How to count chromosomes in a cell: An overview of current and novel technologies

*Bjorn Bakker, Hilda van den Bos, Peter M. Lansdorp and Floris Foijer**

Aneuploidy, an aberrant number of chromosomes in a cell, is a feature of several syndromes associated with cognitive and developmental defects. In addition, aneuploidy is considered a hallmark of cancer cells and has been suggested to play a role in neurodegenerative disease. To better understand the relationship between aneuploidy and disease, various methods to measure the chromosome numbers in cells have been developed, each with their own advantages and limitations. While some methods rely on dividing cells and thus bias aneuploidy rates to that population, other, more unbiased methods can only detect the average aneuploidy rates in a cell population, cloaking cell-to-cell heterogeneity. Furthermore, some techniques are more prone to technical artefacts, which can result in over- or underestimation of aneuploidy rates. In this review, we provide an overview of several ''traditional'' karyotyping methods as well as the latest high throughput next generation sequencing karyotyping protocols with their respective advantages and disadvantages.

Keywords:
aneuploidy; chromosomal instability; karyotyping; next generation sequencing

DOI 10.1002/bies.201400218

European Research Institute for the Biology of Ageing (ERIBA), University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

***Corresponding author:**
Floris Foijer
E-mail: f.foijer@umcg.nl

Abbreviations:
CGH, comparative genomic hybridization; **CNV,** copy number variation; **FISH,** fluorescence in situ hybridization; **MCB,** multicolor banding; **M-FISH,** multiplex FISH; **SAC,** spindle assembly checkpoint; **SKY,** spectral karyotyping.

Introduction

A brief history of aneuploidy

During each cell division, all chromosomes are duplicated and distributed equally over the two emerging daughter cells. Various checkpoints help to ensure that chromosome segregation occurs accurately during the cell cycle. When errors occur during mitosis, cells can end up with an abnormal number of chromosomes, a state called aneuploidy. The relationship between aneuploidy and disease was first proposed early in the 20th century by Theodor Boveri. By injecting multiple sperm cells into sea urchin embryos, he showed that an increased chromosome content can result in abnormal development or death [1–3]. From the mid-20th century on, a large number of assays have been developed to quantify aneuploidy, which allowed linking various human syndromes and diseases to aneuploidy.

Most systemic aneuploidies for human autosomes are incompatible with embryonic development, and those that are viable (trisomies for chromosomes 13, 18, and 21) all result in a wide range of developmental and cognitive defects. The most well-known aneuploidy-related syndrome is Down's syndrome, which was first linked to a trisomy for chromosome 21 in 1959 [4]. One year later, Edward's syndrome (trisomy 18) and Patau's syndrome (trisomy 13) were uncovered as congenital syndromes caused by chromosomal copy number changes [5, 6]. Collectively, these syndromes demonstrate the severe consequences of an additional chromosome at the organismal level.

Aneuploidy accelerates cancer

Cancer cells frequently exhibit errors in chromosome segregation, resulting in chromosomal imbalances [7]. In fact, roughly two out of three human tumors display aneuploidy [8, 9], and genomic instability is considered to be a major enabling characteristic of malignant transformation [10]. Paradoxically, studies in aneuploid yeast strains and mouse embryonic fibroblasts have shown that aneuploidy reduces cell fitness

and leads to growth defects, as well as metabolic and proteotoxic stresses [11–13]. It is therefore remarkable that aneuploid cancer cells can proliferate in vivo despite aneuploidy-induced stress [14], and this suggests that aneuploid cancer cells somehow adjust their physiology to cope with the detrimental consequences of aneuploidy.

While structural genomic rearrangements (local amplifications/deletions and translocations) in cancer have been studied in great detail, we only begin to understand the precise role of whole-chromosome aberrations [15]. One disease that links cancer to aneuploidy is the mosaic variegated aneuploidy syndrome (MVA). MVA is caused by mutations in the spindle assembly checkpoint (SAC) gene *BUB1B*. The SAC monitors kinetochore–microtubule attachment during metaphase, and blocks anaphase onset until all chromosomes are properly aligned and attached to microtubules, thus preventing chromosome missegregation and consequent aneuploidy [16]. MVA patients are indeed characterized by random aneuploidies, suffer from developmental and cognitive defects [17, 18], and are significantly more likely to develop pediatric cancers, including rhabdomyosarcoma, Wilms tumor, and leukemia [17].

Mouse models, in which chromosomal instability (CIN) was provoked in vivo by inactivation of SAC genes (reviewed extensively elsewhere [19–21]), have been instrumental in better understanding the link between aneuploidy and cancer. Briefly summarized, CIN results in four major phenotypes: (i) embryonic lethality when the SAC is fully alleviated in the whole organism; (ii) weak tumor predisposition when SAC genes are heterozygously inactivated; (iii) tumor suppression in some tumor predisposed backgrounds (e.g. knockout of tumor suppressor genes or exposure to carcinogens); and (iv) premature aging [19–21]. These phenotypes even differ between in vivo cell lineages: for instance, the basal layer in mouse epidermis copes much better with CIN than the hair follicle stem cells that reside in the same tissue [22]. While, these data indicate that CIN is an important accelerating factor in cancer, the next challenge will be to understand how CIN contributes to malignant transformation. For this, we need to faithfully quantify the dynamics of chromosome missegregation in individual tumors, analyses that will heavily depend on improved cytogenetic tools, the topic of this review.

