

FISH Analysis System V2.0

User Manual

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Introduction

FISH analysis system is a practical software designed for fluorescence microscope to acquire image and analysis image.

The FISH analysis system is equipment with user friendly visual interface, clear marked icon and identification, make it simple to use and simplified operation process. It greatly reduce user incorrect operation rate, with improvement by professional engineer, the FISH software not only offer user routine necessary image acquire function but also image annotation and image enhancement, etc function.

System requirement

To install and use FISH analysis system, you need below device (or higher configuration) and software:

Configuration	Requirement
CPU	Intel core 2 duo E2140
Memory	2 GB
Free hard disk space	160 GB
Monitor	17 inch (screen resolution 1280×1024 , 24/32 bit true color)
Operating OS	Windows® XP/WIN7/WIN8/WIN10

Installation guide

1. Copy FISH V2.0 software file to PC.
2. Double click icon, system pop out installation windows.
3. Click[Next] (Image 01)

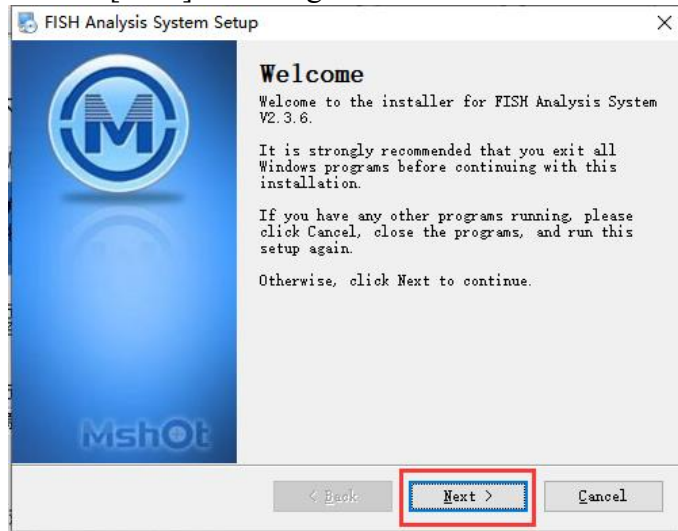


Image 01

4. Choose 'I agree', click [Next].(Image 02)

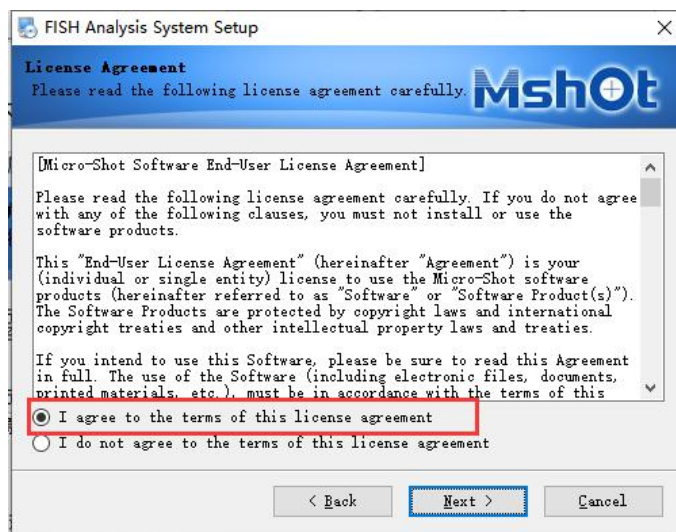


Image 02

5. Click [Next] (Image 03)

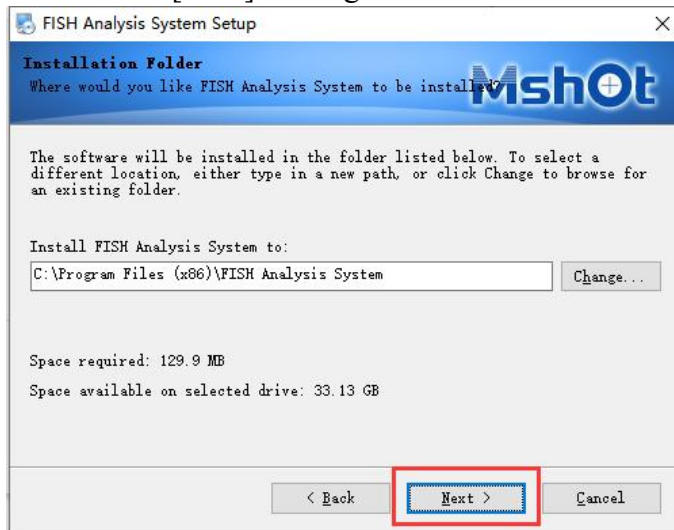


Image 03

6. Click[Next] (Image 04)

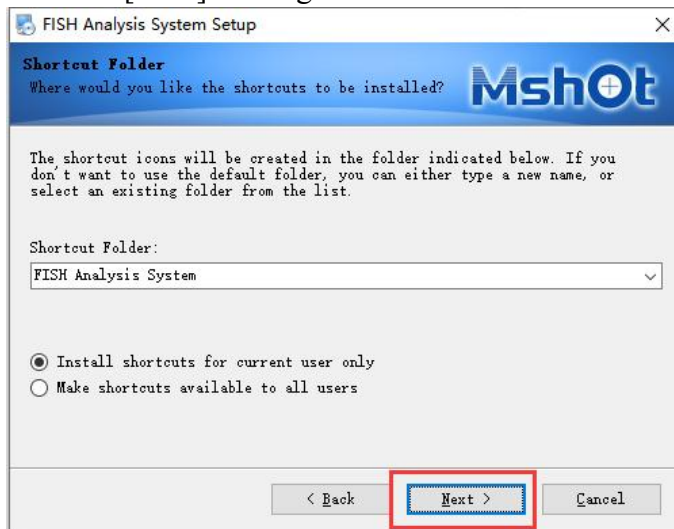


Image 04

7. Click **Finish** (Image 05)

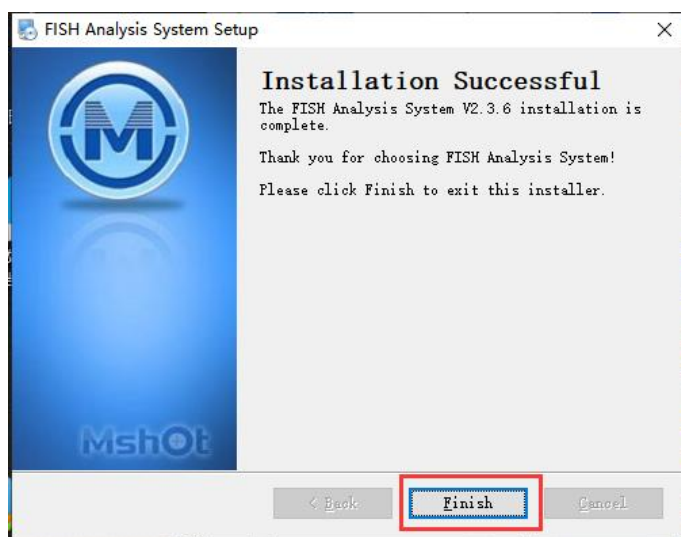


Image 05

8. Installation finished, shortcuts icon created on PC desk, click to run.

Key installation

MSHOT FISH analysis system request to open and use with Key, Windows® XP/Vista/7/8/10 has preset driver of hardware key, user only need to insert key to USB port, system will auto read driver information.

Operation UI

FISH analysis system UI is simple for user to understand. (Image 06)

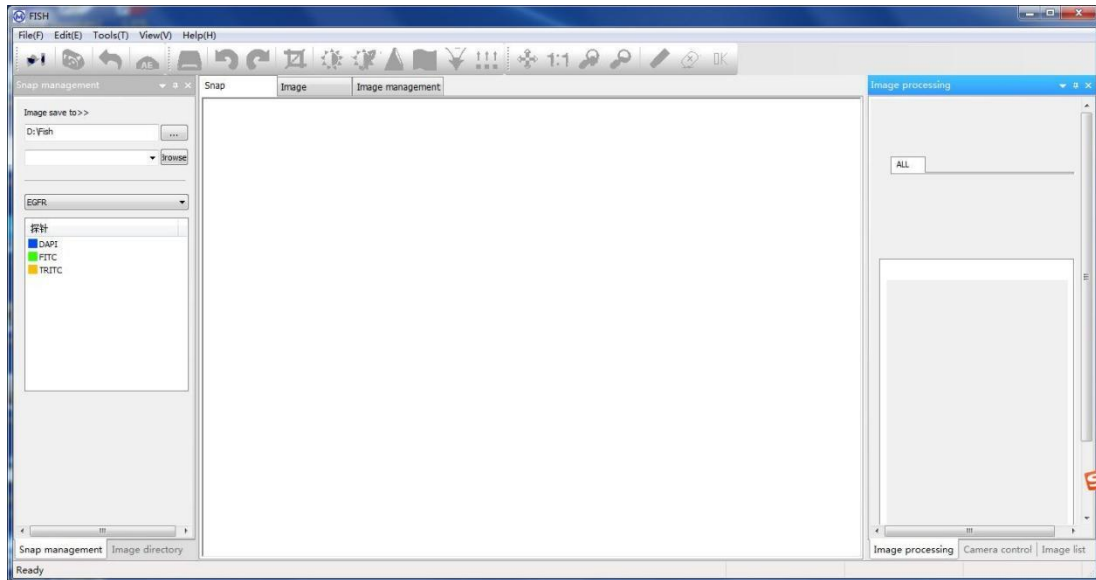
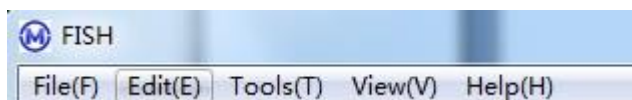


Image 06

Menu bar

When program under view mode, all menus are operable.



Primary Menu	Secondary Menu	Functions
File (F)	Exit system	Exit system
Edit (E)	Step backward	Unavailable
	Crop	Unavailable
	Copy	Unavailable
	Paste	Unavailable

Primary Menu	Secondary Menu	Functions
Tools (T)	Options	Start Snap and Probe functions
View (V)	Camera control	Select or cancel camera control module
