

Design and implementation of a microfluidic chamber for synchronization studies of glycolytic oscillations in yeast cells

Martin Mojica-Benavides, Amin A. Banaeiyan, Caroline Beck Adiels and Mattias Goksör

Department of Physics, University of Gothenburg, Sweden,

www.physics.gu.se/biophotonics

Aim

To detect possible entrainment in a cell-cell level during glycolysis using a microfluidic chamber that by means of diffusion, oscillations are locally triggered in a quasi-static and single layered environment.

Introduction

It has been well proven that single yeast cells can present sustained oscillations under the presence of a cyanide-glucose solution and that periodic variations of this solution entrain the oscillations causing a phase shift¹. Acetaldehyde (Aca) has been shown to be a possible mediator for cell-cell synchronization given its fast membrane transportation dynamics and its role in the NAD-NADH cycle during glycolysis², Fig 1.

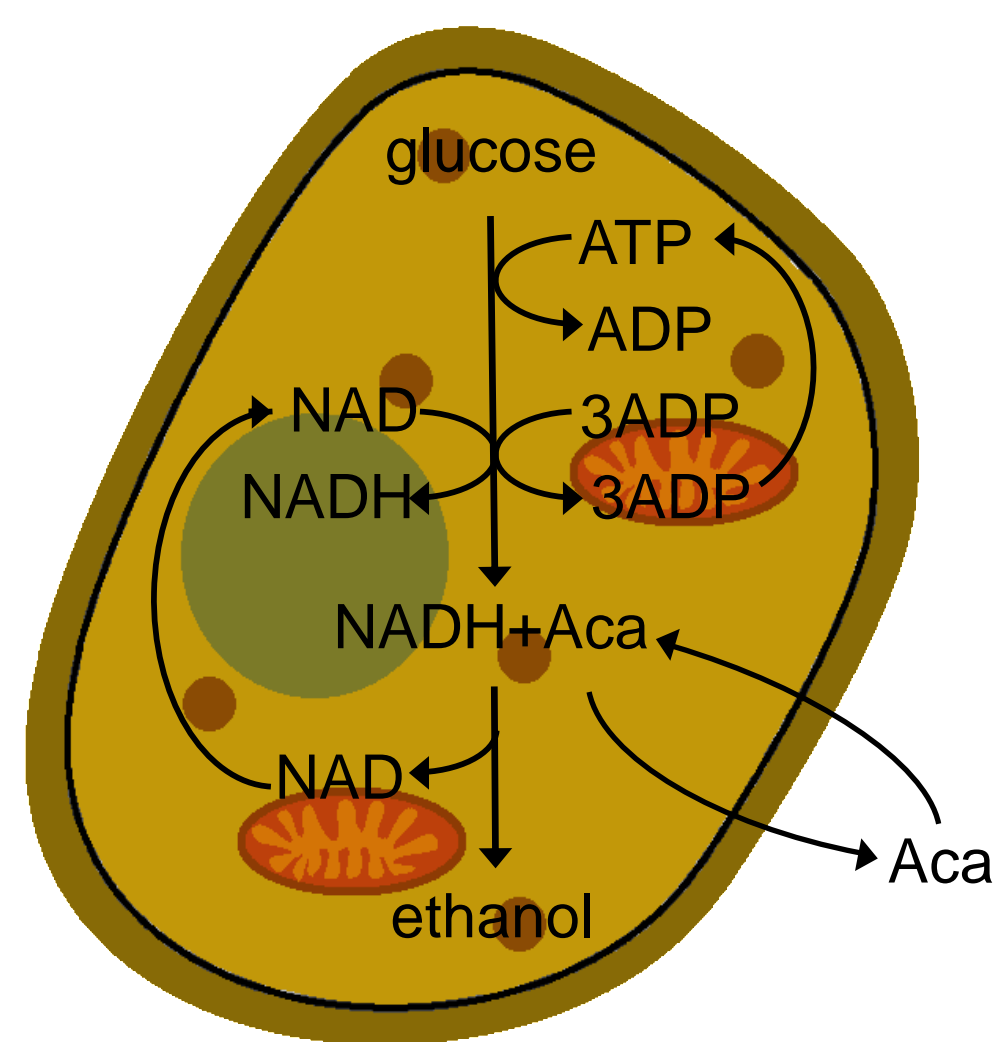


Fig 1. Summarized glycolysis cycle in a yeast cell. The steps that are shown occur in the cytoplasm along the central arrow and show the roll played by the Aca secreted and absorbed in each oscillation.

