Eurachem 5th PT Workshop

Current practice and future directions in PT/EQA: Applications in the medical laboratory field

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EQALM chairman

EQA requirements in ISO 15189

- 5.6.4

…

« External quality assessment programmes should as far as possible, provide clinically relevant challenges that mimic patient samples and have the effect of checking the entire examination process, including pre- and post-examination procedures. »

Example of pre-examination procedures

a sputum sample is submitted with a companion Gram stain that indicates excessive saliva contamination. The sample is intended to challenge pre-analytic rejection criteria. The anticipated report is “Sample is contaminated and inadequate for testing”.

Example of post-examination procedures

- **Sample S/5339 and S/5340** were taken to the same patient with an interval of 4 weeks; Pregnancy wish in the scope of IVF for a woman, 35 years old. Request for CMV diagnosis
- **Requested results**:
- On each sample: total antibodies, IgG, IgG avidity,
- IgM
- **Test interpretation**: positive,negative,borderline
- **Combined interpretation for both samples**:
  - Negative
  - Seroconversion
  - Recent infection (< 3 months)
  - Infection > 3 months
  - Reactivity
  - Others

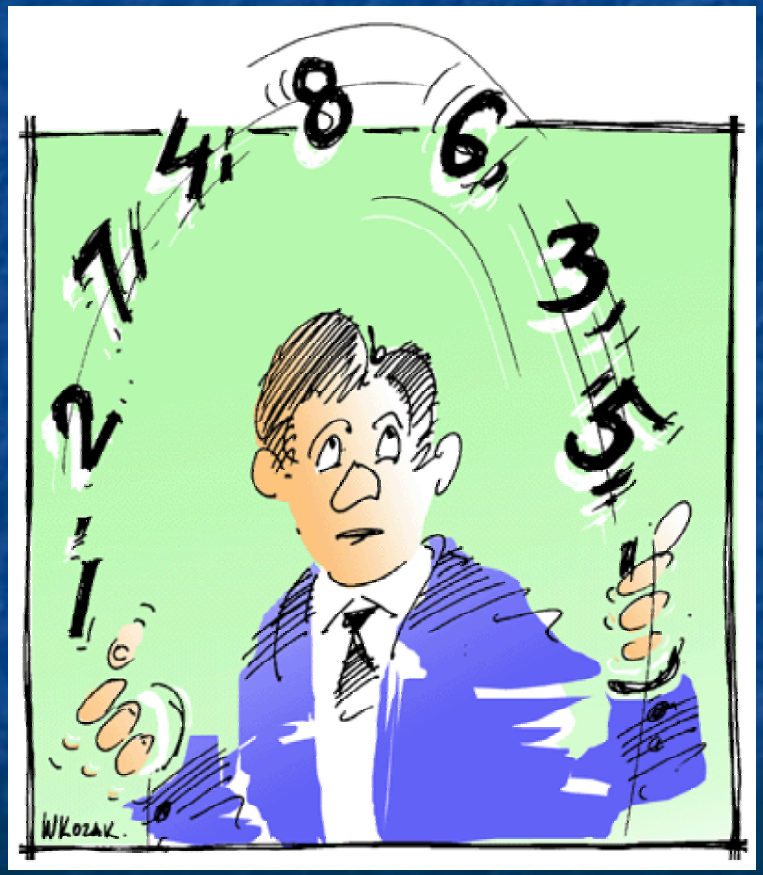
Our EQA schemes focus on

- Performance evaluation of laboratories
  - Analytical performance (quality of results)
  - Clinical performance (quality of information)
- Performance evaluation of used in vitro diagnostics (method performance evaluation)
- Vigilance role
- Education
- Training and help

The cornerstones of good EQA practice

Samples

Statistics



Sample requirements

- Stable
- Homogeneous
- Viable
- Mimic as much as possible real patient material

Virtual samples

- Pictures
  - Relevant pictures are shown with/without possibilities to zoom-in
  - Can be used for educational purposes
  - Cannot be used to evaluate routine performance of a laboratory
- Movies
- Virtual microscopy samples
  - Your PC screen replaces a microscope

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Clinical Laboratory

International

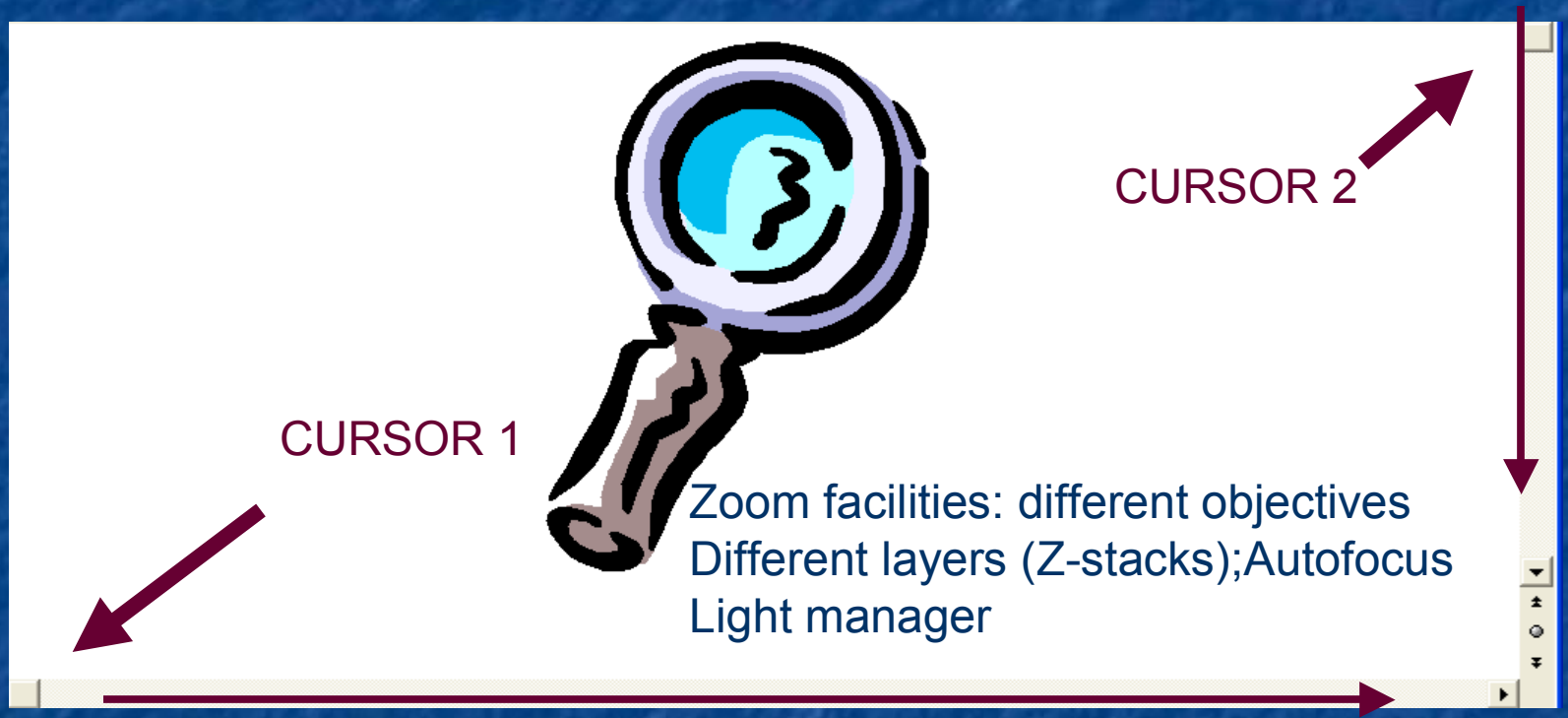
The Malaria P.f/P.v Ag ELISA is a rapid and simple assay to qualitatively determine the presence of the plasmodium species *P. falciparum* and *P. vivax*. This direct sandwich ELISA detects the presence of plasmodium lactate dehydrogenase (pLDH) and is ideal for both establishing an initial diagnosis and monitoring therapy.

STANDARD DIAGNOSTICS, INC.
Kyonggi-do, Korea, Fax +82 31 258 2995

CLI 0010

Your screen is more then a basic microscope

Your screen is more then a basic microscope



Your screen is more then a basic microscope

+ possibility to identify AND STORE interesting fields/cells by using their coordinates

« As computers are getting progressively less expensive and more powerful, and pathologists are becoming more comfortable with their use, virtual microscopy is very likely to mature in the next few years to become the method of choice for proficiency testing in pathology »

A. M. Marchevsky. Arch Pathol Lab Med: 1327-1328;128, 2004

Applications for EQA

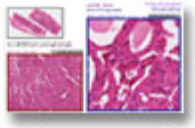
- Use of the technology to prepare virtual samples
  - It is even not needed for the EQA organizer to dispose of the technology
- Electronic mailing of samples to participants or
- Mailing of a CD/DVD
- International collaboration between EQA organisations for digital sample libraries and for software applications

How Our Virtual Slide Scanning Service Works

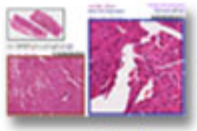
Mail us your glass slides.

We scan them and create virtual slides.

Your virtual slides reside on our servers.



Review your slides using your browser.

