Detection of Lipid Droplets-Mitochondria Contacts through Fluorescence Microscopy and Image Analysis

Gemma Martín[1], Marta Bosch[2], Elisenda Coll[1], Albert Pol[2,3,4] and Maria Calvo[1,2]

Introduction

Lipid droplets (LD) are the major lipid storage organelles of eukaryotic cells. They **are involved in cell metabolism, signaling and the building of organelle membranes.**

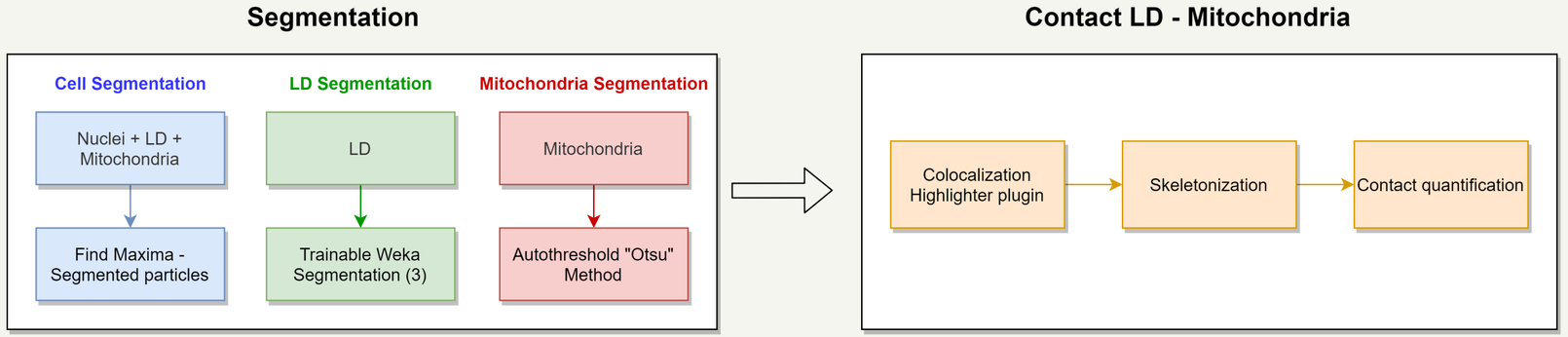
In order to achieve all their functions, **LDs communicate with other organelles** (Endoplasmic Reticulum, LD, Mitochondria, Peroxisomes and Vacuoles) forming extensive contacts to transfer material, mainly proteins and lipids. Contacts between these organelles have been described and characterized by Transmission Electron Microscopy (TEM) as it resolves at the membranes scale where these contacts take place. Nevertheless, this characterization by TEM can only be done for a few cells. **By using fluorescence microscopy and image analysis this characterization can be extended to hundreds of cells.**

Aim

Fluorescence Image analysis allows to segment specifically LD and organelles and quantify their “possible contact” regions, cell by cell, and to obtain cellular population data by the analysis of numerous cells and conditions. **In this work, we describe an image analysis method to identify contacts between LD and mitochondria, and to quantify and express them relatively to these organelles’ morphometry.**

Method

The combination of **standard and machine learning segmentation processes** and the novelty of using **colocalization methods together with skeletonization** allows the automated detection of LD-Mitochondria contacts from a large number of fluorescence images. The following **workflow** was automated in an ImageJ macro script (1,2),



This workflow was used over a set of several confocal images from fluorescently labeled HEK293 cells (Mitochondria (TOM20), LD (BODIPY), PLIN5 and nuclei (DAPI)), acquired to analyze contacts between mitochondria and LD under lipopolysaccharide treatment (LPS), a bacterial component activator of the innate immunity, and PLIN5 expression, a LD-mitochondria tethering protein (4).

Affiliations

1. Advanced Optical Microscopy Facility. Scientific and Technological Centers. University of Barcelona.
2. Department of Biomedical Sciences, Faculty of Medicine, Universitat de Barcelona, 08036, Barcelona, Spain
3. Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010, Barcelona.
4. Cell Compartments and Signaling Group, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), 08036, Barcelona, Spain.

SCAN ME

github.com/UB-BioMedMicroscopy

Detection of Lipid Droplets-Mitochondria Contacts through Fluorescence Microscopy and Image Analysis

Gemma Martín[1], Marta Bosch[2], Elisenda Coll[1], Albert Pol[2,3,4] and Maria Calvo[1,2]

Results

The novel use of skeletonization combined with colocalization methods allows the automatic quantification of LD-Mitochondria contacts together with a complete characterization of cell and organelles parameters (such as perimeter, area, distribution and shape descriptors), in different cell populations.