	Image list	Select or cancel image list module
	Image processing	Select or cancel image processing module
	Image library	Select or cancel to show image library
	Status bar	Select or cancel to show status bar
Help (H)	About FISH analysis system	Open FISH analysis system version information

Tool bar

FISH analysis system shortcuts menus are as below:



Menu bar introduction:



: Connect camera to PC and select 'Snap' windows, click it to open or close camera and show image in main windows.



: Select 'Snap' to view image, click the icon can snap according to image list.



: Select 'Snap' windows to snap image, click 'Retake' image, probe in image list getting back to last probe.



: Select 'Snap' to view image, show auto zone exposure, the probe is default to be selected, 'auto exposure' in right sidebar under camera control is selected; re-click it to cancel auto exposure, 'auto exposure' in right sidebar under camera control is canceled.



: Select 'Image' windows, click pop up 'open' windows, it can open image under default file.



: Select 'Image' windows and image opened, click 'Save' image.



: Select 'Image' windows and processed image opened, click to 'Step backward' image.



: Select 'Image' windows and 'Step backward' image opened, click to 'Step forward' image.



: Select 'Image' windows and image opened, click to crop yellow zone image, right-click to confirm crop.



: Select 'Image' windows and image opened, show contrast adjustment on offside of 'image processing', can adjust image of each probe with brightness, contrast, GAMA and related parameters.



: Select 'Image' windows and image opened, show contrast adjustment on offside of 'image processing', can adjust selected area image of each probe with brightness, contrast, GAMA and related parameters.



: Select 'Image' windows and image opened, sharp current image and show in-time result.



: Select 'Image' windows and image opened, smooth current image and show in-time result.



: Select 'Image' windows and image opened, click to show image in auto adaption.



: Select 'Image' windows and image opened, click to show image in 1:1 proportion.



: Select 'Image' windows and image opened, click to zoom in image.



: Select 'Image' windows and image opened, click to zoom out image.



