Supporting Information

Next-Generation TLC: A Quantitative Platform for Parallel Spotting and Imaging

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I. General Procedure

All materials used in the synthesis of each compound and related tests were purchased from Sigma-Aldrich Chemical Co., Acros Organics, etc. and used without further purification. Solvents (Hexanes, Ethyl Acetate, MeOH and MeCN) were of reagent grade or HPLC grade quality and purchased from Fischer Scientific. NMR solvents (CD_3OD) were purchased from Cambridge Isotope Laboratories

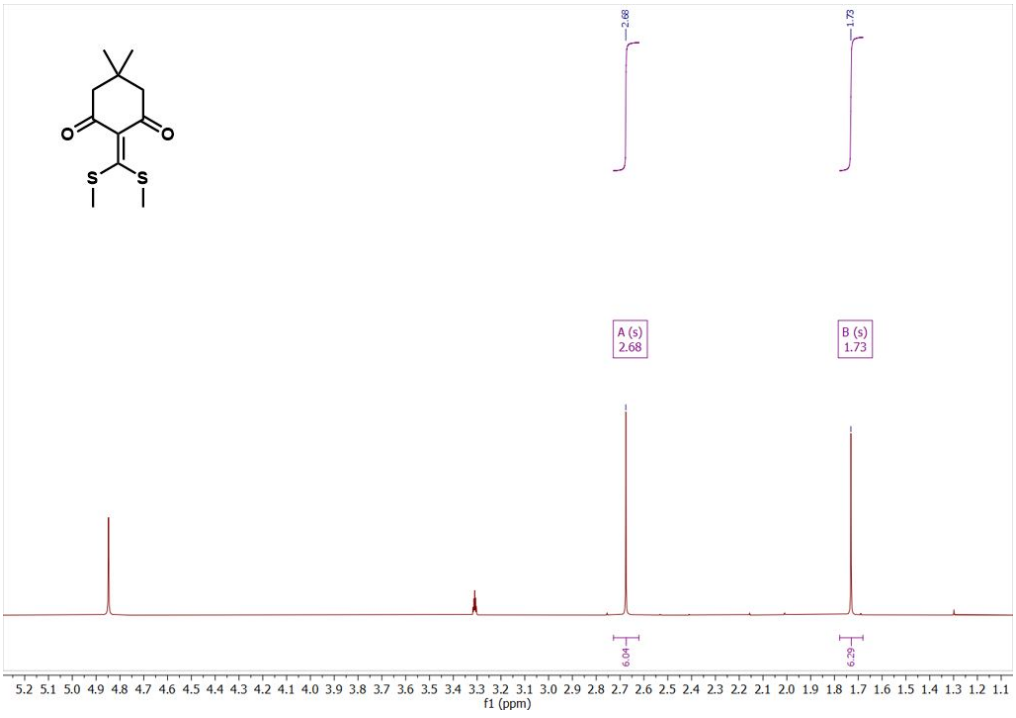
NMR spectra were taken on a Bruker AVANCE III 500 MHz NMR spectrometer.

TLC analyses were carried out using Silica TLC Plates Backing 20 by 20 cm sheet UV active at 254 nm.

Liquid Chomatography/Mass Spectrometry data was recorded on an Agilent Technologies 6120 Single Quadrapole or 6130 Single Quadrapole interfaced with an Agilent 1200 series liquid chromatography system equipped with a diode-array detector. Resulting spectra were analyzed using Agilent LC/MSD ChemStation. All liquid chromatographs were run as 5-95% gradient elution (MeOH/Water or MeCN/Water) over 15 minutes.

II. Synthesis and Characterization

Compound **1** was prepared according to literature procedure.[1] **1H NMR** (500 MHz, CD_3OD) δ 2.68 (s, 6^{i}H), 1.73 (s, 6H).



III. Results of TLC Analysis

Exchangeable Unit	Conversion%	Exchangeable Unit	Conversion%
1,4-phenylenediamine	100	4-methoxybenzenethiol	63
1-dodecanethiol	21	dithiothreitol	*
2-mercaptoethanol	3	isopropylamine	9
1,4-butanedithiol	23	1-propanethiol	3
Control	0	Veratrylamine	100
3,6-dioxa-1,8-octane-dithiol	19	1-octanethiol	27
p-Xylylenediamine	100	Control	3
1-decanethiol	4	dibutylamine	100
4-methoxybenzylamine	100	1,3-propanedithiol	-6
dodecylamine	100	m-Xylylenediamine	100
N-benzylmethylamine	100	isopropylamine	100
1,2-phenylenediamine	49	Oleylamine	100
4-methoxybenzenethiol	39	nonylamine	100
2-methoxyphenethylamine	100	allylamine	100
1,2-ethanedithiol	6	hexylamine	69
1,3-propanedithiol	6	R-Cyclohexylethylamine	100
2-methoxybenzylamine	100	S-Cyclohexylethylamine	100
Control	*	2-Aminoheptane	100
cis-Myrtanylamine	100	Propargylamine	100
decylamine	100	Control	7
2-napthalenethiol	-3	diethylamine	100
1-methyl-3-phenylpropylamine	100	3,4-dimethoxybezylamine	-7
1-octanethiol	*	Oleylamine	33
heptylamine	100	3,6-dioxa-1,8-octane-dithiol	17

Table S1. List of Exchangeable Units and their Resulting Reaction Conversion

*Denotes spot overlap on the TLC. Conversion was unable to be determined.

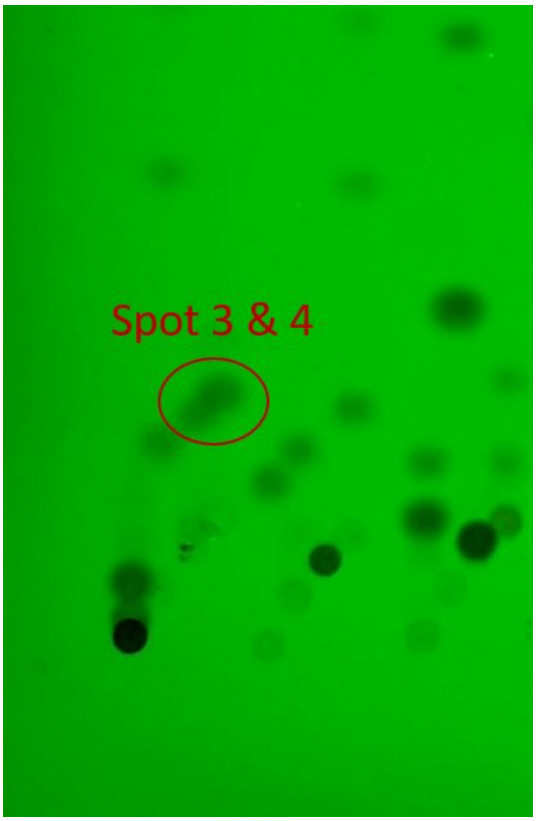


Figure S1. Overlap between spot 3 (2-mercaptoethanol) and spot 4 (1,4-butanedithiol)

IV. ^{1}H NMR and LC/MS spectral data for conversion analysis

Due to the small scale these reactions were run on, the conversion of each control reaction was evaluated using NMR with 1,3,5-Trimethoxybenzene (TMB) as an internal standard. A ratio of peak the peak integrations of (TMB:**1**) were used to calculate conversion. The A summary of the results is given below (Table S3).

Scheme S1. Meldrum's acid derived conjugate acceptor **1** with various thiols or amines as nucleophiles. E.U. = Exchangeable Unit.

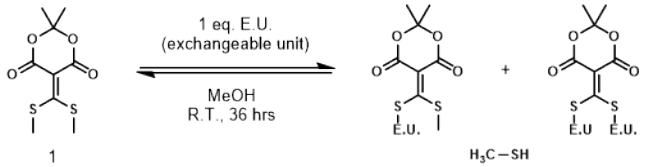
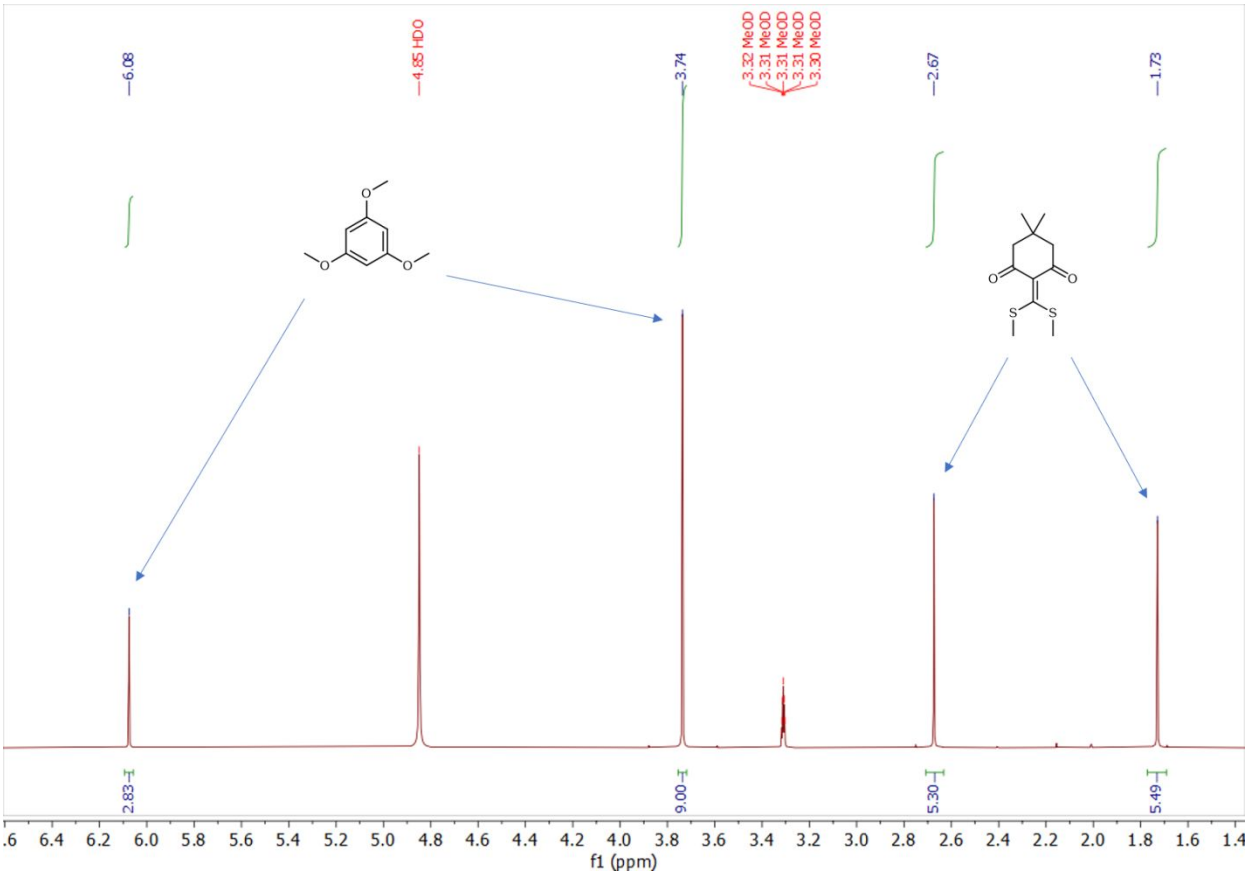


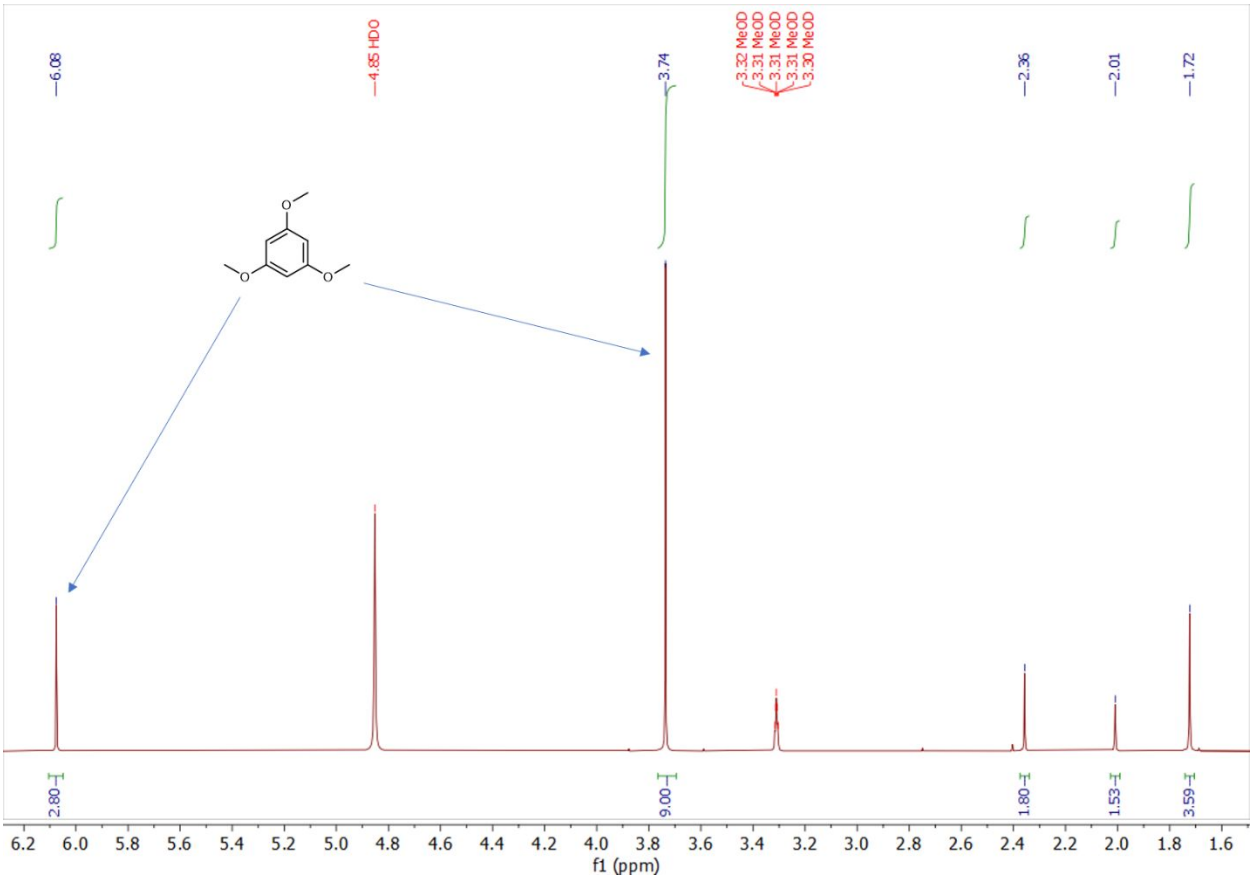
Table S2. Conversion analysis of 10 reactions using ^{1}H NMR and LC/MS.

