Chapter 6

SUMMARY AND CONCLUSIONS

The work in this thesis is based around applying similar phase variance analysis techniques to different imaging situations in order to produce the optimal motion contrast images in the most efficient method. There are many observations that can be summarized for the phase variance contrast experimental data:

1) Waiting longer can help motion contrast measurements

In Chapter 3.3, the phase measurements resulting from several different types of motion were theoretically calculated. For each case, an increase in time between phase measurements resulted in an increase in the measured phase change (up to the limits of phase wrapping and complete randomness in the phase measurements). This fact has been experimentally demonstrated in Figure 3.14 for the case of Brownian motion. The main accuracy limitation of phase measurements is the SNR-limited phase noise, which is a property independent in time. Instead of trying to reduce this lower bound on phase measurements, waiting longer between phase measurements allows for the slow motions to move beyond the accuracy limitations of the system (e.g., if the phase accuracy for a given reflection is 0.5 radians, the time separation between measurements must be large enough that the reflection is able to move more than 0.5 radians for motion to be seen).

Increasing the time separation between phase measurements improves the visualization of slow motions but adds complications to regions of fast flow. With faster motions comes an increased likelihood of phase wrapping, with the possibility that fast transverse motion will move the reflection completely out of the imaging beam waist. In Doppler flow imaging, the maximum axial flow that can be quantitatively measured is reduced when the time separation between phase measurements is increased (due to phase wrapping).

Phase variance calculations do not suffer the same limitations for regions of fast flow in the cases of increased time separation between phase measurements. Phase wrapping and transverse motion out of the imaging beam cause the variance measurement to saturate at a maximum level consistent with a purely random signal. Waiting longer between phase measurements has the added benefit of increasing the ability to observe random occurrences due to the increased time in which the phase measurements are acquired.

2) Waiting longer can increase motion noise in measurements

From the experimental data, the main sources of phase noise observed were SNR-limited phase error and noise created from transverse motion occurring during the contrast image acquisition time. While numerical calculations can remove most of the SNR phase error, the demonstrated removal techniques of the transverse motion error are in the very early developmental stages. It is much easier to simply create a situation in which no transverse motion error occurs within the phase contrast image. The longer the time separation between phase measurements in the contrast image, the more likely that transverse will occur within that image.

It becomes a tradeoff between increasing the observed motion contrast and trying to avoid potential transverse motion of the sample. The optimal imaging parameters depend on the sample properties: the expected motion of the reflections of interest as well as the amplitude and frequency of bulk transverse motion. If the motion signal within the sample is fast enough that the variance measurements become saturated for a given time separation (identical to a completely random phase measurement), increasing time separations further will only increase susceptibility to motion noise in the system without adding any additional motion contrast to the image. Flexibility of the time separation between phase measurements used for the contrast image allows for adjustment to optimize the imaging for a given sample.

3) Minimum BM-scan time is hardware limited

The BM-scan acquires multiple successive B-scans over the same transverse location in order to calculate the phase change that occurs during the time it takes for the acquisition to return to the same original location. The minimum time between phase measurements is defined as the minimum time between B-scans, which is limited by the A-scan acquisition time of the spectrometer and the speed of the transverse scanners. A uni-directional scan pattern for the BM-scan consists of the transverse scan of the B-scan acquisition as well as the fly-back to quickly return to the starting transverse position of the scan. This scan pattern is a very efficient method of ensuring a constant time separation between phase measurements, with the fly-back portion of the scan as the only wasted time within the acquisition. The fly-back time of the transverse scan is limited by the maximum achievable speed of the transverse scanner, which is a hardware limitation of galvanometer scanners like those used in the experimental systems.

The B-scan acquisition time is limited by the number of transverse locations chosen and the A-scan acquisition speed. In some of the presented mouse retinal imaging data, the BM-scan was altered by reducing the number of transverse locations by a factor of two. The majority of the observed flow within the mouse retina was fast enough such that the phase variance measurements were saturated with time separations of 10 ms. In this case a reduction of the time separation to 5 ms did not substantially reduce the observed motion contrast, suggesting that the same motion contrast may be observed in the mouse retina for even shorter time separations of the BM-scan. Technological improvements are allowing faster data transfer rates of SDOCT cameras as well as the development of SSOCT light sources with A-scan rates that are faster than experimentally demonstrated SDOCT. These A-scan speed improvements would allow for shorter BM-scan time separations, potentially maintaining the same motion contrast while reducing the phase noise created by bulk transverse motion from the sample. Sometime in the near future, BM-scan contrast methods can be implemented on systems with different limitations on the minimum time of BM-scans to explore this possible improvement.

Even if the fly-back of transverse scanning was not a problem and the A-scan rate could be as fast as possible, the optimal time separation between phase measurements might be very similar to the currently chosen parameters. In zebrafish imaging, phase variance contrast was visible but not saturated for time separations of 1 ms in the MB-scan. To improve this visualization, an increase in the time separation would be suggested. The minimum time of the BM-scan presented was 5 ms for the 100 transverse pixel repeating BM-scan case. The only case where minimizing the BM-scan time further would be beneficial is when all of the expected motion in the system is relatively much faster than the zebrafish. Without any parameters known of the expected motion of the choroidal neovascularization, it would be premature to predict a benefit to any substantial reduction to the minimum BM-scan time. Hardware improvements resulting in increased A-scan acquisition rate and improved transverse scanning could result in larger B-scans in each BM-scan for the exact same time separation of phase changes. This would improve the scan region and the data throughput for the same phase contrast images if multiple buffered acquisitions were required.