: Select 'Image' windows and image opened, reduce background of current image and show in-time result.



: Select 'Image' windows and image opened, signal enhancement of current image and show in-time result

Start FISH analysis system

1. Connect camera to PC and install driver.
2. Open microscope light source, turn light to camera.



3. Insert Key, double click software icon to run program.

Acquire and probe setting

1. Select [Tools]--[Options], set snap resolution under 'Snap' menu, modify or add need probe and probe kit under 'Probe' menu. (Image 07~09)

Note: This function can be used when the camera preview mode is turned off.

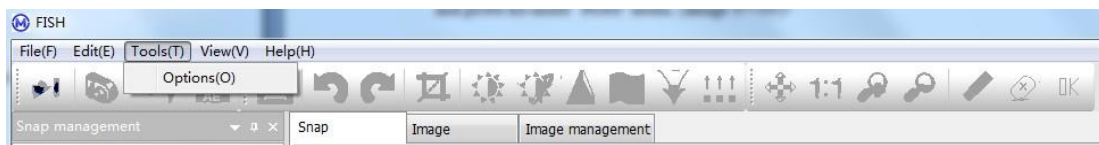


Image 07

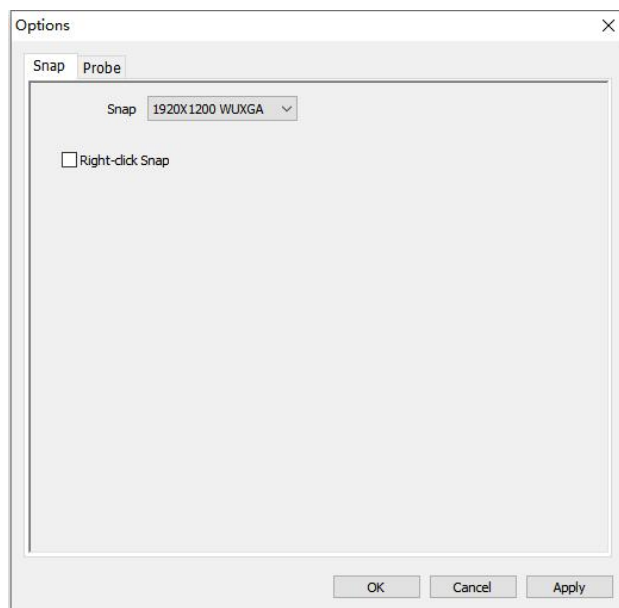


Image 08

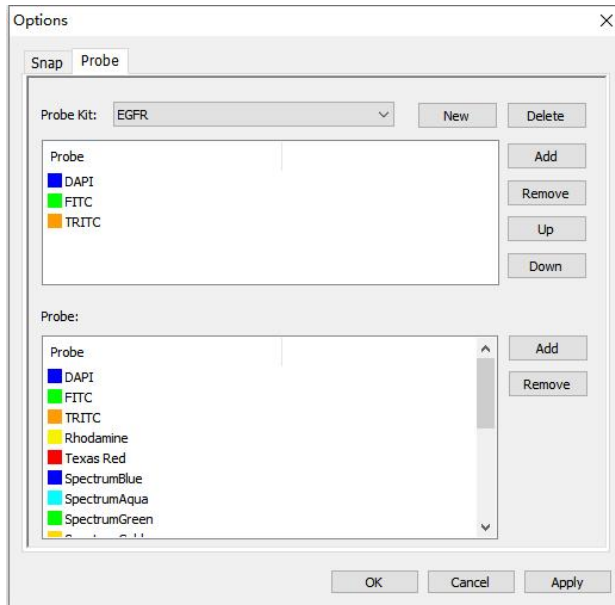


Image 09

2. If there is no matching probe in the default probe set, you can manually add a custom probe set. The operation method is as follows:

2.1 Create a new probe set and select the probe set (Figure 10)

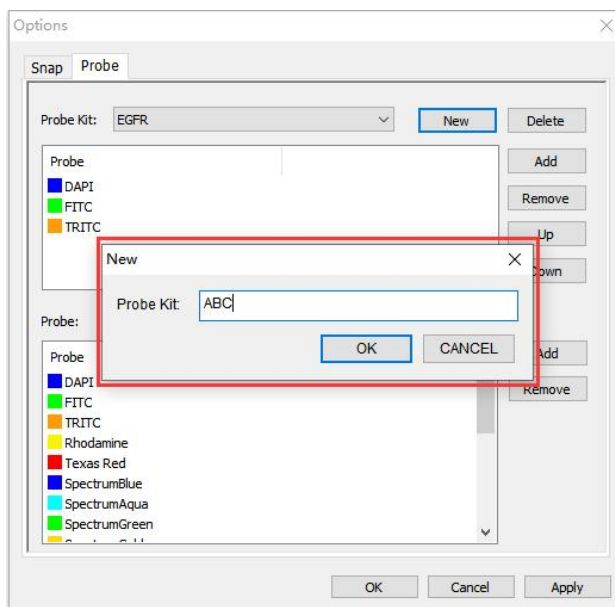


Image 10

2.2 Select the corresponding probe in the probe list, and click Add in the position shown in Figure 11 to add the probe to the custom probe kit.

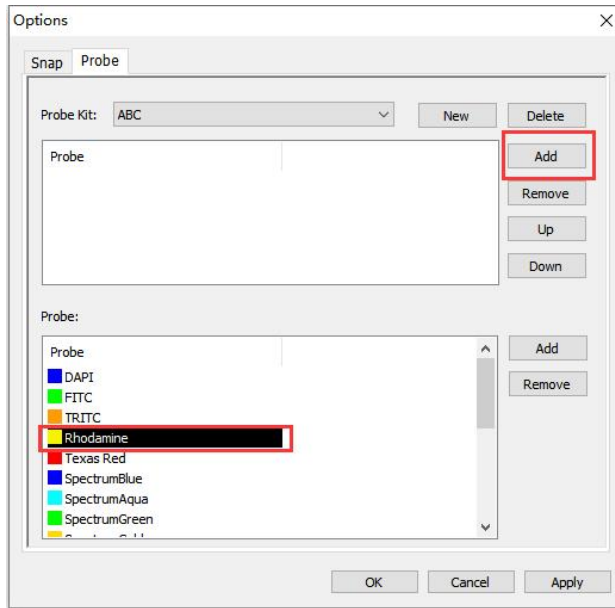


Image 11

2.3 Repeat step 2 to add other probes. If the desired probe is not in the probe list, you can also add it by yourself. Click the Add button at the position shown in Image 12, enter the probe name, select the probe color, click OK to add it to the list, and then follow step 2 to add the custom-added probe to the corresponding probe kit list.

