Computerized Translocation Summation

Image Processing Technique

Wilson To
Hugh Edmondson Research Fellowship
Department of Pathology: University of California, Davis Medical Center

Theory

Shifting from a subjective to an objective quantification of measuring flow velocity with the use of a computerized translocation summation program increases the degree of validity in estimating flow rate.

Design and Concept

A multidisciplinary approach in experiment and design:

A programmed algorithm mimics the concept of a photodiode; photodiodes are used to determine the intensity of light at a single pixel of a vessel in question. The program follows the pattern in light intensities throughout the given segment of the video sequence as a means of tracking blood cell flow. Because light intensities may not be consistently intense throughout the sequence, the vessel segment is isolated and analyzed using a bicubic interpolation algorithm, resulting in a collection of matrices that will estimate the intensities of light of a particular pixel and the area surrounding it. Doing so allows averaged intensities to be easily tracked and analyzed.

The video sequence is also run through a two dimensional image cross correlation matrix and projected onto a one dimensional matrix in order to maximize information supplied by the video clip. The purpose for the two matrices are as follows: the two dimensional matrix calculates the velocity frame by frame as the clip is played; the one dimensional matrix is used to calculate the flow linear change in light intensities while ignoring possible error due to frame jumps. The two means of analyses ensure that noise and artifacts do not alter the correlation peak location, but simply lower its negative effect on the outcome. Furthermore, the convergence of the correlation matrix from the two will create unique correlation peak, which can be monitored by the investigator to ensure accuracy of analysis.

To verify that the AVID program currently identifies, tracks, and processes a consistent and accurate means of object velocity, a software generated animation and mechanically generated movement was used to generate known velocities of movement features similar to that seen in blood vessels. Multiple velocities were generated as well as multiple directions of flow.

Results

In experimental batch runs, consisting of forty different one second video sequences, computations were attempted three times per video sequence to validate the consistency of the velocity program. However, it was only with sequences that were stabilized that the AVID velocity program gave fairly consistent results, with an occasional result out of range. With these results, it is recommended that three measurements be taken per sequence to confirm the relative value of the blood flow velocity. If numbers fall within 10% range of one another, then the average may be taken as the final velocity. This reason for having to take more runs is the fact that boundary selection may differ from pixel to pixel; thus, resulting in different velocities.

Velocities calculated by the previous method of analysis (VASVEL) were similar in many direct comparison tests. In video sequences with slow blood flow rate (< 0.5 millimeters / second), VASVEL was not able to calculate the velocity as accurately due to its design being subjective in analysis (inability to detect and define minimal movement). It is difficult for the investigator to track cell shift frame by frame if there is very little motion to begin with. The AVID program is able to track the cell movement to a much smaller degree than any human would be able to. However, in video sequences with normal blood flow rate or fast blood flow rate, AVID was able to calculate nearly

identical results to the VASVEL program, with differing results less than 5% of the time in trial runs. Because the investigator is able to fully track cell movement, it is easier to identify over successive frames. In doing this direct comparison, validation to a much higher degree for the AVID program's ability to provide a more precise means of estimation can be established.

Conclusions

By running a video sequence through computerized computations of AVID's algorithms for identifying, tracking, processing, and analyzing blood flow velocity, a subjective quantification can be replaced and objective-based results obtained. These results support a more precise measurement in estimating the actual value of the flow rate.

Introduction

In assessing the effects of hemodynamic forces on the endothelial lining of blood vessels seen in the bulbar conjunctiva, the measurement of blood flow dynamics is necessary.

Hemodynamic forces are associated with wall shear rate and shear stress, given by:

Wayland – Johnson Formula:

Wall Shear Rate: $WSR = 8 \times V_m \times D^{-1}$

V_m = Mean Velocity of Centerline Blood Speed

D = Diameter of analyzed vessel

Shear Stress: $SS = WSR \times \eta$

WSR = Wall Shear Rate

η = Whole Blood Viscosity

Shear stress caused by blood flow irritates the endothelial lining of blood vessels, resulting in substantial morphometric, structural, and dynamic alterations and adaptations in blood vessel properties (Cheung et al., 2001). Hemodynamic forces, as described in figures 1 and 2, add stress on the sensitive endothelial lining. Though the human body is able to actively adjust itself to maintain homeostasis with the added transient disturbances temporarily, chronic disturbances upon the endothelial lining results in endothelial dysfunction (Zweifach, 1994). In all vascular diseases, changes in blood flow velocity, blood viscosity, or vessel diameter play a crucial role in tissue physiology.

