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Optimal parameters for near infrared fluorescence imaging of amyloid plaques in Alzheimer's disease mouse models

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Abstract

Amyloid- β plaques are an Alzheimer's disease biomarker which present unique challenges for near-infrared fluorescence tomography because of size ($<50\ \mu\text{m}$ diameter) and distribution. We used high-resolution simulations of fluorescence in a digital Alzheimer's disease mouse model to investigate the optimal fluorophore and imaging parameters for near-infrared fluorescence tomography of amyloid plaques. Fluorescence was simulated for amyloid-targeted probes with emission at 630 and 800 nm, plaque-to-background ratios from 1–1000, amyloid burden from 0–10%, and for transmission and reflection measurement geometries. Fluorophores with high plaque-to-background contrast ratios and 800 nm emission performed significantly better than current amyloid imaging probes. We tested idealized fluorophores in transmission and full-angle tomographic measurement schemes (900 source–detector pairs), with and without anatomical priors. Transmission reconstructions demonstrated strong linear correlation with increasing amyloid burden, but underestimated fluorescence yield and suffered from localization artifacts. Full-angle measurements did not improve upon the transmission reconstruction qualitatively or in semi-quantitative measures of accuracy; anatomical and initial-value priors did improve reconstruction localization and accuracy for both transmission and full-angle schemes. Region-based reconstructions, in which the unknowns were reduced to a few distinct anatomical regions, produced highly accurate yield estimates for cortex, hippocampus and brain regions, even with a reduced number of measurements (144 source–detector pairs).

1. Introduction

Amyloid- β plaques are small, spherical aggregates (5–50 μm diameter) of mis-folded protein (amyloid- β), which accumulate over time in Alzheimer's brain, primarily in the cortex. Along with neurofibrillary tangles (another protein aggregate), amyloid plaques are the principal histo-pathological hallmark of Alzheimer's disease (AD) and therefore a biomarker of great interest (Hyman and Trojanowski 1997). The ability to non-invasively assess brain amyloid could greatly accelerate drug development and aid basic research. To this end, we and others are developing near-infrared (NIR) fluorescence imaging for detecting amyloid plaques in transgenic mouse models of AD.

Although a few amyloid imaging agents emit out to 670 nm (Nesterov *et al* 2005, Hintersteiner *et al* 2005), to date no fluorophore has been developed that emits above 700 nm, binds amyloid plaques and crosses the blood-brain barrier. A fluorophore that emits between 700 and 850 nm is optimal because tissue absorption at these wavelengths is the

lowest, allowing deeper light penetration. The molecular weight of most true NIR fluorophores, which generally increases with emission wavelength, precludes passage across the blood-brain barrier and represents a major hurdle for NIR amyloid probe development. Additionally, amyloid-binding dyes often show affinity to white matter (Raymond *et al* 2008) and thereby exhibit a low plaque-to-background contrast ratio (CR). The trade-off of BBB-permeability and NIR emission, along with propensity for poor contrast, have complicated NIR amyloid probe development and motivate a study of fluorophore properties relative to amyloid imaging capabilities.

In the past, fluorescence tomography (sometimes called fluorescence molecular tomography, FMT) has been used to track tumor growth and localize discrete objects in small animals (Weissleder and Ntziachristos 2003, Montet *et al* 2005, Montet *et al* 2007), but has not been used to measure sub-resolution, widely scattered targets, such as amyloid plaques. FMT has seen rapid development of new imaging hardware, including full-angle measurement schemes (Meyer *et al* 2007, Deliollanis *et al* 2007) and reconstruction software (Joshi *et al* 2004, Boas *et al* 2002, Dehghani *et al* 2008), that may potentially enable 3D tomographic imaging of amyloid in transgenic mouse models. A recent paper demonstrated the ability to detect brain amyloid with a sophisticated FMT-CT measurement scheme, including region-based priors for guiding the reconstruction algorithm (Hyde *et al* 2009). Because the resolution and sensitivity of these techniques will be affected by the partial volume effects of imaging sub-resolution, distributed targets, they should be quantitatively and rigorously tested.

The purpose of this study was, first, to determine the optimal fluorophore parameters for amyloid imaging to guide future fluorophore design and development. Second, we wanted to explore the feasibility of FMT for quantifying amyloid plaque deposition (amyloid burden) in AD mouse models and to establish the resolving power of FMT given realistic fluorophore properties (emission wavelength and contrast ratio) and measurement schemes (transmission and full-angle).

To address these issues, we developed a high-resolution digital model of a mouse with amyloid plaques based on a published cryosection/CT atlas. We simulated fluorescence excitation and detection for a range of amyloid burden, plaque-to-background contrast ratios and excitation wavelengths with different source–detector (SD) measurement schemes. The optimal parameters from these preliminary simulations were tested in tomography simulations with 900 SD pairs. Reconstructions were compared for varying amyloid burden, transmission versus full-angle schemes and anatomically based priors. Finally, we considered the efficacy of region-based inversion for estimating the average amyloid burden across atlas-defined anatomical regions.

