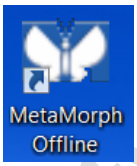
MetaMorph Standard Operation Protocol
Basic Application

Contents

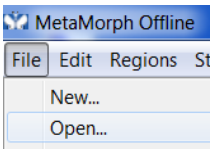
Lauch MetaMorph



- Log in Metamorph computer with account of "**user**".
- Download your data in D/User/Your folder. **No data is allowed to save in C drive or Desktop**.
- Double click "**MetaMorph Offline**" icon on desktop to lauch MetaMorph.

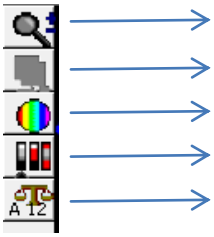
Basic Navigation and Image Handling

Opening Images



- To open images by pressing **File Open.**

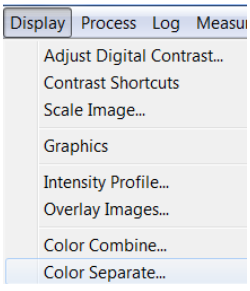
- A toolbar will show on the left side of the image. This tool bar allows you to zoom in an out, turn on and off overlays, change LUT settings (monochrome images only), adjust contrast and set a threshold.

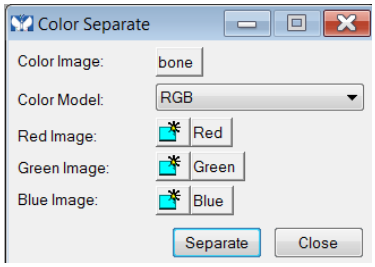


Separating Multichannel Images

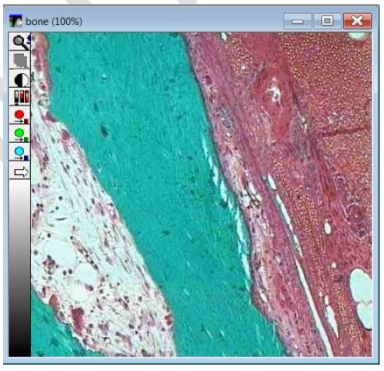
- If you have 24bit RGB image, you may need to separate it into individual component channels for analysis. Open your image with MetaMorph.

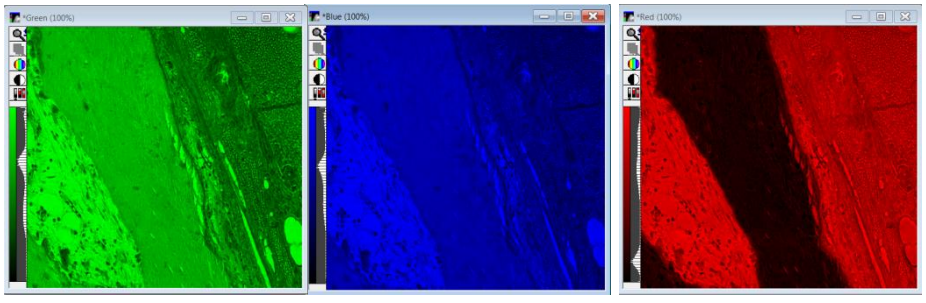
- Go to **Display** → **Colour Separate**





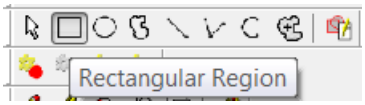
- The resulting dialog lets you configure which channels will be separated out (and what they will be called). Leave everything as default and press **Seperate.**
- 24bit RGB image will result in 3 8bit images.

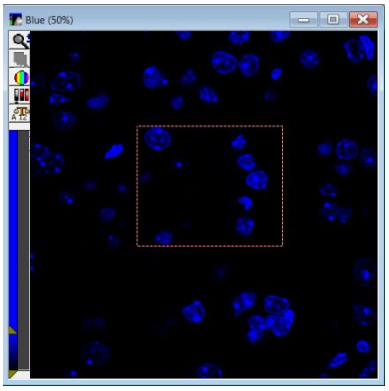




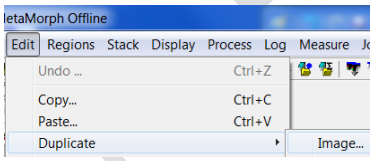
Cropping an Image

- Select the rectangle region tool and draw a region on the image you would like to crop



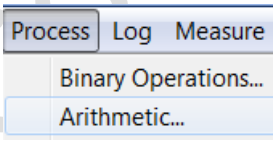


- Go to **Edit** → **Duplicate** → **Image**
  If the file is stack, go to **Edit** → **Duplicate** → **Stack**
- A new image/stack will be generated.