Previous experiments have shown that cyanide reacts with Aca producing lactonitrile³. This reaction must have direct influence on phase shifts in the oscillations based on the Aca-cyanide concentration ratio inside and in the surroundings of the cells. We designed a microfluidic device to observe possible entrainment on a cell-cell level, where a phase shift is triggered on single yeast cells by means of diffusion and the response of the neighboring cells can be tracked.

Methodology

- Design and simulate the microfluidic using COMSOL Multiphysics to assure radial diffusion and absence of flow during the triggering of glycolytic oscillations, Fig 2.
- Fabrication of the system by means of photolithography and PDMS replica molding.
- Loading the yeast cells and experimental evaluation of the microfluidic conditions.
- NADH autofluorescence time dependent measurements in order to follow metabolic oscillations.

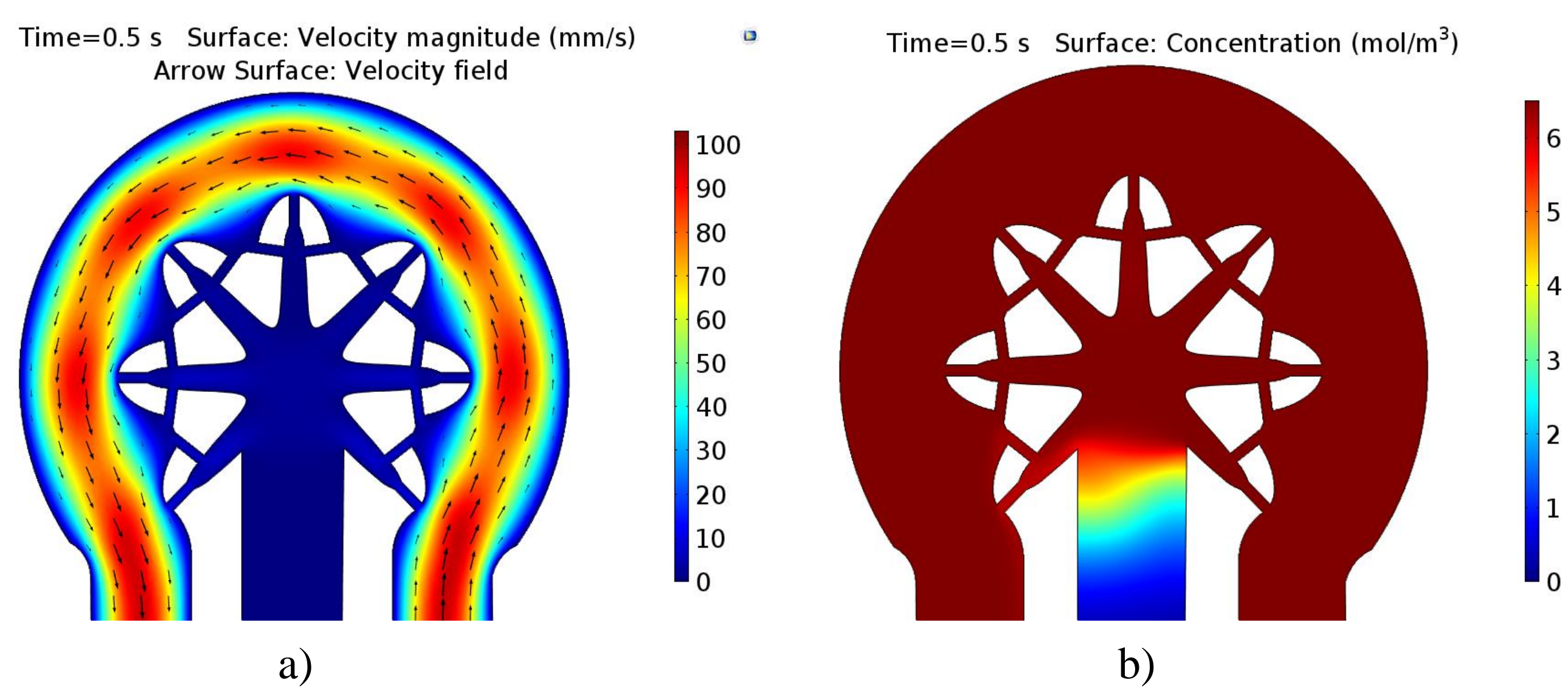


Fig 2. Computational simulations of the "Star" chamber for a) Flow rates and b) diffusion of cyanide in the microfluidic chamber.

Results

After simulating and fabricating, the device was loaded with yeast cells previously starved and induced to the diauxic shift. The main purpose was to achieve a single plane of cells in order to evaluate oscillations in a high 2D cell density but null cell neighboring in the depth direction, Fig 3.

