

Application of Dicentric assay for triage dose estimation in case of large-scale radiation emergencies

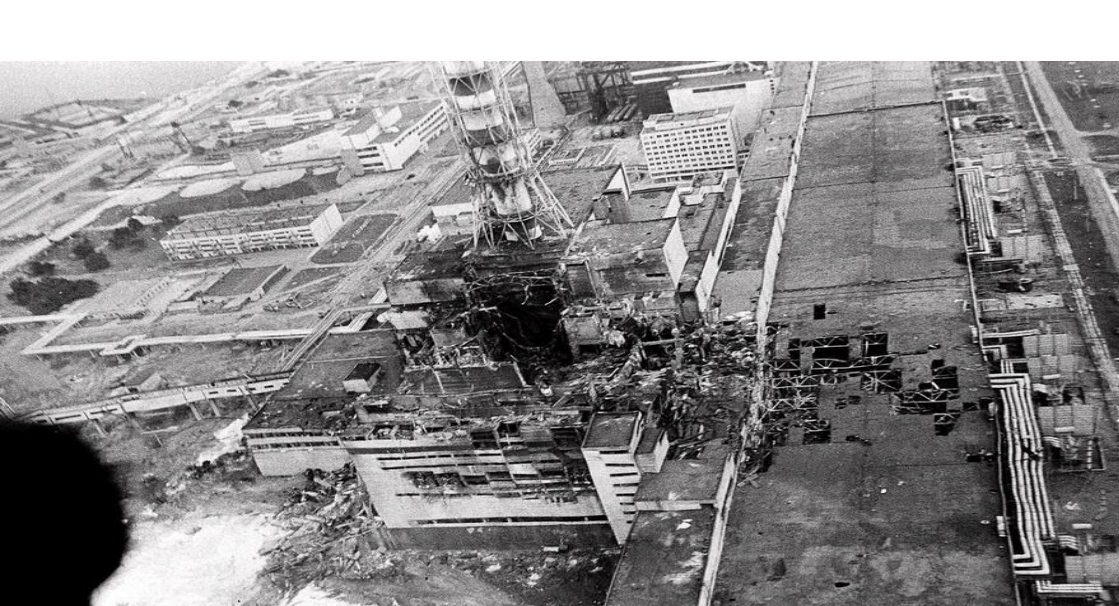
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Introduction

- The use of radioactivity and ionizing radiation has increased in the last decades.
- The risk of overexposure is becoming more likely.



the Chernobyl nuclear power plant
AP Photo



Devastations of the Fukushima tsunami
(2011) www.express.co.uk



Goiânia Accident (1987)
todayinhistory.blog

Introduction

- A first indication of radiation exposure
 - clinical signs and symptoms e.g. nausea and vomiting
 - blood cell counts fluctuations.
- Some of these symptoms are subjective parameters and can be caused due to intense anxiety [1].
- In emergency situation it is necessary to have an independent source of information about individually received irradiation doses to the blood [1, 2] .

[1] E. A. Ainsbury, E. Bakhanova, J. F. Barquinero, M. Brai, V. Chumak, V. Correcher, F. Darroudi, P. Fattibene, G. Gruel, I. Guclu, *et al*, Review of retrospective dosimetry techniques for external ionising radiation exposures, *RadiatProtDosimetry*. 2011 Nov; 147(4): 573–592.

[2] U. Kulka et al. RENEB - Running the European Network of biological dosimetry and physical retrospective dosimetry. *International J Radiat Biol*. 2017;93(1):2-

