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Dynamic contrast-enhanced diffuse optical tomography (DCE-DOT): experimental validation with a dynamic phantom

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Abstract

Dynamic contrast-enhanced diffuse optical tomography (DCE-DOT) can provide spatially resolved enhancement kinetics of an optical contrast agent. We undertook a systematic phantom study to evaluate the effects of the geometrical parameters such as the depth and size of the inclusion as well as the optical parameters of the background on the recovered enhancement kinetics of the most commonly used optical contrast agent, indocyanine green (ICG). For this purpose a computer-controlled dynamic phantom was constructed. An ICG–intralipid–water mixture was circulated through the inclusions while the DCE-DOT measurements were acquired with a temporal resolution of 16 s. The same dynamic study was repeated using inclusions of different sizes located at different depths. In addition to this, the effect of non-scattering regions was investigated by placing a second inclusion filled with water in the background. The phantom studies confirmed that although the peak enhancement varied substantially for each case, the recovered injection and dilution rates obtained from the percentage enhancement maps agreed within 15% independent of not only the depth and the size of the inclusion but also the presence of a non-scattering region in the background. Although no internal structural information was used in these phantom studies, it may be necessary to use it for small objects buried deep in tissue. However, the different contrast mechanisms of optical and other imaging modalities as well as imperfect co-registration between both modalities may lead to potential errors in the structural *a priori*. Therefore, the effect of erroneous selection of structural priors was investigated as the final step. Again, the injection and dilution rates obtained from the percentage enhancement maps were also immune to the systematic errors introduced by erroneous selection of the structural priors, e.g. choosing the diameter of the inclusion 20% smaller increased the peak enhancement 60% but changed the injection and dilution rates only less than 10%.

1. Introduction

Diffuse optical tomography (DOT) is an emerging non-invasive imaging technique that uses near-infrared light to recover the optical parameter maps of the medium under investigation (Gibson *et al* 2005). In general, multiple sources and detectors are placed on the boundary of the region of interest and numerical iterative methods based on the diffusion equation are used to reconstruct the absorption and scattering maps from the detector measurements. In essence, multi-wavelength measurements allow the determination of the spatial distribution of a number of physiologically significant chromophores, mainly water, fat, oxy- and deoxy-hemoglobin (Corlu *et al* 2003). The chromophore maps can be either obtained from the absorption maps at multiple wavelengths or reconstructed directly from the measurements (Li *et al* 2004). Although the DOT cannot compete with anatomical imaging modalities in terms of spatial resolution, it offers functional information such as total hemoglobin and oxygen saturation maps, with high sensitivity. This allows not only the differentiation of tumors from normal tissue but also the separation of malignant tumors from benign ones. Therefore, recent advances in the optical imaging have led to the development of a broad

variety of diagnostic applications, in particular imaging of the breast cancer (Culver *et al* 2003, Dehghani *et al* 2003, Intes *et al* 2003, Groesenick *et al* 2004, Jakubowski *et al* 2004, Pogue *et al* 2004, Schmitz *et al* 2005).

Although efforts have been mostly focused on applications based on endogenous tissue contrast, exogenous contrast agents are already available and used for optical tomography. Administering contrast agents is a widely used way of enhancing contrast between diseased and normal tissue in medical imaging. Two images can simply be acquired before and after the injection of a contrast agent or alternatively, a series of images may be acquired following the administration that is called dynamic contrast-enhanced (DCE) imaging. It is well established that the endogenous contrast between the diseased and normal tissue can be only up to two or three times higher than the healthy tissue for optical imaging (Tromberg *et al* 2000). This relatively low contrast is one of the limiting factors for the widespread use of DOT. Meanwhile, there is already an available FDA approved optical contrast agent namely, indocyanine green (ICG). A dynamic contrast-enhanced DOT (DCE-DOT) system with a high temporal resolution can provide the enhancement kinetics of ICG and hence, additional information for several key applications such as differentiation of benign and malignant lesions.

