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Cell Culture Device Using Spatial Light Modulator

Chung-Jen OU[1,2], Ching-I SHEN[1,2], and Chung-Ming OU[3]

[1]*Department of Electrical Engineering, Hsiuping Institute of Technology, Taichung 400, Taiwan*
[2]*Department of Life Science, National Chung Hsing University, Taichung 400, Taiwan*
[3]*Department of Information Management, Kainan University, Taoyuan 300, Taiwan*

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Spatial light modulator is introduced for cell culturing and related illumination experiment. Two kinds of designs were used. The first type put the cell along with the bio-medium directly on top of the analyzer of the microdisplay and set a cover glass on it to retain the medium environment, which turned the microdisplay into a bio-container. The second type introduced an optical lens system placed below the spatial light modulator to focus the light spots on specific position. Details of the advantages and drawbacks for the two different approaches are discussed, and the human melanocyte cell (HMC) is introduced to prove the feasibility of the concept. Results indicate that the second type is much more suitable than the first for precision required application. © 2009 The Optical Society of Japan

Keywords: spatial light modulator, optical neural guide

1. Introduction

During cell culturing, it is sometimes necessary to provide the appropriate illumination for the cells or tissue within the incubator or the container. Experiments such as optical guiding of neurons, photodynamic therapy of tissue and photosynthesis of plant cells all required a specific illumination condition. Factors such as the wavelength, polarization, spatial intensity distribution and duration are of concerns to the engineers and scientists.

Take an optical neural/cell guiding experiment as an example,[1–6] which is a promising technique for tissue engineering/reconstruction. It requires producing a small light spot near the neuron with appropriate environment for the induction of neural growth. The optical induced chemical effects will lead to growth of the neural system in specific directions.

Inspired by those experiments, this paper explains use of the spatial light modulator (SLM) approach to provide a multi-point control for cell culturing. This apparatus can also be applied to other bio-culturing related applications which require multi-point illuminations on cell or tissue.

2. Setups and Methods

2.1 Basic setups

The principle of this system is shown in Fig. 1. The system's major parts are a microdisplay controller and a microscopy/imaging system with imaging recording function. Latter discussion will reveal details of the system which include an appropriate light source, a well-designed relay optics for SLM illumination, an X-prism along with zoom optics to manage the desired multi-points patterns, a polarization beam splitter (PBS) which can redirect the light energy patterns, and focusing optics to shrink the energy patterns into the incubator. The images of both the cells and energy patterns are examined by a microscope and a CCD camera. During creation of the design, the light energy pattern is made by the SLM directly below the incubators, and a supplementary light source can be added

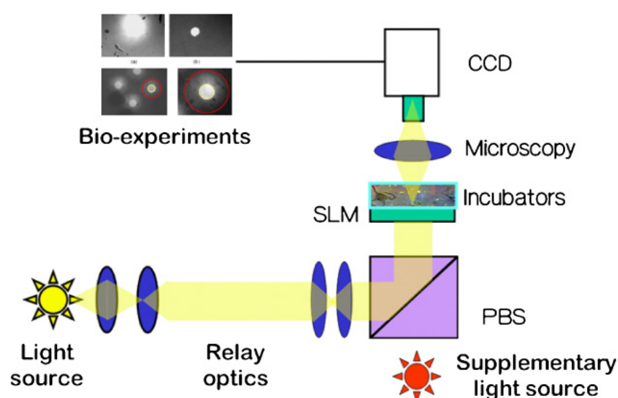

Fig. 1. (Color online) Culturing system layout for configuration 1.

for advanced purposes, such as spectrum mixing and energy enhancement.

Figure 2 shows the practical approach for the proposed cell culture system using SLM. There is a medium injection system to prevent cell degradation by the heating of the light source. Spectrum filters are provided for several wavelength selections. Magnification of the microscope objective lens is from 50× to 150×. Devices that control the temperature, the electromagnetic field and the Ph values, can be integrated into this system, and these can be helpful to study the effects of these factors on optical neural guiding phenomena. Possible negative effects of these devices on the microdisplay must be excluded in advance.

