Technical Explanation

Outline and Features of UF-5000, Fully Automated Urine Particle Analyzer

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INTRODUCTION

UF-5000, the fully automated urine particle analyzer (Sysmex Corporation; hereinafter UF-5000) is a new type of analyzer that is capable of analyzing the birefringence of particles [1)] and the amount of nucleic acid content and size information of the cell, coupled with the complexity of internal structure, using a blue semiconductor laser (488 nm). With an improved optical system, detailed analysis of signal waveforms originating from each particle has been realized, and casts, epithelial cells, etc. can now be analyzed in greater detail.

Major improvements have been incorporated in the stains and in the classification algorithms. Therefore, the UF-5000 is expected to contribute to further enhancement of the clinical value of urinalysis rather than merely being an improved version of the previous models, UF-100 and UF-1000*i*. We describe here the principles of measurement and the main features of the UF-5000.
Comparison of the main specifications between the UF-5000 and UF-1000*i* is shown in ***Table 1***.

Note: This article is described based on the specifications of UF-5000 Ver.00-11 and U-WAM Ver.00-06.

Table 1 *Comparison of the main specifications between UF-5000 and UF-1000i*

	UF-5000	UF-1000*i*
Parameters	RBC, WBC, EC, Squa.EC, CAST, BACT, WBC Clumps, Non SEC, Hy.CAST, Path.CAST, X'TAL, YLC, SPERM, MUCUS	RBC, WBC, EC, CAST, BACT
	NL RBC, Lysed RBC, Tran.EC, RTEC SRC, Atyp.C, DEBRIS, Cond., Osmo.	X'TAL, YLC, SRC, Path.CAST, MUCUS SPERM, Cond.
Research information	RBC-Info. (Red blood cell morphology information) BACT-Info. (Bacterial Gram staining information) UTI-Info. (UTI information)	RBC-Info. (Red blood cell morphology information) BACT-Info. (Information on bacterial morphology) UTI-Info. (UTI information) Cond.-Info. (Urine concentration information)
Body fluid analysis	Available	Unavailable
Principle	Flow cytometry using a blue semiconductor laser (488 nm)	Flow cytometry using a red semiconductor laser (635 nm)
Measured signals	Forward scattered light, side scattered light, side fluorescence, depolarized side scattered light	Forward scattered light, side scattered light, side fluorescence
Detection channels	SF ch (for elements having no nucleus) CR ch (for elements having nucleus)	BACTERIA ch (for bacteria) SEDIMENT ch (for elements other than bacteria)
Throughput	105 samples/hour (max)	100 samples/hour (max)
Aspirated sample volume	0.45 mL (common for all modes)	0.8 mL (manual mode) 1.2 mL (sampler mode)
Required sample volume	2 mL (sampler mode) 0.6 mL (STAT mode)	3 mL (sampler mode) 1 mL (manual mode)

* Categories of the individual parameters (reportable, non-reportable, quantitative, semi-quantitative, research-use only) varies depending on the regulatory requirements of each region or country.

Note: This article is translated and republished from the Sysmex Journal Web Vol.18 No.1, 2017. (Japanese)

MAIN SPECIFICATIONS

1. External appearance of the analyzer

The UF-5000 consists of an analysis section (main unit), a sampler section and a pneumatic unit (***Fig. 1***).

2. Instrument specifications

The main specifications of the instrument are given below (***Table 2***).
As with UF-1000*i*, the UF-5000 utilizes flow cytometry as its measurement principle. The wavelength of the laser

UF-5000

U-WAM (Urinalysis Work Area Information Management System)

Fig. 1 *External appearance of UF-5000 and U-WAM*

Table 2 *Specifications of UF-5000*

Name	The fully automated urine particle analyzer, UF-5000
Principle of measurement	Flow cytometry
Analysis targets	Human urine and human body fluids
Throughput	Urine mode: 105 samples/hour (max) Body fluid mode: 20 samples/hour (max)
Aspirated sample volume	0.45 mL (common for all analysis modes)
Required sample volume	Urine mode: 2 mL in the sampler mode, 0.6 mL in STAT mode Body fluid mode: 0.6 mL in STAT mode
Data storage capacity*	Analysis results: Maximum 1,000 samples (including scattergrams) Quality control: 2 concentrations × 3 lots (120 plots/lot)
Dimensions (mm)	Analyzer (including the sampler (SA-51)): Approx. 760 (W) × 754 (D) × 855 (H) mm Analyzer (including the sampler (CV-11)): Approx. 640 (W) × 901 (D) × 873 (H) mm Pneumatic unit: Approx. 280 (W) × 355 (D) × 400 (H) mm
Weight	Analyzer (including the sampler (SA-51)): Approx. 90 kg Analyzer (including the sampler (CV-11)): Approx. 105 kg Pneumatic unit: Approx. 17 kg
Power source	Analyzer : 100 to 240 V AC, 50/60 Hz Pneumatic unit: 100 to 117 V AC, 50/60 Hz
Power consumption	Analyzer : 600 VA or less Pneumatic unit: 230/280 VA or less (50/60 Hz)

*In U-WAM, the data storage capacity is 100,000 samples and 300 plots × 50 files.

light in the UF-5000 is shorter than the one of the UF-1000*i*. This enables the UF-5000 to detect smaller particles which was not possible with the UF-1000*i*.
The Required sample volume for measurement is also significantly decreased in the UF-5000, compared to the UF-1000*i*.
The UF-5000 does the measurement on the samples and displays the numerical results; the other detailed data including the scattergrams can be viewed only in U-WAM. Most of the operational functions such as monitoring the results and QC charts are available in U-WAM. The system is so designed that the UF-5000 and U-WAM function together as a comprehensive urine analysis screening system.

3. Reportable parameters, measurement ranges and units

Leveraging the vast expansion of available detectable signals and advances in optical analysis technologies, different analytical methods from the conventional ones have been used in the UF-5000. To be more specific, improved measurement accuracy of crystals and red blood cells by detection of depolarized side scattered light [2)], sub-classification of epithelial cells based on the size of the particle, the amounts of nucleic acid content and cumulative side scattered signal providing size information coupled with complexity of internal structure. Analysis of hyaline and non-hyaline casts and mucus elements have been realized by the use of new optical technologies including signal waveform analysis (***Fig. 2***). Moreover, additional new parameters have been added to the menu through the use of the aforesaid new technologies. (***Tables 3 - 5***).

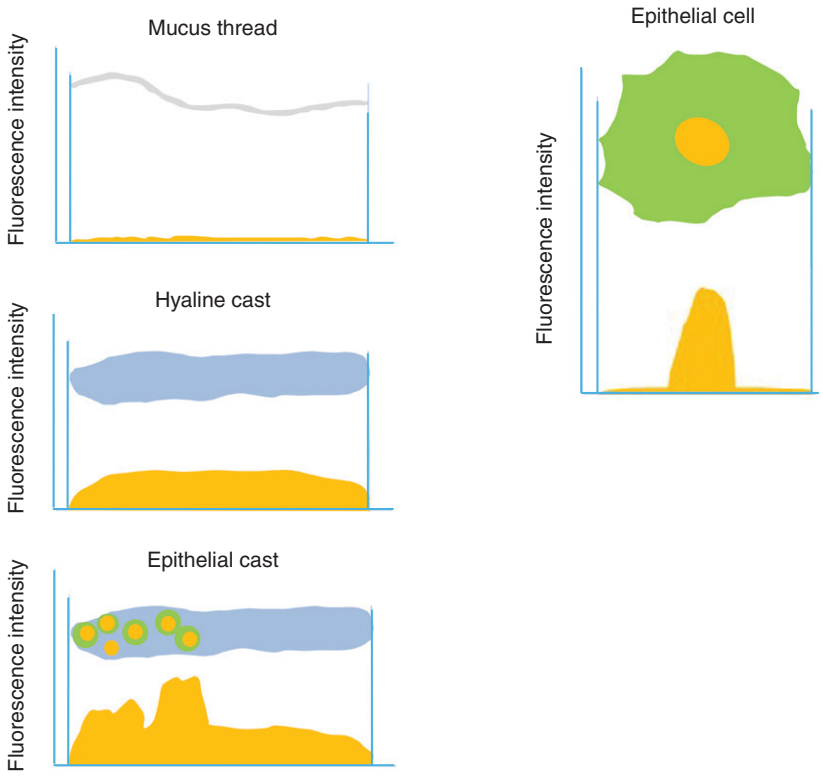


Fig. 2 *Analysis of side fluorescence signal waveforms of various particles*
(The shape of the side fluorescence waveform varies depending on the type of urine particles)

