

Journal of Biomedical Optics

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Abstract. Reorientation of adhering cell(s) with respect to other cell(s) has not been yet possible, thus limiting study of controlled interaction between cells. Here, we report cell detachment upon irradiation with a focused near-infrared laser beam, and reorientation of adherent cells. The detached cell was transported along the axial direction by scattering force and trapped at a higher plane inside the media using the same laser beam by a gravito-optical trap. The trapped cell could then be repositioned by movement of the sample stage and reoriented by rotation of the astigmatic trapping beam. The height at which the cell was stably held was found to depend on the laser beam power. Viability of the detached and manipulated cell was found not to be compromised as confirmed by propidium iodide fluorescence exclusion assay. The reoriented cell was allowed to reattach to the substrate at a controlled distance and orientation with respect to other cells. Further, the cell was found to retain its shape even after multiple detachments and manipulation using the laser beam. This technique opens up new avenues for noncontact modification of cellular orientations that will enable study of intercellular interactions and design of engineered tissue. © 2011 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.3646207]

Keywords: gravito-optical trap; laser detachment; orientation; cellular interaction.

Paper 11246PR received May 17, 2011; revised manuscript received Aug. 25, 2011; accepted for publication Sep. 12, 2011; published online Oct. 31, 2011.

1 Introduction

Interactions between the same or different cell types control different aspects of tumor formation and progression. For example, interactions between the tumor cells with normal cells (such as stromal fibroblasts, cells of immune system, and endothelial cells) influences tumor biology by mechanisms that are not yet fully understood.^{1,2} Since cytoskeletal reorganization is a dynamic process as a consequence of the presence or absence of different binding partners, the study of cell–cell interaction not only requires the ability for micro-organization of different cells of the same or even different types, but also necessitates the removal of targeted cell(s) of the organized assembly away from the extracellular matrix (ECM). Further, interaction between cells and the ECM are critical to the function and development of multicellular organisms as well as in disease progression. The cell-ECM interaction plays an important role in processes including angiogenesis and carcinogenesis.³ Cell migration⁴ is required for various physiological and pathological processes such as gastrulation, immune response as well as cancer migration, involves rapid changes in the dynamics of actin filaments, together with repeated adhesion to and detachment from the ECM-microenvironment.⁵ These events are mainly mediated by integrins, which are a major family of cell-surface-adhesion receptors. While interacting with components of the ECM, integrins reorganize to form adhesion complexes termed as focal adhesions,^{5–7} which are associated with cytoskeleton reassembling. Further, since the interacting cells and intracellular organelles (e.g., Golgi apparatus, microtubule-organization

center, synaptic junctions) get polarized^{8,9} in a specific pattern, detachment and reorientation of the desired adhering cell(s) with respect to each other would provide vital information on change in kinetics of both physical (reorganization of altered morphology, focal adhesions, intracellular motor transport) as well as chemical (calcium and other intracellular signaling)⁹ processes during cellular interactions. Specifically, testing of early adhesion times or weak cell–substrate interactions is vital for the understanding of cell migration and transient interactions between immune cells. Most of the commonly used cell adhesion assays have been developed from qualitative assays that wash off nonadherent cells from culture surfaces with application of hydrodynamic forces to adherent cells and count the remaining cells.^{10,11} However, these shear forces may not represent true cell-ECM interactions. Further, detachment of targeted single cell(s) and reorientation of adhering cell(s) with respect to other cell(s) has not yet been possible, thus limiting the study of controlled interaction between the cells or cell-ECM system. It may be noted that although laser catapulting has enabled noncontact isolation and analysis of a single cell by use of a pulsed laser beam,^{12–14} it lacks the control to reorient and place the catapulted cell back onto the sample plane.

In this paper, we report a new laser-assisted method for the cell adhesion and interaction studies. Microirradiation of the targeted cell with a weakly-focused Ti:Sapphire laser beam resulted in detachment of the cell, followed by tossing into a vertical orientation. The detached cell was transported and trapped inside the media at a plane higher than the focal plane using the same laser beam by gravito-optical trapping. Use of a rotatable astigmatic trapping beam led to reorientation of the detached cell that could be repositioned with respect to desired

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cells-on-the-substrate by translation of the sample stage. Viability of the detached and manipulated cell was found to be not compromised as confirmed by propidium iodide (PI) fluorescence exclusion assay. Further, no noticeable change in the cytoskeletal morphology of the detached cell could be detected. This technique is not only intriguing for the study of cell migration, but also opens up new avenues for noncontact modification of cellular orientations that will enable study of intercellular and cell-ECM interactions.