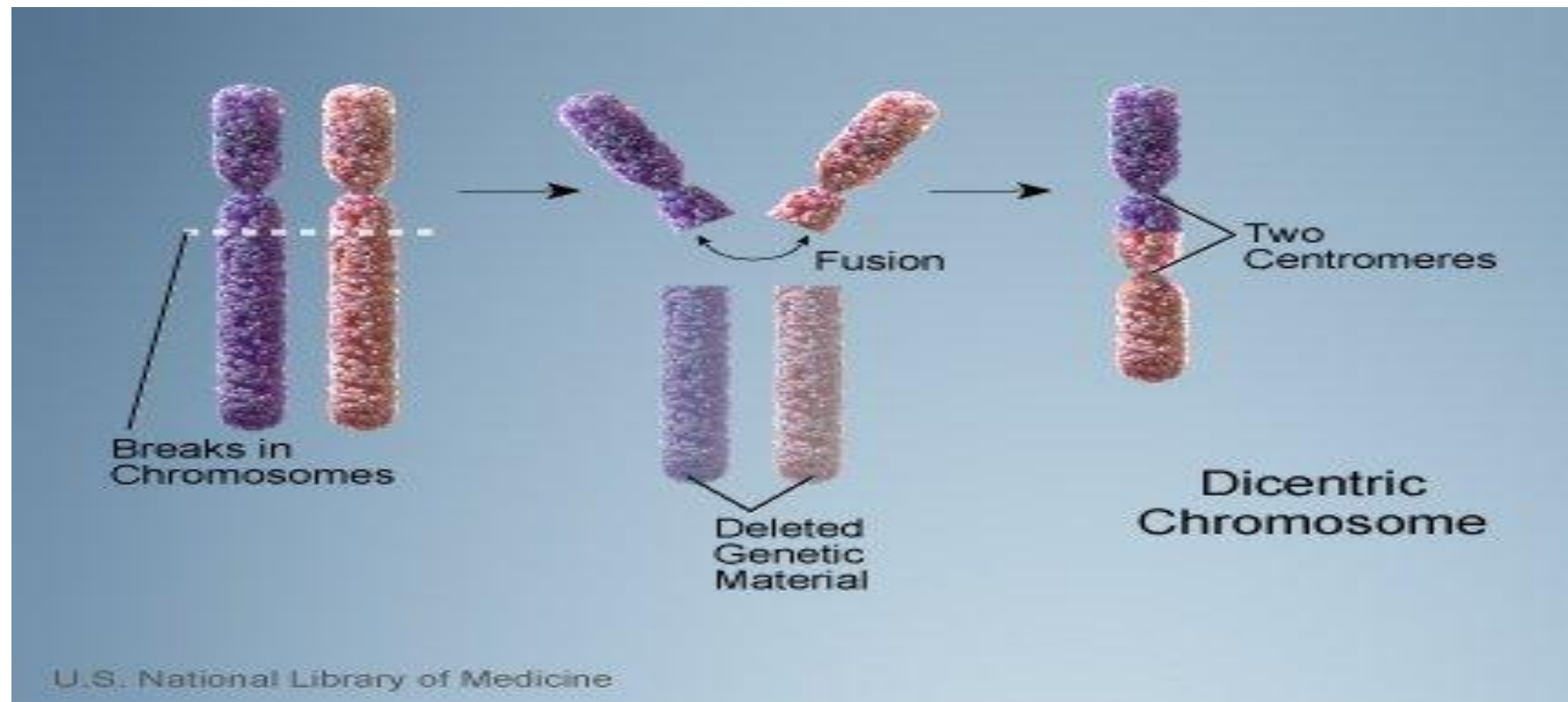
Introduction

- Biological dosimetry provides a useful method to estimate a radiation dose received by individuals.
- The dicentric chromosome assay is considered as the “goal standard” in biological dosimetry
- It is the most reliable and sensitive assay for the assessment of the radiation dose of potentially exposed individuals [1].



Dicentric chromosomes

- DNA double strand breaks can be induced by exposure to ionizing radiation .
- The misrepair of these damages results in the formation of abnormal chromosomes.
- A dicentric chromosome is an abnormal chromosome resulting from the fusion of two chromosome segments, each with a centromere.



Performing the Dicentric Assay



(a)

I. Peripheral blood is withdrawn



II. T-Lymphocytes are culture with phytohaemagglutinin (PHA-L)



III. Addition of potassium chloride (KCl) solution (2) and Fixative (3:1 methanol/acetic acid)



(a)

IV. Cells are dropped onto slides

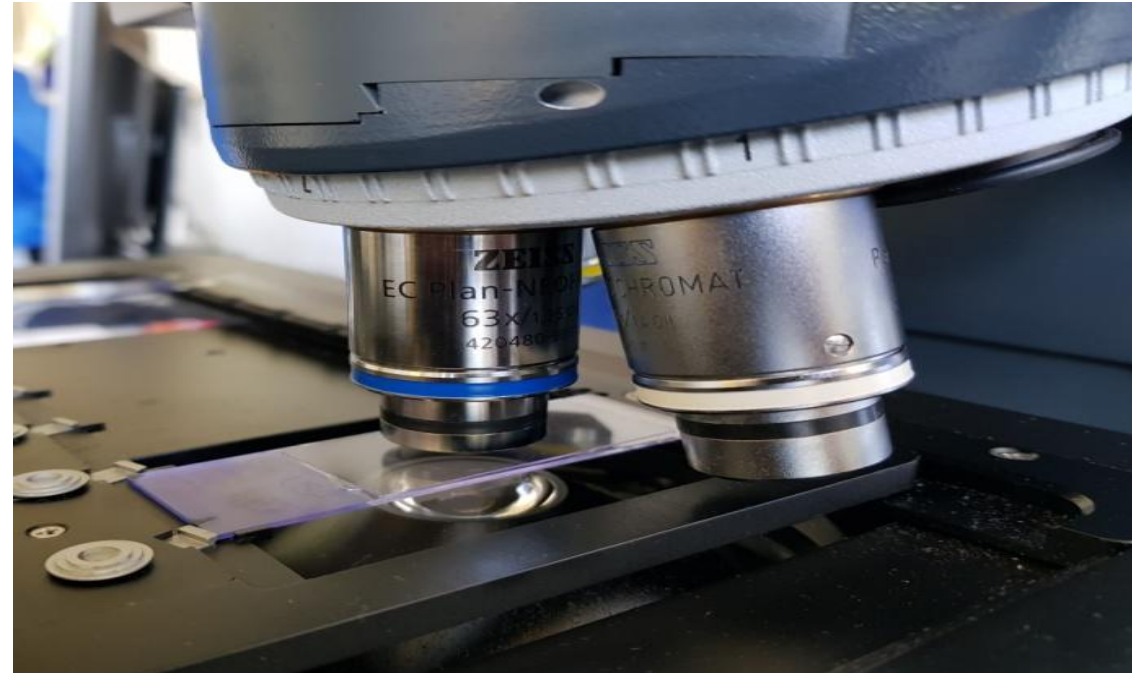


(b)



