Integration of digital microscopy and flow cytometric analysis of solid elements in urine:

The best of both worlds and the gate to total automation

Critically Appraised Topic

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Why this topic?

Replacement of Urinalyzers

Manufacturer // Distributer

Wear and Tear

Wet Overheidsopdrachten
17/06/2016

Current practice?

Test strip & Digital microscopy

Technological innovation

Manual microscopic review rate

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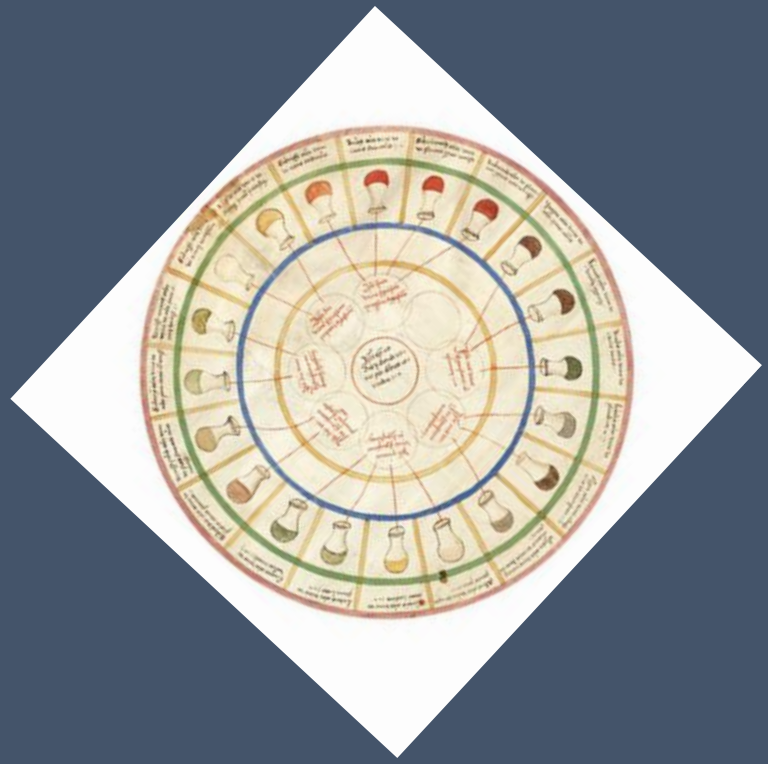
I. **Traditional Urinalysis**

II. Urine Flow Cytometry (UFC)

III. Remarks on UFC

I. Traditional Urinalysis

A. Foreword
B. Chemical Urinalysis
C. Urine Concentration
D. Sediment – Manual Microscopy
E. Automated Urine Particle Analysis



I/A Foreword

- Simple, expeditious, elementary, reliable, safe, cost-effective
- Physical, chemical and microscopic assessment
- Disease screening and diagnosis, therapy effectiveness evaluation
- Color, clarity and odor (historical)
- Pre-analytical conditions
  - Speciment collection, container, transport and storage
  - [-] Light, metabolisation, pH alteration, lysis
  - Freshness (< 2 h)
  - Well-mixed, first morning, uncentrifuged, 15-25°C specimen.

I/B Chemical Urinalysis

Urine component	Testing principle	Indications	Pitfalls
Leukocytes	Leukocyte esterase (lysed WBC) Granulocyte specific	Infections, urinary stones, inflammation	F+: drugs, strongly colored urine (red beets, bilirubin), oxidizing agents, formaldehyde, sodiumazide (preservative) F-: acidic or alkalic pH, ascorbic acid (vitamin C), high protein (>5 g/L) or glucose concentrations, drugs[1]
Nitrite	Nitrate reductase (bacterial)	Infections (E. coli, Klebsiella, Proteus, Citrobacter)	F+: strongly colored urine F-: ascorbic acid, alkaline pH, low urinary nitrate, non-nitrite reducing bacteria (S. saprophyticus, Enterococci, Pseudomonas, Acinetobacter, etc.)
Erythrocytes / Hemoglobin	Hemoglobin peroxidase (lysed RBC) Differential color development due to hemoglobin or intact erythrocytes	Hematuria due to kidney damage (e.g. glomerulonephritis), infection, kidney or bladder stones, malignancies, or blood disorders	F+: myoglobinuria (due to muscle damage), oxidizing agents, bacteria F-: inability to hemolyze RBCs due to acidic or alkaline urine, ascorbic acid, high nitrite concentration, high urine density, formaldehyde
Protein	Presence of albumin (pH indicator error)	Kidney damage, harmless physiological phenomena (posture-related, exercise)	F+: alkaline pH, drugs, heavily pigmented urine, drugs[2], contrast media F-: albumin concentrations below 300 mg/L, microproteinuria, tubular protein, Bence Jones-proteinuria
Glucose	Glucose oxidase/peroxidase	Glucosuria (tubular reabsorption limit in young adults ~ 1.8 g/L), renal diabetes	F+: oxidizing agents F-: ascorbic acid, UTI, acidic urine (keto acidosis, aspirin usage), reducing sugars (galactose, fructose, etc)
Ketones	Legal reaction (Acetoacetate, acetone)	Fatty-acid oxidation (ketosis), ketoacidosis (diabetes, chronic alcoholism, etc.), physiological (exercise, fasting)	F+: drugs[3] F-: pre-analytical storage
pH	Universal pH indicator	Kidney or urinary tract disorder	Alkaline pH due to bacterial growth (bacterial urease), dietary (vegetables), Fanconi syndrome (aminoaciduria), cast-forming due to alkalic urine Acidic pH due to dietary (meat, cranberries)
Urobilinogen	Ehrlich reaction	Impaired liver function, increased hemoglobin degradation (hemolytic anemia)	Decreased urobilinogen may indicate a blockage in the bile duct system or bile production failure.
Bilirubin	Ehrlich reaction	Hemolysis, liver damage or disease (jaundice).	F+: rifampicin
Creatinine	Benedict-Behre method	Kidney diseases	F-: ketone bodies, ascorbic acid (> 200mg/dL)
P/C **A/C**	Protein/Creatinine ratio Albumin/Creatinine ratio	Higher sensitivity for A/C ratio than conventional protein dipstick.	Albumin dipstick ~ 10-150 mg/L
Specific gravity	Refractometry	Urine concentration	F+: intravenous contrast media

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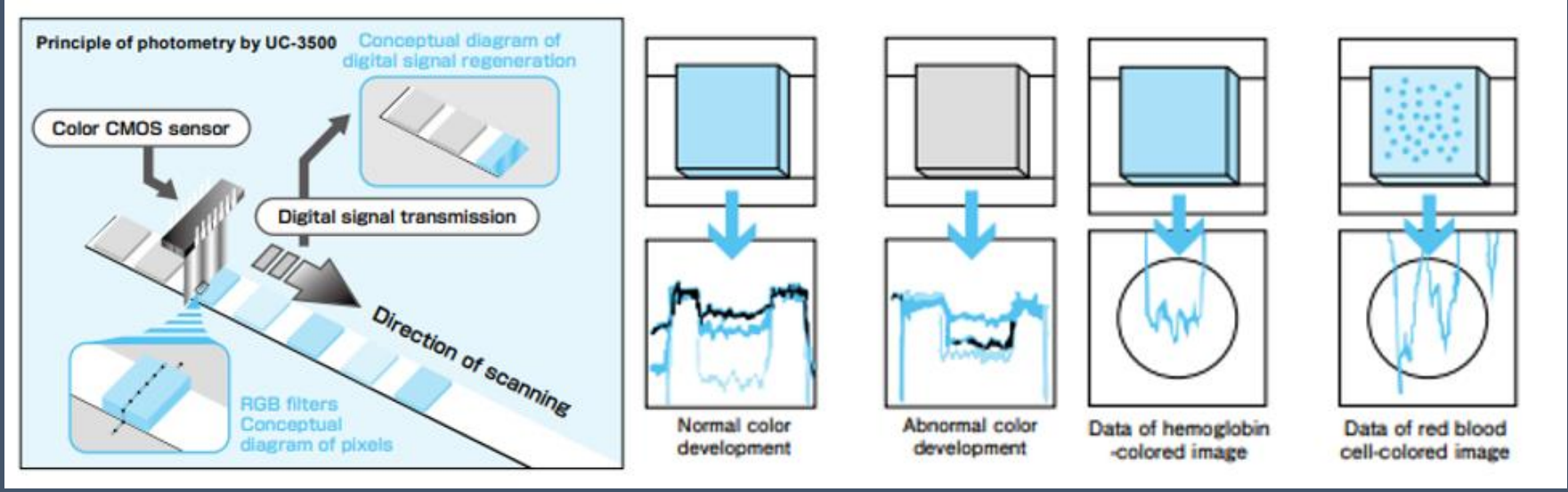
[1]Cefalexine, cephalothin, nitrofurantoin, tetracycline, tobramycin

