Combining quantitative phase microscopy with fluorescent reporters: quantifying the alteration of nuclear structure following radiation damage

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Cell membrane permeabilization alters cell volume, mass, and density

Rationale The successful union of label-free and label-based microscopy requires a quantitative understanding of cellular perturbations arising from cell membrane permeabilization required for intracellular immunolabeling. We quantified the role of membrane permeabilization in altering cellular mass, volume, and density - primary readouts from quantitative microscopy.

Results

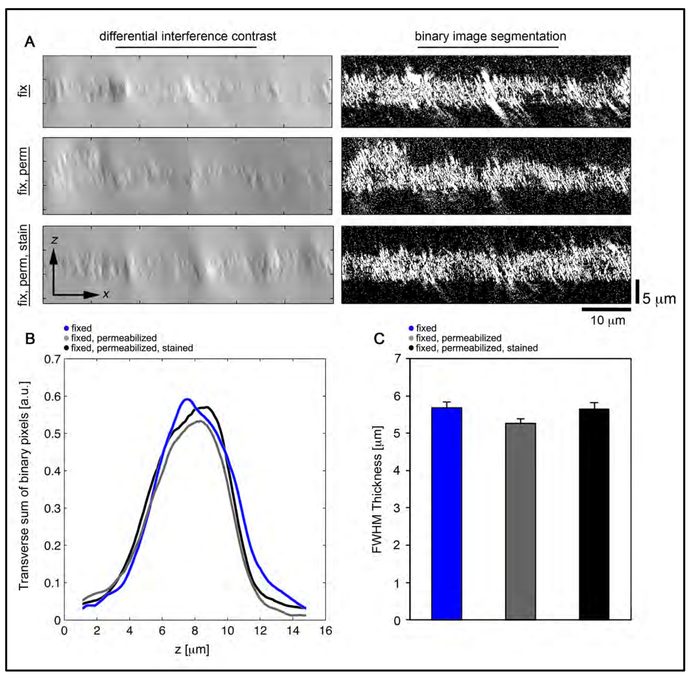

Figure 1 **Cell volume is conserved following cell membrane permeabilization**. (A) Cross sectional DIC imagery, *left column*, and corresponding binary image segmentation, *right column*, of UM-SCC-22A cell monolayers. (B) Transverse sum of binary images, FWHM of these sums reports the mean monolayer thickness of each transverse plane. (C) Quantification of monolayer thickness over 10 fields of view/treatment.

Conclusion Following cell membrane permeabilization with 0.1% Triton X-100, cell dry mass density is reduced by 28%, independent of staining. Visualization of nuclear architecture, regularly obscured by cytoplasmic constituents, is greatly enhanced under differential interference contrast. Cell volume, quantified with femto-liter resolution, is unchanged by permeabilization.

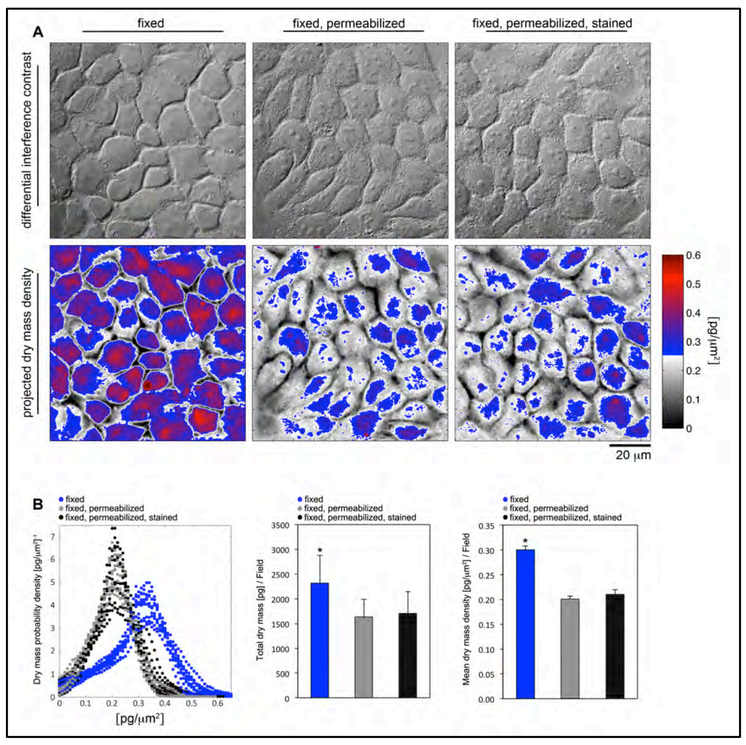

Figure 2 **Cell mass and density are reduced following cell membrane permeabilization**. (A) *En face* DIC imagery, *top row*, and corresponding projected dry mass density maps, *bottom row*, of UM-SCC-22A cell monolayers. (B) Density histograms and corresponding quantification of total mass/field and mean density/field for each treatment group. * $p < 0.05$.

Multiscale characterization of DNA damage repair following x-ray radiation

Rationale Double strand breaks (DSBs) are among the most important lesions in chromosomes resulting from ionizing radiation exposure. Correlation of the nanoscale molecular response of the cell nucleus, mapped via fluorescent reporters, with the organization of cellular density on the micron scale, quantified by QPM, will provide a multiscale understanding of DNA damage repair.

