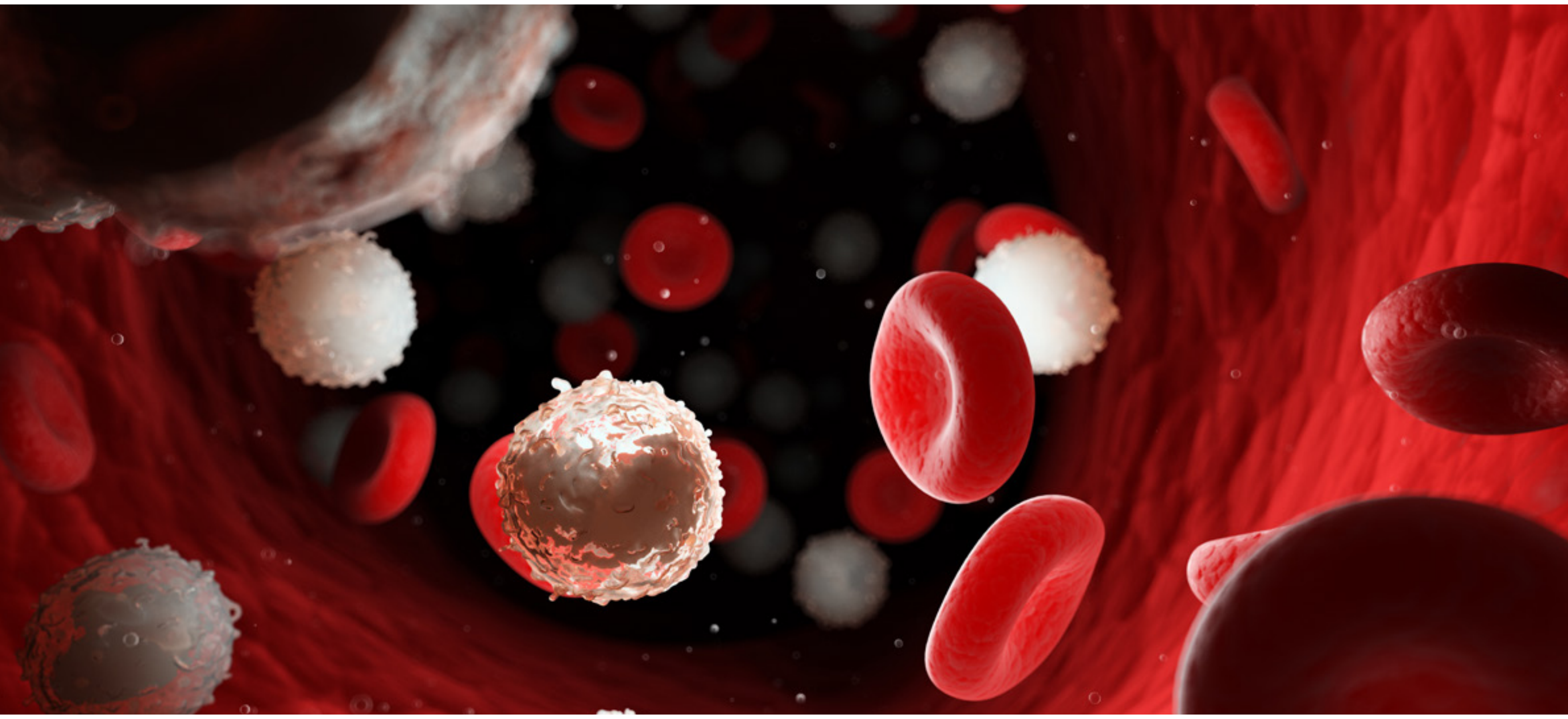
Quick Guide

Microscopy in Hematology



Seeing beyond

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Talking about hematology is talking about blood, blood-forming organs and blood diseases. In clinical laboratories, hematologists diagnose and treat blood disorders and malignancies, including types of hemophilia, leukemia, lymphoma and sickle-cell anemia.
To do so, hematologists routinely investigate peripheral blood smears on glass slides with a microscope to find any abnormalities indicating such hematological diseases or to look for blood parasites, such as those found for malaria and filariasis.

Blood – circulation and blood cells

What is blood?

