Digital Hematology

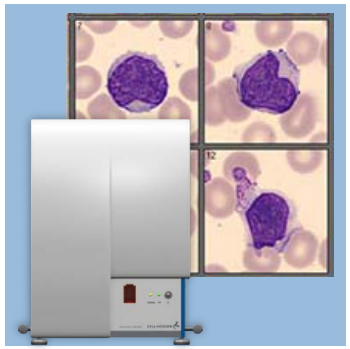
Dr. Jurgen Riedl

Albert Schweitzer ziekenhuis

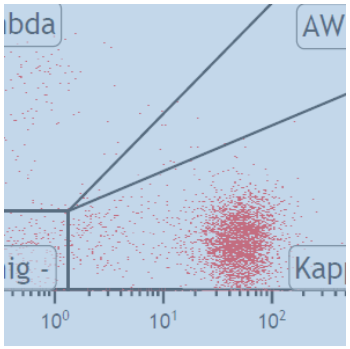
Multidisciplinary

CELLCOUNTERS

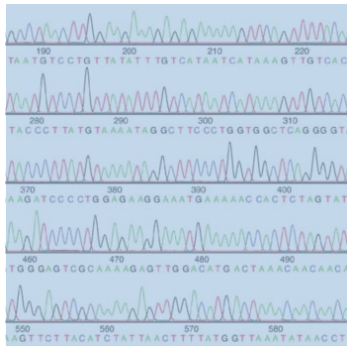
MORFOLOGY



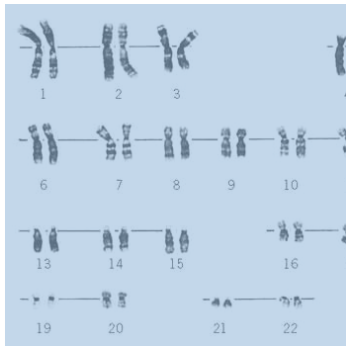
FLOWCYTOMETRY



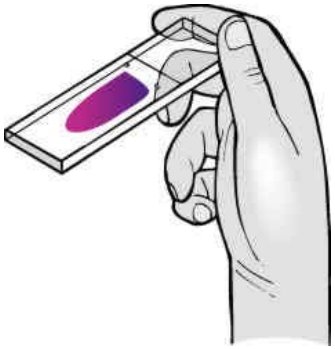
MOLECULAR DIAGNOSTICS

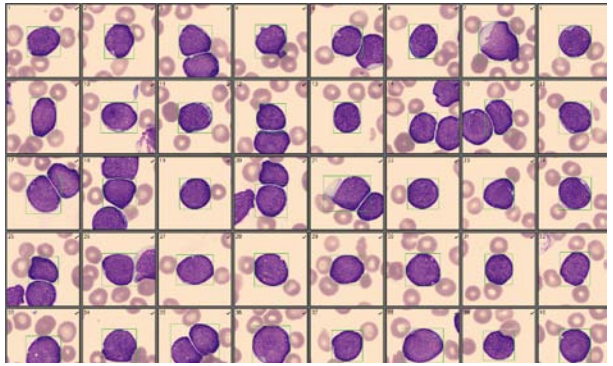


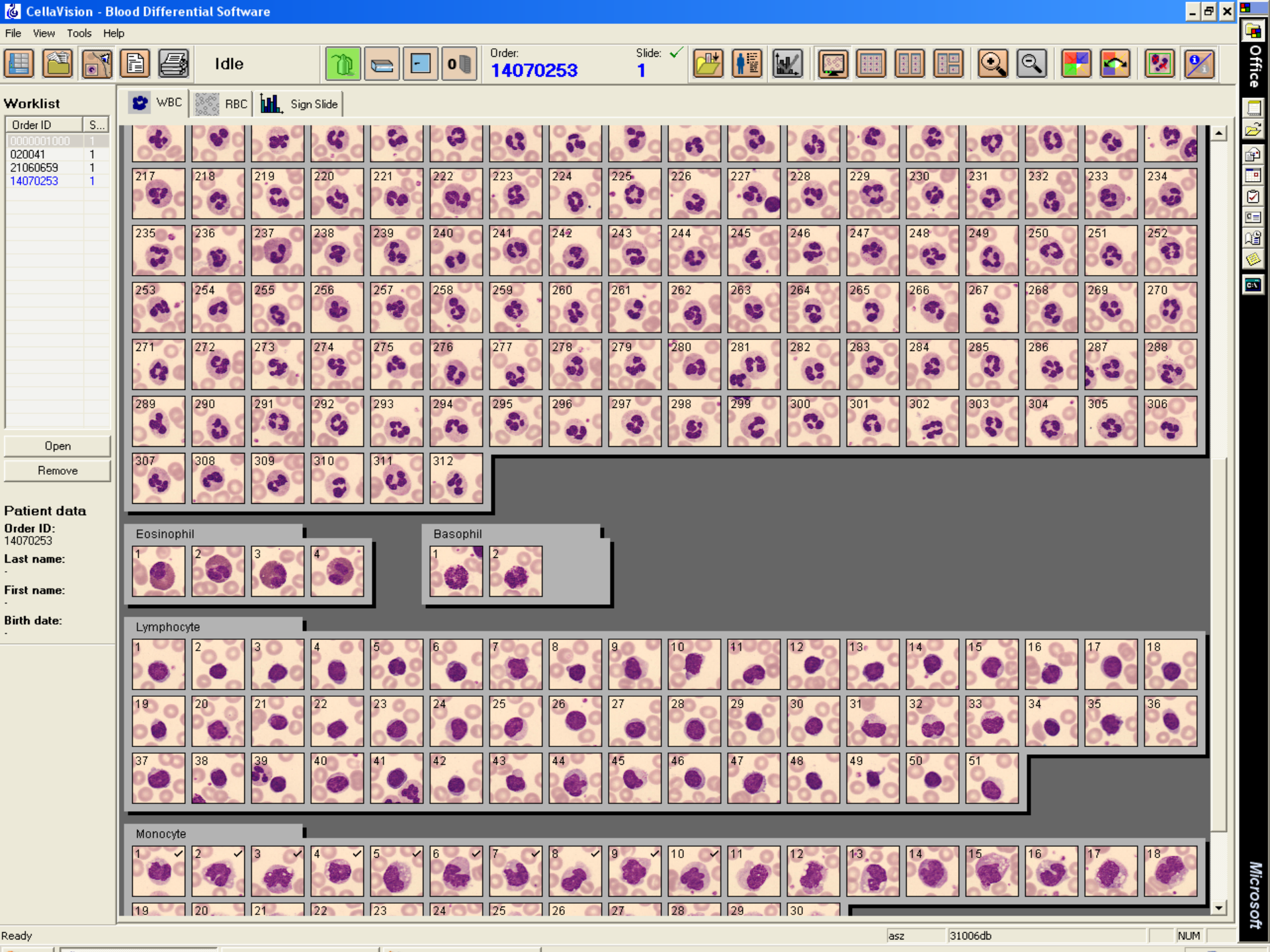
CYTOGENETICS



Digital Imaging/Morfology






CellaVision - Blood Differential Software
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CellaVision - Blood Differential Software (DM96)

File View Tools Help

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230231746	1

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Patient data

Order ID:
230231746

Last name:
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Birth date:
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WBC RBC Sign Slide

WBC	Count
• Unidentified	-
Band neutrophil	-
• Segmented neutrophil	96
• Eosinophil	8
• Basophil	-
• Lymphocyte	76
• Monocyte	2
• Promyelocyte	-
• Myelocyte	-
• Metamyelocyte	-
Promonocyte	-
Prolymphocyte	-
• Blast (no lineage spec)	16
• Lymphocyte, variant form	-
• Plasma cell	-
Hairy cell	-
Cleaved cells	2
Total	200

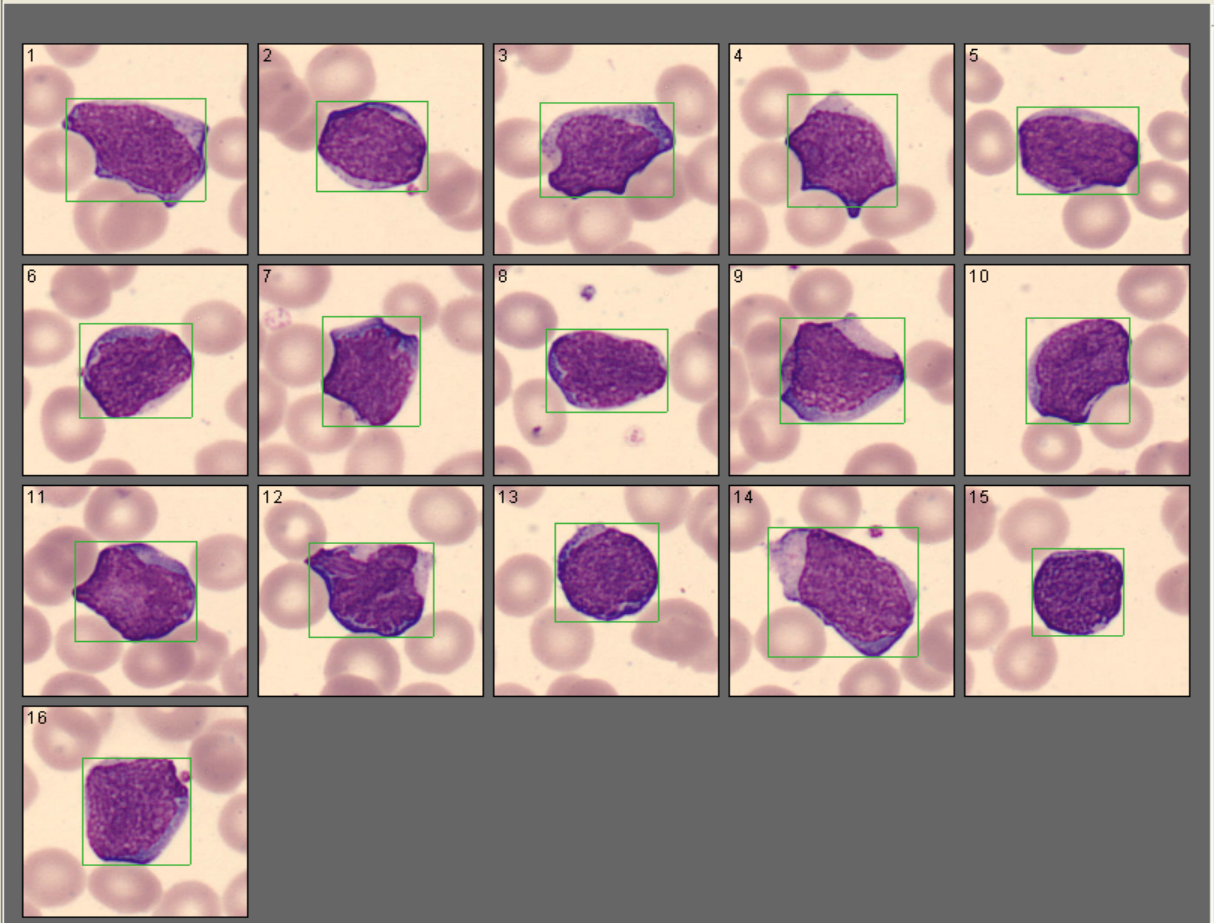
Non-WBC	Count
• Erythroblast (NRBC)	4
• Giant thrombocyte	3
• Thrombocyte aggregation	-
Megakaryocyte	-
• Smudge cell	3
• Artefact	-

Not classed	-

WBC comment

unidentified naar cleaved[jongmans]

Blast (no lineage spec)



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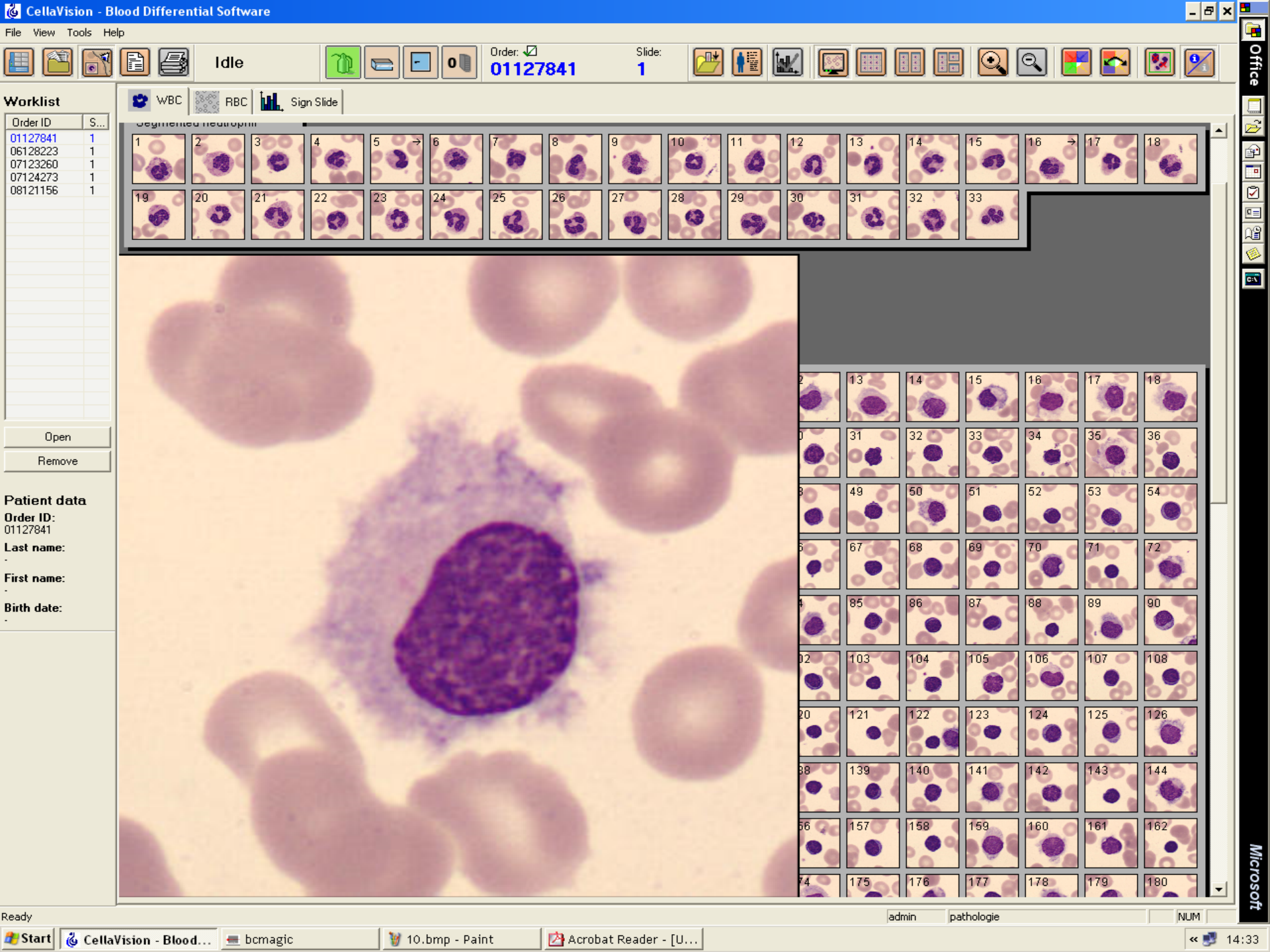
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Microsoft

ORIGINAL ARTICLE

Examination of peripheral blood films using automated microscopy; evaluation of Diffmaster Octavia and Cellavision DM96

H Ceelie, R B Dinkelaar, W van Gelder

J Clin Pathol 2006;**60**:72–79. doi: 10.1136/jcp.2005.035402

See end of article for authors' affiliations

Correspondence to: H Ceelie, Department of Clinical Chemistry, Geïntegreerd Klinisch Chemisch Laboratorium (GKCL), Albert Schweitzer Ziekenhuis Dordrecht and RIVAS Zorggroep Gorinchem, PO Box 444, 3300AK Dordrecht, The Netherlands; h.ceelie@asz.nl

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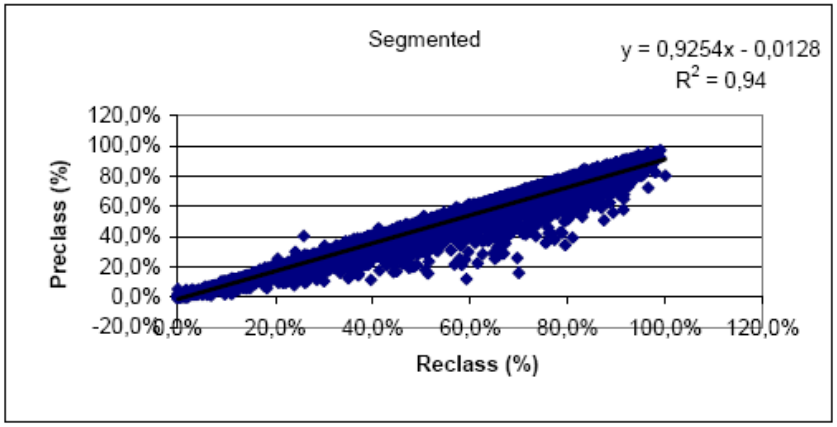
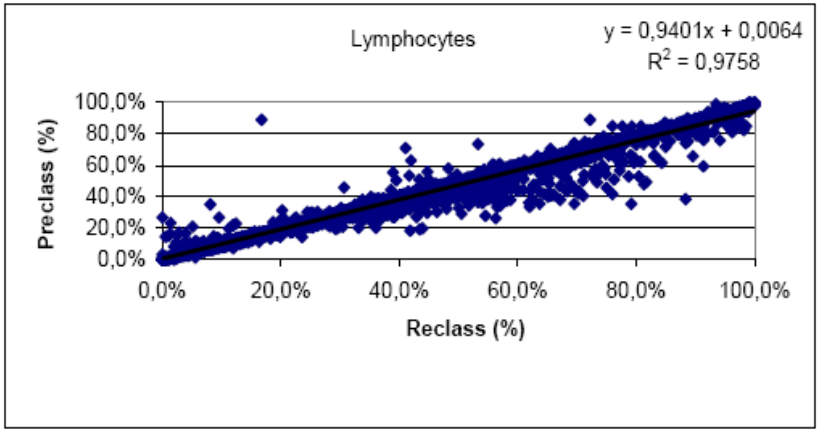
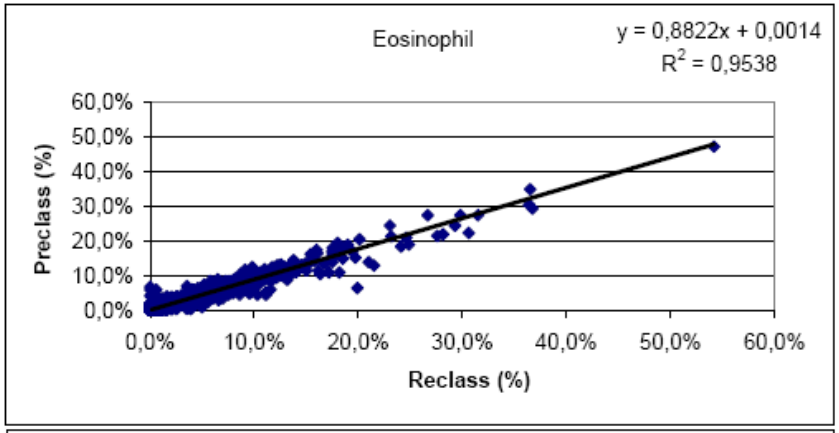
Background: Differential counting of peripheral blood cells is an important diagnostic tool. Yet, this technique requires highly trained staff, is labour intensive and has limited statistical reliability. A recent development in this field was the introduction of automated peripheral blood differential counting systems. These computerised systems provide an automated morphological analysis of peripheral blood films, including a preclassification of both red and white cells (RBCs and WBCs, respectively).
Aims: To investigate the ability of two automated microscopy systems to examine peripheral blood smears.
Methods: Two automated microscopy systems, the Cellavision Diffmaster Octavia (Octavia) and Cellavision DM96 (DM96), were evaluated.
Results: The overall predassification accuracy values for the Octavia and the DM96 systems were 87% and 92%, respectively. Evaluation of accuracy (WBC analysis) showed good correlation for both automated systems when compared with manual differentiation. Total analysis time (including post classification) was 5.4 min/slide for the Octavia and 3.2 min/slide for the DM96 (100 WBC/slide) system. The DM96 required even less time than manual differentiation by an experienced biomedical scientist.
Conclusions: The Octavia and the DM96 are automated cell analysis systems capable of morphological dassification of RBCs and WBCs in peripheral blood smears. Classification accuracy depends on the type of pathological changes in the blood sample. Both systems operate most effectively in the analysis of non-pathological blood samples.

