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# STORM Phosphorimager

**Rm. 5310 MPRB**

-Login
    -your password
    -domain IMNT
-Place screen on glass
    -put in upper left corner under the ledge
-open scanner control
-highlight squares to scan
-click scan
-save to disk before scan will begin
    (USB drive, Zipdisk, or H drive)
-close scan when done

-Open image quant
-Open gel file
-Click on icon at top right for Gray scale change
-□ selection tool to select region of interest
-save area that was selected as a tif
-use red □ on the side for volume quant

Notes:
-Storm is always kept on
-Use the lever to open the lid
-Erase after the scan for 20min and erase just before scanning for 10 minutes on a light box

Analyzing the scanned images from "STORM"
**Software:** ImageQuaNT 5.2
**References:**

ImageQuaNT user's guide, chapter 11 (Molecular Dynamics)
ImageQuaNT Tutorial Book
(Both of them are available on the shelf by the Storm machine at 5th floor)

**Protocol (Quantity the image using Spot Finder)**

**1. Open the scanned image in ImageQuaNT software**

- Click the icon "**ImageQuaNT 5.2**" in Desktop
- Click "**File**" in menu bar, and then go to "**Open**".
- Click the file name

***Results:*** the new window with your image in it

**2. (Optional) Enlarge the image**

Notes: it is better to zoom in the image and bring out the detail of your image before the quantitative process.

- Click "**View**" in menu bar, and then go to "**Zoom in**"

**3. Set up the parameters**

**A. Set the preferences for Background Correction:**

Notes: two choices for the Background Correction: "None" or "Histogram Peak".

In general, the "histogram Peak" is recommended, particularly if the image in the selected rectangle has more pixels in the background area than in the spot area.

- Click "**Preference**" in menu bar, and then go to "**Background Correction**" in the section of "Spot finder"
- Click "**Histogram Peak**"
- Click "**Ok**"

**B. Set the Volume Report Options**

Notes: using this setting to set up the report format.

- Click "**Preference**" in menu bar, and then go to "**Volume Report Set Up**"

(Optional) In the new window popped up, there are three sections, and here are the selection recommended:

*Section "Header":*

Select "Image Name" "Preset time/date"
"Scan time/date"

*Section "Results"*

Select "Object name"

"**Volume**": Integrated intensity of all the pixels in the spots excluding the background. **(Final data!)**.
"Background Value":
"Background type"
"Average": Average intensity of all the pixels in the spot
"Standard deviation": Standard deviation of the pixel intensity in the spot
"Sum": Integrated intensity of all the pixels in the spot including the background
"Area"

*Section "Prints"*

Select "Images & results"

- Click "**Ok**"

**4. Draw a rectangle to define the spot area**

- Click the "**Rectangle**" icon on the tool bar (left side of the screen)
- Draw the rectangle around the entire spot you want to evaluate (using the Mouse):

  Notes: Pick the "fattiest" spot in the image to draw the initial box, if you want to keep the consistence for the area of all the spots to be quantitied.

  To modify the size of rectangle after the initial drawing, click the "**Arrow**" icon (top of the tool bar) at first, and then you can drag or change the rectangle using the Mouse.

- (Optional) Duplicate the rectangle boxes (if you have multiple spots to be quantitied at once)
  Click the "**Arrow**" icon in the tool bar at first.
  Click the initial rectangle box you created
  Push the button "**Control**" + "**C**" in the keyboard to copy
  Push the button "**Control**" + "**V**" in the keyboard to paste
  Notes: Create all the boxes you need at once, and then move them to the individual spot.

**5. Quantitate the spot using Spot Finder Inspector**

- Click "**Analysis**" in Menu bar, and then go to "**Spot Finder**"
- New window "Inspector" opened
- Define the spots in "Inspector" window:
  In the left top of the Inspector window, select all the spot you defined by highlight all of them using your Mouse plus "**Shift**" in the keyboard.
  In the Report section, click "**Display**". (The printer was not connected!)
  In "Algorithem" section, select "**Default**", and make sure the "Intensity threshold" is shown in this section.
- Click "**Compute**"
  Note: the number of rectangle box in the image is changed after click "compute", the program assigns the second set of number to each rectangle box.
- New window "Volume report #..." popped up (Be patient! It takes a while!)
  Note: Check several parameters, such as:
  "Background type", see if it is correct;
  "Area", see if it is consistent for all the spot;
  "results", see if approx. matched the eyeball results.

**6. Save the data**

- Select the "Volume Report #..." window, and new window will pop up
  "Data must be saved from Excel
  Activate Excel"
- Click "**Yes**"
- Save the data in **YOUR** storage disk
- Close all the windows
- Log off