

Flicker Comparison of 2D Electrophoretic Gels

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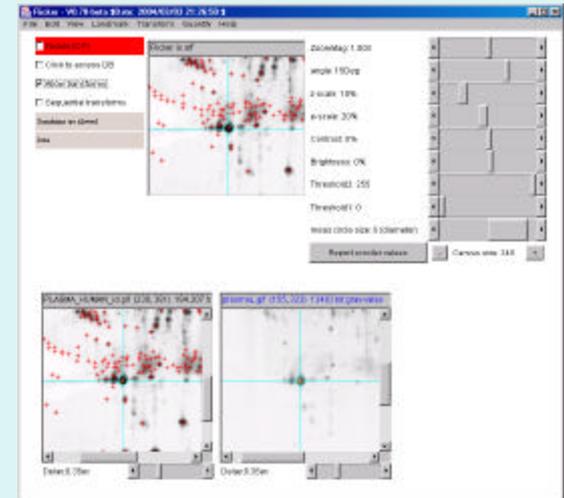
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<http://open2dprot.sourceforge.net/Flicker>

Revised: 09-12-2004



Overview

- Flicker is an open-source stand-alone Java computer program for visually comparing 2D gel electrophoresis images
- 2D polyacrylamide gel electrophoresis (2D-PAGE) gels are often difficult to compare because of rubber-sheet distortions
- Flicker allows you to compare your gel images against each other or against those found in Internet databases
- Many published Internet gels have subsets of spots identified which may make them useful to compare with your gels.

Main Features of Flicker

- Flicker allows comparison of two gels at a time
- Menu system helps organize and access a set of local user gels and access Internet reference database gels
- Built-in demonstration gels with calibration data
- Built-in access to Swiss-2DPAGE active map reference gel database. Easily extendible to other federated databases
- Image enhancement optimizes images - helps support visual comparison: zoom, brightness/contrast, spatial-warping, smoothing, sharpening
- Build lists of spot measurements for estimating spot quantification and annotation

Main Features (continued)

- Calibrate gray scale if OD, CPM, etc. standards are available
- Export measured spot lists and annotated paired spot lists (to Excel, etc.)
- Save/restore data-mining sessions
- Written in Java as open source and is freely available
- Runs on MS Windows, MacOS-X, Linux, Solaris
- Documentation available as HTML on the Web site or as PDFs
- Tutorial vignettes available on using Flickr

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Open2Dprot

open2dprot.sourceforge.net

Powered by

Flicker Comparison of 2D Electrophoretic Gels

Flicker Comparison of 2-Dimensional Electrophoretic Gels

Welcome To Flicker

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

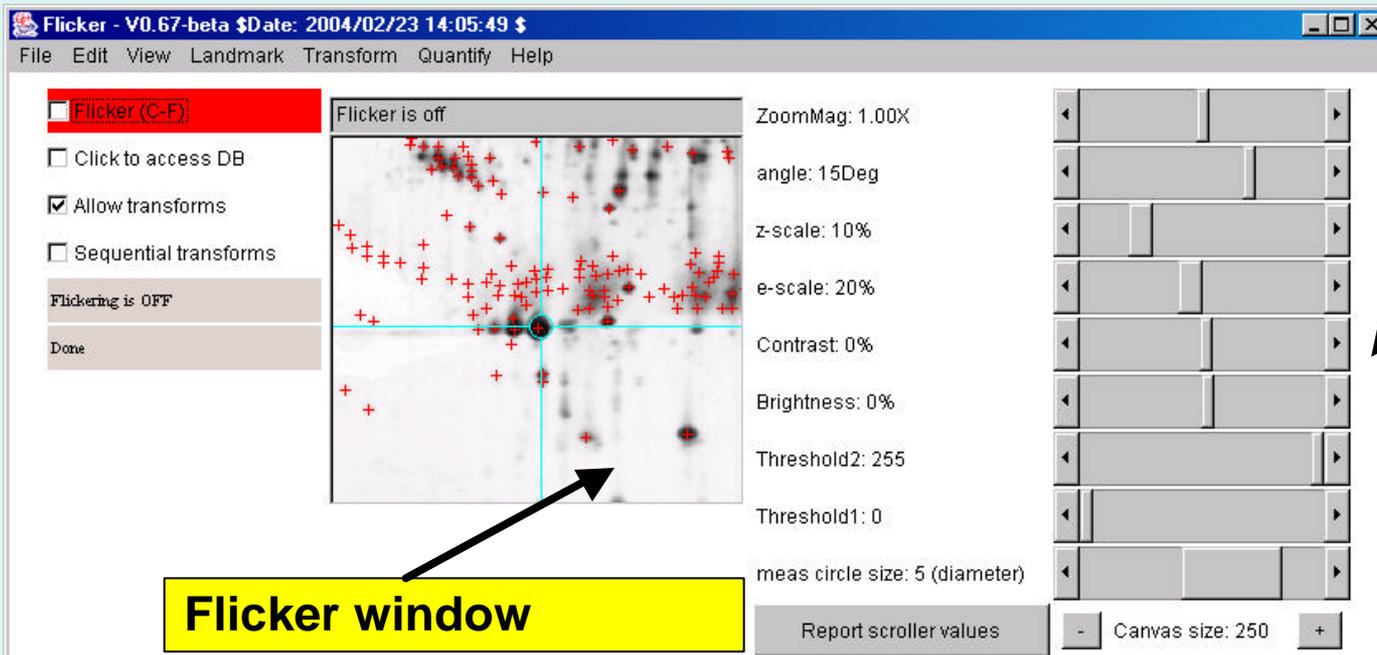
Introduction

Flicker is an open-source stand-alone computer program for visually comparing 2D gel images. Two-dimensional polyacrylamide gele electrophoresis (2D-PAGE) gels are often difficult to compare because of rubber-sheet distortions. Flicker allows you to 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. You may be able to draw putative conclusions as to the identification of some spots in your gels that are visually appear to be the same spot.

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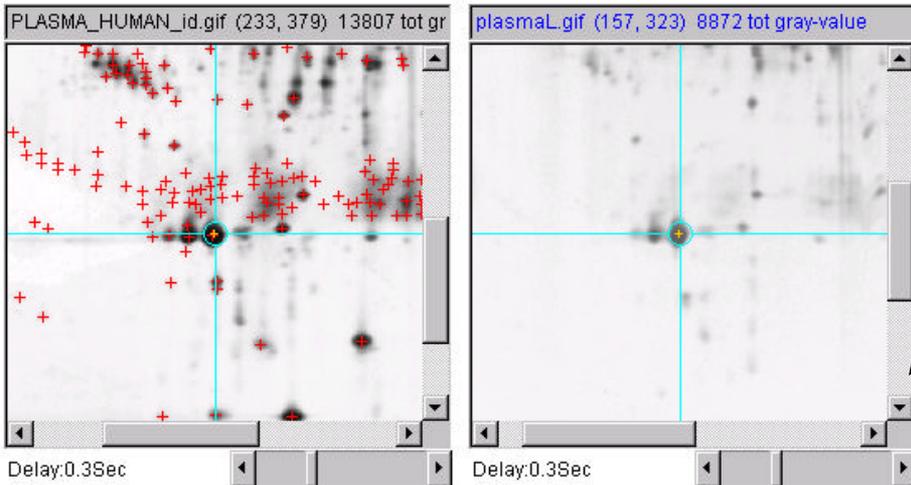
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Flicker Program User Interface



Parameter sliders

Flicker window



Two scrollable images user specifies

Concept of Flicker-Comparison

- Flickering is a dynamic visualization technique
- If two images could 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 non-linear rubber-sheet distortion (i.e., local translation, rotation, and magnification)
- May be more distortion in some parts of the images than in others
- Although it may be impossible to align two whole images at one time, they may be locally aligned piece-by-piece by matching the morphology of local regions
- Alternating two images in the same visual space will “fuse” the aligned regions in your minds-eye when they are optimally aligned

