Darkfield Microscopy Applied to Live Blood Analysis

In researching the subject of live blood analysis with darkfield microscopy and its long relationship to microbiology, I was appalled at the misinformation and false claims posted on the Internet. Some websites completely discounted any clinical value at all to viewing live blood in darkfield and its unique ability to view microorganisms, outright denying evidence-based studies and decades of research; whereas others made inaccurate and false claims beyond its diagnostic limitations. It is obvious that analyzing live blood in darkfield has become a very controversial subject that has medical politics (monetary interest) at its core. Of course, microscopy is the cornerstone diagnostic of microbiology as taught at any reputable medical or biological institution. In the U.S., optical microscopes are primarily utilized in the arenas of research, chemistry, forensic science, and in government-sanctioned CLIA laboratories (Clinical Laboratory Improvement Amendments). Optical microscopy has been reduced in clinical medicine to some specialized practices such as dentistry (viewing plaque) and fertility (sperm counting and allowing gametes and embryos to be visualized) and certain surgical operations. In medicine, optical microscopes used for hematology have become the strictly sanctioned domain of laboratories and generally are no longer utilized by clinicians to view patient's blood. There are still some European doctors who clinically utilize optical microscopy in viewing live blood, but very few.

I have written this monograph as an attempt to illuminate, separate the evidence-based research from biased opinions and false claims, and to help users better understand the history of light microscopy and some basic aspects of live blood microscopy as viewed in the optical mode of darkfield. Additionally, technical aspects of darkfield microscopes and basic information on live blood analysis has been included. Information is based on review of scientific research data and historical practice patterns.

This information should not be interpreted as specific medical advice and is only presented herein as a very basic introduction on this subject. Users should consult with a qualified healthcare provider for specific questions regarding therapies, diagnosis and/or health conditions, prior to making therapeutic decisions.

History of Darkfield Microscopy and its Relationship to Microbiology

Optical microscopy has been a major tool for biological and biomedical research since 1595, when Zacharias Jansen (ca. 1580–1638) of Holland invented a compound light microscope. His simple microscope used two lenses, with the second lens further magnifying the image produced by the first. Antonie van Leeuwenhoek (1632–1724) is credited with bringing the microscope to the attention of biologists, even though simple magnifying lenses were already being produced in the 16th century.

Van Leeuwenhoek's home-made microscopes were simple microscopes, with a single, very small, yet strong lens. He observed what he called “animalcules” (what we call microbes, organisms that can be seen only with the aid of a microscope). Joseph Jackson Lister is credited as the creator of the first darkfield microscope in Germany in 1830. By 1909, the Bausch and Lomb Company was manufacturing darkfield microscopes to assist chemists studying colloidal reactions such as the setting of cement. Syphilis is what made darkfield popular. Fritz Richard Schaudinn discovered Treponema pallidum, the syphilis spirochete, in 1905 using darkfield microscopy. This further promoted the use of darkfield by the medical profession and subsequently by biologists in general. Darkfield microscopy is ideally used to illuminate unstained samples causing them to appear brightly lit against a dark background.

In the 1970s, numerous doctors began clinically using and teaching live blood analysis; i.e. Philip Hoekstra developed a live blood analysis in darkfield technique he termed Hemaview™ and Dr. Robert Bradford developed what he called High Resolution Blood Morphology. Both developed live blood analysis models more in line with orthodox pathology techniques to help alleviate technical issues, improve the validity of the technique, and so that information about the patient could be understood by all types of health practitioners.

Development of Light Microscopy and Darkfield

Over the centuries, optical microscopy developed into what is now compound light microscopes that can achieve magnifications up to 1200x and resolutions down to about 0.25 micrometers. That is, two objects in a cell can be as close as 0.25 micrometers and still detected as separate entities. Such resolution is good enough to see most bacteria and some mitochondria and microvilli.

The utility of light microscopy is governed by its use of visible light, which limits resolution. The shorter the wavelength of the illumination, the better the resolution. Electron beams have shorter wavelengths than photons. The invention of the electron microscope in the late 1930s and its refinement over the next half century permitted vastly improved visualization of cell and tissue fine structure.

Although other techniques such as electron microscopy offer significantly better spatial resolution, light microscopy still occupies a central role in biomedical science because of its ease of use and the potential for noninvasive, live cell imaging. Aside from hematology, numerous other biomedical technologies such as cell cloning and regeneration also need to visualize live cell and cellular mechanisms.

Most compound microscopes today have an illuminator built into the base. A condenser located below the stage has lenses that focus the light on the specimen and a diaphragm that regulates contrast. After passing through the specimen on the stage, the light enters an objective lens. Most light microscopes have three or four objective lenses on a rotating turret. These lenses magnify the image by 4x to 100x. The light then passes up the body tube to an ocular lens that magnifies the image another 10x to 15x. Research-grade microscopes and the better student microscopes have a pair of ocular lenses so that one can view the specimen with both eyes at once.

In order to get a usable image in the microscope, the specimen must be properly illuminated. The light path of the microscope must be correctly set up for each optical method and the components used for image generation. The condenser was invented to concentrate the light on the specimen in order to obtain a bright enough image to be useful. The optimum set-up for specimen illumination and image generation is known as Köhler illumination (after the man who invented it). It is used for most of the optical configurations listed below. The common microscope techniques requiring a transmitted light path include bright field, darkfield, phase contrast, polarization and differential interference contrast optics.

Additionally, many newer techniques have been developed to improve resolution such as near-field scanning optical microscopy, stimulated emission depletion microscopy, photoactivated localization microscopy, stochastic optical reconstruction microscopy, randomly adsorbed molecule microscopy, the “superlens", and “hyperlens", and structured illumination microscopy. However, few techniques other than darkfield microscopy have been developed for high contrast imaging. Darkfield microscopy is perfect for viewing high-contrast imaging objects that have low-contrast in bright-field microscopy.

Darkfield illumination is a technique in optical or light microscopy that eliminates scattered light from the sample image. It works by illuminating the sample with light that will not be collected by the objective lens, and thus will not form part of the image. As a result, the field around the specimen (i.e. where there is no specimen to scatter the beam) is generally dark but the objects that scatter the illumination beam are bright.

Thus, this yields an image with a dark background around the specimen and is the opposite of the brightfield illumination technique.

This is of great help when the objects have refractive indices very close to those of their surroundings and are difficult to image in conventional bright field microscopy. While darkfield microscopy can achieve high contrast imaging, its resolution may also be improved using a high numerical aperture configuration of the condenser/objective pair.

The primary imaging goal of the darkfield illumination technique is to enhance the contrast of an unstained sample, which is incredibly powerful, yet simple, for live blood analysis or other biological samples that have not gone through the staining process.

Darkfield microscopy is a simple technique used in many industrial applications and is particularly well suited for the following medical-related applications:

- Viewing live and unstained biological samples, such as blood, saliva, urine and feces (biological darkfield microscope is best combined with phase contrast).
- Viewing water-borne single-celled organisms; observation at low powers of pond water samples or soil infusions.
- Viewing organisms such as bacteria, yeast and protozoa (biological darkfield microscope is best combined with phase contrast)

The advantage with using darkfield illumination is that unstained specimens can remain alive and vital, whereas their brightfield counterparts must be stained or “treated” and are no longer active. Also, it is possible to acquire more qualitative results with this technique through live cellular analysis.

Darkfield microscopy is a technique not yet widely used that can be very useful in biological sample analysis, since it allows both the observation of live cells without an invasive sample preparation and the use of light scattering properties of cells. Under darkfield conditions, blood cells appear brighter than in other methods due to illumination by a light source at oblique angles, thus removing the need for a staining agent or a contrast agent to visualize more details in samples.

Technical Differences in Brightfield and Darkfield Microscopy

In contrast, brightfield microscopy uses light from the lamp source under the microscope stage to illuminate the specimen. This light is gathered in the condenser, then shaped into a cone where the apex is focused on the plane of the specimen. In order to view a specimen under a brightfield microscope, the light rays that pass through it must be

changed enough in order to interfere with each other (or contrast) and therefore, build an image. At times, a specimen will have a refractive index very similar to the surrounding medium between the microscope stage and the objective lens. When this happens, the image cannot be seen. In order to visualize these biological materials well, they must have a contrast caused by the proper refractive indices or be artificially stained. Since staining can kill specimens, there are times when darkfield microscopy is used instead.

In darkfield microscopy the condenser is designed to form a hollow cone of light (see illustration below), as opposed to brightfield microscopy that illuminates the sample with a full cone of light. In darkfield microscopy, the objective lens sits in the dark hollow of this cone and light travels around the objective lens but does not enter the cone-shaped area. The entire field of view appears dark when there is no sample on the microscope stage. However, when a sample is placed on the stage, it appears bright against a dark background. It is similar to back-lighting an object that may be the same color as the background it sits against - in order to make it stand out.

Immersion oil

In microscopy, more light = clear and crisp images. By placing a substance such as immersion oil with a refractive index equal to that of the glass slide in the space filled with air, more light is directed through the objective and a clearer image is observed. When light passes from a material of one refractive index to another (for example: from glass to air), it bends. In the space between the microscope objective lens and the slide (where air is), light is refracted, the light scatters and is lost. The refractive index of air is approximately 1.0, while the refractive index of glass is approximately 1.5. When light passes through both glass and air, it is refracted. Light of different wavelengths bends at different angles, so as objects are magnified more, images become less distinct.

Basically, when using lower magnification microscope objective lenses (4x, 10x, 40x) the light refraction is not usually noticeable. However, once you use the 100x objective lens, the light refraction when using a dry lens is noticeable. If you can reduce the amount of light refraction, more light passing through the microscope slide will be directed through the very narrow diameter of a higher power objective lens.

When light hits an object, rays are scattered in all azimuths or directions. The design of the darkfield microscope is such that it removes the dispersed light, or zeroth order, so that only the scattered beams hit the sample.

