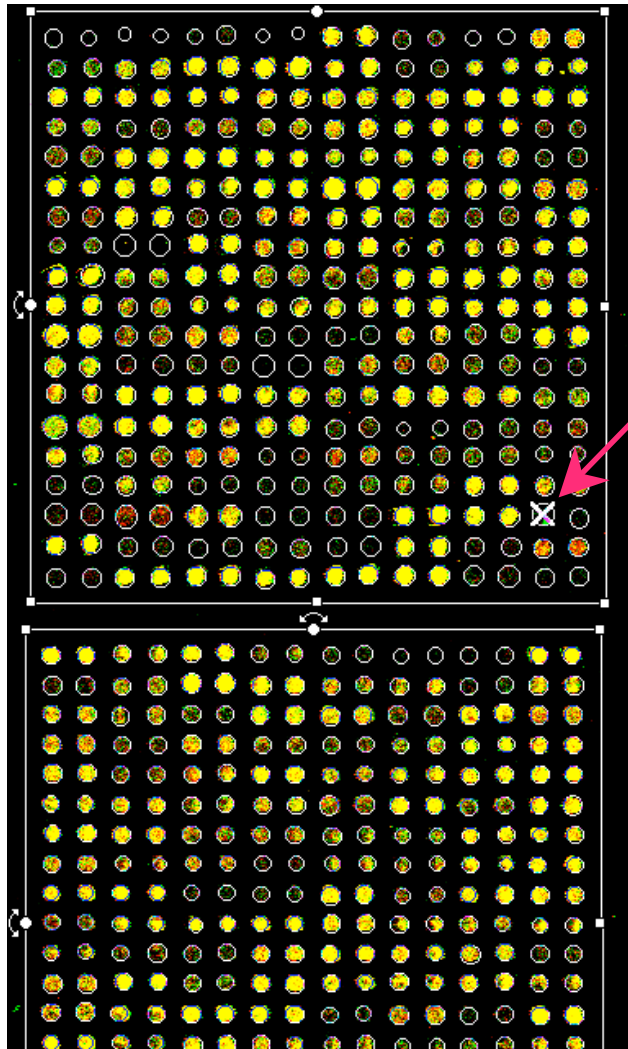
The background of the slide is a high-resolution image of a microarray chip. It features a dense grid of small, circular spots. Each spot is a different color, including yellow, green, orange, and red, set against a dark, almost black background. The spots are arranged in a regular, repeating pattern across the entire frame.

Image analysis using GenePix software program

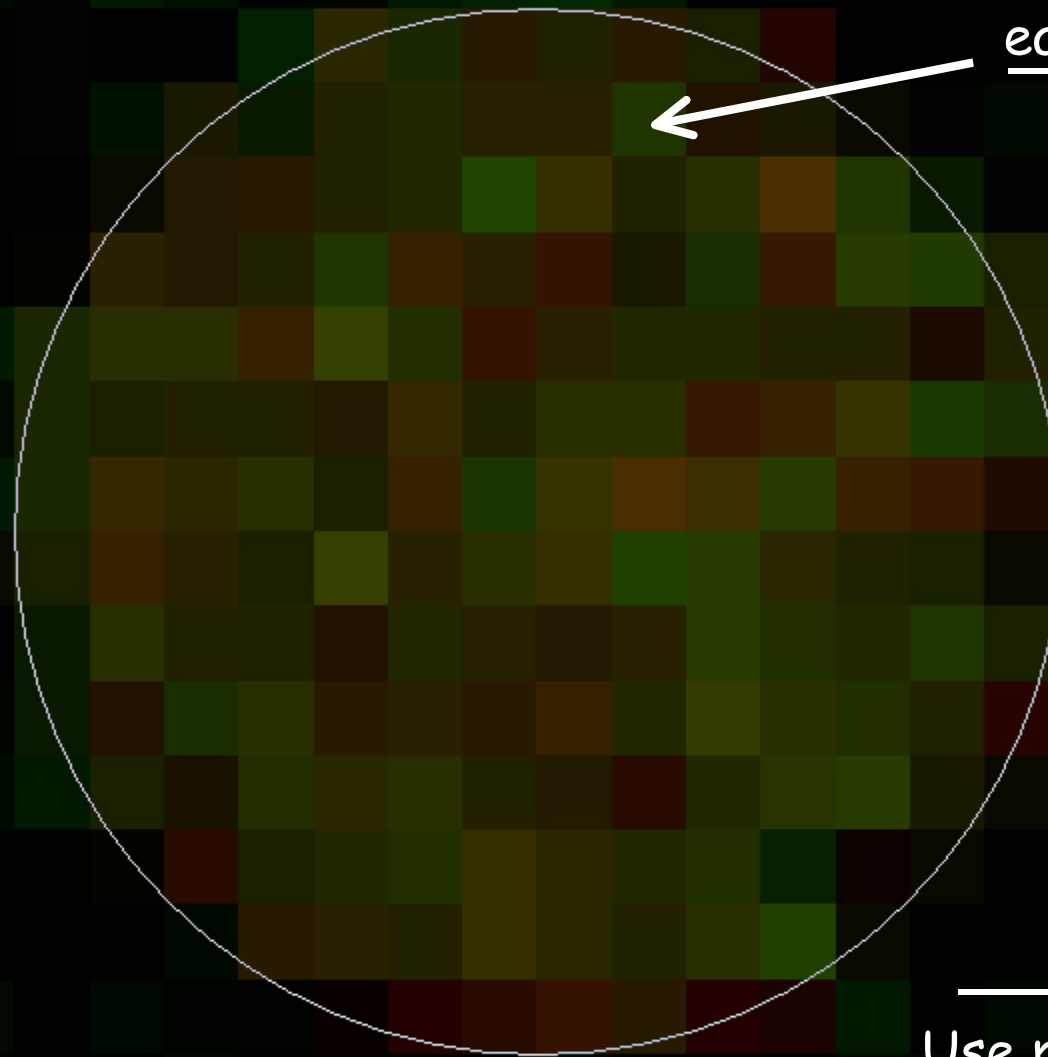
-by Steve Clough

Several key functions of GenePix



1. Locates spots, defines spot area
2. Flags bad spots (ex: dust in spot)
3. Calculates ratio Cy5 fluorescent intensity over Cy3 intensity for each spot
4. Produces tab-delineated tables for import to analysis programs

Value of pixels within spot equals the raw data. Software will give pixel value related to fluorescence from both Cy3 and Cy5 scans



each pixel (16-bit)

$$2^1 = 2$$

$$2^2 = 4$$

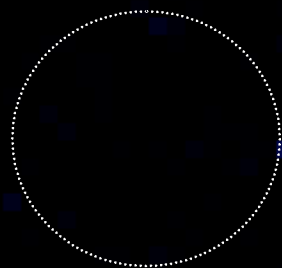
$$2^3 = 8$$



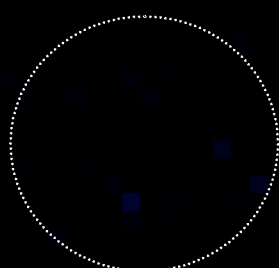
$$2^{16} = 65,536$$

Use median pixel value
of all pixels within circle

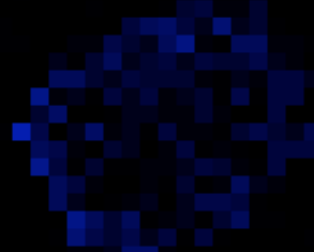
Fluorescent intensities from quality data.
Samples were double spotted. Background was ~80



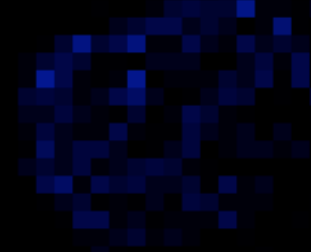
115



126



826



730



50,580



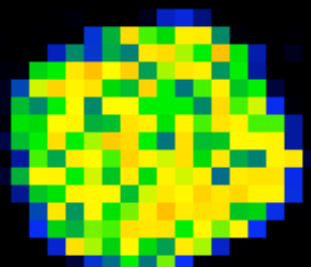
53,485



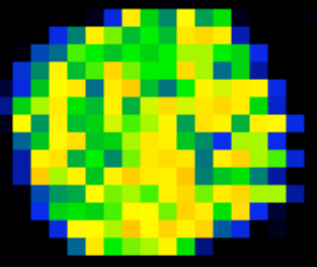
45,239



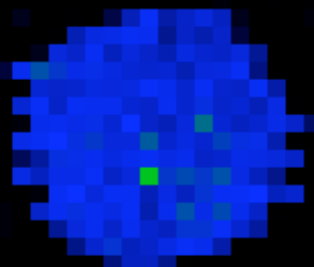
49,334



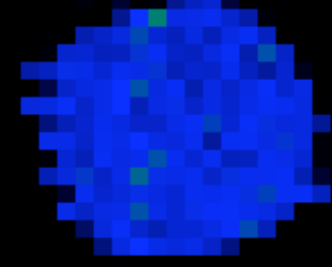
15,916



15,986

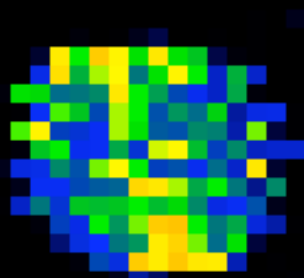


7,327

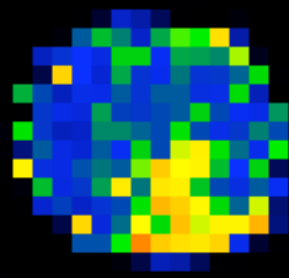


7,577

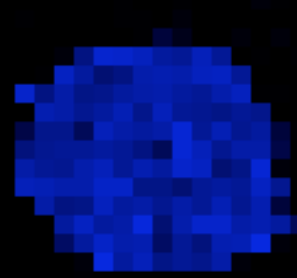
Fluorescent intensities from quality data.
Samples were double spotted. Background was ~80



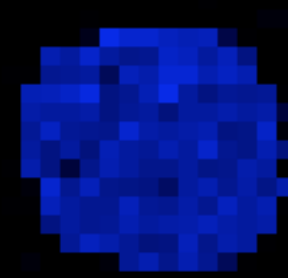
12,605



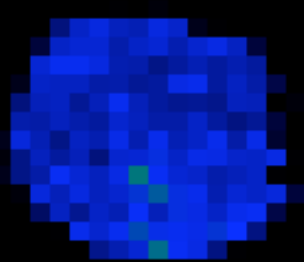
12,338



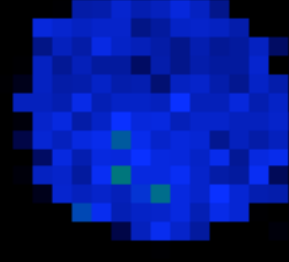
4,455



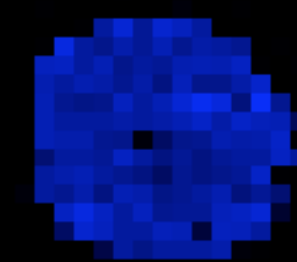
4,560



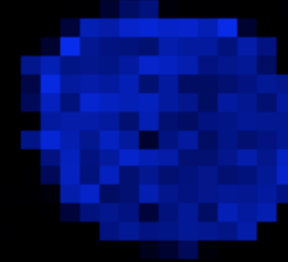
5,989



6,427



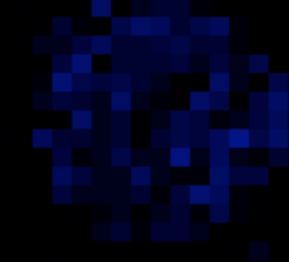
4,552



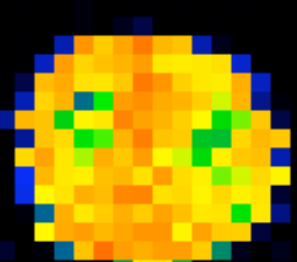
3,824



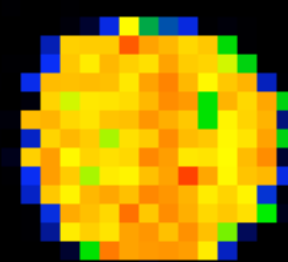
1,262



1,233



19,990

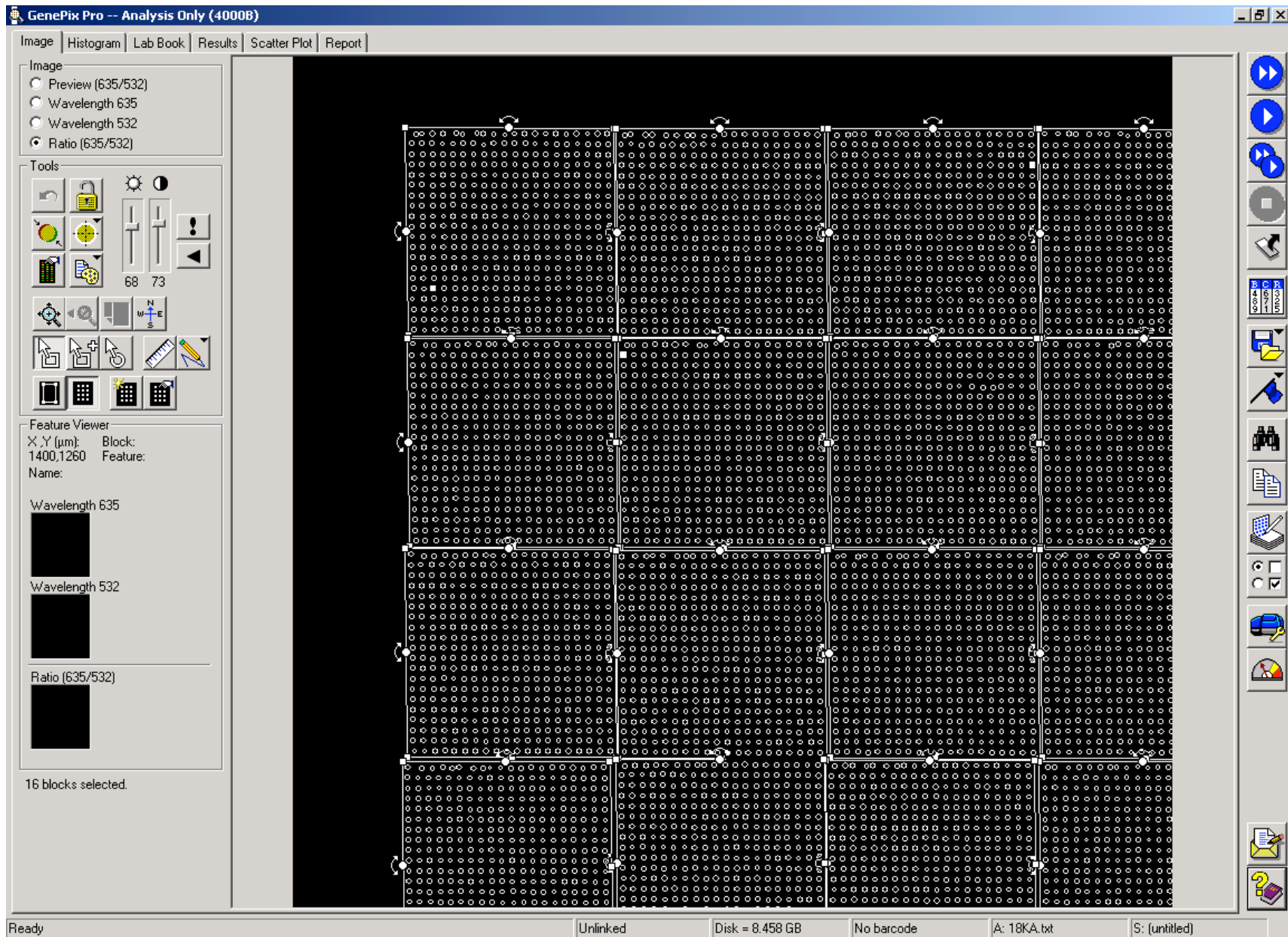


22,899

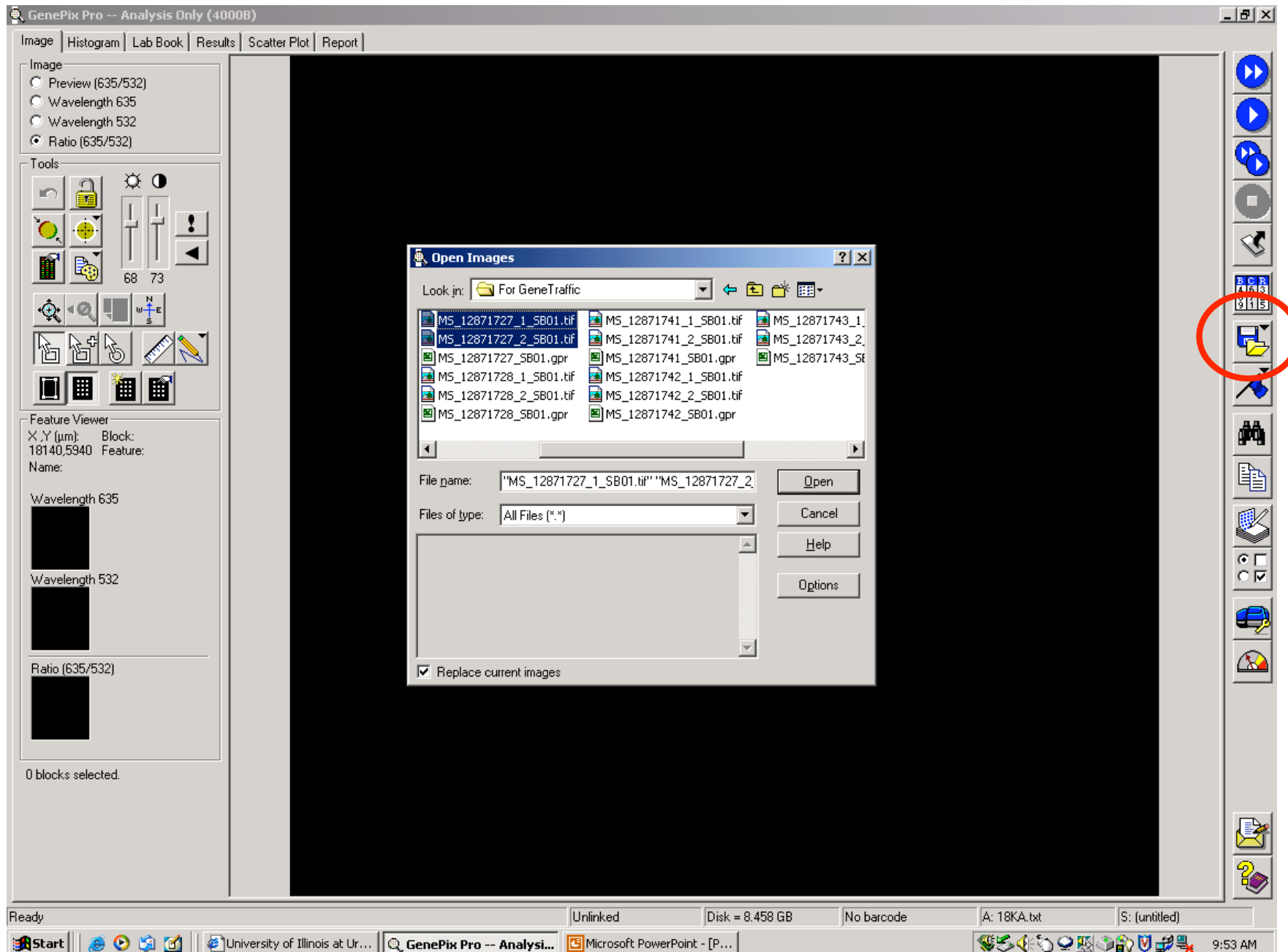
The background of the slide is a microarray image, showing a grid of small, colored spots (yellow, green, orange, and red) on a dark background, representing gene expression data.

