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► **To cite this version:**

Matthias Le Bec, Sylvain Pouzet, Céline Cordier, Simon Barral, Vittore Scolari, et al.. Optogenetic spatial patterning of cooperation in yeast populations. 2023. hal-04288752v1

HAL Id: hal-04288752

<https://hal.science/hal-04288752v1>

Preprint submitted on 16 Nov 2023 (v1), last revised 13 Nov 2024 (v2)

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1 **Optogenetic spatial patterning of cooperation in yeast** 2 **populations.**

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13 **Keywords:** Optogenetics, Yeast, Cooperation, Competition, Spatial Patterning

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15

16 **Abstract**

17 Microbial communities are a sieve of complex metabolic interactions such as cooperation and
18 competition for resources. Methods to control such interactions could lead to major advances in
19 our ability to engineer microbial consortia for bioproduction and synthetic biology applications.
20 Here, we used optogenetics to control invertase production in yeast, thereby creating landscapes
21 of cooperator and cheater cells. Yeast cells behave as cooperators (*i.e.*, transform sucrose into
22 glucose, a public “good”) upon blue light illumination or cheaters (*i.e.*, consume glucose
23 produced by cooperators to grow) in the dark. We show that cooperators benefit best from the
24 hexoses they produce when their domain size is constrained between two cut-off length-scales.
25 From an engineering point of view, the system behaves as a band pass filter. The lower limit is
26 the trace of cheaters’ competition for hexoses, while the upper limit is defined by cooperators’
27 competition for sucrose. Hence, cooperation mostly occurs at the frontiers with cheater cells,
28 which not only compete for hexoses but also cooperate passively by letting sucrose reach
29 cooperators. We anticipate that this optogenetic method could be applied to shape metabolic
30 interactions in a variety of microbial ecosystems.

31

32 Introduction

33 Metabolic interactions, such as competition and cooperation to metabolize nutrients, are central
34 to the development of microbial colonies and biofilms¹⁻³. These metabolic interactions participate
35 in the establishment of complex, spatially structured multicellular systems in which cells located
36 at different positions experience varied microenvironments and can compete or cooperate with
37 each other. Cooperating cells (cooperators) are defined by their capacity to invest resources to
38 promote the proliferation (*i.e.*, increase the fitness) of other cells⁴⁻⁶. Conversely, cells that benefit
39 from other cells without contributing to their metabolic efforts are defined as “cheaters”⁷⁻¹⁰, since
40 they compete for resources without paying the cost required to produce the resources.

41 Cooperation and competition dynamics have been studied in various contexts^{11,12}, particularly
42 using the canonical example of sucrose utilization by the budding yeast *S. cerevisiae*¹³⁻¹⁷. Briefly,
43 yeast cells can produce the invertase Suc2p, which is retained in the periplasmic space and can
44 catalyze the hydrolysis of sucrose into glucose and fructose. Both of these hexoses (glucose and
45 fructose) can then be taken up by cells and metabolized intracellularly (Figure 1). Since hydrolysis
46 of sucrose occurs extracellularly, the hexoses produced by hydrolysis are public goods as these
47 sugars can also diffuse away and be consumed by adjacent cells (including cheater cells). This
48 situation both favors interactions between individual cells and also represents a fitness cost for
49 the cells expressing Suc2p¹⁵. Note that although sucrose can alternatively be taken up via sucrose-
50 proton symporters (Mal11p, Mal31p, and Mph2/3p),¹⁸ usually external hydrolysis is the dominant
51 sucrose uptake process in wild-type yeast^{19,20}.

52 Most studies have focused on competition/cooperation mechanisms in well-mixed populations;
53 thus, it is not known how a spatially structured population — in which cooperators and cheaters

54 are growing in separate domains — develops over time. In the case of sucrose utilization by
55 structured yeast populations, the key parameters that define the length-scales at which
56 cooperation and competition occur should be the diffusion and consumption of both hexoses and
57 sucrose. Simple estimates based on the diffusion-reaction equation indicate that the typical
58 length-scale over which the concentration decreases from a point source at steady state is given
59 by $\sqrt{D/k}$, where D is the diffusion coefficient of the metabolite of interest and k is the rate at
60 which the metabolite is absorbed by cells. In expanding microbial colonies, this distance over
61 which metabolic gradients are formed is of the order of a few hundreds of micrometers²¹. The
62 absorption rate (k) depends on the local cell density, which also varies in time as hexoses are
63 progressively transformed into biomass. Hence, the cell density of cooperators and cheaters is
64 likely to be a key parameter that defines the cooperation potency and indeed, this parameter has
65 been shown to be critical in the case of sucrose cooperation dynamics. For example, in liquid
66 culture, higher population growth was observed when yeast formed multicellular clumps¹⁵.
67 Other researchers showed that — *in silico* — static growth assortment (where daughter cells stay
68 close to their mother) stabilize yeast cooperation for sucrose catabolism²². Taken together, these
69 results suggest that cooperation through production of a diffusible public good appears generally
70 more cost-efficient if the cooperating cells stay close together³.
71 However, most experimental studies have not considered the impact of the spatial organization
72 of cheating and cooperating domains on the dynamics of cooperation and, so far, very few
73 experimental quantitative studies^{23,24} have investigated this problem, mostly due to technological
74 limitations. Indeed, a quantitative method to control the spatial organization of cheaters and
75 cooperators needs to be established to experimentally study the dynamics of populations that
76 compete or cooperate for shared resources.

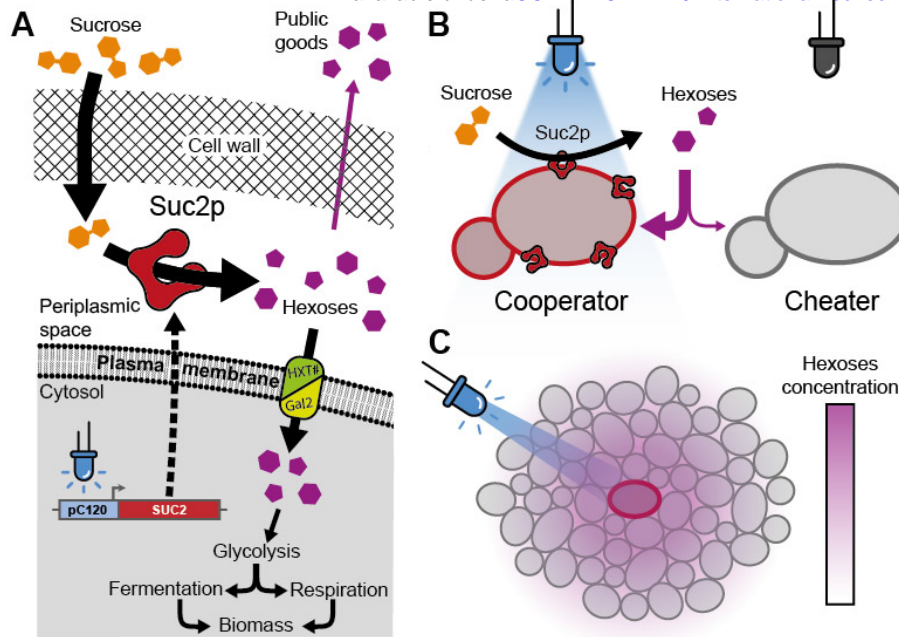


Figure 1 Optogenetic control of yeast sucrose catabolism. (A) Blue light illumination induces transcription of the *SUC2* gene and the production of the invertase Suc2p, which is secreted by exocytosis and retained in the periplasm. There, Suc2p catalyzes the hydrolysis of sucrose into two hexoses (glucose and fructose). These hexoses can be imported by cells via specific transporters (HTX1-4,6-7 and Gal2) to support the growth of yeast cells through

glycolysis. Alternatively, glucose and fructose can also diffuse away from the producing cell into the extracellular environment. (B) If the optogenetic system is tightly controlled, only cells stimulated by light can produce Suc2p, while cells in the dark cannot produce Suc2p. Projecting patterns of light on a yeast assembly induces well-separated spatial domains of cooperators and cheaters: illuminated cells behave as cooperators (*i.e.*, they produce hexoses as public goods), while cells in the dark behave as cheaters (*i.e.*, they rely on cooperators' production of public goods to grow). (C) Illumination induces the local production of hexoses and the establishment of hexose gradients through diffusion and uptake of glucose by both cooperators and cheaters.

