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Review

# Fluorescence *in situ* hybridisation

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**Abstract:** Fluorescence *in situ* hybridisation (FISH) is a rapid and reliable technique for chromosomal investigations that is used for a wide variety of cytogenetic purposes at present. This molecular-cytogenetic method has been developed continuously for many years. As a consequence, various modifications with different kinds of fluorescently labelled probes have been introduced to optimise the detection of DNA and RNA sequences. This review articlepaper presents the general principles of *in situ* hybridisation, probe labelling and examples of proper use of different kinds of probes. In addition, some newer FISH methods and their usefulness in human molecular cytogenetics are described.

**Key words:** fluorescence *in situ* hybridisation; probes; chromosomal investigations.

**Abbreviations:** CCK, colour changing karyotyping; CGH, comparative genomic hybridisation; DIG, digoxigenin; FISH, fluorescence *in situ* hybridisation; M-FISH, multicolour (multiplex) FISH; PNA, polypeptide nucleic acid; SCAN, Spectral colour banding; SKY, spectral karyotyping; WCP, whole chromosome painting.

## Introduction

*In situ* hybridisation is a very effective and fast method that enables specific detection of unique sequences, chromosomal region and entire chromosomes (Nowakowska & Bocian 2004), or chromosomal abnormalities directly on cytological preparations containing morphologically preserved chromosomes, nuclei, or tissue parts. Molecular *in situ* hybridisation uses labelled single-chain hybridisation probes totally or partially complementary to the target sequence of DNA or RNA. Although fluorescence *in situ* hybridisation (FISH) is an extremely useful technique, until recently only a few target sequences could be visualised simultaneously (McNeil & Ried 2000). Comparative genomic hybridisation (CGH), spectral karyotyping (SKY), multicolour FISH (M-FISH), telomere multicolour FISH, colour changing karyotyping (CCK) and multicolour banding that are relatively new FISH-based techniques can, by contrast, detect gains and losses of whole chromosomes, subchromosomal regions (cryptic translocation, small inversion, microdeletion), detect subtelomeric rearrangements, gene amplification in tumours and allow the visualization of the entire human genome in 24 different colours (Kallioniemi et al. 1992; Chang & Mark 1997; McNeil & Ried 2000; Carpenter 2001; Kearny & Horsley 2005; Kolialexi et al. 2005).

The presented review describes the basic concept and methodology of FISH, including different types of probe labelling and detection as well as recent trends in this field.

*Brief historical survey*

*In situ* hybridisation was described for the first time independently by Pardue & Gall (1969) and John et al. (1969). The first successful experiments were performed on toad (*Xenopus laevis*) and vinegar fly (*Drosophila* sp.) chromosomes. Radioactively labelled 5S, 18S, and 28S rRNA was used as a probe detected after hybridisation localised on the chromosome 2 by a photographic emulsion (Gall & Pardue 1969). The first experiments on mammal chromosomes were related to the chromosomal localisation of the satellite DNA in mice that presents about 10% of the genome and takes place in the centromeres of all chromosomes, except for the sex chromosome Y (Pardue & Gall 1970).

Initially, the probes used in the *in situ* hybridisation technique were labelled with the radioisotopes $^{32}$P, $^{125}$I, $^{3}$H, and $^{35}$S (McNeil & Ried 2000). This way of labelling, however, had several disadvantages, such as probe instability, elaborateness, time demand, price and relative strict precaution measures, due to the work with the radioactive material (Levsky & Singer 2003). Therefore a non-radioactive labelling was introduced at the beginning of 80's that consisted of incorporation of signal molecules (chemically modified nucleotides) into the probe sequence by an enzymatic or chemical way (Langer et al. 1981; Pinkel et al. 1988; Trask 1991). The probes can be detected by a fluorescent microscope either directly or using fluorescently labelled antibodies (thus fluorescence *in situ* hybridisation) if fluorochromes are used for labelling.

## Hybridisation principle

Hybridisation is a very dynamic reaction at which a denatured target sequence and a complementary single-stranded DNA or RNA probe form a stable double-stranded hybrid molecule by heat action (Swinger & Tucker 1996).

