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Letter to the Editor

Determination of erythrocyte flow velocity by dynamic grey scale measurement using off-line image analysis

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Determination of erythrocyte flow velocity is an important parameter for microcirculatory research. We established an off-line method for determining erythrocyte velocity in microcirculation by computerized dynamic grey scale analysis. In principle, the method is based on the dual-slit photometric technique [1], and updated by software image analysis. We have applied high speed video recorder (FASTCAM-ultima APX, Photron, San Diego, CA) to capture digital images for mesentery microvessels in rat and rabbit since 2004. Each series of images during 10 seconds is captured at a speed of 2000 frames/s (fps) and is composed of images of 704 $\times$ 576 pixels with 256 grey levels. The video recording is converted to AVI format required by the software of ImagePro Plus (versions 4.5 or 5.0). The video is then investigated frame by frame with the help of the "Sequencer Toolbar" in the menu of ImagePro Plus. The criterion for selecting an investigative segment in a capillary is that the erythrocyte flow keeps smooth and steady for 10 seconds at least. A reference line perpendicular to the axis of the capillary is setup to show the grey scale distribution along the radial line in the first frame of image. Then, a down-stream measuring line perpendicular to the axis of the capillary is setup with a distance about 10 μm apart from the reference line. The dynamic grey scale changes at the measuring line in the subsequent images are monitored frame by frame and the changes are compared with the grey scale distribution at the reference line in the first image (Figs 1 and 2). When the most matchable grey scale with the reference distribution is detected, the time interval for erythrocytes flowing from the reference line in the first frame to the measuring line in the "matchable" frame can be figured out by multiplying the frame difference by the duration between each frame. The erythrocyte velocity can be easily calculated by dividing the distance between the reference line and the measuring line by the time interval (Fig. 2).

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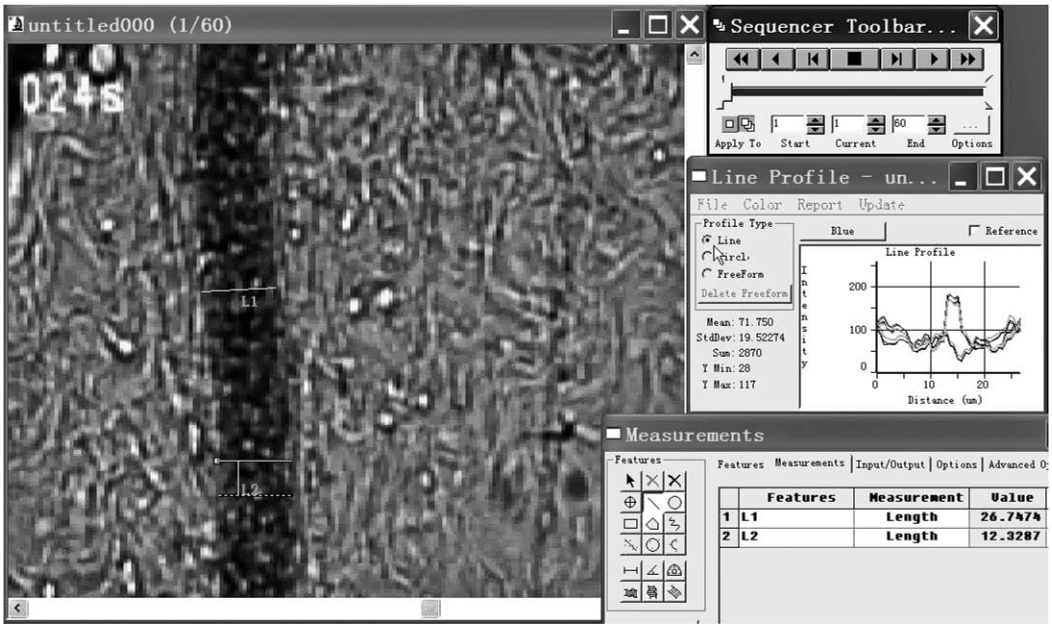

Fig. 1. Example microscopic image of blood flow in a capillary of rat mesentery recorded at 2000 fps. The diameter of the vessel (L1) is 26.7 μm, and the distance (L2) between the radial measuring line (the solid upper line at L2) and the radial reference line (the doted lower line at L2) is 12.3 μm. The grey scale distribution for the reference line is shown as the doted lines in the "Line Profile" (see the middle of the right side in the figure). The grey scale distribution for the measuring line is shown as the solid lines in the "Line Profile". The erythrocyte flow direction is from the bottom to the top.

