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Vessel motion measurement in real-time using movement detection at multiple regions of interest

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Abstract

A vessel motion detection algorithm has been implemented for the measurement of multiple lymphatic chamber diameters via pre-recorded VHS tape (PAL, 25 Hz). The method differs significantly from previous attempts based on edge-detection, in that it employs center of mass of pixel brightness in the vessel wall based on a Kalman filtered image subtracted from a Kalman filtered background. The operation of the algorithm confirms that it is able to detect accurately the full constriction–relaxation cycle of the vessel and produce data that meets high standards of tolerance. The procedure is well suited for real-time measurement of lymphatic and blood vessel constrictions in vitro or in vivo at multiple sites so long as the object under measurement is sufficiently differentiated from its background.

Keywords: LabVIEW; Vessel motion measurement; Lymphatic vessels; ROI; Kalman filter; Video analysis; Real-time

1. Introduction

With the advent of fast, affordable computers, acquiring real-time data from in vitro biological systems via video has become increasingly efficient. If standard techniques such as region of interest (ROI) and Kalman filtering are used, multiple objects can be processed for motion analysis in a timely manner. Further optimization can be achieved through the implementation of a pseudo-parallel architecture. In this design, an indeterminate sub-population of data from each ROI is processed in non-serial fashion. The processing continues until completion of one cycle—one cycle typically being one video frame. In this way, the data from each ROI completes at approximately the same time.

This paper reports a revised method of measuring changes in diameter of lymphatic vessels at multiple selected sites using a PC equipped with a frame-grabber for capturing PAL (25 Hz) images from VHS video. An early simple method demonstrated that by carefully picking the darkest pixels on the vessel wall without any filtering, the edge of the vessel wall could be tracked and plotted (Neild, 1989). This method requires that the vessel be presented to the user in a perfectly horizontal position by rotating the camera. A more recent method used a simplified "radar" tracking technique (Beresford-Smith et al., 1993). Tracking is achieved by estimation of vessel wall motion using a technique similar to that used in tracking aeroplanes on radar screen, and is based on Kalman filtering. The technique is simplified by employing tracking in only one dimension. The position of the darkest pixel in the vessel wall is used to track the movement of the wall. However, the Kalman filter assumes the background is random noise. If differential filtering is not used, the background is not random. Differential filtering is when the image differential (current image *minus* previous image *divided by* the time difference between images) is the significant part of the filter. At subtraction, the signal-to-noise-ratio can significantly increase; therefore, it is necessary to use Kalman filtering on the images before subtractions.

Increased, inexpensive computer processing power has allowed further improvement overcoming present limitations. Here we describe a method to reliably track vessel edges in multiple cross sections (up to 10 are considered here), in this case used to investigate lymphatic vasomotion by showing real-time phase and frequency data. Real-time here is defined after Weeks (1996): "If the processing unit can manipulate the image and store it back in the processing memory at a rate faster than the video frame rate (30 frames per second) and process the

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next frame of image data when it appears, this imaging system is referred to as a real-time imaging processing system." Several critical hurdles had to be surmounted to achieve this aim. First, despite the availability of a powerful processor, the software algorithm had to be optimized to enable timely processing of multiple data inputs. In our case, this was achieved using a pseudo-parallel architecture as described above. Second, ROI selection needed to be flexible; in short, allow the user to position the ROI at any angle they wished, where angle is defined as the longitudinal axis of the vessel. Third, to minimize noise, we needed to use the information from the entire ROI, temporally filter the ROI, and subtract the ROI from the filtered background image. The implementation of this last step meant that we did not have to apply a smoothing filter on the position of the vessel walls to acquire accurate diameter measurements. Finally, to measure vessel wall motion, we employed a center of mass of pixel brightness on the vessel wall. This technique differs significantly from traditional edge-detection methods in that it does not attempt to track the edge of the vessel wall. This technique proved to be very reliable, and has the additional benefit of facilitating measurement down to 0.2 pixels (1.5 μm), neither of which the other methods produce.

2. Materials and methods

2.1. Ethics

This research was approved by the University of Newcastle Animal Care and Ethics Committee.

