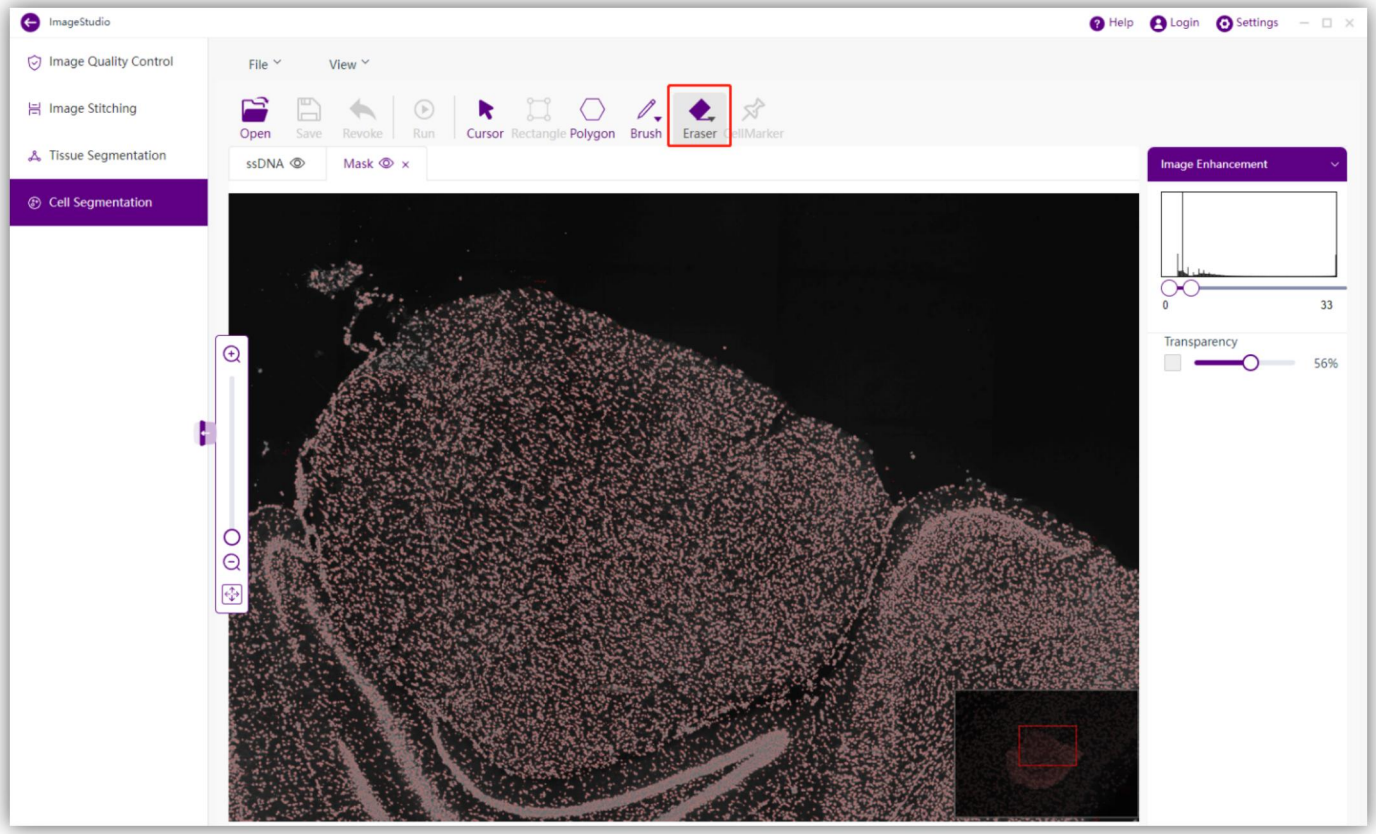
(6) Click 'Cursor' to exit the brush editing mode.

4.5.5 Eraser

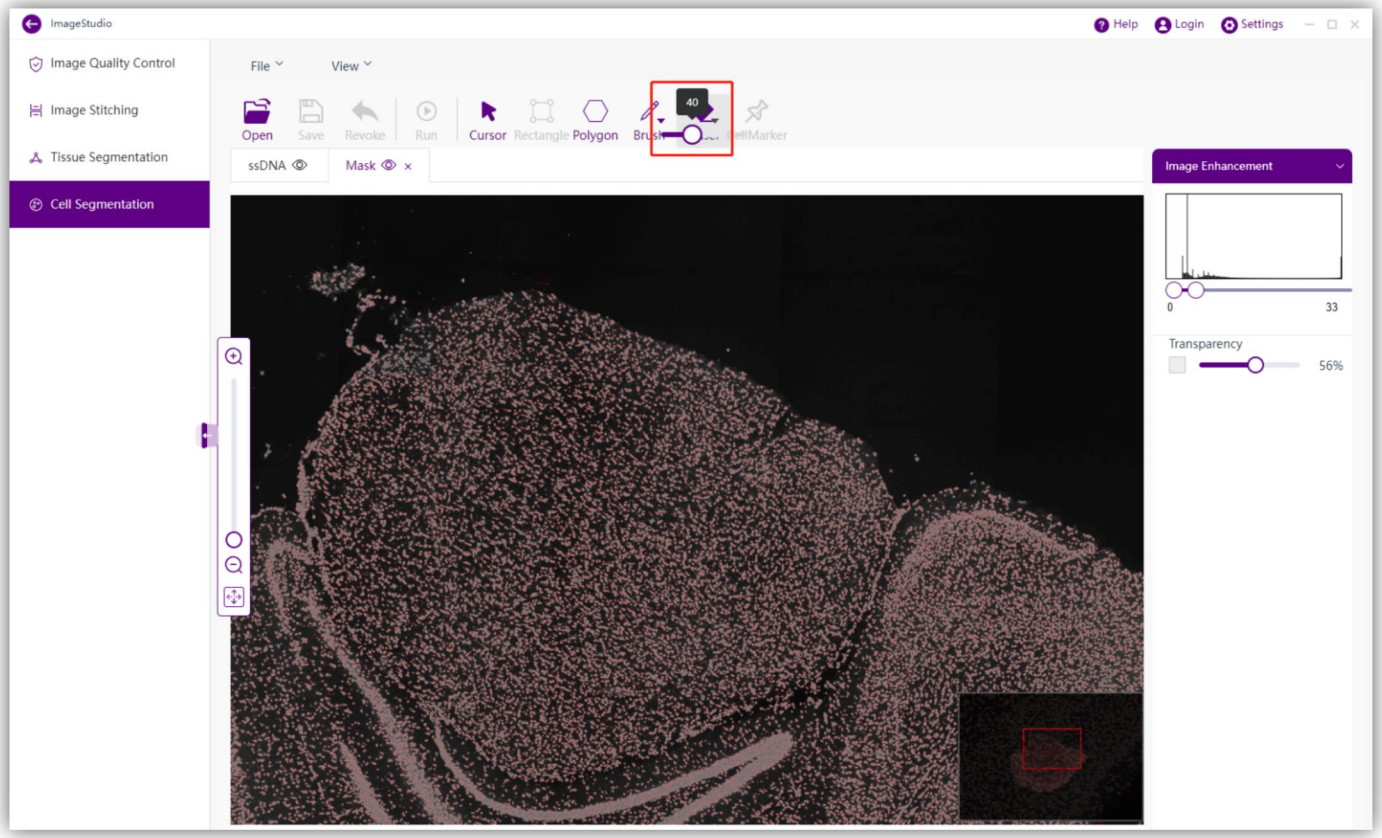
This function mainly operates on the Mask image for modifying algorithm generated cell Mask file

with the eraser tool to manually erase unwanted cell Mask region.

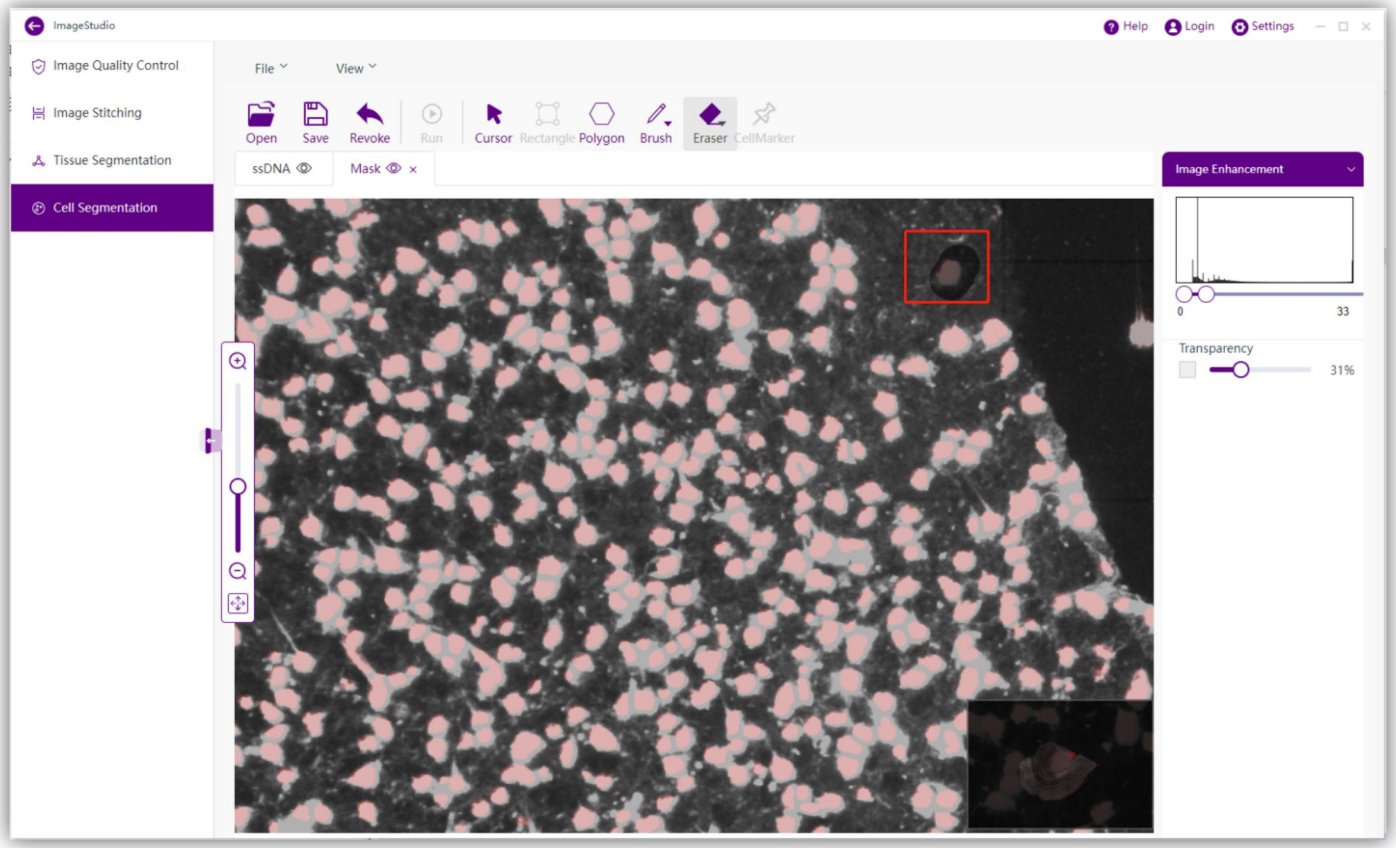
(1)Click 'Eraser' in the Mask image tab.

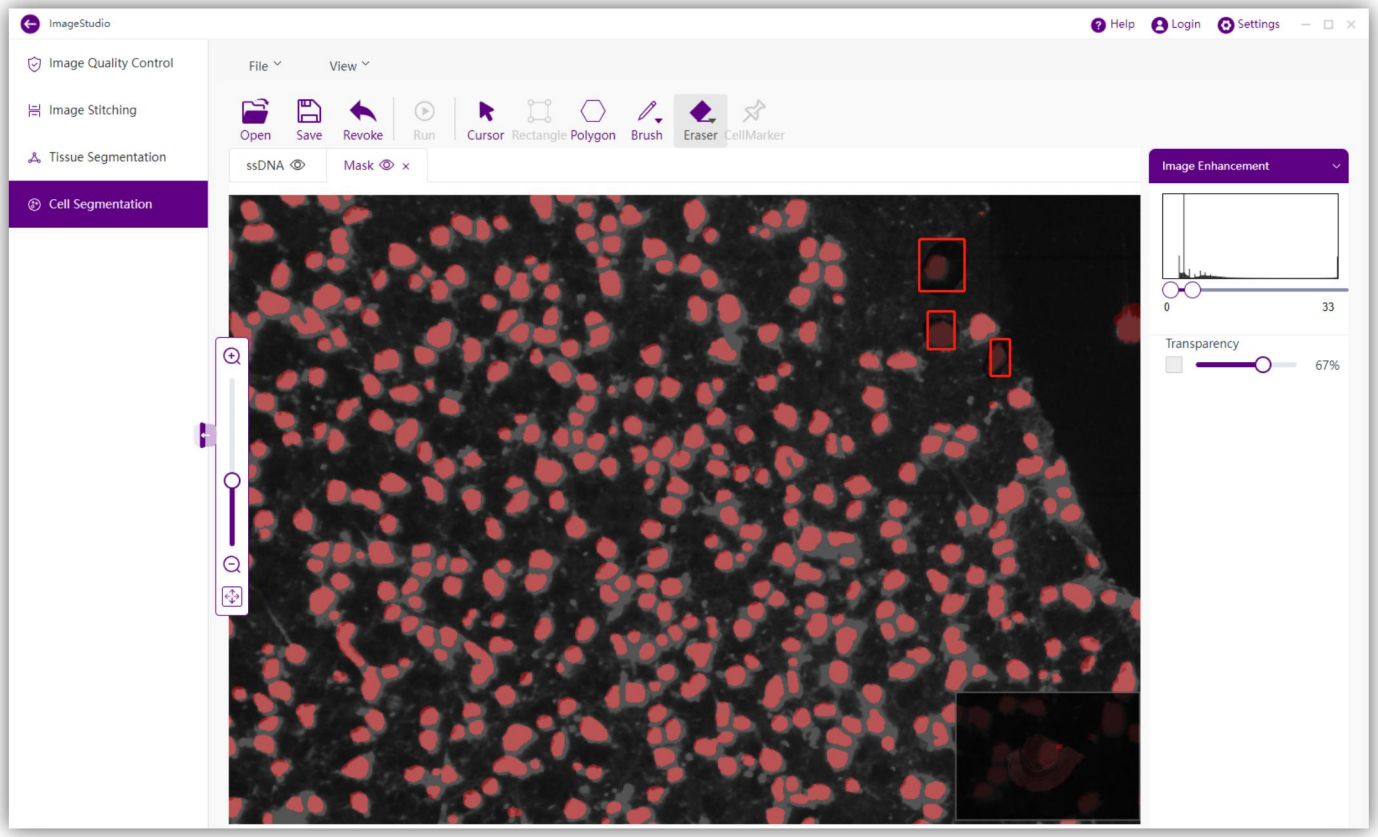


(2)Click on the triangle icon next to 'Eraser' to adjust the size of the eraser. Range: [0, 100], unit: pixel.



