A coherent model for turbid imaging with confocal microscopy

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Abstract: We present an engineering model of coherent imaging within a turbid volume, such as human tissues, with a confocal microscope. The model is built to analyze the statistical effect of aberrations and multiply scattered light on the resulting image. Numerical modeling of theory is compared with experimental results. We describe the construction of a stable phantom that represents the statistical effect of object turbidity on the image recorded. The model and phantom can serve as basis for system optimization in turbid imaging.

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1. Introduction

It is commonly of interest to extract information from within turbid, volume objects. Confocal microscopy in particular can provide sub-cellular lateral resolution and axial sectioning from the sample [1, 2]. This can be comparable to standard histology [3–6]. A primary limiting factor in the use of these technologies is the low signal-to-background ratio experienced when probing deeper or within more heterogeneous regions of tissue [7–9]. When imaging with single-mode illumination each wavelength component of the multiply scattered light from outside the region of interest (ROI) coherently interferes at the pinhole with the attenuated/aberrated light originating from the intended ROI. This gives rise to speckle in the images; these speckles are artifact. This speckle-noise can be somewhat mitigated with suitable choice of the pinhole size [10]. However, this is at the expense of increased background. Other theoretical modeling of the confocal microscope when imaging in this environment has presented many useful insights [11–13]. However, most of these models do not serve as a foundation for optimization of the optical system design and operation for maximum image fidelity. This paper will present novel theoretical [14] and experimental methods to examine how a confocal microscope produces images from a turbid medium. This framework can be used to explore novel imaging modes that can minimize speckle artifact.

2. A new model based on phase plates

We begin the discussion with an idealized confocal microscope, illustrated in Fig. 1. This system can develop an image by either laterally scanning the object relative to the optical axis or by angularly scanning the illumination at a pupil of the system. Both configurations can be represented by Fig. 1. A collimated point-source is incident into the pupil \( \Sigma_{\text{pupil}} \) of the optical system. For optical scanning systems, the illumination beam is angularly scanned in the pupil. The optical field is then focused into the turbid sample, to a depth \( z_d \) below the surface. This point is scanned within a volume of interest, which in our case is scanned in an en-face (x-y) fashion. Light is scattered within the sample, both from regions in the nominal focus and regions outside of focus. A portion of this scattered light is collected by the objective lens, collimated and focused to the pinhole plane, \( \Sigma_{\text{pin}} \), onto a detector that is restricted by a pinhole. The pinhole acts as a spatial filter to increase the average signal-to-background ratio for light that scatters near the nominal focus [1, 2].

The detected signal, as a function of illumination beam waist position \( (x_o, y_o, z_d) \), is an integration of the fields captured in the pinhole. Equation (1) is a scalar model of the light collected within the pinhole:

\[
S(x_o, y_o, z_d) = \alpha \iint_{\text{Pinhole}} |U_p(x_p, y_p; x_o, y_o, z_d)|^2 \, dx_p \, dy_p,
\]

where \( U_p \) is the modeled electric field presented to the detector by the imaging system, \( \alpha \) represents the photoelectric scaling of the detector being used and \( (x_p, y_p) \) are the local coordinates of the pinhole plane. The field in the pinhole is modeled as a coherent sum of light that was scattered at the nominal focus of the objective, \( U_F \) and a multiply scattered background field, \( U_B \).
The collected signal is a two-beam interference between the object beam $U_F$ and the background beam $U_B$. Separating the collected signal into that associated with an object point within a turbid object and that associated with bulk scatter from the bulk turbidity is helpful in developing an engineering model since it allows for the parametric analysis of the detected irradiance versus the relative strengths of the fields from the object and the background.