2 Materials and Methods

2.1 Cell Culture and Incubation

HELA cells were routinely cultured in Dulbecco's modified eagle's medium (DMEM) supplemented with 5% fetal bovine serum. The cultures were maintained at 37°C in a 5% CO₂ humidified atmosphere. For cell adhesion and detachment research, cells were trypsinized, plated on cover slips, and used after 2 h of culturing. DMEM and fetal bovine serum were purchased from Lonza (Maryland); Trypsin was purchased from Mediatech (Manassas, Virginia). All other reagents were purchased from Fisher Scientific except those specifically mentioned. Cells were incubated with PI (5 μm) 10 min before laser manipulation experiments.

2.2 Near-Infrared Laser Manipulation and Cell Imaging Setup

A schematic of the setup for detachment, transportation, and orientation is shown in Fig. 1. A 785 nm Ti:Sapphire laser (Maitai HP, Newport Spectra-Physics Inc.) beam was expanded (BE), and coupled to a low NA (0.5) 20× microscope objective on a

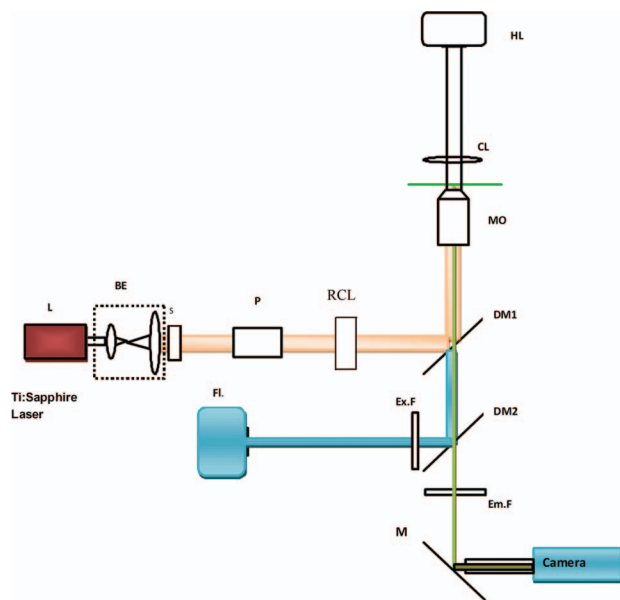


Fig. 1 Experimental setup for laser-assisted detachment, transportation, and orientation. L: Ti: Sapphire laser; BE: beam expander; S: shutter; P: polarizer; RCL: rotatable cylindrical lens; FL: fluorescence excitation source; Ex: excitation filter; Em: emission filter; MO: microscope objective; CL: condenser lens; DM1 and DM2: dichroic mirrors; M: mirror; HL: halogen lamp.

Nikon Ti-U inverted microscope platform. The dichroic mirror 1 (DM1) combined the laser beam and the visible light from the excitation source. The laser beam was made astigmatic by use of a cylindrical lens (RCL), which was rotated for orienting the elliptic beam profile. At the sample plane, the spot size was ~2 μm × 10 μm. The dichroic mirror 2 (DM2) reflects the excitation lamp light and transmits the emitted fluorescence to the CCD camera. Bright field and fluorescence images were captured using a cooled CCD and processed using IMAGEJ software. The power of the laser beam at the focal plane was estimated by multiplying the transmission factor of the objective with the power measured with a power meter (PM100D, Thorlabs Inc.) at the back of the objective.

2.3 Principle

Figure 2 shows the schematic of cellular detachment and follow-up manipulation processes. When the cell is irradiated continuously by a near-infrared (NIR) laser beam with power levels on the orders of magnitude below plasma formation threshold, the mechanism of detachment is primarily due to radiative forces induced by the laser beam,¹⁵ instead of other disruptive forces used in single-pulse laser catapulting experiments.^{16,17} The detachment [Fig. 2(a)] force required for bond breaking could consist of two major components: i. Photothermal interaction and absorption force (F_{ab}); ii. Photomechanical interaction and scattering force (F_{sc}). Using single cell force spectroscopy,¹⁸ the rupture force of integrin-binding with substrate has been measured to be ~30 pN. In the case of early binding events, depending on the binding sites, the required rupture force can be several hundreds of pN. The scattering force by laser beam power (P) at the cell membrane-substrate interface can be estimated using the equation $F_{sc} = Q_s \cdot n_1 P / c$, where Q_s is dependent¹⁹ on relative refractive index (n_2/n_1). Using refractive index of cell (n_1) to be 1.4 and that of surrounding media to be 1.33, Q_s will be ~0.1, which corresponds to scattering force F_{sc} of ~100 pN for a laser power level of 200 mW. This may be sufficient to break