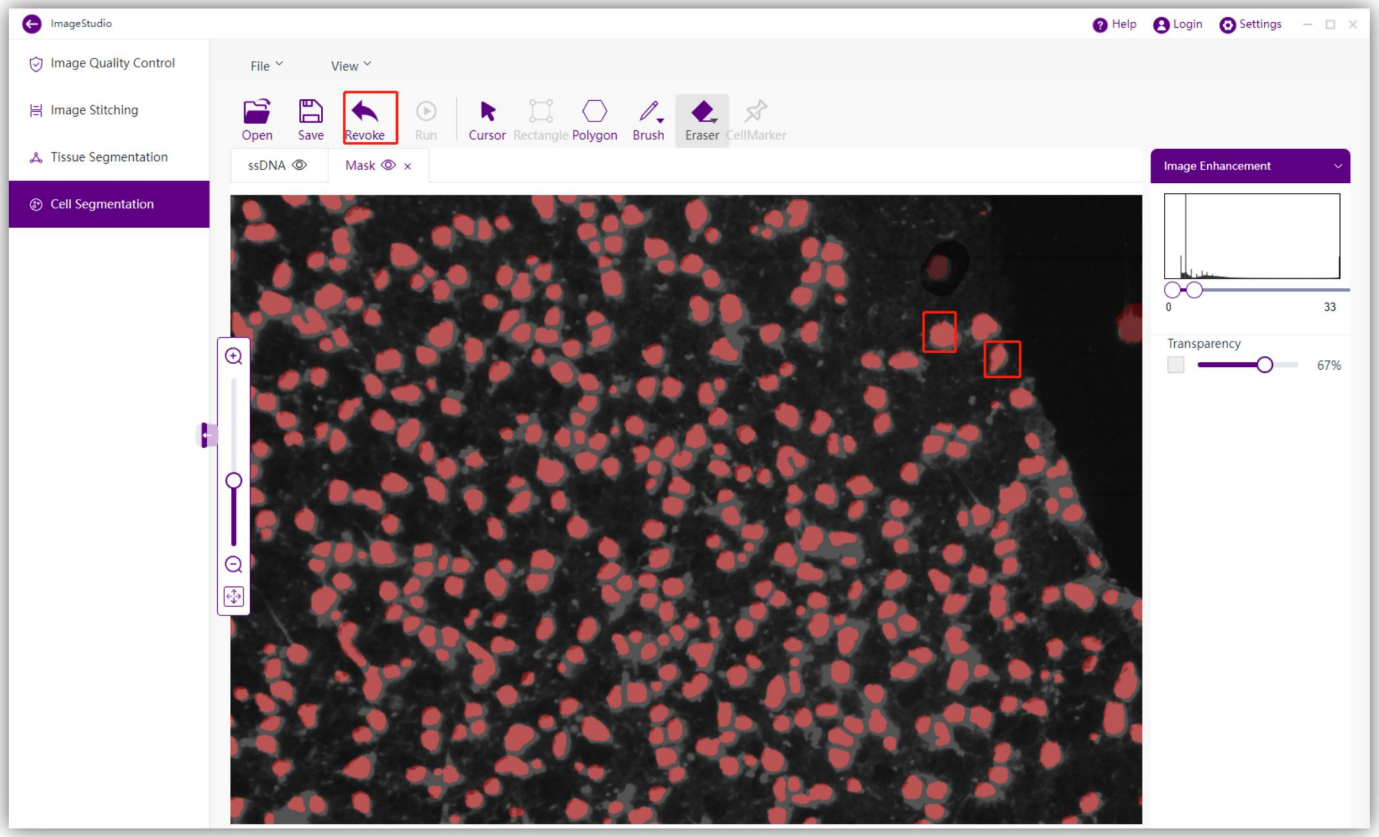
(3) Adjust 'Transparency' to get a clearer view of the cell contour. Left-click and hold to erase unwanted cell Mask region.


ImageStudio
Help
Login
Settings
Image Quality Control
Image Stitching
Tissue Segmentation
Cell Segmentation
File
View
Open
Save
Revoke
Run
Cursor
Rectangle
Polygon
Brush
Eraser
CellMarker
ssDNA
Mask
Image Enhancement
0
33
Transparency
31%

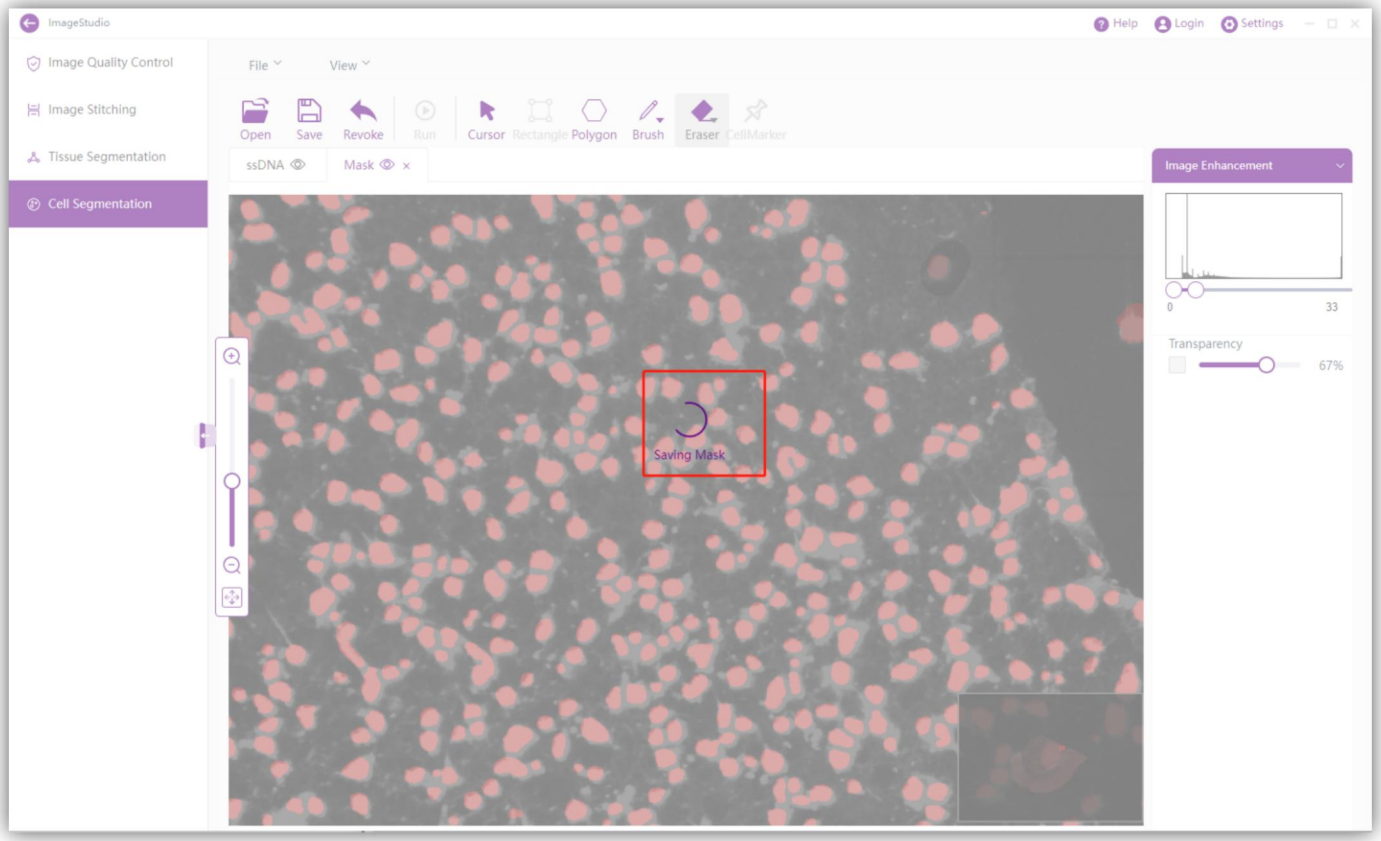


⚠Left-click within the local view window to move the field of view.

(4)If mistakes were made, click 'Revoke' before saving to revoke current action.



(5) After erasing is completed, click 'Save' to update the tissue Mask image and write it into the IPR file.

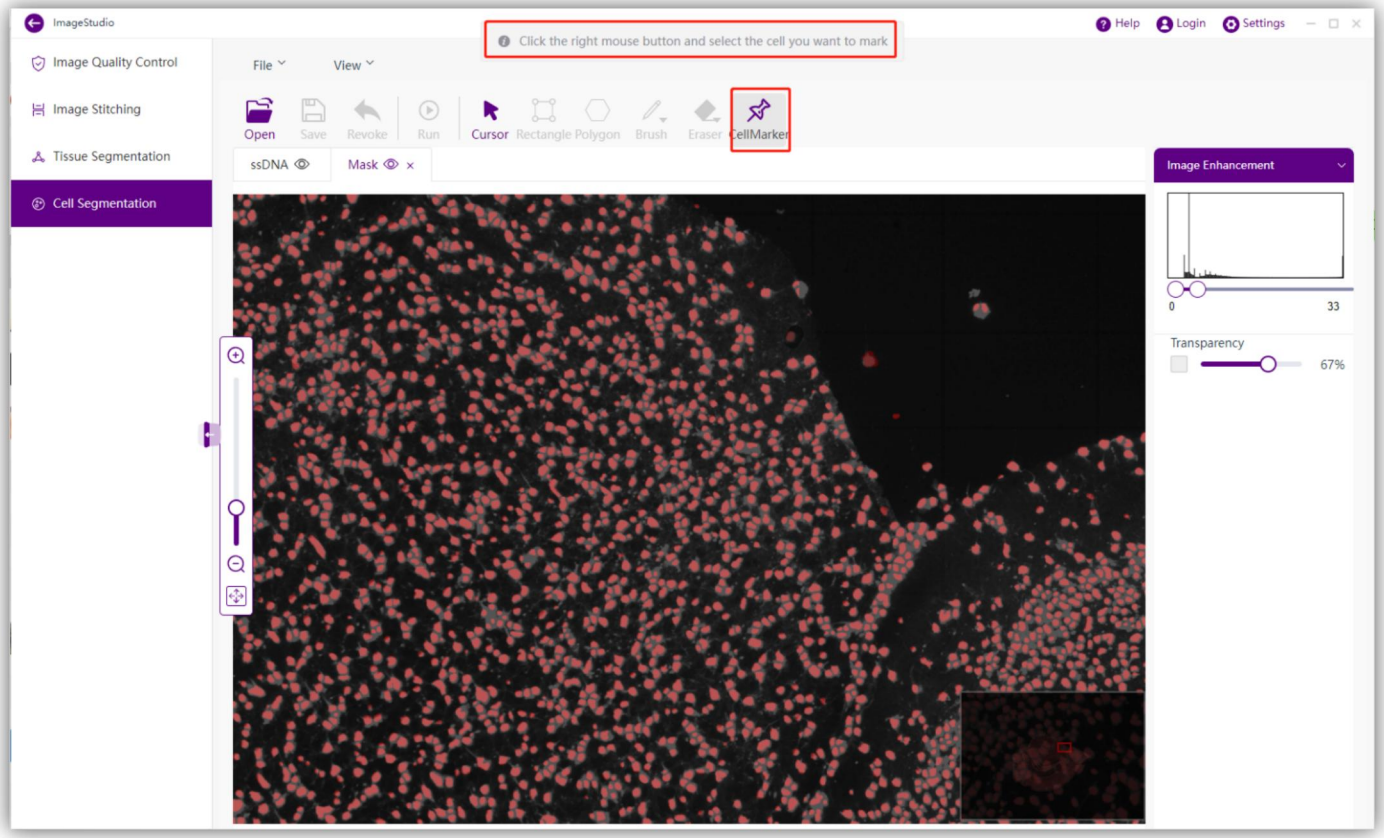


(6)Click 'Cursor' to exit the eraser editing mode.

