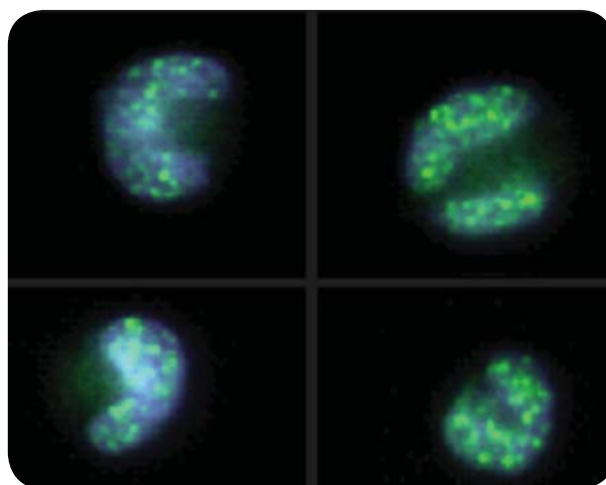


Quantitation of γ -H2AX spots on the ImageStream

IMAGESTREAM CAPABILITIES FEATURED: Internalization / Cell Signaling & Molecular Translocation / Cell-Cell Interaction / Morphology / Cell Cycle & Mitosis / Co-localization / **Spot Counting** / DNA Damage & Repair / Cell Death & Autophagy / Immunology / **Oncology** / Biochemistry / Virology / Microbiology / Parasitology / Hematology / Stem Cell Biology / Oceanography / Toxicology / Drug Discovery /

ABSTRACT

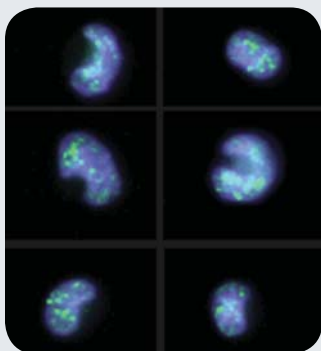
Failure to repair biologic and environmental DNA damage, particularly double strand breaks (DSB), results in mutations and chromosomal aberrations that can lead to cellular dysfunction and death, genomic instability, and carcinogenesis. Radiation-induced DSB induce phosphorylation of histone H2AX to γ -H2AX and subsequent focal recruitment of several factors, such as 53BP1 and MDC, that coordinate the repair process. Immunostaining for these factors thus allows for identification and localization of DSB foci that can be quantitatively analyzed using ImageStream cytometry. In this report we show ImageStream methods for quantifying DSB foci using automated γ -H2AX and 53BP1 spot counting, both in tumor cell lines and in primary murine CD8+ T cells. We also show quantification of MDC and γ -H2AX co-localization at DSB foci following irradiation.



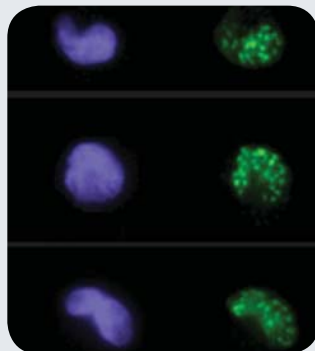
Composite imagery of DAPI nuclear dye (violet) and Alexa Fluor 488-525 53BP1 (green). H1299 human lung carcinoma tissue culture cells.

