STOmics

Microscope Assessment Guideline

Manual Version: A1

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	◆ Stitched large image instruction ◆ 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

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

Chapter 1 Introduction

This document is a guideline for assessing whether a microscope can be used for STOmics imaging and photographing purpose by providing the use of STOmics Test Chip and ImageQC software.

Chapter 2 General instruction

2.1 Microscope Assessment Preparation

Material

STOmics Test Chip (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)

Notes for Imaging

Avoid touching the top active surface of the chip.
Place the Imaging Test Chip on the chip capture area of the object stage before testing and imaging.
The microscope scan area should cover the entire chip surface including the four corners.
Image resolution ≥ 1800 pixels (height) and ≥2000 pixels (width).

The imaging system should have functions of taking sharp images, scanning an area of 10mm×10mm, and stitching FOVs automatically. The computer should be capable of exporting FOV (field of view) raw image and stitching images in TIFF or PNG format. It should also be capable of processing large image (>5 Gb) and installing a third-party image processing software. (e.g., ImageQC)

2.2 Imaging System Configuration

Overview of Microscope Requirements

Index/Parameter	Description
XY stage travel distance of microscope	At least 25*75mm
Target of scanning	STOmics Test Chip
Brightfield	Upright microscope, Reflection mode

Focusing approach	Pre-focus map, Real-time autofocus
Fluorescent channel	DAPI、FITC、TRITC、CY5
Objective lens	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	16bits
Background balance	Adjustable background balancing function required
Distortion correction	Adjustable distortion correction function required
Overlap ratio	Adjustable, 10% by default
File format	Is capable of viewing and exporting stitching (optional) and raw images (mandatory), 8/16 bit, TIF/PNG, grayscale or colored images
Hardware config requirement	Windows10 ×64 system, 16G memory or beyond

Recommended Imaging Configuration:

Recommended Brightfield Configuration (for H&E staining)

Reflective brightfield
Color camera (3x8 bit)
White balancing function
5μm maximum pixel size
Exposure time 0.1-100ms

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)
TRITC filter cube (Excitation 545/25, Emission 605/70)
5μm maximum pixel size

Exposure time 1ms-2s

2.3 Imaging System Recommendations

Supplier	Description
Leica	Leica DM6M
Zeiss	Automated Slider Scanning Microscope Axio Scan Z1 Automated slide scanner Zeiss Axioscan 7

2.4 Test Chip

STOmics Test Chip (Cat. No.1000029779)

2.5 Imaging Test Procedure

Chip staining	• Transfer the chip from the box to a 24-well cell culture plate using forceps. Note that the chips have front and back sides. Pay attention to keep the front side facing up, and do not scratch the surface. • Ensure the top surface of the chip is clean. If a white wave pattern is seen, rinse the chip with 400µL Nuclease free water twice and drain liquid from chip surface, then dry it in the oven at 37°C for 1min before staining. • The chips were placed in a petri dish with a sealing membrane. Add the staining solution which the preparation method referred in Table 1 to the surface of the chip then incubate in dark for 5min. Remove excessive staining solution with a pipette. Rinse with 200µL of Nuclease-free water. Dry the surface of the chip with a dust remover. Then cover the well with foil and keep it in dark before imaging. Table 1 Imaging Test Chip staining solution <table><tr><td>Component</td><td>Volume</td></tr><tr><td>Invitrogen ® Qubit ssDNA Buffer</td><td>199µL</td></tr><tr><td>Qubit ssDNA Reagent</td><td>1µL</td></tr><tr><td>Total</td><td>200µL</td></tr></table>
Channel selection	• Use FITC channel with 10x objective lens.
Fluorescence imaging	• Place the chip on the imaging stage. The chip should be parallel to the stage or with an angle between less than 8°. • image the entire chip including the four corners using the fluorescence channel Clearly.

QC Image	• Download the latest version ImageQC software package, and refer to 2.6 QC software introduction. • QC software can receive the original micrograph path or splice the micrograph of the microscope in 2.3. • If the microscope is not the one recommended in 2.3,a spliced micrograph of TIFF/PNG format can be used for evaluation. Then, the original file of the microscope and the exported FOV TIFF/PNG image original file are packed to the ImageQC developer for the development of the corresponding QC interface. If the original FOV image cannot be automatically saved and manually exported, please contact FAS.

