eReuss user manual

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Software configuration

eReuss requires Python 2.7 and the following libraries:

- Scipy
- Numpy
- Scikit-image
- Matplotlib

If you do not have Python or these libraries installed, a simple option is to use the free Anaconda package distributed by Continuum, Analytics™ which can be downloaded from:

`http://continuum.io/downloads`

After installing Anaconda for Python 2.x (not 3.x) run the `erserver.py` script, either with the console command `python erserver.py` in the eReuss folder or by double-clicking the `run.bat` (Windows) or `run.sh` (Linux) file. This will start the eReuss server.

Once the server is running, open a web browser and connect to the server at this URL:

`http://127.0.0.1:8081`

You should see the starting page for uploading your image file to eReuss.

To terminate the server, simply close the shell window.

Chapter 1

User Manual

At the eReuss starting page (`http://127.0.0.1:8081`) you will be able to upload your image to the server. Note that the server is a local server, so no data will leave your computer. The browser merely serves as an interface for eReuss.

1.1 Image preprocessing

Once you select your image file and press upload, you will be taken to the image preprocessing page.

Invert Image

eReuss assumes the gel image has a dark background and bright bands. If this is not the case, select `yes` for inverting the image. The `auto` option will invert any image for which the average brightness is above the midpoint of the brightness range in the image.

Band Color

In this droplist you should specify the main color of your bands when using colored images with a bright background. This makes eReuss ignore the color channel in which your bands will have a light intensity closer to the background, improving contrast.

Use `average` (default) for grayscale images, if you are using fluorescence images with a dark background or if the bands do not have a color close to red, green or blue. This option uses all the color channels in color images.

1.2 Clipping

In the image clipping page, you will specify the gel dimensions, the number of lanes used and select the region of interest (ROI) for processing. This also allows you to correct any problems with the orientation of the image.

In the `Comb length` box fill in the distance between the *beginning* of the first well and the *beginning* of the last well, in centimetres. This should be measured directly on the comb used to create the gel. Note that the distance is from the beginning of the first and last teeth in the comb and not to the end of the last tooth. Also fill in `Well count` the total number of wells and in `Used wells` the number of wells that will be included in the ROI.

Use the left mouse button to position the image on the right canvas and the mouse wheel to zoom the image so that you can see the line of wells. Position the mouse cursor at the beginning of this line and press `1` on your keyboard. This will move the top-left corner of the ROI to that position. Then move the mouse cursor to the end of the wells line, adjusting the image and zoom as necessary, and press `2` on your keyboard to define the top-right corner. This will place the top of the ROI, which should span over all the wells that were used and start a bit behind the midpoint of the wells to allow a margin for the band detection stage. Figure 1.1 illustrates this procedure.

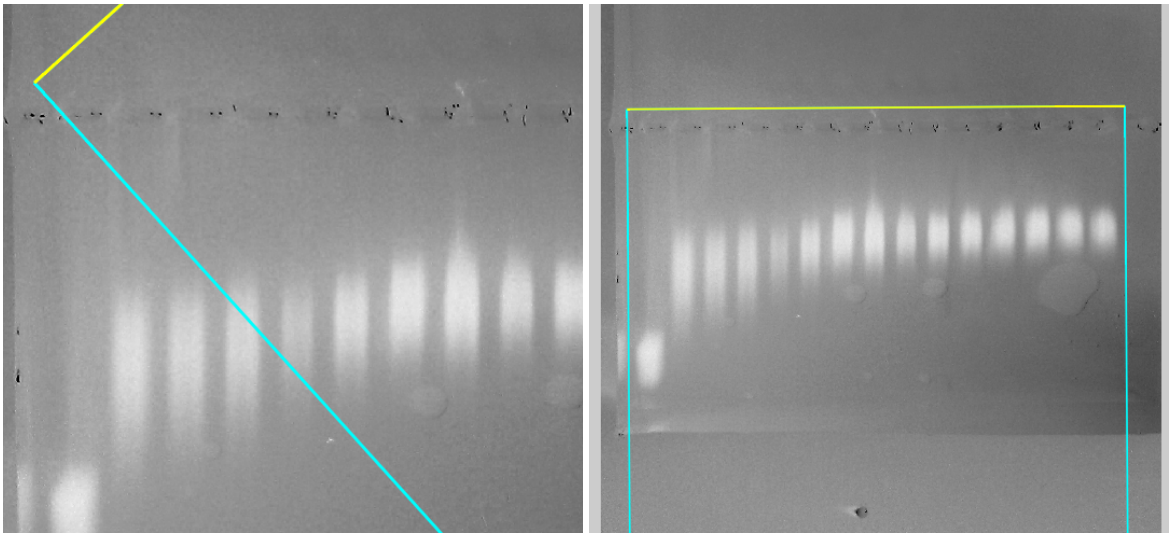

Figure 1.1: Setting the top of the region of interest: the left and right points, behind the start of the wells line. Gel image credits: Marta Giza, 2016

Next, move the mouse cursor to a point a bit farther than the farthest band, but not over the end of the gel, adjusting the image zoom and position as necessary. Then press `3` on your keyboard. This will set the length of the run and finish defining the region of interest. Adjust the rectangle as necessary by moving the mouse cursor slowly around the top-left corner or the top-right corner and repeatedly pressing `1` (for the left side) or `2` (for the right side). Look for the relative orientation of the lanes and the sides of the box, as these should be parallel.