Aneuploidy might lead to neurodegeneration

Various studies have suggested that aneuploidy is not unique to cancer cells. For instance, a large fraction of normal mouse [23] and human [24–26] neurons appear to be aneuploid. Strikingly, these aneuploid neurons seem to be fully functional, because they are integrated into the brain circuitry and can be activated [27]. While the levels of aneuploidy in healthy brain are still under debate [28–30], aneuploidy in the brain could play a role in neurodegeneration [31]. For instance, patients suffering from Alzheimer's Disease (AD) exhibit frequent copy number changes for chromosomes 17 and 21 in buccal cells. Furthermore, post-mortem AD brain tissue appears to be more aneuploid than age-matched control brain [29, 32]. Possibly, aneuploidy contributes to neurodegenerative diseases through proteotoxic stress, leading to misfolded proteins, protein aggregates and thus neurodegeneration. However, most of these studies relied on noisy techniques to quantify aneuploidy in post-mitotic cells (e.g. in situ interphase FISH), which might have resulted in over- or underestimation of the actual aneuploidy in neurons. Therefore, improved methods to measure the number of chromosomes in non-dividing cells are a necessity to further substantiate the role of aneuploidy in neurodegenerative disease.

Accurate karyotyping tools are needed

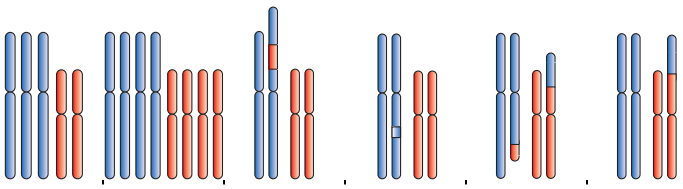
As argued above, accurate karyotyping is a crucial tool to better understand the role of aneuploidy in disease. While various karyotyping methods have been developed since the late 1950s, their accuracy and reliability differ. Importantly, each karyotyping method is limited as to which cytogenetic abnormalities can be detected, and in which cell type (e.g. proliferating vs. post-mitotic). Furthermore, each method is prone to its own technical and biological artefacts that need to be considered. Importantly, few platforms exist that allow for quantifying full karyotypes of non-dividing cells, a type of measurement that is becoming increasingly more important in studying the relationship between chromosomal instability and disease. In this review we provide an overview of the most common cytogenetic techniques, along with a number of new methods, and their associated applications and limitations.

The first important consideration when selecting a protocol for karyotyping is the type of cells to be assessed: dividing cells or non-dividing cells. While essentially all cytogenetic methods can be used to quantify chromosome copy numbers, some are either restricted to detection of only a few chromosomes per cell, reducing the resolution of the analysis, or fail to detect some types of karyotypic abnormalities (e.g. balanced translocations), which we will further discuss below. The detection limits of all of the addressed methods are summarized in Figure 1.

Tools to detect chromosome copy numbers in dividing cells

Traditional karyotyping and fluorescence in situ hybridization (FISH)

Traditional metaphase spread-based karyotyping requires cycling cells [33]. For this, cells are arrested in metaphase, using spindle assembly checkpoint poisons such as colcemid, to simplify chromosome counting. Cells are then incubated in a hypotonic solution followed by fixation. The fixed cells are then dropped onto a microscope slide to spread the chromosomes, and stained with Giemsa or DAPI to visualize the chromosomes. This protocol allows for the detection of whole chromosome copy number gains and losses, large amplifications, insertions, deletions, inversions, translocations, isochromosomes, and ring chromosomes (by assessing Giemsa chromosome banding patterns). Metaphase karyotyping can be combined with fluorescence in situ hybridization (FISH), for instance to detect common copy number variations (CNVs) or translocations such as the BCR-ABL t(9;22)(q34;q11)



	Technique	Whole genome	Aneuploidy	Polyploidy	CNVs (size)	Inversion	Reciprocal translocation	Unbalanced translocation	Heterogeneity	Costs	Reference
Metaphase spread-based (dividing cell populations)	Giemsa staining	+	+	+	5-10 Mb	+	+	+	+	Inexpensive	[41, 57]
	FISH	-	+	+	(+)[1]	+	+	+	+	Inexpensive	[34, 43, 57]
	SKY, M-FISH, COBRA-FISH	+	+	+	-	-	+	+	+	Moderate	[36–42, 44–46]
	mMCB	+	+	+	5-10 Mb	+	+	+	+	Moderate	[47,76, 77]
Non-metaphase spread-based (non-dividing cell populations)	Interphase FISH	-	+	+	(+)[1]	+	+	+	+	Inexpensive	[34, 43, 57]
	CGH & aCGH	+	+	-	10-20 Mb	-	-	+	(+)[2]	Moderate	[34, 41, 52–55]
	SNP array	+	+	-	500 kb	-	-	+	(+)[2]	Moderate	[41, 56, 60–62]
	Flow cytometry	-	(+)[3]	+	-	-	-	-	-	Inexpensive	[50, 51]
	Single-cell sequencing	+	+	(+)[4]	200-500 kb[4]	(+)[5]	(+)[6]	+	+	Expensive	[28, 65, 66]