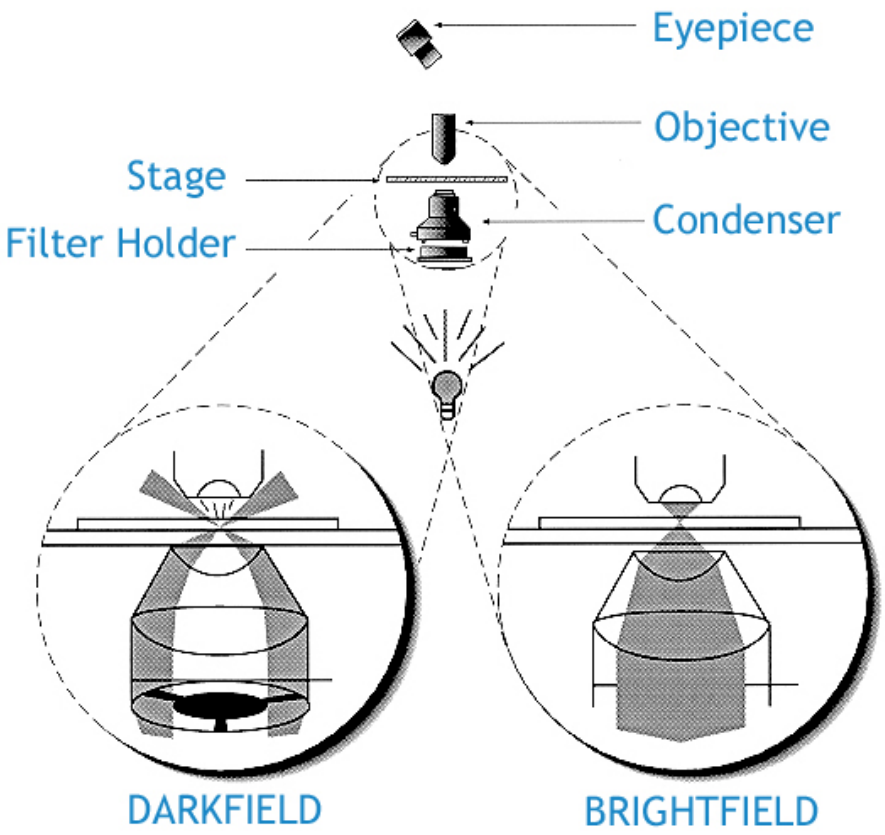


Illustration provided courtesy of Washington State University.

Darkfield is especially useful for viewing cells in suspension. Darkfield makes it easy to obtain the correct focal plane at low magnification for small, low contrast specimens.

Disadvantages of Darkfield Microscopy

A darkfield microscope can result in beautiful and amazing images; however, this technique also comes with several disadvantages.

First, darkfield images are prone to degradation, distortion and inaccuracies.

A specimen that is not thin enough or its density differs across the slide, may appear to have artifacts throughout the image.

The preparation and quality of the slides can grossly affect the contrast and accuracy of a darkfield image.

Essentially, the darkfield is only as good as the technique of the clinician.

Special care is needed to make sure that the slide, stage, nose and light source are free of small particles such as dust - as these will appear as part of the image.

Similarly, if you need to use oil on the condenser and/or slide, it is almost impossible to avoid all air bubbles. These liquid bubbles will cause images degradation, flare and distortion and even decrease the contrast and details of the specimen.

Darkfield needs an intense amount of light to work. This, coupled with the fact that it relies exclusively on scattered light rays, can cause glare and distortion.

It is not a reliable tool to obtain accurate physical measurements of specimens.

Darkfield has many applications and is a wonderful observation tool, especially when used in conjunction with other techniques. However, when employing this technique as part of a research study, it is also necessary to take into consideration the limitations and knowledge of possible unwanted artifacts.

As always, education is key in determining the effectiveness of this tool.

The Major Microscope Manufacturers

The major microscope manufacturers all have devices capable of darkfield illumination. Depending on the make/model, the microscope may come with attachments or have options for darkfield accessories. The major companies are:

- Nikon - https://www.nikoninstruments.com/Products
- Olympus - https://www.olympus-lifescience.com/en/microscopes/
- Zeiss - https://www.zeiss.com/microscopy/int/home.html
- Leica - https://www.leica-microsystems.com/
- Meiji - http://meijitechno.com/

In addition, there are numerous lesser known smaller companies that produce excellent quality video microscope systems with darkfield condensers. Generally, the leading innovators in light microscopes are Nikon, Olympus, and Zeiss offering stereo and compound microscopes with darkfield capability and/or accessories.

Live Blood Analysis in Darkfield

First, the politics and tragic truths. Be advised there are numerous websites critical and skeptical of live blood microscopy. A microscope is an amazing tool, but like a stethoscope, it has its limitations. Many physicians hang a stethoscope around their neck, but this commonly accepted scope requires a technical level of skill to properly use. It, like a microscope, is primarily a screening tool. If properly used, a microscope can become an invaluable research and clinical instrument. Blood is a rich source of all types of information, and this is why clinical laboratories offer hundreds, if not thousands, of blood tests. Over the last several decades, politically and legally, blood testing has become the proprietary domain of government-sanctioned (Clinical Laboratory Improvement Amendments) laboratories.

Live blood has been studied for several centuries and new developments and discoveries in hematology are forever being discovered. Clinical live blood analysis is more common in Europe where doctors still physically examine patients and perform many “in-clinic” evaluations and screening tests. Medicine in North America has become less personalized, more specialized, and more time restrained. Doctors rarely spend more than 15 minutes with patients, if that, and most have physician assistants or nurse practitioners who evaluate and treat most patients. How many doctors would take the time to view the patient’s live blood and educate the patient about their blood in a personalized way?

Few areas of medicine are as controversial as live blood microscopy. This is primarily because the field of live blood microscopy is unregulated, there is no training requirement for practitioners, and no officially recognized qualification. Yes, there are companies that offer instruction on live blood analysis, but these are not government-sanctioned. This certainly doesn’t mean that most live blood analysis is invalid. Much can be observed and learned using live blood microscopy. In short, live blood microscopy has its limitations and should never be used as a solo diagnostic, but rather can be used as an educational tool, or as a screening technique to evaluate, monitor, and demonstrate progress and response to treatment. It is also an invaluable teaching tool for client/patient education, making their own bodies less of a mystery.

Live blood analysis uses a drop of blood from the person's finger or ear lobe and then it is viewed under a light microscope using either phase contrast, differential-interference-contrast, ultraviolet, fluorescence or darkfield. For the limited purposes of this article, only the darkfield optical mode will be discussed. However, most serious researchers and clinicians also utilize some of the other types of light microscopy in visualizing organisms or live blood. Under the coverslip the blood remains alive and active for a brief period, allowing the viewers to see the motility of white cells, the structure, size and

quality of red blood cells, platelets, crystal formations in plasma, blood proteins (fibrinogen strands), microorganisms, and other formed blood elements and inclusions in the plasma.

An advantage of live blood analysis is that the patient can see their blood in a living state through high-definition video microscopy on a monitor. This is an educational experience for the observer, as most people have never seen their blood via video-microscopy. Not only can this experience become a valuable and memorable lesson in hematology, but the examination of live blood is also an excellent screening test for numerous basic cellular features as well as the presence of microbes. Observations are made on variations in number, size, shape, and fine structure and quality of red cells, motility of certain white cells, presence and behavior of platelets, and other formed structures in plasma, such as fats and plaque particles.

The analysis must be done with precision and care as to avoid artifacts and be performed quickly because many of the live blood features will degrade (oxidize) in a short time.

Cells and plasma features as well as abnormalities can be discussed allowing the patient to participate in the experience and become more informed about their blood and health condition. The correction of any abnormalities viewed can be achieved with the adoption of healthier eating habits, lifestyle modifications and various bioregulatory therapies. Most patients, when told to modify their diet and lifestyle, are unlikely to persist with such changes for any significant length of time. With live blood analysis, the patient can see directly the abnormalities that need correction and become more motivated to make the necessary improvements. In short, this procedure can improve patient compliance. With the proper therapeutic approaches, they often start to feel better quickly, and can visually see the changes in the microscope after making the nutritional, lifestyle and bioregulatory changes and appreciate their progress at the next visit.

Taking the Blood Sample

It is best to take the blood sample after the patient has been fasting. Fasting and non-fasting plasma appear very different. It does not matter which hand or finger the sample comes from, but it is useful to do it the same way each time for consistency. It is not necessary to use any alcohol or disinfectant on the finger before taking the sample. However, it is best to have the patient thoroughly wash their hands with soap and water and rinse them well and dry their hands, then wait for 5 minutes or so. With a gloved hand, wipe the fingertip with a clean cotton ball before taking the sample as a precaution against excess sweat or dirt. Wipe the finger with hydrogen peroxide doused

on a clean cotton ball after taking the sample. Always use a new lancet, even when taking a second sample on the same individual.

It is good practice to wipe away the first three drops of blood as these may contain skin cells and debris. However, it is not good technique to strongly squeeze the finger to extract blood. If the finger does not bleed freely, you may need to pressurize the finger with a gentle squeeze. Take special care never to touch the subject's blood, even with your gloved hand, or let the blood roll off the subject's finger. It is important to place the coverslip on the slide in less than 10 seconds. Longer can cause artifacts to form in the RBCs.

Preparation of Slides and Care for the Microscope

The slide and coverslip may include production inclusions which can lead to false observations. Slide artifacts have often been the central focus of critics of live blood analysis and justifiably so. Thus, care needs to be taken in slide preparation because features that lie above and below the plane of focus, such as fingerprints, dust, fibers or cleaning residue can scatter light and contribute to image degradation. Examine the slide and coverslip with a good light for any scratches or defects prior to use. All precautions should be exercised to ensure the sterility of the slide and coverslip. Close the coverslip and slide containers when not in use. Always use the most expensive pre-sterilized slides and coverslips that are available. If cheap slides are used, you could see all kinds of inclusions that look identical to blood crystalline formations, thus potentially making a false observation. The slide should be not thicker than 1 mm. Slides with frosted ends are useful, as they can be written on with a pencil or indelible felt tip with name, date or time. The coverslip must be of the thickness (usually 0.17 mm) for which the microscope objective has been corrected.

Never use a paper towel, a "kimwipe", your shirt, or any material other than good quality lens tissue or a cotton swab (must be 100% natural cotton) to clean an optical surface. Be gentle! You may use an appropriate lens cleaner or distilled water to help remove dried material. Organic solvents may separate or damage the lens elements or coatings. Cover the instrument with a dust jacket when not in use.

Because darkfield uses high-light intensity, attention should be paid to cleanliness of the sample. No cleaning technique can remove all glass blemishes; if these seem excessive, the only recourse is to try a different source of slides. For cleaning, lens paper is best. Blood sample size should be just enough to allow the liquid to spread to the area of the cover slip, since excessive liquid depth lowers image quality by providing a background of out-of-focus scattering sources. Conversely, too little blood can create rouleaux and unwanted thickness to a sample.

The best darkfield microscopy is oil immersion. When oiling the slide and condenser lens, touch the tip of the oil dispenser (Zeiss Immersol 518N) to the slide or condenser and roll out the oil slowly onto the slide so that bubbles are less likely to form in the oil. Bubbles can distort the field of view and cause hazing of the light. An example of this is a very grey background. Oil the top of the condenser lens with 1-2 drops of immersion oil and then rack the condenser up until it is just in contact with the bottom of the slide, which should light up the edges upon contact. Put 2-drops of oil on top of the coverslip and lower the objective until it just touches the slide. Do not try to focus until you have completed this process, to make sure you do not crush the slide.

