doi:10.1017/S1431927618006591
Microsc. Microanal. 24 (Suppl 1), 2018

Correlative Light and 3D Electron Microscopy of Subnuclear Structures.

Claudia S. López[1,2,3], Jessica Riesterer[1,2,3], Kevin Loftis[2,3], Sunjong Kwon[2,3], Guillaume Thibault[2,3], Melissa Williams[1,2,3], Brett Johnson[2,3] and Joe W. Gray[2,3].

[1.]Multiscale Microscopy Core, Oregon Health & Science University (OHSU), Portland Oregon USA.
[2.]Department of Biomedical Engineering, OHSU, Portland Oregon USA.
[3.]OHSU Center for Spatial Systems Biomedicine, Portland Oregon USA.

Subnuclear structures such as the nucleolus have historically been studied mostly for histological interest. However, the pathological significance of the nucleolus has only been appreciated since the eighties. This was mainly due to the fact that this subnuclear organelle is not always visible by routinely used H&E-stained sections. Only enlarged nucleoli also defined as "prominent nucleoli" by pathologists are visualized by this method. In recent years, the presence of prominent nucleolar structures is one of the parameters used to define the nuclear grade of tumors by pathologists [1]. In this regard, many studies done in peripheral neuroblastic tumors have shown that MYCN gene amplification is characterized by the presence of one or more large prominent nucleoli in neuroblastic cells [2, 3]. Regarding function, it is well known that nucleoli manufacture the subunits that combine to form ribosomes; therefore, their size can be a measure of both ribosomal requirements and the physiological state of the cells. In cells synthesizing large amounts of proteins, like in proliferating cancer cells, the size of their nucleolus is considerable and sometimes occupies as much as 25 percent of the total nuclear compartment volume.

We have characterized at the ultrastructural level the morphology, number, and localization of nucleoli in cultured breast cancer cells. MCF7 cells were cultured on IBIDI correlative slides [4] for 3 days, chemically fixed in 4% paraformaldehyde and 0.1% glutaraldehyde in 1X PBS for 1 h at 4°C and stained with DAPI (1µg/mL) for 30 min. at room temperature to evaluate nuclear morphology and nucleoli localization. Cells were screened by optical microscopy, both by bright field and 405nm excitation. The absence of DAPI staining within the nuclei served to reveal the localization of the nucleoli (Fig. 1A-C). Images were acquired on a FEI CorrSight fluorescence microscope using MAPS software at different magnifications. Cells were further processed for 3D FIB-SEM imaging [4]. To confirm that the absence of DAPI staining corresponds to the presence of nucleoli within the nuclear compartment of cells, we generated thin sections (250 nm) from the resulting blocks. These sections were analyzed by scanning electron microscopy (SEM) using backscattered electron (BSE) imaging methods on a FEI Helios Nanolab 660 DualBeam. The absence of DAPI signal, as monitored by fluorescence microscopy (Fig. 2A) provides an indication of nucleoli presence. Over 20 cells were screened to confirm these results. Fig. 2B shows the BSE-SEM image of the plastic section, and Fig. 2C depicts the overlay between the fluorescence and BSE images. BSE-SEM image acquisition and image overlays were performed using MAPS 3.1 software. Subsequently a 3D FIB-SEM dataset was acquired. The bright field and IBIDI grid pattern was used to localize the cells of interest [4]. Fig 3 (A-C) shows the 3D volume acquired at 8 nm voxel resolution.

Our results indicate that there is a clear advantage in initially screening hundreds of cultured cells grown under defined culture conditions (e.g. growth factors, environmental stressors, and exposure to toxin) by fluorescence imaging. This facilitates the reliable selection of those specific cells showing a phenotype of interest, such as the presence of prominent nucleoli, for further examination by 3D SEM methods to

reveal ultrastructural details and cellular contextual information which could otherwise not be obtained.

References:
[1] Derenzini M. *et al*. Histopathology, **54** (2009) p. 753.
[2] Kobayashi C. *et al*. Cancer, **103** (2005) p. 174.
[3] Tornóczky T., *et al.* Pathol Oncol Res., **13** (2007) p. 269.
[4] López C.S. *et al*. Methods Cell Biol., **140** (2017) p. 149.
[5] We thank Dr. Cedric Bouchet-Marquis from Thermo Fisher Scientific for his help with Amira segmentation.
[6] Electron microscopy was performed at the Multiscale Microscopy Core (MMC) with technical support from the OHSU Center for Spatial Systems Biomedicine (OCSSB). This project was also supported by a Pilot Project Grant from the OCSSB to CSL.