Table 3 *Parameters (urine mode)*

RBC		Red blood cells
	NL RBC	Non-lysed red blood cells
	Lysed RBC	Lysed red blood cells
WBC		White blood cells
	WBC Clumps	White blood cell clumps
EC		Epithelial cells
	Squa.EC	Squamous epithelial cells
	Non SEC	Non-squamous epithelial cells
	Tran.EC	Transitional epithelial cells
	RTEC	Renal tubular epithelial cells
	SRC	Small round cells
Atyp.C		Atypical cells
CAST		Casts
	Hy.CAST	Hyaline casts
	Path.CAST	Non-hyaline casts
BACT		Bacteria
X'TAL		Crystals
YLC		Yeast-like cells
SPERM		Spermatozoa
MUCUS		Mucus
DEBRIS		Debris
Cond.		Conductivity
Osmo.		Osmolality

The measured values of semi-quantitatively displayed parameters can be viewed on the research screen.
The measured results are displayed in categories, such as "5-9/HPF" in the semi-quantitative display.
Note 1: Certain parameters have the following relationships:
EC = Squa.EC + Non SEC
Non SEC = Tran.EC + RTEC
SRC = RTEC
CAST = Hy.CAST + Path.CAST
Note 2: WBC Clumps are not included in WBC count
Note 3: Osmolality is calculated from conductivity using a conversion formula.

* Categories of the individual parameters (reportable, non-reportable, quantitative, semi-quantitative, research-use only) varies depending on the regulatory requirements of each region or country.

Table 4 *Parameters (body fluid mode)*

RBC		Red blood cells
WBC		White blood cells
	MN#	Mononuclear cells (count)
	MN%	Mononuclear cells (ratio)
	PMN#	Polymorphonuclear leukocytes (count)
	PMN%	Polymorphonuclear leukocytes (ratio)
EC		Epithelial cells
TNC		Total number of nucleated cells
BACT		Bacteria
DEBRIS		Debris

*Parameter calculated using a formula
WBC = MN# + PMN#
TNC = EC + WBC

* Categories of the individual parameters (reportable, non-reportable, quantitative, semi-quantitative, research-use only) varies depending on the regulatory requirements of each region or country.

Table 5 *Research information (urine mode)*

Parameter		Description
RBC-Info.	Isomorphic?	Particle size distribution suggests that the RBC are not damaged.
	Dysmorphic?	Particle size distribution suggests that the RBC are damaged or small.
	Mixed?	Particle size distribution of the RBC suggests that the RBC are neither of the above types.
UTI-Info.	UTI?	The combination of measured WBC and BACT counts suggests the presence of bacterial urinary tract infection (UTI).
BACT-Info.	Gram Positive?	The scattergram suggests the presence of Gram positive bacteria.
	Gram Negative?	The scattergram suggests the presence of Gram negative bacteria.
	Gram Pos/Neg?	The scattergram suggests the presence of both Gram positive and Gram negative bacteria.
	Unclassified	The type of the bacteria present is not clear from the scattergram.

Note 1:
RBC-Info.: Red blood cell morphology information
UTI-Info.: UTI information
BACT-Info.: Gram staining information about the bacteria
Note 2:
The algorithm for RBC-Info.[3] and the assessment performance[4] are almost the same as in UF-1000*i*.

*Some improvement is expected due to the improvement of RBC analysis. In UF-5000, lysed and non- lysed RBC (total RBC) are used for the determination of RBC-Info.

*Categories of the individual parameters (reportable, non-reportable, quantitative, semi-quantitative, research-use only) varies depending on the regulatory requirements of each region or country.

4. Linearity, limit of blank value (LoB) [5], limit of detection (LoD) [5] and limit of quantification (LoQ) [5] (*Tables 6, 7*)

These are standard values based the assumption of maximum data variation when a sample prescribed by a specification, such as a standard preparation of particles, is analyzed. Actual samples sometimes may not meet these standards because cellular elements in their native state may react differently than prepared particles.

Table 6 *Linearity, limit of blank value (LoB), limit of detection (LoD) and limit of quantification (LoQ) (urine mode)*

Linearity

Urine	The specifications below are indicated as theoretical values or residual percentages of values measured using a reference analyzer.		
	RBC	Correlation coefficient r ≥ 0.975	
		100 to 10,000/μL:	Within ± 10%
		50 to 100/μL:	Within ± 20%
		1 to 50/μL:	Within ± 35%
	WBC	Correlation coefficient r ≥ 0.975	
		100 to 10,000/μL:	Within ± 10%
		50 to 100/μL:	Within ± 20%
		1 to 50/μL:	Within ± 35%
	WBC Clumps	—[*1]	
	EC	Correlation coefficient r ≥ 0.975	
		50 to 200/μL:	Within ± 30%
		1 to 50/μL:	Within ± 35%
	Squa.EC	Correlation coefficient r ≥ 0.975	
		50 to 200/μL:	Within ± 30%
		1 to 50/μL:	Within ± 35%
	Non SEC	—[*2]	
	CAST	Correlation coefficient r ≥ 0.975	
		1 to 30/μL:	Within ± 40%
	Hy.CAST	—[*3]	
	Path.CAST	—[*3]	
	BACT	Correlation coefficient r ≥ 0.975	
		1,000 to 10,000/μL:	Within ± 20%
		5 to 1,000/μL:	Within ± 35%
	X'TAL	—[*4]	
	YLC	—[*5]	
	SPERM	—[*5]	
	MUCUS	—[*4]	

Limit of blank value (LoB), limit of detection (LoD) and limit of quantification (LoQ)

Parameter	LoB	LoD	LoQ
	Urine		
RBC	0.5/μL or less	1.0/μL or less	1.0/μL or less
WBC	0.5/μL or less	1.0/μL or less	1.0/μL or less
WBC Clumps	—[*1]	—[*1]	—[*1]
EC	0.5/μL or less	1.0/μL or less	1.0/μL or less
Squa.EC	—[*2]	—[*2]	—[*2]
Non SEC	—[*2]	—[*2]	—[*2]
CAST	0.50/μL or less	1.00/μL or less	1.00/μL or less
Hy.CAST	—[*3]	—[*3]	—[*3]
Path.CAST	—[*3]	—[*3]	—[*3]
BACT	1.0/μL or less	5.0/μL or less	5.0/μL or less
X'TAL	0.5/μL or less	1.0/μL or less	10.0/μL or less
YLC	0.5/μL or less	1.0/μL or less	1.0/μL or less
SPERM	0.5/μL or less	1.0/μL or less	50.0/μL or less
MUCUS	—[*4]	—[*4]	—[*4]

*1: Based on the total WBC count
*2: Based on the total EC count
*3: Based on the total CAST count
*4: Based on RBC and CAST counts measured by the SF channel
*5: Based on WBC and EC counts measured by the CR channel

Table 7 *Linearity, limit of blank value (LoB), limit of detection (LoD) and limit of quantification (LoQ) (body fluid mode)*

Linearity

Body fluid	The specifications below are indicated as theoretical values or residual percentages of values measured using a reference analyzer.		
	RBC	100 to 99,999/μL:	Within ± 10%
		50 to 100/μL:	Within ± 20%
		15 to 50/μL:	Within ± 35%
	WBC	100 to 10,000/μL:	Within ± 10%
		50 to 100/μL:	Within ± 20%
		2 to 50/μL:	Within ± 35%

Limit of blank value (LoB), limit of detection (LoD) and limit of quantification (LoQ)

Parameter	LoB	LoD	LoQ
	Body fluid		
RBC	2.0/μL or less	15.0/μL or less	15.0/μL or less
WBC	1.0/μL or less	2.0/μL or less	2.0/μL or less

