ACCSCIENCE PUBLISHING

Tumor Discovery

ORIGINAL RESEARCH ARTICLE

Malignant versus normal breast tissue: Optical differentiation exploiting hyperspectral imaging system

Supplementary File

Table S1. Regular diagnostic and identification approaches of breast cancer

Approach	Type of practice	Sensitivity	Specificity	Shortcomings	Time taken
X-ray mammography[77,78]	• Lump screening • Imaging for the soft tissues, blood vessels, and bone density bone instant • Identifying dense tissues	~67.8%	~75%	• Exposure to ionizing radiation • Minimum sensitivity and specificity especially in highly dense tissues	Limited seconds. A few seconds
Ultrasound[79,80]	Capable to assess masses from mammography, but not proper to recognize bones	~83%	~34%	• Qualified user required • Minimum sensitivity • Low image resolution	10~20 min
Magnetic resonance imaging[2,81]	Proper for young females with superior risk, and capable of recognizing tiny soft tissues	~94.4%	~26.4%	• Unable to identify some breast cancers (lobular and ductal carcinoma) • Expensive	40~60 min
Positron emission tomography[6,19]	Proper for organ function and biological procedures in therapy or metastasis	~91%	~93%	• Exposure to ionizing radiation • emitting radiation • Exposure to ionizing radiation which could affect (pregnancy/breastfeeding). • Causing an allergic reaction (very rare and minor).	1.5~4 h
Computerized tomography[14]	To settle and image distant metastasis in a particular investigation	~61%	~80%	• Radiation hazard • Minimum sensitivity • Expensive	~5 min

Sensitivity and specificity varied with respect to the breast cancer type, stage, category, and structure.

Table S2. Characteristics of breast cancer patients investigated in this study

No.	Patient ID	Laboratory no.	Breast type[a]	Gender[b]	Age	Specimen size	Tumor size	Tumor grade[c]	Histology type of cancer[d]	Pathological TNM stage[e]
1	1001	154543	Type C	F	56	9×8 × 3.5 cm	4×2.5×1.5 cm	Garde I	IDC	T2/N3/M_x
2	1002	152567	Type D	F	69	10×8.5×5 cm	4×3 × 2.5 cm	Garde II	IDC	T2/N1/M_x
3	1003	407153	Type C	F	49	17×15×3 cm	1.3×1 × 1 cm	Garde III	IDC	T2/N1/M_x
4	1004	ZK4900-22	Type C	F	60	24×16×9 cm	14×7.5 cm	Garde II	IDC	T4/N3/Ro
5	1005	ZK5435-22	Type D	F	94	14×10×8.5 cm	0.6×0.5 cm, 1.5×1 cm and 2.5×2.5 cm	Garde II	Multifocal IDC	T2/Nx
6	1006	152513	Type B	F	69	10.5×6 × 4.5 cm	4×2.5×1.5 cm	Garde I	IDC	T2/N3/M_x
7	1007	162568	Type D	F	49	9.5×8.5×5 cm	4×3 × 2.5 cm	Garde II	IDC	T2/N1/M_x
8	1008	467151	Type B	F	54	12×15×3 cm	1.3×1 × 1 cm	Garde III	IDC	T2/N1/M_x
9	1009	153511	Type B	F	50	24×16×9 cm	14×7.5 cm	Garde II	IDC	T4/N3/Ro
10	1010	182578	Type D	F	46	17×15×3 cm	1.3×1 × 1 cm	Garde III	IDC	T2/N1/M_x
11	1011	567152	Type B	F	51	16×11×6 cm	6×5.5 cm	Garde II	IDC	T4/N3/Ro
12	1012	ZK4611-12	Type B	F	53	12×9 × 4.5 cm	3.5×3.5×2.5 cm	Garde II	IDC	T3/N3/M_x
13	1013	ZK3635-32	Type C	F	56	11×8.5×5 cm	4×3.5×2.5 cm	Garde III	IDC	T2/N2/M_x
14	1014	132520	Type D	F	58	18×13×3.5 cm	2.3×1.5×2 cm	Garde I	IDC	T3/N1/M_x
15	1015	112567	Type C	F	60	21×14.5×9 cm	2.5×3.5×4.5 cm	Garde II	IDC	T4/N3/Ro
16	1016	367132	Type C	F	55	21×12×6.5 cm	6.5×5.5 cm	Garde II	IDC	T4/N3/Ro
17	1017	123611	Type C	F	59	18×9 × 3.5 cm	2.5×5.5×1.5 cm	Garde II	IDC	T4/N3/Ro
18	1018	192471	Type C	F	72	12×8.5×5 cm	4×2.5×2.5 cm	Garde II	IDC	T2/N3/M_x
19	1019	267132	Type C	F	69	17×13×3.5 cm	2.3×1.5×2 cm	Garde II	IDC	T4/N3/Ro
20	1020	167511	Type C	F	67	16×11×9.5 cm	2.5×3.5×4.5 cm	Garde II	IDC	T2/N3/M_x
21	1021	167571	Type B	F	59	9.5×6 × 3.5 cm	3.5×2.5×2.5 cm	Garde I	IDC	T4/N3/M_x
22	1022	257111	Type D	F	45	6.5×6.5×3.5 cm	4.5×3 × 2.5 cm	Garde II	IDC	T2/N1/M_x
23	1023	ZK6611-01	Type B	F	52	11×11×5.5 cm	3.5×1 × 2.5 cm	Garde III	IDC	T2/N1/M_x
24	1024	ZK3985-02	Type B	F	53	20×14×7.5 cm	11×5.5 cm	Garde II	IDC	T4/N3/Ro
25	1025	122541	Type D	F	49	18×14×3.5 cm	6.5×2.5×2.5 cm	Garde III	IDC	T2/N1/M_x
26	1026	112432	Type B	F	55	19×12×6.5 cm	6.5×5.5 cm	Garde II	IDC	T4/N3/Ro
27	1027	367326	Type B	F	65	16×9 × 4.5 cm	2.5×3.5×1.5 cm	Garde II	IDC	T3/N3/M_x
28	1028	123987	Type C	F	59	10×8.5×5 cm	4×3.5×2.5 cm	Garde III	IDC	T2/N2/M_x
29	1029	192745	Type D	F	58	17×13×3.5 cm	2.3×1.5×2 cm	Garde I	IDC	T3/N1/M_x
30	1030	267102	Type C	F	60	16×11×9.5 cm	2.5×3.5×4.5 cm	Garde II	IDC	T4/N3/Ro

