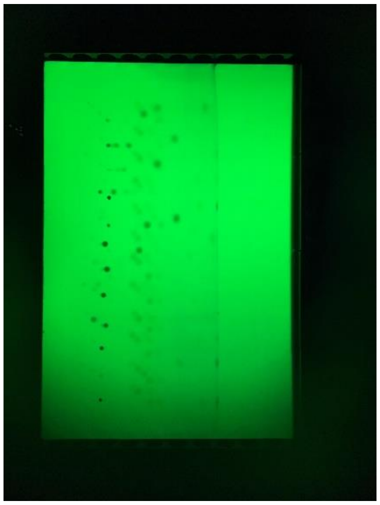
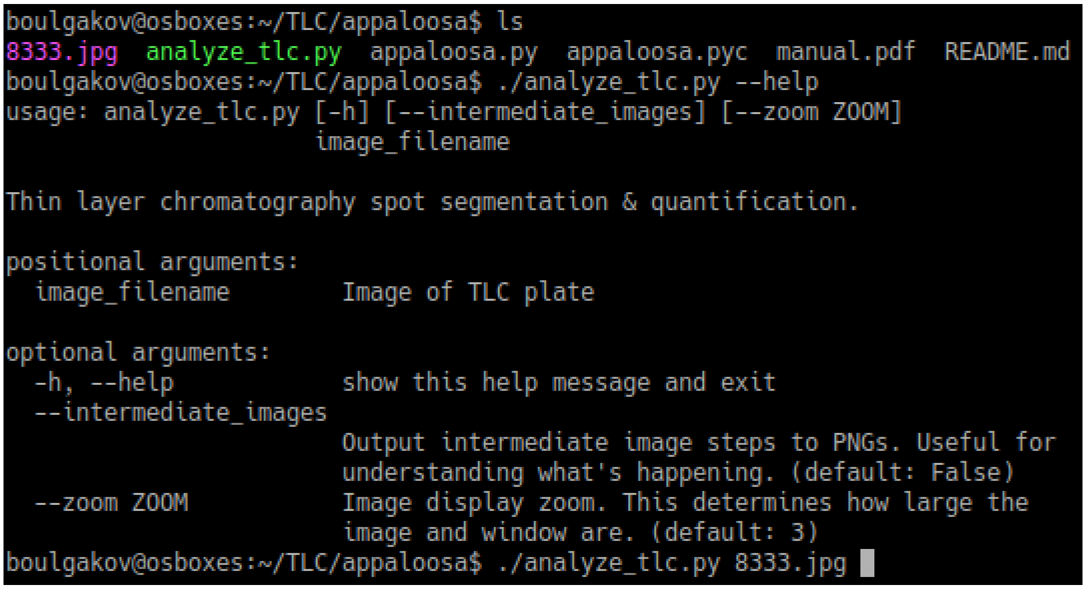
Appaloosa thin layer chromatography wizard user manual

1. Original plate image used in this example:



2. Run analyze_tlc:



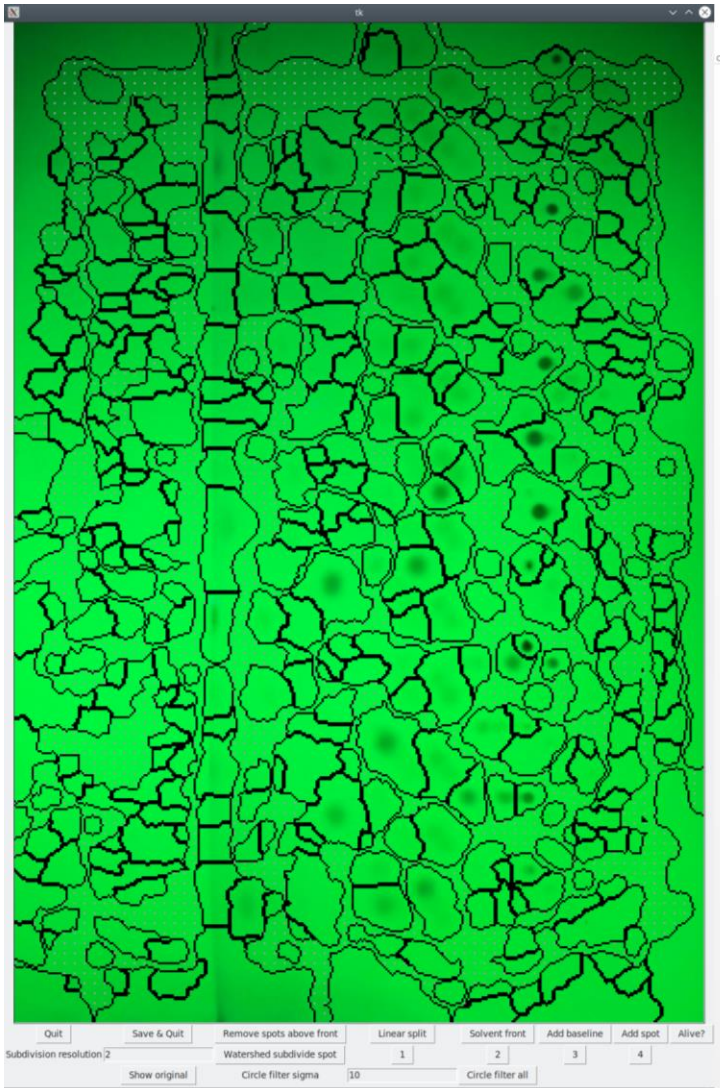
```
boulgakov@osboxes:~/TLC/appaloosa$ ls
8333.jpg  analyze_tlc.py  appaloosa.py  appaloosa.pyc  manual.pdf  README.md
boulgakov@osboxes:~/TLC/appaloosa$ ./analyze_tlc.py --help
usage: analyze_tlc.py [-h] [--intermediate_images] [--zoom ZOOM]
                      image_filename

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
```

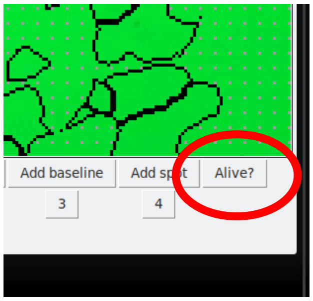
3. The program will identify the plate from the background and perform an initial segmentation of spots. It will then display the results in an interactive window:



Subdivision resolution 2

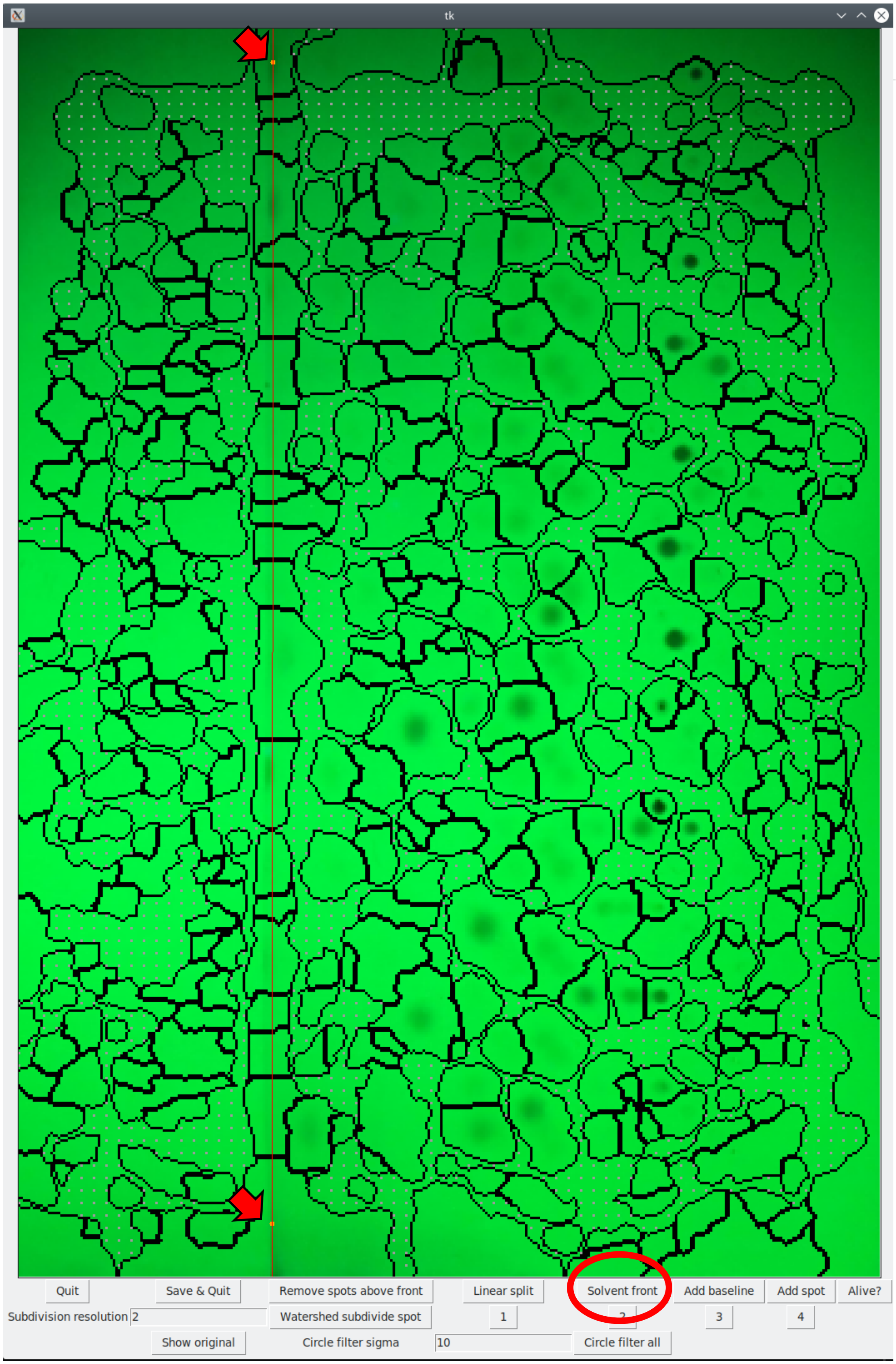
Circle filter sigma 10

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.

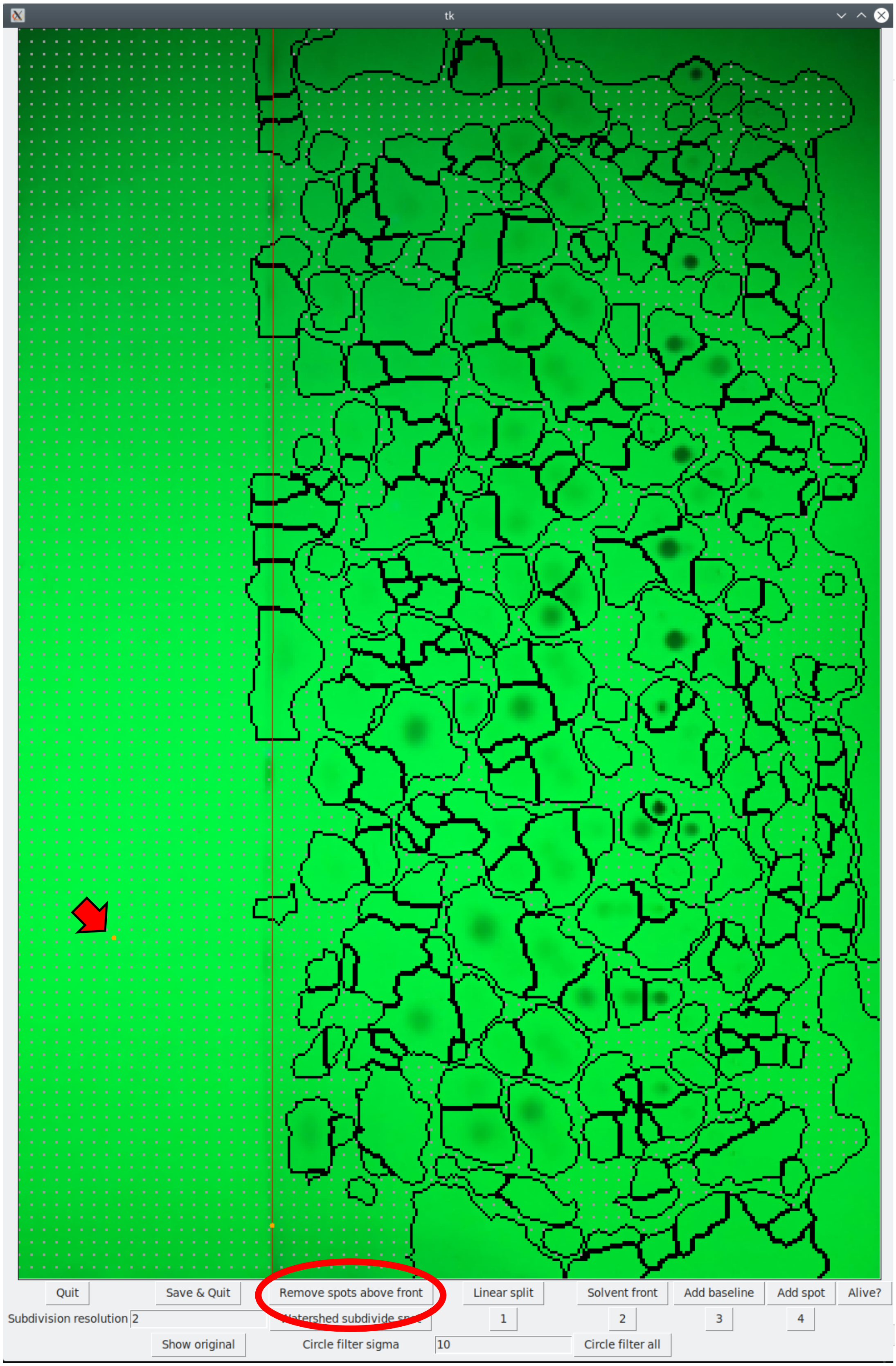
6. You can define the solvent front by clicking on the image at two points along the front, and then clicking the “Solvent front” button.



