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Fluorescence in situ hybridization (FISH): types and application: a review

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A B S T R A C T

Macromolecule identification method known as fluorescence in situ hybridization (FISH) is regarded a recent development in the study of cytology. It was first created as a physical mapping method to identify genes inside chromosomes. Later, other biological and medical studies benefited from the precision and adaptability of FISH. This attractive method offers a level of resolution in between chromosomal studies and DNA analysis. A hybridizing DNA probe used in FISH can either be directly or indirectly labeled. Fluorescent nucleotides are employed in direct labeling, whereas reporter molecules used in indirect labeling are afterwards recognized through affinity molecules, fluorescent antibodies, or other means. An excessively high number of chromosomes in a cell, unique gene fusions, or the loss of a chromosomal segment or an entire chromosome are examples of genetic disorders that can be detected with FISH. It is also used in a variety of scientific projects, including gene mapping and the discovery of fresh oncogenes. This article illustrates the principles of FISH, its use, and its benefits in medical research.

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Introduction

Fluorescence in situ hybridization (FISH) techniques have significantly advanced both the diagnosis and research of solid tumors and haematological malignancies. Advancements in cytogenetic techniques over the past 30 years have made it possible to detect chromosome abnormalities in hematological malignancies with increasing sensitivity [1]. Additionally, chromosome-specific staining methods were developed as a result of the capacity to distinguish groupings of DNA fragments that span particular chromosomes. Researchers were also interested in examining specific chromosomal regions within intact cells' nuclei. Fluorescence in situ hybridization (FISH), a cytogenetic technique, was therefore employed to map DNA sequences to particular human chromosomal locations. In FISH, fluorescently labeled DNA probes are used, and these probes can hybridize to complementary chromosomal regions. A fluorescent dot appears at the chromosomal position where the labeled probe attaches, allowing researchers to visualize the location of a specific gene or DNA sequence under a microscope using this method. Thyroglobulin was the first human gene with a single copy that was mapped by FISH in 1985. Compared to conventional G-banding methods, FISH enables a better degree of resolution [2].

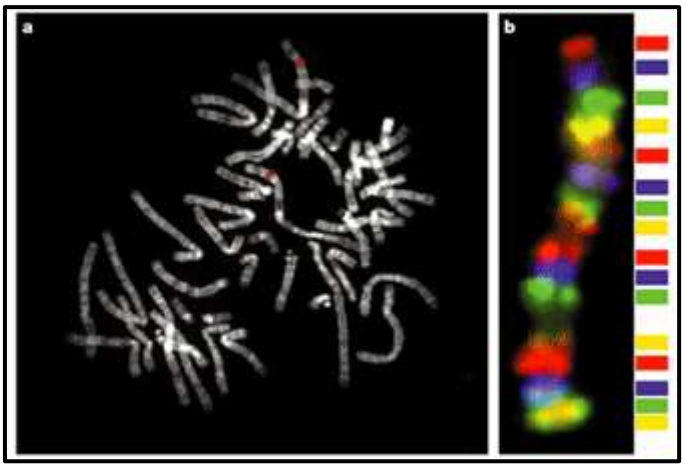

Figure 1. Fluorescence in situ hybridization for cytogenetic localisation of DNA sequences
a) In FISH, a fluorescent signal (red) is produced at the locations of a particular DNA sequence, in this example, a 150 kb section of chromosome 1. b) Multicolor fish may be used to concurrently evaluate and sort a number of probes, each of which corresponds to a distinct genetic section. 2001 Nature Publishing Group BAC Resource Consortium (Panel A), integration of cytogenetic landmarks into the human genome's draft sequence. Nature 409, 953–968 (2001). Reservation of rights. 2002 in Panel B Springer. O'Brien, B., Zitzelsberger, H. F., and Weier, H. U. Inter- and intrachromosomal rearrangement detection using multicolor FISH techniques. FISH Technology, edited by B. Rautenstrauss and T. Liehr, 408–424 (Springer, Heidelberg, 2002). Reserved for all rights