2.2. Tissue preparation

Young guinea pigs (4–12 days of age) of either sex were killed by an overdose of the inhalation anesthetic, isoflurane (5–10% in air) followed by decapitation. The mesenteric lymphatic vessels (diameter <350 μm) from the ileal region of intestine with associated artery, vein and surrounding mesentery were dissected and placed in a dish containing physiological saline solution: NaCl 120 mM, KCl 5 mM, $CaCl_2$ 2.5 mM, $MgCl_2$ 2 mM, $NaHCO_3$ 25 mM, NaH_2PO_4 1 mM and glucose 11 mM. The mesentery was pinned flat onto the Sylgard-coated base of the organ bath (volume 1.0 ml). The mesentery was then transferred to the stage of an intravital microscope. The tissue was continuously superfused at a rate of 6 ml min^{-1} with physiological saline solution maintained at a pH of 7.4 by bubbling with a 95% O_2 and 5% CO_2 gas mixture and heated to 35 °C. After 30 min of incubation with physiological saline solution, the collecting lymphatic vessels were continuously perfused through a glass cannula with low concentration calcium physiological saline solution ($CaCl_2$ 1.25 mM) at a rate of about 2.5 μl min^{-1} to induce rhythmical vasomotion of the lymphatic vessels. The constrictions were observed through an objective lens (4×) and video camera (Panasonic, WV-CP210/G) attached to an inverted microscope (OLYMPUS, IMT-2). They were recorded on a videocassette recorder (Panasonic, AG7355). Changes in the frequency of the lymphatic vessels were measured either visually and/or by the computer edge-detection algorithm.

2.3. Software and hardware

Video data was fed into a PC (Windows XP platform, 2.53 GHz processor) running LabVIEW (National Instruments) software. The user interface and processing algorithms were developed using LabVIEW 7.1 in conjunction with the LabVIEW Vision 7.1 module. Frames were acquired by an IMAQ PCI-1409 (National Instruments) frame-grabber card, producing PAL, 25 Hz 8-bit 768 × 576 grayscale images.

2.4. Region of interest selection

User-selected regions of interest were laid over the 768 × 576 image, producing sub-images which could be efficiently processed. A user ROI is created by two mouse clicks running center-line along the inside of the vessel (Fig. 1). Both height and width can be individually pre-adjusted for each ROI. Multiple diameter measurements are then obtained simultaneously from these ROIs using a pseudo-parallel architecture based on temporal filtered differential image pixel brightness weighted center of mass.

The program design comprises two main modules: ROI filtering (including vessel wall measurement), and interval detection. Finding the vessel wall and measuring its movement was accomplished by use of a simple Kalman filter on temporal image brightness. Rather than taking measurements from the moving edge (usually the leading edge), we chose to use the entire wall based on the calculated pixel brightness. By calculating center of mass of the brightest pixels, we were able to detect movement down to 0.2 pixels (for further discussion on this issue see Section 4.2). To accomplish this, we applied two Kalman filters on the image in parallel: one set at a long duration to create a 'background' image ($K = 1/64$), and another set to a short duration ($K = 1/8$) to create a 'filtered image'. This second faster filter was necessary because we needed to reduce noise from sources such as photon, camera, VHS tape and the digitising process. We then subtracted the filtered image from the

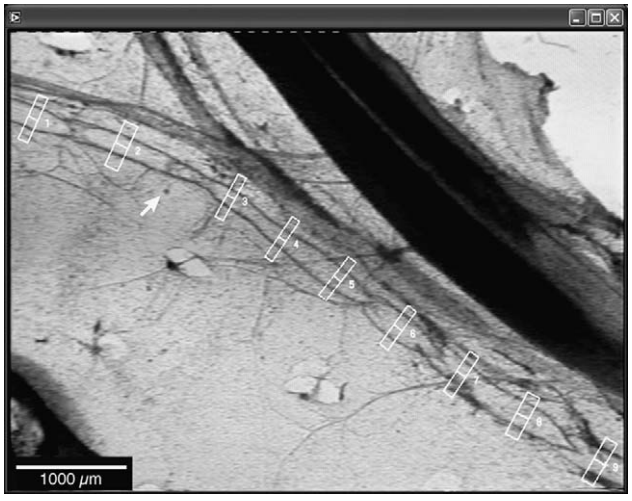

Fig. 1. User selected ROIs (1–9) on the computer monitor generated 768 × 576 grayscale video image. Each ROI is 60 pixel in height (but can be individually adjustable); width also varies according to user selection.