UNC-Roswell Park Prostate Cancer Image Processing Program Manual

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Manual written by
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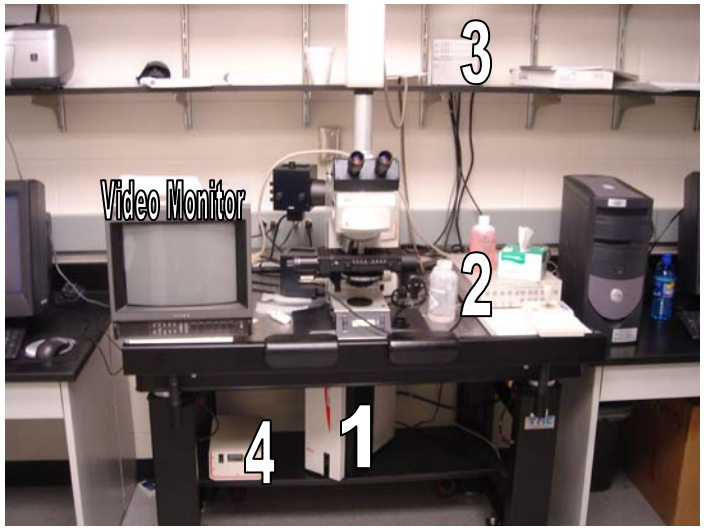
Avaiable at
http://www.med.unc.edu/lccc/iac/Protocols/unc-rpci-cap-ipp-manual.pdf

Image Acquisition

Configuration of the Microscope



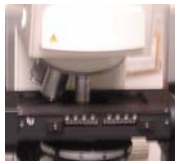
Turning on the Microscope

- Turn on Parts 1,2, 3 and the Video Monitor
  - 4 creates an optional fluorescent light
- Turn on the incandescent light (for white light.)

- The fluorescent and the incandescent lights require time to heat up so that they can emit an accurate light.

Choosing the right lens

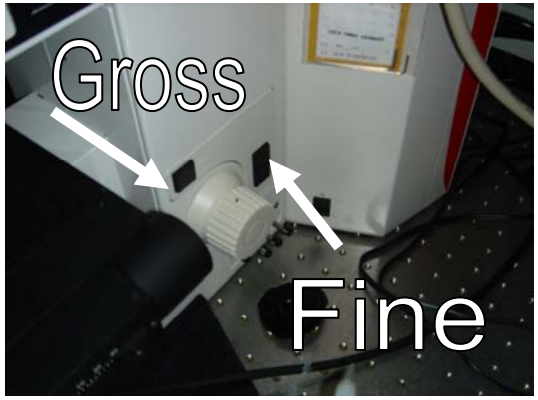
- Select the 40x lens



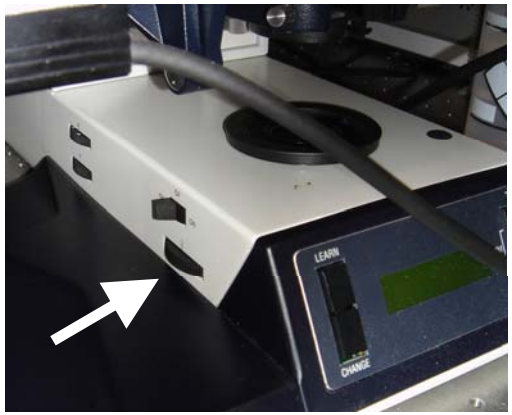
Opening the Shutter and Choosing the Correct Filter (Optional)

The microscope contains fluorescent filters also.

Gross and fine focus adjustment



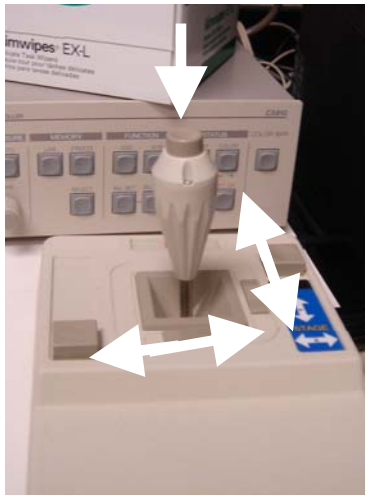
Adjusting the voltage



- Optimal voltage: 7-9V
- If a very high voltage is required (≈11-12V), it may be necessary to replace the bulb.

Movement of the stage using the joystick

- The stage will move slowly when the joystick is moved without pressing any buttons.
- The stage will move quickly if the center button on the joystick is depressed.



Configuration of the video camera

Adjusting the White Balance

- The white balance adjusts for the color temperature of the light (basically a yellow or blue tint.)
- Find an all white portion of the slide with the joystick, and then press the AWB (for Auto White Balance) button.

- Manual Adjustments can be made by pressing the Gain button.
    - Note: the Button takes about 2 seconds to take effect on screen. After being pressed about 10 times it will cycle back to a lower setting and begin increasing incrementally again.

Adjusting the Exposure

- Auto
    - Pressing the “Auto” button will cause the camera to automatically select the correct exposure.

- Manual
    - If one is not pleased with the automatic adjustment, one can use the manual knob to make fine adjustments to the exposure.

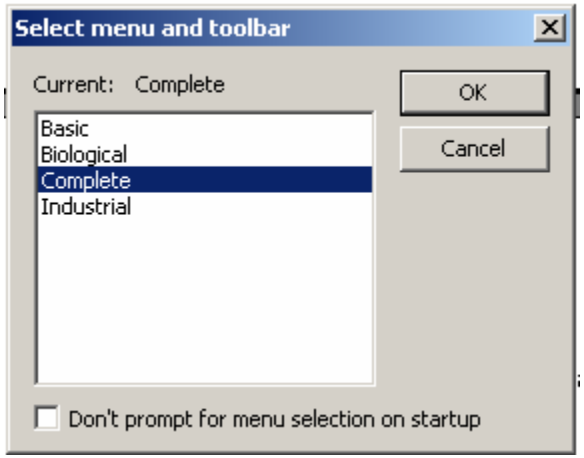
Adding/Removing the Status Text

- Above is an example of the Status Text.

- Press the ON/OFF Button under STATUS on the Camera Controller.
- Press COLOR to change the color of the text.

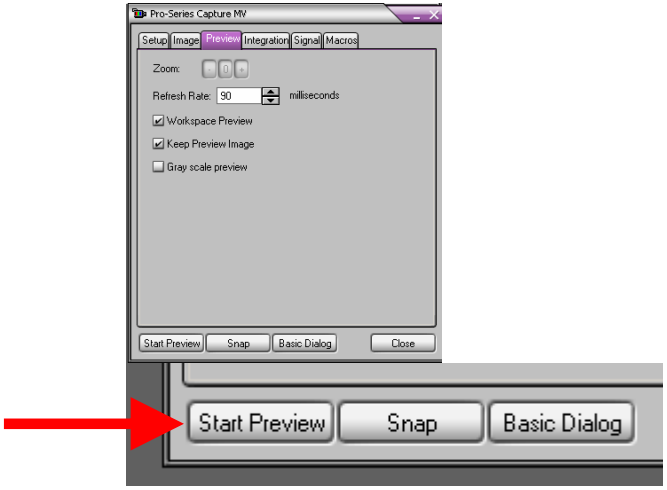
Use of Image Pro Plus 5.0

- Camera MUST be on before using Image Pro Plus 5.0.
- Open Image Pro Plus 5.0.
- When asked what type of toolbar you would like, choose “Complete.”



- Click on the video camera icon.

