10.1148/radiol.2018161041

Appendix E1

Methods

Subjects

All eligible breast cancer patients that fulfilled the necessary requirements were offered the option to participate in this study. 40 out of 44 patients approached agreed to participate. 6 of the patients who initially agree to participate, were eventually excluded because they either chose not to take part in the 2nd imaging session or due to staffing or technical difficulties. Therefore, either the optical imaging machine was not operational or no operator for the imaging system was available at the time of the first imaging session.

A detailed list with clinical information for all 40 subjects enrolled in this study is provided in Table E1. Given are the age, body mass index (BMI), the menopausal status, the type of tumor as determined by pathology, the stage at time of diagnosis, the location of the tumor (left or right breast), ER and PR readings, HER2 status, tumor dimension as determined by palpation at time of diagnosis, and the patient's RCB class at the end of the study, as determined by pathology just before surgery. Table E2 provides additional summary information for the 34 patients that concluded the study.

Imaging System

The optical tomographic imaging system employs light at four different wavelengths (λ = 765 nm, 808 nm, 835 nm, and 905 nm), which is guided to each breast through 32 sources fibers. For each breast the transmitted light is collected by 64 optical fibers that guide the light to 64 Si-photodetectors (64 sources and 128 detectors in total). The detection unit utilizes lock-in detection techniques to decrease noise (14,37). The patient interface consists of two sets of four concentric rings that hold the source and detector fibers. The fibers are placed around the rings in a source-detector-source-detector pattern. The largest ring can be removed to accommodate smaller breast sizes. The ring sets have been placed on camera mounts so that each ring set can move in different angles to provide the most comfortable settings for the patients. Meshes used in the image reconstruction are generated from the known geometry of these rings. During an imaging session, a woman will be seated in front of the system leaning forward to place her breast into the imaging interface (see Fig E1).

Image Reconstruction Algorithm

The raw data were preprocessed by applying a discrete cosine transform filter that uses the first 15 coefficients to reduce high-frequency noise. In a second step, the data belonging to source-detector pairs that showed a signal to noise ratio of less than 10 dB was removed. The resulting data set was input to a diffusion-theory based PDE-constrained multispectral imaging algorithm (38). This algorithm produced 3-dimensional volumetric images of various tissue parameters of both breasts. In this study, we focused on the percent change of oxy-hemoglobin concentration ($\%\Delta[HbO_2]$) and percent change of deoxy-hemoglobin concentration ($\%\Delta[Hb]$) with respect to

values observed at baseline. The baseline values were obtained as the average signal level over a period of 30 seconds prior to a patient's breath hold.

The mesh needed for the reconstructions were generated specifically for each individual subject. The settings for the measurement rings in the imaging interface were recorded prior to the start of the imaging session and subsequently used to generate the patient specific meshes. The meshes extend 2 cm past the largest ring to account for the effects that the chest wall might have on the reconstruction. Each mesh contained approximately 50,000 to 70,000 elements.

Image Analysis and Quantification

Based on reconstructed 3D imaging of the two parameters (%Δ[Hb] and %Δ[HbO2]) for both breasts, we assessed the response to NAC. Since time-dependent dynamic data were obtained during a breath hold, we first focused on time varying features inside the tumor. To identify the area that contains the tumor in the tumor-bearing breast, we average each voxel of the full three-dimensional tomographic data set over the period of the breath hold and half the recovery time (Fig E2, *A*). This results in a single frame of 3D data (Fig E2, *B*). Within this single frame we determine the location $\vec{x}_{max} = (x_{max}, y_{max}, z_{max})$ of the maximum percentage change in deoxyhemoglobin ($\%\Delta[Hb]_{max}$) and the value of half this change, defined as HM = $\%\Delta[Hb]_{max}/2$. All voxels within the vicinity of $\vec{x}_{max}$ for which $\%\Delta[Hb] \geq$ HM are defined as part of the "volume of interest" (VOI) that contains the region with the tumor (Fig E2, *B*). Then for each time point *t* of the original breath-hold data, the %Δ[Hb] values of each voxel within the VOI is averaged to get a single data point $S_{Hb}(t) = \%\Delta[Hb]_{avg}(t)$. This value will change from time point to time point and a time-dependent curve $S_{Hb}(t)$ can be plotted (Fig E2, *C*). The same process was used to determine $S_{HbO2}(t) = \%\Delta[HbO_2]_{avg}(t)$.

