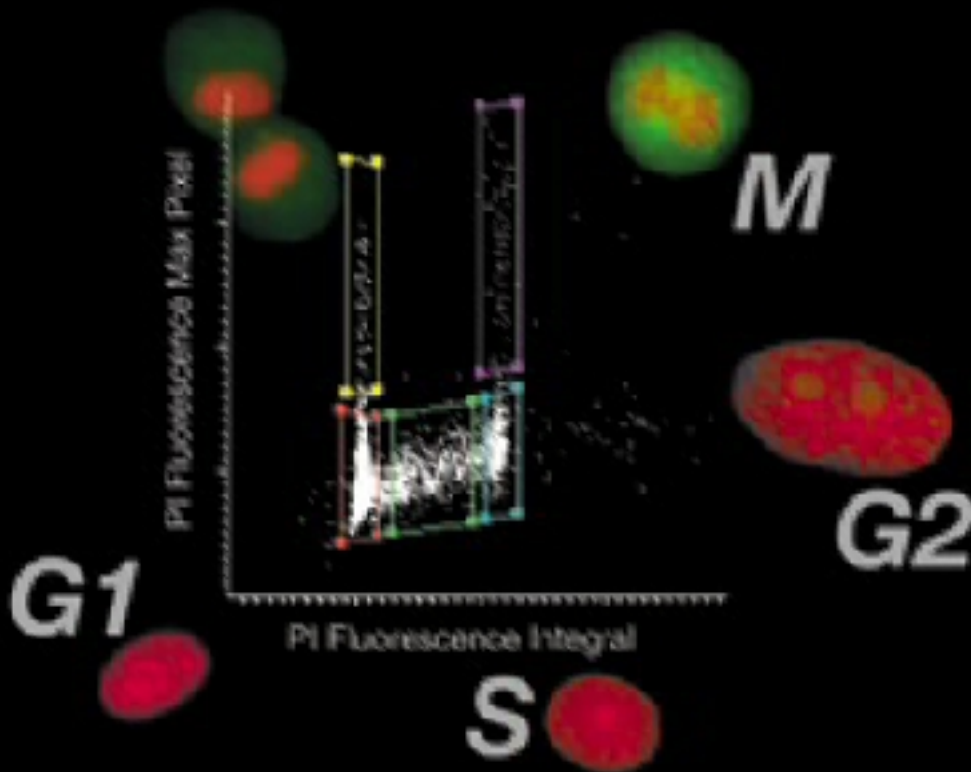
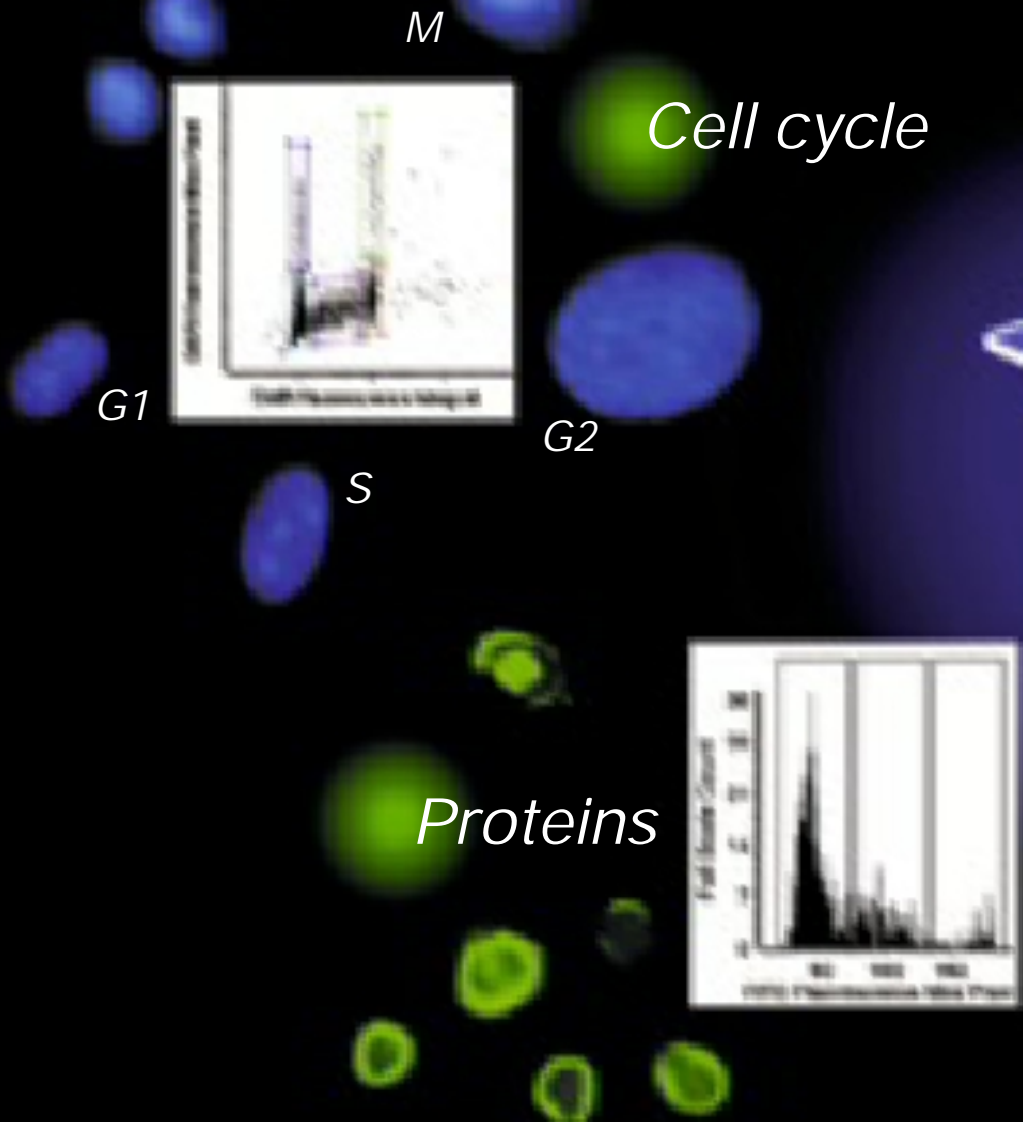


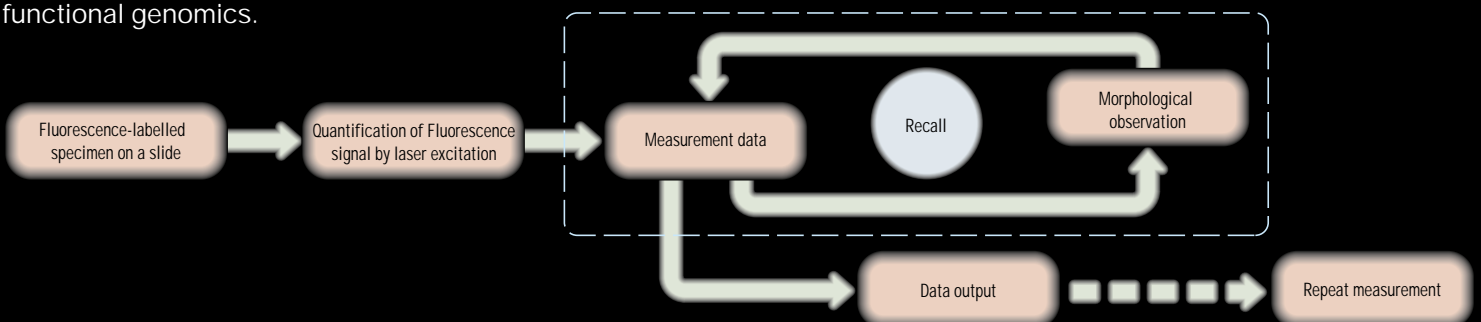
*Slide-based cytometry
for quantitative high content cellular analysis*



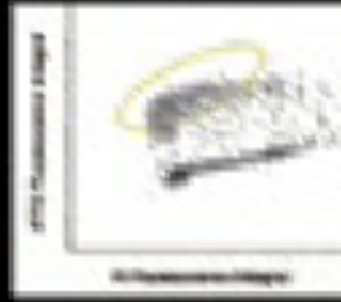


LSC2, the new laser scanning cytometer for precise quantitation

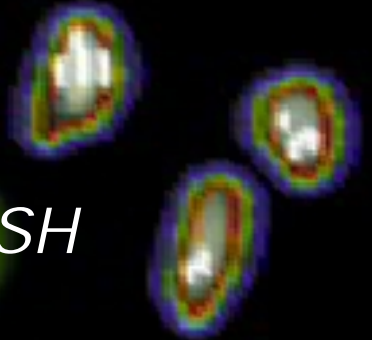
LSC2, the new laser scanning cytometer for precise quantitative multi-colour analysis and morphological observation. The LSC2 is an advanced new cytometer that allows simultaneous acquisition of quantitative data and cell morphology. It scans thousands of fluorescence-labelled cells on a slide, measures simultaneously up to 4 fluorescent colours plus scattered light, and saves both the measurement data and the coordinates of each cell. By recalling these coordinates, closer observation of the target cell's morphology can be performed instantly, either on the monitor or through the eyepiece of the microscope. The coordinates also allow automatic analysis of the same cells, even if subsequently re-stained and re-measured. In this way, far more information can be obtained than with existing analysis techniques. The LSC2 meets users' needs for measurement, analysis and observation at a much higher level than ever before. It brings advanced analysis technologies to areas of life science research such as cancer studies, proteomics, and functional genomics.



