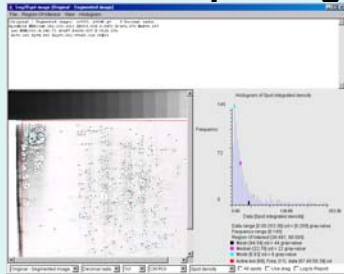


## Seg2Dgel - 2D Electrophoretic Gel Spot Segmenter



<http://open2dprot.sourceforge.net/Seg2Dgel>

Revised: 12-03-2004, P. Lemkin, NCI-Frederick

### Overview

- Seg2Dgel is an open-source Java 2D electrophoresis gel segmentation program for finding and measuring the integrated density and position of spots in a gel. It is a step [2] module in the pipeline analysis for the Open2Dprot project.
- The segmentation is performed on a computing window region of interest (ROI) of a 2D gel image file. It uses the second derivative (laplacian) magnitude and direction of the gaussian-smoothed gel image as well as neighborhood connectivity properties in determining spot extents.
- It handles grayscale TIFF, JPEG and GIF images.
- Images with calibrated grayscale (optical density, counts/min, etc.) may be used if calibrations are available.

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### Overview - GUI Window (2)

- Spot lists are generated in XML, tab-delimited, or human readable formats.
- The program may be run either interactively (-gui) with a graphical user interface (GUI) or under an OS shell command to implement batch (-nogui).
- Options are specified as Unix-style command line '-' prefixed input switches so the program is also usable under batch. These may be set by an Options Wizard window.
- In the GUI version, after the segmentation is finished, the user has the option of interactively viewing any of the images generated by the segmenter with the Image Viewer window.

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### Overview - GUI Window (3)

- It has been extended to also handle low resolution 2D LC-MS images consisting of long narrow spots
- The narrow spots are detected using a horizontal or vertical laplacian filter
- Additional spot sizing can be done using the expected aspect ratio Sx/Sy of spots.

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### Overview - Image Viewer Window (4)

- User may interactively view any of the images generated by the segmenter.
- Query images for quantified spot data or look at small numeric windows (3x3 to 21x21 pixels) of the image data in decimal or optical density (if calibrated).
- View horizontal and/or vertical image density slides at the selected pixel.
- Dynamic histograms of image density or selected spot features with rudimentary data filtering that is useful for setting spot threshold sizing parameters.

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### Overview - Downloading and Installing (4)

- The user may modify the input switch options and save the new options in a "Seg2Dgel.properties" file in the current project directory so that it may be used as the default switch options in subsequent running of the segmenter.
- You may currently download the pre-alpha version and install it on your computer.
- Currently, Seg2Dgel is hardwired to start with the demo gel and with the -gui switch. However, you can override this in the "Edit options" popup wizard.
- The home page contains links to some screen shots of the interactive Image Viewer GUI.

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<http://open2dprot.sourceforge.net/Seg2Dgel>

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- \* Demos & Screen shots
- \* Download

Seg2Dgel - 2D gel Spot Segmenter  
pre-alpha version

Welcome To Seg2Dgel

<http://open2dprot.sourceforge.net/Seg2Dgel> and  
<http://www.lecb.ncifcr.gov/Open2Dprot/Seg2Dgel/>

