Microarrays and Biochips:

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VCSEL Arrays as Micromanipulators in Chip-Based Biosystems

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Abstract. The potential use of vertical cavity surface emitting laser (VCSEL) arrays for applications in cell analysis and tissue engineering is investigated by means of parallel optical trapping and active manipulation of biological cells on microfluidic chips. The simultaneous and independent transport of nine cells using a 3 × 3 array of VCSELs has been demonstrated experimentally; indicating that larger 2-dimensional array transport using individually addressable tweezers is achievable with VCSEL array devices. The transport properties of VCSEL tweezers have been investigated for various types of cells including 3T3 Murine fibroblasts, yeast, rat primary hepatocytes and human red blood cells. Due to the low relative index of refraction between the biological cell and surrounding medium and the relatively low optical power available with present VCSELs, the Laguerre-Gaussian laser mode output of the VCSEL is more favorable to use in an optical tweezer since the highest intensity is located at the outer ring of the optical aperture, producing stronger optical confinement at lower power levels. For larger biological cells or cells with a lower relative index of refraction, the power limitations of a single VCSEL were overcome through the binning of several VCSELs together by combining the outputs of a sub-array of VCSELs into a collective optical tweezer. A comprehensive analysis and simulation of how the VCSELs’ pitch and output beam divergence influence the operation of the resultant optical tweezer array is presented along with our experimental data. Employing the methods of parallel array transport and the binning of multiple VCSEL outputs, allows for the manipulation and spatial arrangement of different types of cells in a co-culture so as to facilitate the formation of engineered tissues.

Key Words. optical tweezers, VCSEL arrays, cell viability, engineered tissue

Introduction

This paper describes a versatile technique of all-optical manipulation of living cells through the use of VCSELs as optical tweezer (Flynn et al., 2002). In this type of device, objects such as biological cells are 3-dimensionally trapped in the focus of the light beam through the interaction of the photons with the relative index of refraction of the cell. The ability to design and create an optical setup, which employs optical tweezers as a means of cell sorting and measurement of cellular interactions and forces, has become a common and useful scientific tool (Ozkan et al., 2001; Wang et al., 2001a; Mehta et al., 1999; Hirano et al., 2002). Substituting VCSELs and arrays of VCSELs in place of the standard diode and gas lasers previously used in optical tweezer designs, provides the ability to meet the demands of current biochip and lab-on-a-chip technologies, which require that the controlling devices be small and have the ability of being replicated in large arrays such that multiple, simultaneous experiments can be performed in parallel and at a low cost (Ogura et al., 2001; Wang et al., 2001b).

Recently it has been reported that an optical tweezer comprised of a higher order Laguerre-Gaussian mode can offer significant advantages in axial stability and trap strength over the fundamental Gaussian mode since the Laguerre-Gaussian modes resemble a “donut” shape with the majority of the optical intensity located in the outer ring of the mode structure (O’Neil and Padgett, 2001). In a tightly focused beam, the largest incident angle photons provide the greatest axial restoring force to the 3-dimensional optical trap. Photons that are more weakly focused reside in the center portion of the beam and have the deleterious effect of producing an axial scattering force along the propagation axis that pushes the object out of the trap. By removing power from the central portion of the beam, the trap strength will improve along the beam axis (Ashkin, 1992; Simpson et al., 1998). To further increase the trapping ability of the VCSEL arrays used in our experiments, we

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implemented a technique developed by Kibar et al. (1999), whereby a standard packaged Hermite-Gaussian spatial mode VCSEL can be converted to a Laguerre-Gaussian mode laser through a post-fabrication current annealing process. The output of the VCSEL before and after the current annealing process is shown in Figure 1.

One of the limitations to using VCSELS as optical tweezers is the relatively low output power in comparison with a diode or gas laser. In order to capture objects of increasing size or lower relative index of refraction, there must be a proportional increase in the optical tweezer power (Ashkin et al., 1986). To overcome the output power constraints of a single VCSEL, a technique has been developed in which multiple VCSELS in the array are binned together to function as a single optical tweezer for the purpose of increasing the amount of power available in the optical trap.

