Characterization of the laser-based release of micropallets from arrays

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Abstract. The micropallet array system uses a pulsed laser to release pallets tens of microns to hundreds of micrometers in size from a larger array, enabling selective isolation of single cells adherent to the pallets. We characterize the laser-based release of pallets with respect to pallet array and laser parameters. The threshold laser energy required for pallet release increases linearly with the area of the pallet in contact with the underlying glass substrate. The spacing of the pallets within an array as well as the thickness or height of the pallet does not impact the energy required to release a pallet. Delivery of multiple laser pulses decreases the energy/pulse required for pallet release when the pallets were 100 μm or greater on a side. In addition to the square pallets, complex structures such as cantilevers and spirals could be released without damage using the pulsed laser. Identification of the pallet-array variables influencing the energy required for pallet release as well as strategies to minimize this energy will prove critical in optimizing the release of pallets with cells on the arrays.
 [DOI: 10.1117/1.2937475]

Keywords: pallets; photoresists; arrays; laser applications; laser-induced forward transfer; laser release.

Paper 07355RR received Aug. 28, 2007; revised manuscript received Jan. 21, 2008; accepted for publication Jan. 22, 2008; published online Jun. 10, 2008.

1 Introduction

The need to sort cells is fundamental to almost all areas of biomedical research.[1–6] A new cell-sorting strategy incorporates a pulsed laser to release microfabricated pallets on which cells are cultured.[7,8] A laser pulse is used to release individual pallets for collection to accomplish the sort. Pulsed lasers have been used in other applications for the direct transfer of cells from one surface or container to another. Chief among these techniques are laser-induced forward transfer (LIFT) and laser microdissection with laser pressure catapulting[9,10] (LMPC). LIFT was first described for the deposition of copper metal patterns inside a vacuum chamber.[11] In LIFT, a laser pulse heats a material past its boiling point so vapor-induced pressure ejects the material from a donor to an acceptor substrate. Modifications of the LIFT process enable the technique to be used to transfer material without subjecting that material to vaporization to transfer delicate substances or structures for deposition of electronic components, biological molecules, or cells.[12–16] LIFT of biomolecules may prove useful in manufacturing DNA and protein microarrays.[12,13] When applying LIFT for microarray spotting, the solvent acts as a transport vector and prevents decomposition of the soluble biomolecules.[17] Damage to biological materials during LIFT can also be mitigated by using a biocompatible sacrificial absorbing layer. It is this approach that has been used to transfer live cells for cell arraying.[14,18] The cells are suspended in a thin fluid layer overlying the sacrificial layer on which the laser pulse is focused. The focused pulse causes transfer of a droplet of overlying fluid containing cells in suspension onto an acceptor substrate. Measures of cell viability, stress, and proliferative ability after LIFT point toward its potential for applications in single-cell studies and tissue engineering.[14,18] However, while the technique is suitable for transfer of random cells suspended in buffer, it is not readily compatible with identification or analysis of unique cells followed by their sorting.

Laser microdissection is used predominantly to obtain tissue sections for genetic and proteomic studies.[10,19–24] The technique works well for fixed or frozen tissue as the laser-cutting systems utilized in these instruments are affected by moisture, and removal of fluid from the specimen is generally

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1083-3668/2008/13(3)/034007/9/$25.00 © 2008 SPIE

required for dissection and collection.[25] Drying of the specimen limits the use of this technique for live cell applications, although protocols for this purpose have been published.[26,27] Zeiss (Göttingen, Germany) markets an instrument for laser microdissection that uses a pulsed UV laser to "catapult" the dissected tissue or cells into an overlying collection device.[28] LMPC has had greater success in live-cell applications than earlier laser microdissection technologies due to the fact that a thin layer of fluid can be present during cutting and laser transfer.[10] A 5-μm-thick UV-absorbing polymer foil is used to protect the specimen from UV-light-induced and thermal damage, but the foil scatters and fluoresces, interfering with histochemical and fluorescence identification techniques for cells of interest. A large number of layers are present in the current technique for live-cell catapulting, making catapulting dynamics and optimization complex.[10]

The aforementioned pallet-array system permits living cells or colonies of cells to be sorted while they remain on their growth surface, thus enabling analysis prior to the sort.[29] Studies to date using this sorting technique have documented a high rate of cell viability after laser-based release, and exceptional success in clonal expansion of sorted cells.[7] Stresses during sorting procedures, as is well known in flow cytometry, can induce apoptosis (programmed cell death) particularly in noncancer cells.[30–34] Although high rates of cell viability have been demonstrated for cancer cell lines, optimization of laser-based pallet release remains a need for more fragile cells, e.g., primary cells, to maximize cell health and minimize cell stress. In addition, the various cell types and applications envisioned for pallet arrays will require a variety of pallet designs, which will impact release parameters, most critically the pulse energy required for pallet removal. This paper seeks to perform quantitative assessment of the effect of laser and array parameters on threshold energies for pallet release to understand and optimize the variables for laser-based release of living cells. A number of variables were examined to determine how they influence the energy required for laser-based pallet release. Pallet size, the distance between pallets, and pallet height were varied on a test pallet array. The laser parameters of pulse duration and the pulse number required for pallet release at a given energy were also investigated. Strategies to minimize the laser pulse energy for pallet release are described as well as alternative uses for the focused laser in the release of complex microstructures. The results of this study should provide a better understanding of the laser release process of pallets and enable the choice of parameters that reduce the exposure of cells to physiologic stresses during the sort.

