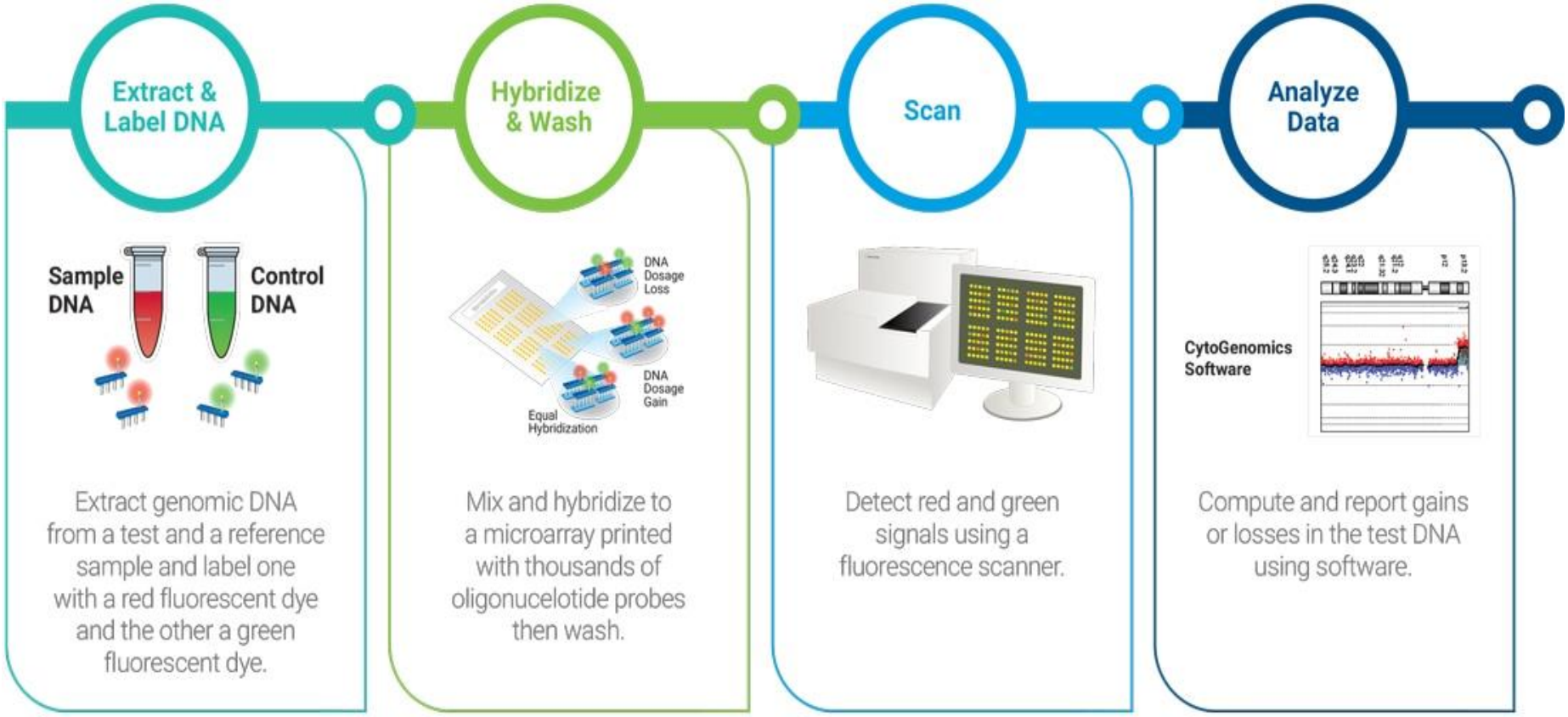
Comparative genomic hybridization (CGH)