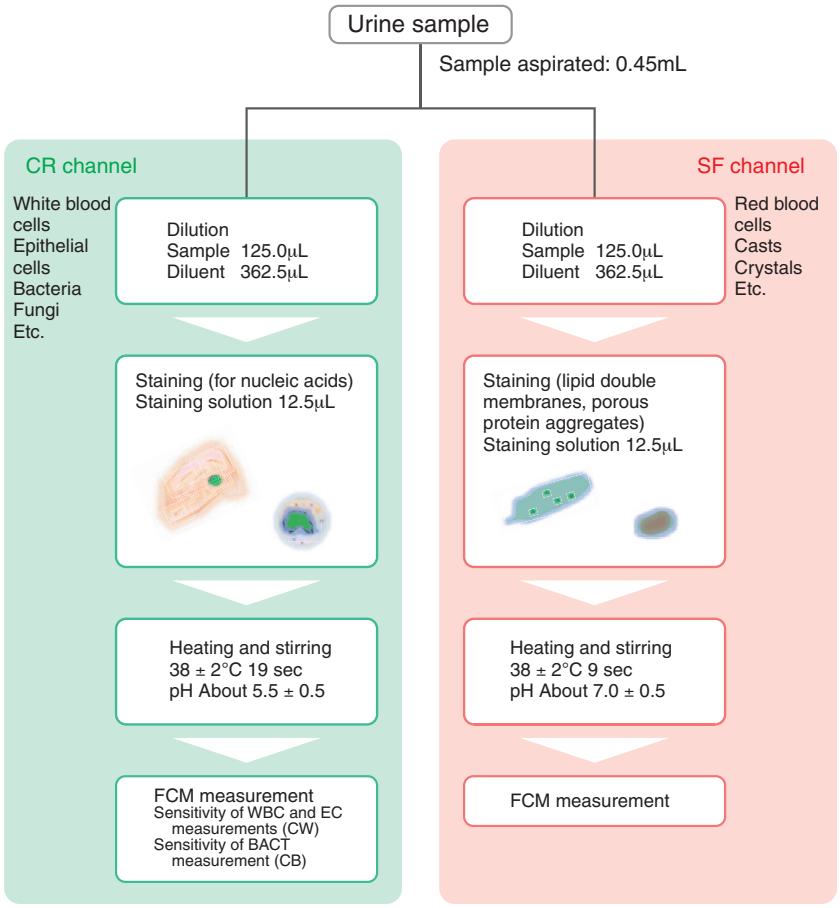
PRINCIPLE OF MEASUREMENT

1. Outline of measurement workflow

The analysis workflow of the UF-5000 is shown in ***Fig. 3*** (Urine mode and body fluid mode).
The aspirated sample is mixed with diluent and staining solution, and then analyzed by flow cytometry. The measurements are made in the newly developed "SF channel" and "CR channel".
The SF channel measures elements that do not have nucleic acids, such as red blood cells, crystals, and casts.
In the CR channel, the red blood cells and crystals are lysed or dissolved, and white blood cells, epithelial cells, bacteria, fungi, etc., all of which have nucleic acids, are analyzed.

2. Functions of the diluent and staining solution in the SF channel

The sample, diluent and staining solution are mixed in the reaction chamber of the SF channel, and heated and stirred. In this process, the amorphous salts that affect the red blood cell analysis are removed by the chelating function [6] of EDTA-2K present in the diluent. Mucus which are mostly attached to bacteria or cells [7] and caused false positive results for CAST and Path.CAST in UF-1000*i*, are dispersed by the surfactant. This has reduced the number of false positive results for CAST. The use of surfactant here does not affect red blood cell morphology, epithelial cells and casts.
In the SF channel, the cellular membranes including internal cellular organisms and porous protein aggregates that constitute the matrix of the cast are stained by a polymethine dye. White blood cells and epithelial cells emit extremely strong fluorescence after this staining, which takes them outside the range of measurement. Casts with inclusions are also strongly stained; however, they can be distinguished from epithelial cells and other elements on the basis of size information coupled with the complexity of internal structure of the particles, staining intensity of the membrane components and the cast matrix, and the results of signal waveform analysis. Thus they remain within the measurement range of SF channel.
Through this mechanism, red blood cells, crystals, hyaline casts and casts with inclusions are measured in the SF channel by flow cytometry.
Table 8 gives an outline of the reagents used in the SF channel.



	CW		CB	
	Urine	Body fluid	Urine	Body fluid
Volume measured by FCM (μL)	31.2	40.0	4.0	24.0
Volume analyzed by FCM (μL)	7.8	10.0	1.0	6.0

	SF	
	Urine	Body fluid
Volume measured by FCM (μL)	31.2	3.9
Volume analyzed by FCM (μL)	7.8	0.975

Fig. 3 *Outline of analysis work flow (urine mode and body fluid mode)*

Table 8 *SF channel reagents*

Diluent	Name	UF-CELLPACK™ SF
	Components	HEPES 1.2% 1,2-Benzisothiazolin-3-one, less than 0.01% (preservative) Chelating agent, surfactant
	Functions	Removes amorphous salts which interfere with RBC analysis when mixed with the chelating agent and heated (the chelating action dissociates the divalent cation from the salts and dissolve them). The surfactant disperses the mucus elements.
Stain	Name	UF-Fluorocell™ SF
	Components	Polymethine dye 0.05% Ethylene glycol 99.9%
	Functions	Stains the lipid double membranes of cells, such as red blood cells, and the porous protein aggregate that constitutes the cast matrix.

3. Functions of the diluent and staining solution in the CR channel

The sample, diluent and staining solution are mixed, heated, and stirred in the reaction chamber of the CR channel. In this process, crystals are dissolved and removed by the mixing of EDTA-2K, which has chelating action, and the acetate buffer, both present in the diluent [8]. Besides this, red blood cells are lysed by the surfactant. The use of surfactant here does not significantly affect the morphology of cellular elements such as white blood cells and epithelial cells.

In the CR channel, the surfactant makes minute holes on the cell membranes to promote the penetration of the polymethine dye into the cells. The polymethine dye stains the nucleic acids in the cells.

White blood cells, epithelial cells, yeast-like cells, spermatozoa and bacteria are measured by flow cytometry in the CR channel. The amount of nucleic acid content is also determined in this channel. There is a vast difference in the amount of nucleic acid content of human derived cells and non-human derived cells in urine specimen of the human. This has made it possible to analyze the cells with greater accuracy (***Fig. 4***). A general outline of reagents used in the CR channel is given in ***Table 9***.

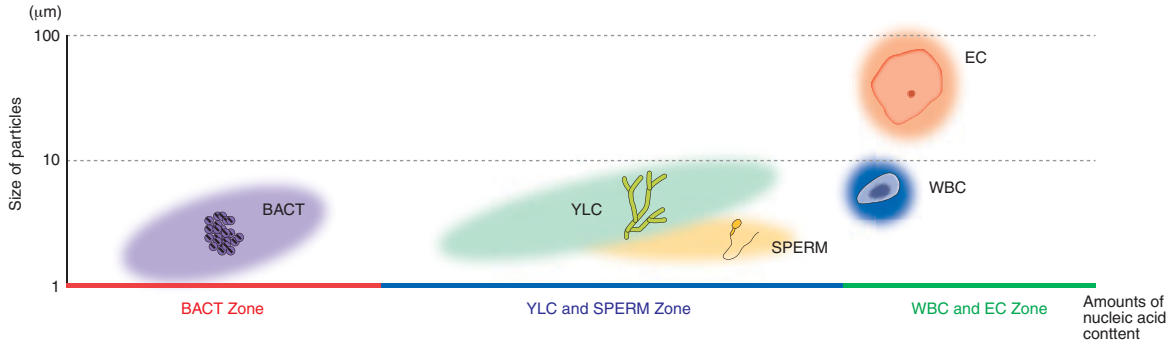


Fig. 4 *Diagrammatic representation of nucleic acid content and the size of particles in urine*

Table 9 *CR channel reagents*

Diluent	Name	UF-CELLPACK™ CR
	Components	Less than 0.1% of acetic acid, surfactant and a chelating agent
	Functions	The surfactant lyses the red blood cells. It makes holes on the cell surface and improves intracellular penetration of the staining solution (cellular elements like white blood cells are not significantly affected). The crystalline elements are dissolved by mixing with a chelating agent and acetic acid, and heating.
Stain	Name	UF-Fluorocell™ CR
	Components	Polymethine dye 0.02% Ethylene glycol 99.9%
	Functions	Staining of nucleic acids

4. Detection and measurement by flow cytometry

Sample that is mixed with diluent and staining solution is introduced to the flow cell after the process of mixing and heating, and measured by flow cytometry. The sheath flow fluid used in the flow cytometry is described in ***Table 10***.

