Advances in fluorescent *in situ* hybridisation

Rosemary Ekong* and Jonathan Wolfe†

Recent advances in fluorescent *in situ* hybridisation included the generation of allele-specific probes, bar-coded chromosomes, and the visualisation of chromosome territories and genes within the nucleus. One major advance has been our ability to visualise and make precise and reproducible measurements from stretched DNA molecules prepared directly from human cells.

Addresses
*MRC Human Biochemical Genetics Unit and †University College London Biology Department, The Galton Laboratory, 4 Stephenson Way, London NW1 2HE, UK
*e-mail: r.ekong@galton.ucl.ac.uk
†e-mail: jwolfe@galton.ucl.ac.uk

Current Opinion in Biotechnology 1998, 9:19–24

http://biomednet.com/elecref/0958166900900019

Abbreviations

DMC	dynamic molecular combing
FISH	fluorescent *in situ* hybridisation
kb	kilobase

Introduction

The fluorescent *in situ* hybridisation (FISH) technique involves the labelling of DNA probes with haptens, such as biotin or digoxigenin, by random priming or by nick translation. After denaturation the probes are applied to chromosomes *in situ*, as metaphase chromosomes or interphase nuclei, and DNA–DNA hybridisation is allowed to occur. Hybrids are then detected by means of fluorescently labelled molecules which have high affinity for the haptens, and the signal may be amplified with simultaneous layers of antibodies and antibodies conjugated to fluorescent molecules. Alternatively, DNA probes may be directly labelled with fluorescent molecules such as FITC, texas red or the recently developed cyanine dyes, and in this instance, visualisation of the DNA–DNA hybrids does not require signal detection and amplification mentioned above.

FISH has become a powerful molecular tool for detecting cytogenetic and molecular genetic alterations, and is widely applied in various fields of study. It has contributed to unravelling cytogenetic alterations in cancer [1] and early human development [2•], molecular descriptions of native nuclear and genome structure [3•–5•], comparative mapping between species [6,7•], countless cases of gene mapping/localisation [8,9] and animal cytogenetics [10,11]. Applications of FISH technology have extended beyond basic research into clinical diagnostics where FISH is fast gaining ground for chromosome analyses. In clinical cytogenetics, FISH undoubtedly allows more cells and chromosomes to be analysed compared to classical karyotyping [12].

While established FISH techniques continue to find wider uses, technical advances have been reported that have potential new applications. The available DNA substrates for FISH have been expanded from fixed chromosomes and interphase nuclei to include stretched single DNA molecules [13••,14,15] and the nucleus and its components in their native forms [3•–5•]. FISH on stretched molecules enables improved resolution by as little as 1–2 kilobases (kb) can be mapped with respect to each other, compared with resolutions of ~1–5 megabases and ~50 kb obtained when mapping on metaphase chromosomes and interphase nuclei, respectively. Such an improved resolution on stretched DNA molecules allows detailed studies of gene structure or intragenic rearrangements. FISH studies on cells maintaining their original shape and on native chromosomes permit investigations on the spatial distribution of nuclear components without inference from computer-assisted analysis of three dimensional image reconstructions. Also, nuclear functions such as the transcriptional activities of two homologous chromosomes can be studied, and one can directly visualise the localisation of nuclear components involved in processes of gene expression.

Probes and targets now include all sizes, from chromosome paints covering parts of, or entire, chromosomes (10–100 Mb) to a few kb or even less [16•,17•]. The level of sensitivity of the FISH technique has increased such that it is now possible to discriminate between single basepair substitutions [18••]. Analysis has been extended from freshly prepared samples to previously analysed and stored archival samples. These archival samples range in age from a few months to several years, and are being used for retrospective chromosome analysis. The samples, when fresh, have previously been subjected to standard cytogenetic analysis, then portions preserved by formalin-fixation and paraffin-embedding. Subsequent FISH analyses of such archival samples have either agreed totally with previous karyotype analysis or provided additional information on aneuploidies and structural aberrations using chromosome-specific probes or paints [19,20].