Compromise of biochemical processes and functions of endothelial cells in arterioles or veins by shear stress is characteristic of endothelial dysfunction. Normal functions of endothelial cells include mediation of coagulation, platelet adhesion, immune function, control of volume and electrolyte content of the intravascular and extravascular spaces.

Hemodynamic Forces: Wall Shear Stress and Shear Stress

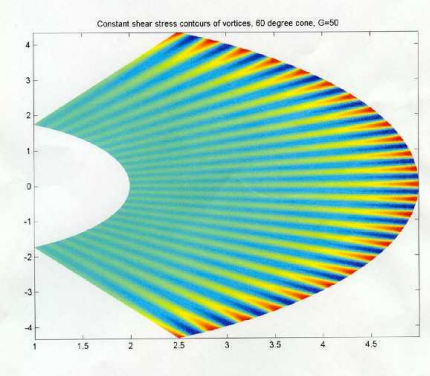


Figure 1

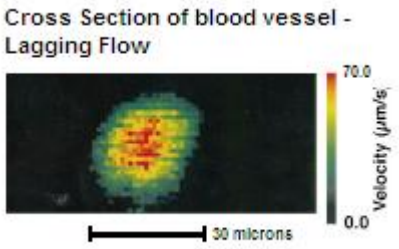


Figure 2

Figure 1. As the result of the shear force acting on the endothelial lining by red blood cells flowing through vessels, the blood cells running alongside the vessel wall begin to drag and lag behind the normal flowing blood (particularly in cases dealing with higher than average blood viscosity).

Figure 2. A cross-section of a blood vessel. Hemodynamic forces act in all directions into the endothelium. Blood closer to the center does not have an adverse effect on the vessel lining. However, the outer cells that drag along the endothelium cause irritation of vessel lining, leading to endothelial dysfunction.

Various approaches have been made to create a user-friendly and accurate system of analysis of blood flow velocity:

1. Imaging using photo by photo analysis
   Description: Overlaying successive photo stills (determined by camera's frames per second) allows the investigator to track movement over the span of one second.
   Pros: Accurate for its simplicity
   Cons: Subjective; tedious and time consuming; inability to track movement of slow flow (due to lack of observable flow movement between photos)

2. Imaging using digitized frame by frame analysis (VASVEL)
   Description: Computer assisted analysis projects frame by frame digitized images (at given standard 29.9700 frames per second) where the investigator is allowed to select points to analyze through a single-step acquisition multiple-frame tracing algorithm.
   Pros: Computer assisted in selecting points
   Cons: Often difficult to select desired pixel (accuracy of selection); inability to track movement of slow flow (due to lack of observable flow movement between frames)

3. Imaging using lasers and ultrasound (Doppler Effect)
   Description: Laser Doppler Flowmetry works by illuminating the tissue under observation with low power laser light from a probe containing optical fiber light guides. Laser light from one fiber is scattered within the tissue and some is scattered back to the probe. Another optical fiber collects the backscattered light from the tissue and returns it to the monitor. Most of the light is scattered by tissue that is not moving but a small percentage of the returned light is scattered by moving red blood cells. The light returned to the monitor undergoes signal processing whereby the emitted and returned signals are compared to extract the Doppler shift related to moving red blood cells.
   Pros: Ability to detect pulsatile feature of blood flow; able to take various readings at the same time (blood pressure, etc.)
   Cons: Inability to differentiate between various cells in the body (red blood cells and white blood cells); patient movement may cause error in reading

4. Imaging using photodiodes and diffraction gradients (Interference Pattern Recognition)
   Description: The concept of a dual slit diffraction gradient utilizing two parallel arrays of photodiodes (one "downstream" from the other) is used. As sets of features pass each slit, the changes in the intensities of light are recorded, and the one dimensional cross correlation between these two arrays is used to compute the time it takes for the same light intensity pattern to be observed by the second array.
   Pros: Objective analysis
   Cons: Possibility of uneven lighting surfaces affecting results in intravital studies; inability to account for possibility of two dimensional shifts during video sequence

Although previous methods of estimating blood flow velocity have been utilized to analyze the microcirculation, all have erred because they were based on subjective analysis. Selected points of a blood cell were chosen by the investigator and followed between subsequent frames. In doing so, only a satisfactory estimate can be obtained because of the lack of consistency in tracking the point over successive frames. Though the estimate may be close to the actual value, the subjective analyses of these tests hinder the possibility of it being accurate.

