

Indian Institute of Technology Kanpur



SURGE INTERNSHIP REPORT

Distribution Analysis of Mitochondria in Confocal Images

Student Name
Vishakha Goyal

Student ID
2430164

Guide


Dr. Tushar Sandhan

Submission Date : 12/07/2024

1 Abstract

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. Mitochondrial clustering patterns were analyzed in confocal microscopy images through K-means clustering and contour detection methods. Angular lines were employed to measure distances between perinuclear, central, radial zones and the nucleus, revealing distinct spatial relationships. This research contributes to understanding mitochondrial dynamics in cellular responses to stress, with implications for biomedical research and therapeutic strategies.

Keywords: mitochondria, image segmentation, density distribution

2 Literature Review

Heat stress refers to the physiological and biochemical challenges that organisms face when exposed to elevated temperatures. This condition can impact cells, tissues, and entire organisms, leading to varying degrees of physiological disruption or damage depending on the intensity and duration of the heat exposure (Kovats & Hajat 2008).

At the cellular level, heat stress can cause several significant effects. High temperatures can induce protein denaturation, causing proteins to unfold and lose their functional conformation, which can lead to toxic protein aggregation (Martin et al. 1992). Additionally, heat stress increases the production of reactive oxygen species (ROS), resulting in oxidative damage to proteins, lipids, and DNA (Yang et al. 2010). This oxidative stress occurs when there is an imbalance between ROS production and the body's antioxidant defenses, causing damage to cells, tissues, and organs (Belhadj Slimen et al. 2014). Furthermore, heat stress can lead to both direct and indirect DNA damage, resulting in mutations and genomic instability (Kantidze et al. 2016).

Cells initiate the heat shock response (HSR), a conserved cellular defense mechanism to counteract heat stress. Key players in the HSR are heat shock proteins (HSPs), which assist in refolding unfolded proteins and preventing the accumulation of damaged proteins (Park et al. 2005). HSP genes are activated by the transcription factor HSF1, a master regulator of the HSR pathway (Park et al. 2005). Despite knowing that various stressors activate HSF1, the precise molecular players and processes involved in HSF1 activation remain unclear (Park et al. 2005).

A functional link exists between oxidative stress and the heat shock response in eukaryotes, though the underlying mechanisms are not yet fully established (Agarwal & Ganesh 2020). ROS are highly reactive oxygen-containing molecules and natural byproducts of cellular metabolism, particularly in mitochondria (Schieber & Chandel 2014). Maintaining a balance between ROS production and detoxification is crucial for cellular homeostasis (Schieber & Chandel 2014). While low levels of ROS are essential for cell signaling and defense against pathogens,

excessive ROS can lead to oxidative stress, contributing to diseases such as neurodegenerative disorders, cardiovascular diseases, and cancer (Schieber & Chandel 2014). Excessive ROS can also damage DNA, proteins, and lipids, and impair mitochondrial function, further increasing ROS production (Schieber & Chandel 2014).

Mitochondria are essential organelles, often referred to as the "powerhouses" of the cell due to their critical role in energy production. They also play roles in ROS generation (Cadenas & Davies 2000) and calcium and iron ion homeostasis (Bawa & Abbott 2008; Upadhyay & Agarwal 2020). Mitochondria are highly dynamic, changing size and shape through fission and fusion and being actively transported within the cytoplasm via motor proteins over the cytoskeleton (Agarwal & Ganesh 2020). Their spatial and temporal positioning meets the functional needs of the cell (Saxton & Hollenbeck 2012). For example, mitochondria can be found at the subplasmalemma during calcium ion deprivation but at the perinuclear space during embryonic development, viral infection, endoplasmic reticulum stress, hypoxia, and apoptosis (Agarwal & Ganesh 2020). Under heat stress, mitochondria may redistribute to regions of the cell that require more energy or are experiencing greater stress, such as near the nucleus or specific subcellular locations where repair processes are active. In this study, we analyze the distribution of mitochondria in eukaryotic cells under heat stress. By examining mitochondrial distribution, we can detect whether a cell is heat-stressed.

