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**SKY and FISH analysis of radiation induced chromosome aberrations:  
a comparison of whole and partial genome analysis**

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**Abstract**

For a retrospective dose estimation of human exposure to ionising radiation, a partial genome analysis is routinely used to quantify radiation-induced chromosome aberrations. For this purpose, fluorescence in situ hybridisation (FISH) with whole chromosome painting probes for selected chromosomes is usually applied covering about 20% of the whole genome. Since genome-wide screening techniques like spectral karyotyping (SKY) and multiplex FISH (mFISH) have now been developed the detection of radiation-induced aberrations within the whole genome became feasible. To determine the correspondence between partial and whole genome analysis of radiation-induced chromosome aberrations they were measured comprehensively in this study on *in vitro* irradiated blood samples from three donors. We could demonstrate that comparable results can be detected with both approaches, however, complex aberrations might be misinterpreted by partial genome analysis. We therefore conclude that whole genome analysis by SKY is useful especially in the high dose range to correct aberration data for complex exchange aberrations.

## Introduction

Since many years fluorescence in situ hybridisation (FISH) and in particular so-called “FISH-painting” has been used for the quantification of radiation induced chromosome aberrations [for review see ref. 1, 2]. For routine application of FISH in biodosimetry, a combination of three whole chromosome-specific DNA probes covering about 20% of the total genomic DNA content is usually used and detected in one or more different colours. Thus, analysis of radiation-induced chromosome aberrations by FISH-painting is restricted to parts of the human genome. For intercomparison of translocation frequencies obtained with different chromosome combinations data have to be extrapolated to whole genomic equivalents [3]. This extrapolation requires the assumption that chromosomes are involved in translocations proportionally to their DNA content which could be demonstrated experimentally not for all human chromosomes [4]. Thus, such an extrapolation can lead to an incorrect estimation of genomic translocation yields, especially if they are part of complex aberrations.

Meanwhile, new techniques for the detection of chromosome aberrations have been established that allow a simultaneous detection of each of the 24 human chromosomes in individual colours. Two methods are available to perform such analysis: the filter-based multi-fluor FISH’ (mFISH) approach [5] and the interferometer-based ‘spectral karyotyping’ (SKY) approach [6]. Both techniques are based on the combinatorial labelling of whole chromosome-specific painting probes for all human chromosomes using five different fluorochromes and the subsequent classification in 24 different false colors. These techniques have been applied very successfully to the analysis of cytogenetic alterations in tumors [e.g., 7, 8] and radiation-induced cytogenetic changes [9, 10].

In this paper, chromosomal aberration yields in peripheral lymphocytes after irradiation with 3 Gy x-rays have comprehensively been measured by SKY- and FISH-painting analysis. Subsequently, resulting whole genomic and partial genomic frequencies have been compared and conclusions about the accuracy of the projection of whole genomic equivalents from partial genome analyses have been drawn.

## **Material and Methods**

### *Lymphocyte culture and in vitro irradiation*

Peripheral blood samples of three healthy donors (two females, 22 and 25 years of age and non-smokers, one male, 33 years of age, smoker) were irradiated with 3 Gy 220 kV x-rays (12.5 mA, 4.05 mm Al+0.5 mm Cu filters, dose-rate 0.5 Gy min<sup>-1</sup>) at 37°C. Immediately after irradiation, whole blood cultures were set up containing 4.5 ml RPMI 1640 plus Glutamax medium supplemented with 15% fetal calf serum, 2.5% phytohemagglutinin, antibiotics and bromodeoxyuridine (final concentration: 9.6 x 10<sup>-6</sup> µgml<sup>-1</sup>). Cultures were incubated at 37°C for 48 hrs involving a colcemid treatment (final concentration: 0.1 µg ml<sup>-1</sup>) for the final 3 hrs.

### *Metaphase preparation*

Metaphase preparation was performed according to standard procedures as described by Huber et al. [11]. Slides were stored under nitrogen atmosphere at -20°C until use.

