Digital Imaging in Hematology

Dr. Jurgen Riedl

Albert Schweitzer ziekenhuis

Financial Disclosure speaker

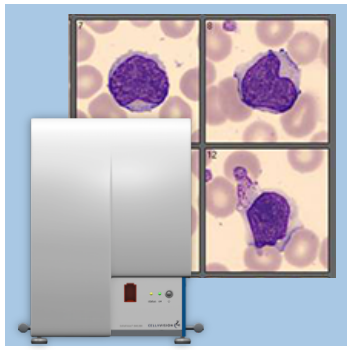
Riedl

Potential conflict of interest	none
Financial partnerships with commercial companies	none
• Sponsor- or research funds • Honoraria or financial compensations • Share-holder • Other relations	• none • none • yes, of Result Laboratory BV • none

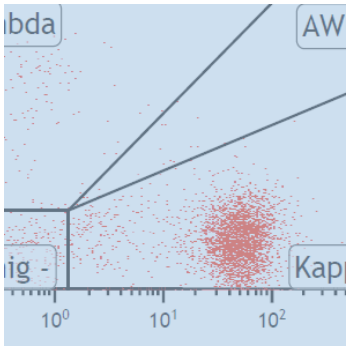
Multidisciplinary

CELLCOUNTERS

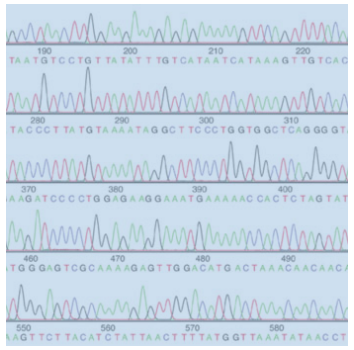
MORFOLOGY



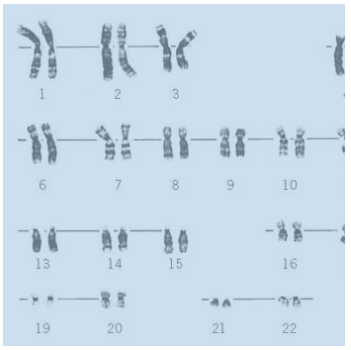
FLOWCYTOMETRY

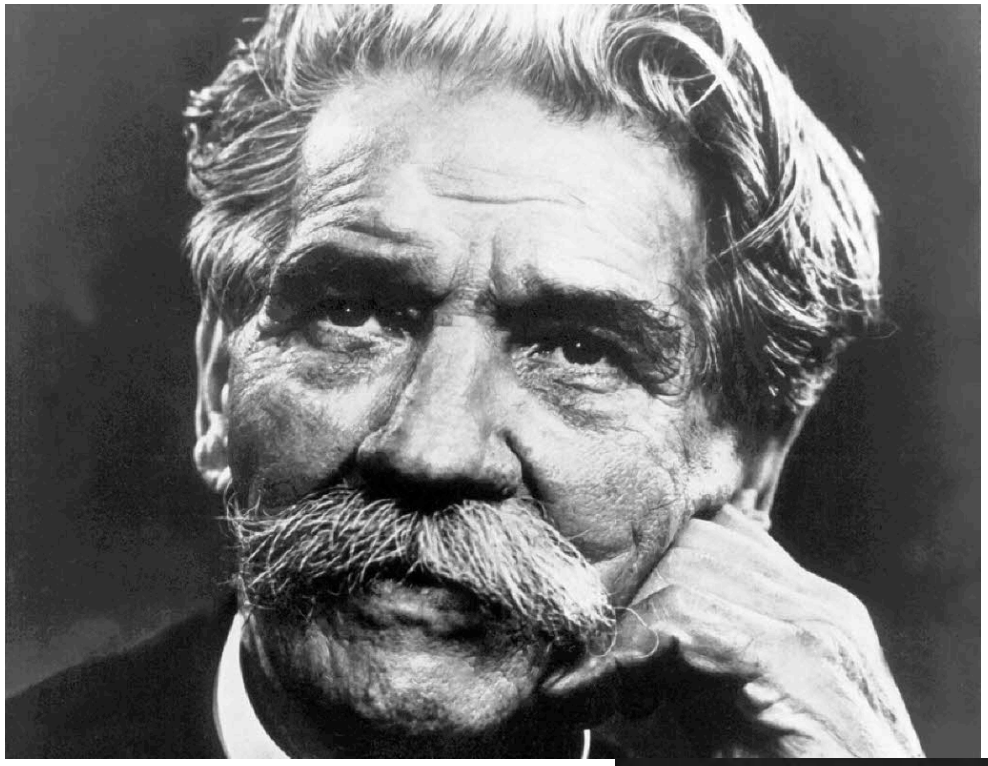


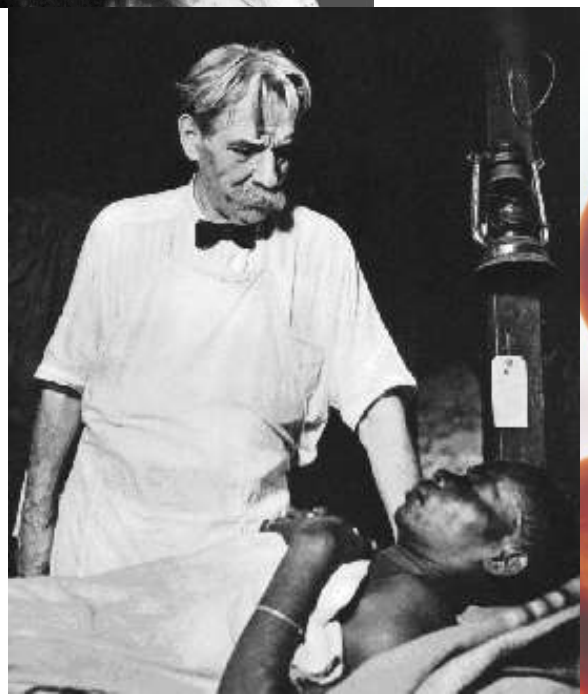
MOLECULAR DIAGNOSTICS

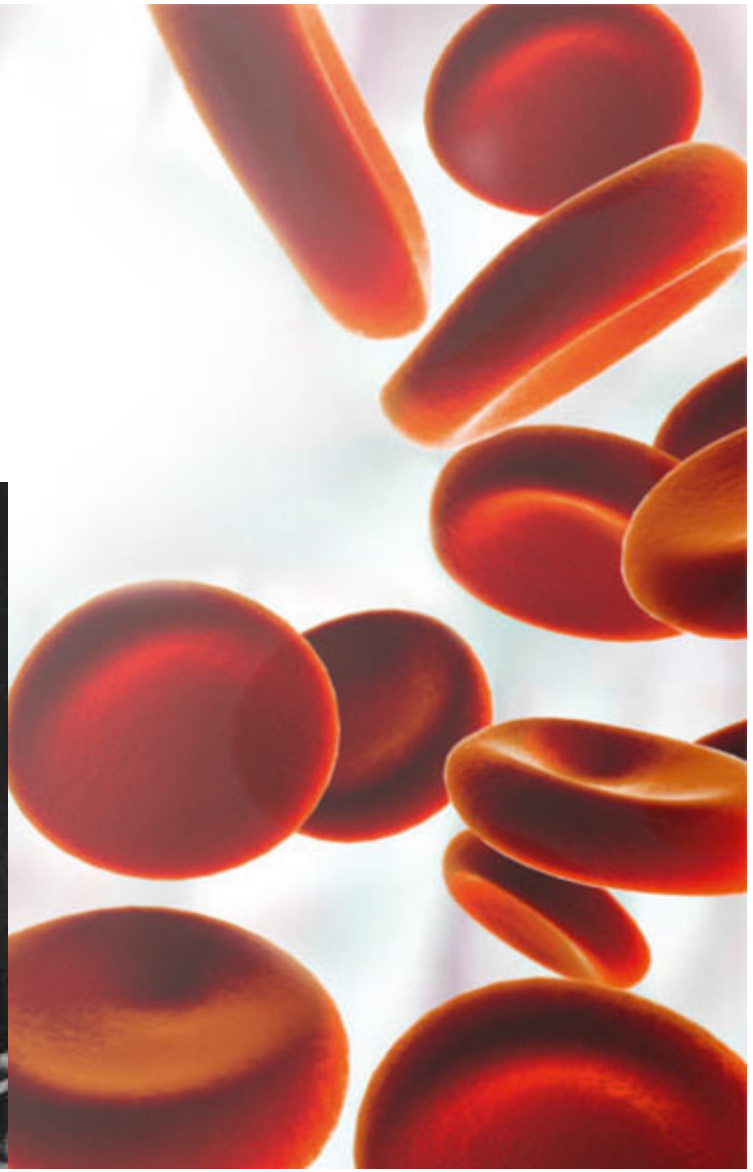


CYTOGENETICS









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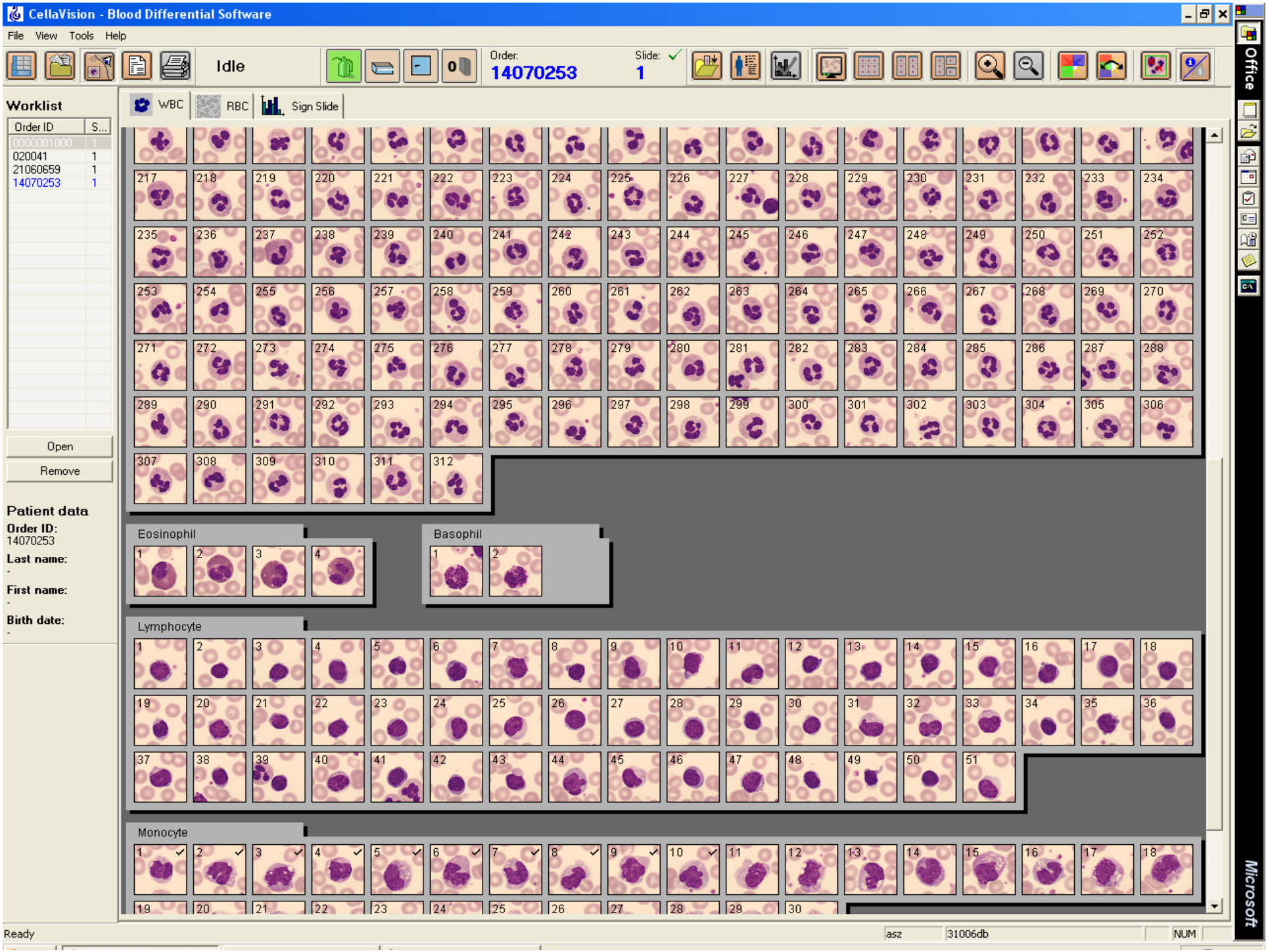
Digital Imaging/Morfology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1
Worklist
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Patient data
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14070253
Last name:
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First name:
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WBC
RBC
Sign Slide
Eosinophil
Basophil
Lymphocyte
Monocyte
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asz
31006db
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Microsoft