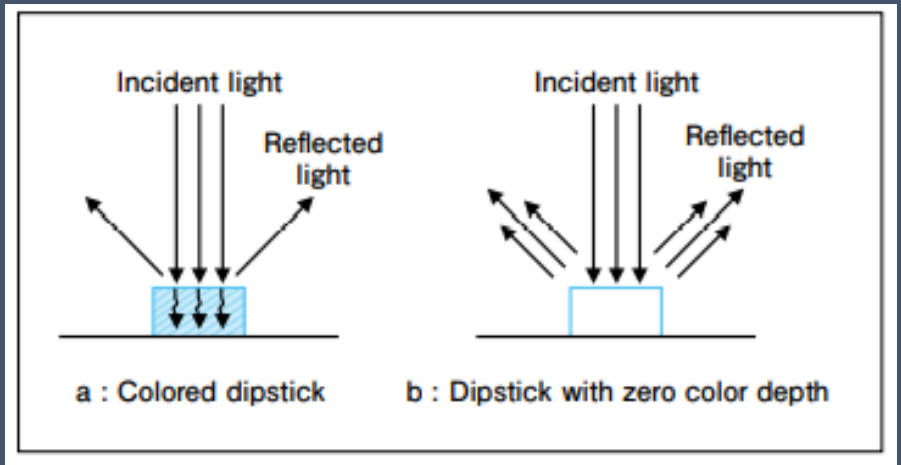
I/B Chemical Urinalysis

- Automated reagent test strip readers (operator subjectivity)
- Reflectometry
- Complementary metal oxide semiconductor (CMOS) technology



I/C Urine Concentration

- Urine creatinine, osmolality, specific gravity
- Hydrometry, harmonic oscillation, osmometry, refractometry



I/D Sediment – Manual Microscopy






Urine component	Indication	Morphology	Note
Erythrocytes	Kidney disease, a blood disorder or another underlying medical condition, such as bladder cancer Temporary erythrocyturia in children not uncommon (unsignificant)	Tonicity of urine: - Echinocyte or burr cell (hypertonic), - Bloating or lysis (hypotonic) Glomerular and non-glomerular hematuria: - Dysmorphic; acanthocyte (renal hematuria; glomerulonephritis), - polymorphic (urologic hematuria)	Lysis due to sample freshness, alkaline pH, low osmolality, casts due to glomerulonephritis
Leukocytes	Infections (UTI)	In conjunction with possible microorganisms. Glitter cells in hypotonic condition; polymorphonuclear neutrophils with granules showing a Brownian movement. Eosinophils in drug induced interstitial nephritis.	Casts due to UTI Sterile pyuria exists in kidney tuberculosis, polycystic kidneys, malignancies Eosinophils require staining (Hansel)
Epithelium	Physiological conditions UTI, inflammation Kidney damage	Superficial urothelium : squamous epithelium Deeper layers urothelium: 'small round cells' Tubular epithelium	
Casts (cylinders)	Kidney disorders, physiological conditions	Hyaline casts: - consist of Tamm-Horsfall protein secreted by urothelium, can be exercise-related Cellular casts: - kidney pathology; erythrocyte cast (glomerulonephritis), leukocyte cast (pyelonephritis) Granular casts: - kidney disorder; due to autolysis (granulation) Wax casts: - severe chronic kidney disease (diabetic nephrosclerosis, nephrotic syndrome); denaturation of plasma proteins in tubuli (associated with proteinuria)	Fragile and brittle particles
Crystals	Kidney stones: trivial, pathological, drug induced	- Trivial crystals and amorph deposits (calcium oxalate, urate, phosphate) - Drug induced: e.g. indinavir, sulfamethoxazole-trimethoprim, ciprofloxacin - Pathological: cystinuria (hexagonal), xanthine, leucine, tyrosine	Diuresis, dietary, urinary pH
Oval fat bodies	Lipoid nephrosis	Oval fat bodies due to leakage of plasma lipoprotein Cholesterol crystals (polarization microscopy)	Also isolated small fat droplets in sediment
Mucine threads	Physiological condition	Urothelium coated with mucin threads	
Organisms	Bacteria, yeast, fungi, parasites	Infections	Contamination, worm eggs

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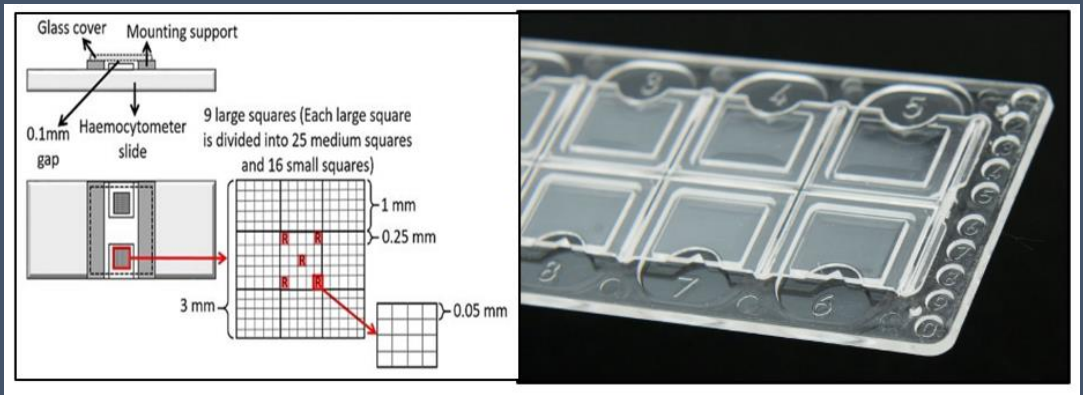
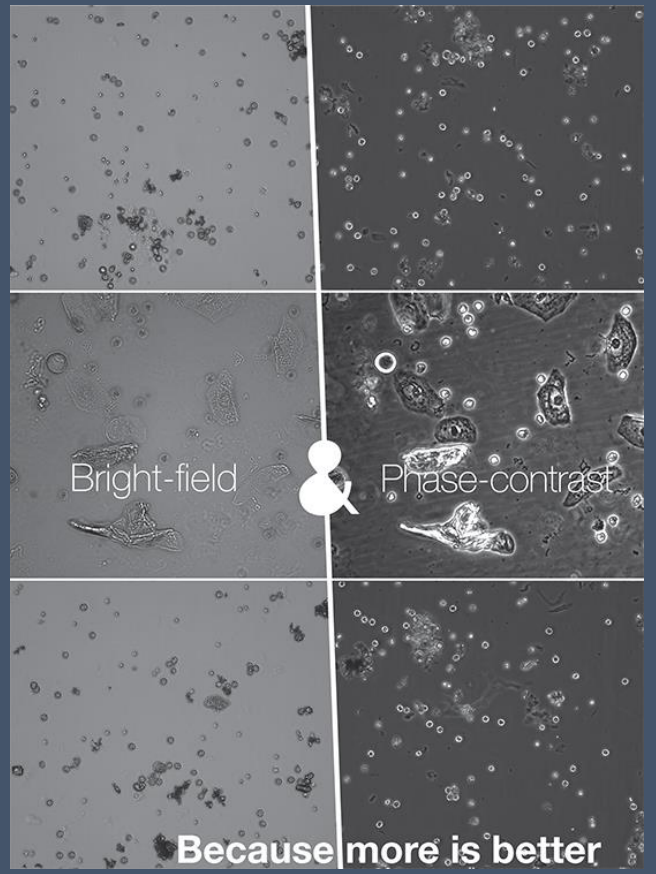
I/D Sediment – Manual Microscopy





