Chapter 6

Quantifying OCT image quality in turbid samples using Gaussian and Bessel beams

6.1 Introduction

In this chapter, we analyse the performance of energy-efficient Bessel beams when used to perform OCT imaging of turbid tissue, and, in particular, whether they have an image quality advantage over imaging with Gaussian beams.

Light interaction with turbid tissue affects the image quality indicators described in Section 2.3. In fact, the presence and relative contribution of the different categories of light described in Section 4.2 in the detected OCT signal determines the precision and ability to localise and discriminate the position and intensity of the backscattering events generated by the tissue refractive index distribution.

More specifically, according to [354], for Gaussian beams, the primary effects of low- and wide-angle multiple scattering (Category II and III signals) are a reduction of the imaging contrast and resolution of the OCT system and a slope of the signal intensity depth profile (signal attenuation) that is less steep than that of the slope given by the single-backscattering (Category I) model.

Among the main findings of [164], obtained by applying a model of OCT image formation, based on the superposition of linear system responses, to a fractal description of the size distribution of scatterers in tissue, are:

- low-angle multiple forward scattered light (Category II) passed through the temporal coherence gate degrades resolution and contrast,
- wide-angle multiply scattered light (Category III) degrades contrast without affecting resolution significantly;
• the maximum penetration depth in tissue is limited by the single-scattering coefficient and the mean scattering angle of the tissue. Estimates of these variables for skin and other highly scattering tissues yield a maximum value of 1.5 mm (5–8 mean free scattering lengths) at a wavelength of 1300 nm;
• the coherence time of the source has a strong influence on the resolution of an OCT scanner because it determines the width of the axial point-spread function as well as the fineness of the grain of the speckle; and
• decorrelation of the incident and returning beams occurs, but its effects are difficult to discern in speckle because the phase (wavefront) aberrations caused by the multiple forward scattering (Category II) and multiple backscattering (Category I) effects superimpose in the speckle formation process.

The main aim of this chapter is to investigate how the performance of OCT imaging systems changes when non-Gaussian beams are employed using beam shaping. We investigated this, both theoretically and experimentally. As a first step, we describe the experimental approach for the case of Gaussian beams, in which case our results verify the points above. We then consider non-Gaussian beams within the same experimental system and compare the performance of these beams with Gaussian beams of equal resolution and power.

6.1.1 Testing arrangement: sample configuration with a Gaussian beam

We experimentally verified the aforementioned points by using the structured phantoms described in Section 4.5, overlaid by the tissue-mimicking phantoms described in Section 4.3. This arrangement allows for the viewing of signal-degrading speckle independently from any signal-bearing speckle [160]. The overlayers are slabs of scattering layers of known constant depth (300 µm) but different scatterer concentration. They were made with silicone and a homogeneous distribution of TiO₂ particles (1 µm mean diameter), in the following concentrations: 0 mg/mL, 6.5 mg/mL, and 13 mg/mL.

The phantoms were imaged using a swept-source OCT system (Thorlabs OCS1300SS). Light was incident from the top in Figure 6-1, in free-space, with the beam focus set to the middle of the letters. The various overlayers were interchanged keeping the structured phantom stationary with respect to the illumination beam. The images are displayed on a logarithmic grayscale with the white level corresponding to a signal strength of approximately 50 dB above the noise floor. As the Structured Phantom I in Figure 6-1(a)-(c) is transparent around the letters, no signal contribution should arise from that sample area. This is the case for the lower concentration overlayers (Figure 6-1(a) and Figure 6-1(b)). Another important observation is that the speckle realization within the
letters changes very moderately (e.g., the normalized cross-correlation coefficient within the letter L between (a) and (b) is 0.7), even though the preceding structure is clearly different, meaning that these two different overlayers introduce a limited amount of wavefront aberration.

On the other hand, Figure 6-1(c) shows a “tail” of signal [266, 354-356] extending from the overlayer into the embedding casting of the structured phantom. At the top of the overlayer, there is predominance of Category I signal; as depth increases, there is an increasing contribution of multiply scattered (Category III) signal. The detected signal will feature only Category III contributions as we move to the embedding casting of the structured phantom.

It is interesting to observe in the letters of the structured phantom, that the speckle realization has changed significantly in going from the case of Figure 6-1(a) to Figure 6-1(c) (the normalized cross-correlation coefficient within the letter L between (a) and (c) is 0.5). The contribution from Category I and Category III add on an amplitude basis, as expected, modifying the position of peaks and troughs in the speckle realization. Nevertheless, the speckle size is consistently the same, within the experimental error, at every depth of the image, in Figure 6-1(c) and across all three parts of Figure 6-1(a)-(c), before noise dominates.

In Figure 6-1(d) to Figure 6-1(f), the Structured Phantom II, which has scattering also in the silicone embedding the lettering, shows in full the confounding and contrast-degrading effect of Category III signal, as the top of the letters in Figure 6-1(f) are buried in the multiple scattering signal tails from the overlayer.

By using overlayers made with polystyrene spheres of increasing diameters, we were able to experimentally verify the claim of contrast and resolution degradation with increasing low-angle forward scattering (Category II) signal, brought by overlayers with
increasing scattering anisotropy. As in Section 4.3.2, we adjusted the scatterer concentration to provide the same scattering coefficient (and for the low concentrations used, the same attenuation coefficient, as shown in Figure 6-2(e)) for all overlayers, but increasing scattering anisotropy.

The results of Figure 6-2 clearly demonstrate the progressive trend of contrast degradation with increasing overlayer anisotropy when imaging with Gaussian beams. In Figure 6-2(d), the relative signal contribution from Category II light is high enough to reduce resolution as well as contrast, confirming what was hypothesised earlier by Schmitt et al. [164].

Figure 6-2. Effect of increasing degree of low-angle forward scattering (Category II) signal on OCT images of the Structured Phantom I overlaid by phantoms containing different diameter polystyrene beads. Polystyrene bead concentration varies between overlayers to give the same attenuation coefficient for all overlayers, as shown in the line average plots in (e). The line averages have been taken over a transverse width of 150 µm as indicated by the colour-coded rectangles in (a)-(d). Bead diameter progression from left to right: 1.9 µm, 3.0 µm, 5.4 µm, 8.6 µm. Contrast and resolution is increasingly deteriorated from left to right.

In our effort to benchmark image quality and to provide strategies for its improvement, we now present our work done in quantifying the influence of Bessel beams on image quality in optical coherence tomography. This analysis will bring together all the tools developed so far: firstly, the beam shaping platform used to produce Gaussian and Bessel beams of equal transverse resolution; secondly, the overlayers and structured phantom technology to test different realistic scattering conditions and to quantify the image quality; and, finally, the 3-D beam propagation simulation to verify the different image-carrying and image-degrading light contributions to the OCT signal. These tools will help us answer the question of which beam type attains better OCT image quality in turbid tissue under general tissue-like scattering conditions.
6.2 Quantifying the influence of Bessel beams on image quality in optical coherence tomography [28]

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Abstract: Light scattered by turbid tissue is known to degrade optical coherence tomography (OCT) image contrast progressively with depth. Bessel beams have been proposed as an alternative to Gaussian beams to image deeper into turbid tissue. However, studies of turbid tissue comparing the image quality for different beam types are lacking. We present such a study, using numerically simulated beams and experimental OCT images formed by Bessel or Gaussian beams illuminating phantoms with optical properties spanning a range typical of soft tissue. We demonstrate that, for a given scattering parameter, the higher the scattering anisotropy the lower the OCT contrast, regardless of the beam type. When focusing both beams at the same depth in the sample, we show that, at focus and for equal input power and resolution, imaging with the Gaussian beam suffers less reduction of contrast. This suggests that, whilst Bessel beams offer extended depth of field in a single depth scan, for low numerical aperture (NA < 0.1) and typical soft tissue properties (scattering coefficient, \( \mu_s = 3.7 \text{ mm}^{-1} \) and high scattering anisotropy, \( g > 0.95 \)), superior contrast (by up to \(~40\%\)) may be obtained over an extended depth range by a Gaussian beam combined with dynamic focusing.

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6.2.1 Introduction

Scattering is the basis of OCT contrast in turbid tissue [10, 174, 357], yet it progressively degrades the image quality with depth [164, 266, 358, 359], causing unacceptable degradation often before the backscattered signal reduces to the system noise floor. In the absence of absorption, the most important factors in determining this degradation are the imaging depth in the tissue, \( z_0 \), the scattering coefficient, \( \mu_s \), and scattering anisotropy, \( g \) [234, 266, 355]. Thus far, studies of their effects on OCT image quality have been confined to the use of Gaussian beams and, although several of these have analysed the effect of scattering anisotropy [360, 361], most have studied the impact of only the scattering coefficient [164, 282, 358, 362] at various depths.

Bessel beams have been proposed and demonstrated for use with OCT to extend the depth of field (DOF) over that of a Gaussian beam, whilst preserving the transverse resolution [27, 76, 184]. An extended DOF is particularly attractive in Fourier-domain OCT, which captures a whole depth scan in a single detector dwell time without axial scanning. One drawback of the Bessel beam is that, in order to maintain a more axially uniform irradiance than the Gaussian beam, energy is necessarily distributed more broadly
transverse to the optical axis. As a consequence, the on-axis irradiance is everywhere below that of the peak Gaussian irradiance, for beams of equal power, thereby causing an inherent SNR penalty in comparison to the Gaussian beam at focus [27]. At the same time, however, in a low scattering sample, the Bessel beam maintains a higher axial irradiance than the Gaussian beam in the out-of-focus region beneath the focus, i.e., it gains an SNR advantage, owing to its extended DOF. For this latter reason, Bessel beams have been used to improve imaging through turbid media, including tissue [184, 219, 363], and the explanation of their performance has been linked to their self-reconstructing property. The self-reconstructing property [218, 227] is stated as follows. A Bessel beam can reconstruct its amplitude profile after a characteristic propagation distance if a part of the beam is obstructed or distorted.

Rigorous analysis of OCT imaging performance in turbid media with Bessel or Gaussian beams has not been reported. Thus, whilst some comparison of OCT images acquired with Bessel or Gaussian beams (or Bessel beam illumination and Gaussian beam detection) has been made [27, 76, 184], definitive testing of the apparent advantages of the self-reconstructing property of a Bessel beam, beyond the simple case of partial transverse beam occlusion, has not been done. The choice of optimal beam type for OCT imaging in turbid tissue requires such an analysis. We report on the first rigorous computational and experimental analysis of the influence of Bessel and Gaussian beams on OCT contrast in turbid media. Our analysis contains a number of novel elements, including use of a sophisticated full-wave electromagnetic simulation, benchmarked against experiment, a novel test phantom containing well-defined structure covering a wide range of spatial frequencies, and probing the effects of a range of scattering anisotropies. Our analysis allows us to draw firm conclusions regarding the use of Bessel and Gaussian beams in OCT.

The basic setup, depicted schematically in Figure 6-3, comprises a Gaussian or Bessel beam (NA = 0.12) passing through a thin scattering overlayer to image a target. The beams have the same input power and the centre of their respective DOFs is located at the same depth in the sample. We used three scattering overlayers, each with the same scattering coefficient (\(\mu_s = 3.7 \text{ mm}^{-1}\)) chosen to be typical of tissue [280], but with different scattering anisotropy (\(g = 0.94 – 0.99\)) and a fourth scatterer-free (SF) overlayer for comparison. The narrow range of high scattering anisotropies was chosen to be representative of tissue and to highlight the high sensitivity to this parameter in imaging in turbid media [44]. We used two imaging targets: one comprising random point scatterers to image the system point spread function (PSF) versus depth, termed the PSF phantom, and another comprising
pillars of varying sizes to measure the OCT contrast of differently sized features, termed the contrast phantom.

The separation of the highly forward scattering layer and imaging target helps to isolate the effect of image-degrading and image-forming contributions on image quality [160], according to the presence or absence of scattering in the overlayer.

![Figure 6-3. Beam and sample configuration. (a) Schematic diagram of the beam, overlayer and imaging target, for transparent (top) and scattering overlayers (bottom). (Not to scale and not all pillars shown.) (b) The configurations implemented computationally (left), by simulating the one-way beam propagation through the overlayers, and experimentally (far right), by acquiring OCT images, here shown from an area bounded by a yellow dotted box in a macro-photograph (middle) of the target illuminated with a red beam.](image)

We seek to assess image quality for Gaussian and Bessel beams versus scattering anisotropy in terms of OCT contrast. Recalling Eq. (2-39), we define OCT contrast [157] as the difference in dB between the square of the average OCT signal amplitude, $\omega_{D_{A1}}^2$, in a homogeneous area $A_1$ of a sample, and the square of the average OCT signal amplitude, $\omega_{D_{A2}}^2$, in a homogeneous area $A_2$ neighbouring $A_1$, near the border between the two:

$$C = 10\log_{10}(\omega_{D_{A1}}^2) - 10\log_{10}(\omega_{D_{A2}}^2).$$

(6-1)

We carried out the assessment of OCT contrast in three steps. We firstly experimentally confirmed that neither the PSF phantom nor the contrast phantom alter the measured degradation due to the overlayer. Secondly, we analysed how the PSF generated using either beam is affected by scattering from the overlayers and, thirdly, we showed experimentally where OCT contrast is better maintained in turbid tissue with either beam.

The first step is achieved by fitting analytic models [262, 263, 306, 354] to the experimental peak and ensemble-averaged OCT signal intensity versus depth to validate the
predominance of single scattering in our imaging targets. In practice, this means that we expect the PSF phantom not to add any OCT signal attenuation, and the contrast phantom to produce a very low attenuation outside the pillars, compatible with single-scattering model fits [364].

Secondly, to test the degradation of the PSF in the presence of tissue-like scattering, we simulate how the illumination irradiance of either beam type is distorted after propagating through the overlayer. We then imaged the PSF phantom, with and without scattering in the overlayers for a qualitative comparison. For a more robust and easily manipulated platform for quantification of the PSF degradation, we used the ensemble-averaged computational results.

The PSF degradation due to the overlayers is produced by the forward-scattered background (image-degrading component) [154] coherently added [365] to the ideally attenuated, diffraction-limited illumination beam (image-carrying component), the irradiance of which is simply the scattering-free illumination irradiance attenuated by $e^{-\mu_s z_0}$. We quantify this degradation by calculating the ratio of the ideally attenuated diffraction-limited irradiance peak and the background at a particular axial location, i.e., the on-axis signal-to-background ratio (SBR) [164, 229, 266, 366]. We, thus, evaluate the SBR as a function of the depth along the optical axis for both beams and all overlayers.

Thirdly, with the PSF degradation, there is a concurrent OCT contrast degradation. To show the link between reduced SBR and OCT contrast, and to compare the contrast performances of both beams, we use the contrast phantom and experimentally analyse the pillar-to-embedding casting OCT contrast, $C$, for a range of overlayer anisotropies.

Novel rigorous electromagnetic (EM) simulations of the two beam types, propagating through the scattering overlayers into the PSF phantom, were used to assess the SBR. Experiments with a dual beam OCT system were carried out on characterisation of the imaging targets, on the experimental validation of the simulation, and on the OCT contrast reduction.

6.2.2 Results

Signal-to-Background Ratio

Figure 6-4 presents the simulated beam field amplitude after propagating through each of the overlayers. The linear field amplitude scale is directly comparable to that of Figure 5-14. The results in Figure 6-4 show a significant increase in image-degrading background around the optical axis with increasing anisotropy, even though all three scattering overlayers equally attenuate the diffraction-limited peak of the illumination beam.
Figure 6-4. Simulated beam profiles after propagation through Overlays 1–3 with increasing anisotropy from left to right compared to (left) propagation through a scattering-free (SF) overlayer. Degradation of a single beam profile versus propagation (DOF centre at 300 microns) for (a) Gaussian, and (b) Bessel beams on a linear amplitude scale, where 1 a.u. represents in each case the peak Gaussian field amplitude at focus after attenuation by the overlayer. Dashed horizontal lines in (a) and (b) show the locations of beam profiles presented in Figure 6-5.

Figure 6-5 shows an ensemble-average of 10 transverse beam irradiance profiles plotted at two different depths (60 µm before the focus and at the focus.) Remarkably, the lateral extent and magnitude of the background are similar for both beams. Also, the sidelobe structure of the Bessel beam becomes increasingly buried in the background at high anisotropies (Cases 2 and 3). We found qualitative agreement between the experimental and simulated transverse PSFs (plotted on a logarithmic scale in Figure 5-16), compatible
with the necessarily different realizations of the coherent (speckled) field between the two. We subsequently evaluated the SBR on the simulated beam irradiances of Figure 6-5.

![Simulated transverse beam profiles. Degradation of an ensemble-average of 10 transverse beam profiles for (a) Gaussian, and (b) Bessel beams on a logarithmic irradiance scale, where 0 dB represents the peak Gaussian irradiance (at focus) attenuated by the overlayers. Transverse profiles are plotted for two depths, shown in Figure 6-4, corresponding to 60 μm before the focus, and at the focus (top and bottom, respectively.]

