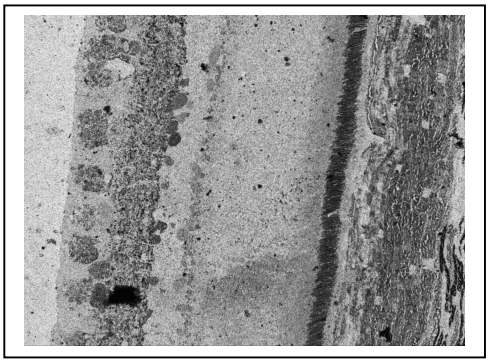
Assigning colors to digital images for Computational Molecular Phenotyping

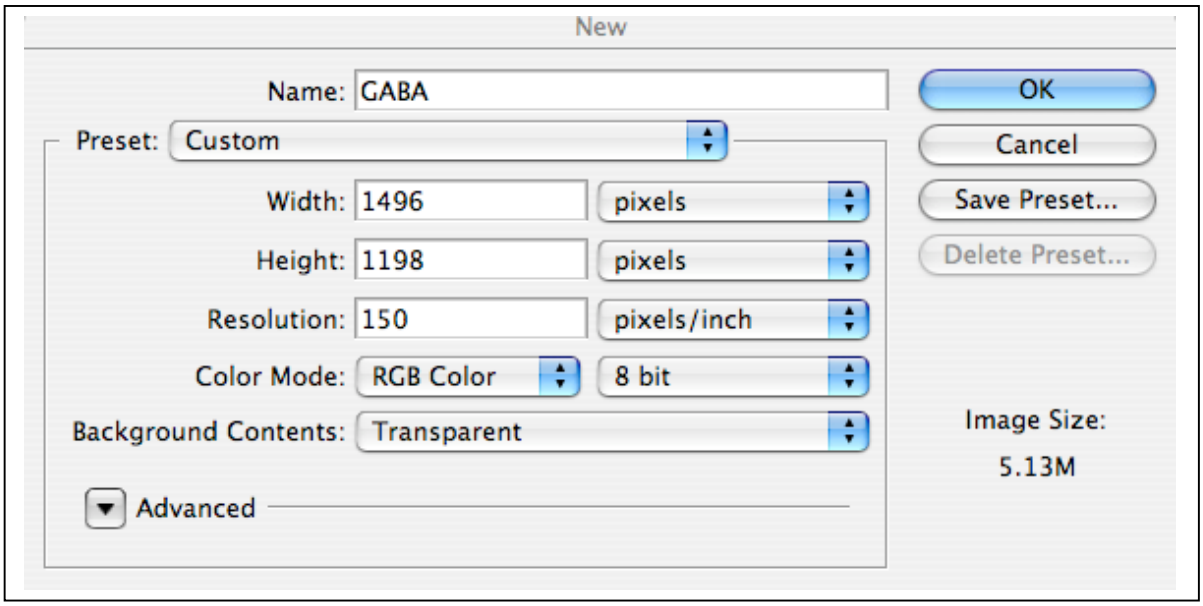
1. Open one of the images, say the one you want to be the red channel in final image (good combo is GABA=R, Glycine=G, Glutamate=B.



3. Take the GABA image and “select all” (Apple + A)

4. Copy the GABA image(Apple + C)

5. Create a new Photoshop file (Apple + N). It should be in RGB color mode. Name it GABA or whatever probe you are going to place in it.

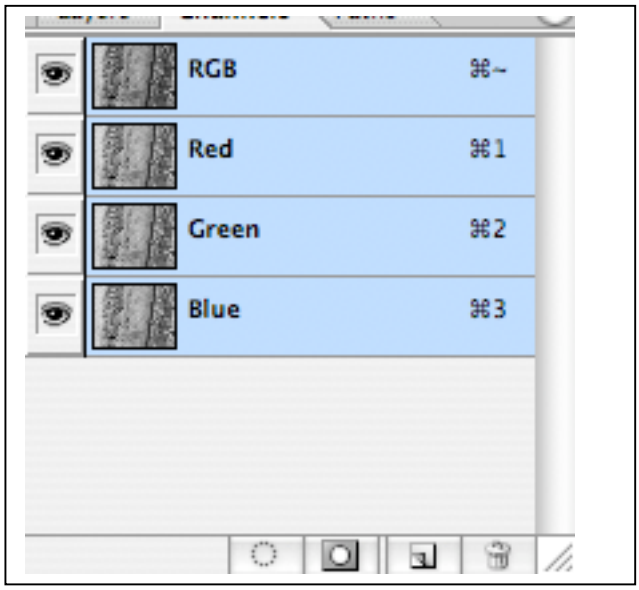


6..Click "OK"

