Fixed-background processing for SD-OCT and implications for scanning probe

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1 Introduction

Measuring the light source spectrum, or *background*, is an important part of the OCT processing pipeline – it is primarily used to deblur the OCT A-Scan, leading to qualitatively better signals and less signal competition. The ThorLabs Telesto SD-OCT (hereafter *bulk optics*) system in our lab can measure the background spectrum by positioning its galvo mirrors so that only the reference arm's signal will be measured. This is typically performed over the course of a 25 ms *flyback time* before each scan (A-, B-, C- or M-mode). Thorlabs' processing algorithm, used in the ThorImage program as well as our own acquisition/processing programs, defaults to using this method.

The OCT probe developed in our lab uses reflection at the probe tip as its reference beam, and is not capable of measuring the background in this fashion. Instead, background shots must be taken separately, with no sample in place, and then used later in the processing step after data has been acquired. Initially, this was done using offline processing: both background and data scans were taken in separate runs, then loaded into MATLAB where a lengthy processing algorithm was performed. Recently, we have moved our processing online for experiments using the bulk optics system, by use of procesing functions in the ThorLabs SpectralRadar software development kit (SDK). This has greatly sped up processing time, but has also put the background processing steps "under the hood."

Using the SpectralRadar SDK, however, we have been able to modify the online processing algorithm so that a previously measured (hereafter *fixed*) background could be loaded and used for processing. This step is necessary for online processing with the probe, but could also be used with the bulk optics system.

Using a fixed background with the bulk optics system avoids the mirror flyback, which could cause some ringing mechanical transient in the mirror at the beginning of our measurements. Until now, we have attempted to avoid the effects of this ringing by ignoring the first 100 samples of each recording, but the transient may still be present past this time. On the other hand, using a fixed background may be worse, on account of the fact that the background spectrum could change due to temperature fluctuations, or similar arbitrary phenomena.

In this document, we explore the impact of using fixed backgrounds with the bulk optics system, comparing it to the standard flyback method, and validating our acquisition/processing software for future use with the OCT probe. We begin with a review of the OCT theory to understand how the background is used in the processing pipeline. Then we present, at a high level, the steps used in the program to measure the fixed background. We then provide an analysis of images and vibration measurements made using different backgrounds. Finally, we discuss the implications these results have for future bulk optics system measurements, and for measurements using the probe.

We find that using a fixed background measured far from any sample achieves slightly worse A-Scan quality and vibration SNR to measurements taken using the standard flyback method, although the difference is not large. However, vibration magnitudes and phases measured using the two methods are identical. We conclude that processing for probe measurements can be moved online using a fixed background, but bulk optics measurements should continue to use the standard flyback method.

2 Theoretical OCT Signal

2.1 The unprocessed A-Scan signal

In SD-OCT we measure the interference pattern between reference light and sample light. Writing the source intensity spectrum as $S(k)$, the electric field of the light in either arm can be written as

$$E(k, z; t) = \sqrt{\frac{S(k)}{2}} e^{j(\omega t - kz)}. \tag{1}$$

Reference light travels some distance $2z_r$ (the factor of 2 is because it must travel both to and from the reference mirror) before reaching the detector, and sample light travels into the sample, being reflected at distances z_n at different surfaces in the sample. The light from the n^{th} reflective object in the sample travels $2z_n$ as it must go to the sample and back to the detector.

The detector measures a time-average of the intensity, $I(k)$, proportional to the square of the electric field. This means the time component of the field is averaged out, so we can write $E = E(k, z)$. We write the reflectivity of the reflector mirror as R_r and that of the sample reflectors as R_n. Now $I(k)$ is given by

$$\begin{aligned}
I(k) &\propto \left| \sqrt{R_r} E(k, 2z_r) + \sum_n \sqrt{R_n} E(k, 2z_n) \right|^2 && (2)\\
&= S(k) \left(\sqrt{R_r} e^{-2kz_r j} + \sum_n \sqrt{R_n} e^{-2kz_n j} \right) \left(\sqrt{R_r} e^{2kz_r j} + \sum_n \sqrt{R_n} e^{2kz_n j} \right) && (3)\\
&= S(k) \left(R_r + \sum_n R_n \right) \qquad \text{(DC Terms)} && (4)\\
&+ S(k) \left(2 \sum_n \sqrt{R_r R_n} \cos\left(2k(z_r - z_n)\right) \right) \qquad \text{(Cross-Correlation Terms)} && (5)\\
&+ S(k) \left(2 \sum_{k,l} \sqrt{R_k R_l} \cos\left(2k(z_k - z_l)\right) \right) \qquad \text{(Autocorrelation Terms)} && (6)
\end{aligned}$$

where we have used the usual complex formula for the cosine as a sum of exponentials, and the fact that complex modulus of an entitity squared is the product of that entity and its complex conjugate. Usually we make the assumption that the reflectivity of the reference mirror is close to unity, and that the reflectivities of the sample reflectors are small so that the autocorrelation term is negligible.

Note that the source spectrum multiplies every term. The inverse Fourier transform of $S(k)$ is usually written as $\gamma(z)$, and is called the *coherence function*. Taking the inverse Fourier transform, ignoring autocorrelation terms, and writing the sum of the DC reflectivities simply as R, we have the raw A-Scan which we will write as $p(z)$:

$$p(z) = R\gamma(z) + \sum_n \sqrt{R_r R_n}\gamma(z) * \big[\delta(z - 2(z_r - z_n)) + \delta(2(z_r - z_n) - z)\big]. \tag{7}$$

2.2 Background subtraction and deconvolution

This is a symmetric function of space, so considering only positive z we have one impulse corresponding to each reflector, convolved with the coherence function. This coherence function *spreads* the response of the *point* reflector, i.e. it is OCT's axial point-spread function. As with any point-spread function, it is desirable to *deconvolve* by it – a process known as deblurring – after the signal is measured.