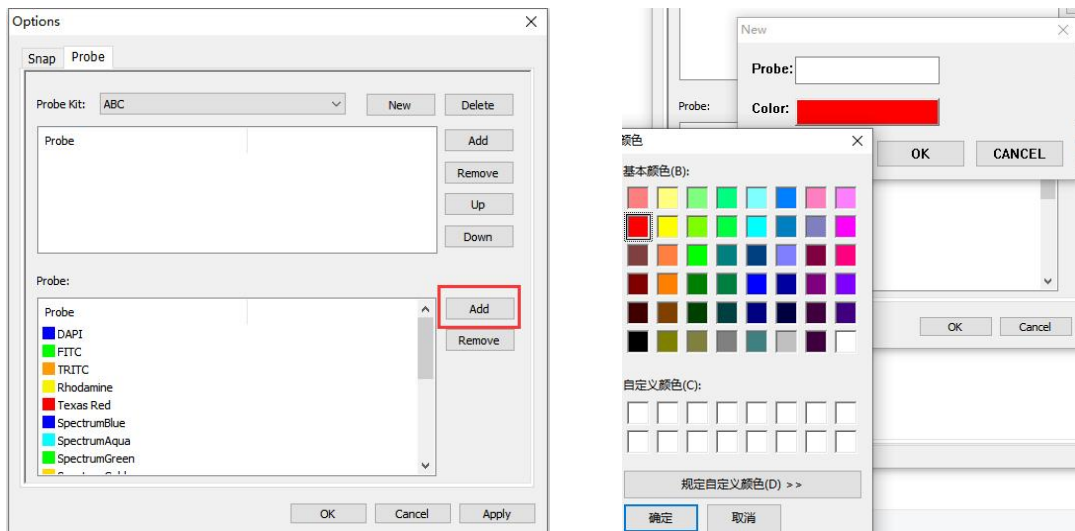


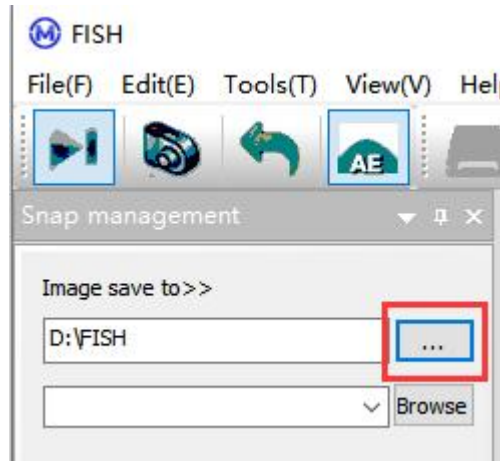
Image 12

2.4 Click OK to complete the addition of the custom probe kit.

Save and file setting

Set image save path to file after installed software, modify save path in below image

bar, default save path is D:\Fish.



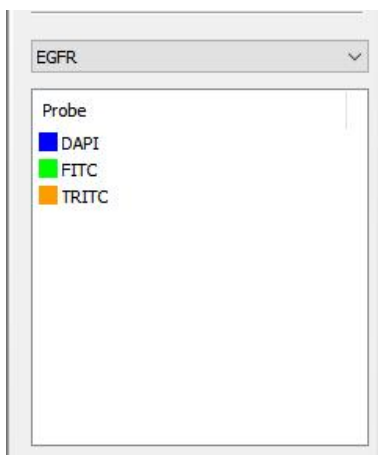
Click the Free Report button in the menu bar to enter the report interface, enter any code in the patient code, and click Save to create a corresponding folder in the save location. You can enter multiple patient codes at one time, and select the file to be saved in the folder selection box.

Image acquire

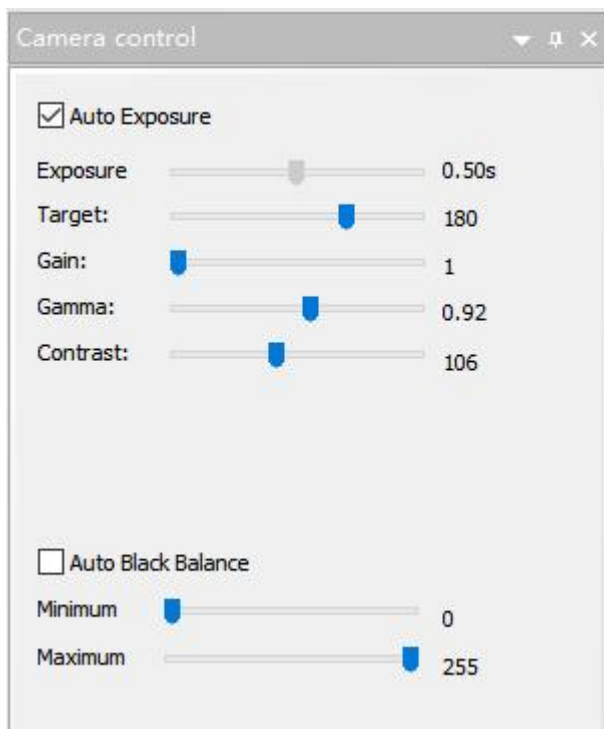
1. Click Play button to open camera.



2. Select settled probe kit and probe.



3. Adjust the corresponding parameters on the right side of the software interface according to the preview real-time image.



4. Select the desired probe set, click to select the corresponding probe, and click the capture button to capture images. The captured images will be displayed in the image list on the right. Switch the channels and probes of the fluorescence module, and then capture images until the fluorescence channels under observation complete the image acquisition.