Blood is a body fluid that performs a variety of transport and regulatory functions. The heart pumps it constantly around the body (fig. 1) of humans and animals providing the cells with necessary substances such as nutrients and oxygen. It also carries carbon dioxide and other waste materials to the lungs, kidneys, and digestive system to be removed from the body. Blood fights infections and carries hormones around the body.

Blood circulation

The circulatory system is made up of two types of blood vessels:

Figure 1 *The circulatory system, courtesy of: CC BY-SA 2.5**

- Arteries carry oxygenated blood from the heart to the rest of the body.
- Veins transport the blood back to the heart and lungs, so it can get more oxygen to send back to the body via the arteries.

How much blood is circulating through the body depends on a person's size. The larger a person is, the more blood volume they have. An average heavy, healthy, adult person has a blood volume of about eight percent of his or her body weight. A person weighing about 70 kilograms therefore has about five to six liters of blood.

The color of blood

Blood in the human body is red, the shade of red may vary. The red color itself comes from the hemoglobin. This is a protein molecule in the red blood cells. Hemoglobin contains iron, which reacts with oxygen, giving blood its typical red color. The level or amount of oxygen in the blood determines the shade of red. As blood leaves the heart and is oxygen-rich, it is bright red. When the blood returns to the heart, it has less oxygen and is darker.

Blood Components and their function

Blood is made up of solid components, the blood cells and a liquid intercellular substance, blood plasma.

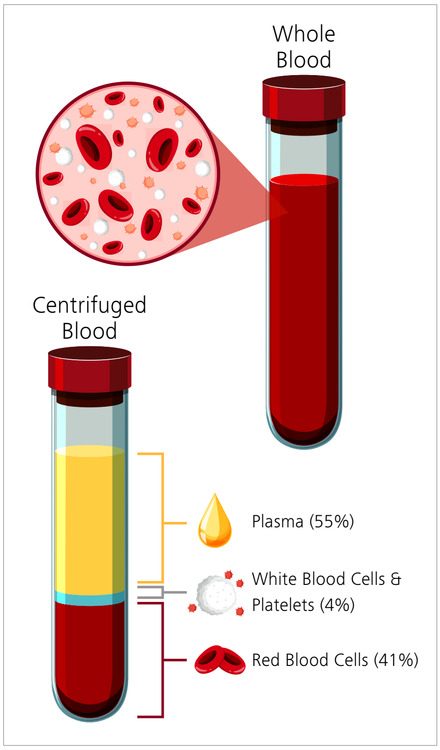

Figure 2 *Composition of blood*

Fun fact – most animals also have red blood. However, there are exceptions:

- Some types of octopus, squid, and crustaceans have blue blood due to a high concentration of copper. When copper reacts with oxygen, it gives their blood its blue color.
- The skink, which is a type of lizard, has green blood due to biliverdin, which is a by-product of the liver.

* *https://commons.wikimedia.org/w/index.php?curid=741255*

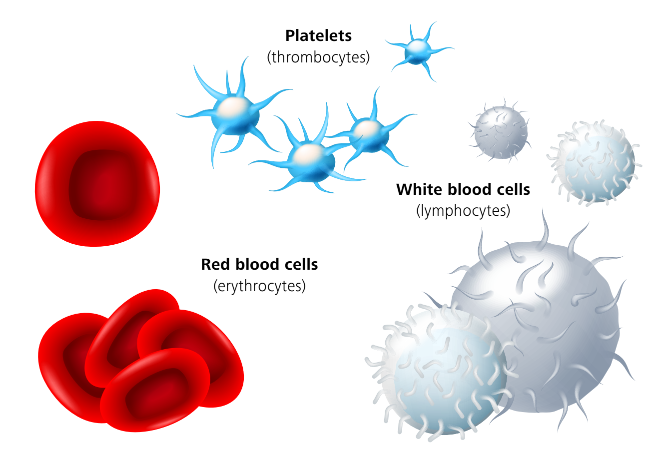

Figure 3 *Blood cells*

The yellowish plasma makes up about half of the content of blood. Composed of 92% water, it also contains nutrients such as glucose, proteins helping blood to clot, hormones, and waste products.

The other half of blood volume is composed of blood cells. There are mainly three types of blood cells.

