Kaunas University of Technology

Faculty of Mathematics and Natural Sciences

Optimization Of Semi-Automatic Dicentric Assay For Triage Dose Estimation In Case Of Large-Scale Radiation Emergencies

Master's Final Degree Project

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Declaration of Academic Integrity

I confirm that the final project of mine, Antonio Jreije, on the topic „Optimization Of Semi-Automatic Dicentric Assay For Triage Dose Estimation In Case Of Large-Scale Radiation Emergencies“ is written completely by myself; all the provided data and research results are correct and have been obtained honestly. None of the parts of this thesis have been plagiarised from any printed, Internet-based or otherwise recorded sources. All direct and indirect quotations from external resources are indicated in the list of references. No monetary funds (unless required by Law) have been paid to anyone for any contribution to this project.

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Kaunas University of Technology

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Topic of the project	Optimization of Semi-Automatic Dicentric Assay for Triage Dose Estimation in Case of Large-Scale Radiation Emergencies
Requirements and conditions (title can be clarified, if needed)	- Evaluation of the time necessary to complete all steps involved in automatic scoring for both 63x and 40x objectives. - Establishment of dose effect curves using different objectives (40 x, 63 x). - Investigation of the influence of the lower magnification (40 x) on the number of dicentic chromosomes detected by the software but not confirmed by a human scorer (False Positives) and undetected dicentrics (False Negative). - Comparison of the dicentric scoring efficiency using 40x and 63x objectives and between the automatic scoring and the full manual scoring.
Supervisor	Prof. Diana Adlienė (position, name, surname, signature of the supervisor) (date)

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Summary

In case of large scale radiation accident, the absorbed dose of potentially exposed individuals should be estimatet as quickly as possible to differentiate individuals with low exposure dose from those requiring urgent medical intervention. The manual dicentric assay remains the most validated and widely used assay in biological dosimetry for individual dose estimation. Moreover, it allows estimation of doses as low as 0.1 Gy and can differentiate between whole and partial body exposures. However, the so called gold standard assay is laborious and time consuming; consequently cannot be applied in case of mass casualty. Therefore, to increase the throughput of the dicentric assay, the automatic dicentric assay was developed and validated as an accurate method for preliminary dose estimation in recent years. In addition, different inter-laboratories studies were conducted and showed that automatic dicentric assay can be used to promote laboratory networking thus enabling mutual assistance in case of emergencies. The automatic dicentric assay involve 3 steps: the automatic metaphase finding (Msearch) which is performed using a 10x objective, capturing images of high resolution at 63x magnification (Autocap) and automatic detection of dicentric chromosomes which is validated by a human scorer. In this study, the automatic dicentrics scoring with 63x objective was compared to scoring with 40x in an attempt to increase the throughput of the automatic dicentric assay. Whole blood samples were irradiated with low LET X-Ray at doses between 0 and 5 Gy. After slides preparation, dicentric scoring was performed both automatically (with 63x and 40x objective) and manually (only for doses of 1 and 4 Gy). The time taken to perform each step of the automatic scoring was recorded for analysis of both possibilities. The results indicate that analysis using the 40x objectives lead to a 45% reduction in the total time needed for dicentrics scoring. In addition, the dose effect curves established in the case of the usage of both objectives merge at low doses; however, the dose effect curve for the 40x objective is significantly lower than for the 63x magnification at doses higher than 2 Gy.In addition, the rate of false positive and false negative evaluations was comparable in both cases. The mean false negative rate was 47.7% for scoring with the 40 x objective as compared to 55% for scoring with the 63x objective. Moreover, the same mean false - positve rate was obtained for both objectives (0.11% with 40x objective and 0.1% with 63x objective). In summary, our results show that automatic scoring with 40x objective can support a large-scale sample analysis and decrease the turnaround time of the dicentric assay while maintaining comparable dicentric detection

efficiency to the scoring done with the 63x objective. The new automatic method, once validated, can become an effective triage tool for large-scale radiation accident.

Antonio Jreije. Pusiau automatinio dicentrinio tyrimo optimizavimas triados dozės įvertinimui didelių radiacinių avarijų atveju. Magistro baigiamasis projektas / vadovė prof. dr. Diana Adlienė; Kauno technologijos universitetas, Matematikos ir gamtos mokslų fakultetas.

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Santrauka

Įvykus didelei spindulinei avarijai, reikia kiek galima greičiau įvertinti potencialiai apšvitintų asmenų dozę, kad būtų galima atskirti asmenis, kurių apšvita nedidelė, nuo tų, kuriems reikia skubios medicininės pagalbos. Rankinis chromosomų dicentrikų tyrimo metodas yra labiausiai patikimas ir plačiausiai naudojama biologinės dozimetrijos metodas, leidžiantis įvertinti nedideles (mažiausia galima dozė - 0,1 Gy) dozes ir vertinti tiek dalinę, tiek viso kūno apšvitą. Tačiau auksiniu standartu vadinams tyrimas yra sudėtingas ir laikui imlus, todėl nėra efektyvus masinės spindulinės avarijos atveju. Siekiant padidinti dicentrikų tyrimo metodo našumą, buvo sukurtas automatinis dicentrikų tyrimo medodas, kuris pastaraisiais metais buvo patvirtintas tinkamu preliminariam dozės įvertinimui. Be to, atlikus palyginamuosius dozių nustatymo metodų tyrimus tarp skirtingų laboratorijų buvo nustatyta, kad automatinis dicentrikų tyrimo metodas yra priimtinas daugeliui laboratorijų, kas leidžia sukurti laboratorijų tinklą, kurios galėtų teikti viena kitai pagalbą ekstremalių situacijų atveju. Automatinis dicentrinis tyrimas apima 3 etapus: automatinį metafazės nustatymą (Msearch), kuris atliekamas naudojant 10x mikroskopo objektyvą; didelės skiriamosios gebos vaizdų gavimą, naudojant 63x objektyvą (Autocap) ir automatinį dicentrinių chromosomų aptikimą, kurį galutinai patvirtina žmogus. Šiame tyrime automatinis dicentrikų vertinimas naudojant 63x objektyvą buvo lyginamas su dicentrikų vertinimu naudojant 40x objektyvą, siekiant įvertinti pastarojo našumą. Kraujo mėginiai buvo apšvitinti mažo LET (ilginis energijos perdavimas=low energy tranfer) rentgeno spinduliuotės dozėmis nuo 0 iki 5 Gy. Parengtuose bandiniuose dicentrikų skaičiavimas buvo atliktas tiek automatiškai (naudojant 63x ir 40x didinimą), tiek ir rankiniu būdu (tik 1 ir 4 Gy dozėms). Skaičiuojant dicentrikus automatiškai, buvo registruojama vertinimo trukmė, esant 63x ir 40x didinimui. Rezultatai rodo, kad naudojant 40x didinimą vietoje 63x, galima 45% sutrumpinti laiką, reikalingą dicentrikams suskaičiuoti. Be to, dozės atsako kreivės mažų dozių intervale sutapo, o esant dozėms, didesnėms už 2 Gy, dozės atsakas buvo gerokai mažesnis 40x didinimo atveju, lyginant su 63x didinimu. Klaidingų teigiamų ir klaidingų neigiamų vertinimų skaičius buvo panašus abiem atvejais. Vidutinis klaidingų neigiamų vertinimų skaičius naudojant 40x objektyvą sudarė 47,7%, o 63x objektyvo atveju - 55%. Abiejų didinimų atveju gautas toks pats klaidingų teigiamų vertinimų rodiklis: 0,11%, esant 40x didinimui, ir 0,1% - esant 63x didinimui. Apibendrinant galima teigti, jog gauti rezultatai patvirtina galimybę didelės avarijos atveju dicentrikų skaičiavimui panaudoti 40x didinantį objektyvą, ženkliai sutrumpinant vieno

bandinio tyrimo laiką ir išlaikant palyginamą su 63x didinimo atveju dicentrikų aptikimo efektyvumą. Tikėtina, kad atlikus visus reikalingus testavimus, šis naujasis automatinis dicentrikų skaičiavimo metodas taps veiksmingu įrankiu vertinant dozes didelės apimties spindulinių avarijų metu.

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Introduction

The use of radioactivity and ionizing radiation has increased in medicine, industry, research and military purposes worldwide in the last decades and therefore the risk of an overexposure of human beings is becoming more likely. In the past, numerous nuclear and radiation accidents have occurred. Between 1952 and 2009, 99 radiation exposure accidents at nuclear power plants have been reported worldwide [1].However, the most devastating incidents are Chernobyl disaster in 1986 and Fukushima Daiichi nuclear disaster in 2011 [1].During these radiation accidents, a large number of persons were exposed to sometimes unknown doses of radiation.

A first indication of an increased radiation exposure can be based on clinical signs and symptoms such as nausea and vomiting in addition to blood cell count fluctuations. However, some of these symptoms are subjective parameters and can be caused as well due to intense anxiety [2]. In emergency situation it is necessary to have an independent source of information about individually received irradiation doses to blood to identify the 'worried well' individuals who have not actually received radiation doses but are extremely distressed [3].Here biological dosimetry provides a useful method to estimate a radiation dose received by individuals. An ideal biomarker has to fulfill different requirements: [4]

- low background level;
- clear dose effect relationship for different radiation qualities and dose rates;
- specific to ionizing radiation;
- non-invasive;
- fast availability of dose estimate;
- good reproducibility;
- comparability of in vitro and in vivo results.

During large-scale accidents, the huge number of 'worried well' individuals should be identified and assured in order not to overwhelm the healthcare facilities in close proximity of the disaster [4]. Although some accidents involve an assumed smaller number of victims, the number of anxious and unsettled individuals can be enormous, as was shown in the Goiania accident in 1987. As a result of this accident, four individual died due to an internal contamination with radioactive Caesium. However, a total of 112,800 persons were extremely anxious and only felt assured after radiation dose estimation [5].

Analysis of cytogenetic damages in peripheral blood lymphocytes (PBL) induced by ionizing radiation is commonly used for biodosimetry purposes [6]. For many years, the dicentric chromosome assay has been considered as the "goal standard" in biological dosimetry and the most reliable and sensitive assay for the assessment of the radiation dose of potentially exposed individuals [7].

Biological dosimetry (dicentric assay) was conducted after the Chernobyl accident for dose assessment of people living or visiting the contaminated sites in addition to evacuees and

liquidators [8; 9; 10; 11]. Furthermore, the monitoring also included people living in European areas where rain was contaminated with radiation [12].

Following the Fukushima nuclear power plant accident, dicentric assay was performed for constructor workers at the site [13] and also for travelers to contaminated areas [14]. However, it should be noted that after these two devastating incidents, the biodosimetry was conducted by single laboratories and included a limited number of examined persons.

Based on the results of biodosimetry, individuals with high doses of radiation necessitating extensive medical care can be distinguished from people with injuries attributed to causes other than irradiation [3]. In those catastrophic situations, the reassurance of highly distressed people is important since it has been proven that the psychological implications of radiological disasters are related to a perceived exposure rather than a measurable dose [3].

However, the dicentric assay is time consuming due to its prolonged scoring procedure and not appropriate for emergency situations where large number of individuals are exposed and exposure status need to be reported quickly. To increase the capacity in case of emergency situations, the conventional method has to be adapted to a large scale scenario. New scoring strategies have been implemented to increase the throughput of the assay by decreasing the number of cells analyzed and by detecting dicentric chromosomes with the help of software supported analyzing tools [15].

In recent studies, the automatic dicentric assay was proven to be as least as accurate in estimating a preliminary exposure dose as the manual assay in triage mode which is based on the analysis of 50 cells or even less [15].

Even though the automatic dicentric is much faster and need less skilled workers than the manual scoring, the time needed for cell culturing and slides preparation is the same for both assays. This time is at least 51 hours and is needed in order to obtain slides with good metaphase quality for accurate dose estimation [4].

The aim of this study here was to optimize the semi-automatic scoring procedure for the dicentric chromosome assay by using a 40x objective for the auto-capturing of metaphase images instead of 63x objective.To achieve this aim, the following tasks were performed:

- Evaluation of the time necessary to complete all steps involved in automatic scoring for both 63x and 40x objectives.
- Establishment of dose effect curves using different objectives (40 x, 63 x).
- Investigation of the influence of the lower magnification (40 x) on the number of dicentic chromosomes detected by the software but not confirmed by a human scorer (False Positives) and undetected dicentrics (False Negative).
- Comparison of the dicentric scoring efficiency using 40x and 63x objectives and between the automatic scoring and the full manual scoring.

The investigations presented in this work were conducted in the Biological dosimetry unit of the BundesamtfürStrahlenschutz (BfS), Munich, with the support of Dr Ulrike Kulka and Dr Ursula Oestreicher.

1. Overview of the Literature

1.1. History of the cytogenetic assays

The conventional cytogenetic assays have a main application in radiation protection for the measurement of the radiation dose in the blood of the exposed subject. These biological dosimetry techniques can be implemented as well in the case of radiation exposure accidents.

Chromosomal aberration induced by X-ray was reported first by Muller following his innovative work in *Drosophila [16]*. In 1960, chromosomes in peripheral blood cultures were successively stimulated into metaphase in vitro. This was the basis for the development of different assays for the detection of radiation exposure through the analysis of chromosome aberration [17].

Early studies were conducted to identify the effect of radiation in patients undergoing radiation therapy [18], radioiodine therapy, and patients exposed to diagnostic X-rays [19]. These studies revealed that radiation induce different types of unstable chromosome aberrations such as dicentric chromosomes, centric ring chromosomes, and acentric fragments among others.

The concept of biological dosimetry originated first from Bender and Gooch in 1962 who established that the quantification of chromosome aberrations can be used for the assessment of radiation doses in exposed individuals [20, 21]. In the studies, which involved 8 individuals exposed to radiation accident, they established a calibration curve that correlated the frequency of dicentric chromosome aberrations to radiation dose and used the calibration curve to estimate to doses of the exposed individuals [21].

Afterward, the dicentric assay was used for the first time in a radiation accident event at the 'Recuplex criticality accident' at Hanford [7].

Later on, fluorescence plus Giemsa (FPG) staining was introduced to the assay which help to differentiate between first and subsequent mitotic divisions. Only cells with first mitotic division should be used for this assay since dicentric chromosomes disappear at the following cell division. Consequently, due to the fact that dicentrics are lost at cell division, this assay is only appropriate for acute radiation exposure [1].

Many years of experience with the dicentric assay made this assay the "goal standard" in biological dosimetry and the most reliable and sensitive assay for the evaluation of radiation exposure. Many factors make this assay a first choice in biological dosimetry including its specificity for ionizing radiation, established dose effect relationship for different dose rates and radiation qualities. In addition, the dicentric assay is a non-invasive method which provides relatively fast estimation of radiation dose. [1].

1.2. Overview of the dicentric assay

After the exposure to ionizing radiation DNA double strand breaks can be induced. The misrepair of these damages results in the formation of abnormal chromosomes. After the unsymmetrical exchange of two chromosome segments, each with a centromere, a dicentric chromosome and an accompanying acentric fragment are formed (figure 1) [22].