Using GenePix software program

When you open the software, you'll get a window that might have a default grid. Use 'Control A' to select and then delete this grid.

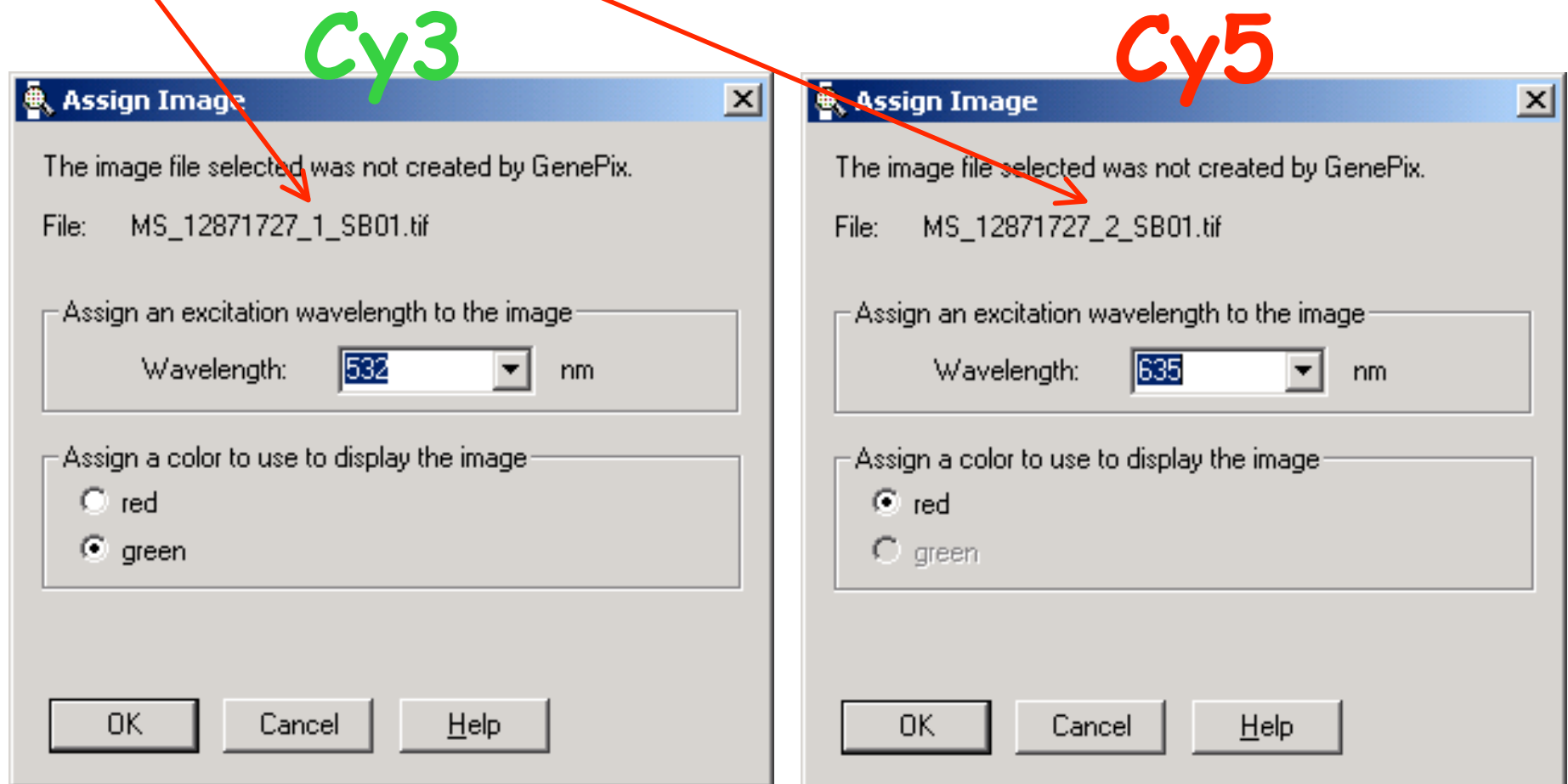


Click on the File Manager icon to open a Cy3 and Cy5 image file.



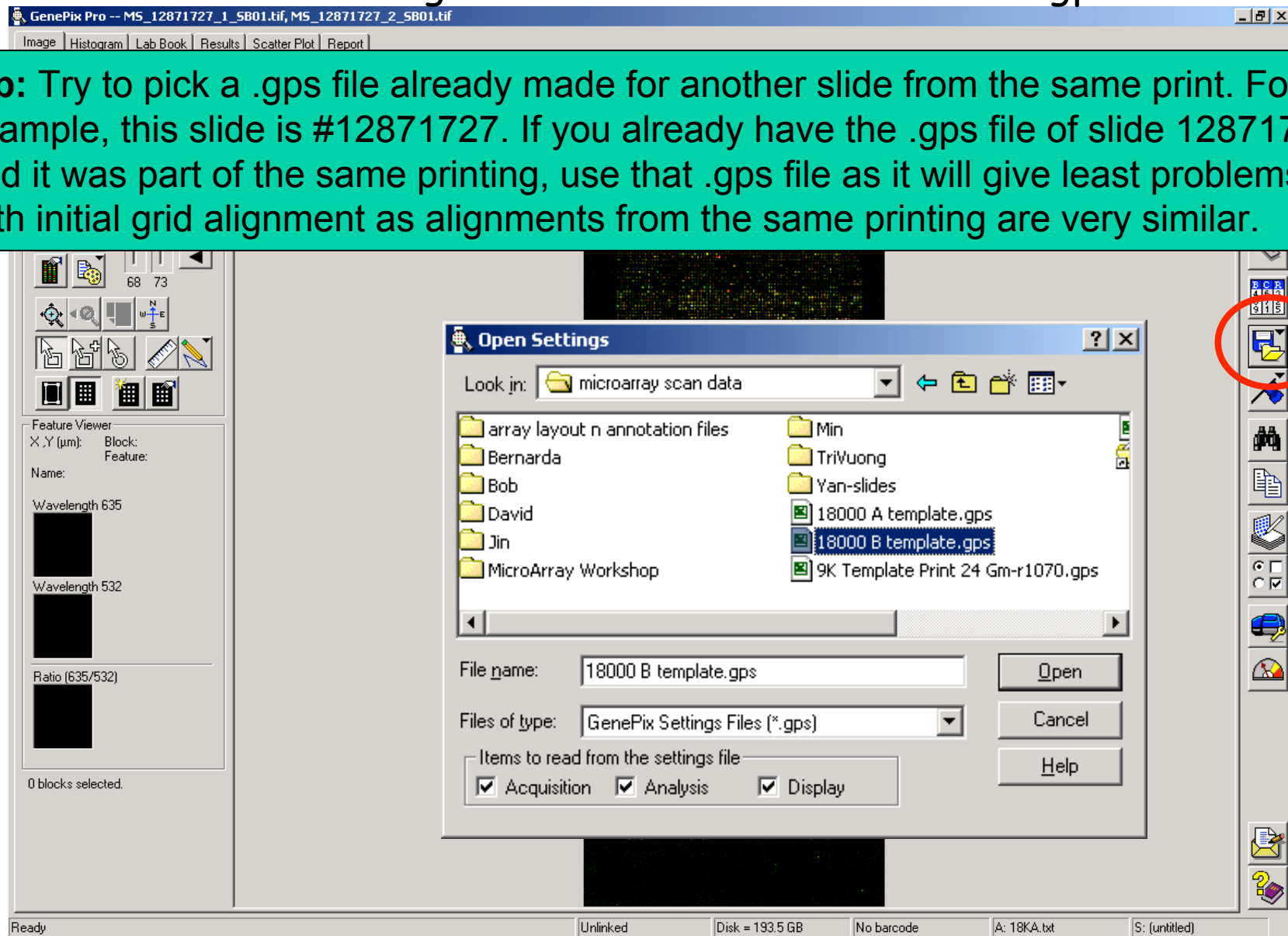
The following 'Assign Image' windows will pop up one after the other to allow one to assign which image is Cy3 (wavelength 532, green) and which is Cy5 (wavelength 635, red).

In the labelling convention of our lab, the slide ID is followed by either a 1 for Cy3 or a 2 for Cy5.

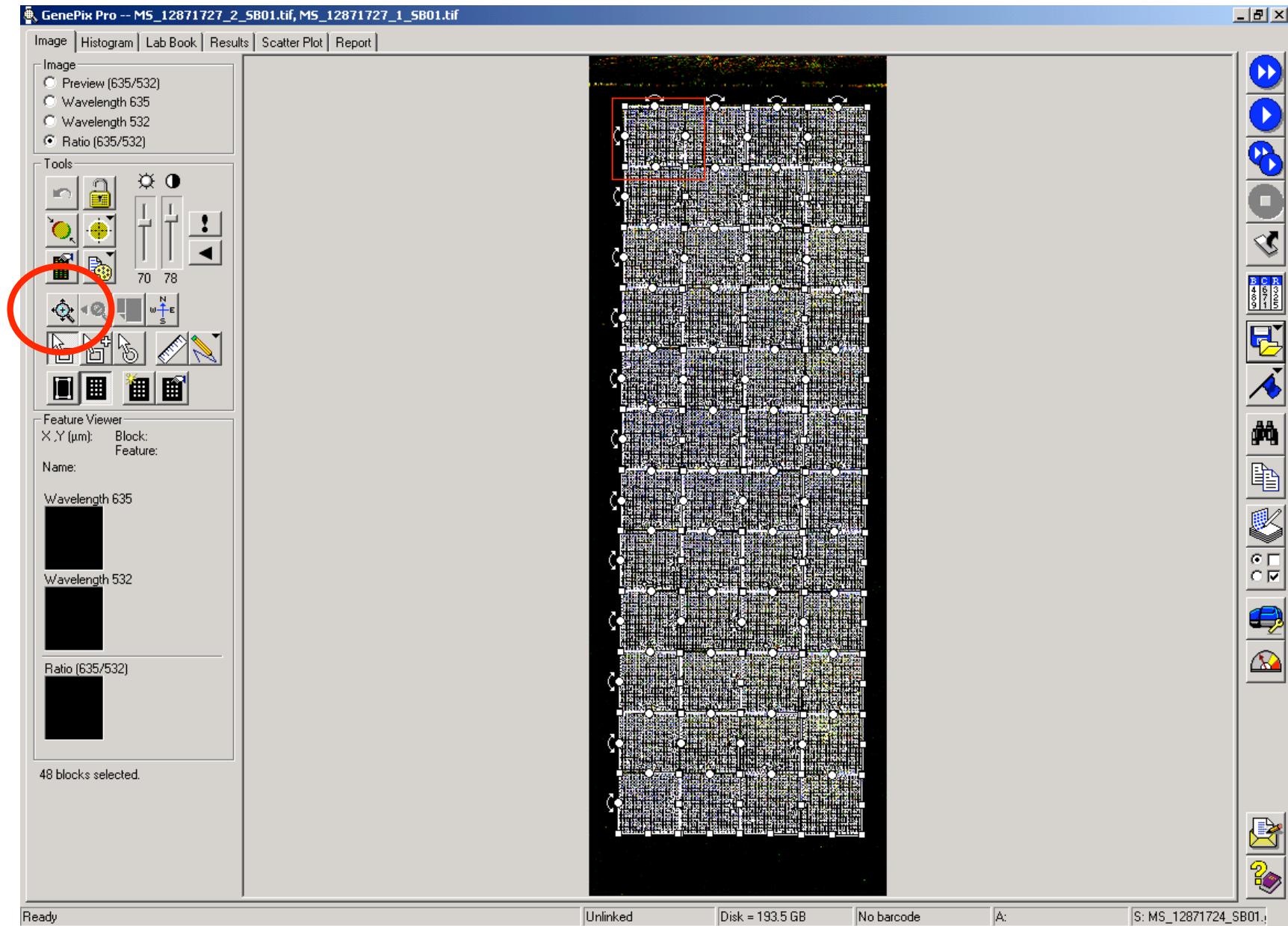


After assigning dyes, the composite image will appear.
Click on File Manager icon to open/select settings (grid template).
GenePix Settings files end with the extension '.gps'

Tip: Try to pick a .gps file already made for another slide from the same print. For example, this slide is #12871727. If you already have the .gps file of slide 12871726, and it was part of the same printing, use that .gps file as it will give least problems with initial grid alignment as alignments from the same printing are very similar.

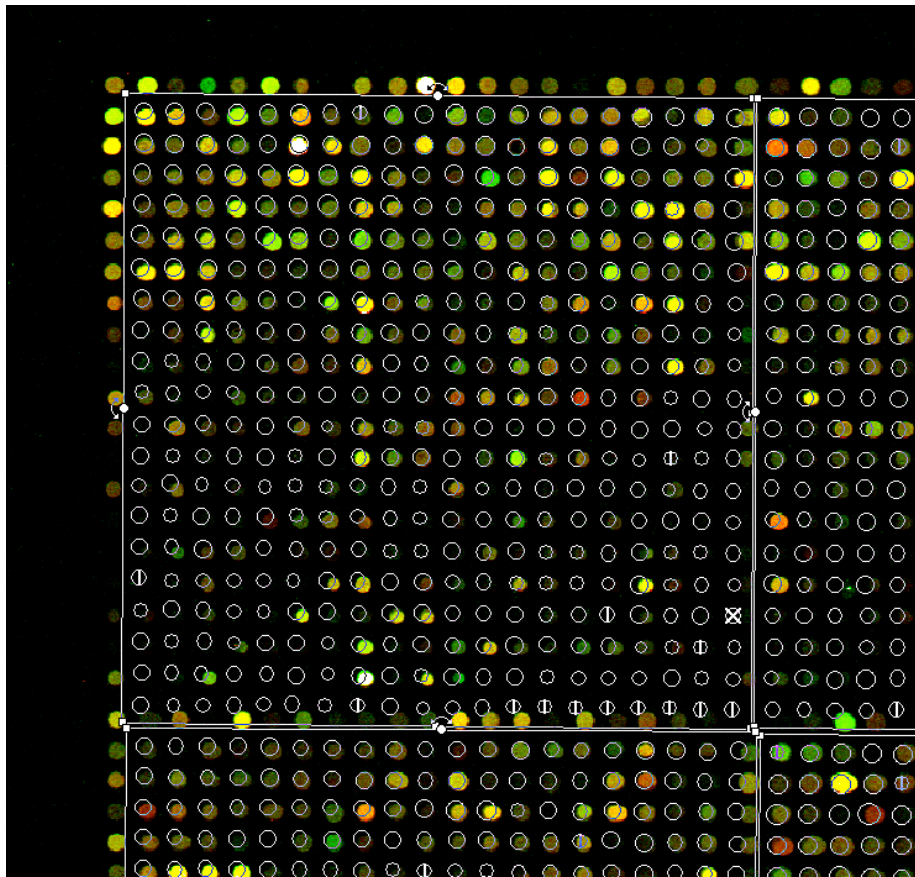


To begin to align the grids, use 'Control A' to select entire grid. Then use the magnifier to select area to Zoom-in upon (keeping the entire grid selected).

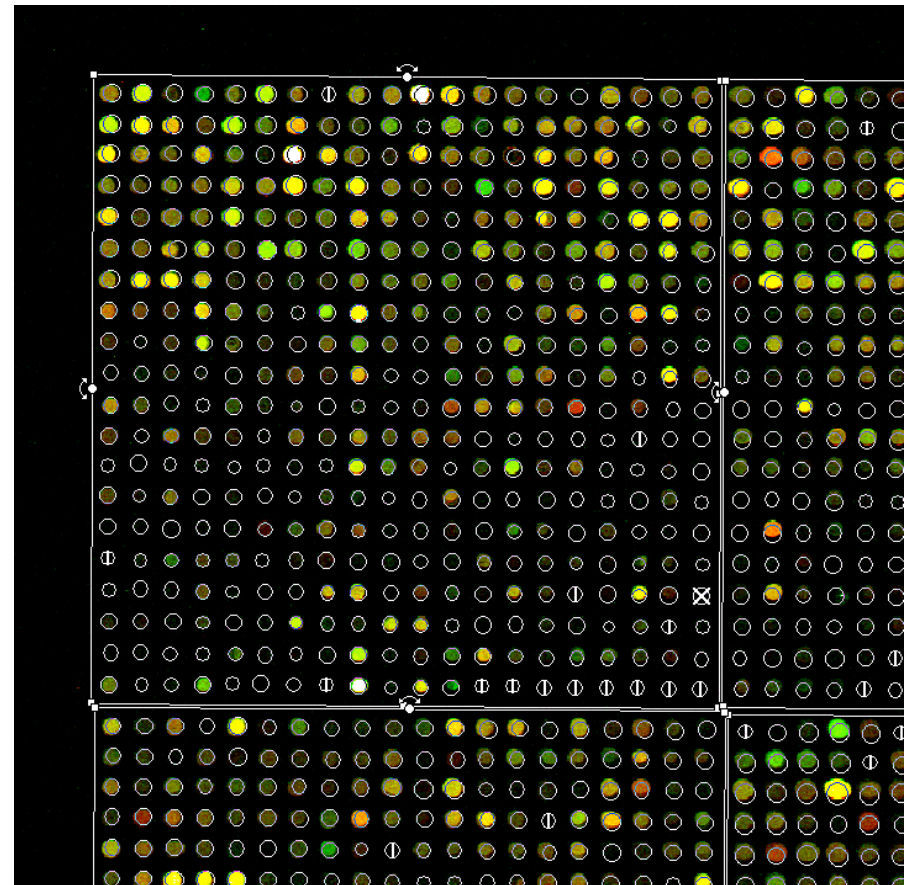


Use the mouse to grab and slide entire grid to approximately line the grids over the spots.