The coherence function also appears as a strong "DC" term centered at $z = 0$, and scaled by R. Importantly, R is not known *a priori*, but is rather a function of the sample reflectivity. The magnitude of the DC component is significantly larger than that of any of the other components, and depending on the breadth of $\gamma(z)$ and the distance of the sample from the OCT system, this DC component may overlap some of the signal of interest. It is thereby desirable to subtract out this component of the signal.

Both deconvolution and background subtraction can be performed easily in the wavenumber domain before the Fourier transform if the spectrum $S(k)$ is known. The convolution theorem tells us that convolution maps to multiplication in the Fourier domain, so *de*convolution maps to division in the Fourier domain. Subtraction maps, by linearity, to subtraction. Using these facts, the Fourier domain processed A-Scan can be written as:

$$A(k) = \frac{I(k) - RS(k)}{S(k)} \approx 2\sum_n \sqrt{R_r R_n}\cos\left(2k(z_r - z_n)\right), \tag{8}$$

where we have, as usual, ignored autocorrelation terms. This gives a spatial domain processed A-Scan of the form

$$a(z) \approx \sum_n \sqrt{R_r R_n}\big[\delta(z - 2(z_r - z_n)) + \delta(2(z_r - z_n) - z)\big]. \tag{9}$$

In this signal, there is no point-spread and there is no large DC background. This is why measuring the background spectrum is important for enhancing the quality of our A-Scan.

When we used offline processing, we let $R = 1$ under the assumption that $R_r \approx 1$ and R_n is small in comparison. This usually underestimates the DC component, which means we would attenuate, rather than eliminate, the DC component. This is sufficient for most images, only becoming a problem if the sample is very close to $z = 0$, i.e. $z_n \approx z_r$. The ThorLabs processing algorithm estimate R via the average of the measured signal, which yields a more complete elimination of the DC component. This method of dynamically choosing R is used in the processing methods described in this document.

2.3 Effects on vibration measurements

Vibration measurements are made using the phase of the spatial-domain A-Scan, as it varies in time. The measured background spectrum is a time-independent object, so it is not immediately clear why background subtraction or deconvolution would have an effect on the phase as a function of time. The answer lies in the subtler problem of signal competition. Whereas ideally, an individual object moving at a certain pixel could be measured on its own, that object is blurred by the coherence function, so that its motion impacts measurements at other nearby pixels in a weighted fashion. This means that failure to deblur (or worse, deblurring by the wrong signal) leads to more signal competition, so that measurements at each pixel are more ambiguous. Quantifying this effect as a function of background is difficult and situation-dependent, but it is worth noting, especially as it comes up in this report.

3 Fixed-background program details

The first two sub-sections of this section are written for lab members who may want to update the code for this program or write related code in the future. It is more technical knowledge than is necessary for anyone who simply wants to use the program. For that, simply consider the last two subsections, titled “Fixed-background routines” and “Suggested use.”

3.1 SpectralRadar SDK Objects

This section uses some technical language surrounding object-oriented programming in the C++ programming language. We have tried to keep this as light as possible while still conveying sufficient information.

Every measurement taken with the OCT uses at least three “handles”, which are pointers to high-level classes with many fields. These objects are useful as they refer to complex systems which we do not want to deal with on a low-level, allowing most of the “nitty gritty” detail to be kept under the hood. The most importan of these are the `OCTDeviceHandle`, the `ProbeHandle` and the `ProcessingHandle`.

The `OCTDeviceHandle` is initialized using `initDevice`, which takes no inputs, and contains all of the information about our OCT system. This includes all of the signals required for the hardware to acquire scans, such as the trigger mode and maximum trigger frequency.

The `ProbeHandle` is initialized using the `initProbe` function, which takes as an input the device handle and the name of a probe configuration ini file. The latter is simply a text file containing important information about the OCT scanning device being used, such as the flyback time of the mirrors. In our case, we use a file given to us by ThorLabs titled “ProbeLSM03.ini”, which contains the necessary information for the Telesto system. It should be noted that ThorLabs programs refer to any OCT scanning device as a “probe,” not to be confused with our fiberoptic probe.

The `ProcessingHandle` object is initialized using `createProcessingForDevice`, which takes the device handle as an input. This contains all of the information about the steps used in the processing algorithm performed when `executeProcessing` is called.

Other important objects include the `ScanPatternHandle`, which is used to define the number and postions of A-Scans in B-, C- or M-mode scans, and the `Coloring32BitHandle` which sets the coloring properties for B-Scans saved as images. Various data handles are also used during the processing pipeline.

The fields in the objects associated with these handles can only be accessed through related functions. For example, to set the flyback time, we must use the `setProbeParameterFloat` function, which takes as inputs 1) the probe handle, 2) the name of the parameter we wish to alter (in this case `Probe_FlybackTime_Sec`), and 3) the value we wish to set it to as a floating point number.

3.2 Methods for measuring, saving and loading the background

The ThorLabs SpectralRadar SDK has a number of ways through which one can measure the background spectrum. The background spectrum is a field in the `ProcessingHandle` object named `Calibration_ApodizationSpectrum`, which is updated automatically by high-level processing functions. This vector is scaled for background subtraction and deconvolution to form a distinct field, `Calibration_ApodizationVector`. This vector is updated during processing automatically as a function of the data and the apodization *spectrum*, so we do not interface directly with this field at all. This is a source of confusion, so again, **we do not interface with the apodization vector**.