2 Materials and Methods

2.1 *Materials*

The SU-8 10 and SU-8 50 photoresist and SU-8 developer were purchased from MicroChem Corp. (Newton, Massachusetts). EPON resin SU-8 was from Resolution Performance Products (Houston, Texas). Precleaned glass slides ($75 \times 25 \times 1$ mm^3) were purchased from Corning Glass Works (Corning, New York).

2.2 *Photomask Fabrication*

Iron oxide photomasks with various micropatterns were fabricated according to the traditional microfabrication process. The masks were used to generate an array with regions containing square pallets with different dimensions (25, 50, 100, and 200 μm) and regions containing pallets with different interpallet spacing (10, 25, 50, and 75 μm).

2.3 *Fabrication of SU-8 Structures*

Glass slides were cleaned by storing them in sulfuric acid (H_2SO_4) for a minimum of a month. The slides were then rinsed with deionized water and dried in a nitrogen stream. The slides were dehydrated in a 180°C oven for 5 min before use. SU-8 films of 25 μm thickness were obtained by spin coating the SU-8 10 resist on the glass slides at 500 rpm for 10 s, followed by 1200 rpm for 30 s using a WS-200-4NPP spin coater (Laurell Technologies Corporation, North Wales, Pennsylvania). The coated slides were baked on a hotplate at 65°C for 3 min, followed by a second bake at 95°C for 5 min to remove organic solvent. After baking, the slides were slowly cooled to room temperature. To prepare structured SU-8 (e.g., micropallets), the SU-8 film was then exposed to UV light through a photomask with the designed features using a 500-W Oriel Flood Exposure Source (Newport Stratford, Inc., Stratford, Connecticut). The exposure time was adjusted to deliver a total of 200 mJ/cm^2. The postexposure baking was performed on a hotplate at 65°C for 1 min and 95°C for 2.5 min. After slowly cooling to room temperature, the SU-8 samples were developed in SU-8 developer for 3.5 min, rinsed with ethanol, and dried in a stream of nitrogen. Arrays were checked for quality, and then hard-baked (cured) at 150°C for 1 h.

SU-8 films of 50 μm thickness were prepared in a similar manner to that for films of 25 μm thickness with the following exceptions. SU-8 50 resist was spin coated on glass slides at 500 rpm for 10 s, followed by 2100 rpm for 30 s. The coated slides were baked on a hotplate at 65°C for 9 min, followed by a second bake at 95°C for 25 min. To prepare structured SU-8 (e.g., micropallets), the SU-8 film was exposed to collimated UV light (400 mJ/cm^2). The postexposure baking was performed on a hotplate at 65°C for 2 min and 95°C for 5 min. The SU-8 samples were developed for 7.5 min, rinsed with ethanol, and dried in a stream of nitrogen. The arrays were then hard-baked.

SU-8 films of 75 μm thickness were prepared in a manner similar to that for films of 25 and 50 μm thicknesses with the following exceptions. SU-8 50 resist was spin coated on the glass slides at 500 rpm for 10 s, followed by 1600 rpm for 30 s. The coated slides were baked on a hotplate at 65°C for 9 min, followed by a second bake at 95°C for 25 minutes. SU-8 films were exposed to collimated UV light (450 mJ/cm^2). The postexposure baking was performed on a hotplate at 65°C for 2 min and 95°C for 9 min. The SU-8 samples were developed for 9.5 min, rinsed, dried, and then hard-baked.

2.4 *Optical Geometry for Plasma Formation*

Light from a pulsed Nd:YAG laser (New Wave Research ACL-1, Fremont, California, 532 nm, 5 ns pulse width) was steered into a beam expander, then directed through an iris to