I/E Automated Urine Particle Analysis

- Automated microscopic pattern recognition (1), Flow cytometry (2)
- Techniques: impedance, digital imaging, light scatter, dyes, fluorescence
- [+] User convenience, productivity, specimen preparation, low sample volume
- [-] Populations with high prevalence of nephropathology

- Combination test strips + automated particle analysis
- Visual microscopy remains necessary (reviews)
- Ongoing morphologist competence assessment

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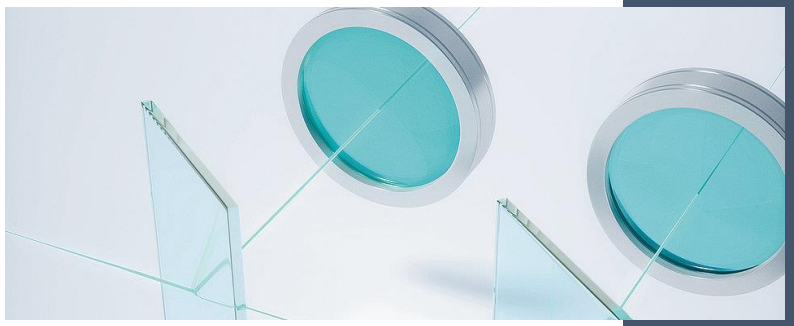


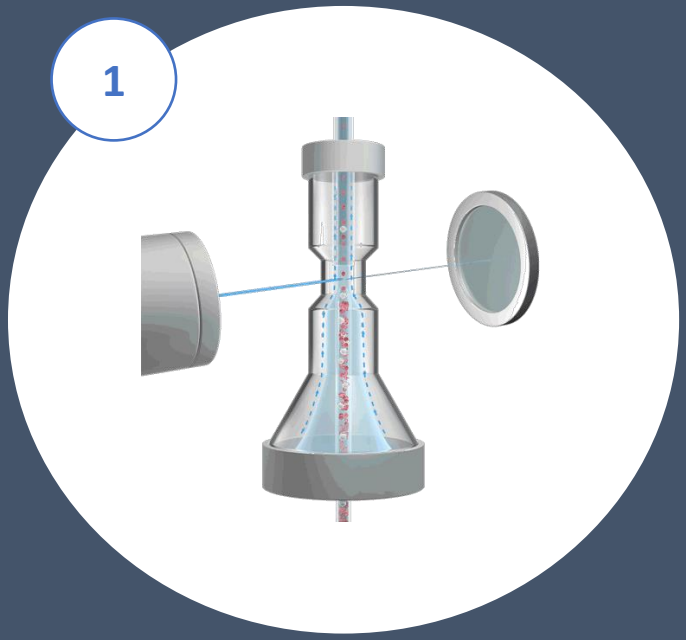
I. Traditional urinalysis

II. <u>Urine Flow Cytometry (UFC)</u>

III. Remarks on UFC

II. Urine Flow Cytometry (UFC)

A. Stains, Channels and Laser Beams
B. Diagrammatic Illustrations
C. Parameters and Properties

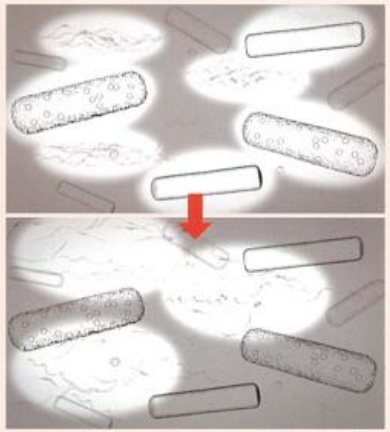


II/A Stains, Channels and Laser Beams

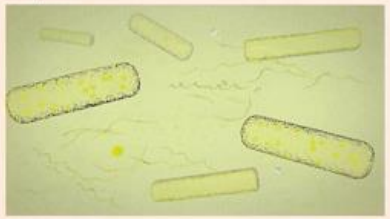
SF ch

The SF channel uses signal waveform information about particles, in addition to the scattered light intensity, signal width, etc., which improves its analysis performance for particles like casts.

The exclusive diluent UF CELLPACK™-SF dissolves amorphous salts and disperses mucus, both of which interfere with the measurements.

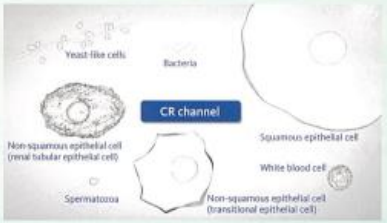


The exclusive stain UF Fluorocell™-SF then stains membrane components of red blood cells, cast matrix, etc.



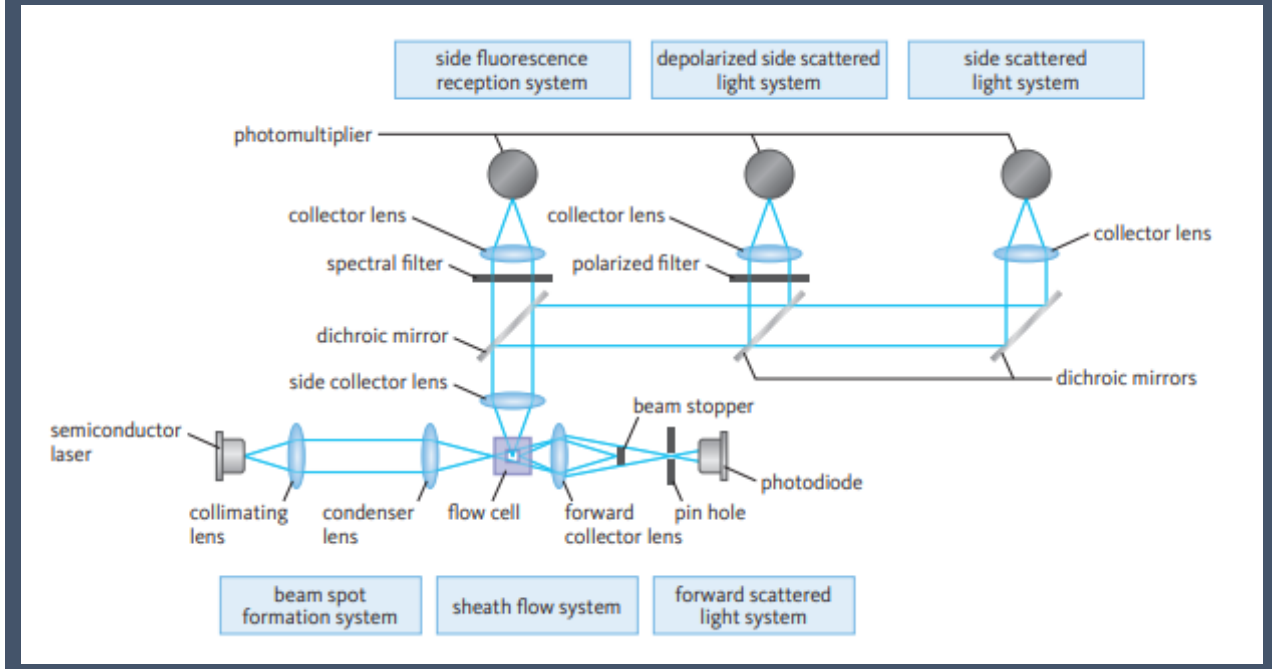
CR ch

The CR channel enables high level cell analysis through the combined use of multiple characteristics like scattered light signal width, scattered light signal waveform area and fluorescent signal waveform area.