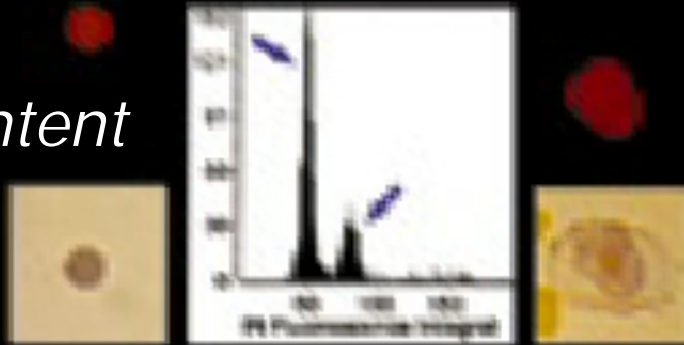
Apoptosis



FISH



DNA content

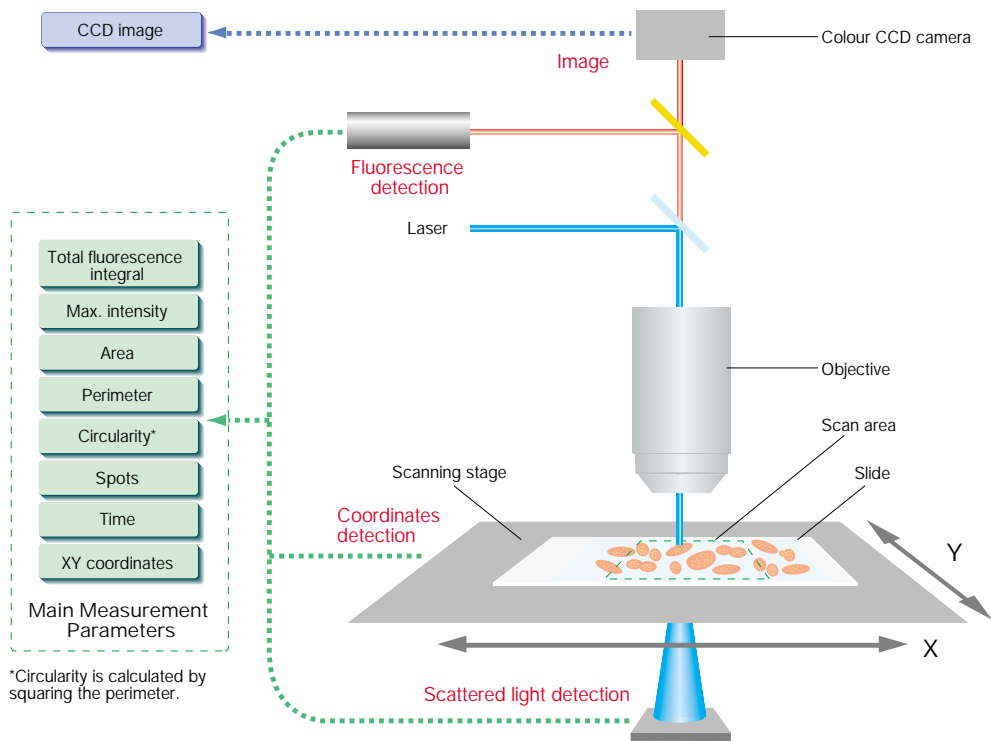


tative multi-colour analysis and morphological observation

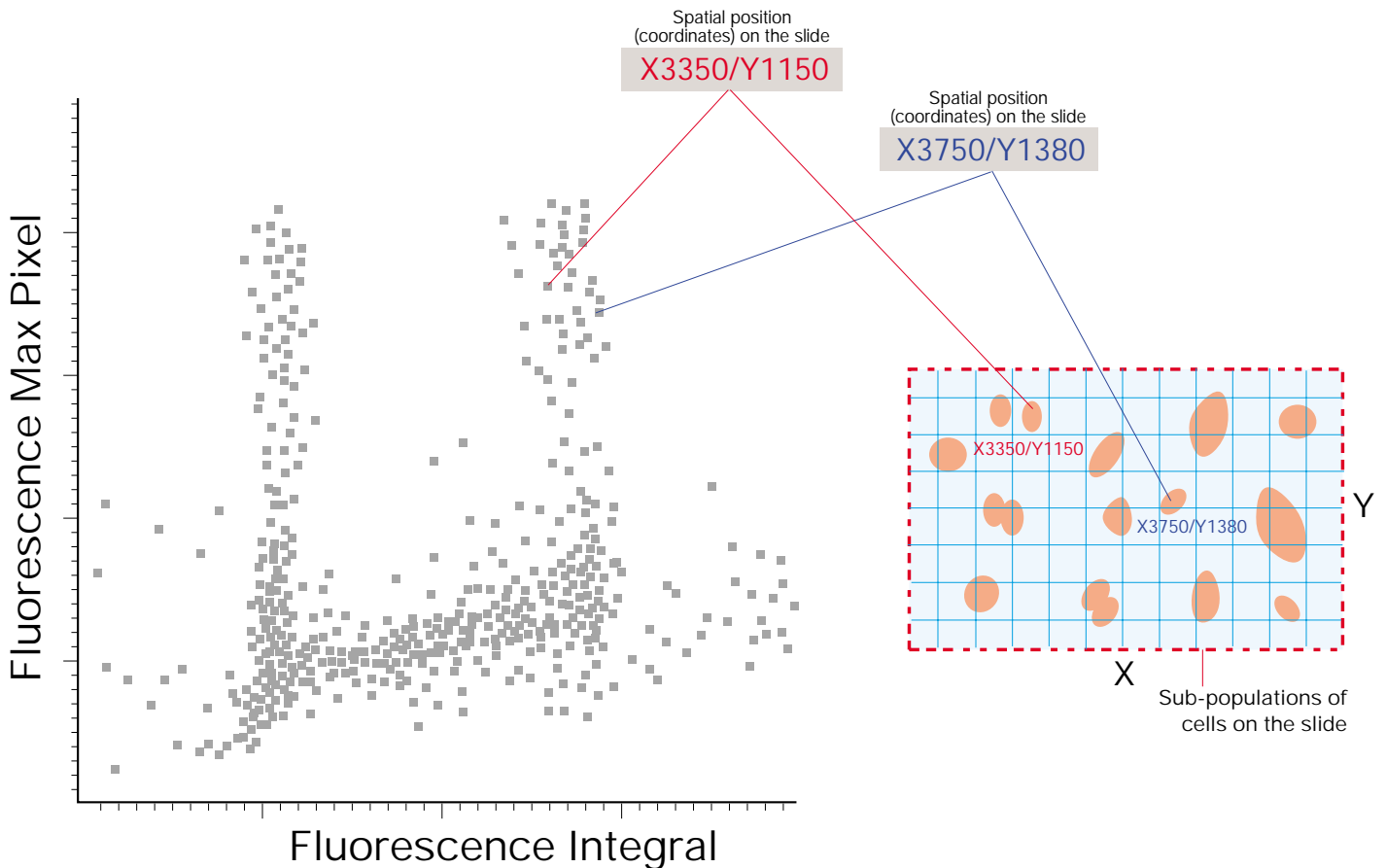


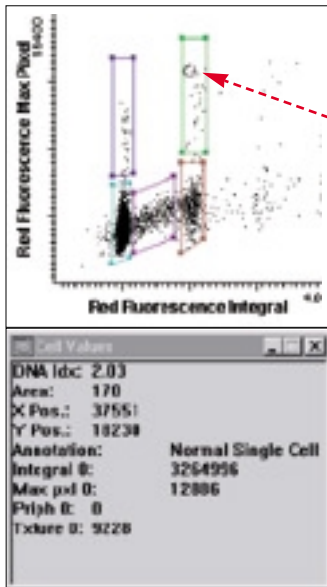
Main Features

- Measures several thousands to tens of thousands of cells on a slide in approximately 10 minutes
- Quantitative analysis of fluorescence staining and labelling with high precision
- Multiplexed data acquisition and on-line processing with up to 3 lasers and up to 4 fluorescence detection channels
- Cross-checking of analysis data and morphological observation, plus two-way recall: retrieves the cell image from measured data and vice versa
- Saving the coordinates of each detected cell on the slide allows measurement data to be cross-checked: after quantification of the fluorescence signal, the specimen can be stained with chromatic dyes and morphology can be observed under transmitted light conditions
- Cells can be classified and their morphological images can be displayed according to their analysis data shown in scattergrams or histograms
- Allows users to select and analyse individual cells
- Scans can be repeated on the same sample, providing high reproducibility of the analysis data
- Different specimen types like suspensions, smears, and monolayer cultures can be analysed
- Includes FISH spot counting functionality
- Only minimal sample volumes are required to perform measurements
- Low running cost