One slide is never enough to give an accurate representation of the blood terrain. Hence, it is best to procure three slides in all. To one of the three, add one drop of bacteriostatic 0.9% physiologic saline (sodium chloride) to the blood sample before placing on the coverslip. The saline solution will cause the blood cells to separate and can reveal any organisms that may be otherwise difficult to visualize. In Enderlein terms, the saline will also stimulate the RBCs to expel *dioekothecits* if they are attached to the cell's membrane. Thrombocyte status may also become better visible. For those true microscopy enthusiasts, another slide can be stained with Loeffer's methylene blue which will reveal other features not normally visible, such as *chondrites, fila* and microorganisms, if present.

Loeffler's is recognized as a simple stain solution used for better determining microorganism morphology - bacteria, fungi and parasites in blood smears. The presence of negatively charged molecules in the cell causes the staining phenomenon, as the positively charged dye is attracted to negatively charged particles, such as polyphosphates like DNA and RNA.

Center the Darkfield Condenser

If using a universal condenser, centering it for Köhler illumination in bright field should be adequate for use in its darkfield setup. If you are using a high numerical aperture darkfield condenser, use the following procedure:

1) Select a very low magnification (e.g. 2X or 5X) objective.

2) Provide all the illumination possible.

3) Oil the condenser and raise it to its uppermost position under a blank slide.

4) If the condenser has a small central bright ring, focus the microscope on this ring and center it by using the condenser centering knobs. This is a spot ring condenser.

5) If there is no ring, do the following steps:

a) Use a slide of the correct thickness with one end frosted on one side.

b) Oil the non-frosted side to the condenser.

c) Image the frosted side.

d) Center the bright spot of light with the condenser centering screws. Some slight adjustment with the centering screws may be necessary when shifting to the high NA objectives.

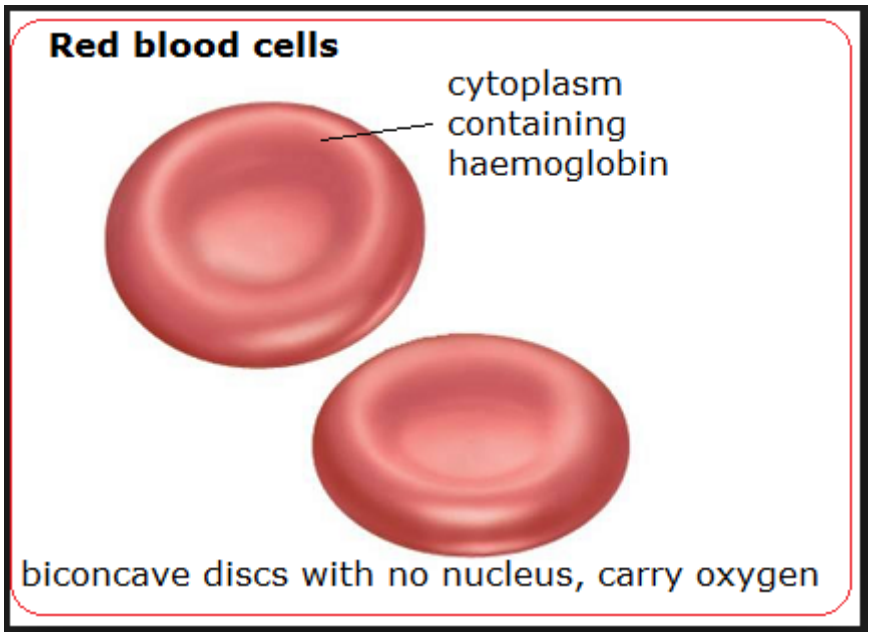
6) Assuming your slides are all the same thickness, you should not move the vertical position of the condenser.

Basic Blood Forms Seen in Darkfield

Formed blood components such as erythrocytes, leukocytes, clotting particles (platelets), lipids, as well as bacteria and other microorganisms are readily visualized with darkfield microscopy.

Erythrocytes (Red Blood Cells)

Structurally, erythrocytes are the simplest cells in the body. Erythrocytes are tiny biconcave disc-shaped cells. They do not have a nucleus or mitochondria. Their cytoplasm is rich in hemoglobin. O_2 binds to the iron in hemoglobin.



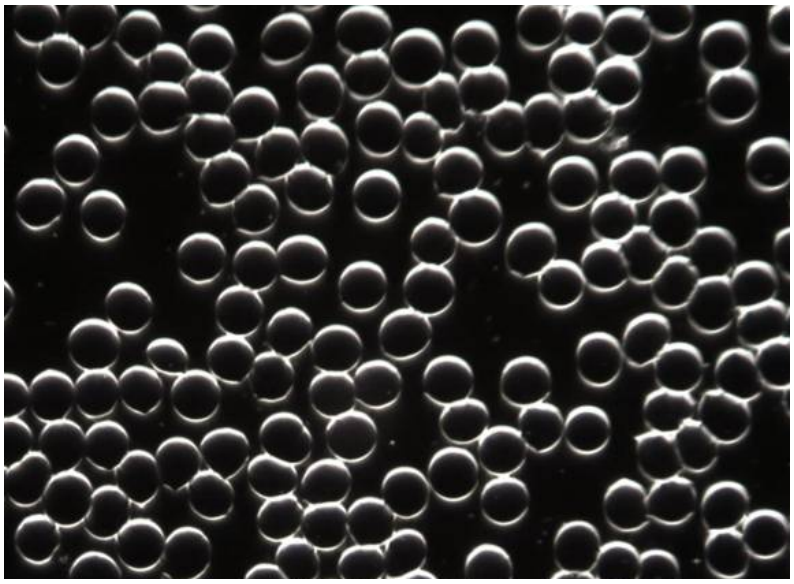
The basic function of the RBC is the creation and maintenance of an environment salutary to the physical integrity and functionality of hemoglobin, the primary carrier of oxygen.

Erythropoiesis is the process which produces red blood cells (erythrocytes). In the normal state, erythrocytes are produced only in the skeleton (in adults only in the axial skeleton), but in certain pathologic states, almost any organ can become the site of erythropoiesis. Numerous nutrient substances are necessary for creation of erythrocytes, including metals (iron, cobalt, manganese), vitamins (B_{12}, B_6, C, E, folate, riboflavin, pantothenic acid, thiamin), and amino acids. Regulatory substances necessary for normal erythropoiesis include erythropoietin, thyroid hormones, and androgens.

Erythrocytes progress from blast precursors in the marrow over a period of five days. Then they are released into the blood as reticulocytes, distinguishable from regular erythrocytes only with special supravital stains. The reticulocyte changes to an erythrocyte in one day and circulates for 120 days (4 months) before being destroyed in the reticuloendothelial system and are recycled by the liver and spleen. They are destroyed because they must constantly change shape to pass through narrow blood vessels. When they die, the hemoglobin is stored in the liver and used to make new blood cells in the bone marrow.

RBC Analysis

First, it is very helpful to have already performed a CBC with differential and platelet count with a laboratory prior to performing live blood microscopy. The CBC gives accurate quantitative information for comparison, whereas live blood analysis may render qualitative and functional information. Number, shape, size and distribution should be observed and noted in the evaluation of the RBCs. Though RBC numbers cannot be accurately counted in darkfield, a deficiency of RBC will appear as extra space between the cells. Generally, women tend to have lower RBC numbers than men, and RBC counts tend to decrease with age. Normal RBC decreases are seen during pregnancy because of normal body fluid increases that dilute the RBCs. Certain nutritional deficiencies that affect the elderly and pregnant may also play a role in causing anemia. When the RBC value in a CBC is decreased by more than 10% of the expected normal value, the patient is said to be anemic. The ratio of cells in normal blood is 600 RBCs for each white blood cell and 40 platelets.



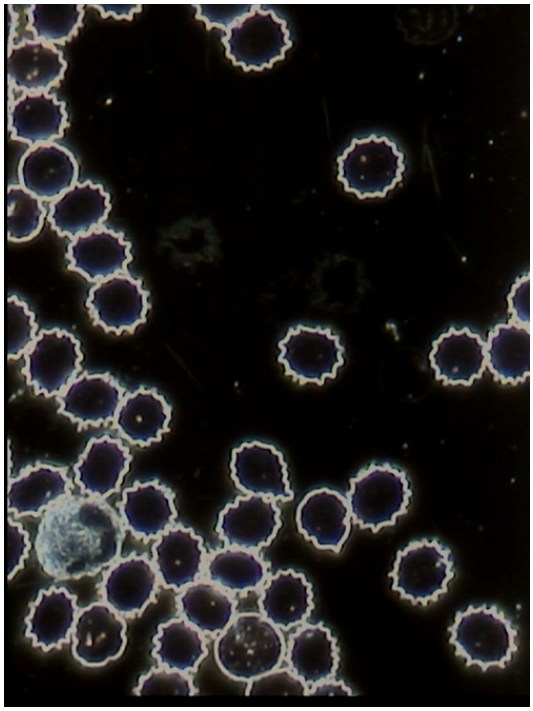
Normal RBC Size, Shape and Distribution

RBC sizes (diameters) should be approximately the same. When there are significant size differences it is called anacytosis. Abnormally small RBCs are microcytes and abnormally large RBCs are called macrocytes. Make note of the red blood cell diameter with (RDW) in the laboratory CBC analysis.

Poikilocytes

One of the key features of RBC observed in darkfield is their lipid/protein membranes. RBC membrane fluctuations have been studied for nearly a century, as they offer a window into understanding the structure, dynamics, and function of this unique cell. Diet and lifestyle play a significant role in the alterations of the lipids both in serum and RBC membranes.

Normal red blood cells are round, flattened disks that are thinner in the middle than at the edges. Abnormal variation in cell shape is called poikilocytosis. Poikilocytes may be oval, teardrop-shaped, sickle-shaped, have pointy projections, bottle-cap shaped or irregularly contracted.