Flow cytometry measurement is performed each in SF channel, and CR channel (CRchannel at the sensitivity level for analyzing WBC and EC, and at the sensitivity level for analyzing BACT). A Total of three measurements are done per sample. The parameters and the methods of analysis obtained from each measurement are shown in ***Table 11*** and ***Fig. 5*** and ***Fig. 6***. The forward scattered light (FSC) provides information about the size and permeability of particles, and the side scattered light (SSC) provides information about the thickness and internal structure of particles. Side fluorescence (FL) provides information about the stainability of particles and depolarized side scattered light (DSS) provides information about the intensity of the birefringence of particles.

Table 10 *Sheath fluid*

Sheath fluid	Name	UF-CELLSHEATH™
	Components	0.14% Tris buffer
	Functions	Creation of sheath flow within the flow cell, washing the hydraulic system of the analyzer

Table 11 *List of optical parameters*

Analysis channel	Optical parameters	Description
SF channel	SF_FSC_P (forward scattered light intensity)	Size / thickness of particles
	SF_FSC_W (forward scattered light signal width)	Length of particles
	SF_FLH_P (side fluorescence intensity (high sensitivity))	Stainability of particles
	SF_FLL_P (side fluorescence intensity (low sensitivity))	
	SF_FLL_W (side fluorescence signal width (low sensitivity))	Length of particles
	SF_FLL_A (side fluorescence signal waveform area (low sensitivity))	Stainability of membrane components and cast matrix
	SF_SSH_P (side scattered light intensity (high sensitivity))	Complexity of internal structure and thickness of particles
	SF_SSL_P (side scattered light intensity (low sensitivity))	
	SF_SSH_A (side scattered light signal waveform area (high sensitivity)	Size information coupled with complexity of internal structure
	SF_DSS_P (depolarized side scattered light intensity)	Intensity of birefringence of particles
CR channel (measured at the sensitivity level for analyzing WBC and EC)	CW_FSC_P (forward scattered light intensity)	Size / thickness of particles
	CW_FSC_W (forward scattered light signal width)	Length of particles
	CW_FLH_P (side fluorescence intensity (high sensitivity))	Stainability of nucleic acids
	CW_FLL_P (side fluorescence intensity (low sensitivity))	
	CW_FLL_A (side fluorescence signal waveform area (low sensitivity))	Amounts of nucleic acid content
	CW_SSH_P (side scattered light intensity (high sensitivity))	Complexity of internal structure and thickness of particles
	CW_SSL_P (side scattered light intensity (low sensitivity))	
	CW_SSH_A (side scattered light signal waveform area (high sensitivity)	Size information coupled with complexity of internal structure
	CW_DSS_P (depolarized side scattered light intensity)	Intensity of birefringence of particles
CR channel (measured at the sensitivity level for analyzing BACT)	CB_FSC_P (forward scattered light intensity)	Size / thickness of particles
	CB_FLH_P (side fluorescence intensity (high sensitivity))	Stainability of nucleic acids
	CB_FLL_P (side fluorescence intensity (low sensitivity))	
	CB_SSH_P (side scattered light intensity (high sensitivity))	Complexity of internal structure and thickness of particles

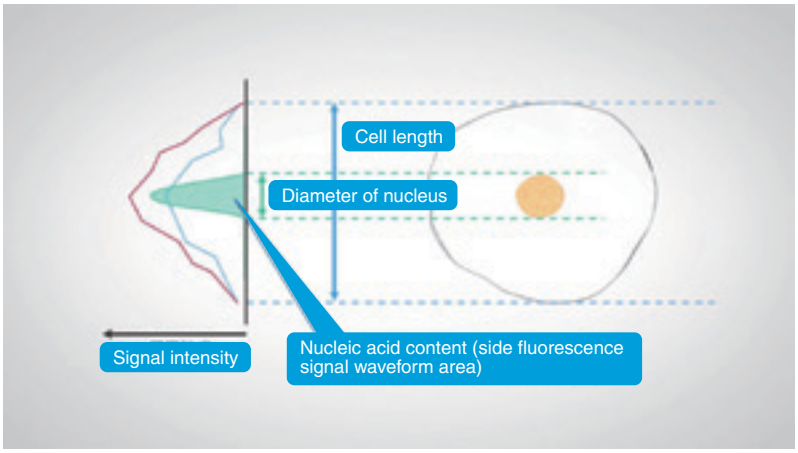


Fig. 5 *Signal waveforms obtained by UF-5000*

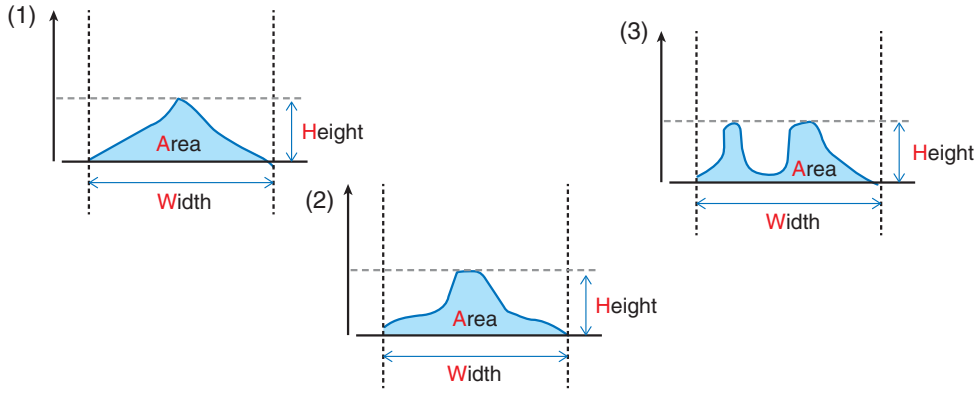


Fig. 6 *Signal waveform analysis by UF-5000*

As described above, the UF-5000 carries out the analysis based on a variety of optical parameters. In particular, these include signals related to birefringence, amounts of nucleic acid content, etc. of the particles which cannot be measured by ordinary microscopic analysis. Thus the analyzer has capabilities that go beyond those of visual observation. In this respect, it is significantly different from the UF-1000*i*.

As can be seen in the example shown in ***Fig. 5***, in which the x-axis is the side fluorescence, the area under the curve reflects the amount of nucleic acid content.

When the x-axis is side scattered light, the area reflects size information of the particle coupled with the complexity of its internal structure.

Even when the height, width and the area of the curve are the same (***Fig. 6*** (1), (2) and (3)), if the shape of the signal waveform is different, the particles are assessed as different. This analytical capability contributes to improved differential classification of CAST from MUCUS.

Information on birefringence of the particle is obtained by analyzing depolarized side scattered light. Polarized light is light in which the plane of oscillation i.e., their plane of polarization of all the light waves, is the same. Laser light is basically polarized light [9]. Many solid substances, including crystals, have the property of birefringence (***Fig. 7***). When such a substance is irradiated, the plane of polarization changes. Therefore, if

we place a polarized filter upstream of the photomultiplier to block light with the same plane of polarization as the original laser beam, we can detect only the light whose plane of polarization has been changed (***Fig. 8***). The UF-5000 captures the same image as the polarized light image seen under a polarized light microscope. Therefore it can obtain information from particles such as crystals that can be observed under polarized light microscopes. As shown in ***Fig. 7***, when a substance having birefringence is irradiated with light having random planes of polarization (such as sunlight) from your side to the opposite side, light with plane of polarization along the direction of the green arrows and those with plane of polarization along the direction of the blue arrows are transmitted differently within the substance.

In this manner, when a polarized light beam with a single plane of polarization, such as a laser beam, is irradiated on such a substance, the light that has passed through the substance emerges with a different plane of polarization.