For the moment, there are only a couple of *in vivo* dynamic imaging studies on breast reported in the literature using the DOT technique. The optical contrast agent ICG was used in these studies. It mostly binds to albumin in blood and act as a blood pool agent (Yoneya *et al* 1998). Therefore, the temporal dynamics of albumin whose molecular weight is 67 kDa govern the kinetics of the ICG (Ntziachristos *et al* 2000, Cuccia *et al* 2003). Ntziachristos *et al* (2000) examined the relative enhancement (the relative change in the optical absorption) using a time-domain DOT system for three cases: a benign tumor, a malignant tumor and a control patient with no disease. The malignant case showed the highest increase in the absorption, while the benign and the normal cases showed relatively lower enhancements. Later, Intes *et al* (2003) used a continuous wave DOT system to explore *in vivo* uptake and clearance dynamics of ICG in breast tumors. Three patients with a fibroadenoma mass, an adenocarcinoma lesion and an invasive ductal carcinoma were enrolled for the study. The pharmacokinetic analysis on both these lesions and nearby healthy lesions showed no differences in the first case. However, the wash-in parameter was different in the adenocarcinoma case, while the invasive ductal carcinoma case exhibited differences in both wash-in and wash-out parameters with respect to the healthy tissue. More recently Rinneberg *et al* (2008) carried out dynamic contrast-enhanced time-domain optical mammography on a small number of patients, but no differences in wash-out kinetics of ICG between tumors and healthy breast tissue were observed. Although their results were not encouraging, it should be noted that the performance of a particular optical tomography system depends on many parameters such as the performance of the instrumentation, detection geometry, compression scheme as well as modeling technique of photon migration in tissue and the selected image reconstruction method. Hence, larger scale clinical studies should be undertaken with different DOT techniques to investigate the true potential of the ICG enhancement kinetics in breast cancer diagnosis.

In addition to these studies, Unlu *et al* (2008a) demonstrated simultaneous acquisition of enhancement kinetics of a MR and an optical contrast agent using a hybrid MR-DOT animal imaging system. Moreover, Cuccia *et al* (2003) used the six-parameter pharmacokinetic model and presented a study of the dynamics of the ICG in an adenocarcinoma rat tumor model by the use of diffuse optical spectroscopy co-registered with MRI. Meanwhile, ICG is also suitable to use as an agent for fluorescence imaging. There are ongoing efforts for monitoring ICG dynamics with fluorescence imaging systems. For example, Sevik-Muraca *et al* (2008) showed the feasibility of near-real-time visualization of ICG trafficking in

lymph nodes in breast cancer patients. However, temporal resolution of currently available fluorescence tomography systems is not high enough for acquiring ICG kinetics in breast lesions yet (Corlu *et al* 2007).

Due to the highly scattering nature of the tissue and limited number of measurements acquired from the boundary of the medium, the DOT inverse problem is ill-posed and underdetermined (Arridge 1999a). Therefore, it suffers from the poor spatial resolution and low quantitative accuracy. As a result, the recovered optical parameters of an inclusion highly depend on its size, location and contrast. In addition, the overall size and the optical heterogeneity of the background medium also affect the recovered parameters. Furthermore, the diffusion approximation fails in modeling of light propagation in non-scattering regions. The presence of such regions in the background also introduces error in estimating the optical parameters of a particular inclusion. Consequently, the same problems exist in the dynamic contrast-enhanced DOT (DCE-DOT) and these factors are expected to affect the recovered enhancement kinetics of contrast agents. In the last couple of years, we have developed a multi-frequency domain DOT animal imaging system (Gulsen 2006a, 2006b). To be able to monitor the enhancement kinetics of ICG, we increased the time resolution of our DOT system to 16 s using a heterodyne detection technique. Later, we constructed a dynamic phantom to investigate the effect of the geometrical parameters such as the depth and the size of the inclusion on the recovered enhancement kinetics. The ICG–intralipid–water mixture was circulated through the inclusion while the DOT measurements were acquired with the high temporal resolution version of the DOT system. The size and the depth of the inclusions were varied and the same dynamic study was repeated circulating the solution through one of the inclusions at a time.

In addition to this, the effect of non-scattering regions was investigated by placing a second inclusion with a very low scattering coefficient in the background. A major problem in optical animal imaging is the presence of non-scattering regions such as the bladder in the imaging volume. Although computationally demanding and difficult, the radiative transfer equation can be used to model the light propagation in these non-diffusive regions. However, here we investigated whether the enhancement kinetics of a region of interest (ROI) can be obtained without proper modeling of the light propagation in a non-scattering background.

Furthermore, *structural a priori* information that can be obtained by another high-resolution imaging modality has been found to be effective in improving the quantitative accuracy by guiding and constraining the DOT reconstruction algorithm (Intes *et al* 2004, Brooksby *et al* 2005a, 2005b, Guven *et al* 2005, Gulsen 2006b). Indeed, the *structural a priori* is very pivotal if the object is small and buried deep in the tissue. In such a case, the DOT by itself may not resolve the object. Nevertheless, if another imaging modality provides the size and location of the object, DOT can provide optical properties of the object and hence the optical enhancement curve in the case of the dynamic imaging. Although the use of *a priori* information produces substantial improvement in the reconstructed images, this approach is susceptible to any potential errors that can occur due to the imperfect co-registration between both modalities, the errors in images segmentation or even the different contrast mechanisms of optical and other imaging modalities (Yalavarthy *et al* 2007).