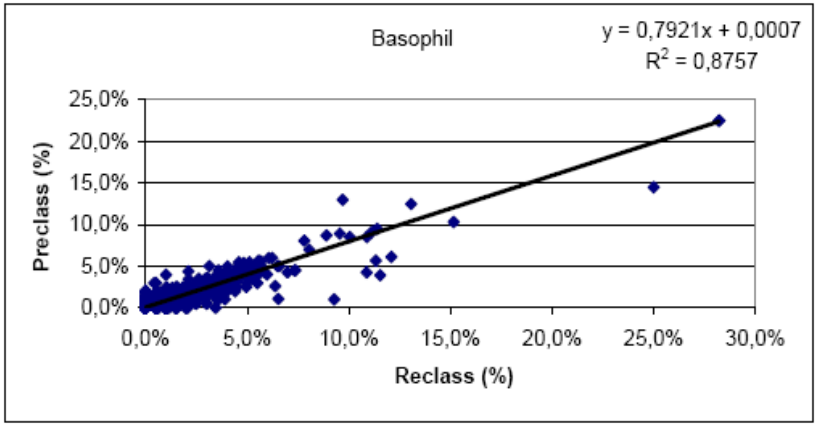
Differential counting of blood cells is an important diagnostic tool for successful treatment and management of patients. Reliable and efficient analysis of patient samples is therefore crucial. Current automated cell counters are based on laser-light scatter and flow-cytochemical princi-

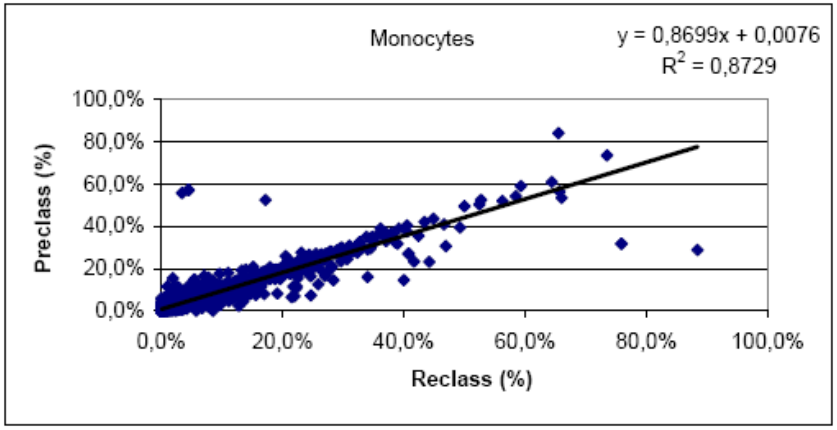
scientist. Coupled with automated sample handling, a "walk-away" system can be created that can partially replace the biomedical scientist. In addition, these systems should be capable of storing relevant morphological data and distribute images to other workstations for review purposes (telehaema-











	R²
segmented neutrophils	0,94
lymphocytes	0,98
eosinophils	0,95
basophils	0,88
monocytes	0,87

Stouten, Riedl et al., Int J Lab Hematol. 2015 Oct;37(5)

Blast cell sensitivity!

DM96/User	Pos	Neg	Total
Pos	241	0	241
Neg	2238	4575	6813
Total	2479	4575	

Specificity	67%
Sensitivity	100%
False pos ratio	33%
False neg ratio	0%

Stouten, Riedl et al., Int J Lab Hematol. 2015 Oct;37(5)

Automated morphological analysis of cells in body fluids by the digital microscopy system DM96

Jurgen A Riedl, Rob B Dinkelaar, Warry van Gelder

Geïntegreerd Klinisch Chemisch Laboratorium, Albert Schweitzer Hospital, Dordrecht, The Netherlands

Correspondence to
Dr Jurgen Riedl, Department of Clinical Chemistry and Haematology, Albert Schweitzer Hospital, Dordrecht, PO Box 444, 3300 AK Dordrecht, The Netherlands; j.riedl@asz.nl

Accepted 18 February 2010

ABSTRACT

Background Differential counting and morphological analysis of nucleated cells in body fluids (eg, cerebrospinal fluid and pleural fluid) are of great diagnostic importance to the clinician. A recent development in this field was the introduction of an application for an automated microscopy system, the DM96 Body Fluid module, enabling the automated analysis of body fluid samples. This computerised system provides an automated morphological analysis of body fluids, including an automated classification of all nucleated cells.

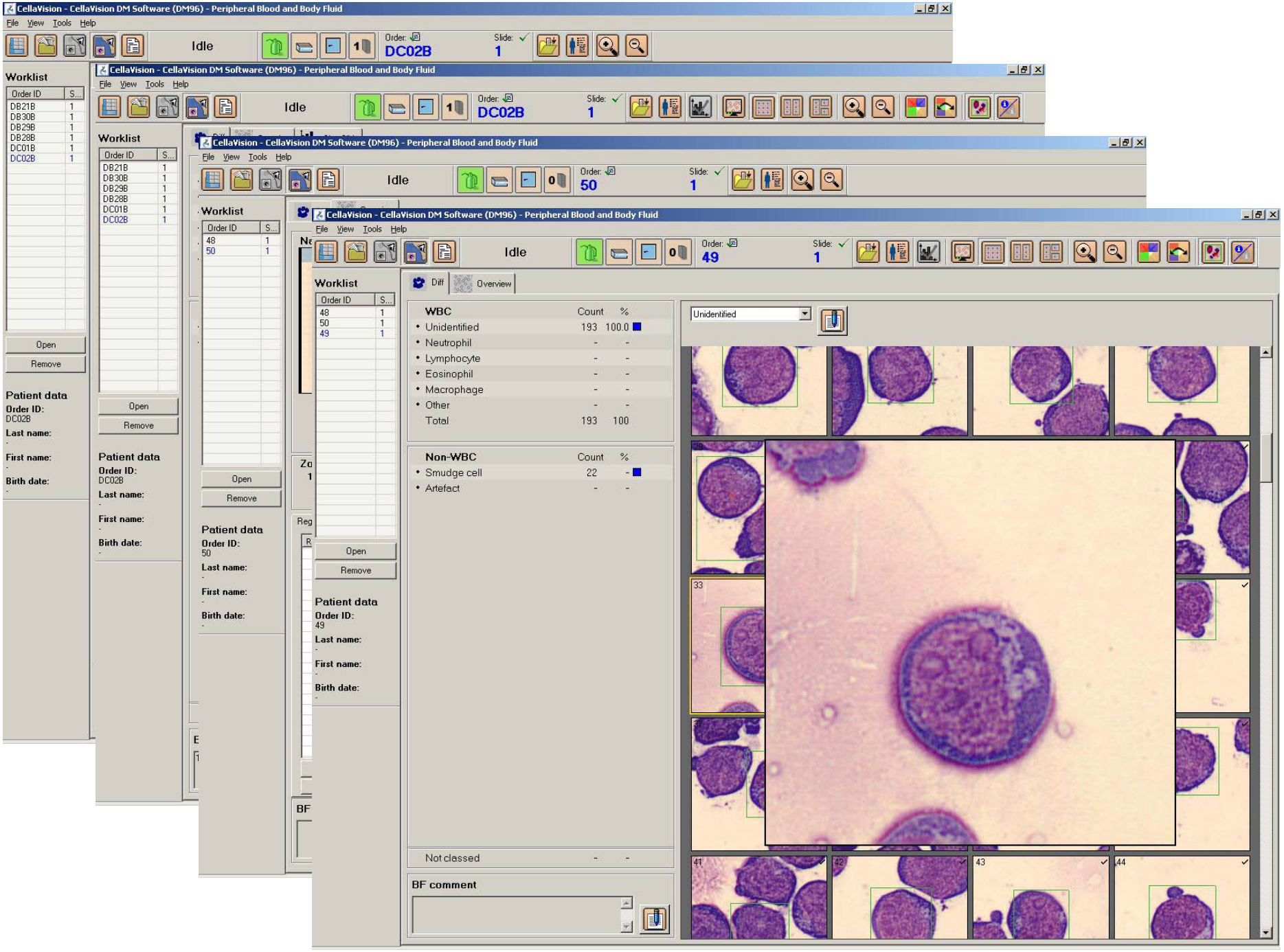
Aims To investigate the ability of the digital microscopy system, DM96, to automatically classify cells in different types of body fluids.

Methods A total of 177 body fluids (including cerebrospinal fluid, abdominal fluid and continuous ambulant peritoneal dialysis fluid) were analysed on the DM96, and results were compared with the manual microscopy method.

Results A study in 177 samples demonstrates an overall preclassification accuracy of 90% in spinal fluid and 83% in other body fluids using the automated system. Correlation coefficients for postclassification as compared with manual review range from 0.92 to 0.99 for spinal fluid sample analyses and from 0.83 to 0.98 for other body fluids. The within-run variation of automated findings and those of previous evaluations of the DM96,[4 5] we decided to put the DM96 into practice in our central laboratory location.

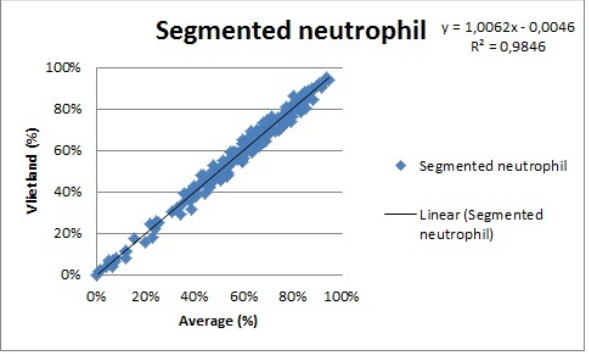
In the past 3–4 years, the DM96 has improved the morphological analysis of blood samples in our laboratory in terms of quality of morphological assessment and turnaround time, and requiring less staff. Moreover, the detection of low percentages of abnormal cells (eg, blasts) in peripheral blood smears has improved using the digital morphology system. This is not only due to increasing the standard differential to 200 cells per sample but also because the system will group cells with similar morphology automatically in one category, thereby facilitating the recognition of pathological cells. Furthermore, the system allows for easy discussion with colleagues about the classification of an individual cell and even with experts in other laboratories using remote access or email. The results of all analyses, including all images, are stored for review purposes.

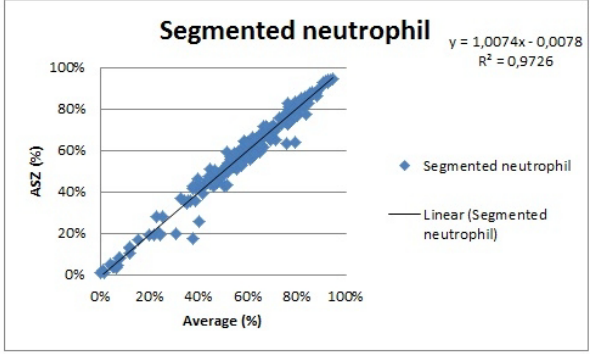
Body fluid material, such as cerebrospinal fluid, requires adequate technical expertise to ensure proper handling and analysis. Samples are collected at the cost of considerable discomfort for the patient and usually yield only a limited volume of material available for analysis. Moreover, there are

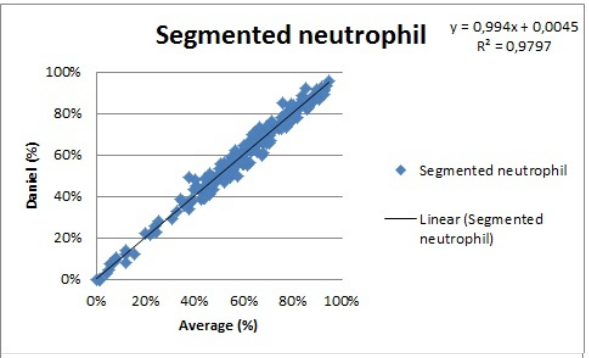

CellaVision - CellaVision DM Software (DM96) - Peripheral Blood and Body Fluid
File View Tools Help
Idle
Order: DC02B
Slide: 1
Worklist
Order ID S...
DB21B 1
DB30B 1
DB29B 1
DB28B 1
DC01B 1
DC02B 1
Open
Remove
Patient data
Order ID:
Last name:
First name:
Birth date:
Order: 50
Slide: 1
48 1
50 1
Order: 49
Slide: 1
49 1
Diff
Overview
WBC Count %
Unidentified 193 100.0
Neutrophil - -
Lymphocyte - -
Eosinophil - -
Macrophage - -
Other - -
Total 193 100
Non-WBC Count %
Smudge cell 22 -
Artefact - -
Not classed - -
BF comment
Unidentified

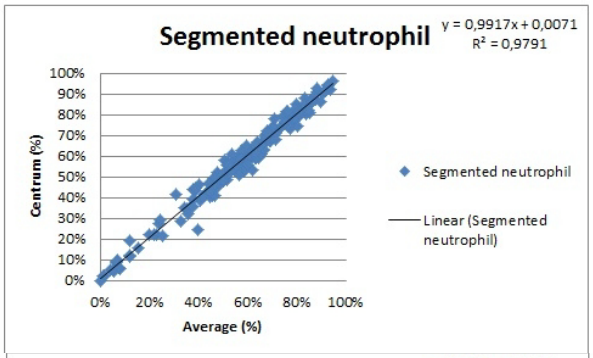
Reproducibility? Inter-Laboratory

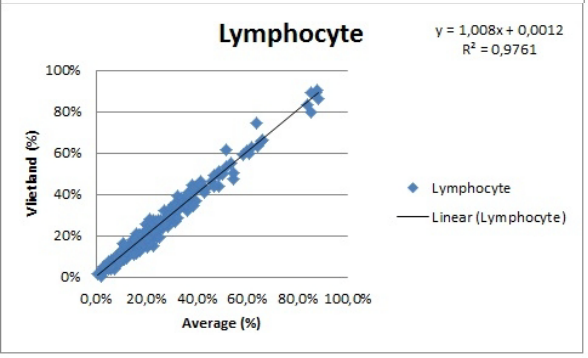
- 200 samples/slides
- 4 different hospital locations
- Comparison of 5-part differential and blasts

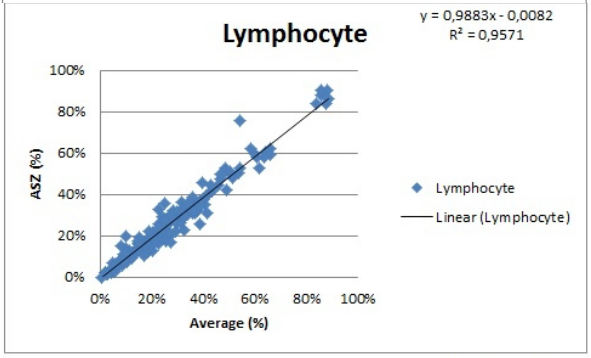


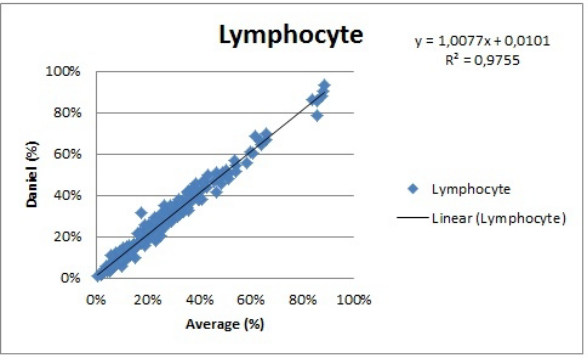


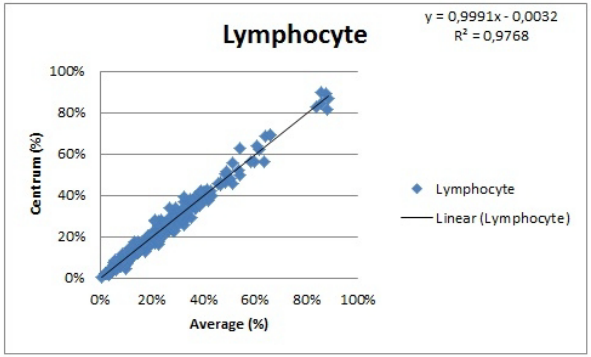












R^{2}-values:

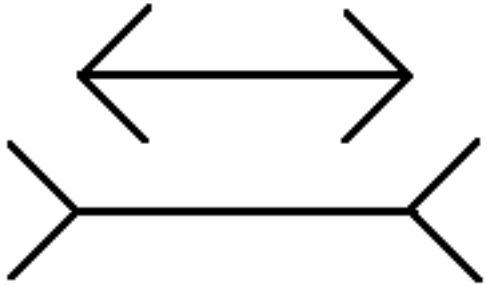
Neutro's:	0,97-0,98
Eo's:	0,82-0,88
Lymfo's:	0,96-0,98
Mono's:	0,89-0,91
Baso's:	0,49-0,59
Blasts:	0,99-1,00