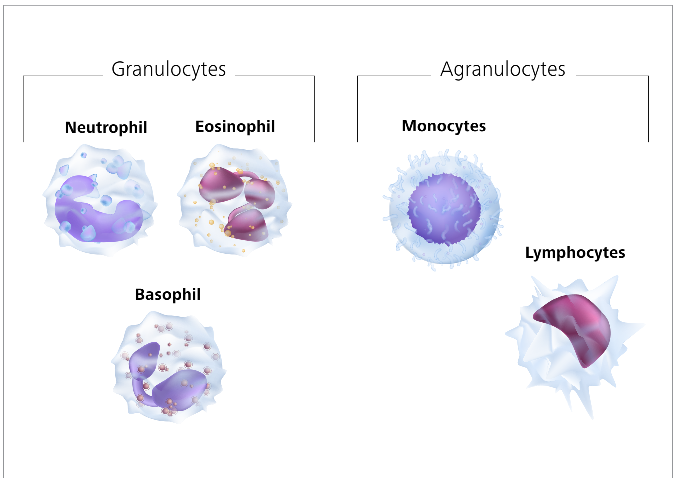

Figure 4 *White blood cell*

What are blood types?

Even though everyone's blood contains the same elements, not everyone's blood is alike. There are different blood types based on the absence or presence of specific antigens and antibodies on the surface of the red blood cells.

There are eight blood types, described using the letters A, B, and O. People with the blood type A have an A-antigen on their red blood cells, people with type B blood have a B-antigen. Some people have both. People with O blood type do not have either A or B antigens on the red blood cells. Type O is the most common blood type in the world. Besides getting a letter or two, a person's blood is either "positive" or "negative." This is a way of keeping track of whether someone's blood has a protein called Rh protein.

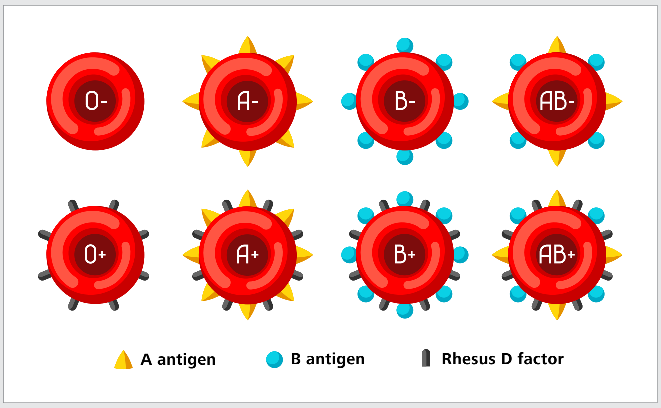

Figure 5 *Blood types*

If your blood is positive, you have this protein. If it's negative, you don't. It is more common to have Rh-positive blood than Rh-negative. Either way is totally fine.

Red blood cells (erythrocytes)	White blood cells (leukocytes)	Platelets (thrombocytes)
Diameter: 7.5 µm	Diameter: 7 – 20 µm	Diameter: 2 – 3 µm
Formed in the bone marrow	Formed in the bone marrow or spleen, thymus, and lymph nodes	
Contain the protein hemoglobin that carries oxygen from the lungs into the organs and tissues and carbon dioxide from the tissues into the lungs. Hemoglobin is responsible for the red color.	Part of the immune system, which helps the body defend itself against infection. Occur in various types including granulocytes, monocytes, and lymphocytes. Different types fight different germs such as bacteria, viruses.	When a blood vessel breaks, platelets gather in the area and help seal off the leak. Platelets work with proteins called clotting factors to control bleeding inside our bodies and on our skin.
Require about 9 days to develop and have a life span of about 4 months	Depending on the type of leukocyte, life span can vary from a few days to years.	Have a life span of about 7 to 9 days.
Insufficient number of red blood cells can cause paleness, fatigue, shortness of breath, and other symptoms. This is referred to as anemia.	Depending on the type of leukocyte, a distinction is made between non-specific (phagocytosis) and specific (formation of antibodies) immunity.	Thrombocytes are responsible for blood coagulation and haemostasis.