Technical advances

Fibre FISH

Physical mapping plays a major role in the cloning of human disease genes and FISH has been important to such studies, for instance, in the cloning of Tuberous sclerosis 1 gene [21,22], for which FISH analysis of cosmid, P1, PAC, YAC and BAC clones aided the unambiguous assembly of the critical region. Although metaphase and

interphase FISH have played their roles in these studies, fibre FISH (i.e. FISH either to extended chromatin or to purified DNA) has become increasingly important. The technique has developed from hybridisation to extended chromatin released from nuclei by detergent or alkaline lysis [23–27], through genomic DNA stretched mechanically by pulling one slide along the length of another [28,29], to novel methods such as molecular combing [14,30], and most recently dynamic molecular combing (DMC) [13••] of purified high molecular weight genomic DNA. All the above techniques permit DNA sequences to be mapped, and the organisation of single DNA molecules to be studied. Fibre-FISH with two cDNA probes was used to detect additional gene duplication or deletion within a region of the MHC that contains a short duplicated gene cluster [31]. Arrays of chromosome 22 rDNA and α-satellite, which span 430 kb and 2.6 Mb respectively, were measured in purified high molecular weight DNA prepared by the halo method, which involves lysing cells with detergent to release the DNA [32•].

The level of resolution depends on the technique used for stretching the DNA molecule. Some problems with mapping on stretched DNA include variability in DNA stretching from one area of the slide to another and too few hybridisation signals. Variability in DNA stretching is a problem when the aim of an experiment is to determine distances, sizes and extent of overlaps, but not order. Corrections in measurements have to be performed using the size of a known control, and this may still yield erroneous results. To obtain reliable size and distance measurements, it is important to perform statistical analysis. This cannot be done with a few hybridisation signals which are variable in size. These drawbacks have now been overcome with molecular combing of cloned DNA [14,30] or DMC of total genomic DNA [13••], making it possible to obtain consistent and uniform stretching of total human genomic DNA at very high density, without excessive DNA shearing. DNA is prepared by a standard pulsed-field gel electrophoresis agarose block method. Silanised coverslips are dipped into a reservoir of the DNA solution and withdrawn using a mechanical device operating at a constant speed. The DNA is stretched by the action of the meniscus, which creates a constant stretching force and this removes the variability in stretching, and irreversibly bound by the instantaneous drying as the hydrophobic surface of the coverslip emerges from the liquid. DNA molecules are thus aligned in the same direction throughout the surface and not in several directions, except at localised points where the treated surfaces are uneven.

Advantages of DMC [13••] over other methods of extending genomic DNA for FISH [23–28] are:

1. Stretching of DNA over the entire surface with a constant stretching factor of 2 kb/μm (2 kb of relaxed DNA would measure 0.68 μm) giving uniform hybridisation signals and hence no need to normalise data [33].

2. No variability in DNA stretching between different batches of surfaces or different DNA solutions. This means reliable measurements of distances with no requirement for internal standards or knowledge of any previous data.

3. High density of total genomic DNA and hybridisation signals permitting statistical analysis on a single 22 × 22 mm coverslip.

4. Limited DNA shearing and, consequently, analysable DNA fragments of several hundreds of kilobases.

5. Many surfaces can be prepared from the same DNA solution.

Using DMC [13••], distances between cosmids from two contigs of the human calcium-activated neutral protease 3 gene were measured within a YAC, without its isolation from the total yeast genome, and a correct re-orientation of one of the contigs achieved. The technique also enabled the precise measurement of uncloned intervals within a contig spanning the Tuberous sclerosis 1 gene using human genomic DNA and the detection and measurement of deletions involving the Tuberous sclerosis 2 disease gene using patient DNA.

It would be of value if comparative genome hybridisation could be extended from its current use on metaphase chromosomes to stretched DNA, where it might be capable of revealing copy number variations in the genes of control and experimental DNA. A first step towards this has been reported [15] using a modification of the molecular combing method [14,30]. Comparative self hybridisations with mixtures of the same cosmid probe labelled with two different haptens were performed and uniform fluorescence ratios over the length of the target molecule were plotted. Ultimately, the goal is to perform comparative genome hybridisation on immobilised clones using complex, whole genome probes.