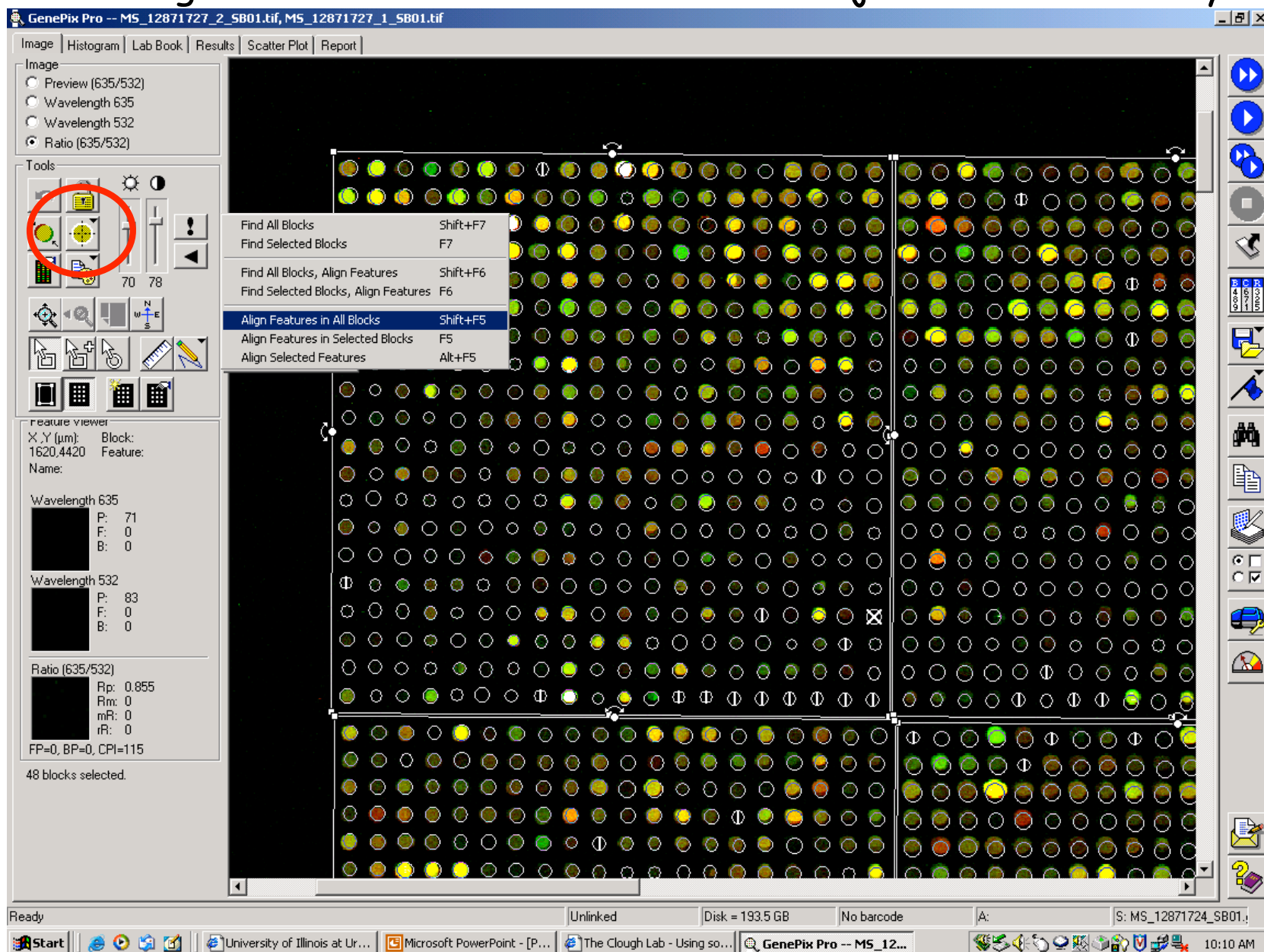
Before



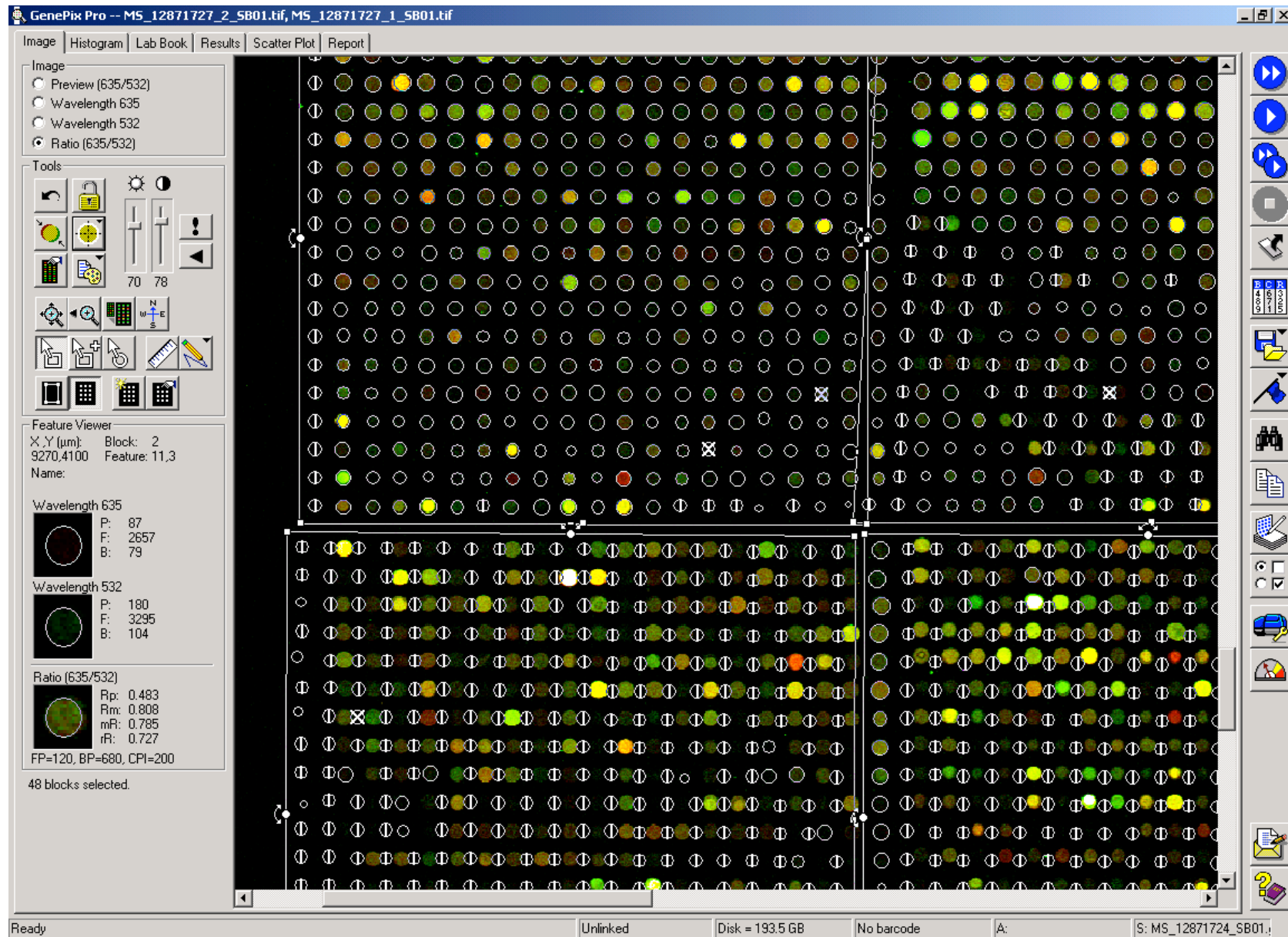
After



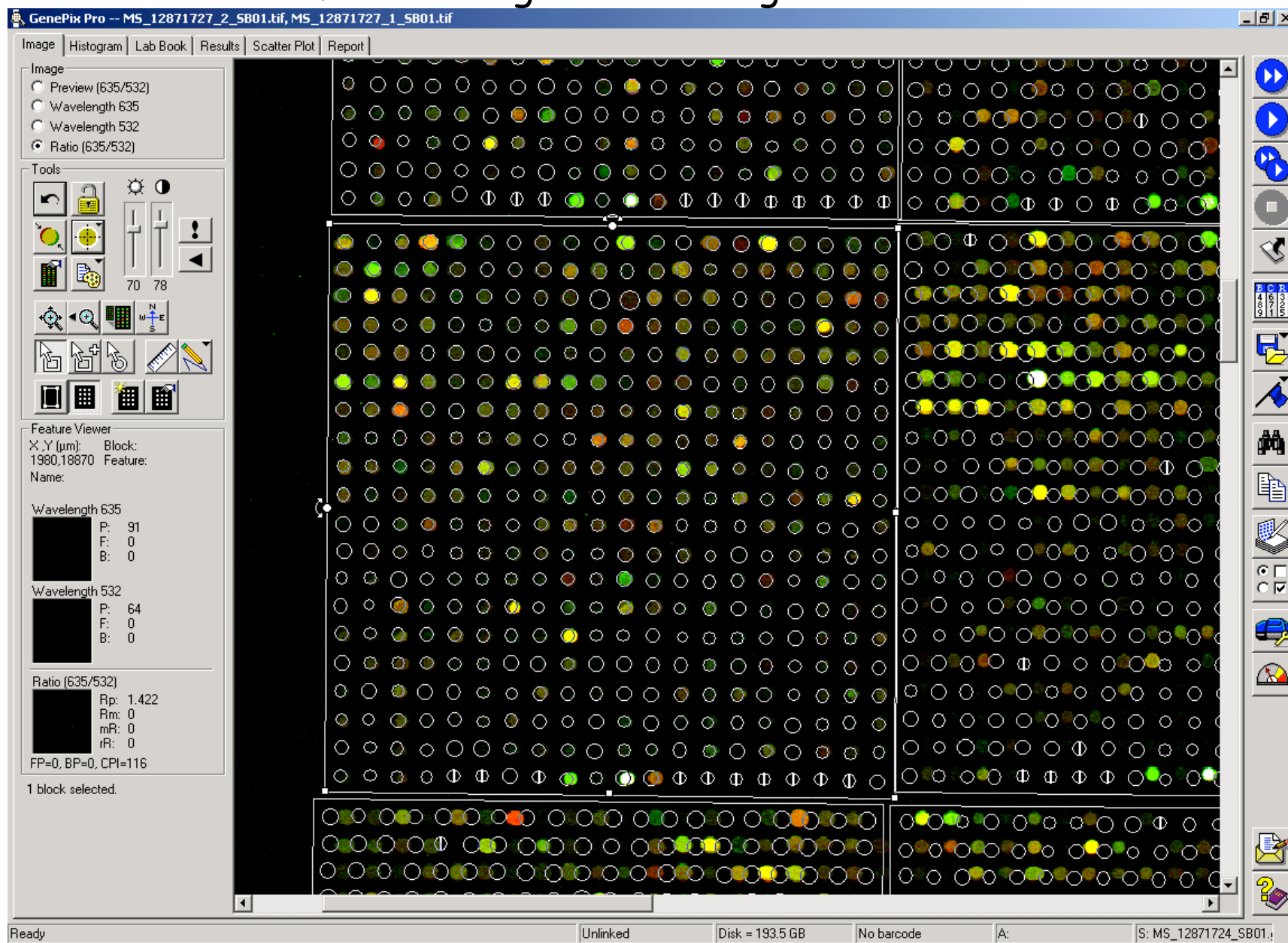
Click on the alignment icon and select any of the align features options, such as 'Align Features in Selected Blocks'. Or just hit the 'F5' key.



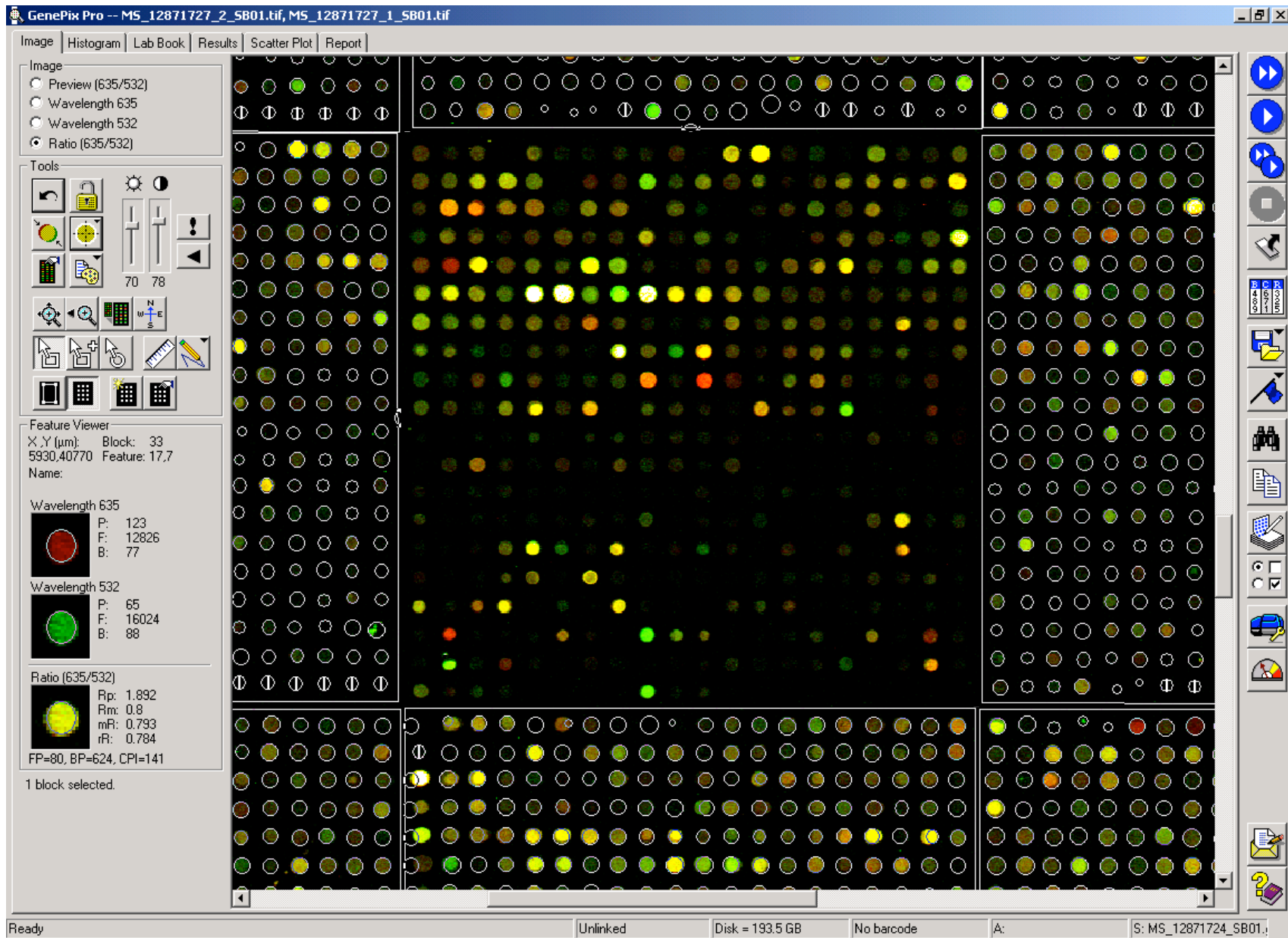
Using this quick method to align works sometimes. But occasionally, one or more of the blocks will be misaligned as seen here in this example.



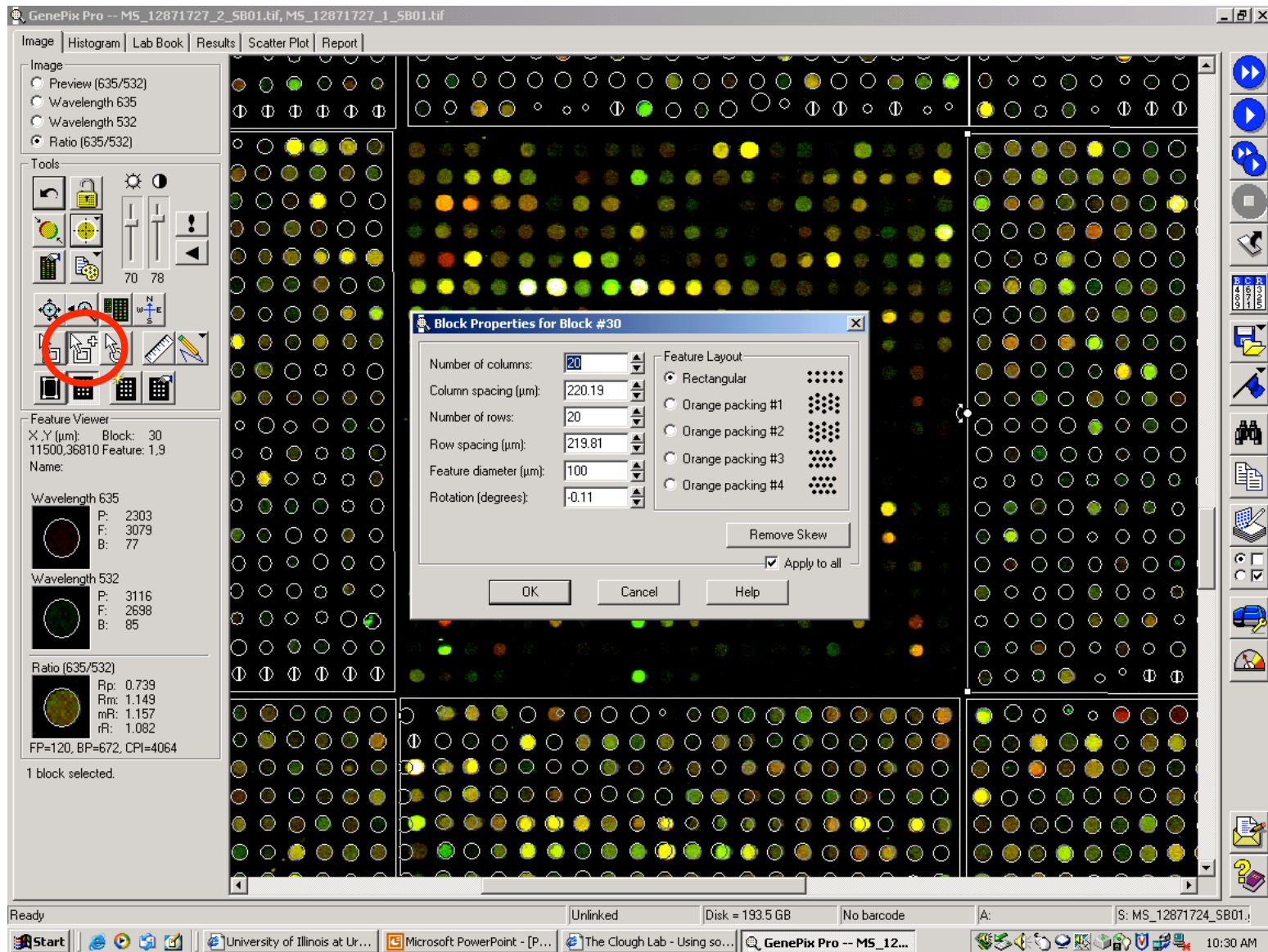
To minimize such misalignments, use the mouse to roughly align each individual block before hitting the auto alignment button.



If a block grid is too messed up, it can be deleted and replaced with a good block. Select bad block and hit 'delete' key.