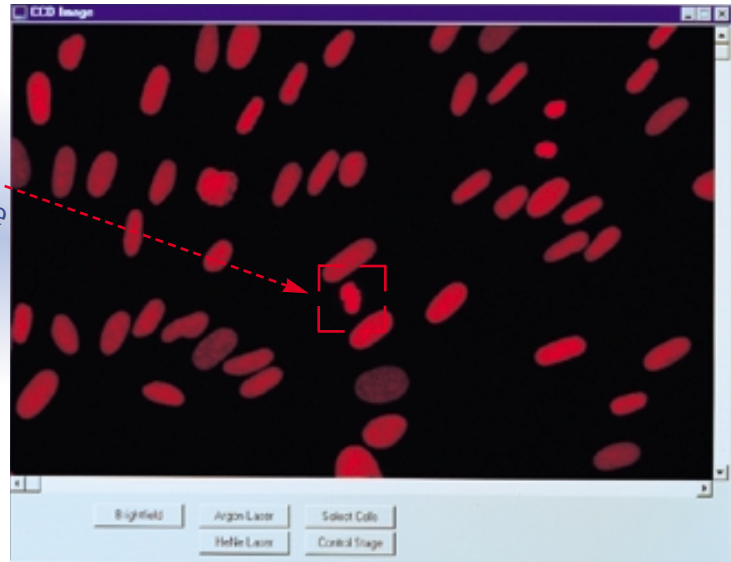


High-speed analysis of cell populations simultaneously measures fluorescence signal and spatial position of individual cells





Interactive recall



Digital image on the PC monitor

Automatically recalling individual cells for morphological observations

DETECT

Measurements on slide makes it easy to prepare specimen even small samples are sufficient for analysis

As measurements are taken from fluorescence-labelled samples, only the smallest amount of material from a biopsy or fine needle aspiration is required to prepare the specimen. Cultured cells and even touch smears can be analysed. Making a specimen is easy; only a small quantity of fluorochrome is needed. Measuring can be done without cell isolation or nuclei stripping, minimising cell damage. Additionally it is also possible to use tissue sections for the LCS analysis. Slide based specimens offer a number of benefits: they are easy to store, easy to carry and there is no risk of cross-contamination during the measuring process.



ANALYSE

High-speed measurement of cells and cell clusters on the slide, and recording the coordinates for each individual cell

Up to three high-speed lasers scan any designated fluorescence stained specimen. Scanning of 5,000 - 10,000 cells within 5 to 10 minutes*, the LCS2 records the total fluorescence signal and scattered light for each individual cell, its maximum fluorescence peak and area, and its coordinates on the slide. Up to 8 analysis parameters per cell or cell cluster can be graphically displayed as scattergram or histogram, or statistically calculated as, for example, a coefficient or variation, cell ratio etc.

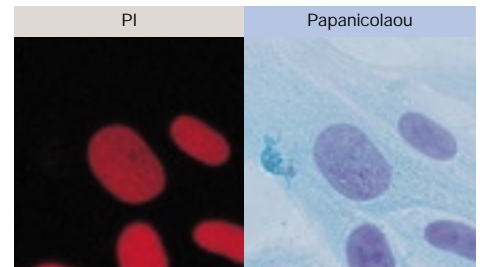
* Measurement time varies depending on the state of the specimen.



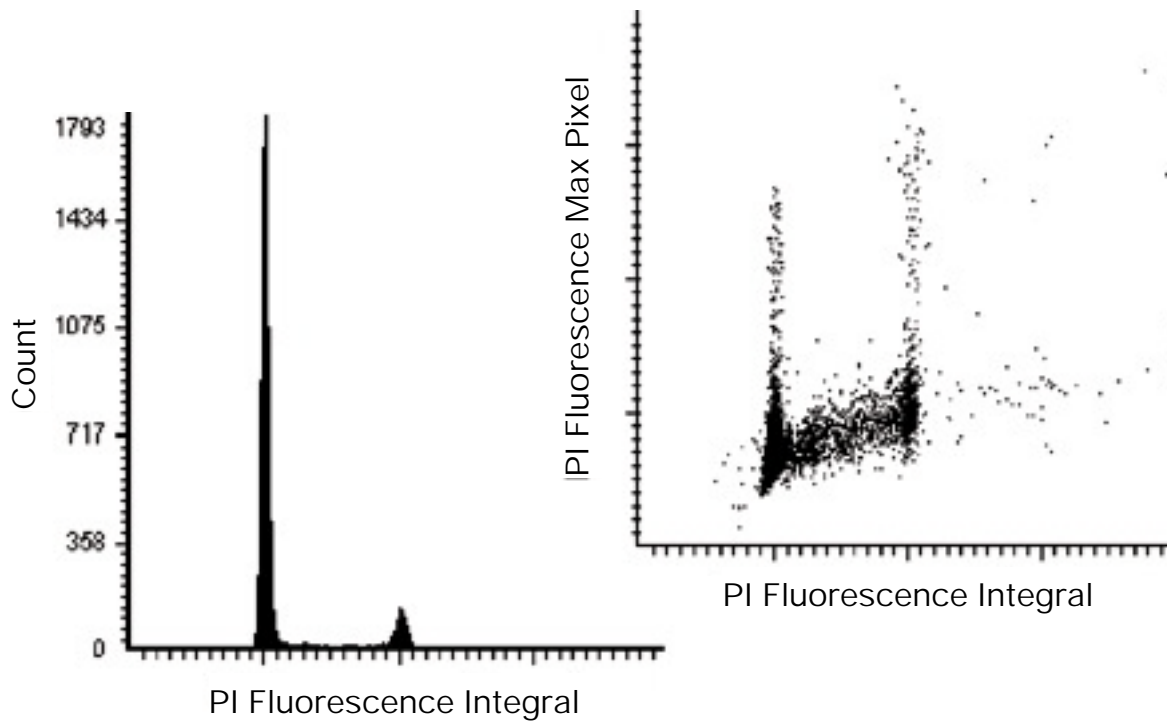
VISUALISE

Recalling views of cells based on their coordinates and carrying out morphological observations under the microscope

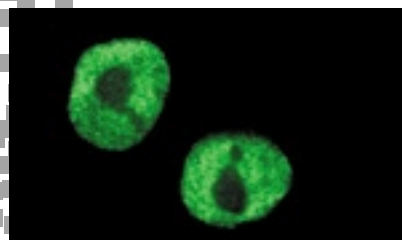
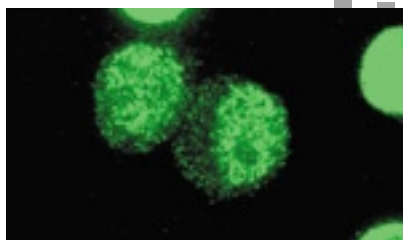
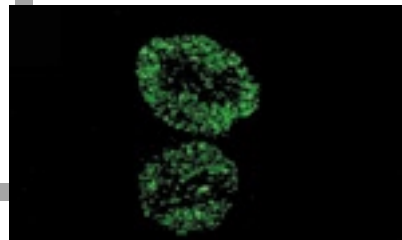
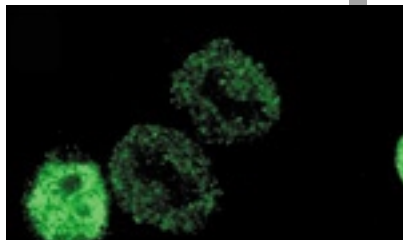
When the cells to be observed are gated in the scattergram, the scanning stage moves according to the cell coordinates; cells in the gating region can be recalled one by one and observed under the microscope or displayed on the monitor. Interactive recalls from the cell image are also possible. Since the measured data and the cell images appear on the same monitor, the data can easily be compared. As all the measured cell coordinates are recorded, it is possible to examine re-stained (e.g. with Papanicolaou stain) specimens not only in fluorescence but also in brightfield observation mode.



