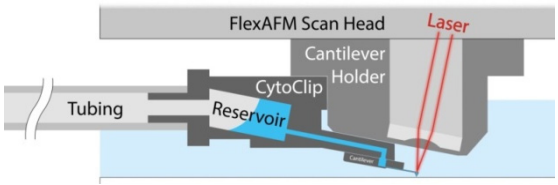
# Spotting and lithography with FluidFM

## Introduction

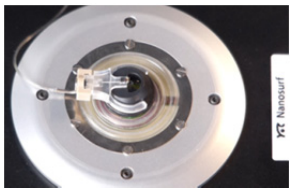
In cell biology, micropatterning is becoming a widely accepted technique to address cellular behavior at the single-cell level. The ability to guide cell growth by local suppression or stimulation, for example, is beneficial for studying cell growth, for developing cell-based sensors, and for tissue engineering applications.

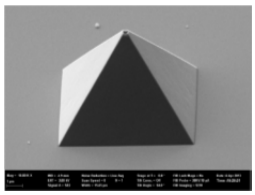
The production of microfabricated biosensors is another application that requires precise placement of biomaterials on a substrate. With the advances of MEMS-based technologies, sensor elements are being reduced in size to a few microns. In order to develop practical applications of these technologies for detection of biological and chemical entities, the sensors must be specifically coated with a capture agent in the same and even smaller size regime.

FluidFM® technology by Cytosurge enables deposition of (bio)molecules and particles at defined locations with micrometer accuracy and with femtoliter volumes[1-3]. The closed channel is capable of depositing molecules from a liquid in both air and liquid environment. This enables numerous applications in biomedicine, cell- and microbiology, as well as non-biological nanolithography.




FluidFM probes have a closed channel connecting the cantilever opening to a fluid reservoir. A controller is used to vary back pressure to the reservoir to allow deposition parameterization. Force control is obtained through laser-based measurement of cantilever bending by the AFM that is an integral part of the FluidFM system.



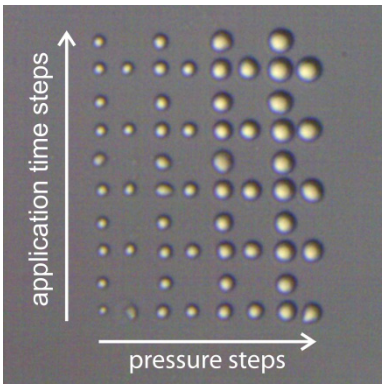


Left: Mounted hollow cantilever with tubing connected to the reservoir.

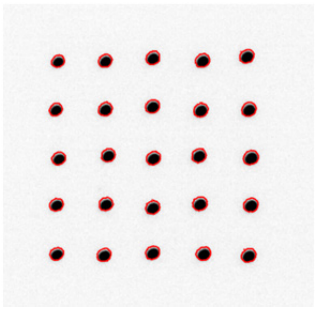
Right: Apex cantilever, featuring a 300nm opening at the end of the pyramid

## Features of FluidFM for spotting and lithography

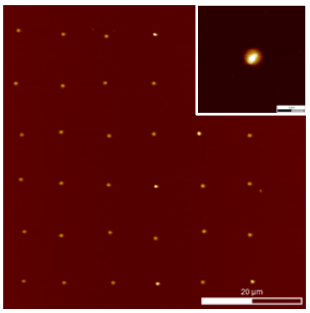
- Flexible and complex definition of spot and line patterns for deposition and nanolithography
- Precise deposition down to femtoliter volumes
- Closed channel enables deposition under liquid and in air
- Force control to prevent sample damage
- Accurate back-pressure, time, force and speed control to optimize feature dimensions
- Can handle high viscosity carriers (e.g. glycerol, low-MW PEG)
- Integrated reservoir on the microfluidic probe for continuous supply of liquid, allowing deposition of spots and lines covering large areas without reloading
- Can be used to deposit material onto or even into single cells[4,5]
- A channel electrode can be used to perform spatially-confined electrochemistry at the surface
- FluidFM can also be used for other applications, such as spatial cell manipulation[6,7], cell adhesion experiments[8], and extraction of intracellular material




Automated grid calibration varying back pressure (left to right with increasing pressure) and contact time (bottom to top with increasing time). For each condition 3 spots were printed.

