Three-dimensional cytometry and reconstruction of the relief of red blood cells in the reflection contrast

Contents:

When reflection contrast is a light microscopic illumination method which was developed in the 70s by the company. Ernst Leitz in Wetzlar. First publications on this at the time, new microscopy method appeared in 1977. The reflection contrast allows the study of biological structures in incident light illumination using immersion objectives with high aperture and magnification. The illuminating rays strike at an angle from the lateral to the object through reflection, absorption and interference Education created the imaging beam path. Illuminating and imaging beam path each extend through the used microscopic lens. Similarly as in the fluorescence microscopy, the contrast of the reflection light efficiency is low. Therefore, high-energy lighting devices have to be used to achieve sufficient image brightness.

Not coplanar opaque structures show when illuminated in reflection contrast interference lines, which can be used for a three-dimensional quantitative analysis of each object.

(: Prof. Dr. med Kurt Fleischhauer, Head Light Microscopy: Director Prof. Franz Pera..) In cooperation with the Institute of Anatomy at the University of Bonn, I have from 1978 - 1982 carried out experimental work with this new light microscopic examination method. Based on wave-optical calculations, a method has been developed to use the reflection contrast to the three-dimensional quantitative analysis of opaque structures and create scale spatial reconstructions of the surface relief of such structures. As a model for the development of this method of measurement, first served Normal human erythrocytes, in the further course also very complex deformed pathological erythrocytes from patients with hereditary spherocytosis, sickle cell disease and thalassemia.

Using the example of the erythrocytes, which were present in stained and unstained Blutausstrichpräparaten, a calculation model was developed to describe the surface relief of the respective cells quantitatively. It was worked out that by using monochromatic green light (wavelength: 546 nm) of the distance between two similar interference lines corresponds to a height difference of 226 nm. Based on this metric methods have been developed by means of cartographic character sets to create three-dimensional reconstructions of cell reliefs.

In addition, a calculation method has been developed to infer the absolute layer thickness by a comparative study of the same object with varying wavelength of the illuminating beam.

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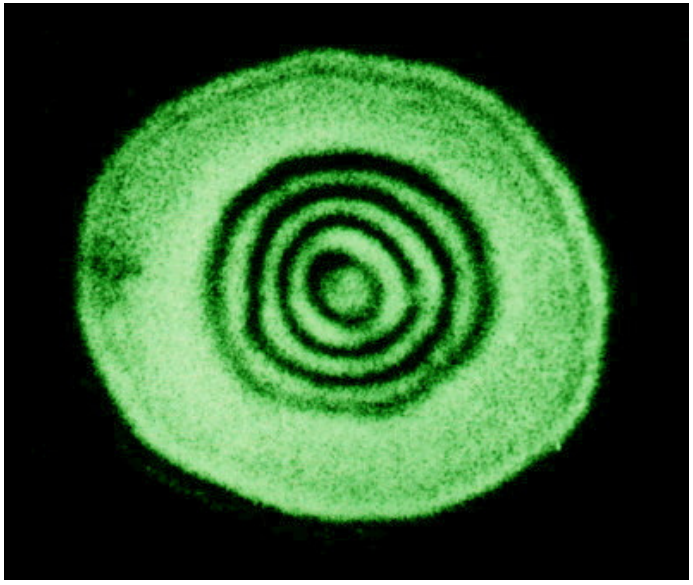
In further work also immediately visible three-dimensional representations of cell reliefs were realized through interventions in the microscopic beam path, which are similar to interference microscopic and scanning electron microscopic images.

The respective individual measurements and three-dimensional reconstructions were in a for light microscopic images remarkably high magnification range of 10,000: 1 rate.

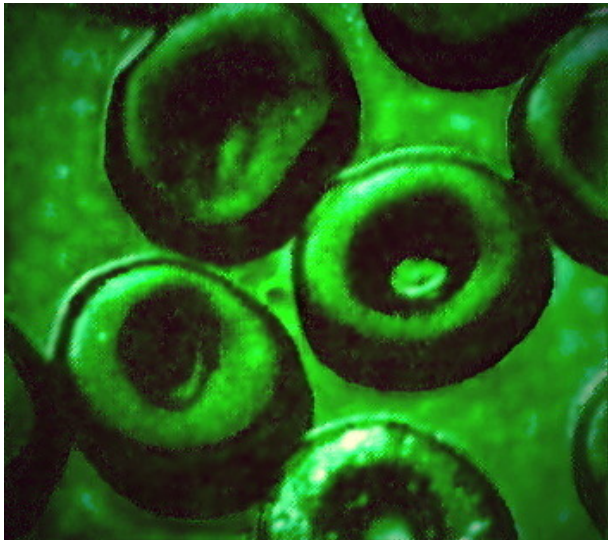
The developed methodology was validated by comparing measurements by scanning electron microscope, transmitted light interference microscope according to Mach-Zehnder and comparative analysis of microsphere of known size.