Another source of confusion here is the use of the word "apodization." The apodization spectrum is just what we have refered to as the background spectrum above, meaning when it is scaled it is used for both background subtraction and deconvolution. The SpectralRadar SDK overuses the word "apodization," which is the process of windowing prior to taking a Fourier transform.

There are multiple ways of measuring the background spectrum. We compare two such methods in this report. The simplest is the `measureCalibration` function, which takes three arguments – 1) the device handle, 2) the processing handle, and 3) the field we wish to measure (in our case, always `Calibration_ApodizationSpectrum`). This measures the background at the current position of the scanning mirrors, which can be set prior using the `moveScanner` function. The second method we consider is to simply take a scan with the probe parameters for apodization set to nonzero values (these are `Probe_FlybackTime_Sec` and `Probe_ApodizationCycles`). Before the scan is taken, the mirrors will fly back to the apodization position and measure the background in what we call the "standard" fashion.

The OCT probe designed by our lab cannot measure the spectrum in the flyback position, so the standard method will not work. Instead, we will use `measureCalibration` while the probe is pointed away from any sample. We use thebulk optics system to compare this method to the standard method to see if it leads to comparable SNR and image quality.

After the apodization spectrum is measured, we can write it to a file using the `saveCalibration` function, which takes three arguments – 1) the processing handle, 2) the field we wish to save (in our case, always `Calibration_ApodizationSpectrum`), and 3) the location where you want to save the file. This will save the background as a text file containing 2048 lines, each of which is a single floating point number representing the charge stored on each CCD sensor. This can be loaded into MATLAB with MATLAB's `load` function, to be viewed as shown in the Results section below.

We can load in an apodization spectrum from a file in the same format as it was saved, using the `loadCalibration` function (which takes the same arguments as `saveCalibration`). However, use of any of the functions described above will overwrite this loaded-in apodization spectrum. Most importantly, **taking a scan without setting the apodization parameters to zero will overwrite the loaded apodization spectrum.** This means the following two lines of code are critical after the initialization of the probe handle:

```
setProbeParameterInt(Probe, Probe_ApodizationCycles, 0);
setProbeParameterFloat(Probe, Probe_FlybackTime_Sec, 0);
```

3.3 Fixed-background routines

We have added three functions to the "OCT with Processing" data acquisition program – one to measure M-Scans at the center location using a fixed background, one to take B-Scans for real-time "semi-video" imaging using a fixed background, and one to measure the fixed background used in these functions. The terminal on startup of this program is shown in Figure 1.

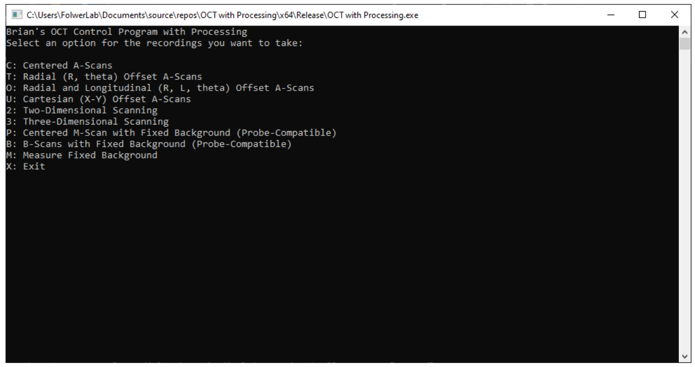

Figure 1: Startup for the OCT acquisition and processing program with options P, B and M added for fixed-background experiments.

Option M, Measure Fixed Background, is a very simple routine. It initializes the device, probe and processing handles, moves the scanner to the center position (0,0), calls `measureCalibration`, and then calls `saveCalibration` to save the measured background to a file specified as a string. This file is titled `spec`, and is overwritten every time this function is called. It lives in the directory `C:/Users/FowlerLab/Documents/Matlab OCT Computer/Scanning Probe SDOCT Files`.

Option P, Centered M-Scan with Fixed Background (Probe-Compatible), is very similar to the commonly used option C, which takes centered M-Scans in the standard fashion. The user inputs the sort of stimulus they are using (e.g. 1 second tones, 5 second Zwuis) and is prompted to begin the stimulus/trigger sequence when the system is ready. The program loads in the `spec` file from the path specified above and uses it as the background. This routine can be used with either the bulk optics or the probe.

Option B, B-Scans with Fixed Background (Probe-Compatible), is designed as a fixed background alternative to the ThorImage B-Scan video mode. This program first asks if you are using the probe or the bulk optics system. With the bulk optics, you can pick the angle, field of view and number of A-Scans in your B-Scan. With the probe, the angle is fixed as the piezo scans along only one axis, and the number of A-Scans in your B-Scan is also fixed as the piezo driver circuit works only for a specific pixel size. The field of view is currently fixed at 360 μm, the maximum field of view for the piezo, but this can be changed in future if smaller B-Scans are desired.

Once these parameters have been set, the program will take a B-Scan whenever a key is pressed, and save it to a png titled `BScan_update.png` in the the directory `C:/Users/FowlerLab/Documents/Matlab OCT Computer/Scanning Probe SDOCT Files`. This process takes about half of a second per B-Scan, so by taken subsequent B-Scans in this way with the png file opened in a photo viewer, one would see a low framerate "video." This would be slower than ThorImage with no real user interface, but produces higher quality images due to the use of fixed background. What is meant by "higher quality" can be seen in the Results section below. The program will exit out of this semi-continuous scanning mode when the `x` key is pressed.

3.4 Suggested use

For use with the OCT probe, one should first position the probe so as to take a background shot.

