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Super Resolution of γH2AX Substructure in Chromatin in GBM cells

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DNA double strand breaks (dsbs) are significant cellular damages that initiate a rapid, coordinated cell signaling cascade called the DNA damage response (DDR). An early event in the DDR is the discontinuous phosphorylation of thousands of histone H2AX molecules upstream and downstream of a DNA dsb, creating microscopic γH2AX foci in the nucleus. Scoring the presence of γH2AX foci is widely used to quantify DNA dsbs and their disappearance measures repair kinetics. However, the actual function of the γH2AX foci remains controversial. Recently, a few studies have revealed novel substructure in the architecture of γH2AX foci and they find that γH2AX foci are composed of numerous elementary structural subunits that have dimensions of 200 nm and these nano-domains are arranged into a larger cluster of nano-domains having a larger diameter of 1 mm. The findings on dimensions of the substructure of γH2AX foci, provide a rationale for the use of the enhanced, super resolution capabilities of a Zeiss LSM 900 with Airyscan 2 given that it achieves resolution of 150 in the lateral dimension and 400 nm in the axial dimension [1]. Moreover, Airyscan 2 technology can also be used to support recent evidence for the nano- γH2AX domains acting to reveal local chromatin decondensation [2]. In this way, this study contributes to new understanding of the sub-structural architecture of γH2AX foci with implications for its function using the advanced super resolution technology introduced in the cutting-edge Zeiss LSM 900 with Airyscan 2 confocal laser scanning microscope.

Human brain cancer, U87 GBM cells were maintained and prepared for immunofluorescence imaging of γH2AX foci as previously described [3] after exposure of the cells to 2 Gy ^{137}Cs γ rays. These irradiated cell samples were then imaged using a 63X 1.4 NA oil immersion objective lens when sampling was optimized for Airyscan so that the calculated size of the image was 14.75 X 15 μm having a pixel sampling of 418 X 425 with a scaling of 35 nm X 35 nm per pixel. z-stack data (0.5 μm) was acquired for each cell nucleus. The super resolution image data was processed and analyzed using Zen Blue 3.3.

Representative images comparing confocal images of gH2AX foci in cell nuclei and super resolution images of γH2AX foci using a Zeiss LSM 900 with Airyscan 2 revealed increased sensitivity to the γH2AX fluorescence signal and increased resolution. The images of elementary structural subunits of γH2AX showed vast improvement over conventional confocal microscopy imaging (Figure 1). Furthermore, the super resolution imaging mode also revealed fine detail of the architecture of γH2AX by detailing the smaller substructures that made up each of γH2AX foci.

The nuclear context of the structural units in γH2AX foci was investigated further using the profile tool in Zen 3.3. Fluorescence intensity data was generated for each pixel along the profile line drawn through a γH2AX cluster containing several nano-domains (Figure 2). In comparison, the fluorescence intensity data for chromatin stained with DAPI was also collected along that line. Knowing that fluorescence intensity of nuclei stained with DNA-specific dyes like DAPI is related not only to the total DNA content but also to the chromatin-DNA structure, we inferred the fluorescence intensity was proportional to chromatin compaction. As can be observed in the plot of the fluorescence intensity data, the line through

the gH2AX foci contained less DAPI fluorescence intensity than the areas outside the foci, supporting previous observations of nano-domains of γH2AX foci localized in areas of lower chromatin compaction than the areas outside the foci.

Herein, we present supportive evidence for novel architectural make-up of γH2AX and describe a new method to probe molecular details of the role of chromatin condensation in an early event in the DDR the cell signaling events triggered by radiation-induced DNA dsbs [4].

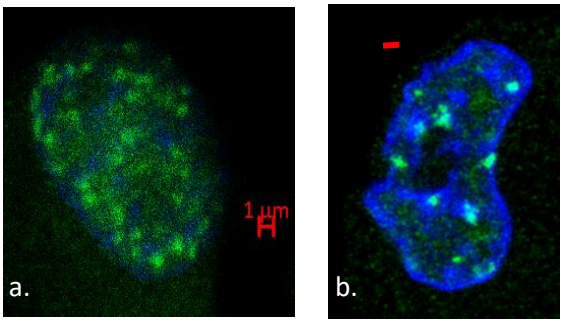

Figure 1. Comparison between confocal (a) and super resolution (b) images of U87 cell nuclei containing gH2AX foci (green). Resolution was visibly increased using super resolution imaging (workflow: the image data was acquired using Airyscan 2 technology underwent Airyscan processing using Zen Blue 3.3). The increased sensitivity of the detectors enabled the collection of the nuclear, blue DAPI fluorescence (b). The micron markers equal 1 mm.

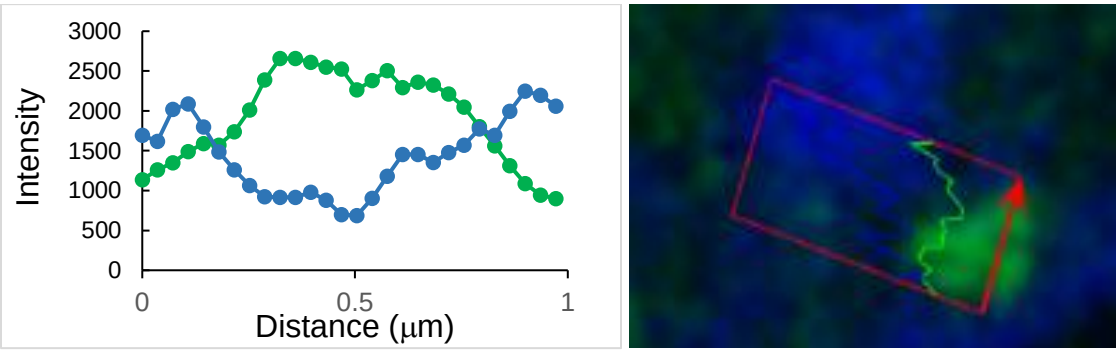

Figure 2. Super resolution nano-domains aggregate together for form a clustered higher-order clustered nano-domain structure shown in the image of 1 gH2AX foci shown the right. The diameter of the cluster equals 1 um. The intensity plot (on the left) shows the intensity of the nano-domain gH2AX structure (green lilne) is in an area of low DAPI staining (blue line) or decreased packaging of DNA compared to the areas around the nano-structure.

References:

[1] S D'Abrantes et al. Radiation Research **189** (2017), p. 19. doi: 10.1667/RR14594.1
[2] F Natale et al. Nature Communications **8** (2017), p. 15760. doi: 10.1038/ncomms15760

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[3] LS Yasui, International Journal of Radiation Biology **80** (2004), p. 895. doi: 10.1080/09553000400017556.
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