

## Flicker Comparison of 2-Dimensional Electrophoretic Gels

Welcome To Flicker

<http://open2dprot.sourceforge.net/Flicker>

### Introduction

Flicker is an open-source stand-alone computer program for visually comparing 2D gel images. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) gels are often difficult to compare because of rubber-sheet distortions. Flicker allows you to [visually compare your gel images](#) against each other or against those found in Internet databases. Many published Internet gels have a subset of spots identified which may make them useful to compare with your gels. Some of these Internet gels are active maps that you can click on a spot to inquire of its identity. You may be able to draw putative conclusions as to the identification of some spots in your gels that visually appear to be the same spots as in reference gels. The Flicker program integrates these various needs to help you try to make putative spot identifications. See the [Quick start examples](#) below for a short list of some of these methods.

Of course Flicker might be useful for comparing other types of images such as 2D-blot, 1D gels, serial microscope sections, time-lapse, microarrays, etc. - any type of image where there are distortions or intensity variation of the images, differing numbers of spots, and other systematic and procedural experimental differences. It can read black and white or color JPEG and GIF images, and black and white TIFF images (some un-banded color TIFF images are available).

The Flicker application is Java program that runs under the MS Windows, MacOS-X, Linux and Solaris operating systems. It is [downloaded](#) to and runs on your computer. Flicker has been made open source and was contributed to the [Open2Dprot](#) project. Both the executable binary as well as the source code is available.

The original version of Flicker was a Java applet that ran in your Web browser ([\[Lemkin97a\]-\[Lemkin97c\]](#), [\[Lemkin99\]](#), [\[Lemkin02\]](#)) and is available at <http://www.lecb.ncifcrf.gov/flicker>. However, being a Java applet, it had many limitations in its capabilities. To resolve these limitations it was converted in 2003 to a Java application by Peter Lemkin and Greg Thornwall with help from Jai Evans. Code was added from the open-source MicroArray Explorer program, <http://maexplorer.sourceforge.net/>, to implement some of the new features in Flicker. The new version of Flicker is much more flexible and makes it easier for you to compare your gels with each other or with reference gels on Internet databases. A limited measurement functionality is available to estimate spot or region quantification.



The following are brief sketches of several ways that Flicker could be used. There are many other ways of using Flicker, and these are detailed in the [Reference Manual](#).

A. Compare two gels to find spot differences.

1. Open 2 gels to compare (your own or gels from the Internet).
2. [Flicker align](#) similar regions for the spot(s) of interest.
3. When they are aligned, you can see local spot differences between the gels.

B. Compare your gel against an Internet reference gel to try to putatively identify the spot.

1. Open 2 gels to compare (let one of them be an [active reference gel](#)).
2. Flicker align similar regions for the spot(s) of interest.
3. If one of the gels is an active reference gel, then you can click on it to putatively identify the protein.

C. Quantitate a list of spots. Flicker has a rudimentary [spot list definition and quantification](#) capability that might be used as follows:

1. Open 2 gels to compare.
2. Flicker align similar regions for the each of the spot(s) of interest.
3. [Add spots of interest to spot lists](#) (a separate list for each gel).
4. Click on corresponding spots in each gel and pair them using a common annotation id.
5. List the spots in the paired spot list (this can be generated as tab-delimited data for export to Excel).

D. [Putatively identify a list of spots](#) in your gel that are identified in an active reference gel by first identifying spots in the reference gel and then using them to identify corresponding spots in your gel.

1. Open 2 gels to compare (let one of them be an [active reference gel](#)).

2. Flicker align similar regions for each of the spot(s) of interest.
3. Add spots of interest to spot lists (a separate list for each gel).
4. Request Flicker to visit the active reference gel Web server and try to lookup the protein id (e.g, Swiss-PROT) for the spots you have defined in the active gel.
5. Then click on corresponding spots in your gel and then [pair them using a common annotation id](#) from the reference gel.
6. List the spots in the paired spot list (this can be generated as tab-delimited data for export to Excel).

## Use of 2D-PAGE in modern proteomics

This approach may be useful for comparing similar protein samples created in different laboratories to help putatively identify or suggest possible protein spot identifications. The gels should be run under similar pH and molecular weight ranges if possible. Although available for over three decades, 2D polyacrylamide gel electrophoresis (2D-PAGE) is still routinely used [1] even considering the now common use of mass spectrometry [2-7] and recently protein arrays [8] for protein identification. If you have [defined a list of spots in an active gel](#) (such as one of the Swiss-2DPAGE gels) and you are connected to the Internet, you can request Flicker to lookup the annotation information (Swiss-Prot id and name) for each spot in the list. Then you can define a list of spots in your gel that correspond to spots in the annotated active reference gel, and then assign these protein identifications to your gel.

Recent advances, such as IEF "zoom" fractionation gels [9] that divide the protein sample by pH range or immunoaffinity subtraction with LC [5], greatly increase the resolution and numbers of spots able to be discriminated by subsequent 2D-gel electrophoresis. Another increasingly common image comparison technique uses 2 to 6 cyanine dyes using dye multiplexing to label multiple control and experimental samples run in the same gel such as DIGE [10] and scanned with very high resolution systems [11]. Multiple scans of the same gel using different color filters can then be color mapped to see the contributions of the different samples. This is useful if one has control over the experimental design when determining the reference gel, set of control gels, and experimental gels. However, it does not solve the problem of trying to putatively compare one's own sample against an Internet reference gel where they have identified protein spots.

## Finding reference gels on the Internet

A number of 2D-gel image databases are available on the Web where some of the proteins are identified for various types of samples. Both [WORLD-2DPAGE](#) and [2D-HUNT](#) on the [SWISS-2DPAGE \[12-15\]](#) server can be used to find Web URL addresses for a number of 2D protein gel databases. The Web site a large number of tissues with databases that include a wide range of human tissues, mouse tissues, *E. coli*, *aribidopsis*, *dictyostelium*, and yeast. You might also try a [Google 2D-gel search](#).

## Active 2D image maps in Internet 2D-gel databases

Some 2D-gel image Web databases have active maps (e.g., SWISS-2DPAGE) where you can [click on a spot](#) in an active gel image to identify the protein if it is in their database (see [WORLD-2DPAGE](#)). Clicking on a spot queries the associated Web server database to determine if the spot you pointed to is in that database. If it is, it then reports the protein identity of the spot with links to SWISS-PROT etc. Alternatively, you can have it report the ID and protein name in the reporting window or assign it to the selected spots annotation.

## Using active maps with Flicker to putatively identify protein spots

We have integrated this capability in Flicker so that if you are viewing an active map (say from SWISS-2DPAGE), you can switch from *Flicker* mode to *Clickable image database* (DB) mode and then just [click on the spot to query the database](#) which then will bring up the [specific protein annotation Web page](#) from the associated active DB server (e.g., SWISS-2DPAGE) in a Web browser. This is most useful after you have flicker-aligned your gel with the reference gel map image. Comparing one's own experimental 2D gel image data with gel images of similar biological material from such Internet reference databases opens up the possibility of using the spots in these reference gels to suggest the putative identification of apparently corresponding spots in your gels. The image analysis method described here allows scientists to more easily collaborate and compare their gel image data over the Web.

## Methods for 2D gel comparison

When two 2D gels are to be compared, simple techniques may not suffice. There are several methods for comparing two gel images: 1) put the images side by side and visually compare them; or 2) slide one gel (autoradiograph or stained gel) over the other while back lighted; or 3) build a 2D gel quantitative computer database from both gels after scanning and quantitatively analyzing these gels using a 2D gel database system; 4) more recently dye multiplexing has been used to label different samples in the same gel. A variant if this is to warp two gels so they are the same geometry, then generate pseudo color images and look for differences by color differences. These methods may be impractical for many investigators since in the first case the physical gel or autoradiograph from another lab may not be locally available. The first method may work for very similar gels with only a few differences. The second method will work better for gels that are not so similar but that have local regions that are similar. The third method may be excessive if only a single visual comparison is needed because of the costs (labor and equipment) of building a multi-gel database solely to answer the question of whether one spot is probably the same spot in the two gels. The fourth method may have some problems if spot sizes vary for similar spots.

Flicker was developed to fill the need for a quick comparison of a researcher's gel image against one of these internet reference 2D-gels. We have also provided a limited quantification facility for manually measuring, annotating, a limited number of spots.

The [Flicker Reference Manual](#) describes the operation of the various commands. The current [status](#) of the program and the [revision history](#) is updated as changes occur in the software. You [download](#) the software to install it on your computer. We use a commercial Java installer ([ZeroG.com](#)) that enables Flicker to be easily installed and run on Microsoft Windows, MacOS-X, Linux and Solaris operating systems.

The old Flicker Web applet is still available on <http://www.lecb.ncifcrf.gov/flicker> and may have some documentation that may be useful for this new downloadable and enhanced application. However, we recommend you use the new version as it is easier to use and has greatly increased functionality.

Please [contact us](#) with suggestions and comments. If you make interesting changes in the source code, please send us a copy and describe your changes so we can merge them in the released version.

[Contact us](#)

Flicker is a contributed program available at  
[Open2Dprot.sourceforge.net/Flicker](http://Open2Dprot.sourceforge.net/Flicker)

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## Flicker Program Contributors

The current Flicker is the result of a collaborative effort with contributions from various groups. Flicker was initially written in 1997 by Peter Lemkin as a [Java applet for use in a Web browser](#) to compare 2D gels residing on the internet with investigators own 2D gels [\[Lemkin97a\]](#). This visual technique was developed as a simple way to compare 2D gels without having to install a complete quantitative 2D gel analysis system to ask the simple question "which spots in my gel are similar to which spots in a reference gel?" The Flicker applet was enhanced with the help of Greg Thornwall and has been applied to 2D gel data from a variety of Internet sources.

In 2003, we decided to create an open-source downloadable Java application version of Flicker that would make it much easier for investigators to analyze their own data [\[Lemkin04\]](#). As a standalone Java application, it is independent of the Web and has greatly increased functionality because of access to the user's local computing environment. The new version was written by Peter Lemkin and Greg Thornwal with help from Jai Evans. It incorporates open-source code from the MAExplorer project (<http://maexplorer.sourceforge.net/>).

The core group developing the Flicker program source currently consists of

- \* Peter Lemkin
- \* Greg Thornwall
- \* Jai Evans

In addition, a few of the individuals who have contributed to the program in terms of ideas, data, ideas, bug reports and documentation and suggestions, including Lew Lipkin, Carl Merrill, Eric Lester, Jim Myrick, Dennis Hochstrasser, Ron Appel, Mike Dunn, and others. A special debt is owed to Lew Lipkin, George Carman, and Carl Merrill who were involved in early implementations of the Flicker technique using image processing hardware. We also want to thank the user community for feed back on enhancements and bugs - especially Enrico De Toni, Stephen Lockett, Joe Creed, Mark Holmes, Neil Kitteringham, Hesham Agrama, and many others.

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[Contact us](#)

Flicker is a contributed program available at  
[open2dprot.sourceforge.net/Flicker](http://open2dprot.sourceforge.net/Flicker)

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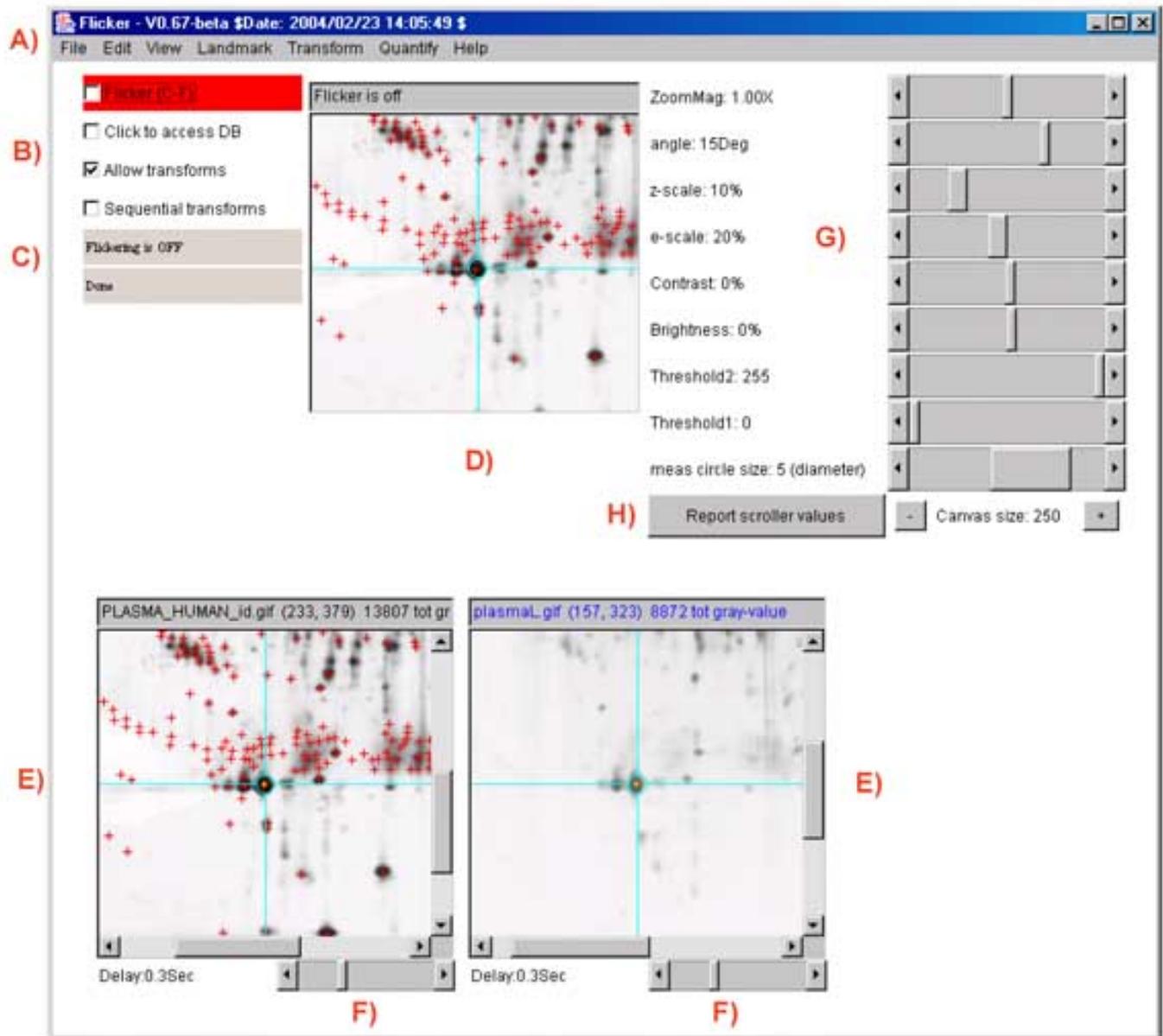
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# Flicker Reference Manual

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This reference manual undergoes revision as the program develops. A short form of the manual will appear as a chapter in the upcoming 3rd edition of The Proteomics Handbook, John Walker (ed) to be published in 2004 [[Lemkin2004](#)].

The following [summaries](#) list some information on using the interactive controls to run the program. You interact with the Flicker program using the [mouse](#) movements, [keyboard](#) commands, [checkboxes](#) states, [slider controls](#) parameter values, and [pull-down menus](#). Figure 1 shows a screen view of the Flicker program.



**Figure.1 Screen view of the Flicker program** This screen shot shows the pull-down menus (A) at the top of the Flicker window used to invoke file operations, editing, view selection, landmarking, image transforms, spot quantification, and help commands. A set of scroll bars on the right determines various parameters used in some of the transforms. The **File** menu options include opening new gel images. Checkboxes on the left (B) activate flickering and active gel map access if the data supports it. A set of status lines (C) appear below the checkboxes and indicate the state of operation and various other messages. The flicker image (D) is in the upper-middle of the frame when it is enabled. The two labeled human blood plasma gel images are shown in the bottom left and right scrollable image windows (E) that may be positioned to the region of interest. These windows also have associated flicker time-delays (F) used when flickering. Image plasmaH is an IPG non-linear gradient gel from [SWISS-2DPAGE](#) and plasmaL is a carrier-ampholyte linear gradient gel from the Merrill Lab at NIMH. Transformed image results are shown in the same scrollable windows. The four checkboxes are: **Flicker** to enable/disable flickering; **Click to access DB** checkbox enables/disables access to a Web server that is associated with a clickable image DB if it exists for the selected image; **Allow transforms** checkbox enables/disables image transforms; **Sequential transforms** checkbox enables/disables using the last image transform output as input for the next image transform. Parameters are directly specified using sliders (G) to adjust zoom magnification, contrast, brightness,

etc. A popup scrollable report window (not shown) logs all text output that appears in the status lines. You can popup the report window with the current scroller values by the **Report scroller values** button (**H**). This text may be saved to a text file on your local disk for printing or further analysis. The size of the three image canvases may be increased/decreased using the "+" / "-" buttons under the parameter scrollbars (**H**).

## 1. Flickering gel images

The basic concept of using flickering as a dynamic visualization technique is simple. If two images may be perfectly aligned then one could simply align them by overlaying one over the other and shifting one image until they line up. However, many images such as 2D PAGE gels have rubber-sheet distortion (i.e., local translation, rotation, and magnification - linear over short distances and non-linear over the entire gel). This means there is more distortion in some parts of the image than in others. Although it is often impossible to align the two whole images at one time, they may be locally aligned piece-by-piece by matching the morphology of local regions.

If it appears that a spot and the surrounding region do match, then one has more confidence that the objects are the same. This putative visual identification is our definition of matching when doing a comparison. Full identification of protein spots requires further work such as cutting spots out of the gels and subjecting them to sequence analysis, amino-acid composition analysis, mass spectrometry, testing them with monoclonal antibodies, or other methods.

### 1.1 Flickering

When flickering two images with the computer, one aligns putative corresponding subregions of the two rapidly alternating images. The flicker window overlays the same space on the screen with the two images and is aligned by interactively moving one image relative to the other using the cursor in either or both of the lower images. Enable the **Flicker** checkbox ([Figure 1.B](#)) to turn on flickering and disable it to shut it off.

[Using the mouse](#), the user initially selects (by clicking on the image - left or right window) what they suspect is the same prominent spot or object in similar morphologic regions in the two gel images. If you click on the spot with the **Control** key pressed, it will center that image at that spot in the window and also center it in the flicker window. You can continuously adjust the position in the flicker window, without changing the selected window position, by selecting the image you want to recenter and dragging the mouse. When these two local regions come into alignment, they appear to pulse and the images appear to fuse together. At this point, differences are more apparent and it is fairly easy to see which spots or objects correspond, which are different, and how they differ. We have found that the user should be positioned fairly close to the flicker window on the screen to optimize this image-fusion effect (i.e., it does not work as well standing back more than a few feet from the screen).

Another useful trick is change the image canvas size after doing the initial alignment. This may help you focus on a smaller region after you have done the rough alignment. You can either use the "+" / "-" buttons under the parameter scrollbars ([Figure 1.I](#)), or you can use the **Control-keypad +** and **Control-keypad -** keys.

### 1.2 Selecting the proper time delays when flickering

The proper flicker delays, or time each image is displayed on the screen, is critical for the optimal visual integration of image differences. We have also found that optimal flicker rates are dependent on a wide variety of factors including: amount of distortion, similarity of corresponding subregions, complexity and contrast of each image, phosphor decay time of the display, ambient light, distance from the display, individual viewer

differences, etc. We have found the process of flickering images is easier for some people than for others.

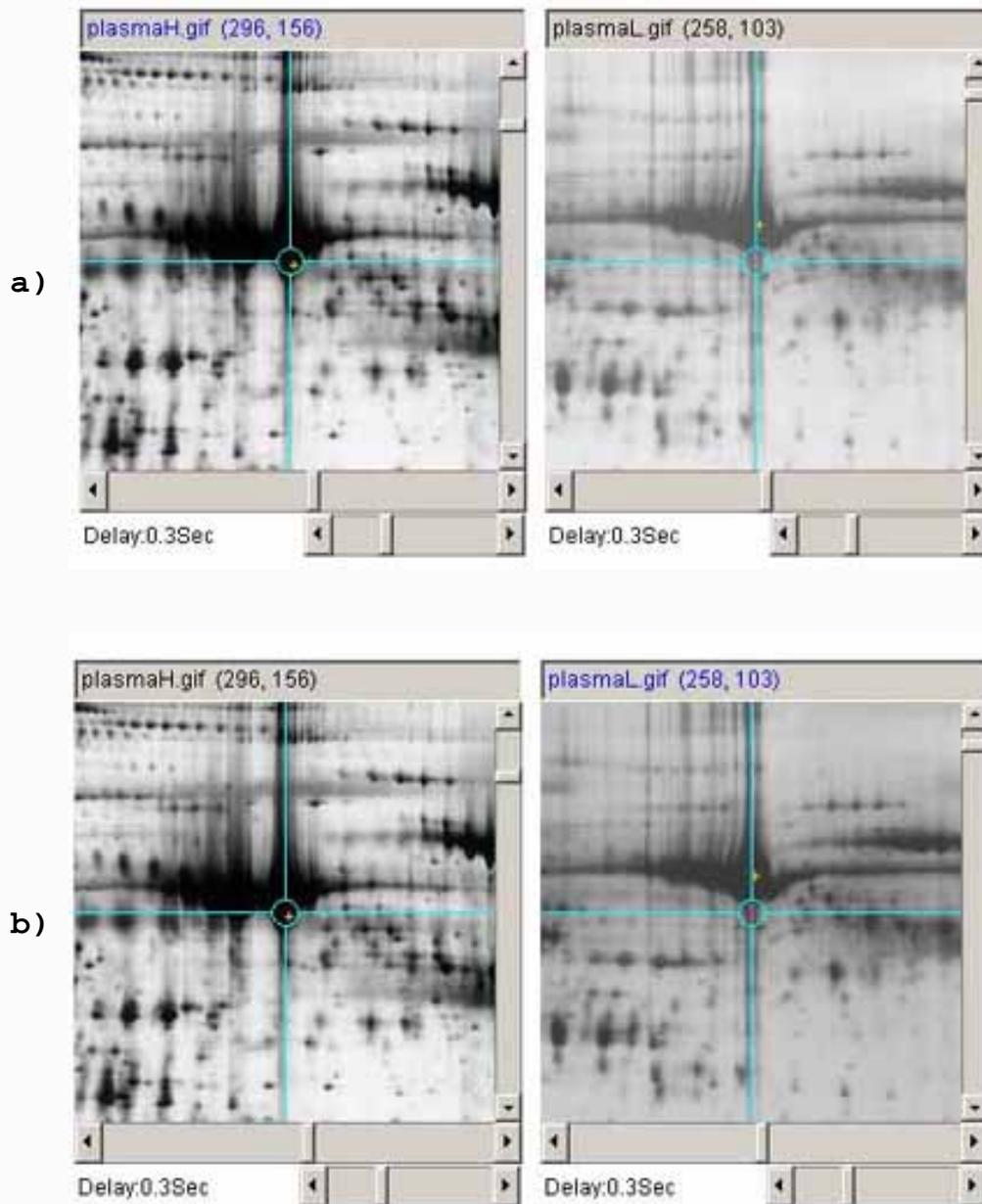
When comparing a light spot in one gel with the putative paired darker spot in the other gel one may want to linger longer on the lighter spot to make a more positive identification. Because of this, we give the user the ability to set the display times independently for the two images (typically in the range of 0.01 second to 1.0 second with a default of 0.30 second) using separate **Delay** scroll bars located under each image ([Figure 1.E](#)). If the regions are complex and have a lot of variation, longer display times may be useful for both images. Differential flicker delays with a longer delay for the light gel are useful for comparing light and dark sample gels. This lets you stare at the lighter spots to have more verification that they are actually there.

These flicker delay values are saved for the left and right images when you save the Flicker state.

### 1.3 Mouse control of images

The following mouse and key-modified mouse operations control various actions.

- **Pressing the mouse** in either the left or right image selects it. If flickering is active, then it will move the flicker image center for the selected image to that position. A little orange "+" indicates the position of the trial object you have selected. If you have enabled the (**View | Display gray value (C-G)**) then it may also show a circle which is the size of the circular mask used in the density measurement. If the Click to access DB checkbox is enabled and the image has an associated active map database server associated with it, then it will request the spot identify when you click on a spot from the map database. Dragging the mouse is similar to pressing it. However, only pressing it will invoke a clickable database. It also displays the cursor coordinates in the image title. If you click on a spot in the active map and the  **Click to access DB** was enabled, it will try to locate that spot in the associate Web server for that image (see [Figure 2](#)).
- **Control/Press** will position the selected image at the trial object so that the point you have clicked on will be in the center of the crosshairs. If you are near the edge of the image, it will ignore this request as it can not put that position in the center of the window.
- **Shift/Drag** activates the brightness-contrast filter with minimum brightness and contrast in the lower left hand corner and maximum brightness/contrast in the upper right hand corner. Alternatively, you can use the brightness and contrast scroller controls ([Figure 1.G](#)) instead after you have selected the image by clicking on it. Hint: try to make the images have similar brightness and contrast to make it easier to see differences when you flicker. [Figure 2](#) shows the effects of contrast adjustment.



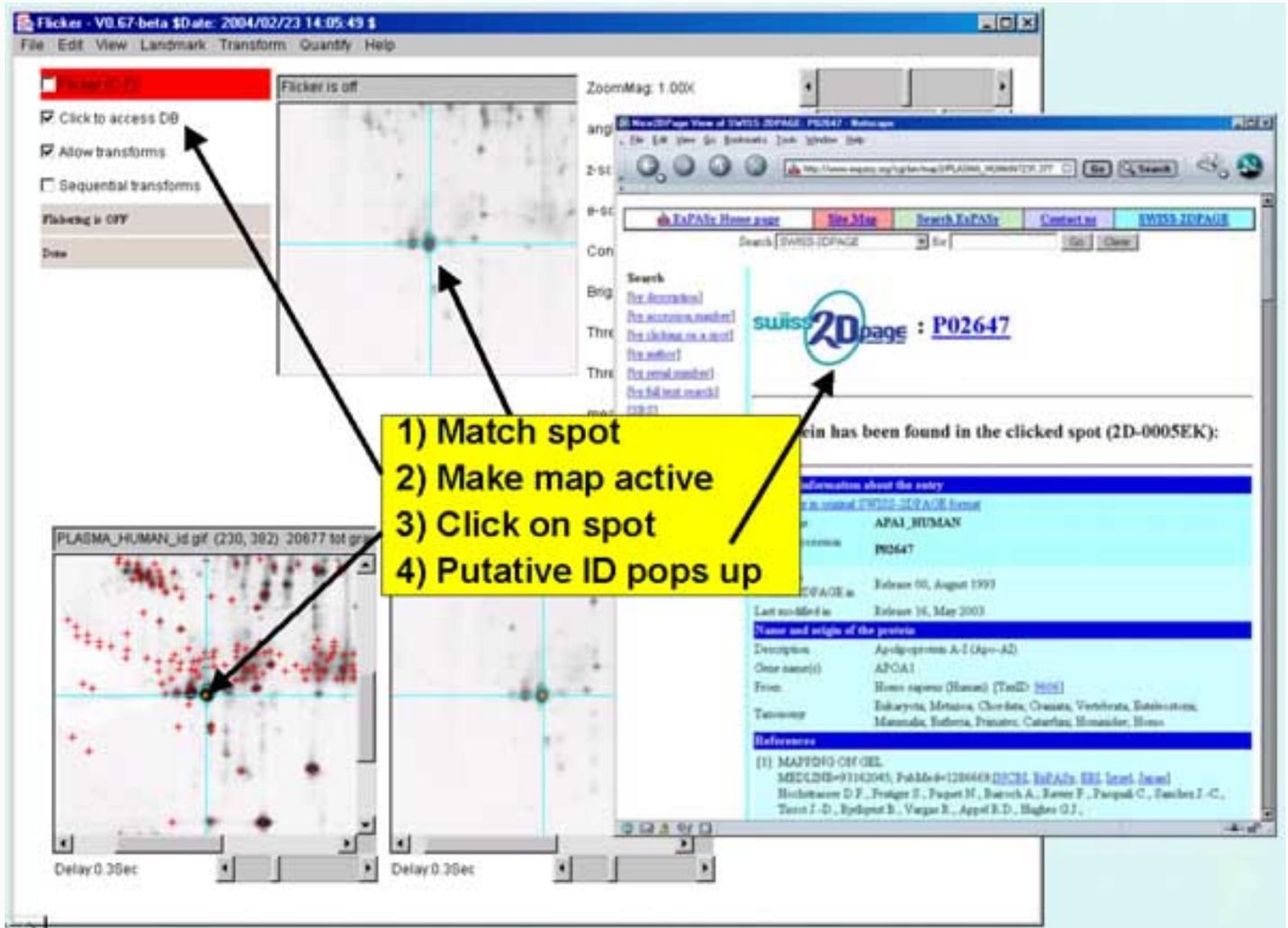
**Figure 2. Example of adjusting the image contrast on one image to match the overall contrast of the other.** A) shows the original plasmaH and PlasmaL images before contrast adjustment b) shows the plasma images after the contrast was adjusted in the plasmaL image.

#### 1.4 Lookup of putative spot identification on Swiss-2DPAGE

Protein spots may be putatively identified using active map images that link to federated 2D gel databases such as [SWISS-2DPAGE](#). First, load an active map image for your type of biological material (if it can be found). Then flicker align the two gels around the spot(s) you are interested in. Then enable the  **Click to access DB** checkbox. Then set the (**View |  Use protein DB browser, else lookup ID and name on active images**) menu checkbox command is enabled. Finally, click on the spot of interest and this brings up the [specific protein annotation page](#) from the active DB server (in this case SWISS-2DPAGE) in a Web browser. [Figure 3](#) shows an illustration of procedure.

In place of the Web browser, you can just have it report the Swiss-Prot ID and the protein name annotation from the Swiss-2DPAGE Web server. Set the (**View |  Use protein DB browser, else lookup ID and name on active images**) menu checkbox command is disabled. Then when you click on a spot, it will now try to get the Swiss-Prot ID and the protein name by the spot's coordinates and display the results in your report window.

The [next discussion](#) describes looking up the (ID, protein names) for a set of spots you have defined.

