Mean Cell Volume Estimation

By: **Olivia Hernandez and Paul Webster**

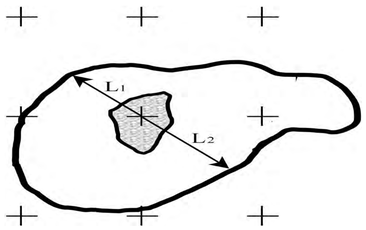
Background

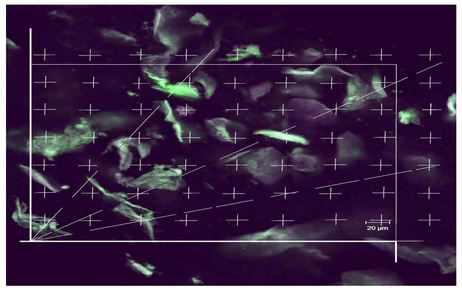
- Serological methods are widely used in pathological research and diagnostics and “How a particular cell size is specified by differentiation programs and physiology remains one of the fundamental unknowns in cell biology.”(M.Ginzberg).
- The methods used in this project allow for “assumption free estimates of mean volume from the usual number distribution of particle size.”(H.J.G. Gundersen).
- This project objective was to use a stereological method, “the nucleator” to quantify cell volumes in human epithelial vaginal cells.

Methods & Materials

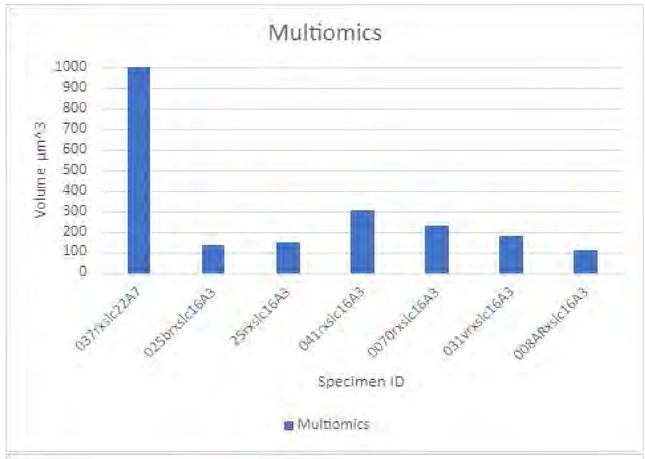
1. Image cells using confocal microscope. Laser Parameters cy3 and cy5 to detect the cell and antibody labeling for CAPRISIA and for Multiomics cells.
2. Apply nucleator overlay with forbidden box on images using adobe photoshop.
3. Open ImageJ to measure lengths of cells in different directions per image. Set distance in pixels to scale using calibration bar and set known unit to 100 um.Leave pixel aspect ratio to 1.0. Measure each cell that is not touching forbidden box lines and record measurements in excel. Find average length of cells per specimen and use in formula Volume = 4/3*π *(l)^3.
4. Repeat steps 2-3 for all images of cell specimen. Graph results.

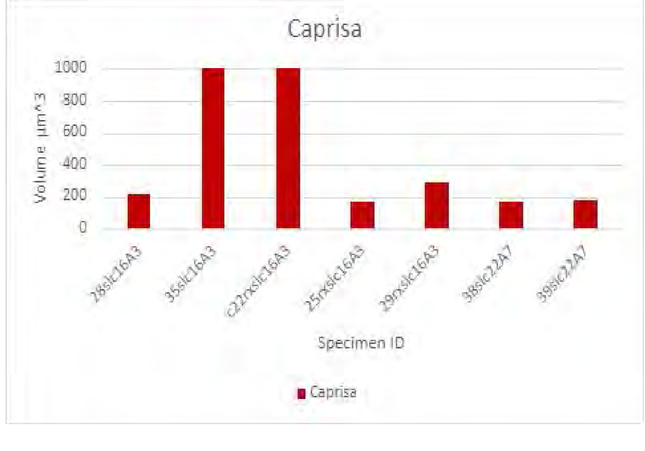
Preliminary Results





- Figure 1.Left. Visualization of method, nuclear profile used to measure length.
- Figure 2. Right. Rabbit antibody bound to drug transporter proteins SLC16A3, SLC22A7 & SLC2A7 proteins. Immunolabeled cells imaged under confocal microscope with nucleator overlay.
- Figure 3. Below. Cell Volume comparison chart trial 2. Caprisa cells were HIV positive, and Multiomics were HIV negative volunteers.





Implications & Future Work

- Cells were measured in four different directions (across, upward and diagonally)and Each picture only one direction was applied to the cell.
- Results may be influenced by their positioning within the agarose on the glass slide. In the Caprisa specimen there is two with outlier volumes this could be due to the virus infection.
- To further this study we could measure more cell samples for further quantitative analysis. We could compare our data results with other stereological methods e.g selector. We could also compare the volume sizes to cells that have been cryosectioned and imaged with a light microscope.

References

- GUNDERSEN, H. J., BENDTSEN, T. F. (1988). Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS, 96(1-6), 379–394. https://doi.org/10.1111/j.1699-0463.1988.tb05320.x
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- Ginzberg, M. B., Kafri, R., & Kirschner, M. (2015, May 15). Cell biology. on being the right (cell) size. Science (New York, N.Y.). Retrieved July 13, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4533982/

Acknowledgments

This project would not be possible without the Oak Crest Institute of Science, Dr. Marianne Smith, Dr. Paul Webster, the Citrus College Summer Research Experience program, and the Project RAISER III HSI-STEM grant. Thank you all.

Alternate Text

Olivia Hernandez

Oak Crest

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