### *SKY analysis*

SKY analysis was performed as described previously [7]. Briefly, metaphase preparations were pretreated with RNase A (0.1 mg/ml in 2 x SSC) and pepsin solution (10 µg/ml in 0.001 HCl) and fixed in 1% formaldehyde. Pepsin pre-treatment was performed under microscopic control. Slides were placed in denaturing solution (70% formamide in 2 x SSC) at 72°C for 2 to 3 minutes and subsequently dehydrated in a 70%, 80%, 100% ethanol series. Metaphase slides were then hybridized with a probe mixture supplied by Applied Spectral Imaging (ASI, Mannheim, Germany). The probe mixture (SKY<sup>TM</sup> mixture) was denatured at 75°C for 7 min. and incubated at 37°C for 1 hr. It was then applied to the denatured metaphase slides and incubated for 48h at 37°C. Posthybridisation washes were performed according to the manufacturer's protocol. Detection of biotinylated and digoxigenin-labeled probes were carried out with avidin-Cy5 and an anti-digoxigenin antibody followed by a goat anti-mouse antibody conjugated to Cy5.5 according to the manufacturer's protocol. Metaphase spreads were stained with 4',6-diamidino-2-phenylindole (DAPI) solution (150 ng/ml in 2 x SSC) and covered with antifade solution (Vectashield mounting medium; Vector Laboratories, Burlingame, CA). Images of metaphase spreads were acquired by use of a SpectraCube system (ASI) and analyzed with the SKYView imaging software (ASI).

### *Scoring of aberrations*

For scoring of aberrations after FISH-painting and SKY hybridizations the PAINT nomenclature [12] have been applied. The description of chromosome aberrations in SKY experiments have been performed using the modified mPAINT nomenclature [10, 13]. According to this nomenclature, chromosomal segments that are involved in visible colour junctions are given in parantheses and separated by dashes. Centromeric segments are designated by an apostroph. For example a dicentric involving chromosomes 'a' and 'b' is described by (a'-b')(a-b). The first paranthesis refers to the rejoined product of the centromeric segments, the second paranthesis to the associated bicoloured acentric fragment. Aberration patterns which appeared visibly unrelated to each other were scored separately, thus assuming that such a cell has two or more independent aberrations. Visibly shortened chromosomes were designated as (a'T) and centric or acentric rings by r(a') or r(a), respectively. According to Cornforth [13] also one-way and incomplete aberrations have been distinguished. Incomplete aberrations represent patterns for which all segments are visible but at least one pair of them appear unrejoined, for example (a'-b')(a)(b) denotes an incomplete dicentric. One-way aberrations represent patterns for which some segments are missing, such as the one-way translocation (a-b').

To assess the degree of complexity of aberrations, the number of colour junctions (cj) and the required minimal number of breaks (mnb) in each aberration were determined. Exchanges were classified as complex, if they require three or more breaks on two or more chromosomes. Complexity was described by the CAB system [14], which assigns to each exchange the minimal required number of chromosomes, chromosome arms and breaks.

### *Mathematical methods*

As established by Lucas et al. [3], the relationship between the so called genomic translocation frequency  $F_G$  and the frequency of colour-reciprocal translocations  $F_p$  involving the painted DNA fraction  $f_p$  is

$$F_p = 2f_p(1-f_p)F_G/T \quad (1)$$

where T represents all symmetrical translocations between any two of 46 chromosomes. This resulted in  $T = 0.9745$  or  $2/T = 2.05$  using chromosomal DNA fractions according to Morton [15]. In SKY analysis it is of interest to figure out the whole genomic colour-reciprocal

translocations only, if comparing translocations from a partial genome analysis. This yields  $T = 0.949$  or

$$F_p = 2.11[f_p(1-f_p) + f_1f_4 + f_1f_{12} + f_4f_{12}]F_{SKY} = 0.35 F_{SKY} \quad (2)$$

for the painted chromosomes 1, 4 and 12, where also exchanges between these chromosomes are taken into account.

SKY-derived patterns (b'-a)(b) (type I), (b'-a) (type II/III), (b'-a')(b) (type IV/VI) and (b'-a)(a'T) (type VII) were categorized as simple types. They may be true incomplete as type I or one-way exchanges. The interrelation to FISH-painting analysis is as follows: SKY-type II/III contributes to FISH-types (b'-a) (III) or (a'-b) (II) each of them to 50%. SKY-type I contributes 50% to FISH-type (b'-a)(b) (I) or II, dependent on which chromosome, b or a, corresponds to the painted chromosome in FISH. Type VII contributes to FISH types II or III, because a terminal deletion is not or very rarely detected in FISH analysis. Thus, equation (2) for the conversion between SKY and FISH is also approximately valid for these types, when they are pooled. Equation (2) is not precise, because some complex aberrations that are visible in SKY analysis may be detected as simple aberrations in FISH.

