STOmics

Guidebook for ImageQC software, Microscope Assessment, and Imaging **(FAS version)**

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Manual Version: A4

Revision History

Manual Version	Software Version	Revision Date	Description
A0	V1.0.1	Aug. 2021	• Initial release
A1	V1.0.6	Nov. 2021	• Stitched large image instruction • Remove instruction (ImageQC is not responding) • Modify chip number and experimenter instructions • Added the exception handling instruction for long QC time • Added installation directory suggestion
A2	V1.0.7	Dec.2021	• Updated the software screenshots up to date • Added install mode suggestion • Deleted the exception handling instruction for long QC time • Added the handling instruction for unexpected error • Update instructions for chip staining, fluorescence imaging, and image QC in the 2.5 Imaging Test Procedure • Added chip scratch and excessive inclination of track line picture examples
A3	V1.0.7	April 2022	• Combine microscope assessment instructions, imaging guidelines, ImageQC software and API development in one guidebook • Added more details about input files used for ImageQC and image registration in SAW • Added more image examples and advice to avoid poor quality images • Added introduction of ImageQC software • Added requirements for microscope Application Programming Interface (API) development
A4	V1.0.8	July 2022	• Added pipelines tailoring ImageQC output files of a stitched image to SAW. • Update illustration diagrams of the ImageQC software interface. • Added a new evaluation score – QC blur

			score to evaluate the cell boundary (not determining the evaluation final pass/fail result) ◆ Compatible with the short code version of STOmics chip numbers. ◆ Compatible with mouse brain and macaque tissue czi data; cancelled reading via a second channel. ◆ The local tar.gz and json files will not be deleted or moved after successful uploading via rsync, in this version.

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

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Contents

Chapter 1. Introduction

The STOmics SpatioTemporal Gene Expression Analysis quantifies mRNA of tissue sections followed by mapping and visualizing the transcriptomic data on an anatomical image. Hence, the tissue image quality determines the success of downstream analysis and spatial clustering of gene expression data. This guidebook provides hardware recommendations, image acquisition and evaluation guidelines, as well as microscope compatibility test procedure. Additionally, a BGI-developed image evaluation software – ImageQC is also introduced in this guidebook.

Chapter 2. Imaging Guidelines

2.1 Imaging System Recommendations

Table 1 shows microscope models used by BGI Research in developing STOmics protocols.

Table 1. Recommended imaging systems

Manufacturer	Model/ Specification
Leica	Leica DM6-M Leica Thunder Leica DMi8
Zeiss	Zeiss Axio Scan Z1 Zeiss Axio Scan 7
Olympus	Olympus FV3000
Nikon	Nikon Eclipse No-E (DS-Qi2)

If none of the above microscopes is available,

1. Ensure that the microscope used fulfill requirements stated in section 3.1.
2. Perform a microscope compatibility assessment following instructions provided in section 3.2.
3. After performing the microscope compatibility assessment, we strongly recommend the user to send the stitched image (TIFF/ PNG) and ImageQC output files (json and tar.gz) to our field application scientist (FAS). A further evaluation will be done by the R&D (IT) team to determine if an application programming interface (API) development is required to tailor the microscope's software to the BGI-developed data analysis pipelines, namely STOmics Analysis Workflow (SAW). This will greatly improve the accuracy in anatomical/ spatial mapping of gene expression data later. However, we will need the user to connect our FAS to the manufacturer's microscope engineer to obtain some information and stitching parameters of the microscope for our R&D team to develop an API. If the microscope can achieve a high stitching accuracy with stitching error of less than 5 pixels, API development is not required.

2.2 Imaging Configurations

Recommended Imaging Configuration:

Recommended Brightfield Configuration:

For H&E staining to assess tissue embedding quality

- Transmitted brightfield
- Color camera (3 x 8 bit)
- White balancing function
- Maximal pixel size of 5 µm
- Exposure time of 0.1 - 100 milli sec

Recommended Fluorescence Configuration:

- Light source with a wavelength range of 380 - 680 nm
- Monochrome camera (≥ 12 bit)
- FITC filter cube (Excitation 470/40, Emission 525/50) – in test chip and S1 chip imaging
- TRITC filter cube (Excitation 545/25, Emission 605/70) – in F1 chip imaging
- Maximum pixel size of 5 μm
- Exposure time 1 milli sec - 2 sec

2.3 Input Files and Requirements for Image Registration

A tissue specimen stitched image (nucleus-stained fluorescent image) is used as an anatomical map on which the STOmics transcriptomic data are visualized. This is carried out by SAW pipelines in a step termed as register (image registration). Different input files are used for image registration depending on the microscope model used (Table 2).

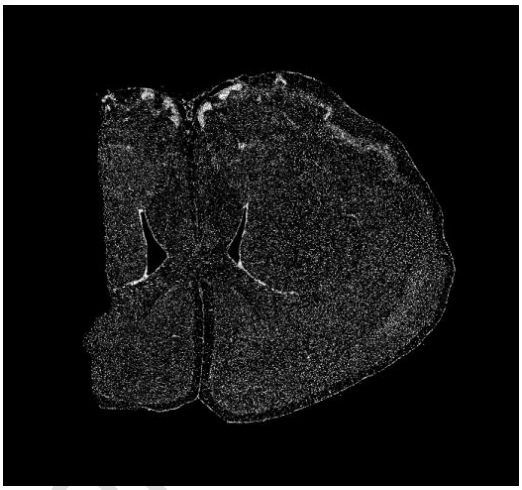
Table 2. Input files for image registration and ImageQC software

Microscope Model	Input file for Image Registration
Zeiss Axio Scan Z1 Zeiss Axio Scan 7	Image raw file in czi
Motic BGI-customized	All tile images in TIFF/ PNG and mdsx file
Leica DM6-M[1] Leica Thunder Olympus FV3000 Nikon Eclipse No-E (DS-Qi2)	Stitched image in TIFF/ PNG
Other Microscope: Before API development After API development[2]	 Stitched image in TIFF/ PNG All tile image in TIFF/PNG or Image raw file in original format

[1] Leica DM6-M's API is only partially developed, please contact BGI IT team at least two weeks before experiments, leaving sufficient time for us to complete the API development.

[2] The required input files and format depend on API development method of the microscope. Please consult the IT team.

An image intended to be used for image registration must be first evaluated using ImageQC software. The input image for the ImageQC software should follow the same file format indicated in Table 2. Refer to section 4.3 for instructions to perform image evaluation via ImageQC software.



Nucleus-stained fluorescent image of a mouse brain coronal section which passed the ImageQC

In general, a qualified image should fulfil the following requirements:

1. High image resolution: ≥ 1800 pixels (height) and ≥2000 pixels (width)

⚠ When exporting an image file into TIFF/PNG format, ensure the file is not compressed to avoid resolution loss.

2. The imaged tissue nuclei should be clear and within focus
3. Track lines on the chip should also be clear and within focus

⚠ To have both the imaged tissue and track lines within focus, select multiple focal points when performing imaging when performing imaging. Set 3 focal points within the tissue and 3 other focal points at the background.

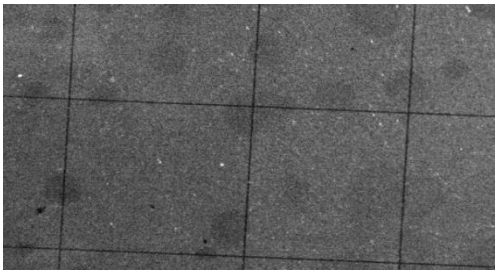
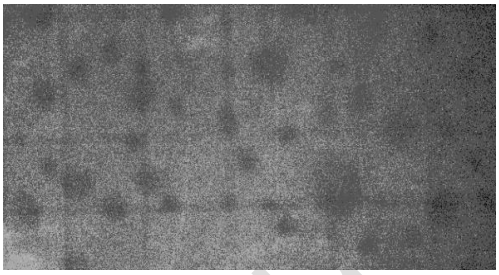
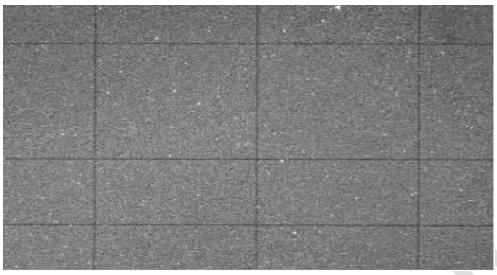
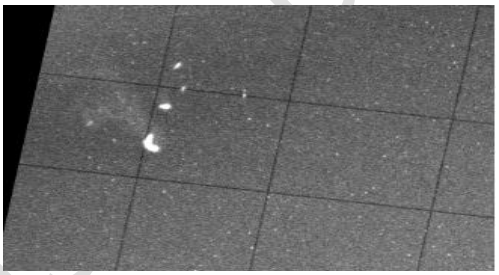
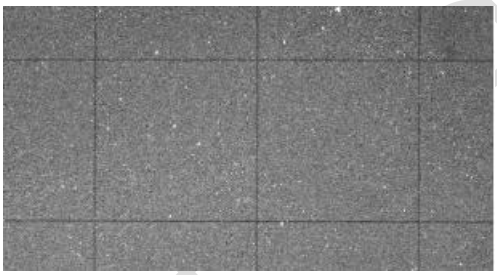
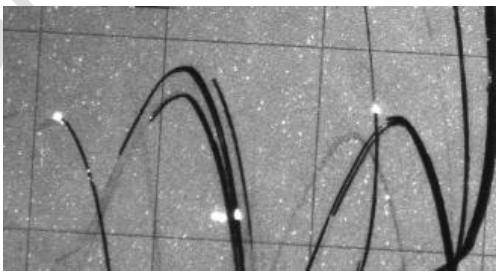
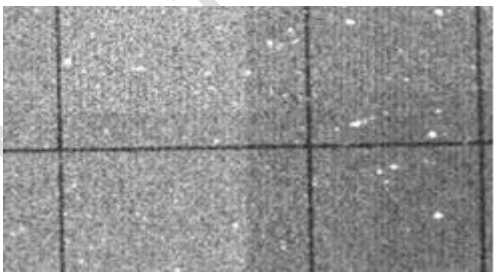
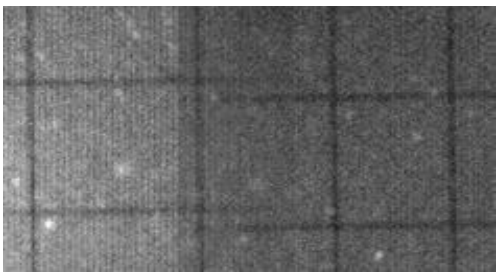
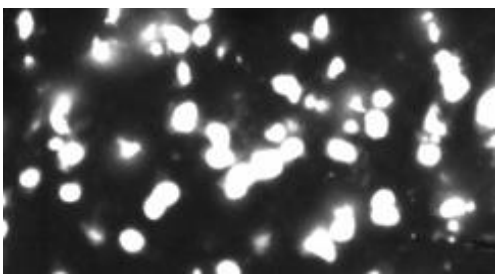
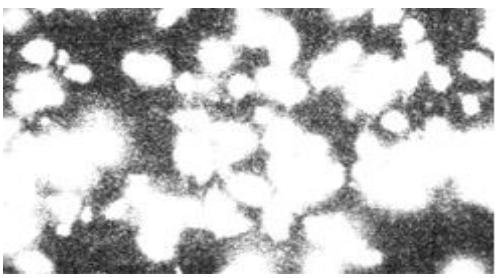
⚠ Adjustment of contrast and brightness might be required post-imaging, to have the track lines clearly visible.

