Supplementary Materials for:

Unveiling microbial single-cell growth dynamics under rapid periodic oxygen oscillations

Keitaro Kasahara[1,2], Johannes Seiffarth[1,2], Birgit Stute[1], Eric von Lieres[1,2], Thomas Drepper[3], Katharina Nöh[1], and Dietrich Kohlheyer[1,*]

[1]IBG-1: Biotechnology, Institute of Bio- and Geosciences, Forschungszentrum Jülich GmbH, Jülich, Germany

[2]Computational Systems Biotechnology (AVT.CSB), RWTH Aachen University, Aachen, Germany

[3]Institute of Molecular Enzyme Technology, Heinrich Heine University Düsseldorf, Forschungszentrum Jülich GmbH, Jülich, Germany

[*]Corresponding author: IBG-1: Biotechnology, Institute of Bio- and Geosciences, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany. Email: d.kohlheyer@fz-juelich.de

This document includes:

Figure S1 - 3

Table S1

Supplementary Materials and Methods

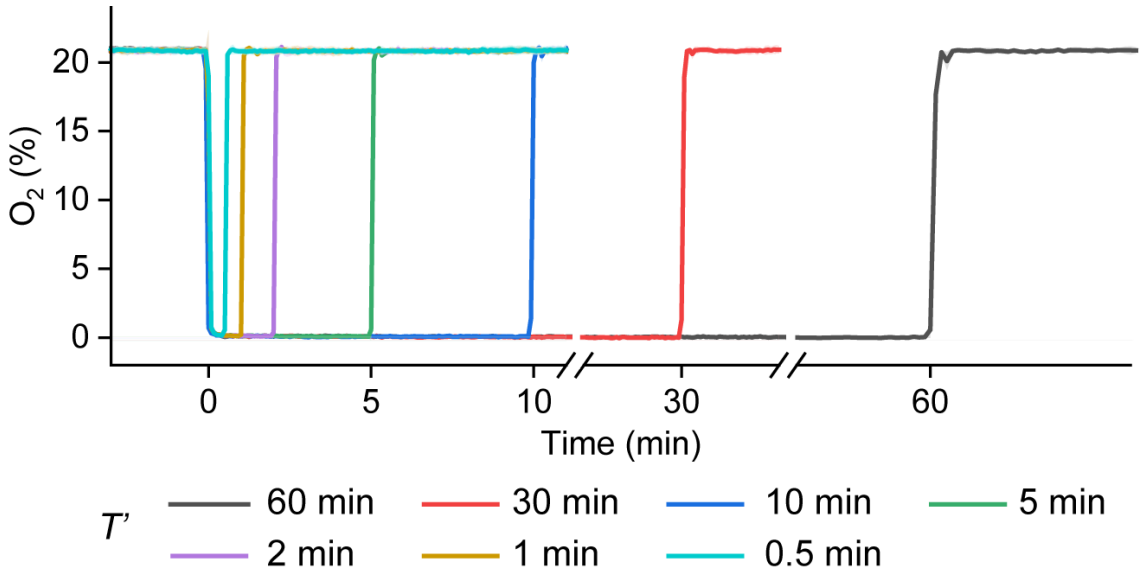

Figure S1. O_2 concentration in the fluid channel of the microfluidic chip determined by RTDP lifetime measurement with FLIM under oscillating O_2 environments at various oscillation half-periods (T').