CellaVision - Blood Differential Software (DM96)

File View Tools Help

Idle

Order: 230231746

Slide: 1

Worklist

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230231746	1

Open

Remove

Patient data

Order ID:
230231746

Last name:
-

First name:
-

Birth date:
-

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

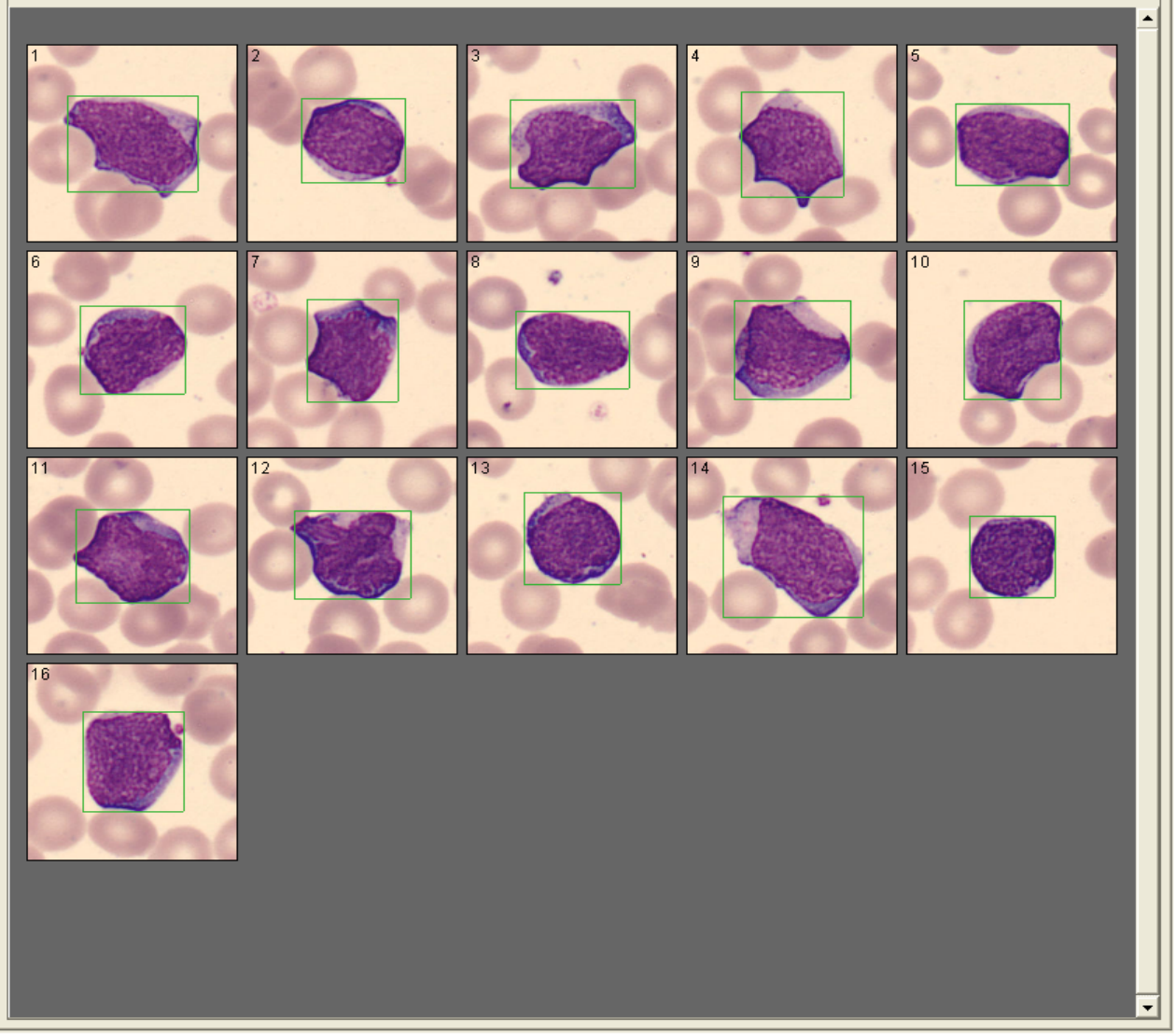
Non-WBC	Count
• Erythroblast (NRBC)	4
• Giant thrombocyte	3
• Thrombocyte aggregation	-
Megakaryocyte	-
• Smudge cell	3
• Artefact	-

Not classed	-

WBC comment

unidentified naar cleaved[jongmans]

Blast (no lineage spec)



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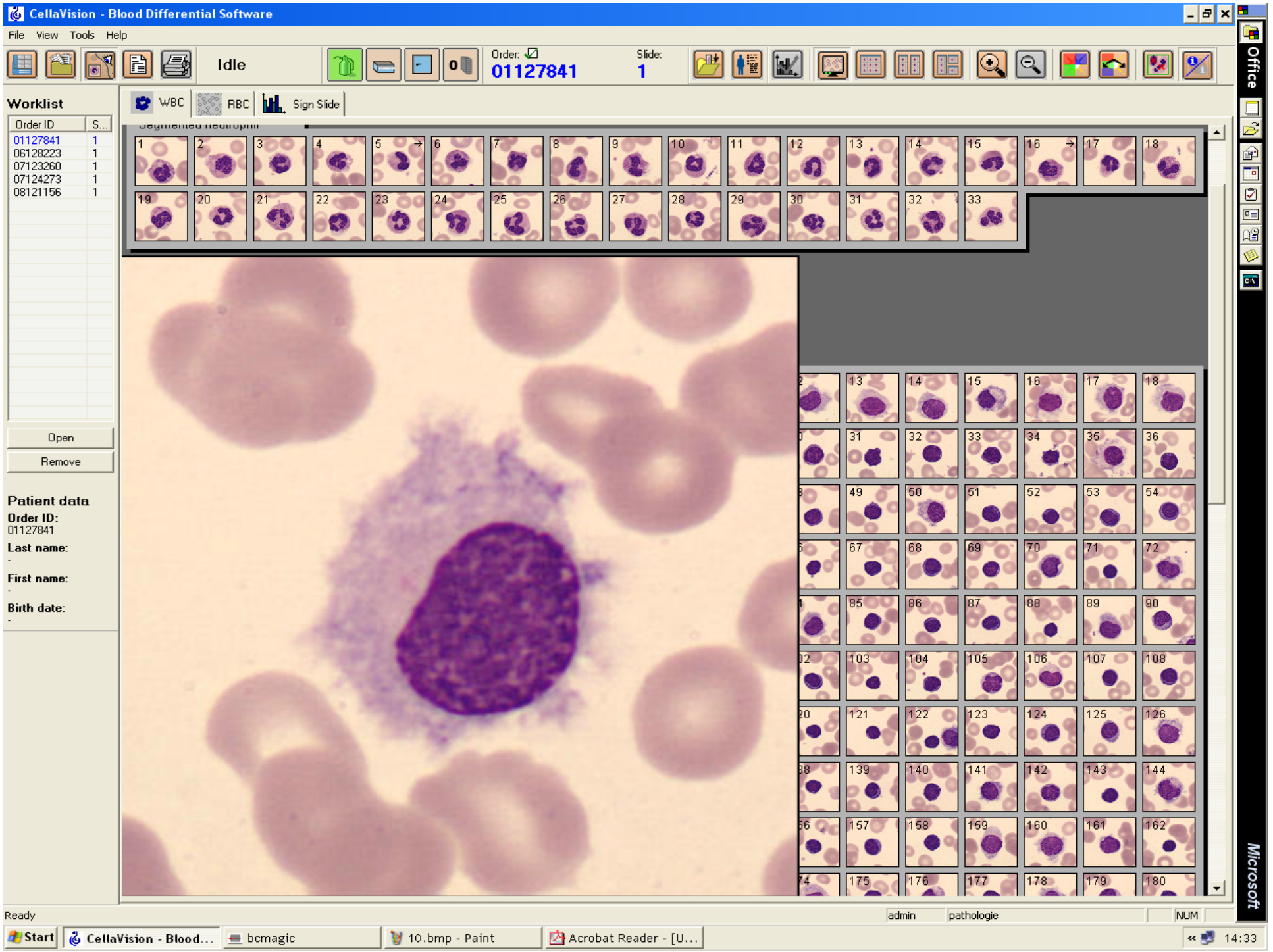
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Last name:
First name:
Birth date:
WBC
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ORIGINAL ARTICLE

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

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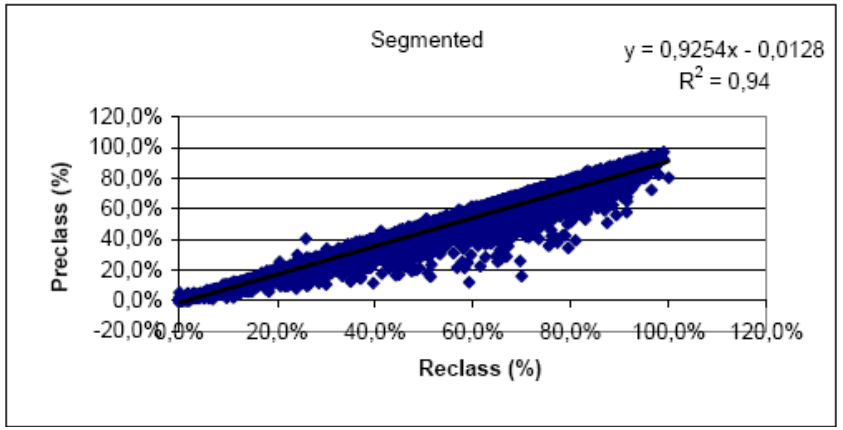
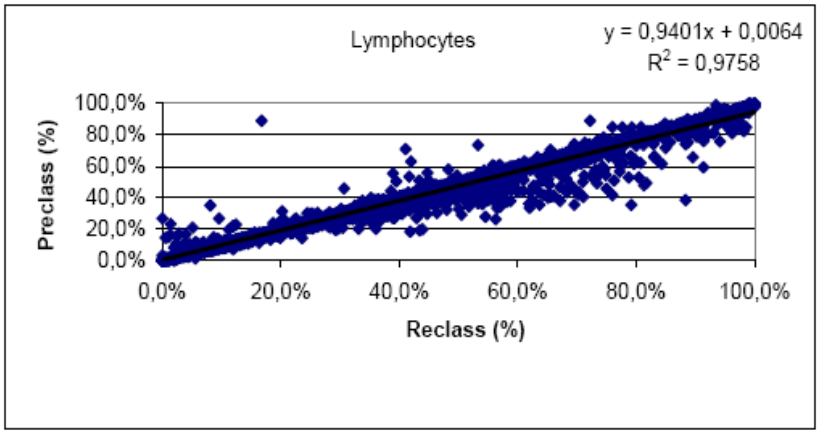
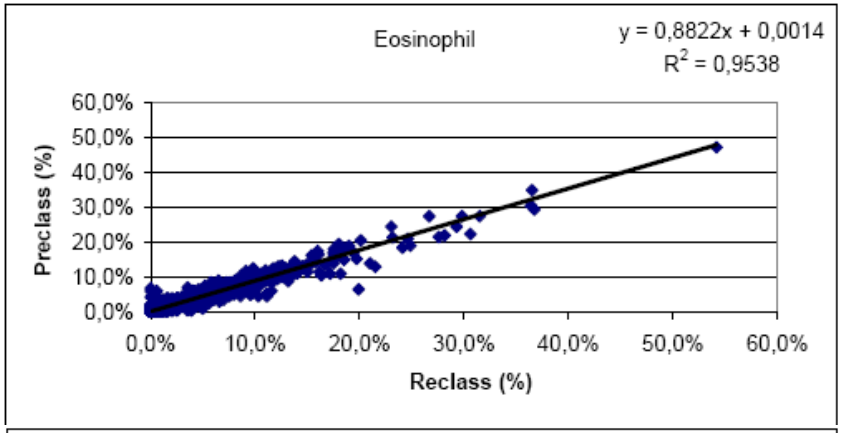
Background: Differential counting of peripheral blood cells is an important diagnostic tool. Yet, this technique requires highly trained staff, is labour intensive and has limited statistical reliability. A recent development in this field was the introduction of automated peripheral blood differential 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 classification 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.