4.5.6 CellMarker

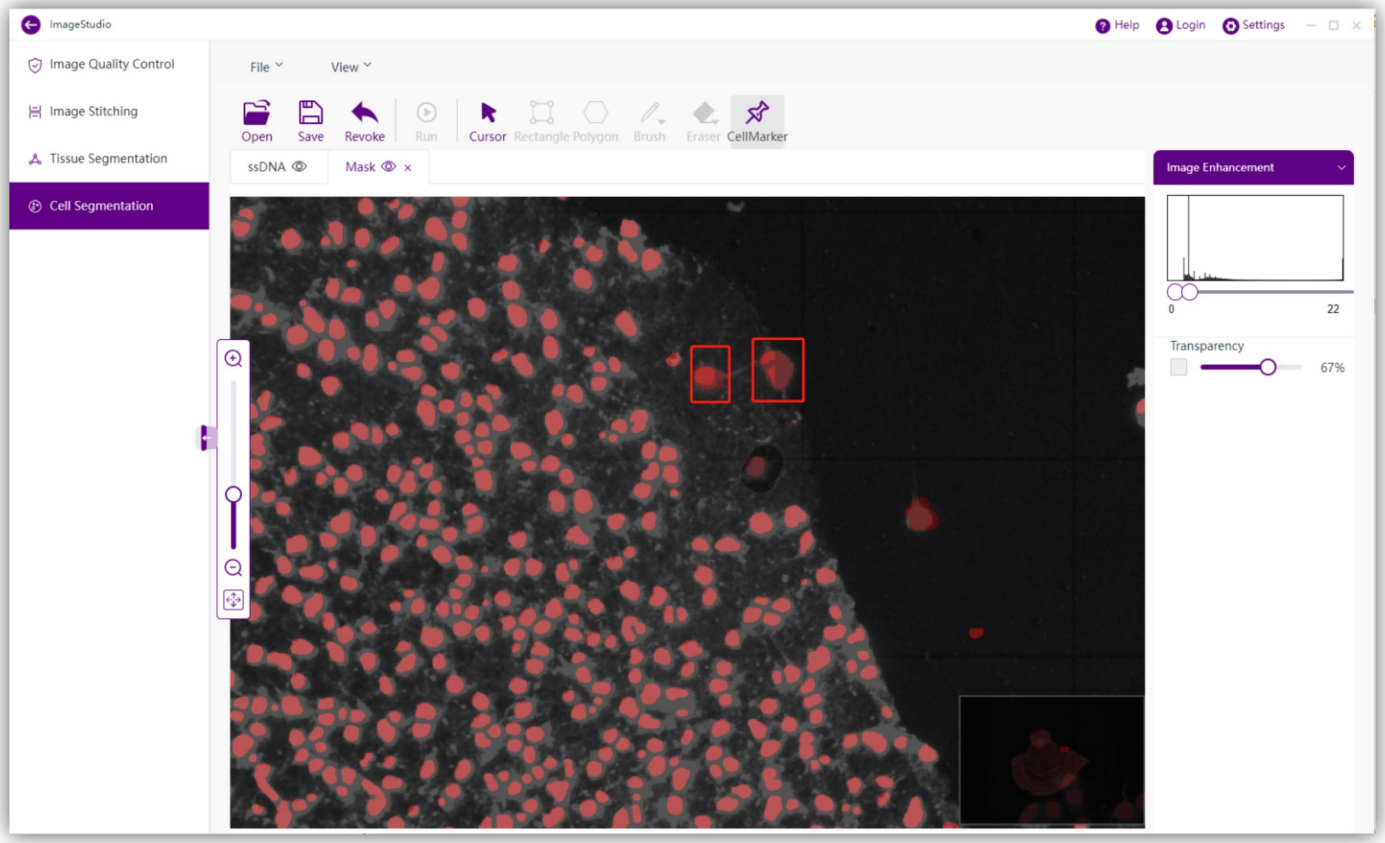
This function mainly operates on the Mask image for rapidly identifying cells in sparse tissue area through clicking on the cell region.

(1) Click 'Cell Marker' in the Mask image tab.

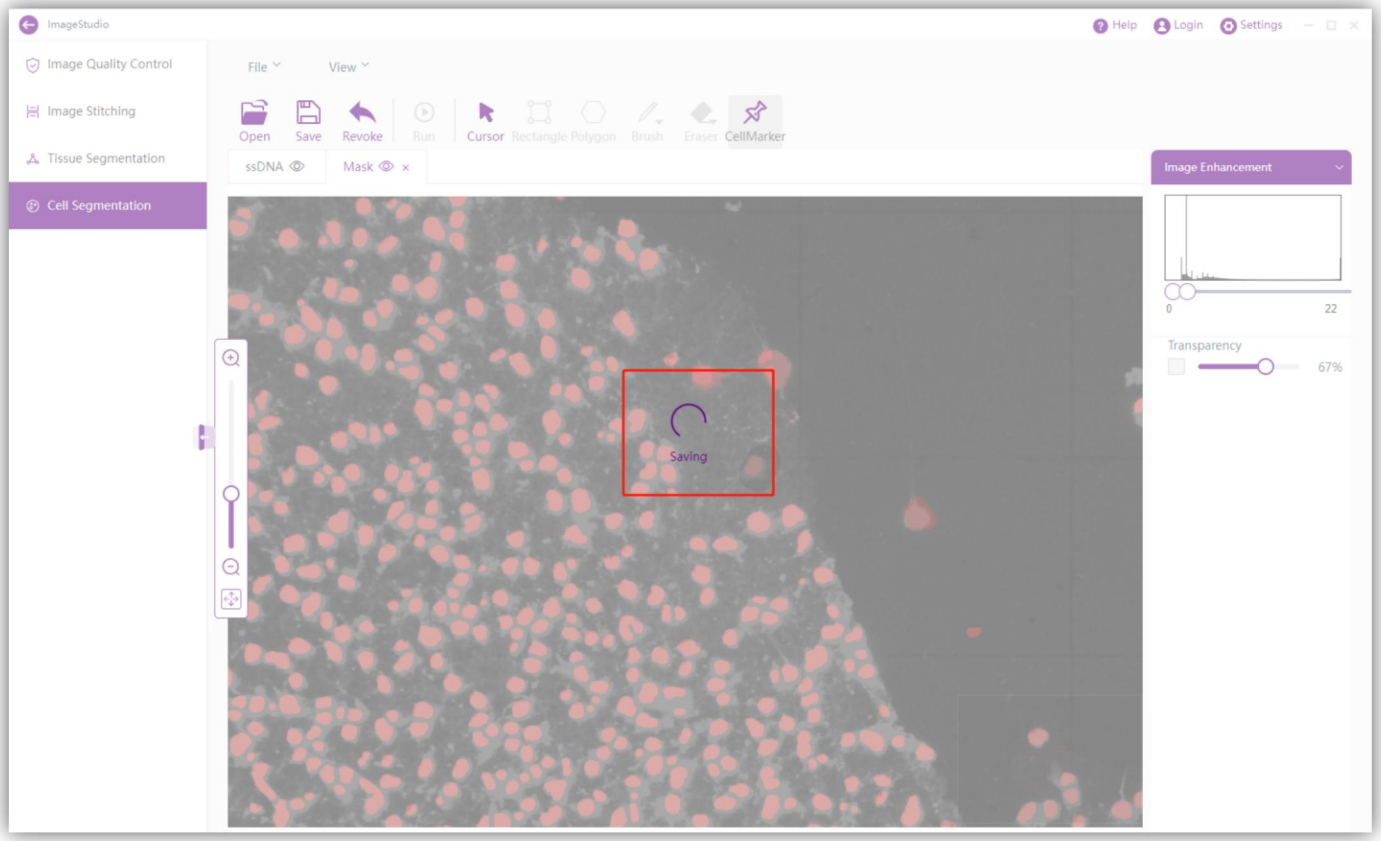


Note: Try to achieve a greater contrast between the tissue area and the background by adjusting image enhancement in the ssDNA tab before using the Cell Marker function.

(2) Right-click on the cell of interest to obtain the cell Mask. Multiple clicks are supported.



(3)Once completed, click 'Save' to update the cell Mask image and write it into the IPR file.



Note: Since it takes some time to save the files, we suggest marking down all the cells of interest before saving.

(4) Click 'Cursor' to exit the Cell Marker editing mode.

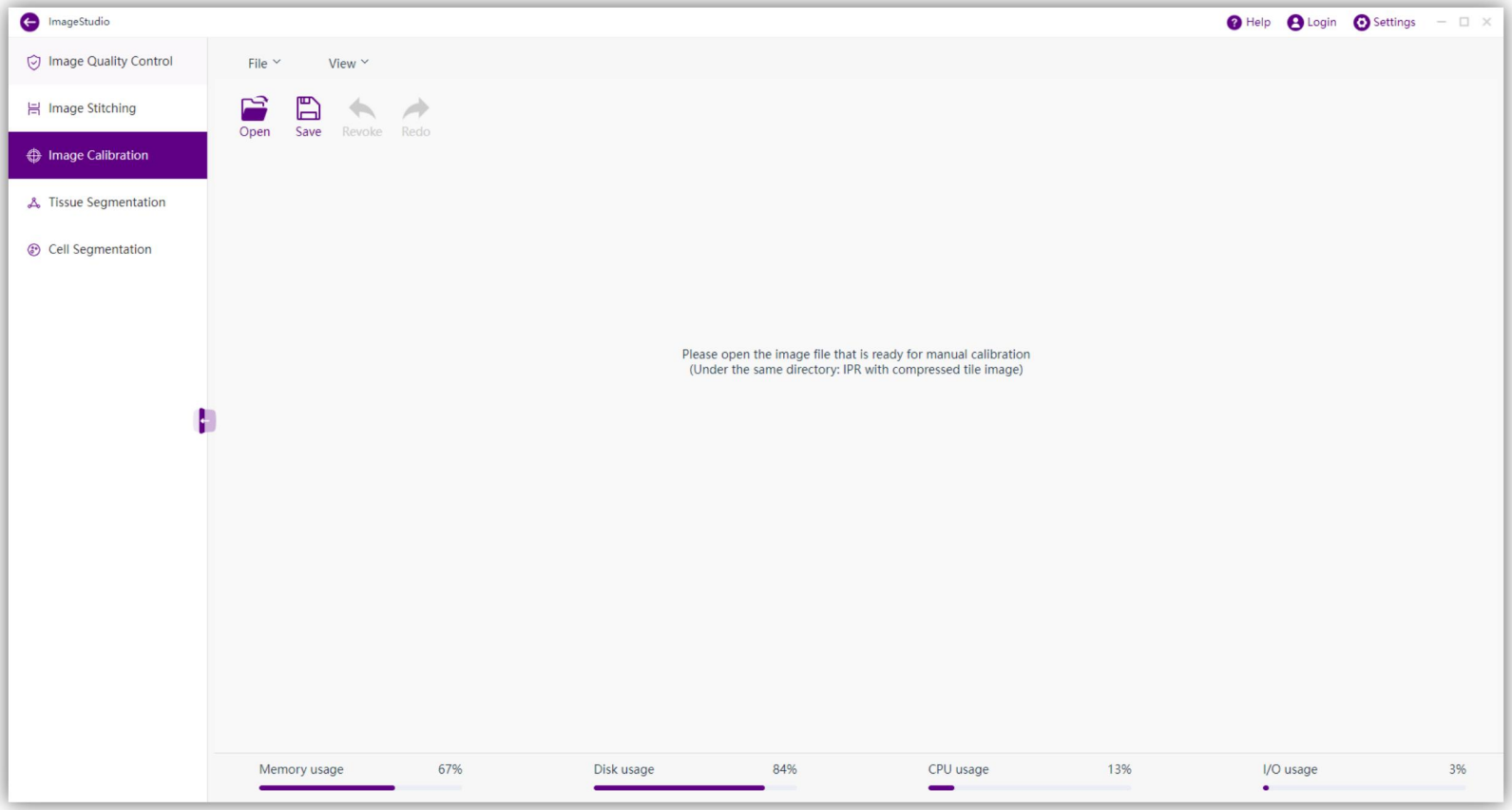
4.6 Image Calibration

The Image Calibration module is designed to align two images into one coordinate system based on their morphological features. The transforming options include pan moving in the horizontal or vertical direction, small angle rotation, scale, etc.

Note: The Image Calibration function in Version 2.1.x-3.0.x is only available for DAPI&mIF images. The input files should be DAPI & mIF images that have been compressed by the QC module.

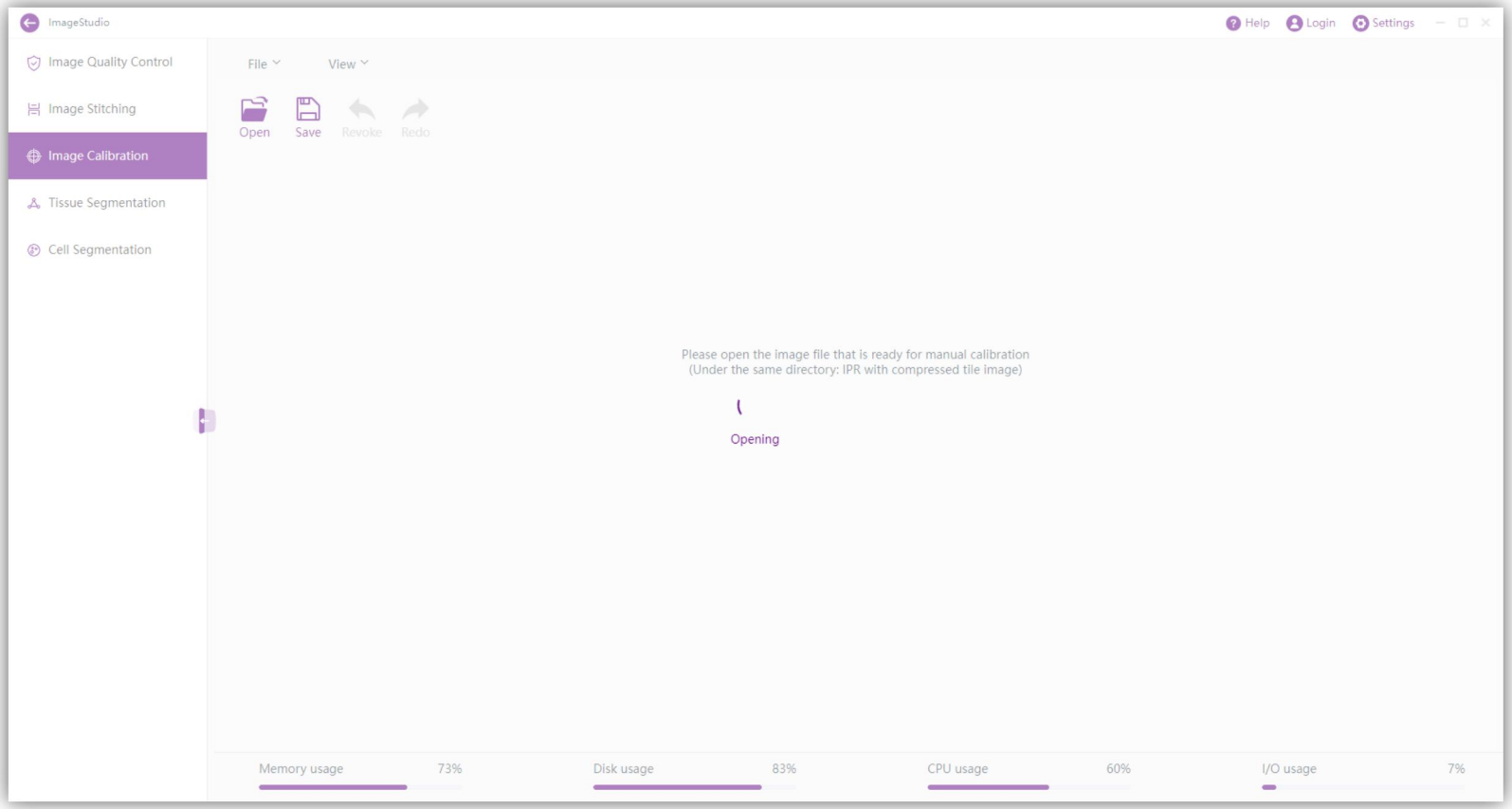
4.6.1 Basic Operation

(1)Initiate the image calibration module.

