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# VESSEL DIAMETER TRACKING IN INTRAVITAL MICROSCOPY IMAGE SEQUENCES

Jaesung Lee<sup>1</sup>, Artit Jirapatnakul<sup>1</sup>, Anthony P. Reeves<sup>1</sup>, William E. Crowe<sup>2</sup>, and Ingrid H. Sarelius<sup>2</sup>

<sup>1</sup>School of Electrical and Computer Engineering, Cornell University, Ithaca, NY

<sup>2</sup>Dept. of Pharmacology and Physiology, University of Rochester Medical Center, Rochester, NY

## ABSTRACT

A new feature-based tracking algorithm was developed for measuring diameter of vessels in intravital video microscopy image sequences. Testing was performed with ten synthetic image sequences with known diameters and ten intravital microscopy sequences. The automatic measurements were compared with the manual measurements using linear regression analysis. The regression analysis indicated that our method is in agreement with manual measurements by human observers. The reproducibility of the method was evaluated based on Bland-Altman analysis. Bland-Altman statistic for the automated method was narrower than for the manual measurements, indicating that the automated method has a better reproducibility than human raters.

Index terms—intravital microscopy, microcirculation, vessel diameter, feature tracking

## 1. INTRODUCTION

In microcirculation studies, the blood vessel diameter is often measured to help quantify the effects of various stimuli [1]. Typically, intravital video microscopy is used to observe blood vessels in vivo [2]; video image sequences are recorded of the subjects and later analyzed to obtain diameter measurements. Image sequences frequently span several minutes of time resulting in the acquisition of several thousand images. The current manual measurement method is for an operator to measure the diameter with electronic calipers in every image frame, a time-consuming task that is prone to measurement error and operator fatigue. In this paper a computer algorithm is presented which, once the diameter is marked in the first image frame, automatically computes the diameter for all subsequent image frames. The automated method uses an image feature tracking algorithm based on the seed points in the first image frame.

While the specific task of measuring vessel diameter in intravital microscopy image sequences using computer vision techniques has not received much attention, there have been many proposed algorithms and techniques for object tracking and vessel measurements for other applications in other imaging modalities. Magers et al. [3] proposed a method for measuring microvascular diameters in video microscopy.

Their algorithm used feature tracking with cross-correlation in one dimensional search space. Their method would not be able to deal with the shifting of the vessel due to the constrained search space. Sonka et al. [4] developed an automated method for analysis of brachial ultrasound image sequences. The vessel borders were detected in the image sequence using the globally optimal graph-search algorithm, and the vessel diameter was estimated from the border locations. Tynml et al. [5] proposed a method to measure arteriolar diameter and hemodynamic resistance in intravital video microscopy images. Their algorithm would require several markings of each edge along the lumen on every image frame, thus making it unsuitable for automatic measurement in a sequence of multiple frames.

The algorithms using edge detection to measure the vessel diameters work well only if there is a clear distinction between lumen and tissues surrounding the vessel. The previous methods using feature-based tracking cannot deal with shifting of the vessels which is common in the intravital video microscopy images. Our work focuses on the accurate measurement of the vessel diameter using image feature tracking that can deal with the shifting vessels and uses the appropriate features.

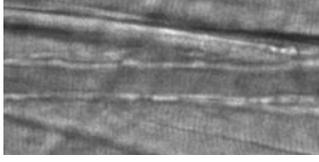
## 2. METHODS AND MATERIALS

### 2.1. Image Acquisition

A set of ten grayscale videos of blood vessels of unknown diameter were obtained from intravital microscopy at different vessel locations. The microvasculature was observed with an Olympus BX50WI microscope. The microscope image was displayed via charge-coupled device camera (Dage MTI CCD72S) and recorded on a DVD-RW using Sony DVO-1000MD. Final magnification of the site was 1420x with a resolution of 3.66 pixels/ $\mu\text{m}$ . The videos were sampled at 30 frames per second to obtain sequences of image frames.

A rectangular region of interest was clipped from each image sequence and rotated to position the vessel horizontally (Figure 1). An interactive user-friendly tool has been developed for preparing the intravital images of vessel.