Study Highlights

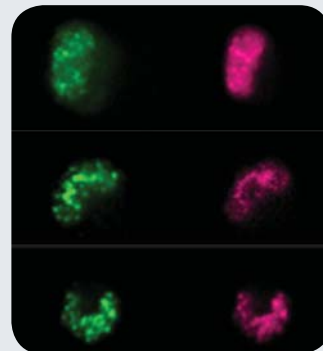
Phospho-53BP1 (S25) Spot Counting



γ -H2AX Spot Counting



MDC1 Spot Count & γ -H2AX Co-localization

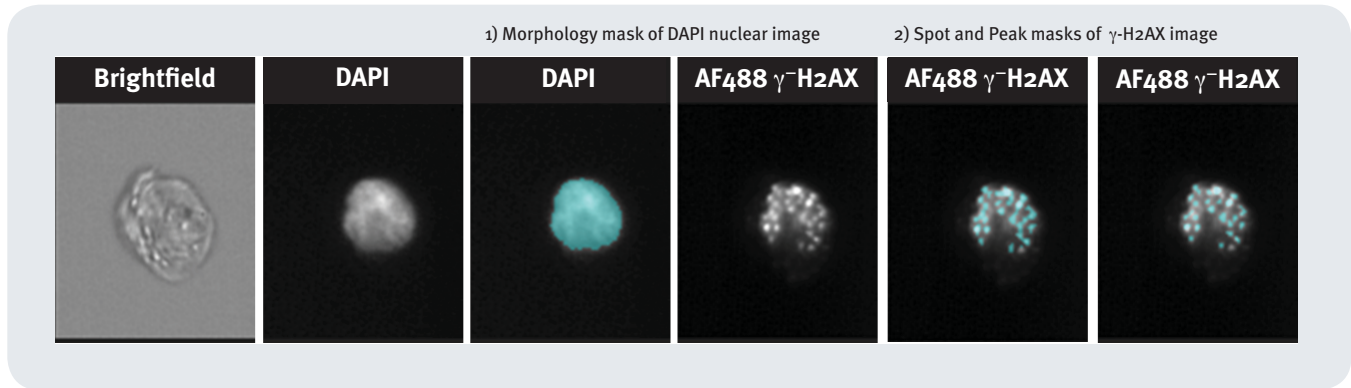


QUANTITATION OF NUCLEAR γ -H2AX SPOTS

Robert Bristow, Shane Harding, and Farid Jalali, Princess Margaret Hospital

To determine the number of γ -H2AX foci in each H1299 human lung carcinoma cell, masks were created which identify the region of interest: in this case Alexa Fluor 488 intensity peaks at least 4 fold greater than

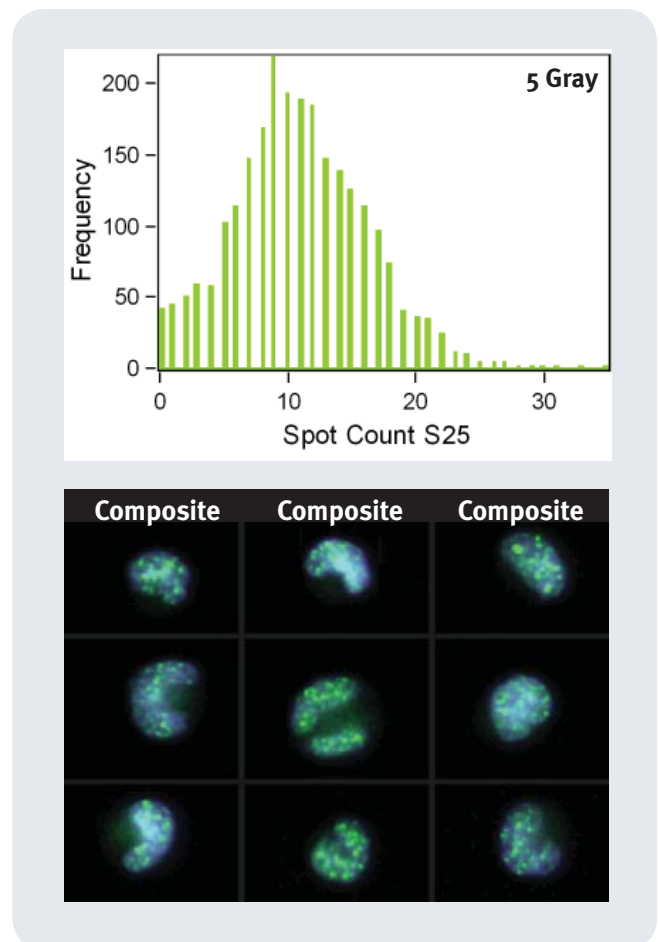
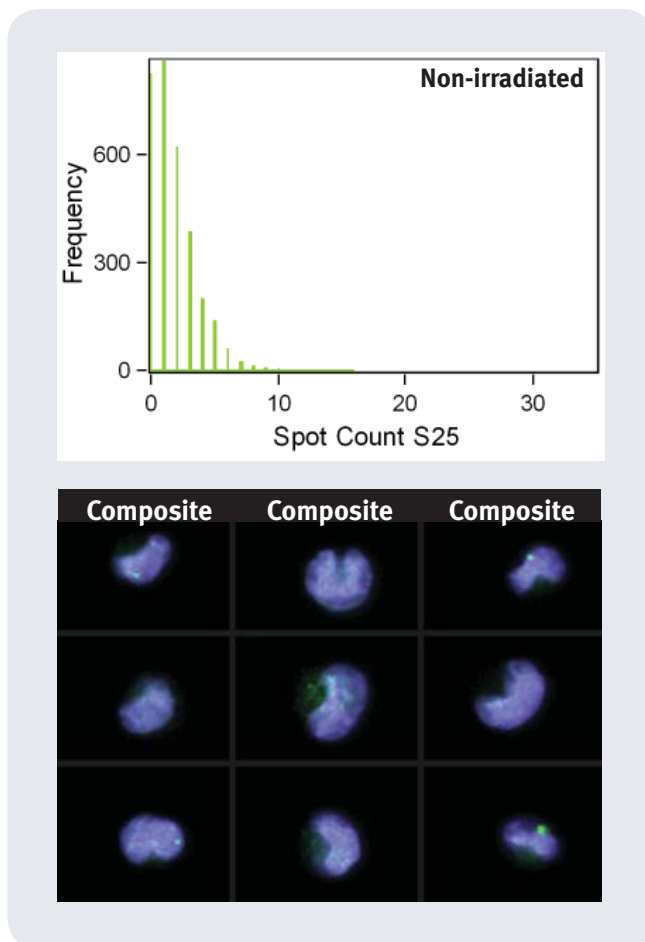
the background with a diameter of 2 to 9 pixels. The number of individual masks in a cell was enumerated using the Spot Count feature. Spot Count is then plotted for every cell in histograms as shown below.



