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An integrated top-stage incubator and lens free holographic imaging system for culture monitoring applications

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ABSTRACT

Lens free inline holographic microscopy was shown to be a potent approach for many applications relying on cellular imaging. Applications requiring a large field of view at moderate resolution are the ones that are most suitable for this platform. Besides, the simplicity of the overall imaging system, which requires only a light source and a camera, positions this approach as an easily accessible one. Acquired holograms from such a system are processed to recover phase images. As an additional advantage on top of the simplicity, phase imaging enables the imaging of otherwise transparent cell samples without any need for labeling or staining. Eventually, such a system can be used for long term imaging of live cell cultures with wide field of view. Up to now, two alternatives were explored for the imaging of live cell cultures for extended duration. In one approach, a portable imaging system was placed inside a standard incubator with cell cultures on top. A stage top incubator was used on a modified microscope in the other approach. In both approaches, the cost of the system grows due to commercial systems, and the overall footprint of the system with incubator is too large to be classified as portable. Here, an integrated portable system is presented that can maintain cell cultures at desired temperature in a 3D printed enclosure while imaging them in lens free inline holographic microscopy modality. Such a system is well suited for tissue culturing and monitoring at limited resources settings.

Keywords: Digital in-line holographic microscopy, cellular imaging systems, lens free imaging, top stage incubator, numerical reconstruction, Gerchberg–Saxton algorithm, time-lapse microscopy

1. INTRODUCTION

Digital in-line holography is an agile method for live-cell imaging. The main reason for that is the capability of digitally capturing and reconstructing the images. Digital holography enables obtaining both amplitude and phase data of the whole field, advanced image processing on attained complex field, and fast acquisition of holograms[1]. Images can be easily collected, and 3-D datasets can be obtained by numerically reconstructing the digitally captured images at desired depths[2, 3]. These datasets or models can be used in numerous areas of research as developmental biology, drug discovery, toxicology, and cancer. In human studies, biologically relevant data, that may potentially shorten the drug development process, can be acquired by using imaging-based 3D models[4, 5].

Furthermore, an imaging incubator is a fundamental requirement for long term observations. Such observations are parts of routine protocols for cell biology laboratories. The ability of incubators to be placed on an off-the-shelf microscope stage positions this type of incubators convenient for live-cell imaging purposes[6]. Imaging is frequently used in the medical field for example in vitro fertilization (IVF) treatment. Incubators are essentially important for IVF, because simulating a close environment with the organism is a must for the embryonic development[7]. Digital holographic process may be divided into two parts, namely recording and reconstruction. In recording, by using the superposition of object and reference beams, it is possible to obtain the holographic interference pattern. This pattern can be captured by using a digital

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camera sensor and processed in the digital environment as a number array[1]. In the digital environment, the hologram can be reconstructed by using numerical propagation between planes with the Gerchberg-Saxton (GS) based algorithms[8, 9].

A low-cost imaging platform, which is based on inline holographic microscopy and a low-cost incubator, is realized for the time lapse imaging of living cells. Figure 1 conceptually represents this platform. The imaging subsystem is simple that is composed of a light source and a camera. Besides, by aiming for long-term live cell imaging applications, an integrated portable system is constructed that can maintain cell cultures at the desired temperature in a 3D printed enclosure. The thermal stabilization requires a duration of 15-20 minutes and sustains the internal temperature with ±1°C peak-to-peak fluctuation. The overall platform is convenient to be used in live-cell imaging applications for few days.