In the development of this program, it was hypothesized that shifting from a subjective to an objective quantification of measuring flow velocity with the use of a computerized translocation summation program would increase the degree of validity in estimating flow rate.

Design and Methods

Obtaining Images / Sequences
Computer Assisted Intravital Microscopy:

The microcirculation of the bulbar conjunctiva was videotaped in each patient using a charge-coupled device (CCD) video camera (COHU Model CCD-6415-3000). A fiber-optics light source (Fiber-Lite Model 3100) with a Kodak #58 Wratten anti-red (green) filter was used as a supplementary light source on the peri-limbal vessels of the bulbar conjunctiva to enhance image display.

The procedure was recorded by a Super VHS video cassette recorder (Panasonic AG5700) onto video tapes (TDK HS Premium) and is kept on permanent record. Processing video clips requires analyzable sequences, which are defined by the investigators during pre-processing. Sequences are transferred from cassettes using a different video cassette recorder (Panasonic AG 3200) through Separate Video (S-Video) connections to the Image Processing Station computer (Dell Dimension 4100).

Hemorheology: Obtaining Viscosity
Venous blood drawn for studies is immediately processed to determine blood viscosity (typically within the first hour). For the purposes of blood studies, a rheometer (Rheologics Rheolog SCV v.1.2) was used to capture blood over a broad range of shear rates. The rheometer measures the rheological properties of whole blood in an environment that mimics blood flow in the human vascular system. Remaining blood is spun down using a centrifuge and is kept in cold storage for further analysis.

Plasma analysis. Blood plasma contains several important components that act as markers for metabolic symptoms that are signs of endothelial dysfunction. C-reactive proteins, soluble adhesion molecules (ICAM-1 and VCAM-1), integrands, and vitamins are measured from the sample plasma.

Plasma Component	Test Purpose
C-Reactive Protein	Elevated levels are signs leading to inflammation
Adhesion Molecules (ICAM-1 and VCAM-1)	Elevated due to shear stress caused by the drag of red blood cells
Integrands	Adhesion Properties
Vitamins (B12)	Adhesion Properties

With various vascular disorders, the levels of each plasma component are seen to be chronically elevated in the patient's blood. It is crucial to evaluate these various components in order to effectively analyze shear stress, in terms of viscosity and correlating it back to original diagnosis.

Acquisition:
Computer Specifications:
Dell Dimension 4100 XPS - Z
996 Megahertz Pentium III x86-based Family 6 Model 8 Stepping 6
523 Megabytes RAM
37.2 Gigabyte Harddrive
Microsoft Windows 2000 Professional
5.0.2195 Service Pack 4 Build 2195
ATI All In Wonder Radeon 9700 Pro

Supplement Storage: Maxtor One Touch II - 100 Gigabyte External Harddrive
Additional Network Drives Available

Necessary Software:

Software: Mathworks Incorporated Matlab 7.0.4 Core
Signal Processing Toolbox

One Dimensional Cross Correlation Algorithm (xcorr):
function [c,lags] = xcorr(x,varargin)
XCORR Cross-correlation function estimates.

C = XCORR(A,B), where A and B are length M vectors (M>1), returns the length 2*M-1 cross-correlation sequence C. If A and B are of different length, the shortest one is zero-padded. C will be a row vector if A is a row vector, and a column vector if A is a column vector.

Two Dimensional Cross Correlation Algorithm (xcorr2):
function c = xcorr2(a,b)

XCORR2 Two-dimensional cross-correlation.
XCORR2(A,B) computes the crosscorrelation of matrices A and B.
XCORR2(A) is the autocorrelation function.