Therefore, the effect of erroneous selection of *structural priors* was investigated as a final step. First, the reconstructions were performed assuming that the true knowledge of the size and location of the inclusion were known. Later, the error in the recovered enhancement kinetics was investigated when the size of the inclusion was estimated larger or smaller, and the inclusion's center was misplaced due to potential imperfect co-registration between different modalities. The phantom studies confirmed that although the peak enhancement

varied for each case, the recovered injection and dilution rates obtained from the percentage enhancement curves were independent of the geometrical parameters as well as the optical properties of the background. If *structural a priori* was used to improve the DOT reconstruction results, the very same parameters were also immune to the errors in estimating the size and or position of the inclusion.

2. Methods

2.1. Modeling of forward and inverse problems

We use the diffusion equation to model the light transport in the tissue-like phantom. The frequency domain representation of the optical diffusion equation is written as (Arridge 1999a)

$$\vec{\nabla} \cdot D(\vec{r}) \vec{\nabla} \phi(\vec{r}, \omega) - \left[\mu_a(\vec{r}) + \frac{i\omega n}{c_0} \right] \phi(\vec{r}, \omega) = -S(\vec{r}, \omega), \quad (1)$$

where $\phi(r, \omega)$ is the optical light fluence rate (W mm^{-2}), $S(r, \omega)$ is the optical light source (W mm^{-3}), ω is the optical light source modulation frequency, $D(r)$ is the photon diffusion coefficient, μ_a is the photon absorption coefficient, c_0 is the speed of the light in vacuum, and n is the index of refraction of the medium. The diffusion coefficient is given as

$$D(\vec{r}) = \frac{1}{3[\mu_a(\vec{r}) + \mu'_s(\vec{r})]}, \quad (2)$$

where μ'_s is the reduced scattering coefficient.

The Robin boundary condition (RBC) relates the optical fluence rate to optical flux at the boundary and can be written as

$$\phi(\vec{\xi}, \omega) + 2AD(\vec{r})\hat{n} \cdot \vec{\nabla} \phi(\vec{\xi}, \omega) = 0, \quad (3)$$

where ξ is any point on the boundary $\delta\Omega$ of the domain Ω , $\hat{n}$ is the outward normal vector for the boundary and A is a constant that accounts for the internal reflection of light due to the index of refraction mismatch.

The image reconstruction for the DOT seeks a minimum between the measured and calculated data:

$$\chi(\mu)^2 = \sum_i^M (\phi_{m,i} - \phi_{c,i}(\mu))^2 + \sum_j^{\text{NE}} \lambda_j (I(\mu_j - \mu_0))^2, \quad (4)$$

where ϕ_m is the measured data, ϕ_c is the calculated data using the forward solver, M is the total number of source–detector combinations, NE is the number of elements and $\mu = \{\mu_a, \mu'_s\}$. I is the identity matrix and λ is the Tikhonov regularization parameter. In order to find the minimum of the error function, the derivative is equated to zero and then the solution is expanded in a truncated Taylor series (Paulsen and Jiang 1995). Equation (4) becomes a matrix equation such that

$$\Delta\mu = (J^T J + \lambda I)^{-1} J^T (\phi_m - \phi_c), \quad (5)$$

where $\sum$ is the sum over the diagonal elements belonging to the $J_{ij} = \frac{\partial \phi_{c,i}}{\partial \mu_j}$ is the Jacobian matrix that can be calculated using the adjoint method (Arridge 1999b), $\Delta\mu$ is the update vector that is the difference between the true value and the estimated value of either absorption or reduced scattering coefficient.

Equation (5) must be solved iteratively since the relationship between the measured data and the optical parameters is nonlinear. A homogenous fitting is performed to find the initial values for the algorithm using the real measurements. The regularization parameter is selected as (Franchois 1997)

$$\lambda_i = \frac{\text{Tr}[(J^T J)_i]}{N} \frac{\text{norm}(\chi_i)}{\text{norm}(\chi_1)}, \quad (6)$$

where i is the iteration number, Tr represents the trace of a matrix and N is the number of unknowns. χ_1 is the norm of the error in the first iteration. This empirical method provides a very fast and efficient way of selecting the regularization parameter in each iteration. We stop the iterations when $\chi(\mu)^2$ error changes less than 2% between two consecutive iterations. The finite element method (FEM) is used to solve the forward problem. The simulated measurement data are generated solving the forward problem on a fine mesh of 6785 nodes and 13 056 elements. Then, a dual mesh scheme is used for all the reconstructions. The forward problem to obtain the fluence rate is solved on a mesh of 1761 nodes and 3264 first-order triangular elements. A denser element distribution is used underneath the boundary to improve the accuracy close to measurement points. The sources are placed at a location $1/\mu'_s$ below the surface. A coarse mesh with 289 nodes and 512 elements is generated for the reconstruction basis in order to reduce the number of unknowns.