In the next step, we tried to identify dynamic features for the volume of interest (VOI). Figure E2, *C*, shows a plot of the time-dependent S_{Hb} values for the VOI in one of our subjects (see also Fig 2c and Fig 3c in the main text). One can see how the S_{Hb} increases before a maximum is reached. Once the patient starts breathing again S_{Hb} decreases.

Feature Calculation

Initial enhancement (IE) is a parameter often used in functional magnetic resonance imaging (MR imaging). It is a measure of how an MR imaging contrast agent increases in signal intensity after injections (39). We adapted this parameter for DDOT by defining it as the ratio of the difference between the peak signal strength, S_{peak}, and the initial minimal signal before the peak $S_{initial}$, divided the peak signal strength (see Fig E3):

$$IE = \frac{S_{peak} - S_{initial}}{S_{peak}} \quad (1).$$

The ***post-initial enhancement (PIE)*** is another parameter employed in contrast-enhanced MR imaging. It is used to determine how fast a contrast agent leaves a region of interest in DCE-MR imaging. For DDOT we define the PIE as

$$PIE = \frac{S_{postBH\ 15\ sec} - S_{peak}}{S_{peak}} \quad (2).$$

In this equation $S_{postBH15sec}$ is the [THb] value 15 seconds post the breath hold mark. In previous studies we found that this value leads to biggest differences between nontumor bearing and tumor bearing breast (15). The post-15-second value was determined by using the value in the trace 15 seconds after the peak and normalizing it by the value of the peak.

The ***rise slope, m_{rise},*** was found by using the formula for slope between two points, where the first point was the minimum value before the peak and the second point was the peak point. The ***fall* slope, m_{fall},** was similarly found using the peak as the first point and the post-15 second point as the second. A visualization of the above features can be seen in Figure E3.

The ***rise rate, q_{rise},*** was determined by fitting the rising portion of the curve to an exponential and taking the rate coefficient by looking at the section between the minimum values before the peak to the peak value. Similarly, the ***washout rate, q_{fall},*** was determined by fitting the recovery portion of the breath hold trace to an exponential and taking the rate coefficient. The recovery portion was defined from the maximum point of the trace to the following minimum position. These portions of the curve were fitted to the following equations:

$$S_X = A_{rise} * e^{q_{rise} * t}$$
$$S_X = A_{fall} * e^{-q_{fall} * t} \quad (3),$$

where A_{rise} and A_{fall} were amplitude coefficients, t was the time that has passed in seconds, and S_X is either S_{Hb} or S_{HbO2}. The parameters q_{rise} and q_{fall} were then used in the current analysis.

The ***normalized maximum peak value (NMPV),*** was calculated using the ***peak value of the tumor trace***, S_{peak}, normalized by the peak value of the healthy breath hold trace. Furthermore, we calculated the ***correlation coefficient (CC)*** between the time traces observed in the healthy and tumor bearing breasts between the corresponding VOI.

Table E3 shows the Pearson correlation coefficients for all 9 features that were used to describe the two signal curves $S_{Hb}(t)$ and $S_{HbO2}(t)$. Note the particularly strong correlation between Hb and HbO_2 pairs.

Results

In addition to the results shown in Tables 2 and 4, we provide here (Fig E4) the related ROC curves for 6 features used to distinguish between 3 differently grouped RCB classes. Two ROC curves to distinguish between classes RCB 0 and RCB I,II,III are shown for feature q_{fall} and Δm_{fall} (left most column). Another two ROC curves to distinguish between classes RCB 0, I and RCB II,III are shown for feature m_{fall} and Δm_{fall} (middle column). Finally two ROC curves to distinguish between RCB 0,I,II from RCB III are shown for feature IE and ΔIE (right column).