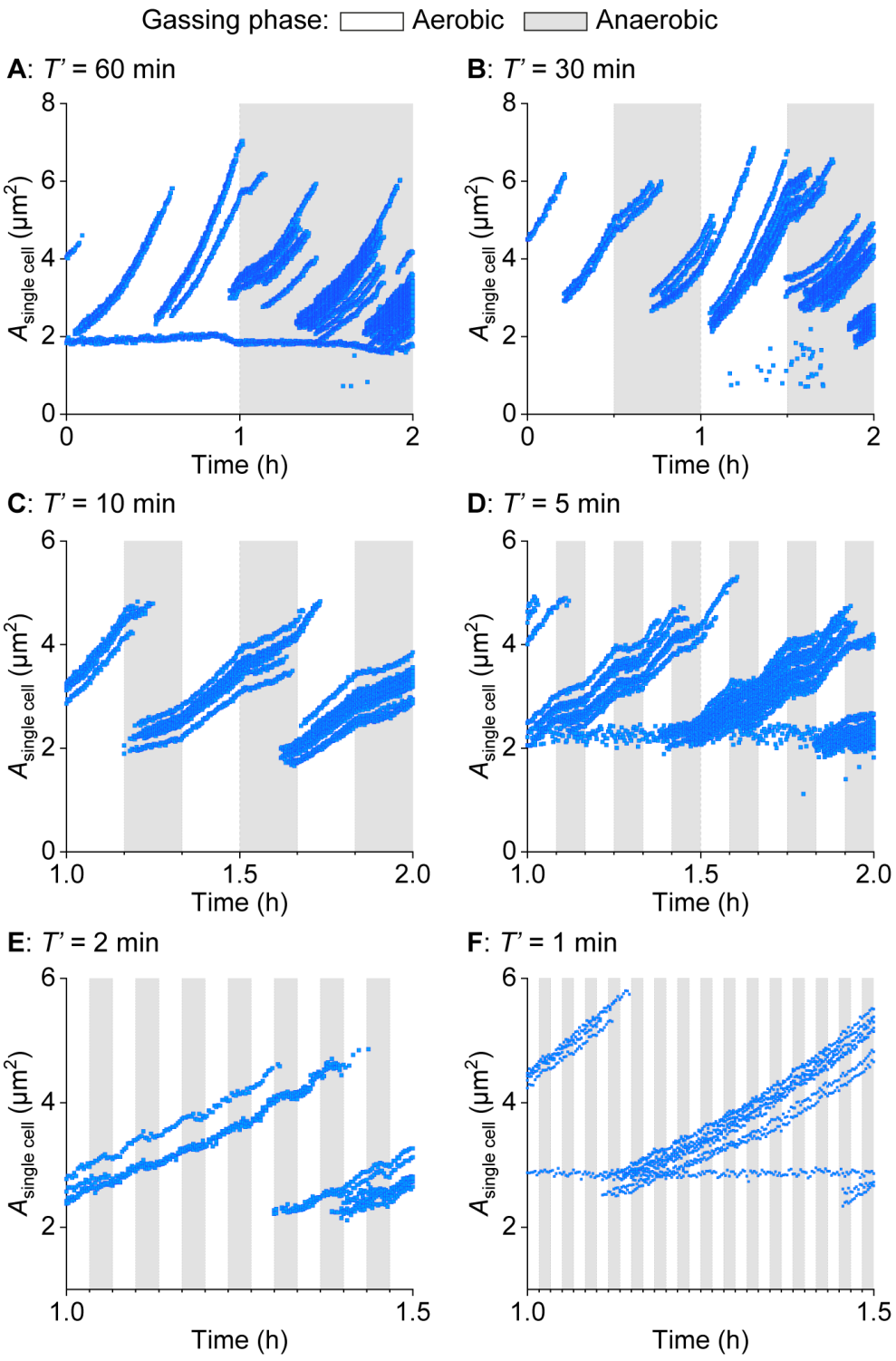

Figure S2. Single-cell area ($A_{singlecell}$) plotted over time under oscillating O_2 environments at various oscillation half-periods (T').

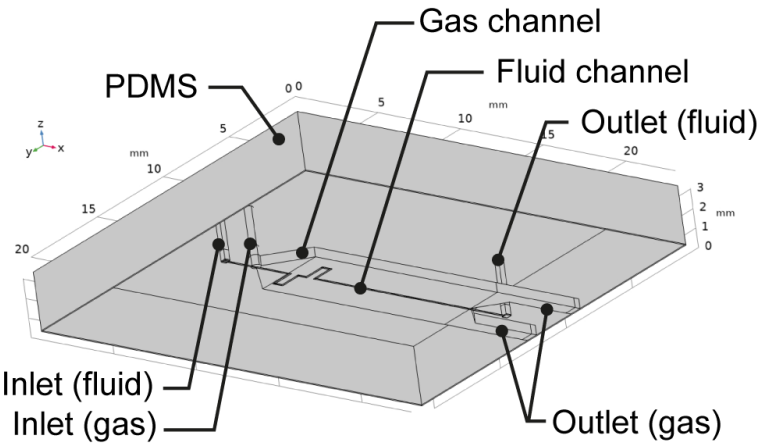

Figure S3. Geometry of the PDMS chip generated for gas diffusion simulation.

