# COMPUTER VISUALIZATION OF THE EXPERIMENTAL COUNT OF CHROMOSOMAL ABERRATIONS

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## OVERVIEW: HOW CAN WE KNOW IF COMPUTER MODELS ARE TRULY IN SYNC WITH EXPERIMENTALIST'S' WORK?

### *COMPUTER VISUALIZATION OF CA MODELING MEANS EXPERIMENTALISTS CAN SEE THE ALGORITHM'S LOGIC AND DETERMINE WHETHER IT MATCHES THEIR CLASSIFICATION*

Researchers in the field of chromosomal aberrations (CA) often use their own classification scheme to classify various chromosome aberrations. A broad and sometimes confusing terminology was developed for naming CAs produced from fluorescence in situ hybridization (FISH) painted chromosomes; some investigators name CAs using an *ad hoc* system. Naming schemes may depend on the experimental techniques used (for example, M-BAND, M-FISH, 3-color FISH [1], though for less frequently used G-banding, such classification is much better defined). Herein, we will attempt to formalize and automate the specific way of counting CAs visualized in 3-color FISH chromosome painting. Although a simple algorithm with random breakage and rejoining can simulate the whole genome [2], under experimental conditions, not all CAs are discernable because some chromosomes are DAPI-stained (a fluorescent stain that binds strongly to adeline) and, therefore, indistinguishable. The discernable chromosomes in the experiment studied are 6 chromosomes (3 chromosome pairs); each pair is painted a distinct color (red, green and yellow). Thus, the exchange of chromosome material due to improper repair, resulting in CAs, is quantified only for the 6 painted chromosomes (more precisely, for 3 distinct pairs). If the painted chromosomes recombine with the same-color DAPI-stained chromosomes, the reported yields of some CAs are inadvertently reduced. The experimentalists may count simple exchanges, complex exchanges, dicentrics, rings, translocations, inversions, deletions, terminal deletions, and many others, by inferring what they can discern, and then extrapolating the result to the whole genome. The experimentalist extrapolates the result to the whole genome equivalents, using a modified version of the so-called geometric factor, or "g-factor" ($F_G$) of the Lucas et al formula [3, 4]:

$$F_G = 2.05*[f_p(1 - f_p) + f_{p1}f_{p2} + f_{p1}f_{p3} + f_{p2}f_{p3}]*F_p.$$

$F_p$ is the combined frequency of exchanges in all painted chromosomes, $f_p$ is the fraction of the whole genome comprised of the painted chromosomes, $f_{p1}$, $f_{p2}$ and $f_{p3}$ are the fractions of the genome for each individual chromosome. In this work, we developed an object-oriented graphical program to count yields of CAs, using the same system of counting CAs, as used by our experimentalist. The program algorithmically extrapolates the result to the entire genome. We found that certain CAs, such as complex aberrations, are difficult to discern, either "manually" or "algorithmically," due to not only the obfuscation of the complex sets of interacting fragments, but also because it is hard to establish a consensus on how to define complex exchanges in a 3-color experiment. To address these issues, we introduced new terminology and performed an analysis to map the data derived from a "human" counter onto the steps taken by the algorithm. We found, for example, that it is better to start the analysis of the observed CAs with, what we call, the "constituent chromosomes," rather than first trying to track misrejoined chromosome breaks. We present a graphical analysis done in the what-you-see-is-what-you-get manner, but still with the strictly reproducible automatic count of CAs, and by the algorithm calibrated by our experimentalist. Using this approach, we can refine the g-factor by developing an algorithmic extrapolation to the whole genome.

[1] Hada M. et al (2014) Radiat Res 182, 368-349.

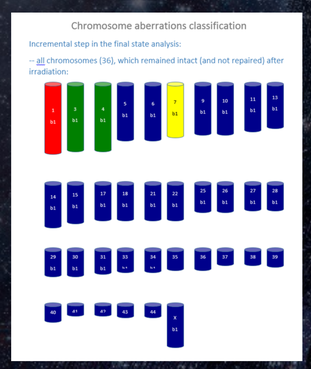
[2] Ponomarev A.L. et al (2014) Radiat Res 181, 284-292.

[3] Lucas J.N. et al (1992) Cytogenet Cell Genet 60, 255-256.

[4] George K.A. et al (2015) Frontiers Oncol 5, 226.

### *NASARTIcs USES THE NASARTI ALGORITHM WHICH HAS BEEN NASA VETTED & VERIFIED, FEATURED IN MANY PUBLICATIONS, AND IS INCLUDED IN THE NASA SPACE RADIATION ELEMENT INTEGRATIVE RISK MODELS TOOLKIT, LOCATED AT HTTPS://SPACERADIATION.JSC.NASA.GOV//IRMODELS/*

### *LET'S GO THROUGH THE STEPS IN THE MODEL: WE BEGIN WITH CHROMOSOME ABERRATIONS PROCESSING DURING ONE MONTE CARLO HISTORY*




Chromosomes are painted red, green & yellow as in the experiment. The graphic analysis to the left represents only one Monte Carlo history. These (remaining) chromosomes have never been broken by radiation (at 1 Gy, Si ions, E= 55 MeV/n). Chromosomes 1 (which was red) and 8 (which was yellow) are missing in this set, as they have been fragmented have DSBs and/or can interact through binary rejoining of DSBs (and shown on the next slide). The program methodically outputs all steps of the chromosome damage analysis, in a graphical manner.