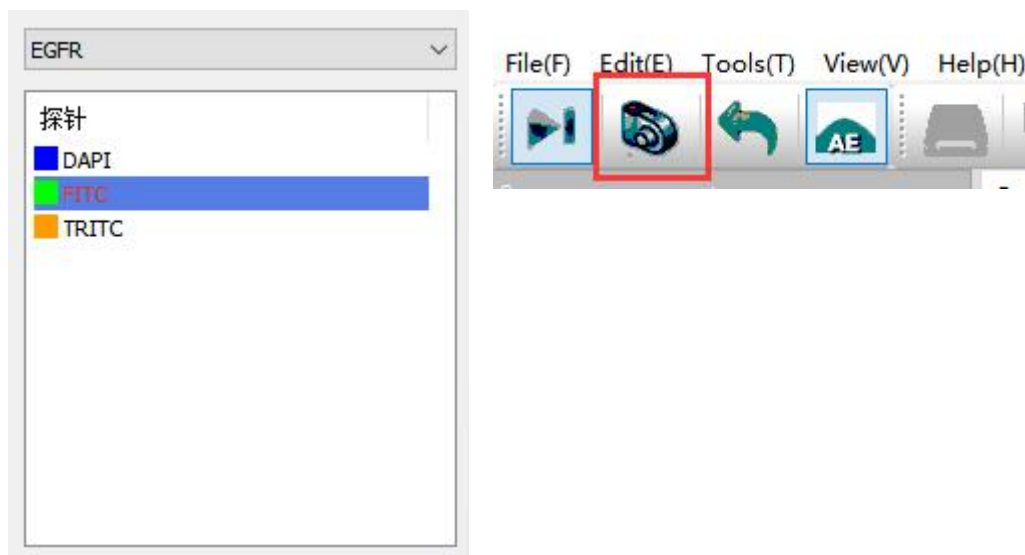


Image processing

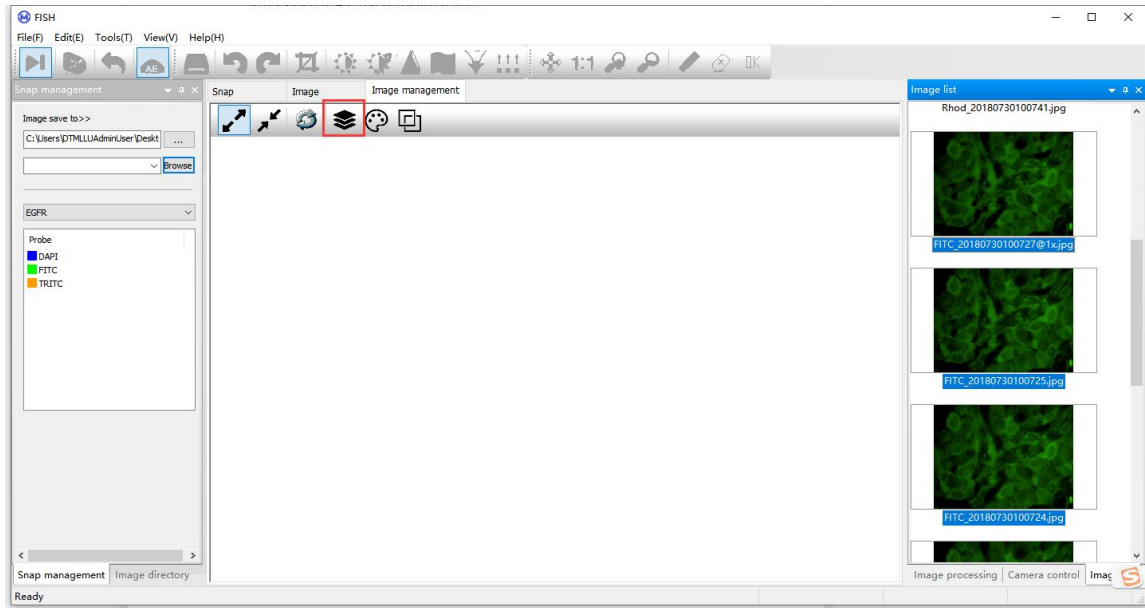
1. Select the folder where the pictures were collected, click the browse button on the right or the image management at the bottom of the interface to open the captured pictures.
2. Depth of field stacking: For some samples, because the signal points are not on the same focal plane, or the signal points of different focal planes cannot be captured clearly at one time under the high-power objective lens, it is often necessary to shoot multiple samples of different focal planes under the same channel and the same probe. photos, and then overlay multiple images into one image. The software provides this function, which can be operated in the image management interface, so that the signal points of the same position and different focal planes are clearly presented.
3. Multi-channel fusion: The images collected by the software are all single-channel monochrome images. This function can synthesize images of different channels in the same area into the same picture, so that users can better evaluate the results.
4. Local image processing: After image synthesis, local image processing functions, such as reducing background noise, enhancing local signals, erasing impurity signals, etc., can be used to optimize the imaging effect.

Detailed operation steps of image processing function

I. Extend DOF

Some sample signal-points are not at one focal surface, one image can not acquire all signal points, it needs acquire multi-images and combine them into one. The software offers this function in image processing window.

1. Enter the image management, long press the left mouse button and drag to select the images to be superimposed or press Ctrl + left mouse button to select the images to be superimposed (usually the same location image taken by the same probe). Click "Overlay" for depth of field overlay operation.



2. The software pops up a query window, you can customize the name of the image to save (if you don't enter it, the superimposed image will default to the probe name). Click the button [Yes] to delete the original image while superimposing, and click [No] to add the image to the overlay. At the same time, save the original picture.