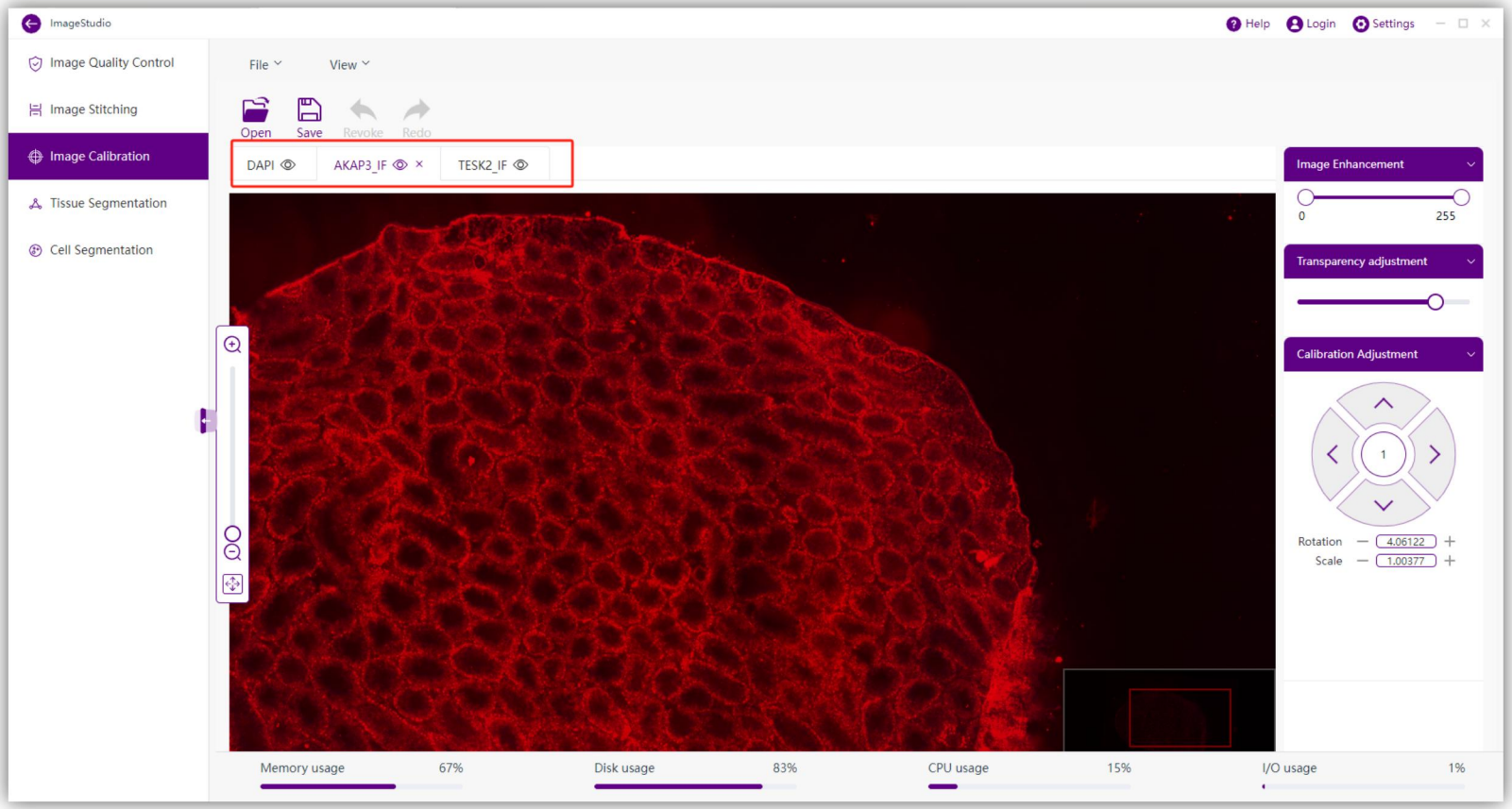


(2) Click 'Open' and select the image file. Output directory:

D:\ImageStudioWorkspace\Calibration

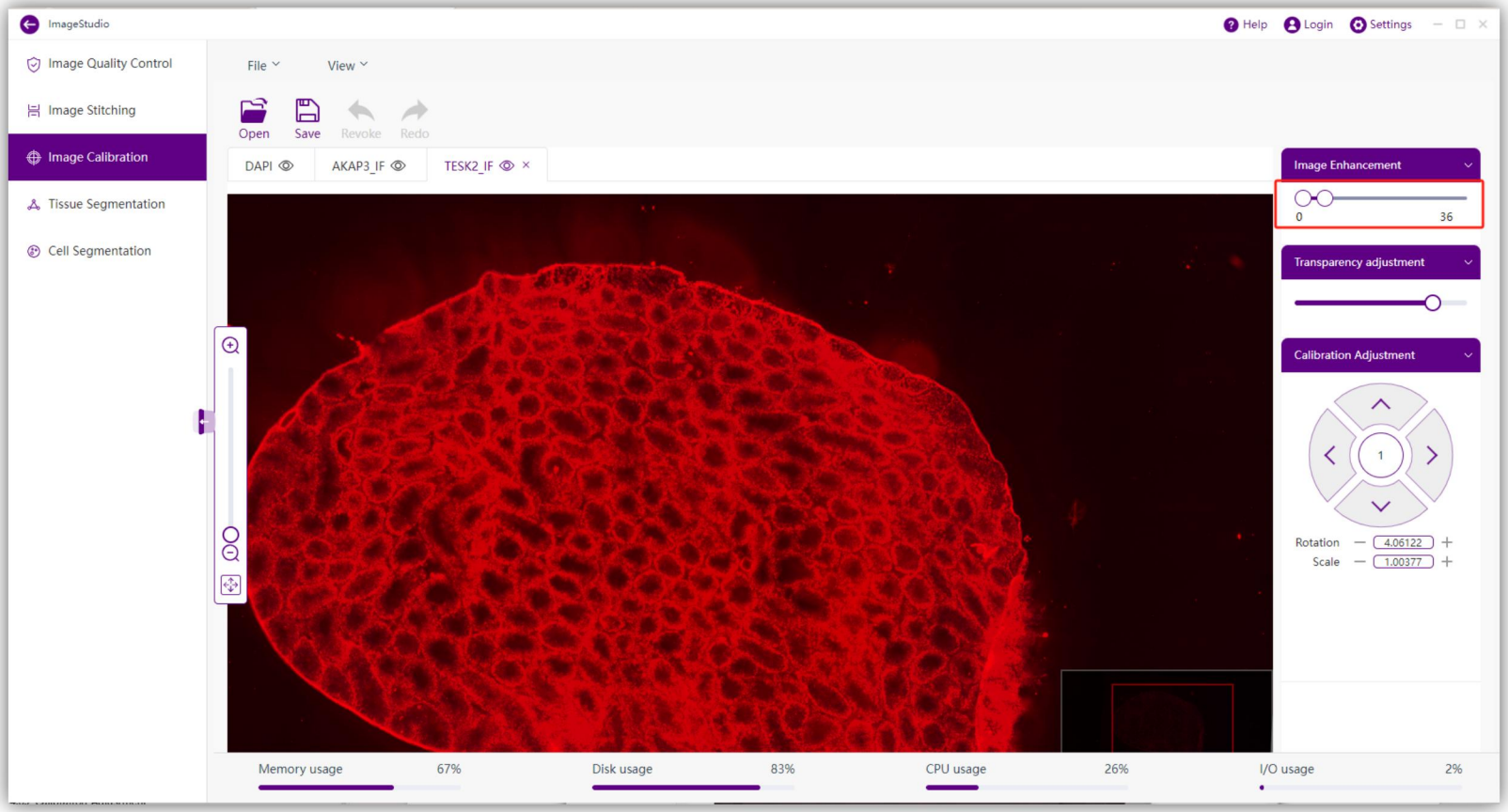


(3) The tabs will be opened and display the DAPI and protein image.

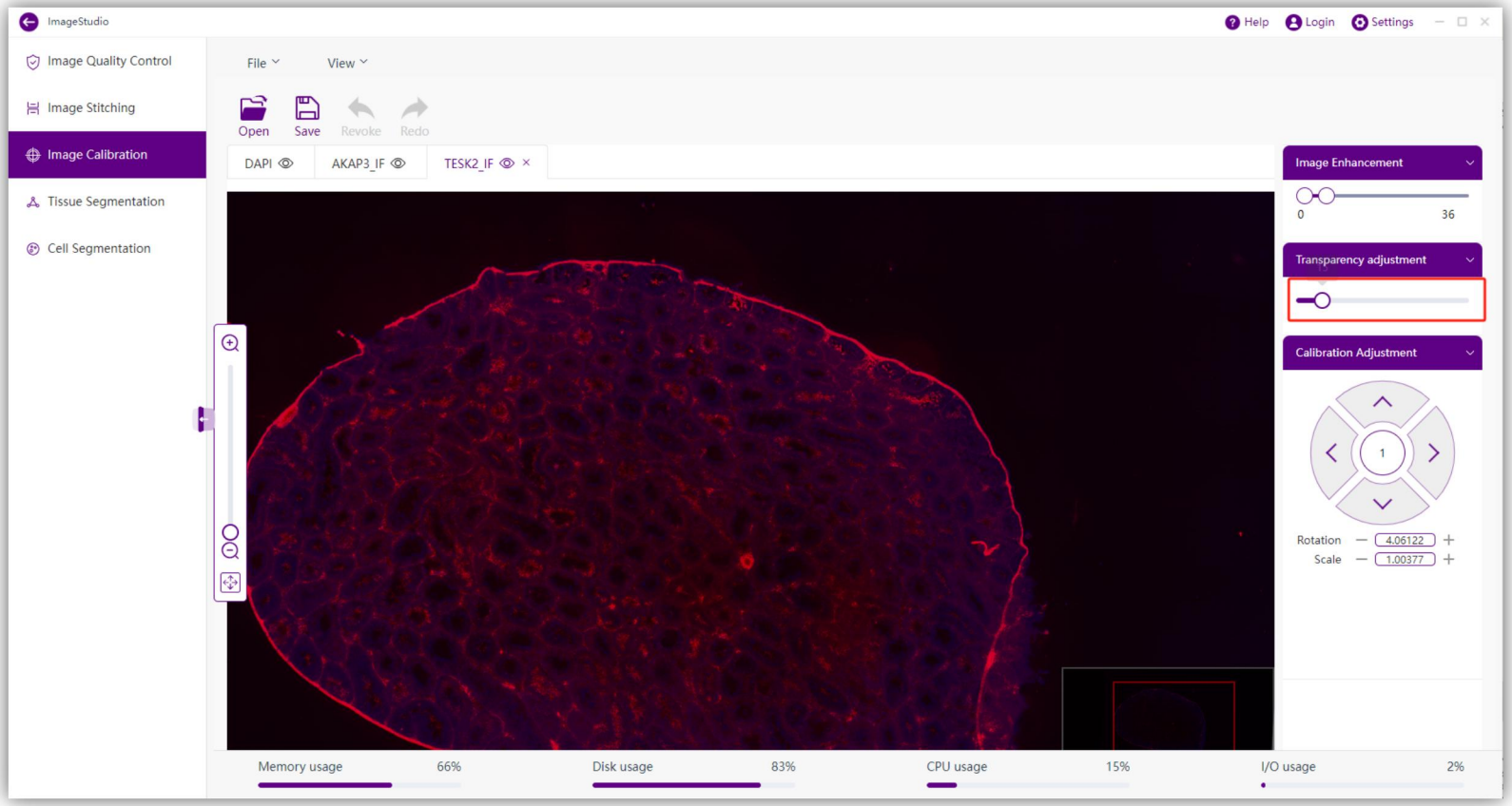


Note: Since generating the image pyramid is time-consuming, please only open one Stereo-seq Chip file at a time. One 1cm by 1cm Chip T will take about 3~5min to open.

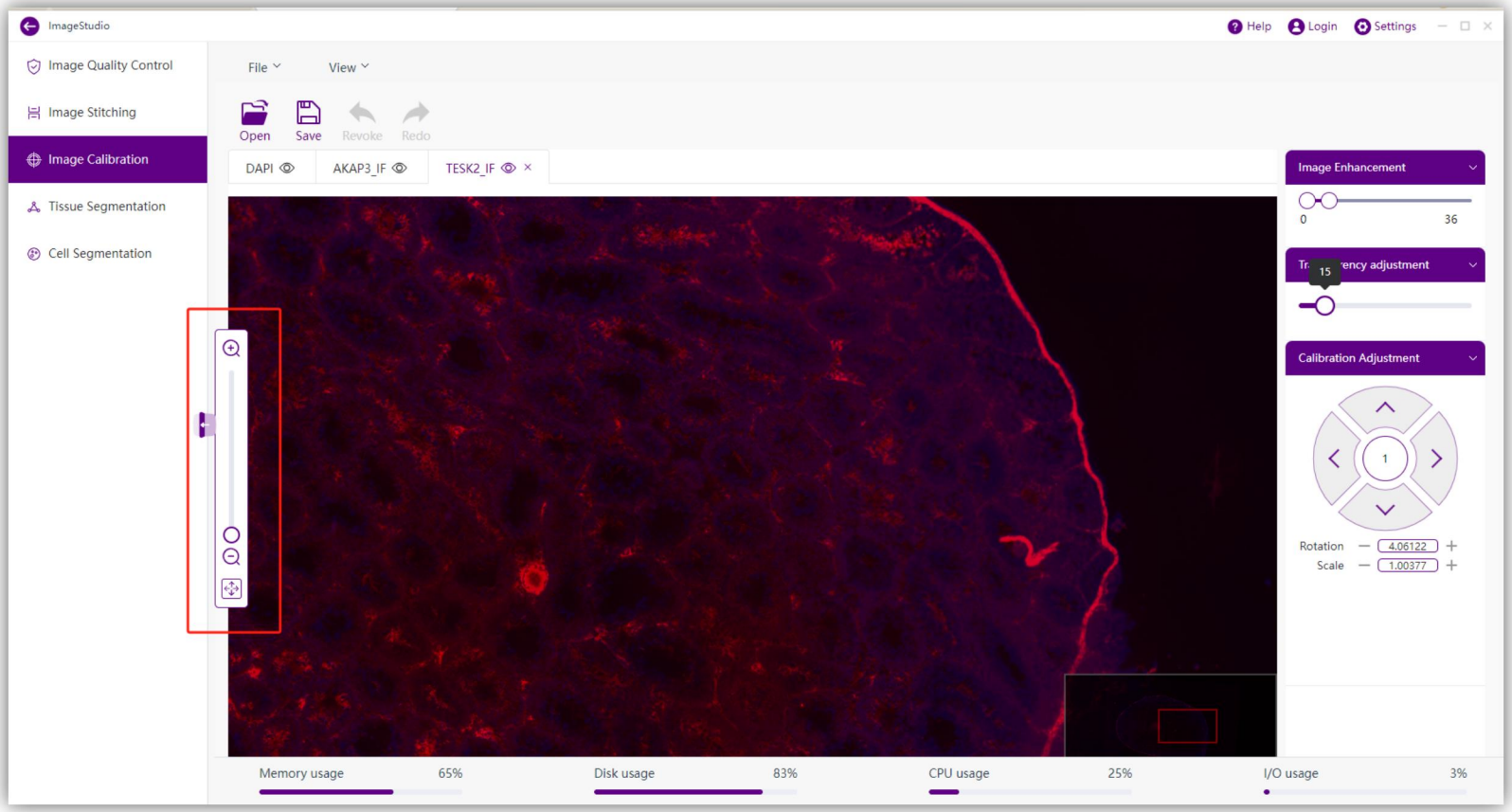
(4) Drag the 'Image Enhancement' slide bar under the image tab to better display the tissue contour and background.