Thus, the user (the experimentalist) can follow the logic of the algorithm to see whether its actions match experimental CA classification. The program adds textual annotation too.

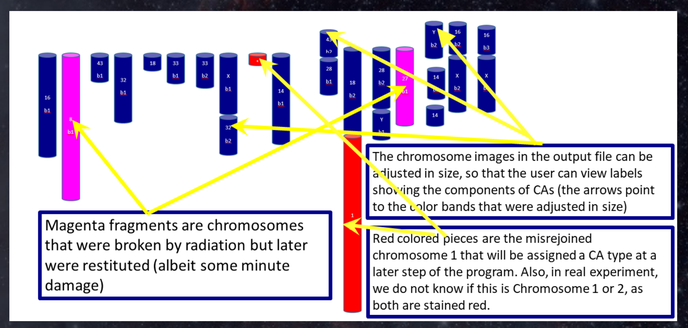
Email artem.l.ponomarev@nasa.gov with questions and comments. Thanks!

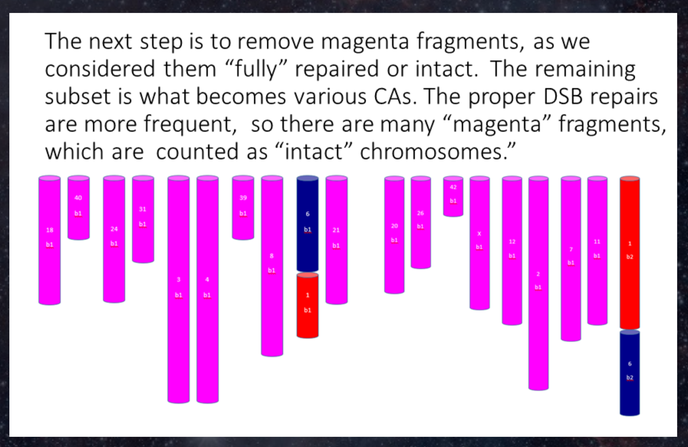
## NASARTIcs ANALYZES SEVERAL DIFFERENT STAGES OF CHROMOSOME REJOINING/MISREJOINING

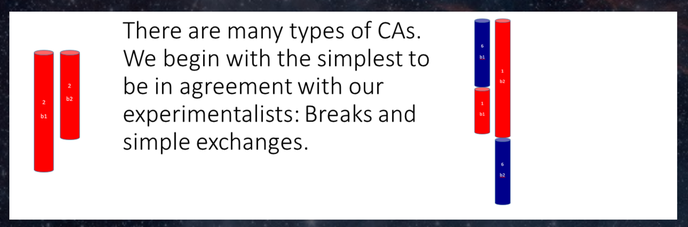
### *FOLLOW THE PROCESS IN A MONTE CARLO SIMULATION: ALL CHROMOSOME MAPS BELOW ARE THE OUTPUT OF THE COMPUTER MODEL FROM JUST 1 HISTORY*

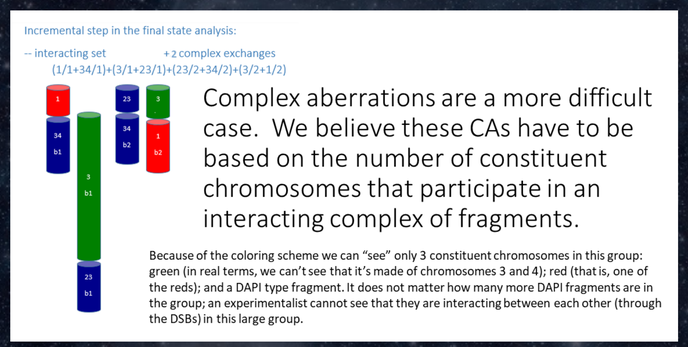
### *INCREMENTAL STEPS IN THE ANALYSIS: REJOINING/MISREJOINING AFTER DNA BREAKAGE*

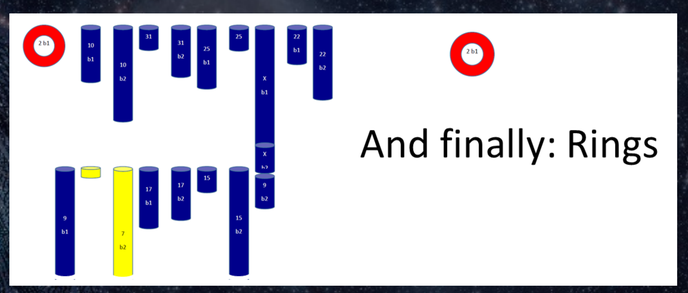
### *(WE CHOSE GOOD EXAMPLES OF THE MODEL'S STAGES FROM DIFFERENT SIMULATED IRRADIATED GENOMES)*



