Supplementary Information

Design and fabrication of nanoengineered Pt electrodes by laser welded CNTs for electrochemical biosensing of cancer lymph nodes

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The mechanisms of tumor invasion to LNs are still unclear. Recent research indicated metabolic shift from hypoxia glycolysis toward FAO in cancer cells when they invade LNs [1]. Hence, our diagnostic approach is tracing FAO process by electrochemical recording the lipidic contents of the free and involved LNs.

Fundamentals of FAO metabolism of malignant cells in LNs were presented in figure S1. FAs consumption by cancer cells could be done by either direct exogenous uptake from the nearby microenvironment or using nutrients (such as glucose or glutamine) to synthesize *de novo*. Alterations in FA transport, *de novo* lipogenesis, storage as lipid droplets (LDs), and β-oxidation to generate Adenosine Triphosphate (ATP) are metabolisms of cancerous cells all known as lipidomic remodeling [2 3].

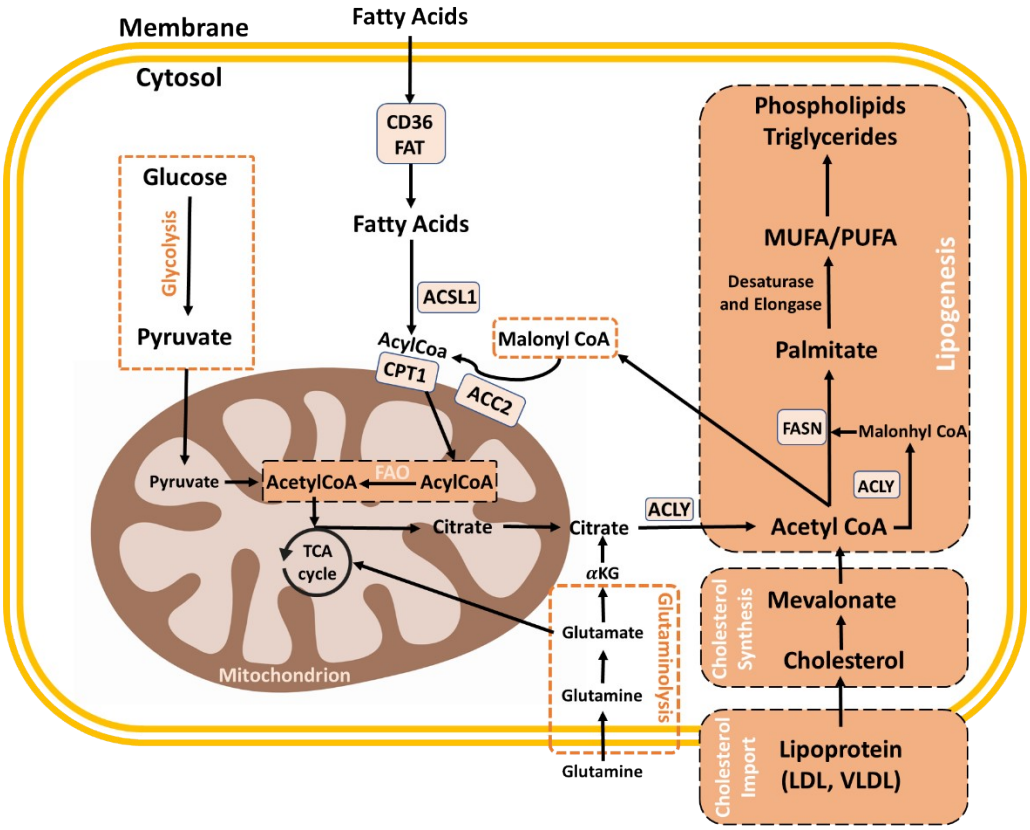

Figure S1, *Cancer cells obtain fatty acids (FAs) in two main ways, direct exogenous uptake and de novo lipogenesis of them. There are different transporters that allow exogenous uptake of FAs from the surrounding microenvironment. CD36, FATPs and FABPpm are the most important ones. Abbreviations: CD36, Cluster of Differentiation 36; ACSL1, Acyl-CoA Synthetase Long-chain family member 1; CPT1, Carnitine Palmitoyltransferase 1; ACC, acetyl-CoA carboxylase; TCA, Tricarboxylic Acid Cycle; LDL, Low-Density Lipoproteins; VLDL, Very Low-Density Lipoprotein; ACLY, ATP–citrate lyase; FASN, fatty acid synthase; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.*

