RELOADING THE CONFOCAL DATA MATRIX: QUANTITATIVE CHARACTERIZATION OF ORGANIC AND BIOLOGICAL ASSEMBLIES BY OPTICAL SECTIONING OF SAMPLES

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by

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CERTIFICATE

This is to certify that the thesis entitled "**Reloading the Confocal Data Matrix: Quantitative Characterization of Organic and Biological Assemblies by Optical Sectioning of Samples**" being submitted by **Ms. Pragya (Entry No. 2017BLZ8282)** to the Indian Institute of Technology, Delhi, for the award of the degree of Doctor of Philosophy is a record of bonafide research work carried out by her, which has been prepared under my supervision and guidance in conformity with the rules and regulations of "Indian Institute of Technology, Delhi". The research reports and the results presented in this thesis has not been submitted in part or full to any other University or Institute for the award of any degree or diploma.

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ABSTRACT

The visualization of cells has significantly advanced since Robert Hooke's first observation of cork cells in the 1600s using a compound light microscope. This led to identification of cells as the basic structural and functional units of life. The mid-20th century saw the advent of fluorescence microscopy (1950s) and confocal microscopy (1970s), which enhanced imaging capabilities by allowing optical sectioning and improving the signal-to-noise ratio. Confocal microscopy, especially, has become a go-to technique for visualizing live cells in their native hydrated state due to its ability to generate three-dimensional data matrices through optical sectioning. However, more often than not the confocal microscopy has only been used as a 2D tool to increase signal:noise ratio in the images. These advancements in light microscopy have allowed researchers to delve deeper into cellular heterogeneity, recognizing that variations within cell populations are not merely noise but are critical for proper cellular function. This work utilizes confocal microscopy as a 3D tool to study, quantitatively characterize, various organic and biological assemblies leading to defining 3D innate morphological heterogeneity in cells at whole cell level and at the level of subcellular components with respect to their distribution within the cells' confinement.

The study begins with an examination of pseudopeptosome, pseudopeptide self-assemblies, in controlled chemical systems to minimize heterogeneity that might affect structural dynamics and then extends to studying morphological variations in RAW264.7 cells in different conditions.

The cells have been studied in their native condition and after synchronization at the G2/M phase. Synchronization reduces overall morphological heterogeneity, giving cells a common purpose of division, allowing for a focused study by minimizing variations in morphology of the whole cell and subcellular component distribution. Furthermore, the study investigates the effects of osmolarity on cellular morphology and organelle distribution. Despite extensive literature on osmolarity, a systematic study quantifying its impact on subcellular organelle distribution and cellular pleomorphism has been lacking. As opposed to the popular belief that the hyperosmolarity increases pleomorphism and hypoosmolarity decrease it, with the help of quantitative image analysis of morphometric parameters,we report that both the conditions decrease morphological heterogeneity in the population. We further report the organelles in

which the whole cell heterogeneity has been translated into. From our repertoire of 7 subcellular components, we report ER, mitochondria, and tubulin to be independent of whole-cell apico-basal heterogeneity of optical density while nuclear, plasma membrane, lysosomal, and actin fluorescence distributions are found to contribute to the apico-basal polarity of the whole cell. We report that the variation in trends of the parameters, related to extracellular osmolarity, observed at whole-cell level are translated into actin and tubulin parameter variations, while nucleus, mitochondria, and endoplasmic reticulum are independent of the whole cell morphology alterations. While doing so, we have also developed an image analysis algorithm utilizing 2D segmentation to analyze the single cells in 3D using confocal microscopy - a technique that allows us to analyze cellular states in their native hydrated state.