For the first set of experiments, the reconstructions are performed without any *structural a priori* information. In the last set, the *a priori* information is incorporated in three steps during the image reconstruction. For this purpose, the finite element mesh is segmented into a background region and ROI. In the first step, the initial optical parameters for different regions are found using a nonlinear least-squares estimate algorithm using MATLAB. In the second step, a two-region regularization method is employed. The selection of the λ values can be achieved modifying equation (6) for multiple regions:

$$\lambda_i^r = \frac{\text{Tr}[(J^T J)_i^r]}{N^r} \frac{\text{norm}(\chi_i)}{\text{norm}(\chi_i)}, \quad (7)$$

where r denotes the region number, $\text{Tr}[(J^T J)_i^r]$ is the sum over the diagonal elements belonging to the region r and N^r is the number of unknowns corresponding to that region. In the third step, the mean value of the optical parameters in each region is assigned to all elements in that region at the end of each iteration. As a result, the values of reconstructed optical parameters are accurate as long as the boundaries of the regions are selected correctly.

2.2. Instrumentation

A multi-frequency DOT system is used in the experiments. Optical fibers are used to conduct light from the sources to the medium under investigation as well as to transfer the collected light from tissue to detectors. The system utilizes eight sources and eight detectors. The detector and source fibers are chosen as 1 mm step-index and 62.5 μm gradient-index fibers, respectively. An 8×32 fiber optic switch (Dicon Fiberoptic, GP700) is used to

multiplex the laser diode outputs through the source fibers. Photomultiplier tubes (Hamamatsu R7400-20) are used at the multiple detection sites. The output of each PMT is amplified by a 65 dB rf-amplifier. The details of the instrumentation can be found in Gulsen *et al* (2006a).

In vivo studies in the literature show that following the administration, the ICG concentration reaches its peak in nearly 1 min (Cuccia *et al* 2003, Intes *et al* 2003). To be able to monitor the enhancement kinetics of ICG, we have increased the time resolution of our DOT system to 16 s using a heterodyne detection technique. Using an interface, any desired modulation frequency can be set promptly as per user's request. Once recorded, the data are post-processed to obtain 64 phase and 64 amplitude data and used as an input for the DOT inverse solver.

2.3. Phantom experiments

A dynamic phantom was designed and implemented for the experimental studies. It consisted of a main container, a computer-controlled syringe, a mixer and a 63 mm diameter cylindrical solid phantom that was constructed using the method described by Firbank *et al* (1995), figure 1. The absorption (μ_a) and reduced scattering (μ_s') coefficients of the solid phantom were set to 0.013 mm^{-1} and 0.85 mm^{-1} , respectively. It had three cylindrical inclusions, figure 2. The diameter of one of the inclusions (1) was 15 mm and it was located 15.75 mm away from the center. The diameters of the other two inclusions were the same, 8 mm. They were located at 15.75 mm (2) and 23.62 mm (3) away from the center, respectively.

There were four different configurations for the dynamic studies, figure 3. For the first three cases, the tubes from the main container were connected to one of these inclusions at a time, while the other two inclusions were filled with an ICG–intralipid–water mixture whose optical properties matched to the solid phantom. For the first case (R15), the solution was circulated through the 15 mm diameter inclusion (1). For the other two cases, the solution was circulated through the 8 mm diameter inclusions. The case in which the solution was circulated through the second inclusion (2) was denoted as 'R8'. The other case in which the solution was circulated through the inclusion closer to the surface (3) was denoted as 'R8side'. Finally, the solution was again circulated through the third inclusion (3); however, the first inclusion was filled with pure water instead of the ICG–intralipid–water mixture whose optical properties matched to that of the background. This last case was denoted as 'R8sideW'. Table 1 also presents the optical properties of each inclusion for all four cases.

Initially, an ICG–intralipid–water mixture, which had optical properties of $\mu_a = 0.03 \text{ mm}^{-1}$ and $\mu_s' = 0.85 \text{ mm}^{-1}$, was circulated through the phantom using a metering pump. The main solution was kept in a container. After the baseline measurements, a computer-controlled syringe pump was used to inject additional ICG into the container to increase the absorption coefficient linearly up to 0.06 mm^{-1} in exactly 1 min, figure 4. Simultaneously, a stirrer was used to mix the solution in the container and the pump continued circulating the solution throughout the experiment. One minute after the end of the injection, the valve connected to a second container that held an additional intralipid–water mixture solution was opened. This solution was continuously added to the main container for nearly 4 min to dilute the main solution. Thirty complete sets of DOT data of 64 measurements were acquired during the experiment with 16 s intervals. To increase the temporal resolution, only a single modulation frequency, 100 MHz, was employed. The images were acquired at 780 nm and the total imaging time was approximately 8 min.