Karyotyping

In clinical cytogenetics, karyotyping by FISH is being developed for use in conjunction with classical karyotyping to enable easier and faster identification of chromosome aberrations. One such technique is the 'chromosome bar code' [34] where each chromosome pair can be identified by a unique set of multi-coloured bands or bars similar to the black and white bar codes found on supermarket products. A recent report [35••] has confirmed that the chromosome bar code used to investigate clinical cases gave the same results as previous classical analysis together with additional information. The difference between the chromosome bar code and classical analysis

is that the chromosome bar code simplifies chromosome identification as opposed to the grey-scale of classical chromosome banding. In the review by Muller *et al.* [35••], 10 clinical cases, previously analysed by standard banding methods, were analysed by chromosome bar coding. Bar coding revealed chromosome aberrations not previously detected by Giemsa-banding, and it also narrowed down sites of rearrangements. Irradiation fragment hybrid cell lines containing between them subregions of each human chromosome were amplified by Alu primed PCR. The resulting PCR products were placed into one of two pools and labelled either with biotin or digoxigenin. The mixed probe was then used in a single hybridisation. Each human chromosome was identified by a unique set of signals and a coloured idiogram of all human chromosomes produced [35••]. Chromosome rearrangements and aneuploidies should be easily detected, and rearrangements will be seen as deviations from the signal sequence of the normal chromosome. The resolution of this technique, however, still needs improvement as some chromosomes are poorly represented in the pool of probes used and rearrangements involving unrepresented chromosome regions will be difficult to identify. Ideally, the resolution of chromosome bar coding should be comparable to classical chromosome banding.

In gynaecology and fetal medicine, multicolour FISH is increasingly being used clinically or as a research tool to investigate fertility [2•,36,37] and to analyse fetal chromosomes/aneuploidies in samples of fetal origin [38,39]. For example, Klinefelter's syndrome in men [37] and endometriosis in women [36] are two conditions which cause infertility. The chromosomal composition and/or alterations in cells from these patients have been analysed by FISH in an attempt to unravel possible causes for the infertile status of these patients.

Another area where FISH is of value is in monitoring the effects of clinical treatments on chromosomes. For example, chromosome abnormalities in germ cells following chemotherapy and/or radiotherapy [40,41] have been examined. In these studies, standard multicolour FISH was applied to sperm samples collected before, during and after treatment. In both studies, high rates of aneuploidy were observed and both concluded that the disease, as well as the treatment, contributed to the observed results.

High-throughput assays/diagnostics

FISH has undoubtedly become an important tool in clinical cytogenetic laboratories but to be used routinely in clinical diagnostics, it is desirable that multiple analyses be performed in a single hybridisation experiment. For instance, the simultaneous assay of rearrangements involving subtelomeric regions of all the human chromosomes has been described [42•]. Telomeric probes for each chromosome arm were dried onto a simple device of 24 panels — one panel (a section of a slide containing a unique probe) for each chromosome probe. The probe device was then placed over a template slide, also divided into 24 squares each containing dried, fixed chromosome preparations, and each square was hybridised to one of the panels. The advantage of this approach over multiple hybridisations with individual telomeric probes is that only a single slide per patient material is needed rather than 24 slides per patient.

A new concept in clinical investigations/diagnostics is to apply multilocus probes in single hybridisations using standard FISH onto metaphase chromosomes or interphase nuclei. Such multiple probes have been specifically targeted to specific subsets of disorders like the microdeletion syndromes [43•], for example, the DiGeorge (or velocardiofacial), Prader–Willi, Angelman and Wiliams syndromes; or single disorders, such as acute myeloid leukaemia or cri-du-chat [44,45]. The absence of a probe at its expected locus indicates the presence of the syndrome to which the probe corresponds.