## Results

SKY and three-colour FISH analysis have been performed on metaphases of three donors. After in vitro irradiation of peripheral blood samples (3 Gy x-rays) 200 metaphases have been scored by SKY and 1770 metaphases by three colour FISH-painting for chromosomes 1, 4 and 12. Chromosomal aberrations have been classified according to PAINT and mPAINT nomenclatures and are summarised in Tables 1 and 2.

### *Simple aberrations*

Simple translocations and dicentrics (complete and one-way) measured with both techniques are demonstrated in Table 1. Examples for simple reciprocal and one-way exchanges are depicted in Figure 1. For comparison with FISH results, SKY data for translocations and dicentrics were converted into partial genomic frequencies according to equation (2).

No significant differences between SKY and FISH could be observed except for translocation frequencies detected by SKY in case 3930 which showed up twice as high compared to three-colour FISH data. Also, the ratio of simple translocations to dicentrics of 2.6 appeared to be very high after SKY analysis. For dicentrics, no difference between SKY and three colour FISH-painting was detected in this case.

### *Complex aberrations*

Complex aberrations detected by SKY are shown in Table 2 and exemplary in Figure 2. Table 2 shows complexes of irregularly patterns each of which occurred only once except the aberration (a'-b)(b'-a)(a) which became apparent five times. In total, 34 complex aberrations (0.25 per cell) could be found. Many of the complex rearranged cells contained further non-complex aberrations which were scored as independent events. Among them there were stable as well as unstable aberrations. In case 3930 showing a high translocation frequency after SKY, most of the simple translocations occurred in cells without complex aberrations. The number of involved chromosomes per complex exchange aberration varied between 2 and 4, the minimal number of breaks between 3 and 6. Eleven complexes were composed of three breaks in two chromosomes (2/A/3)-class. Most complex rearrangements appeared as one-way or incomplete exchanges. Seven of the 34 complexes (0.06 per cell) detected by SKY would occur after FISH-painting for chromosomes 1, 4 and 12 as simple complete



translocations (3906-35, -50, 3960-31) or simple one-way exchanges (3906-37, 3930-15, 3930-17, 3960-43). These cells that result in a complex aberration pattern after SKY but in a simple aberration after FISH-painting are highlighted in Table 2.

### *Statistical analysis*

Table 3 provides an overview on the frequency of damaged cells including complex aberrations as well as resulting colour junctions (cj) and minimal number of breaks (mnb) that are necessary to create the respective chromosome aberrations. It became obvious that FISH-painting for chromosomes 1, 4, 12 detected only a fraction of rearranged cells (in the range between 36.8% and 41.6 %) that are visible after SKY analysis. The proportion of cj present after FISH-painting ranged from 16.9% to 30.6% of the SKY-derived frequencies. Due to the occurrence of one-way exchange patterns a lower number of cj than mnb has been observed. Therefore, the ratio mnb to cj reflects the overall extent of one-way aberrations. If all parts from all aberrations are visible and complete, the mnb:cj ratio should be close to 1.0. For FISH-painting, this ratio was in the range between 1.4 and 1.5, whilst it was larger than 1.5 for SKY-derived aberrations indicating that there is a tendency for more one-way or incomplete aberration patterns after SKY.

## Discussion

We have investigated radiation-induced chromosome aberration frequencies by SKY and FISH-painting analysis after in vitro irradiation of peripheral lymphocytes from three donors. This is the first study which directly compares these two approaches on the same irradiation experiment. So far, for a routine quantification of radiation-induced chromosomal aberrations a partial genomic analysis with FISH-painting probes for selected chromosomes have been applied [for review see 1, 2]. To investigate whole genomic aberration frequencies after radiation exposure first studies have been performed using multiplex FISH (mFISH) [9-10, 16-19]. For this purpose, mFISH and SKY have the same methodological power to quantify radiation-induced chromosome aberrations. Although somebody might argue that the speed of image acquisition is higher for the mFISH approach, it became clear from the present data set that the time-limiting factor for a routine use of whole genome analysis is not the speed of image acquisition but a precise delineation of aberration patterns (especially of complex aberrations) after karyotyping has been performed. Therefore, we have applied the newly introduced terminology of Cornforth [13] that provide a better description of exchange aberrations after whole genome analysis than PAINT [12] which has been specifically developed for FISH-painting analysis of a part of the genome.

