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# **Bq3d Manual**

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# 1. Introduction

The binary, bq3d, is a software platform for quantifying variety protein localizations (e.g., H2AX, ATM) from 2D cell culture models that are imaged in 3D using either confocal or deconvolution microscopy. It is assumed that input images are 16 bits per pixel and each channel is represented in stk format. The target applications are DNA repair proteins.

## 2. Menu

There are three main menus in bq3d. They are “File”, “Operation” and “Mode”.

### 2.1 File

File menu is used for reading and writing files. As seen in Figure 2.1.

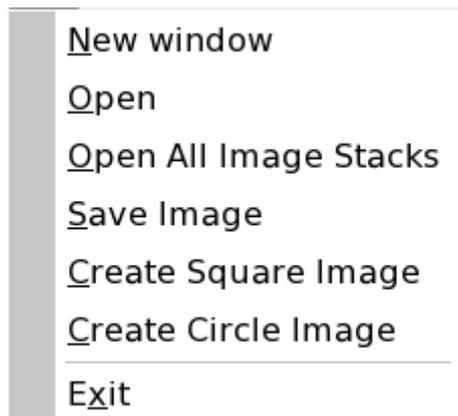


Figure 2.1.1

**New Window** – used to create a new process of bq3d.

**Open** – used to open a single image stack ( the supported formats are: tif , ics, stk). As seen in Figure 2.1.2.

**Open All Image Stacks** – used to load all image stacks such as DAPI, FITC and TXRD. (The names of the corresponding DAPI, FITC and TXRD should have “D”, “F” and “T” at the same place respectively, and the rest of the names should be the same.) The user should select the DAPI stack image and the software is designed to automatically open the corresponding FITC and TXRD stacks if there are. As seen in Figure 2.1.2.

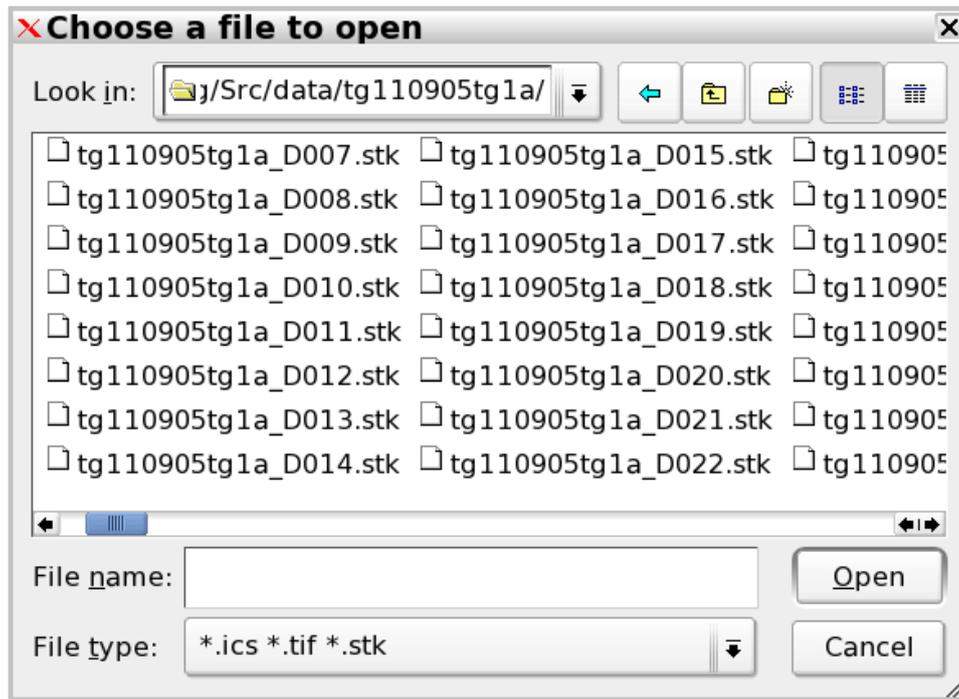


Figure 2.1.2

**Save Image** – used to ...

**Create Square Image** – used to ...

**Create Circle Image** – used to ...

**Exit** – used to terminate the process.

## 2.2 Operation

Operation menu is used for image displaying and processing. As seen in Figure 2.2.1