Figure 6-6 shows the computed beam axial and radial logarithmic irradiance plots, in which the solid lines represent the total irradiance immediately after propagation through each overlayer in the PSF phantom. The black dashed line represents the image-carrying (or single-scattering, SS) component of the total irradiance and, by design, it is the same for all cases within either beam type. The on-axis image-degrading component (or background) is shown for both beams after each overlayer using colour dashed lines in the axial plots in Figure 6-6(a). To estimate the axial background, we used the radial plots in Figure 6-6(b). The radial plots are averages over radial lines for 500 polar angles for each of the transverse plots of Figure 6-5(a)-(b). From the radial plots, for both the Gaussian and Bessel beams, the coherent background is directly identifiable off-axis and can be extrapolated with a quadratic fit to estimate its on-axis contribution. This is even more conveniently done for
6.2 Quantifying the influence of Bessel beams on image quality in optical coherence tomography [28]

the Bessel beam, as we can evaluate the background alone as close as 2.7 µm from the optical axis, where the ideally attenuated diffraction-limited beam irradiance is close to zero. Therefore, we calculated the average axial background irradiance at each depth over the same 200 µm range of propagation in the PSF phantom after the overlayer. The results in Figure 6-6 demonstrate that both beams have similar on-axis image-degrading components, of increasing magnitude with increasing anisotropy. As the PSF phantom bulk provides no attenuation, the contribution of both beams decreases with depth in the PSF phantom due to divergence of the forward-scattered light.

Figure 6-6. Simulated total beam irradiance and image-carrying and -degrading components of the beam versus depth in the PSF phantom after each overlayer. (a) Axial ensemble-averaged profiles, for the (top) Gaussian and (bottom) Bessel beams on a logarithmic irradiance scale. (b) Radial averages of the ensemble-averaged transverse Gaussian and Bessel beam profiles on the same scale for the two depths indicated by the dashed lines in Figure 6-4.

With both the image-carrying and image-degrading axial components, we can plot the on-axis SBR versus depth, \( z \), as:

\[
SBR(z) = 10 \log_{10} \left( \frac{I_{SS}(x = 0, y = 0, z)}{I_B(x = 0, y = 0, z)} \right),
\]

where the image-carrying component for either beam after any overlayer is \( I_{SS}(x = 0, y = 0, z) = I_{SF}(x = 0, y = 0, z)e^{-\mu_s z}, \) i.e., the scattering-free irradiance ideally attenuated by \( e^{-\mu_s z} \).

Figure 6-7 shows the on-axis SBR in the PSF phantom for both beams after each overlayer. An important result confirmed by Figure 6-7 is that a higher anisotropy implies a smaller SBR regardless of the beam type. The smaller the SBR, the smaller the ratio of the image-carrying to image-degrading component of the detected backscattered light. This means that, in a scattering sample (such as the contrast phantom), any sample structure (refractive index variation) illuminated by the much larger background irradiance will
increasingly contribute to the image, even if it falls outside the diffraction-limited spot-size, therefore, reducing the OCT contrast.

Another result taken from Figure 6-7 is that, for any given value of $g$, we observe a higher susceptibility of Bessel beams to turbid tissue scattering, i.e., a lower SBR than that of the Gaussian beam in focus. This is due to the reduced on-axis irradiance of a Bessel beam (see Figure 5-14), which reduces only the image-carrying component, i.e., the numerator of the SBR. This susceptibility is mitigated in regions outside the Gaussian focus, where these power ratio considerations, rather than the self-reconstructing property, enable the Bessel beam to regain a higher SBR than the Gaussian beam.

Figure 6-7. On-axis signal-to-background ratio (SBR) versus depth for Overlayers 1–3.

OCT contrast degradation

Figure 6-8(a) shows experimental OCT images, for both beams, of the contrast phantom for the case with no overlayer (left) and two cases with scattering overlayers. For the case with no overlayer, a neutral density filter with optical density 0.25 was used to attenuate the beam to obtain an SNR in the contrast phantom comparable to that obtained with the scattering overlayers. Figure 6-8(b) shows close-up regions including the 60, 70, and 80 µm-wide pillars, boxed by a yellow line in Figure 6-8(a), for the scattering-free case, and Overlayers 2 ($g = 0.987$) and 3 ($g = 0.993$) (Overlayer 1 omitted for clarity).

Figure 6-8(c) shows transverse lines centred on the first row of pillars, for the three selected pillars, in an area close to the beam focus. The lines are incoherently averaged over 40 µm-long segments of A-scans. To aid the visual comparison of contrast degradation on a logarithmic scale, for each curve the mean signal from the embedding casting is normalized to that of the corresponding beam for Overlayer 2. We performed the contrast assessment by analysing the pillar-to-embedding contrast, $C$, in an area boxed by an orange dashed line, ~80 µm x 40 µm across the left edge of the largest pillar. The maximum contrast, $C_0$, was attained by the Gaussian beam after no overlayer with a value
of \( C_0 = 12.6 \) dB. The reduction in contrast calculated as the ratio of the contrast \( C \) to the best case \( C_0 \), i.e., \( C/C_0 \), expressed on a linear scale, is presented in Table 6-1.

The results quantify the contrast degradation that takes place with increasing anisotropy for both beam types, which reaches, at its worst, \( \sim 60\% \) of the contrast at focus (for a given beam) in the case with no overlayer, for the given parameters.

![Figure 6-8. OCT contrast assessment.](image)

(a) Experimental OCT B-scans of the contrast phantom acquired with Gaussian and Bessel beams propagating through the overlayers (Overlay 1 not shown). (b) Close-up (650 × 250 μm) on selected pillar features, boxed by a yellow line in (a). (c) Transverse line across selected pillars in the beam focal region. Lines are averages over depth within the areas bounded by the respective coloured boxes. Quantitative contrast in Table 6-1 is evaluated using the 80 μm × 40 μm orange box around the pillar edge.

<table>
<thead>
<tr>
<th></th>
<th>Gaussian</th>
<th>Bessel</th>
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<tbody>
<tr>
<td>No overlayer</td>
<td>1</td>
<td>0.87</td>
</tr>
<tr>
<td>Overlay 2</td>
<td>0.79</td>
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<tr>
<td>Overlay 3</td>
<td>0.72</td>
<td>0.51</td>
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Table 6-1. Reduction in contrast
The results highlight that, around the focus, the degradation is lower for the case of the Gaussian beam. This is explained by the higher SBR for the Gaussian beam at focus, which translates to a contrast advantage. In fact, at that depth, both beam types produce a similar image-degrading background, but the relative contribution of this background to the image formation process is smaller for the Gaussian beam at focus, owing to its peak irradiance advantage over the equivalent Bessel beam, making the Gaussian beam produce up to ~40% better contrast than the Bessel beam.

6.2.3 Discussion

In this paper, we have brought a comprehensive suite of tools to bear on the question of the relative performance of Bessel and Gaussian beams in OCT imaging in turbid tissue-like media. Through the combination of a novel, rigorous full-wave electromagnetic simulation, a well calibrated dual-beam OCT setup, and a set of controlled scattering overlayers and highly evolved image target phantoms, we have calculated an important indicative parameter of contrast degradation, the on-axis signal-to-background ratio, SBR, and verified that lower SBR leads to higher contrast reduction in experimental OCT images.

The narrow range of high anisotropies ($g = 0.94 – 0.99$) tested here is in line with previous analytical, simulated and experimental studies in OCT of the image-degrading background due to forward scattered light [361, 367], using micron-sized polystyrene particles. In our case, the lower refractive-index contrast ($\Delta n = 0.06$) and the mixture of particle diameters used ($d = 1.3 – 5.5 \mu m$) more closely approximate cell nuclei and other sub-cellular and cellular tissue constituents that dominate the scattering properties of tissue [44].

The reduction of contrast with increasing anisotropy was postulated by Schmitt et al., considering only the Gaussian beam case [164]. We evaluated the SBR similarly to the ratio of single backscattered-to-multiple backscattered amplitude, $\langle i_{sb} \rangle / \langle i_{ms} \rangle$, in Eq. 29a) of Schmitt et al. [164], which gives a quantitative predictor of contrast reduction. We consider the same scattering coefficient and thickness for all overlayers (same scattering parameter).

Therefore, in our case, that ratio would reduce to $\langle i_{sb} \rangle / \langle i_{ms} \rangle \propto \left( \frac{a_m}{a_s} \right)^2 \frac{2 z_R^2}{R^2} \mu_s z \theta_{rms}^2 + 1$, where $a_m$ is the width of the overall PSF, $a_s$ is the width of the diffraction-limited PSF, $z_R$ is the Rayleigh range of the focused beam, $R$ is the 1/e radius of the collimated beam impinging on the objective lens and $\theta_{rms}$ is the root-mean-squared scattering angle. This is the ratio of the width of the total PSF to the width of the diffraction-limited PSF (image-
6.2 Quantifying the influence of Bessel beams on image quality in optical coherence tomography [28]

carrying), i.e., it scales with the width of the multiply forward-scattered PSF (image-degrading). Due to the energy conservation of the elastic scattering process, a smaller background width leads to a larger on-axis background peak irradiance, which is the parameter we chose to evaluate in the denominator of the SBR. For lower root-mean-squared scattering angles $\theta_{rms}$ (higher $g$), the background width is smaller and so is the ratio $\langle i_{sb} \rangle / \langle i_{ms} \rangle$, or, equivalently, the on-axis background irradiance is higher and so the SBR is reduced. This means that our choice of using the on-axis ratio of image carrying-to-image degrading irradiances for the SBR is consistent with the methods and findings of previous research in the field, but it also enables comparison of SBR with beams that do not have a transverse Gaussian profile.

To answer the question of what beam type attains better contrast in turbid tissue (higher SBR), in the situation where there is no a priori knowledge of the depth-dependent phase and amplitude spatial response of the sample refractive index distribution, and no adaptive beam correction, we have made a few assumptions that we recap here.

We considered Bessel and Gaussian beams of equal power and FWHM width and assumed that their focus (or, more accurately, their DOF centre) is placed at the same depth in the sample. Thus, they experience the same peak attenuation, determined by the effective scattering parameter. Indeed, we demonstrated that the overall scattered power is then very similar for both beams, as visible in Figure 6-6(a). Also, we showed that the spatial distribution and concentration around the optical axis of the forward scattered component is very similar for both beams and determined by the scattering anisotropy.

The components of the Bessel beam’s angular spectrum propagate within a narrow range of angles with respect to the optical axis. This fact has two important ramifications: the Bessel beam’s extended DOF and its ability to self-reconstruct. Firstly, all components maintain a similar phase as they propagate along the optical axis, thus resulting in an extended DOF. An illustration of the angular spectra of the Bessel and Gaussian beams, and the corresponding PSF, is shown in Figure 6-9. We hypothesize that Bessel beams do not reconstruct their profile any better than Gaussian beams in turbid media that exhibit distributed scattering that approximates that of biological tissue. This is because in tissue-like turbid media, all sections of each component of the Bessel beam angular spectrum will be perturbed and so perturbed wavefronts will reach all axial locations, thus preventing self-reconstruction. This situation differs markedly from that in which an isolated point scatterer is encountered, when the self-reconstructing property is in evidence [219].
This hypothesis is supported by the fact that both beams produce a nearly identical background, at least for the case in which the obstructions (PMMA spheres in this case) are distributed throughout an area covering most (>95% here) of the beam input energy. These disrupting backgrounds increase in scale with anisotropy, as visible in Figure 6-4.

Yet Bessel beams have been shown to perform well in turbid tissue [184]. However, we believe that the reported persistence of the central lobe, i.e., its propagation stability [229], is not a result of the self-reconstructing property of the Bessel beam but rather a consequence of the ratio of the power confined within the central lobe (in the absence of scattering) to the scattered power being substantially high. More specifically, the power fraction confined within the central lobe is determined by the Fresnel number [27], and the scattered power is determined by the effective scattering parameter. The combination of low Fresnel number (resulting in a high proportion of the beam power being confined in the central lobe) and low effective scattering parameter is all that is necessary to explain the central lobe propagation stability in distributed scattering media.

Another consequence of this analysis is that the lower peak irradiance of the Bessel beam (see Figure 5-14), relative to a Gaussian beam of the same power, is then buried in a similar image-degrading background, leading to lower on-axis SBR and lower OCT image contrast around focus than those produced by the Gaussian beam.

The Bessel beam also suffers an additional contrast penalty at focus, even in the scattering-free case, as the sidelobe structure of Bessel beams inherently reduces contrast compared to the equivalent Gaussian beam (see Figure 6-8 and Table 6-1). However, below the focal region of the Gaussian beam, the Bessel beam holds an irradiance advantage (see...
Figure 5-14) and, consequently, an SBR advantage (far right side of Figure 6-7), which ultimately translates to a contrast advantage. This can be seen by looking at the second row of pillars in Figure 6-8(b), which shows better delineation of the pillars in images formed by the Bessel beam, both in terms of resolution and contrast. (Contrast quantification in this area is not reliable because of the low SNR at this depth.)

We can readily extend our results on SBR and its relation to contrast degradation when the condition that both beams have the same input power is relaxed. Allowing an increase in relative power of the Bessel beam only affects the relative OCT SNR, but not the contrast between features in the image, as doubling the Bessel beam power, for example, will also double the coherent background produced by the overlayer, and their ratio will be independent of the input power.

One significant advantage of Fourier-domain OCT over other microscopy techniques is the high 3-D acquisition speed afforded by the absence of mechanical movement of the sample arm optics (or sample) required to acquire a depth profile. A great advantage of a Bessel beam is its longer DOF. For these reasons, the use of a Bessel beam may still be advisable, as the contrast reduction around the DOF might be an acceptable trade-off for high-speed acquisition, depending on the OCT system sensitivity. At the same time, this suggests that, if acquisition speed is not critical, and combatting image-degrading tissue scattering is a priority, dynamically focusing a Gaussian beam is preferable to use of a Bessel beam in retaining higher OCT contrast at depth (and the same resolution).

Dynamic focusing removes the condition that the centre of the DOF be aligned at a certain depth inside the sample. From simple geometrical considerations, if the Gaussian beam focus is placed deeper in the sample, the beam will encounter more scatterers at any given depth, due to its larger beam cross-section than in the shallower focus case. Hence, as the Gaussian focus is placed deeper in the sample, the effective scattering parameter increases. With an increasingly higher effective scattering parameter, more and more light is scattered and the background increases, leading to a decrease of the SBR, until the Gaussian beam SBR at focus reduces to that of the Bessel beam with its focus in the original position. Below this depth, there will be no contrast advantage of the Gaussian focus over the Bessel beam. The effect of increasing effective scattering parameter with depth would be more prominent in high-numerical aperture regimes, for a given sample [36], reducing the range over which shifting the Gaussian focus deeper produces any contrast benefit relative to an equal NA Bessel beam. However, our results suggest that for sufficiently low numerical aperture (NA < 0.1) and typical soft tissue properties ($\mu_s = 3.7 \text{ mm}^{-1}$ and high scattering anisotropy, $g > 0.95$), superior contrast (by up to ~40%) may be obtained by a Gaussian beam combined with dynamic focusing over an
Chapter 6 Quantifying OCT image quality in turbid samples using Gaussian and Bessel beams

extended depth range. The depth at which the contrast crossover occurs within representative soft tissues, as a function of the NA, will be the subject of further studies.

In conclusion, we analysed the effect of using Bessel or Gaussian beams on OCT contrast in turbid tissue for a range of scattering anisotropies. We rigorously compared the performance of both beam types using beams of equal resolution, input power, and aligned foci in the sample. We did this by analysing both simulated beam propagation and experimental images of scattering imaging targets through overlayers that produce the strong forward scattering typical of biological tissue.

We demonstrated that, the higher the scattering anisotropy of the overlayers, the lower the OCT contrast, regardless of the beam type. This effect is marked by a reduction of the SBR, the on-axis signal-to-background ratio, which is, in effect, the ratio of the image-carrying to image-degrading signal components. The forward-scattered background has a higher on-axis irradiance with increasing $g$, as its divergence ‘cone’ is increasingly concentrated around the optical axis.

Furthermore, the Gaussian beam in focus suffers less reduction of local contrast than does the Bessel beam. For equal powers, this is caused by the inherent lower on-axis peak irradiance of a Bessel beam necessary for it to have an extended depth of field. On the other hand, the irradiance of the forward-scattered background of both beams is very similar, which means that the Bessel beam is just as sensitive to scattering as the Gaussian beam is, and it does not reconstruct any better than a Gaussian beam in turbid tissue. This implies that the SBR for a Bessel beam anywhere along its optical axis is bound to be lower than the SBR for a Gaussian beam in focus, when their foci are at the same depth in the sample. We conclude that, in cases where Bessel beams have been reported to exhibit propagation stability in turbid tissue, this characteristic is more related to a relatively large fraction of the beam power being confined in the central lobe (achieved by using a low Fresnel number), than to the self-reconstructing property.

6.2.4 Methods

Beam and Sample Configuration

Optical beam characteristics

We computationally and experimentally realized Gaussian and Bessel beams, as described in Section 5.4.1. We recap their features here. The beams have equal centre wavelength (840 nm), numerical aperture (0.12, FWHM resolution 2.6 µm) and power, but with different DOF, and peak irradiance (see Table 6-2, and Figure 5-14.) The centre of the DOF of both beams was located at the same depth in the sample, so that both beams
experienced the same effective scattering parameter, $\mu_s z_0$ [282]. For simplicity, we have referred to the centre of the DOF of both beams as the focus position, even though this definition strictly applies only to the Gaussian beam.

