AUTOMATED ANALYSIS OF URINE SEDIMENT

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CLINICAL AND RESEARCH LABORATORY ON URINARY SEDIMENT

U.O. DI NEFROLOGIA E DIALISI

FONDAZIONE IRCCS CA' GRANDA OSPEDALE MAGGIORE POLICLINICO

MILANO- ITALY

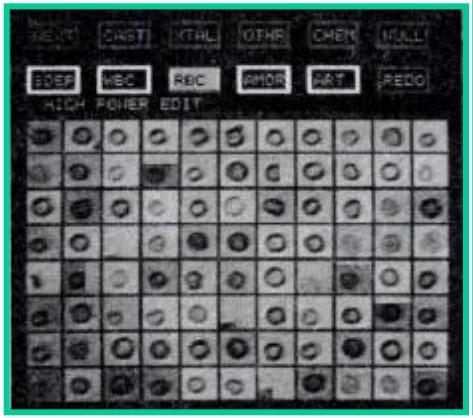
1985: THE FIRST AUTOMATED URINE SEDIMENT ANALYZER

CLIN. CHEM. 31/9, 1491–1499 (1985)

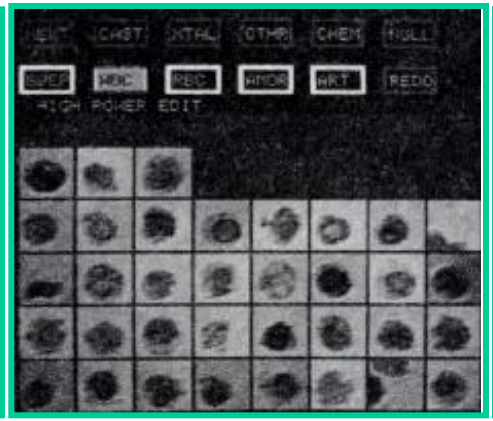
"The Yellow IRIS™" Urinalysis Workstation—The First Commercial Application of "Automated Intelligent Microscopy"

F. H. Deindoerfer, J. R. Gangwer, C. W. Laird, and R. R. Ringold

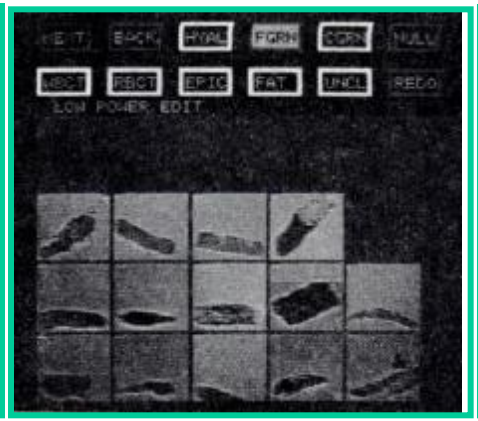
RBC



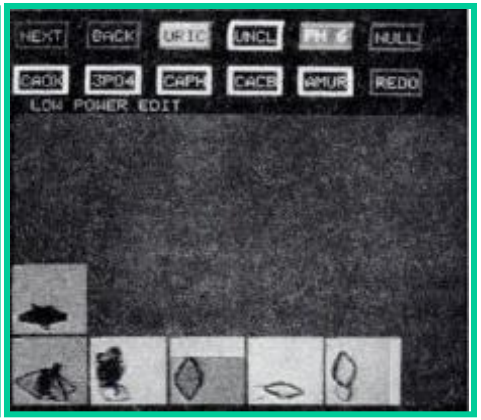
WBC



CASTS



CRYSTALS



3 JULY 1995: 11th IFCC EUROPEAN CONGRESS OF CLINICAL CHEMISTRY, TAMPERE (FINLAND)

Workshop 2: STRATEGIES IN URINALYSIS

Prof Dolphe Kutter

"Automation of urinalysis: possibilities and problems"

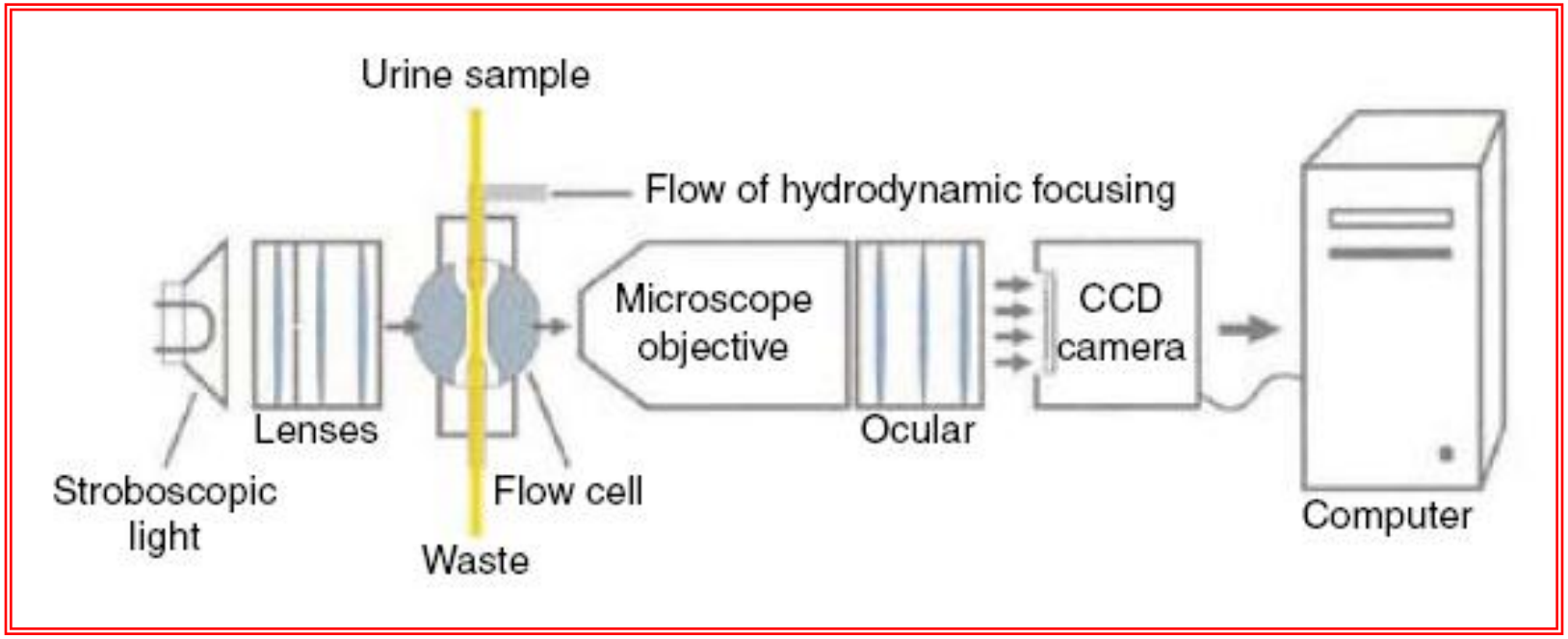
During the discussion that followed, a representative of an international company stated:

"Our company has decided to stop investing in this sector because the technology is not assisting us any further, we feel at a standstill…"

TODAY, 20 YEARS LATER

- In the developed world, automated urine sediment analyzers are in use in *all large laboratories*
- Three types of instruments are on the market, each one being based on its own technology:
- Automated intelligent microscopy (iQ200, Beckmann)
- Flow cytometry (UF-1000i, Sysmex)
- Cuvette-based microscopy (UriSed/sediMAX, 77 Elektronika/A. Menarini Diagnostics)

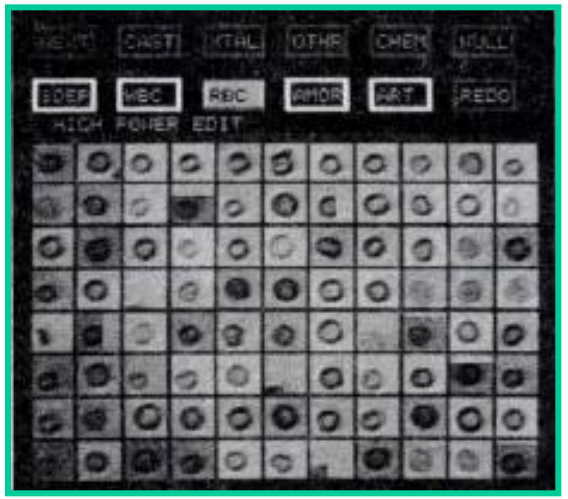
AUTOMATED INTELLIGENT MICROSCOPY: iQ200



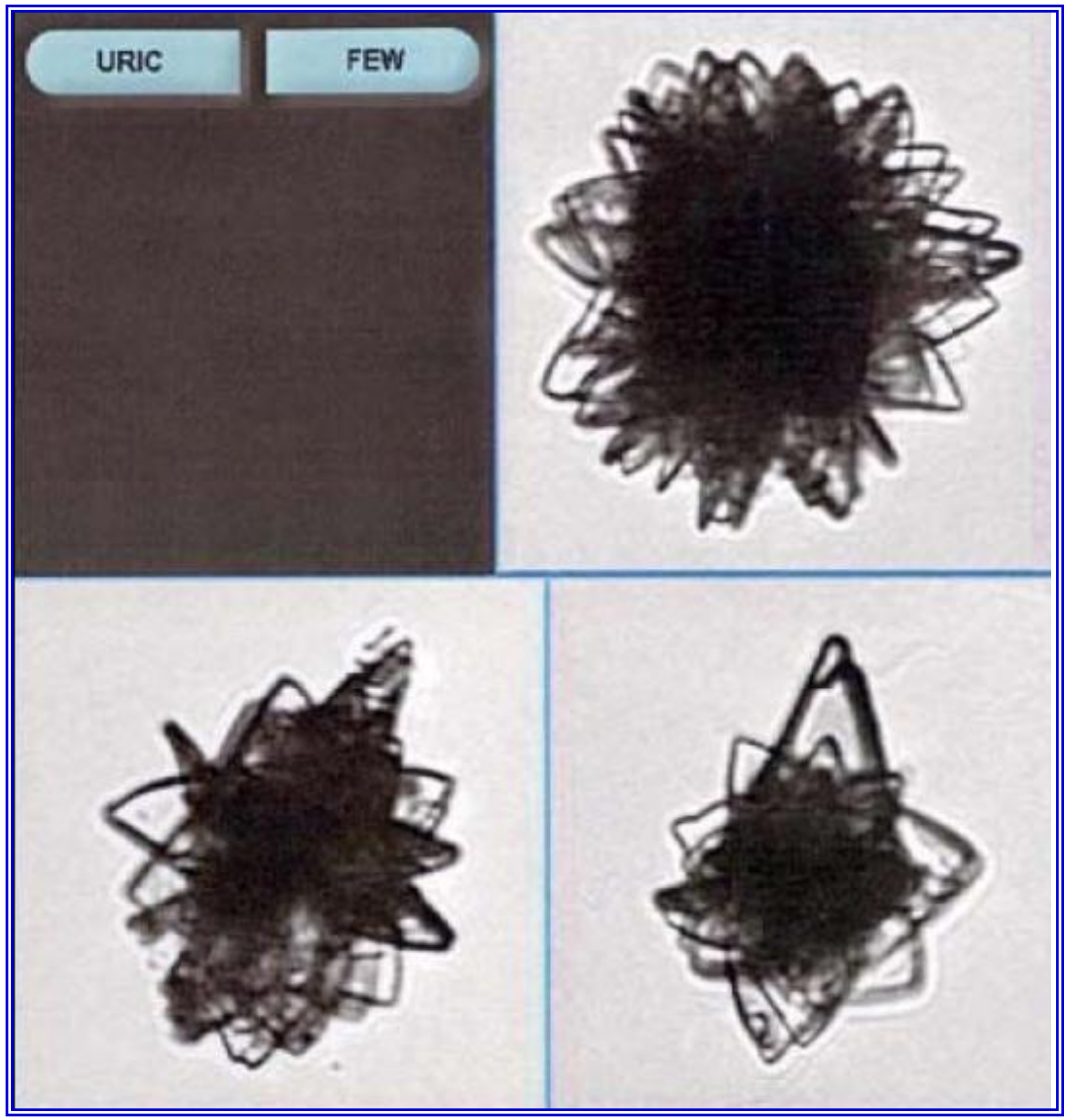
- An automated microscope is focalized on a planar flow cell, in which the particles flow as a sheet, being sandwiched between two layers of an enveloping fluid
- A stroboscopic lamp, firing 24 bursts/second, stops the motion of the particles passing through the camera
- The stopped motion view is observed through magnifying lenses
- The images are collected by a videocamera

- A very high number of images/sample is taken
- For each particle, the background is removed in order to better identify and show the particle
- Each particle is analyzed by a neural network which contains 26,000 reference images
- Each particle is isolated within one image, which is then inserted in one particle category