Introduction

Seg2Dgel Report Window User Interface -gui mode

Pull-down menus

Report window text

Command buttons

Seg2Dgel Command Options Wizard Window

Command help

Parameters

Threshold sliders

Enable switches

Switch choices

Input gel file browser

Seg2Dgel Image Viewer Window - original image - Decimal

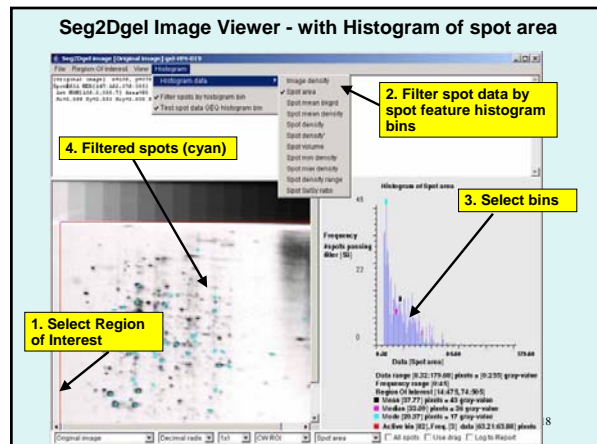
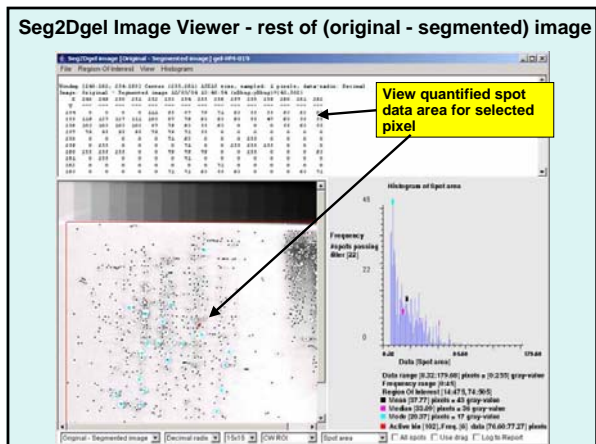
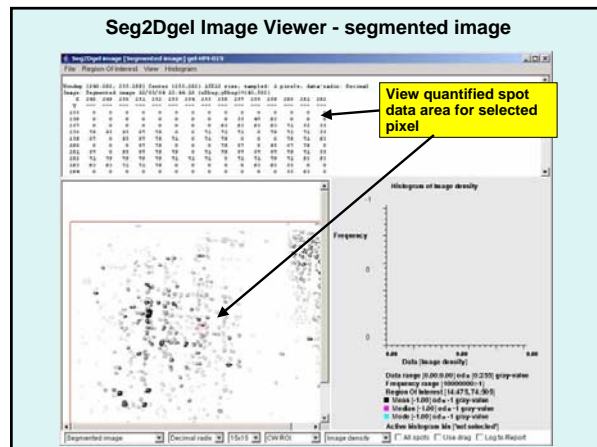
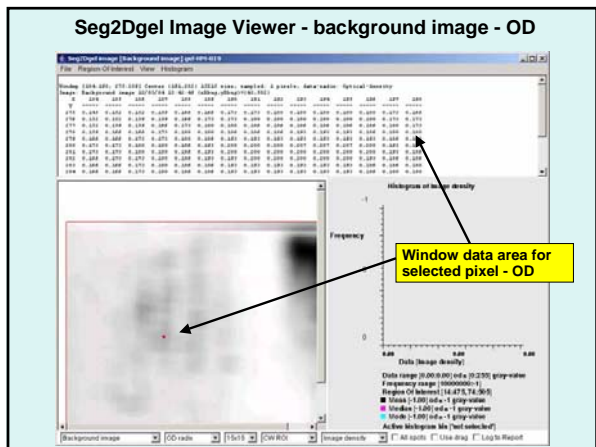
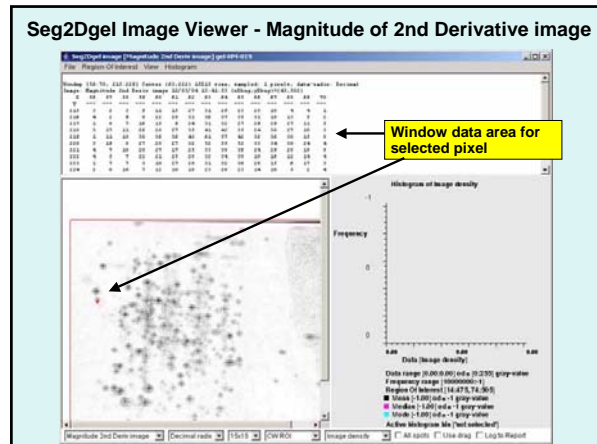
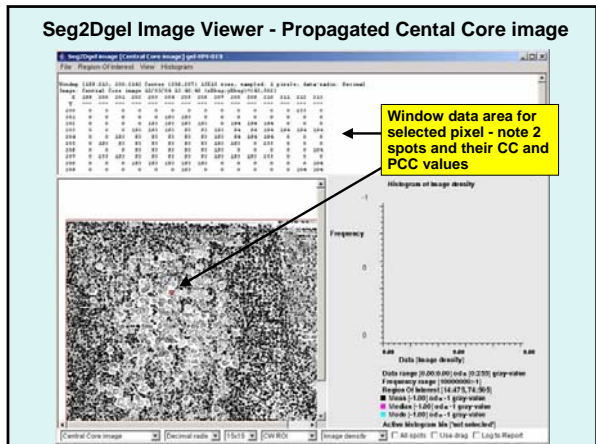
Window data area for selected pixel - Decimal