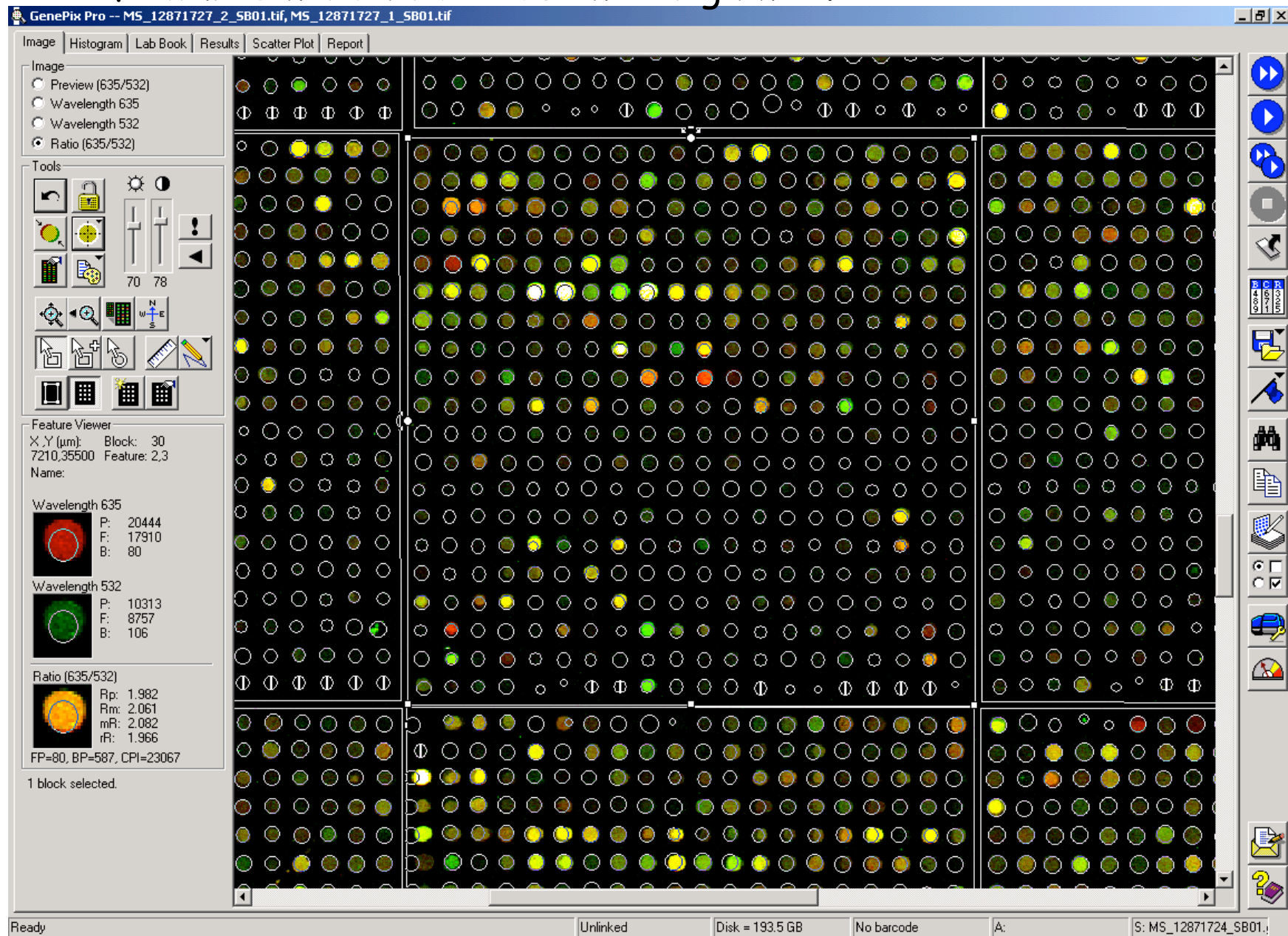
To copy a block, click on the duplicate block icon, and then use the mouse to select the block to duplicate. You'll get a 'block properties' window. Hit 'ok'



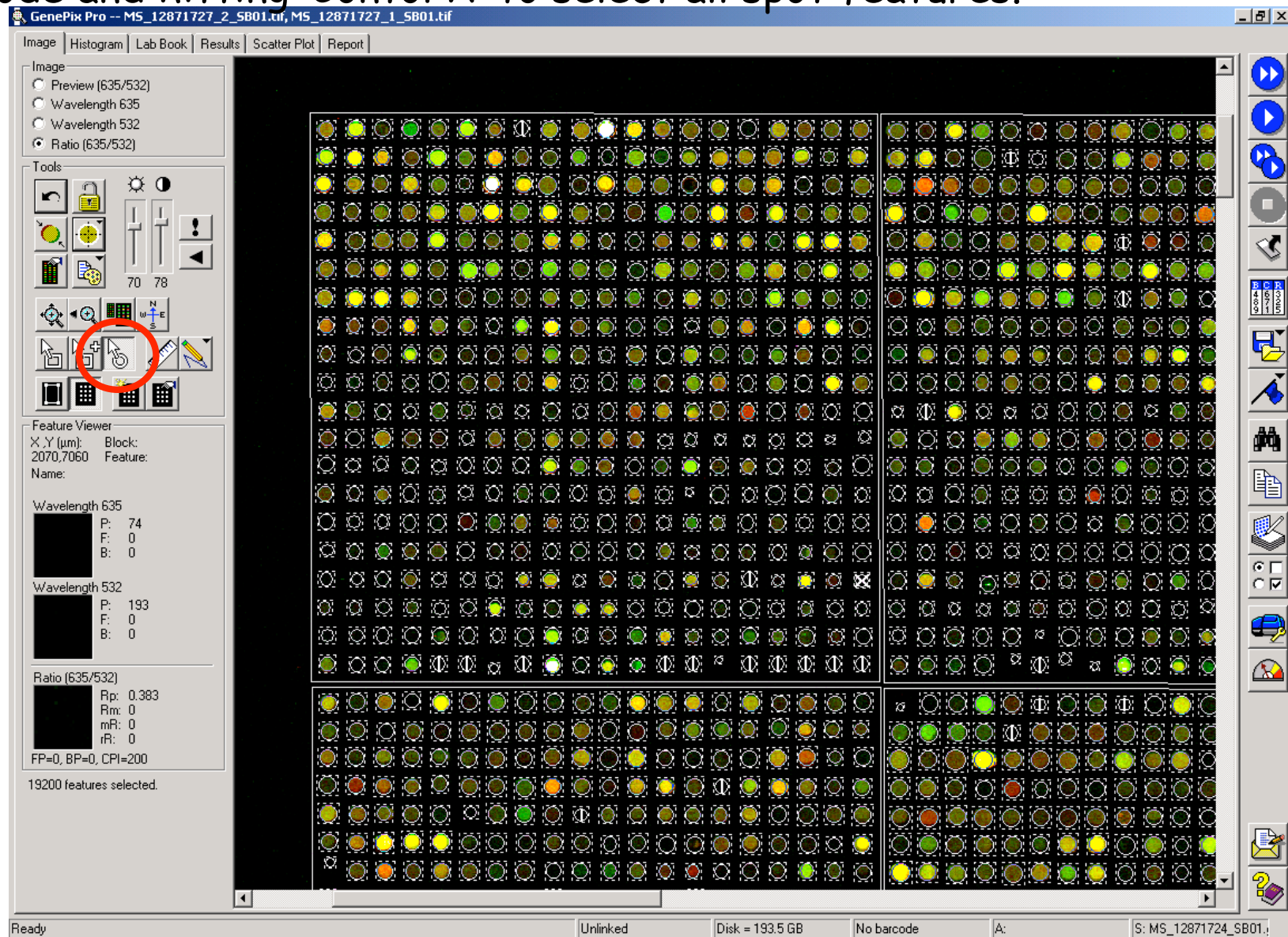
Mouse to where you want to place the duplicated grid and click to place. Then use mouse to position it.



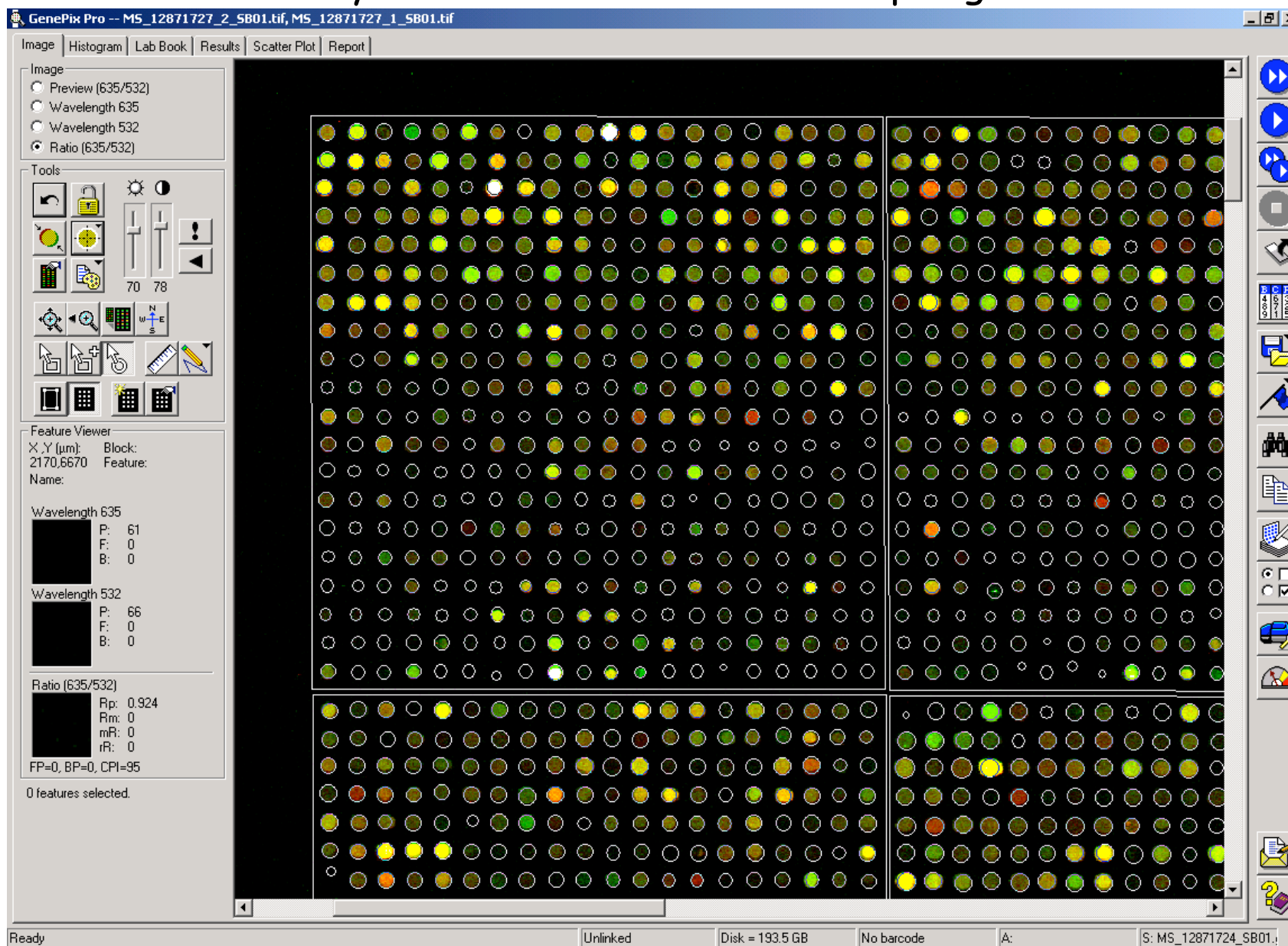
Hit F5 to align and you might get lucky and this time the features within the block will align well.



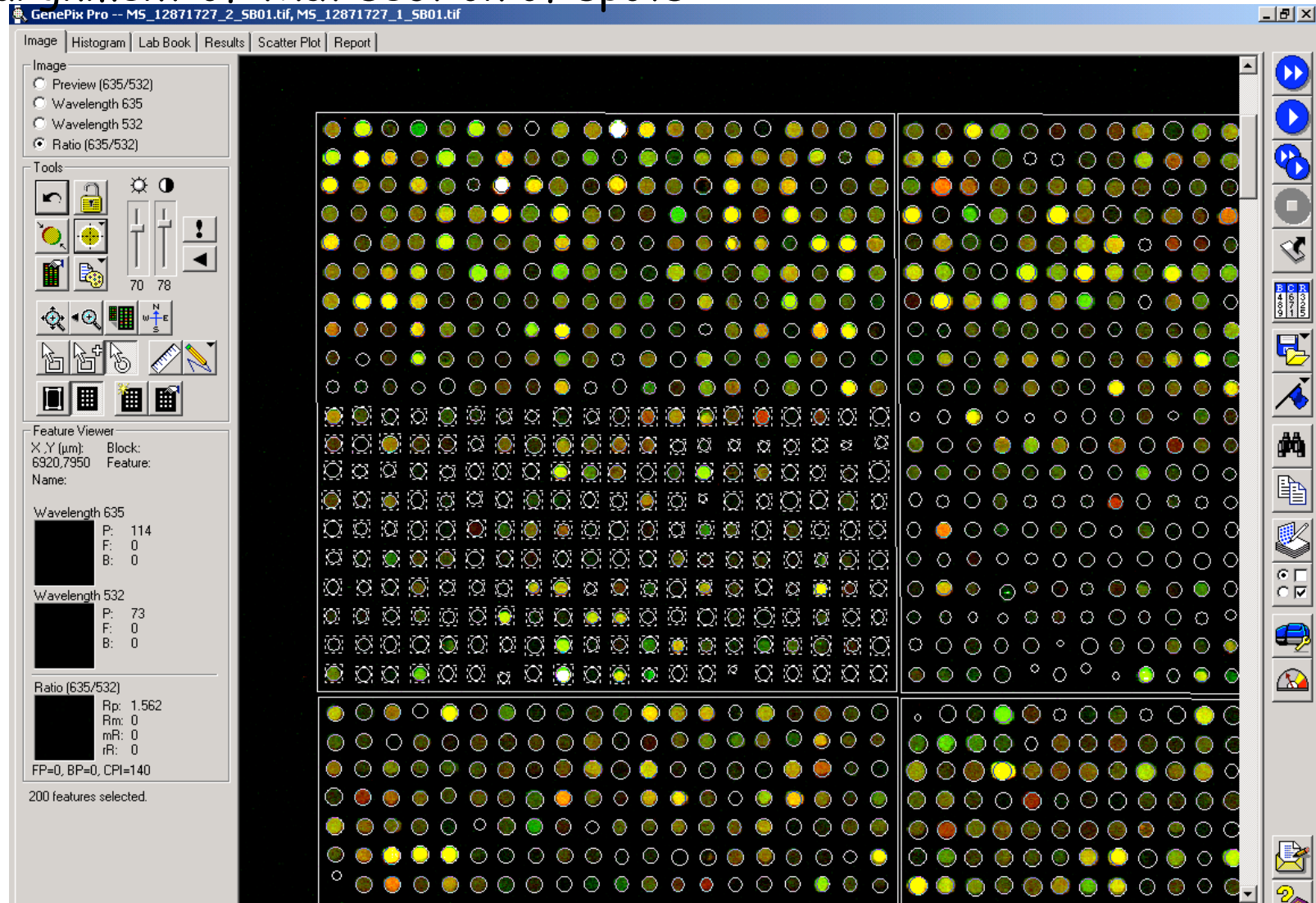
Once you have finished getting a rough overall alignment using the automatic alignment feature, remove all flags by switching to Feature Mode and hitting 'Control A' to select all spot features.



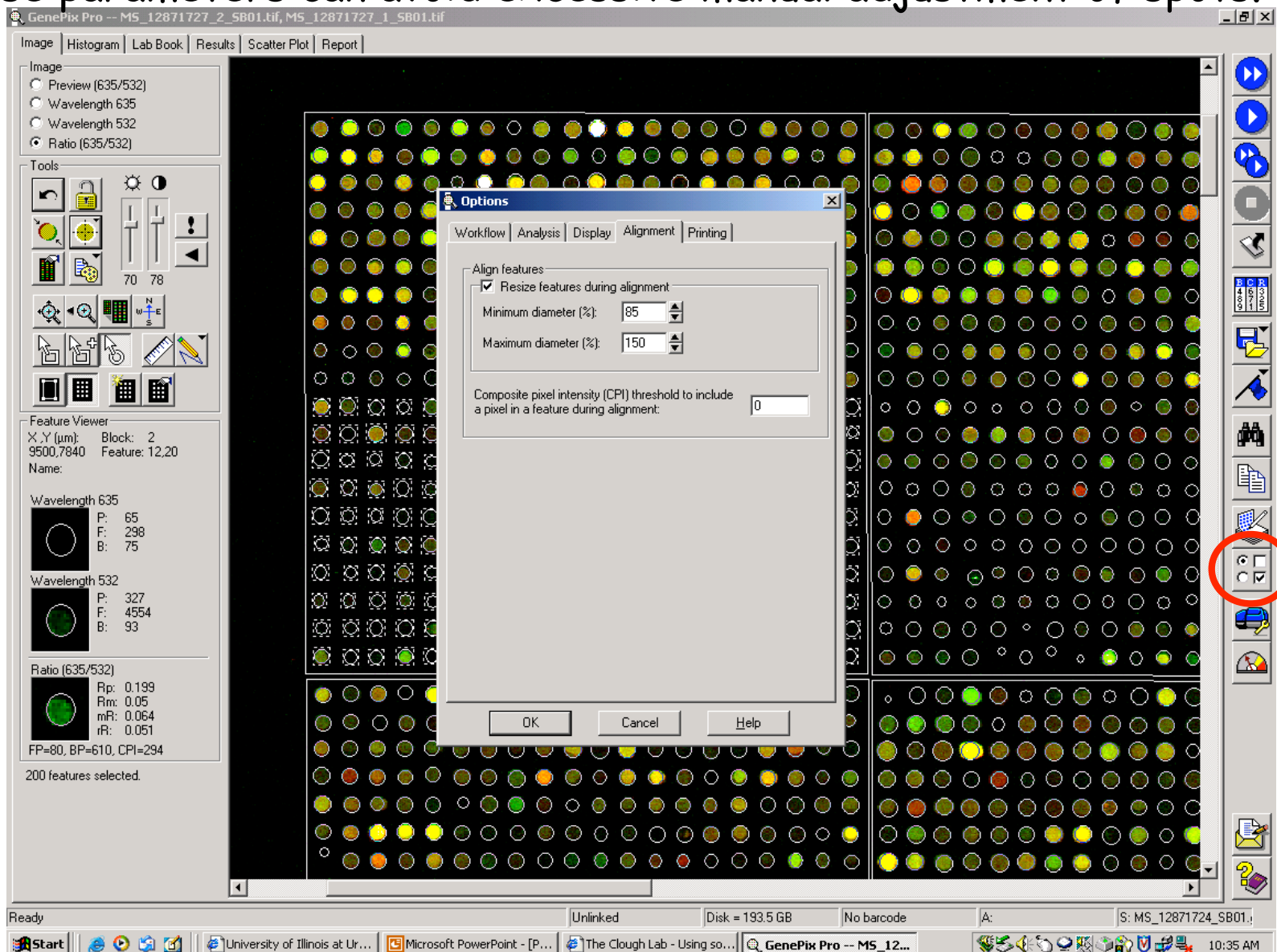
Hitting the 'L' key will remove all flags.
Now you'll have to fine tune the spot grids.