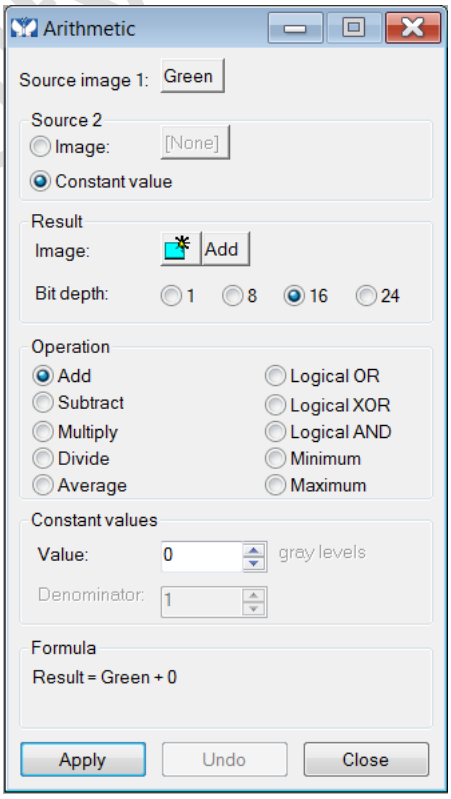


Changing an 8 bit Image into 16 bit

- Open the image and go to **Process** → **Arithmetic....**

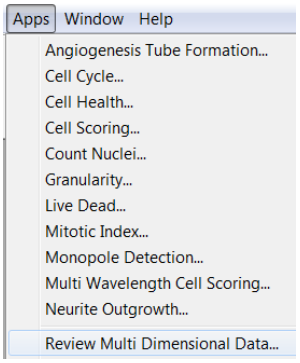


- Configure the dialog box as below. Then press **Apply**. A new 16bit image will be generated.

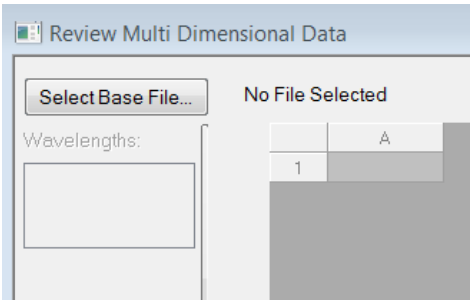


Making Movie

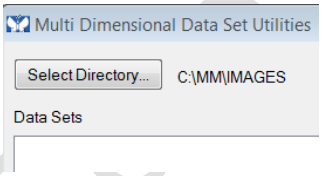
- Go to **Apps** → **Review Multi Dimensional Data**



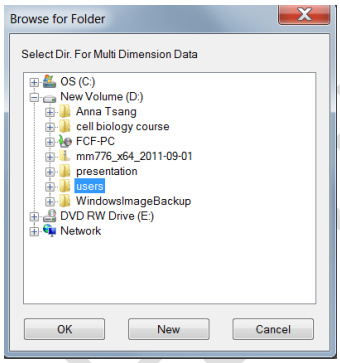
- Press **Select Base file** in dialog window



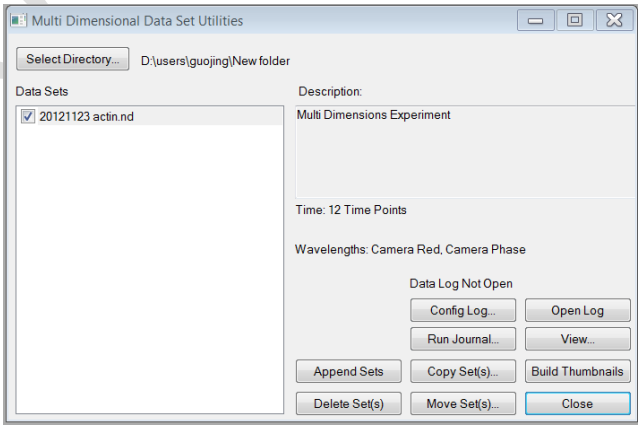
- Press **Select Directory** in dialog window



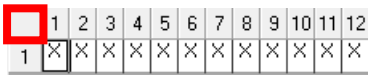
- Highlight the folder of your files in "**Browse for Folder"** window, then press **OK**.