Reaction	Exchangeable Unit	%Conversion
1	1,4-phenylenediamine	100
2	1-dodecanethiol	12
3	2-mercaptoethanol	0
4	1,4-butanedithiol	10
5	3,6-dioxa-1,8-octane-dithiol	0
6	p-xylylenediamine	100
7	1-decanethiol	20
8	4-methoxybenzylamine	96
9	dodecylamine	100
10	N-benzylmethylamine	94

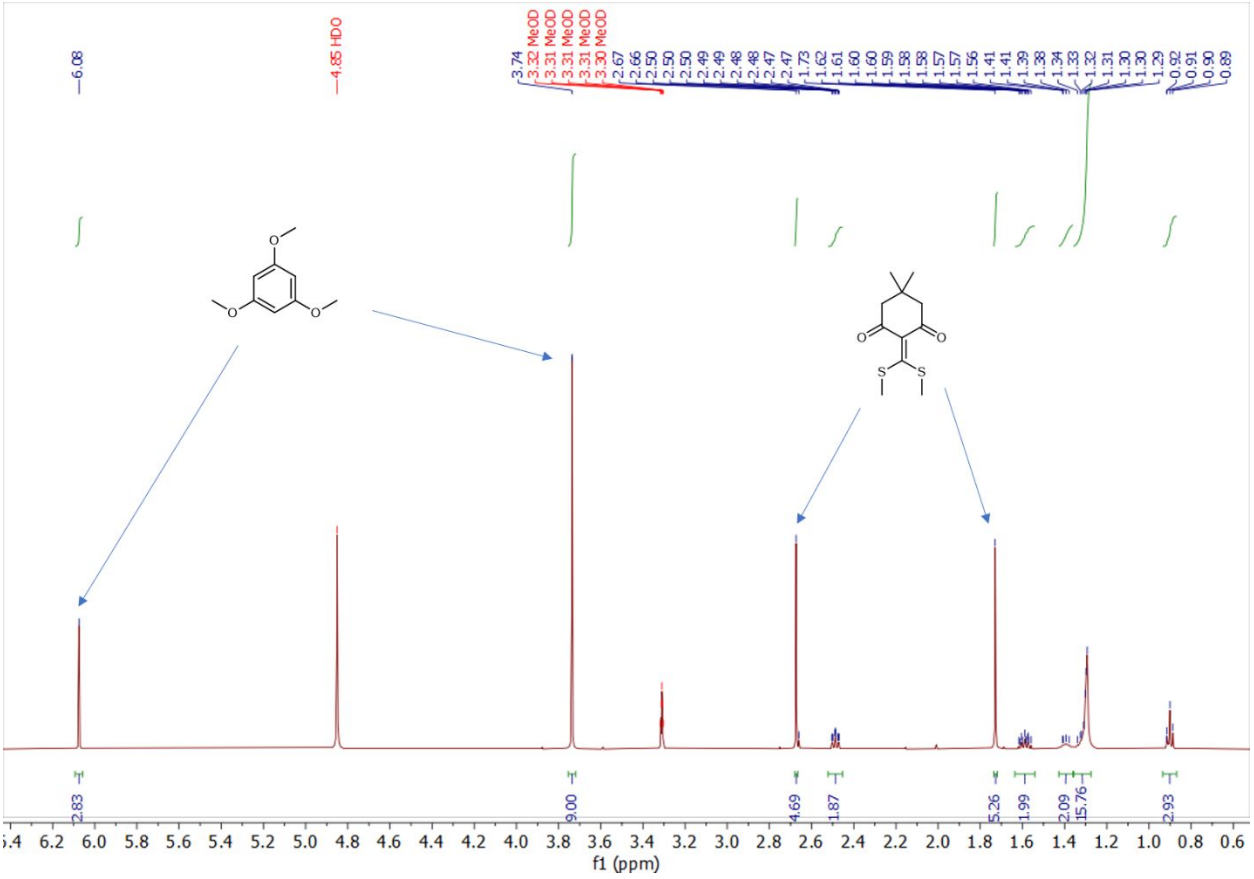
Control Reaction in CD_3OD.



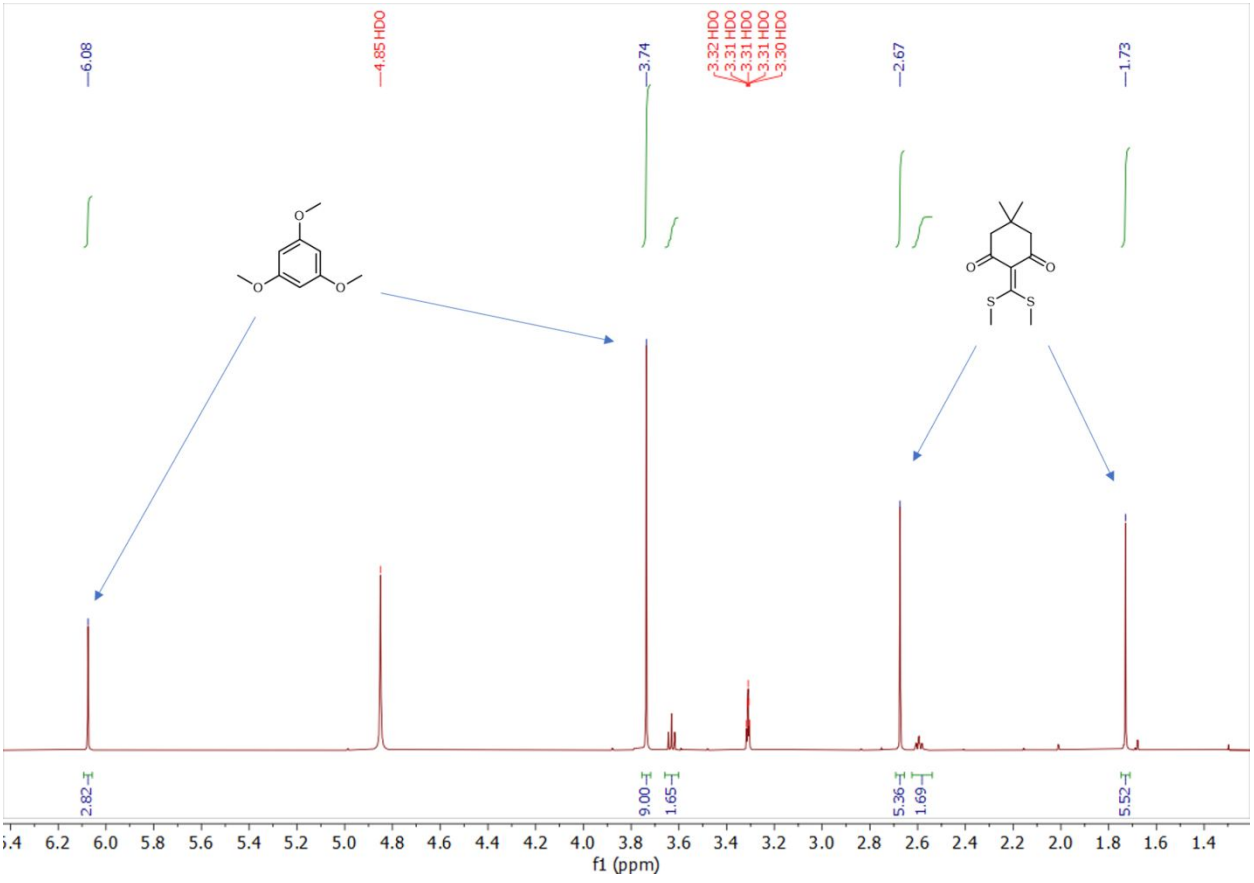
Reaction 1 in CD_3OD. Conversion was determined to be 100%.



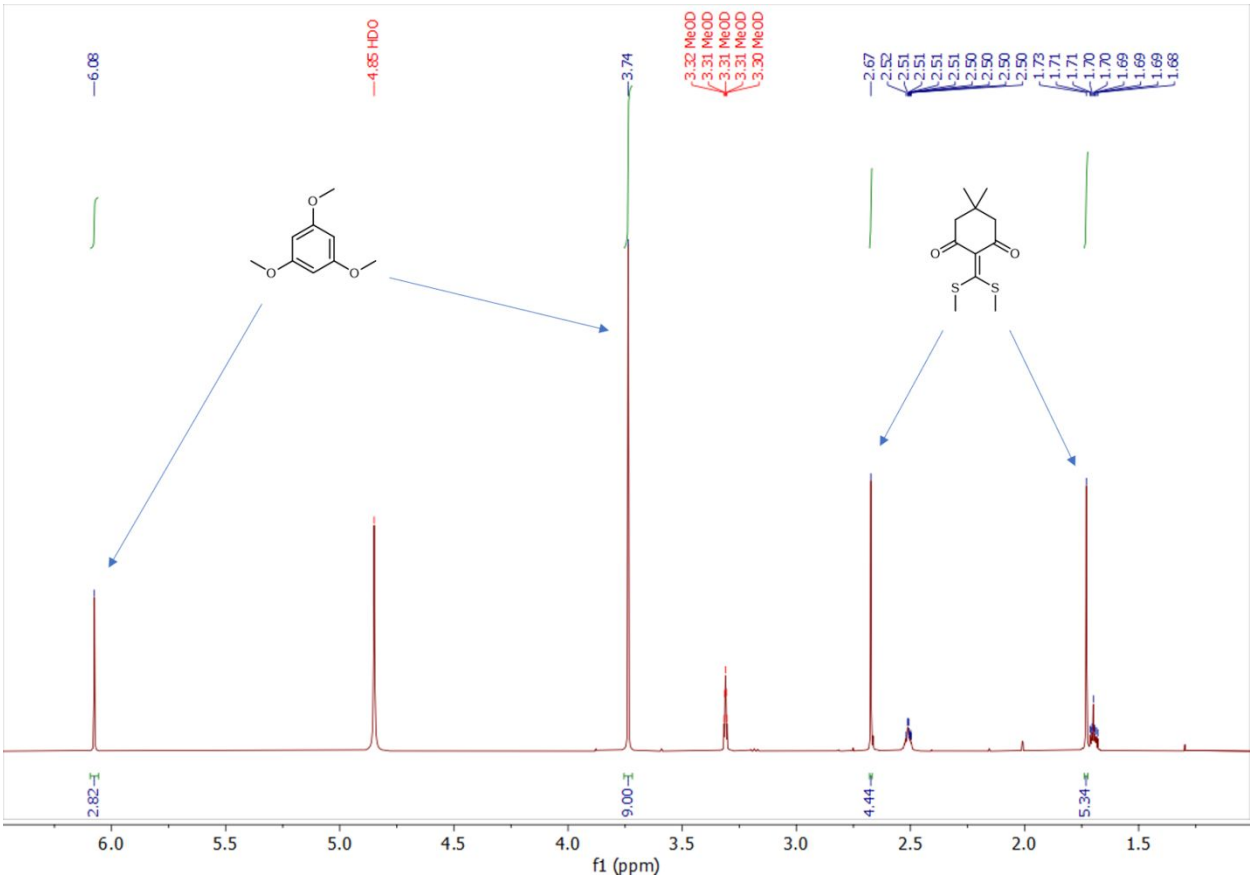
Reaction 2 in CD_3OD. Conversion was determined to be 12%.