Metaphase chromosome spreads are prepared by using a spindle inhibitor such as Colcemid to arrest cultured cells during mitosis. Hypotonic solution (0.075 M potassium chloride) and a fixative (methanol and acetic acid in a 3:1 ratio) are then applied sequentially. The hypotonic solution will cause the erythrocytes to lyse, and in some cases swell up and burst. The fixative must be freshly prepared and kept in cold to prepare a clear suspension with good-quality metaphases. The chromosome suspension is then dropped onto glass slides (Verma & Babu 1989). The metaphase chromosomes are pre-treated with ribonuclease, washed in 2xSSC (Salt Sodium Citrate) and dehydrated in a series of 70%, 80% and 95% ethanol before hybridisation. The pre-treated chromosome preparation must be denatured in a formamide bath. After denaturation, the slides must be immediately immersed in ice-cold ethanol. The probe denatured at high temperature and pre-hybridised at 37 ℃ is then applied to a slide for hybridisation. Hybridisation between the probe and target DNA at 37 ℃ takes place during a ∼16–48 hours incubation period (for details, see http://users.ugent.be/~fspelema/neubla/protocol/fish.htm).

Detection of the probe permits the visualization of the target DNA sequences. It starts with post-hybridisation washes of the slide in formamide and washing buffer (SSC with sodium dodecyl sulphate) at 37 ℃ to remove any excess of the probe that is not specifically bound (Fig. 1). Temperature and buffer concentration (stringency) of hybridisation and washing are important in FISH experiments. Lower stringency can result in non-specific binding of the probe to other sequences; higher stringency can result in lack of signal.

## Probes for *in situ* hybridisation

The target sequences of nucleotides from one to several hundreds of kilobases (kbp) can be visually evaluated; however, it is possible to differentiate two probes only if they are at least 1,000 bp distant each from the other. The differentiating potential of the FISH method depends on the degree of condensation of the target DNA. Chromatin is condensed into well visible chromosomes during mitosis and with increasing degree of its condensation the differentiation decreases (Laan et al. 1995). Regarding sequences, probes are divided to DNA and RNA probes.

*DNA probes*

For preparation of the DNA probes, either the sequences of cDNA, cloned fragments of genomic DNA, or synthetic oligonucleotides are employed. Also whole isolated chromosomes obtained by flow cytometry, somatic hybridisation, or laser microdissection, are used in the *in situ* hybridisation.

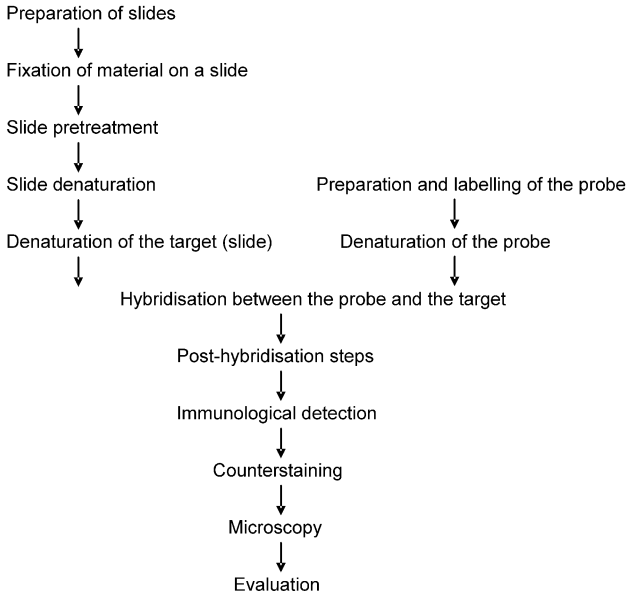


Fig. 1. Diagram for *in situ* hybridisation.

According to the target site, the DNA probes can be divided more precisely to: (1) chromosomal probes; those include whole chromosome and "arm-specific" probes; (2) satellite probes that are divided to $\alpha$ satellite and conventional satellite probes; (3) telomeric and subtelomeric probes; (4) probes for unique sequences; and (5) genome probes.

*RNA probes*

The RNA probes can be used for detection of mRNA in tissues that can be long-term stored frozen or in paraffin. RNA probes are best for high sensitivity detection procedures because hybrids between mRNA and RNA probes are highly stable. Synthetic RNA oligonucleotide probes conjugated with fluorescently labelled hapten are suitable alternative to RNA probes for the detection of abundant mRNA sequences in tissue sections. This type of probes was used by Deo et al. (2006) at the *in situ* hybridisation with micro RNA (miRNA) in the mouse embryos tissues as well as in the organs of adult individuals. The method, developed by the above-mentioned authors, enables a more detailed study of the miRNA function in mammals.