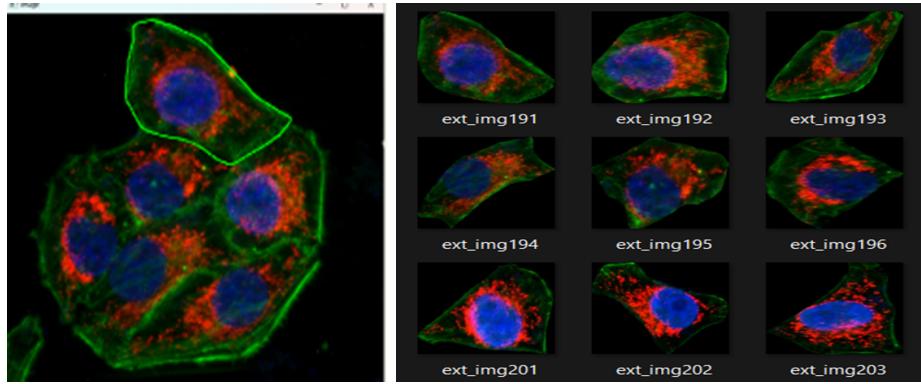
Understanding the distribution of mitochondria in cells is crucial, as mitochondrial dysfunction is linked to various diseases, including metabolic disorders, neurodegenerative diseases, and aging (Orrenius et al. 2007). Measuring mitochondrial activity can help characterize cancer metabolism patterns (Annesley & Fisher 2019), identify metabolic vulnerabilities (Javadov et al. 2020), and discover new drug targets. Maintaining ROS levels is essential for cell signaling and mitigating disease processes (Schieber & Chandel 2014). Heat stress affects mitochondrial distribution by influencing mitochondrial dynamics, transport, and localization. These changes help cells adapt to increased energy demands and stress conditions, supporting cellular survival and function. However, prolonged or severe heat stress can overwhelm these adaptive mechanisms, leading to mitochondrial dysfunction and cellular damage. Understanding these processes is critical for developing strategies to protect cells from heat-induced damage, particularly in the context of diseases, environmental stressors, and climate change.

3 Methodology

3.1 Data Acquisition and Preprocessing

Each confocal microscopy image was standardized to ensure uniformity in cell size and bit depth across the dataset. This initial step involved obtaining a single cell per image, ensuring consistency in experimental conditions. To facilitate accurate analysis, images were cropped interactively with user input, focusing on selecting individual cells of interest within the acquired images.

Figure 1: Cropped Images



3.2 Cell wall, Nucleus and its centre of mass detection

In the methodology employed for nucleus detection and foreground-background separation, an HSV (Hue, Saturation, Value) color space transformation was utilized to distinguish between foreground (cellular structures) and background (surrounding area) within confocal microscopy images. Initially, black pixels were excluded using a threshold mask applied to the value channel of the HSV image, ensuring focus on non-black pixels that primarily represent cellular structures. Subsequently, K-means clustering was applied to these selected pixels, dividing the image into three clusters based on pixel intensity similarities. For nucleus detection, the innermost cluster was identified by examining each pixel's neighborhood to determine its cluster affiliation. Pixels belonging to different clusters were marked as boundary pixels, and the cluster with the fewest boundary pixels was designated as the innermost cluster, indicating minimal influence from neighboring clusters. The center of mass for this cluster was then calculated using weighted averaging of pixel coordinates, providing precise centroid coordinates (x, y) for the nucleus.

Conversely, the outermost cluster, representing the cell boundary, was determined based on the cluster with the highest count of boundary pixels, indicating its distinct separation from neighboring clusters. This approach ensured accurate nucleus detection and delineation of cellular boundaries, critical for subsequent mitochondrial distribution analysis under heat stress conditions.

Figure 2: Original and clustered image of cell

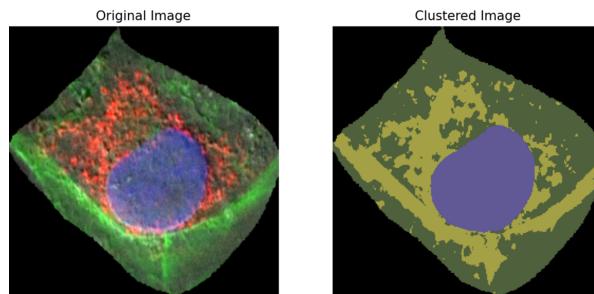
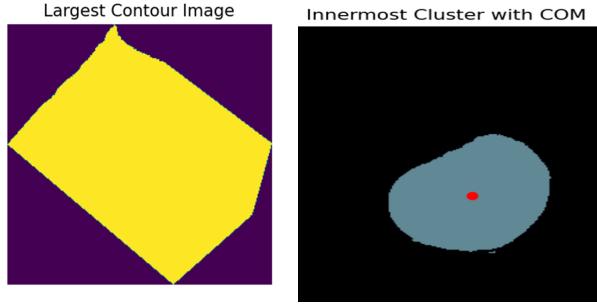


Figure 3: Cell wall and Nucleus with its center of mass



3.3 Dividing the cell into regions wrt nucleus

In the Angular Lines Analysis phase of the methodology, radial lines were systematically drawn outward from a specified center point, typically the centroid of a relevant cluster such as the nucleus or a segmented cell region. These lines extended evenly across a circular range, aiming to capture spatial variations in mitochondrial distribution within the cell.

