Supplementary materials

Advancing Liquid Biopsy: Whispering Gallery Mode Laser Detection of HER2 Cancer Biomarker on Extracellular Vesicles

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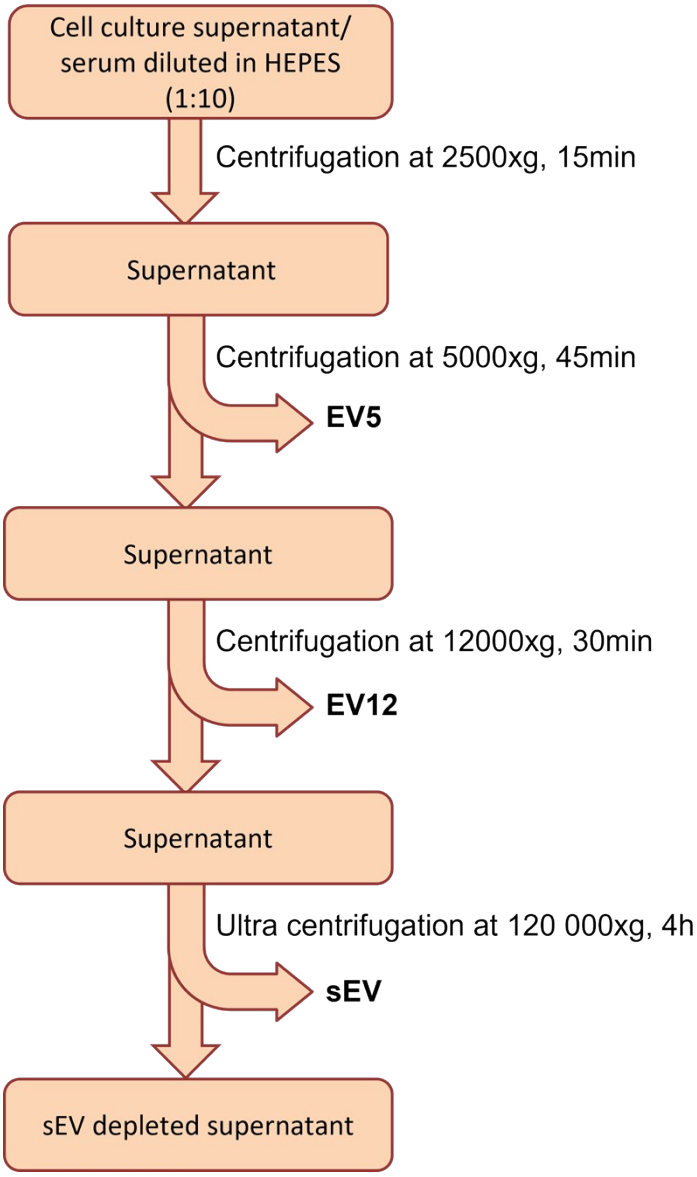
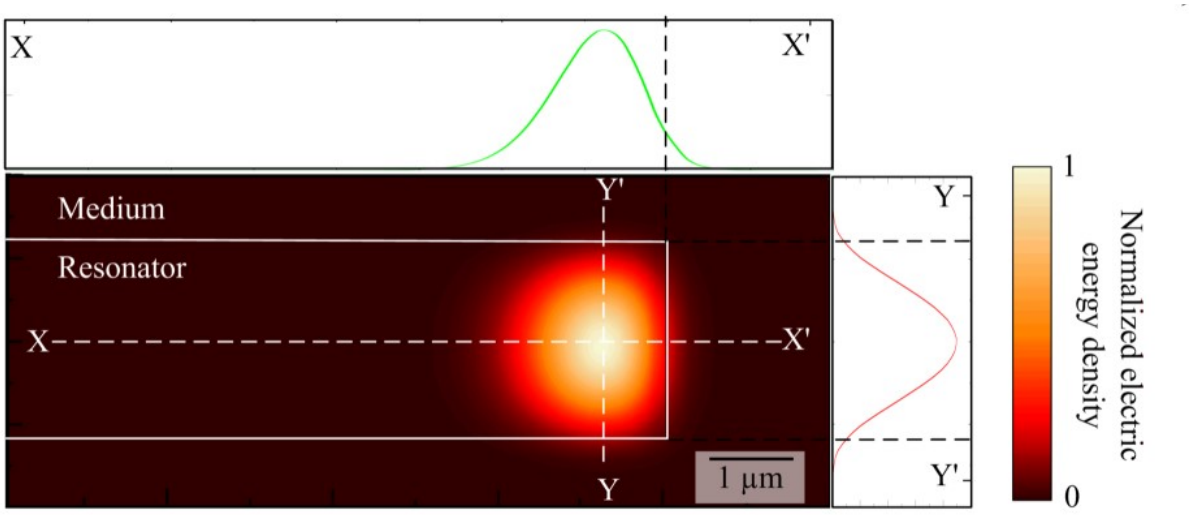
Keywords: extracellular vesicles, breast cancer diagnostics, liquid biopsy, whispering-gallery mode (WGM) resonators, human epithelial growth factor receptor 2 (HER2)



Supplementary Figure S1. Flowchart of EV Isolation Using the Differential Centrifugation Method The flowchart illustrates the sequential steps for isolating extracellular vesicles (EV) from cell culture supernatant and serum via differential centrifugation.

