Colour visualization of red blood cells in native smears by the new method reflected light microscopy

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As a rule to get color contrast image of biological samples under optical microscope need to use a variety of dyes and fluorescent substances but it leads to artificial staining of sample, destructive modification and loss very important structural information its native structure. Usually in medical practice conventional bright-field microscopy let us see black and white image of separate morphological elements of blood smears only (Figure 1a). Proposed in this paper the new nondestructive method of optical microscopy allows to examine the structures of living cells in their natural colors without its staining by using a specially designed substrate for deposition of biological sample and observing a native blood smears in reflected light. This method based on physical phenomena of white light interference reflected from sample surface and special supporter on which this sample is deposited. It allows to occur at the image plane converting previously invisible gradients of refractive index within the specimen in to intensity gradients in the image. Color interference contrast image is achieved due to special condition of experiment is connected with chose of angle of incidental light, wave length of light of reflected ray, chemical composition of sample, thickness of sample, refractive index of sample, refractive index of substrate, chemical composition of substrate [1,2]. If any conditions of the experiment are fixed beside of chemical content of sample we can say that color contrast is caused by chemical compounds. On the Figue1(b) red color of erythrocytes correspond hemoglobin. Therefore from this picture we can see visually content of hemoglobin in blood smear. Similar we can determine other chemical compounds after calibration color scale by alternative methods. To demonstrate the potential usefulness of this method, we provide qualitative data describing color image of healthy and pathological damaged cells for alive and dry blood smears (Figure 1a-1e).

Comparison Figure1a and Figure1b for same samples but obtained by conventional bright-field microscopy and by using new method correspondently showed distinguishing in color not only separate red blood cells but distinguishing difference parts in area separate erythrocytes too.

Usually for healthily individuals a albuminous aureole around the erythrocytes are mainly white-yellow (Figure 1c), but for cancer cells (core rectal cancer) the aureole color is quite different and reflects significant changes in chemical composition of both internal, and external contents of erythrocytes (Figure 1d, 1e).

Easy detection of organic shells around blood cells in our case is evident. Operations by fixing, smear coloring, prolonged processing, the availability for phase-contrast or interference microscope, special illuminators, radiating the exciting short-wave light beams are not required. Interferometric coloring of blood elements occurs on a surface of specially selected substrate. Corresponding colored images of blood elements are formed due to

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interference phenomena occurring under interaction of light beams reflected from front and back surfaces of blood elements, smeared on a substrate.

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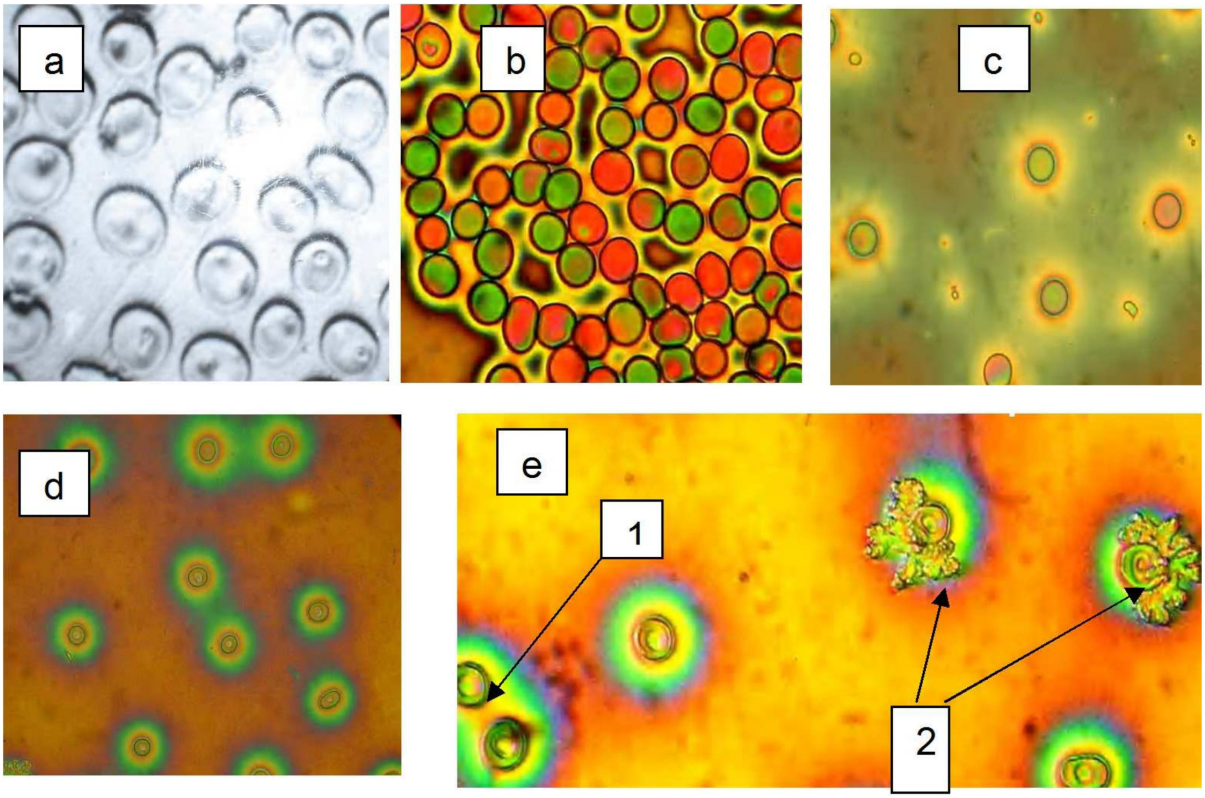

Figure 1. a- bright-field image of erythrocytes of healthy individuals; b-color image same blood sample; c-erythrocytes of healthy individuals. Different colors can be seen in certain areas of an individual red blood cell. d- and e- living erythrocytes of a patient with diagnosed core rectal cancer. Images show a multi-layered pattern of interference picture. Differences between the coloration in left (d) and right image (e) caused by different experimental conditions. We can see double cells (1) and damaged cells (2), both are pathologic.

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