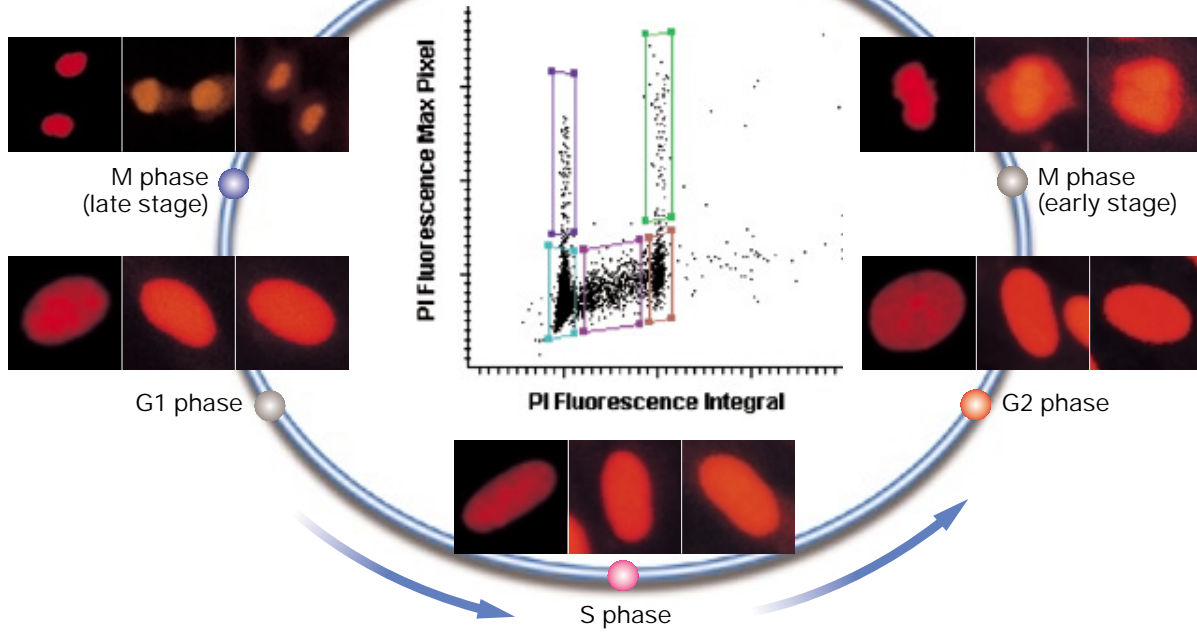
Microscope images of the recalled cells



Data analysis is vital to provide an insight into cellular events but morphological information is essential for the full picture



Cell cycle

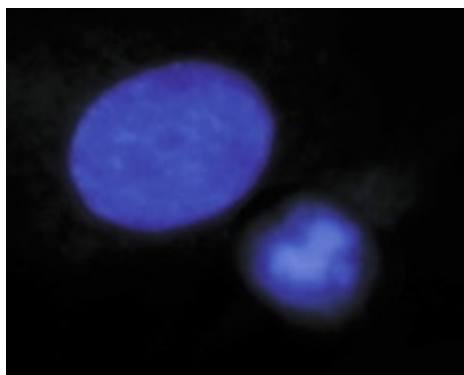


*When, where and what?
The LSC2 answers the key questions*

WHEN

Resolving the cell cycle

The LSC2 can analyse the stages of the cell cycle within a cell population using a single stain, such as PI or DAPI. Based on the analysis of the chromatin condensation (Max Pixel), the DNA content and the nuclear size, the LSC2 is even able to differentiate between the G2 phase and mitosis.

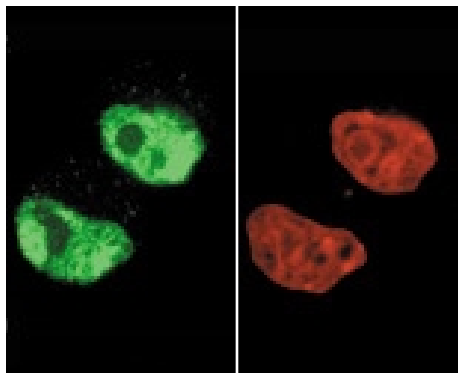


Upper cell: G2 phase, lower cell: M phase

WHERE

Analysing the localisation and expression level of proteins

The analysis data can be obtained from subsequently staining DNA and proteins. Using the recall function to observe both nuclei and proteins, it is possible to define the cell-cycle phase and identify at which stage the proteins are actively expressed.

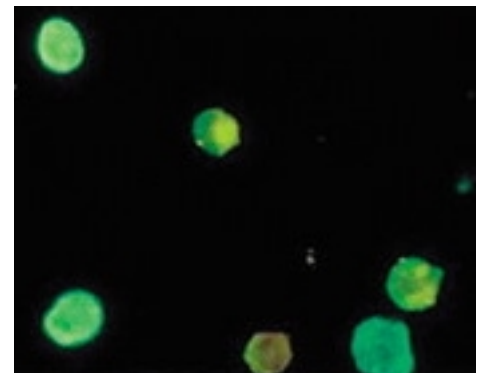


Protein DNA

WHAT

Simultaneous analysis of proteins and nuclear acids

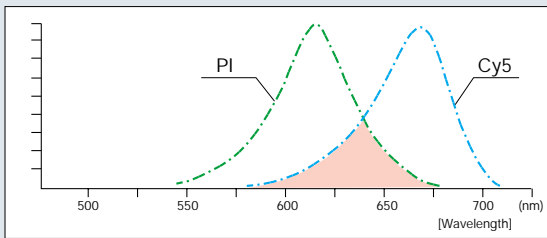
The LSC2 can be equipped with up to 3 lasers and 4 PMTs as detection channels, enabling simultaneous measurement of different fluorochromes. Staining e.g. several proteins differently makes it possible to monitor their expression patterns under various conditions.



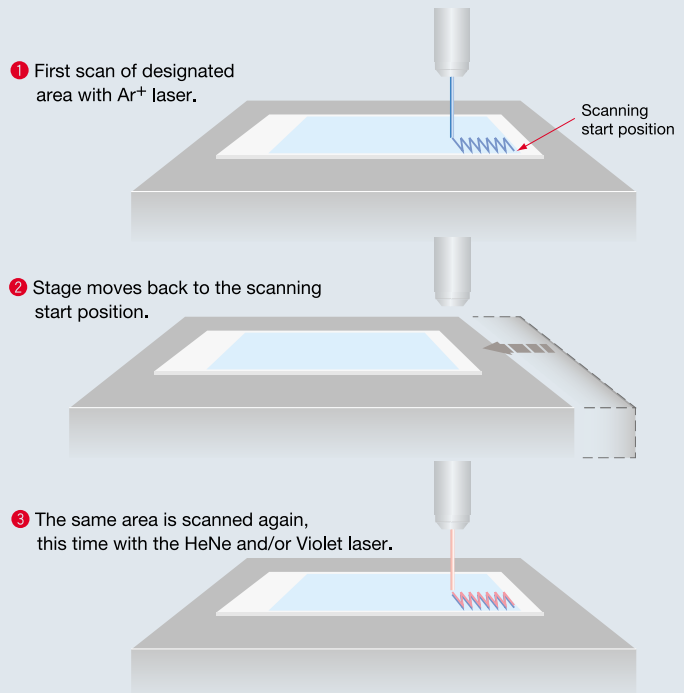
Sequential scanning prevents spectral overlapping

The LSC2 employs sequential scanning, whereby two separate lasers with different excitation wavelengths are used alternately (not simultaneously) to scan the same areas. This provides highly accurate data without interference from the emitted fluorescence signals (2-laser, 3-laser* specifications).

* Sequential scanning where Violet and HeNe lasers oscillate at the same time, and the Ar⁺ laser operates in sequence.



The above graph shows the fluorescence emission spectra of PI and Cy5. When excited simultaneously, even fluorochromes with different emission spectra may tend to overlap.



Merge function unites data from re-staining and re-measuring

Since the LSC2 saves the coordinates of each cell on the slide, the same area can be stained again with a different fluorochrome and measured a second time. The two sets of data obtained together are combined into one, using the

merge function, and analysis can be conducted on the combined data set.

Note: data on some cells might not be mergeable, depending on the specimen condition.

Five colour analysis plus DNA content measurement



The above charts are 6-colour measurement examples, formed by merging 5-colour Immuno-fluorescence analysis data plus DNA-quantification using PI staining. Three different fluorochromes were applied to the same blood specimen in each scan pass, and data from each measurement were ultimately put together as a single data set for analysis.

Laser	1. Scan pass HeNe	2. Scan pass Argon	3. Scan pass Argon
1. Emission filter	Orange —	Orange PE, Ab#2(CD4)	Green FITC, Ab#5(CD8)
2. Emission filter	Red —	Red PE/TR, Ab#3(CD19)	Red* PI, DNA Content
3. Emission filter	Far Red Cy5, Ab#1(CD45)	Far Red PE/Cy5, Ab#4(CD3)	—
Scatter detector	Scatter*	Scatter*	

* =Contouring Parameter

3-lasers plus 4-channel fluorescence detection for the use with a wide range of fluorochromes

Colour CCD camera

Cells can be recalled and the image displayed on an analogue monitor or on one connected to a PC.

3-lasers

Ar⁺ laser (standard), Violet laser and HeNe laser ensures compatibility with a wide range of fluorochromes.