One major discussion with a partial genome analysis of radiation-induced chromosome aberrations was the question whether whole genome aberration frequencies can be reliably calculated using the formula of Lucas et al. [3]. Our comparison of FISH-painting data on chromosomes 1, 4 and 12 and whole genome analysis using SKY contributes to this question. As demonstrated for simple exchange aberrations in Table 1 the frequencies of reciprocal translocations and dicentrics are in good agreement between FISH-painting and SKY and don't show a statistically significant deviation except for the translocation rate of case 3930 which is significantly increased after SKY analysis (standard difference test,  $p < 0.05$ ). This also result in a very high translocation to dicentric ratio of 2.57 compared to ratios resulted from FISH-painting data (Table 1). For this reason we looked in more detail at the chromosomes involved and identified some recurrent translocations between chromosomes 1/6, 4/15, 4/17 and 5/7 after SKY (each translocation became apparent in two cells; data not shown). This might indicate some clonal events which were obviously not detectable by FISH-painting in this case.

Another important result of this study was the frequency of complex exchange aberrations measured by SKY and FISH-painting. In total, 34 complex aberrations have been detected by

SKY. In this frequency of complex aberrations eight insertions are included six of which occurred in case 3030 and one each in cases 3906 and 3960. As demonstrated in Table 2 six cells (3906-35, 3906-37, 3906-50, 3930-17, 3939-23, 3960-31) became apparent that carry complex chromosome aberrations that would not have been visible after FISH-painting of chromosomes 1, 4 and 12 and therefore would have been scored as simple aberrations. Since complex chromosome aberrations are a major problem in scoring of FISH-painted chromosome-type exchange aberrations at higher doses [20], such findings are important at increasing doses and should also be considered if the temporal stability of cells carrying translocations are being investigated. These data also support the idea to use a whole genome analysis approach instead of partial genome analysis for applications in dose reconstruction. However, although complex aberrations might be misinterpreted in FISH-painting analysis the importance of such aberrations in the low dose range is little. Moreover, calibration curves and -most important- control frequencies do not exist for any of the whole genome analysis approaches so far. Although the cell numbers which are necessary for a statistically sound whole genome analysis are obviously lower than for a partial genome analysis such data cannot be established in short time. Thus, whole genome approaches are not applicable for routine analysis, however, it seems reasonable to apply them if high dose exposures can be assumed to complete aberration data obtained from a partial genome analysis (e.g., by correcting complex aberration frequencies).

Our data of a comprehensive SKY and FISH-painting analysis also have been analysed statistically for the frequency of damaged cells, observed colour junctions and resulting minimal number of breaks (Table 3). After *in vitro* irradiation with 3 Gy x-rays more than 80% of cells exhibit chromosomal damages as detected by SKY. Analysing these SKY data it turned out that for each case the minimal number of breaks per cells is much higher than the observed colour junctions per cell. This means that in comparison to FISH-painting analysis more one-way exchange aberrations have been observed. Since it has been reported that SKY and mFISH have a higher resolution being able to detect “hidden aberrations” [9, 21], it can be assumed that one-way exchange aberrations are likely to represent in fact incomplete aberrations with un-rejoined breaks.

In summary, we have demonstrated in this paper that the quantification of radiation-induced chromosome aberrations from the whole genome by SKY revealed comparable results as when calculated from partial genome data by FISH-painting with chromosomes 1, 4 and 12. It turned out that at high doses complex aberrations may be misinterpreted by FISH-painting as

simple aberrations. Therefore, whole genome analysis of radiation-induced chromosome aberrations by SKY is a helpful tool especially in the high-dose range.

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**Captions to Figures:**

Figure 1: SKY analysis of peripheral lymphocytes after *in vitro* irradiation with 3 Gy x-rays showing simple reciprocal aberrations and one-way exchanges:

- a) reciprocal translocation involving chromosomes 2 and 7 (arrows);
- b) incomplete dicentric (one fragment is missing) involving chromosomes 3 and 10 and incomplete translocation between chromosomes 4 and 14.