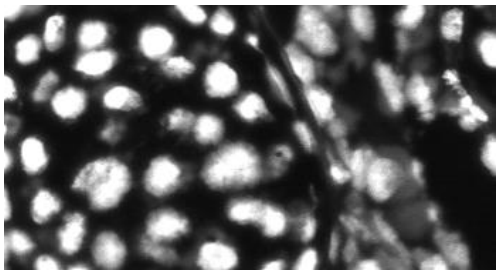
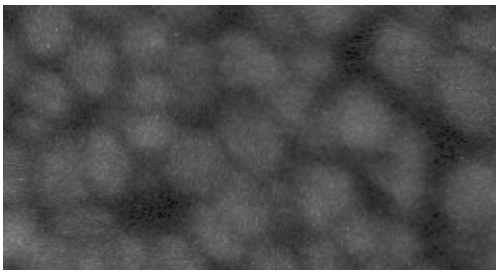
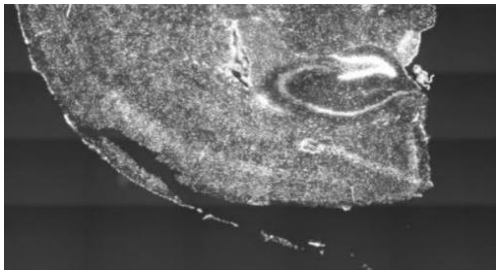
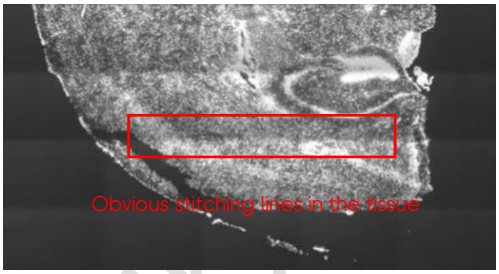
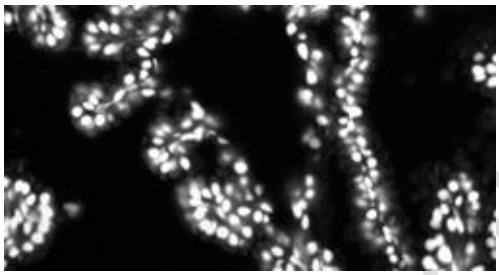
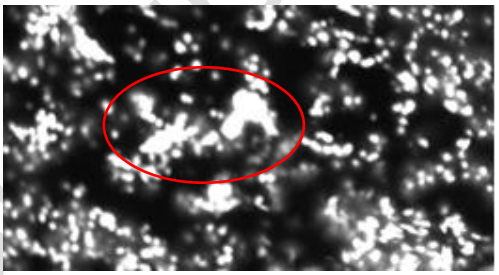
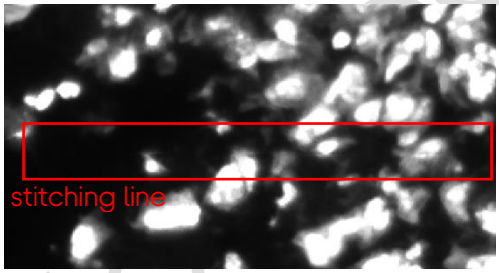
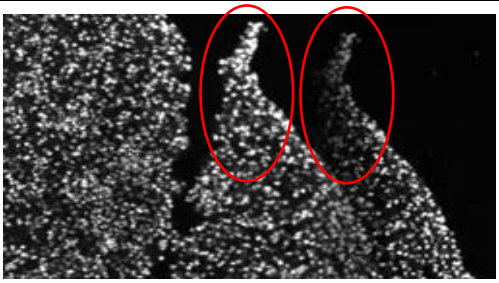
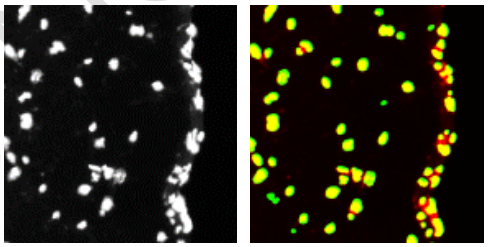
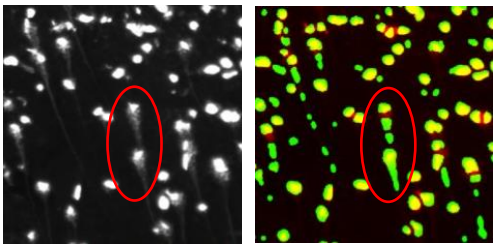
4. No stitching errors
5. Avoid photobleaching
6. Tissue area should not exceed 80% of the chip area

All the factors 3 to 6 will affect the image track line QC score in ImageQC evaluation, which has a scale from 0 to 1. A minimal score of 0.5 is required to pass the evaluation, the higher the score, the more accurate the image registration which has a direct impact on data clustering. Further, the track line QC score of a test chip image is usually higher than that of a S1 chip image because of most the track lines on the S1 chip are covered by the tissue.

2.4 Image Examples

Examples below are images of good and bad qualities for comparison.

Issues	Correct	Incorrect
Out-of-focus track lines		
Track lines tilted more than 8°		
Scratches		
Stitching errors (Track lines) [1]		
Overexposure		

Out-of-focus tissue image		
Exposure imbalance among image tile (FOV)[2]		
Indistinguishable cell clumps[3]		
Stitching errors (Tissue image)[1]	 Adjacent FOVs are precisely stitched	 Repetition due to stitching errors
Tailing[4]	 Nucleus-stained fluorescent image (right) and transcriptomic heatmap (left)	 Presence of "ghost" tails at the cells

[1] To solve stitching errors, please perform a microscope stitching calibration.

[2] The appearance of distinct stitching lines is usually due to a strong brightness contrast among different tile images (FOV). Hardware repair/recalibration might be required, please contact the manufacturer's microscope engineer.

[3] The presence of cell clumps will affect the downstream single cell segmentation analysis. It usually

comes with the nature of a tissue specimen and is hardly avoidable. Lowering the exposure time may help, but this could also compromise the image quality of other regions of the tissue section.

[4] The presence of "ghost" tails at the cells will affect the downstream cell segmentation analysis. Violent sliding of cover slip over the tissue which burst the cells, or vigorous air blowing on the tissue by a power dust-remover can be the cause of this occurrence.

Chapter 3. Microscope Assessment

3.1 Imaging System Requirements

In general, a fluorescent imaging system being used should:

1. Be able to perform tile scanning to image a minimal area of 10mm x 10mm (standard STOmics chip size).
2. Have image stitching function either via the microscope's software or equivalent such as ImageJ.
3. Have an image resolution ≥ 1800 pixels (height) and ≥ 2000 pixels (width).
4. Be able to view and save stitched image in raw file format (e.g., czi in Zeiss) and export it into TIFF/ PNG.
5. Optional: be able to view and perform automatic or manual saving of tile images (individual FOV) in both raw file and TIFF/ PNG formats (required for API development).

Additionally, the computer connected to the microscope should also be able to handle large images (>5 GB) and allow installation of a third-party image processing software such as ImageQC (section 4.1).

If none of the microscopes recommended in Table 1 (section 2.1) is available, the prospective microscope should also have **ALL** the properties and functions listed in Table 3.

Table 3. Overview of imaging system requirements

Index/ Parameter	Description
XY stage travel distance of microscope	At least 25*75mm
Microscope type	Upright microscope
Brightfield (**FAS ver. Only- for H&E staining QC**)	Transmitted mode
Focusing approach	Pre-focus map, Real-time autofocus
Fluorescent channel	DAPI、FITC、TRITC、CY5
Objective lens	4X, 10X (NA ≥ 0.3); 20X, 40X (optional)
Camera resolution	Camera resolution selection depends on objective lens. Please consult your microscope vendor for details
Image bit depth	16 bits
Background balance	Adjustable background balancing function
Distortion correction	Adjustable distortion correction function
Overlap ratio	Adjustable, 10% by default

File format	Is capable of viewing and exporting stitched image, 8bit/16bit, TIFF/PNG, grayscale or colored images
PC requirement	Windows 10 ×64 system, 16G memory or beyond

3.2 Pre-assessment Preparation

Materials

- Slide dryer set to 50℃
- STOmics Test Chip (BGI, Cat. No.1000029779)
- Nuclease-free water (NF water; Ambion, Cat. No. AM9937)
- Staining reagent: Qubit® ssDNA Assay Kit (Invitrogen, Cat. No. Q10212)
- Power dust remover (MATIN, M-6318)
- Parafilm and dust-free paper
- 24-well plate and petri dish
- Aluminum foil or paper box

Tips and Cautions

- Avoid touching the front-side of the chip which has a shiny surface containing DNA nanoball (DNB) molecules.
- The microscope scanning area should cover the entire chip surface including the four corners.

3.3 Microscope Assessment Procedure

Chip staining

1. Transfer a test chip from its original packaging to a 24-well cell culture plate using a pair of forceps.

⚠ Note that every chip has a shiny surface and a matte surface, the former is the active surface of the chip and should be handled carefully. Always keep the active front-side facing upward and avoid scratches on it.

2. Ensure the front-side of the chip is clean. If there are dusts or white wavy patterns on the surface, rinse the chip with 400 μL nuclease-free water twice. After that, remove the water from the chip surface using a power dust remover and dry it on a slide dryer at 50°C for 1 minute before proceeding to the staining step below.

3. Attach a piece of parafilm on the base of a petri dish and place the test chip on it (front-side facing upward). This eases chip handing since it is easier to pick up a chip on a parafilm surface with forceps.

4. Add staining solution prepared according to Table 4 onto the front-side of the chip, followed by an incubation of 5 minutes in the dark (covered with a paper box or aluminum foil).

Table 4. STOmics Test Chip staining solution

Component	Volume
Invitrogen ® Qubit ssDNA Buffer	199 µL
Qubit ssDNA Reagent	1 µL
Total	200 µL

5. After that, remove the staining solution from the chip corner with a pipette. Rinse the chip with 200 µL of nuclease-free water. Dry the surface of the chip with a dust remover. Then, place the chip into a new petri dish and cover it with aluminum foil or paper box to keep it in the dark before imaging.

Fluorescent Imaging

1. Place the chip directly on the imaging stage of a microscope or attach it onto a glass slide before placing onto the stage. For better adherence, simply add a drop of water (3 µL) onto the slide before placing the chip on it. Mind the angle of the chip on the imaging stage, it should not be tilted more than 8°.

2. Use FITC channel and 10x objective lens to capture image of the entire test chip including its four corners using the fluorescence channel.

3. Save the stitched image in both raw file format and TIFF/ PNG formats. Record image parameters (refer to section 4.2) and proceed to image evaluation.

⚠ When exporting an image file into TIFF/PNG format, ensure the file is not compressed to avoid resolution loss.

Evaluation of fluorescent images via ImageQC software

1. Download and install the latest version of ImageQC software. Download links are provided in section 4.1. For installation guidelines, refer to section 4.2.

2. Follow instructions provided in section 4.3 to perform image evaluation via ImageQC software. Different input image files and formats are required for the software, depending on the used microscope, kindly refer to Table 2 in section 2.3.
3. Refer to section 4.4 for the interpretation of the ImageQC evaluation results.

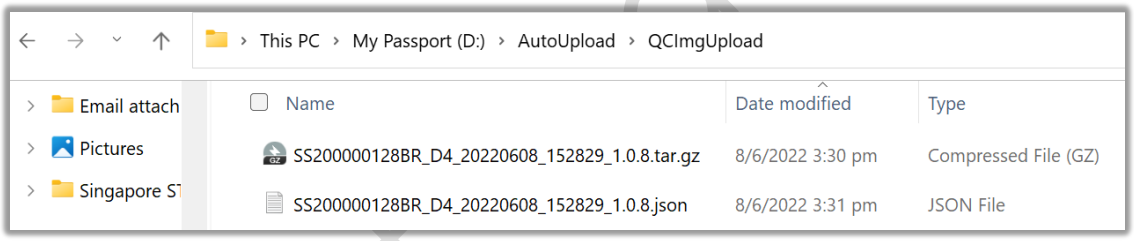
Chapter 4. ImageQC Software

4.1 General Information

STOmics microscope ImageQC software is intended for assessing the quality of microscope images. Besides resolution and sharpness, it also evaluates stitching quality of a STOmics chip image by referring to the background track lines. The ImageQC software has two main applications. Firstly, a fluorescent image of a tissue mounted S1 chip (STOmics, Cat. No.: 1000028487) should pass the ImageQC evaluation to ensure that it fulfills requirements for the downstream data analysis via SAW pipelines. Secondly, with the aid of a STOmics test chip (BGI, Cat. No.: 1000029779), the software can also be used to assess if a microscope is compatible with the STOmics protocols.