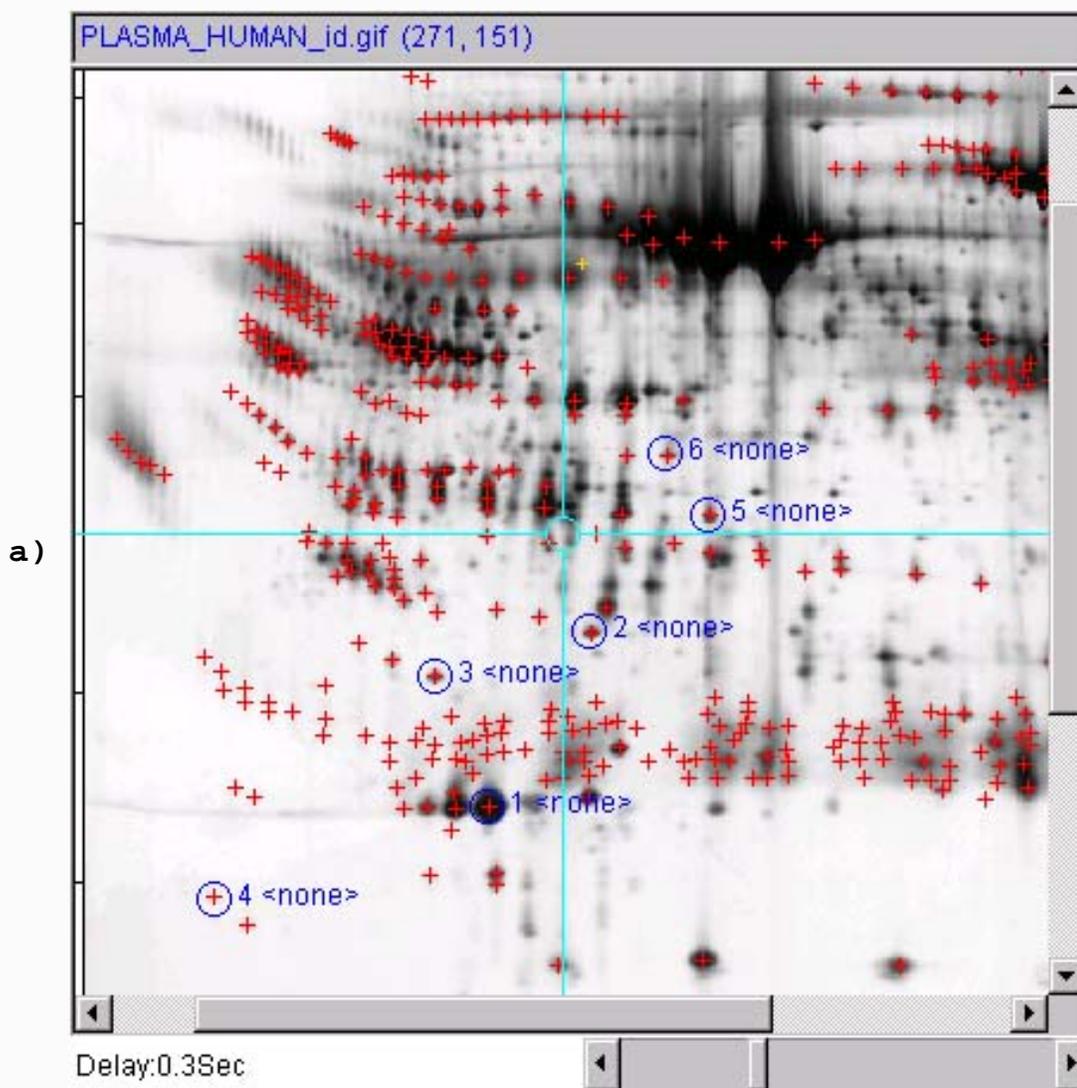


**Figure 3. Protein spots may be putatively identified using active map images that link to federated 2D gel databases.** First, load an active map image for your type of biological material (if it can be found). Then flicker align the two gels around the spot(s) you are interested in. Then enable the  **Click to access DB** checkbox. Finally, click on the spot of interest and this brings up the Web page from the active DB server (in this case SWISS-2DPAGE). If the (**View |  Use protein DB browser, else lookup ID and name on active images**) is enabled, then clicking on a spot will popup a Web browser with the associated database page (as is shown). If the checkbox is off, then it will get the data from that web page and report it. If you have defined a set of spots in a spot list in an active gel, the (**Quantify | Measure by Circle | Lookup Protein IDs and Names from active map server (selected image)**) menu command will try to lookup each spot in the list by its coordinates and will save the results (Swiss-Prot ID and protein name) as the spot annotation's (id,name).

### 1.4.1 Looking up and assigning putative spot IDs for lists of spots

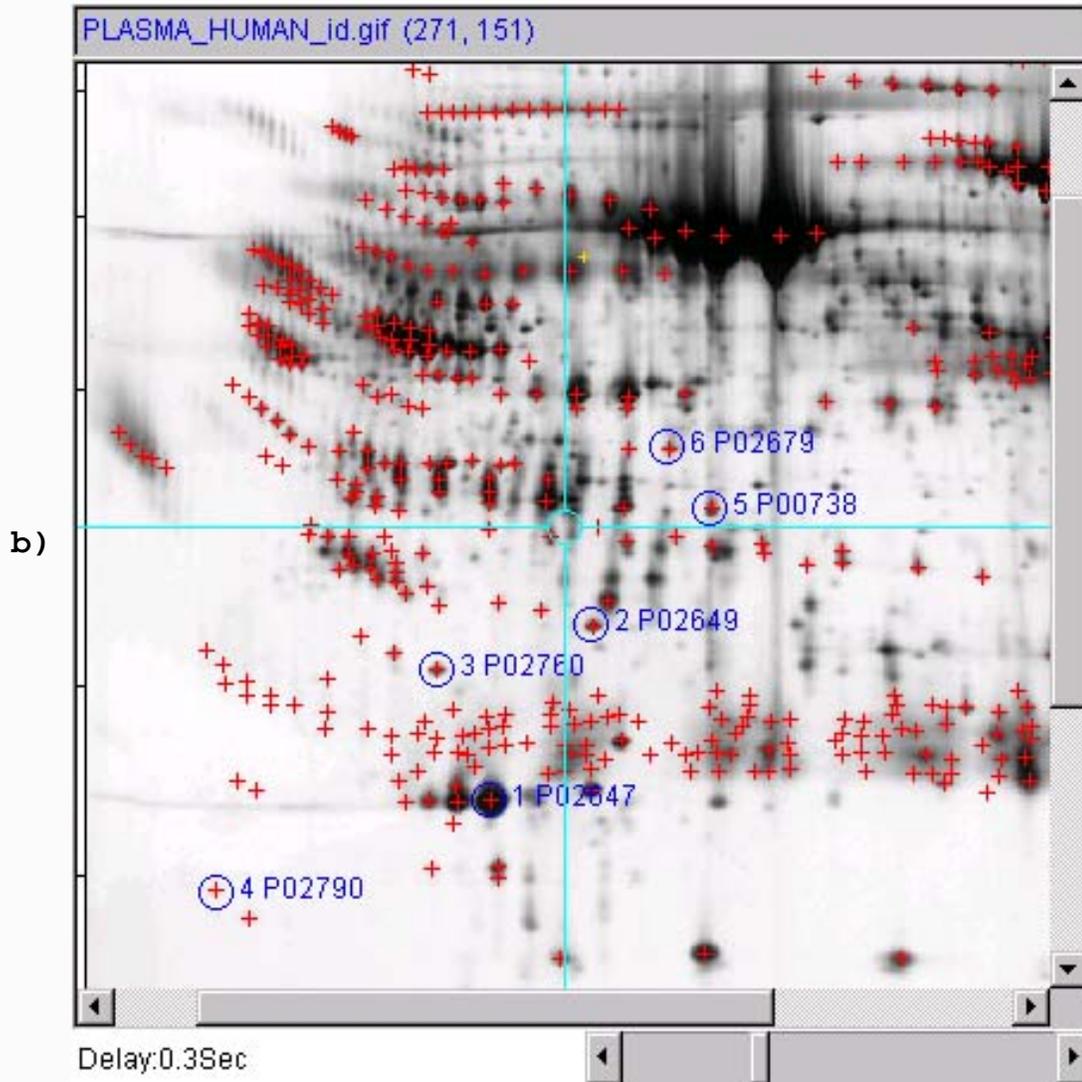
If you have defined a set of spots in a spot list for an active gel, the (**Quantify | Measure by Circle | Lookup Protein IDs and Names from active map server (selected image)**) menu command will try to lookup each spot in the list by its coordinates and will save the results (Swiss-Prot ID and protein name) as the spot annotation's (id,name). Note that you must be connected to the Internet. After this lookup has finished (it may take a while), use the (**Quantify | Measure by Circle | List spots in the spot list for selected image**) to view the filled in table. The (**C-Q**) key-command may be use to stop the lookup procedure for a long list of spots after it finishes the current spot lookup.

If you then have a set of corresponding spots you have defined in your gel, you can then edit their annotation so it matches the reference gel that you just assigned putative spot identifications. This is described in the following Figures **3.1a** through **3.1d** You can lookup the protein (ID,names) for a set of spots you have defined in the active gel map image that is link to federated 2D gel databases. See the vignettes for [query a spot's putative identity](#) and [assigning a spot's putative identity](#) for more details.

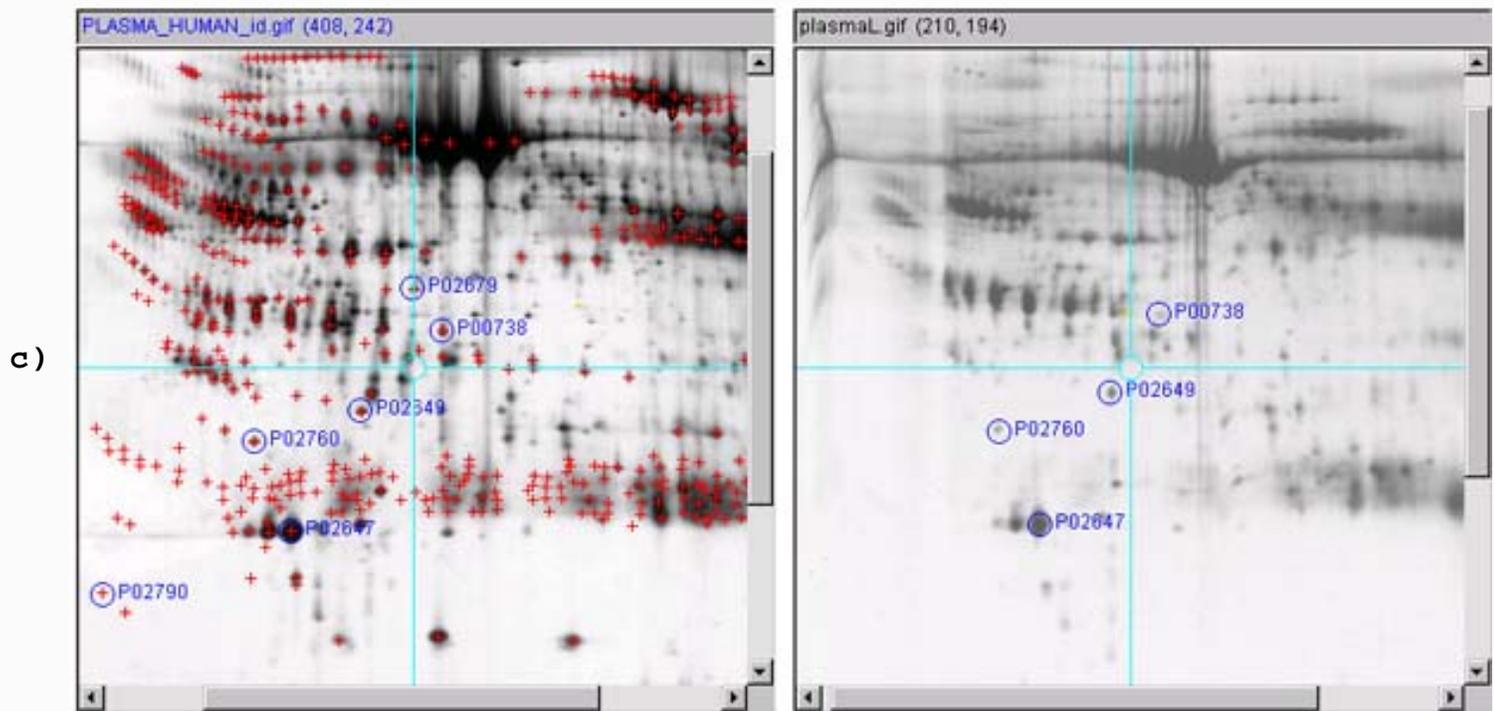


**Figure 3.1a)** First define a set of spots in the active gel (described under the [Quantify menu](#)). You might want to view the spot annotations by setting the (**View | Set view measurement options |  Use 'spot identifier ' for spot annotations**)

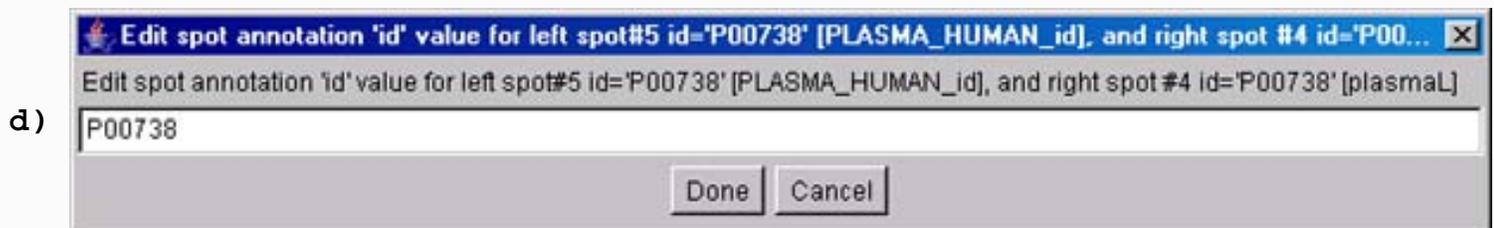
menu checkbox. Then enable the  **Click to access DB** checkbox. Then, if you are connected to the Internet, with the active gel selected, use the (**Quantify | Measure by Circle | Lookup Protein IDs and Names from active map server (selected image)**) menu command. This will try to lookup each spot in the list by its coordinates and will save the results (Swiss-Prot ID and protein name) in each the spots annotation's (id,name).



**Figure 3.1b) Shows the updated spot list after the spots have been identified from the active reference gel database.** Now disable the  **Click to access DB** checkbox so that it does not popup the Web browser when you then click on the image.



**Figure 3.1 c) Then define a list of spots in the other user gel by selecting the spot and typing (C-M).** Repeat the following for each spot in the gel lists. First select a spot in the reference gel, and then select the corresponding spot in the user gel. Assign the same annotation to both by typing (C-I).



**Figure 3.1 d) This will popup "Edit spot annotation" window with the spot identifier from the reference gel.** Just press the Done button and the annotation will be assigned to the user gel spot you are editing.

#### 1.4.2 Accessing PIR UniProt, iProClass and iProLink server Web pages for selected proteins

You may optionally access PIR [UniProt](#), [iProClass](#) and [iProLink](#) server Web pages for selected proteins in the spot list through their Swiss-Prot accession names. This is accomplished in a two-step process enabled using the (**Edit | Select access to active DB server |  ...**) checkbox command. You may select either SWISS-2DPAGE, UniProt, iProClass or iProLink servers - or none of these. If you measure a spot (select a spot in an active image and then type **C-M**) (and are connected to the Internet), it will also lookup the Swiss-Prot protein (accession name, and protein id) on the SWISS-2DPAGE server. Then, if you enable "Click to access DB", it will pop up the particular active DB server you have selected. Figures [3.2a](#) UniProt, [3.2b](#) iProClass, and [3.2c](#) iProLink.

An additional option that makes this easier to use is to enable the (**Edit |  Auto measure, protein lookup and**

**Web page popup**) checkbox command. Then when you click on a spot in an active image (associated with a Web database), it will: 1) measure the spot and add it to the spot list; 2) lookup the Swiss-Prot (name, id); and 3) pop up the Web server on the currently selected active DB server.

a)

The screenshot shows a Netscape browser window displaying the UniProt PIR database entry for protein P02760. The browser's address bar shows the URL: <http://www.pir.uniprot.org/cgi-bin/lupEntry?id=P02760>. The page title is "UniProt Entry - UniProt [the Universal Protein Resource] - Netscape". The main content area displays the UniProt logo and navigation links. Below the navigation, the entry is titled "UniProt Entry: P02760". The entry information is presented in a table format:

ENTRY INFORMATION	
ENTRY NAME	<a href="#">AMBP_HUMAN</a>
ACCESSION NUMBERS	P02760; P00977; P02759
CREATED	Release 01, 21-JUL-1986
SEQUENCE UPDATE	Release 05, 13-AUG-1987
ANNOTATION UPDATE	Release 45, 01-OCT-2004
NAME AND ORIGIN OF THE PROTEIN	
PROTEIN NAME	AMBP protein precursor
DESCRIPTION	Alpha-1-microglobulin; Protein HC; Complex-forming glycoprotein heterogeneous in charge; Alpha-1 microglycoprotein; Inter-alpha-trypsin inhibitor light chain, ITI-LC; Bikunin; HI-30
GENE NAME	AMBP; ITIL; HCP
SOURCE ORGANISM	Homo sapiens
TAXONOMY ID	9606 [NCBI, NEWT]
LINEAGE	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo
REFERENCES	
[1]	Vetr H; Gebhard W <b>Structure of the human alpha 1-microglobulin-bikunin gene.</b> 1990, <i>Biol. Chem. Hoppe-Seyler</i> , 371, 1185-1196 <i>Position:</i> SEQUENCE FROM N.A. <i>PubMed:</i> <a href="#">1708673</a> ; <i>Medline:</i> <a href="#">91214554</a> ;
[2]	Kaumeyer JF; Polazzi JO; Kotick MP <b>The mRNA for a proteinase inhibitor related to the HI-30 domain of inter-alpha-trypsin inhibitor also encodes alpha-1-microglobulin (protein HC).</b> 1996, <i>FEBS Lett</i> , 391, 2929-2930

**Figure 3.2a) Results of accessing PIR UniProt server Web pages for protein 3 P02760 from Figure 3.1b.**

This was accessed using the method describe in section 1.4.2 above by enabling (Edit | Select access to active DB server |  Use

**PIR UniProt DB access**) checkbox command set, enabling (**Edit** |  **Auto measure, protein lookup and Web page popup**) checkbox command, and then clicking on the spot.

b)

PIR Search Results

Site Map Site Search

Text Search Protein Databases:

About PIR Databases Search & Retrieval Download Support

Max Sep 13 10:33:39 EDT 2004

Search NREF for SwissProtAccession AND All Fields AND All Fields AND All Fields  
P02760 not null

1 protein sequences in total

For sequence analyses, pick a method (radio button) below, select a sequence(s) (checkbox) in Protein ID column, and GO.

BLAST  FASTA  HMM Search  Pattern Match  Multiple Alignment  Domain Display

<input type="checkbox"/> Protein ID	Matched	Protein Name	Length	Organism Name /Taxon Group	PIRSF ID /Family ID	Pfam ID	PC Motif ID	PDB ID
<input checked="" type="checkbox"/> NREF <a href="#">NF00080626</a> iProClass <a href="#">P02760</a> PIR-PSD <a href="#">HCHU</a> UniProt <a href="#">AMBP_HUMAN</a>	SwissProt Accession=>P02760	AMBP protein precursor (Contains: Alpha-1-microglobulin (Protein HC) (Complex-forming glycoprotein heterogeneous in charge) (Alpha-1 microglycoprotein), Inter-alpha-trypsin inhibitor light chain (III-	352	<a href="#">Homo sapiens</a> <a href="#">Euk/mammal</a>	<a href="#">SF001622</a> <a href="#">FAM0001605</a>	<a href="#">PF00014</a> <a href="#">PF00051</a>	<a href="#">PCM00213</a> <a href="#">PCM00280</a>	<a href="#">1BIK</a>

**Figure 3.2b) Results of accessing PIR [iProClass](#) server Web pages for protein 3 P02760 from Figure 3.1b.**

This was accessed using the method describe in section 1.4.2 above by enabling (**Edit** | **Select access to active DB server** |  **Use PIR iProClass DB access**) checkbox command set, enabling (**Edit** |  **Auto measure, protein lookup and Web page popup**) checkbox command, and then clicking on the spot.

c)

Figure 3.2c) Results of accessing PIR [iProLink](#) server Web pages for protein 3 P02760 from Figure 3.1b. This was accessed using the method describe in section 1.4.2 above by enabling (Edit | Select access to active DB server |  Use PIR iProLink DB access) checkbox command set, enabling (Edit |  Auto measure, protein lookup and Web page popup) checkbox command, and then clicking on the spot.

### 1.5 Checkbox control of flickering and database access

There are four checkboxes in the upper left part of the window ([Figure 1.B](#)) that control commonly used options. Figure 3 describes these options in more detail.

- **Flicker (C-F)** checkbox enables/disables flickering.
- **Click to access DB** checkbox enables/disables access to a Web database server that is associated with a clickable image - if it exists for the selected image. Turning on this option will disable flickering.
- **Allow transforms** checkbox enables/disables image transforms
- **Sequential transforms** checkbox enables/disables using the last image transform output as input for the next image transform (image composition) if the  **Allow transforms** is enabled

Flicker (C-F)

Click to access DB

Allow transforms

Sequential transforms

**Figure 4. Screen view of the Flicker checkbox controls.** This shows the global checkbox controls that are used to set operational modes for flickering, Web database access and image processing transforms. These values are saved when you save the Flicker state.

## 2. Image enhancement prior to flickering - Transforms

It is well-known that 2D gels often suffer from local geometric distortions making perfect overlay impossible. Therefore, making the images locally morphologically similar while preserving their grayscale data may make them easier to compare. Even when the image subregions are well aligned, it is still sometimes difficult to compare images that are quite different. Enhancing the images using various image transforms before flickering may also help. The **Transforms** menu contains a number of image transforms. Click on the image you want to transform and *then* select the transform from the list of available transforms. If you select neither the left or right image, it will perform the transform on both images.

Some of these transforms involve spatial warping, which maps a local region of one image onto the geometry of the local region of another image while preserving its grayscale values. Another useful operation is contrast enhancement that helps when comparing light or dark regions by adjusting the dynamic range of image data to the dynamic range of the computer display. [Figure 2](#) shows the effects of contrast adjustment. Other transforms include image sharpening and contrast enhancement. Image sharpening is performed using edge enhancement techniques such as adding a percentage of the gradient or Laplacian edge detection functions to the original grayscale image. The gradient and Laplacian have higher values at the edges of objects. In all cases, the transformed image replaces the image previously displayed. Flicker will normally transform the input image to an output image. You can use another transform on the previously transformed image if you had set the  **Sequential transforms** checkbox.

### 2.1 Display models for image transform and brightness-contrast operations

There are several display models for combinations of using image transforms and brightness contrast or zoom filtering. These are applied to the left and right windows and also are shown in the flicker window. Two checkboxes in the upper left of the main window control transforms:  **Allow transform** enables/disables transforms, and  **Sequential transforms** allows using the previous transform as the input to the next transform, i.e., image composition. The original image is denoted *iImg*. If you allow transforms and are also composing image transforms, you may optionally use the previous transformed output image (denoted *oImg*) as input to the next image transform. The output (either *iImg* or *oImg*) is then sent to the output1. Then the output2 which is either zoomed or not is sent to the brightness-contrast filter if active (specified by dragging the mouse in the selected window with the **SHIFT-key** pressed). The output2 of the brightness-contrast filter is denoted as *bcImg*. If you have never used the zoom or brightness-contrast filtering since loading an image, then *zImg* and *bcImg* are not generated and hence not used in the displayed image. Figure 5. illustrates these four cases.

- a) If *no* transforms or brightness-contrast filtering is used on the selected image

**(No transforms)**

*iImg* -----> **output1**

- b) Image transforms may be composed from the original image (*iImg*) or from *Sequential composition* of image transforms on the selected image

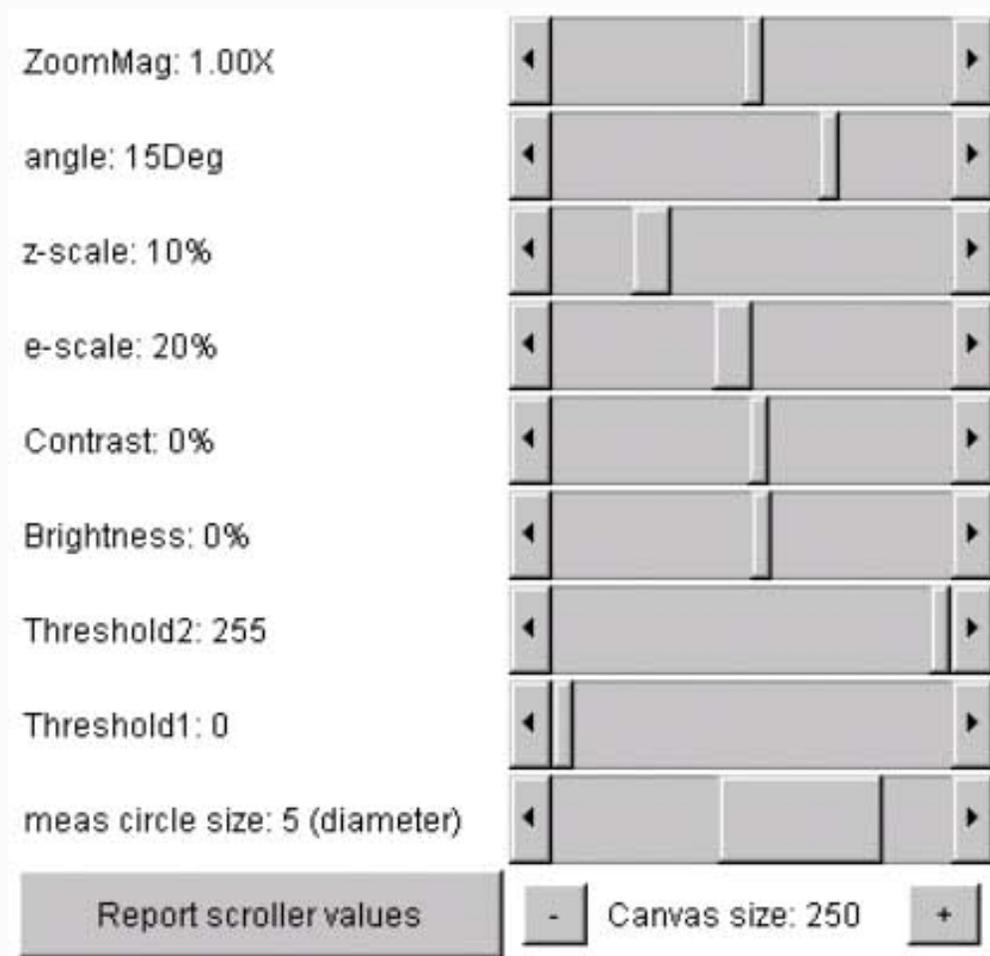


- computing window (see [Quantify menu](#))
- **C-M** measure and show intensity under a circular mask for current image. Report background-corrected value if background was defined (see C-B shortcut and [Quantify menu](#)). An alternative way to measure spots is to hold the **ALT-key** when you press the mouse to select the spot. This combines spot selection and measurement in one operation.
- **C-R** measure and show intensity under a the computing window defined by the ROI (see commands (**C-U**) and (**C-L**)) for the current image. Report background-corrected value if circular mask background was defined (see (**C-B**) shortcut and [Quantify menu](#))
- **C-T** repeat the last Transform used, if one was previously performed else no-op (see [Transform menu](#))
- **C-U** Define the upper left hand corner (ULHC) of the region of interest (ROI) and assign that to the computing window (see [Quantify menu](#))
- **C-V** Show data-window of selected pixel in the popup report window.
- **C-W** Clear the region of interest (ROI) and computing window (see [Quantify menu](#))
- **C-Y** set 3 pre-defined landmarks for demo gels for Affine transform (see [Landmark menu](#))
- **C-Z** set 6 pre-defined landmarks for demo gels for Polywarp transform (see [Landmark menu](#))
- **C-Keypad "+"** increase the canvas size for all three images (see [Edit menu](#))
- **C-Keypad "-"** decrease the canvas size for all three images (see [Edit menu](#))

## 2.3 Sliders for defining transform parameters

The set of parameter sliders are in the upper right part of the window ([Figure 1.G](#) and [Figure 6](#)) are used for adjusting parameters for the various image transforms (see [Transform menu](#)). The size of the three image canvases may be increased/decreased using the "+" / "-" buttons under the parameter scrollbars. The current canvas size for the three image windows is displayed (in pixels).

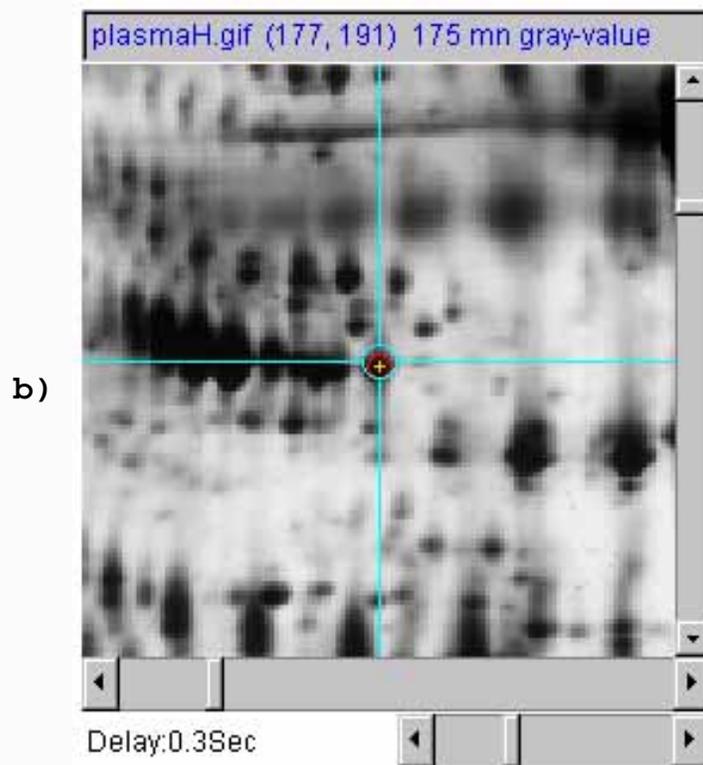
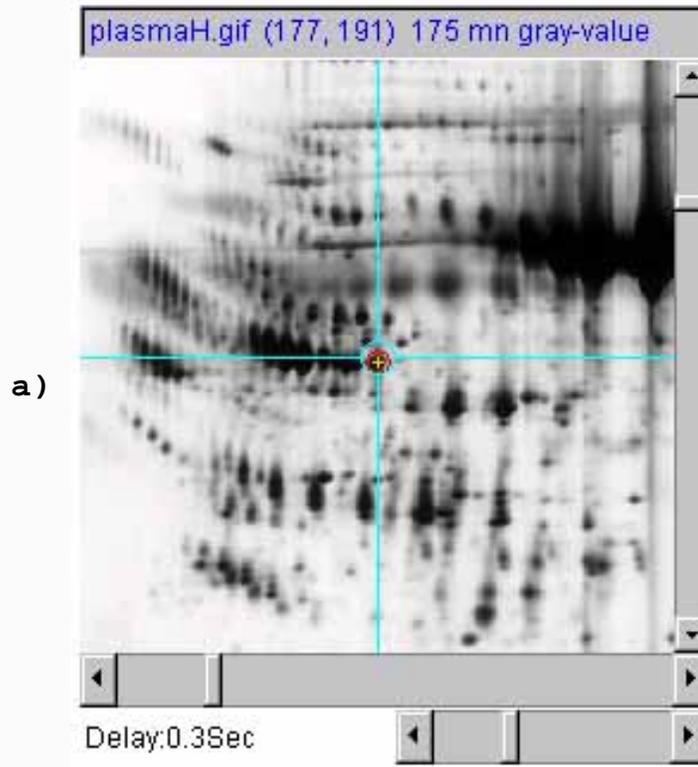
- **zoomMag (X)** to zoom both left and right images from 1X to 16X
- **angle** (degrees) used in the pseudo 3D transform
- **eScale(%)** used in the sharpening transforms
- **zScale (%)** used in the pseudo 3D transform
- **contrast (%)** set by Shift/Drag to change the image contrast
- **brightness (%)** set by Shift/Drag to change the image brightness
- **threshold1** (grayscale or od) is the minimum grayscale value to show pixels otherwise they are shown as white
- **threshold2** (grayscale or od) is the maximum grayscale value to show pixels otherwise they are shown as white
- **measurement circle size** diameter (pixels) of the measurement circle



**Figure 6. Screen view of the Flicker parameter slider controls.** This screen shot shows the parameter slider control that are used to set parameters for the various transforms. Separate parameter values are assigned to the left and right windows and are saved when you save the Flicker state. Pressing the **Report scroller values** button will popup the report window and display the current parameter values for both the left and right windows. The size of the three image canvases may be increased/decreased by pressing the "+" / "-" buttons under the parameter scrollbars. The current canvas size for the three is displayed (in pixels).

## 2.4 Example of changing image magnification

Sometimes when comparing gels from different sources, you want to change the magnification of one of them so they are easier to compare. [Figure 7](#) shows an example of adjusting the magnification of the selected image by 1X, 2X and 0.5X using the zoom mag slider.



