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General Information:
- The current code is uploaded on github.com. By using the provided link, the user can download the recent version of the software.
- The directory image under the directory src contains images collected from 2016/04 and can be used as an sample for the initial test run of the software.
- Once the entirety of the software is pulled from github, compile and run the java file ImageInterface.java which is located within the src directory.

Step 1: File Selection
- Button Explanation:
  - "Add Channel" allows user to add a channel including a "plain image" with visible wells, streets and radioactive markers and a "contrast image" with only visible radioactive markers present.
  - "Choose Overlay Image" allows user to pick one image file that is the overlay of all radioactive colors plus the well and street plain image.
  - "Clear" clears all channel names.
  - "Save Channel Names" saves all channel names.
  - "Choose Plain Image" allows user to pick one image file that present the visible wells, streets and radioactive markers.
  - "Choose Contrast Image" allows user to pick one image file that present only the visible radioactive markers.
  - "Remove Channel" removes the channel including both images and channel name.
- Test Run
  - Since there are 13 images that I need to upload to the software, 2016.04.-1 and through 2016.04.-6, 2016.04.-1-HC through 2016.04.-6-HC, and 2016.04.-1-DP, 6 channels should be created.
  - **Add 2016.04.-1-DP as the overlay image** by clicking the "Choose Overlay Image"
  - After naming Channel #1, add 2016.04.-1 as the plain image and 2016.04.-1-HC as the contrast image.
  - Follow the same pattern with the rest of the 5 channels.
    - User can review the individual files for a better interpretation of the instructions.
  - After the image selection is complete, click "Save Channel Names" to save all inputs.

Step 2: Image Viewer and Image Grid Preparation
Click the Image Viewer tab on the very top to switch.
- Button Explanation:

- ○ The user can click anywhere in the picture to gain information on the row number, column number, and the RGB value of the clicked pixel.
  - ■ This is especially useful when inputting parameters required later in the program.
  - ■ Click and drag is also possible for locating a precise pixel.
- ○ "Clear" clears all inputted parameters.
- ○ "Save and Try Parameters" will save the inputted values and make a grid based on these parameters to let the user evaluate the accuracy of the inputted values. The user can scroll using the scollers to the bottom right of the grid to see if the grid fits or not.
- ○ "Fix Param & Grid Image" will finalize all of the parameters. This should only be clicked if the parameters inputted generated a fitting grid. This button will generate a gridded images for every individual channels (Channel #? (channel name) - EDIT) so the user can come back and evaluate the individual protein expression of the selected cells.
- ● Test Run
  - ○ The default values can generate a fitting grid. Change individual values and click "Save and Try Parameters" to see how that might change the grid on the image. User can always click the info button for more information.
  - ○ When the grid is appropriate, click "Fix Param & Grid Image" to continue to the next step.
    - ■ The program should automatically change back to the first tab when the gridding process is complete. This process might take a while due to the extensiveness of image processing.

Step 3: Scanning Parameters
- ● Button Explanation:
  - ○ "Pick a Color Threshold for the selected Channel" lets user to pick a color with explicit RGB value such that any pixel with a higher RGB will be counted as one pixel of protein expression
  - ○ Two text boxes are for inputs of the lower number and the higher number of the pixel bounds. If the number of pixels of protein expression, which passed the RBG threshold that was set earlier, falls within the pixel bounds, the well contains a cell with positive expression.
  - ○ "Save Pixel Range" saves the pixel range.
  - ○ "Confirm and Continue" saves all inputted parameters and begin scanning the channel with inputted parameters.
- ● Test Run
  - ○ Click the Image Viewer tab to identify a good color threshold and pixel range for the specific channel.
  - ○ Click "Pick a Color Threshold for the selected Channel". Generally, in this specific set of images, R: 0, G: 153, B: 153 is a good color threshold. In other cases where weaker expression of protein can still be categorized as a positive identification, the RGB threshold can be set lower and vice versa.

- - Set the range from 4 to 20. Again, these values can be adjusted for different sizes of cancer cells.
  - Click "Save Pixel Range" and "Confirm and Continue" to the next channel
  - Repeat the process until all channels have their appropriate scanning parameters
    - In actual practice, however, the color threshold and pixel range may vary between each channel.

Step 4: Color Combination Search
- Button Explanation:
  - "Search" will search for cell membrane protein expressions with positive expression of channels that are "Selected for Search" and negative expression of channels that are not "Selected for Search", both requirements must be met to identify a target cell.
    - The change in final cell count indicates that the results are outputted.
- Test Run
  - Choose different combinations and see the cell count at the end of the search.
  - Combination 1,2,4 is epithelial cancer cell type and Combination 2,4 is cancer stem cell (if I recall correctly from last year)
  - After search, an image file named "Combination (number selected)" (Ex. "Combination 1 2 4.jpg" will be generated in the image directory and wells with red boxes around them will be positive IDs that can be further evaluated in separate Channel #? (channel name) - EDIT.jpg files. Also, a word document will also be generated in accompany to the image output.

Important Tips:
- Since the software will render through a number of large files simultaneously, RAM capacity may be an issue. So it is a good idea to first restart the computer before starting the software. (Recommended RAM is 16GB and higher)
- If the software is behaving strangely, there might be an error message somewhere, just recompile and rerun the code, the software is functional as long as all inputs are entered correctly.
- The actual software will only access the high contrast image, so the brightness of the plain image has no effects on the accuracy of the software.