Input and Output files of ImageQC

- Different input files in their corresponding formats are used for ImageQC software based on the microscope used. They are listed in Table 2 of section 2.3.
- Two output files are generated after an image has passed the evaluation – json file and tar. gz compressed file. These ImageQC output files can be found in "QCImgUpload" folder and will also be uploaded to a corresponding computer cluster or cloud and used for data analysis via SAW pipelines. To set up SAW pipelines in a computer cluster or cloud, please contact our IT team.



Software logo

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 (refer to section 4.2, step 10 for details).

ImageQC software Download links

Github: https://github.com/BGIResearch/imageQC

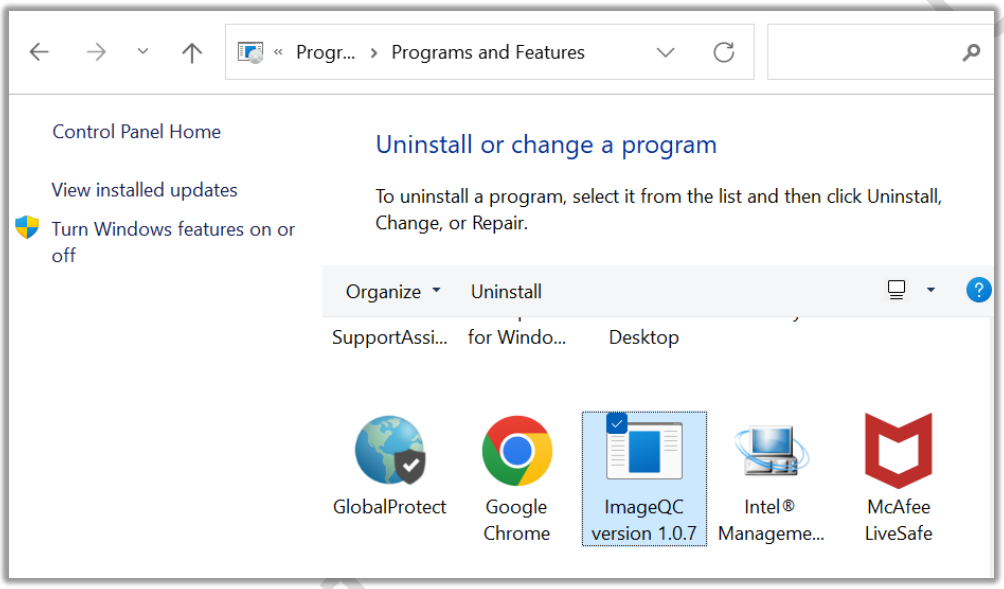
Or https://drive.google.com/file/d/1vT3oHsSlvieg84jliu_dLzYWllqmNyB0/view?usp=sharing

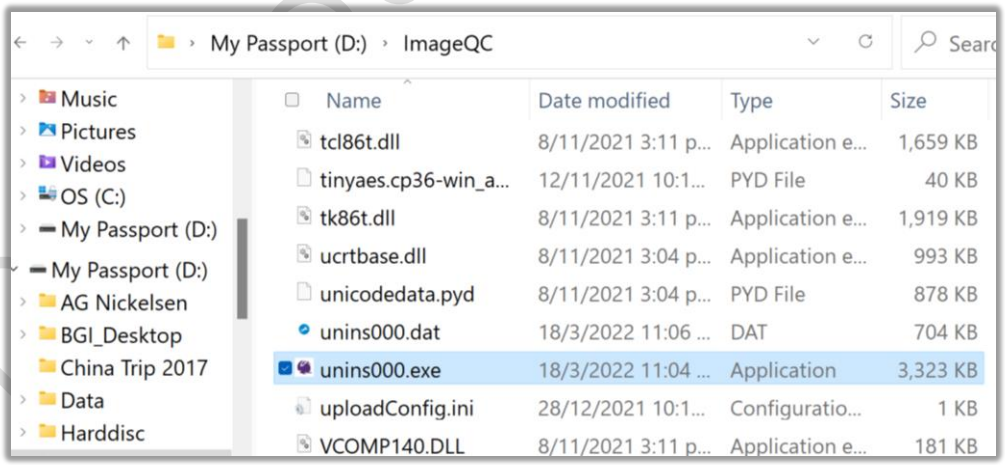
4.2 Software Installation

1. If ImageQC v1.0.7 or an earlier version has been downloaded previously, please refer to step 2. Otherwise, skip step 2 and proceed to step 3.

2. Please uninstall an earlier ImageQC version from the control panel or delete "unis000.exe" file from the same folder in which the ImageQC program is located, as shown in the two screenshots below:

⚠ When updating the software, you may choose NOT to delete the folder "QCImgUpload" where all previous image evaluation result files and information directories are saved.

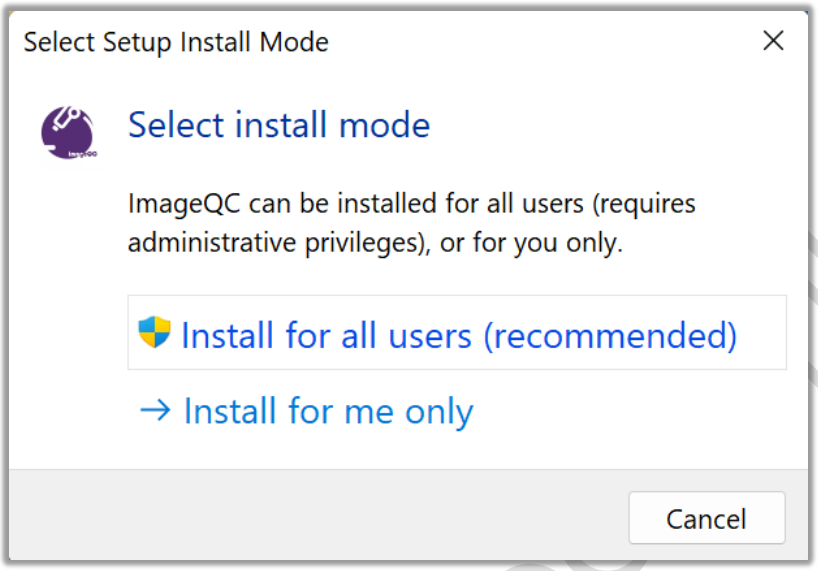




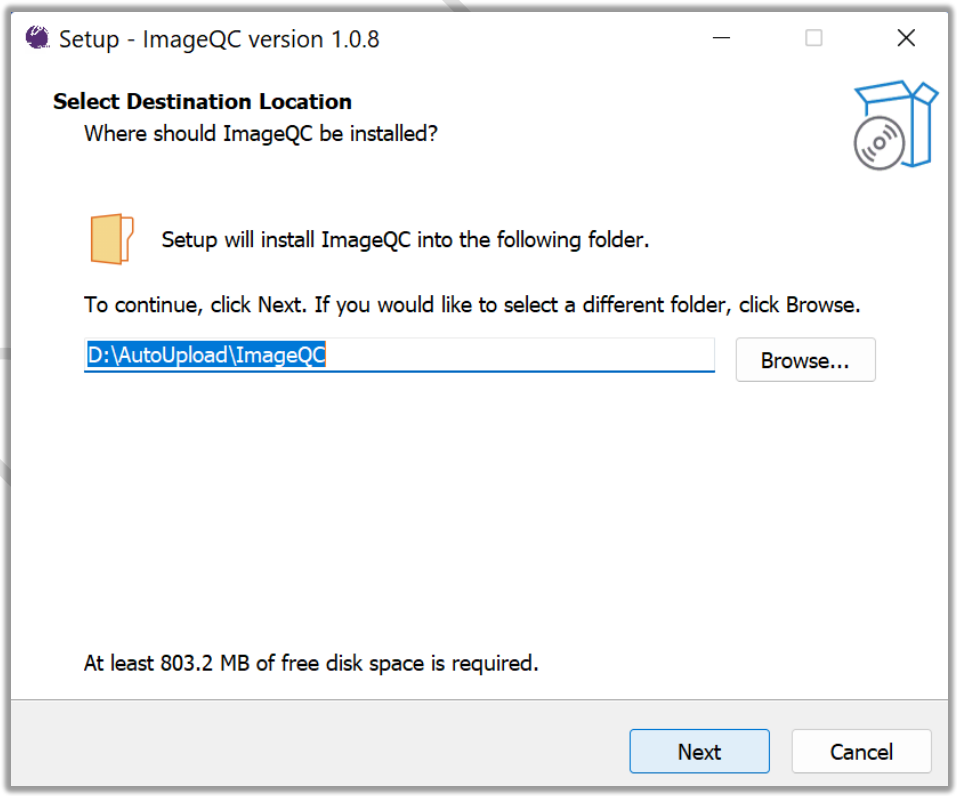
3. Download "ImageQC-Setup v1.0.8.exe" installation package from links given in section 4.1

4. Double click the downloaded file "ImageQC-Setup v1.0.8.exe"

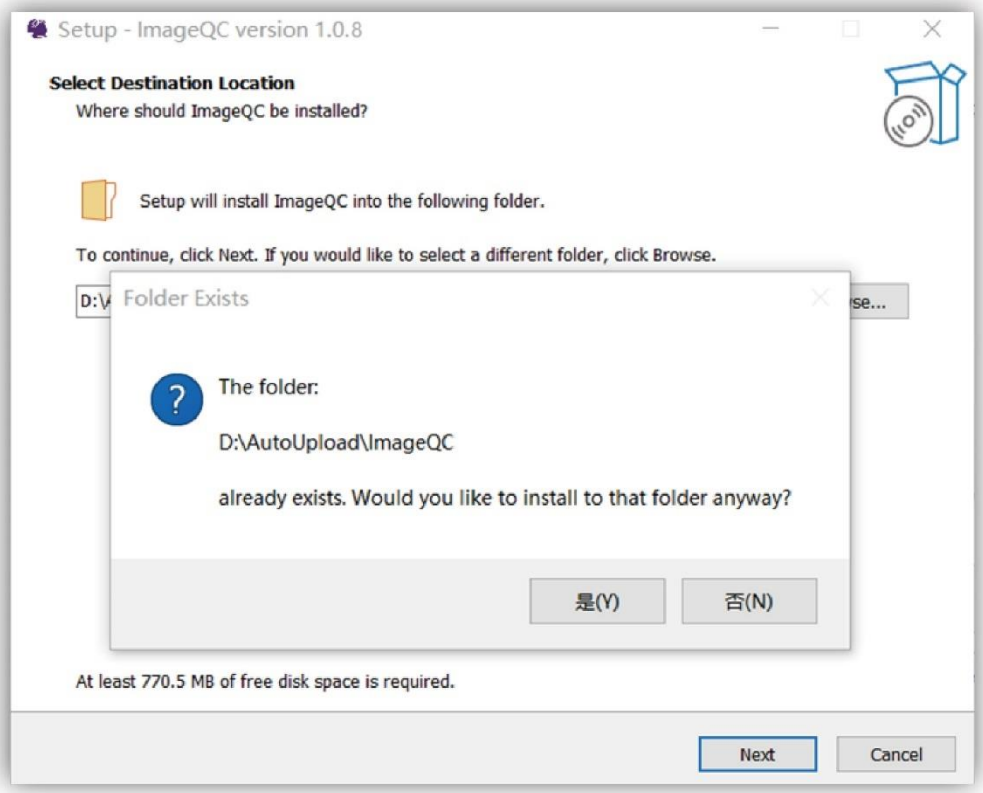
5. Select an install mode. "Install for all users" mode is recommended.



