Supporting Information

Plasmonic Nanobiosensors for Detection of MicroRNA Cancer Biomarkers in Clinical Samples

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Table S1. Clinical sample labeling and histopathological discrimination. Histopathological diagnoses included normal tissue, EAC, esophageal squamous cell carcinoma (ESCC) and BE. Those samples denoted with an asterisk were unable to be tested by iMS assay due to insufficient amount of extracted RNA for triplicate measurement (100 ng required per iMS test).

De-identified Sample ID	Patient Number	Sample Type
Sample 1	5	EAC
Sample 2	10	BE
Sample 3*	6	Normal
Sample 4	5	Normal
Sample 5	4	BE
Sample 6	1	Normal
Sample 7	10	EAC
Sample 8	9	Normal
Sample 9	7	EAC
Sample 10	2	BE
Sample 11	8	EAC
Sample 12*	9	ESCC
Sample 13	3	BE
Sample 14	6	BE
Sample 15	1	EAC
Sample 16	4	Normal
Sample 17	8	Normal
Sample 18	3	Normal
Sample 19	7	Normal
Sample 20*	2	Normal

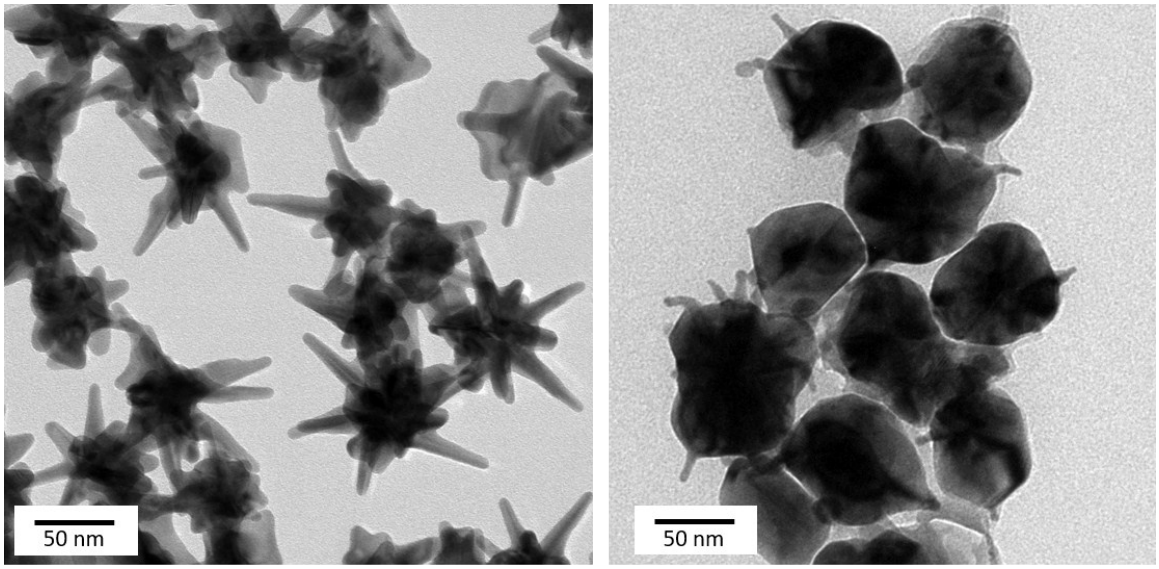

Figure S1. TEM images of gold nanostars (left) and silver coated gold nanostars (right).