V. Slides are stained for 5 min in Giemsa-solution

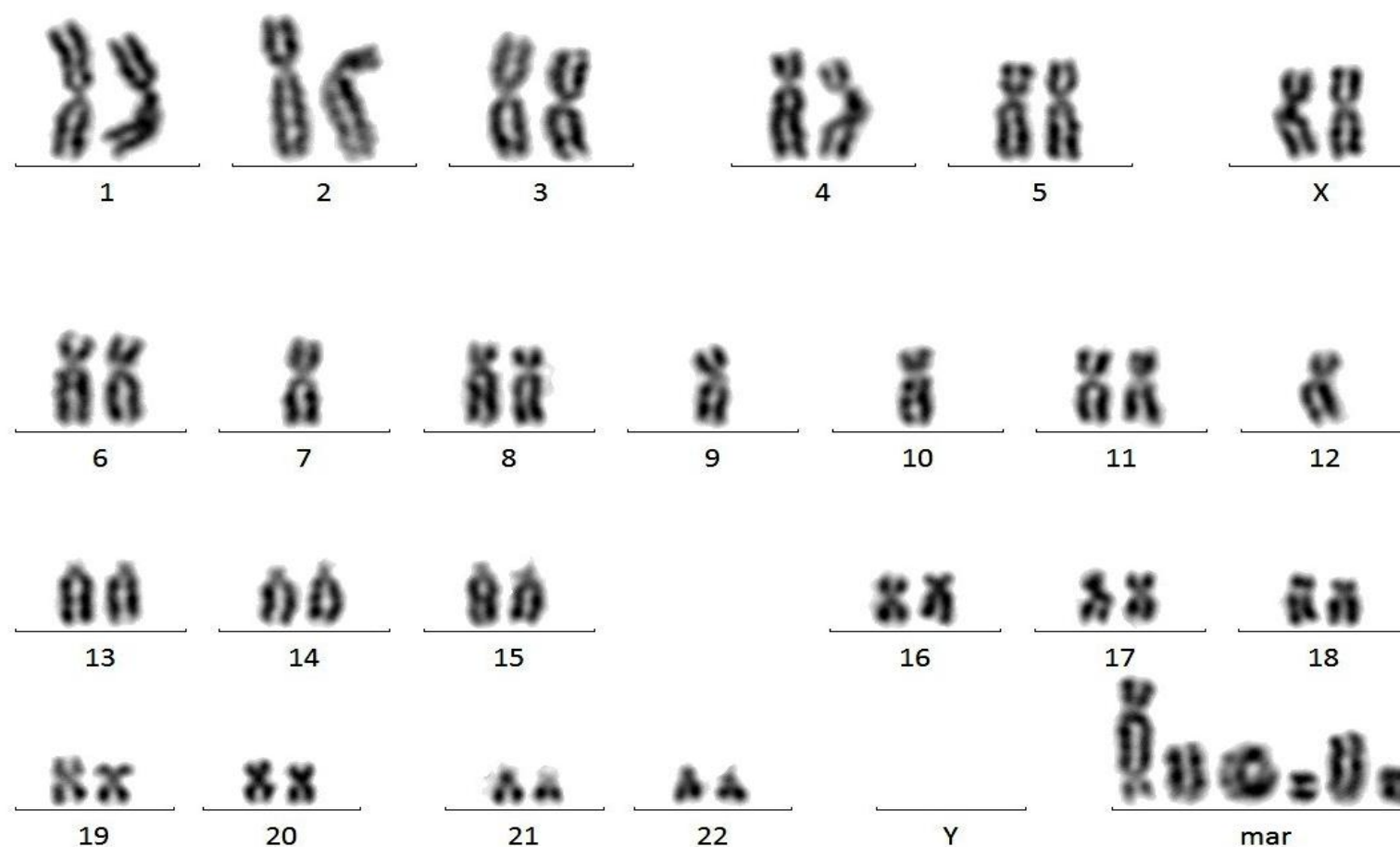




VI. Cells are analyzed for chromosome aberrations

Manual scoring

- Conventional manual scoring is the most accurate method of dose estimation
- For manual dicentric scoring, only complete cells containing 46 centromeres are analyzed.
- Furthermore, metaphases should have good morphology and few overlapping chromosomes.

