A Low-Cost Bio-Imaging and Incubation System

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ABSTRACT

Here we describe an integrated system for biological experiments on dynamic processes on the millimeter length scale. The system permits live imaging under controlled environmental conditions using low-cost technology that is easy to implement. This work was presented at PhysNet 2015.

Categories and Subject Descriptors

B.4.0 [**Hardware**]: Input/output and data communications—*General*; K.3.0 [**Computing Milieux**]: Computers and education—*General*; J.3 [**Computer Applications**]: Life and Medical Sciences—*Biology and Genetics*

Keywords

Physarum polycephalum, Raspberry Pi, Arduino Uno, Educational, Low-cost.

As the fundamental units of life cells take a central role in biological education. However, their cultivation and experimentation requires expensive equipment, such as incubators and microscopes, and is a considerable financial challenge for educational institutions.

The current mass production of ultra low-cost optical and electronic components for computers, webcams, and cell phones facilitates innovative new concepts for economic realizations of experimental equipments. An outstanding example along these lines is the recently developed origami-based one-dollar foldscope, which is a microscope of remarkable imaging capabilities [1]. Further, single-board computers, such as the Raspberry Pi, and freely programmable microcontrollers, such as the Arduino Uno, have successfully been utilized in scientific context [2–4].

Here we present a novel system for biological experiments: the Digital Integrated Imaging Incubation Cube (DI^3). The DI^3 was designed to provide controlled temperature conditions and digital time-lapse imaging for biological experiments. We introduce two different versions of the DI^3 - a standalone solution, based on the Raspberry Pi (Pi-DI^3), and a PC-controlled version, which relies on the Arduino Uno (Uno-DI^3). Both DI^3s are based on low-cost components and can easily be assembled in schools and universities following our outline.

We designed the DI^3 for experiments on the unicellular slime mold *Physarum polycephalum*, but it can also be used for other organisms on the millimeter scale.

P. polycephalum has the unique property to exist as a single cell on different spatial scales, ranging from tens of micrometers to several centimetres. It exhibits various interesting features, such as the formation of transport networks and pronounced motility patterns, including the oscillatory shuttle streaming of its cytoplasm and the formation of growth fronts [5, 6]. Besides the interest from the scientific community, *P. polycephalum* has also been appreciated as a model organism in education [7, 8].

However, in order to fully harness the potential of *P. polycephalum* as a model organism for cell motility it is required to take video recordings, as most of its movements are too slow to be captured by the bare eye. For experiments that can serve the scientific standard of reproducibility the environmental conditions must be kept constant and in a controlled range. *P. polycephalum* is known to be sensitive to, e.g., light exposure [9] and temperature changes, which constituted requirements on designing the DI^3. In the following we evaluate the capabilities of the DI^3 with *P. polycephalum* as a specimen.

Imaging. The sample was imaged from above using a CCD camera and illuminated from below via an array of LEDs (see the schematic of the system in Figure 1*A*). Camera, sample, and LEDs were mounted into a rack consisting of three vertically stacked plates of acrylic glass. The plates had holes at each corner where they were held in position

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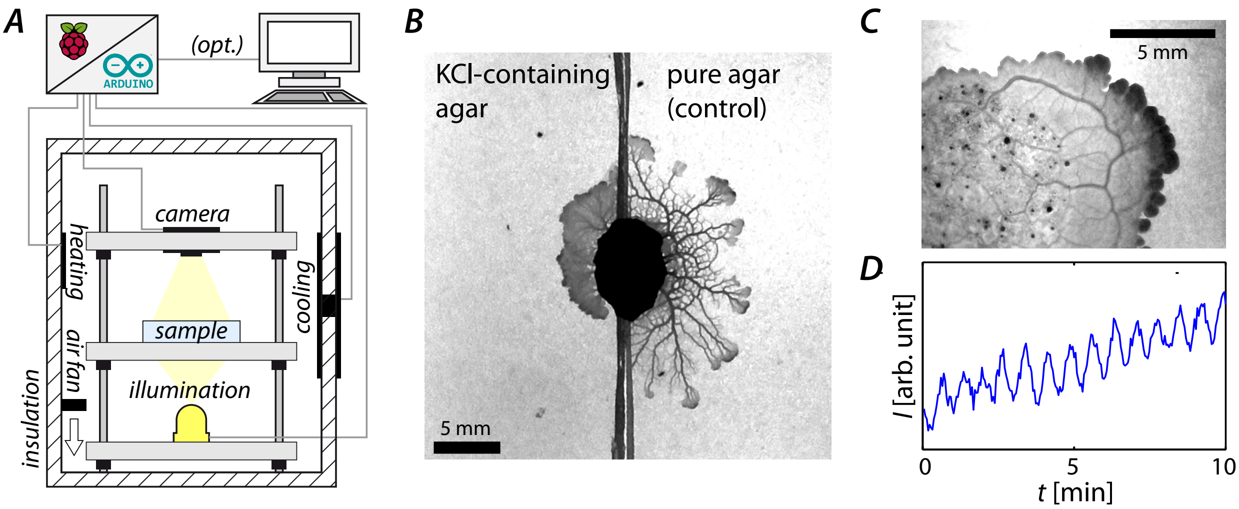