77 Here, we tackle this problem by using optogenetics and spatial light-patterning to activate the
 78 expression of the invertase *SUC2* at selected locations within populations of yeast cells. Yeast cells
 79 could therefore selectively be switched from "cheater" to "cooperator" phenotypes upon light
 80 stimulation, creating spatially structured landscapes of cooperators and cheater cells. Combined
 81 with a dedicated experimental system to track the growth of cell populations with time and a
 82 numerical model, we were able to show the existence of two characteristic length-scales of
 83 cooperation/competition that involve both cheaters and cooperators and drive the emergence of
 84 the spatial landscape within a cooperator/cheater yeast consortium.

85 Results

86 **Light-inducible production of the Suc2p invertase enables extracellular hydrolysis of sucrose.**

87 We built a yeast strain in which SUC2 expression was placed under the control of a light-inducible
88 promoter. We integrated the blue-light sensitive transcription factor EL222 into the genome of the
89 Δ SUC2 yeast strain under the control of a strong constitutive promoter and the *SUC2* gene under
90 the control of the EL222-dependent pC120 promoter²⁵ (Figure 1A, 2A; see Methods). To estimate
91 the expression of SUC2 upon illumination, we fused the gene to a YFP fluorescent reporter with
92 (strain yPH_471) or without (strain yPH_470) the self-cleaving peptide sequence P2A, which
93 ensures that the invertase and the fluorescent reporter are produced (or not) as separate
94 proteins²⁶. Similarly, we built a native SUC2 reporter strain (yPH_484) by fusing P2A-YFP to the
95 *SUC2* gene in the wild-type strain. We measured the invertase activity (Figure 2B) and YFP
96 fluorescence (Figure 2C, 2D) of these strains after 2 h of invertase production under blue light in
97 24-well plates placed in a homemade light plate apparatus (LPA; see Methods and Supplementary
98 Figure S1)²⁷. As expected, the WT and Δ SUC2 strains did not show SUC2 enzymatic activity in
99 response to light, while both light inducible SUC2 strains exhibited marked increases in invertase
100 activity upon blue light stimulation. Quantitatively, yPH_470 and yPH_471 produced 40% and
101 310% of the enzymatic activity of WT, respectively (under 2.8 mW/cm² illumination).
102 Interestingly, the yPH_470 strain only reached ~13% of the invertase activity measured for the
103 yPH_471 strain, despite having the same promoter. Similar trends were observed for the cytosolic
104 fluorescence levels, as the fluorescence value for yPH_470 was only ~20% of yPH_471. These
105 results suggested invertase production/secretion was impaired in the yPH_470 strain, in which
106 the invertase enzyme is fused to a YFP fluorescent reporter. We attributed this effect to protein
107 quality control in the early secretory pathway²⁶, which would limit the export of SUC2-YFP into
108 the periplasmic space.

109 We then investigated the growth rate of both strains in sucrose upon illumination. We performed
110 these experiments in the same way as the previous experiments: 2 h invertase production in 0.05%
111 glucose, followed by cell growth monitoring in 1% sucrose using a plate-reader (see Methods).
112 First, we observed that the Δ SUC2 strain exhibited slow growth in sucrose, with a maximal
113 growth rate around ~30% of the WT (Figure 2E). This unexpected residual growth could be due
114 to the presence of maltose symporters, which may have residual activity for sucrose¹⁸. However,
115 we did not observe any difference when the maltose symporter *MAL11*, *MAL31*, and *MPH2-3*
116 genes were deleted (Supplementary Figure S2), ruling out this hypothesis. We therefore
117 attributed the residual growth of Δ SUC2 in sucrose to spontaneous hydrolysis of sucrose, which
118 is known to occur in acidic environments (the pH of media is ~5)²⁸. In addition, we observed that
119 — even in the absence of blue light illumination — the light-inducible SUC2 strains grew faster
120 than Δ SUC2. Hence, the basal activity of the pC120 promoter was high enough for cells to produce
121 and progressively accumulate Suc2p, resulting in a significant growth rate of 0.11/h (Figure 2E).

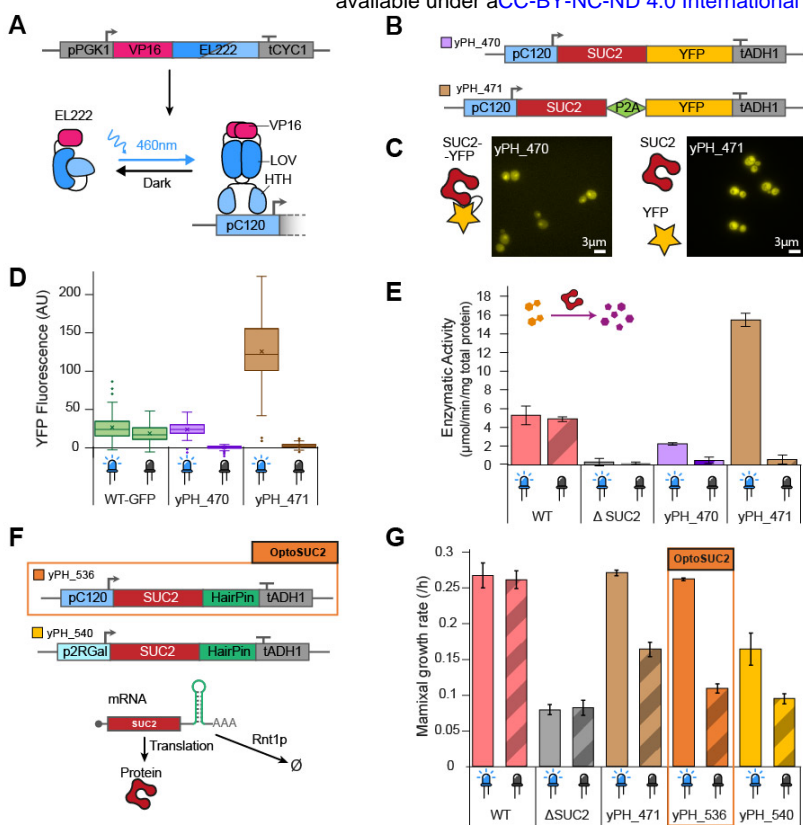


Figure 2. Strain design for tight control of light-induced production of the invertase Suc2p. (A) Schematic of the EL222 light-inducible transcription factor and its corresponding specific promoter pC120 to drive gene expression using blue light²⁹. (B) Construction of strains with or without the P2A self-cleaving peptide to separate the fluorescent protein YFP from the Suc2p invertase. (C) Microscopy images of YFP fluorescence in illuminated cells. (D) Fluorescence measurements of the Suc2p-YFP reporter in the presence or absence of light. (E) Enzymatic activity of Suc2p measured in the dark and upon illumination using a glucose

quantification assay (see Materials and Methods). (F) Improved designs for optogenetically induced expression of Suc2. Reduced mRNA lifetime is accomplished by RNA hairpin-mediated degradation of the transcript (bottom). (G) Maximal growth rate of relevant strains in SC 1% sucrose. The yPH_536 (hereafter, called OptoSUC2) exhibited the best dynamic range for inducible growth on sucrose. Error bars represent \pm the standard deviation for three technical replicates.

122 The long lifetime of the Suc2p protein (*i.e.*, no loss of activity was measured after 48 h of
 123 incubation at 30°C between pH 4 and 6)³⁰ likely enhanced the effect of basal leakage from the
 124 pC120 promoter and led to active accumulation Suc2p in the periplasmic space. Thus, to increase
 125 our capacity to control yeast growth based on light-induced invertase production, we optimized
 126 our strain construct via two complementary strategies by (1) reducing the leakiness of the pC120
 127 promoter and (2) reducing the lifespan of the SUC2 mRNA. As we previously showed that the
 128 yPH_471 strain produced more invertase upon light induction than the levels required to support
 129 yeast growth on sucrose (Figure 2B, 2E), we did not expect these modifications to drastically
 130 reduce growth upon illumination. We modified the pC120 promoter²⁹ by reducing the number of
 131 binding domains repeats from five to two (p2RGal; see Supplementary Information). We also

132 added a hairpin mRNA degradation tag¹ in the 3' untranslated region of the *SUC2* gene. The
133 resulting strains called yPH_536 (hairpin tag) and yPH_540 (changed promoter and hairpin tag)
134 both showed reduced growth rates in the dark compared to yPH_471. When illuminated,
135 yPH_536 had a comparable growth rate to the WT in sucrose, while the maximal growth rate of
136 yPH_540 was 61% of the WT (Figure 2E). We thus selected the yPH_536 strain, which we call
137 **OptoSuc2** in the remainder of this article, since it exhibited the largest contrast in invertase
138 expression between dark and illuminated conditions.