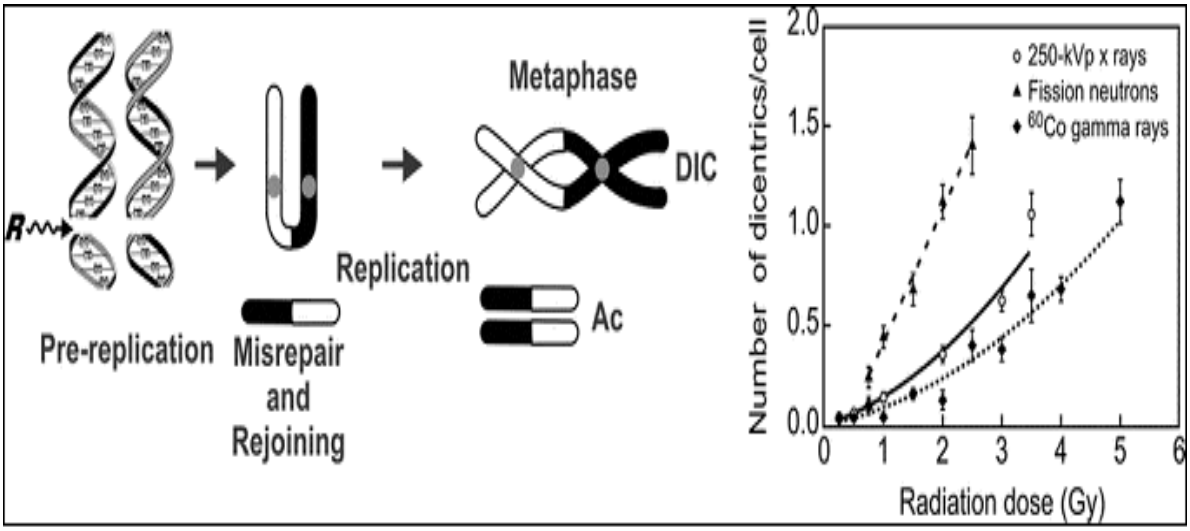

Figure 1: radiation-induced DNA damage in an interphase cell and the resulting formation of dicentric (DIC) chromosome aberration in lymphocytes arrested in metaphase mitosis [23].

In peripheral human lymphocytes dicentric chromosomes are visible in the metaphasestage of the cell division (figure 2).

There is a clear correlation between the radiation dose and the numbers of dicentric chromosomes. Radiation induces other types of chromosomal aberrations in addition to dicentric chromosomes. However, dicentrics are considered until the present the most sensitive and specific types of changes for evaluating radiation dose [7].

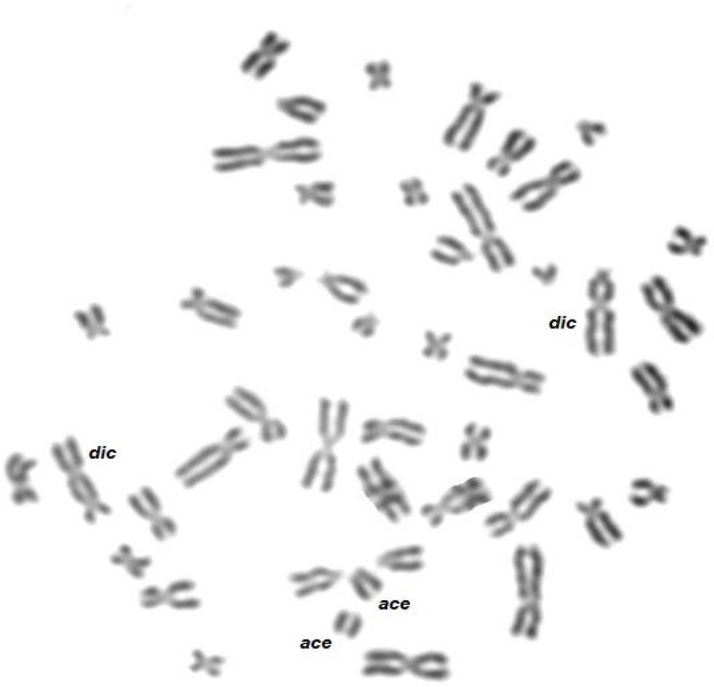

Figure 2: Lymphocyte cell in metaphase with two dicentric chromosomes and two acentric fragments.

The results of Dicentric Chromosome Assay (DCA) depends on the radiation dose, the dose rate, the volume of the body irradiated (whole vs. partial body) and on the radiation quality [7].

The background level of chromosome aberrations has an influence on the sensitivity of the method used for dose estimation. This is the case especially for persons exposed to low doses of radiation. It was established that the Dicentric Assay is specific for radiation since the background level for dicentrics in the regular healthy population is very low, around 1 dicentric chromosome in 1,000 cells. In the literature, this background level is reported between 0.09 and 2.99 dicentrics per 1000 cells [24]. These differences in the background dicentric level result from variations in the method used for dicentric assay preparation and scoring criteria between laboratories and from differences in the natural background radiation level between countries. Since inter-laboratory differences in dicentric assay analysis results exist, each laboratory should establish its own control group and its own dose-response curves [24].

Dicentric aberrations are induced almost exclusively from ionizing radiation. Few chemicals such as pentachlorophenol, formaldehyde or pesticides can lead to dicentric chromosomes formation. However, this excess of dicentrics was only observed in industrial

workers exposed to a high level of these hazardous chemicals. Also some cytostatic agents like bleomycin are known to induce dicentric chromosomes [25].

The lowest dose detectable by the dicentric assay is approximately 0.1Gy. For acute exposures to low LET radiation, the dicentric assay has a good dose-effect correlation in the range of 0.1Gy to 5.0 Gy. Even at low doses of radiation exposure, this assay remains specific and sensitive [7].

The dicentric assay can be applied in case of large-scale radiation emergency to confirm radiation exposure and to identify persons that need urgent medical intervention. There are limitations to the use of this assay in large incident since national laboratory capacity is limited. In addition, at least 3-4 days are needed before the assay results can be obtained. However, in the last year, network has been established between laboratories on the national and international level with a primary purpose of collaboration in case of large scale radiation emergency [3].

Dicentric assay has useful application in acute emergency triage. It can help in the management of exposed individuals with a potential whole-body dose in the range of 1.5-3 Gy. These victims may be at risk of developing acute radiation syndrome (ARS) such as hematological subsyndrome and cytopenias about 21-28 days after exposure. Consequently, this assay has a potential to detect persons who should be transferred for treatment [26].

After significant acute whole body or high-percentage partial-body irradiation, the victim may become symptomatic and pancytopenic. These patients require immediate, intensive and interdisciplinary medical-treatment approaches before the results of dicentric assay become available. In this case, Lymphocyte depletion kinetics are useful even though the dicentric assay can be used to provide confirmatory information regarding radiation doses [26].

Currently, there are no known effective preventive measures against radiation-induced cancer following radiation exposure. However, an estimation of the received dose can help in guiding subsequent monitoring and follow-up [26].

1.3. Performing the dicentric assay

Peripheral blood is withdrawn from individuals with suspected radiation exposure. T-Lymphocytes are stimulated to proliferate in culture by phytohaemagglutinin (PHA-L) during a period of 48 hours. Cells in the first-division metaphase spreads are collected on glass slides and stained. Then, cells are analyzed for chromosome aberrations. Analysis are performed by counting the number of dicentric chromosomes in the sample and then by comparing the frequency of dicentrics counted to an established calibration curve [27].

The blood sampling time post exposure should be taken into consideration since the optimal time for blood sampling is between 24 hours and 4 week after the exposure. After this period the frequency of the dicentric chromosomes starts to decline. Blood sampling should be performed 24 hours post exposure to allow sufficient time for the completion of repair by lymphocytes. In case of partial or non-uniform exposure, this time is needed for the irradiated lymphocytes to be homogenously distributed in the body. It should be noted that samples collected after 6 weeks of radiation exposure can be evaluated if a time correction factors are

used to account for the lymphocyte half-life in the peripheral blood [1]. Over time starting from around 4-6 weeks post exposure, unstable aberrations such as dicentric and ring chromosomes are eliminated which decrease assay sensitivity. Moreover, samples taken from persons exposed before 6 to12 months can be used for the estimation of doses > 0.5 Gy with the use of appropriate corrections [27].

The culture medium is prepared by the addition of 10 ml Fetal Calf serum (FCS); 0.4 ml Penicillin/Streptomycin and 2.4 ml PHA-L to 90 ml of RPMI-1640 medium. PHA-L role is to stimulate lymphocytes into the mitotic phase of the cell cycle. Blood aliquots (0.5 ml) are cultured in 5 ml of the prepared RPMI medium and 0.1 ml of BrdU. Since only cells in the first cell division should be analyzed, Bromodeoxyuridine (BrdU), which is a thymidine analogue, is added to the medium in order to differentiate between first and subsequent cell divisions (M2) [28]. BrdU is taken up into replicating DNA. Fluorescence plus Giemsa (FPG) staining results in a 'harlequin' effect in chromosome of the second metaphase cells. It is important to mention that BrdU is light sensitive, thus, cultures preparation should take place in low lighting. Additionally, cultures should be incubated in the dark (or culture tubes can be wrapped in aluminium foil). Culture tubes are kept in the incubator at 37°C with 5% CO2 for a duration of 24 hours [28]. The temperature of the incubator should be kept stable at 37.0 ± 0.5°C since a temperature lower than the mentioned range will result in a poor metaphase yield after 48 hours. In addition, at high temperature (≥ 38°C), cells will divide more quickly and therefore, a high numbers of metaphases in the second cell division may be present at the end of the culturing period [29].

Mitotic block is induced by the addition of colcemid. In the past years, colcemid was kept with the cells for 3 hours before harvesting. However, there are modification possible involving the addition of colcemid after 24 hours of culture time or for the whole period of culture. This avoids the Fluorescence plus Giemsa (FpG) staining of the cells when high throughput analysis of the cells by automatic procedures is needed [30].

Therefore, 40 µl of colcemid is added after 24 hours of cell culturing at 37°C. The culture tubes are then returned to the incubator for an additional 24 hours of culturing [27].

After 48 hours, the tubes are centrifuged for 10 minutes at 209 times gravity (x g) to separate the cell pellet from the supernatant. After removal of the supernatant by suction, 4-6 ml of 0.075M potassium chloride (KCl) solution is added to the cell pellet and mixed well on a vortex before placing the tubes in a water bath for 15 min of incubation [27].

Then the tubes are centrifuged again for 10 minutes at 209 x g. The supernatant is removed and 1 ml of fixative (3:1 methanol/acetic acid) is dropped slowly and at a constant rate until the pellet color change from red to black. When the black color is reached, an additional 6 ml of fixative are added. Fixative is added will mixing on the vortex thoroughly to ensure uniform dispersion of the cells [27].

The supernatant is removed after 10 min centrifugation at 209 x g and fixation is repeated 2-3 times until the fixative is clear. It should be noted that overnight storage of the fixed cells in a refrigerator at 4°C is possible. If necessary, the cells in fixative can be stored for a long term in a freezer (-20°C) [27].

Before the cells are dispensed onto slides, the tubes are centrifuged one last time and the final wash of fixative is removed, leaving the cell pellet suspended in a sufficient quantity of fixative [27].

Only clean slides are used for dropping. To make sure that slides are clean, they are stored in a degreasing fluid consisting of a mixture of 1:1 acetone/methanol or 1:1 ether/ethanol or 1% hydrochloric acid in methanol. The slides are dried and polished with tissue papers before use [27].

The slides should be kept cold and wet in order to improve the metaphases spreading. This can be achieved by dipping the slides into a beaker filled with distilled water and ice cubes. Some methanol can be added to the iced water without stirring. Directly before dropping, slides should be removed from the cold water and any remaining water should be shaken from the surface of the slide. Other factors that can influence the spreading of the metaphases are the temperature and humidity in the laboratory [27].

The cells are re-suspended in the remaining fixative and two to three drops are dispensed onto the slide. At least two to three slides should be prepared from one culture. After dropping the cells into the first slides, the spreading of the metaphases should be checked under the microscope before dispensing the cells from the same culture onto the remaining slides. In case of very high/low number of metaphases on the first slide, the remaining suspension concentration can be adjusted with fixative. If the appearance of the metaphases under the microscope is poor (e.g. bad metaphase spread or excessive amount of debris), the cell suspension should be washed with fixative one additional time and stored overnight in a refrigerator before cells dropping on the slides. After the dropping, the slides should be allowed to dry at room temperature [27].

Finally, In case of longtime colcemid, the slides are stained for 5 min in Giemsa-solution (20 ml Giemsa and 230 ml PBS buffer). After 5 min, the slides are rinsed 3 times in distilled water. The slides are stored over night to dry and then covered with mounting medium (Eukitt) and coverslips. The slides should be allowed to dry for an additional day before analysis [28].

1.4. Other retrospective dosimetry assays

The following is a summary of the most promising dosimetry techniques other than dicentric assay including a review of their advantages and disadvantages (table 1). The stability of the radiation induced change (i.e. aberrations) detected by the dosimetry method have an influence on the time frame during which an assay can be used post-exposure. The sensitivity and specificity of the assay for ionizing radiation as well as the time window from sample receipt to dose estimation are also factors that affect the suitability of the assay for use in biodosimetry [4].

Cytogenetic techniques

Premature chromosome condensation is a technique used to visualize chromosome aberrations during interphase. Premature chromosome condensation can be accomplished by cell fusion with mitotic Chinese hamster ovary cells. The number of PCC fragments is counted and an excess of 4 to 5 fragments above the number of 46 chromosomes per grey are seen in case of low LET radiation. In order to perform the PCC, blood samples should be withdrawn immediately after radiation exposure. Otherwise, the repair kinetics of the chromosome aberrations must be considered in the analysis. The results of the PCC indicate that the number of fragments is two times more elevated at 4 hours post exposure when compared to 1 and 7 days. The time required to perform the PCC assay including blood collection is approximately 3 hours [4, 31].

The cytokinesis block micronucleus assay (CBMN) is a biodosimetric assay that relies on the detection of small spherical objects called micronuclei (MN) in the cytoplasm of the daughter cell following cellular division. This MN results from chromosomes or acentric fragments that failed to incorporate into the daughter cell during cell division. MNs are radiation non-specific since they can be caused by exposure to different genotoxic agents. The CBMN assay has been validated for individual dose assessment in case of occupational, medical and accidental radiation exposure. MNs are unstable aberrations that are removed with the turnover of lymphocytes and therefore, are not appropriate for use in case of exposure that occurred many years ago. Although scoring of MN is rather simple, dose estimation will be available after at least 75 hours since it takes 3 days for the lymphocytes to reach cytokinesis. The sensitivity of the MN assay is relatively low, being unable to detect doses lower than 0.2-0.3 Gy. This is caused by the high background level of MNs in the general population that increases even more with age [4].

Fluorescence in situ hybridization (FISH) techniques has been used for assessment of exposures that occur many years ago. Different variations of the technique exist such as single color FISH (sFISH) used for the detection of inter-exchanges (e.g. dicentrics and translocations) and multi-color FISH applied when translocations are induced in different chromosomes. FISH is the method of choice for the assessment of protracted and historic exposures. This is due to the fact that translocations occur in the lymphatic stem cells and therefore are stable aberrations that can be detected in peripheral blood lymphocytes many years after exposures. However, the spontaneous frequency of translocations increases with age and is significantly influenced by smoking. Moreover, inter-individual variability in the background frequency can exist between individuals of the same age and dose exposure. All these factors limit the ability of the FISH to detect cumulative lifetime doses lower than 0.5 Gy. Another limitation of the FISH assay is the long duration needed (5 days) to obtain results. Currently, only the single color FISH is applied in biological dosimetry for the identification of two-way or one-way translocations since it’s faster and less expensive than multi-color FISH [4, 31, 32].

