Investigating the Localization of Mitochondrial Proteins, OCIAD1 and Bcl-Rambo, to the Peroxisome; Developing a High-Throughput Biological Image Processor

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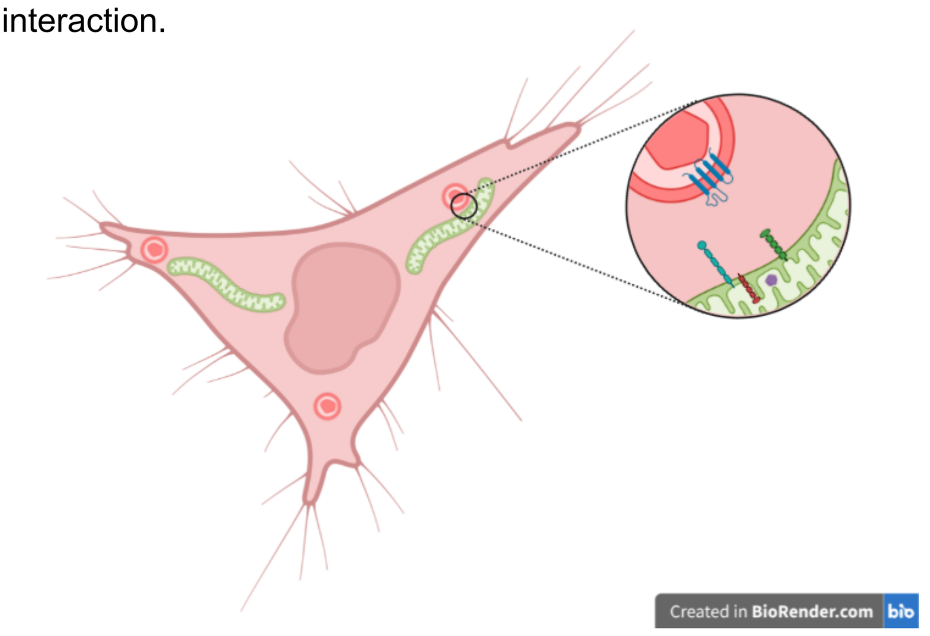
Introduction

The mitochondria is an organelle that is involved in metabolic pathways that lead to the production of ATP, a molecule that provides energy for most cellular processes. The peroxisome is an organelle that performs tasks such as the oxidation of very long and branched-chain fatty acids, which are essential to the body. It has been previously shown that some mitochondrial membrane protein such as USP30 can be recruited to the peroxisome. In a BioID screen, two mitochondrial proteins, OCIAD1 and Bcl-Rambo, had hits with multiple key peroxisomal proteins. Bcl-Rambo is a mitochondrial membrane protein that induces apoptosis in cells via the release of cytochrome c into the cytosol. OCIAD1 is an inner membrane mitochondrial protein that among other functions, integrates the catalytic subunit, CYC1, into CIII of the mETC.

The relative proximity of these protein with peroxisomal proteins suggested some potentially uncharacterized interaction.