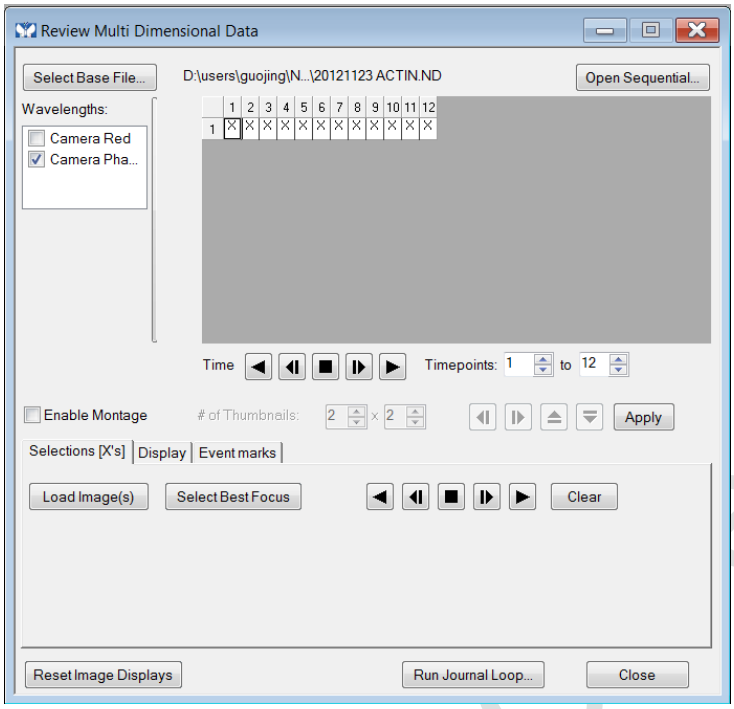


- Tick the .nd file and press **View** in "**Multi Dimensional Data Utilities**" window



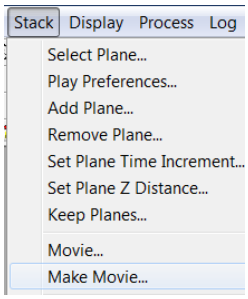
- Tick the **wavelength** and **time points** and press **Load Images**. For select all time points, right click the left corner of time points row.



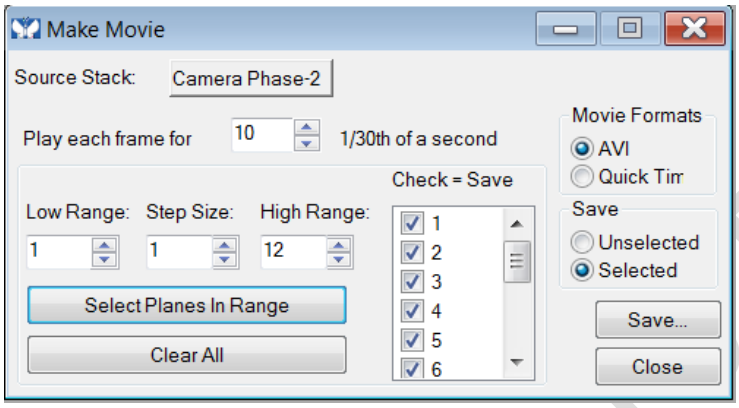


- A new stack file will be generated. Save this stack file as .stk if needed.

- Go to **Stack** → **Make Movie**



- In the **Make Movie** dialog box select **the stack file** as the source stack. The play speed of the movie is set by entering a number in the **Play Each Frame For** box. The value could be set range from 1 to 30000. The play speed will be slower if the value is higher. A value of 8 usually works quite well.
- To make a movie of the whole stack set **Low Range** to 1, **Step Size** to 1 and **High Range** to the number of planes in the stack and press **Select Planes in Range**.
- Chose AVI or Quick Time for movie format then check **Selected** under **Save** and press the **Save** button.

