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If you use this software please cite the following paper:

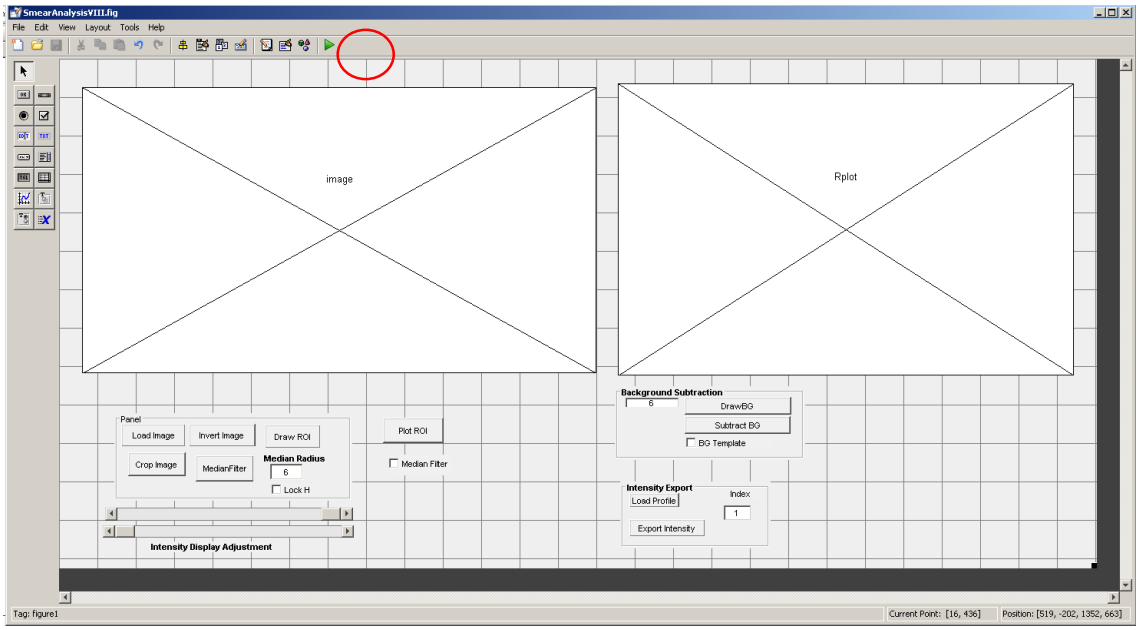
M.A. Koussa*, K. Halvorsen*, A. Ward, W.P. Wong, DNA Nanoswitches: A quantitative platform for gel-based biomolecular interaction analysis, *Nature Methods* (2015)

Overview

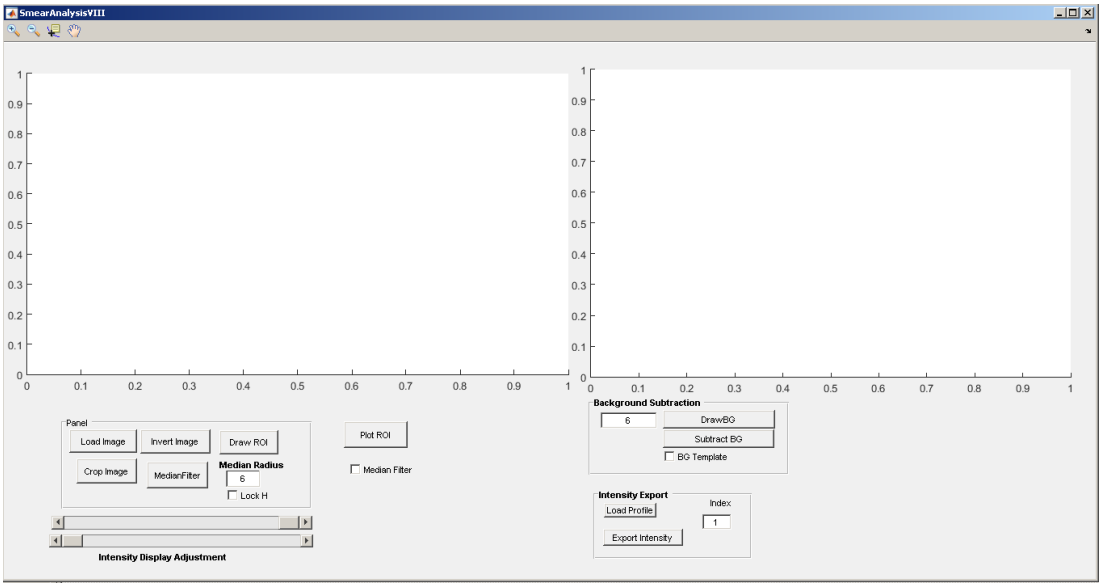
The Gel analysis software consists of three different steps. First the intensity profiles are extracted from the image in the SmearAnalysisVIII GUI. This works similarly to the ImageJ Gel plugin. The profiles are then exported to the matlab workspace. From there the profiles can be fit with Scewed Guassian functions with the FitScewProfilesScript.m file. The areas based on the fits can be calculated using the FindScewAreas function.

