Huron Technologies

Illuminating Pathology™

Chromosome Biodosimetry for Mass Radiation Emergencies

Introduction

Ionizing radiation produces immediate, characteristic chromosome changes. Chromosome biodosimetry studies are a definitive test for occupational radiation exposure, other mass radiation emergency situations, or monitoring long term effects such as cancer. A major change results in chromosomes containing 2 constrictions, termed centromeres, instead of one (dicentric chromosomes [DCs], Figure 1). The analysis requires special expertise and microscopy equipment, is labor intensive and slow. In an emergency response to a mass casualty with exposure to a wide, unknown range of doses, biodosimetry labs will have an acute need for more methods that accurately and rapidly identify DCs within a few days. This proposal will streamline data acquisition using a revolutionary wide-field microscope system developed by Huron Technologies which will feed patent-pending image analysis software developed by Western researchers to automatically interpret DCs. Huron will engineer its system to capture images 10-30 fold more quickly than other existing systems and Western will validate its performance with samples prepared in our laboratory. The goal of this project is to significantly accelerate timely radiation dose estimation, preferably within 48 hrs of exposure or patient ascertainment, whichever occurs first.

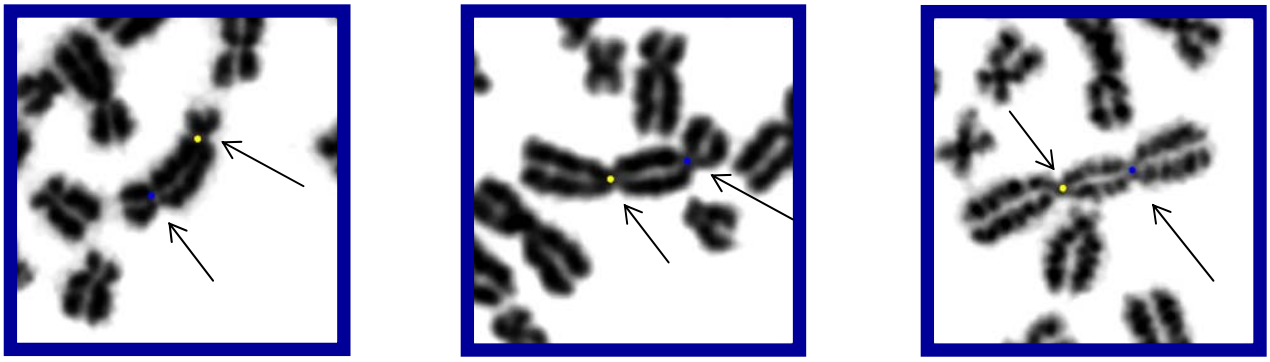

Figure 1. Dicentric chromosomes

Imaging Technology

The project will carry out proof-of-principal engineering and experimental validation of a wide-field epifluorescence microscope with high power magnification capabilities (≥63X objective, 1.4NA). The current wide-field TISSUEscope™ 4000 system offered by Huron Technologies based on the patented MACROscope technology does not have the resolution to detect dicentric chromosomes, a prerequisite for automated

biodosimetry to measure radiation exposures. Huron will modify this existing system to meeting the need to generate data more quickly. Higher data throughput will maximize the benefits of the patent pending image analysis software that Western researchers are developing.

TISSUEscope™ 4000 is a third-generation fluorescence and brightfield scanner that was specifically designed for highly sensitive fluorescence imaging of up to 4 fluorophores simultaneously. The laser scan lens used in this instrument has a 20X equivalent resolution with a viewing area that is at least 10 times that of equivalent microscope objectives. This is a major advantage in whole-slide imaging, with much less potential for stitching artifacts even when imaging very large specimens. Weak and strong fluorophores can be imaged in the same scan, using adjustable gain for each channel with large dynamic range (12 bit) fluorescence detection. Figure 2 (top) shows a DAPI fluorescence image of a biodosimetry slide imaged with the TISSUEscope™ at 10X equivalent resolution. The scan area is 22mm x 46.2mm. Figure 2 (bottom) shows a 100% zoom area of the 10X image where several ROIs of interest are highlighted in green circles.