7. Paste the image that you copied into it (Apple + V)

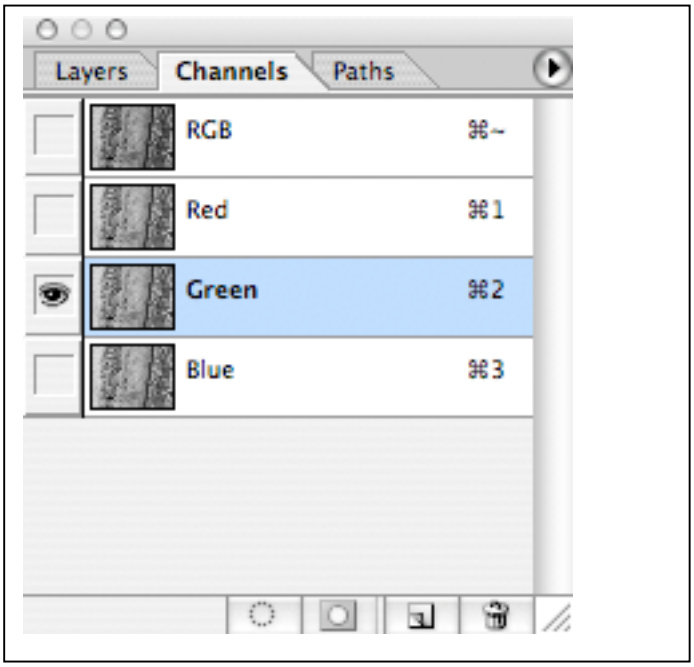
8. You should now have a file with the GABA image in red, green and blue channels. If you look at your little box that has the layers in it, there should be a tab for channels

9. Click the channels tab and you should see the image in Red, Green and Blue channels



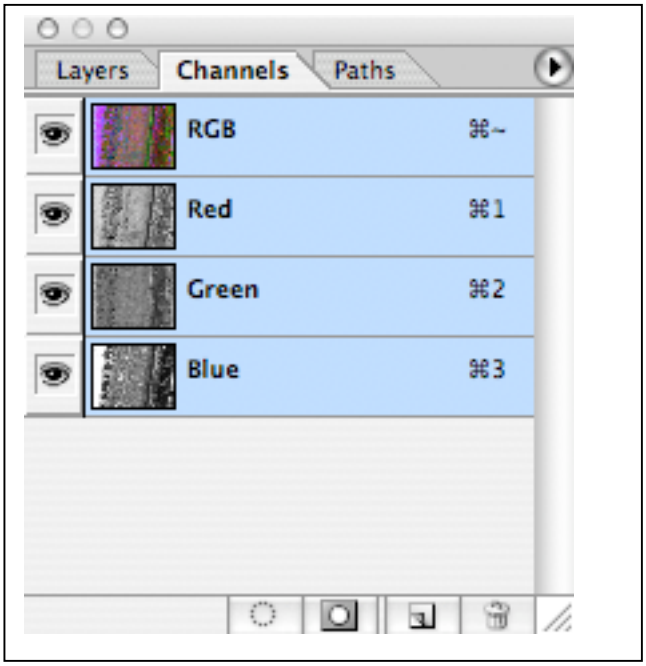
10.. Next, open up the glycine image and Select All and Copy just like you did with the GABA image

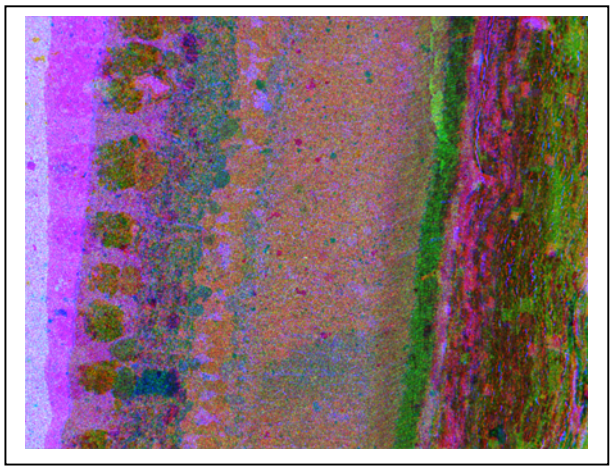
11.Click on the green channel box in the new image file you just created and paste your image there (highlight the green channel in the channel box and then past {Apple + V} and the image will be pasted into the green channel.)



