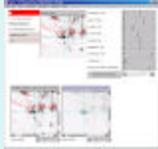


Flicker Comparison of 2D Electrophoretic Gels

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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.

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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

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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 Flicker

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

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Flicker Program User Interface

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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

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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

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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

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Solution: Image Transforms for Better Visualization

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

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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

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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

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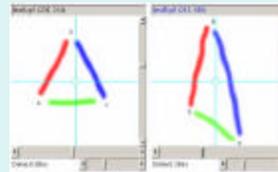
Putative Identification - Click on Active Map

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

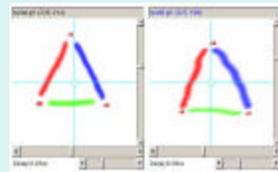
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Warping a Gel to Other Gel's Geometry

Original

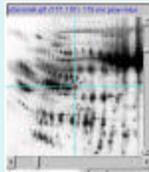


Warped

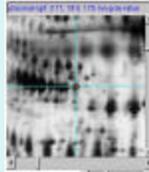


Zooming a Gel to Other Gel's Magnification

Original

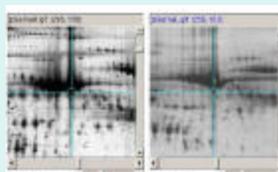


Zoomed

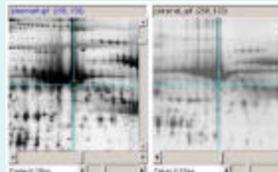


Adjusting Brightness/Contrast So Similar

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 $D_s - D_b$

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- Lists of spots may be created with user-supplied annotation

User Measured Spot Lists

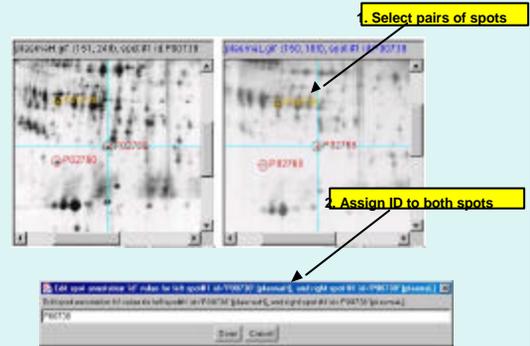
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Spot List Functionality

- 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

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Manually Annotating Paired Spots



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Generate Paired-Spots Reports For Spots with Same IDs

| image1 | image2 | SpotID | Density | Area |
|----------|----------|----------|---------|------|
| plasma.g | plasma.g | 1 P10270 | 1.621 | 1.54 |
| plasma.g | plasma.g | 2 P10270 | 0.805 | 0.7 |
| plasma.g | plasma.g | 3 P10270 | 0.555 | 0.45 |

| image1 | image2 | SpotID | Density | Area |
|--------|--------|----------|---------|------|
| 1 | 2 | 1 P10270 | 1.621 | 1.54 |
| 2 | 3 | 2 P10270 | 0.805 | 0.7 |
| 3 | 4 | 3 P10270 | 0.555 | 0.45 |

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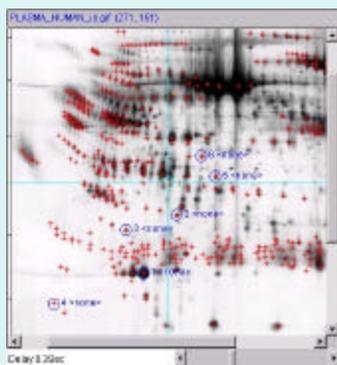
Looking Up Spot ID Annotation At Reference DB

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).