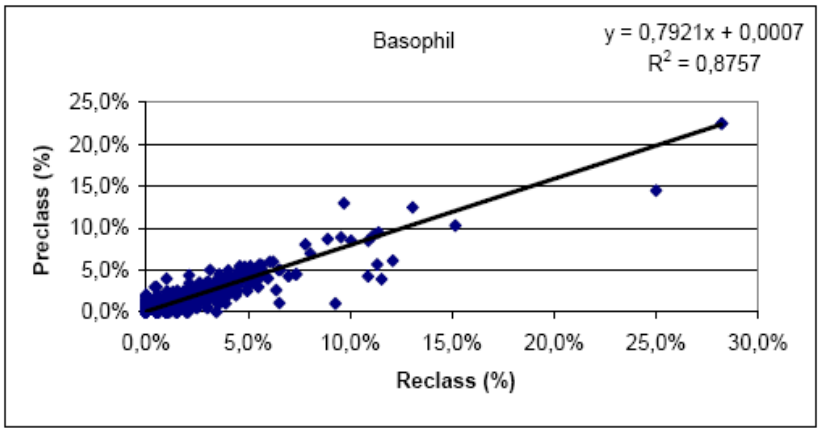
Differential counting of blood cells is an important diagnostic tool for successful treatment and management of patients. Reliable and efficient analysis of patient samples is therefore crucial. Current automated cell counters are based on laser-light scatter and flow-cytochemical princi-

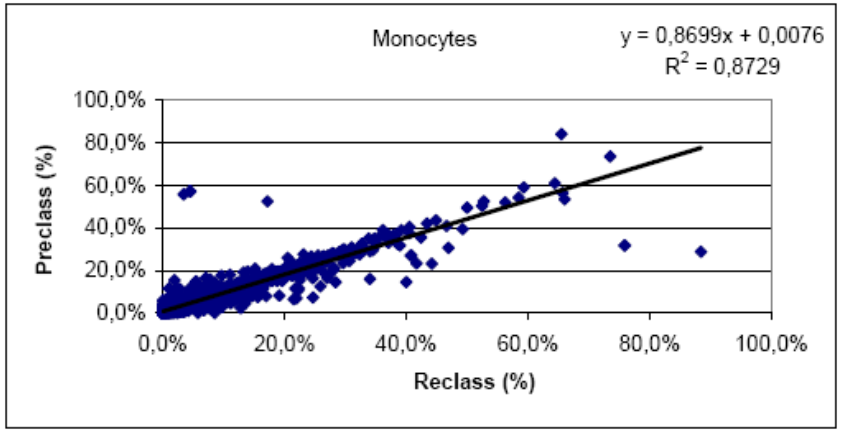
scientist. Coupled with automated sample handling, a "walk-away" system can be created that can partially replace the biomedical scientist. In addition, these systems should be capable of storing relevant morphological data and distribute images to other workstations for review purposes (telehaema-











	R^2
segmented neutrophils	0,94
lymphocytes	0,98
eosinophils	0,95
basophils	0,88
monocytes	0,87

Riedl, Stouten et al., Int J Lab Hem, Oct;37(5):e137-40; 2

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%

Riedl, Stouten et al., Int J Lab Hem, Oct;37(5):e137-40; 2

Original article

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

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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.

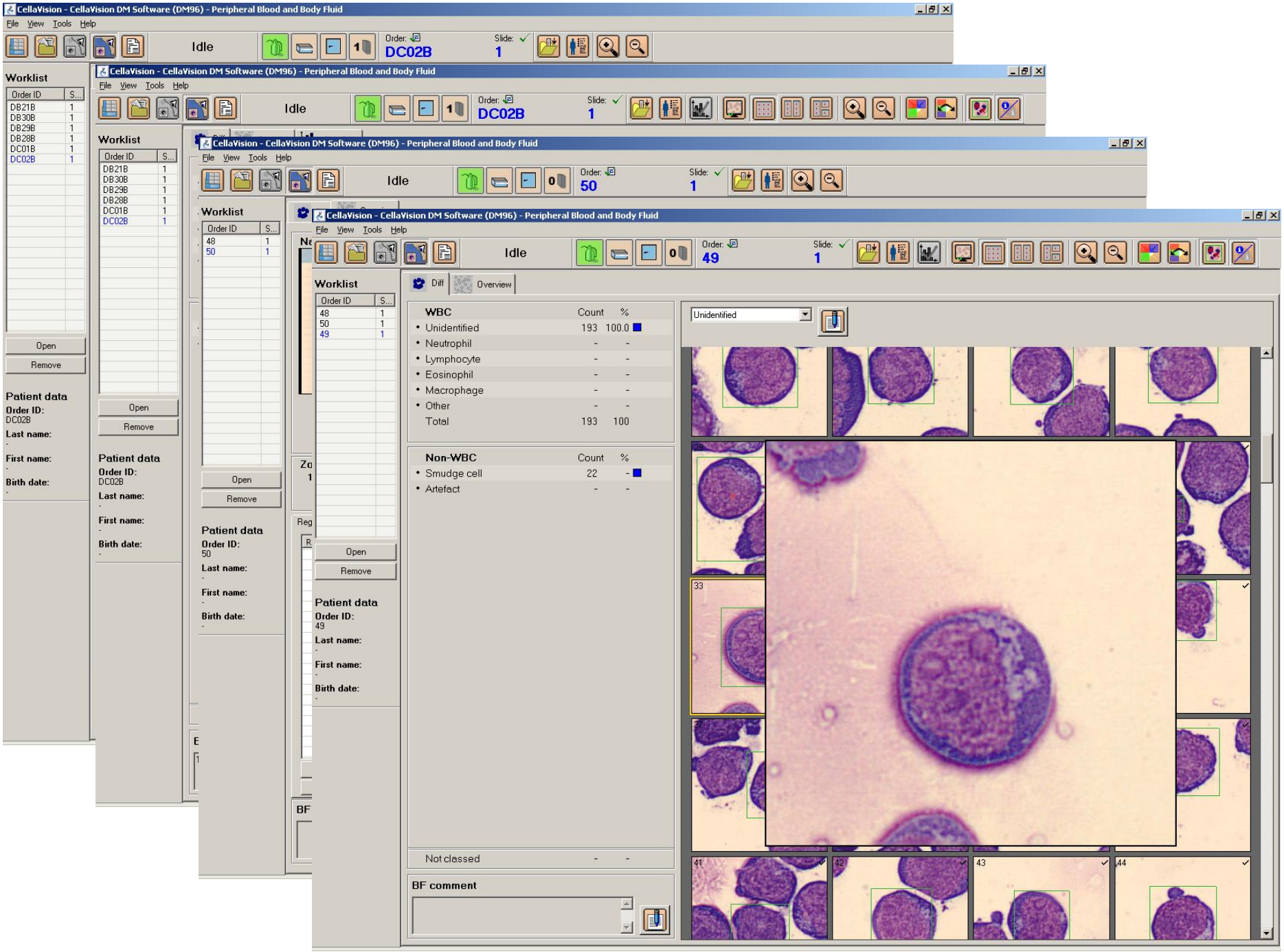
Aims To investigate the ability of the digital microscopy system, DM96, to automatically classify cells in different types of body fluids.

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.

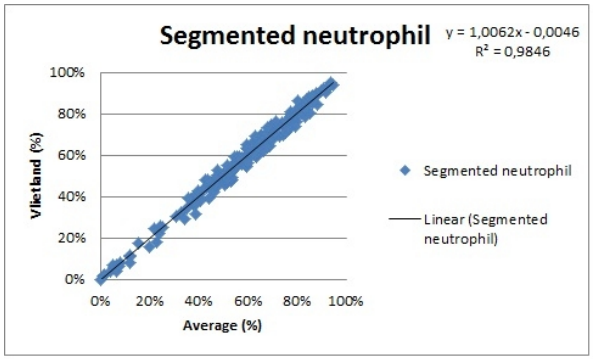
In the past 3–4 years, the DM96 has improved the morphological analysis of blood samples in our laboratory in terms of quality of morphological assessment and turnaround time, and requiring less staff. Moreover, the detection of low percentages of abnormal cells (eg, blasts) in peripheral blood smears 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.

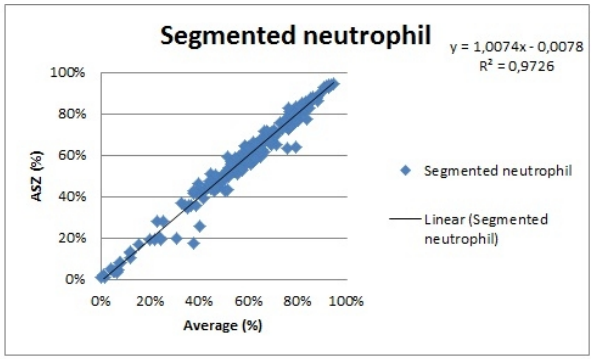
Body fluid material, such as cerebrospinal fluid, requires adequate technical expertise to ensure proper handling and analysis. Samples are collected at the cost of considerable discomfort for the patient and usually yield only a limited volume of material available for analysis. Moreover, there are

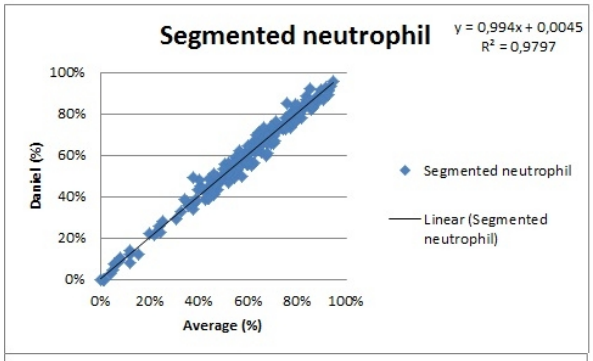

CellaVision - CellaVision DM Software (DM96) - Peripheral Blood and Body Fluid
File View Tools Help
Idle
Order: DC02B
Slide: 1
Worklist
Order ID S...
DB21B 1
DB30B 1
DB29B 1
DB28B 1
DC01B 1
DC02B 1
Open
Remove
Patient data
Order ID: DC02B
Last name:
First name:
Birth date:
Order: 50
Slide: 1
48 1
50 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