FISH studies have also made significant contributions to our knowledge in Prader-Willi and Angelman syndromes [3]. Researchers discovered that the identical loss in chromosome 15 was connected to both Prader-Willi syndrome and Angelman syndrome (from region q11 to q13). However, scientists discovered that children with father-inherited deletion of chromosome 15 causes Prader-Willi syndrome, whereas those with Angelman syndrome inherited it from their mother. This is because of a process known as chromosomal imprinting. Using more or less than two fluorescent "dots" in a somatic cell, FISH may also be used to detect gene loss or to identify genes with increasing copy number. Additionally, nondividing cells can be used for FISH, allowing researchers to analyze non-mitotic cells. This is significant because non-mitotic (interphase) cells' DNA packing is around 10,000 times less dense, enabling scientists to work at a greater resolution. For instance, a duplication of one million base pairs linked to the neurological illness Charcot-Marie-Tooth type 1A may be corrected not by metaphase FISH rather by interphase FISH [4]. Fiber-FISH is a very high-resolution FISH technique that utilizes solitary chromosomes, which exist as long, stretched-out DNA strands and lack nuclear architecture [5]. The fundamentals of how our cells function should be reviewed in order to comprehend fluorescence in situ hybridization (FISH). Each cell has a nucleus in its core. A cell's development, survival, and reproduction are controlled by the 46 chromosomes found in 23 pairs in this region. These guidelines are encoded in our DNA. A single cell splits into two daughter cells, which results in the formation of new cells. For each daughter cell to receive all 46 chromosomes, it must first make a copy of its genetic material. The procedure is extremely controlled. However, cancer cells don't adhere to the guidelines. It is common for chromosomal numbers or

their structural composition to vary. FISH enables us to observe the number of chromosomes and their organization within a cell, as well as to spot mistakes [6].

FISH's mechanism

FISH's fundamental components: DNA target sequence and probe

The first step in FISH is to create short single-stranded DNA sequences, known as probes, that closely resemble a region of the gene of interest. The DNA probe is tagged via PCR using random primed labeling, and nick translation. Indirect and direct labeling are the two labeling methods employed. The probes are marked with fluorophore-containing nucleotides in the case of direct labeling. In indirect labeling, the probes are labeled at modified nucleotides that include haptens. The target DNA is then denatured after the tagged probe. Complementary DNA sequences anneal as a result of the combined denatured probe and target DNA effects. When using an enzymatic or immunological detection method, an extra step is required in order to observe the non-fluorescent hapten after indirect labeling [7]. Furthermore, the illnesses, abnormalities, or anomalies covered by the field of interest determine which FISH probes are used [8]. Figure 2 shows the fundamental steps in the FISH method [2].