Problems with Flicker-Comparison

- Because flickering is a dynamic visualization technique that depends on hand-eye-brain integration, we find that some people are better at using this technique than others
- There is the occasional danger of false alignments when comparing charge-trains of spots if there is not enough local morphology context

Problems with Flicker-Comparison (continued)

- Because it may be difficult to compare a user's entire gel against an Internet database reference gel (e.g., Swiss-2DPAGE) which was run in a quite different way:
IPG vs CA,
linear vs non-linear gradients,
pI isoelectric range,
MW molecular mass range,
etc.
- However, parts of the gels may be comparable
- Even when a comparison is made and a putative correspondence made between the user's and the reference gel, the spot of interest may not be identified in the Internet database reference gel

Solution: Image Transforms for Better Visualization

- It is difficult to visually compare gels of different magnification, contrast, and geometry
- Flickr has a zoom transform to magnify or de-magnify a gel so it is closer to the magnification of the other gel
- Flickr has a brightness-contrast adjustment to adjust one gel to the range of the other gel
- Flickr has geometric correction using spatial warping transforms
- Additional image enhancement transforms are available for smoothing and sharpening images to make them easier to compare

Finding Putative Identifications by Accessing Reference 2D Web Databases

1. First find a putative match between a user's gel and an active map reference gel
2. The user then clicks on the spot in the reference gel to access that spot's identification in the Internet reference gel database
3. The reference database then supplies the identification of the spot selected and by inference the putative identification of the user's spot

Finding Putative Identifications by Accessing Reference 2D Web Databases (continued)

- The active map reference gel must be supported by a federated 2D gel map Internet database such as Swiss-2DPAGE
- Additional lab work can confirm that putative identification of the spot extracted from their gel

Putative Identification - Click on Active Map

1) Match spot
2) Make map active
3) Click on spot
4) Putative ID pops up

The interface includes a 'Flicker' control panel with options: Flicker (C-F), Click to access DB, Allow transforms, Sequential transforms, Flickering is OFF, and Done. A 'ZoomMag: 1.00X' slider is also present.

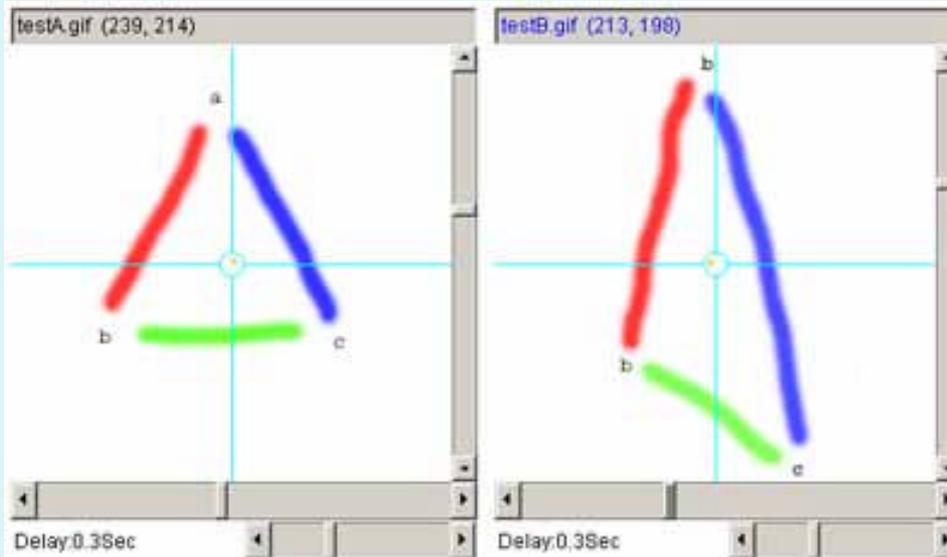
The protein identification page shows search options: [by description](#), [by accession number](#), [by clicking on a spot](#), [by author](#), [by serial number](#), [by full text search](#), and [\[SRS\]](#). The search results for 'SWISS-2DPAGE : P02647' are displayed, including the protein name 'Apolipoprotein A-I (Apo-AI)' and its description.

Name and origin of the protein	
Description	Apolipoprotein A-I (Apo-AI)
Gene name(s)	APOA1
From	Homo sapiens (Human) [TaxID: 9606]
Taxonomy	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Homnidae; Homo

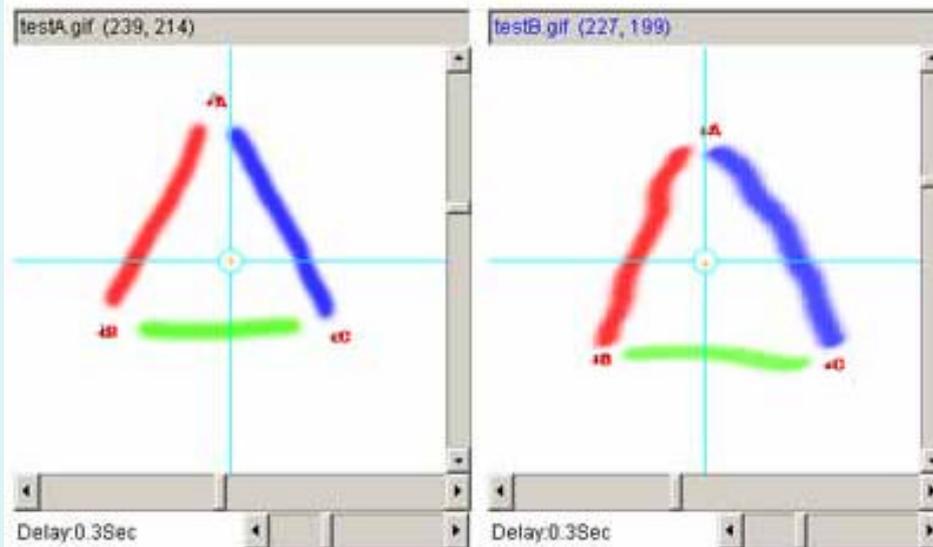
References:
[1] MAPPING ON GEL.
MEDLINE=93162045, PubMed=1286669, [NCBI, ExPASy, EBI, Israel, Japan]
Hochstrasser D.F., Frutiger S., Paquet N., Baroch A., Ravner F., Pasquall C., Sanchez J.-C., Tissot J.-D., Bjellquist B., Vargas R., Appel R.D., Hughes G.J.;