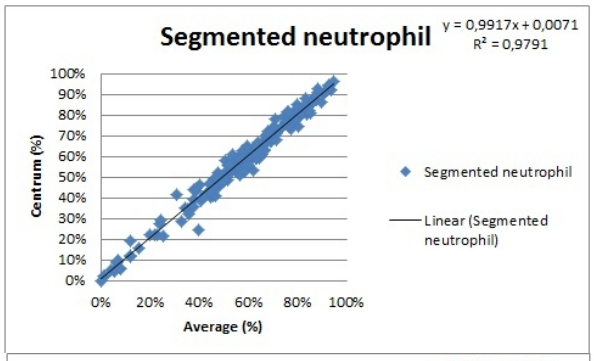
Reproducibility? Inter-Laboratory

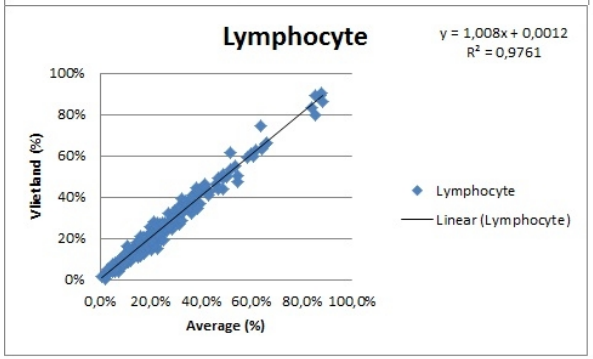
- 200 samples/slides
- 4 different hospital locations
- Comparison of 5-part differential and blasts

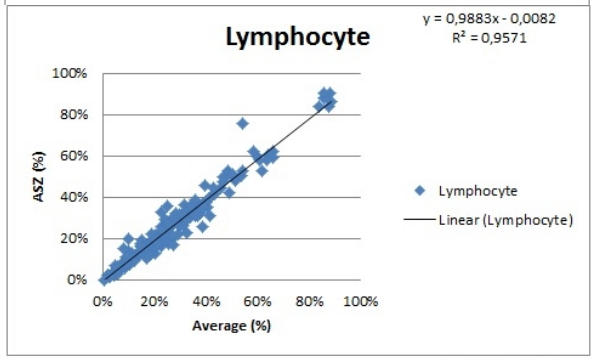


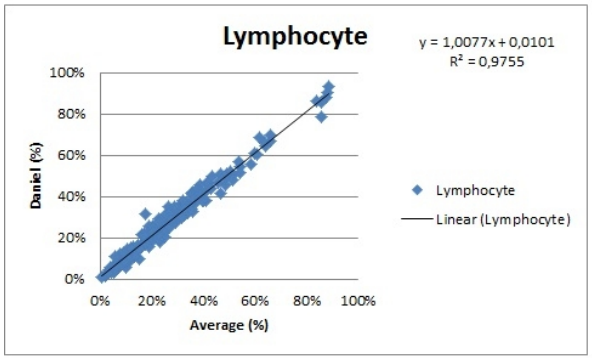


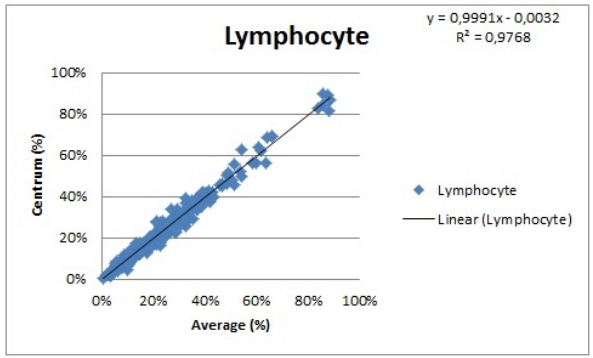












R^2-values:

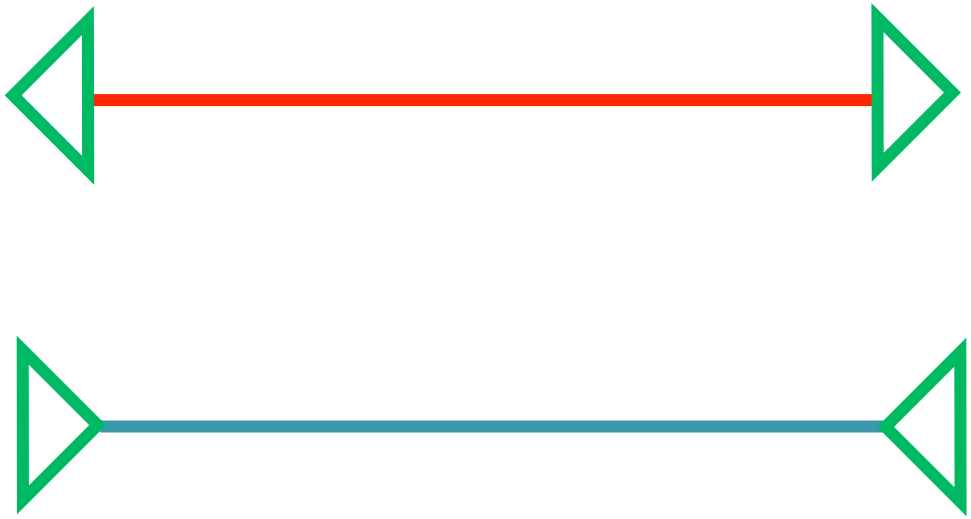
Neutro's:	0,97-0,98
Eo's:	0,82-0,88
Lymfo's:	0,96-0,98
Mono's:	0,89-0,91
Baso's:	0,49-0,59
Blasts:	0,99-1,00

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

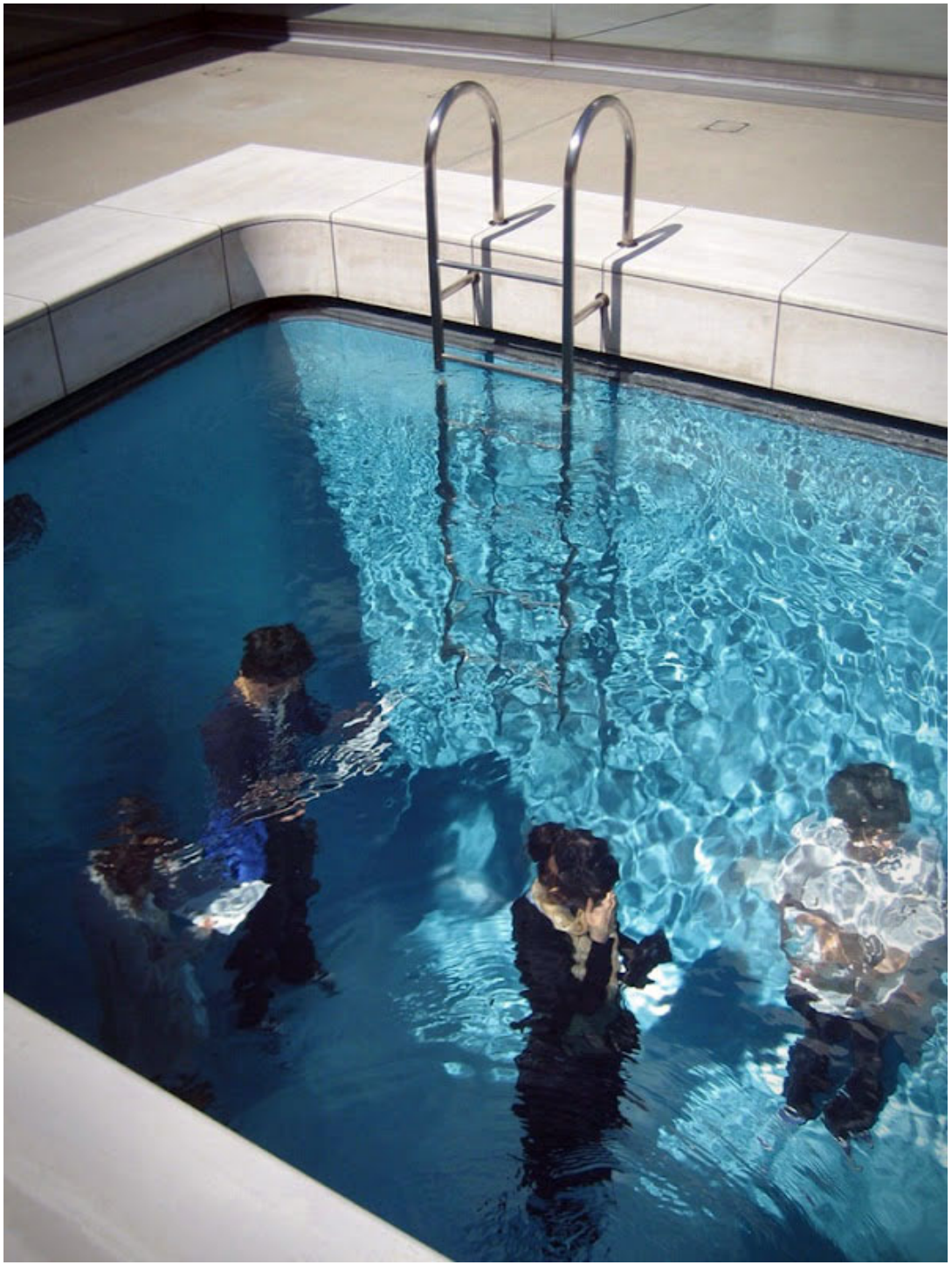
So, Digital Imaging:

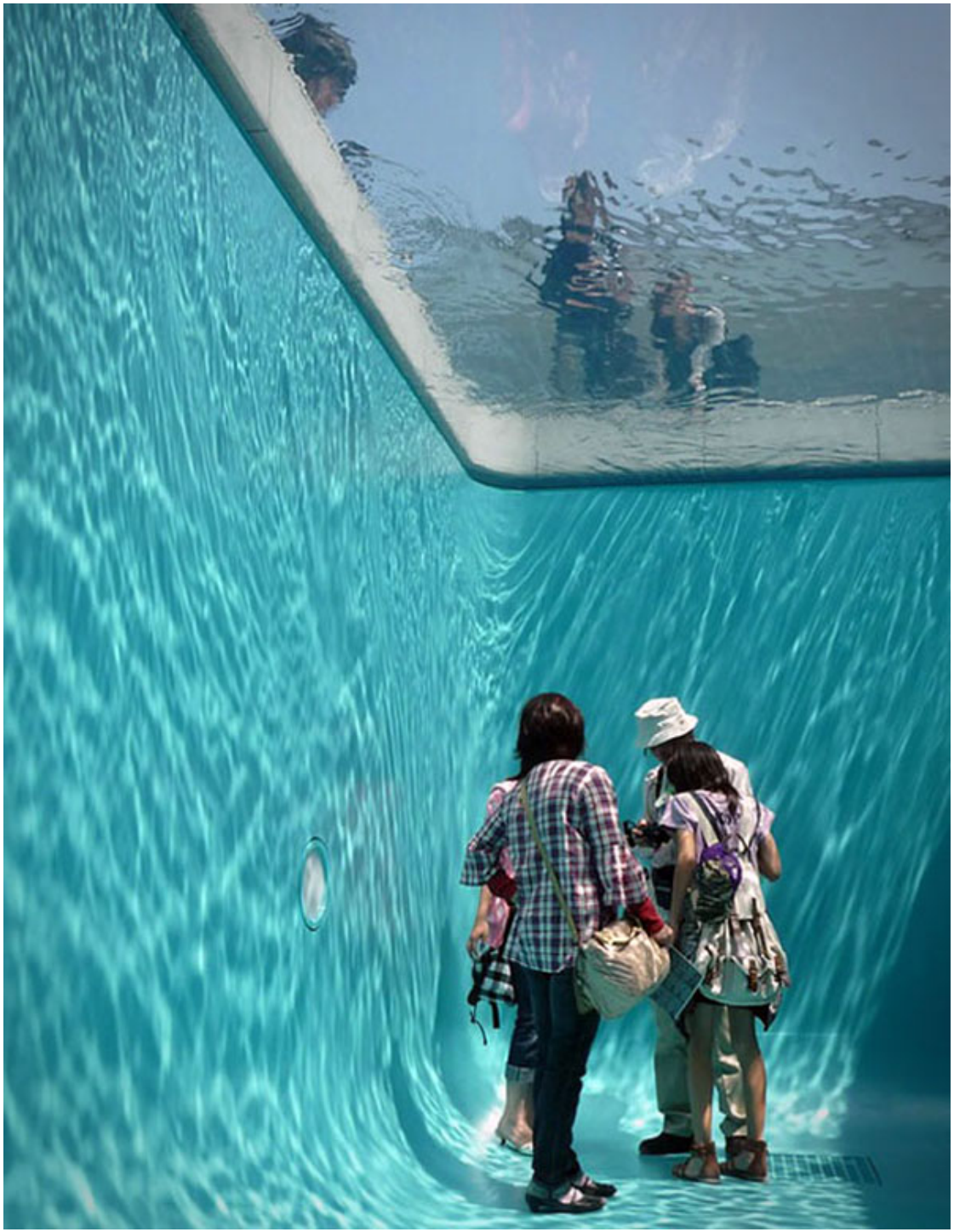
- Works in daily routine for leukocytes in peripheral blood and other body fluids
- Is reliable, accurate and displays a high blast cell sensitity
- Is reproducible
- Excellent learning tool for the next generation

