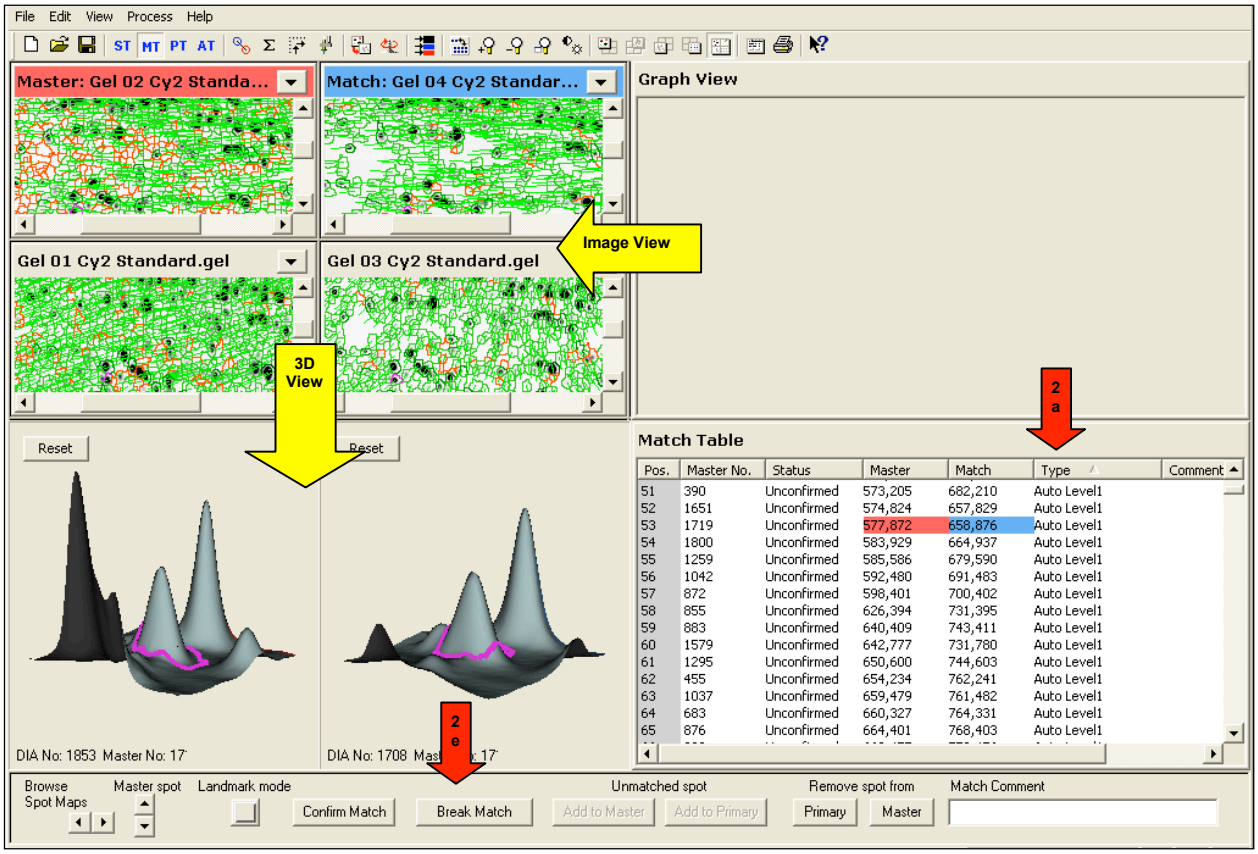
A. Check Spot Matching
    1. Click the Match Table icon or click View – Match Table
        a. Click the Magnify Image View icon or View - Image View
        b. Make sure all of the images are Cy2 images from different gels
        c. Check to make sure that the green vectors in each gel are going approximately the same way
            i. If the vectors do not show up or you want to remove the signature, click the Properties icon and either check or uncheck the items in the Image View tab
    2. Click the Display All Views icon or click View – All Views
        a. In the Match Table select at the Type tab and arrange so the Auto Level1's are at the top
        b. Check randomly about 5 Auto Level 1 matches
        c. Look at the Image View and the 3D view to ensure the spots match
        d. Repeat for about 10 Auto Level 2 spots
        e. If they do not match, use the Break Match button and make the correct match



B. Analysis
    1. Click the Protein Table icon or click View – Protein Table
    2. Click the Protein Statistics icon or click Process – Protein Statistics Σ
        a. Select the following items in the Protein Statistics window:
            i. Independent tests

