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**Citation:** Brito-Pacheco, D. A., Karabağ, C., Brito-Loeza, C., Giannopoulos, P. & Reyes Aldasoro, C. C. (2024). Morphological Analysis of HeLa Cells and their Mitochondria under Electron Microscopy. Poster presented at the Crick BioImage Analysis Symposium 2024, 25-26 Nov 2024, London, UK.

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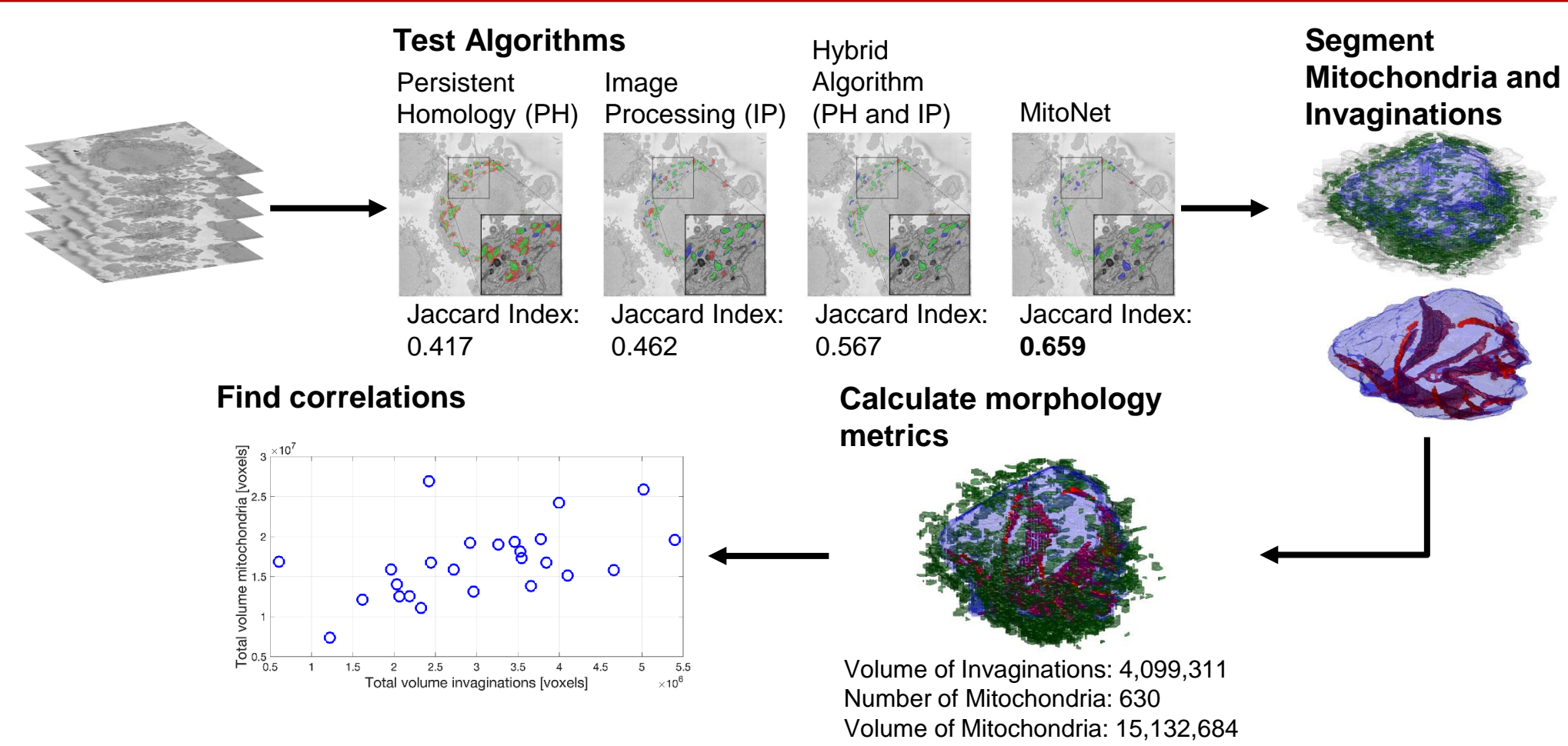
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- Number of mitochondria have a positive correlation with the volume of nuclear invaginations.
- Volume of cytoplasm has negative correlation to aspect ratio of mitochondria.