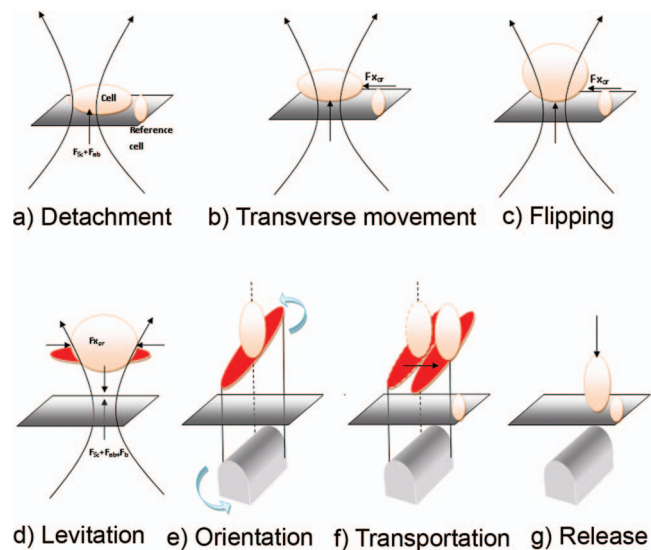


Fig. 2 A schematic process of the cellular manipulations. (a) Detachment; (b) Transverse movement; (c) Flipping; (d) Levitation; (e) Orientation; (f) Transportation; (g) Release.

the focal adhesion sites and detach the early-adhering cells with few binding sites. Further, the required rupture force may be reduced due to an increase in temperature created by the laser beam at cell-substrate interface. For strongly-adhering cells, the laser beam can be made to operate in a pulsed condition leading to higher axial (e.g., scattering) force.^{20,21} Subsequent to detachment, the cells (if not placed at the center of the laser beam) can slide toward the center of the beam as a result of gradient force in the horizontal direction [Fig. 2(b)]. In order to minimize the potential energy, the flattened cell flips into the vertical plane [Fig. 2(c)]. This tilting maximizes the interaction energy with the laser beam, leading to higher axial transport (absorption and scattering) forces by laser beam which can completely break the remaining bonds (if any).

In the absence of tight focusing, the cell can be levitated at a plane higher than the focal plane [Fig. 2(d)]. Stable axial position of levitation is determined by balance of the sum of buoyant and radiation forces in vertical up-direction against the downward gravitation force [Fig. 2(d)]. Figure 2(e) shows the rotation of the cylindrical lens resulting in rotation of the astigmatic (elliptic) beam profile. This results in transfer of orbital angular momentum^{22,23} to the vertically tilted cell, leading to orientation of the cell around the optical axis [Fig. 2(e)]. Transportation of the cell, in its new orientation, is carried out by moving the laser beam or moving the stage to the area of interest [Fig. 2(f)]. Finally, the cell can be brought back to the targeted area on the substrate by reducing the laser beam power [Fig. 2(g)]. The reoriented cell now attaches to the substrate at the desired distance and orientation with respect to other cell(s).

3 Results

3.1 Bond Breaking and Flipping of Cells

When the selected cell was irradiated at a laser power of 90 mW focused to a spot radius of $\sim 1 \mu\text{m}$ by a 0.5 NA objective (intensity $\sim 3.6 \times 10^6 \text{ W/cm}^2$), the processes described in Figs. 2(a)–2(c) was found to occur. After bond breakage, the cell moved toward the center of the beam and got oriented by the laser beam without complete detachment and levitation (2 out of 5 cells). Figure 3 shows manipulation of a cell, irradiated by a 785 nm laser microbeam for 20 s. 4.58 s after laser irradiation, the cell moved in the horizontal plane [Fig. 3(b)]. Nine seconds after irradiation, the cell was seen to be flipped into a vertical plane on the Petri dish [Figs. 3(c) and 3(d)] and maintained this orientation until the laser beam was switched off. With a laser power lower than 70 mW, the cell was not able to flip even after irradiation for a longer duration.