2. Methods

2.1. Digital AD transgenic mouse model

We developed a digital model of transgenic AD mouse head by modifying a publicly available digital mouse atlas (Digimouse (Dogdas *et al* 2007)). We cropped the full mouse to retain only the head, manually determined the hippocampus segmentation with the help of a stereotaxic brain atlas (Paxinos and Franklin 2001), and slightly modified the Digimouse ‘cerebrum’ segment to produce a new ‘cortex’ segment. The hippocampus and cortex were combined to constitute a ‘plaque-prone’ segment. All brain regions besides the hippocampus and cortex were combined into a generic brain segment. With these modifications, the model contained skin, skull, muscle, brain (including eyes and olfactory bulbs) and plaque-prone brain (cortex and hippocampus) in $181 \times 155 \times 167$ voxels at $100 \mu\text{m}$ isotropic resolution.

For a given amyloid burden (% volume occupied by amyloid plaques), plaques were placed randomly in the AD-prone brain segment, assuming uniform probability.

Background optical properties (μ_a , absorption coefficient, μ_s , scattering coefficient and g , anisotropy) were assigned to the different tissue segments according to literature values at 630–650 nm and 800 nm (Barnett *et al* 2003, Firbank *et al* 1993, Simpson *et al* 1998, Yaroslavsky *et al* 2002, Kienle and Glanzmann 1999) (table 1). To estimate the amount of dye in the brain and the effect on absorption, we assumed a dosage of 0.1 mg kg^{-1} , uniform biodistribution, i.e. 100% injected dose index (Mathis *et al* 2004), and a molar extinction coefficient of $100\,000 \text{ cm}^{-1} \text{ M}^{-1}$. With these assumptions, the total dose received by the brain was distributed into the plaque voxels and background voxels based on the contrast ratio (CR), defined as the ratio of fluorescence yield at the plaque to fluorescence yield in the surrounding brain. The total absorption (μ_a) in the simulation was the sum of absorption due to dye and normal brain; the fluorescence quantum yield (Φ) was 1, i.e. every photon absorbed by dye was converted to emission. Although most NIR fluorophores have much lower quantum yield, on the order of 0.1, we chose this value to represent an ideal fluorophore and to allow easy scaling of the simulation results for arbitrary fluorophores in the future.

2.2. Forward problem

In a traditional fluorescence measurement, light from a source enters the tissue at a specific point, excites fluorophores deep in the tissue, which then emit photons that are detected at the surface. Photon propagation, from the source to the fluorophore and from the fluorophore to a detector, is further complicated by tissue scattering and absorption. The so-called forward problem estimates the number of photons that reach a detector, given a known source position and tissue optical properties, and is generally modeled with the transport or diffusion equations. In this formulation, the fluence at a detector, $U(\mathbf{r}_s, \mathbf{r}_d)$, is given by the integral equation

$$U(\mathbf{r}_s, \mathbf{r}_d) \propto \int_V d^3\mathbf{r} G_{\text{ex}}(\mathbf{r}_s, \mathbf{r}) \eta(\mathbf{r}) G_{\text{em}}(\mathbf{r}, \mathbf{r}_d). \quad (1)$$

$\eta(\mathbf{r})$ is the fluorescent yield at a given point $\mathbf{r}$, and is the product of the fluorophore concentration ($[C]$), molar extinction coefficient (ϵ) and quantum yield (Φ); $\mathbf{r}_s$ and $\mathbf{r}_d$ are the source and detector positions; $G_{\text{ex}}(\mathbf{r}_s, \mathbf{r})$ and $G_{\text{em}}(\mathbf{r}, \mathbf{r}_d)$ are Green's functions for light propagation at the excitation and emission wavelengths, respectively. Equation (1), in a discrete form, can be expressed as $\mathbf{y} = A\eta$, where $\mathbf{y}$ is the measurement vector, η is the fluorescence yield vector and A is the sensitivity matrix.

In this study, we used a transport-based Monte Carlo approach (Wang *et al* 1995, Boas *et al* 2002) to calculate photon migration through the digital mouse model, with optical properties specified at each voxel. In brief, individual photons were propagated stepwise through the mouse model based upon voxel optical properties. Escape at the boundaries was determined by Fresnel's equation for internal reflection. For each simulation, excitation (source into medium) and emission (detector into medium) two-point functions were calculated based on the propagation of 10 million photons; the sensitivity matrix, A , was calculated from the excitation and emission two-point functions using the adjoint method. Excitation and emission background optical properties were assumed to be the same, which is true when imaging fluorophores with a small Stoke's shift. Because most NIR fluorophores have Stoke's shift from 10 to 30 nm, this assumption is generally accurate. The excitation two-point, however, included absorption from both the tissue and the fluorophore, whereas the emission two-point considered only intrinsic tissue absorption.

In transmission, photons were launched from the ventral surface and detected at the dorsal surface of the mouse; in reflection, photons were launched from the dorsal surface of the head and detected at the dorsal surface 5 mm rostral, adjacent to the source (see figure 1). The fluorescence yield at each voxel, $\eta(\mathbf{r})$, was calculated by the product of the absorption due to the dye and the quantum yield, i.e. $\eta(\mathbf{r}) = \Phi\mu_{a,\text{dye}}(\mathbf{r})$. The simulated fluence at the detector was then given by $A\eta$.