In this article, we report and analyze the novel use of VCSEL arrays for the independently controlled, parallel capture and combined tweezer capture of biological cells for the purpose of fabricating engineered tissues on a biophotonic chip. In performing these experiments, we have fully characterized and simulated the method in which an array of VCSELS can be incorporated into an optical tweezer system and the tradeoffs that exist between the pitch of the VCSEL array, the VCSEL beam divergence, and the spacing of the optical tweezers in the sample plane. In addition, preliminary tests were conducted to study the physiological effects that VCSEL wavelength and intensity may have on cellular structure and function.

Materials and Methods

Cells and cell cultures

NIH 3T3 murine fibroblast cells grown to pre-confluence were trypsinized in 0.01% trypsin (ICN Biomedicals), 0.01% EDTA (Boehringer Mannheim) solution in phosphate buffered saline (PBS) for 5 minutes and then resuspended in 25 ml media. Approximately 15% of the cells were inoculated into a fresh tissue culture flask. Cells were cultured in 75 cm² flasks (Corning) at 10% CO₂, balance moist air. Culture medium consisted of DMEM (Gibco) with high glucose, supplemented with 10% bovine calf serum (BCS, JRH Biosciences), 200 U/ml penicillin, and 200 μg/ml streptomycin. The NIH 3T3 murine fibroblasts were then diluted in PBS to achieve a final concentration of 1.2 × 10⁶/ml immediately before the experiment was performed. Hepatocytes were isolated from 2–3 month old adult female Lewis rats (Charles River Laboratories, Wilmington, MA) weighing 180–200 g by a modified procedure of Seglen (1976). Detailed procedures for isolation and purification of rat primary hepatocytes were previously described by Dunn et al. (1991). Briefly, 200–300 million cells were isolated with viability between 85% and 95%, as judged by trypan blue exclusion. Nonparenchymal cells (less than 10 μm in diameter) and morphology (nonpolygonal or stellate) made up less than 1% of the sample. The culture medium consisted of Dulbecco’s modified eagle medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Sigma, St. Louis, MO), 0.5 U/ml insulin, 7 ng/ml glucagon, 20 ng/ml epidermal growth factor, 7.5 μg/ml hydrocortisone, 200 U/ml penicillin, and 200 μg/ml streptomycin. Human red blood cells were procured from a whole blood sample (San Diego Blood Bank), diluted in Alsever’s solution to a concentration of 10⁸/ml, and stored at 4 °C until use. Yeast cells were obtained from an active dry mixture (Fleischmann’s) and activated in a solution of distilled water with a 2% sugar concentration. The yeast were allowed to sit 24 hours in the solution at room temperature and were then diluted to a concentration of about 10⁶/ml in DI water prior to use.

Chemicals and materials

PBS and Alsever’s solution were obtained from Sigma Aldrich. The polystyrene microspheres of varying sizes were purchased from Bangs Laboratories (Fishers, IN), the 1.25 NA 100 × oil immersion microscope objectives from Edmund Industrial Optics (Barrington, NJ), the dichroic beam-splitter and antireflection coated lenses from Thor Labs (Newton, NJ), and the 0.1 μm resolution motorized stages and controller from Newport (Irvine, CA). The 4 × 4 arrays of 850 nm, 15 μm aperture, proton-implant VCSELS were obtained from Honeywell EM (Santa Clara, CA) and fitted with microlens arrays from Weible OpTech (Neuchâtel, Switzerland).
Results and Discussion

VCSELs and photon-damage study
Effects of photon absorption on cell fate have been studied with several types of cells. Early work suggested that long term exposure to light at 1064 nm from Nd:YAG lasers produced photodynamic damage to cell, which is believed to be from the optically pumping of singlet molecular oxygen, a toxic free radical (Block, 1990). It was also observed that an intermediate wavelength of 760 nm is found to be highly absorptive for certain cell types (Vorobjev et al., 1993). However, since the mechanism of photo damage is not yet well established, studying optical damage to biological systems may become necessary on a case-by-case basis. In order to prevent damage by light absorption, a VCSEL was chosen for our experiment that operates in the near infrared (850 nm), where a window of transparency exists for biological materials. This underlying natural transparency of the biological materials stems from the knowledge that chromophores, including hemoglobin in red blood cells, specifically absorb less light near the infrared region of the spectrum and that water has a relative absorption minimum at the 850 nm wavelength (Block, 1992; Wright et al., 1990).