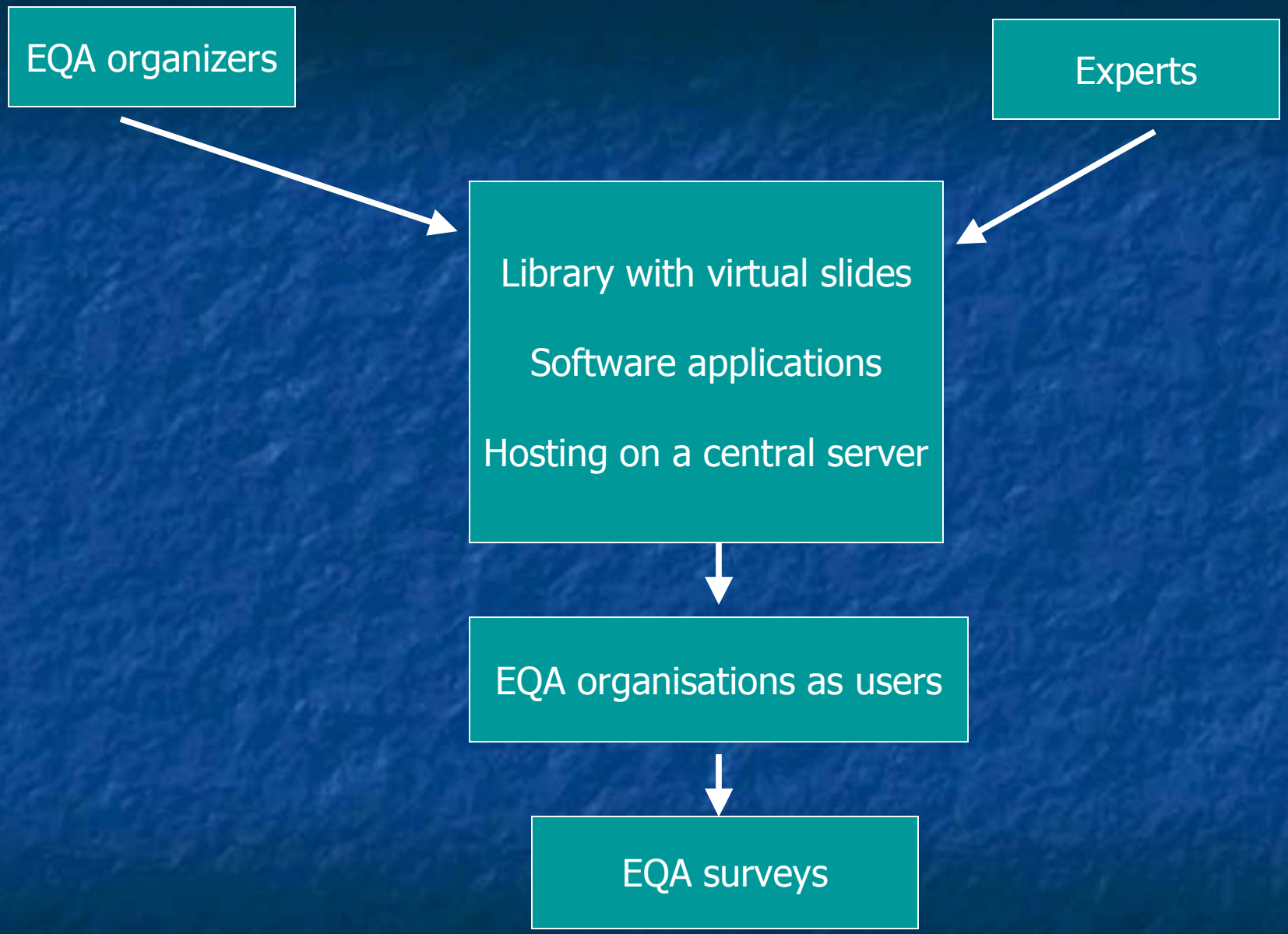


Save custom views of your slides, add comments, bookmark your work, then send direct links of your bookmarks to others.

BLISS

Bacus Laboratories, Inc. Slide Scanner

Next Generation Microscope Imaging Workstation


EQA organizers
Experts
Library with virtual slides
Software applications
Hosting on a central server
EQA organisations as users
EQA surveys

New perspectives

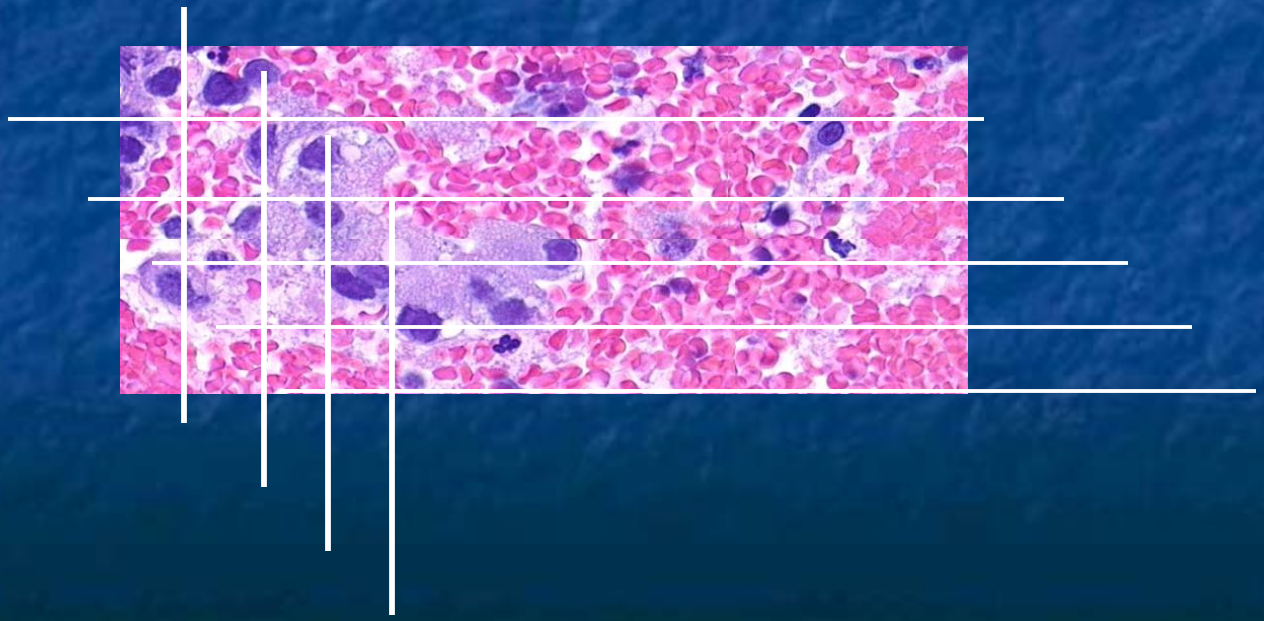
- Histology samples
- Cytology samples
- Sperm motility/ sperm morphology
- Parasitology
- Gram stains
- Urine sediment
- Bone marrow

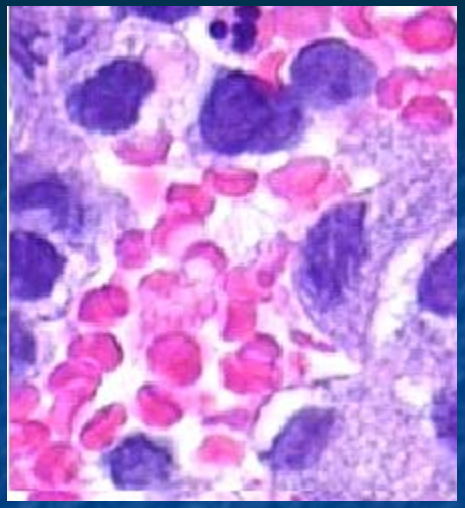
Technology for image capture

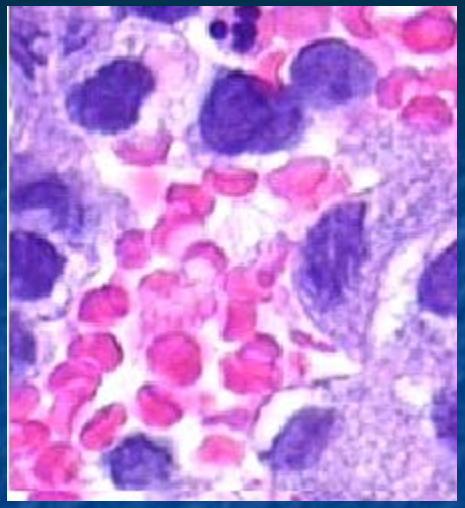
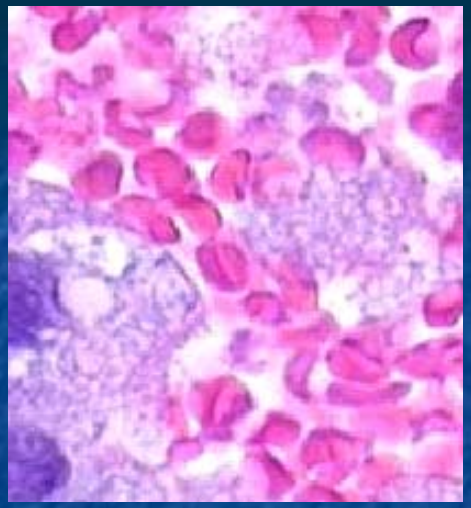
- Tile scan
- Contineous scan bright light
- Contineous scan fluorescent light (under development)

