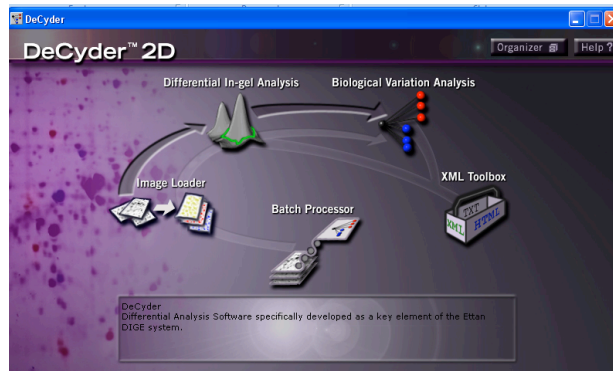


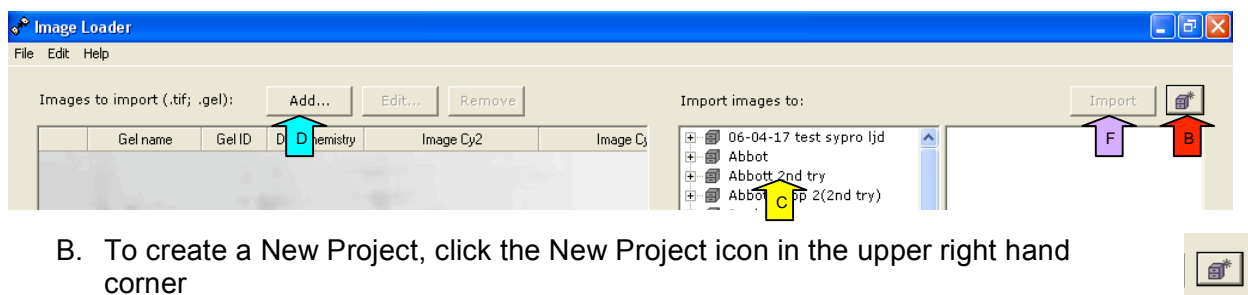
**COMPUTER
ANALYSIS
OF
DIGE GELS**

DECYDER 6.5 CHEAT SHEET



I. Image Loader

A. Open Image Loader









- B. To create a New Project, click the New Project icon in the upper right hand corner
 1. Type a name and description for your project
 2. Click OK
- C. To add images to an existing project, double click on the project in the list provided
- D. Click the Add button
 1. Browse to find your cropped gel images
 2. Open the folder for your gel
 3. Select all three of the images
 4. Click Open
- E. Your images should all appear on one line in the left column. If they appear on multiple lines, you named them incorrectly when scanning and they are no longer linked.
- F. Once you have added all of your gels, click the Import icon at the upper right hand corner. Your images will now be added to your project.
- G. Close Image Loader.

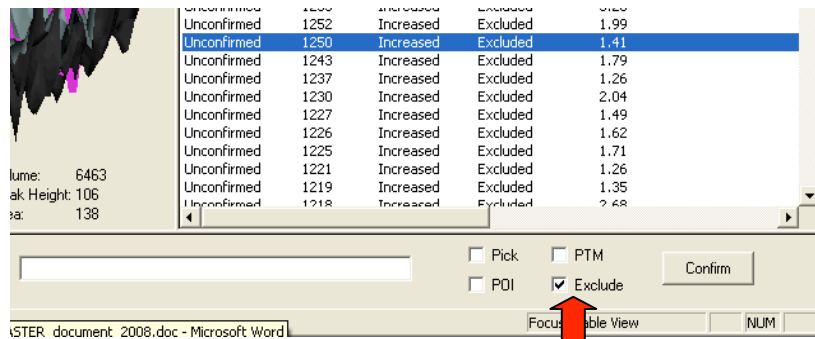
II. Differential In-Gel Analysis (DIA) – Control vs. Experimental

- A. Open DIA
- B. Click the Create Workspace icon or Click File – Create Workspace
 1. Find your project folder in the list and open it
 2. Open the GEL folder
 3. Select your gel and all three images should appear in the right column



4. Click Create
- C. Adjust the Contrast and Brightness, if necessary, by clicking the Contrast and Brightness icon or click View – Contrast/Brightness 
- D. Click the Process Gels icon or click Process – Process Gel Images 
 1. Type 2500 as the estimated number of spots
 2. Click OK
- E. Click the Properties icon (next to the printer icon) or select View – Properties 
 1. Click the Table View tab
 2. Make sure that the Similar, Decreased and Increased boxes are checked under Included Spots
 3. Click OK
- F. Click the Image View icon or click View – Image View 
 1. Make sure all of the obvious protein spots are identified (have a colored outline)
 2. If there are spots not identified, go back to Process Gel Image and increase the number of spots
- G. Click the All Views icon or click View – All Views 
 1. In the Table panel at the bottom right,
 - a. Select the first spot and look at it in the Image View and 3D View panels
 - i. If it does not look like a real spot or it looks like dust, continue down the list
 - ii. Keep going down the list until you find a real protein spot
 - iii. When you find a real protein spot, write down its Max Slope
 2. In the Table at the bottom right, click the Area column heading so that the spots are ordered from the smallest area to the largest
 - a. Select the first spot and look at it in the Image View and 3D View panels
 - i. If it does not look like a real spot or it looks like dust, continue down the list
 - ii. Keep going down the list until you find a real protein spot
 - iii. When you find a real protein spot, write down its Area
 3. In the Table at the bottom right, click the Volume column heading so that the spots are ordered from the smallest volume to the largest
 - a. Select the first spot and look at it in the Image View and 3D View panels
 - i. If it does not look like a real spot or it looks like dust, continue down the list
 - ii. Keep going down the list until you find a real protein spot
 - iii. When you find a real protein spot, write down its Volume
 4. In the Table at the bottom right, click the Peak Height column heading so that the spots are ordered from the shortest to the tallest

- a. Select the first spot and look at it in the Image View and 3D View panels
 - iv. If it does not look like a real spot or it looks like dust, continue down the list
 - v. Keep going down the list until you find a real protein spot
 - vi. When you find a real protein spot, write down its Peak Height
- H. Click the Exclude Filter icon or click Process – Exclude Filter
 1. Check the Slope, Area, Volume, and Peak Height Boxes 
 2. Fill in your recorded values for the Max Slope and minimum Area, Volume, and Peak Height
 3. Click OK
 4. All of the spots that did not meet your requirements have now been excluded from your analysis
- I. In the Table panel, click the Excluded column heading. Now the excluded spots are together and the decreased and increased spots are grouped
 1. Scroll through the excluded spots to make sure none of them are real spots that need to be included
 - a. If a spot needs to be included, uncheck the Exclude box at the bottom of the screen



- J. Click the Save icon or click File – Save Workspace
 1. Name the workspace with the gel number
 2. Click Save