Supplementary Materials and Methods

Simulation setup

Fluid and gas flow through the respective channels were computed by solving the time-dependent Navier-Stokes equations for laminar, incompressible flow, as follows.

$$\frac{\delta \rho}{\delta t} + \nabla \cdot (\rho\, u) = 0$$
$$\rho \frac{\delta u}{\delta t} + \rho (u \cdot \nabla) u = \nabla \cdot [-pI + K]$$
$$K = \mu (\nabla u + (\nabla u)^T)$$

Here, ρ represents density (kg/m^3), u is velocity vector (m/s), p denotes pressure (Pa), I is identity matrix, and μ is dynamic viscosity (Pa $\cdot$ s). Boundary conditions at the PDMS block and the glass plane are defined as follows.

$$u = 0 \qquad \text{(wall)}$$
$$u = -nU_0 \qquad \text{(inlet)}$$
$$[-pI + K]n = -p_0 n \qquad \text{(outlet)}$$

n is the boundary normal vector, pointing outward from the domain, and U_0 is the normal inflow speed.

With u defined by the Navier Stokes equations, O_2 transport via convection and diffusion was simulated using the diluted species transport module in COMSOL, incorporating specific boundary conditions as detailed below. The O_2 concentration in the surrounding air is assumed to remain constant at 21%.

$$\nabla \cdot J_i + u \cdot \nabla c_i = R_i$$
$$J_i = -D_i \nabla c_i$$

i is the index for different domains (water, air, PDMS), and D_i represents the diffusion coefficient for each domain i. The velocity u within the PDMS domain is set to 0. c_i denotes the O_2 concentration (mol/m^3)

Table S1. Parameters used in simulation (1 atm, 310.15 K)

	water	PDMS	air (21% O_2)	air (100% O_2)
S (mol/m^3)	0.218 [1]	1.25 [1]	8.1375	-
D (m^2/s)	2.7×10^{-9} [1]	7×10^{-9} [1]	2.3×10^{-5}	-
ρ (kg/m^3)	993.31	-	1.1383	1.24
μ (Pa · s)	0.00101	-	1.814×10^{-5}	-

in each domain i, and R describes sources or sinks. Boundary conditions between different domains are defined as follows.

$$\frac{c_i}{S_i} = \frac{c_j}{S_j} \qquad \text{(between PDMS and gas / water channel)}$$

$$c_{\text{PDMS}-\text{air}} = S_{\text{PDMS}} = 1.25 \text{ mol/m}^3 \qquad \text{(between PDMS and surrounding air)}$$

$$-n \cdot J_i = 0 \qquad \text{(at the glass plate)}$$

$$c_{\text{water}-\text{air}} = S_{\text{water}} = 0.218 \text{ mol/m}^3 \qquad \text{(between water and air)}$$

S_i represents the solubility of O_2 (mol/m^3) in each domain i at 1 atm and 310.15 K. The initial solubility in air, S_{air}, was calculated to be 8.134 mol/m^3. The parameters used in the simulation were summarized in Table S1.

The mesh for the given geometry was generated using a semi-automated approach. First, a hexahedral mesh was created for the fluid channel. The boundaries adjacent to surrounding parts were then converted to a triangular mesh. Finally, the remaining domains were meshed by a tetrahedral mesh with an "extra-fine" resolution.

References

1. M.-C. Kim, R. H. W. Lam, T. Thorsen and H. H. Asada, *Microfluid. naonfluid.*, 2013, **15**, 285-296.