Native nuclear and chromosome structure

FISH is one of the few methods available for visualisation of the 3D nuclear structure and of genome organisation within cell nuclei. The most important aspect in such studies is the preservation of the original shape of the cell and its components during the experiments. Usually cells are grown directly on coated slides where they are subsequently processed using reagents that maintain the 3D structures [5•]. Two novel approaches for preserving 3D structure involve embedding the cells either in acrylamide [3•] or in agarose [46•]. With the agarose method, template DNA is denatured using NaOH, not heat, in order to avoid melting the agarose. To study chromatin fibres the cells can be subjected to electrophoresis *in situ* in the agarose ('comet' assay) but to study intact nuclei no electrophoresis is applied. The spatial distribution of chromosome-specific sequences in centromeres, telomeres and other regions of specific chromosomes has been determined [46•].

Confocal laser microscopy enables 3D reconstruction of the positions of FISH signals within a nucleus. Such studies have shown, for instance, that the arrangement and peripheral localisation of telomeres within nuclei change depending on the stage of the meiotic cell cycle [3•], that genes, whether active or inactive, are preferentially located in the periphery of chromosome territories, whereas non-coding sequences are randomly distributed in the chromosome territory [5•], and that the active and inactive X chromosomes differ in shape and surface structure, but are similar in volume, and an autosome was found to be similar to the active X chromosome [4•].

The scanning near-field optical/atomic-force microscopy will prove to be an additional new technology for studying native chromosome structures. With this technology samples do not require pre-treatment and its first application has been to detect the topography of fluorescently-stained

native chromosomes [47•]. With the FISH technique, it should be possible to analyse the structure of specific regions of chromosomes.

Probes

Oligonucleotides that differ by just a single nucleotide have been used in FISH experiments [18••] to distinguish repetitive α-satellite sequences in the centromeres of two human chromosomes (13 and 21). Biotinylated or digoxigenin-labelled oligonucleotides were designed so that, following hybridisation to a target, their ends abut at the point of potential sequence mismatch. Here, if the sequence matches, they could be ligated into a closed circle forming a 'padlock probe' locked at the hybridisation site. Although this technique is still in its infancy, it will be interesting to see if it can be developed to the point of detecting sequence variation at single copy DNA targets.

To reduce the cost of COT-1 DNA (COT refers to the degree of association of complex DNA, where CO is the initial DNA concentration, and T is the incubation time in seconds) required for suppressing repetitive sequences in probes and to lower background signal caused by suboptimal suppression of labelled repetitive sequences, it would be advantageous to generate FISH probes lacking repeat sequences. If the repetitive sequences are not suppressed, they give hybridisation signals which could affect the interpretation of results. Subtractive hybridisation with excess biotin-labelled COT-1 DNA has been employed prior to amplification of the remaining probe by PCR and labelling [48]. Another report describes the deliberate choice of small insert (2–4 kb) genomic FISH probes lacking repetitive sequences for mapping small (2–5 kb) deletions within genomic regions on metaphase chromosomes [16•]. These probes were found to give strong hybridisation signals (presumably because they lacked repetitive sequences); however, the hybridisation efficiency of the 4 kb probe (78–90%) was higher than that of the smaller probes (43–81%).

The limit of fibre-FISH mapping has been pushed further by the mapping of 400 bp PCR products of exons onto extended cosmids with hybridisation efficiencies of 70–90% [17•].

Conclusion

'Seeing is believing' says the proverb and although we have many other techniques, which together can accomplish much that fluorescence *in situ* hybridisation can currently achieve, there remains something very satisfying in being able to view directly the relative positions of DNA sequences. Perhaps because of this there is a great deal of interest in FISH, which in consequence is a rapidly evolving technique. A very few years ago FISH could resolve the order of two probes only if they were at least a megabase apart. Now resolutions are measured in hundreds or maybe even tens of basepairs. Probes have shrunk from cosmid inserts to small PCR products and the latest probes are allele specific, recognising single nucleotide substitutions. It will not take a huge step forward in technology to enable the direct reading of a chromosome's haplotype by the use of multiple padlock probes. One capability unique to FISH is the ability to examine DNA (and RNA) structures in their normal surroundings. We will undoubtedly see many more pictures of interphase chromosomes over the next couple of years, particularly when fluorescently tagged antibodies to nuclear scaffold proteins are studied in conjunction with the FISH results. The next year or two promise to be very colourful.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Thein ATA, Han X, Heyderman E, Fox M, Steele SJ, Parrington JM: **Molecular cytogenetic analysis of five newly established cervical cancer cell lines using G-banding and fluorescence *in situ* hybridization.** *Cancer Genet Cytogenet* 1996, **81**:1-9.

