Reflections on the Past, Present and Future of Automated Aberration Scoring Systems for Radiation Dosimetry

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INTRODUCTION

From its inception in the early 60's, an important objective of work on computerised chromosome analysis was the creation of a system for automated recognition and scoring in metaphase cells of environmentally induced chromosome aberrations. Rapid advances were made in deriving the principles upon which such a system might operate, but attempts at implementation in working hardware were unsuccessful. Progress in the intervening decades has been dominantly influenced by the exponential increases in the ratio of power to price (and power to size) of computing electronics. This factor has more than any other determined the design of imaging hardware and algorithms for classical slide-based, absorption mode, aberration scoring. But each decade has also seen the introduction of entirely new technologies, both instrumental and biological, offering different avenues of attack on the problem (Table 1). It is my purpose in this essay to set down their salient features, to attempt to sum up the contribution that each is currently capable of making, and to hazard some thoughts about likely future developments.

Table 1. AUTOMATION OF RADIATION DOSIMETRY-THREE DECADES of ADVANCE in FACILITATING TECHNOLOGIES

- Electro-mechanics
- Electro-optics
- Electronics and computing
- Operating systems/languages
- HCI-Human Computer Interface
- Algorithms/Classifiers
- Cytometry
- Immunology
- Molecular Biology
- Fluorochemistry
The late 1950's and 1960's was the great decade of cytogenetics, beginning with LeJeune's 1959 finding that trisomy 21 was the cause of Down's syndrome. In the next few years the major sex-chromosome variants and their phenotypic expression were catalogued, and within a few years all the major elements of the framework of clinical cytogenetics were in place [1]. In the same period the counting of dicentrics, fragments and ring chromosomes was established as a means of determination of radiation exposure [2]. Attempts at automation of the process of scoring soon followed.

The first was that of Butler and Stroud, at Argonne National Laboratory (near Chicago) with radiation dosimetry an avowed objective. They created a system of hard-wired electronics for picture acquisition in the form of "intervals"—above-background segments of a scan-line—which because of the data-reduction and other processing economies associated with this form of picture encoding, has remained one of the key-approaches to the subject ever since [3.

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Table 2a. Available support technology: 1960's

| ELECTRO-OPTICS |
|----------------|
| Film Scanners |
| Crt Flying Spot (1000×1000 + available)/Photomultiplier |
| Vidicon Cameras (interfaces 256×256) |

| ELECTRONICS AND COMPUTING |
|---------------------------|
| 8 k Memory               |
| 10 μ Second Cycle Times  |
| Millisecond Pixel A-D    |
| 64 k Discs               |

| OPERATING SYSTEMS, LANGUAGES |
|-----------------------------|
| Fortran, Assembler          |

| HUMAN COMPUTER INTERFACE |
|---------------------------|
| Batch Processing          |
| First full-screen editors |

Table 2b. Developments in Associated Disciplines-1960's

| IMAGE PROCESSING ALGORITHMS |
|-----------------------------|
| Cooley and Tukey            |
| The Fast Fourier Transform  |
| Various Authors             |
| Basic Filters, Edge Detectors |
| Blum                        |
| 'Brush-Fire' Transform      |
| Golay & Preston             |
| Golay Logic [precursor of Mathematical Morphology] |

† Refer Table 2.
AUTOMATED ABERRATION SCORING SYSTEMS

CYTOGENETICS

| Down's, XXY, XYY, X0 Etc.... | Aberration Scoring In Homogenously Stained Metaphases |

IMMUNOLOGY

| Radio-immuno Assay |

MOLECULAR BIOLOGY

| On The Way ..... |

CYTOMETRY

| Van-Dilla, Kamentsky et al (Los Alamos) | Flow Sorting |
| Mendelsohn, Mayall, Prewitt | Cydac [first accurate cytometer] |

Table 2c. Developments in Automated Cytogenetics, 1960's

CHROMOSOME ANALYSIS SYSTEMS

| Butler and Stroud, Argonne Labs | 'CHLOE' Marmoset aberration analysis system |
| Ledley et al, National Biological Research Foundation | 'FIDAC' Denver group karyotyping system |

CHROMOSOME ANALYSIS ALGORITHMS

| Ledley et al | ‘BUGSYS’-syntactic analysis of boundary shape |
| Butler and Stroud | ‘interval’ representation of regions |
| Mendelsohn and Prewitt | chromosome and aberrant chromosome classification by feature statistics |
| Rutovitz | local threshold selection algorithms for chromosomes. |
| | Algorithms for precision densitometry. |
| Hiditch | systematic development of interval representation |
| | object-oriented shape and density analysis |
| Hilditch and Rutovitz | the Hilditch skeleton; grey scale skeletons |
| | composite resolution by adjacency graph processing |
| Butler/Rutovitz/ Neurath | “Poor man’s skeleton” |

HARD-COPY

| Mendelsohn and Prewitt | Line-Printer Grey Scale Rendition (!) |
Their work was more or less contemporary with that of R. S. Ledley, at the National Biomedical Research Foundation (Washington D.C.) who encoded the outlines of above-background parts of an image using a Cathode Ray Tube (CRT) flying spot contour follower, and developed a system for genetic karyotyping. Ledley and Butler had based their work on digitisation of film [6]; but in 1964 Mendelsohn, Prewitt and Mayall began work in Philadelphia on a flying spot CRT system for measurement of chromosomes by direct microscopy: this measuring system eventually became the Cydac instrument [7]. In 1965 in the U.S., Neurath started work on cytogenetics automation at the New England Medical Center, and Stone, Stuart and Littlepage for the DoE. Rutovitz, Hilditch and others established the MRC group, then in London, initially using a FIDAC flying-spot film scanner, but by 1968 they had acquired an “image dissector” based direct microscopy system.

Butler and Stroud, rather than attempting to identify specific aberrant chromosomes, set up an aberration scoring system for experiments with marmosets and other mammalian species based on an analysis of the variations in the distribution of moments and other shape parameters.

It was quickly perceived that some aberrations, dicentric chromosomes in particular would be relatively easy to identify; also that a scoring system would not be useful without the capability to locate suitable metaphase spreads. By 1967, Wald at Pittsburgh, and Preston at the Perkin Elmer Corporation, were working on a prototype automated microscope and metaphase finder [8].

Of these early efforts, the greatest practical success was obtained by Mendelsohn, whose objective was not so much automation, as accuracy: within a decade Mendelsohn had shown the enormous potential of precision digital cytometry [9]. Those aiming at rapid throughput and sophisticated automatic pattern recognition were disappointed: partly because of the limitations of the hardware available at that time, and partly because of a failure to understand the real difficulty of building automatic or computer-aided systems to a standard sufficient to achieve operator acceptability. Nevertheless these early workers in the field established the principal methods and algorithms upon which most subsequent work was based (see table 2C: “Developments in Automated Cytogenetics, 1960’s”), though it took 20 years to see their ideas move from the laboratory of the computer scientist to that of the clinical cytogeneticist.