4-channel fluorescence (max.)

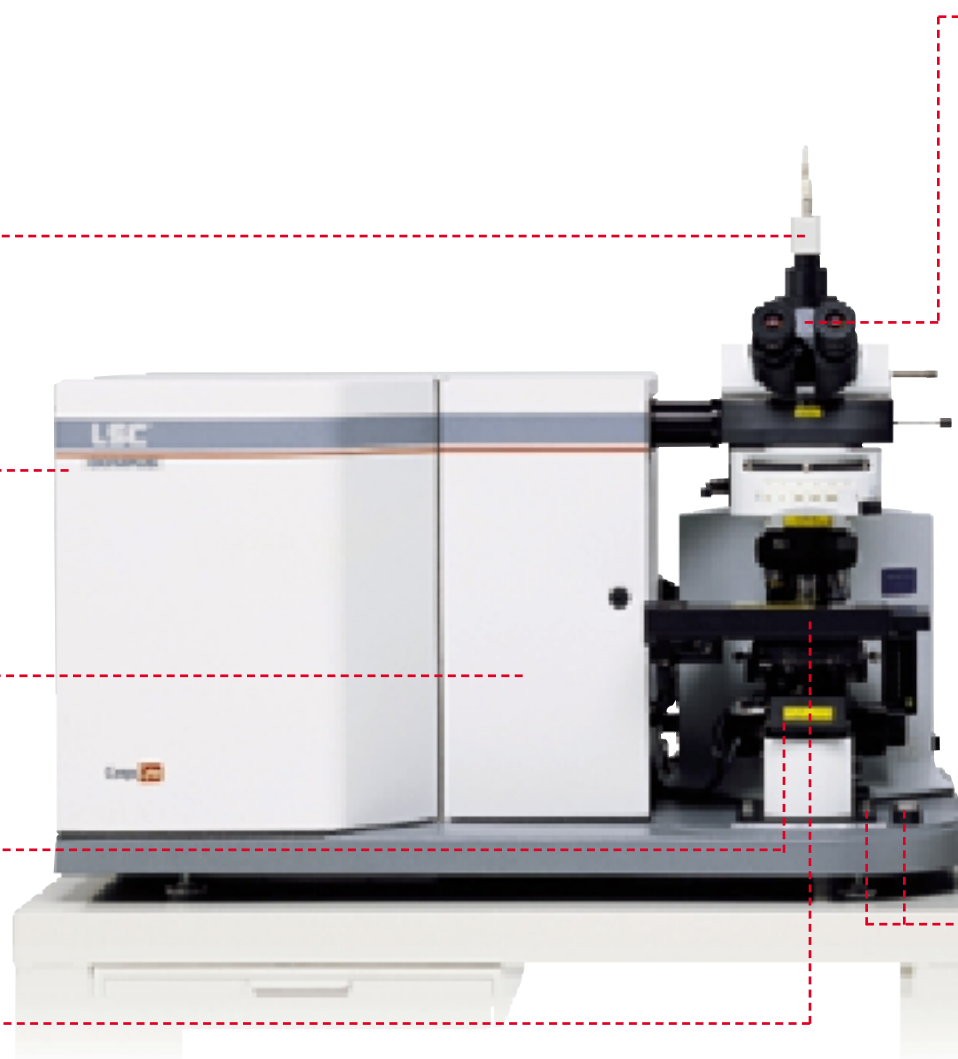
Parallel fluorescence detection with up to 4 photo multipliers and interchangeable filter cubes guarantees highest flexibility.

Front scattered light detection

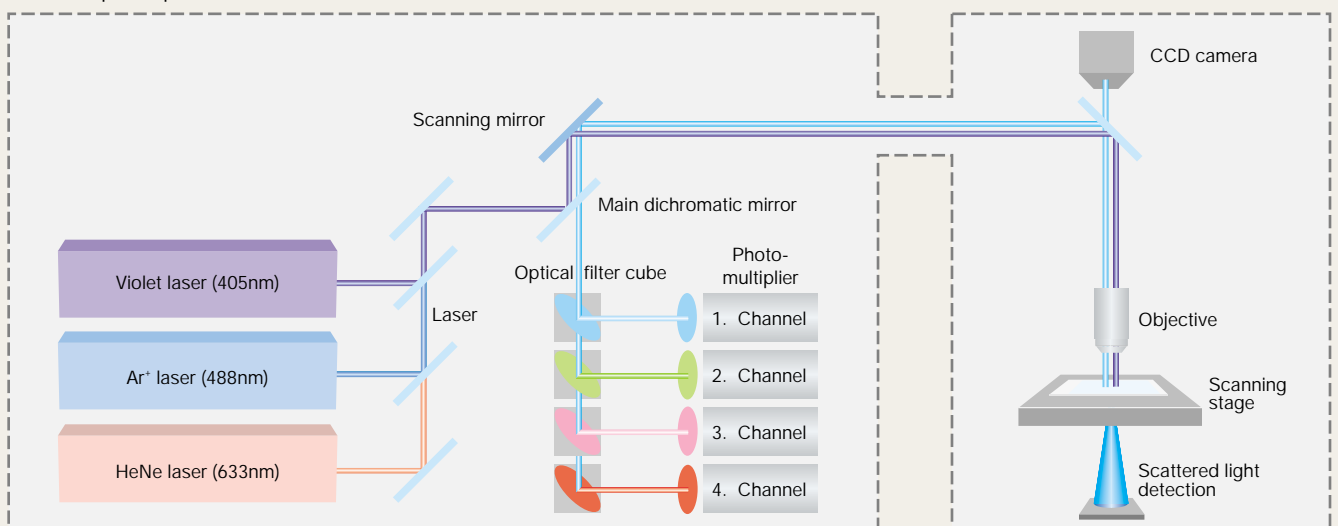
Cell morphology is imaged by a photodiode detecting the front scattered light.

Scanning stage

High-speed scanning of a specimen (from several thousands to tens of thousands of cells) takes approximately 10 minutes.



■ LSC2 optical path of a 3-laser/4-channel instrument





Imaging

Digital and video cameras can be mounted simultaneously by using a double port adapter (U-DPT).

U-DPT+3 Chip CCD camera+DP70



Analogue monitor

Images acquired with an analogue video camera are clearly displayed on the high-resolution monitor.

Data display

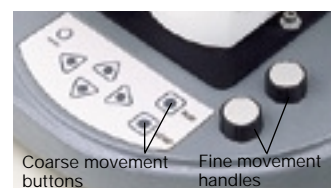
Switchable display of analysis data, cell image or both.

Data processing

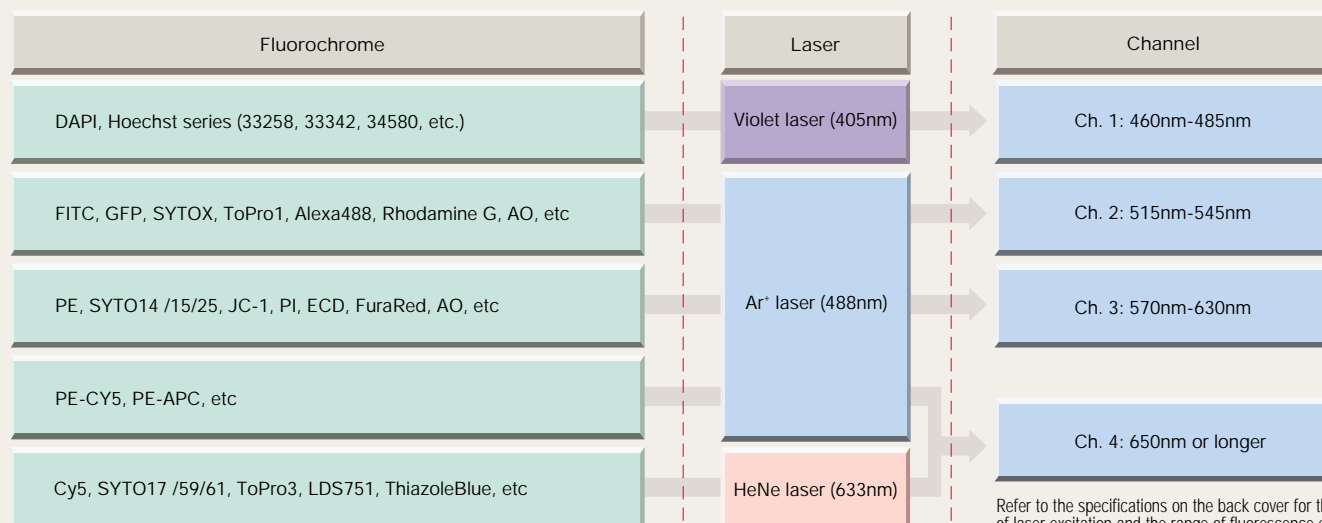
A Windows XP workstation is used for high-speed data processing. The software, WinCyte, controls both data acquisition and data analysis.