This should be either far away from a sample in air if you are scanning in air, or far from any surface in a cup of water if you will be scanning inside the cochlea. Then the M option, Measure Fixed Background, in the OCT with Processing program should be used once.

After this point, the ThorImage software should be used in B-Scan video mode to position the probe near to the sample. The experimenter should be careful to ensure the angle, field of view and pixel size parameters are all set properly for the probe circuitry. Although this will be low resolution on account of using a poor background, the presence of some sample in the image will still be clear. If a high resolution image is desired, use the semi-video mode in option B in the OCT with Processing program once your sample is clearly present in the ThorImage video.

Finally, once the scanner is centered at the desired measurement location, use option P in the OCT with Processing program to measure data using the fixed background measured before.

4 Results

The following data were measured with the bulk optics system. Validation with the probe must still be performed. However, this is sufficient evidence that the software works as expected, and directly answers our questions about use of fixed-background methods in our bulk optics recordings.

4.1 Measured backgrounds

We used the program to measure the background in two ways, as described above – using the standard method where a background is recorded automatically before a B-Scan, and using the `measureCalibration` function. In Figure 2, we show the bckground recorded using the standard method alongside two backgrounds recorded using the `measureCalibration` function. These two differ in that the first was measured with the lens far from any sample, and the second was measured with the lens near a stack of ten pieces of Scotch tape.

Let us first compare the standard method and the fixed background where no sample is in the way (blue and red curves, respectively). The fixed background method produces a spectrum of nearly the same shape, with slightly higher intensity across the whole range of photodetectors. This makes sense – in the standard method, only reference light is being measured, but in the fixed background method, we measure the reference beam *and* whatever is reflected back through the sample arm. As there is no sample in the beam path, this will most likely be due to reflection directly from the scanning lens, but could also be random reflections from particles in the air or other near-infrared light present in the room. The result is a bit more power in the measured spectrum, however the additional power is wavenumber-dependent (which is also reasonable). This means that our fixed background method gives a spectrum that is shaped a bit differently than the light source's spectrum, which could negatively affect our processed signal quality.

The third spectrum shown here (yellow curve) is a "background" measured using the `measureCalibration` function while the OCT lens is pointed at ten stacked pieces of Scotch tape. This is a very poor approximation of the background, which would be very undesirable to use in an experiment. It is presented here because this is similar to what would be used by the probe in the ThorImage program, where the "background" is measured while the probe is pointed at the sample. We can see that this signal has an envelope in the shape of the true background, as expected, but has high frequency components, which as we know from OCT theory encode depth information.

It's instructive to think about how these backgrounds will affect final signal. In the wavelength domain, shown here, we take the data signal and subtract the background, then divide by the background. Using the yellow curve here, for example, subtracting this background from a signal could actually introduce frequency components corresponding to the tape positions to a signal that does not otherwise have such components present.

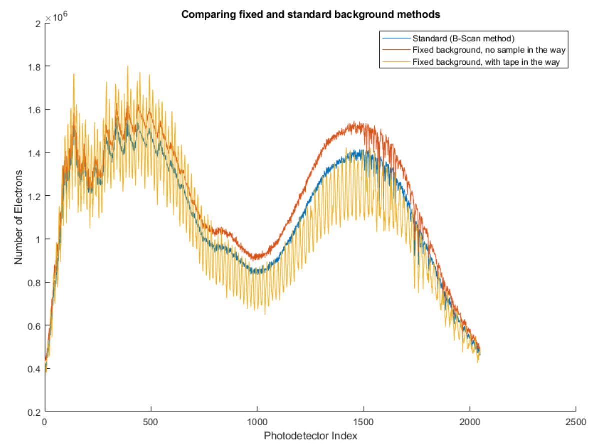

Figure 2: Backgrounds taken using the standard method and `measureCalibration`.

We interpolate these backgrounds to the wavenumber space and take the Fourier transform, then display the first half of the magnitude response in Figure 3. The rectangles emphasize the location where the Scotch tape is in the sample. These are the spatial-domain A-Scans corresponding to the background, which may be a more natural way to consider the effects the background will have on processed data. A raw data A-Scan will have this signal subtracted from it, and it will then be *deblurred* (i.e. deconvolved) by this signal. In the first two curves, we can see almost all of the signal power is concentrated near $z = 0$, as is expected from OCT theory. When we subtract out the background, this DC component which carries no information will be removed from the data A-Scan. Deconvolving by this background can be see as an operation that "converts" signals in the shape of this background to impulses. The idea that this DC shape is the impulse response of the system is clear if we look at the tape A-Scan (the third subplot), where the bright first surface has a similar shape to the DC component.

Again, considering using the signal in the third subplot here as the background, we can see that high-intensity spatial components in the boxed region (similar intensity to the DC component) will be introduced to the data signal. This will reduce the SNR and signal quality, but in a spatially-dependent way. Deconvolution by this signal is a bit harder to picture, but it should be clear that this is not the genuine impulse response of the system. This will introduce blurring in some complicated fashion, which will also be spatially dependent.

We use the three backgrounds described above in our tests on B-Scan image quality for a stack of ten pieces of Scotch tape. In our M-Scan tests, we measure a speaker's displacement as it plays sounds at various frequencies and sound pressure levels. We consider the case of using a sample-like background in these test as well. The speaker background in both the wavelength and spatial domains is shown in Figure 4.

