Novel lab on chip technology for fast 3D particle tracking in living yeasts based on micromirrors

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We present a novel Lab-on-Chip technology for 3D particle tracking in living cells based on V-shaped micromirrors, which are used to observe fluorescent specimens from multiple vantage points, providing stereo-images that can be recombined for 3D reconstruction. We apply our technology to study chromatin dynamics *in vivo* using yeast cell lines with one or two GFP-tagged genes. Gene loci are followed in 3D with acquisition rates as short as 10 ms, providing quantitative information on their dynamics with exquisite temporal resolution.

Confocal microscopy is the standard method to perform fast 3D tracking in living organisms. This technique has however its shortcomings because 3D reconstruction is obtained by acquiring a collection of views of the same specimen, and thus requires high doses of illumination that are associated to photobleaching and potentially to cellular photodamages. A number of alternative approaches based on bright field microscopy have recently been proposed to overcome this limitation, including bifocal microscopes[1], which simultaneously acquire two spatially separated images by insertion of a defocusing lens ahead of the camera, or astigmatic microscopes [2,3], which provide the full 3D position information owing to the introduction of a cylindrical lens in the emission path. These methods involve some modifications in the microscope optical path that are not always feasible on commercial devices. Microfabricated pyramidal mirrors that are placed on fluorescent specimens and used with standard wide-field microscopes have been proposed to perform multiple vantage point imaging[4], and 3D particle tracking in vitro[5]. Here, we report on the design of integrated Lab-on-Chips based on V-shaped micro-mirrors optimized for 3D tracking in living cells.

Our technology is based on V-shaped mirrors, which are fabricated by wet etching of (100) or (110) silicon wafers subsequently covered with aluminum to increase their reflectivity, and finally protected by a glass layer of 1 µm (Fig. 1.1). After silicon drilling to obtain access holes, the device is sealed with a glass coverslip, and facetted mirrors are used as optical and fluidic components.

When fluorescent particles are introduced in the Lab-on-Chip (Fig. 1.2), two reflections through the mirrors appear on each side of the bead. Due to the tilt of the mirrors, vertical displacements of the particle are projected on the y-axis on the reflected views (Fig. 1.3), and 3D tracking is achieved by monitoring the trajectories of the bead and its reflections, which are simultaneously captured by the video camera.

After rational optimization of the geometry of micro-mirrors as well as the microscope optical settings to improve the accuracy of 3D reconstructions, our Lab-on-Chip is applied to study chromatin dynamics *in vivo* using budding yeasts as a model system. Yeasts cells are modified with one or two chromosomes sites fluorescently tagged with bacterial operator sequences, and visualized using GFP fused to the associated repressor protein (Fig. 2.1). We confirm earlier observations that telomeric sequences, *i.e.* located close to chromosome ends, accumulate at the nuclear periphery, whereas genes found midway

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along chromosome arms are mostly present in the nuclear lumen (Fig. 2.2). The dynamics of these sequences is followed in 3D with an unpreceded temporal resolution as short as 10 ms, showing that chromosome dynamics is non linear in the short time regime and switches to a linear regime at larger timescales (dashed and bold lines in Fig. 2.3). Notably, this behavior is reminiscent of universal responses observed in polymer solutions[6], and is related to the confinement and the structure of chromosomes, which is thus probed by a non-invasive technique.

In this study we have designed a novel Lab-on-Chip technology for 3D particle tracking, and demonstrated its performance to study nuclear organization in yeast. This technique shows a great potential for studying dynamic processes in small living organisms.

1. Toprak, E., Hamza, B., Blehm, B.H. & Selvin, P.R., Nano Lett **7**, 2043-2045 (2007).
2. Kao, H.P. & Verkman, A.S., Biophys J **67**, 1291–1300 (1994).
3. Holtzer, L., Meckel, T. & Schmidt, T., Appl Phys Lett **90**, 053902 (2007).
4. Seale, K.T. et al., Journal of Microscopy **232**, 1-6 (2008).
5. McMahon, M.D., Berglund, A.J., Carmichael, P., McClelland, J.J. & Liddle, J.A., acsNANO (2009).
6. Doi, M. & Edwards, S.F. The theory of polymer dynamics, (Oxford University Press, New York, 1987).

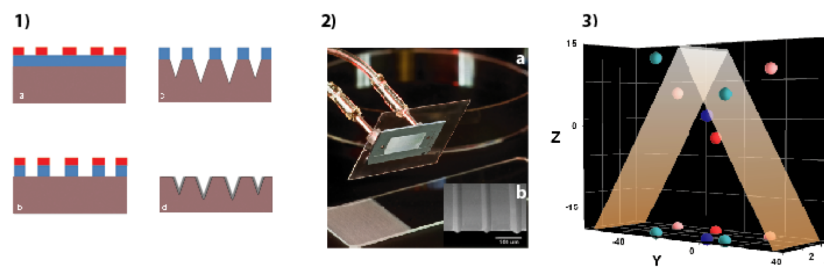

Figure 1. Lab on Chip principle. (1) Micromachining involves deposition of a nitride layer (blue), photoresist masking (red, (a)), followed by reactive ion etching (b), anisotropic wet etching (c), and metal evaporation (gray layer (d)). (2) Reflected images provide a tilted view of the scene, and can be used for 3D reconstructions based on stereovision analysis. (3) Picture of the Lab on chip for 3D particle tracking with the fluidic tubings (the scale is represented with a glass slide). The inset shows an electron micrograph of V-shaped mirrors.

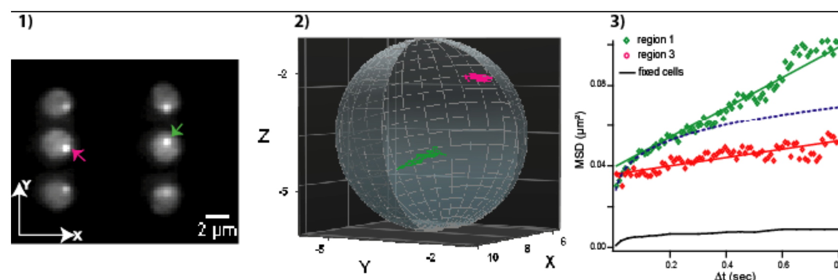

Figure 2. 3D live cell imaging. (1) Yeast nuclei placed in the Lab on Chip containing one fluorescently labeled locus. (2) 3D reconstructions of two DNA trajectories in a realistic nucleus (gray sphere) corresponding to loci positioned in the lumen (green) or close to the nuclear membrane (red). (3) Locus dynamics is studied based on their mean square displacement, which corresponds to the average motion after a given time lag. A two step response is observed with a non linear departure, followed by a diffusive-like linear increase in time.

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