Lipid droplet 3D imaging in living single cell

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Standard Project

Experimental Report

Proposal title: **Lipid droplet 3D Imaging in living single cell**		**Proposal number:** 20140219
Beamline: DISCO	**Date(s) of experiment:** from: 18/09/2014 to: 21/09/2014 from: 10/04/2015 to: 12/04/2015	**Date of report:** 11/08/2015
Shifts: 15	**Local contact(s): Matthieu Réfrégiers; Frédéric Jamme**	*11/08/2015*

Objective & expected results (less than 10 lines):

In cell, oil is stored in lipid droplet (LD). LD and neutral lipid metabolism is abundantly documented but less is known about LD structure and dynamics in the cell. With this project we will obtain an exhaustive picture of the LD intracellular organization using multimodal approaches (fluorescence and absorbance). Deciphering the structure/function relationship between LD morphology and oil content dynamics will allow improving the quantity and the quality of oil produced by cells.

The main objectives of the proposal are:

1. To evaluate the presence of proteins in the central core of LD
2. To visualize the intracellular organization of LD in yeasts with contrasted oil content
3. To decipher the structure/function relationship between LD morphology and oil content dynamics with the aim to improve the quantity and the quality of oil produced by cells.
4. To obtain an exhaustive picture of the various LD intracellular organizations using multimodal approaches (Multiphoton and DUV imaging)

Results and the conclusions of the study (main part):

Biological material

Species	strain	Lipid droplet phenotype
Saccharomyces cerevisiae	WT	Small LD
Saccharomyces cerevisiae	*fld1*Δ	Supersized LD
Saccharomyces cerevisiae	*ino2*Δ	Supersized LD

Cells were grown 24h at 28°C in minimal medium (YNBG) or in complete medium (YPG) in the Biology 2 laboratory at SOLEIL synchrotron.

Lipid staining with Laurdan, TMA-DPH and DPH

500 µL of cell culture was incubated with 10 µM final concentration of 6-Dodecanoyl-2-Dimethylaminonaphthalene (Laurdan), 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) or 1,6-diphenyl-1,3,5-hexatriene (DPH). Incubations were performed 15 minutes at room temperature. Cells were washed once with PBS before observation. Cell suspensions (3 µL) were placed between two quartz slides.

Microscope configuration

TMA-DPH and DPH = excitation: 340 nm; Emission: DM410_420-480
Tryptophan fluorescence = excitation: 275 nm; Emission: DM300_327-353 (Z offset +1 µm)
Absorbance = excitation = 270 nm, Emission: DM5050_nofilter + 285/28

Results for the first session: 18-21 of september

We performed spectra on yeasts using Polypheme microscope. We obtained spectra but the bleaching effect

impairs the fine cartography of cells. Data at the subcellular level could not be obtain. This technique is not suitable for lipid droplets and organelles imaging. Neverthemess we recorded single cell spectra on yeats with lipid contrasted content but we did not see clear differences.

Then, we used the Telemos (full field) microscope for fluorescence protein imaging and lipid imaging after staining with Laurdan, TMA-DPH and DPH. We were not able to record images with Laurdan as the optimal excitation wavelength (360 nm) was out of range of the available dichroics mirrors (Max 340 nm) and a part of the signal was lost due to light polarisation (anisotropy devices on the beamline). Therefore, we observed contrasted staining with TMA-DPH and DPH. Z-stacks (200 nm or 500 nm) were acquired for 3D reconstruction. TMA-DPH stains mainly plamsa membranes whereas DPH stains the lipid droplets (Figure 1). We decided not to continue with i*no2* mutant as we obsverded multiple morphological defects in this strain which will impair image treatment and interpretation of results.