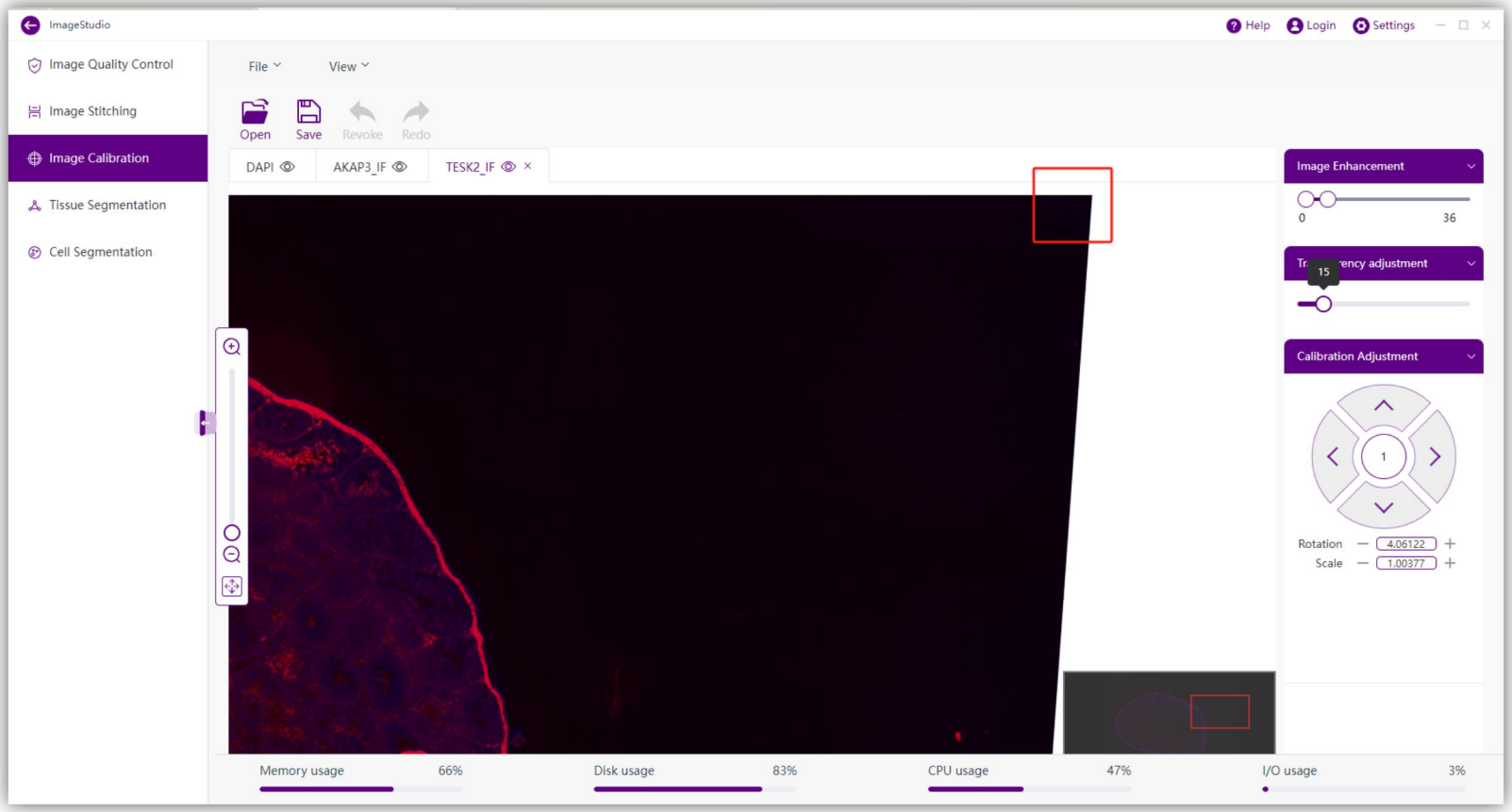
(5) Drag the 'Transparency Adjustment' slide bar under the Mask tab to adjust the transparency of the cell mask image for a clearer view of the overlaying DAPI and protein images.



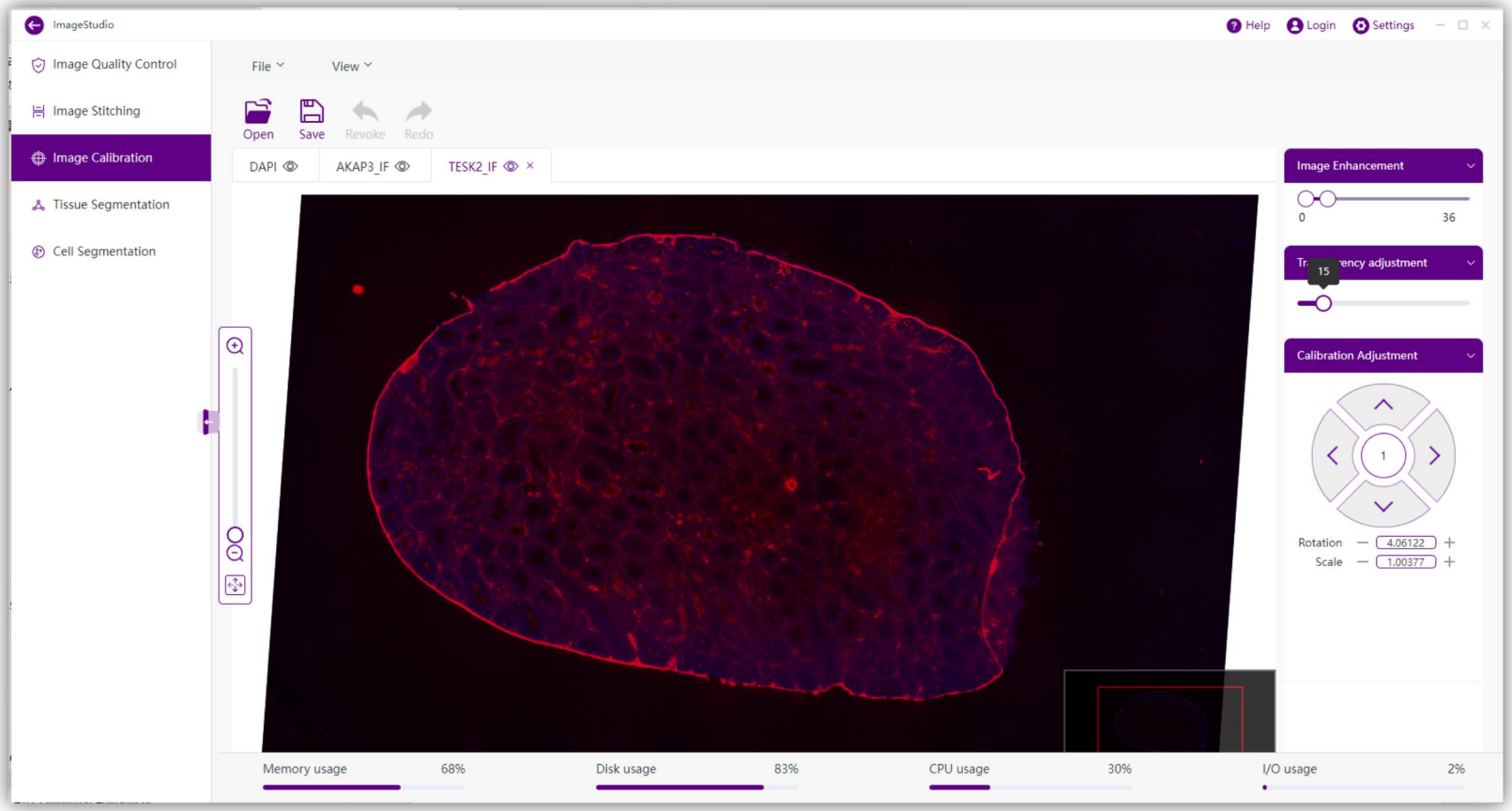
(6) Press and hold Ctrl+ while using the mouse wheel or click on the magnifying glass icon to zoom in or out of the image.



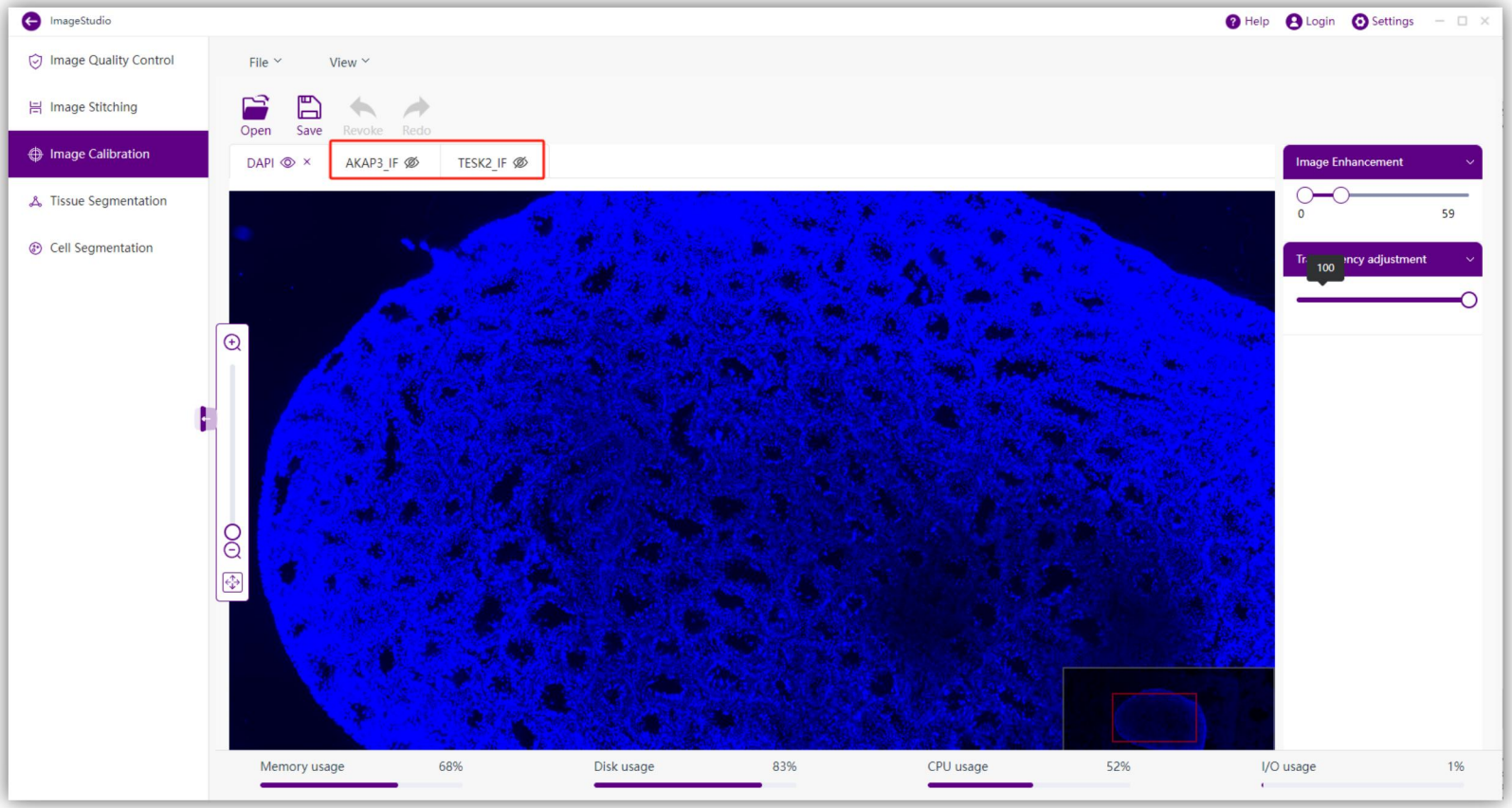
(7) Left click to move around the image.



(8) Left-click within the local view window to move the field of view.