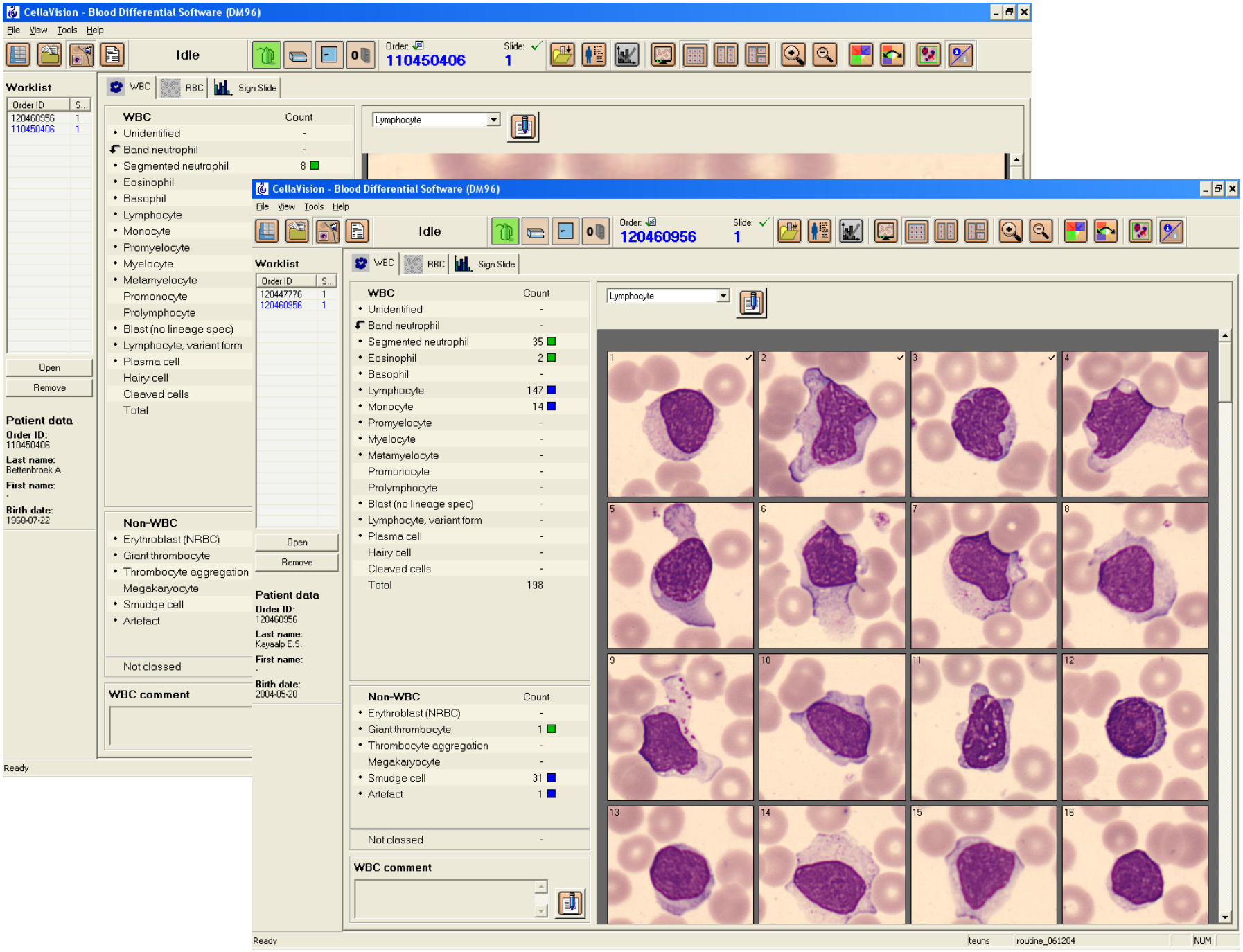
- Not everything is what it seems…



Objectivity?






CellaVision - Blood Differential Software (DM96)
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Slide: 1
Worklist
Order ID S...
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Patient data
Order ID: 110450406
Last name: Bettenbroek A.
First name: -
Birth date: 1968-07-22
WBC RBC Sign Slide
WBC Count
Unidentified -
Band neutrophil -
Segmented neutrophil 8
Eosinophil
Basophil
Lymphocyte
Monocyte
Promyelocyte
Myelocyte
Metamyelocyte
Promonocyte
Prolymphocyte
Blast (no lineage spec)
Lymphocyte, variant form
Plasma cell
Hairy cell
Cleaved cells
Total
Non-WBC
Erythroblast (NRBC)
Giant thrombocyte
Thrombocyte aggregation
Megakaryocyte
Smudge cell
Artefact
Not classed
WBC comment
Lymphocyte
Ready
CellaVision - Blood Differential Software (DM96)
File View Tools Help
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Slide: 1
Worklist
Order ID S...
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120460956 1
Open
Remove
Patient data
Order ID: 120460956
Last name: Kayaalp E.S.
First name: -
Birth date: 2004-05-20
WBC RBC Sign Slide
WBC Count
Unidentified -
Band neutrophil -
Segmented neutrophil 35
Eosinophil 2
Basophil -
Lymphocyte 147
Monocyte 14
Promyelocyte -
Myelocyte -
Metamyelocyte -
Promonocyte -
Prolymphocyte -
Blast (no lineage spec) -
Lymphocyte, variant form -
Plasma cell -
Hairy cell -
Cleaved cells -
Total 198
Non-WBC Count
Erythroblast (NRBC) -
Giant thrombocyte 1
Thrombocyte aggregation -
Megakaryocyte -
Smudge cell 31
Artefact 1
Not classed -
WBC comment
Lymphocyte
1 2 3 4
5 6 7 8
9 10 11 12
13 14 15 16
Ready
teuns
routine_061204
NUM

- 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

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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):

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 – – – >30%	Report if present			
Irregularly contracted red cells (Bite cells) - > 4%				
Stomatocytes – – > 30%				
Teardrop cells* – > 4%				
Agglutination				
Dimorphic red cells				
Dual population				
Howell-Jolly bodies				
Oval macrocytes				
Pappenheimer bodies				
Parasites				
Sickle cells				

pg, picogram; fL, femtoliter.
*Teardrop cells accompanied by NRBCs can be reported even <4%.

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

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

- Despite efforts to standardize RBC morfology and grading there are still inter-laboratory differences…

Digital Imaging of RBC’s

Albert Schweitzer ziekenhuis

Content:

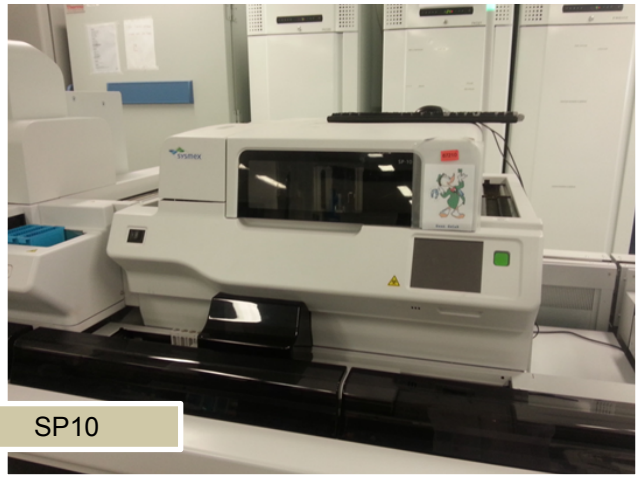
- Introduction
- Material & Methods
- Results
- Conclusion & Discussion

Introduction

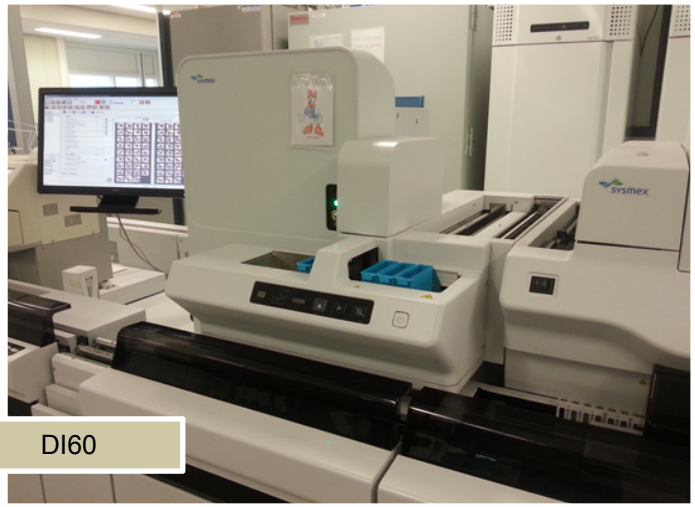
- **Aim of the study:** validate a novel morphological RBC-module which is capable of correct detection and classification of RBC abnormalities.
  - is determination of a cut-off value possible?

- 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).