Table 6-2. Simulated and experimental beam characteristics

<table>
<thead>
<tr>
<th>Beam type (in free space)</th>
<th>Gaussian</th>
<th>Bessel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transverse resolution, FWHM</td>
<td>2.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Depth of field (µm)</td>
<td>40</td>
<td>330</td>
</tr>
<tr>
<td>Irradiance penalty (dB)</td>
<td>0</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Scattering overlayers

We manufactured and simulated three overlayers as sources of controlled, turbid tissue-like scattering, following the method of Bisaillon et al. [263], as described in Section 4.3.2. We recap their features here. The overlayers are 150 µm-thick silicone slabs containing a uniform-density, random distribution of PMMA spheres. The spheres in each overlayer have different nominal diameters and concentration, chosen to produce the same scattering coefficient, $\mu_s = 3.7 \text{ mm}^{-1}$ and different anisotropy $g$ (see Table 6-3, and Figure 4-5), as calculated from Mie theory. The refractive indices, sphere sizes, resulting scattering coefficient, and thickness were chosen to be representative of typical human soft tissue, such as skin, muscle or epithelial tissue [280, 281]. To simulate each overlayer, ten different random sphere arrangements were computationally generated, enabling an ensemble-averaged measure of the irradiance of the beam after propagation through the overlayer.

Table 6-3. Specified and calculated scattering overlayer characteristics

<table>
<thead>
<tr>
<th>Overlayer</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium refractive index</td>
<td>1.42</td>
<td>1.42</td>
<td>1.42</td>
</tr>
<tr>
<td>Sphere refractive index</td>
<td>1.48</td>
<td>1.48</td>
<td>1.48</td>
</tr>
<tr>
<td>Sphere diameter (µm)</td>
<td>1.3</td>
<td>3.36</td>
<td>5.5</td>
</tr>
<tr>
<td>Sphere concentration ($10^9 \text{µm}^{-2}$)</td>
<td>16,700</td>
<td>400</td>
<td>69</td>
</tr>
<tr>
<td>Scattering coefficient $\mu_s$ (mm$^{-1}$)</td>
<td>3.7</td>
<td>3.7</td>
<td>3.7</td>
</tr>
<tr>
<td>Scattering anisotropy $g$</td>
<td>0.946</td>
<td>0.987</td>
<td>0.993</td>
</tr>
</tbody>
</table>

Imaging targets (phantoms)

To test the imaging PSF, we used a target (phantom) consisting of randomly dispersed 300 – 800 nm-diameter, red iron-oxide ($\text{Fe}_2\text{O}_3$) particles ($\bar{n} \approx 3$) embedded in polyurethane resin ($\bar{n} = 1.49$) (National Physical Laboratory, UK) [225], shown in Figure 4-12.

To assess OCT contrast, we obtained a purpose-manufactured imaging target (contrast phantom) with well-specified geometry based on silicone mixed with TiO$_2$ particles, as described in Section 4.6.1. We recap its features here. The average particle size was 1 µm.
Consecutive rows of pillars protrude into an embedding silicone casting, doped with a different concentration of TiO$_2$ particles (see Figure 4-12). The pillars in each row vary from 10 to 90 $\mu$m in diameter and are spaced at a pitch of 200 $\mu$m. Each row is spaced at a pitch in depth of 100 $\mu$m. The manufacturing technique was replica-moulding soft lithography [30].

With regards to Eq. (6-1), we expect a nominal maximum OCT contrast $C = 10$ dB for a 10-fold concentration difference between the pillar and the embedding casting, as the average OCT signal amplitude scales nominally with the square root of the scatterer concentration [25, 263], i.e., $\bar{a} \propto \sqrt{\rho}$, (Table 6-4).

Table 6-4. Contrast phantom nominal characteristics

<table>
<thead>
<tr>
<th></th>
<th>Casting with pillars</th>
<th>Embedding casting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium refractive index</td>
<td>1.42</td>
<td>1.42</td>
</tr>
<tr>
<td>Scatterer (TiO$_2$) index</td>
<td>2.51</td>
<td>2.51</td>
</tr>
<tr>
<td>Scatterer concentration $\rho$ (mg/ml)</td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td>Scattering anisotropy $g$</td>
<td>0.36</td>
<td>0.36</td>
</tr>
<tr>
<td>OCT nominal contrast $C$(dB)</td>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>

**Analysis Implementation**

**Simulation**

As described in Section 5.4.2, the pseudo-spectral time-domain (PSTD) method [338, 352] was used to perform the EM simulations of the two beam types within the overlayers. We then propagated the output field for a further 200 $\mu$m using an angular spectrum method [353], as no significant bulk scattering is expected in the PSF phantom (see Table 5-2, and Figure 5-15). Our computational analysis of the SBR was limited to the illumination irradiances, as adequately representing the trend of PSF degradation with increasing anisotropy and significantly reducing the complexity of the simulations.

**Experimental**

As described in Section 3.3, in the experiment, we used a spectral-domain OCT system (see Figure 3-15) with a superluminescent diode light source (Superlum), centred at 840 nm wavelength with a 3 dB bandwidth of 50 nm. It featured a reconfigurable sample arm to produce both beam types.
Validation

The process and results of the validation are detailed in Section 4.6.2. We confirmed that both the simulated and experimental beams and overlays are in agreement with our specifications, with minor exceptions. In particular, the single scattering predominance in the imaging targets was confirmed (see Figure 4-13).

6.3 Conclusion

In conclusion, the answer to the question of which beam type attains better OCT image quality in turbid tissue, under tissue-like scattering conditions, is not a simple Bessel or Gaussian beam across all image quality indicators.

In general, we demonstrated that, the higher the scattering anisotropy of a sample, the lower the OCT contrast, regardless of the beam type. This phenomenon is, for example, an explanation for the poor image quality and ability to perform reliable speckle correlation measurement [74] below blood vessels in tissue [276], due to the high scattering anisotropy of red blood cells [246].

Bessel beams present the advantage of an extended DOF, but that comes at the expense of reduced peak power [27]. This trade-off makes the OCT contrast for a Bessel beam worse than that attained by an equivalent-resolution and power Gaussian beam within its DOF, when their foci are at the same depth in the sample.

At the same time, for fixed focus beams, the Bessel beam shows better contrast and resolution than the Gaussian beam, at depths where it presents a higher irradiance than the Gaussian beam when compared in free-space. Furthermore, in highly scattering samples ($\mu_s > 3$), we showed that the Bessel beam propagation stability is more related to a relatively large fraction of the beam power being confined in the central lobe (achieved by using a low Fresnel number), than to the self-reconstructing property.

Our results also suggest that for sufficiently low numerical aperture (NA < 0.1) and typical soft tissue properties ($\mu_s = 3.7$ mm$^{-1}$ and high scattering anisotropy ($g > 0.95$), superior contrast (by up to ~40%) may be obtained by a Gaussian beam combined with dynamic focusing over an extended depth range. Further experiments and 3-D simulations using dynamically-focussed Gaussian beams for a range of NA will help to shed additional light on the extent of contrast improvement and maximum depth of contrast advantage achievable over fixed focus Bessel beams.

In the work described so far, we have used sample arms with identical illumination and detection paths. This setup provides a simple and compact sample arm optical layout,
especially required in those applications where a separate detection path is not feasible. Alternatively, it was proposed to employ Bessel beams only in the illumination path and to use a separate detection path with conventional Gaussian beam optics [184] to reach an acceptable compromise between extended DOF and peak irradiance (hence OCT sensitivity) penalty. Similarly, we expect this compromise to apply to the aforementioned image quality considerations.

In the next chapter, we will present an ultrahigh-resolution optical coherence elastography system based on an extended-focus optical coherence microscope [184] employing separate Bessel beam illumination and Gaussian beam detection. We shall see that ultrahigh-resolution circumvents some issues brought about by speckle, and exacerbates others, and we shall see that the combination of ultra-narrow Bessel illumination and Gaussian detection is very effective in extending ultrahigh-transverse resolution to match the axial resolution.
Chapter 7

Improving OCE image quality: strain precision and resolution

7.1 Introduction

In this chapter, we focus on the characterisation and improvement of optical coherence elastography image quality. We present the results of the optical simulations introduced in Chapter 5, and incorporate those in a multiphysics simulation, combining optical and mechanical models, to highlight the influence of acquisition parameters, such as the loading conditions (compression amplitude), on the elastogram image quality. We do so with specific reference to elastogram precision, i.e., the repeatability of the tissue displacement and strain measurement, as determined by indicators such as displacement and strain sensitivity and strain SNR.

With the goal of expanding the capabilities of OCE to serve the field of cellular biomechanics in situ, we then concentrate on improving the elastogram resolution, without compromising its precision. We do so, by designing an ultrahigh-resolution optical coherence microscopy system with an advanced beam shaping setup, combining Bessel beam illumination and Gaussian beam detection, and devising an image acquisition strategy that preserves elastogram precision.

We demonstrate this improvement on both the tissue-mimicking phantoms, described in Section 4.7, and on freshly excised mouse aorta, revealing the mechanical heterogeneities of vascular smooth muscle and elastin sheets in the aorta wall in exceptional details.

7.1.1 Phase-sensitive OCT displacement measurement precision

In phase-sensitive compression optical coherence elastography, as described in Section 2.2.2, the assumption of a linear phase gradient with depth forms the basis of the displacement calculation from the measured phase difference between the complex OCT
scans of the uncompressed and compressed sample. Eq. (2-30) relates the displacement with the OCT phase difference,
\[ d(x, y, z) = \Delta \phi(x, y, z) \lambda_0 / 4\pi. \]

However, as seen in the simple model shown schematically in Figure 5-2, the phase of an OCT A-scan, even before sampling, is not completely linear with depth. In fact, the phase advances (or is retarded) in conjunction with both scatterer density changes and low phasor sum magnitude, i.e., dark speckle (see phase retardation in the dark speckle near 13 \(\mu\)m, in Figure 5-2).

These intrinsic OCT phase non-linearities hinder the precision of the displacement measurement, as they will carry through to the OCT phase difference. Even in the simple case of a “frozen speckle model” [368], where the speckle realisation, and its underlying phase, does not change with displacement, but is only translated according to the scatterer (bulk) displacement, the native OCT phase difference suffers from increasing non-linearity with increasing sample displacement. We term this phenomenon translation-induced phase decorrelation.

In Figure 7-1, we illustrate this phenomenon using the one-dimensional model of Section 5.2, for optical parameters relevant to the ultrahigh-resolution system we will describe in Section 7.3, i.e., a centre wavelength of 785 nm and FWHM bandwidth of 170 nm.
Figure 7-1. Origin of translation-induced phase decorrelation in one dimension. The OCT axial PSF is shown at the top, for a centre wavelength of 785 nm and FWHM bandwidth of 170 nm. The axial backscattering cross-section of a sample is shown below the PSF in three cases: initial, displaced by 0.1 µm, and by 0.3 µm, and colour-coded from dark blue to light blue, respectively. The OCT signal amplitude and phase signals from these three cases are displayed in the following panel, from dark to light blue and green, respectively. The bottom panel shows the OCT phase difference of each case with the undisplaced sample case.

A similar phenomenon takes place when the sample is undergoing compression (or tension) and, as a result, the phase decorrelates due to both the change in phase realization caused by the rearrangement of scatterers under compression and to the increasing displacement with depth, as seen in Figure 7-2.

In Figure 7-2, we can see how the native OCT phase difference suffers from increasing non-linearity with increasing applied sample (bulk) strain. We term this phenomenon strain-induced phase decorrelation.
Figure 7.2. Origin of strain-induced phase decorrelation in one dimension. The OCT axial PSF is shown at the top, for a centre wavelength of 785 nm and FWHM bandwidth of 170 nm. The axial backscattering cross-section of a sample is shown below the PSF in three cases: initial, uniformly compressed by 8 με, and by 24 με, and colour-coded from dark red to light red, respectively. The OCT signal amplitude and phase signals from these three cases are displayed in the following panel, from dark to light red and green, respectively. The bottom panel shows the OCT phase difference of each case with the uncompressed sample case.

These phenomena, together with the influence of the OCT SNR, form the major sources of optically-related noise in the elastography image. We shall expand their description and quantify their influence on OCE image quality when tied to a proper model of tissue mechanical deformation in the following section.
7.2 Analysis of image formation in optical coherence elastography using a multiphysics approach [25]

Image formation in optical coherence elastography (OCE) results from a combination of two processes: the mechanical deformation imparted to the sample and the detection of the resulting displacement using optical coherence tomography (OCT). We present a multiphysics model of these processes, validated by simulating strain elastograms acquired using phase-sensitive compression OCE, and demonstrating close correspondence with experimental results. Using the model, we present evidence that the approximation commonly used to infer sample displacement in phase-sensitive OCE is invalidated for smaller deformations than has been previously considered, significantly affecting the measurement precision, as quantified by the displacement sensitivity and the elastogram signal-to-noise ratio. We show how the precision of OCE is affected not only by OCT shot-noise, as is usually considered, but, additionally, by phase decorrelation due to the sample deformation. This multiphysics model provides a general framework that could be used to compare and contrast different OCE techniques.

7.2.1 Introduction

Optical coherence elastography, as described in Section 2.2.2, is a technique in which an image (elastogram) is formed of a mechanical property of a sample. In OCE, a mechanical load is applied to a sample, and the resulting deformation is detected using optical coherence tomography (OCT) [18]. There are many forms of OCE based on the method of mechanical loading, such as compressive [22, 106, 110], shear wave [143, 144], surface wave [107, 145], frequency-swept [369, 370] and localized loading using magnetic nanoparticles [371]. Similarly, there are several methods for measuring the resulting sample deformation, including phase-sensitive detection [103, 142], speckle tracking [22, 110, 372, 373], and Doppler spectrum analysis [124, 141, 374]. Regardless of the implementation, inherent to OCE is the interaction between two processes: the mechanical deformation of the sample and its detection using OCT. A theoretical framework for describing both processes is vital in understanding elastogram formation, and in establishing the performance of OCE on a variety of samples and for different system parameters. Several groups have previously analysed aspects of the elastogram formation process, including recent studies focused on the detection of sample displacement [106, 140, 375] and earlier studies examining the mechanical deformation of samples [139, 376, 377]. These studies have largely considered the processes of deformation and detection as independent.
In this paper, we present evidence that the coupling between mechanical deformation and its detection by OCT significantly affects the measurement precision of OCE (i.e., the sensitivity to which the sample deformation is detected), to an extent not previously considered. We do this through a multiphysics simulation of OCE, which combines a finite element model of mechanical deformation, capable of simulating complex geometries, and a linear systems model of displacement detection by OCT, incorporating attenuation and shot-noise-limited optical detection. Multiphysics simulation has the advantage that it can model a wider range of sample properties, system parameters and loading conditions than is generally represented with OCE phantom studies[29]. Previous studies have proposed multiphysics models for simulating speckle-tracking based techniques in both ultrasound elastography [378] and OCE [102]. In this study, we demonstrate a multiphysics simulation approach to phase-sensitive OCE, which is the most prevalent signal processing method currently used [18].

We validate this model by simulating elastograms acquired using a specific OCE technique, namely phase-sensitive compression OCE [106, 142], and demonstrate close correspondence with experimental results. We then use this model to analyse the measurement precisions of phase-sensitive OCE. We show that the displacement sensitivity, and hence the precision, is affected not only by optical noise, as is generally considered [103, 106, 139, 142, 291, 370], but additionally by “phase decorrelation noise”, which results from a violation of the assumption, when measuring displacement from OCT scans, that sample deformation preserves phase correlation. Phase decorrelation noise varies throughout an OCE elastogram as a function of the local deformation of the sample. Previous studies on speckle-tracking methods in OCE have shown that there is a threshold level of decorrelation beyond which sample displacement cannot be reliably estimated [102, 373, 379, 380]. Similarly, a recent study on Doppler flow imaging analysed the adverse effect of decorrelation on Doppler phase sensitivity [381]. However, to the best of our knowledge, the effect of decorrelation has not previously been considered in phase-sensitive OCE. We demonstrate that existing techniques of measuring displacement sensitivity in phase-sensitive OCE [103, 106, 139, 142, 291, 370] overestimate system performance as the amount of sample deformation is increased. Additionally, we demonstrate how this variation in measurement precision affects elastogram quality by using our model to analyse how strain signal-to-noise ratio (SNR) varies with sample deformation. Whilst we focus our analysis on phase-sensitive compression OCE, similar relations between displacement sensitivity and decorrelation may hold true in other forms of OCE. In principle, this framework is extendable to other forms of mechanical loading
7.2 Analysis of image formation in optical coherence elastography using a multiphysics approach [25] 179

and OCT-based detection methods, providing a tool for comparing and contrasting the variants of OCE.

7.2.2 Metrics of elastogram quality and precision

In phase-sensitive OCE, the axial displacement within a sample in response to a load, \(d(x, y, z)\), is calculated from the change in the OCT phase, \(\Delta \phi(x, y, z)\), between scans of the loaded and unloaded sample [103, 106, 142], according to Eq. (2-30).