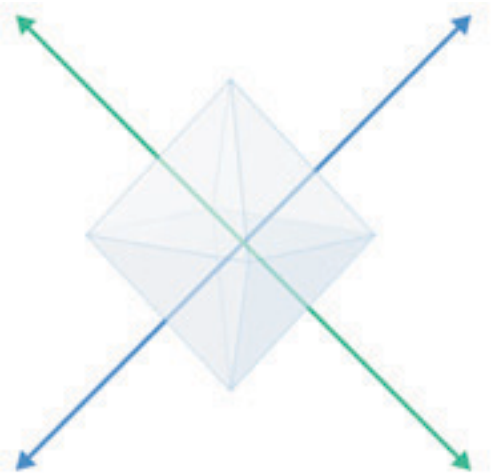


Fig. 7 *A substance having birefringence property*

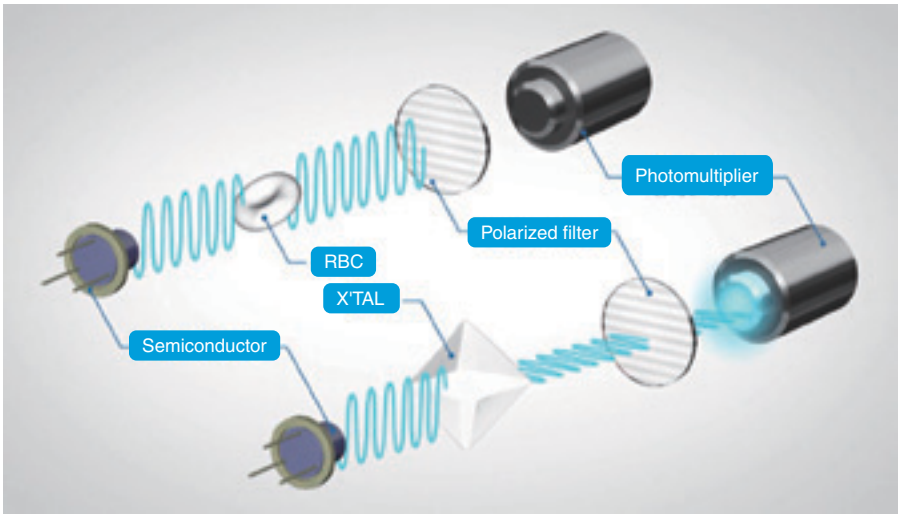


Fig. 8 *Diagram showing the method of detecting depolarized side scattered light*

5. Scattergram analysis (urine mode)

The signal intensity, signal width, signal waveform area, and the signal waveform of forward scattered light, side fluorescence, side scattered light and depolarized side scattered light of each particle obtained in the flow cytometry are analyzed, and classified by applying differentiation algorithms that take this data into account. The results of this analysis (differential classification) are displayed as scattergrams (the scattergrams can be displayed only in the U-WAM).

1) RBC/X'TAL scattergram
[SF Channel SF_SFC_P/SF_FLH_P (Not displayed by UF-5000)]

This scattergram is not shown in UF-5000; however, it was used in UF-1000*i* (***Fig. 9***). In general, crystals show low staining intensity compared to red blood cells. White blood cells and epithelial cells, which get stained strongly, appear on the right side of the scattergram and thus can be separated from particles without nuclei. CAST (including Path.CAST) are distributed at a relatively low intensity of forward scattered light. Although the forward scattered light intensity reflects the size of particles that correspond to its particle type, the actual forward scattered light intensity changes depending on the permeability and the amount of scattered light of the particles. Therefore, the forward scattered light intensity for dehemoglobinized red blood cells is thought to be weaker even if the size is the same. If the particle size is larger than the width of the laser beam, only the irradiated portion (cross section) of the particle will be reflected as its forward scattered light intensity. This is because the central part of the flow cell has the highest flow rate, and the particles present there move with their long axes aligned with the direction of the flow. The likely reasons for the relatively low forward scattered light intensity of CAST are (1) permeability and (2) the fact that the forward scattered light intensity reflects the cross sectional area in the case of large particles.

2) RBC/X'TAL scattergram
[SF channel SF_FSC_P/SF_DSS_P]

This scattergram shows the intensity of the depolarized side scattered light, a new signal detected by the UF-5000. It is mainly used to differentiate RBC from X'TAL (***Fig. 10***). The lateral placement of crystals and red blood cells is reversed compared to the SF_FSC_P/SF_FLH_P scattergram (***Fig. 9***). The RBC appear as red dots at a low intensity zone of depolarized side scattered light on the RBC/X'TAL scattergram. As RBC do not have birefringence, their depolarized side scattered light intensity is low. X'TAL appear as aqua blue dots on the same scattergram. The depolarized side scattered light intensity reflects the birefringence of the crystals. Therefore, the higher the intensity a crystal shows, the more to the right side the dot is displayed on the scattergram. Red blood cells which have very small depolarized side scattered light intensity appear on the left side. In UF-1000*i*, red blood cells and crystals were differentiated using side scattered light intensity, which had limitations because some red blood cells and crystals had similar side scattered light intensity. Depolarized side scattered light measurement was newly introduced in the UF-5000 as a powerful means of resolving this problem. As described above, depolarized side scattered light reflects birefringence of the particle and therefore, more precise differentiation of red blood cells from crystals became possible with the UF-5000. The fluorescence signal is also used in the UF-5000 for this differential classification, as in the UF-1000*i*.

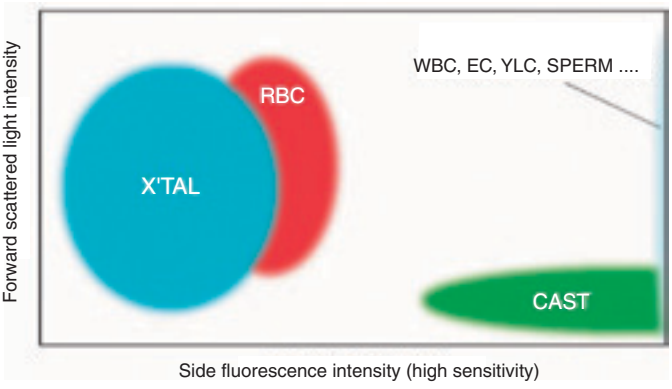


Fig. 9 *The RBC/X'TAL scattergram (not displayed in UF-5000)*

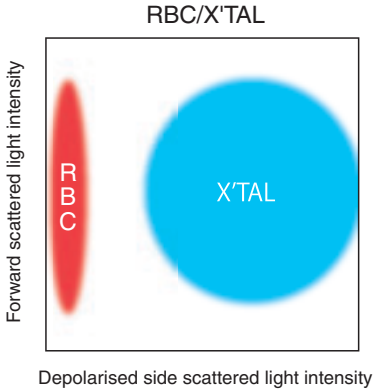


Fig. 10 *The RBC/X'TAL scattergram*

Additionally, as in the UF-1000*i*, the UF-5000 prepares a red blood cell histogram based on forward scattered light intensity and analyzes red blood cell morphology information (RBC-Info.). We will not discuss the method of this analysis here as it is the same as in the UF-1000*i* (***Fig. 11***).

3) CAST scattergram
[SF channel SF_FLL_A/SF_FLL_W]
This scattergram displays the results of differentiation of Path.CAST, Hy.CAST and MUCUS (***Fig. 12***). Hy.CAST appear as dark green dots on the CAST scattergram. As the total dye uptake is smaller in Hy.CAST than in Path.CAST, the side fluorescence waveform area of the former is somewhere intermediate between those of MUCUS and Path.CAST. Path.CAST appear as yellow-ish green dots on the scattergram. The more inclusions there are in a cast, the higher is the total dye uptake. Therefore, the side fluorescence waveform area of a Path.CAST is larger than of a Hy.CAST. MUCUS appear as brown dots on the scattergram. Mucus threads are dispersed by the surfactant in UF-CELLPACK™-SF. Therefore, their side fluorescence signal waveform area, which reflects the total dye uptake, in the SF channel is smaller than that of Hy.CAST.
Here, the side fluorescence signal waveform area represents the sum total of fluorescence intensity. The more inclusions there are in the cast the higher is the total fluorescence intensity. Here, as the mucus threads are dispersed by the diluent, and are thus generally believed to be in an unraveled state, the total fluorescence intensity is also small (***Fig. 13***).