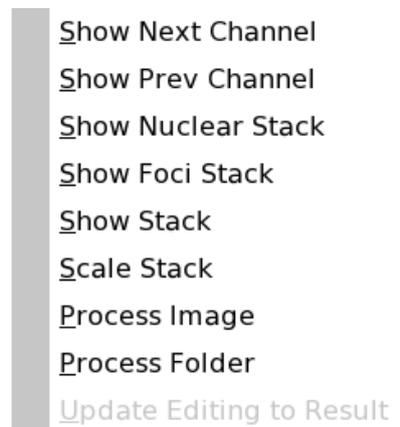


Figure 2.2.1

**Show Next Channel** – used to display the next channel of the current stack.

**Show Prev Channel** – used to display the previous channel of the current stack.

**Show Nuclear Stack** – used to display the DAPI stack from beginning to end.

**Show Foci Stack** – used to display the FITC Channel from beginning to end.

**Show Stack** – used to display the current stack from beginning to end.

**Scale Stack** – used to ...

**Process Image** – used to set the parameters and process a single group of images. The parameter setting interface is shown in Figure 2.2.2.

**Process Folder** – used to set the parameters and process a folder with groups of images. The parameter setting interface is shown in Figure 2.2.2

**XY Scale:**  
0.15

**Z Scale:**  
1.23

**Min Nuclear Area:**  
1000

**Min Foci Diameter:**  
2

**Max Foci Diameter:**  
15

**Min Foci Gradient:**  
1

**Min Foci Voted Threshold:**  
1

Dapi Channel valid

Fitc Channel valid

Txrd Channel valid

Spot in Fitc Channel

Spot in Txrd Channel

OK Cancel

**XY Scale** – The scale of XY coordinate.

**Z Scale** – The scale of Z coordinate.

**Min Nuclear Area** – The minimum volume of nuclear(pixel).

**Min Foci Diameter** – The minimum diameter of spot ( pixel ).

**Max Foci Diameter** – The maximum diameter of spot (pixel).

**Min Foci Gradient** – The minimum gradient for voting.

**Min Foci Voted Threshold** – The minimum voted value of a spot.

**Dapi Channel valid** – This item will be automatically checked if Dapi is loaded.

**Fitc Channel valid** – This item will be automatically checked if Fitc is loaded.

**Txrd Channel valid** – This item will be automatically checked if Txrd is loaded.

**Spot in Fitc Channel** – Select for finding spots in Fitc.This item will be automatically checked if both Dapi and Fitc are loaded.

**Spot in Txrd Channel** – Select for finding spots in Txrd.

Figure 2.2.2

## 2.3 Mode

Edit menu is used for switching between process mode and edit mode. As seen in Figure 2.3.1

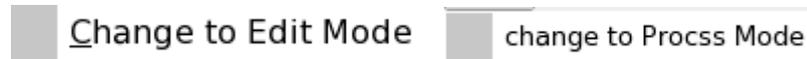


Figure 2.3.1

**Change to Edit Mode** – used to change from process mode to edit mode. It will automatically load the result for editing. When it is selected, there will be sub-window to assist you for editing. As seen in Figure 2.3.2.