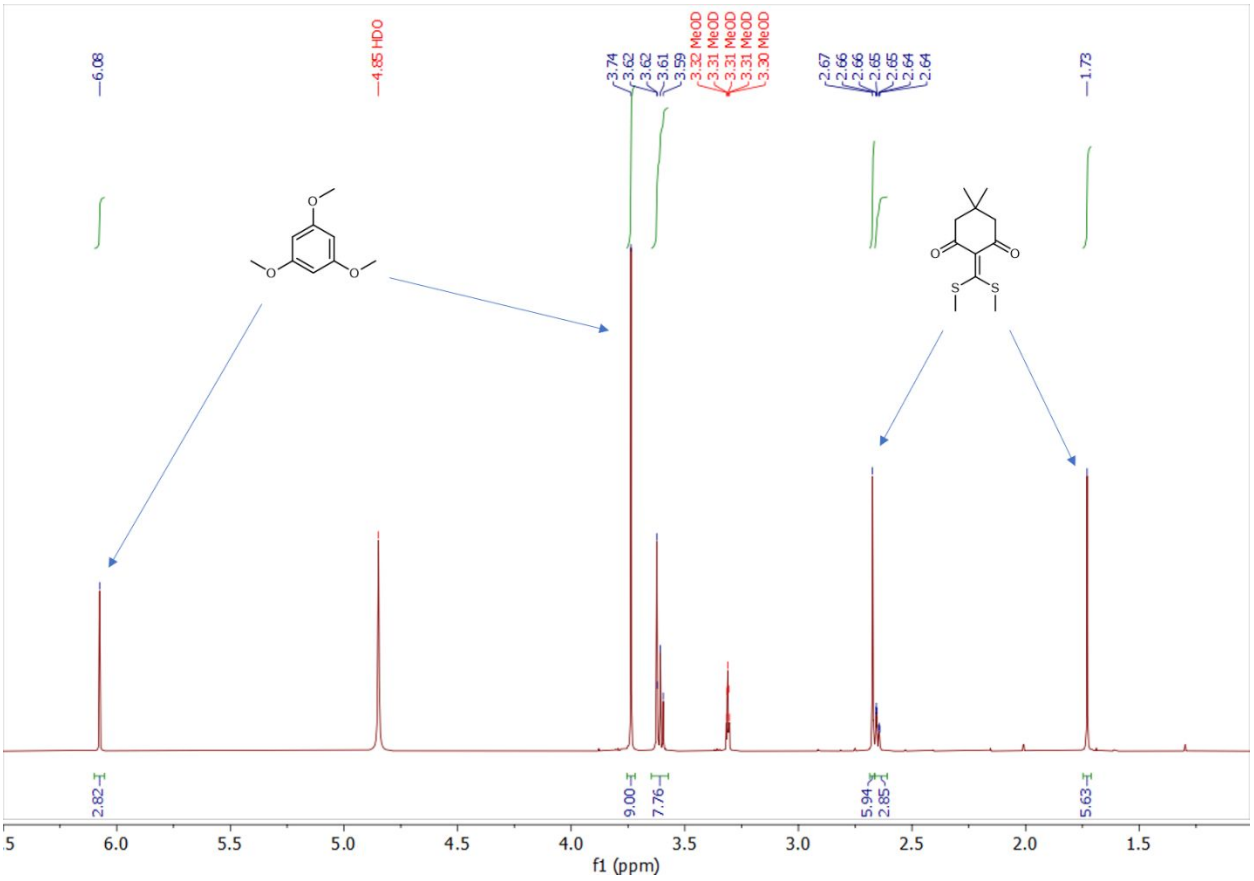
Reaction 3 in CD_3OD. Conversion was determined to be 0%.



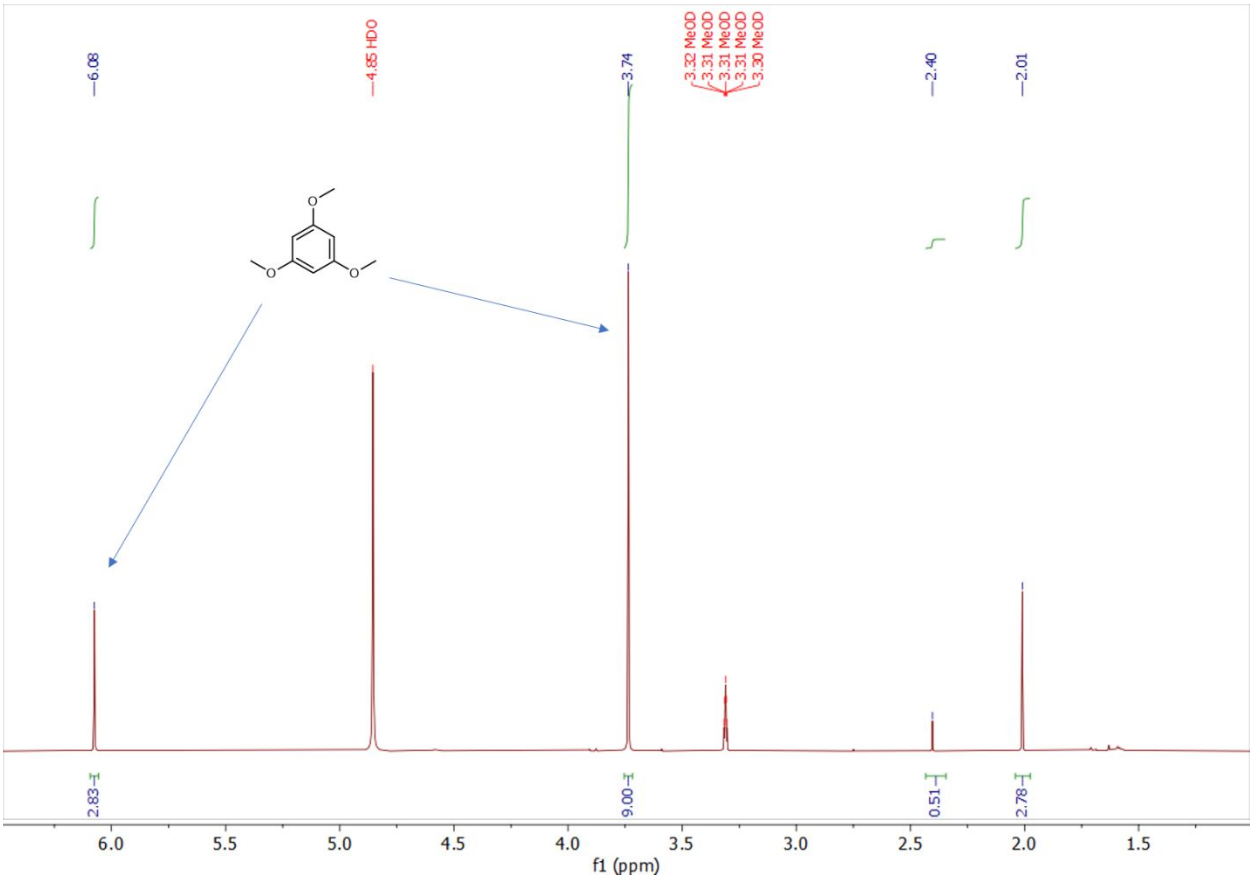
Reaction 4 in CD_3OD. Conversion was determined to be 10%.



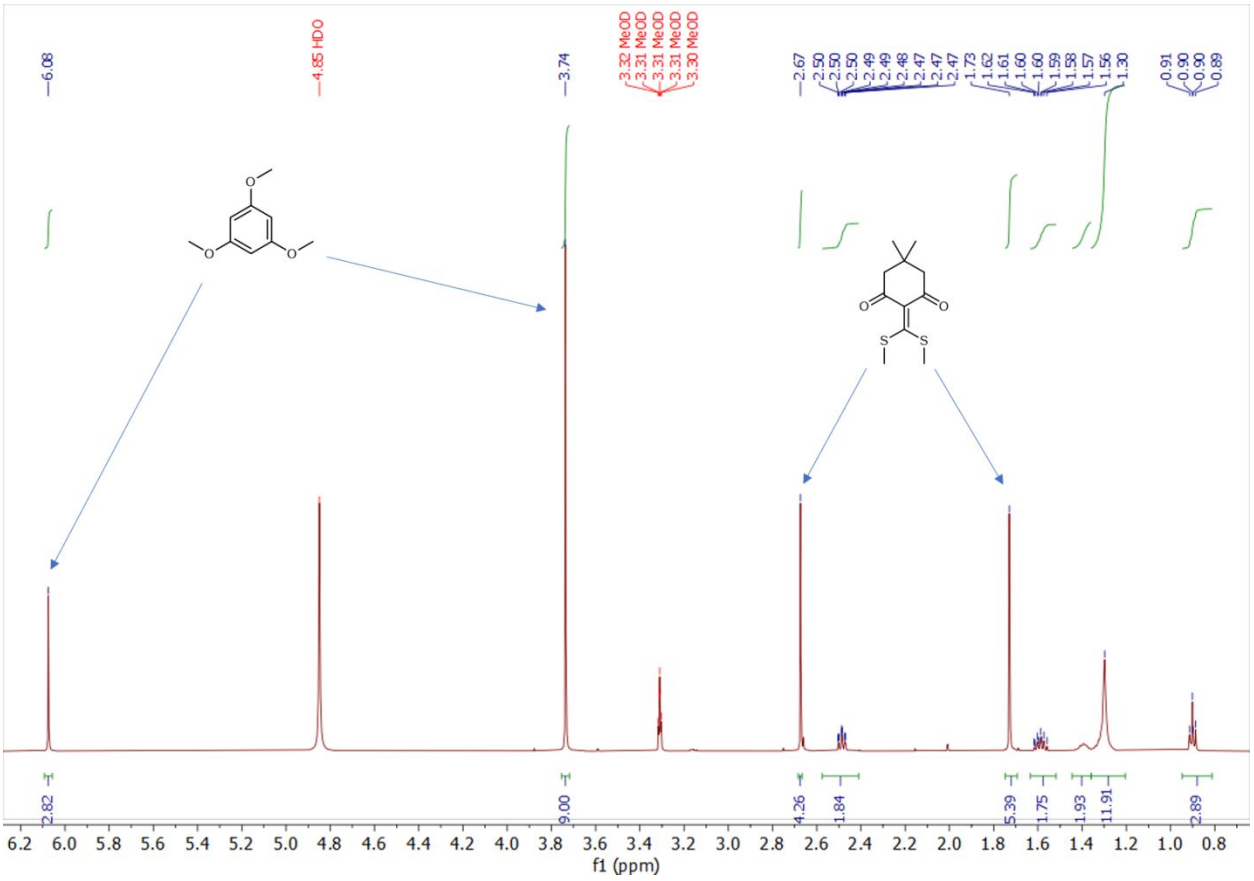
Reaction 5 in CD_3OD. Conversion was determined to be 0%.



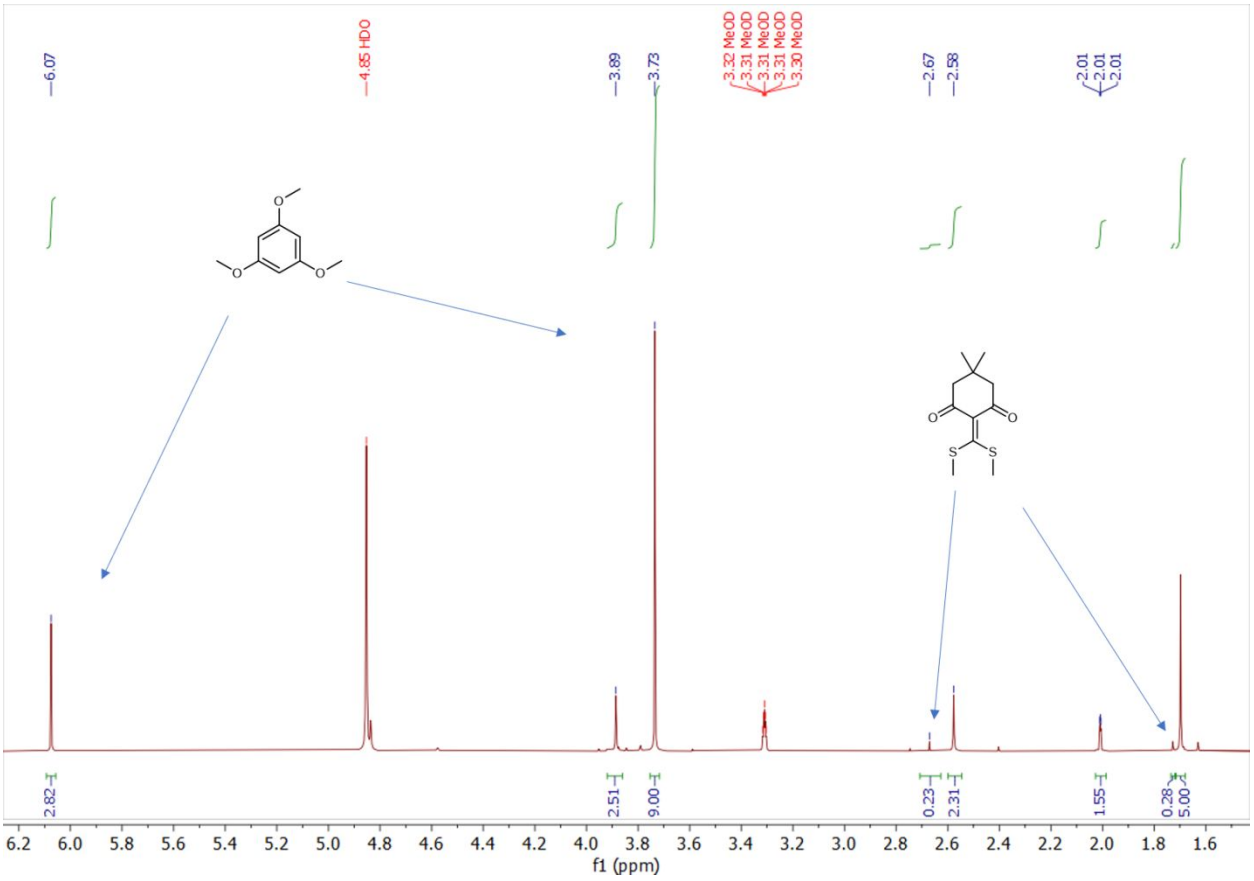
Reaction 6 in CD_3OD. Conversion was determined to be 100%.