सार

रॉबर्ट हुक द्वारा 1600 के दशक में यौगिक प्रकाश सूक्ष्मदर्शी का उपयोग करके कॉर्क कोशिकाओं का पहला अवलोकन करने के बाद से कोशिकाओं के दृश्यीकरण में उल्लेखनीय प्रगति हुई है। इससे कोशिकाओं को जीवन की मौलिक संरचनात्मक और कार्यात्मक इकाइयों के रूप में पहचाना गया। 20वीं सदी के मध्य में प्रतिदीप्ति सूक्ष्मदर्शी (1950 के दशक) और कन्फोकल सूक्ष्मदर्शी (1970 के दशक) का आगमन हुआ, जिसने इमेजिंग क्षमताओं को ऑप्टिकल सेक्शनिंग की अनुमति देकर और सिग्नल-टू-शोर अनुपात में सुधार करके और भी उन्नत बनाया। विशेष रूप से, कन्फोकल सूक्ष्मदर्शी जीवित कोशिकाओं को उनके मूल हाइड्रेटेड अवस्था में देखने के लिए एक प्रमुख तकनीक बन गई है, क्योंकि यह ऑप्टिकल सेक्शनिंग के माध्यम से त्रि-आयामी डेटा मैट्रिक्स उत्पन्न कर सकती है। हालांकि, कन्फोकल सूक्ष्मदर्शी का अक्सर केवल 2D इमेजिंग उपकरण के रूप में उपयोग किया गया है, जिससे छवियों में सिग्नल: शोर अनुपात में सुधार हो सके। प्रकाश सूक्ष्मदर्शी में ये प्रगति शोधकर्ताओं को कोशिकीय विविधता में और गहराई से जाने में सक्षम बनाती है, जिससे यह समझा जा सके कि कोशिकाओं की आबादी में अंतर केवल शोर नहीं है, बल्कि उचित कोशिकीय कार्यों के लिए महत्वपूर्ण है।

यह अध्ययन कन्फोकल सूक्ष्मदर्शी का 3D उपकरण के रूप में उपयोग करता है ताकि विभिन्न जैविक और कार्बनिक संरचनाओं का अध्ययन किया जा सके और कोशिकाओं में 3D आंतरिक रूपात्मक विविधता का मात्रात्मक लक्षण वर्णन किया जा सके, संपूर्ण कोशिका स्तर और उपकोशिकीय घटकों के वितरण के संदर्भ में।

अध्ययन की शुरुआत स्यूडोपेप्टोसोम (स्यूडोपेप्टाइड स्व-असेंबली) की जांच से होती है, जिसमें संरचनात्मक गतिशीलता को प्रभावित करने वाली विविधता को कम करने के लिए नियंत्रित रासायनिक प्रणालियों का उपयोग किया जाता है। इसके बाद विभिन्न स्थितियों में RAW264.7 कोशिकाओं में रूपात्मक भिन्नताओं का अध्ययन किया गया। कोशिकाओं का अध्ययन उनकी सामान्य स्थिति में और G2/M चरण पर सिंक्रोनाइज़ेशन के बाद किया गया है। सिंक्रोनाइज़ेशन समग्र रूपात्मक विविधता को कम करता है, जिससे सभी कोशिकाओं का एक समान विभाजन उद्देश्य बनता है, और इस प्रकार कोशिका और उपकोशिकीय घटक वितरण में विविधता को कम करके अध्ययन को अधिक केंद्रित बनाता है। इसके अतिरिक्त, अध्ययन परासरणीयता (osmolarity) के प्रभावों का भी निरीक्षण करता है, जो कोशिकीय आकृति और अंगक (organelle) वितरण को प्रभावित करता है।

परासरणीयता पर व्यापक साहित्य के बावजूद, उपकोशिकीय अंगक वितरण और कोशिकीय रूपात्मक विविधताओं पर इसके प्रभाव को व्यवस्थित रूप से मापने वाला अध्ययन अब तक अनुपस्थित था। प्रचलित विश्वास के विपरीत, कि हाइपरऑस्मोलारिटी से प्लियोमोर्फिज़्म बढ़ता है और हाइपोऑस्मोलारिटी इसे घटाती है, हमने मात्रात्मक छवि विश्लेषण के माध्यम से मॉर्फोमेट्रिक मापदंडों का उपयोग करते हुए पाया कि दोनों ही स्थितियों में कोशिका समूह में रूपात्मक विविधता कम हो जाती है। हमने आगे यह रिपोर्ट किया है कि किस उपकोशिकीय अंगक में यह विषमता परिलक्षित होती है।