139

140 **Spatial control of yeast growth can be obtained by light activation of SUC2 in a microfluidic** 141 **chamber.**

142 We first tested the OptoSuc2 strain in a microfluidic chamber perfused with media supplemented
143 with sucrose (Figure 3 and Methods). At this small scale (*i.e.*, cells are growing as a monolayer in
144 a chamber of 400 x 400 μm^2), we wanted to evaluate how rapidly the hexoses released by a well-
145 defined spatial domain of Suc2p-producing cells diffuse to non-producing cells at the opposite
146 side of the chamber. The cells were grown in the microfluidic system in glucose, starved for one
147 hour, and then switched to 1% sucrose. We used a digital micro-mirror device (DMD) to
148 illuminate a small patch of ~400 cells while performing timelapse microscopy (Figure 3,
149 Supplementary Movie 1). The acquired images were analyzed by particle image velocimetry (PIV;
150 see methods) to obtain a vector field of the cell displacement (Figure 3D; see Methods) that results
151 from the growth of cells pushing neighboring cells.

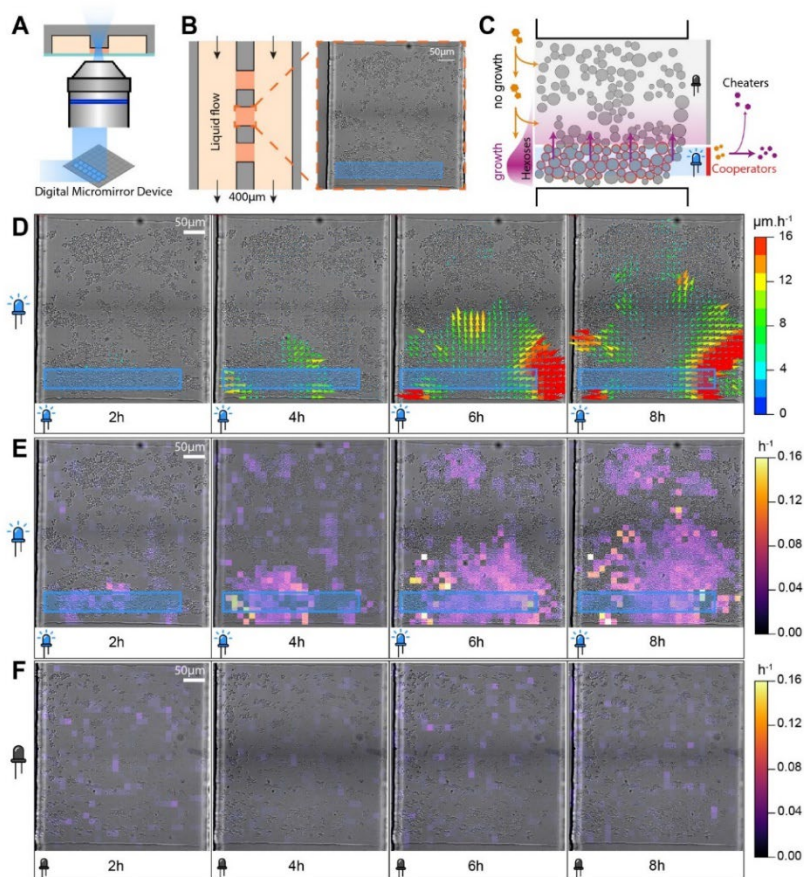


Figure 3. Spatial control of yeast growth in a microfluidic chamber. (A) A microfluidic chip is used to grow a monolayer of yeast cells in a well-controlled chemical environment through perfusion. A digital micromirror device is used to project a pattern of light (with single-cell resolution) onto the field of view. (B, C). Cells are grown in chambers of typically $400 \mu\text{m}^2$ and are perfused via two large channels on the sides of the chambers. The height of the chambers ($3.5 \mu\text{m}$) ensures that cells grow as a monolayer. (C) Bright-field image of the chamber filled with hundreds of OptoSUC2 yeast cells. The blue rectangle represents the area of the field of view that is illuminated at 460 nm for 200 ms every 6 min .

(D) Displacement vector map obtained through analysis of time-lapse images through PIV (particle image velocimetry). Cell motion only occurs due to cell growth; thus, we expect to observe large vectors (large displacement) at the interface between the dark and the illuminated areas (See Supplementary Movie 1). (E) Divergence map of the vector field, which is a proxy of the local cell growth rate. (F) Control without illumination, showing no significant growth throughout the experiment.

152 Computing the divergence of this vector field gives an estimate of the local cellular growth rate
 153 (Figure 3E, 3F). Once we illuminated a selected area, we observed cell growth in the same area
 154 (Figure 3D, 3E, Supplementary Movie 1), demonstrating that cells were indeed producing large
 155 enough quantities of hexoses for cellular growth. With time, we also observed that the “cheater”
 156 cells located in the dark region at the opposite side of the microfluidic chamber started to grow,
 157 but at a slower pace than the hexose-producing cells (Figure 3E). In comparison, cells trapped in
 158 chambers kept in the dark in the control experiments did not exhibit significant growth (Figure
 159 3F). This suggested that the cheater cells used the hexoses that diffused from the illuminated cells
 160 and confirmed that the illuminated cells were acting as cooperators. This experiment

161 demonstrated our capacity to control local growth at a spatial resolution of a few cells by
162 patterning a yeast population into domains of induced cooperators and cheater phenotypes.
163 However, cell growth inexorably pushed cooperating cells (*i.e.*, cells that were expressing the
164 Suc2p invertase) away from the illuminated area (Supplementary Figure S3 and S4). Moreover,
165 because of the long lifetime of Suc2p, the cooperator cells conserved their capacity to produce
166 hexoses even when they were outside of the illuminated area, which effectively blurred the
167 frontier between cooperator and cheater domains and made it difficult to maintain spatial
168 segregation of cooperators and cheaters at such a small scale.

169 **OptoCube: a device for simultaneous light patterning and microbial growth monitoring on** 170 **agar-plates**

171 Therefore, we next focused on yeast growth on solid media at larger scales (from a few
172 millimeters to several centimeters), which is more consistent with the scale of natural microbial
173 populations such as biofilms. To this end, we built the OptoCube, a temperature-controlled
174 incubator equipped with a DMD to project light patterns on a set of six standard (10 cm diameter)
175 or 15 small (6 cm diameter) agar plates. The agar plates are placed on top of a flatbed scanner to
176 record time-lapse images of microbial growth (Figure 4A, Supplementary Figure S5). This setup,
177 which is described in detail in the Supplementary Information, allows high spatial and temporal
178 resolution of light patterning (~ 0.1 mm, ~ 1 s, Figure S8) compared to the dimensions and
179 dynamics of microbial colonies (> 1 mm, > 1 hour). We designed a specific protocol to
180 reproducibly obtain a homogeneous lawn of yeast over the surface of the Petri dishes, by
181 overlaying a soft-agarose layer (0.67 mm) containing yeast on top of a gel containing sucrose
182 (Figure 4C, 4D). This resulted in a thin (~ 2.35 mm) and translucent gel that can be imaged using
183 the scanner. Cells embedded in the gel proliferated and formed clumps of microcolonies (Figure

184 4B) trapped in the gel, which avoids uncontrolled cell displacement over long distances and/or
185 colony expansion that modify the cheater/cooperator domains, as occurred in the microfluidic
186 device. We calibrated the intensity measured by the scanner (Supplementary Figure S6) with gels
187 containing known densities of cells and used this calibration curve throughout our experiments
188 to convert the pixel intensities into cell densities in colony-forming units per milliliters (CFU/mL).
189 We also checked that the illumination did not induce significant phototoxicity (Supplementary
190 Figure S7) and confirmed the spatial sharpness of the DMD illumination in the OptoCube
191 (Supplementary Figure S8). As a first demonstration of the capabilities of the OptoCube, we
192 directly projected images onto a lawn of OptoSuc2 cells (yPH_536) growing on top of a 1% sucrose
193 gel (Figure 4D-G, Supplementary Movie 2).