Warping a Gel to Other Gel's Geometry

Original

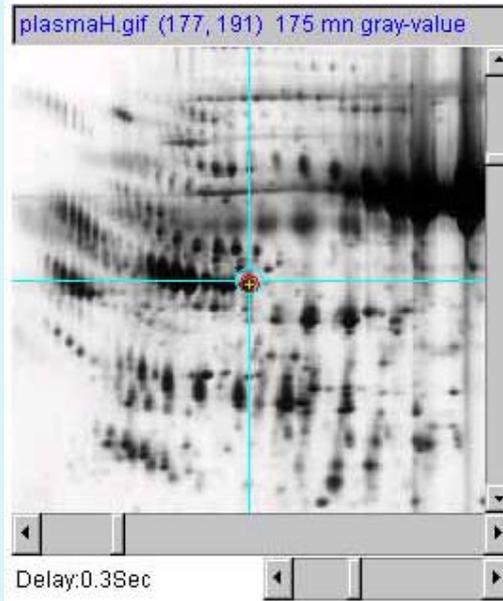


Warped

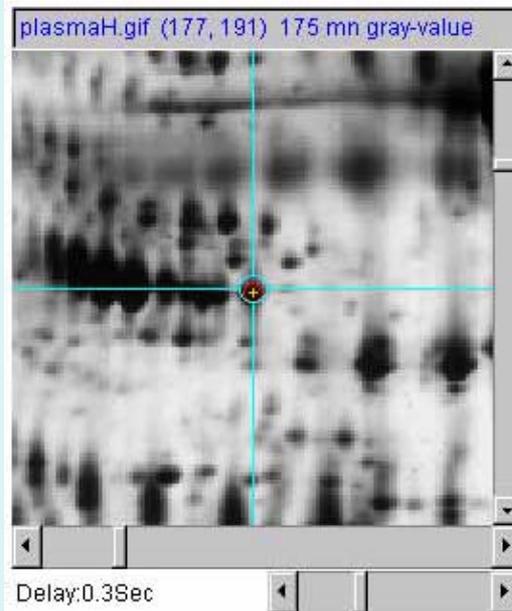


Zooming a Gel to Other Gel's Magnification

Original

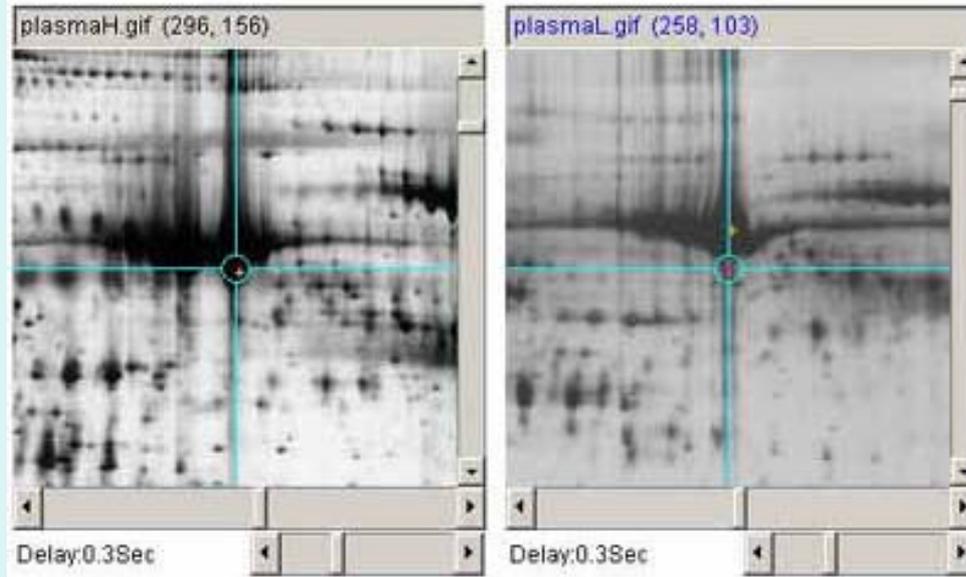


Zoomed

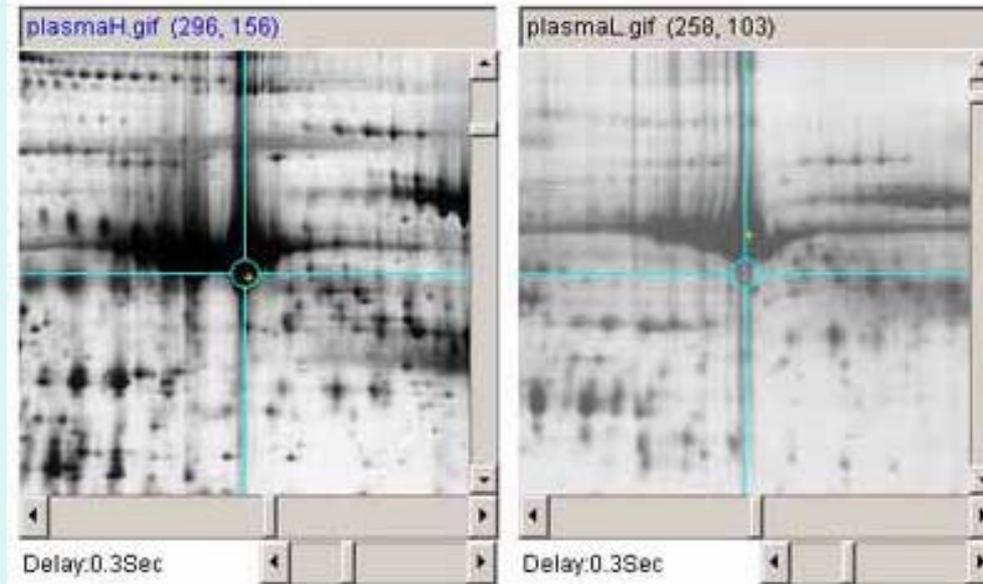


Adjusting Brightness/Contrast So Similar

Original



Adjusted

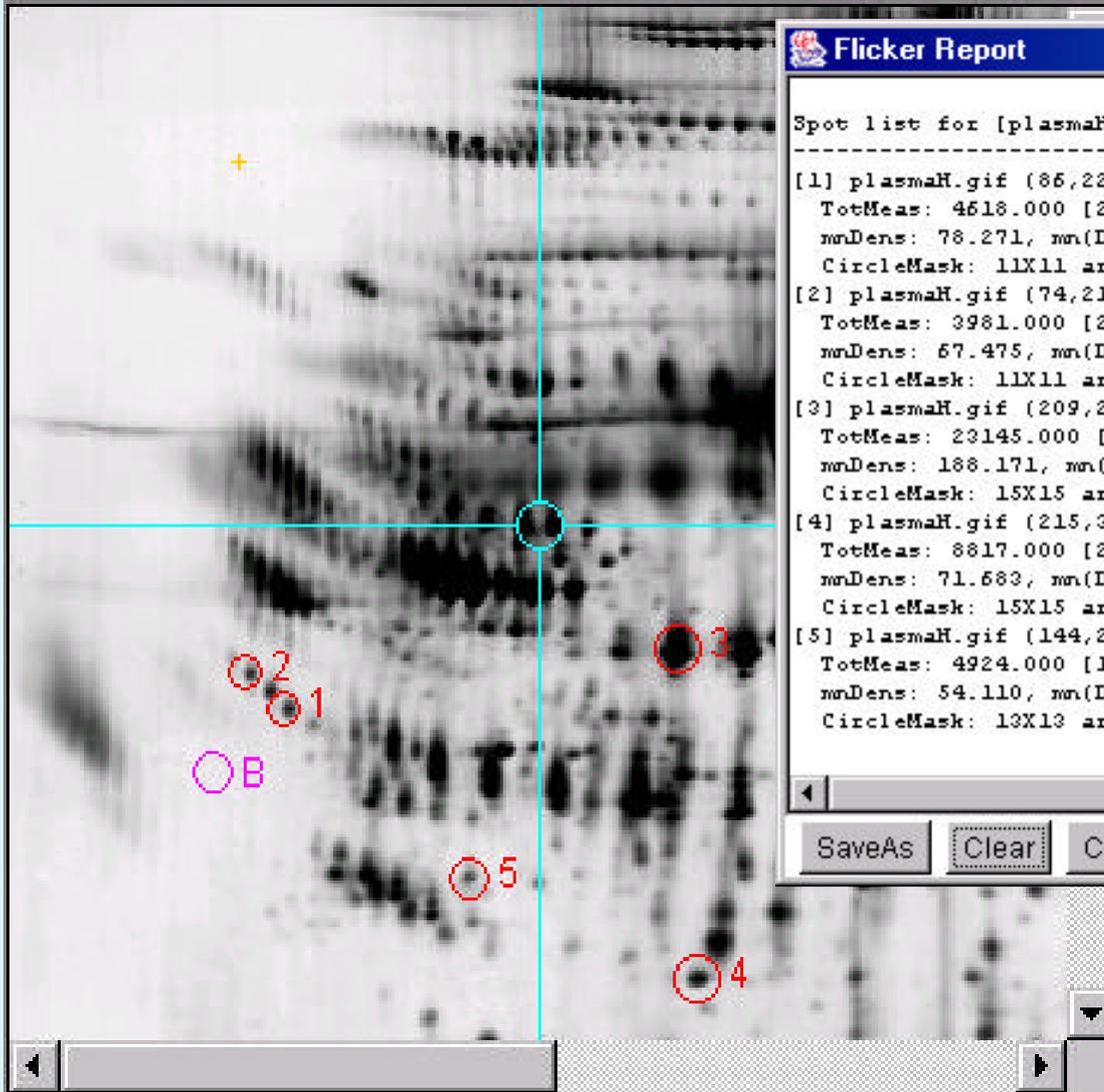


Estimating Spot Quantification

- Flicker provides a *limited* estimated-spot quantification capability to collect a list of manually measured spots that may be reported and saved for further analysis
- Integrated density (grayscale or calibrated OD) may be *estimated* for isolated spots using measurement circle masks (1 to 51 pixels in diameter)
- Background density, D_b , near the spot is measured first
- Then, an isolated spot's density, D_s , is measured and the density corrected for background D'_s is estimated as $D_s - D_b$
- Lists of spots may be created with user-supplied annotation