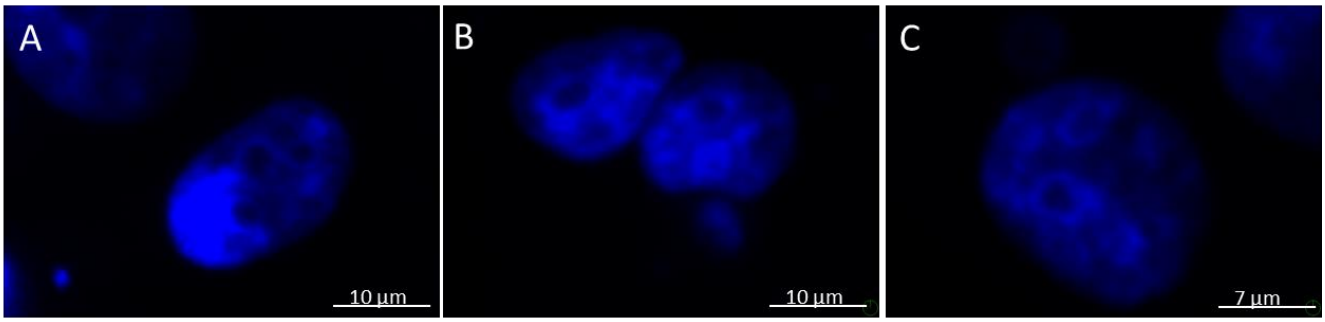

Figure 1. DAPI signal acquired on a FEI CorrSight using MAPS software. **A-C**) representative images are depicted. Fluorescence images were acquired in a single plane.

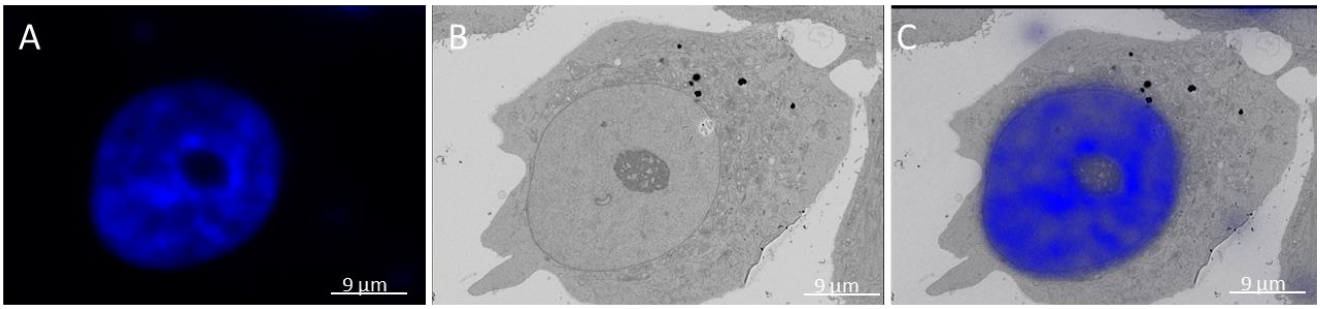

Figure 2. A) DAPI signal on a single cell showing a prominent unstained region within the nucleus. **B)** BSE-SEM image using a dedicated concentric backscatter (CBS) detector and 2.5 keV and 0.2 nA. **C)** Overlay image showing both fluorescence and BSE signals.

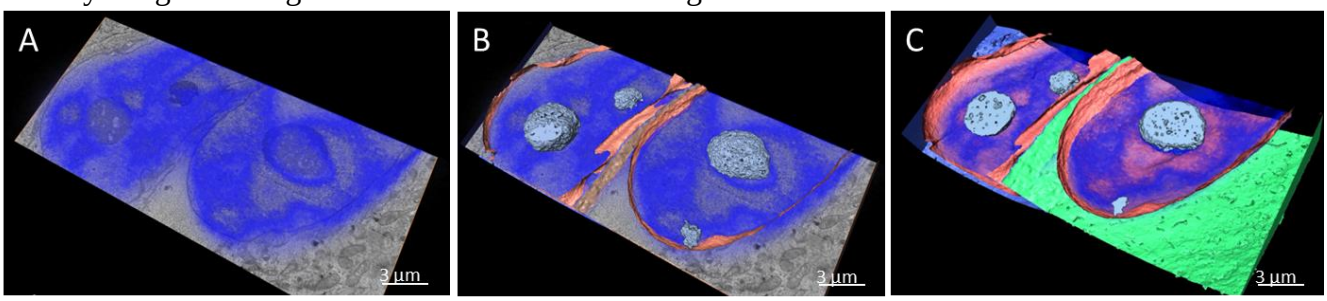

Figure 3. A) Overlay FM and BSE-SEM image displaying the presence of nucleoli in adjacent cells as indicated by the absence of DAPI stain. **B-C)** Volume rendering shows nucleolar components (light blue), nuclear membrane (coral) and plasma membrane (green and blue) of the cells.

https://doi.org/10.1017/S1431927618006591 Published online by Cambridge University Press