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Review Article

A mechanical microcompressor for high resolution imaging of motile specimens

Jessica A. Zinskie [a], Michael Shribak [c], Michael F. Bruist [a], Karl J. Aufderheide [d], Chris Janetopoulos [b,*]

[a] University of the Sciences Chemistry and Biochemistry, 600 S 43rd Street, Philadelphia, PA 19104, United States
[b] University of the Sciences, Department of Biological Sciences, 600 S 43rd Street, Philadelphia, PA 19104, United States
[c] Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543, United States
[d] Texas A&M University, Department of Biology, College Station, TX 77843, United States

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ABSTRACT

In order to obtain fine details in 3 dimensions (3D) over time, it is critical for motile biological specimens to be appropriately immobilized. Of the many immobilization options available, the mechanical microcompressor offers many benefits. Our device, previously described, achieves gentle flattening of a cell, allowing us to image finely detailed structures of numerous organelles and physiological processes in living cells. We have imaged protozoa and other small metazoans using differential interference contrast (DIC) microscopy, orientation-independent (OI) DIC, and real-time birefringence imaging using a video-enhanced polychromatic polscope. We also describe an enhancement of our previous design by engineering a new device where the coverslip mount is fashioned onto the top of the base; so the entire apparatus is accessible on top of the stage. The new location allows for easier manipulation of the mount when compressing or releasing a specimen on an inverted microscope. Using this improved design, we imaged immobilized bacteria, yeast, paramecia, and nematode worms and obtained an unprecedented view of cell and specimen details. A variety of microscopic techniques were used to obtain high resolution images of static and dynamic cellular and physiological events.

Contents

Abbreviations: 3D, three dimensions; MMC, mechanical microcompressor; DIC, differential interference contrast; OI-DIC, orientation- independent DIC; ngm, nematode growth media; DO, drop-out; GFP, green fluorescent protein; CV, contractile vacuole; eMMC, enhanced mechanical microcompressor; CCMM, computer-controlled milling machine; OR, outer ring; BB, brass base; GC, glass coverslip; IR, inner ring; CM, compressor mount; CC, coverslip compressor; AO, acridine orange

* Corresponding author. Fax: +1 215 596 8710.
E-mail address: c.janetopoulos@usciences.edu (C. Janetopoulos).

1. Introduction

Advancements in the optics of fluorescent and contrast-enhancement microscopy have given rise to a demand for a rapid and delicate means of immobilizing ambulatory organisms. Small, jostling specimens are difficult to image because they move in and

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out of the focal plane, prohibiting precise or extended imaging. Other, larger organisms can move out of the field of view if they are not held steady. In these larger specimens, it may be critical to immobilize the organisms precisely so that subcellular organelles can be imaged over time. Only when the movement of the whole organism is inhibited can the best information from dynamic molecules, such as fluorescent proteins and RNAs or DNA in live-cells, be obtained. Efforts to reduce the complexity of imaging moving molecules often presents a significant challenge to experimental design so that one can obtain the optimum spatial and temporal resolution.

A myriad of options exist for immobilization of specimens for imagining, each with their own set of advantages and disadvantages [1]. For instance, chemical immobilization utilizes small-molecule anesthetics that induce paralysis. The use of pharmacological drugs can interfere with physiological or biochemical studies, or slowly kill the specimen. Furthermore, these samples are still subject to Brownian motion. Physical inhibition of a specimen includes its placement in a highly viscous fluid, adherence to, or imbedding in, a surface such as agarose, or uncontrolled squeezing of the sample between a coverslip and slide, often without regard for the amount of distortion. Many times these practices do not affect biochemical processes, however recovery of the specimen can be difficult or impossible. Controlled compression of the sample can be obtained with the use of a rotocompressor or a mechanical microcompressor (MMC), a device that allows for manual compression of a specimen between two glass planes. Although the design has evolved over the last 100 years or so, the basic concept has been consistent [2]. Such devices can also be useful for the identification of new species and even novel organelles in living samples [3–5].

Our group has recently developed a more sophisticated MMC that also has the optional feature of microfluidics [6]. The MMC can be used to gently trap and immobilize a wide range of organisms including bacteria, yeast, small protozoa and larger specimens including fly embryos and nematode worms. This device is non-lethal, reusable, and could be used for either long-term or short-term imaging. The addition of the microfluidics to the MMC allowed for long-term growth and imaging of specimens such as the budding of *Saccharomyces cerevisiae* [6]. The ease by which specimens could be recovered after imaging allowed for future manipulation, analysis, or culture.