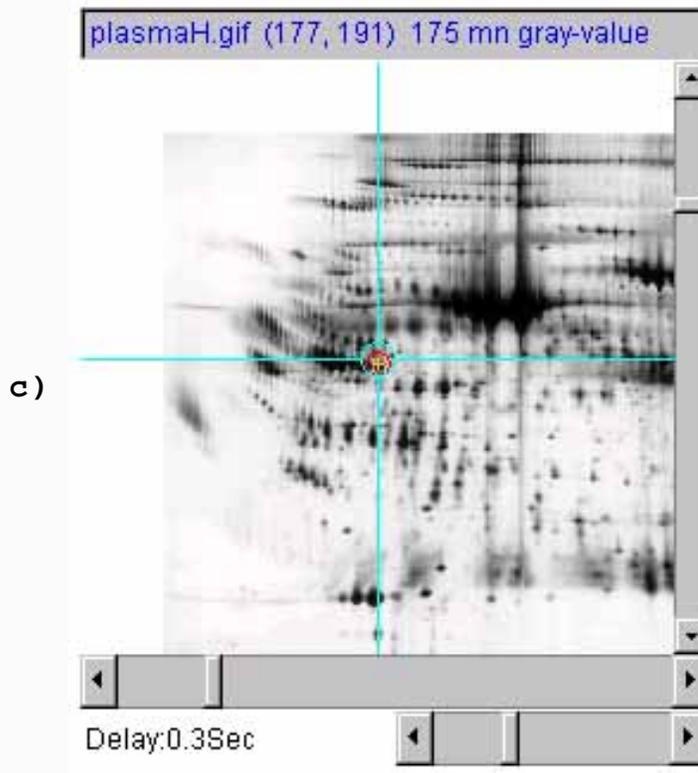


Figure 7 shows an example of adjusting the magnification of the selected image by 1X, 2X and 0.5X using the zoom mag slider. a) is the original image at 1.0X zoom. b) is the original image at 2.0X zoom. c) is the original image at 0.5X (de)zoom.

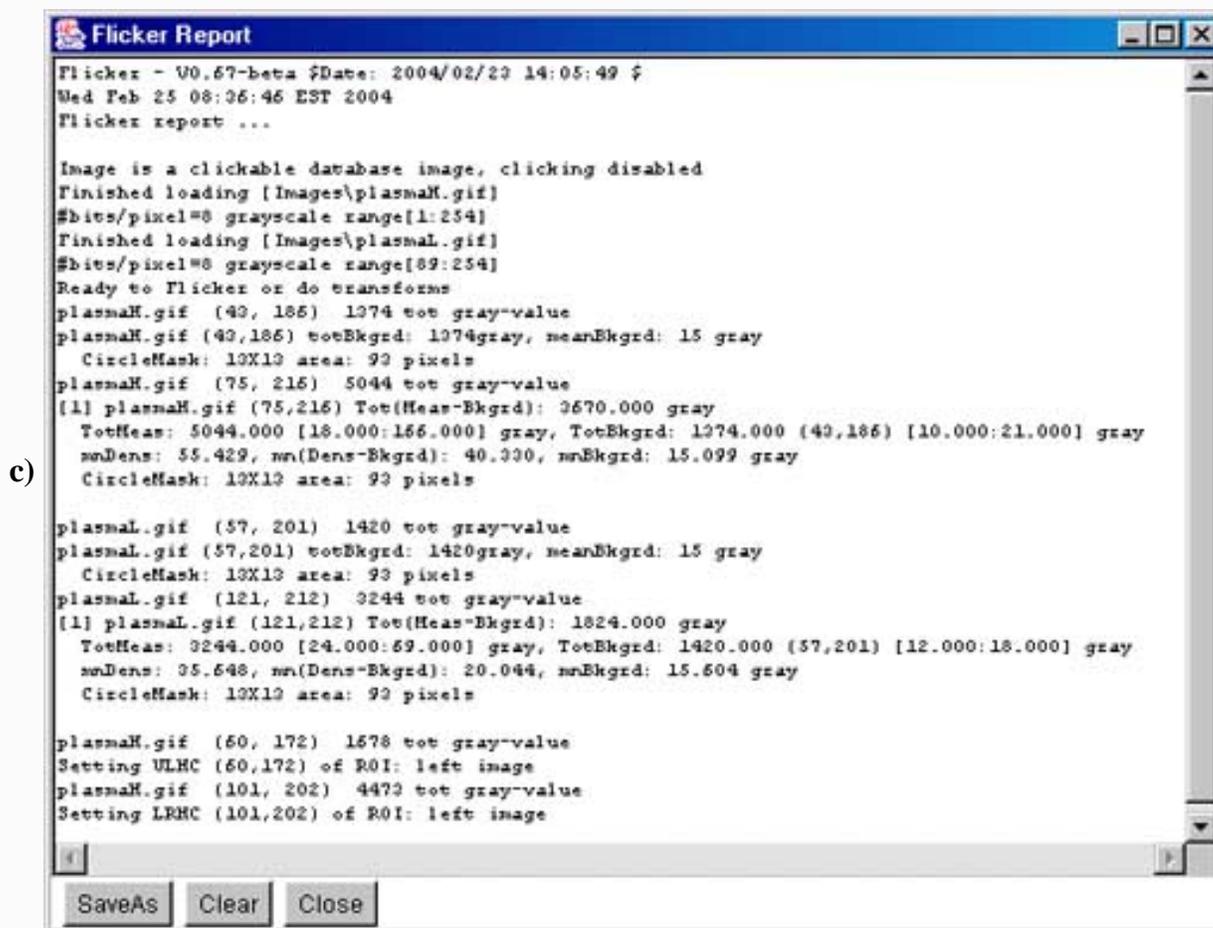
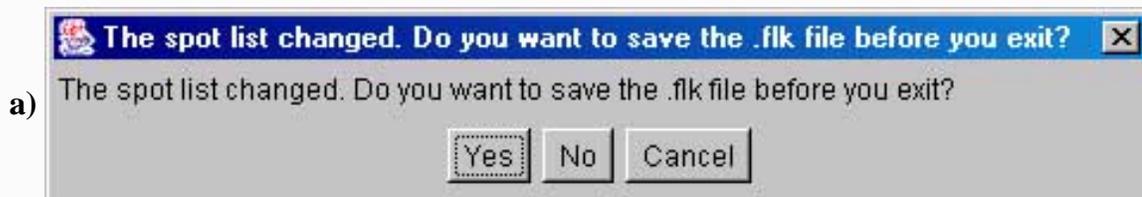
### 3. Reporting the status in the popup status window

The Flicker status information is displayed in several places.

- There are **two status lines** in the upper left part of the main window ([Figure 1.C](#)). The output is also appended to the Popup Report window.
- There are an additional **six status lines** in the upper left part of the main window under the above two status lines. These are used for a few operations to include additional information (e.g., affine transform). The output is also appended to the Report window.
- When you have created data (e.g., spot lists) and attempt to exit, it will ask you if you want to save the data first by popping up a **Yes/No/Cancel window**. Press Yes to save the data and then exit, No to not save the data and then exit, and Continue to not exit.
- When errors or warnings occur, an **alert window** is popped up to bring it to your attention. Press the **Ok** button to continue.
- The **title line** of the selected image (selected by clicking on the left or right image) changes from black to blue ([Figure 1.E](#)). If neither image is selected (click on the top flicker window ([Figure 1.D](#))), then both titles are black. It will also display the (x,y) coordinates of the current cursor position. You may also

display the pixel gray scale value (OD if calibrated) if you enable it with the (**View |  Display gray values (C-G)**) option. It reports the total measurement circle density if the (**Quantify |  Use sum density else mean density**) option is enabled.

- A **report popup window** is created when Flicker is started (see Figure 8 below). It may be temporarily removed by closing it. You can get it back at any time by selecting (**View | Show report popup**) or by pressing the **Report scroller values** button. All text output is appended to this window. The **Clear** button clears all text. The **SaveAs** button lets you save the text in the window into a local text file in the **tmp/** directory or some other file.



---

**Figure 8. Screen view of the popup information windows.** a) **Flicker Yes/No/Cancel window** to give you a chance to save data that you have edited or created. b) **Flicker error or warning alert window** when illegal conditions occur. c) **Flicker Report window** This screen shot shows the popup report window that contains a log of all activity. You can save this window's contents or clear it. If you close the window, it continues to log activity and may be popped up again using the (**View** |  **Show report popup**) command or the **Report scroller values** button underneath the parameter scrollers.

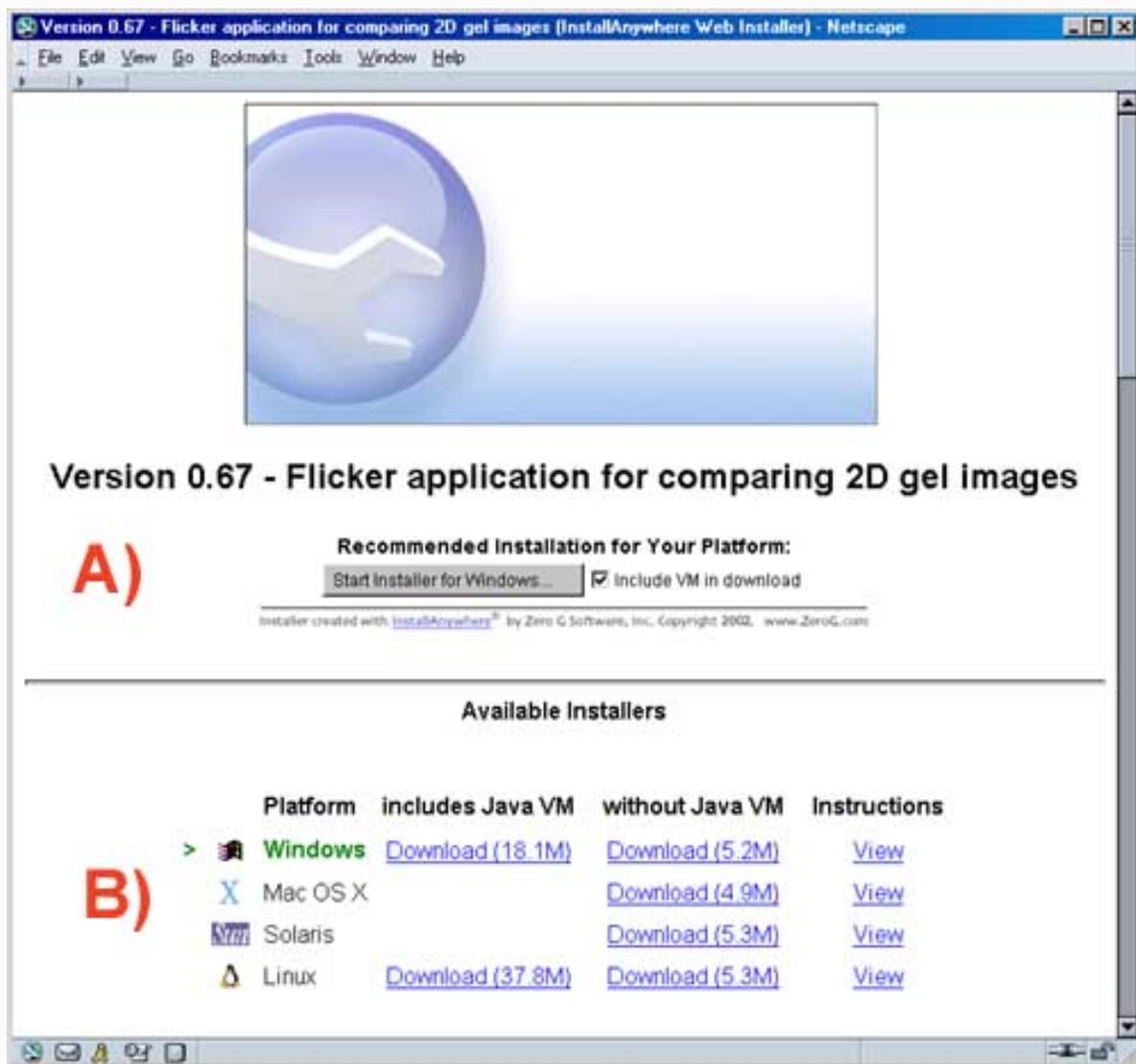
## 4. Downloading, installing and running Flicker

There are two ways to install Flicker: using the [downloadable Java installer](#) (highly recommended) or installing [without the Java installer](#) (not recommended, but it is available for users who have problems with the Java installer).

### Installing Flicker "with" the Java installer

Click on [Download](#) to bring up the Java installer for your computer (we use the commercial InstallAnywhere installer by ZeroG.com) shown in Figure 9 below. (A) You may either click on the "Start installer for *your computer type*" button or (B) click on one of the links in the list of available installers and save the installer as a file on your computer. If you do not have Java applets and Java enabled in your Web browser, you must use the latter method. The latter is useful if you want to save the downloaded installer for later installation or for installing it on another computer. You have the option of downloading the "Java Virtual Machine (JVM)" - which we strongly recommend. This will not interfere with any other JVMs you have already installed or may install in the future. The downloaded JVM is used only by Flicker and guarantees you won't have problems if your computer has an older version of the JVM (Flicker requires a JVM JDK of at least version 1.4 because of the Sun's JAI TIFF reader library.)

Once the installer starts, you may "Select an installation language" (English is the default) and press "OK". Then press the "Next" button after the Introduction window pops up. It then asks you where to install it, suggesting a reasonable default that you may override - then press the "Next" button. For Windows and some of the other systems, it will ask you where you want to put the startup icon - then press the "Next" button. After it finishes the installation, it will show the "Installation Complete" window. Finally, press the "Done" button to finish the installation. For example, in MS Windows systems, a "Flicker startup" icon will appear on your desktop.



**Figure 9. Screen view of the installer you use to download and install Flicker.** You can install it several ways. **A)** You can use the default installer selected by the program "Start installer for (your OS)". **B)** Alternatively, you can download the installer with or without a Java Virtual Machine (JVM). If you do download it with the JVM (recommended to avoid incompatibility problems), it does not affect any other JVM you may have installed previously or in the future. It may ask you if you wish to substitute a JVM you had previously installed or that came with your operating system - so you may override the JVM if you wish.

### Alternate installation of Flicker "without" the Java installer

This method was developed for users who are having problems with the installer or don't want to use it or have long download times. However, if possible we encourage users to use the Java installers discussed above with the full Java JVM download as in general it will be much easier to install and guarantees the proper JVM is used.

This installation method repackages the three Java .jar files required (Flicker.jar, jai\_coded.jar, and jai\_core.jar) into a new .jar file called F.jar. This is then the file that you use to run Flicker.

Note: this method may be used with either Windows or Unix (including MacOS-X) and is discussed in the following README file. First get the two files from the from the Flicker Web server <http://open2dprot.sourceforge.net/Flicker>. Click on "Files mirror" in the Table of contents on the left side of the Flicker home pag and download the file.

README-FlickerNoJavaInstallerNeeded.txt  
 FlickerNoJavaInstallerNeeded.tar.gz

Then read the README-FlickerNoJavaInstallerNeeded.txt file and follow the directions it provides. It also discusses how to start Flicker using scripts since there will be no clickable startup icons described in the following discussion.

### Starting Flicker (if you have used the Java installer)

To start Flicker, click on the startup icon shown in Figure 10 below. For Unix systems including MacOS-X, you can start Flicker from the command line by specifying the path to Flicker.bin. Normally it comes up with the two demonstration human plasma 2D-gel images (plasmaH.gif - an IPG gel from SWISS-2DPAGE on the left) and (plasmaL.gif - a carrier ampholyte gel from Dr. Carl Merril/NIMH on the right).



**Figure 10. Startup icon for Flicker.** This is installed on your computer (default is the desktop) when you install Flicker. Clicking on the icon starts Flicker. When you (**File | SaveAs state file**) to save the state of your session with the gels you have loaded, it will let you name the .flk startup file.

If you have your own gels (JPEG, GIF or TIFF formats), you can try loading them. You may want to limit resolution by first decreasing their size using an image editing program like Adobe PhotoShop or the shareware program ThumbsPlus ([www.cerious.org](http://www.cerious.org)). Large very high resolution images that are 20Mb to 40Mb will not work as well. We suggest reducing the size to about 1Kx1K for good interactivity if you have any problems with running out of memory or very sluggish response. These image editing programs can also be used for converting other formats to JPEG, GIF or TIFF formats that Flicker can read.

### 4.1 Requirements: minimum hardware and software requirements

A Windows PC, MacIntosh with MacOS-X, a Linux computer or a Sun Solaris computer having a display resolution of at *least* 1024x768. We find that a 1024x768 is adequate, but a 1280x1024 screen size much better since you can make the flicker windows larger and see the Popup Report window at the same time. At least 30 Mb of memory available for the application is required and more is desirable for comparing large images or performing transforms. If there is not enough memory, it will be unable to load the images, the transforms may crash the program or other problems may occur.

An Internet connection is required to download the program from the Flicker Web site. New versions of the program and associated demo data will become available on this Web site and can be [uploaded](#) to your computer

using the various (**File | Update | ...**) menu commands. If you have obtained the installer software that someone else downloaded and gave to you, then you do not need the Internet connection to install the program. If you will be using the active gel image maps associated with federated 2D-gel databases, then you will need the Internet connection for accessing those databases. You do not need the Internet for local image comparisons. We currently distribute Flicker so that it uses up to 96Mb. This limit can be lowered (or increased) by editing the `Flicker.lax` file included in the download (MS windows installations). If you want to run it on a computer with with more or with less memory, you can change the startup memory size of Flicker using the (**Edit | Resize Flicker memory limits**) command which edits the LAX file for you. This LAX file is only read by Flicker when it is restarted. So restart Flicker if you change the memory requirements. For very large images you could set it to 512 mb or more if your computer has at least that much memory. The allowable memory range you may set it to is 30 Mb to 1768 Mb.

## 4.2 Files included in the download

The following files are packaged in the distribution and installed when you [download](#) and install Flicker from the Web server.

- **Flicker.jar** is the Java Archive File for Flicker that is executed when you run Flicker
- **jai\_core.jar** is the core Java runtime from SUN's Java Advanced Imaging (JAI) at [java.sun.com](http://java.sun.com)
- **jai\_codec.jar** is the JAI tiff file reader from SUN's Java Advanced Imaging JAI at [java.sun.com](http://java.sun.com)
- **DB/** is a directory containing the set of tab-delimited DB files **Flk\*DB.txt** read at startup
- **Images/** is a directory holding demo .gif, .tif, and .ppx sample files. If you add directories of user files, then Flicker will discover these and you can access them with (**File | Open user images | ...**) submenus
- **FlkStartups/** is empty directory to put the startup Flicker .flk startup files. When you do a (**File | SaveAs state file**), it allows you to name the .flk file (**FlkStartup.flk** is the default). Hint: we find it useful to make short-cuts of some of these saved .flk startup files to our desktop where we can just click on them to start Flicker with those saved states.

## 4.3 Local (Flk...DB.txt) database files

When Flicker is installed, several tab-delimited (spreadsheet derived with column names) .txt files are available in the **DB/** directory (located where the **Flicker.jar** file is installed). These **DB/Flk\*DB.txt** files are read on startup and are used to setup the (**File | Open ... image | ...**) menu trees.

- **DB/FlkMapDB.txt** - contains instances of Web-based active image maps with fields:(*MenuName, ClickableURL, ImageURL, BaseURL, DatabaseName*)
- **DB/FlkDemoDB.txt** - contains instances of pairs of images in the local **Images/** directory and contains fields: (*SubMenuName, SubMenuEntry, ClickableURL1, ImageURL1, ClickableURL2, ImageURL2, StartupData*)
- **DB/FlkRecentDB.txt** - contains instances of recently accessed non-demo images with fields (1 or 2 images/entry): (*DbMenuName, ClickableURL1, ClickableURL2, ImageURL1, ImageURL2, DatabaseName, TimeStamp*)

## 4.4 Local Folders and files created and used by Flicker

When Flicker is first started, it will check for the following folders and files in the installation directory and create them if they can not be found.

- **Flicker.properties** - is the user specific preferences for generic properties such as colors, view options, etc. This file is created when you exit flicker and read (if it exists) when you start it. For example, you may want to use a red trial object cursor instead of yellow, etc. Notes: (1) if you have both a "Flicker.properties" file and are starting Flicker using a .flk startup file, it (1) reads the user preferences first, and then (2) reads the .flk startup file to override these preferences. (2) If you have startup problems when you click on a .flk startup file, try deleting the Flicker.properties file and then restarting. A new one will be created when you exit Flicker.
- **spt/** - contains *{image name}.spt* files. These are the last list of user defined spot measurements that were created using the circular mask Quantify operations for the corresponding images. If there is no spot list or it has been cleared, (**Quantify | Measure by circle | Clear spot list (ask first) for selected image**) command, then the .spt file will be deleted as well.
- **cal/** - contains *{image name}.cal* files. These are the last image grayscale to OD (or other measure) calibrations if they exist. If there is not calibration, then there is no .cal file.
- **Images/** is a directory where the user could put additional directories of images (we suggest you organize the directories by project or experiment) This directory is discussed in more detail below.
- **tmp/** is the user's temporary directory used for miscellaneous output reports and SaveAs files (.txt report files and .gif image files).

#### 4.5 Adding your own image data to the user Images/ database

There is another way for users to add many of their gel images without editing the **DB/FlkDemoDB.txt** file. When you place your image data directories in the **Images/** directory, Flicker will discover them when it starts and add them to the demo menu. It works as follows:

1. You copy or move one or more of your directories of with the images you want to use with Flicker in the **Images/** folder.
2. When Flicker starts, it creates additional submenu entries in the (**File | Open user images | Pairs of images | ...**) and (**File | Open user images | Single images | ...**) submenus that are the names of the user's directories.
3. The first submenu contains unique combinations of pairs of all images within each of the user's directories. Selecting one of these entries will load the pair of images into the left and right Flicker image windows.
4. The second menu command lets you select the right or left Flicker image, and then load a single image from any of the user image directories into that Flicker image window. This would be useful if you wanted to compare one of your images with one of the Internet reference gels.

##### 4.5.1 Example of pairs of images

An example of the (**File | Open user images | Pairs of images | ...**) may help clarify this.

1. Within each submenu, it will generate all unique combinations of the image files within the corresponding directory and denote them as for example, "*image3 vs. image4*", etc.
2. Then just access them from the demo menu as you would with the built-in pairs of images.

For example, if you have four images *image1*, *image2*, *image3* and *image4* in your directory *MyData-* folder, then the submenu entries will be

MyData-folder ▶

- image1 vs. image2
- image1 vs. image3
- image1 vs. image4
- image2 vs. image3
- image2 vs. image4
- image3 vs. image4

Note that it does *not* generate comparisons between directories. You can still do that by clicking on the left (and then later the right) image and using the (**File | Open image file**) command to manually load the file into the image. If you want to do this often, put your images in the same directory.

If you have this type of data, it will also add the (**File | Open user images | List user's images by directory**) command. You can use this to get a report of all of the files in the popup report window.

## 4.6 Updating the latest versions of the program and data from the Web server

You can update your program and active image map and demo image data files using the various Update options in the Files menu. The (**File | Update | Flicker program**) command downloads and installs the latest Flicker.jar file. The (**File | Update | Active Web Maps DB**) command downloads and installs the latest active Web maps database DB/FlkMapDB.txt file. The active maps point to federated 2D-gel web servers with identified spots. The (**File | Update | Demo images DB**) command downloads the latest demo images and the DB/FlkDemoDB.txt file.

### 4.6.1 Instructions for using an older version of Flicker

The version of flicker you use is dependent on the version of the Flicker.jar file.

You will *not* use the (**Files | Update | Flicker program**) command. Using this command will get the latest release which is not what you want if you want an earlier release.

Instead do the following. You copy the version of Flicker.jar you want to the location on your disk where you had installed Flicker overwriting the previous version. For example, on MS Windows this is typically

```
C:\Program Files\Flicker\Flicker.jar
```

If you installed Flicker elsewhere on your computer, then that is where you need to change it. Go to the files distribution in the [File mirror](#) web page.

There are a number of .jar files of the form

```
Flicker.jar-V0.xx
```

Copy the one you want to your computer.

To be used with the Flicker startup process, this file *must* be called Flicker.jar, so you must rename it as

Flicker.jar. The procedure is as follows:

1. copy `Flicker.jar-V0.xx` to your computer (where `V0.xx` is the version number),
2. rename the file to "Flicker.jar"
3. copy it to `C:\Program Files\Flicker\Flicker.jar` or to where it was installed on your computer,
4. then start (or restart) Flicker. It will then get the new version of the `Flicker.jar` file you changed.

#### 4.6.2 Adding your own or another Web sites demo data to your Flicker Demo database

You can add set set of demo gel data to the Flicker demo database from any Flicker demo-data compatible web site. This data must be is exactly the form required that is spelled out in this manual (see [Section 4.3 details on FlkDemoDB file formats](#)). Once you have confirmed that the Web site is compatible, it is simple to add that data to your local Demo database. If the Web site data changes, when you do the update, it will replace existing entries with the new data from that Web site. NOTE: all file names are case-dependent so becareful when building your DB/ tables and in naming the images. The Web site should have 2 folders:

```
DB/
and
Images/
```

The `DB/` folder must have two files `FlkDemoDB.dir` (the list image images in the `Images/` folder, and `FlkDemoDB.txt` which is a tab-delimited spread sheet describing the demo images. NOTE: demo images generally live in the top level of the `Images/` directory. They are not in subdirectories of the `Images/` directory. If you are designing such a remote demo Web site, you could model the files from the current Flicker DB files.

Once you are sure your remote web site is set up correctly, then simply use the **(File | Update | Add user's Flicker Demo Images DB by URL)** command to download and and update the Flicker demo database. After it is successful, it will tell you to exit and restart Flicker. If you go to the **Files | Open demo images| ...** menu, you should see the new demo data you have installed.

## 5. Saving and restoring the Flicker state

Flicker gives you the option of saving the current state of your session including the images your are looking at and the parameter values of the sliders, etc. To save the current state, use the **(File | Save (or SaveAs) state file)** command. This creates a file with a `.flk` file extension in the installation **FlkStartups/** folder (default **DB/FlkStartup.flk**). If you have used the Flicker Web site Java installer (ZeroG.com) for installing Flicker, then it lets you click on a specific `.flk` you have previously saved to restart it where you left off. While running Flicker, you can also use **(File | Open state file)** command to change it to another state.

The **Flicker.properties** file is the user-specific preferences for generic properties such as colors, view options, etc. This file is created when you exit flicker and read (if it exists) when you start it. If you have never run flicker or the file was deleted, Flicker uses the default values. If the **Flicker.properties** file exists, it overrides these files. If you have started Flicker using a `.flk` startup file, this in turn overrides any settings from the **Flicker.properties** file. For example, you may want to use a red trial object cursor instead of yellow, etc. If you have both a `Flicker.properties` file and are starting Flicker using a `.flk` startup file, it (1) reads the user preferences

first from `Flicker.properties`, and then (2) reads the `.flk` startup file to override these preferences. If you want to reset the standard view, use the (**Edit | Reset default view**) command to override the previous view read from `Flicker.properties`. If the you ever have problems starting Flicker because of a corrupted `Flicker.properties` file, just delete it (it is kept in the same directory where you installed Flicker) and restart Flicker. When you exit, it will create a new `properties` file.

## 6. Pull-down menus

The menu bar commands are divided into menus divided by function.

1. [File menu](#) - to load/save the Flicker `.flk` state, load images, active map urls, update (from the server) program, `DB/Flk*DB.txt` database files, demo images
2. [Edit menu](#) - to change various defaults
3. [View menu](#) - to change the display overlay options
4. [Landmark menu](#) - to define landmarks for warping or other operations
5. [Transform menu](#) - contains various image processing transforms
6. [Quantify menu](#) - contains circle and boundary measurements, ROI, gray to OD calibrations
7. [Help menu](#) - popup Web browser documentation on Flicker

### Menu notation

In the following menus, selections that are *sub-menus* are indicated by a '▶'. Selections prefaced with a '☑' and indicate '☐' indicate that the command is a checkbox that is enabled and disabled respectively. Selections prefaced with a '👤' and indicate '👤' indicate that the command is a multiple choice "radio button" that is enabled and disabled respectively, and that only one member of the group is allowed to be on at a time. The default values set for an initial database are shown in the menus. Selections that are not currently available will be grayed out in the menus of the running program. The command short-cut notation **C-key** means to hold the Control key and then press the specified *key*.

### 6.1 File menu

These commands are used to load/save the Flicker `.flk` state, load images, active map URLs, update (from the server) program, `DB/Flk*DB.txt` database files, demo images, and user's images. It also saves and restores the Flicker state, does updates from the server. You can save transformed or overlay images as GIF files in the **tmp/** directory.

- **Open image file** - pop up gel image file browser to load gel to selected image
  - **Open image URL** - pop up gel image URL dialog to load gel to selected image
  - **Open demo images** ▶ - load pairs of demonstration gel images
  - **Open user images** ▶ - load single (selected image) or pairs of user's image data if they had set up the [Images/](#) directory as specified on their data
  - **Open active map image** ▶ - load active gel image from the Internet in selected image
  - **Open recent images** - load an image (or images) you have used recently
  - **Assign active image URL** - to one of the open images to make it active
- 
- **Open state file** - restore the Flicker state of previously saved session `.flk` state file

- **Save state file** - save the Flicker state in current .flk state file
- **SaveAs state file** - save the Flicker state in new .flk state file
- 
- **Update**  - update Flicker programs and data from open2dprot.sourceforge.net/Flicker server
- **Flicker program** - to get the latest program release (Flicker.jar) from Flicker server
- **Active Web Maps image DB** - get latest active maps database from Flicker server
- **Demo images DB** - to get the latest demo images database from Flicker server
- **Add user's Flicker Demo Images DB by URL** - to add additional demo database from [another Web server to the local Flicker demo database](#)
- 
- **Save transformed image** - If you have transformed the selected image and are allowing transforms, then this will save the transformed image in a **tmp/** (default) directory as a .gif file
- **SaveAs overlay image** - If you have transformed the selected image and are allowing transforms, then this will save the transformed image in a **tmp/** (default) directory as a .gif file
- **Reset images** - to the initial state when they were loaded
- **Abort transform** - abort any active image transforms
- 
- **Quit** - exit the program, saving the state in the **Flicker.properties** file of Flicker in the process

## 6.2 Edit menu

These commands are used to change various defaults. These are saved when you save the state and when you exit the program.

- **Canvas size**  - change the size of the 3 canvases and the overall Flicker window
- **Increase size (C-Numpad '+')** - increase the canvas size
- **Decrease size (C-Numpad '-')** - decrease the canvas size
- **Set colors**  - change overlay graphics colors
- **Target colors**  - to change the target color
- **Trial object colors**  - to change the trial object color
- **Landmarks colors**  - to change the landmarks color
- **Measurement colors**  - to change the circle mask measurement color
- 
- **Resize Flicker memory limits** - for the next time it is run. Initial default is 96 Mbytes
- **Use linear else log of TIFF files > 8-bits** - transform the input TIFF image if it is more than 8-bits of intensity.
- **Enable saving transformed images when do a 'Save(As) state'** - If you have transformed the left and/or the right image(s) and are allowing transforms, then this will save the transformed images in a **tmp/** directory as .gif files when you save the Flicker state.
- **Use protein DB browser, else lookup ID and name on active images** - used when click on a spot in an active DB that has  **click to access DB** checkbox enabled. If this command is set, report the protein identification in a popup window. If it is not set, then lookup the protein ID and name and report it in the popup Report window.
- 
- **Auto measure, protein lookup and Web page popup** - if one the following active DB servers is enabled, it will: 1. measure the spot and add it to the spot list; 2. lookup the Swiss-Prot (name, id); and 3. pop up the Web server on the currently selected active DB server.
- **Select access to active DB server**  - SWISS-2DPAGE, UniProt, iProClass or iProLink servers. If you

measure a spot (select a spot in an active image and then type **C-M**) (and are connected to the Internet), it will lookup the Swiss-Prot protein (accession name, and protein id) on the Swiss-2DPAGE server. Then, if you enable "Click to access DB", it will pop up the particular active DB server you have selected.

- Use **SWISS-2DPAGE DB access** - select [SWISS-2DPAGE](#) DB access.
  - Use **UniProt DB access** - select [UniProt](#) DB access.
  - Use **iProClass DB access** - select [iProClass](#) DB access.
  - Use **iProLink DB access** - select [iProLink](#) DB access.
- 
- **Reset default view** - set to the default Flicker view overriding your saved preferences in **Flicker.parameter** file (if it exists)
  - **Clear all 'Recent' image entries** - clear all recent images from the recent images history list.

### 6.3 View menu

These checkbox menu commands are used to change the display overlay options. Some options (e.g., view Landmarks, view ROI, etc.) will not display any overlays until you define the corresponding data. The **Flicker images (C-F)** checkbox menu command can also be accessed from the Flicker checkbox in the upper left corner of the window.