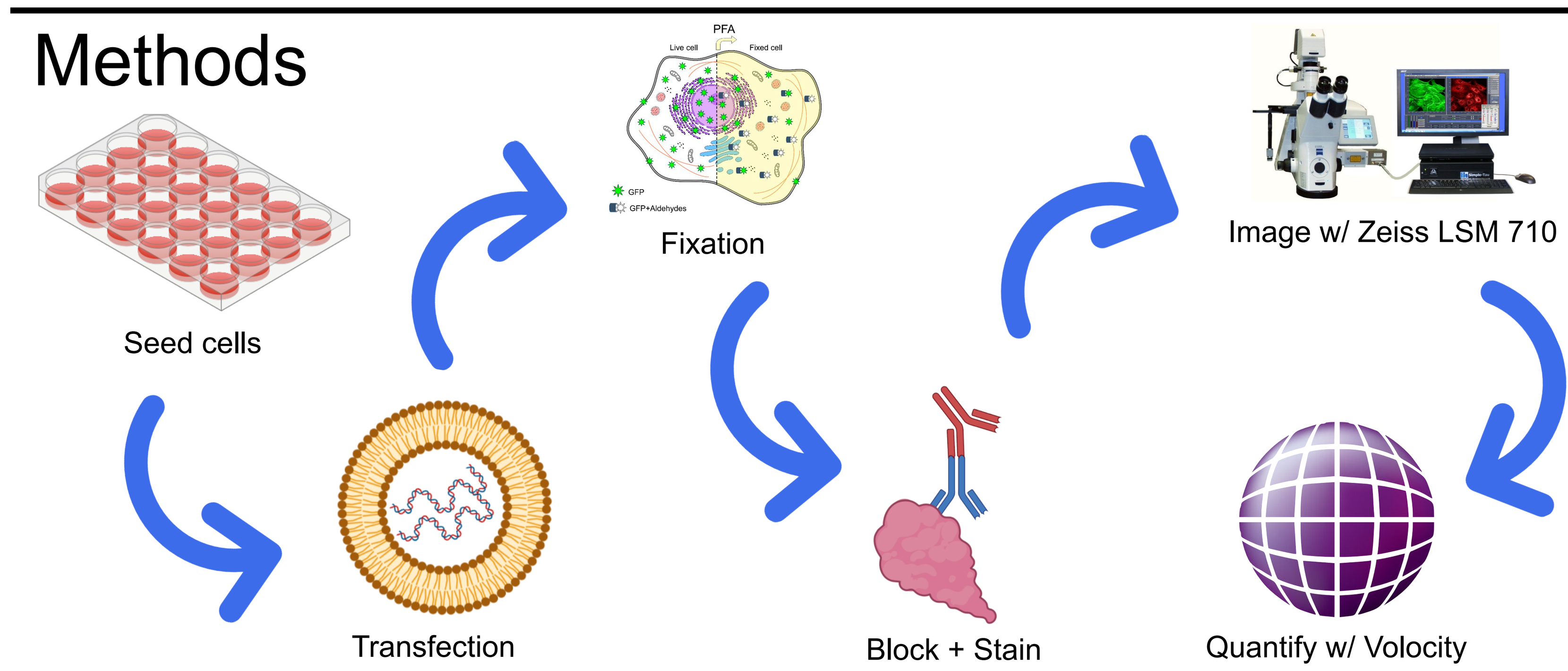


Thus, we posed the question: **do OCIAD1 and Bcl-Rambo interact with the peroxisome and what is their mechanism of interaction?**

We explored the possibility of co-localization. To analyze this, we performed an IF assay with fluorescent constructs of the proteins along with an antibody stain for OCIAD1. HSP60 and PMP70 were used as markers for the mitochondria and peroxisome, respectively, and in over-expression conditions, a fluorescent mitochondrial outer-membrane protein, OMP25-GFP was used as a control.

Figure 1: Schematic of pertinent proteins. Blue TM protein is PMP70 on peroxisome, and teal, red, and green TM proteins represent OMP25, OCIAD1, and Bcl-Rambo, respectively. HSP60 is shown in the mitochondria as purple.

