Wet electron microscopy with quantum dots

Winston Timp[1], Nicki Watson[1], Alon Sabban[2], Ory Zik[2], and Paul Matsudaira[1]
[1]Whitehead Institute, M.I.T., Cambridge, MA, USA and [2]Quantomix Corporation, Nes-Ziona, Israel

BioTechniques 41:295-298 (September 2006)
doi 10.2144/000112239

Wet electron microscopy (EM) is a new imaging method with the potential to allow higher spatial resolution of samples. In contrast to most EM methods, it requires little time to perform and does not require complicated equipment or difficult steps. We used this method on a common murine macrophage cell line, IC-21, in combination with various stains and preparations, to collect high resolution images of the actin cytoskeleton. Most importantly, we demonstrated the use of quantum dots in conjunction with this technique to perform light/electron correlation microscopy. We found that wet EM is a useful tool that fits into a niche between the simplicity of light microscopy and the high spatial resolution of EM.

INTRODUCTION

Microscopy has played a vital role in biology since the days of Robert Hooke, leading to the origin of cell theory (1). First, light microscopy and later, electron microscopy (EM), allowed biologists to explore the cell down to the molecular level. Individual components of the cell, from organelles down to single proteins were imaged using these techniques.

The central issues of microscopy are contrast and resolution. Contrast provides the ability to differentiate structures in the cell from one another and from the background. Resolution refers to the minimum separation between objects required to identify them as individual structures. In order to effectively use microscopy to explore the biological landscape, the sample must have sufficient contrast and resolution.

For light microscopy, the natural contrast of the sample may easily be enhanced through the application of chromophores or fluorophores. This also allows multicolor imaging using differential staining. However, the resolution of light microscopy is physically restricted by the Abbe limit. The Abbe limit dictates a minimum x-y spatial resolution given by $\lambda/2NA$, where λ is the light wavelength, and NA is the numerical aperture of the objective lens (2).

By comparison, EM has extremely fine spatial resolution. Using transmission electron microscopy (TEM), distances on the order of 10^{-10} m or 1 Å may be resolved, and with scanning electron microscopy (SEM), 10^{-8} m or 10 nm is possible (3). However, obtaining contrast in EM is more difficult than the simple staining needed for light microscopy. Complicated preparation techniques, including critical point drying, coating, embedding, and sectioning are required (4). Expensive equipment and extensive training are necessary, creating a barrier that prevent many researchers from using EM.

Correlative light/electron microscopy is the ideal technique for structural work, combining nondestructive, time-lapse light microscopy with high spatial resolution EM (5). Unfortunately, the destructive nature of EM sample preparation, combined with the arduous task of finding the same cell previously imaged—makes this an extremely difficult technique in practice.

A new technology available from Quantomix™ allows for biological EM without laborious sample preparation. Their product consists of a sealable capsule with an electron-transparent membrane. The sealed, fully hydrated sample is then imaged through the membrane, which behaves as a coverslip does in light microscopy. Electron-dense stains may be used to enhance sample contrast, as fluorescent stains are used in light microscopy. This technique, dubbed Wet SEM™, makes EM as easy to use as light microscopy, while retaining a substantial resolution advantage (6).

In addition to its uses for biological SEM, this platform is uniquely suited to correlative microscopy. As the membrane is both photon and electron transparent, it is possible to image the same cells in both light and electron microscopy. To provide contrast in both modes of imaging, an electron dense fluorescent stain was needed. Quantum dots fill that role, being made of a CdSe/ZnS fluorescent material (7,8).

MATERIALS AND METHODS

Cell Culture

The IC-21 cell line (accession no. TIB-186; ATCC, Manassas, VA, USA) used in these experiments is an adherent murine macrophage line. These cells were maintained at 37°C and 5% CO_2 in RPMI media (Mediatech, Herndon, VA, USA), supplemented with 15% fetal bovine serum (FBS; Mediatech) and 50 IU penicillin/50 µg/mL streptomycin (Mediatech). Cells were passaged by detaching in phosphate-buffered saline (PBS) without Ca^{2+} (Mediatech) with a dilution factor from 1:5 to 1:2.5.

IC-21 cells were detached from a culture dish when at approximately 75% confluency. The resulting cells were centrifuged at 600× *g* for 5 min. The supernatant was aspirated, and the cells resuspended at 2×10^5 cells/mL concentration. Fifteen microliters of this cell solution were added to a series of QX-102 capsules (Quantomix, Nes-Ziona, Israel). These capsules were secured in a MP-10 multiwell plate (Quantomix). The multiwell plate's reservoirs were filled with sterilized water, and the plate was incubated overnight at 37°C and 5% CO_2.