Results

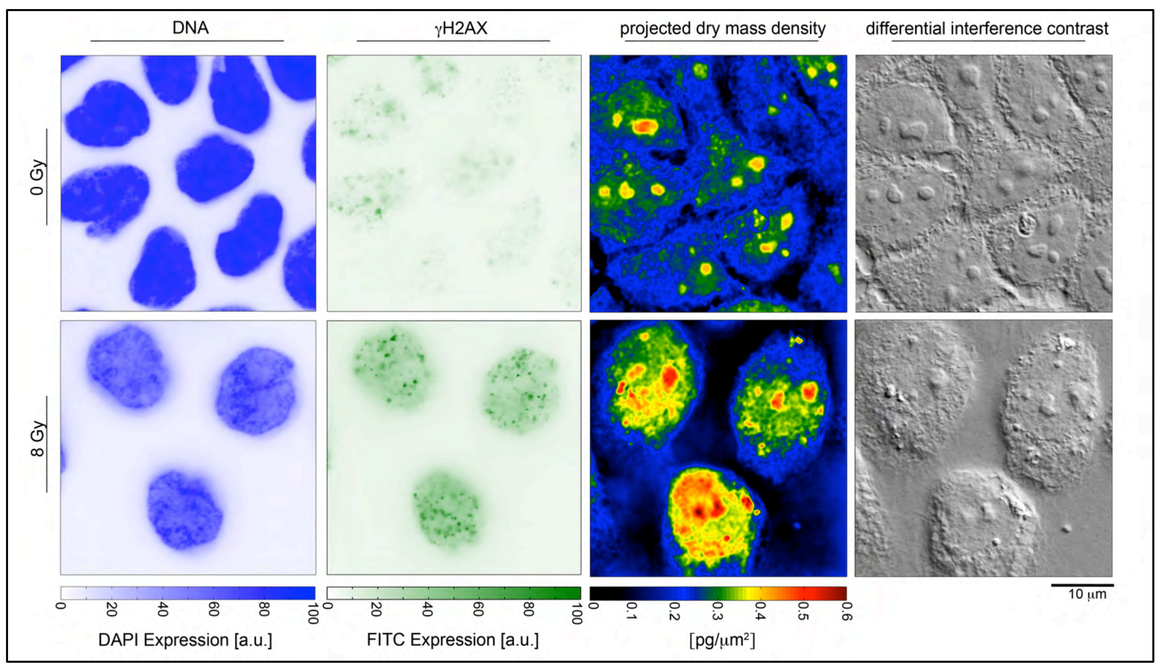

Figure 3 **Label-free and label-based multimodal imaging of UM-SCC-22A cells following 8 Gy x-ray treatment.** (*Left to right*) DNA visualization using DAPI, γH2AX expression visualized with FITC, projected dry mass density map derived from QPM, differential interference contrast imaging. 0 Gy (*top row*), 8 Gy (*bottom row*) treatment groups.

Conclusion The expression of γH2AX foci increased 52% following 8 Gy x-ray treatment while DAPI staining of nuclear DNA revealed condensed chromatin structure. Mean cell density increased across all cellular compartments with the sample skew of the dry mass density distribution increasing in the cytoplasm and nucleus. Together, these results quantify a coordinated multiscale response to damaging x-ray exposure.

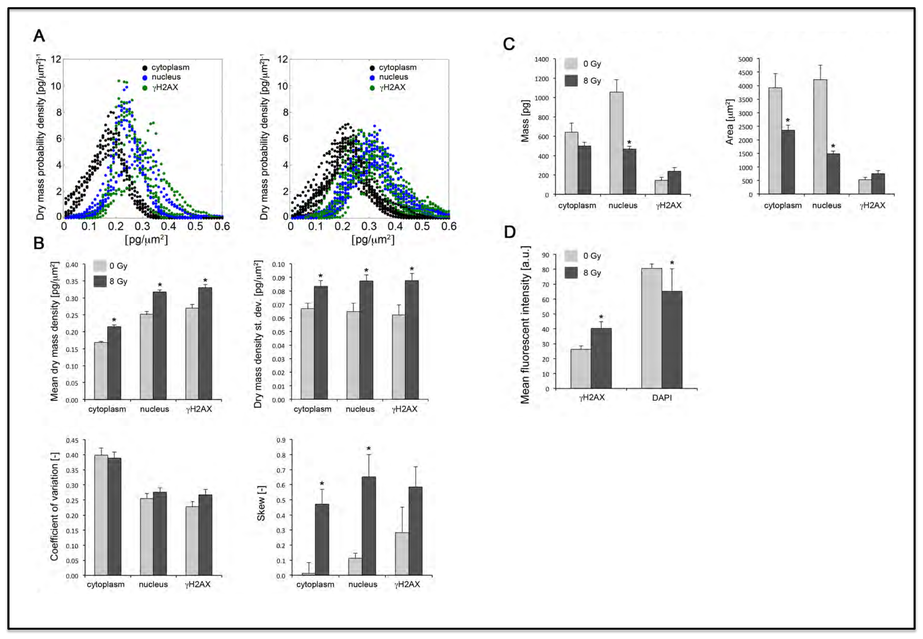

Figure 4 **Quantification of γH2AX, DNA, and cell density following 8 Gy radiation treatment**. (A) Fluorescence-QPM correlated histogram of cell density. (B) Cell density metrics, (C) bulk mass and cell area, (D) quantification of fluorescence expression. * denotes $p < 0.05$ with respect to 0 Gy Tx. Error bars denote SEM (A-C), St. Dev. (D). N = 10 fields/Tx. Each field of size 12507 μm^2.