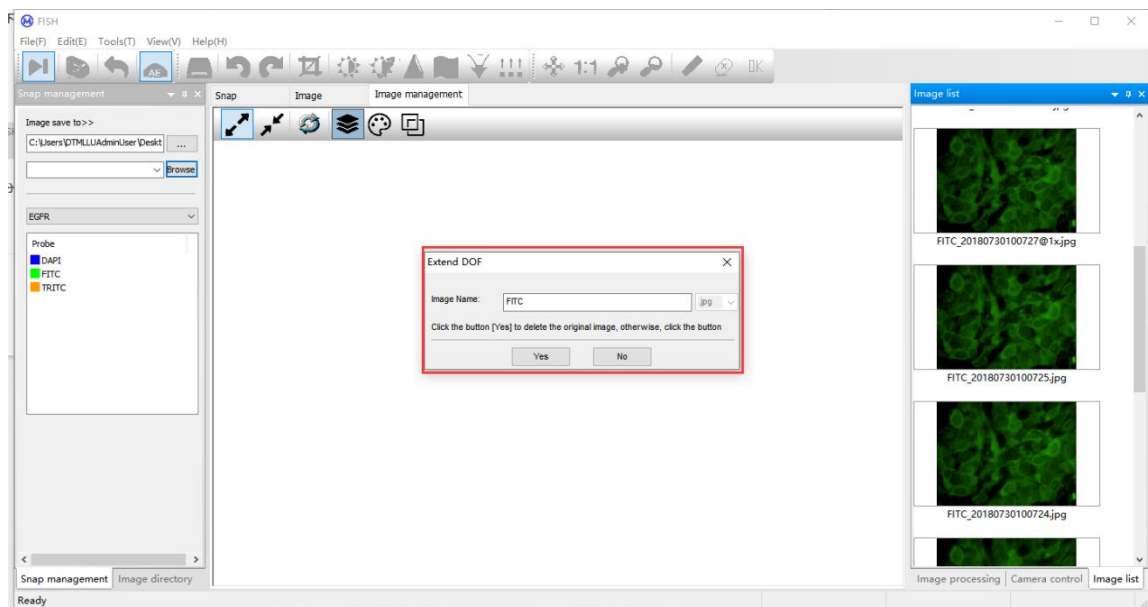
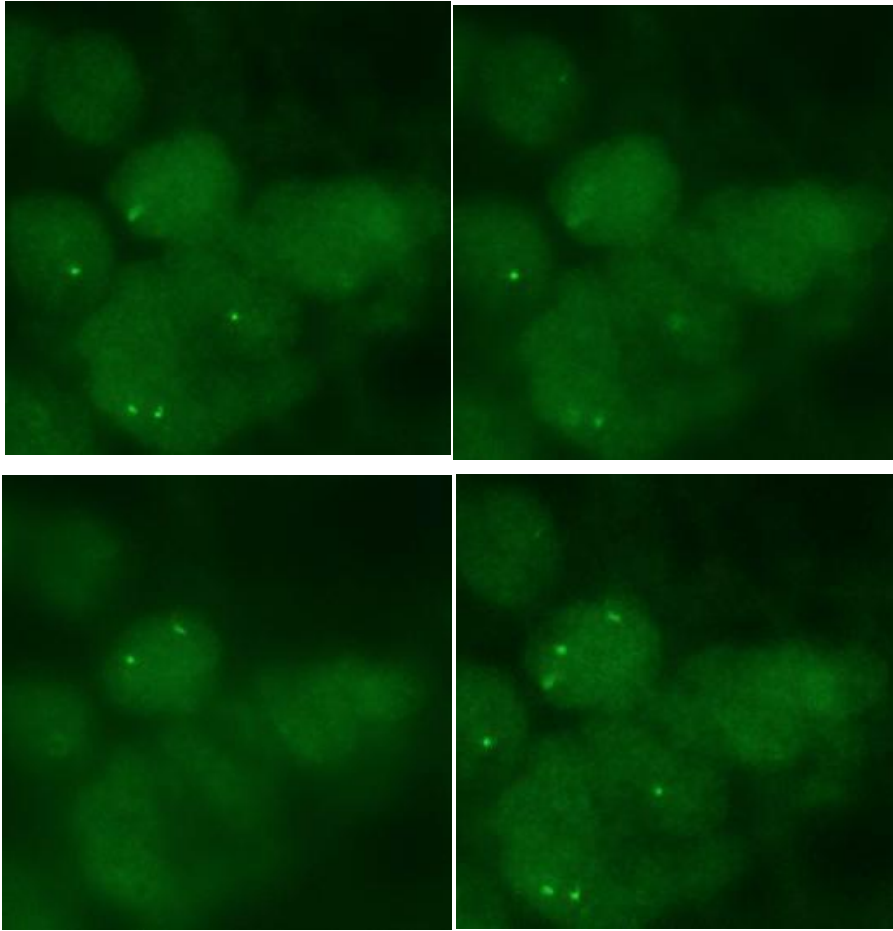


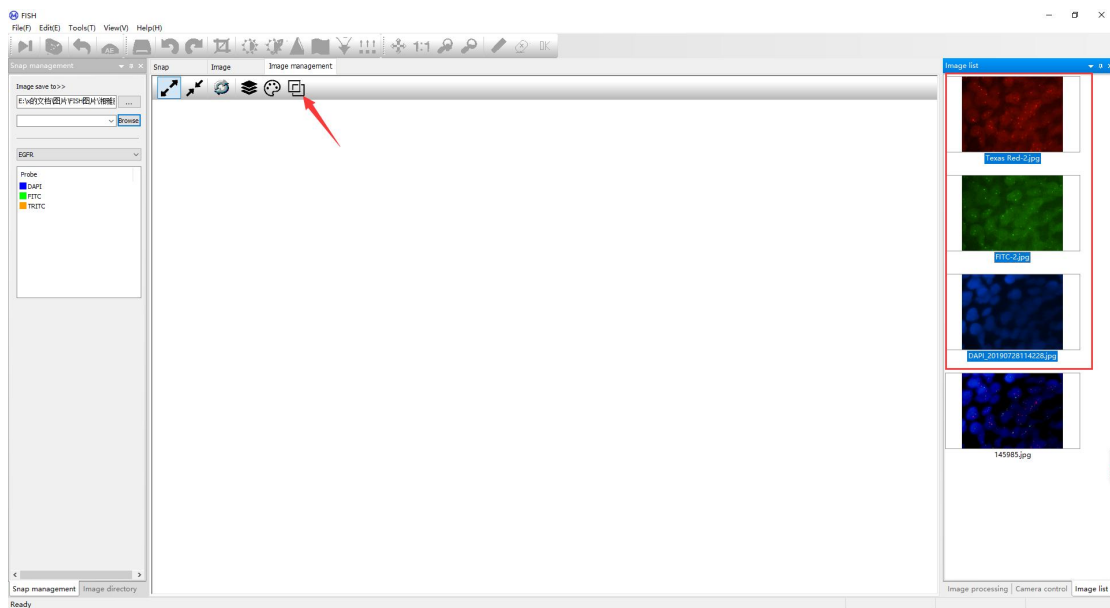
Image 10

After the superposition is completed, the superimposed image will be displayed in the image list on the right. The enlarged effect of the overlay area is as follows:

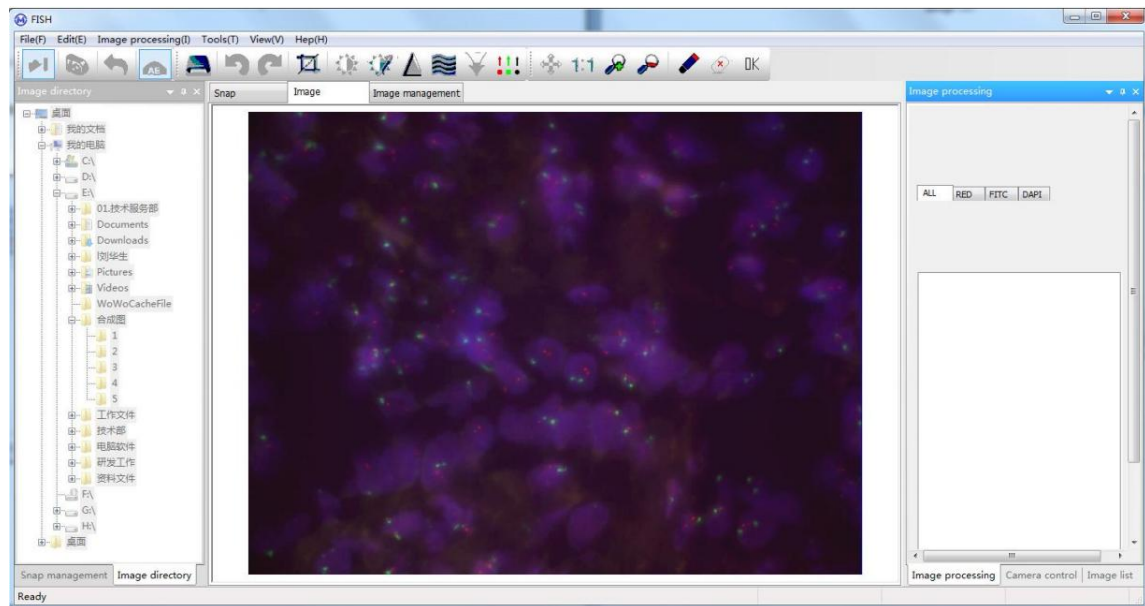


II. Fluorescence multi-channel merging:

1. Long press the left mouse button and drag to select the pictures to be fused, or hold down the keyboard Ctrl + left mouse button to select the pictures to be fused (usually pictures of the same location taken by different probes). Click "Combine" to perform multi-channel merging operation.

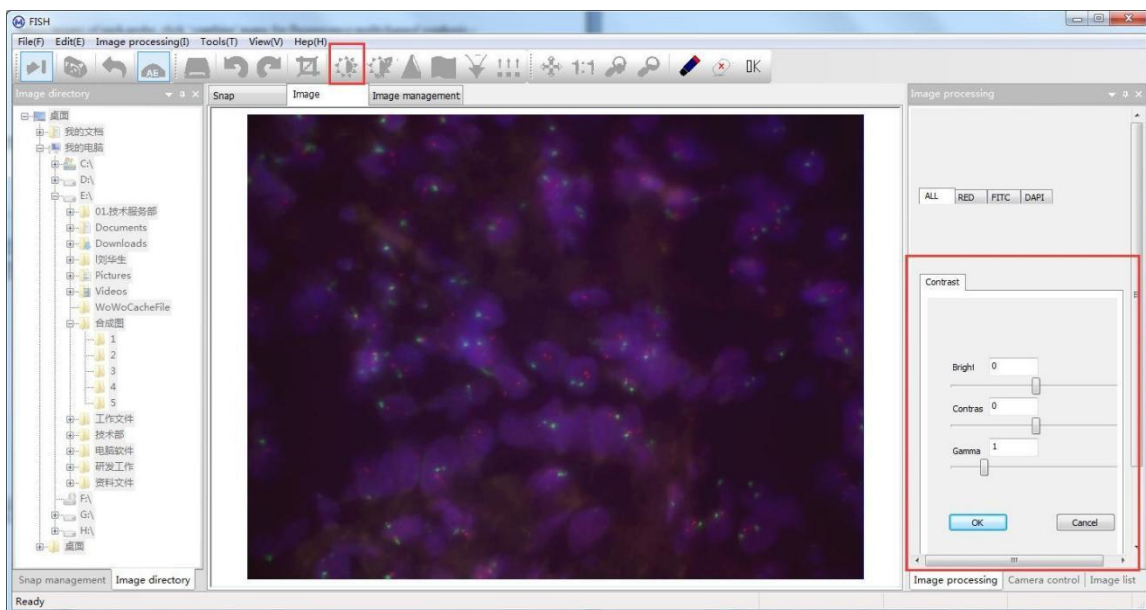


2. After the image fusion is completed, it will automatically enter the image processing interface.



3. Click the "Image Processing" button, the parameter adjustment list will appear on the right, and the image will be adjusted for brightness, contrast and gamma. Click OK after parameter adjustment. The boxes show pictures of different channels, and "ALL" indicates the merged picture. You can select the pictures of different channels to adjust the parameters separately, and click "ALL" to observe the effect of real-time fusion after parameter adjustment.

It is recommended to adjust the parameters of a single channel, the effect of fusion is better.