This procedure was repeated for each inclusion. Between each experiment, the containers, the pump and the tubes were cleaned first by circulating water and then disassembling and

washing the components separately. After all components dried, compressed air was used to extract any remaining solution, especially in the tubes and parts of the pump. Hence, any contamination of remaining ICG from the previous experiment was prevented. To cross validate the change in the absorption coefficient of the circulated mixture, a transparent cuvette was placed at the pump output and illuminated from one side with a white light source. A fiber coupled to the other side collected the transmitted light through the cuvette and transferred it to a spectrometer that was used to measure the transmittance. The transmittance data at 780 nm were acquired at every 5 s during the dynamic study. At the end of the study, a homogeneous solid phantom was used to obtain calibration data that would be used to calibrate the dynamic measurements.

3. Results

During the study, four different dynamic phantom experiments are conducted. For the first three cases, the solution is circulated through one of the three inclusions, while the other two inclusions are filled with a matching solution that brings their optical properties to the same levels of the solid phantom (R15, R8 and R8side). For the last case, the 15 mm diameter inclusion is filled with pure water while the main solution is circulated from the 8 mm diameter inclusion that is located 24 mm away from the center (R8sideW). For this particular case, the third inclusion is again filled with a matching solution that brings its optical properties to the background levels. Figure 3 presents a schematic representation while table 1 gives the description of each case. The spectrometer data that are collected from the beginning to the end of each experiment confirmed that the absorption coefficient of the initial solution and the change in the absorption coefficient of the circulated mixture is the same for each run, figure 5.

Once calibrated, the DOT data are used to reconstruct the absorption and scattering maps. For each case, 30 maps are reconstructed. The reconstructed images for the first case (R15) without and with the *structural a priori* is given in figure 6 for several time points. The mean absorption coefficient in the ROI is calculated from the reconstructed images for each time point. Figure 7 presents the plot of the mean absorption coefficient in the ROI with respect to the time for each case when the reconstructions are performed without the *structural a priori* information. Meanwhile, table 2 exhibits the maximum enhancement values together with the injection and dilution rates for each case calculated from the relative or percentage enhancement maps. The injection rate is defined as the slope of the absorption change from baseline to the 90% of the peak value. Similarly, the dilution rate is estimated as the slope during the dilution phase. The addition of the intralipid–water mixture solution is completed around the 390th s that corresponds to the 26th DOT frame. Please note that the absorption in the ROI increases after this frame due to the mixing of the remaining solution in the tubes that has higher concentration of the optical dye into the main container. Hence, the dilution rate is specifically estimated between the time point when the absorption coefficient reduces to 90% of its peak value and the 26th data point that is acquired nearly 1 min before the end of each experiment.

Figure 8 shows the relative enhancement curve that is calculated by subtracting the baseline values from each curve when the reconstructions are performed without the *structural a priori* information. As expected, the peak value of each relative enhancement curve is different. For example, maximum relative enhancement for the case R15 is 0.0033 mm^{-1} and nearly three times higher than the maximum relative enhancement for the 8 mm diameter object located deeper (R8). Nevertheless, even if the inclusion in the former case is the largest (R15), the recovered relative enhancement only reaches to nearly one-tenth of the true value, which is 0.03 mm^{-1} . These results confirmed that peak enhancement cannot be used as a measure due to its dependence on the size and location of the inclusion as well as

background optical properties. However, when normalized with respect to their own peak, curves overlap with each other completely, figure 9. This shows that the injection and dilution rates obtained from the percentage enhancement curves are immune to the variance in the size and location of the inclusions. As seen from table 2, if the relative enhancement curves are used, the relative errors of the injection and dilution rates with respect to the first case (R15) are quite high and reaching up to 76%. However, the errors in the injection and dilution rates for the second (R8) and third (R8side) cases were substantially lower (5–15%), when the percentage enhancement curves are used. Furthermore, the percentage enhancement curve obtained in the presence of a non-scattering medium (R8sideW) is also overlapping with the others. This verifies that quantitatively correct injection and dilution rates can be obtained even if the propagation of the light is incorrectly modeled at some part of the background medium. Indeed, even when the 15 mm inclusion is filled with pure water, both rates for the 8 mm object located closer to the boundary (R8sideW) agree with the first case (R15) within 7% error.