2.6 QC Software Introduction

2.6.1 Software Installation Instruction

1.Run ImageQC-Setup.exe in the minimum configuration for Windows 10 x64 system, memory 16G.

 ImageQC-Setup.exe

2.Select install mode. You are advised install for all users.
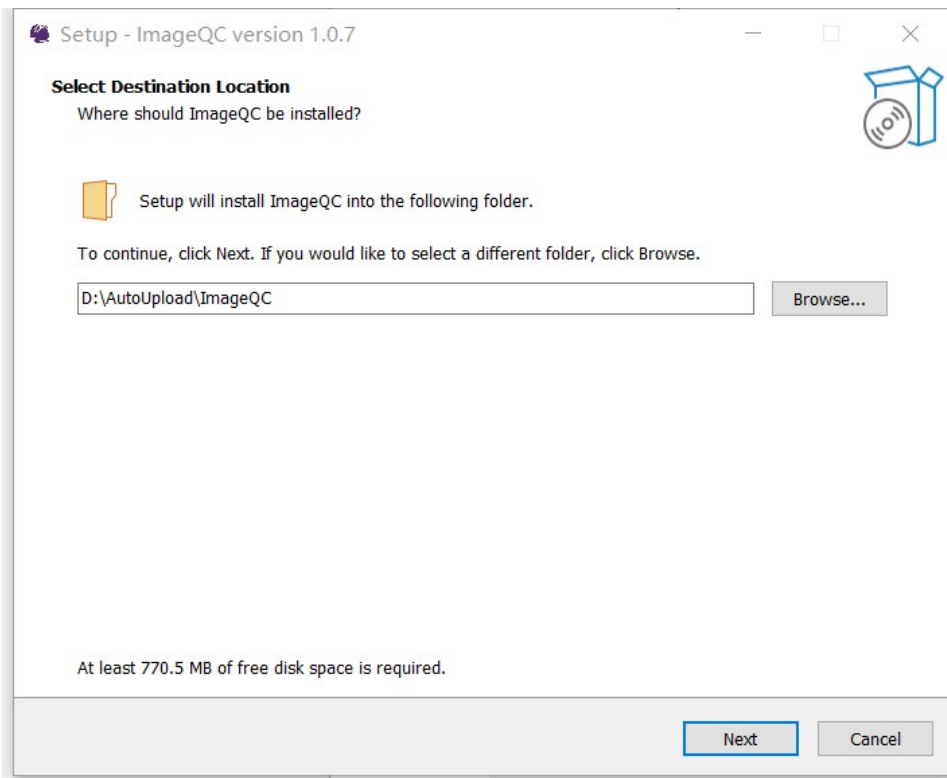
 Select install mode