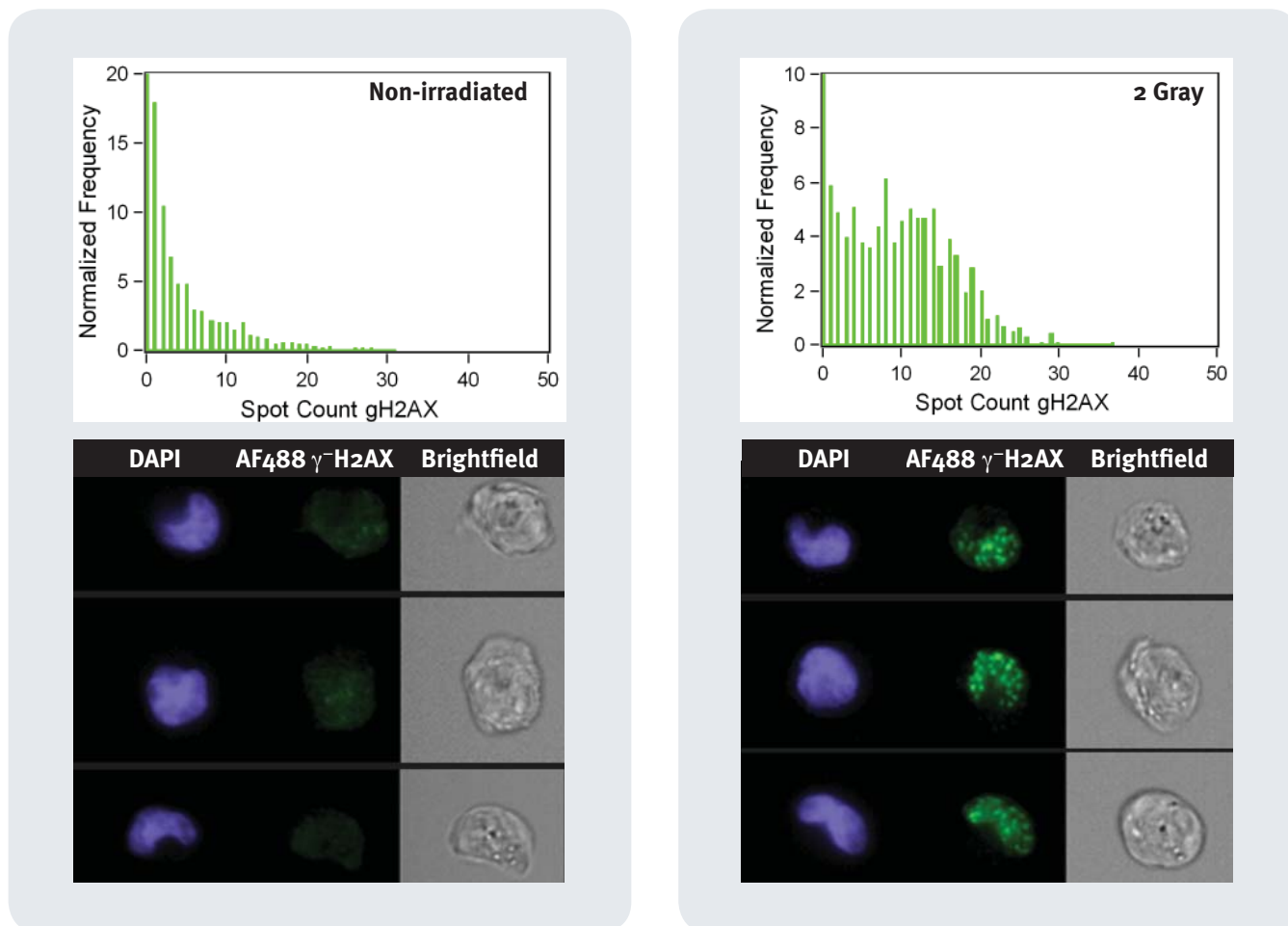
QUANTITATION OF PHOSPHO-53BP1 (S25) FOCI IN A HUMAN LUNG CARCINOMA CELL LINE

Foci were enumerated for S25 phosphorylated 53BP1 (S25), a mediator of the DNA damage response. S25 is

labeled with Alexa Fluor 488 (green) while the DAPI nuclear image is shown in violet.