2.1. The focal phase plate

The object beam $U_F$ is found by propagating the illumination wavefront through the tissue, reflecting it off an object located at the nominal focal plane and then propagating the light back out of the tissue. The tissue aberrates the focusing illumination wavefront as it propagates from the tissue surface to the nominal beam waist. An aberrator can be associated with those aberrations that affect both the light that reaches the nominal focus at $z_d$ and that returns for collection by the objective. We approximate the effect of the aberrations by consolidating them in a single surface we call the focal phase plate (FPP). It is routine practice in optical engineering and lens design to place all system aberrations at the pupils for analysis and calculations [15, 16] and so we place the FPP into the pupil plane of the focusing objective (Fig. 2). This allows us to use standard, paraxial (Fourier transform) techniques to propagate fields [15]. Each point on the object will in general have a different FPP due to the different volumes of the tissue that are illuminated as the spot scans. We note that the FPP model only considers pupil aberration experienced by the ballistic and quasi-ballistic light as it proceeds to the nominal focus. The aberrated light scattered well outside the beam waist or multiply scattered is not considered by the FPP. Because the FPP induces a variable amount of aberration at each scan position, the peak irradiance at the pinhole will vary even when scanning a uniform object.

The resultant field at the pinhole due to the FPP of a perfectly uniform object can be shown to follow simple propagations through the FPP. We will use $G = \mathcal{F} \{ g \}$ to describe the far-field propagation transform of $g$ [15]. Beginning with the illumination field in the aperture stop of the objective, $U_{ill}$, the paraxial field at the nominal object plane, $U_o$ is:

$$U_o (x_o,y_o;z_d) = \varepsilon F \mathcal{F} \{ U_{ill} (x_A,y_A) \cdot \text{FPP} (x_A,y_A;x_o,y_o,z_d) \}$$

(3)
Fig. 2. (a) Planar illumination (solid-black) wavefronts are focusing into the turbid medium, scattered near the focus and returned (dashed-red) to the objective. (b) Physically and mathematically, an equivalent diffuser (FPP in blue) can be placed into the pupil that represents the aberrations of turbid medium.

where $\epsilon_F$ is a complex constant that will envelope all prefactors, nominal object reflectance and energy scaling and $(x_o, y_o)$ are the coordinates in the aperture or pupil. To calculate the image of an area object, the object is sampled into an $n \times m$ grid, with $(\Delta x, \Delta y)$ sampling pitch. For convenience, here forward we will use scan coordinates to represent object position where

$$(x_s, y_s, z_d) = (x_o + m\Delta x, y_o + n\Delta y, z_d). \quad (4)$$

For a general object, the reflected field in the aperture, $U_{A, refl}$ is:

$$U_{A, \text{refl}}(x_A, y_A; x_s, y_s, z_d) = \epsilon_F \mathcal{F} \{ U_o(x_s, y_s, z_d) \cdot \text{Object}(x_s, y_s, z_d) \} \quad (5)$$

The electric field at the pinhole plane is calculated via another propagation integral, but the reflected aperture field is again acted upon by the FPP with appropriate coordinate reversal:

$$U_F(x_p, y_p; x_s, y_s, z_d) = \epsilon_F \mathcal{F} \left[ U_{A, \text{refl}}(x_A, y_A; x_s, y_s, z_d) \cdot \text{FPP}(-x_A, -y_A; x_s, y_s, z_d) \right] \quad (6)$$

For a uniformly reflecting object, $\text{Object}(x_s, y_s, z_d) = r_o$, where $r_o$ is the amplitude reflectivity of the light backscattered from the object, the recursive propagations simplify to:

$$U_F(x_p, y_p; x_s, y_s, z_d) = \epsilon_F r_o \mathcal{F} \left[ U_{A, \text{refl}}(x_A, y_A; x_s, y_s, z_d) \cdot \text{FPP}(-x_A, -y_A; x_s, y_s, z_d) \right] \quad (7)$$

In our model we numerically calculate $U_F$ at each scan position and store it for later interference with the background component, $U_B$. 

