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**SPIE.**

# Custom incorporation of DMD-based photolithography and photopatterning techniques in soft condensed matter research

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## ABSTRACT

Research with soft materials, that is, polymeric gels, colloidal suspensions, liquid crystals, and most biomaterials often involves the need for microfabrication of confinement channels, cells, and lab-on-a-chip devices. Photolithography techniques are often chosen, as they offer the combination of versatility, precision, and quick delivery demanded by researchers. Beyond fabrication, stimulus-responsive systems, such as photosensitivity biomaterials, are the object of broad study within a very interdisciplinary community. Here, we show that a standard laboratory microscope can be quickly and economically transformed into a powerful maskless photo-fabrication/photoexcitation station using off-the-shelf DMD development modules and simple optomechanical components allowing real time observation of the fabrication process.

**Keywords:** DMD, photolithography, soft matter, microscopy

## 1. INTRODUCTION

Soft material science and in-vitro biomedical studies typically demand sample confinement, miniaturization or the development of lab-on-a-chip devices. The required micropatterning methods typically rely on photolithography processes, where a thin, photosensitive layer is exposed to light irradiation (typically in the UV range) through a mask, resulting in selective polymerization of the desired motifs. This process has been in use in the microelectronics industry for more than 50 years. In general, the steps leading to the manufacture of the mold require specialized equipment and are typically performed in clean room conditions. Moreover, alteration of the devices requires a redesign of the mask and fabrication of new molds.

More recently, the same protocols were adapted to manufacture molds to cast curable elastometic materials, such as the celebrated polydimethylsiloxane (PDMS), which has been widely employed to build microfluidic chips for lab-on-a-chip applications. Alternatively, these devices can be directly fabricated by exposure of suitable photoresins to UV light through the mask.[1] Although the latter steps can be performed in a standard laboratory or in a simple laminar flow cabin, having to rely on a mask becomes unpractical for changing experimental conditions or to tailor the device to real time applications.

In a different context, micropatterning is also employed in the development of liquid crystal (LC) devices. These are thin layers of anisotropic liquids that can be controlled through the application of local electric fields. To be of practical use, design of a robust zero-field configuration requires patterning of the bounding surfaces of the LC layer. Among the different physico-chemical methods developed over the decades, the use of photosensitive polymeric layers are lately favored, as the zero-field arrangement can be tuned by optical patterning combined with suitable polarization of the light irradiation.[2]

In-situ patterning using maskless photolithography in an in-vitro biological system was demonstrated more than ten years ago in the context of localized protein micropatterning (LIMAP),[3,4] and it has become a standard tool for the placement of proteins or cells using complex dedicated[5] or commercial devices.[6–8] In a broader
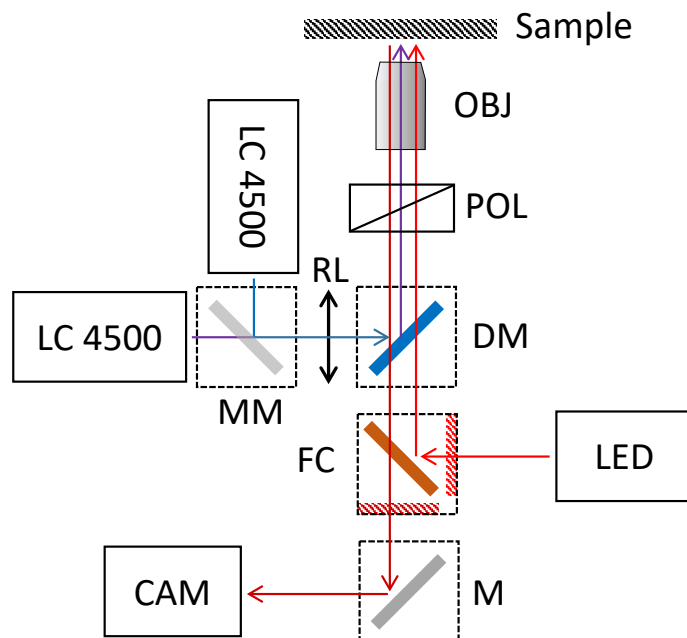
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Figure 1. Sketch on the incorporation of DMD photo-patterning, based on 2 units the Light Crafter 4500 DMD device (LC 4500), coupled to a standard inverted fluorescence microscope. OBJ: microscope objective; DM: dichroic mirror; FC: filter cube for fluorescence; LED: led light source for fluorescence; M: mirror that bends the light 90° towards the camera (CAM); MM: movable mirror; RL: relay lense.

context, this technique can be used not only to build channels, obstacles, or otherwise pattern a device, but also for the in-situ synthesis of microparticles,[9] or for the manufacture of complex LC devices.[10]

Here, we show an implementation of a maskless system that is directly integrated in a standard fluorescence research microscope. The implementation relies on easy to use, cost effective, and readily available DMD devices, and combines two of these instruments to broaden the range of usable photosensitive materials, allowing simultaneous observation of the irradiated substrate for real time user feedback.