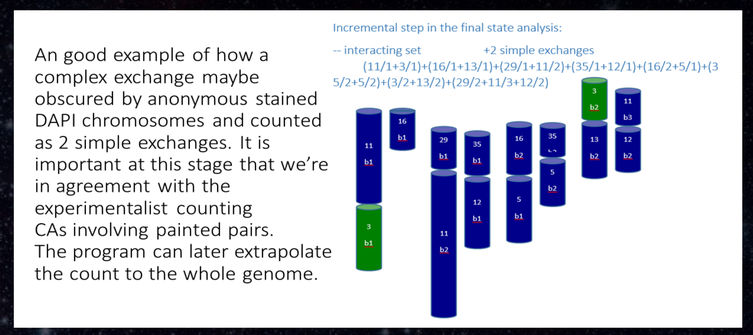




## INNOVATION: CHROMOSOME ABERRATIONS COMPUTER MODEL OF MIXED FIELD CHRONIC EXPOSURE WITH VISUALIZATION WHICH CAN BE CUSTOMIZED TO EXPERIMENTALISTS' WORK

### *COMPUTER VISUALIZATION ALLOWS US TO CALIBRATE WITH EXPERIMENTALISTS' INDIVIDUAL CLASSIFICATION ROUTINES, WHICH DON'T FOLLOW SIMPLE RULES*




**The biggest difference between the model and the experiment is that, programmatically, we can keep track of each chromosome, as their numbering shows in the Figs. In contrast, in the experiment, DAPI stained chromosomes are "anonymous," or in other words, indistinguishable except by color. As a result, sometimes there is no way experimentalists can distinguish between two simple exchanges and one complex exchange. M. Hada's photos of CAs show this.**

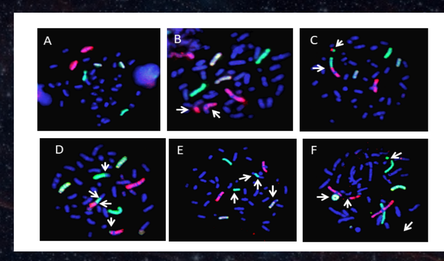



**Figure 2.** Example of chromosome painting in human fibroblast cells: Chromosome 1 (red), chromosome 2 (green), and chromosome 4 (yellow). Chromosome aberrations were identified as simple (reciprocal exchanges between two chromosomes) or complex-type exchanges (exchanges involving a minimum of 3 breaks in 2 or more chromosomes). A: normal; B: simple exchange in chr 1; C: simple exchanges (dicentric ) in chr1 & 2; D: complex exchange in chr 1 & 2; E: complex exchange (insertion) in chr 2 & 4; F: Ring (chr 4) and breaks (chr 1 & 2).

**Algorithm description: We learned that to properly match the experimentalist's classification scheme, we needed to focus on constituent chromosomes and bind them into interacting-via-DSBs groups of chromosomes. Although the program produces visual output, it bases its analysis on the numerical data describing each chromosome group (fragment sizes, DSB locations and patterns, tracks structures, restitution kinetics with the adjustable *P* (=0.08) and *I*, which are probabilities of rejoining/misrejoining, DSB complexity, etc.). An interacting set can have 4 visible colors of objects: red, green, yellow and blue. It does not matter how many actual constituent chromosomes are in it. DAPI fragments and the painted fragments are anonymous (indistinguishable) since, for example, the experimentalist can't tell if a red fragment is from chromosome 1 or chromosome 2. Anonymity of chromosomes obscures the real count, so the program has to count like the experimentalist.**

**Algorithm execution: The program gradually subtracts each thus processed group from the remaining unclassified damaged chromosomes and repeats this procedure until all broken chromosomes are bound in their individual fragment groups and thus classified.**

**Advantages: With NASARTIcs, the model can have better synchrony with experimentalists because it can be responsive to the experimentalist's systems. Showing the images to an experimentalist allows the developer to "calibrate the logic" of their CA classification.**

**Conclusions: Because the anonymity of chromosomes in experiments obscures an accurate count, it appears that the complex exchanges, as defined by the rule "at least 2 chromosomes and at least 3 DSBs", are more frequent than previously thought. In M_FISH experiments, complex exchanges always lose counts because of DAPI fragments. Most importantly, the interacting sets often "fall apart," since the experimentalist cannot connect fragments interacting through fragments with only DAPI staining, and are counted as simple exchanges.**

**A similar, albeit less pronounced, anonymity exists in painted pairs as the experimentalist cannot see which of the two painted chromosomes is interact in a CA. The program also takes this effect into account.**

**Because complex exchanges appear to be more frequent than previously thought, we believe the g-factor used to extrapolate the data to the whole genome is underestimated and should be higher than it's traditional value. The g-factor should be higher for complex exchanges than for simple exchanges. We observed that complex exchanges are more sensitive to the presence of "anonymity" in this 4-color scheme, as the interlinked sets are missed in a higher proportion, than just the proportion of DAPI stained chromosomes in the cell culture.**

**Future work: Refine g-factor for complex exchanges, using this model.**