Biology 324 **NIH Image and Gel Analyses** **Leaf/Moyer**
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NIH image is a freeware image analysis program developed by Wayne Rasband at the National Institutes of Health. A PC version is available from Scion. Another freeware image analysis program called Image Tool is also available from the University of Texas. Recently, a JAVA version of NIH image has also been released. Links to the NIH Image Home page and Image Tool Home page can be found from the Biology Department Home page.

You will be extensively using NIH Image to analyze your gels. You can accurately calculate the relative distance a DNA fragment migrates and, if careful, quantitate the amount of DNA in a gel.

Accessing NIH Image
To access NIH Image, go to any Mac that is connected to the Biology network. NIH Image is found at Mendel/Courses/324/NIH Image. NIH image is also found locally on many macs. To connect to Mendel on a Mac, go to **Chooser** under the ***Apple*** menu, select the **Appleshare** icon and then select **Mendel** in the box on the right hand side. This will bring up an icon on the desktop called Mendel. Double-click on the icon, open the **Courses** folder, then the **Biol 324** folder, then the **NIH Image** folder. To launch NIH image, double click on the microscope icon.

Opening your TIFF files
To open your a TIFF file of your gel, go to **Open** command under the ***File*** menu of NIH Image. Note that the top of the drop down menu is the folder in which you are presently in with the program (Desktop/Mendel/Courses/324/NIH Image). To navigate to your gel, which is in the 324.00 folder, select the 324 folder in the navigator and select 324.00. This will bring up a folder for each student pair. Select your folder and this will bring up your gel TIFF file.

Analyzing your gel
To compare the relative distance of your known samples (Hi Lo markers, and Lambda Hind III DNA fragments) you can measure the number of pixels from the bottom of the well to the middle of each DNA fragment.

1) Under the ***Analyze*** menu, select the **Options** command and <u>length</u> as the measurement parameter.

2) Then go to the TOOLS BOX and select the teeter/totter device on the right column next to the paintbrush. This is the measuring tool. Use this tool draw a line from the bottom of the well to the middle of the DNA fragment of interest.

3) To view the measurement, select **Measure** under the ***Analyze*** menu, and then select **Show Measurements** under the ***Analyze*** menu. (An easier of doing this is to simply select *Command 1* and then *Command 2*. (Command keys are directly to the space bar and have an apple on them).

To delete measurements, select **Reset** under the **Analyze** menu (or command 3) and it will clear it will the window with measurements.

Note that you can drag on end of the line to the DNA fragment of interest, select *Command 1*, drag the end of the line to the next DNA fragment, select *Command 1* and so on until all the DNA fragments in the gel have been measured. Then you can show the data by a *Command 2*. This data can be exported to Excel or simply recorded and manually plotted out on semi-log paper.