- **Flicker images (C-F)** - enables/disables flickering.
- 
- **Set view overlays options**  - enable/disable various overlays for the images.
    - **View landmarks** - add landmarks to the overlay display in images
    - **View target** - add target to the overlay display images
    - **View trial object** - add trial object to the overlay display images
    - **View boundary** - add boundary to the overlay display images
    - **View Region Of Interest (ROI)** - add ROI to the overlay display images
- 
- **Set view measurement options**  - set various measurement overlay options. Some of these require the (**Quantify | Measure by circle |  List-of-spots else trial-spot measurement-mode (C-J)**) checkbox command be set.
    - **View measurement circle** - add measurement ("M") and background circles ("B") to the overlay display images. The ("M") is replaced with a series of spot measurements. These are visible if the list-of-spots measurement mode is set.
    - **Use 'circle' for measured spot locations** - if you have measured spots, show the location with a circle. These are visible if the list-of-spots measurement mode is set. This will show the actual circle masks (of different sizes if you changed the sizes) around each measured spot. Otherwise, it shows it as a '+' marker.
    - **Use '+' for measured spot locations** - if you have measured spots, show the location with a '+'. These are visible if the list-of-spots measurement mode is set.
    - **Use 'spot number' for measured spot annotation** - if you have measured spots, add the spot measurement number annotation. These are visible if the list-of-spots measurement mode is set.
    - **Use 'spot identifier for measured spot annotation** - if you have measured spots, add the spot identifier (if it exists else <none>) annotation. These are visible if the list-of-spots measurement mode is set. You may edit the spot identifier using the (**Quantify | Measure by circle | Edit selected spot from spot list (C-E)**).
- 
- **Set gang options**  - enable/disable various multiple "ganged" image options where performing a change

will do the same change in several images simultaneously. This is most useful after doing an initial alignment, zoom, etc.

- **Multiple popups** - make multiple popup windows instead of reusing one window
  - **Gang scroll images** - move left and right images scrolling together
  - **Gang zoom images** - zoom left and right images together. This is useful if they are the same exact magnification
- 
- **Display gray values (C-G)** - show pixel gray values of cursor trial object in the left and right image titles as well as (x,y) coordinates. Also display the measurement circle if it is > 1 as you move the mouse.
  - **Show report popup** - display the report popup window again if needed

## 6.4 Landmark menu

These commands are used to define landmarks for image warping or other operations. First select the image, then click on the position you want to use as a landmark, then do **Add landmark (C-A)**.

- **Add landmark (C-A)** - add trial objects (in images) as landmark
- **Delete landmark (C-D)** - delete the last landmark defined
- **Show landmarks similarity** - compute a least-square error similarity measure of the two sets of landmarks
- **Set 3 pre-defined landmarks for demo images (C-Y)** - only for plasmaH/plasmaL or testA/testB (triangle) images
- **Set 6 pre-defined landmarks for plasmaH/plasmaL demo (C-Z)** - only for plasmaH/plasmaL or testA/testB (triangle) images

## 6.5 Transform menu

These commands are used contains various image processing transforms. First select the image you want to transform. For some transforms, if you don't select either image it will do the transform on both images. Then select the transform from the Transform menu.

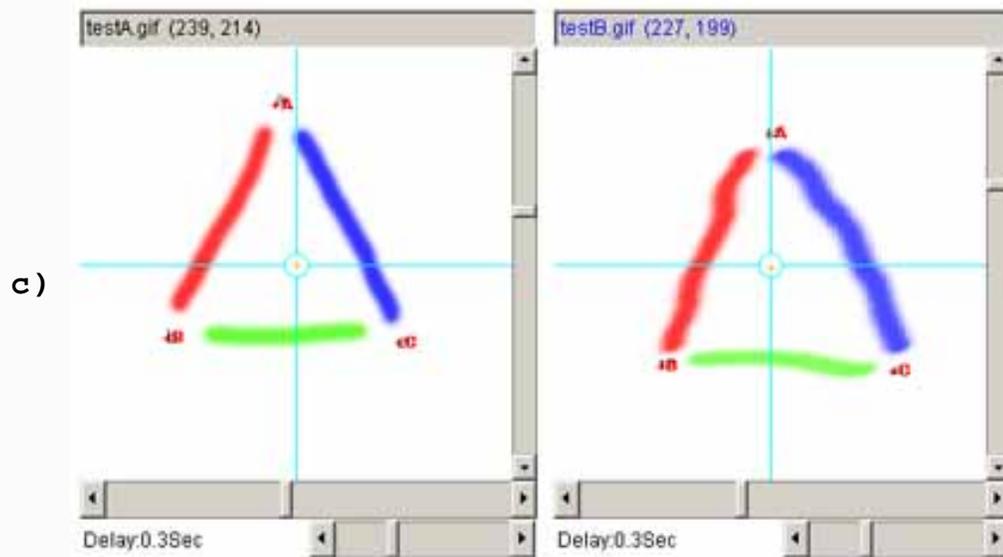
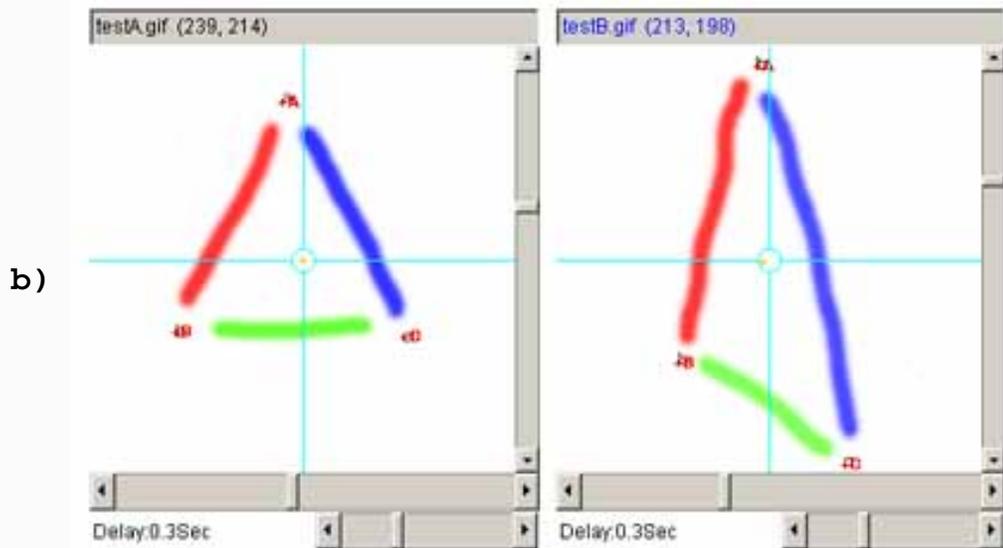
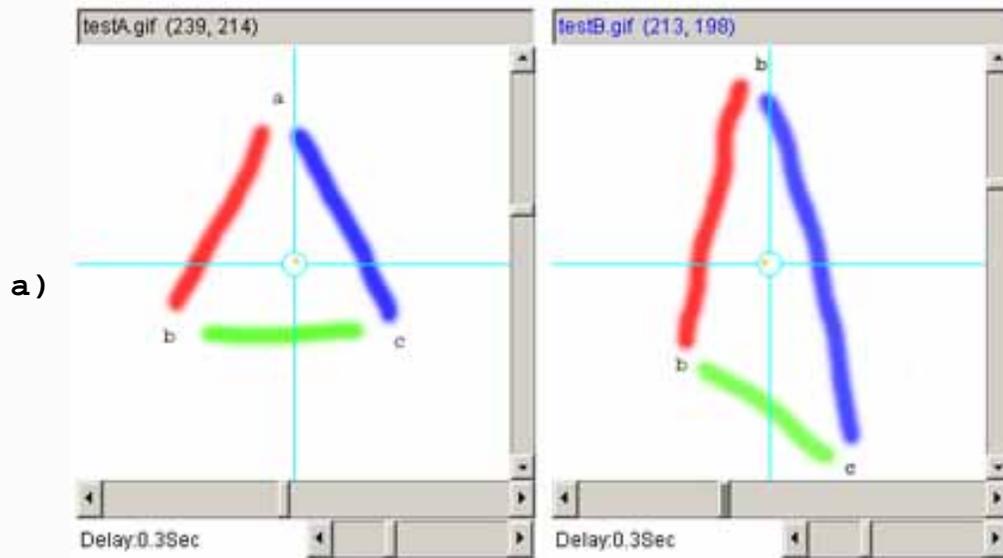
- **Affine Warp** - warp selected image using first 3 pairs of landmarks
  - **Polynomial Warp** - warp selected image using first 6 pairs of landmarks
  - **Pseudo 3D transform** - do pseudo 3D scaling based on image intensity
- 
- **Sharpen Gradient** - gradient + gray scale sharpen selected image
  - **Sharpen Laplacian** - Laplacian + gray scale sharpen selected image
  - **Gradient** - gradient of the selected image
  - **Laplacian** - Laplacian of the selected image
  - **Average** - average selected image
  - **Median** - median of selected image
  - **Max 3x3** - max of 3x3 neighborhood of selected image
  - **Min 3x3** - min of 3x3 neighborhood of selected image
- 
- **Complement** - complement selected image
  - **Threshold** - threshold the selected image by gray values in [T1:T2]. This is also invoked by clicking on an image and moving the threshold 1 or 2 parameter scrollers
  - **Contrast Enhance** - Contrast enhance selected image

- **Histogram equalize** - histogram equalize selected image  
-----
- **Original color** - Restore original data for selected image
- **Pseudo color** - compute pseudo color scaling for selected image
- **Color to grayscale** - compute NTSC RGB to grayscale transform for selected image (gray= red\*0.33 + green\*0.50 + blue\*0.17)  
-----
- **Flip Image Horizontally** - flip image horizontally selected image
- **Flip Image Vertically** - flip image vertically selected image  
-----
- **Repeat last transform (C-T)** - repeat last transform, if any
- **Use threshold inside [T1:T2] filter** - filter by pixels inside the range [T1:T2], otherwise pixels outside of [T1:T2]

### 6.5.1 Example affine warping an image to the geometry of the other image

This example warps the right image to the geometry of the left image to make them easier to flicker compare. There are two warping transforms - affine that requires 3 landmarks. You first define the required 3 pairs of corresponding landmarks between the triangular region you are interested in warping (see the [landmark](#) vignette and the [using warping](#) vignette for more info). Then select the gel you wish to warp. You then apply the warp transform from the Transform menu. We illustrate this with the two triangle test images testA and testB. Flicker has predefined landmarks for the plasmaH/plasmaH and testA/testB demo images that you can easily invoke for this example (see step [2]).

1. Load the test images using (**File menu | Open demo images | Test images | Affine warp test A and B**) command. The images are shown in Figure 11a.
2. Define the landmarks. You can do this using the Landmark commands or use a demo short cut (**Landmark menu | Set 3 pre-defined landmarks for demo images (C-Y)**) command. The images are shown in Figure 11b.
3. Set  **Allow transforms** checkbox enabled.
4. Set  **Sequential transforms** checkbox disabled.
5. Select the right image window you want to warp.
6. Perform the transform you want to try in the (**Transform | Affine warp**) or (**Transform | Poly warp**) command.
7. Reposition the transformed image and the other image to the spot of interest inside of the landmark region. The recentered warped images are shown in Figure 11c.
8. Enable flickering. It should then be easier to see the cooresponding spots.



**Figure 11. Example using the affine transform.** **a)** shows the original left and right images. **b)** shows the left and right images with 3 pairs of landmarks after they are added (large red A, B and C labels). **c)** shows the two images after applying the affine transform to the right image and recentering it.

## 6.6 Quantify menu

These commands are used to define and measure integrated density (grayscale or calibrated OD if calibrated) for circle, boundary, region-of-interest (ROI) measurements. Integrated density measurements are made on the 8-bit (0:255 gray-value) data of pixels. It is only valid on grayscale data since we do the measurements on the least significant 8-bits (blue channel of the 24-bit RGB data). If you have color data, you can use the NTSC color to gray-scale transform to convert it to gray scale first. If you have calibrated grayscale to optical density (OD), you get the measurement in terms of OD rather than grayscale. The calibration wizard also lets you define the calibration in terms of other units.

In the mean time, we refer to "density" as either of these two measurements. You may measure the total integrated density (summing the pixel gray values or OD), or mean density (the total density/#pixels in the region). This background density is used to correct the measurement density if it was defined (otherwise 0.0 is assumed). Note that you may clear the background measurement circle using the **(C-W)** command.

## Creating lists of spots

Flicker was not designed to measure very large numbers of spots. That said, it could be used that way but with a lot of manual work. We provide some commands to make it easier to quantify **(C-B)** and **(C-M)** or **(ALT-click)**, annotate **(C-I)**, edit **(C-E)**, and delete **(C-K)**. You can also list and save the measured spot lists for the two images. The measured spot lists automatically get saved if you save the .flk startup state (**File | SaveAs state file**). Opening Flicker using that startup state file will restore the measured spot list.

You can save the spot list in a tab-delimited file suitable for import to Excel, by 1) clearing the Popup Report Window; 2) select the image you want and use **(Quantify | Measure by circle | List spots in the spot list (tab-delimited))** command; 3) then, Save the text in the Popup Report Window or cut and paste it into Excel, etc.

It is possible to generate a list of paired spots between the two images using the **(Quantify | Measure by Circle | List paired 'id' annotated-spots in both spot lists (tab-delimited))** command. It lists paired spots that occur in the spot lists of both gels and have the same annotation 'id' values (case-sensitive). The user assigns the spot 'id' annotations using the **(C-I)** spot editing command to assign the case-sensitive spot identifiers. **(C-E)** command may also be used to edit the ids'. The paired spot data list may be exported to Excel similar to what was described in the last paragraph for spot lists.

Note that the background density is *not* the same everywhere on the gel image. Therefore if you are measuring spots in regions of the gel with quite different background (e.g., tail streaks from other spots, etc.), to get better estimated quantification you need to redefine the background adjacent to where you are making measurements. Recent versions of Flicker track the most recent background **(C-B)** estimate and associate it with new measured spots **(C-M)** until you redefine it.

- **Measure circle**  - measure intensity/density within the circular mask
- **Capture background (C-B)** - get background measurement at current position for a circular mask. It

draws a **+B** at that position. It uses the currently measurement circle diameter for that image.

- **Capture measurement (C-M)** - get measurement at current position for a circular mask and report background-corrected results (if background was defined). It uses the currently measurement circle diameter for that image. It draws a **+M** at that position or **+1, +2, +3, ...** for a series of measurements if the (**Quantify** |  **Use measurement counter**) is enabled. An alternative way to measure spots is to hold the **ALT-key** when you press the mouse to select the spot. This combines spot selection and measurement in one operation. Note that you can toggle between trial- mode and spot-list measurement mode using the (**Quantify** |  **List-of-spots else trial-spot measurement-mode (C-J)**) checkbox command.
  - **Clear measurement** - clear measurement and background values
  - **Edit selected spot(s) 'id's from the spot list(s) (C-I)** - edit the spot annotation 'id' field for the selected spot(s) you previously measured with the circular mask if you are within 2 pixels. It will edit one or both spots from the two images if they are selected (see [additional discussion](#)).
  - **Edit selected spot(s) from the spot list(s) (C-E)** - edit all of the fields of the selected spot(s) you previously measured with the circular mask if you are within 2 pixels. It will edit one or both spots from the two images if they are selected (see [additional discussion](#)).
  - **Delete selected spot from the spot list (C-K)** - delete the selected spot you previously measured with the circular mask if you are within 2 pixels.
- 
- **List spots in the spot list** - list spot list report in the popup report window if there are any spots in the list (see [additional discussion](#))
  - **List spots in the spot list (tab-delimited)** - list spot report in the popup report window if there are any spots. The data is listed in a tab-delimited format suitable for importing into Excel or other software (see [additional discussion](#)).
  - **List 'id'-paired annotated mean norm. spots in both spot lists (tab-delimited)** - list paired spots that occur in the spot lists of both gels. The pairing is defined by assigning the same annotation spot 'id' using the (**C-E**) spot editing command to assign the case-sensitive spot identifiers. You must use the same calibration (if any), total or mean density mode for both gels. Data is mean-spot normalized over the gels by dividing by the mean-spot density in each gel. Spot report appears in the popup report window if there are any spots. The data is listed in a tab-delimited format suitable for importing into Excel or other software (see [additional discussion](#)).
  - **List 'id'-paired annotated spots in both spot lists (tab-delimited)** - list paired spots that occur in the spot lists of both gels. The pairing is defined by assigning the same annotation spot 'id' using the (**C-E**) spot editing command to assign the case-sensitive spot identifiers. You must use the same calibration (if any), total or mean density mode for both gels. Data is not normalized over the gels. Spot report appears in the popup report window if there are any spots. The data is listed in a tab-delimited format suitable for importing into Excel or other software (see [additional discussion](#)).
  - **Clear spot list (ask first)** - clear spots in the spot list, but ask them if they are sure first.
- 
- **Measure boundaries**  - measure intensity/density within user-drawn boundary
  - **Define background** - start measuring background region in image
  - **Define object** - start measuring object region in image
  - **Done boundary** - finished measuring region in image and report results
- 
- **Print-data window**  - Print data-window
  - **Show data-window of selected pixel (C-V)** - print a 5x5 through 40x40 gray-scale window (in decimal, octal, or hex number radix) in the popup report window.
  - **Set print-window size** - set the print window size
  - **5x5** - window size

- **10x10** - window size
- **15x15** - window size
- **20x20** - window size
- **25x25** - window size
- **30x30** - window size
- **35x35** - window size
- **40x40** - window size
- **Set print-data radix** - set the data format radix
- **Decimal** - data format radix
- **Octal** - data format radix
- **Hexidecimal** - data format radix
- **Optical density** - data format radix (if calibrated)

- 
- **Calibrate**  - calibrate optical density or other step wedge
  - **Optical density by step wedge** - calibrate optical density from ND step wedge. This will popup a calibration wizard which includes a histogram and peak table that you can edit. This assumes you have set up a computing window ROI over the step wedge scanned with the data. You must also know the OD values corresponding to the step wedge which you must enter into a peak table.. It will compute a histogram over the ROI, find the peaks and insert them into the peak table and extrapolate the grayscale to OD curve over the dyanmic range of the histogram (see [additional discussion](#)).
  - **Use demo leukemia gels ND wedge calibration preloads wedge** - used to demonstrate the ND step wedge calibration process. We have built in four sample ROI's and the corresponding OD values corresponding to the step wedge scanned with the four demo gels (Leukemia-AML, Leukemia-ALL, Leukemia-CLL, Leukemia-HCL). Enable this option *before* invoking the **Optical density by step wedge** command.

- 
- **Optical density by spot list** - similar to the above calibration, except that you specify the corresponding values by measuring a set of spots that cover the corresponding calibration values so there is no histogram analysis (see [additional discussion](#)).

- 
- **Molecular mass** - calibrate MW from MW standards
  - **pIe** - calibrate pIe from pIe standards

- 
- **Region of Interest (ROI)**  - region of interest operations
  - **Set ROI ULHC (C-U)** - define upper left hand corner of ROI
  - **Set ROI LRHC (C-L)** - define lower right hand corner of ROI
  - **Clear (ROI) (C-W)** - delete ROI
  - **Show grayscale ROI histogram (C-H)** - popup a histogram of the computation region ROI. If you redefine the ROI, it will update the histogram. If the ROI is not defined, it uses the entire image.
  - **Capture measurement by ROI (C-R)** - measure integrated density inside the computation region ROI. Use the circular mask mean background for background correction

- 
- **Use sum density else mean density** - use sum of the density else mean density within the region (circular mask or ROI)
  - **List-of-spots else trial-spot measurement-mode (C-J)** to either measure spots or use a trial measurement mode. In measurement mode, add spot label overlays consisting of measurement locations (either "+" or circles or neither) followed by measurement annotation (measurement # and/or spot specific identifier (if edited), or neither). As specified by the (**View | Set view measurement options | ...** checkbox

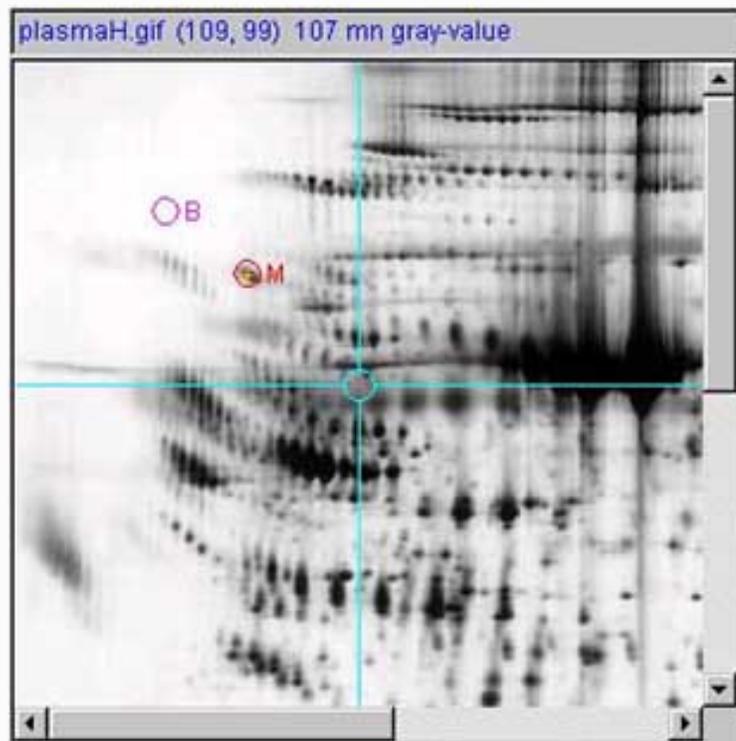
options. In measurement mode it will number the measurements 1, 2, 3, ... etc, whereas in trial-measurement mode it will show a single "+M" instead. Note that in trial mode, it will redraw the measurement circle as you change the size with the slider. Note that if you are measuring several different ROI's with (C-R), then it will assign sequential measurement numbers to these ROI measurements.

Figures 12a-12h show various permutations of the spot list overlay options. Figure 13 shows an example of a histogram of the Region Of Interest (ROI). Figure 14 shows examples of calibrating gray scale a step wedge scanned with the image using the step wedge calibration wizard. Figure 15 shows calibrating grayscale where there are calibration spots in the image by using the spot list calibration wizard.

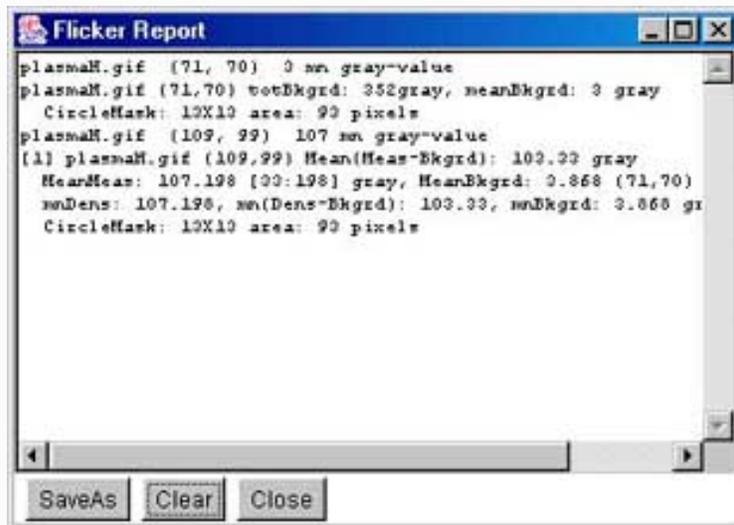
### 6.6.1 Spot measurements

Figures 12a-12h show various permutations of the viewing spot quantification using the various spot list overlay options.

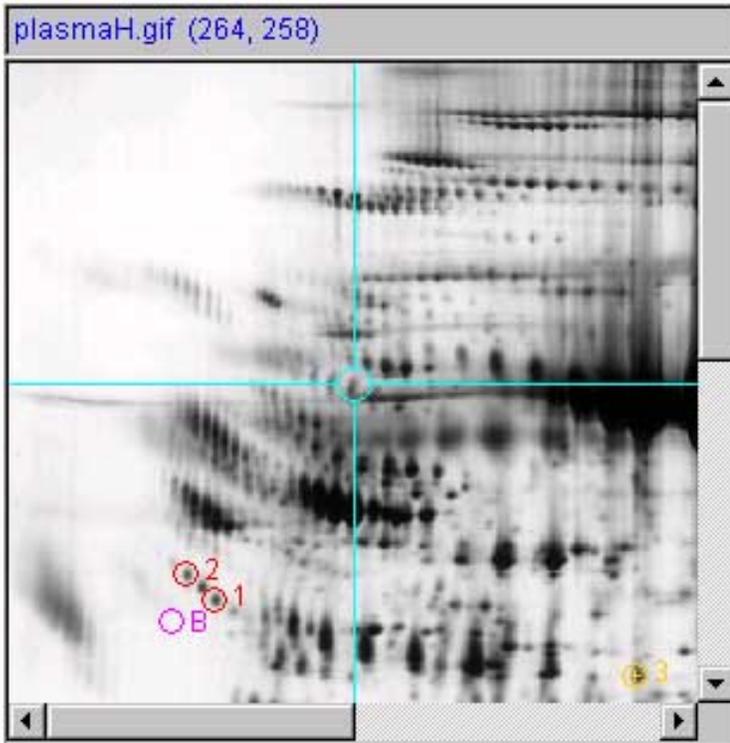
a.1)



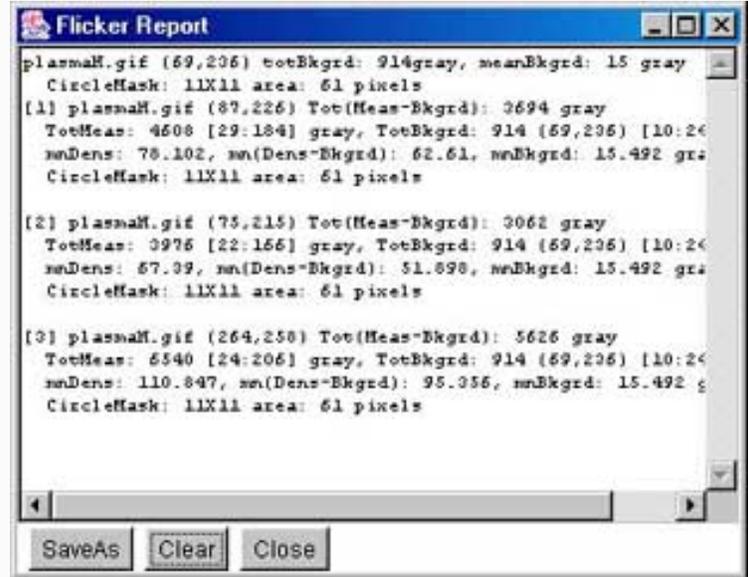
a.2



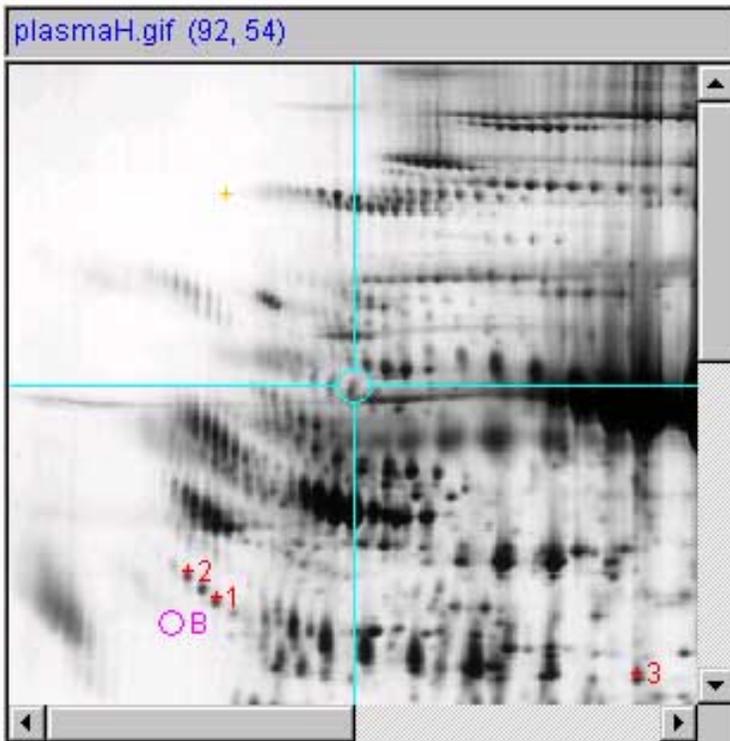
b.1)



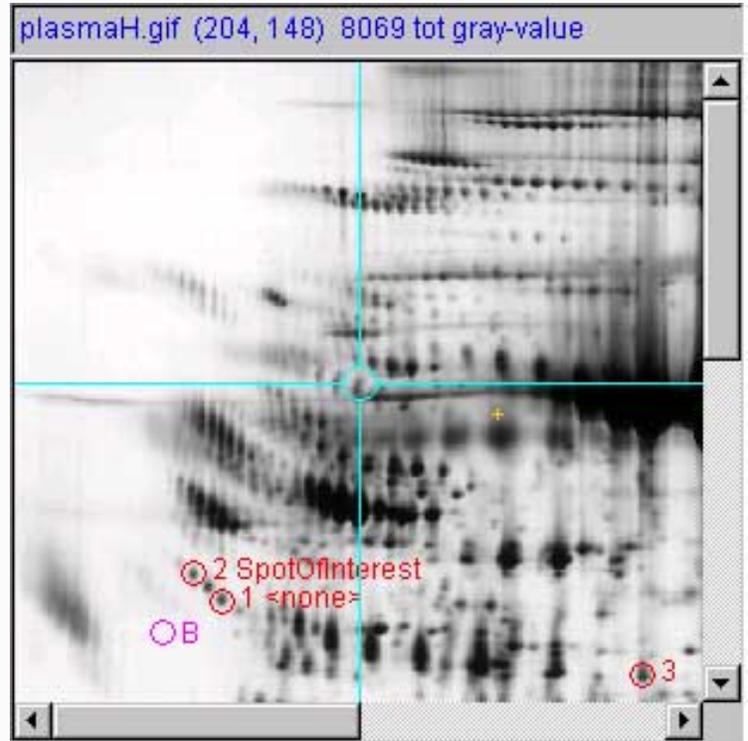
b.2)

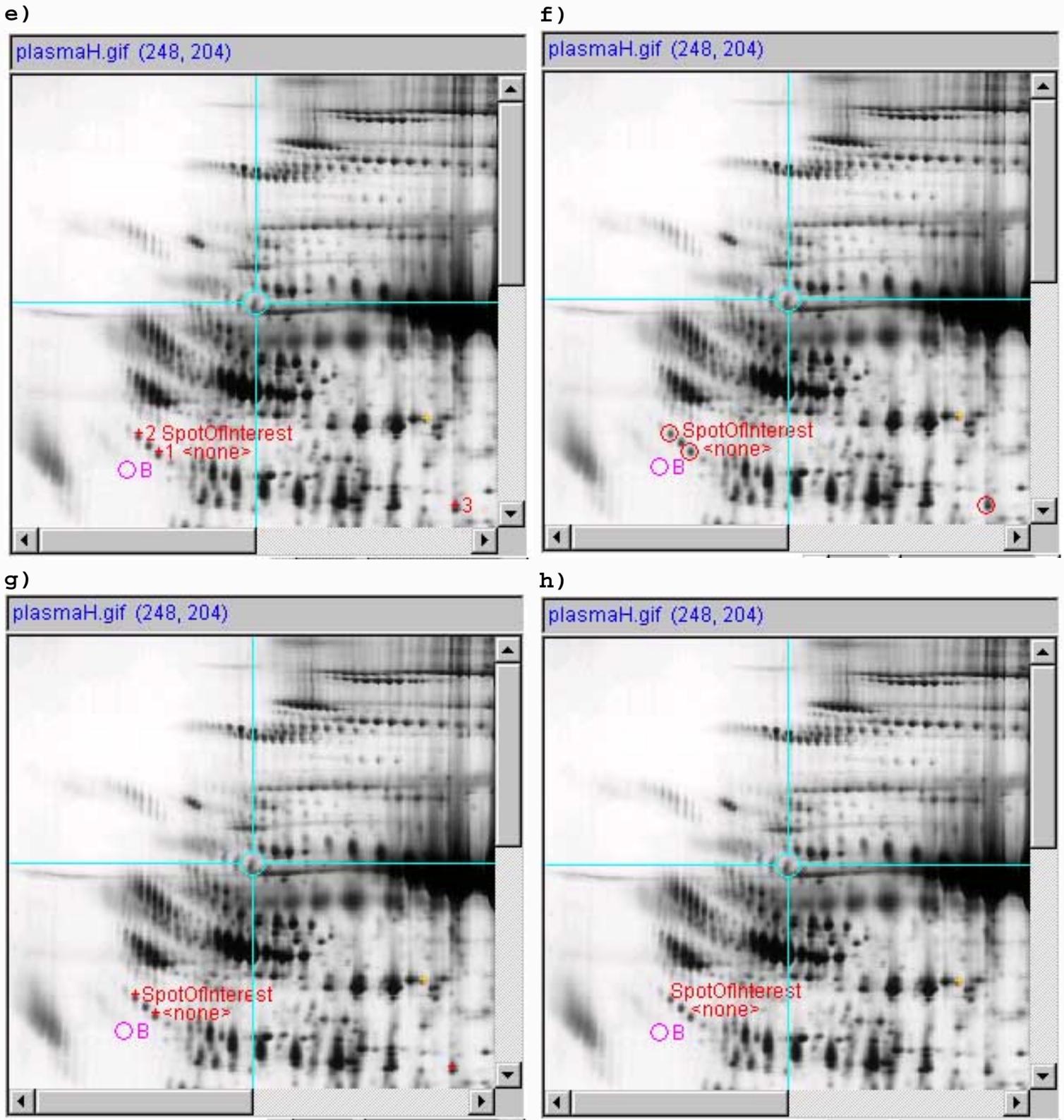


c)



d)





**Figure 12. Examples of the measured spotlist.** This figures show various options in displaying the measured spots. As a shorthand for this legend, **VM:** is the (**View | Set view measurements options | ...**) submenu.