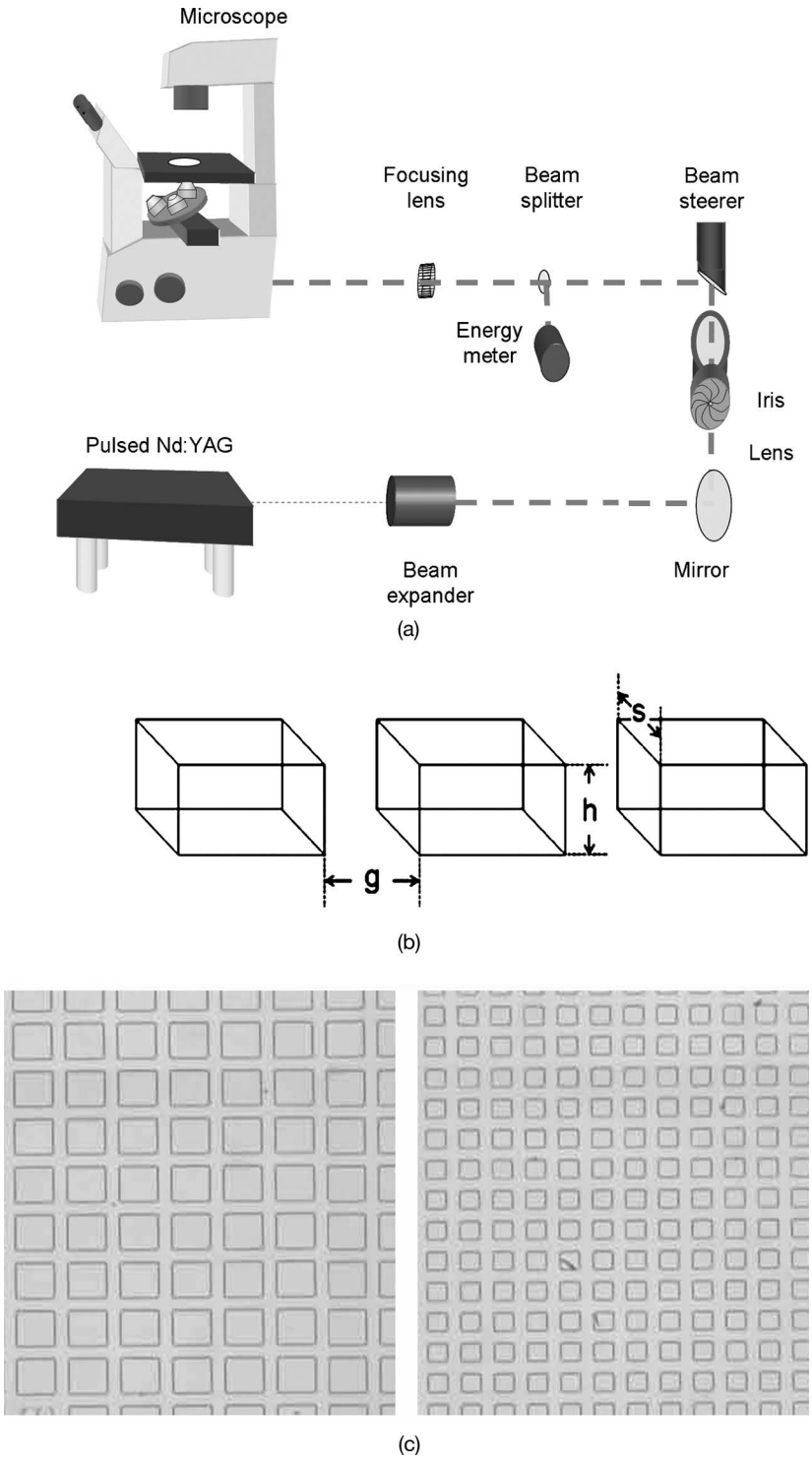

Fig. 1 Experimental system for laser-based pallet release: (a) optical system; (b) schematic of three pallets in an array with dimensions of height (*h*), size (*s*), and inter-pallet gap (*g*); and (c) image of two sections in the pallet array. The right and left panels are a section of pallets with a side (*s*) of 200 and 100 μm, respectively. Both arrays possess other dimensions of 50 (*h*) and 50 (*g*) μm.

yield a beam diameter of 6 mm [Fig. 1(a)]. The light then passed through a lens (150-mm focal length) into the rear port of an inverted microscope (Nikon TE 300, Melville, New York). Arrays were imaged using a CCD camera (CCD Camera Model KP-M1AN, Hitachi, Brisbane, California, or CoolSNAP™ *fx*, Photometrics, Portland, Oregon). Images were captured using MetaFluor (Universal Imaging, Downingtown, Pennsylvania). An objective with a magnification of 20× [numerical aperture (NA) 0.5, Nikon Plan Fluor] was used to focus the laser beam in order to release pallets. Beam intensity distribution on sample was TEM_{00} Gaussian. Beam diameter at the focal point was approximately 1 μm. A

coverslip was placed into the path of the laser beam prior to the back entrance of the microscope. The light from the coverslip was directed to an energy meter (J4-09 probe, Molectron EPM 1000, Santa Clara, California) and used to measure the energy of each laser pulse.