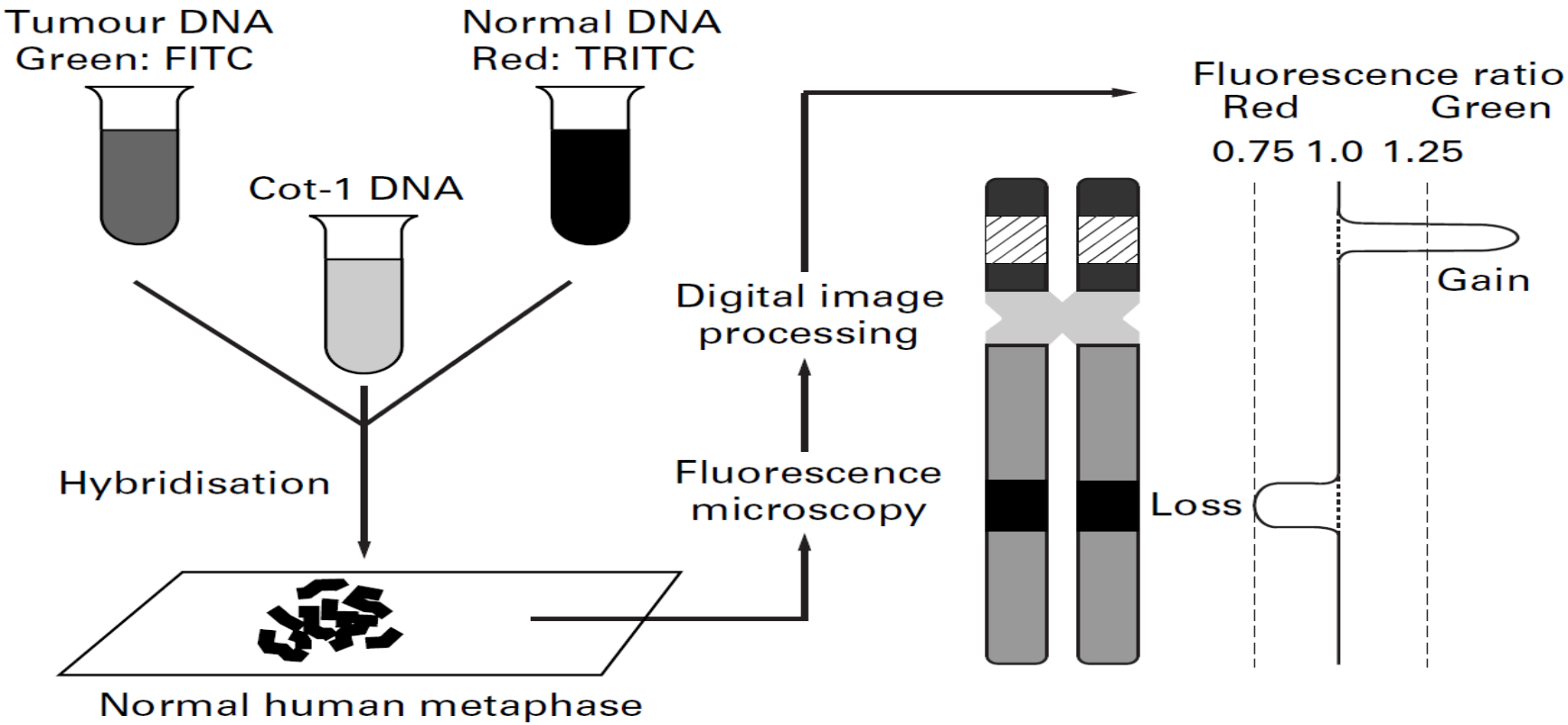
What is Comparative genomic hybridization (CGH) ?

Is a technique that permits the detection of chromosomal copy number changes without the need for cell culturing.

Kallioniemi et al at the University of California, San Francisco were the first to report CGH as a new chromosome analysis technique in 1992,1 shortly followed by du Manoir et al.

Principle of CGH

The principle of CGH is that, the tumour DNA is labelled with a green fluorochrome, mixed (1:1) with red labelled normal DNA, and hybridised to normal human metaphase preparations. The normal reference DNA and the metaphases are obtained from a healthy volunteer and do not need to be from the patient. The green and red labelled DNA fragments compete for hybridisation to their locus of origin on the chromosomes. The green to red fluorescence ratio measured along the chromosomal axis represents loss (ratio < 1) or gain (ratio > 1) of genetic material in the tumour at that specific locus.



Applications of CGH?