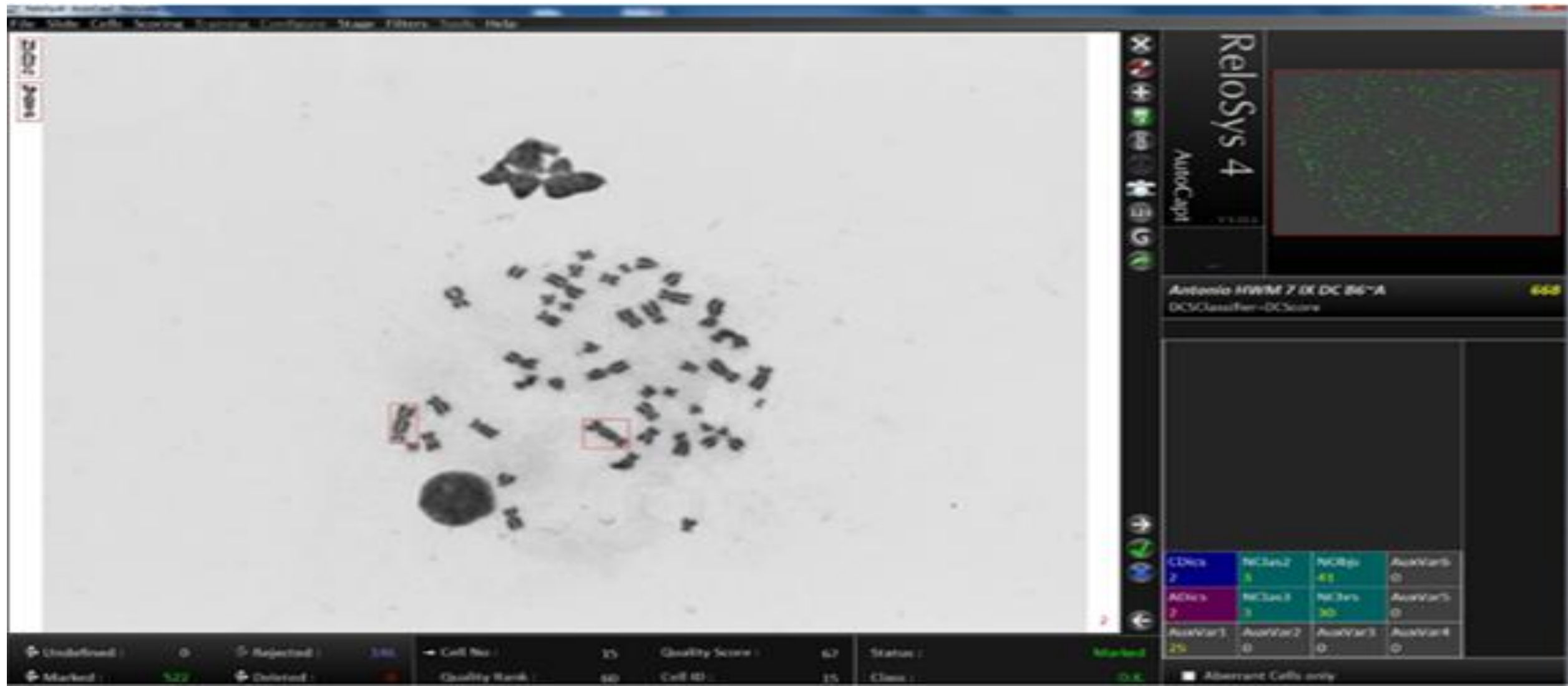


Manual scoring

- The dicentric assay is time consuming and not appropriate for emergency situations.
- New scoring strategies have been implemented to increase the throughput of the assay [1]
 - By decreasing the number of cells analyzed
 - By detecting dicentric chromosomes with the help of a software [1].

Semi-automatic scoring

- Automatic scoring involves
 - Metaphase finding (10 x magnification)
 - Capture cells (63 x and 40x magnification)
 - Automated scoring
 - Evaluation of dicentric candidates



Aim

- The aim was to optimize the semi - automatic scoring procedure for the dicentric chromosome assay by applying a 40x objective without oil.
 - Evaluation of the time to complete the automatic scoring for both 63x and 40x objectives.
 - Establishment of dose effect curves using different objectives (40 x, 63 x).

Method

Irradiation

- Blood samples (10 ml heparinized tubes) from one healthy donor (female) were irradiated with ^{137}Cs gamma rays (dose rate 0.495 Gy/min).
- 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.
- After irradiation the samples were incubated for 2 h at 37 ° C before culture initiation.

Cell Cultures

- Blood cultures was set up according to the protocol which follows the IAEA recommendations [1] and the ISO standards [2].

