Interactive and scalable biology cloud experimentation for scientific inquiry and education

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A real-time interactive, fully automated, low-cost and scalable biology cloud experimentation platform could provide access to scientific experimentation for learners and researchers alike.

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Many access barriers to life-science experimentation exist for academic and commercial research, mainly due to professional training needs, equipment purchase and operation costs, and safety considerations[1]. Computational cloud and time-sharing paradigms[2,3] have recently inspired the development and deployment of cloud-based experimentation labs for biology research, such as commercial platforms that can execute experiments semiautomatically[1,4] and the browser-based puzzle game EteRNA, which provides experimental feedback for citizen scientists[5]. However, these platforms still face limitations, such as relying on batch processing with no opportunity for real-time interaction while the experiment is running, hindering the exploration that hands-on experimentation allows and taking days to return results due to long experimental turnaround times.

Cloud labs are also poised to help solve significant educational challenges. Familiarity with advanced scientific practices and 'authentic inquiry'[6–8] are imperative for K–12 and college education (for example, Next Generation Science Standards)[8,9] but are difficult to achieve in real-world classrooms given logistics and cost[6,10]. In addition to traditional physical hands-on labs, virtual and remote labs have recently been successfully deployed, with each modality having its distinct advantages given educational goals and situational context[11–15]. Physical remote labs for life science education are comparably underdeveloped[12], in large part because of the associated logistics of specimen handling. We have previously developed, demonstrated and deployed the first educational biology cloud lab with slime mold chemotaxis experiments[16], which was suited for non-real-time interactions but did not scale cost-effectively, given back-end logistics and turnaround time.

Here we conceptualize, implement and validate a biology cloud experimentation platform (**Fig. 1**) that (i) enables the types of inquiry mandated for professional science and educational purposes; (ii) has a low entry barrier and can be used even at the middle-school level; (iii) is real-time interactive; (iv) has a fast result turnaround time (within minutes); (v) is fault tolerant against biological variability and failure; (vi) scales to millions of users worldwide from a design as well as an economic viewpoint; (vii) has a large exploration and discovery space; and (viii) generalizes to many other experiment types.

Interactive biology experimentation online

Our cloud platform focuses on the photoresponsive behavior of *Euglena gracilis*, a single-celled organism ~50 µm long (**Supplementary Text 1–3**). While swimming forward, it rolls and wobbles around its long axis to scan all directions for light with its single eye spot (**Fig. 1a**). *Euglena* are commonly used in hands-on biology education[17–20] and are relevant for basic research[21–23]; for food, chemical and fuel production[24]; and as biosensors[25].

Experiments are executed on a cluster of biotic processing units (BPUs)[16], instruments that combine sensors, biological material, actuators and a microcontroller (**Fig. 1b** and **Supplementary Figs. 1–5**). Each BPU consists of a webcam microscope containing a microfluidic chip with four attached light-emitting diodes (LEDs) that provide directional light stimuli to *Euglena* (**Supplementary Video 1**), which are cultured in reservoirs and supplied to the microfluidic chips via automated valves as needed. The microcontroller controls the LEDs, streams live video, postprocesses data and communicates with the central server (**Supplementary Text 2** and **Supplementary Fig. 5**). We adopted the task scheduling concepts of high-performance computing[26] to design the central server. This server assigns BPUs and remote users according to a non-exclusive group allocation policy (**Fig. 1c** and **Supplementary Text 2.4**), handles distinct BPU types, routes experiments to the best-suited BPU and optimizes wait times through load balancing.

Via a web interface, users choose a specific BPU or are autorouted (**Fig. 2a**) to execute experiments in real-time live mode or in asynchronous batch mode (**Supplementary Video 2**). The live mode user interface (**Fig. 2b**) employs a virtual analog joystick to control intensities of four LEDs (**Fig. 1b**) to induce directional light stimuli to *Euglena*; two live video streams show the microscopic *Euglena* responses and the macroscopic LED actuation. In batch mode (**Fig. 2c**), the user designs and uploads a program that contains