Finally, set the cursor in the mid-line of a well and press `4` on your keyboard to set the starting line (represented in yellow). This is the line from which migration will be measured. Figure 1.2 illustrates these final two steps.

Note that the `1` key sets the point outside the first (leftmost) lane and the `2` key sets the point at the other extreme. Normally, this means pressing `1` for the left side and `2` for the right side. But if the imate was taken with the wells down and the bands above them, it's the other way around.

Once your are satisfied with the clipping rectangle, press the `Submit` button.

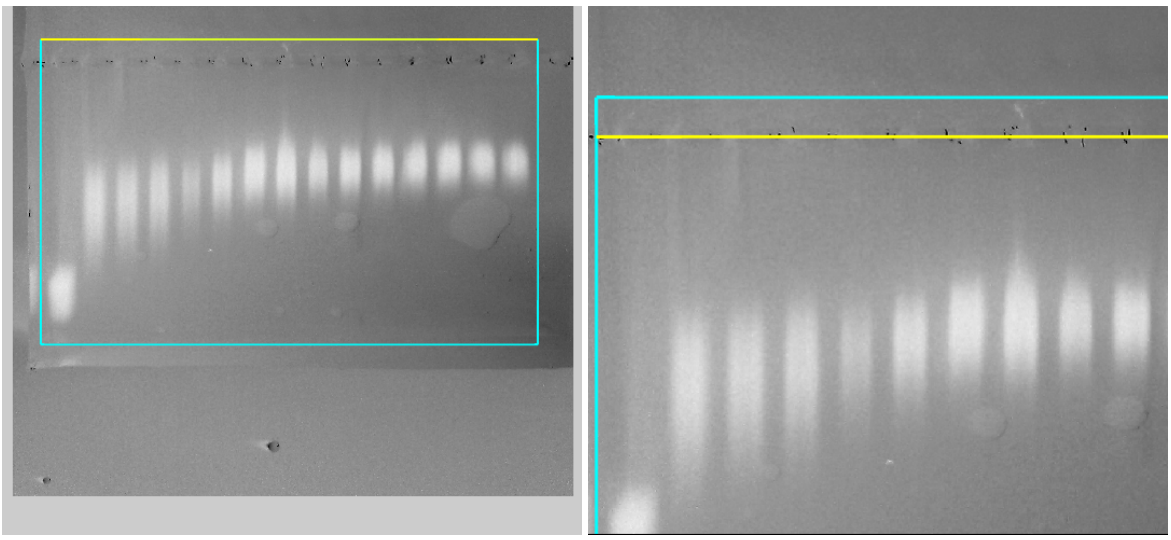

Figure 1.2: Setting the length of the region of interest and the midpoint of the wells line. Gel image credits: Marta Giza, 2016

1.3 Lane identification

This step identifies the lanes where the intensity values will be measured. Here you can correct the number of used wells, if it was wrong, and change the degree of the polynomial used in the baseline. The baseline is important to fit the lower intensity values corresponding to the gaps between the lanes. Figure 1.3 shows the lane identification graph. The green lines indicate the bands (higher values) and gaps (lower values). If the bands are not correctly identified because of excessive variation in the brightness of the gaps, you can try increasing the degree of the baseline. Press the `Submit` button to recompute the lanes if necessary.

You can also assign concentration or concentration ratio values to the `X vals` text box. These should be formatted one per line and the number of lines must match the number of lanes used. If these values are provided, eReuss will compute Langmuir and Hill curve fits based on the relative migrations of the strongest band in each lane.

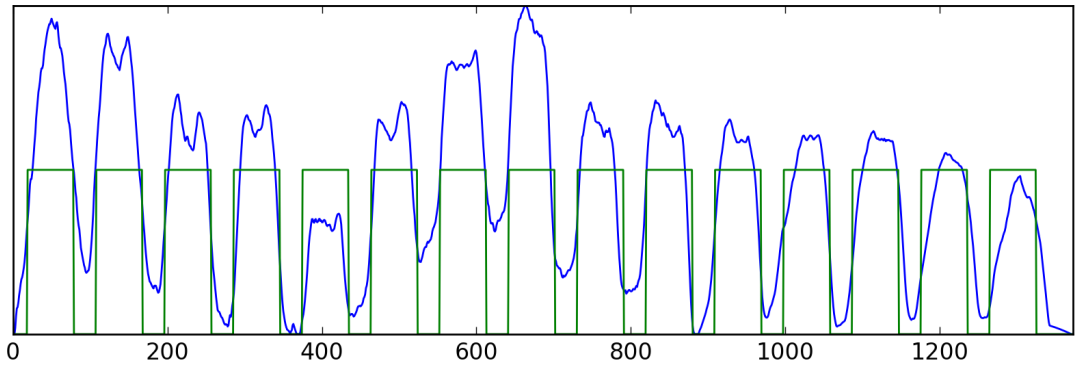

Figure 1.3: Lane identification

Once you are satisfied with the identification of the lanes, follow the link `Proceed to lane profiling` below the form.