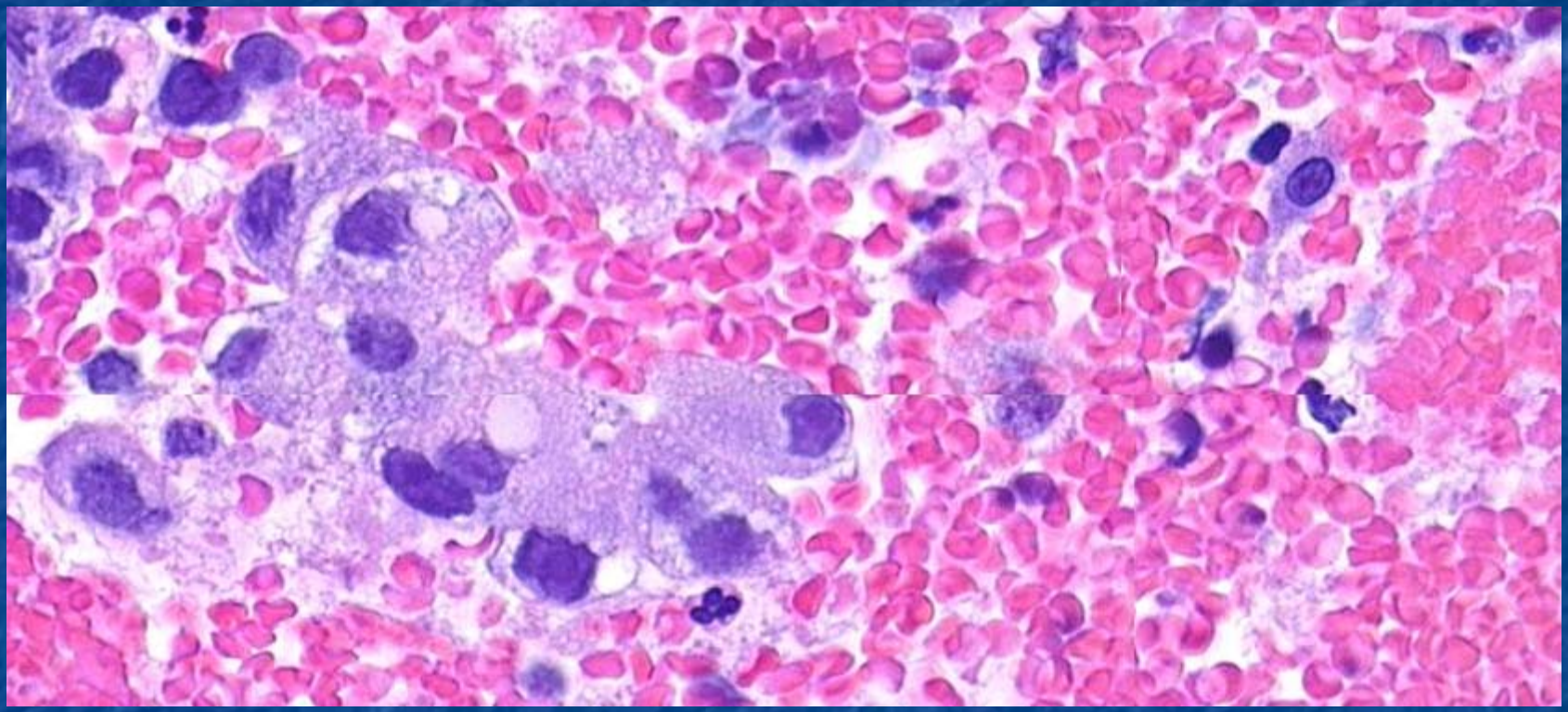
Tile scan technology

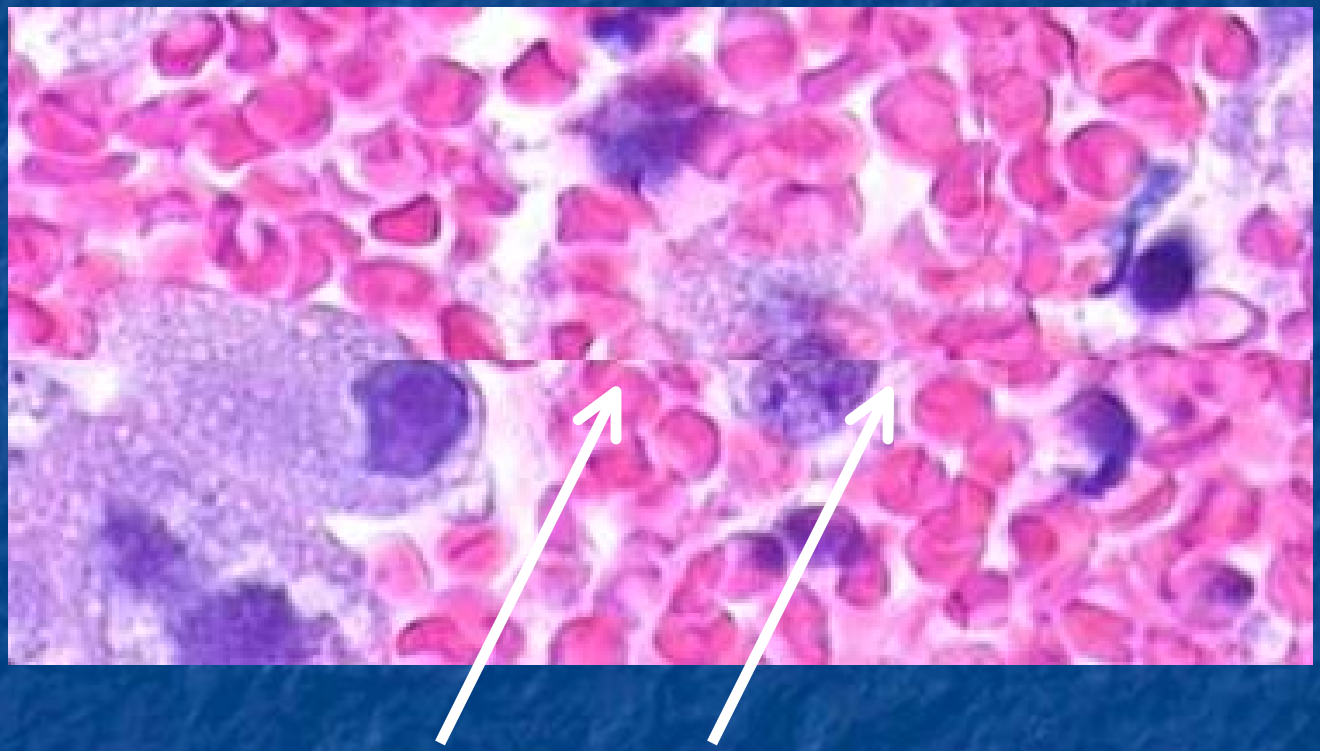
- Re-assembled total image from scanned tiles