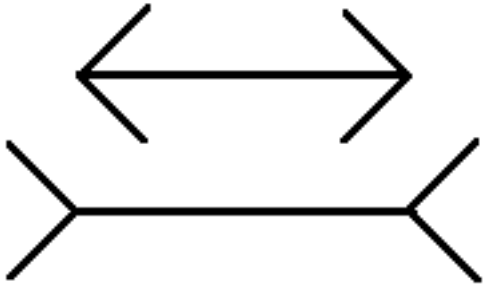
Riedl et al., J Lab Autom. 2015 Apr 29

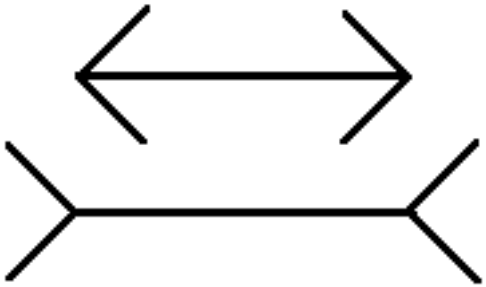
So, Digital Imaging:

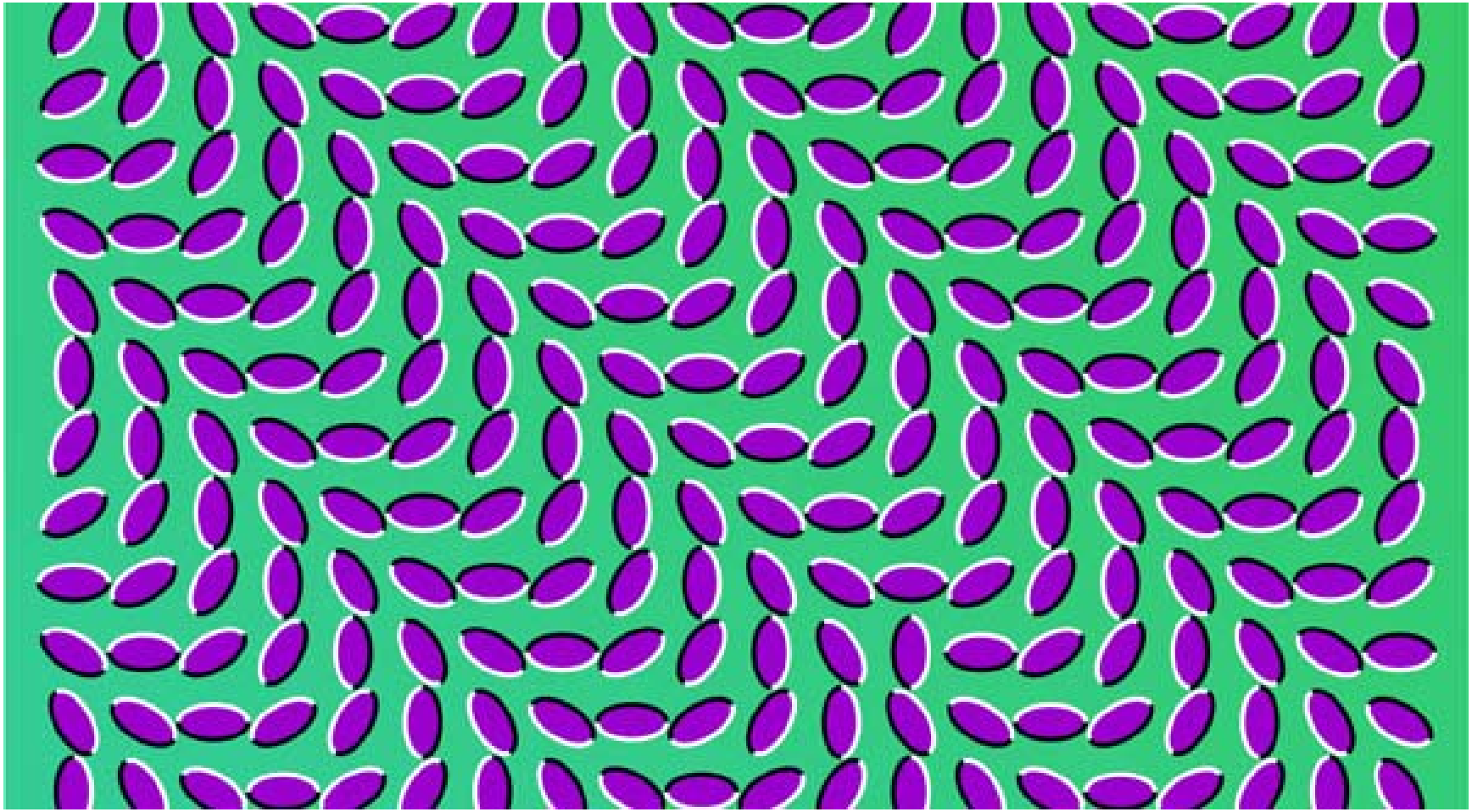
- Works in daily routine for leukocytes in peripheral blood and other fluids
- Is reliable, accurate and displays a high blast cell sensitity
- Is reproducible
- Excellent learning tool for the next generation

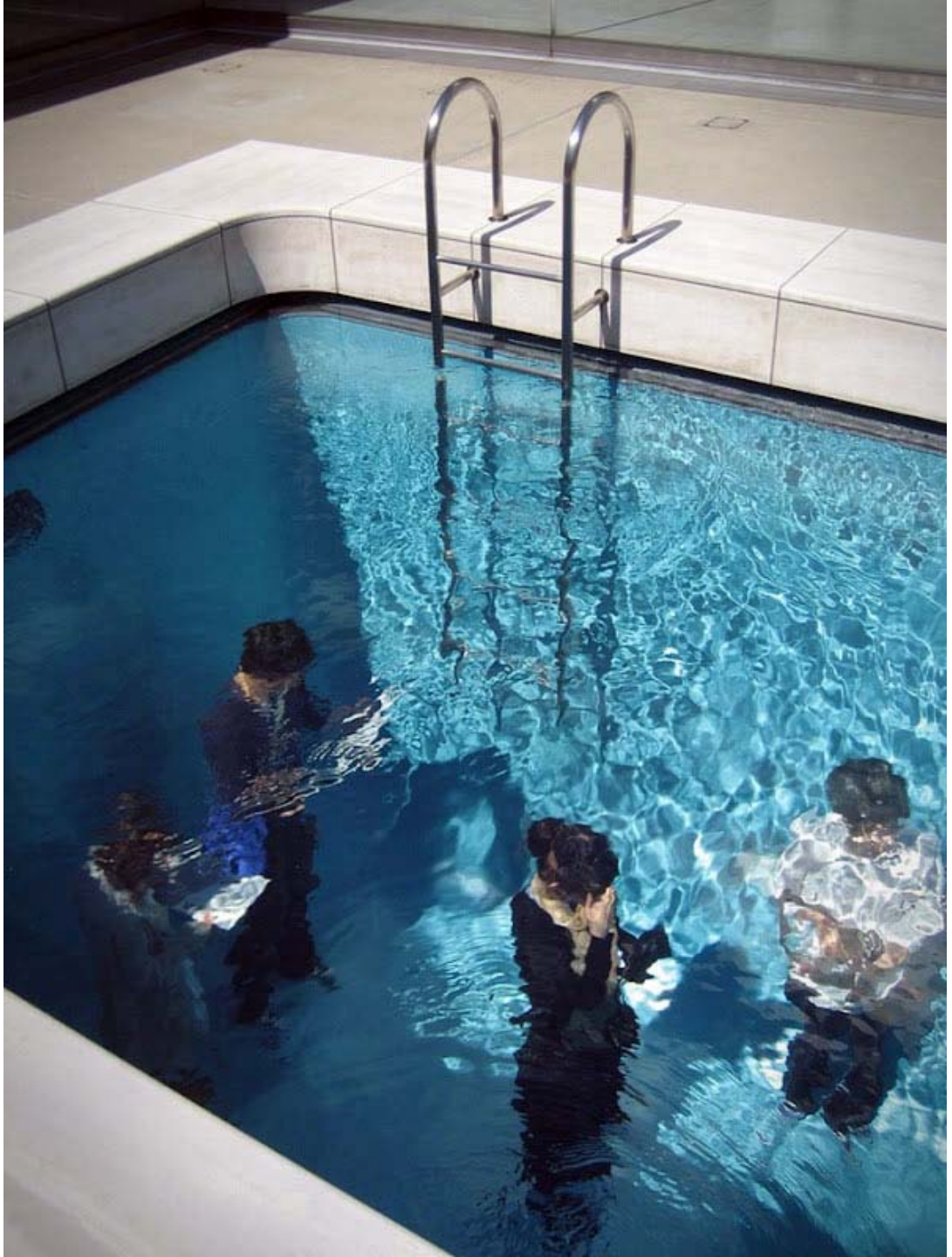
- Not everything is what it seems…











- So, the human eye is far from objective
- And…context matters

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REVIEW INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

Reporting and grading of abnormal red blood cell morphology

B. T. CONSTANTINO

Mississauga, ON, Canada

Correspondence:
Benie T. Constantino, 5591 Haddon Hall Road, Mississauga, ON L5M 5G4, Canada.
E-mail: btconstantino@hotmail.com

doi:10.1111/ijlh.12215

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Keywords
Red blood cell morphology, grading system, standardization

SUMMARY

In spite of the continual standardization of test result formats, the improvements of laboratory technologies, publications of reference guidelines, and the advancements in hematology analyzers, the methods of reporting or grading abnormal red blood cell morphology still vary among laboratories everywhere. This article describes the methods or systems of reporting abnormal red cell morphology and the conditions associated with the abnormalities.

Constantino; Int J Lab Hematol. 2015 Feb;37(1):1-7.

Table 1. Reference guide for grading red blood cell morphology [1–4, 9–12]

Red blood cells (cell type)	Normal (nonspecific)	1+ (%) (Slight/few)	2+ (%) (Moderate)	3+ (%) (Marked)
Hypochromasia		5–15	16–40	>40
(MCH – pg)	27–34 pg	22–26	18–21	<18
Polychromasia		3–5	6–20	>20
Microcytes (MCV – fL)	80–99 fL	70–79	60–69	<60
Macrocytes (MCV – fL)	80–99 fL	100–110	111–125	>125
Schistocytes (Fragments)		1–5	6–15	>15
Elliptocytes/Ovalocytes		6–20	21–50	>50
Rouleaux			11–50	>50
Spherocytes		1–5	6–20	>20
Target cells		5–10	11–25	>25
Acanthocytes		1–10	11–30	>30
Burr cells – – – >				

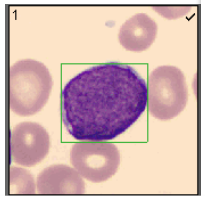
Constantino; Int J Lab Hematol. 2015 Feb;37(1):1-7.

Table 2. Conditions associated with abnormal RBC morphology based on their grading [4, 8, 13–20]

Cell type	Slight* (1+) to Moderate* (2+)		Marked 3+
Schistocytes (Fragments)	Hypersplenism Myeloid metaplasia Megaloblastic anemia Iron deficiency anemia Cancer cytotoxic chemotherapy Enzymes deficiencies Premature infants Renal graft rejection Infection Severe sepsis Myelofibrosis	Thalassemia major Severe burns Mechanical hemolytic anemia (prosthetic heart valve) Hereditary pyropoikilocytosis Metastatic carcinoma Chronic renal failure Unstable hemoglobin Malignant hypertension	Microangiopathic hemolytic anemia Disseminated intravascular coagulation Vasculitis syndromes
Elliptocytes/ Ovalocytes	Megaloblastic anemia Severe iron deficiency anemia Sickle cell anemia Hypersplenic state Metastatic carcinoma Sideroblastic state Thalassemia trait	Hereditary pyropoikilocytosis Myelofibrosis Hemoglobin C trait South East Asian ovalocytosis	Hereditary elliptocytes
Rouleaux		Hyperfibrinogenemia Hyperglobulinemia Chronic inflammatory Disorders	Multiple myeloma Waldenstrom's Macroglobulinemia
Spherocytes	Post splenectomy Liver disease Hemoglobinopathies Older population of transfused red cells Heart valve prosthesis Heinz body hemolytic anemia Premature infants Mvelofibrosis	Microangiopathic hemolytic anemia Hereditary pyropoikilocytosis Severe burns Hypersplenism Clostridium perfringens	Hereditary spherocytosis Autoimmune hemolytic anemia Hemolytic transfusion reaction ABO incompatibility

Constantino; Int J Lab Hematol. 2015 Feb;37(1):1-7.

- Despite efforts to standardisation of RBC morfology and grading there are still inter-laboratory differences…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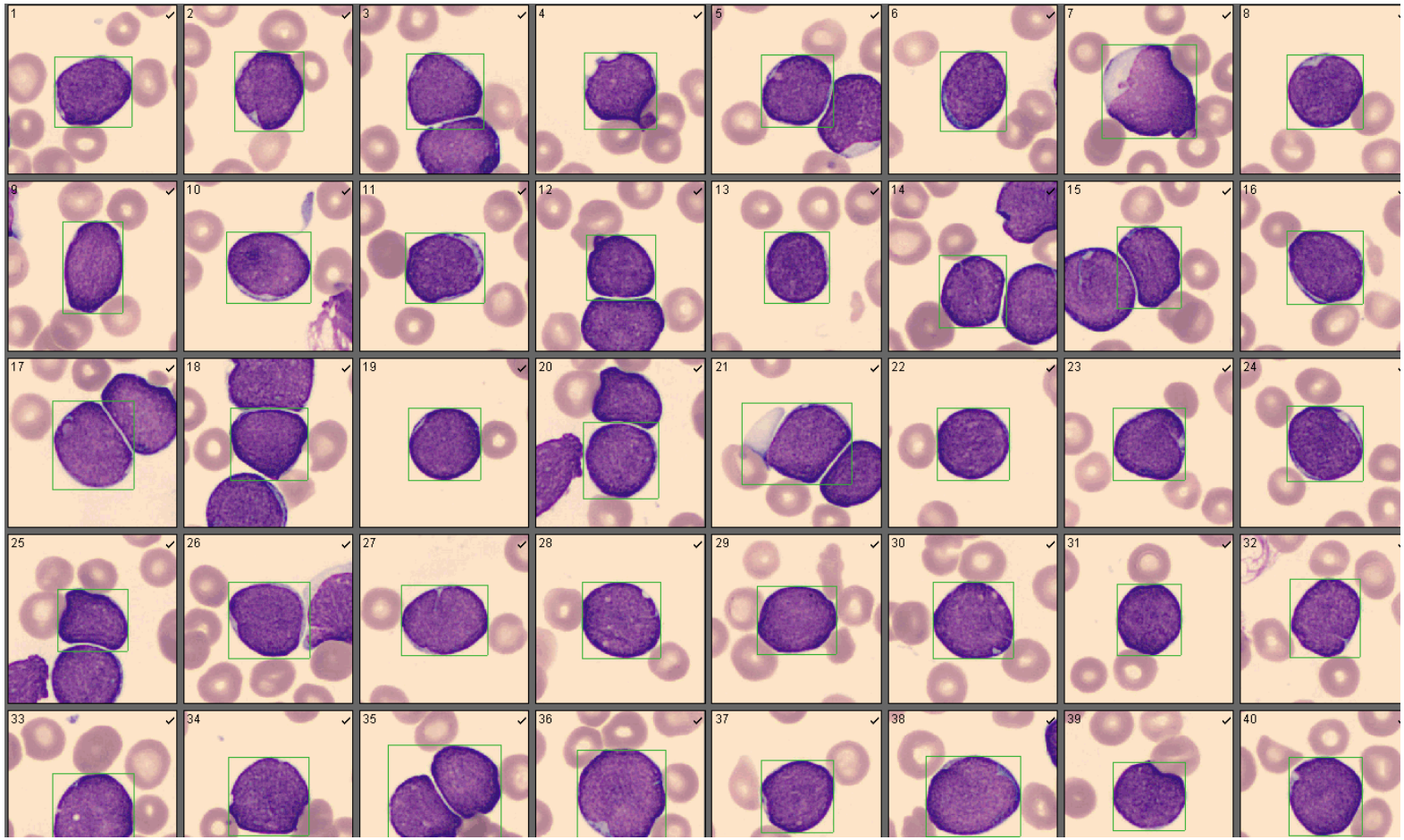
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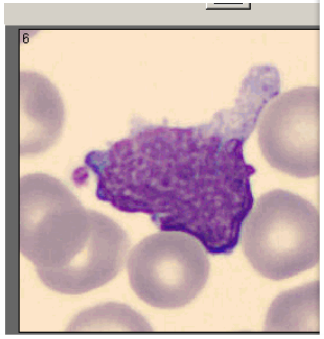
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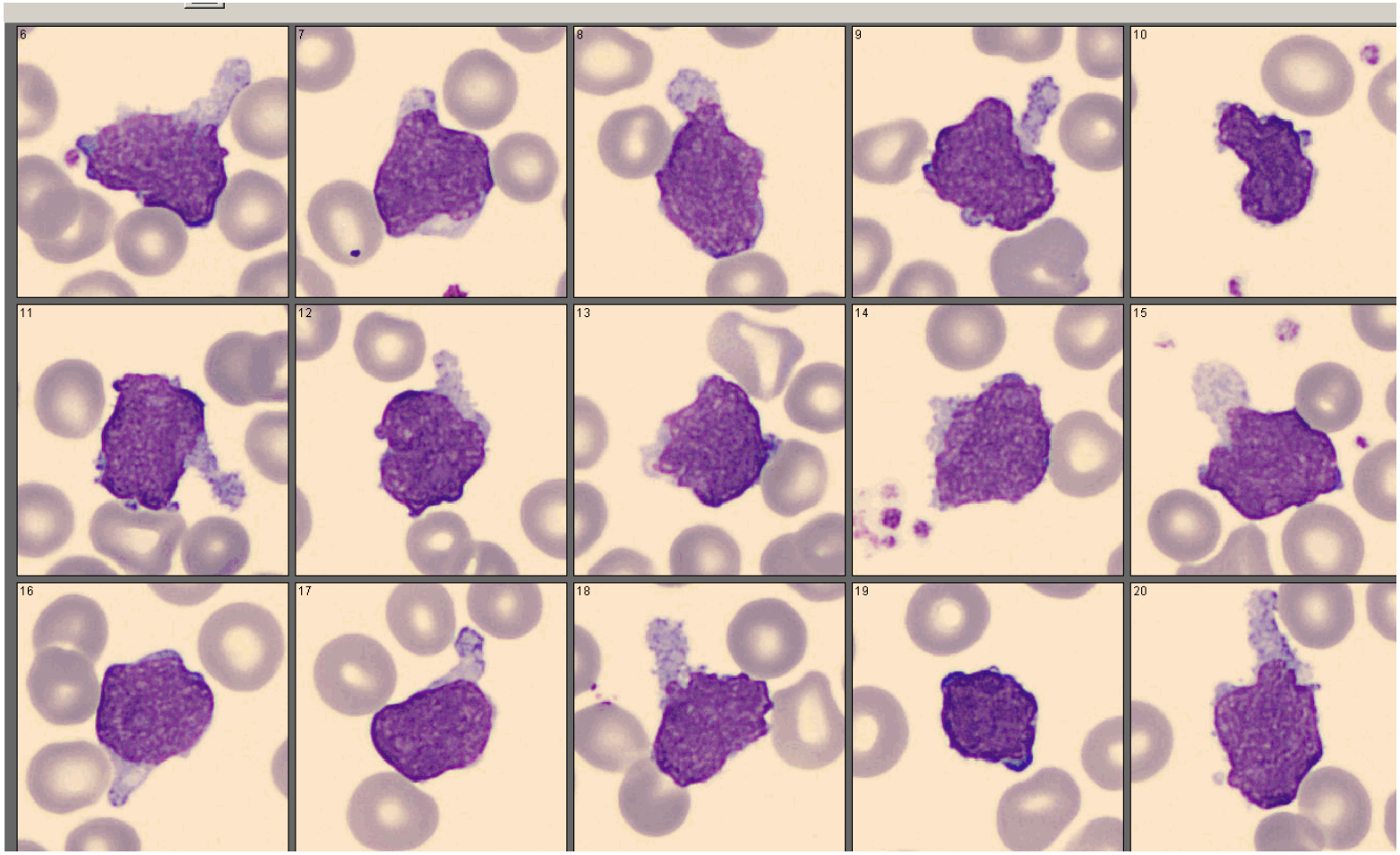
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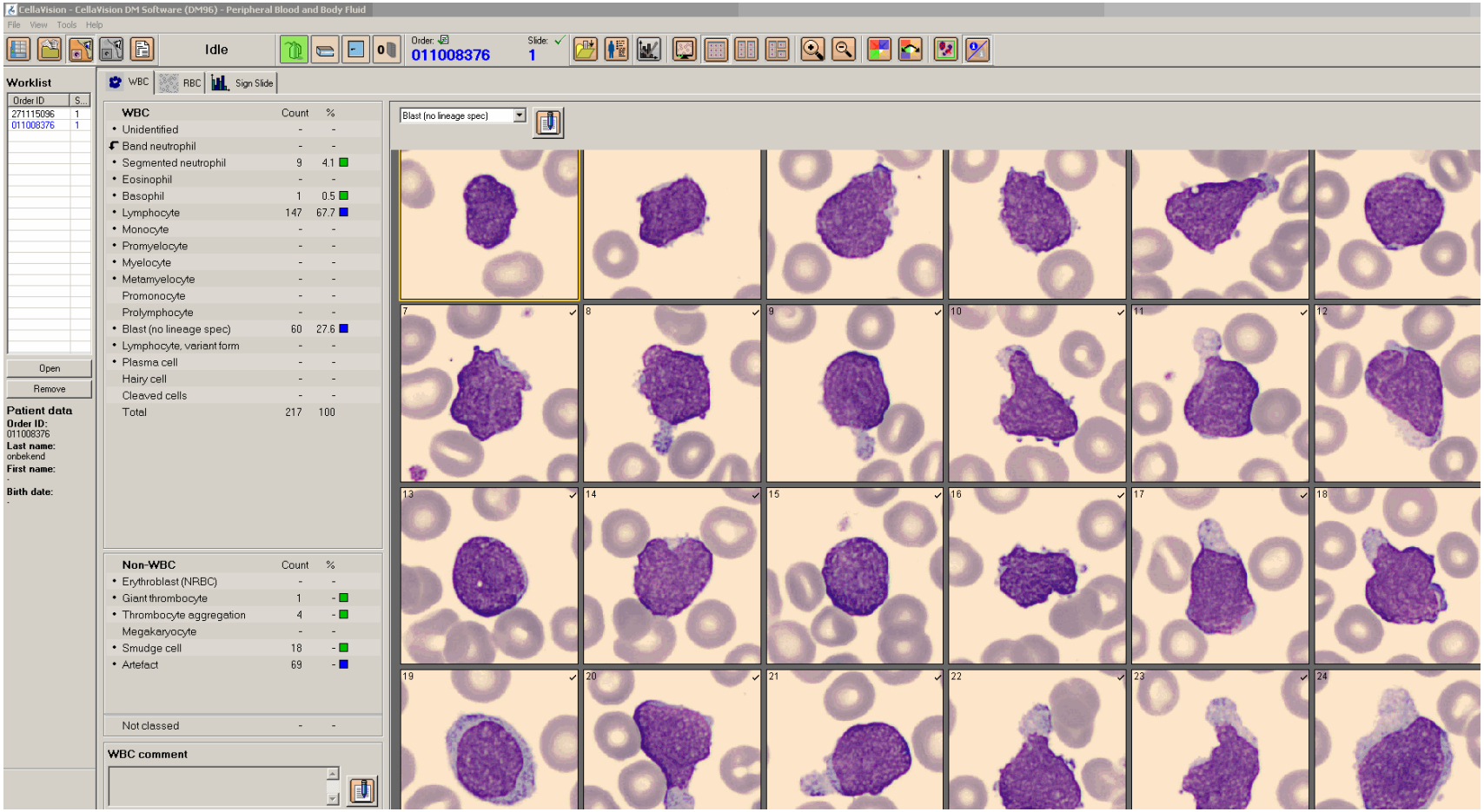
CellaVision - CellaVision DM Software (DM96) - Peripheral Blood and Body Fluid
File View Tools Help
Idle
Order: 011008376
Slide: 1
Worklist
Order ID S...
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011008376 1
Open
Remove
Patient data
Order ID:
011008376
Last name:
onbekend
First name:
-
Birth date:
-
WBC
RBC
Sign Slide
WBC Count %
Unidentified - -
Band neutrophil - -
Segmented neutrophil 9 4.1
Eosinophil - -
Basophil 1 0.5
Lymphocyte 147 67.7
Monocyte - -
Promyelocyte - -
Myelocyte - -
Metamyelocyte - -
Promonocyte - -
Prolymphocyte - -
Blast (no lineage spec) 60 27.6
Lymphocyte, variant form - -
Plasma cell - -
Hairy cell - -
Cleaved cells - -
Total 217 100
Non-WBC Count %
Erythroblast (NRBC) - -
Giant thrombocyte 1 -
Thrombocyte aggregation 4 -
Megakaryocyte - -
Smudge cell 18 -
Artefact 69 -
Not classed - -
WBC comment
Blast (no lineage spec)
1 2 3 4 5 6
7 8 9 10 11 12
13 14 15 16 17 18
19 20 21 22 23 24