User Measured Spot Lists

plasmaH.gif (72, 62) 323 tot gray-value



Flicker Report

Spot list for [plasmaH.gif] with 5 spots

[1] plasmaH.gif (86,226) Tot(Meas-Bkgrd): 3943.000 gray
TotMeas: 4618.000 [26.000:184.000] gray, TotBkgrd: 675.000 (64,246)
mnDens: 78.271, mn(Dens-Bkgrd): 66.831, mnBkgrd: 11.441 gray
CircleMask: 11X11 area: 61 pixels

[2] plasmaH.gif (74,215) Tot(Meas-Bkgrd): 3306.000 gray
TotMeas: 3981.000 [22.000:166.000] gray, TotBkgrd: 675.000 (64,246)
mnDens: 67.475, mn(Dens-Bkgrd): 56.034, mnBkgrd: 11.441 gray
CircleMask: 11X11 area: 61 pixels

[3] plasmaH.gif (209,208) Tot(Meas-Bkgrd): 22470.000 gray
TotMeas: 23145.000 [50.000:237.000] gray, TotBkgrd: 675.000 (64,246)
mnDens: 188.171, mn(Dens-Bkgrd): 176.730, mnBkgrd: 11.441 gray
CircleMask: 15X15 area: 125 pixels

[4] plasmaH.gif (215,307) Tot(Meas-Bkgrd): 8142.000 gray
TotMeas: 8817.000 [21.000:207.000] gray, TotBkgrd: 675.000 (64,246)
mnDens: 71.683, mn(Dens-Bkgrd): 60.242, mnBkgrd: 11.441 gray
CircleMask: 15X15 area: 125 pixels

[5] plasmaH.gif (144,277) Tot(Meas-Bkgrd): 4249.000 gray
TotMeas: 4924.000 [13.000:150.000] gray, TotBkgrd: 675.000 (64,246)
mnDens: 54.110, mn(Dens-Bkgrd): 42.669, mnBkgrd: 11.441 gray
CircleMask: 13X13 area: 93 pixels

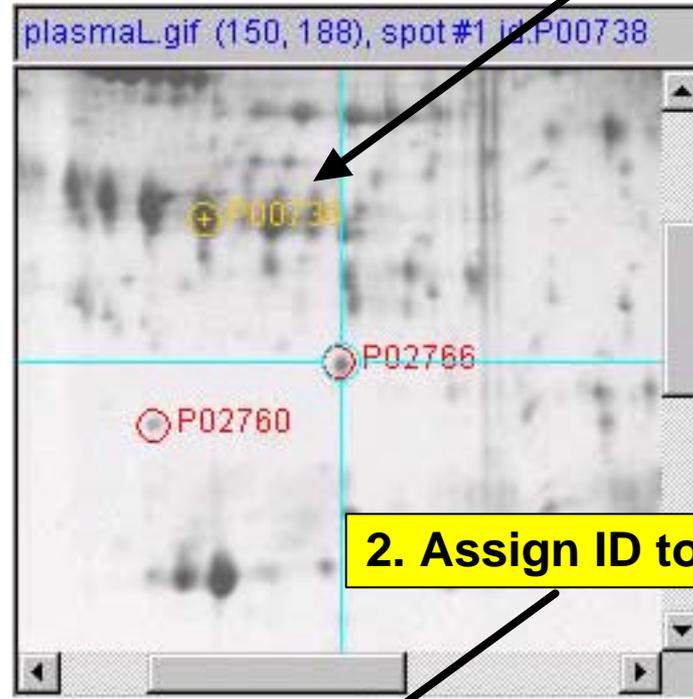
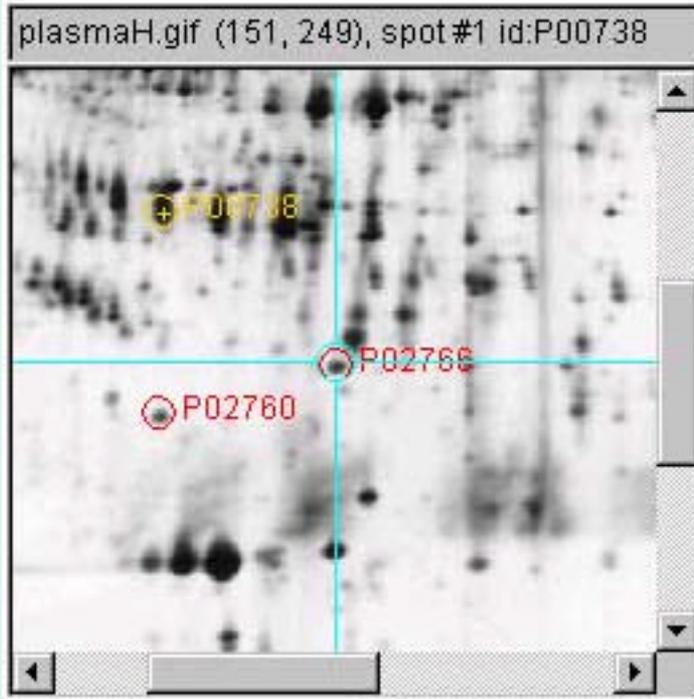
SaveAs Clear Close

Spot List Functionality

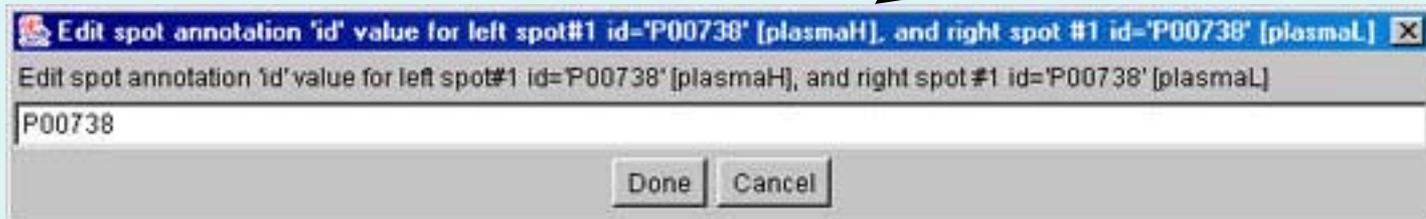
- Define, delete, annotate, edit spots in the spot list
- View spots with various overlay options
- List spots in report-form or tab-delimited form suitable for export to Excel or other analysis programs
- List paired-spots reports with same IDs in tab-delimited form
- Save spot lists for further use when exit Flicker and reload them when reload those gel images

Manually Annotating Paired Spots

1. Select pairs of spots



2. Assign ID to both spots



Generate Paired-Spots Reports For Spots with Same IDs

Microsoft Excel - Flicker-SpotListData-demo.xls

File Edit View Insert Format Tools Data S-PLUS Window Help Acrobat

Arial 10 B I U \$ % , +.00 +.00

	A	B	C	D	E	F	G	H	I	J	K	L
1	Image1	Image2	SpotNbr1	SpotNbr2	ID	DensityMode	Units	Dm1	Dm2	(Dm1/Dm2)	(Dm1-Bm1)	(Dm2-Bm2)
2	plasmaH.g	plasmaL.g	1	1	P00738	Total	gray	1.641	1.84	0.89	1.641	1.843
3	plasmaH.g	plasmaL.g	2	2	P02766	Total	gray	0.805	0.7	1.144	0.805	0.703
4	plasmaH.g	plasmaL.g	3	3	P02760	Total	gray	0.555	0.45	1.224	0.555	0.453