Figure 1. Comparison of cytogenetic methods. Cytogenetic techniques to detect chromosomal abnormalities are listed, as well as their ability to detect various chromosomal aberrations. A ''+'' indicates the technique is able to detect the abnormality, a ''−'' indicates an inability to detect. A (+) signifies that the method can detect the aberration only under certain conditions or in a limited fashion, the details of which are listed below. If a technique can detect local CNVs, whenever possible the minimum detectable CNV size is given in kb (kilobases) or Mb (megabases). (1) CNVs can only be detected when probes specific to the amplified or deleted region are used. (2) Multiple experiments have to be performed on subpopulations of the same sample in order to identify heterogeneity. (3) Bulk aneuploidy can be detected using flow cytometry, i.e. a deviating DNA content from the haploid genome or a multiple thereof. Heterogeneity and specific copy number changes cannot be determined. (4) Polyploidy using single-cell sequencing can only be detected using a non-WGA approach in which more than two identical reads are mapped to the reference genome. (5) The minimum detectable CNV size is heavily influenced by the coverage. Here, we provide a conservative estimate based on 1% coverage. (6) Both inversions and reciprocal translocations may only be detectable with single-cell sequencing if sufficient coverage over the breakpoint region can be achieved. Alternatively Strand-seq can be used.

translocation [34]. FISH is a powerful tool for establishing cytogenetic abnormalities in patients and in pre-implantation embryos in the clinic. Unfortunately, FISH can only detect a small number of features per cell, as a result of limitations in the number of fluorescent labels. Also, a duplication or deletion of the probe-binding region can lead to falsely called gains or losses of chromosomes. Furthermore, technical artefacts such as probe clustering, failure of hybridization, or incomplete spreads, can result in an over- or underestimation of the targeted feature or chromosome. Finally, FISH requires technical expertise, and quantification is labor intensive because it is technically difficult to automate. This makes FISH a powerful tool for detecting recurrent chromosomal abnormalities in a standardized setting, but less suitable for the detection of random aneuploidies [34–36].

Karyotyping dividing cells using whole chromosome paints

Multiplex FISH (M-FISH) and spectral karyotyping (SKY) are FISH-adapted protocols that can be used to detect both chromosome copy number changes as well as gross translocations within the entire genome. For this, metaphase chromosome spreads are prepared on a microscope slide, similarly to FISH. Instead of one labeled probe per chromosome, chromosome-specific probe sets consisting of up to five distinct fluorescent dyes are hybridized to metaphase chromosomes, resulting in chromosome-specific, unique combinations. This allows for simple detection of all chromosomes in a metaphase spread. The main difference between M-FISH and SKY is the detection method of the labeled chromosomes: a fluorescence microscope for M-FISH and an interferometer for SKY. Both measurements require further computer post-processing of the imaging data, resulting in false-colored images in which the whole chromosomes are ordered in numerical order [37, 38]. This allows for simple detection of structural as well as numerical aberrations. Importantly, chromosome fragments can also be identified as individual fragments, a limitation of next-generation sequencing-based karyotyping tools (see below). This makes M-FISH and SKY ideal tools for detecting gross chromosomal instability in dividing cells. The technical limitations of M-FISH and SKY are similar to those of FISH, discussed above [36, 39–43].

An even more sensitive technique is COBRA-FISH: COmbined Binary RAtio labeling. COBRA-FISH combines combinatorial fluorescence labeling with ratio labeling, thereby increasing the resolution. Three fluorophores are paired in five different ratios, providing a total of 12 unique signatures. Addition of two more binary fluorophores added to each fluorophore-ratio pair increases the total number of possible unique combinations fourfold to 48, further increasing the number of targets than can be labeled. As such, COBRA-FISH allows for the detection of all chromosome arms individually [44–46].

An alternative to COBRA-FISH is multiplex multicolor banding (mMCB). In this platform, metaphase chromosomes are hybridized to a probe collection detecting different chromosomal regions, resulting in multi-banded patterns for each chromosome [47, 76, 77].

Limitations to metaphase-spread based karyotyping: FISHy business

While metaphase-spread based karyotyping is a powerful technique to detect aneuploidy, the key limitation is the requirement of dividing cells. In some cases, dividing cells are not available, e.g. in the case of paraffin-embedded material, post-mitotic cells, or primary tumor material. While tissue culture cells typically divide at least once per day with about 50% of cells in S phase at any time, the doubling time of primary breast cancer cells, for example, can be as low as ~1–10 months, and at a given time only 2–5% of cells are in S phase [33]. Furthermore, when harvesting primary tumor material, colcemid-mediated enrichment of mitotic cells to obtain condensed chromosomes is not possible, which, together with low proliferation rates, disqualifies metaphase-dependent aneuploid-quantification.

Even more important, because aneuploidy has such detrimental consequences for cell fitness and proliferation [13, 48] and cancer cells appear to select for some chromosome combinations [49], it is likely that the observed aneuploidy in the mitotic cell population is not fully representative of the total (tumor) cell population. In addition, metaphase-dependent methods preclude analysis of post-mitotic cells, such as neurons or quiescent stem cells. Therefore, to reliably quantify aneuploidy, protocols are required that do not depend on mitotic chromosomes: we will discuss these further below.

Detecting chromosome copy numbers in non-dividing cells

Interphase FISH

The classical way to quantify aneuploidy in non-dividing cells is by interphase FISH [77]. Like metaphase FISH, interphase FISH (I-FISH) relies on chromosome-specific probes, which are now hybridized to uncondensed chromosomes in interphase, followed by counting the resulting foci per nucleus for each probe. Therefore, the limitations of interphase FISH are similar to metaphase FISH: under and over-quantification of aneuploidy due to failure of probe hybridization or probe clustering, respectively. Similarly, the number of available fluorophores limits the number of quantifiable chromosomes.

An adapted version of this technique is multicolor banding (MCB). MCB reduces the technical noise of interphase FISH, by hybridizing multiple probes to one chromosome, resulting in a colored banding pattern for the assessed chromosome, increasing reliability. The flip side of the increased reliability is loss of resolution per cell, because only one chromosome can be quantified per analysis.