ImageQC can be installed for all users (requires administrative privileges), or for you only.
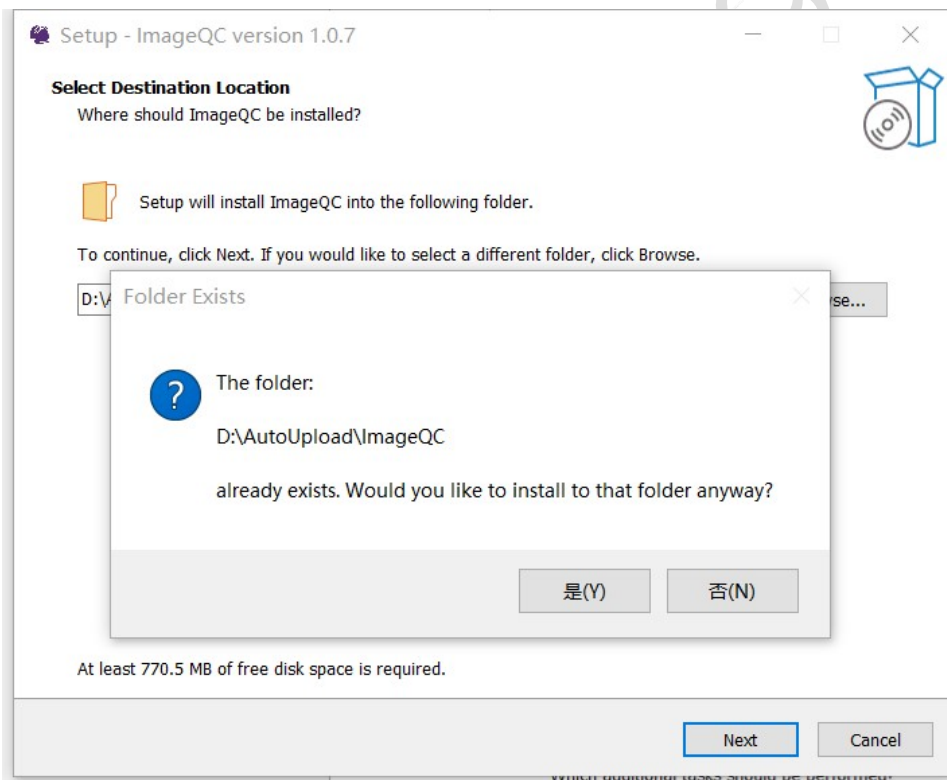
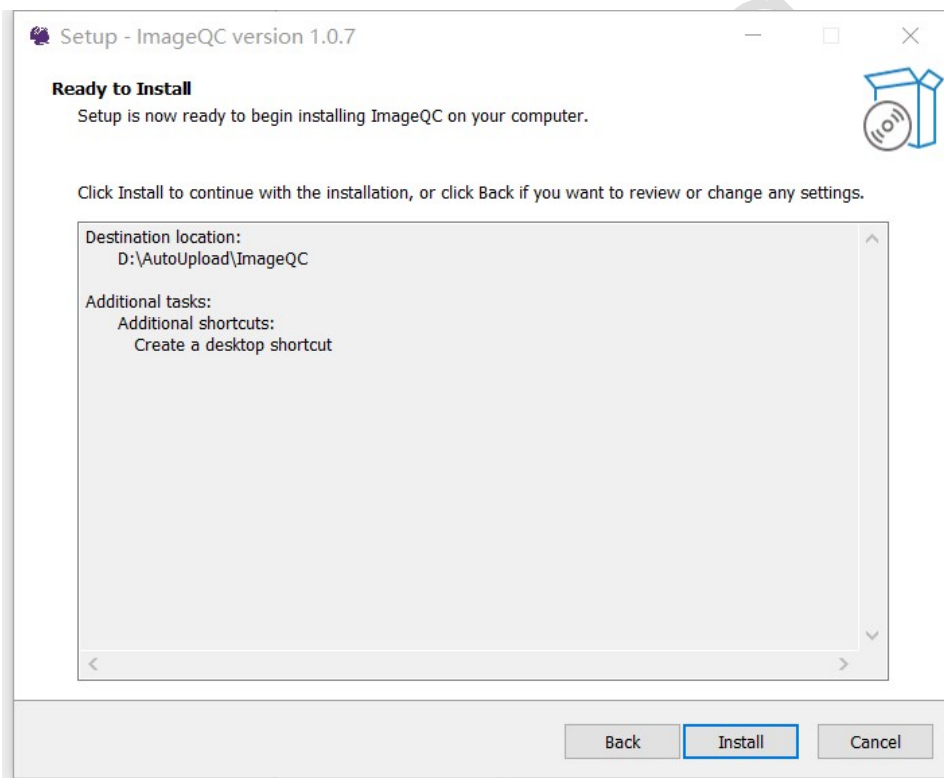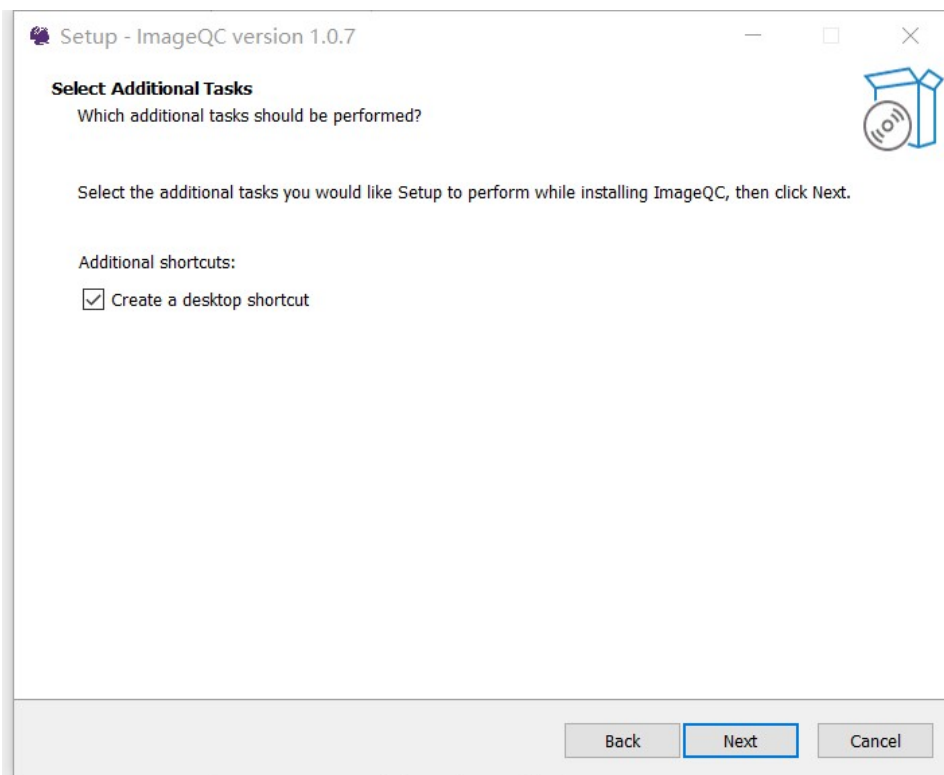 Install for all users (recommended)