3.2 Levitation of the Detached Cells

Figure 4 (Video 1) shows complete detachment and levitation of a targeted cell [marked by a circle, Fig. 4(a)] cell irradiated by a 785 nm laser at power of 141 mW. Figure 4(b) shows propidium iodide fluorescence before laser irradiation. The targeted cell first feels the force of attraction toward the center of the focused laser beam (marked by the arrow) as shown in Figs. 4(c) and 4(d). This can be attributed to the transverse gradient force exerted by the laser beam. During process of detachment, the cell experienced torque around the horizontal axis leading to flipping

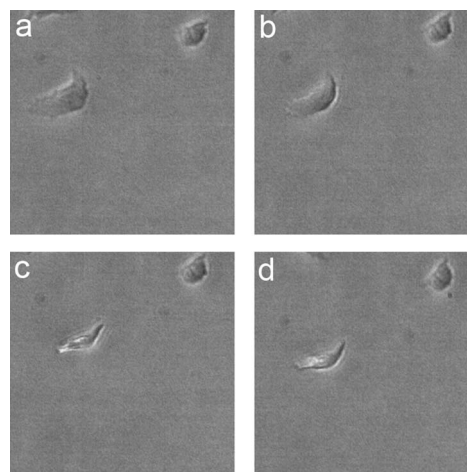


Fig. 3 Flipping of cell by 785 nm laser beam at a power of 110 mW. (a) Before irradiation of cell. (b) 4.58 s, (c) 9.08 s, and (d) 130 s of laser irradiation.

of the cell in the vertical plane [Fig. 4(e)]. After 4.17 s of laser irradiation, the cell was completely detached and levitated up in the media to a height of $\sim 110 \mu\text{m}$ [Fig. 4(f)]. By translating and orienting the astigmatic laser beam ($2 \mu\text{m} \times 10 \mu\text{m}$), the cell was transported to the desired location and reoriented anticlockwise at a higher plane [Fig. 4(g)]. Decreasing the laser beam power led to bringing the cell down to the substrate [Figs. 4(h)–4(j) 97.00, 111.67, and 121.58 s, respectively]. The reattached cell on the substrate is shown in Fig. 4(k). Propidium iodide exclusion in the targeted cell after laser manipulation [Fig. 4(l)] shows that the viability of the laser manipulated cell is not compromised. No fluorescence emission from the cell demonstrates that the cell membrane is intact after detachment.

3.3 Dependence of Height of Levitation on Laser Power

76.93% of cells were detached by different powers of laser beam (20 out of 26). The effect of laser power on the distance of levitation was studied by varying the laser power up to

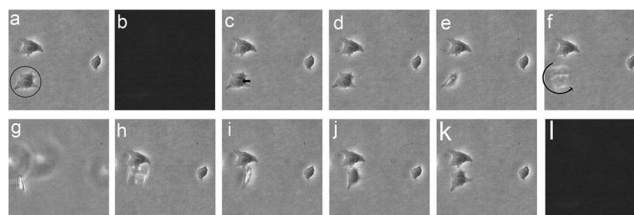


Fig. 4 Cell detachment, levitation, and orientation by the NIR laser beam at 141 mW. (a) Targeted cell (marked by a circle) before irradiation (0 s); (b) Propidium iodide fluorescence before irradiation; [(c) and (d)] Cell movement to the left toward the center of the laser spot (1.17 s, 2.83 s); (e) Flipping of the cell in a vertical plane (3.87 s); (f) Levitation of the cell at a higher plane (4.17 s). (g) Anticlockwise orientation of the cell at a higher plane (12.75 s). [(h)–(j)] Bringing the cell down to the substrate (97.00, 111.67, and 121.58 s, respectively). (k) Cell reattached to the bottom. (l) Propidium iodide staining of cells after laser manipulation. All images are in the same magnification. Scale bar: $50 \mu\text{m}$. (AVI, 1.8 MB). [URL: <http://dx.doi.org/10.1117/1.3646207.1>]

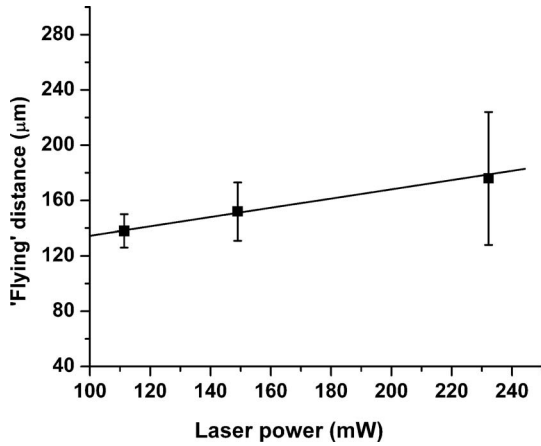


Fig. 5 Effect of laser power on cell levitation height. The error bars represent standard error of the mean, $n = 4$. The straight line represents the linear fit to the data.

