Coming Events

Inter/Micro
July 11–15, 2011
Chicago, IL
www.mcri.org/home/section/101/inter-micro

Denver X-ray Conference
August 1–5, 2011
Colorado Springs, CO
www.dxcicdd.com

2011
Microscopy & Microanalysis 2011
August 7–11, 2011
Nashville, TN

Microscopy Conference MC 2011
August 28–September 11, 2011
Kiel, Germany
www.mc2011.de

Multinational Congress on Microscopy
September 4–9, 2011
Urbino, Italy
www.mcm2011urbino.it

ICXOM21
September 5–8, 2011
Campinas, Brazil
icxom21.lnls.br

EMAG 2011
September 6–9, 2011
Birmingham, UK
www.emag-iop.org

National Society for Histotechnology
September 16–21, 2011
Cincinnati, OH
www.nsh.org

FEMMS 2011
September 18–23, 2011
Sonoma County, CA
www.femms2011.llnl.gov

CIASEM 2011
September 25–30, 2011
Mérida, Mexico
www.ciasem.com

Neuroscience 2011
November 12–16, 2011
Washington, DC
www.sfn.org

2012
Microscopy & Microanalysis 2012
July 29–August 2, 2012
Phoenix, AZ

2013
Microscopy & Microanalysis 2013
August 4–8, 2013
Indianapolis, IN

2014
Microscopy & Microanalysis 2014
August 3–7, 2014
Hartford, CT

More Meetings and Courses
Check the complete calendar near the back of this magazine and in the MSA journal *Microscopy and Microanalysis*.

Carmichael's Concise Review

Wrapped for Accurate Imaging

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Since transmission electron microscopy (TEM) was developed about 80 years ago, numerous strategies have been attempted to visualize living cells at high resolution. The harsh environment within the TEM (mostly the vacuum and damage from a fixed beam of electrons) presents challenges. Some approaches have been to fabricate chambers within the TEM that provide a more "friendly" environment for living cells (that is, less stringent vacuum), but they have limitations. Impressive images have been generated with various cryogenic techniques, but frozen cells are not alive or in their native state in the traditional sense. Nihar Mohanty, Monica Fahrenholtz, Ashvin Nagaraja, Daniel Boyle, and Vikas Berry have developed an ingenious solution to the problem by "wrapping" cells with modified graphene [1].

Graphene is an allotrope of carbon composed of single sheets of graphite (the carbon form found in pencil lead). The Nobel Prize in Physics last year went to Andre Geim and Konstantin Novoselov for what the Nobel Committee termed "groundbreaking experiments regarding the two-dimensional material graphene." The point here is that graphene is a material that has a lot of potential uses, and Berry's group has discovered a novel one.

Sheets of modified graphene can be used to confine bacterial cells within an easy-to-apply impermeable and electron-transparent encasement that retains the cellular water, while enabling imaging by TEM. The layer is just a few atoms thick of an element of low atomic number. The outer shells of electrons (the π electrons) of the carbon atoms are so close that even small atoms cannot pass through the graphenic sheets, yet it is strong enough to contain an internal pressure when the wrapped cell is in a vacuum. The modified graphene is flexible, allowing wrapping to conform to the cell surface. Finally, the graphenic sheets have a high electrical conductivity (again, due to the π electrons) to significantly reduce electrostatic charge buildup and a high thermal conductance to dissipate heat while in the electron beam.

Specifically, Mohanty et al. demonstrated that protein-functionalized graphene (PFG) can wrap bacteria in such a way as to enable wet-phase TEM imaging. In a proof-of-concept study, they used an unstained Gram-positive bacteria (*Bacillus subtilis*), which is about 70 percent water (by volume) with a wall thickness of 16 to 30 nm. An aqueous suspension of graphene oxide (GO) sheets was covalently bonded to Concanavalin-A, which has a specific affinity for the polyteichoic moieties on the bacterial cell wall. When this was mixed with a purified bacterial suspension, the mixture clouded, and this is attributed to the encasement of the bacteria by the PFGs. This interpretation is supported by control experiments in which GO not functionalized with Concanavalin-A did not yield wrapped bacteria.