In collaboration with the department of medical angiological University Polyclinic Bonn (Director: Prof. Dr. G. pulp stone) the methodology developed in patients with arterial occlusive disease, Raynaud's syndrome and pernicious anemia was used. In these studies, a semi-automatic image analysis has been implemented. It could be shown that the deformability of the erythrocytes increases in the aforementioned disease patterns. This could be interpreted as a possible cellular compensation mechanism in order to contribute through optimized erythrocyte rigidity to improve disease caused disturbed microcirculation.

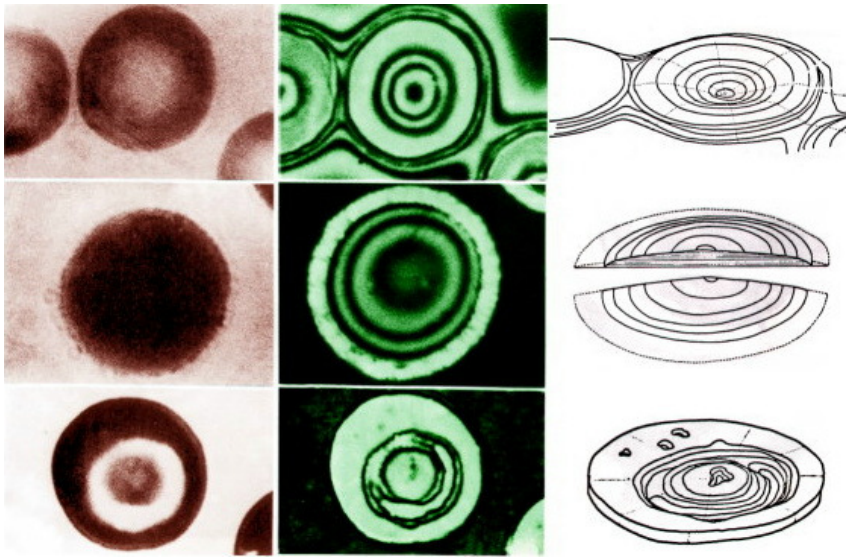


Dyed in erythrocyte reflection contrast (default) Magnification of the original image: 10000: 1, monochromatic green light, wavelength: 546 nm

Dyed in erythrocyte reflection contrast (default)
Magnification of the original image: 10000: 1,
monochromatic green light, wavelength: 546 nm.
The distance between two similar, ie lighter or darker
Interference fringes corresponding to a height difference of 226 nm.
From bead to the center this results in the present
Case, a depth of the central indentation von4x226 = 904 nm.



Colored erythrocytes in modified reflection contrast
(Real three-dimensional representation of cell reliefs)
monochromatic green light (see above).



Three-dimensional reconstructions of normal and pathological erythrocytes
Left Column: Conventional transmitted light illumination (phase contrast)
Middle column: Reflection Contrast (Standard view)
Right column: Scale-based cartographic Rekonsruktionen
Above: undyed normocyte
Middle: Dyed Sphaerozyt (spherocytosis)
Bottom: target cell (thalassemia).

Meaning:

Conventional light microscopy allow only a two-dimensional measurement of biological structures. A three-dimensional quantification of the surface relief of cells is not possible with this method. Even real three-dimensional considerations on the type of scanning electron microscopy does not allow the conventional light microscope illumination method. Only in transmitted light interference contrast can be achieved three-dimensional relief-like images; Here is but pseudo-three-dimensional effects, which are determined by the optical density of the object.

First time since the reflectance contrast there was a method available that enabled real spatial representations in the light microscope according to the laws of reflection and absorption. The developed optical wave calculation methods formed the basis to realize by means of the reflection contrast real measurements of vertical height differences in the end zone by light microscopy of magnifications. In contrast to the electron microscopic method of reflection contrast basically allows the study of living cellular structures.

In this way, the reflection contrast was suitable, the gap between light - close and scanning electron microscopy.

Project related publications:

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Morphological Quantitative Analysis of Erythrocytes by Reflection Contrast Microscopy.
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Leitz-msg-Wiss. . u Tech, Vol VII, No. 7, 230 -... 234 (1980)

Pera, F. Piper, J., customer, V .:
Morphometric studies of deformation of blood cells during Ausstrichpräparation.

Pera, F. Piper, J., customer, V .:
Morphometric studies of deformation of blood cells during Ausstrichpräparation.
. Verh Anat Ges 76, 147 -.. 148 (1982)

Wilgalis, M., Pera, F., cloudy stone, G. Ludwig, M., Piper, J .:
Erythrocytenverformbarkeit with semiautomatic image analysis and reflection contrast technique in arterial occlusive disease, Raynaud's syndrome and pernicious anemia.
German Society of Angiology (1982)

Piper, J .:
Qualitative and quantitative morphological analysis of normal and pathological erythrocytes in reflection contrast microscopy - Dissertation