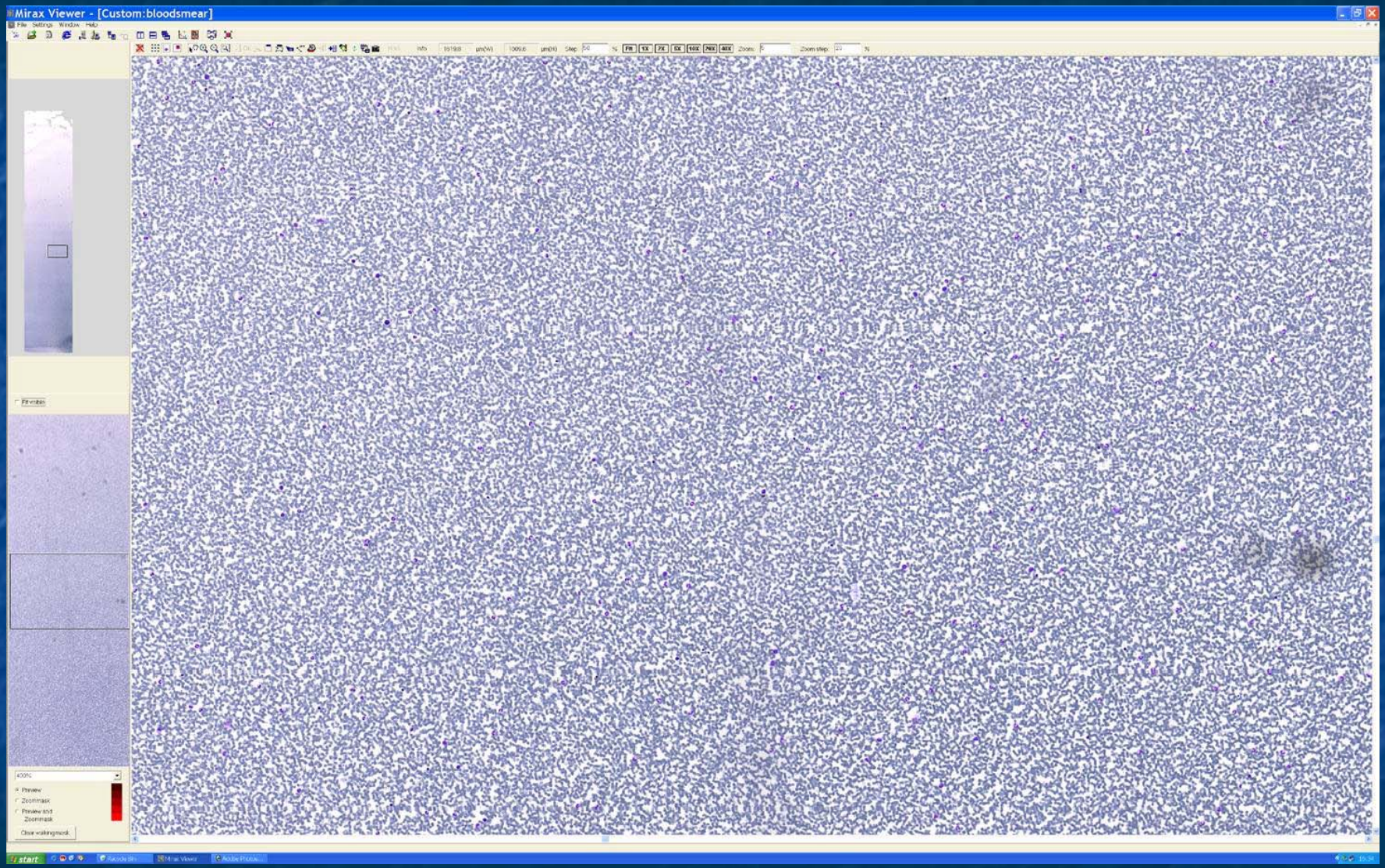




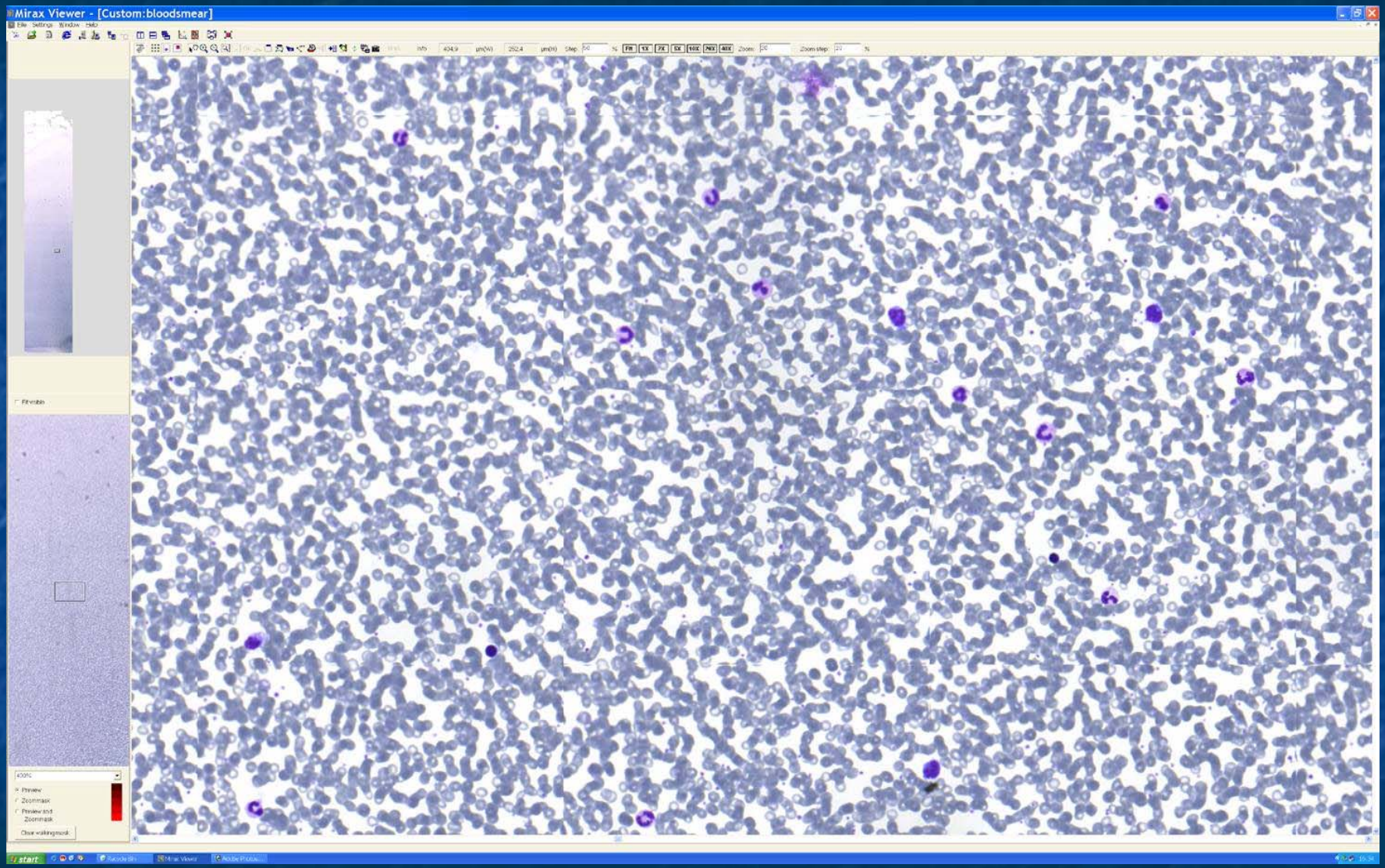





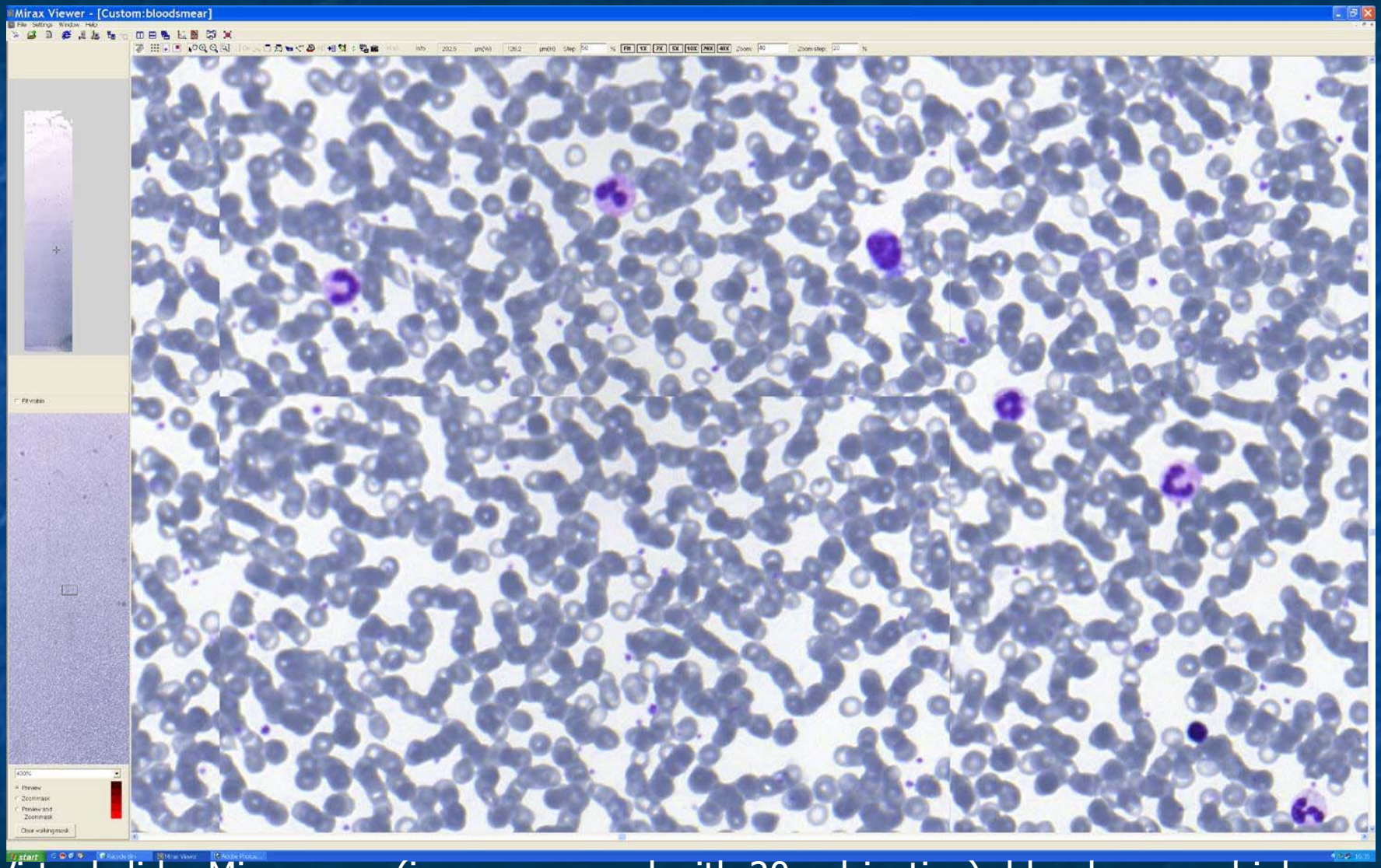




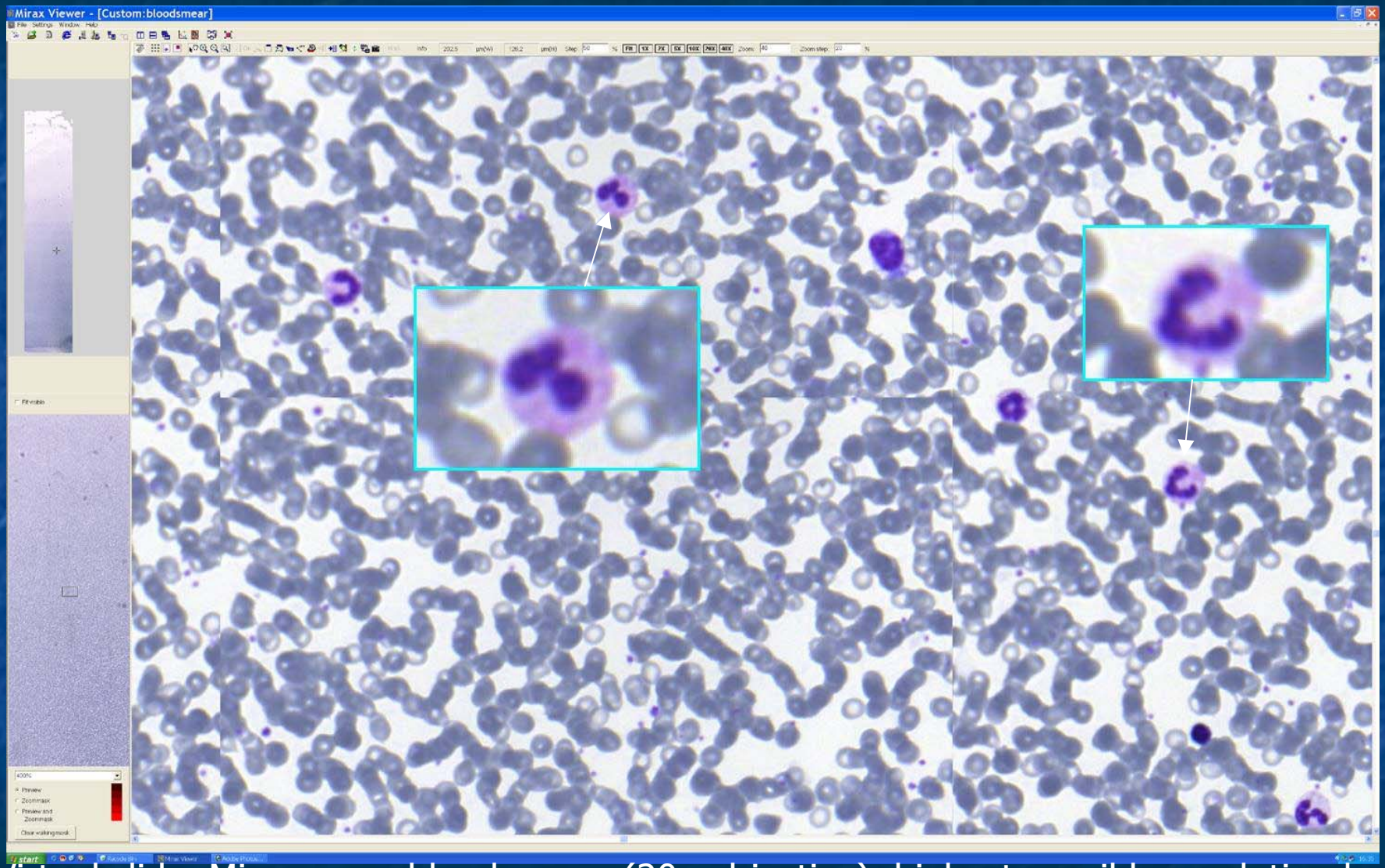
Virtual slide, Mirax scan, blood smear, low power view, 7596 image tiles



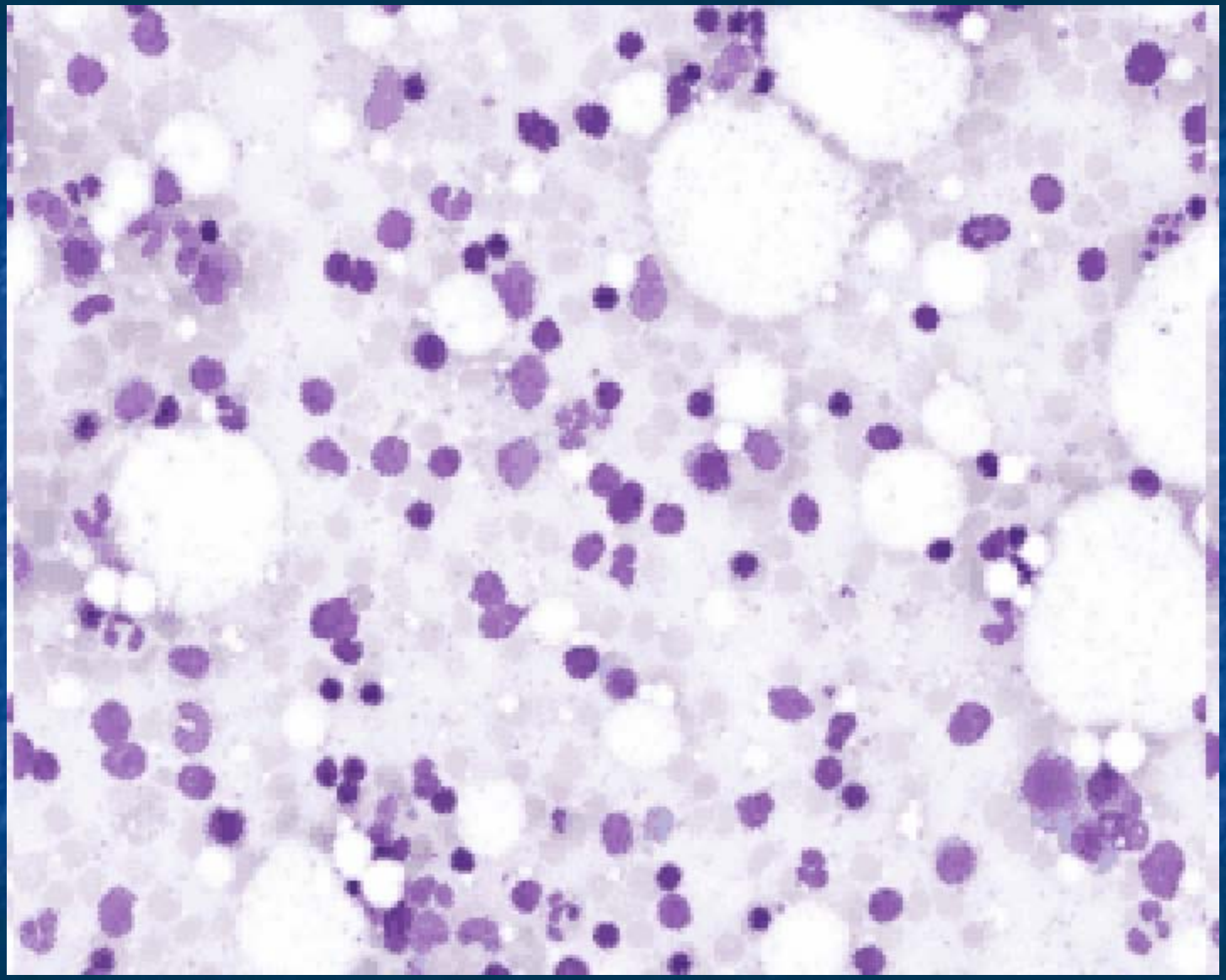
Virtual slide, Mirax scan (image scanned with 20x objective), blood smear, medium power view