RBC



EXAMPLE OF IMAGES SUPPLIED BY iQ200 (URIC ACID)



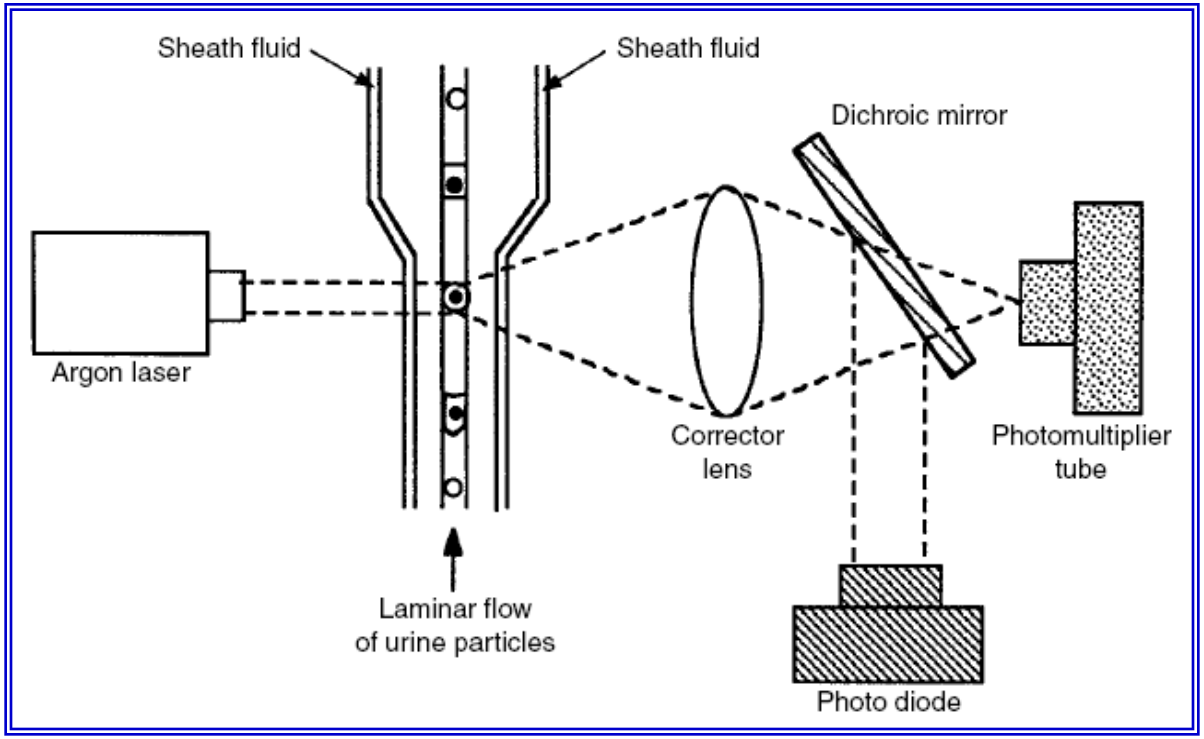
PARTICLES IDENTIFIED

- Erythrocytes
- Leukocytes
- Leukocyte clumps
- Squamous epithelial cells
- Non-squamous epithelial cells
- Hyaline casts
- Pathological casts
- Crystals
- Bacteria
- Yeasts
- Spermatozoa
- Mucus
- Unclassified particles (= all the individual images which cannot bye recognized confidentially by the software and need to be reclassified by the operator)

OTHER FEATURES OF iQ200

- The minimum urine volume required = 3 mL
- 1 mL is aspitrated
- 2 µL are used for analysis
- Quantitative results as No/µL, No/HPF, No/LPW or class intervals
- Throughput: 60 samples/hour