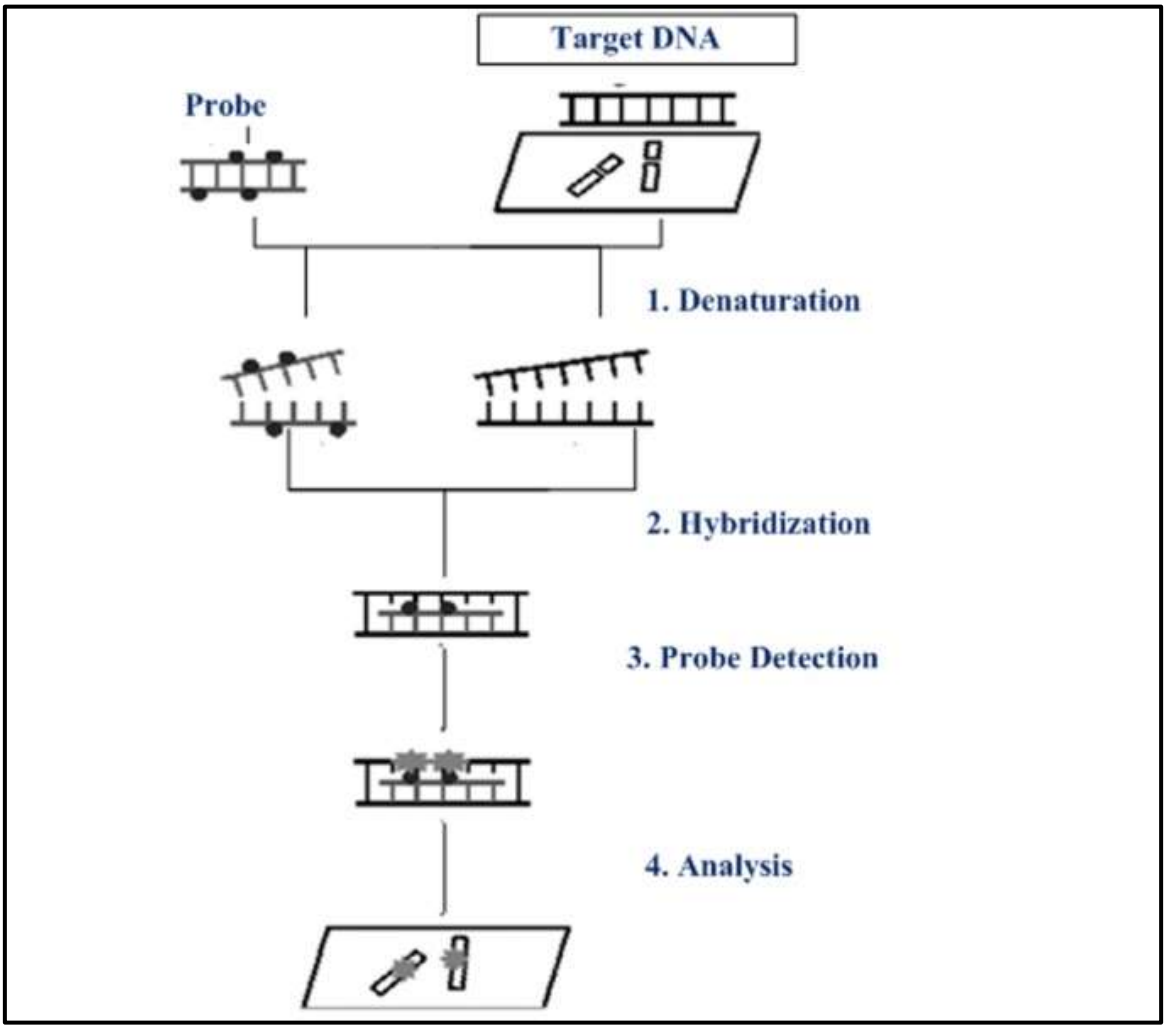

Figure 2. The fundamental steps in the FISH method

Genetic anomalies

DNA, histones, and other proteins that are not histones make up chromosomes, which are arranged into domains during interphase. One DNA molecule makes up each G0/G1 chromosome, and this one DNA molecule is duplicated twice during the mitotic cell cycle's S phase to produce two sister chromatids, each of which contains a copy of the original DNA molecule. Topoisomerases are widely distributed enzymes that conveniently facilitate DNA double-strand breaks to alter the negatively supercoiled state of DNA during the cell cycle. For topoisomerases to perform their vital biological tasks, such as those involved in DNA replication, transcription, chromosomal condensation and segregation, and the preservation of genome stability, a brief cleavage is required. Nevertheless, the activity of these enzymes may encourage unauthorized recombination, which may result in chromosomal abnormalities [9].

Aneuploidies

Studies clearly showed that nondisjunction occurring during maternal meiosis is the primary cause of age-related aneuploidies [10]. However, we may identify aneuploid oocytes in IVF patients by analyzing the first and second polar bodies using one of the cytogenic approaches. It can thus help in the creation of a plan to prevent the transfer of embryos produced by aneuploid oocytes. It could lessen the likelihood that an IVF couple would give birth to a kid who has Down syndrome or another frequent aneuploidy. The identification of chromosomal signals in interphase nuclei is made feasible by FISH. Therefore, this might be an effective way to identify common aneuploidies before implantation [11].

FISH application in oncology

1. **Chronic Myeloid Leukemia (CML):**

In both the initial diagnosis and ongoing monitoring of CML, FISH is employed as a quick and trustworthy molecular cytogenetic approach. FISH, on the other hand, is used to examine both metaphase and interphase cells. FISH is therefore an effective technique to utilize when there aren't enough metaphases [12]. Leukemia's molecular process has been significantly aided by aberrant fusion proteins caused by chromosomal rearrangements. The BCR/ABL1 translocation commonly occurs in chronic myeloid leukemia (CML). When choosing a targeted therapy for certain leukemias, the FISH test is the gold standard [13].

2. **Multiple Myelomas(MM)**

Multiple myelomas (MM) are formed from heterogeneous B cells that have undergone terminal differentiation. According to molecular research, primary translocations in the early stages of MM are followed by a massive number of secondary translocations as the tumor progresses [14]. The most rigorous genetic test for characterizing cytogenetic abnormalities in MM, which are outlined by cytogenetic testing using FISH, is acknowledged for its effectiveness in analyzing interphase nuclei and minor chromosomal aberrations [15].

3. **Pulmonary Adenocarcinomas**

Anaplastic lymphoma kinase (ALK) rearrangements are related to pulmonary adenocarcinomas. FISH may be used to detect the EML4-ALK gene fusion. EML4-ALK rearrangements are mostly brought on by the fusion of ALK and EML4, which occurs at chromosome 2p23 in echinoderms [16]. Immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), and other techniques are now available to identify individuals with ALK-positive NSCLC. FISH is regarded as the "gold standard" to direct the use of ALK inhibitors in therapeutic settings [17]. This method can also be used to identify the diseases of prostate Cancer [18], Breast Carcinomas and Renal Mesenchymal Neoplasm[19].

