Strategies to Efficiently Locate Cultured Cells of Interest on Transmission Electron Microscopy Grids

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Introduction

In transmission electron microscopy (TEM), finding a cell of interest can be time-consuming and, given the high cost of beam time, expensive. The difficulty is exacerbated if each grid has only *one* cell of interest. An example might be when a particular cell has been recorded with time-lapse microscopy before and during an experimental treatment that culminated in fixation. The sample may even have been subjected to post-fixation immuno-TEM procedures, increasing the importance of locating the cell. Here we report a strategy for finding our targets expeditiously.

Materials and Methods

Grids. To assure the most efficient use of beam time, we have developed a method of culturing cells on special formvar-coated electron microscopy (TEM) grids and pre-examining them with light microscopy. It is crucial to use gold grids when seeding live cells (copper and nickel grids are toxic to cells) and to have some asymmetry that is visible both in EM and light microscopy. Grids are available in which each square of the grid is numbered, but we eschewed these because we thought that the numbers occluded too much of the field of view. Instead, we use grids in which the center is marked with an "**L**" shape (PELCO 50 mesh gold grids from Ted Pella, Inc.; #1GG-50; Redding, CA).

Grid preparation. To prepare the grids, they are first coated with 0.5% formvar (Electron Microscopy Sciences, Hatfield, PA) by inverting a cluster of 2–3 grids onto an island of formvar that is floating on sterilized water [1]. Each island of formvar with grids attached is then picked up upside-down with an acid-washed 22 × 22 mm coverslip (Gold Seal #3406) such that the formvar-coated grids are facing upward (Figure 1A). The coverslips, draped with the formvar-coated grids are allowed to air dry overnight in a dust-free environment. Thirty-five millimeter polystyrene Petri dishes (BD Falcon #351008) are prepared by punching a 14 mm hole in the bottom with a punch-and-die press. Each grid/formvar/coverslip assembly is attached, formvar-side up, using a non-toxic medical silicone adhesive (#A-100, Factor II, Inc., Lakeside, AZ; Figure 1B). The adhesive is allowed to cure for at least 24 hours at room temperature. The hole in the plastic Petri dish with the glass coverslip glued to the bottom creates a well in the dish, which holds about 0.2 ml. To prepare the dish for cell culture, the insides of the assembled Petri dishes are sterilized for 15 minutes in a biosafety cabinet equipped with an ultraviolet germicidal light, and a suitable substrate is applied for cell survival. Because we examine the cell biology of neuronal growth cone motility, for our purposes we first coat the sterile dishes containing formvar-coated grids with 0.2 ml of sterile 0.01% poly-L-ornithine for 1 hour at room temperature (Sigma, St. Louis, MO), gently rinse with water, coat with 20 µg of laminin (Invitrogen/ Life Technologies, Grand Island, NY) in 0.2 ml sterile phosphate-buffered saline for 1 hour at room temperature, and finally gently wash with three changes of sterile phosphate-buffered saline. The last rinse is left on the dishes so that the laminin will not dry out. A sterile transfer pipet or sterile pipet tip is used rather than using a vacuum device to *gently* remove the rinses because the force of the suction can easily remove the formvar film. These assemblies could potentially be used for any cell type and are also suitable for time-lapse microscopy using an inverted light microscope or for correlative light and electron microscopy.

For our studies, we remove the last saline rinse and seed a triturated suspension of chicken embryo dorsal root ganglion neurons in cell culture medium into the center of the coverslip and allow the cells to attach overnight. The cells on the grids are fixed while attached to the coverslips in the Petri dishes, then they are removed for processing, either for TEM or for

