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Microscopy pixel classification of intracellular sites of triglycerides and cholesteryl esters formation and storage through a machine-learning assisted polarity-driven segmentation

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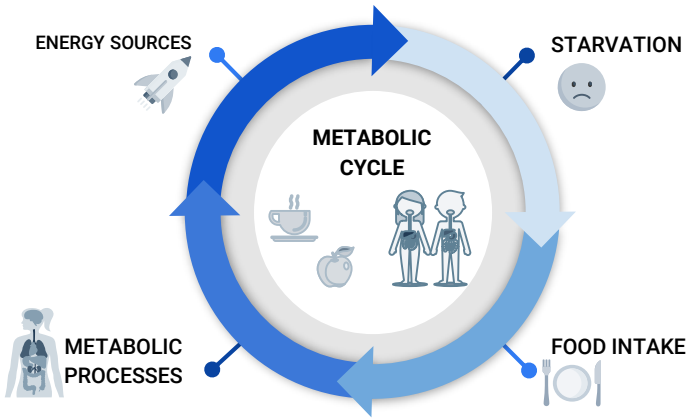
Introduction

Background:

- Lipid pathway consists of a complex network of metabolic reactions
- Impairment in metabolic cycle can promote neurodegeneration
- Non-polar (NP) lipids can accumulate in organelles other than lipid droplets (LD)
- Mechanisms of LD biogenesis are unclear

Open problem

⇨ Individuation and localization of LD formation sites



Aims

Develop a combined machine-learning polarity-driven segmentation workflow to

- Detect LD number and size with high selectivity
- Localize and quantify triglycerides/cholesteryl esters (TAG/CE) deposits

Materials and methods (I)

Sample preparation and image acquisition

1. Cell culture and differentiation

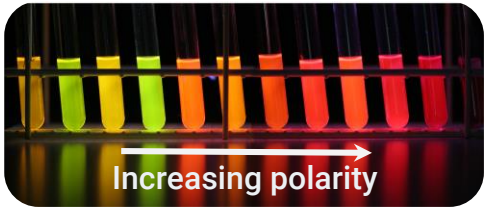
PC12 cells

Nerve Growth Factor (NGF)

2. Cell staining

Nile Red

- High specificity for LD
- Solvatochromic probe



3. Image acquisition

Confocal microscope

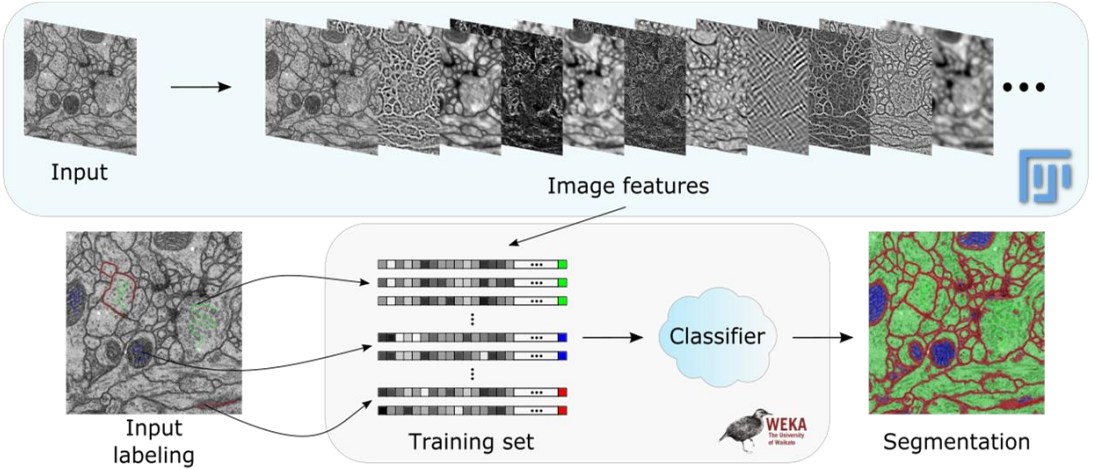
- Spectral detector
- Emission range: 498-684 nm
- 32 separated channels

Materials and methods (II)

Trainable Weka Segmentation (TWS)

- Based on pixel classification

1. Image features are extracted from an input image
2. A set of pixel samples is defined and represented as feature vectors
3. A WEKA learning scheme is trained on those samples
4. Finally it is applied to classify the remaining image data