The next step involved detecting intersections between these angular lines and the contours present in the image, which could include either the largest outer contour or boundaries of inner clusters like the nucleus. This process was carried out by iterating through each line segment defined by consecutive endpoints and assessing potential intersections with contour edges using computational geometry principles.

When valid intersections were identified within the defined contour bounds, the coordinates of these intersection points were recorded. Typically, the endpoint of each angular line served as a default intersection point unless a valid intersection was detected along the line segment.

To further refine the spatial analysis, the length between pairs of intersection points along each line segment was divided into three equidistant points, labeled as p1, p2, and p3. These points were strategically chosen to capture key spatial characteristics and were subsequently connected using a closed polyline, effectively outlining the distinct regions of interest within the cell. This approach facilitated a detailed examination of mitochondrial distribution patterns relative to cellular structures under heat stress conditions, enhancing the study's ability to discern spatial relationships and dynamics crucial for understanding cellular responses to environmental stressors.

Figure 4: Angular lines and intersection points with contour

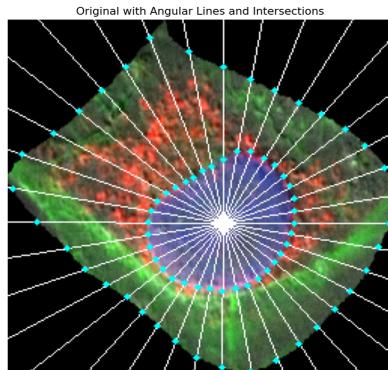


Figure 5: Intersection points with nucleus and cell boundary and length between the endpoints

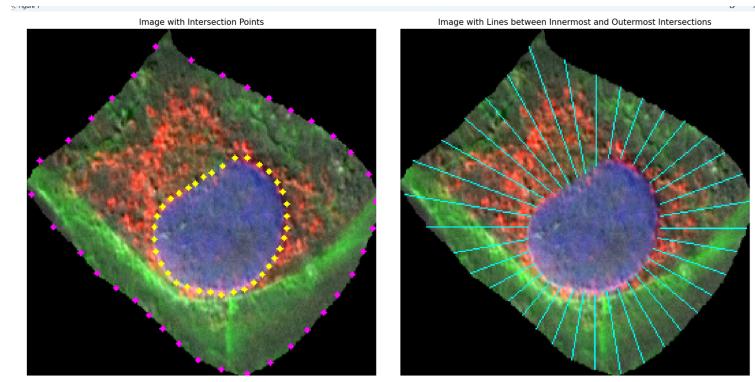


Figure 6: The endpoints of line divided into three points(p_1, p_2, p_3) at equal distance wrt each other

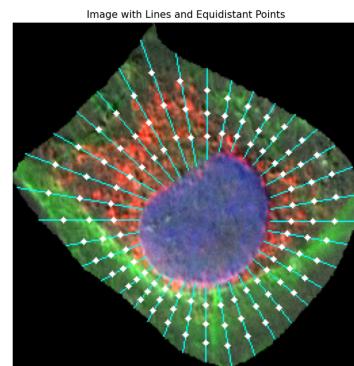
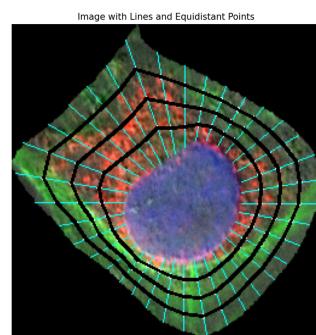


Figure 7: Polygon connecting a point on each line on basis of proximity from COM



4 Discussion

The analysis of mitochondrial distribution provides valuable insights into cellular organization and adaptation mechanisms. Our study confirms the feasibility of dividing the cell into distinct regions with respect to the nucleus, supporting the accurate definition of perinuclear, central, and peripheral areas. This precise segmentation allows for detailed examination of mitochondrial clustering patterns, which is crucial for understanding cellular dynamics and functions.

The methodological advancements presented in this study, including the integration of K-means clustering and angular line analysis, offer a detailed and accurate framework for examining cellular structures and their spatial relationships. By dividing the cell into multiple regions and capturing 3D distribution patterns, our approach surpasses traditional density-based analyses, providing a more nuanced understanding of mitochondrial dynamics.