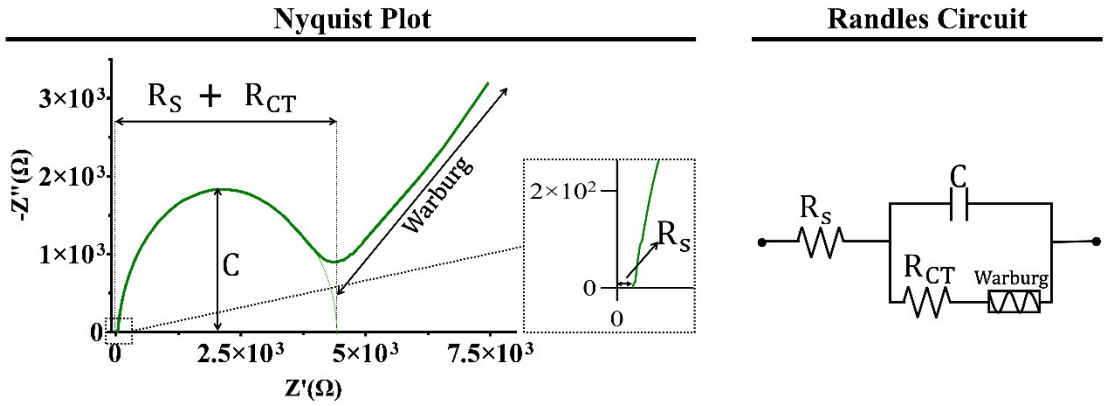

Figure S2, *Nyquist diagram. LDP response is a nyquist diagram which its equivalent circuit is of importance to analyze the data.* R_{CT}*, charge transfer resistance, is the most important element of an equivalent circuit.* R_S *(solution resistance), C (double layer capacitance), and Warburg (diffusion element) are the other elements of the equivalent circuit.*

80 ***Table S1,*** *Principal demographic characteristics of the patients, the origin of disease, number of dissected LNs, IHC*
81 *tumor markers, and serum tumor markers.*

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	Patient ID#	1	2	3	4	5	6	7	8	9	10	11
	Age	58	31	46	42	44	352	51	42	44	32	39
	Height (m)	1.62	1.59	1.50	1.67	1.73	1.61	1.74	1.58	1.52	1.65	1.62
	Weight (Kg)	69	72	60	68	108	86	73	81	89	93	88
	Sex	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female	Female
	Cancer Type	MetastaInvasive Ductal Carcinoma	Invasive Ductal Carcinoma	Invasive Ductal Carcinoma	Invasive Ductal Carcinoma	Invasive Ductal Carcinoma	Invasive Ductal Carcinoma	Invasive Ductal Carcinoma	Invasive Ductal Carcinoma	Invasive Ductal Carcinoma	Invasive Ductal Carcinoma	Invasive Ductal Carcinoma
	Primary Tumor Site	Right Breast; 4-5 O'clock Retroareola.	Left Breast Mass; 11 O'clock	Left Breast Mass; Upper Outer Quadrant	Right Breast Mass, 9-10 O'clock	Right Breast; 12 O'clock	Right Breast Mass; Mid Zone	Right Breast Mass, 10 O'clock	Right Breast	Left Breast Mass; 2 O'clock	Left Breast Mass; Upper Outer Quadrant	Left Breast Mass; 3-5 O'clock
	Tumor Size (Largest Tumor)	19×19×5.5 mm^3	42×17 mm^2	40×25 mm^2	30×11 mm^2	18×8 mm^2	21×11 mm^2	26×20×17 mm^3	40×40 mm^2	26×25 mm^2	34×25 mm^2	26×19×12 mm^3
	Lymph Node Sentinel	1	1	1	2	1	1	1	1	2	2	1
	Lymph Node Non-Sentinel	2	1	1	0	3	2	1	1	0	1	3
IHC Tumor Markers	HER2/neu	Positive (Score 3+)	Negative	Negative	Positive (Score 3+)	Negative	Negative	Negative (Score 1+)	Positive (Score 2+); 10%	Positive (Score 3+); 90%	Positive (Score 2+)	Positive (Score 3+)
	ER	Positive	Negative	Negative	Negative	Positive 90%	Positive 10%	Positive 95-99%	Positive 100%	Negative	Positive 25%	Positive 10%
	PR	Positive 40%	Negative	Negative	Negative	Positive 30%	Positive 10%	Positive 2-3%	Positive 80%	Negative	Positive 15%	Positive 10%
	Ki67	20%	50-55%	80%	30-35%	18-20%	35%	8-10%	40%	90%	40%	30-35%
Tumor Markers	CA125	9.2	13.2	7.7	3.6	8.2	19.1	5.6	11.0	3.9	8.8	6.8
	CEA	6.3	3.5	2.3	11.4	10.2	5.7	1.1	<0.1	2.3	9.0	7.2
	CA15-3	13	62	52	48	33	67	14	8.61	27	51	47