Cell Staining

After the overnight incubation, the capsules were removed from the incubator. The samples were washed with 1× PBS and fixed in a solution of

4% formaldehyde/0.1% Triton® X-100 in 1× PBS for 15 min. The samples were then washed again with PBS and stained with a contrast-enhancing agent.

We used three different types of electron dense materials in our experiments. The first stain was a weak solution of uranyl acetate (UA). UA stains required a preparatory tannic acid treatment, 1% tannic acid in double-distilled water for 5 min. A 1% solution of UA was then used to stain the sample for 1 min.

The other two stains were colloidal gold particles (Molecular Probes™, Invitrogen, Carlsbad, CA, USA) and quantum dots (Qdot®; Invitrogen). These particles were both available conjugated to streptavidin. To apply these stains, the samples were first blocked with a solution of 2% BSA/PBS for 1 h. The samples were then incubated overnight with biotin-XX-phalloidin (Molecular Probes, Invitrogen) at 4°C. The next day, the samples were blocked again with 1× Sigma Blocking Buffer (Sigma, St. Louis, MO, USA) for 30 min. Finally, the sample was incubated with colloidal gold or quantum dots for 1 h. To aid in light microscopy, the cells were also stained with 1 µg/mL Hoechst 33342 (Molecular Probes, Invitrogen) as a nuclear counterstain.

To prepare for imaging, we first washed with double-distilled water and then filled the capsules with QX-102 imaging buffer. The aqueous imaging buffer has a backscattering coefficient equivalent to water, its sole effect to protect the sample from the damaging effects of electron beam. The sealing stubs were screwed back onto the QX-102 capsules, and the capsules were placed in the microscope. When sealed, the capsules may be preserved for months at 4°C.

Imaging

Capsules were imaged on a JSM-7401F scanning electron microscope (JEOL-USA, Peabody, MA, USA) using a backscatter detector. The microscope was operated at a working distance of around 8 mm and an acceleration voltage of 15–25 kV. For light microscopy, a DeltaVision® microscope (Applied Precision, Issaquah, WA, USA) was used with a 10× objective (0.5 NA; Olympus America, Center Valley, PA, USA).

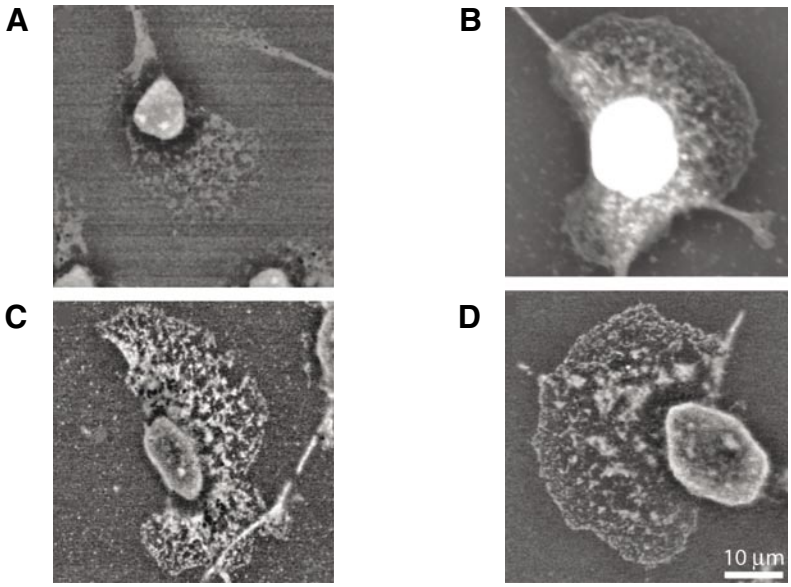

Figure 1. Electron micrograph montage of different staining methods. IC-21 cells (A) unstained [25 kV; 11 mm working distance (WD)], (B) uranyl acetate (UA) stained (25 kV; 7.8 mm WD), (C) actin stained with colloidal gold (15 kV; 7.8 mm WD), and (D) actin stained with quantum dots (15 kV; 7.8 mm WD).