Cross sections of wrapped bacteria cut at 90 nm were examined in the TEM, and about 90 percent were fully wrapped, with the

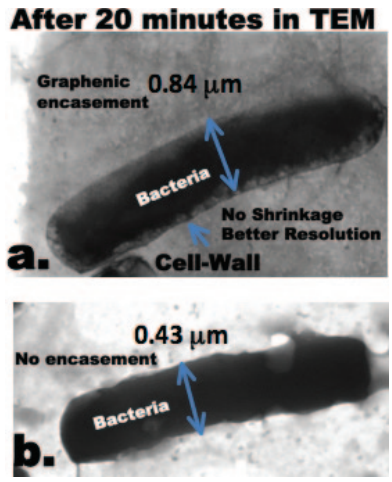

Figure 1: Wrapped bacterium. (a) Representative TEM images of wrapped bacterium exhibits no shrinkage from the original size after 20 minutes inside the TEM chamber at ~10^{-5} Torr. (b) Representative unwrapped bacteria (UWB) exhibit ~76% shrinkage after 20 minutes under TEM vacuum. Note that in (a) under the same conditions, the cell wall of the wrapped bacteria is clearly discernible. Courtesy of Dr. Vikas Berry and the American Chemical Society.

doi:10.1017/S1551929511000423

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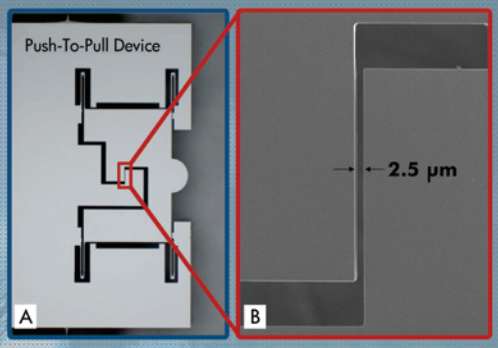

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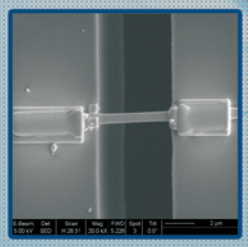

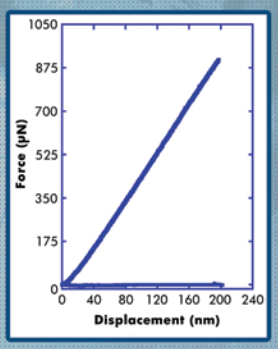

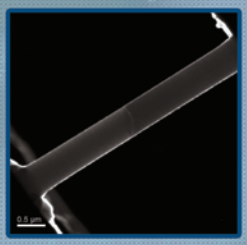

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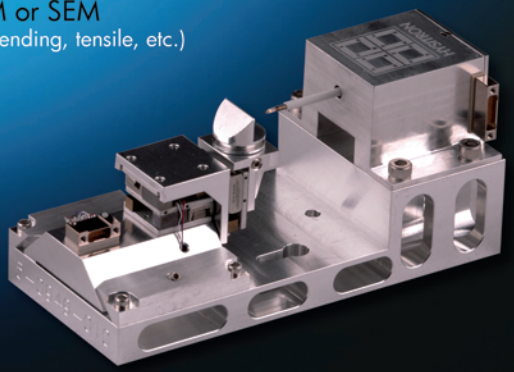

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remainder partially wrapped. A majority of cells were wrapped in as many as 7 layers of PFG. Tests confirmed that cells that were alive when they were wrapped. Wrapped and unwrapped cells were immobilized on silicon nitride windows and examined in the TEM for up to 20 minutes. TEM micrographs of the wrapped cells clearly showed the bacterial cell wall and intracellular structure, whereas micrographs of unwrapped cells had unrecognizable intracellular structure and evidence of charging that distorted the image (see Figure 1). Also, much of the water was retained within the wrapped cells, and there was no discernable volume change. The unwrapped cells quickly shrank by 76 percent and were apparently dry after 20 minutes in the TEM.

Berry's group has developed yet another use for graphene and its modifications, one that can be helpful to microscopists. This is reinforced by the supplemental material (such as videos of wrapped bacteria) that accompanied their article. They envision that encasing wet samples within graphenic chambers could enable real-time imaging of fluid dynamics, proteins, liquid suspension of nanoparticles, and the biochemical activity within living cells [2]!

References

[1] N Mohanty, M Fahrenholtz, A Nagaraja, D Boyle, and V Berry, *Nano Letters* 11 (2011) 1270–75.

[2] The authors gratefully acknowledge Dr. Vikas Berry for reviewing this article.