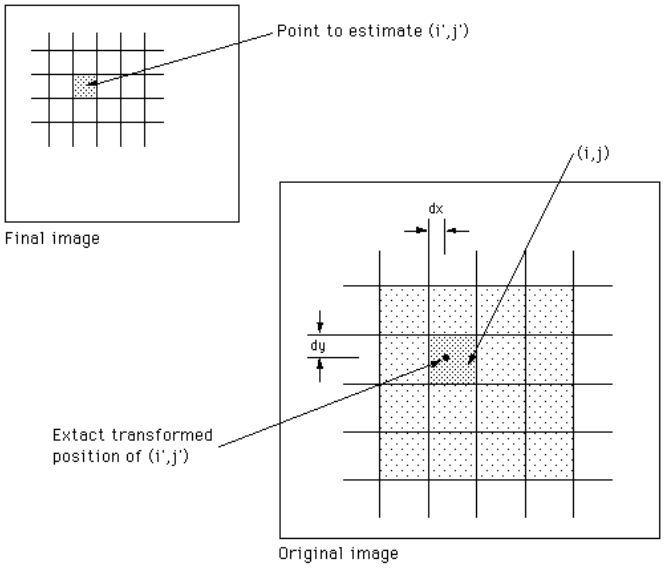
Modified Supplement of xcorr2.m:
This allows for specific tracking as the bicubic interpolation algorithm processes the sequence.

```
if nargin == 1
 b = a;
end
c = conv2(a, rot90(conj(b),2));
```

_____

Image Processing Toolbox

Bicubic Interpolation Concept:



Interpolation Function:

$$F(i',j') = \sum_{m=-1}^{2} \sum_{n=-1}^{2} F(i+m, j+n)\, R(m-dx)\, R(dy-n)$$

Cubic Weighting Function:

$$R(x) = \frac{1}{6}\left[P(x+2)^3 - 4\,P(x+1)^3 + 6\,P(x)^3 - 4\,P(x-1)^3 \right]$$

$$P(x) = \begin{cases} x & x > 0 \\ 0 & x \le 0 \end{cases}$$

Stabilization Algorithm:

function [success,totaladj] = stabvidnew(hObject,handles,finp,foutp)

A stabilization algorithm was added to the original program as a means of making video sequences analyzable by having the program track certain features of the sequence and overlaying them to create a solid stream for analysis.

Software: VirtualDub 1.6.15 Build 24442
- Serial Video Input
- Capture Pin:
  - Video Standard: NTSC_M
  - Frame Rate: 29.9700 frames per second
  - Color Space: YUY2
  - Output Size: 720 x 480 pixels
- Process Amplification: 128 Default
- No Video Vertical Reduction
- No Noise Reduction Function
- No compression / decompression module
- RGB Filtering
  - Reduce color variance caused by the VCR during acquisition
- Format: Audio/Video Interleave (.avi)

Uncompressed Audio Video Interlace:

In creating the final video for analysis, no compression schemes were introduced because many compression/decompression schemes introduce spatial distortions to which the image processing algorithm is sensitive to. Uncompressed video formats also prevent the likelihood that acquisition would result in any dropped frames.

Procedure

TO CAPTURE THE CLIP:

1. Run the Virtual Dub program from the desktop. The connection should already be set to S-Video mode by default. Click on File / Capture AVI… to run the capture function.
2. Place Tape into VCR and find the sequence needed for capture.
3. Click on File / Set Capture File. Designate the desired folder and type in the BASE NAME for the video files (WT.01.00, for example). The ".00" at the end will allow the program to automatically forward to the next file number (.01, .02, etc) for easier use.
4. Press F5 or F6 to begin capture.
5. Press Esc to end capture.
6. To view clip, go to the Desktop / Acquisition Folder. Double Click on the file to view via Windows Media Player. If the capture is sufficient, then you can begin editing. If not, recapture.