The lipid profile of the patients, including total cholesterol, triglyceride, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very low-density lipoprotein (VLDL) has been checked by a blood test. The normal distribution of each factor and the linear correlation between the R_{CT} and lipid profile elements were checked by Kolmogorov-Smirnov test, and a simple scatter graph, respectively. Afterward, the correlation between each lipid profile factor and R_{CT} value was analyzed using Pearson Correlation Coefficient at the 2-tailed significance level of 0.01. The results demonstrate that there is no significant correlation between the lipid profile status of the patients (including total cholesterol, triglyceride, LDL, HDL, and VLDL) and their R_{CT} value ($P > 0.01$).

Table S2, *Lipidic profile of four selected patients. Blood characterizations include complete blood count (CBC), blood biochemistry, serum electrolytes, tumor markers, and liver enzymes have been analyzed in order to find any correlation between the LDP response and blood analyses.*

Elements	Patient #3	Patient #4	Patient #6	Patient #9
CBC				
W.B.C	5.2	3	7.9	3.1
R.B.C	5.18	4.41	4.38	3.04
Hb	13.3	13	13	8.3
Hct	40	37.8	38	25.9
M.C.V	77.22	85.71	86.76	85.2
M.C.H	25.68	29.48	29.68	27.3
M.C.H.C	33.25	34.39	34.21	32.05
RDW	13.6	15.6	13.5	18
PLATELETS	219	427	296	162
Blood Biochemistry				
FBS	96	91	88	109
Urea	31	28	50	31
Creat	0.8	0.9	1.2	0.8
Chol	222	173	179	191
TG	177	92	29	370
HDL	44	54	57	33
LDL	89	89	93	75
VLDL	2.023	1.648	1.632	2.273
LDL/HDL	2.023	1.648	1.632	2.273
SGOT(AST)	28	37	18	32
SGPT(ALT)	35	18	7	24
ALP	268	173	222	187
CPK	51	97	50	67
LDH	256	389	252	339
Protein (Total)	7.8	7.1	7.7	6.9
gGT	32	20	15	16
Albumin	4.6	5.5	5.1	3.8
Bilirubin T&D	0.13	0.6	0.5	0.54
ESR 1h	20	33	10	61
BD	0.13	0.18	0.2	0.14
Electrolytes				
Na	142	138	140	139
K	4.59	4.8	4.7	4.29
Ca	9.8	9.9	9.8	9.1
Ph	4.1	3.4	3.3	3.4
Mg	1.8	1.9	1.9	1.6
CRP (Quant)	Negative	Negative	Negative	Negative
Tumor Marker				
CEA	2.3	11.4	1.1	2.3
CA15-3	52	48	14	27
CA125	7.7	3.6	5.6	3.9
Immunoassays-Thyroid Function				
Anti TPO		178		
Liver Enzymes				
PT	13	12.5	13	12
P.T.T	33	31	33	27
INR	1	1.1	1	1.1