12. OK, now do the same for the glutamate image, only now in the blue channel

13. OK, so now if you select channel view as RGB image, you should have an RGB image of the three





(It will not be terribly beautiful because they aren't registered)

And they are density scaled images as opposed to brightness scaled

Yeah, this is where you really see registration problems

(You can also refine the registration in the channels)

Usually you would register the 3 as separate files and save t hose before doing the step we just did.

14. How do we change to brightness scaling?

Ah, that is pretty straight forward

15. You select the channel you want to work on, say the red one

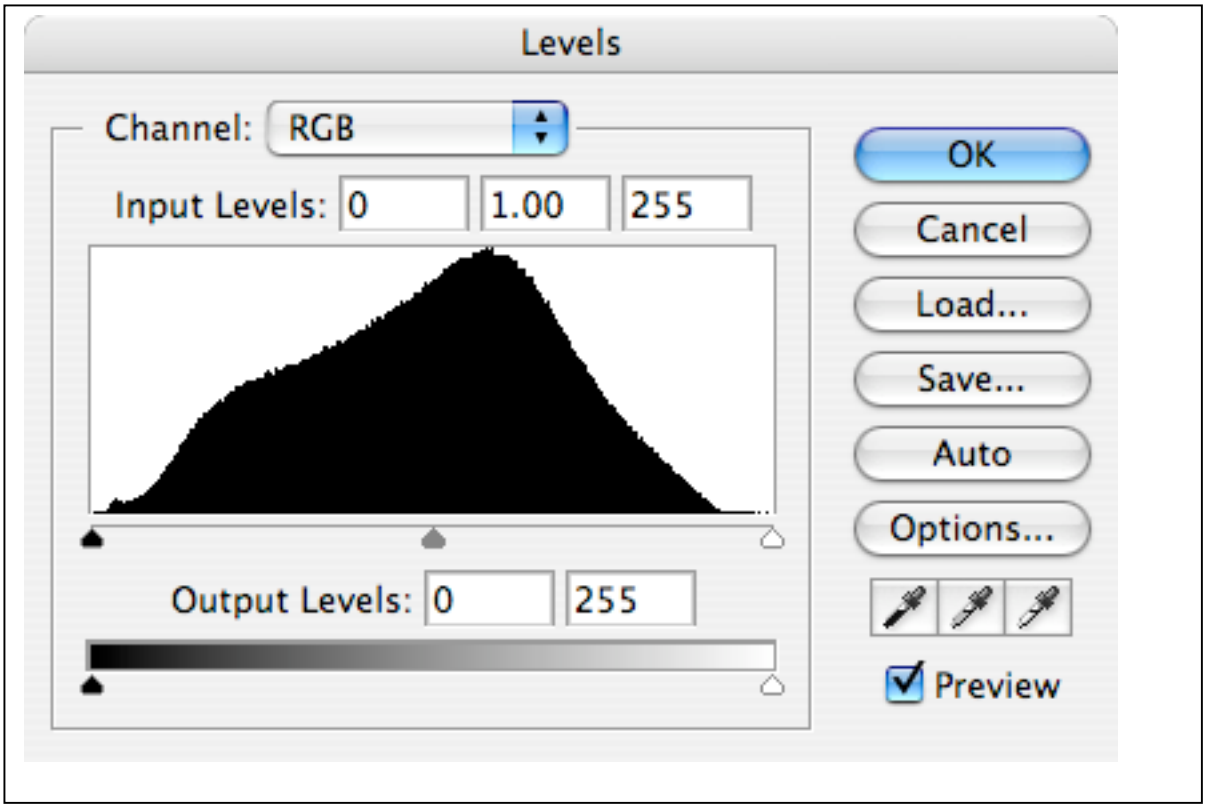
16. just click on the channel box to highlight it

17. and then Apple + I (for Invert) or use the menu command to invert.

