# DISCOmega 2: Application of DISCO beamline technical developments for membrane fluidity measurements on yeasts with Omega3 contrasted content

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# Experimental Report on proposal 20150869 (Standard)

**Proposal title:** DISCOmega 2: Application of DISCO beamline technical developments for membrane fluidity measurements on yeasts with Omega3 contrasted content.
**Proposal ID:** 20150869 (Standard)
**Beamline:** DISCO IMAGING
**Shifts:** 15
**Experiment date:** from: 2016-06-08 08:00:00.0 to: 2016-06-13 08:00:00.0
**Local contact:** Dr. JAMME Frédéric
**Date of report:** 02/02/2018

## Objective and expected results:

We will use DUV DISCO beamline to perform measurement of fluorescence anisotropy using TMA-DPH membrane staining. With fluorescence anisotropy data on yeast strains with contrasted omega 3 content, we will explore the link between omega 3 content and membrane fluidity. This work will provide fundamental data on the physiology of Debaryomyces species which are key yeasts for food and non-food biotechnologies. Membrane fluidity data obtained on DISCO beamline, combine with a set of phylogeny, genomic and biochemical existing results will allow major advances on omega 3 metabolism in Debaryomyces species and will contribute to our largest project on the study of the Saccharomycotina subphylum.

## Results and the conclusions of the study:

Background

Our consortium performed pioneer work on DISCO beamline for single microorganisms (yeasts and bacteria) study using DUV. A tight collaboration between beamline scientists and biologists lead to the great upgrade of the end station (motorization of polarizers, homogenization of light, etc) and establishment of protocols (staining conditions, acquisition conditions, etc) for performant analysis of membrane fluidity (fluorescence anisotropy) in living cells (Report 99140076 and Report 20141082). We determined that TMA-DPH is the best staining for membrane fluidity measurement. We also observed high level of non-specific fluorescence (cytoplasm autofluorescence) when cells were in bad physiological conditions. To date, this signal impairs anisotropy calculation.

Biological Material

*Saccharomyces cerevisiae; Debaryomyces species 1298; Debaryomyces species 1277; Yarrowia lipolytica*

Cells were grown 24h at 28°C or one week at 4°C in minimal medium (YNBG) or in complete medium (YPG) in the Biology 2 laboratory at SOLEIL synchrotron.

Membrane staining with TMA-DPH

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

Microscope configuration

TMA-DPH = excitation: 340 nm; Emission: DM410_420-480

Results

During this session we encountered many technical impairments such as failure of synchrotron light (first day), dirty camera that required to stop experiment for a careful cleaning procedure (second day), unexplained stray light (third day), etc.
Furthermore, the Telemos microscope had to be upgraded with an OptoSplit system allowing simultaneous acquisition of parallel and perpendicular images for anisotropy calculation but, due to DUV technical incompatibility (glass lenses in the commercial system), we could not performed the planned experiments.
For these reasons, in accordance with the beamline local contact, we modified our program and we performed technical improvement of multimodal imaging of intracellular compartments in living yeast, a new technique we developed on DISCO beamline (see Report 20142019). The results are subjected to publication under revision.

Conclusions

At this time, we did not obtained anisotropy data convenient for publication as we had to deal with lot of technical bottlenecks.

## Justification and comments about the use of beam time:

The beam time allowed for this experiment was 15 shifts and was not efficiently used to study fluorescence anisotropy on yeast cells. We encountered to much technical bottlenecks for acquisition of a convenient set of data. After 3 days, we decided to modify our technical approach (multimodal imaging vs anisotropy) in order to perform valuable yeast imaging during this beam time.

## Publication(s):

Froissard et al., 2016. Lipid cell structure imaging and membrane fluidity measurement in single living microorganisms. INRA SOLEIL 10 ans. Froissard et al., 2016. Lipid droplet heterogeneity revealed in living Saccharomyces cerevisiae. Scientific Reports. Under revision.