## Fluorescence *in situ* hybridisation

In case of the FISH technique, the probe detection is based on fluorescence observed by means of an epifluorescence microscope. There are two ways of labelling and detection of a probe for fluorescence *in situ* hybridisation: (i) indirect; and (ii) direct ways.

*Indirect way of labelling*

At the indirect way of labelling, chemically modified nucleotides are incorporated into DNA probes (Rudkin &

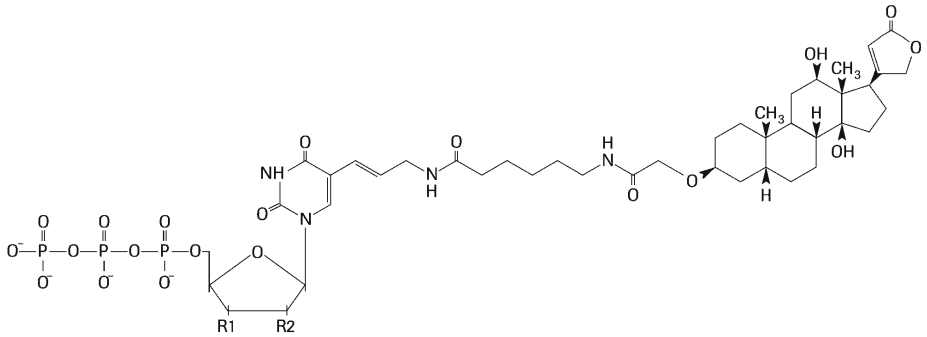


Fig. 2. Structural formula of digoxigenin-UTP/dUTP/ddUTP. Digoxigenin-UTP (R1=OH, R2=OH); digoxigenin-dUTP (R1=OH, R2=H); digoxigenin-ddUTP (R1=H, R2=H).

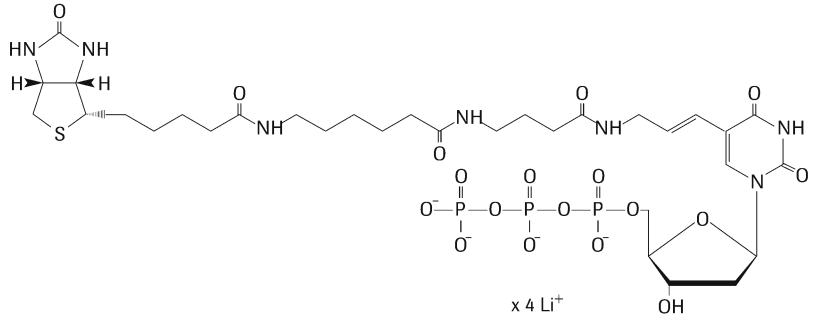


Fig. 3. Structural formula of Biotin-dUTP.

Stollar 1977; Langer et al. 1981; Raap et al. 1989). The basic principle is that biotin or digoxigenin are covalently bound to dUTP that incorporates into the probe sequence instead of thymine. After hybridisation with such labelled probe, the immunodetection is carried out by means of antibodies conjugated with an appropriate fluorochrome (Rosypal et al. 2002).

*Labelling with digoxigenin (DIG).* The digoxigenin labelling method uses fytosteroid digoxigenin isolated from the foxglove plant (*Digitalis purpurea*, *Digitalis lanata*). The digoxigenin is bound to the fifth carbon of uridine (nucleotide) by an 11-carbon chain (Fig. 2). The DIG-labelled nucleotides could be incorporated, at defined density, into probes by DNA polymerase as well as RNA polymerase and terminal transferase. Labelling with digoxigenin is done by nick translation methods, random priming, PCR, 3'-end-labelling, or *in vitro* transcription.

Hybridised DIG-labelled probes are detected by anti-digoxigenin (anti-DIG) high affinity antibodies that are conjugated with an appropriate fluorochrome (fluorescein, rhodamin, aminomethyl-cumarin, etc.).