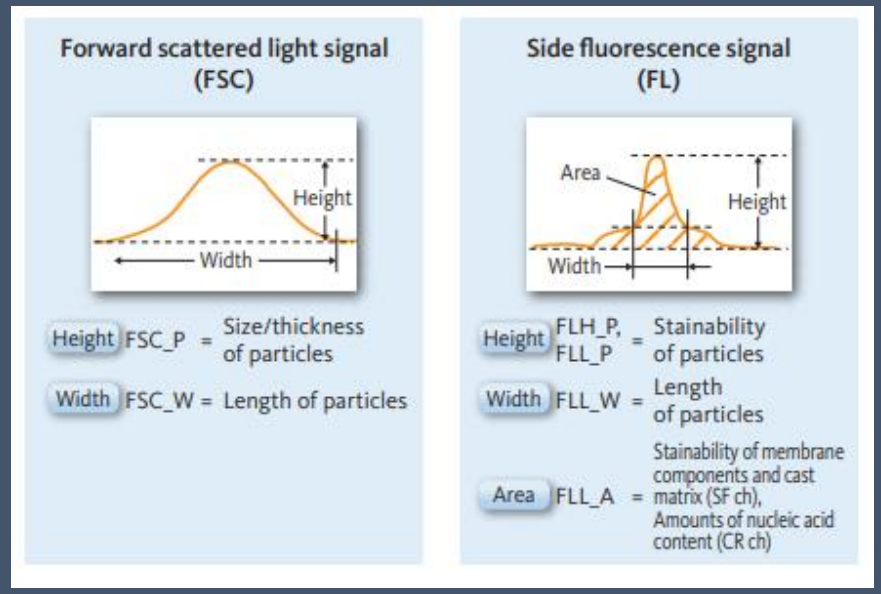


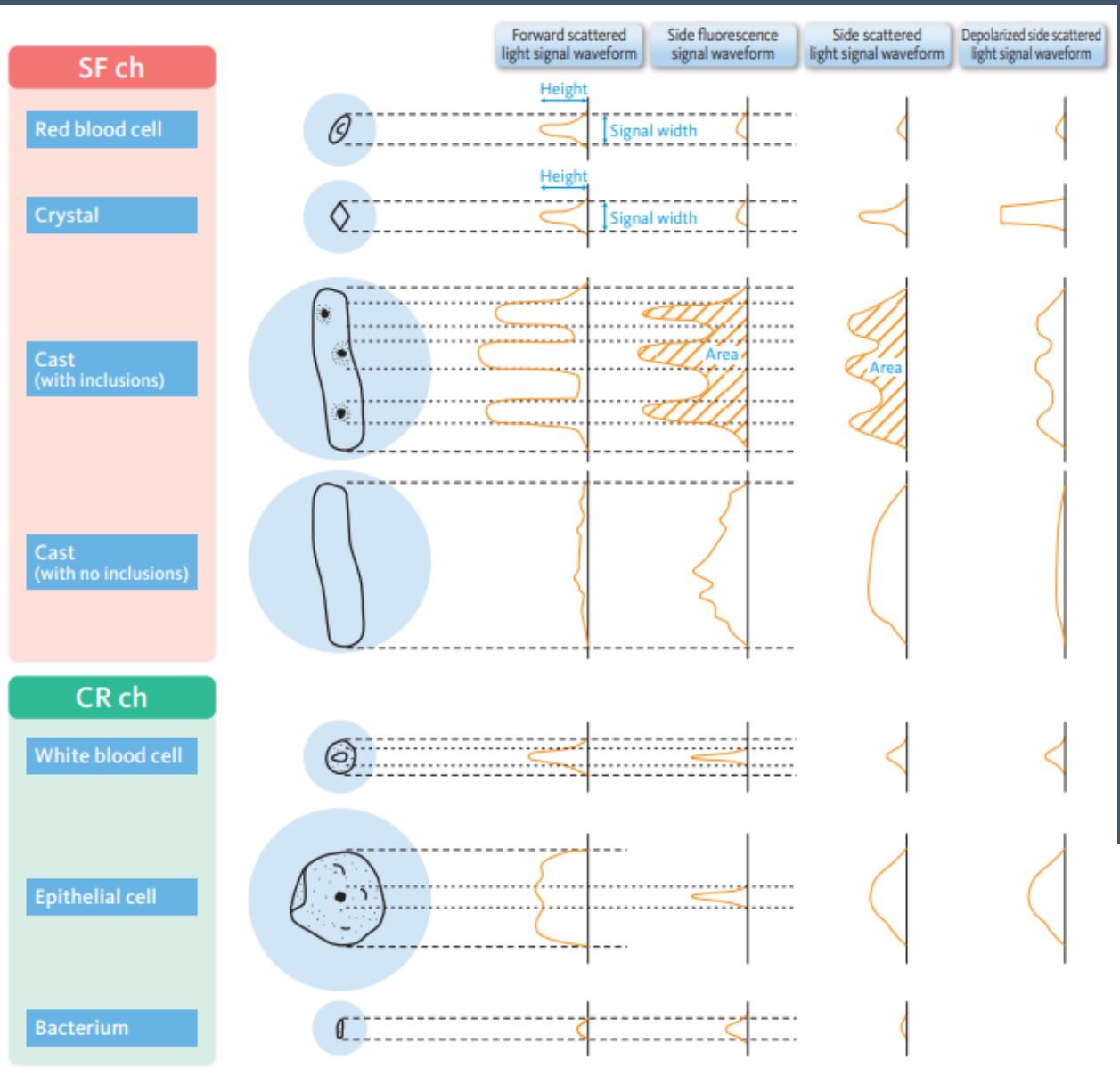
The exclusive diluent UF CELLPACK™-CR lyses or dissolves red blood cells and crystals, which interfere with the classification of particles in urine. On the other hand, white blood cells and epithelial cells are not lysed.

The surfactant in this diluent creates fine pores on the cell membranes and the polymethine dye in UF Fluorocell™ CR enters through these pores to stain nucleic acids in the cells.