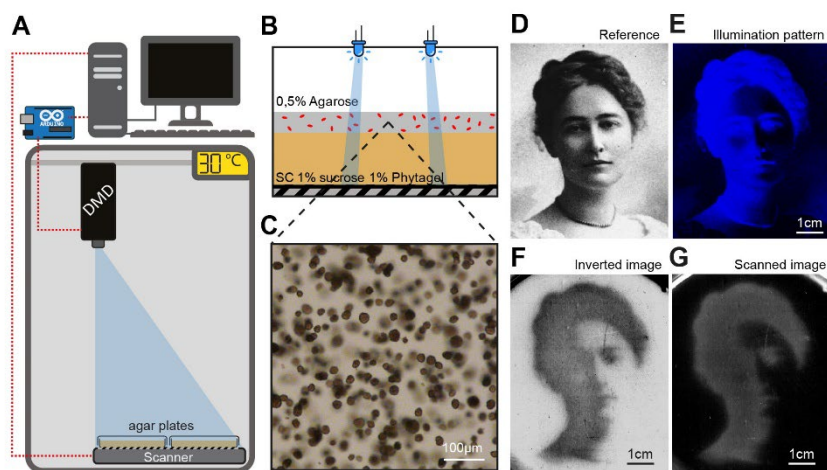


Figure 4. The OptoCube, a home-made device for optogenetic spatial patterning and yeast growth monitoring on solid media at “large” scale. (A) The OptoCube is composed of a DMD (digital micromirror device) fixed at the top of a temperature-controlled incubator and calibrated to illuminate a scanner placed under an array of Petri dishes. The DMD and the

scanner are controlled by a computer and a microcontroller. Under our tested conditions, the light intensities of the DMD pattern on the Petri dishes ranged from $0.0014 \text{ mW}\cdot\text{cm}^{-2}$ to $1.13 \text{ mW}\cdot\text{cm}^{-2}$. (B) Cells were grown in a layer of 0.5% agarose gel on top of a layer of Phytigel containing 1% sucrose and yeast SC media (see Methods). (C) Under these conditions, cells develop into microcolonies with diameters of a few tens of μm . In contrast to the microfluidic device (Figure 2), hexoses and sucrose can diffuse and cell growth is constrained within the gel. (D) To illustrate the patterning ability of this device, we projected an image of Maud Menten as a small tribute to her work on the Michaelis-Menten enzymatic kinetic equation, which was developed using invertase as the model³². (E) Blue light pattern projected by the DMD on top of a Petri dish containing OptoSuc2 cells for 45 h. (G) Scan of the Petri dish showing the regions where yeast have grown (grey areas). The first image of the timelapse was subtracted as the background. (F) Inverted image of the resulting yeast growth, revealing the image of Maud Menten developed through OptoSUC2-induced cooperation (see also Supplementary Movie 2).

194 As expected, growth mostly occurred within the illuminated areas, in which cells were producing
195 Suc2p and were therefore able to hydrolyze sucrose. The resolution of the image produced at the
196 surface of the gel was slightly blurred. We hypothesized that blurring occurs due to metabolic
197 cooperation between cells at the frontier between the dark (cheaters) and illuminated (light-
198 induced cooperators) domains. Indeed, cells in the dark near an illuminated area could grow
199 using the hexoses (public good) produced in an illuminated domain by cooperators.

200 **Modelling of yeast growth on sucrose**

201 To better understand the roles of the key physical and chemical ingredients involved in this light-
202 induced cooperator/cheater landscape, we built a simple model of partial differential equations
203 (PDEs) based on the work of Koschwanez et al.¹⁵. This model used: (1) Michaelis–Menten kinetics
204 for enzymatic reactions—*i.e.*, invertase catalysis for hydrolysis of sucrose and high- and low-
205 affinity glucose transporters for yeast consumption of hexoses; and (2) a Monod equation for the
206 dependency of the growth rate on the concentration of hexoses. Crucially, the model accounts for
207 diffusion of the sugars in space. Given the small gel thickness, diffusion equilibrates the
208 concentrations of the sugars much faster in the vertical dimension than horizontally; thus, we
209 restricted the model to the two horizontal dimensions only. The set of equations and related
210 parameters are described in the Supplementary Materials. We did not account for other nutrients
211 (notably nitrogen sources) in our model, as we assume that their availability was not limiting.
212 Importantly, we retrieved most of the parameters of the model from the literature and manually
213 tuned only three parameters — the invertase production rates α_{coop} and α_{cheat} and the maximal
214 growth rate μ_{max} — so that the numerical simulations fit both the dynamic and final densities of
215 our experimental observations (light dose response and spatial wavelength experiments, Figure
216 5 and Figure 7). Note that we attributed a small invertase production rate to the cheater cells (in

217 the dark) to account for leakage of the pC120 promoter. We found α_{coop} to equal $1.8\text{e-}24 \text{ mol.s}^{-1}.$
218 cell^{-1} , $\alpha_{\text{cheat}} = 1.5\text{e-}25 \text{ mol.s}^{-1}.\text{cell}^{-1}$, and $\mu_{\text{max}} = 0.27 \text{ h}^{-1}$. This model was numerically solved using a
219 PDE solver in Python (see Methods).

220 **Varying the light intensity enables quantitative control of the level of invertase and the yeast** 221 **biomass yield**

222 Next, we investigated how the rate of invertase production influences the growth of yeast on
223 sucrose on agar plates. We thus examined the light-dose response of the OptoSuc2 strain by
224 applying homogeneous and constant light stimulation over the agar plates (Figure 5,
225 Supplementary Figure S9). The resulting growth curves (Figure 5A) were used to extract the
226 maximal growth rates (Figure 5C) and the final cell densities in the stationary phase (Figure 5D).
227 By varying the light intensity, we were able to tune the maximal growth rate to between 0.12 h^{-1}
228 and 0.27 h^{-1} (Figure 5C), with the growth rate increasing with the intensity of illumination — as
229 expected. We also observed that the final cell density depended on how fast the cells consumed
230 the sucrose stock (Figure 5D), with faster growth leading to lower yields. As expected, the
231 simulated growth curves (Figure 5B) recovered the observed final densities (Figure 5D) but did
232 not fully fit the experimental growth rate, especially for slow dynamics (Figure 5C). However,
233 the model sufficiently replicated the experimental observations.

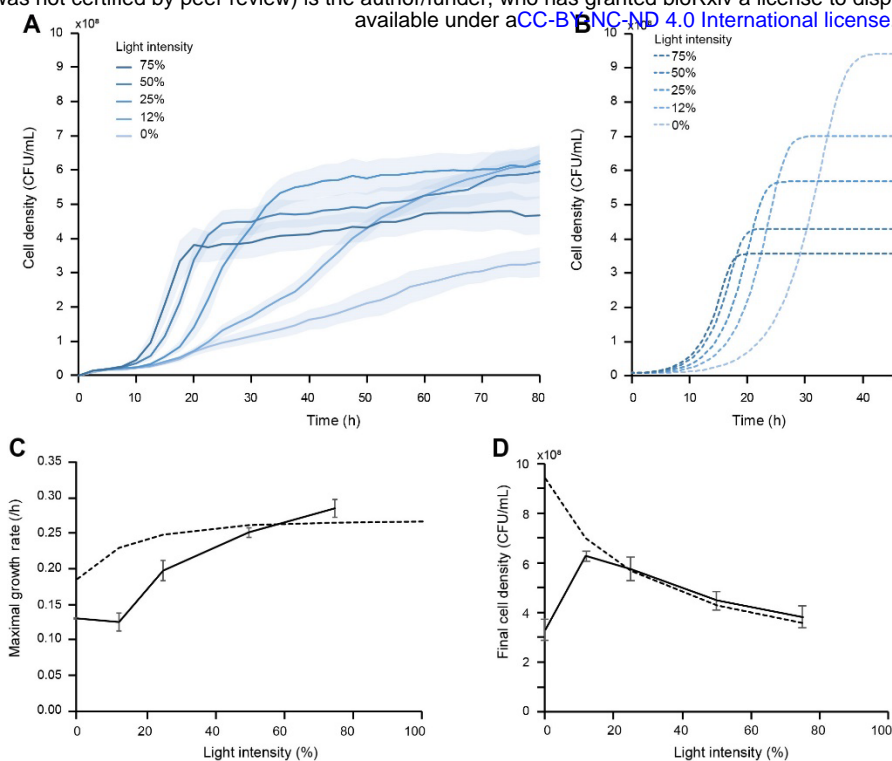


Figure 5. Light-dose response of the OptoSUC2 strain in the OptoCube in response to homogeneous light illumination. (A) Experimental measurements of the cell density as a function of time at varied light intensities. (B) Corresponding simulated growth curves obtained with our model. More intense illumination resulted in faster induction of growth and more rapid saturation of cell density. (C) Maximal growth rate determined from the growth curves presented in (A). (D) Dependence of the final cell density on the intensity of light. Solid lines represent experimental results, dashed lines represent simulated data. The shaded blue areas and error bars represent \pm one standard deviation for three technical replicates.