Flow cytometry

A simple but low resolution method for determining the ploidy of many cells simultaneously is fluorescent labeling of all the DNA at once with one dye, followed by flow cytometry. By comparing the fluorescence of the sampled cells of unknown ploidy to diploid reference cells' fluorescence, ploidy of the assessed cells can be deduced. The key advantages of flow cytometry-based karyotyping are throughput (large numbers of cells can be assessed at once), and speed (preparation time is minimal). The downside is that the resolution is very low: individual chromosome copy number gains or losses cannot be detected, let alone small CNVs or other genomic aberrations [50, 51].

(Array) comparative genomic hybridization

Another more high-resolution method for quantifying aneuploidy in non-dividing cell populations is comparative genomic hybridization (CGH). For this, genomic DNA of the sample to be assessed (e.g. a tumor) is fragmented and labeled with a green fluorescent dye, and DNA from a normal diploid (isogenic) reference control is labeled in red. Both sample and reference DNA are next hybridized to a diploid metaphase spread from a cell line from the same organism. Fluorescence ratios are then determined through fluorescence microscopy. In this example, an increased green signal implies amplification of a specific region or a gain of a whole chromosome in the tested sample, and red implies a deletion. Finally, a DNA stain is used to identify the individual chromosomes. The approximate resolution of CGH is ~10–20 Mb [52].

CGH has now mostly been replaced by a more sophisticated and higher resolution adapted platform employing microarrays (array CGH or aCGH). For aCGH distinctly fluorescently labeled DNA from a sample in one color and a reference in another are hybridized competitively to a reference genome. However, instead of using a metaphase spread, an array chip containing defined genomic probes encompassing the whole genome is used for hybridization. Fluorescence ratios are then determined through a microarray scanner. Depending on the probe density/sizes used, the resolution can be as high as ~400 kb, sufficient to quantify copy alterations for individual loci.

One important limitation of both CGH and aCGH is that neither method can detect reciprocal translocations or inversions, since such abnormalities do not result in changes in the chromosomal content. Furthermore, as typically the genomic DNA of tumor fragments and not individual cells is hybridized, only copy number changes that affect the bulk of the tumor will be detected [34, 41, 52–57], unless expensive single cell array CGH platforms are used [58, 59].

Single nucleotide polymorphism array

Another array-based technique for detecting chromosome copy numbers is the single nucleotide polymorphism array (SNP array). Instead of competitively hybridizing sample and reference genomes, only the sample-labeled genome is hybridized to an array with roughly 100,000 SNP probes.

Copy number changes are then determined by comparing the fluorescent signal from the labeled sample to an independently hybridized control. In addition to assessing copy number changes, SNP arrays can also be used to detect ratios between parental chromosomes. This permits for instance the detection of copy neutral loss of heterozygosity evidenced by uniparental disomies or gene conversions in for instance leukemia. [41, 56, 60–62]. The limitations of SNP arrays are similar to those of array CGH.

A need for improved karyotyping platforms

So far, we have discussed most common techniques that are currently used to quantify aneuploidy. Each of these techniques comes with advantages and disadvantages. Whole chromosome paints are an extremely powerful tool to determine full karyotypes of individual cells, but can only be used when dividing cells are available and results will therefore only be representative for the dividing cell population. Interphase FISH can detect chromosomal abnormalities at the single cell level of all cells, but for only few chromosomes per analysis. Finally, array-based karyotyping does allow for high resolution karyotyping of non-dividing cells, but not at the single cell level. However, to understand how chromosomal instability contributes to the development of disease, we need karyotyping platforms that combine single cell resolution with complete karyotyping. Recently, a number of such platforms have been developed, and are discussed below.

Emerging technologies: Sequencing-based karyotyping allows for quantification of karyotype heterogeneity

While aCGH and SNP array protocols can measure aneuploidy at much higher resolution than for instance interphase FISH, they are commonly less suitable for measuring karyotype heterogeneity (i.e. the differences among the cells' karyotypes within one sample). An ideal karyotyping method would therefore combine the best of both worlds: an affordable single cell approach with high resolution. Such an innovation might arise from new sequencing-based karyotyping methods. Next-generation sequencing (NGS) technology has opened up new possibilities for exploring both the human and the mouse genome, hence allowing us to map mutations in various oncogenes and tumor suppressors at the single base level. Other than determining the DNA nucleotide sequence, NGS can also be used to study tumor evolution. For example, high-throughput and high coverage sequencing allows the use of SNPs to map the clonality of tumors [63, 64]. It is also possible to karyotype cells using NGS.

Single-cell sequencing is a powerful tool for high resolution single cell karyotyping

Single-cell next generation sequencing is a recently developed platform for quantifying karyotypes of single cells, and has been used to quantify aneuploidy levels in liver, brain, and skin from both humans and mice [28]. Depending on the desired cell type or sample, single cells can be collected using cell pickers, serial dilutions, or FACS. As the input DNA from a single cell is very limited, library preparation often starts with a whole genome amplification (WGA) step. Here we describe a typical library preparation protocol. The DNA is fragmented, end-repaired, phosphorylated, and A-tailed to prepare the DNA for adaptor ligation, to make the DNA fragments compatible with the sequencing platform used. Following adapter ligation, the library fragments are PCR-amplified. To allow for multiplexing of libraries in individual sequencing lanes, barcodes can either be included in the adapter sequences or added during the PCR amplification using indexed primers followed by NGS. Following NGS, the libraries are demultiplexed, run through a quality control pipeline, and reads are mapped to the reference genome [28, 65].