Sheet1 Sheet2 Sheet3

Microsoft Excel - Flicker-SpotListData-demo.xls

File Edit View Insert Format Tools Data S-PLUS Window Help Acrobat

Arial 10 B I U \$ % , +.00 +.00

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

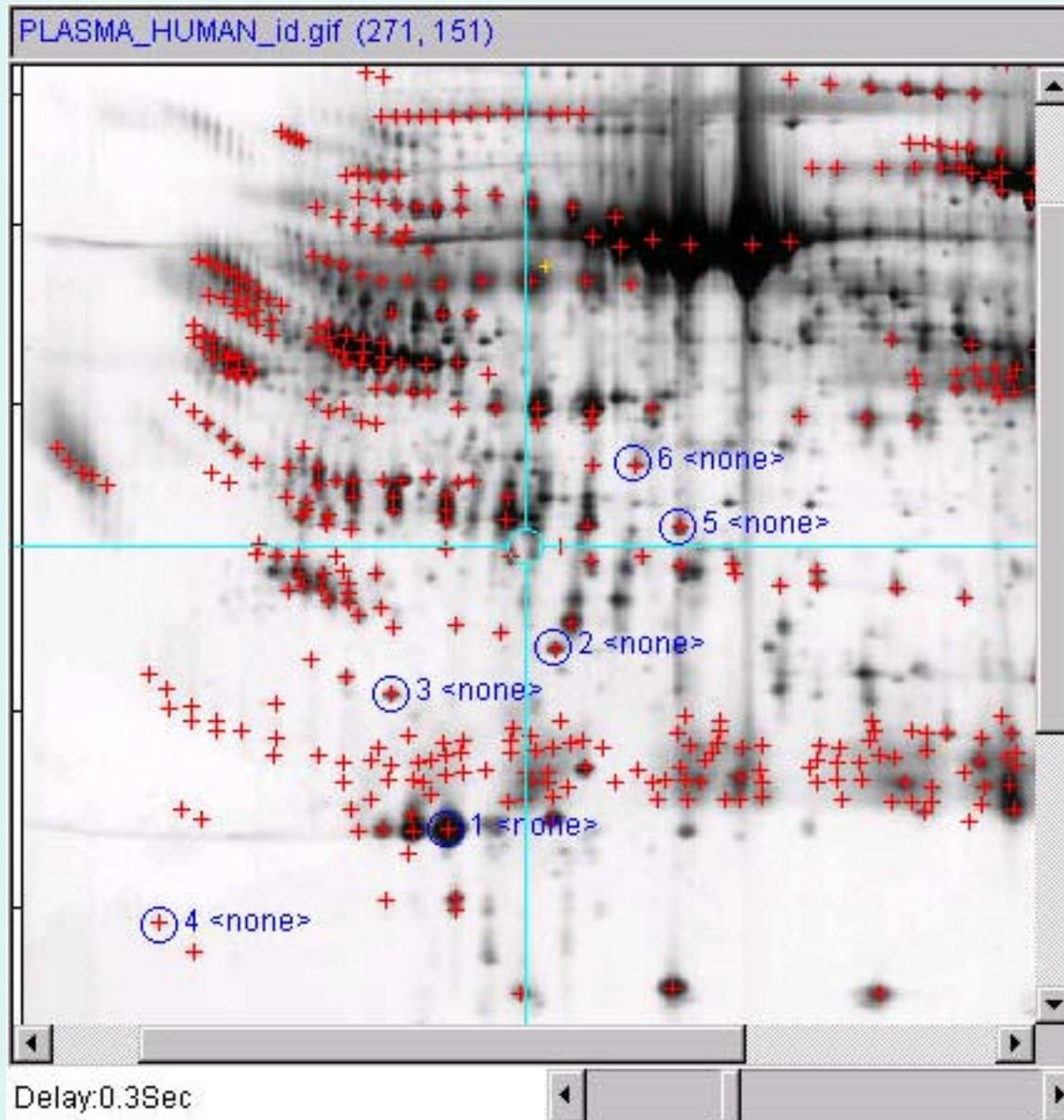
Sheet1 Sheet2 Sheet3

Looking Up Spot ID Annotation At Reference DB

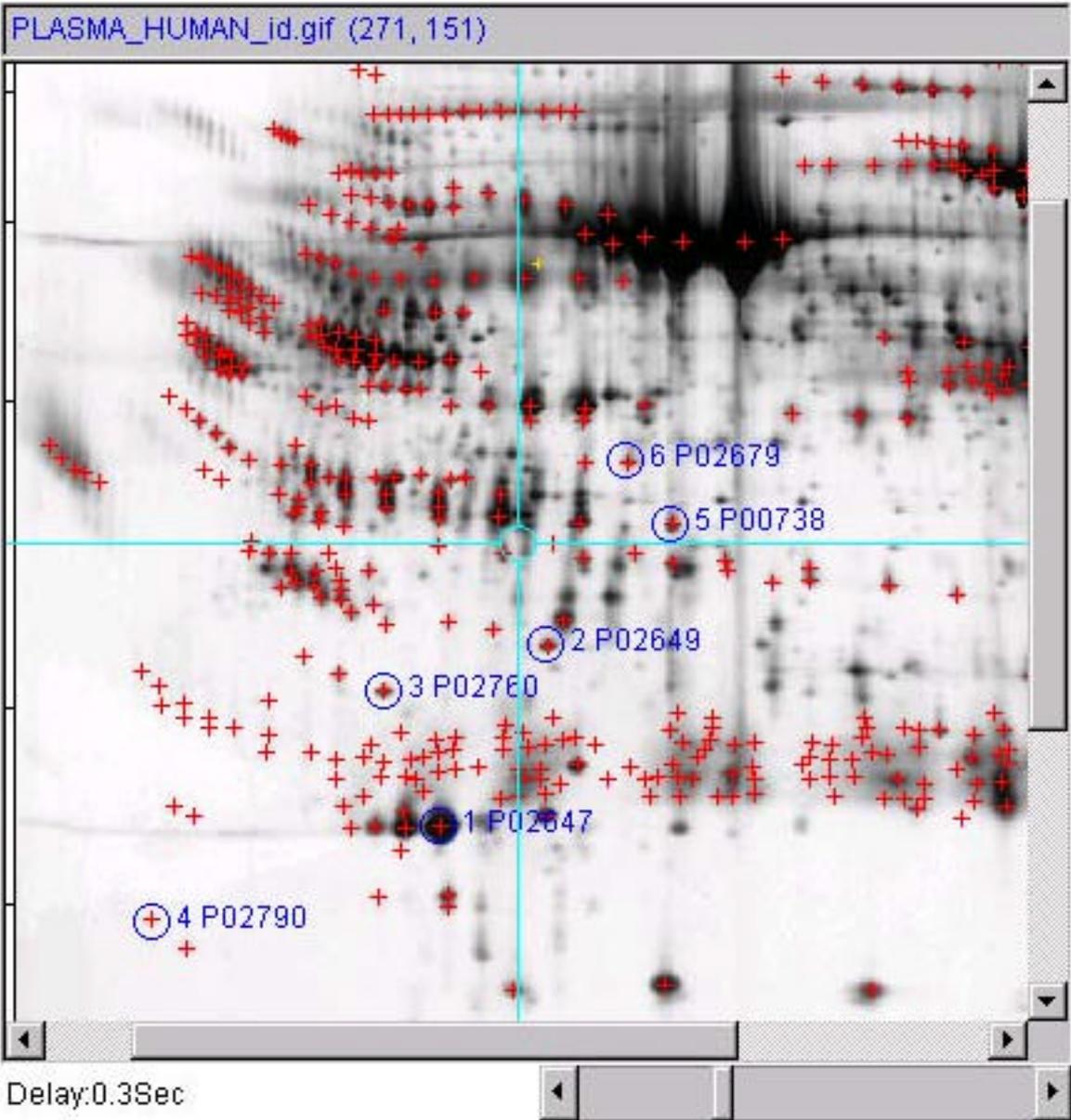
Putatively identify a list of spots in your gel that are identified in an active reference gel by first identifying spots in the reference gel and then using them to identify corresponding spots in your gel.