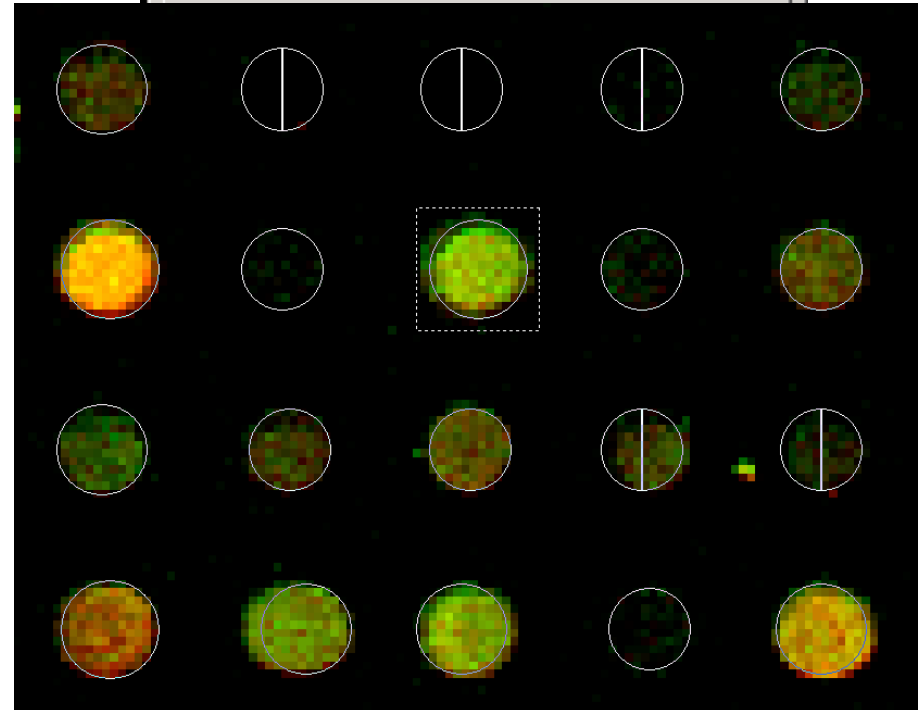
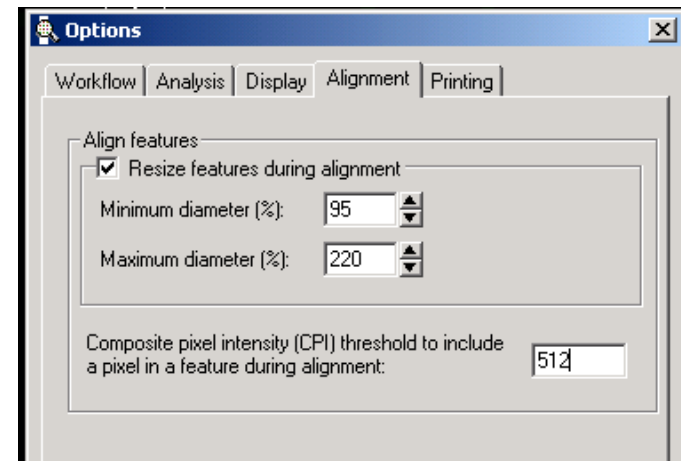
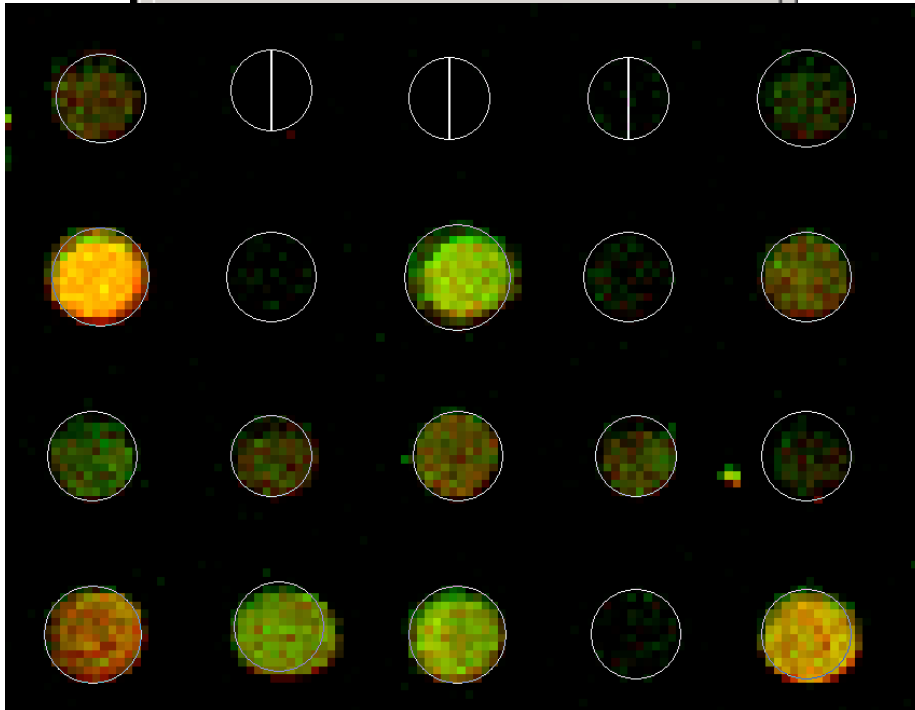
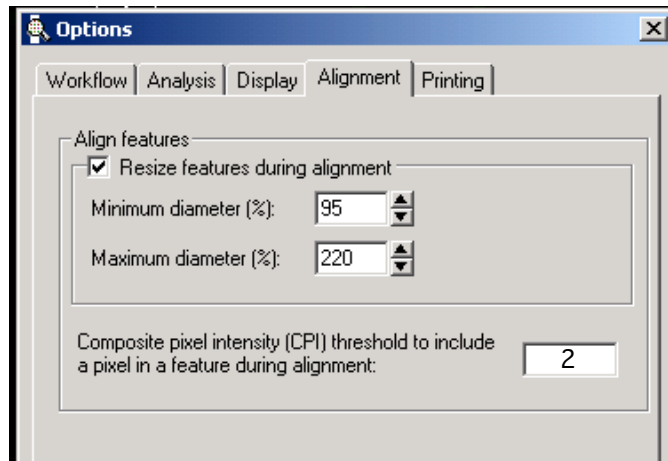
If the printing produces uneven grids, one can use the mouse to select the part of the subgrid that is uniform (as in the lower half of this example), and then hit the F5 key to try to improve the alignment of that section of spots



One can readjust the auto align parameters to improve the auto alignment function for specific sections of the slide. Playing with these parameters can avoid excessive manual adjustment of spots.

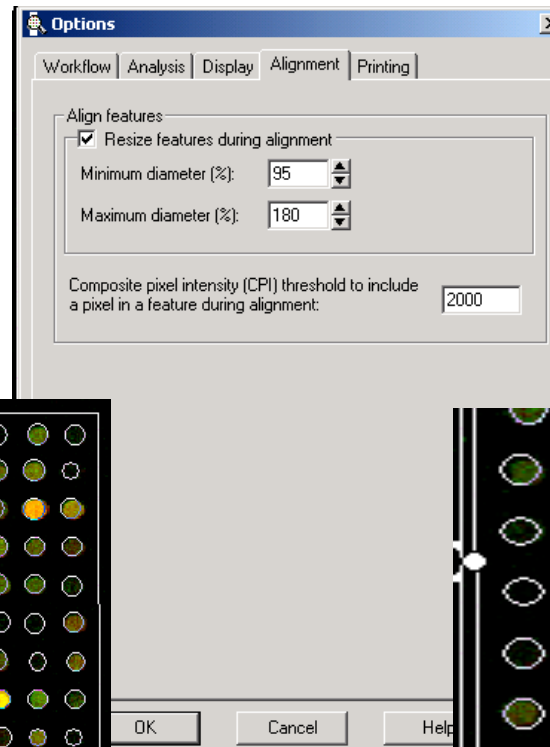
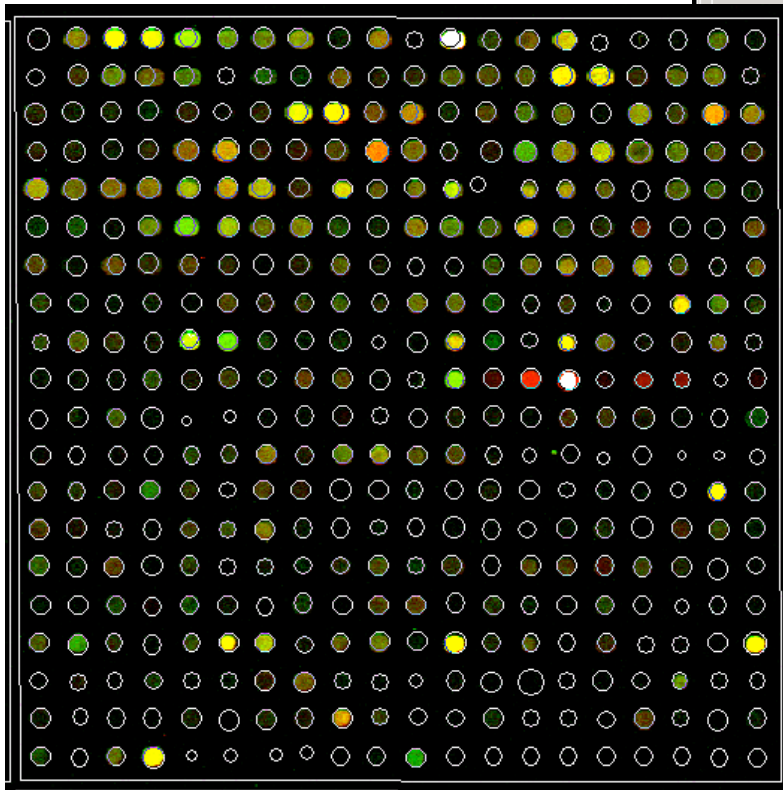


Play with the parameters to see how the changes affect the spot alignment.

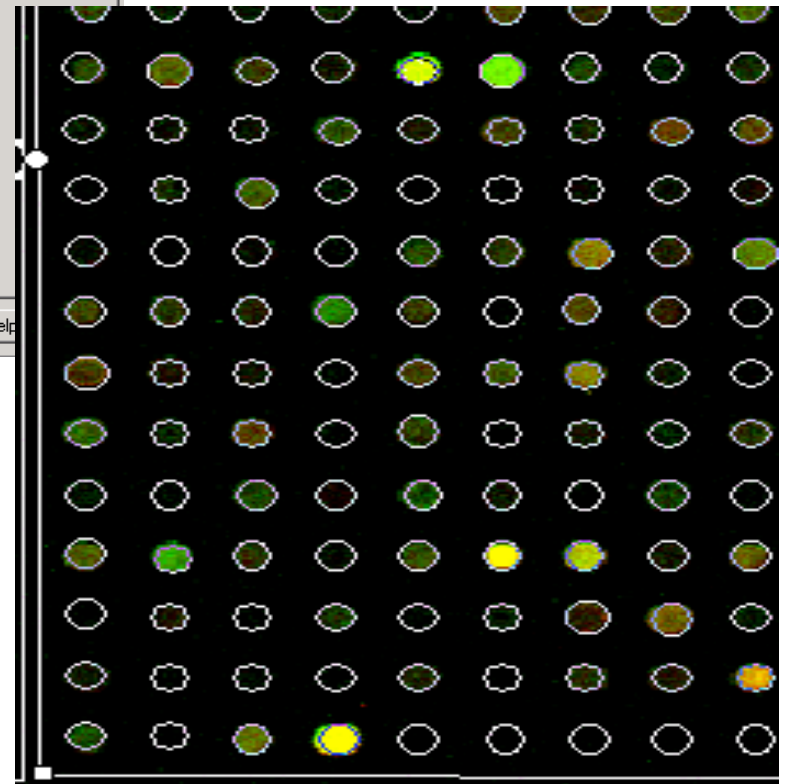


Before and After changing the alignment parameters.
Previous setting were min 50 and max 200 with CPI set to 0.

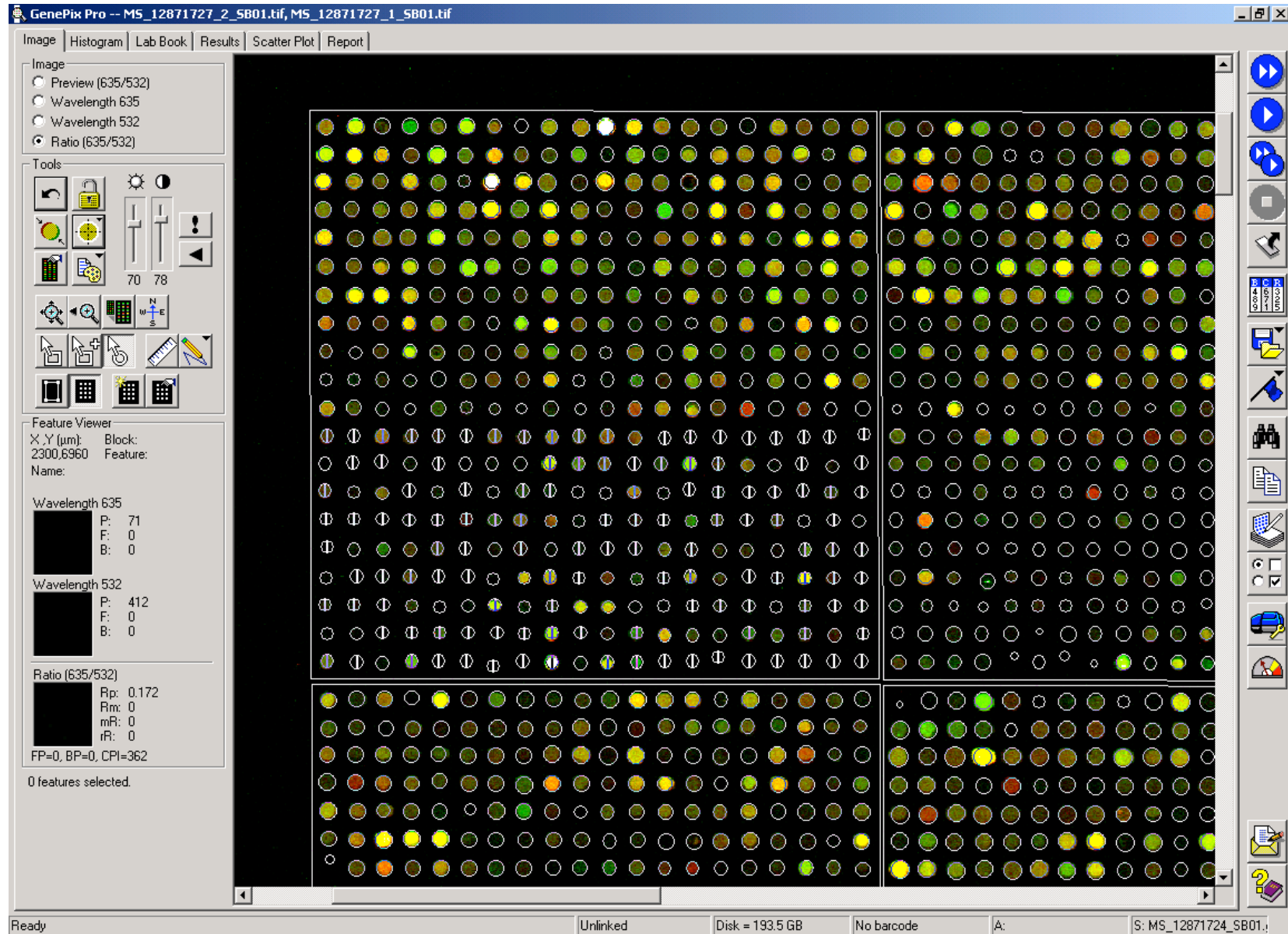
Before



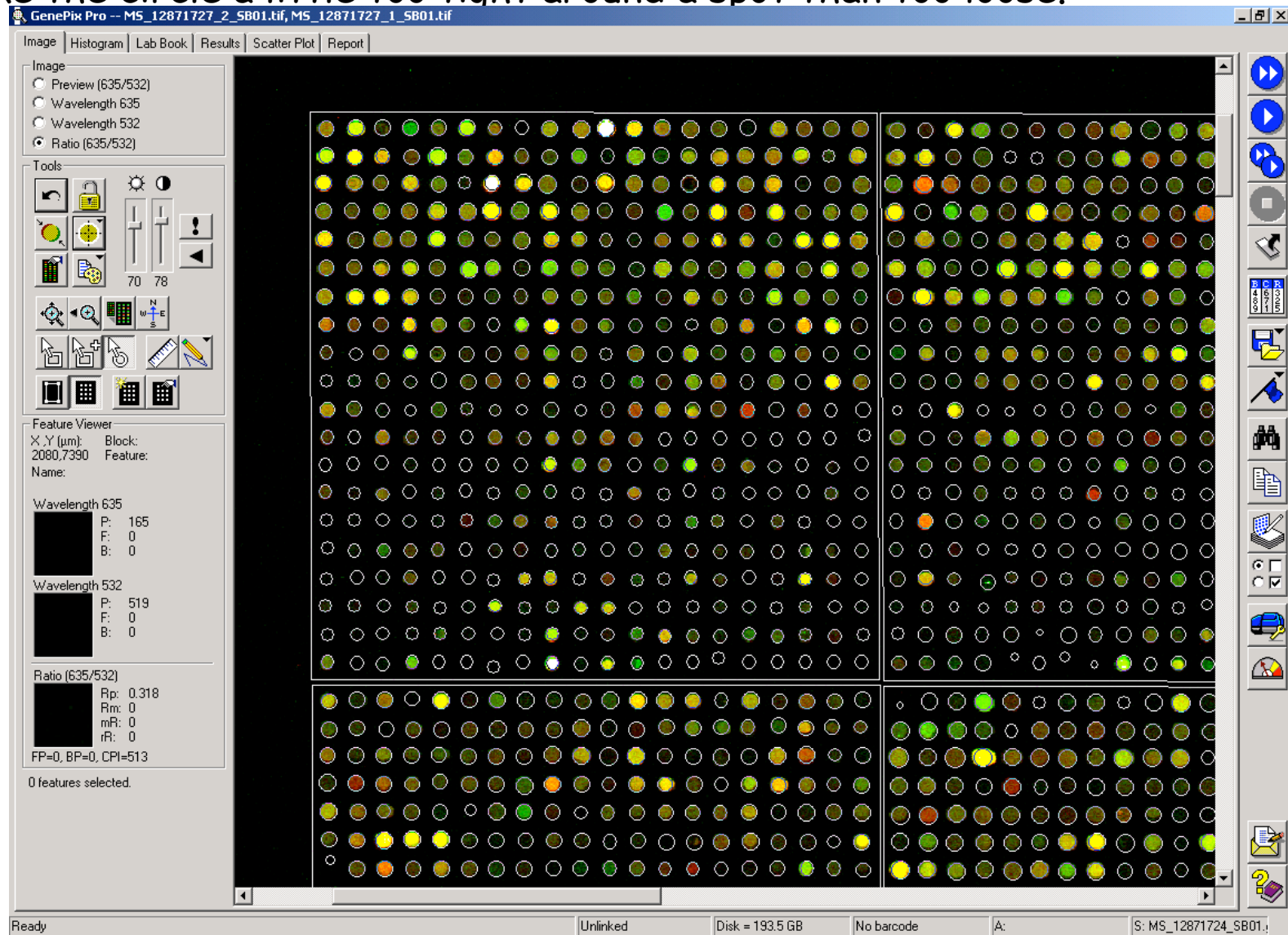
After



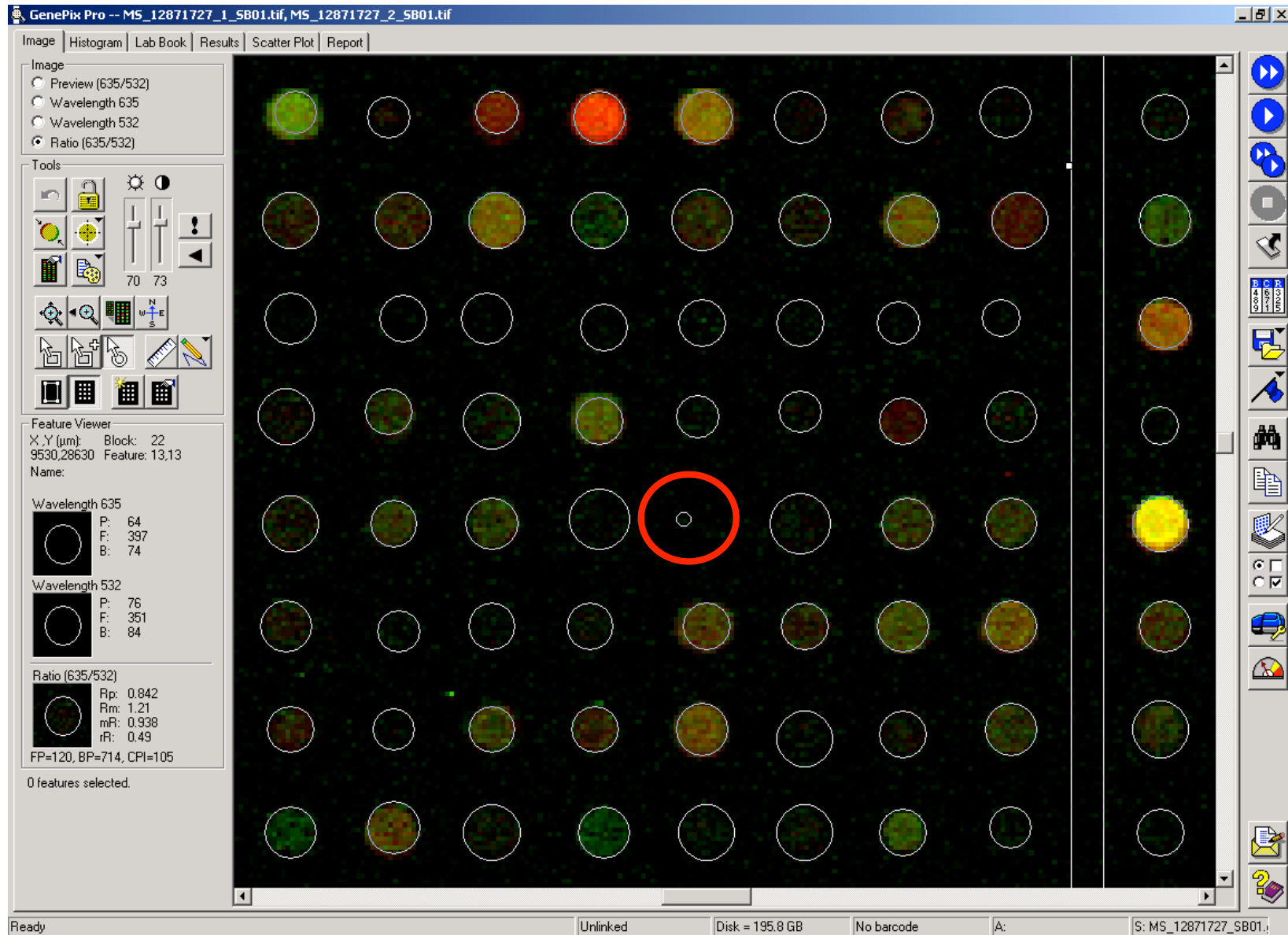
Select these spots again and hit the 'L' key to remove flags.