To test whether VCSEL beams induce irreversible damage to NIH 3T3 murine fibroblast cells, several NIH 3T3 murine fibroblasts were individually exposed to a VCSEL beam of 850 nm wavelength for 5 minutes at a power of 7 mW. The cells were subsequently plated in a tissue culture dish with 10% DMEM and stored in a 37 °C incubator. Cellular functions such as attachment, spreading, and mitosis were observed over a one-week period. Figure 2 shows the observed morphology using phase contrast microscopy at several time points. After 1 week, the NIH 3T3 murine fibroblasts were confluent, suggesting that many rounds of cell division had taken place, which is a predominant indicator that no critical photo damage physiologically occurred from the laser light exposure at 850 nm. Our observations, however, do not rule out subtle differences in gene expression patterns in the cells that may be induced by the incident light exposure.

Characterization of the VCSEL array for optical tweezing
An understanding of the intricacies involved in the incorporation of a VCSEL array into an optical tweezer system is needed in order to optimize its use for cell transport and tissue engineering. A representative diagram of our optical setup is shown in Figure 3 wherein a microlens array is used to collimate the output of the VCSELs, which then undergo a magnification

Fig. 2. Viability test of the NIH 3T3 murine fibroblast post exposure to the 850 nm wavelength light output of the VCSEL. During the time period of (A) one day, (B) three days, and (C) one week, it can be seen that the NIH 3T3 murine fibroblast cells are exhibiting normal cellular functions such as attachment, spreading and mitosis in the culture. From this experiment we can conclude that exposure to the VCSEL wavelength does not impair the normal cellular processes exhibited by the NIH 3T3 murine fibroblasts.
\(\frac{D_2}{D_1}\) before reaching the microscope objective. The use of a high numerical aperture (NA) microscope objective (100\(\times\), 1.25 NA) is necessary in order to focus the laser beam and produce the high incident angle photons required to create a fully 3-dimensional optical trap.

A key factor in the analysis of an optical tweezer system is the precise measurement of the optical tweezer power in the sample plane. Since the optics contained inside our microscope objective were antireflection coated for the IR spectrum, it was necessary to measure the optical power loss due to the microscope objective. Assessing the efficiency of the microscope objective is accomplished by coupling a duplicate 1.2 NA, 100\(\times\) microscope objective to the first, measuring the ratio of the input power and coupled output power, and adjusting this ratio to account for any power clipped at the back-aperture of the first microscope objective. Employing this method, the efficiency of the microscope objective was calculated to be 74.9\% at a wavelength of 850 nm.

The relationship between the divergence of the VCSEL beams after the microlenses and the fixed VCSEL pitch (4° half angle, 250\(\mu\)m in our setup) is the factor that affects: the spacing of the optical tweezers in the sample plane, the amount of power clipped at the microscope objective back-aperture, and the ratio of the optical tweezer’s gradient force relative to the photon scattering force. Adjusting the distances \(D_1\) and \(D_2\) in Figure 3 allows for the changing of the optical tweezer pitch in the sample plane. Utilizing the Gaussian lens formula (Hecht, 1990), an equation can be derived for the overall magnification factor that relates the pitch of the VCSEL array to the optical tweezer pitch in the sample plane:

\[
\Lambda_{OT} = \left(\frac{D_2f_3}{D_1D_3}\right)\Lambda_V,
\]

where \(\Lambda_{OT}\) and \(\Lambda_V\) are the pitch of the optical tweezers in the sample plane and the pitch of the VCSEL array respectively; \(D_1, D_2, D_3\) are distances represented in Figure 3, and \(f_3\) is the focal length of the microscope objective, which is approximated by the ratio of the distance to the secondary conjugate plane and the microscope magnification (\(M\)) such that:

\[
f_3 \approx \frac{160\text{ mm}}{M}.
\]

There is a limit to which the optical tweezer pitch in the sample plane can be adjusted though, and as seen in Figure 4, sufficiently decreasing the tweezer pitch will increase the amount of power clipped at the microscope back-aperture and diminish the optical tweezer power in the sample plane. On the other hand, greatly increasing the tweezer pitch leads to an under filling of the microscope back-aperture that results in a degradation of the optical gradient trapping force.