Methods



Results

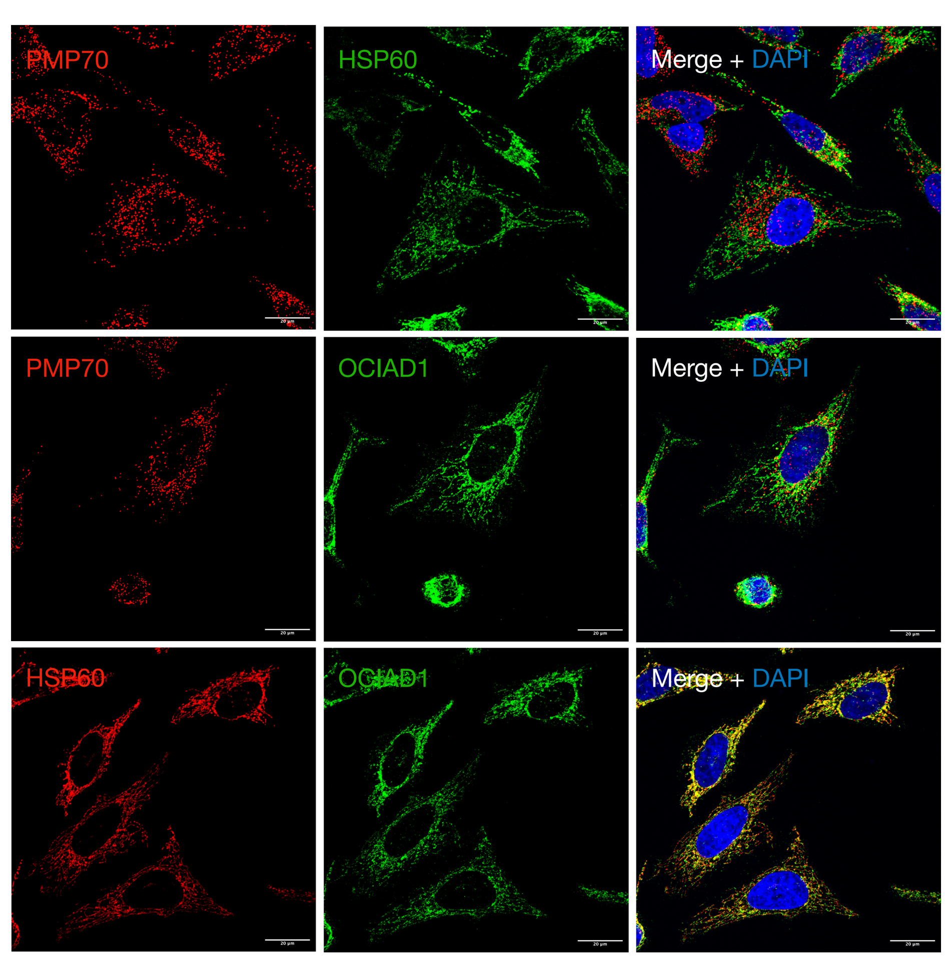


Figure 2: Representative images of cells stained for PMP70 and HSP60. This is the negative control for the stain condition.

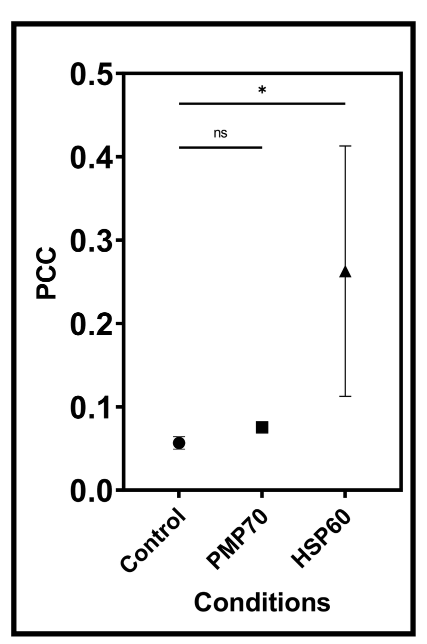


Figure 3. To the left are representative images of cells stained for OCIAD1 and either PMP70 or HSP60. To the right is a plot showing the PCC averaged from three trials between a negative control, a PMP70 and HSP60 double-stain, and the previously mentioned conditions. Statistical significance in the data is shown by asterisks.