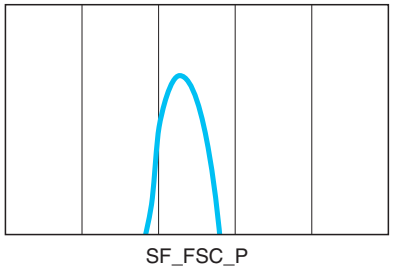


Fig. 11 *The RBC histogram*

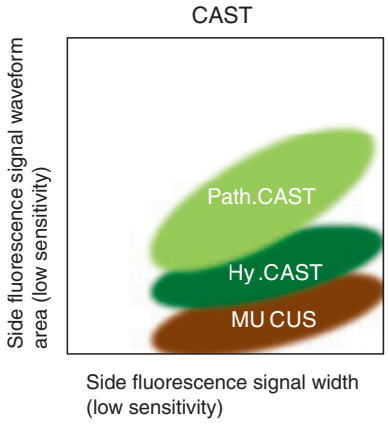


Fig. 12 *The CAST scattergram*

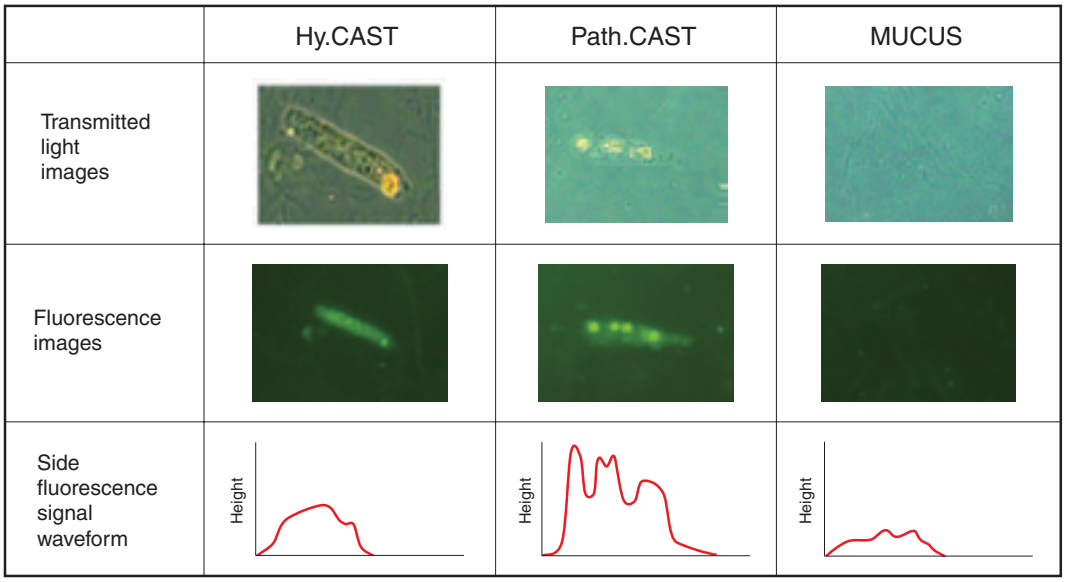


Fig. 13 *Stained images and side fluorescence signal waveforms of casts and mucus*

The side fluorescence signal width can be practically taken as reflecting the length of the particles. Because of the high fluorescence intensity in the SF channel, the entire cast is stained even if it is a hyaline cast. The method of displaying the side fluorescence signal width as the length of the particles is the same as in the UF-1000*i*. The Path.CAST, Hy.CAST and MUCUS are distributed diagonally towards the top right in the scattergram because the accumulated fluorescence intensity of the particle becomes larger as the length increases.

Signal waveform analysis and side scattered light information are also used for the actual differentiation of Path.CAST, Hy.CAST and MUCUS. However, for the sake of ease of interpreting the plots, the same type of scattergram as used in the UF-1000*i* have been also used in the new analyzer .

4) WBC/EC1 scattergram
[CR channel CW_FSC_W/CW_SSH_A]
WBC/EC2 scattergram
[CR channel CW_FSC_W/CW_FLL_A]

EC and WBC are displayed in these scattergrams (***Fig. 14*** and ***Fig. 15***).

The forward scattered light signal width reflects the length of the particles and the side scattered light signal waveform area reflects size information that is coupled with the complexity of the internal structure. The side fluorescence signal waveform area reflects the amount of nucleic acid content. (As nucleic acid is present not only in the nucleus but also in the mitochondria, in cells with an abundance of intracellular organelles the value of the side fluorescence signal waveform area becomes slightly larger.)

The WBC/EC1 scattergram (***Fig. 14***) reflects cell morphology. It gives size information that is coupled with the complexity of the internal structure of the cell, in relation to the length (long axis dimension) of the particle.

WBC appear as blue dots on the WBC/EC1 and -/EC2 scattergrams where they converge at bottom left.

In the above-mentioned scattergrams, EC appear as orange, pale orange or reddish brown dots, and they represent the total counts of all kinds of epithelial cells that measured by the analyzer.

Squa.EC appear as orange colored dots on the scattergrams. The Squa.EC counted by this analyzer mainly consist of superficial layer squamous epithelial cells with the size in range of about 60 - 100μm. The amount of nucleic acid contents is low and the size information is small relative to the length of particles (dimension along the long axis of the cell). Therefore, compared to other kinds of epithelial cells, their side fluorescence signal waveform area and side scattered light signal waveform area are relatively smaller with respect to their forward scattered light signal width which reflects the length of particles. Non SEC, on the other hand, appear as reddish brown or pale orange dots on the scattergrams. Their size data is large relative to the length of the particles.

The WBC/EC2 scattergram (***Fig. 15***) reflects the length of the particles and the amounts of nucleic acid content.

Squa.EC have small amounts of nucleic acid content relative to the length of the particles where as Non SEC have high amounts of nucleic acid content relative to their length.

WBC Clumps appear as pale blue dots on the WBC/EC1 and -/EC2 scattergrams. WBC clumps have a larger side fluorescence signal waveform area than WBC because the clump as a whole has higher nucleic acid content. The larger the clumps the greater would be the amount of nucleic acid content and the size. Therefore, the distribution of the dots on the scattergram spreads upward towards the right.

As described above, the WBC, Squa.EC and Non SEC are displayed on these two scattergrams.

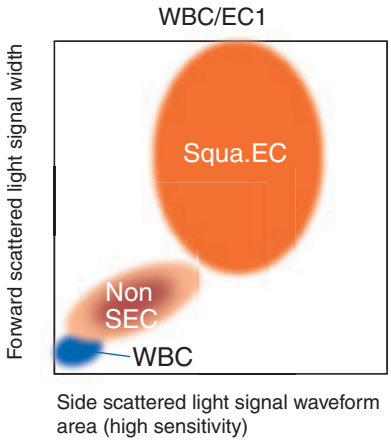


Fig. 14 *The WBC/EC1 scattergram*

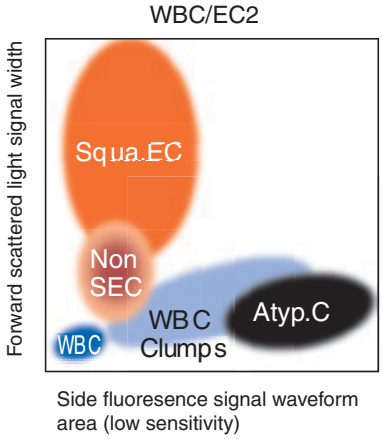


Fig. 15 *The WBC/EC2 scattergram*

Non SEC are further classified into Tran.EC and RTEC. Tran.EC appear as pale orange dots and RTEC appear as reddish brown dots on the WBC/EC1 and /EC2 scattergrams. Tran.EC and RTEC appear in the same area of the scattergram using the length of particles (forward scattered light signal width) and the amounts of nucleic acid content (side fluorescence signal waveform area) or the length or particles (forward scattered light signal width) and the size information coupled with complexity of internal structure (side scattered light signal waveform area). However, both Tran.EC and RTEC can be differentiated based on the amount of nucleic acid content (side fluorescence signal waveform area) and the size information coupled with complexity of internal structure (side scattered light signal waveform area). This principle is based on the fact that Tran.EC tend to have a greater amount of nucleic acid content than RTEC for a given cell size.

Apart from Tran.EC and RTEC, Atyp.C (atypical cells) is also a research parameter that is a part of Non SEC. These appear as black dots on the right side of the WBC/EC2 scattergram. These Atyp.C include cells with abnormally increased nucleic acids such as atypical cells, cells with cytoplasmic inclusions, and virus infected cells, and side fluorescence signal waveform area becomes larger. Compare to Tran.EC and RTEC as previously described, the length of particles (forward scattered light signal width) and the size information coupled with complexity of internal structure (side scattered light signal waveform area) of Atyp.C are about equivalent.

5) YLC/SPERM scattergram
[CR channel CW_FSC_P/CW_FLH_P]

This scattergram displays YLC and SPERM (***Fig. 16***).