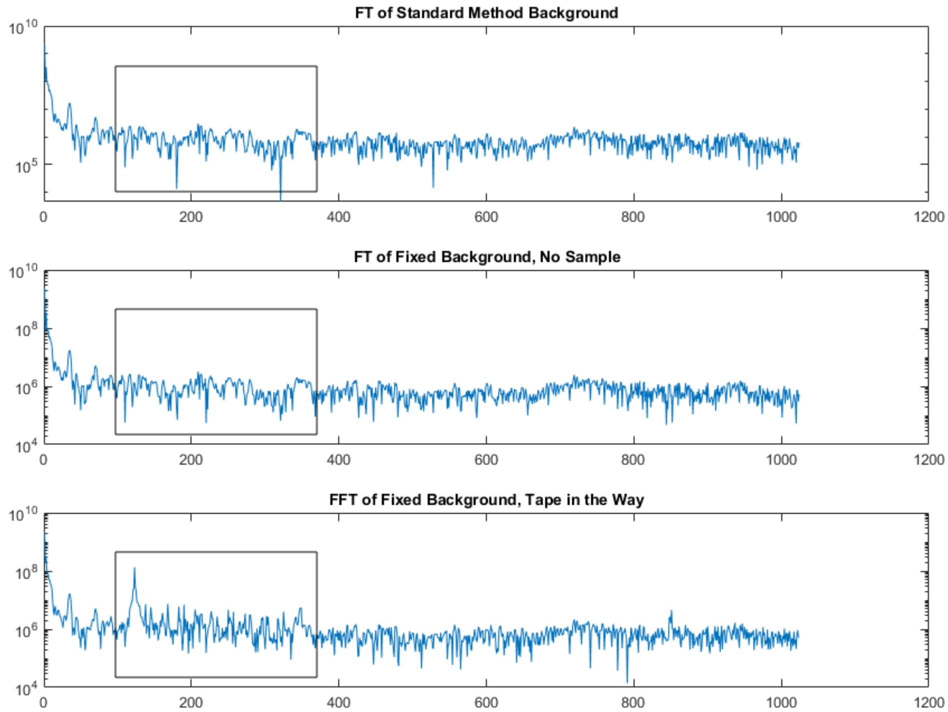

Figure 3: Spatial domain forms of the backgrounds shown in Figure 2.

4.2 B-Scans

We use the three backgrounds shown in Figure 2 to process B-Scans of ten pieces of Scotch tape stacked atop one another. In panel A, where we used the standard method, we can clearly make out the ten pieces of tape. In panel B, where we used a fixed background taken while no sample was present, we again see a very clear picture. It is difficult to say if either of these two pictures is better than the other. This is good news for the probe – the method of fixed background, necessary for the OCT probe, seems to produce good B-Scans.

In panel C, we used the fixed background taken while the lens was pointed at the tape, which corresponds to what might be seen with the probe when using ThorImage. This means we used something similar to the center A-Scan of this B-Scan as the background. The slight tilt of the tape in the image helps us understand the effects this has. By subtracting this false background, we add in the spatial components corresponding to the positions of the tape at the center. Looking at the left and right sides of the image, we see that the true positions of the tape are joined by phantoms of the positions of the tape at the center, making it impossible to tell how many pieces of tape there are and how thick the tape is.

One might be surprised to see that there is any signal at all in the center, where we have

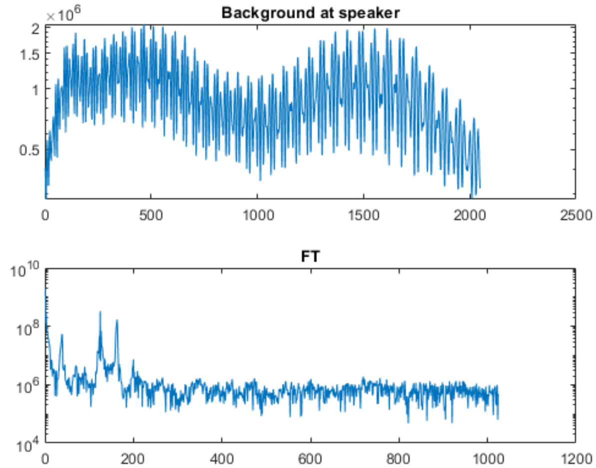

Figure 4: Background taken by using `measureCalibration` while the speaker was near the lens.