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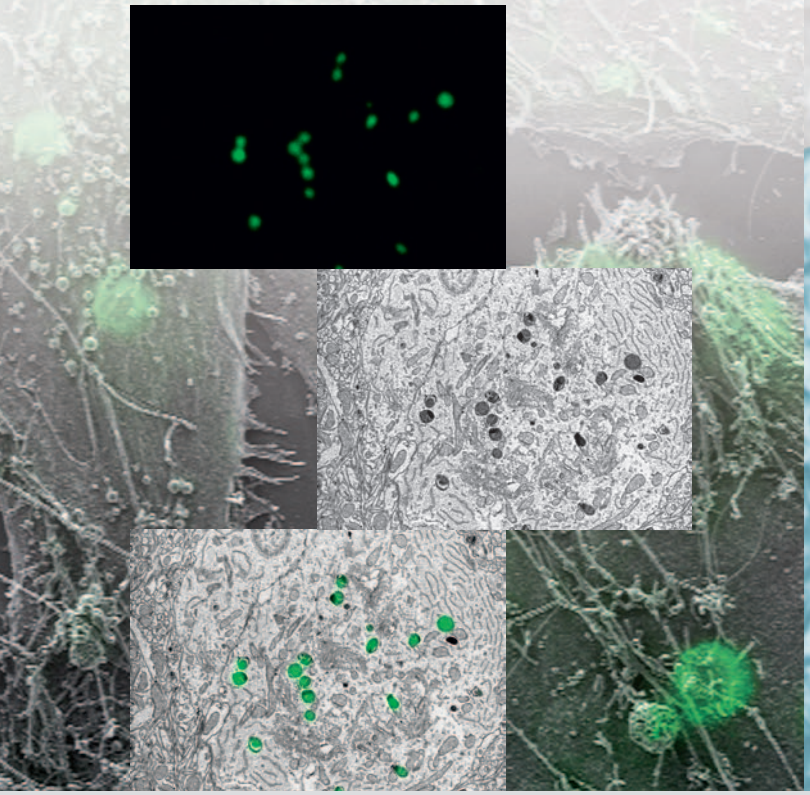

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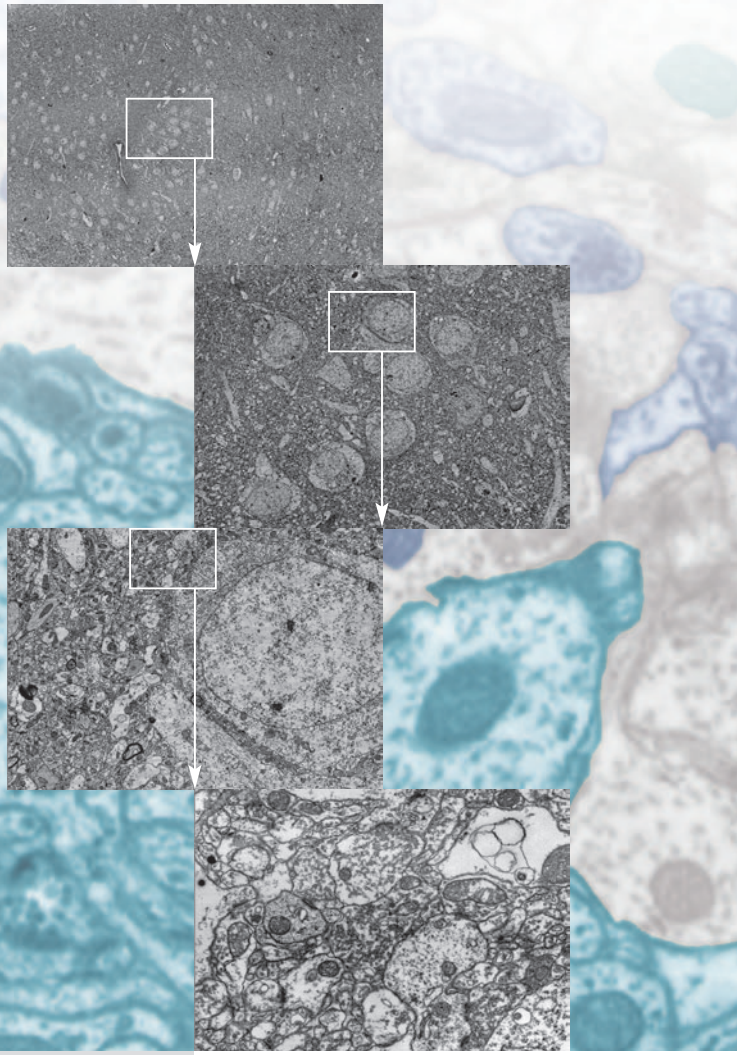

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