In compression OCE, the axial displacement can be used to calculate the local axial strain (i.e., the strain defined over a finite range), according to Eq. (2-31) [103, 106, 142], and in shear wave and surface acoustic wave OCE, it can be used to calculate the phase velocity of the propagating wave [107, 143-145].

We consider the precision of OCE as quantified by three metrics: displacement sensitivity, strain sensitivity, and strain SNR. In phase-sensitive OCE, we define the phase difference sensitivity of the OCT system, \(s_{\Delta \phi_m}\), as directly proportional to the minimum measurable displacement. As we saw in Eq. (2-40), assuming shot-noise-limited detection, and OCT SNR \(\gg 1\), \(s_{\Delta \phi_m} \approx \sigma_{\Delta \phi_m} = (\text{SNR})^{-1/2}\) [106]. The displacement sensitivity due to optical noise, \(s_{do}\), is then

\[
s_{do} = s_{\Delta \phi_m} \lambda_0/(4\pi n). \tag{7-1}
\]

The typical method of calculating the displacement sensitivity of an OCE system is to first measure the phase difference sensitivity (the standard deviation of the phase difference) from OCT scans of a stationary, unloaded reflector, then apply Eq. (7-1) [103, 106, 139, 142, 291, 370]. However, because of phase decorrelation noise, this does not quantify the true displacement sensitivity in OCE. Phase decorrelation noise is caused by both strain-induced and translation-induced decorrelation. Strain-induced decorrelation, \(\phi_{de}\), arises from mechanical deformation, or strain, of the sample, which causes the sub-resolution scatterers in a particular region to move closer or further apart, which in turn causes decorrelation of the OCT speckle pattern, and its phase, introducing additional errors into the displacement measurement. Translation-induced decorrelation, \(\phi_{dt}\), arises from shifts in the mean location of scatterers in a particular region due to the loading. The true displacement sensitivity, \(s_d\), is then a function, \(f_d(Q)\), of three terms,

\[
s_{do} = f_d(s_{do}, \phi_{de}, \phi_{dt}). \tag{7-2}
\]

In this study, we measure the displacement sensitivity of a sample under varying loads. The method of measuring displacement sensitivity described above works well for point measurement techniques, such as Doppler OCT; however, in OCE, a collection of
displacement measurements acquired from different spatial locations is required to estimate
the mechanical parameter of interest [382]. For example, compression OCE requires
displacement measurements from a number of axial points to calculate the local strain [106,
382]. To take this into account, and to more accurately define displacement sensitivity in
the context of compression OCE, in this study we use a measure for displacement
sensitivity by considering multiple points within an OCT scan which are all undergoing the
same displacement but have a range of OCT SNR values. In a homogeneous sample
subject to uniform, uniaxial compression, all lateral points at a given depth undergo the
same axial displacement. Thus, we calculate displacement sensitivity as the standard
deviation of the measured displacement over a lateral extent at a given depth in the loaded
sample. As the relationship between displacement sensitivity and OCT SNR is non-linear,
the displacement sensitivity of a collection of points with varying OCT SNR, with a mean
of $\text{SNR}_\mu$, is less than the displacement sensitivity of a single point measurement with OCT
SNR equal to $\text{SNR}_\mu$.

The displacement varies with depth, so taking measurements at different depths allows
us to analyse the effects of translation-induced decorrelation on the displacement
sensitivity. Similarly, all points in a homogeneous sample will experience the same axial
strain, so taking measurements under different loads allows us to analyse the effects of
strain-induced decorrelation on the displacement sensitivity. In addition, the strain
sensitivity is measured as the standard deviation of the strain over a mechanically
homogeneous region. The strain SNR is then given by the ratio of the mean strain, $\mu_e$, over
the strain sensitivity, $s_e$, expressed using a log scale as [106, 139]:

$$SNR_e = 20\log_{10}(\frac{\mu_e}{s_e}).$$ (7-3)

7.2.3 Multiphysics model of optical coherence elastography

In this study, the precision of phase-sensitive OCE is analysed using a multiphysics
simulation model. The model we present comprises four components: simulation of the
optical image formation using a linear systems model of OCT, simulation of the sample
mechanical deformation using the finite element method (FEM), combining the mechanical
deformation with the detection by OCT, and generation of the OCE elastogram.

**OCT image formation**

The model for OCT image formation and the OCT image simulation was described in
Section 5.2.2.
Mechanical deformation

Mechanical deformation of the sample under an applied load is modelled using the finite element method, a numerical method for computing approximate solutions to boundary value problems by subdividing the problem into a finite number of discrete, homogeneous elements [46]. The coupled equilibrium equations are then solved at each element, given a set of model parameters [46]. A FEM model is constructed by:

1. Defining the geometry of the sample;
2. Assigning material properties to each of the deformable regions of the sample;
3. Subdividing the model into discrete elements – this is referred to as “meshing” the model, and the resulting elements as the “FEM mesh”;
4. Applying boundary conditions, such as surface friction; and
5. Defining a known load or displacement introduced to the sample, corresponding, in this case, to the load applied to the sample during OCE imaging.

The solution of the FEM model then provides the displacements, strains and stresses experienced by each of the elements in the mesh. Specifically, the displacements are evaluated at the vertices of the mesh elements, and the strains and stresses are evaluated at the integration points which lie within the mesh elements [46]. Figure 7-3 shows an example of a FEM simulation of compressive loading applied to a sample comprising a stiff rectangular inclusion embedded, at a slight angle to the surface, in a softer bulk surrounds. The size of the FEM mesh, and the size of the displacements, has been exaggerated for visibility.

![Figure 7-3. FEM simulation of a sample containing a stiff inclusion under quasi-static compression. Black lines indicate the FEM mesh (x, z), arrows indicate the magnitude and direction of the computed local displacements under uniform compressive loading from above.](image)

Combining optical and mechanical models

Simulating OCE requires application of the mechanical deformations from the solution of the FEM model to the map of optical scattering potentials to generate an OCT simulation of the sample under load. We accomplish this using linear interpolation, with barycentric coordinates, of the locations of the scattering potentials. In particular, the displacements
calculated from the FEM model, which are known only at the vertices of the FEM mesh, are interpolated throughout the computational space to all locations where scatterers reside.

The interpolation method uses, in general, a different mesh to that used by the FEM model, allowing the FEM model to be solved using an element shape which is optimal for the sample geometry. Once the FEM solution is obtained, Delaunay triangulation [383] is used to obtain a second mesh, upon which the interpolation is based, which uses the same vertices as the FEM mesh but simplex elements (triangles in 2-D, tetrahedra in 3-D). Delaunay triangulation is a common triangulation method with optimized implementations in many programming environments. Barycentric interpolation is then used to linearly interpolate the calculated displacements from the vertices of the FEM mesh to any required location.

For ease of notation, we consider the case of a 2-D field of scattering potentials, however, the same methodology applies in 3-D. The location of a scattering potential, \( \mathbf{r}_s = (x, z) \), can be expressed in terms of a linear combination of the three vertices \((\mathbf{r}_1, \mathbf{r}_2, \mathbf{r}_3)\) of the triangle which surrounds the scattering potential, \( \mathbf{r}_s = \lambda_1 \mathbf{r}_1 + \lambda_2 \mathbf{r}_2 + \lambda_3 \mathbf{r}_3 \). The real-valued coefficients \( \lambda_1, \lambda_2, \lambda_3 \) are called the barycentric coordinates of \( \mathbf{r}_s \) with respect to \((\mathbf{r}_1, \mathbf{r}_2, \mathbf{r}_3)\), and fulfill the constraint that \( \lambda_1 + \lambda_2 + \lambda_3 = 1 \) [384],

\[
\begin{bmatrix}
\lambda_1 \\
\lambda_2 \\
\lambda_3
\end{bmatrix} = \left( \mathbf{r}_1^T - \mathbf{r}_3^T : \mathbf{r}_2^T - \mathbf{r}_3^T \right)^{-1} \left[ \mathbf{r}_s^T - \mathbf{r}_3^T \right]
\]

(7-4)

where \(^T\) corresponds to the transpose operator.

Given a general function \( f() \) that transforms \( \mathbf{r}_1, \mathbf{r}_2, \mathbf{r}_3 \) to \( f(\mathbf{r}_1), f(\mathbf{r}_2), f(\mathbf{r}_3) \), the barycentric interpolation of \( f(\mathbf{r}_s) \) is then given by \( f(\mathbf{r}_s) = \lambda_1 f(\mathbf{r}_1) + \lambda_2 f(\mathbf{r}_2) + \lambda_3 f(\mathbf{r}_3) \), where the barycentric coordinates \( \lambda_1, \lambda_2, \lambda_3 \) are the same as those computed from Eq. (7-4). Let \( f() \) be the function which takes \( \mathbf{r}_i \), the location of the \( i \)-th mesh vertex in the unloaded sample, and returns \( f(\mathbf{r}_i) \), the location of the same vertex in the loaded sample; \( f(\mathbf{r}_i) = \mathbf{r}_i + \mathbf{u}_i \), where \( \mathbf{u}_i \) is the FEM computed displacement of the \( i \)-th vertex from the unloaded to the loaded sample. Applying this transformation to the location, \( \mathbf{r}_{sj} \), of the \( j \)-th scattering potential in the sample then gives the location, \( f(\mathbf{r}_{sj}) \), of the scattering potential in the loaded sample. Figure 7-4 shows a schematic of this process. Applying Eq. (5-12) to the new locations of the scattering potentials, it is then possible to generate a simulated OCT scan of the sample under the applied load.
7.2 Analysis of image formation in optical coherence elastography using a multiphysics approach [25]

Figure 7-4. Computing the new location of the scattering potentials under an applied load. (a) FEM provides a mesh of the sample geometry, and the local displacements evaluated at the vertices of the mesh. (b) Delaunay triangulation remeshes the FEM vertices using triangular elements. (c) Barycentric interpolation uses the relative locations of the scattering potentials with respect to the triangulation to obtain the locations of the scattering potentials in the loaded sample.

A flowchart illustrating the simulation process is shown in Figure 7-5. The inputs and simulation parameters are summarized in Table 7-1. The outputs are the simulated OCT scans of the sample before and after loading, and the simulated OCE elastogram.

Figure 7-5. Flowchart of the multiphysics simulation of OCE. Blue boxes denote inputs to the simulation, green boxes denote particular processes, detailed in Section 7.2.3, and red boxes denote simulation outputs.
7.2.4 Experimental procedure

Phantom fabrication and characterisation

To validate the OCE simulation, we used the cylindrical silicone tissue-mimicking phantom (diameter \( \approx 2.5 \text{ cm} \), thickness \( \approx 1 \text{ mm} \)) with controlled optical and mechanical properties, comprising a stiff inclusion in softer bulk surrounds, described in Section 4.7.

Table 7-1. OCE simulation inputs and parameters

<table>
<thead>
<tr>
<th>OCE system properties</th>
<th>Sample properties</th>
<th>Simulation parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Optical</strong></td>
<td><strong>Structural</strong></td>
<td><strong>Discretization</strong></td>
</tr>
<tr>
<td>( \lambda_0 ), light source free-space mean wavelength</td>
<td>Sample geometry</td>
<td>( \delta z / \delta x_i ), axial/lateral spacing of the initial positions of the scattering potentials</td>
</tr>
<tr>
<td>( \Delta \lambda ), source 3-dB bandwidth</td>
<td></td>
<td>( \delta z / \delta x_j ), axial/lateral spacing of sampling points in the simulated OCT scans</td>
</tr>
<tr>
<td>( f_0 ), objective lens free-space focal length</td>
<td>( n ), sample group refractive index</td>
<td>Spacing between the vertices of the FEM mesh</td>
</tr>
<tr>
<td>( R ), 1/e radius of irradiance of the beam before the objective</td>
<td>Map of relative scatterer densities</td>
<td></td>
</tr>
<tr>
<td>( \sigma^2 ), variance (power) of the optical shot-noise</td>
<td>( \mu(x, y, z) ), map of attenuation</td>
<td></td>
</tr>
<tr>
<td><strong>Mechanical</strong></td>
<td><strong>Optical</strong></td>
<td><strong>OCE processing</strong></td>
</tr>
<tr>
<td>Surface friction</td>
<td>( n )</td>
<td>Axial fitting range for WLS regression [106]</td>
</tr>
<tr>
<td>Applied load</td>
<td>Map of Young’s modulus</td>
<td></td>
</tr>
</tbody>
</table>

In addition, to enable analysis of the effects of mechanical deformation on the precision of OCE measurements, a homogeneous phantom was simulated using properties similar to those of the soft bulk silicone in the inclusion phantom.

Compression OCE system and optical parameters

OCE measurements were performed using a fibre-based Fourier-domain OCT system [24, 106, 139]. The light source is a superluminescent diode with a mean wavelength of \( \lambda_0 = 835 \text{ nm} \), and a 3-dB spectral bandwidth of \( \Delta \lambda = 50 \text{ nm} \). The objective lens in the sample arm has a focal length of \( f_0 = 35.8 \text{ mm} \) in air, and the 1/e radius of the intensity of the Gaussian beam before the lens was measured to be \( R = 793 \mu\text{m} \). The measured free-space axial/transverse resolution (full width-at-half maximum irradiance) is \( 8.5 \mu\text{m} / 11 \mu\text{m} \). The group refractive index of the samples is approximately \( n = 1.42 \). The interference spectrum for each A-scan is captured over 1,300 pixels of a CMOS line-scan camera, resulting in a free-space axial imaging range of 3.13 mm, a free-space axial sampling density of \( 4.8 \mu\text{m} / \text{pixel} \), and an effective imaging range and sampling density in the sample of
2.20 mm and $\delta z_j = 3.4 \, \mu m/\text{pixel}$, respectively. The lateral sampling density was $\delta x_j = 4 \, \mu m/\text{pixel}$. Scans were acquired with a lateral range of 4 mm, cropped to 2.5 mm.

The sample attenuation coefficient, $\mu_t$, was measured from experimental scans of the silicone phantoms, and $\sigma^2$, the variance (power) of the optical shot-noise, was chosen such that the resulting average OCT SNR matched the experimental scans in selected corresponding areas. The relative concentrations of the scattering potentials in the model were chosen to match the ratios of TiO$_2$ particles used in the different parts of the phantoms, and the initial positions of the scattering potentials were discretised to a grid spacing of $\delta z_i = \delta x_i = 0.5 \, \mu m$. For the inclusion phantom, the attenuation was measured to be $\approx 3.69 \, \text{mm}^{-1}$ in the inclusion and $\approx 1.18 \, \text{mm}^{-1}$ in the bulk. The average OCT SNR was 18.6 dB in an area $325 \, \mu m \times 50 \, \mu m (x \times z)$ at the top of the phantom. The relative density of scattering potentials in the inclusion:bulk was 3.125:1. In the homogeneous phantom, the attenuation coefficient was 1.5 $\text{mm}^{-1}$. The noise level was chosen to produce an average OCT SNR of 15 dB at the top of the homogeneous phantom.

The sample arm contained an imaging window rigidly fixed to a piezo-electric ring actuator, enabling imaging and mechanical loading of the sample from the same side [139, 141]. A preload, generating 9–22% bulk strain, was applied to each sample using a rigid brass plate, of larger surface area than the sample, to ensure uniform contact between the brass plate, the sample, and the imaging window [139]. The system operated in a common-path configuration, and the imaging window itself, a partial reflector, acted as the OCT reference arm mirror.

The piezo-actuator was driven by a 5 Hz square wave, slow enough that the sample could be considered to be under quasi-static load. This was synchronized to the OCT B-scan acquisition rate of 10 Hz, ensuring consecutive B-scans were acquired in the loaded/unloaded state [106]. Axial displacements in the sample were calculated from Eq. (2-30) using the unwrapped phase difference between consecutive (loaded minus unloaded) OCT B-scans. A schematic of our phase-sensitive compression OCE setup, and example displacement and strain A-scans are shown in Figure 7-6. The zero-phase reference coincides with the position of the imaging window (labelled “reference reflector” in Figure 7-6(a)) in this common-path setup. The phase difference, $\Delta \phi$, and hence the relative displacement between the reference reflector and the sample, $d$, are both zero at the imaging window, and maximal at the rigid plate and the measured displacement and strain values are negative under compressive loading. The local strain was calculated using weighted-least-squares linear regression over a fitting range of 100 $\mu m$ [106].
Mechanical parameters

The FEM models were constructed in the Abaqus simulation software package (Dassault Systèmes, Providence, USA, v6.12). The geometry of the inclusion phantom was determined from structural OCT scans of the phantom. The elasticity of the samples was modelled using a linear, isotropic, elastic model. Using the stress/strain curves obtained from the bulk compression testing of the constituent phantom materials, a value for Young’s modulus was calculated from a tangent fitted to the curve about a quiescent point specified by the bulk strain applied during the preload stage of imaging [139]. The inclusion phantom was subject to a preload displacement of ≈ 270 µm from an initial thickness of ≈ 930 µm, corresponding to an initial pre-strain of 22%. This gives a Young’s modulus of 830 kPa in the inclusion, and 33.3 kPa in the bulk, comparable to inclusion phantoms examined in previous studies [139]. Both the inclusion and the bulk are modelled as being nearly incompressible, with Poisson’s ratio close to 0.5 [139], and the displacement introduced to the surface of the phantom by the piezo-actuator is 0.93 µm. The homogeneous phantom was simulated with an initial pre-strain of 9% and a thickness under preload of ≈ 1060 µm, giving a Young’s modulus of 19.2 kPa, comparable to homogeneous phantoms examined in previous studies [139]. The Poisson’s ratio is also taken to be close to 0.5, and the displacement introduced to the surface of the phantom was simulated for 23 discrete steps between 0.21 µm and 15 µm.