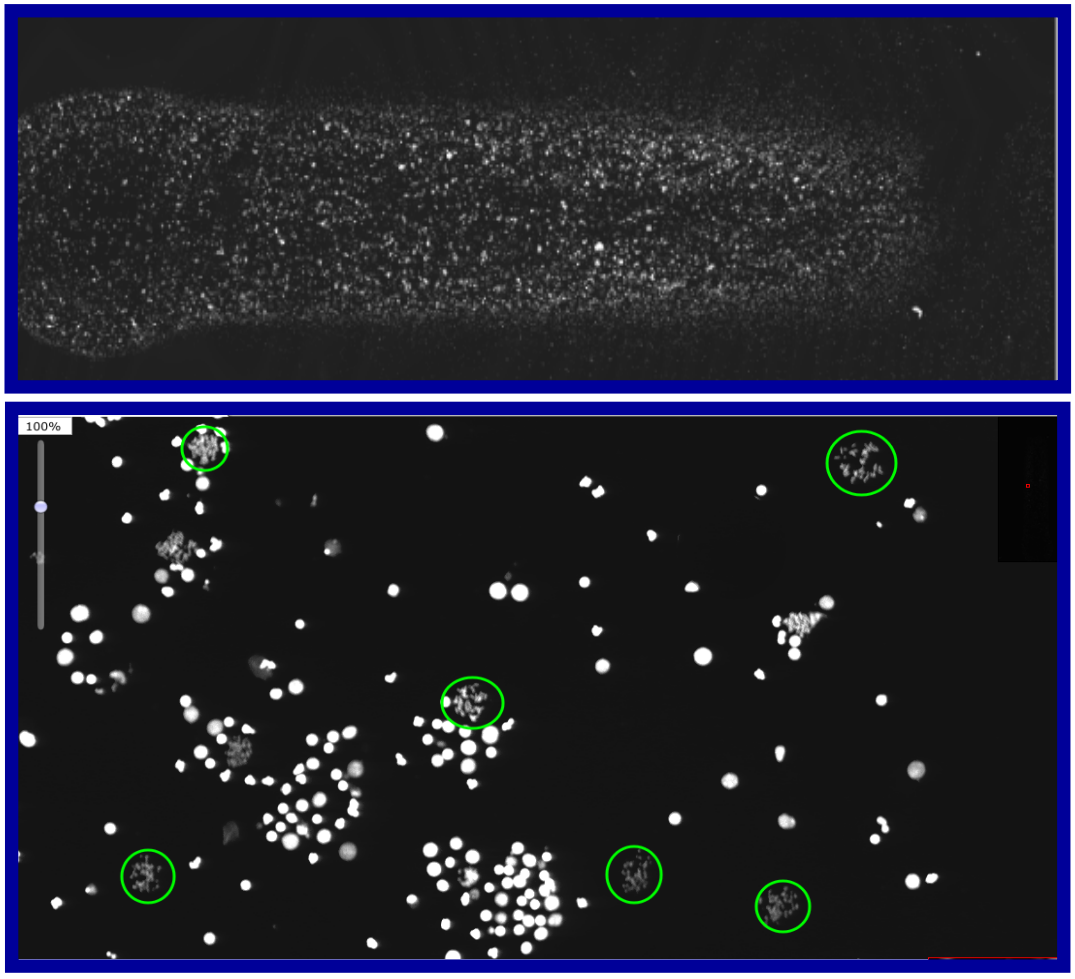

Figure 2. (Top) 10X DAPI image of a biodosimetry slide; (Bottom) 10X image zoomed at 100% showing an area with several ROIs of metaphase cells.

The ROIs once identified from the 10X image are imaged at 63X with a 1.4NA oil objective to clearly identify the chromosomes and using image analysis software developed by Western researchers to automatically interpret DCs. In Figure 3 images of two ROIs acquired with a microscope at 63X, at Western, showing dicentric chromosomes.

In the event of a mass casualty radiation incident, expedited preparation of samples, microscope hardware acceleration, and automated interpretation of the resulting chromosome images will be necessary to handle to overwhelming demand for biodosimetry testing. Huron's intention, in close collaboration with Western university, is to develop a high speed hybrid imaging system that uses the MACROscope scanning technology and recently patented imaging technology to build a hybrid imaging system to automatically image and interpret (by implementing Western's image analysis algorithms) chromosome images. Western is focusing on expediting preparation of samples and microscope hardware acceleration, and integration of these elements with automated software to interpretation of dicentric chromosome images, the latter which is currently supported by pilot funding from NIH. Western and Huron collaboration is partially funded by FedDev ARC.