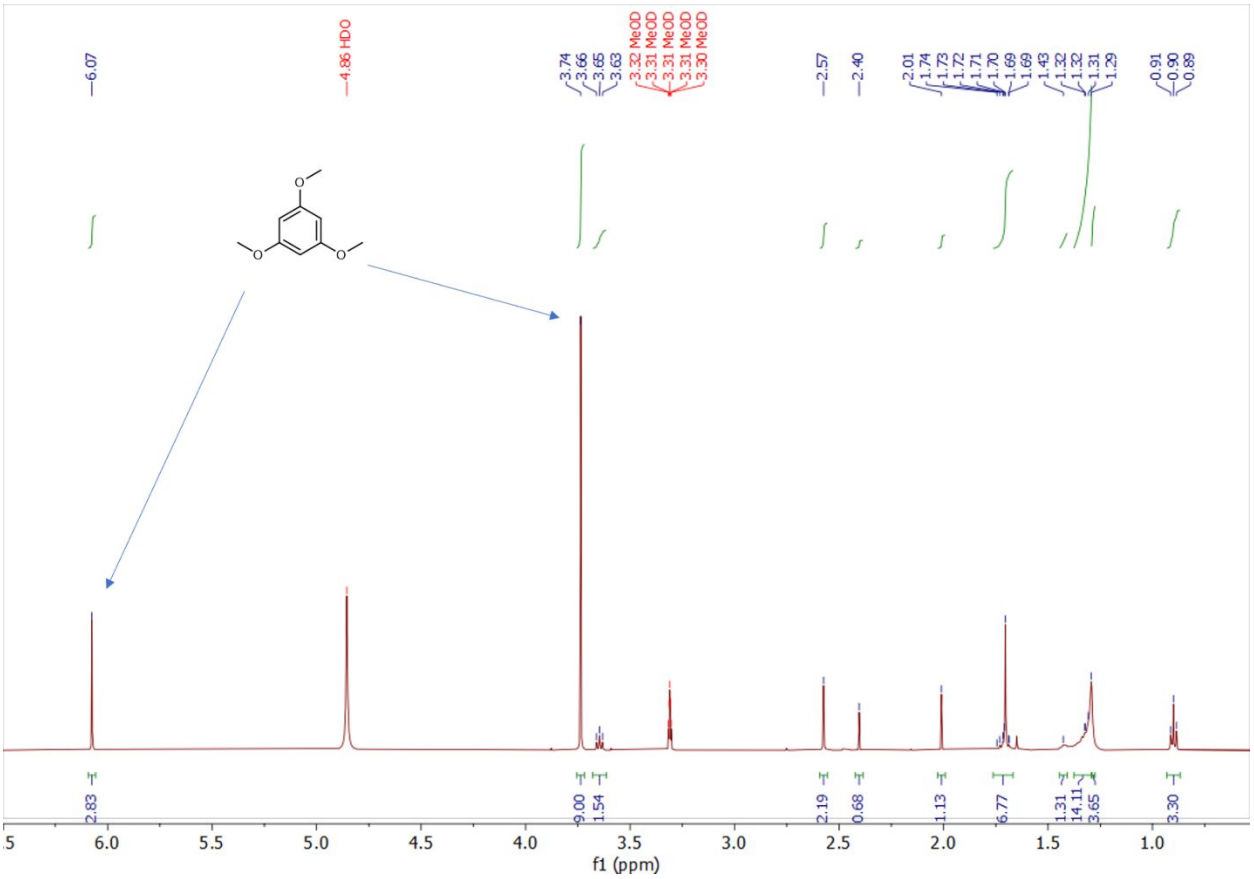
Reaction 7 in CD_3OD. Conversion was determined to be 20%.



Reaction 8 in CD_3OD. Conversion was determined to be 96%.



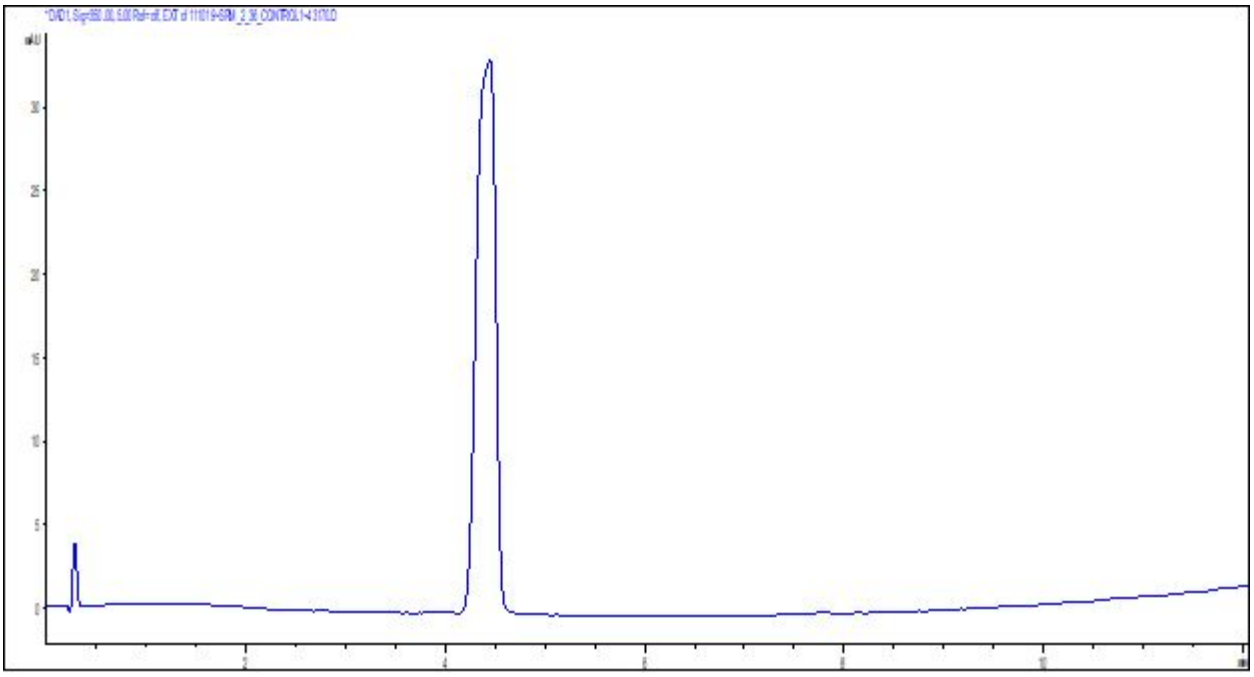
Reaction 9 in CD_3OD. Conversion was determined to be 100%.

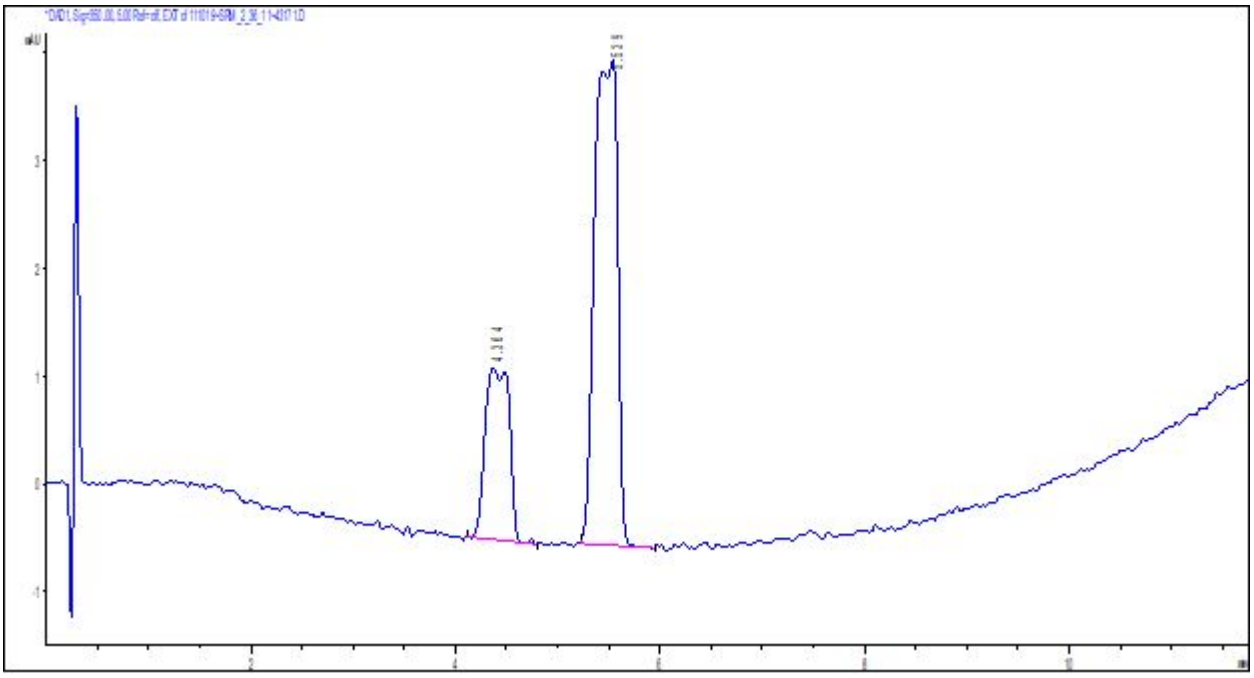


Reaction 10. Conversion was determined to be 94%

Table S3. Summary of LC/MS run data and integrations.

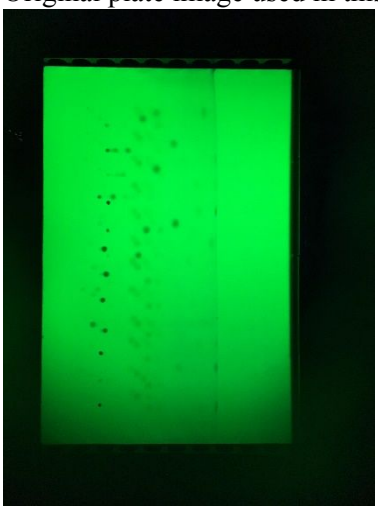
Run	Area	Height (mA)	Ret. Time (min)
Control	432	32	4.44
Reaction 10	25.3	1.5	4.35





V. Appaloosa Thin Layer Chromatography Wizard User Manual

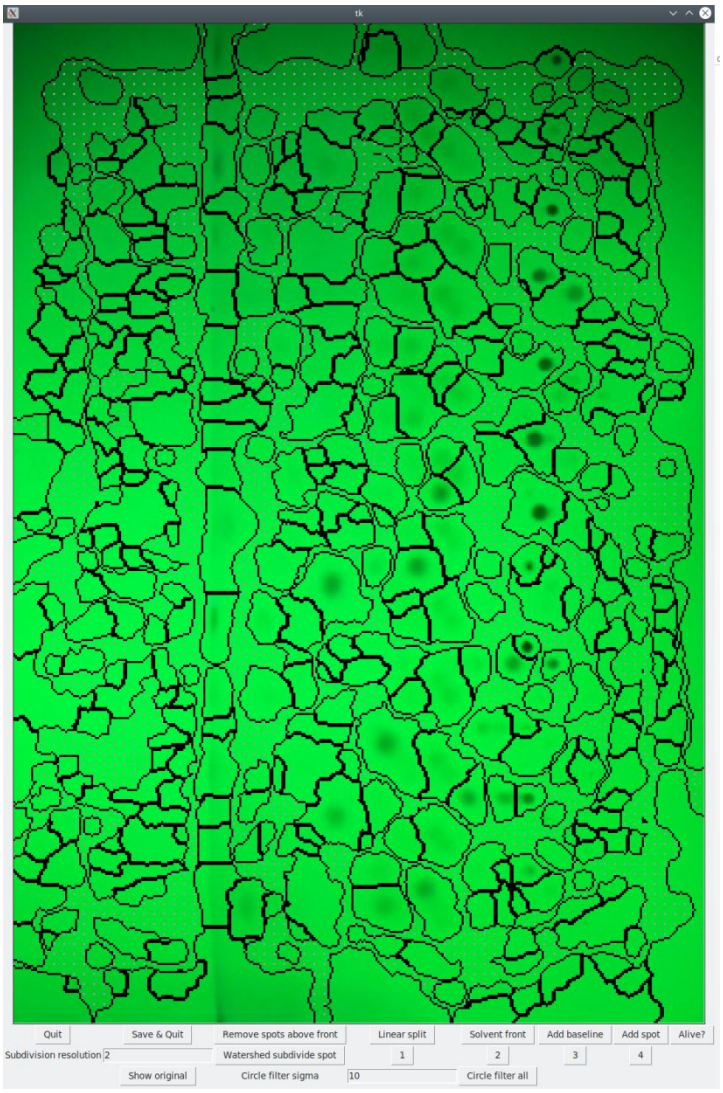
1. Original plate image used in this example:



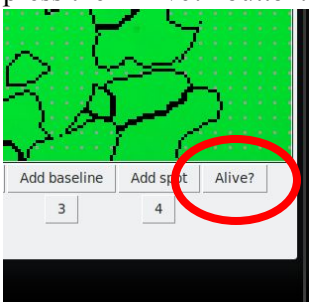
2. Run analyze_tlc:

```
boulgakov@osboxes:~/TLC/appaloosa$ ls
8333.jpg  analyze_tlc.py  appaloosa.py  appaloosa.pyc  manual.pdf  README.md
boulgakov@osboxes:~/TLC/appaloosa$ ./analyze_tlc.py --help
usage: analyze_tlc.py [-h] [--intermediate_images] [--zoom ZOOM]
                      image_filename

Thin layer chromatography spot segmentation & quantification.

positional arguments:
  image_filename        Image of TLC plate

optional arguments:
  -h, --help            show this help message and exit
  --intermediate_images
                        Output intermediate image steps to PNGs. Useful for
                        understanding what's happening. (default: False)
  --zoom ZOOM           Image display zoom. This determines how large the
                        image and window are. (default: 3)
boulgakov@osboxes:~/TLC/appaloosa$ ./analyze_tlc.py 8333.jpg
```

3. The program will identify the plate from the background and perform an initial segmentation of spots. It will then display the results in an interactive window:

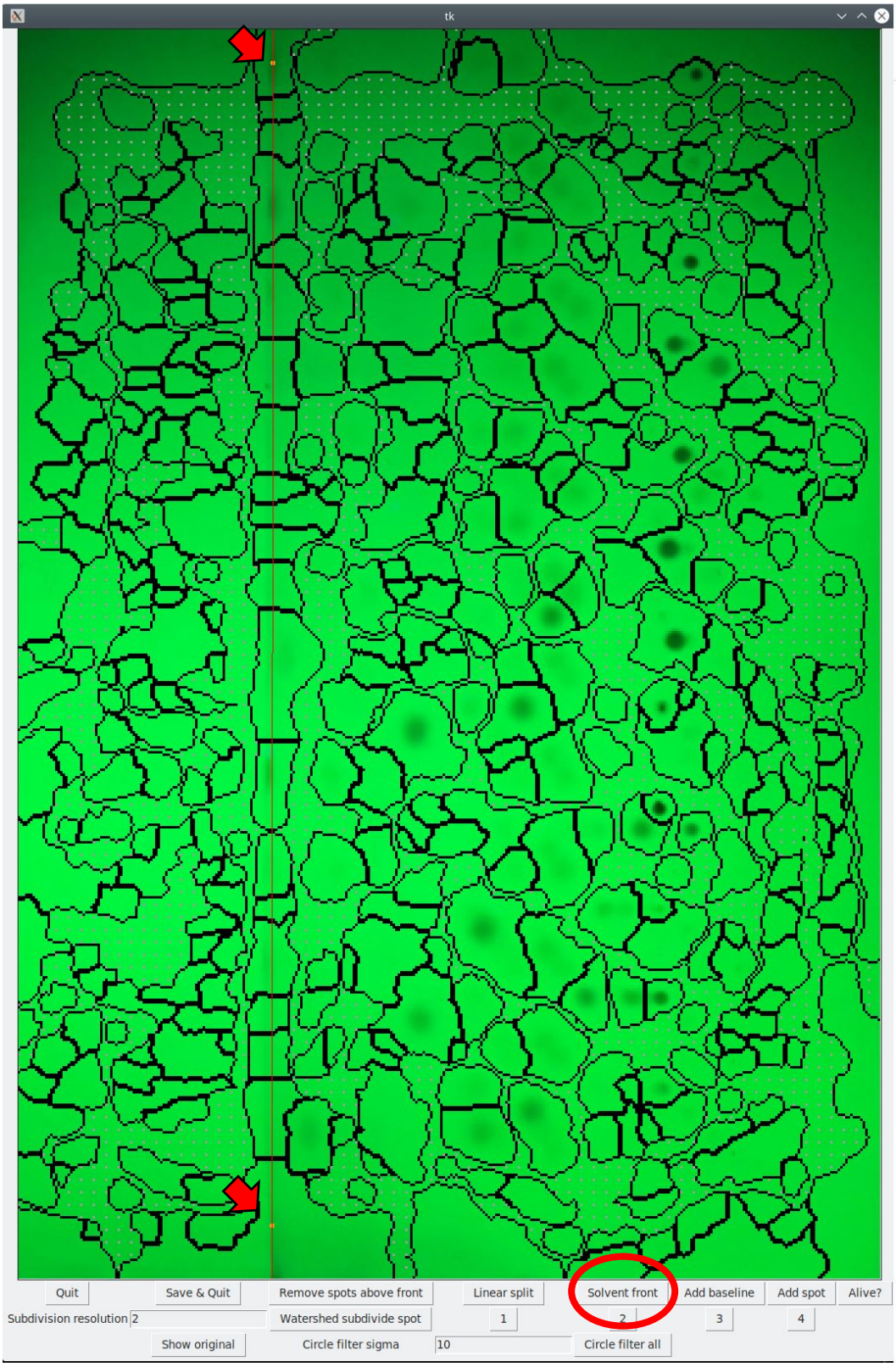


4. The program will sometimes output useful information to the original shell. To test this, you can press the “Alive?” button.

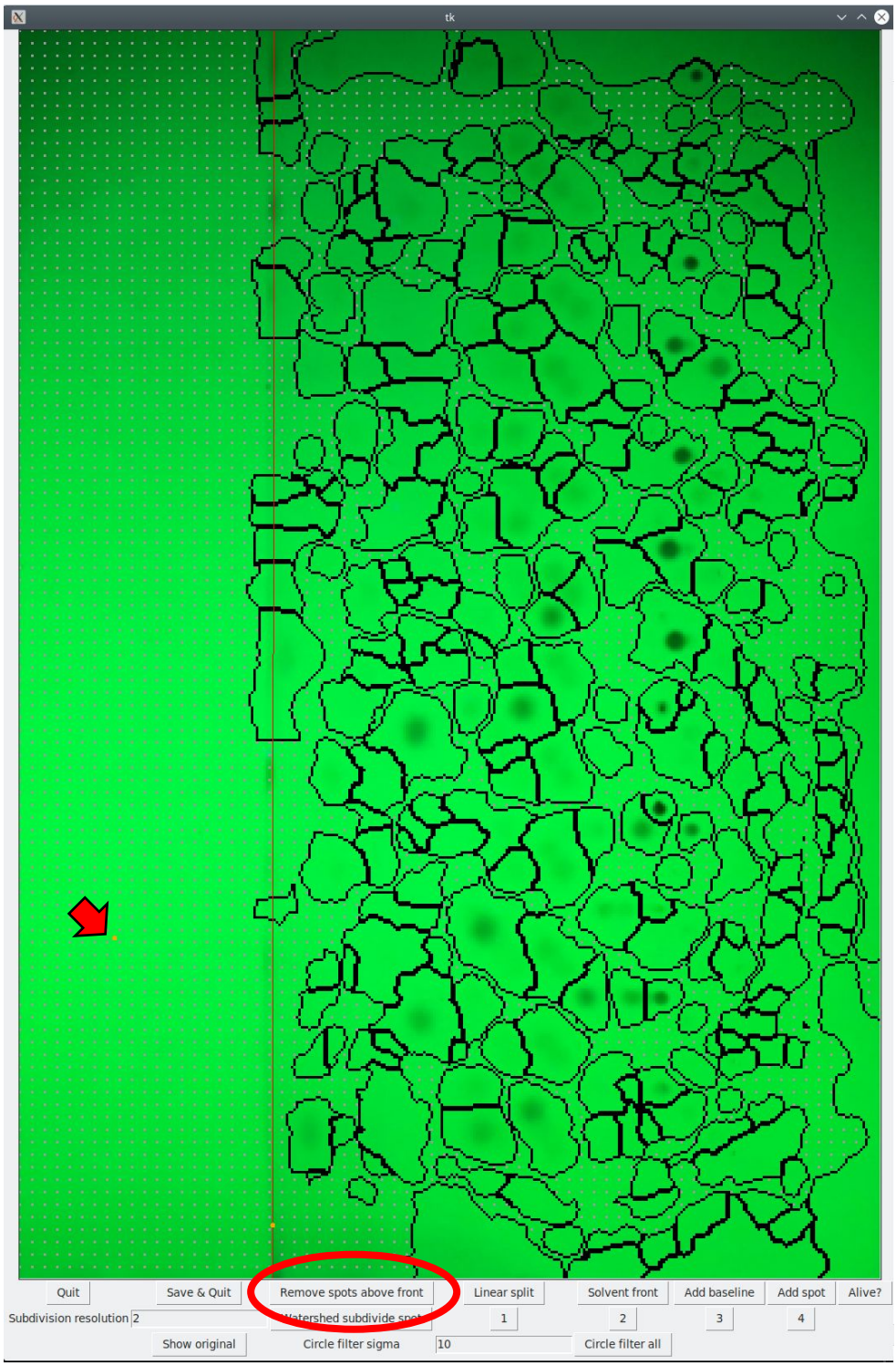


5. The plate background is overlaid by a grid of gray squares. Each individual spot is shown with a black outline. You can see the original image without the overlaid basins at any time by pressing and holding the “Show original” button.

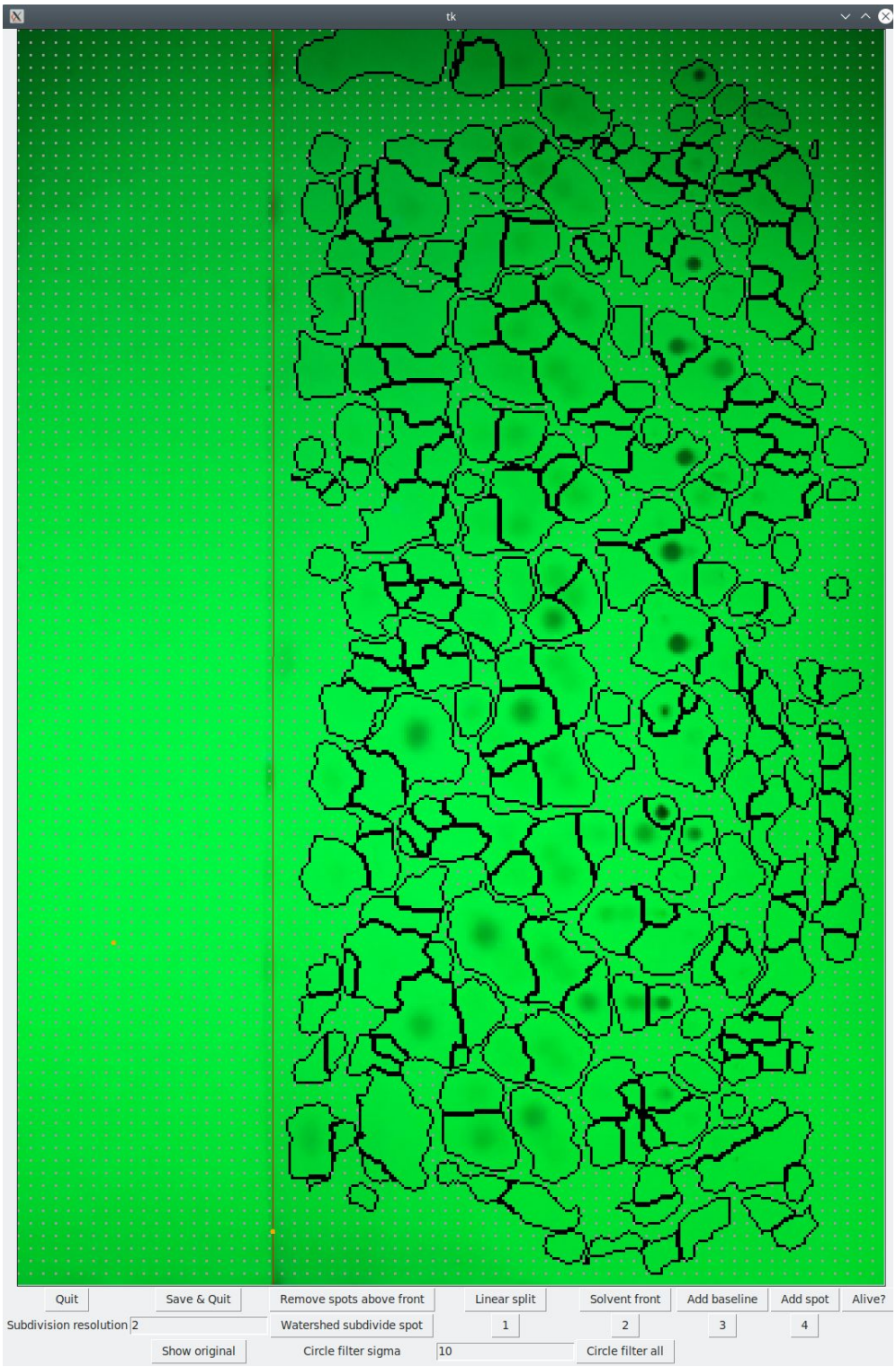
6. You can define the solvent front by clicking on the image at two points along the front, and then clicking the “Solvent front” button.