*Labelling with biotin.* Enzymatic labelling of nucleic acids with biotin-dUTP (Fig. 3) was introduced by Langer et al. (1981). Biotin is a vitamin of the group B known also as vitamin B7 or vitamin H. Other biotin nucleotides such as biotin-labelled adenosine and cytosine triphosphate (Gebeyehu et al. 1987) were synthesised and used later, too.

In principle, biotin can be used in the same way as digoxigenin and can be detected by fluorescently labelled antibiotin antibodies. However, streptavidin and avidin are used more often because their molecules have higher biotin-binding capacity.

*Direct way of labelling*

In the direct method, the probe is labelled with a dUPT-bound fluorochrome. The hybridised probe could be observed under microscope immediately after the hybridisation reaction. For such methods it is inevitable that both the binding probe and the detected molecule tolerate relatively rough hybridisation and washing conditions (Swiger & Tucker 1996). The procedure of the RNA end-labelling fluorochrome probe was developed by Bauman et al. (1980). Direct enzymatic labelling of nucleic acids that meets Bauman's criteria was described by Renz & Kurz (1984).

## Use of the fluorescence *in situ* hybridisation

*Whole chromosomal painting probes (FISH-WCP)*

FISH with the whole chromosomal painting (FISH-WCP) enables both to detect chromosomal aberrations induced by various clastogenes (Marshall & Obe 1998; Natarajan 2002; Holečková et al. 2004; Bonassi et al. 2005; Holečková 2005; Natarajan & Kesavan 2005; Lakatošová & Holečková 2006) and to complete conventional cytogenetic assays studies (Šiviková et al. 2005). It is suitable mainly for detection of structural rearrangement, especially for analysis of induced translocations (Pinkel et al. 1988; Natarajan et al. 1991; Tucker et al. 1993, 1994, 1995).

This method uses the so-called "illumination" of chromosomes by fluorescent probes. The whole-

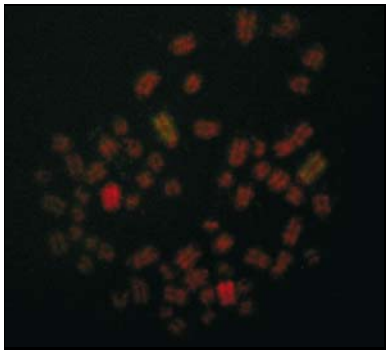

Fig. 4. Bovine whole chromosome 1 (green) and 5 (red) painting probes (Institute of Veterinary Genetics, University of Veterinary Medicine, Košice, Slovakia).

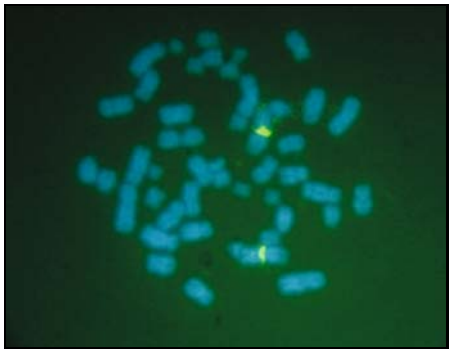

Fig. 5. Chromosome 1 satellite probe, direct green labelled (Institute of Veterinary Genetics, University of Veterinary Medicine, Košice, Slovakia).

chromosome painting probes hybridise with the whole chromosomes in metaphase. Every such probe is in fact a set of probes that specifically hybridises with many unique sequences along the target chromosomes highlighting thereby the fluorescence on both homological chromosomes (Fig. 4). The contrast background enables to differentiate the remaining chromosomes in metaphases while the chromosomes with exchanges appear as two-coloured. The FISH-WCP is thus based on visualisation of specific, whole chromosomal chromatically distinguishable, hybridised sequences (Tucker 1994, 1995).

The WCP probes are used for identification of chromosomes, for searching for structural chromosomal aberrations and marker chromosomes. The FISH-WCP has found its application in clinical cytogenetics (prenatal and postnatal) where it is used for elucidation of translocations and insertions, in tumorous cytogenetics, in *in vitro* studies for detection of mutagenic effects of a wide spectrum of clastogenes, and in *in vivo* biodosimetric studies (Tucker et al. 1995).

*Satellite probes*

The satellite probes are specific for a certain chromosome and bind themselves into relatively long repetitive regions where they provide well visible signal.