**Array simulation results**

Simulating our optical setup in Code V (Optical Research Associates, Pasadena, CA) confirms our experimentally
measured clipping results for the $2 \times 2$ and $4 \times 4$ VCSEL arrays as the optical tweezer pitch in the sample plane is varied. The slight offset in magnitude between the experimental and simulated clipping values in Figure 4 can be attributed to the limitations of the Code V model in which a Gaussian beam is used to approximate the Laguerre-Gaussian modes of the VCSEL array. Using the results from Figure 4, the optical distances $D_1$, $D_2$, $D_3$ (see Figure 3) were set to reflect the optical tweezer separation (4 μm in this case) in the sample plane that yielded minimal clipping loss while still filling MO back aperture. Taking into account the 25% power loss incurred by the MO and the power loss due to clipping at the MO back-aperture, we calculated that for a $2 \times 2$ array of VCSELs with a 4 μm optical tweezer pitch in the sample plane, the average power of each optical tweezer is 4.2 mW when the output power from each VCSEL in the array is maximized at 7.14 mW.

As the last part of our optical system characterization, we performed another Code V simulation (see Figure 5) to calculate the amount of clipping loss that occurs as the dimensions ($N \times N$) of the VCSEL array (250 μm standard pitch) are increased while maintaining a constant optical tweezer separation in the sample plane of 4 μm. As seen in Figure 5, if we choose to limit the clipping loss to 25%, for a total power loss of 50% including the MO, it is possible to use an $9 \times 9$ array of VCSELs to generate 81 optical tweezers with a pitch of 4 μm.

**VCSEL tweezers for parallel array transport of cells**

To demonstrate the capability of our VCSEL array tweezer system, we used a $3 \times 3$ array of VCSELs to capture and relocate nine individual yeast cells within the microfluidic chip. Adjusting the magnification of the optical system, the spacing of the optical tweezers in the sample plane was set to 4.25 μm in order to accommodate the size of the yeast cells whose diameters averaged between 2 μm and 3 μm. For the purposes of demonstrating parallel array transport, the $3 \times 3$ optical tweezer array was loaded with yeast cells on a one-by-one basis by using a single optical tweezer to move the cells into the same vicinity. This loading method proved to be easily reproducible although somewhat time consuming and alternative methods are currently being investigated by which the VCSEL tweezer array is combined with dielectrophoretic cell aggregation to increase the loading efficiency. Figure 6 shows sequential images captured by a CCD camera of the optically trapped yeast cells undergoing simultaneous translation with a velocity of 8 μm/s for a total distance of 400 μm. As is evidenced from the images, the relative spacing between the yeast cells remains constant and each cell is

![Fig. 5. Code V simulation showing the fractional clipping at the microscope back aperture as the dimensions ($N \times N$) of the VCSEL array (250 μm standard pitch) are increased while maintaining a constant optical tweezer separation in the sample plane of 4 μm. The optical tweezer pitch in the sample plane was determined using the optimized results from the $2 \times 2$ and $4 \times 4$ VCSEL arrays (see Figure 3) that minimized clipping yet still filled the MO back aperture.](image)

![Fig. 6. Sequence of images showing the trapping and simultaneous translation of nine yeast cells using a $3 \times 3$ VCSEL array as optical tweezers. The cells are moving to the right and the position of the reference cell remains stationary.](image)
sustained in a fully 3-dimensional optical trap as the yeast cells are positioned past a reference cell. Along with the array capture of yeast cells, we have also previously performed experiments that use a 2 × 2 array of VCSEL optical tweezers to trap human red blood cells\(^1\) that ranged in diameter from 5 μm to 6 μm.

**Optical traps using combined VCSEL beams**

For the arrangement and transport of cells, the force exerted upon the cell by the optical tweezer will vary according to the cell size, cell shape, and relative index of refraction between the cell and its medium. To correct for a low relative index of refraction, larger cell size, or non-spherical cell shape requires an increase in the optical tweezer power. One of the limitations to using VCSELs as optical tweezers is the low amount of output power provided by each VCSEL. We were able to compensate for the low individual VCSEL power by decreasing the distance between the optical tweezers in the sample plane to a point where multiple VCSELs can be focused to trap a larger sized object (> 10 μm in diameter). Figure 7 shows a plot of the resultant increase in optical trapping force (with respect to the force of a single VCSEL tweezer) when additional VCSEL outputs are binned together to function as a single optical tweezer.

As shown in Figure 7, there is more than a two-fold increase in the optical trapping force when four VCSELs in a 2 × 2 array pattern are used to capture 10 μm and 20 μm polystyrene microspheres. This method of binning multiple VCSEL tweezers proved to be successful for biological cells and we were able to effectively capture cells such as NIH 3T3 fibroblasts and rat primary hepatocytes that were too large to be optically trapped with a single VCSEL alone. Figure 8 demonstrates the results of VCSEL tweezer binning in which a 3 × 3 array of VCSEL beams with an inter-tweezer spacing of 2.5 μm are combined to optically trap in 3-dimensions and translate with a velocity of 10 μm/s, a 23 μm diameter rat primary hepatocyte cell suspended in a PBS solution.