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 visit the active reference gel Web server and try to lookup the protein IDs (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).

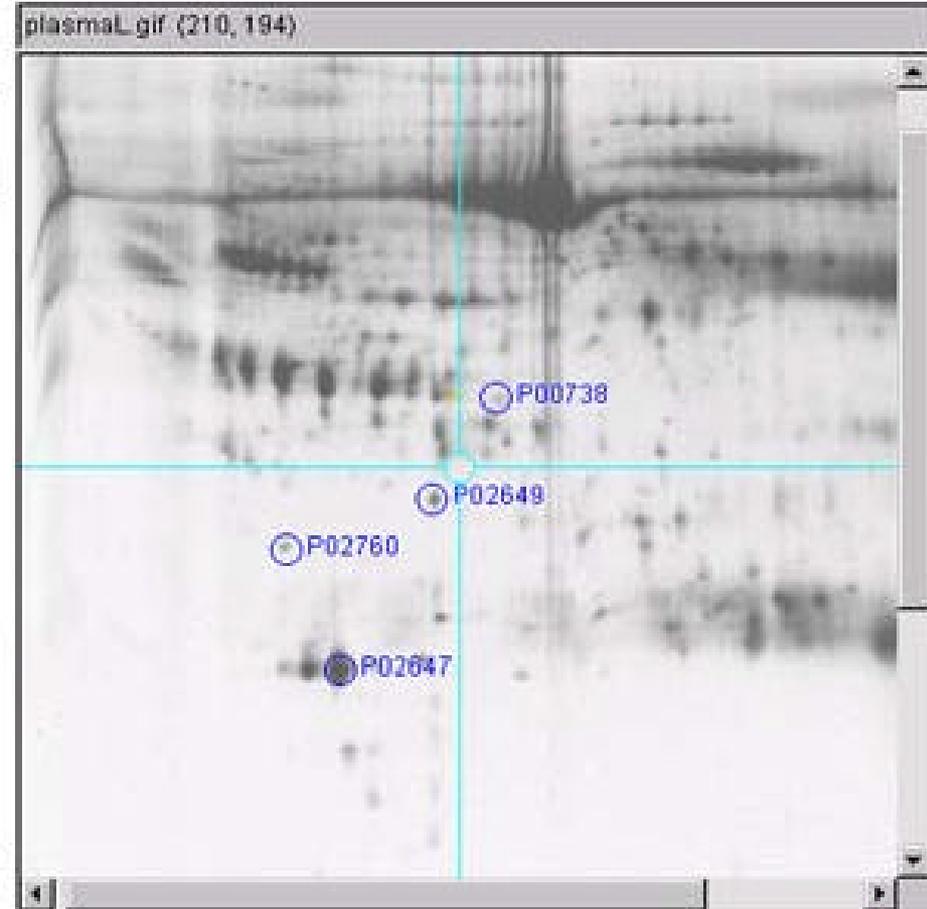
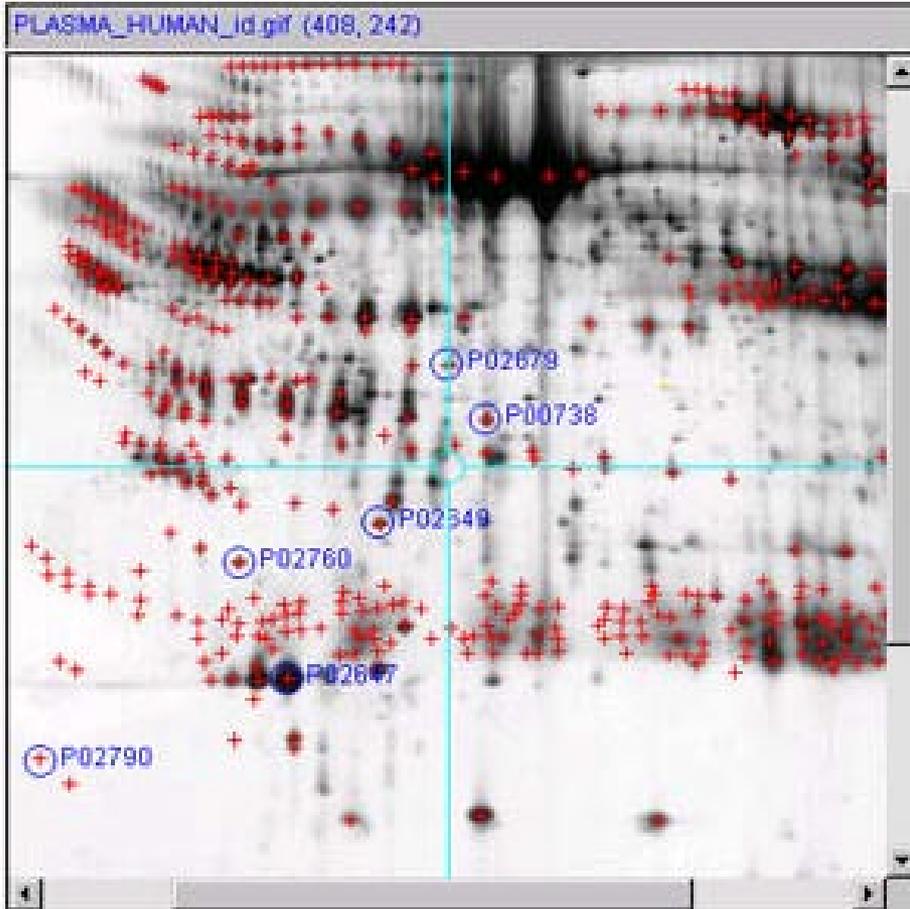
Define Reference Gel Spot List



Look Up Reference Gel Spot ID Annotation



Assign Reference Gel Annotation to User Gel



Edit spot annotation 'id' value for left spot#5 id='P00738' [PLASMA_HUMAN_id], and right spot #4 id='P00...

Edit spot annotation 'id' value for left spot#5 id='P00738' [PLASMA_HUMAN_id], and right spot #4 id='P00738' [plasmaL]

Done Cancel

Lookup PIR Database Pages for Identified Proteins

- Access PIR (pir.georgetown.edu) UniProt, iProClass and iProLink server Web pages for selected proteins in the spot list through their Swiss-Prot accession names.
- A two-step process enabled using the (**Edit | Select access to active DB server | ...**) checkbox command to select either SWISS-2DPAGE, UniProt, iProClass or iProLink servers.
- When you measure a spot (select a spot in an active gel image and add it to the spot list by typing **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 PIR DB server you have selected.

PIR UniProt Web Page for Identified Protein

UniProt Entry - UniProt [the Universal Protein Resource] - Netscape

http://www.pir.uniprot.org/cgi-bin/upEntry?id=P02760

UniProt
the universal protein resource

Home > Databases > UniProt

hosted by PIR

Text Search UniProt Knowledgebase

Home About UniProt Getting Started Searches/Tools Databases Support/Documentation

UniProt Entry

PIR View Niceprot View | SRS View

UniProt Entry: P02760

ENTRY INFORMATION	
ENTRY NAME	AMBP HUMAN
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 Structure of the human alpha 1-microglobulin-bikunin gene. 1990, <i>Biol. Chem. Hoppe-Seyler</i> , 371, 1185-1196 <i>Position:</i> SEQUENCE FROM N.A. PubMed: 1708673 ; Medline: 91214554 ;

PIR iProClass Web Page for Identified Protein

PIR Search Results - Netscape

File Edit View Go Bookmarks Tools Window Help

http://pir.georgetown.edu/cgi-bin/textsearch.pl?s_field=SWACCS&query=P02760

Search

pir.georgetown.edu

PIR Search Results

Site Map Site Search

Text Search Protein Databases: GO

About PIR Databases Search & Retrieval Download Support

Mon Sep 13 11:08:48 EDT 2004

Search NREF for SwissProt Accession AND All Fields AND All Fields AND All Fields

P02760 not null

1 protein sequences in total

[HELP](#)