Since the phantoms are relatively wide compared with the imaging field-of-view (FOV), we approximated the 3-D sample deformation by a 2-D plane strain model, which set the displacement and strain in the y direction to zero [385]. Similarly, lateral symmetry conditions were defined at the left and right edges of the FEM model, which set lateral (x) displacement at the edges of the OCT FOV to zero. The silicone phantoms were relatively sticky (likely due to a portion of the silicone remaining uncured), and not lubricated during OCT scanning, so friction at the top and bottom surfaces of the FEM simulation was taken to be infinite (no lateral motion at these surfaces) during loading. The average spacing...
between vertices of the FEM mesh was set to \( \approx 5 \, \mu m \). Initial tests showed that this gave comparable numbers, with much shorter processing time, than finer mesh spacing.

7.2.5 Results

Inclusion phantom

Figure 7-7 shows the simulation of phase-sensitive compression OCE compared to experimental scans of the silicone inclusion phantom. Overall, the results show good agreement between the experiment and simulation. Variations in the axial strain can be seen surrounding the inclusion (Figure 7-7(e) and Figure 7-7(f)) due to non-uniform stresses caused by the inclusion geometry [139]. Figure 7-8 shows five regions used for numerical comparisons of the mean OCT SNR, mean displacement \( (\mu_d) \), displacement sensitivity \( (s_d) \), mean strain \( (\mu_s) \), strain sensitivity \( (s_s) \), and strain SNR \( (SNR\varepsilon) \) between the experimental and simulated scans. The results of these comparisons are shown in Table 7-2. OCT and strain measurements were averaged over each entire region; displacement and displacement sensitivity were evaluated along a line at the bottom of each region. The numbers are comparable between the experiment and simulation, although the displacement and strain sensitivity are less in the experimental scans in all cases. These results are discussed in more detail in Section 7.2.6.
experimental scans (blue) compared to the simulation (red). A-scans are averaged over a 30 µm lateral region indicated by the blue and red dotted boxes in (a)–(f).

![Image](image.png)

Figure 7-8. Regions used for comparing experiment to simulation, shown on (a) the simulated OCT image, and (b) the simulated strain elastogram. (c)–(g) Zoomed views of the 325 µm × 50 µm \((x \times z)\) regions 1–5, respectively, from the experimental and simulated OCT B-scans, and the experimental and simulated OCE strain elastograms.

Table 7-2. Numerical comparison of experimentally acquired vs. simulated elastograms for the regions marked in Figure 7-8.

<table>
<thead>
<tr>
<th>R</th>
<th>OCT (dB)</th>
<th>(\mu_x) (nm)</th>
<th>(s_x) (nm)</th>
<th>(\mu_z) (µε)</th>
<th>(s_z) (µε)</th>
<th>SNR (_x) (dB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18.6</td>
<td>18.6</td>
<td>-94.5</td>
<td>-115</td>
<td>-2.9</td>
<td>-2.4</td>
</tr>
<tr>
<td>2</td>
<td>20.7</td>
<td>20.8</td>
<td>-52.7</td>
<td>-164</td>
<td>-2.0</td>
<td>-2.4</td>
</tr>
<tr>
<td>3</td>
<td>23.2</td>
<td>20.2</td>
<td>-414</td>
<td>-411</td>
<td>-0.34</td>
<td>-0.26</td>
</tr>
<tr>
<td>4</td>
<td>7.4</td>
<td>6.2</td>
<td>-659</td>
<td>-683</td>
<td>57.8</td>
<td>45.1</td>
</tr>
<tr>
<td>5</td>
<td>13.2</td>
<td>13.5</td>
<td>-860</td>
<td>-805</td>
<td>39.7</td>
<td>28.6</td>
</tr>
</tbody>
</table>

Displacement sensitivity

Displacement sensitivity was estimated using simulations of the homogeneous phantom detailed in Section 7.2.4. If calculated in the usual manner, from multiple measurements of a single point with high OCT SNR in a stationary, unloaded sample, the displacement sensitivity is 2.2 nm from a point with an OCT SNR of 27 dB. This is comparable to previous experimental measurements of 1.2 nm at an OCT SNR of 50 dB [139], which, although calculated at a higher OCT SNR, is less than would be expected from Eq. (7-1) due to phase noise caused by environmental conditions and galvanometer mirror noise. As detailed in Section 7.2.2, in this study we calculate the displacement sensitivity as the standard deviation of the displacement over a 500 µm lateral extent, as this better reflects that OCE elastograms generally require multiple phase or displacement measurements to estimate strain [382].

Figure 7-9 shows plots of the displacement sensitivity, calculated as defined above, as a function of strain and depth in the sample. For this homogeneous sample, the axial strain is constant throughout the sample for a given load. The axial displacement is likewise
The average OCT SNR at the top of the phantom was 15 dB (min ≈ 0 dB, max ≈ 27 dB). Even in the absence of noise (blue curves), the displacement sensitivity degrades, due to phase decorrelation, with both increasing strain (Figure 7-9(a)) and depth (Figure 7-9(b)) into the sample. In both plots, the displacement sensitivity decreases dramatically as the combined phase decorrelation (strain-induced and translation-induced) reaches a threshold.

The black lines show the displacement sensitivity calculated using the method described in Section 7.2.2, from the simulation of a stationary, unloaded sample; the sensitivity was calculated to be 14.8 nm at a mean OCT SNR of 15 dB. This value provides an upper bound on the plots of displacement sensitivity in the presence of both optical noise and phase decorrelation (red curves). There is a strain threshold below which the displacement sensitivity is limited by optical noise (solid red curve in Figure 7-9(a)); however, decorrelation with depth always causes the displacement sensitivity to decrease, regardless of the initial displacement sensitivity.

Figure 7-9. Displacement sensitivity ($S_d$) vs. (a) local strain at various depths in the sample, and (b) vs. depth at selected values of strain in the sample. Blue lines are simulation results without optical noise. Red lines are simulation results with optical noise and attenuation. Black lines are displacement sensitivity values calculated at zero strain, assuming only optical noise.
Strain signal-to-noise ratio

Figure 7-10 shows plots of the strain SNR calculated over 500 µm × 50 µm (x × z) regions in the simulated homogeneous phantom. With no optical noise (blue curves), strain SNR is constant with mean strain at a particular depth, until a threshold is reached at which the strain SNR decreases sharply (Figure 7-10(a)). When optical noise is included, increasing strain increases strain SNR until the same threshold is reached. Similar to the displacement sensitivity, even without including optical noise, the strain SNR decreases with depth into the sample, until a point at which it drops abruptly (Figure 7-10(b)). The higher the applied strain, the greater is the axial displacement at a particular depth, and the closer is the point of strain estimation failure to the sample surface.

Figure 7-10. Strain SNR (SNR_\text{\varepsilon}) (a) vs. local strain at various depths in the sample, and (b) vs. depth at selected values of strain in the sample. Blue lines are simulation results without optical noise. Red lines are simulation results including optical noise and attenuation.

7.2.6 Discussion

This study has demonstrated the first multiphysics simulation of phase-sensitive OCE. Related models have previously been proposed for simulating speckle-tracking based methods in OCE. Zaitsev et al. [379, 380] described a method of simulating OCE images by generating a map of random values, filtered such that the correlation resembled an actual OCT irradiance image. Sample deformation was simulated using a simple analytical expression of axial and lateral displacements given an applied strain. Fu et al. [373] used a more rigorous optical simulation of OCT in their model of OCE, generating a 2-D speckle map using a random distribution of scattering particles and a linear systems model of OCT
Analysis of image formation in optical coherence elastography using a multiphysics approach [25]

[375]; however, their study of mechanical deformation only considered homogenous samples. Chan et al. [386] used both a linear systems model of OCT and a FEM model of mechanical deformation; however, they simulated a time-domain OCT system, and implemented the displacement of the scattering potentials using a finely spaced grid. This implementation limits the resolution of the scatterer locations to the size of the grid spacing. Finer resolution of the scatterer locations in the optical simulation of the loaded sample requires finer grid spacing, with a corresponding increase in the computational memory requirements, reducing the practicality of this method to simulating larger, or 3-D, samples.

In this paper, we used our simulation to assess the effect of sample deformation on the precision of measured displacement and strain. A related study by our group examined the accuracy of OCE strain elastograms to the elasticity distribution of samples [139]. That study showed how uneven stress distributions surrounding a stiff inclusion can lead to “wings” of high strain around the inclusion, which matches what we observe in Figure 7-7(e) and Figure 7-7(f). However, this previous study did not consider the interaction between sample deformation and detected optical signal. In this paper, we have demonstrated that this interaction sets bounds on measurable displacement and strain in phase-sensitive OCE. A major implication, not considered in the previous study, is that the precision of OCE strain elastograms, and hence the precision to which we can infer the sample elasticity distribution, is heavily dependent on the value of the applied load.

It should be noted that the phase decorrelation noise described in this study is not noise in the conventional sense of being random with each measurement, but rather, like speckle, it is a deterministic result of the particular arrangement of sub-resolution scatterers. Measuring displacement with phase-sensitive OCE is only valid under the assumption that the speckle pattern in the scans used to determine phase shift is fully correlated, analogous to the frozen speckle model described previously by Duncan and Kirkpatrick [368]. Phase decorrelation noise is then the degradation in precision that results when this assumption is no longer valid. Consider the case of a single scattering particle at the focus of the OCT beam, as it moves a small distance from \( z = 0 \) to \( z = \delta z \). From Eq. (5-11), the phase shift resulting from this motion is

\[
\Delta \phi(\delta z) = 2k\delta z + 2\arctan\left(\frac{2z_0\delta z}{f^2 - f\delta z}\right),
\]

approximated in Eq. (2-30) by

\[
\Delta \phi(\delta z) \approx 2k\delta z.
\]

It is clear that the error in this approximation will increase as \( \delta z \) increases, contributing to what we have termed, in this study, translation-induced decorrelation noise. In addition, when we consider the case of multiple scattering particles within the OCT resolution volume, displacement of the scatterers will also lead to a shift or translation in the speckle pattern,
which implies that the phase difference is calculated between phase realizations comprising partially decorrelated areas of non-overlapping speckles. Similarly, strain will not only cause the mean location of the particles to displace, but will also alter the distance between the particles. In this case, the error caused by using the approximation in Eq. (2-30) will increase with the amount of strain, leading to what we have termed strain-induced decorrelation noise.

In Section 7.2.5, we demonstrated the ability of our multiphysics simulation framework to model elastograms generated using phase-sensitive compression OCE. Scans of the inclusion phantom shown in Figure 7-7 demonstrate good agreement between the experimental data and the simulation; however, the speckle patterns clearly differ between the two OCT B-scans in Figure 7-7(a) and Figure 7-7(b). This is due to the infeasibility of matching the exact locations of the sub-resolution scattering particles in the experiment and the simulation [26]. The current optical simulation also does not take into account effects such as the confocal function, or wave-front distortion in the turbid medium, which may be present in the experimental scans. Nevertheless, provided that the statistics of fully developed speckle patterns match, as is the case here, the differences in the specific speckle realizations do not affect the validity of the multiphysics simulation framework in modelling elastograms. In Table 7-2, the displacement sensitivity and strain SNR are slightly inferior in the experimental data, acquired from scans of a 3-D phantom, compared to the 2-D simulation. This is likely caused by sample motion out-of-the-plane (along the \(y\)-axis) in the experimental scan that could result in additional phase decorrelation. The largest discrepancies between the experiment and simulation are in the regions closest to the boundaries (top and bottom) of the phantom. This is likely due to the effects of friction [139], which is assumed to be infinite in the simulation, but in the experimental scan is likely to be finite; the exact value is, however, unknown.

Section 7.2.5 also demonstrates the effects of phase decorrelation on the displacement sensitivity of phase-sensitive OCE. In the absence of optical noise, the displacement sensitivity degrades approximately linearly with the amount of strain in the sample (Figure 7-9(a)), although at lower strains the displacement sensitivity is limited by the optical noise rather than by strain-induced decorrelation. However, from Figure 7-9(a), it is clear that this best-case sensitivity degrades rapidly when the sample is subject to loading, i.e., the displacement error is increasing with strain. Without phase unwrapping of the phase difference, \(\Delta \phi\), the maximum detectable strain for a 1 mm thick sample is \(\ll 1 \text{ m\varepsilon}\), which, combined with lower OCT SNR in real samples, is likely the reason previous studies on OCE have not encountered this issue. The translation-induced decorrelation is likely to be a more significant factor in practice. As Figure 7-9(b) demonstrates, the displacement
sensitivity degrades approximately exponentially with depth into the sample, which, in our system, corresponds to increasing relative displacement between the reference reflector and the sample. Translation-induced decorrelation degrades the displacement sensitivity regardless of the OCT SNR; beyond a depth of \( \approx 800 \mu m \) into the sample, the displacement sensitivity can have degraded by a factor in the range of 2 to 5.

The last part of Section 7.2.5 shows the corresponding effects of phase decorrelation on the strain SNR (Figure 7-10). Even without optical noise, strain-induced decorrelation limits the maximum strain SNR to approximately 40 dB for this system, which occurs close to the zero-phase reference at the top of the phantom. Without noise, the ideal scenario is reached with low levels of applied strain, as these achieve the best displacement and, hence, strain sensitivity, whilst retaining the maximum achievable strain SNR at the surface. With noise, there is a trade off between increasing strain SNR with higher applied loading, and achieving better displacement and strain sensitivity with lower applied loading. This suggests that for optimum strain SNR in phase-sensitive compression OCE, the applied load should be below the strain threshold, but otherwise as large as possible.

The results shown in Figure 7-9 and Figure 7-10 were acquired from simulations of homogeneous samples in which the strain resulting from uniform compressive loading is uniform across the sample. In more complex geometries, such as the inclusion geometry shown in Figure 7-7, or in tissue, the strain can vary greatly throughout the sample. In particular, the strain in areas surrounding a stiff inclusion, such as in a phantom or around a region of fibrosis in tissue, can be much higher than the strain in the surrounding bulk. Nevertheless, the general conclusions to be drawn from the results shown in Figure 7-9 and Figure 7-10 still hold, as can be seen in Table 7-2. Regions 1 and 2 in Table 7-2 have comparable OCT SNR, but Region 1 has a higher mean strain, just past the threshold of \( \approx 2 \mu \varepsilon \), and therefore a moderately lower strain sensitivity, but still higher strain SNR than Region 2, as would be expected; similarly for Regions 2 and 3.

A common method to improve OCT SNR is to average multiple scans. Improvement by averaging assumes that the acquired data consists of a noise component, which is isotropic and independent with each measurement, and a signal component, which is constant over all the averaged points. These conditions hold in the case of OCT shot-noise, but not for decorrelation noise, which, for the same loading conditions, does not change between OCT measurements. Thus, averaging of the OCT scans will improve the shot-noise contribution to displacement and strain sensitivity, bringing the red curves of Figure 7-9 and Figure 7-10 closer to the blue ones, but we expect it not to have any effect on the decorrelation noise contribution. A possible method to minimize decorrelation noise could be to sample the displacement at multiple points during loading, such that the
displacement, and hence relative strain, between any two sample points is minimized. However, this would require many more OCT acquisitions and, thus, slow the OCE scan speed.

This study has focused on phase-sensitive compression OCE; however, the multiphysics simulation framework we have presented here is readily extendable to other forms of mechanical loading and optical detection. For example, needle OCE [387, 388] could be simulated by restricting the simulation output to a single A-scan. Dynamic loading methods such as shear wave [143, 144], surface wave [107, 145], or frequency-swept loading [369, 370], could be modelled by running the simulation for each time step. Similarly, the simulation framework is extendable to modelling deformation in 3-D.

7.2.7 Implications for the ultrahigh-resolution regime

Our analysis of image quality in elastography showed the interplay between the mechanical and optical contributions to the image formation process and the influence of the loading conditions on the displacement sensitivity and ultimately on the strain SNR.

With resolution of tens of micrometres, OCE shows promise in visualizing mechanical contrast in tissue on a scale intermediate between that of cells and organs. This resolution is yet too coarse to probe changes to the mechanical properties of tissue on the smaller length scales required to study the onset and development of disease [19], restricting the opportunity for OCE to impact on the field of cell mechanics. This field focuses, in part, on pathogenesis studies at the cellular scale and on the characterisation of the mechanical signatures of healthy and diseased cellular tissue constituents [20].