2.2 Light source and spatial light modulator

The light source for present study that described here could be projection lamp, light emitting diode (LED) or other incoherent light emitting element/device, using the SLM is to modulate the intensity of the illumination energy within a specific spatial region. Using a coherent light source will result in diffraction patterns. The light source for the authors' experiment was a metal halide light source (135W

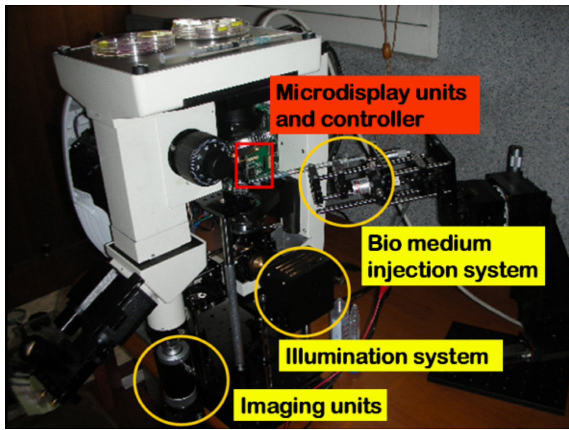

Fig. 2. (Color online) Practical approaches for culturing system.

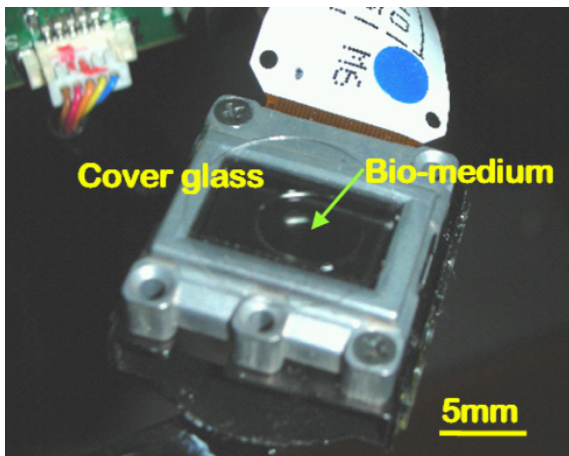

Fig. 3. (Color online) Microdisplay for first configuration.

UHE). By putting the SLM below the culturing receptacle, the appropriate light energy could modulate the illumination condition for the medium environment. The SLM was a microdisplay device, which was a transmissive liquid crystal based intensity modulator made by EPSON with 1024×768 pixels on a 0.55″ diagonal with more than a 1500 contrast ratio. Figure 3 gives a close look at this device. There were polarization plates incorporated with the SLM. Polarization effects are still not perfectly clear for different kinds of tissues and cell samples. Therefore, the advantages of an additional polarization element could make the polarization properties become the controlling factors in these cell illumination experiments. Other kinds of SLM developed for display applications, either transmissive or reflective type, with appropriate optical layouts can also serve for this culturing notion. It is also feasible to use an organic light emitting diode (OLED) or a digital mirror device (DMD) from Texas Instruments.

Wavelength filters (for example, color filters for visible light) and polarization elements (such as $1/2\lambda$ and $1/4\lambda$ wave-plates) can work together to provide a specific wavelength/polarization state on the tissue and cell for studying the photo-biochemical reaction. The overall contrast ratio of the whole system (along with these additional optical plates and a UV–IR filter) is around 600, and the wavelength range for the overall system is from 455 to 645 nm.

2.3 Configurations

To meet the requirements for cell culturing with accurate illumination, stability for the microscopy system become critical. If the system is designed for only short time (low energy) multi-regional illumination, then the *first configuration* (Fig. 1) is our recommendation for its simplicity. We put the cell along with the bio-medium directly on top of the analyzer of the microdisplay. A cover glass is set on it to retain the medium environment (Fig. 3), which turns the SLM into a bio-container. The advantage of this configuration is that it is easy to achieve and set up, but there are focusing problems and environmental control capability. These focusing problems are caused by the F/# of the illumination system, the microlens within the microdisplay and the distance issue (focusing position of the microdisplay and the exact position where the cell lies do not coincide). Figure 4 demonstrates the image of the cell and the single light spot at the same position under different focusing conditions. Figure 4(a) is the focal image of the cell along with the defocusing of the light spot. On the other hand, Fig. 4(b) is a clear image of the light spot but loses the focus of the cell at that image plane. The reason is that the two focal planes are not at the same spatial position. Similar situations are also demonstrated through the multi-point illuminating techniques. Figure 4(c) is the two images taken from the different focal planes overlapping, so we can compare the focusing spots (yellow circle) and the defocusing spots (red circle) simultaneously.