Digital Imaging of RBC’s

Albert Schweitzer ziekenhuis

Content:

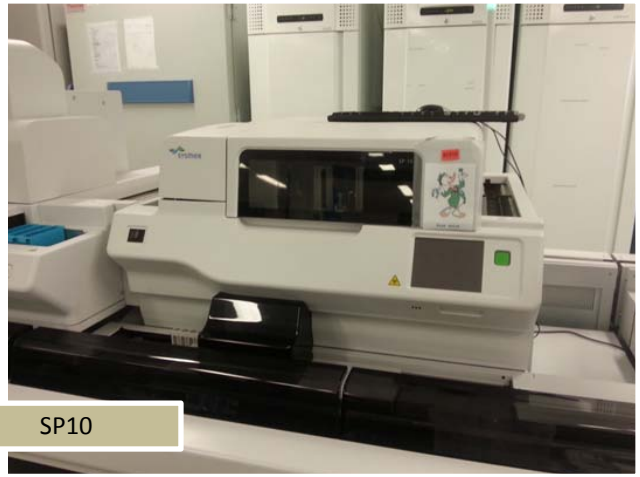
- Introduction
- Material & Methods
- Results
- Conclusion & Discussion

Introduction

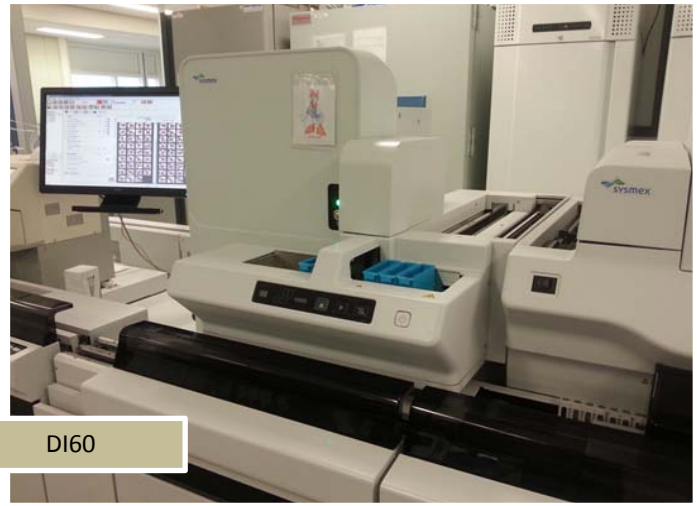
- **Aim of the study:** validate a novel morphological RBC-module which is capable of correct detection and classification of RBC abnormalities.
  - is determination of a cut-off value possible?

- Automatic classification of leukocytes is already used on an routine basis in variety of labs around the world.
  - now also possible in a total lab automation setting (e.g. DI-60, Sysmex).

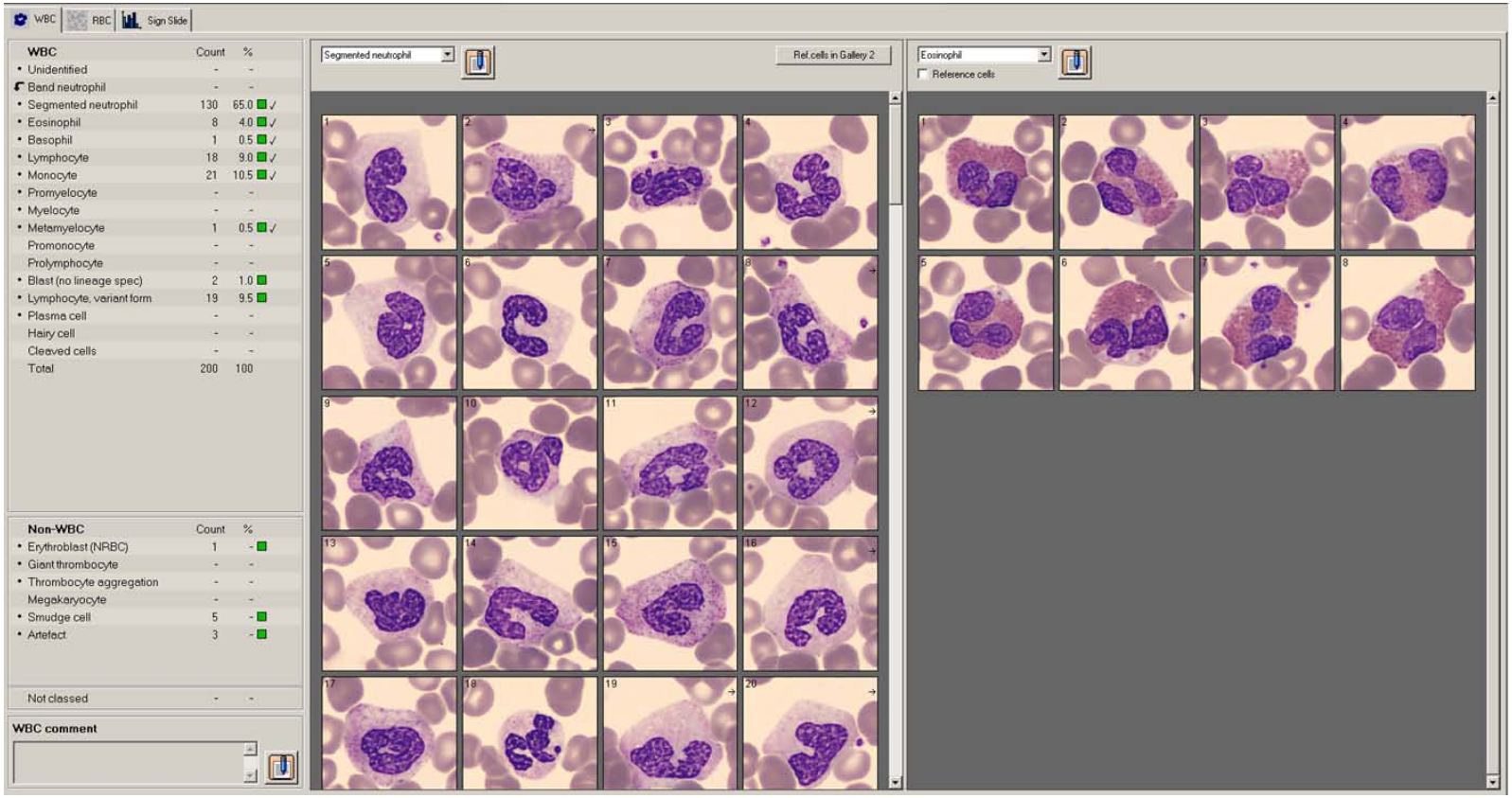
Cellcounter



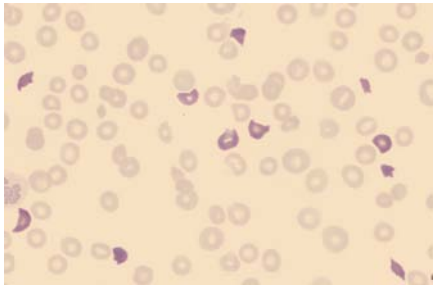
SP10



DI60

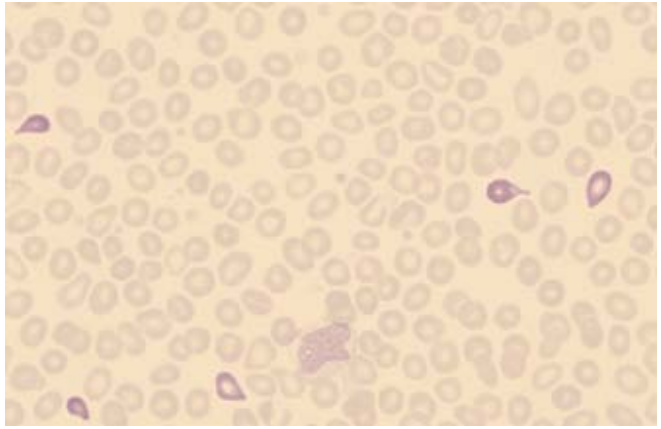

WBC
RBC
Sign Slide
WBC Count %
Unidentified - -
Band neutrophil - -
Segmented neutrophil 130 65.0
Eosinophil 8 4.0
Basophil 1 0.5
Lymphocyte 18 9.0
Monocyte 21 10.5
Promyelocyte - -
Myelocyte - -
Metamyelocyte 1 0.5
Promonocyte - -
Prolymphocyte - -
Blast (no lineage spec) 2 1.0
Lymphocyte, variant form 19 9.5
Plasma cell - -
Hairy cell - -
Cleaved cells - -
Total 200 100
Non-WBC Count %
Erythroblast (NRBC) 1 -
Giant thrombocyte - -
Thrombocyte aggregation - -
Megakaryocyte - -
Smudge cell 5 -
Artefact 3 -
Not classed - -
WBC comment
Segmented neutrophil
Ref.cells in Gallery 2
Eosinophil
Reference cells

- Detection of morphological RBC abnormalities is essential in the diagnostic process of several diseases:
  - TTP; fragmentocytes/schistocytes
  - Myelofibrosis; teardrop cells
  - Thalassemia; target cells

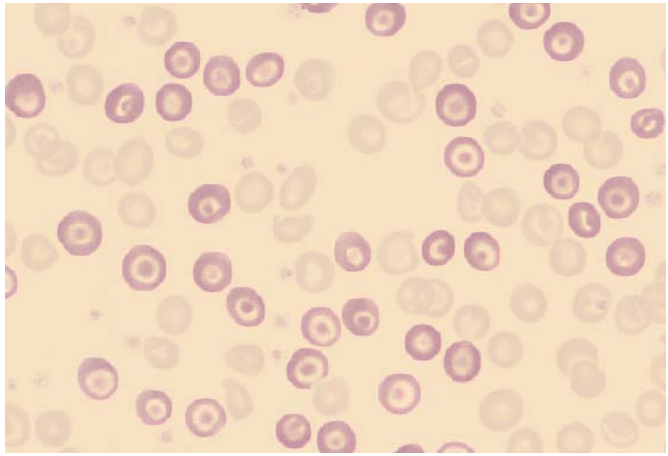


- **TTP (Trombotische trombocytopenische purpura):**
  - Acute, medical emergency
  - ADAM-TS 13 enzyme non-functional (no cleaving of vWF)
  - Hemolytic anemia & trombocytopenia
  - Patients display:
    - Fever, neurological symptoms, kidney failure, hemolytic anemia & tromocytopenia & fragmentocytes/schistocytes.