The $\alpha$ satellite probes consist of $\alpha$ satellite DNA sequences located in centromeres (Fig. 5). These probes are based on a tandemly repeating monomer of about 170 bp that contains conserved sequences present in all chromosomes as well as variable regions specific for certain chromosomal pairs. By means of the satellite probes it is possible to detect centromeres of individual chromosomes both in the metaphase and in interphase nuclei. The chromosome specific $\alpha$ satellite probes are used for a fast and easy detection of numerical aberrations (trisomies, monosomies) (Tönnies 2002; Holečková et al. 2004) and for the identification of the marker chromosomes (McNeil & Ried 2000). Fast detection of aneuploid constitution by means of the satellite probes is used in the prenatal diagnostics, genetic toxicology and oncocytogenetics.

The conventional satellite probes hybridise with a short repeating sequence AATGG, which is localised near the centromere in the heterochromatin regions of the human chromosomes 1, 9, 15, 16, and on the long arm of the Y chromosome.

*Telomeric and subtelomeric probes*

These probes distinguish telomeres (end sequences) of short and long arms of chromosomes. The telomeres protect ends of linear eukaryotic chromosomes, preserve their stability, participate in the regulation of cellular proliferation and protect them against terminal fusions. They consist of complex regions of DNA sequences. These sequences can be detected by using FISH with DNA or peptide nucleic acid (PNA) (pan)telomeric probes (Bolzan & Bianchi 2006).

The first telomere component in vertebrates is universal, about 20 kb long, tandemly repeating sequence $(TTAGGG)_n$ (Ballif et al. 2000). When this sequence was used as a probe for *in situ* hybridisation, it was found that it forms chromosome ends of a lot of species. It serves for simultaneous identification of all telomeres in the metaphase.

The sequences specific for the individual chromosomes are the second component of the telomeric regions. Probes containing these sequences are suitable for detection of cryptic translocations, for determination of terminal deletions, acentric fragments, telomeric associations and fusions (Bolzan & Bianchi 2006). Recently Desmaze et al. (2004) have attained an interesting conclusion that sequences of telomeric repetitions usually localised on the ends of eukaryotic chromosomes are in many species also detected in the intrachromosomal regions.

Chromosomal rearrangements involving the chromosome ends (the telomeres) are a significant cause of human genetic diseases (Knight & Flint 2000). Terminal chromosomal deletions and rearrangements are the cause of some hereditary diseases. These probes are used for specific chromosomal analysis and identification of marker chromosomes. The FISH studies using specific telomeric probes/clones have suggested that these probes are suitable diagnostic tool at examination of the mental retardation, infertility, congenital anomalies, at investigation of rearrangements in the haematological malign tumours, and in the pre-implanting

diagnoses as well as in other fields of clinical interest (Ning et al. 1996; Bacino et al. 2000; Knight & Flint 2000; Caliskan et al. 2005). The submicroscopic telomeric deletions, that are not well visible after FISH, could be, according to Wong et al. (2005), detected more precisely by means of an array-based comparative genomic hybridization (CGH).

*Unique-sequence DNA probes*
The unique-sequence DNA probes hybridise with the specific regions of chromosomes, locuses or individual genes (Tönnies 2002). In dependence on the cloning vector, probe can be a plasmid (500 bp to 5 kb), cloned by the bacteriophage lambda (8 to 15 kb), a cosmid (20 to 50 kb), cloned by artificial bacterial chromosomes (about 1000 kb) or by artificial yeast chromosomes (50 to 1000 kb). They are used for detection of individual genes, for determination of amplification, deletions (deletion probes), or specific translocations both on metaphase chromosomes and in interphase nuclei. The signal quality of the probes for unique sequences depends, above all, on their length. In shorter probes (from 500 bp to several kb) the use of the FISH technique does not necessarily give satisfactory results. These probes are used in clinical cytogenetics, tumour cytogenetics, at gene mapping, and at the study of genetically conditioned diseases.

The unique sequence DNA probe p53 is an example of a useful probe for identifying specific genomic sequences that include p53 gene. This gene codes for tumour suppressor protein involved in the regulation of cell proliferation and is normally present in quantities too small for detection by immunochemistry (Harris 1992).

*Genome probes*
In certain cases the whole genome DNA can serve as a probe. These probes are used for the complete detection of chromosomal changes in the whole genome by the CGH method (Tönnies 2002).