Subdivision resolution 2

Circle filter sigma 10

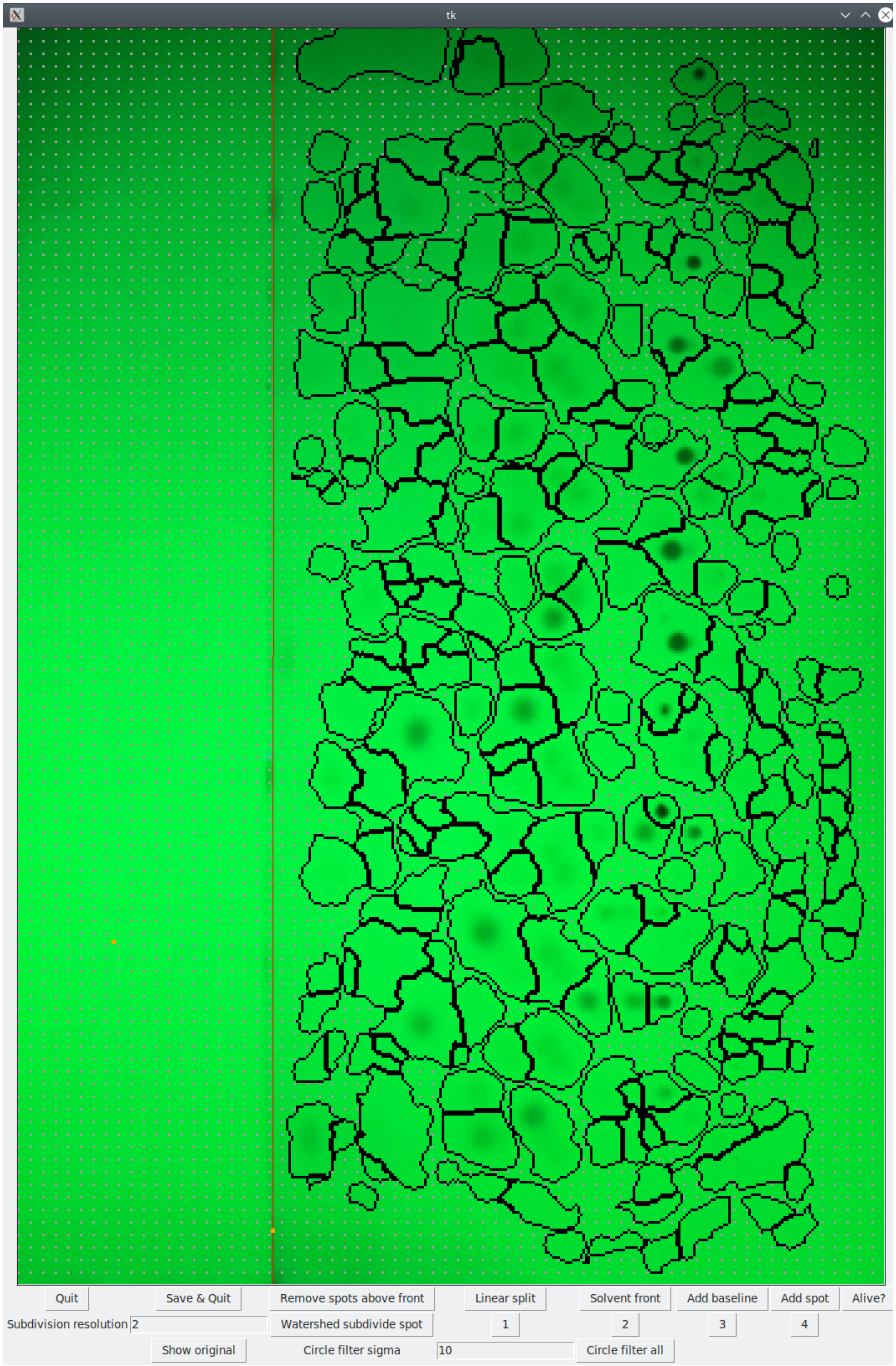
7. There are many spots identified above the solvent front. These are false positives. The default algorithm parameters are set to be relatively sensitive. You can eliminate all spots whose centroids are on one side of the solvent front by clicking on a point above the front and pressing the “Remove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