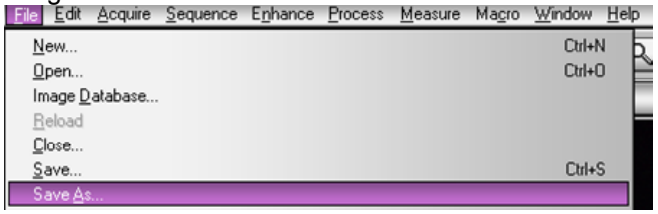
- Click “Start Preview.”

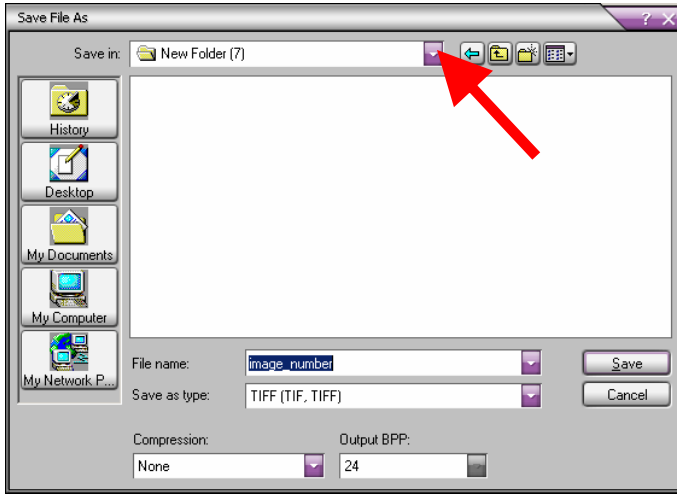


- Once image has been selected, click “S” meaning “Stop.”

- Click “Snap.”

- Click File > Save As and give a name to your image.



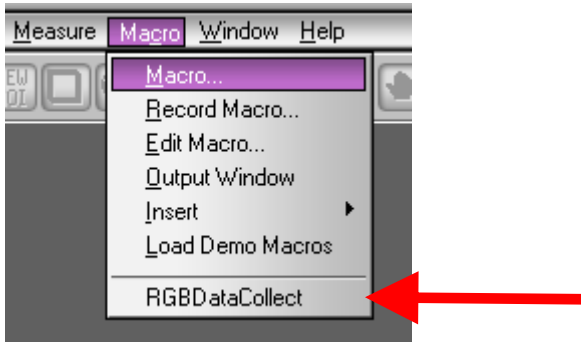


- Make note of where you save the images. You can see where the images are by looking at the top of the window. This will be important later.

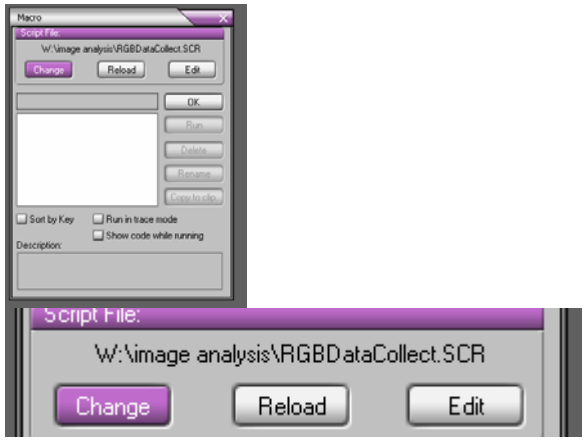
Running RGB Data Collect Macro

Open the Macro

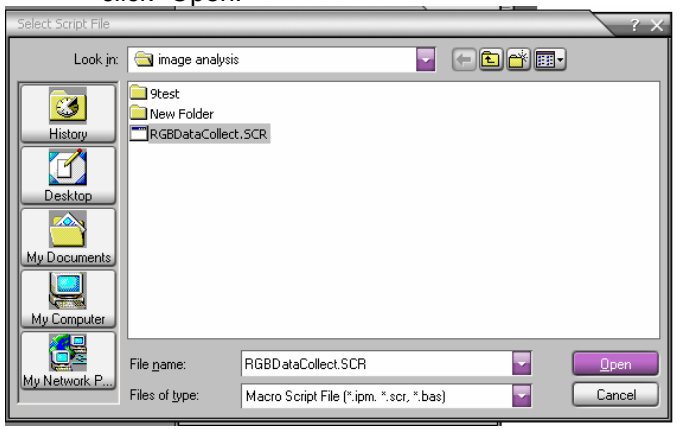
- Choose Macro > Macro



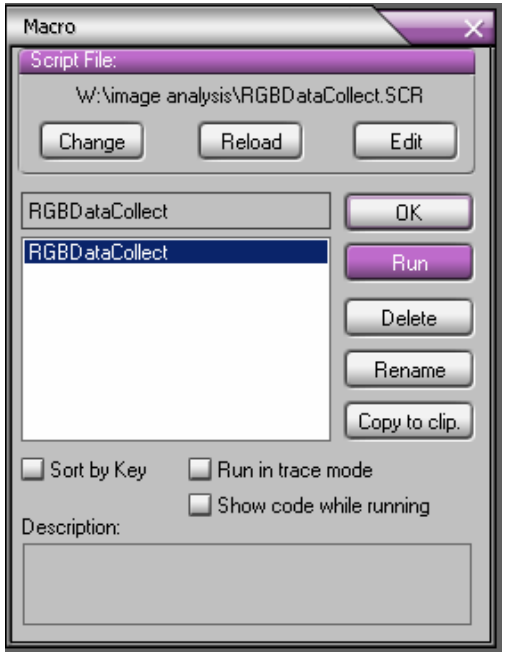
- If RGBDataCollect appears, click RGBDataCollect and skip to page 17. Otherwise, Click “Change” and follow these instructions.



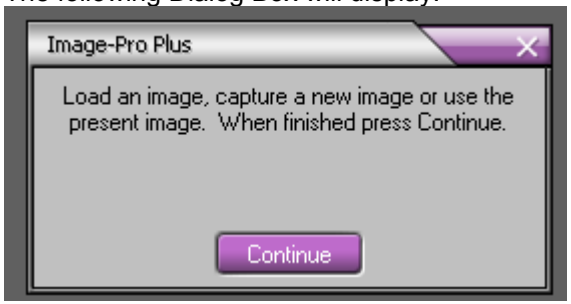
- Find the location of RGBDataCollect.scr and click “Open.”



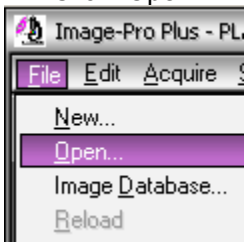
- Highlight RGBDataCollect.scr and choose "Run."



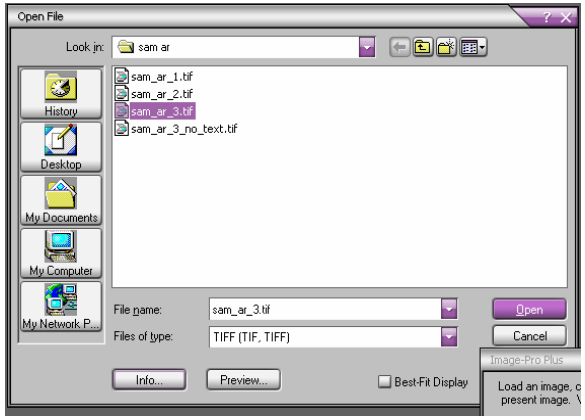
- The following Dialog Box will display:



- Click "Open."

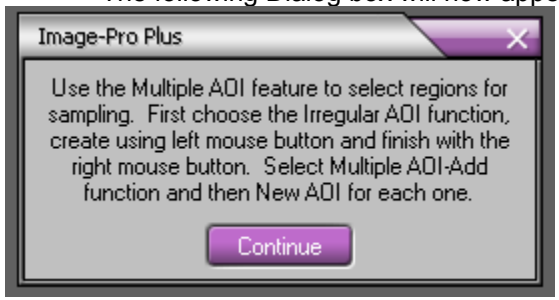


- Select one of your already saved images and click "Open."