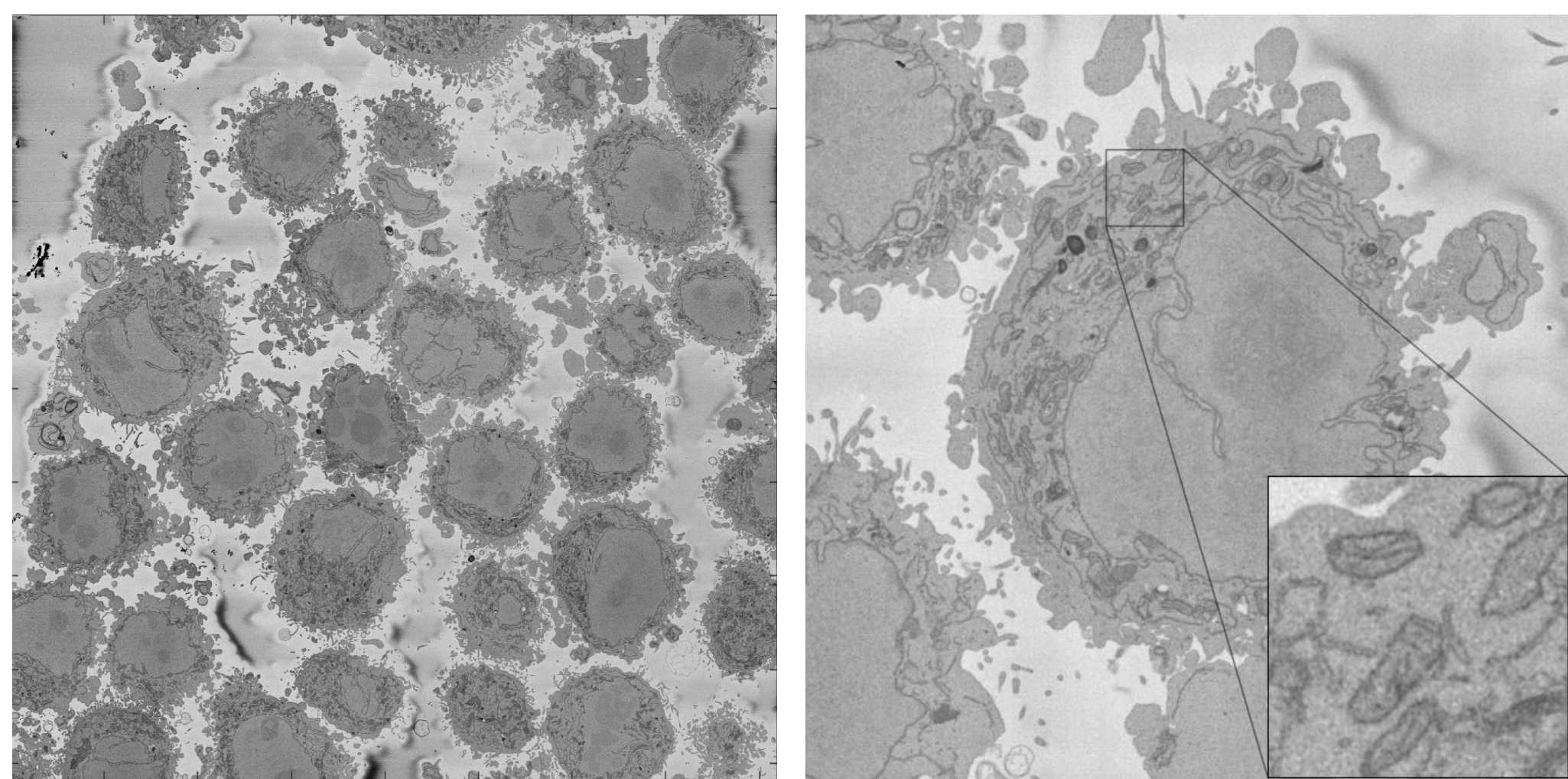


**Figure 1.** HeLa cells are imaged using serial block face scanning electron microscopy, segmentation algorithms are tested to find the best. The mitochondria, nucleus, and invaginations are segmented. Finally, morphology metrics of the structures are calculated and correlations between the features are found.

## Materials and Methods

### Electron Microscopy images of HeLa cells are acquired.

The sample, immobilized in Durcupan resin, contains 25 cells complete enough that segmentation of their nucleus and cytoplasm can be performed. This is done through classic image processing (IP) techniques [1].



**Figure 2.** Images of HeLa Cells are obtained used serial block face scanning electron microscopy (SBF SEM). The initial 3D volume of images was made up of 517 slices of 8192 x 8192-pixel wide images. Regions of Interest (ROIs) of dimensions 2000 x 2000 x 300 are centred over the nucleus of individual cells. Shown in a zoom box are multiple mitochondria.