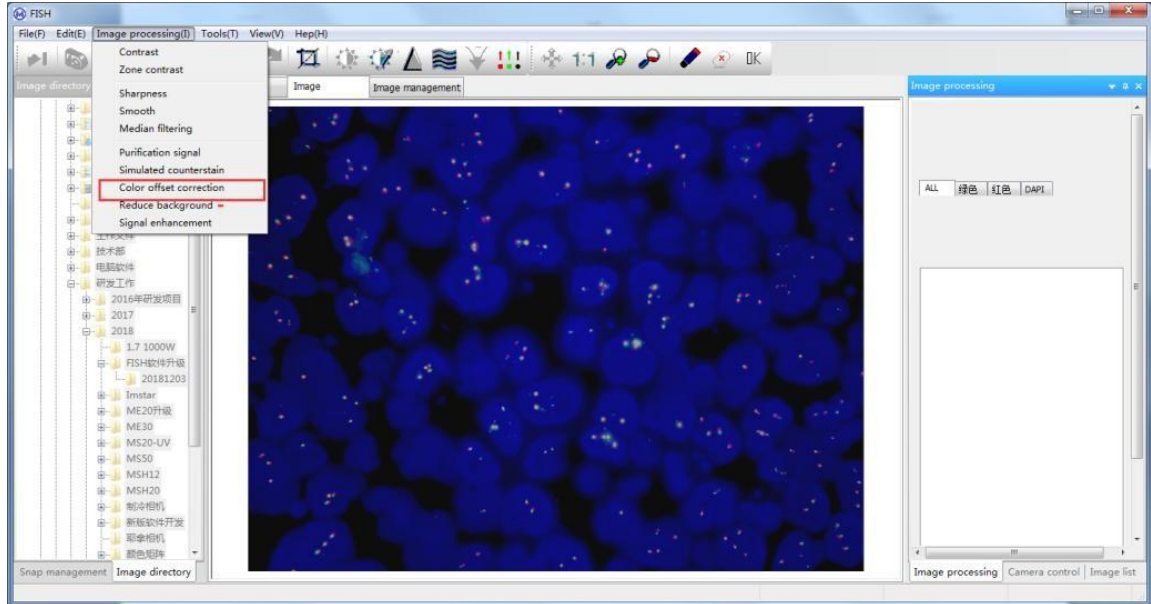


Click  to save image.

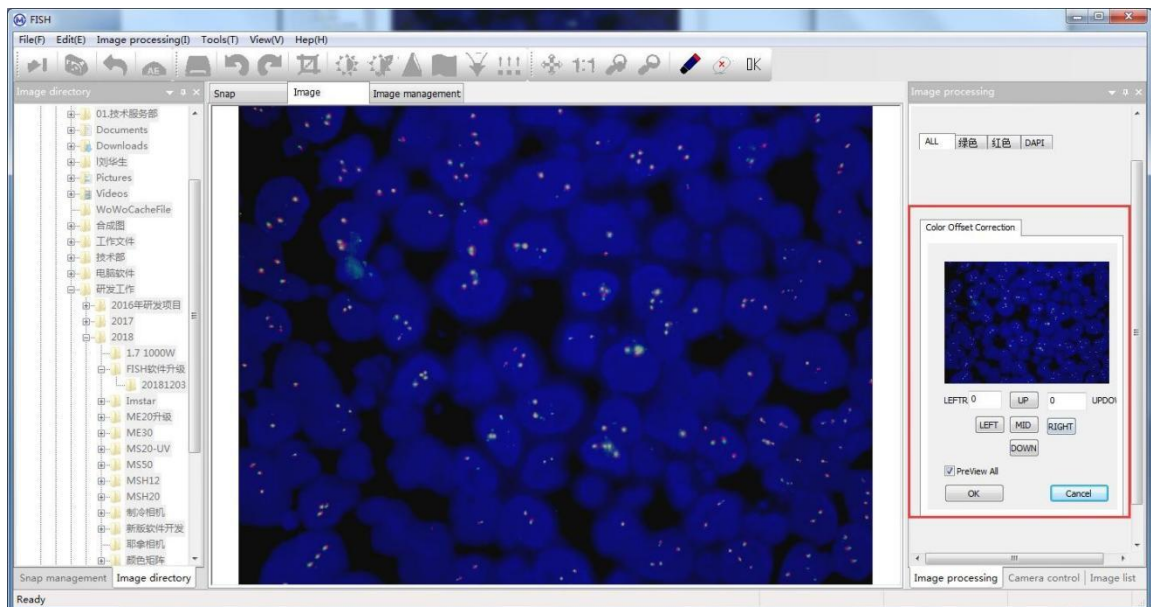
III. Color shifting correction

When some samples are taken, the relative positions of the images captured by different probes are shifted due to hardware optical path displacement or external vibration, and cannot be combined at the same position. At this time, the offset correction function is needed to adjust the relative position of the picture. The operation method is as follows:

In the image processing interface, select the image offset correction function.



Select the offset probe image, click up, down, left, and right to adjust the displacement, and click OK after the adjustment is complete.



IV. Manual image processing

After multi-channel merging, use the manual adjustment tool on the toolbar for image optimization



:Available after switching to the "Image" tab and opening a picture, click the "Image Smoothing" button to smooth the currently displayed image once, and display the smoothing result in real time.



:Available after switching to the "Image" tab and opening the picture, click the "Reduce Background" button to perform a background reduction operation on the currently displayed image, and display the background reduction result in real time.



:Available after switching to the "Image" tab and opening the picture, click the "Signal Enhancement" button to perform a signal enhancement on the currently displayed image, and display the signal enhancement result in real time.



:It is available after switching to the "Image" tab and opening the picture. Click the "Manual Enhancement" button, move the mouse cursor to paint the area where the signal needs to be enhanced several times to enhance the signal, and display the signal enhancement result in real time.



:It is available after switching to the "Image" tab and opening the picture, click the "Deletion Erase" button, move the mouse cursor to smear the area to be erased multiple times to remove the impurities, and display the result of the removal of impurities in real time.



:After the manual processing is over, click the "Confirm" button to save the processing results.

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