Panels (a.1) and (a.2) show a single spot measurement image and report and does not create a list of measured spots. The (**Quantify |  List-of-spots else trial-spot measurement-mode (C-J)**) should be turned off. The **VM:  View measurement circle** option should be enabled.

---

Panels (b.1) and (b.2) show a measured spot list with three spots, image and report respectively. Spots are shown with circles and spot number annotation. Since the user had clicked on the third spot, it is considered the "current" spot in the spot list and is indicated in yellow. The rest are indicated in red. The **(Quantify |  List-of-spots else trial-spot measurement-mode (C-J))** should be turned on. The **VM:  Use 'circle' for measured spot locations** option should be turned on, **VM:  Use 'spot number, for spot annotations** option should be turned on.

---

Panel (c) shows the measured spot list with a "+" instead of a circle with spot numbers. Since the user had clicked on a different part of the screen, there is no "current" spot in the spot list so all spots are shown in red. The **(Quantify |  List-of-spots else trial-spot measurement-mode (C-J))** should be turned on. The **VM:  Use '+' for measured spot locations** option should be turned on, **VM:  Use 'spot number, for spot annotations** option should be turned on.

---

Panel (d) show a measured spot list with three spot measurements image and report. Spots are shown with circles, spot numbers and spot annotation. The **(Quantify |  List-of-spots else trial-spot measurement-mode (C-J))** should be turned on. The **VM:  Use 'circle' for measured spot locations** option should be turned on, **VM:  Use 'spot number, for spot annotations** option should be turned on, and **VM:  Use 'spot identifier, for spot annotations** option should be turned on. The user had edited the spot identifiers by (1) clicking on a spot, (2) doing the **(Quantify | Measure by circle | Edit selected spot from spot list (C-E))** command, and 3) typing a spot "identifier" for that spot and pressing "Done". Spots where the 'id was not edited are indicated by "<none>". For this example, we set 'id' to "SpotOfInterest" for spot #1 and a space for spot #3. Spaces make the identifier disappear.

---

Panel (e) show a measured spot list with three spot measurements image and report. Spots are shown with pluses, spot numbers and spot annotation. The **(Quantify |  List-of-spots else trial-spot measurement-mode (C-J))** should be turned on. The **VM:  Use '+' for measured spot locations** option should be turned on, **VM:  Use 'spot number, for spot annotations** option should be turned on, and **VM:  Use 'spot identifier, for spot annotations** option should be turned on.

---

Panel (f) show a measured spot list with three spot measurements image and report. Spots are shown with circles and spot annotation. The **(Quantify |  List-of-spots else trial-spot measurement-mode (C-J))** should be turned on. The **VM:  Use 'circle' for measured spot locations** option should be turned on, and **VM:  Use 'spot identifier, for spot annotations** option should be turned on.

---

Panel (g) show a measured spot list with three spot measurements image and report. Spots are shown with pluses and spot annotation. The **(Quantify |  List-of-spots else trial-spot measurement-mode (C-J))** should be turned on. The **VM:  Use '+' for measured spot locations** option should be turned on, and **VM:  Use 'spot identifier, for spot annotations** option should be turned on.

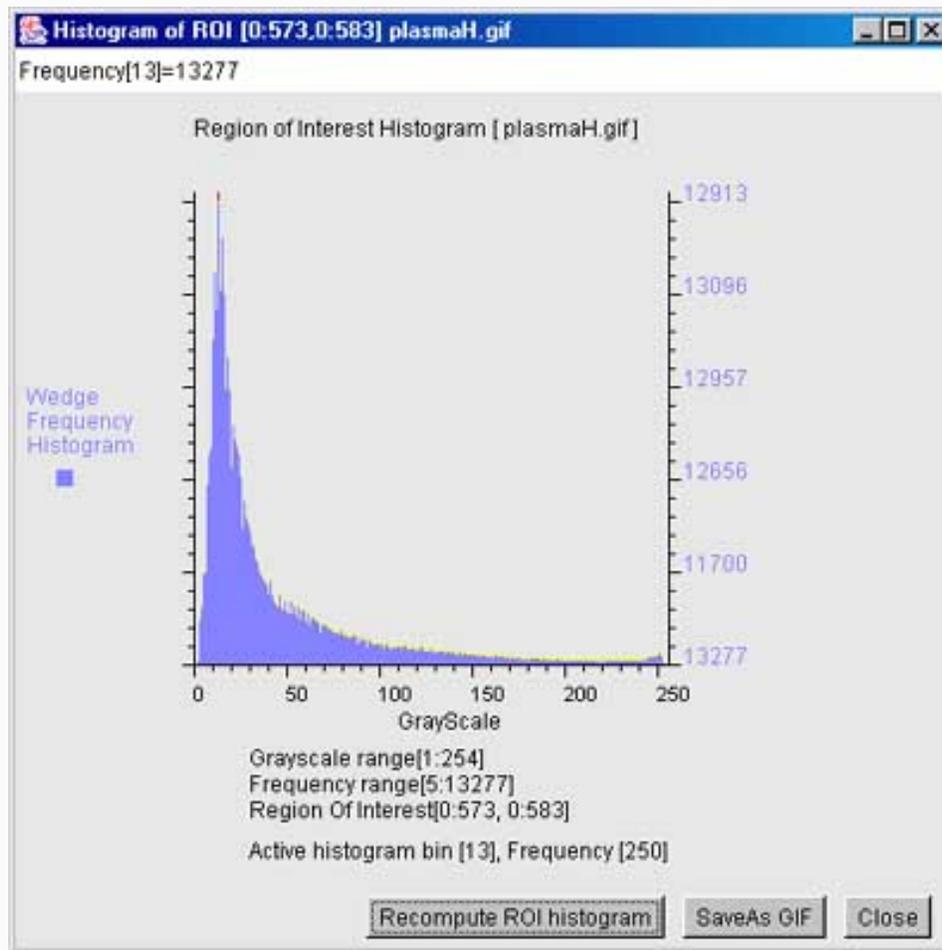
---

Panel (h) show a measured spot list with three spot measurements image and report. Spots are shown with just spot annotation. The **(Quantify |  List-of-spots else trial-spot measurement-mode (C-J))** checkbox option should be turned on. The **VM:  Use 'spot identifier, for spot annotations** option should be turned on.

---

## 6.6.2 Region of Interest histogram display

You can compute a histogram of the current region of interest in the selected image. Figure 13 shows an example of a histogram of the Region Of Interest (ROI).



**Figure 13. Example of a grayscale histogram of a region in the image.** This popup window shows the grayscale histogram under the computing window region of interest (ROI). If no ROI was defined (the case shown here), it computes the histogram over the entire image. It is invoked by (**Quantify | Region Of Interest (ROI) | Show RPI grayscale histogram (C-H)**). Clicking on a value of the histogram will show the frequency of the specified gray value at the top of the display.

### 6.6.3 The ND step wedge grayscale calibration **[ALPHA-level code]**

It is useful and sometimes essential to [calibrate an image using known standard density values](#). If the gel stain is stoichiometric within limits, density corresponds to protein concentration. Then within the constraints of linearity of the staining, range and saturation of the scanner, it is possible to get more accurate spot density measurements. One way to do this is to scan the gel (or image) with a neutral density step wedge (Suppliers: such as Stauffer Graphic Arts or similar) with known optical density (OD) values for each step. Note that if you have step wedges calibrated in other units (e.g., counts-per-minute or CPM, then you can specify that to the calibration wizard). If the gray values are mapped to the corresponding optical density values, the wizard interpolates the intervening values constructing a translation table to map gray scale (in the range of [0:255] used in Flicker) to the corresponding OD or other calibration values. Then when measurements are made using the Quantify menu commands (circle mask or ROI), the data is first mapped to the calibrated units rather than grayscale. The sum of the pixel gray values is computed using this map so the measurement is the sum of the optical densities or integrated density/spot measurement area. This gives a better estimate of the protein concentration.

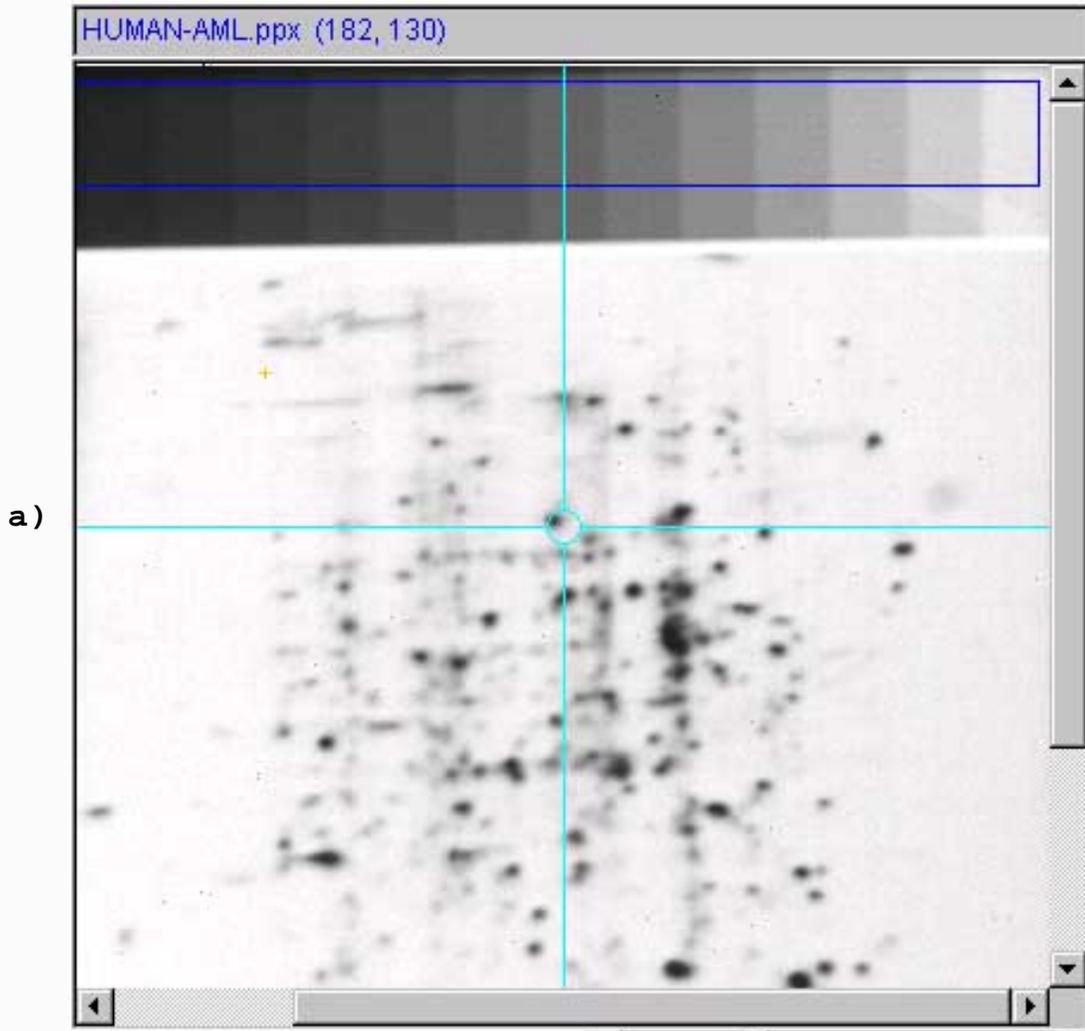
Once an image is calibrated and the calibration saved (in the {installation directory}/cal/{imageFile}.cal file), it may be reused everytime the image is loaded into Flicker.

The initial calibration is created from the step wedge data and known corresponding OD values using a histogram specified by a region of interest (ROI) specified as the step wedge region.

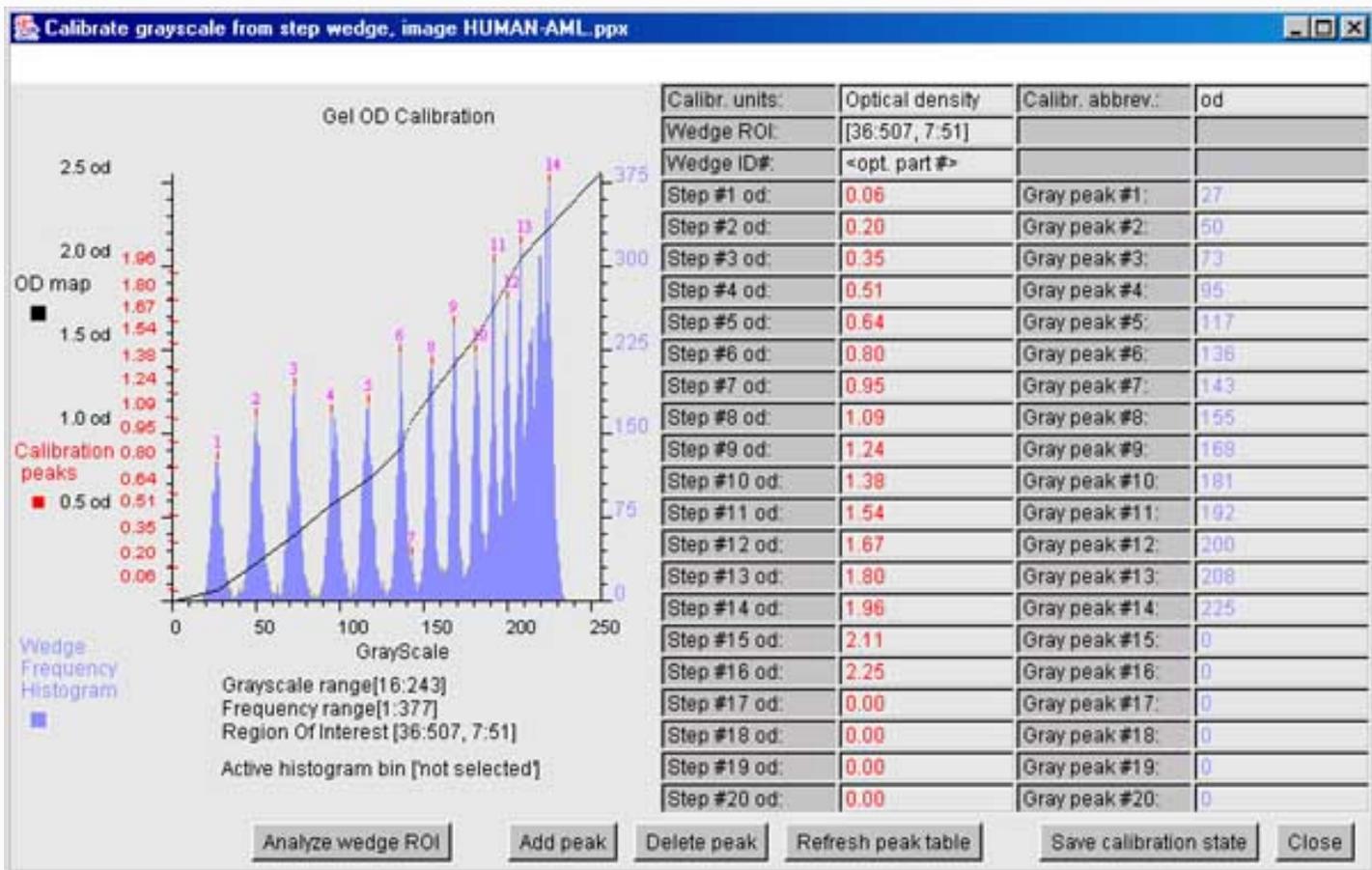
To make it easier to demonstrate this process, we have set up special demo calibration data for the four demo Leukemia gels. If you have enabled (**Quantify | Calibration | Use demo leukemia gels ND wedge calibration preloads**) checkbox, it will preload the OD values and step-wedge ROI for demonstration purposes. If you are using any other data, you must specify the OD values and step-wedge ROI. You invoke the wizard by the (**Quantify | Calibration | Optical density by step wedge**) command. This will popup the calibration wizard window.

### Procedure: for calibrating the ND step wedge

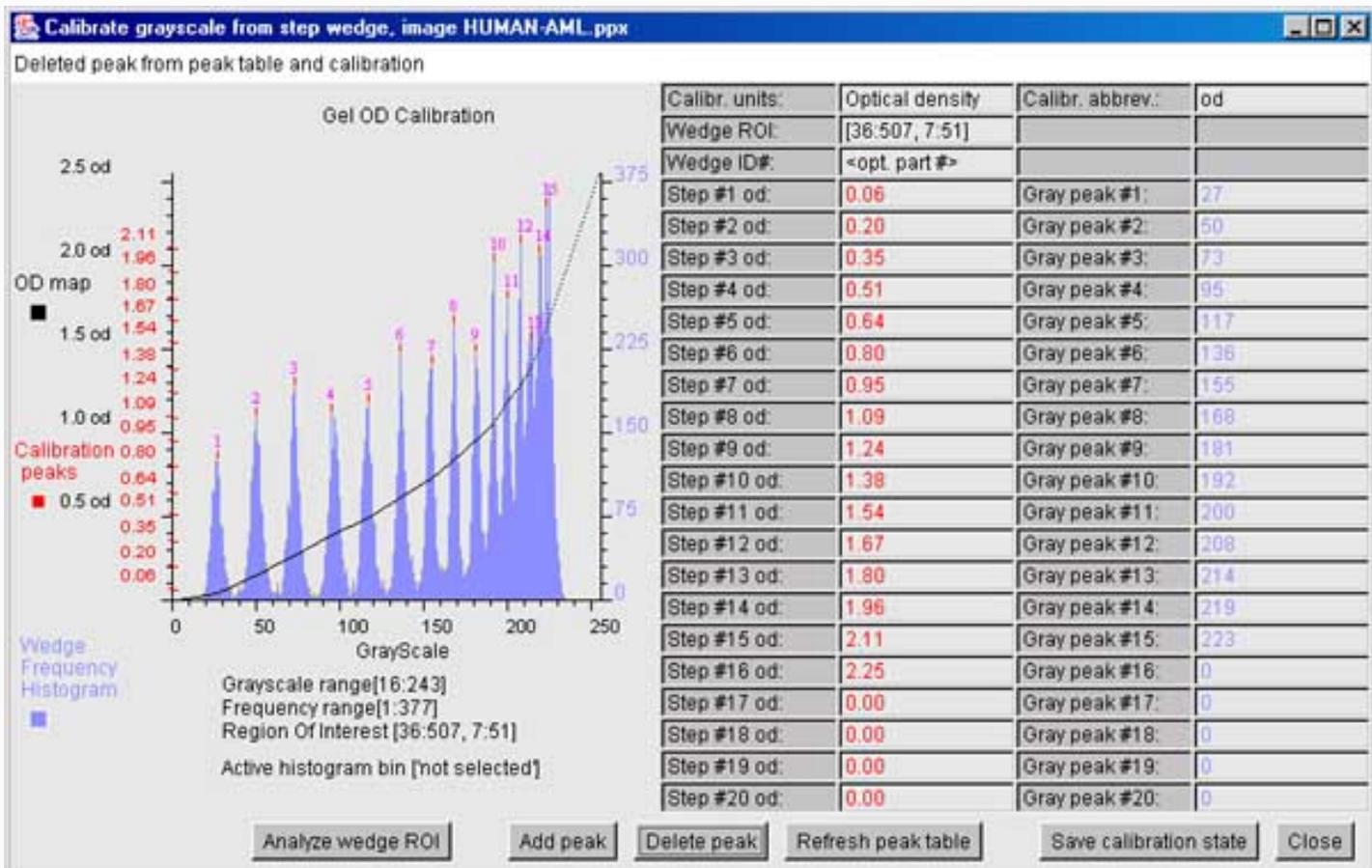
1. If you are using the preloaded demo leukemia gels data specified above, then this step is done for you automatically. Setup a well-define ROI region around the ND step wedge in the image using (C-U) upper left-hand corner and (C-L) lower right-hand corner. Then press the **Analyze wedge ROI** button in the wizard. This computes the histogram of the step wedge region and attempts to find the peaks in the histogram corresponding to the mean wedge values. It will then put them in the peak table on the right of the wizard.
2. If there are no OD data in the peaks table, then enter the OD calibration values into the red Optical-Density fields. [If the calibration was set previously and we read them in from the .cal file, then these files are preset as well as the corresponding grayscale Gray-peak field values if you know what the values are. Press the **Update peak table** button in the wizard to recompute the OD calibration and update the calibration plot. **Note:** If the current gel is not calibrated and the other gel has a calibration step list of OD values, then it will use it to save you having to type it in.
3. To force it to analyze the ROI wedge area, click on the **Analyze wedge ROI** button. This recomputes the histogram on the ROI (which may have changed) and the recomputes the calibration curve.
4. This will also update the Calibration Peak Table and generate the extrapolated gray to OD translation map calibration. The new histogram will show the Gray-peak values cooresponding to the OD values with red tick marks on the peaks as follows:
  - 4.1 It tries to find the peaks and copies the peaks into the Calibration Peak Table Gray-peak fields.
  - 4.2 It then generates a piecewise-linear extrapolation of the peak-table data to generate the translation map.
  - 4.3 It then redraws the histogram plot with **a**) overlay calibration curve which is the gray to OD map, **b**) updates the peaks table of (OD values, peak gray values) and draws this data into the plot.
5. You may edit the peak list, by selecting a peak with the mouse and then pressing either **Add peak** or **Delete peak** button in which case it redoes the step 4.
6. When you are done editing you may save the calibration by pressing the **Save calibration state** button. This saves the calibration data in the `cal/{imageFile}.cal` and makes the gray to OD calibration available to the image. You can then make measurements calibrated in OD.
7. Press **Done** to exit the calibration wizard. If you have not saved the calibration and do not want to, it will prompt you to save it and you should press the **No** button.



b)



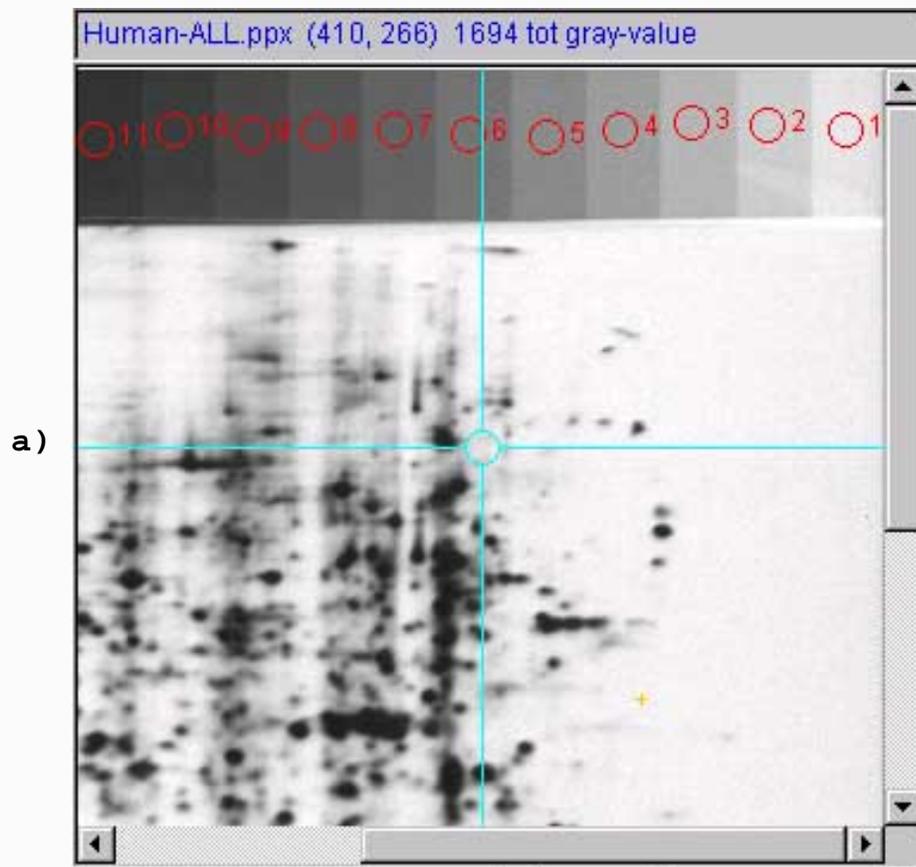
c)

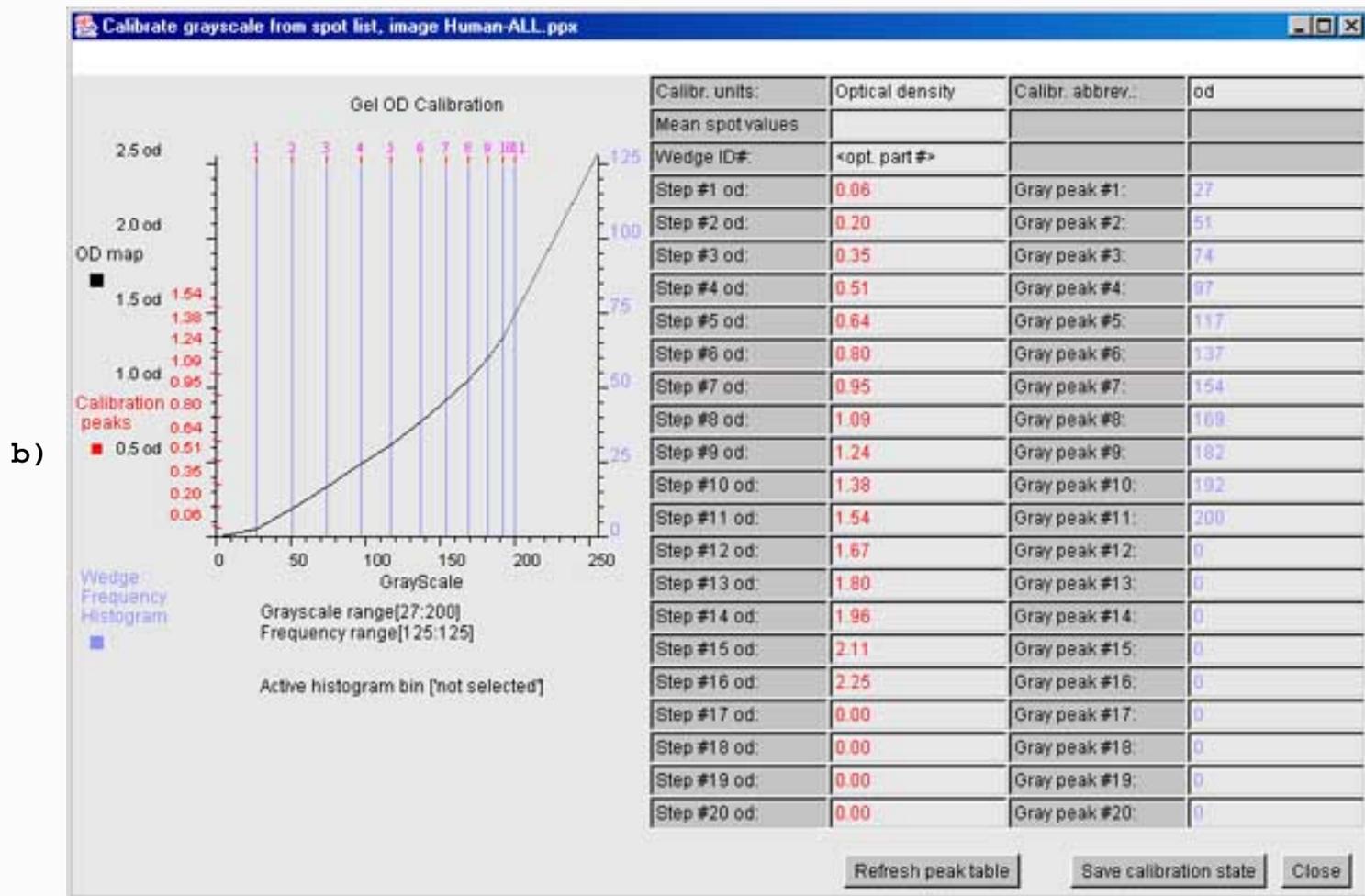


**Figure 14. Example of calibrating grayscale using the step wedge calibration wizard.** This shows an example of calibrating the gray scale using a ND step wedge that was scanned with the image. The OD values corresponding to the steps are known and are entered into the peak table. **a)** The ROI region over the ND step wedge was defined. In this example using one of the demo leukemia gels (AML), we used the preload option (**Quantify | Calibration | Use demo leukemia gels ND wedge calibration preloads**) command. Then, startup the wizard by using the (**Quantify | Calibration | Optical density by step wedge**) command. If you did not do this, then you must define the ROI for the wedge region yourself and then press the **Analyze wedge ROI** button. **b)** Shows the calibration wizard window with the histogram on the left, the peak table on the right and control buttons on the bottom. This is before editing the histogram. If you were defining the calibration values from scratch, enter them in the peak table and define the calibration units and units abbreviation. Then press the **Update peak table** button. **c)** Because there are some bad peaks (#7, grayscale of 143) and some that were missed (grayscale peaks at 214 and 220), we need to edit the peak table. Click on each peak in the histogram you want to delete and then press the **Delete peak** button. To add peaks, click on each peak in the histogram you want to add and then press the **Add peak** button. When you are satisfied with the calibration, press the **Save calibration file** to save the calibration in the `{installation directory}/cal/{imageName}.cal` file and then press **Close** to exit the wizard. A good calibration should be a smooth continuous function with no kinks. If you forgot to save the calibration when you leave the wizard, it will ask you if you want to save it. If you don't, it will revert to the previous calibration if any.

#### 6.6.4 The Spot list grayscale calibration [ALPHA-level code]

There is an alternative calibration method. You can calibrate grayscale if have a set of calibrated spots or regions in the image instead of a calibration step-wedge. You do not use the ROI, but instead will specify a set of spot measurements of increasing darker regions that correspond to your calibration values (such as OD, CPM, etc). (**Quantify | Calibration | Optical density by spot list**) command. This will popup the calibration wizard window.





**Figure 15. Example of calibrating grayscale using the spot list calibration wizard.** This shows an example of calibrating the gray scale using a set of known calibrated regions or spots that were scanned with the image. The OD or other calibration values corresponding to the spots are known and are entered into the peak table. **a)** We selected a set of spots over the ND step wedge but any set of known regions could be used. In this example using one of the demo leukemia gels (AML), we used the preload option (**Quantify | Calibration | Use demo leukemia gels ND wedge calibration preloads**) command. Then, startup the wizard by using the (**Quantify | Calibration | Optical density by spot list**) command. If you did not do this, then you must define the OD list of calibration values yourself and then press the **Update peak table** button. **b)** Shows the calibration wizard window with the histogram on the left, the peak table on the right and control buttons on the bottom. If you are defining the calibration values from scratch, enter the OD (or other calibration values) into the peak table and define the calibration units and units abbreviation. Then press the **Update peak table** button. You may edit the peak table (just as you can for the ND wedge calibration). When you are satisfied with the calibration, press the **Save calibration file** to save the calibration in the `{installation directory}/cal/{imageName}.cal` file and then press **Close** to exit the wizard. A good calibration should be a smooth continuous function with no kinks. If you forgot to save the calibration when you leave the wizard, it will ask you if you want to save it. If you don't, it will revert to the previous calibration if any.

