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Fluorescent two-photon nanolithography

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Summary

Improving the excitation conditions in two-photon fluorescent lithography reduces the size of the fabricated structures to nanometre scales. We demonstrate that a precise control of the illumination profile and the scanning speed of the laser beam is enough to decrease the photo-polymerization volume of resins by orders of magnitude. This work also shows experimental evidence of surface effects that yield a different polymerization intensity threshold compared with bulk. Such phenomenon enables to perform a non-linear optical nanolithography in a simple way, allowing fast-prototyping procedures. We present a detailed study of the polymer growth process using fluorescence and atomic force microscopy.

Introduction

Total internal reflection fluorescence microscope (TIRF) (Axelrod, 2001), scanning near-field optical microscopes (SNOM) (Betzig & Trautman, 1992) and other super-resolution techniques (Gustafsson, 2000; Hofmann *et al.*, 2005; Betzig *et al.*, 2006; Willig *et al.*, 2006) have provided ways to look at the fluorescence in a very small scale of tens of nanometres. In this manner, tagged samples can be analyzed beyond the diffraction limit using optical microscopies.

In a recent paper, a way to produce fluorescent patterns has been presented based on the two-photon polymerization of a resin loaded with the desired fluorescent dye or particle (Costantino *et al.*, 2005). Micrometre-sized lines and spots could be grown following any pattern, thus serving as landmarks to locate specific regions, as fiduciary reference frames to look at cells or particle movements, and to cancel the thermal drift of the microscope. Furthermore, the fabricated fluorescent structures have proven compatible with primary cell cultures.

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Single-photon lithographies as projection and contact lithography (Schmid *et al.*, 1998) have proven useful to engineer nanostructures, but these techniques use UV light and therefore they cannot be used to fabricate fluorescent structures because the dye photobleaches. Two-photon lithography without fluorescence properties has been reported for several different applications in optics and material science (Maruo *et al.*, 1997; Cumpston *et al.*, 1999; Kawata *et al.*, 2001; Sun *et al.*, 2001). Moreover, the use of commercially available UV-cure resins was shown to fabricate complex micro-machines for laser tweezers manipulation (Galajda & Ormos, 2001).

In this article, we extend their work to the nanometre scale that can now be used for higher-resolution fluorescence microscopes, thus opening the door to new nanotechnological applications.

Materials and methods

The method is basically analogous to the one reported previously (Costantino *et al.*, 2005), but the exposure conditions were improved using lower powers or higher scanning speeds to yield a reduction of orders of magnitude in the height of the fabricated structures. The technique is illustrated in Fig. 1, where a schematic of the set-up is presented. A mode-locked Ti:Sapphire laser emitting trains of pulses 100 fs wide at a repetition rate of 94 MHz is focussed into the sample by means of a high numerical aperture air objective (Olympus UplanSapo 40×, NA = 0.9, Olympus, Tokyo, Japan) placed in an inverted microscope configuration. A blend of the UV-cure adhesive (NOA 60, Norland Products, Cranbury, NJ, USA) with a highly concentrated solution of fluorescent dye (ADS675MT) in ethanol is prepared and a drop is placed on top of a standard glass cover slip or mica sheet to begin the photo-polymerization process. The power of the laser is regulated by controlling the pump laser power and by inserting losses within the trajectory of the infrared beam. The laser central wavelength is tuned to 750 nm in order to allow a