234 Overall, we succeeded in building an experimental system and a mathematical model to
 235 quantitatively explore the spatial interactions between cheater/cooperator cells in spatially
 236 extended yeast populations. Importantly, using these tools, we can now determine the impact of
 237 the relative size of the domains of cheaters and cooperators on their respective growth.

238 **Emergence of cheater-cooperator and cooperator-cooperator competition depends on the**
 239 **population domain sizes.**

240 To start, we projected single lines of blue light with varying widths (w) on Petri dishes containing
 241 homogeneous lawns of OptoSuc2 cells (Figure 6A, Supplementary Movie 3). As expected,
 242 significant growth occurred in the cooperator domains induced in the illuminated areas.
 243 However, growth inhomogeneities became evident when these cooperator domains were wider,
 244 with most of the growth occurring in the border zones between cooperator and cheater domains
 245 rather than at the center of the cooperator domains. We measured the final cell density profile in

246 the region of interest (at $t = 85$ hours, Figure 6B) and obtained the mean cell density profile along
247 the horizontal axis. Cell density decayed exponentially at the border between illuminated and
248 non-illuminated areas, confirming that cheaters (in the dark) were growing on the glucose
249 produced by the cooperators (illuminated area) and that the cheater cells were growing more near
250 the frontiers of the cheater/cooperator domains.

251 To estimate how cooperator and cheater populations share the available sugars, we measured the
252 cell densities at the center of the illuminated area (cooperator domain) and 1 cm away from this
253 area (cheaters) as a function of the width of the illuminated area (Figure 6C). Interestingly, we
254 observed that increasing the width of the illuminated area decreased the density of cooperators
255 and increased the density of cheaters. The final densities of cooperators were the highest for thin
256 lines of light; this can be explained by the fact that sucrose diffused from the dark (cheaters)
257 domain and supported the growth of cooperators in the illuminated area. This influx of sucrose
258 was mostly used up close to the interface of the dark/illuminated domains and could not reach
259 the center of cooperator areas if the cooperator domains were too large. This led to lower densities
260 at the center of large, illuminated areas and more growth of cooperators (and cheaters) near the
261 frontiers of the domains. This demonstrated that cooperators are competing for sucrose within
262 the illuminated areas, even though they cooperate by sharing another resource (hexoses) as a
263 public good. For large cooperator domains ($w > 5$ mm), this phenomenon led to the highest final
264 cell density profiles at the frontiers of the lines instead of their center (Figure 6B).

265 To better understand this process, we simulated one of these experiments ($w = 5.6$ mm wide blue
266 line) and numerically examined the concentration profiles of sucrose and glucose (Figure 6D)
267 after 15 and 25 h of illumination. These numerical simulations showed that the concentration of
268 hexoses initially increases in illuminated areas, promoting cooperator growth. This is the result

269 of the production and consumption of hexoses in the proximity of its production site. However,
270 the presence of higher numbers of cells that can hydrolyze sucrose depletes sucrose, which in
271 turn reduces the hexose production rate. This mostly occurs for cells that are far from a source of
272 sucrose (*i.e.*, cells at the center of the cooperator domain), which can no longer sustain their
273 growth. The cheaters in the dark domain are unable to metabolize sucrose, thus sucrose
274 continually diffuses from the cheater domain towards the cooperator domain; thus, the
275 cooperator domain can be viewed as a sink for sucrose. Sucrose is primarily hydrolyzed at the
276 frontiers, which promotes the growth of both cooperators (and cheaters through diffusion of
277 hexoses) at this interface. In other words, the maintenance of cooperator growth (and their
278 cooperation phenotype) is only guaranteed by the near lack of sucrose uptake by cheaters. Indeed,
279 the cheater domain is a reservoir of sucrose and its size relates to the amount of sucrose that can
280 feed the cooperators in their own domain. Thus, both the cheater and cooperator domain sizes
281 are needed to explain the landscape of microbial growth.

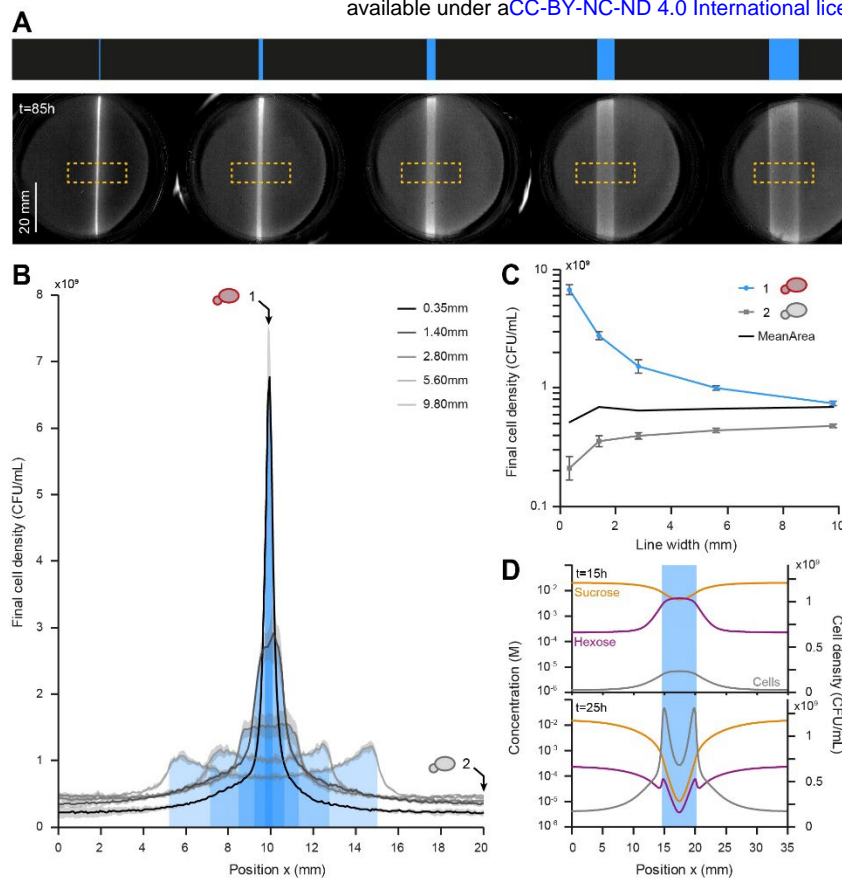


Figure 6. Varying the size of the cooperator domain impacts the density of cooperators. (A) Single lines of light of varying widths (top) were projected on cells embedded in a thin layer of gel. The growth of cells in the cooperating (illuminated) and cheating (dark) domains was monitored over time. The bottom images show the cell densities measured by the scanner after 85 h of growth. The background of the first image in the timelapse was subtracted from subsequent images. (B) Cell density profiles (averaged from the dashed orange squares in A) at $t = 85$ h. Increasing the width of the cooperator domain both decreases the final density of cooperators at the center of the line and increases the density of

cheaters at the frontier of the dark and illuminated domains. (C) Density of cooperators in the center of the illuminated area ($x = 10$ mm) and density of cheaters far from the line ($x = 20$ mm). The mean final density over the entire selected area is nearly constant (black trace). Concentration values are plotted on a logarithmic scale. Shaded grey areas and error bars represent the \pm standard deviation for three technical replicates. (D) Simulated concentration and cell density profiles obtained numerically for a 5.6 mm wide illumination line at $t = 15$ h and $t = 25$ h. At 15 h, hexoses are produced everywhere in the cooperating domain, which promotes the growth of cooperating cells, decreases the sucrose concentration, and creates a source of glucose (public good). This leads to competition for glucose and an increase in the cell density of cheaters located at the frontiers of the cooperating domain. Within the cooperating domain, competition for sucrose leads to a decay in the sucrose concentration towards the center and an increase in the density of cooperating cells at the frontiers with the cheater (dark) domain.