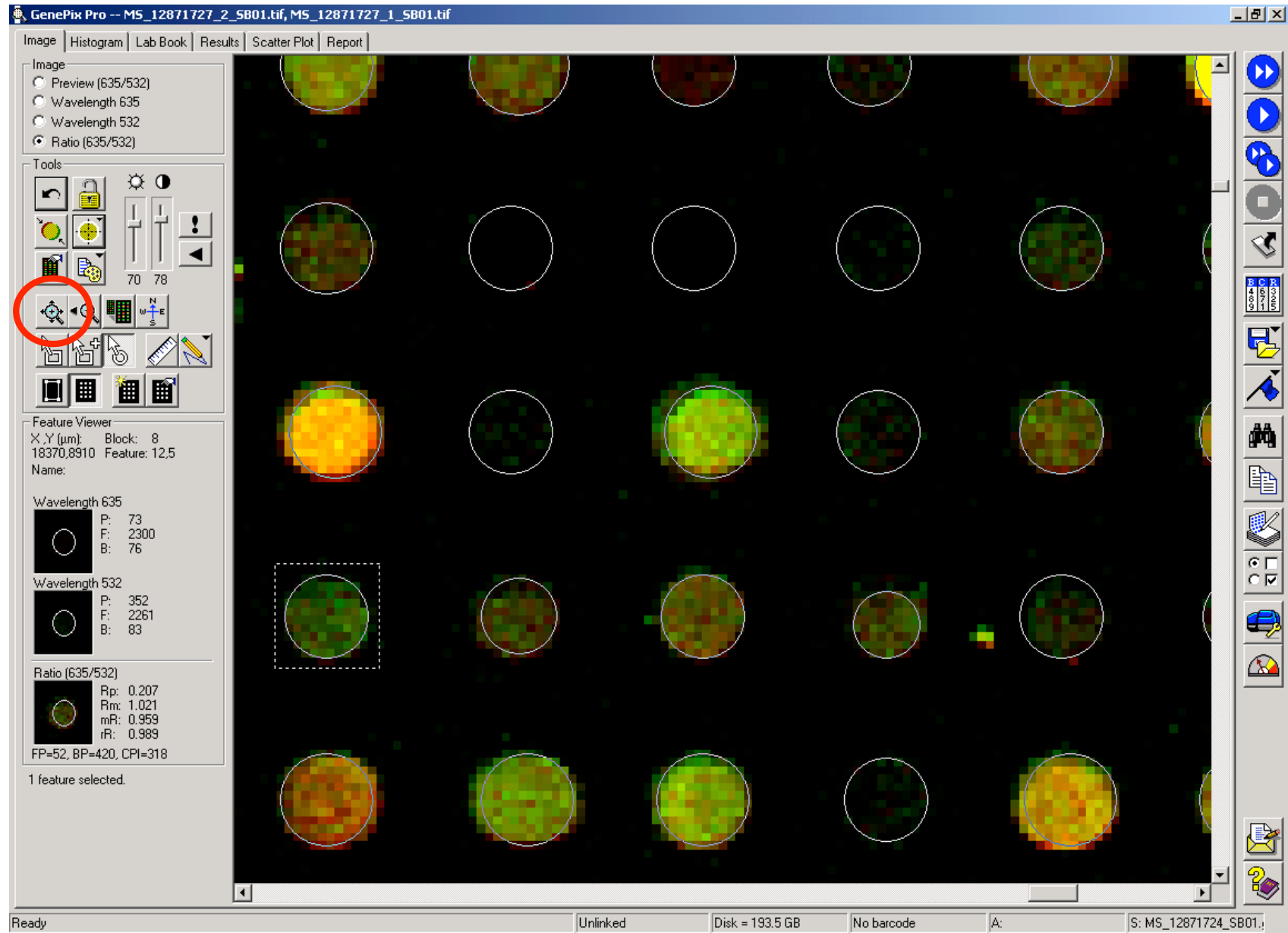
This first grid is now almost perfect. Can manually adjust the few remaining misaligned spots by selecting individual circles. Use arrows to move circle, and 'CTRL Arrows' to resize. As we are ignoring background, it is best to make the circle a little too tight around a spot than too loose.



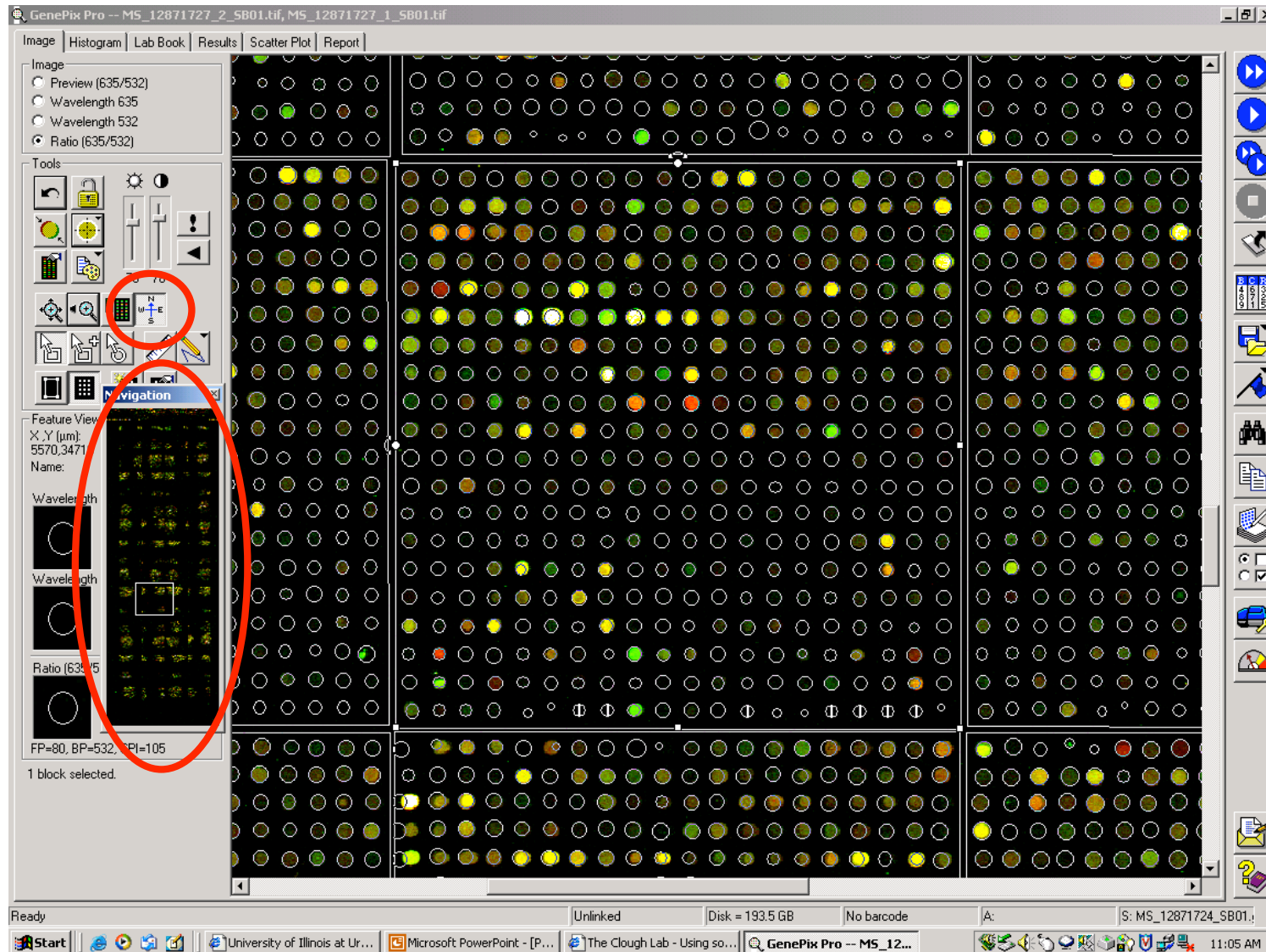
Small spots need to be re-sized. Use arrow keys while holding down the control key.



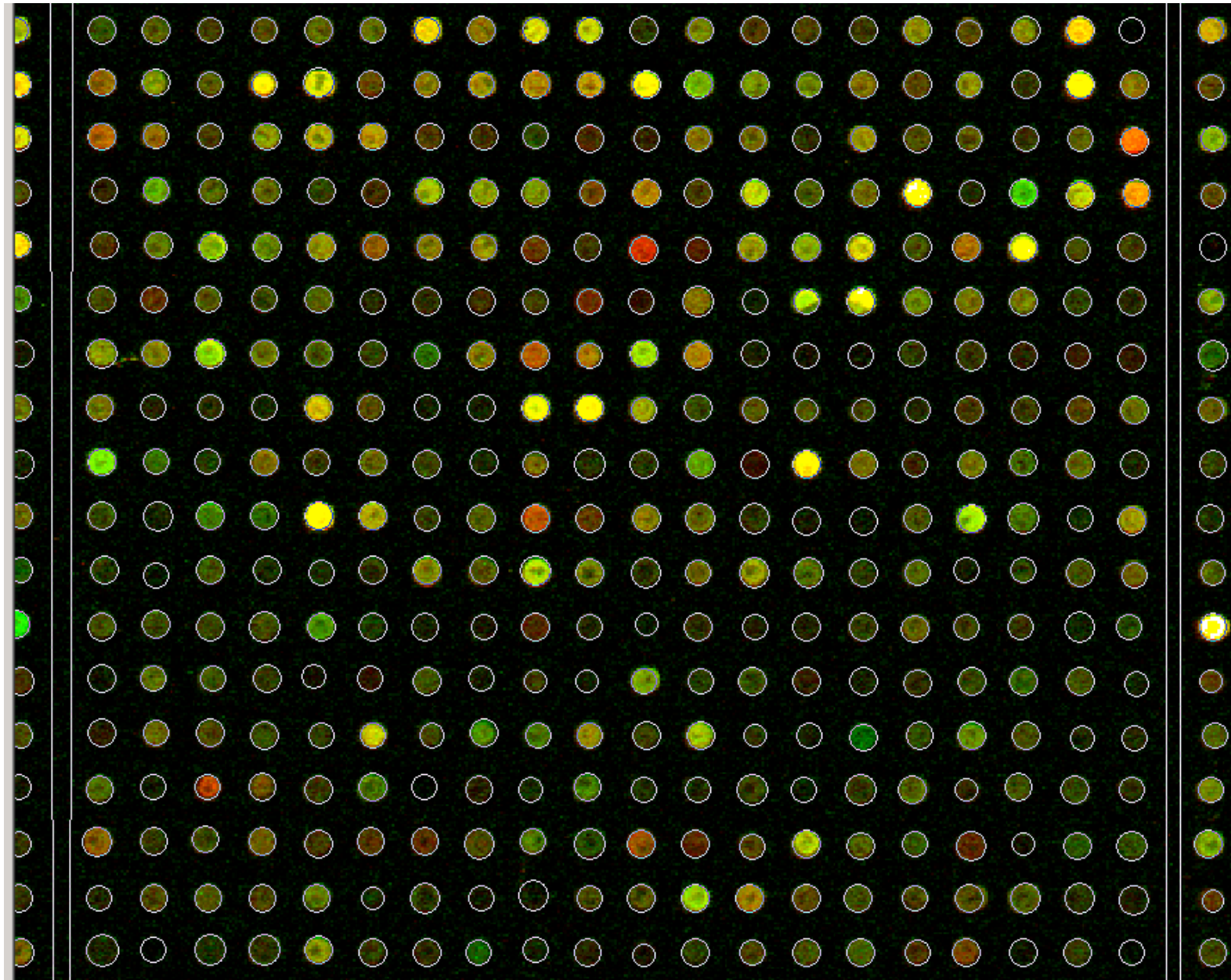
Using the magnifier can make it easier to align some spots.



Use the 'Navigation' window to help track the location of the subgrid being worked on.