Here, we show further uses for the MMC by immobilizing several other specimen types and using an advanced form of DIC optics and polarization microscopy [7–9]. The remarkable optics obtained through the gentle flattening of organism allowed us to obtain unprecedented images of several structures in species of *Paramecium* and *Stentor,* as well as several other microbes and pond water specimens. While this new generation of MMCs could be used on inverted microscopes, they were designed mainly for use on an upright microscope. Here we describe a new MMC that takes advantage of several of the updates in the Yan et. al design [6] and is modified specifically to be used on inverted microscopes. The new design allows the user to adjust the degree of compression from above the stage. Additionally, the location of the adjustable components is no longer close to the objectives or inhibited by the size of the objectives. This modification increases the area that may be scanned for imaging. As is the case with the previous MMC, this microcompressor is simple to use, and allows the gentle and reversible trapping of numerous organisms.

2. Materials and methods

2.1. Cell growth and specimen acquisition

Stentor polymorphus, as well as the rotifers, were collected from a small pond in Falmouth, MA. Specimens were mechanically compressed in the pond water at room temperature.

Stentor coeruleus was provided by Wallace Marshall, University of California, San Francisco, and also mounted in Falmouth, MA pond water at room temperature.

The *Caenorhabditis elegans* was maintained at 18 °C in Nematode Growth Medium(NGM) on plates seeded with OP-50 strain of *Escherichia coli*, as previously described [10]. The strain LX929 (Punc-17::GFP(vsIs48)) was provided by Dr. Samuel Caito (Albert Einstein College of Medicine, Bronx, NY). After compression, the *C. elegans* were returned to the NGM plates and grown successfully for 1–6 days, compressed again, and imaged.

Paramecium sonneborni [5] was grown at 17 °C in baked lettuce media inoculated with *Klebsiella pneumoniae* (ATCC #27889) as food.

Saccharomyces cerevisiae strand CY6002 (MATa his3Δ1 leu2Δ0 lys2Δ 0 ura3Δ0 SEC61-yEpolylinker-yEGFP::kanMX) [11] was transformed with PhyB-mCherry-NLS, a gift from Chao Tang, (Addgene plasmid # 51571) using the Frozen-EZ Yeast Transformation II Kit (Zymo Research). Transformants were plated on the appropriate synthetic dropout(DO) media (Sigma Aldrich) with kanamycin (50 μg/mL, Sigma-Aldrich). The cells were cultured in the appropriate DO media and grown at 30 °C to mid log phase.

Escherichia coli strain DH5α (Invitrogen) was grown in 2xYT media (Sigma-Aldrich) at 37 °C overnight shaking at 200 rpm.

2.2. Live-cell imaging

For, OI- DIC the light source used was a 100 W halogen lamp. The bandpass interference filter had a central wavelength of 546 nm and 40-nm full width at half maximum, Chroma Technology, Rockingham, Vermont. We selected the green light for illumination. Magnification and image collection used an Olympus BX61 Upright Microscope using either a 10X/0.30NA or 100X/1.3NA lens and an Infinity 3M CCD camera (Lumenera, Ottawa, Ontario, Canada).

For the true OI polarized light microscope technique for real-time birefringence imaging, we used a video-enhanced polychromatic polscope. This was performed on an Olympus IX-81 microscope. Low magnification used a 10X/0.30NA lens, while high magnification was with a 40X/0.75NA lens.

L3 and L4 stage *C. elegans* were picked from NGM plates and washed in a well of NGM media. The worm was then transferred with a pipetter from the well onto the viewing area of the microcompressor in a small droplet no more than 2 μm in diameter (~1 μL of liquid). *C. elegans* images were taken on a Marianas Imaging Workstation from Intelligent Imaging and Innovations Inc (3I), Denver, CO, USA. This consists of an inverted Zeiss-Axiovert 200M epifluorescence microscope (Zeiss Inc., Oberkochen, Germany). The lens used was a 40X PlanNeofluar 1.3NA oil immersion objective. A FITC cube (Semrock) was used to excite the green fluorescent protein (GFP) and image acquisition was performed with SlideBook6 software from 3I.

Paramecium were transferred from the lettuce media, stained with DAPI (Life Technologies, 20 ng/mL) and washed with media. One paramecium was placed on the microcompressor in 1 μL media and the top coverslip was lowered until the paramecium was immobilized. It was imaged with the 63X PlanNeofluar 1.4NA oil objective on the Marianas system (3I).

Imaging of *S. cerevisiae* was performed on a spinning disk (Yokagawa CSU-X1; Andor Technology) confocal microscope using a 100X PlanNeofluar 1.4NA oil immersion objective lens on a TiE