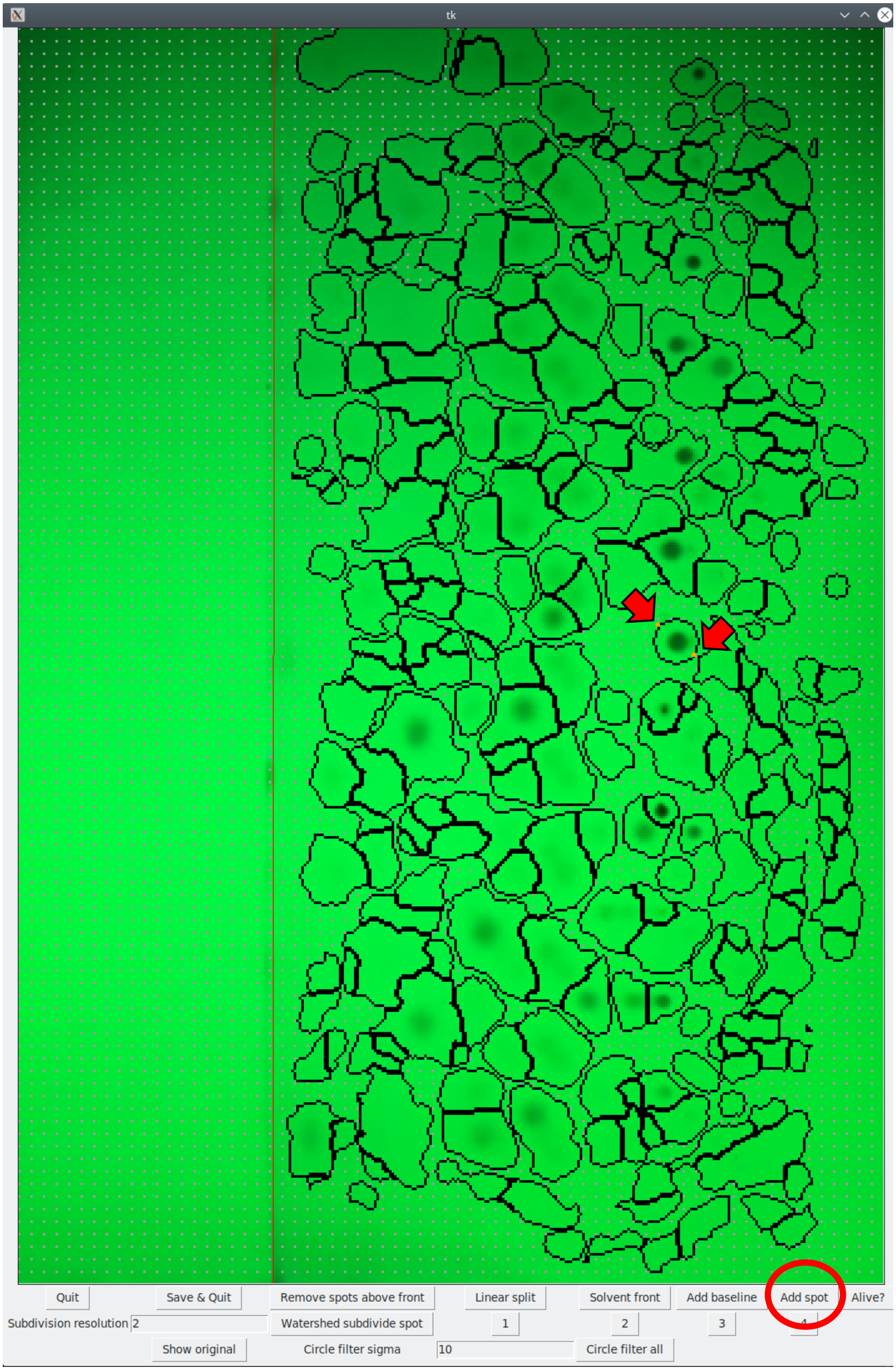
Subdivision resolution 2

Circle filter sigma 10

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



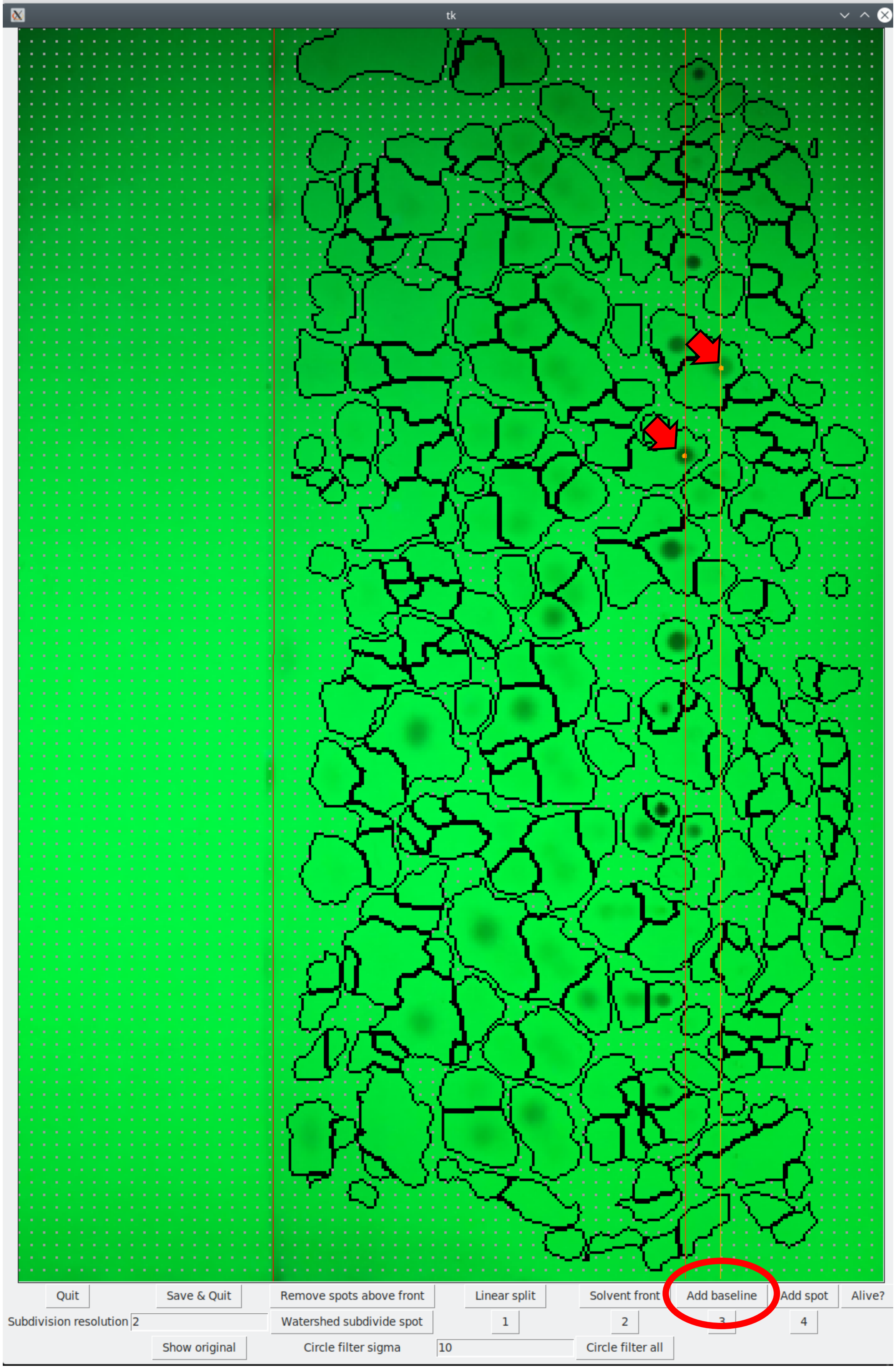
9. It is possible that some spots were not identified. To manually add them, place two points around them on the opposite sides of a circle centered on the spot. (Note: that spot was indeed identified as shown in the images above, but for illustrative purposes it was “removed” via right-click.)