Intensity Profile extraction

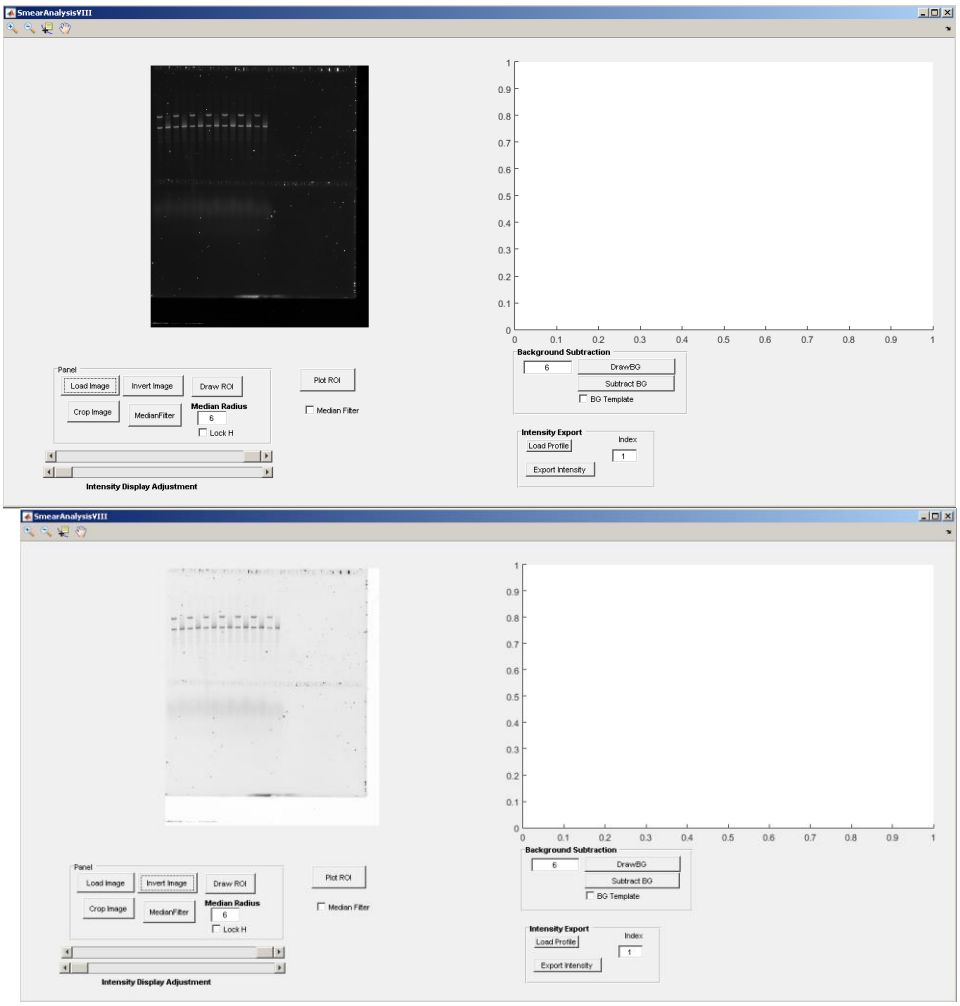
Open SmearAnalysis VIII.fig by calling guide in the command line, browsing to the file and opening.



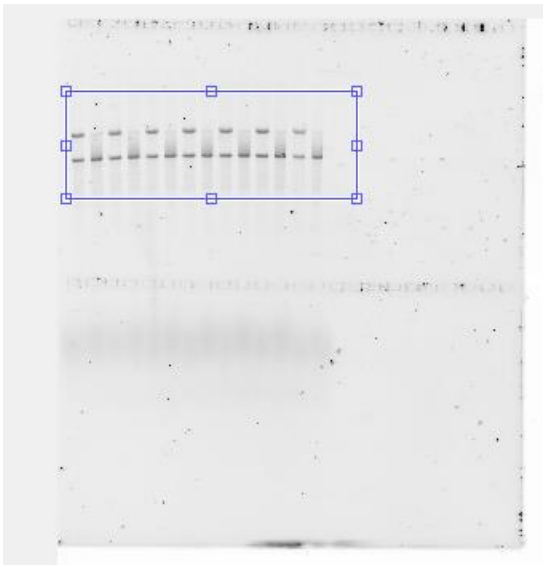
Run the GUI, by clicking the green run button.