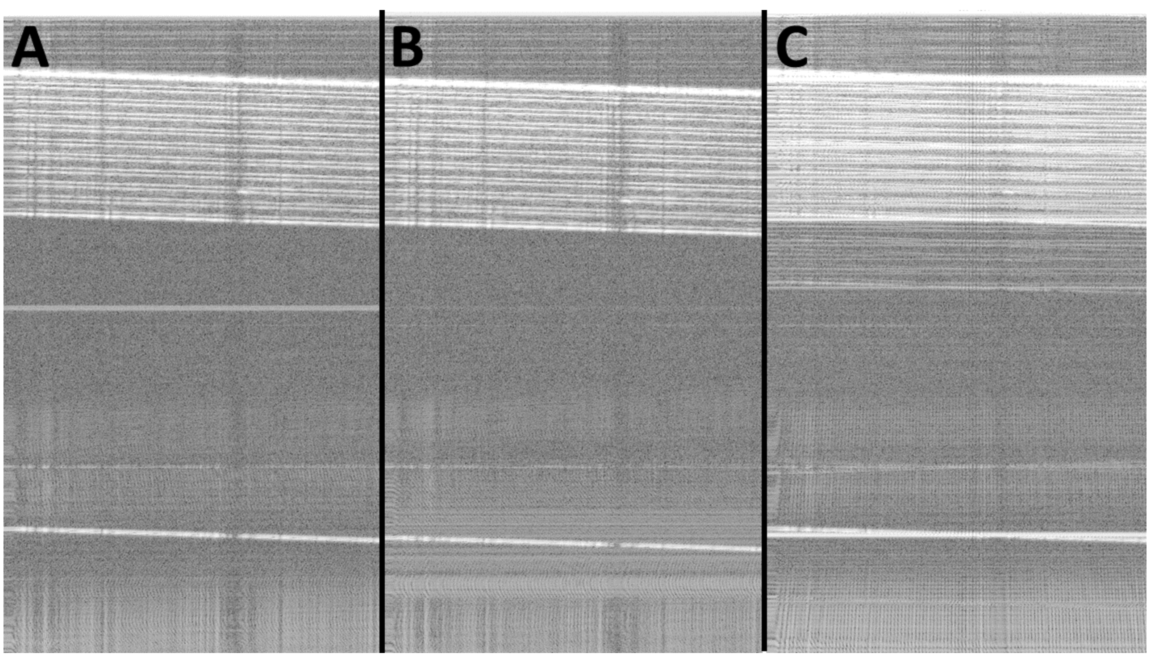

Figure 5: B-Scans of ten layers of Scotch tape, taken using **A** – the standard method; **B** – a fixed background taken far from the sample; **C** – a fixed background taken with the sample in the way.

subtracted out the "background" that ought to look just like these center signals. In fact, we do see a range of dark and light A-Scans near the center. Small variations from the background seem to yield some A-Scans where almost no signal is present while others have (less bright than the rest of the image) signal near where the tape is. At each A-Scan near the center, you have something close to, but not equal to, the background signal. Some high-intensity light will be subtracted out, while others will remain, and blurring effects of the deconvolution operation will amplify this slightly offset signal at certain small variations from the background. This is why we see this "dark-light" spatial modulation.

4.3 A-Scans

We now move on to the speaker, from which we took M-Scans to test the effect of background subtraction on vibration measurements. The A-Scans shown in Figure 6 are the average A-Scan magnitudes from 1-second M-Scans (approximately the average of 100,000 A-Scans) taken using four background signals. The A-Scan in panel A used the standard method, where the mirror flew back right before the M-Scan was recorded. Panel B used the "probe method," where we measured the background using `measureCalibration` far from the sample. Panel C used a saved background using the B-Scan method from about an hour prior. This is similar to what we have been told other labs do, where they do not fly back the mirrors but instead use saved backgrounds taken in the apodization position of the galvos. In panel D we use the background taken in which the tape was present.

We see that the A-Scan quality is best using the standard method, with the fixed background methods in panels B and C underperforming in terms of SNR. However, these fixed-background A-Scans still capture the character of the signal magnitude quite well, as the sample is reflective. These two fixed background methods yield acceptable A-Scans. This is also good news for the probe – the method of fixed background seems to produce good averaged A-Scans.

This is a point against the use of a fixed background in bulk optics experiments, as use of recorded backgrounds from even the recent past does seem to reduce the SNR. Because of this, bulk optics experiments ought to use the standard method of flying back prior to each M-Scan.

In the case where we used the background in which tape was near the lens, we see that there are "ghost peaks" present in the A-Scan which correspond to the location of the tape in the background (see boxed region in panel D). These create the illusion of a sample at a position where nothing is actually present. In this case, we know the actual depth profile of our sample so the effect is very clear. If we did not know what our sample ought to look like, this would ruin our perception of the sample's depth profile.

In Figure 7, we see the average A-Scan where the background used was also an A-Scan of the speaker (as seen in Figure 4). This has strange effects, decreasing the SNR and creating many phantom peaks which are likely a product of deconvolution. This of course gives a remarkably poor picture of the actual depth profile of the sample, but does present peaks at the position of the speaker membrane in the other A-Scans. We will now consider what happens when we measure motion in each of these M-Scans

4.4 M-Scan magnitudes

Figures 8, 9 and 10 show the displacement at the speaker membrane as a fuction of frequency for a 1-second 15-tone Zwuis complex at 20, 40 and 60 dB SPL respectively. We compare the use of all five backgrounds considered in the previous subsection.

All methods seem to agree very well except that which uses the speaker A-Scan as a background.

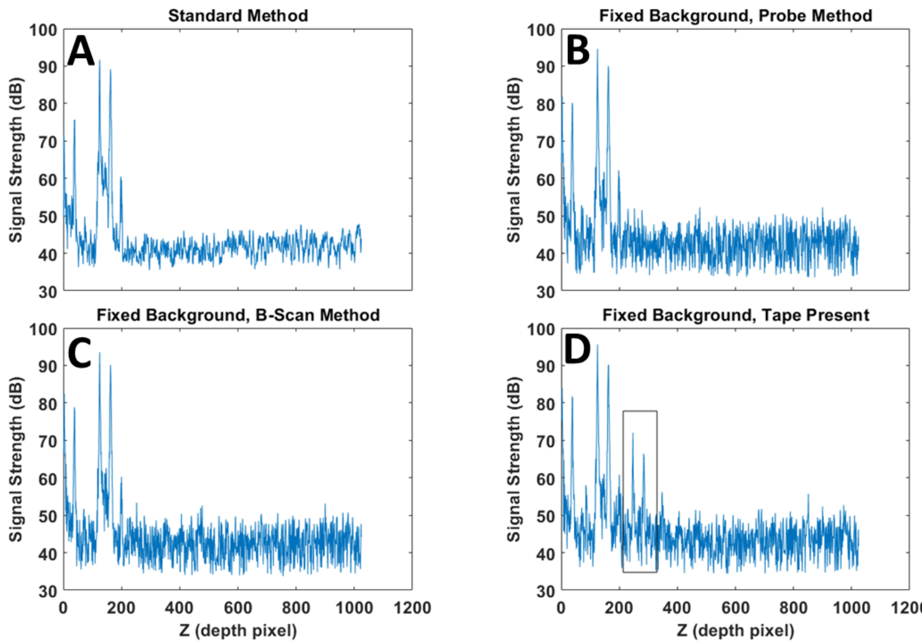

Figure 6: Average of about 100,000 A-Scan magnitudes of the speaker taken over a one-second period, using **A** – the standard method; **B** – a fixed background taken far from the sample; **C** – a fixed background taken in the standard fashion an hour prior; **D** – a fixed background taken with tape near the lens.