For determination of accuracy, ten synthetic image sequences were created. The synthetic sequences were created



**Fig. 1.** Intravital microscopy image prepared for tracking. First frame of the sequence is shown.

by taking a portion of the background from a real image sequence and overlaying two higher intensity lines meant to represent the vessel walls. The vessel wall width and diameter were varied along the vessel. To better model the intensity profile of the real vessel walls, Gaussian blurring with a  $\sigma$  of 1.0 was applied to each image. Gaussian noises with varying variances were then applied to the images.

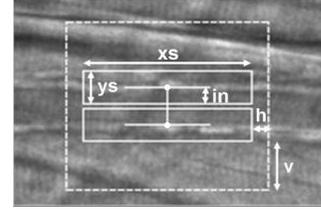
## 2.2. Image Registration

Once an image sequence is prepared, image registration is performed to reduce shifts among image frames. Each image frame is shifted appropriately so that it yields the least difference when compared to the first frame of the sequence. The sum of squared differences in pixel values was used to compare image frames. Registering the image sequence results in less movement of the vessel and therefore allows for smaller search space and faster tracking.

## 2.3. Patch Definition

After the image registration process, the user must define two starting points in the first frame, one point on each side of the vessel lumen. These seed points should form a line perpendicular to the lumen. For each seed point, a surrounding box is established, and we refer to this box as “patch” throughout this paper. The properties of the patches are determined by the user-specified parameters (Figure 2). The algorithm attempts to locate the same two patches in new frames based on the reference patches. The reference patch can be the corresponding patch in the first frame of the sequence, previous frame, or the blend of both. The frame to use as a reference is also a parameter that can be specified by the user.

The Euclidean distance between the two patches is considered the vessel diameter. It should also be noted that most of the interesting features lie outside of vessel walls. The blood may be flowing within the vessels, or the vessel may be too narrow so that the patches include the opposite vessel wall. The patches are shifted outward to reduce errors due to these distractions. The amount of shifting is user-specified with the parameter  $in$ , as shown in Figure 2.



**Fig. 2.** User-specified parameters. The size of patches are determined by  $xs$  and  $ys$ , and the search area is determined by  $h$  and  $v$ . The parameter  $in$  is used to offset the patches.

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### Algorithm 1 Linear exhaustive search with linked patches

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 $MSE_{min} = MAXVAL$ 
 $p1 = p(x, y_1)$ 
 $p2 = p(x, y_2)$ 
for all  $x_i \in$  search area do
   $y_{1j} = \operatorname{argmin}_y \{MSE(p1, p(x_i, y)) \mid y \in \text{search area}\}$ 
   $y_{2j} = \operatorname{argmin}_y \{MSE(p2, p(x_i, y)) \mid y \in \text{search area}\}$ 
   $MSE_i = MSE(p1, p(x_i, y_{1j})) + MSE(p2, p(x_i, y_{2j}))$ 
  if  $MSE_i < MSE_{min}$  then
     $MSE_{min} = MSE_i$ 
     $x' = x_i; y'_1 = y_{1j}; y'_2 = y_{2j};$ 
  end if
end for
 $p1' = p(x', y'_1)$ 
 $p2' = p(x', y'_2)$ 

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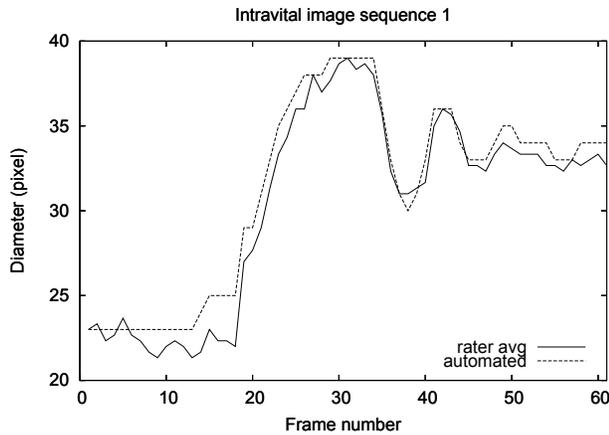
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## 2.4. Tracking