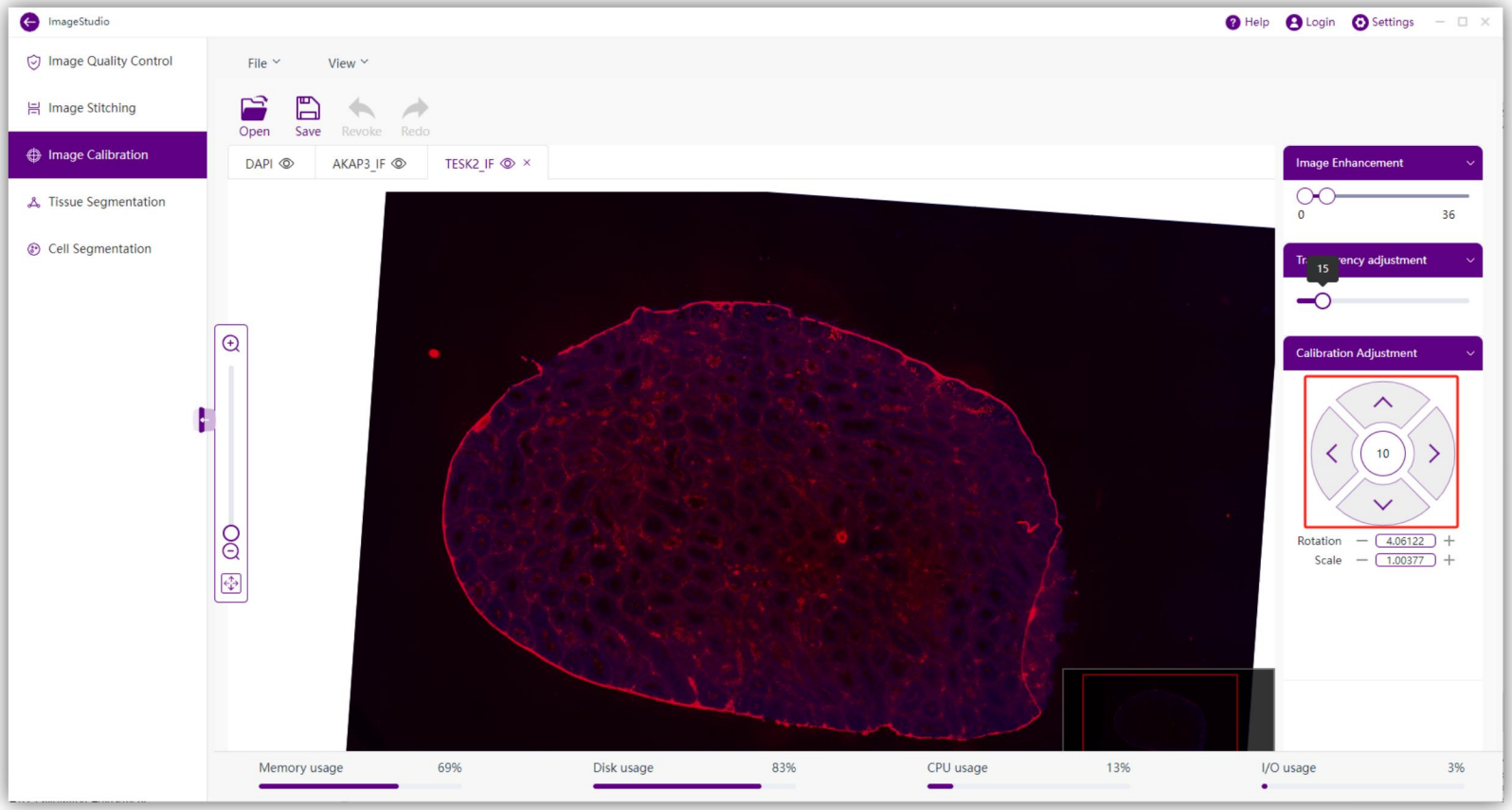


(9)Click on the 'eye icon' to hide or display the image under the tab.

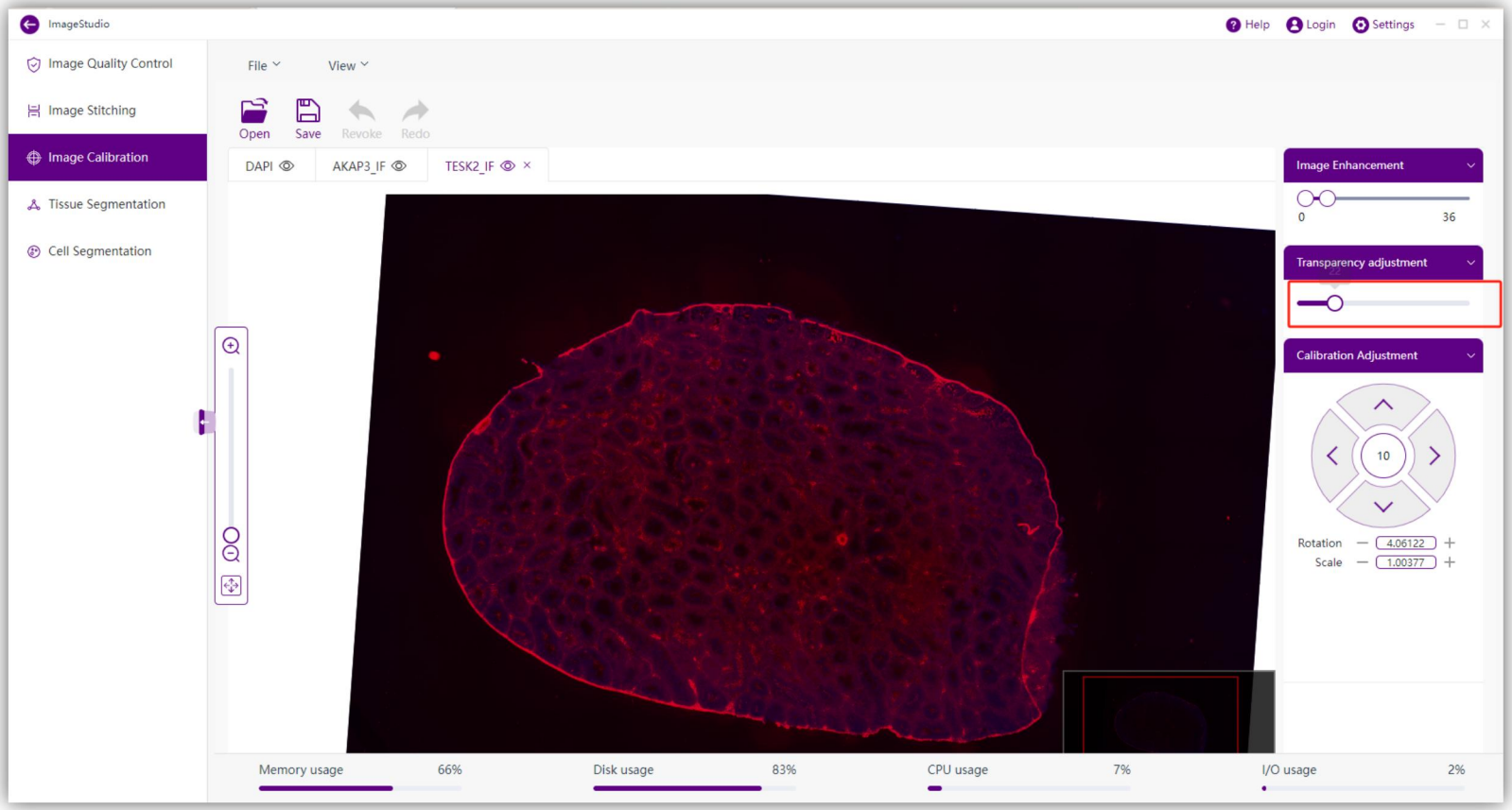


4.6.2 Calibration Adjustment

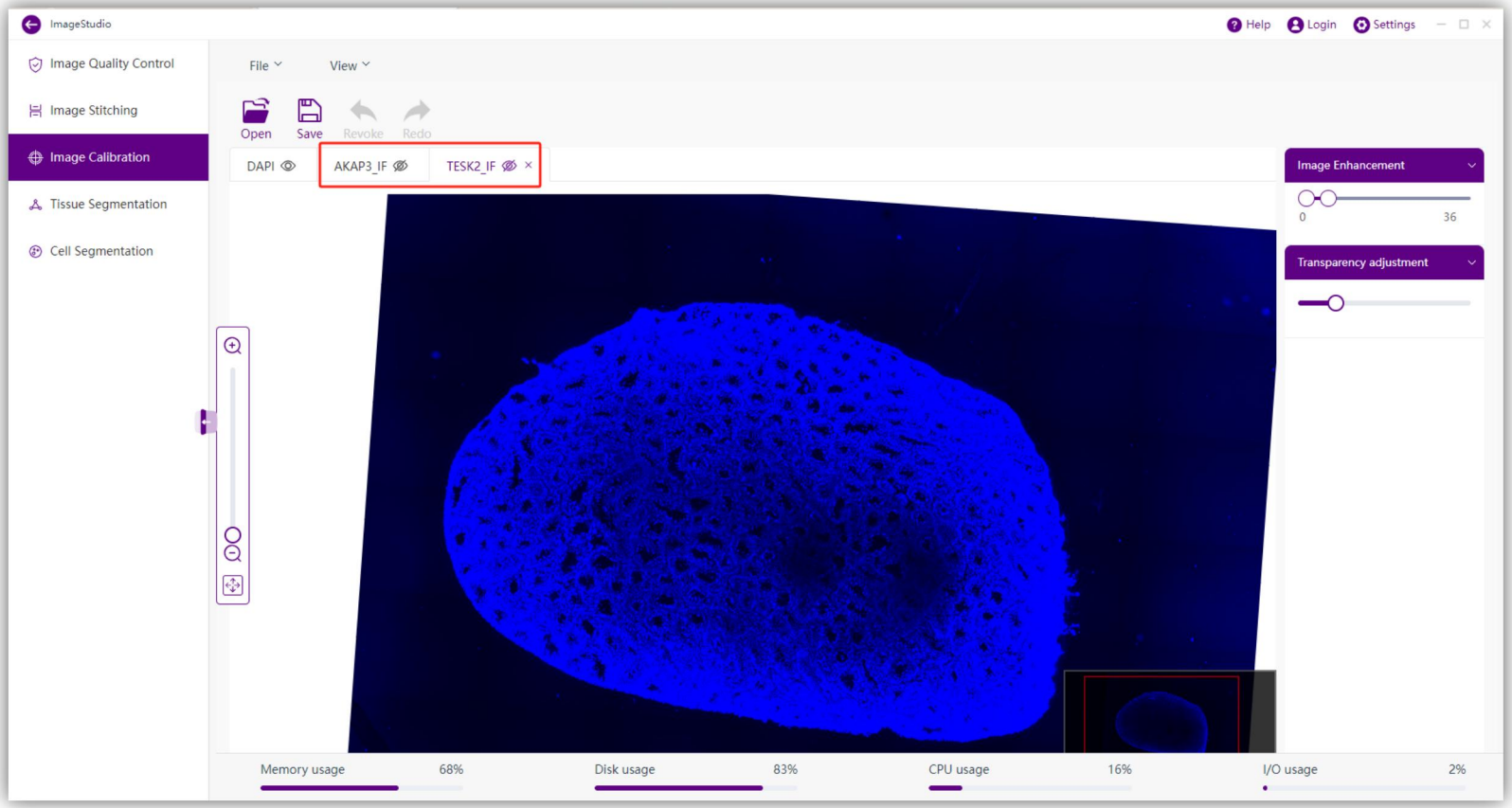
(1)Open the file and the "Calibration Adjustment" panel on the protein page.



(2) Drag the 'Transparency Adjustment' slide bar under the Mask tab to adjust the transparency of cell mask image for a clearer view the reflect the degree of overlap between protein and DAPI.