Figure 2: Partial spectral karyotypes from peripheral lymphocytes after *in vitro* irradiation with 3 Gy x-rays showing complex aberrations that would appear as simple aberrations after FISH-painting analysis for chromosomes 1, 4 and 12 (Table 2):

- a) metaphase 3906-35 showing  $(2'-4)(2-4')(2)$
- b) metaphase 3906-37 showing  $(2'-12)(2-13')(12T')$
- c) metaphase 3906-50 showing  $(1'-16)(1-16')(16), (2'-5-12)(5-12')(5T')$
- d) metaphase 3960-31 showing  $(9'-12)(12'-16)(X'-16)$ .

## References

1. M. Bauchinger, Retrospective dose reconstruction of human radiation exposure by FISH/chromosome painting, *Mutat. Res.* 404 (1998) 89-96.
2. A.T. Natarajan, J.J.W.A. Boei, Formation of chromosome aberrations: insights from FISH, *Mutat. Res.* 544 (2003) 299-304.
3. J.N. Lucas, A. Awa, T. Straume, M. Poggensee, Y. Kodama, M. Nakano, K. Othaki, H.-U. Weier, D. Pinkel, J. Gray, G. Littlefield, Rapid translocation frequency analysis in man decades after exposure to ionizing radiation, *Int. J. Radiat. Biol.* 62 (1992) 53-63.
4. H. Braselmann, U. Kulka, R. Huber, H.M. Figel, H. Zitzelsberger, Distribution of radiation-induced exchange aberrations in all human chromosomes, *Int. J. Radiat. Biol.* 79 (2003) 393-403.
5. M.R. Speicher, S.G. Ballard, D.C. Ward, Karyotyping human chromosomes by combinatorial multi-fluor FISH, *Nat. Genet.* 12 (1996) 368-375.
6. E. Schröck, S. du Manoir, T. Veldman, B. Schoell, J. Wienberg, M.A. Ferguson-Smith, Y. Ning, D.H. Ledbetter, I. Bar-Am, D. Soenksen, Y. Garini, T. Ried, Multicolor spectral karyotyping of human chromosomes, *Science* 273 (1996) 494-497.
7. H. Zitzelsberger, L. Lehmann, L. Hieber, H.U.G. Weier, C. Janish, J. Fung, T. Negele, F. Spelsberg, E. Lengfelder, E.P. Demidchik, K. Salassidis, A.M. Kellerer, M. Werner, M. Bauchinger, Cytogenetic changes in radiation-induced tumors of the thyroid, *Cancer Res.* 59 (1999) 135-140.
8. H. Zitzelsberger, J. Bruch, J. Smida, L. Hieber, C.M. Peddie, P.E. Bryant, A.C. Riches, J. Fung, H.-U.G. Weier, M. Bauchinger, Clonal chromosomal aberrations in simian virus 40-transfected human thyroid cells and in derived tumors developed after in vitro irradiation, *Int. J. Cancer* 96 (2001) 166-177.
9. K.M. Greulich, L. Kreja, B. Heinze, A.P. Rhein, H.-U.G. Weier, M. Brückner, P. Fuchs, M. Molls, Rapid detection of radiation-induced chromosomal aberrations in lymphocytes and hematopoietic progenitor cells by mFISH, *Mutat. Res.* 452 (2000) 73-81.
10. B.D. Loucas, M.N. Cornforth, Complex chromosome exchanges induced by gamma rays in human lymphocytes: An mFISH study, *Radiat. Res.* 155 (2001) 660-671.