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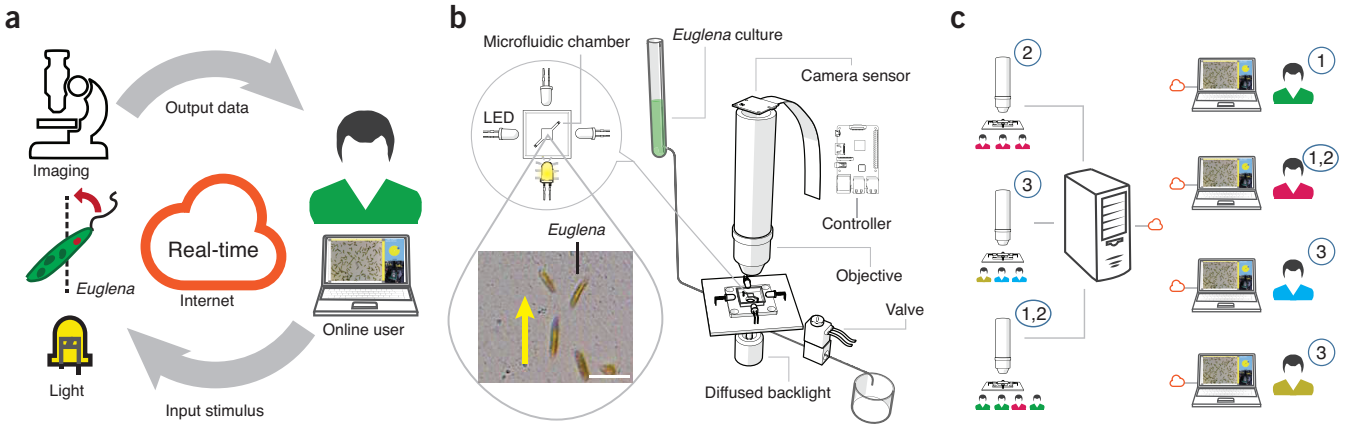

Figure 1 A biology cloud experimentation platform that is real-time interactive and scales cost-effectively to large user numbers and versatile applications. (**a**) The experimental model allows online users to send light stimuli to biological substrates, such as phototaxic *Euglena* cells, and observe the response in real time. (**b**) The back-end hardware consists of a webcam microscope targeted at a microfluidic chip positioned between four LEDs. The chip contains *Euglena*, which can be replenished automatically from an upstream reservoir via an electronic valve. This stimulus-biology-sensor module includes its own microcontroller and is conceptualized as a BPU. Scale bar, 50 µm. (**c**) An array of BPUs is monitored and managed by a central server. Both users and BPUs belong to different groups (circled numbers), and users are routed to the appropriate BPU (same group), optimizing for wait time and BPU quality (**Supplementary Text 2** and **Supplementary Fig. 5**).

instructions for time sequences of LED intensities. The back-end server automatically tracks shape and motion of all motile cells and overlays these data on the captured videos (**Fig. 2d** and **Supplementary Text 2.5**). Video, stimulus and track data are stored for future download and analysis.

Live mode enables open-ended, real-time-interactive exploration of *Euglena* biophysics followed by quantitative substantiation in batch mode. A user can test *Euglena*'s response to changes in light direction and intensity and then observe variability among traces (**Fig. 2d**). The prevalent behavior is negative phototaxis, but localized tumbling and changes in cell morphology are also observable (**Supplementary Video 3**). We characterized the system by executing periodic light on–off experiments in batch mode, measuring the time constants (τ)for cell alignment with light on, $\tau_1 = 6.7 \pm 2.4$ s ($n = 6$; mean ± standard deviation throughout), and subsequent light-off orientation decay, $\tau_2 = 9.9 \pm 2.6$ s (**Fig. 2e**). We defined responsiveness to quantify how well *Euglena* aligned with light after 15 s of light exposure (on a scale of 0–1, for random (0) to perfect (1) alignment; **Supplementary Text 2.2** and **Supplementary Video 4**). This responsiveness score also depends on light intensity and exhibits Hill-equation-type characteristics (**Fig. 2f**). Hence, experimenters can investigate *Euglena*'s response to changes in light direction and intensity on the time scale of seconds, study its long-term behavior over weeks (**Fig. 3**), and record and download this data for offline analysis (**Supplementary Video 2**).