**Change to Process Mode** – used to change from edit mode to process mode. All unsaved editing results will be lost.

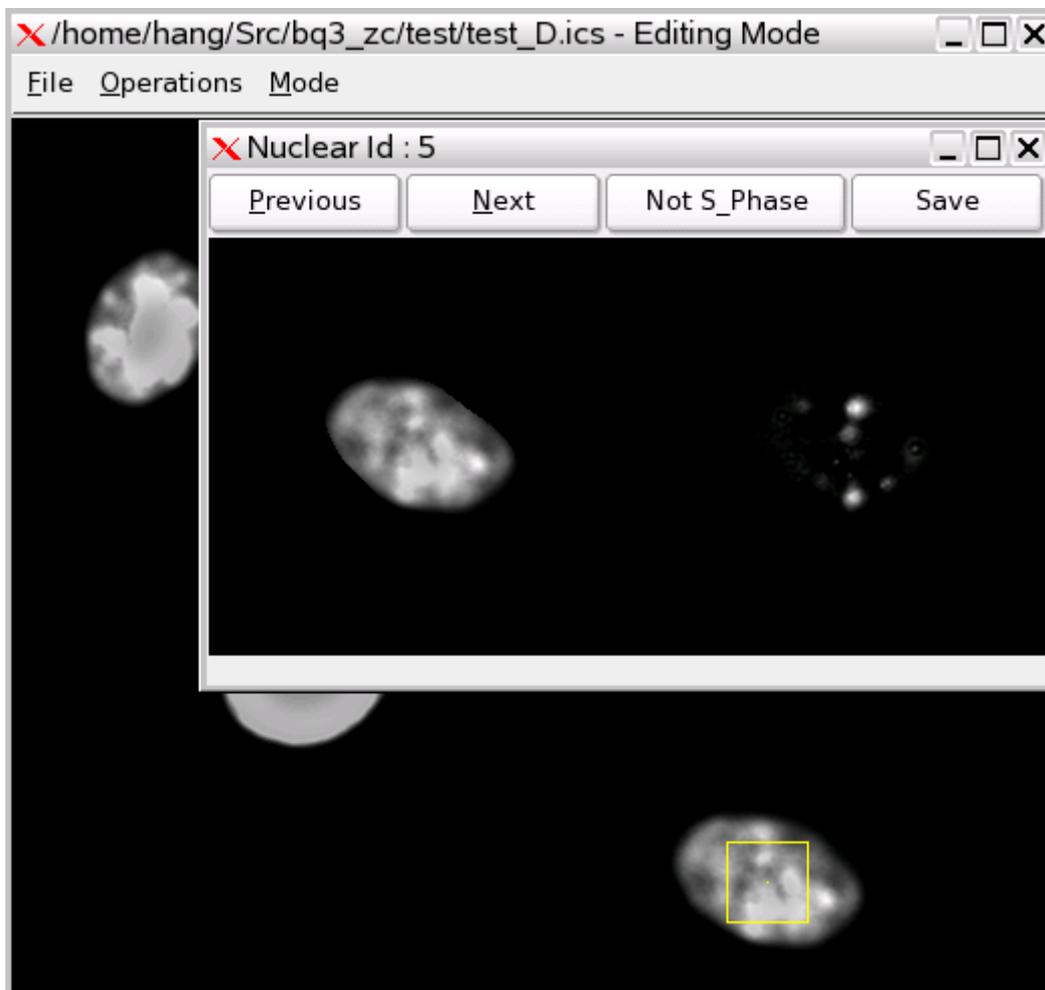


Figure 2.3.2

In Edit mode, when you left click on a nuclear, the Dapi channel and Fitc channel of the nuclear will be showed in the sub-window. The mean of each button in the sub-window is :

**Previous** – used to view the previous channel of both Dapi and Fitc.

**Next** – used to view the next channel of both Dapi and Fitc.

**S\_Phase(Not S\_Phase)** – used to mark the status of the nuclear. The nuclear in S\_Phase is marked with a yellow rectangle.

**Save** – used to save the nuclear region in both Dapi and Fitc channel.

**Note:** After editing, user should use “Operations->Update Editing to Result” to update the edited result to disk files.

## 3. Output

### 3.1 Output items

After processing, bq3d record rich information for analysis, including images and txt files. The naming of these files is : **filename(image) + version + extension**. The list of output items:

Filename\_version\_nuclear\_mask.ics

Filename\_version\_nuclear\_fitcSpot.ics ( if “Spot in Fitc Channel” is valid)

Filename\_version\_nuclear\_txrdSpot.ics ( if “Spot in Txrd Channel” is valid)