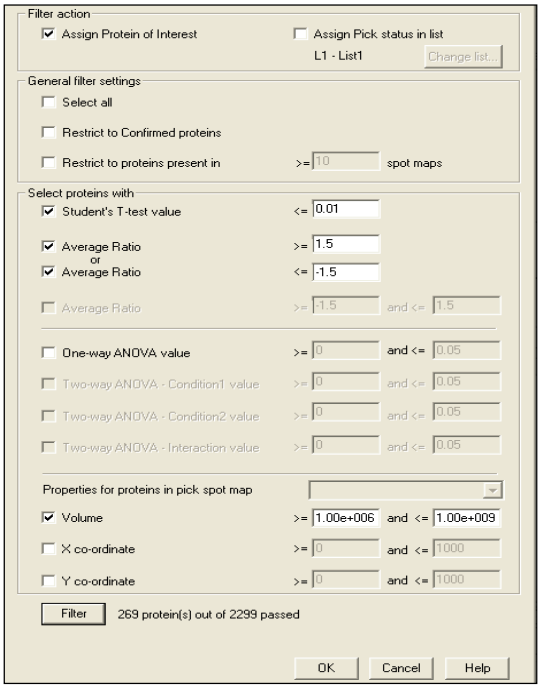
ii. Average Ratio
iii. Student's T-test
iv. Select Control in Population 1
v. Select Experimental in Population 2
vi. One-Way ANOVA
vii. Apply false discovery rate

b. Click Calculate

3. Click the Properties icon or click View – Properties
   a. Select the Protein Table tab and check the following in the Table Column Order and Visibility list:
      i. Pos
      ii. Master No
      iii. Appearance
      iv. Av. Ratio
      v. T-test
      vi. 1-Anova
      vii. Pick
      vii. Pick Spot Vol.
      ix. POI
   b. Click OK
4. In the Protein Table click the T-test tab so that the smallest T-test value is at the top
   a. Select the first spot and look at it in the Image View and the 3D View

C. Selecting Proteins of Interest

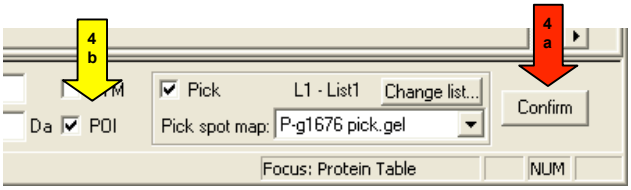
1. Click the Protein Filter icon or click Process – Protein Filter 
   a. Check the following items and enter the appropriate values into the Protein Filter window:
      i. Assign Proteins of Interest
      ii. Student's T-test – 0.01
      iii. Average Ration >=-1.5 and <=1.5
      iv. Volume 1.00e+005 to 1.00e+008
   b. Click Filter
   c. Check the number of spots generated and change the values above if necessary to allow more or less spots to pass the filter
   d. Click OK



D. Confirmation

1. Click the Multiple Image View icon or click View – Display Multiple Gel Views
   a. Select an appropriate number so you can view all of your images with the Control images on top and Experimental Images on bottom

2. Click the Display All Views icon or click View – All Views
3. Click the Properties icon or click View – Properties
    a. Select the Protein Table tab
    b. In the Protein Table Filter View box select Protein of Interest
    c. Click OK
4. Select the first spot in the Protein Table
    a. If the spot looks good, click the Confirm button at the bottom right of the screen
    b. If the spot does not look good, uncheck the POI box at the bottom of the screen
    c. Continue through all of the POI's in the Protein Table



5. Click the Save icon or click File – Save

IV. Adding A Preparative Gel Image and Creating a Pick List

A. Open Image Loader
    1. Highlight your project in the list
    2. Click Add
    3. Find your cropped pick gel image and click Open
    4. Your pick gel image should now appear on line one in the left panel
        a. In the Dye Chemistry column it should say PostStain
        b. If it does not, click Edit and select PostStain from the Dye Chemistry drop down menu
    5. Click Import
    6. Close Image Loader
B. Open DIA
    1. Click the Create Workspace icon or click File – Create Workspace
        a. Open your project folder
        b. Open your GEL folder
        c. Select your pick gel image
        d. Click Create
C. Spot Detection
    1. Adjust the Contrast and Brightness if necessary
    2. Click the Process Gels icon or click Process – Process Gel Images
        a. Set the Estimated number of spots to 2500
        b. Check the Autodetect Picking references box
        c. Click OK
    3. Click the Image View icon or click View – Image view
        a. Verify all spots have been detected (have a signature)

b. Zoom in and verify the picking references have been detected and their signatures are lined up correctly
    i. If they need to be moved, click Edit – Edit Picking Reference
    ii. You will now have a hand as your mouse courser
    iii. Position this hand over the center of the picking reference signature and press the left button
    iv. Hold the left button down and drag the signature to the correct location and release the left button
    v. Repeat for the other picking reference if necessary
    vi. If something happens and you want to start from scratch, click Edit – Clear All Picking References
    vii. Then click Edit – Define Picking Reference
    viii. Move the signature over the **LEFT** picking reference and click the mouse button to place it
    ix. You must start with the left because it will direct the spot picker where to pick your gel
    x. Repeat with the right picking reference

D. Exclude Filter
1. Click the All Views icon or click View – All Views
2. In the Table panel at the bottom right, click the Max Slope column heading so that the spots are ordered from the largest slope to the smallest
    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
        1. If it does not look like a real spot or it looks like dust, continue down the list
        2. Keep going down the list until you find a real protein spot
        3. 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
        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 Peak Height
5. Click the Exclude Filter icon or click Process – Exclude Filter
    a. Check the Slope, Area, Volume, and Peak Height Boxes
    b. Fill in your recorded values for the Max Slope and minimum Area, Volume, and Peak Height
    c. Click OK
    d. All of the spots that did not meet your requirements have now been excluded from your analysis
6. Click the Save Workspace icon or click File – Save Workspace
    a. Note that you can do the above for a Coomassie gel to create a pick list
7. Close DIA