Chromosome copy numbers and local CNVs per cell can then be extracted from the NGS data by examining the number of reads per chromosome or region, for instance using a Hidden Markov model [28]. This yields data with a resolution comparable to aCGH or even higher (resolutions up to 20–50 kb are feasible depending on coverage) and, importantly, at the single cell level. High resolution sequencing data of the assessed genome is not required for determining chromosome copy numbers faithfully: for this, 0.5 - 1% coverage per cell is more than sufficient. A lower coverage threshold also permits multiplexing of hundreds of single cell sequencing libraries, reducing sequencing costs per individual cell. Furthermore, the entire library preparation process can be automated using robotic pipetting system, thereby further reducing costs.

A major advantage of single cell sequencing over FISH is the ability to look at all chromosomes simultaneously in a single cell. Therefore, single cell sequencing combines the best of interphase FISH (single cell analysis) with the resolution of array based karyotyping. Furthermore, the risk of over- or underestimation of copy numbers is greatly reduced because thousands of reads are sequenced for each chromosome, instead of assessing only a few loci per chromosome. Indeed, several recent reports on copy number variation and aneuploidy in normal brain cells using single cell sequencing have also emphasized the advantages of using whole genome single cell sequencing over FISH [28, 66, 67].

Single cell NGS also comes with disadvantages. As mentioned above, for now NGS is still expensive, especially when compared to lower resolution karyotyping protocols. Furthermore, it is not possible to reliably detect balanced translocations and inversions, especially at lower coverage, precluding the mapping of for instance chromothripsis [68] at the single cell level. However, if coverage is very high, translocations can be identified from ''chimeric'' sequencing reads (i.e. one read aligning to two chromosomes) that bridge the translocation breakpoint. Acquiring the required coverage for a single cell to map these reads is not trivial, and heavily depends on the efficiency of the library preparation and the sequencing platform parameters (for instance whether it includes paired end reads [69]) and bioinformatical tools used. Unbalanced translocations on the other hand can easily be detected, although its relative position in the genome (i.e. to which chromosome the extra fragment is ligated) can only be

identified with sufficient sequencing coverage when the sequencing data includes chimeric reads that can be mapped to the reference genome. However, when such alterations are suspected, other karyotyping tools, such as FISH or G-banding can be employed for further confirmation. Last, but not least, sequencing of minute amounts of DNA using next generation sequencing platforms also comes with the risk for sequencing artefacts, such as GC bias, or whole genome amplification PCR bias, which, for which can at least be partially corrected for using new bioinformatical tools [70].

When needed, single cell NGS coverage can be increased, for instance by reducing the number of sequencing libraries per sequencing run. This will not limitlessly increase resolution though, because the library complexity of a single cell library is limiting. To increase library complexity, single cell genomes can be amplified through WGA before library preparation, which, in theory, should allow for mapping e.g. chromothripsis events at the single cell level. WGA does come with a risk of amplification bias, which can result in under- and overrepresented genomic regions in the final alignment [71, 72]. Such regions, or even whole chromosomes, could then incorrectly be called as aneuploid: therefore optimization is required before using WGA in single cell sequencing. Furthermore, increased coverage will literally come at a price as fewer cells/libraries can be analyzed per sequencing lane.

Single-cell strand sequencing (Strand-seq)

An alternative method for mapping translocation breakpoints and inversions is Strand-seq. This method was originally developed to study sister chromatid inheritance patterns in asymmetrically dividing cells [65, 73]. During cell division, each daughter cell inherits one sister chromatid from each parental chromosome pair. Strand-seq enables researchers to uncover this inheritance pattern. For this purpose, cells are cultured in the presence of bromodeoxyuridine (BrdU), a thymidine analogue, for exactly one cell cycle resulting in BrdU to be incorporated only in the newly synthesized DNA strands. Libraries are then prepared from FACS-sorted single cells followed by a UV-Hoechst treatment step to induce nicks at the sites of BrdU incorporation in the newly formed strand. Therefore, only the original template strand is amplified in the subsequent PCR amplification. Importantly, the resulting libraries maintain directionality so that the reads map to their parental strand after sequencing. The bioinformatic pipeline BAIT (Bioinformatic Analysis of Inherited Templates) can then be used to further annotate the reads [74]. Maintaining directionality allows for the detection of sister chromatid exchanges that occurred during the cell division in the presence of BrdU, which can be visualized by BAIT. These sister chromatid exchanges are visible as switches in the template strand inheritance pattern. Besides sister chromatid exchanges, Strand-seq can also be used to map translocations and inversions and to identify chromosomal or localized amplifications or deletions, hence allowing more detailed karyotyping, even at lower sequencing depth. Because upfront WGA cannot be used to identify DNA template strands, typically only a few percent of the DNA in a cell is captured in Strand-seq libraries. However, the lack of amplification in Strand-seq avoids amplification bias in single cell sequencing libraries. As a result the number of reads per chromosome typically shows a good correlation with the chromosome size and copy number. Since directionality is not needed for plain karyotyping, BrdU can be omitted from the Strand-seq protocol to get accurate karyotype information.

Taken together, single-cell sequencing provides opportunities to accurately karyotype all chromosomes on single cell level, although the use of Strand-seq is limited to dividing cells [65, 75]. For now, sequencing costs and the need for bioinformatics expertise might limit the use of single-cell sequencing karyotyping to research laboratories, but with the rapidly decreasing NGS costs and development of user-friendly bioinformatical tools, NGS platforms might soon become standard tools used in a diagnostic setting.