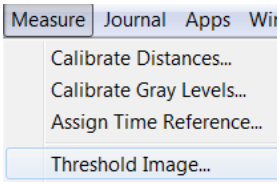


- Choose where to save and a name for the movie file and press **Save.**
- You will be presented with a **Video Compression** dialog box. It is recommended to save the movie as **Full Frames (Uncompressed)** and compress it later if required.

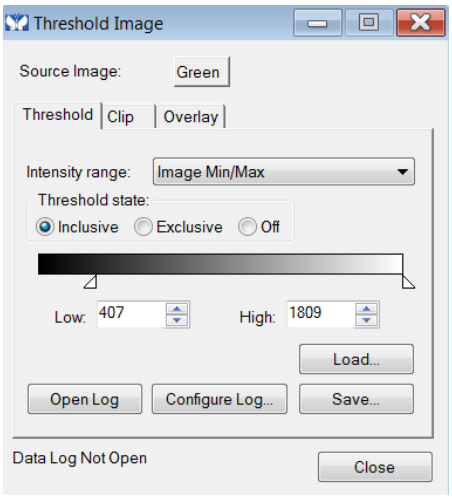
Intensity measurement

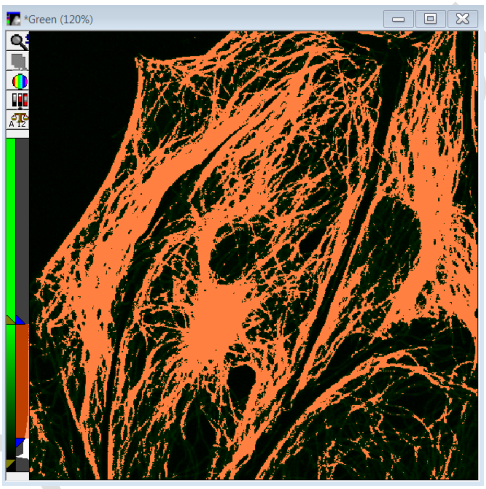
Entire image measurement

- Open the image and go to **Measure** → **Threshold Image**

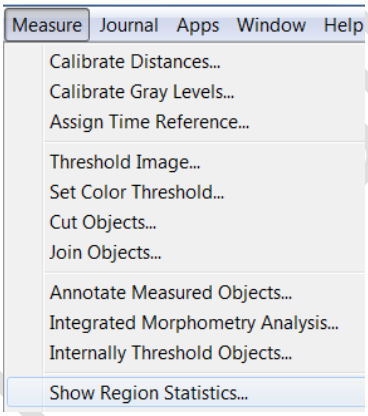


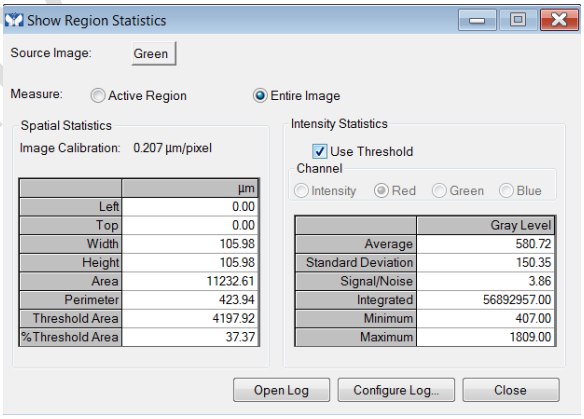
- Use the inclusive option under **Threshold state** and move the white triangle sliders (or type values into the **Low** and **High** boxes) to set the desired threshold range. The image will be thresholded as the desired threshold range.



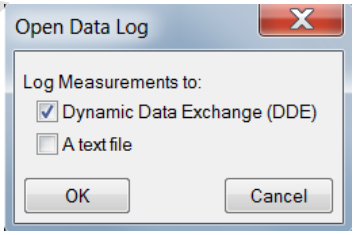


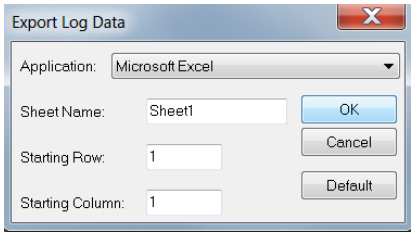
- Go to **Measure** → **Show region statistics**
- Select the **Entire Image** and check **Use Threshold**.