Robust, cost-effective and dynamic scaling of BPU clusters

To make this BPU cluster cost-effectively scalable and tolerant against failures in hardware, software and 'bioware' (**Supplementary Fig. 5**), we extended high-performance computing concepts to include biology by automonitoring its state: the system submits batch experiments to each BPU every hour (**Fig. 2e**) to measure three variables—cell density, motility and light responsiveness. Population density and responsiveness monitored over 10 d can be stable (**Fig. 3a**), undergo microecological fluctuations (**Fig. 3b**) or be susceptible to external ambient light cycles (**Fig. 3c**). This biological variability emphasizes a key challenge of any cloud lab, i.e., to consistently provide a prespecified experimentation experience. User testing revealed that responsiveness above ~0.4 was easily recognizable (**Supplementary Text 2.2** and **Supplementary Table 1**), providing a quantitative target for good BPU performance. (Even lower responsiveness highlights interesting and noticeable *Euglena* behavior; **Fig. 2f**). BPUs not meeting specifications can often be recovered by automated flushing (**Fig. 3b**); organisms and chips are replaced every ~4 weeks, leading to a maintenance burden of ~10 min per week per BPU (**Supplementary Text 3.2**). Under current maintenance protocols, individual BPU performance was good ~61% of the time, and continued experimentation did not decrease BPU performance or *Euglena* responsiveness (**Supplementary Text 3.1** and **Supplementary Fig. 6**). Each BPU can handle >100,000 experiments per year for ~$0.01 per experiment (~4 min per experiment; setup and maintenance cost of ~$1,000 per BPU per year), with negligible wait times for randomly accessing users. Dynamic addition of BPUs ('hot swapping'; **Fig. 3d**) or queuing of batch experiments increases throughput (**Supplementary Text 3.3**). Running a cluster with six BPUs guarantees the availability of at least one good BPU 99.5% of the time at an average availability of 3.6 BPUs, with users automatically routed to good BPUs.

Educational use cases

We evaluated the platform in three educational contexts encompassing and illustrating various aspects of future usage in education and even research (**Fig. 4**). We primarily assessed (i) whether the technology works robustly, (ii) whether it can be operated even by middle-school students and (iii) whether it achieves the key elements of best laboratory practice as described in America's Lab Report[8], i.e., integration into the flow of instruction, alignment with process and content learning goals, and engagement of students in reflection and discussion. The cloud lab was embedded into regular instruction and scaffolded along the main phases of the inquiry cycle[7]; more details on study design and outcomes are provided in **Supplementary Text 4**.

First, we studied whether university students taking a professor-led theory-based biophysics class could successfully carry out experiments and sophisticated quantitative data analysis from home in a self-paced manner (**Fig. 4a**,**b** and **Supplementary Video 2**). Working individually over 14 days, ten students completed a homework project focusing on concepts regarding microswimmers, diffusion and low-Reynolds-number hydrodynamics[27]. Using the live mode (**Fig. 2d**), students explored *Euglena*