To be able to show the effect of erroneous *structural a priori* information on the recovered enhancement kinetics, initially the absorption map for the first case is reconstructed at each time point assuming that the exact position and size of the inclusion were known (R15A). Later, the size of the inclusion is chosen 20% larger (R15Lar) and 20% smaller (R15Sma) during the reconstructions. Finally, the absorption maps are reconstructed while misplacing the center of the inclusion by 20% of the actual distance (R15Off) from the center of the phantom. Table 3 presents the location and the size information for each case. Figure 10 shows the recovered enhancement curves, while figure 11 shows the relative enhancement curve that is calculated by subtracting the baseline values from each curve for all four cases. Again, the peak values vary substantially among these four cases. The peak enhancement reaches up to 0.0519 mm^{-1} when the actual location and size of the inclusion are used to guide and constrain the reconstruction algorithm for the 15 mm diameter object (R15A). However, the recovered value reduces down to 0.0397 mm^{-1} and increases up to 0.0828 mm^{-1} when the size of the object is estimated larger (R15Lar) or smaller (R15Sma), respectively. Similarly, the injection and dilution rates obtained from the relative enhancement curves also differ significantly, table 4. For example, the injection and dilution rates for the case R15Sma are nearly twice of the rates obtained with the use of the actual *structural a priori* information (R15A) and nearly three times higher than the rates obtained when the center of the inclusion is misplaced by 20% of the actual distance (R15Off).

On the other hand, the percentage enhancement curves overlap with each other as seen in figure 12. Hence, even when the erroneous *a priori* information is utilized, the relative errors in the injection and dilution rates with respect to the first case with actual *structural* information are all less than 10%, table 4. These results show that these rates are immune to the erroneous selection of *a priori* information, which can be the result of wrong segmentation or inaccurate co-registration between the optical and high-resolution anatomical imaging modalities.

4. Discussion and conclusion

Diffuse optical tomography studies reported in the literature have generally focused on the identification of malignant tumors based on endogenous absorption and scattering parameters. Indeed, there are several dynamic contrast-enhanced *in vivo* optical imaging studies reported in the literature for animal or human breast cancer imaging (Ntziachristos *et al* 2000, Cuccia *et al* 2003, Intes *et al* 2003, Rinneberg *et al* 2008, Unlu *et al* 2008b). There are a number of parameters that can be extracted from the optical enhancement curve either in the qualitative form, such as maximum (relative) enhancement and initial slope of increase, or in the quantitative form using two compartmental models. For example,

Ntziachristos *et al* and Intes *et al* both used DOT to examine the contrast-enhancement of ICG in breast tumors (Ntziachristos *et al* 2000, Intes *et al* 2003). Although the relative enhancement was compared between different cases in the former study, a two-compartment model was used to analyze the pharmacokinetics of ICG in the latter one. Rinneberg *et al* also carried out dynamic contrast-enhanced DOT on 11 patients and concentrated on the pattern of ICG enhancement curves between tumor and normal tissue. Cuccia *et al* on the other hand, used a six-parameter pharmacokinetic model to investigate the dynamics of the ICG with DOS in an adenocarcinoma rat tumor model.

This paper provides the first experimental phantom studies evaluating the accuracy of contrast enhancement kinetics. It was found that monitoring enhancement kinetics is inaccurate unless percent enhancement is used. DCE-DOT is particularly challenging due to its poor spatial resolution and low quantitative accuracy apart from the difficulty in interpreting the enhancement kinetics of the contrast agent. The recovered optical parameters of an inclusion highly depend on its size, location and contrast as well as the size and the optical heterogeneity of the background medium. In our previous simulation study (Unlu *et al* 2008a), we studied the variations in the recovered enhancement kinetics of different sized inclusions located in different depths. Using the two-compartment model, we showed that the accuracy of the recovered pharmacokinetic parameters was improved when *a priori* information was used especially for small objects located deep in a large medium. Here we present the first systematic phantom study to investigate not only the effect of the size and depth of the inclusion but also the presence of non-scattering regions in the background. Non-scattering regions are especially important for animal imaging. Organs such as bladder do not present enough scattering for proper modeling of light propagation with diffusion approximation in these organs. Using a water-filled inclusion in the background (R8sideW) allowed us to investigate the effect of non-scattering regions in the background on the recovered enhancement kinetics of the ICG circulated through another inclusion.

The temporal resolution of our multi-frequency DOT animal imaging system was 16 s. The dye injection was completed in 60 s. After that, the absorption change in the main solution occurred due to fact that it took sometime for the solution to reach the phantom from the main container and the change was not linear anymore. The upper level used in injection rate calculations, 90% of the peak value, corresponded to the nearly first 50 s of the injection, in which the rate was linear. During the dilution, on the other hand, the absorption coefficient of the solution reduced down to 60% levels. To calculate the dilution rate, we again used 90% of the peak value, which gave the largest range in the linear regime to be able to estimate accurate dilution rates.