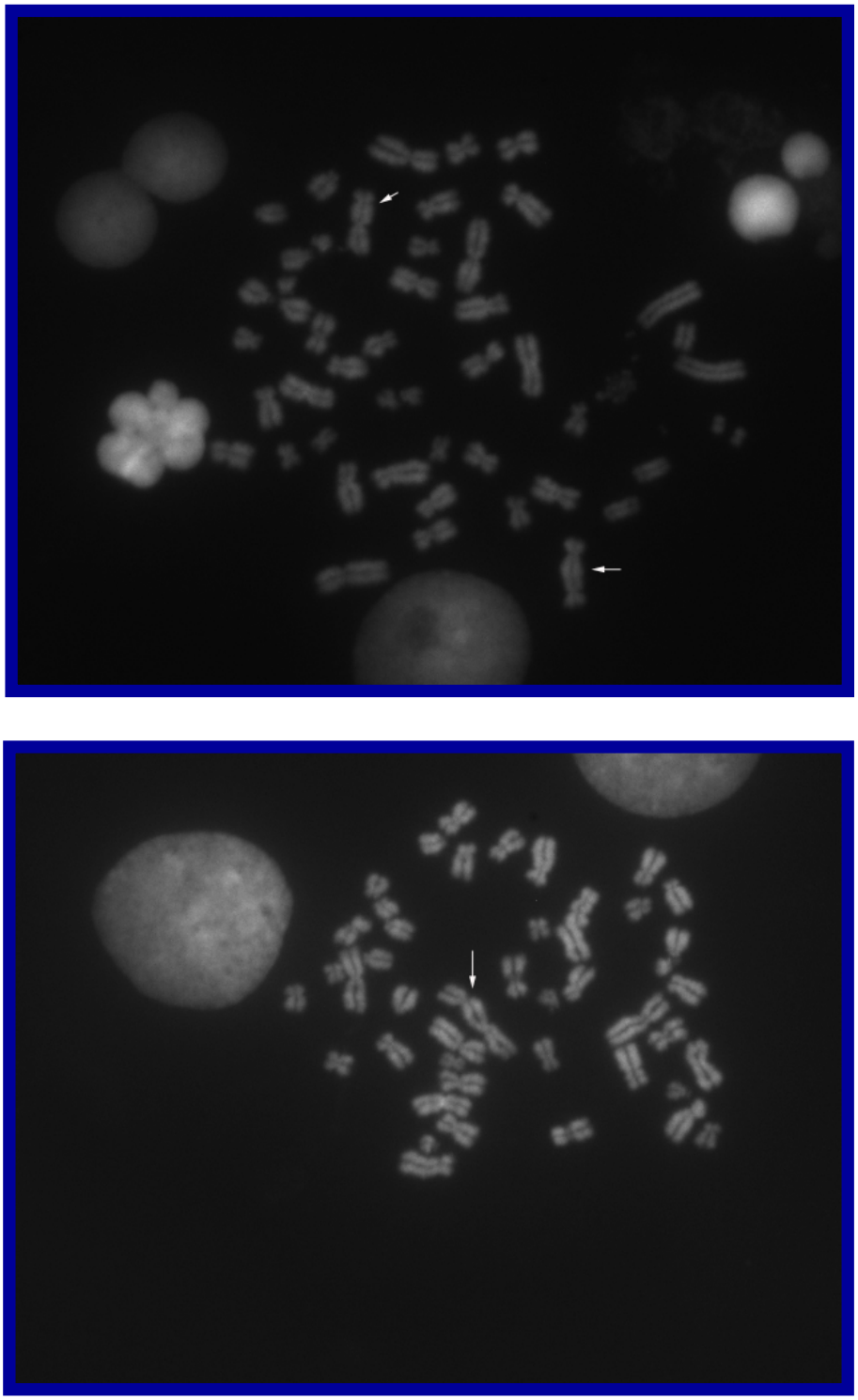

Figure 3. Microscope images at 63X of cells with dicentric chromosomes

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