Results Cont'd

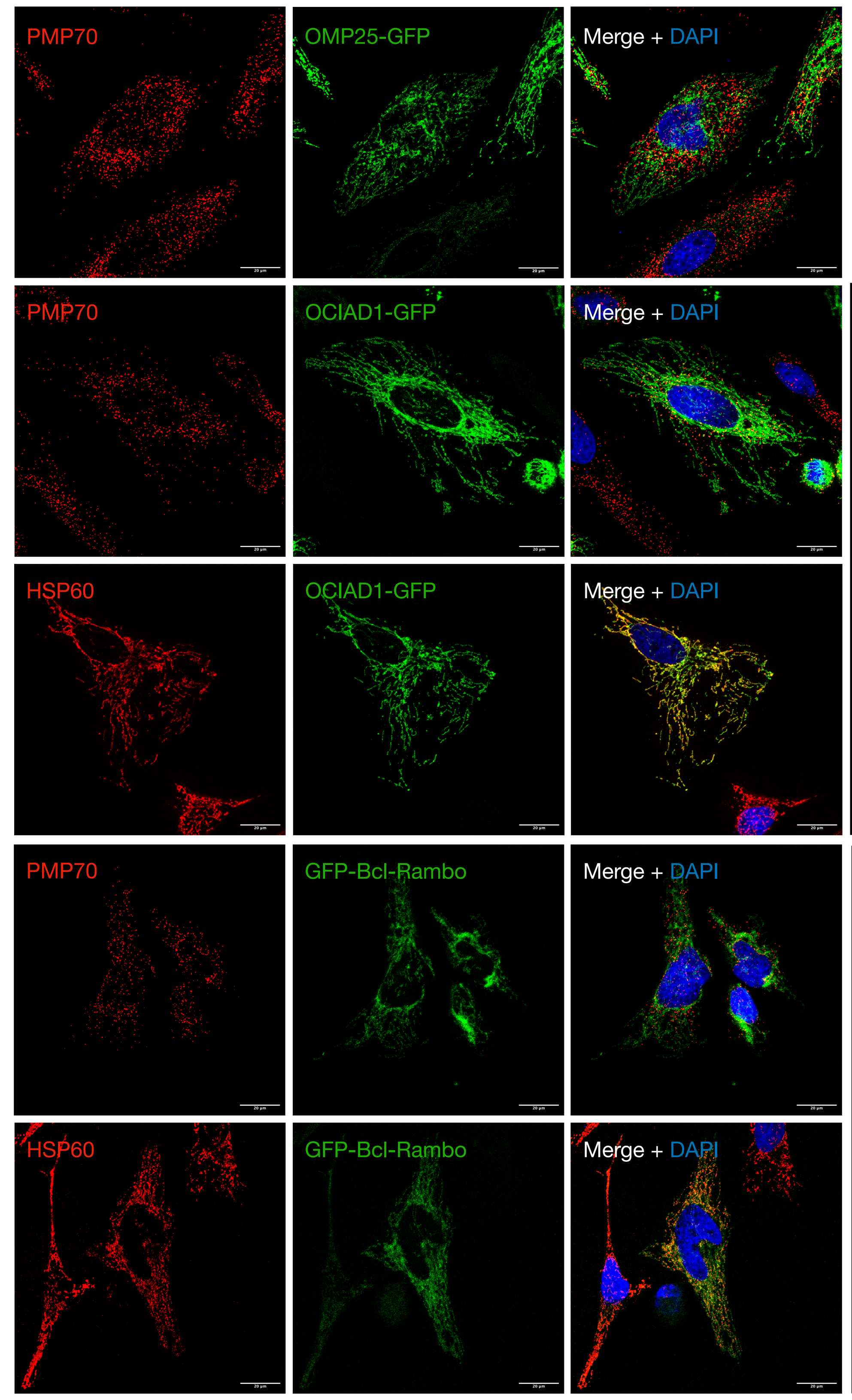


Figure 4: Representative images of cells stained for PMP70 and over expressing OMP25. This is the negative control for the OE conditions.