- Because **no cell culturing is required** for CGH, this technique has enabled tremendous progress in the analysis of chromosomal changes in **solid tumours**.
- Applied in cancer research include screening of tumours for genetic aberrations, searching for **genes involved** in the carcinogenesis of particular subsets of cancers, analyzing tumours in experimental models to obtain an insight into **tumour progression, diagnostic classification, and prognosis assessment**.
- Apart from these oncological applications, CGH analysis has also been used to study **chromosomal aberrations in fetal and neonatal genomes.**

Disadvantages of CGH

- CGH is a relatively **time consuming** and difficult technique with a number of limitations.
- It cannot detect structural chromosomal aberrations without copy number changes, such as **balanced chromosomal translocations**, **inversions**, or **ring chromosomes**
- It also has the disadvantage of being **less sensitive** than PCR based methods in detecting deletions.
- Theoretically, the loss or gain of one copy of a certain chromosome in a diploid genome should result in a fluorescence ratio of 0.5 or 1.5. However, in experiments comparing male and female DNA, where the fluorescence ratio for the **X chromosome** should be 0.5 or 2.0, these ratios are never found in practice.

Steps for preforming CGH

1. METAPHASE SLIDE PREPARATION

Metaphase chromosomes are prepared according to standard protocols using phytohaemagglutinin stimulated peripheral blood lymphocytes from a karyotypically normal man or woman. Because women have two X chromosomes and the Y chromosome does not contain much genetic information, it is preferable to use metaphase spreads from women.

2. DNA ISOLATION FROM TUMOUR TISSUE

Appendix 1

DNA isolation protocol for formalin fixed, paraffin wax embedded tissue (10 µm sections, haematoxylin stained) based on affinity columns (QIAamp tissue kit; Qiagen, Valencia, California, USA)

(1) If the tissue contains water, spin down for five minutes at maximum speed and decant the supernatant.

The next four steps are for unmicrodissected paraffin wax sections only (to remove the wax)

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(2) Add 1 ml xylene, incubate for 10 minutes at 55°C, spin down, and decant supernatant (×3).
(3) Add 1 ml methanol, incubate for five minutes at room temperature, spin down, and decant supernatant (×2).
(4) Spin down for five minutes and decant the supernatant.
(5) Add 1 ml 100% ethanol, vortex, spin down for five minutes, decant the supernatant (×2), and air dry the pellet.

