Microbeam-initiated foci formation

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To demonstrate the impact of the microbeam at RARAF, the ion beam will be used to initiate DNA-damage repair foci and the dynamics (onset and fading) of these foci will be observed in real time.

OBJECTIVE: Irradiate a single cell nucleus using the microbeam to produce prompt and visible proof that the cell was irradiated.

BACKGROUND: The cells for this demonstration are HT1080 human Fibro Sarcoma cell nuclei with green fluorescent protein (GFP) tagged to XRCC1 (single-strand DNA repair protein). As DNA damage occurs in these cell nuclei, XRCC1 repair protein migrate to the damage sites, forming foci, and then dissipate as the repair process nears completion. The process is observed by imaging the distribution of the GFP-tag. Acknowledgement: these HT1080 cells were provided by David Chen, UT Southwestern, Dallas, Texas.

PROCEDURE: The cells will be plated on microbeam dishes and maintained under physiological conditions during the irradiation and imaging phases. Cells will be placed over the predetermined location of a focused 6-MeV alpha-particle beam using the nano-positioning stage then irradiated during a time interval corresponding to a predetermined number of particles. An EMCCD camera and imaging software will gather time-lapse images (movies) in the follow-up observations.

Irradiations:

1. Irradiate one cell nucleus with 200 alpha particles
2. Irradiate one cell nucleus with other particle counts
3. Irradiate a cell nucleolis, expect no response because there is minimal DNA
4. Irradiate a cell nucleus with a pattern

As an example, a single cell nucleus was irradiated in an “NIH” dot-matrix stage-motion pattern with ~200 6-MeV alpha particles per spot. Typical spacing between points was 1 μm. Immediately after the irradiation, a Z-stack of 21 wide-field fluorescent images was acquired using a water-dipping objective (60X 1.0NA), and a step size of $\Delta Z = 0.5$ μm, to reveal GFP concentrations in the nucleus exposed with the “NIH” pattern and in the neighboring cell nuclei. Autoquant software was used to deblur the image.