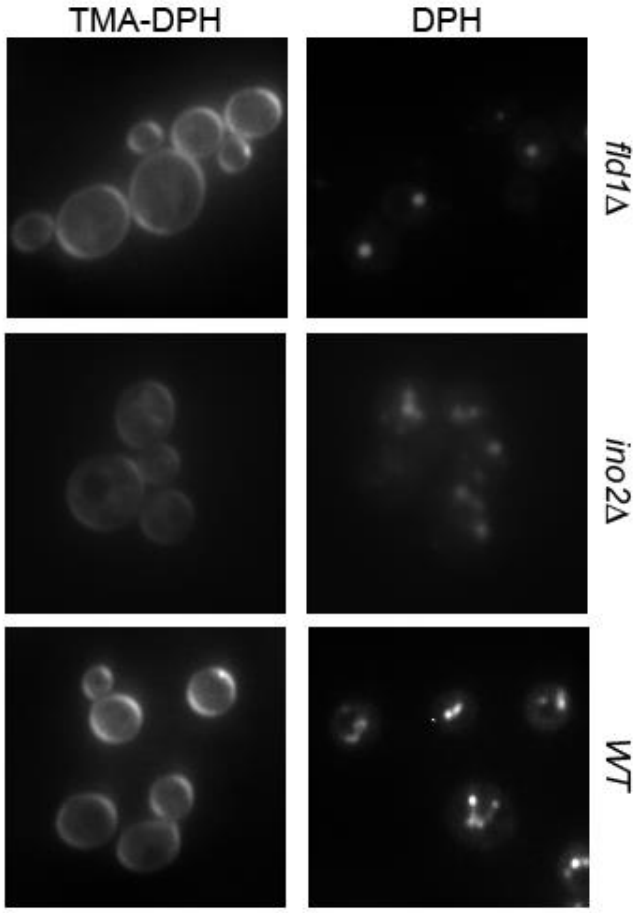

Figure 1 : yeasts stain with TMA-DPH (left panels) or DPH (right panels). TMA-DPH is mainly associated with plasma membrane but we observed unspecific fluorescence in the cytoplasm (cell autofluorescence?). DPH is mainly associated with round intracellular structures corresponding to lipid droplet. LD morphologies are different in all the strains with a unique LD in fld1, many and big LD in ino2 and many and small in WT.

We kept DPH stain to develop methodology combining LD staining and fluorescence imaging of proteins. We performed data acquisition on *fld1* mutant and WT cells (Figure 2). Surprisingly we observed subcellular heterogeneity in the cells. The cytoplasm is highly fluorescent whereas the vacuole is devoited of signal, as lipid droplets.

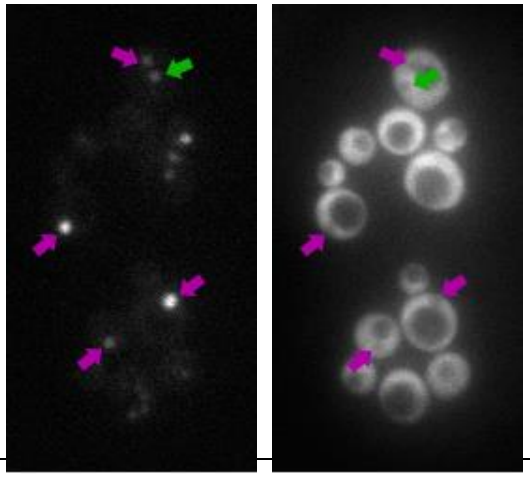

Figure 2 : fld1 mutant stains with DPH (left panel) and monitored for fluorescence after 275nm DUV exposition (right panel). We observed the region corresponding to LD are devoided of protein signal (pink arrows). We also observed LD moving during acquisition (green arrows)

F. Jamme performed bioinformatic image analysis. First, he performed PSF deconvolution of images (Figure 3). Then, he performed 3D reconstruction but infortunately it is not possible to obtain convenient images, the round shape of LD and cells were lost after recontruction due to refractive index mismatch (Fishtank effect). However, The PSF deconvolution allowed to increase the spacial resolution and image contrast (see Figure 3).