To overcome the focusing problem mentioned above, a *second configuration* is proposed in Fig. 5. The solution is to modify the optical layout by placing a precision lens system below the SLM, and to refocus the light spots. One needs to estimate a higher safety factor for the illumination

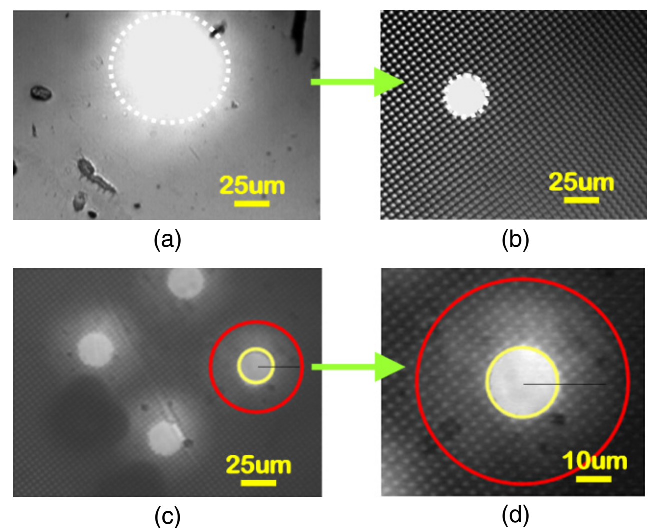

Fig. 4. (Color online) Defocusing problems from configuration 1.

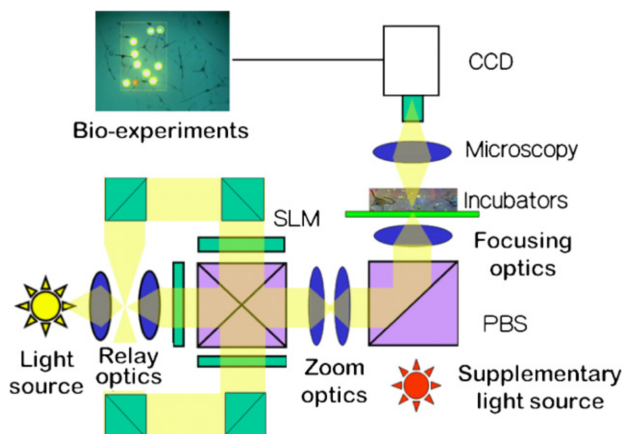

Fig. 5. (Color online) Optical layouts for second configuration.

energy, since there are imaging components during the image acquisition process. The advantage is that the focusing problem for the first configuration can be eliminated. However, compared to Fig. 1, a more complicated optical system is truly a disadvantage.

The minimum spread of the light spot can be controlled by the smallest pixel image (not the actual physical size of the pixels on the microdisplay) that is produced by the projection system, which is around 2 µm. However, after the elimination of the ghost and the stray light issues to remain the best cell illumination spot, the total energy is too weak (less than 0.005 mW) for the appropriate cell illumination experiments. A larger spot is introduced in the present study to provide sufficient energy around the cell activities region. Whether the key factor for bio-chemical reaction is the total accumulated energy or the energy intensity remains to be investigated, but the feasibility of this system to serve as a multipoint illumination experiment are proved.

There are still ghost and stray-light problems for both configurations, especially for a multi-point illumination mode. However, the appropriate operating procedures can probably get around these annoying areas for the culturing system, and serve in specific photo-biochemical experiments. We can evaluate the system performance by plotting the cross section of the energy plot for these cell/spots image and calculate the capability of the spatial energy modulation.[7] There are also two other issues, aberrations and image blurring, which need to be considered for improvement of the system performance. Aberrations are caused by the arrangement of the optical components, and the blurring phenomena are unavoidable results from the scattering of the cell/medium complex.

2.4 *Cell culture and characterization*

In order to prove the feasibility of this proposed SLM based cell culturing device, the human melanocyte cell (HMC) is introduced. There are similar morphologies between the HMC cell and the neural dendrites, and HMC is much more cost effective. The medium was provided by Cascade Biologics (M-254-500). This basal media contains essential and nonessential amino acids, vitamins, other organic compounds, trace minerals and inorganic salts. The primary HMC was isolated from human foreskin. The materials were donated with patient agreements and with approval of the Research Ethics Committee in Taichung Hospital, Department of Health (Taiwan). Supplements included fetal bovine serum (0.5%), basic fibroblast growth factor (3 ng/ml), bovine pituitary extract (0.2%), heparin (3 µg/ml), hydrocortisone (0.18 µg/ml), insulin (0.5 µg/ml), transferring (5 µg/ml), and endothelin-1 (10 nM). HMC was characterized by the expression of melanocyte specific genes, including tyrosine hydroxylase, transcriptional factor Sox10 and intermediate filament S100.

3. Results and Discussion

We found that the materials in the medium would not harm to the analyzer on the microdisplay in this experiment. Results for the two configurations are discussed below.