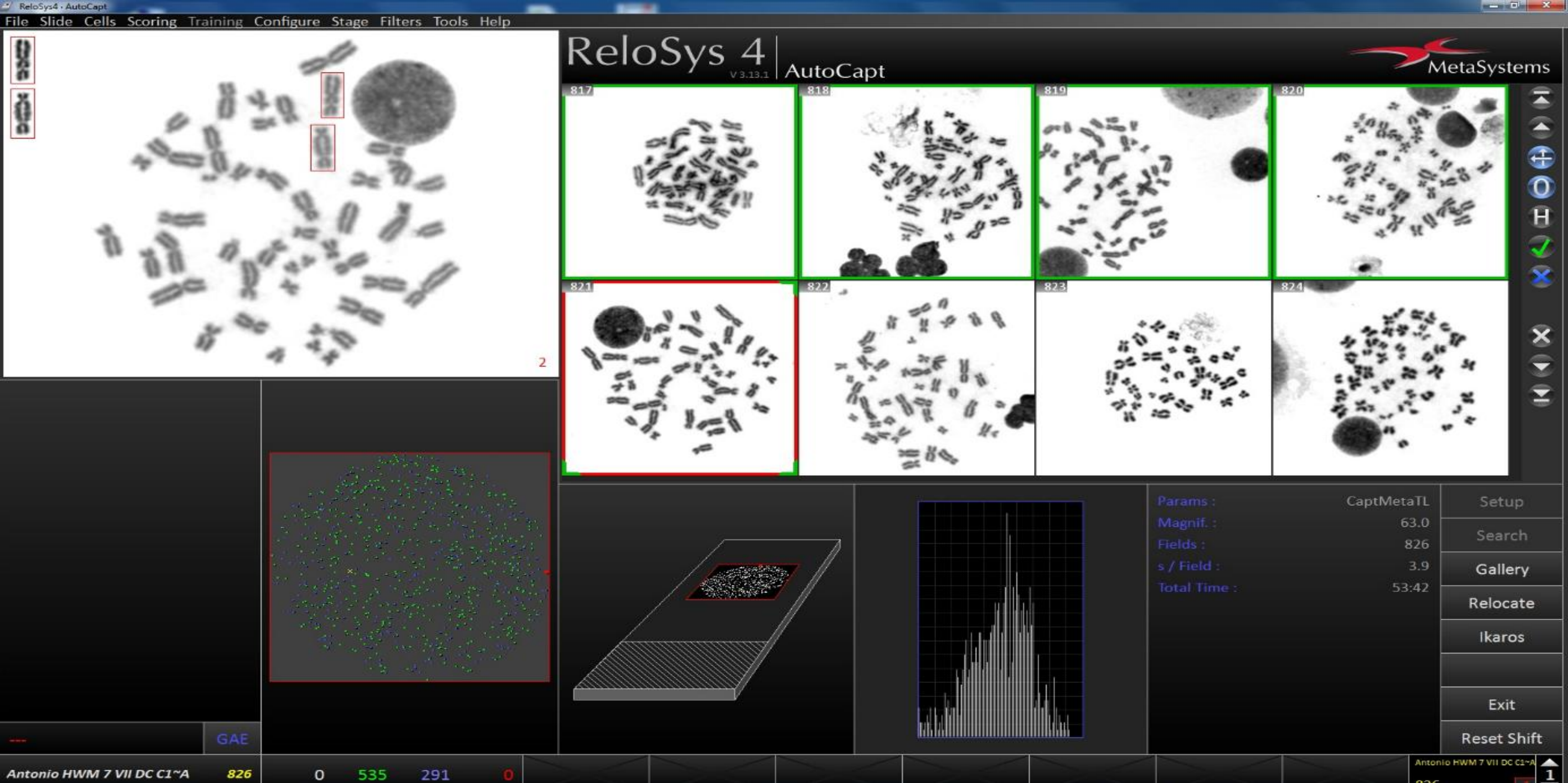
[1] ISO 21243, Radiation protection - Performance criteria for laboratories performing cytogenetic triage for assessment of mass casualties in radiological or nuclear emergencies - General principles and application to dicentric assay, International

[2] Organization for Standardization, Geneva, Switzerland, 2008. World Health Organization. *WHO BioDoseNet*. Geneva: WHO. Available from:

www.who.int/ionizing_radiation/a_e/biodosenet/en/index.html.

Semi-automatic scoring (60x VS 40x objectives)