- **Myelofibrosis:**
  - Myeloproliferative disorder
  - Uncontrolled fibrosis of bone-marrow
  - No room for normal hematopoiesis -> extramedullary hematopoiesis
  - hallmarks:
    - Progressive anemia, pancytopenia, splenomegaly of hepatomegaly (extramedullary hematopoiesis), leuko-erytroblastosis, myeloïd precursors and teardropcells in the peripheral blood smear.
    - Usually begins with leuko- & trombocytosis.
    - JAK2- and recently discovered CALR-mutation



- **Thalassemia:**
  - Hemoglobinopathies
  - Thalassemia is caused by variant or missing genes that affect how the body makes hemoglobin
  - Normal hemoglobin -> 4 globin chains; 2 alpha en 2 bèta-globin chains
  - Two main forms; alpha- en bèta-thalassemia
  - Autosomal recessive disorder
  - Diagnostics:
    - Usual low MCV and high erytrocyte count
    - Mild anemia usually
    - Target cells



Materials and methods

- Peripheral blood smears of 316 patiënt samples and 10 healthy individuals (determination of a “cut-off” value)
  - 80 samples were used containing fragmentocytes, targetcells en teardrop cells
- May-Grünwald Giemsa staining using SP-10 (automatic slidemaker/stainer)
  - 2000-4000 erytrocytes were analysed/bloodsmear
  - Pre-classification by the RBC module and post-classification manually
- Statistical analysis using EP-evaluator (Passing-Bablok)

CellaVision - CellaVision DM Software (DM96) - Peripheral Blood and Advanced RBC

File View Tools Help

Error

Order: 030951026 Slide: 1

Worklist

Order ID	S...
130649146	1
080770966	1
140703356	1
160948676	1
030951026	1

Open

Remove

Patient data

Order ID:
030951026
Last name:
-
First name:
-
Birth date:
-

RBC | Sign Slide

Report all as 0 - normal
Use characterization

	0	1	2	3	%
COLOR					
• Polychromatic cells	0	●	○	○	3.6
• Hypochromatic cells	1	○	●	○	11.7
SIZE					
• *Anisocytosis*	1	○	●	○	11.1
• Microcytes	0	●	○	○	6.5
• Macrocytes	0	●	○	○	4.7
SHAPE					
• *Poikilocytosis*	1	○	●	○	19.5
• Target cells	0	●	○	○	2.6
• Schistocytes	2	○	●	●	3.7
• Helmet cells	0	●	○	○	0.4
• Sickle cells	0	●	○	○	0.1
• Spherocytes	1	○	●	○	1.0
• Elliptocytes	1	○	●	○	7.6
• Ovalocytes	0	●	○	○	0.4
• Tear drop cells	0	●	○	○	0.8
• Stomatocytes	0	●	○	○	2.2
• Acanthocytes	0	●	○	○	0.0
• Echinocytes	0	●	○	○	0.7
INCLUSIONS					
• Howell-Jolly	0	●	○	○	0.1
• Pappenheimer	0	●	○	○	0.1
• Basophilic stippling	0	●	○	○	0.0
• Parasites	0	●	○	○	0.0
TRASH					

Number of RBCs used for pre-characterization: 2359

Reset to Precharacterization

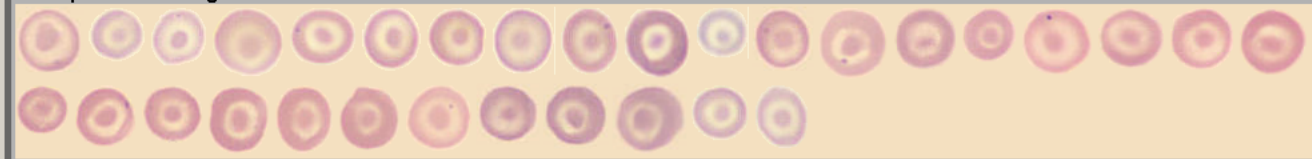
Exclude RBC Analysis

RBC comment

Overview Individual Cells

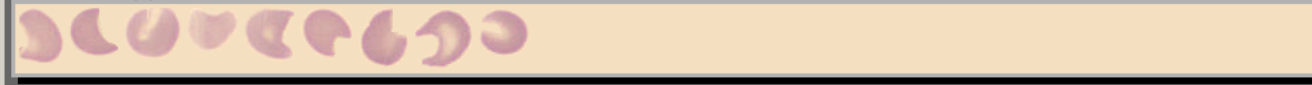
Target cells (61)

Example Cells : Target cells

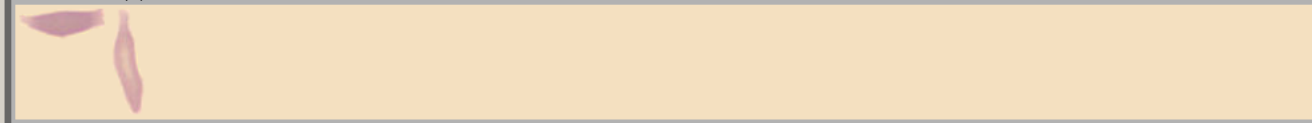


Schistocytes (88)

Helmet cells (9)



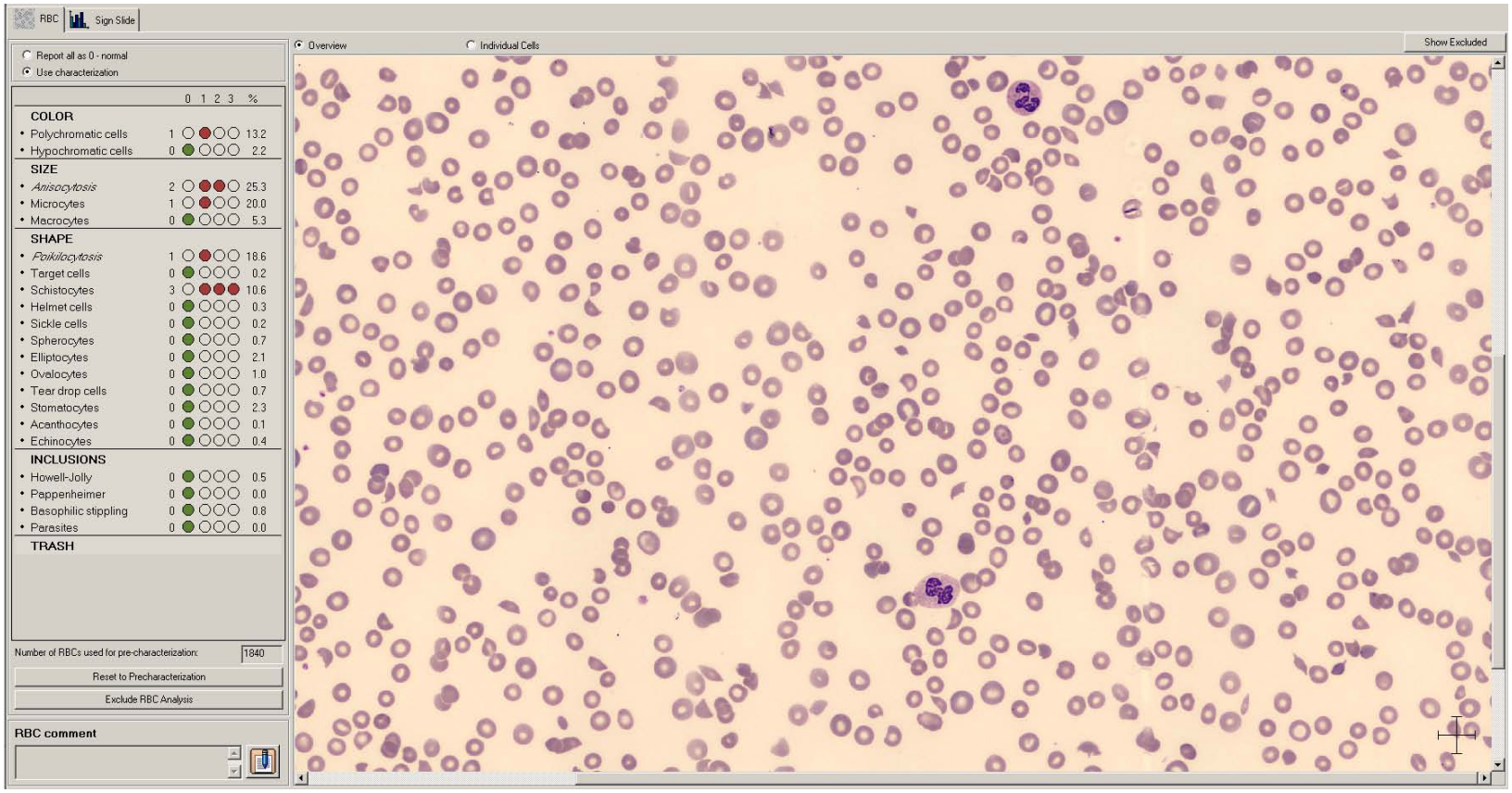
Sickle cells (2)

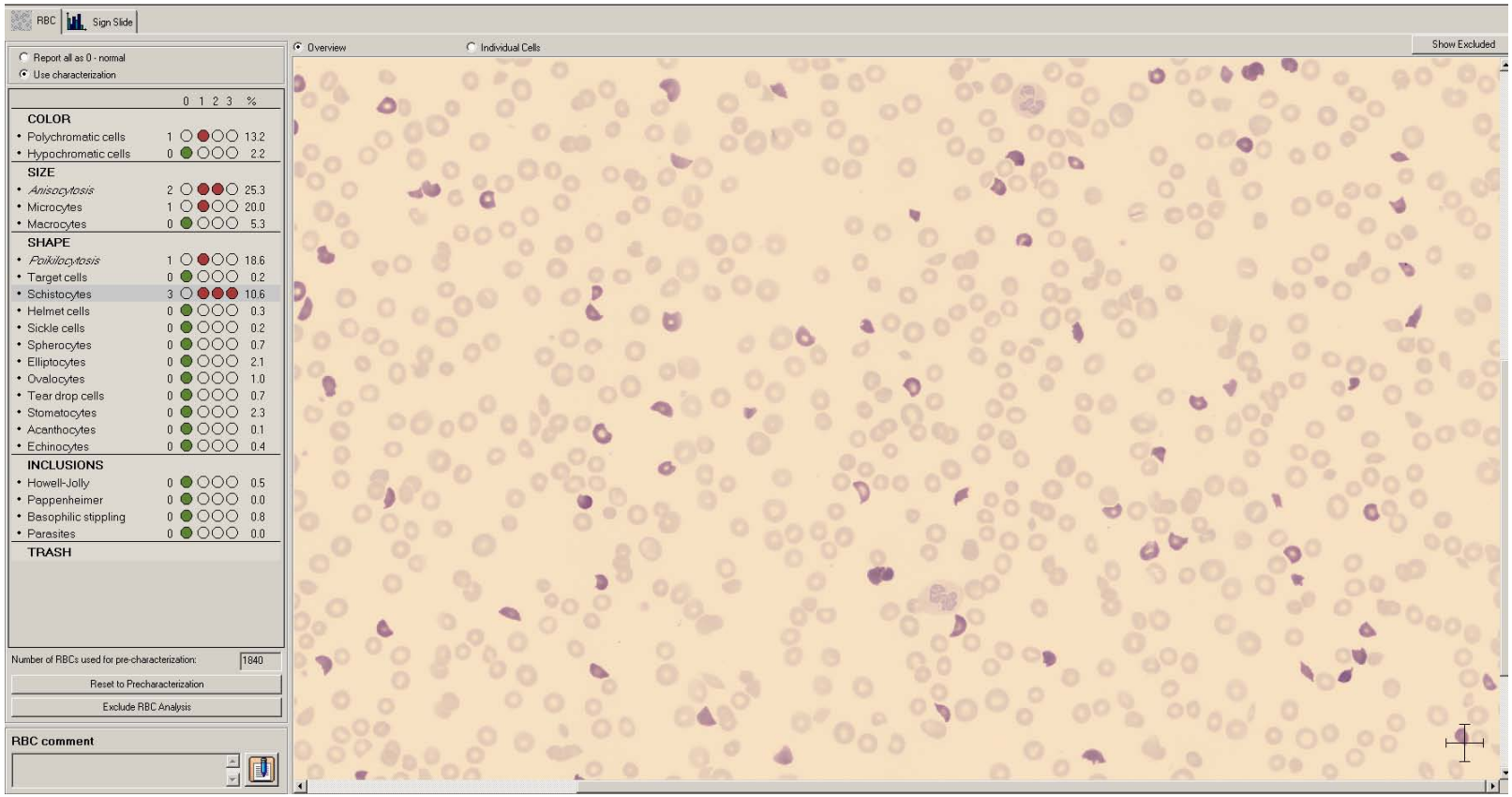


Spherocytes (24)

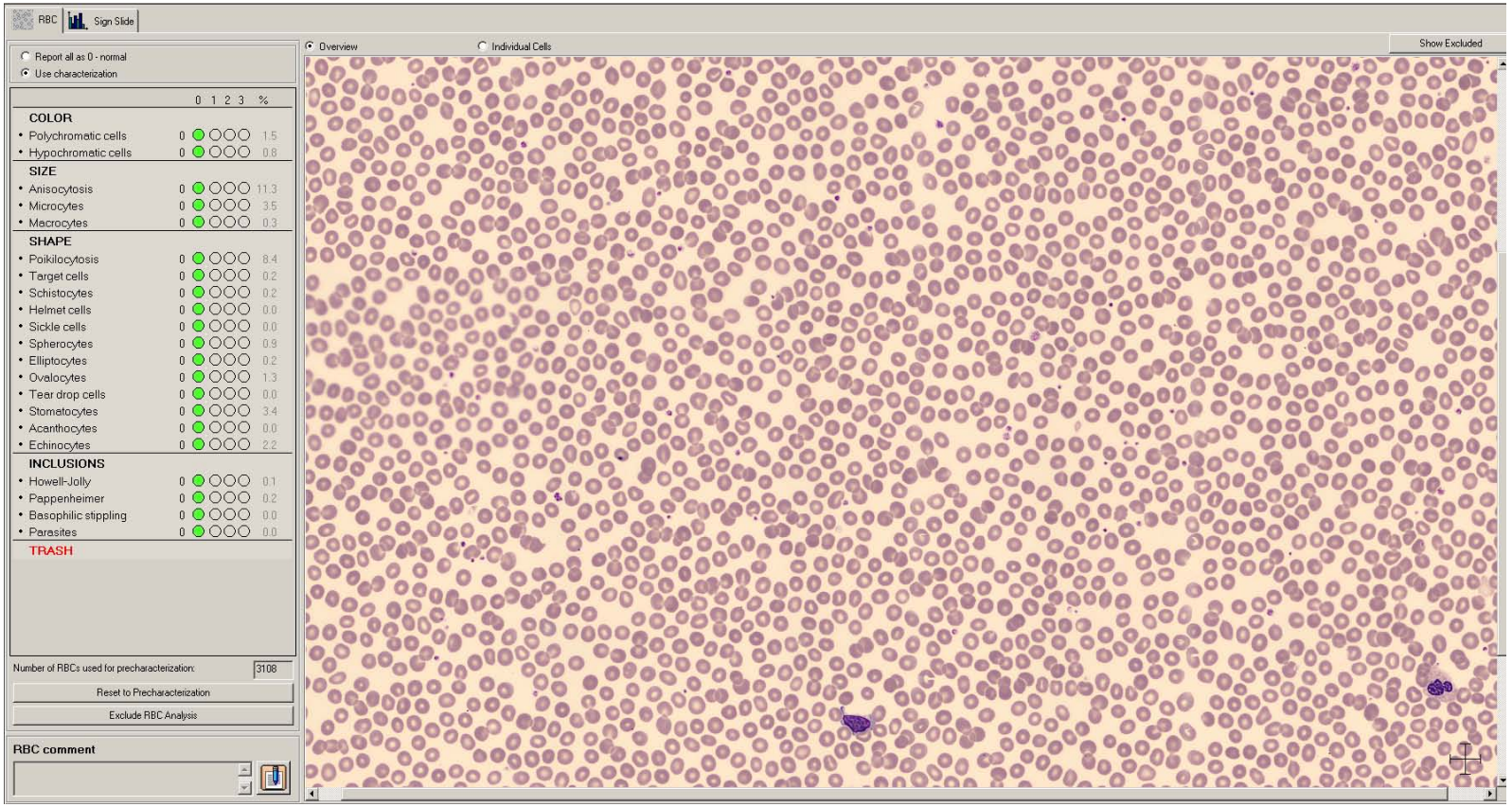
Ready

Start | CellaVision - CellaVisi... | target.bmp - Paint


RBC
Sign Slide
Report all as 0 - normal
Use characterization
0 1 2 3 %
COLOR
• Polychromatic cells 1 13.2
• Hypochromatic cells 0 2.2
SIZE
• Anisocytosis 2 25.3
• Microcytes 1 20.0
• Macrocytes 0 5.3
SHAPE
• Poikilocytosis 1 18.6
• Target cells 0 0.2
• Schistocytes 3 10.6
• Helmet cells 0 0.3
• Sickle cells 0 0.2
• Spherocytes 0 0.7
• Elliptocytes 0 2.1
• Ovalocytes 0 1.0
• Tear drop cells 0 0.7
• Stomatocytes 0 2.3
• Acanthocytes 0 0.1
• Echinocytes 0 0.4
INCLUSIONS
• Howell-Jolly 0 0.5
• Pappenheimer 0 0.0
• Basophilic stippling 0 0.8
• Parasites 0 0.0
TRASH
Number of RBCs used for pre-characterization: 1840
Reset to Precharacterization
Exclude RBC Analysis
RBC comment
Overview
Individual Cells
Show Excluded


RBC
Sign Slide
Report all as 0 - normal
Use characterization
0 1 2 3 %
COLOR
Polychromatic cells 1 13.2
Hypochromatic cells 0 2.2
SIZE
Anisocytosis 2 25.3
Microcytes 1 20.0
Macrocytes 0 5.3
SHAPE
Poikilocytosis 1 18.6
Target cells 0 0.2
Schistocytes 3 10.6
Helmet cells 0 0.3
Sickle cells 0 0.2
Spherocytes 0 0.7
Elliptocytes 0 2.1
Ovalocytes 0 1.0
Tear drop cells 0 0.7
Stomatocytes 0 2.3
Acanthocytes 0 0.1
Echinocytes 0 0.4
INCLUSIONS
Howell-Jolly 0 0.5
Pappenheimer 0 0.0
Basophilic stippling 0 0.8
Parasites 0 0.0
TRASH
Number of RBCs used for pre-characterization: 1840
Reset to Precharacterization
Exclude RBC Analysis
RBC comment
Overview
Individual Cells
Show Excluded


RBC
Sign Slide
Report all as 0 - normal
Use characterization
Overview
Individual Cells
0 1 2 3 %
COLOR
Polychromatic cells 1 13.2
Hypochromatic cells 0 2.2
SIZE
Anisocytosis 2 25.3
Microcytes 1 20.0
Macrocytes 0 5.3
SHAPE
Poikilocytosis 1 18.6
Target cells 0 0.2
Schistocytes 3 10.6
Helmet cells 0 0.3
Sickle cells 0 0.2
Spherocytes 0 0.7
Elliptocytes 0 2.1
Ovalocytes 0 1.0
Tear drop cells 0 0.7
Stomatocytes 0 2.3
Acanthocytes 0 0.1
Echinocytes 0 0.4
INCLUSIONS
Howell-Jolly 0 0.5
Pappenheimer 0 0.0
Basophilic stippling 0 0.8
Parasites 0 0.0
TRASH
Number of RBCs used for pre-characterization: 1840
Reset to Precharacterization
Exclude RBC Analysis
RBC comment
Target cells (3)
Show Example Cell
Schistocytes (195)
Show Example Cell
Helmet cells (6)
Show Example Cell
Sickle cells (4)
Show Example Cell
Spherocytes (12)
Show Example Cell

