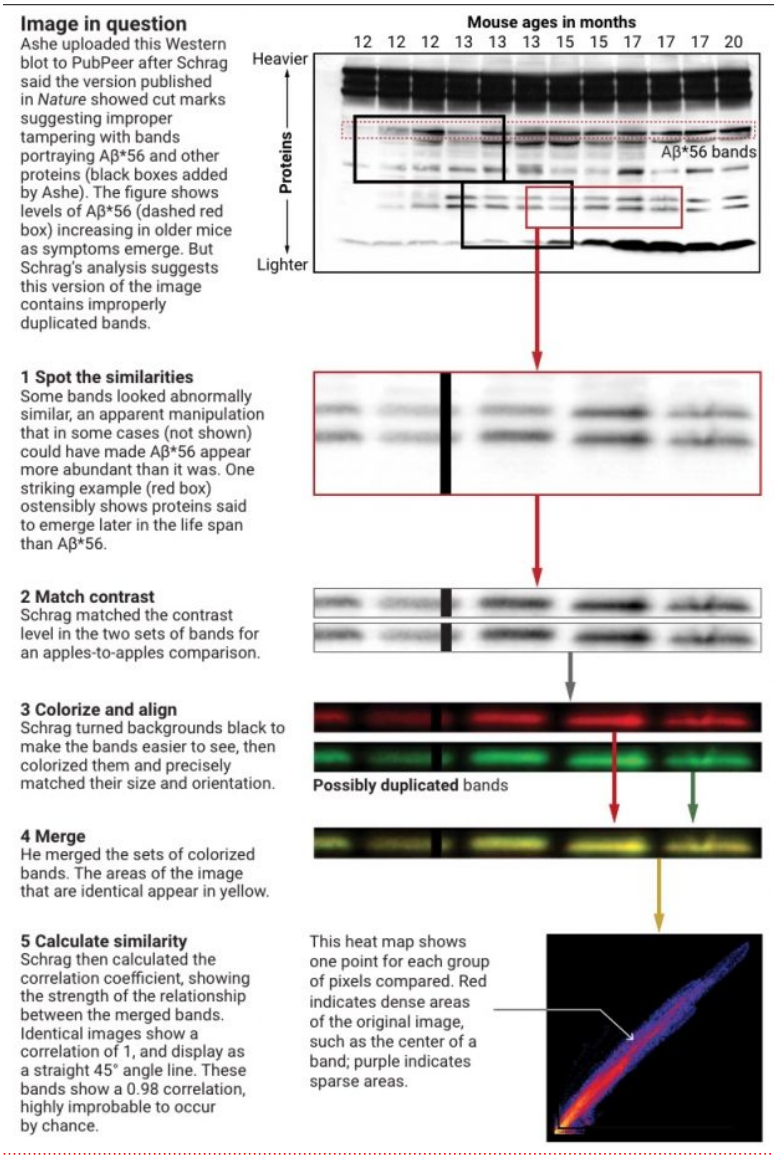
SOFTWARE

THE AMYLOID WESTERN BLOT: SCHRAG VS LESNÉ

1.09.2022

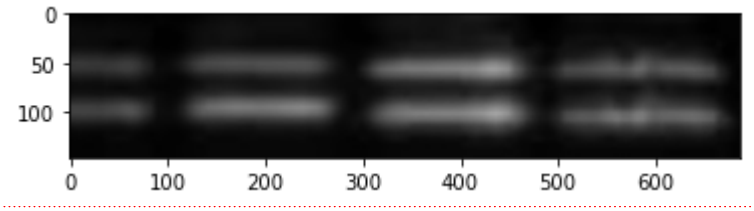
Similar bands are worrying of course, but as Ashe writes on PP “bands that migrate close to each other may differ in intensity but appear similar in shape”. Unfortunately the Piller article did not respond to this argument raising doubts not only on the Lesné paper but also on the Schrag analysis in Science.