19. Do the same for the other two channels

What you will get is not quite the most pretty image, so what we can do is intensify the image by throwing away some pixels

20.. To do that, you highlight each channel like before and then press Apple + L this will bring up a histogram box



21. Now is slide the little triangles at the bottom of the histogram to closely approximate the ends of the histogram

22. What this operation does is take all the pixels that are on either side of the histogram and throw them out and then re-map the histogram over your 8 bit range. it "brightens" the image, but it also throws pixels out

so you would not want to use these processed images for classification

23. Do the same for the other channels (adjust level histogram)
(I do this for images that I want to survey or publish)

24. you can then flatten the image and save it in any form you want

REGISTRATION

1. I'm still not clear on registration

2. The registration part in Photoshop takes some doing it will not get you as clean results as a direct method of establishing GCPs or ground fiducial points

3. Do we say start with glut, and paste gaba over it and change the transparency setting?

4. How do we register an image that is in 1 file with an image that is in another?

5. What I do when registering in PS is take the image with the most information in it (like glutamate or taurine) and then place the image that I want to register to it in another layer on top of it

6. I then adjust the transparency of the image in the more superficial layer to say 50-70%

7. And then slide it around until it approximates the position I want
it may require rotation

(so then will I copy each layer into the new file like we just did to make the RGB image?) (ah yes)

and then you can use the free transform tool (Apple + T) to warp

8. Layer 1 into red, layer 2 into blue, layer 3 into green ...
you can then selectively show layers or hide them by clicking on the little "eye" box to the left of the layers

Oh, use layers for this, not channels

Yes, its actually easier to work in greyscale space for this type of thing
color can confuse

9. Let me get it straight...start with grayscale images....paste image 1 into a new file, new layer...then image 2 into the file, new layer, same for image 3

yes

10. but I would register 1 and 2

(then when everything is registered, you can then bring them into the color channels)

Then do 3 on top of those 2 or do I want to do 3 relative to 1 or display
3 relative to 1

11.Always pick one image that will be your master image

and register everything to it

12. Do I bring them into the color channels like we just did...make new file, but copy in the layers into t he channels?

yes.

Now....I have a taurine image...a 4th one but I'm out of color channels. Now what?

well, you can always simply replace one of the color channels with your new image or create an Alpha Channel and assign a color to it.

ALPHA CHANNELS

But that typically works only for data that is sparse, like glycine or even more sparse like TH

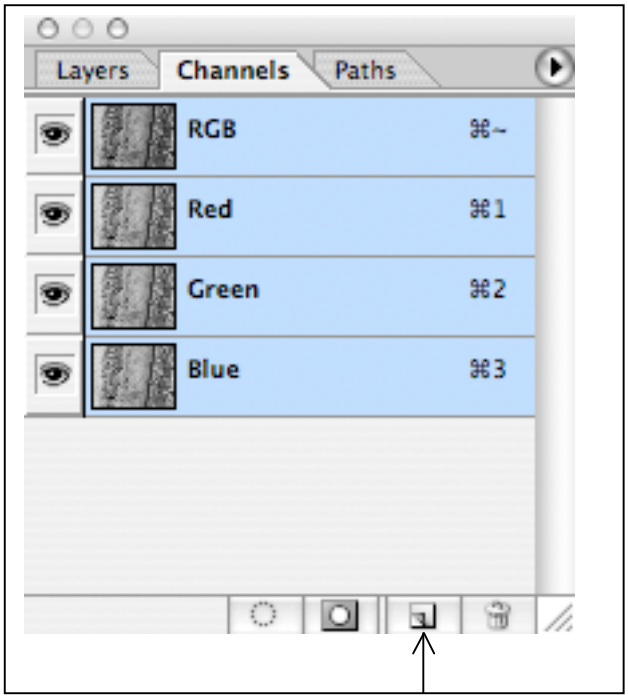
how do I create this alpha channel?

1. Go to the bottom of your channel/layers box

when you have selected the channel tab, there should be a little box at the bottom that has a little page icon

If you hover your cursor over it you will read: ”create new channel”

2. clicking it will allow you to create a new channel



3. How do I assign a new color to it?

4. Click on the little black box in the new channel window next to the name, double click and then it will bring up a channel options box

5.. you can then select masked areas, selected areas or spot color and also choose your color by cilcking in the color window you can also select the opacity there

6. So paste an image into the alpha channel
.

Voila.