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Optimization and validation of automated dicentric chromosome analysis for radiological/nuclear triage applications

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ABSTRACT

Dicentric Chromosome Assay (DCA) is the most preferred cytogenetic technique for absorbed radiation dose assessment in exposed humans. However, DCA is somewhat impractical for triage application owing to its labor intensive and time consuming nature. Although lymphocyte culture for 48 h *in vitro* is inevitable for DCA, manual scoring of dicentric chromosomes (DCs) requires an additional time of 24–48 h, making the overall turnaround time of 72–96 h for dose estimation. To accelerate the speed of DC analysis for dose estimation, an automated tool was optimized and validated for triage mode of scoring. Several image training files were created to improve the specificity of automated DC analysis algorithm. Accuracy and efficiency of the automated (unsupervised) DC scoring was compared with the semi-automated scoring that involved human verification and correction of DCs (elimination of false positives and inclusion of true positives). DC scoring was performed by both automated and semi-automated modes for different doses of X-rays and γ-rays (0 Gy–5 Gy). Biodoses estimated from the frequencies of DCs detected by both automated (unsupervised) and semi-automated (supervised) scoring modes were grossly similar to the actual delivered doses in the range of 0.5 to 3 Gy of low LET radiation. We suggest that the automated DC tool can be effectively used for large scale radiological/nuclear incidents where a rapid segregation is essential for prioritizing moderately or severely exposed humans to receive appropriate medical countermeasures.

1. Introduction

Ionizing radiation (IR) is a well-known carcinogen and exposure to IR generates DNA single strand breaks, double strand breaks, base damage and DNA-protein crosslinks. Among these lesions, DNA double strand break (DSB) is the most lethal lesion and mis-rejoining of DSBs results in the formation of stable and unstable chromosomal aberrations. Therefore, analysis of chromosomal aberrations can be a powerful tool not only for monitoring the extent of chromosomal damage but also for predicting some of the stochastic health risks such as cancer. Dicentric chromosome (DC), a type of unstable chromosomal aberrations, is formed in cells after IR exposure in a radiation dose dependent manner. Bender and Gooch [1] first demonstrated the utility of DCs for estimating the absorbed radiation dose in a few exposed people during the Recuplex criticality accident in Hanford, WA, USA. Since then, Dicentric Chromosome Assay (DCA) has become the method of choice for radiation dose estimation and its utility as a radiation biodosimeter has been well documented by several studies performed on the victims of large scale accidents such as Chernobyl [2–4], Goiania [5–7], and Fukushima-Daiichi [8–10]. DCA is considered to be the "gold standard" for dose estimation because the baseline frequency of DCs in humans is extremely low (1–2 dicentrics per 1000 metaphase cells) without any bias for gender and age. Further, DC formation is fairly specific to ionizing radiation with a clear dependence on dose, dose-rate and radiation quality.

Performance of DCA is laborious and time consuming with a turnaround time of 72–96 h for dose estimation. These attributes make DCA somewhat unsuitable for radiological/nuclear triage where several hundreds and thousands of people may require biodosimetry in a timely manner for any clinical/medical intervention. Automation has been developed for some of the procedures to make DCA suitable for radiological/nuclear triage application: (I) lymphocyte culture and harvesting, (II) chromosome preparation, (III) metaphase chromosome image capture and analysis. There is hardly any room for minimizing

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the culture time as human lymphocytes need to be stimulated/grown for 48 h *in vitro* for obtaining first division metaphases. However, automation of procedures for lymphocyte harvesting, chromosome preparation and DC analysis can reduce the turnaround time considerably for biodosimetry.

Manual DC analysis takes an enormous amount of time and an experienced scorer may take anywhere between 6–8 h for analyzing 500 metaphase cells. In case of radiological/nuclear mass casualty incidents, manual scoring of large number of samples is likely to create a bottleneck and therefore automated DC scoring is desirable. Several efforts have been made to develop an automated DC scoring tool for large scale radiation accidents. Using the "semi-automated" DC scoring (where automatically detected dicentric chromosomes are verified and validated by human scorers), Romm et al. [11] reported that the time required for scoring 150 metaphase cells was only 2 min relative to 60 min for complete manual scoring of 50 metaphases. One of the major advantages of semi-automated scoring over manual scoring is that it enables a rapid analysis of a large number of cells which can alleviate some of the statistical uncertainties in radiation dose estimation. Oestreicher et al. [12] recently utilized the automated DC scoring to analyze the radiation sensitivity of young children after *ex vivo* exposure of lymphocytes to low radiation doses (41 mGy and 978 mGy). As chromosome image quality is critical for automated DC detection, Rogan and his colleagues have developed improvements in image segmentation methods for selecting high quality metaphase chromosome spreads for an expedited radiation biodosimetry application [13–16]. All the published reports [11–17] have unequivocally established that the automated dicentric chromosome scoring certainly enhances the rapidity of radiation dose assessment but accuracy of dose prediction needs further optimization. In this study, we attempted to optimize and validate automated DC scoring under supervised and unsupervised conditions for reliability and reproducibility of radiation dose assessment for radiological/nuclear triage scenarios.