1970’s*

Metaphase Finding

By 1970 a version of the Preston-Wald-Renshaw-Herron device was operational. To find the dividing cells it utilised the Fraunhoffer diffraction image of a 50 μ-diameter coherent laser beam, in effect a frequency domain representation of the image rather than the image itself. With an optical arrangement of this type annular or radial stops can be used to filter out all but the spatial frequencies of interest. To detect the magnitude of the chosen frequency components, it is then only necessary to measure the total intensity of the signal transmitted through

* see Table 3.
Table 3a. Available support technology: 1970's

| ELECTRO-OPTICS            |
|--------------------------|
| Quality Vidicons, Plumbicons | Frame-rate interfaces (custom) |
| Laser scanners            | Microscope-based scanning systems |

| ELECTRONICS AND COMPUTING                  |
|--------------------------------------------|
| 2 μ sec cycle time processors              | 128 K memory |
| Mb Discs                                   |

| LANGUAGES/OPERATING SYSTEMS               |
|-------------------------------------------|
| Unix and C Developed                      | Rtl1, DEC TOPS10, Multics |

| HUMAN COMPUTER INTERFACE                 |
|------------------------------------------|
| Full-screen Interactive Control and Editing |
| Experimental Windows Systems             |

| ELECTROMECHANICAL                        |
|------------------------------------------|
| Reliable accurate stepping motors        |
| 0.1 μ-step microscope stages and controllers |

Table 3b. Developments in Associated Disciplines-1970's

| IMAGE PROCESSING ALGORITHMS               |
|------------------------------------------|
| J. Serra (Ecole Superieure des Mines)    | Mathematical Morphology |
| C. J. Taylor (Manchester)                | ‘Point-set’ based object calculus |
| J. Brenner (New England Medical Center)  | Object separation by minimal-cost path tracing |

| CYTOMETRY                                 |
|-------------------------------------------|
| Livermore Group                           | Hamster Chromosomes Sorted (1974) |
| Wheeless                                  | Slit scan/image capture flow system design |
| Beckton, Coulter, others                  | Commercial sorters and cytometers |

| IMMUNOLOGY                                |
|-------------------------------------------|
| Monoclonal Antibodies                     |
| Biotin-Avidin-Fluorescein system          |
Table 3c. Developments in Automated Cytogenetics, 1970’s

| IMAGE PROCESSING ALGORITHMS | |
|----------------------------|----------------------------|
| Agarwhal and Fu            | Threshold-independent ‘poor man’s skeleton’ |
|                            | Classification of aberrations/chromosomes by feature-statistics |
| Gallus (New England        | Quantitative boundary curvature analysis |
| Medical Center)            | |
| Caspersson et al (Karolinska Institute) | Characterisation of band patterns by Fourier spectrum |
| Castleman and Wall (Jet Propulsion Laboratory) | Various band pattern characterisations |
| Granlund (MIT & Linkoping) | Gaussian decomposition of band patterns |
| Piper (MRC)/others         | Chromosome width, density and moment profiles; interpolative rotation; chromosome straightening |
| Various authors            | Chromosome straightening |

| PREPARATION SYSTEMS | |
|---------------------|----------------------------|
| Melnyck, City of Hope Hospital, LA | automatic culture and staining machine |

| CHROMOSOME ANALYSIS SYSTEMS | |
|----------------------------|----------------------------|
| Castleman, (J.P.L.)        | Demonstration complete interactive system, with hard copy output and metaphase finding capability |

| FLOW SYSTEMS | |
|---------------|----------------------------|
| Mendelsohn, Mayall, Carrano, Gray | Experiments in histogram-degeneration dosimetry |
the stop; the process is instantaneous and appears to offer a ready-made means of metaphase spread detection. This is because metaphase cells, in contradistinction to most other material present on a metaphase slide, have strong spatial frequency components corresponding to the average inter-chromosome distance, to the average chromosome width and to the average inter-chromatid gap. Pulse-width measurement was later added to the system to enable selection of objects on the basis of size as well as frequency content. It worked!—a complete sq. cm. of slide could be scanned in 1.5 minutes, a speed not exceeded by many subsequent instruments. Unfortunately the size, cost and complexity of the apparatus, and a tendency to become confused by dirt and debris (the slides had to be very carefully cleaned and free of crystalline impurities), in the end rendered the device unsuitable for routine laboratory use [10].

Meanwhile, others had set about the construction of metaphase finding equipment in more conventional ways. In 1972 Green and Cameron [MRC Edinburgh] published a description of an experimental metaphase finding system which used analogue circuitry to locate chromosome-sized dark intervals within scan lines, and hard-wired digital electronics to detect fields containing numbers of such signals consistent with the presence of metaphases [11]. By 1974 Green reported tests of a second system capable of continuous scanning with a Plumbicon camera operated at 22.5 frames/sec, using a synchronised flashing light source to strobe the images onto

| Table 3c. Developments in Automated Cytogenetics, 1970's, ctd. |
|---------------------------------------------------------------|
| **METAPHASE FINDERS**                                        |
| Preston (Perkin Elmer), Wald, Renshaw, Herron (Pittsburgh)   |
| 1971                                                         |
| ★ Optical Filtering                                          |
| ★ Rotating Platen                                             |
| ★ Performance ......                                          |
| ● 1 cm²/(23 Sec)                                              |
| ● 50% FN 10% TP                                               |
| Green and Cameron (MRC) 1972                                  |
| ★ analogue detection ‘right-sized’ above threshold intervals |
| ★ clustering into preset number of boxes                     |
| Green (MRC), Neurath (New England Med. Centre) (separately)   |
| 1974                                                         |
| ★ custom digital logic, find ACP’s                           |
| ★ cluster by minicomputer                                    |
| ★ rescan/re-assess                                            |
| ★ Performance ......                                          |
| ● speed 1 cm²/45 secs+second pass                             |
| ● 10–50% high quality true-positives                         |
| R.J.P Le Go (Commissariat a l’Energie Atomique)               |
| ★ detect 10 sq. micron areas of intermediate greyness        |
| ★ cluster.                                                    |
| ★ Performance ......                                          |
| ● speed 1 cm²/25 secs+second pass                             |
| Castleman (J.P.L.)                                           |
| demonstration system                                         |
| Neurath/Oosterlinck /Ledley/Others                           |
| experimental Systems                                         |

| **SISTER CHROMATID EXCHANGE**                                |
|--------------------------------------------------------------|
| Zack                                                         |
| Feasibility of automatic SCE scoring demonstrated            |
the camera. Instead of direct use of the count of above-background $x$-intervals of the desired length and separation, Green now used digital logic to detect $y$-adjacent strips of these: more precisely, to detect the termination points of such strips. This idea had previously been incorporated into the Quantimet range of machines by Metals Research Ltd, (later Cambridge Instruments, now Cambridge Leica) who also provided Green's automatic microscope. The termination points were clustered by a PDP9 computer into a number of rectangular subdivisions of the scan frame; the counts and spatial relationships of these rectangles were used to generate an indication of the likely presence of metaphases. In a second pass, sites flagged during the first were revisited and re-analysed in somewhat greater detail, in order to cull the less likely candidates from the list. The initial search pass was effected at 1 cm$^2$/45 secs; the timing of the second pass depended on slide density, but did not significantly increase overall time in most preparations. In an evaluation exercise conducted jointly at Tufts New England Medical Centre and the MRC Edinburgh laboratory, between 50% (Orcein) and 10% (Giemsa) of the cells found by the device were judged complete and suitable for karyotype analysis, a standard which can be bettered by later instruments, but which approaches the acceptable [12]. By this time metaphase finders had been constructed by other groups also, notably R. Ledley at the National Biomedical Research Foundation; R. J. P. Le Go, Commissariat a l’Energie Atomique, Fontenay aux Roses and K. Castleman [13] at the Jet Propulsion Laboratories, Pasadena. Le Go observed that viewed at low magnification, metaphase cells were characteristically lighter than interphases and yet rather darker than background. He built up an array of photodetectors onto which were imaged 10×10 μ$^2$ areas of the slide. A procedure was used to determine an appropriate detection level for metaphases, and electronics provided to give a binary output signal; in effect, an optical detection system working in the density rather than the Fourier domain. Accompanied by software to sort out clustering and coincidence of detected regions in successive swatches, the device worked rapidly and well, but due to commercial or bureaucratic rather than technical problems, never entered routine laboratory use or commercial production [14].