Subdivision resolution 2

Circle filter sigma 10

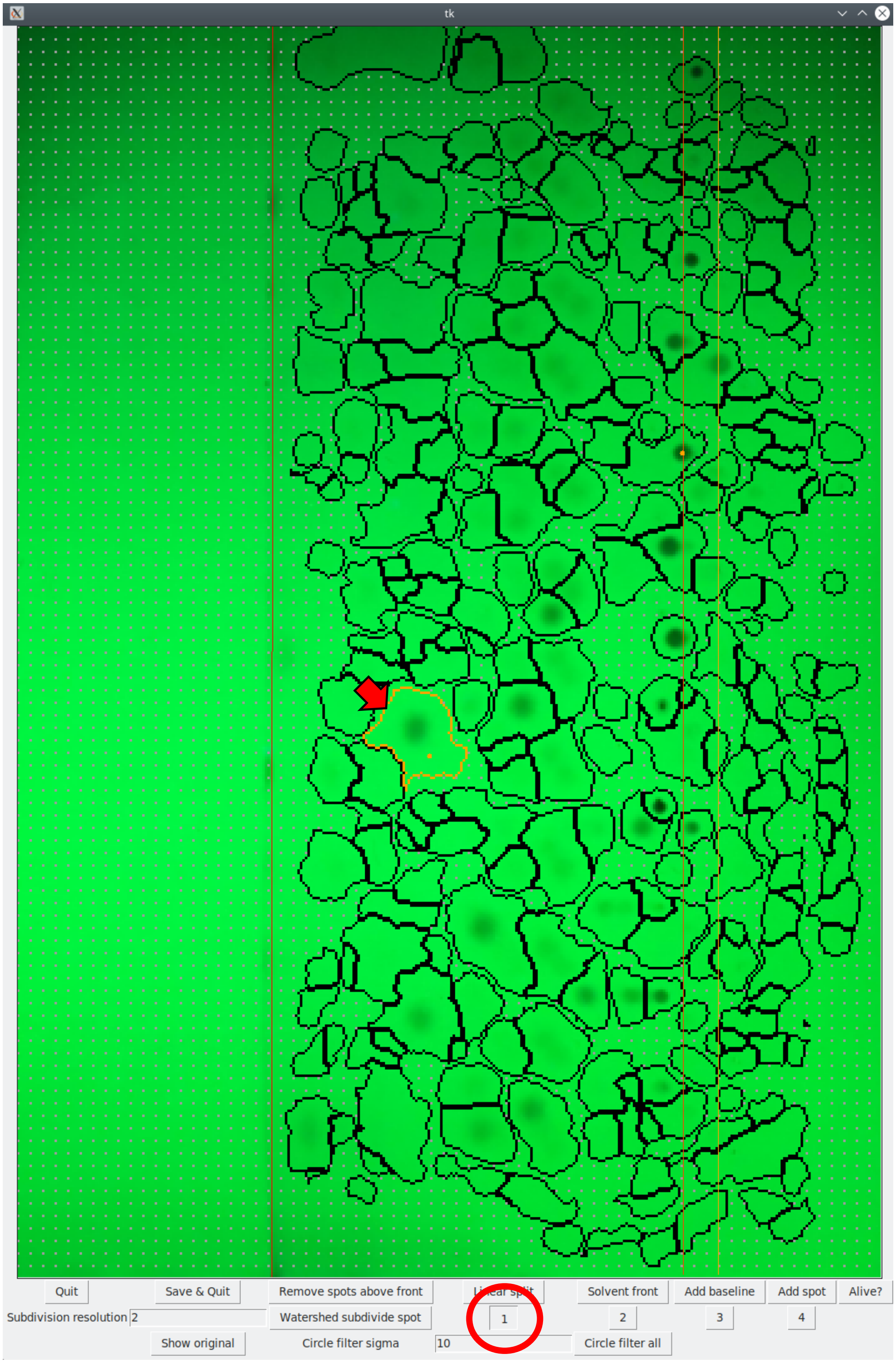
10. You can now assign baselines where the samples were loaded. You can assign up to four baselines. Assign a baseline by clicking on a spot, and the clicking on the “Add baseline” button. The baseline is automatically parallel to the solvent front. You must first define a solvent front before assigning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