4. **Cholangiocarcinoma**

Cholangiocarcinoma (CC), a rare digestive system cancer, has a poor prognosis. Due to the tumor site's restricted access, preoperative diagnosis is based on interventional imaging techniques, particularly endoscopic retrograde cholangiopancreatography (ERCP). However, obtaining appropriate tissue samples is often challenging. Therefore, it is difficult to learn about CC's genetic makeup. Studies shown that individuals with extrahepatic CC, Four chromosomal structural and numerical aberrations can be found using an alternate FISH method on brushing smears [11].

Melanoma

Melanocytes, pigment-containing cells, are the source of the cancer known as melanoma. It can be precisely found using FISH. Four probes that target 6p25 (RREB1), 6q23 (MYB), 11q13 (CCND1), and centromere 6 (CEP6) are used for the diagnosis. On the basis of these four probes, the best algorithms for identifying positive FISH findings are also developed [20]. One of the first uses of FISH was chromosome painting, which is competitive hybridization employing whole human genomic DNA serves as the rival and chromosome-specific libraries serve as the probes [2]. It enabled the unique identification of chromosomes involved in intricate rearrangements by providing strong and precise fluorescence labeling of human chromosomes in metaphase spreads and interphase nuclei. Due to the availability of a variety of single-locus probes made possible by the Human Genome Project, gene mapping methodologies have seen a major improvement and the breakpoints of consistent translocations have been found [4, 5, 9].

Multiplex-FISH and Spectral Karyotyping (SKY)

Based to the ability to extract individual human chromosomes using flow cytometry and knowledge of the human genome sequence, cytogeneticists have developed 24-color probe sets that are used to identify each human chromosome with a different color. Chromosome-specific probes are created by adding a brightly colored fluorescent dye to DNA segments that span the length of each individual chromosome. The labeled DNA probes are then combined and employed in hybridization studies using metaphase chromosome spreads. Each chromosome may be marked with a distinct fluorescent hue over its whole length, by virtue of the labeled DNA probe sets' ability to connect to their counterpart chromosomes. Each chromosome's maternal and paternal copies will have the identical color labels in a somatic cell. Either multiplex-FISH (M-FISH) or spectral karyotyping (SKY) are two names for this potent technique that allows for the simultaneous monitoring of all human chromosomes [21]. These methods are most likely the most important advancement in molecular cytogenetics discovered in the last ten years. Since each chromosome has its unique color, chromosomal translocations may be quickly identified when a chromosome displays an area that is colored differently. Additionally, the second color indicates which other chromosome is involved in the translocation. The use of SKY/M-FISH methods has made it possible for researchers to accurately identify cytogenetic abnormalities in individuals and to identify minor chromosomal rearrangements in people with karyotypes that appear to be normal.

Comparative Genomic Hybridization

Comparative genome hybridization is a method created by researchers (CGH). This method entails isolating and fragmenting the genomic DNA of an experimental participant as well as a control subject. While the fragmented experimental DNA sample is marked with red fluorescence, the fragmented control DNA sample is marked with green fluorescence. In research involving hybridization with typical chromosomes, the two DNA samples are combined and utilized as DNA probes. Then, the red and green probes compete with one another to attach to the chromosomes [2]. Standard CGH techniques are labor-intensive and rely on metaphase chromosomes, which results in low resolution. However, a more modern CGH technique based on microarrays does not need to employ metaphase chromosomes [22]. Thousands of base-pair portions of the human genome are assembled into arrays that are attached to microchips for use in this method. Each individual DNA fragment resides in a specific place on the chip and is correlated to a known DNA sequence that has been allocated to a certain chromosomal region. Then, using the same colored probes, hybridization tests are conducted on the CGH microarray platform, which may be automatically scanned (green for the control group and red for the experimental group). As with typical CGH tests, unaltered chromosome regions exhibit equal binding of the green and red probes and a consequent orange/yellow hue, but amplified and deleted chromosomal regions in the experimental group have red and green, respectively, appearances. The particular chromosomal areas and genes that are amplified or absent can be identified by researchers utilizing microarrays. In actuality, the knowledge gained from a single CGH experiment using an array is equivalent to that gained from tens of thousands of FISH tests.

FISH test outcomes

Researchers examine the number of chromosomes or their structural makeup within a cancer cell using a FISH test. There are a few genetic errors that might happen:

- Duplication/amplification - Researchers discover more copies of genes, chromosomes, or chromosomal segments.
- Deletion - Chromosomes or portions of chromosomes are missing.
- Translocation: When a section of one chromosome splits off and rejoins another chromosome. The Philadelphia chromosomal rearrangement is a condition in which, for instance, a portion of chromosome 9 joins chromosome 22 and a portion of chromosome 22 joins chromosome 9. Acute lymphoblastic leukemia (ALL) patients may develop it [6].