Permanent pathology has been considered as the gold standard test for cancerous and normal lymph nodes diagnosis. As shown in the crosstab table (Figure S3, b), positive predictive value, negative predictive value, sensitivity, and specificity of the LDP were 97%, 100%, 100%, and 98%, respectively. Consequently, to evaluate these parameters' balance and determine how accurate and predicting LDP is, the receiver operating characteristics (ROC) test has been performed. The area under the curve (AUC) of the LDP was 0.986 (P-value < 0.001, 95% CI: 0.964-1.0). As the AUC of frozen pathology was 0.969 (P-value < 0.001, 95% CI: 0.93-1.0), it seems that LDP could be used as an appropriate diagnostic tool, same as frozen. It should be noted that the obtained values may be due to the small sample size, and more clinical trials with a higher number of participants should be conducted to identify these particular characteristics accurately.

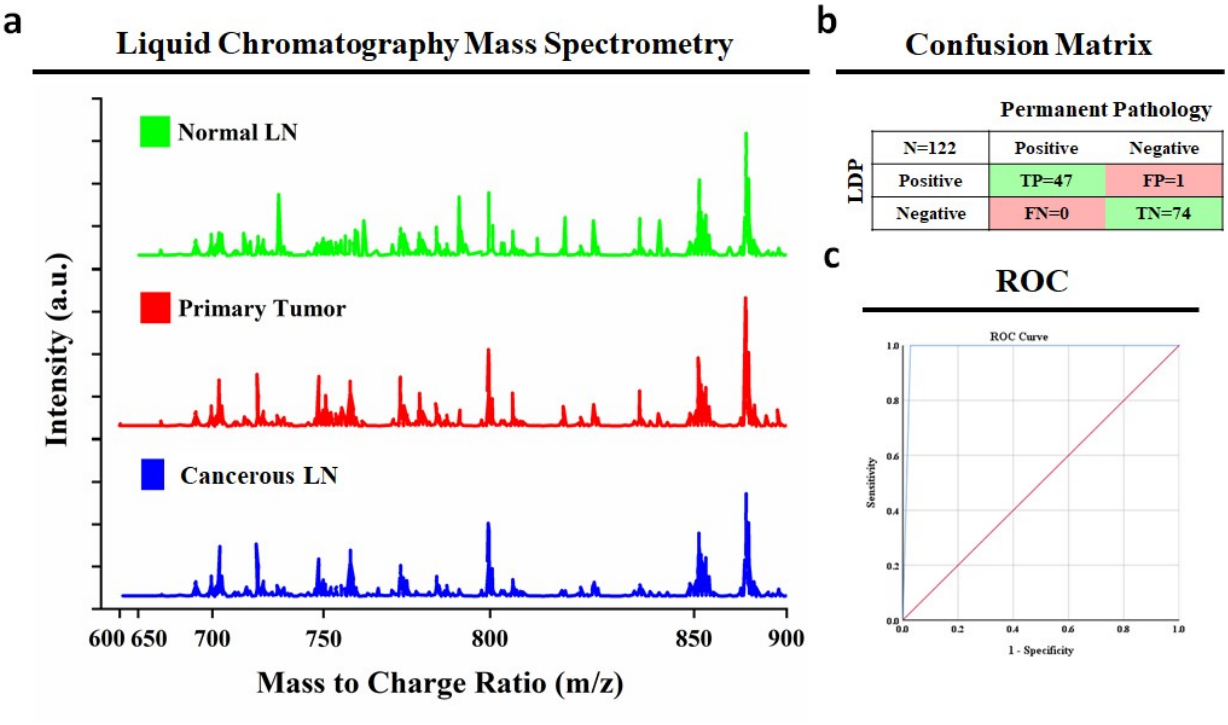
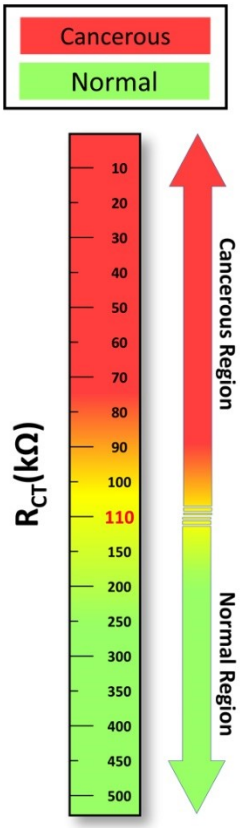