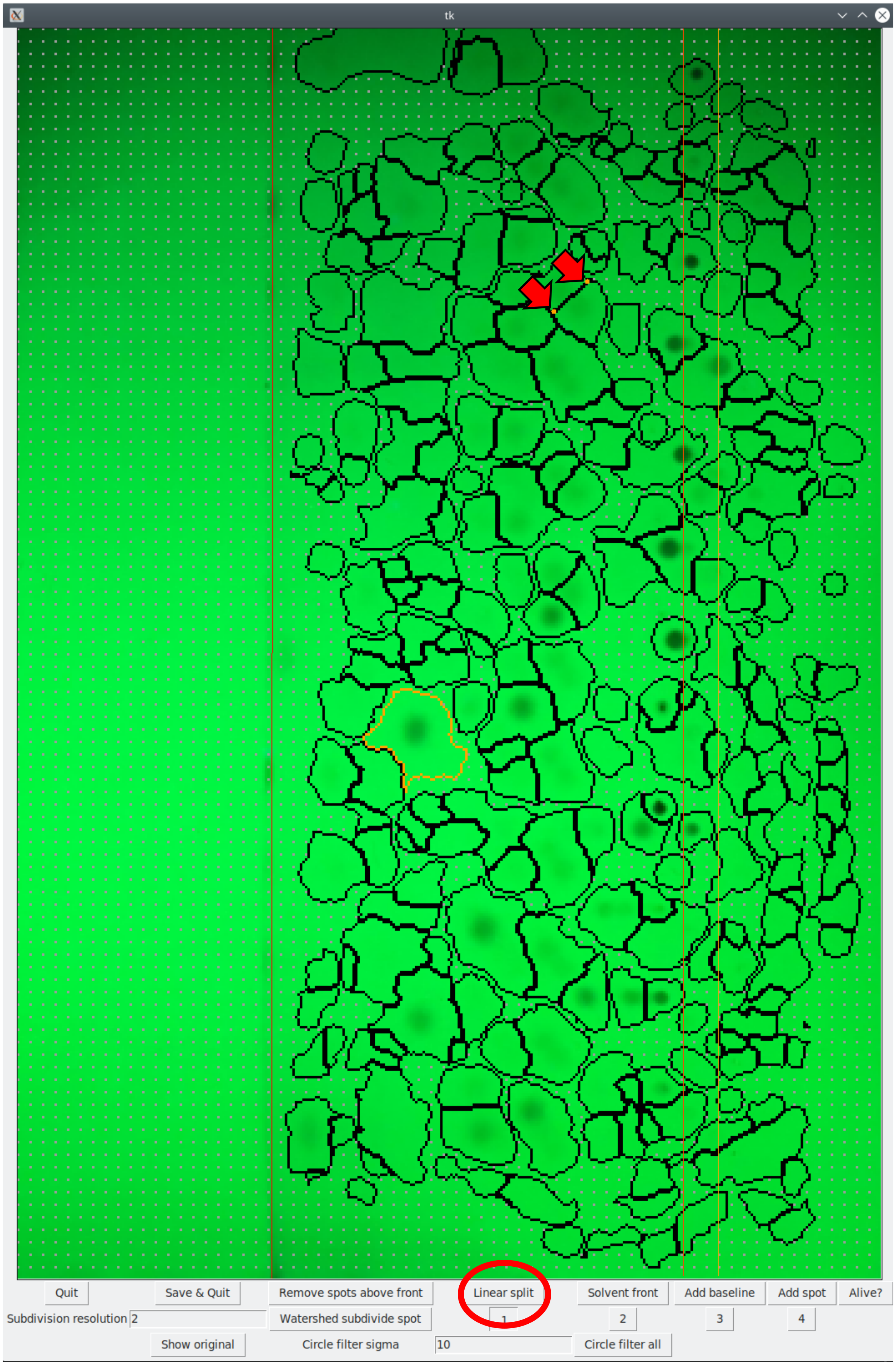
Subdivision resolution 2

Circle filter sigma 10

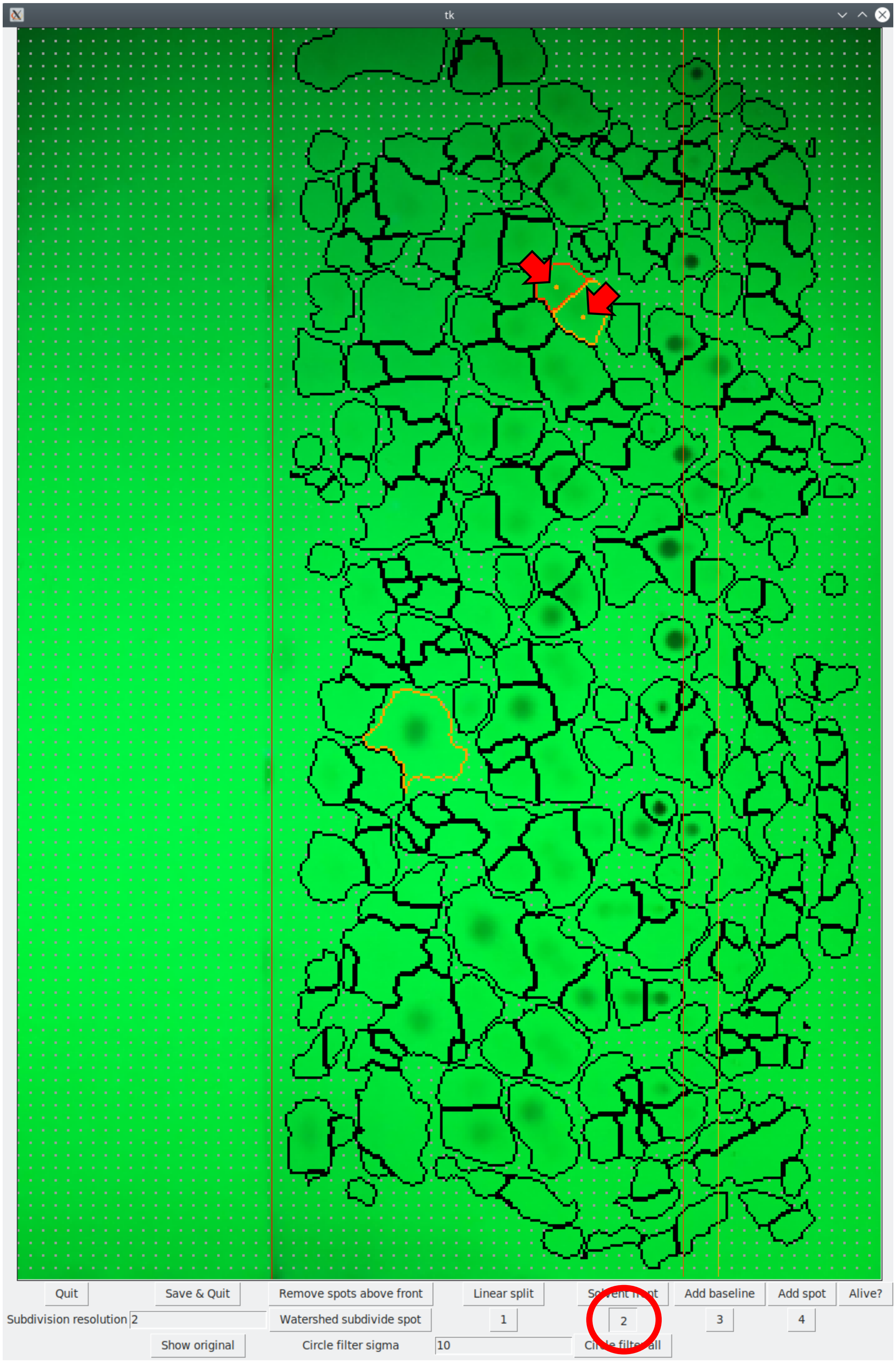
11. We can now assign samples to the baselines they came from. First, click on the button labeled “1”. Then double-click on a spot to assign it to the first baseline.



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.



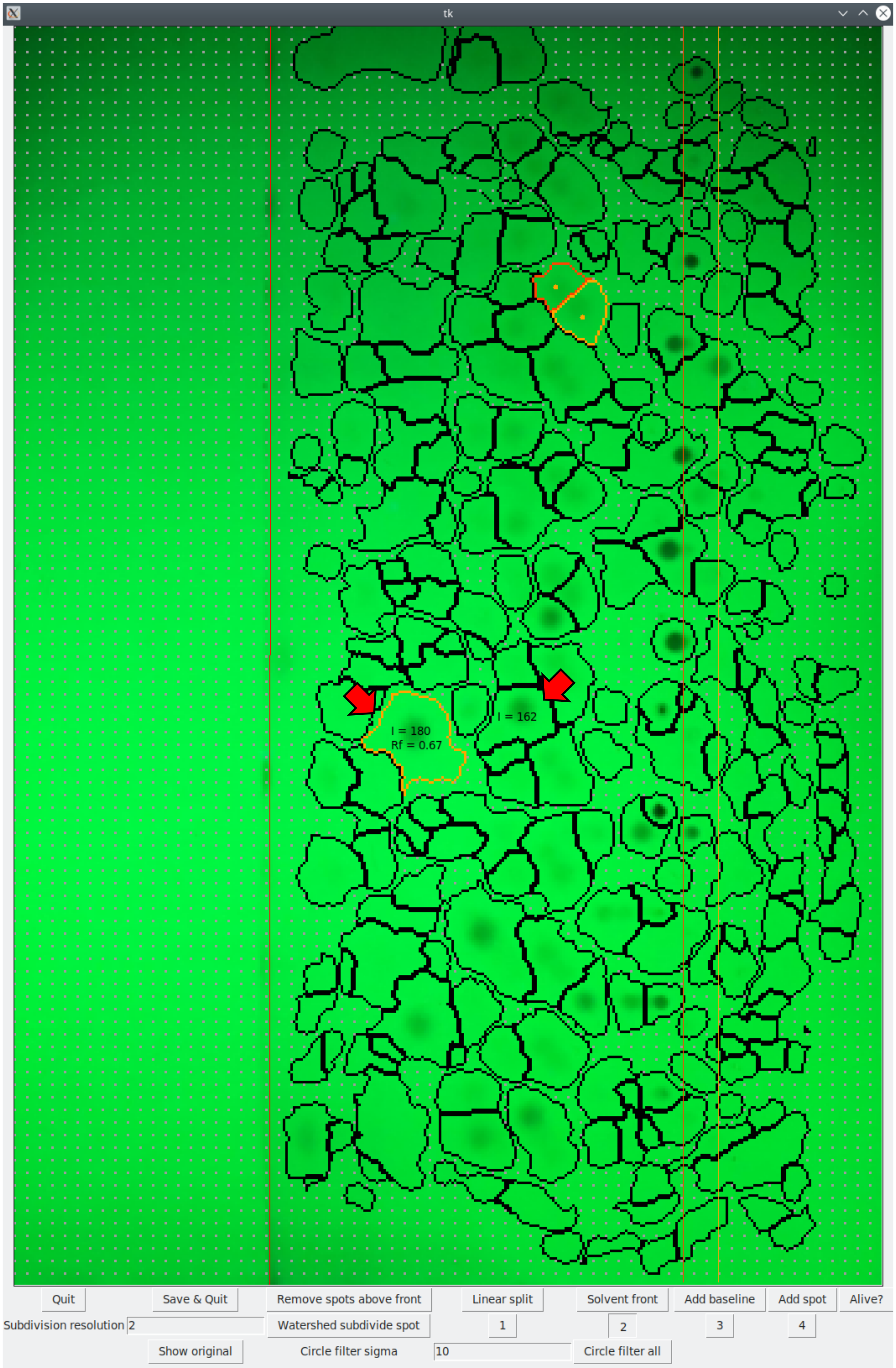
13. Now we can assign each spot to a different baseline. First double-click on one (because "1" has already been depressed), then click the "2" button, and double click on the other. The selections are color-coded.