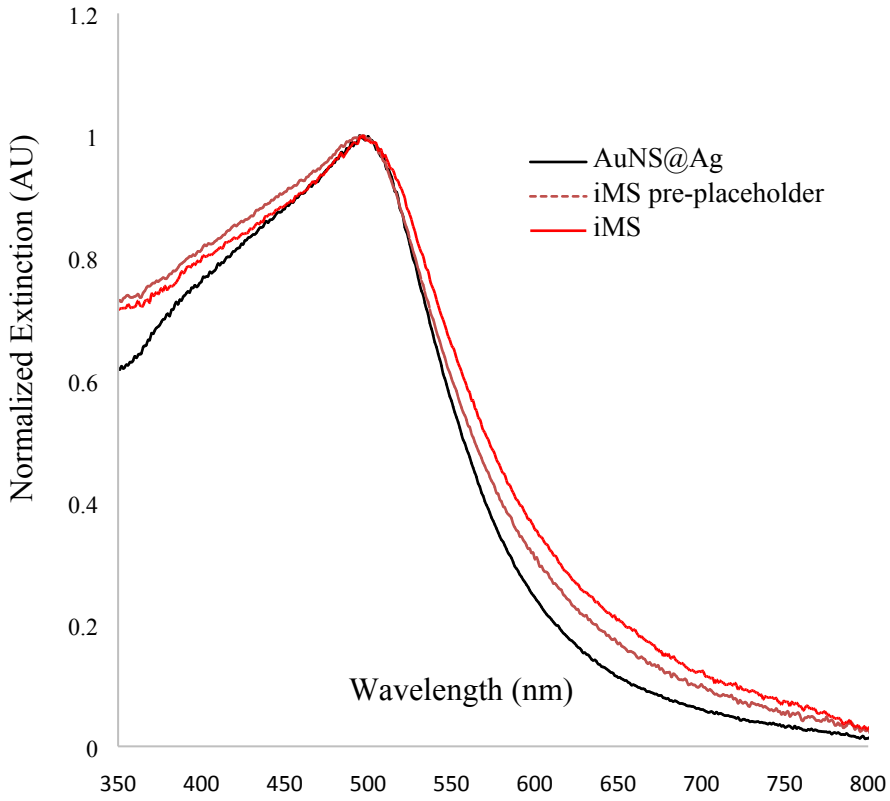

Figure S2. Absorbance of silver-coated gold nanostars (AuNS@Ag) before modification with DNA sequences, after modification with stem-loop probe (iMS pre-placeholder) and following addition of placeholder to achieve fully prepared nanoprobes (iMS). The absence of a shift in absorbance peak demonstrates that no particle aggregation occurred during iMS synthesis.

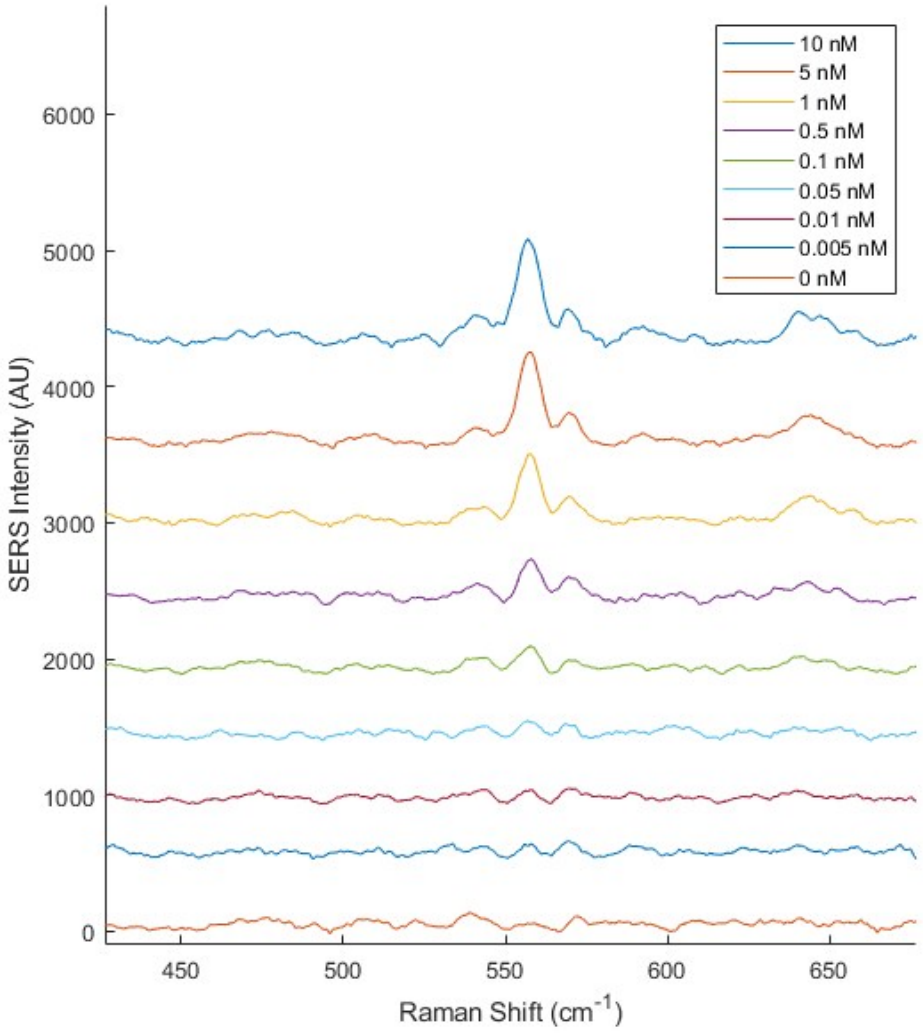

Figure S3. Detection sensitivity of the iMS nanobiosensors for miR-21 detection. Spectra offset for clarity.