### To segment the mitochondria, four segmentation models are tested:

- **Persistent Homology (PH):** uses PH to extract features from patches of an image and determine if they are part of a mitochondria.
- **Image Processing (IP):** based on traditional morphological operations to isolate closed, dark regions inside the cytoplasm.
- **Hybrid Algorithm (PH & IP):** combines the segmentations produced by the previous models to improve performance.
- **MitoNet:** state-of-the-art deep learning model based on a U-Net architecture, trained on CEM1.5M and CEM-MitoLab.

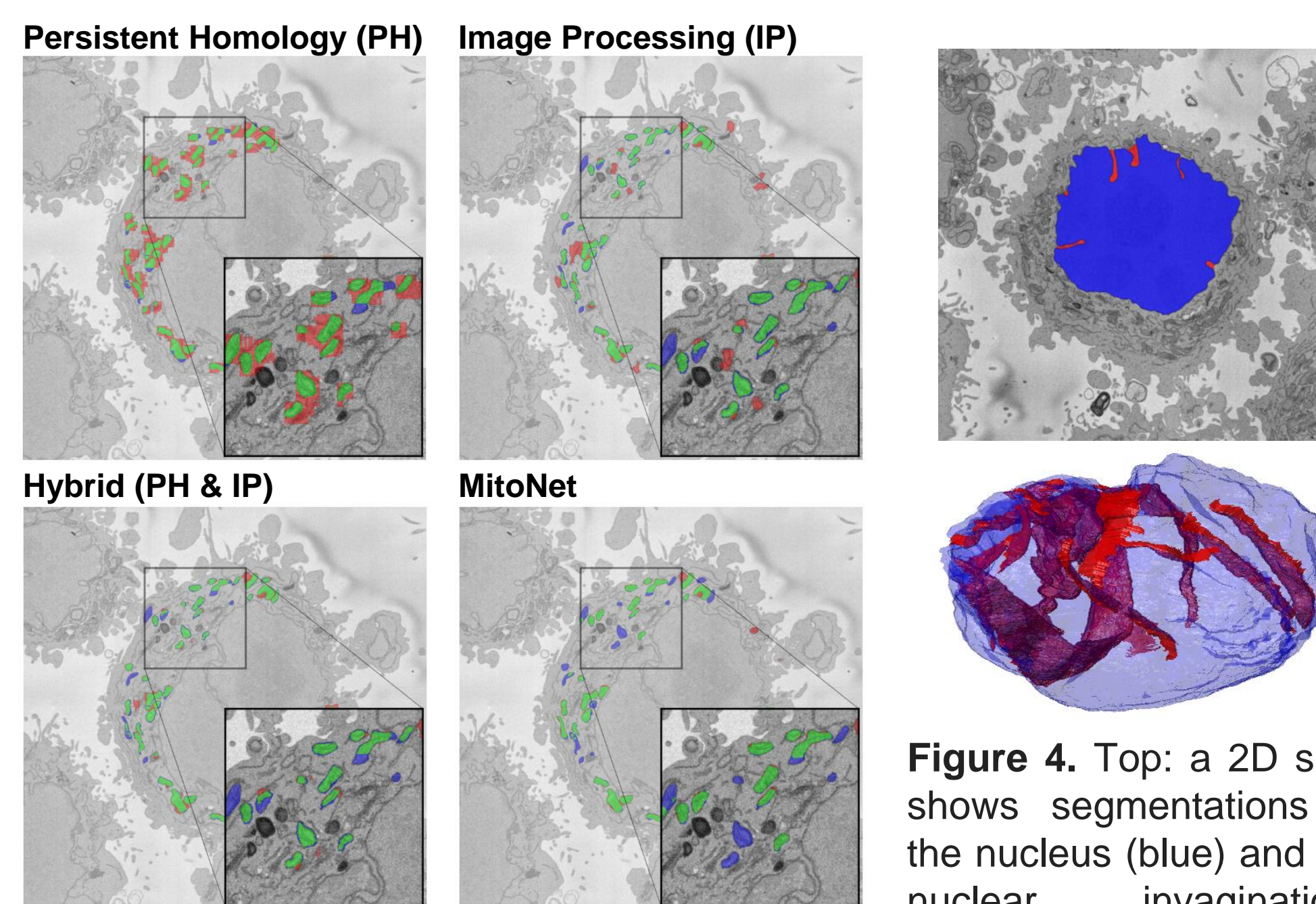
**Invaginations are segmented slice-by-slice** using Image Processing procedures. Morphological operations such as filling holes and image closing are involved in the segmentation process.