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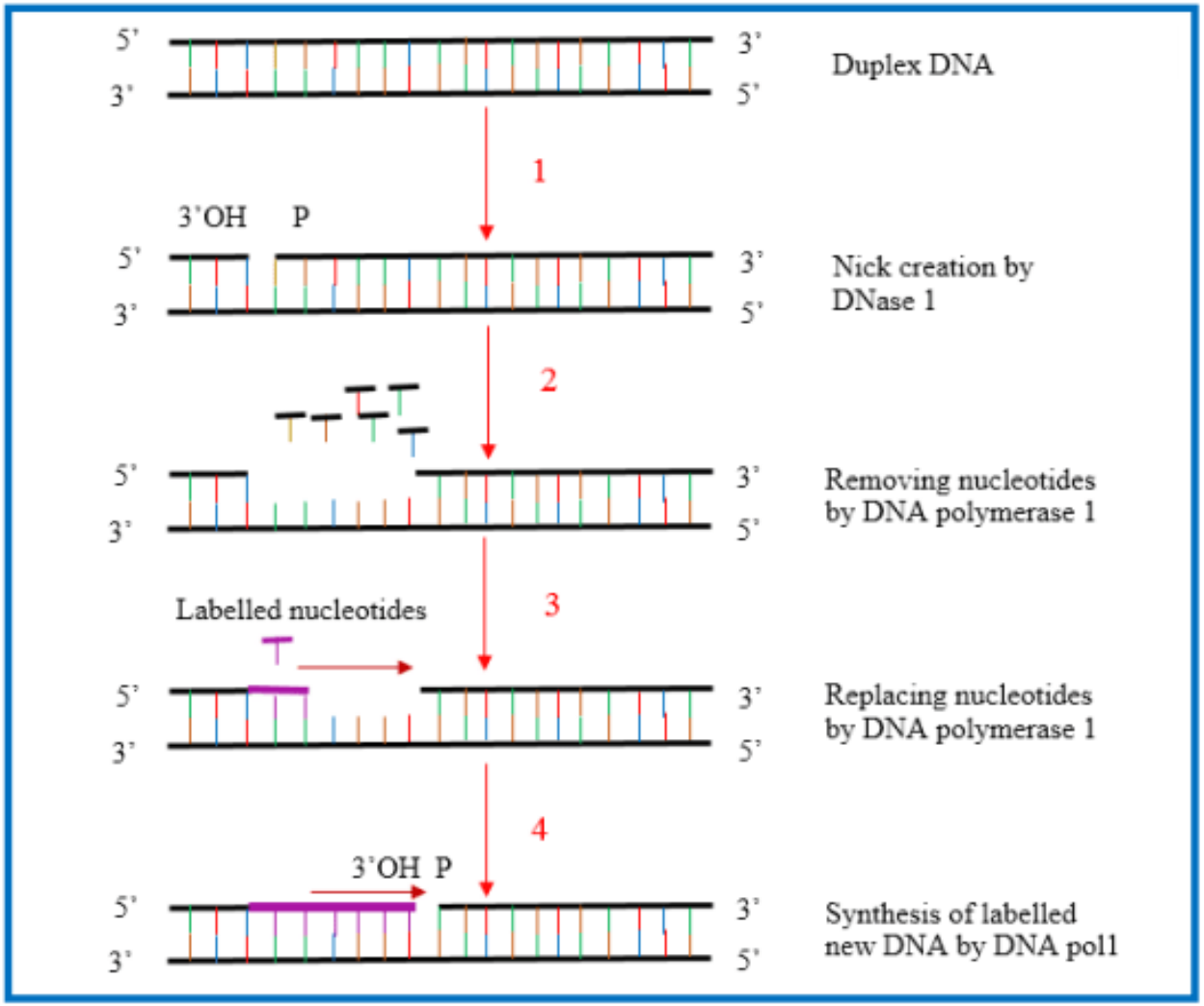
(6) Add 1 ml 1 M NaSCN and incubate at 37°C overnight.
(7) Spin down for five minutes and decant the supernatant.
(8) Add 1 ml phosphate buffered saline (PBS), vortex, spin down, and decant the supernatant (×2).
(9) Spin down for five minutes, decant the supernatant, and air dry the pellet.
(10) Add 200 µl ATL buffer and 40 µl proteinase K (10 mg/ml), vortex, and incubate overnight at 55°C.
(11) Add 40 µl proteinase K (10 mg/ml) and incubate at 55°C all day, vortex approximately every hour during the day.
(12) Add 40 µl proteinase K (10 mg/ml) at the end of the day, incubate overnight at 55°C.
(13) Add 40 µl RNase A (20 mg/ml), vortex, and incubate for two minutes at room temperature.
(14) Add 400 µl AL buffer, vortex, and incubate for 10 minutes at 70°C.
(15) Add 420 µl 100% ethanol and vortex well.
(16) Place a QIAamp column in a QIAamp tube, apply the total volume of the sample (in steps of maximum 500 µl) on to the column, spin for one minute at 3000 ×*g*, decant the filtrate.
(17) Add 500 µl AW buffer and spin down for one minute at 8000 rpm, decant the filtrate.
(18) Add 500 µl AW buffer, spin down for three minutes at maximum speed and decant the filtrate.
(19) Place column into a new Eppendorf tube (not provided).
(20) Elute DNA with 75 µl AE buffer at 70°C, incubate for one minute at room temperature, and spin down for one minute at 3000 ×*g*.
(21) Measure the DNA concentration the next day.

3. DNA LABELLING (NICK TRANSLATION)

Is the, cutting DNA and substituting unlabelled nucleotides with digoxigenin, biotin, or fluorochrome labelled nucleotides)



3. DNA LABELLING (NICK TRANSLATION)

Appendix 2

Nick translation for comparative genomic hybridisation (CGH)

For a 30 µl reaction:

(1) Combine 1 µg of probe DNA, 3 µl of dNTP reaction mixture (0.2 mM dATP, dCTP, dGTP; 500 mM Tris/HCl, pH 7.8; 50 mM $MgCl_2$; 100 µM dithiothreitol; 100 µg/ml bovine serum albumin), 0.5 µl of dTTP (0.2 mM), 1 µl of digoxigenin or biotin conjugated dUTP (1 ng/µl), 3 µl of DNA polymerase I/DNase I (Gibco BRL, Breda, The Netherlands), 0–1 µl diluted DNase I (Gibco BRL). Adjust volume to 30 µl with double distilled water. Note, the amount of DNase I added varies for each DNA sample and is dependent upon the DNase I batch (dilute freshly before use).