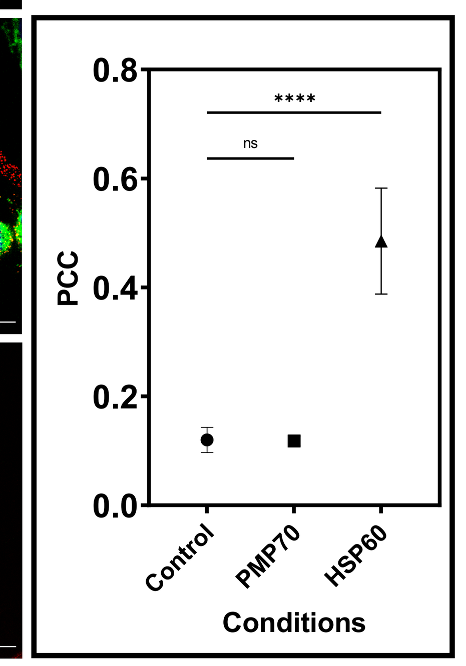


Figure 5. To the left are representative images of cells over-expressing OCIAD1-GFP and stained for either PMP70 or HSP60. To the right is a plot showing the PCC averaged from four trials between a negative control, OMP25-GFP and PMP70, and the previously mentioned conditions. Statistical significance in the data is shown by asterisks.

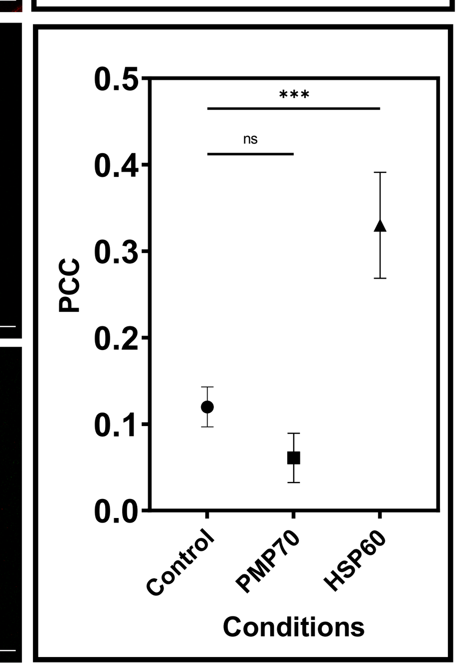


Figure 6. To the left are representative images of cells over-expressing GFP-Bcl-Rambo and stained for either PMP70 or HSP60. To the right is a plot showing the PCC averaged from four trials between a negative control, OMP25-GFP and PMP70, and the previously mentioned conditions. Statistical significance in the data is shown by asterisks.

Conclusion & Next Steps

Statistical analyses of the data showed that there was no significant difference in PCC comparing co-localization of OCIAD1 and Bcl-Rambo with PMP70 to their respective controls. The data also showed that OCIAD1 and Bcl-Rambo had a statistically significant increase in PCC with HSP60 compared to the control, implying co-localization. This was expected since these are mitochondrial proteins.

These quantitative co-localization studies shows that OCIAD1 and Bcl-Rambo are mainly localized to mitochondria and not on the peroxisome in HeLa cells.

Analysis in other cells lines might yield different results.

Therefore, it is possible that OCIAD1 and Bcl-Rambo may interact with peroxisomal proteins through other mechanisms such as mediating contact sites.

Processor Introduction

This is all well and good, but this analysis was a rather labour-intensive process. Through a combination of image filtering techniques and mathematical morphology, the image segmentation work can be greatly reduced!

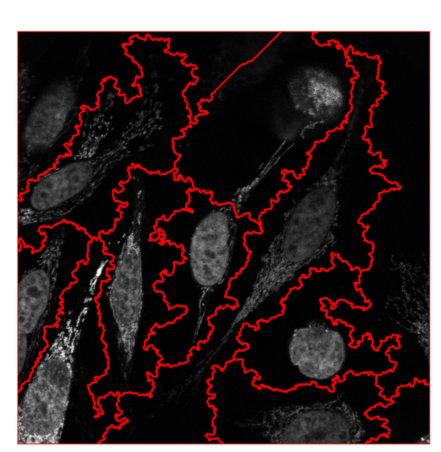


Figure 7. Results of the SegmentationEngine of the HTPIP. The separation of cells are depicted in red.