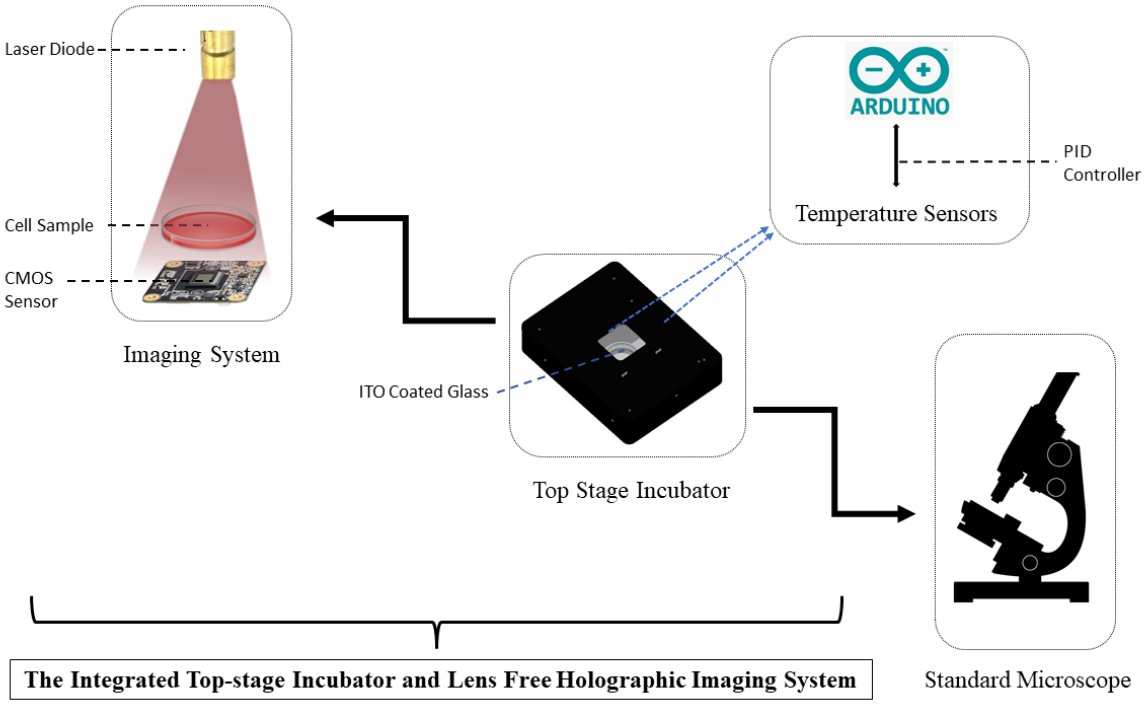


Figure 1. Schematic of the integrated system.

2. METHODS

This live-cell imaging platform has two separate subsystems. These are top-stage incubator and lens-free holographic imaging system. These subsystems are detailed in Sections 2.1 and 2.2.

2.1 Top stage incubator

In the first stage, a practical, portable, standalone, and low-cost top-stage incubator is 3D printed. Sustaining the internal temperature at the required value for cell viability is the essential function of the incubator. An ITO-coated glass window on the top lid provides thermal regulation while enabling optical access. The ITO-coated glass pane is chosen because it can prevent water vapor condensation by forming a vertical temperature gradient. Additionally, a 50mm x 50mm sized pane is employed at a close distance to have a uniform temperature distribution in a culture dish[6]. An Arduino Uno microcontroller regulates the temperature inside the incubator through a PID (Proportional–Integral–Derivative) loop. The microcontroller generates PWM signals. The signals switch on or off the heating of the ITO-coated glass pane with the help of a solid-state relay. A PID algorithm implementation varies the PWM signal utilizing the feedback from the temperature sensors placed inside the incubator. The incubator system is an alternative to costly off-the-shelf top-stage incubators. Fused deposition modeling-based 3D printing is a well-suited technology for the manufacturing of the body of such low-cost alternatives. The incubator that perfectly fits the XY stage of microscopes subassembly is designed to be compatible with 35 mm petri dishes. The top-stage incubator that is shown in Figure 2 can work in tandem with the imaging system or standalone.

Figure 2. The final form of the imaging system and top-stage incubator.

2.2 Imaging System

In the second stage, an imaging system based on digital in-line holography was developed. For this purpose, a 3D printed body keeps the optical elements in alignment. The setup of the digital in-line holographic imaging system is shown in Figure 1. By digitization of the holograms, it became possible to capture, process, and reconstruct images in digital environment.