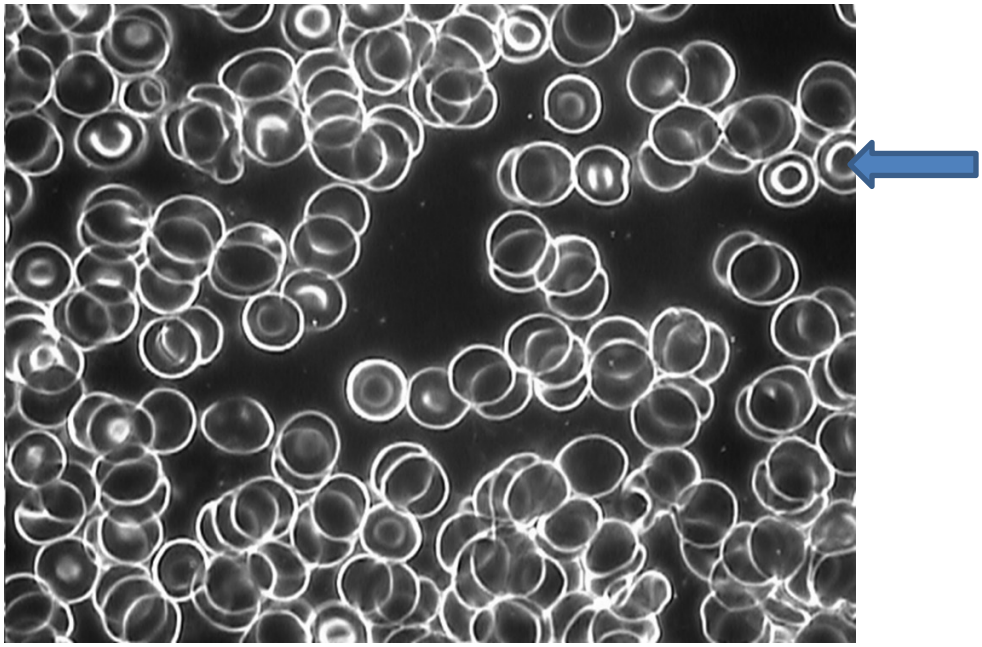
Poikilocytosis: bottle-cap shaped RBCs

There are several reasons RBCs membranes may become damaged and exhibit poikilocytosis. A common cause is elevated oxidative stress (reactive oxygen species) that oxidizes the RBC membrane prematurely. Other causes are types of anemia, certain prescription drugs, cytotoxic chemotherapy, toxic elements such as mercury or lead, and or liver and kidney disorders – to name a few. Studies show elliptocytes and tailed poikilocytes correlate with severity of iron-deficiency anemia.

Be advised that this damage may also be an artifact caused by compressing the sample by pressurizing it with the objective, thereby rendering damaged membranes. Look at the entire sample for RBC poikilocytes. If there is a question as to pressure damage of the sample, procure another sample.

Target Cells

The RBC lipid membranes may also appear as “target cells” with light shining through the center of the cell. This occurs usually due to the lipid membranes being excessively thin and may be caused by a deficiency of essential fatty acids or problems with digestion and metabolism of lipids. RBC target cells are commonly seen in individuals who have had their gall bladder removed yielding an insufficiency of bile and an inability of the small intestine to adequately emulsify lipids.



RBC Target Cells

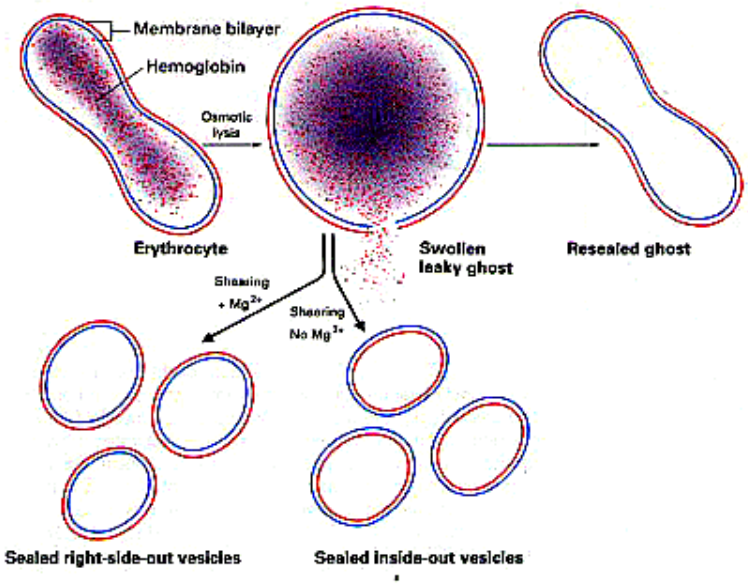
Another abnormal feature of RBCs in live blood is represented by the stacked erythrocytes. These refer to erythrocytes that stick together, yielding an appearance like a stack of coins (rouleaux). The formation of these aggregates can be a phenomenon induced by biological factors, i.e. due to excess blood plasma proteins and loss of cellular RBC voltage (low electrolytes). However, it is important to know that rouleaux can also be an artifact of poor blood collection technique that does not allow the blood drop to disperse evenly and fully under the cover slip. A clean slide and proper collection technique are essential to the observation quality of the blood.

Ghost or Shadow Cells

Another feature that may be seen is the presence of pale cells with no internal content. This feature is typically indicative of certain milieu conditions that causes altered permeability of the RBC membranes resulting in rupture. These cells occur by *hemolysis* through which hemoglobin and cytoplasmic material “leak out”. These empty-looking red blood cells have been named “RBC ghosts” or “shadow cells” based on their appearance under the microscope. When numerous ghost cells are present, it may be due to abnormal toxic antibodies, reactive proteins, or certain membrane-invasive microorganisms that damage the lipid/protein membrane.

Exposure to too much ambient air in the process of taking the sample can contribute to this appearance. This is a primary reason for taking multiple samples in order to

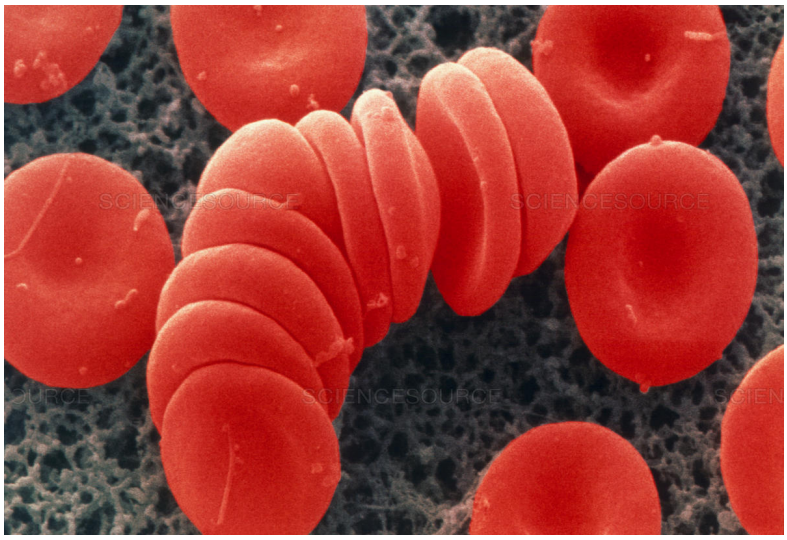
compare cellular features for consistency. If this picture is inconsistent in the sample and does not appear in all fields, take another sample with expediency to get the coverslip on the slide within 10 seconds. It can also be revealing to view the slide over an extended period (12 hours or longer) in order to view the hemolysis process and how quickly it occurs.



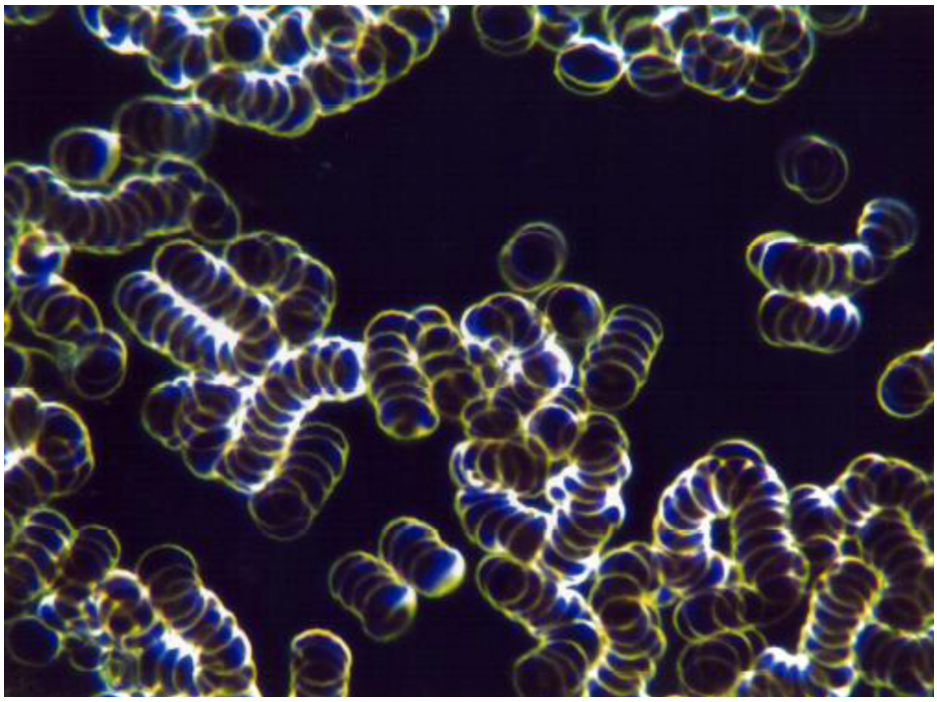
RBC ghosts may also be artificially produced to study membrane integrity. When red cells are placed in a solution with lower solute concentration (hypotonic) than found in the red cells, by osmosis, water moves into the cells and causes them to swell and then burst. Such cells may show only the empty membrane and are called “ghost” cells. The following video shows ghost cells forming from a hypotonic solution (distilled water) - https://www.youtube.com/watch?v=LSDLFZPa9BU

RBC Rouleaux

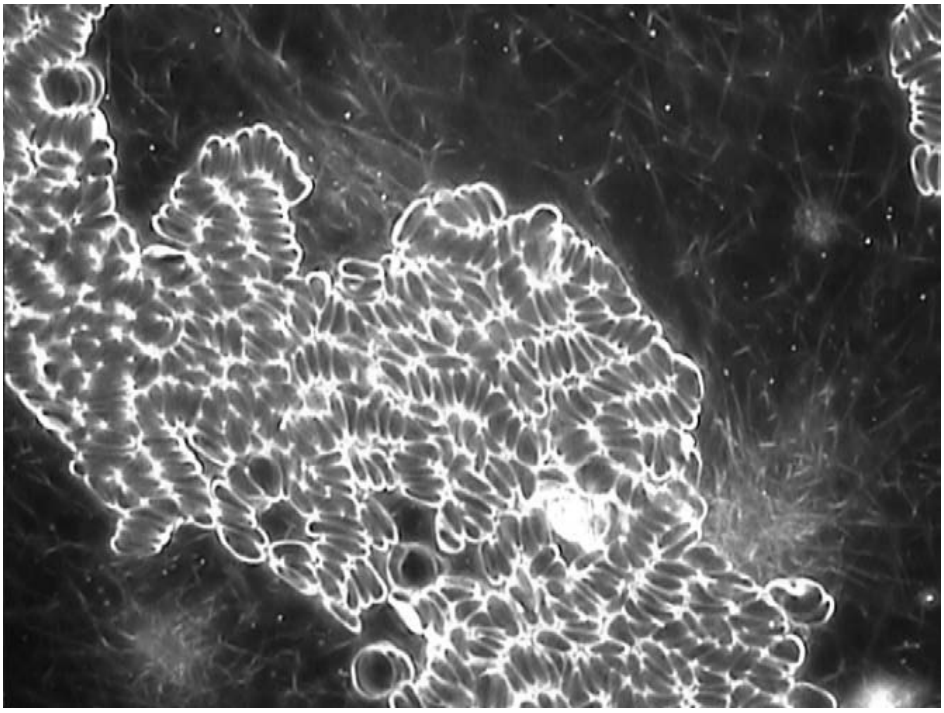
Distribution of the RBCs across the slide should be spaced and not stacked. When RBCs are stacked together in long chains it is known as "rouleaux formation". This can happen with increased serum proteins, particularly fibrinogen and globulins that interact with sialic acid on the surface of RBCs to facilitate the formation of rouleaux. Such long chains of RBCs sediment more readily. This is the mechanism for the sedimentation rate, which increases non-specifically with inflammation and increased "acute phase" serum proteins.