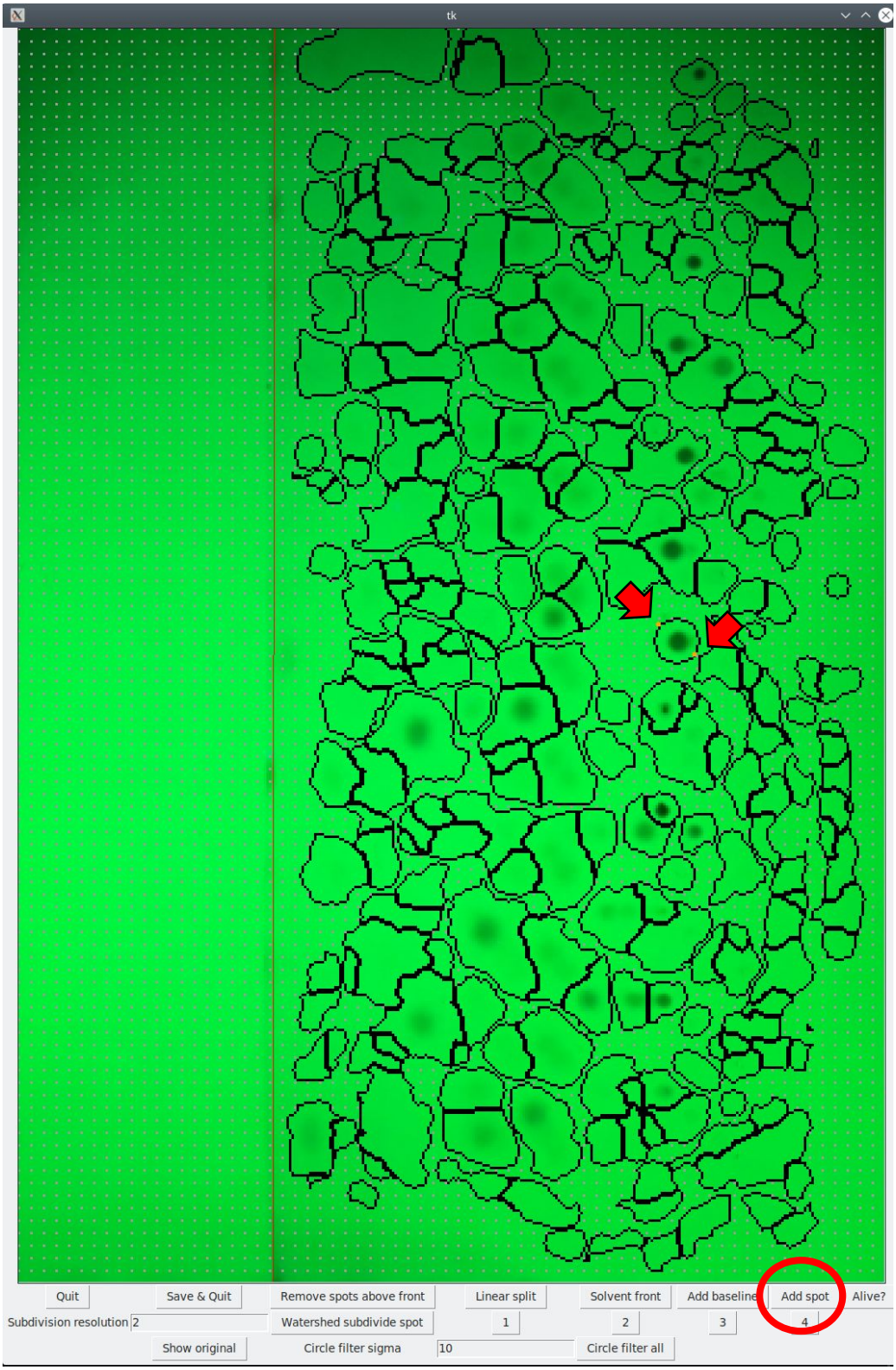
7. There are many spots identified above the solvent front. These are false positives. The default algorithm parameters are set to be relatively sensitive. You can eliminate all spots whose centroids are on one side of the solvent front by clicking on a point above the front and pressing the “Remove spots above front” button.



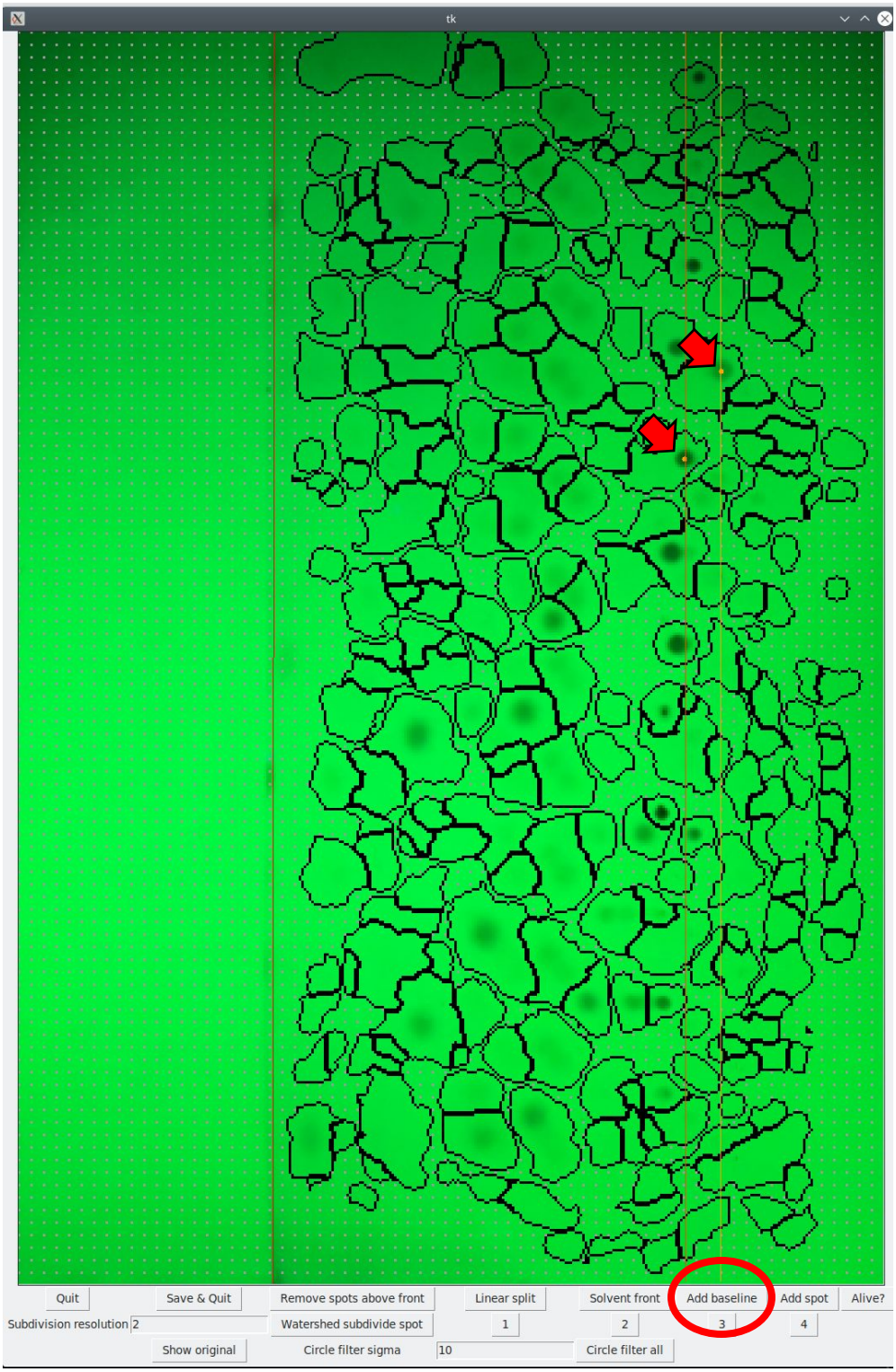
8. The solvent front is still identified as a cluster of spots. You can delete any spot by right-clicking on it.



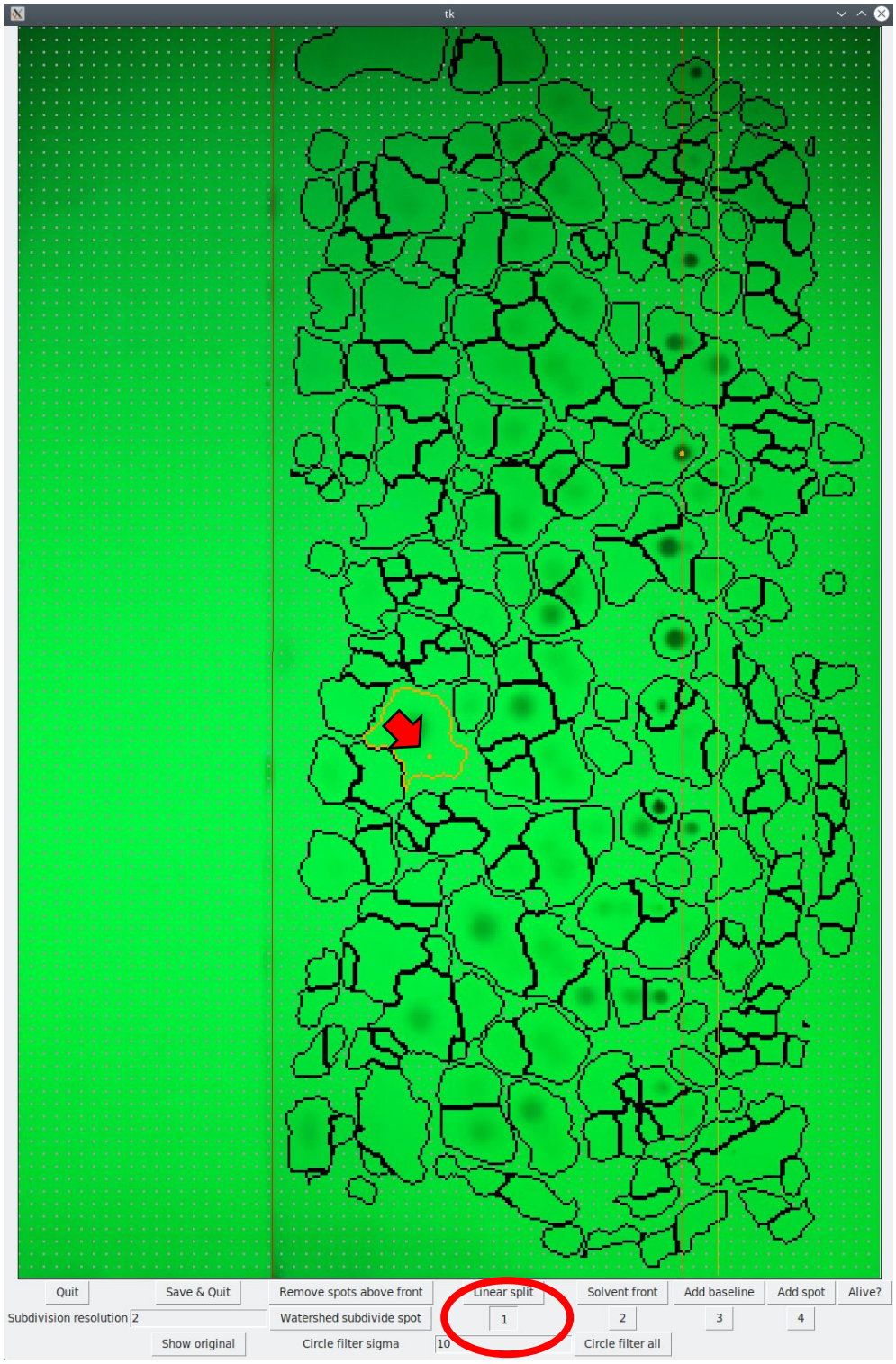
9. It is possible that some spots were not identified. To manually add them, place two points around them on the opposite sides of a circle centered on the spot. (Note: that spot was indeed identified as shown in the images above, but for illustrative purposes it was “removed” via right-click.)