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Neglecting absorption, the FPP consists of a pure phase term containing the tissue aberrations:

$$\text{FPP}(x_A, y_A; x_s, y_s; z_d) = \exp(i \cdot \varphi(x_A, y_A; x_s, y_s; z_d))$$  \hfill (8)

The important characteristic of the FPP is the variation in the phase error across the pupil. This can be mathematically expressed with the first two moments and the autocorrelation profile of its phase error, $\varphi$. We assign the first moment, its mean, to be zero for convenience since a uniform phase does not affect the irradiance calculations. In this case, the second moment is identically the square of the standard deviation which is a function of depth into the turbid volume: (angle brackets $\langle \rangle$ denote ensemble average)

$$\langle \varphi \rangle = 0$$  \hfill (9)

$$\langle \varphi^2 \rangle = \left[ \sigma_{\varphi}(z_d) \right]^2$$  \hfill (10)

The autocorrelation profile, $R_\varphi$, illustrates the lateral size of the aberrations in the pupil plane. The statistics are assumed to be stationary and, thus, only a function of the difference between scanned points or equivalently the difference in their respective grid coordinates $(m, n)$:

$$R_\varphi(\delta x_s, \delta y_s) = \langle \varphi(x_A, y_A; x_s, y_s; z_d) \varphi(x_A, y_A; x_s + \delta x_s, y_s + \delta y_s; z_d) \rangle$$  \hfill (11)

where $\delta x_s = x_{s,1} - x_{s,2} = \Delta m \Delta x$, $\delta y_s = y_{s,1} - y_{s,2} = \Delta n \Delta y$.

We choose to ignore the low-order aberrations associated with the optical system or any bulk index of refraction mismatch. These low-order aberrations are more or less constant and can be removed with fixed aberration control if needed; \textit{in-vivo} imaging is often done at pixel rates greater than 1 MHz, therefore real-time correction cannot be made to the high-spatial frequency aberrations that make up the FPP.

For the computer simulations of the effect of the FPP on the interpreted image, a random phase distribution was generated that has the same statistics ($\sigma_{\varphi}$ and $R_\varphi$) as what would be encountered in tissue. To arrive at reasonable waveform deformations for skin tissue, we used a previously developed finite-difference-time-domain FDTD algorithm for the propagation of focused electric fields in tissue [17]. A randomly generated 2D index of refraction map using known values [18] for cellular components was used as the simulation environment. To approximate the inhomogeneity in skin cell structure, we placed a 10 \(\mu\)m thick layer of stratum corneum (SC) cells (flat, scale like, 1.5 index of refraction) atop a 60 \(\mu\)m thick layer of spinous-like cells (ellipsoidal, 10-15 \(\mu\)m diameter, nucleus and other cellular components with an average index of refraction of 1.35). A converging 830 nm wavelength cylindrical wave with a focus point 100 \(\mu\)m below the surface of the tissue interface was used as illumination. The lines of constant phase were extracted from the electric field map within the epithelial cells at a level of approximately 70 \(\mu\)m below the SC layer (10 \(\mu\)m) and after approximately 60 \(\mu\)m of epithelial cells. Adding $N$ statistically independent realizations increases the variance of the ensemble by $N$ and the standard deviation by $\sqrt{N}$. Regression fitting this profile to the RMS phase errors in the simulations we found $0.10\lambda$ RMS aberration per 10 \(\mu\)m of tissue:

$$\sigma_{\varphi}(z_d) = 0.10\lambda \sqrt{\frac{z_d}{10\mu m}}$$  \hfill (12)

Total single-pass RMS phase errors were found to be on the order of 1/10 to 1/4 $\lambda$ (at 830nm) for the skin depths typically investigated with confocal microscopy. Figure 3 illustrates the FDTD simulation results and the fitted trend line. The 1/e correlation lengths of the phase profiles averaged to approximately 5 \(\mu\)m (scaled to the dimensions at the surface of the tissue interface). Figure 4 shows the correlation profiles of the phase aberrations for three realizations of the simulated tissue environment.
Fig. 3. RMS wavefront error calculated at an illumination wavelength of 830 nm and NA = 0.9. Each ‘x’ represents one realization of the simulated tissue environment, at the indicated axial depth.