Table S2. True positive (sensitivity) and true negative (specificity) of discriminating esophageal cancer (EC) from normal tissue.

	EC	Normal	Total
Total	5	7	12
Test Positive	5	0	5
Test Negative	0	7	7
	True Positive 5/5 = 100%	**True Negative** 7/7 = 100%	

Table S3. True positive (sensitivity) and true negative (specificity) of discriminating EC & Barrett's esophagus (BE) from normal tissue

	EC & BE	Normal	Total
Total	10	7	17
Test Positive	9	0	9
Test Negative	1	7	8
	True Positive 9/10 = 90%	**True Negative** 7/7 = 100%	

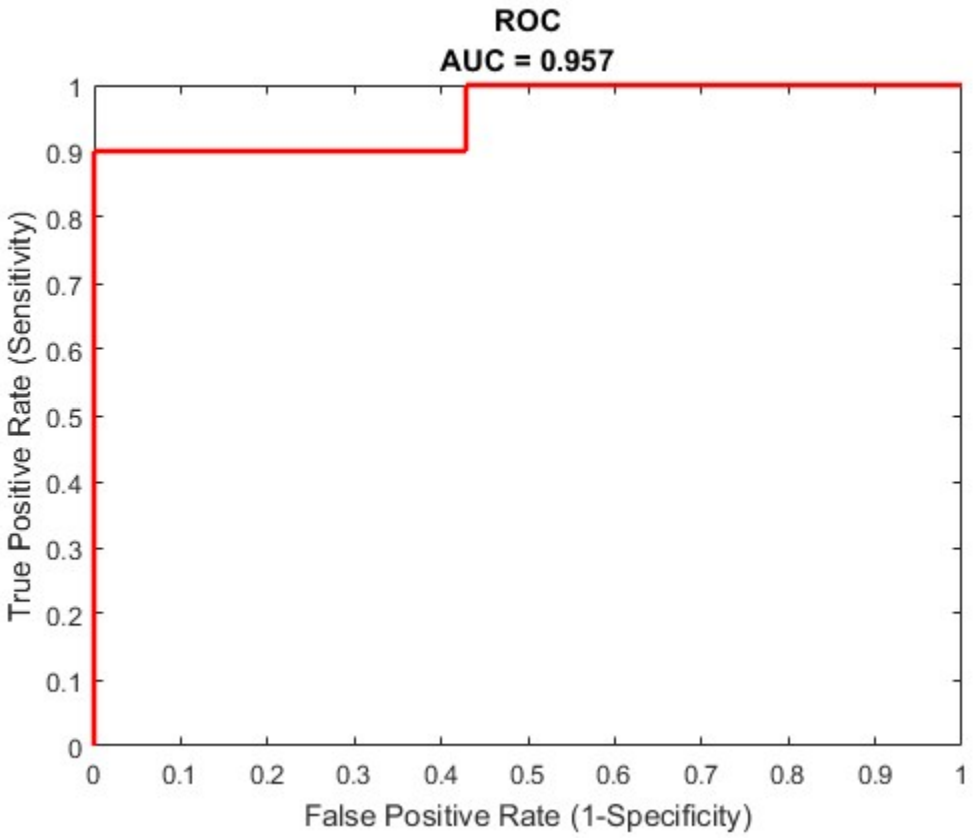

Figure S4. Receiver Operating Characteristics (ROC) curve for iMS response of patient samples diagnosed as normal vs. unhealthy (Barrett's esophagus and esophageal cancer). The area under the curve (AUC) indicates how well the iMS technique can distinguish between the two diagnostic groups.