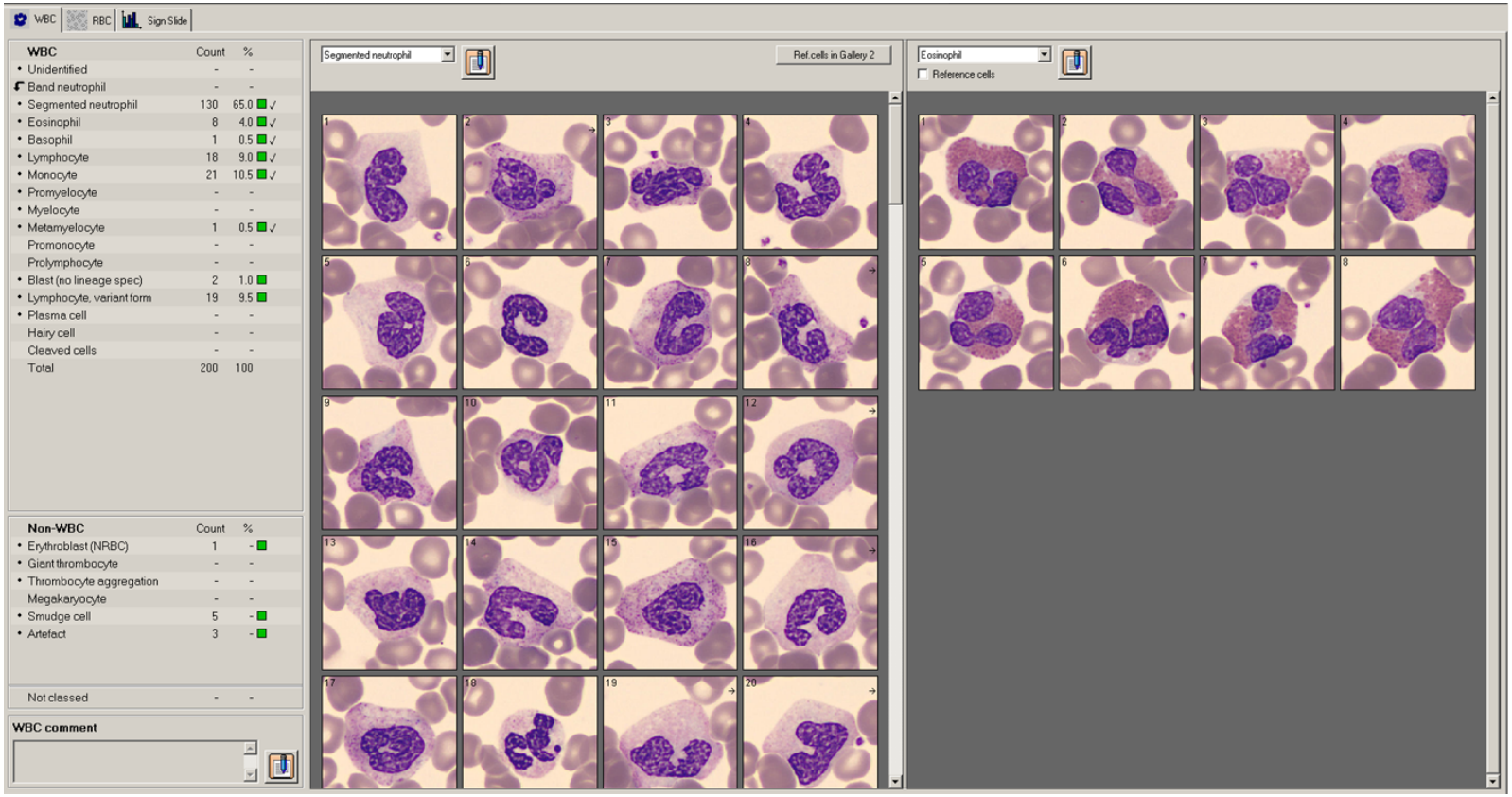
Cellcounter



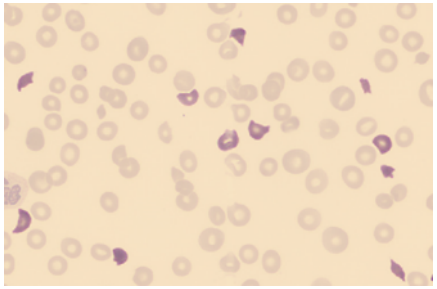
SP10



DI60

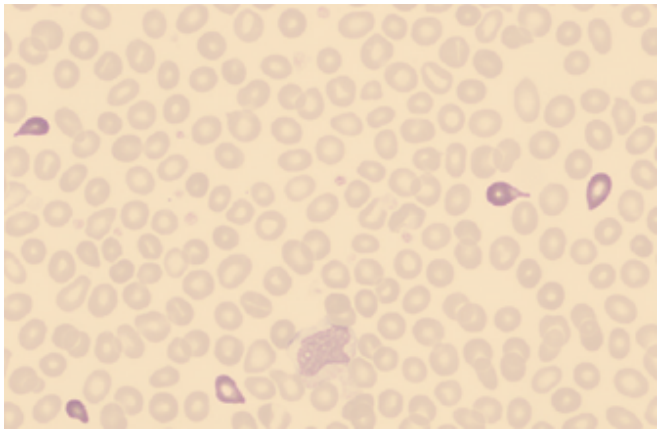

WBC
RBC
Sign Slide
WBC Count %
Unidentified - -
Band neutrophil - -
Segmented neutrophil 130 65.0 ✓
Eosinophil 8 4.0 ✓
Basophil 1 0.5 ✓
Lymphocyte 18 9.0 ✓
Monocyte 21 10.5 ✓
Promyelocyte - -
Myelocyte - -
Metamyelocyte 1 0.5 ✓
Promonocyte - -
Prolymphocyte - -
Blast (no lineage spec) 2 1.0
Lymphocyte, 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

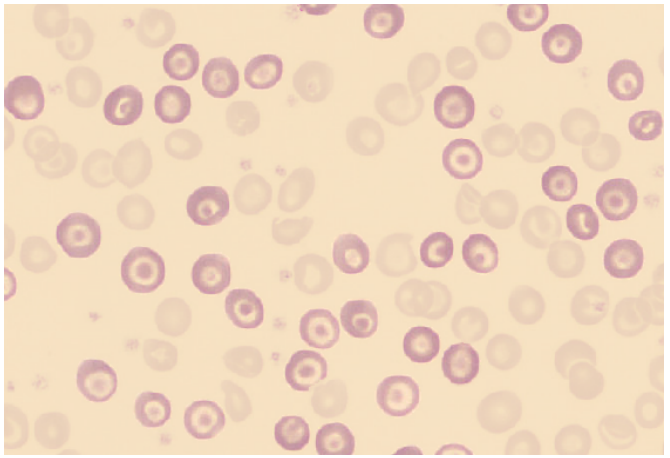


- **TTP (Trombotische trombocytopenische purpura):**
  - Acute, medical emergency
  - ADAM-TS 13 enzyme non-functional (no cleaving of vWF)
  - Hemolytic anemia & trombocytopenia
  - Patients display:
    - Fever, neurological symptoms, kidney failure, hemolytic anemia & tromocytopenia & fragmentocytes/schistocytes.

- **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

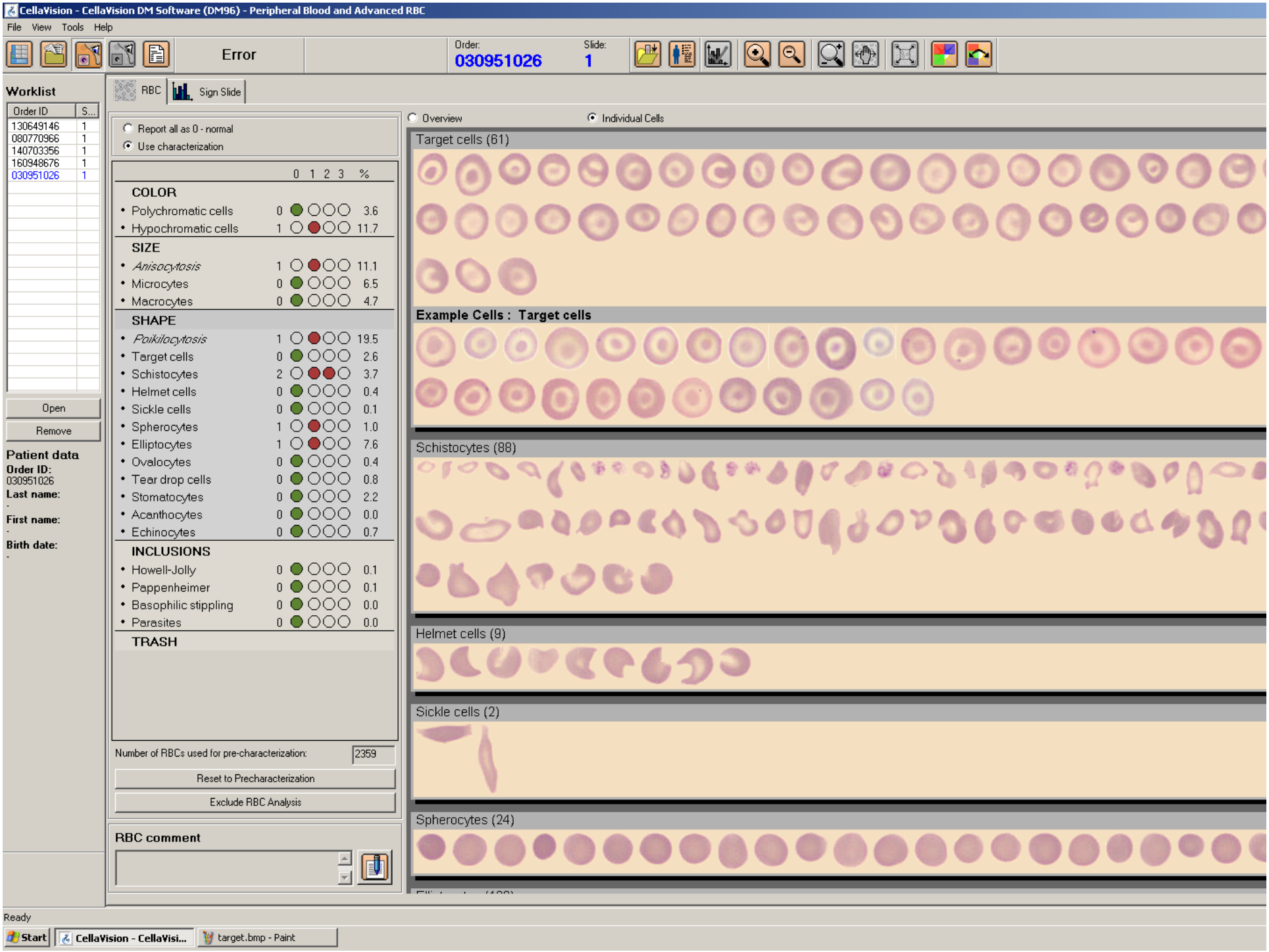


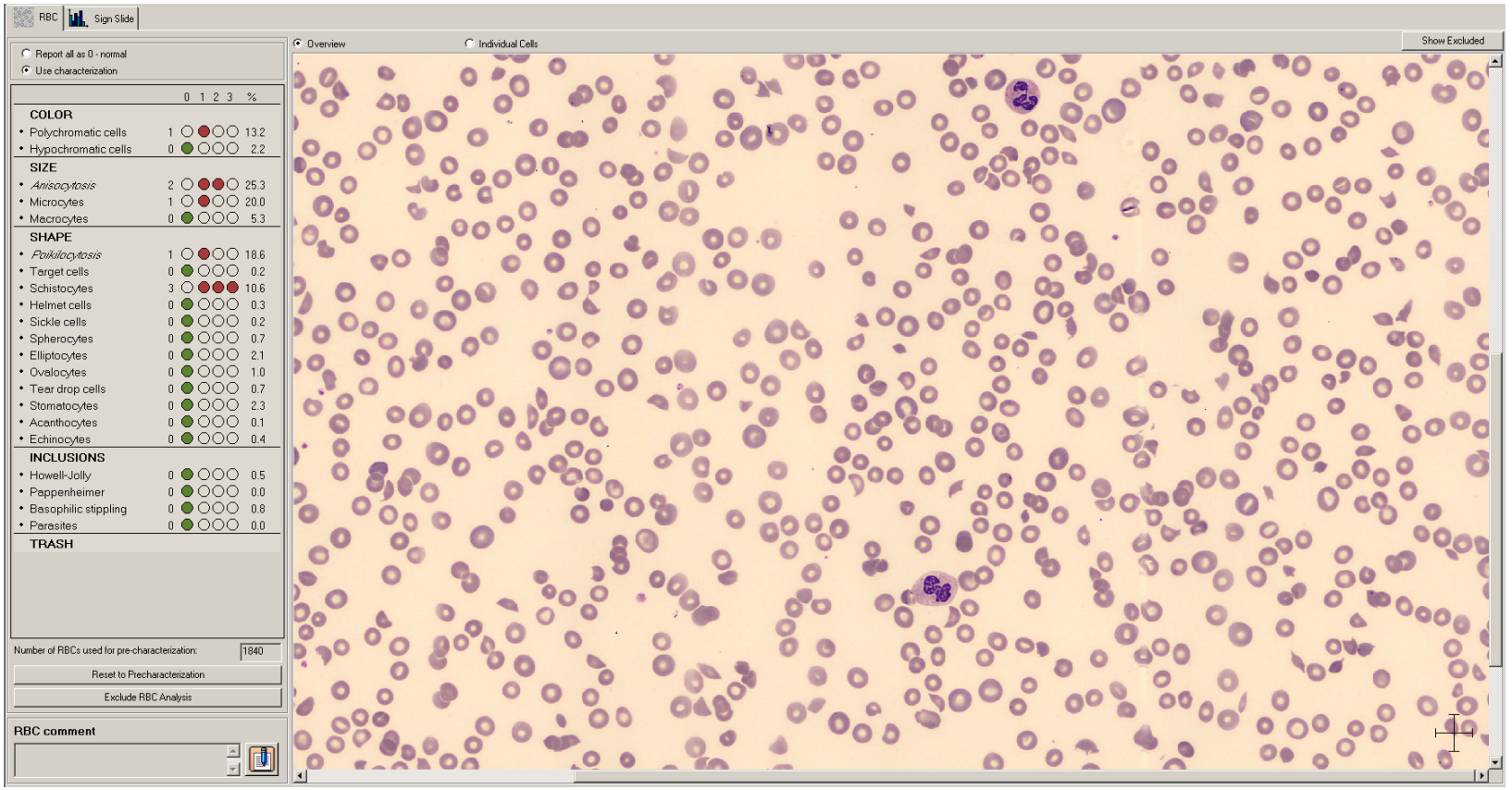
- **Thalassemia:**
  - Hemoglobinopathies
  - Thalassemia is caused by variant or missing genes that affect how the body makes hemoglobin
  - Normal hemoglobin -> 4 globin chains; 2 alpha en 2 bèta-globin chains
  - Two main forms; alpha- en bèta-thalassemia
  - Autosomal recessive disorder
  - Diagnostics: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