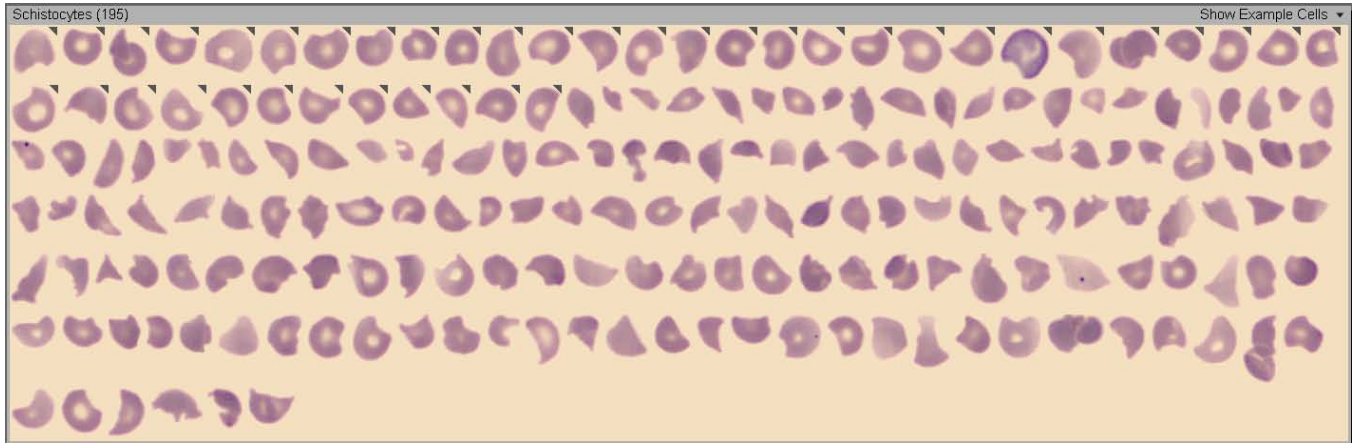

RBC
Sign Slide
Report all as 0 - normal
Use characterization
0 1 2 3 %
COLOR
• Polychromatic cells 0 1.5
• Hypochromatic cells 0 0.8
SIZE
• Anisocytosis 0 11.3
• Microcytes 0 3.5
• Macrocytes 0 0.3
SHAPE
• Poikilocytosis 0 8.4
• Target cells 0 0.2
• Schistocytes 0 0.2
• Helmet cells 0 0.0
• Sickle cells 0 0.0
• Spherocytes 0 0.9
• Elliptocytes 0 0.2
• Ovalocytes 0 1.3
• Tear drop cells 0 0.0
• Stomatocytes 0 3.4
• Acanthocytes 0 0.0
• Echinocytes 0 2.2
INCLUSIONS
• Howell-Jolly 0 0.1
• Pappenheimer 0 0.2
• Basophilic stippling 0 0.0
• Parasites 0 0.0
TRASH
Number of RBCs used for precharacterization: 3108
Reset to Precharacterization
Exclude RBC Analysis
RBC comment
Overview
Individual Cells
Show Excluded

Results

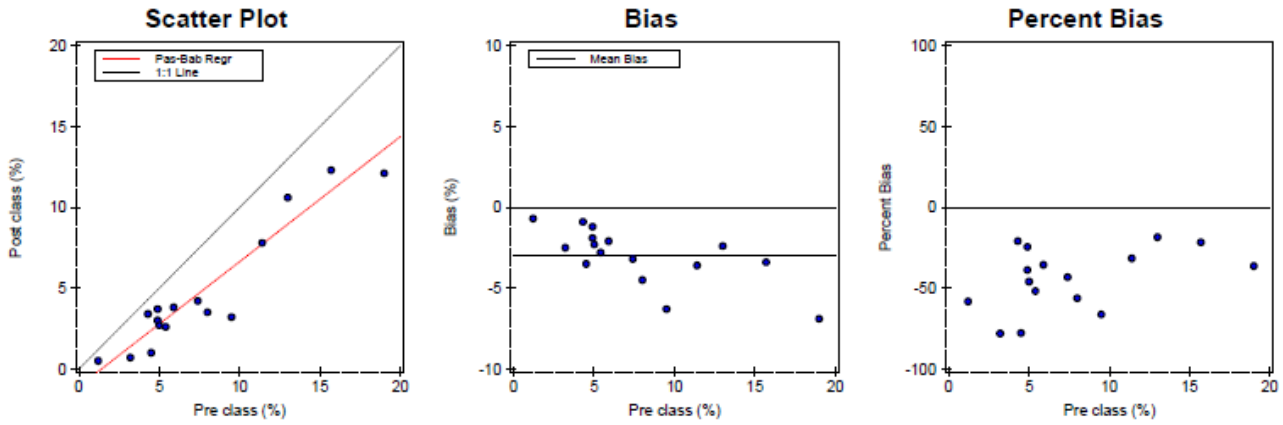
Fragmentocytes/schistocytes:

- 16 samples resulting in a pre-classification between 1.2% and 19.0%
- Between 0.5% and12.3% in the post-classification

- Pre-classification healthy individuals (n=10) < 1%
- Post-classification < 0,5% fragmentocytes/schistocytes



Egele, Riedl et al., Journal of Hematology; 2015; 4(2): 184-186.



Regression Analysis

	Deming	Passing-Bablok	Regular
Slope	0.785 (0.636 to 0.934)	0.773 (0.579 to 0.938)	0.752 (0.604 to 0.900)
Intercept	-1.36 (-2.70 to -0.01)	-1.09 (-2.37 to 0.04)	-1.10 (-2.44 to 0.24)
Std Err Est	1.31	--	1.30

95% Confidence Intervals are shown in parentheses

Figuur 1. Figure showing comparison of pre- and post-classification of fragmentocytes.

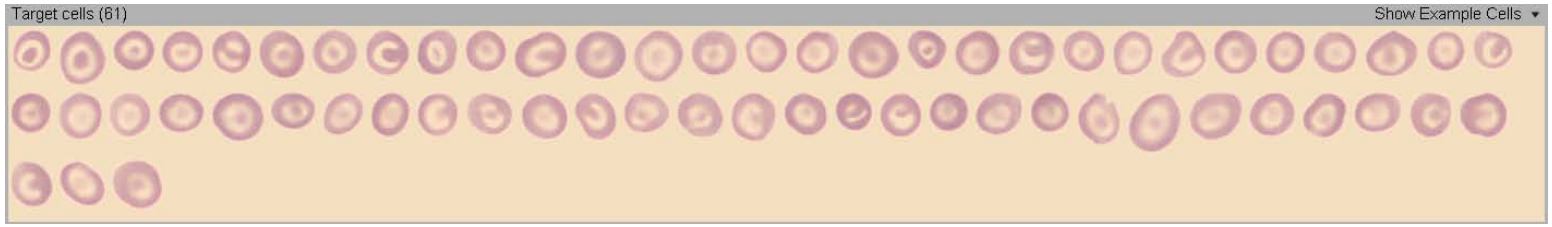
- y=x line of y=0,8x-1
- Correlatiecoëfficiënt of 0,95

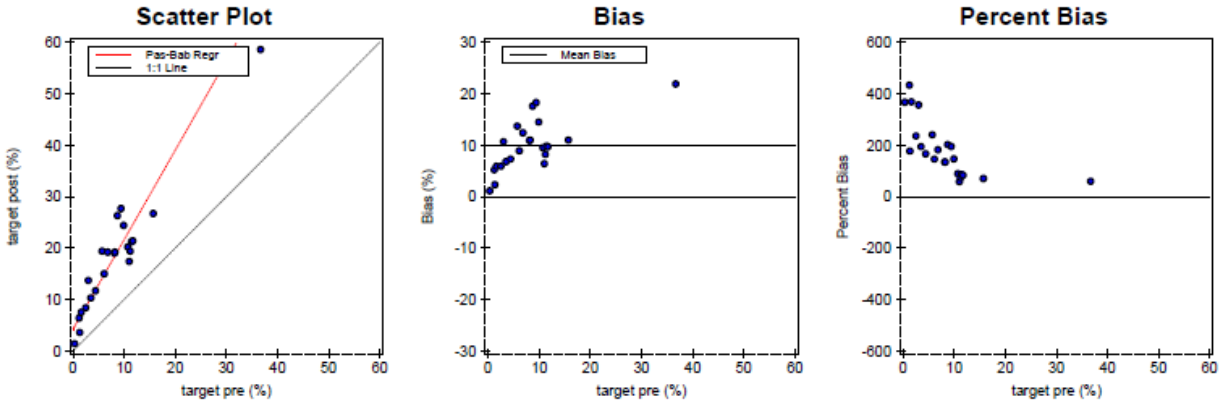
Egele, Riedl et al., Journal of Hematology; 2015; 4(2): 184-186.

Targetcells:

- 23 samples resulting in a pre-classification between 0.3% and 36.7%
- Between 1.4% and 58.6% targetcells in the post-classification

- Pre-classification healthy individuals ≤ 0.5%
- < 1.4% targetcells in the post-classification





Regression Analysis

	Deming	Passing-Bablok	Regular
Slope	1.570 (1.349 to 1.792)	1.743 (1.425 to 2.265)	1.462 (1.246 to 1.678)
Intercept	5.27 (2.83 to 7.71)	4.20 (1.19 to 5.31)	6.16 (3.78 to 8.54)
Std Err Est	3.74	--	3.65

95% Confidence Intervals are shown in parentheses

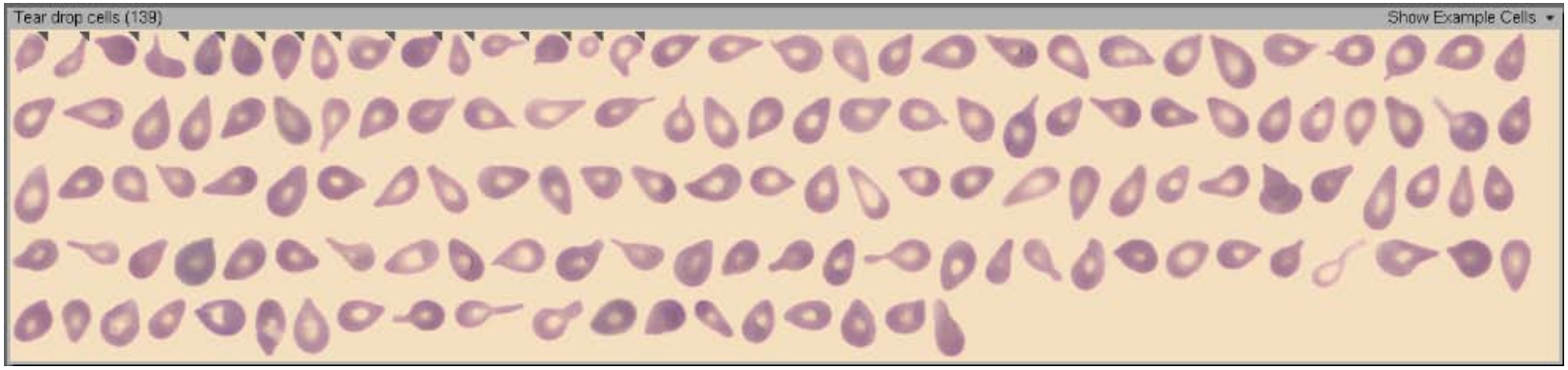
Figuur 2. Figure showing comparison of pre- and post-classification of targetcells.

- y=x line of y=1,7x+4
- Correlatiecoëfficiënt of 0,95

Teardropcells:

- 41 samples resulting in a pre-classification between 0.5% and 10.4%
- Post-classificatie between 0.2% and 6.8%

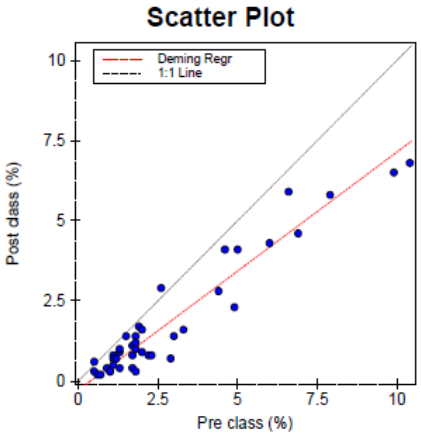
- Pre-classification healthy individuals ≤ 0.5%
- < 0.1% teardropcells in the post-classification

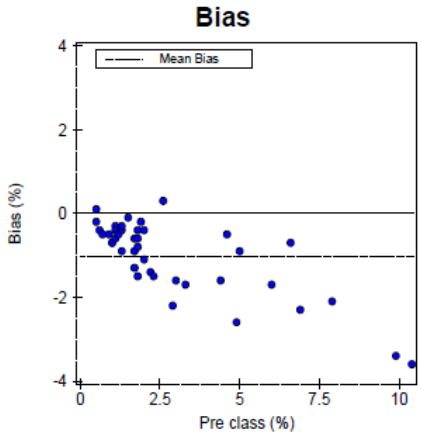


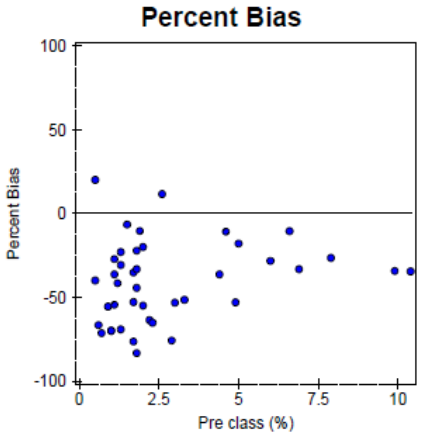
Egele, Riedl et al., Int J Lab Hematol. 2015 Jun 29.

Diagnosis	Number of samples
Myelofibrosis	13
Myelofibrosis evolved from PV	2
MDS	6
CLL	3
Iron deficiency anemia	3
ET	1
PV	1
Varying from solid tumors to sickle cell anemia and thalassemia	12
Total	**41**

Egele, Riedl et al., Int J Lab Hematol. 2015 Jun 29.







Regression Analysis

	Deming	Regular
Slope	0.743 (0.673 to 0.814)	0.720 (0.650 to 0.791)
Intercept	-0.29 (-0.56 to -0.02)	-0.22 (-0.49 to 0.04)
Std Err Est	0.55	0.55

95% Confidence Intervals are shown in parentheses

Figuur 3. Figure showing comparison of pre- and post-classification of teardropcells.

- y=x line of y= 0,8x
- Correlatiecoëfficiënt of 0,95

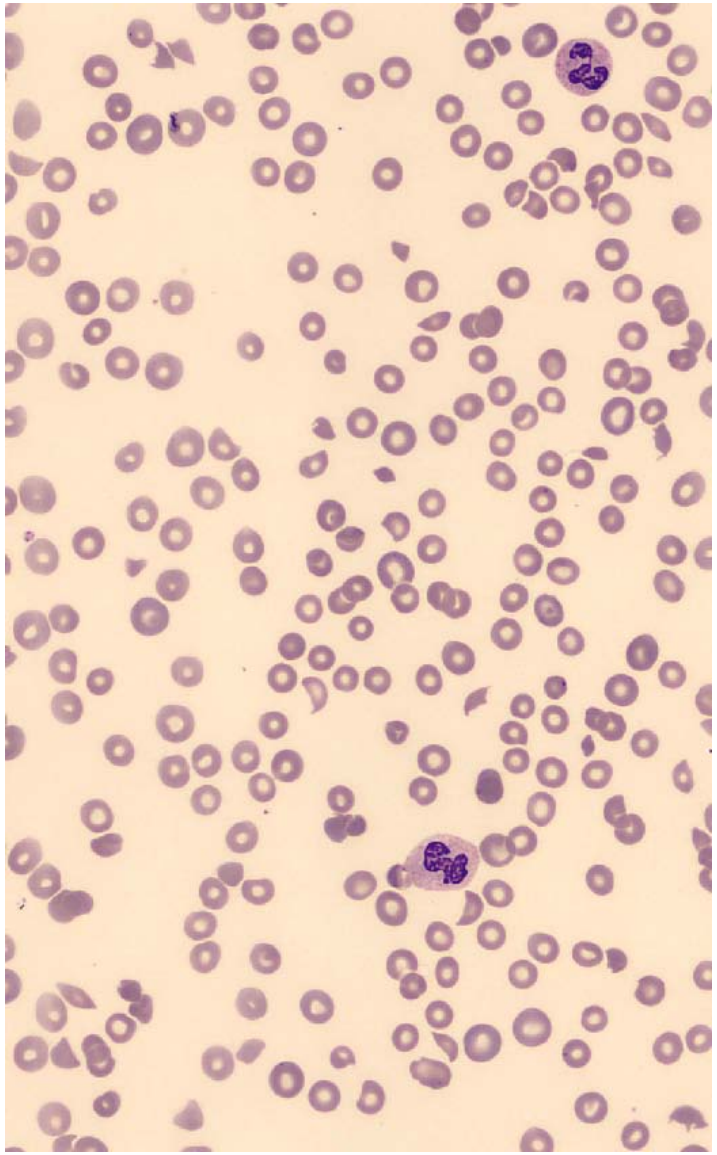
Egele, Riedl et al., Int J Lab Hematol. 2015 Jun 29.

Conclusion

- Correlation between pre- and post-classification in the detection and quantification of fragmentocytes, targetcells and teardropcells in this study.
- Morphological RBC abnormalities are now depicted with 1+, 2+ or 3+ scores
  - Can now be scored/displayed in percentages.
- “cut-off” values for:
  - Fragmentocytes/schistocytes; > 1% - abnormal
  - Targetcellen; > 0,5% - abnormal
  - Traandruppelcellen; > 0,5% - abnormal
- The time is ripe to only report clinical relevant RBC morphological abnormalities.
- Cellcounters & Digital microscopes are pathology filters!

Discussion & Future perspectives

- Integration of cell counter flaggings with DM96/DI-60 flaggings!
- Gold standard? Will digital microscopy become the gold standard?
- Exciting clinical studies possible! Diagnosis of diseases in an earlier stage possible? Example MPN?
- Malaria?
- Morphological detection and classification of leukocytes and red blood cells possible……trombocytes?
- In the nearby future complete digital imaging of a peripheral blood smear possible!!



Questions?