An example of a nicely aligned grid

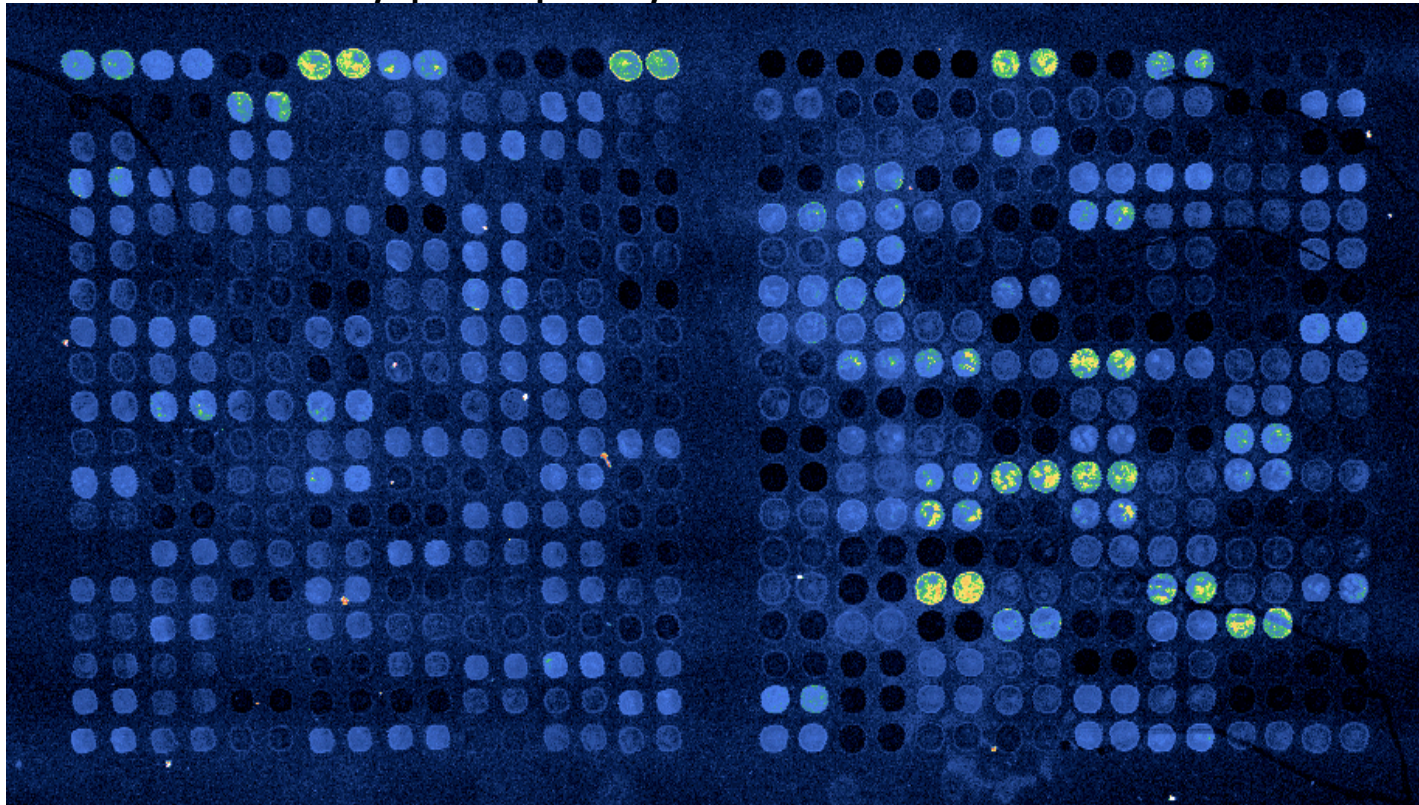




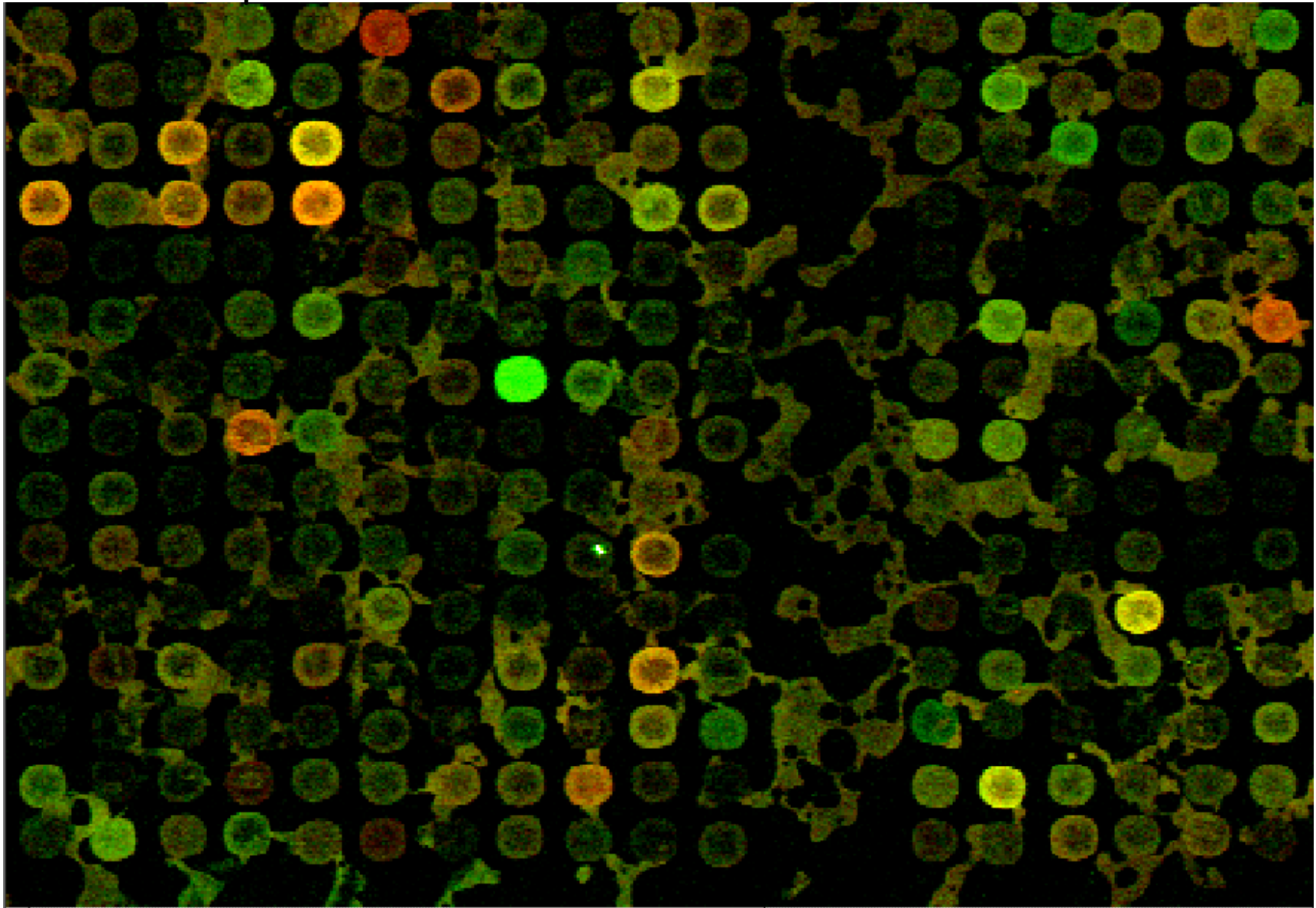
Background and Flagging

Subtracting Background

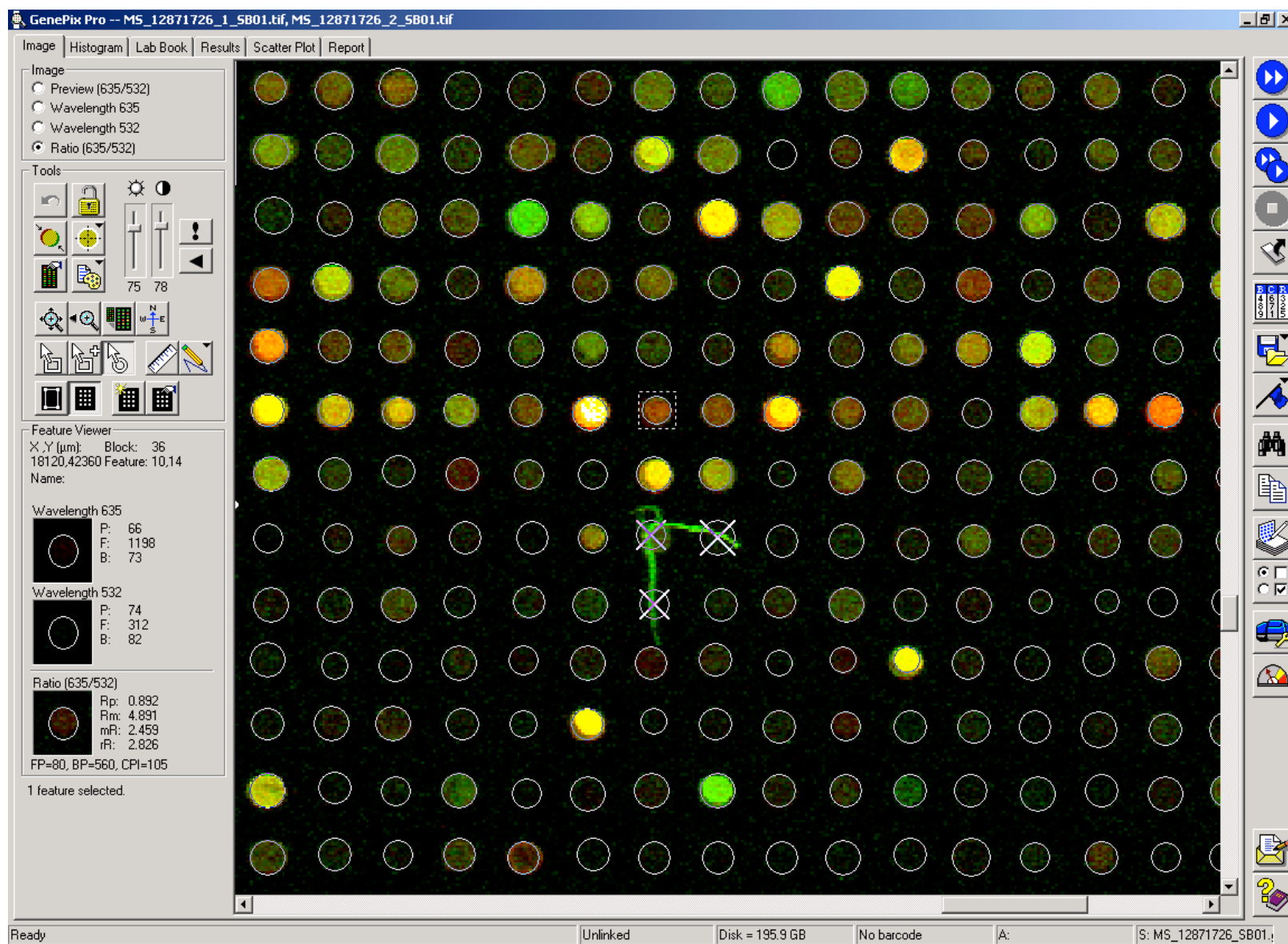
Currently we do not subtract background, but manually flag spots that are obviously affected by it. We take this approach because background is not necessarily related to non-specific binding to spotted DNA, but often is due to non-specific binding to the slide coating. As an example, notice all the 'black holes' in this fairly poor quality slide.



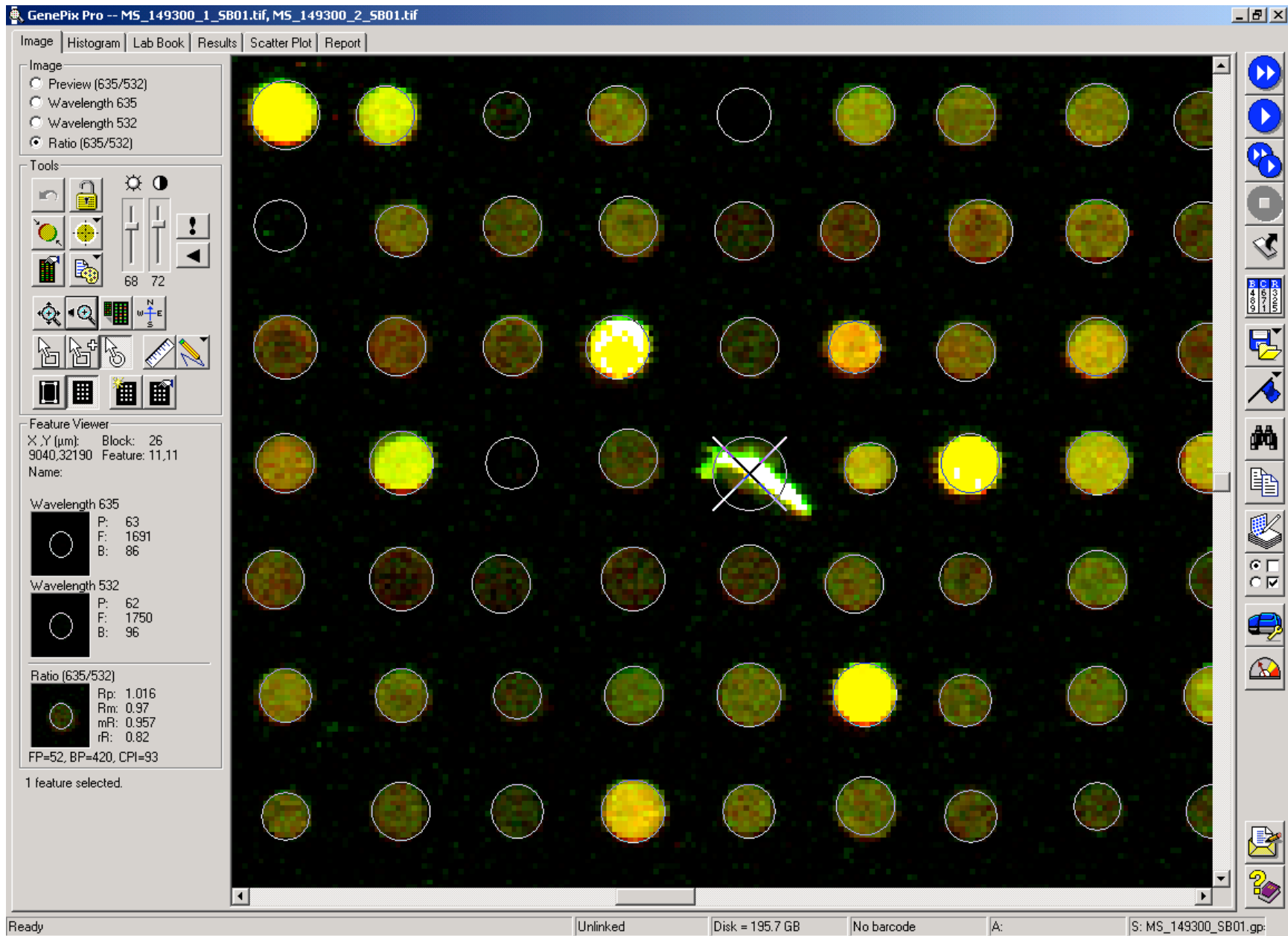
Occasionally you will have a slide with very nasty or weird background, such as this one, that will have to be discarded as you will not be able to trust the spot data.



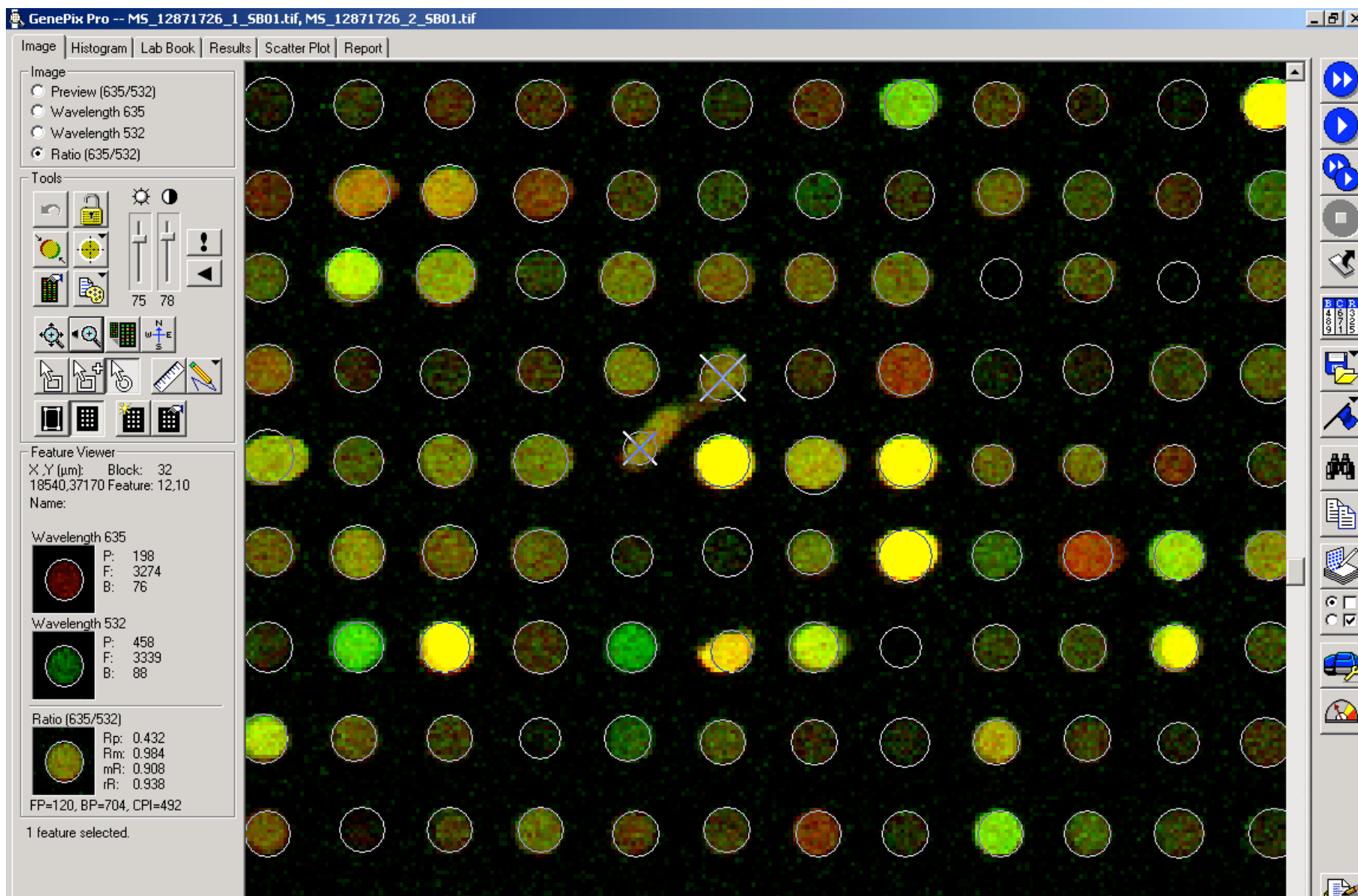
Manually flag spots that are obviously bad using the 'A' key.
Here a piece of lint is laying across 3 spots.



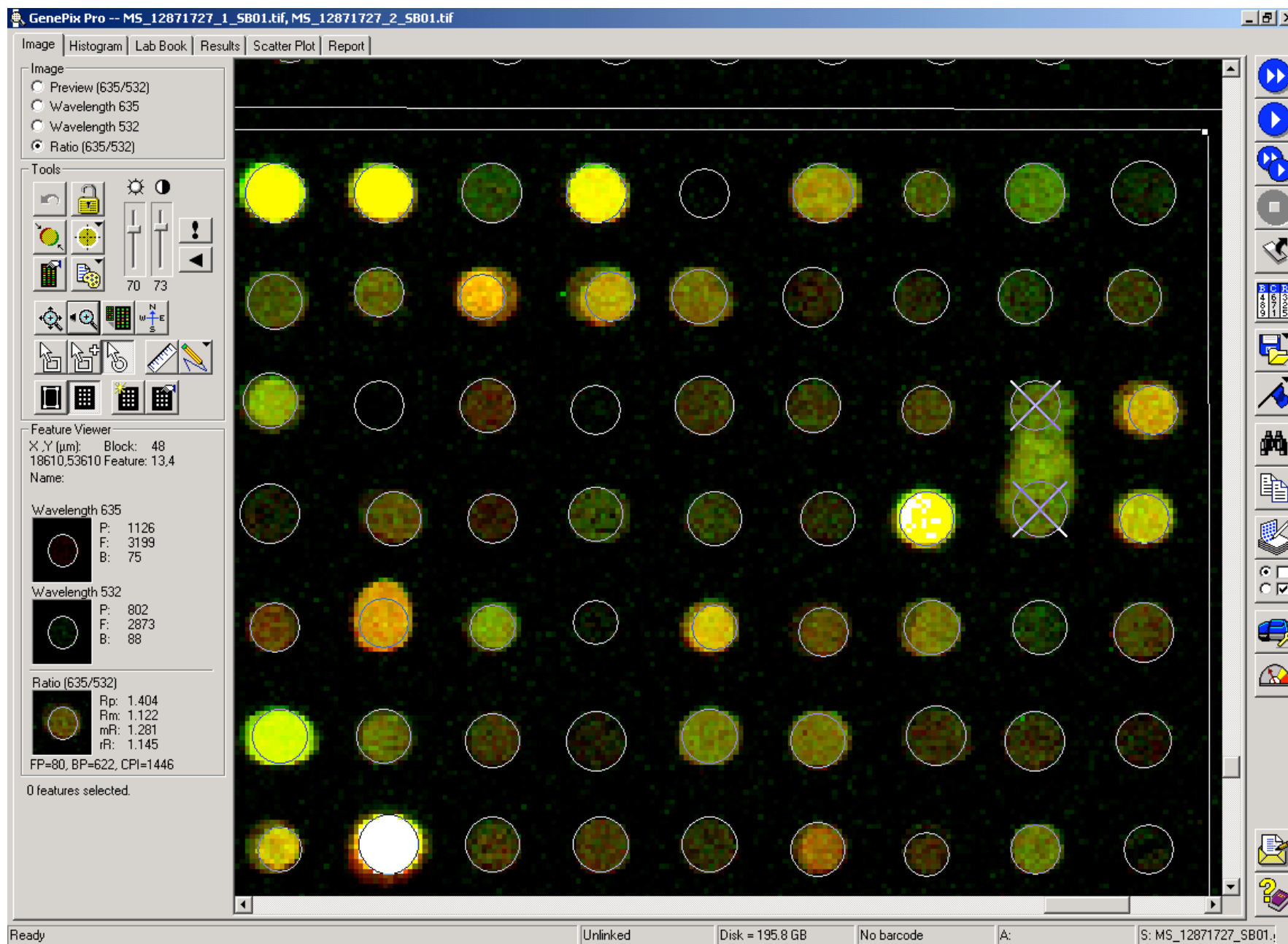
This spot also seems to contain a piece of lint.



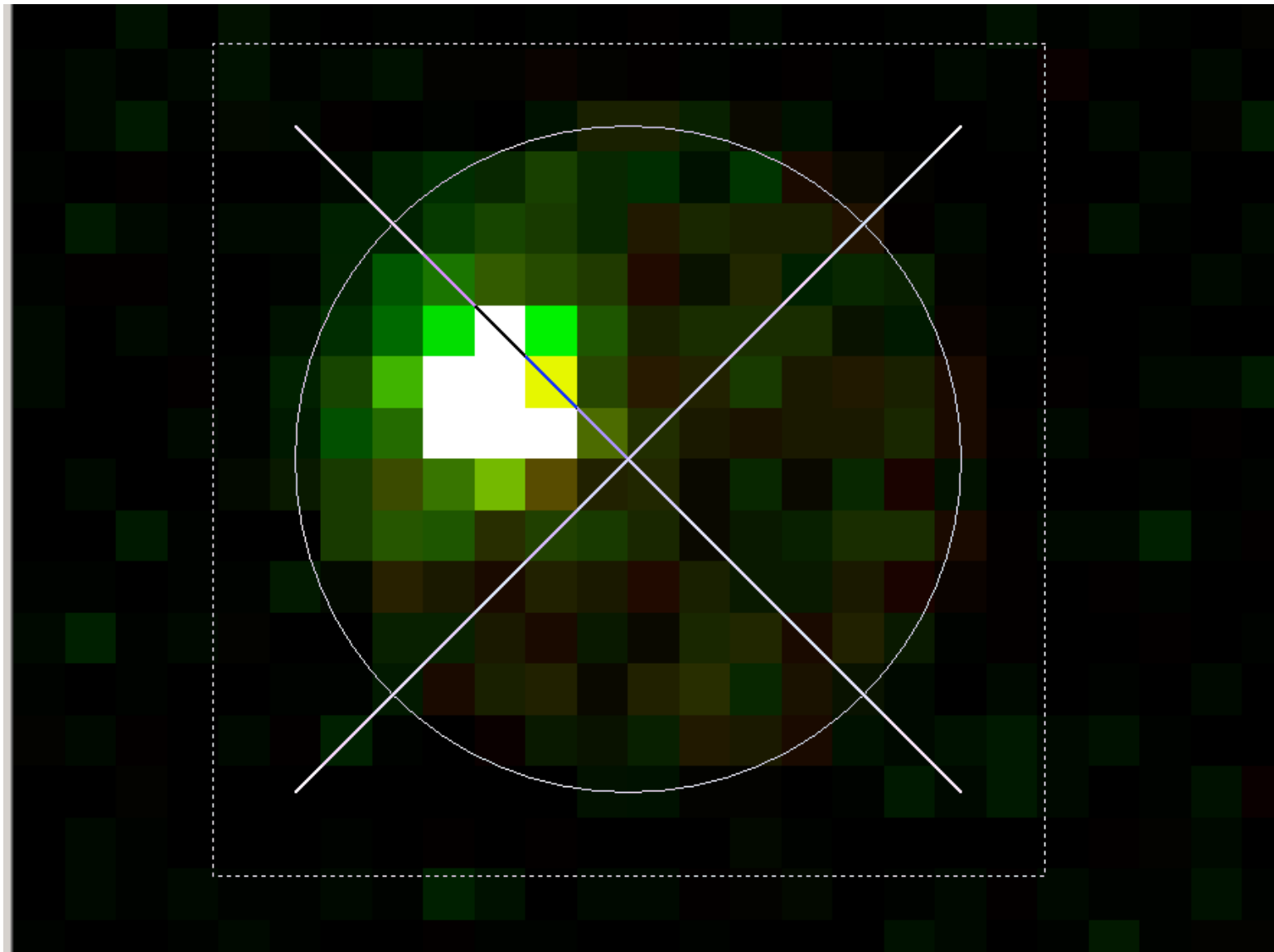
These 2 spots have smeared into each other and should be flagged.