Fig. 4. Autocorrelation profiles of wavefront error after 80 μm of simulated tissue. Each profile is one realization of the simulated tissue environment.
2.2. The background phase plate

The background field, $U_B$, is caused by light that is scattered within the tissue, from outside the nominal focus, but due to the re-direction of multiple scattering, is collected by the objective and focused to the pinhole. All the multiply scattered light that will arrive at the pinhole must have left the surface of the tissue. Since after this surface the light experiences deterministic imaging, we choose to represent the scattering within the tissue as a secondary source in the form of a disk bounded by the cone of illumination, lying at the surface of the tissue (a distance $z_d$ from the nominal focus). We choose to call this the background phase plate (BPP). Prior work supports the notion that most of the background scattered light that does reach the detector originates near the surface of the medium [11]. Though we are neglecting light from outside this region, these extra source points would only add to the strength of the BPP, which we account for in a normalization presented shortly. Figure 5 illustrates the imaging relationships between the object, BPP and the pinhole. The geometric image of this source is located a distance $z_d$ from the pinhole plane. The propagation of this image field to the pinhole plane results in the background fields, $U_B$. We note that by geometrical modeling, the NA of the $U_F$ component in the pinhole is equal to the $U_B$ components propagating back from the imaged BPP. Because the angular size of the BPP as viewed from the pinhole is equal to the NA of the object beam as it converges on the pinhole, the feature sizes of the point-spread-function (PSF) of the focal information and of the BPP-induced “speckle” are equal. The interference of the speckle field from the BPP and the PSF over the pinhole aperture modulates as the object is scanned and the interference features are the same size as the diffraction-limited PSF. This modulating pattern is the cause of the large signal variations seen when imaging with coherent light in a turbid medium.

For our computer modeling, the phases of the BPP source are assumed to have a uniform distribution over $2\pi$ [15, 19]. The FDTD simulations used to derive the statistics for the FPP calculation also support a fully random phase distribution for the light reflected from the turbid medium. The background field $U_B$ is found by Huygens-Fresnel propagation [15] from the tissue surface to the pinhole plane:

$$U_B(x_p, y_p; x_s, y_s, z_d) = \varepsilon_B \iint_{\text{BPP}} \exp\left(\frac{i k r_B'}{|r_B'|^2}\right) dx'_B dy'_B,$$

$$r_B' = \sqrt{(x'_B - x_p)^2 + (y'_B - y_p)^2 + (z'_d)^2},$$

where $\varepsilon_B$ envelopes all constants, $(x'_B, y'_B, z'_d)$ are the coordinates in the imaged BPP offset a distance $z'_d$ from the pinhole. These coordinates are simple magnifications of the object-centered background-coordinates: $(x'_B, y'_B, z'_d) = (-Mx_o, -My_o, M^2z_d/n_o)$ where, for the idealized confocal microscope depicted in Fig. 1, the magnification is a ratio of the pinhole and objective-lens focal-lengths: $M = f_{pin}/f_{obj}$ and $n_o$ is the average index of refraction in the object.

For each scanned position $(x_s, y_s)$, we assume that a new distribution of random phasors replaces the previous position’s BPP. This is equivalent to saying the correlation length of the BPP is less than the distance between adjacent data recordings. The resulting pinhole field, $U_B$, is stored for later interference with the focal component, $U_F$.

2.3. Coherent interference

The theoretical model was coded numerically in MATLAB [Mathworks. Natick, MA]. We simulated scanning a single line of an object area with the confocal microscope with a line of calculated pixel values. For each data point in the line, we numerically translated the FPP by a distance equal to our experimental microscope’s lateral pixel-pitch (described shortly); the BPP
Fig. 5. The BPP is geometrically imaged (blue lines) through the confocal system. The NA of the $U_F$ fields (dashed red) at the pinhole is equal to the NA of the $U_B$ fields propagating back to the pinhole. Inset: The fields across the BPP are propagated to the pinhole plane to model the interference between the $U_B$ field with the $U_F$ field.
was randomly generated for each data point in a line scan. Fast-fourier-transform (FFT) kernels were used to calculate the $U_F$ component and $U_B$ components were numerically calculated using Eq. (13). All calculations were done on an IBM BlueGene cluster at the University of Rochester. The calculation of the FPP and BPP fields at the pinhole plane took approximately 2 seconds for each object point position using partial parallelization of the code. The interference and integration of $U_B$ and $U_F$ fields in the pinhole yield the final signal for a particular image location, and a collection of such image point calculations are assembled up to form an image (or in our case, a line of signals from a representative image). Ensemble averaging the focal and background components over a large number of image lines and taking their ratio yields a ratio of the constant pre-factors $\varepsilon_F$ and $\varepsilon_B$. These pre-factors can be used to control the modeled signal to background (SNB) ratio:

$$SNB = \frac{\left\langle \iint_{\text{Pinhole}} |U_F|^2 \right\rangle}{\left\langle \iint_{\text{Pinhole}} |U_B|^2 \right\rangle} = \frac{|\varepsilon_F|^2}{|\varepsilon_B|^2}$$  \hspace{1cm} (15)

Figure 6 contains a movie (Media 1) of the resulting pinhole irradiance for the focal signal $U_F$, the background signal $U_B$ and the coherent interference $U_F + U_B$ for a uniform object subject to the distortions of a FPP and BPP. The variations in the irradiance due to the FPP and BPP interference distort the true representation of the object. It is the goal of this model to account for this distortion and consider the effects that system aberrations, tissue aberrations and amount of collected backscatter have on the statistics of the image. It should be restated that the object within our model has a constant reflection. This allows us to investigate signal variations that are solely due to the aberration and background components of the FPP and BPP. To characterize these variations, we used the normalized standard deviation of the detected signal as our characterization of the deviation from the ideal object field without the interference induced distortion:

$$\bar{\sigma}_S = \sqrt{\frac{\langle S^2 \rangle - \langle S \rangle^2}{\langle S \rangle^2}}$$  \hspace{1cm} (16)

This is essentially a measure of the width of the distribution of normalized signal. In the absence of distortions, the image of a uniform object would report only a single signal value. With interference, the image will report a range of values. The width of the distribution is a
Fig. 7. Cross-sectional schematic of the objective lens focusing the illumination wavefronts into the phantom. i) Cover glass with rear-side ground forming the FPP height profile; ii) Immersion medium, typically water; iii) Uniform reflective surface spaced a distance $z_d$ from the FPP surface. The rear-side of the uniform reflector is ground to form the BPP height profile; iv) Mirror or high reflector. Converging waves (red) are aberrated by the FPP surface on (i). Light transmitted by the uniform object is scattered by the BPP and mirror surface (dashed red). The BPP generated light is further dephased by the FPP surface before collection by the objective.

measure of the effect of the interference and can be correlated to the average amount of tissue aberrations and tissue back scatter. As a validation of this model, we now present experiments and simulation results.

3. Experiment

3.1. A new turbid phantom

To validate the model, a tissue phantom with known statistics was needed. The typical spheres-in-solution or fixed-media phantoms used for turbid imaging studies make it difficult to measure the wavefront scattering statistics. We chose to build a phantom using rough glass surfaces. We used standard glass grinding techniques to impart known, measurable statistics onto glass surfaces. By grinding surfaces with a particular choice of grit we were able to control the height profile and the autocorrelation length. The statistics of this profile was measured with optical profilometry. This phantom assembly was repeatable and temporally stable. Figure 7 illustrates the phantom construction. We chose to incorporate the cover slip used for spherical aberration correction as part of the phantom assembly. The far side of the cover-slip, that would otherwise be in contact with the tissue, was ground. When an immersion medium is used with the modified coverslip, the transmitted wavefront statistics of the ground surface approximate the effect of the tissue aberrations, or similarly, the FPP in the model. Choice of the immersion medium, index of refraction of the cover-slip glass and ground surface statistics could be used to tailor the wavefront deformations. The mean thickness of the overall coverglass is still set to minimize spherical aberration. Spaced a distance from the aberrator, at the nominal focus of the objective, we chose to use a polished, uniformly reflecting piece of glass as the object surface. This allowed us to measure the deviations from an ideally perfect signal. On the rear-side of the
uniform object surface, is a second ground surface. With air immersion on its exit surface and coupling to a high-reflector, this secondary diffuser provides the source of multiply scattered background, or similarly, the BPP in the model. Changing the distance of the high reflector for the focal point varied the amount of backscatter that was collected at the pinhole. Listed in Table 1 are the statistics and parameters of the phantoms used in the experiments corresponding to the regions noted in Fig. 7. These parameters were also used as inputs for the mathematical modeling. All ground surfaces were made with 9 μm silica grit; statistics were measured with a calibrated white light interferometer \([\text{NuView600, Zygo, Middlefield, CT}].\) Figure 8 shows images taken with a confocal microscope of the phantom in various configurations.