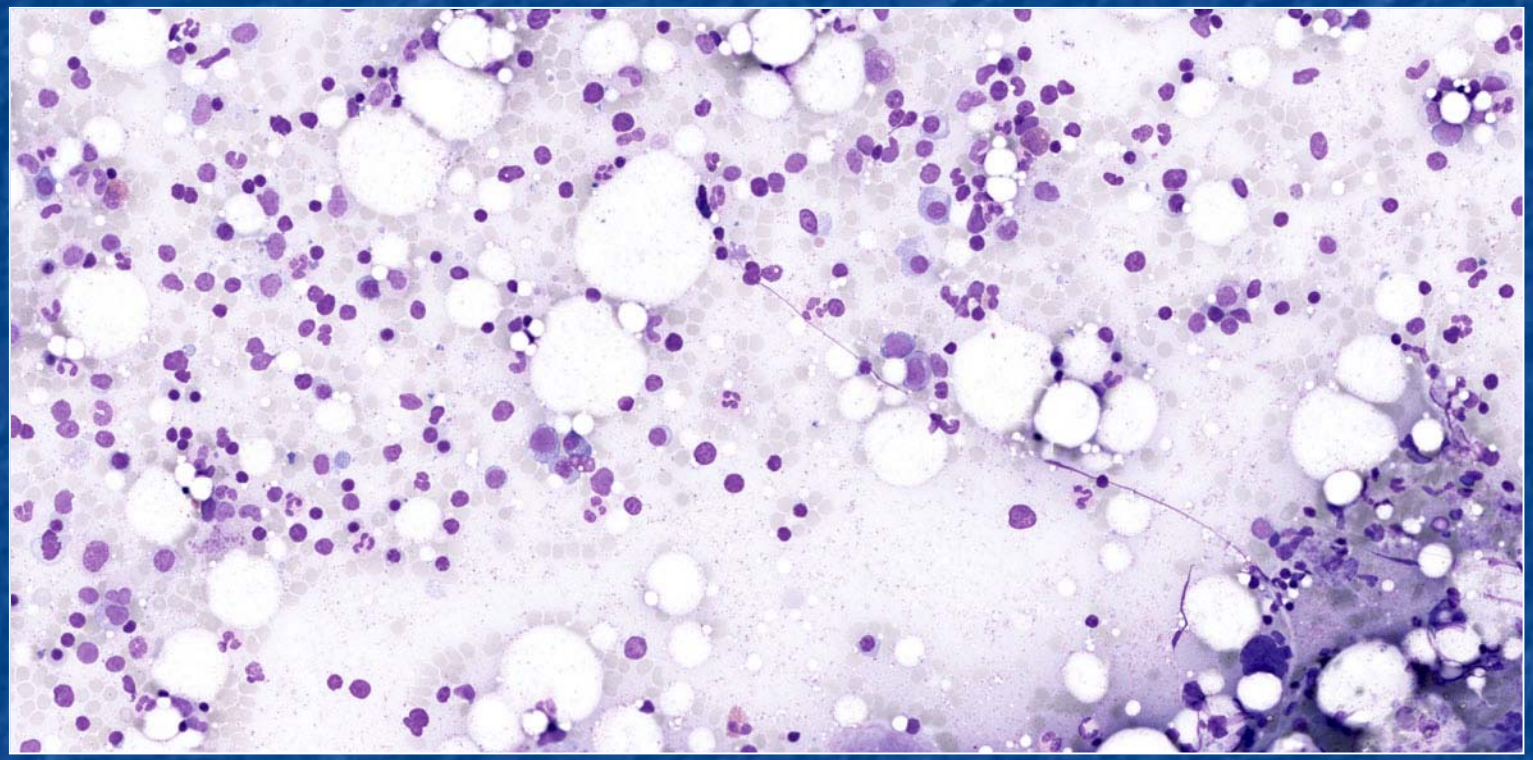
Virtual slide, Mirax scan (image scanned with 20x objective), blood smear, higher Power view



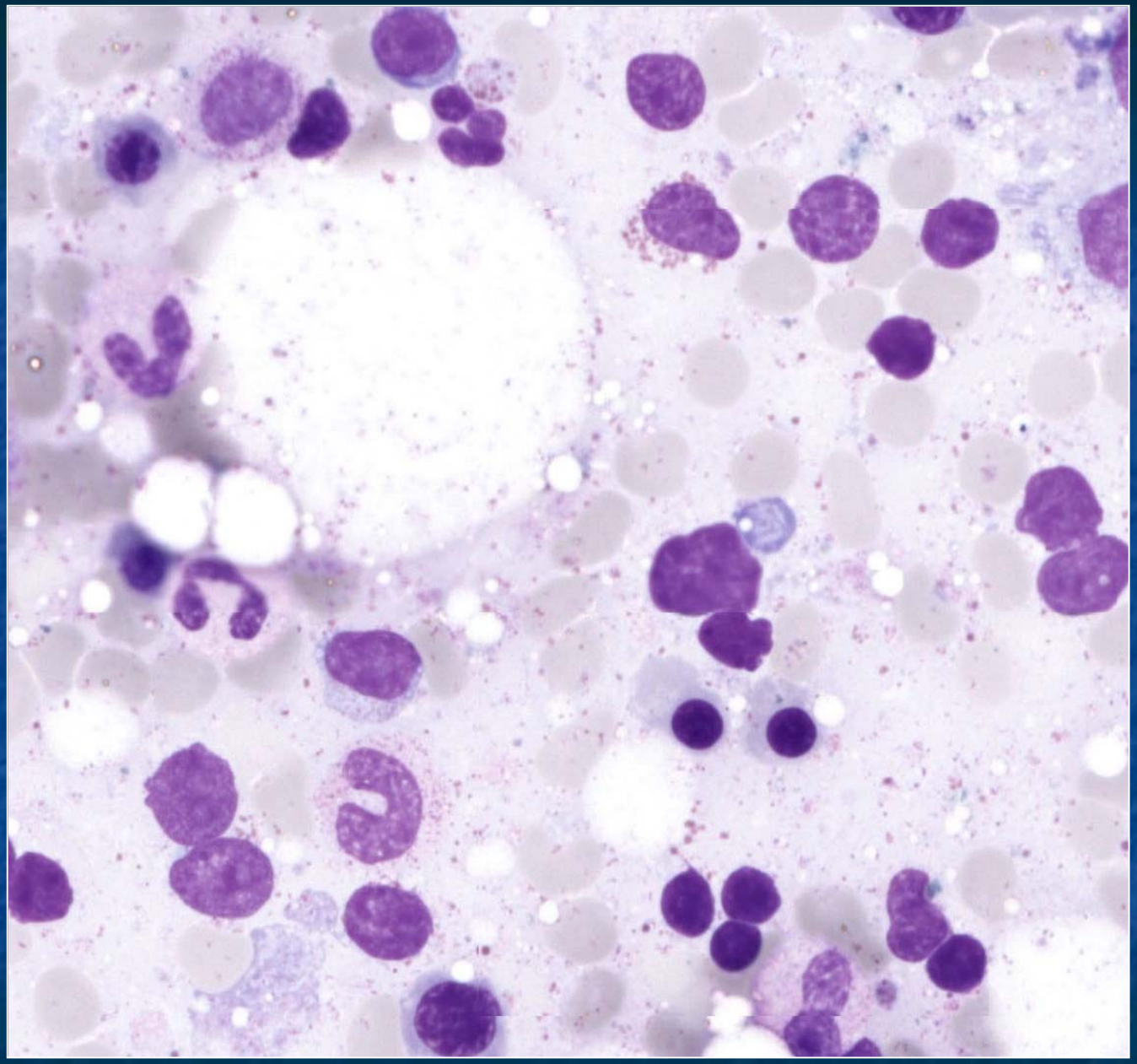
Virtual slide. Mirax scan, blood smear (20x objective), highest possible resolution but not sufficient for the detection of fine granular intracellular components



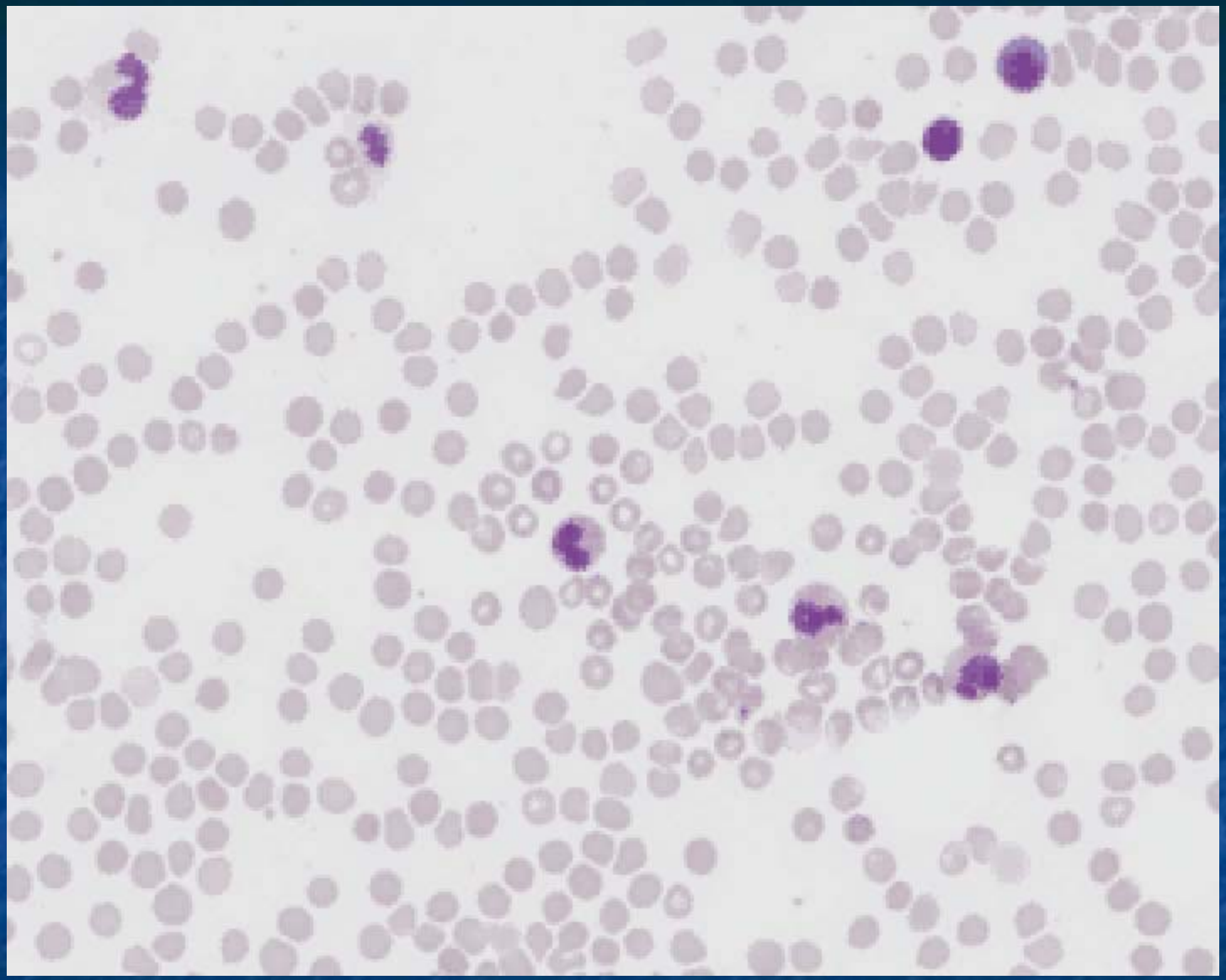
Bone marrow smear (Axioplan 2 image) with Axiovision software 100x, 104 tiles
Quality is rather low by the high compression of the video file (204 MB)



