Nano-aquariums from ultrashort laser pulses

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Microchambers in photostructurable glass enable the dynamic observation of aquatic microorganisms.

A large variety of organisms are presently living on the earth. Among these, microscopic-sized aquatic microorganisms have been surviving for more than 500 million years. Some of these microorganisms exhibit extremely rapid motion, which is unusual in the macro world in which we live, and can show unique 3D movements that appear to contradict gravity. Most are composed of a unit cell. Understanding the abilities and functions of these unit cells may offer insight into the cells that comprise more complex organisms, including human beings. The observation of microorganisms is currently a challenging subject for cell biologists. In the conventional microscopic observation system, a glass slide with a cover glass is generally used. However, the high numerical aperture of the objective lens limits both the field of the image and the depth of focus, thereby making it difficult to capture images of moving microorganisms. Indeed, it can take a very long time until a clear image is obtained. A method to reduce the amount of tracking required or the reliance on chance movements of the organisms is needed.

To solve these problems, we applied our technique for fabricating 3D hollow microstructures inside photostructurable glass using femtosecond lasers to the manufacture of a special microchip, referred to as a nano-aquarium.1 The nano-aquarium can scale down the observation site by encapsulating the microorganism in a limited area while still providing enough space for motion. This makes it much easier to capture images of moving organisms in freshwater with a standard objective lens (see Figure 1). The nano-aquarium structures also have the advantage of keeping living microorganisms fresh for a long time since such a structure prevents the evaporation of water as seen under the glass slide/cover glass system.

To fabricate the nano-aquarium, we developed a technique that can be used to directly form 3D hollow microstructures with smooth internal surfaces inside photostructurable (Foturan) glass via femtosecond laser direct writing followed by annealing and successive wet etching.1–3 A 1mm-long microchannel with an almost constant width of 150µm was fabricated at a depth of 150µm below the glass surface (see Figure 2). In addition, the top internal wall of the microchannel is flat and smooth and is parallel to the glass surface. The average roughness of the etched surfaces was previously evaluated to be 0.8nm.3

Using the nano-aquarium, we succeeded in recording movies of the 3D flagellum movement of Euglena gracilis, a single-celled ovoid protist about 50µm long (see Figure 3 and videos4–6). The time required to obtain a clear image can be reduced by a factor of more than 10 compared with the conventional method. In addition, water in the embedded channel does not evaporate or leak as is the case with a cover glass or for bonded transpar-

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Figure 2. Optical microscope images of (a) the top view and (b) the side view of the nano-aquarium used for observing the motion of Euglena gracilis.

Figure 3. Still images of (a) Euglena gracilis in the microchannel, (b) enlarged picture of (a), and (c) the front view of Euglena gracilis swimming upward in the reservoir (see videos 4–6).

Figure 4. Sequential microscope images of Pleurosira laevis in the nano-aquarium before, during, and after contact stimulation by the integrated microneedle (see video 7).

As demonstrated, the use of nano-aquariums for the dynamic analysis of microorganisms offers advantages over conventional observation methods. In particular, observation time is significantly reduced, movement can be controlled, and the ability to carry out 3D observations is enhanced. Furthermore, the chamber structure prevents water vaporization and leakage resulting in the microorganism remaining fresh for a longer time. Femtosecond laser direct writing is an effective and powerful technique for rapid manufacturing of nano-aquariums, generating a variety of 3D microstructures and allowing for the incorporation of functional microcomponents with little difficulty. The next steps will be to fabricate and integrate new components to expand application of the devices.

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References

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4. http://spie.org/documents/newsroom/videos/1453/Hanada-Fig3a.avi
Movie of *Euglena gracilis* in the microchannel. Credit: Yasutaka Hanada, RIKEN.

5. http://spie.org/documents/newsroom/videos/1453/Hanada-Fig3b.avi
Close-up of *Euglena gracilis* in the microchannel. Credit: Yasutaka Hanada, RIKEN.

6. http://spie.org/documents/newsroom/videos/1453/Hanada-Fig3c.avi
The front view of *Euglena gracilis* swimming upward in the reservoir. Credit: Yasutaka Hanada, RIKEN.

7. http://spie.org/documents/newsroom/videos/1453/Hanada-Fig4.mpg
Movie of contact stimulation of *Pleurosira laevis* in the nano-aquarium by integrated microneedle. Credit: Yasutaka Hanada, RIKEN.