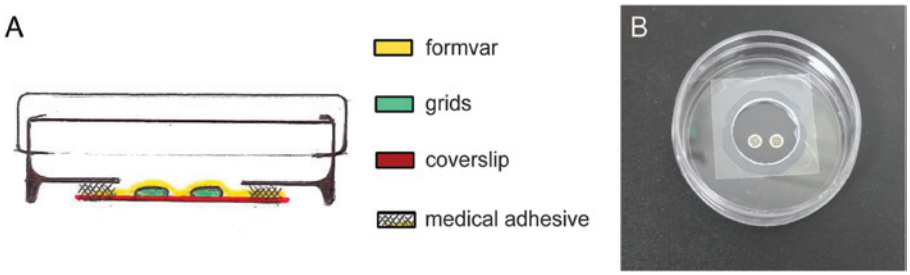

Figure 1: (A) A sketch of the side view of a Petri dish/coverslip/formvar-coated grid assembly. Medical adhesive is placed around the bottom edge of the hole punched in a 35 mm plastic Petri dish. Then a coverslip containing formvar-coated grids is glued grid side up over the hole, creating a small well in the center of the dish containing the formvar-coated grids. The Petri dishes are stackable and have a small outer rim around the bottom so that the Petri dish does not rest on the coverslip. (B) A photograph of a completed dish assembly.

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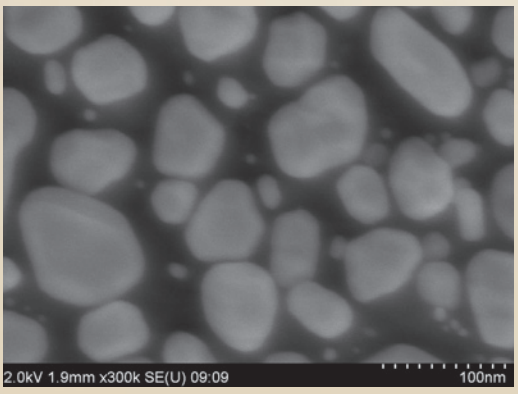

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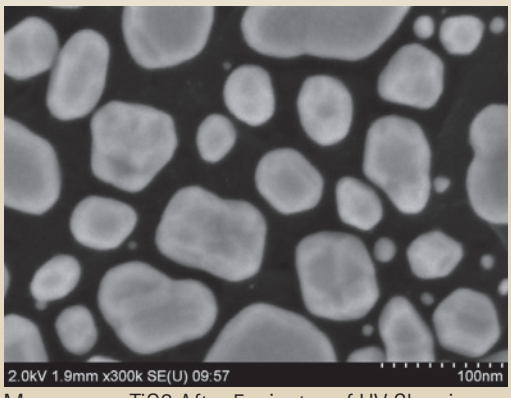

Mesoporous TiO2 After 5 minutes of UV Cleaning

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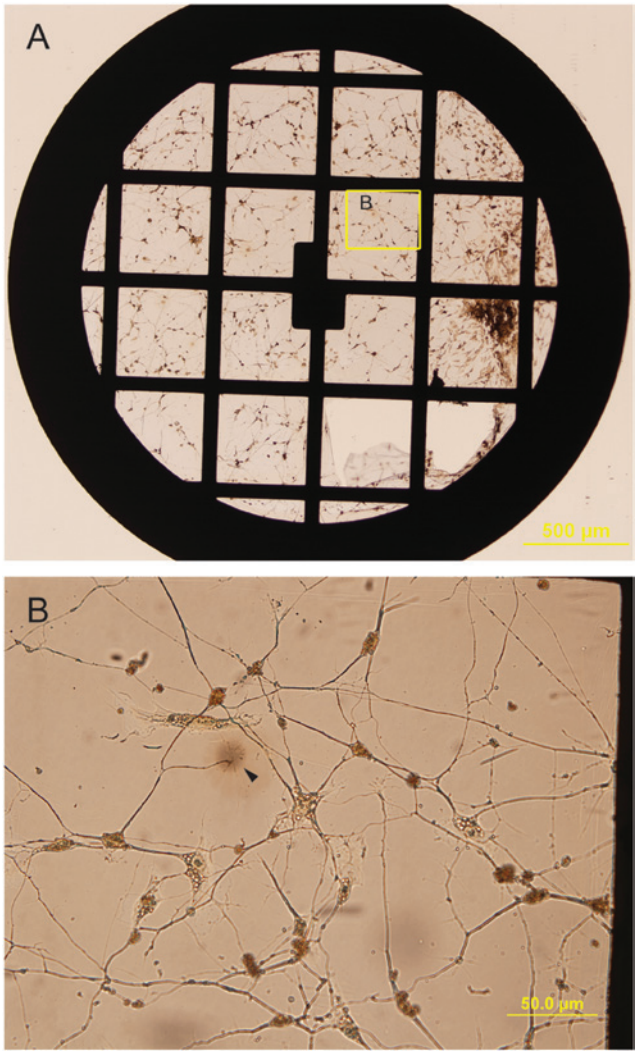

Figure 2: (A) A processed, formvar-coated grid (note the "**L**" in the center) with neurons and glial cells, as viewed with phase optics. (B) The insert in 2A is shown here at a higher magnification that allows neuronal growth cones to be easily visualized. Note the brown "burned in" spot (arrow) where a growth cone has been viewed with TEM.