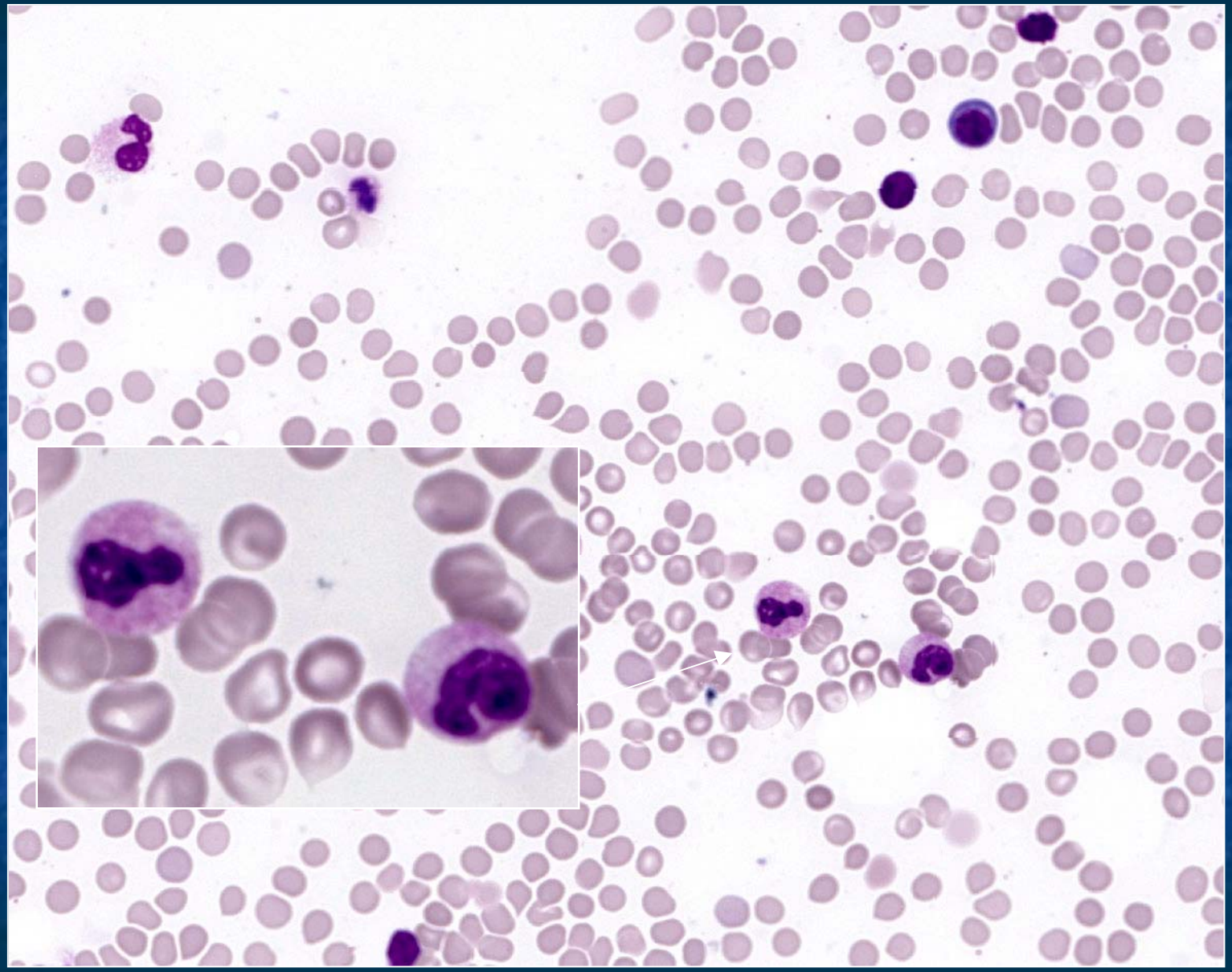
Virtual slide from the same image as in the previous slide. Low power view, 100x objective, 104 tiles



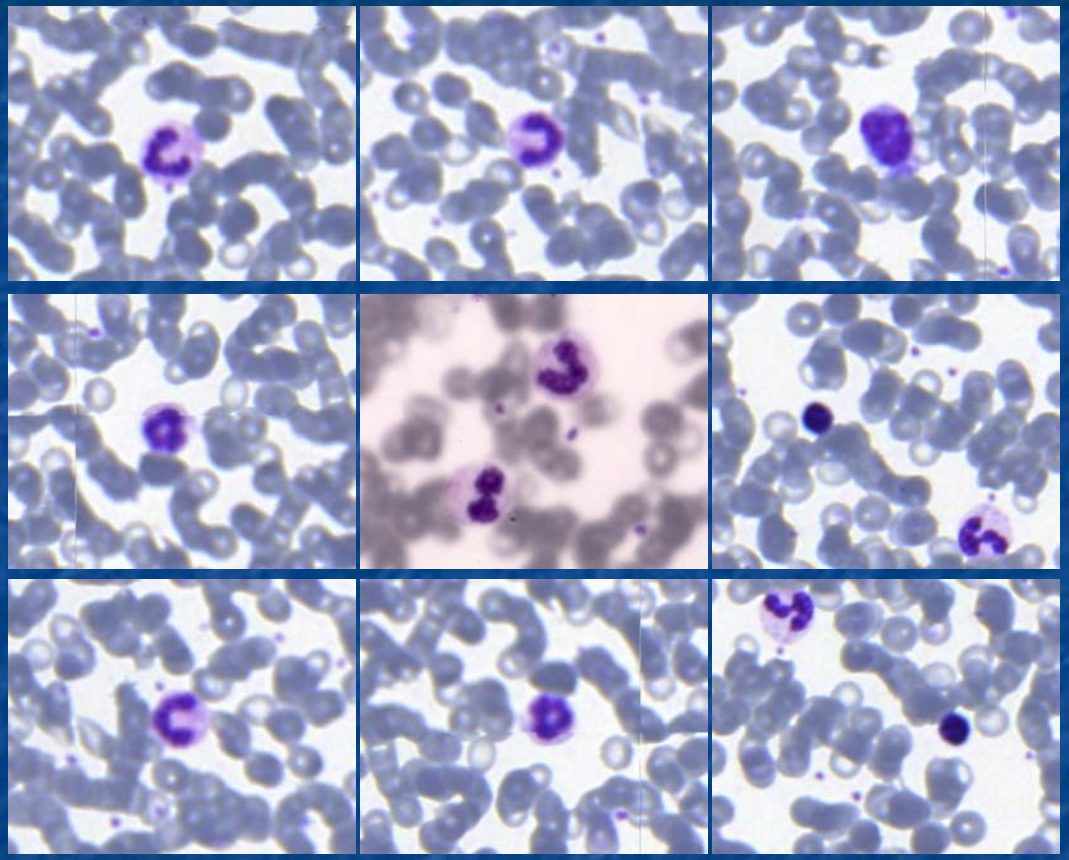
Same at higher magnification



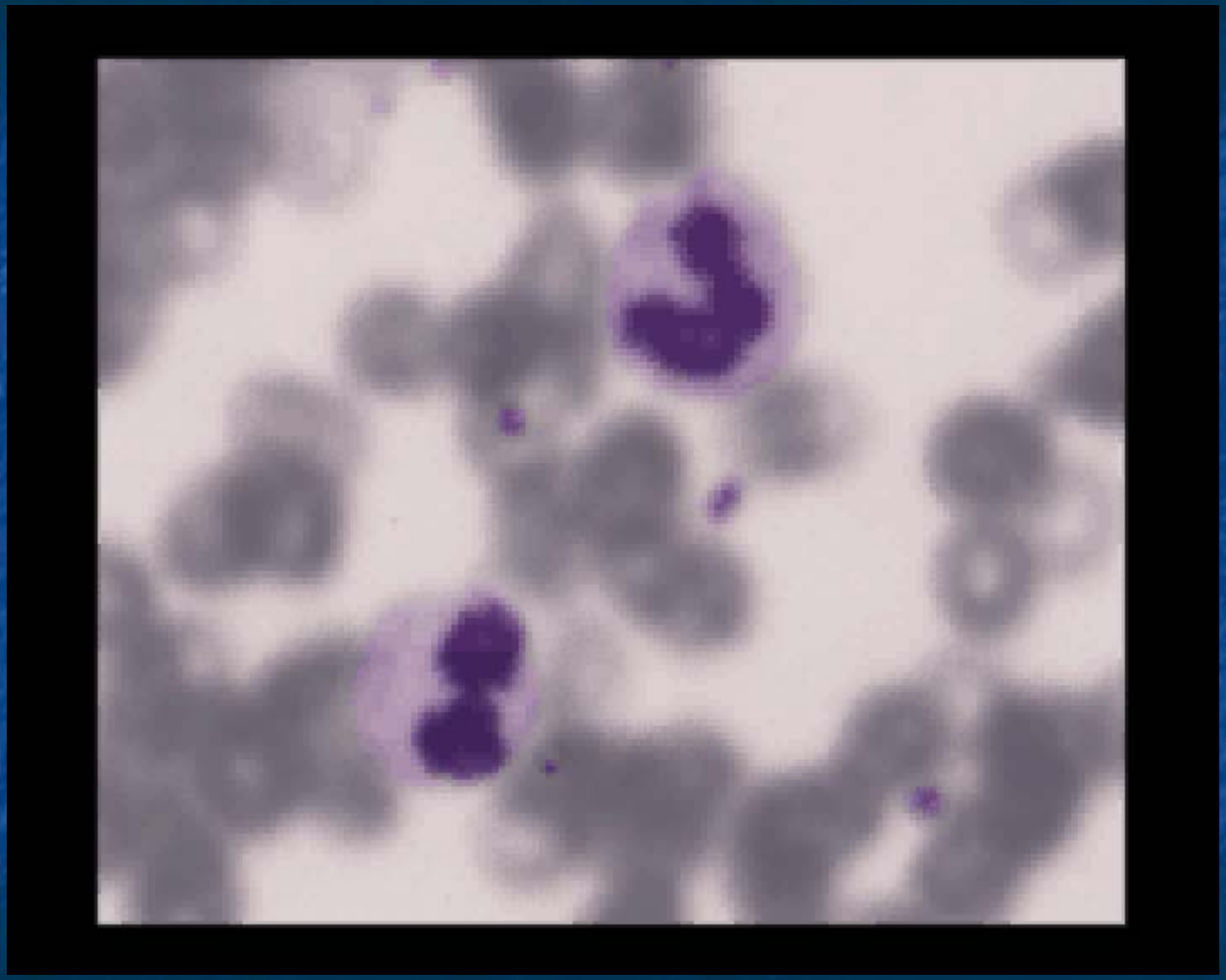
Blood smear. Images stitched with the MosaiX module of the Axiovision Software, 25 tiles, 100x objective
The AVI image quality is rather low by the high compression of the video file