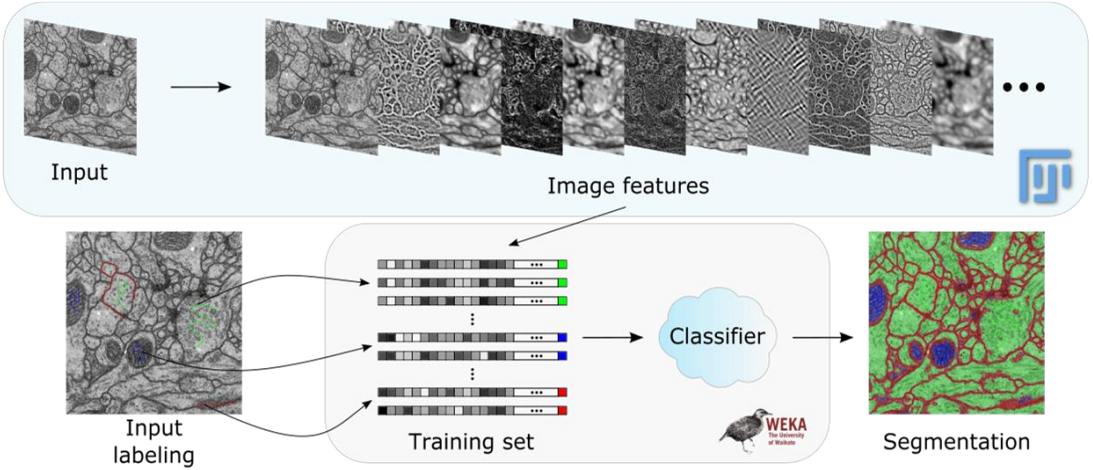


Bioinformatics, Volume 33, Issue 15, 01 August 2017, Pages 2424–2426, https://doi.org/10.1093/bioinformatics/btx180

Materials and methods (II)

Trainable Weka Segmentation (TWS)

- Based on pixel classification
  - Two classes: LD and background
  - Probability value: 90%



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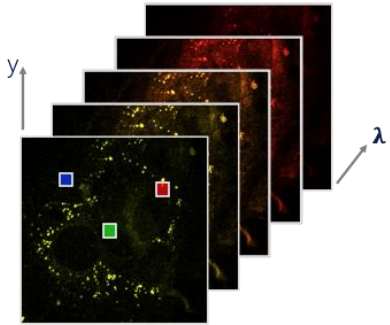
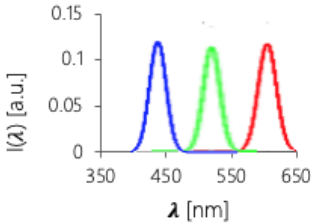
Materials and methods (III)

Spectral phasors – Polarity-driven Segmentation (PDS)

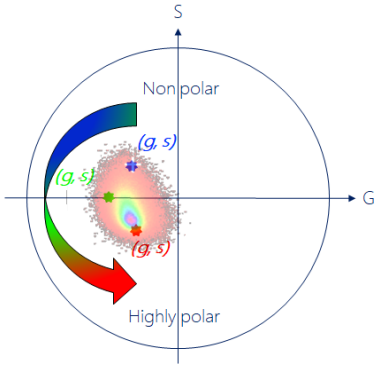
- Based on the Fourier representation of spectral properties
- Emission spectrum converted into a **phasor**, made up of two numbers: ***g*** and ***s***
- ***g*** and ***s*** are the coordinates in the phasor plot
- Remap regions of the phasor plot to the original fluorescence image
- Segmentation based on pixels with similar spectral properties





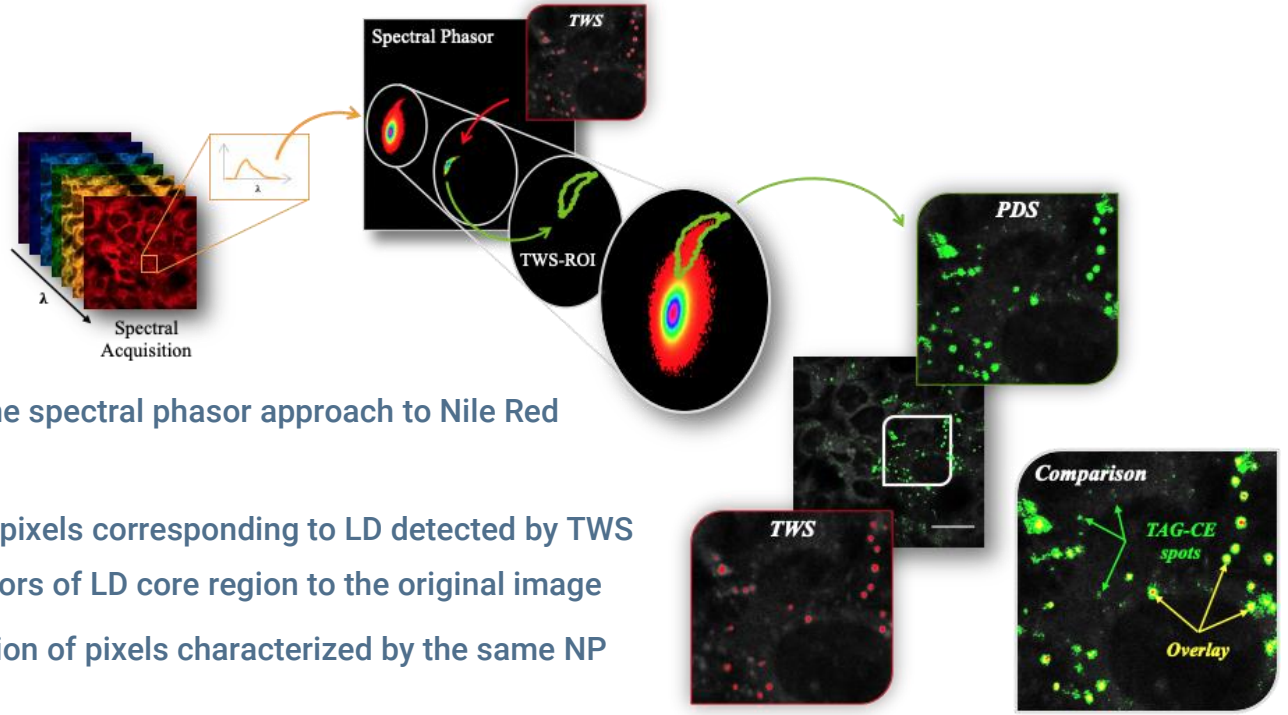
$$g = \frac{\sum_\lambda I(\lambda)\cos(2\pi n\lambda/L)}{\sum_\lambda I(\lambda)}$$