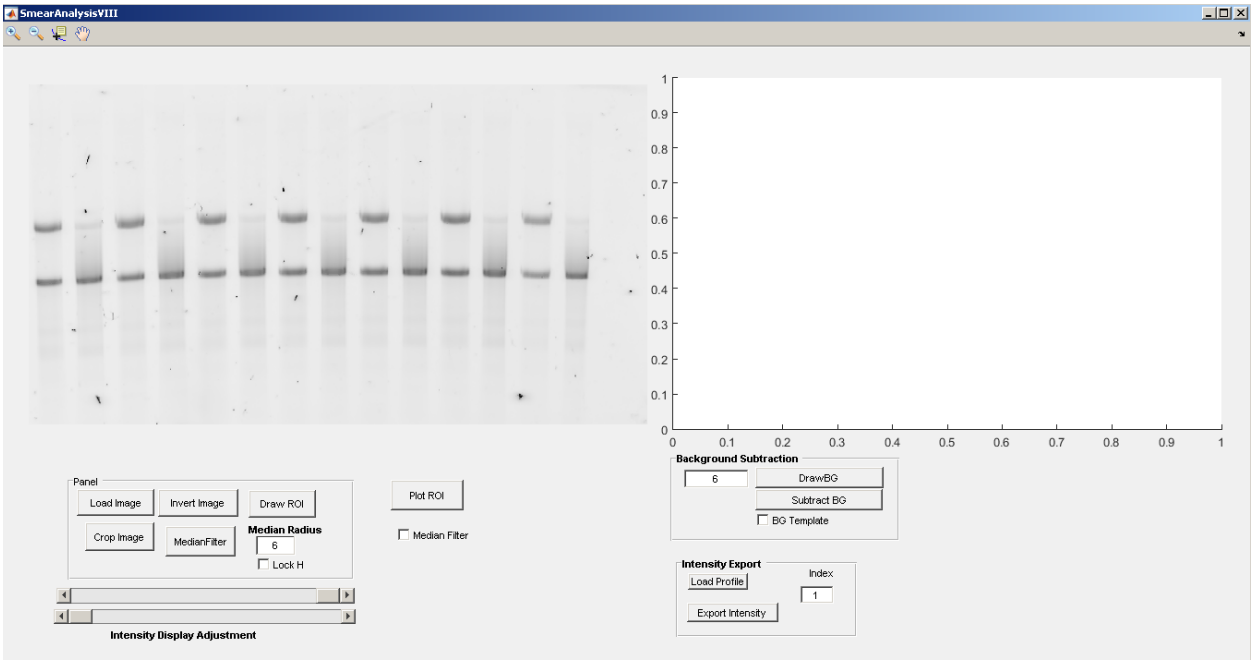
To load an image click the load image button. **Remember to use linear Tif files, .Gel files usually do not have a linear intensity scale, this can mess up quantitative analysis.** Everything in the analysis assumes that the bands should be dark and the background light. So there is an invert image button to invert your image accordingly.



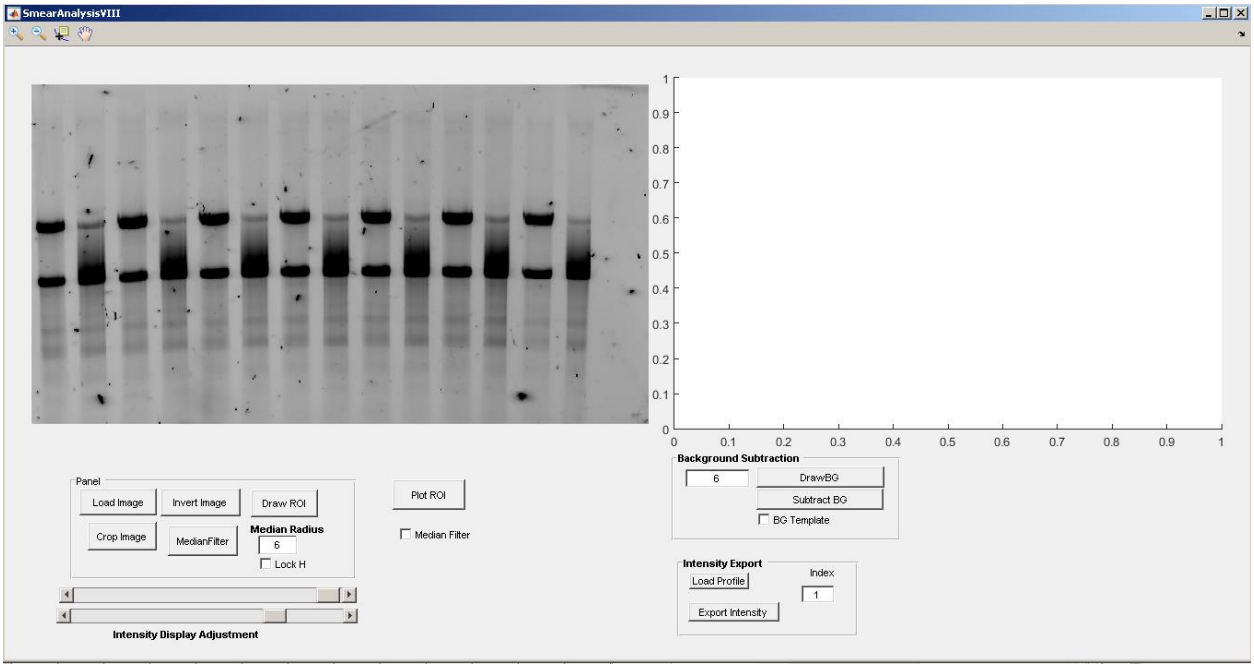
This is a very large image, to make it easier to work with you can crop the image down. To Crop and image click the Draw ROI button, that allows you to draw a region of interest(ROI) around the area you want to crop. Once clicked a the mouse will turn into a crosshair that you can draw a resizable ROI on the image with



Once drawn, then click the crop button.