Table 1: Comparison of different dosimetric assays [4].

	Time since exposure			Exposure			Time (h) from sample receipt to dose estimate	Agent specificity	Sensitivity of the assay (dose range in Gy)	Triage use	Automated analysis
	Days	Months	Years	Acute	Protracted	Partial body					
Dicentrics, full	✓	✓	-	✓	✓	✓	55	IR	0.1-5	-	✓
Dicentrics, triage	✓	✓	-	✓	✓	-	52	IR	0.5-5	✓	✓
PCC fragments	✓	-	-	✓	-	✓	2	IR	0.2-20	✓	✓
Micronuclei	✓	✓	-	✓	✓	-	75	Genotoxins	0.2-4	✓	✓
FISH	✓	✓	✓	✓	✓	-	120	IR	0.25-4	-	✓
GPA	-	✓	✓	✓	-	-	3	Mutagens	.1	-	✓
HPRT	✓	✓	-	✓	-	-	400	Mutagens	>1	-	-
Haematology	✓	-	-	✓	-	-	(1	Wide range	>1	✓	✓
g-H2AX	✓	-	-	✓	-	✓	3	Genotoxins	0.5 to >8	✓	✓
CRP	✓	-	-	✓	-	-	1	Wide range	>1	✓	✓
SA	✓	-	-	✓	-	-	1	Wide range	>1	✓	✓

Protein Biomarkers

Irradiation causes biological changes on the level of proteins abundance and enzymatic activities. These protein biomarkers can be identified in blood and urine. The most promising markers for dose assessment are γ-H2AX, C-reactive protein and Serum amylase. Ionizing radiation induced DNA double strand break result in the activation of the DNA damage signaling factor γ-H2AX. These foci of γ-H2AX are expressed within minutes following radiation exposure in a dose dependent manner. Their level peaks in less than 1 hour but drastically decreases afterward and reach baseline within one to several days. The rapid losses of γ-H2AX foci in a short time interval decrease the sensitivity of the assay when used in exposures that occurs before one or more days [4].

C-reactive protein (CRP) is a well know biomarker for inflammation with wide clinical applications. Exposure to high dose of radiation induces an increase in the level of CRP for several days. Since CRP is triggered by many medical conditions, it is not a specific marker of radiation exposure [4, 31].

Serum amylase is another inflammatory marker which increases as a result of salivary gland irradiation. Consequently, this biomarker can only provide information regarding the dose received by the salivary glands. Serum amylase level reach a maximum 18 to 30 hours post radiation exposure and then return to normal within few days [4, 31].

The protein biomarkers discussed here have common disadvantages such as radiation non-specificity and inter-individual variation of the biomarker baseline level. Thus, they are unsuitable as a stand-alone biodosimetric method. However, they can be used as a fast tool for preliminary dose estimation in case of mass causalities while waiting for the results of more accurate assays [4, 31].

1.5. Dose response relationship

Influence of radiation quality

Ionizing radiation ejects electrons from atoms through which it passes leading to ionizations and excitations along the track of an ionizing particle. Linear energy transfer (LET) is defined as the amount of energy deposited by a particular radiation per unit of track length and is important in determining the effectiveness of a particular radiation type in inducing various biological effects [27].

Radiations of different LET (i.e. radiation quality) have different distribution of ionization which leads consequently to a change in the frequency of chromosome aberrations distribution between cells. In the case of low LET, the ionization will be distributed randomly between cells at any dose, given that a large number of tracks is induce by these sparsely ionizing radiation. Since the resulting DNA damage will be distributed randomly between cells and while assuming that an equal probability of any damage can be converted into an aberration, thus, the aberrations will be distributed randomly between cells as well. This is the case for X or γ irradiation, where the generated chromosome aberrations follow a Poisson distribution. However, as opposed to low LET, high LET or densely-ionizing radiation will induce non-randomly distributed ionization tracks between cells since the energy will be deposited in more 'discrete packets'. Therefore, the number of tracks will be much lower than with low LET radiation and the induced aberrations will be non-randomly distributed between cells [27].

Dicentric aberrations are exchange between two damaged chromosomes that result from radiation induced misrepair of DNA strand breaks or misrepair during the excision repair of base damage. Therefore, in order for dicentric aberrations to take place, the lesions should occur in closely located chromosomes within a so called 'rejoining distance' which is generally less than 1.0 μ m in diameter [27].

Since X-rays have low LET and consequently will induce a lower frequency of ionization per unit track length, the probability that a single track will produce two ionizing events within the target is low. In order to produce a dicentric, two different ionizations are needed in two neighboring chromosomes. The probability that ionization from two independent tracks will cause two lesions is much higher. The frequency of dicentrics resulting from ionization from one track is proportional to a linear function of dose, whereas the frequency of dicentrics induced by two different tracks is proportional to the square of the dose [27].

The probability that two tracks will traverse a target at similar time interval is low for doses below 0.5 Gy. As a consequence, dicentrics will be induced by one ionization track and at a low frequency. As the dose increases, the number of dicentric aberrations induced by two tracks will also increase. Thus the dose–response curve representing the yield of dicentrics induced by low LET (figure 3) is a combination of one- and two-track events [27].

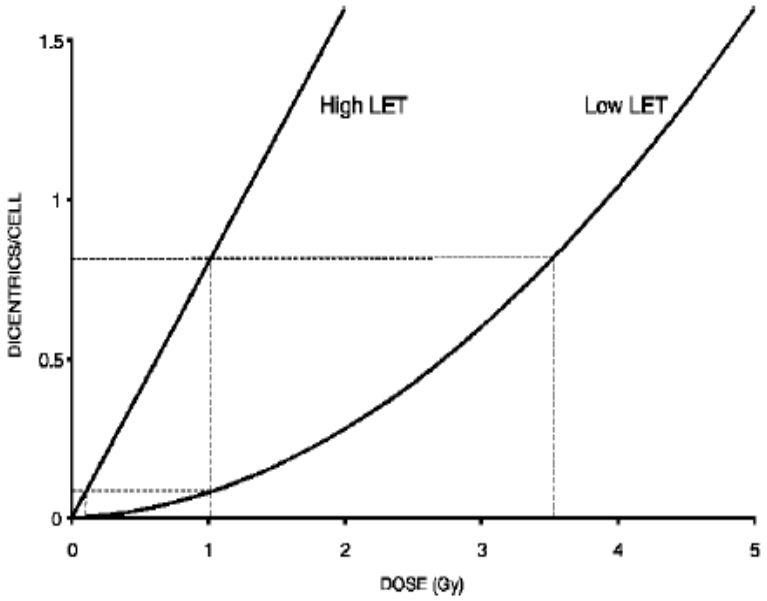

Figure 3: linear and linear quadratic dose response curves for different radiation qualities [27]

The dose–response curve for the yield of exchange aberrations is given by the equation $Y = C + \alpha D + \beta D^2$.

Where *Y* is the yield of dicentrics, *D* is the dose, *C* is the control (background frequency), α is the linear coefficient, and β is the dose squared coefficient [27].

The α / β ratio or cross-over dose is the dose at which both the linear and quadratic components lead to the formation of dicentrics equally. As the LET of the radiation increases, the probability of two lesions produced by two ionizing events within the same track increases as well [27].

The radiation quality (LET) has an influence on the on the shape of the dose–response curve. Therefore, the reference dose–response curve used for the estimation of an exposure dose should be that of a radiation quality which is similar to the type of radiation involved in the emergency [27].

Relative biological effectiveness (REB)

The relative biological effectiveness (RBE) of the higher LET radiation, defined as the effectiveness of radiation in producing a particular biological endpoint (in this case for inducing dicentrics), increases with increasing LET due to the increased probability that the two damages will be induced by one track. It is more efficient to induce two lesions by one track than by two random tracks that take places in a close distance especially at low doses since the track density is low. RBE is maximal for LET radiation where ionization is optimal and induces damage in the two DNA helices involved in the dicentrics formation without wasting any additional energy/ionization in the target. However, above this optimal value, RBE will decrease as LET increases since excess of energy will be deposited in the target [27].

Influence of the dose rate

DNA lesions that are converted into dicentrics can be repaired in a few minutes up to several hours. If the two lesions leading to dicentric are induced by separate tracks, there is a probability that the dicentric will not be produced if the dose rate is reduced. This is due to the fact that the lesion produced by the first track may be repaired before the second lesion is formed by the second track. Therefore, the probability of two lesions interaction resulting in dicentric production decreases. Moreover, for low dose rate, there is a longer time frame available for the repair of the first lesion before the second is produced since the frequency of ionization tracks per unit time is lower. For low LET radiation decreasing the dose rate results in a reduction in the frequency of dicentrics per unit dose [27].

However, in case of high LET radiation, both lesions that results in the production of dicentrics are induced by a single track, and therefore, decreasing the dose rate have no influence on the frequency of dicentrics. This can be explained by the fact that lesions repair mechanism during longer exposures does not have an influence since both lesions are induced by a single track [27].

1.6. Application in MASS CASUALTY radiation accident

Different studies confirm that the dicentric assay provides accurate dose estimation that can be used in assessing health risk and guiding medical treatment decisions during large scale radiation accidents. Furthermore, the dicentric assay is referred to as the gold standard technique of all biological dosimetric assays for use in large scale accidents. Partial body and protracted exposures can be derived from the dicentric assay through mathematical models [27].

In the case of an emergency situation, the biological dosimetry can be used to distinguish between exposed individuals who require urgent medical intervention and the “concerned public” [28]. In the early stage of response to a radiation emergency, the purpose of cytogenetic assays is to provide a rapid triage of victims into different categories of radiation exposure (1 Gy

to 2 Gy, 2 Gy to 4 Gy, 4 Gy to 6 Gy and > 6 Gy) in order to supplement physicians with clinical assessment. In addition, biological dosimetry can help in identifying false positive samples i.e. patient that are falsely diagnosed with radiation exposure due to symptoms that can be caused by other factors such as stress and anxiety [26]. Different strategies were developed to help cope with a high number of exposed individuals, such as restrictive analysis of only 20 to 50 metaphases or 30 dicentrics [33]. This analysis technique provides dose estimation with an uncertainty of ±0.5 Gy which is a satisfactory confident interval for the estimation of exposure level in a triage mode [27; 34]. Once the exposed victims are identified and people with severe injuries are treated, more accurate dose assessment can be provided for patients with high preliminary doses [3].

Since a large number of individuals are analyzed during an emergency, a single cytogenetic laboratory is not capable of analyzing the exposure level of all the victims. Hence, collaboration between different laboratories is vital in these situations where the capability of individual laboratories is likely to be overwhelmed and has been established in recent years on both national and international level [33].

Different networks have been established on the European and global level. In Europe, RENEB project (Running the European Network of Biodosimetry) was established in 2012 by 23 organizations from 16 European countries with the aim of initiating a European Biodosimetry network to increase the capacity of performing rapid individual dose estimation in case of large scale radiation emergency. The network operates five biodosimetric tools (i.e. dicentric assay (DCA), FISH assay (FISH), micronucleus assay (MN), premature condensed chromosome assay (PCC) and gamma-H2AX assay) and two retrospective physical dosimetric tools (i.e. electron paramagnetic resonance (EPR) and optically stimulated luminescence (OSL)) [3]. The RENEB project insures the harmonization and standardization of the dosimetry assays between the participating laboratories [3, 35]. Other objectives of the RENEB are the continuous education and training of existing as well as new members in order to ensure the preparedness of the network for any potential event. Furthermore, this network encourages the development of new triage tool for individual dose estimation [3, 35].

On the global level, International Atomic Energy Agency (IAEA) launched a Response Assistance Network (RANET) in 2006. RANET which includes biodosimetry, was the result of the work of the global Emergency Response Network (ERNET) to increase preparedness to radiation emergencies [36].

In 2008, World Health Organization (WHO) established a global Network of biodosimetry laboratories called WHO *BioDoseNet*whose main role is to promote collaboration in cases of large scale radiation emergencies [37].

In order to make laboratories networking possible, the dicentric assay should be standardized and harmonized between different laboratories and frequent inter-comparisons should be performed to confirm good consistency in the results of dose assessment [27].

1.7. Overview of the automatic dicentric assay

First attempt to establish the automatic dicentric scoring was in the 1980s with the development of a software capable of detecting dicentric chromosomes [38]. However, the first scoring system had very restrictive scoring criteria such as only complete metaphases with 46 centromeres were included in the analysis. The restrictive requirements were not fulfilled by the software because of the various differences in the size and position of chromosomes in metaphase between different cells. Therefore, the qualities of the metaphase spreads have an important influence on the number of chromosomes detected by the software per image. The human scores have better analytical skills and consequently are able to detect more dicentrics than automated machines. Another problem of the automated scoring is the inability to recognize which chromosomes belong to which cell when two adjacent cells are captured in one image. Instead, the system assumes that all the chromosomes belong to a single cell. For this reason, the system is instructed to reject images with more than 55 objects. The number was chosen to be 55 objects to take into consideration some cell nuclei in the image that will be considered as chromosomes by the software [38]. More improvements have been introduced to the automatic scoring procedure over time. Also comparison of manual and automated scoring was an important aspect to investigate.

The automatic dicentric analysis was used for the first time for biological dosimetry to estimate dose in a triage mode during the Dakar accident that occurred in 2006. The accuracy and reliability of the automatic assay were investigated by comparing the estimated exposure dose of 63 victims obtained from the automatic and manual scoring. It was concluded that the automatic scoring provides more accurate dose estimating due to the fact that the number of scored cells is higher. In addition, dose estimations were obtained in a shorter time frame than those based on the manual analysis [39]. When the automatic scoring was used, a fewer number of individuals were misclassified as compared to the triage mode of the manual scoring during which only 50 cells are analyzed. Another study showed that automatic dicentric scoring give similar dose estimating as manual scoring in case of partial body exposures [40].

In recent years, several researches have focused on optimizing the semi-automatic dicentric assay. There have been improvements to the classifier used for the automatic detection of dicentric over the years. The first classifier used at the BfS was established in 1980s based on 1200 Giemsa stained metaphases containing 1304 dicentric chromosomes. This classifier was able to detect 50.2% of the dicentrics. In 2008, another classifier was developed at IRSN according to the scoring procedure applied and the classifier at the BfS was improved in 2010. A comparison of the three different classifiers was done as part of the MULTIBIODOSE EU FP7 project. Both new classifiers were better at detecting dicentrics, with a decrease in the number of false positive dicentrics by a factor of two. This result in a reduction in the time needed to score dicentric which allows dose estimates to be available faster. However, the dicentric assay should not be performed in a fully automatic manner, since there is a high number of not true dicentric chromosomes (false positive) detected at low dose which should be rejected by the human

scorer. The number of undetected dicentric chromosomes (false negative) increases with higher dose. The two new classifiers have the same rate of FN dicentrics detection [38].

The Multibiodose project done by a network of six European biodosimetry laboratories also concluded that software based automatic scoring of dicentric is a reliable and reproducible method of dose assessment in case of MASS CASUALTY. Despite inter-laboratories variation in culturing and slide preparation as well as the use of two different classifiers for automatic dicentric detection, there was no statistically significant difference in the dose effect curves of the six laboratories that participated in the study. The study showed that similarly to manual scoring, dicentric detection by semi-automatic scoring is also governed by the Poisson distribution. Automatic scoring can be used for the detection of partial body exposure. Moreover, a common calibration curve was established in this study, which can lead to inter-laboratories cooperation in case of large scale radiation incidences and a faster dose assessment [38].