2.3. System calibration and noise model

To translate simulated photons into CCD counts, we calculated a scaling factor based on measurements taken with our fluorescence tomography system. As described elsewhere (Kumar *et al* 2008), our system consists of a translatable fiber-based source, a 60-mm lens (AF Nikkor, f2.8, Nikon) mounted to a CCD camera (Picostar HR-12 CAM 2, LaVision GmbH, Goettingen, Germany), and a photogrammetric camera (3D Facecam 100, Genex Technologies Inc., Kensington, MD) for capturing 3D boundaries. In this work, the time-gated intensifier was removed for continuous-wave (CW) imaging.

We estimated system noise and sensitivity using intralipid in a 1.9 cm deep petri dish (1% Intralipid, 0.02% Indian Ink, estimated $\mu_a = 0.05 \text{ mm}^{-1}$, $\mu_s = 1.257 \text{ mm}^{-1}$, $g \approx 0$). A single tube (0.4 mm inner diameter) transversing the dish at a height of 1.5 cm was filled with Alexa Fluor 647 at 500 nM and imaged in transmission for 11 source positions (2 mm spacing). Analogous to the tube phantom measurements, we simulated fluorescence coming from a virtual tube placed 0.4 cm below the surface of a 1.9 cm slab. The simulation scaling factor was then calculated by comparing CCD fluorescence counts with the Monte Carlo simulated fluorescence intensity for corresponding detectors.

Given these parameters, simulation results were scaled according to

$$\mathbf{y} = S \frac{A_{\text{CCD}}}{A_{\text{det}}} \mathbf{y}_{\text{sim}} \quad (2)$$

where S is the scaling factor, $\mathbf{y}_{\text{sim}}$ is the simulated fluence at the detector, A_{CCD} is the surface area for the virtual detector in the CCD image and A_{det} is the simulated detector area. The scaling factor was calculated assuming laser power at 50 mW and detector exposure time of 1 s, which are reasonable acquisition parameters for most FMT systems. Detector counts were estimated assuming only emission photons, without considering excitation leakage across the emission filter which can generally be eliminated with emission filters ($\text{OD} > 6$) and subtraction techniques (Soubret and Ntziachristos 2006).

System noise was assumed to be shot noise limited, following Poisson statistics,

$$\sigma^2 = \beta \bar{y} + \sigma_r^2 \quad (3)$$

where $\bar{y}$ is the data mean, β is a scaling coefficient specific to the camera and σ_r^2 is the CCD readout noise (Hyde *et al* 2007). In order to estimate these parameters, we took 100 images of a 0.275 mW 650 nm diode laser directed in the transmission geometry (from below) and calculated σ and $\bar{y}$ for each pixel in the image. The resultant data were fit according to (3), yielding a best fit for $\beta = 0.67$ and $\sigma_r^2 = 11.45$ (figure 2). These parameters were independent of the integration time, filters or lasers used (data not shown).

Given (3), it was possible to explicitly calculate the signal-to-noise and contrast-to-noise ratios (SNR and CNR). We calculated the SNR as $\text{SNR} = \bar{y}/\sigma$, or, expressed in dB, $\text{SNR}_{\text{dB}} =$

$10 \log_{10}(y^2/\sigma^2)$. The contrast-to-noise ratio (CNR) was estimated by the change in the SNR for a 1% change in amyloid burden (from 5 to 4%), i.e. $\text{CNR} = \text{SNR}_{5\%} - \text{SNR}_{4\%}$.

2.4. Tomography measurement geometries

We investigated the capabilities of three different acquisition geometries for tomography: transmission, full-angle and reduced-measurement. For each transmission and full-angle simulation, forward data were generated for 900 source–detector (SD) pairs. The transmission geometry consisted of 25 sources at the ventral surface and 36 detectors at the dorsal surface; the full-angle geometry consisted of 5 sources and 30 detectors in transmission across the mouse, rotated every 60° surrounding the mouse (see figure 3(a,b)). The reduced-measurement geometry consisted of 12 sources at the ventral surface and 12 detectors at the dorsal surface, and was used for region-based inversion (figure 3(c)). Forward data were generated as described above and noise was added assuming that each measurement set utilized the full dynamic range of the CCD (12-bit, i.e. $\max(\text{data}) = 4096$).

2.5. Inverse problem

To begin the inverse problem, we calculated a sensitivity function, A , assuming homogeneous optical properties throughout the mouse at the voxel-averaged μ_a and μ_s . For inversion, we used a generalized objective function (Yalavarthy *et al* 2007, Tarantola 2004) given by

$$\Omega = \|A\eta - \mathbf{y}\|_{C_y}^2 + \|L(\eta - \eta_o)\|_{C_\eta}^2, \quad (4)$$

$$= (A\eta - \mathbf{y})^T C_y^{-1} (A\eta - \mathbf{y}) + (\eta - \eta_o)^T L^T C_\eta^{-1} L (\eta - \eta_o). \quad (5)$$

Minimizing (5) gives

$$\eta = \eta_o + L^{-1} C_\eta L^{-1^T} A^T (A L^{-1} C_\eta L^{-1^T} A^T + C_y)^{-1} (\mathbf{y} - A\eta_o). \quad (6)$$

In this expression, C_η and C_y are the state and measurement covariance, respectively, L is a spatially-variant regularization term and η_o is the initial guess for η . This approach is advantageous because the regularization is explicitly determined by the state and measurement noise (Yalavarthy *et al* 2007).