2. • Delhanty JDA, Harper JC, Ao A, Handyside AH, Winston RML: **Multicolour FISH detects frequent chromosomal mosaicism and chaotic division in normal preimplantation embryos from fertile patients.** *Hum Genet* 1997, **99**:755-760.

Three satellite DNA probes specific for chromosomes 1, X and Y were used to examine interphase nuclei of 6–10 cell stage human embryos by three colour FISH. The embryos were spare embryos resulting from *in vitro* fertilisation. The study revealed a surprisingly high level of aneuploidy.

3. • Bass HW, Marshall WF, Sedat JW, Agard DA, Cande WZ: **Telomeres cluster *de novo* before the initiation of synapsis: a three-dimensional spatial analysis of telomere positions before and during meiotic prophase.** *J Cell Biol* 1997, **137**:5-18.

A novel technique for the preservation of the three dimensional structure of nuclei and chromosomes: meiocytes were embedded in acrylamide before hybridisation to a telomere oligonucleotide probe.

4. • Eils R, Dietzel S, Bertin E, Schrock E, Speicher MR, Reid T, Robert-Nicoud M, Cremer C, Cremer T: **Three-dimensional reconstruction of painted human interphase chromosomes: active and inactive X chromosome territories have similar volumes but differ in shape and surface structure.** *J Cell Biol* 1996, **135**:1427-1440.

Serial optical sections allow the reconstruction of the volume occupied by X chromosomes and chromosomes 7. The volumes occupied by active and inactive X chromosomes are similar but the surface area of the active X is much greater. The active X has a similar 'roundness factor' to that of chromosome 7.

5. • Kurz A, Lampel S, Nickolenko JE, Bradl J, Benner A, Zirbel RM, Cremer T, Lichter P: **Active and inactive genes localize preferentially in the periphery of chromosome territories.** *J Cell Biol* 1996, **135**:1195-1205.

Using similar techniques as described by Eils *et al.* 1996 [4•], the spacial localizations of three X-linked genes and two noncoding X-linked sequences were examined with respect to the territory occupied by the whole X chromosome. The genes are preferentially localised on the periphery of the chromosome.

6. Frengen E, Thomsen PD, Brede G, Solheim J, de Jong PJ, Prydz H: **The gene cluster containing the LCAT gene is conserved between human and pig.** *Cytogenet Cell Genet* 1997, **76**:53-57.

7. • Yang F, Muller S, Just R, Ferguson-Smith MA, Wienberg J: **Comparative chromosome painting in mammals: human and the Indian muntjac (*Muntiacus muntjak vaginalis*).** *Genomics* 1997, **39**:396-401.

Human chromosome specific paints used to paint muntjack chromosomes show some regions of conservation but an enormous amount of genome reshuffling.

8. Woodward K, Nahmias J, Hornigold N, West L, Pilz A, Benham F, Kwiatkowski D, Fitzgibbon J, Wolfe J, Povey S: **Regional localization of 64 cosmid contigs including 18 genes and 14**

markers to intervals on human chromosome 9q34. *Genomics* 1995, **29**:257-260.

9. Yamamoto K, Kobayashi H, Miura O, Hirosawa S, Miyasaka N: **Assignment of IL12RB1 and IL12RB2, interleukin-12 receptor β1 and β2 chains, to human chromosome 19 band p13.1 and chromosome 1 band p31.2, respectively, by *in situ* hybridization.** *Cytogenet Cell Genet* 1997, **77**:257-258.

10. Sebastian S, Hoebee B, Hande MP, Kenkare UW, Natarajan AT: **Assignment of hexokinase types 1, 2, 3 (*Hk1,2,3*) and glucokinase (*Gck*) to rat chromosome band 20q11, 4q34, 17q12 and 14q21 respectively, by *in situ* hybridization.** *Cytogenet Cell Genet* 1997, **77**:266-267.