Castleman’s metaphase finder utilised principles rather similar to that of the first Green machine, and Ledley’s to the second. Indeed, albeit with numerous embellishments, all subsequent machines have been based either on the detection of areas evidencing spatial frequencies in a specified range, or containing clusters of small objects or terminal points of objects. So far as I am aware, only Le Go based detection primarily on optical density rather than spatial frequency, which may have been a costly oversight by the rest of the community.

**Image Recognition**

There were important developments in relevant algorithms in the 70’s, though mostly not inspired directly by the requirements of chromosome recognition. Probably the most significant of these was the development of the subject of “Mathematical Morphology” at the École Supérieure des Mines (Fontainebleau, France). Mathematical morphology, as a calculus of local operators, had many precursors—perhaps most notably the “Golay Logic” developed by Preston and Golay in the 60’s [15]—nevertheless Serra and Matheron’s systematisation and tremendous development of the subject were most important [16, 17]: in particular, in its digital
form, it provided a mathematically rigorous basis for, for example, the theory of skeletonisation, which is a basic tool in chromosome recognition. Mathematical morphology was also used, perhaps not wholly to advantage, as the operating principle for the TAS machines developed for cytometry by Ploem, Meyer and others [18, 19] at Leiden.

Of a very different nature, also important in the context of chromosome analysis, was the work of Chris Taylor and colleagues at the University of Manchester who developed what they called a point-set based image processing calculus, one of whose objectives is the same as that of the interval-coding representation mentioned earlier: i.e. the achievement of convenience and economy of processing by formal separation of aspects of representation concerned with pixel-density values from those concerned only with domain outlines, enabling either the one or the other to be taken into consideration as appropriate. What is particularly important about Taylor's work is that it was the basis for the design in hardware of Joyce Loebl's "Magiscan" range of machines, which played a signal role in cytogenetics automation [20].

Another algorithmic development at the time which has perhaps not been accorded the recognition which it should have been, was the work by Brenner and colleagues at the New England Medical Centre, of a method of separating touching objects by tracing out minimal cost paths with appropriate definition of cost function [21]. This of course is very relevant to the fundamental problem of separation of composite groups of touching chromosomes, and distinguishing them from dicentrics.

Gallus, working first in Milan, later with Neurath at the New England Medical Centre, had another look at boundary curvature analysis, using this time not the syntactic style of Ledley but a more quantitative approach which has been used in one form or another by many workers since [22].

The decade also saw a number of publications outlining schemes for recognition of aberrations in chromosome spreads. Amongst these were papers by Agarwhal and Fu [23], who discussed the recognition of aberrant chromosomes by the use of feature statistics.

Automatic slide preparation

Melnyck, at the City of Hope Hospital in Los Angeles began work on an automatic culture and staining machine, which took a long time to mature. It was eventually evaluated in Chicago in the 80's; but by then it seemed too cumbersome, inflexible and difficult to maintain and was eventually abandoned [24].

Flow

In 1974 flow systems were used to measure and sort isolated chromosomes for the first time [25]. In a paper in the 1975 Asilomar Workshop on Automated Cytogenetics, van Dilla, Carrano and Gray (Lawrence Livermore Laboratory) reported, inter alia, experiments carried out on irradiated Chinese hamster chromosomes, which showed that the chromosome-emission peaks in the flow histogram broadened and flattened with increasing radiation dose [26]. They also proposed the construction of a slit-scan flow cytometer for centromere detection. Dosimetry by histogram degeneration was pursued further by the Livermore group [27] and later by Green and Fantes at Edinburgh [28] and others. But it turned out that histogram perturba-
tion did not lend itself to dose-response calibration; and although highly accurate measurements of karyotypes and certain mosaics could be obtained, individual aberration events (at low frequencies) were in effect always swamped in the noise of debris on the one hand and adhering groups of chromosomes on the other.

Flow systems are an attractive option for aberration scoring, because of the very high data rates that can be achieved; but, often described as zero-(spatial) resolution devices, they normally cannot achieve the degree of shape recognition required for recognition of aberrations. Aiming at improved spatial resolution, Wheeless in 1975 embarked on what was to be a long-running program in the construction of slit-scan and image-capture flow devices [29], others likewise (see: 1980s).

**ALTERNATIVE BIOLOGICAL SYSTEMS**

**Micronuclei**

Micronuclei are small, generally round, conspicuous objects found in the cytoplasm of cells, outside the main nucleus, which they resemble in their properties. They form during division of cells from acentric fragments or aberrant chromosomes which fail to attach to the mitotic spindle and are left behind during anaphase. This results in the formation of miniature self-contained cell "nuclei". Normally they share the cytoplasm of the daughter cells, but should the cytoplasm be disrupted by hypotonic overexposure, they may become separated from these cells. Both clastogenic agents (i.e., agents which induce the formation of chromosome aberrations) and mitotic inhibitors can bring about the formation of micronuclei. Determination of the ratio of the number of micronuclei formed to the number of cells undergoing division can constitute a simple and fast method of evaluating chromosome breakage and spindle apparatus disturbances in routine genotoxic screening.

Their use for biological dosimetry was first proposed at this time by Heddle [30] and Schmid [31]. Attempts have been made ever since that date to automate their scoring; but despite encouraging starts the problem remains with us to this day (see 1990s, below).

**1980's†**

**Signal acquisition**

The 80's saw the emergence and eventual ascendance of the solid state Charge-Coupled Diode (CCD) camera—that is, cameras using the same technology as microchips, rather than vacuum tubes and electron guns. Beginning as single line cameras which increased in size from 256×1 up to 4096×1; they then progressed to rectangular arrays, which by the end of the decade were available in inexpensive units in 512×512 formats, at intermediate cost at 630×720, and at a price, in formats of 1024² and above. These give great geometric accuracy and reproducibility, good dynamic range and linearity, and, particularly if cooled to overcome dark-current problems, are suitable for use in long integration mode for low-light level signal acquisition.

