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Cardiovascular research Institute Maastricht

Towards the complete picture: computational strategies to identify Mass Spectrometry Imaging derived molecular fingerprints associated with inflammatory cell components

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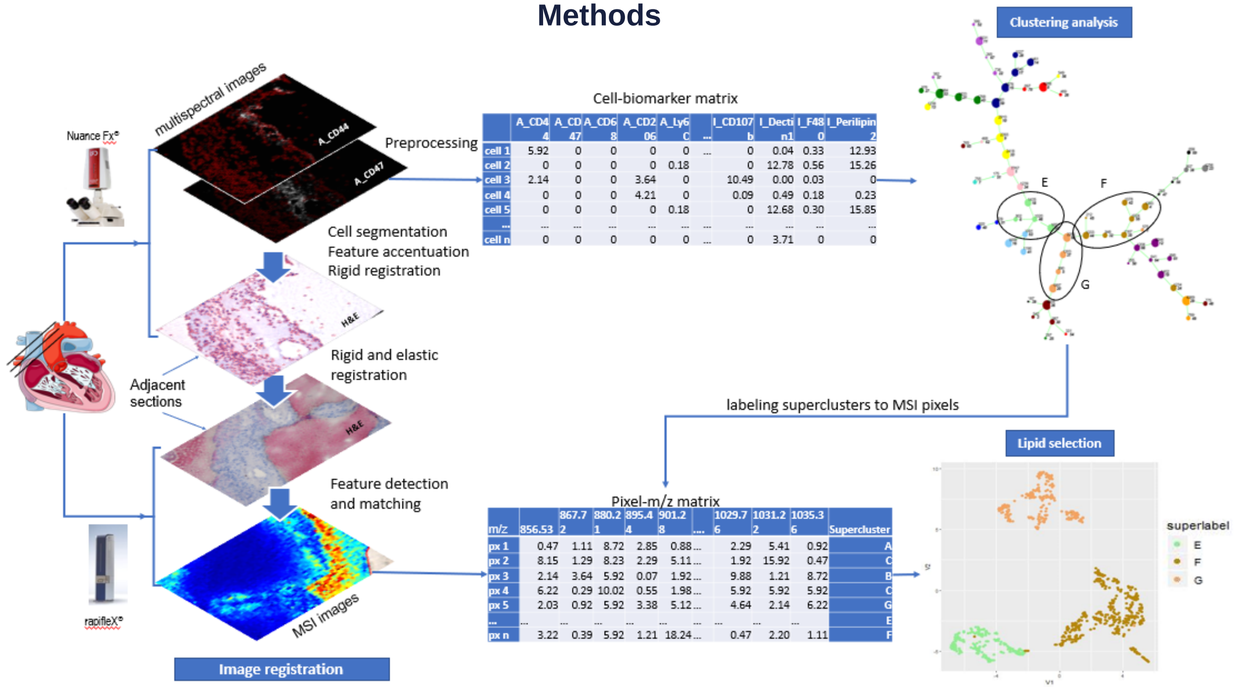
Background and Objective

Macrophages can adapt to stimuli in their direct environment, adopting a functional phenotypic spectrum throughout the disease course of atherosclerosis. While this suggests significant plaque macrophage heterogeneity, it has thus far only been poorly described, largely due to technical constraints. Here we present a new strategy to dissect macrophage heterogeneity in its molecular context at high spatial resolution.