RESULTS AND DISCUSSION

Samples in QX-102 capsules were imaged through the detection of backscattered electrons. Briefly, the excitation beam passed through the membrane of the QX-102 capsule and struck the sample. Some of the electrons were absorbed, stimulating the emission of secondary electrons. These electrons no longer had sufficient energy to travel through the membrane and were not detected. Other electrons elastically scattered off the sample, returned through the membrane, and were collected by the backscatter detector. Only electrons that scattered from near the membrane surface retained enough energy to reach the detector, limiting the effective focal depth to a few microns (6). The biological sample in this case was composed of adherent cells firmly attached to the membrane, within the effective focal depth. As the beam is scanned across the sample, the image is formed from the intensities of the backscattered electrons.

First, a sample was imaged in its natural state, without any contrast enhancing agents. This was intended as a control for baseline contrast provided by cells, demonstrating that any added contrast was due to the stain. After fixation, the sample was imaged, as seen in Figure 1A. The contrast from the sample was quite low, making it difficult to resolve internal features of the cell. General morphology, as well as the location of the nucleus, was all that could be determined from the unstained sample.

Next, a standard EM stain was used as a positive control, to calibrate the amount of signal expected from the cell sample. UA, a reactive heavy metal stain, was used at several different concentrations and times. The optimal conditions for imaging cytoskeletal structure were found to be a 1% (w/v) solution for 1 min (Figure 1B). UA seemed to give contrast to fibrous-like structures in the cell. Unfortunately, as UA is a nonspecific stain, it bound indiscriminately to proteins and nucleic acids. There is no way of knowing if the filaments visible are actually cytoskeletal in origin.

It was uncertain whether the quantum dots would show up using wet EM—so a calibration with a similar particulate stain was performed. Colloidal gold particles are the same size as the quantum dots and are known

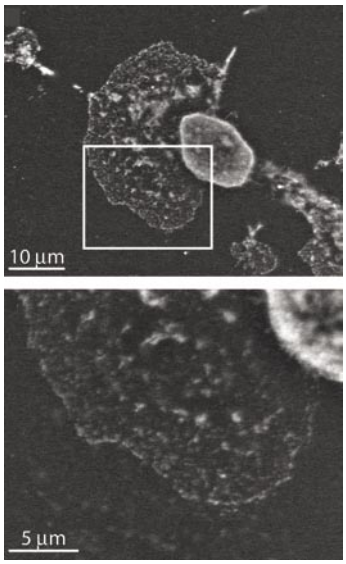

Figure 2. Electron micrograph of a quantum dot stained cell. Images taken at (A) 2000× [15 kV; 7.6 mm working distance (WD)] and (B) 5000× (15 kV; 7.6 mm WD). These images demonstrate the superior spatial resolution possible with scanning electron microscopy.

to produce high contrast in EM. These particles were 10 nm in diameter, bound to streptavidin, which in turn binds to biotinylated phalloidin, staining filamentous actin in the cell. Extensive blocking steps were found necessary to prevent nonspecific binding, including both bovine serum albumin (BSA) and Sigma Blocking Buffer.

Samples treated and stained in this manner were imaged in the electron microscope (Figure 1C). The stain appeared punctate, not bound continuously along actin filaments as expected. However, it did label a structure that resembles the actin cytoskeleton and generated high contrast as expected.

Next, streptavidin-conjugated quantum dots were substituted for the colloidal gold particles. The quantum dots are made of high atomic number material, CdSe, with a ZnS outer coating, approximately 10 nm in diameter. The resulting particle should be strongly visible from backscattering. Indeed, the quantum dot sample (Figure 1D) looks very similar to the colloidal gold sample. The only significant difference between the two was a smaller signal-to-noise ratio from the quantum dot sample.

The quantum dot sample shows a clear, though not overwhelming stain. The morphology of the cell is very clear, as the stain is well confined to the cell body. The technique does not seem capable of resolving individual quantum dots, as the signal is probably too weak. However, small clusters and strands were visible (Figure 2A). The highest useful magnification of the image (Figure 2B) demonstrates the resolution possible using this technique. The sample displayed no perceptible thermal or Brownian motion while imaging, probably due to cross-linking from the fixation process.

The fluorescent properties of the quantum dots allowed imaging of the sample using light microscopy. Using the grid that is on the bottom of the QX-102 capsules, it proved easy to find the same cell sample on both scopes. Epifluorescent images of the cells were taken using a 10× objective, as the current capsule construction prevents the use of higher magnification objectives. Using MATLAB, electron and light microscopy images were rotated, flipped, and rescaled properly to create Figure 3, demonstrating quite clearly that it is easily possible to image the same sample in both electron and light microscopy.