TO EDIT THE CLIP:

1. Since the clip may contain involuntary movement (jumps, skips, etc), we must now edit it to maintain stability of the clip. Run the Virtual Dub software that is situated on the desktop (or if Virtual Dub is still running in capture mode, click File / Exit Capture Mode).
2. Go to File / Open Video. Select your clip (which should be in the Acquisition Folder).
3. When your clip opens, there should be two boxes, and a frame bar underneath.
4. Scan your entire sequence, looking for your desired consecutive frames.
5. Click at the first frame of your specified sequence. Click Home on the keyboard to set this frame as the beginning of your new sequence.
6. Next, click move the cursor to the last desired frame, and hit END on the keyboard. This should highlight the specified frame sequence (shown by the bar) skyblue.
7. Go to File / Save Segmented AVI. It will save as a 'VirtualDub/AVI_IO video segment' file format. Name the clip and save it on the desktop. NOTE: After saving it, the file will be named ###.00.avi (### representing your input).

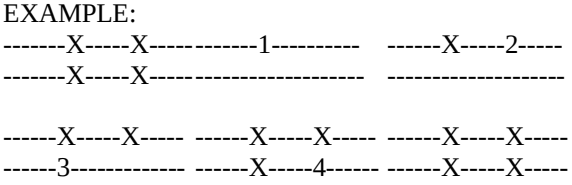
TO RUN THE VELOCITY SOFTWARE:

1. Drag the new file into the SHORTCUT TO AVID folder on the desktop (C:/programfiles/matlab7.0.4/work/avid).
2. Run the Matlab software.
3. On the upper lefthand box, there is a folder labeled WORK or AVID. Open the folder; inside this folder you will see various files, including the clip you just dragged in.
4. Right Click on AVID.m and click RUN.
5. The program will start and a window will open. This is the velocity program.

ANALYZING VELOCITY:

1. In the upper left hand side, there will be a text box with the words .avi in it. In front of the avi, type in the ###.00 (note that the .avi that was in the name is already in the box).
2. Click on COMPUTE!
3. Figure 21 Window will open. You will choose the vessel of study.
4. Along the vessel, you will be given a chance to select four points. Select these points alongside the vessel wall. When placing the points, put two on each side before going to the other side.

   EXAMPLE:

5. After selecting the points, right click inside the window. The program will start calculations.
6. Figure 41, Figure 333, and Figure 33 will open (these are not needed at this time).
7. On the orginal Avid window, the calculations will be listed. On the bottom left hand corner under RESULTS. If the converging speed and mean nonzero speed are similar, find the average of the two numbers, this is your velocity. The diameter is listed under DONE CALCULATING (near the COMPUTE button). It will also give you the number of frames and the length of your selection.

Accuracy of Measurement

Figures displaying the correlation matrix and the one dimensional projection are also provided to subjectively assess the degree of validity of the estimate (Rahaghi et al., 2006). In the cases that correct detection and processing occur, investigators could examine the degree of "accuracy" by comparing the peak of the one dimensional correlation with the next highest peak as a means of assessing the validity of the software's ability to generate the correct velocity. This relative magnitude serves as a measurement of the degree of "strength" of the detection.

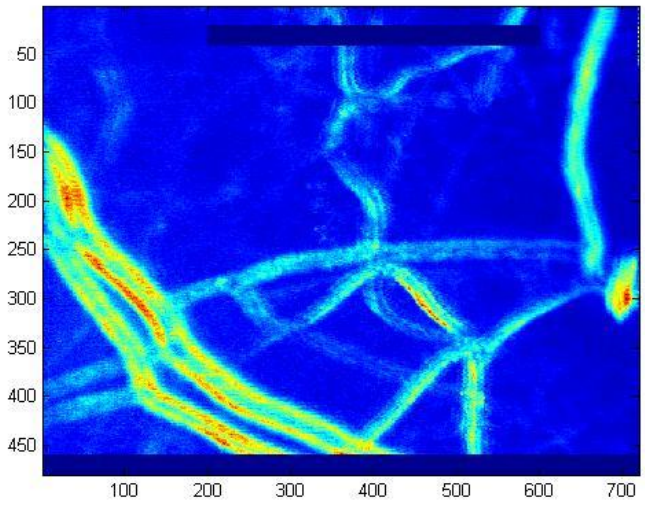
Special Features of AVID

Frame Separation

A special function in the program allows the user to adjust the frame numbers that are read by the program. By doing so the distance that an artifact is moving is exaggerated to better emphasize movement while retaining the accuracy of the calculated velocity. However, if the frame separation is set too high, the program may not be able to detect the same artifact due to deformation.