For this study, we assumed that the noise was uncorrelated between SD pairs, and set the diagonal of to the simulated variance, i.e. $C_{yij} = \sigma_i^2$. C_η was estimated as $\sigma_\eta^2 I$, where σ_η was the estimated standard deviation in the fluorescence yield, chosen here to be 0.0056 mm^{-1} (the volume-averaged yield in plaque-prone regions and $2\times$ the background fluorescence), and I was the identity matrix; η_o was 0.0028 mm^{-1} , which was the background fluorescence yield in the input model. L was a diagonal matrix whose elements were given by the diagonal elements of $\sqrt{A^T A}$, normalized to have mean of 1, since the regularization was explicitly set by C_η and C_y . Anatomical priors were introduced by multiplying the $L^{-1} C_\eta L^{-1^T}$ term by the prior matrix, P , where the diagonal elements of P are the probability that a certain tissue voxel is brain. We estimated this probability by first generating an atlas with brain voxels set to 1 and all other tissue types set to 0, and then applied a Gaussian smoothing filter ($5 \times 5 \times 5$ kernel). This allowed uncertainty in the tissue type at the edges of the brain.

Tomographic reconstructions were evaluated by comparing the fluorescence yield in reconstruction voxels with the input model voxels. Before comparison, the input model was volume-averaged and down-sampled from 0.1 to 0.5 mm, corresponding to the spatial resolution of the reconstructions. We estimated correlation using linear regression between reconstruction and input voxels (R^2), as well as the entropy correlation coefficient (ρ^2), which is a model-independent measure of correlation utilizing mutual information, calculated as $\rho^2 = 2 - H_{XY}/(H_X + H_Y)$, where H_X and H_Y are the Shannon entropies of $\mathbf{x}$ and $\mathbf{y}$ and H_{XY} is the joint entropy. ρ^2 allows for nonlinearities in the reconstruction due to inhomogeneities in the sensitivity matrix.

2.6. Region-based inversion

In cases where fluorescence yield can be assumed homogeneous over distinct anatomical regions, the tomography problem can be reduced to solving for the fluorescence yield within a limited set of tissue regions. In this case the sensitivity matrix is reduced as

$$A_{i,t} = \sum_{j \in \mathcal{R}} A_{i,j}, \quad (7)$$

where i are the measurements and t are voxels in a specific region, which are a subset of all tissue voxels, j . For this simplification, tissue regions are specified by a co-registered CT or other anatomical model and the fluorescence yield at each region, η_t , represents a volume-averaged solution over the region.

We tested this approach with our digital model by simulating fluorescence for varying amyloid burden in the hippocampus (1–10%), while keeping the amyloid burden in the cortex at 1, 5 or 10%. Forward data were generated for 11 different scenarios using the adjoint method for 144 SD pairs in transmission geometry (12 sources and 12 detectors, see figure 3(c)); tissue optical properties and fluorophore parameters were the same as for the tomography simulations. Noise was added to forward data before inversion using the noise model derived above. We used a least-squares solution to solve $\mathbf{y} = \mathbf{A}\boldsymbol{\eta}$.

3. Results

3.1. Fluorophore parameters

To determine the optimal fluorophore parameters, we simulated CCD counts for two emission wavelengths (630 and 800 nm), two measurement schemes (transmission and reflection), plaque-to-background contrast ratio (CR) from 1 to 1000 and amyloid burden ranging from 0 to 10% (figure 4(a)). For these simulations, background optical properties were assumed to be the same for both emission and excitation photon propagation, as in the case of a fluorophore with a small Stoke's shift. Fluorophore concentration in brain and plaque voxels was determined from the contrast ratio as follows: the total amount of fluorophore in the brain was constant, but distributed in plaque and brain voxels as dictated by the contrast ratio (see the Methods section).

As might be expected, CCD counts increased linearly with increasing amyloid burden for all parameters tested. The highest CCD counts were achieved for 800 nm emission, in reflection geometry, with the greatest sensitivity to change in amyloid burden at the highest CR (1000). For a given measurement scheme and emission wavelength, the average CCD counts increased with decreasing CR (more fluorophore was in the background and less at plaques), but the change in counts with respect to amyloid burden (slope) increased with increasing CR (see figure 4(a)). CCD counts were significantly higher at 800 nm compared to 630 nm for both reflection (27-fold) and transmission (370-fold). Reflection signal was much

stronger than transmission (88-fold at 630 nm and 6-fold at 800 nm). Using the noise characteristics of our system, we estimated the 10, 20 and 30 dB SNR lines ($\text{SNR in dB} = 10 \log_{10}(y^2/\sigma^2)$) and plotted these thresholds relative to the simulated CCD counts for the various fluorophore parameters (figure 4). The 630 nm, transmission scaled simulation SNR was below 20 dB for all CRs, and below 10 dB for many measurements; in comparison, almost all measurements at 800 nm and most of the 630 nm, reflection measurements had an SNR greater than 30 dB.

We estimated the sensitivity to changes in amyloid burden with the contrast-to-noise ratio ($\text{CNR} = \text{SNR}_{5\%} - \text{SNR}_{4\%}$). The CNR was highly dependent upon measurement geometry, emission wavelength and CR, as shown in figure 4(b). For the parameters tested, CNR was greater than 2 only for $\text{CR} \geq 50$ at 630 nm, reflection; $\text{CR} \geq 20$ at 800 nm, transmission; and $\text{CR} \geq 5$ at 800 nm, reflection.