2.5 *Measurement of the Probability of SU-8 Structure Release by a Single Laser Pulse*

To release individual pallets, a pallet array was first placed on a microscope stage, and then the laser focus was set at the interface of the glass substrate and SU-8 pallet. A solution of polystyrene beads (approximately 1 μm diameter) in water was placed over the array, and beads were allowed to settle on the top surface of the glass to facilitate accurate and consistent focusing at the interface. Laser energies were chosen so at least two energies resulted in 0% release of targeted pallets, at least two energies resulted in release of 100% of targeted pallets, and at least two energies yielded between 0% and 100% release of targets. For each laser energy 10 pallets were targeted with each pallet receiving only a single pulse. For each laser energy, the fraction of pallets released (out of 10 targeted) and the average energy of the 10 pulses fired were determined.

2.6 *Measurement of the Probability of SU-8 Structure Release by Multiple Laser Pulses*

For experiments using multiple pulses for release of a single pallet, the pallet array was first placed on the microscope stage and laser focus was set at the interface between SU-8 and glass, as for release with a single pulse. Multiple pulses fired manually at a frequency of 1 Hz were then used to release the structures as described here.

2.7 *Fit of the Data to a Gaussian Error Function*

To determine the threshold energy for pallet release, the pallet release frequency was plotted as a function of incident pulse energy calculated to reach the microscope stage. A Gaussian error function was fitted to this data. Fitting was performed using the nonlinear least-squares fitting capability of the software Origin 7.5 SR6 (OriginLab Corporation, Northhampton, Massachussets). The Gaussian error function was

$$p(E_p) = 0.5\{1 + \mathrm{erf}[P_1(E_p - P_2)]\},$$

where $p(E_p)$ is the probability of pallet release at a laser energy E_p. The values P_1 and P_2 were the fitted parameters, where P_1 determined the sharpness of the Gaussian error function and P_2 was the threshold energy, the pulse energy that resulted in a 50% probability of pallet release.

3 Results and Discussion

3.1 *Characterization of the Likelihood of Pallet Release with Respect to Pallet Size*

The size of the pallet used when sorting cells will depend on the cell type and the desired number of cells per pallet. Typically smaller pallet sizes ($\leq$50 μm) will be suitable for single cells while larger sizes ($>$50 μm) are more appropriate for cell colonies. The required laser energy for pallet release may depend on the size of the pallet. Thus, it is important to understand how the laser energy increases with the size of the pallet. To determine how the laser energy required for release scaled with the pallet size, pallets were released from an array of square pallets with a sides (s) of 25, 50, 100, or 200 μm [Fig. 1(b) and 1(c)]. For these pallets, the height (h) was 50 μm and the interpallet gap (g) was 50 μm. Six laser energies ranging from less than 1 to greater than 10 μJ were chosen for pallet release. The pulses were aimed at the center of the targeted pallets, at the interface between the glass and SU-8. To minimize the effects of batch-to-batch variability in pallet release, the data for all sized pallets was obtained from a single array. The fraction of pallets released was recorded, along with the average energy of the ten pulses aimed at the pallets. The probability of pallet release as a function of pulse energy was fit to a Gaussian error function [Fig. 2(a)]. The Gaussian error function describes the stochastic nature of the

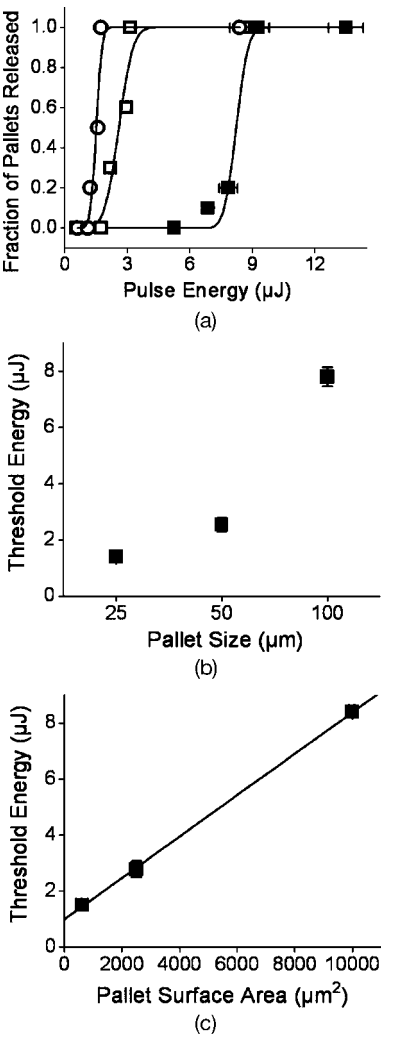

Fig. 2 Dependence of release energy on pallet size: (a) the probability of pallet release for different sized pallets was plotted against the average laser energy. The pallet side (s) was 100 (solid squares), 50 (open squares), or 25 (open circles) μm. The error bars represent the standard deviation of the laser energies. The solid lines represent fits of the data points to an error function. The threshold energy for pallet release is plotted as a (b) function of pallet size and (c) function of the pallet surface area. The solid line is a straight line fit to the data points. For both (b) and (c), each data point is the average of three measurements and the error bars represent the standard deviation.

