Structural study of lipid droplet using synchrotron label-free multimodal imaging

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STRUCTURAL STUDY OF LIPID DROPLET USING SYNCHROTRON LABEL-FREE MULTIMODAL IMAGING

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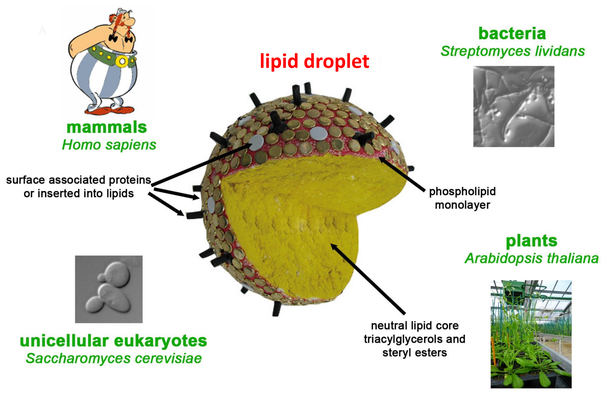
LIPID DROPLETS

Lipid droplet: a complex and dynamic organelle

In cells, neutral lipids (triglycerides TG and steryl esters SE) are stored in organelles called **lipid droplets (LD)** [1]. They are present in all organisms, from bacteria to plants and animals.



Lipid droplets: not well known but with rising interest

From biologists

- LD is not an inert fat depot but a **dynamic organelle** which regulates cell metabolism and signaling

From medical field

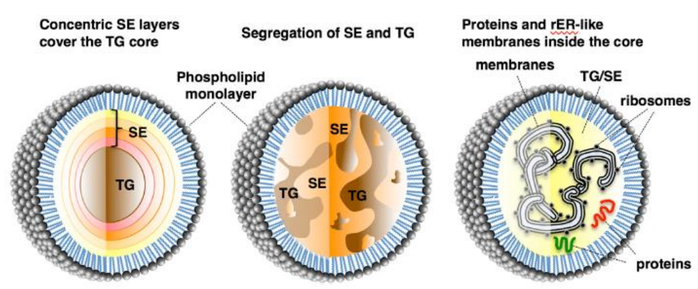
- LDs have a crucial role in **diseases with increasing prevalence** (obesity, diabetes) [2]
- **Oleosins** (from peanut and hazelnut), seed LD associated proteins are **allergens** [3].

From industrials

- **crushing**: oils for food and non food industries are extracted from seed LDs
- **food processing industry, cosmetic and health**: oleosins harbor interfacial properties and could be use as emulsifying agents or in drug delivery systems [4]

Lipid droplet structure under debate

Many fundamental questions about LD biology remain unanswered [5] . How LDs form and grow? How proteins move to and from LDs? How LDs are related to protein degradation? **How LD core is structured?**

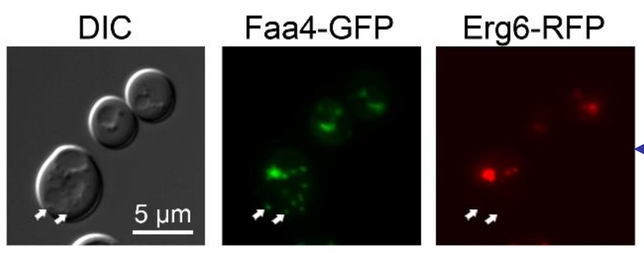


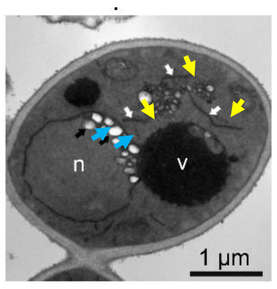
IMAGING LIPID DROPLETS IS NOT A TRIVIAL ISSUE

LD subpopulations in yeast

Various LD populations revealed using epifluorescence microscopy with tagged protein

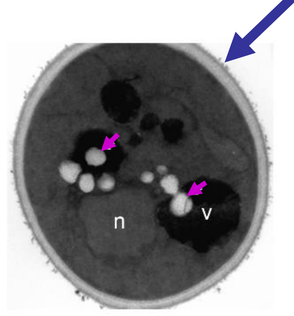
- with long chain fatty acyl-CoA synthetase Faa4p
- with more or less (white arrows) sterol-Δ24-methyltransferase Erg6p





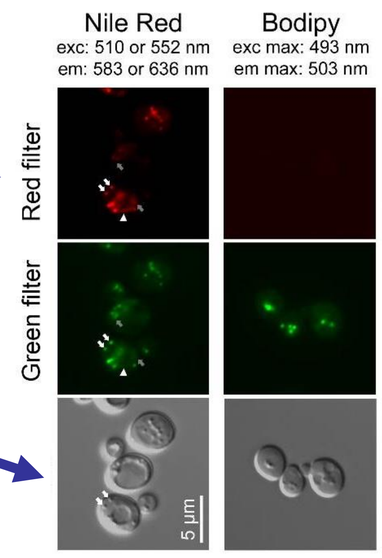
Various LD populations revealed using TEM

- Nucleus-associated (blue arrows)
- ER-associated (yellow arrows)
- Vacuole-associated (purple arrows)



Advantages and disadvantages of some LD imaging techniques

APPROACH	ADVANTAGES	DISADVANTAGES
Lipid probes	Living cells, fast and easy protocol, dynamic studies	Modified LDs and lipid environment
Proteins with fluorescent tag	Living cells, dynamic studies	Modified LDs, GMOs, non exhaustive labeling
AFM-IR	Label-free, lipid quantification	Dried cells
Electron microscopy	High resolution, visualisation of subcellular structures	Fixed cells, fastidious protocol
Soft X-ray microscopy	**30 nm resolution, Label-free**	Cryofixed cells, soft X-ray source and microscope
Differential Interference Contrast (DIC)	Living cells, visualisation of subcellular structures	structure identification
Raman (CARS, SRS)	Label-free, living cells, dynamic studies	Resolution, structure identification
Deep UV imaging	**Label-free, living cells, 110 nm resolution, dynamic studies**	DUV source and microscope