2. Materials and methods

2.1. Sample collection and irradiation

Human whole blood samples (~10 ml) were collected from 6 healthy volunteers and the blood collection was performed with the written consent of donors in compliance with the Institutional Review Board (IRB) protocol (ORAU 000,349). Aliquots of 1 ml of samples were either irradiated with X-rays (0 Gy–5 Gy) or γ-rays. X-rays irradiation was performed at the University of Tennessee, Knoxville (RS-2000, Atlanta, GA, USA; Dose rate 2 Gy/min) while γ-rays (Atomic Energy of Canada, Ottawa, CA; Co-60; dose rate 1.2 Gy/min) irradiation was performed at the Yale University Medical Center, New Haven, CT.

2.2. Dicentric chromosome assay (DCA)

All the tissue culture reagents used in this study were purchased from Invitrogen, Carlsbad, CA, USA. DCA was performed essentially as described before [18–20]. Briefly, 0.5 ml aliquots of mock and irradiated samples were mixed with 9.5 ml of complete growth medium (PBMAX, GIBCO). To promote an optimal growth of lymphocytes, an additional 1 ml of Phytohaemagglutinin (PHA) was added for every 100 ml of PBMAX. Bromodeoxyuridine (BrdU; Sigma-Aldrich, St. Louis, MO, USA) was added to the cultures at a final concentration of 10 μM to identify first division metaphases. The cultures were incubated at 37 °C for 48 h in a 5% CO_2 incubator. Colcemid (0.1 μg/ml) was added for the last 4 h and the cultures were harvested using a standard procedure. Cells were treated with 0.56% KCl (Fisher Scientific, Fair Lawn, NJ, USA) for 18 min at 37 °C and fixed in three changes of fixative (acetic acid: methanol 1:3; Fisher Scientific, Fair Lawn, NJ, USA). An aliquot of fixed cell suspension (30–40 μl) was placed at the center of a glass slide, air dried and subsequently stained with 5% Giemsa in a buffered solution (EMD Millipore Corporation, Billerica, MA, USA).

2.3. Metaphase chromosome image capture and analysis

Metaphase chromosome spreads initially detected by 10X objective lens using the metaphase finder algorithm of Metafer (MetaSystems, Boston, MA, USA) were subsequently captured with an immersion oil lens objective (63X). The captured images were subjected to the DCScore algorithm for the automated DC detection. For optimizing the automated DC detection for triage mode (analysis of 50 metaphase cells or 30 DCs), both chromosome quality and metaphase cell number were determined by testing various sensitivities for metaphase cell selection. For optimizing the triage mode of automated dicentric chromosome analysis, region of interest was chosen at the center covering 50% of the slide. Automated DC scoring accuracy was optimized for both supervised (correction of dicentric chromosome frequency through manual verification) and unsupervised conditions. Automated DC analysis was performed without metaphase selection and dicentric chromosome correction. Calibration (radiation dose response) curves generated for X-rays and γ-rays in our laboratory were utilized to compare the physical doses (actual dose delivered) with the biodoses (detected by dicentric chromosome frequency). In addition to the accuracy and efficiency, turnaround time for radiation dose assessment was also evaluated between manual and automated DC scoring modes.

2.4. Evaluation of metaphase spreads selected for automated DC detection and analysis

Metaphase selection based on chromosome quality is absolutely critical for a reliable and accurate estimation of radiation dose. According to DC scoring criteria set by the International Atomic Energy Agency, Vienna, Austria (IAEA manual on Cytogenetic Dosimetry:Applications in Preparedness for and Response to Radiation emergencies, 2011), metaphase cells with less than 46 centromeres or overlapping chromosomes masking the centromeric regions have to be excluded from analysis. Also, anaphase cells with well separated sister chromatids are excluded from analysis. Inclusion of these "unsuitable" metaphase cells is likely to result in an erroneous dose estimation. To verify the efficiency and consistency of metaphase cell selection by Metafer, manual evaluation was performed on Metafer selected metaphase spreads for their suitability for automated DC analysis. Slides prepared from X-rays treated (1 Gy, 2 Gy and 3 Gy) human lymphocytes were utilized to get a snapshot of how many metaphase cells were either selected or rejected by Metafer relative to manual analysis. Since our intention was to utilize the automated mode for triage scoring (50 metaphase cells or 30 dicentrics), analysis was restricted to ~100 cells per radiation dose. Manual verification revealed that 47–67% of the cells analyzed by Metafer automated mode fulfilled the IAEA criteria for metaphase selection for DC analysis (85 cells out of 126 cells for 1 Gy; 61 cells out of 130 cells for 2 Gy and 69 cells out of 121 cells for 3 Gy).