→ Install for me only

取消

3.Select destination location. Installation on drive C may cause errors due to permissions. You are advised to install it on drive D

If ImageQC has been installed before, select "yes" for the pop-up window shown as below.

4.Select additional shortcuts and install.

Setup - ImageQC version 1.0.7

Select Additional Tasks
Which additional tasks should be performed?

Select the additional tasks you would like Setup to perform while installing ImageQC, then click Next.

Additional shortcuts:

☑ Create a desktop shortcut

Back | Next | Cancel

Setup - ImageQC version 1.0.7

Ready to Install
Setup is now ready to begin installing ImageQC on your computer.

Click Install to continue with the installation, or click Back if you want to review or change any settings.

Destination location:
    D:\AutoUpload\ImageQC

Additional tasks:
    Additional shortcuts:
        Create a desktop shortcut

Back | Install | Cancel

5. After the installation is complete, select "Launch ImageQC" and click "Finish" to launch the program.
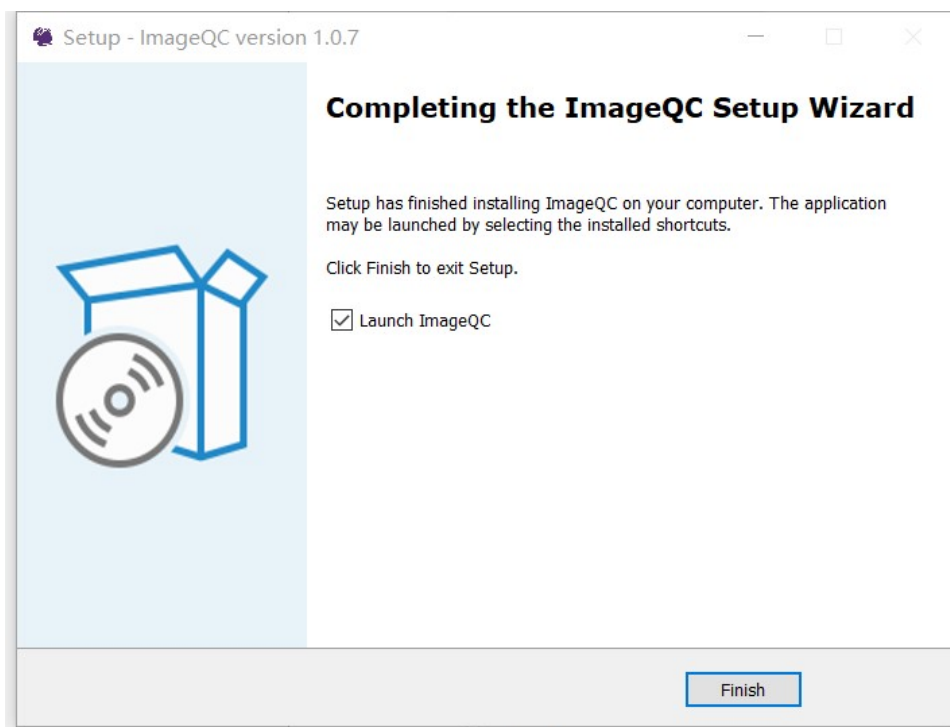
2.6.2 Software Operation Instruction

1. Open the ImageQC software after imaging (you can create a desktop shortcut, and keep it the program running) .

2. Drag and drop the file or folder into the program window

Motic microscope: drag the folder in which the mdsx file is located.