$$s = \frac{\sum_\lambda I(\lambda)\sin(2\pi n\lambda/L)}{\sum_\lambda I(\lambda)}$$



Results (I)

Detection of intracellular LD and other non-polar deposits

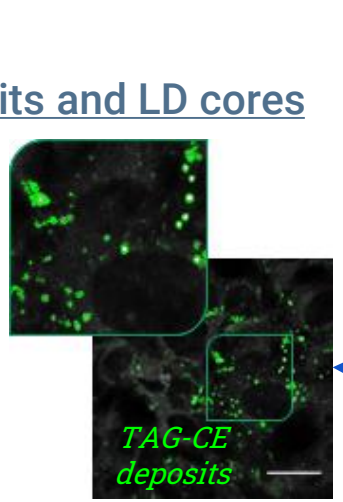


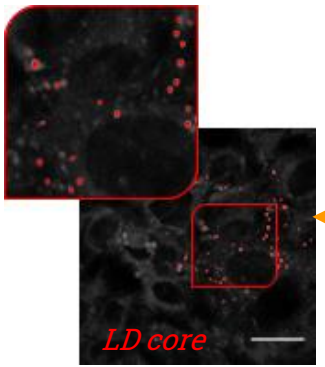
1. Application of the spectral phasor approach to Nile Red fluorescence
2. Selection of the pixels corresponding to LD detected by TWS
3. Remap the phasors of LD core region to the original image
4. Spatial localization of pixels characterized by the same NP spectrum

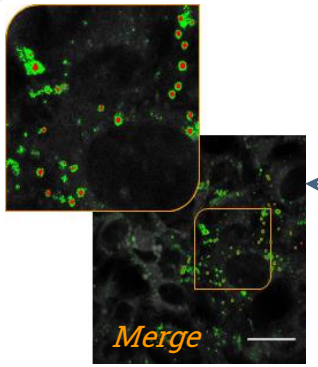
Results (II)

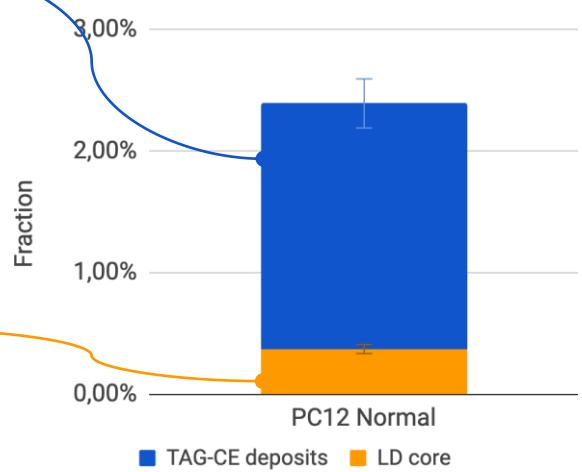
Discrimination of TAG/CE deposits and LD cores

1. The combination of TWS and PDS allows to distinguish LD core from other NP deposits
2. Definition of a Segmentation Quality Factor, *Q**
3. NP spots evaluated as the difference between PDS and TWS*
4. Quantification of LD cores and TAG/CE deposits in PC12 cells