We expect this to behave the worst, as the deconvolution should introduce more complicated signal competition. Interestingly, the effect seems to be unpredictable, overestimating the magnitude at 20 and 60 dB SPL but underestimating at 40 dB SPL.

The background taken with the tape in the way was a bit of a “wild card”, where it was not clear what sort of effect it might have on motion measurements. Interestingly, it seems to match the standard method well, suggesting that while the background was incorrect, its being sufficiently different from the sample A-Scan at which we measure motion seems to make this issue matter less for motion measurement.

This is, again, good news for the probe – the use of a reasonable fixed background seems to provide accurate motion measurements.

4.5 M-Scan phase

We are interested also in the phase of the motion measurement, not because we expect it to be impacted by background subtraction, but because we want to be able to interpret motion phase re ear canal pressure in experiments. The motion phase for recordings at 60 dB using three of the fixed backgrounds – the saved B-Scan background from an hour prior, the background taken far from a sample, and the background in which the speaker was present – are compared to the standard method in Figure 11.

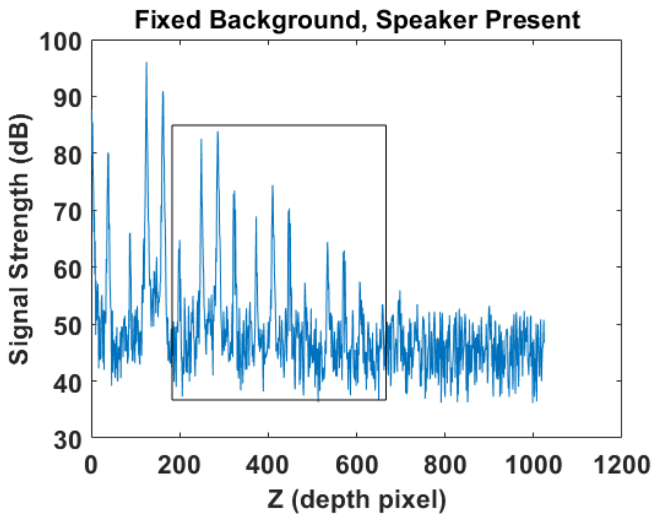

Figure 7: Average of about 100,000 A-Scan magnitudes of the speaker taken over a one-second period, using a fixed background similar to the data scan itself.

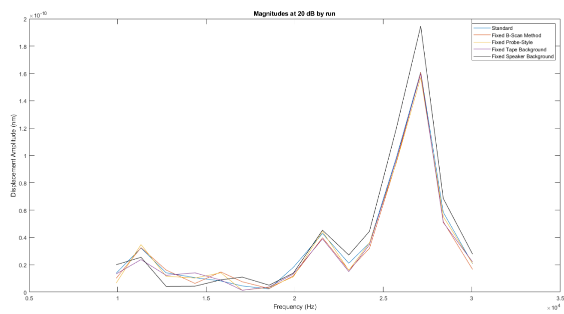

Figure 8: Displacement magnitude response at speaker membrane for a one-second zwuis complex of 15 frequencies at 20 dB SPL, processed with five different backgrounds. All methods agree well except that which uses an A-Scan of the speaker as a background.

Here the fixed background methods all seem to agree in phase, with the standard method being off in a manner that is not immediately recognizable. Considering the same trigger sequence is used to take all three backgrounds, we believe the difference is due to the fact that some number of samples at the beginning of the recording are used to measure the background in the standard method. This means that the standard method should be delayed by some integer multiple of the sampling period.

When we add a 319 sample delay to any of the phases measured with fixed background, we see agreement with the phase measured using the standard method as seen in Figure 12. This corresponds to 319 samples being used to measure the background in the standard method. It is possible that this number may be different for different recordings (i.e. 5 second Zwuis, tones) and

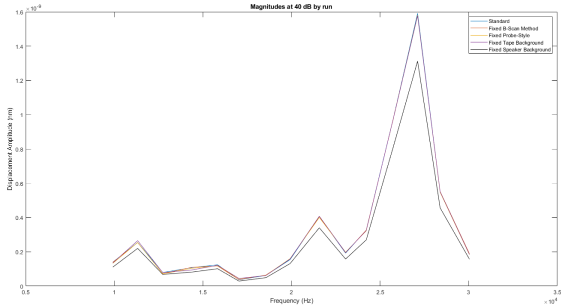

Figure 9: Displacement magnitude response at speaker membrane for a one-second zwuis complex of 15 frequencies at 40 dB SPL, processed with five different backgrounds. All methods agree well except that which uses an A-Scan of the speaker as a background.