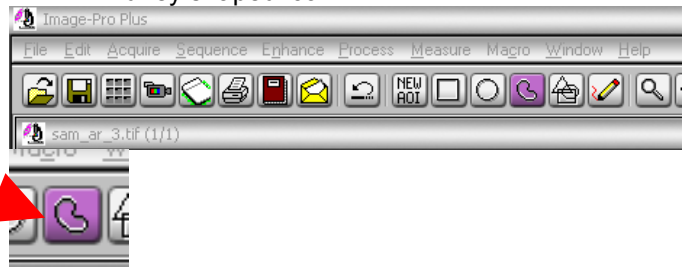


- Once an image is displayed, Press “Continue.”

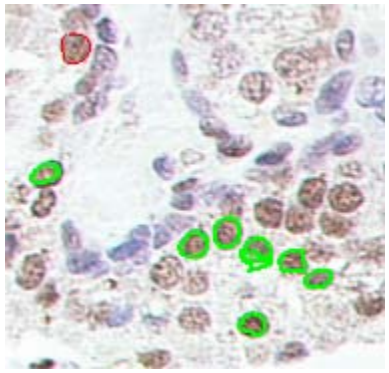
- The following Dialog box will now appear:



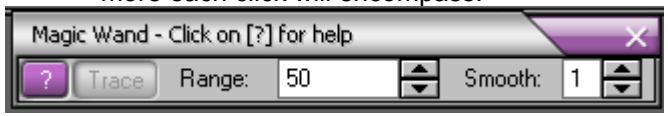
- Select the “Magic Wand” Tool by clicking the kidney shaped icon.



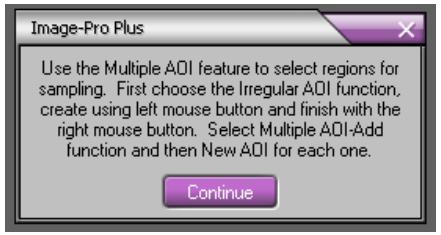
- Select Positive or Negative cells with “Magic Wand” tool. If you like the selections in red, hold down CTRL, and make another selection. If a selection is in red and you are unhappy with it, click another cell. Once you are happy with what is in red, hold down CTRL and it will be made green and added to the selection.



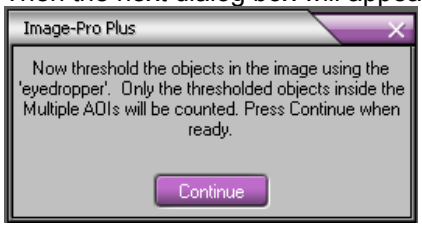
- Adjust the sensitivity of the magic wand with the following dialog box, the greater the number the more each click will encompass.



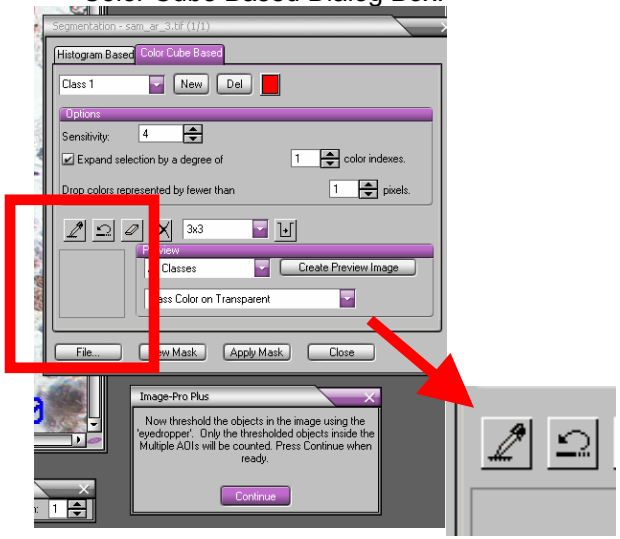
- Click continue



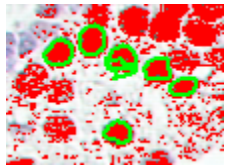
- Then the next dialog box will appear:



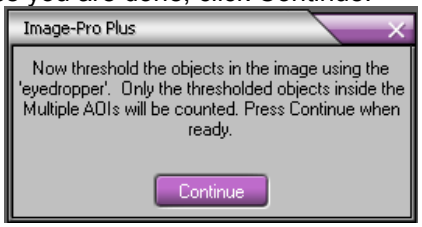
- Click the eyedropper tool in the Segmentation > Color Cube Based Dialog Box.



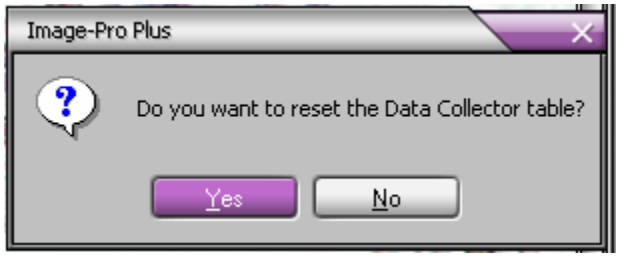
- Click inside the selected cells (green circles) so that they become entirely red. If everything is working, the cells should look like this:



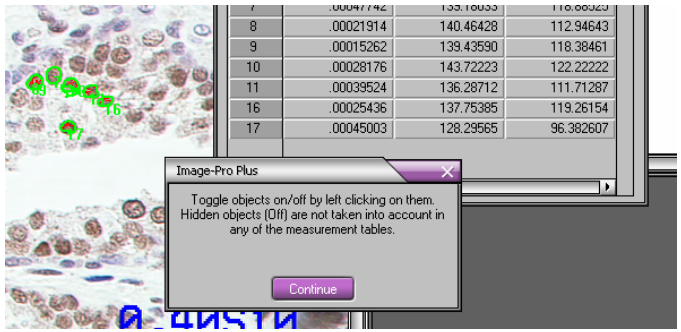
- Once you are done, click Continue.



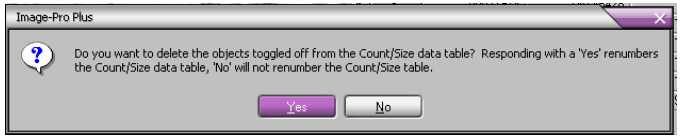
- Click "Yes."



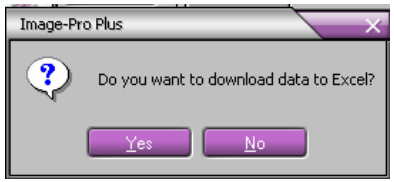
- Click on Objects (in the picture) you do not want to count in the final analysis – a number will not appear next to them if they are not being counted.



- Click "Yes."



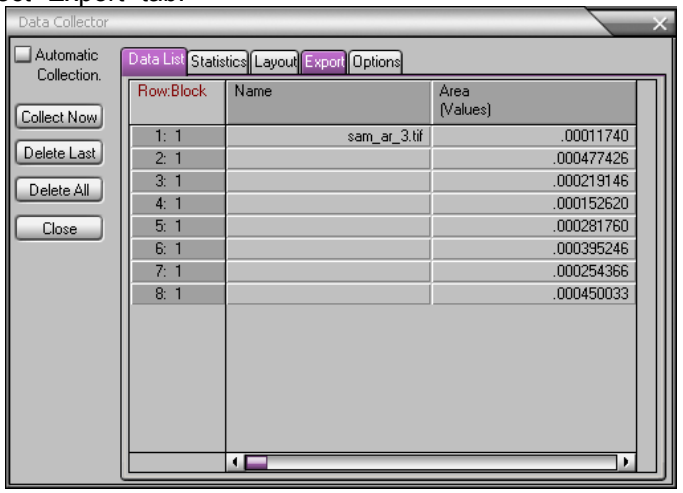
- Click “Continue.”
- Then click “no.”