YLC appear as pale green dots on the YLC/SPERM scattergram. Several nucleus are included by a chain of YLC and the amount of nucleus is depending on the state of budding. The size of the cell and its amount of nucleic acid content increase by the advancement of budding process. Both forward scattered light intensity and side fluorescence intensity increase as budding progresses further, and this leads to the distribution of the dots spreading diagonally upwards towards the right on the scattergram.

SPERM on the other hand appear as pale yellow dots on the YLC/SPERM scattergram. The heads of spermatozoa are normally uniform in size. Therefore, the forward scattered light intensity and staining intensity are constant. The horizontal spread of the distribution of spermatozoa on the scattergram is due to the characteristic features of the YLC/SPERM scattergram. Waveform analysis is also used for differentiating SPERM.

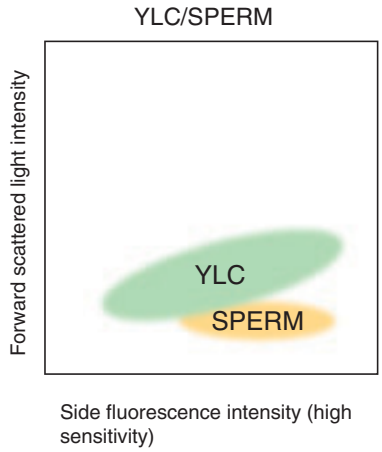


Fig. 16 *The YLC/SPERM scattergram*

6) BACT scattergram
[CR channel CB_FSC_P/CB_FLH_P]
This scattergram displays BACT and DEBRIS (***Fig. 17***). The sensitivity of detection of forward scattered light has been enhanced by the use of the blue semiconductor laser. Besides this, the use of a new stain has increased the difference between BACT and DEBRIS in their stainability (side fluorescence intensity). As a result of this, the new analyzer has an improved ability to detect small bacteria such as *Pseudomonas aeruginosa* compared to the UF-1000*i*. It has been confirmed that the shape (angle of distribution of the dots) of clusters in the BACT scattergram varies depending on the type of bacteria present. BACT appear as purple colored dots on the BACT scattergram. Measurement is done at the sensitivity level used for detection of bacteria in the CR channel.
DEBRIS are fine components such as cell fragments. They are counted as DEBRIS to separate them from the BACT count in order to strengthen the analytical accuracy of the BACT parameter. DEBRIS appear as gray dots on the BACT scattergram.
The UF-5000 displays Gram-staining information of bacteria (BACT-Info.) estimated from the scattergram. The forward scattered light intensity reflects the differences in the cell wall constituents, such as the peptidoglycan layer of the bacteria cells. Gram positive bacteria which have a thick peptidoglycan layer generally show higher intensity of forward scattered light than Gram negative bacteria. The side fluorescence intensity reflects the amount of dye that has penetrated into the bacterial cell, which is affected by differences in the cell wall structure. For Gram positive bacteria, intensity of side fluorescence is lower due to the lesser amount of dye that penetrates into the bacterial cell. This is caused by the difference of bacterial cell wall structure. On the other hand, intensity of side fluorescence for Gram negative bacteria is higher due to a large amount of dye penetrates into the bacterial cell. This BACT-Info. is displayed as one of the following 4 messages: "Gram Positive?", "Gram Negative?", "Gram Pos/Neg?" and "Unclassified".

6. Scattergram analysis (body fluid mode)

1) SF channel
In the body fluid mode, red blood cells are analyzed in the SF channel (***Fig. 18***). The method of fractionation used here is the same as in the urine mode.
2) CR channel (body fluid mode)
Unlike in the urine mode, the epithelial cells are not sub-classified and the white blood cells are differentially classified into MN and PMN (***Fig. 19***). MN appear as light green dots on the MN/PMN scattergram. MN contains lymphocytes and monocytes and present on the diagonal line from lower left to upper right on the MN/PMN scattergram. PMN appear as blue dots on the MN/PMN scattergram. Side scattered light intensity is higher and side fluorescence intensity is lower due to the presence of granules and segmented nucleus.

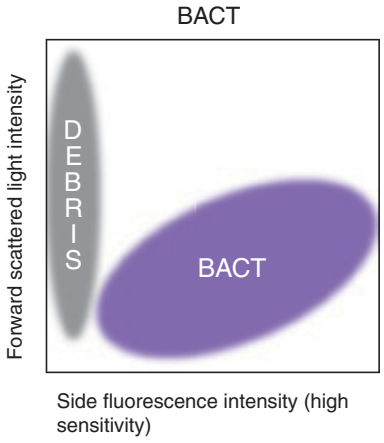


Fig. 17 *The BACT scattergram*

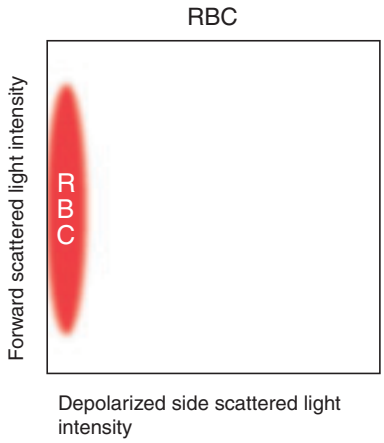


Fig. 18 *Body fluid mode scattergram (SF channel)*

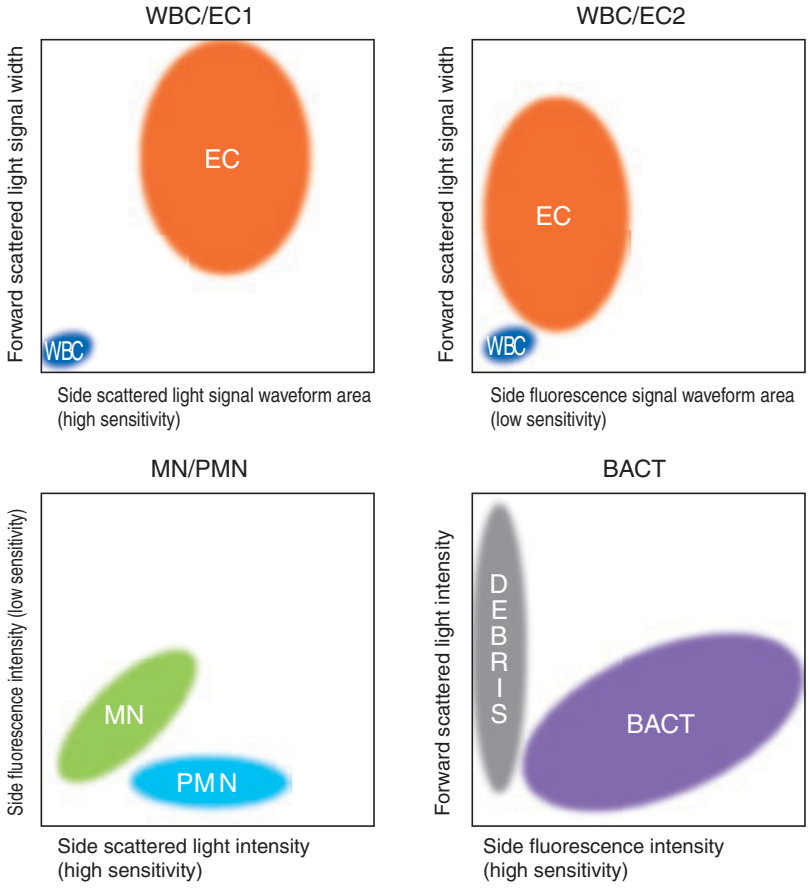


Fig. 19 *Body fluid mode scattergram (CR channel)*

DISPLAY AND OUTPUT OF ANALYSIS RESULTS

As described above, the intensity, signal width, signal waveform area and the signal waveform of forward scattered light, side fluorescence, side scattered light and depolarized side scattered light obtained by flow cytometry for each particle are analyzed and the particles are differentially classified using algorithms that take into account all these results.

The results screen of the analyzer main unit displays the results of the analysis (quantitative and semiquantitative), comments (***Fig. 20***), and the results for research parameters, etc. The analyzer is designed to be used with U-WAM for review of the scattergrams (***Fig. 21***). The main unit can also be connected to a ticket printer to which selected analysis results can be output (analysis results of both reportable parameters and research parameters can be output, but the output of the research parameters is restricted by access authorization).