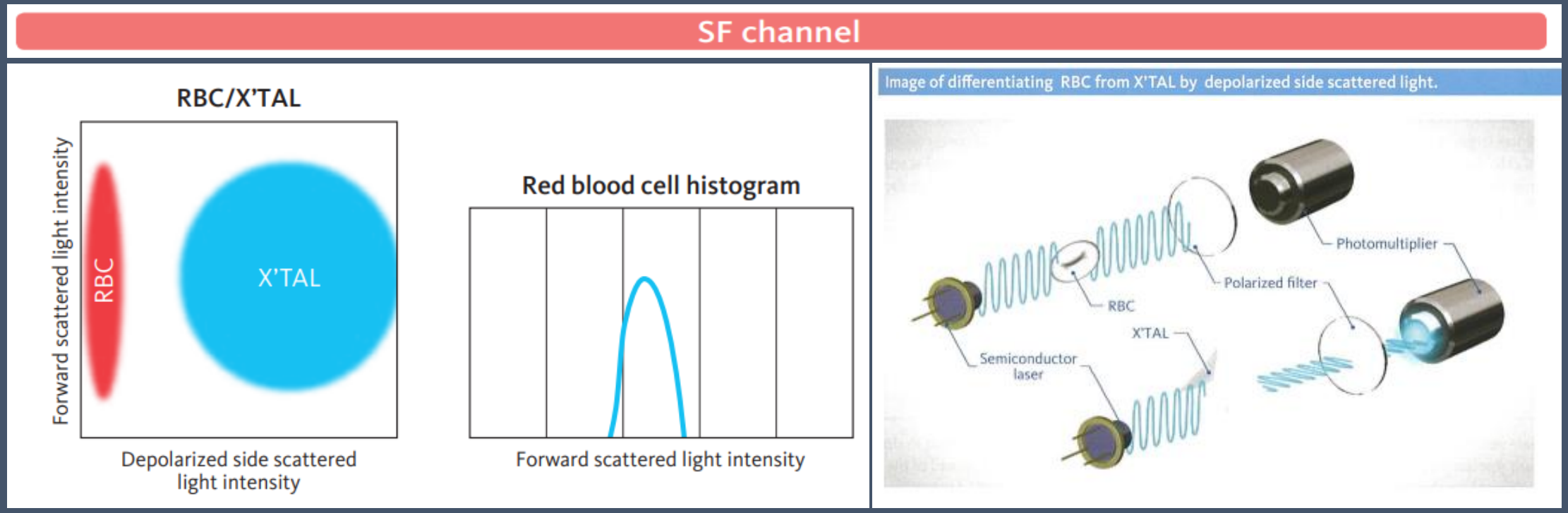
II/B Diagrammatic Illustrations





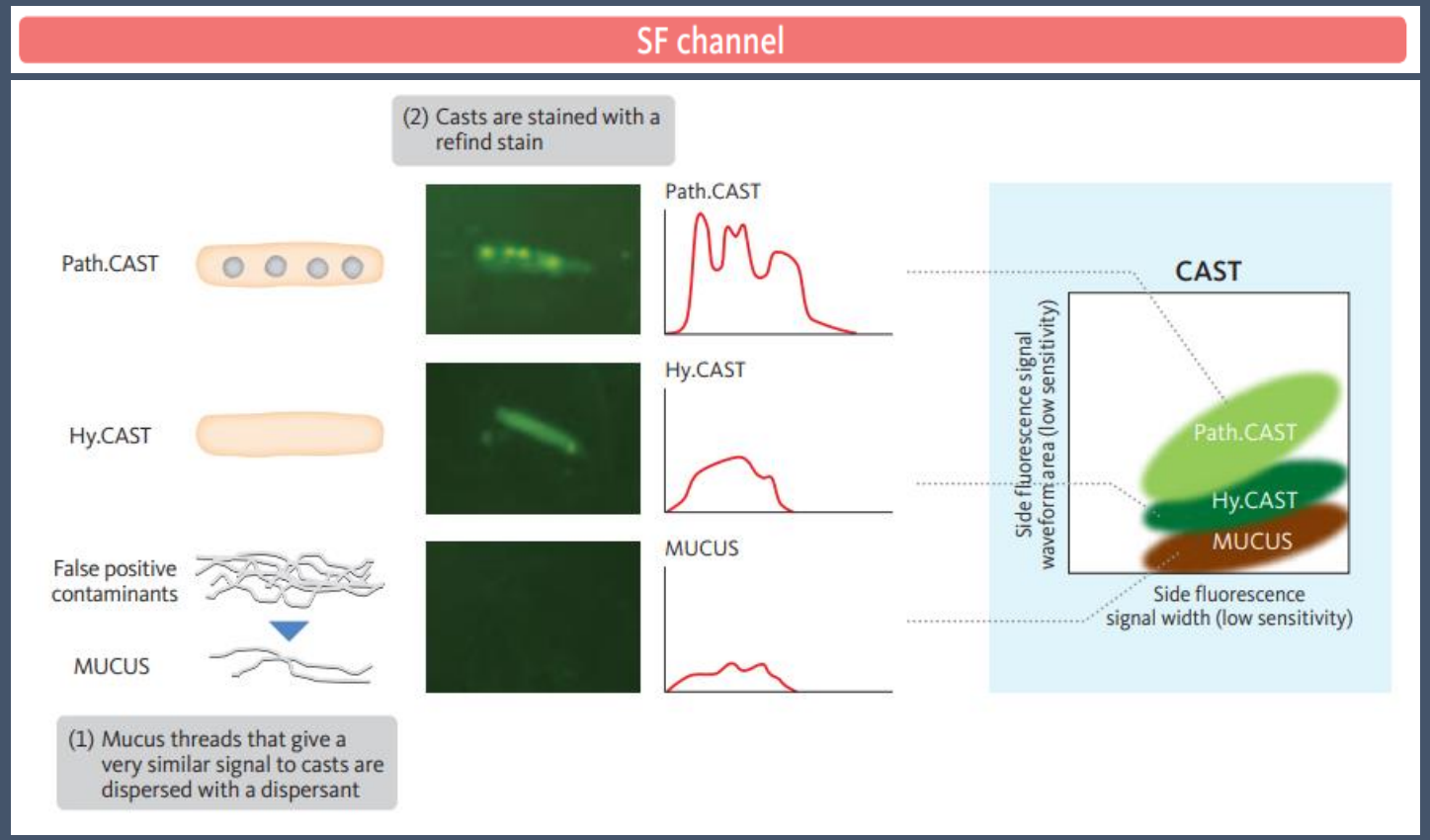
II/C Parameters and Properties

- Red blood cells & Crystals



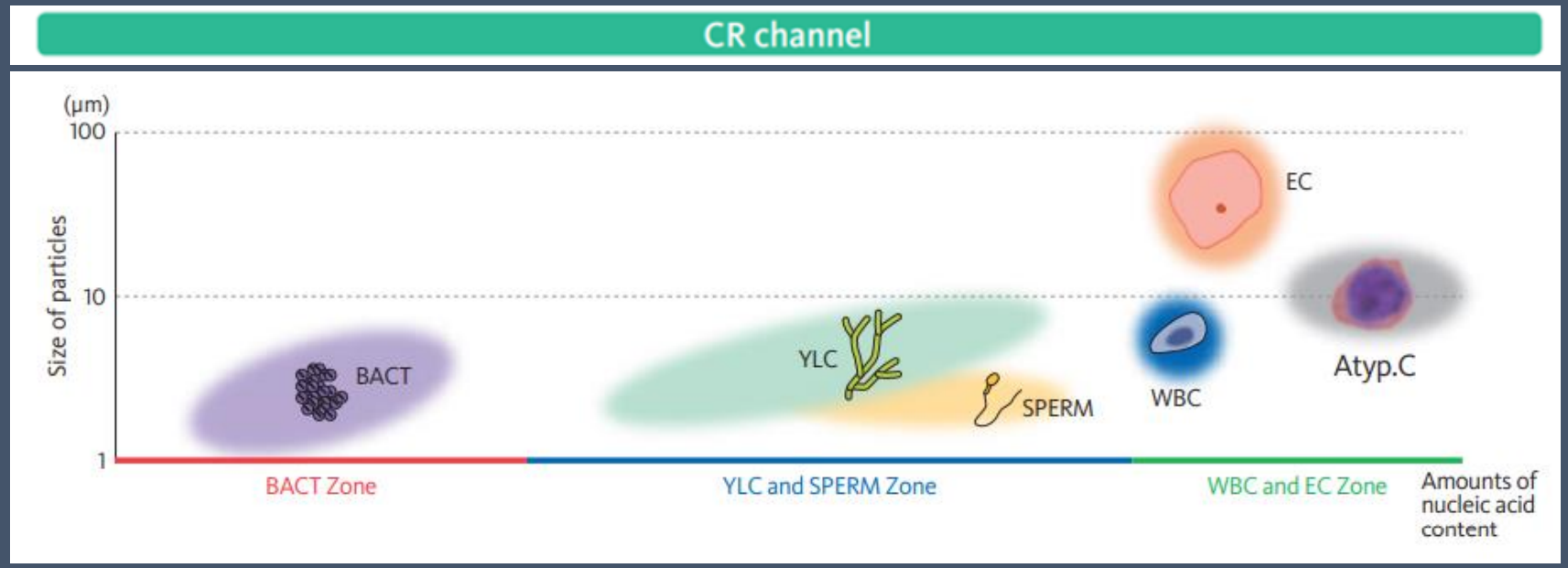
II/C Parameters and Properties

- Casts



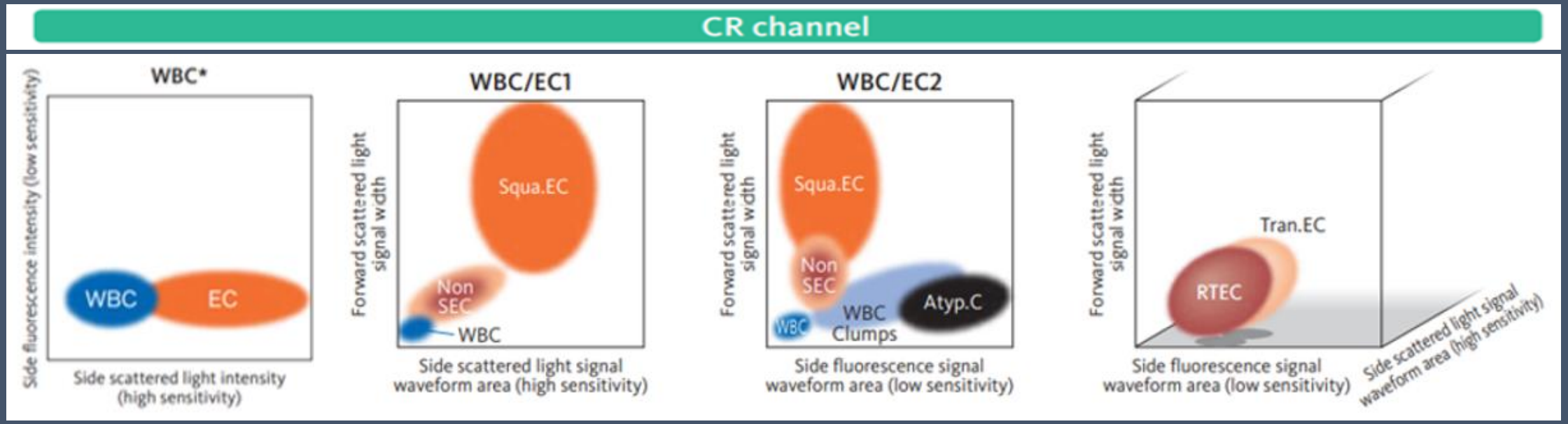
II/C Parameters and Properties

- Nucleic acid containing particles



II/C Parameters and Properties

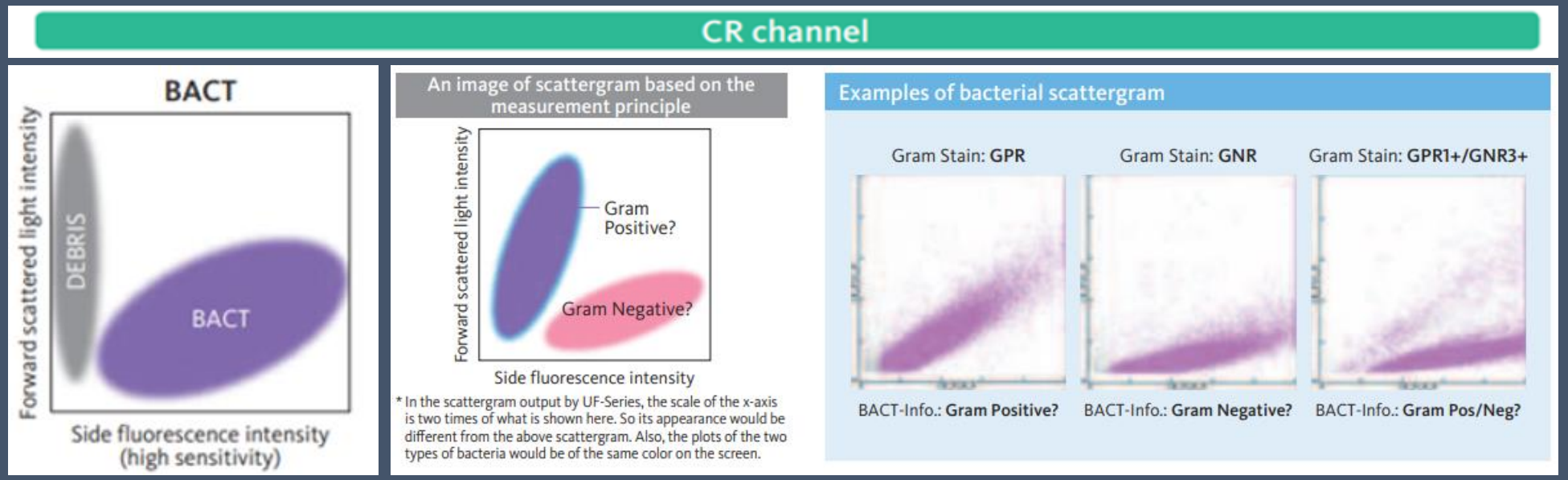
- White blood cells & Clumps, Epithelial cells

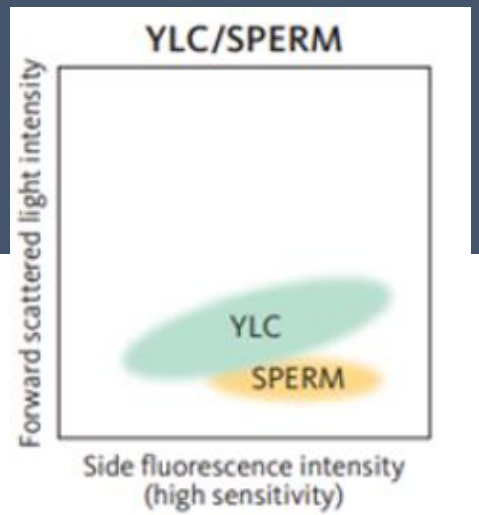


- Atypical Cells

II/C Parameters and Properties

- Bacteria, Yeast-like cells and Sperm





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I. Traditional urinalysis

II. Urine Flow Cytometry (UFC)

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Pro & Con

A. Next-generation particle analyzer
B. Applications
C. Value of (digital) microscopy
D. Implementation

III/A Next-generation particle analyzer

- Clinical application: User-defined rules & Guideline-based disease profiles
- Precise and accurate counting, Capacity and quality (> manual microscopy count)