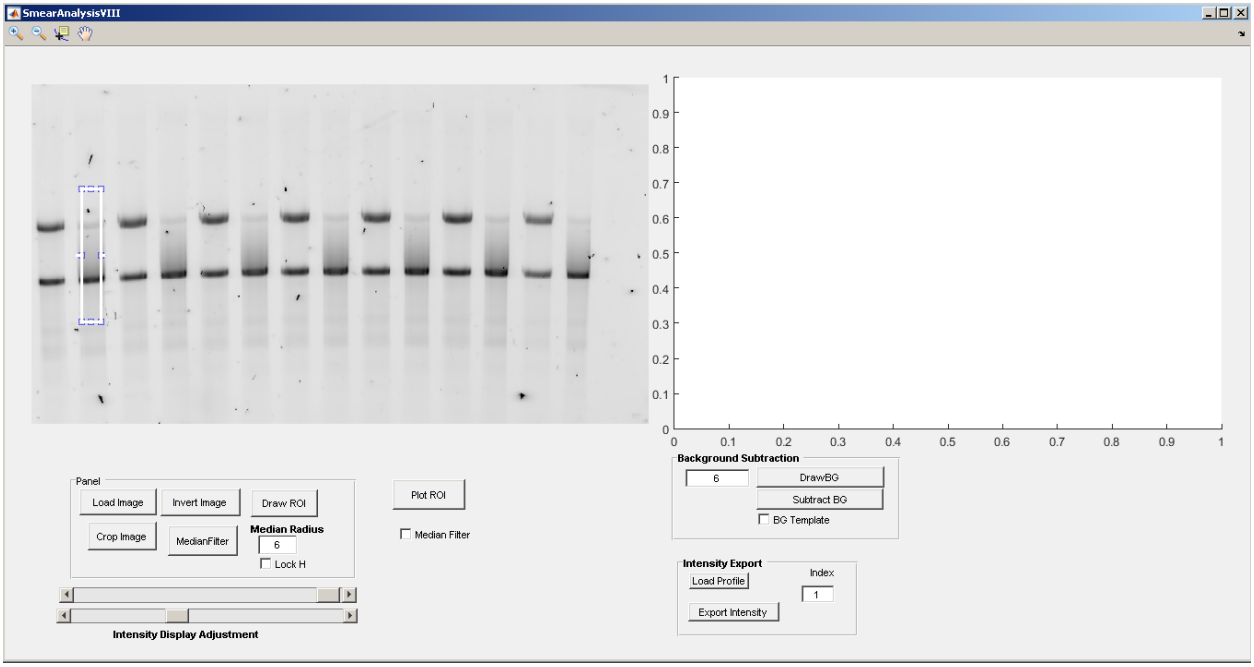


If the ROI remains on the screen you can right click, and delete to remove it.



You can also adjust the image contrast by moving the sliders.

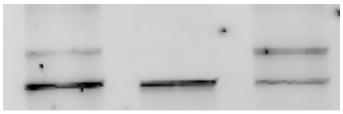
To plot the intensity profile of a lane. Click the Draw ROI button again and draw a box around the gel you want to analyze.



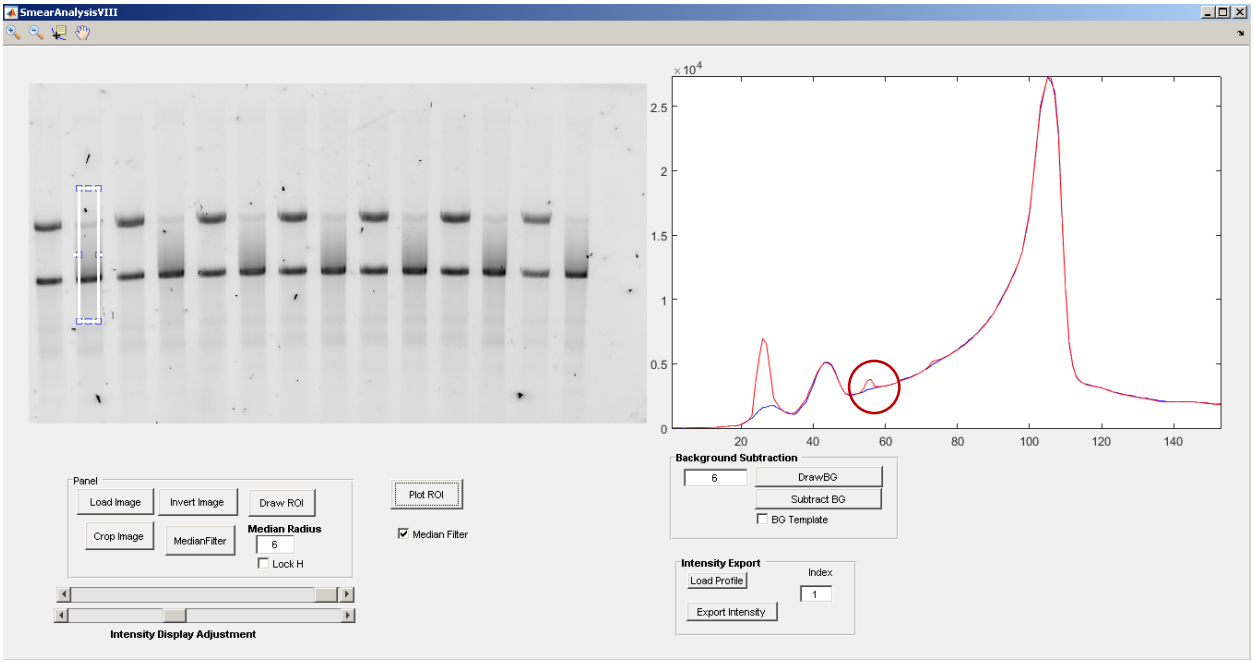
Make sure the box is not wider than the lane width. In can always be thinner. To plot this profile click the Plot ROI button. The 2D ROI is converted into a 1D intensity profile by either taking the mean of each row, or the median (if the Median Filter box is checked). The median filter is helpful for removing some image

speckle. Doing the median filtering only along the row allows you to preserve the resolution along the band migration direction while still removing the image speckle. If the median filter is checked the unfiltered profile is plotted in red, and the filtered profile is plotted in blue.