The imaging system includes only the light source and the CMOS camera sensor. The laser diode is used as the light source. In this in-line holographic imaging configuration, under the weakly scattering object assumption, the unscattered light is treated as the reference beam. The hologram can be formed by the superposition of object and reference beams[10]. The hologram can be captured using digital cameras and displayed on a computer. After the captured hologram is digitized using the camera, the amplitude and phase information of the object can be obtained by numerical reconstructions[1]. To obtain amplitude and phase information, reconstruction approaches with numerical Fresnel transform are used. Gerchberg-Saxton (GS) algorithm is preferred to obtain phase information[8, 9]. Since this algorithm is implemented depending on various parameters: the pixel size of the camera sensor, the wavelength of the light source, and the numerical propagation distance[11], it provides more flexible and more reliable results compared to other algorithms. The GS algorithm aims to reach phase information with the least error rate with repeated back and forth propagation[9].

As a result of numerical reconstruction, the amplitude and phase information of the object can be obtained in the digital environment. One of the most important advantages of digital in-line holography is that it can be performed without the need for an external optical system[12].

In addition to the reconstruction operations carried out in MATLAB, a user interface was designed to track the imaging process. Thanks to the user interface, the holograms can be recorded at desired time intervals and then can form a time-lapse video. The interval of capturing the images will be determined by the user. The developed user interface is shown in Figure 3. This interface instantly calls the GS algorithm with the captured images and the parameters determined by the user in accordance with the experiment. GS algorithm returns the amplitude and phase images, and they are displayed on the application window instantly. Besides the holograms, amplitude, and phase images can be recorded with current time information using the save feature. These recordings can be converted into a time-lapse video by using their time information after an experiment is completed.

Unlike the classical imaging systems, the setup does not rely on imaging optics. Thus, this system is appropriate for the low-cost imaging system purpose. The final form of the systems is shown in Figure 2. The imaging system can work integrated with the top-stage incubator or standalone.

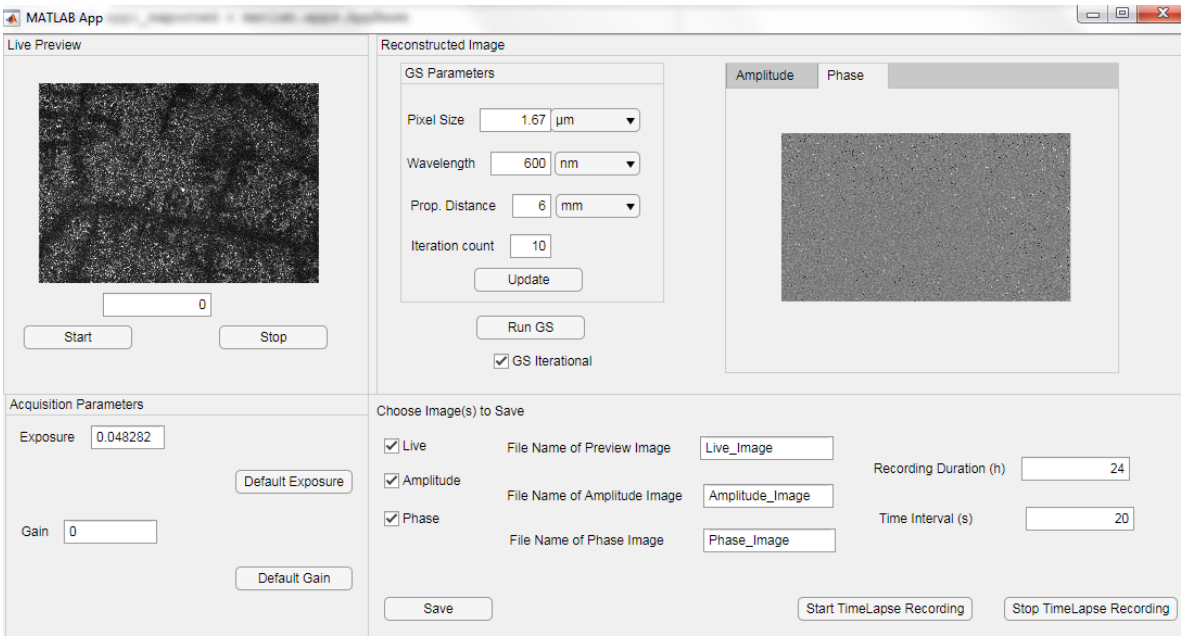
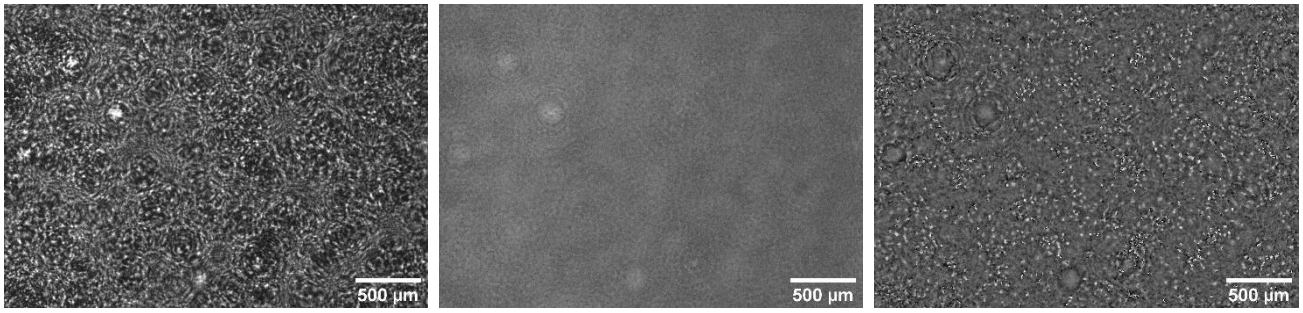