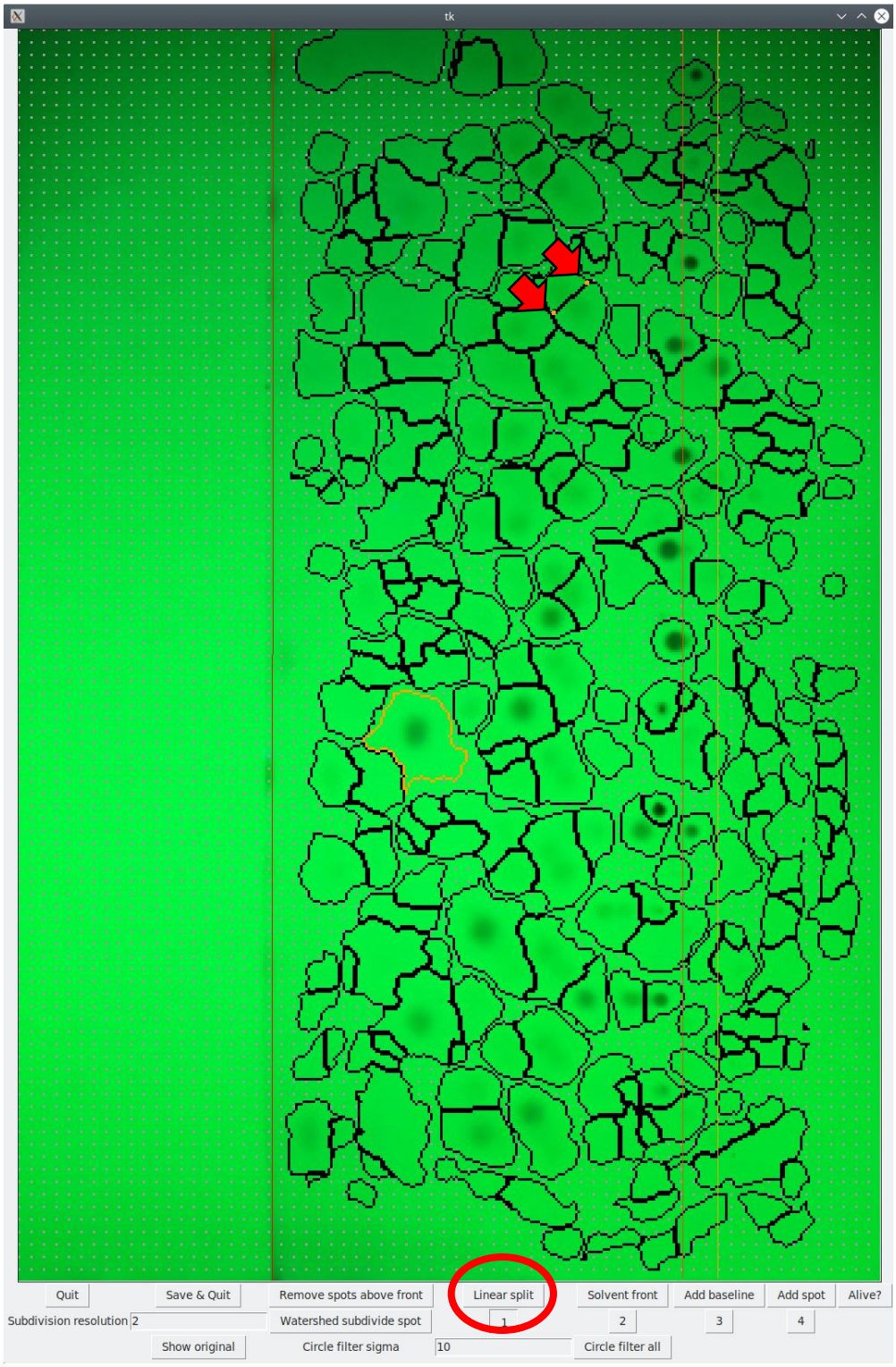
10. You can now assign baselines where the samples were loaded. You can assign up to four baselines. Assign a baseline by clicking on a spot, and the clicking on the “Add baseline” button. The baseline is automatically parallel to the solvent front. You must first define a solvent front before assigning baselines. Here we have defined the first two baselines.



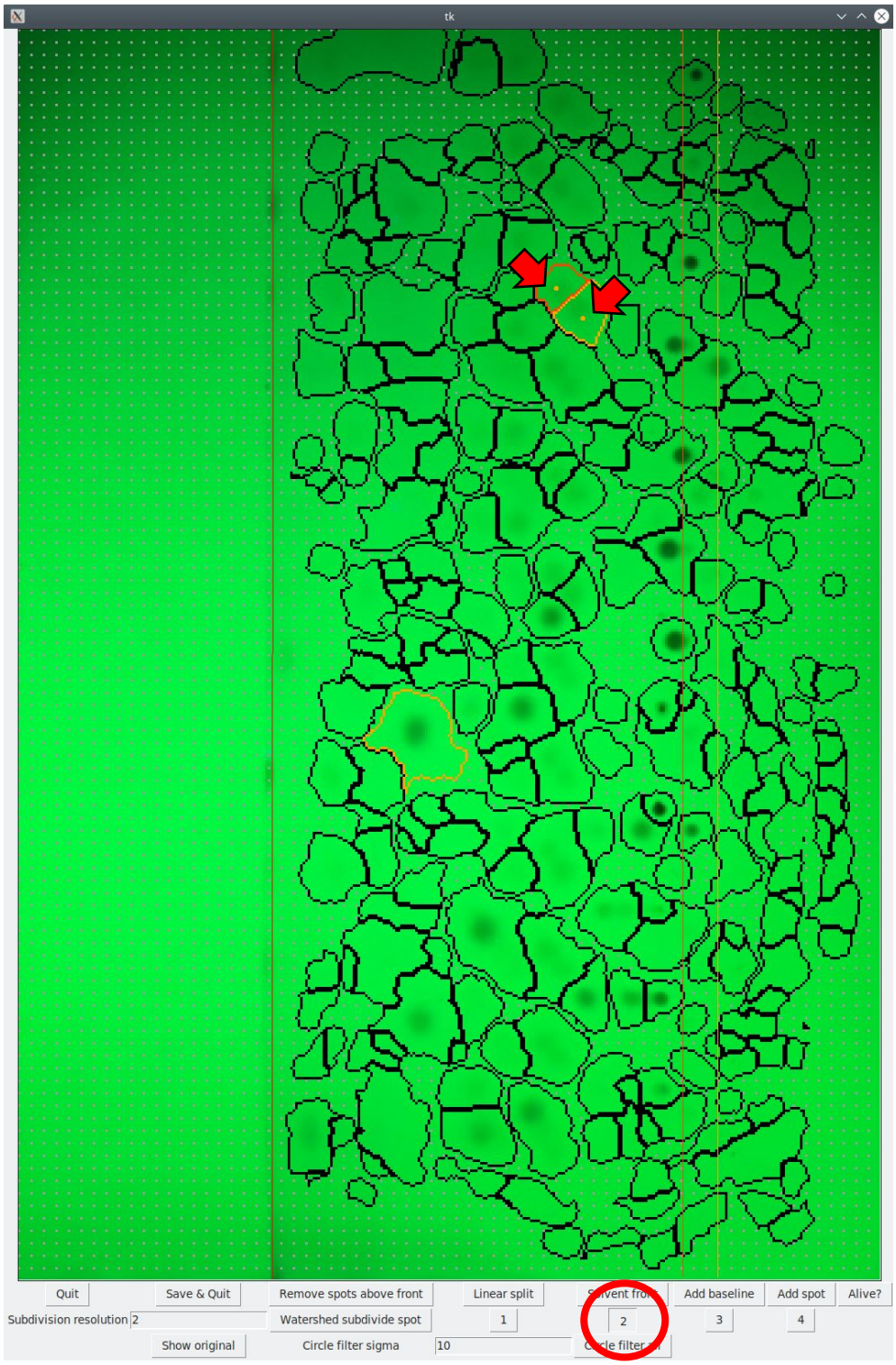
11. We can now assign samples to the baselines they came from. First, click on the button labeled "1". Then double-click on a spot to assign it to the first baseline.



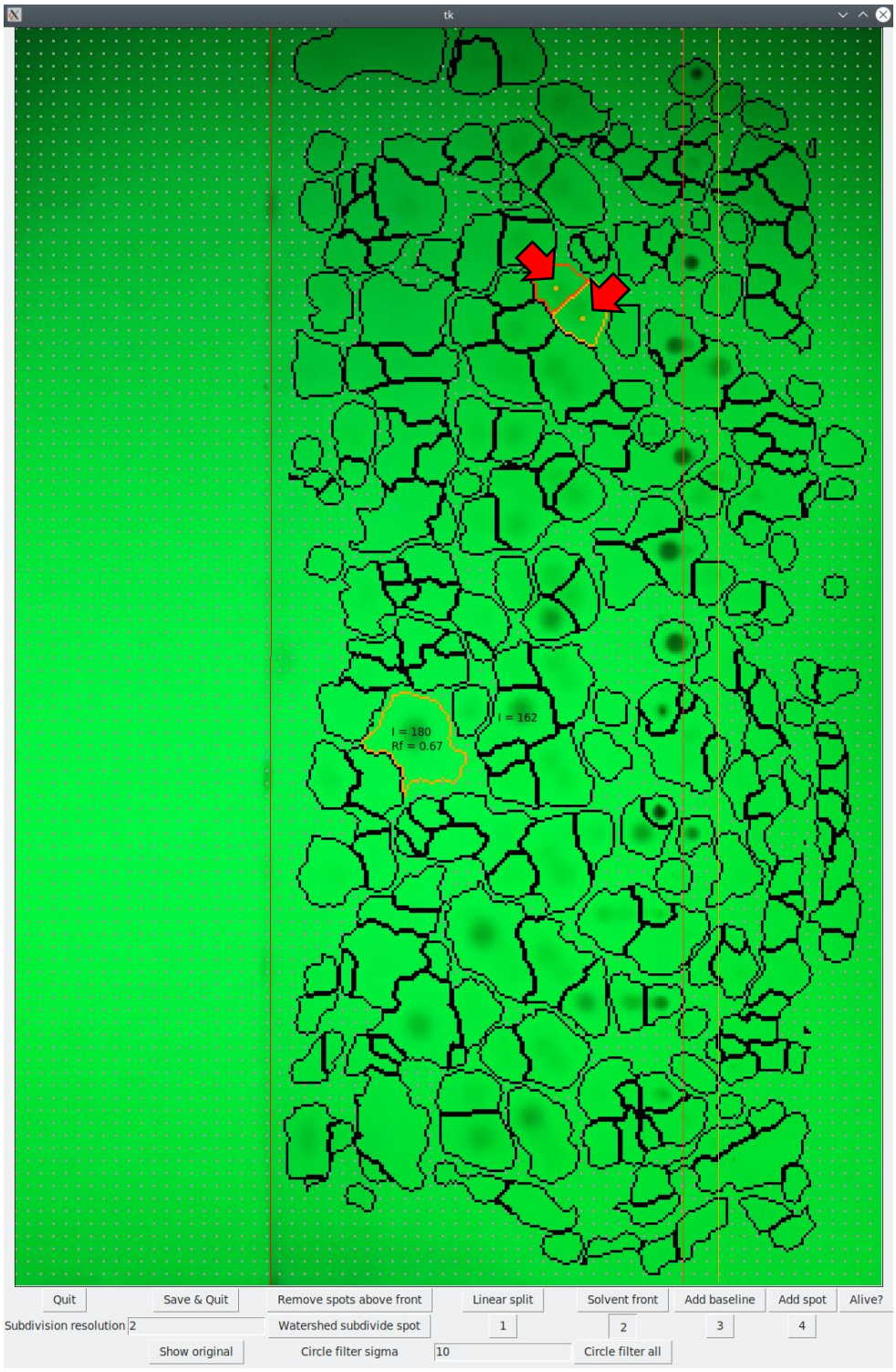
12. Suppose a pair of spots were not split properly. You can bisect any spot with a line by first clicking on the spot in two locations, and then clicking the “Linear split” button.