11. Elduque C, Laurent P, Hayes H, Rodellar C, Leveziel H, Zaragoza P: **Assignment of the beta-nerve growth factor (NGFB) to bovine chromosome 3 band q23 by *in situ* hybridization.** *Cytogenet Cell Genet* 1997, **77**:306-307.

12. Guttenbach M, Engel W, Schmid M: **Analysis of structural and numerical chromosome abnormalities in sperm of normal men and carriers of constitutional chromosome aberrations. A review.** *Hum Genet* 1997, **100**:1-21.

13. •• Michalet X, Ekong R, Fougerousse F, Rousseaux S, Schurra C, Hornigold N, van Slegtenhorst M, Wolfe J, Povey S, Beckmann JS, Bensimon A: **Dynamic molecular combing: stretching the whole human genome for high-resolution studies.** *Science* 1997, **277**:1518-1523.

Uses purified high molecular weight DNA stretched onto a coverslip by a receding meniscus to achieve such uniform tension of the DNA strands that no normalisation is required. Both yeast artificial chromosome and total human genomic DNA are assayed. Deletion mutations (38–135 kb) are visualised and accurately measured, as well as the genomic distance between several pairs of cosmid clones.

14. Weier H-UG, Mang M, Mullikin JC, Zhu Y, Cheng J-F, Greulich KM, Bensimon A, Gray JW: **Quantitative DNA fiber mapping.** *Hum Mol Genet* 1995, **4**:1903-1910.

15. Kraus J, Weber RG, Cremer M, Seebacher T, Fischer C, Schurra C, Jauch A, Lichter P, Bensimon A, Cremer T: **High-resolution comparative hybridization to combed DNA fibers.** *Hum Genet* 1997, **99**:374-380.

16. • Dreyling MH, Olopade OI, Bohlander SK: **Generation of small insert genomic FISH probes with high signal intensity suitable for deletion mapping.** *Cytogenet Cell Genet* 1997, **76**:202-205.

Repeat free fragments (2–4 kb) of cosmid clones were amplified by sequence-independent PCR and used as FISH probes. They gave good metaphase signals but were said to be no good on interphase nuclei. Their principle use may be in detecting small deletions.

17. • Florijn RJ, van de Rijke FM, Vrolijk H, Blonden LAJ, Hofker MH, den Dunnen JT, Tanke HJ, van Ommen G-JB, Raap AK: **Exon mapping by fibre-FISH or LR-PCR.** *Genomics* 1996, **38**:277–282.

Hybridisations with 400 bp PCR products of exons onto extended cosmids are shown as intensity profiles along the length of the cosmid. Hybridisation efficiencies drop from 70–90% to only 30% when fragments less than 300 bp were mapped. When mapping cDNAs (3.3 kb, 3.6 kb, 4.6 kb) with exon sizes varying from 25 bp to 860 bp onto their respective cosmids the exons could not be distinguished as each cDNA was seen as a single hybridisation spot. The inability to distinguish individual exons of each cDNA was attributed to the limited spatial separation between the exons.

18. •• Nilsson M, Krejci K, Koch J, Kwiatkowski M, Gustavsson P, Landegren U: **Padlock probes reveal single-nucleotide differences, parent of origin and *in situ* distribution of centromeric sequences in human chromosomes 13 and 21.** *Nat Genet* 1997, **16**:252-255.

The authors uses DNA ligase to concatenate a 'padlock probe' to a target sequence, having the astonishing ability to discriminate DNA sequences which differ by only a single basepair. Demonstrated here with the tandemly repeated α-satellite but with potential to view polymorphism directly on the chromosome. Using this technique we might one day be able to see a haplotype directly without needing to infer it.

19. Blough RI, Smolarek TA, Ulbright TM, Heerema NA: **Bicolor fluorescence *in situ* hybridization on nuclei from formalin-fixed, paraffin-embedded testicular germ cell tumors: comparison with standard metaphase analysis.** *Cancer Genet Cytogenet* 1997, **94**:79-84.