Another study was performed by the NATO Research Task group RTG-003 to compare the manual dicentric scoring in the triage mode with the automated scoring with regard to the precision and reliability of the dose estimates and the time required to provide the results. This study was done in 6 different laboratories and inter-comparison of the results of the dose assessment was performed. X-Ray irradiated blood samples stimulating whole body irradiation (0.25-5 Gy) and blind samples (0.1-6.4 Gy) were sent to all laboratories. The study concluded that both scoring procedures have similar accuracy, sensitivity and specificity [41].

Gruel et al. 2013 evaluated the capability of automatic scoring in a simulated emergency involving whole and partial body exposures to classify victim in the correct dose range based on data obtained from the first slide. The study indicates that ADS provide preliminary triage dose estimations after the analysis of 300 to 400 cells. In a triage mode, no major different were shown between ADS (automatic dicentric scoring) of 300-400 cells and manual scoring of 50 cells. An important disadvantage of the quick dose estimation provided in a preliminary triage is its associated uncertainty. However, to obtain more accurate dose estimation for triage using the ADS a minimum of 1000 cells should be analyzed. While taking into account that the time needed to manually score 50 cells is equivalent to the analysis time of 1000 cells using the automatic scoring, ADS gives more precise dose estimation. The results indicate that partial exposure to doses lower than 2.5 cannot be detected when automatic scoring is used for triage. This is also the case when analysis is done using manual scoring of 50 cells. However, at high doses, both manual and automatic scoring were able to detect partial body exposure. In this case, 3000 cells should be analyzed for preliminary dose estimation [42].

2. Methods

2.1. Irradiation

Blood samples (10 ml heparinized tubes) from onehealthy donor (female) were irradiated with 137-Cs gamma rays (dose rate 0.495 Gy/min) in a HWM D 2000 unit (Wälischmiller Engineering GmbH, Markdorf, Germany; formerly Hans Wälischmiller GmbH). Peripheral blood samples were obtained, with informed consent, from a healthy adult donor. The whole blood samples were irradiated with 0.1; 0.25; 0.5; 0.75; 1; 1.5; 2; 3; 4; 5 and 6 Gy. One unirradiated sample served as control. After irradiation the samples were incubated for 2 h at 37 ° C before culture initiation.

2.2. Cell cultures

Blood cultures were set up according to the protocol established at Bundesamt für Strahlenschutz (BfS) [43] which follows the IAEA recommendations [44] and the ISO standards [37].

Whole blood was transferred to culture tubes (10 ml) containing RPMI-1640 culture medium (Biochrom, Berlin, Germany) supplemented with 10% FCS (Biochrom, Berlin, Germany), 2% PHA (Biochrom, Berlin, Germany) and antibiotics (Biochrom,Berlin, Germany). For cell cycle controlled scoring long-term Colcemid treatment (Roche, Mannheim) with a final concentration in culture of 0.08 mg/ml was added 24 h after culture set up. Blood samples were cultured in total for 48 h. The hypotonic treatment of cells was carried out with 75mM KCl. Cells were then fixed in methanol:acetic acid (3:1) three times and the suspension was stored in the freezer (–18 °C) [27].

For slide preparation, the cell solution was concentrated according to the cell yield. The quality and quantity of the metaphases were checked under the microscope. The slides were stained for 5 min in Giemsa-solution (20 ml Giemsa and 230 ml PBS buffer). After 5 min, the slides were rinsed 3 times in distilled water. The slides were stored overnight to dry and then covered with mounting medium (Eukitt) and coverslips. The slides were allowed to dry for an additional day before analysis [27].

It should be noted that cell culturing and slides preparation technique have an impact on the quality of the slides. A good spreading of the chromosomes is needed to make analyzing easier and more effective. Especially in the case of automated scoring procedure, the quality of preparation has a major impact on the quality of the results. Therefore, Preparation with less background dirt and chromosomes with clear centromeres are required. Figure 4 present few examples of metaphases that should be rejected [27].

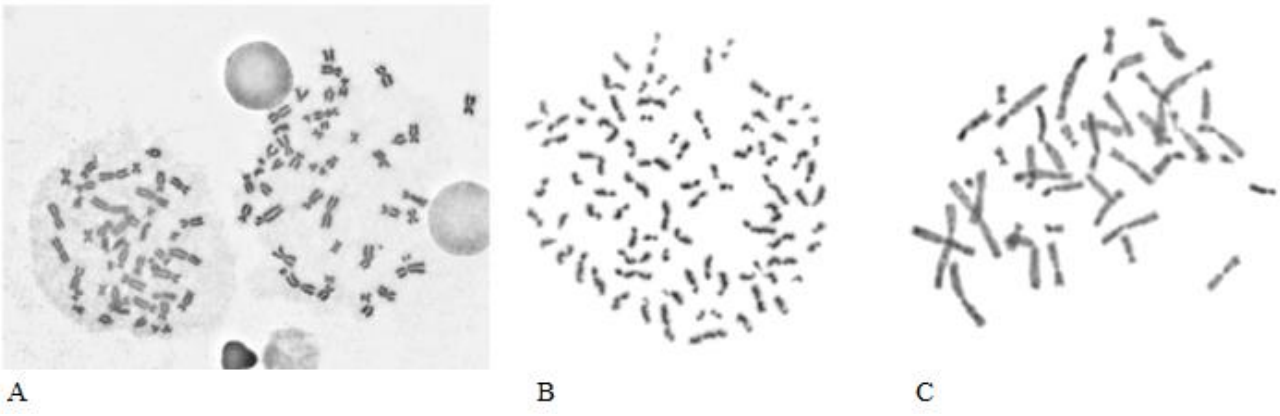

Figure 4: examples of cells that should be rejected; (A) two cells are located very close to each other (cells not spread properly); (B) and (C) chromosomes did not reach the metaphase and the centromere is not visible.

2.3. Scoring methods

2.3.1. Establishing dose effect curves by automatic scoring procedure

Analysis of the slides was done using the automatic scoring system Metafer 4 by MetaSystem (Altlussheim, Germany). Three slides were scored per dose point. Automatic scoring involves metaphase finding (Msearch), auto-capturing of high resolution images (Autocap) (figure 4) and finally automatic detection of dicentric (DCScore). Images were auto-captured at both 63x magnification (with oil) and 40x magnification (without oil).

The same cells were captured with both 63x and 40x objectives in order to perform a direct comparison of the behavior of both objectives. Out of 41168 captured images, 27992 cells were semi-automatically scored for the presence of dicentrics using the 63 x magnification and 23224 cells were scored with the 40 x objective (table 3).

According to the parameters of the DCScore Classifier used at BfS (table 2), images with less than 9 and more than 55 objects were automatically rejected by the system. Other important parameters to be fulfilled by the metaphase chromosomes in order to be accepted for analysis by the DCScore classifier are summarized in table 2. This classifier was established at BfS in 2010 and was based on 3804 Giemsa stained metaphases with 2303 marked dicentrics [38].

Table 2: the parameters of the DCScores classifier at BfS [38]

DCScore classifier, version 3.6.7	
Minimum chromosome width (pixel)	14
Maximum chromosome width (pixel)	32
Minimum chromosome length (pixel)	16
Minimum chromosome area (pixel)	200
Maximum chromosome area (pixel)	3000
Minimum chromosome aspect ratio (in/100)	126
Minimum chromosome area/contour length ratio (in/100)	229
Minimum number of chromosomes in metaphase cell	9
Maximum number of chromosomes in metaphase cell	55

There is one major difference between the semi-automatic scoring and conventional manual scoring. In manual scoring, the human score will count the chromosomes and only complete cells with 46 centromeres are included in the analysis of dicentric frequency. In semi-automatic scoring, the software decides which cells are included in the analysis according to the established classifier parameters and searches for dicentric candidates. Then, an experienced human scorer validates the dicentric candidates detected by the software (which are marked in a red frame) in a short interaction and decides to accept them as dicentric chromosomes or reject them as false positive (Figure 5). This procedure is well established in the biodosimetry laboratory of the BfS.

It should be noted that the difference between automatic and semi-automatic scoring is that the later involve the validation of the automatically detected dicentrics by a human scorer. This means that semi-automatic scoring doesn't rely completely on the software for the analysis since machine algorithms are not 100% accurate.

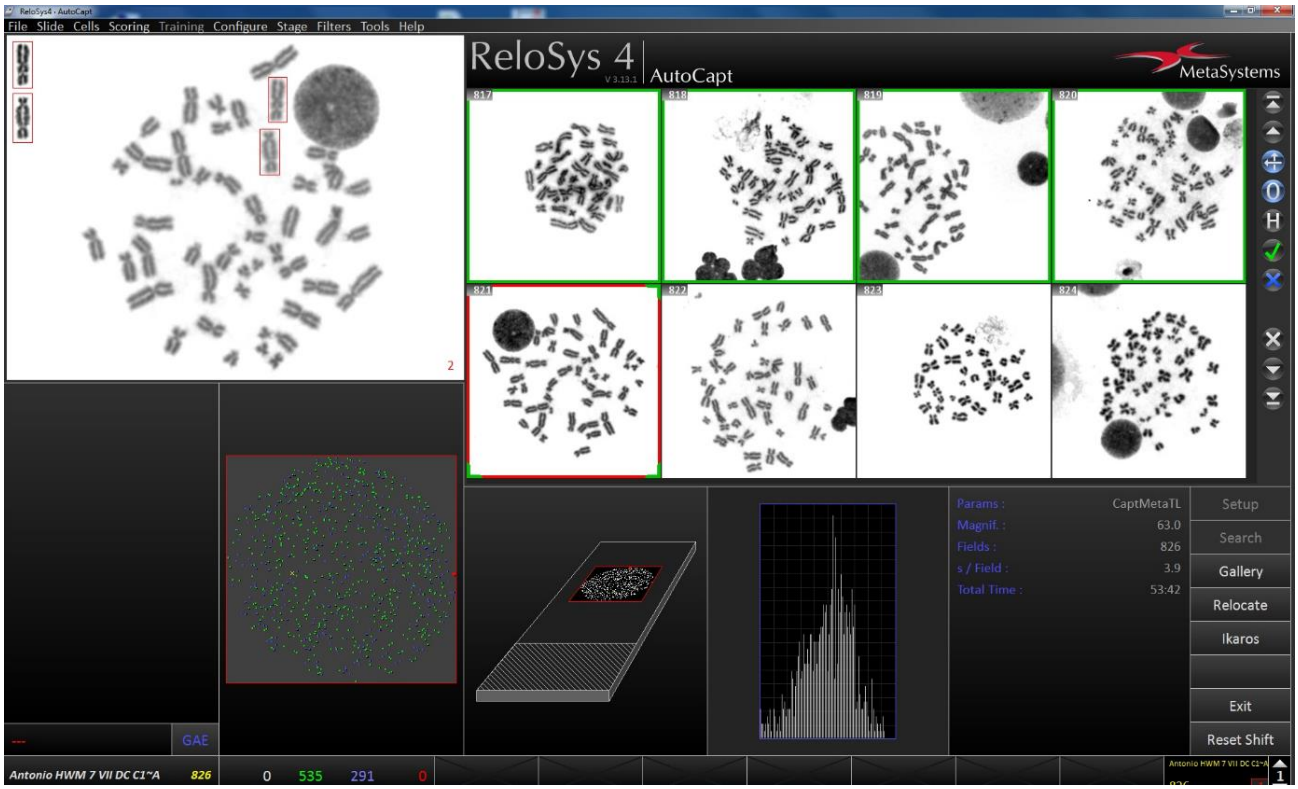

Figure 5: Captured metaphases used for the detection of dicentric candidates with DCScore software tool are shown. On the left, the current metaphase is shown with the detected dicentrics marked in a red frame. These dicentrics are also shown upright on the left edge of the image. The right part of the figure represents the gallery of acquired metaphases. All images in this figure have been captured with a 63x oil objective.

For the 40x objective the DCScore software tool is not yet fully developed by Metasystems and needs to be adapted to the lower magnification. Therefore the software was unable to mark correctly the detected dicentrics with a red frame (figure 6).

To overcome this problem and to have the possibility to compare the results, the scoring methods for both magnifications were adjusted as follows.

First, all the cells that were marked with aberrant chromosomes by the software were evaluated for the presence of dicentrics by the human scorer and the number of dicentrics detected by the scorer was included in the protocol as confirmed dicentrics (CDics). This was performed for all dose points (i.e. 0 to 6 Gy) and for both magnifications (40x and 63x objectives).

In a second scoring mode, all the cells that were accepted for scoring by the software were analyzed by the human scorer; dicentrics were recorded and included in the protocol. This was done to identify false negative (undetected dicentrics) and also false positive dicentrics (not true dicentrics). The evaluation of false negative was done only for 1 and 4 Gy dose points. This same scoring procedure was also performed for images captured with the 63x magnification.

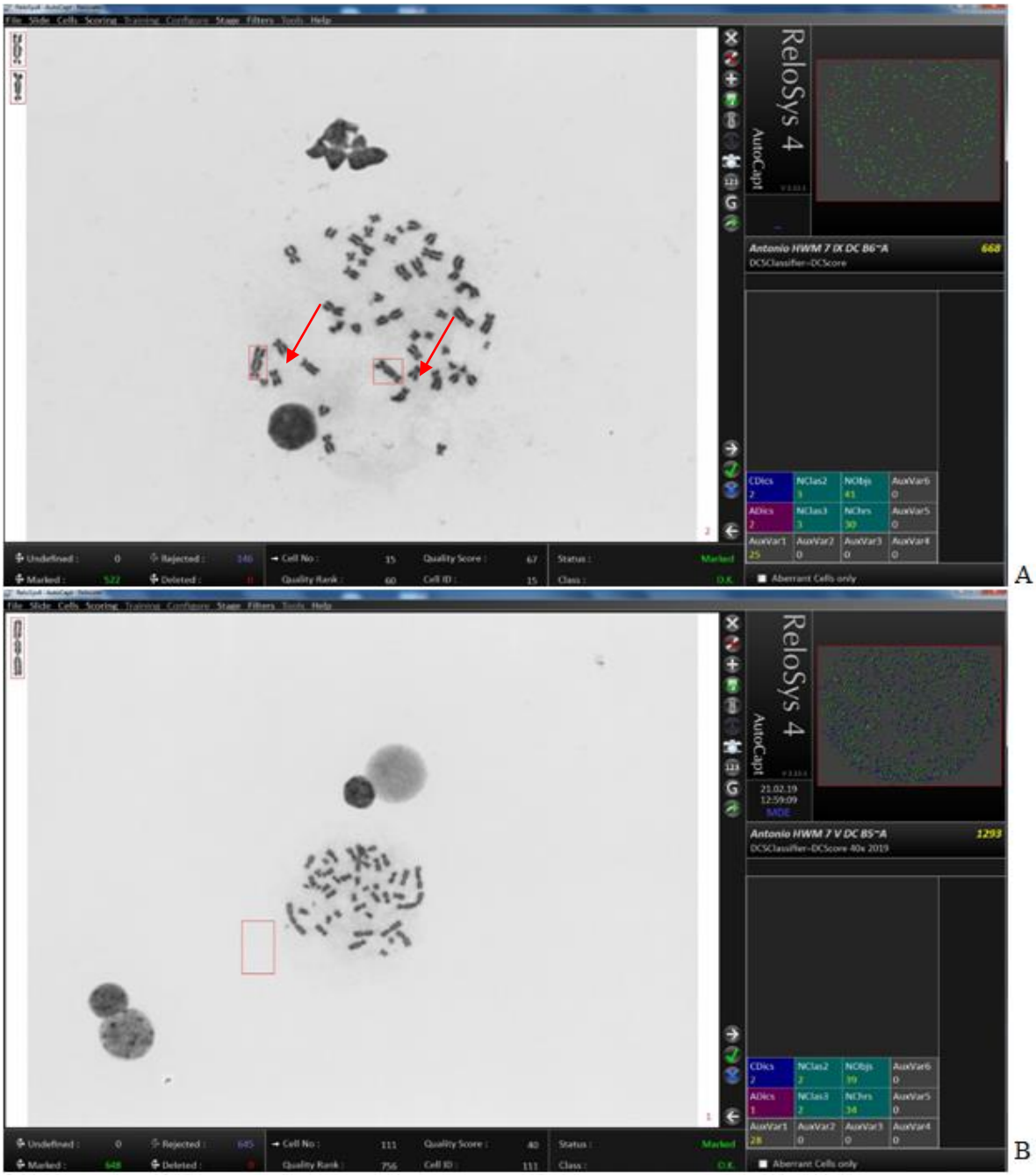

Figure 6: (A) Two dicentrics detected by the DCSscore software are marked by a red frame. These dicentrics are also shown upright on the left edge of the image. The image is acquired by with a 63x oil objective. (B) For images acquired by 40x objective (without oil), the frame is not located on the dicentric although the dicentric was shown on the left edge of the image. The recorded scoring includes the number of automatically detected dicentrics (ADics) as well as the number of dicentrics confirmed by the scorer (CDics).