Advancement in techniques

In metaphase or interphase cells, Fluorescence In Situ Hybridization (FISH) may identify certain DNA sequences at precise places. Schwarzacher et al. (1989) were the first to use this method on plant chromosomes after it was first created for mammalian chromosomes. In plant chromosomes, FISH has identified repetitive DNA sequences and 18S and 26S rRNA in a variety of plant species, including those of Aegilops, Hordeum, Oryza, Arabidopsis, Brassica, soybean, and barely.

Genomic in situ hybridization, also known as GISH, uses genomic DNA as a probe. In this procedure, unlabeled DNA from the other species being examined serves as the competition at a considerably greater quantity while genomic DNA from one species serves as the labeled probe. The method is extremely useful for cytologically recognizing foreign chromatin in interspecific hybrids at the molecular level. Aegilops triuncialis, Millium montianum, Triticum aestivum, and Nicotiana tabacum are examples of naturally occurring allopolyploid species. GISH has also been used in plant molecular cytogenetics to detect parental genomes in these species as well as foreign segments in translocations [23].

Principle Involved in Fish

An interphase cell's nuclear DNA or metaphase chromosomes attached to a microscope slide are hybridized with a nucleic acid probe in this process. Either a fluorophore is directly incorporated into the probes or an indirect hapten label is applied to them. Once the target DNA has been denaturized, the labeled probe and the target DNA are combined, allowing complementary DNA sequences to anneal. In the case that the probe has been indirectly labeled, an extra enzymatic or immunological detection step will be required for the observation of the non-fluorescent hapten. The enzymatic detection method makes use of fluorochrome, which produces colored signals at the hybridization site. Following that, fluorescence microscopy is used to analyze the data. The immunological detection approach is based on the binding of certain antigens by specific antibodies, which is then demonstrated by a colored histochemical reaction visible under a light microscope or by fluorochromes under UV light [6].

Diversification of fish Techniques

FISH has become more practical and well-liked in other biological and medical research areas, such as clinical genetics, neuroscience, reproductive medicine, cellular genomics, and chromosome biology, ever since it was widely acknowledged as a physical mapping technique to support massive nucleotide sequencing in the context of the Human Genome Project. The development of the technique's sensitivity, specificity, and resolution has led to the diversification of the basic FISH protocol into a number of noteworthy techniques throughout time [24]. The chemical and physical characteristics of nucleic acids and chromatin have been better understood because to the development of these enhanced methods, fluorescence microscopy, and digital imaging. The following strategies demonstrate the adaptability of FISH and were partly inspired by the glossary of Volpi and Bridger [24].