2.5. Construction of X-rays and γ-rays calibration curves for automated dicentric scoring

Calibration curves for X-rays and γ-rays were constructed by both semi-automated and automated DC scoring modes using the **C**hromosome **AB**erration c**A**lculation **S**oftware (CABAS). A minimum of 100–200 metaphase cells were used for each radiation dose for constructing the calibration curves. Standard calibration curves for X-rays and γ-rays generated by semi-automated scoring were utilized to estimate the accuracy and efficiency of radiation dose prediction by automated and semi-automated scoring modes for triage.

2.6. Statistical analysis

A paired *t*-test analysis was performed for statistical significance and

Table 1
Timeline for single metaphase cell image scanning, capture and dicentric chromosome analysis.

	Manual	Automated
Metaphase finding (10X objective lens)	10–15 s	~0.4 s
Analysis (63X objective lens)	Capture/ analysis time	
Cell with no dicentrics	30 s	8.5 ± 0.1 s
Cell with 1–2 dicentrics	35 s	8.5 ± 0.1 s
Cell with 2–4 dicentrics	45 s	8.5 ± 0.1 s
Cell with 6–8 dicentrics	60 s	8.5 ± 0.1 s
Cell with > 8 dicentrics	75 s	8.5 ± 0.1 s
Average time for a single metaphase	49 s	8.5 ± 0.1 s
Average time for sample analysis of 100 individuals for triage (50 cells/individual case)	68.05 h	12.35 h

a p value of < 0.05 was considered to be significant.

3. Results

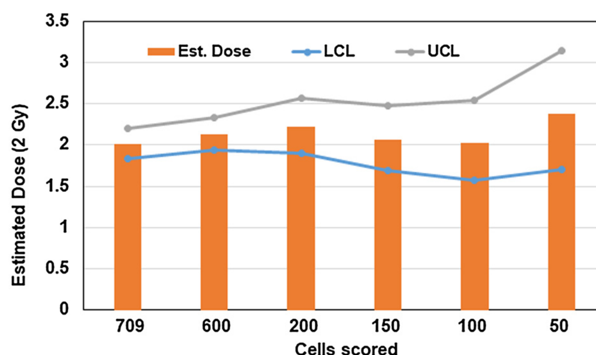
3.1. Automated DC scoring substantially reduces the analysis time

We utilized the metaphase chromosome finder and DCScore algorithm of MetaSystems to compare the analysis time required for manual and automated DC scoring modes. Analysis time was estimated for automated (unsupervised) detection of DCs without considering DC detection accuracy. Table 1 shows the time line for analysis of a single metaphase cell with varying number of dicentric chromosomes by both manual and automated scoring modes. The analysis time required for manual scoring of a single metaphase cell ranged from 30 to 75 s depending on the number of DCs in a cell. In contrast, time for automated analysis of a single cell was estimated to be around 8.5 s including image capture (Table 1). We also estimated the analysis time required for scoring the samples of 100 exposed individuals in a triage scenario by both manual and automated modes and found that the turnaround time was reduced by 4–5 folds by the automated scoring compared to manual scoring (Table 1).

3.2. Optimization and validation of automated DC detection for triage

To optimize the metaphase cell selection at 10X objective, several image files were generated for machine training to improve the specificity of DCScore algorithm. Additionally, different levels of detection sensitivities (6–10) as well as scanning speed (low, medium and high) for metaphase cells were tried to evaluate the quality of metaphase cells selected. An inverse correlation was found between metaphase quality and sensitivity level of detection. Sensitivity level of 8 was found optimal for metaphase cell selection. We next determined the minimum number of cells required for an optimal triage mode of automated DC scoring. Automated DC scoring on varying number of 2 Gy X-rays treated cells indicated that 100–200 cells were sufficient for a reliable radiation dose assessment. However, increasing the number of cells for analysis considerably improved the dose prediction with a narrow range of lower (LCL) and upper (UCL) confidence limits at 95% (Fig. 1).