2.3.2. Comparing automatic and conventional manual scoring

In addition to automatic scoring, conventional manual (by eye) scoring was performed for samples irradiated with 1 and 4 Gy. For the manual scoring, a total number of 1154 cells were analyzed. For each dose point, 2 slides were scored manually by eye. For 1 Gy, 1054 cells were analyzed; however, a smaller number of cells (100 cells) were scored for the slides containing cells irradiated with 4 Gy since at this high dose a larger number of dicentric aberrations are produced (table 6).

Conventional manual scoring is the most accurate method of dose estimation and in routine 500 cells are analyzed in the frame of biological dosimetry. Nonetheless, scoring can be terminated after the detection of 100 dicentrics in the case of high doses. For manual dicentric scoring, only complete cells containing 46 centromeres were analyzed. Furthermore, metaphases should have good morphology and few overlapping chromosomes.

First, the chromosomes were scored in the microscope. Then, for cells with 46 centromers, the chromosomes were rearranged in a karyogram based on their size and the position of centromere for chromosomes of the same size. All observed aberrations such as dicentric chromosomes, excess acentric fragments, centering rings were recorded (figure 7). The IKAROS software was used to perform karyotyping.

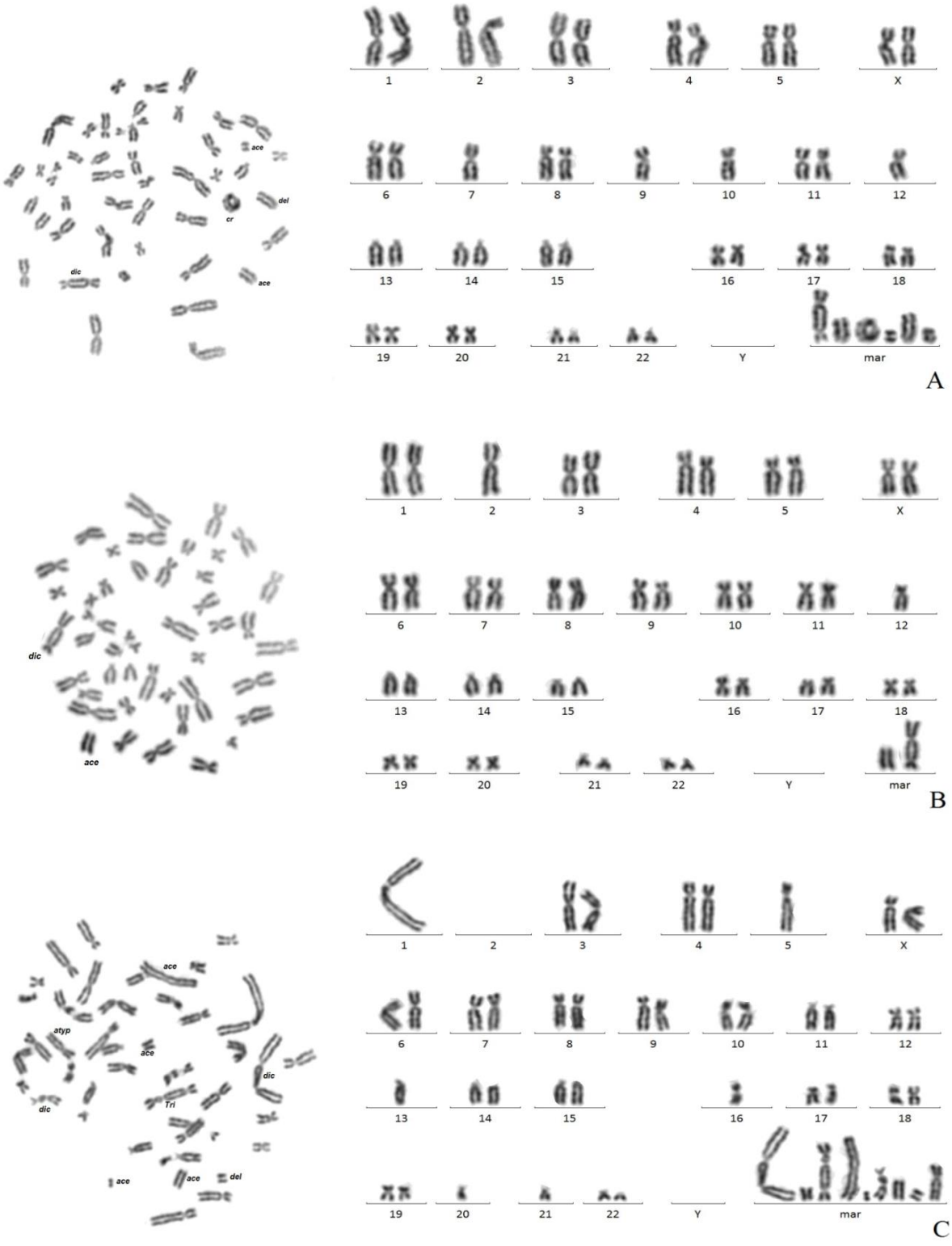

Figure 7: Karyogram of human using Giemsa staining representing cells with different types of chromosome aberrations. (A) Cell with 48 pieces containing one dicentric chromosome with an acentric fragment, one chromosome ring with an acentric fragment and two deletions. (B) Cell with 47 pieces containing one dicentric chromosome with an acentric fragment. (C) Cell with 47 pieces containing two dicentric chromosome with their corresponding acentric fragments, one tricentric chromosome with two acentric fragment and two deletions.

3. Results:

3.1. Comparison of time required for automatic scoring between 63x and 40x objective

When a 63x oil object is used for auto-capturing of the images, the total time necessary to analyze 100 metaphases is approximately 8 minutes (figure 8). The majority of this total time is required for capturing of high resolution images (6.49 minutes/ 100 images) while the two other steps involved in the automatic analysis (metaphase finding and image analysis) are accomplished in a small portion of time. However, using a lower magnification (40x objective) decreases the images acquisition time drastically to 2.93 minutes. Consequently, this leads to a reduction in the total time for the analysis of 100 metaphases up to approximately 4.4 minutes. The results show that if a 40x objective is used, approximately 45% of the time required for automatic dicentrics scoring will be saved.

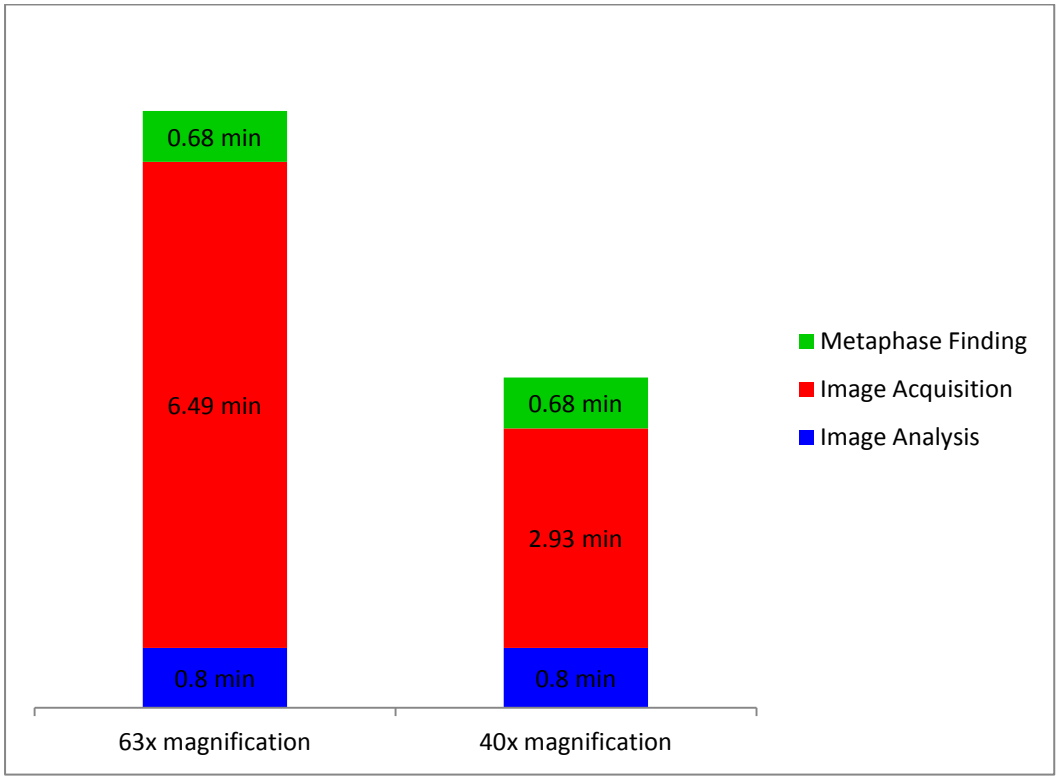

Figure 8: Time required for automatic dicentrics scoring when a 63x oil objective and 40x objective are used for image acquisition. Total time for metaphase finding, image acquisition and image analysis of 100 metaphases are shown.

Table 3: Number of analyzed images and observed yield of dicentrics following automatic dicentric scoring with 63 x and 40 x objectives. DA: automatically detected dicentrics, DC: confirmed dicentrics; RD: rejected dicentric candidates.

	Dose (Gy)	Captured images	Scored cells	Rejected cells	Rejected cells %	DA	DC	Dic/cell	SE	Distribution of dicentrics									Var(x)/x	u test
										0	1	2	3	4	5	6	7	8		
Semi-automatic (63 x)	0	3145	1931	1214	38.6	110	8	0.004	0.001	1923	8								1	-0.12
	0.1	5207	3164	2043	39.2	288	17	0.005	0.001	3147	17								0.99	-0.21
	0.25	5075	3414	1661	32.7	171	15	0.004	0.001	3399	15								1	-0.18
	0.5	5993	4268	1725	28.8	342	57	0.013	0.002	4213	53	2							1.06	2.66
	0.75	5807	3789	2018	34.7	350	89	0.023	0.002	3706	77	6							1.11	4.89
	1	4150	2731	1419	34.2	291	126	0.046	0.004	2618	100	13							1.16	5.96
	1.5	2610	1869	741	28.4	251	193	0.103	0.007	1703	139	27							1.18	5.43
	2	2179	1527	652	29.9	299	253	0.166	0.01	1320	164	40	3						1.22	6.16
	3	2896	2018	878	30.3	719	760	0.377	0.014	1491	343	145	31	6	2				1.4	12.64
	4	1839	1449	390	21.2	822	1032	0.712	0.022	875	269	183	97	20	4	1			1.55	14.71
	5	877	704	173	19.7	459	676	0.96	0.037	403	94	103	61	28	10	4	1		1.92	17.28
	6	1390	1128	262	18.8	1087	1549	1.373	0.035	481	181	202	153	68	29	11	2	1	1.69	16.3
Semi-automatic (40 x)	0	3145	1611	1534	48.8	88	2	0.001	0.001	1609	2								1	-0.02
	0.1	5207	2658	2549	48.9	213	13	0.005	0.001	2645	13								1	-0.17
	0.25	5075	2800	2275	44.8	130	17	0.006	0.001	2783	17								0.99	-0.22
	0.5	5993	3058	2551	42.6	212	45	0.015	0.002	3015	41	2							1.07	2.95
	0.75	5807	3318	2489	42.9	268	99	0.03	0.003	3224	89	5							1.07	2.93
	1	4150	2242	1908	46	209	101	0.045	0.004	2148	87	7							1.09	3.16
	1.5	2610	1601	1009	38.6	200	134	0.084	0.007	1484	101	15	1						1.19	5.27
	2	2179	1351	828	38	262	230	0.17	0.011	1163	147	40	1						1.2	5.33
	3	2896	1712	1184	40.9	483	485	0.283	0.013	1367	238	82	19	4	2				1.47	13.83
	54	1839	1265	574	31.2	669	800	0.632	0.022	806	227	150	63	12	6	1			1.58	14.69
	5	877	565	312	35.6	350	475	0.841	0.039	346	67	79	47	21	5				1.83	13.95
	6	1390	1043	347	25	894	1351	1.295	0.035	479	156	171	142	62	24	5	3	1	1.74	16.94

3.2. Establishment of dose effect curves by automatic scoring using 63x and 40x objective

In the current study, the automatic detection of the dicentric candidates was performed using the DCScore software module with the new BfS classifier (BfS-CLASS). This classifier was established at BfS in 2010 and was based on 3804 Giemsa stained metaphases with 2303 marked dicentrics. Out of 41.168 captured images, 27.992 cells were semi-automatically scored for the presence of dicentrics using the 63 x magnification (table 3). Additionally, from the same poor of captured cells, 23224 cells were scored with the 40 x objective. Thus, a higher number of cells were rejected when the analysis was performed with the 40 x objective.

Dicentrics detected by the software were either accepted as True Positives (TP) or rejected as False Positives (FP). Normal chromosomes are detected by the software as Dicentrics (FP) due to different reasons presented in figure 9. On the other hand, undetected chromosomes were noted as False Negative (FN) (Figure 10).