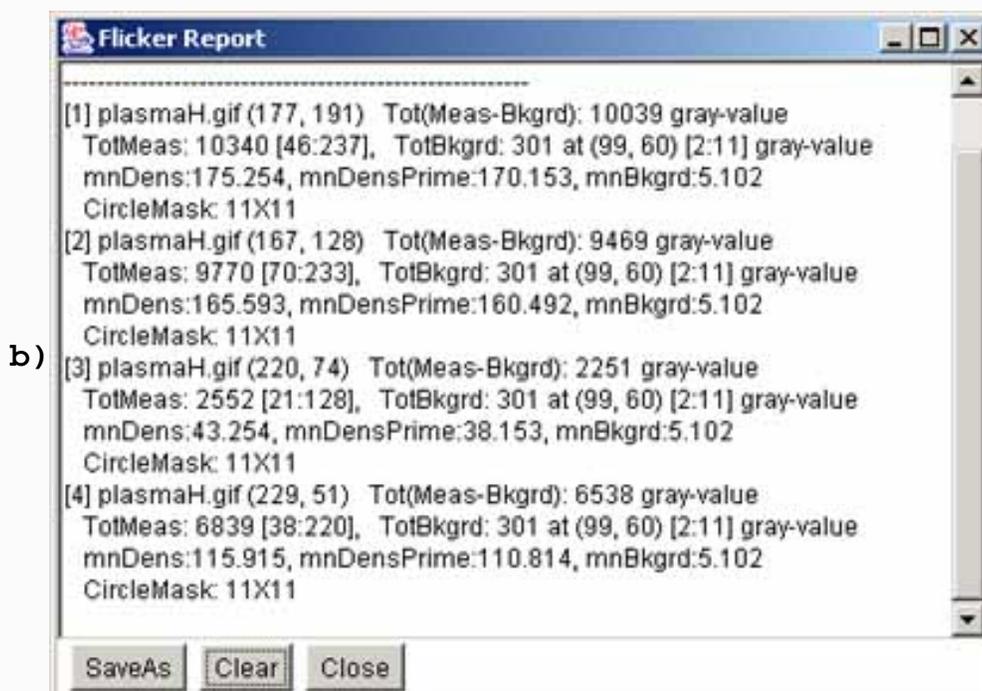
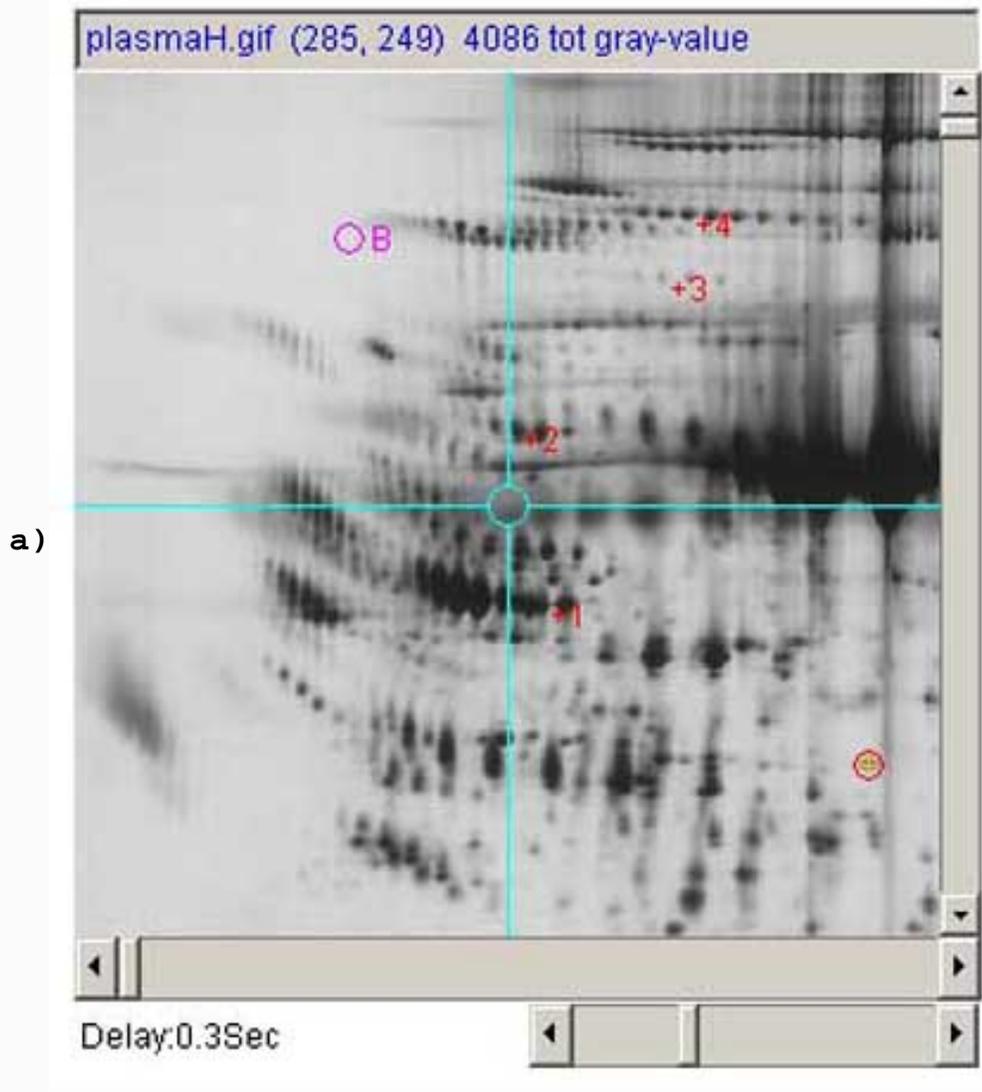
### 6.6.5 Example of measuring multiple spots using the circular mask

This gives an example of how do you can measure spot intensity under the circular mask. First, the background corrected measurement estimate is computed under the circular mask (**C-M**). This background estimate is then used for multiple measurements until you change this background estimate. The current background intensity measurement is associated with all spots measured since it was defined or redefined and is saved in the spot list. You number the spots by enabling the (**View menu | Set view measurement options |  View measurement**

**circle**) option. The numbered image is shown in Figure 16a.

You may also list the saved spots in the report window (shown in Figure 16b). Hint, press the **Clear** button in the report window first. Note that if you save the flicker state, then the spot lists will be saved in the .flk file state and associated .spt spot list files in the **spt/** directory. Flicker will restore the spot lists if you start flicker on the saved .flk startup file.

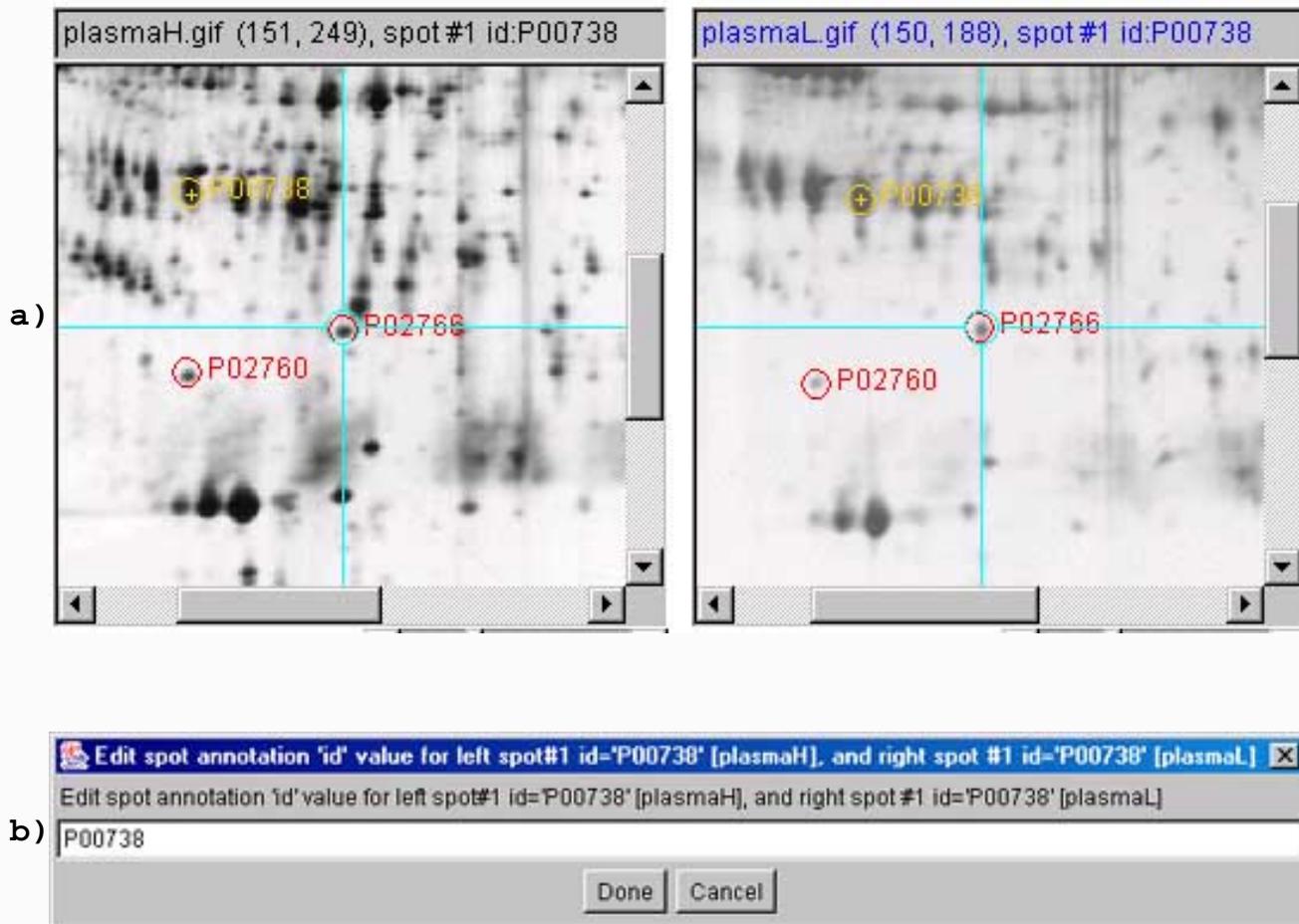
1. Enable the multiple spot list measurements using the (**Quantify** |  **List-of-spots else trial-spot measurement-mode (C-J)**) menu checkbox.
2. Set the measurement circle size for the size spots you want to measure using the **meas circle diameter** scroller. This sets the box around the circle as  $N \times N$  to 1x1, 3x3, 5x5, ..., or 51x51.
3. Select the (**Quantify** | **Measure by circle** | **Capture background (C-B)**) or type (**C-B**) to capture the background value
4. Select the (**Quantify** | **Measure by circle** | **Capture measurement (C-M)**) or type (**C-M**) to capture the spot measurement value. You may use (**ALT-key** click) to both select the spot and add it to the measurement list in one operation.
5. To delete a spot, click on the spot. Then use the (**Quantify** | **Measure by circle** | **Delete selected spot from spot list(C-K)**) command. The next spot you measure will get the next spot measurement number - it does not reuse measurement numbers.
6. To edit a spot's annotation 'id' data, click on the spot. Then use the (**Quantify** | **Measure by circle** | **Edit selected spot(s) 'id' field from spot list(s) (C-I)**). If spots are selected in both images, then you can edit both spots together.
7. To edit all of a spot's data, click on the spot. Then use the (**Quantify** | **Measure by circle** | **Edit selected spot(s) from spot list(s) (C-E)**). If spots are selected in both images, then you can edit both spots together.
8. Repeatedly measure the spots you want using steps [2-7] as required.
9. See the [discussion](#) on the various spot overlay options.
10. You can review the list by first clearing the popup report window and then doing a (**Quantify** | **Measure by circle** | **List spots in the spot list**). You can also view the list as tab-delimited data that you can then either cut and paste into Excel.



**Figure 16. Example of measuring multiple spots.** a) shows the left image window with 4 spots measured. The background spot has a magenta circle and "+B". The 4 spots are numbered "+1", "+2", to "+4". The current cursor position is shown with the red circle with an orange "+" inside of it and the total gray value shown in the image title is the data under the circular mask at this position. b) shows the popup report window with the 4 spot measurements. We "Clear"ed the report to before posting the data to have it contain just the spot data.

### 6.6.6 Examples of annotating spot 'ID's in the spot lists

Flicker allows you to annotate spots in the spot list and to then generate paired-spot tables (tab-delimited) that you can export (to Excel etc.). [Figure 17](#) shows 3 spots that were putatively identified by flickering the right gel against the Swiss-2DPAGE database image and then clicking on each of the corresponding spots to bring up the putative identifications (see [Figure 2](#)). Once the paired spot identifications are assigned,



c)

hGel	1	2
nbr	1	1
id	P00738	P00738
nCirMask	6	6
circleRadius	6	6
xC	150	150
yC	248	188
xB	150	150
yB	0	0
area	113.0	113.0
isCalibFlag	false	false
useTotDensityFlag	true	true
density	17762.0	15355.0
densPrime	17762.0	15355.0
bkgrd	0.0	0.0
mnDens	160.01802	138.33333
totDens	17762.0	15355.0
mnDensPrime	161.01802	139.33333
mnBkgrd	-1.0	-1.0
dMax	232.0	162.0
dMin	40.0	70.0
dMaxBkgrd	-1.0	-1.0
dMinBkgrd	-1.0	-1.0

Done Cancel

**Figure 17. Example of assigning spot annotation 'id's.** a) shows the labeled spot annotations for three spots previously putatively identified (not shown here) using flickering against the SWISS-2DPAGE clickable database (see [Figure 2](#)). The spots were added to the spot lists in each of the two gels using the (**Quantify | Measure by circle | Capture measurement (C-M)**) command on each spot. Then, b) shows the prompt for assigning the paired-spot identifier that is popped up by first selecting the corresponding measured spots in each gel (shown in yellow) and then using the (**Quantify | Measure by circle | Edit selected spot(s) 'id' field from spot list(s) (C-I)**) command. An alternate editing method, c) shows the prompt for editing all fields of the paired-spots that is popped up by first selecting the corresponding measured spots in each gel (shown in yellow) and then using the (**Quantify | Measure by circle | Edit selected spot(s) from spot list(s) (C-E)**) command.

Flicker can then use the annotated spot lists you have generated to create a paired spot table shown below.

a.1)

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	<b>Image</b>	<b>SpotNbr</b>	<b>ID</b>	<b>Density_Mode</b>	<b>Density_meas</b>	<b>Units</b>	<b>MinDensity</b>	<b>MaxDensity</b>	<b>Bkgd</b>	<b>MinBkgd</b>	<b>MaxBkgd</b>	<b>xC</b>	<b>yC</b>
2	plasmaH.gif	1	P00738	Total	17762	gray	40	232	0	0	0	150	248
3	plasmaH.gif	2	P02766	Total	8710	gray	22	207	0	0	0	215	306
4	plasmaH.gif	3	P02760	Total	6003	gray	11	179	0	0	0	149	324
5													
6	<b>Image</b>	<b>SpotNbr</b>	<b>ID</b>	<b>Density_Mode</b>	<b>Density_meas</b>	<b>Units</b>	<b>MinDensity</b>	<b>MaxDensity</b>	<b>Bkgd</b>	<b>MinBkgd</b>	<b>MaxBkgd</b>	<b>xC</b>	<b>yC</b>
7	plasmaL.gif	1	P00738	Total	15355	gray	70	162	0	0	0	150	188
8	plasmaL.gif	2	P02766	Total	5860	gray	21	127	0	0	0	200	242
9	plasmaL.gif	3	P02760	Total	3775	gray	17	89	0	0	0	131	266

a.2)

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	<b>Image</b>	<b>SpotNbr</b>	<b>ID</b>	<b>Density_Mode</b>	<b>Density_meas</b>	<b>Units</b>	<b>MinDensity</b>	<b>MaxDensity</b>	<b>Bkgd</b>	<b>MinBkgd</b>	<b>MaxBkgd</b>	<b>xC</b>	<b>yC</b>
2	plasmaH.gif	1	P00738	Total	17762	gray	40	232	0	0	0	150	248
3	plasmaH.gif	2	P02766	Total	8710	gray	22	207	0	0	0	215	306
4	plasmaH.gif	3	P02760	Total	6003	gray	11	179	0	0	0	149	324
5													
6	<b>Image</b>	<b>SpotNbr</b>	<b>ID</b>	<b>Density_Mode</b>	<b>Density_meas</b>	<b>Units</b>	<b>MinDensity</b>	<b>MaxDensity</b>	<b>Bkgd</b>	<b>MinBkgd</b>	<b>MaxBkgd</b>	<b>xC</b>	<b>yC</b>
7	plasmaL.gif	1	P00738	Total	15355	gray	70	162	0	0	0	150	188
8	plasmaL.gif	2	P02766	Total	5860	gray	21	127	0	0	0	200	242
9	plasmaL.gif	3	P02760	Total	3775	gray	17	89	0	0	0	131	266

b.1)

	A	B	C	D	E	F	G	H	I	J	K	L
1	<b>Image1</b>	<b>Image2</b>	<b>SpotNbr1</b>	<b>SpotNbr2</b>	<b>ID</b>	<b>DensityMode</b>	<b>Units</b>	<b>Dm1</b>	<b>Dm2</b>	<b>(Dm1-Dm2)</b>	<b>(Dm1-Bm1)</b>	<b>(Dm2-Bm2)</b>
2	plasmaH.gif	plasmaL.gif	1	1	P00738	Total	gray	1.641	1.843	0.89	1.641	1.843
3	plasmaH.gif	plasmaL.gif	2	2	P02766	Total	gray	0.805	0.703	1.144	0.805	0.703
4	plasmaH.gif	plasmaL.gif	3	3	P02760	Total	gray	0.555	0.453	1.224	0.555	0.453
5												

b.2)

	L	M	N	O	P	Q	R	S	
1	(Dm2-Bm2)	(Dm1-Bm1)	(Dm2-Bm2)	CircleMask1	CircleMask2	MnDspotList1	MnDspotList2	MnDBspotList1	MnDBspotList2
2	1.843		0.89	6X6	6X6	10825	8330	10825	8330
3	0.703		1.144	6X6	6X6	10825	8330	10825	8330
4	0.453		1.224	6X6	6X6	10825	8330	10825	8330

**Figure 18. Example of paired spot lists exported to Excel.** a.1-a.2) shows data exported to Excel from the above example (Figure 17). The annotated (ids') spot lists were generated by clicking (for each gel) on the image then using the (**Quantify | Measure by circle | List spots in the spot list (tab-delimited)**) command to generate a tab-delimited spot this. This was then cut and pasted into Excel and then reorganized to better show the data for this Web page. Then, b.1-b.2) shows the paired spot table generated using the (**Quantify | Measure by circle | List 'id'-paired annotated mean norm. spots in both spot lists (tab-delimited)**) command. The table was then cut and pasted into Excel and then reorganized to better show the data for this Web page.

### 6.6.7 Examples of typical print-data windows

A spot was first selected by clicking on it. The print window size was set to 20x20 and the data radix to **Decimal** for grayscale values. Then the following output was generated in the popup Report window using the (**Quantify menu | Print data-window | Show data-window of selected pixel (C-V)**) command. Normally, first set the print-window size and data radix then use the (C-V) short-cut key after you have selected the spot you want to view. The following shows typical output for an uncalibrated image where you can see tow spots in the lower left quadrant:

```
Windmp [183:203, 168:188] Center (193,178) 20X20 size,
sampled: 1 pixels, data-radix: Decimal
Image: Images\plasmaH.gif
  X 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203
  Y --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
168  71  61  57  55  54  54  56  57  56  50  46  42  42  43  45  42  43  48  53  67  80
169  71  53  50  48  45  48  50  55  60  56  46  42  40  43  42  40  37  41  47  54  65
170  88  49  44  42  40  42  45  53  56  61  50  41  40  39  39  37  34  37  38  45  54
171  97  49  41  39  39  40  43  53  60  61  54  43  39  38  37  36  34  36  37  43  50
172 106  47  39  37  36  37  39  53  72  78  72  50  43  38  37  35  33  34  35  39  44
173  84  41  38  37  34  33  35  50  82  94  84  58  48  41  40  37  34  35  35  39  44
174  66  39  38  37  35  33  35  50  84  96  84  59  48  41  40  39  35  37  37  39  44
175  47  41  43  40  36  34  34  49  78  84  71  56  50  43  44  43  38  37  37  47  46
176  50  46  46  46  39  34  35  56  91  95  77  61  58  54  57  54  47  43  41  43  47
177  53  49  51  50  42  37  38  69 117 122  98  74  66  62  65  62  51  47  44  45  50
178  58  59  65  65  53  43  46 106 171 174 139  94  78  71  67  65  57  54  50  48  54
179  63  77  97  97  82  59  55 104 163 164 120  76  64  58  62  62  61  58  55  52  56
180  67  90 122 124 106  72  60  86 132 132  95  64  59  55  64  62  61  59  57  53  57
181  76 140 189 193 174 128  94  59  70  68  60  55  55  54  59  63  60  60  58  55  58
182  82 167 214 215 195 137  97  50  47  49  52  53  53  53  53  56  55  57  59  57  61
183  73 142 194 194 160  97  67  42  44  46  51  52  52  50  53  52  54  57  60  62  63
184  51  62  81  80  60  46  39  38  44  46  51  52  52  51  54  54  66  69  78  80  77
185  48  50  52  52  46  40  39  40  45  48  51  52  51  53  56  57  56  65  70  72  72
186  49  49  50  50  46  41  40  40  42  46  49  51  51  53  55  56  53  62  60  61  61
187  51  55  63  62  56  46  43  40  40  41  45  51  53  57  58  55  50  48  52  49  50
188  70  90  97  90  71  57  52  47  41  40  46  56  61  66  64  60  51  46  48  47  50
```

## Example of a print-data window from an OD calibrated gel

If you have a calibrated image (see (**Quantify menu | Calibrate | ...**)), then you can view the calibrated pixels in an OD radix. The following AML demo leukemia gel was calibrated in optical density. The spot was first selected by clicking on it. The print window size was set to 20x20 and the data radix to **Optical-density** for od values. Then the following output was generated in the popup Report window using the (**Quantify menu | Print data-window | Show data-window of selected pixel (C-V)**) command. Normally, first set the print-window size and data radix then use the (**C-V**) short-cut key after you have selected the spot you want to view. The following shows typical output for an uncalibrated image:

```
Windmp [183:197, 322:336] Center (190,329) 15X15 size,
sampled: 1 pixels, data-radix: Optical-density
Image: Images\HUMAN-AML.ppx
  X    183    184    185    186    187    188    189    190    191    192    193    194    195    196    197
  Y    -----
322  0.022  0.022  0.027  0.033  0.038  0.056  0.066  0.072  0.084  0.056  0.049  0.047  0.033  0.040  0.047
323  0.018  0.022  0.020  0.042  0.058  0.058  0.097  0.139  0.090  0.066  0.053  0.029  0.027  0.029  0.036
324  0.020  0.013  0.024  0.040  0.049  0.051  0.127  0.133  0.072  0.060  0.058  0.038  0.036  0.042  0.033
325  0.018  0.027  0.038  0.038  0.078  0.127  0.151  0.157  0.145  0.072  0.049  0.040  0.042  0.033  0.038
326  0.024  0.022  0.033  0.066  0.139  0.188  0.291  0.304  0.265  0.115  0.056  0.040  0.038  0.031  0.033
327  0.018  0.029  0.047  0.109  0.265  0.401  0.534  0.495  0.337  0.194  0.078  0.031  0.029  0.036  0.029
328  0.038  0.029  0.058  0.182  0.394  0.665  0.766  0.648  0.466  0.252  0.097  0.058  0.033  0.022  0.022
329  0.027  0.031  0.066  0.239  0.495  0.724  0.816  0.674  0.522  0.272  0.121  0.051  0.027  0.024  0.024
330  0.036  0.044  0.127  0.220  0.394  0.557  0.622  0.557  0.372  0.246  0.139  0.040  0.033  0.038  0.027
331  0.027  0.036  0.060  0.145  0.291  0.394  0.394  0.357  0.291  0.176  0.066  0.038  0.031  0.029  0.031
332  0.024  0.033  0.044  0.090  0.133  0.233  0.285  0.246  0.207  0.151  0.090  0.058  0.056  0.042  0.049
333  0.027  0.018  0.042  0.060  0.115  0.170  0.239  0.239  0.246  0.182  0.157  0.188  0.145  0.145  0.182
334  0.018  0.031  0.044  0.051  0.097  0.157  0.252  0.304  0.272  0.291  0.246  0.233  0.291  0.317  0.365
335  0.033  0.031  0.042  0.058  0.151  0.176  0.259  0.311  0.343  0.343  0.324  0.298  0.291  0.350  0.430
336  0.018  0.036  0.056  0.090  0.139  0.252  0.291  0.304  0.357  0.298  0.265  0.246  0.272  0.265  0.317
```

## 6.7 Help menu

These commands are used to invoke popup Web browser documentation on Flicker. The documentation is kept on the Internet at <http://open2dprot.sourceforge.net/Flicker>. Normally, these help commands should pop up a Web browser that directly points to the Web page on the <http://open2dprot.sourceforge.net/Flicker> site. If your browser is not configured correctly, it may not be able to be launched directly from the Flicker program. Instead, just go to the Web site with your Web browser and look up the information there.

- **Flicker Home** - the [open2dprot.sourceforge.net/Flicker](http://open2dprot.sourceforge.net/Flicker) home page
- **Reference Manual**  - this reference manual
- **How-To use controls** 
  - **Keyboard shortcut options** - list of options
  - **Mouse controls** - list of options and using the controls
  - **Parameter sliders** - list of options and using the sliders
  - **Checkbox options** - list of options
  - **Menu command list** - list of menus and their commands
  - **Image transforms operation** - how to use image transforms
  - **Adding your images to DB** - how to add your image data
  - **Updating the program and data** - how to use the update commands

- **Vignettes** - short demos to answer to specific "How do I ...?" questions
- **Sun's JAI license** - the sun.com Java Advanced Imaging (JAI) license
- **Version on Web site** - display the version numbers available on the Web site.
- **About Flicker** - information about the Flicker program
- **Old Flicker applet documentation** ▶
- **Flicker applet home page** - <http://www.lecb.ncifcrf.gov/flicker>
- **EP97 paper** - the 1997 Electrophoresis J. paper describing the old Flicker applet
- **Poster 1 pIe** - poster describing Flicker applet
- **Poster 2 pIe** - poster describing Flicker applet
- **Poster 3 pIe** - poster describing Flicker applet
- **Poster 4 pIe** - poster describing Flicker applet

## 7. Demonstrations

The Flicker program integrates these various procedures to help you try to make putative spot identifications. See the [Quick start examples](#) on the home page for a short list of some of these methods.

As part of the demonstrations, we present a series of as [short vignettes](#) that have answers to specific "How do I do ...?" types of questions. There are several demonstration images that are available when you download and install Flicker. Look in the (**File | Open demo images**) menu. These commands will load pairs of demonstration images.

## 8. Flicker References

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Flicker is a contributed program available at  
[open2dprot.sourceforge.net/Flicker](http://open2dprot.sourceforge.net/Flicker)

[Contact us](#)

Revised: 09/12/2004

Powered by

SourceForge

Logo

## Flicker - (PDF) documents

There are a number of Adobe Acrobat PDF formatted documents and slide shows available for the Flicker project.

If you do not have Adobe Acrobat, you can download it for free from Adobe.  The following lists some of the PDF documents you may download. As others are created, they will be added to this list.

1. [Flicker Overview slide show](#) for use as a printable document (full size), [6 slides/page](#)
2. [Flicker Web site as a PDF document](#) for use as a printable document
3. [Vignettes for Flicker as a PDF document](#) for use as a printable document
4. [Comparing Two-Dimensional Electrophoretic Gels Across The Internet](#), (10/1998).  
Revised version of [Lemkin P](#), *Electrophoresis* **18**(3-4):461-470.

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Flicker is a contributed program available at  
[open2dprot.sourceforge.net/Flicker](http://open2dprot.sourceforge.net/Flicker)

[Contact us](#)

Revised: 09/12/2004

## FlickerJarVersion.txt

---

```
| Note: as of Flicker version 0.58, the Flicker web site was moved |
| from from Open2Dgel to Open2Dprot as part of the migration of |
| Open2Dgel to Open2Dprot. If you have an existing version of |
| Flicker, doing the (File | Update | Flicker program) menu command |
```

```
| will install the new version that points to the new Open2Dprot Web |  
| site at http://open2dprot.sourceforge.net/Flicker |  
-----
```

There are two versions of Flicker available on Open2Dprot at  
<http://open2dprot.sourceforge.net/Flicker>

The first is the installer version that you download from the Web server.  
The second is the possibly latest version that you update by having  
Flicker copy the Flicker.jar file from the Web server using  
the (File | Update | Flicker program) menu command. After you do the  
update, you need to restart Flicker to use it.

Until further notice, Flicker is to be considered Beta-level code. This  
means that there may be some functionality not fully implemented, that  
works incorrectly, or that has changed. New commands and functionality  
are in the process of being added. Please report problems and suggestions  
to us.

1. Full download installation Flicker version 0.83.6

Revised: 09-12-2004

2. Update Flicker program (Flicker.jar) version 0.83.6

Revised: 09-12-2004

## Flicker Revision History

This describes the revision history. Generally, only the most recent versions are kept on the Web site (see [Version](#)).

- **V0.83.6 09-12-2004:** Added optional Web access to PIR [UniProt](#), [iProClass](#) and [iProLink](#) Web pages through their Swiss-Prot accession names. This is accomplished in a 2-step process. This is enabled in the (**Edit | Select access to active DB server |  ...**) checkbox command to select either Swiss-2DPAGE, UniProt, iProClass or iProLink servers. If you measure a spot (select a spot in an active image and then type **C-M**) (and are connected to the Internet), it will lookup the Swiss-Prot protein (accession name, and protein id) on the Swiss-2DPAGE server. Then, if you enable "Click to access DB", it will pop up the particular active DB server you have selected. An additional option is to enable the (**Edit |  Auto measure, protein lookup and Web page popup**) checkbox command. Then when you click on a spot in an active image (associated with a Web database), it will: 1. measure the spot and add it to the spot list; 2. lookup the Swiss-Prot (name, id); and 3. pop up the Web server on the currently selected active DB server.
- **V0.83.5 08-25-2004:** Fixed but where active image name status was not being set properly. Improved documentation in Reference Manual [section 1.4.1](#) and added a [Vignette on assigning putative spot ids](#).
- **V0.83.4 07-21-2004:** If you saved the Flicker state in a .flk file having zoomed one or both images, it does now rezooms the image(s) when you restart Flicker on that startup file.
- **V.0.83.3 7-03-2004:** Fixed remaining problems with zoom overlays (see 0.83.1).
- **V.0.83.2 6-21-2004:** Fixed another MacOS-X path bug on startup, and added Quickstart and other Help menu entries.