Accuracy of automated DC detection was evaluated by subjecting the metaphase cells captured at different levels of sensitivity (6, 7, 7.5 and 8). Automated DC analysis was performed on a 2 Gy X-rays irradiated sample as the cut off dose for triage is considered to be 2 Gy for low LET radiation. For reliable comparison, images of ~200 metaphase cells were captured from the same slide at different sensitivity levels by setting the maximum cell count at 200. The number of total metaphase cells captured at different sensitivity levels ranged from 184 to 195 (Table 2). For the actual delivered dose of 2 Gy, radiation doses estimated by the automated (unsupervised) DCScore were 1.63 Gy, 2.03 Gy, 1.73 Gy and 2.05 Gy for sensitivity levels of 6, 7, 7.5 and 8



Cells scored	Cell selected	Cells Rejected	No. of Dicentrics	LCL 95%CI	Estd. Dose (Gy)	UCL 95%CI
709	582	127	226	1.8374	2.0133	2.1966
600	518	82	219	1.9439	2.1313	2.3266
200	180	20	81	1.9034	2.2216	2.5613
150	129	21	52	1.692	2.0645	2.4692
100	84	16	33	1.5699	2.0291	2.5387
50	40	10	20	1.709	2.3811	3.1448

Delivered dose: 2 Gy of X-rays

Fig. 1. Analysis of accuracy and efficiency of automated DC scoring for absorbed radiation dose prediction as a function of cell number. Increasing the cell number dramatically reduced the lower and upper confidence limits (LCL and UCL) of the predicted dose estimated at 95%.

respectively. The radiation doses estimated by the CABAS software at lower and upper confidence limits (LCL and UCL) at 95% using our standard X-rays calibration curve are shown in Table 2. Upon manual verification of the automated dicentric detection method (semi-automated), the estimated doses were 1.81 Gy, 2.27 Gy, 1.91 Gy and 2.08 Gy for sensitivity levels of 6, 7, 7.5 and 8 respectively. The estimated doses were highly similar between automated (2.05 Gy) and semi-automated (2.08 Gy) modes at the sensitivity level of 8 and the number of DCs detected by both automated and semi-automated modes was 52 and 53 respectively. Collectively, the radiation doses estimated by both automated and semi-automated modes differed only by 0.03 Gy when the initial selection of metaphase cells was performed at the sensitivity level of 8 (Table 2). A p value of approximately 1 by the paired t-test showed that the difference between automated and semi-automated modes was not statistically significant. In this study, we estimated that an average of 5 min is required for the manual verification/correction of DC frequency in 100 metaphases.

In case of radiological/nuclear mass casualty incidents, automated DC detection with minimal human intervention is preferred. Therefore, efficiency of automated (without manual verification/correction) and semi-automated (with manual verification/correction) scoring was next evaluated using the images captured at two different levels of sensitivity (7 and 8). These metaphase images, after manual selection or without selection, were subjected to automated DCScore algorithm (Table 3). Manual selection of metaphases prior to automated DC analysis did not make any significant impact on estimated biodoses. In general, biodoses estimated by the automated analysis correlated well with the actual delivered doses of 1 Gy and 3 Gy. At radiation doses exceeding 3 Gy, accuracy of automated detection of dicentric chromosomes was found to be 83.33% of the semi-automated mode when all the captured cells were subjected to DCScore without manual selection.

Table 2
Comparison of dicentric chromosome detection efficiency by automated and semi-automated modes using metaphase cells captured at different sensitivity levels.

Mode	Sensitivity	Cells captured[a]	Cells scored	Cells rejected	No. of Dicentrics	Estd (Gy)	LCL 95% CI (Gy)	UCL 95% CI (Gy)
Automated	6	195	129	66	37	1.63	1.27	2.03
Semi-auto	6	195	129	66	43	1.81	1.44	2.21
Automated	7	188	124	64	49	2.03	1.65	2.45
Semi-auto	7	188	124	64	58	2.27	1.89	2.69
Automated	7.5	185	122	63	38	1.73	1.35	2.14
Semi-auto	7.5	185	122	63	44	1.91	1.53	2.33
Auto	8	184	130	54	52	2.05	1.68	2.45
Semi-auto	8	184	130	54	53	2.08	1.70	2.48

[a] Metaphase images were captured from human lymphocytes irradiated with 2 Gy of X-rays.

3.3. Comparison of DC detection efficiency by automated and semi-automated modes for different doses of X-rays

The maximum cell count was set at 200 cells for triage mode since the DCScore algorithm rejects 10–35% of the captured cells. Slides prepared from the samples irradiated with different doses of X-rays (0 Gy, 0.1 Gy, 0.25 Gy, 0.5 Gy, 0.75 Gy, 1 Gy, 2 Gy, 3 Gy, 4 Gy and 5 Gy) were utilized (Table 4). Comparison of automated (unsupervised) and semi-automated (manual verification/correction) modes revealed a general trend where false positives were more pronounced at low radiation doses (0-0.5 Gy) while an underestimation of truly positive dicentrics (false negatives) was noted at radiation doses higher than 3 Gy (Fig. 1). Of interest, number of DCs detected by both automated and semi-automated modes was grossly similar for radiation doses of 0.75 Gy (28 dicentrics for automated and 22 dicentrics for semi-automated), 1 Gy (26 dicentrics for both modes) and 2 Gy (47 dicentrics for automated and 50 dicentrics for semi-automated).