(2) Incubate for 1.5–2 hours at 15°C.

(3) Inactivate enzymes at 70°C for 15 minutes.

(4) Check fragment length by gel electrophoresis on an ethidium bromide stained 1% agarose gel (5 µl/sample). Run at 100 V for 30 minutes. Inspect DNA fragment lengths with a UV transilluminator.

(5) For optimum hybridisation conditions, the probe (which is visible as a smear) should be between 500 and 1500 kb in length. If the probe size is larger, add more DNase I and 3 µl DNA polymerase I, incubate at 15°C for 15–30 minutes, and repeat steps 3 and 4.

3. BLOCKING

Chromosomal regions with **short repetitive DNA** sequences occur throughout the whole genome, but in a high number at all **centromeres**, **telomeres**, and some specific regions (chromosome arms **1p** and 16p, and chromosomes **19 and 22**). The lengths of these regions are highly variable between individuals (and thus between tumour and reference DNA), and this can interfere with CGH analysis. Therefore, repetitive DNA regions are blocked with unlabelled Cot-1 DNA (placental DNA from 50 to 100 bp, which is enriched for repetitive DNA sequences). Suboptimal blocking, seen as non-black centromeres, can lead to a reduced amplitude of the green to red ratio and gains and losses may go undetected. As an alternative to blocking, removal of repetitive sequences from the probe may be another solution to this problem.

3. HYBRIDISATION

Appendix 3

Hybridisation

(1) Mix 8–12 µl of the labelled tumour (biotin) and labelled reference (digoxigenin) DNA.
(2) Add 40 µg Cot-1 DNA to the mixture of labelled DNAs.
(3) Ethanol precipitate sample: add 0.1× volume of 3 M NaAc and 2× volume EtOH, spin at 10 000 ×*g* for 30 minutes.
(4) Decant supernatant and air dry pellet.
(5) Dissolve pellet in 6 µl hybridisation mixture (50% deionised formamide, 10% dextran sulphate in 2× SSC, pH 7.0. Store at −20°C).
(6) Denature metaphase slides in a jar with 70% formamide/2× SSC (20× SSC: 0.3 M sodium citrate, 3 M NaCl, pH 7.0) in a waterbath at 80°C for five minutes (dependent on batch metaphase slides, the temperature in the jar is ~ 72°C).
(7) Dry slides in an ethanol series (70%/96%/100%).
(8) Denature DNA mixture in water bath (80°C) for 10 minutes and transfer to metaphase slide immediately.
(9) Cover with a sealed coverslip (18 × 18 mm).
(10) Hybridise for two to four days in a humid chamber at 40°C.
(11) Remove coverslips carefully.
(12) Wash for 5 minutes in 2× SSC at room temperature.
(13) Wash three times for five minutes each in 0.1× SSC at 45°C.
(14) Wash for five minutes in TNT (TNT: 50 ml 10× TN, 450 ml milli-Q, 1.25 ml 20% Tween-20; 10× TN: 1 M Tris/HCl, 1.5 M NaCl, pH 7.5 at room temperature).
(15) Preincubate for 10 minutes in 100 µl TNB (TNB: 0.5% blocking reagent (Roche Diagnostics, Almere, The Netherlands) dissolved in 1× TN; before use, spin down undissolved particles and take the clear supernatant) under a coverslip (24 × 50 mm).
(16) Incubate for 60 minutes in 100 µl TNB with avidin–fluorescein isothiocyanate (FITC) (1:200) and sheep antidigoxigenin–tetramethyl rhodamine isothiocyanate (TRITC) (1:50) under a coverslip (24 × 50 mm) in a humid chamber at 37°C.
(17) Wash three times for five minutes each in TNT at room temperature.
(18) Wash for five minutes in 2× SSC at room temperature.
(19) Dry slide in an ethanol series (70%/96%/100%).
(20) Cover with 20–25 µl antifade containing DAPI (0.35 µg/µl) and seal with a coverslip (24 × 50 mm).
(21) Analyse slides with a fluorescence microscope and a digital image analysis system.