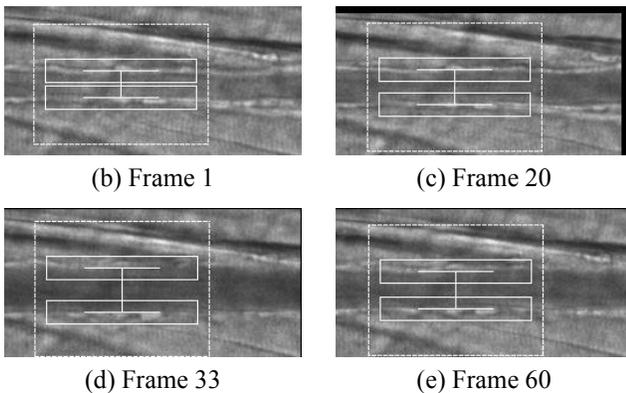
After two patches have been established, we search for a new patch in the next frame that best matches the patch from the current frame. The mean squared error (MSE) of gray levels within patches is used as a matching metric for locating the patches in image frames. The implicit assumptions are that the gray level pattern is approximately constant between successive frames and that local texture contains sufficient unambiguous information [6].

If separate feature tracking is used for each patch, it is possible that two patches will drift toward opposite directions as image frame progresses. This is especially true for image sequences with shifting vessel. This will incur inaccurate estimation of vessel diameter, as we want to measure the length of a line perpendicular to vessel walls.

To address this problem, the horizontal motion of the two patches is linked. Because the patches are linked, a new matching metric has to be introduced. Our algorithm uses the sum of MSE for two patches being considered. Instead of finding the best  $x$  and  $y$  coordinates separately for each patch, the best  $x$  coordinate is sought to optimize the matching metric for both patches, while for each patch the best  $y$  coordinate is sought separately within each  $x$  coordinate in the search space. In other words, given two reference patches



(a) Plot of diameter vs. frame

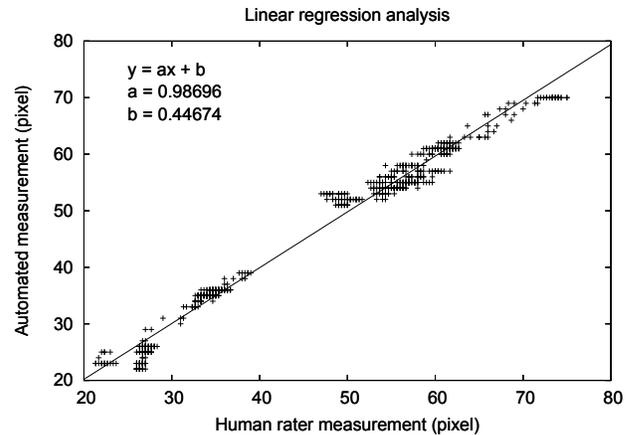


**Fig. 3.** Result for an intravital microscopy sequence. The black edge on (c) is the result of image registration. The automated measurements are within 1 pixel from the manual measurements in most frames.

$p1(x, y_1)$  and  $p2(x, y_2)$ , the best matching patches  $p1'(x', y'_1)$  and  $p2'(x', y'_2)$  in each frame are found using linear exhaustive search. The procedure for carrying out linear exhaustive search is shown in Algorithm 1. Linking the patches in this way prevents them from drifting toward opposite directions. If the patches of interest have shifted horizontally in the following image frame, the patches will both shift horizontally by the same amount.

### 3. RESULTS

The tracking result for each case was visually inspected using an image viewing tool to evaluate the correctness of the tracking (i.e. whether the algorithm tracked the vessel walls or tracked other structures). Out of ten intravital microscopy sequences, there was one case in which the algorithm failed to track the vessel walls, and this case was excluded from the analysis of the result. This particular case had a strong back-



**Fig. 4.** Regression analysis of manual and automated measurements,  $r^2=0.982$  and  $n=1022$ . The regression plot indicates the strong agreement between manual and automated measurements.