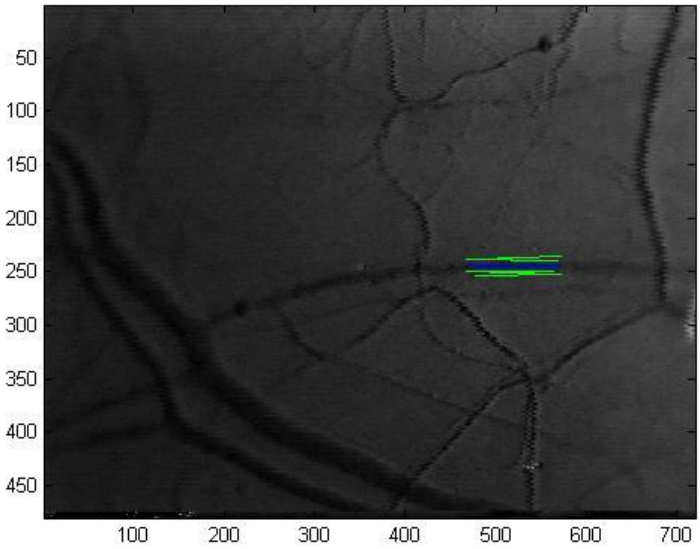
Generate Video Statistics

This function allows the investigator to determine areas of high movement or velocity on a software generated map by having the program overlap frames to determine high areas of concentration within the map and obtain an accurate, consistent calculation.



Session Reviews

This feature allows users to track the boundaries they have defined on the video analysis in order to judge the degree of subjective error. By doing so, the investigator is able to view the consistency of boundary selection.



Conversion Factor Table

Results from the AVID velocity program are measured in pixels. The conversion from pixels to physical units involved the use of a micro-ruler placed in front of the conjunctiva while filming. The ruler was captured and processed using the exact procedures as would be used for blood flow determination. This was done both in the x and y axes (as pixel dimensions are not always squares). With this method, a calibration conversion factor was done to produce usable numbers. The ability to adjust the horizontal and vertical dimensions of pixels, allows for calibrations to be made.

Calibrated Conversion Factors Table			
Magnification Index	10X	3.4X	Separation
Horizontal	3.603	0.27 pix / micron	10
Vertical	3.603	0.27 pix / micron	5

Results

UC San Diego Results using AVID:

Video Segment	Frame Seperation	Reading 1	Reading 2	Reading 3	Reading 4	units in pixels/second
neweyestud1.avi	10	39	39	39	39	
neweyestud2.avi	10	57	60	57	57	
neweyestud3.avi	10	45	45	45	45	
neweyestud4.avi	10	60	60	60	60	
neweyestud5.avi	10	120	120	120	120	
neweyestud6.avi	10	150	150	150	180	
neweyestud7.avi	10	60	60	60	60	
neweyestud8.avi	5	78	78	78	84	
neweyestud9.avi	5	192	192	198	198	
neweyestud10.avi	5	108	108	108	108	
neweyestud11.avi	5	282	294	282	576	
neweyestud12.avi						not stablized
neweyestud13.avi	5	102	102	108	102	
neweyestud14.avi	5	252	258	264	258	
neweyestud15.avi	5	30	30	30	30	
neweyestud16.avi	5	54	60	60	60	
neweyestud17.avi	5	42	36	36	42	
neweyestud18.avi	5	42	42	42	42	
neweyestud19.avi	10	42	42	42	42	
neweyestud20.avi	10	66	66	171	66	

UC Davis Lab Results using AVID:

Video Segment	Frame Separation	Reading 1	Reading 2	Reading 3	Reading 4
wtest1.00.avi	10	26	26	24	26
wtest2.00.avi	10	9	9	11	11
wtest3.00.avi	10	13	12	13	13
wtest4.00.avi	8	12	14	14	14
wtest5.00.avi	8	33	33	33	33
wtest6.00.avi	8	30	30	30	30
wtest7.00.avi	6	54	54	50	55
wtest8.00.avi	6	39	40	40	41
wtest9.00.avi	6	22	22	22	21
wtest10.00.avi	4	3	5	4	5
wtest11.00.avi	4	21	20	20	20

wtest12.00.avi	4	71	70	71	72
wtest13.00.avi	2	55	54	54	50
wtest14.00.avi	2	36	33	34	35
wtest15.00.avi	2	21	21	20	21
wtest16.00.avi	1	39	39	39	39
wtest17.00.avi	1	18	18	19	19
wtest18.00.avi	1	31	33	34	35
wtest19.00.avi	1	26	28	25	28
wtest20.00.avi	1	13	13	14	13

UC Davis Study in Calibration

Frame Separation	Pixel Length (in microns)	Reading 1	Reading 2
5.00	0.27	12.96	11.34
4.00	0.27	18.225	18.23
6.00	0.27	9.45	9.45
2.00	0.27	16.20	18.10
10.00	1.00	27.00	28.00

Discussion

Determining blood flow velocity is important because it is a major factor in determining hemodynamic force on the endothelial lining of blood vessels. Because complications arising from the irritation of vessel lining can lead to endothelial dysfunction, it is crucial to be able to determine trends in velocity, viscosity, and vessel diameter resulting in substantial morphometric, structural, and dynamic alterations and adaptations in blood vessel properties. The newly developed AVID program is able to track and analyze blood velocity and measure vessel diameter. Using these data as basis, the AVID program calculates results that are accurate and consistent. Test readings are calculated and determined to be within less than 5% of all other readings, with an occasional outlier. Bench tests have confirmed the accuracy of these readings within limitations using separate software generated video sequences (both at the University of California, Davis laboratory as well the University of California, San Diego) and mechanically generated movement (conducted at the University of California, San Diego). Results from these bench tests confirm that for velocities traveling less than 300 mm/sec, the chance for errors in calculated results are less than 0.1%. The development of this program has increased the accuracy of measurement by nearly eliminating the subjective nature of measurement.

Practical applications that AVID may help advance are the computer assisted intravital microscopy studies that are conducted using a similar velocity program (VASVEL):

1. Contact Lens
    a. Detecting changes in velocity on contact edge as a means of determining pressure on the conjunctival surface
2. Sickle Cell Anemia
    a. Ability to detect changes in blood flow to test efficacy of various drug treatments; observe vaso-constriction during Sickle Cell crisis to examine velocity changes.
3. Shock: Hemorrhagic and Endotoxemic
    a. In animal models, ability to detect changes in velocity as subject crashes during shock (hemorrhagic or sepsis); vasodilatation and velocity

During the development of the AVID program, a number of complications were addressed and are subject to be integrated in the next revision of AVID:

1. Foreign Artifacts
    a. Noise or motion artifacts from patient and experimental conditions
    b. Solution : Averaging of the 2D correlation matrix in time helps counter artifacts in the direction of the flow and natural variations and noise in each frame.
2. Stabilization
    a. Bugs in the stabilization algorithm allows for certain sequences to be stabilized
3. Assumptions: rectangular boundary for defining segment length and width
    a. Does not take into account three dimensional aspect of vessel and velocity flow
4. Deformability of individual cells in flow
    a. The features that are effectively tracked by the algorithm (such as red blood cells) are highly deformable; they do not appear to be the exact same between frames. This has the added difficulty that the improvement in detection theoretically expected by tracking features across a longer vessel segment is reduced because the longer an object is tracked, the more likely that it will have been deformed; but if the vessel segment is not long enough, then the velocity program would not be able to track it as it moves (if high enough velocity).
5. Limits to velocity in terms of boundary size
    a. The maximum velocity that can be analyzed is restricted to the boundary frame which the investigator defines. For example, if the specified segment is 29 pixels in length, but the vessel is traveling 30 pixels / second, then the program would not be able to detect the velocity.
6. Subjective analysis in that the investigator defining the points in which the program should analyze
    a. The usage of four points to define the region of interest instead of simply two points to define the axis of flow lowers this error by averaging the two parallel borders of the vessel area. The investigator must select the optimal vessel to analyze. Color inversion method to highlight areas that are veins/venules/arterioles (can also be used for A/V ratio)