Figure 3. User interface.

3. RESULTS AND DISCUSSION

The proposed imaging system and integrated top-stage incubator were utilized to observe the human osteosarcoma U2OS cells on a 35 mm glass bottom dish with 150000/dish seeding concentration. A cropped sample frame of the input holograms, corresponding amplitude, and phase images are represented in Figure 4.



(a) (b) (c)

Figure 4. (a) The acquired hologram within the first 4 hours of the test. (b) The amplitude and (c) phase images are reconstructed after performing 10 iterations of back-and-forth propagation at 6 mm and using 650 nm of laser light source.

To investigate the viability of the U2OS cells over time, optical flow vectors are analyzed between each consecutive frame. Optical flow computations are performed using the Farneback method through the full size (3872 x 2764 pixels) phase images at an acquisition rate of a hologram per ten minutes. Thanks to Farneback's multi-level resolution pyramid parameter, the algorithm can be optimized to track the motion at the desired level of resolution[13]. In our case, 3 layers are used to perform quantitative motion estimation of the morphological dynamics of the cells. As a result, the polar histogram plots are constructed to illustrate both the direction and magnitude of the optical flow vectors. The magnitude values are expressed in terms of migration velocity and converted to micron-scale. Several examples of polar histograms acquired at different timestamps are shown in Figure 5. Accordingly, a significant decrease in the amplitude of motion vectors over time can be easily observed. However, the cells do not exhibit a clear preference for flow in any direction. At least when the migration of all cell clusters inside full-size frames is included, no appreciable bias in any direction is realized.