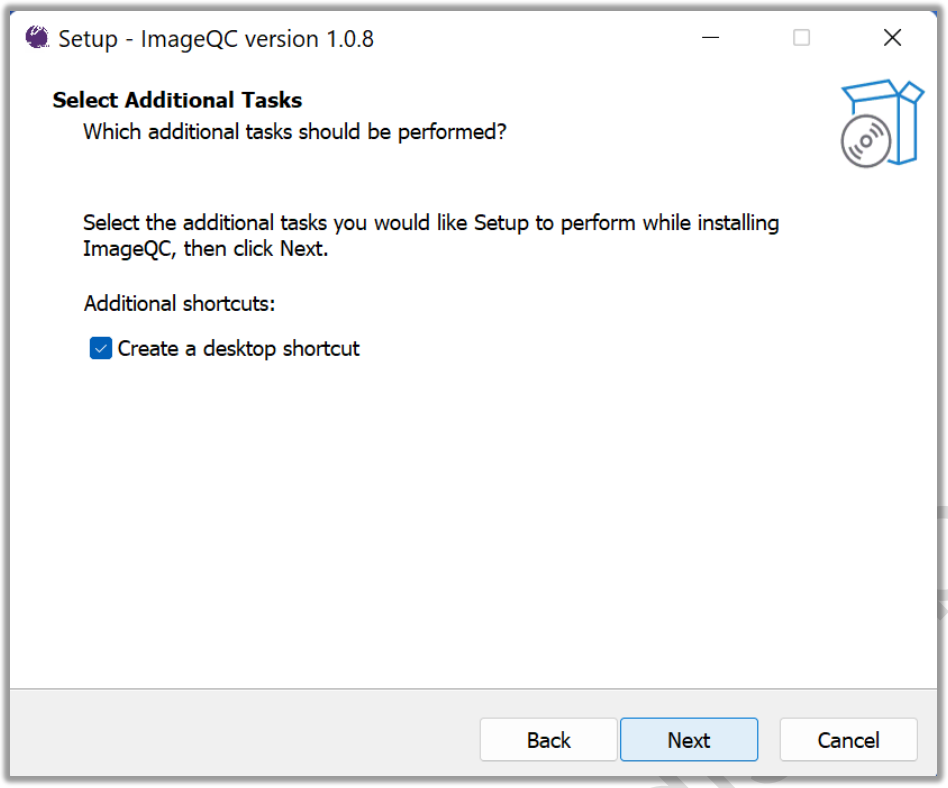
6. Select a destination location. Installation into the C: Drive may require administrative permission. Hence, you are advised to install it into the D: Drive.

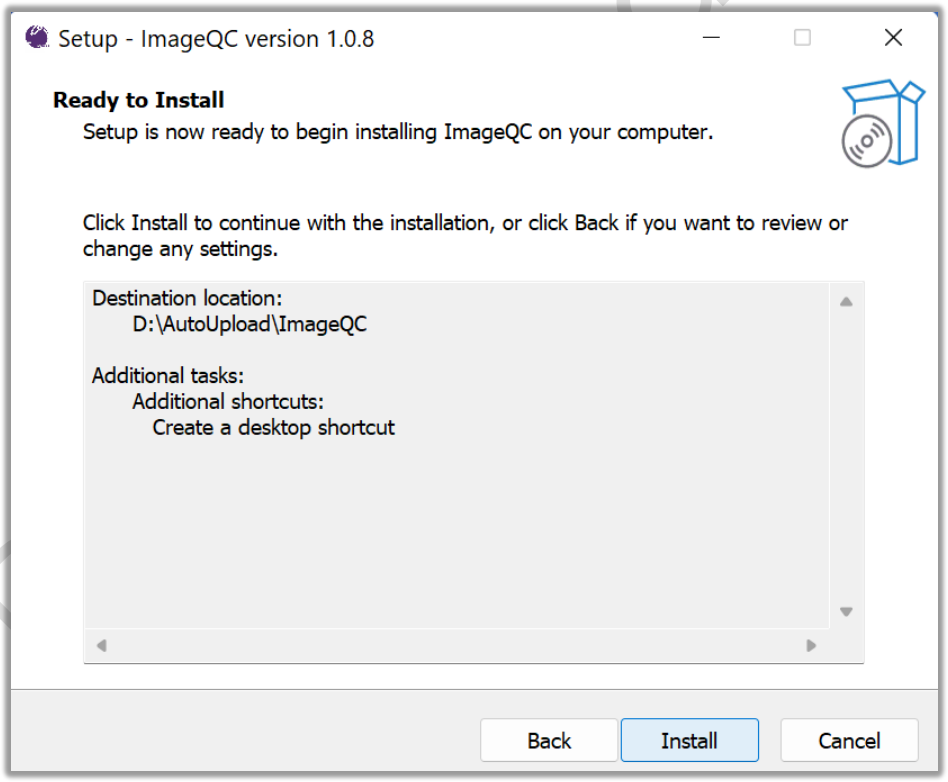


7. If an earlier version of ImageQC software has been installed previously, select "yes" in the pop-up window shown below:

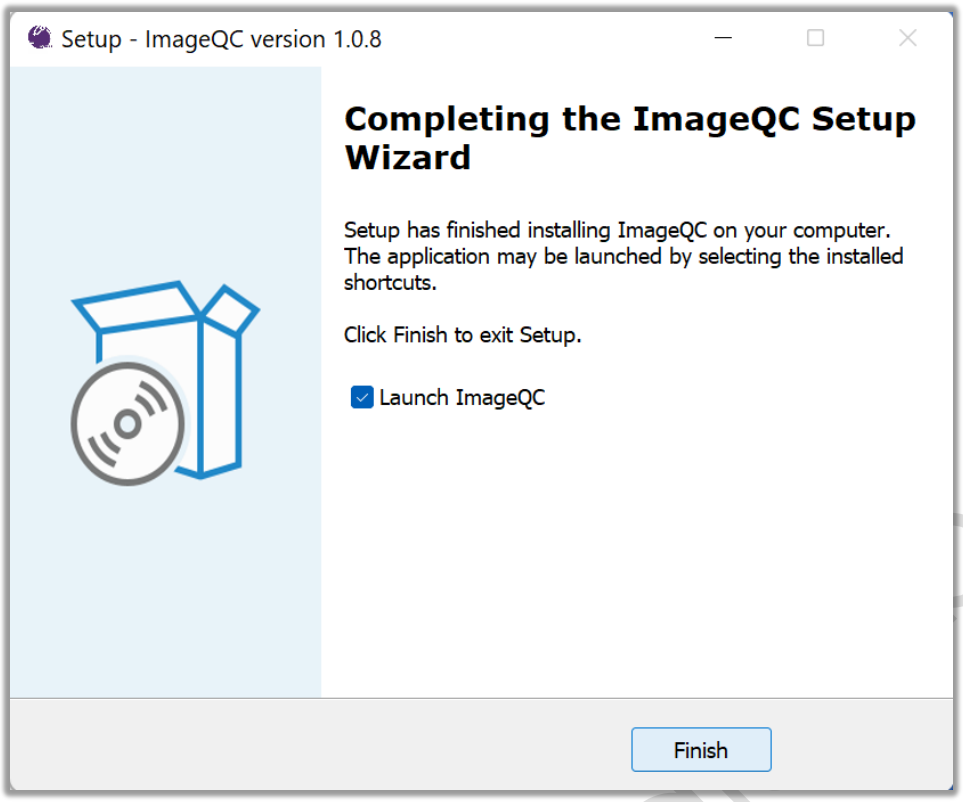


8. Select if a shortcut should be created and click "install" to start the software installation.

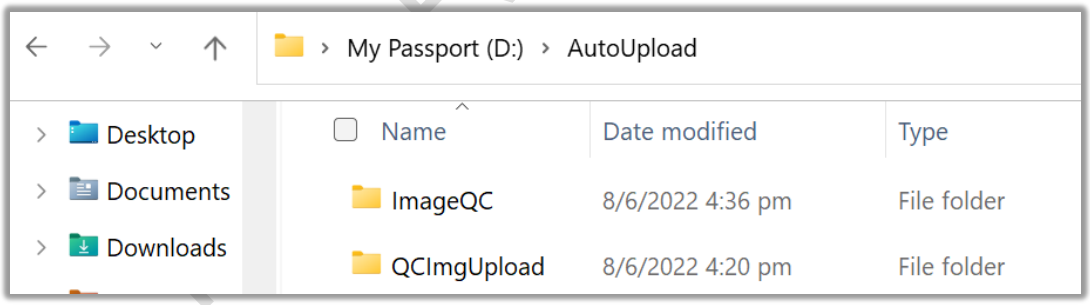




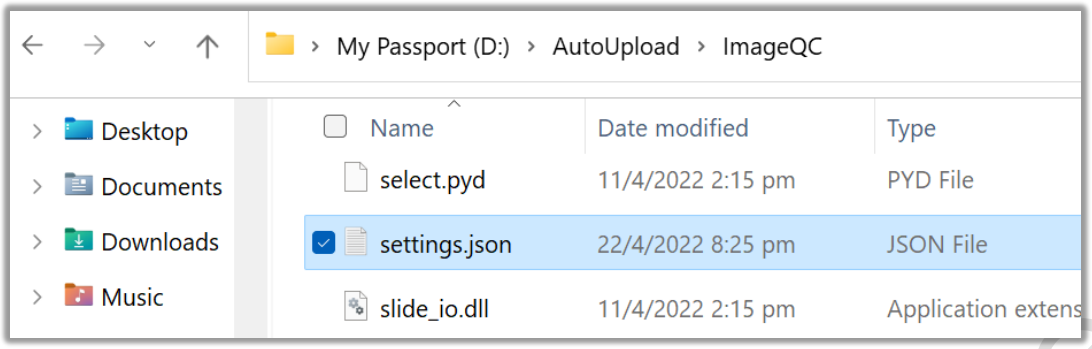
9. After installation is completed, tick the option "Launch ImageQC" and click "Finish" to launch the program.

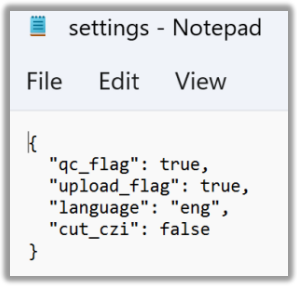


Once the installation is completed, there will be an "**ImageQC**" folder generated in the installation destination chosen in step 7. Another folder – "**QCImgUpload**" will appear immediately upon the first run of image evaluation by the program.



10. If this is the first installation or an update from v1.0.7 or earlier version, please check if there is a file named "settings.json" in "ImageQC" folder. If there is not, please open the newly installed ImageQC program, the file will be auto generated immediately.

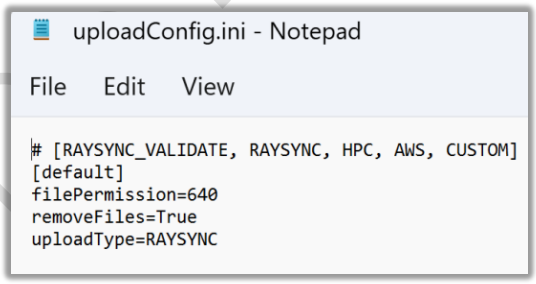




The "setting.json" file is a configuration file for ImageQC output file uploading to a corresponding computer cluster or cloud, upon evaluation. Open this "setting.json" file with Notepad application; there are 4 clauses in the file, edit them if necessary:

- **qc_flag**: Clause for the operation of image evaluations. Do not make any changes.
- **upload_flag**: Clause that control ImageQC output file auto-uploading. "True" is set as the default, implying an image is going to be uploaded automatically once it has passed the evaluation. If auto-uploading is not required, change it into "false".
- **language**: Clause for the program interface language. The default is "chn" which stands for Chinese. If an English interface is preferred, please change it to "eng".
- **cut czi**: Clause controlling czi file processing. Do not make any changes.

11. Please also open with Notepad application "uploadConfig.ini" file which can be found in "ImageQC" folder, and check the image uploading method:



There are three clauses:

- **filePermission**: Permission to access ImageQC output files uploading
- **removeFiles**: Deletion of intermediate files generated in the local system during image evaluation
- **uploadType**:

1. HPC is for local upload of ImageQC output files to the computer cluster in Shenzhen. Please set up the network configuration in advance, BGI intranet is required.
2. RAYSYNC and RAYSYNC_VALIDATE, both uploads ImageQC output files via Raysync software to the Shenzhen's computer cluster. It requires only internet, and no additional network configuration. RAYSYNC_VALIDATE is for external ImageQC users, the parameter will be changed to RAYSYNC automatically by the program once an image has passed the evaluation and the output files have been uploaded successfully.
3. AWS stands for Amazon Web Service. ImageQC output files will be uploaded to Amazon cloud. AWS account password and Python language installation are required. Later versions of ImageQC software will have AWS path configuration function.