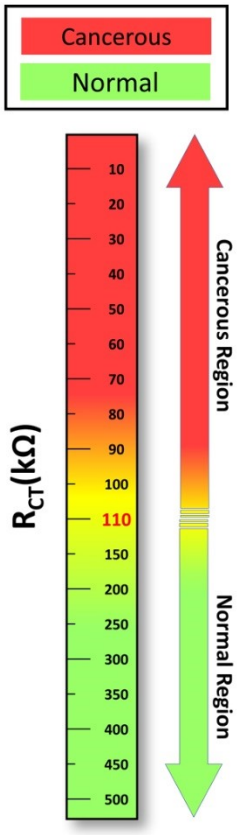
Figure S3, *a) Comparing lipidomics mass spectral profiles (m/z 600-900) of normal LN, primary tumor, and involved LN. b) Confusion matrix for LDP predicted results based on pathological assays (as gold standard) for 122 in-vivo samples from 41 patients. (TP: True Positive, FP: False Positive, TN: True Negative, and FN: False Negative). c) Receiver operating characteristics (ROC) curve of LDP; the area under the curve (AUC) was 0.986, P-value < 0.001, 95% CI: 0.964-1.0.*

Table S3, *Patients' LNs LDP results, frozen section, permanent pathology section, and LDP result evaluation in comparison to permanent histopathological diagnosis in 45 SLNs and 77 non-sentinel LNs and a comparative chart for the measured R_{CT} of patients' LNs. The red dashed line defines the border of normal and involved LNs at 110 kΩ. True-positive and false-positive results have been specified in the entire green and red rows, respectively. Each test was repeated for 5 times with STD: ± 5%.*