**Morphology metrics are calculated** from the segmented structures: volume of invaginations, volume, number and aspect ratio of mitochondria.

## Results

Segmentation Algorithm	Jaccard Index	Pixelwise F1-Score
Persistent Homology (PH)	0.417	0.582
Image Processing (IP)	0.462	0.626
Hybrid (PH and IP)	0.567	<b>0.724</b>
MitoNet	<b>0.659</b>	0.709
Inter-Observer (IO)	0.696	0.582

**Table 1.** All four segmentation models were tested on five benchmark images, and precision metrics were calculated. The best performer overall was MitoNet. It is notable that the IO score reveals that a “perfect” Jaccard index of 1 is nearly impossible, since mitochondria are small compared to the whole image.



**Figure 3.** Mitochondria segmented by each of the four models. True Positive pixels are shown in **Green**, False Positive pixels are shown in **Red**, and False Negative pixels are shown in **Blue**.

**Figure 4.** Top: a 2D slice shows segmentations of the nucleus (blue) and the nuclear invaginations (red). Bottom: a 3D reconstruction of the segmented nucleus (blue) and the nuclear invaginations (red).

### Correlations in the morphology of invaginations and mitochondria were found:

- The total volume of invaginations and the total volume of mitochondria are positively correlated ( $r = 0.5067$ ).
  - The number of mitochondria and the average volume of mitochondria are negatively correlated ( $r = -0.4466$ ).
  - The volume of the cytoplasm and the aspect ratio of the mitochondria are negatively correlated ( $r = -0.4407$ ).
  - The number of mitochondria and the aspect ratio of mitochondria are negatively correlated ( $r = -0.1466$ )
- (All correlations were found to be significant,  $p < 0.05$ ).

## REFERENCES

[1] - C Karabağ, ML Jones, CC Reyes-Aldasoro Volumetric Semantic Instance Segmentation of the Plasma Membrane of HeLa Cells J. Imaging 2021, 7(6), 93; <https://doi.org/10.3390/jimaging7060093>

## CONTACTS

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