Table 1 Threshold energy (in microjoules) for pallet release.

		10-μm gap	25-μm gap	50-μm gap	75-μm gap
25-μm squares	25 μm tall	1.8±0.2	1.7±0.1	1.7±0.1	1.7±0.3
	50 μm tall	—	1.8±0.4	1.4±0.1	1.4±0.1
50-μm squares	25 μmtall	4.3±0.6	4.7±0.2	5.2±0.7	4.6±0.5
	50 μm tall	—	2.4±0.2	2.5±0.3	2.9±0.2
	75 μm tall	—	4.1±0.1	4.4±0.1	3.8±0.1
100-μm squares	50 μm tall	—	8.0±0.5	7.8±0.3	8.0±0.4
	75 μmtall	—	13±1	12±1	11±1

Thresholds are calculated from data of triplicate experiments and shown as mean ± standard deviation.

plasma assumed to be the mechanism of laser-based pallet release. Comparison of thresholds for pallets of different sizes revealed a significant increase in threshold energy with increase in pallet size. The threshold energy required to release 25-μm squares, 50-μm squares, and 100-μm squares increased nonlinearly with size [Fig. 2(b), Table 1]. The 200 μm size pallets could not be released when tested with the highest laser energy available on the current system (14 μJ). To determine whether the threshold energy was linearly related to the surface area, the surface area of the pallet was plotted against the threshold energy [Fig. 2(c)]. The data points fell on a straight line with a y intercept of 1.0 μJ. Since the release energies are proportional to the surface area, the 200-μm pallets would likely require about 28 μJ to be released. As the pallet surface area decreases to zero, a finite amount of energy is still required to release the pallet since the y intercept is not zero. It is likely that this is the energy (1 μJ) required to form a plasma at the SU-8-glass interface. This threshold energy for plasma formation acts as a necessary condition for pallet release to occur. For small pallet size ($<$25 μm), the magnitude of plasma formed at the threshold for plasma formation is sufficient to disrupt the adhesive forces between the glass and SU-8. As pallet size increases ($>$25 μm), the threshold energy for pallet release will be increasingly higher than the threshold for plasma formation, as larger plasmas will be required to disrupt the larger adhesive forces corresponding to larger SU-8 to glass contact area. Prior work studying the laser-induced forward transfer of liquids demonstrated that droplet volume displayed a linear dependence on laser pulse energy.[17] Furthermore, in similarity to our work, a threshold energy density was a necessary condition for transfer. In the presented experiments, the laser is focused to a spot size smaller than the pallet area interface with the glass substrate. Similar to the mechanism described for LMPC, it is likely that the mechanism of pallet release relates to the generation of plasma with a concomitant shock wave and cavitation bubble.[10] The mechanical forces created by these phenomena are the probable source of energy used to dislodge the pallet.

3.2 *Dependence of Pallet Release Energy on the Inter-pallet Spacing*

Different applications of the pallet array system may be best served by different interpallet spacings. For cell sorting, the distance between pallets on arrays will be optimized for cell isolation and the stability of air virtual walls between the pallets. To determine whether interpallet spacing influenced thresholds for pallet release, experiments were performed on an array with regions containing square pallets spaced 10, 25, 50, or 75 μm from their neighbors. The height of the pallets was either 25 or 50 μm and the side of the pallet was 25, 50, or 100 μm. The probability of pallet release at different energies was measured and the threshold energy determined. For each pallet height, the data were determined from a single array to eliminate array-to-array variability. The pallets with a 50-μm height and a 10-μm interpallet gap could not be released due to residual SU-8 in the regions between the pallets. For all other pallets, comparison of the threshold energies for pallet release revealed no significant difference in threshold energy with respect to the inter-pallet spacing [Fig. 3(a), Table 1]. Since interpallet spacing does not affect the energy required for laser release, the interpallet gap can be optimized to improve other array qualities. Air pockets (virtual walls) placed between the pallets are used to direct cells to the pallet tops. The stability of these air pockets is directly related to the size of the interpallet gap and pallet height. In future studies, the interpallet gap can be optimized for virtual wall stability with no influence on the required laser energy for pallet release.