In a set of our early data of forty investigations for rat mesentery, the diameters of the selected capillaries are distributed from 20 to 55 μm, the mean and standard deviation are 30.7 ± 11.0 μm. The average distance between the reference line and the measuring line is 11.5 ± 3.93 μm. In fact, the distance between the two lines cannot be arbitrarily expended since the grey scale distribution of erythrocyte at the measuring line could not be matchable with the one at the reference line when the distance is extended too much. Therefore, a distance of about 10 μm is recommended. The erythrocyte velocities in that data set are determined by the suggested method, showed in the range of 714 to 2637 μm/s. There appears to be a linear correlation between the erythrocyte velocity (Y) and the vessel diameter (X): $Y = 937.9 + 16.2X$ ($r = 0.3439$, $p = 0.03$).

Although dual-slit is an old principle for measuring erythrocyte velocity [1], and some new real-time and automated approaches with hybrid analog–digital systems are recently developed (such as the instrument described in reference [2]), the videomicroscopic methods with off-line computerized analysis of microcirculatory parameters still have some advantages for *in vivo* microvascular research [3]. However, a limitation of the general videomicroscopic methods is that higher velocities of erythrocytes cannot be measured using standard video framing rate (25 fps). For the method described here, high speed video recording is the basic requirement. As estimation, the measurable high limits for erythrocyte velocity will be 20,000, 10,000, 1000 and 250 μm/s when video framing rates of 2000, 1000, 100 and 25 fps are used accordingly. On the other hand, the video framing rate does not need to go beyond 2000 fps in general cases since a unnecessary high speed may cause difficulties in fitting grey scales at the measuring line with that of the reference line: there may appear a number of images at the measuring line with similar grey scale distribution "matchable" with the reference.

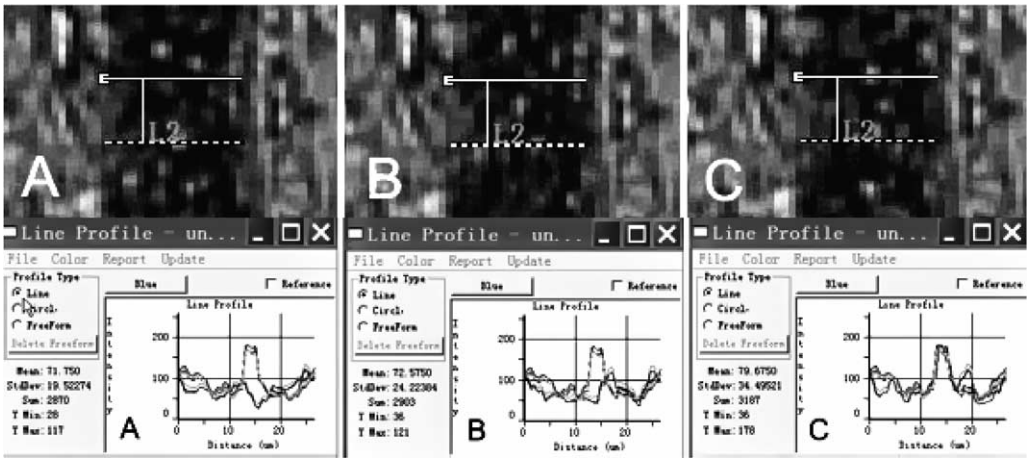

Fig. 2. The matching process of grey scale distribution at the measuring line with that at the reference line for Fig. 1. For better view, the microscopic images are locally enlarged and put in the top row (A, B and C in white). The measuring line is indicated as the white solid lines in the images, and the reference line is expressed as the doted white line in the images. Selected from the sequence of the recorded images, the 1st image (A), the 14th image (B) and the 26th image (C) are at the lift, the middle and the right positions in the top row. The grey scale distribution at the measuring line and the reference line are in A, B and C (in black) in the bottom row accordingly. The fixed reference grey scale distribution is the doted curves with a middle peak in the bottom profiles, showing the grey scale at the radial reference line of the 1st image. The grey scale distributions at the measuring line are expressed as the solid lines in those profiles. The grey scale profile at the measuring line for the 14th image is un-matchable with the reference profile as illustrated in "Line Profile" B. The grey scale profile at the measuring line of the 26th image is basically matchable with the reference profile as illustrated in "Line Profile" C. Therefore, the reappearance of the grey scale at the measuring line indicates that the local erythrocytes in blood at the reference line in the 1st image flow to reach the measuring line in the 26th image. The interval between the 1st image and the 26th is 0.0125 s (0.0005 s $\times$ 25). Finally, the erythrocyte velocity can be calculated by dividing 12.3 μm by the interval of 0.0125 s, i.e., an erythrocyte velocity of 984 μm/s.

Apparently, the measuring principle of the above method is reasonable since it is based on the original definition of velocity. The method allows the routine frame by frame method to be objectively judged with computerized evidences. We believe that the method is practical for determining erythrocyte velocity when a high speed video recorder and a suitable image software are ready in a laboratory. As some recently designed consumer cameras can take high speed digital video at 1000 fps, the method appears to be more feasible in practice.

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