Zeiss microscope: drag the czi file.

Other microscopes: enter the path where the exported images are saved.

Notice：For microscope types that cannot automatically save the FOV images and cannot be manually exported, please ask FAS for assistance.

Chip Number.: Enter the chip number of the captured image. The QC program can only support standard STOmics chip number.

Experimenter: enter the mailbox prefix of the person who perform the test.

Notice: If the chip number and the mailbox of the experimenter have been entered when imaging, these two fields will be filled automatically, or the newly entered information will overwrite the ones entered during imaging.

Remark: Record additional descriptive information at the remark field if needed.

ExtraInfo: When dragging a tif, czi image without any image information, the QC program will pop out a window like the screenshot below

- Microscope: enter the name of the microscope
- Chip type: enter the standard STOmics chip number
- Magnification: enter the magnification
- Image Ratio: enter the ratio of the image, the unit is μ m/pixel, please enter at least 3 decimals,
- FOV width and FOV height: enter the size of one FOV
- Overlap: enter the overlap between the adjacent FOVs (if the overlap of height is different than the overlap of the width, please use the largest one)
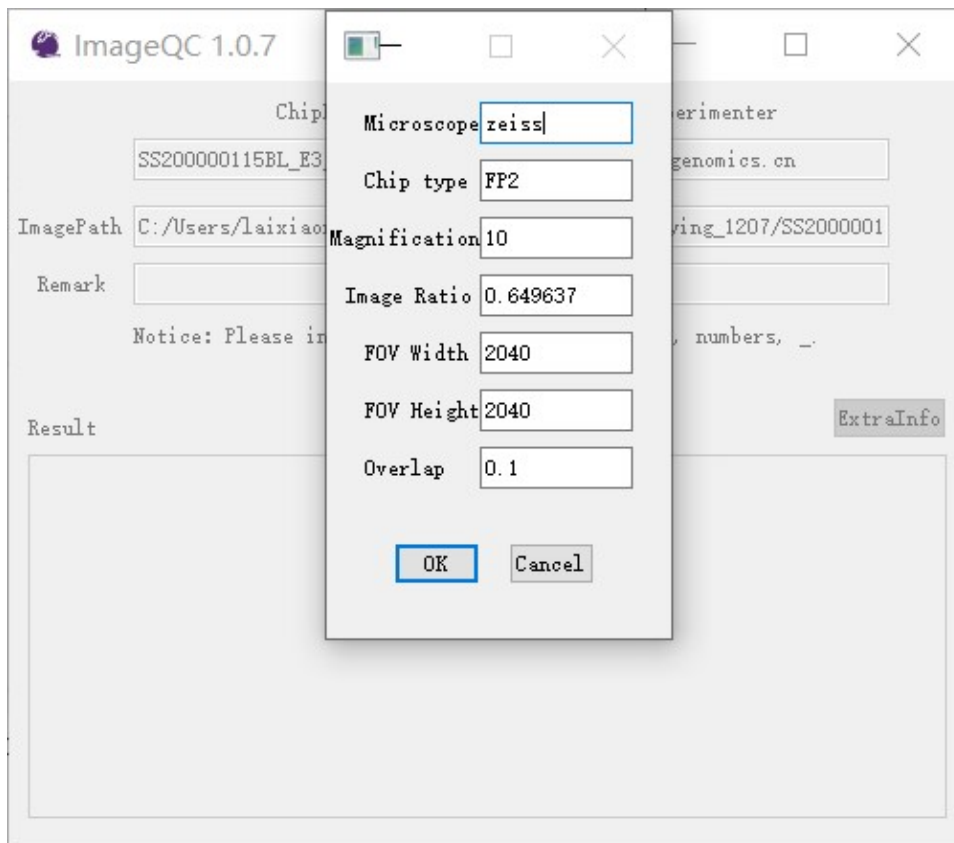
3. Click the "Start" button to start QC, then "Analyzing..." will be displayed. The QC process is now in progress.