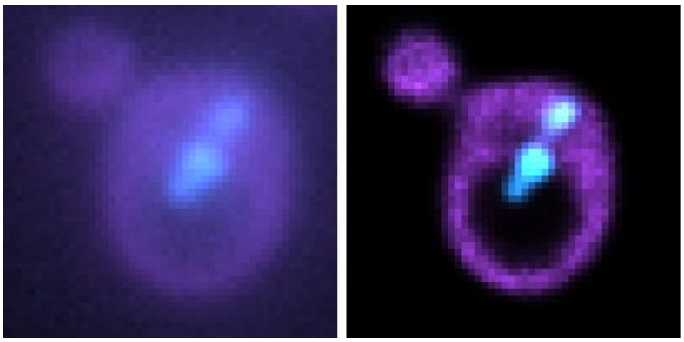

Figure 3 : Images of yeast before (left panel) and after (right panel) deconvolution.

Results for the second session: 10-12 of april

During this session we investigated a new technique, absorbance imaging, proposed by the DISCO beamline scientists. This new technique available on the Telemos microscope generates contrats from variations in cell absorbance. We also used TRP auto-fluorescence. We evaluated if this multimodal label free approach gives information on cell structure at the subcellular level. For this prupose, we designed a protocol for living cell imaging on silicium mirror. We used *fld1* mutant as the strain contains big LD.

The visualization of contrasted signals (fluorescence and absorbance) inside the cells confirmed chemical heterogeneity at the subcellular level. Absorbance could discriminate vacuoles and LDs, two low fluorecent compartments after 270-nm or 280-nm excitation. We demonstrated that some intracellular compartments can be identified thanks to their DUV signature (Figure 4):

Cell compartment	Fluorescence	Absorbance
Cytoplasm	High	High
vacuole	Low	Low
Lipid droplet	Low	High

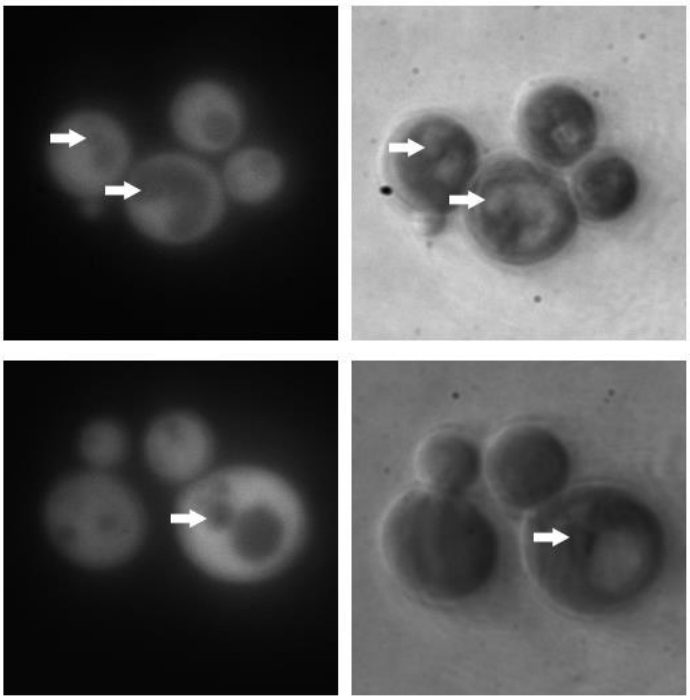

Figure 4 : Fluorescence (left panels) and absorbance (right panels) images of *fld1* cells. Arrows indicate the LD position.

Conclusion

Deep UV light provided by the DISCO beamline enabled imaging of organelles in the living unicellular yeast. The quality of the synchrotron light offered a good spatial resolution permitting the visualization of structures like contact zones between two organelles. For the first time, we designed and constructed dedicated devices for

absorbance imaging on living cells and combined it with fluorescence imaging. This new powerful and original technique led us to obtain images of intracellular compartments at the subcellular level based on their chemical properties.

Justification and comments about the use of beam time (5 lines max.):

The beamtime allowed (15 shifts) was sufficient to settle up a new label free multimodal approch combining fluorescence and absorbance. We obtained a set of data convenient for the redaction of an article describing this work :

Marine Froissard, Isabelle Bouchez, Caroline Pénicaud, Stéphanie Passot, Fernanda Fonseca, Yann Gohon, Matthieu Réfrégiers, Frédéric Jamme. **Synchrotron label free multimodal imaging to explore lipid droplet structure in living cells**. En préparation

Publication(s):
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