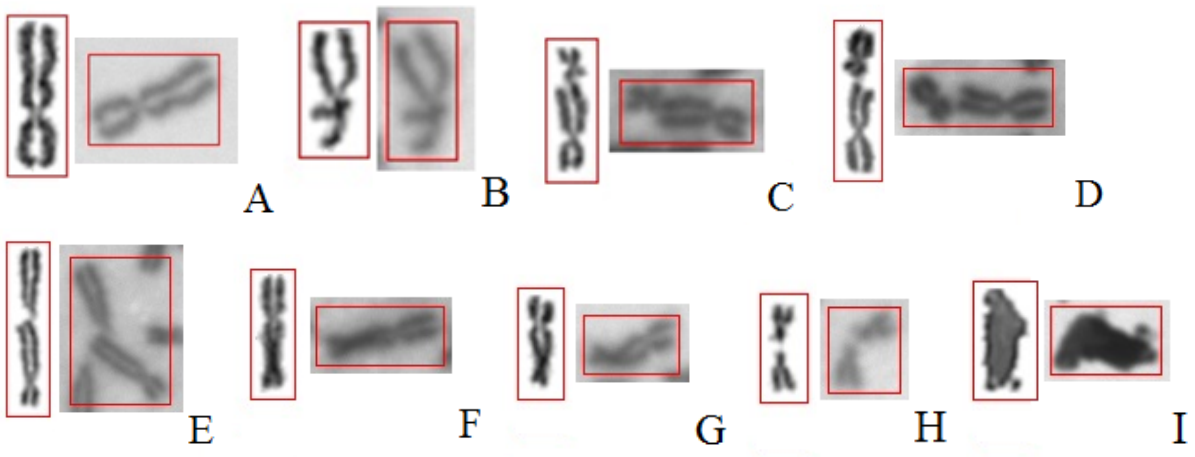

Figure 9: False Positive dicentric candidates that are rejected by the human scorer. (A, B) normal chromosome, (C, D, E, H) touching chromosomes, (F, G) chromosome with overlapping chromatids, (I) dirt detected as chromosome.

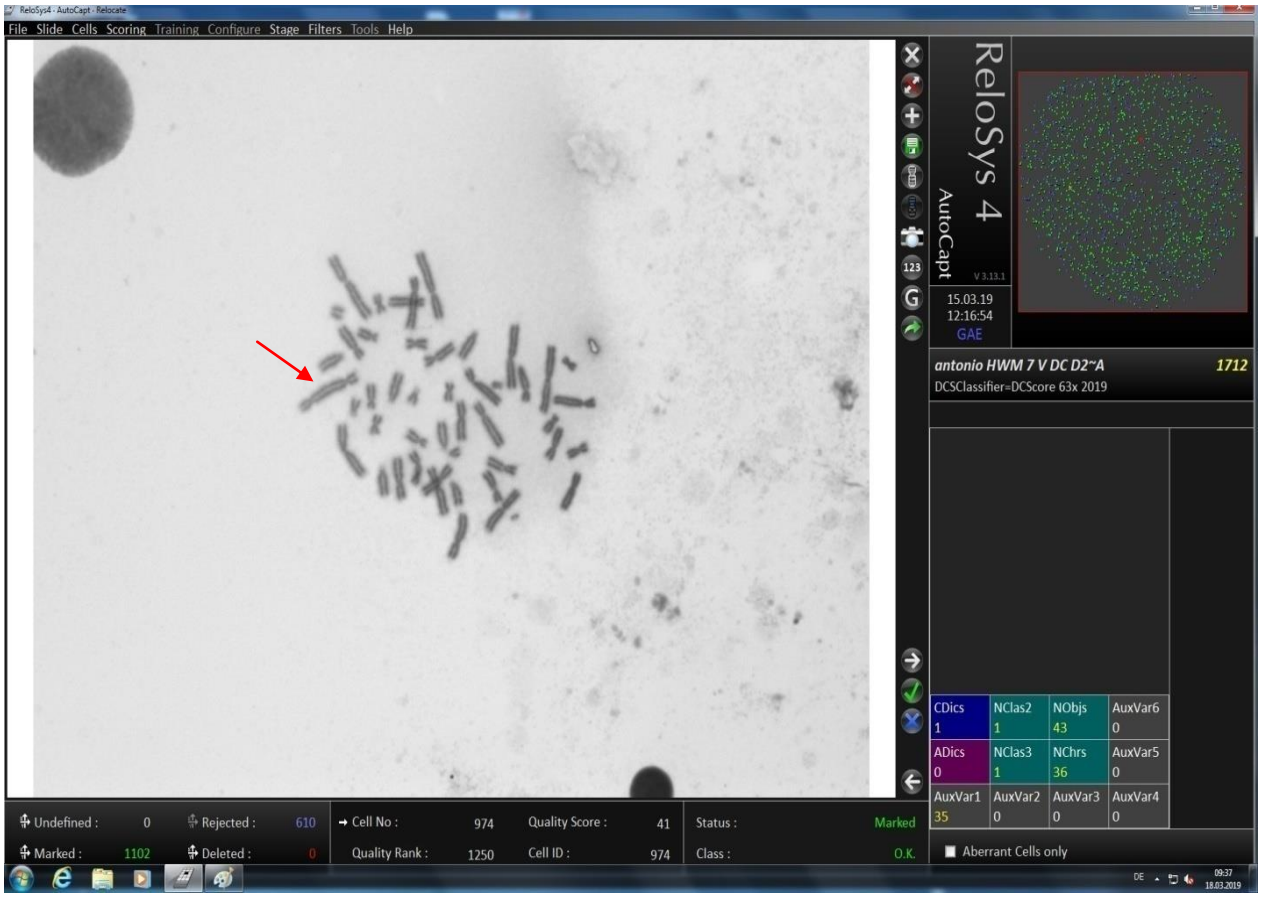

Figure 10: Example of undetected dicentric (FN) that is confirmed by the scorer (CDics 1)

All the cells that were marked with aberrant chromosomes by the software were evaluated for the presence of dicentrics by the human scorer and the number of dicentrics detected by the scorer was included in the protocol as confirmed dicentrics (CDics).

The dose effect curves resulting from automatic dicentric scoring using 63x and 40x objectives (figure 11) can be described by a linear quadratic curve of the form $Y = C + \alpha D + \beta D^2$ where *Y* is the yield of dicentrics, *D* is the dose, *C* is the control (background frequency), α is the linear coefficient, and β is the dose squared coefficient. The coefficient values of the curves derived from dicentric scoring with the 63 x and 40 x objectives are shown in table 4. While both curves merge at low exposure dose, the dose effect curve obtained from dicentrics scoring with the 40x objective is significantly lower than the 63x magnification at doses higher than 2 Gy (figure 11). A statistical comparison of the regression coefficients of the two curves was performed. The Z-Score p value for the beta coefficient (<0.0001) suggest a significant difference between the two curves fitted quadratic coefficients (table 5). The beta coefficient is significantly higher for the 63x objective. This indicates that at higher doses, a higher number of dicentrics were detected by the software and validated by the human scorer when analysis was

performed with the 63x magnification. The alpha coefficient is lower for 63x magnification, without any statistical significance. Thus, at lower dose, there is no significant difference in the yield of dicentrics between the two objectives.

Table 4: Estimated values of the coefficients of the dose effect curves obtained after semi-automatic scoring using 63x and 40x objectives.

		Estimate	SE	P-value
Semi-automatic (40 x)	C	0.0025	0.0009	0.0089
	α	0.0082	0.0033	0.0137
	β	0.3389	0.0011	<0.0001
Semi-automatic (63 x)	C	0.0036	0.0009	<0.0001
	α	0.0007	0.0031	0.8120
	β	0.4004	0.0011	<0.0001

Table 5: Statistical comparison of the model coefficients based on Z-Scores

	Z-Score	P-value
C	0.873	0.383
α	-1.627	0.104
β	4.014	<0.0001

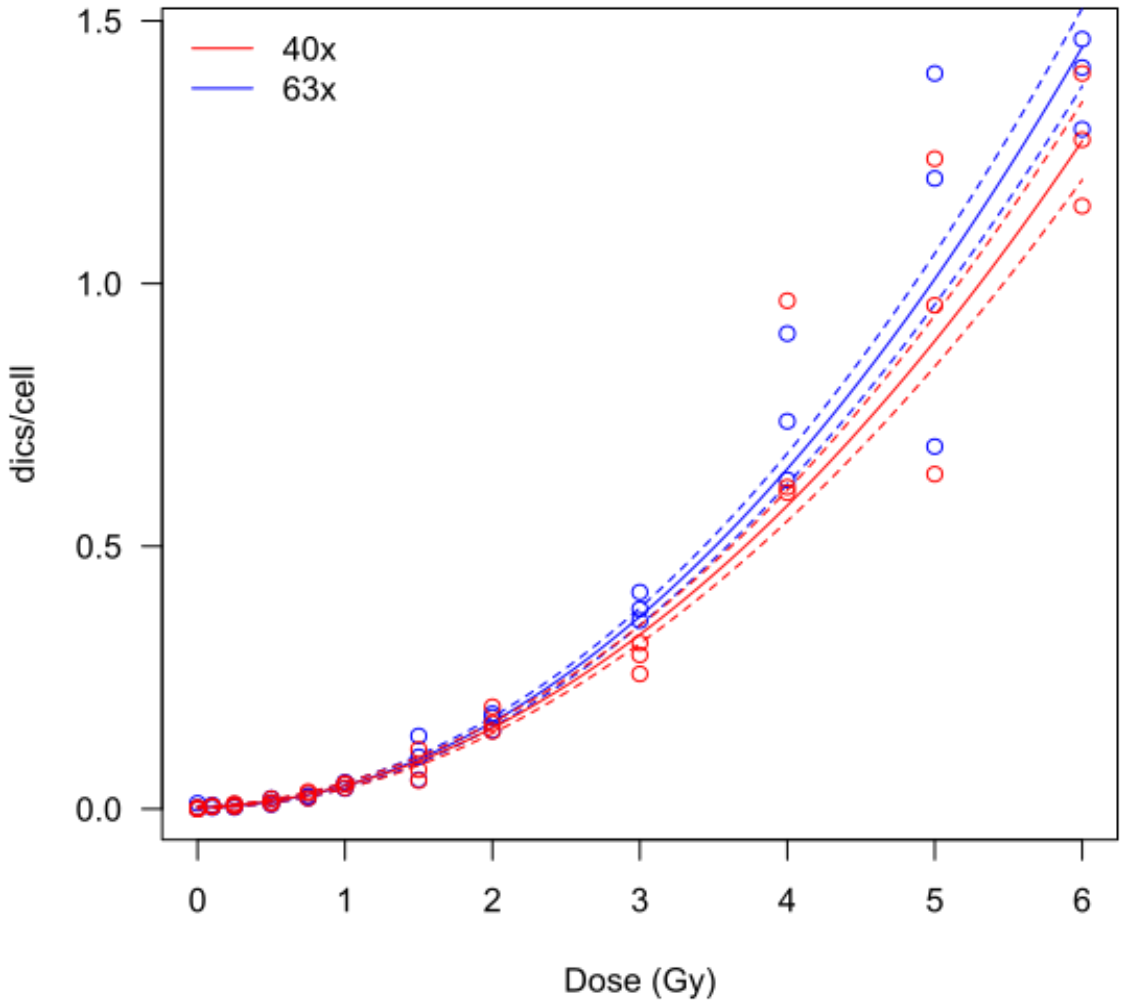

Figure 11: Dose effect curves of semi-automatic dicentric analysis using two different objectives for image capturing (63x objective with oil and 40x objective without oil). There is no significant difference between the curves while the curve of the 40x objective is slightly lower for doses higher than 2 Gy. Each dot represents the dicentric yield per slide. Three slides where evaluated for every dose.

Figure 12 indicates that the data are over-dispersed since the ratio of variance/mean is different than 1. In addition, this over-dispersion can be detected by u test values higher than 1.96 (P value < 0.05). It should be noted that since the data is over-dispersed, calibration curves were fitted based on the Quasipoisson model instead of the Poisson distributions.

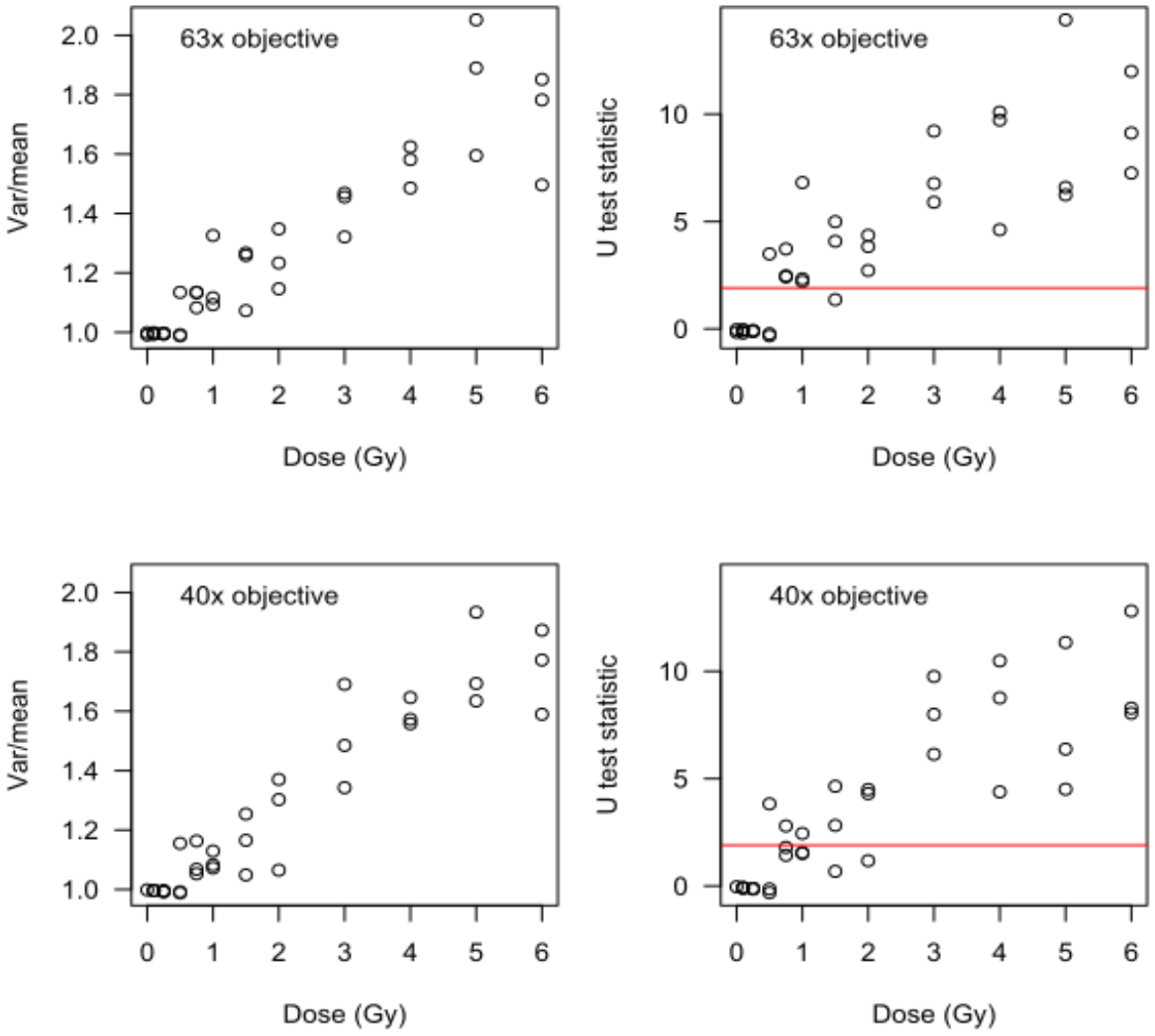

Figure 12: Over-dispersed of the data sown by the result of the variance/mean and u test statistic.

In order to determine if the two dose effect curves that were derived from scoring dicentrics from images captured in different magnifications can be used interchangeably, the doses for 63x objective were estimated based on the curve of 40x objective and vice versa. This was accomplished by using the number of dicentrics/cell derived from scoring done using the 63x objective to estimate the dose based on the dose effect curve of the 40x objective. The estimated dose - true dose was plotted (figure 13). If the 95% CI in the plot contains 0, the true dose was included in the CI and therefore, the estimated dose provides an accurate estimation of the true dose.