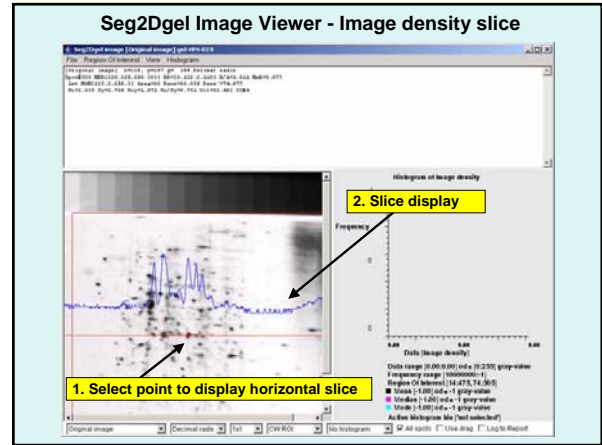
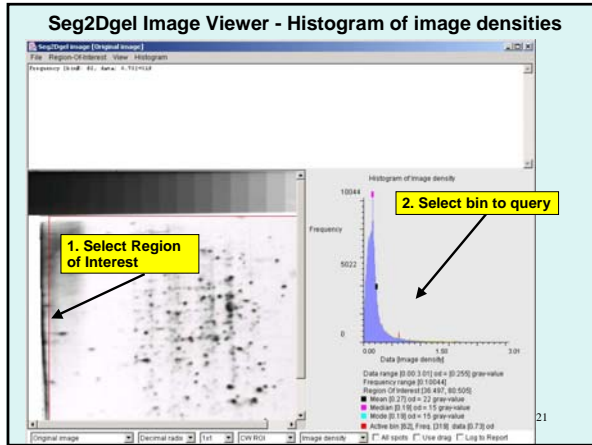
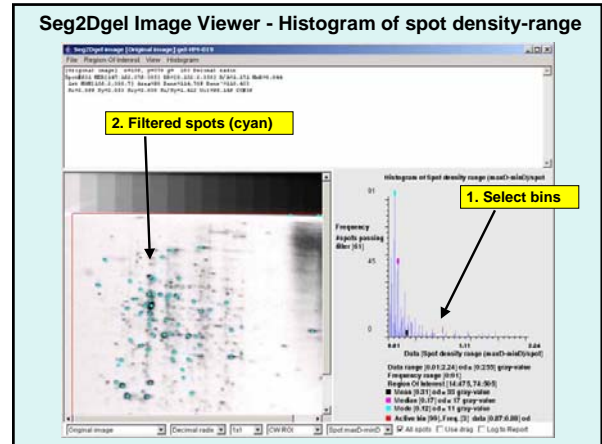
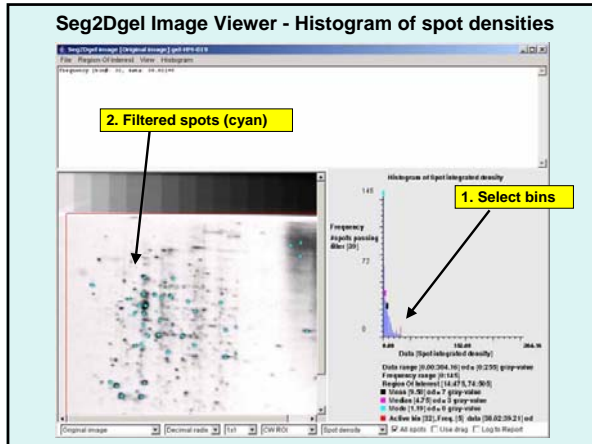
Seg2Dgel Image Viewer - original image - OD