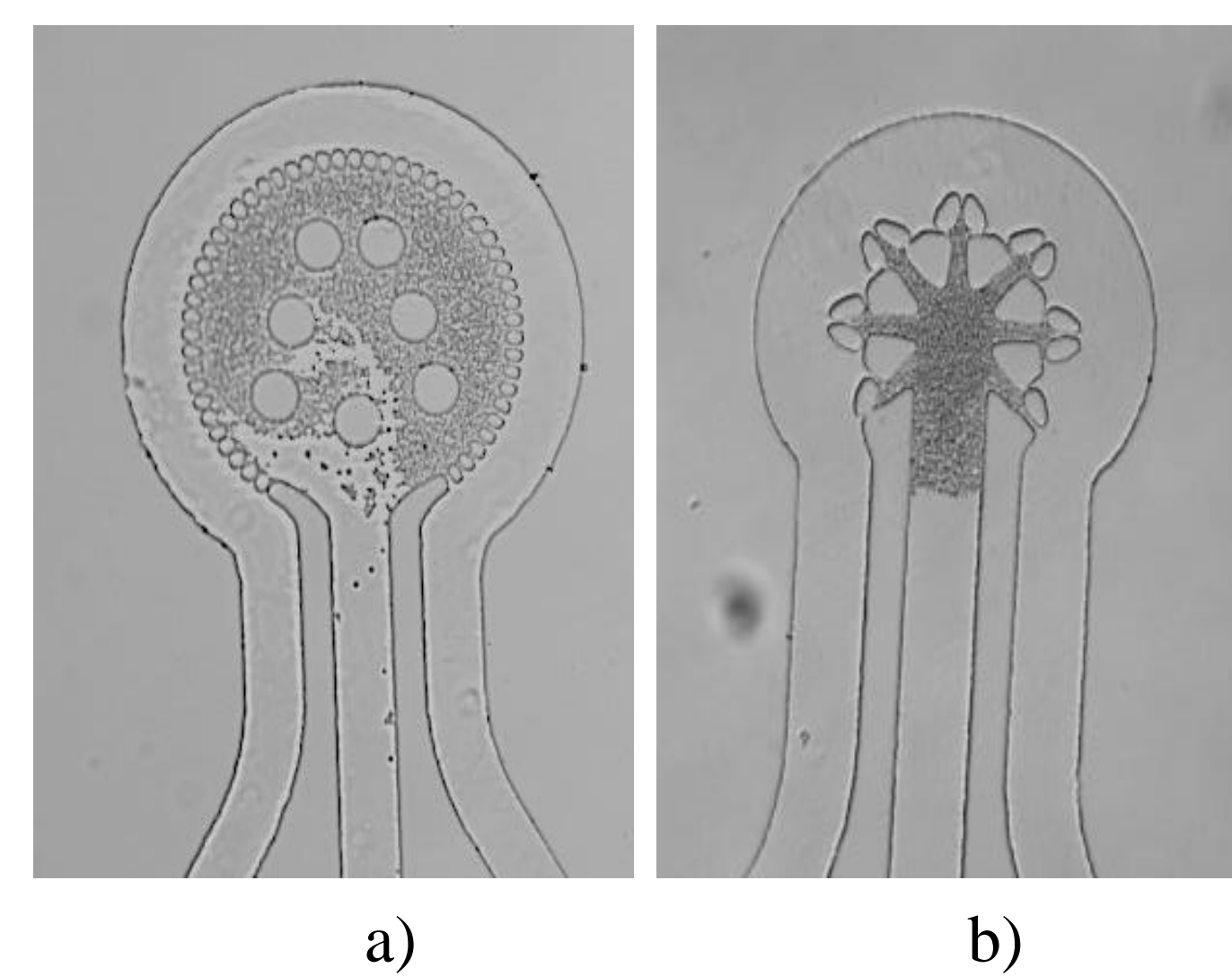


Fig 3. Cell loading in the two fabricated devices, a) Circle and b) Star chambers. The height of the devices was adjusted to the average diameter of a single yeast cells to pursue a single plane loading.

By means of epi-fluorescence microscopy, NADH time-dependent auto-fluorescence was acquired and oscillating cells could be identified. When observing the relation between neighboring cells oscillations, it is possible to notice the influence from the initially triggered cells towards the subsequent ones. This gives an idea of local Aca and cyanide concentrations due to the different trigger thresholds and secretion-absorption rates among the cells in the chamber. It is important to remark that even cells that don't show oscillations, do secrete Aca and influence synchronization between oscillating neighbors, Fig 4.