Figure 1: *The DI³ system and its imaging capabilities.* *A*: Schematic of functional elements. *B*: *P. polycephalum* growing on a bi-domain agar substrate. *C*: Close-up view of a growth front. *D:* Oscillation of image intensity at the position of a vein indicating thickness variations with a period of 0.7 min.

by nuts on four threaded bars. This design permitted us to adjust the focal plane. To avoid permanent exposure of the sample to illumination light the LEDs were turned on shortly before image acquisition and off immediately after each shot of the camera via custom-written C routines and bash- (Uno-DI³) or Python scripts (Pi-DI³).

The minimal spatial scale exhibited by a fully grown network of *P. polycephalum* is given by the diameter of its veins, which are on the order of 0.1 mm (Figure 1*B* & *C*). The fastest temporal time scale is provided by the oscillatory shuttle streaming, which has a period of typically 1 min (Figure 1*D*). A frame rate of 0.2 Hz has proven very suitable for demonstrational purposes. Further, with this choice of frequency one is beyond the time scale of minutes at which *P. polycephalum* is known to be able to anticipate oscillatory stimulation events [10].

The Pi-DI³ utilizes the Raspberry Pi Camera Module, whereas the Uno-DI³ relies on a webcam. Both systems can easily account for the temporal sampling. The Raspberry Pi Camera Module of the Pi-DI³ system is further able to fully resolve the spatial scale of *P. polycephalum* (all images in Figure 1 were taken with the Pi-DI³). In contrast, the Uno-DI³ setup cannot fully resolve the spatial scale of *P. polycephalum*, as webcams utilize hardware implemented image compression, which typically leads to a loss of details on a scale below 1 mm in our setup.

In summary, both systems are suitable for qualitative live imaging of *P. polycephalum*. Further, the dynamics of larger scale-structures, such as growth fronts, can sufficiently be quantified with both systems. However, only the superior imaging quality of the Pi-DI³ additionally permits quantitative image analysis on the spatial scale of the vein diameter.

Incubation. To provide both, protection from light and insulation from outside temperature, we constructed a Styrofoam box for housing (Figure 1*A*). The box was equipped with a Peltier cooling plate and a heating foil, yielding constant optimal temperature (24°C for *P. polycephalum*). Both elements were connected to an external power supply and controlled via an Arduino Uno or a Raspberry Pi with custom-written C / Python routines. Temperature was sensed via a digital thermometer placed close to the sample. To ensure thermal convection a computer fan was used. The outside part of the Peltier cooler plate was connected to a computer heat sink, which was attached to an additional computer fan to prevent over-heating of the Peltier cooling plate.

Experiments. The DI³ permits various experiments, ranging from qualitative demonstrations such as, e.g., chemotactic impact on growth (Figure 1*B*), to quantitative experiments, such as measurements of oscillation periods (Figure 1*D*) or velocities of growth fronts. Further, the increase of biomass of growing networks can easily be quantified via segmentation-based area measurements.

The system can also be used in scientific laboratories. Due to its low price and easy manufacturing multiple DI³ systems can be used in parallel. Since experiments on the dynamics of *P. polycephalum* usually have a runtime of several days this approach is highly favourable for gaining high data yield.

Despite its intended range of application, the system is versatile and can also be used for studies on other biological systems that have comparable sizes and time scales of dynamics. These comprise but are not limited to, e.g., colonies of bacteria, degrading food, yeast activity, growth of plant calli - including the temperature dependency of these processes.

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