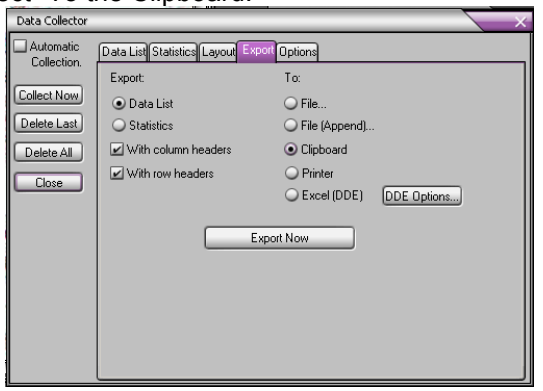
Parameter Training

Exporting the Data to Excel

- Select “Export” tab.

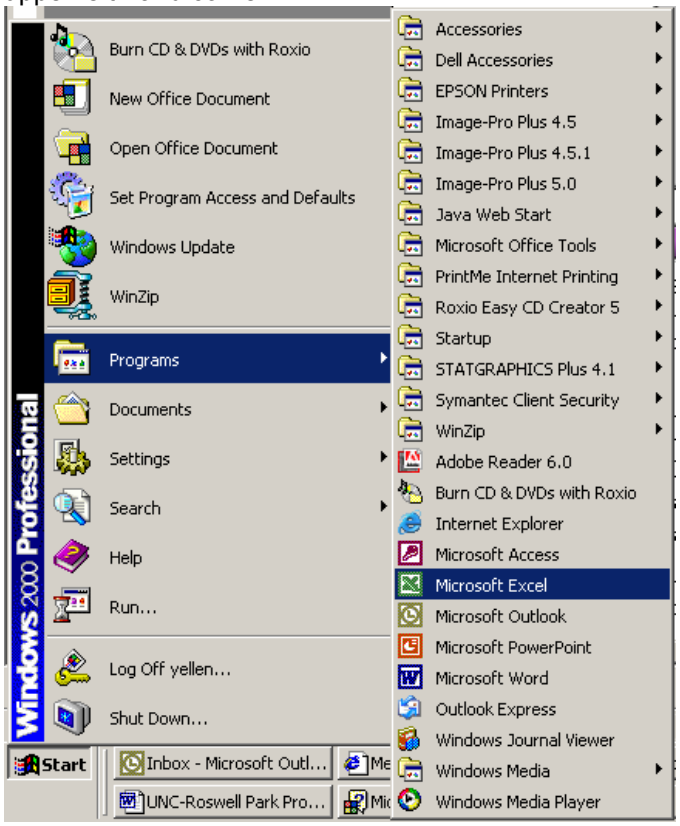


- Select “To the Clipboard.”

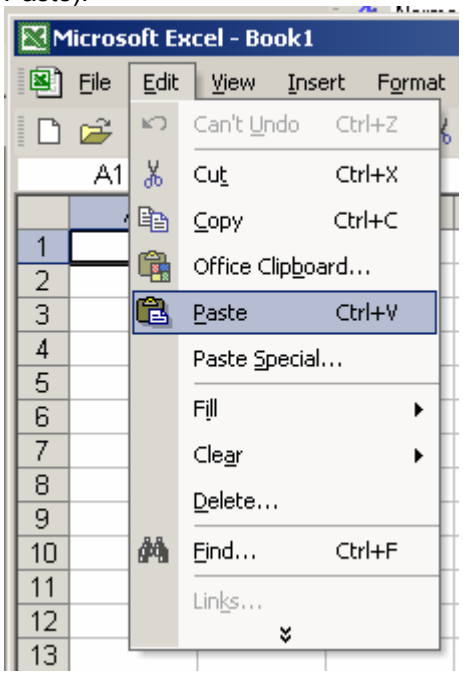


- Click “Export Now.”

- Open a new spread sheet in Excel and click the upper left-hand corner.



- Go to the edit menu and click paste. (Edit > Paste).



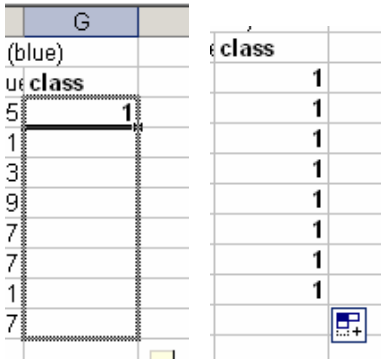
- Go back and run the **RGBDataCollect** macro again (**page 14**,) collecting more data from positive (or if you have just done negative, negative) nuclei. For best results, one would have data from 10 positive nuclei from 10 different images and 10 negative nuclei from 10 different images.

Labeling the data positive or negative

- Create a new column to the right of the RGB Data, called class.
- 1 will be used to denote positive.
- 2 will be used to denote negative.

	A	B	C	D	E	F	G
1	Row:Block	Na	Are	Density (	Density (gr	Density (blue)	
2			(Value	(Value	(Value	(Value	class
3	1: 1	sam_ar_	0.000117	141.5333	123.6	116.5	1
4	2: 1		0.000477	139.1803	118.8852	113.7951	
5	3: 1		0.000219	140.4643	112.9464	107.3393	
6	4: 1		0.000153	139.4359	118.3846	114.7179	
7	5: 1		0.000282	143.7222	122.2222	119.5417	
8	6: 1		0.000395	136.2871	111.7129	106.6337	
9	7: 1		0.000254	137.7538	119.2615	120.5231	
10	8: 1		0.00045	128.2957	96.38261	92.4087	

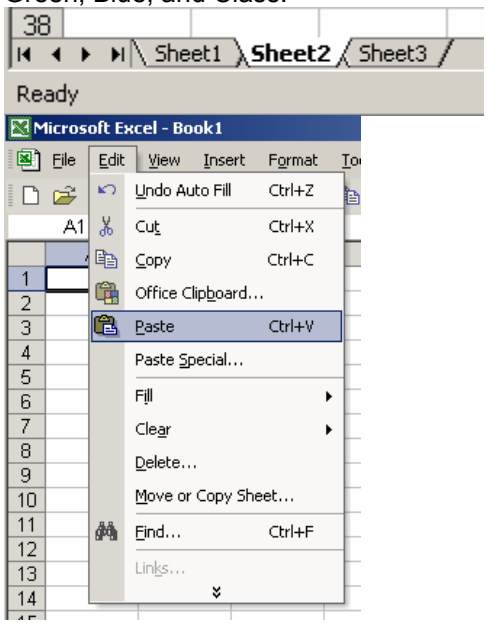
- click the black corner of the cell and drag it down so that all of the positive samples are marked “1.”



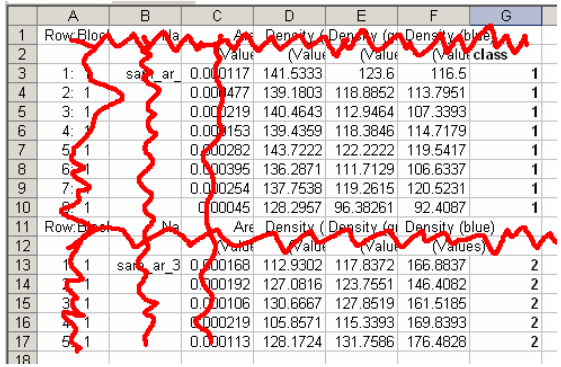
- Continue to add data by running the **RGBDataCollect** macro on **page 27** until you have a data set of an appropriate size. Add Negative data after the Positive data and label it with a 2.