You can also sum the intensity along the X direction. This can be helpful if you want to fully quantify all of the material in each lane, for comparisons to other lanes, and the lanes do not all run the same. To do this check the Sum Intensity box (This is a newer feature so it is not in all of the screen shots). You cannot currently do the median filter and the Sum intensity.



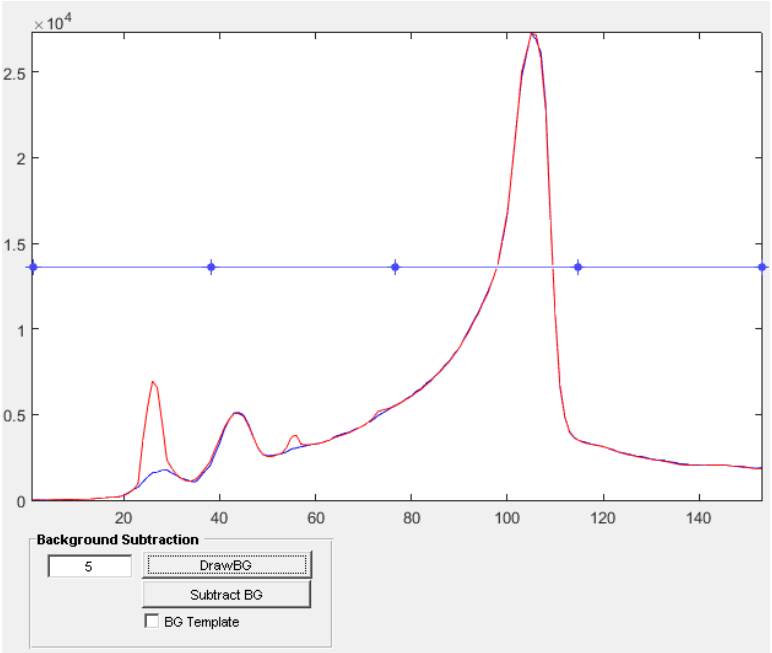
Example where you may want to sum the intensity rather than take the mean or median

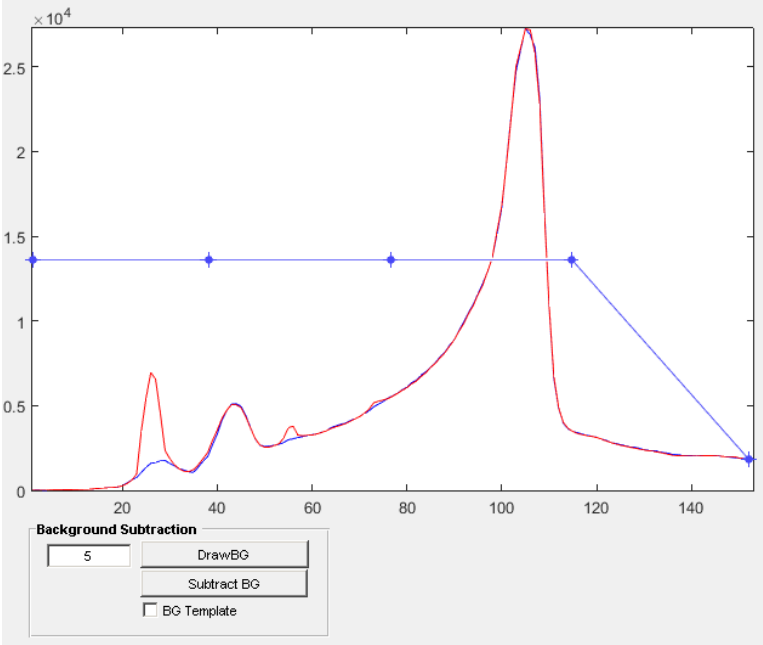


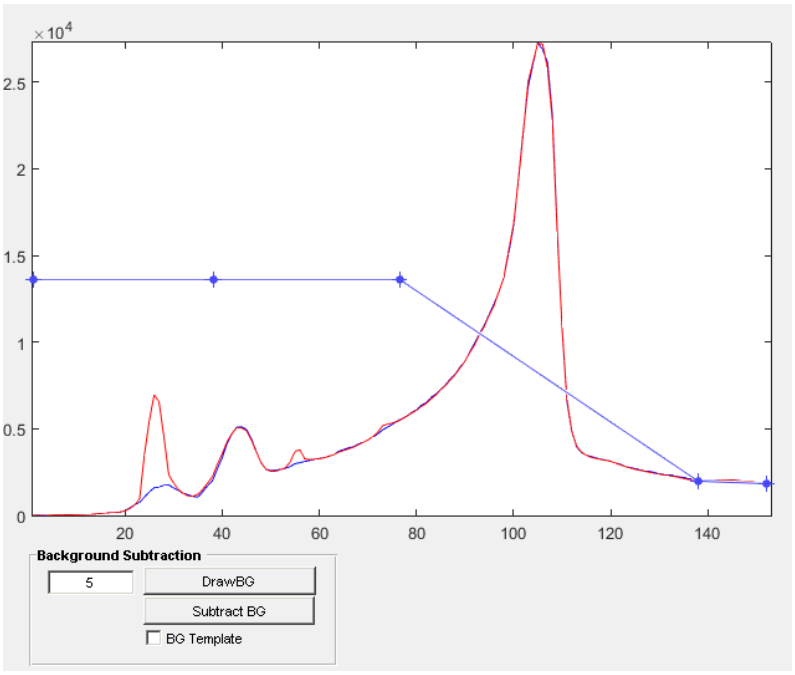
Above is shown how the median filter has nicely removed the speckle in this profile (red circle).