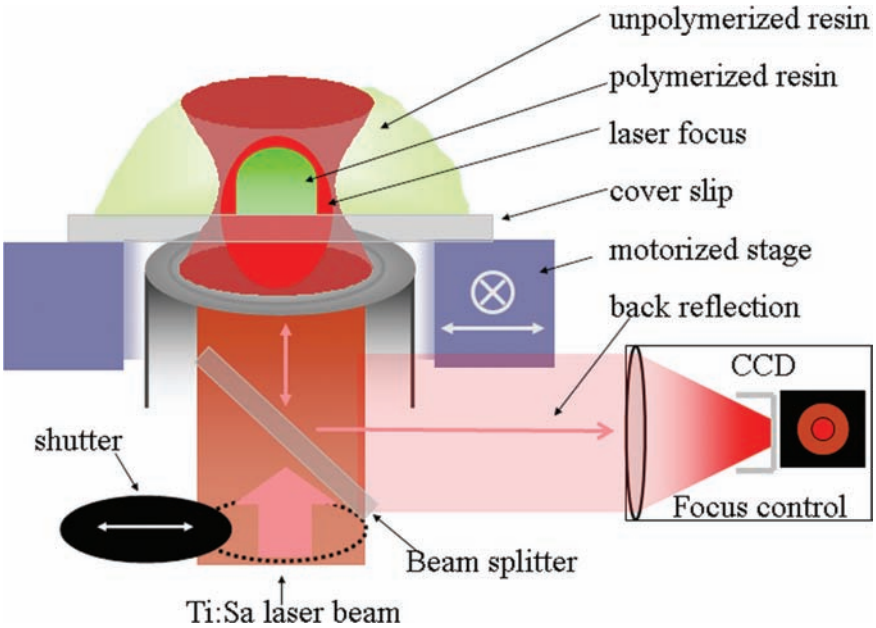

Fig. 1. Schematics of the set-up used for the two-photon lithography.

two-photon absorption process to reach the required energy for the polymerization. A shutter is inter-posed in the beam way in order to control the pattern design as desired. The sample holder is motorized in the three axes by means of closed-loop actuators of 25 mm of travel with a speed range between 50 μm s^{-1} and 400 μm s^{-1}. The smallest step accessible for the motors was 50 nm.

The control of the focus was made by means of a CCD camera imaging the back reflection of the laser beam. As the focus of the beam approaches the surface of the cover slip, the back reflection propagates along the same path that the input beam and the imaging point at the CCD gets the smaller. A kinematic mirror mount used as a platform allowed the tilt correction of the cover slip plane respect to the translation stage axes in order to avoid focus walk off during the sample scan. As the beam was focussed down to less than a micrometre in diameter, the focal depth resulted in a few micrometres, thus rendering this focus control essential for a homogeneous treatment along the scan. A 1 μm control over a scan of 1 mm requires setting the sample within 1 mrad of the scanning axis.

As the sample is scanned, the polymerization reaction takes place within the focal volume defined by the two-photon absorption cross-section. The polymerization depends on both laser power and the time the beam excites a given location. Hence, the width and height of the rigid line created can be controlled by the laser power and the speed of the scan. However, when the laser-scan rate is sufficiently fast, a qualitatively different behaviour is detected. An abrupt reduction in the height of the fabricated lines appears, not correlated with a reduction in their width. This result is an indication of a different threshold for the bulk two-photon polymerization and the reaction starting at the surface.

To measure the height of the polymer lines and their detailed structure with nanometre resolution, an atomic force microscope (AFM, Veeco-Digital Instruments NanoScpope IIIa – Quadrex, Woodbury, NJ, USA) was used in tapping mode. The surface photo-polymerization threshold was found to be at powers an order of magnitude lower than the bulk process and this fact allowed the fabrication of nanometre high lines as described in the next section.

Results and discussion

To work near the surface polymerization threshold within adequate scanning speeds, the average laser power was reduced to 1.5 mW before the objective. At a scanning speed of 100 μm s^{-1} the lines obtained were not continuous, but instead they presented an aspect of isolated islands of different diameters ranging from 1 nm to 100 nm as seen in the AFM image presented in Fig. 2. The topographic image acquired in tapping mode clearly shows the line formed by non-contacted islands that we interpret as growing from isolated surface-active regions (probably some digs or other defects) acting as nucleation sites. The phase mode AFM image, which is sensitive to different surface stiffness, shows a 500-nm-wide continuous line in the background of the islands. This is indicating that a very thin layer of polymer has been deposited along the region radiated by the laser beam, but only at selected spots the polymer has grown.

Structures of different heights in the nanometre range can be produced by scanning at different speeds. In Fig. 3, four different lines are shown within a single AFM image. As the scanning speed is increased, their width is always

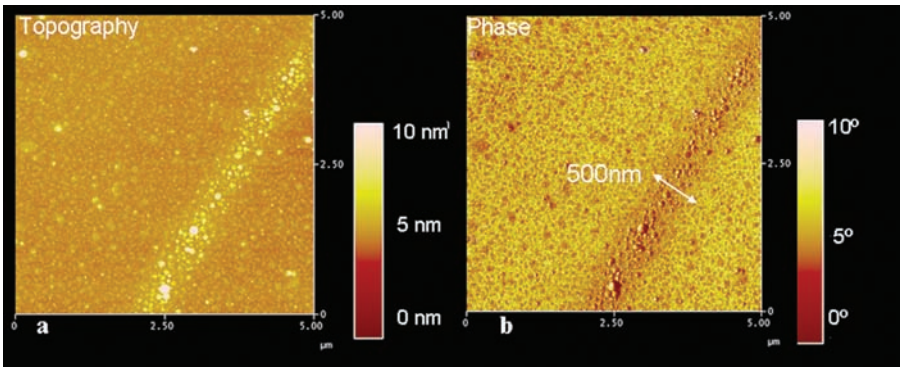

Fig. 2. AFM images of lines produced close to the surface polymerization threshold, where can be seen that the polymer growth starts on the surfaces of the substrate. The laser power was 1.5 mW and the scanning speed 100 μm s^{-1}. (a) Topography and (b) phase image.

approximately 500 nm and the height of their main features decrease from 2.8 nm, down to the noise limit of the AFM image in the vertical direction. This indicates that a very shallow structure probably about one molecule high is deposited even at the higher speeds.