Window data area for selected pixel - Optical Density

Seg2Dgel Image Viewer - Gaussian averaged image

Window data area for selected pixel

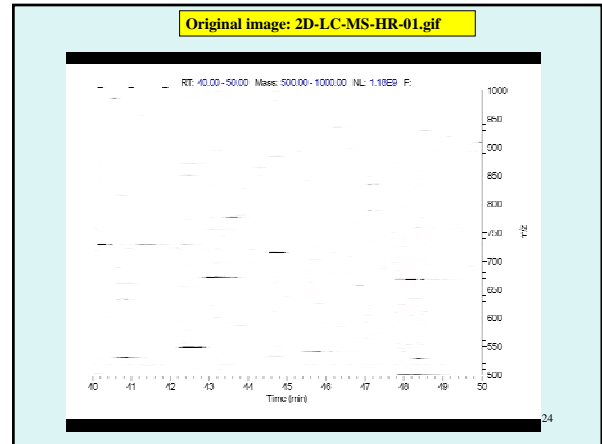


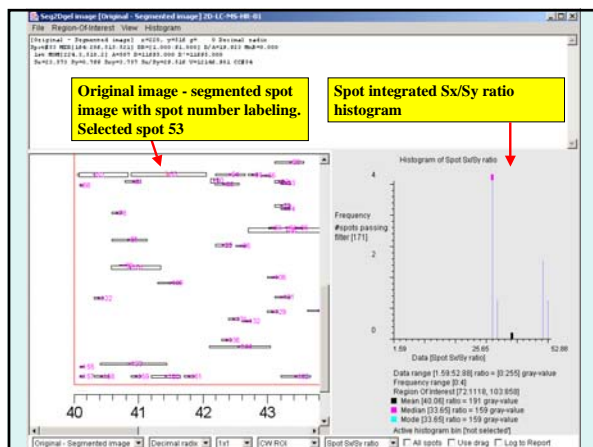
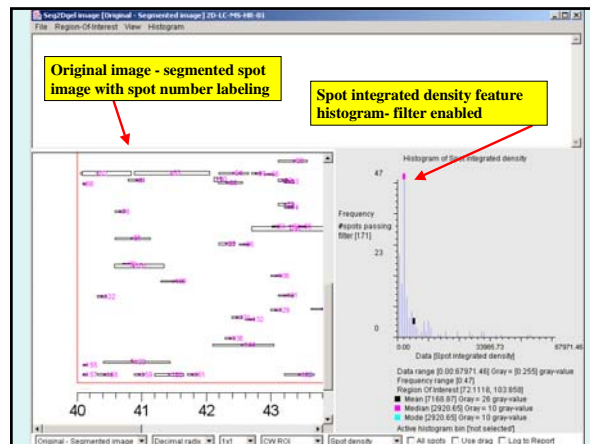
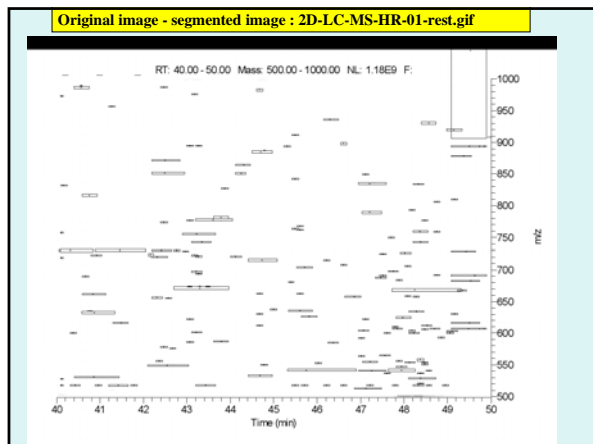


### Using the Segmenter on 2D LC-MS Data

- The following slides show that the segmenter can be used with low resolution (~800x600 pixel image shown here) 2D LC-MS images
- Uses `-laplacian:H,3,9` (horizontal Laplacian line filter)
- Pre-filter with 3x3 Gaussian filter (`-lowPass:3`)
- View spot rectangles using `-drawMinEnclosingRect`
- Use `-thrSxSyRatio: 2.5,100` to filter out small noisy line segments. This uses a density weighted spot X and Y deviation ratio  $Sx/Sy \geq 2.5$

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## Summary

- Seg2Dgel is an open-source 2D gel spot segmentation Java program freely available at <http://open2dprot.sourceforge.net/Seg2Dgel>
- Useful for segmenting spots in 2D gels and other images with similar types of data.
- It will be used as one of the step [2] alternative modules in the analysis pipeline in the Open2Dprot project at <http://open2dprot.sourceforge.net>