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A Low Cost Correlative Technique for Cell Imaging via Confocal and Scanning Electron Microscopy

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Increasingly, researchers are required to use multiple imaging approaches to solve a scientific problem. For example, confocal microscopy, atomic force microscopy and electron microscopy are commonly used to evaluate biological samples [1]. However, each technology has its own limitations and on their own often do not fully answer the question [2]. It can be argued that correlative microscopy is the ideal method to employ looking at the same exact cell across several imaging platforms. Two primary challenges with correlative microscopy include finding the microscopic object under very different imaging conditions and maintaining sample integrity [3]. Here we will present a correlative method using an SEM finder grid that has been modified from a previously reported TEM grid method to produce a mask for relocating cells quickly, effectively and inexpensively over large areas [4].

SEM finder grids (Electron Microscopy Sciences, cat# 80101-Cu) were placed on top of 13mm round Thermanox® (NALGE® Nunc®, cat# 174950) coverslips. The coverslips were placed flat in a Denton Vacuum Bench Top Turbo III and sputter-coated with Au/Pd for three minutes to insure a clear outline of the SEM finder grid was produced. The SEM finder grid was removed revealing an inverted image of the grid and sterilized prior to incubating with RAW264.7 macrophage cells. Grids were saved and could be reused indefinitely if they retained their flat profile. The cells were prepared for confocal microscopy on the masked coverslip using a 4% paraformaldehyde fixation. Subsequently, coverslips with cells were washed in 0.1M sodium cacodylate buffer, stained with a 1:1000 dilution of Syto® 13 (Invitrogen, cat# S7575), washed and inverted onto a drop of water inside a Nunc® chamber slide (NALGE® Nunc®, cat# 155360) to maintain hydration during imaging. Samples were imaged on a Zeiss LSM 510VIS with a 10x C-Apochromat 0.45 N.A. water immersion objective lens using 488nm Argon-ion (505 long pass emission filter) and HeNe 543nm (no emission filter) laser lines for fluorescence and reflected light, respectively. After the cells of interest were identified, they were fixed in 2% glutaraldehyde, rinsed three times in 0.1M sodium cacodylate buffer, post fixed with 1% osmium tetroxide, rinsed with distilled water and dehydrated in a graded series of ethanol for critical point drying in an Autosamdri-815B, Series A. The coverslips were mounted onto aluminum stubs with silver paint and the entire Au/Pd masked coverslips were sputter-coated again and viewed with a Hitachi S-4700 FESEM at 3.0kV.

This correlative method is a low cost and simple way to image large areas of cells with both light and confocal microscopy prior to viewing them at the electron microscope level. The SEM finder grid pattern produced by the Au/Pd shadowing made locating the same cells easy and took little effort. The letters, numbers and grid spaces containing the Au/Pd coating were easily observed as light areas in secondary electron mode (Fig. 1). Spacing of the finder grid letters and patterns were well suited for cell sized objects (Fig. 2). Various coatings of other cell

supporting materials will be further explored using this effective technique to optimize contrast and cell coverage and viability.

References

[1] K. Jahn et al., Modern Research Topics and Educational Materials in Microscopy, Formatex, Badajoz, (2007) 203
[2] S.C. Mohapatra and H.E. Pattee, Correlative Microscopy in Biology Instrumentation and Methods, Academic Press, Orlando, (1987) 409
[3] T.M. Svitkina et al., *J Structural Bio* 115 (1995) 290
[4] T.M. Svitkina and G.G. Borisy, *Cell Bio* (2005) 277

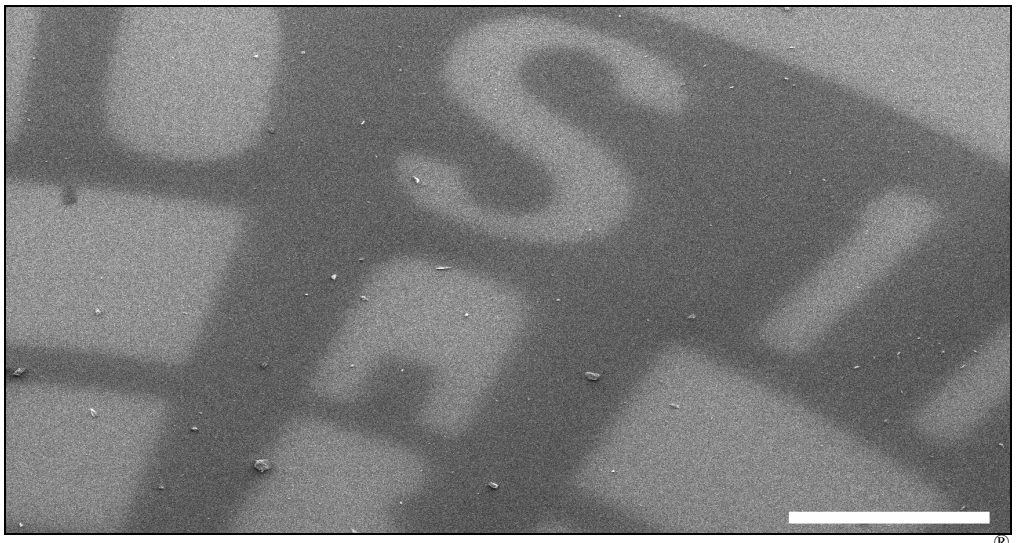

Fig. 1. An image of an SEM finder grid mask made from coating a Thermanox® coverslip with Au/Pd for three minutes. The dark areas are representative of where the grid bars were during the sputtering process. Scale bar = 500um.

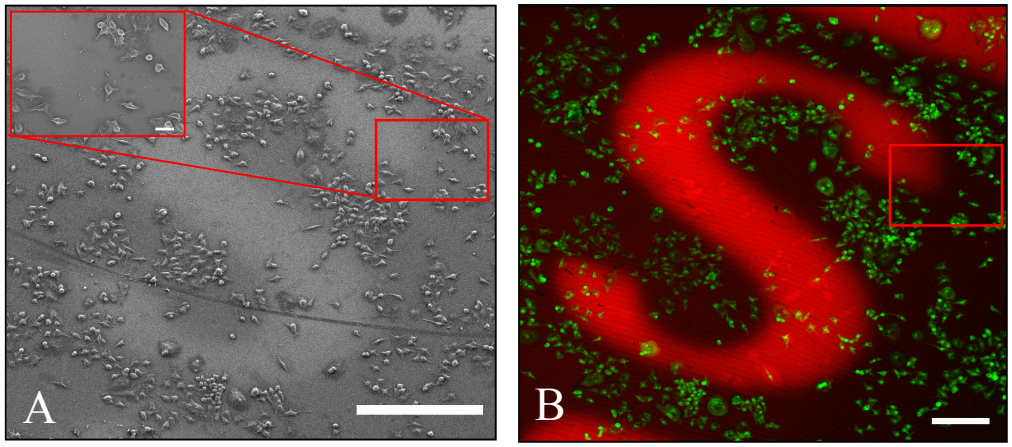

Fig. 2. A correlative SEM and confocal image using the SEM finder grid mask to rapidly relocate the same cells. An area was selected and found by both methods as indicated by the red square in Figs. 2A & 2B with a magnified SEM view in insert Fig 2A.
Scale bar 2A = 250um, 2B = 100um, 2A Insert = 25um.

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