Scanning stage control

Use of the coarse movement buttons allows quick observation of the whole specimen. Specific areas are observed by means of the fine movement dials: which is particularly useful when taking digital images.



Fluorochrome analysis chart (3-laser/4-channel configuration)

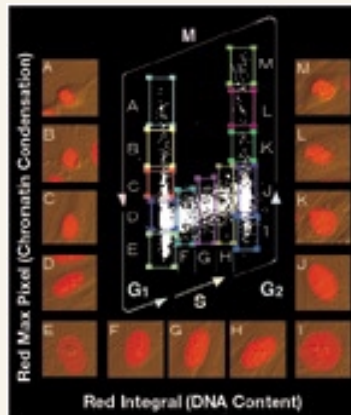


Refer to the specifications on the back cover for the combination of laser excitation and the range of fluorescence emission detection.

Cell cycle

Cell cycle analysis

The cell cycle can be studied using only the single fluorochrome PI. From the relationship between DNA content (X-axis) and the chromatin condensation (Y-axis), the cell nuclei of each phase in the cell cycle can be identified. The highly precise analysis not only allows differentiation between the G₀/G₁, S, and G₂/M phases (based on DNA content analysis), but also between G₂ and M, and the phase after-cell division and G₁.



Inducing cell division of cardiac myocytes by gene transfection

Cardiac myocytes are known to stop growing in any way as soon as they are differentiated. It has been established that after differentiation cyclin D1, a cell cycle control protein, is located in the cytoplasm and not translocated into the nucleus. Therefore an attempt was made to induce cell division of cardiac myocytes by introducing the recombinant gene DINLS to induce the translocation of cyclin D1 to the nucleus. Analysis of the cell cycle using LSC2 showed that cultured cardiac myocytes are arrested in the G₀/G₁ phase, and even the stimulation with 10% FCS serum failed to induce cell division. However, the transfection with the recombinant DINLS gene successfully induced the progress in cell cycle to the S and G₂ phases (Chart C), cell division (M phase) (Chart A-18 etc., Chart B-vi) and subsequent development (Chart A-4,5 etc, Chart B-i). These results prove that the intracellular localisation of the protein cyclin D1 plays a central role in the arrest of cell growth and division of cardiac myocytes.

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 Masa-Aki Ikeda
 Section of Molecular embryology, Graduate School,
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Chart A

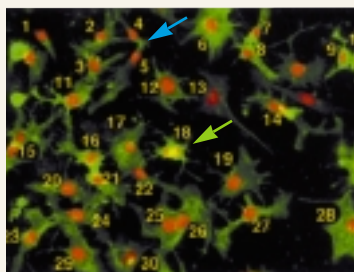
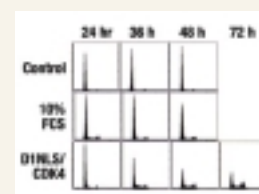
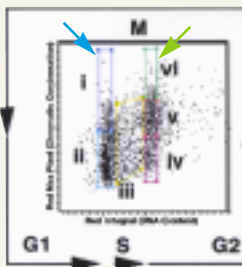


Chart C



i (Immediately after cell division): 4,5
 vi (M phase): 18

Chart B



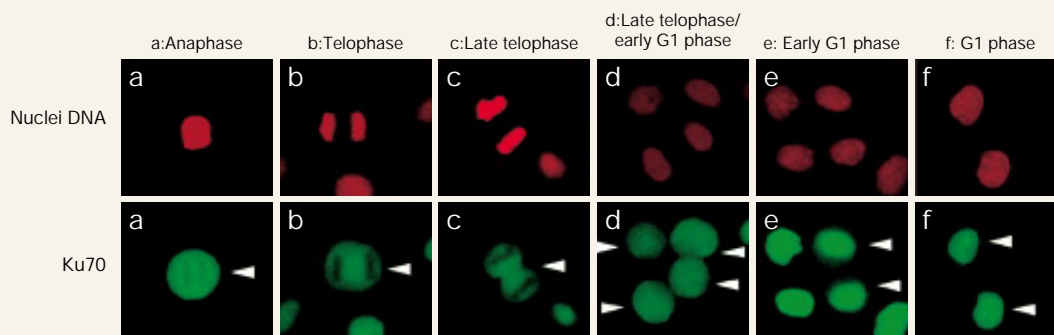
i	4, 5, 7, 8, 9, 10
ii	1, 2, 3, 13, 17, 22
iii	20, 25, 24, 30,
iv	12
v	6, 11, 14, 19, 25, 26, 27, 28, 29
vi	15, 16, 18, 21

Repair of double-strand DNA breaks relating to the intracellular localisation of the protein Ku70

Ku70 is a protein identified as a self-antigen in human scleroderma and multiple myositis overlapping syndrome. Previous studies have also indicated that Ku70 plays an important role in the repair mechanism during recombination, and may also be a contributory factor in the control of cell growth. To obtain more information on the functions of this protein, an analysis was conducted on the

localisation of Ku70 in the cell. The cell cycle was identified from the DNA content and the concentration of chromatin, and the intracellular localisation was checked at each phase.

These studies revealed that Ku70 is localised in both the nucleus and the cytoplasm during the late telophase/early G₁ phase.



Intracellular localisation of Ku70 at each phase of the cell cycle

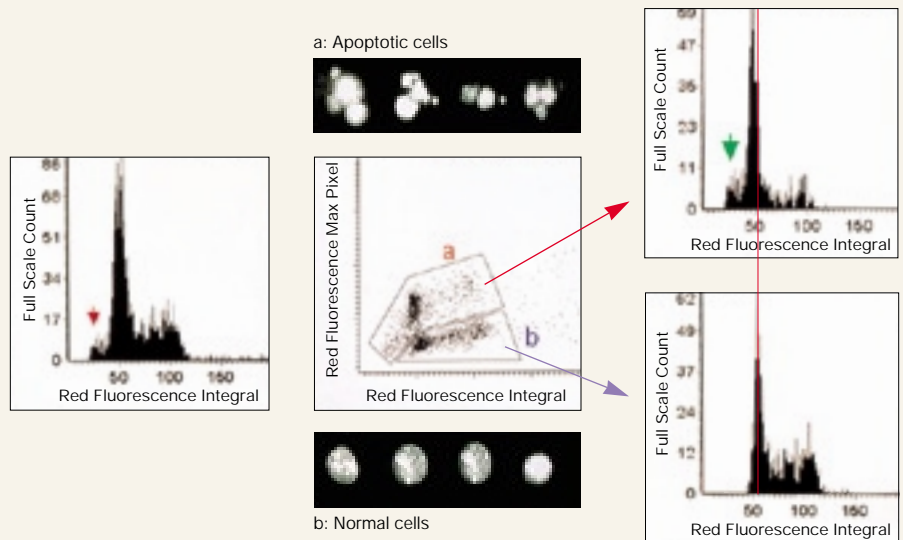
Manabu KOIKE
 National Institute of Radiological
 Sciences,
 Radiation Hazard Research Group

Apoptosis

Adriamycin on HeLa cells using the fluorochrome PI

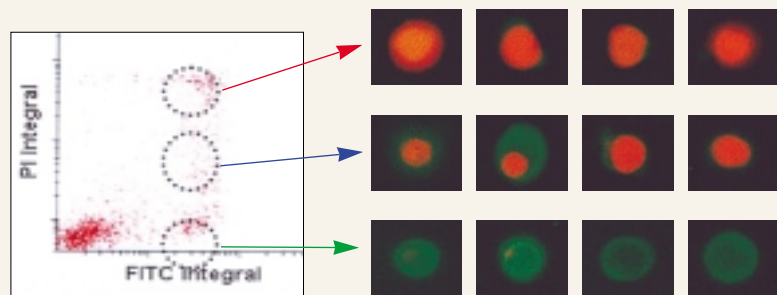
The DNA content of HeLa cells was measured after treatment with the anti-cancer drug Adriamycin. Data derived exclusively from the histogram seemed inconclusive, due to high CV values at the peak of G1 phase. However, in the scattergram the cells could be divided into two subpopulations with different Max Pixel values. By recalling images of the cells it could be shown that cells with a high DNA condensation (Max Pixel high value) did undergo apoptosis while the cells with low values were interphase cells. Separation of the histogram results also demonstrated that the DNA content of the apoptotic cells was lower than that of the normal cells.