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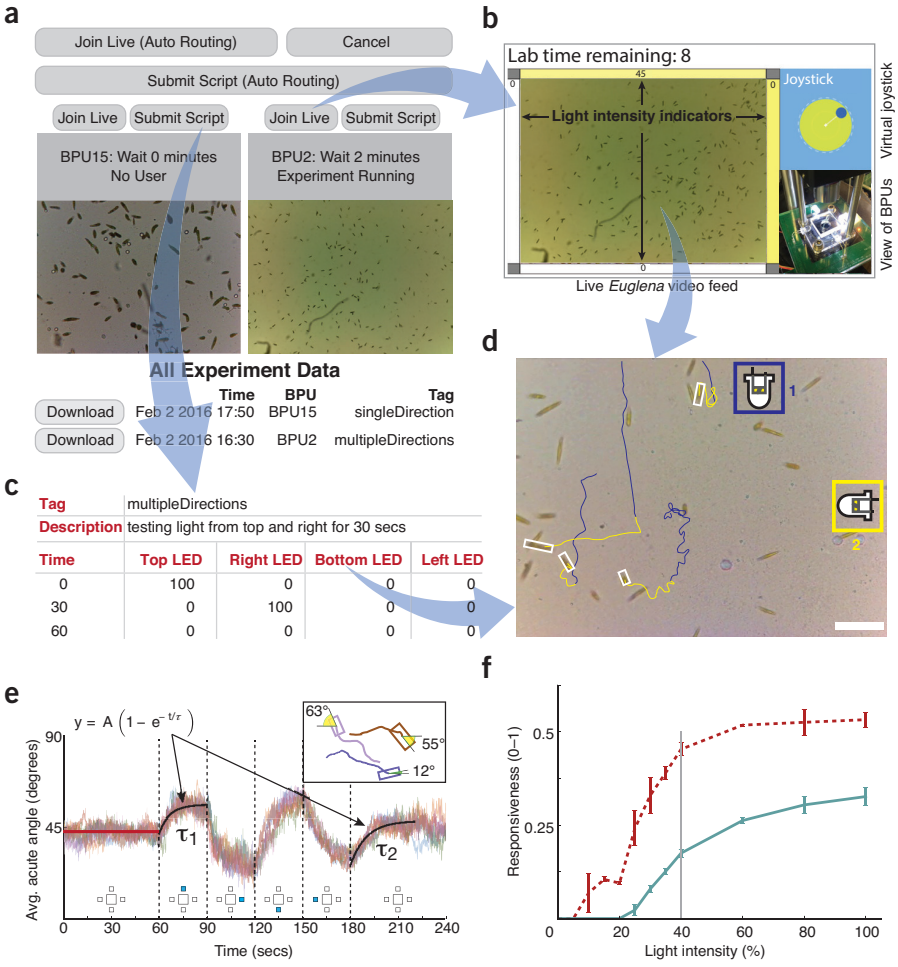

Figure 2 The cloud lab enables biophysics experiments on timescales from seconds to minutes in live and batch modes. (**a**) Schematic of landing webpage presenting choice of available BPUs, choice of interactive ("Join Live") or batch mode ("Submit Script"), as well as previously recorded experimental data ("Download"). (**b**) The live mode provides a virtual joystick to control the intensities of the four LEDs. *Euglena* response is fed back in real time through a live video stream; a secondary live video stream shows the BPUs in action. (**c**) Example of preprogrammed instructions for batch mode. (**d**) Example of an experimental result in response to a light stimulus sequence from top to right (blue and yellow, respectively), using the script in **c**, and showing *Euglena* swimming traces extracted automatically. Scale bar, 100 μm. (**e**) Response and relaxation time constants of *Euglena* to align with light direction at maximum light intensity (orientation measured in acute angles 0–90°; see inset: 0 and 90° correspond to perfect alignment; A, acute angle amplitude; t, time; τ, time constant). (**f**) Population level orientation responsiveness (0–1, from random to perfect response) to varying light intensities; procedure as in **e**. Results for two BPUs highlight biological variability when cultures are in different states; vertical bar marks approximate transition toward saturation (three trials performed for each of the ten data points in each of the two curves; error bars are 1 standard deviation).

light-response behavior and made cells swim along geometric paths (**Fig. 4a**). Students were able to self-discover semiquantitative relationships, for example, reporting that the "fraction of *Euglena* participating in the directed motion seems to increase as you hold the joystick longer, and depending on the intensity of the light." They performed back-of-the-envelope analyses regarding *Euglena* size (~50 μm), speed (~50 μm/s), and drag and propulsion forces (~10 pN)[27], experimentally confirming theoretical lecture content. Students then analyzed self-generated large-scale batch data (**Fig. 2c**; typically hundreds of autotracked cell traces in a 1-min movie; **Supplementary Video 2**) in Matlab, testing two hypotheses. (i) *Euglena* behave like active particles opposed to passive Brownian particles. Ninety percent of students found that the expected relationship of root-mean-square displacement versus time was violated and the apparent diffusion coefficients (D) were too high given cell size (student example: expected: D ~0.01 μm^2/s; measured D ~2,000 μm^2/s; **Fig. 4b**). (ii) The population average velocity changes between dark and light conditions. Sixty percent reported that cells slowed when the light was on, 10% reported that cells sped up and the others found no significant differences (student example: 26 ± 12 μm/s (n = 389) for light off versus 13 ± 10 μm/s (n = 431) for light on, respectively; **Fig. 4b**. A decrease in velocity for increased light is expected[22], but results may vary given experimental conditions. These results demonstrate that 1-min experiments provide students with hundreds of autotraced cells supporting sophisticated statistical analysis. The logged data revealed that students accessed the system at their own convenience at day and night (**Supplementary Fig. 7**) and engaged in different modes of experimentation, from "playful" (as self-described by multiple students) to more systematic testing of one or multiple light directions and intensities (**Supplementary Figs. 8** and **9**). Student's feedback and the fact that they each ran 11 ± 6 experiments (three were sufficient for the assignment) indicated that the platform affords ease of experimentation and incentivizes self-driven exploration. Students' feedback also captured many items that motivated this project, including ease of exploration and of gaining intuition (30%); ease of obtaining and analyzing large batch data sets (30%); and minimal manual labor, logistic effort and need for technical understanding, which allowed more focus on thinking (50%). Examples of feedback include, "It was fun to play around with real organisms … didn't require thinking about the set up"; "Playing for a few minutes gave me some intuition"; "Text mode allows more detailed and controlled tests"; "Very little rote labor time, spent most time thinking!"