† see Table 4.
### Table 4a. Available support technology: 1980's

**ELECTRO-OPTICS**

| Technology                              |
|-----------------------------------------|
| CCD (microchip technology) solid state cameras |
| Digital-output cameras                  |
| Megapixel cameras                       |
| Low-light cameras                       |
| Confocal microscopy                     |

**ELECTRONICS AND COMPUTING**

| Technology                              |
|-----------------------------------------|
| Sub-microsecond cycle times             |
| Mb memories                             |
| Gb discs                                |
| ‘off-the-shelf’ frame-speed interfaces |
| numerous IP boards                      |
| turnkey parallel systems                |

**LANGUAGES/OPERATING SYSTEMS**

| System                      |
|-----------------------------|
| Unix/VMS                    |
| OS9/DOS/Flex                |
| MS Windows/X windows        |
| C, C++ widely available     |

**ELECTROMECHANICAL**

| Technology                              |
|-----------------------------------------|
| Laboratory robot technology well-established |
| Quality stage drives (Merzhauser)        |

**HUMAN COMPUTER INTERFACE**

| Technology                              |
|-----------------------------------------|
| Window-menu-mouse control commonplace  |

**HARD-COPY**

| Technology                              |
|-----------------------------------------|
| Xerox and photographic style laser printers |
| Colour laser and thermal printers and copiers |

### Table 4b. Developments in Associated Disciplines-1980's

**MOLECULAR BIOLOGY**

| Technology                              |
|-----------------------------------------|
| U.S. national and other gene library projects |
| In-situ hybridisation techniques well-established-demonstrated in paraffin sections, amnions |
| Heidelberg, Leiden, Livermore            |
| Simultaneous multi-probe multi-colour systems |
| Harvard, Heidelberg, Livermore, Leiden   |
| Chromosome Painting                      |

**DOSIMETRY**

| Technology                              |
|-----------------------------------------|
| Fenech and Morley                       |
| Cytokinesis-blocking for micronucleus assay |
### Table 4c. Developments in Automated Cytogenetics, 1980's

#### IMAGE ALGORITHMS

| Granum, Piper (MRC Edinburgh)/Lörch (Metasystems, FRG) | WD functions for band-pattern classification adaptive centromere classifiers for dicentric detection |
| Ji Liang (MRC Edinburgh) | composite recognition by hypothesis-and-test, features, paths |

#### COMMERCIAL CHROMOSOME ANALYSIS SYSTEMS

| Taylor, Graham, Lundsteen, Joyce Loeb | 1'st Clinical Laboratory system "Magiscan", for Copenhagen Rigshospitalet (JPL concept) |
| Piper, Rutovitz, Shippey | prototype 'Fast Interval Processor', marketed by Image Recognition Systems as "Cytoscan" |
| Castleman | 'Perceptive Systems' machine and company. |
| Zeiss, Leitz, Kontron, Athena, others | Misc. Commercial Machines |
| Chromoscan, Applied Imaging, others | low cost PC based "electronic scissors" systems |

#### METAPHASE FINDERS

| Magiscan | spatial frequency detection (hardware)+other features |
| 1981 | ★ Performance ..... |
| | ● 1 cm²/(6 mins) |
| | ● FP/FN ratios acceptable |
| Cytoscan | ACP detection (hardware), computer clustering of ACP's; line array with autofocus device |
| 1983 | ★ Performance ..... |
| | ● 1 cm²/(35 secs) |
| | ● FP/FN ratios acceptable |

#### SPECIMEN PREPARATION

| Vrolijk, Korthof et al (Leiden) | Programmable culture, stain and slide deposition system |

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Table 4c. Developments in Automated Cytogenetics, 1980's, etc.

#### FLOW SYSTEMS

| Cremer, Cremer et al (Heidelberg) Aten (Amsterdam) | experimental scoring of hybrid 'painted' translocations by slit-scan system |
| Langlois, Bigbee (Livermore) | glycophorin assay for radiation dosimetry |

#### MICRONUCLEI

| Romagna and Staniforth | automatic system for erythrocyte micronucleus assay |
Computing
The 80's also saw a progression from microsecond to nanosecond cycle-time processors and memories, at affordable prices. By the end of the decade 8–16 Megabyte memories had become a commonplace on workstations, as also gigabyte-measure magnetic and optical discs.

Hard Copy
Good hard copy reproduction is critical to the achievement of acceptability in clinical karyotyping: the 80's was the decade of emergence of the relatively cheap 300 DPI laser-xerographic printer, but the "dotty" appearance of dithered-grey scale representation has militated against their use in this context. Two other solutions are employed: relatively cheap thermal printers giving analogue video reproduction, and rather expensive digital film-writers giving near photographic quality.

Operating Systems
Unix became firmly established as the system for workstations: OS9, which is in some ways Unix-like, but more efficient in use of resources, and better adapted for real time working, was adopted for the Cytoscan. Also, C (to some extent, later C++) emerged as the de-facto standard for image processing (for better or, in some people's view (not shared by the author) for worse). DOS, IBM's operating system for PC's was perforce used for the PC-based systems, with considerable disadvantage.

Man-machine interface
Mouse-driven interaction and windowing systems became established, and the one or the other or both are now almost universal in cytogenetics systems. Microsoft Windows in particular was used as the basis for interactive control of some of the low-end karyotyping systems.

Slide-based karyotyping
As already noted, Castleman had during the 70's developed an interactive karyotyping system at the Jet Propulsion Laboratory. The system included a metaphase finder; from a list of cells found, high resolution television-camera metaphase images could be acquired under operator control. The machine would attempt to analyse the image, but rely on the operator to correct any errors of segmentation, centromere positioning or classification on a screen with light pen. Finally, a karyogram would be printed. This machine served as a model for many systems for clinical cytogenetics developed in the early 80's. The first company to build a commercial device of this type was Joyce Loebl (Gateshead, UK) inspired by and under contract from Lundsteen in Copenhagen. The same route was soon followed by others, such as Castleman's own Perceptive Systems, and Kontron. Image Recognition Systems were the first in the field with a fast effective metaphase finder (the MRC device already referred to) and also the MRC interactive karyotyping system. There was a flurry of activity in the installation of systems for general cytogenetics, especially ante-natal diagnosis, beginning in the middle eighties and continuing to the present day.
**Automatic Preparation Systems**

Vrolijk and colleagues at Leiden investigated a programmable machine for culturing and harvesting of metaphase preparations. As with the machine by Melnyk [13], experience was gained but there was no practical outcome [32].

**Slide-based aberration scoring**

The emphasis in aberration scoring systems had shifted decisively away from the concept of automatic detection of aberrations towards instruments in which it was intended that a metaphase finder would be coupled to an interactive system of scoring the spreads once located. At the start of the decade a number of machines (such as the Quantimet 720) were on offer by commercial suppliers, but performance left much to be desired. It was not until 1983 that a metaphase finder with an interactive reporting system was installed and working effectively in a high-throughput dosimetry laboratory (MetaFip). This was a successor to Green's MRC machine, in fact a prototype version of what later became the Cytoscan (Image Recognition Systems and Applied Imaging). This machine was installed and tested in Lloyd's National Radiological Protection Board Laboratory in Didcot, UK and has been in continuous use ever since. Its search speed was $1 \text{ cm}^2/25 \text{ sec}$, 90% of the objects found (in 'good' homogeneously stained material) were scorable metaphases, and only a small proportion of usable cells was missed. Previous to MetaFip, speed had been obtained by use of at least some analogue pattern analysis. MetaFip converted the output of a line camera to digital form at the very beginning of the processing chain. Custom electronic logic was then used to produce an "interval coding" representation of the image: that is, the original 8-bit pixels were replaced by information about "intervals"—above background sections of scan line (in a manner developing the work of Butler and Stroud in 1963, and which has been reinvented by many others since [5]). This reduces a 6 MHz pixel stream to a much more manageable 10–50 KHz interval stream; a fast (for its day) general purpose micro-computer, the Plessey Miproc was then used to articulate the intervals into metaphase clusters. Interactive recall and reporting was also extremely effective: using the automatic relocation facilities of the device, Lloyd's staff could score metaphases, once located, at rates of up to 100/hour (unfortunately, due to various alterations to both hardware and software, Cytoscan proper never quite achieved the speed of the original) [33].