20. Ghazvini S, Char DH, Kroll S, Waldman FM, Pinkel D: **Comparative genomic hybridization analysis of archival formalin-fixed paraffin-embedded uveal melanomas.** *Cancer Genet Cytogenet* 1996, **90**:95-101.

21. Nahmias J, Hornigold N, Fitzgibbon J, Woodward K, Pilz A, Griffin D, Henske EP, Nakamura Y, Graw S, Florian F *et al.*: **Cosmid contigs spanning 9q34 including the candidate region for TSC1.** *Eur J Hum Genet* 1995, **3**:65-77.

22. The TSC1 Consortium: **Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34.** *Science* 1997, **277**:805-808.

23. Fidlerova H, Senger G, Kost M, Sanseau P, Sheer D: **Two simple procedures for releasing chromatin from routinely fixed cells for fluorescence *in situ* hybridization.** *Cytogenet Cell Genet* 1994, **65**:203-205.

24. Haaf T, Ward DC: **Structural analysis of α-satellite DNA and centromere proteins using extended chromatin and chromosomes.** *Hum Mol Genet* 1994, **3**:697-709.

25. Heng HHQ, Squire J, Tsui L-C: **High-resolution mapping of mammalian genes by *in situ* hybridization to free chromatin.** *Proc Natl Acad Sci USA* 1992, **89**:9509-9513.

26. Parra I, Windle B: **High resolution visual mapping of stretched DNA by fluorescent hybridization.** *Nat Genet* 1993, **5**:17-21.

27. Weigant J, Kalle W, Mullenders L, Brookes S, Hoovers JMN, Dauwerse JG, van Ommen GJB, Raap AK: **High-resolution *in situ* hybridization using DNA halo preparations.** *Hum Mol Genet* 1992, **1**:587-591.

28. Heiskanen M, Karhu R, Hellsten E, Peltonen L, Kallioniemi OP, Palotie A: **High resolution mapping using fluorescence *in situ* hybridization to extended DNA fibers prepared from agarose-embedded cells.** *Biotechniques* 1994, **17**:928-934.

29. Yokota H, Johnson F, Lu H, Robinson RM, Belu AM, Garrison MD, Ratner BD, Trask BJ, Miller DL: **A new method for straightening DNA molecules for optical restriction mapping.** *Nucleic Acids Res* 1997, **25**:1064-1070.

30. Bensimon A, Simon A, Chifaudel A, Croquette V, Heslot F, Bensimon: **Alignment and sensitive detection of DNA by a moving interface.** *Science* 1994, **265**:2096-2098.

31. Suto Y, Tokunaga K, Watanabe Y, Hirai M: **Visual demonstration of the organisation of the human complement C4 and 21-hydroxylase genes by high-resolution fluorescence *in situ* hybridization.** *Genomics* 1996, **33**:321-324.

32. • Shiels C, Coutelle C, Huxley C: **Analysis of ribosomal and alphoid repetitive DNA by fibre-FISH.** *Cytogenet Cell Genet* 1997, **76**:20-22.

DNA arrays up to 2.6 Mb measured.

33. Florijn RJ, Bonden LAJ, Vrolijk H, Weigant J, Vaandrager J-W, Baas F, den Dunnen JT, Tanke HJ, Van Ommen G-JB, Raap AK: **High-resolution DNA fiber-FISH for genomic DNA mapping and colour bar-coding of large genes.** *Hum Mol Genet* 1995, **4**:831-836.

34. Lengauer C, Speicher MR, Popp S, Jauch A, Taniwaki M, Nagaraja R, Reithman H, Donis-Keller H, D'Urso M, Schlessinger D, Cremer T: **Chromosomal bar codes produced by multicolor fluorescence *in situ* hybridization with multiple YAC clones and whole chromosome painting probes.** *Hum Mol Genet* 1993, **2**:505-512.

35. •• Muller S, Rocchi M, Ferguson-Smith MA, Wienberg J: **Towards a multicolor chromosome bar code for the entire human karyotype by fluorescence *in situ* hybridization.** *Hum Genet* 1997, **100**:271-278.