Patient ID#	Patient Sample#	Type of Lymph Node	R_{CT} (Ω)	Frozen Pathology	LDP Response	Permanent Pathology	Response Compared to Permanent Pathology	Percentage of Cancerous Cells (%)
1	i	Sentinel	5.85E+04	+	+	+	TP	
	ii	Non-sentinel	9.82E+04	-	+	+	TP	<10
	iii	Non-sentinel	1.43E+05	-	-	-	TN	
2	i	Sentinel	4.00E+05	-	-	-	TN	
	ii	Non-sentinel	4.95E+05	-	-	-	TN	
3	i	Sentinel	4.00E+05	-	-	-	TN	
	ii	Non-sentinel	3.76E+05	-	-	-	TN	
4	i	Sentinel	1.30E+04	+	+	+	TP	
	ii	Sentinel	1.43E+05	-	-	-	TN	
5	i	Sentinel	1.44E+04	+	+	+	TP	
	ii	Non-sentinel	2.52E+04	+	+	+	TP	
	iii	Non-sentinel	4.90E+05	-	-	-	TN	
	iv	Non-sentinel	5.04E+05	-	-	-	TN	
6	i	Sentinel	2.55E+04	+	+	+	TP	
	ii	Non-sentinel	1.57E+05	-	-	-	TN	
	iii	Non-sentinel	1.45E+05	-	-	-	TN	
7	i	Sentinel	3.66E+04	+	+	+	TP	
	ii	Non-sentinel	3.49E+05	-	-	-	TN	
8	i	Sentinel	6.80E+04	+	+	+	TP	
	ii	Non-sentinel	7.40E+04	+	+	+	TP	
9	i	Sentinel	5.13E+05	-	-	-	TN	
	ii	Non-sentinel	4.95E+05	-	-	-	TN	
10	i	Sentinel	1.60E+05	-	-	-	TN	
	ii	Sentinel	1.85E+05	-	-	-	TN	
	iii	Non-sentinel	1.41E+05	-	-	-	TN	
11	i	Sentinel	1.90E+04	+	+	+	TP	
	ii	Non-sentinel	1.40E+04	+	+	+	TP	
	iii	Non-sentinel	1.49E+04	+	+	+	TP	
	iv	Non-sentinel	4.98E+05	-	-	-	TN	
12	i	Sentinel	1.34E+04	+	+	+	TP	
	ii	Non-sentinel	1.44E+05	-	-	-	TN	
	iii	Non-sentinel	4.94E+05	-	-	-	TN	
	iv	Non-sentinel	4.99E+05	-	-	-	TN	
	v	Non-sentinel	1.46E+05	-	-	-	TN	
13	i	Sentinel	1.33E+04	+	+	+	TP	
	ii	Non-sentinel	1.63E+05	-	-	-	TN	
	iii	Non-sentinel	1.87E+05	-	-	-	TN	
	iv	Non-sentinel	4.88E+05	-	-	-	TN	
14	i	Sentinel	1.37E+04	+	+	+	TP	
	ii	Non-sentinel	1.45E+05	-	-	-	TN	
	iii	Non-sentinel	1.65E+05	-	-	-	TN	
	iv	Non-sentinel	1.46E+04	+	+	+	TP	
	v	Non-sentinel	3.96E+05	-	-	-	TN	
15	i	Sentinel	1.29E+04	-	+	-	FP	
	ii	Sentinel	1.41E+05	-	-	-	TN	
16	i	Sentinel	1.44E+04	+	+	+	TP	
	ii	Non-sentinel	1.42E+05	-	-	-	TN	
	iii	Non-sentinel	6.94E+04	+	+	+	TP	
	iv	Non-sentinel	3.42E+05	-	-	-	TN	
17	i	Sentinel	3.62E+04	+	+	+	TP	
	ii	Non-sentinel	1.05E+04	+	+	+	TP	
	iii	Non-sentinel	3.46E+05	-	-	-	TN	
	iv	Non-sentinel	1.44E+05	-	-	-	TN	
18	i	Sentinel	5.03E+05	-	-	-	TN	
	ii	Non-sentinel	1.89E+05	-	-	-	TN	
19	i	Sentinel	1.92E+04	+	+	+	TP	
	ii	Non-sentinel	3.52E+05	-	-	-	TN	
	iii	Non-sentinel	7.33E+04	+	+	+	TP	
	iv	Non-sentinel	1.40E+05	-	-	-	TN	
20	i	Sentinel	4.08E+05	-	-	-	TN	
	ii	Non-sentinel	4.12E+05	-	-	-	TN	