RBC Rouleaux



Rouleaux in Darkfield



RBC Agglutination in Darkfield

Normally, red cells pass through capillaries in a single-file fashion. With rouleaux, the cells being stacked cannot freely pass through the capillary beds. This may cause a microcirculatory insufficiency and result in inadequate oxygen delivery to the tissues. A poor oxygen tissue milieu also allows for microorganisms to become more pathogenic.

Careful attention should be made to not confuse rouleaux with RBC agglutination. Rouleaux are orderly linear stacks of RBCs, much like a stack of coins, whereas RBC agglutination is much worse and is formed by grapelike RBC aggregates and often clumps together as one large mass. To aid in differentiating between rouleaux and agglutination, a saline dilution test is useful. Rouleaux may be easily dissociated by dilution of RBCs in saline, whereas true agglutination persists despite saline dilution.

The main cause of rouleaux is over-proteinization of the blood. If rouleaux is confirmed, it is recommended to further evaluate the plasma or serum total protein, albumin, globulin, fibrinogen values, and investigate potential causes of any abnormalities found.

A false appearance of rouleaux can be artificially caused by expelling the blood from the pricked finger by pressurizing it. If the sample does not bleed easily, take another sample, making sure to prick the finger deeply enough to allow it to bleed freely.

Leukocytes (White Blood Cells)

There are five basic types of leukocytes: neutrophils, monocytes, eosinophils, lymphocytes and basophils. These are divided into two main classes:

- **Granulocytes** (includes Neutrophils, Eosinophils and Basophils)
- **Agranulocytes** (includes Lymphocytes and Monocytes).

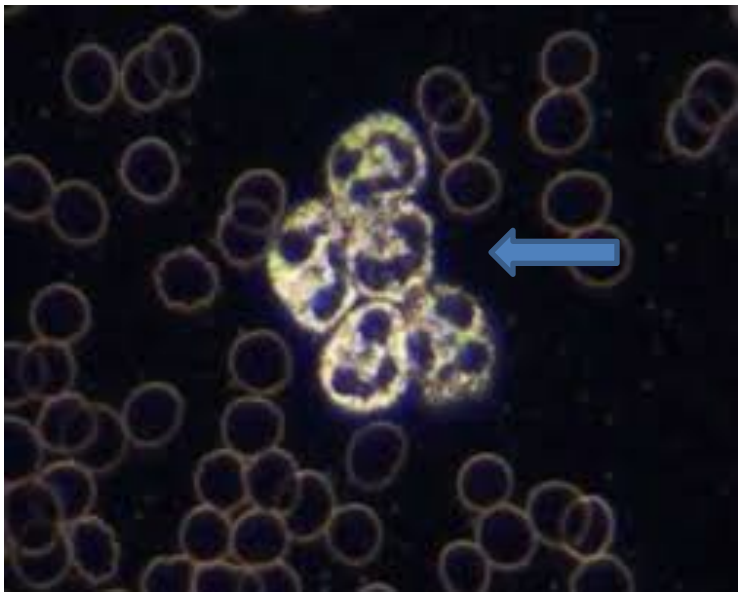
This classification depends on whether granules can be distinguished in their cytoplasm using a light microscope and conventional staining methods. It is easy to confuse the different leukocytes in blood smears. To identify them, you need to look for the shape of the nucleus, and compare their size, relative to that of a red blood cell.

Neutrophils

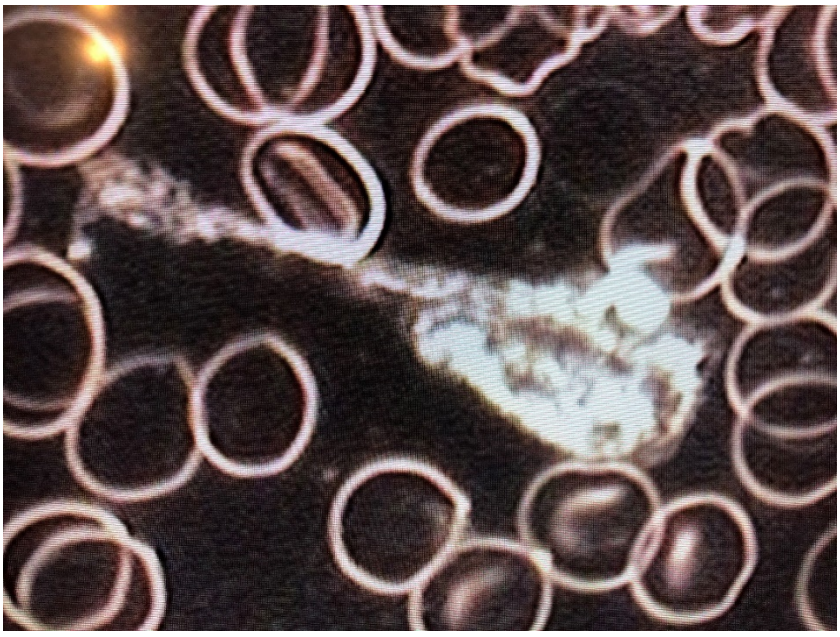
Neutrophils are the most populous of the circulating white cells and they are also the most short-lived in circulation. After production and release by the marrow, they only circulate for about eight hours before proceeding to the tissues (via diapedesis), where they live for about a week, if all goes well. They are produced as a response to acute body stress, whether from infection, trauma, emotional distress, or other noxious stimuli. When called to a site of injury, they phagocytize invaders and other undesirable substances and usually kill themselves in the action, and thereby become autolytic – breaking apart.

The neutrophils are 12-14 μm in diameter, and so look bigger than the surrounding red blood cells. The nucleus has from 2-5 finely connected nuclear lobes that are rarely uniform in size. Normally, the circulating neutrophil series consists only of band neutrophils and segmented neutrophils, the latter being the most mature type. In stress situations (i.e., the "acute phase reaction"), the immature cells called “band” or “stab cells” can be seen in the blood. This picture is called a "left shift”. The band count has been used as an indicator of acute stress and a sign of a bacterial terrain. Often this shift is accompanied with neutrophil aggregation (see below).

With live blood, the motility of the neutrophil may be viewed and is an important indictor of immune health. Small, non-motile neutrophils are an indication of a weakened cellular immune function.



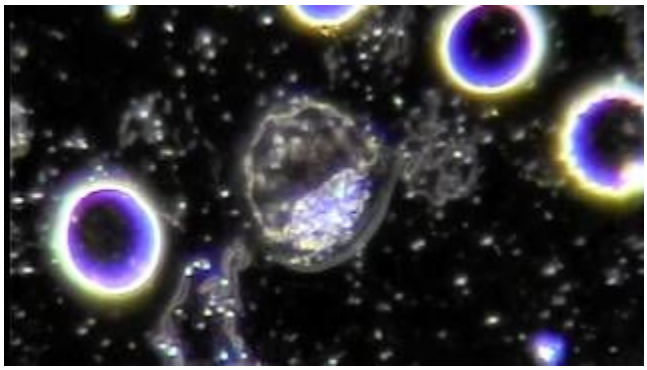
Neutrophil Aggregation



Neutrophil Extruding Pseudopod

Neutrophils extrude pseudopods to clean the blood of debris. Pseudopods have a variety of functions for leukocytes: they aid in mobility, crawling, phagocytosis, and transport out of the blood vessels. Circulating neutrophils move around, cleaning RBC and the plasma and eventually tether to the endothelial lining of blood vessels, allowing them to exit the bloodstream and enter the surrounding tissue to target pathogens.

Lymphocytes



After neutrophils, lymphocytes are the most numerous of the circulating leukocytes. An optimum range for lymphocytes is up to 24 to 44% of the total WBC population (1000 - 4800/μL). Most of the lymphocytes are small; a bit bigger than red blood cells, at about 6-9μm in diameter. The rest (around 10%) are larger, about 10-14μm in diameter. These larger cells have more cytoplasm, more free ribosomes and mitochondria. Lymphocytes can look like monocytes, except that lymphocytes do not have a kidney-bean shaped nucleus, and lymphocytes are usually smaller. Larger lymphocytes are commonly activated lymphocytes.

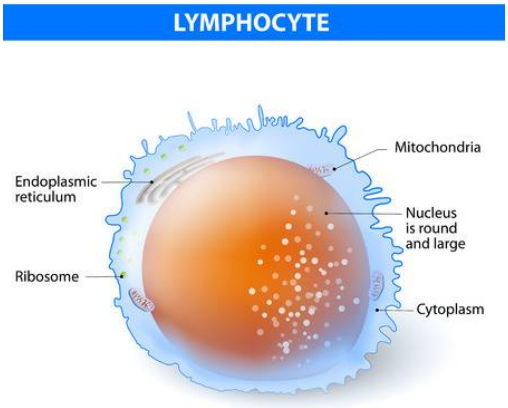
Their lifespan may vary from several days to a lifetime (as for memory lymphocytes). Unlike neutrophils, monocytes, and eosinophils, the lymphocytes

1) can move back and forth between the vessels and the extravascular tissues,

2) are capable of reverting to blast-like cells, and

3) when so transformed, can multiply as the immunologic need arises.

Lymphocytes assist in destroying the toxic products of protein metabolism. Lymphocytes originate in the lymphoblast of the bone marrow, spleen, lymph glands, gut associated lymphoid tissue (GALT), tonsils and the appendix.