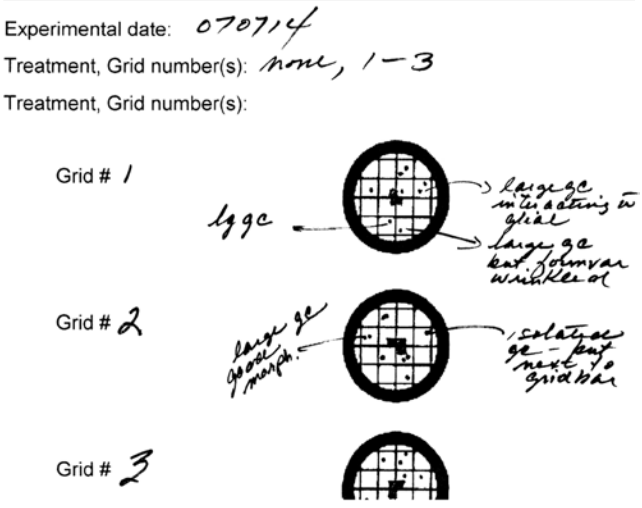

Figure 3: A portion of a grid "map" in which the center "**L**" shape has been recorded. All cells of interest are indicated, as well as notes on experimental details and the sample condition.

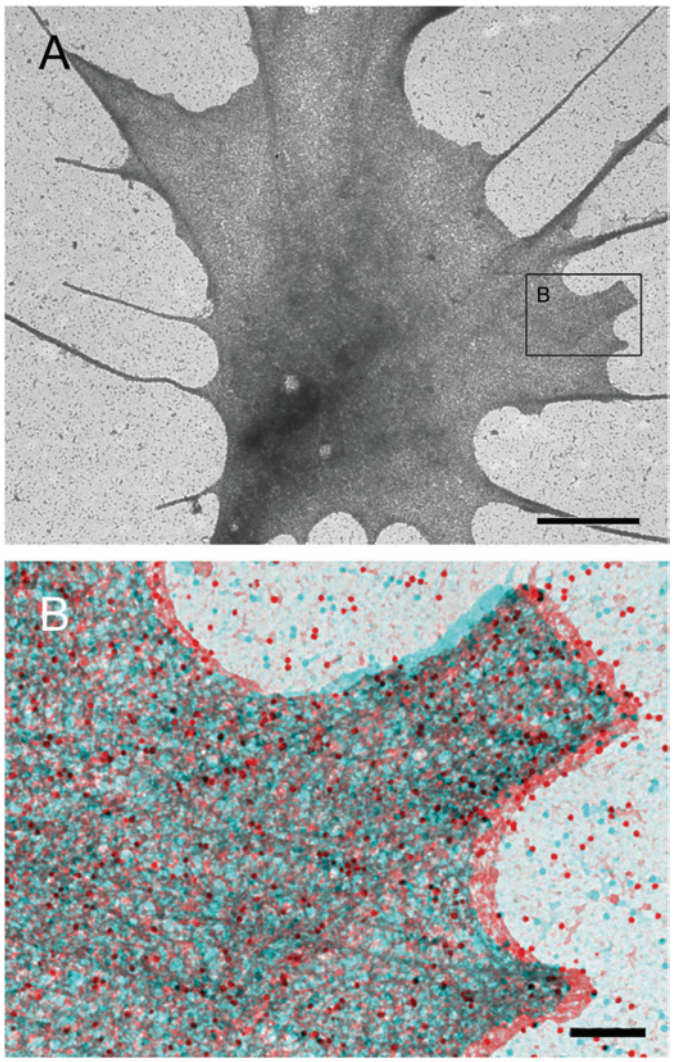

Figure 4: (A) Low magnification of a growth cone with region of interest (B) indicated. (B) Stereo pairs of the growth cone in (A) were taken at a magnification of 40,000 at +6° and -6° angles by tilting the goniometer. The resulting stereo pairs were used to produce an anaglyph by placing one image in the red channel and the other in the blue channel using Adobe Photoshop software (Adobe Systems Incorporated, San Jose, CA) according to [3]. View the anaglyph image in 3D using red (left lens)/cyan (right lens) glasses. Scale bars are 2 µm (A) and 0.2 µm (B).

immuno-TEM. The grids are filed in numbered slots in a grid box labeled with the date of the experiment.

Map making. To map the location of cells of interest, we view each grid with light microscopy and enter the specific cell positions on a "grid map" that is preprinted with a pictorial representation of a 50 mesh grid, repeated in a column of six per page. Each grid is placed on a clean slide and viewed using phase optics with an inverted microscope (Figure 2). This examination reveals information that is useful for prioritizing samples, for example, sample quality and holes or wrinkles in the formvar. On each grid map, we record the grid identity, the orientation of the grid's **L** shape, the location of growth cones of interest relative to the **L**, and notes on sample quality (Figure 3). When viewing the grid in TEM, the map is turned to reflect the orientation of the grid as seen on the screen. We can then quickly toggle over to any cells we wish to view on that grid.

Stereo pairs. Producing stereo pairs requires taking paired photos in which the sample has been tilted. We use a JEOL 1400 TEM with a side-entry goniometer; the specimen height (*Z* height; eucentric height [2]) is adjusted by selecting the standard focus button and using the *Z* controls and the *X* or *Y* wobble keys and the focus knob to bring the image into fine focus.