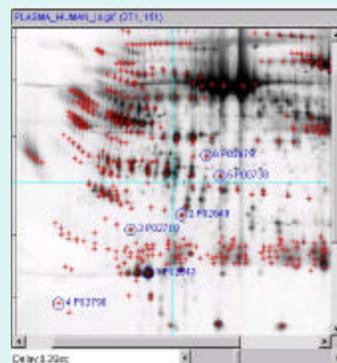
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Define Reference Gel Spot List



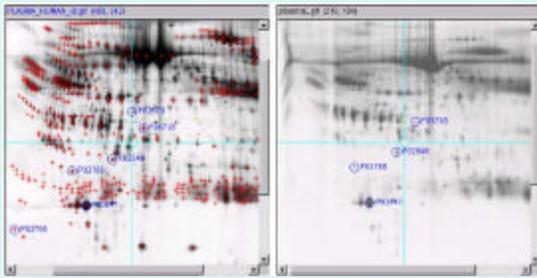
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Look Up Reference Gel Spot ID Annotation



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Assign Reference Gel Annotation to User Gel



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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.

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PIR UniProt Web Page for Identified Protein



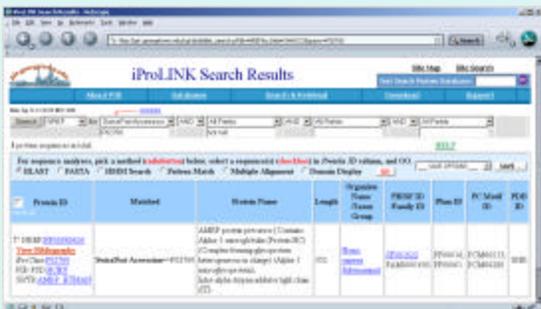
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PIR iProClass Web Page for Identified Protein



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PIR iProLink Web Page for Identified Protein



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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

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Calibrating Grayscale with a ND Step-Wedge

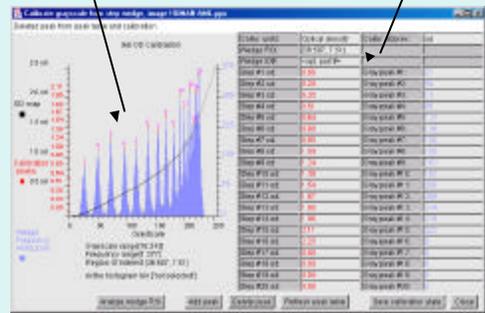
1. The ND step wedge must be scanned with the image and the corresponding OD values known for each step
2. A region of interest (ROI) is overlaid on the step wedge
3. The ND wedge calibration wizard is invoked to analyze the data and estimate the calibration



Calibrating Grayscale from ND Wedge Data

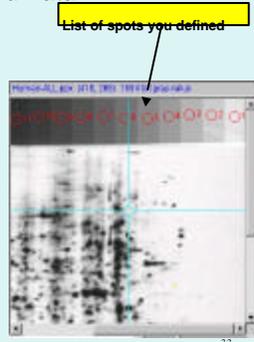
ROI histogram, peaks found and extrapolated calibration curve

OD vs gray-peaks table



Calibrating Grayscale with a Spot List of Calibrated Data

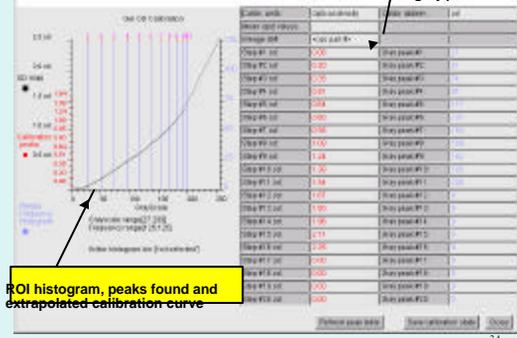
1. The image must contain calibrated regions with known concentrations or corresponding OD values known for each spot
2. You define a set of spots using (C-M) or (ALT-click)
3. The Spot List Calibration wizard is invoked to analyze the data and estimate the calibration



Calibrating Grayscale from Spot List Data

ROI histogram, peaks found and extrapolated calibration curve

OD vs gray-peaks table



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.)
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