GenePix Analysis- Scanning Protocol

1. Open Tiff image from the corresponding assay folder
2. Open GAL file from respective PR Date
   1. Folder Icon on Right 🡪 “Load Array List”
3. Make sure the correct wavelength is selected with no more than one ratio selected
4. Auto-contrast image to desired brightness
5. Align the grid using the landing lights as guidelines
   1. Make sure to click on block mode, select one block then “Ctrl, A” to select all
   2. Move individual blocks so that the top right corner has three lights and the bottom left corner has two lights
   3. \*Note CFG slides only have landing lights in the 488 Channel\*
6. Once grid is set, auto-align features to the positive binders on the array
   1. For more accuracy, start by selecting the OPTIONS button on the right side of screen (silver square with 4 blocks in it-2 checked off), and then going to the “Alignment” tab
      1. Place your mouse over where there is no glycan printed anywhere on the array (dotted circle) in order to get a read of the background fluorescence
      2. Plus that number in as “Composite pixel intensity (CPI) Threshold”
   2. Go to the Align Spots tab on the left side of screen which looks like a cross in the center of a circle and click on the downward arrow in order to select “Align features in all blocks” (can also just align in selected blocks)
      1. Another way to improve accuracy of alignment, right click on any of the spots within the subarray
         1. Select “Block Properties” and enter in a set feature diameter to make all the spots the same size so it will be easier to adjust
7. These strategies don’t do the job fully... MUST manually resize and move the spots to better align the positive binders
   1. To resize:
      1. Ctrl + arrow keys to make bigger or smaller
         1. Ctrl + mouse wheel also works but make sure “Ctrl” is pressed when going to resize using the wheel
8. Once spots are tightly aligned, save settings as a GPS file
9. Next, click the DATA button on the right hand side panel- this will generate a GPR file for data analysis
   1. Make sure only one ratio is selected before generating the GPR
   2. If this is a subarray, can save separate GPR files per block (click this option before clicking save)
10. Save your GPR file in the corresponding file, with the same name as the image file