Fabrication analysis by as reported in https://www.science.org/content/article/potential-fabrication-research-images-threatens-key-theory-alzheimers-disease I have no idea why they left the black vertical bar there?

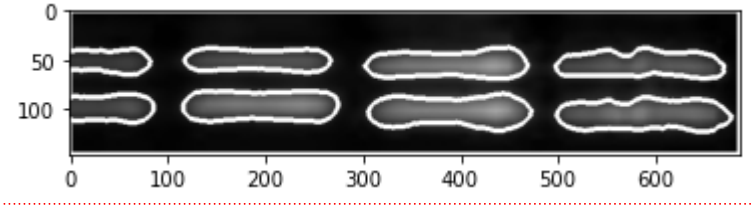
According to Piller Schrag had only 4 weeks of PP experience with image analysis and is using here a method of Western blot alignment that has never been validated before – most likely a manual analysis of undocumented pixel shuffling.

As Schrag did not respond to my email for technical details, I am trying now to repeat his analysis. So we read the images first and find the contours of the bands.



Analysis of the 3 and 4mer band of Fig 2 A at 600px.

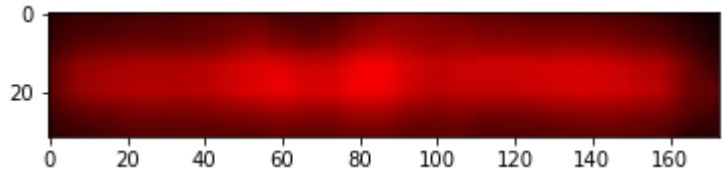
```
im = cv2.imread( "schrag.jpg")
# I am not adjusting background to keep the image as natural as
possible
# im = cv2.pyrMeanShiftFiltering(im, 25, 70)
# converting to grayscale and applying threshold
im = cv2.cvtColor(im, cv2.COLOR_BGR2GRAY)
thresh = cv2.adaptiveThreshold(im,255,cv2.ADAPTIVE_THRESH_MEAN_C,
cv2.THRESH_BINARY,101,10)
# find contours
contours, _ = cv2.findContours(thresh, cv2.RETR_LIST,
cv2.CHAIN_APPROX_NONE)
for i in range(len(contours)):
    x,y,w,h = cv2.boundingRect(contours[i])
    out = im[y:y+h,x:x+w]
```

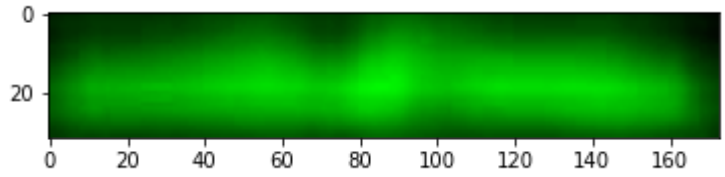


Analysis of the 3 and 4mer band of Fig 2 A: Contours are similar but not identical.

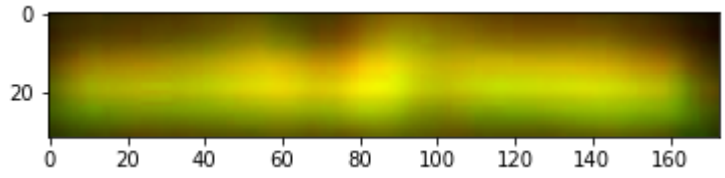
We then focus on the most distinct two bands on the right, correct their size and adjust contrast as done by Schrag.

```
# change crop to make out1 comparable to out2
# compressing eg cv2.resize(i2, (h,w), interpolation = cv2.INTER_AREA)
seems to invasive
out1 = out1[0:32,2:175]
h,w = out1.shape

# Michelson contrast
def getcontrast(im):
    min,max =(int(np.min(im)),int(np.max(im)))
    return( (max-min)/(max+min) )
print( getcontrast( out1) ) # 0.31
out2 = cv2.addWeighted(out2, 1.2, out2, 0, -50)
print( getcontrast( out2) ) # 0.33 which is acceptable

# display both bands
im = np.ones([w,h,3], dtype=np.uint8)
im[:,:,0] = 255-out1
plt.imsave("out1.jpg", im)
im = np.ones([h,w,3], dtype=np.uint8)
im[:,:,1] = 255-out2/0.5
plt.imsave("out2.jpg", im)
# heatmap seems overkill to me but Spearman's R is nice to know
r,_ = stats.spearmanr( im[:,:,0].flatten(), im[:,:,1].flatten() )
print(r) # 0.92
# no change also after combining neighboring pixel
im = cv2.resize(im, (int(w/4), int(h/4)),
interpolation=cv2.INTER_NEAREST)
stats.spearmanr( im[:,:,0].flatten(), im[:,:,1].flatten() )
```



