

# **Quantitative chemical imaging of breast calcifications in association with neoplastic processes**

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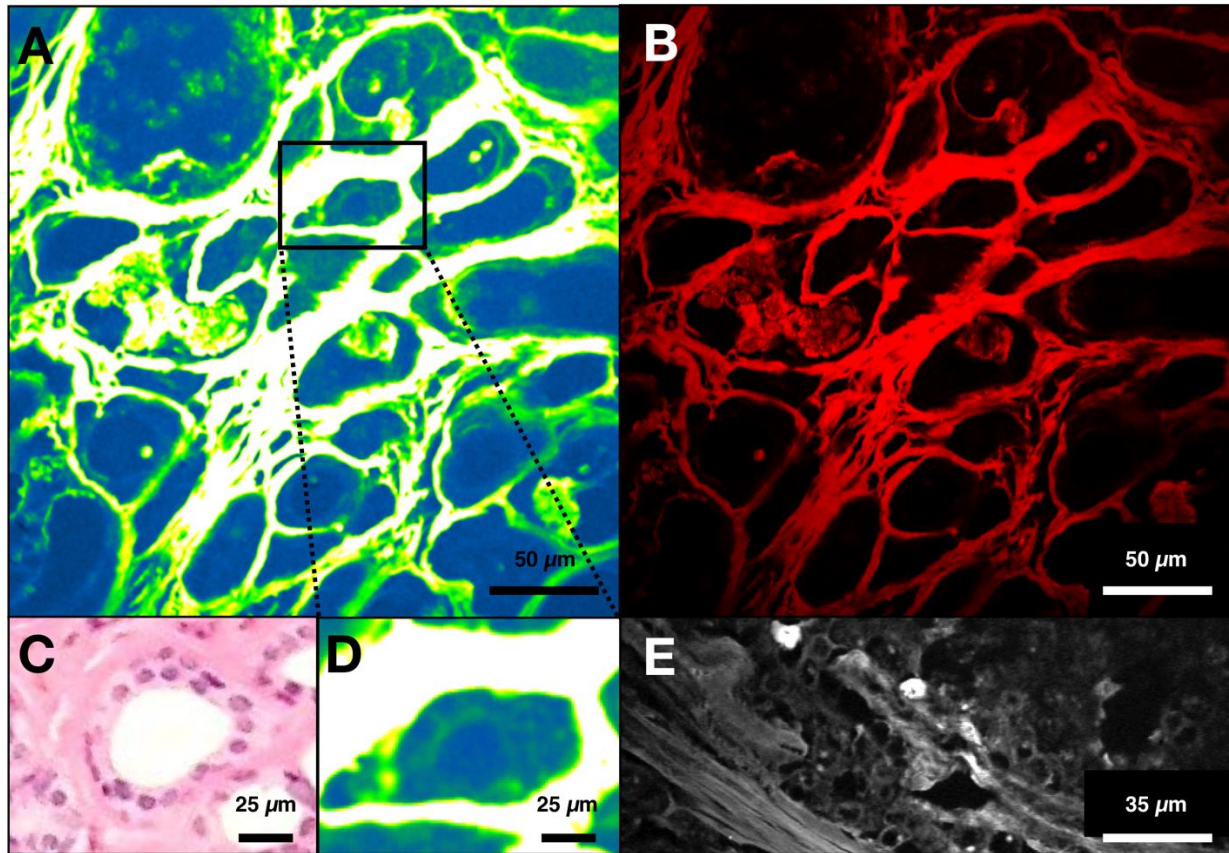
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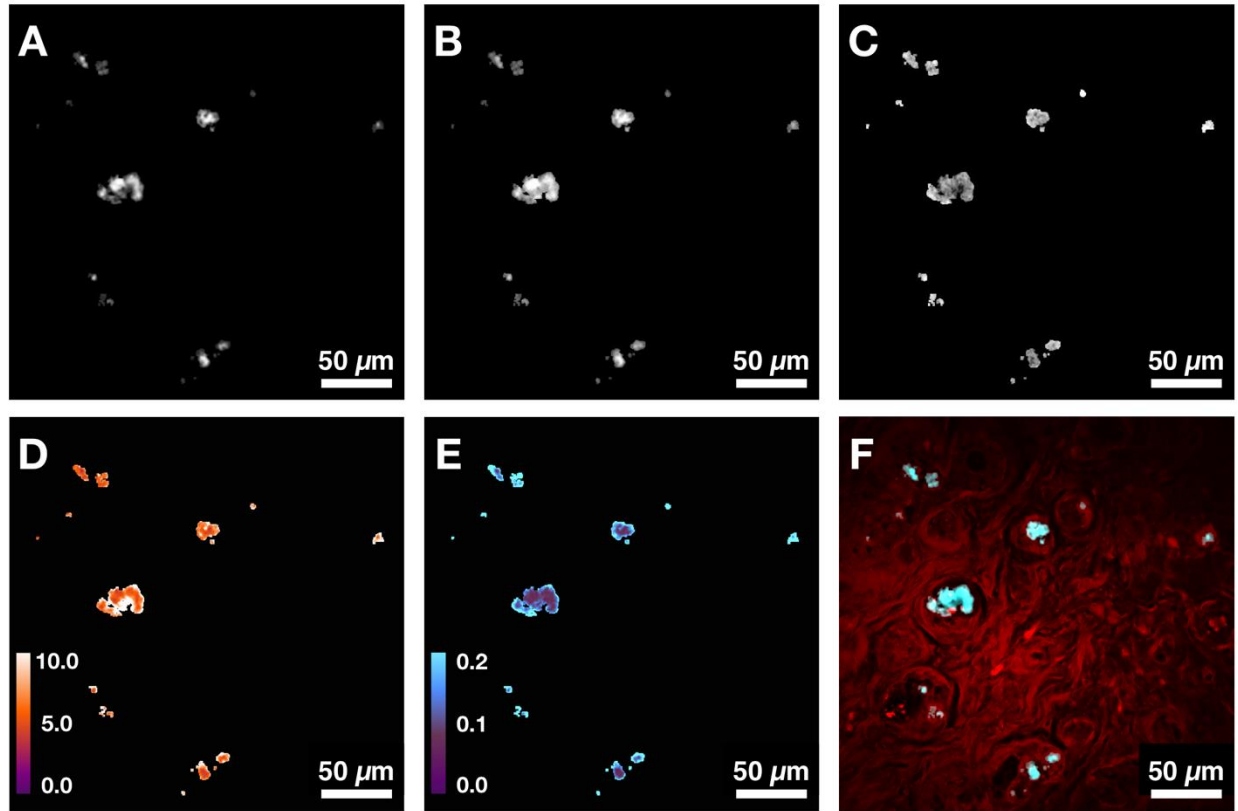
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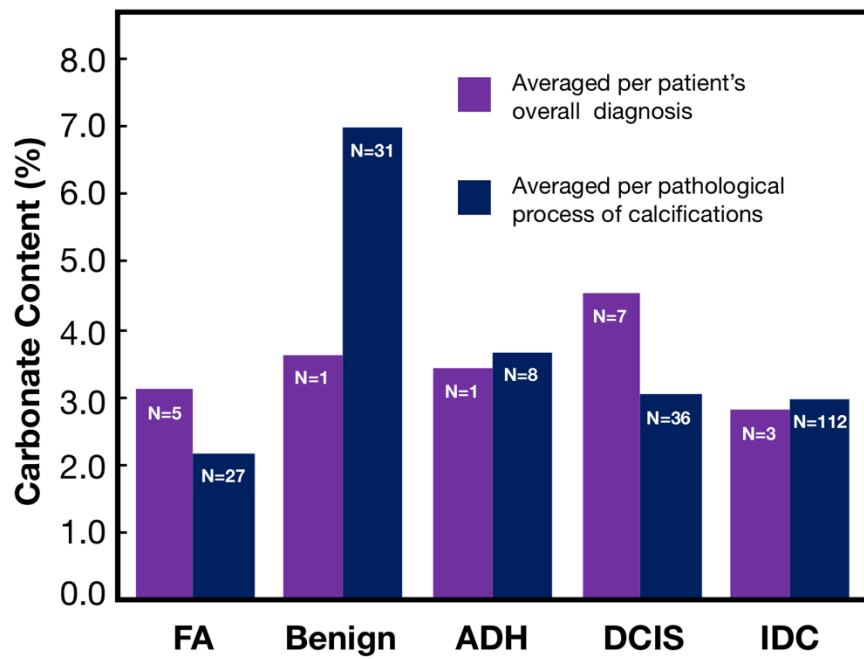
## SUPPLEMENTARY MATERIAL



Supplementary Figure 1: Visualizing cellular material in presence of strong CH signal from stromal collagen. A) Adenosis case presented in Figure 3 of main manuscript with adjusted intensity and color scale to assist with visualizing cellular material. B) Adenosis case as presented in Figure 3 of main manuscript. C) A close up of a duct on H&E. D) A close up of a duct from image in A). E) Additional example with easier to visualize cellular material (DCIS case).