By means of sweeping the scanning speed and excitation power, the conditions for fabricating continuous lines were analyzed and found. In Fig. 4a, a three-dimensional topographic AFM image reconstruction of three continuous lines is shown. A cross-section of them is presented in Fig. 4b,

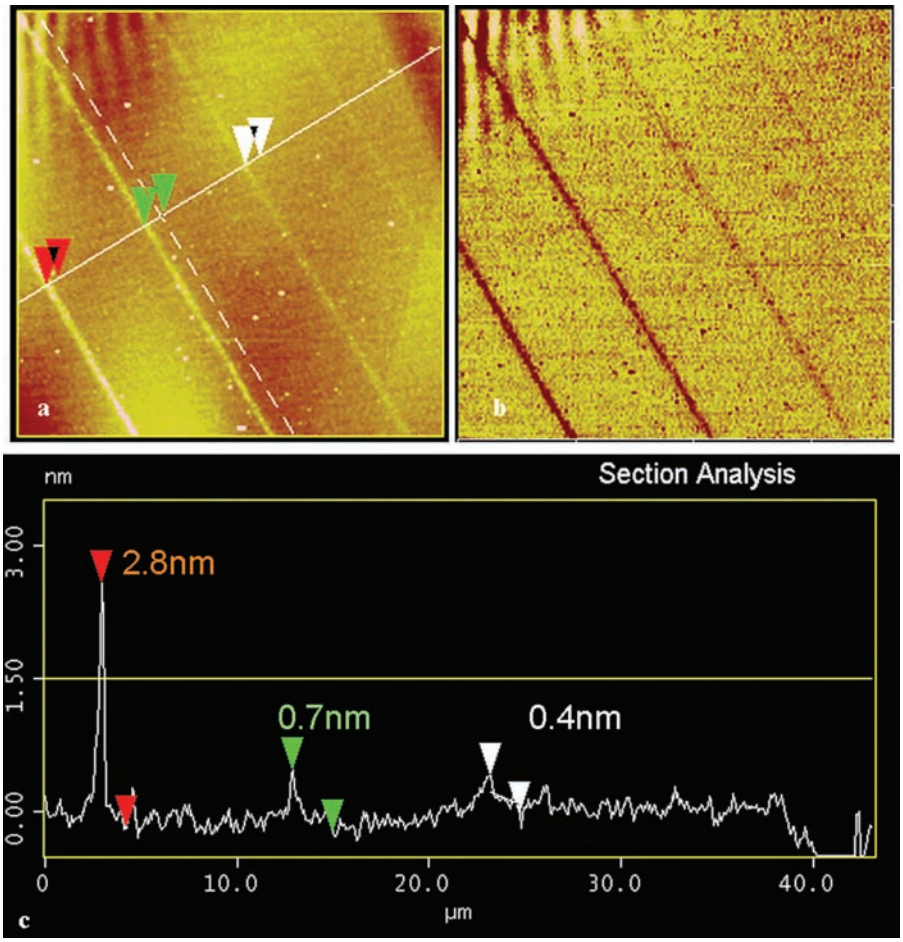

Fig. 3. Lines produced at increasing scanning speeds, yielding structures with decreasing characteristic height. (a) Topography acquired in tapping mode. (b) Phase image. (c) Cross-section of the lines. The scanning speed was 120 μm s^{-1}, 130 μm s^{-1}, 140 μm s^{-1} and 150 μm s^{-1}, respectively.