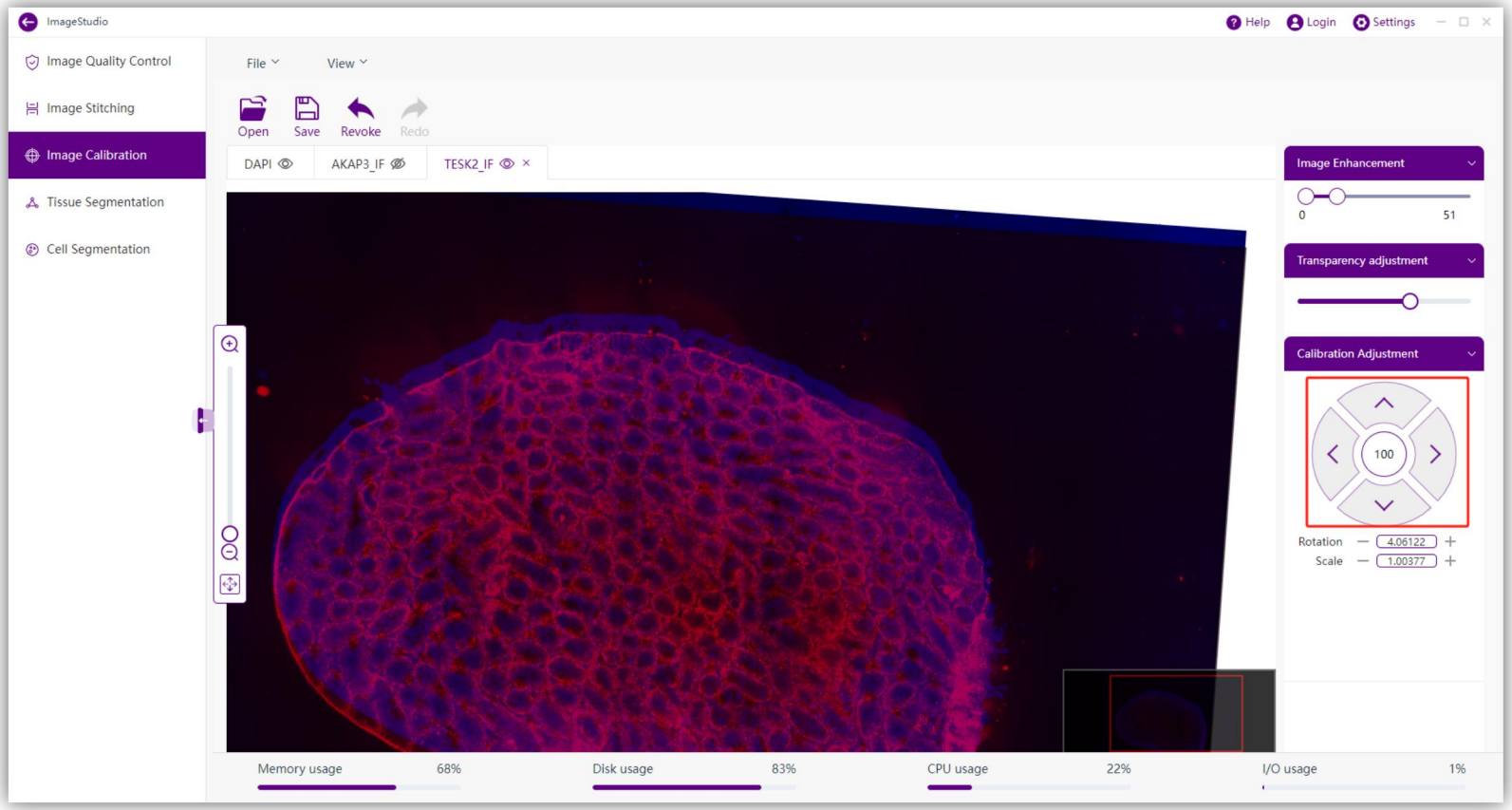


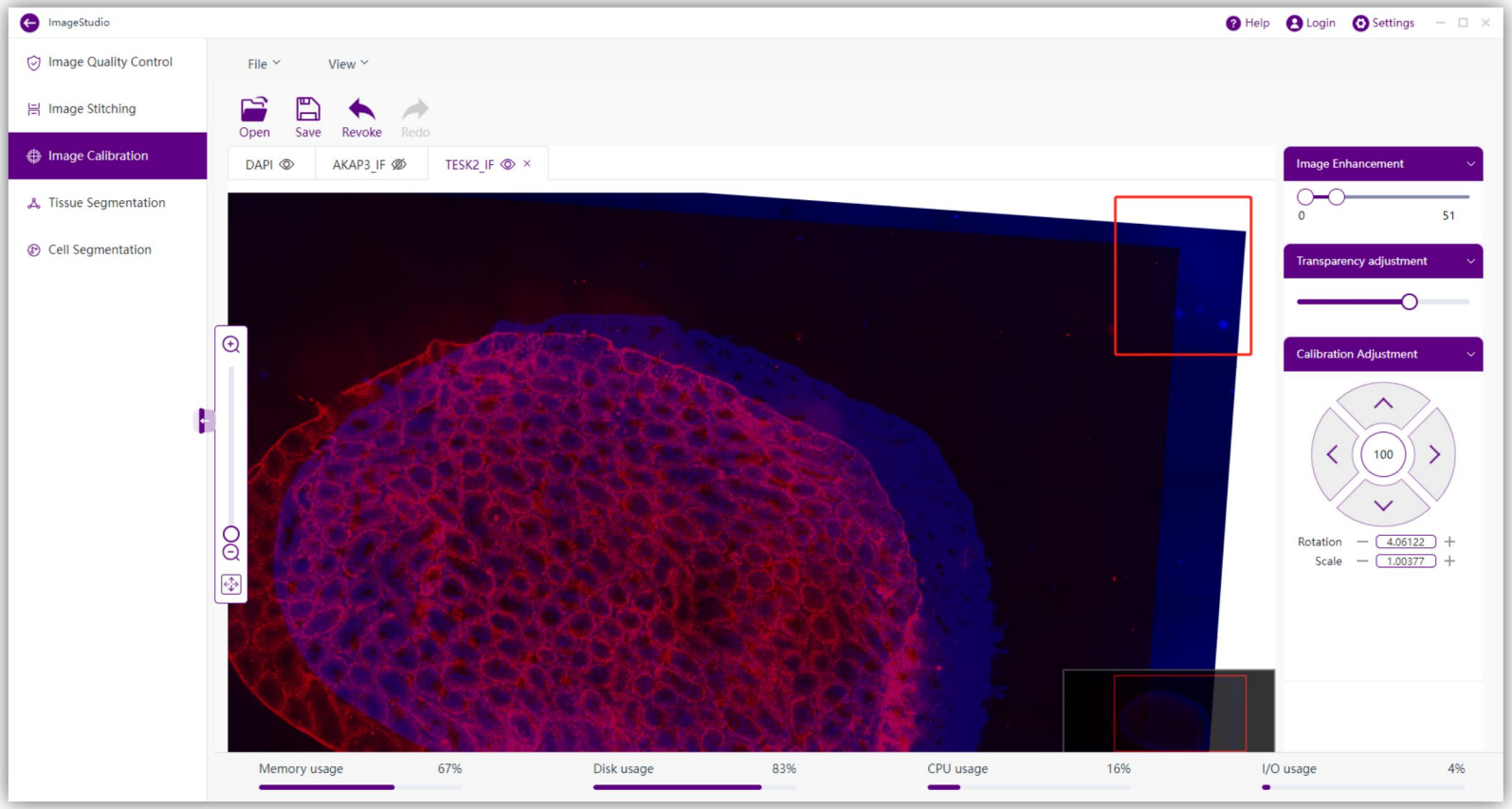
(3) Click on the 'eye icon' to hide or display the image under the tab, avoid the presence of other proteins that may cause background noises on the current protein image.



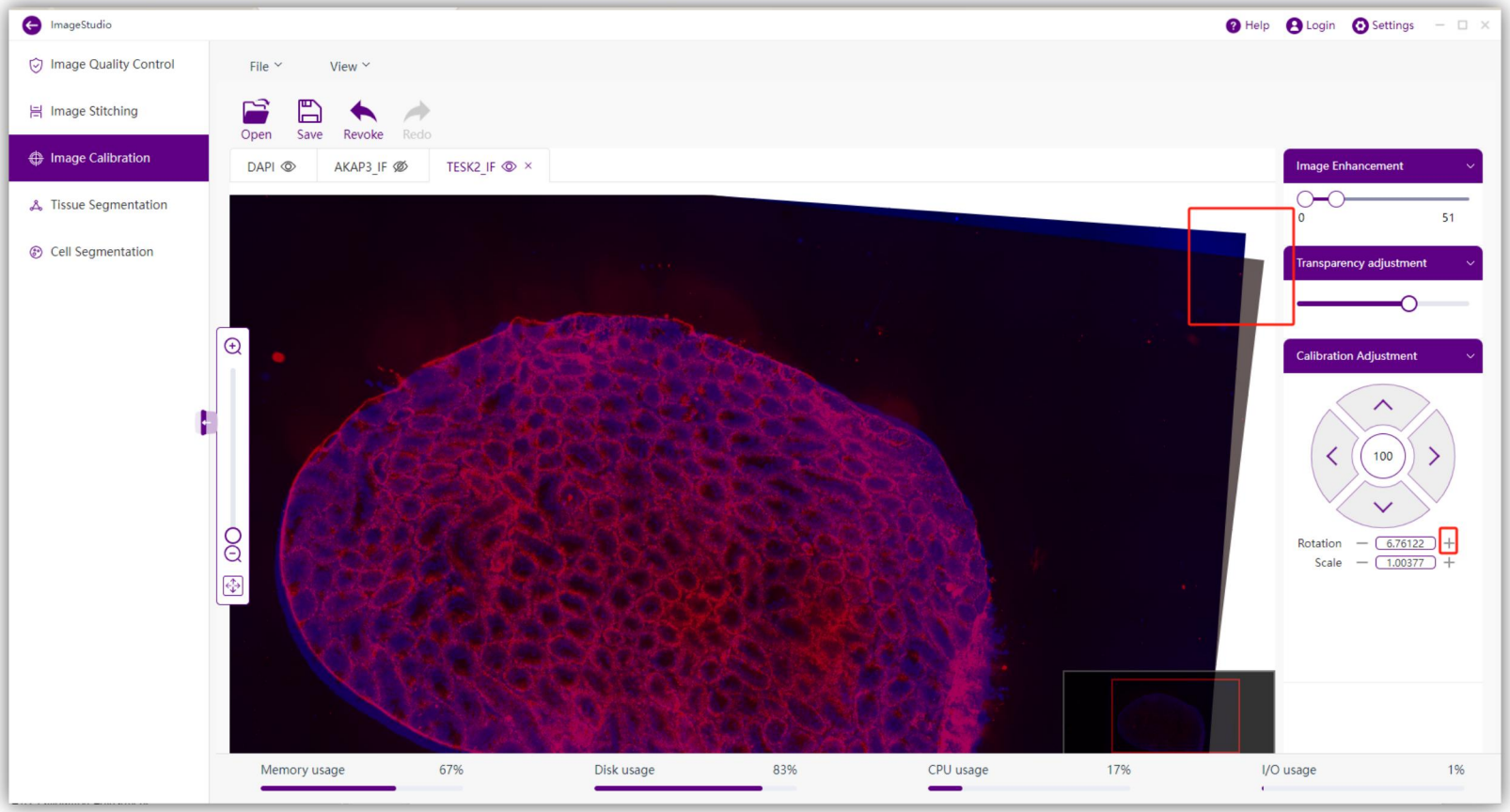
(4) For the protein page:

- If the protein image has offset with DAPI in the horizontal or vertical direction, click the arrows in the "Calibration Adjustment" panel on the right to move the protein image.

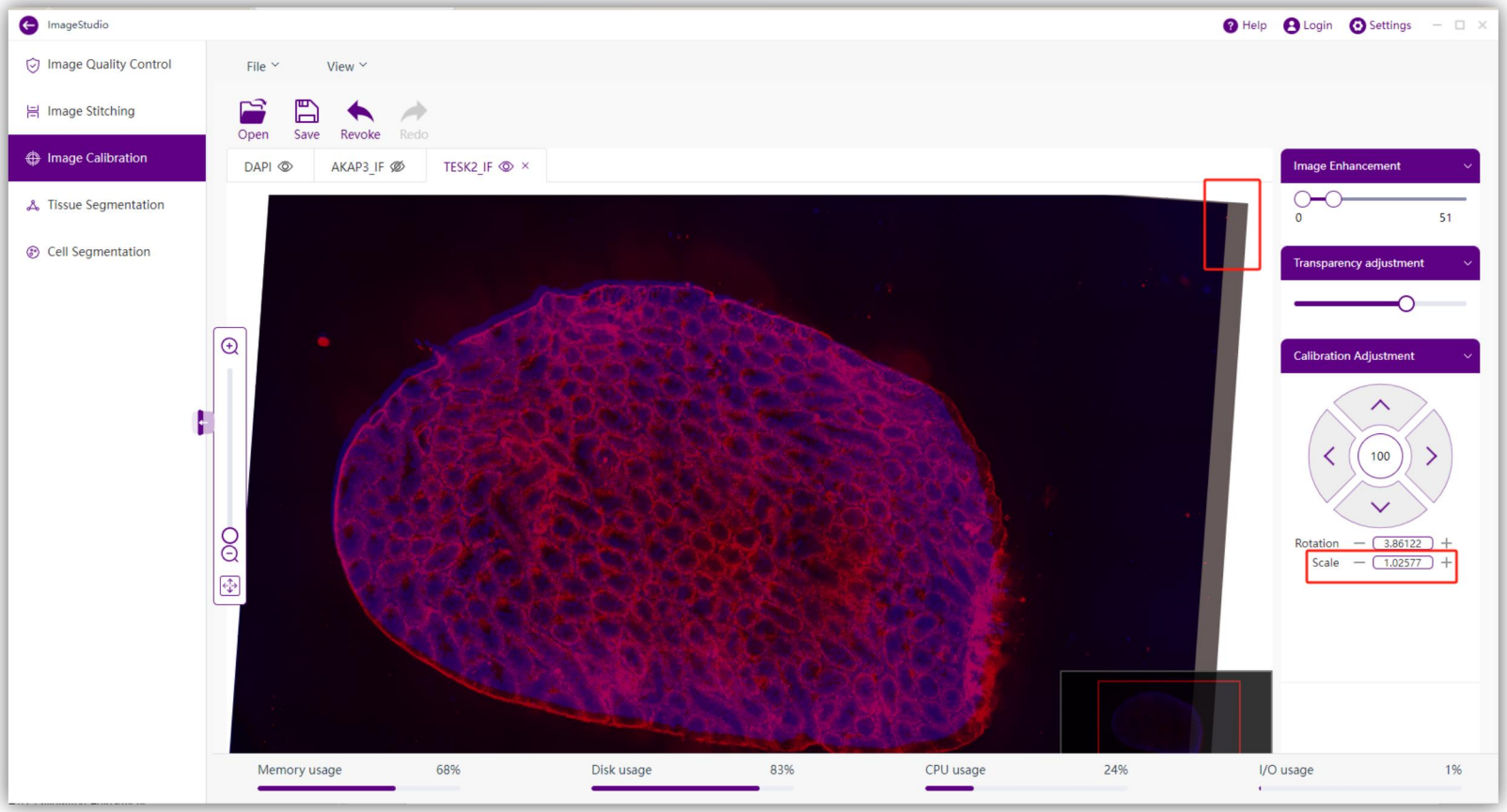

ImageStudio
Help
Login
Settings
Image Quality Control
Image Stitching
Image Calibration
Tissue Segmentation
Cell Segmentation
File
View
Open
Save
Revoke
Redo
DAPI
AKAP3_IF
TESK2_IF
Image Enhancement
0
51
Transparency adjustment
Calibration Adjustment
100
Rotation
4.06122
Scale
1.00377
Memory usage
68%
Disk usage
83%
CPU usage
22%
I/O usage
1%



- If the protein image needs to rotate a small angle to match with DAPI, adjust the image with the "Rotation adjustment" function. "+" rotate the image counterclockwise, and "-" rotate the image clockwise.