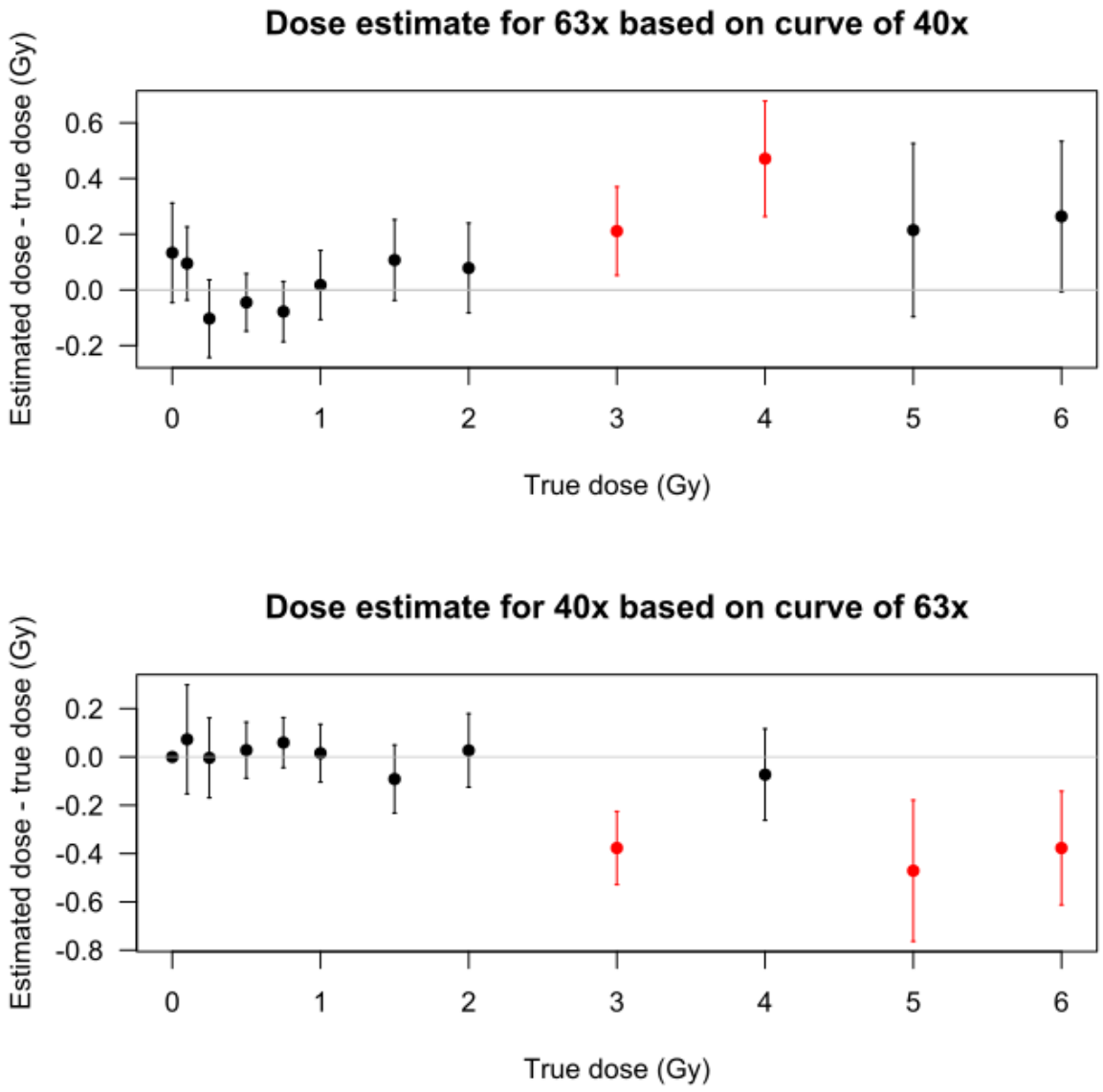

Figure 13: Plot of the estimated doses for 63x based on the curve of 40x and vice versa. If the 95% CI in the plot contains 0, the true dose was included in the CI.

The results shows that when the doses were estimated for samples scored with the 63x objective based on the 40x objective calibration curve, the data obtained from the analysis provided an accurate estimate of the actual dose except for radiation doses of 3 and 4 Gy. The data from scoring performed with the 40x can also be used to establish dose based on the calibration curve of the 63x objective except for doses of 3, 5 and 6 Gy.

3.3. Comparison of the rate of false positive and false negative between the two objectives

For the automatic capturing of images, two diferent objectives were applied (63x objective with oil and 40x objective without oil). In addition, two different DCScore classifiers were used for the automatic detection of dicentric candidates according to the objective applied. The efficiency of software in detecting dicentrics based on images captured in different resolutions was compared. In this step, all the cells that were accepted for scoring by the software were analyzed by the human scorer; dicentrics were recorded and included in the protocol. The rate of false positive (not true dicentric detected by the software) and false negative (undetetced dicentrics) obtained from scoring with 63x and 40x objectives are presented in figure 14. This evaluation was only done for slides irradiated with 1 and 4 Gy because of time constraint. It should be noted that 3 slides were iradiated with a dose of 1 Gy (HWM 7 V DC B5, D2 and D4) and another 3 slides with 4 Gy (HWM 7 IX DC B6, C2 and D2). For both magnifications, the same slides were used to have the possibility to compare the results directly. The results show that the mean false negative rate was 47.7% when the analysis was done with the 40x objective as compared to 55% with the 63x objective. On the other hand, the same mean false positve rate was achieved with both objectives being 0.11% with 40x objective and 0.1% with 63x objective.

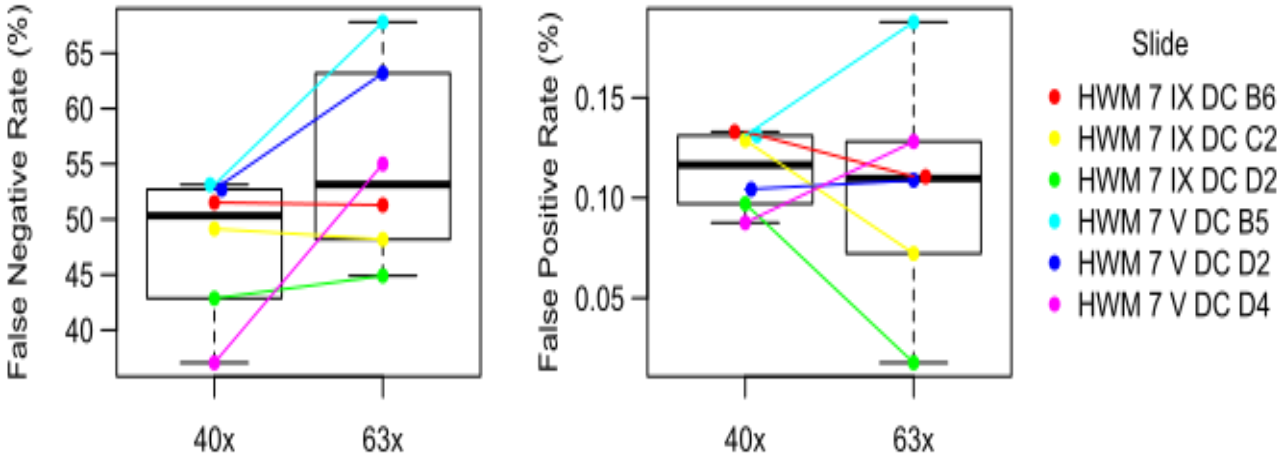

Figure 14: False positive and false negative rates for63x and 40x objectives. Results are presented for cells exposed to 1 Gy (slides HWM 7 V) and 4 Gy (slides HWM 7 IX)

3.4. Comparison of the percentage of cells accepted for analysis for both objectives

In the step involving automatic dicentric detection, images with more than 55 objects as well as metaphase spreads of poor quality are automatically rejected by the software. Although a

normal cell has 46 chromosomes, the number of objectives accepted for analysis is taken as 55 to account for chromosome deletions as well as cell nuclei present in the background. The number of cells accepted for dicentric analysis was compared between 63x and 40x objectives. About 80% of the cells from 63x magnification were also found in 40x magnification and about 97% of the cells found in 40x magnification were also found in 63x magnification (Figure 15).

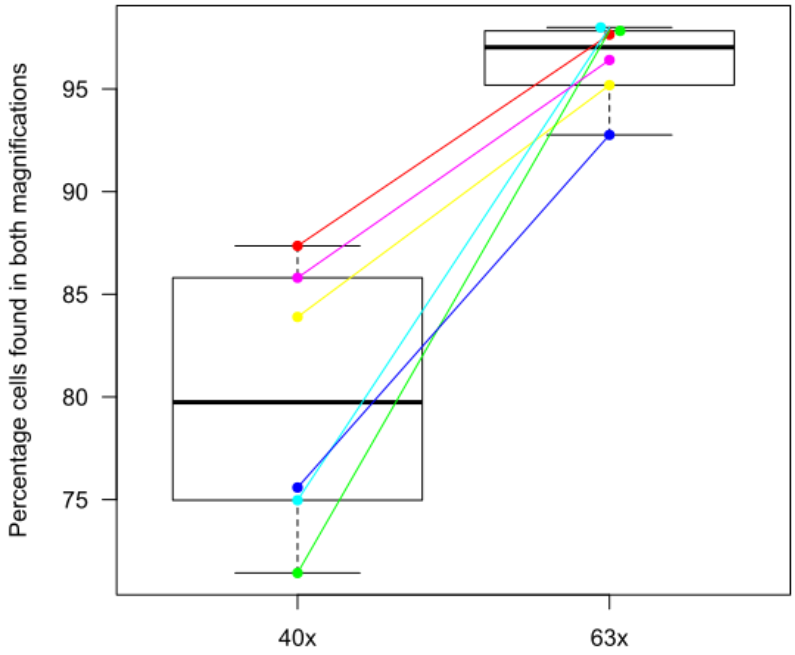

Figure 15: Percentage of cells found in both objectives for 1 Gyy and 4 Gy irradiated cells.

3.5. Comparison between full manual, automatic and semi-automatic scoring

For slides containing cells irradiated with 1 and 4 Gy, dicentrics were scored manully in addition to automatic scoring. For each dose, two slides were scored by the full manual mode. The number of cells selected by the human scorer for analysis was compared to the cells selected by the software. Between 68% and 94% of the cells from full manual mode were also detected by the software (Figure 16). For slides irradiated with 1 Gy, a higher number of cells accepted for the manuals scoring (i.e. cells with 46 centromers) was also detected automatically by the software in the case of analysis with 63x objective. The same result was obtained for slides irradiated with 4 Gy (e.g. for the slide HWM 7 IX D2, 76.5% of the cells overlap between manual and automatic scoring in case of scoring with the 40x objective as compared to 92.2% overlap for the 63x objective)

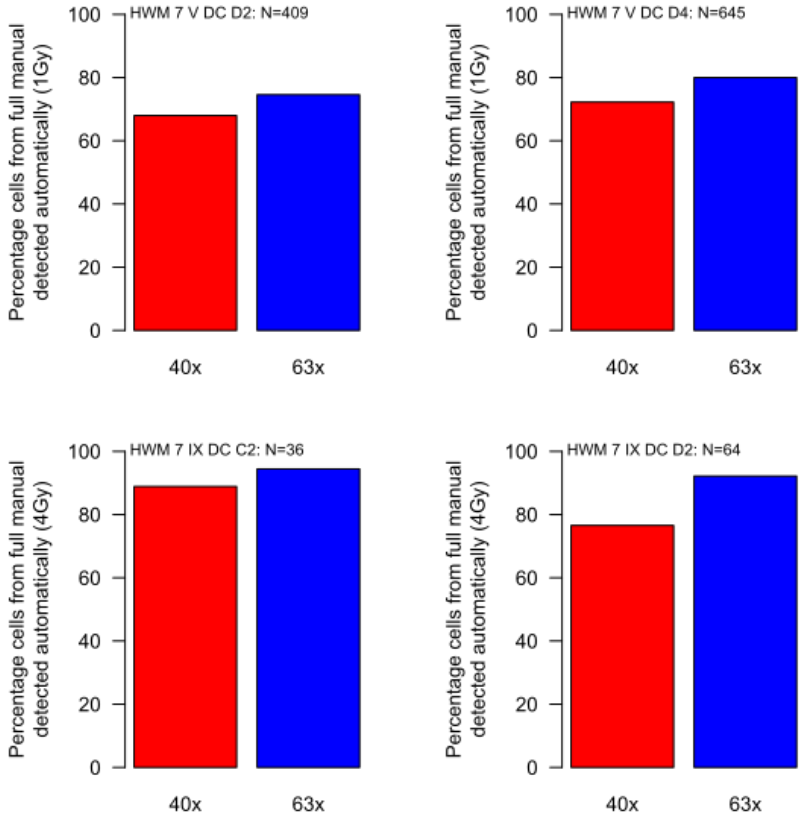

Figure 16: Percentage of cells from full manual scoring detected automatically for slides with ells irradiated with 1 Gy (HWM 7 V) and 4 Gy (HWM 7 IX).

Table 6: Number of analyzed images and frequencies of different types of aberrations following full manualldicentric scoring. Dic dicentric chromosome; tri: tricentric chromosome; Cr: chromosome ring; Del: deletion.

Dose (Gy)	Slide	Scored cells	Rejected cells	Dic+	Dic-	Dic/cell	Tri	Cr+	Cr-	Del	Distribution of dicentrics					
											0	1	2	3	4	5
1	HWM 7 V DC D2	409	308	51	2	0.13	0	2	0	25	363	40	5	1	0	0
	HWM 7 V DC D4	645	498	60	1	0.09	0	2	0	62	588	53	4	0	0	0
4	HWM 7 IX DC C2	36	17	58	0	1.6	3	2	0	29	6	15	5	7	3	0
	HWM 7 IX DC D2	64	103	103	0	1.6	7	0	0	61	12	20	18	11	1	2

As stated previously, the manual scoring is the most accurate method for detecting dicentrics since it relies solely on the detection of chromosome aberrations by a skilled human scorer. Therefore, to get reliable information concerning the accuracy of dicentric detection by both automatic and semi-automatic scoring, the rate of false negative or rate of undetected dicentrics after automatic and semi-automatic scoring was compared to the conventional manual scoring (figure 17). The results show that for 1 Gy, 76.3% of the dicentrics scored manually were undetected by the automatic software with 40x objective (slide HWM 7 V DC D2). However, this number decreases to 36.8% in case of semi-automatic scoring. When automatic analysis was performed with 63x objective, the rate of false negative as compared to full eye manual scoring was 66.7% and decreased to 10.3% when the automatically detected dicentrics were validated by the scorer (i.e. semi-automatic scoring). Similar results were obtained for the second slide irradiated with 1 Gy and the two slides exposed to 4 Gy. The figures 17 shows that in all cases except for slide HWM IX DC C2, the rate of false negatives vs full manual scoring is higher when scoring was done with 40x objective. This is true for both full automatic and semi automatic scoring.