*Technology of polypeptide nucleic acids*
Polypeptide nucleic acids (PNAs) are analogues of DNA that have (2-aminomethyl)-glycine instead of the deoxyribosophosphate backbone. They therefore quickly form thermally stable duplexes with the complementary DNA. Its structure gives the PNAs the capacity to hybridise with the high affinity and specificity to the complementary RNA and DNA sequences, as well as a great resistance to nucleases and proteinases. Fluorescently labelled PNA oligomers were introduced by Landsdorp et al. (1996) who used them for a simultaneous detection of all 96 telomeres of human chromosomes, improving thus the fluorescent signal quality in comparison with conventional telomeric probes.

PNA exhibits superior hybridisation characteristics and improved chemical and enzymatic stability compared to nucleic acids, which is why PNAs find major use in the diagnostic and pharmaceutical areas (Shakeel et al. 2006). Recent studies have reported a successful use of the chromosome-specific PNA probes in human lymphocytes, amniocytes, and spermatozoa as well as in isolated oocytes and blastomeres (Pellestor et al. 2005). Moreover, Pellestor (2006) formulated that the PNA-FISH adaptation to human spermatozoa has allowed the development of a new and fast procedure for the chromosomal screening of male gametes and has provided an efficient complement to the conventional FISH.

*"Padlock" probes*
During the last 2 years a breakthrough in genetic analysis has been reached using the application of the technologies based on the analytic reactions of DNA circulation (Nilsson 2006). In these technologies, the so-called "padlock" probes are used that accelerated a progress in genotyping of single-nucleotide polymorphisms of DNA as well as in the *in situ* genotyping of DNA molecules in individual cells. The "padlock" probes are oligonucleotides with probe sequences of about 20 nucleotides on the 3' and 5' ends (Raap 1998) that also contain a spacer serving for binding haptens (biotin, digoxigenin) that are necessary for detection. After hybridisation to the target sequence the probes are circularised (Larsson et al. 2004). Formation of the circular DNA is catalysed by a DNA ligase that manages to differentiate variants of individual nucleotides. The DNA polymerase, that replicates probes and forms product visible under a fluorescent microscope, is added to the reaction (Nilsson et al. 2006).

Christian et al. (2001) demonstrated that rolling circle amplification *in situ* can detect the gene copy number and single base mutations in fixed cells and can also detect and quantify the transcribed RNA in individual cells, making it a versatile tool for cell-based assays. Using the PCR-generated padlock probes, homologous chromosomes were quantitatively distinguished by Antson et al. (2003), who observed the transmission of the chromosomes by the *in situ* analysis during three consecutive generations. According to Zhang et al. (2006), the method of amplification of "padlock" probes (sometimes also called C-probes, i.e. circular probes) is useful especially in those fields of research and molecular diagnostics in which the other methods have failed.

## New trends in FISH

*Multicolour techniques based on FISH*
The multicolour karyotyping procedures, such as the multicolour (multiplex) FISH (M-FISH), spectral karyotyping (SKY), colour changing karyotyping (CCK) as well as multicolour banding (mBAND) techniques were introduced as the modifications of hybridisation techniques in the past several years. The multicolour FISH assays are nowadays indispensable for a precise description of complex chromosomal rearrangements (Liehr et al. 2004).

*SKY and M-FISH.* The SKY (Schröck et al. 1996) and M-FISH (Speicher et al. 1996) were reported as successful methods that allow visualising of all 22 pairs of

autosomes and sex chromosomes X and Y of the human karyotype in individual colours during one hybridisation experiment.

The sensitivity of these methods depends on the location and despiralisation of chromosomes and ranges from 1.0 to 2.5 Mb (Lichter 1997). They are used particularly for detection of the complex chromosomal rearrangements unidentified with G-banding, which is mainly the case of the cryptic translocations and insertions (Michalová et al. 2001). 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 into 24 different computer-generated colours. Using this strategy, the total number of colours achievable (N) is given by the equation $N = 2^{n-1}$, where n is the number of spectrally separated fluorochromes (Kearney 2006).

The M-FISH and SKY differ only in the method that is used to discriminate the differentially labelled probes. The M-FISH uses specific narrow bandpass fluorescence filter sets and a digital imaging equipment as a part of a conventional epifluorescence microscope, with appropriate computer software (Kearney 2006). The microscopical image is gradually shot by a CCD camera using six different optical filters. The analysis of the individual six pictures is followed by a complex analysis of the whole image by means of a specific software.