- Fragile particles (e.g. casts) are not damaged

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III/B Applications

- Screening of urinary tract infections
- Diagnosis and monitoring of nephrological/urological conditions
  - Origin of hematuria, renal disease
- Bacteria differentiation scattergram
  - [+] Preliminary Gram differentiation
  - [-] Viable and dead bacteria / non-culturable bacteria.
- Reduction of bacterial cultures (+/- 75%)
  - [+] Negative predictive value ~> cultuur (CLED)
  - [-] Rudimentary, subjective, time-consuming and having poor NPV

  → Acceptance ratio false-negatives?

III/C Value of (digital) microscopy

- Microscopic examination
  - Physician's request
  - Laboratory-defined abnormalities
  - Analyzer indicated abnormalities
- Unambiguous identification of parameters (Microscopy vs UFC)
  - Casts, non-squamous epithelial cells, dysmorphic erythrocytes, fungi, parasites and clinically significant crystals

Indications & Publications

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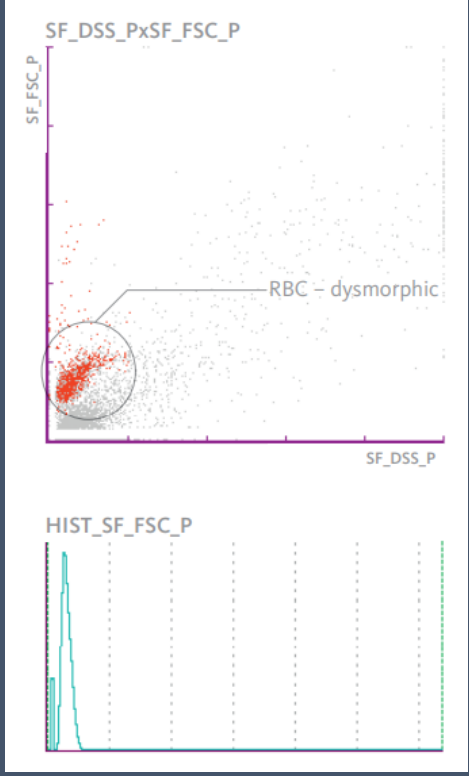
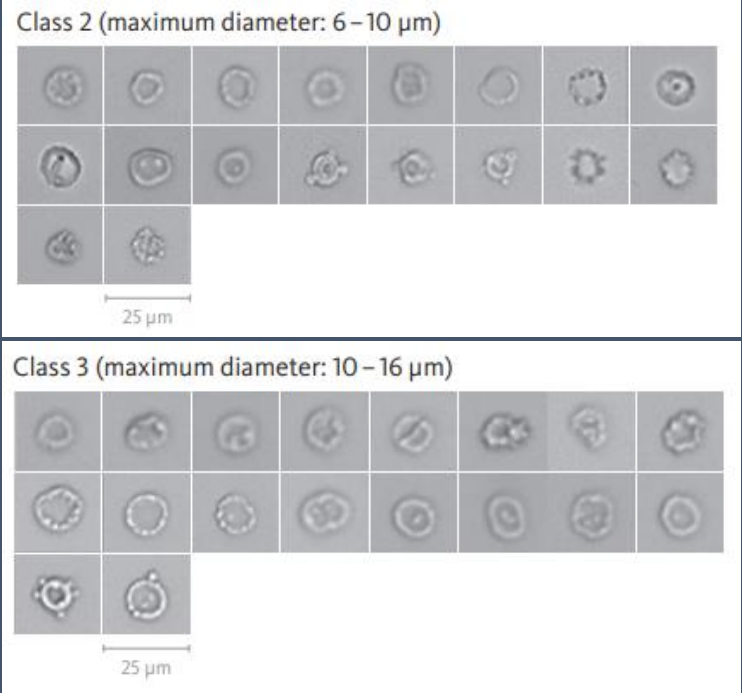
III/C Value of (digital) microscopy