11. R. Huber, U. Kulka, T. Lörch, H. Braselmann, D. Engert, M. Figel, M. Bauchinger, Technical Report: application of the Metafer2 fluorescence scanning system for the analysis of radiation-induced chromosome aberrations measured by FISH-chromosome painting, *Mutat. Res.* 492 (2001) 51-57.
12. J.D. Tucker, W.F. Morgan, A.A. Awa, M. Bauchinger, D. Blakey, M.N. Cornforth, L.G. Littlefield, A.T. Natarajan, C.A. Shasserre, A proposed system for scoring structural aberrations detected by chromosome painting, *Cytogenet. Cell Genet.* 68 (1995) 211-221.
13. M.N. Cornforth, Analyzing radiation-induced complex chromosome rearrangements by combinatorial painting, *Radiat. Res.* 155 (2001) 643-659.
14. J.R.K. Savage, P. Simpson, FISH "painting" patterns resulting from complex exchanges, *Mutat. Res.* 312 (1994) 51-60.
15. N.E. Morton, Parameters of the human genome, *Proc. Natl. Acad. Sci. USA* 88 (1991) 7474-7476.
16. M. Durante, K. George, H. Wu and F.A. Cucinotta, Karyotypes of human lymphocytes exposed to high-energy iron ions, *Radiat. Res.* 158 (2002) 581-590.
17. M. Vazquez, K.M. Greulich-Bode, J. Arsuaga, M.N. Cornforth, M. Bruckner, R.K. Sachs, L. Hlatky, M. Molls, P. Hahnfeldt, Computer analysis of mFISH chromosome aberration data uncovers an excess of very complicated metaphases, *Int J Radiat Biol* 78 (2002) 1103-1115.
18. B.D. Loucas, R. Eberle, S.M. Bailey, M.N. Cornforth, Influence of dose rate on the induction of simple and complex chromosome exchanges by gamma rays, *Radiat. Res.* 162 (2004) 339-349.
19. J. Arsuaga, K.M. Greulich-Bode, M. Vazquez, M. Bruckner, P. Hahnfeldt, D.J. Brenner, R. Sachs, L. Hlatky, Chromosome spatial clustering inferred from radiogenic aberrations, *Int. J. Radiat. Biol.* 80 (2004) 507-515.
20. J.J. Boei, S. Vermeulen, J. Moser, L.H. Mullenders, A.T. Natarajan, Intrachanges as part of complex chromosome-type exchange aberrations, *Mutat. Res.* 504 (2002) 47-55.
21. T. Veldman, C. Vignon, E. Schröck, J.D. Rowley, T. Ried, Hidden chromosome abnormalities in haematological malignancies detected by multicolour spectral karyotyping, *Nat. Genet.* 15 (1997) 406-410.



**Table 1:** Simple or apparently simple exchanges detected by SKY or FISH-painting with chromosomes 1, 4, 12.

Cells scored		Translocation types				Dicentric types				Ratios	
Case		2B <sup>1</sup>	Per cell <sup>2</sup>	I-III <sup>3</sup>	per cell <sup>2</sup>	2A <sup>4</sup>	per cell <sup>2</sup>	IV-VI <sup>5</sup>	per cell <sup>2</sup>	2B:2A <sup>6</sup>	2B <sub>tot</sub> :2A <sub>tot</sub> <sup>7</sup>
3930 SKY	59	36	0.214	13	0.077	14	0.083	15	0.089	2.57	1.69
3930 FISH	633	63	0.100	28	0.044	52	0.082	32	0.051	1.21	1.07
3906 SKY	37	11	0.104	6	0.057	8	0.076	8	0.076	1.38	1.06
3906 FISH	554	40	0.072	17	0.031	38	0.069	30	0.054	1.05	0.82
3960 SKY	38	8	0.074	3	0.028	10	0.092	8	0.074	0.80	0.61
3960 FISH	583	53	0.091	37	0.063	32	0.055	30	0.051	1.66	1.45

<sup>1)</sup> 2B = reciprocal translocation (a'-b)(b'-a), <sup>2)</sup> Frequencies from SKY analyses multiplied with factor 0.35 according to equation (2),

<sup>3)</sup> I-III = one-way aberration types (b'-a)(b), (b'-a) or (a'-b), <sup>4)</sup> 2A = two-way dicentric (a'-b')(a-b) or (a'-b')(a)(b), <sup>5)</sup> IV-VI = one-way aberration types (b'-a')(b) or (b'-a'), <sup>6)</sup> ratio of two-way aberrations, <sup>7)</sup> ratio of total one-way and two-way aberrations