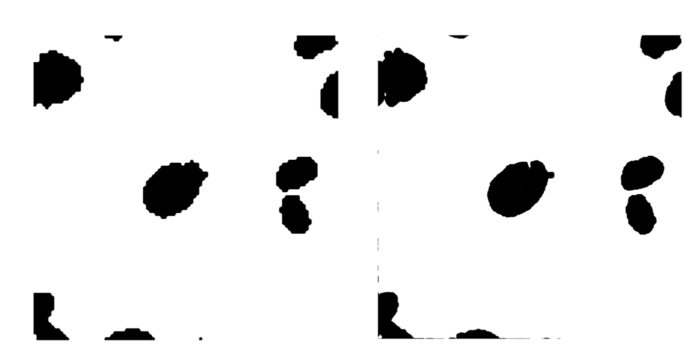
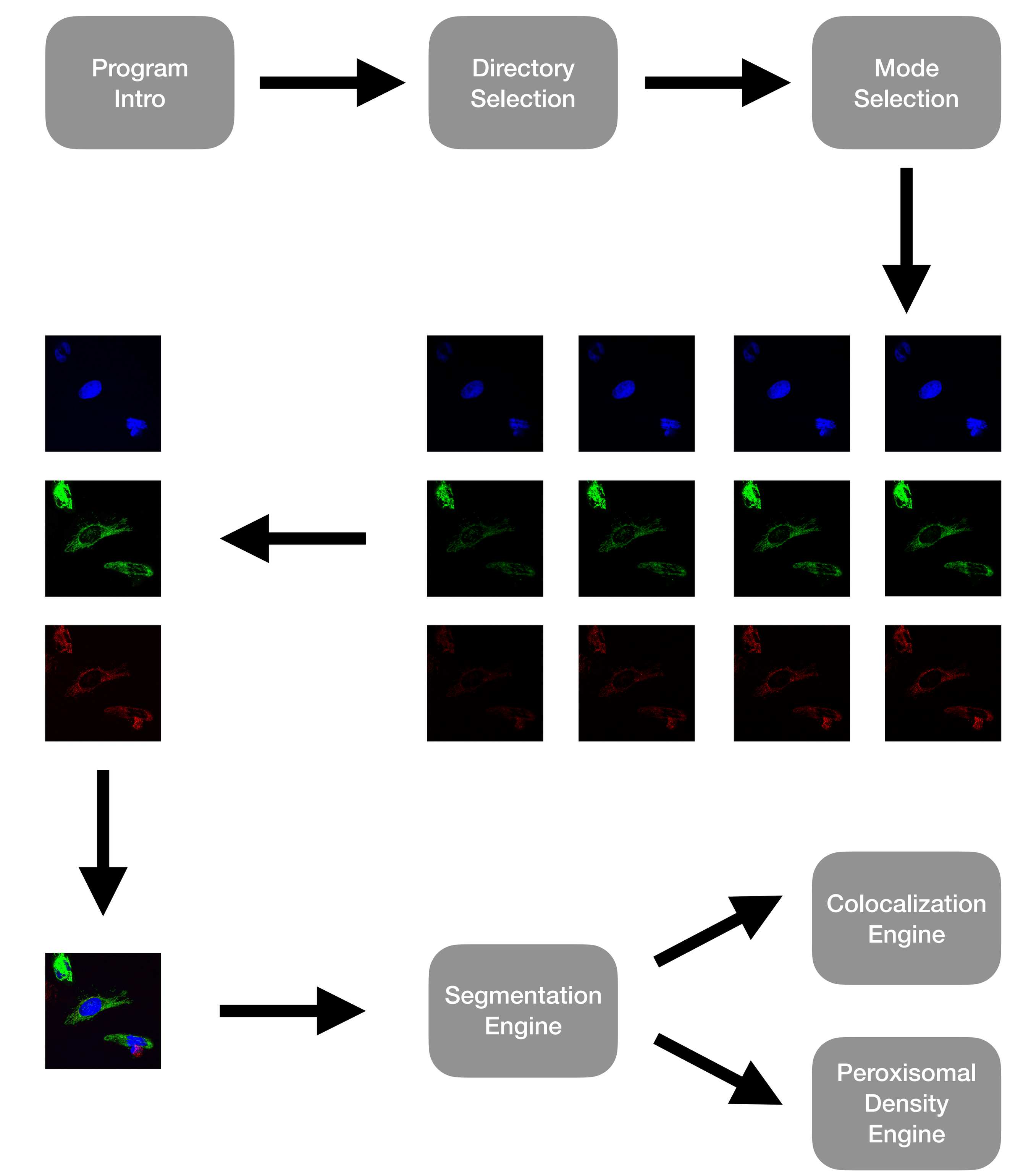