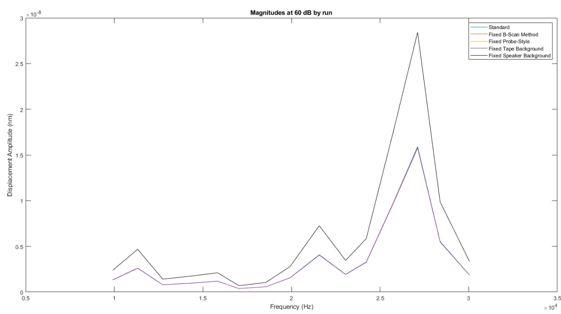

Figure 10: Displacement magnitude response at speaker membrane for a one-second zwuis complex of 15 frequencies at 60 dB SPL, processed with five different backgrounds. All methods agree well except that which uses an A-Scan of the speaker as a background.

this should be tested. However, it seems that we can reliably relate the phases using the standard method to those using the fixed background method, which is important for quantitative phase analysis.

4.6 Summary of results

We have found that the method of using a fixed background taken far away from any sample, as must be done in the probe, yields A-Scans, B-Scans and M-Scans that agree with those using the standard method with only slightly worse SNR. The phase of the motion measured using this method can be reliably related to that of the standard method by a 319-sample delay. This is promising for use with the OCT probe developed by our lab.

We have also found that use of a saved background taken in the standard method (i.e. with the galvo mirrors at their apodization postion) yields slightly lower SNR A-Scans than the standard method. This suggests that we should not pursue this method, which other labs have used, in our experiments.

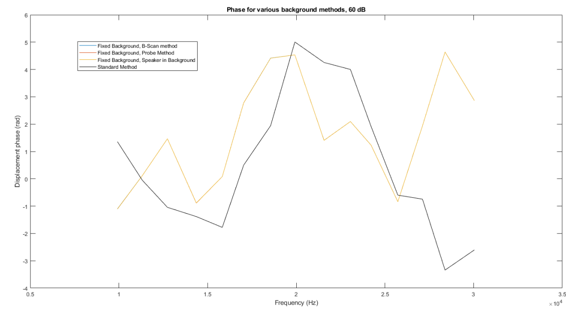

Figure 11: Displacement phase response at speaker membrane for a one-second zwuis complex of 15 frequencies at 60 dB SPL, three of which are processed using the fixed background method and the fourth of which is processed with the standard method. All methods agree well except the standard method.

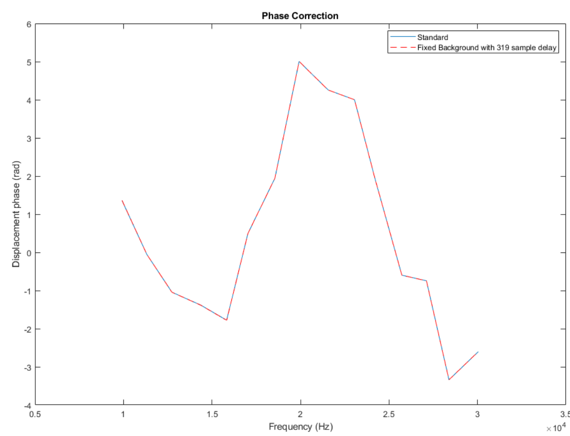

Figure 12: Displacement phase response at speaker membrane for a one-second zwuis complex of 15 frequencies at 60 dB SPL, one of which is processed using the fixed background method while the other is processed with the standard method. We have added a 319-sample delay to the fixed-background measurement to arrive at an agreement between the two.

We have also found that use of an incorrect background can be very detrimental for all scanning modes, but B-Scans taken using poor backgrounds still maintain enough basic characteristics to make first approximations at where you are in a sample. This is important, as the probe will use sample A-Scans as backgrounds in ThorImage's B-Scan mode.

5 Implications for the OCT probe

5.1 Use with the ThorImage software

When using the bulk optics system, we usually use the ThorImage software to acquire B-Scans in real-time which orient us within the cochlea. The background is acquired before each B-Scan using the standard flyback method. ThorImage is easy to use, as it acts as an OCT video camera allowing us to adjust the position of the animal until a good image can be seen. Of course, we would like to be able to similarly use ThorImage with the OCT probe.

When the ThorImage software is configured properly, it can be used with the probe to generate real-time B-Scan videos. This is achieved by feeding the Y-Galvo scanning output of the OCT into a circuit which converts this waveform to one capable of driving the probe. We can even adjust the field of view of the B-Scan, up to 360 μm. However, as the probe cannot take backgrounds using the flyback method, the flyback signal (a 25 ms rectangular high-voltage spike in the Y-Galvo scanning signal) is removed by our circuit. In this time, background scans are being taken to be used in ThorImage's processing algorithm. While the bulk optics system would take these scans with the mirrors positioned properly to record backgrounds, the probe is in the process of moving back across the sample to reach its starting position during this acquisition. This means that an incorrect background is being used in processing the B-Scans.

As we've seen above, use of an incorrect background is quite detrimental to resolution, but does not completely eliminate the character of the signal. The effect is more detrimental in A-mode, where the background is esentially "the same" as the scan of interest. Instead, the background here is some average of scans taken while moving across the sample. This average will look more like the signal than the true background spectrum, but will not be identical to any individual A-Scan in the image. The result is a worse, but recognizable image.

If this is insufficient, the fixed-background B-Mode program described above has been tested with the OCT probe and is capable of generating B-Scans in about half of a second. While this program is not as accessible or fast as ThorImage, it yields higher resolution and higher SNR images quickly.

5.2 Use with our processing programs

As the outputs of the fixed-background programs are fully processed complex A-Scans, the existing M-Scan processing programs (i.e. the MATLAB programs) will function identically on data acquired using the standard or fixed-background method. The only important difference to note is the phase difference of 319 samples described above, which arises from the fact that about this many samples are usually used for background acquisition. A one-line function titled `probe_correct` has been written to correct for this phase difference, and can be added into any of the processing programs as desired, or used after procesing.