Cell Culture Protocol: Cells were cultured in 14.5 cm dishes with complete medium until they reached 70–80% confluency. Cell viability was monitored microscopically to ensure the absence of apoptotic bodies, which could contaminate the EV preparation. Cells were then washed with 5 mL of 1X PBS, followed by the addition of 15 mL starvation medium (complete medium without FBS). After 48 hours of serum starvation, the supernatant was collected and processed for EV isolation through differential centrifugation, as depicted.

Serum Preparation Protocol: For serum-derived EV, 1 mL of serum was diluted in 9 mL of sterile-filtered HEPES buffer (25 mM HEPES, 50 mM NaCl, 5 mM $MgCl_2$, pH 7.4) prior to centrifugation. Protease inhibitors were added to all preparations to prevent proteolytic degradation of EVs. Then the samples were processed as described for cell culture-derived EV.

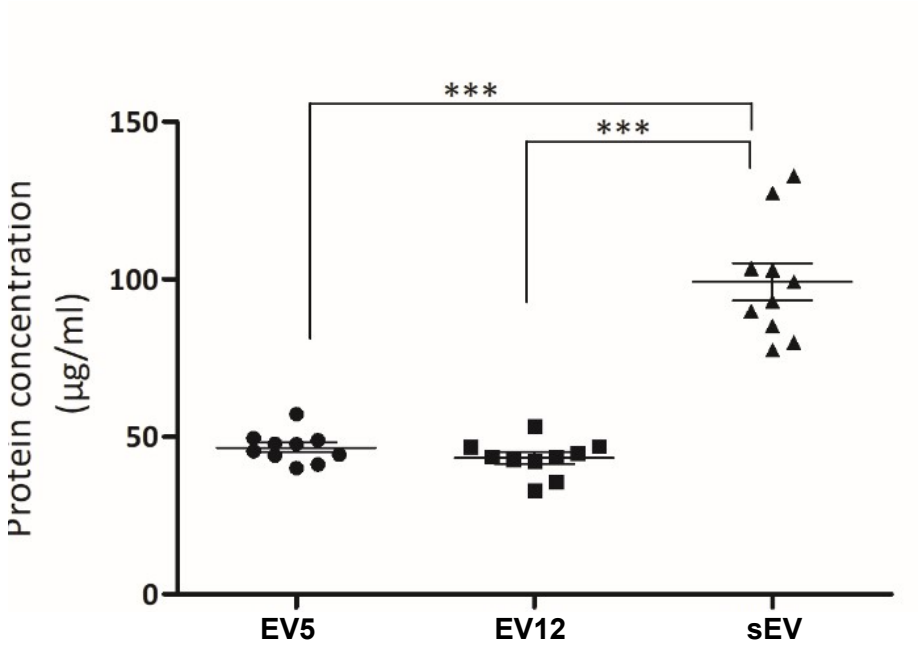


Supplementary Figure S2. WGM laser sensor principle Axial section of the outer most periphery of a WGM-laser showing electric energy density distribution of the fundamental whispering-gallery mode. A part of the electric field which is predominantly guided in the resonator interacts evanescently with the surrounding medium. Profiles of the energy density along the diameters XX' and YY' are shown in the graphs, where a small part of the field extends outside the resonator. This interaction would lead to a shift of the resonance wavelength, which would lead to a shift of the emission spectrum of the sensor, thereby enabling detection of molecular binding to the resonator or surface. The shift in wavelength depends on the size any concentration of the bound molecule or particle. In our case, the surface is functionalized such that only specific target molecules could bind to the lasers.