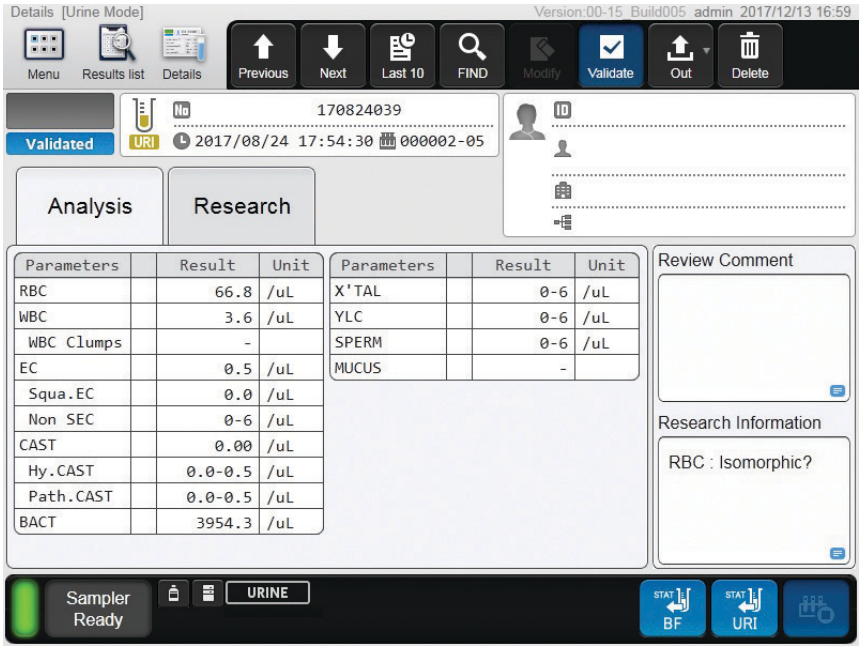


Fig. 20 *Analysis results screen (UF-5000)*

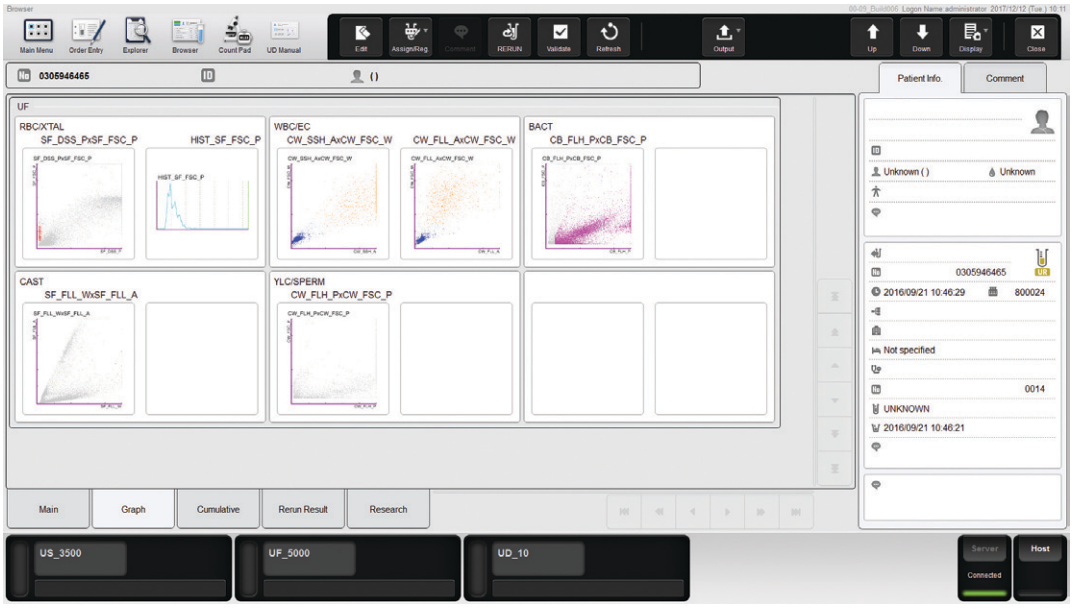


Fig. 21 *Analysis results (scattergram) screen (U-WAM: Ver.00-05)*

FUNCTIONS OF ANALYZER

The functions of the analyzer main unit are as follows. Many of these functions are common with functions provided in Sysmex hematology analyzers, etc.

* Sampler measurement and stat measurement
* Data storage capacity for analysis results
* Quality control
* Anti-carryover
  In cases where RBC or WBC is ≥ 10,000/μL or BACT ≥ 1,000/μL, the anti-carryover function is activated to perform auto rinses (these thresholds can be changed through analyzer setting).
* Flagging of low reliability data (abnormal classification, abnormal conductivity, etc.)
* Threshold setting and flagging functions for abnormal (positive) samples and REVIEW samples
* Automatic recognition of the lot number and validity from the IC tag of the reagents (staining solutions)
* Barcode recognition for reagents and controls (reading of lot number and reference values)
* Automatic monitoring of remaining reagent volumes

QUALITY CONTROL AND CONTROL MATERIALS

As in the UF-1000*i*, the UF-5000 has quality control functions. X-bar control or L-J control can be implemented. UF-CONTROL™ (***Table 12***) is a quality control material for use with the analyzer. It is available in two levels, UF-CONTROL™-H and UF-CONTROL™-L. The lot information of a new control material can be registered by reading the barcode on the assay sheet that comes with the UF-CONTROL™, using a hand-held barcode reader.

For implementing quality control, the control material and its lot that will be used are first selected on the analyzer screen. The control material is then placed in the sample cup, set in the STAT sample holder, and the measurement is made.

The analysis results can be viewed on the QC chart screen and the radar chart screen. The analyzer main unit can retain in its memory the results of 2 concentrations × 3 lots, and the U-WAM can memorize 300 plots × 50 files.

The purpose of internal quality control using the quality control function of the analyzer is to verify the repeatability of analysis by the analyzer of each laboratory. The analyzer is calibrated during its production and at the time of periodic maintenance. The internal quality control is to verify whether the analyzer continued to function within the specifications and the specified performance level. The target values and the upper and lower limits given in the assay sheet provided with the control material are to be used as indices of accuracy while conducting routine quality control. However, strictly speaking, these cannot be used as indices for evaluating the routine quality control results. The internationally recognized CLSI C24-A3 [10] guideline of Clinical and Laboratory Standards Institute (CLSI) recommends that "the assay values provided by the manufacturers should be used only at the start of internal quality control, and ideally, the target values and limits may be determined using 20 measurements made on different days". Therefore, for internal quality control of the UF-5000, the assay sheet that comes with each control material may be taken as the approximate guideline and the actual target values and limits should preferably be set on the basis of actually measured values. Considering the principle of measurement used by this analyzer, the analysis results obtained from sensitivity parameters, such as SF_FSC_P, have greater importance than the results of particle count analysis of control materials. This is because when a sensitivity parameter shows a large change, the distribution of the dots on each scattergram changes, making it difficult to achieve the designed differential classification performance. All the particle counts measured by the analyzer are believed to be sufficiently guaranteed by the measured RBC and WBC counts of the controls.

Table 12 *UF-CONTROL™*

Name	UF-CONTROL™
Components	UF-CONTROL™-H: Particulate component 0.4% (W/W)
Functions	UF-CONTROL™-L: Particulate component 0.1% (W/W)
	Control materials for quality control of Sysmex fully automated urine particle analyzer and fully automated urine particle digital imaging device
Remarks	Contains latex particles

CONCLUSION

The UF-5000 is a new urine particle analyzer, which is capable of analyzing the birefringence of particles, amount of nucleic acid content of cells, size information due to the installation of new technologies such as a blue semiconductor laser, an improved optical system and signal waveform analysis. As the technology to differentially classify particles has also been improved in this new analyzer, it may be considered a next generation urine particle analyzer and not merely an improved version of the previous models. The new analyzer not only has better performance in differentiating between the conventional parameters such as mucus threads from casts and red blood cells from crystals but also has the newly introduced research parameter Atyp.C, by utilizing information for the amounts of nucleic acid content of the particle.

Possibly, with future advancement of clinical application research, such as direct comparison of information obtained by the analyzer with guideline-based clinical profile of the disease, analyzers are expected to provided new evidence-based test information to the point of routine medical care and not be merely positioned as a screening devices before deciding to undertake microscopic urine sediment analysis.

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