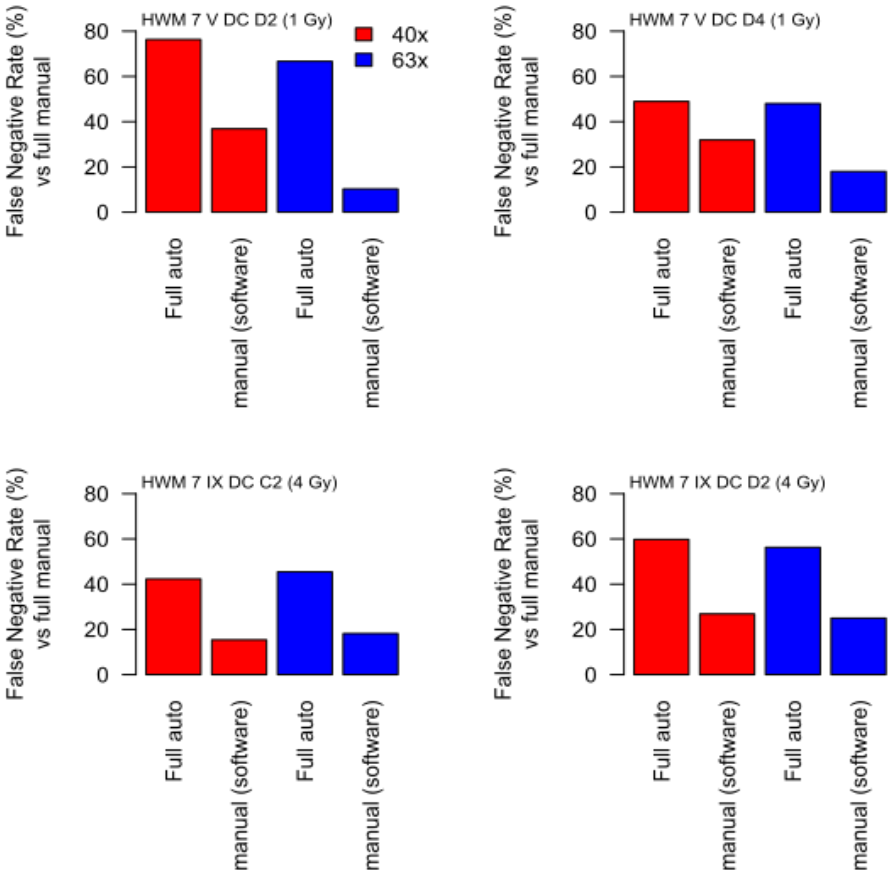

Figure 17: Rate of false negative dicentrics resulting from full automatic and semi-automatic (manual software) scoring as compared to full manual scoring by eye. Results are presented for two slides exposed to 1 Gy (slides HWM 7 V) and 4 Gy (slides HWM 7 IX)

Finally, the yield of dicentrics was compared between the three modes of scoring (figure 18). In the case of 1 Gy, this yield was 0.1 (DICs/1 cell) for full manual scoring, 0.17 (DICs/1 cell) for semi-automatic scoring with 63x objective and 0.08 (DICs/1 cell) for semi-automatic scoring with 40x objective. However, for cells exposed to 4 Gy, the dicentric yields were 1.61, 1.08 and 0.94 (DICs/1 cell) when analysis was performed manually, semi-automatically with 63x and 40x objective respectively.

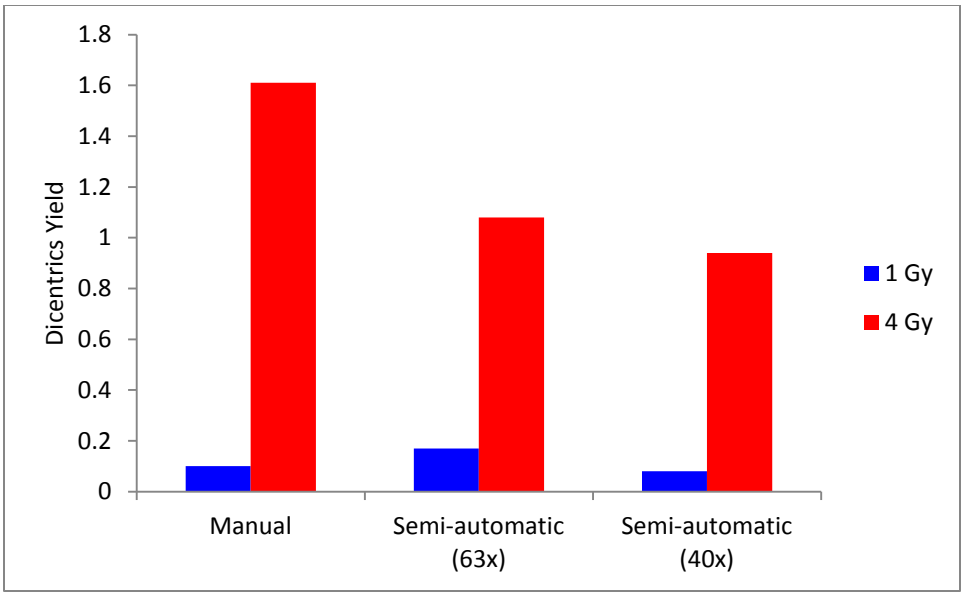

Figure 18: Comparison of the yield of dicentrics between full manual scoring and semi-automatic scoring (with both 63x and 40x objectives). Results are presented for cells exposed to 1 Gy (slides HWM 7 V) and 4 Gy (slides HWM 7 IX).

4. Discussion:

In large-scale emergency situations, estimates of radiation dose should be available as soon as possible to differentiate people with moderate or severe exposures from the worried well (i.e. anxious victims with no exposure). The conventional manual dicentric scoring is labour intensive and does not provide dose estimates in a short time frame to support clinical decision making. Therefore, in the last years, new strategies have been developed to adopt the dicentric assay for large scale accidents. One possibility is "triage" mode for manual dicentric scoring which involves dose estimations based on 50 or even 20 metaphase spreads. Another way to increase the throughput is the automation of the dicentric scoring procedure. [38].

Time factor is especially important in case of MASS CASUALTY since dose estimates need to be available as soon as possible in order to guide medical decision. The time needed for each laboratory involved in MULTIBIODOSE to report dose estimates was presented in the study results. The data shows that dose estimate can be provided within 2.4 days after arrival of the samples if manual scoring in the triage mode was used (one laboratory needed 6.1 days to establish dose estimates) [38].

For manual scoring in a triage mode, a human scorer needs approximately 60 minutes to analyze 50 cells. This is highly influenced by the quality of metaphase captured in the images [27]. Since automatic scoring has a 50% efficiency of dicentric detection and a 33% rate of cell rejection, 150 cells scored automatically provide a similar amount of information as compared to 50 cells scored in the manual system. The semi-automated analysis require less human involvement; a human scorer has a single role of validating the dicentric candidates which leads to a reduction of the time needed to generate a dose estimate to approximately 20 minutes per sample. The output of one human scorer can be increased by a factor of 30 if several scoring systems are used simultaneously [41].

According to Gruel et al. 2013, the time required for the automatic detection of dicentric chromosomes and their validation by the human scorer is approximately 0.5 hour. This time increases for the analysis of low dose exposure because a higher number of false positive dicentrics must be rejected. Since the manual scoring of 50 cells necessitate 1 hour, the results of the triage would be available 1 day in advance if the ADS was used [42].

The aim of the current study was to further optimize the semi-automated scoring procedure by the use of a 40 x objective for the AutoCapt procedure without oil .The time needed for dose estimation when automatic dicentric scoring was performed with two different objectives (63x objective currently in used vs 40x objective) was compared. Automatic dicentric analysis involves metaphase finding, capturing and analysis. The use of the 40x objective for image acquisition results in reducing the total analysis time by approximately 45% as compared to images captured with 63x oil objective. If a 63x oil objective is used for auto-capturing, the total time required to search, capture and analyze 100 metaphases is approximately 8 minutes (figure 8). The majority of this time is required for the image acquisition step. This can be explained by the fact that metaphases should be focused by several focus steps (fine and coarse focus) in order to obtain images of high resolution.

The time required for image acquisition can be significantly reduced when a 40x objective is used. Since the 40x objective has a much higher depth of focus than the 63x oil objective, the fine focus step can be avoided completely while achieving well-focused images. Moreover, immersion oil, which increases the slides scanning time by a few seconds even when an automated dispenser is used, is not used with 40x objective. Oil immersion is used with objectives in the magnification range between 60x and 100x (and higher) to increase the resolving power of the microscope.

The dicentric assay has been standardized to insure harmonization of the results obtained from different laboratories. Laboratory networks between different European and non-European countries were established to enable mutual assistance in case of large scale radiation emergencies. The study done by H. Romm et al. 2013 in the frame of the MULTIBIODOSE project concluded that the manual dicentric scoring performed fully by a human scorer detects almost twice the number of dicentrics than the semi-automatic scoring [38]. This indicates that the dose effect curve established from the manual scoring cannot be used to estimate dose from the yield of dicentrics obtained from the semi-automatic scoring. Therefore, a new calibration curve should be established by each laboratory for automatic scoring. In our study, comparison of the semi-automatic dose effect curves obtained from analysis with 63x and 40x objectives shows that both curves are almost similar while the one for the 40x objective is slightly lower for doses higher than 2 Gy (figure 11). This lower yield of dicentrics when scoring is done with the 40x objective can be attributed to different factors. First, dicentrics are more difficult to be detected by the software since images acquired by the 40x objective have a lower resolution. Additionaly, since the captured chromosomes are smaller in size, the human scorer failed to identify some true dicentrics in the validation step which contributed as well to this slightly lower curve for the 40x objective.

In the case of low LET irradiation, it is generally assumed that the dicentric chromosomes distribute among all cells following a Poisson distribution and this can be deducted by the results of manual scoring. However, the data was over-dispersed (figure 12) which lead to the used of the Quasipoissonmodel to achieve the calibration curves. Over-dispersion indicates that the irradiated blood samples contain mixtures of normal cells with no dicentrics and cells with two or more dicentric aberrations. Our results are not in accordance with the results of H. Romm et al. 2013 that reported that the distribution of dicentrics in case of automatic scoring is governed by Poisson distribution [38]. Moreover, The curves can be used interchangeably to estimate doses (except for doses of 3 and 4 Gy in case of estimates for 63x based on curve of 40x and 3, 5 and 6 Gy for estimates done following analysis with the 40x based on the curve of 63x objective). The previous results indicate that similar yield of dicentrics can be detected by scoring with both the 63x and 40x objectives.

Moreover, the results indicate that when images were captured with the 40x objective, the rate of false negative (47.7%) was lower as compared to images captured with 63x objective (figure 14). However, analysis done by both objectives lead to similar rates of false positive (mean of ~ 0.1%). For both objectives, almost all dicentrics detected by the software are true

dicentrics since the rate of false positive is very low (mean of ~ 0.1%). The surprisingly lower rate of false negative achieved by scoring with the 40xobjective indicates that the classifier used with the 40x objective was able to detect a slightly higher number of dicentrics. Nevertheless, the number of normal chromosomes detected by the classifiers as dicentrics (FP) is comparable for both objectives. A high number of cells passed the selection criterias given by the software for dicentric evaluation in both magnifications (figure 15). These selection criterias are cells with no more than 55 obectives and metaphase spreads of good quality. Almost all cells (~97%) found in 40x magnification were also found in 63x magnification. However, a slightly lower number of cells (~80%) from 63x magnification were also found in 40x magnification. This can be explained by the fact that images acquired by 40x objective have a lower resolution. Consequently, more cells did not pass the selection parameters of the classifier and instead were automatically rejected (table 3).

The percentage of cells automatically accepted for analysis was also compared to the percentage of cells manually scored. In the case of manual scoring, only complete cells with 46 centromeres are analysed (figure 16). For 1 Gy, approximately 70% of the cells from full manual mode were also accepted for analysis by the software. However, in the case of 4 Gy,almost all cells were analysed by both manual and automatic system. Moreover, for both doses this percentage is slightly higher when automatic scoring is performed with the 63x objective.

Fully manual scoring is the most reliable mode of dose assessment since only complete cells with 46 centromeres are analysed. The percentage of cells automatically accepted for analysis was compared to the percentage of cells manually scored. For both 1 and 4 Gy, the automatic software was able to identify a high number of cells adequate for analysis (i.e. cells with 46 centromers). This was deducted from the high percentage of cells from full manual mode that were also accepted for analysis by the software. Furthermore for the 4 slides analyzed, this percentage is slightly higher when automatic scoring was performed with the 63x objective. This can be attributed to the parameters of the classifiers which were developped to only accept cells with specified chromosome number and characteristics (i.e. chromosome width, length, area, etc). The classifier used with the 63x objective has detected a higher number of appropriate cellsfor analysis as compared to the 40x objective classifier.

The data wasalso used to establish the rate of false negative dicentric obtain after automatic and semi-automati analysis with both objectives as compard to full manual scoring (figure 17). For both full automatic and semi automatic scoring, the rate of false negatives vs full manual scoring was higher when scoring was done with 40x objective as compared to the 63x objective (except for slide HWM IX DC C2). In addition, the rate of false negative decreased when the dicentricswere validated by the human scorer (i.e. semi-automatic scoring) and this decrease is higher for semi-automatic scoring of cells captured by the 63x objective. The false negative dicentrics are undetected dicentrics by the software and their number is higher for image captured with the 40x objective. This can also be attributed to the lower image resolution and smaller size of chromosomes captured by the 40x objective which make it difficult for the classifier to detect dicentrics. Further, when semi-automatic scoring was performed, the scorer

identified some dicentrics undetected by the software and therefore the rate of false negative decrease. This decrease was higher for analysis with the 63x objective. Thus, for analysis with the 40x objective, the scorer was unable to identify some dicentrics that were confirmed in the scoring with the 63x objective and this can be caused by the same factors listed before (image resolution and chromosome size).

Conclusion

1. Overall, the results obtained in our study are very encouraging. The automatic dicentric scoring with 40 x objective has a potential in increasing the throughput of the assay by decreasing the turnaround time for individual dose assessment in case of mass casualty incident.
2. The use of the 40x objective for image acquisition results in dicentric analysis time reduction by approximately 45% as compared to images captured with 63x oil objective.
3. Even though the resolution of the image captured, the parameters of the classifier as well as the ability of the human scorer to validate true dicentrics were slightly better with the 63 x objective, the dose effect curves established with both objectives are almost similar while the one for the 40x objective is slightly lower for doses higher than 2 Gy. However, there was a statistically significant difference between the beta coefficient of the two curves (p-value <0.0001).
4. The rates of false positive and false negative were comparable between both objectives.
5. About 80% of the cells accepted for dicentric analysis with the 63x magnification were also found in 40x magnification and about 97% of the cells found in 40x magnification were also found in 63x magnification.
6. When automatic analysis was performed with 63x objective, the rate of false negative as compared to full eye manual scoring was high and drastically decreased when the automatically detected dicentrics were validated by the scorer.
7. In order to establish the semi-automatic dicentric scoring with the 40x objective as routine tool for biodosimetry, further investigation and validation of this method should be performed on different qualities of radiation. Since the DCScore software tool is not yet fully developed by Metasystems for the 40 x magnification, some adjustment of the software should be implemented in order for the dicentrics to be marked correctly with a red frame. For the validation step, the dose of single-blind irradiated blood samples should be estimated using the newly established dose effect curve. Moreover, inter-comparison should be planned between different biological dosimetry laboratories in order to confirm the reproducibility of this new assay assuming large-scale radiation emergency scenario.

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