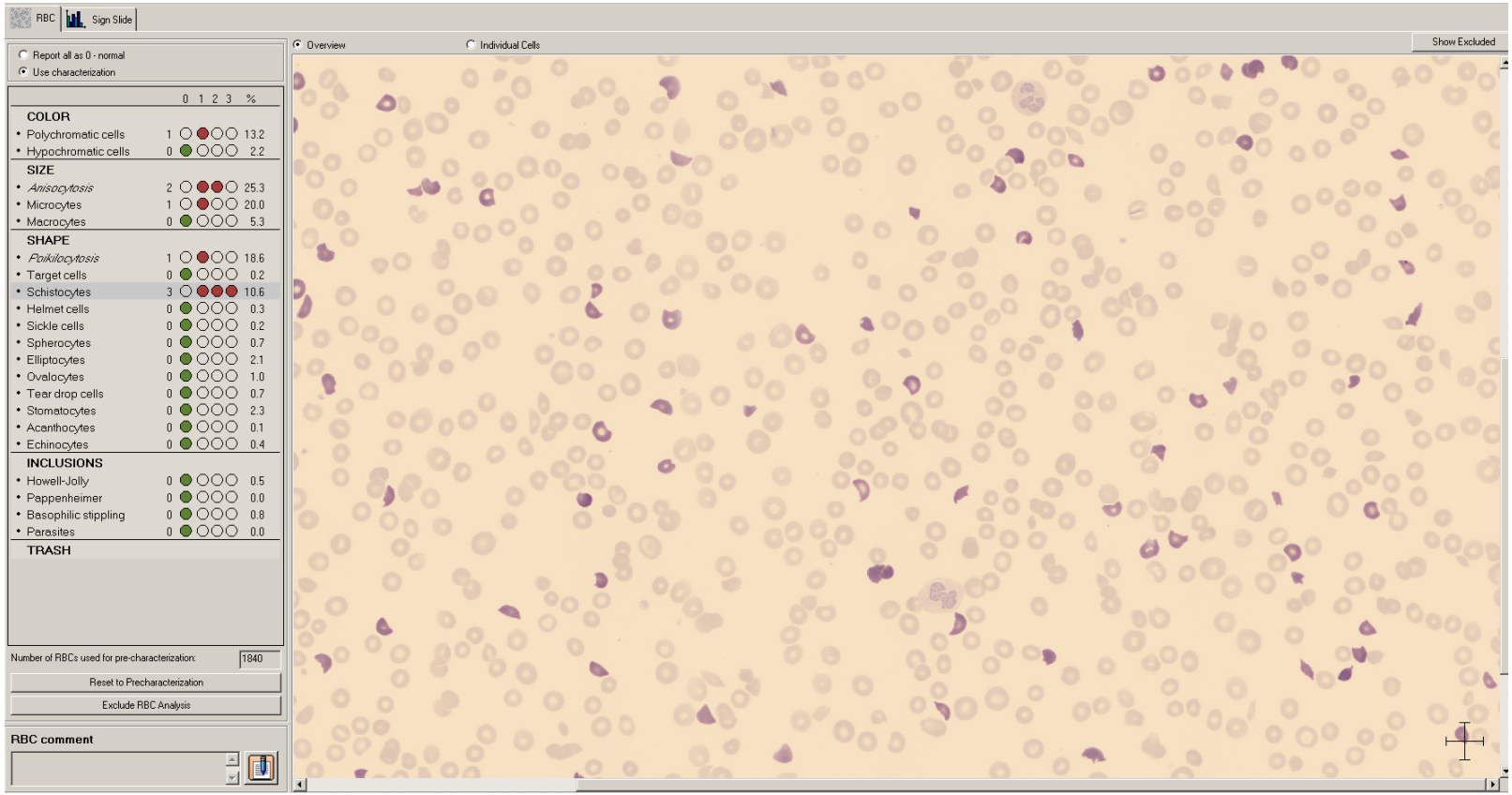


Materials and methods

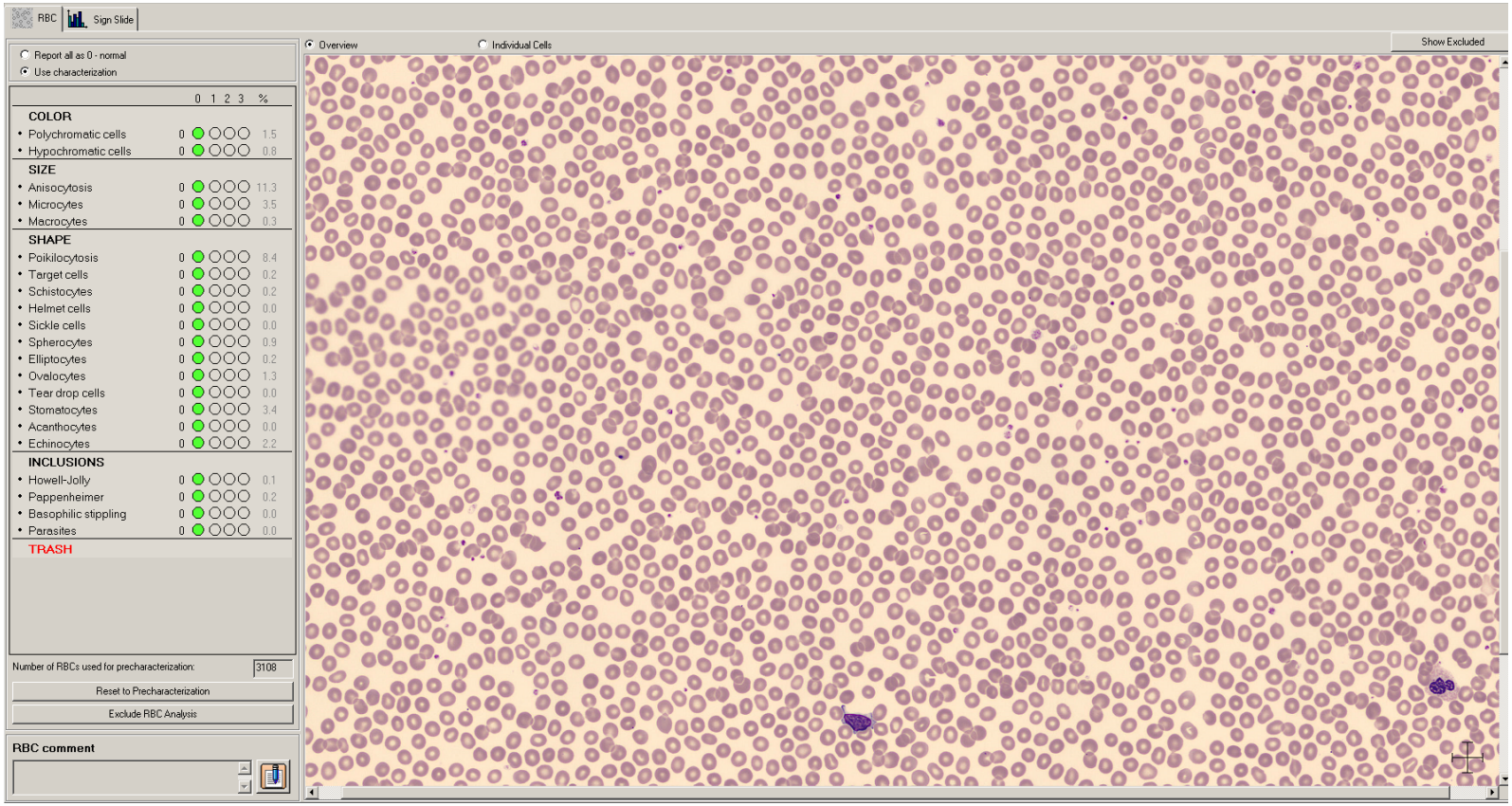
- Peripheral blood smears of 316 patiënt samples and 10 healthy individuals (determination of a “cut-off” value)
  - 80 samples were used containing fragmentocytes, targetcells en teardrop cells
- May-Grünwald Giemsa staining using SP-10 (automatic slidemaker/stainer)
  - 2000-4000 erytrocytes were analysed/bloodsmear
  - 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
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
Target cells (3)
Show Example Cells
Schistocytes (195)
Show Example Cells
Helmet cells (6)
Show Example Cells
Sickle cells (4)
Show Example Cells
Spherocytes (12)
Show Example Cells

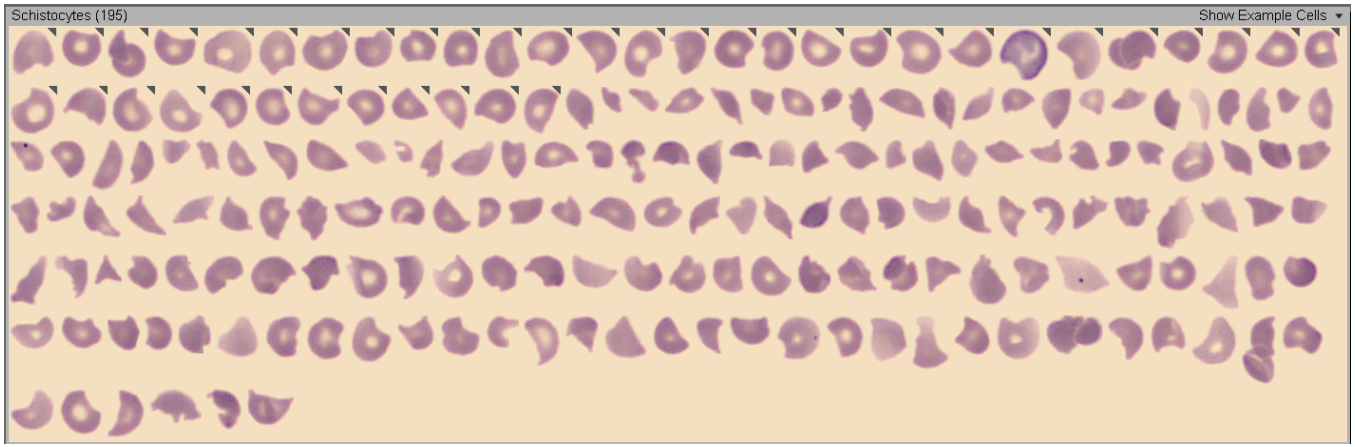

RBC
Sign Slide
Report all as 0 - normal
Use characterization
0 1 2 3 %
COLOR
• Polychromatic cells 0 1.5
• Hypochromatic cells 0 0.8
SIZE
• Anisocytosis 0 11.3
• Microcytes 0 3.5
• Macrocytes 0 0.3
SHAPE
• Poikilocytosis 0 8.4
• Target cells 0 0.2
• Schistocytes 0 0.2
• Helmet cells 0 0.0
• Sickle cells 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