3.2. Tomography

We chose $\text{CR} = 20$ at 800 nm as an ‘idealized’ fluorophore to test tomographic reconstruction techniques. This contrast ratio is likely the highest achievable with current fluorophore design (see the Discussion section). We simulated fluorescence for 900 SD pairs in transmission geometry (see figure 3) for increasing amyloid burden (0, 1, 5 and 10%) and reconstructed the simulated data using equation (6) without anatomical priors or an initial guess, η_0 .

Reconstructions qualitatively resembled the input model of plaque-prone brain regions (figure 5). Yield reconstructions displayed increasing fluorescence yield with increasing amyloid burden, and correctly localized maximum yield to the plaque-prone cortex. However, there was significant off-target reconstructed yield (i.e. outside of the brain) and spatial heterogeneity. For example, the reconstructions did not accurately reconstruct yield in the center of the head (e.g. thalamus) and on the ventral aspect of the brain. Reconstructions were also confounded by plume artifacts which descended inferior from the most lateral aspects of the cortex along SD axes. The global fluorescence yield averaged over the plaque-prone brain regions increased linearly with increasing amyloid burden, and was highly correlated with the input model yield ($R^2 = 0.9991$), but underestimated input model yield by roughly 2-fold (figure 6). Semi-quantitative analysis showed that fluorescence yield in individual voxels only weakly correlated with the input model yield (see table 2).

Motivated by these initial results, we investigated more sophisticated reconstruction methods and measurements schemes in order to improve on the reconstruction accuracy. We developed a ‘full-angle’ measurement scheme with 900 SD pairs encircling the mouse head (figure 3(b)), similar to the recently reported 360° FMT systems (Deliolani *et al* 2007, Meyer *et al* 2007). Simulated data for 10% amyloid burden were reconstructed for the transmission and full-angle schemes, with and without anatomical and initial guess priors (see equation (6)). The performance of these schemes and reconstruction techniques was evaluated with linear regression and mutual information on a voxel-to-voxel basis.

The full-angle scheme did not dramatically improve upon the transmission scheme, in the basic reconstruction without priors (compare figure 7(b, g) with (d, i)). Like the transmission scheme, the full-angle scheme localized the highest yield to the dorsal aspect of cortex and suffered from plume artifacts along the source axes. The full-angle reconstruction, however, did more accurately capture fluorescent yield in the center and ventral portions of the brain, as well as the cerebellum and olfactory bulbs. Additionally, the full-angle reconstruction had slightly less off-target yield; the average yield for plaque-prone regions was not significantly more accurate for full-angle compared to transmission reconstructions. In semi-quantitative

analysis, the full-angle scheme without priors performed equivalently to the transmission scheme, and slightly poorer when considering just the brain or plaque-prone region (table 2).

The use of anatomical priors noticeably improved the qualitative accuracy of both reconstruction schemes. The reconstructions had reduced plume artifacts, less off-target yield and more reasonable yield estimates in the plaque-prone regions (figure 7(c, e, h, j)). The transmission scheme was poorer at estimating yield in the center and ventral regions of the brain compared to the full-angle scheme. The two schemes had very similar performance on semi-quantitative measures of correlation (table 2).

All measurement schemes and reconstruction approaches under-estimated yield in the plaque-prone regions (table 2, last column). Furthermore, although voxel-to-voxel correlation was high for some schemes (see e.g. table 2, Trans., w/priors, all tissue), this correlation was primarily a result of low reconstructed yield in the non-brain tissue. When individual regions, such as the brain or plaque-prone tissue, were considered, the correlation between the reconstructed yield and the input model was significantly reduced. For example, R^2 was <0.3 in the plaque-prone regions for all schemes tested.

3.3. Region-based inversion

Finally, we considered a reduced tomographic problem, where we assumed that fluorescence yield was homogeneous across specific anatomical regions. In this case, the inverse problem was reconsidered in terms of average fluorescence yield across a few specific regions (non-plaque bearing brain, cortex and hippocampus), rather than for every voxel. We generated transmission forward data for a reduced set of sources and detectors (144 SD pairs, see figure 3(c)) for 11 scenarios with varying amyloid burden in the hippocampus and cortex. The region-wise inversion yielded accurate estimates of average yield in these three regions (figure 8). The correlation between 'reconstructed' fluorescence yield and input model yield was very high ($R^2 = 0.9860$) when considering all regions and scenarios, and was strongest for the largest regions (brain and cortex); the hippocampus displayed the highest variability in yield estimates, although it still had high correlation ($R^2 = 0.9986$ for cortex, $R^2 = 0.9502$ for hippocampus).

4. Discussion

The recent developments of digitized and segmented mouse atlases (Dhenain *et al* 2001, Dogdas *et al* 2007, Segars *et al* 2004) and software for generating forward solutions in heterogeneous tissue (Boas *et al* 2002) have enabled high-resolution, realistic simulations of photon propagation in tissue. These resources are now freely available to the public and can be used to provide insight on disease-specific imaging questions. Others have used atlas-based simulations to test and optimize measurement geometries and reconstruction algorithms (Joshi *et al* 2008, Bourayou *et al* 2008, Dogdas *et al* 2007). In this work, we used a digital mouse model of Alzheimer's disease to establish optimal fluorophore parameters and imaging capabilities for detecting amyloid- β plaques in transgenic mouse models.

