Quantification of Cell-to-Cell Variations in Target Molecules Identified by Immunofluorescence

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Purpose: Methods to predict the radiation response in tumors are critical for optimization and planning of therapy. An important example clinically is the detection of tumor hypoxia. Surprisingly, such detection in animal tumors has been much less successful in predicting tumor response. It is important to resolve this discrepancy since pre-clinical research rests on the foundation of understanding tumor model response. We hypothesize that a quantitative description of the DNA damage resulting from a radiation dose, plus a similar description of the cellular pO_2, both at cell-to-cell resolution should help to clarify this important issue. Such descriptions require substantial improvements in both the stability of immunofluorescence staining techniques as well as their analysis.

The latter requires the ability to accurately quantify the location and DNA content of individual nuclei (for whole cells) and nuclear fragments (for normal and tumor tissue sections). This has been studied extensively in the past, but the methods we were able to find were not very successful. This in essence requires an accurate nuclear mask that can be segregated into components representing individual cells or cell fragments. This work illustrates our development of a quantitative toolbox for cell and tissue segmentation and analysis to facilitate the radiobiological studies.

Methods: The toolbox was developed under Matlab software environment. To generate the nuclear mask, we focused our attention on the nuclei, as stained by use of Hoechst 33342. The original immunofluorescence images were modified by subtracting background and field flattening. Then, a threshold was determined to isolate the background from the nuclei. They were initially separated with the watershed function. The touching and clumped cells were further assessed using a 2D Laplacian operator for separation. Iterations continue until no further cells could be separated. The pixels related to each cell were collected for mask generation and individual nucleus characteristic analysis including centroid and mean intensity.

Results: The developed toolbox was applied to several series of immunofluorescence images obtained from the same sample, including flooded Hoechst, γH2Ax antibody and EF5 binding antibody images. In each of the 400x600 tiff images, the toolbox robustly and accurately detected ~1500 individual cell nuclei, with automatically generated individual cell mask and characteristics.

Conclusion: A quantitative toolbox and procedure was developed to segment and analyze the radiobiology immunofluorescence of cells (nuclei) and we are now extending this to the more complex case of tissue sections with the goal to automate the assessment of tumor radiation responses.