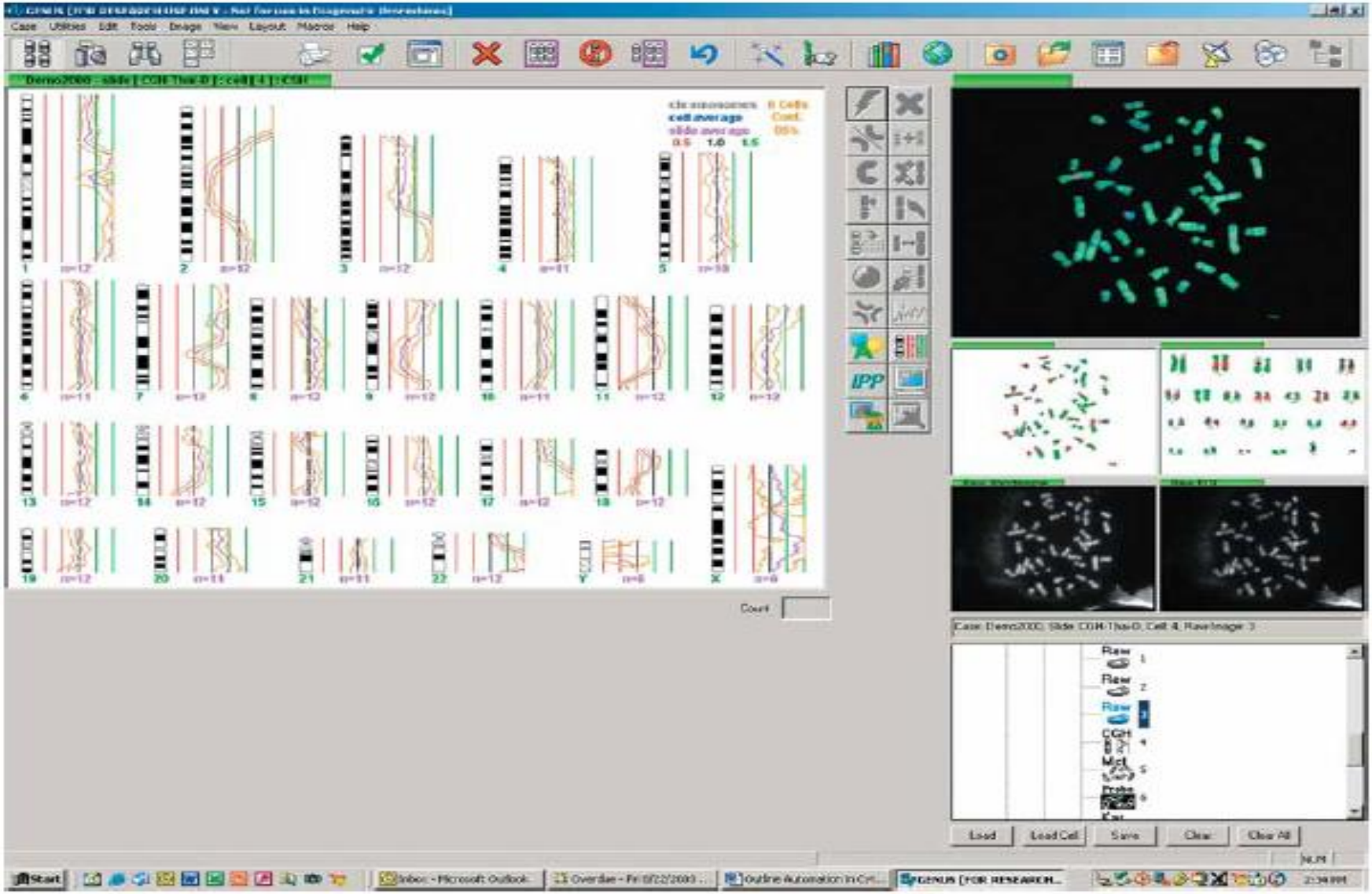


Fig. 17. Software interface of a CGH analysis system, showing the gain and loss profiles of the chromosomes. See text for details. (Courtesy of Applied Imaging.)