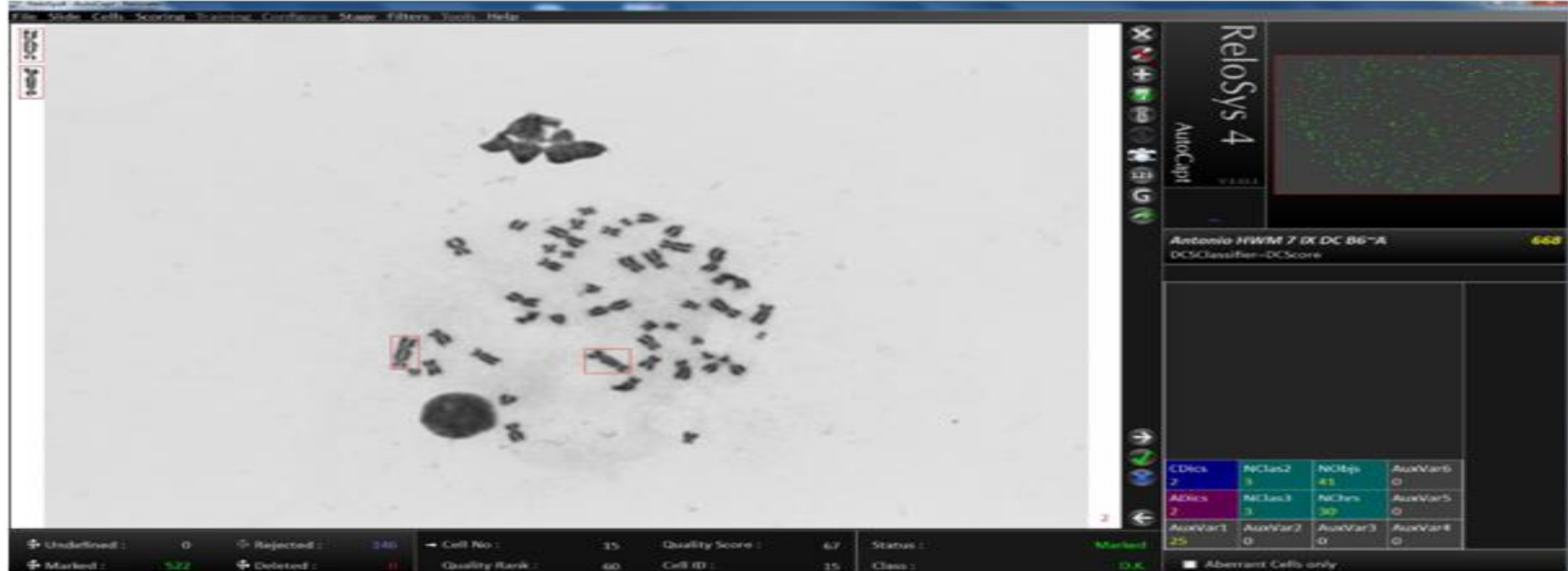
- Analysis of the slides was done using the automatic scoring system Metafer 4 by MetaSystem (Altlussheim, Germany).
- Three slides were scored per dose point.



Captured metaphases used for the detection of dicentric candidates with DCSScore software tool are shown.

Semi-automatic scoring (40x objective)

- For the 40x objective, the DCScore software tool is not yet fully developed by Metasystems
- Therefore the software was unable to mark correctly the detected dicentrics with a red frame



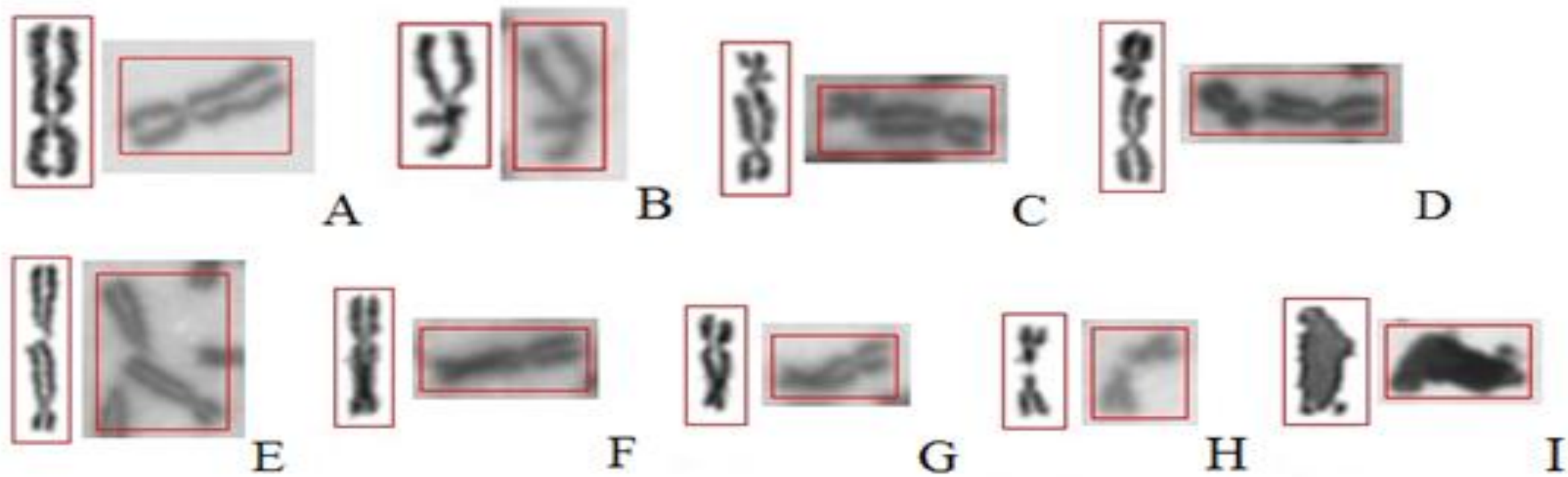
A



B

Semi-automatic scoring (60x VS 40x objectives)

- Dientrics detected by the software were either accepted as True Positives (TP) or rejected as False Positives (FP).