[a]Breast type according to the American College of Radiology (ACR): Type A, fatty breast; Type B, scattered density breast; Type C, heterogeneously density breast; and Type D, extremely density breast.
[b]Gender: F, female; M, male.
[c]Tumor grade: Grade I, well differentiated; Grade II, moderately differentiated; and Grade III, hard to be differentiated.
[d]Histology type of cancer: IDC: Invasive ductal carcinoma; DCIS: Ductal carcinoma *in situ*; TNBC: Triple-negative breast cancer; IBC: Inflammatory breast cancer.
[e]Pathological TNM Stage (refers to tumor, node, and metastasis): T_X represents that the cancer size cannot be evaluated; T1 represents that the cancer size is ≤ 2 cm diameter; T2 represents that the cancer size is 2~5 cm in diameter; T3 represents that the cancer size is ≥ 5 cm in diameter; N_X represents that the lymph node cannot be evaluated; N1 indicates cancer cells in 1 to 3 lymph nodes; N2 indicates cancer cells in 4 to 9 lymph nodes; and N3 indicates cancer cells ≥ 10 lymph nodes.

Table S3. Novel techniques in breast cancer investigation exploiting the HSI

Author	Type of investigation	Sample	Light source	Spectrum range (nm)	Spatial image size (pixels)	Acquisition mode	Study objective
McCormack[5,68]	*In vivo*	Mice	Halogen	500 ~ 600	1040×1392	LCTF	Segmentation and classification of SO_2 microvessels
Aref[47,67]	*Ex vivo*	Human	Halogen 20 W	400 ~ 1000	696×520	Silicon/CCD (traditional line scanning or internal scanning modes)	Discrimination between normal and malignant breast cancer tissue
Pourreza-Shahri[68,70]	*Ex vivo*	Human	Xenon	380 ~ 780	768×1024	--	Improvement of a classification algorithm for identification of breast cancer margins measured by HSI
Aref[37,69]	*Ex vivo*	Human	Halogen 20 W+ Monochromatic LEDs (415,565,660)	400 ~ 1000	696×520	Silicon/CCD (traditional line scanning or internal scanning modes)	Proposing a commercial low-cost optical imaging system to assist the surgeons in the BCS
Kim[69,70]	*Ex vivo*	Human	Xenon	380 ~ 780	--	--	Automated extraction of the ROI for the breast cancer from the HS images
Sterenborg[71]	*Ex vivo*	Human	Halogen	900 ~ 1700	Pixel size=30×30 μm Pixel image=320×256	--	Investigation of the fresh resection specimen from 1 mm to ≥ 1 cm with respect to the wavelength variation
Ortega[72]	*Ex vivo*	Human	Halogen (12 V, 100 W)	400 ~ 1000	826 spectral channels and 1004 spatial pixels and 20×magnification	Silicon/CCD (traditional line scanning)	HSI is attached with a microscope to discriminate between the normal and tumor (breast cancer) cells exploiting the deep learning neural network on the HS images.

BCS: Breast-conserving surgery; CCD: Charge-coupled device; HSI: Hyperspectral imaging; LCTF: Liquid crystal tunable filter; ROI: Region of interest.