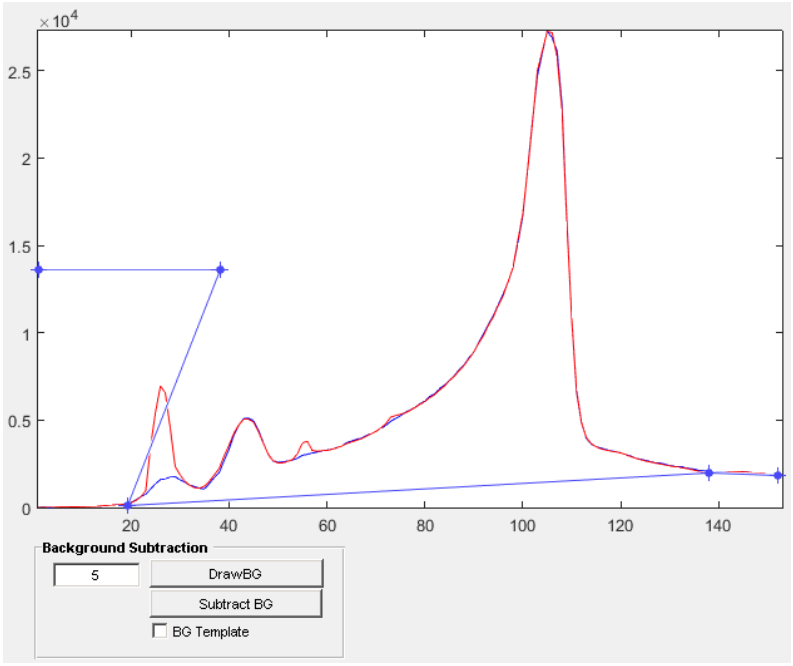
For proper quantification of the gel band the background signal needs to be subtracted out. This is done manually by defining points along the profile. The software then assumes that the background signal is goes through those points linearly. The number of points to use can be input in the Background

Subtraction box (here it is 5). To select the points click the Draw BG button. Then move each point to the desired location.






×10^4
2.5
2
1.5
1
0.5
0
20
40
60
80
100
120
140
Background Subtraction
5
DrawBG
Subtract BG
BG Template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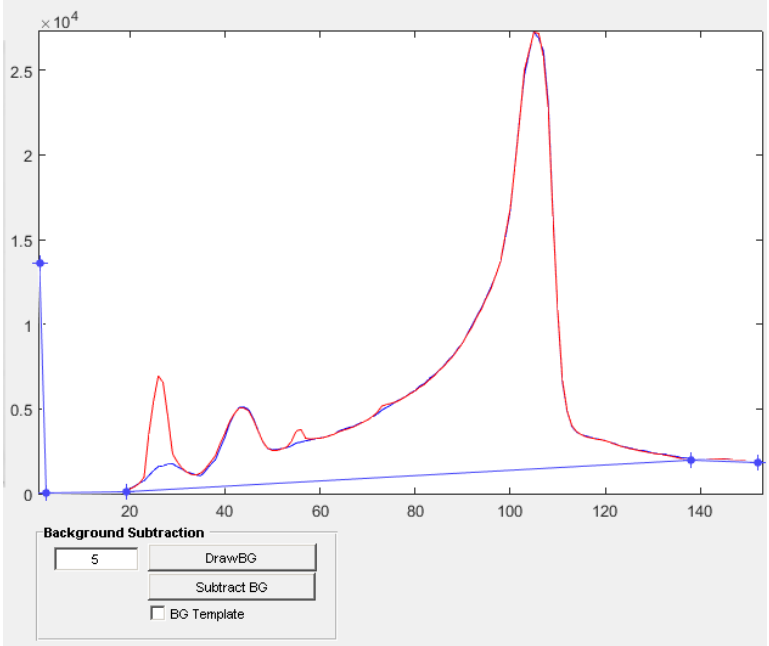
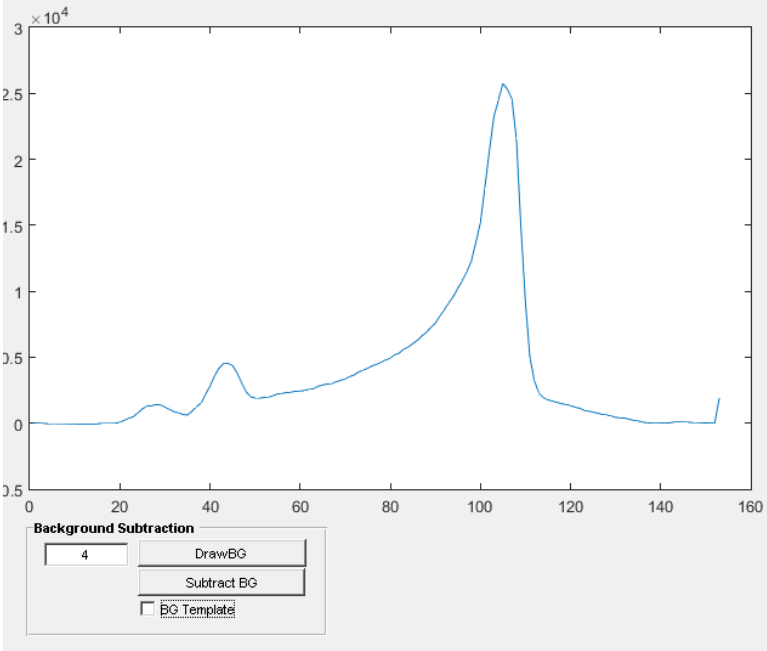