<u>Improvement</u>

1. Maximum resolution acquisition can only be made on a computer with sufficient data write speeds such that data can be written quickly enough as it is acquired in real time.
    a. Update computer hardware: CPU and Hard drive (important features: capacity and RPM)
    b. Selection of a more powerful acquisition program (ie. Adobe Premiere Pro 2.0 or Microsoft Movie Maker v2.0)
    c. Graphics Accelerator Upgrade
2. Digital acquisition
    a. Higher transfer quality : no dropped frames during acquisition
    b. Easier storage, organization, transfer, and backup
    c. High quality, loseless sequences
        i. Cassettes degrade over time, especially after repeated processing
    d. Ability to use other means of analysis (since already digitized)
3. Diastolic / Systolic
    a. Pulsatile rate vs. velocity analysis
    b. Is blood flow constant?
4. Patient History
    a. Rest period prior to videotaping (10 - 15 minutes) in a quiet room
        i. Strenuous activities can account for increased blood flow velocity
    b. Caffeine Intake
    c. Time of day of study
5. Update Matlab software to network license
    a. Includes all toolboxes necessary at cost efficient licensing price

In conclusion, by running video sequences through computerized computations of AVID's algorithms for identifying, tracking, processing, and analyzing blood flow velocity, a subjective quantification can be replaced and objective-based results obtained. These results support a more precise, and consistent means of measurement in estimating the actual velocity of the flow rate.

Acknowledgements

Department of Medical Pathology, University of California, Davis School of Medicine, Davis, California 95616
Dr. Anthony T. W. Cheung
Patricia L. To
The Hugh Edmondson Research Fellowship

Department of Bioengineering, University of California, San Diego, La Jolla, California 92122
Dr. Peter C. Y. Chen
Farbod (Nick) Rahaghi

References

Aaslid R., Markwalder T. M., Nornes H. (1982)
Non-invasive Transcranial Doppler Ultrasound Recording of Flow Velocity in Basal Cerebral Arteries
Journal of Neurosurgery. 57 : 769 – 774.

Chen, P. C. Y., Kovalcheck, B. W. Zweifach, B. W. (1987)
Analysis of Microvascular Network in Bulbar Conjunctiva by Image Processing
International Journal of Microcirculation, Clinical and Experimentation. 6, 245.

Chen, Z., Milner T. E., Srinivas, S., Wang, X., Malekafzali, A., Van Gement, M. J. C., Nelson, J. S. (1997)
Noninvasive Imaging of In Vivo Blood Flow Velocity Using Optical Doppler Tomography
Optical Society of America. 22: 1119-1121.

Cheung, A. T. W., Harmatz P., Wun T., et al. (2002)
Correlation of abnormal intracranial vessel velocity (measured by transcranial Doppler ultrasonography) with abnormal conjunctival vessel velocity (measured by computer-assisted intravital microscopy) in sickle cell disease
Blood. 97: 3401-3404.

Cheung, A. T. W., Price, A. R., Duong, P. L., Ramanujam, S., Gut, J., Larkin, E. C., Chen, P. C. Y., Wilson, D. M. (2001)
Microvascular Abnormalities in Pediatric Diabetic Patients
Microvascular Research. 63: 252-258.

Nagaoka, T., Yoshida, A. (2006)
Noninvasive Evaluation of Wall Shear Stress on Retinal Microcirculation in Humans
Investigative Ophthalmology and Visual Science. 47:1113-1119.

Rahaghi, F. N., Goor, J. B., Chen, P. C. Y., Gough, D. (2006)
Bench-Top Light Microscopy Based Video Flow Measurements In Microcirculation
University of California, San Diego Department of Bioengineering

Sarelius I. H., Duling B. R. (1982)
Direct Measurement of Microvessel Hematocrit, Red Cell Flux, Velocity, and Transit Time
American Journal of Physiology. 243: H1018-H1026.

Zweifach, B. W. (1994)
Microcirculatory Homeostasis 1930 – 1990: Insight into Microcirculatory Readjustments Provided by Studies on the Peripheral Circulatory Insufficiency of the Shock Syndrome.
Microcirculation. 14: 122-131.