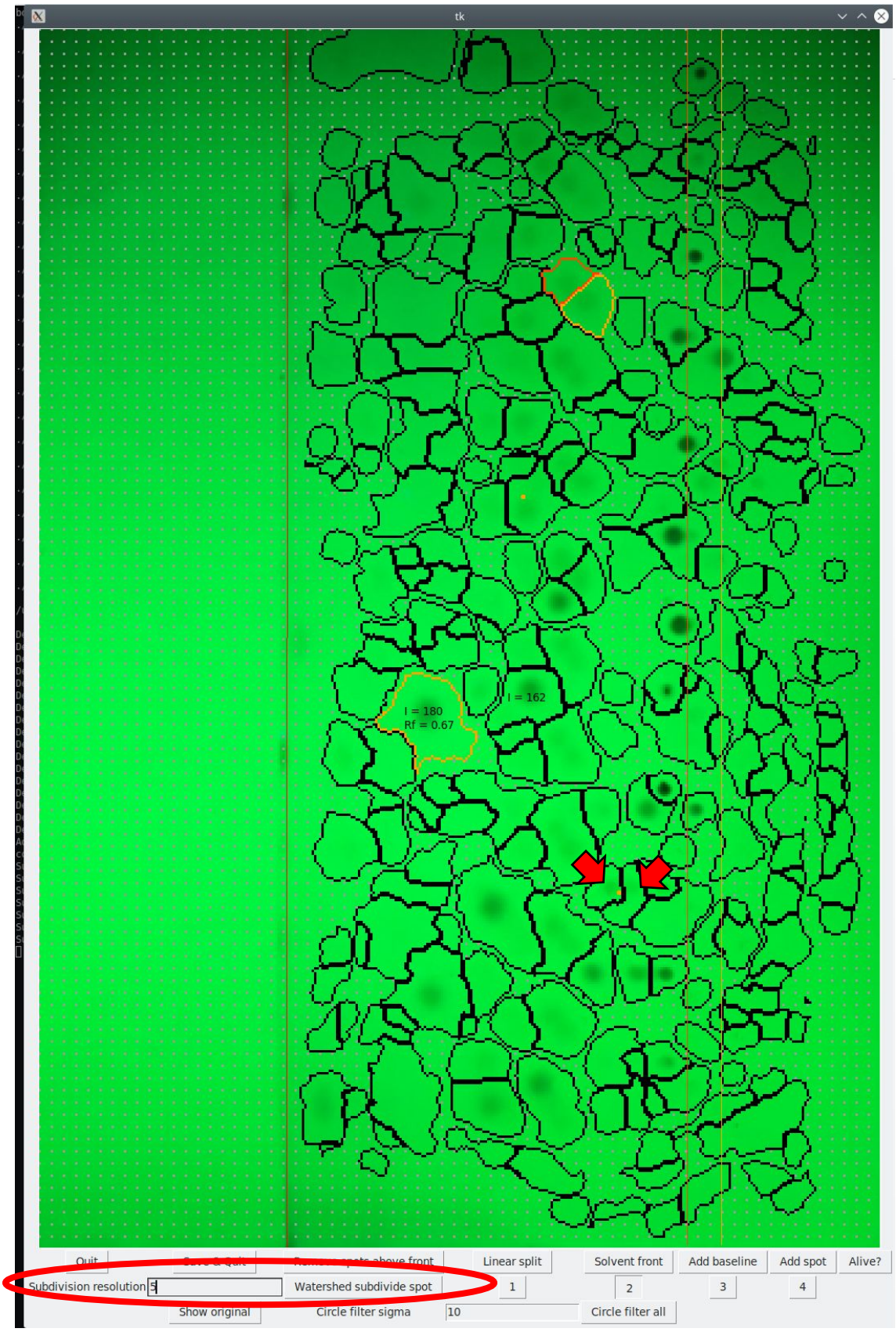


13. Now we can assign each spot to a different baseline. First double-click on one (because “1” has already been depressed), then click the “2” button, and double click on the other. The selections are color-coded.

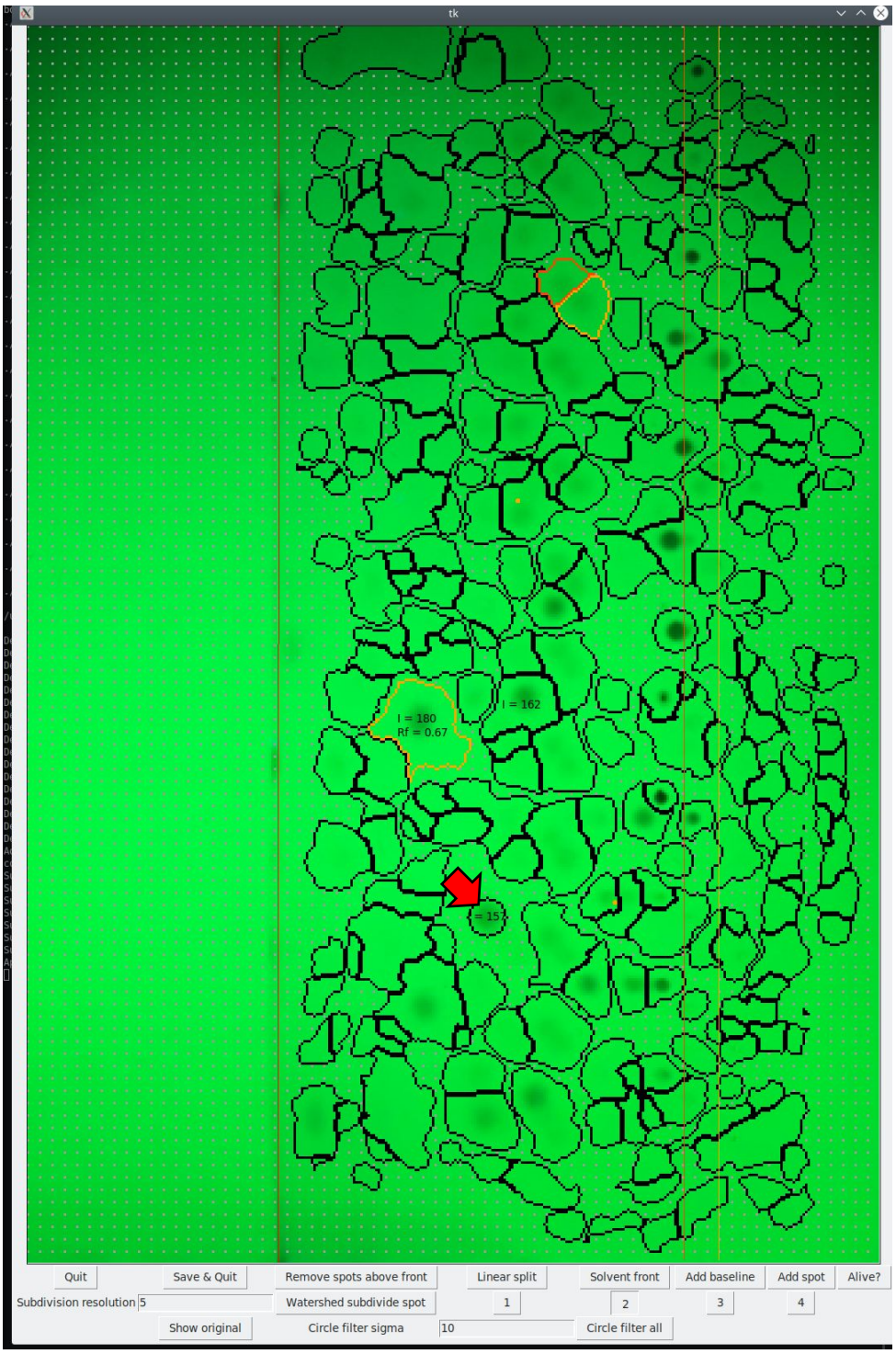


14. When your mouse hovers over a spot and you press “d” on your keyboard, you will get a heads-up display of the spot’s intensity and, if it has been assigned to a baseline, its Rf value.
15. Instead of manual bisection, you can also attempt to subdivide a spot using a watershed algorithm. First, enter in the subdivision resolution value: the lower, the finer the resolution, and the more “sub-spots” the algorithm will find. After entering the value, click on the spot you want to subdivide and click the “Watershed subdivide spot” button. Use this option with caution as it may generate many meaningless subdivisions. In this example, using resolution 5, it worked.

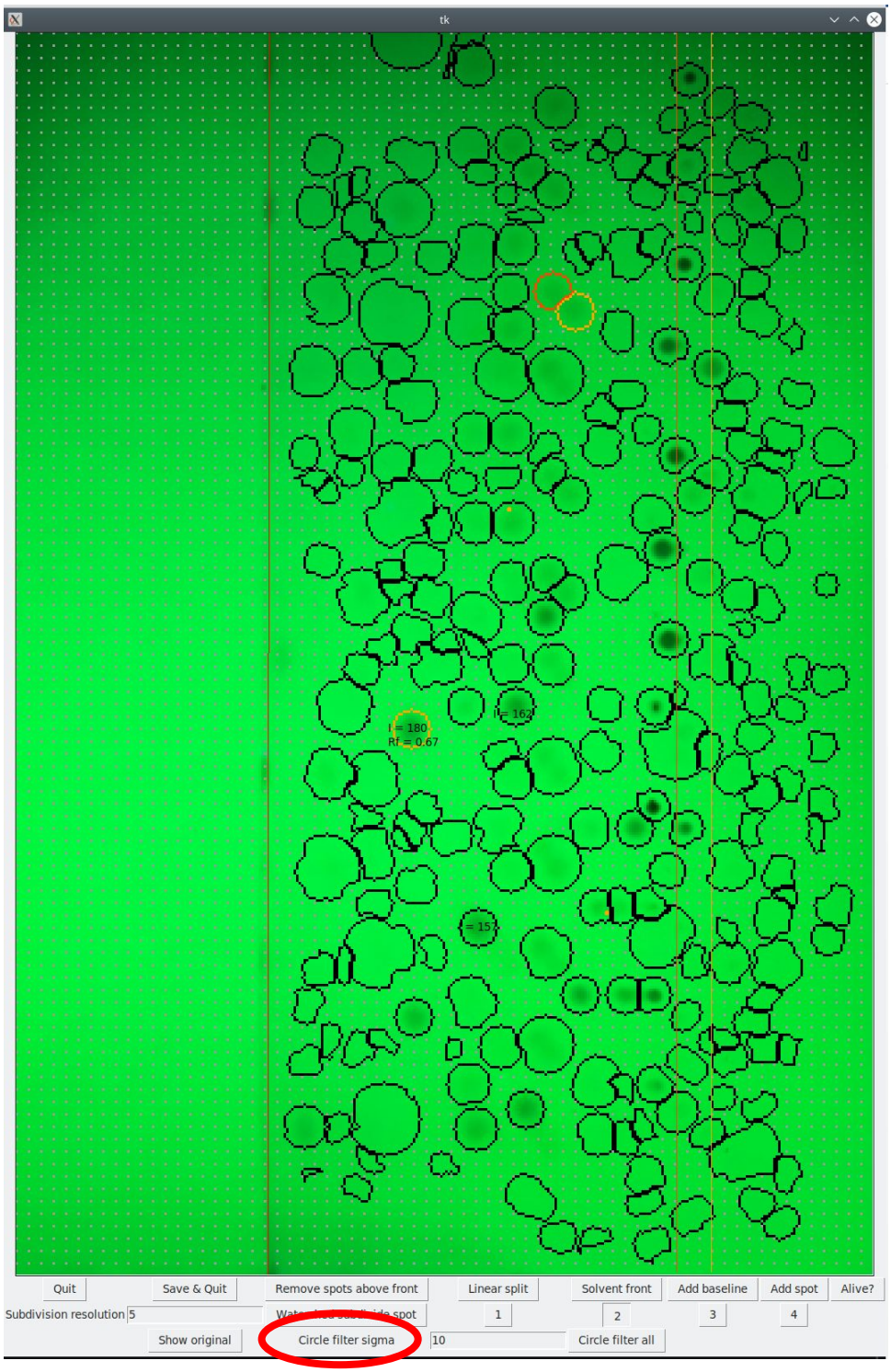



tk
I = 180
Rf = 0.67
I = 162
Quit
Linear split
Solvent front
Add baseline
Add spot
Alive?
Subdivision resolution 5
Watershed subdivide spot
1
2
3
4
Show original
Circle filter sigma
10
Circle filter all

16. If your spots are circular, you can try shrinking the identified areas around them to a tighter, circular fit using the circle filter. First, choose the upper size bound of the circle(s) you want the software to try to locate using the “Circle filter sigma” entry box. The program will try to find circles up to that radius. If found, it will shrink the spot area to within a circle of radius 1.5 x sigma. Note that this is an “intersect” function: the program basically discards any already identified area outside of this radius, but that does not mean that it will add any pixels that were not previously identified to the circle. There are two approaches to using the circle filter, with the one-by-one approach performed by hovering the pointer over a spot and pressing “c” on the keyboard. (Note that if you are keying in a value into the value entry box, you will want to tab over to the image; otherwise you will just type a “c” into the entry box.)


tk
I = 180
Rf = 0.67
I = 162
= 15
Quit
Save & Quit
Remove spots above front
Linear split
Solvent front
Add baseline
Add spot
Alive?
Subdivision resolution
5
Watershed subdivide spot
1
2
3
4
Show original
Circle filter sigma
10
Circle filter all

17. The other way to apply the circle filter is to apply it to all spots. Warning: if you have not properly separated all spots in an image into distinct identified area, the filter will attempt to treat them as one. You can see some examples here. Also note that this operation takes a little time. It is multiprocessor enabled, so having more cores will help you run it faster. You can see a "complete" message on your shell terminal when it is done.



18. Finally, after you have done all your interactive work, you can Save & Quit the program using the button of the same name. The results will be saved as a PNG file color-coding all the individual spots and assigning them a unique tag, and a CSV file with each spot's intensity and R_f (if assigned to a baseline), using the tags shown in the PNG.

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[1]- Diehl, K. L.; Kolesnichenko, I. V.; Robotham, S. A.; Bachman, J. L.; Zhong, Y.; Brodbelt, J. S.; Anslyn, E. V. Click and Chemically Triggered Declick Reactions through Reversible Amine and Thiol Coupling via a Conjugate Acceptor. *Nature Chem* **2016**, *8* (10), 968–973. https://doi.org/10.1038/nchem.2601