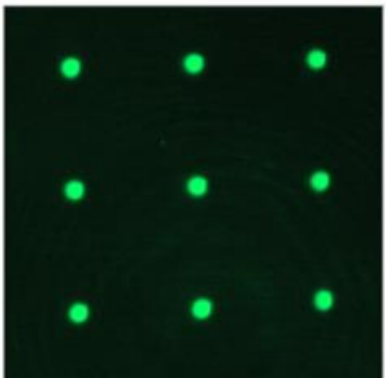


Air environment, 250 mbar back pressure, 1 s contact time. Diameter: 4.83 ± 0.17 µm

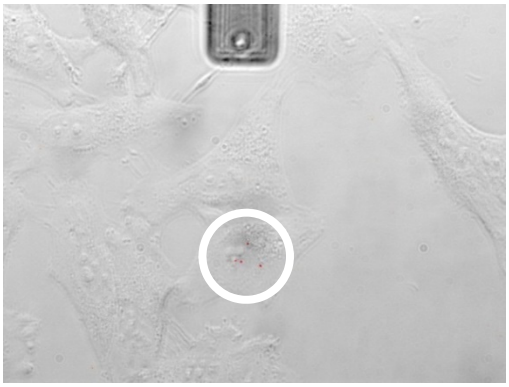


Air environment, 100 mbar back pressure, 50 ms contact time. Diameter: 615 ± 60 nm

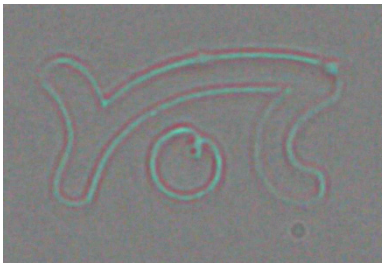
Diameter reproducibility better than 5% standard deviation can be reached for micrometer-sized spots. Sub-micrometer spot sizes can be achieved under optimized conditions.



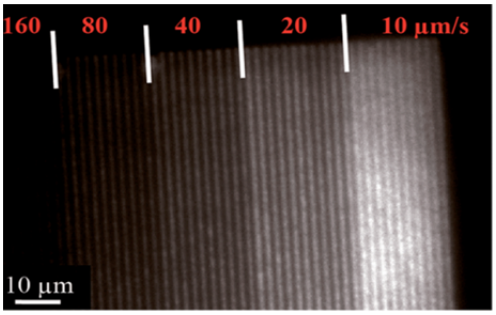
Spotting under liquid environment of Alexa 488 labeled streptavidin on a biotin modified substrate[1]



Deposition of virus particles (red dots in white circle) under liquid to study the cooperativity between virus particles at the single cell level[2]



Nanosurf logo written in in air with a solution containing approximately 50% glycerol, back pressure 200 mbar




Line widths in liquid can be adjusted by variation of speed, pressure and force[3]

[1] Meister et al. 2009, *Nanoscale dispensing in liquid environment of streptavidin on a biotin-functionalized surface using hollow atomic force microscopy probes*, Microelectr.Eng. 86: 1481-1484.
[2] Stiefel et al. 2012, *Cooperative Vaccinia Infection Demonstrated at the Single-Cell Level using FluidFM*, Nano Lett. 12: 4219-4227.
[3] Grüter et al. 2012, *FluidFM as a lithography tool in liquid: spatially controlled deposition of fluorescent nanoparticles*, Nanoscale 5: 1097-1104.
[4] Meister et al. 2009, FluidFM: *Combining Atomic Force Microscopy and Nanofluidics in a Universal Liquid Delivery System for Single Cell Applications and Beyond*, Nano Lett. 9: 2501-2507.
[5] Guillaume-Gentil et al. 2012, *Force-Controlled Fluidic Injection into Single Cell Nuclei*, Small 9: 1904-1907.
[6] Dörig et al. 2010, *Force-controlled spatial manipulation of viable mammalian cells and micro-organisms by means of FluidFM technology*, Appl. Phys. Lett. 97 : 023701.
[7] Guillaume-Gentil et al. 2013, *Isolation of Single Mammalian Cells from Adherent Cultures*, Lab Chip: Accepted manuscript.
[8] Potthoff et al. 2012, *Rapid and Serial Quantification of Adhesion Forces of Yeast and Mammalian Cells*, Plos One 7: e52712.