

Tomographic imaging of Diatoms using digital holographic microscopy

Semester/ Master Thesis @ Stockerlab

An important group of unicellular organisms in the oceans are diatoms, ubiquitous photosynthetic eukaryotes. Diatoms are at the base of marine food webs carrying out ~20% of the yearly photosynthesis on Earth[1]. They are responsible for the conversion of large amounts of inorganic carbon into organic matter, serving as food for many other marine organisms.

Based on recent work [2]–[5], we want to use a Digital holographic camera attached to an inverted microscope(available in the lab) to image singular living diatoms in high temporal (on the order of minutes) and spatial resolution (on the order of microns) and investigate the topics listed below. Digital Holography Microscopy (DHM) quantifies the optical path difference of a sample compared to the surrounding media based on refractive index differences and allows the extraction of density and dry mass measurements. Figure 1 shows how QPI allows the measurement and tomographic reconstruction of chloroplasts in a *T.rotula* cell. The eight chloroplasts (shown in red) are detected based on their refractive index differences compare to the rest of the cell, their volume can be extracted (V_{chl}) and their 3D position within the cell can be determined.

We aim at developing a new method to visualize and track subcellular structures such as organelles in 3-D in single-cell diatoms. The diatoms might be fixed at the end of a glass capillary, inside a capillary, or rotated by flow to extract a full 3D scan of cells. (see [2]). The target is to image *Thalassiosira pseudonana*, *Thalassiosira rotula*, and *Ditylum brightwellii*.

A prospective student will develop the tomographic imaging setup in the lab, automating the image acquisition and the tomographic reconstruction. By staining different organelles and using fluorescent imaging, we will establish protocols how to image and differentiate the various organelles.

You can do this project as a semester or master thesis. The scope of the project will vary accordingly.

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Background information

Compared to other eukaryotic organisms, diatom life history and cell cycle is unique because their cell wall is composed of two rigid silica valves. Diatoms are diploid and for a majority of their life history divide via mitotic asexual division. However, because one valve is slightly larger compared to the other, and vegetative valve enlargement is mostly not possible (only observed in few species), with each cell division one of the two daughter cells is slightly smaller compared to the other. With each cell division, the mean size of the population will decrease. With some exceptions, diatoms need to undergo sexual reproduction when a critical size is reached and environmental factors are favorable. In order to restore cell size, vegetative cells undergo meiosis, form male and female gametes that can then fuse and form a new diploid initial cell with restored cell size [6]. While the gametes are motile in order to find a suitable counterpart for sexual reproduction, diatoms are non-motile during their asexual life cycle stage and therefore subjected to sinking in the water column [7]. In order to counteract this passive sinking, diatoms are able to regulate their buoyancy and therefore their sinking speeds in response to environmental cues such as light and nutrient availability [8], [9]. One such mechanism has been described in the diatom *D.brightwellii*, which can move up and down in the water column by changing the composition of one its organelles, the vacuole. Additionally, this regulation of buoyancy has also been linked to sexual reproduction in some diatoms, where sinking from turbulent surface water to more stable environments

facilitates pairing and sexual reproduction [8], [10]. Besides the vacuole, another important organelle in diatoms is the chloroplast, which is the site of photosynthesis where the energy of light is used to convert inorganic into bioavailable organic carbon (carbon fixation). Chloroplast movement, division and development are tightly linked to cell cycle progression in diatoms [11]. Additionally, silica cell wall formation plays a vital role in mitotic cell cycle progression; for example, *T.pseudonana* cells arrest their cell cycle in early G1 if starved of silica [12].

In the past, the diatom cell cycle has mostly been studied using transmission light or fluorescence microscopy, e.g., focusing on the morphogenesis of the silica cell wall, a unique feature of diatoms [12], [13]. Additionally, some studies have investigated the localization and distribution of organelles such as the chloroplast within algae cells [14]. However, these methods do not capture out-of-plane (3-D) effects, such as an increase in organelle density or positional and morphological changes in the z-direction. Alternatively, high-resolution 3D images of diatoms have been measured using cryoFIB-SEM, characterizing the relationship between total cell volume and the volume of different organelles in diatoms [15]. Although high in spatial resolution, this approach lacks temporal resolution given its destructive nature on the sample.

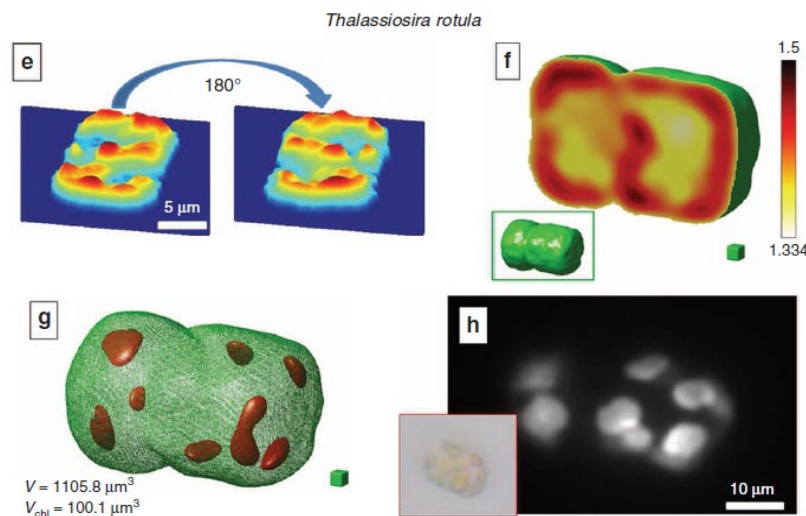


Figure 1 Taken from [2], QPI imaging of *T. rotula* and Tomographic reconstruction of the cell. Panel E shows the raw DHM images with higher optical densities shown in red. F is a cut through the tomographic reconstruction. G shows the chloroplasts inside the diatom and H shows the corresponding fluorescence and brightfield image.

Research directions

Overcoming these limitations and developing an approach to measure subcellular structures in both high spatial and temporal resolution would allow to further investigate open questions in diatom physiology and biology, such as

- 1) Investigating the formation, morphology and composition of vacuoles as well as their response to environmental cues such as light, nutrient or temperature and their coupling to different stages the asexual and sexual life cycle, as wells as different stages of growth (e.g exponential vs. stationary).
- 2) Formation, movement and distribution of chloroplast across mitotic and meiotic cell divisions and 3D positional responses to changes in e.g light conditions
- 3) Formation the silica cell wall during cell division via formation, movement and maturation of silica contain vesicles, which deposit silica at the site of cell wall formation. Can we observe differences in e.g. different sized species with different amounts of silicified cell walls?

References

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