Our experimental studies verified that the injection and dilution rates for all cases agreed only if the percentage enhancement maps were used during the analysis. As expected, the relative enhancement values were substantially different for all cases. The same conclusion was true in the case of utilizing *structural a priori* information. The *a priori* information improved the quantitative accuracy of the recovered optical parameters as demonstrated in the literature. For example, the recovered absorption coefficient for the case R15 at the peak enhancement increased from 0.0234 mm^{-1} up to 0.0519 mm^{-1} when the *structural a priori* information was used to guide the reconstruction algorithm. However, the recovered absorption coefficient was still 13.5% lower than the actual value, 0.060 mm^{-1} . In essence, the recovered optical properties of an inclusion depended on the size and location of the inclusion even if the *structural priori* was used to guide the reconstruction algorithm. Furthermore, together with the peak enhancement, the injection and dilution rates were strongly dependent on the *structural a priori* information. On the other hand, these rates

were immune to the errors in the *structural priori* only if they were calculated from the percentage enhancement maps.

In summary, these results demonstrated that although DCE-DOT was particularly challenging due to its poor spatial resolution and low quantitative accuracy, particular dynamic parameters still could be used to compare the lesions *in vivo* in clinical or animal studies successfully. Mainly, the uptake and wash-out rates could provide meaningful comparison between different tissue types only if percentage enhancement maps were used. The same parameters could also be used even if diffusion approximation was being used to model the propagation of the light in the presence of non-scattering objects in the background. Furthermore, if *structural priori* were available and used, the very same parameters would also be independent of the errors in the *priori* information due to imperfect segmentation or co-registration. Due to the availability of the FDA-approved blood pool agent ICG, DCE-DOT may be a good candidate modality as an adjunct to MRI that currently uses smaller molecular weight MR contrast agents. It should be noted that a dynamic contrast-enhanced DOT system could monitor any contrast agent that absorbs in the near-infrared wavelengths. Although the potential of the ICG enhancement kinetics in breast cancer diagnosis still needs to be investigated with larger scale clinical studies, DCE-DOT can also be used together with a more effective agent that may be developed in the future.

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Figure 1.
The schematic representation of the dynamic phantom. It consisted of a main container, a computer-controlled syringe, a mixer and a 63 mm diameter cylindrical solid optical phantom.

Figure 2.

The solid phantom had three different inclusions. The diameter of one of the inclusions (1) was 15 mm and it was located at 15.75 mm away from the center. The diameters of the other two inclusions were the same, 8 mm. They were radially located at 15.75 mm (2) and 23.62 mm (3) away from the center.

Figure 3.

The schematic representation of four cases. For the first three cases, the solution was circulated through one of the three inclusions, while the other two inclusions are filled with a matching solution that brings their optical properties to the same levels of solid phantom (R15, R8 and R8side). For the last case, the 15 mm diameter inclusion is filled with pure water while the main solution is circulated from the 8 mm diameter inclusion that is located 24 mm away from the center (R8sideW). For this particular case the third inclusion is again filled with a matching solution that brings its optical properties to the background levels.

Figure 4.

The actual absorption change for the dynamic phantom. After the baseline measurements (1), μ_a in the ROI was linearly increased from 0.030 to 0.060 mm $^{-1}$ in 1 min by injecting ICG into the main solution (2–3). After waiting for a minute (3–4), it was linearly decreased from 0.060 to 0.045 mm $^{-1}$ in 4 min by adding the intralipid–water mixture into the main container (4–5). The data acquisition was stopped 1 min after the dilution process ended (6). The red circles show the selected data points (A–D) for which the reconstructed absorption maps are given in figure 6.

Figure 5.

The spectrometer transmittance data at 780 nm were acquired at every 5 s during the dynamic study. The spectrometer data were used to monitor the absorption change of the circulating liquid and verify that the same pattern was repeated in each experiment. The curves present the raw data acquired from the spectrometer without any normalization and all the settings including the integration time was kept the same for all experiments. Please note that the transmittance data were not used to calculate the absorption coefficient of the solution due to the presence of the intralipid.

Figure 6. Reconstructed absorption maps at four time points for the case R15. The first and second rows show the reconstructions without and with *a priori* knowledge of the location and size of the inclusion, respectively. The corresponding time points (A–D) are indicated by red circles in figure 4.

Figure 7.

The recovered mean absorption coefficient in the ROI at each time point for all four cases when the reconstructions are performed without the *structural a priori* information. As expected, the recovered absorption coefficient was the lowest for the small object (8 mm in diameter) located closest to the center of the phantom (R8).

Figure 8.

The relative enhancement for each case calculated without any *structural a priori* information. To calculate the relative enhancement, the first baseline measurement was subtracted from the recovered mean absorption curve for each case separately

Figure 9.
The percentage enhancement for each case calculated without any *structural a priori* information. Each relative enhancement curve was normalized with respect to its own peak value.

Figure 10.