4. After QC is finished, the program will display the QC results. "PASS" will be displayed if QC passes; "FAILED" will be displayed if QC fails. If the QC fails, please check the images, chip number, or redo the imaging test again. If it still fails, please contact FAS. The last line showing "QC finished" means that QC process is complete.
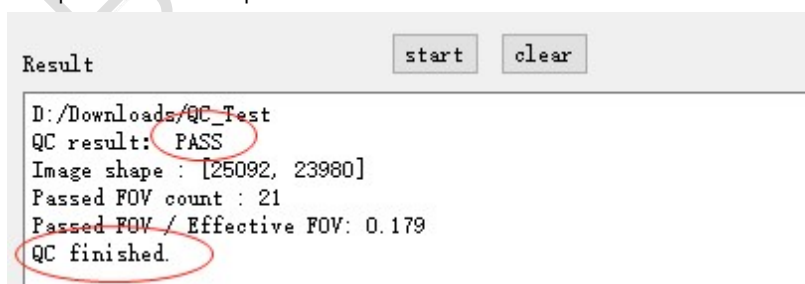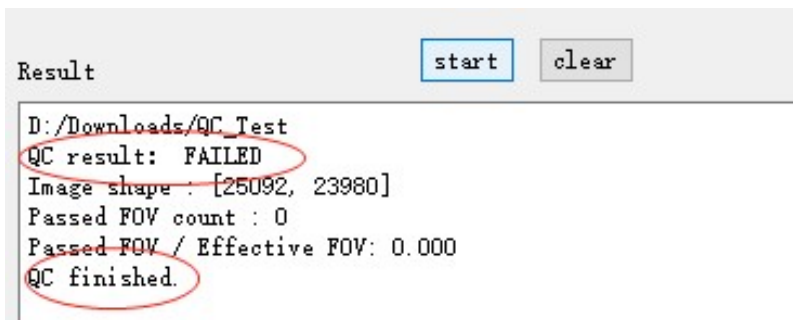
5. After QC is finished, the program will compress the files into zip files. The program will display "Packaging and zipping needed files". "Zip finished" will be displayed when the files are compressed.
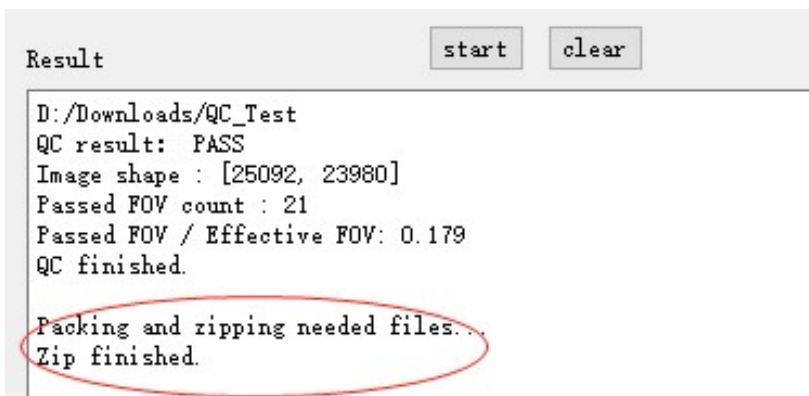
If data upload is not required, click the "clear" button to clear the content in the program window. Then the program is now ready for the next image QC.

6. Description for exceptions

1) If Chinese character is entered as chip number or other information, the program will pop up the following window:
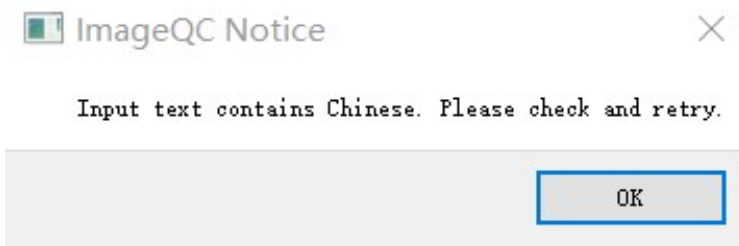
Please confirm that the input content and image path do not contain Chinese, and then restart QC.

2) Motic error: If the info.ini file output by the microscope contains Chinese, the program will pop up the following window.

ImageQC Notice ✕

D:/Downloads/QC_Test
There is Chinese in info.ini, please fix it and retry.

OK

Please check and modify the content of the file and restart QC.