An important consequence of the analysis shown in the preceding sections is to highlight the link between increasing OCT axial resolution and precision of the axial displacement measurement from the OCT phase difference. Figure 7-11 illustrates the effect of halving the resolution on the precision in the one-dimensional noiseless simulation presented in Section 7.1.1.
Figure 7-11. Effect of the OCT axial resolution on the precision of the sample axial displacement measurement. (a) The axial resolution is 3.2 µm, a factor 2 less than that used in (b). An increased axial resolution leads to decreased precision of the axial displacement measurement, for the same bulk compression, as seen in the bottom right plot, compared to the bottom left plot.

Already in this optically and mechanically simplified simulation, the effect of increasing the axial resolution is a visible degradation of the precision of the axial displacement measurement, for resulting bulk strain, i.e., a given loading condition for the sample.

This is a great challenge to overcome in the pursuit of designing and building the first precise ultrahigh-resolution optical coherence elastography system, which we tackle in the following section.
7.3 Ultrahigh-resolution optical coherence elastography [31]

Andrea Curatolo, Martin Villiger, Dirk Lorenser, Philip Wijesinghe, Alexander Fritz, Brendan F. Kennedy, and David D. Sampson

Abstract: Visualising stiffness within the local tissue environment at the cellular and sub-cellular level promises to provide insight into the genesis and progression of disease. In this paper, we propose ultrahigh-resolution optical coherence elastography, and demonstrate three-dimensional imaging of local axial strain of tissues undergoing compressive loading. We combine optical coherence microscopy and phase-sensitive detection of local tissue displacement to produce strain elastograms with resolution \((x,y,z)\) of \(2\times2\times15\) \(\mu\text{m}\). We demonstrate this performance on freshly excised mouse aorta and reveal the mechanical heterogeneity of vascular smooth muscle cells and elastin sheets, otherwise unresolved in a typical, lower resolution optical coherence elastography system.

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OCIS codes: (110.4500) Optical coherence tomography; (140.3300) Laser beam shaping; (100.2980) Image enhancement; (170.6935) Tissue characterisation.

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Disease often changes the mechanical properties of tissue, providing a source of contrast for diagnosis distinct to that provided by optical properties [389]. Physical palpation, the most commonly used diagnostic method based on mechanical properties, enables physicians to detect abnormalities, but is subjective and provides very coarse resolution. Elastography is a medical imaging technique based on the spatially resolved response of tissue to mechanical loading and can provide a map of mechanical properties. Elastography based on magnetic resonance imaging or ultrasound imaging [16] provides better resolution than manual palpation and is emerging as a clinical tool in the diagnosis liver fibrosis and breast cancer [17]. Optical coherence elastography (OCE) [23], a form of elastography based on optical coherence tomography (OCT), shows promise in visualizing mechanical contrast in tissue with resolution of tens of micrometres, enabling the probing of mechanical properties on a scale intermediate between that of cells and organs. However, disease changes the mechanical properties of tissue on even smaller cellular to sub-cellular length scales [19]. Indeed, the field of cell mechanics seeks to study the onset and development of disease on the cellular scale and to characterize the mechanical signatures of healthy and diseased cellular tissue constituents [20]. Ideally, such studies would include the ability to provide \textit{in situ} images at cellular resolution in live tissues in their native environment.
Atomic force microscopy [390] can be used to probe the mechanical properties on this and still finer scales, but it has a small field of view (FOV), typically hundreds of µm², providing measurements from the tissue surface only, and is not compatible with \textit{in vivo} imaging.

Optical coherence microscopy (OCM), in both its Fourier-domain and full-field implementations [10], is a high-resolution version of OCT providing spatially isotropic resolution below 2 µm. Elastography techniques based on OCM have the ability to probe sub-surface mechanical contrast overcoming a main limitation of AFM [391, 392].

A limitation of elastography based on Fourier-domain OCM is the technique’s small depth of field (DOF), imposed by the high numerical aperture required to achieve ultrahigh in-focus resolution [10]. On the other hand, the ability to acquire a depth scan of the sample structure, with a single spectral acquisition, is an attractive feature that enables phase-sensitive displacement measurements. The dynamic focusing ability of full-field OCM overcomes the DOF limitation, but at the expense of severely restricting accurate phase-sensitive displacement detection. To date, the employment of a digital volume correlation (DVC) method [110] on a rather large 3-D sliding window has limited the strain resolution of full-field OCM.
In this paper, we seek to address the above shortcomings by achieving simultaneously high strain sensitivity and high spatial resolution. We do so via ultrahigh-resolution optical coherence elastography (UHROCE), which combines extended-focus Fourier-domain optical coherence microscopy (xf-FDOCM) [184], phase-sensitive detection, and compressive mechanical loading [24]. Bessel beam illumination and Gaussian mode detection [393] maintain sub-2\( \mu \)m transverse resolution over a nearly 100\( \mu \)m DOF. Phase-sensitive detection enables nanometre-scale displacement sensitivity over a wide displacement dynamic range from less than 1 nm to \( \sim 2 \mu m \) [106]. We demonstrate UHROCE in three spatial dimensions on a structured silicone phantom and on freshly excised mouse aorta, comparing UHROCE images with co-located typical, lower resolution OCE images [24]. The comparison highlights the unprecedented strain resolution of UHROCE and its ability to visualise the distribution of features, such as 10\( \mu \)m-thick elastin sheets in the tunica media of mouse aorta, otherwise unresolved in the lower resolution strain elastograms.

In the UHROCE system, broadband light from a supercontinuum source (SuperK Extreme EXR-1, NKT Photonics, Denmark) is spectrally shaped to produce a spectrum with central wavelength \( \lambda_0 = 785 \) nm and 3 dB bandwidth of 200 nm. The source output is coupled to one arm of a 2×2 broadband fibre coupler forming part of a Mach-Zehnder interferometer. The optical design of the sample arm employs point-spread function (PSF) engineering and beam scanning in a three-stage telescope system, depicted in Figure 7-12(a). A Bessel beam (Fresnel number \( N = 10.5 \)) generated by a spatial light modulator (SLM) (Pluto NIR II-HR, Holoeye Photonics AG, Germany) provides a good trade-off between improved DOF and reduced peak intensity compared to an equal resolution Gaussian beam [27]. The Bessel beam is relayed from the intermediate image plane 3 (IIP 3) to the object plane (OP) within the sample, with an effective demagnification of \( \sim 21 \)x. The illumination path (effective numerical aperture, \( NA_{eff} = 0.27 \)) and separate detection path (\( NA_{eff} = 0.16 \)) result in a Bessel illumination DOF of 94\( \mu \)m and a Gaussian detection DOF of 21\( \mu \)m. Figure 7-12(b) shows the axial plane and in-focus transverse plane illumination and detection irradiance profiles, acquired using a beam-profiling camera (SP620U, Ophir-Spiricon, USA). Figure 7-12(c) shows cross-sections of a PSF near focus, \( i.e., \) the OCM signal generated by imaging a phantom comprising red iron oxide (Fe₂O₃) 300 – 800 nm-sized particles (refractive index, \( n \approx 3 \)) embedded in polyurethane resin (\( n = 1.49 \)) (National Physical Laboratory, UK). The measured axial and transverse resolutions are 1.5 and 1.6\( \mu \)m, respectively.
7.3 Ultrahigh-resolution optical coherence elastography [31]

Measurements of the phase stability were carried out to benchmark the displacement sensitivity of the xf-FDOCM system against an in-house, common-path OCT system [24]. The best displacement sensitivity calculated between groups of four A-scans, at an A-scan frequency of 20 kHz, is 2.3 nm. The best displacement sensitivity for the common-path OCT system, at an A-scan frequency of 10 kHz, is 0.34 nm [24]. Such comparatively large phase fluctuations in the xf-FDOCM system are due to the relatively long path lengths employed in the Mach-Zehnder interferometer setup. A common-path configuration would reduce these fluctuations, but it could not be implemented due to the effectively “dark-field” design [393], necessary to reduce the otherwise saturating signal from the sample coverslip. Mechanical loading was introduced using a piezoelectric transducer [24] to compress the sample, from the opposite side to that imaged.

The signal processing chain involves calculating the phase difference between a pair of complex xf-FDOCM B-scans of the uncompressed (pre-loaded) and compressed sample acquired at the same \( y \)-location. Axial unwrapping of the resulting phase difference [24] allows for unambiguous calculation of the local displacement in the sample. Local strain was estimated from the displacement map following the weighted-least squares (WLS) method described in [106]. To reduce the WLS regression window on the measured axial displacement for a given strain sensitivity, \( i.e. \), to exploit the xf-FDOCM resolution improvement, additional steps in the acquisition and signal processing scheme were required. Phase averaging was performed to improve the displacement sensitivity [24]. At each transverse \( x \)-location, four A-scans were acquired for each of the uncompressed and compressed sample states, enabling the averaging of their respective phase differences, thereby providing increased displacement sensitivity with no loss of spatial resolution. Similarly, two pairs of uncompressed–compressed B-scans per \( y \)-location were acquired and the resulting co-located B-scans strain elastograms were averaged.

A structured tissue-mimicking phantom with well-determined optical and mechanical properties was fabricated using two-component, room-temperature vulcanizing silicone (Elastosil®, Wacker, Germany) [29]. It comprised two stiff inclusions separated laterally by less than 20 \( \mu \)m, in a 325 \( \mu \)m-thick soft bulk. The optical properties were controlled by adding titanium dioxide (\( \text{TiO}_2 \)) nanoparticles (refractive index \( n \approx 2.51 \), average diameter 25 \( \text{nm} \)) to the silicone. A scatterer volume ratio of 3:1 between the inclusion and the bulk provided optical contrast. A stiffness (Young’s modulus) ratio of 3:1 between the inclusion and the bulk provided mechanical contrast. The Young’s modulus of the inclusion was 150 kPa and that of the bulk was 50 kPa. These values were measured for a preload of 4 kPa applied to the bulk, corresponding to 10% strain.
We acquired UHROCE images of this phantom. Comparative images of the same phantom were taken with the lower resolution OCE system, with an OCT resolution of 7.8 μm (axial, in air) by 11 μm (transverse) [24]. The amount of phase averaging applied was similar to that used in the UHROCE system and the same axial fitting range window of 20 μm (15 μm FWHM equivalent) was used for strain estimation. For both systems, the additional bulk compression of the inclusion phantom applied during acquisition produced ~0.3% strain.

The results of this comparison are presented in Figure 7-13: for the OCE system at left; and for the UHROCE system at right. Figure 7-13(a) presents OCT B-scan sections of the phantom cutting through the two inclusions. The two inclusions are much better resolved in the xf-FDOCM image, as expected, and clumps of TiO₂ nanoparticles can be clearly observed. Figure 7-13(b) shows the displacement B-scans. The UHROCE displacement B-scan (at right) appears smoother over the chosen axial fitting length. This is related to the higher spatial frequency of the “granularity” in the image. This factor depends on the interplay between the noise sources in the displacement measurement and the axial pixel sampling density. Slightly lower OCT SNR, and higher strain- and displacement-induced decorrelation [25], due to the smaller xf-FDOCM average speckle size (i.e., the higher resolution at focus [26]), introduce more phase (difference) noise per pixel in the UHROCE displacement image. Nevertheless, the higher axial pixel sampling density within the given axial fitting length is the reason for the higher strain sensitivity attained with the UHROCE system, visible in the B-scan strain elastograms shown in Figure 7-13(c).
A better appreciation of the transverse resolution improvement can be gained from the en-face strain elastograms in Figure 7-13(d).

We also demonstrated the value of such resolution improvement by capturing strain elastograms of an ex vivo mouse aorta. A healthy mouse aorta wall is composed of three layers [394], progressing from the lumen outwards: tunica intima; tunica media; and tunica adventitia. The tunica intima is composed of one layer of endothelial cells followed by a layer of smooth muscle cells embedded in an extracellular matrix. The tunica media is made up of multiple smooth muscle layers interleaved with elastin sheets (lamellae) and fibres. The tunica adventitia is rich in collagen and is in contact with connective tissue, such as adipose cells. In our experiment, we imaged an excised aorta, compressed to flatten its lumen so that the tunica adventitia was lying flat against the compressing actuator and coverslip.

Figure 7-14 shows images of the mouse aorta acquired with both the OCE and the UHROCE systems, and compares them to histology of the same tissue. Figure 7-14(a) shows en-face OCT images taken mainly within the tunica media. The adipose tissue on the left aided in the co-location within a few hundred microns of images acquired on the two independent systems. The xf-FDOCM image (right) reveals sections of elastin sheets (lamellae), providing higher backscattering than the vascular smooth muscle, unresolved in the OCT image (left). The UHROCE strain elastogram (right) shows that the strain within the elastin sheets is low, whilst the smooth muscle layers in between them are subject to compressive strains of up to 0.4% in some areas. The improvement over the OCE image (left) is substantial, as features clearly resolved in the UHROCE image are only partially resolved at the lower resolution. Figure 7-14(c)-(d) show in detail the undulating structure of the elastin lamellae (black in the histology image with Verhoeff-Van Gieson (VVG) staining) sandwiching the smooth muscle, and resulting in a muscle layer of only a few cells in thickness experiencing high strain, as detected by the UHROCE system. (In Figure 7-14(b), a phase unwrapping artefact in the UHROCE image has been removed by fusing an image at the same depth processed from the same data with different phase unwrapping parameters.)
Figure 7-14. OCT images and strain elastograms of a mouse aorta taken with the two systems: OCE and UHROCE, compared with histology. (a) En-face OCT images within the tunica media. (b) Corresponding en-face strain elastograms. (c) OCT B-scan images of the aorta cross-section (taken with OCE – top – and UHROCE system – middle), and a VVG-stained histology image (bottom panel) from a closely located but not corresponding section. (d) Speckle-averaged magnified portion (top inset) of the structural B-scan image in (c), corresponding B-scan strain elastogram (middle inset), and representative histology section (bottom inset) showing interleaved elastin sheets and smooth muscle cell layers.

The system and results presented here fall within the greater scope of utilizing OCM to measure tissue deformation. In this space, two groups [391, 392] have used magnetic fields to actuate exogenous agents (magnetic micro beads) dispersed in the tissue. They used Fourier-domain OCM systems for their inherent ability to detect nanometre-range tissue motion. The results were promising, however, tissue displacement was plotted only for specific points of interest in cell cultures, without providing an image of mechanical contrast. Another group [110] performed high-resolution compression OCE by using a piston to load the tissue and full-field OCM to detect the local displacement, from which they derived the strain tensor. They presented images of the axial strain experienced by compressed biological ex vivo tissue, but, as mentioned above, the strain resolution suffered from a rather coarse DVC method of evaluating tissue displacement.
We have proposed and demonstrated compression UHROCE and compared it to a typical, lower resolution phase-sensitive, compression OCE system. The spatial resolution \((x, y, z)\) of the strain elastogram, at \(2 \times 2 \times 15 \, \mu\text{m}\), is the highest reported to date in optical elastography. We have demonstrated the advance in terms of resolution by imaging an inclusion phantom and an \textit{ex vivo} mouse aorta. The results show the ability of UHROCE to resolve mechanical heterogeneity at the micrometre scale, suggesting the method could be suitable for imaging of cell mechanics \textit{in situ} in tissues [395]. A challenge for stiffness quantification at the cellular level with the proposed technique is the uneven stress distribution within the flattened sample. The addition of methods of estimating local stress [23] would allow this technique to be made quantitative along the axial direction and produce elastograms representing tissue stiffness (Young’s modulus), similar to those obtained with dynamic full-field OCE [21], but at a higher spatial resolution. Such a combination of resolution, depth of field, and quantitative mechanical properties would represent a unique combination in the study of mechanobiology.

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### 7.4 Conclusion

In this chapter, we focused on optical coherence elastography image quality. We first analysed the factors influencing displacement and strain sensitivity, and strain SNR. We did so by presenting a multiphysics model for elastogram formation in OCE, which incorporates the mechanical deformation of the sample in response to a load, the detection of the resulting sample motion using OCT, and the combined effect of both processes on the final OCE elastogram. The model links a finite element model of mechanical motion to a linear systems model of OCT image formation, using barycentric interpolation to calculate the displacement of the scattering potentials, which comprise the sample. We have validated this model by comparing simulated strain elastograms of a silicone inclusion...
phantom against experimental data obtained using phase-sensitive compression OCE. Using this model, we have shown that the coupling between mechanical deformation and its optical detection impacts the precision of phase-sensitive OCE. We have shown how phase decorrelation, due to both local strain and to displacement, adversely affects the displacement sensitivity and leads to an optimum value of applied load. We have shown how this change in precision in turn affects the strain SNR.

We then concentrated on improving the OCE resolution with the goal of visualizing stiffness within the local tissue environment at the cellular and sub-cellular level, as this promises to provide insight into the genesis and progression of disease.

In this space, we proposed ultrahigh-resolution optical coherence elastography, and demonstrated three-dimensional imaging of local axial strain of tissues undergoing compressive loading. The technique employs a dual-arm extended focus optical coherence microscope to measure tissue displacement under compression. The system uses a broad bandwidth supercontinuum source for ultrahigh axial resolution, Bessel beam illumination and Gaussian beam detection, maintaining sub-2 μm transverse resolution over nearly 100 μm depth of field, and spectral-domain detection allowing high displacement sensitivity.