Overview and high power view (insert). Not all cells are perfectly focused. Therefore Z-stacks are used

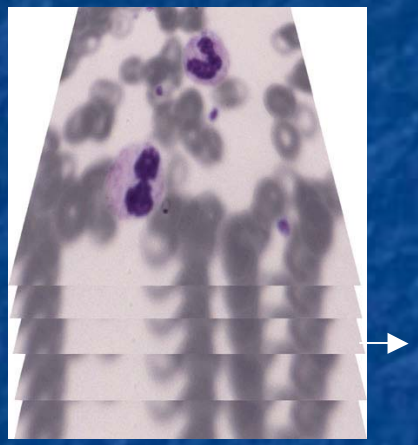


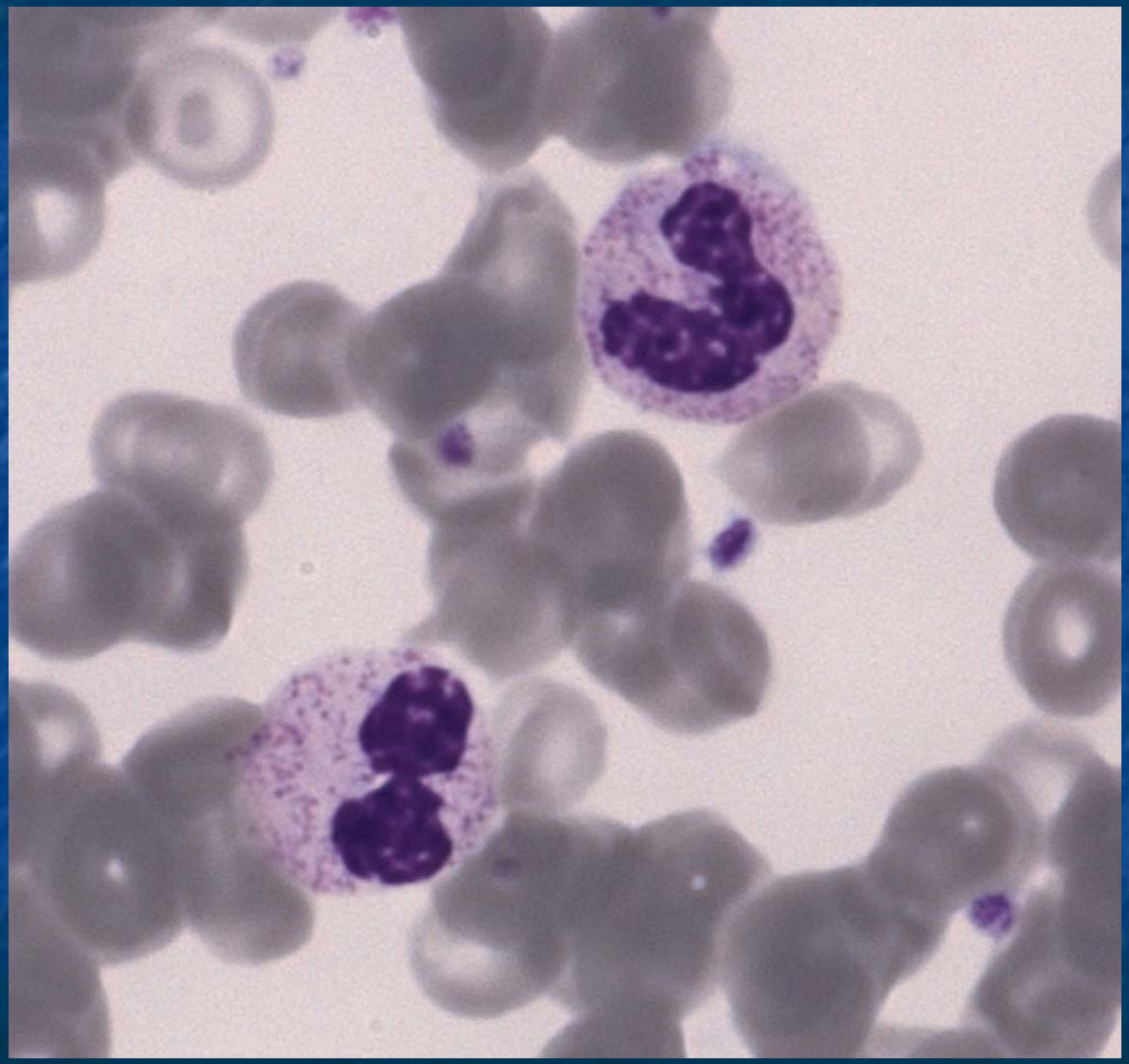
Series of images of a blood smear. The central image is a Z-stack



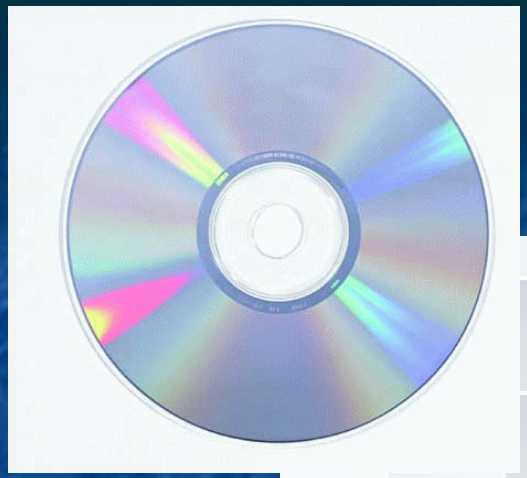
Effect of focusing: Z-stack of 20 images, Z-step: 0.175 µm

Z-stack of images





Original high quality image of previous Z-stack



Our experience

Evaluation externe de la qualité
en hématologie : frottis sanguin

FR

NL

Externe kwaliteitsevaluatie
hematologie : bloeduitstrijkje

Institut Scientifique
de Santé Publique

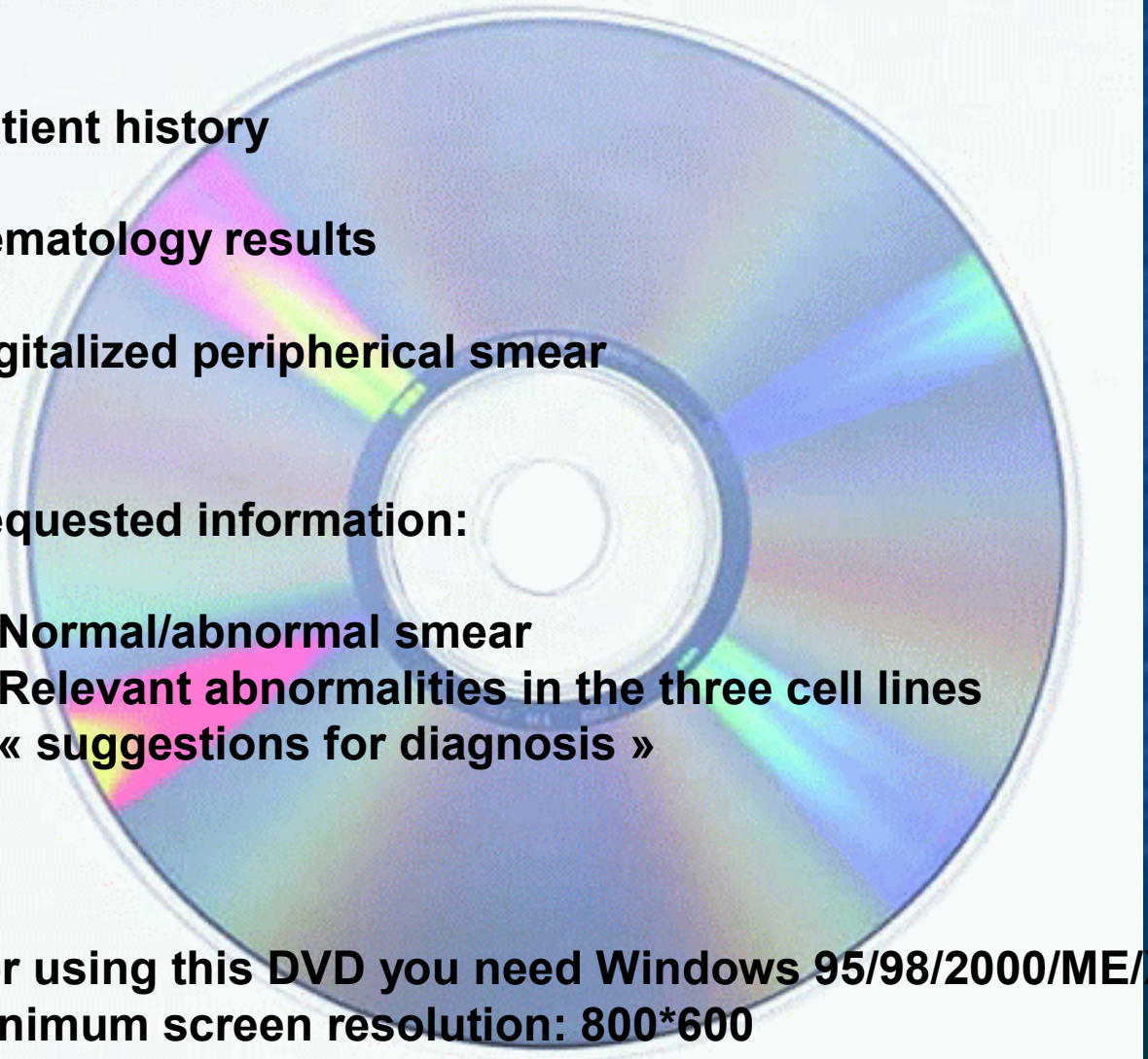
Wetenschappelijk Instituut
Volksgezondheid

Quitter
Afsluiten

Future developments

- Technological improvement will continue
- Cytology applications become possible by new scan technologies and software for Z-stacks stitching
- New possibilities for smart EQA applications