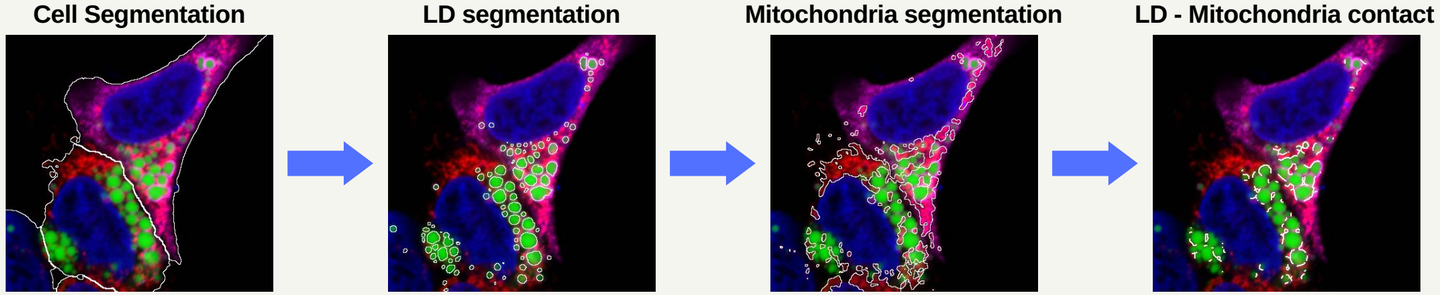


Figure: *LD (green), Mitochondria (red) ,Nuclei (Blue) and Plin5-Flag tag (purple) (4)*

Conclusions

The described image analysis workflow unveils a wide range of possibilities in the **automatic quantification of LD contacts with other organelles**, allowing the normalization and correlation with multiple morphometric parameters of the cell.

Moreover, its application in a large number of fluorescence images enables the use of HCS and HCA, **highly increasing the quality and statistical confidence** of the results (4).

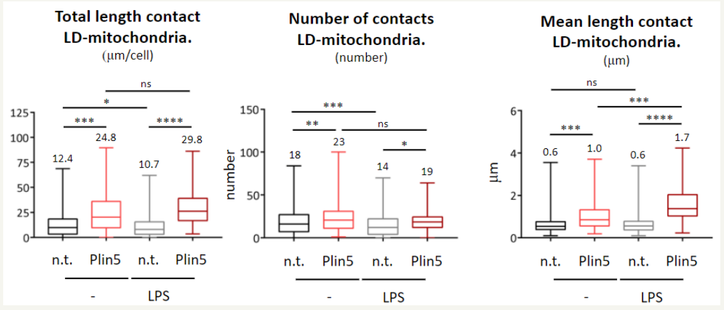


Figure: *Published results obtained using the novel method presented through fluorescence microscopy and image analysis. LPS treatment reduced contacts between LDs and mitochondria, while PLIN5 expression increase LD-mitochondria contacts, even in the presence of LPS(4).*

Acknowledgements

We acknowledge NEUBIAS Cost Action NEUBIAS - COST Action CA15124 www.neubias.org for Image Analysis courses, tutorials and repositories and their important task in training Image Analysts. This work is part of the published article (4).

References

1. Schneider, C. A.; Rasband, W. S. & Eliceiri, K. W. (2012), "NIH Image to ImageJ: 25 years of image analysis", Nature methods 9(7): 671-675, PMID 22930834 (on Google Scholar).
2. Schindelin, J.; Arganda-Carreras, I. & Frise, E. et al. (2012), "Fiji: an open-source platform for biological-image analysis", Nature methods 9(7): 676-682, PMID 22743772, doi:10.1038/nmeth.2019
3. Arganda-Carreras, I.; Kaynig, V. & Rueden, C. et al. (2017), "Trainable Weka Segmentation: a machine learning tool for microscopy pixel classification.", Bioinformatics (Oxford Univ Press) 33 (15), PMID 28369169, doi:10.1093/bioinformatics/btx180 (on Google Scholar).
4. M. Bosch et al., “Mammalian lipid droplets are innate immune hubs integrating cell metabolism and host defense,” Science, 370:eaay8085, 2020.

Contact information

mariacalvo@ub.edu (MC)
martabosch@ub.edu (MB)
apols@ub.edu (AP)

SCAN ME

github.com/UB-BioMedMicroscopy