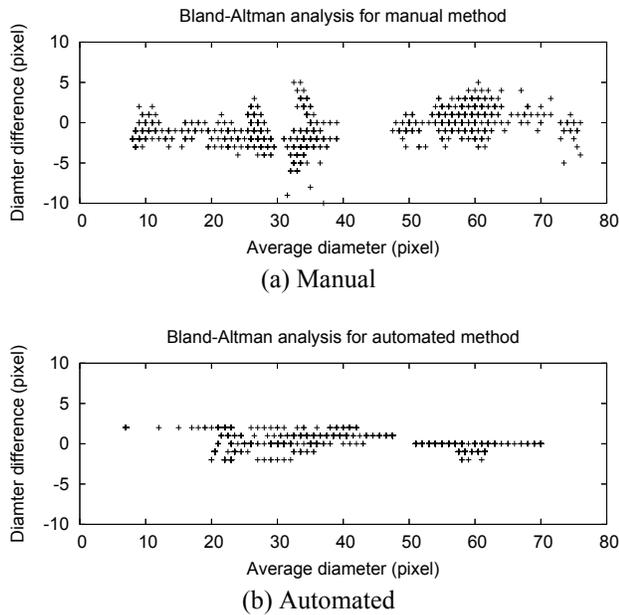
ground patterns across the lumen which interfered with the correct tracking of the vessel walls.

The resulting plot of a successful diameter measurement along with the screenshots of different frames is shown in Figure 3. To establish a point of reference for the intravital microscopy sequences, three raters manually marked intravital microscopy sequences. The interactive web-based marking interface was developed for this purpose. The rater's task was to identify the same two points in all frames for each intravital sequence.

Better tracking of vessel diameter should result in more accurate and consistent determination of the diameter. Two methods were used to compare the manual and automated measurements.

A regression analysis was used for the intravital sequences since the true diameter was not known (Figure 4). The average of measurements by three raters was plotted against the automated method's measurement. The plotted points included 1022 frames from intravital image sequences that had manual measurements by the raters. The slope of the fitted line was 0.987, and the intercept was 0.447. The correlation coefficient ( $r^2$ ) for the model was 0.982.

The reproducibility of the automated method was compared with that of the manual measurements by the raters. Bland-Altman analysis [7] was used to compare the reproducibility (Figure 5). Two automated measurements were generated with the same algorithm using different seed points and parameters. Then, using two sets of measurements, the diameter differences were plotted against the average of two. The plot for manual measurements was generated in a similar way. 95% of the measurement differences for the automated method lied between -1.97 and 2.2 pixels, compared to -4.473 and 3.1384 pixels for the raters.



**Fig. 5.** Bland-Altman analysis for two raters' measurements (a) and two automated measurements (b) using different seed points and parameters. Automated method has narrower range of diameter difference, indicating better reproducibility.

All synthetic image sequences were tracked successfully with the automated method. The ground truth diameters are known for the synthetic image sequences, and the average MSE from ten synthetic image sequences was calculated. The average MSE for the automated method was 0.0 pixel while the human raters performed with the average MSE of 1.74 pixels and the variance of 1.93.

#### 4. CONCLUSIONS AND FUTURE WORK

An accurate, robust automated method to measure blood vessel diameter is crucial in obtaining quality results in microcirculation studies. Very little previous work has been done on this task, though there has been much work on measuring diameters in other imaging modalities for applications such as measuring arteries in ultrasound images, retinal vessels, and airways in CT. A feature-based algorithm was developed for an accurate measurement of the vessel diameter in intravital video microscopy image sequences.

The preliminary results from our algorithm are encouraging. Our method was able to successfully track all synthetic image sequences in the presence of random translational movement and varying amounts of noise, yielding better MSE than human raters for all sequences. This suggested that the automated measurement is more accurate than the manual measurement. Further, the regression analysis using

the intravital image sequences indicated that the automated method gave comparable measurements as the raters. The Bland-Altman analysis was used to evaluate the reproducibility and showed that the automated method had better reproducibility than the manual measurements.

The automated diameter measurements were successful in majority of cases, and we believe that it will soon replace the current manual method with calipers. However, there are still many opportunities for improvements. The algorithm was sensitive to the background patterns from tissues above or below the focal plane of interest. Another unsolved problem with the automated method is an inability to recover from errors; once the algorithm loses track, it is unable to correct itself.

In the future, we plan to enhance the algorithm by resolving these issues. One possible solution for the background pattern is an automated patch size selection to reduce the amount of background included. To rectify the propagation of error, an error correcting mechanism with user involvement will be employed.

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