Second, we studied whether real-time experimentation could be integrated into middle-school classroom settings and whether it could be combined with simulation-based platforms to support sophisticated model exploration practices as prescribed by the Next Generation Science Standards[9,28] (**Fig. 4c**,**d** and **Supplementary Video 5**). During a 50-min class period, 27 students (7th and 8th grades, three classes total) working in pairs executed the following activities (**Supplementary Table 2**). In one class all pairs ran their own live experiments, while in two classes the live experiment was projected to the front wall and operated by one student while the whole class discussed and suggested joystick movements. This generated the hypothesis that *Euglena* move away from light. Then, student pairs tested this hypothesis by measuring the percentage of *Euglena* cells moving away from light in previously recorded movies. The entire class discussed possible mechanisms by which *Euglena* may perceive and respond to light. Student pairs then engaged with a 3D biophysics modeling environment (**Fig. 4c**) in which a *Euglena* cell was represented as

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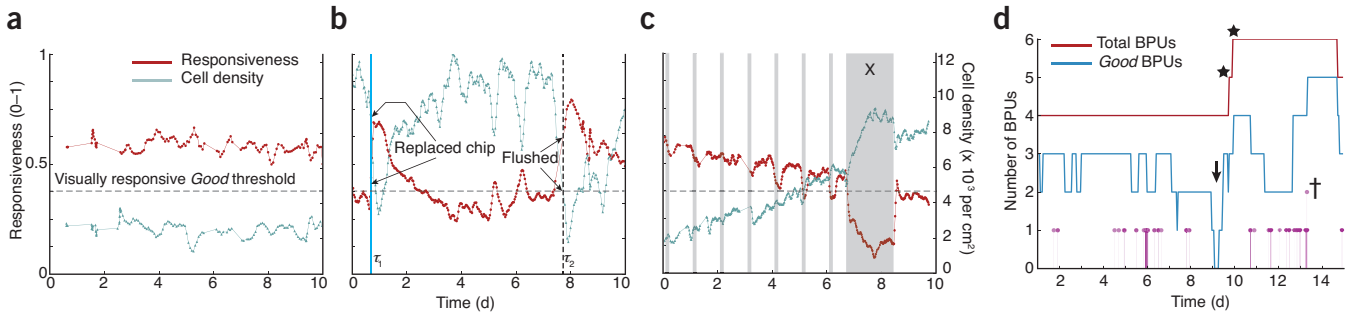

Figure 3 Automonitoring framework and BPU multiplexing enable scaling and robustness of the cloud lab over a timeframe of weeks and demonstrate its potential applicability for long-term microecological studies. (**a**) Example of ideal BPU performance over 10 d. All BPUs are automonitored by a central server that runs automated experiments hourly, measuring cell density and light responses (protocol as in **Fig. 2e**). (**b**) Example of BPU in which chip and organisms were renewed (t_1; arrows indicate levels of responsiveness (top) and cell density (bottom) at t, followed by slow deterioration over 1 week; subsequent flushing (t_2) recovered cell density and responsiveness. (**c**) Example influence of ambient external light on a BPU. Either lights were shut off at night (gray bar), leading to periodic cellular responses, or the whole setup was shielded from external light (X), leading to a more pronounced effect followed by recovery. (**d**) Performance summary of BPU cluster over 14 d (deployment during user study shown in **Fig. 4a–c**), number of total BPUs (red) versus good BPUs (blue). Note performance dip (arrow), followed by self-recovery; BPUs were added dynamically to the cluster (stars). Cluster capacity was ~10,000 experiments over 2 weeks (area under blue curve); only 116 experiments were executed (purple lines indicates student activity; note occurrence of simultaneous demand for two BPUs, marked by dagger). Even during peak demand, availability was never exceeded. A 5-h time-weighted filter was applied to data in **a–c**. (BPU cluster was run continuously over more than 2.5 months, during which 3–6 BPUs were online all the time; BPU availability and 'goodness' of behavior are as described in the text.)