We generally tilt a total of twelve degrees; 6 degrees in the *X* direction; then 6 degrees in the *Y* direction at a magnification of 40,000. Depending on the magnification, the sample can be tilted from 4 to 20 degrees total. Because distortion might be present at the edges of the field at higher magnifications, the second photo should be in exactly the same orientation and cover the same field as the first so that the photos will be in full alignment. If the pairs are not in alignment, some of the periphery of the image may be distorted when the pairs are assembled, rendering them unusable. The problem of achieving good alignment expeditiously is even more challenging when more than one stereo pair is needed of the same cell.

To minimize the laborious tracking of a sample, we use a cell phone camera to record the precise orientation of each photographic field. Because each field is accurately recorded, we can save a great deal of time between series of stereo pairs. We begin by finding the desired growth cone at a low magnification and photographing it for reference (Figure 4A). Keeping the growth cone in view, we tilt the stage -6°. We raise the magnification, again keeping the area of interest in view, and take a whole series of photos along the growth cone's perimeter and interior, all taken at a stage tilt of -6°. For each TEM image taken, the operator takes a cell phone photo of the display screen. Once the entire growth cone has been documented, the stage is tilted +6°. Using the cell phone photos as references for framing the fields of view, the second photo of each stereo pair is taken. Stereo pairs can be used to prepare an anaglyph [3] to demonstrate, for example, the three-dimensional distribution of localized protein using antibody-coated gold beads at the edge of the growth cone (Figure 4B).

In summary, in our experience the small difference in price of the center-marked grids (which can be cleaned and reused) and in the time taken to scan the grids using light microscopy is more than compensated by the time and money saved during the TEM session, making time on the instrument less tedious and more productive. Once we adopted this method for photographing stereo pairs, our sessions on the microscope decreased from an hour to about fifteen minutes for each growth cone. Using a cell phone camera to assure that stereo pairs align suitably saves a great deal of time and prevents the frustration of having taken stereo pairs and later finding that they fail to overlap properly, necessitating another session on the microscope.

References

[1] Kent Makes Formvar Coated Grids: www.youtube.com/watch?v=u2auPtTtroM.

[2] J Rodenburg, "Learn to use the TEM; The Specimen Height and Eucentric Height," http://www.rodenburg.org/guide/t800.html.

[3] F Korobova and T Svitkina, *Mol Biol Cell* 19(4) (2008) 1561–74.

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