Subdivision resolution 2

Circle filter sigma 10

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’s been assigned to a baseline, its Rf value.



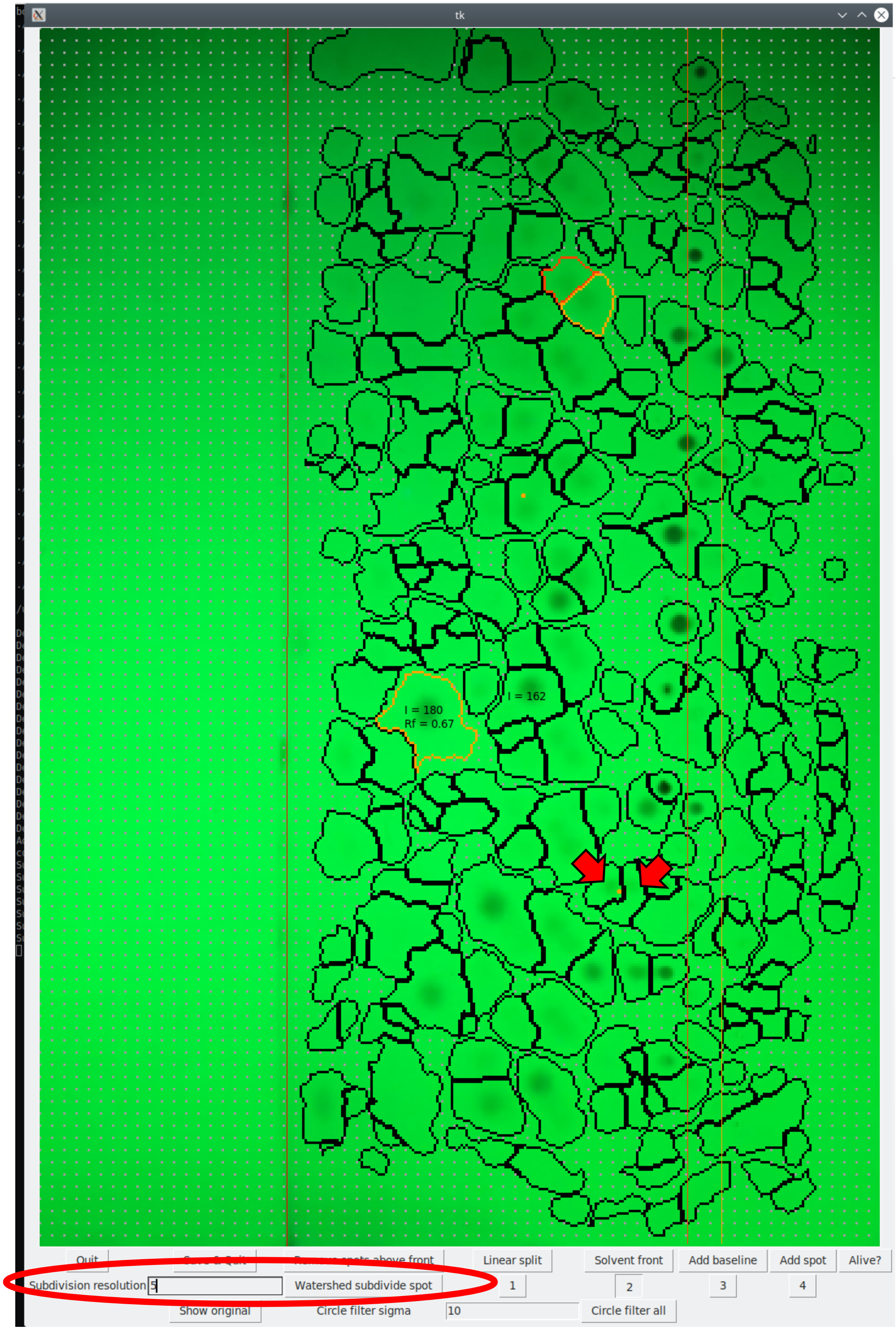
I = 180
Rf = 0.67

I = 162

Subdivision resolution 2

Circle filter sigma 10

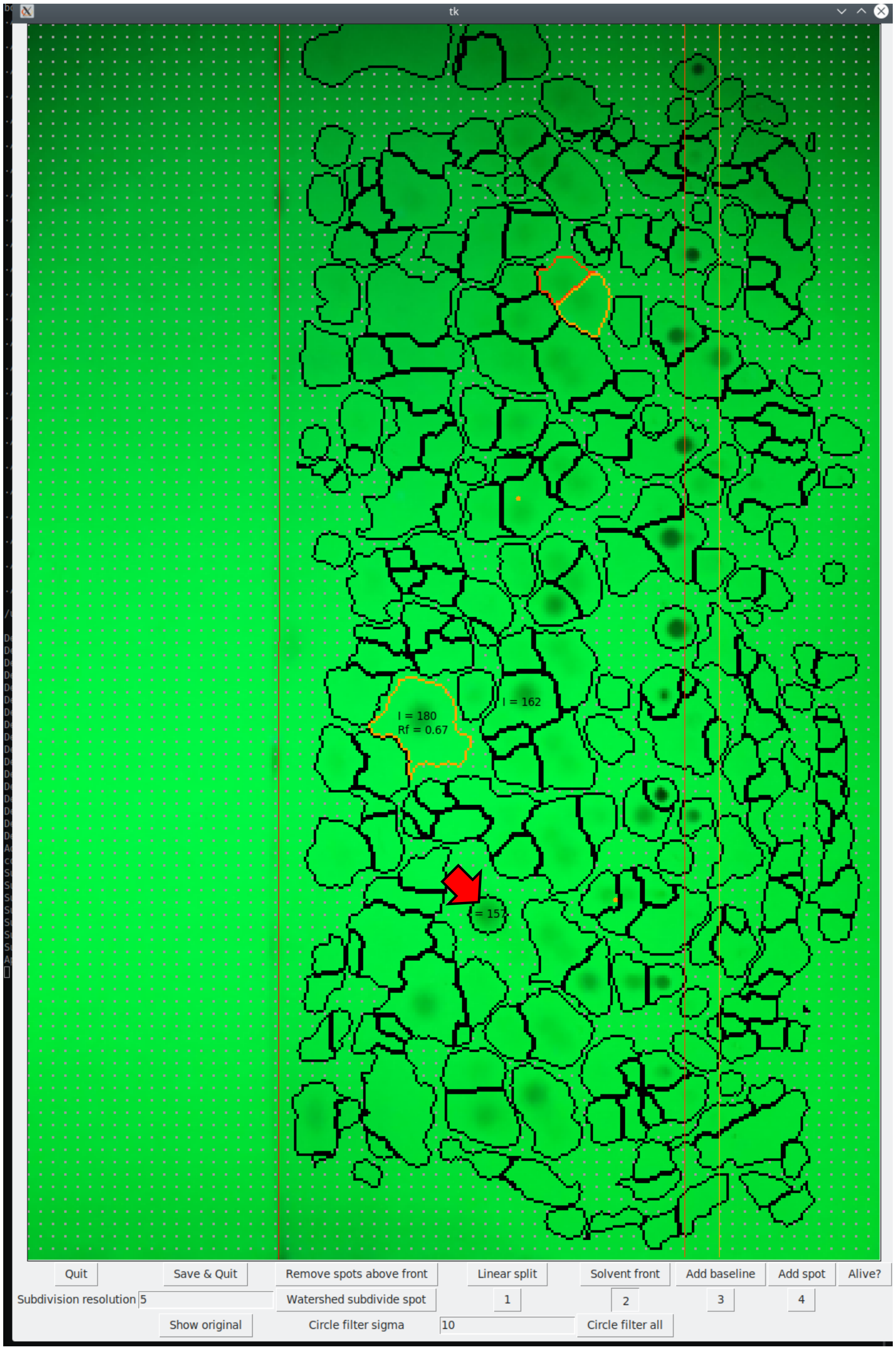
15. Instead of manual bisection, you can also attempt to subdivide a spot using a watershed algorithm. First, enter in the subdivision resolution value: the lower, the finer the resolution, and the more “sub-spots” the algorithm will find. After entering the value, click on the spot you want to subdivide 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.



Subdivision resolution 5

Circle filter sigma 10