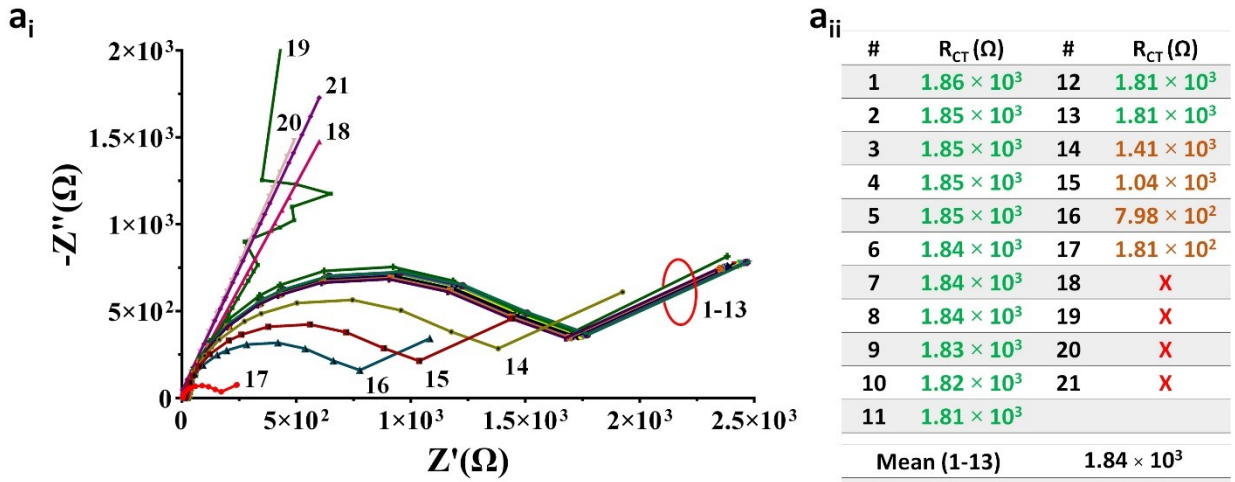


Patient ID#	Patient Sample#	Type of Lymph Node	R_{CT} (Ω)	Frozen Pathology	LDP Response	Permanent Pathology	Response Compared to Permanent Pathology	Percentage of Cancerous Cells (%)
21	i	Sentinel	1.38E+05	-	-	-	TN	
	ii	Non-sentinel	1.47E+05	-	-	-	TN	
22	i	Sentinel	1.40E+05	-	-	-	TN	
	ii	Non-sentinel	4.04E+05	-	-	-	TN	
23	i	Sentinel	1.86E+04	+	+	+	TP	
	ii	Non-sentinel	1.52E+04	+	+	+	TP	
	iii	Non-sentinel	1.40E+05	-	-	-	TN	
	iv	Non-sentinel	1.42E+05	-	-	-	TN	
24	i	Sentinel	1.81E+05	-	-	-	TN	
	ii	Non-sentinel	5.08E+05	-	-	-	TN	
25	i	Sentinel	1.94E+04	+	+	+	TP	
	ii	Non-sentinel	1.48E+04	+	+	+	TP	
	iii	Non-sentinel	1.44E+05	-	-	-	TN	
	iv	Non-sentinel	5.00E+05	-	-	-	TN	
26	i	Sentinel	1.62E+05	-	-	-	TN	
	ii	Sentinel	3.96E+05	-	-	-	TN	
27	i	Sentinel	3.80E+05	-	-	-	TN	
	ii	Non-sentinel	1.44E+05	-	-	-	TN	
28	i	Sentinel	1.03E+04	+	+	+	TP	
	ii	Non-sentinel	7.47E+04	+	+	+	TP	
	iii	Non-sentinel	1.41E+04	+	+	+	TP	
	iv	Non-sentinel	1.40E+05	-	-	-	TN	
29	i	Sentinel	1.00E+03	+	+	+	TP	
	ii	Non-sentinel	9.40E+04	-	+	+	TP	≤5
	iii	Non-sentinel	1.04E+04	+	+	+	TP	
30	i	Sentinel	1.31E+04	+	+	+	TP	
	ii	Non-sentinel	2.60E+04	+	+	+	TP	
	iii	Non-sentinel	1.42E+05	-	-	-	TN	
	iv	Non-sentinel	1.38E+05	-	-	-	TN	
31	i	Sentinel	9.62E+04	+	+	+	TP	
	ii	Non-sentinel	1.58E+05	-	-	-	TN	
	iii	Non-sentinel	3.87E+05	-	-	-	TN	
32	i	Sentinel	1.07E+04	+	+	+	TP	
	ii	Non-sentinel	1.39E+04	+	+	+	TP	
	iii	Non-sentinel	1.47E+05	-	-	-	TN	
33	i	Sentinel	2.60E+04	+	+	+	TP	
	ii	Non-sentinel	3.39E+05	-	-	-	TN	
	iii	Non-sentinel	4.89E+04	+	+	+	TP	
	iv	Non-sentinel	3.59E+05	-	-	-	TN	
34	i	Sentinel	1.65E+05	-	-	-	TN	
	ii	Non-sentinel	5.13E+05	-	-	-	TN	
35	i	Sentinel	3.65E+05	-	-	-	TN	
	ii	Non-sentinel	1.45E+05	-	-	-	TN	
36	i	Sentinel	3.55E+04	+	+	+	TP	
	ii	Non-sentinel	7.18E+04	+	+	+	TP	
	iii	Non-sentinel	5.10E+05	-	-	-	TN	
	iv	Non-sentinel	1.47E+05	-	-	-	TN	
37	i	Sentinel	1.96E+04	+	+	+	TP	
	ii	Non-sentinel	1.39E+05	-	-	-	TN	
	iii	Non-sentinel	1.34E+04	+	+	+	TP	
	iv	Non-sentinel	1.39E+05	-	-	-	TN	
38	i	Sentinel	5.67E+04	+	+	+	TP	
	ii	Non-sentinel	1.48E+04	+	+	+	TP	
	iii	Non-sentinel	1.79E+05	-	-	-	TN	
39	i	Sentinel	1.30E+04	+	+	+	TP	
	ii	Non-sentinel	1.45E+05	-	-	-	TN	
	iii	Non-sentinel	3.88E+05	-	-	-	TN	
40	i	Sentinel	4.80E+05	-	-	-	TN	
	ii	Non-sentinel	1.47E+05	-	-	-	TN	
41	i	Sentinel	2.60E+04	+	+	+	TP	
	ii	Non-sentinel	3.39E+05	-	-	-	TN	