3.3 *Evaluation of Interarray Variability*

In the preceding experiments, all pallets were fabricated on the same array to eliminate variability occurring at different fabrication times. However, it is not always possible to use pallets fabricated at identical times. Pallets with identical dimensions but fabricated at different times may have different threshold release energies since the adhesiveness of SU-8 to glass depends on multiple variables. These variables include the glass-cleaning procedure, the SU-8 baking parameters, the UV exposure time, and the developing parameters of the SU-8

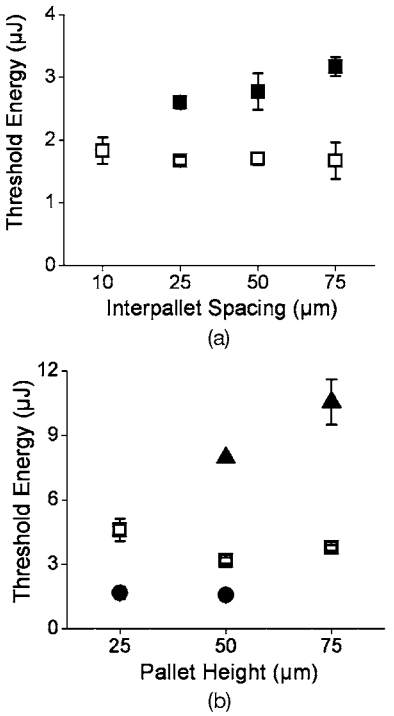

Fig. 3 Influence of inter-pallet gap and pallet height on the threshold energy for pallet release. (a) The average threshold energy needed to release pallets from arrays with different inter-pallet gap was measured. Arrays with pallets of 25 (*h*) and 25 (*s*) μm (open circles) or 50 (*h*) and 50 (*s*) μm (solid squares) is shown. The error bars represent the standard deviation ($n=3$). (b) The average threshold energy to release pallets of different heights was measured. The length of the pallet sides (*s*) were 25 (solid circles), 50 (open squares), and 100 (solid triangles) μm.

structures. Since these variables can be difficult to control precisely during manual fabrication of arrays, array-to-array variability in the SU-8:glass adhesion, and therefore, the threshold pallet release energies may occur. To identify the variation in release energies associated with arrays from different batches, threshold energies were measured for pallets [50 (*h*), 50 (*s*), and 75 (*g*) μm] on arrays from four different fabrication batches. The average release threshold and standard deviation were 4.0 ± 0.9 μJ, thus array-to-array variability can be substantial and must be taken into account in experiments that utilize arrays fabricated at different times. Further optimization of the pallet manufacturing variables as well as automation of the manufacturing process will likely decrease this variability.

3.4 *Characterization of Pallet Release with Respect to Pallet Height*

Pallet height is an important design parameter of the pallet array system. SU-8 fluoresces in the green wavelengths so that an increased pallet height results in greater fluorescent background. This increased background may interfere with the detection of very low intensity fluorescence. For effective live cell sorting using laser-releasable pallets, the pallets must be thick enough to protect the cells during release, but thin enough to produce the least possible amount of background fluorescence. To determine how increasing pallet height affects the energy required for pallet release, arrays with pallets of differing heights (25, 50, and 75 μm) were fabricated. The probability of pallet release at different energies was determined and the threshold energy for pallet release was determined from the fit to the Gaussian error function, as already described. For the 25-μm-size squares, no significant difference in release threshold was observed between pallets 25 and 50 μm in height [Fig. 3(b), Table 1]. Pallets 25 μm in size and 75 μm in height were not manufactured due to the excessive aspect ratio required. For 50-μm squares, 25-, 50-, and 75-μm-tall pallets had similar release thresholds [Fig. 3(b)]. For pallets of 100 μm (*s*) and an interpallet gap of 50 μm, the variations in release thresholds for pallets of 50 μm (*h*) (7.8 μJ ± 0.3) and pallets of 75 μm (*h*) (12 ± 1) were within the range of the variability between batches of arrays (see above). Pallets of 100 μm (*s*) and 25 μm (*h*) did not release with a single laser pulse due to the flexibility of these very thin pallets. The interpallet gap did not introduce variation in the release energy thresholds (Table 1). These data suggest that pallet height and mass do not play a significant role in the threshold release energy. The energy required to disrupt the adhesion of the SU-8 to the glass is far greater than the energy required to lift the small mass. However, further decreases in the array-to-array variability might enable the detection of slight differences in the threshold energy of pallet release with respect to height. It is possible that differences in the curing of the SU-8 near the glass surface vary as the pallet height changes. Given the energy for laser release does not depend on the pallet height, this variable can be optimized to enhance other pallet array properties. For example, the viability of the cells on the pallets may depend on the height of the pallet since the pallet acts to shield the cells from the laser-generated phenomena at the glass:pallet interface. The stability of virtual walls between pallets is also directly related to the height of pallets.[35] In future studies, pallet height can be optimized for cell viability and virtual wall stability with minimal or no influence on the required laser energy for pallet release.