- Press **Configure Log** to select the measurement parameters for log file.
- Select **Open Log** and **DDE** to connect MM to excel. An Excel worksheet will now open.





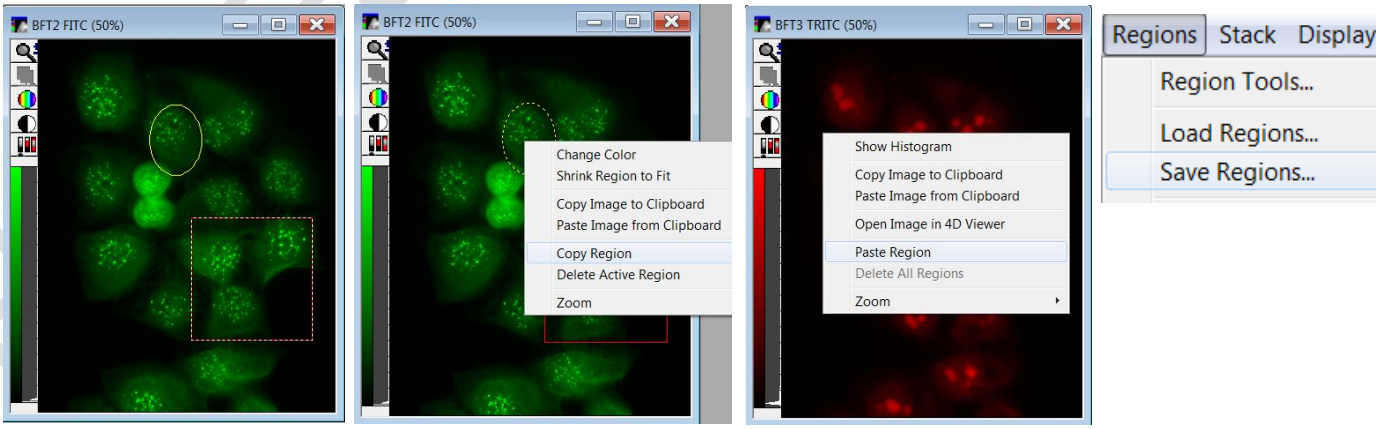
- The Open Log button now reads **F9: Log Data**. Select **Log Data** to copy data in Excel worksheet.
- Save the excel file if needed.

Region measurement

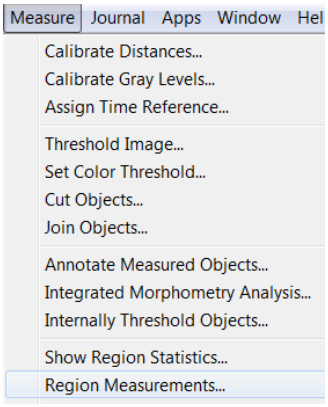
- Various region shapes or types are available in MetaMorph. The **Region Tools** palette is located in the MetaMorph toolbar.

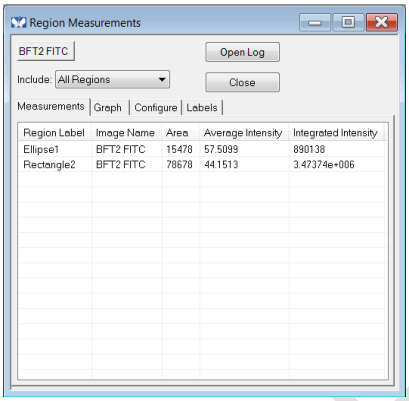
- **Locator** – Selects inactive regions or moves or resizes the active region.
- **Rectangle** –Draws rectangle/square shaped regions.
- **Ellipse** – Draws circular regions.
- **Trace** – Draws a region freehand. Hold down the mouse button to create a freehand shape. Clicking the mouse button will create connected lines. Double clicking will join the current point up with the first point thereby closing the region.
- **Single Line** – used to draw a single line region.
- **Multi-Line** – Used to draw a segmented line. Each mouse click defines the end of each segment. Double click defines the end of the line.
- **Trace Line** – As for the **Trace** region command but does not close upon double clicking
- **Auto Trace** – Automatically traces a region around features of interests. Works similar to the magic wand tool in Photoshop.
- **Region Properties** –Configures region colours.