Following the installation of machines for interactive karyotyping, interest developed in adapting them for aberration scoring, and most of the metaphase finder/karyotyper combinations now offer interactive scoring as an option. The middle 80's metaphase finders were rather slow (typically an hour or so a slide) except for Cytoscan (under 5 minutes per slide). Lately this performance gap has closed, although it has not entirely disappeared: instruments from Perceptive Systems and Joyce Loebl for example, can now search a slide in between 15 and 30 minutes. However, human interaction takes at least 30 seconds per metaphase, so a slide containing 100+ scorable cells will not be processed in under an hour and a half. It follows that metaphase finding overnight on an eight slide capacity magazine, leaving the cells to be scored during the day, can be a satisfactory way of working; but if the case load justifies it, a fast metaphase finder can serve several satellite reporting stations.
The technology employed in these instruments has of course evolved in step with the general advance of electronic technologies and computing. It is a curious anomaly that earlier machines (the Preston/Wald device, the MRC/IRS Cytoscan) have been faster than later ones. The reason of course is that the later machines use less, or no, custom built hardware, whereas the Wald/Preston machine for example was all hardware (and analogue at that). Lörch (Metasystems GmbH) has now developed a metaphase finder which uses just one special purpose image processing accelerator board, and for the rest uses a standard IBM 386 machine, and yet can scan a six square centimetre coverslip in under 15 minutes. The Cytoscan in contrast has an elaborate preprocessor containing at last count, 12 custom made boards.

In sum, past development has resulted in machines with good metaphase finding capability, and efficient interactive scoring regimes, whose combination can double or treble the scoring-throughput of a laboratory concerned with radiation dosimetry. Such machines are now in routine use at a number of centres carrying out either radiation dosimetry or accounting for other forms of environmental damage.

Flow Systems

In parallel with the 1980s development of interactive slide-based scoring systems, there was a great interest in attempting to apply the 1970s developed flow technology to this problem. In the preceding decade Lawrence Livermore Laboratory [27] showed that simple measures of histogram quality, such as standard deviation of peaks fitted to chromosome size histograms, could be shown to be dose-related. However, despite determined attempts by Green [28] and others, it proved difficult to translate such measures into dose response curves that were not particular to individuals, and also to reach into lower exposure ranges. Efforts then turned to

Recognise dicentrics? What's the problem?

Chromosome  dicentric  Composite  ???

Chromosome with secondary constriction

Fig. 1a.
the development of flow systems with a degree of shape resolving capacity, sufficient to enable centromere detection, with the hope that this would in turn lead to methods for dicentric recognition. Several effective slit scan systems were developed, and targeted at dicentric counting [34]. But the results remained somewhat ambiguous, and again low dose range
sensitivity did not seem to be achievable: the problem of distinguishing pairs of touching chromosomes and other artefacts from the small number of true dicentrics present in low-dose specimens remained intractable (see Fig. 1a).

Other Biological Systems

Micronuclei

Micronuclei have been looked at long and hard as a means of dose evaluation, and systems have been and are being developed specifically for micronuclei scoring. There seems however to be some considerable difficulty here also, on the one hand as regards the relationship with radiation dose, and on the other, the development of a technique of preparation and staining which is practicable for a routine laboratory and which results in micronuclei and associated divisions recognisable to an image processor. For standardisation of results in lymphocytes, it is important to restrict scoring of the aberration-ratio to cells in the first division cycle after exposure, because the proportion of aberrant cells is likely to decrease by a factor of two at each cycle. In 1985, Fenech and Morley found that the addition of Cytochalasin-B to the culture medium prevents cells from completing the division cycle by inhibiting the separation of the cytoplasm of the daughter nuclei. By counting only binucleate cells and micronuclei associated with them one can then ensure that analysis is restricted to cells which have undergone only one division cycle since addition of Cyt-B.

The use of Cyt-B also simplifies the task of automatic scoring: since micronuclei of interest must lie within the cytoplasmic envelope of binucleate cells, automatic scoring can proceed in two phases. The first comprises the location of binucleate objects; the second, the identification of micronuclei within their cytoplasm. The count of the former gives the denominator, the count of the latter the numerator, of the desired ratio [35]. However, the problem of segmentation of the micronuclei from the cytoplasmic envelope of the binucleate cells is by no means trivial, and requires the preparation process to deliver good cytoplasm morphology. Another difficulty is that it seems that Cyt-B itself induces micronuclei formation [36].

Micronuclei evaluation in erythrocyte systems seems to be a solved problem, following the development by Romagna of a multi-colour stain system: several effective systems for automation are now available [37].

Glycophorin-A

The state-of-the-art in this approach to dosimetry, developed by Richard Langlois and William Bigbee at Lawrence Livermore Laboratory during the 80's [38], is discussed in the 1990s section, below.

1990's (late 80's, early 90's)\textsuperscript{7}

In the last few years there has been a resurgence of activity in this field, inspired in part by the availability of power to price ratios in computer equipment orders of magnitude better than

\textsuperscript{7} see Table 5.
anything previously seen, thereby putting powerful analytical capabilities within reach of even modestly endowed laboratories. Another reason for renewed interest lies in the possibilities for exploitation of the ever-growing repertoire and steady improvements in the reliability and accessibility of multi-colour molecular probes. Finally, the Chernobyl disaster has refocussed attention on the importance of rapid evaluation of received dose.

**Absorption-based scoring of unstable aberrations**

The advance of electronic technology has been such that it is now possible to process a metaphase spread after initial segmentation has been carried out, on a 25 MHz PC costing a few thousand dollars, in 5 seconds or so. If the segmentation task is also included, allowing indeed the maximum degree of sophistication in current use in karyotyping systems for this purpose, a similar time can be achieved on, say a Sun Sparc2, costing about $25K dollars. Fast metaphase finding still requires the use either of an image processing accelerator board (as in the Metasystems finder), or rather more computing power. I estimate that to attain Cytoscan speed (approximately 1 cm²/40 secs) about 250 million integer instructions per second are required. This is beyond the range of a 386PC, but can be achieved without vast expense by either a transputer based or other parallel hardware machine; and will certainly be achieved by the next generation of work station (the HP 700 series already offers more than 80 Mips, which should be enough for a search speed of 1 cm²/120 secs, sufficient for most purposes).