The SKY uses an image acquisition based on a spectral imaging system with an interferometer and a CCD camera. The light that is emitted from each point of sample is collected by the microscope objective. The collected light is transferred by the CCD camera to the interferometer in which the optical path difference is produced. The emission spectrum is retrieved using Fourier transformation and processed by a computer. The measurement creates the basis for chromosome classification by assigning all pixels with identical spectra-unique colours (McNeil & Ried 2000).

The strength of the M-FISH is in defining translocations and marker chromosomes in complex karyotypes, however, M-FISH and SKY using the whole chromosome painting probes are not suitable for discriminating intrachromosomal rearrangements such as duplications, deletions or inversions. To overcome these limitations, Kakazu et al. (2001) developed a novel chromosome banding technique – spectral colour banding (SCAN). This technique is based on SKY, combined with simultaneous hybridisation of labelled chromosome-band specific painting probes. The method simultaneously identifies the origin of chromosome bands by a unique spectrum for each band. SCAN detected the duplicated segment of chromosome 3 and identified the origin of the chromosome band in a diffuse large B-cell lymphoma case (Kakazu et al. 2003) and was able to analyse the chromosomal aberrations in tumours that have not been fully characterized by G-banding or SKY (Kakazu & Abe 2006).

A range of other specific M-FISH assays is available to look at chromosomal sub regions, including centromere FISH (Henegariu et al. 2001), subcentromere-specific FISH and telomere M-FISH.

*CCK.* An alternative multicolor karyotyping technique, called CCK, was described by Henegariu et al. (1999). This procedure allows simultaneous hybridisation and separate detection of up to 41 different DNA probes using only three fluorescent dyes and an epifluorescence microscope equipped with only three filters. The additional information about this method can be found at: http://info.med.yale.edu/genetics/ward/tavi/CCKprinciple.html.

*mBAND.* For detection of intrachromosomal rearrangements, several approaches to multicolour banding have been developed. The mBAND (Chudoba et al. 1999) is based on the region-specific chromosome paints that hybridise with the specific regions of the individual chromosomes. Detection of the breakpoints and intrachromosomal inversions in human tumours is possible by means the mBAND analysis.

M-FISH, SKY, mBAND and other molecular methods are widely used in clinical and tumour cytogenetics. The aim of these techniques in cancer cytogenetics is a much more detailed description of the highly abnormal karyotypes, a reliable characterisation of complex chromosomal rearrangements in tumour karyotypes, screening for new tumour-specific chromosomal aberrations, identifying specific chromosomal abnormalities, that may provide with the insight to the genes involved in the disease process and identification of new target regions for gene identification strategies (Schrock & Padilla-Nash 2000; Bavani & Squire 2002; Schrock et al. 2006). Detection of subtle karyotype rearrangements (small translocations, hidden or cryptic structural aberrations) and marker chromosomes is possible in clinical cytogenetics (Bavani & Squire 2001).

*Comparative genomic hybridisation*

CGH (Kallioniemi et al. 1992) using the whole-genomic DNA probes is a method of entire genome analysis that involves the differential labelling of test and reference DNA to measure genetic imbalances. The method is based on two-coloured FISH and is useful for the analysis of chromosomal gains and losses in solid tumours. A major advantage of CGH techniques is that for the analysis only DNA is needed from tumour samples, instead of difficult preparation of tumour metaphase chromosomes (McNeil & Ried 2000). However, the resolution of CGH method is limited. Consequently, microassay-based techniques using large insert genomic clones, cDNAs or oligonucleotides have replaced metaphase chromosomes as DNA targets (Albertson & Pinkel 2003). Array CGH can reveal hidden deletions and amplifications, and according to Kearney (2006), in combination with M-FISH it is a promising and a very powerful approach to gene discovery.

## Conclusions

The use of the variable FISH techniques enhances the

interpretation of numerical and complex chromosome aberrations and successfully complements conventional cytogenetic analysis. The nearer future will probably include the specific diagnostic probe sets detecting cryptic subtelomeric chromosome aberrations, microdeletions, specific chromosomal translocations and other aberrations by newly developed sophisticated methods, such as microarray-based techniques.

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