- Open the image and select the region tool to draw regions on the image.
- Region could be copied by right clicking **Region** and selecting **Copy Region** from the menu or deleted by selecting **Delete Active Region** from the menu.
- Copied region could be pasted to a new image by right clicking the new image and selecting **Paste Region** from the menu.
- Region could be saved by selecting **Regions→ Save Regions.**
- Saved Region file could be applied to a new image by clicking **Regions → Load Regions**
- Delete all regions from each image by right clicking and selecting **Delete All Regions** from the menu.

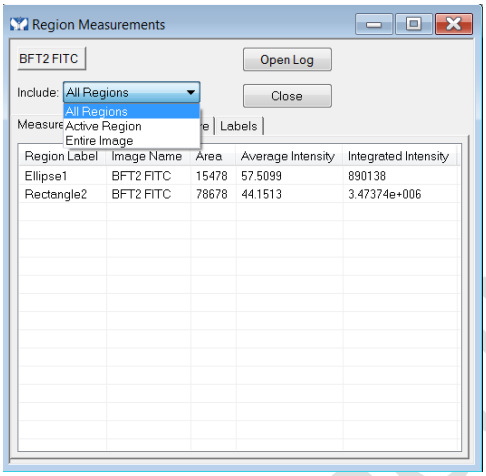


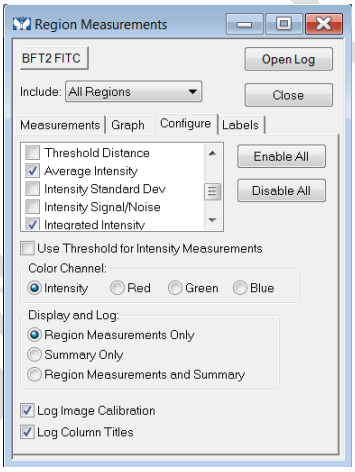
- To measure the intensity in regions, go to **Measure** → **Region Measurements**

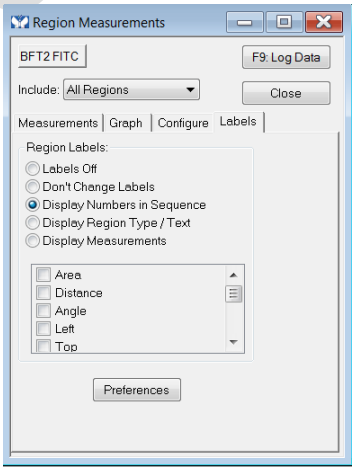




- Select **All Regions** to measure all regions on the image.
- Use the **Configure** tab to select the measurement parameters for log file.
- The **Labels** tab sets what labels are added to the image (if any).



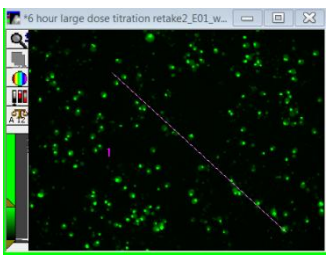


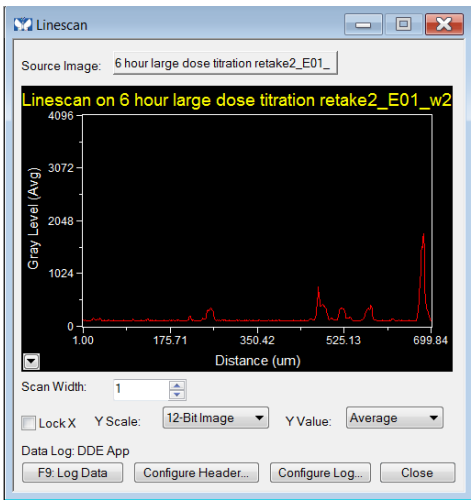


- To log the information in excel file, press the **Open Log** button and tick the **Dynamic Data Exchange (DDE)** box and press **OK.**
- Log the data by clicking **Log Data** as demonstrated previously.