3) If the QC result output directory already has a QC passed file with the same chip number, the program will display the following content:

Result   start   clear

D:/Downloads/QC_tst_data/FP2_QC_Test
Find existed files with pass flag of same chip number, QC stopped.

The program will automatically skip the QC process and proceed directly to step 5.

Notice: If there is an image with the same chip number in the directory, you need to move the result files corresponding to the same chip number to other places or delete the result files under the QCImgUpload folder to rerun QC.

📁 ImageQC
📁 QCImgUpload

The result files corresponding to one chip number includes a json file and a tar. gz file, as shown below:

FP200000430BL_C2_20210601.json

FP200000430BL_C2_20210601.tar.gz

Move these two files to the other location or delete them.

4) If you forget to enter the chip number and experimenter information, or if you enter the unsupported chip type, the information in the info file will be empty or wrong.
If the chip number is not entered in QC either, the program will pop up the following window:

ImageQC Notice ✕
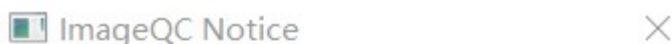
D:/Downloads/QC_tst_data/QC_Test
Cannot find chip number , Please input chip number.

OK

If wrong chip number is entered, the program will pop up the following window:

```
D:/Downloads/QC_tst_data/QC_Test
Chip number input is not supported, Please retry.
Supported chip numbers: DP84, SS84, FP2, SS2, FP1, SS1
```
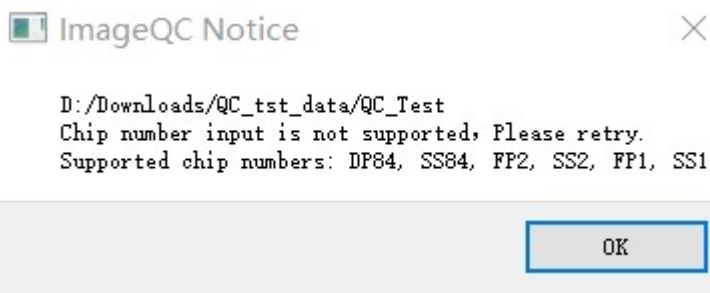
The last line shows the supported chip types.