282 **Light spatial patterning is processed by cooperators as a spatial pass-band filter that filters out**
 283 **too-small or too-large cooperator domains**

284 Finally, we investigated the behavior of the cooperators/cheater landscapes created using periodic
 285 illumination patterns. Cells were illuminated by periodic lines of blue light (cooperator domains)
 286 separated by non-illuminated regions (cheater area; Figure 7A, Supplementary Movie 4). We
 287 chose to vary the wavelength of the patterns (corresponding to the sum of the width of a blue line

288 and a dark line) while maintaining a constant light-to-dark area ratio of 25% to 75% to keep the
289 global illumination constant. As a result, all experiments received the same illumination on
290 average, but the width of the cooperator and cheater domains varied. As previously observed,
291 cells mostly grew in the illuminated areas (cooperator domains); cells also grew in dark areas
292 (cheater domains), but only close to the illuminated domains. In this way, we could determine
293 the dependence of the density of cells across the cheater domains and the cooperating domains
294 as a function of the illumination wavelength. Varying the wavelength provides a way to model
295 the typical patch sizes of populations that can be found in real ecosystems. Specifically, at small
296 wavelengths, the separation between cheaters and cooperators was blurred. To analyze these
297 observations, we computed the cell densities at the center of the illuminated area (1) and the dark
298 area (2), which represent the final densities of the subpopulations of cooperators and cheaters,
299 respectively (Figure 7C). We also computed the difference between these two values as a proxy
300 of the cooperator benefit, which indicates how much cooperators grew compared to cheaters. We
301 obtained an asymmetric bell-shaped curve when we plotted the cooperator benefit as function of
302 the wavelength of the illumination pattern (Figure 7D).

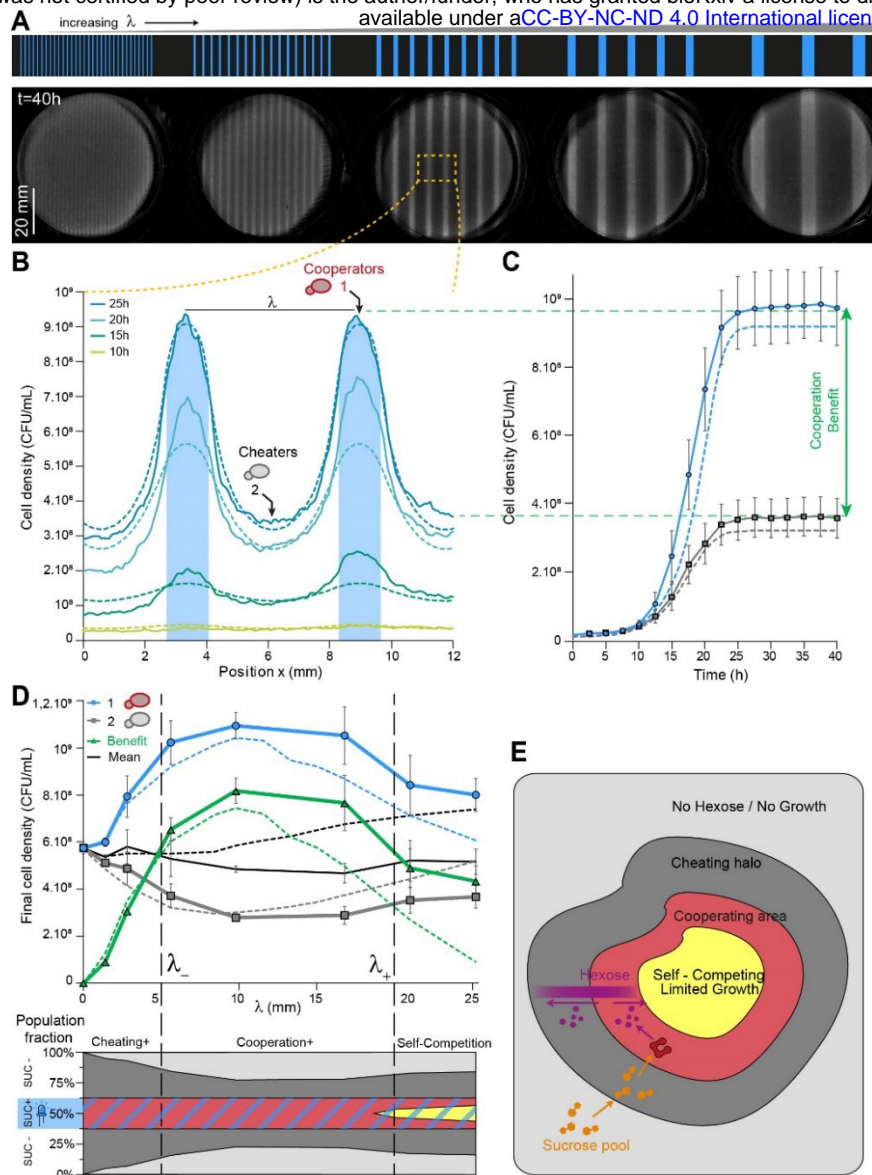


Figure 7. Periodic patterning of cooperator and cheater domains provides a quantitative measurement of cooperation and competition length-scales. (A) Periodic lines of light of various widths were projected, keeping a constant ratio between dark and illuminated domains (75% dark, 25% illuminated). Cell density was measured after 40 h of growth. (B) Averaged cell density profiles of a selected area (dashed orange rectangle) at different times for the wavelength of 5.6 mm for both experimental (solid lines) and numerical data (dashed lines). This illustrates that cooperators and cheaters grow in their respective domains, but the cooperator cells have a marked fitness advantage. (C) Evolution of cell densities over time in the center of a cooperator domain (illuminated) and the center of a cheater domain (dark). (D) Maximal cell density as a function of the wavelength of the illumination pattern for cooperators (blue), cheaters

(grey), and the averaged population (black). Dashed lines represent numerically simulated data. The green curve corresponds to the cooperator benefit, *i.e.*, the difference in cell density between cells in the cooperator domain and cells in the cheater domain. All data were obtained after $t = 40$ h. The bell shape curve of the cooperator benefit (green) represents a band pass filter with two cut-off wavelengths. Below the lower cut-off ($\lambda_- \sim 5$ mm, corresponding to illuminated lines of ~ 1.25 mm), the cooperator domain is too small to retain glucose for its own profit and cells in the dark areas can grow. Above the larger cutoff ($\lambda_+ \sim 20$ mm, corresponding to illuminated lines of ~ 5 mm), the cooperator domain is too large to be sustainable throughout the domain given the limited influx of sucrose crossing its frontier. Cells in the center of the cooperating area are now in competition for both sucrose and glucose (self-competition), which decreases the final cell density. (E) Sketch of typical growth domains of cheaters and cooperators for large domains, illustrating that cooperation and growth primarily occur at the frontiers between cooperators and cheaters.

- 303 Below a certain cut-off (< 5 mm), the smaller the wavelength, the smaller the cooperator benefit:
- 304 the cheaters are too close to the cooperators, and as such the cheaters have access to hexoses under
- 305 the same conditions as cooperators. Thus, cheaters have the advantage — even though they

306 continue to need cooperators to grow. Conversely, and in agreement with our previous
307 observations (Figure 6B), there is also an upper cut-off (> 20 mm), above which the cooperator
308 benefit decreases as the wavelength increases further.

309 The upper cut-off can be explained by self-competition between cooperators for sucrose. Indeed,
310 sucrose diffuses from dark areas to illuminated areas and cannot reach the center of the
311 cooperator area if the cooperator area is too large. More generally, we can interpret these results
312 using an analogy with spatial filters: the system behaves as a bandpass filter, whose transfer
313 function (the cooperator benefit), has a low cut-off wavelength ($\lambda_- \sim 5$ mm) due to cheater-
314 cooperator competition for hexoses and a high cut-off wavelength ($\lambda_+ \sim 20$ mm) due to cooperator
315 competition for sucrose (and hexose). Using our numerical model, we could further check that
316 each cut-off wavelength correlates to the diffusion of the corresponding sugars (low hexoses; high
317 sucrose) by artificially tuning the diffusion coefficients of these nutrients independently. Indeed,
318 from the set of equations of our model, we expected the cut-off wavelengths to increase when the
319 corresponding sugar diffusivity increases and is what we obtained numerically (Figure S10). The
320 diffusion of sucrose (a reserved carbon source for only cooperators) and glucose (a public good
321 for every cell) define the cut-off dimensions of the bandpass filter. Taken together, the data in this
322 study demonstrate that the size of cheater and cooperator domains is a key determinant of the
323 cooperators' benefit and the population growth.

324 Discussion

325 In this study, we built a yeast strain that produces the sucrose invertase Suc2p upon illumination
326 with light and tested the population growth dynamics and the benefits of cooperation in various
327 artificially shaped landscapes of cooperators and cheaters. The OptoSuc2 strain indeed acts as a

328 cooperating cell when illuminated but remains a cheater when kept in the dark because non-
329 illuminated cells cannot metabolize sucrose and instead rely on the glucose produced by adjacent
330 cooperating cells. We were therefore able to quantitatively explore the spatial metabolic
331 interactions between cooperators and cheaters.