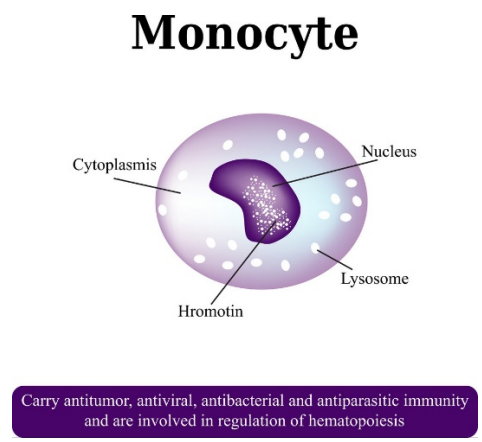


The two major types of lymphocytes in blood are B-cells, which produce a specific antibody when activated, and T-cells, which have various roles in cell-mediated immunity and in immunoregulation. In darkfield, and blood smears in general, T- and B-cells cannot be distinguished from one another, so the differential counts the combination of the two. Immunostains for specific membrane antigens must be used to determine the difference. B-cells work chiefly by secreting soluble substances called antibodies into the body's fluids, or humors. This is known as humoral immunity. Antibodies typically interact with circulating antigens such as bacteria and toxic molecules but are unable to penetrate living cells. T-cells, in contrast, interact directly with their targets, attacking body cells that have been commandeered by viruses or have become neoplastic. This is termed cellular immunity. Although small lymphocytes may look identical, even under the microscope, they can be told apart by means of distinctive molecules they carry on their cell surface. Not only do such markers distinguish between B-cells and T-cells, they distinguish among various subsets of cells that behave differently.

When activated by whatever means, lymphocytes can become very large (approaching or exceeding the diameter of monocytes) and basophilic (reflecting the increased amount of synthesized cytoplasmic RNA and protein). The cytoplasm becomes finely granular (reflecting increased numbers of organelles), and the nuclear chromatin becomes less clumped. Such cells are called "transformed lymphocytes", "atypical lymphocytes", or "viral lymphocytes". Although such cells are classically associated with viral infection, they may also be seen in bacterial and other infections and in allergic conditions.

Monocytes

Monocytes usually make up 3-8% of the total white cell differential count in normal blood and are the largest of the leukocytes. An optimum range for monocytes is up to 7% of the total WBC population. These are the largest type of white blood cells and can be up to 20μm in diameter. They have a large eccentrically placed nucleus, which is kidney-bean shaped. Monocytes are spherical in shape or may appear amoeboid with a central oval or kidney-shaped nucleus with clumped chromatin. This characteristic differentiates monocytes from neutrophils, which have several lobes with a divided nucleus. Monocytes in darkfield appear as dull *white* grey cells, 2-3 times the size of a red blood cell with a nucleus that occupies 50% of the cell.

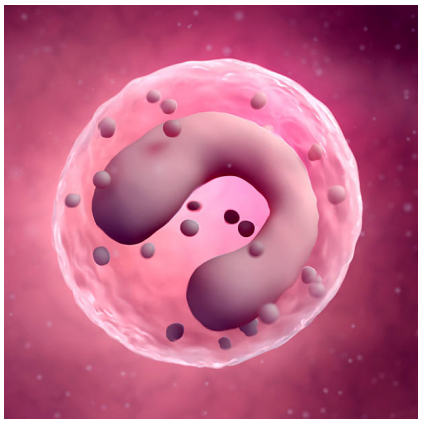


They develop from myeloid stem cells and are released into the circulation for 1-3 days before migrating into tissues, where they transform into macrophages. Undifferentiated monocytes reside in the spleen in higher numbers than in circulation. In response to injury, splenic monocytes increase their motility, exit the spleen, accumulate in injured tissue, and participate in wound healing. In the inflammatory process, neutrophils predominate for about 72 hours; they then break up and the monocytes remain to phagocytize the cell fragments. Hence, monocytes are usually increased during the recovery phase of infection.

Monocytes are important accessory and regulatory cells in humoral and cellular immunity. As macrophages, monocytes play an important role in eliminating foreign material, tumorous cells and microorganisms through a process known as phagocytosis. These large cells are more closely related to neutrophils than are the other “granulocytes", the basophil and eosinophil. Monocytes and neutrophils share the same stem cell. Monocytes are histiocytes or macrophages. They are produced by the marrow, circulate for five to eight days, and then enter the tissues where they are mysteriously transformed into histiocytes. Here they serve as the welcome wagon for any outside invaders and are capable of "processing" foreign antigens and "presenting"

them to the immunocompetent lymphocytes. They are also very capable of phagocytosis. Monocytes-macrophages phagocytose micro-organisms or cellular debris, play a role in the inflammatory reaction and participate in fibrinolysis by secreting plasminogen activators. They secrete TNF and specific interleukins that potentiate B and T lymphocytes.

Monocyte Showing Characteristic Shape of Nuclear Lobes



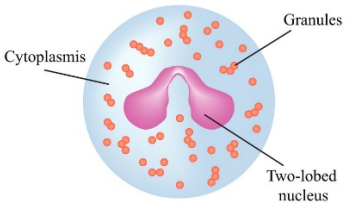
Eosinophils

Eosinophils are the fourth most abundant type of white blood cell, accounting for 0-4% of the total white cell differential count in a normal human blood sample. They differentiate from myeloid precursor cells in the bone marrow.

These intriguing cells are traditionally grouped with the neutrophils and basophils as "granulocytes". Given all their unique and dissimilar abilities, this is a rather meaningless terminological grouping. Current thinking is that eosinophils and neutrophils are derived from different stem cells, which are somewhat difficult to distinguish from each other by currently available techniques of examination.

These cells are 12 - 17 μm in diameter - larger than neutrophils, and about 3 times the size of a red blood cell. Although the hallmark of the eosinophil in darkfield is the presence of bright, golden, large, refractile granules, another feature helpful in identifying them is that they rarely have more than two nuclear lobes (unlike the neutrophil, which usually has three or four). Additionally, the nuclear lobes tend to be more rounded and uniform than found in neutrophils.

Eosinophil



Neutralize parasific infections

Eosinophils have a circulating half-life of approximately 18 hours and a tissue lifespan of at least 6 days. They are capable of locomotion and phagocytosis and can enter inflammatory sites but do so less readily than neutrophils. In tissues the primary location for eosinophils is in the epithelial barriers to the outside world, such as lungs, skin and GI tract. They can return to the circulating blood and bone marrow after they enter the tissues.

Eosinophils are multifunctional leukocytes involved in initiation and propagation of diverse inflammatory processes including parasitic, bacterial and viral infections, tissue injury, tumor immunity, and allergic diseases. Eosinophils have also been shown to respond to foreign protein substances such as allergic antigens, fungal infections, some drugs and exposure to certain chemicals. Hence, a high eosinophil blood count may indicate an allergic reaction. Increased numbers are also found after exercise and numbers are also affected by menstrual patterns in women.

Eosinophils can serve as major effector cells killing pathogens, particularly parasites, but also inducing tissue damage of healthy cells by releasing toxic granule proteins and lipid mediators. They produce cytokines that contribute to their pro-inflammatory functions. Alongside their role in acute inflammation, eosinophils are recognized as immunomodulatory cells interacting with B and T lymphocytes. Eosinophils can act as antigen presenting cells and activate antigen-specific responses.

Eosinophils are capable of ameboid motion (in response to toxic substances released by bacteria and components of the complement system) and phagocytosis. They are often seen at the site of invasive parasitic infestations and allergic (immediate hypersensitivity) responses. Individuals with chronic allergic conditions (such as atopic rhinitis or extrinsic asthma) typically have elevated circulating eosinophil counts. The eosinophils may serve a critical function in mitigating allergic responses, since they can:

1) inactivate slow reacting substance of anaphylaxis (SRS-A),

2) neutralize histamine, and

3) inhibit mast cell degranulation.

These cells largely reside in the tissues where they can live for up to two weeks. Most eosinophils are found in the lamina propria of the gastrointestinal tract except the esophagus, thus increased presence in the blood is acknowledged as clinically significant. Following a classic acute phase reaction, as the granulocyte count in the peripheral blood drops, the eosinophil count temporarily rises.

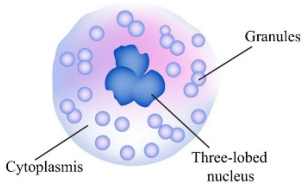
Eosinophils are suppressed by cortisol; thus, numbers are suppressed with stress and concentrations can rise throughout the day. Cortisone also suppresses eosinophils.

Basophils

The most aesthetically pleasing of all the leukocytes, the basophils are also the least numerous of all leukocytes in the blood, making up less than 1% of the total white cell differential count. They have large granules, which contain heparin and histamine and become mast cells in the tissue.

They are 14-16 μm in diameter. In darkfield, they are easily recognized by their very large, blueish or purplish cytoplasmic granules, which overlie, as well as flank, the nucleus (eosinophil granules, by contrast, only flank the nucleus but do not overlie it). The granules contain heparin, histamine, serotonin, prostaglandins and leukotrienes. It is tempting to assume that the basophil and the mast cell are the blood and tissue versions, respectively, of the same cell type. It is controversial as to whether this concept is true or whether these are two different cell types.

Basophil



Generation of histamine for inflammatory responses

In active allergic reactions, blood basophils decrease in number, while tissue mast cells increase. This reciprocal relationship suggests that they represent the same cell type (i.e., an allergen stimulates the passage of the cells from the blood to the site of the allergen in the tissues). Some experiments with animals have also shown that mast

cells are marrow-derived and can differentiate into cells that resemble basophils. Conversely, some recent evidence suggests that basophils (as well as eosinophils) can differentiate from metachromatic precursor cells that reside among epithelial cells in the nasal mucosa. With inflammation, basophils deliver heparin to the affected tissue to prevent clotting.