- **V.0.83.1 6-17-2004:** Fixed problem with truncation of zoom function by complete rewrite of Zoom methods and ImageScroller. There may still be a few problems with overlays when zoomed greater or less than 1.0X. These problems may sometimes include: landmarks, measurement in lower quadrant, ROI in lower quadrant, edit measurement spots.
- **V.0.82.4 6-10-2004:** Fixed MacOS-X path bug on startup. and added additional Help menu entries.
- **V.0.82.4 5-25-2004:** Changed Help menu documentation link.
- **V.0.82.1 5-01-2004:** Fixed non-fatal bug that occurred sometimes when restarting Flicker by clicking on a .flk file. It would incorrectly report that you had run out of memory. However, it was incorrect since clicking OK would let Flicker startup with the correct data.
- **V.0.81.5 4-21-2004:** Also fixed problems with image zoom and dezoom.
- **V.0.81.4 4-21-2004:** Fixed bug in naming of calibration files so it now generates for the demo images, "cal/{demo-images}.cal" file names; and for sub image directories in Images/{sub-dirs}/(images) it generates "cal/{sub-dirs}-DIR-{images}.cal" files.
- **V.0.81.3 3-24-2004:** Final fix for bug where Flicker would crash on startup if you started it with an empty user directory in the Images/ directory. We thought this was fixed on V0.81.2 but it wasn't :-). [Thanks to Neil Kitteringham for helping find this bug.]
- **V.0.81.2 3-23-2004:** Fixed bug where Flicker would crash on starting if you had too many subdirectories of images in the user's Images/ directory.
- **V.0.81 3-19-2004:** Misc bug fixes. The color TIFF image reader is now working for un-banded TIFF images (including uncompressed, JPEG compressed and packed bits - but not for LempleZev compression because Sun's JAI TIFF reader avoids the copyright issue). It does not work with banded images at this time. If you want to read a banded or LempleZev compressed image, then convert it using a program such as ThumbsPlus or other image manipulation program.
- **V.0.80 3-18-2004:** Added command (see [discussion Section 4.6.2](#)) to allow groups to setup a Flicker compatible database and then import the data into the Flicker "open demo images" menu. The new (**File | Updates | Add user's Flicker Demo Images DB by URL**) command adds additional demo database from a another Web server to the local Flicker demo database [suggested by Mark Holme]. Also fixed problem where images were sometimes clipped if you read a larger image than the default image or there was a incorrect region at the bottom of the new image if it were smaller than the previous image.
- **V.0.79 3-17-2004:** We reorganized and simplified some of the View menu commands. First, we moved and renamed the (**View | Set view overlay | View measurements | ...**) menu tree to (**View | Set view measurement options | ...**). Second, for consistency, the (**View | Set view overlay | ...**) command was renamed (**View | Set view overlay options | ...**). ">
- **V.0.78 3-16-2004:** Changed state scrollers (upper right) so that they are inoperable unless you select (click on the left or right image) and it alerts you to this. Also disabled menu commands that require you to

select the right or left image until you do so. Added default save files for the "Save overlay image" and "Save transformed image". Cleaned up the design so it is now easier and more consistent to switch between list-of-spots measurement mode and trial-measurement mode using the new (**Quantify | List-of-spots else trial-spot measurement-mode (C-J)**) command. Removed the old commands which were equivalent but were confusing (**Quantify | Set view overlay | View measurements | View list-of-spots else trial-spot measurement-mode (C-J)**) and (**Quantify | Measurement counters**). The visualization of zoomed (dezoomed) spots, ROI, landmarks are now generated correctly. Note: you must currently define these objects in 1.0X unzoomed mode.

- **V.0.77 3-15-2004:** Fixed bug when clearing spot list(s) on data from spot list(s) loaded with a .flk file. It was not deleting the spot list and resetting the spot counter.
- **V.0.76 3-14-2004:** Changed the way the circle mask size may be defined. We now only allow it to be set from the scroller "meas circle: XXX (diameter)" - the checkboxes previously used are no longer available. The maximum size is now 51x51 which should make it easier to measure larger spots. If you attempt to delete a spot using the (**C-K**) command, it will ask you if you are sure since there is no "undo" command.
  - 1. NOTE: we changed the old (C-I) short-cut command to (C-K).** The (**C-I**) is *NOW* used to bring up a short edit form to define or change the spot annotation 'id' fields for the measured spot(s) selected in the image(s). The same id value you enter is assigned to both spots if you are editing two spots (selected from the left and right images). The full command is (**Quantify | Measure by circle | Edit selected spot(s) 'id' fields from spot list(s) (C-I)**). We fixed some bugs where the circle size shown was different between the circles in a list of measured spots and in the non-list spot measurement. The data was the same.
  - 2. NOTE:** we find it useful to adjust the circle size around a spot, by temporarily turning off the (**View | Set view overlays | View measurements | View list-of-spots else trial-spot measurement mode (C-J)**) while leaving the (**CView | Set view overlays | View measurements | View measurement circle**) on. Then click on a spot and type (**C-M**) to get a trial spot measurement. As you adjust the "meas circle" size diameter scroller, it will redraw the circle around the spot as well as display the new integrated density in the image title (if (**C-G**) was enabled).
  - 3. NOTE:** the (**View | Set view overlays | View measurements | View list of spot measurements**) has been moved to the Quantify menu and renamed (**Quantify | View list-of-spots else trial-spot measurement mode (C-J)**) and given a (**C-J**) short-cut key to make toggling between modes easier. Fixed problem of trying to save an unsaved spot list on exit if you had never defined the .flk startup file (it now prompts you for a .flk file name). Fixed the problem where the scroller states for the images was not setup in the correct order and was being overloaded with the default state.
- **V.0.75 3-10-2004:** Fixed fixed several bugs: 1) problem where loading spot lists takes a long time when restarting from .flk file, 2) sorted spot list data for calibrate OD by spot list, various error msgs, added un-norm paired spot list table command (in addition to norm paired spot list table), 3) synchronized circle mask size menu items with scrollable circle mask size slider.
- **V.0.74 3-9-2004:** Fixed bug where it prompted whether you wanted to clear the spot list if you started Flicker by clicking on a .flk file which had spot lists. Changed the Alert window color to a less obtrusive pink-gray instead of red. Added (**Quantify | Measure by Circle | List paired 'id' annotated-spots in both spot lists (tab-delimited)**) - list paired spots that occur in the spot lists of both gels. The pairing is defined by assigning the same annotation spot 'id' using the (**C-I**) spot editing command to assign the case-sensitive spot identifiers. (**C-E**) command may also be used to edit the ids'.
- **V.0.73 3-8-2004:** Additional cleanup and fixed bug where screen size grew when longerer messages were

introduced. Alpha-level implementation of TIFF images (24-bit RGB).

- **V.0.72 3-5-2004:** This version is the alpha-level release for the the new calibration by measured spot list. This uses the (**Quantify | Calibrate | Optical density by spot list**). Also added an alternative way to measure spots - in addition to using the (**C-M**) *after* you have selected the spot using the mouse, you can alternatively hold the **ALT-key** when you press the mouse to select and measure the spot. This is easier to use when measuring many spots. It combines spot selection and measurement in one operation. [Thanks to Enrico DeToni and Greg Thornwall]
- **V.0.71 3-4-2004:** Changed the file name convention for calibration files. Previous to this version, the "cal/{imageFile}.cal" files were not unique if the same file name appeared in multiple user image file directories using the "Images/{user directories}/{image files}". This problem has been fixed by prepending the directory name with a "-DIR-" separator before the cal file so the new .cal file name is "cal/{user image subdirectory}-DIR-{image file}.cal".
- **V.0.70 3-3-2004:** Fixed bug with false popup alert if did not find Flicker.properties file the first time it was run. Also fixed some of the bugs with the new spot list calibration (not enabled yet). Working on new .cal file naming fct so the .cal files will be unique.
- **V.0.69 3-1-2004:** Fixed calibration ND wedge wizard problems with aborting a calibration by answering NO on close. Fixed OD values inherited from paired gel that was previously calibrated. This saves having to type it in again.
- **V.0.68 2-26-2004:** Fixed "Optical-density" radix format for the (**Quantify menu | Print data-window | Show data-window of selected pixel (C-V)**) command. Fixed format for precision of floating point data to exactly the number of digits in the fraction required. Added new command (**File | Open user images | ...**) so split it into two menu trees. The first, (**File | Open user images | pairs of images | ...**) is the same as the previous functionality. The second, (**File | Open user images | single images | ...**) lets you load any single image from any of the user's image directories into either the selected left or right image. Fixed peak table update and display problem in ND wedge calibration plot and table.
- **V.0.67 2-22-2004:** Fixed bug in (**Quantify | Calibrate | Optical density by step wedge**) command. Added (**Quantify | Calibrate| Clear spot list (ask first)**) command. Updated the Reference manual with examples on spot list overlay views and the (**Quantify | Calibrate | Optical density by step wedge**) command. Fixed bug to cancel save .flk file on exit if you were prompted with Yes/No/Cancel on saving the edited spot list(s).
- **V.0.66 2-21-2004:** Fixed various minor bugs. Changed the spot list display paradigm so that the user has more control over the spot labeling that is useful for crowded regions. You can do this via the (**View | Set view overlays | Set view measurements (submenu)**). These let you customize the displays. The submenu commands include:
  - **View measurement circle** for (C-B and (C-M) trial spots
  - **View list of spot measurement** for viewing spot lists
  - **Use 'Circle' for spot locations** OR
  - **Use '+ for spot locations** OR neither.

- Use 'spot number' for spot annotations AND/OR
- Use 'spot identifier for spot annotations OR neither.

You can select the "current" measured spot (click the mouse on it) and the spot labels and annotation will change to the trial object color while you have selected it. Two new commands were added to make it easier to edit and delete spots in the spot list. We added a string "spot identifier". You can set this identifier by selecting the spot and then using the (**Quantify | Measure by circle | Edit selected spot from spot list (C-E)**) command. Similarly you can delete any spot by selecting it and then using the (**Quantify | Measure by circle | Delete selected spot from spot list (C-I)**) command. [Thanks to suggestions by Enrico De Toni].

We added a new scroller "measure circle size" to the parameter scrollers. This is equivalent to going into the (**Quantify | Measure by circle | Circular mask size (pixels) | ...**) and selecting a size. However it is more convenient and gives you better visual feedback.[Thanks to suggestions by Enrico De Toni].

### Calibration of grayscale in other units (e.g., optical density)

We have activated the grayscale calibration using an neutral density (or other known standard) step-wedge scanned with the material (e.g., 2D-gel) when it was scanned. You must know the calibration values (e.g., OD or CPM, etc.) corresponding to the step wedge. You set the computing window Region Of Interest (ROI) over the step wedge and then use the (**Quantify | Calibrate | Optical density by step wedge**) command. To demonstrate this process, we have built in four sample ROI's and the corresponding OD values corresponding to the step wedge scanned with the four demo gels (Leukemia-AML, Leukemia-ALL, Leukemia-CLL, Leukemia-HCL). To use this demo, first set the (**Quantify | Calibrate | Use demo leukemia gels ND wedge calibration preloads**) first. Then, use the (**Quantify | Calibrate | Optical density by step wedge**) command. We will be adding discussion and documentation on calibration in the reference manual and we plan to add vignettes.

- **V.0.65 2-16-2004:** Fixed various minor bugs and refactored code. Reset the scroller and trial spot position to the upper left hand corner when load a new image. Previously it was using the same coordinates at the previous image or illegal coordinates [Bug reported by Enrico De Toni].
- **V.0.63 02-11-2004:** Added grayscale histogram for the computing window region of interest using the (**Quantify | Region Of Interest (ROI) | Show RPI grayscale histogram (C-H)**) command. Changed error paradigm so that it is easier to see error messages. Error messages are shown in red. If the Popup Report window is not visible, it is forced to be visible to call attention to the error message. Reorganized the [View menu](#) so it is more logical. Added (**Quantify | View measurements | Use 'circle'# else '+'# for meas. list**) - if both  **View spot measurements** and the  **View measurement circle** are enabled, this will show the actual circle masks (of different sizes if you changed the sizes) around each measured spot. Otherwise, it shows it as a '+' marker. Fixed various other minor bugs.
- **V.0.62 02-02-2004:** Added commands to print a 5x5 through 40x40 gray-scale window (in decimal, octal, or hex number radix) in the popup report window. The (**Quantify menu | Print data-window | Set print-window size**) lets you specify the window size. The (**Quantify menu | Print data-window | Set print-data radix**) set the data format radix. The (**Quantify menu | Print data-window | Show data-window of selected pixel (C-V)**). Normally, set the print-window size and data radix then use the (C-V) short-cut key after you have selected the spot you want to view. The following shows typical output:

```
Windmp [183:203, 168:188] Center (193,178) 20X20 size,
```

sampled: 1 pixels, data-radix: Decimal

Image: Images\plasmaH.gif

X	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203
168	71	61	57	55	54	54	56	57	56	50	46	42	42	43	45	42	43	48	53	67	80
169	71	53	50	48	45	48	50	55	60	56	46	42	40	43	42	40	37	41	47	54	65
170	88	49	44	42	40	42	45	53	56	61	50	41	40	39	39	37	34	37	38	45	54
171	97	49	41	39	39	40	43	53	60	61	54	43	39	38	37	36	34	36	37	43	50
172	106	47	39	37	36	37	39	53	72	78	72	50	43	38	37	35	33	34	35	39	44
173	84	41	38	37	34	33	35	50	82	94	84	58	48	41	40	37	34	35	35	39	44
174	66	39	38	37	35	33	35	50	84	96	84	59	48	41	40	39	35	37	37	39	44
175	47	41	43	40	36	34	34	49	78	84	71	56	50	43	44	43	38	37	37	47	46
176	50	46	46	46	39	34	35	56	91	95	77	61	58	54	57	54	47	43	41	43	47
177	53	49	51	50	42	37	38	69	117	122	98	74	66	62	65	62	51	47	44	45	50
178	58	59	65	65	53	43	46	106	171	174	139	94	78	71	67	65	57	54	50	48	54
179	63	77	97	97	82	59	55	104	163	164	120	76	64	58	62	62	61	58	55	52	56
180	67	90	122	124	106	72	60	86	132	132	95	64	59	55	64	62	61	59	57	53	57
181	76	140	189	193	174	128	94	59	70	68	60	55	55	54	59	63	60	60	58	55	58
182	82	167	214	215	195	137	97	50	47	49	52	53	53	53	53	56	55	57	59	57	61
183	73	142	194	194	160	97	67	42	44	46	51	52	52	50	53	52	54	57	60	62	63
184	51	62	81	80	60	46	39	38	44	46	51	52	52	51	54	54	66	69	78	80	77
185	48	50	52	52	46	40	39	40	45	48	51	52	51	53	56	57	56	65	70	72	72
186	49	49	50	50	46	41	40	40	42	46	49	51	51	53	55	56	53	62	60	61	61
187	51	55	63	62	56	46	43	40	40	41	45	51	53	57	58	55	50	48	52	49	50
188	70	90	97	90	71	57	52	47	41	40	46	56	61	66	64	60	51	46	48	47	50

- V.0.61 01-31-2004:** Fixed problem that if you started Flicker from a previously saved .flk state file by double clicking on it's icon, it did not completely restore the state. However, if you restored the state using (**File menu | Open state file**) it did it correctly. This fixed the problem that measured spot lists were not restored with the former, but were with the latter [reported by Enrico De Toni]. Also fixed the graphics problem where spot positions of "+#" markers in measured circles were correctly measured, but the position was off a few pixels.
- V.0.60 01-28-2003:** Fixed bug that if you saved a Flicker state in a .flk file that pointed to some images that you subsequently moved or deleted on your disk, Flicker would hang and you would need to kill the process (e.g., Task manager in Windows). This has been fixed by reporting the problem and then exiting flicker. It is up to the user to either restore the image to the previous location, or to not use the .flk file. [Reported by Enrico De Toni]. Also fixed bug in (**Quantify menu | Measure by circle | List spots in the spot list (tab-delimited)**) where the table header was not aligned with the data. [Reported by Enrico De Toni].
- V.0.59, 01-24-2004:** Fixed bug where data could not be added to the circle measurement list. The problem occurs if you defined a set of circle measurements, then saved the Flicker state (File | Save(As) State file), and then restored this state in a later session. The original spots were there, but you could not add additional spots. Note, you can currently measure up to 1000 spots. [Thanks to Enrico De Toni for reporting this bug.]
- V.0.58, 01-19-2004:** Moved Flicker from the Open2Dgel project to the new Open2Dprot.
- V.0.57, 01-13-2004:** Fixed bug when accessing Swiss-2DPAGE database where it sometimes does not generate the correct URL for clickable spots.
- V.0.56, 01-07-2004:** Fixed bug in the image pipeline processing where if you zoom first and then do an image transform, it did not zoom the transform.

- **V.0.55, 12-27-2003:** Fixed bug in the image pipeline processing where if you zoom first and then use the brightness/contrast filter, it did not use the zoomed image. Now you can use both zoom and brightness contrast and any other transform and it will do a (iImg > oImg > zImg > bgImg) pipeline processing. Of course, doing both zoom and brightness/contrast filtering will take longer. Doing a Reset images will clear the zoom and BC filtering.
- **V.0.54, 12-22-2003:** Fixed the recent images database that updates DB/FlkRecentDB.txt save and restore state. It limits the history to the last 10 images. The (**File | Open recent images**) lists the images. It records single images from the (**File | Open file image**), (**File | Open URL image**). It also records pairs of images from (**File | Open URL user images**) and when you load a pair of images using the (**File | State | Open state file**).
- **V.0.53, 12-19-2003:** Added (**View | Gang zoom images**) command to zoom the left and right images together. This is useful if the images have exactly the same magnification. Added an alternate way of listing the measured spots list as (**Quantify | Measure by circle | List spots in the spot list (tab-delimited)**). This lets you cut and paste it or read the SaveAs text file from the popup report window into Excel or other software. (Thanks to Stephen Lockett for suggestions). Also fixed problem where it would jitter if you tried to flicker the images after doing a (**File | Reset images**).
- **V.0.52, 12-17-2003:** fixed drawing error with numbered spots where is was off a few pixels when it drew "+1", "+2", "+3", etc.
- **V.0.51, 12-13-2003:** Moved the (**File | Update | ...**) server from the mirror (<http://www.lecb.ncifcrf.gov/Flicker>) to the SourceForge server <http://open2dprot.sourceforge.net>.
- **V.0.50, 12-12-2003:** Fixed bug where the "Flicker.parameters" file was generated correctly using the (**File | Quit**) command, but not if you close the window from the GUI. Also, added a command (**Edit | Reset default view**) to set the view to the initial value without reading your "Flicker.parameters" file. This lets you override your saved parameter default.
- **V.0.49, 12-12-2003:** Added additional user settable parameters to the state that is saved with (**File | Save(As) state file**). Also save a subset of these parameters that are generic user preferences in a "Flicker.properties" when you Quit the program. When you next start Flicker, it will attempt to read and use these user preferences. For example, you may want to use a red trial object cursor instead of yellow, etc. Fixed bug so can now save and restore correctly circular-mask measured spot lists you generated a series of circular mask spot measurements and saved with the (**File | State | Save(As)...**) command.
- **V.0.48, 12-11-2003:** Added (**File | SaveAs overlay image**) menu command to save the selected image including overlays as a GIF image you specify. If you are displaying the gray value as you move the mouse (**View | Display gray values (C-G)**) and the size of the circle mask is > 1, then draw the circle mask as you move the mouse so the user can see the area being measured. Added "tot" or "mn" prefix to this displayed data values depending on whether the (**Quantify |  Use sum density else mean density**). Added (**Quantify | Measure by circle | Delete selected spot from the spot list**) so you can edit the list. Changed the default trial object color from yellow to orange which is a little easier to see. Changed the default circular mask size from 5x5 to 11x11. Changed the default measurement circular mask color from yellow to red.

- **V.0.46, 12-10-2003:** Move the user's image discovery menus from (**File | Open demo images | ...**) to (**File | Open user images | ...**) since they are not really part of the demo images. Added ability to save measured circular mask spots in a spot list that is saved (when you do a (File | State | SaveAs ...)) in the "spt/" directory as a file with the same name as the image but with a ".spt" file extension. You can now sequentially label a series of circular mask spot measurements and it labels them with "+1", "+2", etc. You can also make a report of all spots. They will be saved when you save the state and are restored when you restore the state. You can now adjust the trial object color through the (Edit | Set colors | Trial object color).
- **V.0.45, 12-09-2003:** Added extension to demo images. If the user had put one or more directories of the image data in the Images/ folder. Then when Flicker starts, it will create additional submenu entries in the (**File | Open demo images | ...**) that are the names of the user's directories. Within each submenu, it will generate all combinations of the image files within the corresponding directory and denote them as for example, "image3 vs. image5", etc. The maximum measurement circle size has been increased from 11x11 to 15x15. A new measurement option, (**Quantify |  Use measurement counter**) has been added to let you track your measurements in the report log more easily.
- **V.0.44, 12-01-2003:** Only has the zoom/dezoom method transform. It is ONLY invoked from the ZoomMag scroller - not from the Transform menu.
- **V.0.43, 11-28-2003:** New zoom/dezoom transform method that covers 1/10X to 10X zoom. Fixed the Median filter. Added "Max 3x3" and "Min 3x3" filters. This version has both AWT zoom and transform zoom.
- **V.0.42, 11-26-2003:** Added ((**File | Save Transformed image**) and ((**Edit | Enable saving transformed images when do a 'Save(As) state'**)) to save transformed images to a "tmp/" directory as .gif files.
- **V.0.41, 11-24-2003:** Fixed bug introduced in version 0.37 when clicked on canvas, it failed under some conditions. Fixed the SaveAs state so that it saves more of the state and also restore the landmarks and position properly. Added log(ayscale) option to use when reading grayscale images more than 8-bits (e.g., 10-bit, 12-bit or 16-bit tiff grayscale images).
- **V.0.37, 11-17-2003:** Added Resize canvas menu and code to resize the three windows up or down using either menu commands or the "-" "/" "+" canvas resize buttons. The menu commands are (**Edit | Canvas size | in(de)crease size**). Added (**Edit | Resize Flicker memory limits**) to let the user change the initial startup memory limits over the range of 30Mb to 1768 Mb.
- **V.0.36, 11-14-2003:** Fixed subtle error in ROI rectangle drawing routine. Added code to let you define the ROI as between the C-U and C-L marks and it will flip their definitions if required.
- **Version 0.35, 11-14-2003:** fixed minor bugs, added (C-R) to (Quantify | Region of interest (ROI) Capture measurement by ROI (C-R)) command
- **Version 0.35, 11-13-2003:** fixed ROI display and measurement, misc. minor bugs.
- **Version 0.34, 11-12-2003:** fixed misc. minor bugs.
- **Version 0.33, 11-11-2003:** fixed misc. minor bugs.
- **Version 0.32, 11-10-2003:** Implemented the (File | Update | Update Demo Images DB)
- **Version 0.31, 11-10-2003:** Refactored affine warping and fixed various other commands including Quantify Measure by circles and added Measure by ROI. Added C-T command and a few other commands. Fixed thresholding automatic transforms when move sliders on the selected image. Avoid race

condition if adjusting threshold and angle sliders by ignoring command if already doing a transform. Other misc. fixes.

- **Version 0.30, 11-07-2003:** Affine working.
- **Version 0.29, 11-03-2003:** Addressed misc. minor bugs
- **Version 0.28, 10-31-2003:** This is the initial alpha-level release. It is functional however with a few problems which are in the process of being addressed

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[Contact us](#)

Flicker is a contributed program available at  
[open2dprot.sourceforge.net/Flicker](http://open2dprot.sourceforge.net/Flicker)

Revised: 9/12/2004

## Flicker Program Status

The new version of Flicker which now runs as a stand-alone Java application is beta-level code. The following are known bugs, suggested features, functions that are being debugged or being developed, and resolved bugs.

- [Revision history](#)
- [Known bugs](#)
- [Suggested Features/FAQ](#)
- [Resolved bugs](#)

### Known Bugs

- **03-20-2004:** There is no Flicker.lax file for the MacOS-X, Linux and Solaris systems. So the (**Edit | Resize Flicker memory limits**) command does not work on these systems at this time - it does on Windows.
- **02-23-2004:** The NTSC (R,G,B) image to grayscale option needs to be debugged. This will allow users to do quantification on the color image after it has been converted to grayscale. Otherwise, it uses the blue channel which of course biases the results.
- **02-23-2004:** Problem with Flicker not finding an image when you restart it with a previously saved .flk file if you have moved the image files to a different directory. This is because the .flk file saves the path and by moving the files, you have changed the path. Flicker has no way of knowing that you changed the path. You can either specify the files again, or edit the .flk file.
- **02-23-2004:** Problem where the Help menu entries that are supposed to pop up a Web browser - don't. This is because the browser is not configured correctly with the users operating system. The solution is to just go to the Flicker Web site through a separately launched browser. All of the links in Help are available through the Web site.

- **12-17-2003:** Allow gang-control of brightness/contrast range for both left and right images. This is useful if they were taken under the same conditions and have about the same initial contrast/brightness [Suggested by S. Lockett].
- **12-17-2003:** Need to zoom the positions of landmarks, and measured spots and circular mask in the new image scroller.
- **11-10-2003:** the (**Transform | Poly Warp**) menu command is not available. It needs to be debugged.
- **11-09-2003:** The (**Transform | Pseudo3D**) command does not remap the landmarks correctly after doing the pseudo 3D transform.

## Suggested Features / FAQ

- **03-16-2004:** Note on screen size. Flicker will work with a 1024x768 size screen. However, we find it **MUCH** easier to use with a larger screen (e.g. 1280x1024 works nicely). Because we are using AWT, it is difficult to optimize wasted whitespace in the main window. Until this is resolved, we suggest trying to run Flicker on a computer with higher resolution.
- **03-16-2004:** Gang scrolling only works if you use the **Control-click** method for scrolling the images. It does not currently work if you use the scrollbars. Normally, you would align the two images to the same spot using flickering, then enable gang scrolling. Then use the **Control-click** to scroll both images together.
- **12-17-2003:** Extend the brightness/contrast range [Suggested by S. Lockett].
- **12-17-2003:** Add option so that when do a (**File | Open user images**) command that presents images from a user directory, you can either generate pairs of serially comparable images (e.g., an ordered set of images from an optical or E.M. serial sectioning) or all pairs of images (the current default) [Suggested by S. Lockett].
- **12-17-2003:** Sometimes it does not restore the spot list that you have saved with a (**File | SaveAs state file**) and restored with a (**File | Open state file**) command.
- **12-10-2003:** The way we are implementing the image scroller does not allow you to center spots you select (Control-Press) with the mouse that are close to the edge of the image. Currently these spots will not be centered since there is no image beyond the edge. We will be resolving this by adding a white space guard region around the edge of the image.
- **12-09-2003:** (GT) Images in all three canvases are getting cropped off at the bottom and right sides. When one compares the original image with the one displayed in Flicker (scroll to the bottom right) part of the image is missing. Changing the pagestep parameter in ImageScroller.java to 1 from 15 seems to help but there is still 5 pixels width missing. (JC) Related to this is that if you shrink the canvas size, it seems to readily cut off what is scrollable.
- **10-31-2003:** The (**File | Open image file**) and (**File | Open image URL**) menu commands are not available for

color TIFF images at this time. It does work with grayscale TIFF images. Note that images with more than 8-bits of grayscale are scaled down to 8-bits losing the least significant bits.

- **11-10-2003:** Fix blackIsZeroFlag EVERYWHERE... need to have XxxLoaders set it.
- **11-10-2003:** If do full quantification of 16-bit TIFF images, rethink keeping or re-reading 16-bit TIFF image data as (char iPix[]) if we are going to do quantification on that type of image without losing grayscale resolution.
- **11-10-2003:** Check and debug ImageXform.remapLMS() for Pseudo3D and PolyWarp transforms.
- **11-10-2003:** Check data structures, implementation, and debug the boundary drawing code for (**Quantify | Measure by boundary**) methods.
- **11-10-2003:** Check and debug measurements made on the Zoom image for magnifications different than 1X. It currently performs the measurements as if it were 1X.
- **11-10-2003:** Add vertical and horizontal 1D gaussian-like line filters for use with other types of data. E.g, here is an example of a filter to look for horizontal lines:

```

0 0 0 0 0 0 0 0 0 0 0 0
1 1 1 1 1 1 1 1 1 1 1 1
2 2 2 2 2 2 2 2 2 2 2 2
1 1 1 1 1 1 1 1 1 1 1 1
0 0 0 0 0 0 0 0 0 0 0 0

```

- **11-10-2003:** Get some better edge enhancement filters.
- **11-10-2003:** Add interactive rubber-band ROI window definition GUI.

## Resolved Bugs

- **V.0.83.4 07-21-2004:** If you saved the Flicker state in a .flk file having zoomed one or both images, it does now rezooms the image(s) when you restart Flicker on that startup file.
- **V.0.78 03-16-2004:** When using a zoom factor different from 1.0X, measured spots positions are not generated using (C-B) and (C-M) commands. However, if the spots were generated at 1.0X, they do zoom correctly.
- **V.0.68 02-23-2004:** The paradigm was changed in the (**File | Open user images | ...**) so that it handles both "pairs of images" and "single images". This makes make it easier for a user to load say a standard active map gel (i.e. Swiss-2DPAGE map) in the left image, and then individual user gels into the right image for comparison.
- **V.0.68 11-10-2003:** Added PopupODcalib() to popup a spreadsheet of values of (Step, OD value, Gray Value) editing window. This includes a histogram and OD vs gray Peak table of the step wedge pixels values inside of

the Step Wedge ROI. You can mouse over and capture the peaks to edit the values.

- **V.0.68 11-10-2003:** Saved an image calibration in a separate .cal file. This avoids having to recompute the calibration if we change the images. When you load an image, it can check for the corresponding .cal file and then load it and indicate that it exists.
- **V.0.66 2-14-2004:** Modify spot circle overlays options so that it is easier to edit spots close together. Options being investigated include: a) showing only the last current spot; b) showing "+" for all spots except for the current spot; c) other options. [Suggested by Enrico De Toni]
- **V.0.61 01-31-2004:** Fixed problem that if you started Flicker from a previously saved .flk state file by double clicking on it's icon, it did not completely restore the state. However, if you restored the state using (**File menu | Open state file**) it did it correctly. This fixed the problem that measured spot lists were not restored with the former, but were with the latter [reported by Enrico De Toni].
- **V.0.60 12-09-2003:** (JC) Fixed a problem that the image is cropped when scrolling is that if you shrink the canvas size, it seems to cut off what should be scrollable on the right and bottom edges.
- **V.0.60 01-26-2004:** Fixed bug that if you saved a Flicker state in a .flk file that pointed to some images that you subsequently moved or deleted on your disk, Flicker would hang and you would need to kill the process (e.g., Task manager in Windows). This has been fixed by reporting the problem and then exiting flicker. It is up to the user to either restore the image to the previous location, or to not use the .flk file. [Reported by Enrico De Toni].
- **V.0.56, 01-07-2004:** Fixed bug in the image pipeline processing where if you zoom first and then do an image transform, it did not zoom the transform.
- **V.0.55, 12-27-2003:** Fixed bug in the image pipeline where if you zoom first and then use the brightness/contrast filter, it did not use the zoomed image. Now you can use both zoom and brightness contrast and any other transform and it will do a (iImg > oImg > zImg > bgImg) pipeline processing. Of course, doing both zoom and brightness/contrast filtering will take longer. Doing a Reset images will clear the zoom and BC filtering.
- **V.0.54, 12-22-2003:** Fixed the recent images database that updates DB/FlkRecentDB.txt save and restore state. It limits the history to the last 10 images. The (**File | Open recent images**) lists the images. It records single images from the (**File | Open file image**), (**File | Open URL image**). It also records pairs of images from (**File | Open URL user images**) and when you load a pair of images using the (**File | State | Open state file**).
- **V.0.53 12-18-2003:** Fixed problem where it would jitter if you tried to flicker the images after doing a (**File | Reset images**).
- **V.0.49 12-12-2003:** Fixed bug so can now save and restore correctly circular-mask measured spot lists you generated a series of circular mask spot measurements and saved with the (**File | Save(As) state file**) command.
- **V.0.43, 11-29-2003:** Implemented fractional zoom using image sampling (no bin-linear averaging at this time) and changed the scrollbar so the center is 1X, left is fractional to 1/10X and the right is magnification to 10X.
- **V.0.41, 11-24-2003:** Fixed bug introduced in version 0.37 when clicked on canvas, it failed under some

conditions. Fixed the SaveAs state so that it saves more of the state and also restore the landmarks and position properly. When using the ZeroG installer, you need to enable Java applets and Java in order to use the default installer. The other other is to download the installer and save the file. Then run it on your computer.