Two systems now exist which effectively exploit this access of computing power, the one by Lörch at Metasystems [38, 39], the other by MRC Edinburgh. Both have demonstrated (table 6) a capacity for fully automatic metaphase finding, image acquisition and cell analysis. Operator interaction is still required, but only to confirm which of a list of candidate dicentrics are in fact true dicentrics. Metasystems have demonstrated a system capable of scoring at the rate of about 500 cells/hour, and MRC have tested all elements of a similar system, though not on the final hardware. A joint study by the Edinburgh team and Wald’s Pittsburgh group has shown that a good dose response relationship can be obtained, and that this can be inverted to

| Table 5a. Available support technology: 1990's |
|------------------------------|
| **ELECTRONICS and COMPUTING** |
| <.05 µsec instruction times | >16 Mb memory |
| Gb magnetic discs<$3000 | Gb optical discs<$1000 |
| **ELECTRONICS-OPTICS** |
| Digital output CCD cameras, fields>1000×1000 |
| Cooled CCD low-light cameras | Image intensified cameras |
| Wide variety image processing boards |
| **ELECTROMECHEANICS** |
| Robotic components and assemblies readily available |
Table 5b. Developments in Automated Dosimetry, 1990's

**SLIDE-BASED ABERRATION SCORING**

| Developer                                    | Description                                                                 |
|----------------------------------------------|-----------------------------------------------------------------------------|
| Lörrch (Metasystems)                         | metaphase finder, automatic digitisation cycle, auto determination of dicentric: chromosome ratio, interactive selection of true positives  |
|                                              | ★ Performance:                                                              |
|                                              | ● metaphase finder: 1 cm²/(100 secs)                                        |
|                                              | ● 5 secs/metaphase cell, digitisation and analysis                           |
|                                              | ● detection sensitivity ca. 40% of dicentrics                                |
|                                              | ● false positives: 1-2% of chromosomes                                      |
| Piper, Ji, Stark, Rutowitz (MRC)             | metaphase finder, automatic digitisation cycle, auto determination of dicentric: cell ratio, interactive selection of true positives |
|                                              | ★ Performance:                                                              |
|                                              | ● metaphase finder: 1 cm²/(25 secs)                                        |
|                                              | ● 20 secs/metaphase cell, digitisation and analysis                         |
|                                              | ● detection sensitivity ca. 40% of dicentrics                                |
|                                              | ● false positives: 0.5% of chromosomes                                      |

FLOW

| Researcher                                | Description                                                                 |
|-------------------------------------------|-----------------------------------------------------------------------------|
| Lucas, Mullikin, Gray (Livermore)         | Definitive experiment on slit-scan dicentric detection (no probes)           |
| Straume, Langlois, Bigbee et al           | Extensive demonstrations of Glycophorin-A based flow assay of radiation exposure |

MICRONUCLEI

| Researcher                                | Description                                                                 |
|-------------------------------------------|-----------------------------------------------------------------------------|
| Yamamoto, Hayata, Yamada et al (Chiba)    | effective automated erythrocyte micronucleus assay                          |
| Tates, van Weille, Ploem (Leiden)/Ji (MRC Edinburgh) | Cytochalasine blocked lymphocyte system components tested                   |

PREPARATION SYSTEMS

| Researcher                                | Description                                                                 |
|-------------------------------------------|-----------------------------------------------------------------------------|
| Hayata et al (National Inst. Radiological Sciences, Chiba) | automatic culture and deposition on slide in controlled environment |

Table 6. Dicentric Scoring Rates

The rates given here are percentage true positives (TP) found in a known set of dicentrics, and false positive rate (FP) measured per 100 cells.

| Group          | TP  | FP   | Reference           |
|----------------|-----|------|---------------------|
| Edinburgh      | 52* | 11   | Piper et al (1988)  |
| Edinburgh      | 40  | 30-50| Bayley et al (1991) |
| Neuherberg     | 84**| 34   | Lörrch et al (1989) |
| Neuherberg     | 50  | 46   | Lörrch et al (1990) |

(*) Manually segmented data set
(***) Excludes unsegmented dicentrics in data set
estimate dose from yield; table 7 and figure 2 summarise the experimental data and results [41].

For the first time therefore something approaching an automated system, as distinct from a computer aided one, has been produced for cytogenetic applications. Whereas in diagnostic cytogenetics the best that can be expected from current systems is probably a 2 to 1 gain in productivity, the new automated aberration scoring systems will bring at least a ten-fold gain. When fully operational either of these systems should allow the scoring of about 10,000 cells a day, with the help of a single operator. There are some reservations: firstly, these systems are not able to detect very small dicentrics, nor dicentrics where the two centromeres are very close together, nor, for the most part, can they handle acrocentric dicentrics. They are not able to detect other classes of aberrations. In consequence, the yield is 40–50% of that of a human operator engaged in dicentric scoring, and lower again than that which would apply if we asked the operator to score all aberrations. The effect of lower yield is to require a greater number of cells to be done, but my calculation of a tenfold gain in operator productivity takes that into account.

**Flow**

Lucas and coworkers at Livermore have conducted what seems a definitive experiment on low dose range evaluation using slit-scan dicentric scoring. They showed that the necessary specificity can be obtained, but only at the cost of a swinging reduction in sensitivity; in order to exclude touching pairs from being classed as dicentrics, very stringent acceptance conditions had to be imposed [42]. Their results are summarised in the table 10: Current Systems Compared. The conclusion seems to be that, although it can be done, the loss in sensitivity, and the care that has to be taken in preparation and setting up, may make this route less attractive than that

### Table 7. The Edinburgh-Pittsburgh Dicentric Scoring Experiment

**The data, and detected dicentrics**

The data is comprised of two calibration sets ("Immediate" and "48 hour") and a test set. The "dicentrics" row gives, in each case, the number of operator-confirmed dicentrics detected by the machine.

| Dose (rads) | 0  | 10 | 20 | 50  | 100 | 150 | 200 | 300 |
|-------------|----|----|----|-----|-----|-----|-----|-----|
| Cells       | 319| 500| 1068| 790 | 733 | 515 | 668 | 476 |
| Dicentrics  | 0  | 3  | 1  | 7   | 12  | 12  | 34  | 46  |

| Dose | 0  | 10 | 20 | 50  | 100 | 150 | 200 | 300 |
|------|----|----|----|-----|-----|-----|-----|-----|
| cells| 221| 1432| 2406| 964 | 982 | 941 | 1802| 713 |
| dicentrics | 0  | 1  | 6  | 8   | 24  | 56  | 173 | 112 |

| Dose | 0  | 10 | 25 | 50  | 100 | 150 | 225 | 375 | 450 | 600 |
|------|----|----|----|-----|-----|-----|-----|-----|-----|-----|
| Cells| 989| 954| 825| 1131| 808 | 818 | 558 | 835 | 572 | 419 |
| Dicentrics | 0  | 0  | 9  | 15  | 18  | 35  | 35  | 130 | 154 | 152 |
now offered by slide based systems. However work continues (by, amongst others, Cremer and Cremer and Aten at Heidelberg and Amsterdam) and the possible contribution of newer markers (below) to this type of system has yet to be examined.