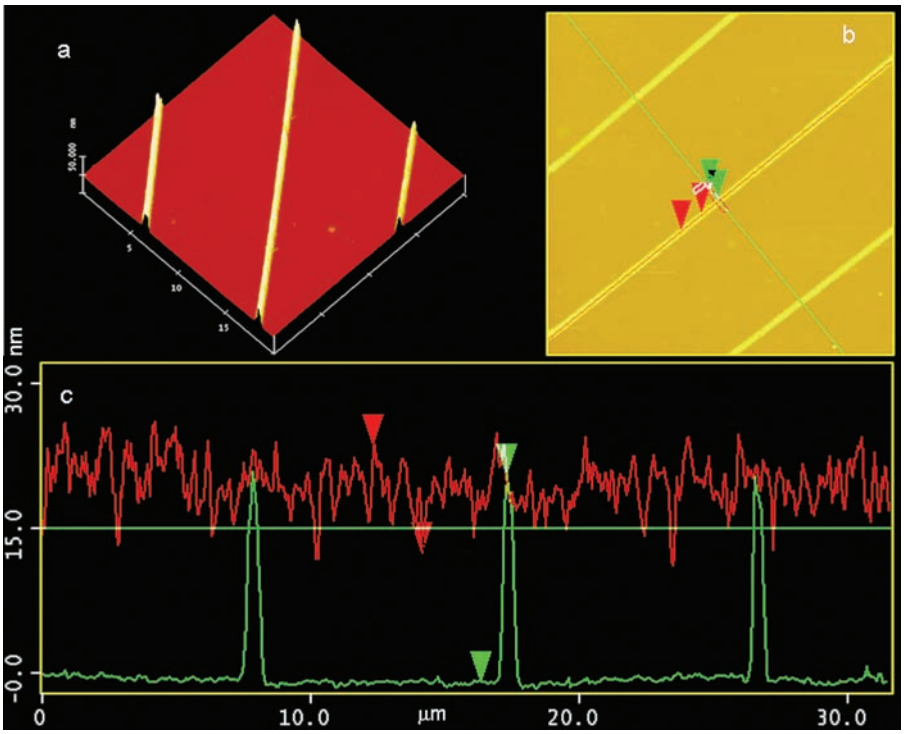

Fig. 4. Lines produced above the threshold for continuous line generation. (a) Topographic image in tapping mode. (b) Z projection of these three lines and (c) Cross-section (green) and longitudinal view (red) of the three lines. The width remains always about 500 nm, given by the focal spot size, and the height is 20 nm. The laser power was 3 mW and the scanning speed 100 μm s^{-1}

where it can be noticed that the height is 20 nm and the 500 nm width is preserved.

To determine if these shallow lines could still be used as fluorescent references an image was obtained with a confocal microscope (Olympus FluoView-1000, Olympus, Tokyo, Japan). In Fig. 5a, we show a projection of seven images Z stack with optical sections separated by 0.1 μm and their average cross-section in Fig. 5b. The fluorescence of these lines can be clearly distinguished from the background noise even using analog detection (without photon counting) indicating that the lines should be even more visible in other microscopy schemes such as TIRF or SNOM.

Conclusions

Our study shows that surface effects reduce the threshold for two-photon polymerization of the resins at the surface of glass and mica substrates. When the polymer growth process

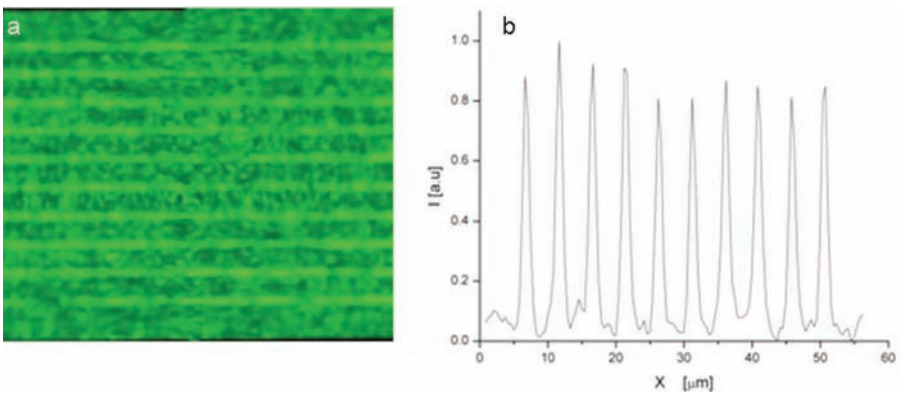

Fig. 5. Confocal image of nanofabricated 20-nm-high lines on a cover slip. (a) Projection of seven images Z stack with optical section separated by 0.1 μm. (b) Average fluorescence cross-section.

is arrested, a series of isolated islands of less than 5 nm high can be observed using force microscopy. In addition, the nanometre-sized structures generated mixing fluorescent molecules in the resin can be used as references and calibration targets for TIRF microscopy and SNOM. Furthermore, the fabrication of fluorescent nanostructures can be performed in less than 15 min and given the simplicity of the set-up even commercial two-photon microscopes can be used as nanolithography stations.

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