Type of fish Techniques

1. **Centromere-FISH (ACM-FISH)** is a multicolor FISH technique used to identify chromosomal aberrations in sperm cells (23). ACM, which stands for simultaneous hybridization of DNA probes for the alpha (centromere), classical (1q12), and midi (1p36.3) satellites of the chromosome, is used to specifically detect duplications and deletions of 1pter and 1cen as well as to identify chromosomal breaks within the 1cen-1q12 region. Prior FISH studies on chromosomal rearrangements in sperm led to the development of the ACM approach, which combined technological elements with biological discoveries [24,25].
2. **ArmFISH:** The p- and q-arms of all 24 human chromosomes, with the exception of the Y and acrocentric chromosomes, may be used to detect chromosomal abnormalities. ArmFISH is a 42-color M-FISH version [23]. In glioma cell lines, the test has been effectively used to demonstrate extensive chromosomal instability [24].
3. **Catalyzed Reporter Deposition-FISH:** Fluorescein tyramine signal amplification is carried out using a technique called catalyzed reporter deposition-FISH (CARD-FISH), which employs an oligonucleotide probe that has been HRP-labeled. With this method, it is quite possible to detect, identify, and quantify microorganisms involved in bioleaching processes [23].
4. **Cytochalasin B:** CB-FISH entails hybridization on binucleated cells whose cytokinesis has been stopped by cytochalasin B treatment (CB). By integrating the 24-color SKY technology with the common CB-FISH methodology, analysis of the chromosomal content of micronuclei may be eased [23]. A study team examining the process by which the percentage of mosaic diploid cells in vivo rose in trisomy 21 instances created the term "CB-FISH" [25].
5. **Chromosome Orientation:** In CO-FISH, strand-specific hybridization is achieved by using single-stranded DNA probes that have been 5-bromodeoxyuridine-labeled during S phase. The relative orientation of two or more DNA sequences along a chromosome can be determined using this method. The original purpose of this method was to identify the orientation of tandem repeats inside centromeric regions of chromosomes. Assessing chromosomal translocations and inversions has also been aided by this method [23,24].
6. **Combined Binary Ratio:** With the use of COBRA-FISH, all human chromosome arms may be identified based on color, and the mapping of gene and viral integration sites can be done in the context of chromosomal arm painting. Combinatorial labeling, which uses variable ratios of label to discriminate between probes, is a component of the COBRA-FISH process. This makes it possible to create up to 48 color combinations for differential painting of human chromosome arms inside a specimen while using fewer fluorochromes [23].
7. **Chromosome Orientation and Direction:** The information about the directional structure of telomeric sequences differs between this procedure and CO-FISH. The term can also refer to concurrent oncoprotein detection-FISH, which enables the observation of locus signals for a specific oncogene as well as the protein product produced from this gene. The combined CaCO3 optical detection-FISH method is another method that has been dubbed COD-FISH that uses FISH to find calcifying microorganisms in open ocean water [23]. Concomitant oncoprotein detection-FISH (COD-FISH) is another name for a technology that allows researchers to see both the loci signals for an oncogene's protein product and its loci. This protocol's creation was intended to quantify gene copy number and protein production in order to shed light on intriguing processes that take place during the transcription and translation of a specific message [26].
8. **Combinatorial Oligonucleotide (COMBO)-FISH:** The combined binary ratio is the prefix COBRA. Ratio labeling and combinatorial labeling are combined in this specific FISH procedure (27). When marking specific genetic locations, COMBO-FISH is employed. It uses homopurine/homopyrimidine oligonucleotides, which when combined with duplex genomic DNA that is still intact, produce triple helices. The target sequence won't need to be denaturized beforehand, as is typically required in conventional FISH techniques for probe binding. One to two percent of the human genome is made up of homopurine or homopyrimidine segments of DNA, which are often longer than 14 base pairs. On average, a 250 kilobase tract of the genome has 150 to 200 such lengths. In light of this, customized probe sets may be created to focus on genomic areas of interest in that size range [23].
9. **Comet-FISH:** By transferring the DNA from the nucleus into an agarose gel, the comet assay, also known as single-cell gel electrophoresis or the single-cell gel test, is used to gauge the degree of DNA breakage within single cells [24]. Combining comet assay with FISH analysis is known as comet-FISH. It is used to find certain DNA damage in specific genomic regions. Prior to in situ hybridization, DNA is attached to an agarose-coated microscope slide, allowing for the delineation of particular sequences in the comet head or tail. This will make it possible to evaluate the genomic region's vulnerability to DNA damage or breaking, which has been found to be related to

the gene density rather than the size of a chromosome. The vulnerability of telomeres to damage has been effectively determined using this method [25].