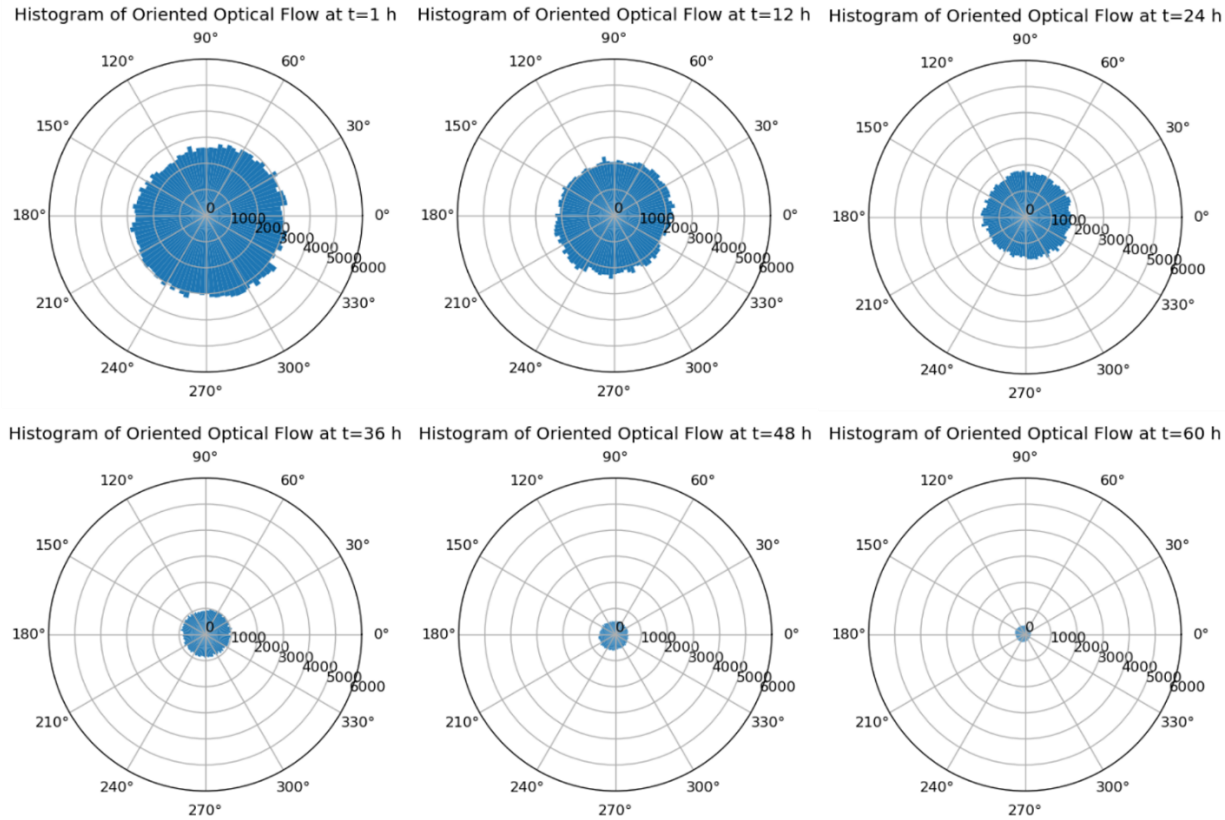


Figure 5. The angular distribution of cell migration velocity at different time points. The plots are constructed for each migration between 2 consecutive frames recorded at 10 minutes time intervals.

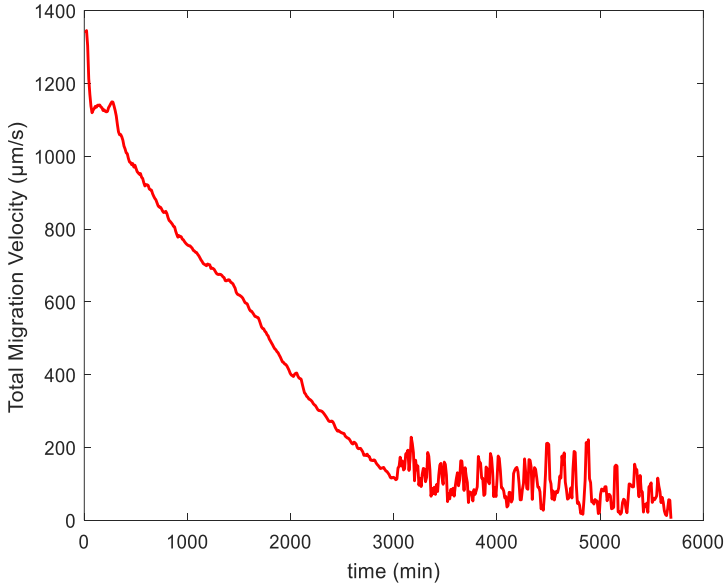


Figure 6. The cell migration velocity variations for the entire duration of the experiment. The sampling time is adjusted as 10 minutes and the line plot is filtered by a 3-point moving average filter to suppress the effect of disturbances due to phase discontinuity.