Formatting the data from excel into a text document the analysis program can understand.

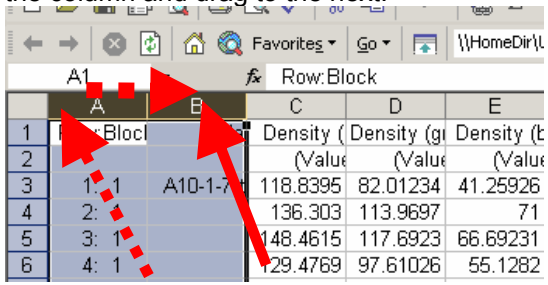
- First, copy all the data into sheet 2 by hitting CTRL + A then going to edit > copy. Then delete all rows of text. Also Delete the Area column and the number column. That should leave 4 columns of data: Red, Green, Blue, and Class.



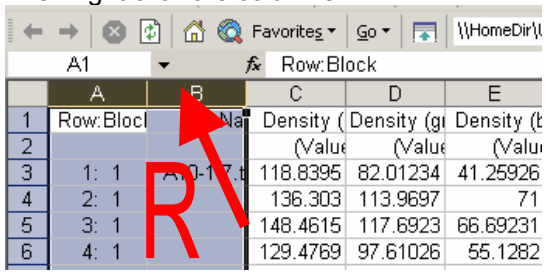
- Then delete all rows of text. Also Delete the Area column and the number column. That should leave 4 columns of data: Red, Green, Blue, and Class.



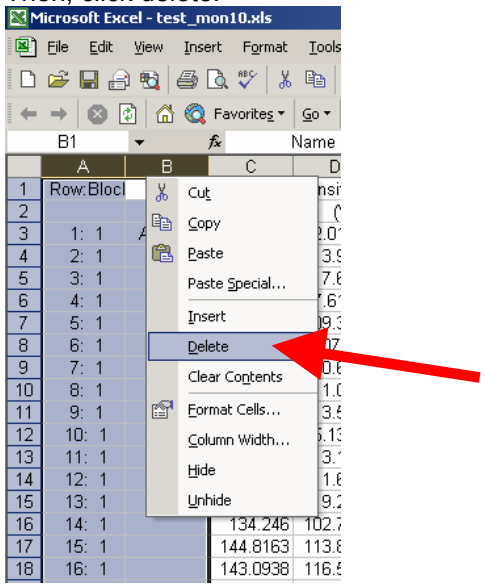
- To delete the rows of text, click the top of the column and drag to the next.



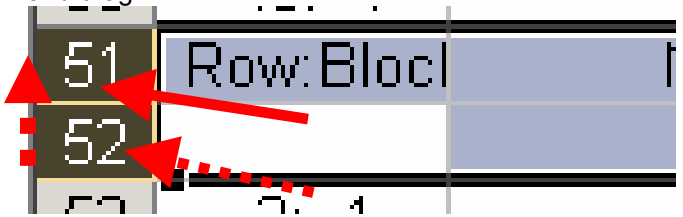
- Then right click the columns.



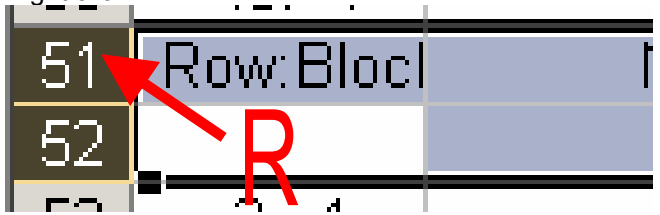
- Then, click delete.



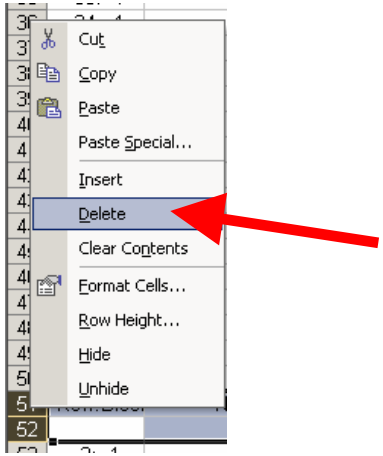
- Unwanted rows are deleted similarly: Click and drag.



- Right click.



- Choose delete.



- When done, your table should appear like this: 4 columns, the first three for Red, Green, and Blue data respectively (values between 0 and 255,) and the last for class data with a value of 1 or 2.

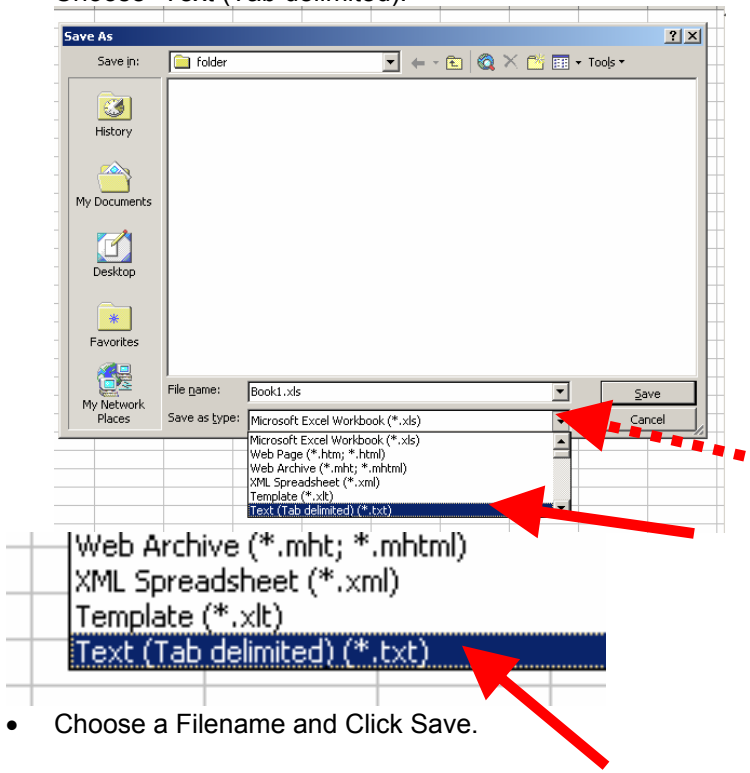
	A	B	C	D
1	141.5333	123.6	116.5	1
2	139.1803	118.8852	113.7951	1
3	140.4643	112.9464	107.3393	1
4	139.4359	118.3846	114.7179	1
5	143.7222	122.2222	119.5417	1
6	136.2871	111.7129	106.6337	1
7	137.7538	119.2615	120.5231	1
8	128.2957	96.38261	92.4087	1
9	112.9302	117.8372	166.8837	2
10	127.0816	123.7551	146.4082	2
11	130.6667	127.8519	161.5185	2
12	105.8571	115.3393	169.8393	2
13	128.1724	131.7586	176.4828	2

Saving from Excel or Copying to Notepad

- There are two methods to take the data from Excel and get it ready for the UNC-Roswell Park Prostate Cancer Image Processing Program: 1) Save the data from Excel as a tab-delimited text file, 2) Copy the data into Notepad and save as a text file.