- Example: iso- vs dysmorphic RBCs

Flag? → Review

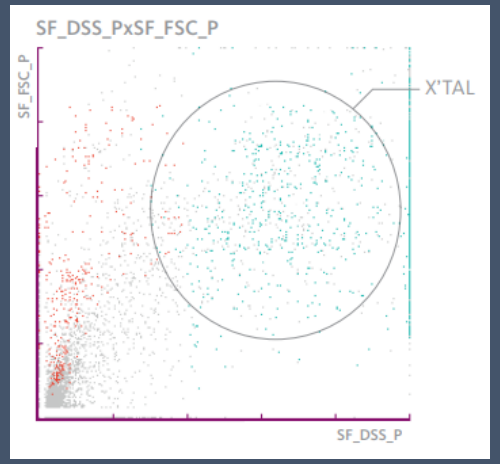


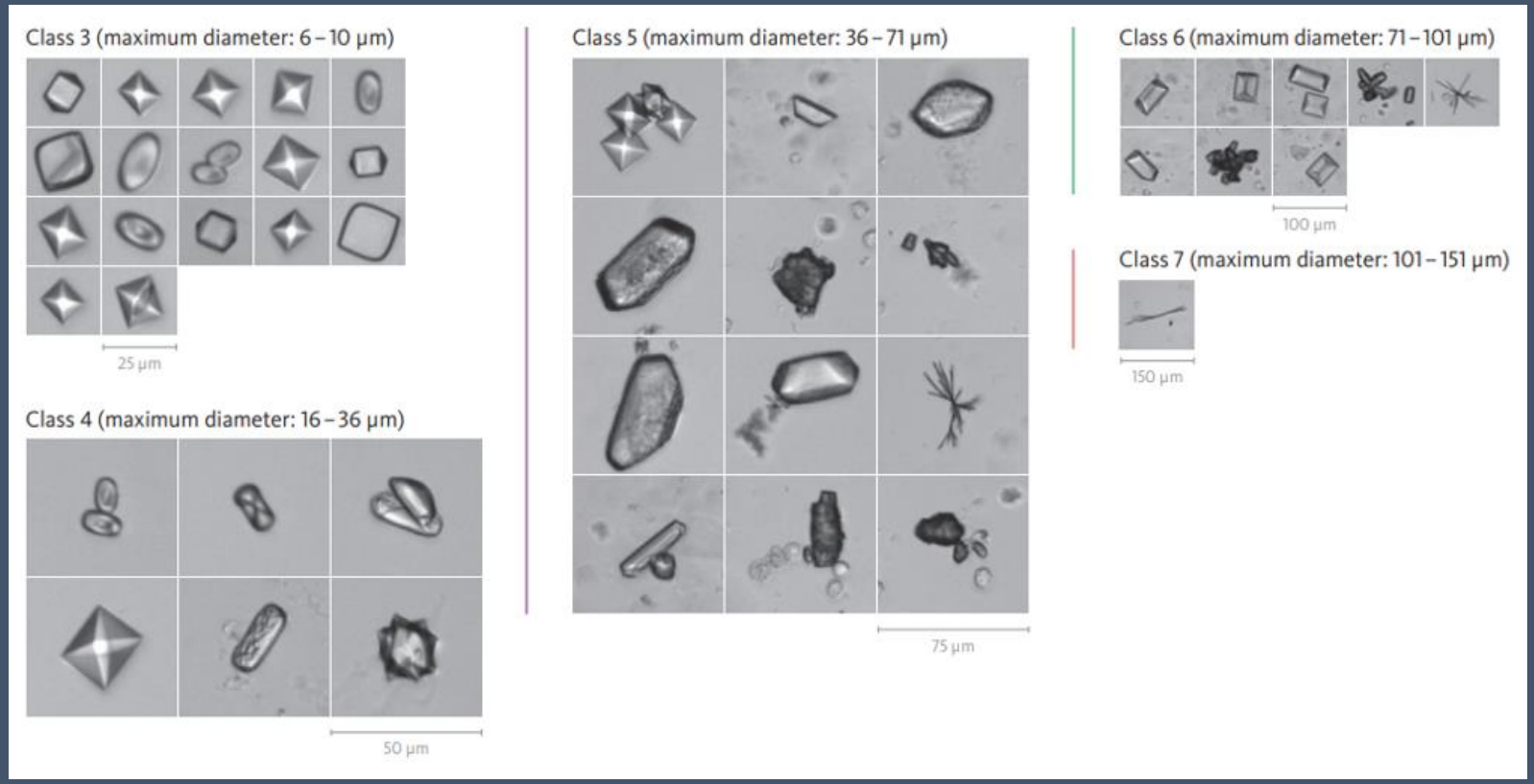


III/C Value of (digital) microscopy

- Example: Crystals

Flag? → Review

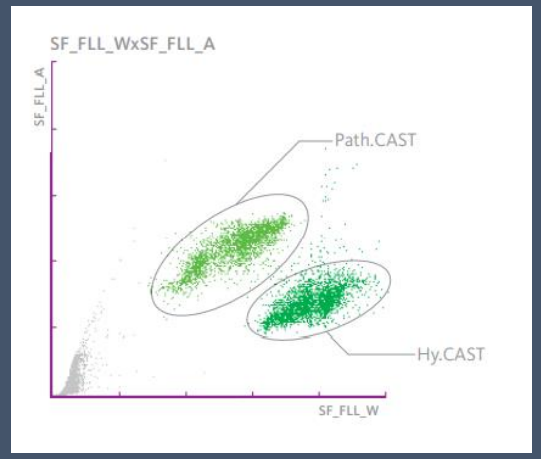


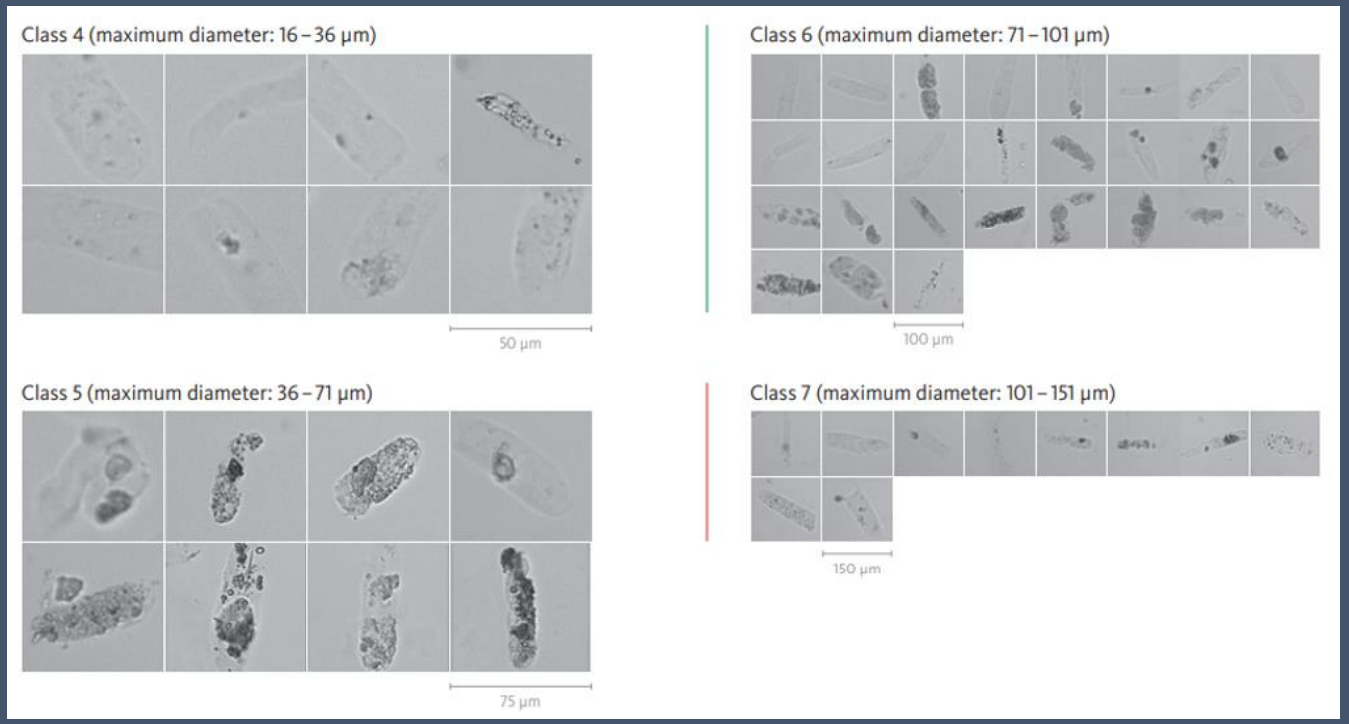


III/C Value of (digital) microscopy

- Example: Casts

Flag? → Review

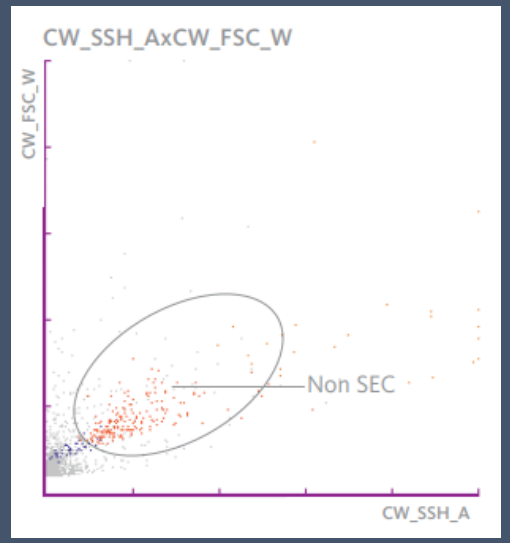


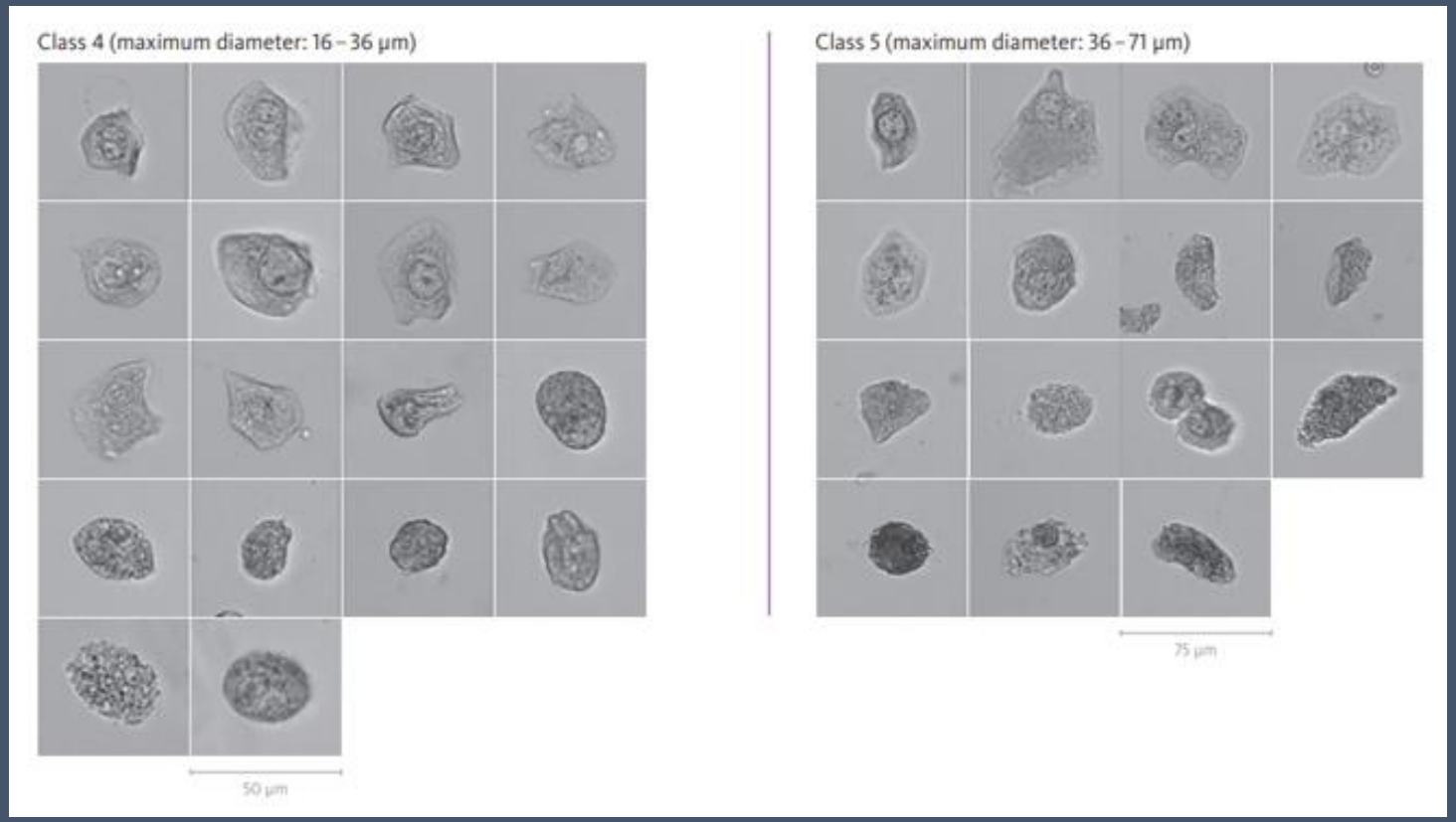


III/C Value of (digital) microscopy

- Example: Non-squamous epithelial cells

Flag? → Review





III/D Implementation

- [+] Reduction of microscopic reviews based on user-definable decision-rules
- [+] Quality of test results
- [+] Reduction time (workload) and cost (unnecessary empirical treatment)

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Conclusion

Improvements and Innovation in Urinalysis

Improvements and Innovation in Urinalysis

- Pre-analytical conditions
- Integration of techniques
  - Test strip: Reflectometry / CMOS technology
  - Concentration parameters
  - Digital microscopy
  - Flow Cytometry
- Automated analytical systems
  - Reduction of turnaround time
  - Avoid treatment
  - Time-saving effect frees resources for additional samples (expertise samples)
  - Generation of reproducible results with standardized procedures
  - Laboratory workflow

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Actions / To Do’s

1. Validation and implementation of new analyzers
2. Determination of screening method to rule out an UTI
3. Reviewing current modality of requesting and reporting
    - Reimbursement test strip and pitfalls
    - Trivial urinalysis vs Nephrological/Urological conditions

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Integration of digital microscopy and flow cytometric analysis of solid elements in urine:

The best of both worlds and the gate to total automation

Critically Appraised Topic