Malaria

Albert Schweitzer ziekenhuis

CellaVision - CellaVision DM Software (DM96) - Peripheral Blood and Advanced RBC

File View Tools Help

Idle

Order: A003

Slide: 1

Worklist

Order ID	S...
A003	1

Open

Remove

Patient data

Order ID:
A003
Last name:
-
First name:
-
Birth date:
-

RBC | Sign Slide

Report all as 0 - normal
Use characterization

	0	1	2	3	%
COLOR					
• Polychromatic cells	0	●	○	○	1.9
• Hypochromatic cells	0	●	○	○	1.9
SIZE					
• *Anisocytosis*	0	●	○	○	3.2
• Microcytes	0	●	○	○	2.2
• Macrocytes	0	●	○	○	1.0
SHAPE					
• *Poikilocytosis*	0	●	○	○	8.1
• Target cells	0	●	○	○	0.0
• Schistocytes	1	○	●	○	1.1
• Helmet cells	1	○	●	○	1.4
• Sickle cells	0	●	○	○	0.0
• Spherocytes	1	○	●	○	2.3
• Elliptocytes	0	●	○	○	0.0
• Ovalocytes	0	●	○	○	0.2
• Tear drop cells	0	●	○	○	0.4
• Stomatocytes	0	●	○	○	1.3
• Acanthocytes	0	●	○	○	0.1
• Echinocytes	0	●	○	○	1.2
INCLUSIONS					
• Howell-Jolly	0	●	○	○	0.4
• Pappenheimer	0	●	○	○	0.0
• Basophilic stippling	0	●	○	○	0.0
• Parasites	0	●	○	○	0.0
TRASH					

Number of RBCs used for pre-characterization: 2553

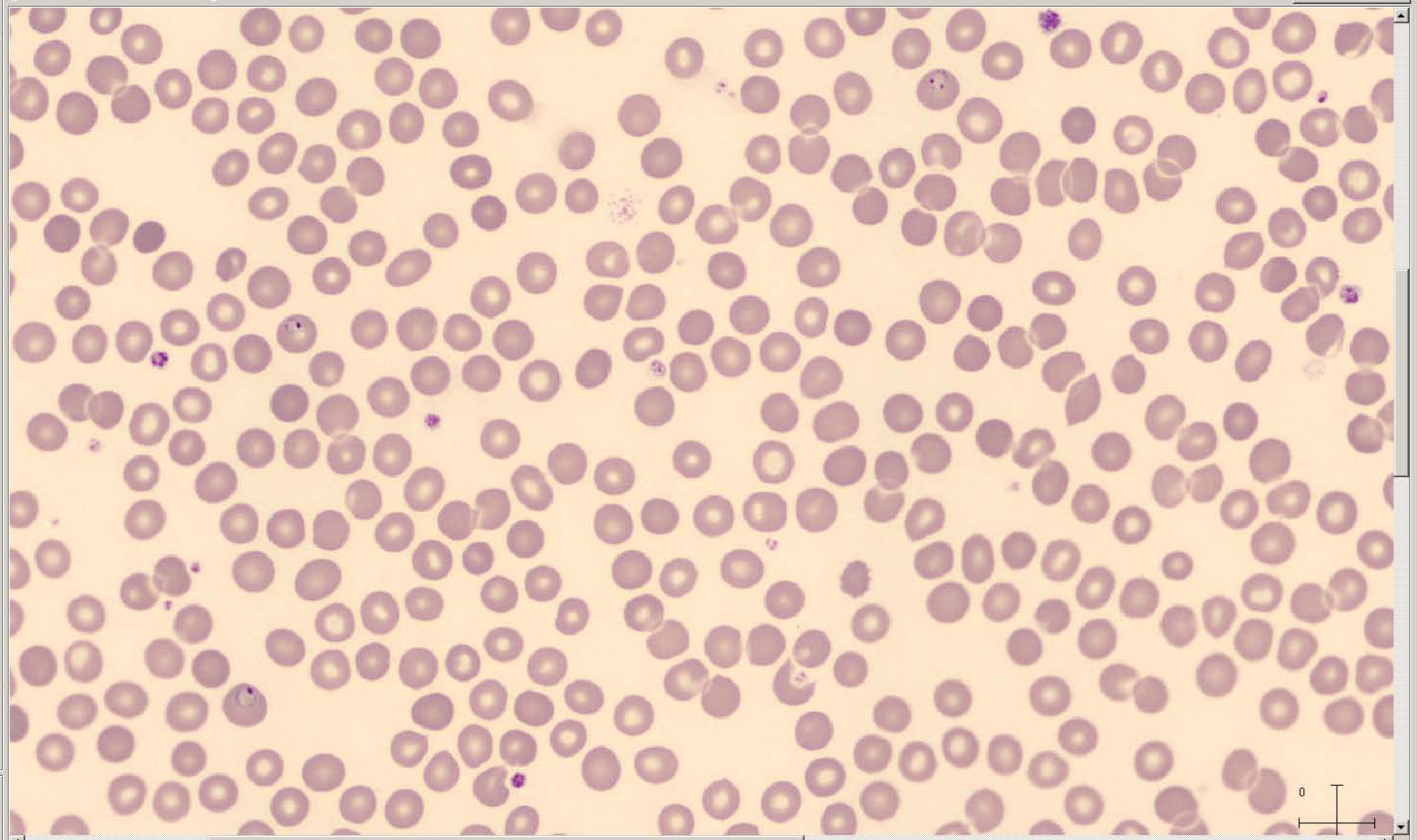
Reset to Precharacterization

Exclude RBC Analysis

RBC comment

Overview | Individual Cells

Show Excluded



Ready

Angelique | RBC_1 | NUM

Start | malaria.bmp - Paint | CellaVision - CellaVisi... | 13:38

CellaVision - CellaVision DM Software (DM96) - Peripheral Blood and Advanced RBC

File View Tools Help

Idle | Order: A003 | Slide: 1

Worklist

Order ID	S...
A003	1

Open | Remove

Patient data

Order ID: A003
Last name: -
First name: -
Birth date: -

RBC | Sign Slide

Report all as 0 - normal
Use characterization

	0	1	2	3	%
COLOR					
• Polychromatic cells	0	●	○	○	1.9
• Hypochromatic cells	0	●	○	○	1.9
SIZE					
• *Anisocytosis*	0	●	○	○	3.2
• Microcytes	0	●	○	○	2.2
• Macrocytes	0	●	○	○	1.0
SHAPE					
• *Poikilocytosis*	0	●	○	○	8.1
• Target cells	0	●	○	○	0.0
• Schistocytes	1	○	●	○	1.1
• Helmet cells	1	○	●	○	1.4
• Sickle cells	0	●	○	○	0.0
• Spherocytes	1	○	●	○	2.3
• Elliptocytes	0	●	○	○	0.0
• Ovalocytes	0	●	○	○	0.2
• Tear drop cells	0	●	○	○	0.4
• Stomatocytes	0	●	○	○	1.3
• Acanthocytes	0	●	○	○	0.1
• Echinocytes	0	●	○	○	1.2
INCLUSIONS					
• Howell-Jolly	0	●	○	○	0.4
• Pappenheimer	0	●	○	○	0.0
• Basophilic stippling	0	●	○	○	0.0
• Parasites	0	●	○	○	0.0
TRASH					

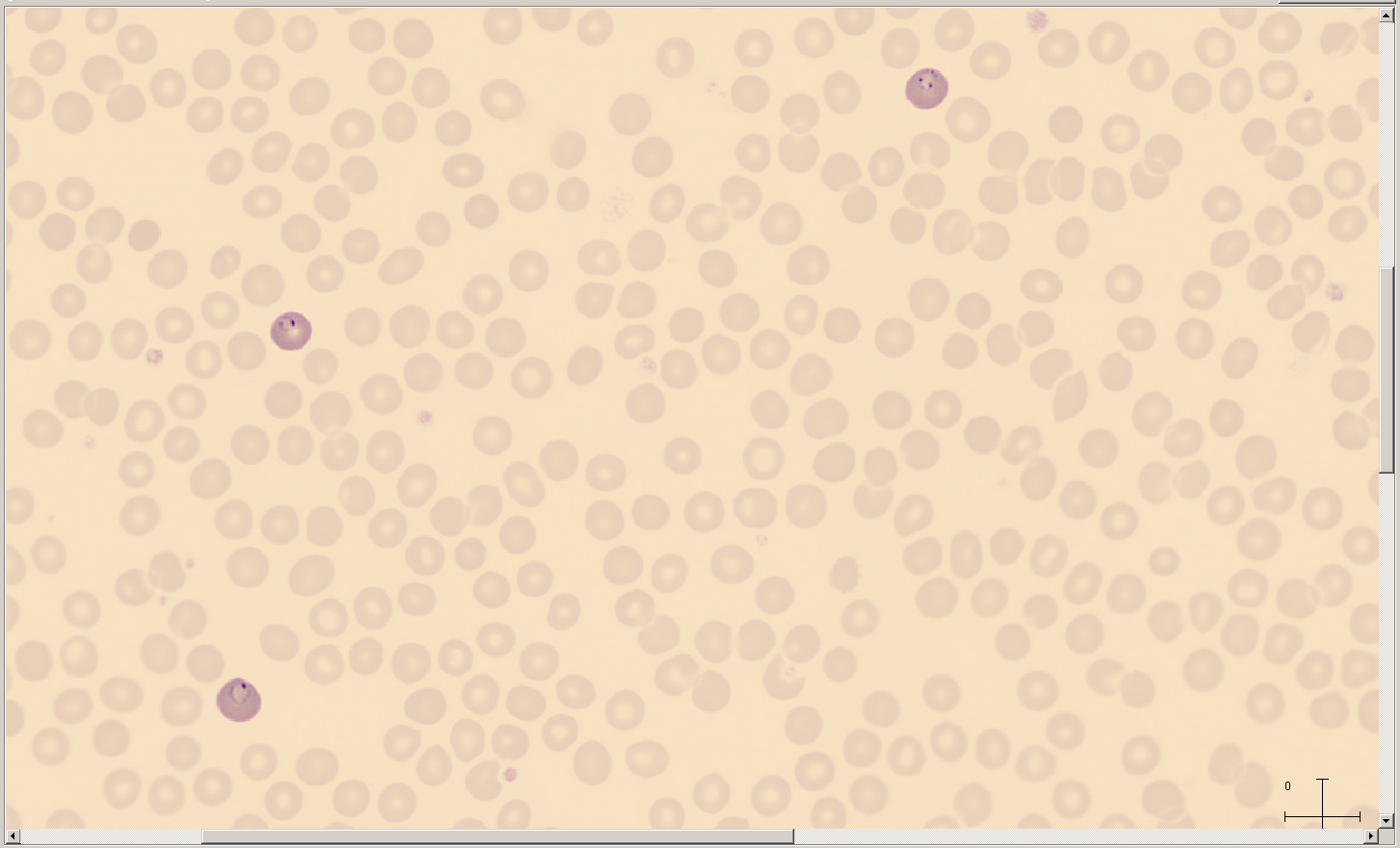
Number of RBCs used for pre-characterization: 2553

Reset to Precharacterization

Exclude RBC Analysis

RBC comment

Overview | Individual Cells | Show Excluded



Ready

Angelique | RBC_1 | NUM

Start | untitled - Paint | CellaVision - CellaVisi... | 13:38

CellaVision - CellaVision DM Software (DM96) - Peripheral Blood and Advanced RBC

File View Tools Help

Idle

Order: A003 Slide: 1

Worklist

Order ID	S...
A003	1

Open Remove

Patient data

Order ID: A003
Last name: -
First name: -
Birth date: -

RBC Sign Slide

Report all as 0 - normal
Use characterization

	0	1	2	3	%
COLOR					
• Polychromatic cells	0	●	○	○	1.9
• Hypochromatic cells	0	●	○	○	1.9
SIZE					
• *Anisocytosis*	0	●	○	○	3.2
• Microcytes	0	●	○	○	2.2
• Macrocytes	0	●	○	○	1.0
SHAPE					
• *Poikilocytosis*	0	●	○	○	8.1
• Target cells	0	●	○	○	0.0
• Schistocytes	1	○	●	○	1.1
• Helmet cells	1	○	●	○	1.4
• Sickle cells	0	●	○	○	0.0
• Spherocytes	1	○	●	○	2.3
• Elliptocytes	0	●	○	○	0.0
• Ovalocytes	0	●	○	○	0.2
• Tear drop cells	0	●	○	○	0.4
• Stomatocytes	0	●	○	○	1.3
• Acanthocytes	0	●	○	○	0.1
• Echinocytes	0	●	○	○	1.2
INCLUSIONS					
• Howell-Jolly	0	●	○	○	0.4
• Pappenheimer	0	●	○	○	0.0
• Basophilic stippling	0	●	○	○	0.0
• Parasites	0	●	○	○	0.0
TRASH					

Number of RBCs used for pre-characterization: 2553

Reset to Precharacterization

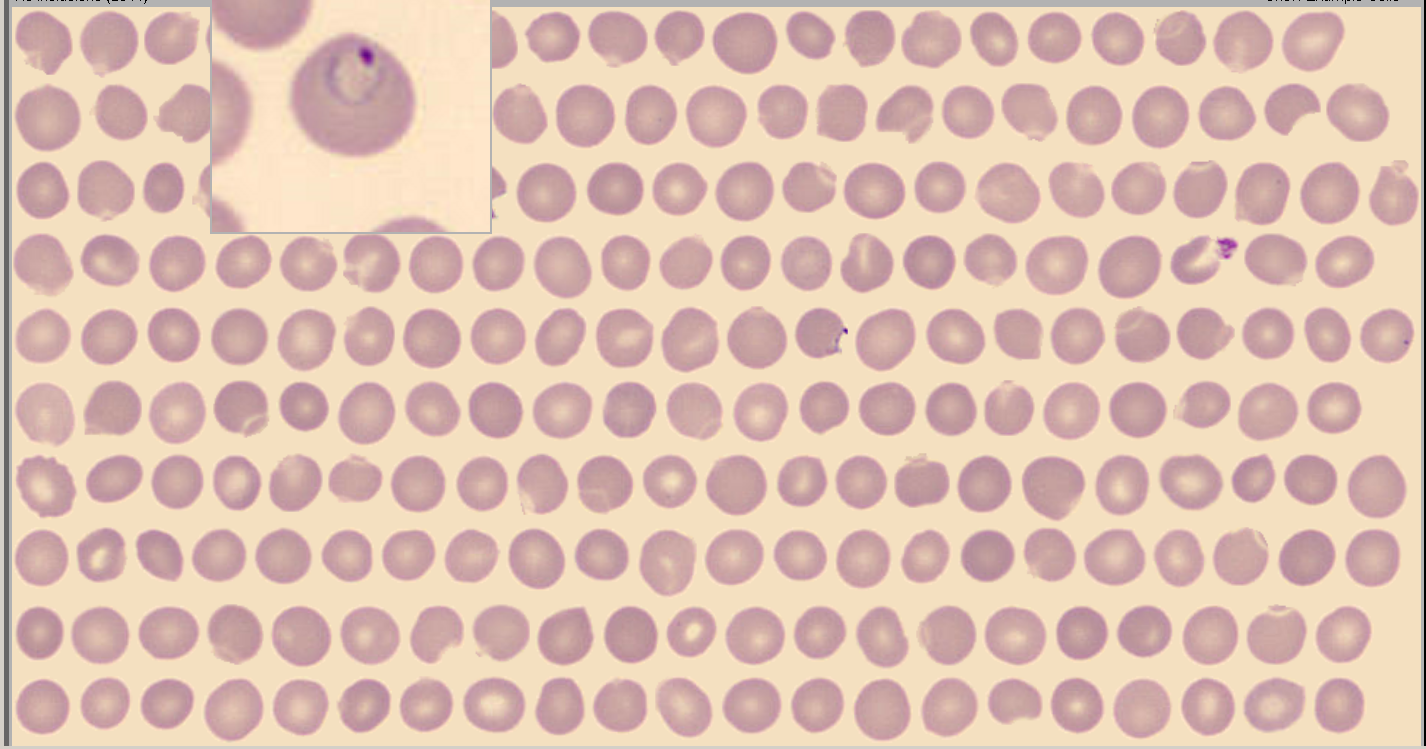
Exclude RBC Analysis

RBC comment

Overview Individual Cells

Howell-Jolly (9) Show Example Cells

No inclusions (2544) Show Example Cells



Ready

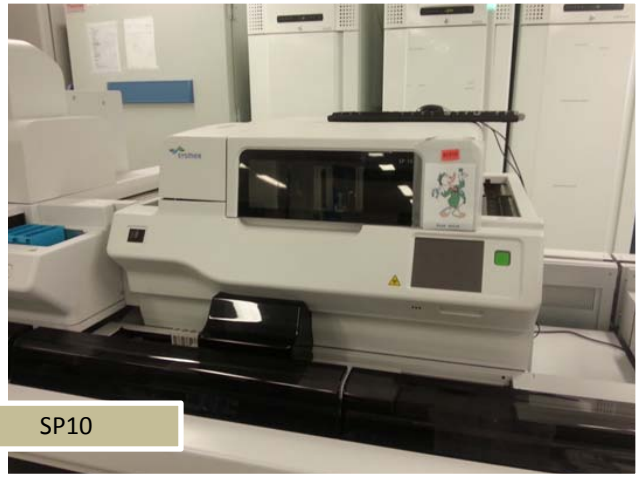
Angelique RBC_1 NUM

Start malaria2.bmp - Paint CellaVision - CellaVisi... 13:39

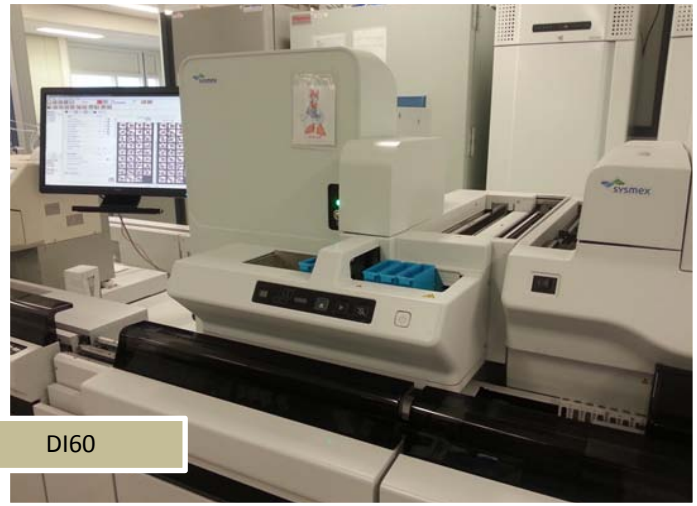
Integrative case:

- 22-year-old-boy (prisoner at that time) was admitted to our emergency ward
- He had been suffering from malaise
- At the emergency ward he displayed neurological symptoms
- Brain CT-scan showed no abnormalities

Cellcounter



SP10



DI60

Cumulatieve uitslag: Uitsl. 64 Tot 68 Van 68

Standaard cumulatieve bevind.
1000 Albert Schweitzer Ziekenhuis
1111 AA Dordrecht

Rapport RN2LN212
Gebruiker RIEDL

Datum 28.11.2014 19:16:43
Pagina 1
Eval.tijdvak 24.11.2014 28.11.2014

Verpleegk. OE: SEH Dow

Ordernummer				1418097364	1418097341	1418097279	1418097262	1451065449
Datum				18.09.2014	18.09.2014	18.09.2014	18.09.2014	18.09.2014
Tijd				22:58	21:44	18:37	17:18	08:15
Hematologie/serologie								
(	- 15	mm/uur	Bezinking					15
(8.5	- 11.0	mmol/L	Hemoglobine		4.1 L		4.5 L	5.1 L
(0.41	- 0.51	L/L	Hematocriet		0.18 L		0.21 L	0.25 L
(80	- 100	fL	MCV				95	101 H
(4.60	- 6.20	10e12/L	Erytrocyten		1.89 L		2.19 L	2.49 L
(0.0	- 2.5	%	Reticulocyten					12.3 H
(11.0	- 16.0	%	RDW		16.2 H		16.0	15.9
(0.3	- 2.0	g/L	Haptoglobine				< 0.1 L	
(130	- 700	pmol/L	vitamine B12					302 *
(5	-	nmol/L	foliumzuur					9
(25	- 250	ug/L	Ferritine					188
(14	- 28	umol/L	IJzer					20.5
(2.00	- 3.60	g/L	Transferrine					2.31
(45	- 80	umol/L	TIJBC					58
(20	- 60	%	Verzadigingspercentage					36
(150	- 400	10e9/L	Trombocyten		8 L		9 L	23 L
(	- 32	sec	APTT		28			
(8	- 11	sec	Protrombinetijd		13 H			
		INR	PT-INR		1.2			
(4.3	- 10.0	10e9/L	Leukocyten		9.6		11.5 H	7.0
(0.0	- 0.15	10e9/L	Basofielen				< 0.1	< 0.1
(	- 0.7	10e9/L	Eosinofielen				< 0.1	0.2
(1.0	- 4.0	10e9/L	Lymfocyten				0.5 L	1.1