3.4. Generation of calibration curves based on automated and semi-automated DC detection modes

Suitable calibration curves based on automated and semi-automated scoring are required for estimating the absorbed radiation dose. For this purpose, metaphase images captured from mock (0 Gy) and irradiated (γ-rays and X-rays; 0.1–5 Gy) samples were subjected to DC analysis by both automated and semi-automated modes. The dose response curves generated from automated and semi-automated dicentric chromosome scoring after X-rays and γ-rays irradiation are shown in Fig. 2A and B respectively. Data on the frequency of DCs detected by both automated and semi-automated scoring modes as well as the dose estimates of X-rays and γ-rays treated cells are summarized in Tables 5A and 5B. Consistent with our observations before, frequency of false positives increased at doses below 0.75 Gy and true positives decreased above 2 Gy for both γ-rays and X-rays treated samples. Interestingly, the frequency of DCs/cell detected by the automated analysis was grossly similar to the semi-automated analysis (manual verification) for the delivered doses of 0.75 Gy, 1 Gy, 2 Gy and 3 Gy. The absorbed radiation doses in *ex vivo* irradiated lymphocytes were estimated using our standard X-rays and γ-rays calibration curves generated by semi-automated scoring. The radiation doses predicted by the dicentric chromosome frequencies were 1.01 Gy, 1.31 Gy, 1.99 Gy and 2.57 Gy for the automated mode and 0.51 Gy, 0.73 Gy, 1.88 Gy and 3.25 Gy for the semi-automated mode for the actual delivered doses of 0.75 Gy, 1 Gy, 2 Gy and 3 Gy. Similar to γ-rays, false positives were observed below 0.75 Gy (4.00% for 0 Gy, 10.20% for 0.1 Gy, 4.80% for 0.25 Gy, 7.40% for 0.5 Gy) of X-rays. Likewise, detection efficiency for true dicentrics showed a radiation dose dependent decline at X-rays doses higher than 2 Gy (98.1% for 2 Gy, 86.90% for 3 Gy, 79.50% for 4 Gy and 35.70% for 5 Gy). A remarkable similarity in the frequency of DCs was observed at the triage cut off dose of 2 Gy (0.4 per cell for automated mode and 0.38 per cell for semi-automated mode). Our results indicate that the automated DC analysis can be effectively utilized for radiological/nuclear triage scenarios (Fig. 3).

4. Discussion

In the event of large radiological/nuclear mass casualties, rapid dose assessment is required to prioritize moderately or severely exposed people for clinical treatment. As manual analysis of DCs is labor intensive and tedious, automated DC analysis is often preferred especially when several hundreds and thousands of people require biodosimetry aftermath of any large scale radiological or nuclear incident(s). In this

Table 3
Evaluation of DC scoring by automated and manual modes using metaphase images captured at different sensitivity levels.

Mode	Delivered Dose (Gy)	Sensitivity Level	Cells captured	Cells rejected	Cells selected	No. of Dicentrics	Estd.Dose (Gy)	LCL 95% CI (Gy)	UCL 95% CI (Gy)
Auto	1	7	176	49	127	17	0.93	0.60	1.33
Semi	1	7	176	49	127	15	0.85	0.53	1.24
Auto	1	8	173	47	126	16	0.89	0.57	1.29
Semi	1	8	173	47	126	13	0.76	0.45	1.15
Auto	3	7	182	61	121	58	2.31	1.92	2.73
Semi	3	7	182	61	121	74	2.71	2.31	3.13
Auto	3	8	171	39	132	70	2.47	2.09	2.87
Semi	3	8	171	39	132	84	2.78	2.40	3.18
Auto[a]	1	7	158	36	122	14	0.83	0.50	1.23
Semi[a]	1	7	158	36	122	11	0.68	0.38	1.07
Auto[a]	1	8	159	47	112	11	0.73	0.41	1.15
Semi[a]	1	8	159	47	112	11	0.73	0.41	1.15
Auto[a]	3	7	158	37	121	59	2.34	1.95	2.76
Semi[a]	3	7	158	37	121	78	2.80	2.41	3.22
Auto[a]	3	8	138	30	108	59	2.34	1.95	2.76
Semi[a]	3	8	138	30	108	78	3.01	2.59	3.46

[a] Metaphase cells unsuitable for scoring were eliminated manually before subjecting to DC analysis by automated (auto) and semi-automated (manual correction) modes. Auto-Automated. Semi-Semi-automated.