In the ultrahigh-resolution regime, the value of applied load to attain the same displacement sensitivity (and strain SNR) as with a lower resolution system, has to be reduced, because the higher resolution reduces the precision of the axial displacement measurement, as seen in Section 7.2.7. Alternatively, phase averaging techniques have to be used to increase the precision. On the other hand, reducing the axial displacement fitting range for strain estimation, to exploit the increased resolution, enables higher displacement precision for the ultrahigh-resolution system, owing to the higher axial pixel sampling density. We benchmark the advances in terms of resolution and strain sensitivity by imaging a suitable inclusion phantom.

The system produces strain elastograms with a record resolution \((x, y, z)\) of \(2\times2\times15\) μm. We also demonstrate this performance on freshly excised mouse aorta and reveal the mechanical heterogeneity of vascular smooth muscle cells and elastin sheets, otherwise unresolved in a typical, lower resolution optical coherence elastography system, opening up new avenues in the study of mechanobiology.

We achieved this promising result only by combining the findings of our study on OCE image formation through simulations, together with the resolution improvement, depth of field extension and contrast penalty mitigation using optical beam shaping. These image quality improvements contribute to advance OCE as the technique of choice for \textit{in situ} analysis of the mechanical properties of biomedical tissues.
Chapter 8

Conclusions

Microscopic structural and functional information is vital in the understanding of many biological and physiological processes, and in the early diagnosis and management of diseases. Despite optical coherence tomography (OCT) and optical coherence elastography (OCE) having shown promise in imaging tissue structure based on backscattered infrared light and in imaging localised tissue stiffness; these techniques still require fundamental image quality characterisation and improvements to expand the range of in vivo or in situ applications, where high-resolution optical imaging can have a competitive advantage over other biomedical imaging techniques. Characterising and improving these techniques, in terms of depth of field, contrast and resolution, will ultimately increase the sensitivity and specificity of identification of tissue-type, leading to their use with higher clinical and scientific relevance in the medical diagnostics and biomechanics fields.

In this thesis, we have characterised and improved image quality in OCT and OCE, performed at millimetre depth in turbid tissue, by means of optical beam shaping and optical simulations. More specifically, we designed and built an OCT system, using beam shaping to provide a variety of sample illumination/collection geometries. We showed that, in a double-pass reflection (identical illumination/collection) geometry (such as is required practically in needle-based and endoscopic probes), for Bessel beams, the Fresnel number governs the trade-off between the DOF improvement and the resulting OCT sensitivity penalty, over a Gaussian beam of equal transverse resolution. We designed and manufactured novel tissue phantoms and image test targets, providing controlled optical, mechanical and structural properties, all aimed at quantifying OCT and OCE image quality in turbid tissue. To strengthen our quantitative assessment of image quality, we developed imaging and optical simulations. We presented a suite of OCT simulations based on the image-carrying signal alone and on a more rigorous (but more computationally intensive) full-wave numerical solution of Maxwell’s equations in two dimensions, and we extended the simulation of the beam propagation in turbid tissue to three dimensions. We analysed the improvement of OCT image quality in turbid tissue, using Bessel and Gaussian beams.
of equal transverse resolution and input power. We showed that, for an energy-efficient low-Fresnel number Bessel beam, the depth of field gain over the Gaussian beam comes, not only at the expense of a reduced OCT sensitivity, as in free-space, but also at reduced contrast compared with that attained by the Gaussian beam at focus. As the image-degrading background, generated by multiple scattering of either beam in turbid tissue, is very similar in terms of irradiance and physical extent (or angular spectrum), for a given set of tissue optical properties \((\mu_s, g)\), we questioned the self-reconstructing property of the Bessel beam as the reason behind the propagation stability of its central lobe in turbid tissue. We pointed out that the combination of low Fresnel number and low effective scattering parameter provides a better explanation for the higher SNR and contrast attained by the Bessel beam at depths below the Gaussian focus. We also showed that a higher scattering anisotropy, e.g., encountered when imaging through blood vessels, degrades contrast, regardless of the beam type. Finally, we improved the OCE resolution, without compromising strain sensitivity, by studying the OCE image formation process and then designing and building an extended depth-of-field optical coherence microscopy system, based on Bessel beam illumination and Gaussian beam detection, capable of performing OCE at the cellular level, with applications in cell mechanics \textit{in situ}.

Below, we summarize the key results of this research, discuss some limitations of the current studies, and provide our perspectives on areas for further development in this field.

### 8.1 Research contributions and significance

In the following section, we describe in more detail the contributions to the research goals of this thesis and their significance. The first objective of this research is restated here:

1. Providing the tools needed to improve and quantify OCT and OCE image quality in turbid tissue, \textit{i.e.}, the tools to alter it (beam shaping), measure it (tissue phantoms), and analyse it (simulations).

The objective is of high importance, as most of the work reported in the literature assessing image quality using different hardware techniques [76, 157, 208, 209, 396] lacked the tools needed to compare their effects in turbid tissue in a rigorous way. To alter image quality, we designed and built an OCT system, using beam shaping to provide a variety of sample illumination/collection geometries. We also designed and manufactured tissue phantoms to simulate realistic tissue scattering conditions. To measure image quality, we designed and used image test targets and structured tissue-mimicking phantoms. Finally, to analyse image quality, we developed imaging and optical simulations.
8.1 Research contributions and significance

More specifically, one fundamental hardware technique to alter image quality in a raster scanning imaging system is the use of different optical beam shapes. Among the multiple beam shapes our system could generate, we investigated quasi non-diffracting Bessel beams [218]. This is because Bessel beams are of great interest in biomedical imaging [307], as they feature a very large DOF compared to conventional (Gaussian) focusing schemes. However, their central lobe carries only a small fraction of the total beam power, leading to a strongly reduced peak irradiance. This is problematic for power-limited applications such as optical coherence tomography or optical coherence microscopy as it can result in a prohibitive reduction of the signal-to-noise ratio. In Chapter 3, for Bessel beams in the context of OCT [27], we have demonstrated that low-Fresnel-number ($N < 10$) Bessel beams in the regime can provide substantial DOF gains of up to 13x for a sensitivity penalty of less than 20 dB.

Together with developing the ability to alter image quality, we pursued the development of tissue phantoms, mimicking optical, mechanical and structural properties. This development was fundamental in order to provide durable and repeatable test targets for system characterisation, for intra- and inter-system comparison, and to study image formation and demonstrate ways to improve it.

In Chapter 4, we covered all aspects and provided details on the characteristics and methods that we used to fabricate and characterise phantoms for OCT and OCE. We presented the world’s first three-dimensional structured phantom for use in OCT [30], produced with a two-stage silicone casting procedure and a soft-lithography patterning technique, called replica moulding. We then designed and manufactured a further-developed structured phantom used to determine which beam type provides better OCT contrast under various turbid tissue scenarios, determined by three custom-made overlayers with varying scattering anisotropy.

We also designed and fabricated structured phantoms, with silicone inclusions presenting different stiffness to the embedding silicone, and used them to validate our simulation of OCE images and better understand noise sources in OCE, and ultimately to help validate our effort to improve resolution in OCE.

The ability to characterise and improve image quality in OCT and OCE is greatly aided by the ability to control beam shapes, power, and tissue optical, structural and mechanical properties to the finest degree, and repeat measurements on the images several times with a variety of different parameters. While this, in principle, can be done experimentally, in Chapter 5, we developed and used various optical simulations in order to generate and study the image formation process in both OCT and OCE in a more accurate way.
For the understanding and description of the speckle phenomena in OCT, we used a two-dimensional model based on local sums of random phasors [26]. For modelling phase-sensitive OCT measurements, which is required to understand OCE image formation and improve its image quality, we coded a more advanced linear systems model providing realistic OCT amplitude and phase images from numerical scattering phantoms [25]. Finally, to assess and improve OCT image quality in scattering turbid media, we developed and expanded a novel full wave model [324], using FDTD or PSTD [338] numerical solutions of Maxwell’s equations in two and three dimensions. We presented the first realistic OCT image simulator in two dimensions [32], given a beam type and a deterministic refractive index distribution, which by its own nature, implicitly includes phenomena, such as speckle and multiple scattering, and explicitly models coherence of the incident beam and scattered light. We wrote code and executed it on a supercomputer to find the field distribution of selected Gaussian and Bessel beams propagating through the scattering overlayers, and inform us of the level of image-degrading contribution to the OCT signal and the ability to reconstruct the amplitude profile in turbid tissue of either beam.

With all the previous tools, i.e., beam shaping, tissue-mimicking phantoms, and light-tissue interaction and image formation simulations, we were able to fulfil the second objective of this research, namely:

2. Analysing OCT image quality in turbid tissue, altered using Bessel and Gaussian beams, and measuring the relative improvements.

In Chapter 6, in fact, we answered the question of which beam type attains better OCT image quality in turbid tissue, under tissue-like scattering conditions. In general, we demonstrated that, the higher the scattering anisotropy of a sample, the lower the OCT contrast, regardless of the beam type. This is, for example, an explanation for the poor image quality and ability to perform reliable speckle decorrelation measurement [74] below blood vessels in tissue [276], due to the high scattering anisotropy of red blood cells [246].

Bessel beams present the advantage of an extended DOF, but that comes at the expense of peak power [27]. This trade-off makes the OCT contrast for a Bessel beam worse than that attained by an equivalent-resolution and power Gaussian beam within its DOF, when their foci are at the same depth in the sample.

At the same time, for fixed focus beams, the Bessel beam shows better contrast and resolution than the Gaussian beam, at depths where it has an irradiance superior to the Gaussian beam when compared in free-space. Furthermore, in highly scattering samples ($\mu_s > 3$), we showed that the Bessel beam propagation stability is more related to a relatively large fraction of the beam power being confined in the central lobe (achieved by
using a low Fresnel number), than to the self-reconstructing property. Incidentally, this means that the higher the tissue scattering coefficient, the lower the Fresnel number needed to maintain the propagation stability of the beam central lobe. However, the lower the Fresnel number, the smaller the attainable DOF gain relative to an equal resolution Gaussian beam.

Our results also suggest that for sufficiently low numerical aperture (NA < 0.1) and typical soft tissue properties ($\mu_s = 3.7\ mm^{-1}$ and high scattering anisotropy, $g > 0.95$), superior contrast (by up to ~40%) may be obtained by a Gaussian beam combined with dynamic focusing over an extended depth range.

Dynamic focusing removes the condition that the centre of the DOF be aligned at a certain depth inside the sample. However, when the Gaussian focus is placed deeper in the sample, the effective scattering parameter increases. With an increasingly higher effective scattering parameter, more and more light is scattered and the background increases, leading to a decrease of the signal-to-background ratio (SBR), and therefore a decrease of contrast. Below the depth at which the Gaussian and Bessel beam SBR are equalised, there will be no contrast advantage of the Gaussian focus over the Bessel beam. The effect of increasing effective scattering parameter with depth would be more prominent in high-numerical aperture regimes, for a given sample [36], reducing the range over which shifting the Gaussian focus deeper produces any contrast benefit relative to an equal-NA Bessel beam.

The third objective of the research described in this thesis is:

3. Improving OCE image resolution without compromising other image quality descriptors (e.g., depth of field, strain sensitivity), in order to perform OCE at the cellular level, with applications in cell mechanics in situ.

High OCT resolution and contrast were sought in order to obtain the high-resolution tissue displacement image needed to derive the tissue mechanical properties using phase-sensitive compression OCE. We aimed at a target resolution (both axial and transverse) below 2 $\mu$m, achievable with NA > 0.25 and a supercontinuum source.

In light of our findings in Chapter 6, we chose to discard the option of using dynamic focussing, both for the limited contrast gain with high NA and because it is unsuited to phase-sensitive compression OCE. In fact, to obtain a high strain sensitivity, a fast A-scan rate, compatible only with a FD-OCT acquisition scheme, is required, as it provides high phase (and displacement) sensitivity.

In the work described so far, we have used sample arms with identical illumination and detection paths. This setup provides a simple and compact sample arm optical layout, especially required in those applications where a separate detection path is not feasible.
Alternatively, it was proposed to employ Bessel beams only in the illumination path and to use a separate detection path with conventional Gaussian beam optics [184] to reach an acceptable compromise between extended DOF and peak irradiance (hence OCT sensitivity) penalty. Similarly, we expect this compromise to apply with respect to the contrast penalty when extending the DOF for high resolution.

In Chapter 7, we presented the highest resolution optical coherence elastography system reported to date, based on an extended-focus optical coherence microscope [184] employing separate Bessel beam illumination and Gaussian beam detection. To properly understand the challenges of the ultrahigh-resolution regime, we focused on optical coherence elastography image quality first, by studying the image formation process. We first analysed the factors influencing displacement and strain sensitivity, and strain SNR. We did so by presenting a multiphysics model for elastogram formation in OCE, which incorporates the mechanical deformation of the sample in response to a load, calculated using a finite element model, the detection of the resulting sample motion using OCT, as simulated by our second model, and the combined effect of both processes on the final OCE elastogram. Using this multiphysics model, we showed that the coupling between mechanical deformation and its optical detection impacts on the precision of phase-sensitive OCE. We showed how phase decorrelation, due to both local strain and to displacement, adversely affects the displacement sensitivity and leads to an optimum value of applied load to maximise the strain elastogram precision, i.e. the strain SNR.

With these findings in mind, reported in [25], we then concentrated on improving the OCE resolution with the goal of visualizing stiffness within the local tissue environment at the cellular and sub-cellular level, as this promises to provide insight into the genesis and progression of disease.

The ultrahigh-resolution optical coherence elastography system we designed uses a broad bandwidth supercontinuum source for ultrahigh axial resolution, Bessel beam illumination and Gaussian beam detection, maintaining sub-2 μm transverse resolution over nearly 100 μm depth of field, and spectral-domain detection allowing high displacement sensitivity.

In the ultrahigh-resolution regime, the value of applied load required to attain the same displacement sensitivity (and strain SNR) as a lower resolution system had to be reduced, because the higher resolution reduced the precision of the axial displacement measurement. We managed to increase the precision by using some phase averaging techniques. On the other hand, by reducing the axial displacement fitting range for strain estimation, to exploit the increased resolution, we attained overall a higher displacement precision for the ultrahigh-resolution system, owing to the higher axial pixel sampling density. We
8.2 Study limitations and future work

8.2.1 Current limitations

The research presented in this thesis achieved the goals of characterising and improving image quality in OCT and OCE. However, some remaining limitations still need to be addressed, and several follow-up studies naturally lead on from the current research. In this section, we list the main limitations, suggest possible solutions, and recommend future work.

In the dual-beam OCT setup, presented in Section 3.3 and used in Section 6.2 to address the question of which beam type attains better OCT image quality in turbid tissue, we had to use two different paths for the generation of the equal-resolution Gaussian and Bessel beams, limiting the ability to quickly toggle between beams. The easy and rapid switching between beams was a desirable feature that we envisaged to have in our system at the time of acquiring the SLM. However, as explained in Section 3.1.3, due to the low fill factor and diffraction efficiency of the SLM, and due to the chromatic aberration possessed by SLM-generated Gaussian beams, it was necessary to separate the Gaussian and Bessel beam paths. The combination of more powerful lasers and higher fill factor SLM could enable the use of the SLM in the pupil plane of the objective lens for the shaping of Gaussian and Bessel beams on the same path.

The three-dimensional structured phantom proved invaluable in benchmarking OCT contrast between Gaussian and Bessel beams in Chapter 6. Nevertheless, experimental uncertainties resulted in sub-optimal accuracy in the quantification of the contrast. Manufacturing tolerances may have led to a difference in concentrations between the pillars and the embedding casting slightly larger than the design value, i.e., a higher pillar-to-embedding casting contrast in the images than expected. This is because, as the concentration of TiO$_2$ in the embedding casting was very low, in some instances, areas of non-fully developed speckles and in others areas absent of signal were observed in the benchmarked the advances in terms of resolution and strain sensitivity by imaging a suitable inclusion phantom.

With this system, we produced strain elastograms with a record resolution $(x, y, z)$ of $2\times2\times15$ μm. We also demonstrated this performance on freshly excised mouse aorta and revealed the mechanical heterogeneity of vascular smooth muscle cells and elastin sheets, otherwise unresolved in a typical, lower resolution optical coherence elastography system. This tremendous enhancement in resolution and image quality strongly suggests that ultrahigh-resolution OCE will be a valuable new tool in the study of mechanobiology.
Gaussian images around the focus. This added variability in the low signal is the reason why the speckle contrast ratio [26] seems higher in some places outside the pillars than within them, and it reduced the accuracy of the contrast degradation measurement.

As previously stated, we supported our experimental claims with novel imaging and beam propagation simulations, where the parameter-space control is much more accurate. Yet, it is still worth reminding the reader of the assumptions and limitations for each of our simulations.

For the model presented in Section 5.2.1 used for the understanding and description of the speckle phenomena in OCT, the confocal effect, light attenuation, multiple scattering and the influence on OCT phase of the relative scatterer position were not taken into consideration. The model presented in Section 5.2.2 and used in Section 7.2.3 to estimate phase-sensitive OCT displacement measurements, only took into account the image carrying signal (i.e., Category I), and it required an estimation of the local sample attenuation. It included a noise model, but it also assumed that the PSF does not vary with depth. Therefore, this model is only accurate for low-NA systems imaging low-scattering samples.