3.1 *Configuration 1*

Figure 6 shows the images of single spot guiding results for configuration 1, which appears in the gray scale intensity plot. The three small pictures indicate the image processing procedure. The first image is the initial state image. We impose the inverse grey scale to the initial state image and overlap it together with the image after the cell illumination (final state). Differences between the two images then will be addressed through this approach, and the tiny variations of the cell can be examined. The dark grey scale indicates the formation of the materials at that position—whatever the attributes of the materials are. The meaning of the formation did not always imply how the material or cell tissue was created. A mechanism-like extension, migration that can result in variations of the material at that location is generalized as the term “material formation”. Since there are only tiny variations for these results, it required a careful calibration to demonstrate the comparison on both the spatial position and grey scale intensity. In the present imaging system, the tissue is represented as a dark object (lower grey

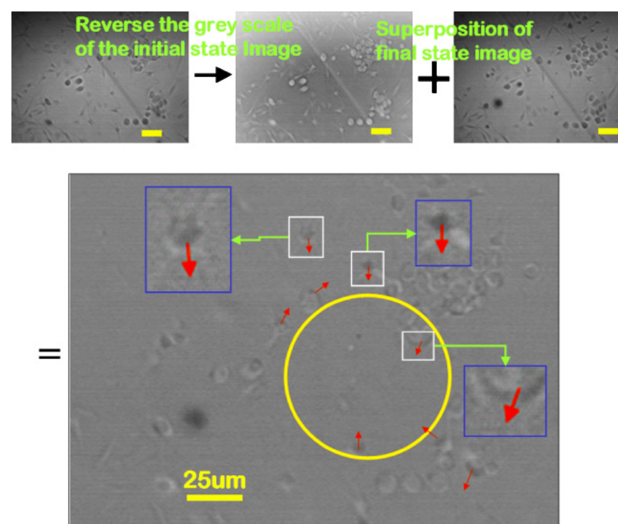

Fig. 6. (Color online) Single spot experiment for configuration 1.

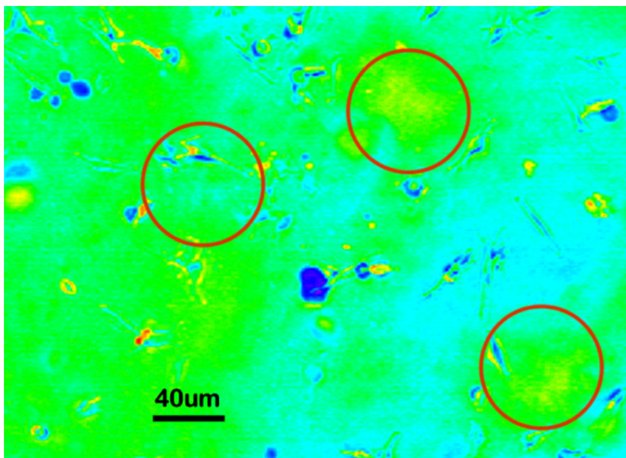

Fig. 7. (Color online) Multi-spot experiment for configuration 1.

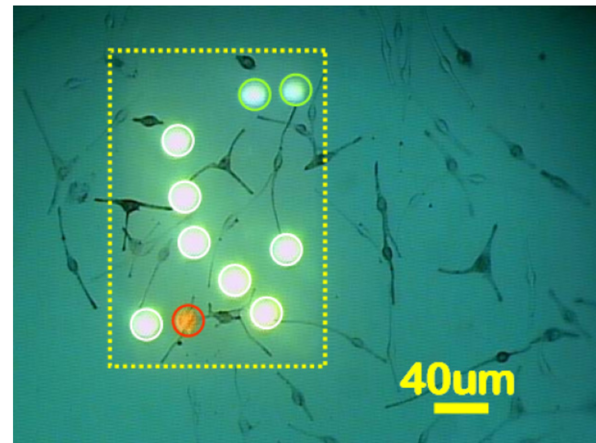

Fig. 8. (Color online) Multi-spot experiment for configuration 2.

scale). Determination of the arrow direction is based on the maximum extension of the material formation within that direction, which obviously appears as the dark pattern after the image operation mentioned above. This can also be achieved by examining the first order gradient of the grey scale from the successive accumulated images. Although not all of the arrows pointed toward exactly the illumination region, we still plotted it based on the experiment fact and studied the factors that might influence or cause such diversity. However, one can observe that five of the eight arrows significantly point toward the yellow region. Therefore, evidence suggests that there are materials toward (red arrows) the illuminated region (yellow circles).