4.3 Software Operation Instructions

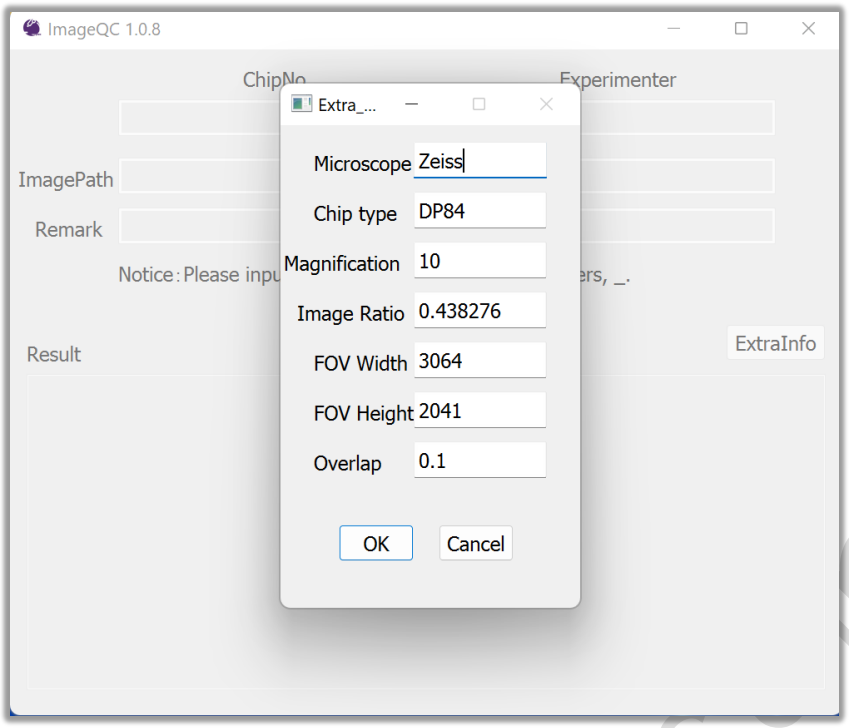
Follow the procedure below to run an image evaluation via ImageQC:

1. Click and open "ImageQC.exe" in "ImageQC" folder to run the ImageQC program after imaging.

2. Drag and drop image files or folders into the program window.
   - Motic BGI-customized: drag and drop the folder in which all tile images in TIFF/ PNG format and mdsx files are located.
   - Zeiss Axio Scan Z1 or Axio Scan 7: drag and drop a raw image file in czi format.
   - Leica DM6-M: drag and drop a stitched image in TIFF/ PNG format
   - Other microscopes (before API development): drag and drop a stitched image in TIFF/ PNG format
   - Other microscopes (after API development):
     - enter the path where exported tile images (individual FOV) in TIFF/ PNG format are saved.
     - drag and drop a raw image file in the original format generated by the microscope software.

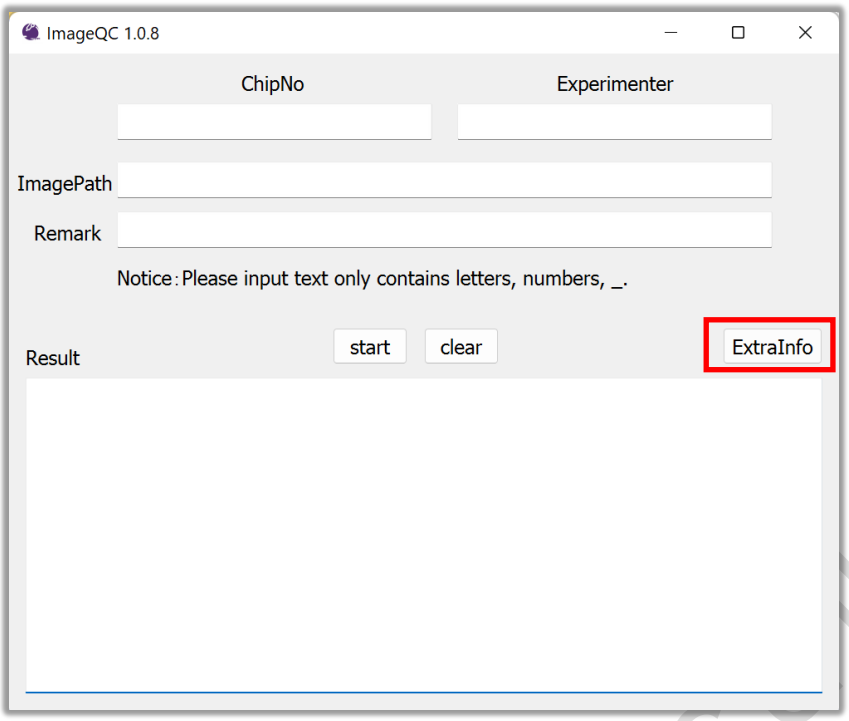
   ⚠ The required input files and format depend on which of the API development options in Appendix B has been used for a particular microscope. Please consult the corresponding BGI FAS.

3. Users will be requested to fill in image parameters manually in the pop-up window shown in the screenshot below:
   Note: These parameters are not required if exported **tile** images in TIFF/ PNG format are used.

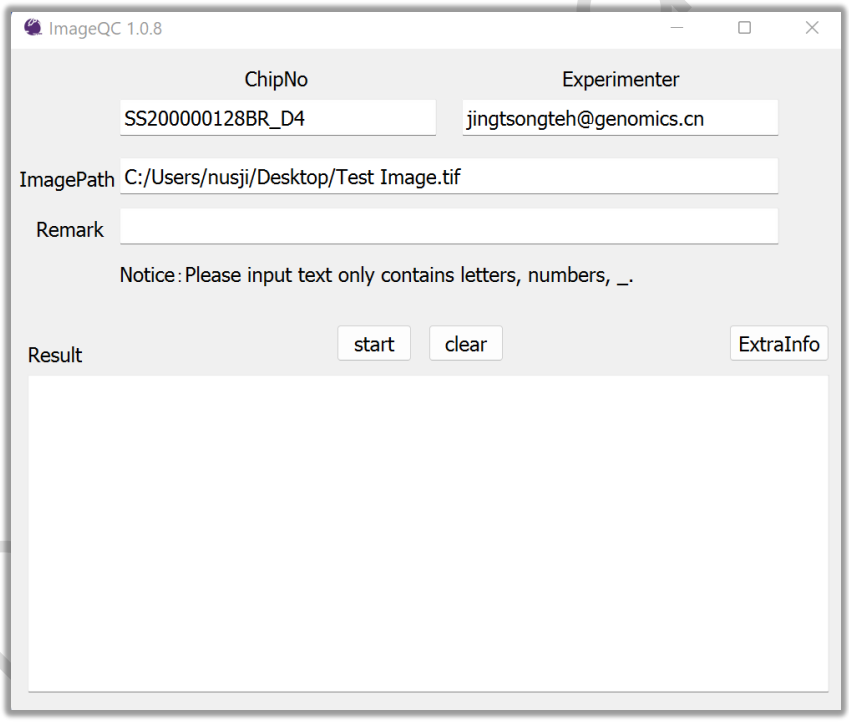


- **Microscope**: Brand name of the microscope such as Zeiss, Leica, Motic, Olympus, etc.
- **Chip type**: It is the first 3 to 4 letters or numbers of a chip number. For examples, FP2, SS2, DP8, DP84.
- **Magnification**: Image magnification. It should be 10, if a 10x lens was used to take the image.
- **Image ratio**: Image ratio can also be defined as 1/ resolution, and hence can derive from the image resolution. It should be in the unit of µm/ pixel, with at least 3 decimal places.
- **FOV width** and **FOV height**: Width and height of a tile image or individual FOV (in pixel)
- **Overlap**: Overlap ratio between two adjacent FOVs (if the overlap ratios at the width and height are different, take the bigger value). The value should be 0.1, if there is a 10% overlap.

The program will memorize the image parameters and fill them in automatically in the next visit. Overwrite them if needed. If any of these details is wrong or missing, especially when the input file is a stitched image, the program will not be able to perform an evaluation properly. For parameter editing, click "**ExtraInfo**" at the bottom right corner as illustrated in the screenshot below:

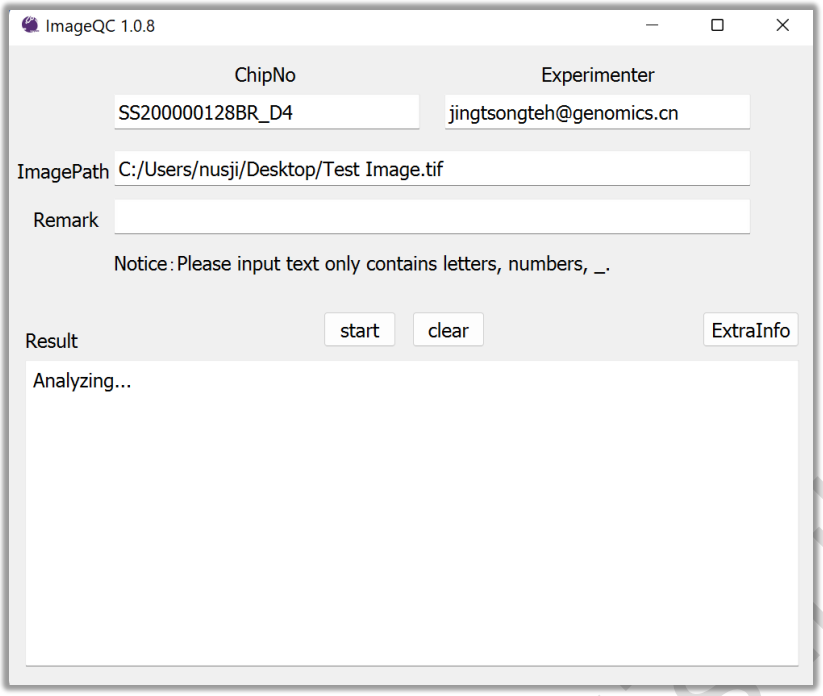


4. Key in the chip number and the operator's email address.

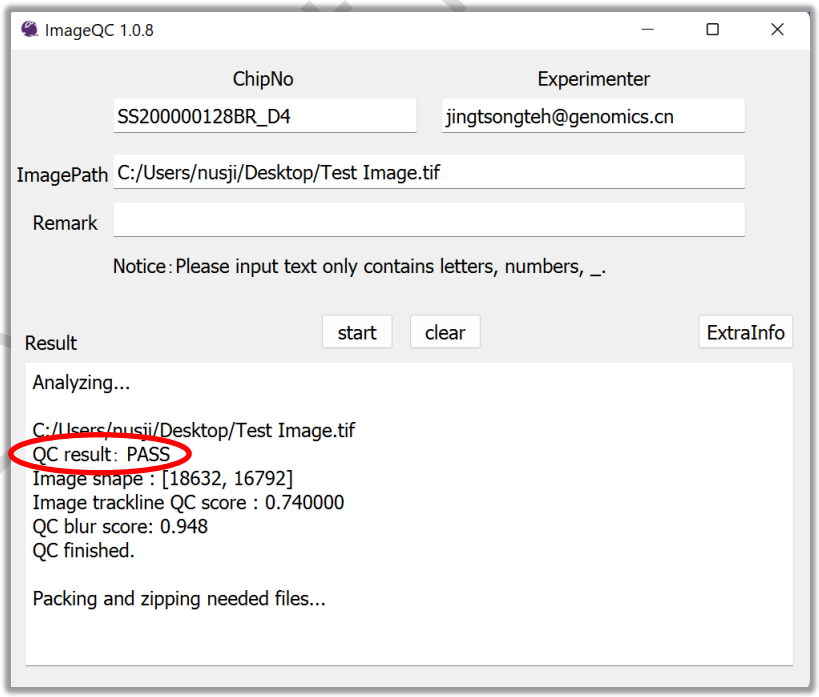


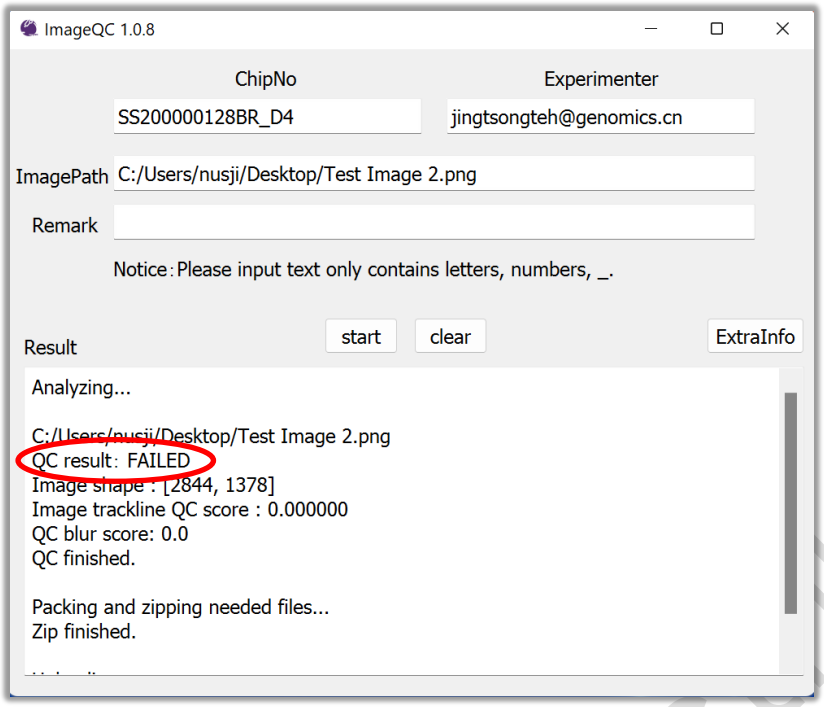
- **Chip Number:** Chip number of a STOmics test chip or S1 chip used in imaging. ImageQC software only recognize a chip number of BGI STOmics.
- **Experimenter:** Full email address of the operator, or mailbox prefix of a BGI employee.
  Note: The chip number and the experimenter's email will be recorded and filled automatically in the next visit. For a new user, please type in new information to overwrite the previous ones.
- **Remark:** Record additional details in the remark field if needed.

5. Click the "Start" button to start the evaluation

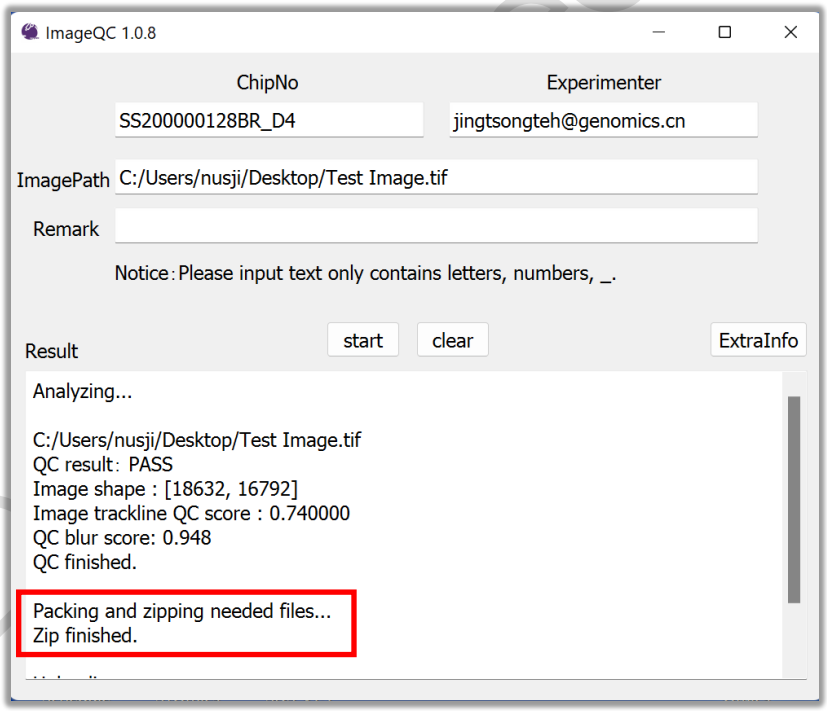


6. When an evaluation is completed, it will be indicated as "QC finished" in the last line of the window. The program will also display the evaluation result as either "PASS" or "FAILED". If the latter result is obtained, please check if the correct chip number and input image files were used and try again. The user may also consider recapturing a new image. Contact a BGI FAS for assistance if necessary.





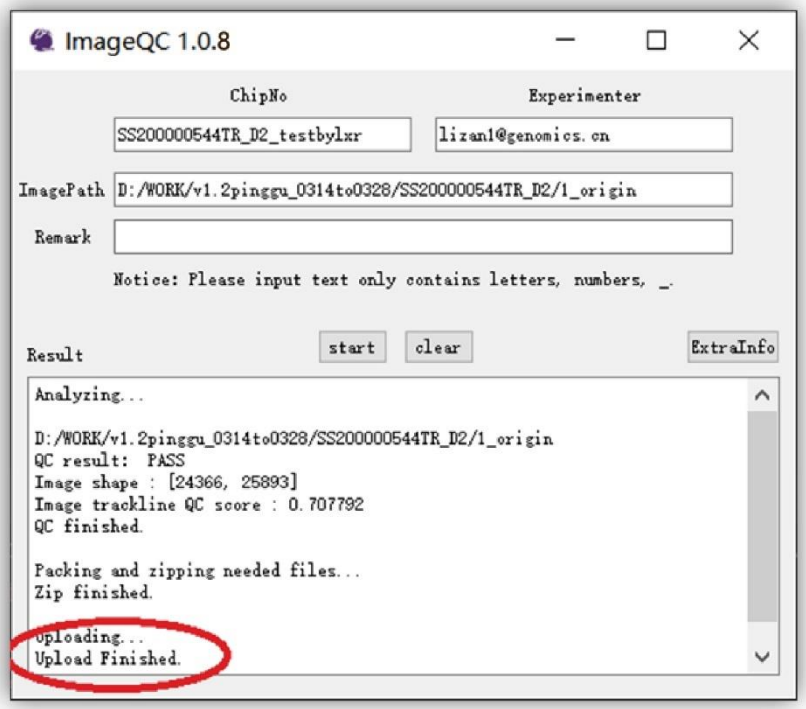
7. Once an image has passed the evaluation, the program will compress ImageQC output files (json and tar.gz files) as demonstrated in the screenshot below:



8. The ImageQC output files will then be automatically uploaded (if auto-uploading function is preset in section 4.2, step 10) to a corresponding computer cluster or cloud as shown in the screenshot below:

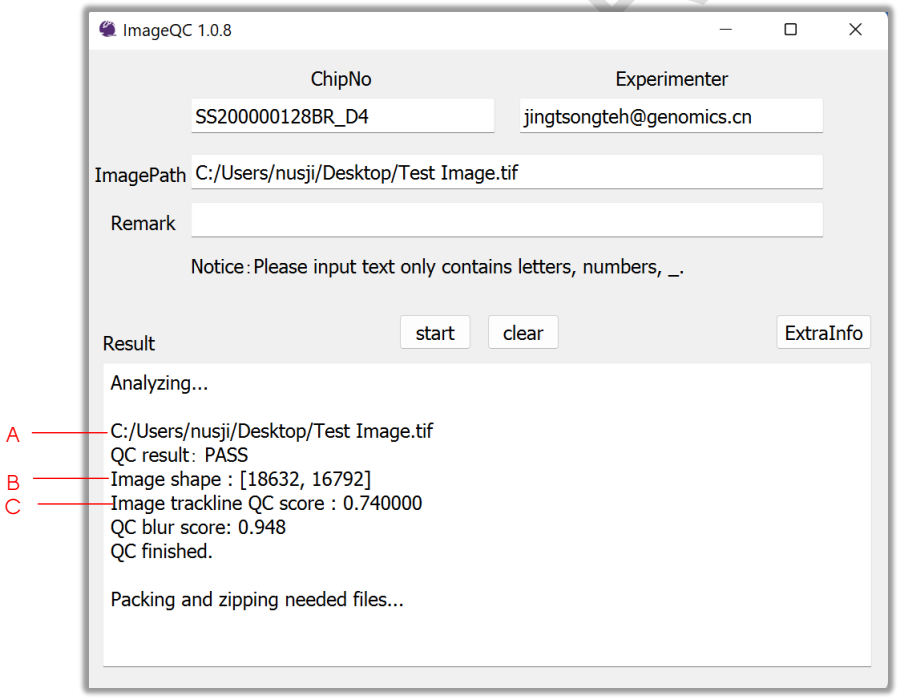
   Note: If data upload is not required, click "clear" to discard contents in the program window. The

program is then ready for a new image evaluation.



4.4 Interpretation of ImageQC Evaluation Results

An image evaluation result of the ImageQC software consists of a few components. As shown in the diagram below, they are QC result, Image trackline QC score, and QC blur score.



A. QC result:

The QC result is the overall result of the evaluation. In the case of tissue mounted S1 chip image, it

indicates if the image is qualified to be used as a map on which the transcriptomics data are overlayed and visualized. On the other hand, in the evaluation of a test chip image, it tells if a microscope is compatible to the STOmics protocols. There are two possible outcomes: PASS or FAILED. 'PASS' indicates a qualified image or a compatible microscope, while 'FAILED' means the opposite.

B. Image trackline QC score:

Track lines on a S1 chip is important for calibration and registration of a tissue stitched image to the transcriptomic data. Hence, when performing tissue mounting onto a S1 chip, users should ensure that at least 20% of the chip area remains uncovered by the tissue.

Tile images which constitute a stitched image can be classified based on the number of track lines that are clear and detectable by the ImageQC software.

1. **Model tile image**: a tile image with at least three vertical and three horizontal track lines detected.
2. **Qualified tile image**: a tile image with at least one vertical and one horizontal track lines detected.
3. **Unqualified tile image**: a tile image which does not fulfil the criterion 2 stated above.

To pass the evaluation, a stitched image of a test chip or a tissue mounted S1 chip should have at least one model tile image and hence has an Image trackline QC score of 0.5 and above, as shown in Table 5. Further, the track line QC score of a test chip image is usually higher than that of a S1 chip image because most of the track lines on the S1 chip are covered by the tissue.

Table 5. Derivation of different Image trackline QC scores. A score of 0.5 is the threshold to pass an ImageQC evaluation.

Image trackline QC score	Criteria	ImageQC Result
< 0.50	No detection of any model tile image	Fail
= 0.50	Detected one model tile image	Pass
> 0.50	Detected at least one model tile image and one or more qualified tile images Score = $0.5 + 0.5 \times \frac{\text{Number of qualified tile images}}{\text{Total number of tile images}}$	Pass

Note: If there are three vertical and three horizontal track lines can be clearly seen with naked eyes on a tile image, yet the image failed in the evaluation, please feedback to the IT team or the software developers. They could perform a more thorough evaluation for the image.