FLOW CYTOMETRY: UF-1000i

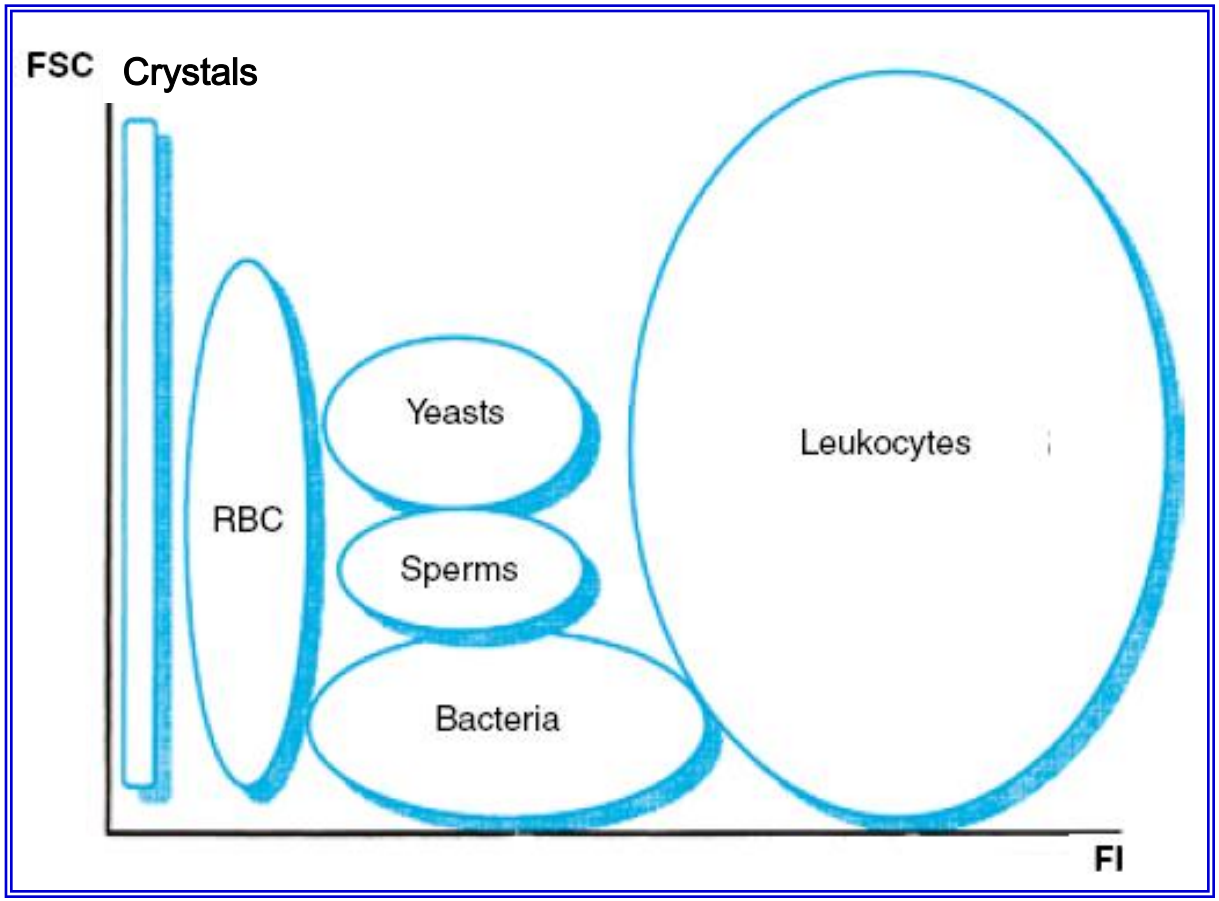


- Passage of the sample into two laminar flow cells (one for bacteria, one for the other particles) obtained by passing a sheath liquid around the sample
- Automatic staining of the particles with two fluorochromes, one for nucleic acid and the other for cell membranes
- Irradiation of the sample with an argon laser beam
- Detection of both scattered light and fluorescence, which are converted into the 4 following parameters:

UF-100: DISTRIBUTION OF THE U-sed PARTICLES

FSC =

Forward scattered light intensity

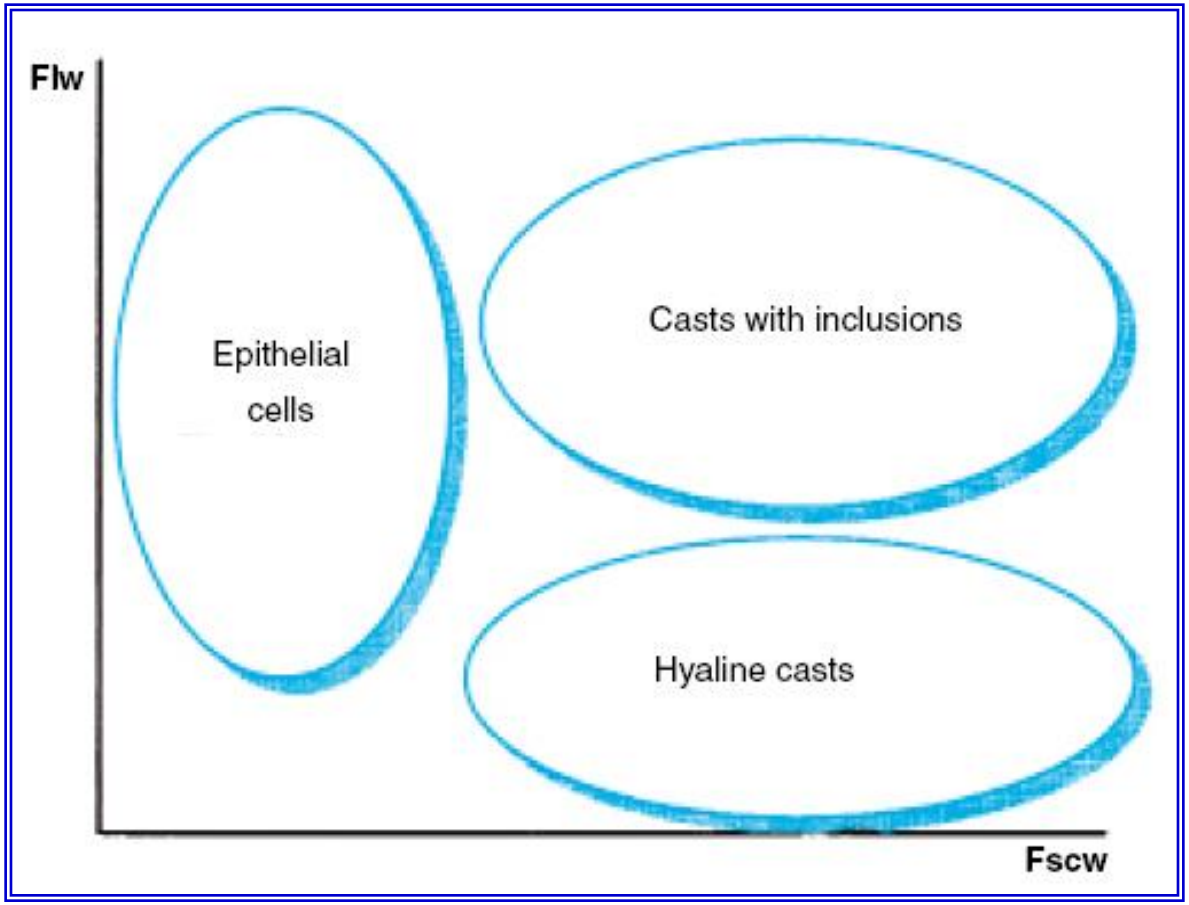


FI = fluorescence intensity

UF-100: DISTRIBUTION OF THE U-sed PARTICLES

Flw =

Fluorescence pulse width



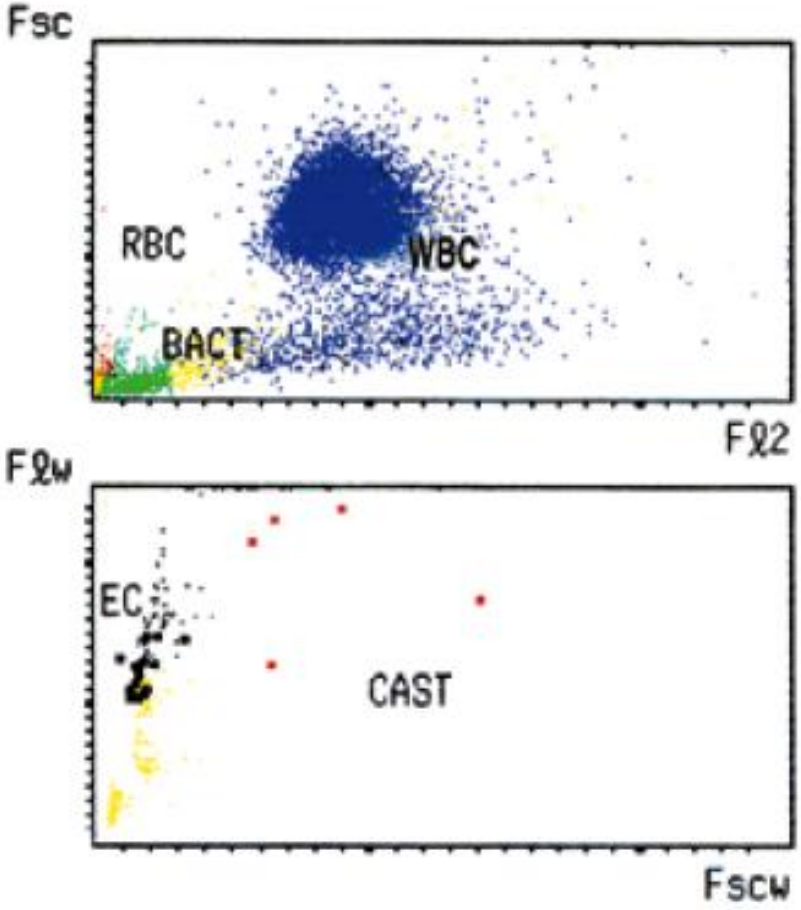
Fscw = forward scattered light pulse width