**Other Biological Systems**

**Micronuclei**

Solutions to the problems of automated scoring of micronuclei in lymphocyte preparations now seem to be in sight: table 8, Counting Micronuclei in Binucleate Cells, presents recent
results by Ji [MRC, Edinburgh] on material from the laboratory of Dr. M. Boavida, Lison. Similar results have been obtained by Walle, Ploem et al in Leiden [43].

### Table 8. Counting Micronuclei in Binucleate Cells
(results of Ji, Edinburgh/Boavida, Lisbon)

| Binucleate Cell Finding (5 slide test) |  |
|---------------------------------------|--|
| Found manually | Found by machine |
| 202 | 128 (63%) |

| Micronuclei scoring (4 slide test) |  |
|------------------------------------|--|
| Found manually | Found by machine |
| 85 | 61 (73%) |

### Table 9. GPA-M/N variant Scoring System
(Straume et al. Livermore)

| Preparation |  |
|-------------|--|
| • formalin fixed “sphered” erythrocytes (red blood cells) |
| • overnight fixation |
| • 4 centrifugation wash cycles and resuspensions |
| • 1 hour immunolabelling |

| Cytometer |  |
|-----------|--|
| • FACScan single-laser flow cytometer (Beckton) |
| • Gating: Forward and side scatter |
| • Speed: 3000–4000 cells/sec |

### Probe Separation

| Mean Position (Log10): | red channel 6A7-B/AV/PE | green channel BRIC157-F |
|------------------------|--|-------------------------|
| MN | 3 | 2.7 |
| MM | 3.3 | 1.7 |
| NN | 0 | 3.1 |
| NO | 0 | 2.8 |

| Count CV’s (%), 10⁶ cells normal donors | Theoretical (Poisson) | Actual |
|----------------------------------------|--|---------|
| NN | 15 | 19 |
| NO | 18 | 30 |

### Gozyana Incident

| | NN, NO counts | MN count | Time |
|-----------------|---------------|----------|------|
| 1 case, exposure >1 Gy (?) | 176 | 5×10⁶ | 25 minutes |
| Control | 192 | 24×10⁶ | 114 minutes |
Glycophorin-A

Glycophorin-A (GPA) is a cell-surface protein occurring in about $10^5$ copies in human erythrocytes. Two forms are found, the M- and N- types. About 50% of the population is heterozygous for the two forms, half the cells carrying one allele, half the other [44]. If a deletion or other transformation resulting from ionising radiation (or other mutagenic agency) should inactivate or remove a portion of a chromosome carrying one of the loci, the heterozygosity will be lost. Monoclonal antibodies are available for both forms: Pharmacia’s GA7 antibody recognises the M-form, the UK Blood Transfusion Service’s BR1C157 recognises the N-, albeit with a lesser specificity. The former, conjugated with biotin can be used with streptavidin phycoerithrin to give a red signal of approximately equal strength to that of a green signal obtainable from the latter antigen directly conjugated to fluorescein isothiocyanate. This enables a flow-cytometer to distinguish unambiguously between MN-cells and NN and NO-cells (due presumably to the lesser specificity of the BR1C157 antibody, the MN-/MO- distinction is somewhat less effective). For a given mutagenic insult, the frequency of deletions at any particular locus should be similar to that of any other two-event phenomenon involving that particular part of a chromosome: probably about two orders of magnitude less frequent than, say, dicentric chromosomes. But since a flow cytometer running at 3–4000 cells/second can be used to evaluate the proportions of homozygous cells in a heterozygous individual, the relative loss of sensitivity is easily made good [45]. These authors have been able to show a strong exposure related effect, with the mean Variant Frequency (VF) in normal donors $6.2 \times 10^{-6}$.

### Table 10. Current Systems Compared

| System                          | Machine                  | Sensitivity | Cells | Slides | Time (hours) |  |
|---------------------------------|--------------------------|-------------|-------|--------|--------------|---|
| Homogenously stained Dicentrics | Mechanised Stage, Autofocus, Fast metaphase finder, Automatic image acquisition, Efficient interaction, 5 sec/cell duty cycle | 0.5         | 3400  | 17     | 5            | 3 |
| Painted Chromosomes, as Straume et al | 50 sec/cell cycle ??     | 0.3         | 5600  | 28     | 31           | ?? |
| FPA M, N Variants               | Flow Cytometer           | 5×10⁶       | 0.5   | 0      | 72           |   |
| Suspension, PI stained          | Flow Cytometer           | 0.0033      | 500,000 | 2    | 0           | ?? |

**Assumptions**
1. true rate of dicentric and translocation occurrence is 6 per 100 cells
2. 200 usable metaphases per slide (conservative)
3. painted translocation detection rate 30%: Straume’s paper indicates approx 35% dicentric detection possibility, allowing 5% machine failure (optimistic) gives 30%
4. capture of multicolour fluorescent image estimated to take 20 seconds (possibly optimistic)
with an observed CV of 30 or so (as against 18 predicted by a Poisson model). In a study of a number of individuals exposed in the Goiania incident, 132 NO events were scored in a count of $10^6$ cells in an individual thought to have been exposed to over 1 Gy, with correspondingly elevated counts in others. Table 9 summarises some of the GPA assay findings of the Livermore authors [46].

Unfortunately, the opposite transformation of homozygous to heterozygous cells requires a point mutation, not a deletion spanning the area, and is several orders of magnitude less likely. In consequence, this interesting assay is at present useful only in the case of heterozygous individuals. Further, although dramatic elevations of variant frequency have been demonstrated, there seems as yet to be too much variability to permit determination of a dose response function.