False Positive dicentric candidates that are rejected by the human scorer.

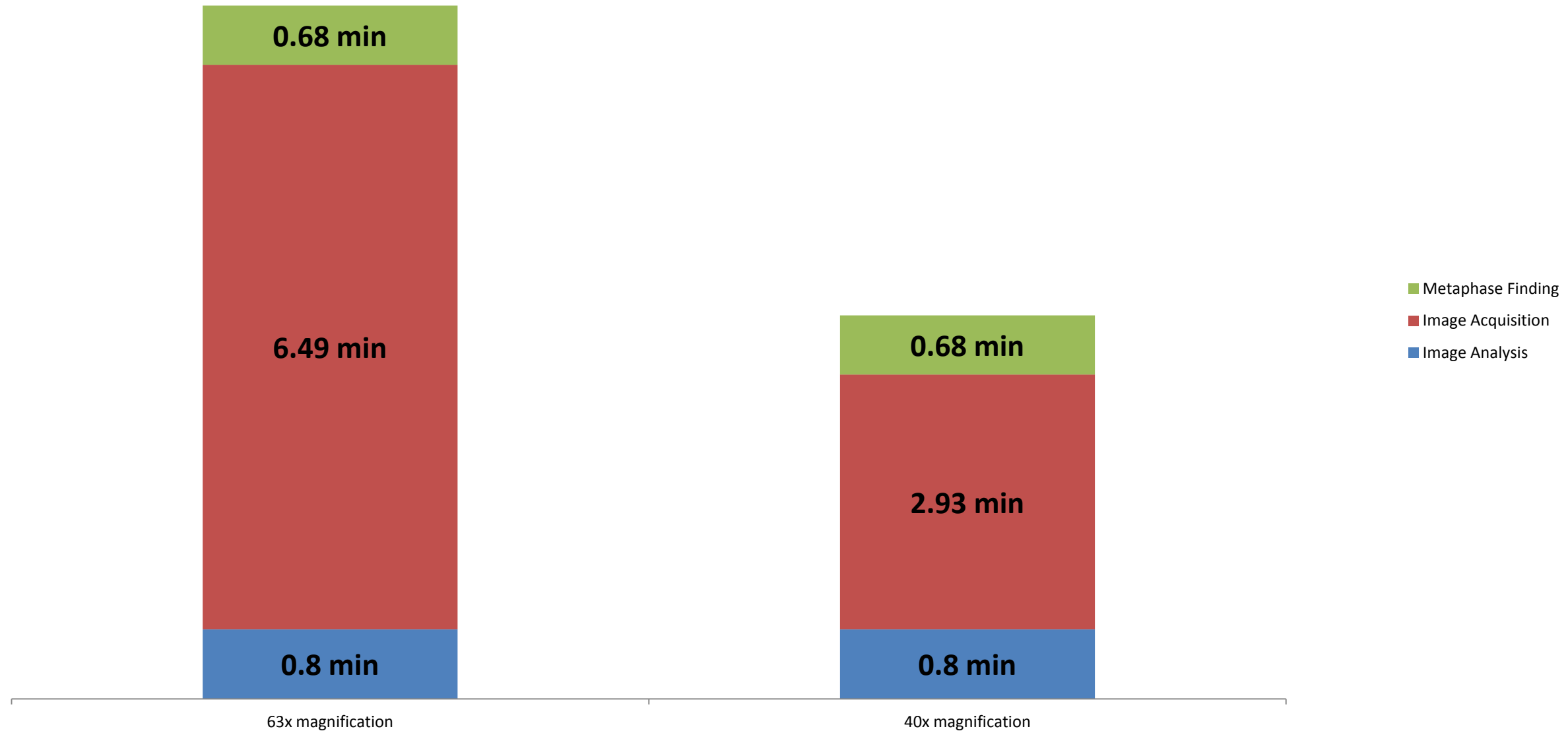
Results

	Dose (Gy)	Captured images	Scored cells	Rejected cells	Rejected cells %	DA	DC	Dic/cell
Semi-automatic (63 x)	0	3145	1931	1214	38.6	110	8	0.004
	0.1	5207	3164	2043	39.2	288	17	0.005
	0.25	5075	3414	1661	32.7	171	15	0.004
	0.5	5993	4268	1725	28.8	342	57	0.013
	0.75	5807	3789	2018	34.7	350	89	0.023
	1	4150	2731	1419	34.2	291	126	0.046
	1.5	2610	1869	741	28.4	251	193	0.103
	2	2179	1527	652	29.9	299	253	0.166
	3	2896	2018	878	30.3	719	760	0.377
	4	1839	1449	390	21.2	822	1032	0.712
	5	877	704	173	19.7	459	676	0.96
	6	1390	1128	262	18.8	1087	1549	1.373
Semi-automatic (40 x)	0	3145	1611	1534	48.8	88	2	0.001
	0.1	5207	2658	2549	48.9	213	13	0.005
	0.25	5075	2800	2275	44.8	130	17	0.006
	0.5	5993	3058	2551	42.6	212	45	0.015
	0.75	5807	3318	2489	42.9	268	99	0.03
	1	4150	2242	1908	46	209	101	0.045
	1.5	2610	1601	1009	38.6	200	134	0.084
	2	2179	1351	828	38	262	230	0.17
	3	2896	1712	1184	40.9	483	485	0.283
	54	1839	1265	574	31.2	669	800	0.632
	5	877	565	312	35.6	350	475	0.841
	6	1390	1043	347	25	894	1351	1.295

Number of analyzed images and observed yield of dicentrics following automatic dicentric scoring with 63 x and 40 x objectives.