These limitations were overcome in our third framework of a rigorous, full-wave model of OCT, presented in two dimensions in Section 5.3.1. Its module solving Maxwell’s equations was used to calculate the light distribution scattered from tissue in three dimensions, in order to assess and improve OCT image quality in turbid media. At the core of the model are rigorous, time-domain electromagnetic scattering solvers, such as the FDTD or PSTD [338] methods, which implicitly included phenomena such as multiple scattering and vectorial effects. Yet, the model currently does not include any noise: neither quantum (shot-noise), nor optical (excess noise), nor electronic (receiver noise) sources. Consequently, the images are on a reflectance scale rather than on a SNR scale.

Also, the computational effort for extending the three-dimensional module into an OCT image simulator in 3-D, even just to produce a simulated B-scan, is currently substantial, requiring on the order of days of computation time to simulate volumes of the size considered in this work. This can be addressed in the future using advanced computational hardware such as graphical processing units on high power institutional computers.

In Section 6.2, we limited our analysis of image quality with Bessel and Gaussian beams to a single focus position inside the sample, set to around 300 µm below the tissue (or phantom) surface. OCT acquisitions with the Gaussian focus at several other depths could be performed to fully validate our claims regarding the preference for dynamically focusing
a Gaussian beam, instead of using a Bessel beam, to retain higher OCT contrast at depth (and the same resolution).

In our multiphysics simulation of the OCE image formation process, presented in Section 7.2, the experimental values, acquired from scans of a 3-D phantom, for displacement sensitivity and strain SNR are slightly smaller than in the 2-D simulation. This is likely caused by sample motion out-of-the-plane (along the y-axis) in the experimental scan that could result in additional phase decorrelation. The largest discrepancies between the experiment and simulation are in the regions closest to the boundaries (top and bottom) of the phantom. This is likely due to the effects of friction [139]. The friction coefficients in the simulation and in the experimental scan are likely to be different, as we assumed a fully bounded surface in the simulation; the exact value in the experiment is, however, unknown. This means that, in an actual OCE experiment, the applied load that leads to the best image quality for the strain elastogram, i.e., the highest strain SNR, is going to be slightly lower than that predicted by our simulation.

The issues of friction and the issue of the uneven stress distribution within the sample pose a challenge to stiffness quantification at all resolution levels, but even more at the cellular level. Our results from the ultrahigh-resolution OCE system, presented in Section 7.3, showed strain elastograms which are only qualitative images of the mechanical heterogeneity at the cellular level of the vascular tissue presented. However, they might not correlate directly with stiffness, without an accurate estimation of local stress. Without stiffness quantification, the power of our method to suitably image cell mechanics in situ in tissues [395, 397] cannot be fully utilised.

8.2.2 Proposed future work

The capability of modern desktop (and superior) computers, along with the emergence of open source FDTD implementations [350, 351] mean that the kind of rigorous full-wave OCT simulation we implemented will eventually be accessible to non-specialists. Access to institutional computer clusters will enable volume scans to be evaluated in on the order of a day, which is a time short enough to be of practical use. In fact, work is underway in presenting the first three dimensional rigorous OCT simulator using a supercomputer.

In general, the modelling of dispersive and anisotropic media can be included in FDTD and PSTD simulations. Sources of noise can also be incorporated. In fact, one of the prime strengths of our method is that the modular nature of the model allows phenomena arising in, for example, a sample, optical elements and detector, to be modelled by modifying only one component (module) of the model.
With such model in the future, we plan to examine a range of phenomena arising from OCT imaging and consider applications such as displacement measurement using phase sensitive detection [25], parametric imaging [74], the use of non-Gaussian beams, such as Bessel beams [27, 183], and to test hypotheses regarding unresolved features observed in a variety of medical and biomedical OCT images [326]. For example, a thorough theory of the combined effect of scattering coefficient and anisotropy on the OCT backscattering intensity and attenuation were beyond the scope of this thesis. However, with such model, we could verify any prediction of the effect of increasing anisotropy on the measured OCT attenuation coefficient, $\mu_t$, for any given scattering coefficient, $\mu_s$.

We could also investigate the depth of the Gaussian focus in tissue at which the Gaussian and Bessel beam SBR are equalised, to find where the contrast advantage of the Gaussian focus over the Bessel beam will cease to be, and do this for a range of beam numerical apertures.

To extend the capabilities of the ultrahigh-resolution OCE system in the study of mechanobiology, we are and will be working on allowing this promising technique to measure the tissue mechanical properties. To do so, we will add methods of estimating local stress [148], which would make this technique quantitative along the axial direction. In such a case, elastograms would directly represent tissue stiffness (Young’s modulus), similarly to those obtained with dynamic full-field OCE [397], but at a higher spatial resolution. Finally, the combination with endoscopic probes will allow the translation of this technique into the realm of in situ and potentially in vivo imaging of cell mechanics in small animals and possibly in humans. With such a technology, access to the tissue microenvironment and the structural and mechanical interactions at the cellular scale will not be confined to just below the tissue surface, making a breakthrough in the study of disease genesis and progression.

8.3 Final remarks

In conclusion, the tools developed for image quality analysis, the characterisation of the influence of Bessel beams on contrast in OCT and the resolution improvement demonstrated in ultrahigh-resolution OCE, provide important contributions to the ability of OCT and OCT-based techniques to provide better microscopic structural and functional information on biological tissue. With better contrast and higher resolution, these techniques will set new standards in the study and diagnosis of diseases and treatment guidance, and will open up to new applications in biomedicine.
Appendix A

Spatial light modulator characterisation

A.1 Beam shaper choice and characterisation

The reconfigurable beam shaping device we selected was a liquid crystal on Silicon (LCoS) phase-only spatial light modulator (SLM). We had the option to purchase one of two different Pluto NIR-II models from Holoeye Photonics, Germany and tested them both against their specifications and for suitability in this project.

The principal specifications for an SLM are: spatial resolution, fill factor, light utilization efficiency, diffraction efficiency, wavelength range, flatness, input frame rate, phase stroke, switching frequency, number of linear phase levels, phase stability and price. Table A-1 provides the design specification with the relevant definitions for both models that were tested, and Figure A-1 shows the basic operating principle of the SLM as a phase only wavefront-shaping device.

Table A-1. Design specification for the tested spatial light modulators

<table>
<thead>
<tr>
<th>Specification</th>
<th>Units</th>
<th>Definition</th>
<th>Pluto-HES 6010 NIR-II</th>
<th>Pluto-HED 6010 NIR-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLM type</td>
<td></td>
<td>Reflective Nematic LCoS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pixel number</td>
<td>pixels</td>
<td></td>
<td>1920x1080 (2,073,600)</td>
<td></td>
</tr>
<tr>
<td>Pixel pitch</td>
<td>mm</td>
<td>Distance between consecutive pixel centres</td>
<td>8 x 8</td>
<td></td>
</tr>
<tr>
<td>Effective Area</td>
<td>mm</td>
<td>Area occupied by the addressable pixel matrix</td>
<td>15.4 x 8.6</td>
<td></td>
</tr>
<tr>
<td>Spatial resolution</td>
<td>lp/mm</td>
<td></td>
<td>62.5</td>
<td></td>
</tr>
<tr>
<td>Mirror material</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fill factor</td>
<td>%</td>
<td>Ratio of addressable pixel area to total display area</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Light utilization efficiency</td>
<td>%</td>
<td>Ratio of the 0th order diffraction to the input light level</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Diffraction efficiency</td>
<td>%</td>
<td>Ratio of the 1st order to the 0th order diffraction light level</td>
<td>&gt; 80 @ 21 lp/mm with 16 level blaze</td>
<td></td>
</tr>
<tr>
<td>Wavelength range</td>
<td>nm</td>
<td></td>
<td>700-1000</td>
<td></td>
</tr>
<tr>
<td>Flatness</td>
<td>nm</td>
<td></td>
<td>lambda/10</td>
<td></td>
</tr>
<tr>
<td>Interface</td>
<td></td>
<td>DVI · HDTV res</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drive scheme</td>
<td></td>
<td>digital (Pulse width modulation)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Input signal phase levels</td>
<td></td>
<td></td>
<td>256 phase levels</td>
<td></td>
</tr>
<tr>
<td>Effective phase levels with 18/6 modulation scheme</td>
<td></td>
<td></td>
<td>1024</td>
<td></td>
</tr>
<tr>
<td>Effective phase levels with 5/5 modulation scheme</td>
<td></td>
<td></td>
<td>192</td>
<td></td>
</tr>
</tbody>
</table>
The physical difference between the two models was the higher retardance of the LC, leading to lower drive voltages, and the higher viscosity of the LC cell of the HED model,
which resulted in a smaller phase instability but also a slower response time (typically 20 Hz for a full $2\pi$ stroke) than the HES model (typically 60 Hz for a full $2\pi$ stroke).

Figure A-2 shows photographs of the SLM incorporated on an optical bench.

![Figure A-2](image)

Figure A-2. Installed and connected SLM. The phase mask on the computer screen is replicated onto the LCoS screen, and to make it visible in this photo, a 45° Polarizer-Analyser has been put in front of the device, to transform the phase-modulation into amplitude modulation, as implemented in a standard monitor.

We tested the phase instability and used configurations to mitigate it. We measured the diffraction efficiencies, and we calibrated the device for use in the zeroth (or first) order. We planned to use it as a diffractive optical element (DOE) in the zeroth order to avoid the issues brought by the use of broadband light: the chromatic aberration and dispersion (in the various diffractive orders). The testing setup is depicted in Figure A-3, and it allowed for the analysis of the phase mask on the SLM screen and of the diffracted beams.

![Figure A-3](image)

Figure A-3. Testing setup for the SLM characterisation. Test for: phase stroke/modulation and linearity with grey levels using polarizer alignment (i); and a) diffraction efficiencies or b) beam profile using polarizer alignment (ii).

The phase mask on the SLM screen was imaged with a CCD camera through a 45° oriented polarizer that transformed the phase modulation into amplitude modulation.
When the polarizer was set to 0°, the power (Figure A-3(a)) or an image/profile (Figure A-3(b)) of the diffracted beams was measured in the focal plane of an external lens (Figure A-3(a)), or in/around the focal plane created by a superposed diffractive Fresnel lens.

### A.1.1 Phase-only device: absence of amplitude modulation

The SLM devices were tested in three configurations: standard factory default digital drive (bitplane addressing) scheme for the Pluto HES, and two different digital drive schemes for the HED. The two drive schemes differed in the internal addressing rate, with the 5:5 scheme having a refresh period of 2.96 ms, versus the standard 8.33 ms of the 18:6 scheme, but trading-off accessible phase levels, reducing them from 1472 virtual grey levels (256 effective) of the latter to the 192 grey levels of the former scheme.

We measured the power of a monochromatic collimated beam, reflected off the SLM, over time, as a function of the phase level we set the uniform phase mask to. We covered a 2π phase range in steps of π/4 and reported a nearly constant power level over a phase modulation cycle. Therefore, we verified the phase-only characteristics of the device.

### A.1.2 Temporal stability of the LCoS device

We tested the device-induced aberrations, especially due to phase instability over time. We also characterised the effect of phase droop (drop from the initially set phase level) on the diffractive power in the different orders of a grating as function of its period and the discretization of its modulation depth. Finally, the phase droop magnitude and its dependency on different grey levels were tested for all models and configurations to determine the device and configuration to keep.

**Effect of phase droop on device aberrations (recurring defocus)**

Figure A-4 presents the setup to test the effect of phase droop on a Gaussian beam focus. Figure A-5 shows the time-varying image on the SLM screen of the Fresnel lens phase mask, and the time-varying focus produced. The instability of the phase mask and the corresponding beam defocus over a period of 8.34 ms are evident.
A.1 Beam shaper choice and characterisation

Figure A-4. Setup to test the effect of phase droop on the Gaussian beam focus. (a) Phase mask of a Fresnel lens with the inner circle of $2\pi$ modulation having a 608 $\mu$m diameter. (b) Representative transverse focal intensity at a distance 78 cm from the SLM.

Figure A-5. Time sequence over 8.34 ms of 8 images of the SLM screen and corresponding transverse focal intensity. The instability of the phase mask and the corresponding beam defocus over such period are evident.

3-D Beam profiles of a Gaussian beam gated at two opposite points in the phase droop cycle are also presented in Figure A-6, and they clearly show the effect of recurring defocus that affects the 18:6 scheme, especially for the HES model tested here.
Figure A-6. Recurrent defocus. (a),(b) In focus transverse intensity profiles and (c),(d) 3-D beam profiles of the Gaussian beam produced by the SLM gated at two opposite points: unaberrated – (a),(c), and aberrated – (b),(d) in the phase droop cycle.

Effect of phase droop on diffraction efficiency

The first order diffraction efficiency is highly time-dependent in the case of the HES model with the 18:6 drive scheme, as can be seen in Figure A-7, where in some cases it can vary by up to 50% or more from its default value. Fortunately, for the HED model with the 5:5 drive scheme, this variation is only subtle.

Figure A-7 also provides a snapshot of the reduction of efficiency with lower numbers of discrete levels or smaller grating period.
Visualization of grey-level dependency of phase droop

The extent of phase droop depends on the grey level in a non-trivial way, meaning that, for complex phase masks, the SLM phase mask can deviate significantly, over time, from the one programmed onto it. This problem cannot be avoided by simply triggering the acquisition in sync with the addressing period.

Figure A-8 shows the phase droop for the two models and configurations over their respective addressing time periods, when programming a blazed grating with a period of 256 different grey lines.
We decided to keep the HED model, and use the 5:5 drive scheme, as it demonstrated constancy of the phase pattern (Figure A-8) and the diffraction efficiency did not seem affected by the lower number of accessible phase level (Figure A-7).

### A.1.3 Calibration

After choosing the model and digital drive scheme, we ensured the device was calibrated for the appropriate operating wavelength $\lambda_0$, such that a 0 to 255 grey level transition corresponded to a $2\pi$ phase shift, *i.e.*, to a optical pathlength difference $\Delta_{\text{opt}} = \lambda_0$. Subsequently, we verified the linear relationship between grey levels and phase retardation.
Figure A-9. SLM phase calibration to obtain a linear conversion from grey scale to phase retardation. A 256 grey level offset corresponds to a $2\pi$ phase shift for a given wavelength. (a) Vertical binary grating, with a modulation depth of 128 grey levels at the top and of 256 grey levels at the bottom (0 and 255). Corresponding image of the SLM screen producing the maximum and minimum amplitude modulation. Images produced using: (b) a monochromatic 633 nm HeNe laser and (c) a 50-nm bandwidth SLD centred at 840 nm. This is achieved when (d) the SLM had been loaded with the 5:5 drive scheme, and the digital potentiometers have set the phase modulation range to $2\pi$, with the proper calibration (gamma) curve to translate grey levels into a linear voltage for the different wavelengths in (e) and (f).

We set the range of the digital potentiometers in the drive scheme to a value corresponding to a phase stroke of $2\pi$, with the technique shown in Figure A-9, for a monochromatic 633 nm laser, and for the 840 nm central wavelength of a broadband SLD. Then we loaded an appropriate gamma curve, preset from Holoeye, which defines a look-up table for the grey level to the proprietary pulse-width modulation (PWM) signal value of the drive scheme that results in a linear phase retardation relationship. We checked the linearity, as shown in Figure A-10, by fitting a sinusoid to the amplitude modulation response recorded on the image of the SLM screen, when programmed as a blazed grating. This technique is advantageous because it allows the simultaneous estimation for all grey levels.
Figure A-10. Characterisation of the linearity of the phase retardation with grey level. (a) Uniform blank screen phase mask used as baseline and (b) corresponding image of the SLM screen illuminated by a 633 nm laser with cross-sectional intensity along the blue line displayed in the inset. (c) Vertical blazed grating shown in Figure A-8, and (d) corresponding image of the SLM screen. The two insets show the cross-sectional intensity along the blue line at the top, and the ratio of that curve to the baseline curve of (b) at the bottom. (e) From the bottom inset in (d) we extracted the experimental sinusoidal curve (blue) against the theoretical cosine fit (red). In (f) the measured phase retardation (in blue) is plotted on top of the expected phase retardation line (in red) in a region corresponding to a 256 grey level sequence in a blaze. Good linearity is achieved.

The result of Figure A-10 shows a good linearity of the phase retardation with grey level after the appropriate calibration has been loaded and verified.

In summary, we used a phase-only spatial light modulator from Holoeye Photonics, Germany. We selected the linear nematic liquid crystal on Silicone Pluto-HED 6010 NIR-II HR model. This model features a higher retardance cell than its HES 6010 NIR-II counterpart, i.e., a more viscous liquid crystal cell, which helps reducing the phase instability caused by the relatively low refresh frequency (120 Hz) of the digital scheme driving the LCoS backplane. We decided to trade-off 832 addressable phase levels (from 1024 to 192) in order to increase the refresh frequency of the LCoS backplane to 360 Hz. We achieved and measured good linearity of the phase retardation with grey level over a $2\pi$ phase modulation range at 840 nm.
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