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cuboid surging along and rolling around its long axis. This model had three user-defined parameters (Surge, Coupling, Roll), with the instantaneous pitch velocity being proportionally coupled to the amount of light entering through one body side. Depending on parameter choices, the model mimics many light-responsive behaviors, including positive and negative phototaxis, straight travel versus meandering swimming paths, and even chaotic behavior (**Supplementary Video 5**). Students explored the function of these three parameters by iterating among self-chosen parameter configurations and then running and stimulating their model through joystick operations, with the overall goal of matching a prerecorded swimming path. Students ran 19 ± 4 simulation experiments; all students found fitting parameter configurations[29]. Cluster analysis of the activity logs (**Fig. 4d**) suggests three dominant strategies of students' model exploration: (i) systematic change of one parameter at a time followed by exactly one test experiment (40%); (ii) alternating between multiple cycles in this systematic stage, followed by extended experimentation with a fixed parameter configuration (30%); and (iii) unstructured transition between changing zero, one or multiple parameters simultaneously (30%). These patterns are consistent with the literature on students' productive model explorations[30]. Students engaged in generative and productive discussions, which led to content-aligned discoveries such as that the roll parameter is required for the cell to "[see] in every direction" or methodological discussion about how the real *Euglena* differs from its model (**Supplementary Tables 3–5**). Post-tests revealed that students learned the concept of *Euglena* phototaxis (90% correct) and engaged in scientific argumentation.

Third, we studied whether this cloud lab could be operated and curated through existing third-party educational content management systems that would allow its wider dissemination (**Fig. 4e,f, Supplementary Data 1,** and **Supplementary Video 6**) and whether the batch mode feature would be suitable for middle school. We chose the iLabStudio.org (http://www.ilabstudio.org/labjournal) platform[31], which enables teachers to create personalized lesson content around online physics and chemistry experiments and to manage student progress. We implemented a general application programming interface (API) and a corresponding iLab batch interface (**Fig. 4e**). During two 50-min class periods on successive days, 34 students working individually or in pairs (8th grade, two classes) carried out the following activities. Students watched prerecorded videos of interactive experiments and then engaged in an open classroom discussion, generating hypotheses about how *Euglena* would react to student generated light stimuli. Students responded with "moving to the light" (60%)" or moving "away from light" (20%), or described more complex behaviors (20%); some provided an explanation, such as the "need for photosynthesis" or that the "light might cook them" (both are correct depending on light intensity). To test their hypotheses, students then designed and ran batch experiments (29 total), i.e., entering intensity, duration and direction of light stimulus. The chosen stimulus sequences revealed versatile experimental designs, including systematic variation of light direction or intensity, testing of multiple variables in sequence and seemingly less-structured designs (**Fig. 4f** and **Supplementary Fig. 10**). Students provided justifications for their rationales, ranging from "raise the intensity" to "put random numbers." We characterized 60% of the designs as sufficiently systematic to test for the influence of light intensity, direction or both. Students and teacher discussed experimental designs and results as they were delivered sequentially from the experimental queue. Based on their own data, students reported moving to the light (25%); away from light (45%); and no directional response (30%). These heterogeneous results arose in part because some students did not choose high enough light intensity levels to induce noticeable negative phototaxis. When students afterwards considered how to improve their experimental designs, 50% suggested investigating the effect of light intensity more closely. When asked their opinion of this experiment, 85% expressed liking it, and 30% explicitly mentioned *Euglena* or living organisms.