~240 mW. The height of the cell was measured by first switching off the laser beam and then moving the microscope objective upward to refocus the cell. The translation of the objective was measured from the rotational displacement (number of divisions) of the focusing knob. As shown in Fig. 5, the height of levitation (flying distance) increased from $138 \pm 12 \mu\text{m}$ (laser power of 111 mW) to $152 \pm 21 \mu\text{m}$ when the laser power is increased to 149 mW. At a laser power of 232 mW, the flying distance was $176 \pm 48 \mu\text{m}$. It may be noted that the levitation distance was found to vary linearly with laser power (Fig. 5). This can be attributed to the fact that the levitating scattering force depends linearly on incident laser power.

3.4 Orientation, Transportation, and Repositioning of the Detached Cell

Figure 6 shows laser assisted multiple cell detachment–attachment cycles with controlled orientation, transportation, and repositioning of the targeted cell. PI fluorescence and a bright field image of cells before laser irradiation is shown in Figs. 6(a) and 6(b), respectively. Figure 6(c) shows the first detachment and levitation of the targeted cell [marked by a circle in Fig. 6(b)] using laser beam power of 138 mW. By lowering the laser power, the cell was brought back to the Petri dish [Fig. 6(d)]. Figure 6(e) shows the second detachment of the same cell after an adhesion period of 5 min. Transportation of the levitated cell and the second attachment close to another cell was carried out by translation of the stage [Fig. 6(f)]. Figures 6(g) and 6(h) show the third detachment and attachment of the cell, respectively. After the fourth detachment, reorientation of the cell was carried out by rotation of the cylindrical lens as described in Fig. 2(e). The major axis of the cylindrical lens and orientation of the levitated cell is marked by a double-sided arrow in Figs. 6(e) and 6(i). Attachment of the cell for the fourth time was carried out by lowering the laser beam power [Fig. 6(j)]. Each intermediate adhesion period was ~5 min and the detachment period was ~1 min. After four attachment–detachment cycles lasting for ~30 min, the cell was still viable as confirmed by PI exclusion assay. Figure 6(k) shows no detectable PI fluores-

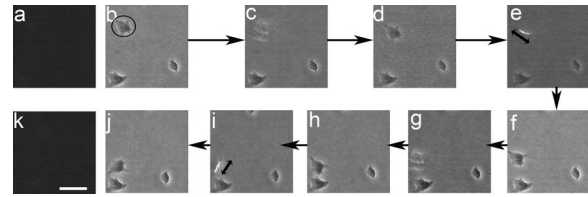


Fig. 6 Laser assisted multiple cell detachment–attachment cycles with controlled orientation, transportation, and repositioning of the targeted cell. (a) Propidium iodide fluorescence of cells before laser irradiation (–50 s); (b) Bright field image before irradiation (0 s); (c) Levitation of detached cell (15 s); (d) Reattachment of the cell on the Petri dish (60 s); (e) Second-detachment of cell (400 s); (f) Transportation and second attachment of the cell (460 s); (g) Third detachment of the cell (800 s); (h) Third attachment of the cell (850 s); (i) Fourth detachment and reorientation of the cell (1200 s); (j) Fourth attachment of the cell (1270 s); (k) Propidium iodide fluorescence of cells after multiple attachment–detachment cycles (1700 s). All images are in same magnification. Scale bar: 50 μm .

cence of cells at the end of laser manipulation. Further, the cell was found to retain its shape even after multiple detachments and manipulation using the laser beam. Compared to the first detachment of the cell, the second detachment required significantly lower irradiation time (or dose) ($p < 0.05$, $n = 4$ for laser powers between 111 to 149 mW). This is indicative of the fact that the force required for breaking early adhesion bonds between the cells and the Petri dish is significantly lower than that of long-term adhesion.

As shown in Fig. 6, the NIR laser beam is able to detach the cell multiple times from the Petri dish. Comparing the second detachment of cells with the first time, the irradiation time required for detachment decreased significantly when laser powers were low ($p < 0.05$, $n = 4$ when laser powers were 111 and 149 mW, Fig. 7). This indicates that the second detachment requires a lower laser dose. These results suggest to us that the adhesion force between the cells and the Petri dish during the second attachment is not as strong as the adhesion before laser irradiation. Further, the time required for the second cell detachment was found not to depend significantly on the laser power.

