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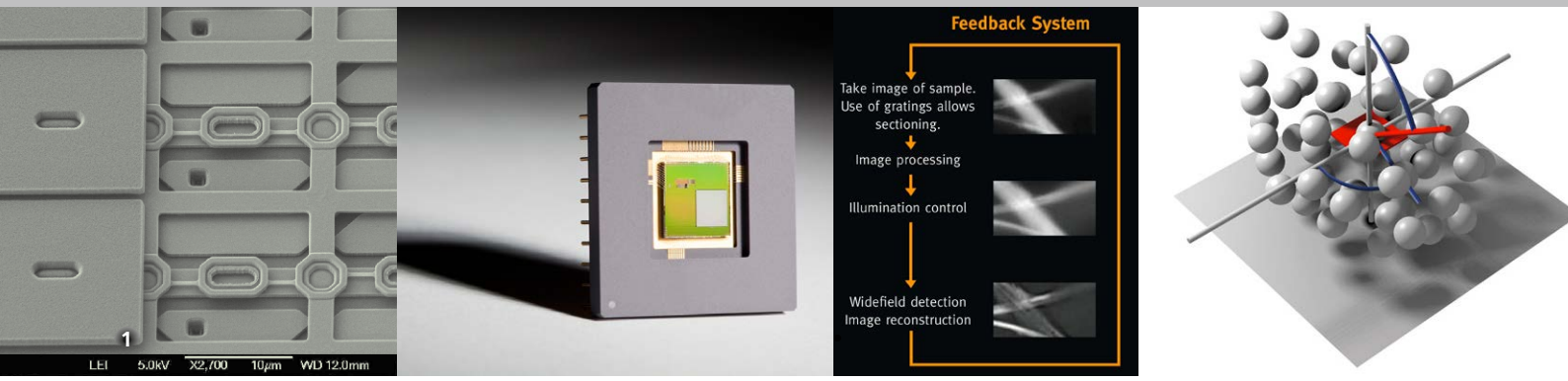

1 *System elements for spatio-angular illumination*

MICROMIRROR ENHANCED MICROSCOPIC IMAGING – MEMI-OP

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For optical microscopy programmable, ultra-fast Micro Mirror Arrays (MMA) can replace mechanical-apertures, and serve as high-resolution Spatial Light Modulators (SLMs). More recently it has become clear that micromirror array (MMA) devices also enable new, and significantly improved functionalities for optical microscopes.

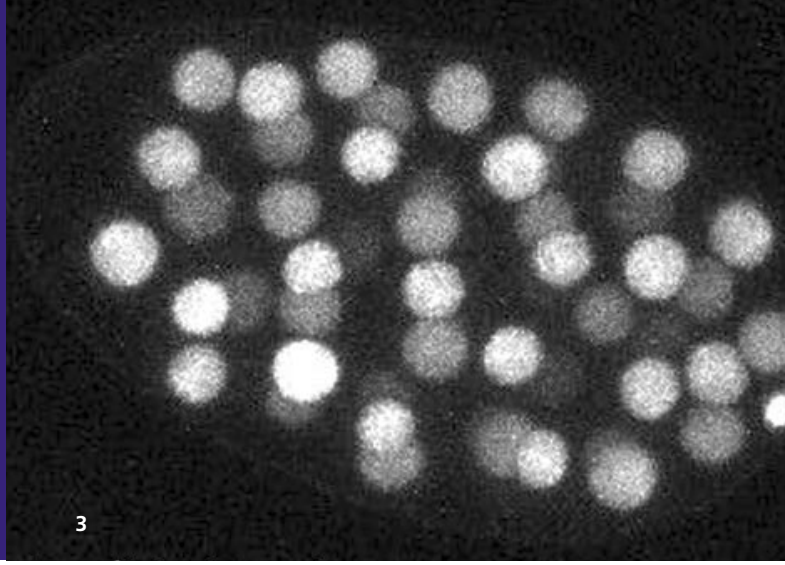
Motivation

Optogenetics is a newly identified application area in life sciences imaging that uses MMA/SLM to achieve fast, compartmentalized and minimally invasive modulation of cellular activity. With the introduction of new genetically encoded optogenetic probes, and spectral chemistry of inorganic and organic probes, optogenetics and photo-activation (PA) is emerging as a burgeoning field of interest.

Currently, however, no MMA/SLM devices have been designed and developed specifically for the Life Sciences applications domain. Consequently existing MMA technology does not match the increasing needs of emerging life sciences field.

Technology

MEMI-OP – a joint programme of Fraunhofer Institute for Photonic Microsystems IPMS and the Carnot Institute Pasteur Maladies Infectieuse – will implement a new and unique MMA/SLM technology developed and optimized in the context of previous works. As such it provides unprecedented and outstanding performance compared to existing commercial SLM devices, notably providing for higher frequency operation. The diffractive MMA technology distinguishes the MEMI device as exceptional for many reasons. Its key feature is the ability to impose continuous



deflection over its whole matrix. It allows, with a remarkable degree of performance, high-speed angular and spatial control of light illumination by modulating the deflection of the 65536 micro-mirror elements. Attached to a microscope, angular light control using MEMI is based on an adaptive object illumination, with variable angle of incidence that relies on diffractive effects. The MMA is embodied into a specialized optical sub-system designed and built by "IN-VISION Digital Imaging Optics GmbH (Austria)" that makes it adaptable for Life-Science microscopes.

MEMI-OP will re-engineer this system to optimize critical performance parameters:

- Implement higher order MMA operation mode and redesign system optics to enlarge the field of view and light throughput,
- Introduce two MMAs into the optical sub-system for improved synchronization and speed,
- Increase the MMA reflectivity above 70% to maximize system efficiency,
- Add laser light sources allowing a broad wavelength selection (DUV-VIS-NIR),
- Systems algorithm development and programming,
- Demonstrate 2D and 3D photoactivation performance in vitro,
- Establish methods and demonstrate 2D/3D photomanipulation in vitro and in living cells,
- Ultrastructural analysis of cells and micro-organisms following MEMI-OP manipulation using Correlative Light Electron Microscopy.

With these improvements, the performance of the system will be validated and benchmarked in real applications using experimental optogenetics paradigms in living cells.

Application

The MEMI-OP illumination module will help to benchmark a "best-in-class" system capable of unprecedented performance (speed, spectral bandwidth, spatial & angular light parameters) and should provide immediate utility for experimental approaches using FRAP (fluorescence recovery after photobleaching), FLIP (Fluorescence light induced photoactivation), and optogenetics (subcellular and cellular biochemical signalling cascade activation). The last possibility is by far the most exciting for studies on microbiology since it opens the doorway to precise subcellular activation, or inhibition of host-pathogen signalling cascades.

Acknowledgement

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2 *MMA chip with address electronics*

3 *Microscopy low light intensity image of a cell ensemble*