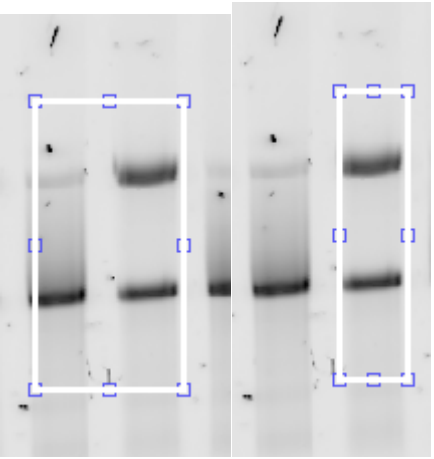
Here there are more points than necessary you can remove any point by right clicking on, and selecting delete vertex. Once you are satisfied with the selection you can click subtract BG



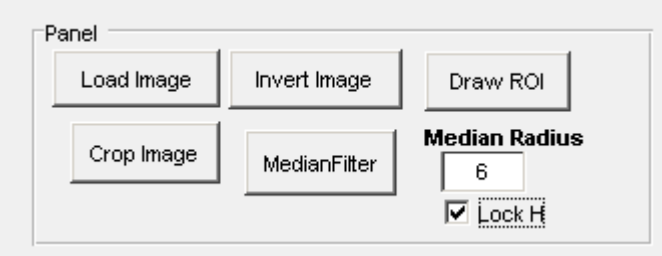
The BG has now been subtracted. You can keep the background subtraction consistent for each lane by checking the BG Template box after you have successfully subtracted the background. This will always use the same vertex points for each lane. Of course if the gel is not homogenous you need to redraw the BG subtraction lines. This profile is ready to be saved now. To save it click the Load Profile button

Once clicked it will save the current profile in the current index number and increment the index number.

If you realize you made a mistake you can manually change the index number redo the lane analysis and Load the profile into that index. To analyze the next lane you need to move the ROI. You can do this many ways. You could just draw the entire box over, or delete and redraw the box. To keep the x coordinates of the each intensity profile the same you can move the ROI in a two-step fashion by first moving the right edge over and then moving the left edge over



You can also click the lock H box, to lock the height position of the box this allows you to move the box in the x direction without worrying about accidentally changing the y position.



At any time you can click the export intensity button to export the profiles to the Matlab Workspace for analysis. The Profiles and X coordinates for each lane are stored in two cell arrays TempIall(Intensity) and TempXall (XCoordinates).

To fit the intensity profiles for each lane first run the following command:

```
Iall=TempIall;
Xall=TempXall;
Mod=0;
Start=1;
```