3.5 *Release of Pallets by Multiple Laser Pulses*

Lower pulse energies for pallet release may lead to higher cell viability during cell sorting. One strategy for lowering the pulse energies is to deliver a train of pulses with each pulse disrupting a portion of the SU-8:glass bond. To determine whether a series of pulses could release a pallet at lower energies/pulse, a pulse was delivered to each corner of a pallet. No more than four pulses were delivered to a pallet. The average energy of the pulses delivered to the pallet was recorded as the release energy. The probability of releasing a pallet versus the average laser pulse energy was fitted to an error function to determine the release energy threshold. Release of the 25-μm-size pallets (*h* of 50 μm, gap of 50 μm) by multiple pulses manually fired at a frequency of 1 Hz required a threshold energy of 1.4 ± 0.3 μJ, while release by a single pulse required a threshold energy of 1.4 ± 0.1 μJ. Similarly, multiple pulses released a 50-μm-pallet (*h* of 50 μm, gap of 50 μm) with a threshold of 1.7 ± 0.5 μJ, and a single pulse required a threshold of 2.5 ± 0.3 μJ. Thus, for small pallets, release thresholds achieved by aiming a pulse at each corner were similar to those obtained by aiming a single

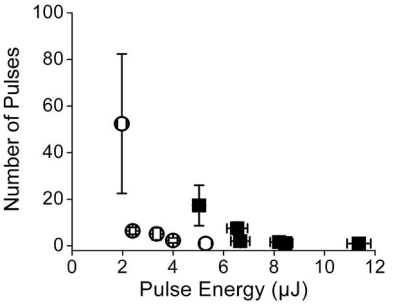

Fig. 4 Release of pallets with a train of laser pulses. The average number of laser pulses required to release a pallet is plotted against the average energy/pulse. The *y* axis error bars represent the standard deviation in the number of pulses utilized to release ten different pallets. The *x* axis error bars represent the standard deviation of the laser pulse energy. The pulses were directed at the pallet corners (open circles) or at the center of the pallet (solid squares).

pulse at the center of the target pallet. For large pallets, 100 and 200 μm in dimension, the minimum energy needed to release a pallet was substantially lower for multiple pulses than for a single pulse. Release thresholds for 100-μm-sized pallets (h of 50 μm, gap of 50 μm) were reduced almost threefold (release threshold 2.9 ± 0.2 μJ) when four pulses were used to achieve release rather than a single pulse (release threshold 8.4 ± 0.2 μJ). Thus, the energy per pulse for pallet release was decreased although the total energy delivered was not decreased for the multiple pulse protocol. Pallets with dimensions of 100 μm (s) with 25 μm (h) or 200 μm (s) with 50 μm (h) could not be released with a single laser pulse aimed at their center due to the flexibility of these very thin pallets. However, pallets of 100 μm (s) with 25 μm (h) were easily released with a threshold energy of 5.4 ± 1.0 μJ when four pulses, one at each corner, were utilized. Pallets with dimensions 200 μm (s) and 50 μm (s) could be released with a threshold of 11.7 ± 1.6 μJ when a pulse was aimed at each corner of the target pallet. For large pallets, multiple pulses lower the required energy per pulse and may be required for the release of very thin, flexible pallets.

An alternative strategy to lower the energy/pulse for pallet release is to deliver a large number of subthreshold pulses, most of which will not impact the SU-8:glass bond. However, a small portion may initiate a plasma leading to SU-8:glass separation. A train of pulses was fired at either the center or corners of a pallet [100 μm (s) and 50 μm (h)] until the pallet was released (Fig. 4). The average number of pulses required to release a pallet was plotted against the energy/pulse of the laser. When directed at the pallet corners, as little as 2-μJ energy pulses could be used to release a 100-μm pallet. Although on average 50 pulses were required. When the laser pulses were targeted to the center of the pallet, 20 5-μJ pulses were required to effect pallet release. It may be possible to further reduce the energy/pulse for pallet release by firing even larger numbers of pulses with a high frequency. While the energy/pulse was lowered by delivering a series of laser pulses, the total energy of all of the laser pulses exceeded that when a single laser pulse was used to initiate pallet release. A key future goal will be to determine whether cell health is most closely tied to the energy/pulse or the total energy of all pulses.

3.6 *Characterization of Pallet Release with Respect to Pulse Duration*

A likely mechanism for pallet release is the formation of a plasma by the focused laser beam at the interface of the SU-8 and glass substrate. The ensuing mechanical shock wave and cavitation bubble might also contribute to the disruption of the SU-8:glass adhesion. Since plasma formation depends more on the critical irradiance (power/unit area) rather than the critical radiant exposure (energy/unit area), the pulse energy needed to form a plasma decreases as the pulse duration decreases. Thus, single laser pulses with a duration of 500 ps might mediate pallet release at lower energies than that of the 5-ns pulses. Pallets of 50-μm size were released with a single laser pulse of 5 ns or 500 ps and the energy threshold for release was measured. The release thresholds calculated for picosecond-laser-based release (1.4 ± 0.3 μJ) were not significantly lower than those for nanosecond-laser-based release (1.5 ± 0.1 μJ) for the tested pallet array. It is likely that the total energy necessary to release a pallet is dominated by that energy needed to disrupt the SU-8:glass adhesion rather than that required to form a plasma.