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Ube*



Living cell analysis using Annexin V and PI

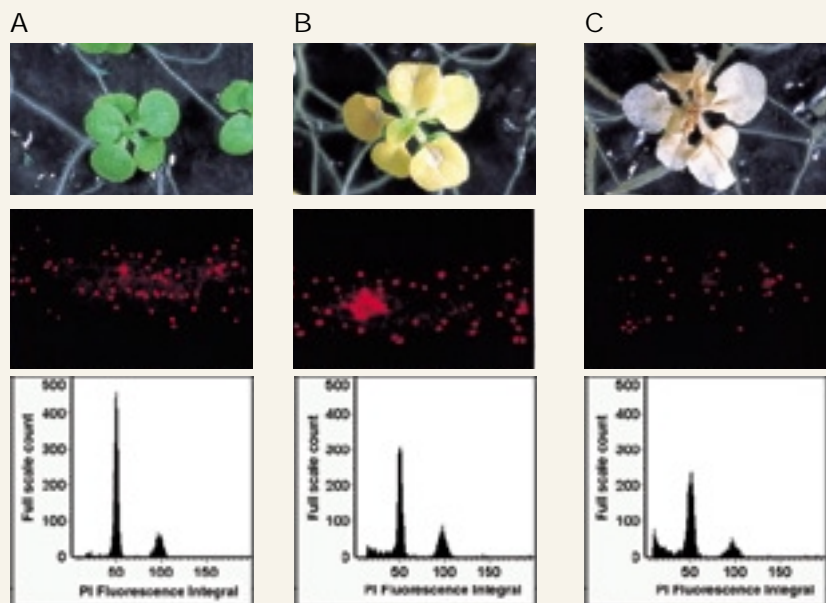
One of the earliest indications of apoptosis is the translocation of the membrane phospholipid phosphatidylserine (PS) from the inner to the outer leaflet of the plasma membrane. Once exposed to the extracellular environment, binding sites of PS become available for FITC-labelled Annexin V. A co-staining with the vital dye PI was used as an indicator for cell viability and the cell morphology was observed by dividing the apoptotic cells into 3 groups according to the intensity of the PI positive staining. It was shown that the binding of Annexin V to the cell membrane was specifically indicating changes in the membrane and its associated loss of integrity which leads to an increased staining of nuclear DNA with PI.



Induction of lethality in interspecies hybrids of tobacco

Analysis was carried out on a tobacco hybrid of *N. glutinosa* x *N. repanda*, which shows lethality in an early developmental stage. The images on the right show leaves at 10 (A), 15 (B) and 30 (C) days after germination. The quantity of DNA was determined by isolating nuclei from the leaves and staining them with PI. Leaves in the first group (10 days after germination) neither show any abnormality in the DNA histogram nor in the chromatin structure. In the second group, nucleic fragmentation (sub G1 peak) occurs indicating a progression in apoptosis and leading to an increase in symptoms of lethality. The analysis demonstrates that the lethality of cross species hybrids is induced by apoptosis.

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Ibaraki University*



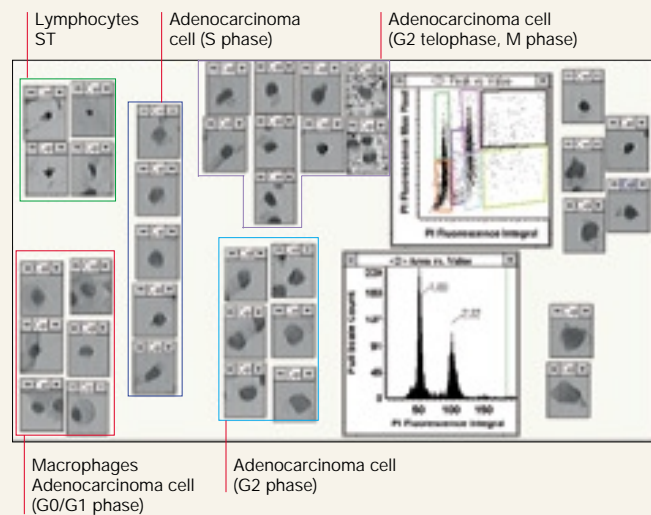
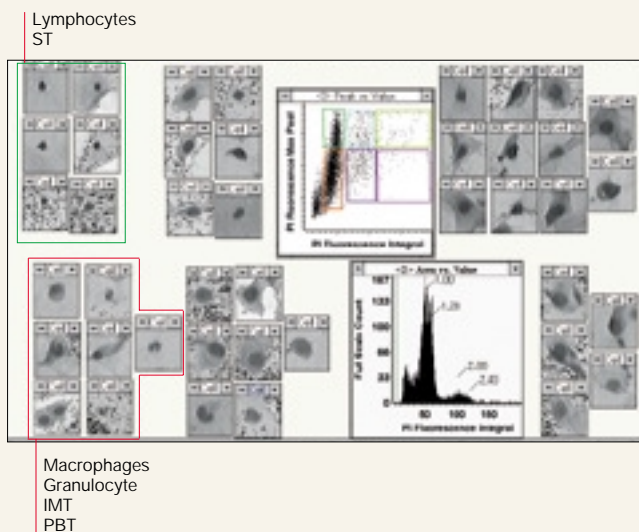
DNA content

Measurement of cytology sample from uterine cervix

Cytology samples from the uterine cervix with a high grade of dysplasia (left chart) and with adenocarcinoma (right chart) had been analysed with the LSC2. Scattergrams had been defined using chromatin condensation (Max Pixel) and DNA content (Fluorescence Integral) as parameters. The samples were stained with Papanicolaou stain and the fluorescence events had been gated into different cell populations (see below). Cells of each gated region were recalled and the Papanicolaou stained images were displayed together with the scattergram and histogram. The cells in the gating region marked

green could be identified as lymphocytes and ST, as they show 2n DNA content and a high degree of chromatin condensation. In the cervix samples with a high grade of dysplasia, abnormal IMT and PBT nucleus cells with more than 4n DNA content were found. The samples from the cervix with adenocarcinoma showed, morphologically clear adenocarcinoma cells with more than 3n DNA content.

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Sasaki Institute Kyoundo Hospital

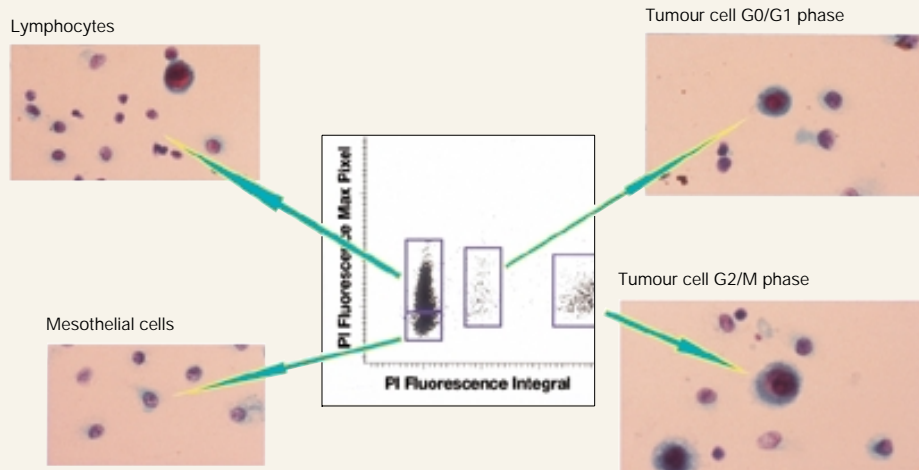


Analysis of pleural effusion cells

This experiment measured the nucleic DNA content of pleural effusion cells taken from the lung of a patient suffering from both adenocarcinoma and carcinomatous pleurisy. According to the analysis of the DNA content (fluorescence integral) cells were gated into three major cell populations. The microscopic observation confirmed that the diploid cells were normal (non-neoplastic). After further dividing this population of diploid cells according to the chromatin condensation (Max Pixel) it was found that the cells in the cluster with high PI Max value (above left) were lymphocytes, while those in the gated region with lower values (bottom left) were mesothelial cells. The microscopic observation

furthermore confirmed that the gated region with the tetraploid cells were tumour cells, at the G0/G1 phase (above right) but not doublet normal cells. The octaploid cells were considered as tumour cells at G2/M phase (bottom right). Further applications can be performed such as observation and quantification of the effect of therapeutic treatments on the cell cycle of tumour cells, or determination of the degree of cell damage caused by tumour cells.