Profile measurement (Line Scan Mode)

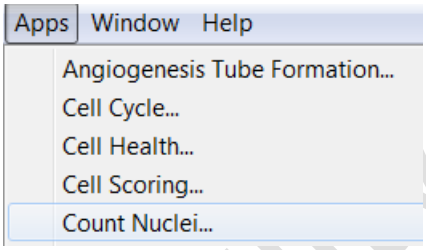
- Open the image and go to **Measure** → **Linescan...**
- A line region will automatically be added to the image. Double clicking on the region will allow size and angle of the line to be changed.
- The scale and graph type can be changed by selecting **Graph Settings** from the menu that appears when the triangle button in the bottom left of the graph is clicked

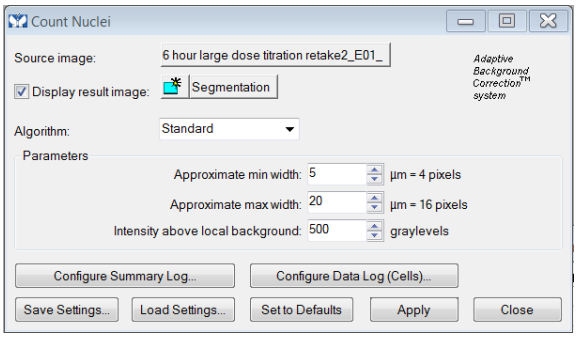




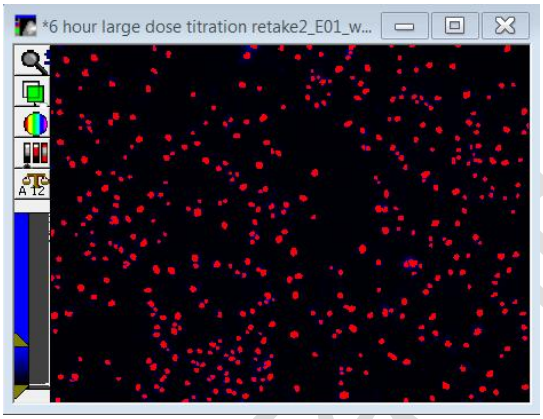
App- Count Nuclei

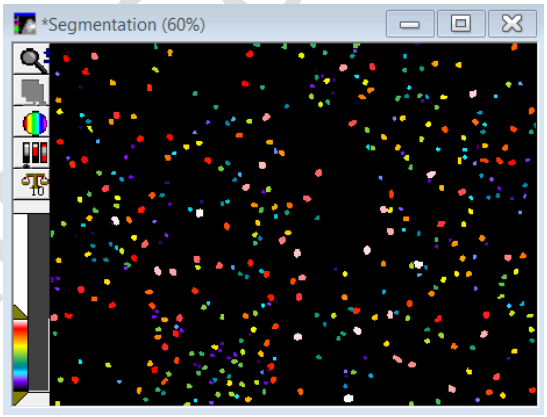
- Open the image and go to **Apps** → **Count Nuclei**
- Using the line region tool, measure the width of the smallest and largest nuclei. Enter the smallest figure in **Approx. min. width** and the largest figure in the **Approx. max. width**.
- Hover the mouse cursor over nuclei and read the intensity displayed at the bottom of the screen. Do the same for the background and insert the difference in the **Intensity above the local background** box.

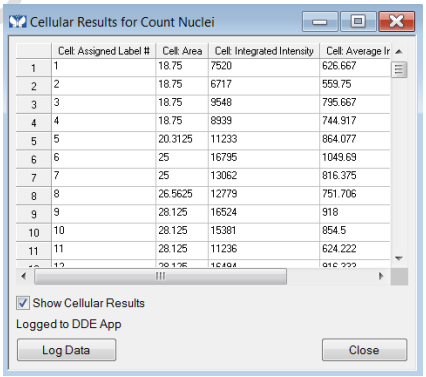




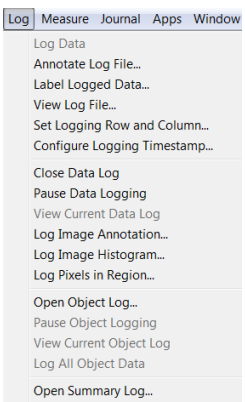
- Configure summary log and data log to select the interested parameter for log files.
- Click **Apply**. A mask image will be applied on the original image and a segmentation image will appear.