From these use cases, we conclude that this platform ran robustly and that we successfully deployed an experimentation model that did not exist in the classroom before, i.e., real-time interaction with microscopic cells on a timescale of seconds, which additionally supported complex, quantitative data analysis and modeling. This should be contrasted with current instructional standards and school lab

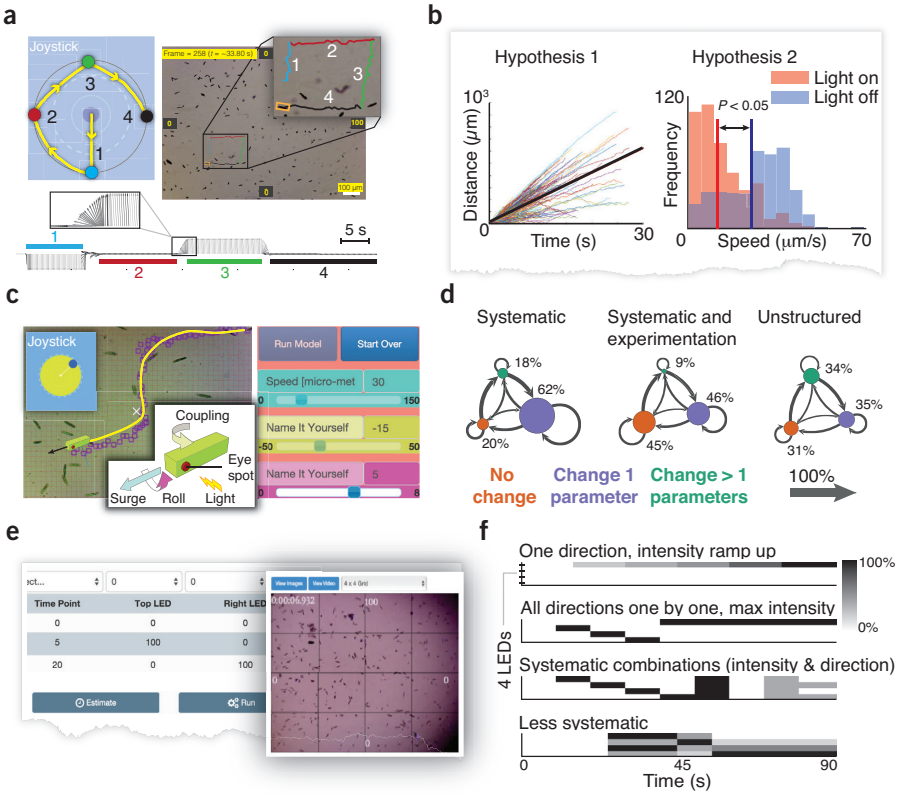

Figure 4 User studies in middle-school and college settings demonstrate utility of platform for face-to-face and online education. (**a**) University students performed exploratory joystick-based experiments from home, gaining intuition about *Euglena*'s phototaxic behavior by making it swim in geometric trajectories such as a rectangle. The yellow line on the joystick traces the motion of the joystick with dots indicating holding positions. The gray plot (below) shows joystick directions with respect to time (holding positions color code). The inset shows zoomed in view of an *Euglena* path due to the light induced by this joystick motion (path segments color coded). Scale bar: 100 μm. (**b**) Automatically generated large-scale data (hundreds of cells) using batch mode allowed students to test two hypotheses. Automatically generated large-scale data (hundreds of cells) using batch mode allowed students to test two hypotheses. Hypothesis 1: *Euglena* behave like active particles opposed to passive Brownian particles. (Colored lines, individual traces; black line, average). Hypothesis 2: The population average velocity changes between dark and light conditions. (Light off, blue, $n = 389$; light on, orange, $n = 431$; Students performed Kolmogorov–Smirnov tests that rejected the null hypothesis—i.e., that the distributions are the same—at a 95% confidence level). (**c**) Middle-school students engaged in modeling *Euglena* phototaxis after in-class joystick experimentation. *Euglena* is modeled as a cuboid surging and rolling around its long axis; pitch velocity is coupled proportionally to the amount of light entering through one side. Three sliders enable setting surge, roll and coupling, followed by virtual experimentation with a joystick (equivalent to **a**) to explore the model's 3D behavior. (**d**) Clusters (silhouette score = 0.47 in 0–1 range) of student approaches to model exploration (5, 4 and 4 student pairs adopted systematic, systematic and experimental, or unstructured approaches, respectively. (**e**) Middle-school students designed batch experiments via the third-party platform interface iLab and analyzed generated movies. (**f**) Examples of student's main experimental design categories. (Number of students for each study: see text.)