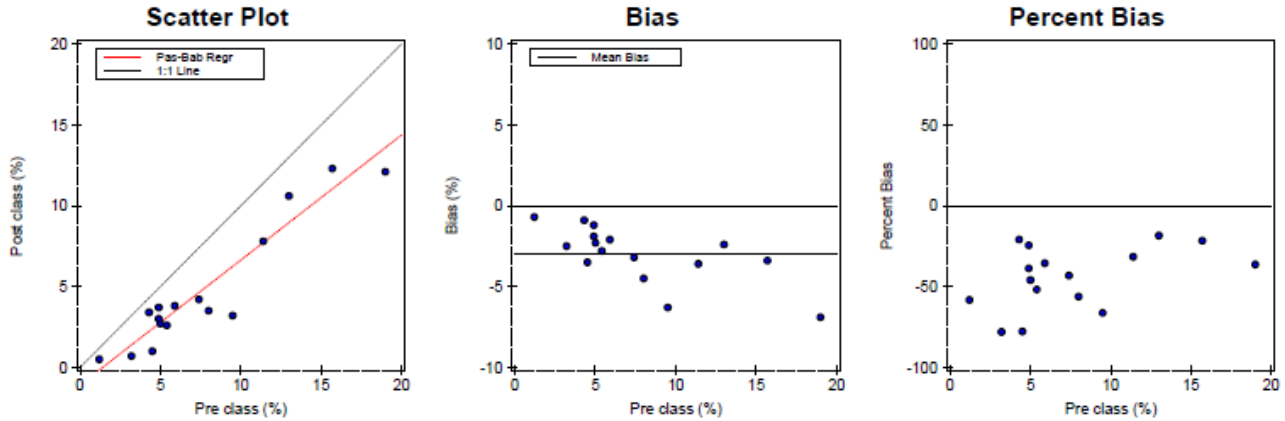
Results

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





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.

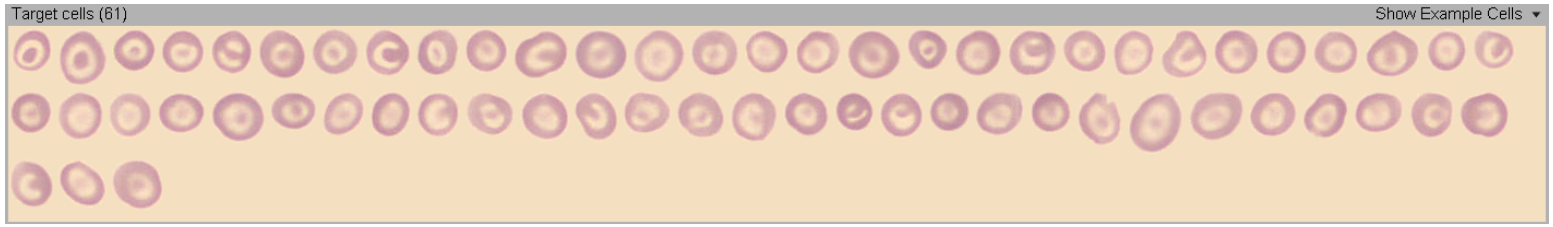
- y=x line of y=0,8x-1
- Correlatiecoëfficiënt of 0,95

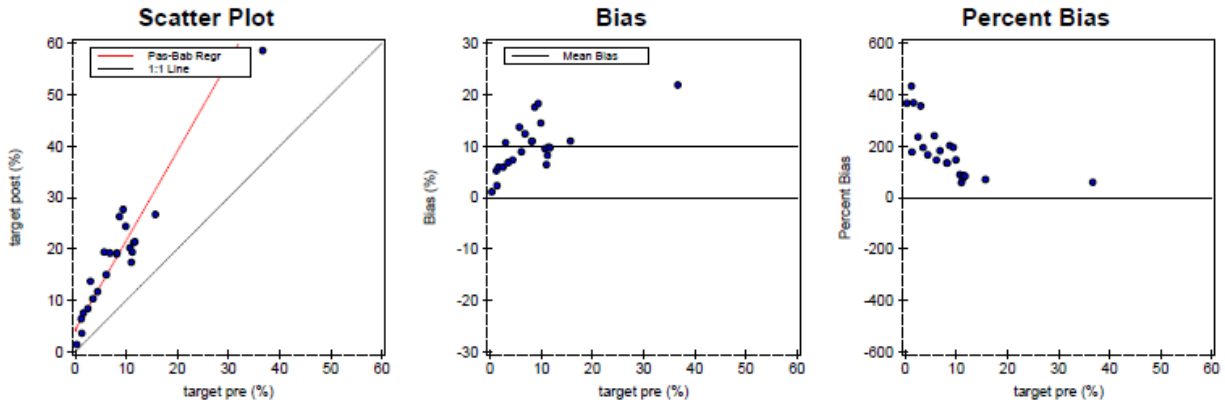
Riedl et al., J. Hematology; 2015;4(2):184-186

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

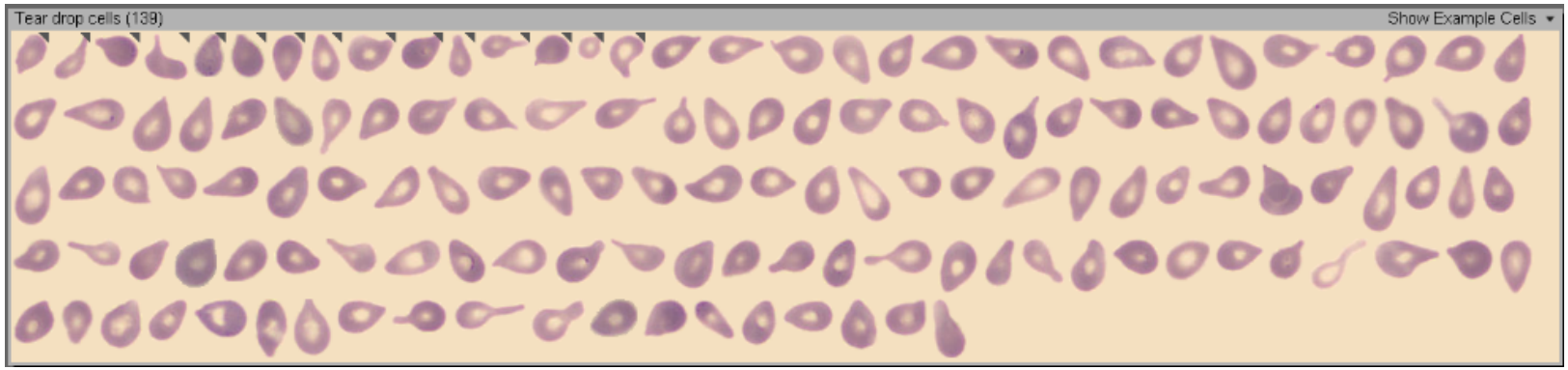
Figuur 2. Figure showing comparison of pre- and post-classification of targetcells.

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

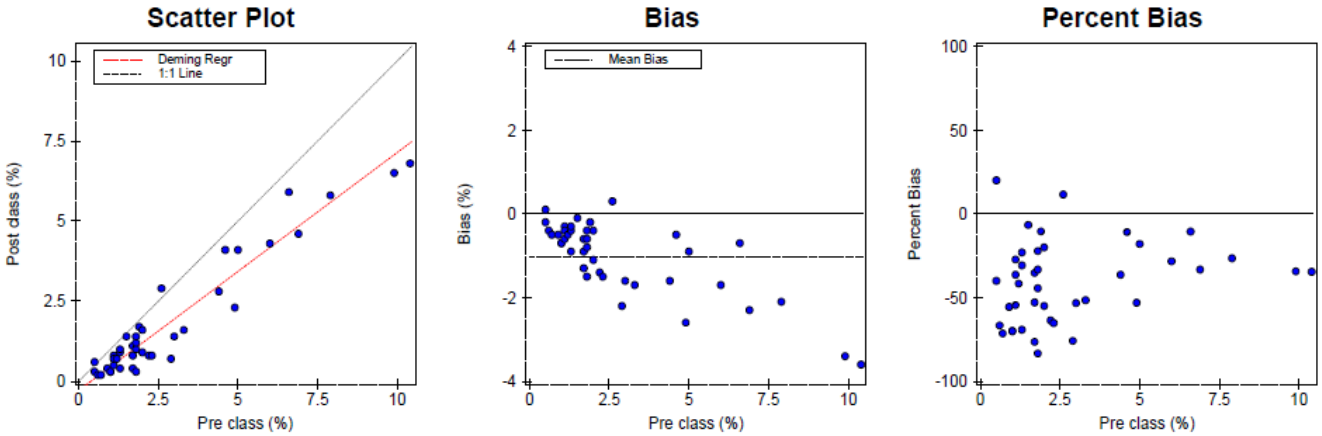
Teardropcells:

- 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



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**



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

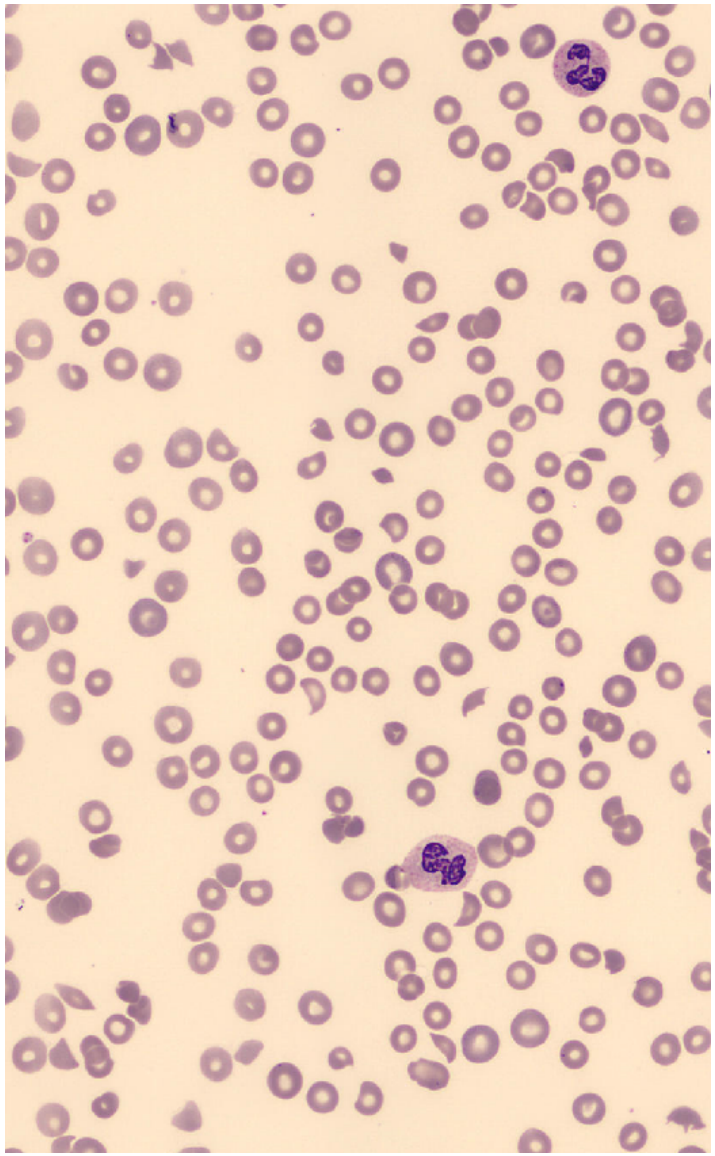
Riedl et al., Int J Lab Hem., Dec;37(6):e153-6; 2

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
  - Teardropcells; > 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?