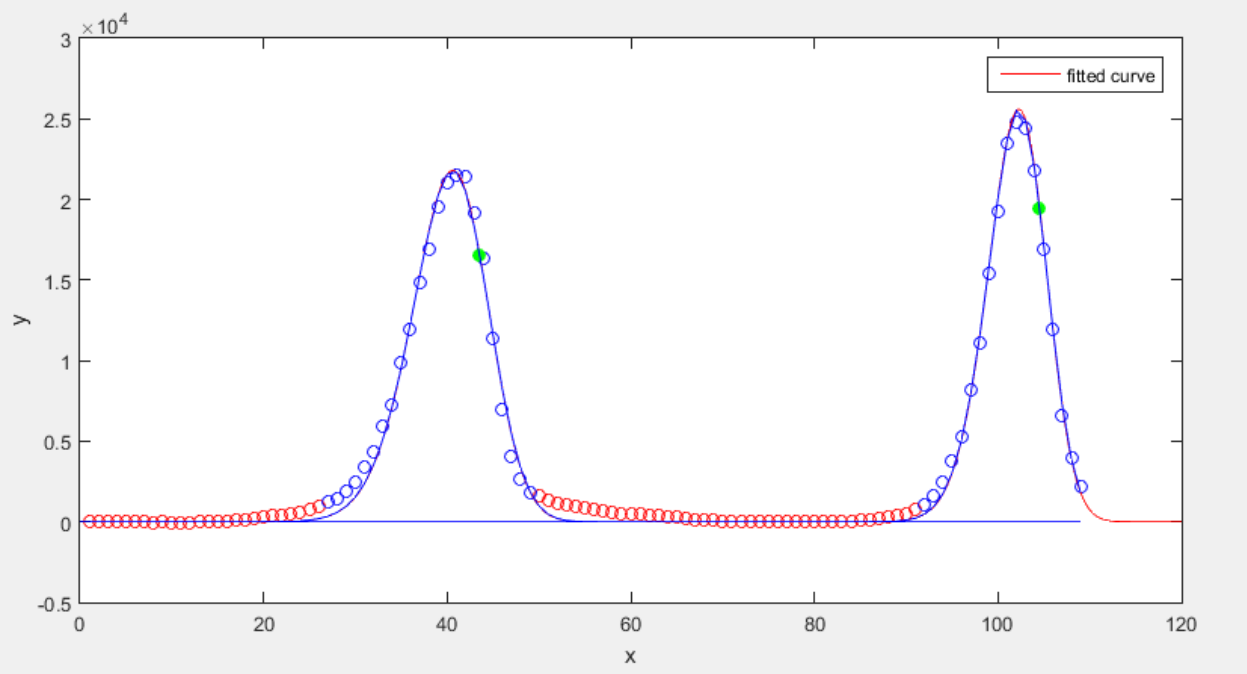
Next run FitScewProfilesScript.m

You will be prompted in the command window for the fitting parameters:

```
>> FitScewProfilesScript
Enter number of peaks to fit with Gaussian: 2
Format is a*exp(-((x-b)/c)^2)*ScewFunction
Enter a vector of starting positions for peaks centers [b1 b2...]: [41 103]
Enter a starting guess for the width: 5
Enter a starting guess for skew, Upper, Lower: [-1,0,-10]
Change in fit parameters absolute change for db [da db dc]: [5000 10 5]
Enter vector desribing fit region in even pairs [xb1 xe1 xb2 xe2..]: [27 49 92 109]
Enter number of refits: 2
```

The for a scew to the left the scew parameter is negative, and for the right it will be positive. [da db dc] set the fit bounds for a, b and c.

Once entered the the profile will be fit, and the results will be displayed on the screen




```
fdt =

     General model:
     fdt(x) = A1*exp(-(x-b1)^2/c1^2)*(1+erf(a*(x-b1)/c1))+A2*exp(-(x-b2)^2/c2^2)
                   *(1+erf(a*(x-b2)/c2))
     Coefficients (with 95% confidence bounds):
       A1 =    1.652e+04  (1.522e+04, 1.783e+04)
       A2 =    1.941e+04  (1.787e+04, 2.094e+04)
       a =       -1.325  (-1.666, -0.9844)
       b1 =        43.55  (43.06, 44.04)
       b2 =        104.5  (104.2, 104.9)
       c1 =        7.742  (7.099, 8.386)
       c2 =        5.983  (5.487, 6.478)
Is fit acceptable? (1 or 0):
```

The fit can be sensitive to starting guess and bounds so you may need to tweak the parameters by selecting entering 0. Once satisfied enter 1, and it will automatically fit the next lane with the previous lanes parameters, you may need to change the parameters. Most commonly you need to shift the start guesses for the peak locations (b) This can be done be manullay reentering the vector of start positions, or shifting each guess by a given amount (shown below)

```
Is fit acceptable? (1 or 0): 0
Change bounds (b) Change region (r) Change Number Peaks(p): b
Change b(b), c(c), delta (d), scew (s) or all (all): b

Q =

b

New Vector (n) or delta (d) ?: d
Enter shift distance: 1
```

At any time you can stop the script (Ctrl-C) and restart it. If you want to start at the 10th profile set Start=10. Also make sure you set Mod=1.

Once finished you have a cell array of the fit parameters named fd.

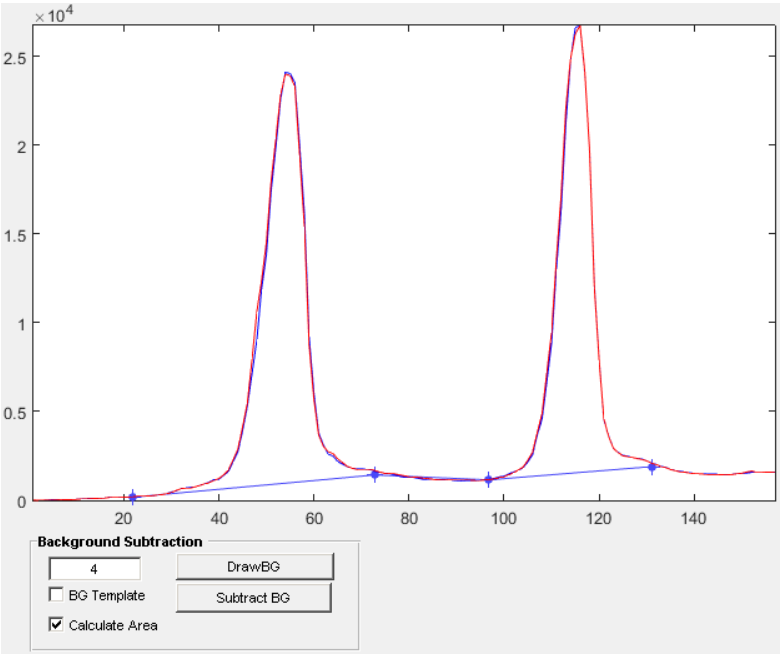
To compute the areas from the fit parameters call the FindScewAreas function.

There is also a function to calculate the areas manully without fitting by selecting points.

ManualAreas.m

New Feature:

Added in the manual Area functionality to the GUI. If you only want to draw the lines to calculate the areas without fitting, set the BG subtraction number to 2Xnumber of peaks. And put each set of vertices around the peaks you want to calculate the area for.



Make sure the Calculate Area button is checked. Now when you click the Subtract BG button it will subtract off the background according to the vertex regions you have selected, and compute the area between each set of vertices.

When plotting new lanes you can still keep the BG Template button selected and it will calculate the Areas for each lane using the same points. You can of course leave the BG Template button unselected and move the vertices for each lanes.

Now when you click the Load Profiles button it also loads the areas as well.

When you click the Export Intensity button it will also export the Areas into the workspace as two variables. AreasAll, which is the areas for each lane by row, and AreasNormAll which is the normalized areas.