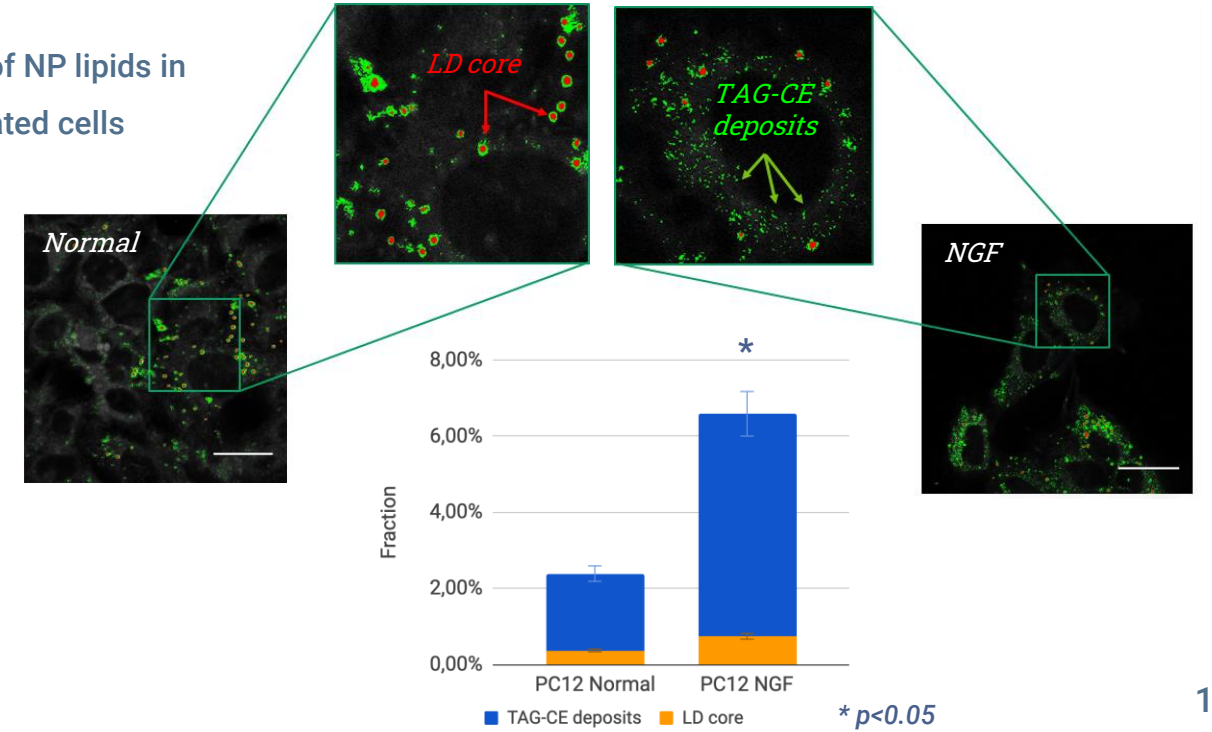




Results (III)

Effects of NGF treatment on PC12 lipid metabolism

- Different spatial distribution of NP lipids in undifferentiated and NGF-treated cells
- Treatment with NGF induces an increase of both TAG/CE deposits and LD
- Enhancement of the lipid turnover to support the reorganization of cells during differentiation



Conclusions, limits and future perspectives

- Improvement in the isolation of LD core through the application of a machine learning-based tool
- Ability to monitor in real-time the overall process of the TAG-CE turnover
- Validation of the method by testing changes in the level of activation of biosynthetic pathways in response to neuronal differentiation

  - ❑ Requirement of a spectral detector
  - ❑ Staining protocol

➢ Clinical application as a powerful personalized medicine tool to follow the progressive accumulation of NP lipids

References

- Bianchetti et al. (2019). ***Machine-learning assisted confocal imaging of intracellular sites of triglycerides and cholesteryl esters formation and storage***. Analytica Chimica Acta

Metabolic Functional Imaging

- Maulucci et al. (2008). *High-resolution imaging of redox signaling in live cells through an oxidation-sensitive yellow fluorescent protein*. Science Signaling
- Maulucci et al. (2009). *Investigation of the spatial distribution of glutathione redox-balance in live cells by using fluorescence ratio imaging microscopy*. Biosensors and Bioelectronics
- Maulucci et al. (2015). *Quantitative analysis of autophagic flux by confocal pH imaging of autophagic intermediates*. Autophagy

Spectral Phasors

- Maulucci et al. (2018). *Real time quantitative analysis of lipid storage and lipolysis pathways by confocal spectral imaging of intracellular micropolarity*. BBA - Molecular and Cell Biology of Lipids
- Di Giacinto et al. (2018). *Quantitative imaging of membrane micropolarity in living cells and tissues by spectral phasors analysis*. MethodsX

Thanks!

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DO YOU HAVE ANY QUESTIONS?

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