332 Our main finding is the existence of two typical length-scales that set the domain size of both
333 cooperators and cheaters. Both length-scales are defined by the diffusion and uptake properties
334 of glucose and sucrose. The first length-scale is that of competition between cheaters and
335 cooperators and can easily be understood as the typical length over which glucose diffuses away
336 from cooperators. As exemplified in Figure 7E, if cooperator domains are smaller than this
337 wavelength, the number of cheaters that benefit from the produced glucose is comparable to the
338 number of cooperators, and cooperating provides no clear benefit. In other words, cooperator
339 domains need to be larger than λ_c to be distinguishable from cheater cells: too-small domains are
340 equivalent to what would be obtained with a homogenous mixture of cooperators and cheaters.

341 The existence of the second, upper length-scale, λ_+ , was unexpected and demonstrates the benefit
342 of grouping cooperators decreases when their domain is too large. We attribute this decrease to
343 the fact that cooperators not only interact by producing glucose that benefits their neighbors
344 (cooperation) but also by competing for the basal carbon source, in this case, sucrose. This
345 competition means that cells far away from the sucrose source obtain less sucrose and, as such,
346 produce less glucose. This is similar to the growth dynamics observed in any extending colony,
347 for which growth occurs mostly at the edge of the colony where nutrients are abundant.
348 Therefore, in a spatially structured cheater/cooperator system, the existence of large domains of
349 cheater cells (which cannot hydrolyze sucrose) ensures the presence of secured pools of sucrose
350 that can diffuse towards cooperator islands and be used first by the cooperator cells located at the

351 frontier between cheater and cooperator domains. Competition for sucrose takes place within a
352 cooperator domain, and the cells closest to the cheater domains are at an advantage. Said
353 differently, cooperating cells benefit from proximity to cheater cells, and cheater cells not only
354 function as cheaters but also as key actors that facilitate the growth of cooperating cells at the
355 domain frontiers. Therefore, cheater cells also help the cooperators to grow faster in the vicinity
356 of the cheaters' domain of existence. Furthermore, as exemplified by our study, this beneficial
357 role of cheater cells is only apparent when the domains of both cheaters and cooperators are large
358 enough.

359 As we proposed in the Results, this relationship can be summarized by an analogy with a spatial
360 bandpass filter, in which critical wavelengths are linked to the typical distances of the metabolic
361 interactions in the SUC2 yeast system: a lower cut-off wavelength ($\lambda_- \sim 5$ mm) due to cheater-
362 cooperator competition for hexoses and a higher cut-off wavelength ($\lambda_+ \sim 20$ mm) due to
363 cooperator self-competition for sucrose. We confirmed this analysis with our numerical model.
364 Thus, thanks to the ability to artificially create cooperator/cheater landscapes with light, we
365 defined the optimal range of domain sizes that create cooperating microbial niches. We anticipate
366 that our approach could be applied to other microbial ecosystems to explore the parameters that
367 define the landscape of metabolic interactions.

368 Our study illustrates the power of optogenetics and spatial patterning to decipher the metabolic
369 interactions at play in spatially complex multicellular assemblies such as colonies, biofilms, and
370 engineered consortia. There is a growing interest in engineering microbial consortia³³⁻³⁵, in which
371 different types of cells cooperate to more efficiently achieve specific biological functions
372 (bioproduction³⁶, living materials³⁷, or live therapeutics^{38,39}). Thus, it is essential to better grasp the
373 physical limitations of such systems — in particular, the impacts of chemical diffusion, the

374 composition of consortia, and their metabolic interdependence — on the dimensions of microbial
375 niches in such applications. We anticipate that optogenetics could be used to locally change the
376 cellular metabolic capabilities of microbial consortia by controlling the size of the domains of the
377 species. Such experiments will help to better understand cooperation and competition
378 mechanisms in microbial ecosystems and how to control complex synthetic microbial consortia
379 in real time. Importantly, we showed that intrinsic dimensions exist for microbial niches and can
380 be played with to optimize cheating and/or self-competition for resources. This study can guide
381 synthetic biologists to appropriately set the dimensions of Engineered Living Materials^{40,41} (ELM)
382 in microbial niches to sizes that are compatible with the desired properties of the ELM, which is
383 a crucial step required to obtain precise functionalities and efficient external control. We
384 extrapolate that such spatial constraints could also be considered when studying the spatial
385 organization of imbalanced microbiomes (dysbiosis), which are linked with major problems such
386 as human obesity, diabetes, skin disease and a myriad of other diseases due to alterations in the
387 human gut microbiome⁴², or unsustainable farm soil fertility associated with a high need for
388 nitrogen fertilization⁴³.

389 **Materials and Methods**

390 All materials described here and in the Supplementary Information are available upon reasonable
391 request. Datasets used to generate the figures can be found on the public Zenodo archive
392 10.5281/zenodo.7908455 while the code for numerical simulations can be found on Github
393 https://github.com/Lab513/Code_YeastCoop.

394 **Yeast strain construction**

395 All yeast strains used in this study are derived from the BY4741 yeast background (EUROSCARF
396 Y00000) and are listed in Table S1. All strains have the nuclear marker mApple-HTB2 and an
397 EL222 expression cassette. The marker and cassette were integrated using a classic lithium acetate
398 transformation protocol at the HTB2 or HIS3 locus using kanamycin G418 resistance and histidine
399 auxotrophy, respectively (using plasmids pPH_330 and pPH_297, respectively). All other genetic
400 modifications were undertaken using the CRISPR/Cas9 system⁴⁴. Guide RNA sequences (gRNA)
401 were obtained from oligo synthesis (IDT) and integrated into the plasmid pML104, which already
402 possesses a *Cas9* expression cassette and URA3 marker for auxotrophic selection. The repair
403 strands were obtained from either oligo hybridization for deletion or were PCR-amplified from
404 appropriate plasmids for integration (Table S2).

405 **Plasmid construction**

406 Plasmids were built using a custom Modular Cloning (MoClo)⁴⁵ approach, in which standard
407 genetic parts are assembled via GoldenGate assembly. The YTK kit (Addgene; Kit #1000000061)
408 was used as a source for all relevant non-coding DNA sequences — including promoters and
409 terminators, as well as pre-assembled integration vectors — and was supplemented with our own
410 components: the pC120 promoter, the SUC2 protein, the self-cleaving peptide P2A, the minimal
411 promoter p2RGal, and the mRNA HairPin degenon tag.

412 **Growth conditions**

413 Cultures (2 mL) were grown overnight (~18 hours) in 14 mL culture tubes (Falcon) in YPD media.
414 Day cultures (5 mL) were grown to exponential phase in filtered synthetic complete media (SC)
415 supplemented with 2% glucose. All cultures were incubated in a Innova 4230 incubator at 30°C
416 with orbital shaking at 250 RPM. SC media is composed of 6.7 g Yeast Nitrogen Base without

417 amino acids (Difco 291940) and 0.8 g complete supplement mixture drop-out (Formedium
418 DCS0019) in 1 L. Care was taken to reduce unwanted light exposure as much as possible before
419 the start of the experiments: thawed strains on agar plates and precultures were covered in
420 aluminum foil, and all cell handling procedures were conducted without direct exposure to light
421 (low ambient light). To prevent uncontrolled hydrolysis of sucrose in water, we used 20% *w/v*
422 sucrose stock solution buffered at pH = 8 with 1 mM Tris buffer and stored the solutions at 4°C.

423 **Enzymatic quantification**

424 We measured the fluorescence and invertase activity of cells after a 2 h enzyme production phase.
425 To compare the expression of invertase in the synthetic strains and WT strain, the production
426 phase was performed in a 0.05% glucose liquid media to repress the native SUC2 promoter.
427 Overnight cultures were washed with 10 mL of sterile water, resuspended in 10 mL of SC media
428 at 0.05% glucose to obtain a final OD₆₆₀ of 0.5, then 1 mL aliquots were placed in triplicate in
429 plastic-bottomed black 24-well plates (Eppendorf Cell Imaging Plates ref. 0030741005), and the
430 plates were placed onto a custom made light plate apparatus (LPA)²⁷ to control blue light
431 illumination in each well ($\lambda = 460$ nm, $I = 2.8$ mW/cm²). After 2 h of illumination at 30°C with
432 orbital shaking (at 225 RPM), 500 μ L aliquots of the cultures were removed, placed on ice to
433 inhibit yeast growth, then washed with 500 μ L of 10 mM sodium azide solution to block further
434 glucose import. The aliquots were then washed with 500 μ L of 50 mM sodium acetate buffer (pH
435 = 5.1) and resuspended in 300 μ L of the buffer; 100 μ L was placed into a PCR tube for the
436 enzymatic activity assay and 200 μ L was incubated on ice for the Bradford protein quantification
437 assay.