Table 1

Microscopic Blood Examination

Role of microscopy

Laboratory blood analysis is one of the most important routine diagnostic procedures in a clinical lab. Hematologists routinely investigate peripheral blood smears on glass slides with a microscope to find abnormalities in morphological characteristics of cells and tissues indicating hematological diseases or to look for blood parasites, such as those found for malaria and filariasis. A microscopic image can deliver information on cell types based on their morphology, about the quantity and composition of blood cells. Light microscopy with a magnification of up to 1,000× is employed to recognize and count the various cell types within a monolayer and document the results with a digital camera. This way, many types of blood diseases or the developmental stages of parasites can be visualized. In certain cases, the microscopic examination of peripheral blood smears is complemented with a bone marrow examination. Key microscopy techniques include brightfield, darkfield, DIC, fluorescence, immunocytochemistry and immunohistochemistry.

Blood smear

A blood smear is used to look for abnormalities in blood cells, be it their morphology or quantity. It can help to detect, diagnose, and monitor deficiencies, diseases, and disorders involving blood cell production, function, and lifespan. Normally a microscopic analysis of a thin blood smear is done when the complete blood count or the differential white blood count deliver abnormal results. [1]

1. Place a clean glass slide on a flat surface. Add a small drop of blood from, for example, the fingertip.
2. Place the cover slip in an angle of about 30 – 45°.
3. Gently wipe the edge of the coverslip over the blood and produce a smear.
4. The smear is air-dried, fixed to the slide with methanol, and stained to distinguish the various cell types.

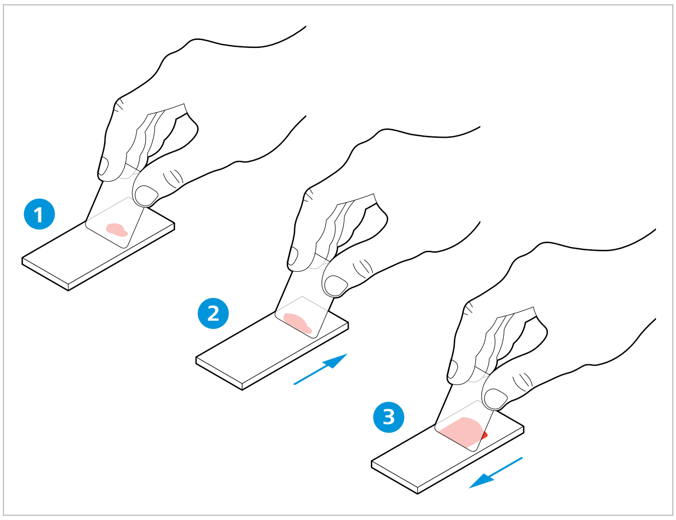

Figure 6 *Preparation of a blood smear*

It is imperative to use the proper technique when preparing the smear. If it is poorly made the stained smear will have no value. If the spreader is moved too slowly or the angle used is too small, a thick blood film will be produced, and it will not be readable. If the spreader is moved too rapidly or the angle is too large, a thin film will be produced, resulting in many of the leukocytes becoming aggregated at the feathered edge. Other causes of poor blood smears are Hemolysis of cells due to moist fingers, scratches or holes.

Bone marrow examination

In situations where a blood test is abnormal or does not provide enough information about the suspected problem, a bone marrow examination can be required. It is used to diagnose and monitor certain diseases of the blood or the blood building system. Fragments from the bone marrow are typically separated or concentrated and then aspirated on several glass slides. These fragments are squeezed gently to generate the smears. Complementary, bone marrow blood smears are prepared.

Figure 7 *Representative image of a Auer rods visible in a bone marrow sample ZEISS Axioscope 5*