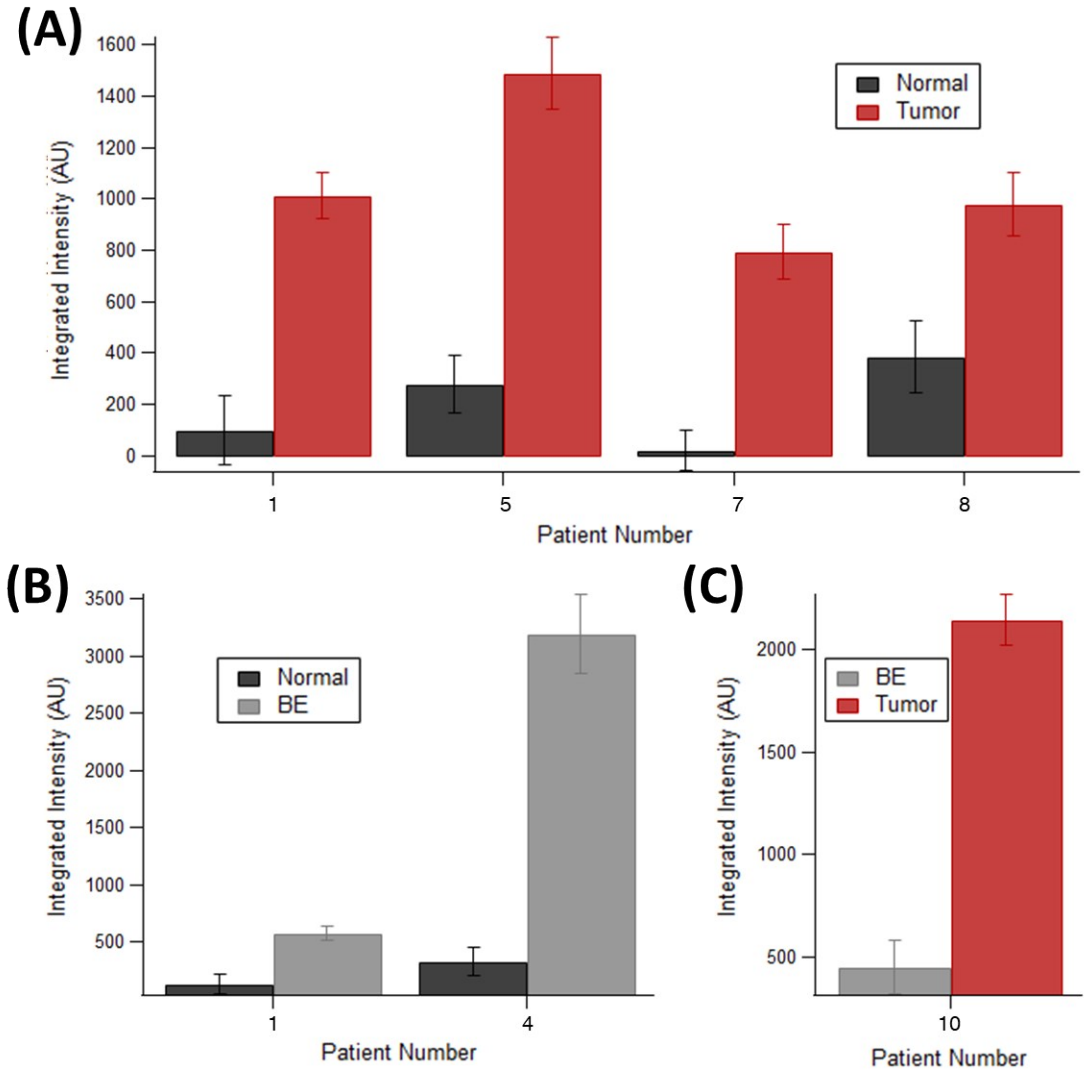

Figure S5. Detection of miR-21 within paired tissue biopsies. SERS intensities (area-under-curve of the 557cm-1 peak; arbitrary units) are given for patient pairs diagnosed as (A) normal and tumor, (B) normal and Barrett's esophagus (BE), and (C) BE and tumor.

Table S4. Concordance (Kendall Tau) and correlation (Pearson correlation) between miR-21 PCR results (normalized by cel-39) and SERS intensity (area-under-curve of the 557cm^{-1} peak) within each tissue type as determined by histopathological diagnosis.

	n	τ	p-value	ρ	p-value
All types combined	17	0.8	<0.001	0.63	0.007
Normal	7	1.0	<0.001	0.93	0.003
Barrett's Esophagus	5	0.6	0.23	0.40	0.51
Tumor	5	0.6	0.23	0.70	0.19

References

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