**Table 2:** Complex aberrations detected by SKY in metaphases from three donors irradiated *in vitro* with 3 Gy X-rays

Cell	Number of simple aberrations <sup>1</sup>	Complex aberrations	Assumed CAB <sup>2</sup>	Missing counterparts <sup>3</sup>	Incomplete items (open ends) <sup>4</sup>	Expected appearance with FISH for chromosomes 1, 4 and 12
3906-4	1	(14'-13)(8'-13')(18'-13)	4/5/5	8, 14, 18		
3906-8	1	(19'-7)(19-7')(19)	2/.3		(19)	
3906-11	1	(11'-7-3)(3T')(3T')(7T')(7T')	3/3/5	11	3, 7	
3906-14		(6'-8')(8-6-17)(17'-6)(6)	3/4/5		(6), (6'-8')	
3906-21		(11'-13)(11-13')(11)	2/.3		(11)	
3906-30	1	(6'-12')(6-12)(12)	2/.3		(12)	(12'-a'), (12-a), (12)
<b>3906-35</b>	<b>2</b>	<b>(2'-4)(2-4')(2)</b>	<b>2/.3</b>		<b>(2)</b>	<b>(4'-a), (a'-4)</b>
<b>3906-37</b>	<b>1</b>	<b>(2'-12)(13'-2)(12T')</b>	<b>3/3/3</b>	<b>(13)</b>	<b>(12' T)</b>	<b>(a'-12)(12T')</b>
3906-41		(4'-13)(4-13')(4-5')	3/3/4	(5)	(4)	(4'-a), (a'-4), (a'-4)
<b>3906-50*</b>	<b>1</b>	<b>(1'-16)(1-16')(16)</b>	<b>2/.3</b>		<b>(16)</b>	<b>(1'-a), (a'-1)</b>
<b>3906-50*</b>	<b>1</b>	<b>(2'-5-12)(5-12')(5T')</b>	<b>3/3/4</b>	<b>(2)</b>	<b>(5' T)</b>	<b>(12'-a), (a'-12)</b>
3930-15	2	(1)(1'-X)(X'-3)(12'-3'-12)(12)	4/5/6	(3)	(1), (12)	(1), (1'-a), (12'-a'-12), (12)
<b>3930-17</b>		<b>(13'-5')(13)(1'-5)(1)</b>	<b>3/3/3</b>		<b>(1), (13)</b>	<b>(1'-a), (1)</b>
3930-18	4	(16-2)(2'-10)(10'-14')(14)	4/4/4	(16'T)	(14)	
<b>3930-23-1</b>	<b>1</b>	<b>(3'-4')(3-4)r(3)</b>	<b>2/2/3</b>			<b>(4'-a'), (4-a)</b>
3930-23-2	4	(6'-1'-21)(6)	3/4/4	2x(1), (21'T)	(6)	(a'-1'-a)
3930-31	3	(14'-2)(2'-20')(20-8')(8-15')(15)	5/5/5	(14)	(15)	
3930-32	3	r(4')(4)(13'-4-13)	2/3/4			r(4'), (4), (a'-4-a)
3930-33	2	(14'-5'-14)(5)	2/3/3			
3930-46	2	(6'-2'-6)r(2)2x(2)(6'-2)	3/4/5	(6)	(2)	

Cell	Number of simple aberrations <sup>1</sup>	Complex aberrations	Assumed CAB <sup>2</sup>	Missing counterparts <sup>3</sup>	Incomplete items (open ends) <sup>4</sup>	Expected appearance with FISH for chromosomes 1, 4 and 12
3960-4		(11-12')(11-12)(11T')	2/2/3		(11-12), (11')	
3960-5		(8T')(8-14')(14-5)(10'-5)(5T')	4/4/5	(10)	(5'T), (8'T), (5-14)	
3960-31*		(3'-17')2x(5-17)r(5')	3/4/4	(5), (3)		
<b>3960-31*</b>		<b>(9'-12)(12'-16)(X'-16)</b>	<b>4/4/5</b>	<b>(9), (X), (16')</b>	<b>(X'-16)</b>	<b>(12'-a), (a'-12)</b>
3960-43	2	(1'-X)(1-X)	2/2/3	(X'T)	(1-X)	(1'-a), (1-a)
3960-49	1	(1'-13)(1-13')(1)	2/2/3		(1)	(1'-a), (a'-1), (1)

<sup>1</sup> not shown in detail, <sup>2</sup> CAB is a short term for number of chromosomes/ number of chromosome arms / number of breaks, <sup>3</sup> chromosome parts that cannot be seen because they are cryptic or hidden, <sup>4</sup> chromosome parts of the aberration with apparently un-rejoined ends, <sup>5</sup> a: all chromosomes except chromosomes 1, 4, 12

\*) Two complex aberrations in one cell

**Table 3:** Summary of statistical analysis

Case	Cells	Cells with detected damage (%)	Complex C.A. (per cell)	Colour junctions (cj per cell)	Minimal number of breaks (mnb per cell)	mnb/cj
3930 SKY	59	81.4	0.15	2.80	4.22	1.51
3930 FISH	633	37.3	0.070	0.654	0.938	1.43
FISH/SKY (%)		<i>41.6</i>		<i>18.8</i>	<i>16.9</i>	
3906 SKY	37	91.9	0.30	2.41	3.84	1.60
3906 FISH	554	33.8	0.049	0.565	0.823	1.46
FISH/SKY (%)		<i>36.8</i>		<i>23.4</i>	<i>21.4</i>	
3960 SKY	38	86.8	0.16	1.79	3.05	1.70
3960 FISH	583	31.9	0.060	0.547	0.81	1.48
FISH/SKY (%)		<i>36.8</i>		<i>30.6</i>	<i>26.6</i>	