10. **Cryo-FISH:** Ultrathin cryosections of cells immersed in sucrose (150 nm thick) are used in cryo-FISH. This method has been used to successfully study the spatial relationships between chromosomal territories and the arrangement of the genome in the cell nucleus [23].
11. **Double Fusion FISH (D-FISH):** Due to the overlap of the neighboring hues in this FISH, a secondary color is seen. Detection of BCR/ABL translocations is one instance in which illness is indicated by the secondary color. An example of the opposite scenario, in which the absence of secondary color is pathogenic, is provided by a translocation test in which only one of the breakpoints is known. One side of the breakpoint and the other intact chromosome are targeted by locus-specific probes. In healthy cells, secondary color is visible, but when a translocation occurs, only the main colors are seen. "break-apart FISH" is the name of this method [23].
12. **DNA Breakage Detection FISH:** Sperm DNA fragmentation levels have been assessed using DBD-FISH. The process of creating single-stranded DNA in the sample that can be hybridized with the proper probes usually involves stabilizing cells in agarose beads and incubating them with the unwinding buffer [25].
13. **e-FISH:** The results of hybridization tests can be predicted using the BLAST-based FISH simulation tool known as e-FISH. As a bioinformatics tool for choosing the best genetic probes for hybridization investigations, this application was created [23].
14. **Fiber-FISH:** Prior to hybridization, DNA or chromatin fibers in the fiber-FISH technique are stretched on a microscope slide after being dissociated from cell nuclei by salt or another solvent. With the use of this technique, DNA or chromatin fibers may be mapped with high resolution, allowing for the analysis of copy number changes as well as gaps and overlaps in contigs [23]. The ability to determine the relative positions of two or more genes or repetitive DNA sequences (fiber-FISH) has greatly enhanced physical gene mapping [28].
15. **Flow-FISH:** The telomeric signals from cells in suspension are measured using this method, which combines in situ hybridization with flow cytometry. Telomere repeat lengths are seen and measured using PNA-labeled telomere probes. In aging research, this method has been employed [23].
16. **Fusion-Signal FISH:** In order to identify the 9;22 Philadelphia translocation in peripheral blood and bone marrow cells of CML patients and identify minimum residual illness following bone marrow transplantation, this approach was first applied. Fusion-signal FISH is the term for a technique that uses BCR and ABL gene segments flanking each of the two breakpoints to identify the BCR/ABL fusion product [25].
17. **Halo-FISH: For halo-FISH**, soluble proteins are removed from the cells by first permeabilizing them and then extracting them with a lot of salt. A halo surrounds a remaining nucleus as the chromatin/DNA that is not attached to an internal structure within the cell nucleus is therefore freed. Then, using any kind of probe, FISH may be done on these preparations to identify particular DNA sequences such telomeres, scaffold attachment regions (SARs), matrix attachment regions (MARs), gene loci, and whole chromosomes [24].
18. **Harlequin-FISH:** In order to accurately measure the amount of induced chromosomal damage for human biodosimetry, chromosome analysis in human cells under cell cycle control using the Harlequin-FISH technique is used. Using cell cultures that have received a BrdU treatment, this method combines FISH painting with differential replication labeling of sister chromatids using either Giemsa or fluorescent dyes. The name "harlequin" refers to the appearance of the chromosomes after a few cell divisions, which is an uneven striping pattern [26].
19. **Immuno-FISH:** Standard FISH plus either direct or indirect immunofluorescence are combined to create immuno-FISH. This method makes it possible to see the antigens in the sample. Furthermore, the same sample may be used to examine both proteins and DNA. It is frequently used to examine how genomic areas and proteins co-localize in interphase nuclei, such as nucleoli or promyelocytic leukemia (PML) bodies [23].
20. **Locked Nucleic Acids (LNAs)-FISH:** Using oligodeoxynucleotide probes with locked nucleic acid (LNA) inserted (LNA/DNA probes), the in situ hybridization efficiency is significantly increased without sacrificing specificity. LNA/DNA oligonucleotide heteroduplexes exhibit a structural change from an A-type helix, which has superior heat stability, to a B-like helix. The identification of mRNA and genes on the chromosomes is better accomplished using LNA/DNA probes [24].
21. **Multiplex (M)-FISH:** Using distinct colors to concurrently identify many areas or genes is one of the most intriguing features of FISH technology. By labeling with a distinctive mixture of fluorophores, the entire chromosome may be painted in a single hybridization. Multiple loci or ML-FISH [23].