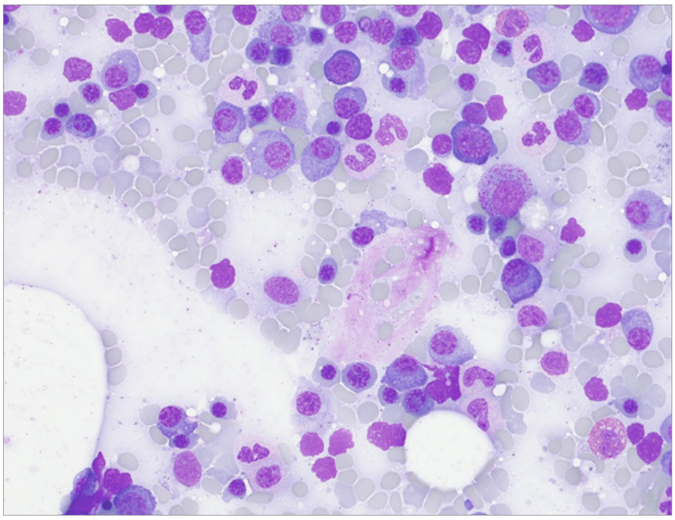

Figure 8 *Representative image of bone marrow in brightfield, ZEISS Axioscope 5, ZEISS Axiocam 208 color, 40× objective*

Staining methods in hematology

In hematology most staining methods are based on panoptic stains according to Pappenheim and Romanowski stains such as Wright's stain, Leishman stain, or Giemsa stain. These allow for the detection of erythrocyte, leukocyte or thrombocyte abnormalities. It is imperative that the slides are dried completely before any staining. Otherwise there is the risk of denaturation of the white blood cells, which can hamper a proper differentiation. The drying should be completed as soon as possible after the blood smear is produced.

Blood components	Coloration
Erythrocytes	Pink
Nuclei of leukocytes and nucleated erythrocytes	Reddish purple
Eosinophilic granules	Brick red to reddish brown
Basophilic granules	Dark purple to black
Neutrophilic granules	Light purple
Lymphocyte cytoplasm	Light blue
Monocyte plasma	Gray-blue

Table 2

Pappenheim staining*

Artur Pappenheim (1870 – 1916) was a german hematologist. The Pappenheim or May-Grünwald-Giemsa staining is a panoptic differential staining, which makes use of the behaviour of the Giemsa and May-Grünwald staining. It depicts all microscopic phenomena used in routine diagnostics, strongly and differently colored on a clean background, so that even fine deviations can be easily detected. In Pappenheim staining, the preparations are fixed with concentrated May-Grünwald solution, stained with diluted May-Grünwald solution and, after rinsing with Aqua destillata, counterstained with Giemsa solution. The cell nuclei appear reddish-purple in the sample, the plasma of lymphocytes and monocytes bluish, the plasma of granulocytes pale pink.

Giemsa staining*

Giemsa is a Romanowsky type stain, named after Gustav Giemsa, a german chemist. It is a differential stain, containing a mixture of Azure, Methylene blue, and Eosin dye. The stain is used to obtain differential white blood cell counts, to differentiate nuclear and cytoplasmic morphology of the various blood cells. It stains red blood cells pink, platelets pale pink, lymphocyte cytoplasm blue and leucocyte chromatin magenta. Giemsa can also be used to stain blood parasites such as malaria and other spirochete and protozoan microorganisms.

Wright's staining*

This technique is named for James Homer Wright, who modified the Romanowsky stain. For Wright's staining there are several variations in technique available because of different behavior of the stain and buffer and because of differences in film thickness. So, the choice of recipe is not as important as the consistency in the staining technique. It is classically a mixture of eosin (red) and methylene blue dyes and offers a coloration similar to the panoptic method. If performed correctly, the red blood cells should look yellowish-red and the neutrophils should have dark purple nuclei, reddish-lilac granules, and pale pink cytoplasm. This method is widely used to perform differential white blood cell counts, which are routinely ordered when conditions such as infection or certain diseases are suspected.

Application Examples

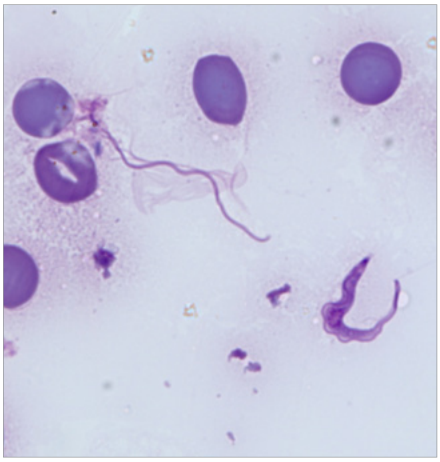

Figure 9 *Representative image of* Trypanosoma brucei gambiense *parasite in human blood smear. Giemsa staining.*