**FLUORESCENT IN-SITU HYBRIDISATION**

*Chromosome Painting*

In the 60's, engineers working on chromosome recognition used to joke about chromosome painting: if only the biologists could paint each one a different colour, how much simpler it would all be... In the late 80's we woke up to discover that it could be done. The principals in these developments have been (with many colleagues) Lichter and Ward (Yale) [47], Gray and Pinkel (Livermore) [48] Landegent and van der Ploeg (Leiden) [49] and Cremer and Cremer (Yale and Heidelberg) [46, 49]. The importance of the development to dosimetry is that it offers for the first time the possibility of rapid conventional scoring of stable aberrations (translocations in which total DNA is conserved), and obvious possibilities for automation: a translocation involving chromosomes 'painted' in different colours should announce itself clearly enough (Fig. 1b), subject to some reservations about the possibility of overlapping chromosomes being confused with positive. The general idea in chromosome “painting” (which could perhaps more accurately be termed “stippling”) is to find a large number of probes which hybridise to sites on a particular chromosome and no other; then if a suitable fluorescent reporting system is added to the probe before hybridisation, the chromosome, when illuminated with light of wavelength in the excitation spectrum of the dye, will “light up” along most of its length. To make a painting probe, the chromosome itself if used as a template: the process usually begins with physical isolation of the chromosome, e.g., by flow sorting. The next step is to cut it into small fragments, which may be done using enzymes which attack and break the DNA at numerous points. The fragments must now be multiplied up: usually this is done by inserting them into vectors, “plasmids” (small ring-shaped bacterial DNA molecules) which can be cultured in profusion. A more recently introduced alternative to the above approach is based on the use of “primers”—short repetitive DNA sequences—which are known to match to sites distributed regularly through the DNA of the chromosome. It is possible to cause transcription to begin at such primers and continue a short distance into the, usually, unique sequence DNA, flanking the primer. These transcribed fragments can then be multiplied up by PCR (the Polymerase Chain Reaction). By either approach, we obtain a probe “library”
capable of recognising all the sites of origin of the fragments, covering all of the original chromosome. However, the system as described so far won’t work too well, because many of the fragments will contain repetitive sequences of DNA which will not be specific to the chosen chromosome. This problem can be overcome by so-called “competition” or “suppression” hybridisation:—a probe is obtained from the DNA of the whole genome in much the same way as is the specific chromosome probe. This is now hybridised, unlabelled, in controlled amount, to the specific probe, after the latter has been labelled. Sites in the specific chromosome probe, essentially repetitive DNA, recognised by fragments occurring on more than one chromosome in the whole genome will be many orders of magnitude more strongly represented in the competitor probe: these will be effectively blocked by the competition hybridisation. Some of the probe fragments occurring on the selected chromosome will be blocked too, because they are of course represented in the whole-genome DNA: but most of the unique sequence sites, as distinct from the repetitive sequences, will still be available for hybridisation. On introducing the partially blocked probe into the target preparation, under correct conditions of annealing, the “painting” effect is found to be convincing and specific. There are still difficulties: for example, chromosome ends do not generally show well: but with increasing experience and attention to detail, the painting systems are becoming ever more effective. Probe libraries for most chromosomes are now obtainable from the US National Gene Library, and other sources. Pre-prepared, and in some cases, ready-labelled painting kits are planned to be marketed by Imagenetics (Chicago), Bohringer, and others.

Using painting probes to score stable aberrations

Probably, at least 3 or 4 different colours can be used simultaneously. Thus by dividing the genome into a number of different colour groups, it should be possible to pick up translocations rather easily by looking to where different colours abut. Some morphological understanding is still required because it will still be necessary, albeit somewhat easier, to differentiate between touching pairs of different chromosomes and actual translocations. Gray and co-workers at Livermore have now demonstrated convincingly that proportions of stable aberrations can be estimated well by use of a two-colour system [51].

Fluorescence probes to score unstable aberrations

Painting could likewise simplify the task of dicentric site detection, but it is not clear how much benefit would result in this case. The human eye can rather easily tell the difference in most cases between touching and translocated or dicentric chromosomes. The presence of different colours would facilitate distinction for both human and machine between a dicentric chromosome and one with a secondary constriction. But the major problem (for flow systems also), to which chromosome painting hardly contributes, is that of false clues occasioned by touching groups of separate chromosomes. Given the additional difficulties of scanning under fluorescence and simultaneous colour and morphology detection, experiment will be required to determine the gains, especially the time gains, which can be obtained.

The stringency requirements for obtaining reliable statistics of low-frequency events such as dicentrics have often not been fully appreciated: many of the proposed solutions (such as slits
can flow cytometry) tend to overlook the all-important question of how artefacts are to be distinguished from true positives, when the artefact frequency may greatly exceed that of the signals to be counted. Fig. 1 illustrates the problem, and shows how a solution could be obtained were strong and consistent telomeric probes available [52] for use in conjunction with existing centromere probes [53]: then centromeres could be marked in one colour and ends of chromosomes (telomeres) in another. This would not pick out balanced translocations, but would facilitate dicentric recognition, because the telomeric markings will enable an easy distinction between the composite group and the integral chromosome; likewise the centromere marker will simplify and reduce errors in centromere identification (Fig. 1c).

The present

Table 10 is an attempt to sum up the present position in terms of the investment of operator and machine time needed to score 100 events from a patient assumed exposed to a radiation source giving rise to about 6 dicentrics in 100 cells—about 1 Gy for most sources. It is apparent that there are now several routes by means of which such an evaluation can be done at least semi-automatically, at a reasonable cost in time and money. I infer from the table that the best present or soon-to-be available option is the slide based aberration scoring system for unstable aberrations: this fits best with established practice, offers prospects of substantial performance upgrade by use of in-situ probes and likewise the possibility of conversion to a translocation evaluation function. Currently, in the projected scenario, about 3 hours of interaction time, and perhaps 2 hours of preparation time would be required for this approach, plus some 5 hours of machine time. Because of lower sensitivity, “painted translocations” would certainly require a great deal more machine time (31 hours!); estimation of the interaction time requires experimental evaluation of the specificity of automatic analysis; and preparation would be considerably more arduous. Dicentric scoring by flow cytometer is another possibility: the sensitivity is very low and preparation exacting, but a careful evaluation of overall costs is warranted. The Glycophorin variant system is also interesting: apart from preparation costs, the obvious negative factor is the requirement that the subjects be heterozygous.

The future

There will soon be a choice of systems capable of carrying out the equivalent of a 5,000-cell conventional aberration scoring study which would currently take several weeks work to effect, in a matter of a few hours of machine time, and perhaps half an hour of operator's time. Several technologies will compete for attention: for unstable aberrations the traditional absorption/morphology based dicentric scorer will certainly be one of the competitors, for a time, perhaps for the next decade—the biological technique involved is simple and well understood, expensive molecular reagents are not required, and the throughput, at least in the short term is likely to compare favourably with anything which can actually be achieved by other systems. The introduction of telomere and centromere markers may well enable an improvement in the sensitivity and accuracy of the slide based techniques, bringing about a further reduction in the amount of interaction needed (which will also be reduced by software improvements) and increase the proportion of dicentrics which can be found by the systems.
Some alteration to the scanning technologies used will be necessary, even if absorption end points rather than fluorescent ones can be used for the markers, as at present such systems are essentially monochrome. There may well be a trade off also between increased sensitivity and the time taken to acquire multi colour images, which initially could offset the other gains. A further development which has been discussed but not explored in this connection is the possibility of using ‘chromosome soups’ rather than intact cells in slides. It would certainly be possible to lay down a fairly uniform monolayer of chromosomes on a slide, which would bring the likely chromosome processing rate with a modern megapixel camera into the region of 400/500 a second, comparable with that presently achieved on slit scan flow systems. Combined with multicolour painting and telomere/centromere marking, we might see another quantum leap in performance, in cases where scores per cell are not thought important. Automated and environmentally controlled preparation systems as being developed by Yamamoto, Hayata and colleagues [54] might also be expected to bring increased repeatability and specificity.

For stable aberrations, the bicolour painted-chromosome system used by Livermore [51] is likely to prove much more practical, as regards reliability to both preparation and analysis systems, than the multi-hued and colour-ratio systems proposed elsewhere [55] (colour-ratio proposals beg the question of distinguishing between overlaps of different colour objects and single objects carrying the intended colour ratios). For interphase-based scoring, I think we must look to micronuclei systems: the uncertainty of interpretation of even colour coded translocations in interphase is likely to preclude low dose work (but may be suited to counting clones of substantial percentage occurrence in tumour cytogenetics). In the longer run however I think we can expect that scoring of disturbances of gene-expression (similar to or reinforcing the glycopherin assay), or newer molecular based methods, will take over entirely from morphology and even from morphology plus colorimetry.

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