Filename\_version\_nuclear.txt

Filename\_version\_fitcSpot.txt

Filename\_version\_txrdSpot.txt

Filename\_version\_params.txt

### 3.2 Output format

**Filename\_version\_nuclear.txt :**

1. nucl\_id;
2. nucl\_x;
3. nucl\_y;
4. nucl\_z;
5. nucl\_volume;
6. nucl\_aspectRatio;
7. nucl\_mean;
8. nucl\_sd;
9. fitcSpots\_num;
10. avg\_fitcSpots\_volume;
11. avg\_fitcSpots\_intensity;
12. fitc\_mean\_withoutSpots;
13. fitc\_sd\_withoutSpots;
14. fitc\_mean;
15. fitc\_sd;
16. txrdSpots\_num;
17. avg\_txrdSpots\_volume;
18. avg\_txrdSpots\_intensity;
19. txrd\_mean\_withoutSpots;

20. txrd\_sd\_withoutSpots;
21. txrd\_mean;
22. txrd\_sd;
  
23. num\_co\_spots;
24. validation;

**Filename\_version\_fitcSpot.txt (Filename\_version\_txrdSpot.txt)**

1. nucl\_id;
2. nucl\_x;
3. nucl\_y;
4. nucl\_z;
5. nucl\_volume;
6. nucl\_aspectRatio;
7. nucl\_mean;
8. nucl\_sd;
9. num\_spots;
10. spot\_id;
11. spot\_x;
12. spot\_y;
13. spot\_z;
14. spot\_volume;
15. spot\_fore\_mean;
16. spot\_fore\_sd;
17. spot\_back\_mean;
18. spot\_back\_sd;
19. spot\_contrast;
20. mean\_nucl\_atSpot;
21. validation;

**Filename\_version\_params.txt**

1. software
2. xy\_scale
3. z\_scale
4. min\_nucl\_size
5. min\_spot\_diam
6. max\_spot\_diam
7. min\_spot\_grad
8. min\_spot\_voted\_thresh