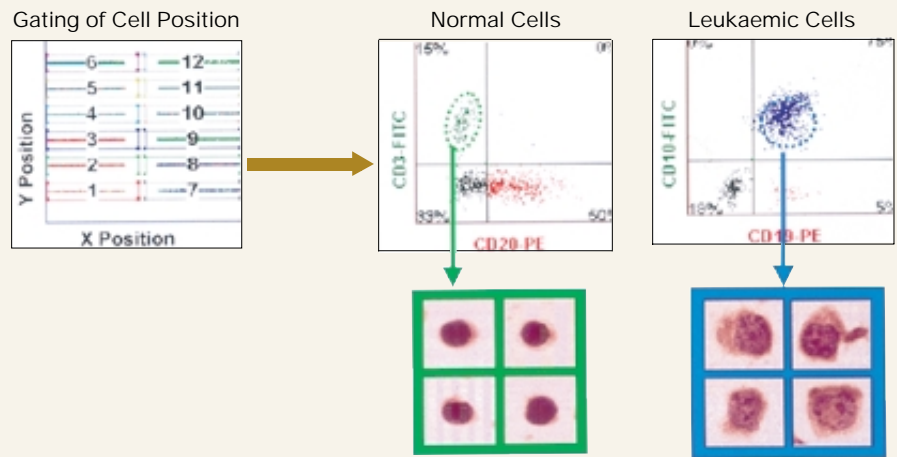
Dr. Hiroshi Isobe
Respiratory organs, National Sapporo Hospital



Immuno fluorescence

Immuno fluorescence analysis using morphology

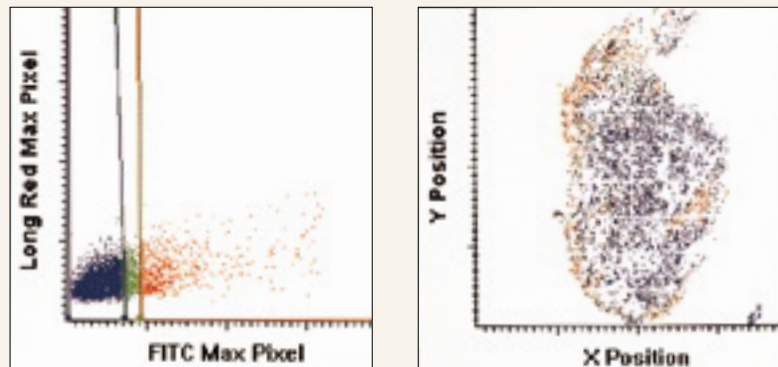
A single blood sample was fractionated into 12 aliquotes on a single slide. Each fraction was incubated with different antibodies. The diagrams on the right show the results of normal blood cells in comparison to leukaemia blood cells stained with respectively CD3-FITC and CD20-PE, and CD10-FICT and CD19-PE. Comparing the data of the fluorescence integral for each fraction of the blood sample with the morphological images of the cells clearly shows the different states of the samples.



Tissue

Mapping of apoptosis (TUNEL) in murine tissue section

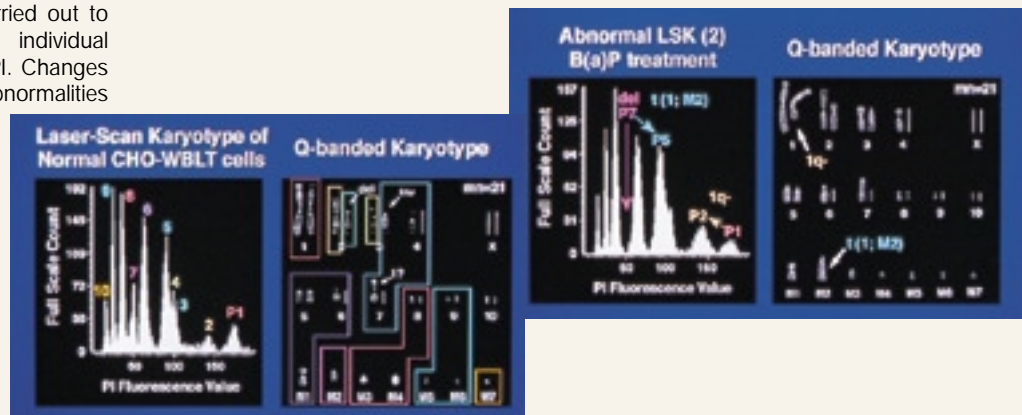
Tissue sections (4-6µm) can be analysed for DNA fragmentation as an indicator of apoptosis using the TUNEL assay with FITC-dUTP, and staining the nuclei with PI. The fluorescence integral values are determined by simultaneous measurement of FITC and PI in the nuclei. Colouring the data points allows the confirmation of the relationship between the fluorescence integral values and tissue mapping data. Cells with a strong apoptotic reaction were found on the surface of the tissue, reflecting the depth of penetration of an apoptosis-inducing compound.



Chromosomes

Detection of chromosomal translocations in CHO cells

Histogram-based analysis was carried out to measure the DNA content of individual chromosomes after staining with PI. Changes reflecting chromosomal abnormalities (chromosomal translocations) were confirmed by comparing normal cells with cell samples treated with carcinogenic substances.



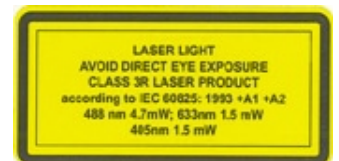
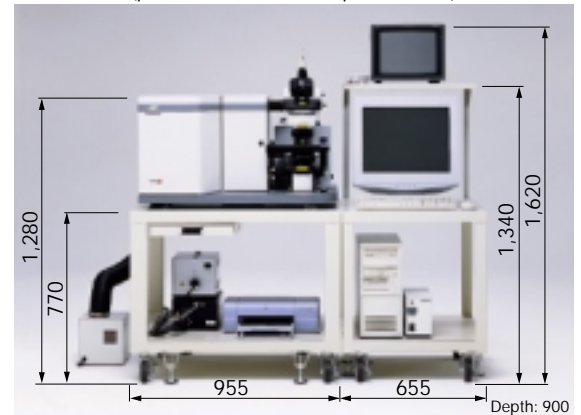
Specifications

Mechanical system		Laser scanning using galvanometer mirrors on a single axis (Y-axis) 2-channel fluorescence detection optical system (optional extendable up to 4 channels) Front scattered light detection optical system via transmitted light detection unit plus sensor stop X-Y scanning stage Specimen : Single slide (75mm x 26mm, thickness 1.0mm —1.2mm) Stroke : 110mm (X) x 51.5mm (Y) Replicate of coordinate position : ±5µm (stage)
Laser		Air cooled Blue Ar-488nm, variable type with maximum 20mW (standard) HeNe Red 633nm, fixed type with maximum 5mW (option) LD Violet 405nm, fixed type with maximum 18mW (option)
Standard wavelength range for detection	4-channel fluorescence	Channel 1: 515-545nm (detection for FITC etc.), Channel 2: 565-595nm (detection for PE etc.) Channel 3: 611-639nm (detection for PI etc.), Channel 4: 650nm and longer (detection for Cy5 etc.)
	3-channel fluorescence	Channel 1: 515-545nm, Channel 2: 570-630nm, Channel 3: 650nm and longer
	2-channel fluorescence	Channel 1: 515-545nm, Channel 2: 570nm and longer
Standard wavelength range for detection (with optional violet laser)	4-channel fluorescence	Channel 1: 460-485nm (DAPI), Channel 2: 515-545nm, Channel 3: 570-630nm, Channel 4: 650nm and longer
	3-channel fluorescence	Channel 1: 460-485nm, Channel 2: 515-545nm, Channel 3: 570nm and longer
Detection wavelength range for near-infrared light (option)		650-690nm, 750-800nm
LSC optical system		Objectives for fluorescence photometry: UPlanFL10x, UPlanFL20x, UPlanFL40x Spot diameter: Approx. 5µm (with 20x objective) Sampling spacing: Approx. 0.5µm (with 20x objective)
Optical path changeover		Changeover between photometry path for LSC2 and microscope observation path (100/0%, 0/100%)
Microscope		BX51TF body 100W halogen transmitted light source BX-RFA reflected light fluorescence illuminator: 100W mercury excitation light source U-MWIB, U-MWIG, U-MNIBA (only for G fluorescence observation in IB excitation and multiple-staining) (Fluorescence attachment is available as an option) U-TR30 observation tube: WH10x, CROSS WH10x eyepiece
Video camera (optional)		1/2 colour CCD camera, Magnification 1x, C-mount, gain on/off possible
Analysis equipment		PC/AT compatible computer Pentium 4, 512 MB RAM, 120 GB HDD, 10/100 NIC, Windows XP Professional
Monitor		20,1" Flat Panel Monitor (NEC or equivalent)
Printer (optional)		Colour ink jet printer
System desk		Desks for LSC2 and computer
Working conditions		Performance guarantee temperature: 15-25°C, Operation guarantee temperature: 10-30°C, Humidity: 30-85% (non condensing)
Power consumption		3,500VA (Max)
Total weight		Approx. 280kg

Optional items

Sensitive colour CCD camera set	Colour 3CCD camera (including adapter)
Video monitor for CCD camera display	14-inch colour monitor
DIC observation set	U-AN, U-DICT, U-DP10,20,40, U-DPA60
Microscope digital camera set	DP70, Photo eyepiece, U-DTP (double port), adapter
Fluorescence imaging set	Cooled CCD, Photo eyepiece, U-DTP
Objectives for low magnification observation	UPlanFL4x
Objectives for high magnification observation	UPlanApo60x
High-resolution, high-performance printer	Colour video printer

Dimensions (photo shown includes optional items) (unit: mm)



Specifications are subject to change without any obligation on the part of the manufacturer.

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* Manufacturer: CompuCyte Corporation, U.S.A.

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