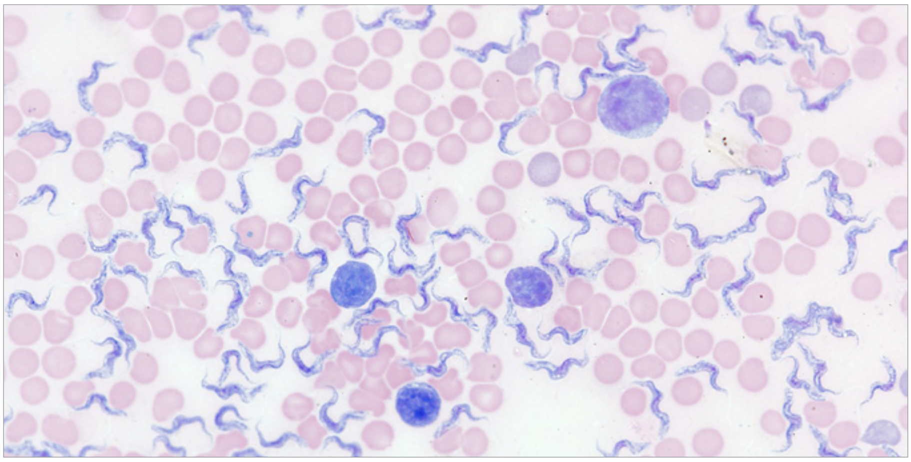

Figure 10 *Representative image of blood smear, Giemsa staining*

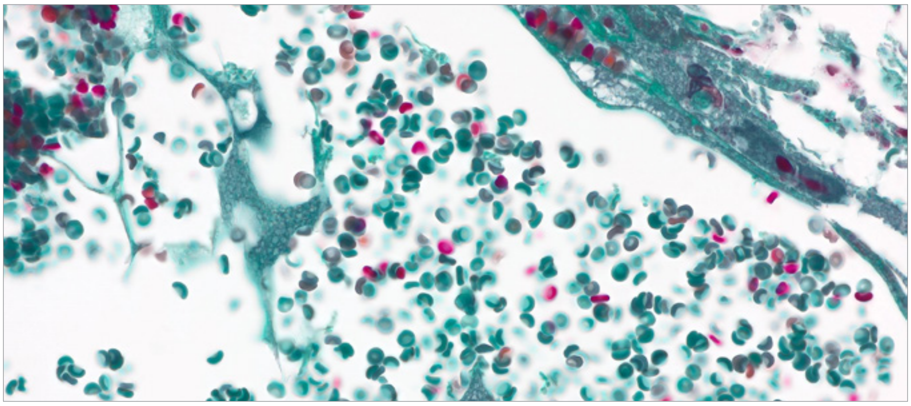

Figure 11 *Representative image of blood vessels*

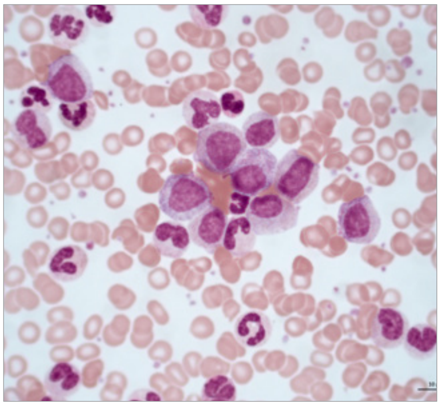

Figure 12 *Representative image of blood with leucocytes*

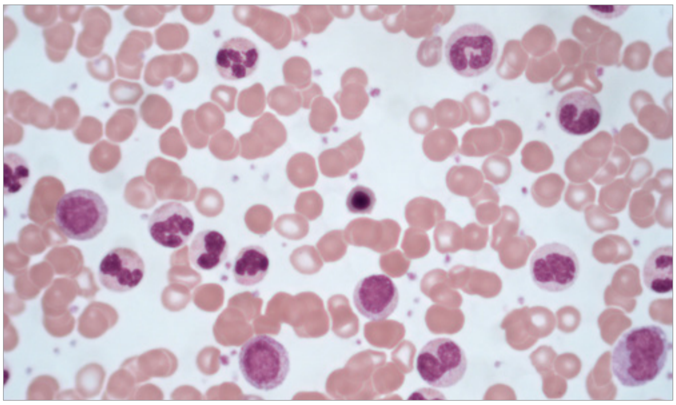

Figure 13 *Representative image of polychromatic erythrocytes, abnormal as this should be part of the bone marrow and not the blood*