The best NIR amyloid probe to date is AO1987, an oxazine-derived amyloid-binding fluorophore that exhibits absorption and emission peaks at 650 and 670 nm (Hintersteiner *et al* 2005); in initial studies, AO1987 pharmacokinetics were used to distinguish wild-type from aged AD transgenic mice. Based on our simulations, a NIR amyloid-binding fluorophore emitting at 800 nm would improve current NIR amyloid imaging capabilities beyond AO1987. For the parameters tested, fluorophores with 800 nm emission resulted in greater signal (27 to 370-fold greater CCD counts) and were more sensitive to changes in amyloid burden than fluorophores emitting at 630 nm, which had much lower CCD counts and SNR (figure 4). Measurements in reflection geometry (similar to planar fluorescence

imaging) resulted in much higher signal than transmission (at both 630 and 800 nm), because amyloid plaques are predominantly located in the cortex (a few millimeters below the dorsal surface of the head); the SNR for 630 emission in reflection was > 20 dB for almost all contrast ratios and amyloid burden tested.

It is important to note that CCD counts, SNR and CNR are affected by a number of imaging hardware considerations, including CCD noise and exposure time, laser power and excitation and emission filters. The CCD counts and SNR values in this work were calculated assuming standard measurement parameters (1 s exposure time; 50 mW laser power; CCD noise characteristics as described above) without tissue autofluorescence or excitation contamination of the emission signal. For some parameters (exposure time, laser power, quantum yield and molar extinction coefficient), the signal scales linearly and the SNR is trivially related via the scaling coefficient. For example, detector counts (y_f) acquired for a fluorophore with non-unity quantum yield, f , are related to the original counts (y) as y_f

$= fy$, and $SNR_f = fy / \sqrt{fy + \sigma_r^2}$, which is simplified further for $fy \gg \sigma_r^2$ to $SNR_f \approx \sqrt{f} SNR$. Thus, these simulations provide guidelines for detection feasibility but should be scaled appropriately when considering different measurement and fluorophore parameters.

To quantitatively assess the sensitivity of fluorophores to changes in amyloid burden, we estimated the contrast-to-noise ratio (CNR) for a 1% change in amyloid burden (from 4 to 5%). Amyloid burden in aged transgenic mouse models is between 1 and 10%, and changes of 1% are not uncommon for preclinical therapeutic trials (Sadowski *et al* 2006). We anticipate that, given inter-animal variability and system noise characteristics, CNR = 2 is a generous criterion for sensitivity to 1% change in burden. To meet this criterion, future fluorophore design should aim for an amyloid-binding probe that emits at 800 nm and has a contrast ratio ≥ 5 for planar imaging and ≥ 20 for tomography (see figure 4(b)).

Meeting these contrast ratio targets will require an advance in current probe characteristics. Contrast ratios have not traditionally been reported, but it is likely that the plaque-to-background contrast for most amyloid imaging fluorophores is <10. Methoxy-X04, a blue-green fluorophore with excellent amyloid specificity (Klunk *et al* 2002), has plaque-to-background contrast of ~9 in an aged transgenic mouse, as measured by two-photon microscopy of post-mortem tissue. The plaque-to-background contrast of AO1987, although not measured by Hintersteiner *et al*, was estimated in our laboratory at ~5, using confocal microscopy of post-mortem tissue taken from aged AD transgenic mice that received 1 mg kg⁻¹ AO1987. For biomarkers besides amyloid, target to background ratios (TBR) have been reported in the literature from 1.5 to 10 (Frisoli *et al* 1993, Figueiredo *et al* 2005, Houston *et al* 2005, Leevy *et al* 2008). Of these, 'smart' or activatable probes, which are enzymatically or otherwise turned on at the target (Weissleder *et al* 1999), generally have the best TBR. New smart amyloid probes could utilize the unique environment around amyloid plaques, such as oxidative stress (McLellan *et al* 2003) or amyloid binding itself (Nesterov *et al* 2005), in order to turn on fluorophore emission. Other smart approaches using lifetime and spectral contrast may offer additional improvements in TBR (Raymond *et al* 2008). Future probe design will most likely require smart capabilities to improve the plaque-to-background contrast.

In addition to testing fluorophore parameters, we also compared the imaging capabilities of transmission and full-angle tomographic reconstructions for amyloid imaging.

Transmission-based reconstructions showed an increased yield in a regional pattern similar to plaque deposition, with an average cortical fluorescence yield that was linearly correlated with average cortical yield in the input model. However, the reconstructed yield was dramatically lower than the input model, and suffered from poor sensitivity in the middle of

the mouse, especially at the ventral region of the brain. Although this is not a critical region for AD, reduced sensitivity in the middle of the head could be problematic for imaging other disease targets in the mouse head.