हमारे 7 उपकोशिकीय घटकों के अध्ययन में हमने यह पाया है कि एंडोप्लाज्मिक रेटिकुलम (ER), माइटोकॉन्ड्रिया, और ट्यूबुलिन संपूर्ण कोशिका की एपिको-बेसल ऑप्टिकल घनत्व विषमता से स्वतंत्र हैं, जबकि न्यूक्लियस, प्लाज्मा मेम्ब्रेन, लाइसोसोम, और एक्टिन का वितरण संपूर्ण कोशिका की एपिको-बेसल ध्रुवीयता में योगदान करता है।

हम यह भी रिपोर्ट करते हैं कि संपूर्ण कोशिका के स्तर पर देखी गई परासरणीयता से संबंधित मापदंडों में बदलाव को एक्टिन और ट्यूबुलिन के मापदंडों में परिलक्षित किया गया है, जबकि न्यूक्लियस, माइटोकॉन्ड्रिया, और

एंडोप्लाज्मिक रेटिकुलम संपूर्ण कोशिका की आकृति-विज्ञान में हुए परिवर्तनों से अप्रभावित हैं। साथ ही, हमने 2D विभाजन का उपयोग करते हुए एक इमेज एनालिसिस एल्गोरिदम विकसित किया है, जो कन्फोकल माइक्रोस्कोपी का उपयोग करके 3D में एकल कोशिकाओं का विश्लेषण करने की क्षमता रखता है, जिससे कोशिकाओं का अध्ययन उनकी प्राकृतिक हाइड्रेटेड अवस्था में किया जा सकता है।

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ABBREVIATIONS

TEM: Transmission Electron Microscopy

SEM: Scanning Electron Microscopy

EM: Electron Microscopy

STED: Stimulated emission depletion

PBS: Phosphate Buffered Saline

NaCl: Sodium Chloride

HBSS: Hank's Balanced Salt Solution

PMT: Photomultiplier Tube

DIC: Differential Interference Contrast

ROI: Region of Interest

NR: Nile Red

RB: Rhodamine B

Trp: Tryptophan

CorTS1: Corneal Targeting Sequence 1

APV: Average Pixel Values

TPN: Total Pixel Number

3D: 3 Dimensional

CSE: Cell Surface Excesses

ER: Endoplasmic Reticulum

ATOM: Asymmetric-detection Time-stretch Optical Microscopy

DAPI: 4′,6-diamidino-2-phenylindole

DCIS: Ductal Carcinoma in situ

DIC: Differential interference contrast

DNA: Deoxyribonucleic acid

DOPC: 1,2-Dioleoyl-sn-glycero-3-phosphocholine

ESC: Embryonic Stem Cells

FB: Fibroblasts

GUV: Gaint Unilamellar Vesicles

HASM: Human Airway Smooth Muscle

HDF: Human Dermal Fibroblasts

HMEC: Human Mammary Epithelial Cells

HMF: Human Mammary Fibroblasts

HTDIC: Hilbert Transform Differential Interference Contrast

iPSC: induced Pleuripotent Stem Cells

MSC: Mesenchymal stem cells

NHDF: Normal human dermal fibroblasts

NIQPM: Non-Interferometric Quantitative Phase Microscopy

NO: Nitric oxide

PDAC: Pancreatic ductal adenocarcinoma

PSC: Pleuripotent Stem Cells

QPM: Quantitative Phase microscopy

RBC: Red Blood Cells

RCM: Reflectance Confocal microscopy

RNA: Ribonucleic Acid

SEFC: Spectrally Encoded Flow Cytometry

SLIM: Spatial light interference microscopy

TF: Transcription Factor

TIR: Total Internal Reflection

VAMPIRE: Visually-Aided Morpho-Phenotyping Recognition