Distribution Analysis of Mitochondria in Confocal Images

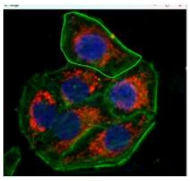
STUDENT: VISHAKHA GOYAL, DEPARTMENT OF MECHANICAL ENGINEERING, IIT KANPUR
MENTOR: DR. TUSHAR SANDHAN, DEPARTMENT OF ELECTRICAL ENGINEERING, IIT KANPUR

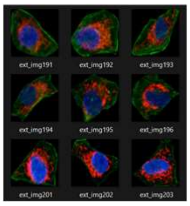
Introduction

- Mitochondria are essential organelles involved in energy production, ROS generation, and ion homeostasis, with their distribution in cells dynamically changing in response to stress conditions. The spatial and temporal position of the mitochondria perhaps meets the functional needs of the cell
- Previous research supports that heat shock causes perinuclear mitochondrial accumulation in mammalian cells. This study aims to enhance our understanding of mitochondrial distribution.
- Understanding these distribution patterns can provide insights into cellular states, contributing to early disease detection and therapeutic strategies.
- We employ image processing techniques to analyse confocal images of cells, categorizing them as perinuclear, central, or radial zones and constructing a pipeline for mitochondrial distribution in each.

01 Data Acquisition and Preprocessing

- Obtained a cell per image with homogeneous size and bit depth.
- Cropping image with interactive input from the user as cell selection





02 Cell wall, Nucleus and its centre of mass detection

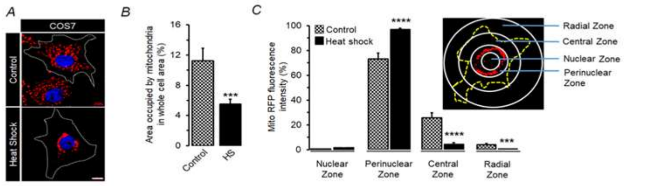
- Foreground background separation
  - Exclude black pixels using a threshold mask on the value channel of the HSV image
- Apply K-means clustering to the non-black pixels to segment the image into three clusters
- Nucleus : innermost cluster; for each pixel in the image:
  - Examine the neighboring pixels to determine if they belong to a different cluster.
  - If a neighboring pixel belongs to a different cluster, increment the boundary pixel count for the current pixel's cluster.
  - Identify the innermost cluster as the one with the fewest boundary pixels, indicating that it is least influenced by neighboring clusters.
- Calculate the center of mass for the innermost cluster
  - Assign pixel intensities as weights based on the mask values.
  - Compute the center of mass using weighted averaging of pixel coordinates, yielding the centroid coordinates (x, y) for the innermost cluster.
- Cell Boundary : outermost cluster
  - Define the outermost cluster based on boundary pixels, as it likely represents the most distinct and separated cluster within the image.
  - Identify the cluster with the highest count of boundary pixels, as this indicates its perimeter is most exposed to neighboring clusters.

03 Dividing the cell into regions wrt nucleus

- Angular Lines Analysis:
  - Angular lines are drawn radially from a specified centre point (such as the centroid of a cluster). These lines extend outward in evenly spaced intervals across a circular range.
- Detection of intersections between the drawn angular lines and contours
  - The code iterates through each line segment defined by consecutive endpoints.
  - It calculates potential intersections between the angular line and the contour (either the largest outer contour or the innermost cluster boundary).
  - Using computational geometry principles, specifically checking for intersection points between line segments (angular lines) and the contour edges.
  - For valid intersections found within the defined contour bounds, the code records the intersection point.
  - The endpoint of the angular line is considered as the default intersection point.
- Dividing the length between the two intersection points on each line into three points (p1,p2,p3) and store them.
- Closing Polyline: Draws a closed polyline connecting the equidistant points p1, p2, and p3 to form a closed shape

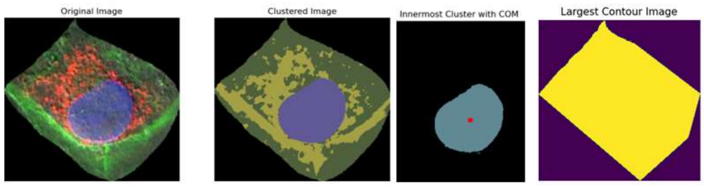
Literature Review

- Perinuclear mitochondrial clustering, increased ROS levels, and HIF1 are required for the activation of HSF1 by heat stress



. (A) Representative images showing the distribution pattern of mitochondria in COS-7 cells at 37°C and upon 1 h heat shock at 42°C. The mitochondria were visualized using red fluorescent protein targeted to mitochondria (Mito-RFP). The cell boundary is identified by the dotted line. (B) Relative cytoplasmic area (%) occupied by the mitochondria in COS-7 cells at physiological temperature or during the heat shock (HS), as shown in A (n=4; 15 cells per set). (C) Relative fluorescence intensity of the mitochondria (%) in the four demarcated zones indicated in cells at physiological temperature or after a heat shock. The dotted line identifies the cell boundary and the circles define the zones

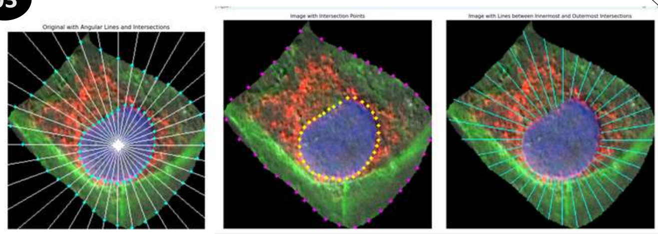
02 Output



Cell wall and Nucleus with its center of mass

Original and clustered image of cell

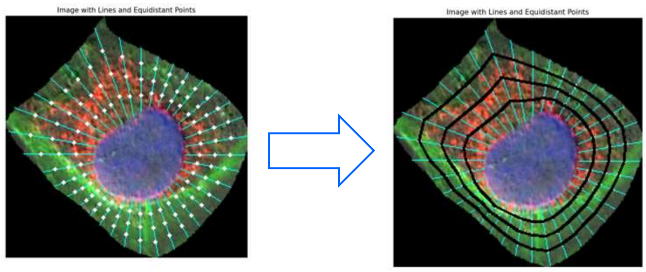
03



Angular lines and intersection points with contour

Intersection points with nucleus and cell boundary

Length between the endpoints and



The endpoints of line divided into three points(p1,p2,p3) at equal distance wrt each other

Polygon connecting a point on each line on basis of proximity from COM

Conclusion:

Divided the cell into three or more regions accurately with respect to nucleus, as per the definition of perinuclear region

Future Scope:

- Getting a 3-D distribution based on the length between intersection points, the percentage distance travelled from COM and the number density of mitochondria present. This will give a robust distribution analysis which can be have wide areas of application, instead of being limited to calculating density distribution in three regions only, i.e. Perinuclear, Central and Peripheral.
- To achieve distribution the Parzen estimation and DBSCAN can be employed after detecting presence of mitochondria.