Table 4
Evaluation of DC scoring efficiency and accuracy by automated and semi-automated modes for triage following exposure to different doses of X-rays.

Mode	Delivered Dose (Gy)	Cells captured	Cells rejected	Cells selected	No. of Dicentrics	Estd. Dose (Gy)	95% CI (Gy)
Automated	0	188	62	126	5	0.34	0.11–0.70
Semi-auto	0	188	62	126	0	0	0
Automated	0.1	181	15	166	18	0.79	0.51–1.12
Semi-auto	0.1	181	15	166	1	0.05	0–0.29
Automated	0.25	182	14	168	10	0.48	0.25–0.80
Semi-auto	0.25	182	14	168	2	0.11	0.01–0.36
Automated	0.5	188	12	176	22	0.88	0.61–1.21
Semi-auto	0.5	188	12	176	9	0.42	0.21–0.72
Automated	**0.75**	**188**	**29**	**159**	**28**	**1.15**	**0.84–1.50**
Semi-auto	**0.75**	**188**	**29**	**159**	**22**	**0.96**	**0.66–1.30**
Automated	**1**	**184**	**15**	**169**	**26**	**1.04**	**0.74–1.37**
Semi-auto	**1**	**184**	**15**	**169**	**26**	**1.04**	**0.74–1.37**
Automated	**2**	**180**	**19**	**161**	**47**	**1.65**	**1.33–2.01**
Semi-auto	**2**	**180**	**19**	**161**	**50**	**1.72**	**1.4–2.08**
Automated	**3**	**184**	**39**	**145**	**70**	**2.32**	**1.97–2.70**
Semi-auto	**3**	**184**	**39**	**145**	**89**	**2.71**	**2.35–3.10**
Automated	4	186	30	156	117	3.08	2.73–3.45
Semi-auto	4	186	30	156	149	3.58	3.23–3.95
Automated	5	180	23	157	178	3.98	3.62–4.35
Semi-auto	5	180	23	157	279	5.20	4.84–5.57

For triage mode of scoring, cell count was set at 200 with a scanning area of 50% at the center of the slides as the region of interest (ROI). Numbers in bold indicate that the radiation doses estimated by both automated and semi-automated modes are grossly similar.

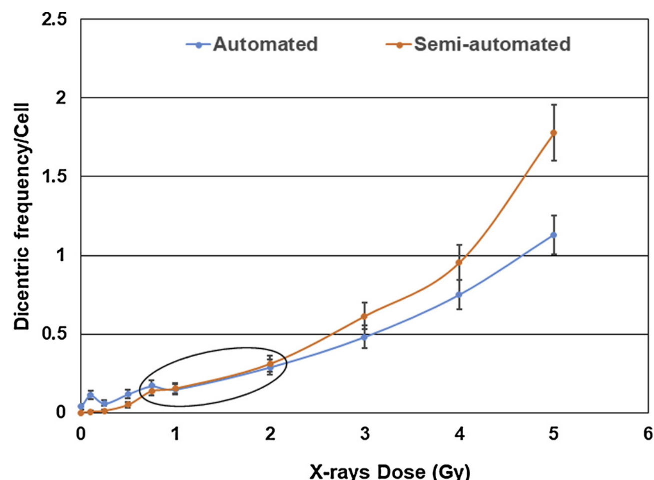


Fig. 2. Comparative analysis of automated and semi-automated scoring of dicentric chromosomes detected in human peripheral lymphocytes after exposure to different doses of X-rays. The cell number was set at 200 for the analysis. Detection of DC frequency without (automated) and with manual correction of false positives and true positives (semi-automated) are shown. Note that the DC frequency detected by both automated and semi-automated scoring modes were similar for the actual delivered doses of 0.75 Gy, 1 Gy and 2 Gy (circle). Bars indicate SEM.

study, automated DC analysis was optimized and validated with the sole objective of providing a rapid radiation dose assessment on exposed humans. As metaphase cell selection is absolutely critical for automated DC detection, varying levels of sensitivity and scanning speed (low, moderate and high) were tested. It was found that the sensitivity level of 8 worked optimally for metaphase selection without much dependence on the scanning speed. To improve the specificity of DC detection, several training files with hundreds of metaphase images were added for improving the selection of high quality metaphases. Selection of high quality metaphases is likely to minimize the time required for manual elimination of undesirable cells prior to automated DC analysis.