1) Saving as a tab-delimited text file

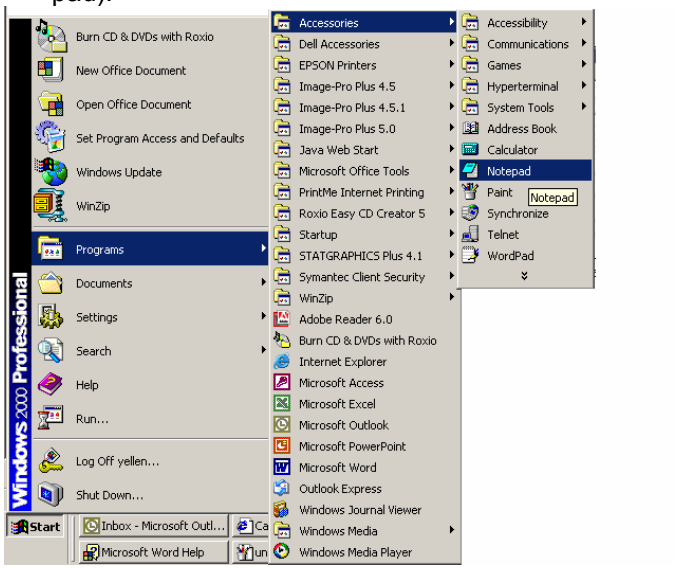
- Go to File>Save As
- Next to “Save as Type.” Choose “Text (Tab delimited).”



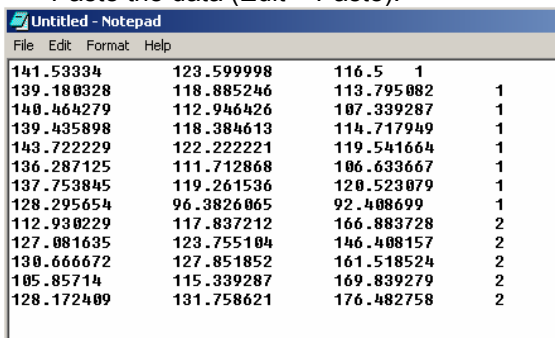
- Choose a Filename and Click Save.

2) Copying the data to Notepad

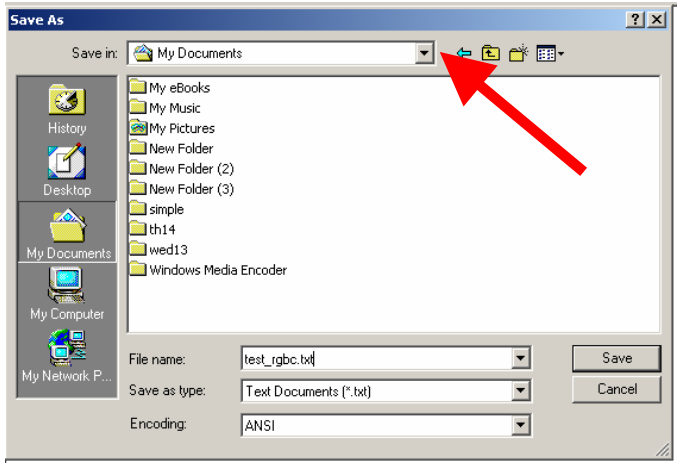
- Highlight all of the data. Then Copy the data (Edit > Copy).
    - Open Notepad (Start>Programs>Accessories>Notepad).



- Paste the data (Edit > Paste).

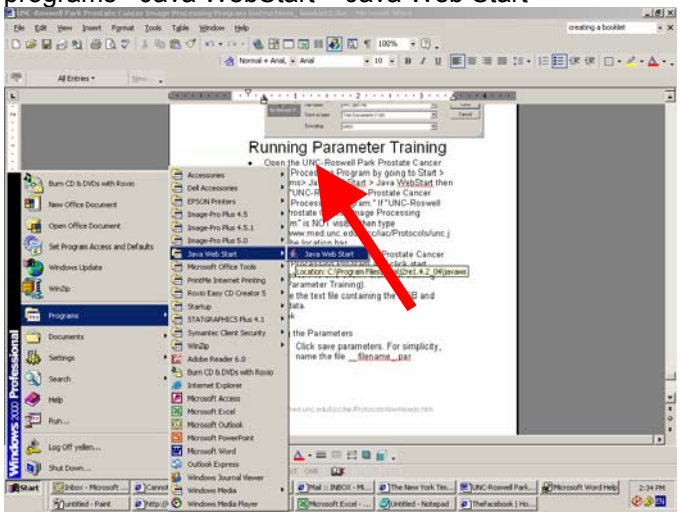


- Save the data with a recognizable filename in a location where it can be found. Check the location by looking at the top of the box. Then Click Save.

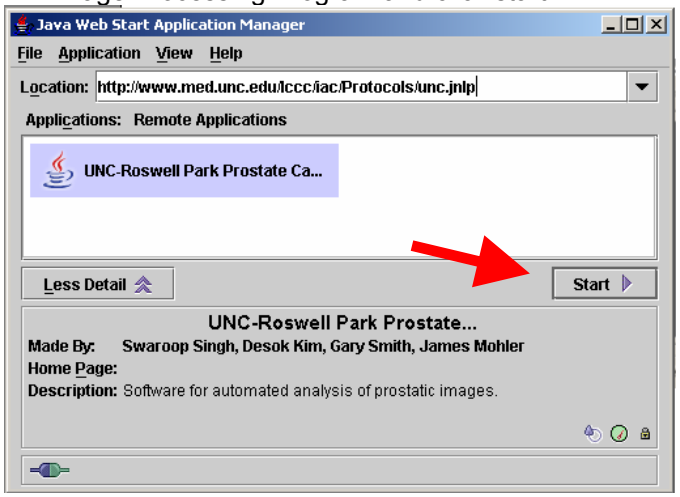


Running Parameter Training

- Open the UNC-Roswell Park Prostate Cancer Image Processing Program by going to Start > programs> Java WebStart > Java Web Start



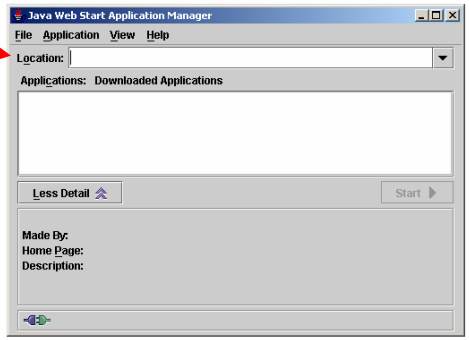
- Select the UNC-Roswell Park Prostate Cancer Image Processing Program and click start.



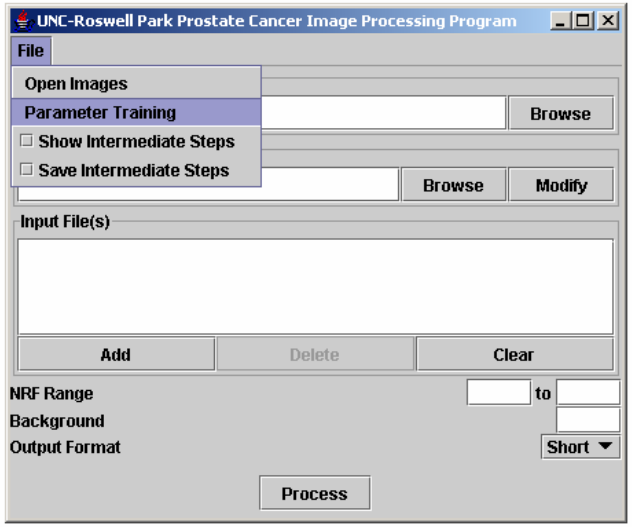
- If the “UNC-Roswell Park Prostate Cancer Image Processing Program” does not appear in the window as it does above, Type the following URL into the Location Bar:

http://www.med.unc.edu/lccc/iac/Protocols/unc.jnlp

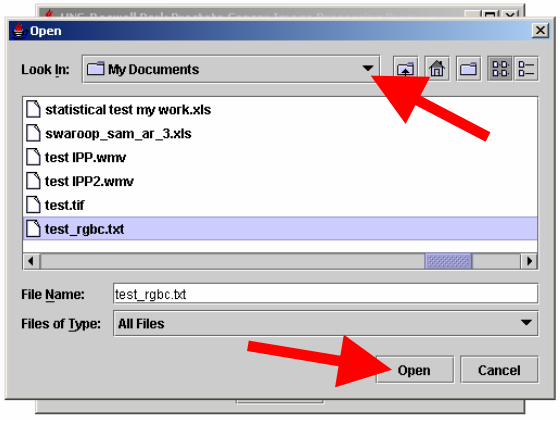
- **NOTE:** The UNC web server is case-sensitive. Therefore, the P in protocols must be capitalized.