Discussion of Related Studies

Given the noninvasive character of this imaging modality and its sensitivity to blood-dependent parameters and tissue vascularity, several groups have started to evaluate DOT or nontomographic diffuse optical spectroscopy (DOS) for monitoring response to NAC. For example, Ueda et al correlated the pCR rates to baseline tumor oxygen saturation measurements obtained with a handheld spectroscopic measurement system (20). The handheld probe had only one source and one detector that were scanned across a region of interest to take both frequency-domain and steady-state measurements. No optical tomographic reconstruction of the entire

breast was performed. Other groups used tomographic breast imaging systems (21–25). This includes the Zhu group at the University of Connecticut, who performed ultrasound guided DOT NAC monitoring studies (26–30), and Dr. Yodh's team at UPENN, who performed a pilot study in 3 patients undergoing NAC. They used a combined frequency and continuous-wave system that employed two parallel plates to hold one of the breasts in place inside a "breast box." The box was filled with a solution of Intralipid and India Ink, which acted as matching fluid and reference medium (24,25). Several groups (18,21–23,26,28–35) reported that [HbT] levels seem to significantly decrease in patients that show a pCR, in which the changes could occur as early as a few days within commencement of the NAC. As a result, it was suggested that DOT could be used to predict treatment outcome. A somewhat different observation was made by Soliman et al and Falou et al. Employing DOS, they found an initial increase followed by a decrease in [Hb] and [HbT] during the first four weeks of treatment in patients that achieved a pCR after 6 months of NAC (32,34). However, it should be noted that Fallou et al did not calculate RCB scores and used a different criterion to identify pCR patient. Using that criterion they report on 100% sensitivity and specificity when considering [Hb] increases at 4 weeks after treatment initiation ($P < .002$, analyzing a total of 10 features).

Table E1: List with Clinical Information for All 40 Subjects Enrolled in This Study

Age	BMI	Menopausal Status	Type	Stage at diagnosis	Location	ER	PR	HER2	Tumor Size [cm]	RCB Class
47	27.32	Pre	IDC	T2 N1 M0 (Stage IIB)	Right	0	0	pos	3.1	0
66	29.1	Post	IDC	T1c N1 M0 (Stage IIA)	Right	0	0	pos	2.1	0
45	32.07	Pre	IDC	T3 N1 M0 (Stage IIIA)	Left	5	10	neg	12	0
46	31.98	Post	IDC	T2 N1 M0 (Stage IIB)	Left	2	3	pos	5.5	0
54	32.76	Pre	IDC	T2 N1 M0 (Stage IIB)	Left	5	80	pos	3	0
55	34.78	Post	IDC	T2 N1 M0 (Stage IIB)	Left	0	0	pos	2.2	0
29	27.12	Pre	IDC	T2 N1 M0 (Stage IIB)	Left	2	5	pos	2	0
29	32.23	Pre	IDC	T2 N1 M0 (Stage IIB)	Left	0	5	pos	3	0
65	29.87	Post	IDC	T2 N1 M0 (Stage IIB)	Left	0	0	neg	3.5	0
56	38.83	Post	IDC	T2 N1 M0 (Stage IIB)	Right	75	90	pos	1.7	0
62	29.9	Post	IDC	T1 N1 M0 (Stage IIA)	Left	0	0	neg	1.1	0
41	20.07	Post	IDC	T2 N0 M0 (Stage IIA)	Left	0	0	neg	2.6	0
41	23	Pre	IDC	T2 N1 M0 (Stage IIB)	Left	75	0	neg	2.4	0
31	32.4	Pre	IDC	T1c N1 M0 (Stage IIA)	Left	0	0	neg	2	1
30	22.47	Pre	IDC	T1c N1 M0 (Stage IIA)	Right	30	90	pos	3	1
33	23.9	Pre	IDC	T2 N0 M0 (Stage IIA)	Right	0	0	neg	5.6	2
60	26.95	Pre	IDC	T2 N1 M0 (Stage IIB)	Right	100	100	neg	5.5	2
52	25.4	Pre	IDC	T2 N0 M0 (Stage IIA)	Left	15	50	pos	5	2
60	34.8	Post	ILC	T3 N0 M0 (Stage IIB)	Left	100	51	neg	7	2
40	34.55	Pre	IDC	T2 N1 M0 (Stage IIB)	Left	99	10	pos	5	2
59	28.34	Post	ILC	T3 N0 M0 (Stage IIB)	Right	90	15	neg	6	2
61	27.49	Post	IDC	T3 N1 M0 (Stage IIIA)	Left	5	0	neg	10.5	2
55	31.65	Post	IDC	T1 N1 M0 (Stage IIA)	Left	50	50	neg	2	2
46	20.66	Pre	IDC	T2 N1 M0 (Stage IIB)	Left	70	50	pos	3.8	2
62	22.1	Post	IDC	T2 N1 M0 (Stage IIB)	Right	0	0	neg	3.5	2
49	43.98	Pre	IDC	T1c N1 M0 (Stage IIA)	Right	95	90	neg	4.4	2
50	31.31	Post	IDC	T3 N1 M0 (Stage IIIA)	Left	85	50	neg	5.2	2
36	29.4	Pre	IDC	T3 N2 M0 (Stage IIIA)	Left	95	90	neg	2	2
73	25.69	Post	IDC	T2 N1 M0 (Stage IIB)	Left	100	15	neg	3	3