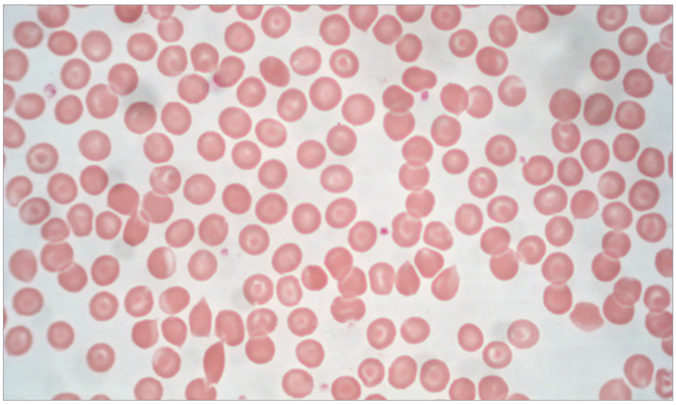

Figure 14 *Representative image of erythrocytes*

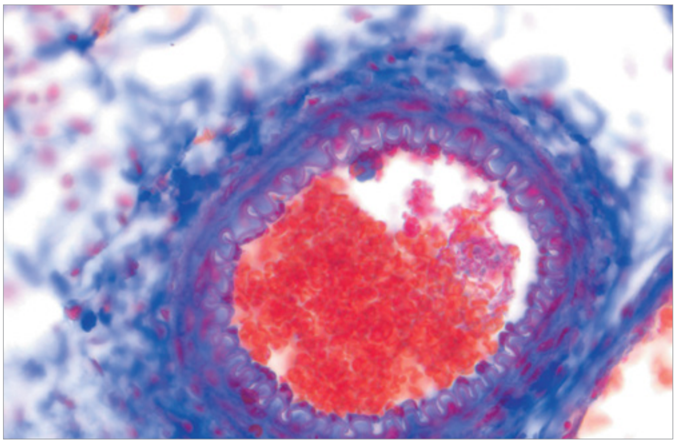

Figure 15 *Representative image of blood vessel. Azan stain; Orange: cytoplasm, Red: nuclei, Blue: collagen, Plan-Apochromat 20×/0.8*

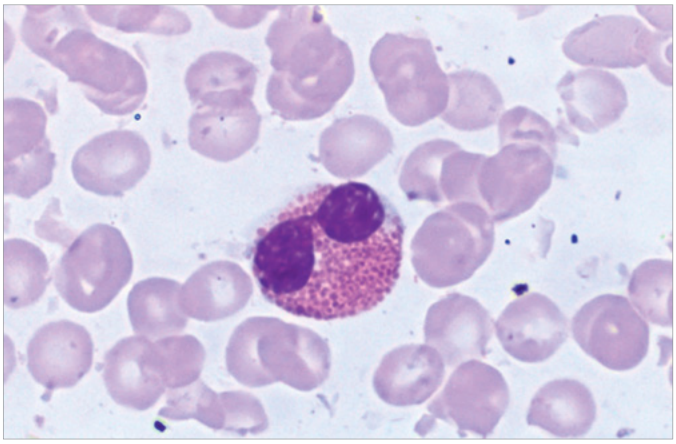

Figure 16 *Representative image of uncovered blood smear (human). Wright stain, Achroplan 100×/1.25 Oil*