PARTICLES IDENTIFIED

- The measured parameters are converted into electric signals that allow the identificaton of the following particles:
  - Erythrocytes
  - Leukocytes
  - Squamous epithelial cells
  - Small round epithelial cells
  - Hyaline casts
  - Casts with inclusions
  - Crystals
  - Bacteria
  - Yeasts
  - Spermatozoa

EXAMPLE OF REPORT (1)

NO. 8 2/ 4/1999 Rack 0001 Tube 08



RBC	18.7	[/μL]	3.4[/HPF]
WBC	3460.3+	[/μL]	622.9[/HPF]
EC	36.5	[/μL]	6.6[/HPF]
CAST	0.64	[/μL]	1.85[/LPF]
BACT	514.2+	[/μL]	92.6[/HPF]

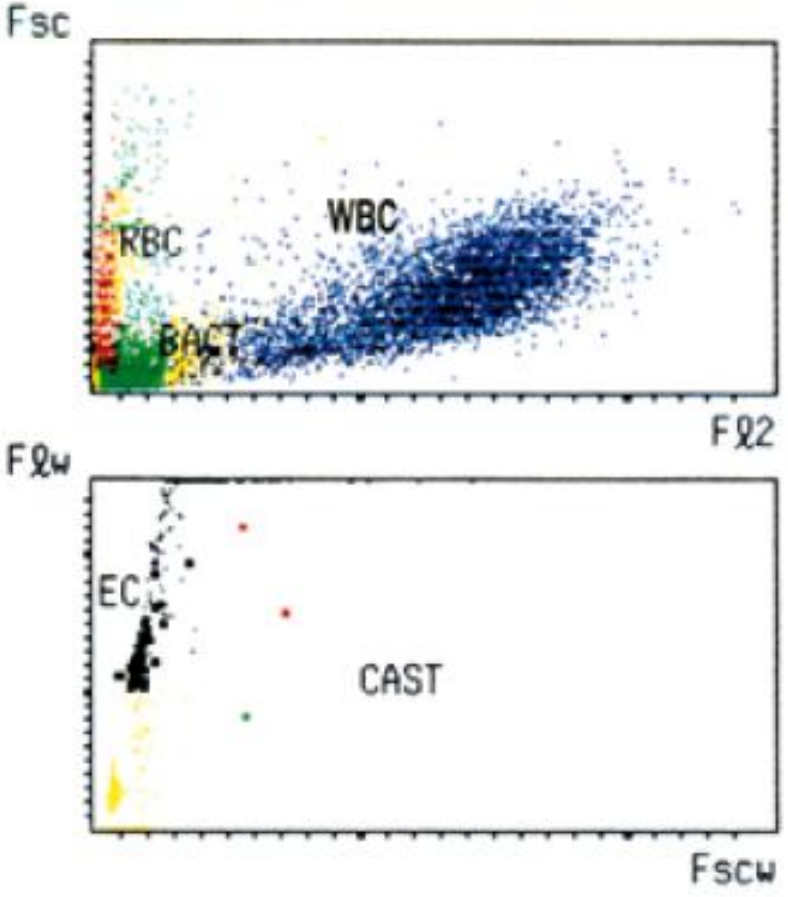
Path.CAST +	X'TAL
SRC	SPERM
YLC	

RBC-Info.

OB/Hb	PRO
L.Est.	NIT

EXAMPLE OF REPORT (2)

NO. 44 23/ 4/1999 Rack 0005 Tube 04



RBC	146.0+	[/μL]	26.3[/HPF]
WBC	1434.8+	[/μL]	258.3[/HPF]
EC	93.4+	[/μL]	16.8[/HPF]
CAST	0.38	[/μL]	1.11[/LPF]
BACT	1243.9+	[/μL]	223.9[/HPF]

Path.CAST		X'TAL
SRC	+	SPERM
YLC		

RBC-Info. Dysmorphic ?

OB/Hb	PRO
L.Est.	NIT

OTHER FEATURES OF UF 1000i

- The urine volume required = 0.8-1.2 mL
- 9 µL are used for analysis
- Quantitative results as No/µL & No/HPF
- Throughput: 100 samples/hour

CUVETTE-BASED MICROSCOPY: UriSed/sediMAX

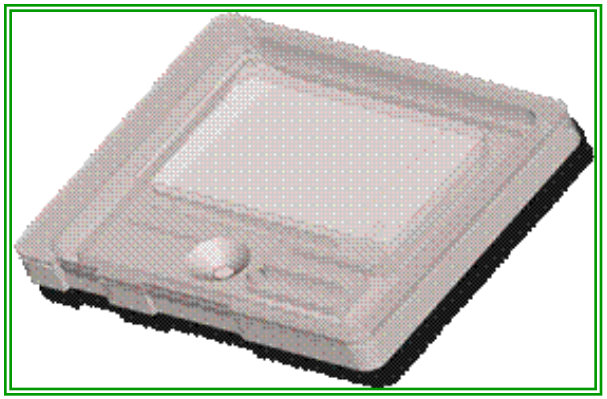
- A walk-away automatic urine sediment analyzer, which has been developed since 2008 by 77 Elektronika, Budapest Kft, Hungary (and distributed as sediMAX in several European countries by A.Menarini Diagnostics, Florence, Italy)

- It supplies B/W images of particles within *whole fields of view*

- These are similar to the microscopic fields seen with manual microscopy

WORKFLOW (1)

- A single-use *patented cuvette*



is filled with automatically mixed native urine (volume aspirated: 2.0 mL, volume examined: 2.2 µL)

- The sample is centrifuged within the instrument (10 seconds at 260 g)
- The cuvette is forwarded to the microscope table
- An automatic focusing at different levels is performed

WORKFLOW (2)