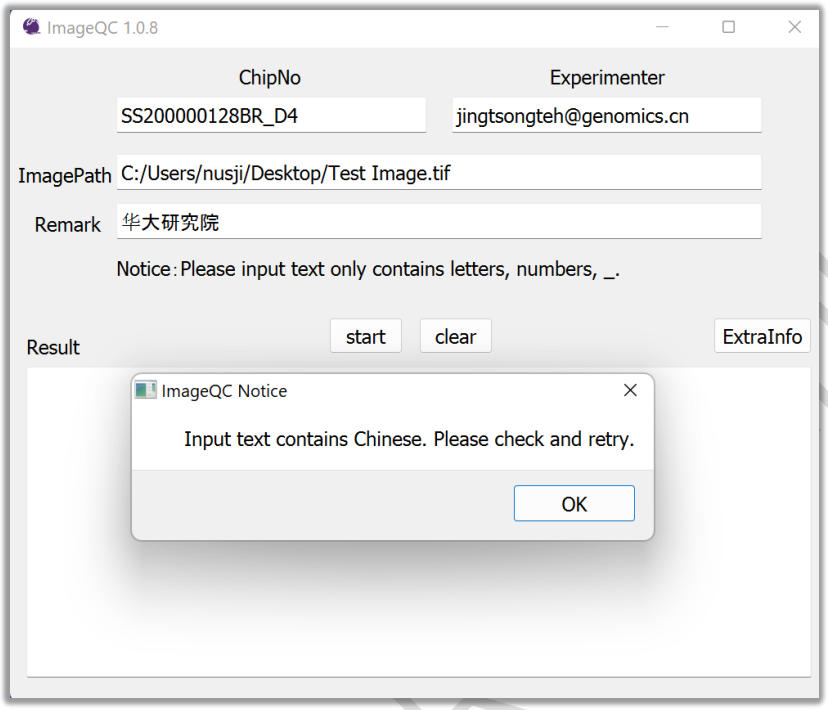
C. QC blur score:

The evaluation is performed by a deep learning model based on convolutional neural network, developed by BGI Research. The score presents the clarity and sharpness of a tissue image on a S1 chip. A distinct separation between cells (nuclei) is a key criterion to perform single cell segmentation analysis. A higher score represents a better tissue image quality. A score of zero is obtained for a test chip image

which contains no mounted tissue. Please take note that the QC blur score will not affect the final result of the evaluation – QC result, it only serves as a reference to assess the feasibility of performing single cell segmentation based on the nuclear stained image.

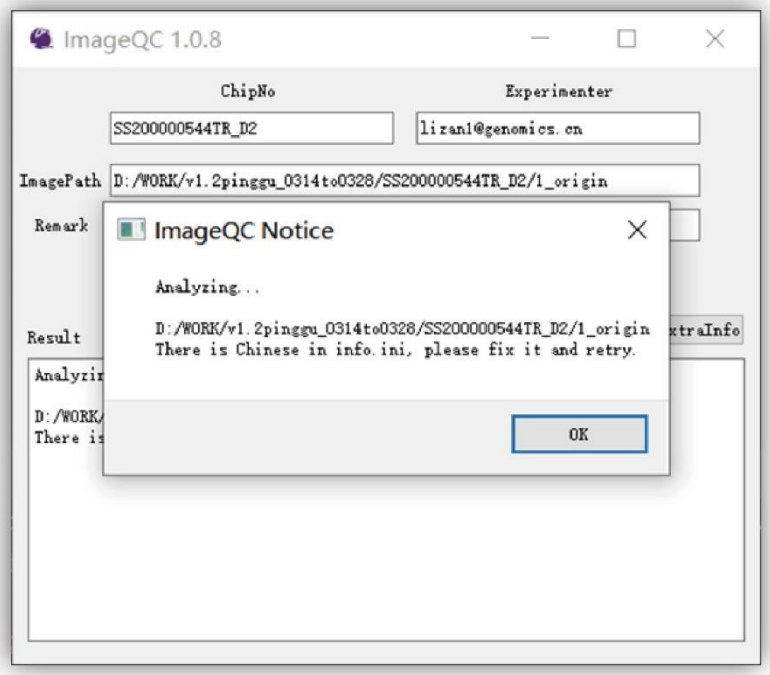
4.5 Errors & Troubleshooting

1. If there is a Chinese character in the input text, an error massage will appear as shown below:



Solution: Please make sure that the input content and image path do not contain any Chinese character. Remove the character to proceed.

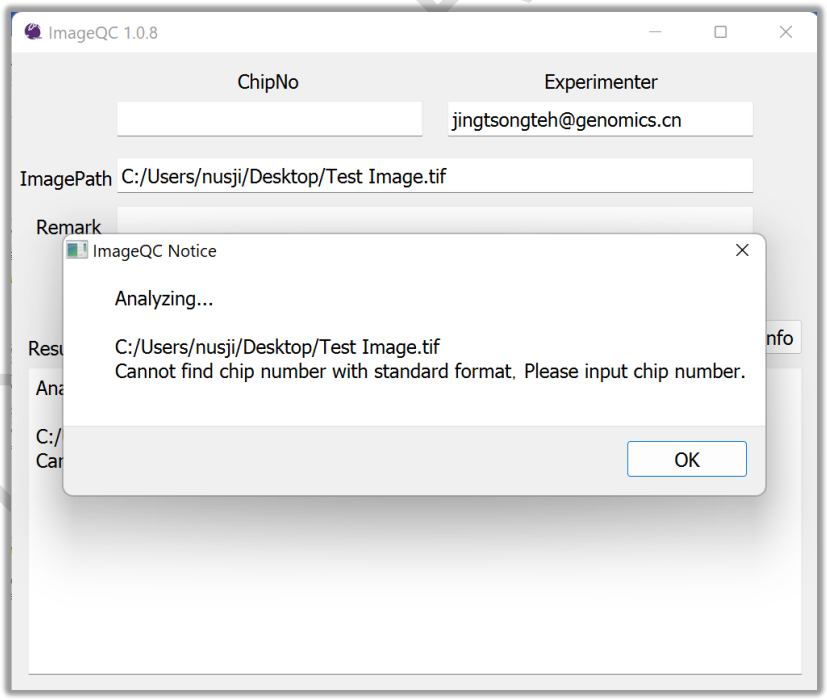
2. If "info.ini" file generated from a Motic imaging system contains a Chinese character, the message window below will appear in the program:



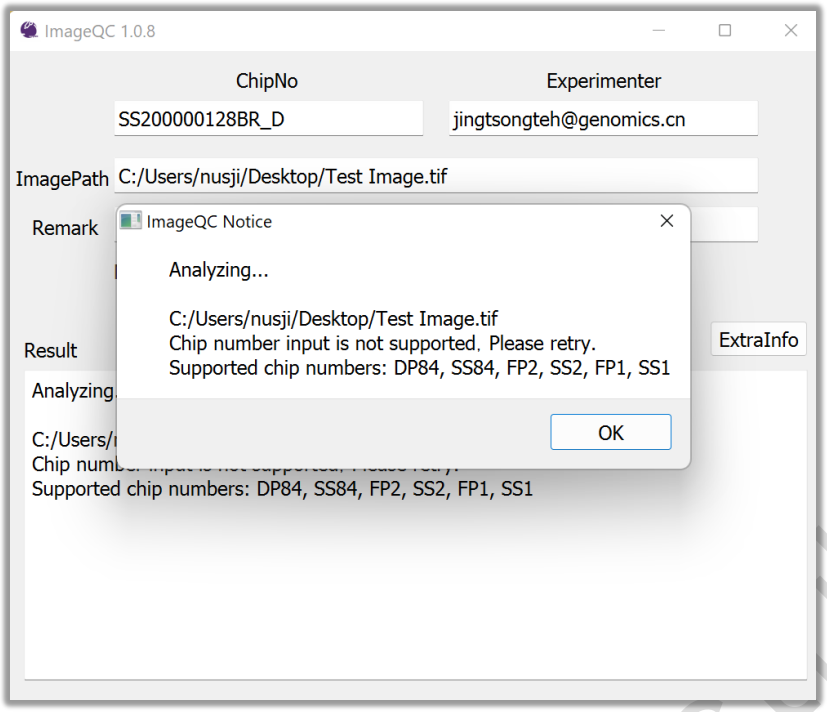
Solution: Please check and modify the content of the file and restart the evaluation.

3. If the chip number is missing or an unrecognized chip type has been entered.

A) If the chip number is missing, the following window will pop up:



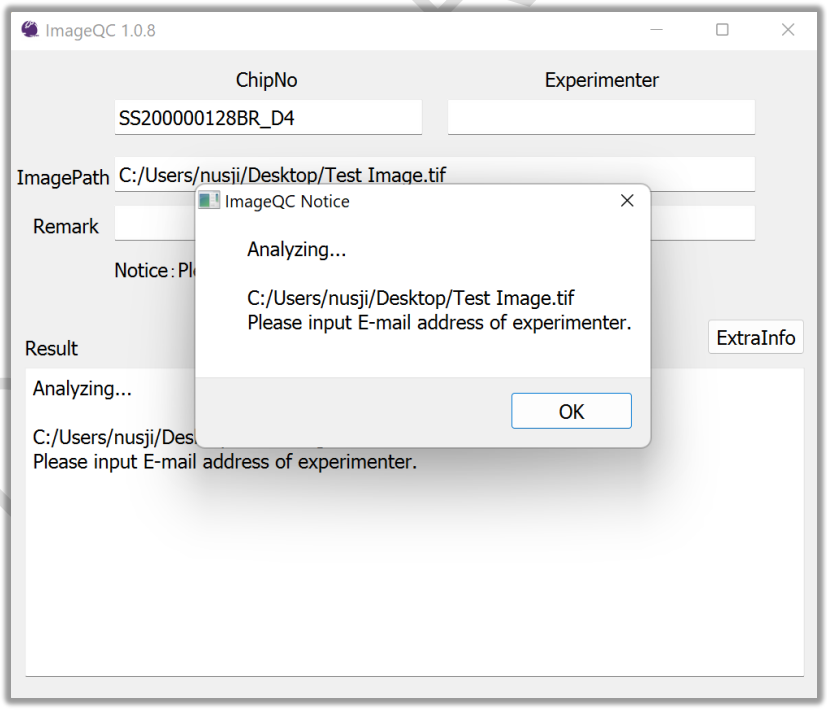
B) If a wrong chip number is entered, the following window will appear:



Note: The last line shows chip types supported by ImageQC software.

Solution: Fill in or correct the chip number and resume the evaluation.

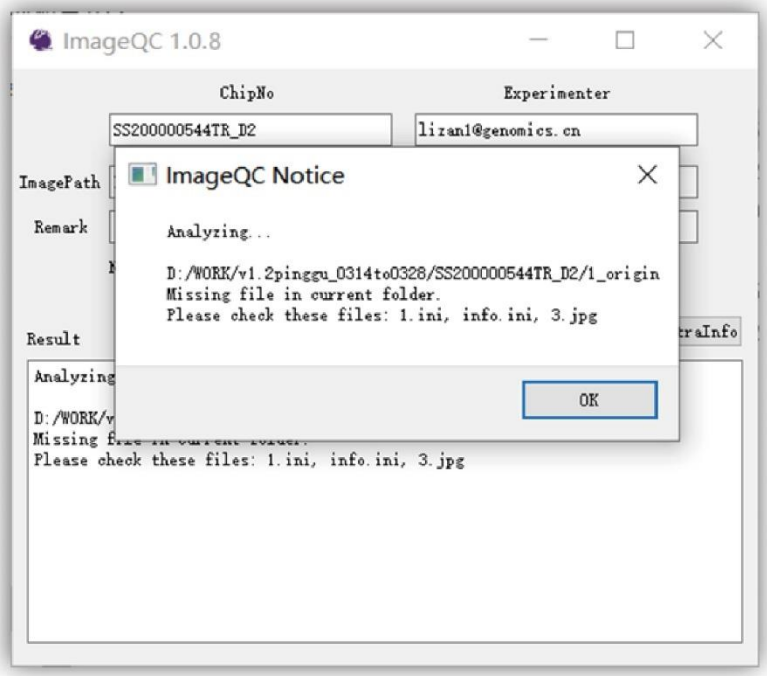
4. If the experimenter's email address is missing, the following window will appear:



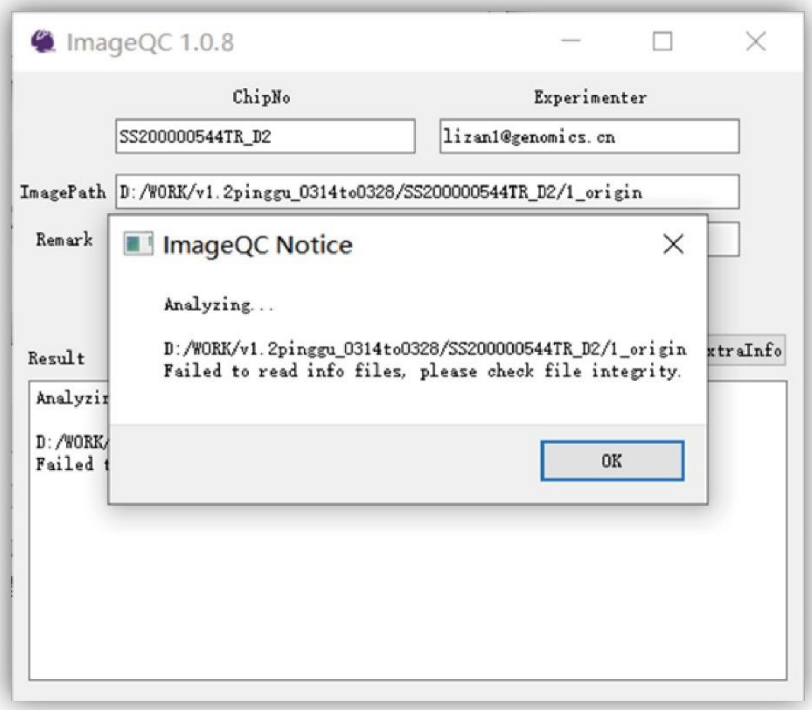
Solution: Fill in the email address and resume the evaluation.