Supplementary Figure 2: Correlating images at phosphate, carbonate, and phenylalanine Raman transitions to resulting carbonate content % and phenylalanine to phosphate ratio maps (calcifications associated with benign process). A) Image at phosphate Raman transition ( $\sim 960\text{ cm}^{-1}$ ). B) Image at carbonate Raman transition ( $\sim 1070\text{ cm}^{-1}$ ). C) Image at phenylalanine Raman transition ( $\sim 1005\text{ cm}^{-1}$ ). D) Carbonate content % map. E) Phenylalanine to phosphate ratio map. F) Image at CH Raman transition (red) together with image at phosphate Raman transition (cyan) to highlight calcification presence in tissue.



Supplementary Figure 3: Comparing the averages for carbonate content % as calculated per patient's overall diagnosis *versus* per pathological process of calcifications.

Patient	Sex	Age	Specimen type	Overall diagnosis	Number of calcifications associated with specified pathology					Total number of calcifications per patient	Carbonate content % per patient
					Benign	ADH	DCIS	IDC	FA		
1	F	75	Excisional biopsy	FA	0	0	0	0	9	9	2.3±1.1
2	F	64	Needle core biopsy	FA	0	0	0	0	11	11	2.4±0.8
3	F	49	Needle core biopsy	DCIS	0	0	8	0	0	8	4.4±1.8
4	F	56	Needle core biopsy	DCIS	0	0	1	0	0	1	3.5
5	F	57	Needle core biopsy	DCIS	0	0	4	0	0	4	3.5±0.5
6	F	58	Excision	ADH	0	3	0	0	0	3	3.4±0.5
7	F	51	Excision	DCIS	0	3	0	0	0	3	3.9±0.3
8	F	60	Needle core biopsy	DCIS	3	0	0	0	0	3	7.7±0.3
9	F	40	Needle core biopsy	DCIS	1	2	0	0	0	3	5.7±3.9
10	F	59	Needle core biopsy	FA	0	0	0	0	4	4	1.6±0.6
11	F	57	Needle core biopsy	FA	18	0	0	0	0	18	6.4±1.6
12	F	51	Excision	Adenosis	7	0	0	0	0	7	3.6±0.8
13	F	60	Needle core biopsy	DCIS	2	0	19	0	0	21	3.8±1.2
14	F	56	Unilateral mastectomy	IDC	0	0	1	76	0	77	2.3±0.7
15	F	68	Lumpectomy	IDC	0	0	0	11	0	11	3.6±1.1
16	F	56	Unilateral mastectomy	IDC	0	0	3	25	0	28	2.5±0.8
17	F	32	Lumpectomy	FA	0	0	0	0	3	3	2.9±0.8
<b>Total number of calcifications per each category</b>					<b>31</b>	<b>8</b>	<b>36</b>	<b>112</b>	<b>27</b>		

Supplementary Table 1: A patient clinical history summary together with detailed account of calcification number in each category. Carbonate content % average for each patient is provided.

Categories Compared	Two-sample T-test P-value	
	Carbonate Content %	Phenylalanine/Phosphate Ratio
Benign <i>versus</i> DCIS	< 0.001	0.013
Benign <i>versus</i> ADH	< 0.001	< 0.001
Benign <i>versus</i> IDC	< 0.001	< 0.001
Benign <i>versus</i> FA	< 0.001	< 0.001
DCIS <i>versus</i> IDC	< 0.001	0.002
ADH <i>versus</i> DCIS	0.059	0.021
Benign <i>versus</i> all neoplastic	< 0.001	< 0.001
FA <i>versus</i> IDC	0.395	0.001

Supplementary Table 2: Summary of t-test p-values for various categories compared (for both carbonate content % and phenylalanine/phosphate ratio). P-value of <0.005 is considered statistically significant and P-value of <0.001 is considered statistically highly significant.