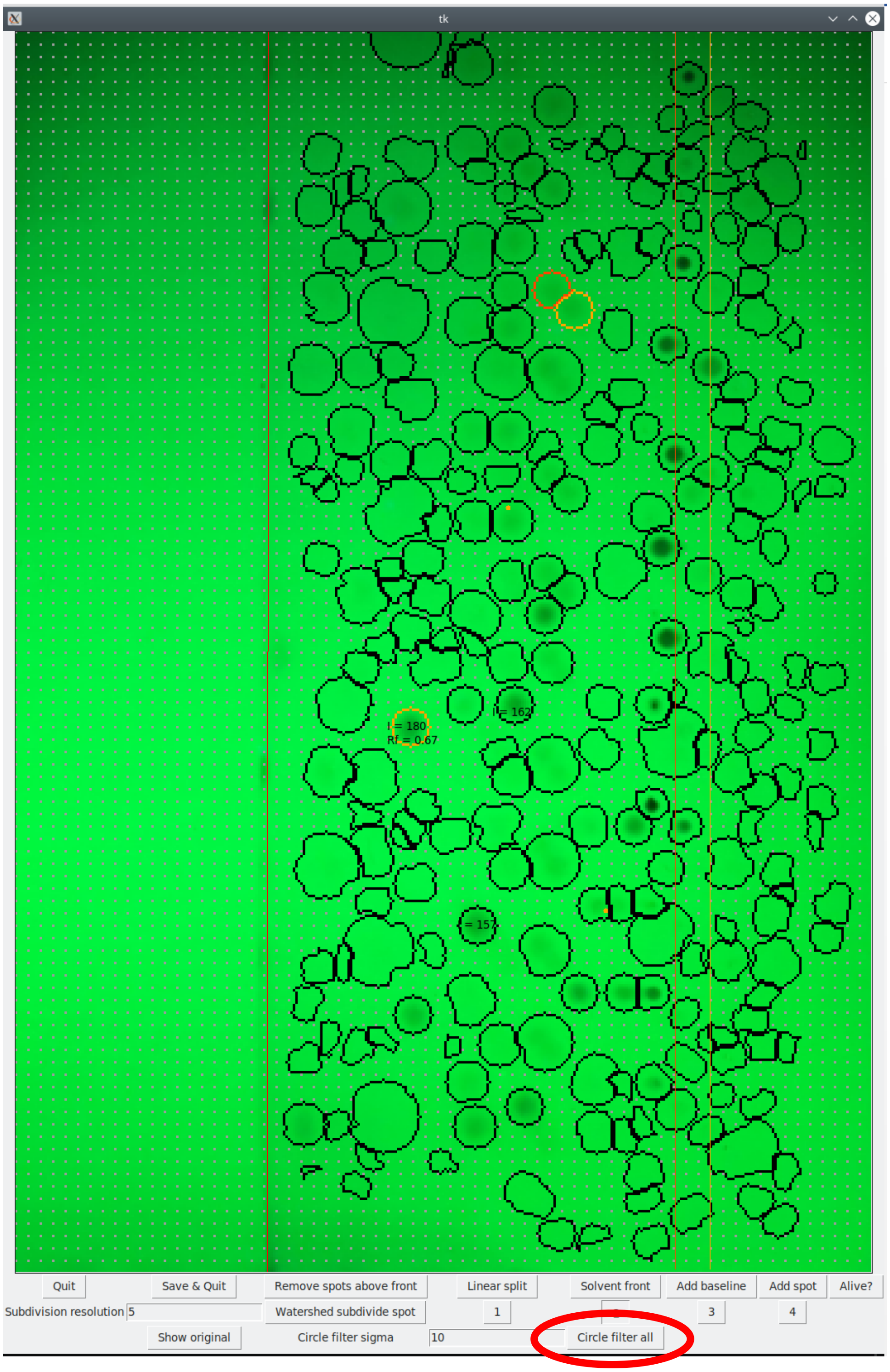
16. If your spots are circular, you can try shrinking the identified areas around them to a tighter, circular fit using the circle filter. First, choose the upper size bound of the circle(s) you want the software to try to locate using the “Circle filter sigma” entry box. The program will try to find 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.)



Subdivision resolution 5

Circle filter sigma 10

17. The other way to apply the circle filter is to apply it to all spots. Warning: if you haven't 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's done.



Subdivision resolution 5

Circle filter sigma 10

18. Finally, after you’ve 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.