Cumulatieve uitslag: Uitsl. 64 Tot 68 Van 68

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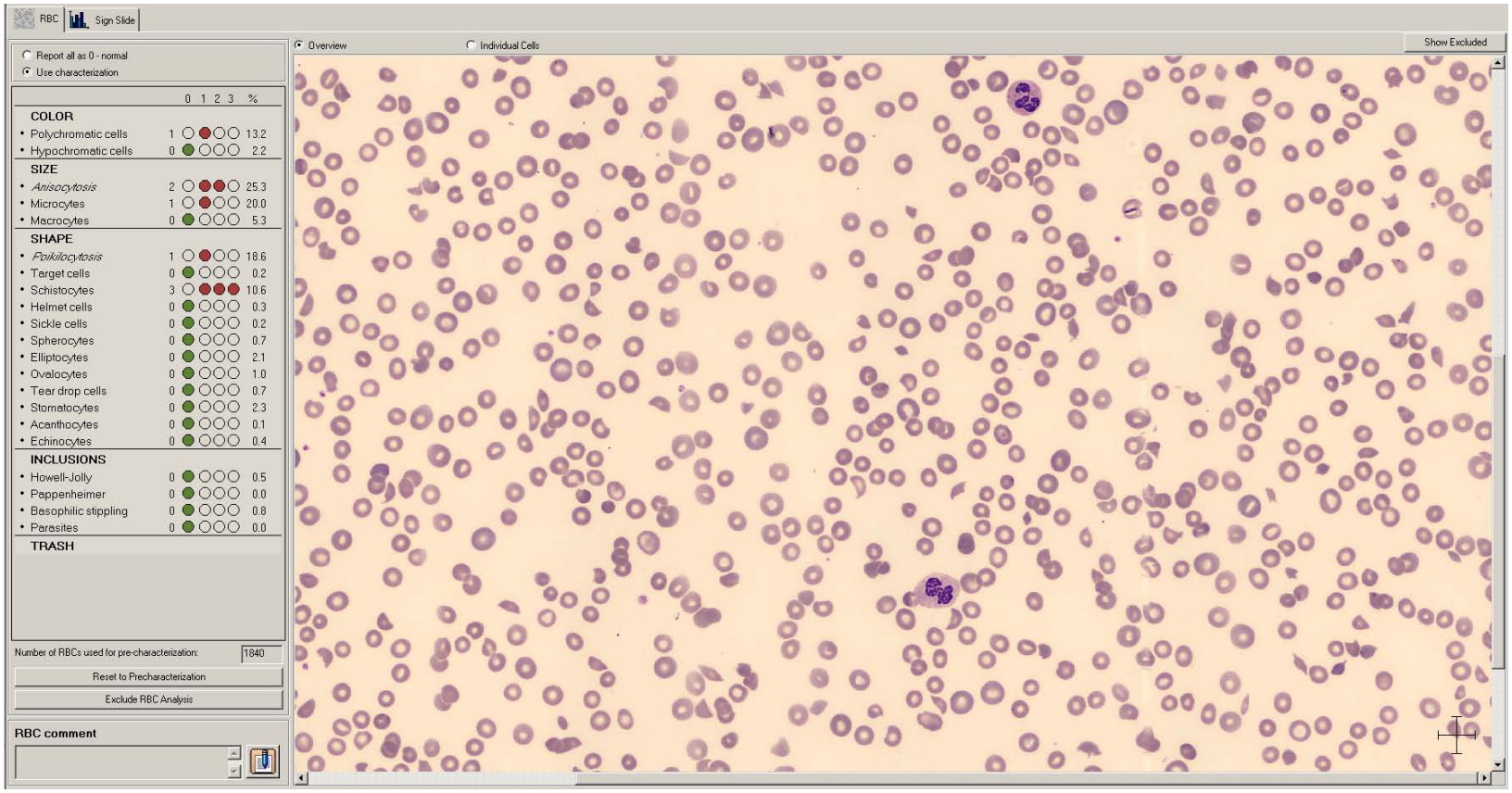
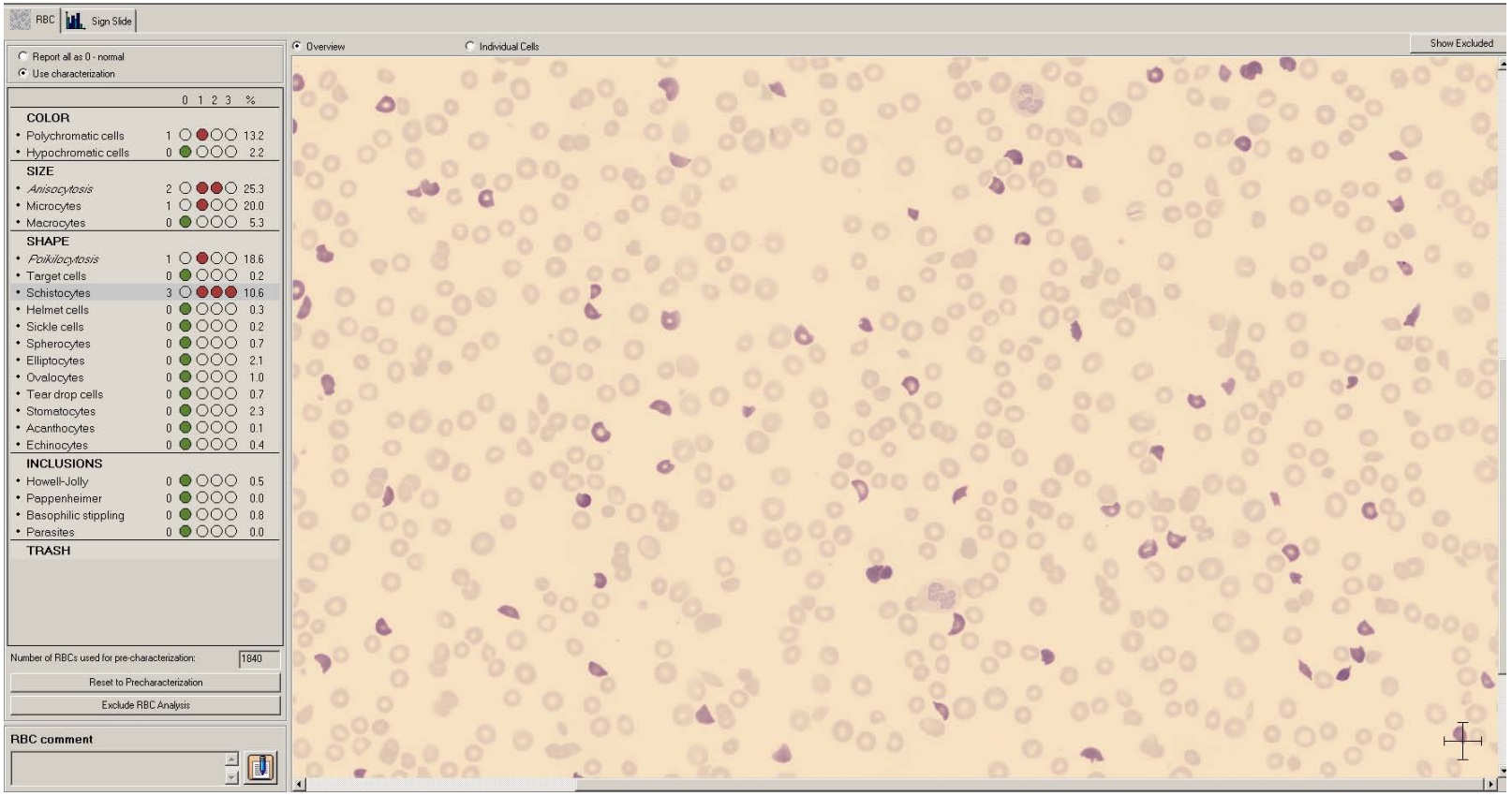
Rapport RN2LN212
Gebruiker RIEDL

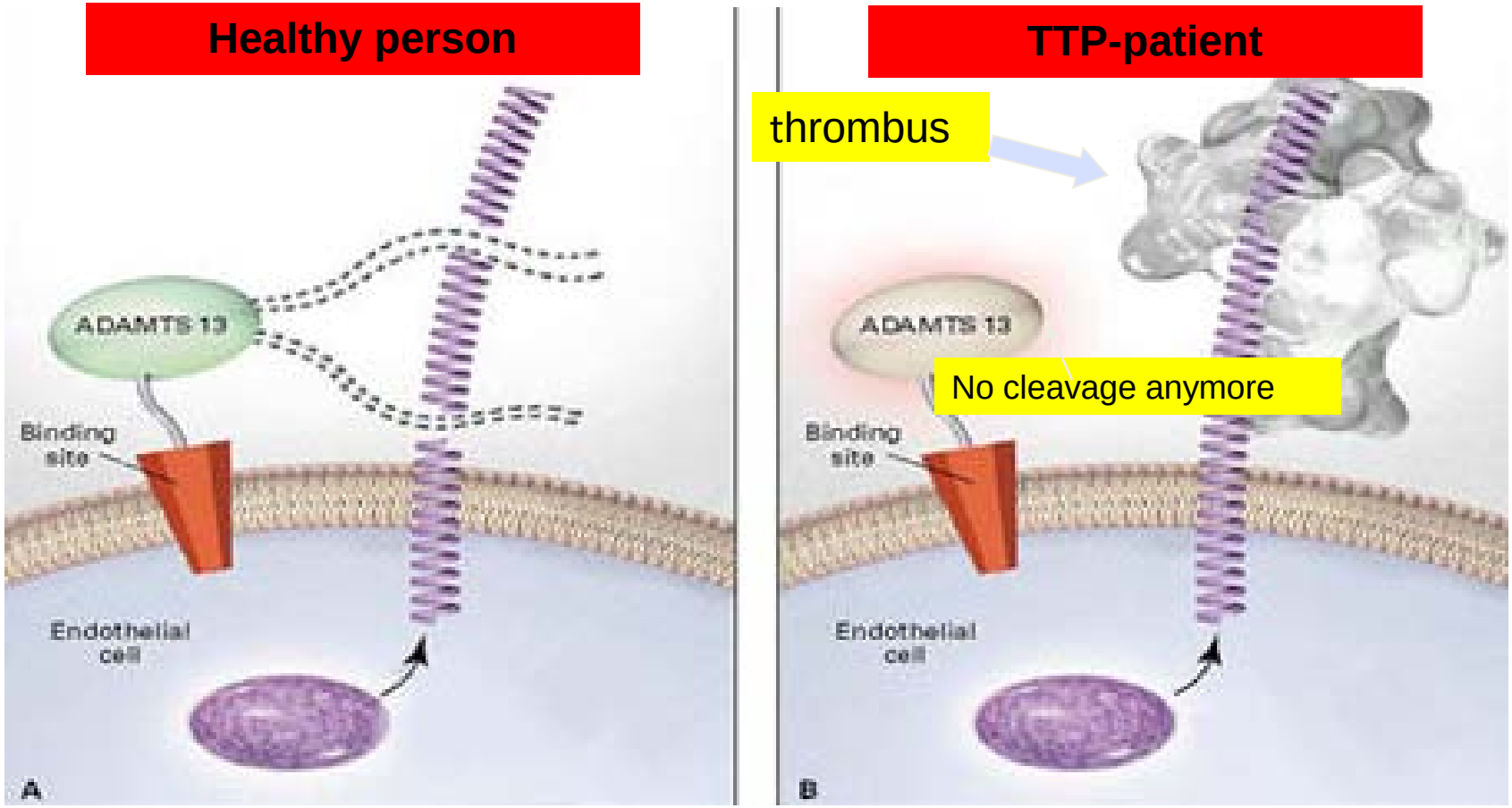
Datum 28.11.2014 19:16:43
Pagina 1
Eval.tijdvak 24.11.2014 28.11.2014

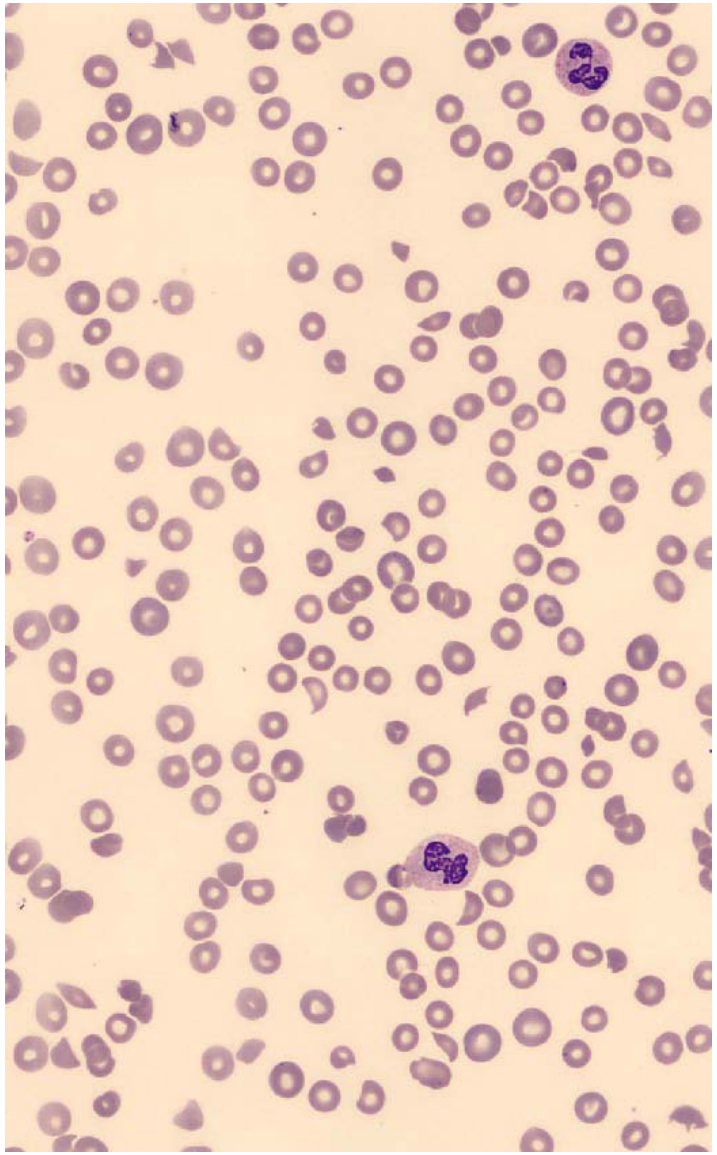
Verpleegk. OE: SEH Dow

Ordernummer				1418097364		1418097341		1418097279	1418097262		1451065449	
Datum				18.09.2014		18.09.2014		18.09.2014	18.09.2014		18.09.2014	
Tijd				22:58		21:44		18:37	17:18		08:15	
(59 -	104	umol/L	Creatinine			84			97		70	
(60 -		mL/min	MDRD (e-GFR)			> 60			> 60		> 60	
(2.5 -	6.4	mmol/L	Ureum			6.5	H		6.2		4.9	
(135 -	145	mmol/L	Natrium			139			138		141	
(3.5 -	5.0	mmol/L	Kalium			3.7			4.0		3.4	L
(96 -	107	mmol/L	Chloor			104			106			
(8 -	16	mmol/L	Anion gap			12						
(2.20 -	2.65	mmol/L	Calcium	2.31		2.07	L		2.31			
(1.12 -	1.32	mmol/L	Geioniseerd calcium	1.09	L	1.22						
(0.80 -	1.40	mmol/L	Fosfaat	0.87		0.84						
(0.70 -	1.05	mmol/L	Magnesium			0.67	L					
(64 -	82	g/L	Totaal eiwit	68								
(35 -	50	g/L	Albumine	40		42			47			
(-	17	umol/L	Bilirubine			26	H		49	H		
(0 -	5	umol/L	Bilirubine direct						7	H		
(-	50	E/L	Gamma GT			14			15		16	
(-	120	E/L	Alkalische fosfatase			61			71			
(-	200	E/L	CK						148			
(-	37	E/L	ASAT			59	H		70	H	38	H
(-	41	E/L	ALAT			24			24		22	
(-	250	E/L	LDH			1225	H		1486	H	876	H
(-	115	E/L	Amylase (totaal)						42			
(4.0 -	7.8	mmol/L	Glucose			7.6			6.0		4.9	
(0.5 -	2.2	mmol/L	Lactaat			2.0			2.6	H		


RBC
Sign Slide
Report all as 0 - normal
Use characterization
0 1 2 3 %
COLOR
• Polychromatic cells 1 13.2
• Hypochromatic cells 0 2.2
SIZE
• Anisocytosis 2 25.3
• Microcytes 1 20.0
• Macrocytes 0 5.3
SHAPE
• Poikilocytosis 1 18.6
• Target cells 0 0.2
• Schistocytes 3 10.6
• Helmet cells 0 0.3
• Sickle cells 0 0.2
• Spherocytes 0 0.7
• Elliptocytes 0 2.1
• Ovalocytes 0 1.0
• Tear drop cells 0 0.7
• Stomatocytes 0 2.3
• Acanthocytes 0 0.1
• Echinocytes 0 0.4
INCLUSIONS
• Howell-Jolly 0 0.5
• Pappenheimer 0 0.0
• Basophilic stippling 0 0.8
• Parasites 0 0.0
TRASH
Number of RBCs used for pre-characterization: 1840
Reset to Precharacterization
Exclude RBC Analysis
RBC comment
Overview
Individual Cells
Show Excluded


RBC
Sign Slide
Report all as 0 - normal
Use characterization
0 1 2 3 %
COLOR
Polychromatic cells 1 13.2
Hypochromatic cells 0 2.2
SIZE
Anisocytosis 2 25.3
Microcytes 1 20.0
Macrocytes 0 5.3
SHAPE
Poikilocytosis 1 18.6
Target cells 0 0.2
Schistocytes 3 10.6
Helmet cells 0 0.3
Sickle cells 0 0.2
Spherocytes 0 0.7
Elliptocytes 0 2.1
Ovalocytes 0 1.0
Tear drop cells 0 0.7
Stomatocytes 0 2.3
Acanthocytes 0 0.1
Echinocytes 0 0.4
INCLUSIONS
Howell-Jolly 0 0.5
Pappenheimer 0 0.0
Basophilic stippling 0 0.8
Parasites 0 0.0
TRASH
Number of RBCs used for pre-characterization: 1840
Reset to Precharacterization
Exclude RBC Analysis
RBC comment
Overview
Individual Cells
Show Excluded


Healthy person
TTP-patient
thrombus
ADAMTS 13
No cleavage anymore
Binding site
Endothelial cell
A
B



Questions?