Figure 8. Smoothing effects of Gaussian Blur and Image resizing on nuclear stain

Processor Flowchart



Processor Introduction

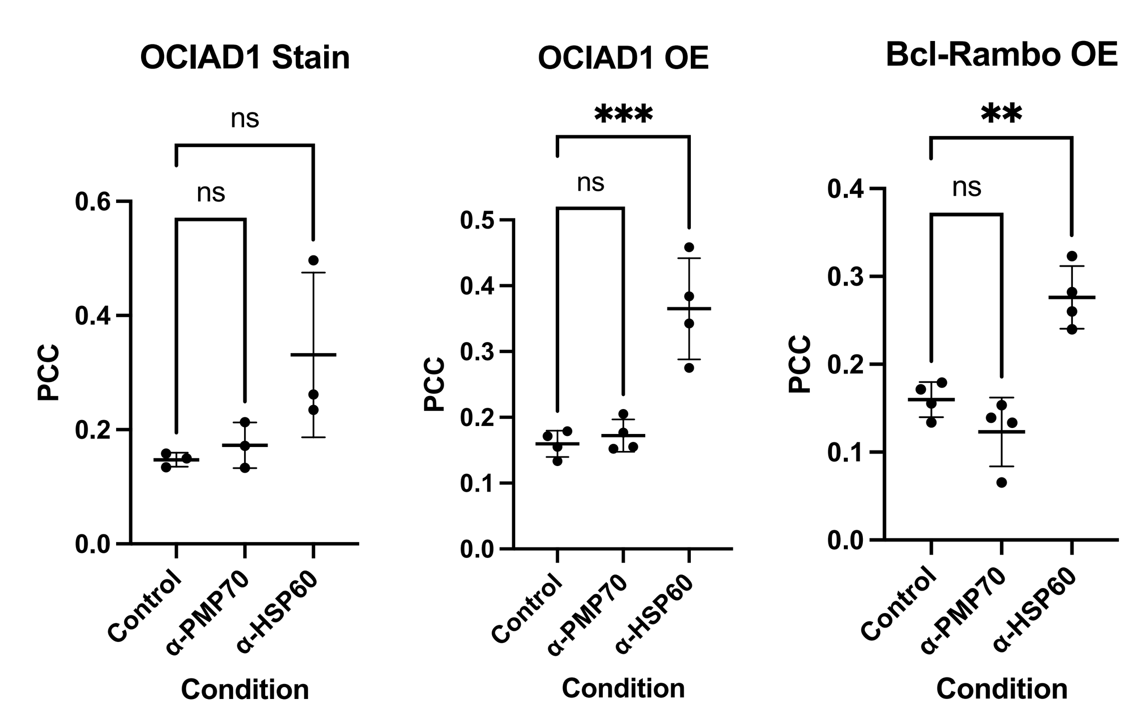


Figure 9. The PCC analysis yielded somewhat similar results to the manually-done colocalization measurements. The statistically significant difference for the control compared to HSP60 in the OCIAD1 is incorrect.

Processor Conclusions

The processor shows a potentially solid foundation in cell segmentation to get colocalization values, but it requires a great deal of fine-tuning to become more accurate.

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