Despite the more complete angular coverage, full-angle measurements did not dramatically improve the qualitative appearance of the reconstructions or semi-quantitative measures of accuracy (see figure 7 and table 2). Full-angle reconstructions did not improve the recovery of average cortical yield and only slightly increased sensitivity at the center of the mouse. Both transmission and full-angle reconstructions were improved by the use of atlas-based and initial guess(η_0) priors. Analysis using linear (R^2) and model-independent, entropy-based (ρ^2) correlation confirmed that transmission and full-angle reconstructions were roughly equivalent, at least for this specific problem of distributed fluorophores. These observations are consistent with other simulation studies which demonstrated improved sensitivity to the rodent brain with transmission versus full-angle SD schemes (Xu *et al* 2003). Priors improved linear correlation and mutual information primarily for non-brain regions, with only a moderate improvement in brain voxel-to-voxel correlation (table 2). In general, reconstructed yield was poorly correlated with the input model yield, and thus individual voxels in high-resolution reconstructions (0.5 mm) did not contribute additional information beyond volume-averaged metrics.

The relatively poor correlation at the voxel level was in contrast to the strong linear correlation for global or volume-averaged fluorescence yield in the plaque-prone regions (see e.g. figure 3). Based on this observation, we hypothesized that a region-based, reduced-unknown inversion, utilizing atlas-generated tissue regions, would provide accurate yield estimates. We observed very good fluorescence yield estimates with region-based inversion of 144 SD pairs (12 sources and 12 detectors) over three regions (cortex, hippocampus and plaque-free brain) for a variety of amyloid burdens in the cortex and hippocampus. Thus, in the case of a relatively uniform target distribution across tissue regions (as simulated here), the non-uniform sensitivity of the tomography measurement could be circumvented by reducing the unknowns from tissue voxels to a few regions.

These results corroborate a recent study on combined FMT-CT imaging of amyloid plaques (Hyde *et al* 2009) using AO1987 (Hintersteiner *et al* 2005). Hyde *et al* used a region-based approach to generate average yield estimates for major brain regions, which were then input as soft spatial priors in a full-angle reconstruction. They observed strong linear correlation between average reconstructed cortical yield and the post-mortem average cortical fluorescence. However, qualitative analysis of the reconstructions revealed a yield distribution within the cortex (favoring high yield near the dorsal surface) which was inconsistent with the post-mortem tissue fluorescence. Based on our findings, it is likely that the strong average correlation demonstrated in the study by Hyde *et al* was primarily a result of the initial region-based inversion and not the sophisticated full-angle, high SD number measurement scheme.

We conclude that although reliable voxel-to-voxel correlation may not be achievable for tomography of distributed, sub-resolution targets in the brain, region-based average yield can be accurately estimated from a small number of measurements, given known anatomical regions. Mouse anatomical regions can be determined from a co-registered CT scans of the same animal (Hyde *et al* 2009), or might be obtained from a generic mouse atlas that is transformed to map onto each FMT-imaged mouse. In preliminary experiments, we have been able to map the Digimouse head atlas onto 3D boundaries of a mouse imaged in our FMT system using an affine transformation (data not shown). Others have shown that reconstructions from ‘hard’ anatomical priors, as in the region-based averaging presented here, are more susceptible to errors in the anatomical priors than reconstructions utilizing

‘soft’ priors (Yalavarthy *et al* 2007). Thus, future work is needed to quantify the accuracy of using a generic atlas for specifying tissue type.

In conclusion, we used a high-resolution model of a transgenic Alzheimer’s disease mouse to determine optimal NIR fluorophore parameters for imaging amyloid- β plaques. We found that fluorophores with 800 nm emission and higher plaque-to-background contrast would significantly improve amyloid detection beyond current amyloid imaging fluorophores. Simulated tomographic imaging of amyloid in transmission geometry demonstrated that large changes in amyloid could be detected tomographically, albeit with limited voxel-to-voxel correlation and artifact-degraded localization. Qualitative image reconstruction and voxel-to-voxel correlation was improved with the use of anatomical priors, but a full-angle measurement scheme did not perform significantly better than the canonical transmission scheme. Highly accurate region-based yield estimates were achievable by reducing the unknowns from all tissue voxels to a small number of anatomically distinct tissue regions.

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Figure 1.

Digital model of transgenic Alzheimer's mouse. (a) 3D rendering of digital mouse head with skull (gray), brain (red), cortex (green) and hippocampus (blue) segments. Arrows depict source (s) and detector (d) placement for fluorophore parameter simulations. (b) Coronal view through digital AD mouse model populated with plaques (10% by volume) in the cortex and hippocampus; tissue types (segments) are shown in grayscale. Arrow indicates source position for transmission geometry. (c) Sagittal view through model.

Figure 2.

System noise model. System noise was estimated by imaging a 0.3 mW, 650 nm laser source projected through a 1.9 cm-thick intralipid phantom. $n = 100$ images were taken to generate a mean and standard deviation (σ) for each pixel (data). The data were fit to a simple shot noise model, $\sigma^2 = \beta \bar{y} + \sigma_r^2$, with $\beta = 0.67$ and $\sigma_r^2 = 11.45$.

Figure 3.

Source and detector configuration for transmission (a), full-angle (b) and (c) reduced region-based measurement schemes. Sources are shown in red 'x' and detectors in black 'o'; arrows denote the source or detector orientation relative to the surface. (a) Transmission reconstructions used 25 sources in the ventral plane directed up and 36 detectors in the dorsal plane directed down (900 SD pairs). (b) Full-angle reconstructions used 5 sources and 30 detectors in transmission geometry, rotated every 60° about the principal axis to cover the entire mouse geometry (900 SD pairs). (c) Reduced region-wise reconstructions used 12 sources and 12 detectors in transmission (144 SD pairs).