The migration velocity is tracked for a four-day experiment where the incubation conditions (37 °C) are maintained. The change in the total magnitude over time is plotted in Figure 6. A linearly decreasing trend in the magnitude of velocity is realized till the end of the second day. As seen, the migration velocity is halved within the first 27 hours approximately. Since the doubling time for U2OS cells is around 29 hours, the reduction in migration activity can be explained by the increase in the number of viable cells. After the 50th hour of test duration, unexpected noisy behavior is realized. The spikes are observed because of the sudden changes in the pixel values of the acquired hologram images. The primary reason for the jumps will be investigated, and a solution will be proposed in the following studies.

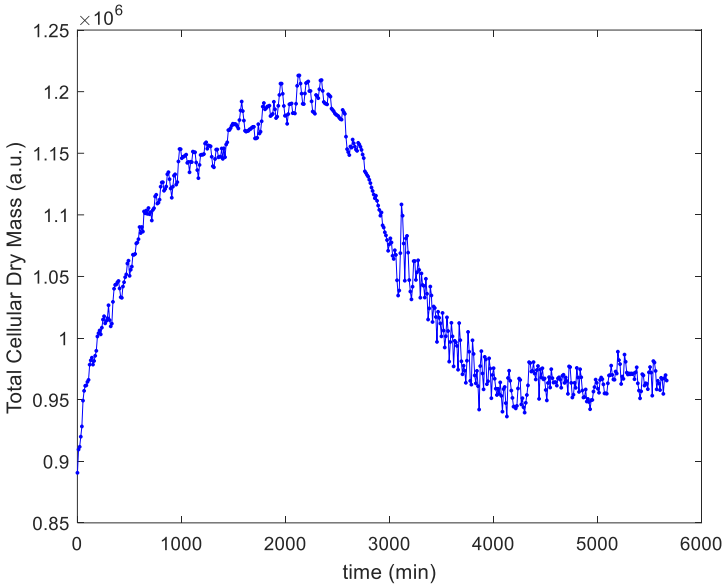


Figure 7. The plot represents the change in cell dry mass over time. The vertical axis denotes the sum of phase values in radians greater than a threshold. The plot is filtered by a 3-point moving average filter.

Figure 7 is presented to estimate the change in cellular dry mass that is related to proliferation. The plot shows that the concentration increases up to the 43rd hour of the test duration. The initial logarithmic growth can be explained by the expected behavior of cellular proliferation. After that, the total dry mass starts to decrease distinctively which can be only explained by the cellular death at a large scale.

The overall behavior is confirmed by the previous analysis in Figure 6. For the logarithmic phase, the cells migrate relatively long distances at the beginning. Moreover, the migration velocity is halved due to the cell cultures being doubled during this time interval. Both plots obtained from 2 different perspectives indicates the active proliferation of U2OS cells.

Nevertheless, the relative confluency value decreases towards the end of the second day and stabilizes after around 66th hours. Meanwhile, the migration velocity value converges to zero. Thus, we can infer that the cells start to cease to carry out the vital functions during the related time interval. Since the fresh medium is not supplied in the desired phase, the lack of nutrients and inadequate living conditions result in cell death. Additionally, having high initial confluency causes to become over confluent within a shorter time duration.

4. CONCLUSION

In this work, an integrated portable system targeting long-term live-cell imaging is presented. The overall system was utilized for monitoring human osteosarcoma U2OS cells. The vital activities of cells were monitored without the need for an external system. Thanks to the integrated top stage incubator, the proper environmental conditions (37°C) were maintained. We have shown the viability of cells by tracking optical flow vectors on the acquired phase images. The quantitative motion estimation analysis such as migration velocity were performed in micron scale. The activities of the cells were tracked successfully for a duration of 4 days. After assessing the population growth in the logarithmic phase, stationary, and death phases are inferred. Since the culture was highly confluent, the capacity of the medium was exceeded and cell proliferation was greatly reduced, then ceases entirely. As a result of the optical flow vectors, the migration velocities were analyzed for the whole duration of the experiment. It was concluded that no appreciable bias in any

direction is tracked by the cells. However, a significant drop in the magnitudes was analyzed and discussed. The consistency between the consequences was realized. The overall system including the post-processing operations offers a quick tool to be used in the many applications where long-term imaging of live cell cultures with a wide field of view is required such as tissue culturing and monitoring.

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