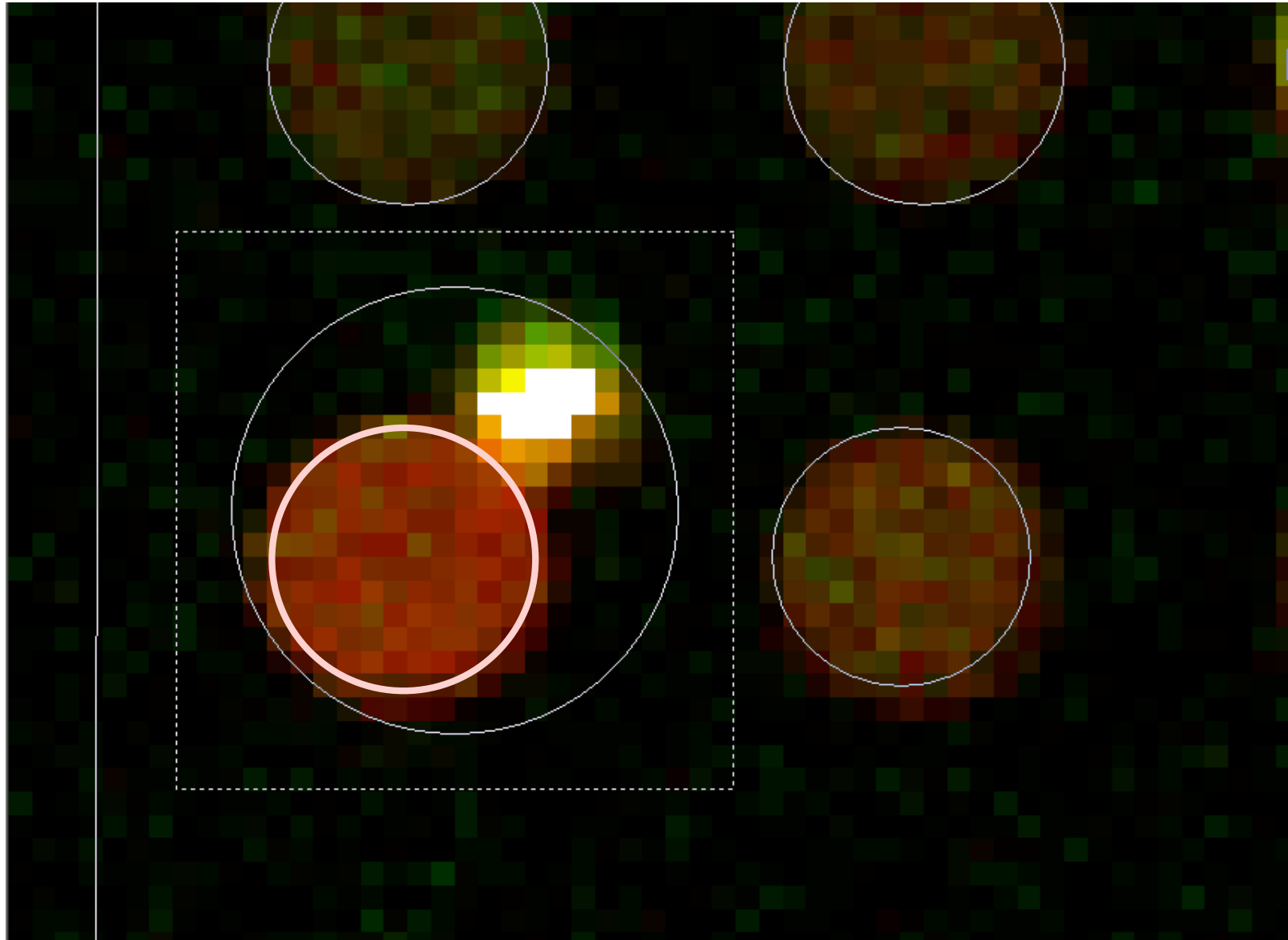
Another example of smeared spots that need to be flagged.



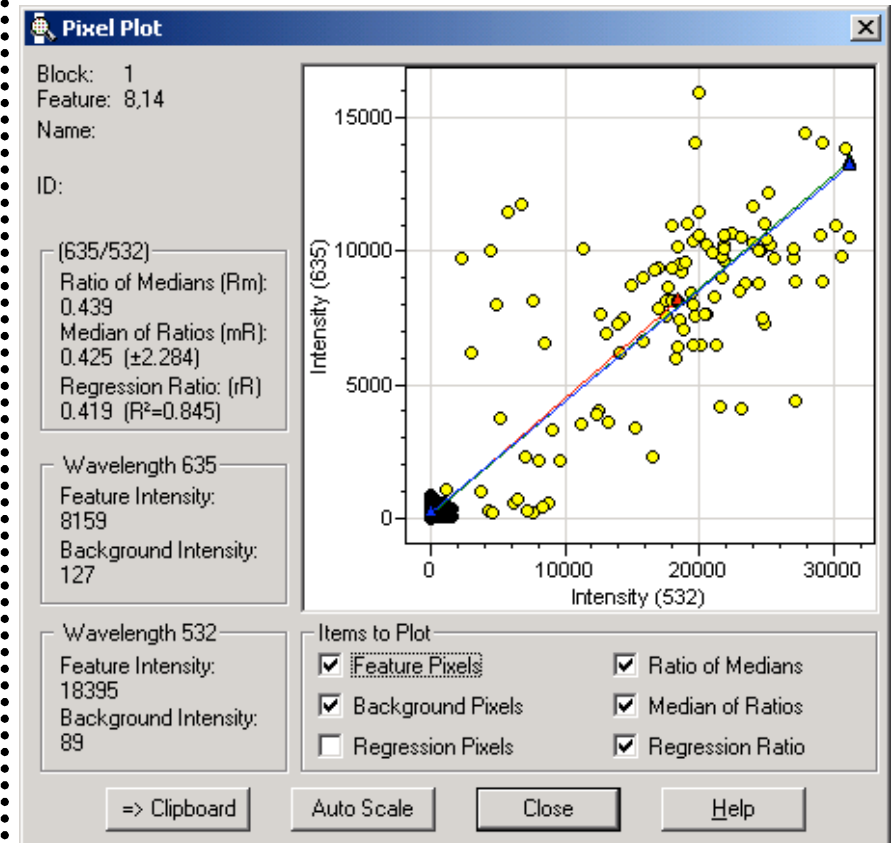
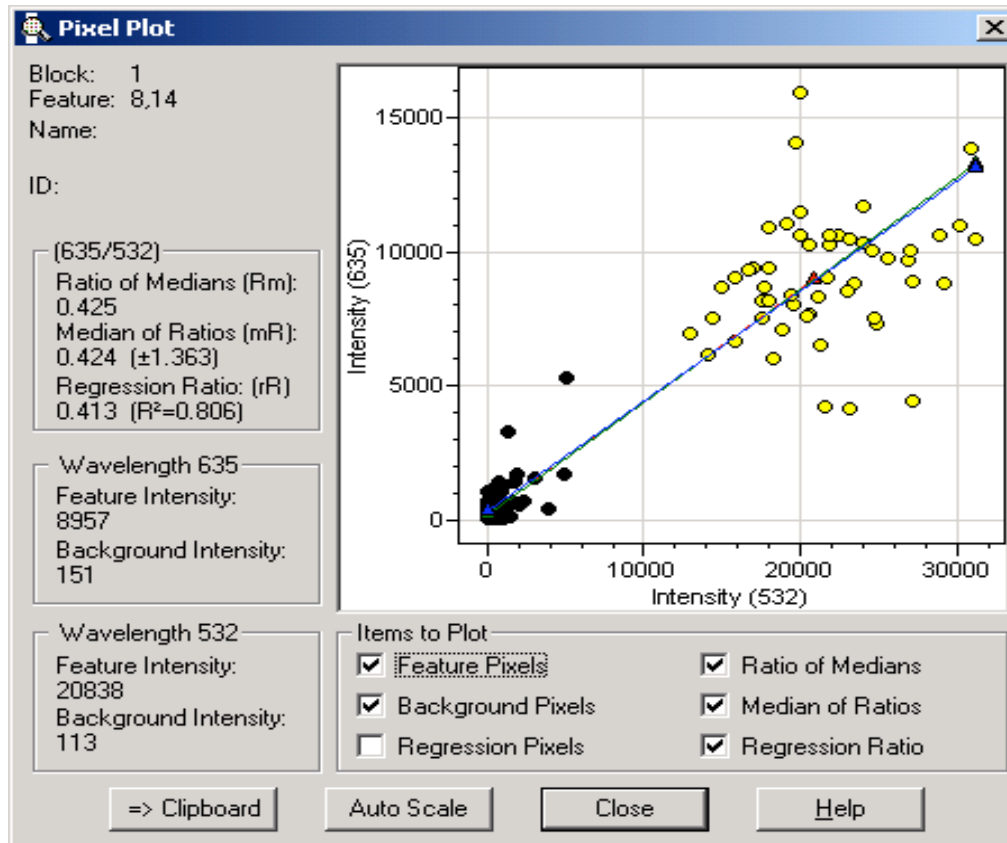
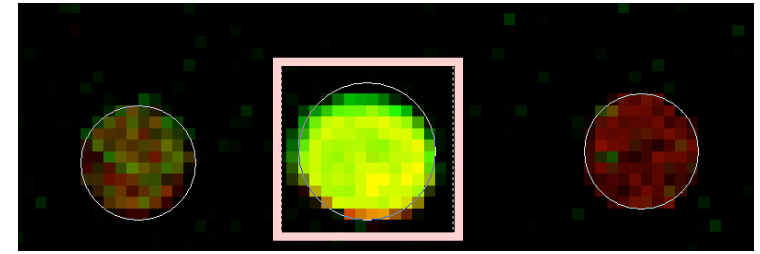
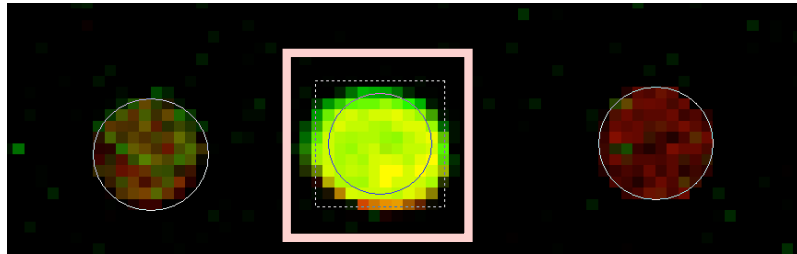
This spot is either poorly dispersed on the slide or it contains some dirt. It should be flagged.



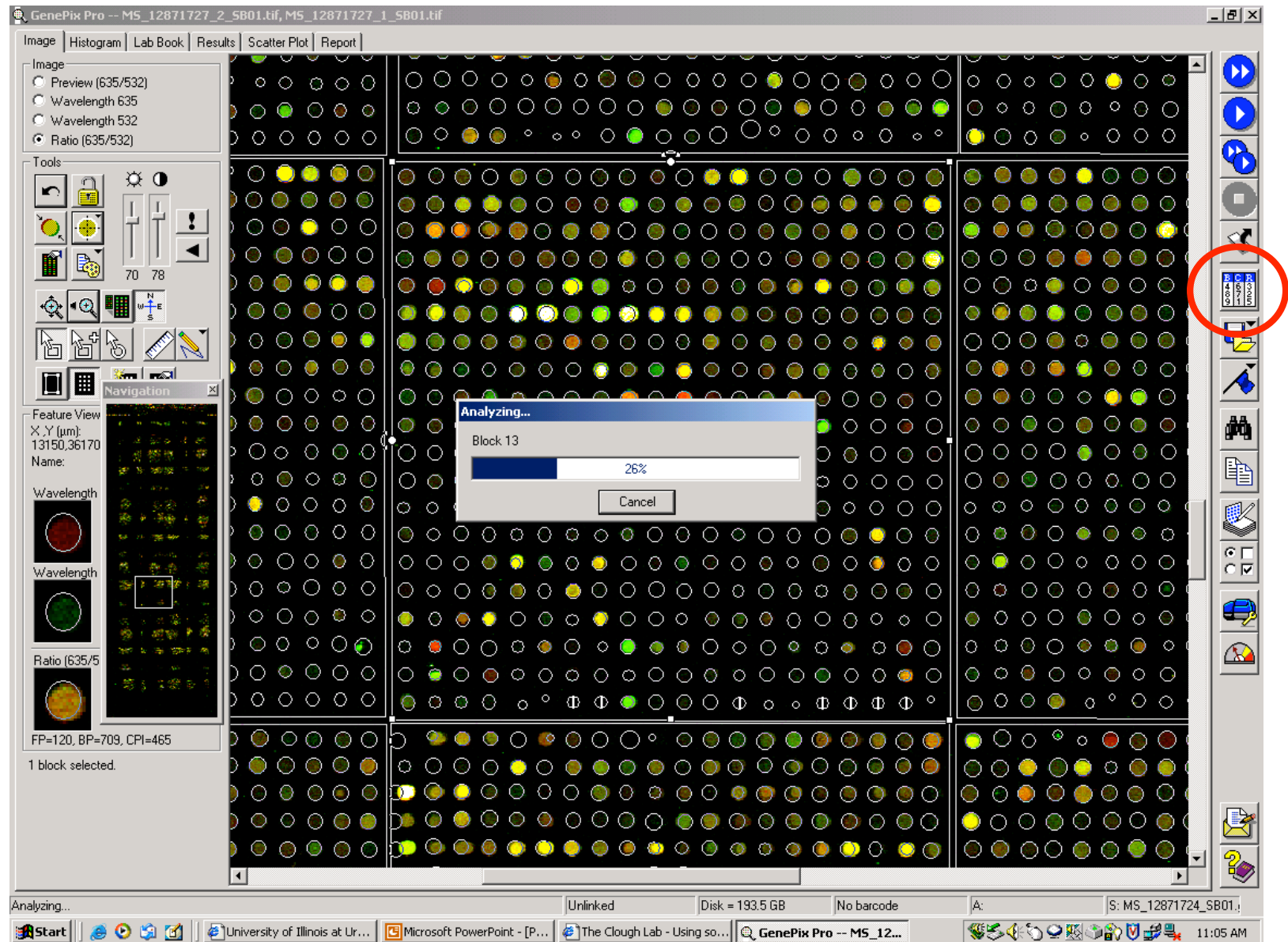
If the dirt is just outside the spot, the grid circle can be resized to exclude the dirt avoiding the need to flag.



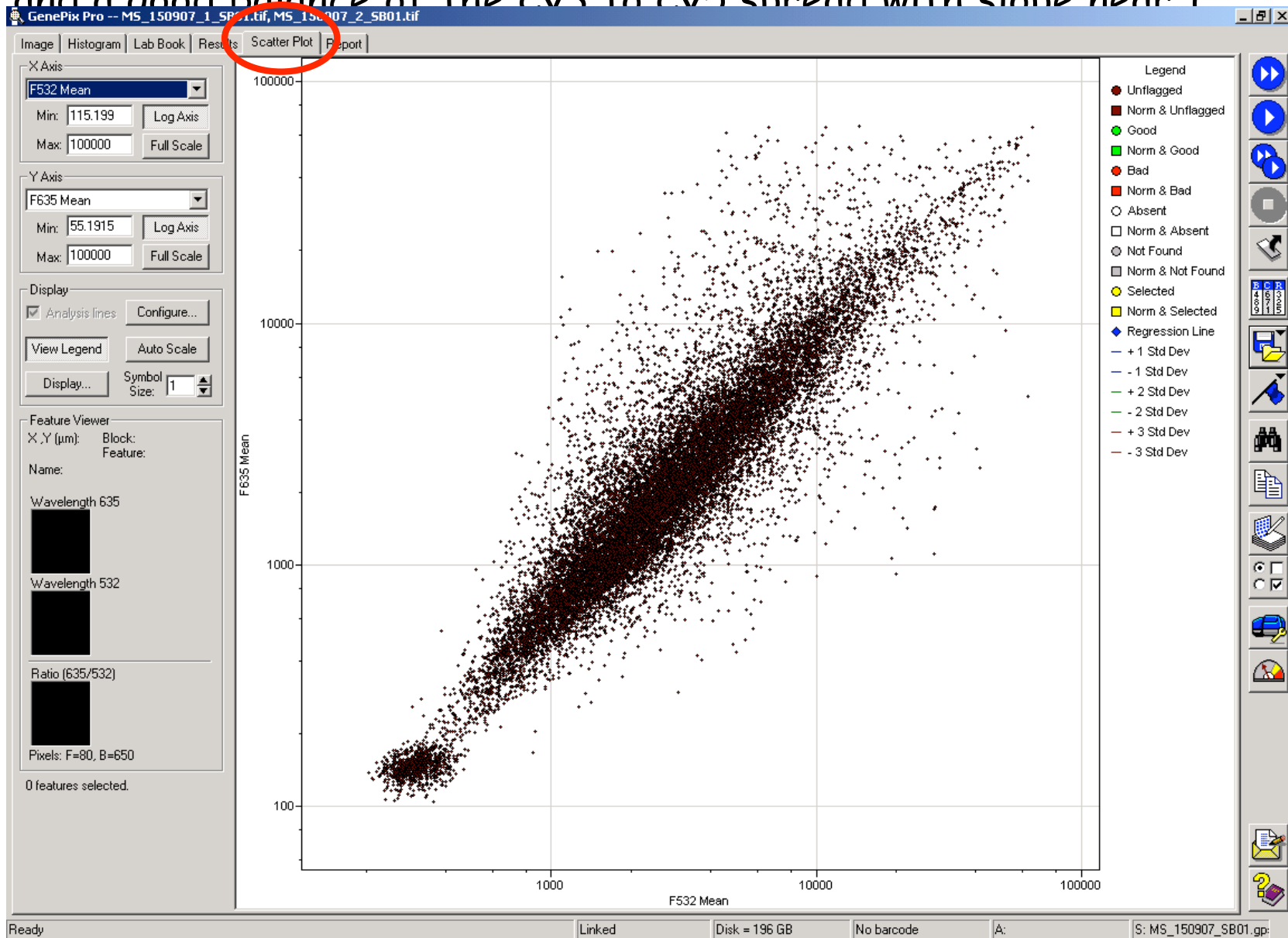
Right clicking on a spot will give a pulldown menu allowing you to see the 'Pixel Plot' window giving details of the spot. Notice how the slightly larger circle around the spot affects these numbers.



Once you finish aligning all the spots, hit the 'Analysis' icon to extract data from the image.



Clicking on the 'Scatter Plot' tab will give you an idea of the quality of the slide. A good slide will have low number of saturated spots, and a good balance of the Cy3 to Cy5 spread with slope near 1



Save results as well as settings using the 'Files' icon.