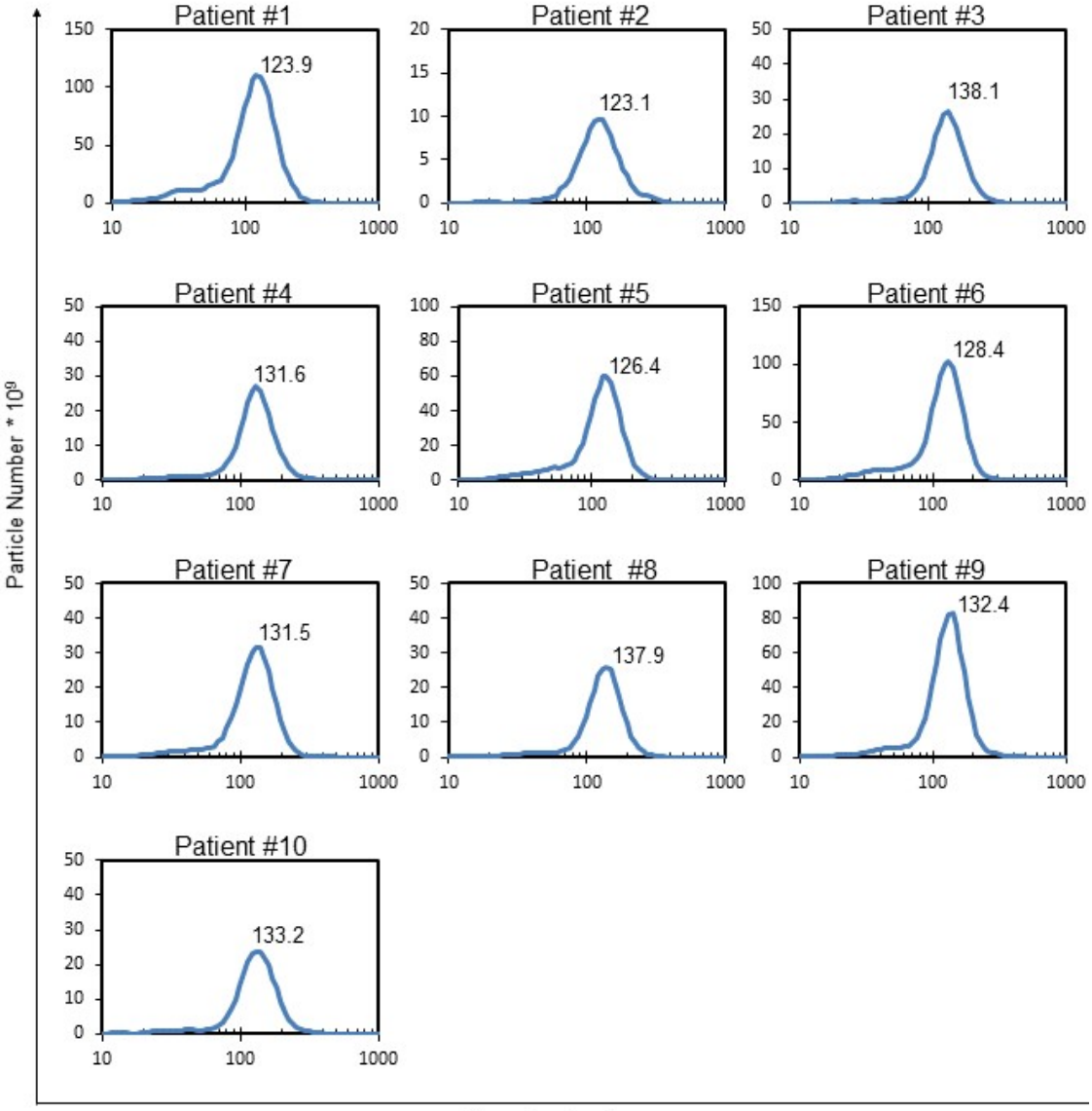
This image is used for the demonstration of the WGM laser -based sensing and presented elsewhere [29]

Supplementary Table S1. Protein concertation measured in the sEV preparations from serum of the breast cancer patients

Pt No.	EV5	EV12	sEV
1	42.70	23.96	92.53
2	33.41	29.55	75.40
3	57.18	53.43	132.87
4	49.59	32.89	85.13
5	47.82	42.24	89.98
6	25.43	28.07	129.79
7	55.17	54.29	82.82
8	54.14	41.67	126.18
9	60.53	51.51	128.99
10	48.92	43.65	103.40



Supplementary Figure S3. Statistical analysis of protein amounts in the EV fractions. Protein concentration of EV-enriched preparations from the patients serum was measured using microBCA Protein Assay kit (Thermo Fisher Scientific). While comparable amounts of protein were detected in the EV5 and EV12 fractions, substantially higher amounts of protein were separated in the sEV.



Supplementary Figure S4. Nanoparticle tracking analysis of sEV isolated from serum of breast cancer patients. The sEV-enriched crude fractions were analyzed using nanoparticle tracking analysis (NTA). The results demonstrate a relatively homogeneous size distribution of particles in all patients, ranging from 123 to 137 nm.

Supplementary Table S2: Characterisation of serum samples

Patient number	Particle count (x10^11/ml)	sEV HER2 (pg/µl)	cf HER2 (ELISA) (pg/µl)	HER2 score (IHC) by p.d.
1.	6.41	0.09	0.12	2
2.	7.48	0.18	0.05	3
3.	5.51	0.24	0.05	3
4.	14.21	0.105	0.07	2
5.	5.92	0.045	0.09	0
6.	14.49	0.045	0.08	0
7.	5.91	0.15	0.11	0
8.	10.49	0.15	0.12	0
9.	6.50	0.09	0.09	0
10.	10.34	0.12	0.08	3
h1	3.4	0.004	0.005	-
h2	2.9	0.02	0.003	-

The patient samples were characterized using TNA, and HER2 ELISA: additionally, the data regarding HER2 score identified by primary diagnosis was provided by the clinical partners. Among 10 patients, 3 were diagnosed with the score 3, two with the score 2, and 5 were considered as HER2 negative. It is to mention, that the blood samples were collected after the primary diagnosis and several months after surgery. Consequently, HER2 status may be different from the primary diagnosis. p.d. – diagnosis before primary tumour surgery and therapy