A pretty paper. Irradiation fragment hybrid cell lines containing between them subregions of each human chromosome were amplified by Alu primed PCR. The resulting PCR products were placed into one of two pools which were labelled either with biotin or digoxigenin. The mixed probe was then used in a single hybridisation. Each human chromosome is identified by a unique set of signals and a coloured idiogram of all human chromosomes produced.

36. Shin J-C, Ross HL, Elias S, Nguyen DD, Mitchell-Leef D, Simpson JL, Bischoff FZ: **Detection of chromosomal aneuploidy in endometriosis by multi-color fluorescence *in situ* hybridization (FISH).** *Hum Genet* 1997, **100**:401-406.

37. Guttenbach M, Michelmann HW, Hinney B, Engel W, Schmid M: **Segregation of sex chromosomes into sperm nuclei in a man with 47, XXY Klinefelter's karyotype: a FISH analysis.** *Hum Genet* 1997, **99**:474-477.

38. Sherlock J, Halder A, Tutschek B, Delhanty J, Rodeck C, Adinolfi M: **Prenatal detection of foetal aneuploidies using transcervical cell samples.** *J Med Genet* 1997, **34**:302-305.

39. Wegner RD, Schrock E, Obladen M, Becker R, Stumm M, Sperling K: **Partial trisomy/monosomy 6q in foetal cells and CVS long-term culture not present in CVS short-term culture.** *Prenat Diagn* 1996, **16**:741-748.

40. Monteil M, Rousseaux S, Chevret E, Pelletier R, Cozzi J, Sele B: **Increased aneuploid frequency in spermatozoa from a Hodgkin's disease patient after chemotherapy and radiotherapy.** *Cytogenet Cell Genet* 1997, **76**:134-138.

41. Robbins WA, Meistrich ML, Moore D, Hagemeister FB, Weier H-U, Cassel MJ, Wilson G, Eskenazi B, Wyrobek AJ: **Chemotherapy induces transient sex chromosomal and autosomal aneuploidy in human sperm.** *Nat Genet* 1997, **16**:74-78.

42. • Knight SJL, Horsley SW, Regan R, Lawrie NM, Maher EJ, Cardy DLN, Flint J, Kearney L: **Development and clinical application of an innovative fluorescence *in situ* hybridization technique which detects submicroscopic rearrangements involving telomeres.** *Eur J Hum Genet* 1997, **5**:1-8.
Cleverly uses a single slide with 24 separate hybridisations.

43. • Ligon AH, Beaudet AL, Shaffer LG: **Simultaneous, multilocus FISH analysis for detection of microdeletions in the diagnostic evaluation of developmental delay and mental retardation.** *Am J Hum Genet* 1997, **61**:51-59.
Probing for many syndromes simultaneously can reduce the risk of misdiagnosis.

44. Fischer K, Scholl C, Salat J, Frohling S, Schlenk R, Bentz M, Stilgenbauer S, Lichter P, Dohner H: **Design and validation of DNA probe sets for a comprehensive analysis of acute myeloid leukaemia.** *Blood* 1996, **88**:3962-3971.

45. Gersh M, Grady D, Rojas K, Lovett M, Moyzis R Overhauser J: **Development of diagnostic tools for the analysis of 5p deletions using interphase FISH.** *Cytogenet Cell Genet* 1997, **77**:246-251.

46. • Santos SJ, Singh NP, Natarajan AT: **Fluorescence *in situ* hybridization with comets.** *Exp Cell Res* 1997, **232**:407-411.
Cells embedded in agarose before lysis and DNA stretched by electrophoresis prior to FISH.

47. • Iwabuchi S, Muramatsu H, Chiba N, Kinjo Y, Murakami Y, Sakaguchi T, Yokoyama K, Tamiya E: **Simultaneous detection of near-field topographic and fluorescence images of human chromosomes via scanning near-field optical/atomic force microscopy (SNOAM).** *Nucleic Acids Res* 1997, **25**:1662-1663.
A new microscopy method. Has it a future in this field?

48. Craig JM, Kraus J, Cremer T: **Removal of repetitive sequences from FISH probes using PCR-assisted affinity chromatography.** *Hum Genet* 1997, **100**:472-476.