Conclusions and outlook

Aneuploidy is a feature of several syndromes associated with developmental and cognitive defects, a hallmark of cancer, and a potential protagonist in neurodegenerative disease. To better understand the relationship between aneuploidy and these pathologies, reliable methods for analyzing karyotypes are a necessity. In this review, we have provided an overview of existing techniques for karyotyping, and highlighted their strengths and limitations. Typically, the most affordable methods require dividing cells, in which case aneuploidy rates are only representative of the dividing subpopulation, which might not represent aneuploidy rates in the whole cell population. Furthermore, dividing cells are sometimes simply not available, for instance when assessing post-mitotic tissues. While methods that can quantify aneuploidy rates in interphase cells can be used to circumvent this bias, most of these methods cannot detect aneuploidies at the single cell level, or are limited to analysis of a few chromosomes per cell, hence obscuring karyotype heterogeneity. Therefore, novel techniques such as single-cell sequencing might combine the best of both worlds: the ability to determine karyotypes at high resolution in an unbiased and high throughput fashion.

Acknowledgments

We thank the members of the Lansdorp and Foijer labs for fruitful discussion, and Jorge Garcia Martinez, Klaske Schukken, and Diana Spierings for critically reading the manuscript. FF is supported by Stichting Kinder Oncologie Groningen (SKOG) and Dutch Cancer Society grant RUG 2012-5549.

The authors have declared no conflicts of interest.

References

1. **Boveri T, Manchester KL**. 1995. Theodor Boveri—the origin of malignant tumours. *Trends Cell Biol* **5**: 384–7.
2. **Bignold LP, Coghlan BLD, Jersmann HP**. 2006. Hansemann, Boveri, chromosomes and the gametogenesis-related theories of tumours. *Cell Biol Int* **30**: 640–4.