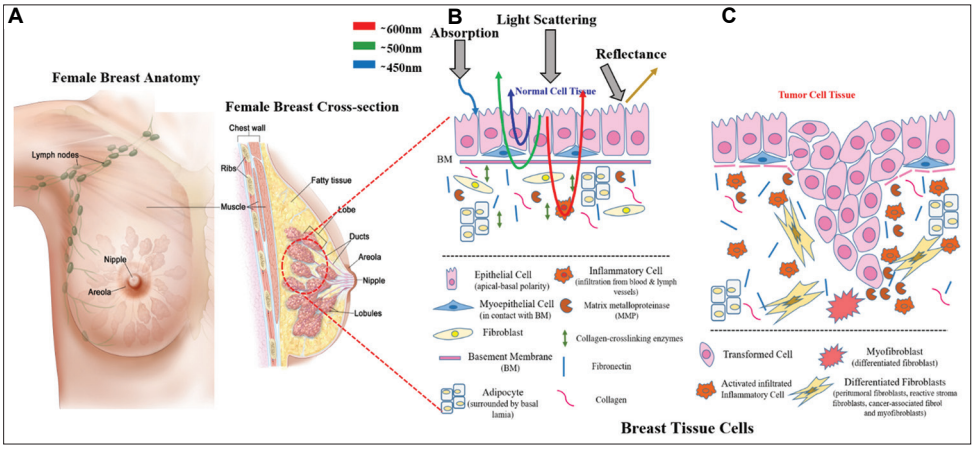

Figure S1. (A) The female breast anatomy illustrating its position in the female body and the location of the lymph nodes; the cross-section view highlighting muscle tissues, fatty tissues, lobular ducts, areola, and nipple. (B) The light interaction with the normal breast tissue highlighting the variation of light penetration depth regarding the wavelength (red, green, and blue wavelengths). (C) The abnormal breast tissue (tumor) with cell tissue variations, which lead to variation of the tissue's optical properties and its interaction with light.

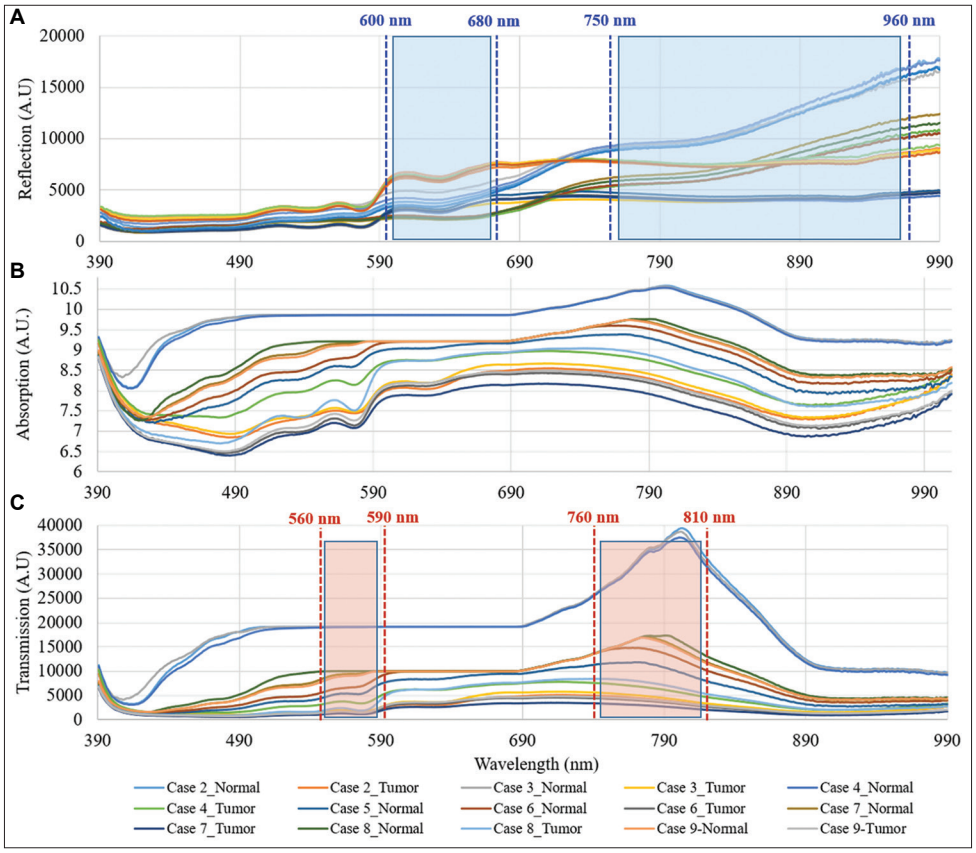

Figure S2. (A) The measured diffuse reflection (R_d) regarding the first approach (reflection) for both the normal and tumor of the whole investigated *ex vivo* breast samples over the spectrum range of 400 – 1000 nm highlighting the peaks which could visually identify between both normal and tumor tissues at wavelengths of 600 ~ 680 nm and 750 ~ 960 nm at the visible and near-infrared (VIS and NIR) spectra, respectively. (B) The measured T_r regarding the second approach of both the normal and tumor of the investigated *ex vivo* breast samples over the spectrum range of 400 – 1000 nm. (C) The sample absorption coefficient (μ_a) from the T_r Measurements regarding the second approach of both the normal and tumor of the investigated *ex vivo* breast samples over the spectrum range of 400 – 1000 nm highlighting the peaks which could visually identify between both normal and tumor tissues at wavelengths of 560 ~ 590 nm and 760 ~ 810 nm at the VIS and NIR spectra, respectively.