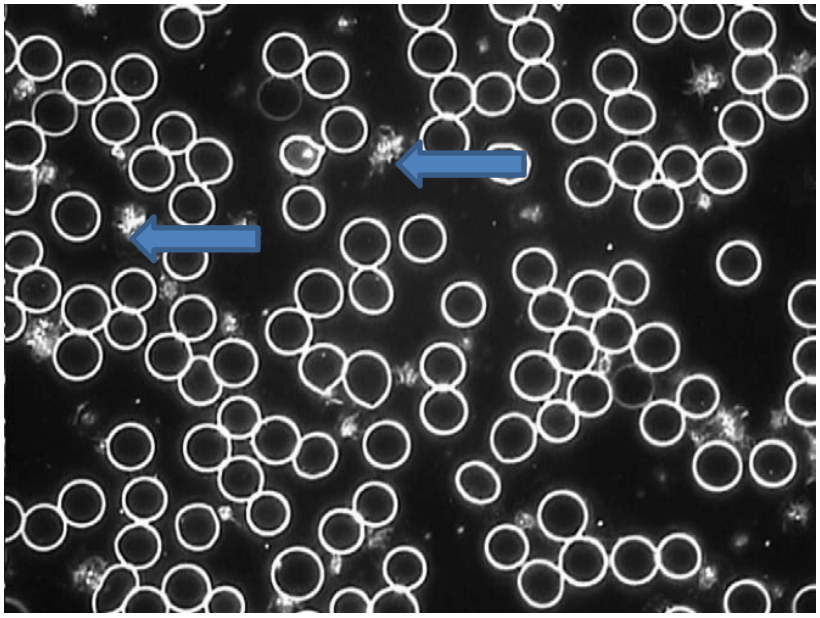
Blood Platelets (Thrombocytes)

Blood platelets are not true cells, but rather cytoplasmic fragments of a large cell in the bone marrow, the megakaryocyte. Platelets have several types of membrane-bound granules, which contain many constituents including fibrinogen and several growth factors (e.g., Platelet-derived growth factor). Approximately 2/3 of the platelets circulate in the blood and 1/3 is stored in the spleen. Normally, one cubic millimeter of blood contains between 150,000 and 400,000 platelets. If the number drops below this range, uncontrolled bleeding becomes a risk, whereas a rise above the upper limit of this range indicates a risk of uncontrolled blood clotting.

Each megakaryocyte can produce 5000–10,000 platelets. Old platelets are destroyed by phagocytosis in the spleen and liver (Kupffer cells). Survival of platelets is measured in days (average 7 to 9 days). Primarily, platelet activity is associated with the initiation of coagulation cascades. Damage in blood vessels makes the subendothelial surface the primary target site of platelet action, where it establishes the hemostasis.

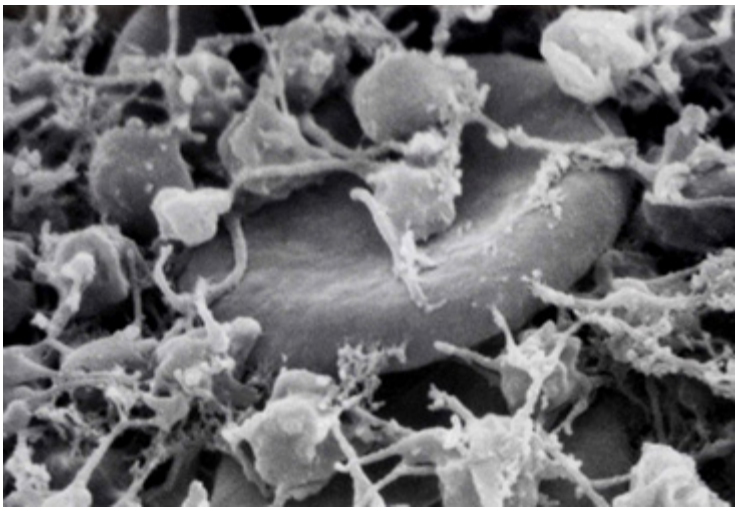
Platelet activation occurs when injury to the vessel wall exposes sub-endothelial components, especially collagen. Platelets adhere to the damaged area and become cohesive to other platelets. This aggregation leads to the formation of a platelet plug, which prevents further blood loss and allows the repair process to begin. Platelets and their behavior can be clearly observed in darkfield microscopy.

Platelets depicted below.



In recent times, platelets have emerged to be important markers for disease pathophysiology. They are multifunctional blood particles and very important clinical targets for many disease pathologies. Being important inflammatory markers, they play important roles in atherosclerosis and cardiovascular disorders which are correlated with type-2 diabetes. They also play a profound role in tumor biology as well as allergic inflammation.

One of the most common abnormal changes in platelet behavior observed in live blood is platelet hyper-aggregation. Hyper-aggregated platelets tend to block the blood vessels leading to circulatory impairment.

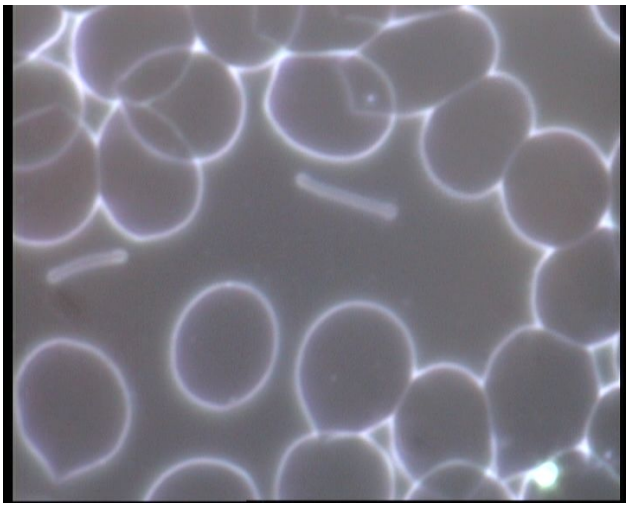


Platelets Surrounding an RBC

Microorganisms in Blood

Blood Bacteria

Scientists and clinicians have been observing bacteria in human blood and body fluids with microscopy since the 1600's. Using darkfield microscopy, Antoine Béchamp in the mid-1800's was able to show that animal and plant cells contain tiny particles which he called microzymas. According to Béchamp, these microzymas were the living remains of plant and animal life of which, in either a recent or distant past, they had been the constituent cellular elements, and that they were in fact the primary anatomical elements of all living beings. He demonstrated that upon the death of an organ its cells disappear, but the microzymas remain and are imperishable. In his book, Béchamp laid the foundation for the concept of pleomorphism. This view stood - and continues to stand - diametrically opposed to the prevailing ideas of monomorphism. At the time, Béchamp's work was controversial; eventually, however, the scientific community came to favor the monomorphism concept of Louis Pasteur over pleomorphism.

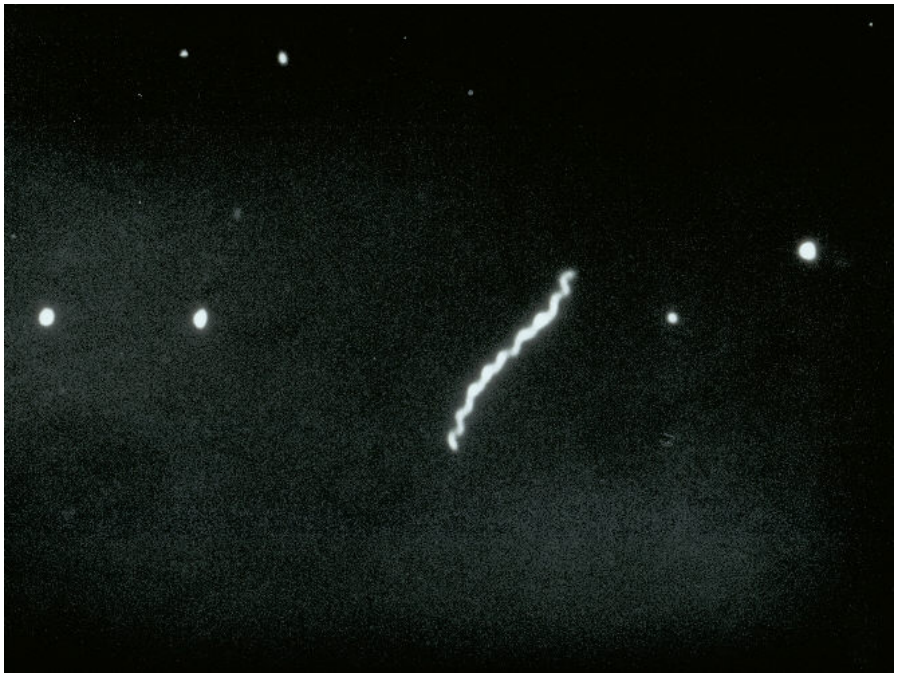


Rod Bacteria

The term pleomorphism refers to the ability of bacteria to change shape, or to exist in several morphological forms. By contrast, the monomorphic perspective is that each bacterial cell is derived from a previously existing cell of practically the same size and shape. Cocci generally beget cocci, and rods give rise to rods. The monomorphism view, later propounded by Rudolf Virchow, Ferdinand Cohn, and Robert Koch, is that by binary fission most bacteria divide transversely to produce two new cells which eventually achieve the same size

and morphology of the original. Koch, famous for his tuberculosis discoveries, was rigid in his belief that a specific germ had only one form (monomorphism). Consequently, he opposed all research showing some germs had more than one form (pleomorphism) and complex “life cycles”.

In 1906, Landsteiner and Mucha were the first to recommend the use of darkfield microscopes to identify the presence of the spirochete Treponema pallidum, which is responsible for causing syphilis. Within a year, the darkfield technique became the routine investigation for syphilis diagnosis in Europe. It is still utilized today and there are many current studies that still highlight the usefulness of darkfield in both syphilis as well as the spirochete Borrelia that causes Lyme disease.



Borrelia Spirochete

See: Bacteria Spirillum in Darkfield Video

https://www.youtube.com/watch?reload=9&v=R99T08FjTkc

In 1925, Zoologist Günther Enderlein published a comprehensive work entitled Bakterien-Cyklogenie based on his research with live blood analysis in darkfield, outlining his theory on microbial life cycles (pleomorphism). Using darkfield microscopy he observed phenomena that could not be seen in stained blood samples, and from

observations he hypothesized that under conditions of environmental stress, pH and nutritional variables, many microbes and cells changed form in consistent ways. Thus, Enderlein followed Antoine Béchamp's theory of pleomorphism. Enderlein believed that some of the structures in the blood he observed were microbes that caused illnesses. He called this group of microbes endobionts. The smallest particles were called "protits", which he believed to be small colloids of proteins, then in increasing size were "symprotits" and "macrosymprotits".

Through many decades of research, Enderlein observed that these small particles were necessary for health homeostasis. His theories postulated that under pathological conditions such as intoxication or high acid load, these particles would polymerize into more complex units that he could observe in the blood. He believed certain microorganisms would undergo a development-cycle, that he called "cyclode" (bacterial cyclode) and that specific diseases were related to particular cyclodes. His research mainly focused on two cyclodes: the cyclode leading to the fungus *Mucor racemosus* that he linked to diseases concerning the blood, spine and rheumatism, and the cyclode leading to the fungus *Aspergillus niger* and diseases of lung, tuberculosis and cancer.

Today, most microbiologists have been trained within the monomorphic doctrine. They accept that, apart from minor variation, each bacterial cell is derived from a previously existing cell of practically the same size and shape. Cocci generally beget cocci, and rods give rise to rods. The monomorphic view is that by binary fission most bacteria divide transversely to produce two new cells which eventually achieve the same size and morphology of the original. In the same way, a single spore germinates to give rise to a vegetative cell essentially the same as the cell from which the spore originated. (This monomorphic view limits the disease state into a linear, mechanistic model.) Exceptions to this rule (of linear monomorphic lines of bacterial growth in the body) are reported in certain so-called higher bacteria, but most pleomorphic observations are ignored and generally regarded as diagnostically insignificant staining artifacts or debris by most monomorphic biologists.