## 4. Example

1. Load All image stacks. As seen in Figure 4.1.

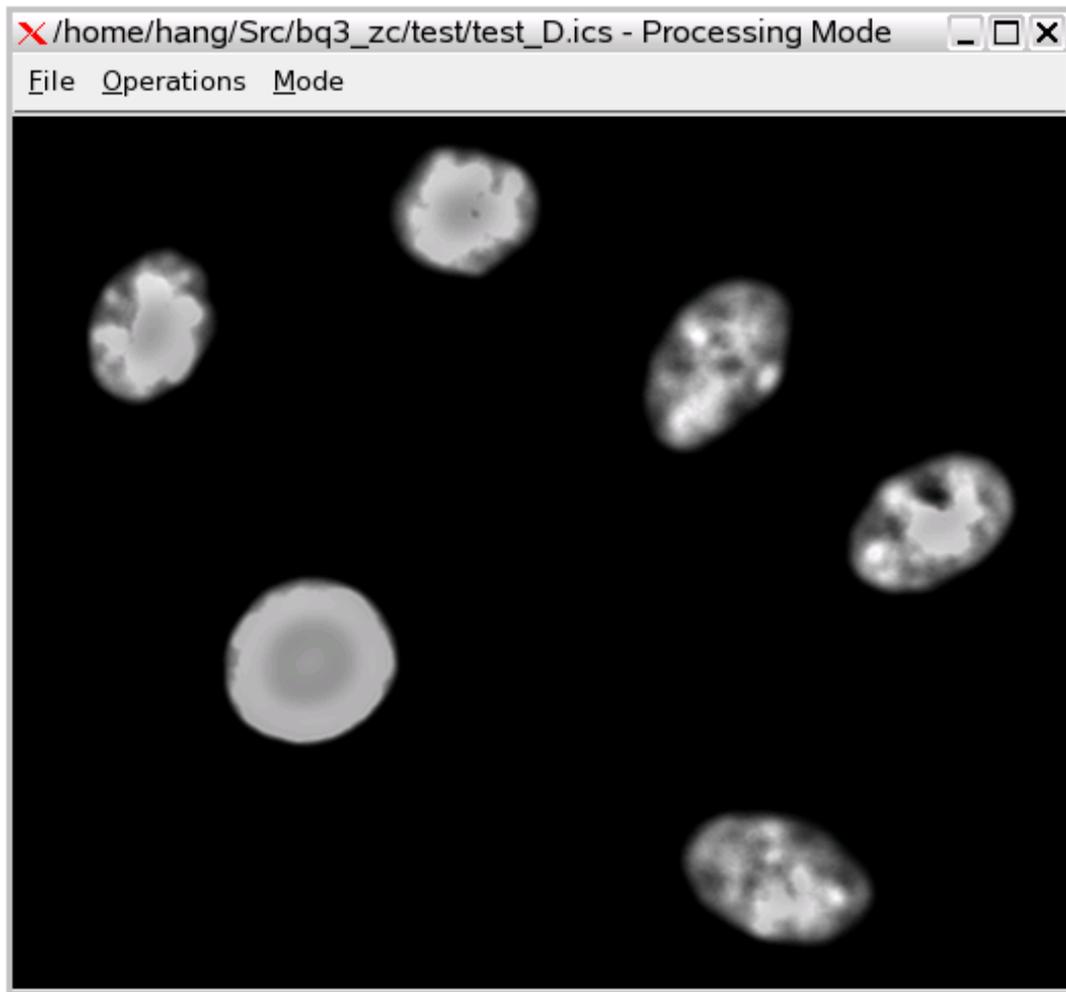


Figure 4.1

2. Set parameters for processing( As seen in Figure 2.2.2 )
3. Edit processed result.(Optional, As seen in Figure 2.3.2)
4. Output list
  - test\_D.ics\_FFv2\_nuclear\_mask.ics (As seen in Figure 4.2 )
  - test\_D.ics\_FFv2\_nuclear\_fitcSpot.ics (As seen in Figure 4.3)
  - test\_D.ics\_FFv2\_nuclear.txt
  - test\_D.ics\_FFv2\_fitcSpot.txt
  - test\_D.ics\_FFv2\_params.txt

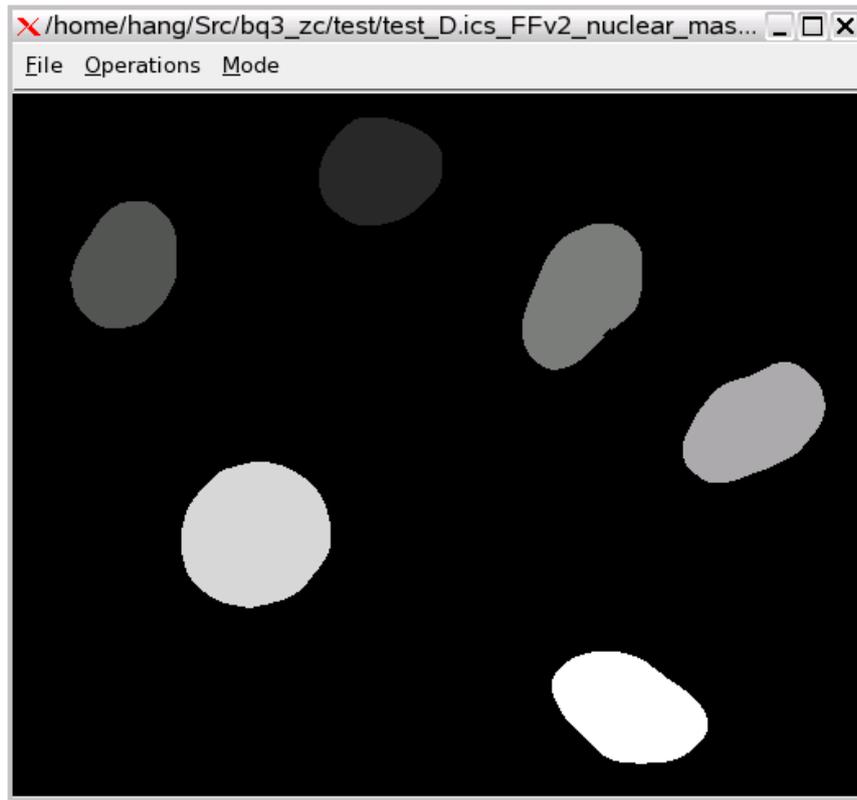


Figure 4.2



Figure 4.3