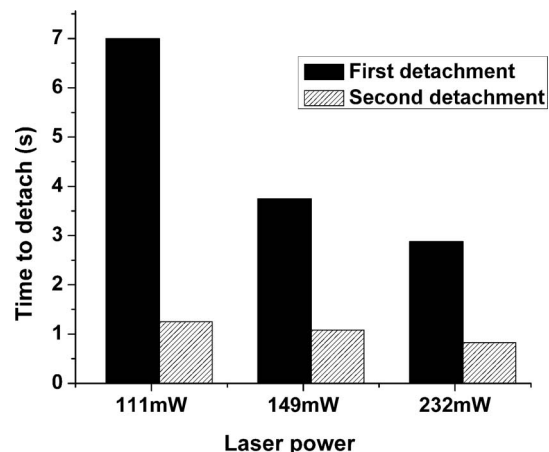


Fig. 7 Effect of different laser power on time required for first and second detachment ($n = 4$). The cell was allowed to adhere for 5 min after the first detachment.

4 Discussions

Though we clearly demonstrated the potential of detachment and controlled manipulation of adhering cells, the mechanism of a weakly-focused NIR laser beam in eliciting the detachment events is still under investigation. While scattering and absorption forces can account for detachment of cells under an early-adhesion phase, significant photothermal or photomechanical thrust is necessary to propel the adhering cell out of the Petri dish surface. Indeed, a high energetic pulsed laser beam has been used in literature to catapult the cells out of the medium.^{16,17} This has been attributed to plasma formation via multiphoton absorption and an avalanche ionization process that results in large energy densities far above the vaporization enthalpy of the target medium. High temperatures and large pressures in the focal volume are considered to play a key role for catapulting of cells from the attached polymer foil.^{17–21} However, it lacks the control necessary for orienting and placing the cell in a new orientation. Further, viability of a catapulted cell under high intensity and disruptive events remains to be delineated.²⁴ The method presented in this paper applies primarily to early-adhesive cells, with significant potential for cellular organization in tissue engineering research. Our future goal is to be able to extend the presented method to detach cells incubated for several hours after seeding. Tilting and reorientation can prove to be very useful in the study of cell–cell interaction in addition to the ability to design engineered tissue.

A complex interplay between the actin cytoskeleton and focal adhesion sites leads to the generation of membrane protrusions and traction forces.^{3,25} Therefore, testing early adhesion times or weak cell–substrate interactions is vital for understanding cell migration. Further, interactions between immune cells are transient. The finding that the shape of the cell was not altered after detachment(s) for approximately 60 s allows estimation of shape memory time constant of the cytoskeletal architecture of the cell. In traditional nontargeted detachment procedures (e.g., Trypsin), the detachment time is more than a few minutes, leading to complete loss of shape of the cell. It is noteworthy that the presented laser-based detachment technique applies normal force between the cell and the substrate contrary to that exists for most of the commonly used cell adhesion qualitative assays that use hydrodynamic shear forces. Thus, the method presented here represents direct perturbation of cell-ECM interactions. The fact that the dose required for subsequent detachments was significantly less than that required for the initial detachment indicates that focal adhesion complex is not completely formed after the first detachment. This suggested to us that formation of focal adhesion complex is a relatively long-term process which is consistent with a previous study.²⁶ Furthermore, since time duration of detachment and reorientation of the cells is controllable down to a few seconds with this technique, it will be valuable for the study of cell polarization in response to various attractants including chemotaxis research.

5 Conclusions

We demonstrated detachment and reorientation of adherent cells using a focused astigmatic near-infrared laser beam and delineated the parameters for various processes. The early adhesive interaction of living cells with substrates was examined. The

detached cell could be transported along the axial direction by scattering force and trapped at a higher plane inside the media by a gravito-optical trap. The height at which the cell was stably held was found to depend on the laser beam power. The trapped cell could then be transported in a transverse plane by movement of the sample stage and reoriented by rotation of the astigmatic trapping beam. The cell could be brought back and repositioned on the substrate by controlling the laser beam power. Viability of the detached and/or manipulated cell was found not to be compromised as confirmed by PI fluorescence exclusion assay. Further, the cell was found to retain its shape even after multiple detachments and manipulation using the laser beam. This technique opens up new avenues for noncontact modification of cellular orientations that will enable study of intercellular interactions and design of microengineered tissue.

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