practices, i.e., passive and qualitative observation of living cells under a microscope, with fixed slide samples, videos or pictures being even more common; in the most sophisticated and rare scenario, students observe a population-level aggregation of *Euglena* in a petri dish under external light over the course of 15–30 min[18]. Ideally, five to ten live and batch mode experiments could be combined to enable initial free-form exploration followed by controlled experimental design. We note that new opportunities for mining of educational data sets are emerging ('learning analytics')[32,33] as logging user activity data on such platforms is easier and more scalable than in traditional physical labs. For example, revealing differences in student strategy and systematicity (**Figs. 4d,f**) is useful for instructors to help their students and also for educational research in general. The user numbers in our studies are too small to draw more specific conclusions, but this work only marks the beginning of future extended design-based research and wider dissemination[34].

Discussion

The experiment throughput and cost of this platform scales to serve massive user numbers and diverse curricular demands, from middle-school to college and massive open online courses[35]. There are more than 15 million high-school students in the United States alone[36], and hundreds of millions in developing countries or remote locations could access such platforms via increasingly ubiquitous smartphones[37]. We estimate that providing lesson plans similarly to Study 1 (**Fig. 4a,b**) to 1 million users per year could be achieved with ~250 BPUs, a modest back-end footprint of ~10 m^2 and a regular 1 Gb/s internet connectivity; cloud lab access for all students in a class at ~1 cent per experiment would cost instructors less than the price of one living *Euglena* sample (**Supplementary Text 3.4**).

This technology also has significant potential for primary life-science research. It already supports complex investigations of microswimmers (**Fig. 2e,f**) and microecology (**Fig. 3**) of current interest to the biophysics community[22,23,38]. Image data is information rich, e.g., unexpectedly we captured cell-division events (**Supplementary Video 3**); given that there is also a rich stimulus space many phenomena can be identified and systematically studied. Because of its domain-specific design[39], this platform is expandable beyond *Euglena* and light stimuli to a general class of increasingly automated and low-cost, high-throughput experiments, such as experiments involving valve-switching in microfluidic devices[40] and cloud chemistry[41]. The ability to support theoreticians carrying out their own investigations, as well as large-scale citizen science[5], is within reach.

In conclusion, we demonstrate a new online access and scientific inquiry model that turns observational microbiology into an interactive experience. This enables (i) interaction with living cells in real time, (ii) complex microscopic inquiry practices, (iii) learning analytics for life science experimentation and (iv) improved in-class time use, logistics, costs and safety. The key technical contribution was to extend the distributed computing concept to include unreliable biological specimens while maintaining quality of service. This approach makes complex biological experiments and modern biotechnology accessible to and interactive for multiple currently underserved audiences, such as students, teachers, scientists and the general public. Although the needs for education and research are not identical, they may synergistically drive technology development and its economics. All code and BPU designs are released open source (**Supplementary Text 5** and **Supplementary Figs. 1–5**), enabling wider dissemination and development, and we invite

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the life-science community to adapt its protocols and technology to make them interactive and available online.

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AUTHOR CONTRIBUTIONS

I.H.R.-K.: project idea and coordination. I.H.R.-K.: engineering conceptualization; P.B., I.H.R.-K.: educational conceptualization. Z.H., A.M.C., C.L., I.H.R.-K.: hardware, biology and experiments; Z.H.: software system architecture; Z.H., A.M.C., C.L.: software implementation at Stanford site; S.N.P.: software implementation at Northwestern site. User study design, execution, and data evaluation: Study 1: I.H.R.-K., H.K., Z.H.; Study 2: P.B., E.W.B., I.H.R.-K.; Study 3: K.J., A.D.W., I.H.R.-K. Manuscript preparation: I.H.R.-K. and Z.H. with creative input from all authors.

COMPETING FINANCIAL INTERESTS

The authors declare no competing finanicial interests.

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