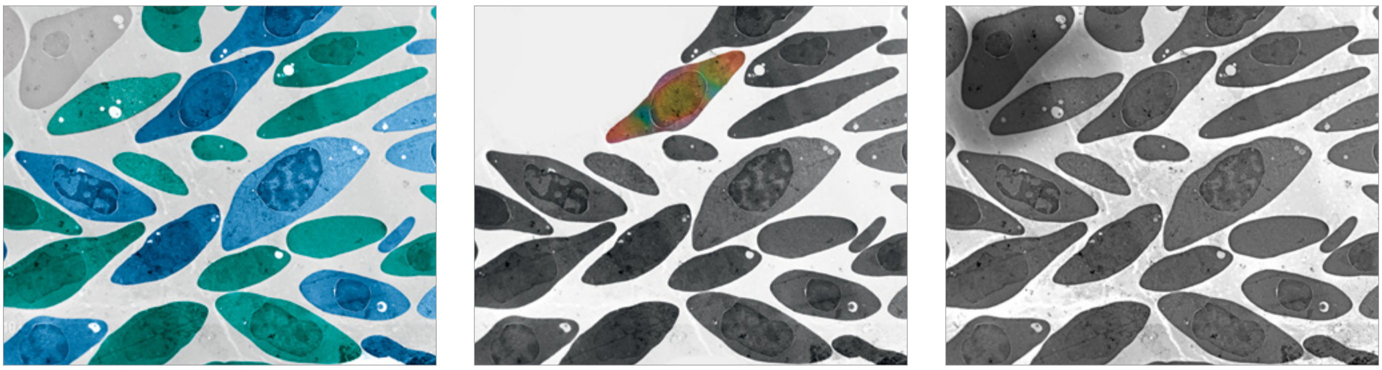

Figure 17 *Representative images of blood cells shown in a STEM image taken with the EVO® STEM detector at 20 kV.*

Suitable Microscope Equipment

When examining a peripheral blood smear, a hematologist starts with a low magnification objective, typically a 20× or 10×. The hematologist gets an overview of red and white blood cell densities, the number of erythrocytes, its color and rough morphology and if obvious cellular inclusions are present. In higher magnification (typically 60× or 100×, sometimes 40× for bone marrow), a manual differential cell count is performed and red and white blood cell morphology including the presence of inclusions or pathogens are visualized. A very good differentiation of cell types and clearly visible cellular details are absolute prerequisites in hematology. Hematologists rely on crystal-clear images to visualize morphological details such as delicate granules, rod-shaped inclusions, irregularities of the nuclear membrane or clefted nuclei. Furthermore, highest color fidelity is key when examining blood smears and bone marrow preparations. Apart from brightfield microscopy, phase contrast and polarizing microscopy techniques are also used for certain types of samples. While hematological stains* result in a good transparency of the sample and specific staining of cellular features, it is the optical quality of the microscope, the fidelity of the attached camera for digital documentation, and the ergonomic design of the instrument that can make all the difference when examining patient samples.

ZEISS Axiolab 5

Axiolab 5 is made for the clinical routine work that goes on every day in your hematology lab. The white LED illumination with very high color rendering index is ideal to visualize peripheral blood smears and bone marrow samples in true color.

The constant color temperature of the LED facilitates system operation and digital documentation. Combine Axiolab 5 with the microscope camera Axiocam 208 color and take full advantage of the smart microscopy concept: you'll be experiencing a completely new form of digital documentation in your daily clinical routine. Just focus your sample and press a single button for crisp images in true color. The 4k live image will look exactly like you see it through the eyepieces, with all the details and subtle color differences clearly visible. Manual camera parameter adjustments are not necessary which saves time. Discussions of critical cases with a colleague can now be done with ease using the high resolution live image.

ZEISS Axioscope 5

Axioscope 5 with Axiocam 208 color makes documenting your hematology samples very efficient. Plan Neofluar objectives provide the necessary visualization of subtle color nuances, even for challenging samples such as bone marrow smears. The color impression in the camera image shows up exactly the same as it appears through the eyepieces. This smart microscope makes automatic adjustments for brightness and white balance to keep digital documentation tasks in your clinical routine easy.

All you have to do is focus on your sample, press the ergonomic Snap button on the microscope, and that's it. To store images with the respective patient ID the camera can be integrated with laboratory information systems that support Twain.

* The user is responsible for correct sample preparation and application of staining methods.

References

[1] https://www.cdc.gov/dpdx/diagnosticprocedures/blood/specimenproc.html

Related White Papers

[1] https://p.widencdn.net/rg433b/EN_wp_microscopic-examination-blood

[2] https://p.widencdn.net/8azb8g/EN_wp_Axio-Lab-A1-Malaria

[3] https://p.widencdn.net/zuwbem/EN_wp_EVO_python-blood-analysis

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