During the study, we found that the DCScore algorithm did not distinguish DCs from tri- or tetracentric chromosomes. Therefore, either a tricentric (an aberrant chromosome with three centromeres) or a tetracentric chromosome (an aberrant chromosome with 4 centromeres) will be counted as one dicentric chromosome by Metafer instead of two and three dicentrics respectively (a tricentric chromosome should be counted as two dicentrics and a tetracentric chromosome should be counted as three dicentrics). In the current study, approximately 10% of the cells (17 of 157 cells analyzed) in 5 Gy X-rays irradiated sample contained both dicentrics (31 in 157 cells) and tricentrics (19 in 157 cells) resulting in a dicentric yield 35 by the automated mode instead of 69 by the semi-automated mode. Further, only 7 out of 19 (36.84%) tricentric chromosomes were detected by Metafer.

Table 5A
Comparative analysis of radiation dose estimation by automated and semi-automated modes of dicentric chromosome detection in human lymphocytes after exposure to different doses of X-rays.

X-Rays (Gy) (Delivered)	Cells Imaged	Cells selected	Dicentrics (Automated)	Estd. Dose (95% CI) (Automated)	Dicentrics (Semi-auto)	Estd. Dose (95% CI) (Semi-auto)
0	985	860	45	0.49 (0.38–0.62)	0	0
0.25	1166	1037	92	0.75 (0.63–0.87)	23	0.23 (0.15–0.33)
0.5	**809**	**681**	**59**	**0.73 (0.59–0.89)**	**30**	**0.42 (0.30–0.57)**
1	**674**	**568**	**106**	**1.29 (1.12–1.47)**	**71**	**0.97 (0.80–1.15)**
2	**653**	**581**	**234**	**2.17 (1.99–2.35)**	**226**	**2.12 (1.94–2.30)**
3	**921**	**73**	**473**	**2.82 (2.60–3.05)**	**526**	**3.01 (2.79–3.24)**
4	349	286	238	3.40 (3.14–3.67)	294	3.86 (3.60–4.13)
5	255	215	272	4.36 (4.06–4.67)	377	5.27 (4.84–5.71)

Radiation doses were estimated using a standard X-rays calibration curve generated by semi-automated mode of dicentric scoring using the fit coefficients: Numbers in bold indicate that the radiation doses estimated by both automated and semi-automated modes are grossly similar. $Y = 0.04 \pm 0.005 D^2 + 0.08 \pm 0.017 D$. Y – Yield of aberrations. D – Radiation dose.

Table 5B
Comparative analysis of radiation dose estimation by automated and semi-automated modes of dicentric chromosome detection in human lymphocytes after exposure to different doses of γ-rays.

γ-Rays (Gy) (Delivered)	Cells Imaged	Cells selected	Dicentrics (Automated)	Estd. Dose (95% CI) (Automated)	Dicentrics (Semi-auto)	Estd. Dose (95% CI) (Semi-auto)
0	1933	1590	211	1.14 (0.72–1.64)	6	0.05 (0.02–0.11)
0.1	1412	1095	172	1.26 (0.83–1.76)	9	0.11 (0.05–0.21)
0.25	1260	980	127	1.01 (0.60–1.51)	11	0.15 (0.08–0.26)
0.5	1347	1086	167	1.26 (0.83–1.76)	29	0.33 (0.23–0.45)
0.75	**1702**	**1502**	**168**	**1.01 (0.60–1.51)**	**66**	**0.50 (0.41–0.61)**
1	**1668**	**1380**	**228**	**1.31 (0.88–1.81)**	**98**	**0.73 (0.62–0.85)**
2	**1074**	**921**	**282**	**1.99 (1.54–2.49)**	**253**	**1.88 (1.73–2.03)**
3	1041	878	403	2.57 (2.11–3.06)	558	3.15 (2.68–3.64)
4	931	744	439	3.06 (2.59–3.55)	550	3.46 (3.00–3.96)
5	360	316	281	3.86 (3.39–4.35)	473	5.20 (4.73–5.69)

Radiation doses were estimated using a standard γ-rays calibration curve generated by semi-automated mode of dicentric scoring using the fit coefficients: Bold letters indicate the similarities in the predicted doses. $Y = 0.04 \pm 0.005 D^2 + 0.06 \pm 0.022 D$. Y – Yield of aberrations. D – Radiation dose.

Therefore, manual correction for the actual number of DCs is inevitable for high radiation doses where tricentric and tetracentric chromosomes occur frequently. To our knowledge, this issue has not been adequately addressed/considered in any of the earlier studies when assessing the absorbed radiation dose by the automated mode.