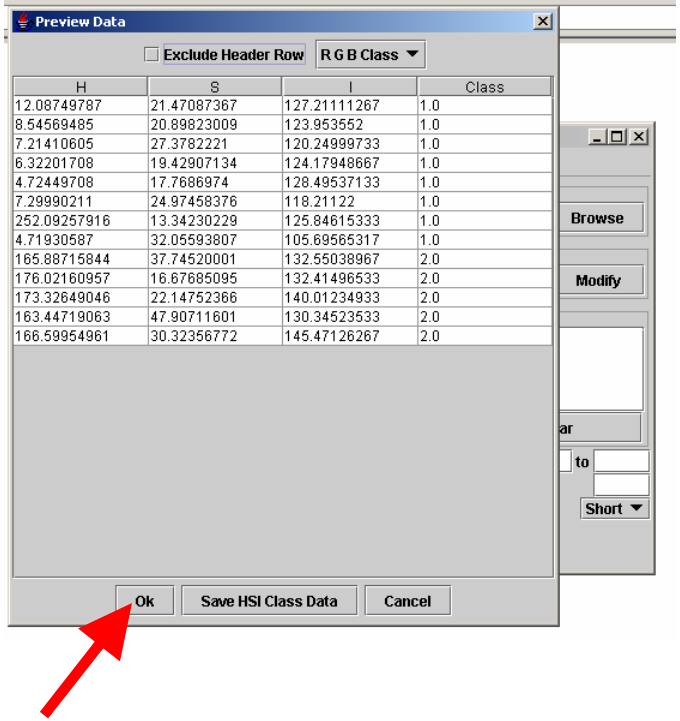
Then go to File and Click Parameter training (File>Parameter Training).



- Choose the text file containing the RGB and class data and click “Open.” Click the top of the box to find the location where the data was saved. (See page 32 or 34.)

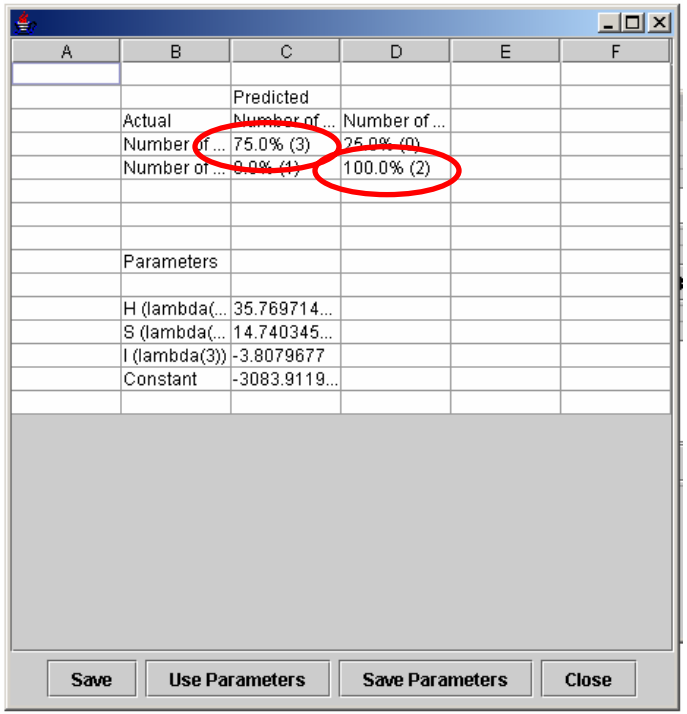


- Click “Ok.”



Saving the Parameters

- Click save parameters. For simplicity, name the file your_filename_here.par
- You can examine the numbers on this screen to see if your parameters are acceptable. The two circled numbers should be greater than 85%. The number on the left shows that the data set must be added to and the parameters retrained.



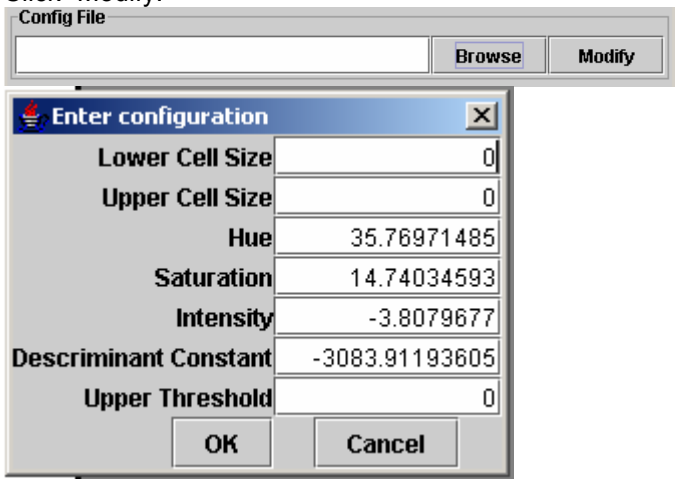
- Then click “Use Parameters.”
- Click “Close.”

Loading the Parameters

- If you have already clicked use parameters, it is not necessary to load the parameters.
- To load the parameters click browse near “config file.”

Modifying the Parameters before performing Analysis

- Click “Modify.”



- Then Change “Upper Cell Size” to 400 and change “Lower Cell Size” to 100.
- Click “Ok.”

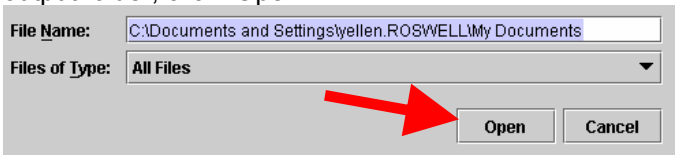
Running the UNC-Roswell Park Prostate Cancer Image Processing Program

Choosing an Output Directory

- The “Output Directory” is the directory where the program will place your results.
- To choose an output directory click browse in the section of the program for the output directory.

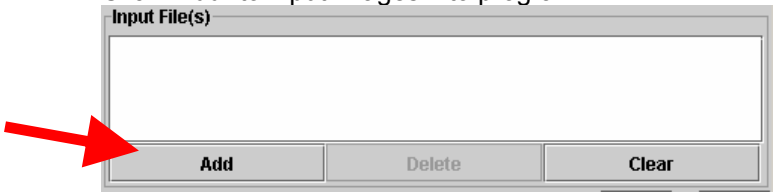
- After clicking browse, you can create a new folder by clicking the following icon.

- Once you are content with the location of your output folder, click “Open.”