For sequence analyses, pick a method (radiobutton) 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 <small>check all</small>	Matched	Protein Name	Length	Organism Name /Taxon Group	PIRSF ID /Family ID	Pfam ID	PC Motif ID	PDB ID
<input type="checkbox"/> NREF: NF00080626 iProClass: P02760 PIR-PSD: HCHU UniProt: AMBP HUMAN	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 (ITI-	352	Homo sapiens Euk/mammal	SF001622 FAM0001605	PF00014; PF00061	PCM00213; PCM00280	1BIK

PIR iProLink Web Page for Identified Protein

iProLINK Search Results

Text Search Protein Databases: **GO!**

[About PIR](#)
[Databases](#)
[Search & Retrieval](#)
[Download](#)
[Support](#)

Mon Sep 13 11:10:09 EDT 2004 [examples](#)

Search: NREF for SwissProt Accession AND All Fields AND All Fields AND All Fields

P02760 not null

1 protein sequences in total. [HELP](#)

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

BLAST
 FASTA
 HMM Search
 Pattern Match
 Multiple Alignment
 Domain Display

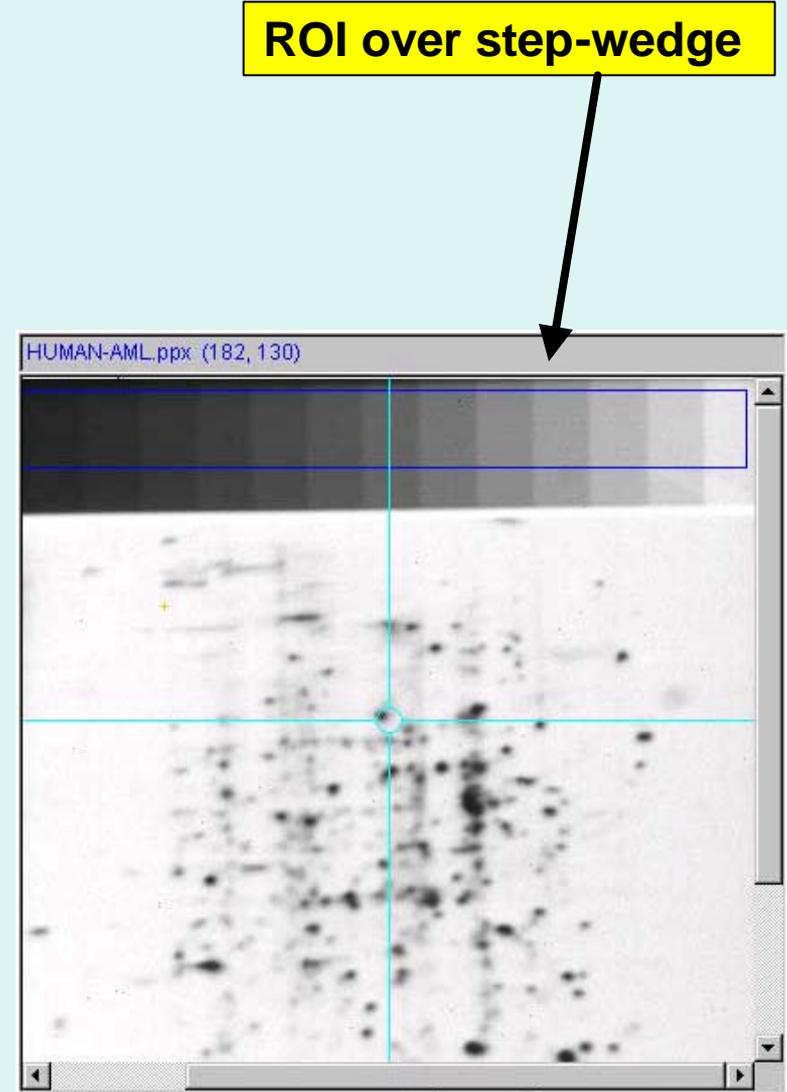
<input type="checkbox"/> Protein ID <small>check all</small>	Matched	Protein Name	Length	Organism Name /Taxon Group	PIRSF ID /Family ID	Pfam ID	PC Motif ID	PDB ID
<input type="checkbox"/> NREF: NF00080626 View Bibliography iProClass: P02760 PIR-PSD: HCHU SP/TR: AMBP HUMAN	SwissProt Accession=>P02760	AMBP protein precursor [Contains: Alpha-1-microglobulin (Protein HC) (Complex-forming glycoprotein heterogeneous in charge) (Alpha-1 microglycoprotein); Inter-alpha-trypsin inhibitor light chain (ITI-	352	Homo sapiens Euk/mammal	SF001622 FAM0001605	PF00014; PF00061	PCM00213; PCM00280	1BIK

Calibrating Grayscale for Better Quantification

- If the gel's stain/detection method is stoichiometric, then integrated density can correspond to protein concentration in a non-saturating range
- The scanner and other systematic sources of non-linearity can be corrected to some degree by calibrating the image against a calibration standard and mapping grayscale to that standard (e.g., optical density, CPM, etc.)
- Their subsequent spot quantification measurements will then be more accurate

Calibrating Grayscale with a ND Step-Wedge

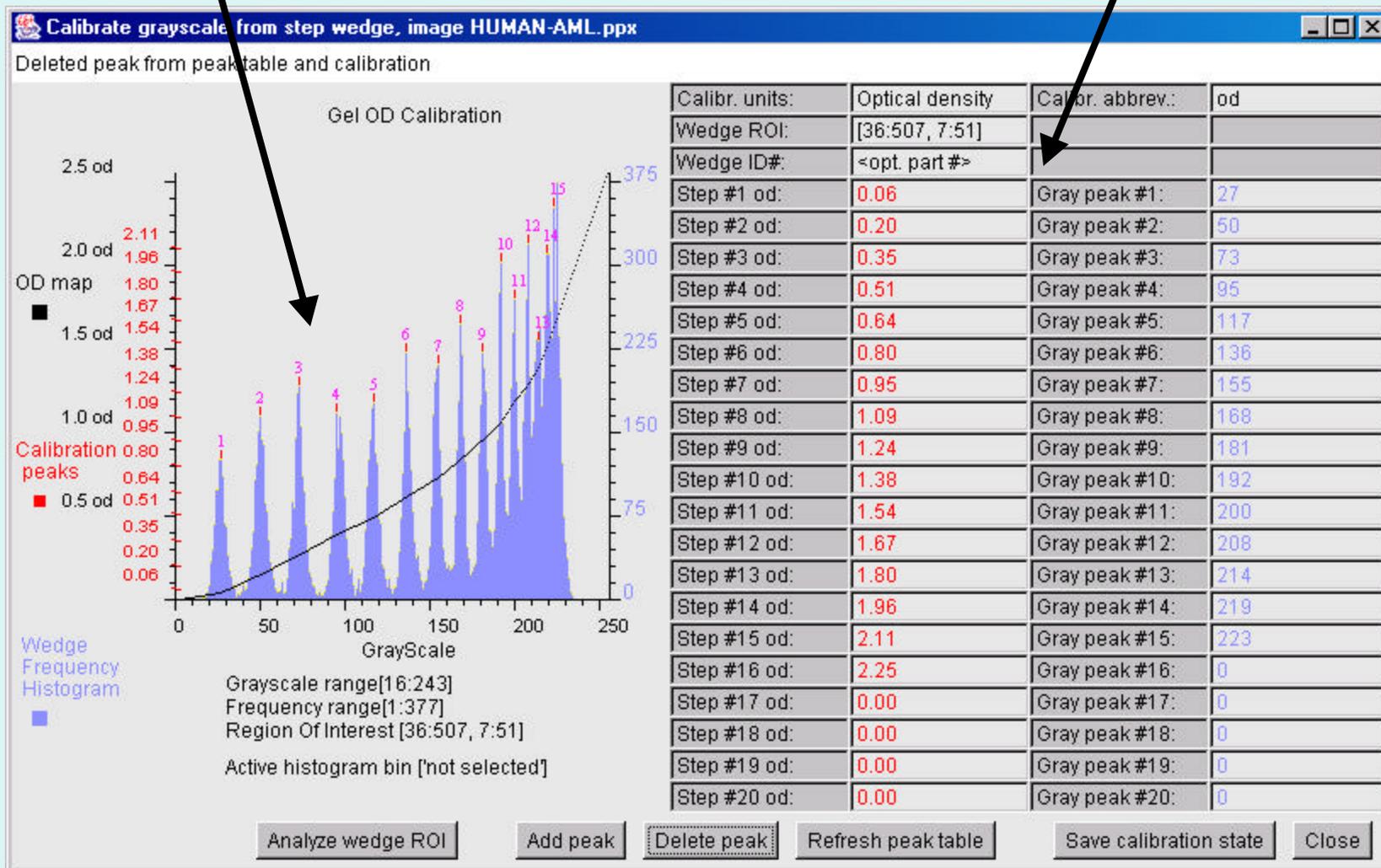
1. The ND step wedge must be scanned with the image and the corresponding OD values known for each step
2. A region of interest (ROI) is overlaid on the step step-wedge
3. The ND wedge calibration wizard is invoked to analyze the data and estimate the calibration