1.4 Profiling

This step measures the peaks in the different lanes. The `Smoothing` parameter sets the size of the window (in pixels) to smooth the lane profiles with a moving local average. The `Degree for baseline` parameter sets the polynomial degree for the baseline.

Peaks are identified by iteratively fitting a gaussian distribution to the maximum value in the curve, subtracting that distribution and repeating until either the `Number of Gaussians` was reached or the maximum value falls below the `Minimum height` parameter, which is a percentage of the image brightness range. Figure 1.4 shows the result of fitting one Gaussian curve to each profile.

Press `Submit` to recompute the peaks after changing any parameter and follow the `Final report` link when satisfied with the results.

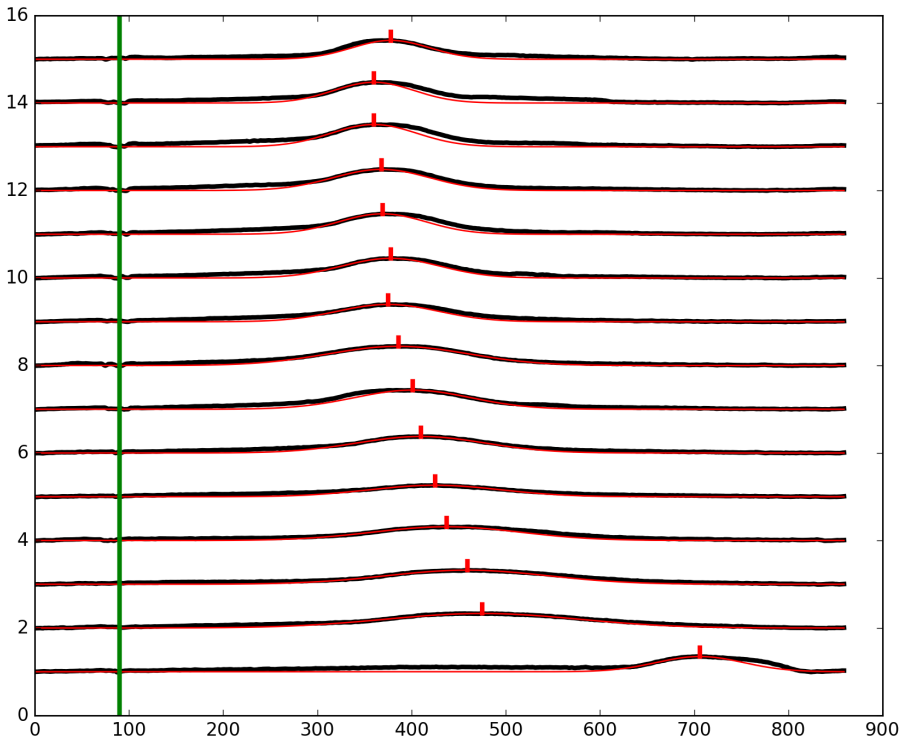

Figure 1.4: Peak identification. The green line shows the starting line for migration. The black plots represent the profile for each band, with the red lines showing the Gaussian curves and the red markers the peak positions.

1.5 Final report

This page summarizes the results and allows you to choose the base file name for the final report. By default, this is the base file name of the image uploaded. However, all spaces in this name will be replaced by the underscore character.

This file name will be used to create a zip archive with the following files:

- `image.png`: clipped and preprocessed image

- `bands.png`: plot of the band profiles and fitted gaussians
- `[base name].csv`: table with peak positions, heights and areas
- `[base name].xml`: detailed data on band profiles. This file is meant for future use when aggregating data from different gels.
- `langmuir.png`: plot of the Langmuir isotherm fit if concentrations or concentration ratios are given.
- `hill.png`: plot of the Hill curve if concentrations or concentration ratios are given.

If concentrations or concentration ratios were given in the band identification step, then eReuss will compute Hill and Langmuir curves and the computed parameters and residual errors will be included in the `csv` file. Note that the units will depend on the units of the values given in the band identification step.

1.6 To Do

- Fine tuning of band identification (scanning different gap and width combinations for each band)
- Quantification (if necessary): currently all brightness values are scaled during image processing, so absolute values are not preserved.
- Peak position: band positions correspond to the peak values. Should a different value be used (10
- Other plots