### Table 1. Phantom construction specifications

| Region | Material | Nominal thickness (μm) | Index of Refraction | RMS height \(σ_h\) (μm) | RMS wavefront \(σ_p(λ)\) | Auto-correlation length \(L_{ac}\) (μm) |
|--------|----------|------------------------|---------------------|-------------------------|------------------------|----------------------------------|
| i      | BK7      | 750                    | 1.510               | 0.58                    | 0.13                   | 3.8                              |
| ii     | SF2      | 620                    | 1.633               | 0.73                    | 0.27                   | 5.8                              |
| iii    | water    | 200                    | 1.33                | –                       | –                      | –                                |
| iv     | BS³      | 150                    | 1.51                | 0.78                    | 0.68                   | 4.2                              |
| iv     | EA⁴ mirror | –                     | –                   | –                       | –                      | –                                |

1With water immersion for region (i), with air immersion for region (iii). ²All autocorrelation profiles were exponential for these ground surfaces; correlation length is the \(1/e\) distance. ³Borosilicate cover slip. ⁴Newport enhanced-aluminum coated mirror.

### 3.2. Optical system and methods

A confocal microscope [Vivascope 1000, Lucid, Rochester, NY] was used to acquire x-y (2D) scanned images of the tissue phantom. A water-immersed, NIR objective [Photon Gear, Rochester, NY] with an adjustable iris for an NA range of 0.5 to 0.9 was used; we took data at each of these two limits. For the 2D images, the field of view with this objective is 0.49 x 0.37 mm, with the images being 640x480 pixels, this gives a pixel pitch of 0.77 μm. A 830nm laser diode [Micro Laser Systems, Inc., Garden Grove, CA] was used to provide TEM\(_{00}\) illumination. The \(1/e^2\) diameter of the illumination mode at the objective is 9.25 mm, just overfilling the 0.9 NA diameter of 8.55 mm. With a 100 μm pinhole, the full-width at half-maximum (FWHM) lateral resolution was measured to be 0.84 and 1.1 μm at 0.9 and 0.5 NA respectively [20]. The axial resolution was 3.3 and 7.6 μm at 0.9 and 0.5NA respectively. It is understood that as the NA is changed, the number of resolvable spots in the pinhole also changes. Defining a 1-resel pinhole as one where the diameter is equal to the FWHM of a diffraction limited point-spread-function (PSF), our microscope operates at 2.7 and 4.8 resels for 0.5 and 0.9 NA respectively, with the 100 μm pinhole (in the standard confocal metric, this is equivalent to 4.5 and 8.1 normalized pinhole radius, respectively). An avalanche photo diode (APD) was used as the detector behind the pinhole [C5460, Hamamatsu, Japan].

A region that represents the aplanatic patch was selected from the images for processing. This is done so as to not bias the data analysis by field dependent aberrations, predominately field-curvature effects of imaging a flat object with a curved focal surface. By itself, field-curvature increases \(σ_δ\) due to the reduced image brightness when the longitudinal field-curvature exceeds the depth-of-focus. This region is approximately 100 x 100 μm (or 130 x 130 pixels) at 0.9NA; we used the same 100 μm regions at 0.5NA to keep the data sets equal in size.
Fig. 8. Images of the turbid phantom with a confocal microscope at 0.9NA: (a) Uniformly reflecting surface; (b) With the BPP; (c) With only the SF2 FPP aberrator; (d) With a combination of FPP and BPP. The calibration bar represents 50 μm.