Results

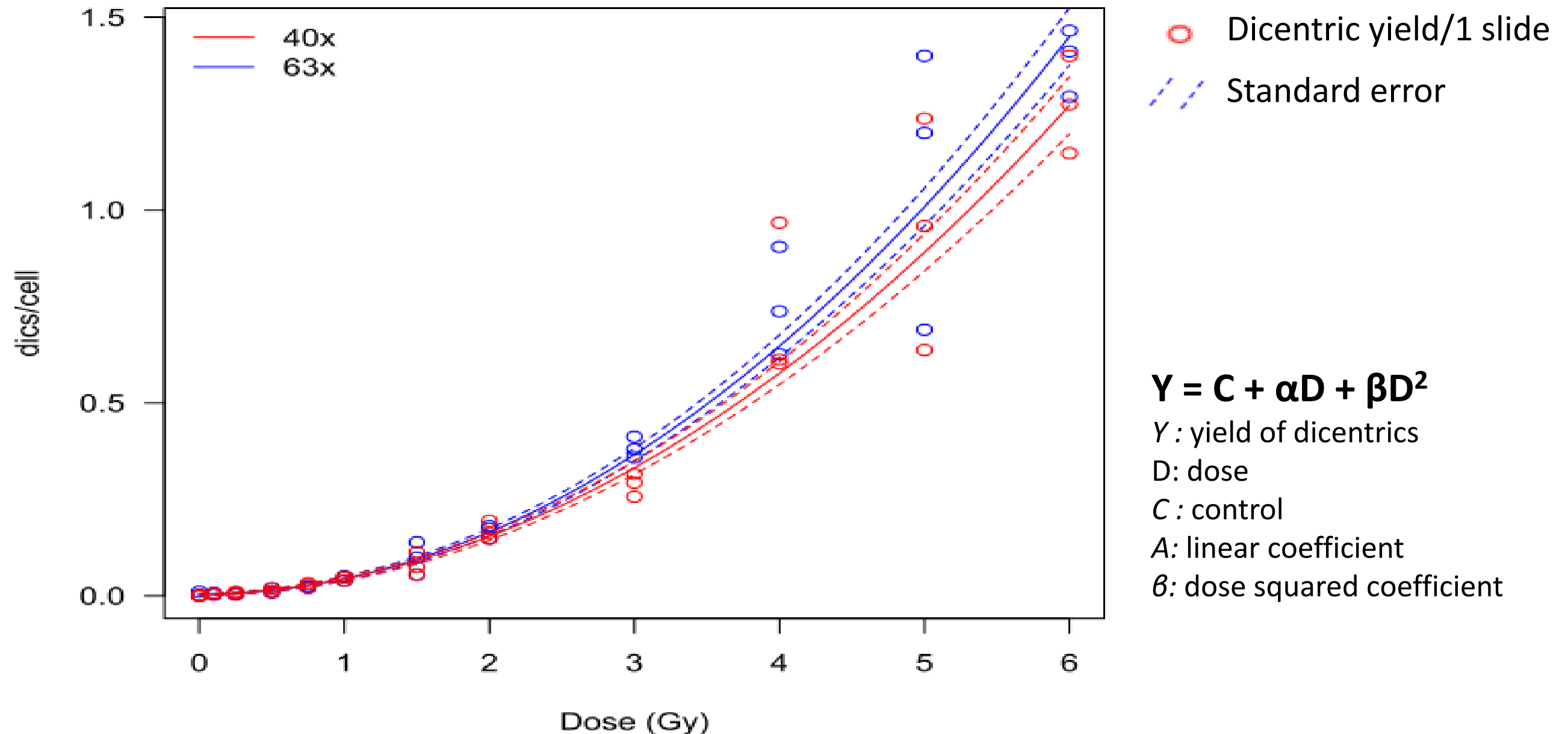
Comparison of time required for automatic scoring



Time required for automatic dicentric scoring when a 63x oil objective and 40x objective are used for image acquisition.

Results

Establishment of dose effect curves by automatic scoring



Dose effect curves of semi-automatic dicentric analysis using two different objectives for image capturing

Results

Establishment of dose effect curves by automatic scoring

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

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

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

Statistical comparison of the model coefficients based on Z-Scores

Conclusion

- The 40x objective results in dicentric analysis time reduction by 45%.
- 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.
- Further investigation and validation of this method should be performed on different qualities of radiation.

Thank you for your attention