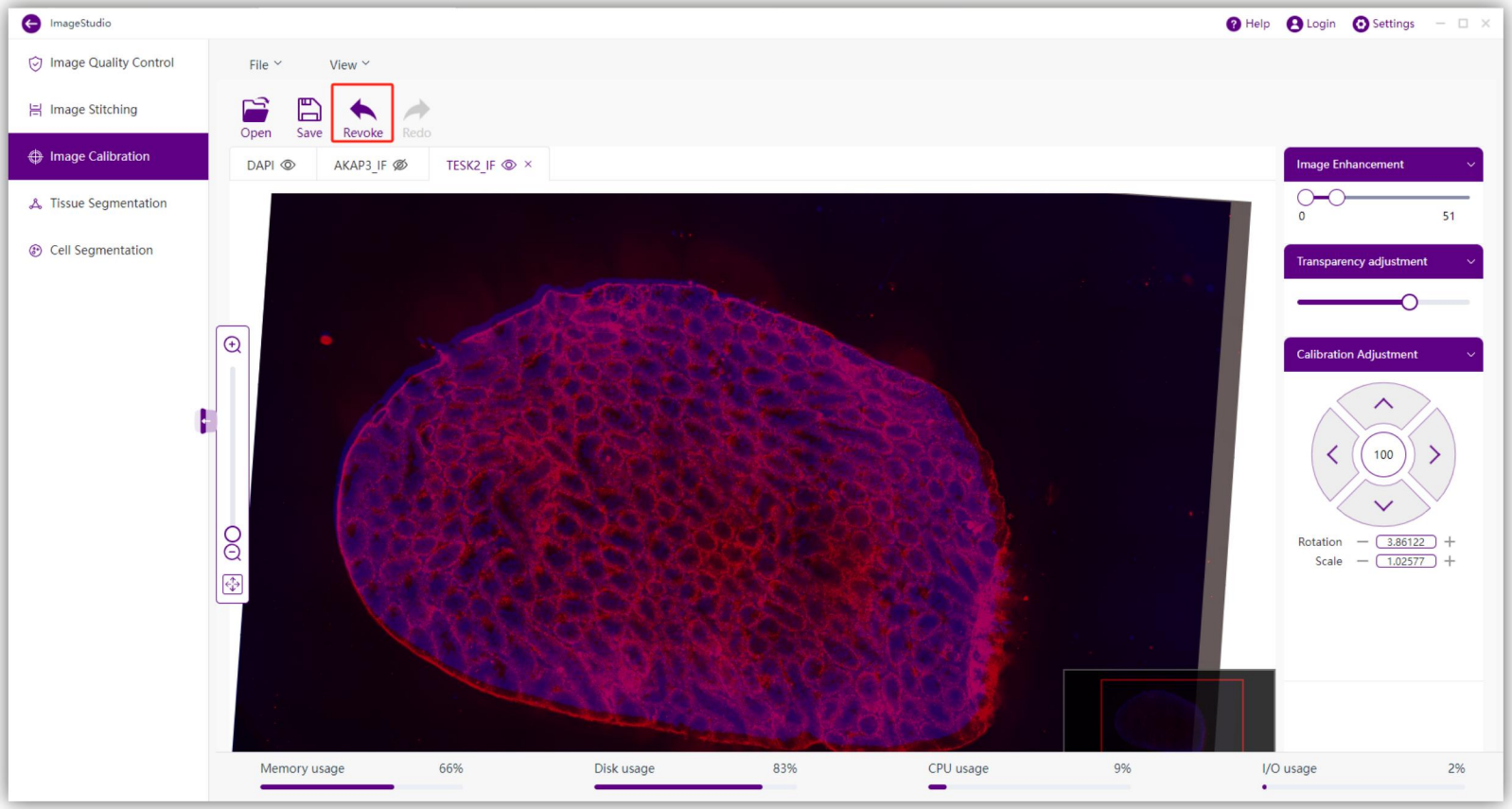
- If the protein image needs to be resized to match with DAPI, adjust the image with the "Scale adjustment" function. "+" will size the image up, and "-" will size the image down.



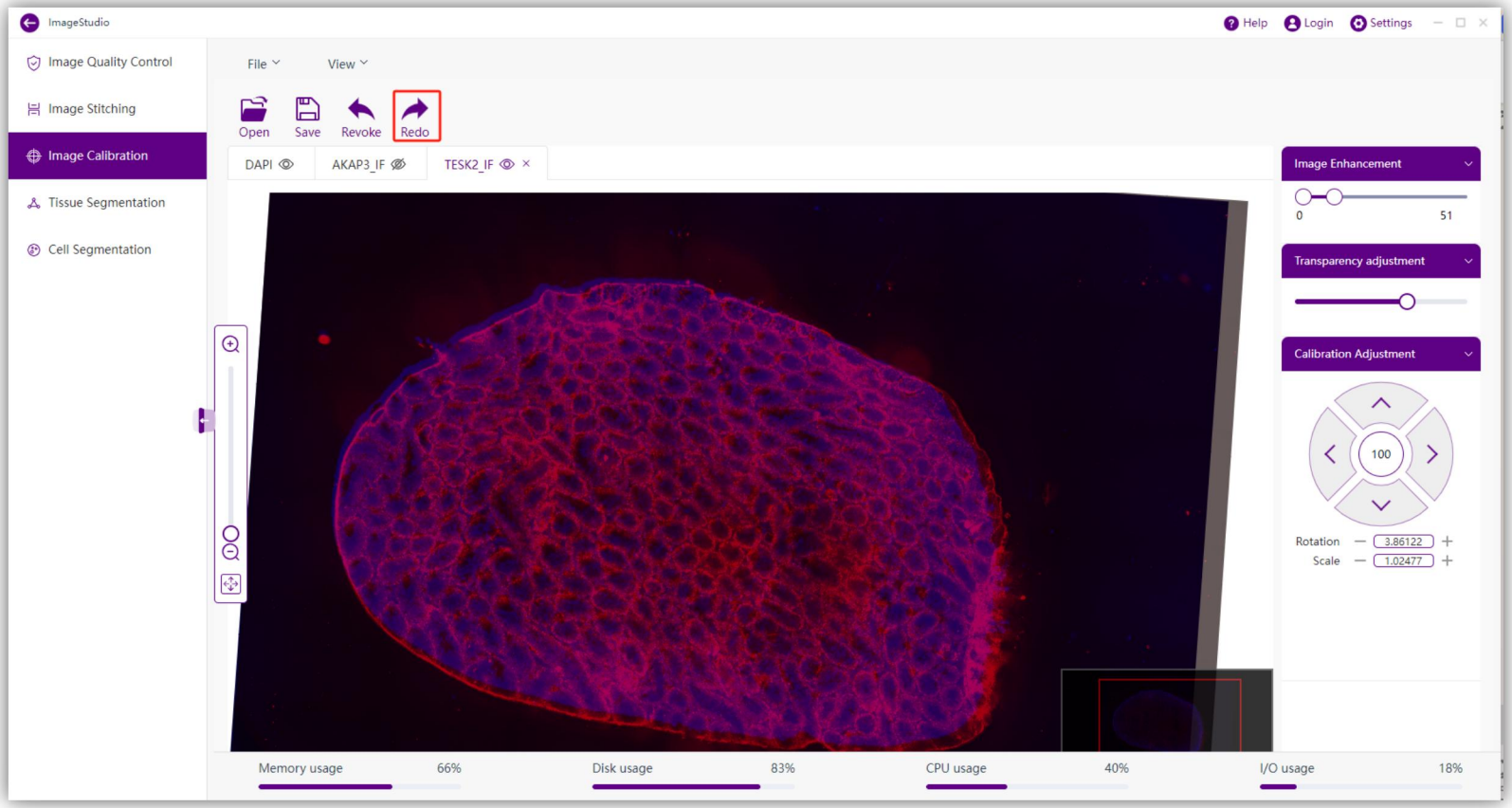
Note:

- The user can enter the step size as required. The default step size of "Calibration Adjustment" is 1 pixel.
- Users can press 'up and down', 'left and right' keys on the keyboard to move the position of the protein in the horizontal and vertical directions.

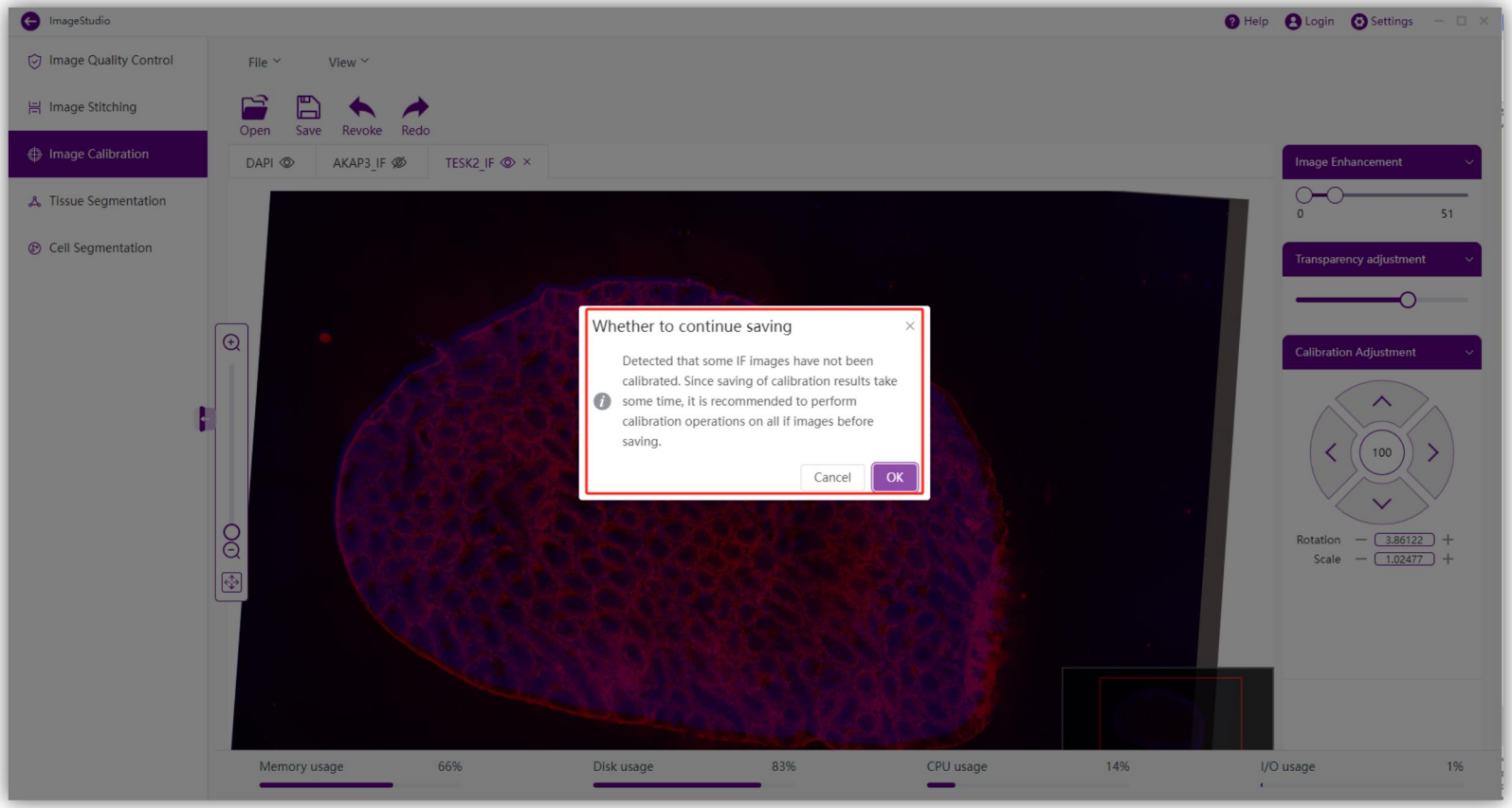
(5) If mistakes were made, click 'Revoke' before saving to revoke current action.



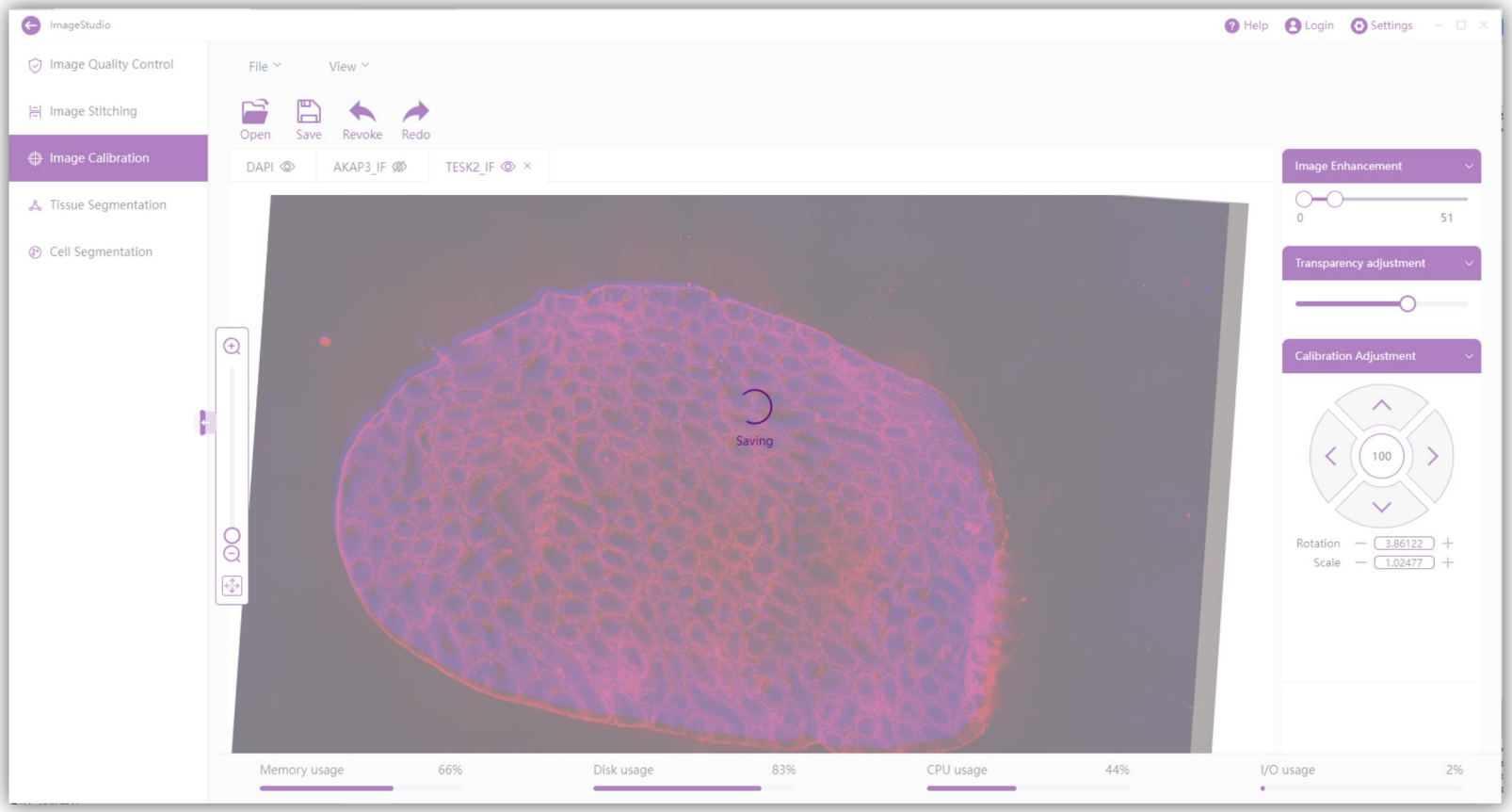
(6)If you want to redo the previous operation after multiple revoke, click "Redo".



(7) If some proteins are not calibrated, click "Save". The system will prompt you whether all proteins are calibrated.



- If you click "Cancel", the system returns to the screen before saving.
- If you select "Confirm", the updated information will write it into the IPR file



Note: Since generating the image pyramid is time-consuming during the saving process, it is recommended to save after all protein adjustment is completed.