Digitalized EQA



Patient history

Hematology results

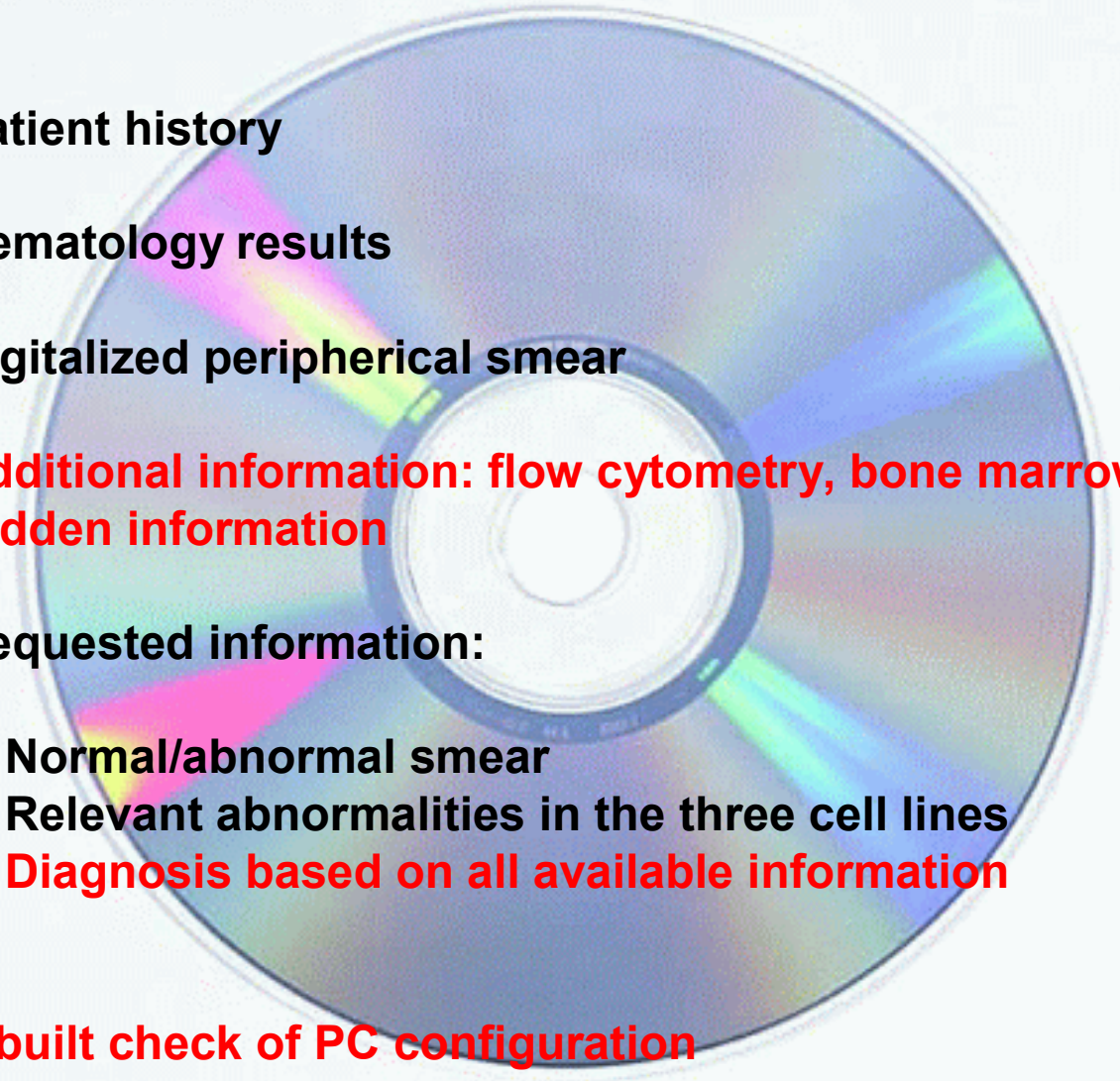
Digitalized peripherical smear

Requested information:

- **Normal/abnormal smear**
- **Relevant abnormalities in the three cell lines**
- **« suggestions for diagnosis »**

For using this DVD you need Windows 95/98/2000/ME/XP/NT
Minimum screen resolution: 800*600
Min processor speed 133 Mhz

Digitalized « Smart »EQA (1)



Patient history

Hematology results

Digitalized peripherical smear

Additional information: flow cytometry, bone marrow,…
Hidden information

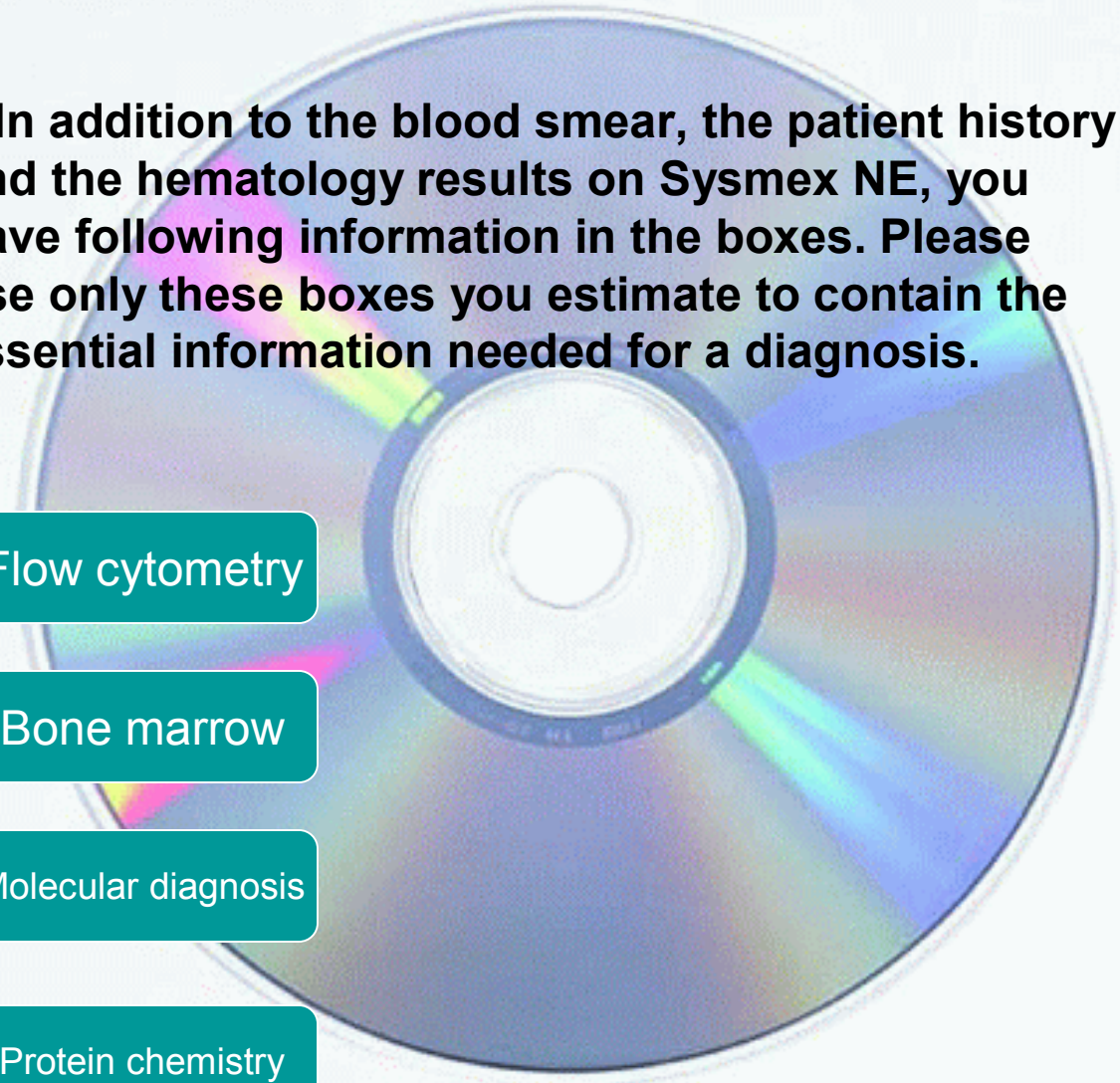
Requested information:

- **Normal/abnormal smear**
- **Relevant abnormalities in the three cell lines**
- **Diagnosis based on all available information**

Inbuilt check of PC configuration

Digitalized « Smart » EBLM EQA (2)

« In addition to the blood smear, the patient history and the hematology results on Sysmex NE, you have following information in the boxes. Please use only these boxes you estimate to contain the essential information needed for a diagnosis.



- Flow cytometry
- Bone marrow
- Molecular diagnosis
- Protein chemistry

EQA organizer

Logging who has opened which box

WBC differentiation in smear H 3456

Neutrofiele segmentkernigen	55	55 %
Neutrofiele staafkernigen	6	0 %
Eosinofiele segmentkernigen	3	3 %
Basofiele segmentkernigen	2	2 %
Lymfocyten	26	26 %
Reactionele lymfocyten	0	0 %
Monocyten	8	8 %
Promyelocyten	0	0 %
Neutrofiele myelocyten	0	0 %
Neutrofiele metamyelocyten	0	0 %
Blasten	0	0 %
Lymfomateuze cellen	0	0 %
Andere cellen	0	0 %
Erytroblasten	0	0 %
TOTAAL	100	100 %

details

Lymphocytes:

A4c, A4d, A7a, B2e, ……;

Monocytes:

A6e, D2f,…

Every cell can be checked by the EQAS organizer

Current format of reporting

- Sample 3652: The correct answer for this smear is « presence of *Plasmodium vivax* ». Following development stades could be detected:

trophozoites
Immature schizonts (rare)
Mature schizonts (rare)
gametocytes

New format of reporting

- Sample 3652: The correct answer for this smear is presence of *Plasmodium vivax*. Following development stades could be detected:

trophozoites	Positions:xxx/yyyy
Immature schizonts (rare)	Positions:xxx/yyyy *** Some participants have difficulties to distinguish mature and immature schizonts**
Mature schizonts (rare)	Positions:xxx/yyyy
gametocytes	Positions:xxx/yyyy

Current format of reporting

Sperm morphology

	Your results	All participants
Sample 421		mean
% Normal	22	6
% Head defects (H)	64	90
% Nec/Mid piece defects (M)	44	33
% Tail defects (T)	6	12
% Cytoplasmic droplets (C)	8	3
Overall impression:	NORMAL	ABNORMAL

New format of reporting

Sperm morphology

Sample 421: details of your results

Cell coord	Normal (N)	Abnormal (H,M,T,C)	Consensus
x1y1	N		H, T
x2y2		H	H
……			

Summary

Number of normal cells in consensus: ….
% of normal cells in consensus: ….

Number of abnormal cells in consensus: ….
% of abnormal cells in consensus: ….

Conclusions

- New EQA applications, not possible until now will be available very soon
- Smart applications of existing EQA schemes are possible
- Enforced EQA on relevant clinical information
- New training and education possibilities