Calibrating Grayscale from ND Wedge Data

ROI histogram, peaks found and extrapolated calibration curve

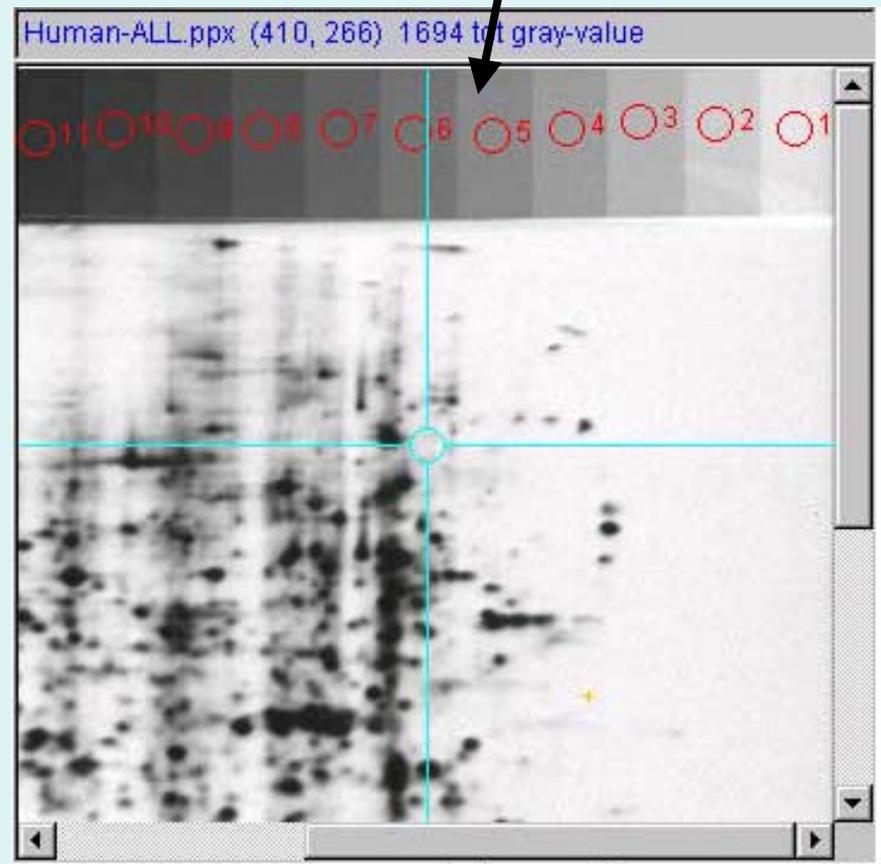
OD vs gray-peaks table



Calibrating Grayscale with a Spot List of Calibrated Data

1. The image must contain calibrated regions with known concentrations or corresponding OD values known for each spot
2. You define a set of spots using (C-M) or (ALT-click)
3. The Spot List Calibration wizard is invoked to analyze the data and estimate the calibration

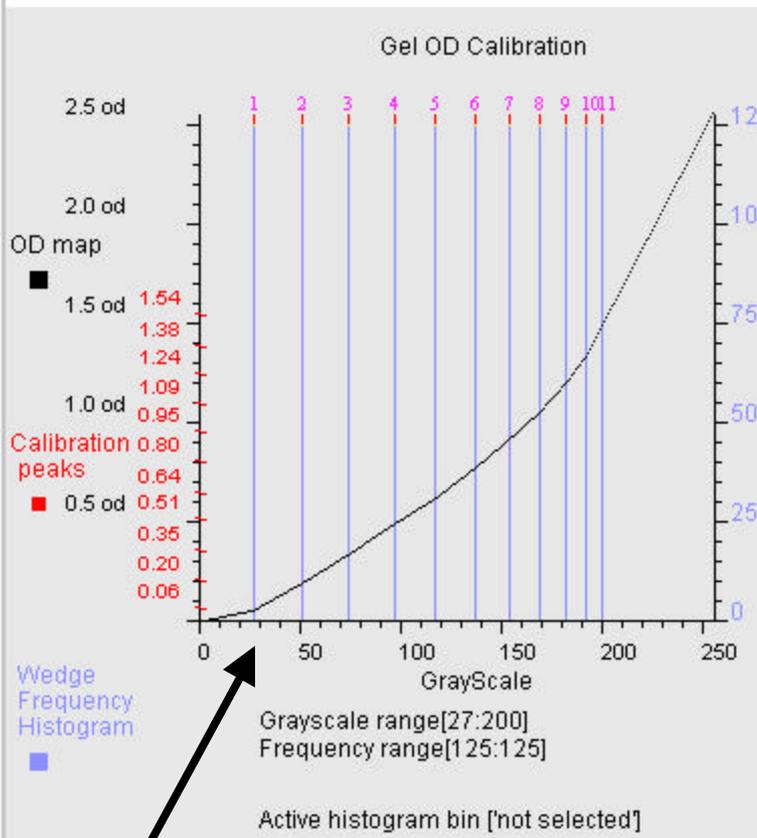
List of spots you defined



Calibrating Grayscale from Spot List Data

Calibrate grayscale from spot list, image Human-ALL.ppx

OD vs gray-peaks table



Calibr. units:	Optical density	Calibr. abbrev.:	od
Mean spot values			
Wedge ID#:	<opt. part #>		
Step #1 od:	0.06	Gray peak #1:	27
Step #2 od:	0.20	Gray peak #2:	51
Step #3 od:	0.35	Gray peak #3:	74
Step #4 od:	0.51	Gray peak #4:	97
Step #5 od:	0.64	Gray peak #5:	117
Step #6 od:	0.80	Gray peak #6:	137
Step #7 od:	0.95	Gray peak #7:	154
Step #8 od:	1.09	Gray peak #8:	169
Step #9 od:	1.24	Gray peak #9:	182
Step #10 od:	1.38	Gray peak #10:	192
Step #11 od:	1.54	Gray peak #11:	200
Step #12 od:	1.67	Gray peak #12:	0
Step #13 od:	1.80	Gray peak #13:	0
Step #14 od:	1.96	Gray peak #14:	0
Step #15 od:	2.11	Gray peak #15:	0
Step #16 od:	2.25	Gray peak #16:	0
Step #17 od:	0.00	Gray peak #17:	0
Step #18 od:	0.00	Gray peak #18:	0
Step #19 od:	0.00	Gray peak #19:	0
Step #20 od:	0.00	Gray peak #20:	0

ROI histogram, peaks found and extrapolated calibration curve

Refresh peak table

Save calibration state

Close

Summary

- Flicker is an open-source 2D gel visual image comparison Java program freely available at <http://open2dprot.sourceforge.net/Flicker>
- Useful for visual comparison of 2D gels and other images
- Putative spot identification made by comparison with reference 2D gel databases
- Manual creation of lists of estimated quantified spot densities can be exported (to Excel, etc.)