Deeper insight with MEMS based illumination

C.Skupsch[1], J.Heber[1], F.Rückerl[2], D.Berndt[1], J.Y.Tinevez[2], S.Shorte[2], and M.Wagner[1]

[1]Fraunhofer Institute for Photonic Microsystems (IPMS), 01109 Dresden; Germany
[2]Institut Pasteur, Imagopole, Plateforme d'Imagerie Dynamique (PFID), 75015 Paris, France
email: skupsch@ipms.fraunhofer.de

Summary

Integrating MEMS in an optical beam line allows the precise *steering* and *structuring* of light. We present diffractive micro-mirror arrays for enhanced illumination in a microscope setup. The illumination pattern, the beam angle, the intensity, and the pulse duration can be modulated continuously and independently in the kHz range. This new modulator prepares striking enhancements in fluorescence microscopy.

Introduction

Since the advent of optical microscopy more than 300 years ago the market for optical sensing in biology grows with enormous rates, driven by a continuous series of innovative microscopy techniques. Decisive movers of the past 30 years are inspection techniques that increase the spatial resolution even beyond the Abbe limit. One representative technique is structured illumination microscopy (SIM), which primarily modifies the illumination path to enhance the microscope resolution [1]. SIM illustrates the importance of outstanding illumination for progressive microscopy.
Today, modern microscopy research accompanies a prominent field of technology: optical lithography. Landmarks in microscopy, like phase shifting and shadow masks have been part of micro-lithography for many years. Moreover, an analogy of beam-steering techniques known from microscopy was presented in a high-end lithographic stepper recently [2]. The setup realizes the beam shaping with novel diffractive micro-mirror arrays (MMA). This technology step marks one highlight of the MEMS development initiated by lithography. A development that started more than 10 years ago [3] continues to pave the way for further increasing resolution and speed [4].

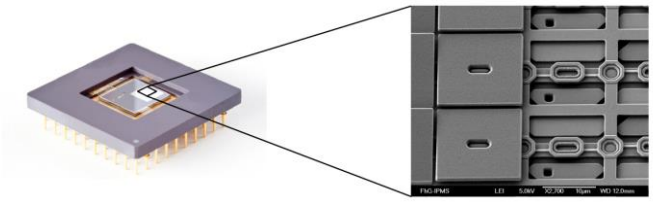

Fig. 1. Micro-mirror array (MMA) manufactured at the Fraunhofer IPMS (left) and close-up showing single micro-mirrors with 16µm pitch (right).

Diffractive micro-mirror arrays for microscopy

Using MMAs in microscopy is not a new approach. For instance, MMAs are used for sectioning in programmable array microscopes (PAM) [5] or structuring in SIM [6]. The MMAs used therein are said to work *digital.* The mirrors only accept two fixed states of deflection, corresponding to an ‘on’ or ‘off’ state. In contrast, MMAs with continuously deflectable mirrors are said to be *analog*. Those *analog* MMAs were originally developed for optical lithography (e.g. [2] and [4]), but their usage is not restricted to this field. A joint project named MEMI-OP between Institut Pasteur and Fraunhofer IPMS is focusing on *analog* MMAs for microscopy [7]. *Analog* MMAs give precise control of the diffraction of light. As a consequence, the intensity (a function

of the angle of deflection) and the pulse duration of the illumination can be adjusted independently [8]. Further, an MMA located in a plane conjugated to the pupil plane of the illuminating optics provides angular control. A second MMA located in a plane conjugated to the sample controls the illumination pattern [7].

Results

In the MEMI-OP project a microscope illumination has been developed for the use in biology and neurophysiology [7]. The MEMI-OP illumination module is based on two *analog* MMAs, each consisting of 256x256 mirrors with a 16µm pitch. Each of the 65.000 micro-mirrors can be actuated individually. CMOS circuits below each micro-mirror enable an actuation accuracy of λ/100. The MMAs can steer and structure shortest possible light pulses at kHz repetition rate. The usable spectrum ranges from NIR to the deep UV.
The capability of selective illumination is demonstrated at B lymphocyte CL-01 cells shown in Fig. 2 [7]. In Fig. 2 MMA1 gives angular control, while MMA2 masks desired regions of interest within the field of view.

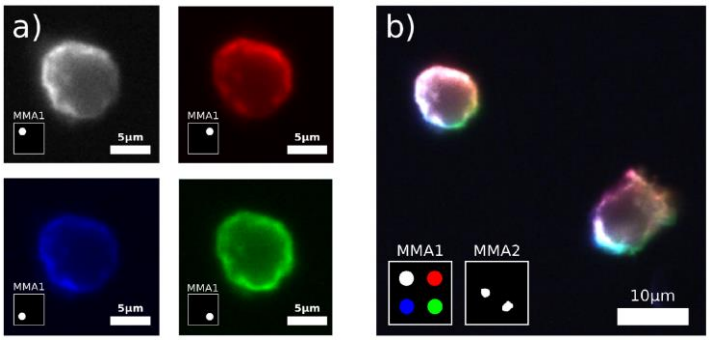

Fig. 2. (a) Illumination of the same B lymphocyte (CL-01) under four different angles, using a 40x water immersion objective. The pattern on MMA1 is shown as an inset. The change of the directionality of the light can clearly be seen from the differences in the intensity patterns. (b) Two cells masked by the MMA2 and illuminated under the four angles shown in (a) at the same time.

Conclusions

For decades microscopy techniques have been influencing optical lithography. New lithographic applications pushed the development of diffractive MEMS to be used as light modulators. Continuously deflectable (i.e. *analog*) micro-mirror arrays (MMAs) are developed for lithographic beam steering. The devices do not only enable spatio-angular control, but also allow setting the intensity and the duration of the illumination independently. An illumination module basing on a*nalog* MMAs is now used for applications in biology and neurophysiology. Precise control over the illumination reduces phototoxicity and photobleaching artifacts. Advantages are expected in fluorescence recovery after photobleaching (FRAP), fluorescence loss in photobleaching (FLIP) and photo activation (PA).

References

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