- A built-in camera takes a digital image of each field of view (magnification: ~400x)
- For each sample 15 images are taken
- Identification and quantitation of the particles (as No/µL or No/HPF) is carried out by Auto Image Evaluation Module (AIEM), a complex artificial neural network structure which has specifically been developed for the instrument
- Throughput: 100 samples/hour

PARTICLES IDENTIFIED (1)

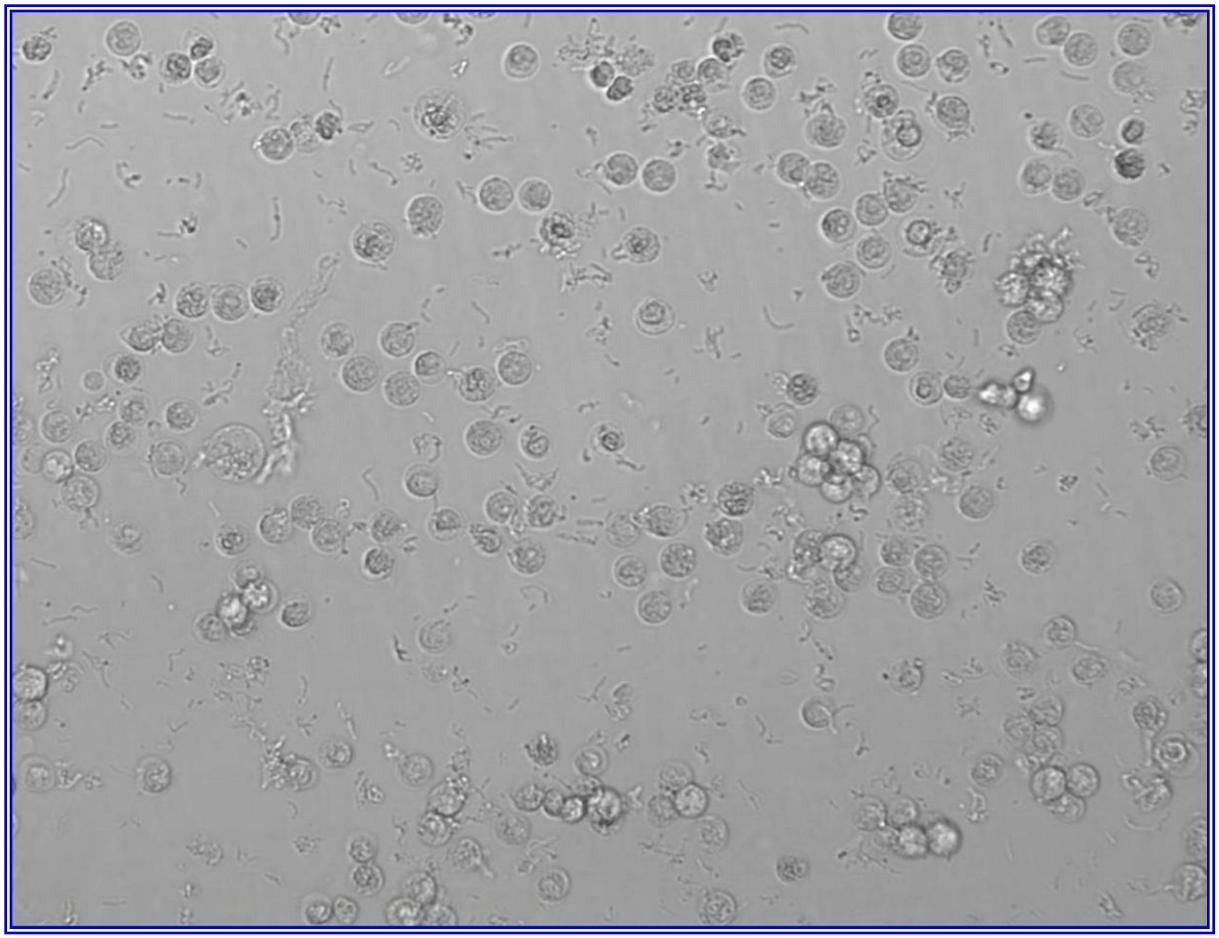
- Erythrocytes
- Leukocytes
- Squamous epithelial cells
- Non-squamous epithelial cells
- Hyaline casts
- Pathological casts
- Crystals: CaOx, UA, struvite
- Bacteria
- Yeasts
- Spermatozoa
- Mucus

PARTICLES IDENTIFIED (2)

- Other particles which might be present in the whole field of view but are not recognized by the instrument may be identified by the operator
- Due to this unique feature, urinary profiles - and the clinical diagnoses associated with them - can be identified (see the three following examples)

WHOLE FIELD OF VIEW:

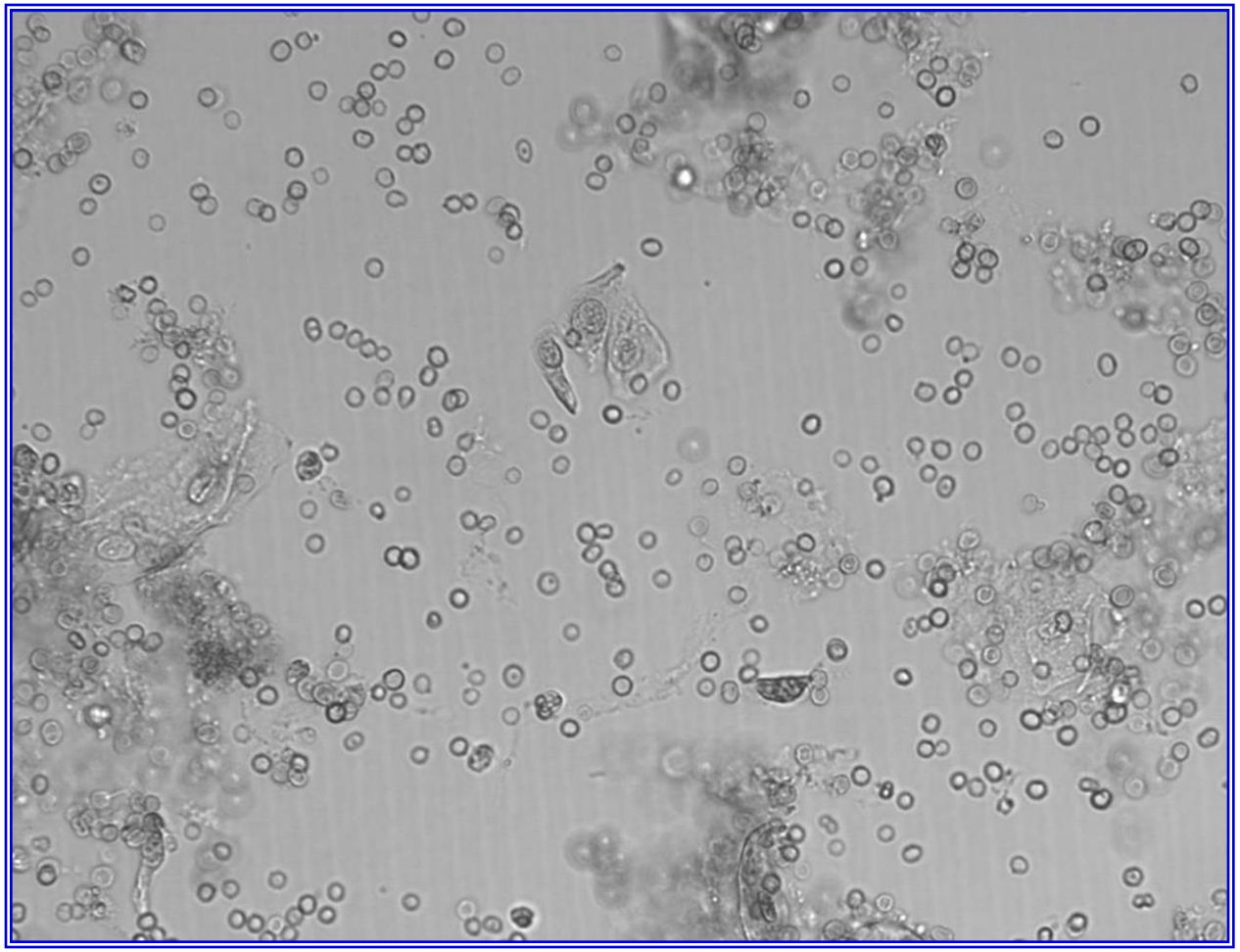
Many WBCS and bacteria



URINARY TRACT INFECTION

WHOLE FIELD OF VIEW:

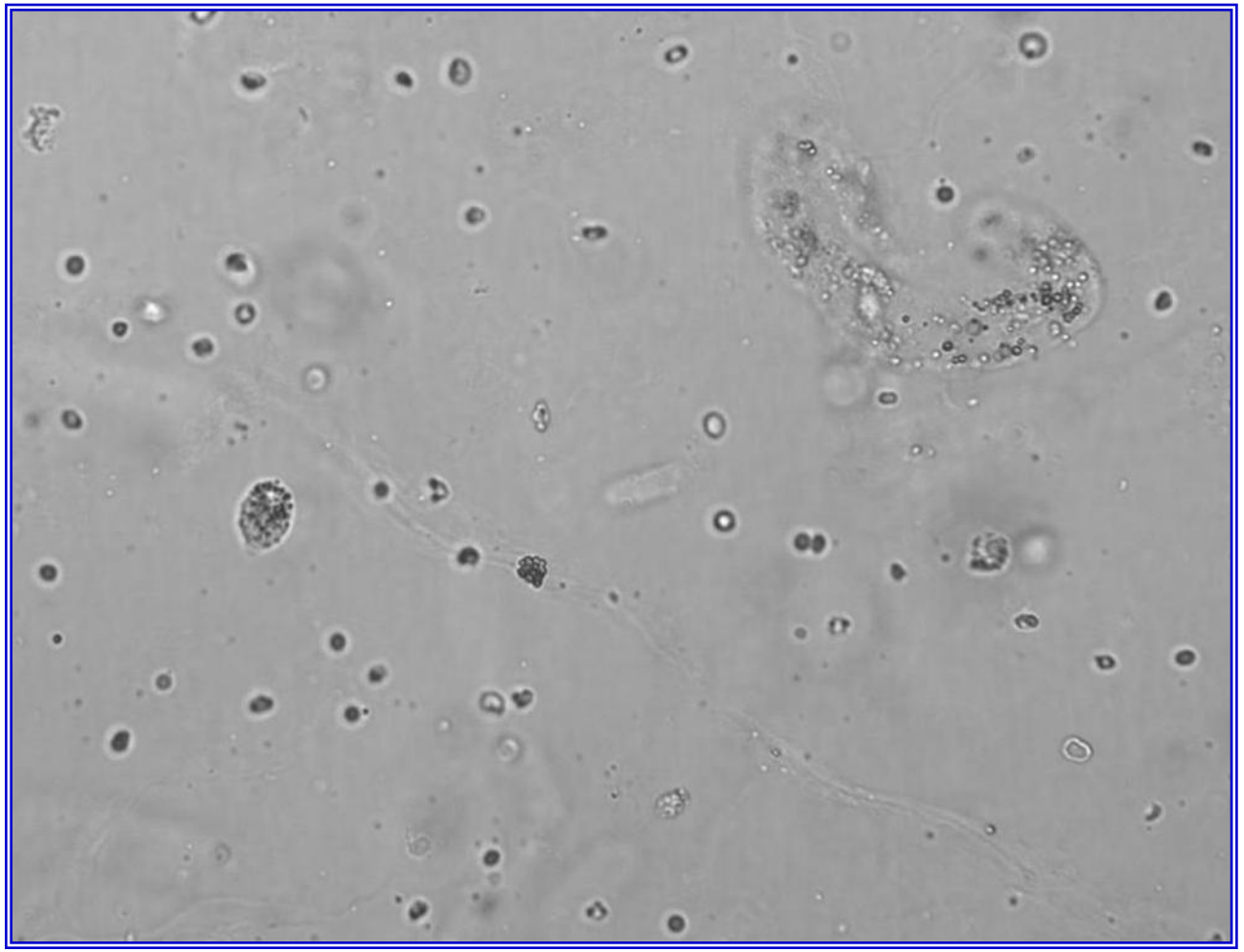
Isomorphic RBCs and deep transitional cells



UROLOGICAL DISEASE

WHOLE FIELD OF VIEW:

Dysmorphic RBCs and fatty particles

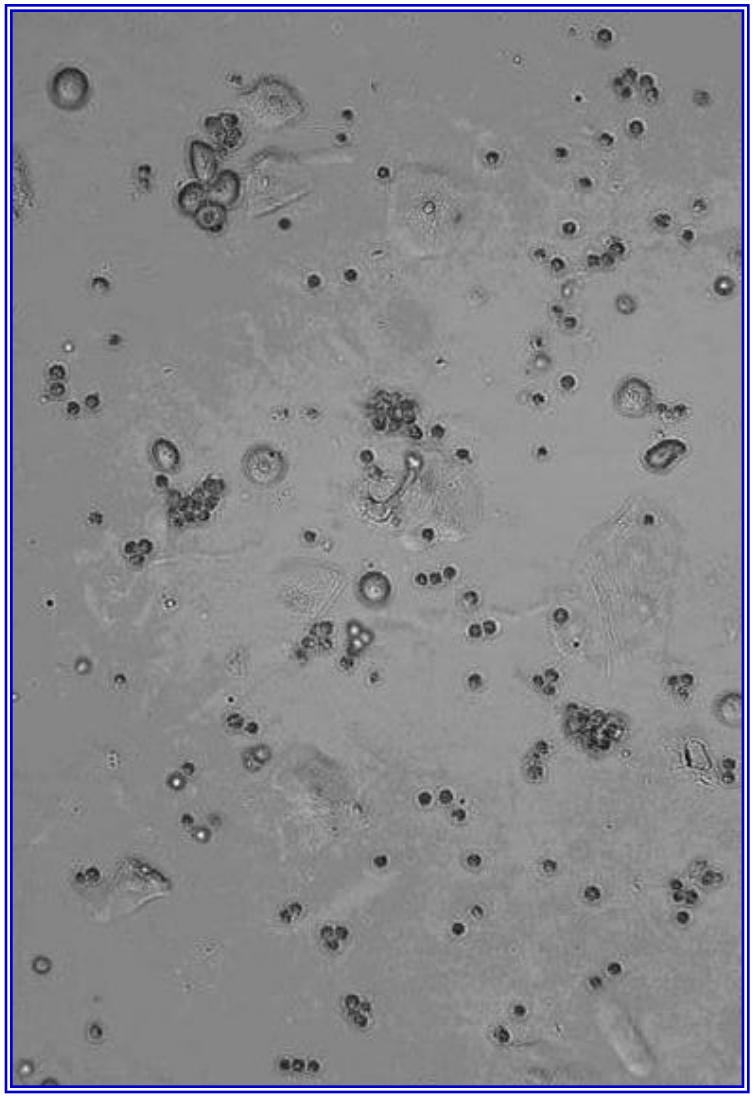


NEPHROTIC SEDIMENT

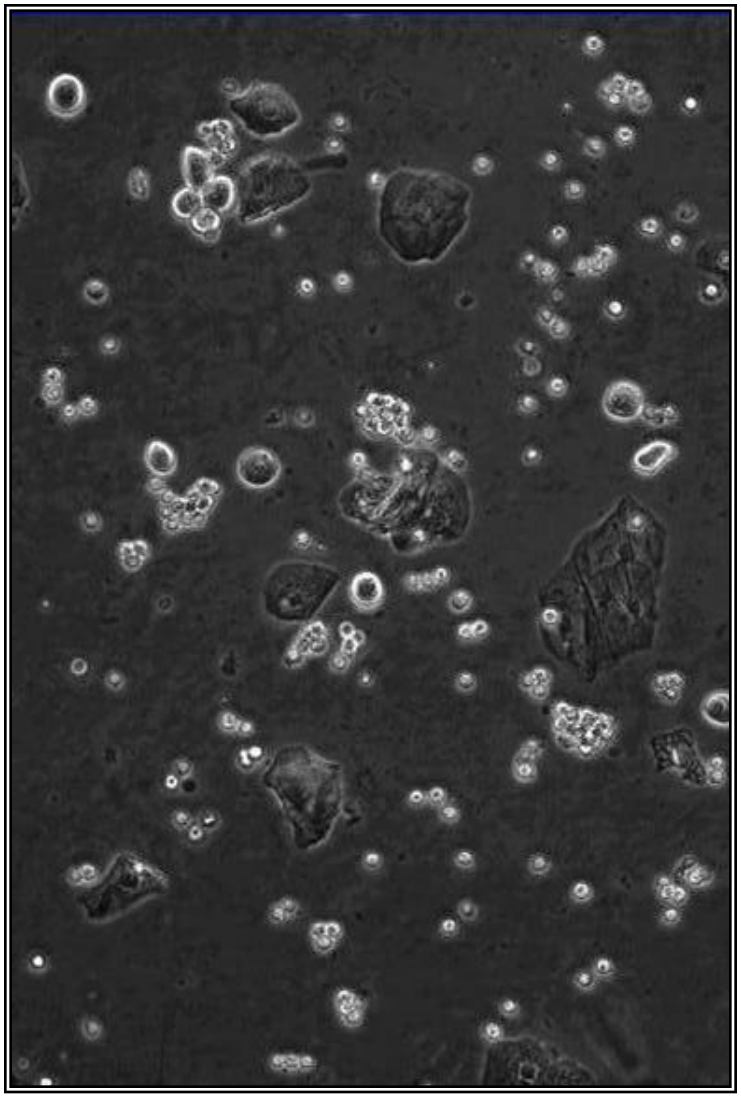
sediMAX DEVELOPMENTS OVER TIME

- sediMAX
- sediMAX 2
- sediMAX LITE (semi-automated)
- sediMAX conTRUST
  Supplies both bright field and phase contrast microscopy images (a further progress in automated urinary sediment examination)

sediMAX conTRUST



Bright field



Phase contrast

CONCLUSIONS

AUTOMATED Used ANALYZERS: ADVANTAGES

- Walk-away instruments
- Examine high numbers of samples in short time
- Require small volumes of urine
- Abolish the problems caused by centrifugation
- Achieve acceptable accuracy for some particles (RBCs, WBCs, squamous epithelial cells)
- Supply quantitative results with small variation coefficients
- Leave time for the manual examination of the more complex samples

AUTOMATED Used ANALYZERS: LIMITATIONS

- Include in one category only renal tubular epithelial cells and transitional epithelial cells, which have totally different clinical implications
- Underestimate casts, of which, in addition, they can identify only hyaline and "non hyaline" (or "pathologic") subtypes
- Identify only a few types of crystals
- Miss lipids completely
- For all tese reasons not yet qualified to investigate complex renal and non-renal samples

AUTOMATED Used ANALYZERS: THEIR PLACE IN LABS

- They supply an acceptable accuracy for the negative samples and those with minor changes, which represent the vast majority of samples examined in central labs
- Therefore, they are very useful/recommended for labs with >100 samples/day
- Their utility is greatly increased if, for selected cases, their use is integrated with manual microscopy performed in a proper way by motivated and trained personnel

THANK YOU FOR YOUR KIND ATTENTION