Methods

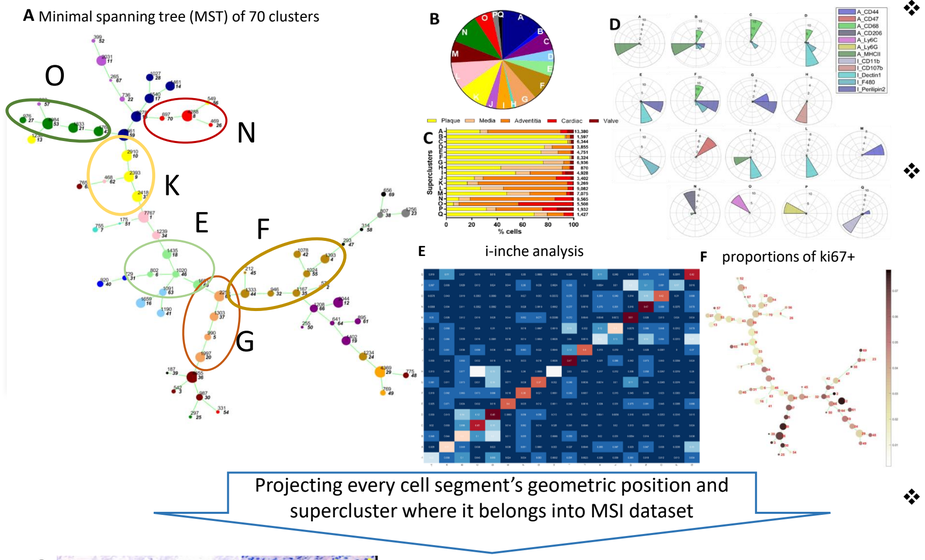


	A_CD44	A_CD47	A_CD68	A_CD206	A_Ly6C	A_Ly6G	...	I_CD107b	I_Decitin1	I_F480	I_Perilipin2
cell 1	5.92	0	0	0	0	...	0	0.04	0.33	12.93
cell 2	0	0	0	0	0.18	...	0	12.78	0.56	15.26
cell 3	2.14	0	0	3.64	0	...	10.49	0.00	0.03	0
cell 4	0	0	0	4.21	0	...	0.09	0.49	0.18	0.23
cell 5	0	0	0	0	0.18	...	0	12.68	0.30	15.85
...	...	...	...	...	...	...	...	...	...	...
cell n	0	0	0	0	0	...	0	3.71	0	0

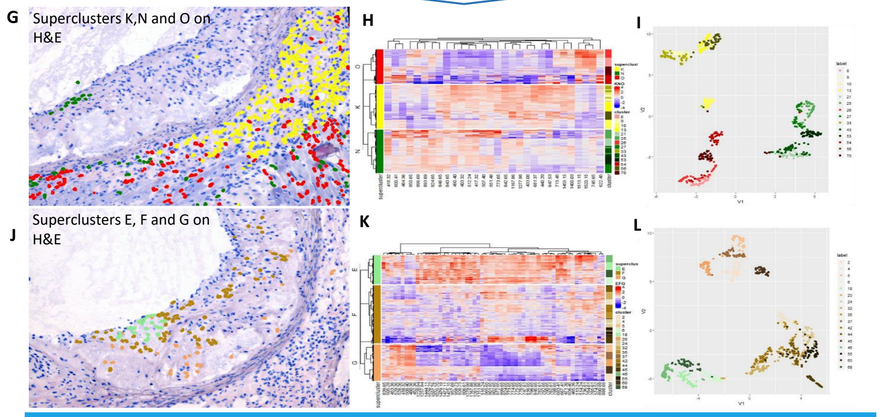
m/z	856.53	867.72	880.21	895.44	901.28	....	1029.76	1031.22	1035.36	Supercluster
px 1	0.47	1.11	8.72	2.85	0.88	...	2.29	5.41	0.92	A
px 2	8.15	1.29	8.23	2.29	5.11	...	1.92	15.92	0.47	C
px 3	2.14	3.64	5.92	0.07	1.92	...	9.88	1.21	8.72	B
px 4	6.22	0.29	10.02	0.55	1.98	...	5.92	5.92	5.92	C
px 5	2.03	0.92	5.92	3.38	5.12	...	4.64	2.14	6.22	G
...	...	...	...	...	...	...	...	...	...	E
px n	3.22	0.39	5.92	1.21	18.24	...	0.47	2.20	1.11	F

- Plaque sections from mouse aorta were analysed by multispectral imaging microscopy to concomitantly visualize up to 12 myeloid markers per section.
- Cell segments were grouped into 70 clusters, and then assigned to 17 phenotypic “superclusters”(named A–Q, colour-coded), based on pathological inspection and analysis of typical tissue localization of these superclusters.
- Adjacent sections were aligned by a series of (rigid and elastic) image registration algorithms, mapping the positions of the superclusters on the mass spectrometry imaging (MSI) dataset to label each MS pixel a certain phenotypic population.
- Top k MSI peaks with excellent classification performance were selected from the peak importance ranking list as the final m/z values for lipid identification

Results



Projecting every cell segment’s geometric position and supercluster where it belongs into MSI dataset



- Figure A to F: Visualizations and analysis of 17 superclusters based on multispectral imaging data (expressions of myeloid markers)
- Figure G to L: Visualizations and lipid selection of superclusters based on MSI data (signal intensities of lipids). We zoomed in on three distinct subsets in the adventitia (superclusters K, N and O) and three foam cell phenotypes (superclusters E, F and G), to reveal differences in lipid profiles based on top k selected MSI peaks.
- **The cell segments from the same supercluster (based on myeloid markers) also demonstrate similar expressions on the crucial MSI peaks!**

Conclusion

We have developed an innovative new approach to define not only macrophage heterogeneity in the atherosclerotic plaque, but also to link this to the microenvironment. This methodology is broadly applicable to other cell types and tissue and may lead to a breakthrough in linking molecular context to cellular phenotype and function, in healthy and diseased tissues.

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