## 2. EXPERIMENTAL IMPLEMENTATION

Our target system is a research microscope, in this case a Nikon Ti series microscope. This is an inverted microscope whose modularity allows both commercial and custom-made add-ons. These types of microscopes are often found in soft matter, biomaterials, and biomedical research laboratories. We use a microscope equipped with a fluorescence module and a stage-up accessory that enables about 70 mm of space between the objective back focal plane and the output from the fluorescence filter cube. We show how to easily introduce into the microscope light path user-specified images projected by two DMD development modules, one featuring UV light for microfabrication, the other featuring blue light for photoexcitation or for microfabrication on alternative substrates. Computer-generated images are fed, in real time, on the DMD modules through the available HDMI port without any additional software or hardware requirements. Assembly requires mostly off-the-shelf commercially available optomechanical components. We also employ some custom parts manufactured by a fused filament 3D-printer made with the thermoplastic PLA.

We use Ti LightCrafter 4500 DLP development modules purchased from EKB Technologies, Ltd. These modules are customized by the provider to include a high power 385 nm LED source in one case, and a liquid light guide coupling in the other case. For the current assembly, the latter device is coupled to a Thorlabs SOLIS 445 nm LED via a liquid light guide.

Coupling between the DMD and the microscope is achieved by means of a dichroic mirror secured in a Thorlabs 30 mm cage cube, placed behind the microscope objective turret and oriented at 45 degrees with

respect to the light path (Fig. 1). The mirror has a cut-off wavelength of 500 nm for the reflection band, thus reflecting light from both DMD modules, but allowing fluorescence imaging using common far-red fluorophores (in our case, we use a CYT5 filter set). No modification is performed on the microscope other that installing the above-mentioned stage-up accessory provided by the manufacturer.

The DMD modules are secured onto optomechanical hardware that allows three-axis linear positioning and also tilt and yaw adjustment, for proper alignment and focus of the projected images. We removed the original focusing optics incorporated in the DMD modules (designed as standalone image projectors) and use a single $f = 150$ mm, $\phi = 50$ mm plano-convex lens to collimate the image from the DMD chip while focusing the LED light to the back focal plane of the microscope objective. With this, the DMD pattern is in focus on the microscope image plane and illumination intensity is homogeneous. The two DMD devices can share the same optomechanical assembly, as they are easily interchanged, or can be assembled independently along orthogonal optical paths using either a second dichroic mirror or a user switchable metallic mirror (this is the configuration shown in Fig. 1), as photofabrication and photoexcitation will seldom be needed simultaneously. Additionally, an adjustable polarizer can be placed between the dichroic mirror and the objective for the patterning of polarization-sensitive films.[10] When in-place, this polarizer will halve the light intensity from the DMD device reaching the sample and divide by four the intensity of fluorescence light reaching the camera. We have verified that such light loss does not significantly hamper the performance of either irradiation or fluorescence as the different light sources typically operate at a fraction of their maximum power.

Next, we will demonstrate the use of this equipment in the study of an active soft matter system where microfabrication and photoexcitation are both employed.

## 3. RESULTS

We demonstrate the power of this setup with an example from active soft matter research by the authors. The system is an in-vitro aqueous reconstitution of cytoskeleton proteins: tubulin that polymerizes into microtubules and the complementary molecular motor kinesin that, through consumption of adenosine triphosphate, walks along the filaments. In this biomaterial, kinesins are dimerized so that adjacent microtubules are pulled away by pairs of motors. The dispersed filaments are brought together using the non-adsorbing polymer poly-ethylene glycol (PEG) that triggers a depletion mechanism, which induces the aggregation of the larger filaments. Furthermore, this resulting active gel is condensed at the interface between the aqueous medium and an inert oil, leading to the self-assembly of long filament bundles that actively move within a quasi-two-dimensional layer (Fig. 2a). This system has been called two-dimensional active nematic liquid crystal, as it is an active counterpart of nematic liquid crystals, whose micro or nanoscale components feature long-range orientational order.[11] This system is considered a paradigm in the study of active soft matter within a broad scientific community as it may help to understand, among others, the mechanisms of intracellular transport and tissue formation. Recently, research in this field has focused on the evolution of these active materials under confinement, requiring the use of high precision crafting of microchannels.[12] This process incurs in the usual difficulties associated with the construction of microstructures, and is further hampered by wetting-related distortions of the planar interface and by potential incompatibilities between the channel wall material and the delicate biopolymers under study.

Within this context, we have incorporated a variant of the depleting agent that can be reticulated through a chain polymerization that is driven by means of a UV-activated photoinitiator. Irradiation of an arbitrary pattern with the 385 nm DLP leads to the transformation of the illuminated regions into a solid gel whose stiffness depends on the local dose of light. Indeed, a grayscale pattern will result in a material with non-homogeneous stiffness. In Fig. 2b we show an example where the original unbound kinesin/tubulin active nematic is in-situ confined into a ring-shaped channel obtained through the photopolymerization of the surrounding active material. Intense photobleaching results in the solidified irradiated patterns to feature a very low fluorescence. In this system, the originally chaotic flows of the active nematic are transformed into a flow with a net circulation along the channel. This can be visualized by constructing a kymograph where the fluorescence profile along a circumference inside the channel is stacked vertically for different times. A net circulation is observed as tilted gray scale patterns, where the tilt angle is proportional to the average flow speed (Fig. 2c).