3. **Boveri T**. 2008. Concerning the origin of malignant tumours by Theodor Boveri. Translated and annotated by Henry Harris. *J Cell Sci* **121**: 1–84.
4. **Neri G, Opitz JM**. 2009. Down syndrome: comments and reflections on the 50th anniversary of Lejeune's discovery. *Am J Med Genet A* **149A**: 2647–54.
5. **Patau K, Smith D, Therman E, Inhorn S**, et al. 1960. Multiple congenital anomaly caused by an extra autosome. *Lancet* **275**: 790–3.
6. **Edwards J, Harnden D, Cameron A, Mary Crosse V**, et al. 1960. A new trisomic syndrome. *Lancet* **275**: 787–90.
7. **Duijf PHG, Benezra R**. 2013. The cancer biology of whole-chromosome instability. *Oncogene* **32**: 4727–36.
8. **Duijf PHG, Schultz N, Benezra R**. 2013. Cancer cells preferentially lose small chromosomes. *Int J Cancer* **132**: 2316–26.
9. **Weaver BAA, Cleveland DW**. 2006. Does aneuploidy cause cancer? *Curr Opin Cell Biol* **18**: 658–67.
10. **Hanahan D, Weinberg RA**. 2011. Hallmarks of cancer: the next generation. *Cell* **144**: 646–74.
11. **Torres EM, Williams BR, Amon A**. 2008. Aneuploidy: cells losing their balance. *Genetics* **179**: 737–46.
12. **Pfau SJ, Amon A**. 2012. Chromosomal instability and aneuploidy in cancer: from yeast to man. *EMBO Rep* **13**: 515–27.
13. **Torres EM, Sokolsky T, Tucker CM, Chan LY**, et al. 2007. Effects of aneuploidy on cellular physiology and cell division in haploid yeast. *Science* **317**: 916–24.
14. **Sheltzer JM, Amon A**. 2011. The aneuploidy paradox: costs and benefits of an incorrect karyotype. *Trends Genet* **27**: 446–53.
15. **Gordon DJ, Resio B, Pellman D**. 2012. Causes and consequences of aneuploidy in cancer. *Nat Rev Genet* **13**: 189–203.
16. **Musacchio A, Salmon ED**. 2007. The spindle-assembly checkpoint in space and time. *Mol Cell* **8**: 379–93.
17. **Hanks S, Coleman K, Summersgill B, Messahel B**, et al. 2006. Comparative genomic hybridization and BUB1B mutation analyses in childhood cancers associated with mosaic variegated aneuploidy syndrome. *Cancer Lett* **239**: 234–8.
18. **Hanks S, Coleman K, Reid S, Plaja A**, et al. 2004. Constitutional aneuploidy and cancer predisposition caused by biallelic mutations in BUB1B. *Nat Genet* **36**: 1159–61.
19. **Schvartzman J-M, Sotillo R, Benezra R**. 2010. Mitotic chromosomal instability and cancer: mouse modelling of the human disease. *Nat Rev Cancer* **10**: 102–15.
20. **Holland AJ, Cleveland DW**. 2009. Boveri revisited: chromosomal instability, aneuploidy and tumorigenesis. *Nat Rev Mol Cell Biol* **10**: 478–87.
21. **Foijer F, Draviam VM, Sorger PK**. 2008. Studying chromosome instability in the mouse. *Biochim Biophys Acta* **1786**: 73–82.
22. **Foijer F, DiTommaso T, Donati G, Hautaviita K**, et al. 2013. Spindle checkpoint deficiency is tolerated by murine epidermal cells but not hair follicle stem cells. *Proc Natl Acad Sci USA* **110**: 2928–33.
23. **Yurov YB, Iourov IY, Monakhov VV, Soloviev IV**, et al. 2005. The variation of aneuploidy frequency in the developing and adult human brain revealed by an interphase FISH study. *J Histochem Cytochem* **53**: 385–90.
24. **Mosch B, Morawski M, Mittag A, Lenz D**, et al. 2007. Aneuploidy and DNA replication in the normal human brain and Alzheimer's disease. *J Neurosci* **27**: 6859–67.
25. **Westra JW, Peterson SE, Yung YC, Mutoh T**, et al. 2008. Aneuploid mosaicism in the developing and adult cerebellar cortex. *J Comp Neurol* **507**: 1944–51.
26. **Pack SD, Weil RJ, Vortmeyer AO, Zeng W**, et al. 2014. Individual adult human neurons display aneuploidy: Detection by fluorescence in situ hybridization and single neuron PCR. *Cell Cycle* **4**: 1758–60.
27. **Kingsbury M, Friedman B, McConnell M, Rehen S**, et al. 2005. Aneuploid neurons are functionally active and integrated into brain circuitry. *Proc Natl Acad Sci USA* **102**: 6143–7.
28. **Knouse KA, Wu J, Whittaker CA, Amon A**. 2014. Single cell sequencing reveals low levels of aneuploidy across mammalian tissues. *Proc Natl Acad Sci USA* **111**: 13409–14.
29. **Iourov IY, Vorsanova SG, Liehr T, Yurov YB**. 2009. Aneuploidy in the normal, Alzheimer's disease and ataxia-telangiectasia brain: differential expression and pathological meaning. *Neurobiol Dis* **34**: 212–20.
30. **Yurov YB, Vorsanova SG, Iourov IY**. 2009. GIN'n'CIN hypothesis of brain aging: deciphering the role of somatic genetic instabilities and neural aneuploidy during ontogeny. *Mol Cytogenet* **2**: 23.
31. **Thomas P, Fenech M**. 2008. Chromosome 17 and 21 aneuploidy in buccal cells is increased with ageing and in Alzheimer's disease. *Mutagenesis* **23**: 57–65.
32. **Yurov YB, Vorsanova SG, Liehr T, Kolotii AD**, et al. 2014. X chromosome aneuploidy in the Alzheimer's disease brain. *Mol Cytogenet* **7**: 20.
33. **Mitchison TJ**. 2012. The proliferation rate paradox in antimitotic chemotherapy. *Mol Biol Cell* **23**: 1–6.
34. **Das K, Tan P**. 2013. Molecular cytogenetics: recent developments and applications in cancer. *Clin Genet* **84**: 315–25.
35. **McGranahan N, Burrell RA, Endesfelder D, Novelli MR**, et al. 2012. Cancer chromosomal instability: therapeutic and diagnostic challenges. *EMBO Rep* **13**: 528–38.
36. **Pellestor F, Anahory T, Hamamah S**. 2005. The chromosomal analysis of human oocytes. An overview of established procedures. *Hum Reprod Update* **11**: 15–32.
37. **Bayani J, Squire J**. 2004. Multi-color FISH techniques. *Curr Protoc Cell Biol* Chapter 22: Unit 22.5.
38. **Liehr T, Starke H, Weise A, Lehrer H**, et al. 2004. Multicolor FISH probe sets and their applications. *Histol Histopathol* **19**: 229–37.
39. **Bayani JM, Squire JA**. 2002. Applications of SKY in cancer cytogenetics. *Cancer Invest* **20**: 373–86.
40. **Padilla-Nash HM, Barenboim-Stapleton L, Difilippantonio MJ, Ried T**. 2006. Spectral karyotyping analysis of human and mouse chromosomes. *Nat Protoc* **1**: 3129–42.
41. **Speicher MR, Carter NP**. 2005. The new cytogenetics: blurring the boundaries with molecular biology. *Nat Rev Genet* **6**: 782–92.
42. **Imataka G, Arisaka O**. 2012. Chromosome analysis using spectral karyotyping (SKY). *Cell Biochem Biophys* **62**: 13–7.
43. **Jiang J, Gill BS**. 2006. Current status and the future of fluorescence in situ hybridization (FISH) in plant genome research. *Genome* **49**: 1057–68.
44. **Szuhai K, Tanke HJ**. 2006. COBRA: combined binary ratio labeling of nucleic- acid probes for multi-color fluorescence in situ hybridization karyotyping. *Nat Protoc* **1**: 264–75.
45. **Szuhai K, Bezrookove V, Wiegant J, Vrolijk J**, et al. 2000. Simultaneous Molecular Karyotyping and Mapping of Viral DNA Integration Sites by 25-Color COBRA-FISH. *Genes Chromosomes Cancer* **97**: 92–7.
46. **Kuźniacka A, Mertens F, Strömbeck B, Wiegant J**, et al. 2004. Combined binary ratio labeling fluorescence in situ hybridization analysis of chordoma. *Cancer Genet Cytogenet* **151**: 178–81.
47. **Liehr T, Heller A, Starke H, Rubtsov N**, et al. 2002. Microdissection based high resolution multicolor banding for all 24 human chromosomes. *Int J Mol Med* **9**: 335–9.
48. **Williams BR, Prabhu VR, Hunter KE, Glazier CM**, et al. 2008. Aneuploidy affects proliferation and spontaneous immortalization in mammalian cells. *Science* **322**: 703–9.
49. **Foijer F, Xie SZ, Simon JE, Bakker PL**, et al. 2014. Chromosome instability induced by Mps1 and p53 mutation generates aggressive lymphomas exhibiting aneuploidy-induced stress. *Proc Natl Acad Sci USA* **111**: 13427–32.
50. **Tribukait B, Granberg-Ohman I, Wijkström H**. 1986. Flow cytometric DNA and cytogenetic studies in human tumors: a comparison and discussion of the differences in modal values obtained by the two methods. *Cytometry* **7**: 194–9.
51. **Doležel J, Vrána J, Safář J, Bartoš J**, et al. 2012. Chromosomes in the flow to simplify genome analysis. *Funct Integr Genomics* **12**: 397–416.
52. **Weiss MM, Hermsen MA, Meijer GA, van Grieken NC**, et al. 1999. Comparative genomic hybridisation. *Mol Pathol* **52**: 243–51.
53. **Oostlander A, Meijer G, Ylstra B**. 2004. Microarray-based comparative genomic hybridization and its applications in human genetics. *Clin Genet* **66**: 488–95.
54. **Brady PD, Vermeesch JR**. 2012. Genomic microarrays: a technology overview. *Prenat Diagn* **32**: 336–43.
55. **Kallioniemi A**. 2008. CGH microarrays and cancer. *Curr Opin Biotechnol* **19**: 36–40.
56. **Bignell GR, Huang J, Greshock J, Watt S**, et al. 2004. High-resolution analysis of DNA copy number using oligonucleotide microarrays. *Genome Res* **14**: 287–95.
57. **Riegel M**. 2014. Human molecular cytogenetics: From cells to nucleotides. *Genet Mol Biol* **37**: 194–209.
58. **Cheng J, Vanneste E, Konings P, Voet T**, et al. 2011. Single-cell copy number variation detection. *Genome Biol* **12**: R80.
59. **Konings P, Vanneste E, Jackmaert S, Ampe M**, et al. 2012. Microarray analysis of copy number variation in single cells. *Nat Protoc* **7**: 281–310.
60. **Raghavan M, Lillington DM, Skoulakis S, Debernardi S**, et al. 2005. Genome-wide single nucleotide polymorphism analysis reveals frequent partial uniparental disomy due to somatic recombination in acute myeloid leukemias. *Cancer Res* **65**: 375–8.

