

# The Fabrication of Multifaceted Patterned Surfaces Using Laser Scanning Lithography

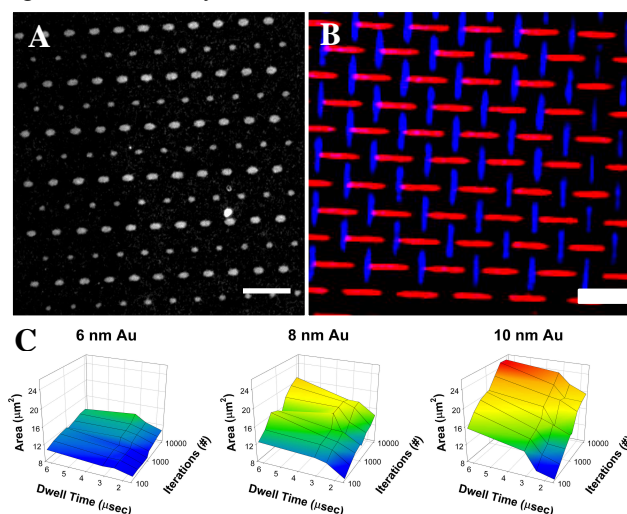
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**Statement of Purpose:** Surfaces presenting patterned arrays of cell adhesive protein that restrict cell spreading or provide precise control over focal adhesion formation have demonstrated the ability to engineer cell behavior via cell-surface interactions alone.<sup>1</sup> Existing patterning techniques such as microcontact printing provide a platform for the patterning of one type of biomolecule but do not allow for the patterning of multiple molecules. To address this limitation we have developed Laser Scanning Lithography (LSL), a thermal-based desorption technique in which focused laser light is raster scanned across a chosen region of interest (ROI) inducing localized heating of the underlying Au substrate. The heat is transferred to attached alkanethiol molecules and induces disulfide bond formation between adjacent thiols and subsequently their removal from the surface.<sup>2</sup> We demonstrate the versatility of LSL through the creation of both nano- and micropatterned surfaces and show the ability to pattern multiple ligands with each ligand confined to its own pattern array.

**Methods:** Piranha-cleaned glass coverslips were coated with 2 nm of Ti and 6-10 nm of Au with an e-beam evaporator, cleaned in TL1 (6:1:1, H<sub>2</sub>O: NH<sub>4</sub>OH: H<sub>2</sub>O<sub>2</sub>), and functionalized with 2 mM oligo(ethylene glycol)-terminated alkanethiol (OEG). ROIs of the OEG were thermally desorbed from the surface using a Zeiss 5 Live confocal microscope equipped with either a 405 or 532 nm laser operating from 5 to 8 mW/ $\mu\text{m}^2$  focused through a 20X (NA 0.8) or 63X (NA 1.4) objective with a pixel dwell time varied from 0.95-6.29  $\mu\text{sec}$  and iterations ranging from 1-10,000. For pattern characterization studies, the surfaces were exposed to 2 mM hexadecanethiol (HDT) followed by adsorption of human plasma fibronectin (HFN). To create multifaceted surfaces, the first patterns were created with 2 mM GRGDS-OEG terminated alkanethiol (RGD) followed by a second round of patterning and exposure to HDT and HFN. Surface functionalization and the ability of OEG and RGD regions to retain their protein repulsive properties were assessed with contact angle, ellipsometry, and X-ray photoelectron spectroscopy (XPS) measurements. Surfaces exposed to HFN were seeded with NIH 3T3 Fibroblasts (Fb) that were fixed, stained with DAPI and phalloidin, and imaged with fluorescent and scanning electron microscopy (SEM).

**Results:** We demonstrate that LSL (1) can be implemented to create patterns as small as 0.17  $\mu\text{m}^2$  (465 nm diameter) in size (Fig 1A), (2) allows for arbitrary pattern shapes to be created “on the fly” without the need for photolithographic masters, (3) provides tight control over pattern size through varying both the laser properties and Au substrate thickness (Fig 1C), (4) allows for both destructive and chemically-specific patterning, and most importantly, (6) provides a platform for the fabrication of multifaceted surfaces displaying patterned arrays of multiple biomolecules (Fig 1B).



**Figure 1:** LSL Pattern Characterization

(A) Circular and oval patterns of HFN ranging in size from 0.17 to 0.68  $\mu\text{m}^2$ . SB = 5  $\mu\text{m}$ . (B) 1 by 8  $\mu\text{m}$  ellipses of an AF633 labeled RGD SAM (red) interwoven with Pacific blue labeled HFN (blue) on a HDT SAM. SB = 10  $\mu\text{m}$ . (C) Pattern area of 1 by 20 pixel ROIs as a function of Au substrate thickness, pixel dwell time, and number of iterations at a constant wavelength (532 nm) and power (8 mW/ $\mu\text{m}^2$ ).

The LSL process requires that previously formed OEG and RGD SAMs are exposed to HDT and HFN. Analysis of the data from contact angle, ellipsometry, and high resolution XPS spectra demonstrate that there is some interaction of the HDT with the OEG and RGD SAMs and allowed for optimization of the functionalization conditions to minimize these interactions. The analysis indicates that some HDT can fill into the OEG SAM yet the OEG still retains its protein repulsive properties; most likely due to its longer chain length and increased mobility compared to the HDT. Furthermore, it appears that some HDT can fill into and actually displace some of the RGD SAM yet the RGD still minimally interacts with HFN most likely due to its long OEG<sub>11</sub> spacer.

## Conclusions:

We have demonstrated that LSL is a versatile surface patterning technique that allows the creation of nano- to micrometer sized patterns in any arbitrary shape without the need for photolithographic masters and that pattern size can easily be tuned by simply varying the laser parameters and/or substrate conductivity. We also demonstrate that cells recognize, adhere to, and align their cytoskeletons to the long axis of elliptical HFN patterns affirming bioactivity. These multifaceted surfaces provide a platform for regulating adhesion site composition and maturation and will provide detailed insight into the use of patterned biomaterials for cell engineering applications.

## References:

1. Chen CS. MRS Bull. 2005;30:194-201.
2. Shadnam MR. J Phys Chem B. 2005;109:11996-12002.