The recovered absorption coefficient for the 15 mm inclusion at each time point when *structural a priori* information was used during the reconstruction (R15A). The effect of the erroneous *priori* information was also investigated. For this purpose, the absorption map was reconstructed when the size of the inclusion was estimated larger (R15Lar) or smaller (R15Sma), and the inclusion's center was misplaced (R15Off) due to potential imperfect co-registration between different modalities.

Figure 11.
The relative enhancement for case R15 with correct and erroneous *structural a priori* information.

Figure 12.

The percentage enhancement for case R15 with correct and erroneous *structural a priori* information. Each relative enhancement curve was normalized with respect to its own peak value.

Table 1

The optical parameters for each case. During the experiments the mixture was circulated through one inclusion at a time (marked as 'Dynamic'), while the other two inclusions were filled with an ICG–intralipid–water mixture whose optical properties matched to the solid phantom. As an exception, the large inclusion was filled with water during the last experiment. Please refer to figure 2 for the geometrical parameters of each inclusion and figure 3 for schematic representation of each case.

Case	Absorption (mm^{-1})		
	Inclusion 1	Inclusion 2	Inclusion 3
R15	Dynamic	0.0132	0.0132
R8	0.0132	Dynamic	0.0132
R8side	0.0132	0.0132	Dynamic
R8sideW	Water	0.0132	Dynamic

The important parameters that are obtained from the recovered enhancement kinetics without any *a priori* information for each case. The first column presents the maximum enhancement values for the mean absorption curve in the region of interest (ROI). The second column presents the maximum values for the relative enhancement curves. The third and fifth columns show the injection and dilution rates that are estimated from the relative enhancement curves. The fourth and fifth columns give the percentage errors in the estimated rates for the last three cases with respect to the first one. Similarly, the last four columns show the injection and dilution rates that are calculated from the percentage enhancement curves as well as the errors in these rates. All the percentage errors are calculated with respect to the first case (R15) and therefore, there are no error values given for this case.

Table 2

Case	Enhancement Max (mm ⁻¹)	Relative enhancement			Percentage enhancement		
		Max (mm ⁻¹)	Injection	Error	Dilution	Error	Dilution
R15	0.0234	0.0033	5.61×10^{-5}	–	6.86×10^{-6}	–	0.205
R8	0.0167	0.0010	1.53×10^{-5}	72.7	1.67×10^{-6}	75.6	0.175
R8side	0.0226	0.0040	5.94×10^{-5}	5.9	7.20×10^{-6}	4.9	0.179
R8sideW	0.0191	0.0026	4.03×10^{-5}	28.1	4.93×10^{-6}	28.2	0.191

Table 3

Different *structural* information parameters were used for each case. The first two columns give the coordinates of the center of the circular region of interest (ROI) that was chosen during the reconstruction. Besides, the third column presents the diameter of the ROI. First, the exact position and size of the inclusion were assumed to be known (R15A). Later, the size of the inclusion was chosen 20% larger (R15Lar) and 20% smaller (R15Sma) during the reconstructions. Finally, the absorption maps were reconstructed while misplacing the center of the inclusion by 20% of the actual distance (R15Off) from the center of the phantom.

<u>Structural <i>a priori</i> information parameters</u>			
Case	x (mm)	y (mm)	Diameter (mm)
R15A	15.75	0	15
R15Lar	15.75	0	18
R15Sma	15.75	0	12
R15Off	12.60	0	15

The important parameters that are obtained from the recovered enhancement kinetics without any *a priori* information for each case. The first column presents the maximum enhancement values for the mean absorption curve in the region of interest (ROI). The second column presents the maximum values for the relative enhancement curves. The third and fifth columns show the injection and dilution rates that are estimated from the relative enhancement curves. The fourth and fifth columns give the percentage errors in the estimated rates for the last three cases with respect to the first one. Similarly, the last four columns show the injection and dilution rates that are calculated from the percentage enhancement curves as well as the errors in these rates. All the percentage errors are calculated with respect to the first case (R15) and therefore, there are no error values given for this case.

Table 4

Case	Enhancement Max (mm ⁻¹)	Relative enhancement			Percentage enhancement		
		Max (mm ⁻¹)	Injection	Error	Dilution	Error	Dilution
R15A	0.0519	0.0193	2.47×10 ⁻⁴	–	3.72×10 ⁻⁵	–	0.193
R15Lar	0.0397	0.0120	1.57×10 ⁻⁴	36.4	2.13×10 ⁻⁵	42.9	1.9
R15Sma	0.0828	0.0358	4.39×10 ⁻⁴	77.9	6.98×10 ⁻⁵	87.6	4.3
R15Off	0.0390	0.0116	1.46×10 ⁻⁴	40.8	2.08×10 ⁻⁵	44.2	1.7