Earlier studies have utilized the automated DC scoring for estimating absorbed radiation dose in *ex vivo* irradiated lymphocytes involving various levels of human verification [17,21–23]. Accuracy of automated DC analysis can be substantially enhanced by improving the quality of metaphase images selected for analysis [24–27]. Various improvements have been recently reported for increasing the accuracy of automated dicentric chromosome analysis [13,14,16]. In a recent report, Li et al. [13] utilized a series of image selection models to improve the accuracy of dose estimation. In this method, metaphase images are evaluated and ranked based on Z-scores of filters (I–VI) that are based on various morphological and numerical features such as average length and width of chromosome objects, centromere density of objects, concavity of objects, total number of chromosome objects, number of segmented objects and ratio of classified objects to segmented objects. Utilizing the image selection models, the accuracy of estimated doses have been reported to be within 0.5 Gy of the actual delivered doses. In the current study, using metaphase finder and Metafer DCScore algorithm, we have demonstrated that most of the biodoses predicted by the automated dicentric chromosome scoring mode was within 0.5 Gy of the actual delivered doses of X-rays with the exception of doses below 0.5 Gy. In a recent study, Dai et al. [21] utilized a 3-gradient scanning system for a rapid dose estimation involving the analysis of 120, 480 and 960 cells for radiation dose estimation using the DCScore algorithm of Metafer with manual correction of false/true positives. In this system, if more than one DC is observed, then the cell number for analysis will be extended to 480 and 960 for further verification and confirmation of radiation dose. In a previous study, it was demonstrated that 300–400 cells were found sufficient for radiation dose prediction in the case of simulated whole-body exposure [23]. In our study, we found that 200 cells are optimum for triage mode of scoring as 70–80% of the captured cells are usually selected for analysis by the DCScore algorithm.

In an earlier study, comparison of automated and manual scoring of 500 metaphases showed only 4.35% of dose misclassification demonstrating the effectiveness of automated scoring [28]. Unlike the earlier studies that involved some degree of human intervention, our study has clearly demonstrated that the completely automated dicentric chromosome scoring works efficiently for the actual delivered doses of 0.75 Gy–3 Gy of low LET radiation (see Tables 5A and 5B) without human intervention either in metaphase cell selection or in DC correction. The automated DC scoring will be particularly useful for rapid segregation of people exposed to either below or above 2 Gy of radiation exposure. Since false positives and true positives increase and decrease respectively at low and high radiation doses, a suitable calibration curve generated by automated scoring mode needs to be utilized to increase/improve the precision of predicted dose. Development of improved chromosome preparation techniques coupled with further optimization of automated DC scoring can make DCA an effective triage tool for rapid radiation biodosimetry.

5. Conclusion

In the present study, we have clearly demonstrated that the automated dicentric chromosome analysis is fairly accurate in predicting the absorbed radiation dose in the range of 0.5 Gy–3 Gy of low LET radiation. The automated dicentric analysis tool can be an effectively used for large scale radiological/nuclear incidents where a rapid biodosimetry is critically required for an appropriate medical management of several hundreds and thousands of exposed people.

Declaration of Competing Interest

The authors declare no conflict of interest.

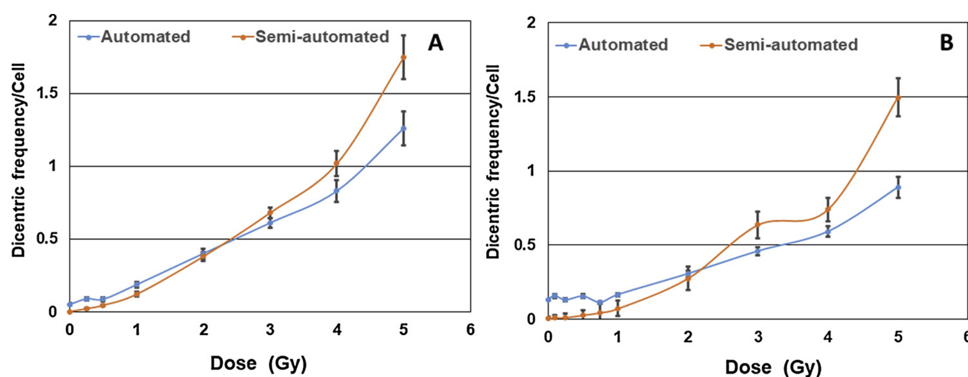


Fig. 3. Comparative analysis of automated and semi-automated DC scoring in human lymphocytes after exposure to various doses of X-rays (A) and γ-rays (B). With the exception of delivered doses of 0.75 Gy, 1 Gy and 2 Gy, a biphasic pattern of dicentric frequencies was observed for the automated scoring: Increased number of false positives at doses less than 0.75 Gy and decreased number of true positives at doses higher than 2 Gy. Bars indicate SEM.

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