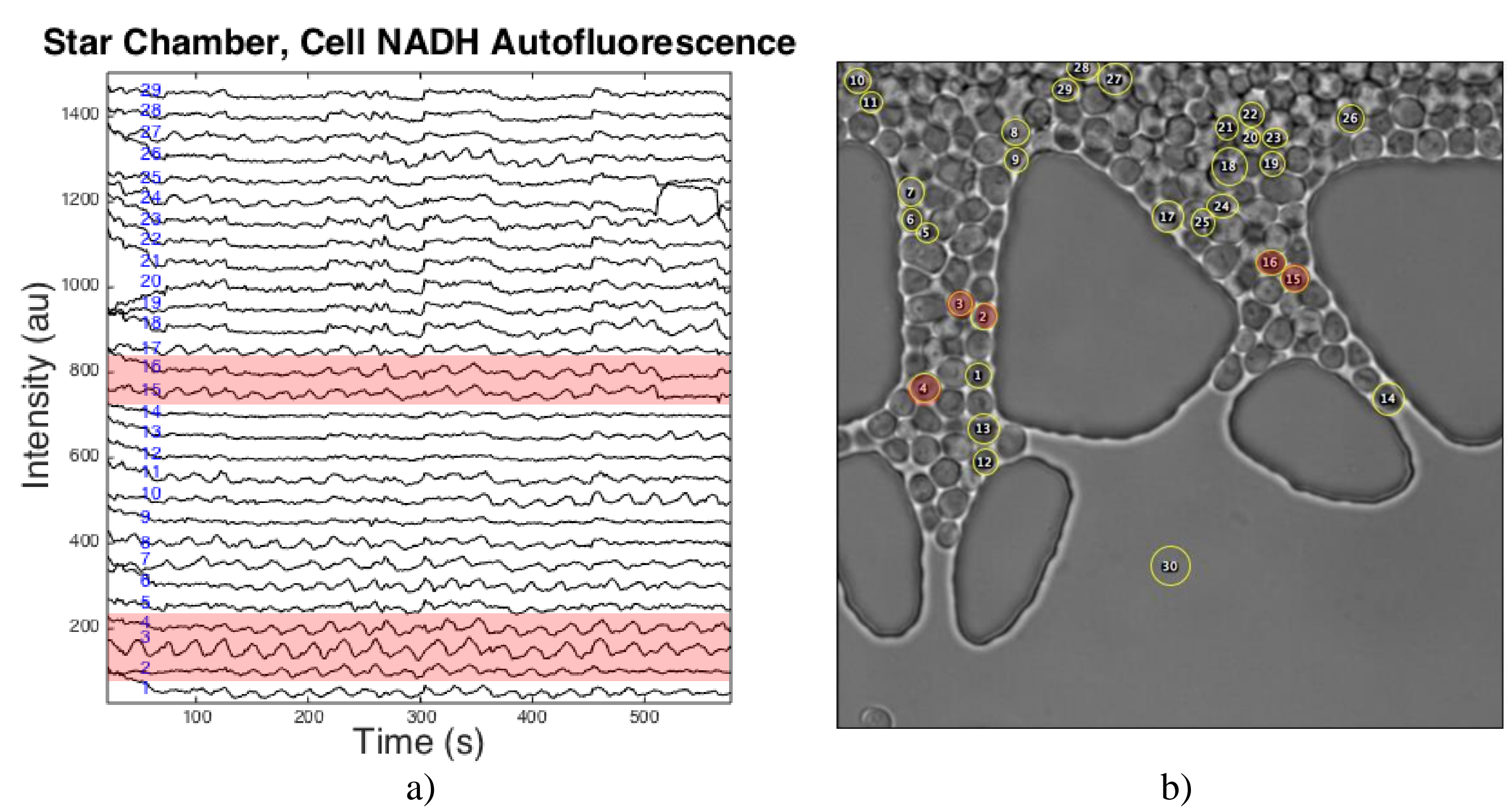


Fig 4. a) Auto-fluorescence signals for each of the tagged cells in b). Highlighted signals suggest cell-cell communication and local concentrations of Aca and cyanide.

Conclusions

- Two different designs of microfluidic chamber were fabricated that allowed the triggering of glycolytic oscillations by means of diffusion in a single 2D configuration of yeast cells.
- NADH auto-fluorescence measurements showed local cell-cell phase influence during glycolytic oscillations.
- Obtained results lead to further system optimization for phase distribution studies.

1. Gustavsson A-K. et al., *Scientific Reports* **5** 9404 EP. (2015)
 2. Bier M. et al., *Biophysical Journal* **78** 1087-1093. (2000)
 3. Danø S. et al., *Proceedings of the National Academy of Sciences* **104** 12732-12736 (2007)