References to pleomorphism disappeared in biology textbooks starting in the 1920s up to the present date. However, during the 1960's, work on L-forms bacteria appeared to substantiate some of the claims made by earlier pleomorphists. Hieneberger-Noble, for example, suggested that L-forms correlated with the "symplasm" observed by Felix Löhnis. Bacterial conjugation (the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells), an idea that had been scoffed at by many monomorphists, was now being taken seriously. Bacterial conjugation is often regarded as the bacterial equivalent of sexual reproduction or mating since it

involves the exchange of genetic material. However, it is not sexual reproduction, since no exchange of gamete occurs, and indeed no generation of a new organism: instead an existing organism is transformed. Conjugation is used in nature to share beneficial genetic material between bacteria, such as antibiotic resistance.

Previously, Löhnis had been mocked when he had claimed that he, and numerous other workers including Potthoft, had observed conjugation tubes connecting two bacterial cells. Ann Wood and Donovan Kelly recently showed that the morphology of a species of *Thiobacillus* varied in response to environmental conditions, while limited pleomorphism in *Bradyrhizobium* was reported by H. Keith Reding and Joe Eugene Lepo to be induced by dicarboxylate.

From the pleomorphic perspective, the internal environment (milieu) is largely responsible for the establishment of the pathogenicity of fungi, viruses and bacteria. This leads to the conclusion that pathogenic organisms that participate in chronic inflammatory and degenerative diseases can be reversed by modulating their milieu, thereby changing the microbiome's pathogenicity. In other words, the environment dictates the direction of the ever-morphing house of organisms toward health or toward disease.

Currently, the concept of pleomorphism means the existence of irregular and variant forms in the same species or strain of microorganisms, a condition analogous to polymorphism in higher organisms. Pleomorphism is particularly prevalent in certain groups of bacteria and in yeasts, rickettsias, and mycoplasmas and greatly complicates the task of identifying and studying them.

It is hypothesized that these pleomorphic forms are truly not staining artifacts or cellular debris, but instead represent various stages in the life cycle of stressed bacteria: cell wall-deficient/defective (often called L-forms) that are difficult-to-culture or nonculturable. When bacteria become wall deficient, the adjustment, as shown by fine structure, is often systemic, with changes throughout the cell. Biochemical alterations known to occur in the cytoplasmic membrane are also reflected in the thickness and other fine structure characteristics of this lamella which may assume new functions previously assigned to the wall. The changes in the distribution and density of the nuclear material with changes in the wall are often dramatic.

Essential to the thesis is that small, electron dense, non-vesiculated L-forms are the central (core) element in bacterial persistence. Depending on the stimulus received, these dense forms might be considered as undifferentiated cells, with the capacity to develop along several different routes. This might translate into

an etiology for chronic inflammatory diseases, when the stressed bacteria increase in numbers and overwhelm the normal biological functions of the host.

Recent reports showing that very small bacteria can be isolated from environmental samples and human blood have recently caused considerable excitement and controversy. Called nanobacteria (or nannobacteria), these very small bacteria appear as spheres and ellipses of a diameter between 0·03 and 0·2 μm, often occurring in chains or groups of similar-sized forms. Nanobacteria have been isolated from blood as clusters of coccoid cell-walled organisms (0·08–0·5 μm) and associated "elementary particles" (0·005–1 μm) which together produce biofilms containing carbonate or hydroxyapatite. Research by Kajander and colleagues released gene sequence data that have positioned bloodborne nanobacteria in the α-2 subgroup of the Proteobacteria. Such isolates are extremely resistant to heat and certain antibiotics and exhibit a "bizarre morphology" (i.e. extreme pleomorphism).

Dr. Gaston Naessens of Canada has continued this tradition with life-long research observing these microscopic pleomorphic particles he calls "somatids". He describes the somatid as an elementary particle that can transform into pathogenic bacteria under certain toxic environmental terrain circumstances.

In 1997, Prusiner, professor of neurology and biochemistry at the University of California, San Francisco, was awarded the Nobel Prize for Medicine or Physiology for his discovery of “Prions – a new biological principle of infection.” A Prion, as defined by Prusiner, is a “small proteinaceous infectious particle, which resists inactivation by procedures that modify nucleic acids.” Unlike known infectious agents, prions contain no genetic material and are simply proteins. Gene encoding for prions is found in all mammals, including humans. Under normal conditions, prions are innocuous, but they have an innate ability to convert into harmful structures. This is exactly how Béchamp’s microzyma, Enderlein’s protit and Naessens somatid were all described.

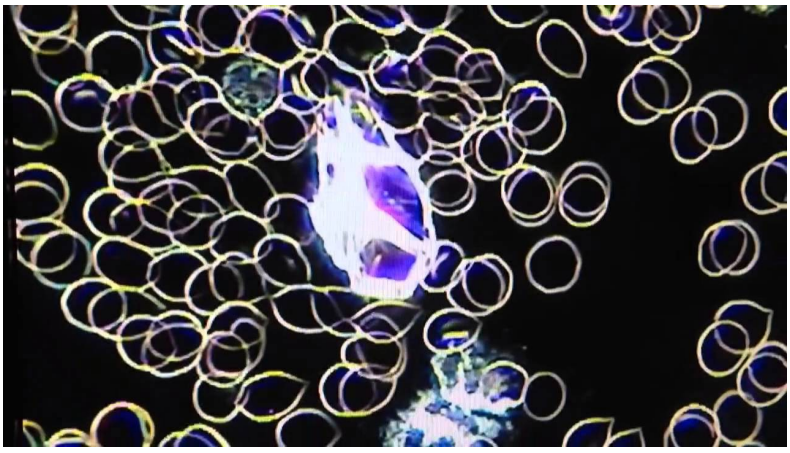
Clinically, darkfield microscopy has also been used since the 1970’s in dentistry for evaluation of dental plaque. Bacteria in plaque are difficult to distinguish with phase contrast, but darkfield enables the bacteria to be clearly identified and the patient treatment adjusted accordingly. Some dentists use darkfield as a tool to inform patients about dental plaque with the aim of improving oral hygiene habits.

Darkfield microscopy is recommended as an adjunct to the IgM ELISA test for leptospirosis diagnosis and darkfield examination of diarrheal stool specimens is useful in the diagnosis of Campylobacter enteritis (Paisley, Mirrett, Lauer, Roe, & Reller, 1982). Darkfield microscopy is also being successfully used to monitor commercial yeast cultures and Wei et al. (Wei, You, Friehs, Flaschel, & Nattkemper, 2007) state “the

contrast of the images is higher than those taken by a light field in situ bright field microscope” allowing for more accurate monitoring.

Crystals

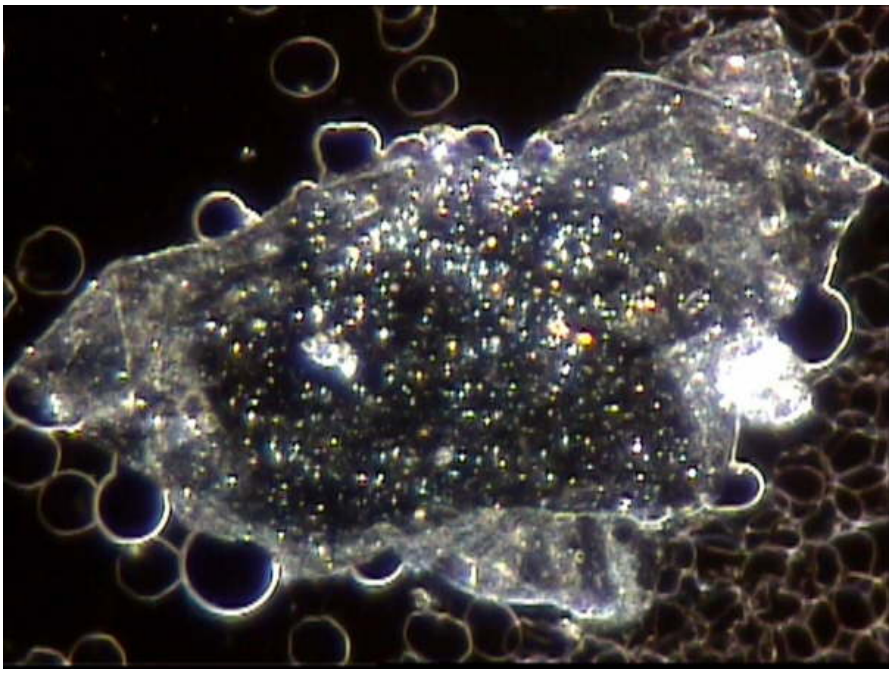
“Crystals” seen in the blood that appear like chards of glass are usually composed of various lipids (i.e. cholesterol and triglycerides). However, some may be made up of metabolic acids, nucleic acids, and cellular waste deposits (uric acid) or an amalgamation of some of the above. Crystal formations vary in size, shape and color depending on their composition. One can reasonably assume that the more debris in the blood, the more potential debris that has deposited into the vessel walls and extracellular matrix.



Crystal in Darkfield

Symplast

The aggregation of any combination of blood elements is simply called a symplast. Colloid symplast are often seen and are a combination of excess unassimilated nutritional reserves, free colloids, chemicals, toxins and any cellular waste that is occurring in the blood. Another type of symplast are RBC symplast where the RBCs are being pulled together by microbes that have invaded them. There are also thrombocyte symplast where the platelets create a large aggregation.



Colloid Symplast

This monograph has attempted to review some basic aspects of live blood examination in darkfield. Entire fields of medical specialization deal exclusively with blood – foremost among them being hematology. No tissue and no other body fluid has been studied more closely than blood. Few medical techniques permit deeper insights into the functions and dysfunctions of the human organism than the microscopic analysis of a person’s blood. Live blood analysis has established itself as an independent field of hematologic specialization that allows for quick review of health status and numerous functional and qualitative blood properties.

Most modern microbiologists, being monomorphists, would doubtless assume the examples of bacterial life cycles and pleomorphism described herein are merely the result of a mixture of wild speculation and contaminated cultures. However, recent research and literature on pleomorphism remains intriguing, and some aspects are certainly worthy of reappraisal. Furthermore, the use of modern molecular techniques will no doubt help clarify any lingering uncertainties arising from the historical literature on pleomorphism, although those certain of the phenomenon's validity would doubtless argue that their claims could be confirmed by simple, if thorough, microscopy.

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