50	18.3	Pre	IDC	T2 N1 M0 (Stage IIB)	Right	100	50	pos	3.7	3
61	43.07	Post	ILC	T3 N1 M0 (Stage IIIA)	Left	90	50	neg	9.5	3
47	40.12	Pre	IDC	T3 N3b M0 (Stage IIIC)	Left	0	5	neg	7	3
33	29.97	Pre	IDC	T3 N1 M0 (Stage IIIA)	Left	99	99	neg	7	3
40	28.16	Pre	ILC	T2 N1 M0 (Stage IIB)	Right	96	99	neg	10	3
Excluded										
53	31.8	Post	IDC	T2 N1 M0 (Stage IIB)	Left	0	5	neg	7.5	N/A
40	30.18	Post	IDC	T4b N3 M0 (Stage IIIC)	Right	<1	10	neg	7	N/A
42	23.32	Pre	IDC	T2 N1 M0 (Stage IIB)	Right	99	80	neg	3.2	0
43	26.81	Post	IDC	T3 N1 M0 (Stage IIIA)	Right	0	0	neg	2.6	0
52	26.81	Pre	IDC	T2 N1 M0 (Stage IIB)	Right	90	90	neg	8	2
66	31.28	Post	IDC	T2 N1 M0 (Stage IIB)	Left	100	95	neg	3.5	2

Given are the age, body mass index (BMI), the menopausal status, the type of tumor as determined by pathology, the stage at time of diagnosis, the location of the tumor (left or right breast), ER and PR readings, HER2 status, tumor size as determined by palpation at time of diagnosis, and RCB class at the end of the study, as determined by pathology just before surgery.

Table E2: Clinical and Pathologic Features

	Average ± SD			Range
Age (Years)	49 ± 12			(29–73)
BMI	29.9 ± 6.1			(18.3–44)
Tumor Size (cm)	4.5 ± 2.7			(1.1–12)
Menopausal State	Pre-Menopausal			Post-Menopausal
Number of Subjects	19			15
Invasive Cancer Histology	Ductal	Lobular		Mixed Ductal & Lobular
Number of Subjects	29	4		1
RCB Class	RCB-0	RCB-I	RCB-II	RCB-III
Number of Subjects (Percent of subject population)	13 (38.2%)	2 (5.9%)	13 (38.2%)	6 (17.7%)

BMI = body mass index, RCB = residual cancer burden.

Table E3: Pearson Correlation Coefficients for 9 Parameters That Describe $S_{Hb}(t)$ and $S_{HbO2}(t)$