Figure 4.

Fluorophore parameters and amyloid sensitivity. Fluorescence was simulated in the AD mouse model for different measurement geometries (transmission versus reflection), wavelengths (630 and 800 nm) and plaque-to-background contrast ratios (1, 2, 5, 10, 20, 50, 100, 1000). The simulation results were scaled to estimate actual CCD counts. (a) CCD counts for various properties. Plaque-to-background contrast ratio is printed over each plot (on left). 10, 20 and 30 dB SNR lines (dashed) were estimated using experimentally determined system noise statistics. (b) contrast-to-noise ratio (CNR) for the different measurement parameters.

Figure 5.

Fluorescence tomography for increasing amyloid burden. Near-infrared fluorescence tomography was simulated for increasing plaque burden (0–10%, shown along bottom) assuming an optimal NIR fluorophore with $CR = 20$ and emission at 800 nm. Measurements were taken for 900 SD pairs in transmission and reconstructed at 0.5 mm resolution using equation (6) without priors. Coronal (a–e) and sagittal (f–j) sections are shown relative to the input mouse model (a,f). Scale bar in (f) is 10 mm. The fluorescence yield ($\eta(\text{mm}^{-1})$) in the input model was $2.8 \times 10^{-3} \text{ mm}^{-1}$ in the brain and $5.3 \times 10^{-2} \text{ mm}^{-1}$ in plaques.

Figure 6.

Average reconstructed yield for plaque-prone regions. The reconstructed fluorescence yield was compared averaged over all plaque-prone and compared with the average input model fluorescence yield.

Figure 7.

Comparison of reconstructions using transmission or full-angle measurements. Transmission and full-angle simulated measurements were reconstructed at 0.5 mm using equation (6) and compared to the input model. (a,f) Input fluorescence yield model, mean-filtered and down-sampled to the reconstruction resolution, 0.5 mm. Fluorescence yield (η (mm⁻¹)) was 2.8×10^{-3} mm⁻¹ in the brain and 5.5×10^{-2} mm⁻¹ in plaques. (b,g) Transmission reconstructions without priors. (c,h) Transmission reconstructions with priors. (d,i) Full-angle reconstructions without priors. (e,j) Full-angle reconstructions with priors. Scale bar in (f) is 10 mm.

Figure 8.

Region-based fluorescence yield estimates. Fluorescence yield was estimated for non-plaque bearing brain, cortex and hippocampus using inversion of 144 measurements over a reduced, region-wise sensitivity matrix. 11 different scenarios were considered, with varying amyloid burden in the cortex (1, 5 or 10%) and hippocampus (1–10%). (a) Input geometry with selected regions outlined in white and labeled: b, brain; c, cortex; h, hippocampus. Scale bar is 1 cm. (b) Correlation between average input fluorescence yield with 'reconstructed' yield for three regions.

Table 1

Optical properties from the literature for different tissue types.

Tissue	Species	λ (nm)	μ_a (mm ⁻¹)	μ_s (mm ⁻¹)	g	References
Brain	Human	630	0.1000	40.0	0.900	(Yaroslavsky <i>et al</i> 2002)
Brain	Human	800	0.0178	12.5	0.900	(Barnett <i>et al</i> 2003)
Skull	Pig	650	0.0400	35.0	0.925	(Firbank <i>et al</i> 1993)
Skull	Human	800	0.0100	10.0	0.900	(Barnett <i>et al</i> 2003)
Skin	Human	633	0.0330	27.3	0.900	(Simpson <i>et al</i> 1998)
Skin (scalp)	Human	800	0.0149	8.0	0.900	(Barnett <i>et al</i> 2003)
Muscle	Human	633	0.1211	8.9	0.900	(Simpson <i>et al</i> 1998)
Muscle	Human	800	0.0240	4.0	0.900	(Kienle and Glatzmann 1999)

Semi-quantitative analysis of various reconstruction schemes. The correlation between the fluorescence yield in the input model (mean-filtered and down-sampled) and the reconstructions were estimated using linear regression (R^2) and model-independent, mutual information (ρ^2). Correlations were calculated for all tissue, brain or plaque-prone voxels. The average fluorescence yield ($\bar{\eta}$) was estimated for plaque-prone voxels and compared with the average yield in the plaque-prone regions of the input model, $\bar{\eta} = 0.0062 \pm 0.0014 \text{ (mm}^{-1}\text{)} (\pm\sigma)$.

Table 2

Reconstruction scheme	All tissue		Brain		Plaque-prone	
	R^2	ρ^2	R^2	ρ^2	R^2	$\bar{\eta} \pm \sigma \text{ (mm}^{-1}\text{)}$
Trans., w/o priors	0.5272	0.1827	0.5482	0.3398	0.2050	0.4260 0.0028 ± 0.0007
Full-angle, w/o priors	0.5238	0.2177	0.4043	0.3289	0.0676	0.4464 0.0031 ± 0.0008
Trans., w/priors	0.8087	0.4412	0.5107	0.3401	0.2850	0.4351 0.0047 ± 0.0016
Full-angle, w/priors	0.7521	0.4329	0.4331	0.3314	0.1788	0.4296 0.0042 ± 0.0012