22. **The ML-FISH:** is a term used to describe the simultaneous usage of many probes in multicolor FISH. In individuals with unexplained developmental delay and/or mental impairment, this FISH technique was first created to check for numerous microdeletion disorders [25].
23. **Premature Chromosome Condensation (PCC)-FISH:** Chromosome damage following radiation exposure is assessed using PCC-FISH. Chromosome-specific painting probes are necessary for it. In this method, prematurely compacted chromosomes of cells in the G1 and G2 phases are treated with phosphatase inhibitors or virus-mediated cell fusion, respectively. To gauge or anticipate the in situ radiation sensitivity of certain human malignancies, PCC-FISH was first developed as a test. The impact of whole-body high- or low-dose exposure to human peripheral lymphocytes has since been calculated using this method [23].
24. **eptide Nucleic Acid (PNA)-FISH:** The deoxyribose phosphate backbone of PNAs, which are synthetic counterparts of DNA, has been changed to a noncharged peptide backbone. The hybridization of PNA oligomers to complementary DNA or RNA sequences is free of electrostatic repulsion as a result of this special structural characteristic. Over time, the PNA-DNA and PNA-RNA duplexes become more stable than the natural homo- or heteroduplexes. The first time telomere lengths on metaphase chromosomes were measured, FISH with PNA probes was utilized [24].
25. **Quantitative-FISH (Q-FISH):** PNA-conjugated probes have been employed in this approach primarily to count the number of telomere repeats on a certain chromosome [23]. The principal use of this technique, which makes use of PNA-conjugated probes, has been to count the telomere repeats on a certain chromosome. Usually, metaphases are photographed, and then software like TFL-TELO is used to analyze them [26].
26. **Quantum Dot (QD)-FISH:** Inorganic fluorophores with a nanometer-sized size known as quantum dots have narrow emission spectra and are photostable. These have been utilized effectively for FISH investigation on human sperm cells, human metaphase chromosomes, and bacterial cells. In tissue slices, subcellular mRNA distribution has also been identified using QD-FISH [23].
27. **Rainbow-FISH:** Through the use of rainbow-FISH, up to seven distinct microbial groups may be simultaneously detected and quantified in a tiny area. To distinguish between several phylogenetic groupings of bacteria, this procedure utilizes unique 16S rRNA-targeted oligonucleotide probes. Seven different types of microbial strains may therefore be identified at once by using a combination of seven DNA probes, each tagged with up to three fluorochromes [23].
28. **Raman-FISH:** It is a method for investigating the ecophysiology of intricate microbial communities that combines FISH and Raman microspectroscopy [23]. The method takes use of the "red shift phenomenon," or the considerable modification of the resonance spectra that results from the anabolic incorporation of the 13C isotope into microbial cells as opposed to normal 12C, as seen by Raman microscopy. For structural and functional investigations of microbial communities at a single-cell resolution, stable isotope incorporation and in situ hybridization with a particular 16S rRNA probe are coupled with metabolic labeling [26].
29. **Replicative Detargeting FISH (ReD-FISH):** ReD-FISH may be used to detect when a certain sequence will replicate. The newly generated DNA strand will be detargeted if BrdU is integrated into the sequence of interest. As a result, each oligonucleotide probe will only be able to hybridize to one of the parental strands, and only one chromatid will show a signal. The normal double signal will be visible on both chromatids of the metaphase chromosome, though, if the sequence of interest has not replicated and has not incorporated BrdU. [23].
30. **Reverse-FISH:** is the process by which DNA from the target material is incorporated into the FISH probe. For identifying marker chromosomes and chromosomal amplifications in cancer, reverse-FISH has proved helpful [24].
31. **Recognition of Individual Genes (RING)-FISH:** Regardless of copy number, RING-FISH uses large concentrations of polynucleotide probes to improve the visibility and sensitivity of any portion of the genetic material in a bacterial cell. Because of the distinctive ring-shaped, halo-like hybridization signal that this technique produced on the cell perimeter, it was given the name "ring-FISH" [25].
32. **RNA-FISH:** in fixed samples at the cellular level, RNA-FISH enables the simultaneous detection, localization, and quantification of individual mRNA molecules in the nucleus or cytoplasm. A way to obtain allelic-specific expression on a single-cell basis is provided by this RNA FISH technique. It may allow for the investigation of single-cell gene expression profiling [23].
33. **Cross Species Color Banding (Rx)-FISH:** The basis for RxFISH, often referred to as chromosomal bar coding, is the 98% sequence similarity between humans and primates like gibbon. In hematological malignancies, it is a dual-color FISH technique for the identification of commonly occurring chromosomal translocations that impact certain genes [23].

34. **Stellaris RNA FISH (Single-Molecule RNA FISH):** It is a technique for mRNA and other long RNA molecule identification and quantification in a thin layer of tissue samples [25].
35. **T-FISH:** In accordance with the chronology of their introduction to the field, the three T-FISH variants—tyramide-FISH, tissue-FISH, and telomere-FISH—are described [24].
36. **Tyramide-FISH:** Tyramide increases the sensitivity of FISH assays dramatically with just one or two layers of visualizing reagents by binding to peroxidase. Tissue-FISH: Tissue samples taken from patients or test animals are fixed or embedded in paraffin wax for the purpose of FISH analysis [25].
37. **Telomere-FISH:** T-FISH stands for tyramide, tissue, or telomere. In chronological sequence of their introduction to the field, the three T-FISH variants are covered. Tyramide-FISH: Tyramide, which binds to peroxidase readily, has been used to significantly boost the sensitivity in FISH investigations, requiring just one or two layers of reagents for visualization [26].
38. **3-D FISH:** To examine the relative placement and spatial positioning of chromosomes and sub-chromosomal areas inside cell nuclei, 3-D FISH has been established [23].
39. **Zoo-FISH:** The process of identifying syntenic areas using zoo-FISH, sometimes referred to as cross-species chromosome painting, which entails blending DNA sequence libraries from different species' chromosomes [25].
40. **Comparative Genomic Hybridization (CGH):** Comparative genome hybridization (CGH), which was introduced in 1992, was one of the most important advancements in FISH technique in terms of genome-wide screening [23,29].

Conclusion:

The FISH is much more accurate and consumer than other molecular profiling methods, such as array-based comparative genomic hybridization, single nucleotide polymorphism (SNP), etc., as compared to a routine application of clinical diagnosis. FISH also became well known as a physical mapping method to enable extensive mapping and sequencing initiatives connected to the human genome project. Through FISH, we can see and understand how genes, chromosomes, transcription, and nucleic acid motions work. FISH also offers trustworthy biomarker data.

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