61. **Gijsbers ACJ, Ruivenkamp CAL**. 2011. Molecular karyotyping: from microscope to SNP arrays. *Horm Res Pædiatr* **76**: 208–13.
62. **Lönnstedt IM, Caramia F, Li J, Fumagalli D**, et al. 2014. Deciphering clonality in aneuploid tumors using SNP array and sequencing data. *Genome Biol* **15**: 470.
63. **Fan HC, Wang J, Potanina A, Quake SR**. 2011. Whole-genome molecular haplotyping of single cells. *Nat Biotechnol* **29**: 51–7.
64. **Navin N, Kendall J, Troge J, Andrews P**, et al. 2011. Tumour evolution inferred by single-cell sequencing. *Nature* **472**: 90–4.
65. **Falconer E, Hills M, Naumann U, Poon SSS**, et al. 2012. DNA template strand sequencing of single-cells maps genomic rearrangements at high resolution. *Nat Methods* **9**: 1107–12.
66. **McConnell MJ, Lindberg MR, Brennand KJ, Piper JC**, et al. 2013. Mosaic copy number variation in human neurons. *Science* **342**: 632–7.
67. **Cai X, Evrony GD, Lehmann HS, Elhosary PC**, et al. 2014. Single-cell, genome-wide sequencing identifies clonal somatic copy-number variation in the human brain. *Cell Rep* **8**: 1280–9.
68. **Korbel JO, Campbell PJ**. 2013. Criteria for inference of chromothripsis in cancer genomes. *Cell* **152**: 1226–36.
69. **Voet T, Kumar P, Van Loo P, Cooke SL**, et al. 2013. Single-cell paired-end genome sequencing reveals structural variation per cell cycle. *Nucleic Acids Res* **41**: 6119–38.
70. **Macaulay IC, Voet T**. 2014. Single cell genomics: Advances and future perspectives. *PLoS Genet* **10**: e1004126.
71. **Frumkin D, Wasserstrom A, Itzkovitz S, Harmelin A**, et al. 2008. Amplification of multiple genomic loci from single cells isolated by laser micro-dissection of tissues. *BMC Biotechnol.* **8**: 17.
72. **Dean FB, Hosono S, Fang L, Wu X**, et al. 2002. Comprehensive human genome amplification using multiple displacement amplification. *Proc Natl Acad Sci USA* **99**: 5261–6.
73. **Lansdorp PM**. 2007. Immortal strands? Give me a break. *Cell* **129**: 1244–7.
74. **Hills M, O'Neill K, Falconer E, Brinkman R**, et al. 2013. BAIT: organizing genomes and mapping rearrangements in single cells. *Genome Med* **5**: 82.
75. **Falconer E, Chavez EA, Henderson A, Poon SSS**, et al. 2010. Identification of sister chromatids by DNA template strand sequences. *Nature* **463**: 93–7.
76. **Weise A, Heller A, Starke H, Mrasek K**, et al. 2003. Multitude multicolor chromosome banding (mMCB) - a comprehensive one-step multicolor FISH banding method. *Cytogenet Genome Res* **39**: 34–9.
77. **Vorsanova SG, Yurov YB, Iourov IY**. 2010. Human interphase chromosomes: a review of available molecular cytogenetic technologies. *Mol Cytogenet* **3**: 1.