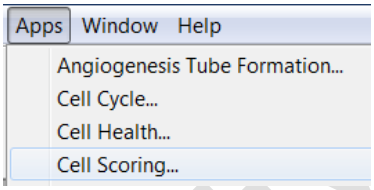




- Adjust the figures for min. width, max. width and intensity above local background if necessary.
- Open **Summary Log** by clicking **Log** → **Open Summary Log**
- Click **Apply** and data is automatically logged into excel.

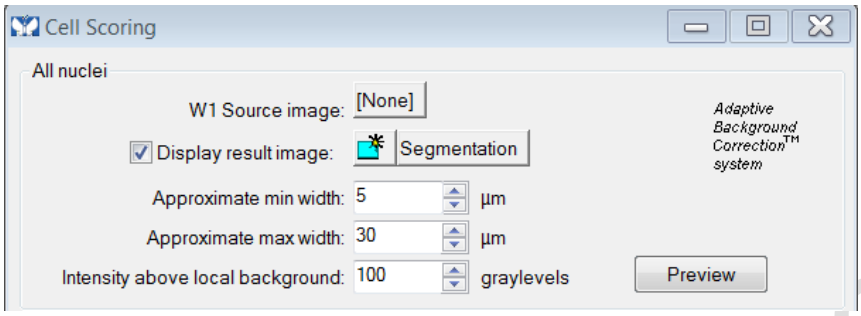


App- Cell Scoring

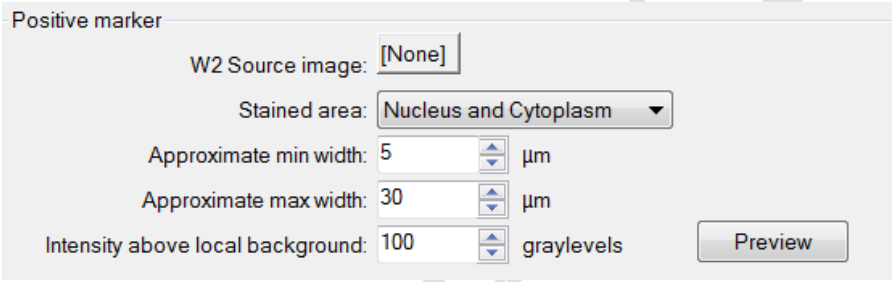


- Open the image and go to **Apps** → **Cell Scoring**

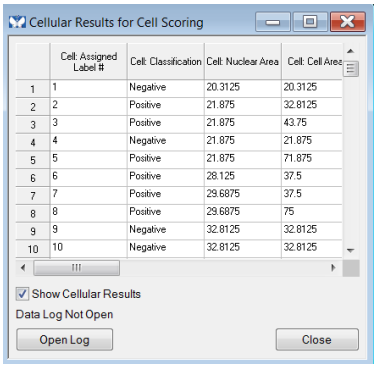
- Select the **W1 Source** image for **All Nuclei.** Measure the **Approximate min. width, Approximate max. width**, and **intensity above local background** in the relevant boxes (line region tool). Press **Preview** button to check settings. Adjust setting if necessary.

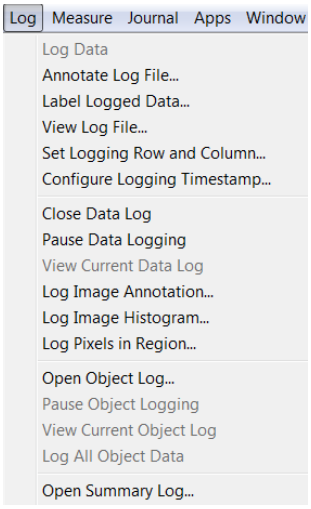


- Select the **W2 Source** image for **Positive marker.** Measure the **Approximate min. width, Approximate max. width**, and **intensity above local background** in the relevant boxes (line region tool). Press **Preview** button to check settings. Adjust setting if necessary.



- Configure summary log and data log to select the interested parameter for log files.
- Click **Apply**. A mask image will be applied on the original image and a segmentation image will appear.
- Adjust the figures for min. width, max. width and intensity above local background if necessary.
- Open **Summary Log** by clicking **Log** → **Open Summary Log**
- Press the **Open Log** button in **Cellular Results for Cell Scoring** window and tick the **Dynamic Data Exchange (DDE)** box and press **OK** to connect cellular result to excel file**.**





- Click **Apply** and data is automatically logged into excel.