One of the key achievements is the ability to detect distinct spatial relationships between mitochondria and the nucleus, revealing the organizational structure of cells. This has broad applications in understanding mitochondrial behavior in various pathological conditions, such as neurodegenerative diseases and cancer, where mitochondrial dysfunction plays a critical role. Additionally, our methodology can be adapted for other cellular structures and conditions, further extending its utility.

Future work can build on this study by employing advanced techniques such as Parzen estimation and DBSCAN for more sophisticated distribution analysis, enhancing the accuracy and depth of mitochondrial distribution studies. Moreover, exploring the functional implications of observed distribution patterns, such as their impact on cellular metabolism and signaling pathways, will provide deeper insights into the role of mitochondria in cell biology.

5 Conclusion

This study successfully developed and applied a robust methodology for analyzing mitochondrial distribution in eukaryotic cells using confocal microscopy images. By leveraging K-means clustering, contour detection, and angular line analysis, we achieved precise segmentation and delineation of cellular structures, including the nucleus and cell boundary. Our innovative approach divided the cell into distinct regions—perinuclear, central, and peripheral—accurately with respect to the nucleus. This methodological framework provides a comprehensive examination of mitochondrial clustering patterns, enabling detailed spatial analysis.

Overall, this research contributes significantly to the field of cell biology, offering a robust and versatile tool for analyzing mitochondrial distribution. It provides a foundation for future studies aimed at understanding cellular organization and disease mechanisms, facilitating advancements in biomedical research and therapeutic strategies.

References

- Agarwal, Saloni & Subramaniam Ganesh. 2020. Perinuclear mitochondrial clustering, increased ros levels, and hif1 are required for the activation of hsf1 by

- heat stress. *Journal of cell science* 133(13). jcs245589.
- Annesley, Sarah J. & Paul R. Fisher. 2019. Mitochondria in health and disease. *Cells* 8(7). <https://doi.org/10.3390/cells8070680>. <https://www.mdpi.com/2073-4409/8/7/680>.
- Bawa, Bhupinder & Louise C Abbott. 2008. Analysis of calcium ion homeostasis and mitochondrial function in cerebellar granule cells of adult ca v 2.1 calcium ion channel mutant mice. *Neurotoxicity research* 13. 1–18.
- Belhadj Slimen, Imen, Taha Najar, Abdeljelil Gham, Hajar Dabbebi, Moncef Ben Mrad & Manef Abdربbah. 2014. Reactive oxygen species, heat stress and oxidative-induced mitochondrial damage. a review. *International journal of hyperthermia* 30(7). 513–523.
- Cadenas, Enrique & Kelvin JA Davies. 2000. Mitochondrial free radical generation, oxidative stress, and aging. *Free radical biology and medicine* 29(3-4). 222–230.
- Javadov, Sabzali, Andrey V. Kozlov & Amadou K. S. Camara. 2020. Mitochondria in health and diseases. *Cells* 9(5). <https://doi.org/10.3390/cells9051177>. <https://www.mdpi.com/2073-4409/9/5/1177>.
- Kantidze, OL, AK Velichko, AV Luzhin & SV Razin. 2016. Heat stress-induced dna damage. *Acta Naturae* () 8(2 (29)). 75–78.
- Kovats, R Sari & Shakoor Hajat. 2008. Heat stress and public health: a critical review. *Annu. Rev. Public Health* 29. 41–55.
- Martin, Jörg, Arthur L Horwich & F Ulrich Hartl. 1992. Prevention of protein denaturation under heat stress by the chaperonin hsp60. *Science* 258(5084). 995–998.
- Orrenius, Sten, Vladimir Gogvadze & Boris Zhivotovsky. 2007. Mitochondrial oxidative stress: implications for cell death. *Annu. Rev. Pharmacol. Toxicol.* 47. 143–183.
- Park, HG, SI Han, SY Oh & HS Kang. 2005. Cellular responses to mild heat stress. *Cellular and Molecular Life Sciences Cmls* 62. 10–23.
- Saxton, William M & Peter J Hollenbeck. 2012. The axonal transport of mitochondria. *Journal of cell science* 125(9). 2095–2104.
- Schieber, Michael & Navdeep S Chandel. 2014. Ros function in redox signaling and oxidative stress. *Current biology* 24(10). R453–R462.
- Upadhyay, Mamta & Saloni Agarwal. 2020. Ironing the mitochondria: Relevance to its dynamics. *Mitochondrion* 50. 82–87.
- Yang, Lin, Gao-Yi Tan, Yu-Qiang Fu, Jin-Hai Feng & Min-Hong Zhang. 2010. Effects of acute heat stress and subsequent stress removal on function of hepatic mitochondrial respiration, ros production and lipid peroxidation in broiler chickens. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 151(2). 204–208.