**Spatial arrangement of cells in co-cultures**

Cell organization into structured tissues has been an ongoing question for the field of tissue engineering. Heterotypic cell interaction includes the communication between parenchymal cells and nonparenchymal neighbors with resultant modulation of cell functions such as
growth, migration and/or differentiation. Recently, the synergy of combining MEMS (micro-electro-mechanical systems) with biological systems had led to the development of new microfabrication technologies such as microstamping (Xia and Whitesides, 1998; Jackman et al., 1995; Folch and Smith, 1999), fabrication of biologically compatible microfluidic channels (Folch et al., 1999; Delamarche et al., 1997), and photolithographic (Bhatia and Chen, 1999; Bhatia et al., 1998) and stencil patterning (Folch et al., 2002; Schwarz et al., 1998; Duffy et al., 1999) for structuring cellular microarrays. In general, it is difficult to fabricate structurally and topographically sophisticated structures using these methods because these techniques require chemically pre-defined patterns for cell adhesion (e.g., cell adhesion proteins, adhesion peptides or bioactive molecules) on the substrate of choice. We believe the use of VCSEL-based optical tweezer arrays can lead to biophotonic chip systems where, for example, the desired 2-dimensional spatial arrangements of parenchymal cells and nonparenchymal neighbors can be easily realized in order to provide a means of producing arbitrary user-defined arrangements of cells, which can then be used to study how cell-to-cell interactions modulate tissue function. Specifically, these interactions are of a fundamental importance in liver tissue (Ozkan et al., 2002), making it one of the main targets of the instrumentation described in this paper.

Using the VCSEL array to produce single and combined optical traps, we have successfully been able to arrange NIH 3T3 fibroblasts and rat primary hepatocytes in an arbitrary, predefined pattern to demonstrate that VCSEL arrays can be used in an optical tweezer system to create primitive engineered tissues. Figure 9 shows a picture of a repeated chain of a single NIH 3T3 fibroblast and two rat primary hepatocytes that is assembled in a PDMS (polydimethylsiloxane) micro-fluidic channel as an engineered co-culture. This controlled cell-cell interaction between hepatocytes and nonparenchymal cells can provide insights about the modulation of the hepatocyte phenotype and the precise ordering of these cells can elucidate the modes of cell signaling and the nature of cell-cell interaction. In addition, the patterning of cells using VCSEL tweezers can straightforwardly be extended to 2- and 3-dimensions with an unlimited number of different cell types, which currently is not possible using existing cell patterning techniques. Looking towards the future, the implementation of VCSEL array tweezers on a large scale has the possibility of providing an immediate impact in current research areas such as the analysis and design of tissue engineered cartilage (Martin et al., 2000) and the precise arrangement of nerve cells for the in vitro recreation of neural networks (Brekenridge et al., 1992).

Conclusions

We have shown that VCSEL arrays can be used to generate arrays of optical tweezers and may play an important role in the newly developing field of biophotonic chips. Although the output power of a VCSEL is lower than that of a traditional gas or diode laser, the capability of the array addressing of multiple cells in parallel makes the use of VCSEL arrays as optical tweezers a powerful and low cost technique that is difficult to reproduce using traditional lasers. We have additionally demonstrated that for the case of larger cell capture or trapping of a cell with a lower relative index of refraction, the VCSEL optical tweezers can be binned and function as a single trap by having their pitch decreased in the sample plane. Binning the optical tweezers in this manner provides the necessary power to optically trap the cell in 3-dimensions and relocate it to another place in the microfluidic sample. The significance of VCSEL array transport can be fully realized when this technique is used to assemble artificial tissues using cells grown in co-cultures. Once the artificial tissues have been assembled using VCSEL array tweezers, it will then be possible to use these tissues for studying the functional significance of tissue architecture at the cellular level and the molecular interactions between these cell types. Whether operated in a parallel array formation or used in combination, VCSEL tweezers provide a powerful complement to and extension of existing techniques used in biochip array technologies such as cellular assays and the creation of engineered tissues via cell manipulation.
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References