5) If the email address of experimenter is not entered during or after QC, the program will pop up the following window:


```
D:/Downloads/QC_Test
Please input E-mail address of experimenter.
```
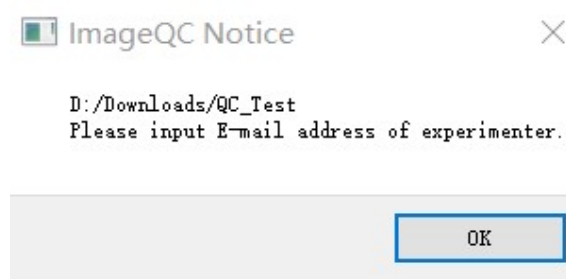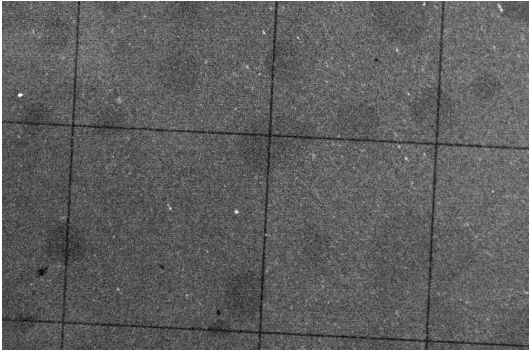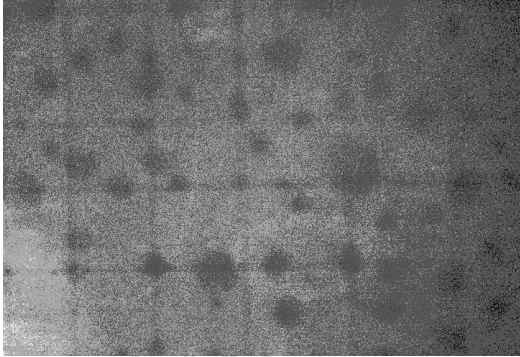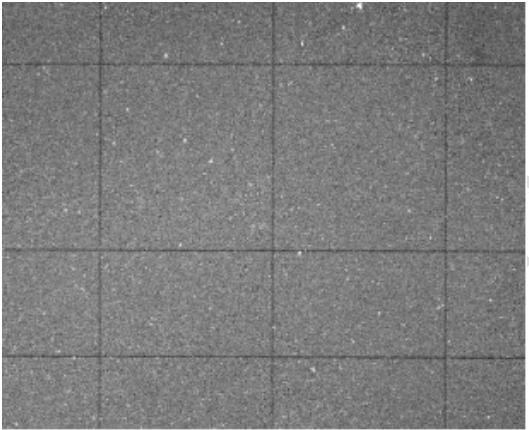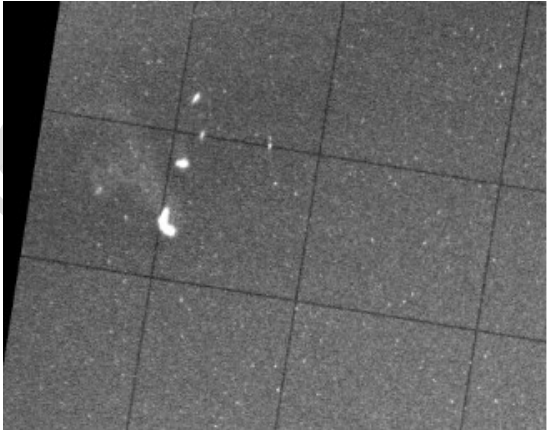
6) If an unexpected error has occurred, the program will display the following content. Please fed back to the developers the params you entered together with sample data if the error has occurred again after you checked the params.
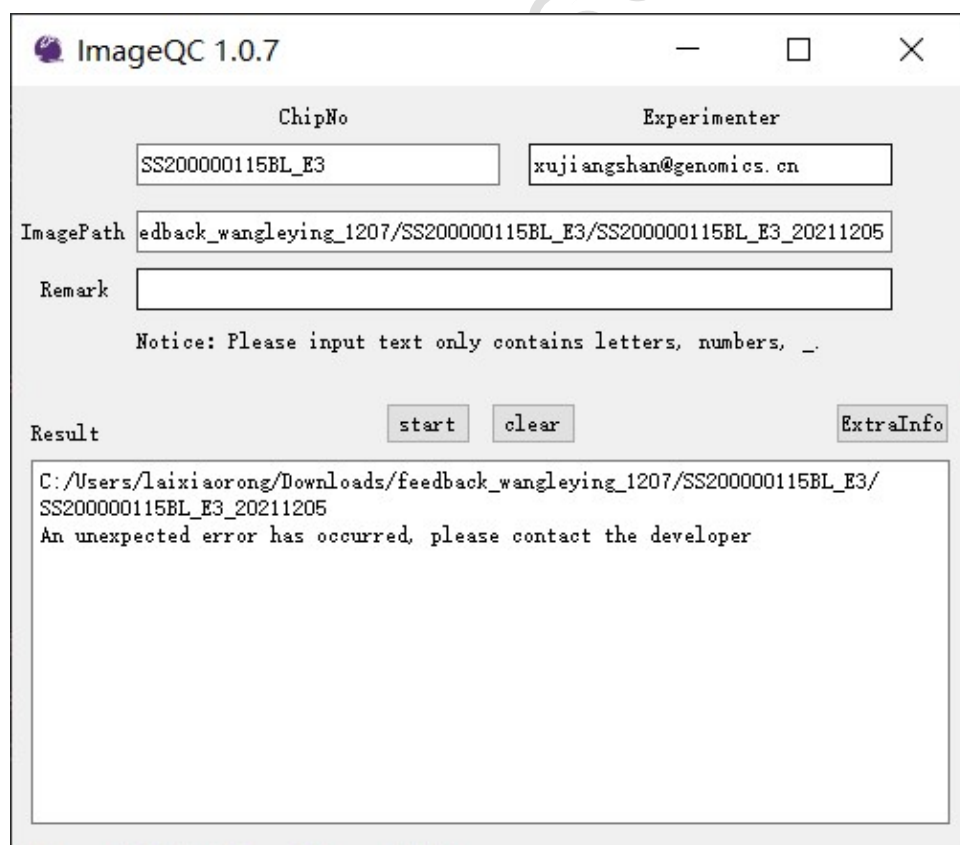
2.7 Image Example

Please refer to the images shown below for QC pass example on the left and QC fail example on the right.

Parameter	QC pass example	QC fail example
Track line		
Inclining Angle of track (less than 8°)		
The scratch		