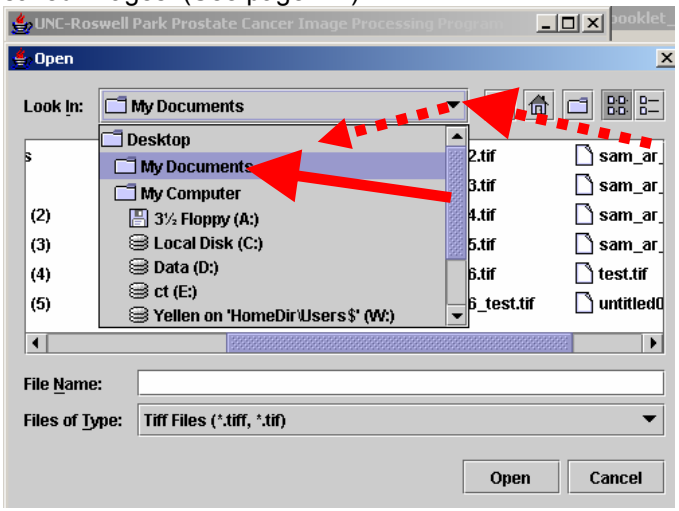


Adding Images to be processed

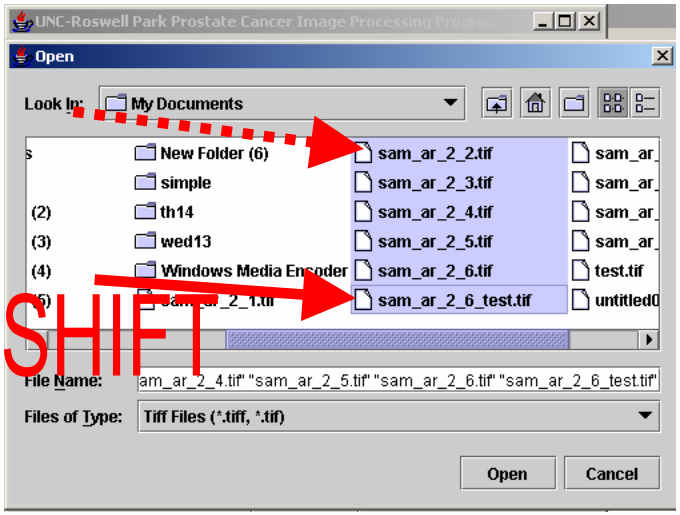
- Click “Add” to input images into program.



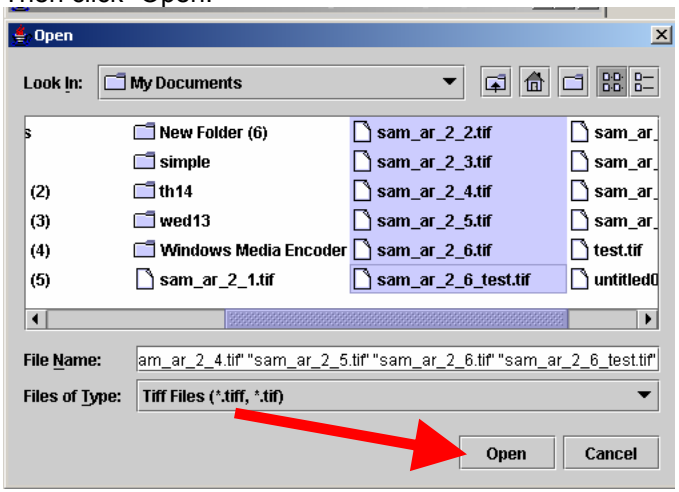
- Click up at the top of the window near to “Look In” to choose the folder where you previously saved images. (See page 14.)



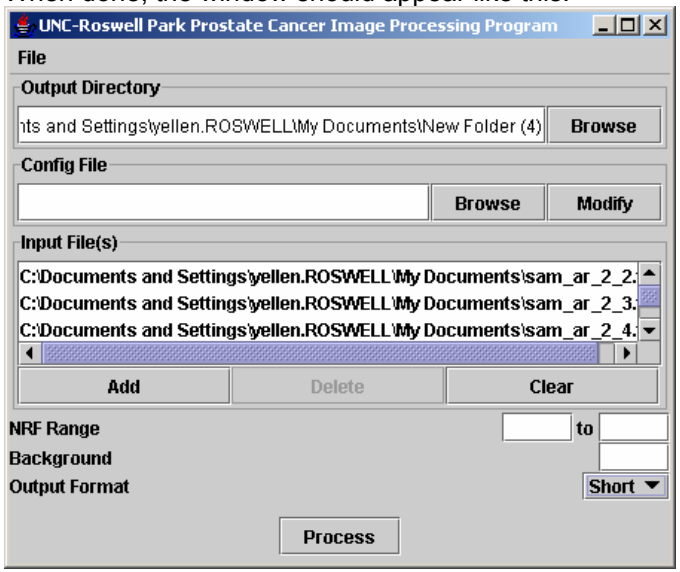
- Then select multiple images by first clicking an image at the top of the box, then while holding down shift, click an image at the bottom of the box.



- Then click “Open.”

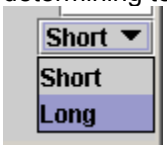


- When done, the window should appear like this:

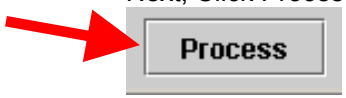


Processing the Images

- Once the output directory has been selected and the images have been loaded, choose the output format. The “Short” format simply provides the end results for each entire image. The “Long” format provides data for each individual scored point. Long is probably best for determining technique.



- Next, Click Process.



- The results will be placed in the “output directory.”

Troubleshooting

Using the color bars

- To make sure that the camera is communicating with the computer, press the “COLOR BAR” button. This will generate a color bar pattern coming out of the Camera Controller which should be able to be seen on the computer and the Video Monitor.

- When viewing the colors bars on the Video Monitor, the brightness and contrast could be configured so that a very slight gray bar can be seen underneath the red bar.

Installing the IPP Environment

- If RGBDataCollect outputs Red, Green, and Blue data outside of the 0 to 255 range, it is necessary to install the Image Pro Plus Environment File, “Swaroop01.env”.
- Installing the file requires downloading the file from this URL
- http://www.med.unc.edu/lccc/iac/Protocols/Swaroop01.env
- NOTE: The UNC web server is case-sensitive, meaning that the P in “protocols” and the S in “Swaroop01.env” must be capitalized.
- Then place the file into the directory containing “IPWIN32.exe” (most likely the C:\IPWIN5\ directory.)

References

Kim D, Gregory CW, Smith GJ, Mohler JL. Immunohistochemical Quantitation of Androgen Receptor Expression Using Color Video Image Analysis. Cytometry 1999;35:2-10.

Kim D, JD Charlton, Coggins JM, Mohler JL. Semiautomated nuclear shape analysis of prostatic carcinoma and benign prostatic hyperplasia. Anal Quant Cytol Histol 1994;16:400–14.

Kim D, Gregory CW, French FS, Smith GJ, Mohler JL: Androgen receptor expression and cellular proliferation during transition from androgen-dependent to recurrent growth after castration in the CWR22 prostate cancer xenograft. Am J Pathol 2002;160:219-226.

Singh SS, Kim D, Ford OH, 3rd, Mohler JL. Automated nuclear analysis of prostate cancer and benign prostate hyperplasia. Proceeding of Biomedical Engineering, IASTED International Conference on Software Engineering 2005; Innsbruck, Austria. ACTA Press, 95-98.

Singh SS, Kim D, Mohler JL. Java Web Start based software for automated quantitative nuclear analysis of prostate cancer and benign prostate hyperplasia. Biomed Eng Online 2005;4:31.

Smitherman AB, Gregory CW, Mohler JL. Apoptosis levels increase after castration in the CWR22 human prostate cancer xenograft. Prostate 2003;57:24-31.