4. Results

Here we compare the experimental results to the theoretical model for the BPP alone, then the FPP alone and finally with the combination of the BPP and FPP. Six images were recorded experimentally and simulated with the model. From each experimental image, 10 lines of 100 pixel length were extracted. For each “image” of theoretical data, 10 lines of 100 pixel length were simulated. For each line, $\bar{\sigma}_S$ was measured. The error bars on both the experimental and modeled data are the standard deviations of $\bar{\sigma}_S$ across all the lines measured. The experimental system had a baseline $\bar{\sigma}_S = 0.02$; this is caused by the non-identical mirror surfaces of the polygon and digitization of the APD voltage. This error was added to the modeled signals.

4.1. BPP

The configuration for BPP validation was with an in-focus, uniformly reflecting object surface but without the FPP present; a standard cover glass was used for our objective in the place of the FPP. In this configuration we determine if the coherent interference of a nominal PSF field reflected from the uniform object surface with the BPP generated field matches observation. The signal to background ratio was measured by calculating the mean pixel signal with and without the high reflector on the BPP, namely 50:1 and 26:1 for 0.9 and 0.5 NA respectively. Using these ratios for the simulations, comparisons of the fidelity metric for modeled and experiment can be made and are in Figs. 9(a)-9(b).
4.2. FPP
Without the background generating BPP in place, images of the uniformly reflecting surface were taken for the two diffusers listed in Table 1 at 0.5 and 0.9NA. The corresponding situation was also modeled. The results are in Figs. 9(c)-9(f).

4.3. FPP+BPP
When imaging in turbid media there is both an aberrated component and a background component, and the coherent interference of the two at the pinhole plane. The aberrating FPPs were used in conjunction with the background generating BPP. The SNB ratio was measured by calculating the mean pixel signal with and without the high reflector on the BPP. These were: 10:1 and 9.2:1 for the BK7 diffuser for 0.5 and 0.9 NA respectively; 1.2:1 and 1.1:1 for the SF2 diffuser for 0.5 and 0.9 NA respectively. These SNB ratios were used for the modeled results. The results are in Figs. 9(g)-9(j).

The width of the normalized signal distribution is not the only factor that can affect the interpretation of what is “truth” in the images. In other words, the fidelity of the image (and the imaging system itself). The shape of the distribution is also important. This shape is dependent on the optical system parameters, such as the effective pinhole size, in addition to the relative strengths of the FPP and BPP. Figure 10 contrasts the distributions for two scenarios between experiment and modeling: configurations (g) and (j) from Fig. 9.

5. Discussion and conclusion
We can see that the modeled and experimental results are in good agreement across the range of situations tested. In particular, the model can also accurately represent the distributions expected when phase errors and background are affecting the resultant image. For example, the
effect of pinhole-size on the detected irradiance and confocal resolution parameters is well known [2]. Smaller pinhole sizes gain sharper axial sectioning. However, this model shows that smaller effective pinhole sizes result in image data that is skewed into an asymmetric distribution when coherent light is used and there are unwanted pinhole contributions from the turbid environment; a larger effective pinhole has a more uniform (and narrower) distribution. The tradeoff between resolution and accuracy in representing the object can now be weighed.

Within the model, some disagreement begins to appear for stronger FPP phase errors (SF2). The wavefront slopes for exponential diffusers theoretically approaches infinity. In trying to keep the model FFT based for speed considerations, the numerical FPP generated for the model may have too few points across a correlation length for accurate modeling. Further refinements to the model can include dynamic scaling for these situations or more advanced propagation techniques.

Framing the problem as a simple two component interference allows easy testing and/or optimization of novel illumination modes that may better represent the true state of the object. The model and corresponding experimental phantom presented here allow easy, repeatable and quantitative analysis of pupil-engineered systems for scanning reflectance confocal microscopes.

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