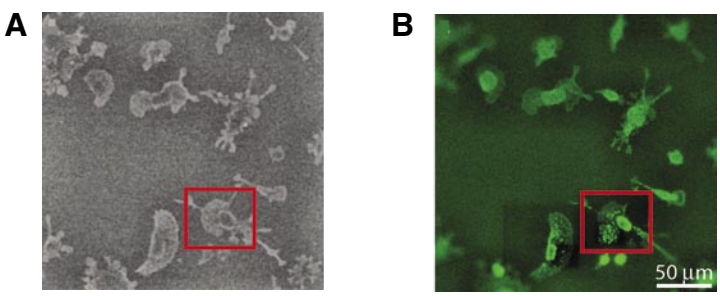

Figure 3. Electron and light micrographs for electron/light correlation microscopy. Image (A) represents a group of cells imaged at 250× [15 kV; 8.1 mm working distance (WD)] with image (B) showing the same cells imaged on a light microscope using a 10× (0.5 NA) objective. The sample required only one stain for both electron and light microscopy. The red box indicates the same cell imaged in Figure 2, also apparent in panel B, is damaged from the electron microscopy (EM) imaging.

It is important to note, however, that EM was destructive to the sample at certain intensities. When imaging at high magnification, the rapid scanning of the beam over the same area seems to damage the cellular structure. The quantum dots are not visibly damaged, retaining their fluorescence, though there is a slight decrease in the background signal around the cells. The damage is likely caused by thermal effects from the electron beam energy, but this is by no means certain. This suggests that samples should first be imaged with the less destructive light microscopy.

In conclusion, we have successfully demonstrated the utility of quantum dots in EM using the Wet EM technique. Using only one stain, samples may be imaged in both electron and light microscopy. This protocol allows for the high spatial resolution of EM to be easily combined with time-lapse light microscopy. This ability may prove extremely useful in further studies. Light microscopy may be used to determine which cell is showing a specific marker or behavior, then placed in the electron microscope to obtain a high resolution image.

ACKNOWLEDGMENTS

We thank James Evans for help with IC-21 cells. We also thank Vern Robertson and Toshi Kanazawa of JEOL USA, Inc. for their help with electron microscopy. This work was supported in part by a grant from the National Institutes of Health (NIH) to P.M. (NIH-57418).

COMPETING INTERESTS STATEMENT

A.S. and O.Z. are employees of Quantomix Corporation, which produces the capsules used in this paper. The other authors declare no competing interests.

REFERENCES

1. **Hooke, R.** 1665. Micrographia: Or, Some Physiological Descriptions of Minute Bodies Made by Magnifying Glasses. J. Martyn and J. Allestry, London.
2. **Pawley, J.B. (Ed.).** 1995. Handbook of Biological Confocal Microscopy. Plenum Press, New York.
3. **Hayat, M.A.** 1978. Introduction to Biological Scanning Electron Microscopy. University Park Press, Baltimore.
4. **Bozzola, J.J. and L.D. Russell.** 1999. Electron Microscopy: Principles and Techniques for Biologists. Jones & Bartlett, Sudbury, MA.
5. **Svitkina, T.M. and G.G. Borisy.** 1998. Correlative light and electron microscopy of the cytoskeleton of cultured cells. Methods Enzymol. *298*:570-592.
6. **Thiberge, S., O. Zik, and E. Moses.** 2004. An apparatus for imaging liquids, cells, and other wet samples in the scanning electron microscopy. Rev. Sci. Instrum. *75*:2280-2289.
7. **Michalet, X., F.F. Pinaud, L.A. Bentolila, J.M. Tsay, S. Doose, J.J. Li, G. Sundaresan, A.M. Wu, et al.** 2005. Quantum dots for live cells, in vivo imaging, and diagnostics. Science *307*:538-544.
8. **Giepmans, B.N.G., T.J. Deerinck, B.L. Smarr, Y.Z. Jones, and M.H. Ellisman.** 2005. Correlated light and electron microscopic imaging of multiple endogenous proteins using Quantum dots. Nat. Methods *2*:743-749.

Received 8 March 2006; accepted 21 June 2006.

Address correspondence to Paul Matsudaira, 9 Cambridge Center, WI-661, Cambridge, MA 02142, USA. e-mail: matsudaira@wi.mit.edu