4) MB-scan can be useful – just not as a screening tool

In all of the demonstrated 3D contrast images, no MB-scan data was shown at all. This is largely due to the inefficiency of data acquisition with this method, requiring multiple buffered acquisitions to acquire any 3D contrast data. Also, the contrast images were frequently unable to produce strong phase variance contrast from the flow within vessels of zebrafish, which was approximately 0.5 mm/s. The inefficiency of this acquisition technique and the requirement of fast flows for the phase variance contrast severely limit the usefulness of the MB-scan as a vascular screening tool. Once vascular events have been identified within a sample using the BM-scan techniques, the MB-scan can act as a quantitative diagnostic of the vasculature. The MB-scan has the ability to measure axial flow and incorporate increased statistics to the flow and variance calculations. With vascular directionality extrapolated from the 3D BM-scan data, flow velocity can be extracted from the axial component measured with the MB-scan data. As a companion technique to the BM-scan vascular screening capabilities, the MB-scan can find its place within diagnostics.

One of the main issues with the MB-scan is that transverse motion cannot be easily dealt with in this case. While it becomes less likely that motion will occur for a given M-scan due to the shortened time separation compared to the BM-scan, when transverse motion does occur, the developed techniques for the BM-scan can not be applied. BM-scan transverse motion analysis relies on the fact that the entire BM-scan experiences approximately the same transverse motion during the phase contrast acquisition. This correlation provides the basis for the additional noise removal, which is information not available in the MB-scan data. Further work would be required to develop removal techniques of any transverse motion occurring during an MB-scan.

The situation in which the MB-scan variance contrast method might have potential as a screening tool is in cases with extremely stable sample position and no restrictions on total data acquisition time. With all of the available statistics acquired in this method, more analysis techniques become available including the analysis of the evolution of phase contrast for different time separations. For the agarose/Intralipid images, increasing time separation between phase measurements in the phase variance contrast image changed the total image contrast. The functional form of these changes can provide another type of information that can be the basis of additional image contrast.

Retinal imaging does not have the transverse motion stability required to produce a 3D phase contrast data set from MB-scans of sequential memory buffers. While choroidal flow motion contrast was visible with some of the larger time separations of the MB-scan, the susceptibility of the method to transverse motion noise reduces the feasibility of use in retinal imaging situations.

5) Human retinal contrast imaging looks very promising

Experimental systems producing aberration-corrected high-transverse resolution confocal images of the human retina have demonstrated images without any obvious transverse motion at an image rate of approximately 20 Hz, equaling 50 ms image acquisition time. This means that the average human can be considered essentially transverse motion-free for

any given 50ms time period. Each BM-scan contrast method presented for the mouse retinal imaging acquired each 2D phase variance contrast images data in 25 ms or 50 ms for 100 and 200 transverse pixels, respectively. This suggests that the current contrast parameters implemented directly into human retinal imaging should produce contrast images without much transverse motion noise.

The three main sources of transverse motion of human retinal imaging are tremor, drift, and microsaccades. Tremors are usually small-amplitude, high-frequency motions of the eye that will most likely limit the ultimate performance of the imaging for a given patient. With enough information, improved noise removal might be able to deal with this completely. Drift is a slow motion of the fixation and transverse position of the eye. Fixation targets for the patient to focus on, as well as head rests reduce a lot of these factors over the course of the imaging session. Microsaccades are sharp jerks of the transverse position of the eye that can occur randomly in time for human eyes. This motion is a major issue in retinal imaging and requires additional work to identify and re-align the images when microsaccades occur. For contrast imaging with OCT, each microsaccade will likely cause contrast noise within several BM-scans that will require them to be thrown out of the data set due to the excess phase noise. While increased statistics and phase analysis can reduce the effect of these motions, implementing realignment techniques previously developed for confocal retinal imaging can further improve the imaging capabilities of the system.

Conclusions

Phase changes calculated from OCT measurements are capable of determining very small motions on the scale of nanometers. Phase change variance calculations have demonstrated motion contrast for several types of motion including mobility and flow, independent of the flow orientation. Within the memory buffer limitations of the acquisition system used in the experiments, 3D phase contrast has been demonstrated in 5.2 seconds of acquisition time. Within the microscopy imaging of the zebrafish and within the mouse retinal images, the calculated phase contrast data represents 3D vascular visualization. Future directions on

phase contrast techniques can refine the analysis procedure, focusing on improving the noise removal techniques associated with transverse motion. These techniques have excellent potential for transitioning into human retinal imaging, which may have new forms of motion noise within the sample that require removal.

The major source of motion expected within retinal imaging is microsaccades, fixation adjustments of the eye. With the reduction of acquisition time of the 3D phase contrast data set, the noise created due to the microsaccades of the patient is reduced as well. Increased statistics and re-alignment can be used with other improved analysis tools to improve the motion contrast visualization of the vasculature within the human retina. The resulting contrast data has the potential of matching the visualization capabilities produced by fluorescein angiography images, but with the added benefit of 3D visualization.

3D vascular visualization capabilities using motion contrast techniques with OCT imaging of retina are ideal as a screening tool. With some development, these techniques can be implemented for early identification of wet age-related macular degeneration. Doppler flow imaging capabilities of the OCT system were previously determined not to be ideal as a screening tool but combined with the visualization capabilities of the phase variance contrast techniques, it can be a useful diagnostic. With the location and directionality of vasculature determined using vascular visualization, Doppler flow imaging techniques can quantify vascular flow within the images, which is an important metric for a variety of retinal diseases beyond AMD. 3D screening and diagnostic capabilities of the OCT imaging system offers many possibilities for improvements to retinal disease diagnosis and treatments. In time, the techniques developed in this thesis may become standard for clinical diagnosis of retinal vascular diseases.