The repeatability and stability of the LDP senor were investigated using different methods of *in-vitro* and *in-vivo* tests. For the *in-vitro* investigation, the sensor was tested by a lipidic solution (MDA-MB-231 media) 21 times. The sensor was gently rinsed using sequential washing with 70% methanol and deionized (DI) water, respectively, and then dried in nitrogen flow. Figure S4-a depicts the results of LDP repeatability tests with the lipidic solution. The LDP repeated the same results for 13 tests; afterward, the results (R_{CT}) decreased till test #18 that the senor lost its semi-circle response at all. The given results depict the LDP repeatability and stability in its response.

Animal lymph node (10 times, Figure S4-b) and dissected human lymph node (2 times, Figure S4-c) were examined for investigating *in-vivo* repeatability tests. Rabbit popliteal lymph node was tested 10 times. The LDP sensor was gently rinsed using sequential washing with 70% methanol and deionized (DI) water, respectively, and then dried in nitrogen flow. LDP results of animal lymph node depict the sensor repeat its response 8 times. LDP in the human axillary lymph node copied its first response exactly. Additionally, to follow the legal considerations on testing human samples *in-vivo* for our future aims, we will not use a sensor twice.



#	R_{CT} (Ω)	#	R_{CT} (Ω)
1	1.86×10^3	12	1.81×10^3
2	1.85×10^3	13	1.81×10^3
3	1.85×10^3	14	1.41×10^3
4	1.85×10^3	15	1.04×10^3
5	1.85×10^3	16	7.98×10^2
6	1.84×10^3	17	1.81×10^2
7	1.84×10^3	18	X
8	1.84×10^3	19	X
9	1.83×10^3	20	X
10	1.82×10^3	21	X
11	1.81×10^3		
Mean (1-13)		1.84×10^3	
STDEV (1-13)		1.8×10	

#	R_{CT} (Ω)	#	R_{CT} (Ω)
1	2.26×10^5	6	2.21×10^5
2	2.25×10^5	7	2.19×10^5
3	2.24×10^5	8	2.18×10^5
4	2.23×10^5	9	1.71×10^5
5	2.22×10^5	10	8.21×10^4
Mean (1-8)		2.22×10^5	
STDEV (1-8)		2.8×10^3	

Figure S4, *a_i) LDP response for the lipidic solution. The sensor was tested 21 times to investigate the repeatability of the responses. a_{ii}) The responses were almost the same as the first result till test #14. b_i) The responses of the LDP in testing animal lymph node. The responses were almost the same for 8 tests. b_{ii}) The results depict the same R_{CT} which shows the reliability of the LDP sensor. c) The results of LDP in testing human axillary lymph node.*

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