Various structures revealed using epifluorescence microscopy with lipid probes

- LDs (white arrows)
- Endoplasmic reticulum (white arrow head)
- Unidentified structures (grey arrows)

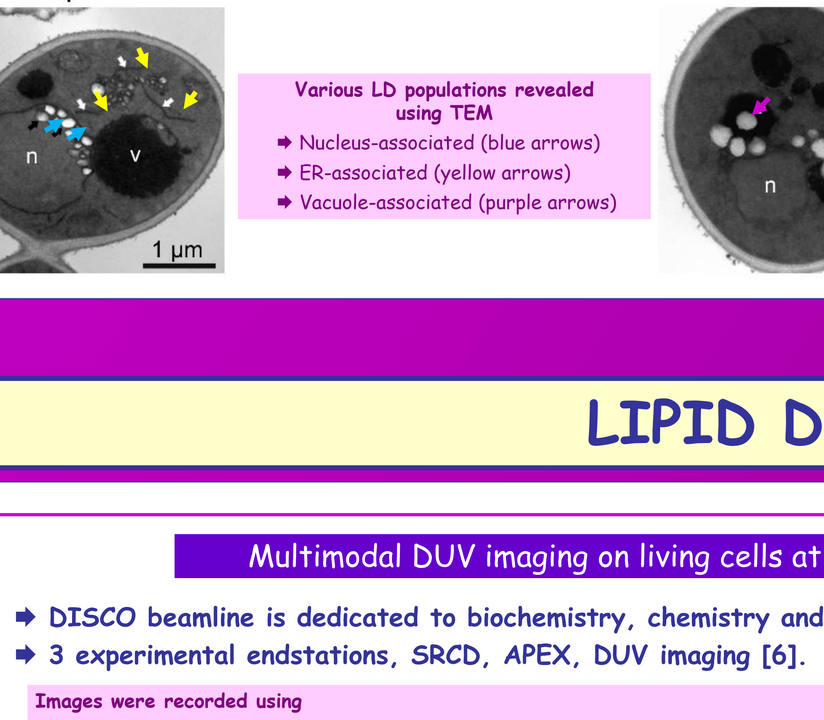
LIPID DROPLET LABEL-FREE IMAGING

Multimodal DUV imaging on living cells at Soleil Synchrotron

- **DISCO beamline is dedicated to biochemistry, chemistry and cell biology.**
- **3 experimental endstations, SRCD, APEX, DUV imaging [6].**

Images were recorded using

- TELEMOS, a full field, fully automatized inverted microscope, for fast imaging of live cells
- *sei1*Δ mutant cells with supersized LDs (1µm vs 250 nm for WT), containing TG and ES such as WT cells [7]



Step 1 : Identification of LDs (white arrow) using DPH, a vital neutral lipid probe with an excitation spectrum compatible with DUV (340 nm). LDs are low autofluorescent organelles.

Step 2: combination of autofluorescence and transmittance without lipid probe under 275 nm excitation. LDs (white arrows) show heterogeneous transmittance properties from surface to central core

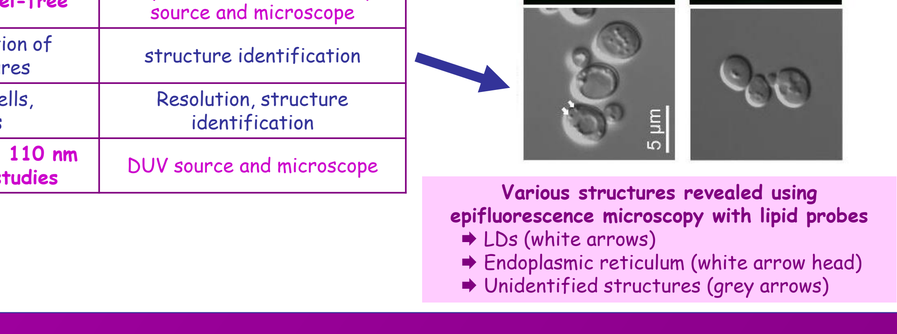
Step 3: LD structural analysis (slices and plot profile) revealed an concentric layer organization, with a low 1/TRANS central core and a high 1/TRANS (absorbance) external ring, which could correspond to ergosterol species (absorbance main peaks at 270 and 280 nm)

Soft X-ray imaging at Alba Synchrotron

- **MISTRAL beamline is devoted to cryo nano-tomography in the water window for biological applications [8].**

Images were recorded using

- Transmission X-ray Microscope (TXM), 270 eV to 1200 eV, cryo-temperature, rotation from -65° to +65°
- Mutant cells with LD contrasted content (TG triglycerides, SE steryl esters, TG+SE) [9]



LDs appear as black round structures corresponding to high absorbance structures.

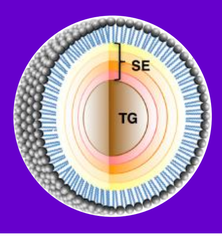
- TG+SE containing LDs and TG containing LDs are homogeneous (pink arrows)
- SE containing LDs appear as "donuts" with high absorbance material (SE) at the periphery and a central "hole"

CONCLUSION ON LD STRUCTURE

DUV fluorescence and absorbance on living cells + **Soft X-ray tomography**

LDs with contrasted signals

LD chemical heterogeneity



Our results are in favor of the concentric layer model

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