Pearson Correlation	m_{rise_Hb}	m_{rise_Hb02}	m_{fall_Hb}	m_{fall_Hb02}	q_{rise_Hb}	q_{rise_Hb02}	q_{fall_Hb}	q_{fall_Hb02}	IE_{Hb}	IE_{HbO2}	PIE_{Hb}	PIE_{Hb02}	S_{peak_Hb}	S_{peak_Hb02}	$NMPV_{Hb}$	$NMPV_{Hb02}$	CC_{Hb}	CC_{Hb02}
m_rise_Hb	1	0.944**	–0.482**	–0.478**	0.339*	0.23	0.064	–0.046	0.365*	0.328	0.162	0.201	0.674**	0.662**	0.228	0.225	–0.22	–0.326
m_rise_Hb02	0.944**	1	–0.525**	–0.541**	0.226	0.199	0.072	–0.1	0.388*	0.357*	0.137	0.218	0.720**	0.772**	0.04	0.037	–0.234	–0.314
m_fall_Hb	–0.482**	–0.525**	1	0.829**	–0.137	–0.154	–0.429*	–0.208	–0.294	–0.269	0.500**	0.520**	–0.285	–0.364*	0.047	0.046	0.132	0.19
m_fall_Hb02	–0.478**	–0.541**	0.829**	1	0.01	0.016	–0.365*	–0.147	–0.319	–0.205	0.346*	0.448**	–0.278	–0.541**	0.083	0.082	0.04	0.077
q_rise_Hb	0.339*	0.226	–0.137	0.01	1	0.854**	–0.03	–0.038	0.323	0.324	–0.032	0.024	–0.061	–0.101	0.546**	0.547**	–0.082	–0.053
q_rise_Hb02	0.23	0.199	–0.154	0.016	0.854**	1	–0.058	–0.049	0.318	0.335	0.044	0.04	–0.103	–0.135	0.355*	0.357*	–0.189	–0.148
q_fall_Hb	0.064	0.072	–0.429*	–0.365*	–0.03	–0.058	1	0.750**	0.439**	0.382*	–0.707**	–0.632**	–0.223	–0.132	–0.152	–0.144	–0.092	–0.064
q_fall_Hb02	–0.046	–0.1	–0.208	–0.147	–0.038	–0.049	0.750**	1	0.408*	0.486**	–0.375*	–0.689**	–0.343*	–0.338	–0.144	–0.14	0.083	0.077
IE_Hb	0.365*	0.388*	–0.294	–0.319	0.323	0.318	0.439**	0.408*	1	0.934**	–0.109	–0.092	–0.095	0.028	–0.026	–0.024	–0.067	–0.009
IE_Hb02	0.328	0.357*	–0.269	–0.205	0.324	0.335	0.382*	0.486**	0.934**	1	–0.024	–0.117	–0.07	–0.033	–0.043	–0.043	–0.091	–0.047
PIE_Hb	0.162	0.137	0.500**	0.346*	–0.032	0.044	–0.707**	–0.375*	–0.109	–0.024	1	0.753**	0.352*	0.235	0.078	0.065	–0.067	–0.084
PIE_Hb02	0.201	0.218	0.520**	0.448**	0.024	0.04	–0.632**	–0.689**	–0.092	–0.117	0.753**	1	0.410*	0.31	0.111	0.102	–0.155	–0.13
S_{peak}_Hb	0.674**	0.720**	–0.285	–0.278	–0.061	–0.103	–0.223	–0.343*	–0.095	–0.07	0.352*	0.410*	1	0.893**	–0.035	–0.043	–0.149	–0.272
S_{peak}_Hb02	0.662**	0.772**	–0.364*	–0.541**	–0.101	–0.135	–0.132	–0.338	0.028	–0.033	0.235	0.31	0.893**	1	–0.086	–0.091	–0.106	–0.193
NMPV_Hb	0.228	0.04	0.047	0.083	0.546**	0.355*	–0.152	–0.144	–0.026	–0.043	0.078	0.111	–0.035	–0.086	1	0.999**	0.038	–0.013
NMPV_Hb02	0.225	0.037	0.046	0.082	0.547**	0.357*	–0.144	–0.14	–0.024	–0.043	0.065	0.102	–0.043	–0.091	0.999**	1	0.038	–0.012
CC_Hb	–0.22	–0.234	0.132	0.04	–0.082	–0.189	–0.092	0.083	–0.067	–0.091	–0.067	–0.155	–0.149	–0.106	0.038	0.038	1	0.910**
CC_Hb02	–0.326	–0.314	0.19	0.077	–0.053	–0.148	–0.064	0.077	–0.009	–0.047	–0.084	–0.13	–0.272	–0.193	–0.013	–0.012	0.910**	1

Note the very strong correlation between Hb and HbO_2 pairs.

* Denotes that correlation is significant at the 0.05 level (2-tailed).

** Denotes that correlation is significant at the 0.01 level (2-tailed).