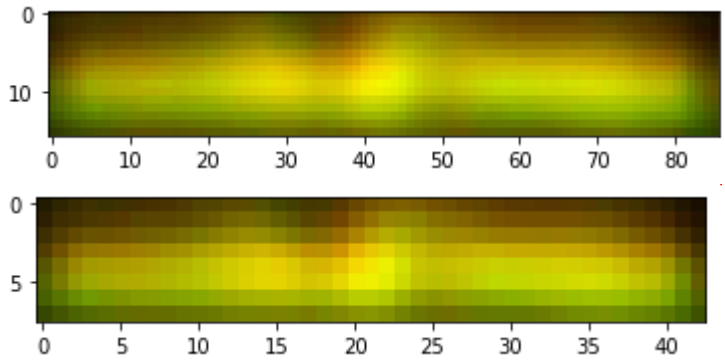


These are the colorized bands basically as doen by Schrag. There is no need to construct the red + green = yellow overlay as the bands are clearly different. Anyway here is it just for completeness.

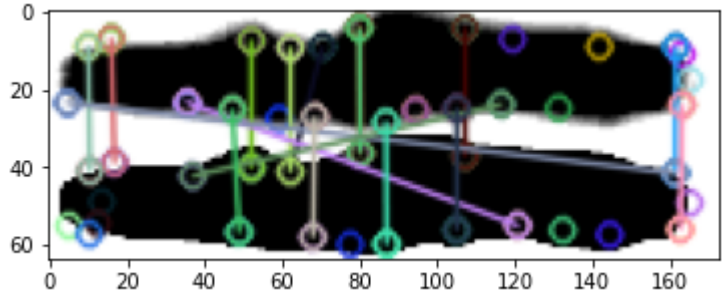


I can't replicate the results – neither the high correlation coefficient nor the shape of bands . There are also no splice marks although there should be some if Lesné would have used version Photoshop CS2 (9.0) at that time.

Is my alignment wrong? In don't think so by visual eyeballing. Also comparing a larger area doesn't change so much.

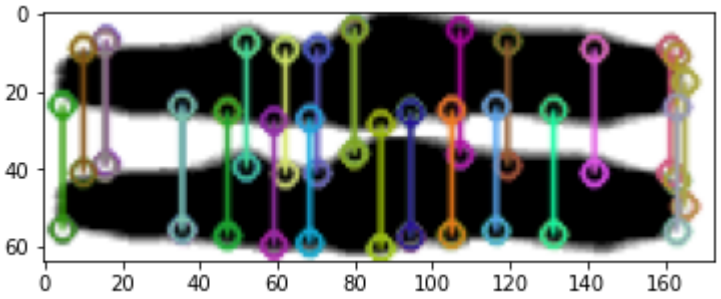


Keypoints also do not match, excluding largely any scaling and alignment issue.



Side by side comparison of both bands after highlight clipping and spreading brightness values before constructing and matching key-points. There are 27 in the first and 23 keypoints in the second

band – so there is clearly a high similarity.



Here is how a cloned band would look like.

Of course I have also doubts on many Lesné images including the duplication identified by Elisabeth Bik shown under #15.

Verdict: It seems that Piller has again produced another one of his dubious stories while the original Lesné images need to be examined in more detail.