In this example, we have also employed a variation of the usual kinesin/tubulin active nematic in which the kinesin dimers are themselves photosensitive: their dimerisation constant increases upon irradiation with blue
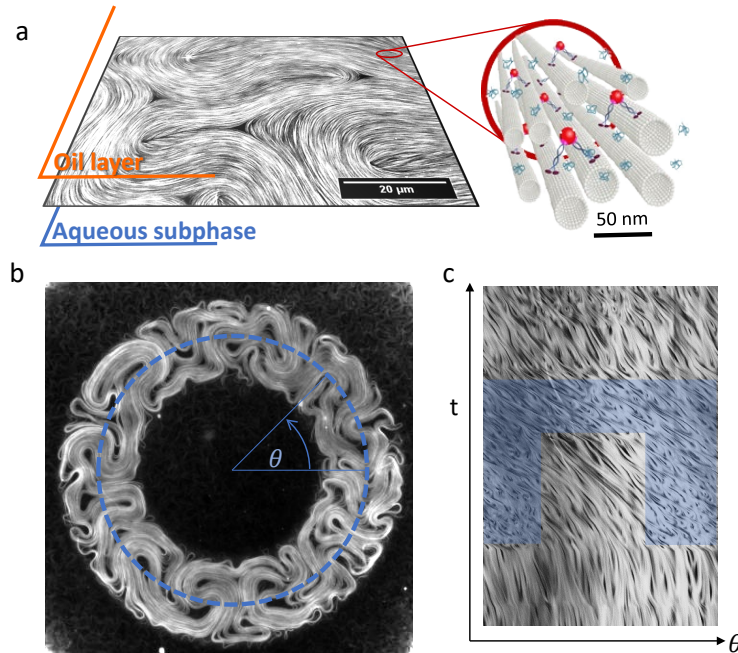
3

Figure 2. a) Fluorescence micrograph of the active nematic layer forming at the aqueous/oil interface. In the inset, a sketch of the underlying biomaterial, as described in the text. b) Active nematic confined in a ring-shaped channel that has been in-situ polymerized. c) Kymograph of the fluorescence intensity along the center of the channel as a function of time. The shading indicates the angular positions and times at which photoexcitation is applied.

light.[13] We take advantage of this property by selectively irradiating a region of the fabricated ring channel with the second DLP, this time the one that uses a 445 nm source. Note that this wavelength is outside of the absorption band of the photoinitiator of the gelation reaction. In the example in Fig. 2b, we have irradiated a section of the ring channel with 455 nm light, resulting in an increase circulating speed of the irradiated region, as enhanced kinesin dimerisation results in a more efficient microtubule sliding. This can be observed in the kymograph (Fig. 2c) where the slope of the irradiated region (shaded in that figure) is significantly higher, *i.e.*, the material is more active. We then extend the irradiation to the full ring, and the speed is homogeneously high. Finally, we extinguish the irradiation, and the whole system returns to the low activity state. Both the microfabrication and the photoexcitation are performed while concurrent fluorescence microscopy is underway, thus allowing to study transient phenomena and to tailor the processes in response to the local state of the continuously-changing active material.

## 4. CONCLUSIONS AND OUTLOOK

In this article, we have described the facile and economical implementation of a photopatterning/photoexcitation system that can be coupled to a standard research microscope that has a modular design. This makes the new microfabrication abilities compatible with existing imaging protocols, such as the fluorescence microscopy that we have demonstrated here. Patterns are generated with off-the-shelf cost-effective DLP demonstration modules, which can be employed without significant engineering challenges. Alternatively, for enhanced modularity, the system could be set up as an independent microfabrication station employing commercially-available optomechanical hardware.

In terms or performance, the resolution of the photopatterning process depends on the pixel size and density of the DMD chip, and the corresponding field of view onto which it is projected. With the demonstrated experimental system, the size of the projected pattern and the corresponding resolution are $3.35 \times 2.15$ mm$^2$ ($2.69 \mu$m/px) using the 4x objective, $1.38 \times 0.86$ mm$^2$ ($1.08 \mu$m/px) using the 10x objective, and $690 \times 430 \, \mu$m$^2$

4

(0.54$\mu$m/px) using the 20x objective. The resolution of projected patterns is enough for the typical microfabrication/micropatterning needs in most soft matter and microfluidic projects, although the size of the field of view may be small for the latter applications. For these needs, a motorized sample stage and custom software could be coupled to the photopatterning system to stitch multiple exposures into arbitrarily large patterns.

Experiments are currently under way to employ this system for the maskless fabrication of microfluidic devices based on the photopolymerizable glue Norland NOA-81 and to fabricate patterned liquid crystal devices when the irradiation is modulated by an adjustable polarizer, as described above. In the latter case, custom software is needed to properly synchronize the pixel-level irradiation intensity and polarization, which will determine the local orientation of the optical axis in the final device. In general, the system could be tailored for any custom microfabrication or micropatterning needs, both in materials or biomedical science research, compatible with the achieved spatial resolution.

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5