438 For the enzymatic activity, 10 μ L of fresh 1 M sucrose solution was simultaneously added to all
439 samples using a multichannel pipette and the reactions were incubated at 37°C for 10 min in a
26

440 thermocycler and then heated to 99 °C for 5 min to denature the invertase. Glucose concentrations
441 were determined using the colorimetric enzymatic Glucose (HK) Assay Kit (Sigma) and a Cary
442 50 Scan spectrophotometer.

443 To determine the corresponding total protein contents for normalization, glass beads were added
444 to the 200 µL aliquots and the cells were lysed by three rounds of 3 min vortexing separated by 1
445 min incubation on ice to prevent overheating. The supernatants were serially diluted with water
446 and 100 µL samples were mixed with 100 µL of Bradford assay solution in 96-well plates. The
447 plate was placed in an EnSpire plate reader (PerkinElmer), incubated for 5 min at 25°C with mild
448 shaking, and the absorbance values were measured at 595 nm.

449 **Microscopy**

450 Cells were placed on agar pad on a glass slide and covered with coverslip for microscopic
451 observation. Fluorescence images for quantification were captured using an inverted Olympus
452 IX81 epifluorescent microscope, an Xcite exacte light source (I = 30%), and a filter cube (EX) 514
453 nm/10 (EM) 545 nm/40 (49905 – ET) with a 1000 ms exposure time. Yeast cells were segmented
454 using Yeast-spotter⁴⁶. To image cell colonies embedded in the gel, we used a Nikon AZ100
455 macroscope equipped with a Nikon camera Color DS-Ri1 and a 2x objective AZ-Plan Apo (NA:
456 0.2/WD: 45 mm).

457 **Growth rate quantification**

458 After the 2 h invertase production phase described above, we added 25 µL of 20% *w/v* sucrose
459 stock solution (1 mM Tris buffer pH = 8) to obtain a final sucrose concentration of 1%. The plate
460 was placed in a Spark plate reader (Tecan) at 30°C and the OD was measured at 660 nm every 5
461 min (average of five measurements at different locations within one well). In between these

462 measurements, cells were kept in suspension by 240 s of orbital shaking with an amplitude of 4.5
463 mm followed by 60 s of linear shaking with an amplitude of 6 mm. The resulting growth curves
464 were fitted using the function smooth spline in R with 15 degrees of freedom. The derivative of
465 the fitted curves was obtained, and its maximum value was used as the maximal growth rate per
466 well.

467 **Microscopy imaging in microfluidic chambers**

468 We used an automated inverted epifluorescence microscope Olympus IX83 equipped with a
469 Motorised stage (Prior Pro scan III), a Zyla 4.2 sCMOS Andor camera with an Olympus 20X Plan
470 (N.A. 0.4) objective, a DMD (MOSAIC3 Andor) and a pE-4000 CoolLed as a light source to achieve
471 spatially resolved optogenetic activation. For optogenetic activation, we used a 460 nm LED at
472 20% intensity through a filter cube (EX) 470 nm/40; (EM) 525 nm/50 (49002) with a 200 ms
473 exposure time every 6 min. Brightfield images were also captured every 6 min. Microscopy
474 experiments were carried out in a thermostat chamber set to 30 °C. Liquid perfusion was
475 delivered using an Ismatec IPC (ISM932D) peristaltic pump at 50 µL/min. Particle image
476 velocimetry (PIV) analysis was performed using the open-source JPIV software
477 (<https://github.com/eguvep/jpiv/>) for an interrogation window containing 128 pixels. Isolated
478 non-significant vectors were removed from the velocity vector map. We retrieved the growth rate
479 map by determining the divergence of the velocity field. We did not display the negative values
480 in the divergence map as negative values are due to unchanged pixels in the areas that do not
481 contain cells at the border of the microcolonies.

482 **OptoCube**

483 The OptoCube is composed of a static incubator (Memmert UF 160) equipped with a prototyping
484 DMD projector for light stimulation (DLP® LightCrafter™ 4500 TexasInstrument) and a flatbed
485 scanner (Canon LiDE400) for image acquisition. VueScan© software running on a Windows
486 computer is used to control the scanner to perform periodic scans to measure cell growth in the
487 Petri dishes over time. The DMD pattern sequence is controlled by a microcontroller board
488 (Arduino Uno) that is synchronized with the image acquisition. This sequence can stimulate and
489 monitor up to 15 Petri dishes (6 cm diameter) per experiment. The light intensities of the DMD
490 pattern range can be adjusted from 0.0014 mW/cm² to 1.13 mW/cm² using a blue LED at $\lambda = 460$
491 nm. The incubator was set to 50% internal ventilation and 50% air inlet. More information on how
492 to build and use the OptoCube is provided in the Supplementary Information. For the data
493 analysis, only the central part of the plate was assessed to avoid boundary effects, and
494 background subtraction was performed using the first image in the timelapse.

495 **Plating procedure**

496 Bilayered agar plates were prepared before each experiment. The bottom layer was composed of
497 3.8 mL SC media with 1% *w/v* Phytigel and 1% *w/v* sucrose. To avoid hydrolysis and
498 caramelization of the sucrose, the SC media and Phytigel solution was autoclaved, then buffered
499 20% *w/v* sucrose stock solution (1 mM Tris buffer pH = 8) warmed to room temperature was added
500 to the hot gel. The solution was poured into small Petri dishes (60 mm; TPP ref. 93060) using 5
501 mL serological pipettes under a laminar flow-hood (Thermo Fisher MSC-Advantage™ 0,9) and
502 allow to solidify for 5 to 10 min. The top layer was made of 0.5% *w/v* agarose D5 (Euromedex ref.
503 D5-C) mixed with a concentrated solution of washed cells at a 40:1 ratio to obtain a final OD of
504 0.1 in the agarose gel. Briefly, the agarose was melted in a microwave, aliquoted into 15 mL Falcon
505 tubes, equilibrated at 46°C in a water-bath, then the cell solution was added and 1.5 mL aliquots

506 were immediately plated on top of the bottom layer using a 5 mL serological pipette. The plates
507 were allowed to solidify for 5 min, then the lids were added, and the plates were sealed with
508 Parafilm.

509 To avoid issues due to light reflection from the scanner, we held the lids at a 5° angle using a
510 home-made 3D printed adapter. To reduce droplet formation due to condensation, both sides of
511 the lids were washed with 1 mL of 0.05% (*v/v*) Triton 100X in 20% ethanol. For the image
512 patterning (Figure 4E-G) we used a standard sized Petri dish (external diameter 94 mm Greiner
513 ref. 633180) with 10 mL of SC media containing 1% *w/v* Phytigel and 1% *w/v* sucrose overlaid
514 with 4 mL of agarose cell suspension. All plates had a total gel thickness of 2.35 mm (1.68 mm
515 nutritive layer and 0.67 mm yeast growing layer).

516 **Simulations of yeast growth on sucrose**

517 All simulations were performed by numerically solving a set of partial differential equations
518 (PDE). The model and its parameters are detailed in the Supplementary Material. The numerical
519 solution was obtained in Python using the PDE solver scikit-finite-diff package⁴⁷.

520

521 **Acknowledgments**

522 The authors would like to thank J. Avalos (Princeton) for kindly providing the original EL222
523 plasmids, as well as the Nikon platform of the PICT IBISA imaging platform at the Institut Curie.
524 The authors thank their colleagues for critical reading of this manuscript. This work was
525 supported by the European Research Council grant SmartCells (724813) and received support

526 from ANR grants ANR-16-CE12-0025-01, ANR ANR-16-CE33-0018, ANR-11-LABX-0038, and
527 ANR-10-IDEX-0001-02.

528 Author Contributions

529 MLB performed all experiments. SP, AB, CC, and SB performed the molecular biology
530 experiments. VS analyzed the mathematical model. MLB and PH conceived and designed the
531 study. MLB, BS, AB, and PH wrote the manuscript.

532 Supplementary Information

533 The Supplementary Information contains two extended materials and methods describing the
534 mathematical model and the OptoCube, eleven supplementary figures, and four supplementary
535 movies. Data used to generate the figures and analysis of this article are available in a public
536 repository (10.5281/zenodo.7908455).

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