3.7 *Laser-Based Release of Complex Structures*

The pallet material SU-8 is used widely to microfabricate high-aspect-ratio structures. Frequently, all or portion of a complex SU-8 structure must be released from the substrate on which it was fabricated. These SU-8 components are often synthesized on a sacrificial layer, which can be removed using a chemical etchant. However, wet etching can chemically contaminate or degrade coatings on the SU-8 microstructures. Dry release processes use an antiadhesion layer, such as Teflon or a self-assembled monolayer, between SU-8 and its substrate, enabling SU-8 microstructures to be mechanically lifted from a substrate without immersion in a fluid. However, all of these methods require bulk treatments, are relatively time-consuming, or cannot be spatially localized. To determine whether laser-based release of SU-8 could detach complex microstructures from an underlying surface, a variety of microcomponents (cantilever, anteater, and spiral) were fabricated in an array format [Fig. 5(a)]. The cantilever-shaped structures were 1.5×0.5 mm with 20-μm-wide arms, while the anteaters were 650×250 μm and the spirals were 350 μm in diameter with 20-μm-wide arms. Each of the microstructures was released using a series (20 to 100) of focused pulses (3 to 5 μJ). No fragmentation of any of the structures occurred and only the targeted structure in the array was released [Fig. 5(c)]. In contrast, mechanical scraping with a spatula led to both release and extensive fragmentation of the components [Fig. 5(b)]. Laser-based release of SU-8 from surfaces may be of utility in applications requiring localized, precise release of microstructures. Structures with dimensions of micrometers to millimeters can be released using the appropriate number and energy pulses.

4 Conclusions

The threshold energy for pallet release was shown to be linearly related to pallet surface area. This analysis also showed

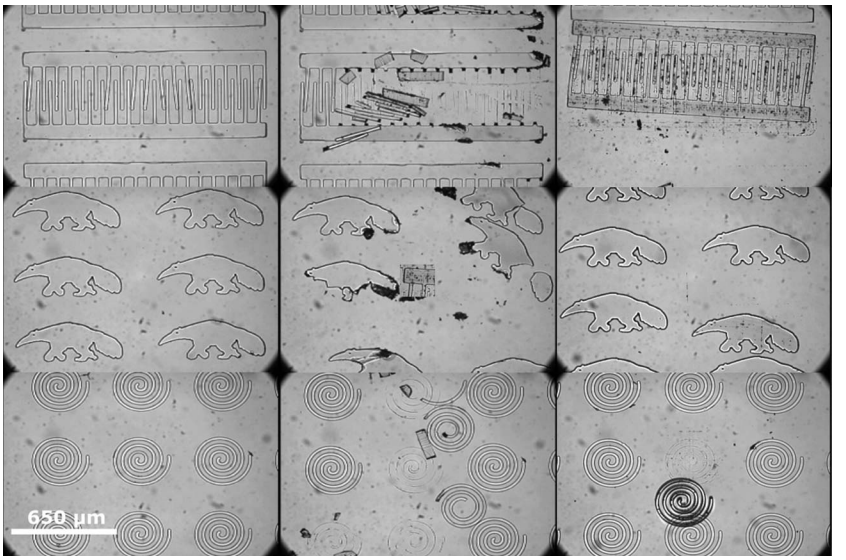

Fig. 5 Laser-based release of complex structures: (a) arrays of cantilevers (top panel), anteaters (middle panel), and spirals (lower panel) were fabricated, a transmitted light image; (b) structures released by mechanical scraping; and (c) single structure released using a train of focused laser pulses.

that a finite amount of energy would be required to release a pallet whose surface area was zero. These data are consistent with a threshold energy requirement that acts as a necessary, but not sufficient, condition for pallet release to occur. This energy is interpreted as the threshold for plasma formation at the focal point of the laser. This process creates a cavitation bubble and shock wave, which likely generate the mechanical forces to drive pallet release. The results further suggest that the optimal strategy for laser-based pallet release depends on the size of the pallet. Small pallets ($s \leq 50\ \mu$m) are most efficiently released by a single, centered laser pulse of low energy. Larger pallets, especially when thin ($h < 50\ \mu$m), may not be releasable with a single pulse, but can be released with multiple pulses aimed near the corners of the pallet. Each of these corner pulses likely detaches a quadrant of the pallet. A series of focused pulses of a few microjoules per pulse can be utilized to release not only pallets, but also complex, millimeter-sized structures with little to no damage. In addition to uses in the sorting of cells using pallet arrays, this method may find use when small regions of a larger structure must be detached, for example, the building of 3-D microstructures.

Acknowledgments

This work was supported by grants from the National Institutes of Health (NIH).

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