6. If there is a missing or damaged file in a Motic image folder, the following window will appear, and

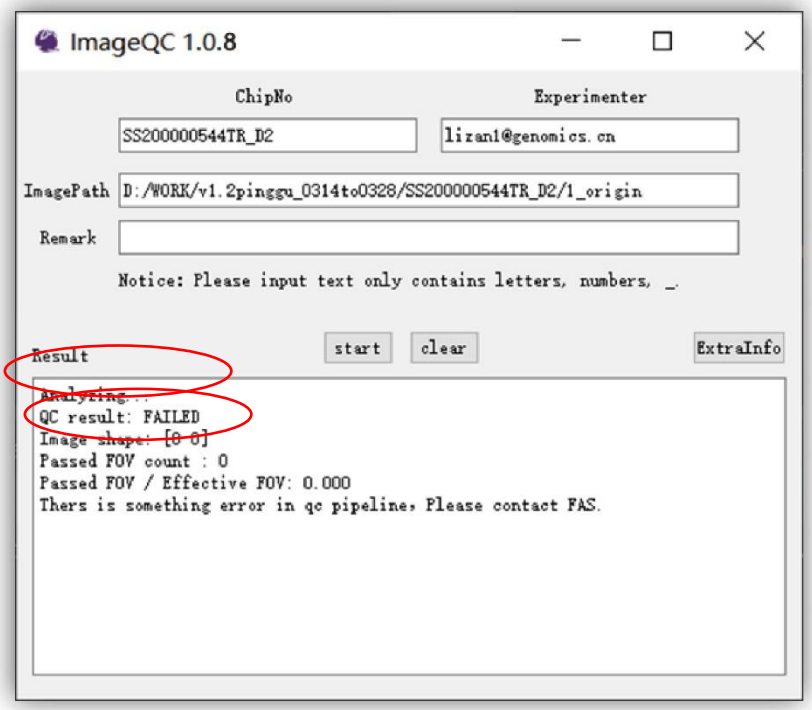
the evaluation will be halted:



Solution: Follow the instructions in the pop-up window. Taking the case above as an example, one of these files: "1.ini", "info.ini" and "3.jpg" are missing. The user may have to re-capture a new image since these files are auto-generated by the microscope during imaging. In the other case shown below, the "1.ini" or "info.ini" file is incomplete or damaged. It is probably due to a missing information clause in the "info.ini" file. Check if all image parameters are entered correctly during imaging, the user may need to redo imaging if necessary.

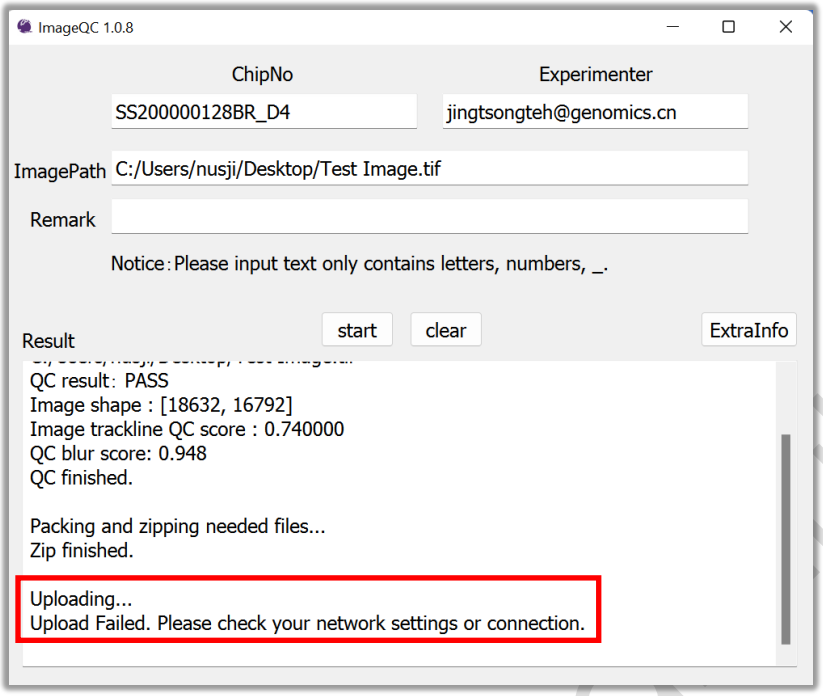


7. If it is indicated in the QC result that the image shape is [0 0] as shown in the following:



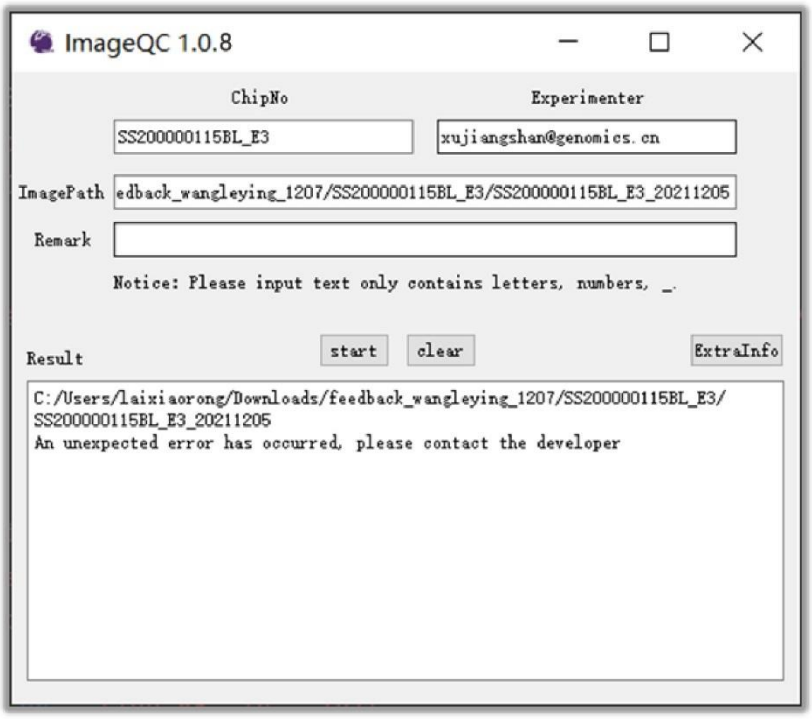
Solution: This is probably due to the program's internal errors. Make a screenshot of the window and contact a BGI FAS for assistance.

8. When failed to upload the ImageQC output files, the following window will pop up:



<u>Solution:</u> Please check the internet connection to resume the evaluation

9. If an unexpected error occurs, the program will display the following content:



<u>Solution:</u> Please check all image parameters entered. If the issue is still not resolved, please send

the image and the corresponding parameters to a FAS of BGI, they will provide feedback to the software developers.

Appendix. Microscope API (Application Programming Interface) Development

A. Introduction

Automated microscopy can capture specimens larger than the microscope's field of view (FOV) by stitching overlapping image tiles. In STOmics analysis, a tissue specimen stitched image (ssDNA-stained fluorescence) is used/ registered as an anatomical map on which transcriptomic data are visualized. Image stitching accuracy is therefore an important factor in spatial visualization of the data, and improving it has always been a challenge in the field.

We improve the accuracy by re-stitching tile images of a specimen with corrections based on the images' background track lines (originated from STOmics chips). The process also requires the **native stitching parameters** which are microscope-specific. Hence, an application programming interface (API) must be developed to tailor the microscope's software to SAW, a set of analysis pipelines to process STOmics transcriptomic data. Refer to section B for API development requirements. Once an interface has been developed, with the post-imaging correction algorithm, we can significantly improve the accuracy in anatomical mapping of gene expression data. This allows spatial clustering of gene expression data at single cell level – **Cell-bin***.

* Most microscopes in the market have a stitching accuracy of around 10 pixels. However, to achieve single cell segmentation (Cell-bin), an accuracy of ≤ 5 pixels is required.

B. Image Files, Parameters and Information needed in Microscope API Development

Provide one of the following options to help our R&D team to retrieve sufficient information from an image raw file or exported tile images (TIFF/ PNG):

Option 1:

- Open-source library, written in python language
- Image raw files (in original format, e.g., vsi format from Olympus VS200)
- Parameters of image raw files stated in the table below:

Parameter	Corresponding file name	Field name in the file
TempPath		
Manufacturer		
Scale		
BitDepth		
OverlapX		
OverlapY		
ScanRows		
ScanCols		

FOVHeight		
FOVWidth		
SlideInfo		
Channel		
Experimenter		
RemarkInfo		
ImagePath		
DeviceSN		
ControllerVer (Imaging Software)		
CameraID		
CameraName		
ComputerName		
AppBuildDate		
AppName		
AppFileVer		
GUID		
ScanTime		
ScanObjective		
ExposureTime		
RGBScale		
Brightness		
ColorEnhancement		
Contrast		
Gamma		
GammaShift		
Sharpness		
DistortionCorrection		
BackgroundBalance		

** Bold parameters in the table are necessary, the rest are optional.

Option 2:

- Microscope's auto-stitched image exported in TIFF/ PNG format
- All tile images (individual FOVs) exported in TIFF/ PNG format, and
  - Information files of the exported tile images
  - Parameters of the exported tile images stated in the table below:

Parameter	Corresponding file name	Field name in the file
TempPath		
Manufacturer		
Scale		
BitDepth		

OverlapX		
OverlapY		
ScanRows		
ScanCols		
FOVHeight		
FOVWidth		
SlideInfo		
Channel		
Experimenter		
RemarkInfo		
ImagePath		
DeviceSN		
ControllerVer (Imaging Software)		
CameraID		
CameraName		
ComputerName		
AppBuildDate		
AppName		
AppFileVer		
GUID		
ScanTime		
ScanObjective		
ExposureTime		
RGBScale		
Brightness		
ColorEnhancement		
Contrast		
Gamma		
GammaShift		
Sharpness		
DistortionCorrection		
BackgroundBalance		

** Bold parameters in the table are necessary, the rest are optional.

- Stitching Information
  - How to retrieve the column and row positions of each image tile (individual FOV) in an auto-stitched image generated by the microscope.

    ** If there is no serial number assigned to each image tiles, kindly tell us the arrangement mode (e.g., linearly from left to right, or in a zig-zag pattern).
  - How to retrieve the coordinate of each image tile's origin (i.e., the top left corner pixel) in the auto-stitched image, and the computational logic behind.
    - Optional: Coordinate of each image tile's origin in the auto-stitched image, refer

to the table below:

Individual image file path	FOV_X (stitched image)	FOV_Y (stitched image)