- **V.0.41, 11-24-2003:** Added log(grayscale) option to use when reading grayscale images more than 8-bits (e.g., 10-bit, 12-bit or 16-bit tiff grayscale images). This maps it in the TiffLoader.
- **V.0.37, 11-17-2003:** Added Resize canvas menu and code to resize the three windows up or down using either menu commands or the "-" "/" "+" canvas resize buttons.
- **V.0.36, 11-14-2003:** Fixed subtle error in ROI rectangle drawing routine. Added code to let you define the ROI as between the C-U and C-L marks and it will flip their definitions if required.
- **V.0.35, 11-13-2003:** Fixed ROI rectangle drawing routine in ImageScroller where the ROI rectangle is now drawn correctly if move the image scroller in and out of where the rectangle should be. Also fixed bug in copying the ROI to the computing window.
- **V.0.33, 11-11-2003:** Implemented the (**File | Update demo images DB**) command that downloads the latest demo images and DB/FlkDemoDB.txt.
- **V.0.32, 11-11-2003:** fixed bug where the (**Quantify | Measure by circle | ...**) and (**Quantify | Background by circle | ...**) menu commands generated negative background corrected data. Fixed use of blackIsZero.
- **V.0.31, 11-09-2003:** the (**Transform | Affine Warp**) menu command was fixed. It also correctly remaps the displayed landmarks in the output image (oImg) where the transform resides.

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Revised: 07/21/2004

## Flicker Vignettes - Short Examples

Vignettes are short examples that pose and answer questions of the form "how do I do xxxx?". We list the vignettes below. Each vignette describes the problem using demonstration data, the method suggested to solve the problem, any additional setups required, and links of related vignettes that may be referenced.

### List of Vignettes



1. [Vignette](#): How do I get on-line help?
2. [Vignette](#): How do I select an image window? window?
3. [Vignette](#): How do I position a gel image within the left or right scrollable window?
4. [Vignette](#): How do I flicker compare two images?
5. [Vignette](#): How do I query a spot's putative identity?

6. [Vignette](#): How do I assign a spot's putative identity?
7. [Vignette](#): How do I load an active map image from the Web?
8. [Vignette](#): How do I load an image from the Web or from my disk?
9. [Vignette](#): How do I compare my own gel images?
10. [Vignette](#): How do I add my own image directories to Flicker?
11. [Vignette](#): How do I reset the state for the currently loaded gel images?
12. [Vignette](#): How do I reset the standard images view?
13. [Vignette](#): How do I change the colors of various overlays?
14. [Vignette](#): How do I view spot lists with various overlay options?
15. [Vignette](#): How do I change the canvas size?
16. [Vignette](#): How do I change other parameters?
17. [Vignette](#): How do I change the image overlays preferences?
18. [Vignette](#): How do I change the magnification (i.e. zoom) of a gel image?
19. [Vignette](#): How do I improve visualization using image transforms?
20. [Vignette](#): How do I warp one image to the geometry of the other?
21. [Vignette](#): How do I define landmarks used in image warping?
22. [Vignette](#): How do I display a spot's intensity?
23. [Vignette](#): How do I measure background intensity near a spot?
24. [Vignette](#): How do I measure spot intensity?
25. [Vignette](#): How do I mark and measure intensity for multiple spots?
26. [Vignette](#): How do I review a list of all measured spots?
27. [Vignette](#): How do I set the circular mask used in measuring spot intensity?
28. [Vignette](#): How do I set and measure a Region Of Interest (ROI)?
29. [Vignette](#): How do I save the state of Flicker so I can continue at a later time?
30. [Vignette](#): How do I restart Flicker on a previously saved session so you can continue?
31. [Vignette](#): How do I save transformed or overlay images?
32. [Vignette](#): How do I save text in the popup report window?
33. [Vignette](#): How do I view a small window of numeric pixel data in a table?
34. [Vignette](#): How do I show a gray scale history in a region of interest (ROI)?
35. [Vignette](#): How do I calibrate grayscale using a ND calibration step wedge?
36. [Vignette](#): How do I calibrate grayscale using a spot list of known calibrations?

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[open2dgel.sourceforge.net/Flicker](http://open2dgel.sourceforge.net/Flicker)

Revised: 08/26/2004

## Flicker Vignette - How do I on-line help?

This vignette shows how do you can get on-line help from the Flicker Web site.

### Method

The reference manual and vignette examples etc. are available on the <http://open2dprot.sourceforge.net/Flicker> Flickr Web site.

1. You can look at the reference manual on the [Web site](#).
2. If you are running Flicker, you can access the reference manual and other parts of the Web site from the [Help menu](#).
3. Look at the list of [Vignettes](#) in the reference manual.

## Related vignettes

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Flicker is a contributed program available at [open2dgel.sourceforge.net/Flicker](http://open2dgel.sourceforge.net/Flicker)

Revised: 01/19/2004

# Flicker Vignette - How do I select an image from the left or right images?

This vignette shows how do you select either the left or right images. In order to do many operations, you must first select an image to operate on.

## Method

1. Select the image you want to position by clicking on it. The title of the window will change from black to blue indicating it is selected.
2. To deselect an image so there are no images selected, then click on the Flicker window outside of either the right or left image. Both titles will now appear in black indicating neither is selected.

## Setup for the demo

You need to have two images loaded in Flicker. The default when you start Flicker is to load the two human plasma gel images.

## Related vignettes

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Revised: 01/19/2004

# Flicker Vignette - How do I position gel images in the

# scrollable windows?

This vignette shows how do you position gel images in the left and right scrollable windows so the spot appears in the center of the cross-hairs.

## Method

### Positioning window and trial object by Control-key/Mouse-Press

1. Select the image you want to position by clicking on it. The title of the window will change from black to blue indicating it is selected.
2. Press the **CONTROL**-key and then click on the spot you want to position in the cross-hairs. This will scroll the image window to the position.
3. This **DOES** position the *trial object* used when you flicker.

### Positioning image window by image window scrollers

1. Select the image you want to position by clicking on it. The title of the window will change from black to blue indicating it is selected.
2. Move the image window horizontal and/or vertical position scrollers to change the position you want to view.
3. This **DOES NOT** position the *trial object* used when you flicker.

### Positioning the trial-object by dragging the mouse

1. Select the image you want to position by clicking on it. The title of the window will change from black to blue indicating it is selected.
2. Press or drag the mouse to the spot you want to position in the flicker window. It will not change the position in the selected window.y
3. This **DOES** position the *trial object* used when you flicker.

## Setup for the demo

You need to have two images loaded in Flicker. The default when you start Flicker is to load the two human plasma gel images.

## Related vignettes

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[open2dprot.sourceforge.net/Flicker](http://open2dprot.sourceforge.net/Flicker)

Revised: 01/19/2004

# Flicker Vignette - How do I compare two images?

This vignette shows how do you can compare two images.

## Method

1. Load the two images you want to compare (see vignettes to load gel images if you want different images).
2. Position the left or right image to the spot you are interested in the scrollable image window (see vignette for positioning gel images).
3. Then position the other image so it is positioned at the spot you think corresponds or the region roughly corresponds
4. Enable flickering by enabling the  **Flicker (C-F)**, typing (C-F), or selecting (**View** |  **Flicker images (C-F)**)
5. Fine-tune the position of the left and right images as required.
6. When the two images are aligned, the spots that are optimally aligned may appear to pulse.
7. When you are done flickering, you can disable flickering by the same controls in step [4].

## Setup for the demo

You need to have two images loaded in Flicker. The default when you start Flicker is to load the two human plasma gel images.

## Related vignettes

- [Vignette](#) for loading gel images
- [Vignette](#) for positioning gel images in the left and right scrollable windows

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Revised: 01/19/2004

# Flicker Vignette - How do I query a spot's putative identity?

This vignette shows how do you can query a spot's putative identity.

## Method

1. Load the two images you want to compare (see vignettes to load gel images if you want different images). One of the gels must be an active clickable map linked to a Web server. You could use, for example, (**File** | **Open demo images** | **Human Plasma** | **(Swiss-2DPAGE vs. Merril) gels clickable**).
2. Alternative, you can explicitly load one of the over 30 active map gel images in the left or right image. Select the left or right image. Then select the active map image using the (**File** | **Open active map image** | ...) command.

3. Flicker align the gels in the region you are interested in.
4. Enable the  **Click to access DB** checkbox. IF flickering was enabled, it will disable flickering.
5. Then select the image with the red "+"s on spots that are in the active Web database.
6. Click on a red "+" for the spot you are interested in. This will popup a Web browser that links to the spot you have selected in the associated Web database. Each time you click on a spot, it will post the new data in the popup browser window.

## Setup for the demo

## Related vignettes

- [Vignette](#) for loading gel images
- [Vignette](#) for loading active map gel images
- [Vignette](#) for positioning gel images in the left and right scrollable windows
- [Vignette](#) for flicker aligning two gels

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Revised: 01/19/2004

# Flicker Vignette - How do I load an active map image from the Web?

This vignette shows how do you can load an active map image from the Web.

## Method

1. Select the image you want to replace with an active map image
2. Select the active map image with (**File | Open active map image | ...**) command. This will replace the selected image with the Web image. It also enables your access (in step [3]) to the  **Click to access DB** checkbox.
3. Enable the  **Click to access DB** checkbox when you want to access the active Web site.

## Setup for the demo

## Related vignettes

- [Vignette](#) for querying a spot's putative ID

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[open2dprot.sourceforge.net/Flicker](http://open2dprot.sourceforge.net/Flicker)

Revised: 01/19/2004

# Flicker Vignette - How do I load gel images from my disk or from the Web?

This vignette shows how do you can load a gel image into the selected left or right image window. Individual gel images may reside on your local disk, on the the weg. You can also load pre-defined pairs of gel images into the left and right image windows from the demo gels provided with Flicker or for the user's own gels. The user's gel images are in directories that they should copy to the installation **Image/** directory.

## Method

### Loading an image into the selected image window

1. Select the left or right image window by clicking on it.
2. Load the image from your file system by the (**File | Open image file**), or
3. Load the image from the Internet by the (**File | Open image URL**), or
4. Load the image from the Internet by the (**File | Open active map image**)

### Loading a pair of images into both left and right image windows

1. Load the image from your file system by the (**File | Open demo images | ...**) submenu to select the image pair you want to use, or
2. Load the image from your file system by the (**File | Open user images | ...**) submenu to select the image pair you want to use

## Related vignettes

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Revised: 01/19/2004

# Flicker Vignette - How do I compare my own images?

This vignette shows how you can compare your own gel images using Flicker.

## Method

1. Flicker is able to handle GIF, JPEG or B&W TIFF formated images. If your images are in another format, then you must convert them one of these formats.
2. First you must copy the directories of your images to the **Images/** directory where you installed Flicker.

For a MS Windows system, it is generally located at

C:\Program Files\Flicker\Images\

3. Then restart Flicker. This will read your image file directories to discover what you are making available.
4. Go to the (**File | Open user images | ...**) to access pairs of your images. Note that the images appear as pairs of images as described in the [Reference Manual Section 4.5](#). Select the pair of images you wish to analyze.
5. After Flicker has loaded the images, proceed to compare them using the method described in the [Vignette](#) for comparing gel images.

## Setup for the demo

Flicker is currently able to handle GIF, JPEG or B&W TIFF formatted images. If your images are in another format, then you could convert them using Photoshop, ThumbsPlus ([www.cerious.com](http://www.cerious.com)) or a similar tool to one of these formats.

## Related vignettes

- [Vignette](#) for comparing gel images

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Revised: 01/19/2004

# Flicker Vignette - How do I reset the state for current images?

This vignette shows how do you can reset the state for the currently loaded gel images.

## Method

1. Select the (**File | Reset images**) command.
2. This will remove the transformed and brightness/contrast images and any landmarks you may have defined for both the left and right image. It will not change your original preferences.

## Setup for the demo

## Related vignettes

- [Vignette](#) for resetting the view and color preferences.

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Revised: 01/19/2004

# Flicker Vignette - How do I change the colors of various overlays?

This vignette shows how do you can change the colors of the various overlays including target, trial object, landmarks and measured spots.

## Method

1. You can change the colors for the target, trial object, landmarks and measured spots using the (**Edit | Set colors | *object-type* | *specific colors***)
2. You can reset the colors and view overlay options using the (**Edit | Reset default view**)

## Setup for the demo

## Related vignettes

- [Vignette](#) for resetting the current gel images
- [Vignette](#) for saving the Flicker state in a .flk file

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Revised: 01/19/2004

# Flicker Vignette - How do I set the image window canvas size?

This vignette shows how do you can set the image window canvas size. When you change the canvas size, it changes all three left, right and flicker windows to the same size. It will also resize the main window.

## Method

1. You can reset the colors, view overlay options, and canvas size using the (**Edit | Reset default view**)
2. You can change the canvas size for the left, right and flicker windows and measured spots using the (**Edit | Canvas size | Increase size (C-Numpad '+')**) and (**Edit | Canvas size | Decrease size (C-Numpad '-')**) commands.

## Setup for the demo

## Related vignettes

- [Vignette](#) for resetting the current gel images
- [Vignette](#) for saving the Flicker state in a .flk file

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Revised: 01/19/2004

# Flicker Vignette - How do I change parameters?

This vignette shows how do you can change parameters. You can reset and change various parameters.

## Method

1. You can reset the colors, view overlay options, and canvas size using the (**Edit | Reset default view**)
2. You can change the colors for the target, trial object, landmarks and measured spots using the (**Edit | Set colors | *object-type* | *specific colors***)
3. You can change the canvas size for the left, right and flicker window and measured spots using the (**Edit | Canvas size | Increase size (C-Numpad '+')**)and (**Edit | Canvas size | Decrease size (C-Numpad '-')**) commands.
4. You can change the transform parameters including the zoom using the slider bars in the upper right part of the main window.
5. You can change the flicker delay rate using the [Delay slider bars](#) below the left and right images.

## Setup for the demo

## Related vignettes

- [Vignette](#) for resetting the current gel images
- [Vignette](#) for changing the overlay colors
- [Vignette](#) for changing the overlay views
- [Vignette](#) for changing the canvas size
- [Vignette](#) for changing the magnification of the left or right image
- [Vignette](#) for saving the Flicker state in a .flk file

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Revised: 01/19/2004

# Flicker Vignette - How do I change the image overlays preferences?

This vignette shows how do you can change the image overlays preferences.

## Method

1. You can reset the colors, view overlay options, and canvas size using the (**Edit | Reset default view**)
2. You can change the overlay for the left, right and flicker windows and measured spots using the (**View | Set view measurement options | ...**) options.

## Setup for the demo

## Related vignettes

- [Vignette](#) for resetting the current gel images
- [Vignette](#) for saving the Flicker state in a .flk file

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Revised: 01/19/2004

# Flicker Vignette - How do I change the image magnification (i.e. zoom)?

This vignette shows how do you can change the magnification (i.e. zoom) of a gel image. Using the [zoom mag slider](#) you can magnify the selected image from 1X to 10X or demagnify it from 1X to 1/10X..

## Method

1. Select the image you want to zoom.
2. To magnify the image, move the zoom scroller to the right.
3. To demagnify the image, move the zoom scroller to the left.
4. To magnify the other image, select it and then do steps [2-3].

## Setup for the demo

## Related vignettes

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# Flicker Vignette - How do I use image processing transforms?

This vignette shows how do you can improve the visualization using [image processing transforms](#).

## Method

### Using the Transform menu commands (C-T)

1. Select the image you want to position by clicking on it. The title of the window will change from black to blue indicating it is selected.
2. Make sure that you have  **Allow transforms** checkbox enabled.
3. Make sure that you have  **Sequential transforms** checkbox disabled.
4. Perform the transform you want to try in the (**Transform** | ...) submenu. You might try "SharpenGradient", "Average" or some of the other transforms.
5. Now, enable sequential transforms by setting the  **Sequential transforms** checkbox to enabled.
6. Then perform the set of sequential transforms you want to try as in step [4].
7. Now turn off the  **Sequential transforms** checkbox and you can no longer compute the sequential transform.

### Using the repeated Transform menu command

1. Select the image you want to position by clicking on it. The title of the window will change from black to blue indicating it is selected.
2. Make sure that you have  **Allow transforms** checkbox enabled.
3. Make sure that you have  **Sequential transforms** checkbox enabled.
4. Perform particular transform you want to repeat in the (**Transform** | ...) submenu. You might try "SharpenGradient", "Average" or some of the other transforms.
5. Then use the (**Transform** | **Repeat last transform (C-T)**) command or type **C-T** repeatedly to perform that operation repeatedly as the sequential transform.

## Setup for the demo

## Related vignettes

- [Vignette](#): to change parameters used in the transforms

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[open2dprot.sourceforge.net/Flicker](http://open2dprot.sourceforge.net/Flicker)

Revised: 01/19/2004

# Flicker Vignette - How do I warp a gel image's geometry?

This vignette shows how do you can warp one image to the geometry of the other to make them easier to flicker compare. There are two warping transforms - affine that requires 3 landmarks and 6 for the polywarp. (The polywarp is not available yet- [FUTURE]). You first define the required number of N pairs of corresponding landmarks between the triangular region you are interested in warping. Then select the gel you wish to warp. You then apply the warp transform from the Transform menu. See the [example](#) in the Reference Manual.

## Method

### Defining the N landmarks

1. Select the left or right gel image window by clicking on it. Then select one of the N landmarks you want to use by clicking on it.
2. Select the other gel image window by clicking on it. Then select the corresponding landmark. You may have to Flicker the gels to ensure they are in fact the correct spots.
3. Add the landmark to the landmark database by using the (**Landmark | Add landmark (C-A)**) or type **C-A**.
4. If you do not like the landmark, you can delete the landmark from the landmark database by using the (**Landmark | Delete landmark (C-D)**) or type **C-D**. You only need to do this step if you are not happy with the landmark you just created.
5. Repeats steps [1] through [4] until you have the N landmarks.

### Warping the image

1. Select the left or right gel image window you want to warp.
2. Make sure that you have  **Allow transforms** checkbox enabled.
3. Make sure that you have  **Sequential transforms** checkbox disabled.
4. Perform the transform you want to try in the (**Transform | Affine warp**) or (**Transform | Poly warp**) command.
5. Reposition the transformed image and the other image to the spot of interest inside of the landmark region.
6. Enable flickering. It should be now easier to see the corresponding spots.

## Setup for the demo

## Related vignettes

- [Vignette](#) for positioning gel images in the left and right scrollable windows  
See the [example](#) in the Reference Manual.

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Revised: 01/19/2004

## Flicker Vignette - How do I define landmarks used in image warping?

This vignette shows how do you can define landmarks used in image warping. See the [warping vignette](#) for more information on warping.

### Method

1. Select the left or right gel image window by clicking on it. Then select one of the landmarks you want to use by clicking on it.
2. Select the other gel image window by clicking on it. Then select the corresponding landmark. You may have to Flicker the gels to ensure they are in fact the correct spots.
3. Add the landmark to the landmark database by using the (**Landmark | Add landmark (C-A)**) or type **C-A**.
4. If you do not like the last landmark you just added, you can delete the landmark from the landmark database by using the (**Landmark | Delete landmark (C-D)**) or type **C-D**. You only need to do this step if you are not happy with the landmark you just created.
5. Repeats steps [1] through [4] until you have the number of landmarks you want.
6. You can get an idea of the distortion between the two gels by having Flicker compute the least square error between the landmark sets. Use the (**Landmark | Show landmarks similarity**) command.

### Setup for the demo

### Related vignettes

- [Vignette](#) for warping gel images
- [Vignette](#) for positioning gel images in the left and right scrollable windows

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Revised: 01/19/2004

## Flicker Vignette - How do I display a spot's intensity?

This vignette shows how do you can display a spot's intensity in the title bar of the left or right window. The default is to just show the (x,y) coordinates (raster coordinate system with (0,0) in the upper left hand corner).

The density measurement is taken under the circular mask. So that needs to be set accordingly.

## Method

1. Enable displaying the grayscale using the (**View** |  **Display gray values (C-G)**).
2. This will then change the title display for the selected image to show for example, plasmaH.gif (105, 45) 469 tot gray-value  
or  
plasmaH.gif (177, 84) 53 mn gray-value
3. To show the total integrated gray-value, (**Quantify** |  **Use sum density else mean density**) is enabled.
4. To show the mean integrated gray-value, (**Quantify** |  **Use sum density else mean density**) is disabled.
5. To show the circular mask, make sure the two options (**View** | **Set view measurement options** |  **View measurement circle**). Make sure the (**Quantify** |  **List-of-spots else trial-spot measurement-mode (C-J)**) is set to list-of-spots mode. This area is the area of pixel integration used in computing the integrated density measurement.
6. To change the new circular mask size, use the **measure circle (diameter)** slider. Note: the  $N \times N$  is one of 1x1, 3x3, 5x5, ..., 51x51 options which is the size of the rectangle enclosing the circular mask.
7. As you move the mouse in the selected image, it will display the data in the image at that position.

## Setup for the demo

## Related vignettes

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Revised: 01/19/2004

# Flicker Vignette - How do I measure background intensity near a spot?

This vignette shows how do you can measure background intensity near a spot. The background estimate is computed under the circular mask. A separate background estimate is defined for the left and right images. Note the background measurement is used for computing background corrected density for measurements made by circular mask (**C-M**) and by region of interest (**C-R**). It will be used for multiple measurements until you change it. If you are measuring a series of spots by circular mask, then if you change the background intensity measurement, it will be associated with all spots measured since then and saved in the spot list until (or if) you change it again.

## Method

1. To show the circular mask, make sure (**View** | **Set view measurement options** |  **View measurement circle**) is enabled.

2. Select the new circular mask size in the **measure circle (diameter)** slider. Note: the  $N \times N$  is one of 1x1, 3x3, 5x5, ..., 51x51 options which is the size of the rectangle enclosing the circular mask.
3. Select the spot you want to use to estimate the background by clicking on it in either the left or right image.
4. Capture the background estimate using the (**Quantify | Measure by circle | Capture background (C-B)**).

## Setup for the demo

## Related vignettes

- [Vignette](#) for measuring circular mask intensity
- [Vignette](#) for measuring ROI intensity

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Revised: 01/19/2004

# Flicker Vignette - How do I measure spot intensity?

This vignette shows how do you can measure spot intensity under the circular mask. The background corrected measurement estimate is computed under the circular mask. Note the separate background measurement (**C-B**) is used for computing background corrected density for measurements made by circular mask (**C-M**). The background estimate will be used for multiple measurements until you change it. If you are measuring a series of spots by circular mask, then if you change the background intensity measurement, it will be associated with all spots measured since then and saved in the spot list until (or if) you change it again. You can number the spots if the you have enabled the (**Quantify |  List-of-spots else trial-spot measurement-mode (C-J)**) option should be enabled. You may also list the saved spots in the report window. Note that if you save the flicker state, then the spot list will be saved in the .flk file state and an associated .spt spot list file in the **spt/** directory. You can restore the spot list if you start flicker on the saved .flk startup file.

## Method

1. To measure the total integrated gray-value, (**Quantify |  Use sum density else mean density**) is enabled.
2. To measure the mean integrated gray-value, (**Quantify |  Use sum density else mean density**) is disabled.
3. To show the circular mask, set (**View | |Set view measurement options |  View measurement circle**) to enabled.
4. Select the new circular mask size in the **measure circle (diameter)** slider. Note: the  $N \times N$  is one of 1x1, 3x3, 5x5, ..., 51x51 options which is the size of the rectangle enclosing the circular mask.
5. To sequentially number and capture a series of spots, enable list-of-spots mode by using the (**Quantify |  List-of-spots else trial-spot measurement-mode (C-J)**) checkbox. Then it will number a series of (**C-M**) spot measurements as as "+1", "+2", "+3", etc. overlays. Otherwise, the measurement will be

- numbered as a "+M" overlay and data is not saved in the spot list.
6. If you want to do background correct, capture the background estimate using the (**Quantify | Measure by circle | Capture background (C-B)**). This is valid for multiple (**C-M**) measurements.
  7. Select the spot you want to use to estimate the background by clicking on it in either the left or right image.
  8. Capture the background corrected estimate using the (**Quantify | Measure by circle | Capture measurement (C-M)**).
  9. To measure a set of spots, just repeat subset of these steps for each new spot.
  10. To list the set of saved spots in the popup report window, use the (**Quantify | Measure by circle | List spots in the spot list**). Hint: clear the popup report window first. You can save this list from the popup report window using its **SaveAs** button.
  11. To export a spot list to Excel, after clearing the report window, use the (**Quantify | Measure by circle | List spots in the spot list (tab-delimited)**). Then cut and paste the data into Excel.

## Setup for the demo

## Related vignettes

- [Vignette](#) for measuring background intensity
- [Vignette](#) for setting the circular mask

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[open2dprot.sourceforge.net/Flicker](http://open2dprot.sourceforge.net/Flicker)

Revised: 01/19/2004

# Flicker Vignette - How do I generate a list of all measured spots?

This vignette shows how do you can mark spots and generate a list of all measured intensity values for these spots. It is similar to the vignette for [measuring spot intensity](#) for a single spot.

## Method

1. Enable the multiple spot list measurements using the (**Quantify |  List-of-spots else trial-spot measurement-mode (C-J)**) menu checkbox.
2. Set the measurement circle size for the size spots you want to measure using the **meas circle diameter** scroller. This sets the box around the circle as  $N \times N$  to  $1 \times 1$ ,  $3 \times 3$ ,  $5 \times 5$ , ..., or  $51 \times 51$ .
3. Select the (**Quantify | Measure by circle | Capture background (C-B)**) or type (**C-B**) to capture the background value
4. Select the (**Quantify | Measure by circle | Capture measurement (C-M)**) or type (**C-M**) to capture the spot measurement value. You may use (**ALT-key** click) to both select the spot and add it to the measurement list in one operation.
5. To delete a spot, click on the spot. Then use the (**Quantify | Measure by circle | Delete selected spot**)

- from spot list(C-K))** command. The next spot you measure will get the next spot measurement number - it does not reuse measurement numbers.
6. To edit a spot's annotation 'id' data, click on the spot. Then use the (**Quantify | Measure by circle | Edit selected spot(s) 'id' field from spot list(s) (C-I)**). If spots are selected in both images, then you can edit both spots together.
  7. To edit all of a spot's data, click on the spot. Then use the (**Quantify | Measure by circle | Edit selected spot(s) from spot list(s) (C-E)**). If spots are selected in both images, then you can edit both spots together.
  8. Repeatedly measure the spots you want using steps [2-7] as required.
  9. See the [discussion](#) on the various spot overlay options.
  10. You can review the list by first clearing the popup report window and then doing a (**Quantify | Measure by circle | List spots in the spot list**). You can also view the list as tab-delimited data that you can then either cut and paste into Excel.
  11. See the [discussion](#) on the various spot overlay options.
  12. You can review the list by first clearing the popup report window and then doing a (**Quantify | Measure by circle | List spots in the spot list**). You can also view the list as tab-delimited data that you can then either cut and paste into Excel.

## Setup for the demo

## Related vignettes

- [Vignette](#) for measuring background intensity
- [Vignette](#) for measuring spot intensity under circular mask
- [Vignette](#) for setting the circular mask

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## Method

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2. Set the measurement circle size for the size spots you want to measure using the **meas circle diameter** scroller. This sets the box around the circle as  $N \times N$  to  $1 \times 1$ ,  $3 \times 3$ ,  $5 \times 5$ , ..., or  $51 \times 51$ .
3. Select the (**Quantify | Measure by circle | Capture background (C-B)**) or type (**C-B**) to capture the

background value

4. Select the (**Quantify | Measure by circle | Capture measurement (C-M)**) or type (**C-M**) to capture the spot measurement value. You may use (**ALT-key click**) to both select the spot and add it to the measurement list in one operation.
5. To delete a spot, click on the spot. Then use the (**Quantify | Measure by circle | Delete selected spot from spot list(C-K)**) command. The next spot you measure will get the next spot measurement number - it does not reuse measurement numbers.
6. To edit a spot's annotation 'id' data, click on the spot. Then use the (**Quantify | Measure by circle | Edit selected spot(s) 'id' field from spot list(s) (C-I)**). If spots are selected in both images, then you can edit both spots together.
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8. Repeatedly measure the spots you want using steps [2-7] as required.
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## Setup for the demo

## Related vignettes

- [Vignette](#) for measuring background intensity
- [Vignette](#) for measuring spot intensity under circular mask
- [Vignette](#) for setting the circular mask

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Revised: 01/19/2004

# Flicker Vignette - How do I set the circular mask size?

This vignette shows how do you can set the circular mask size. The circular mask is used in measuring spot intensity.

## Method

1. Select the new circular mask size in the **measure circle (diameter)** slider. Note: the  $N \times N$  is one of 1x1, 3x3, 5x5, ..., 51x51 options which is the size of the rectangle enclosing the circular mask.

2. To show the circular mask in the left and right images, make sure (**View | Set view measurement options |  View measurement circle**) option is enabled.
3. You can view the size of the actual circle by turning on the trial-spot mode by disabling the (**Quantify |  List-of-spots else trial-spot measurement-mode (C-J)**) checkbox.
4. Then click on a spot and type (**C-M**) to show the circle.
5. Adjust the circle until the spot fits just inside it.

## Setup for the demo

## Related vignettes

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# Flicker Vignette - How do I set and measure a Region Of Interest (ROI)?

This vignette shows how do you can set and measure a Region Of Interest (ROI). If you have defined the circular mask background density estimate, using **C-B**, then it will be used to do the background correction for the ROI measurement by subtracting the ROI area\*MeanBackgroundDensity.

## Method

## Setup for the demo

1. Select the (**Quantify | Measure by circle | Capture background (C-B)**) or type **C-B** to capture the background value
2. Select the (**Quantify | Region Of Interest (ROI) | Set ROI ULHC (C-U)**) or type **C-U** to define the upper left hand corner.
3. Select the (**Quantify | Region Of Interest (ROI) | Set ROI LRHC (C-L)**) or type **C-L** to define the lower right hand corner.
4. Select the (**Quantify | Region Of Interest (ROI) | Capture measurement by ROI (C-R)**) or type **C-R** to capture the background corrected density within the ROI.

## Related vignettes

- [Vignette](#) for measuring background intensity
  - [Vignette](#) for setting the circular mask
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## Flicker Vignette - How do I save the state of Flicker?

This vignette shows how do you can save the state of Flicker so you can continue your data-mining session at a later time. The state is saved in a .flk file in the **FlkStartups/** subdirectory in the Flicker installation directory. You can later restart Flicker to return to this state by either clicking on the .flk file which starts Flicker on this data, or using the (**File | Open state file**) command which requests the name of the .flk state file. In addition, every time you exit Flicker, it automatically saves the current user preferences in the **Flicker.properties** file. This file is read each time Flicker is restarted. If you are starting Flicker with a .flk startup file, it will override these user preferences and use the values in the .flk file.

### Method

1. Select the (**File | SaveAs state file**) command
2. Specify a .flk file (e.g., "MyData.flk").
3. When you are done, exit Flicker.
4. You may restore when the state when Flicker is run in the future either by clicking on the "MyData.flk" or by specifying the file in (**File | Open state file**) command.

### Setup for the demo

### Related vignettes

- [Vignette](#): restarting Flickering using the .flk startup file

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## Flicker Vignette - How do I restart Flicker on a saved state?

This vignette shows how do you can restart Flicker on a previously saved session so you can continue your data mining session. The state should have been saved in a .flk file in the **FlkStartups/** subdirectory in the Flicker installation directory using the (**File | Save(As) state file**) commands.

### Method

1. Save the state of a Flicker session in a named file using the (**File | Save(As) state file**) command. It will

ask you for a file name (e.g., "MyData.flk").

2. You may restore when the state when Flicker is run in the future either by clicking on the "MyData.flk" or by specifying the file in (**File | Open state file**) command.

## Setup for the demo

## Related vignettes

- [Vignette](#): saving the Flickering state in a .flk startup file

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# Flicker Vignette - How do I save transformed or overlay images?

This vignette shows how do you can save transformed or overlay images.

## Method

### Save the transform image

1. Select the (**File | Save transformed image**) command. This saves the transformed image in **tmp/** as a .gif with the same name of the input image.

### Save the overlay image

1. Select the (**File | SaveAs overlay image**) command to save the overlay image as a .gif image. You must specify the .gif image file name.

## Setup for the demo

## Related vignettes

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# Flicker Vignette - How do I save text in the popup report

# window?

This vignette shows how do you can save text in the popup report window.

## Method

1. Press the **SaveAs** button. This will request a file for you to save it as. The default file is **tmp/FlickerReport.txt**.

## Setup for the demo

1. If the popup report is not visible, select (**View | Popup scrollable report**) to pop up the window.

## Related vignettes

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