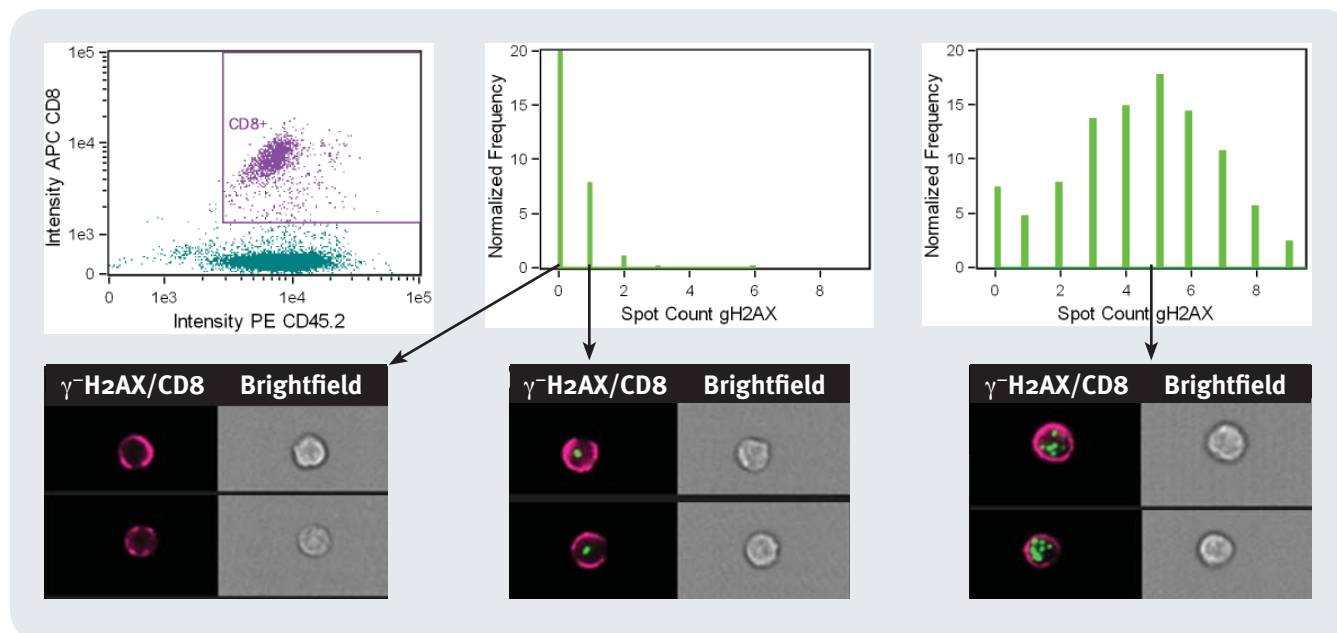


COMPARISON OF γ -H2AX SPOT COUNT IN NON-IRRADIATED AND IRRADIATED SAMPLES



COMBINED IMMUNOPHENOTYPING & γ -H2AX SPOT COUNT IN PRIMARY CELLS

Martin Prlic, University of Washington

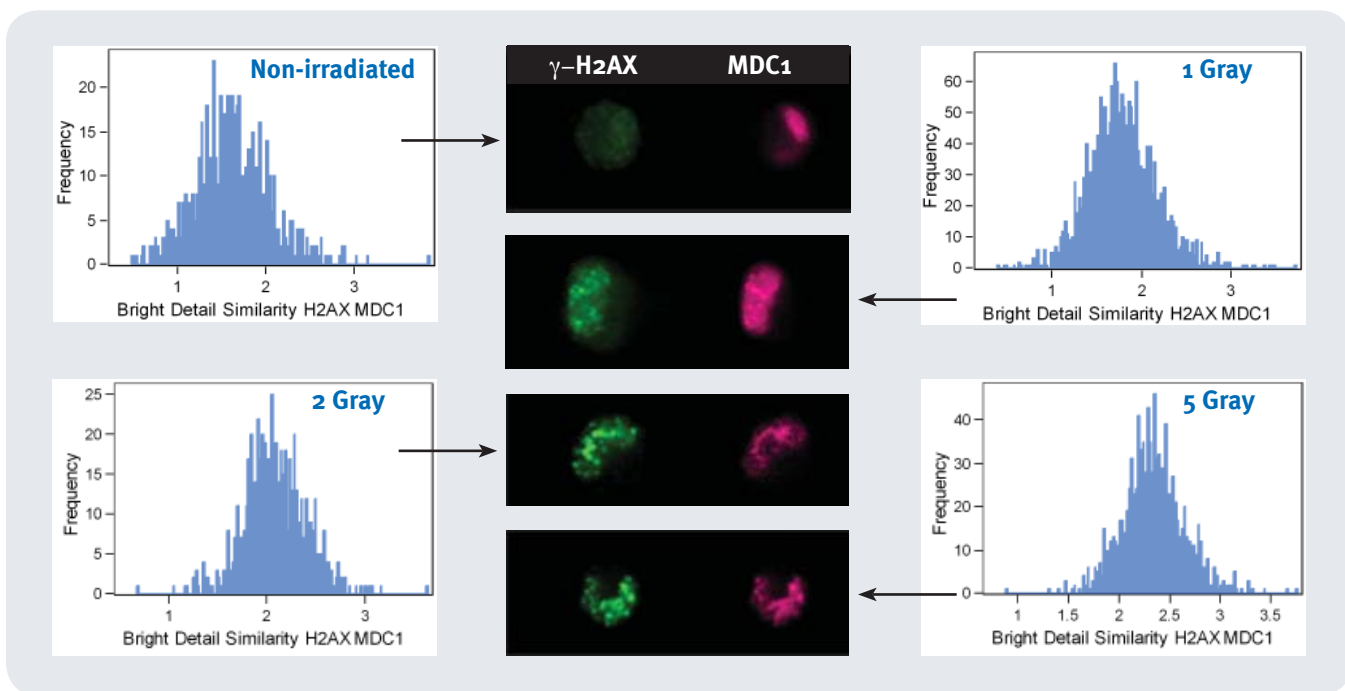


MEASUREMENT OF MDC1 CO-LOCALIZATION WITH γ -H2AX

Robert Bristow, Shane Harding, and Farid Jalali, Princess Margaret Hospital

Here we show quantitation of MDC1- γ -H2AX co-localization in H1299 lung carcinoma cell line following irradiation. Co-localization is measured using the Bright Detail Similarity feature which correlates the bright regions of the MDC1/H2AX image pair for

each cell. Those cells with co-localized molecules will have MDC1 and H2AX images that look alike, and consequently will have high Bright Detail Similarity values.



ImageStream^x Specifications



EXCITATION SOURCES

LASER (NM)	EXAMPLE DYES
405	DAPI, Pacific Blue™
488	FITC, PE, ECD, PE-Cy5
560	Alexa Fluor® 546, Cy3
592	Texas Red®, Alexa Fluor® 594
658	Cy5, Alexa Fluor® 647, APC, APC-Cy-7

IMAGING PERFORMANCE

Magnification	20X	40X	60X
Numeric Aperture	0.5	0.75	0.9
Field of View (μ m)	120 x 512	60 x 256	40 x 170
Imaging Rate (cells/sec)	2,000	1,000	600

INSTRUMENT CAPABILITIES

Images per Cell	Up to 12
Imaging Modes	Brightfield, SSC, and fluorescent
Sample Throughput	1 sample/min nominal
Automated Processes	Startup, shutdown, and self-calibration

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