Another experiment preceded the multi-points illumination in configuration 1, and the result is revealed in Fig. 7. Here we not only introduced followed the similar image processing procedure used in Fig. 6, but also used the color plot to represent the value of the grey scale after the operation. Blue regions indicate the formation of the materials in that area and the red/orange/yellow color indicate that the materials at those positions are now vanishing. Red circles indicate the illumination position using the red wavelength (620 nm). Results indicate that there are changes of the HMC morphologies around (and very close to) the spotting position, even though the defocusing and the scattering phenomena of this system blur these spots. More experiments and explanations are required by the biologist. However, from the engineer's point of view, the first configuration has already shown its capability.

3.2 Configuration 2

The second configuration removes the possible blur of the energy patterns, and can cover a wider spectrum of the energy pattern. Figure 8 shows the multipoint spotting experiment on the HMC cell with better energy delivery. Two green spots with 560 nm wavelength are located in the upper part and emphasized by a green circle. A red spot (circled by a red line with 640 nm wavelength) is designed to hit the core of the cell directly. These functions indicate the capability of the system for cell illumination experiments.

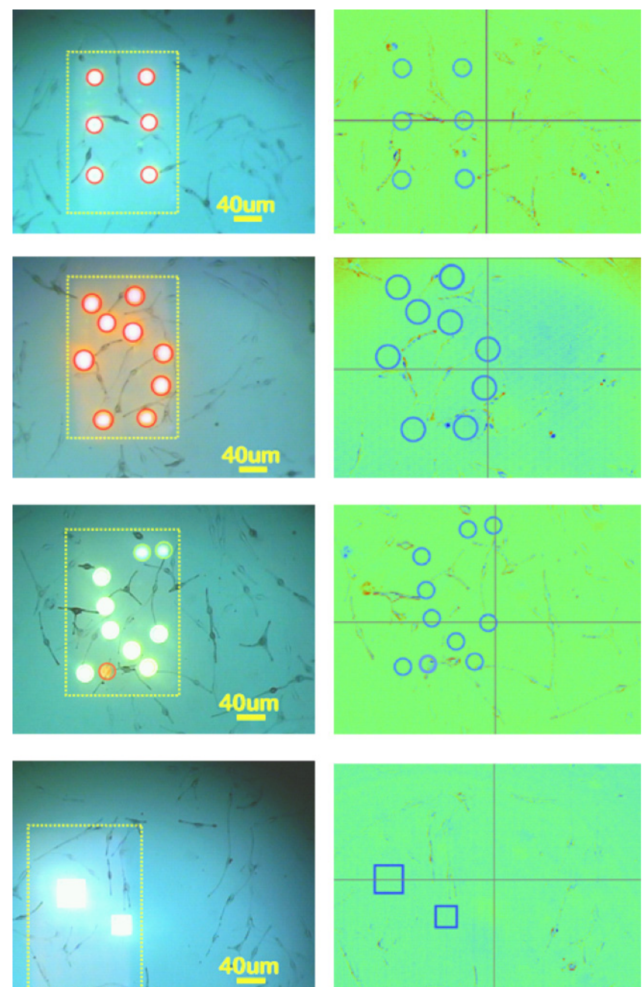

Fig. 9. (Color online) Multi-spotting experiments under different illumination conditions.

Figure 9 demonstrates the flexibility of this device on different types of spotting patterns in various shapes and wavelengths. The definition of pseudo spectrum plot is the same as before, which proves the advantages of this

configuration to study the optical guiding phenomena. The yellow broken lines in the figure indicate the maximum illumination area in this system, which is an image of the micro-display on the detector plane. The right portion is the images corresponding to the experiment shown at the left part. The protocol for producing these color images is the same as the procedure demonstrated in Fig. 6. All the color scales are normalized, and there are color variations over the images in Fig. 9. We are sure about that the blue color near the cells is correlated with the cell activities (either biochemical or physical, even mechanical) directly. The mechanisms that cause the color variation for specific figure are still under investigation. The advantage of this configuration (Fig. 8) over the configuration demonstrated in Fig. 4 is based on the fact that it achieves a clear image for both the cells and the spots.

During the experiments, there are other mechanisms that might result in the cell movement and elongation phenomena. These include the thermal contraction of the tissue or the possible induced flow caused by vaporization of the medium above the microdisplay. In spite of these, however this paper has already revealed the basic design of the present device for bio-related applications.

4. Conclusions

The spatial light modulator is proven here to be a practicable solution for cell culturing if spatial illumination conditions are required. We report the usages for liquid crystal based SLM. Drawbacks and disadvantages for two types of configurations are discussed, and demonstrations of the HMC guiding experiments suggest a promising future. Other types of spatial light modulator, such as liquid crystal on silicon (LCoS), digital mirror device (DMD) and organic light emitting device (OLED), can also be applied for this purpose. We expect that the flexible display can also play a major part in this application.

Acknowledgements

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