Figure 1:

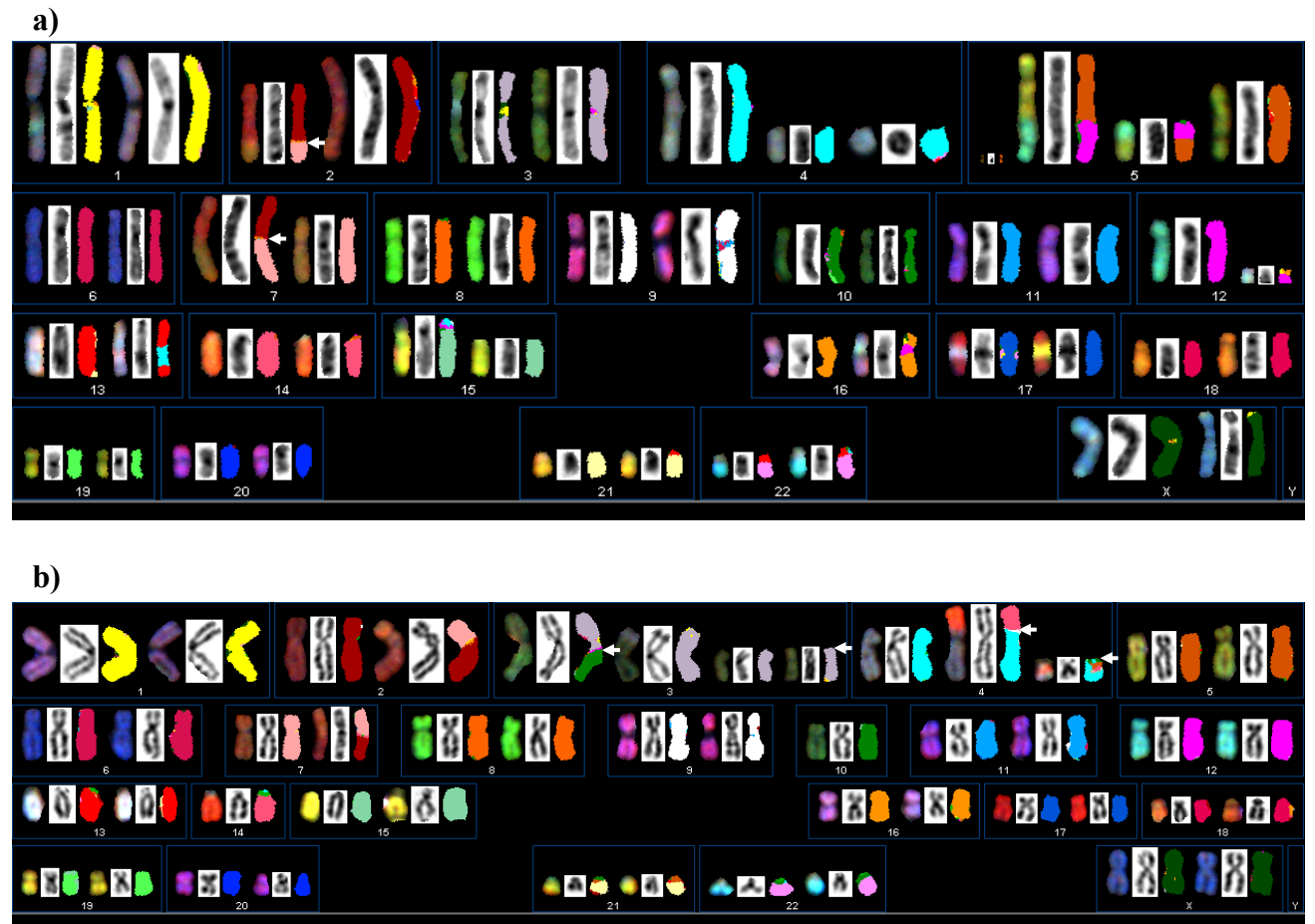


Figure 2:

