Mass Spectrometry Imaging - new approach in molecular imaging of cancer

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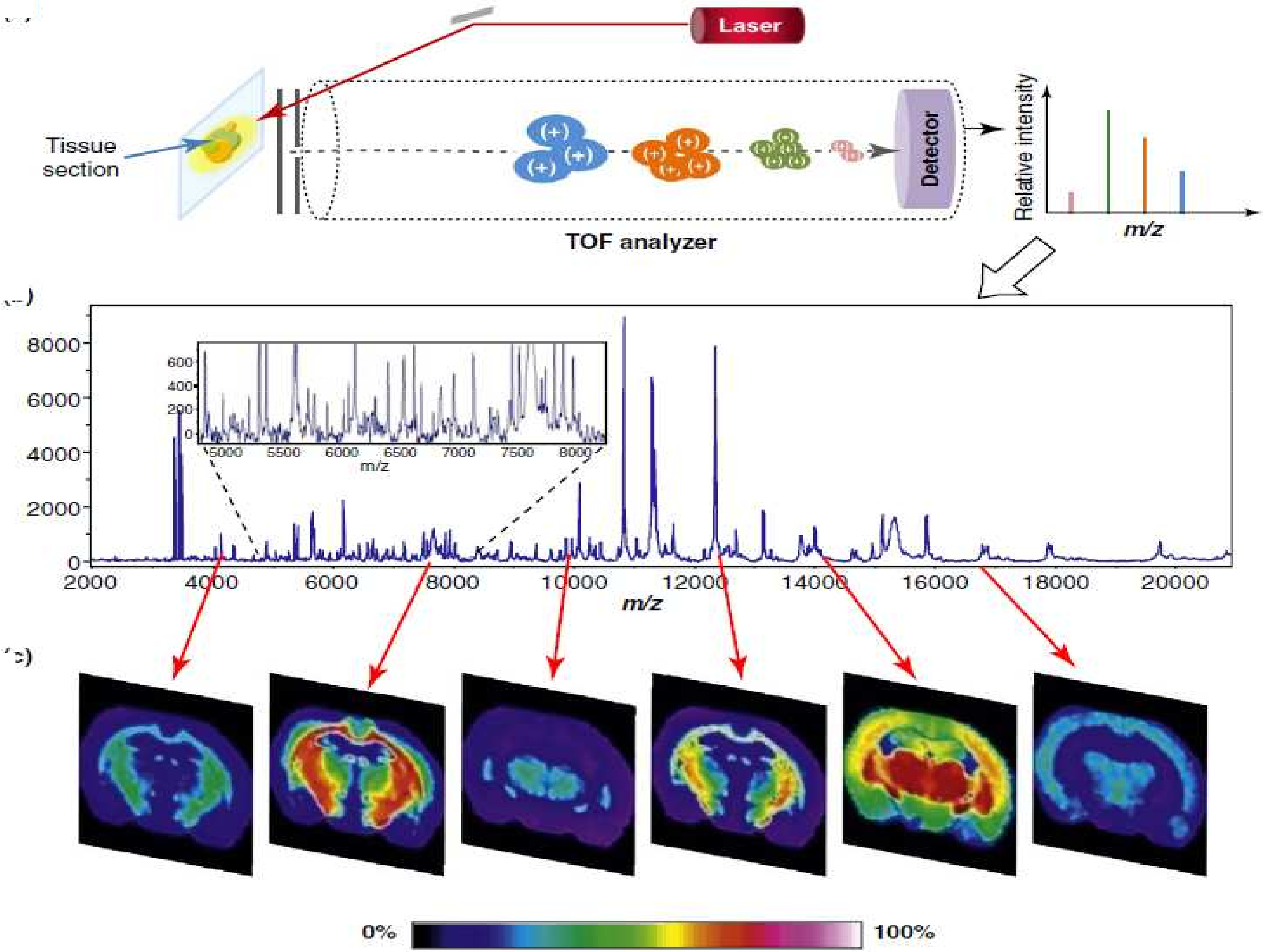
A picture is worth a thousand words

Molecular Imaging of Tissue

MALDI Mass Spectrometry Imaging

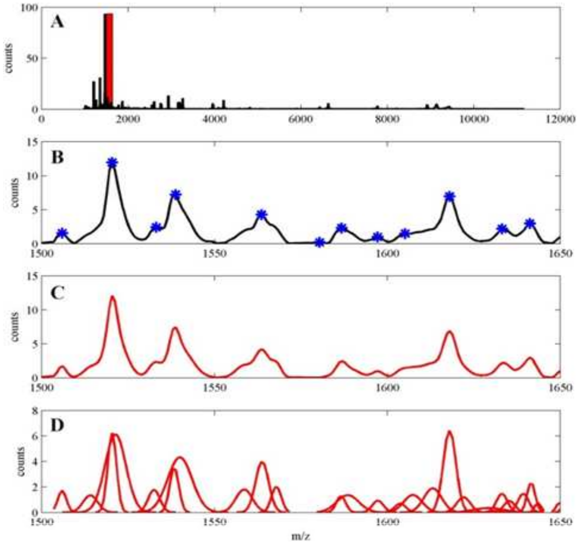


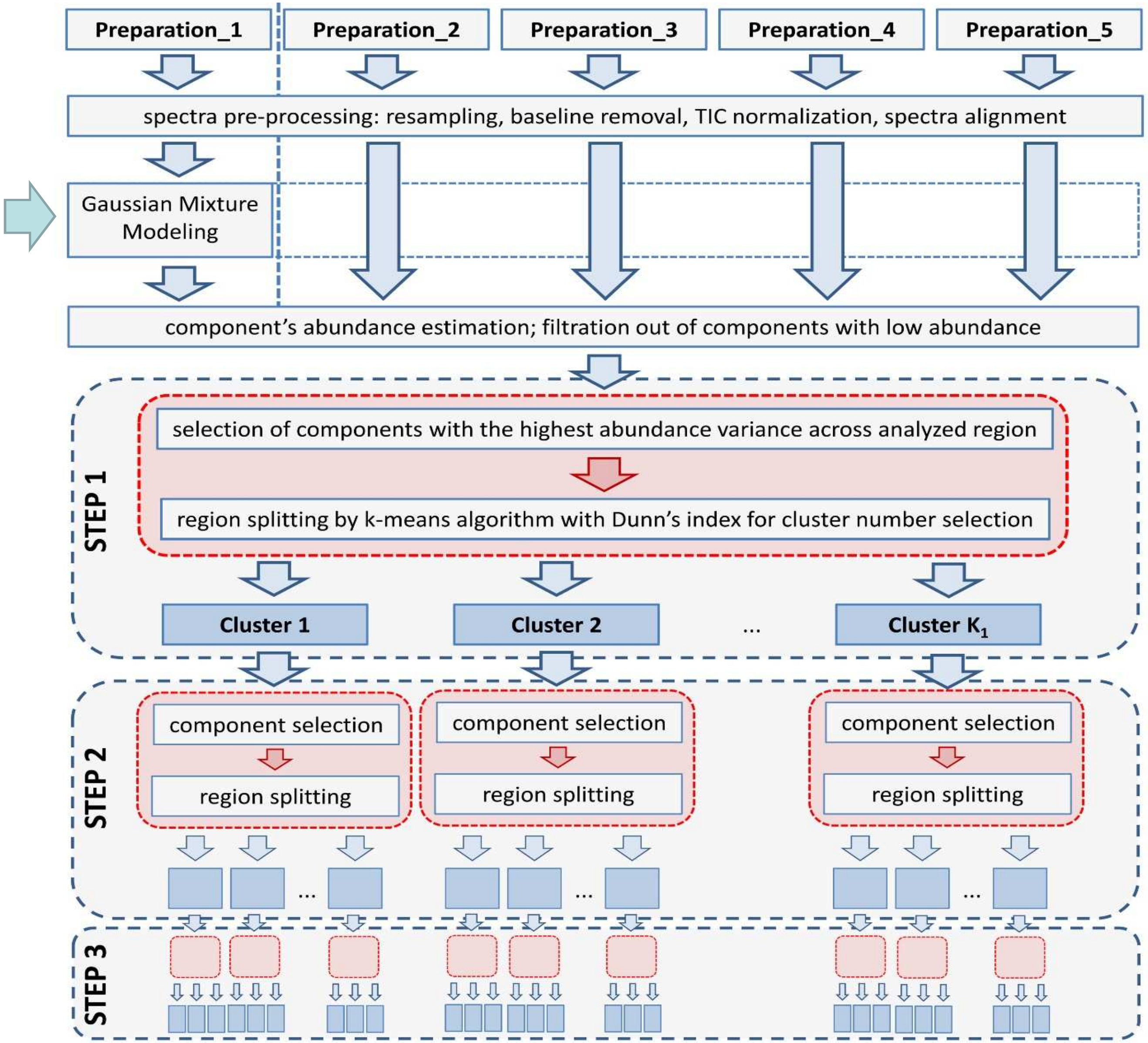
MSI Flowchart

1) selection of tissue specimens (frozen or FFPE) and preparation tissue section with histopathological examination by expert;
2) processing of tissue sections (incl. deparaffinization in case of FFPE material);
3) in-tissue digestion with trypsin;
4) matrix application
5) registration of spectra (usually 10,000-100,000 individual spectra for each tissue specimen with 50-100 µm spatial resolution)
6) detection of spectral components
7) supervised/unsupervised data analyses (incl. image/data segmentation)

Data analysis

Spectral component detection by GMM





Segmentation of MSI maps by the iterative divisive ik-means segmentations (DivIK) algorithm

Example 1 – Head & Neck Cancer

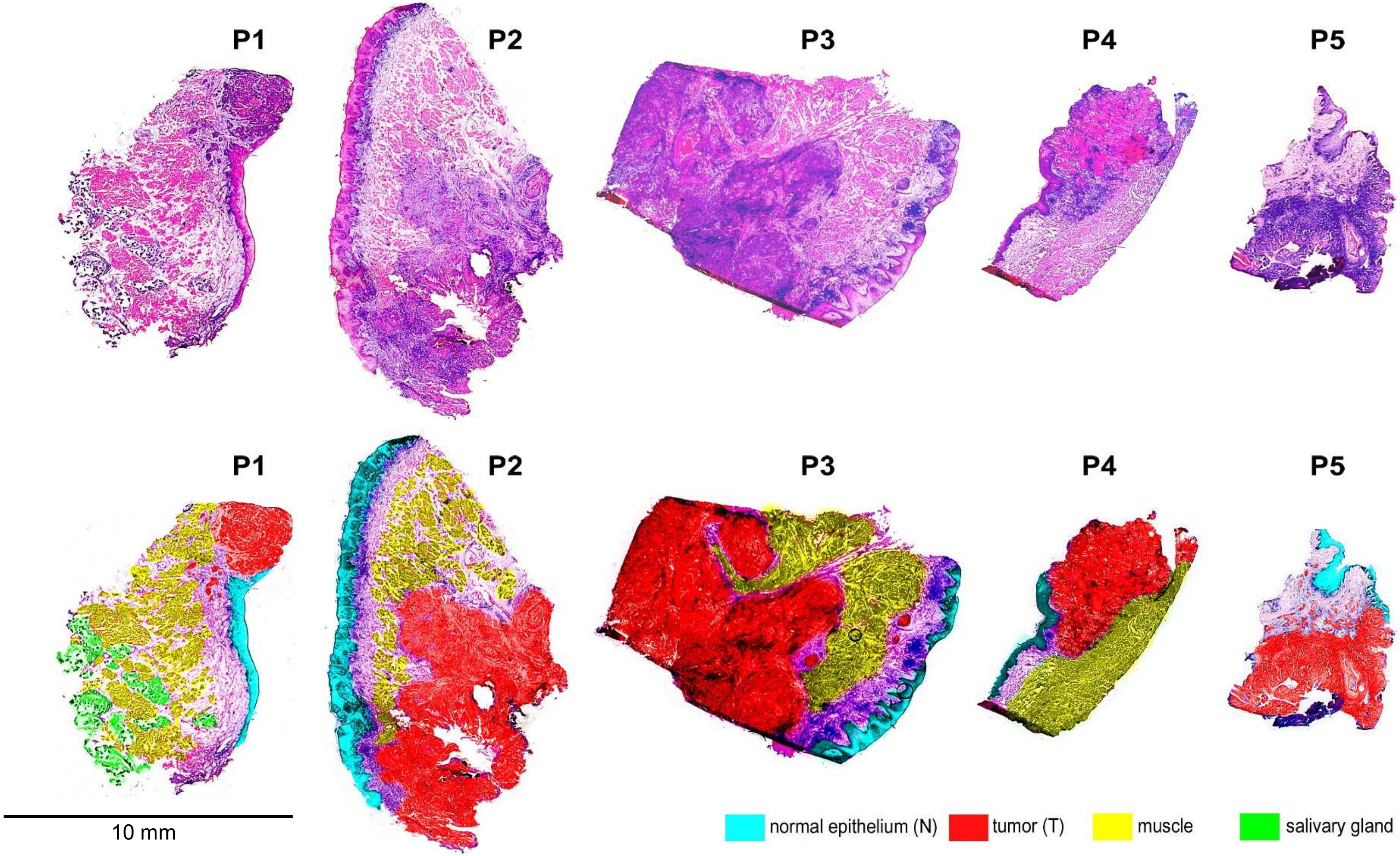
Aim 1:

- to detect peptide/protein components discriminating between oral cancer and normal mucosa

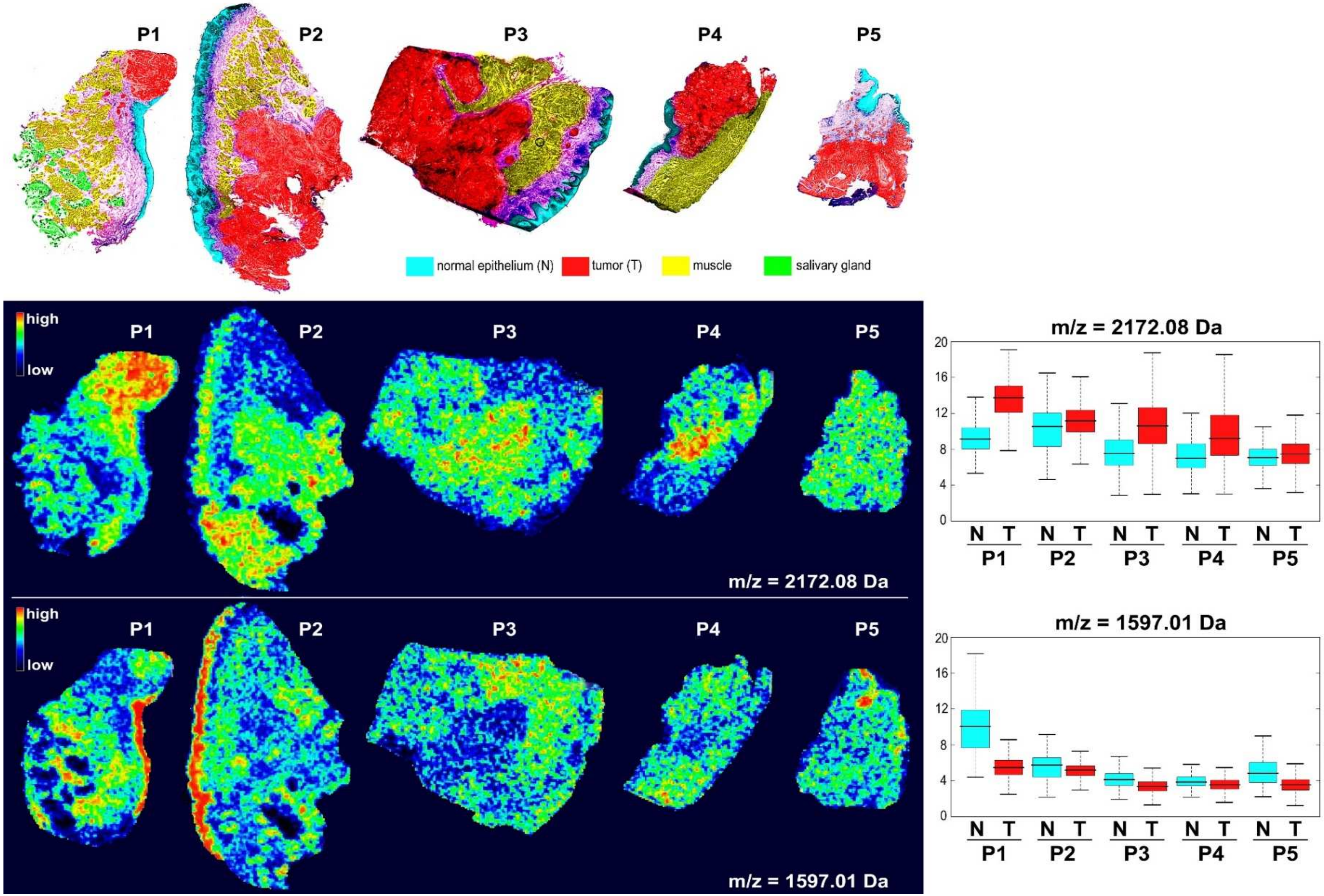
- to identify molecular sub-regions of oral cancer

Clinical material

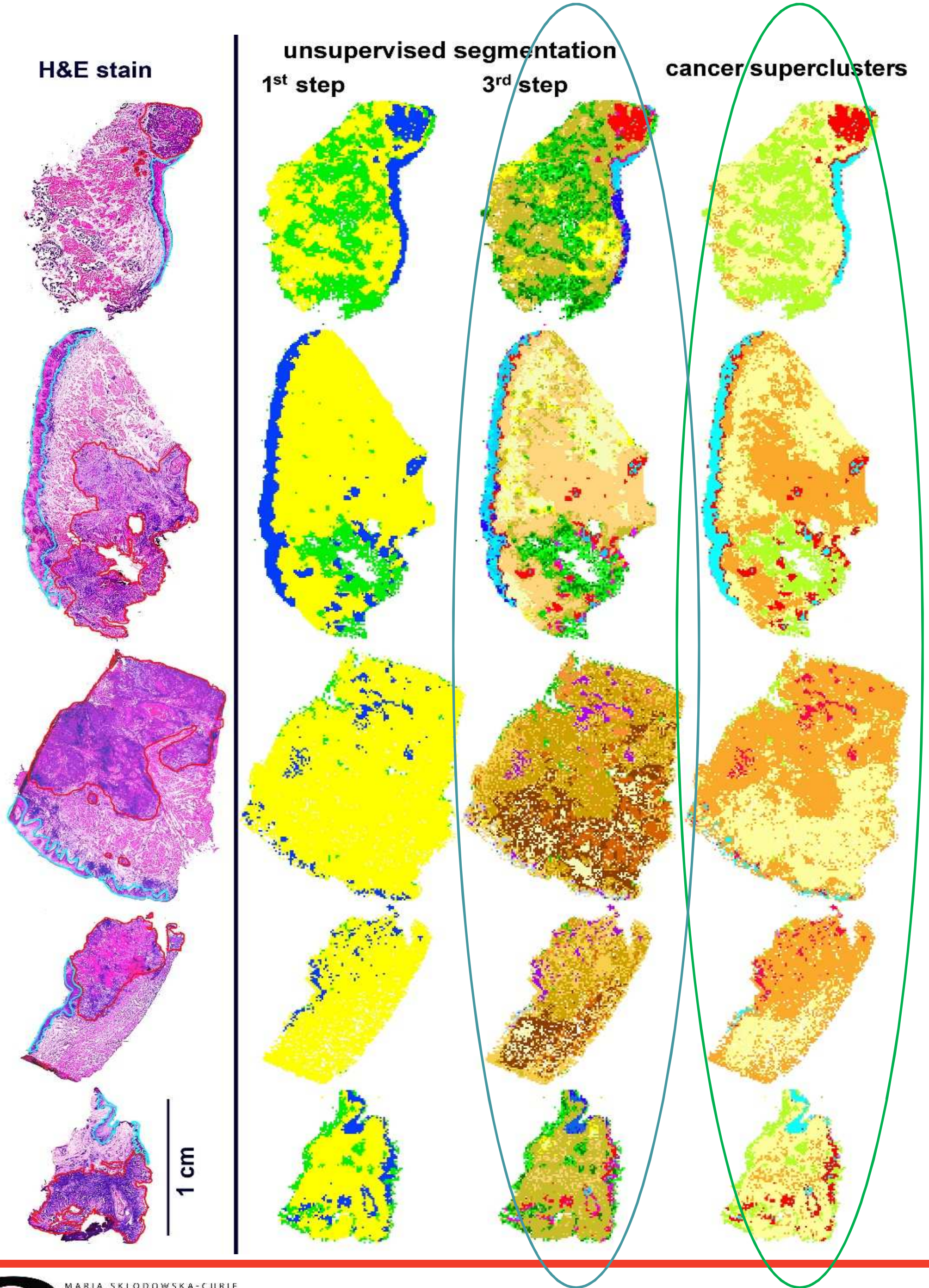
Samples of squamous cell carcinoma located in oral cavity were analyzed. Cancer tissue, together with adjacent tissues, was resected during surgery (without neoadjuvant treatment), and fresh-frozen. Major regions of interest (tumor, normal epithelium, muscle and salivary gland) was determined superficially by an experienced pathologist after HE staining.

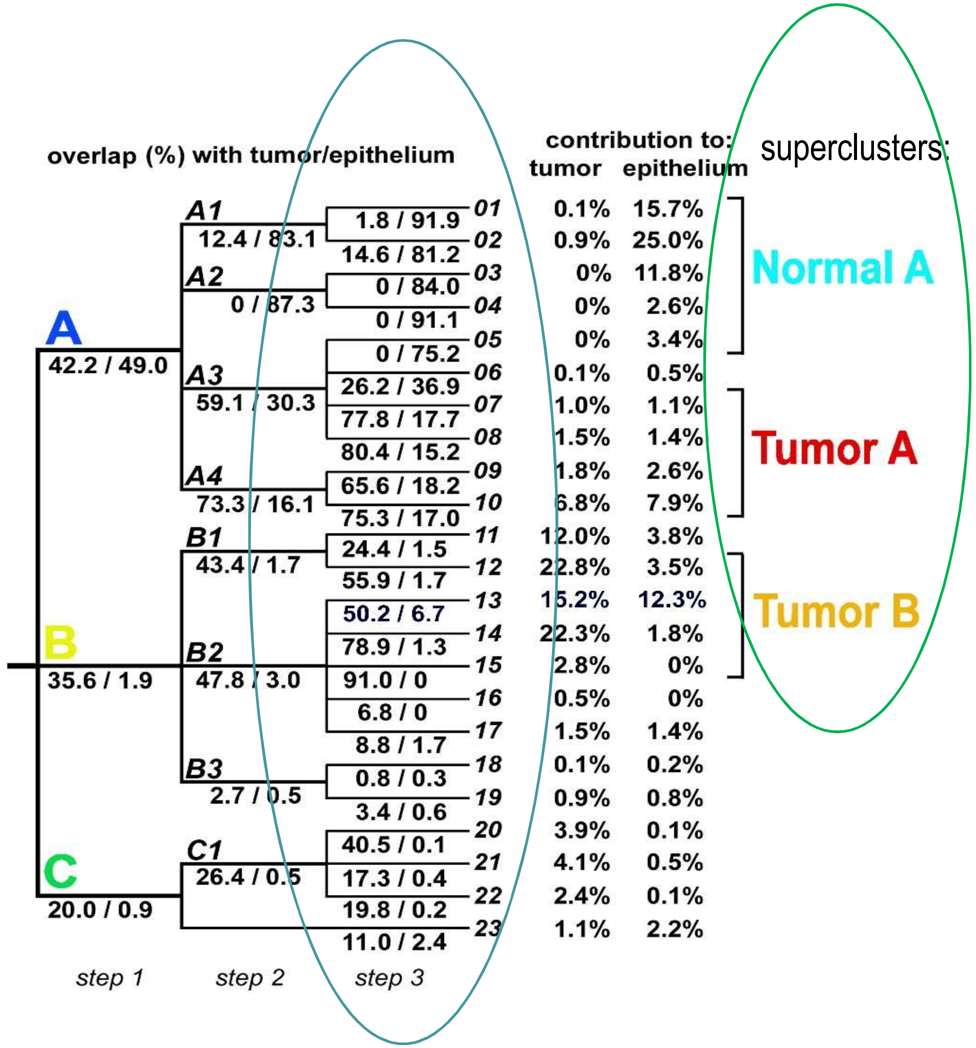


Supervised analysis:
detection of components discriminating cancer ROI and epithelium ROI



Data-driven image analysis





Conclusions (1)

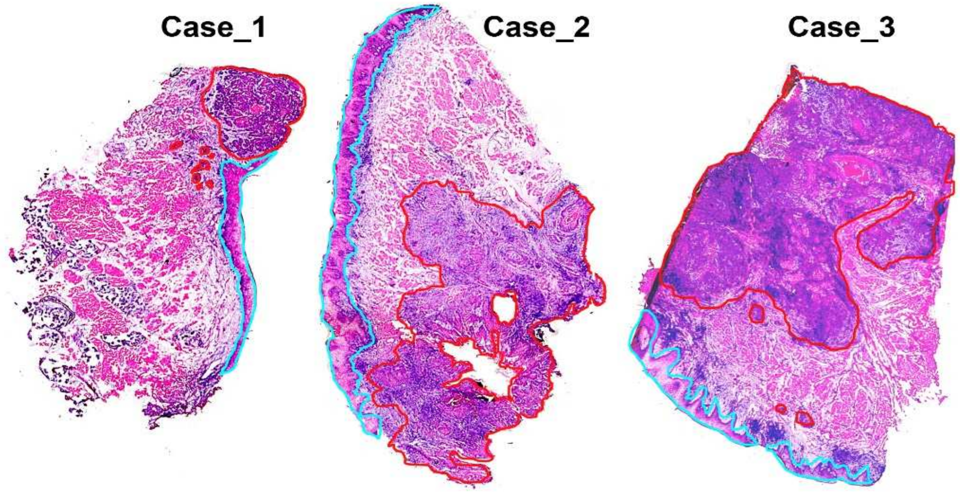
- Mass spectrometry imaging enables discovery of molecular components discriminating normal and cancerous mucosa of oral cavity
- Two sub-regions of cancerous tissues demonstrating different molecular signatures were discovered by unsupervised image segmentation

Aim 2:

- to compare directly the ability of proteome and lipidome components to discriminate oral cancer from normal mucosa

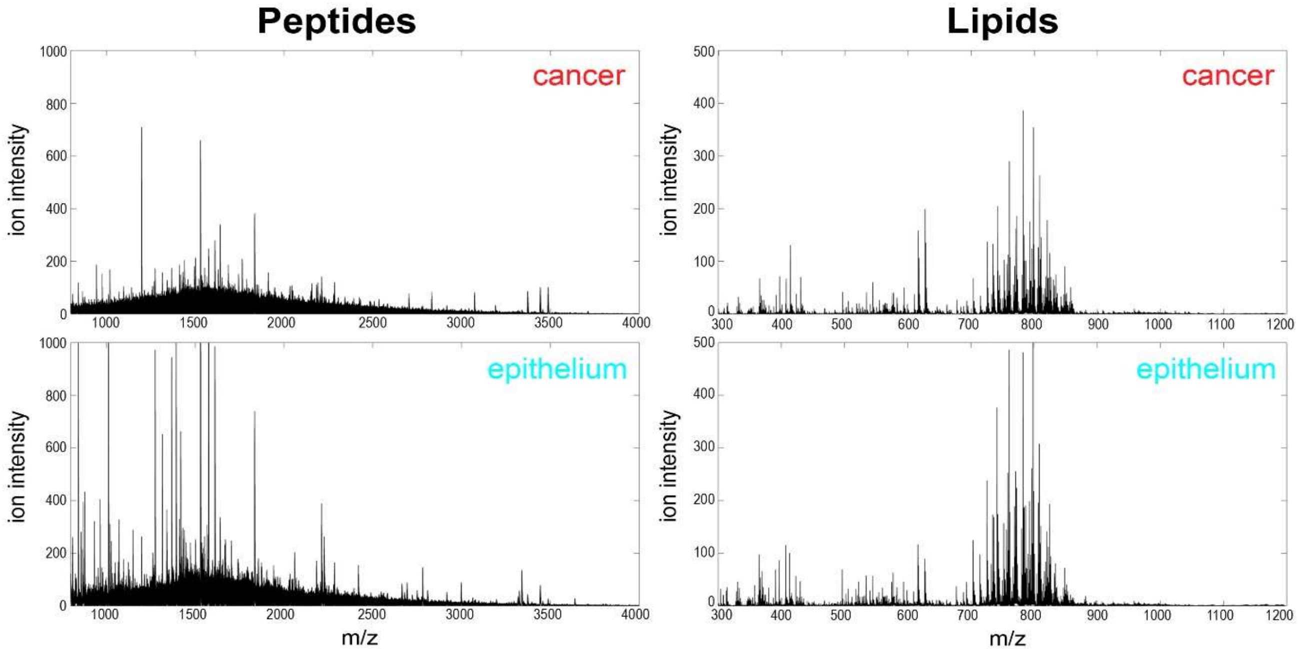
Analyzed tissue specimens
(training set)

cancer ROI
epithelium ROI



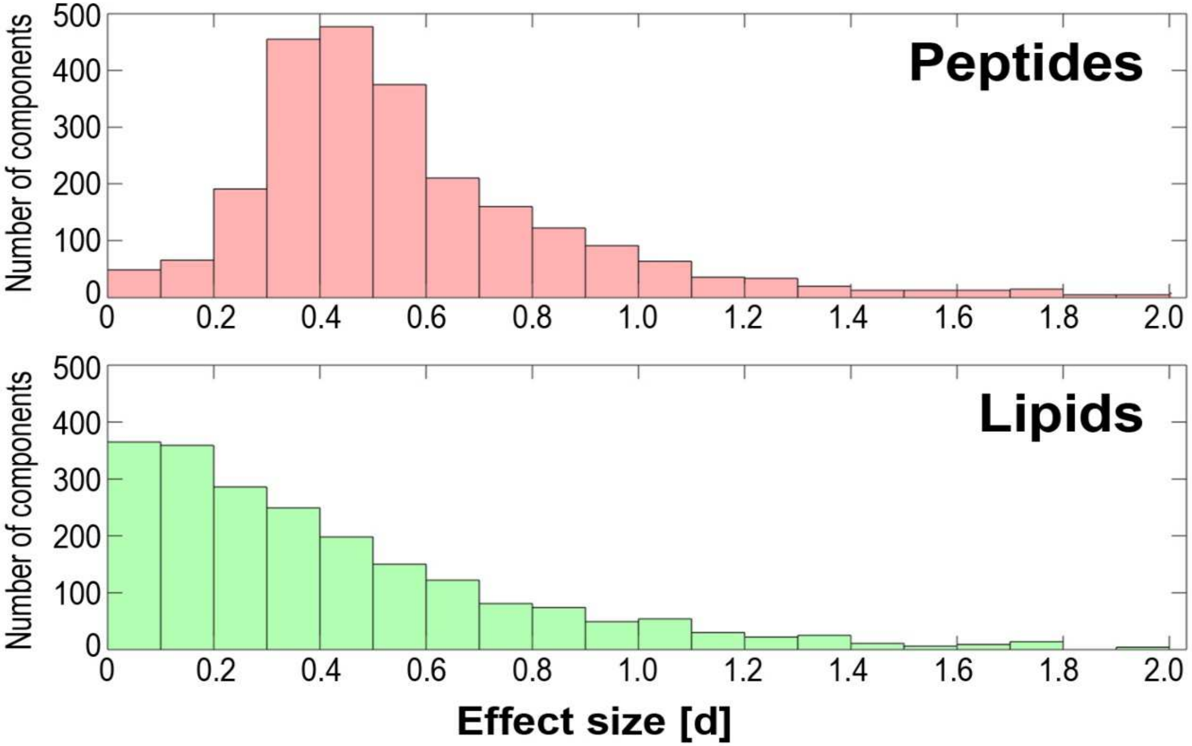
Average spectra

cancer ROI
epithelium ROI



Components discriminating cancer and normal epithelium

Number of discriminatory components (based on the effect size)

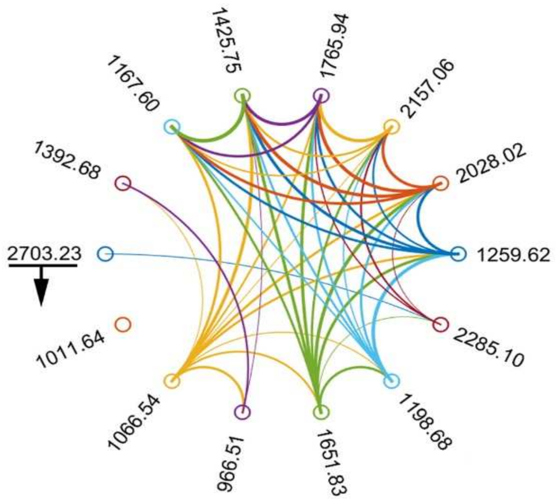


median: 0.49

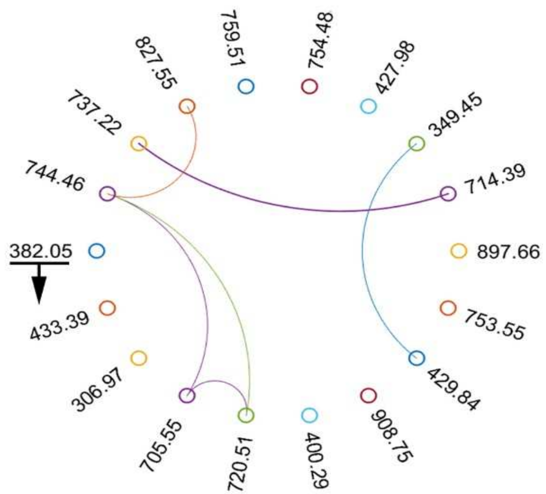
median: 0.31

Classifiers discriminating cancer and normal epithelium

Peptides



Lipids

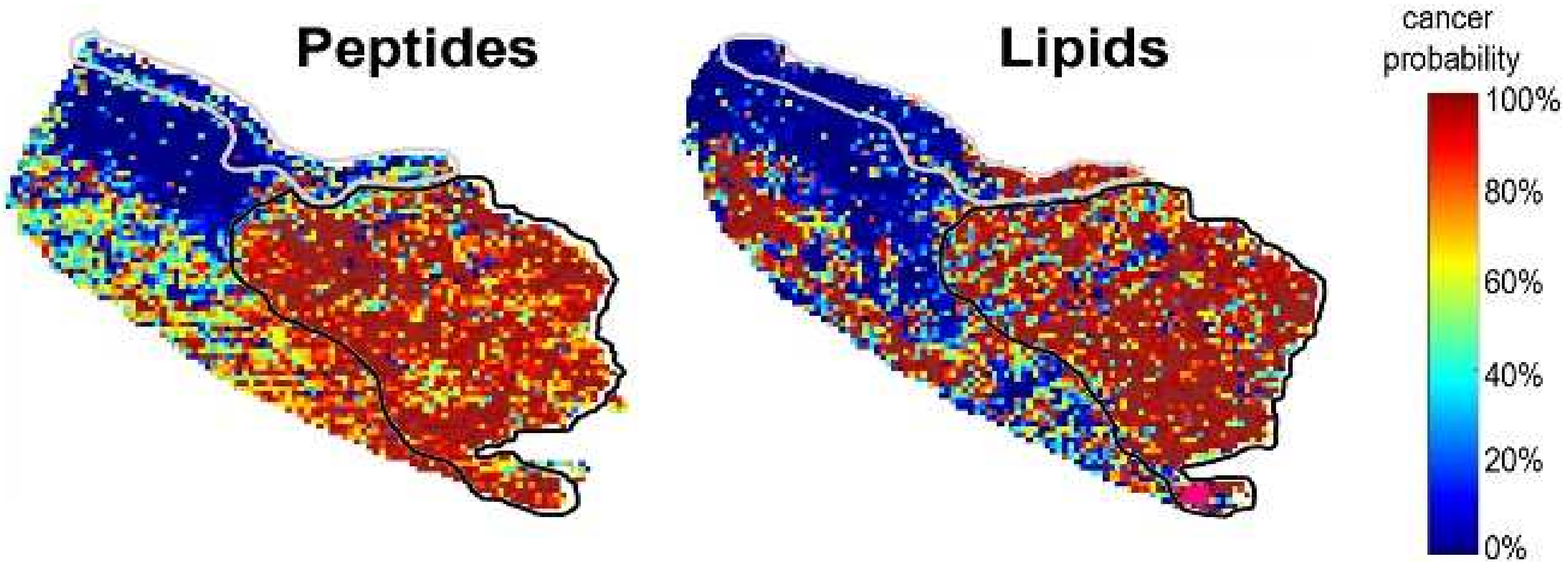


Performance of molecular cancer classifiers based on peptide and lipid components
(validation with independent tissue specimen)

Classifier indices	Peptide classifier (14 components)	Lipid classifier (18 components)
sensitivity	78.7%	56.0%
specificity	90.7%	82.4%
accuracy	89.5%	79.8%
weighted accuracy	84.7%	69.2%
precision	97.5%	94.4%
F-measure	93.9%	87.9%

Classifiers discriminating cancer and normal epithelium

Validation of cancer classifiers with an independent tissue specimen



cancer ROI
epithelium ROI

Conclusions (2)

In general, molecular differences between cancerous and normal mucosa are higher in the proteome domain than in the lipidome domain:

However, imaging of lipidome components also enabled discrimination of oral cancer and normal epithelium.

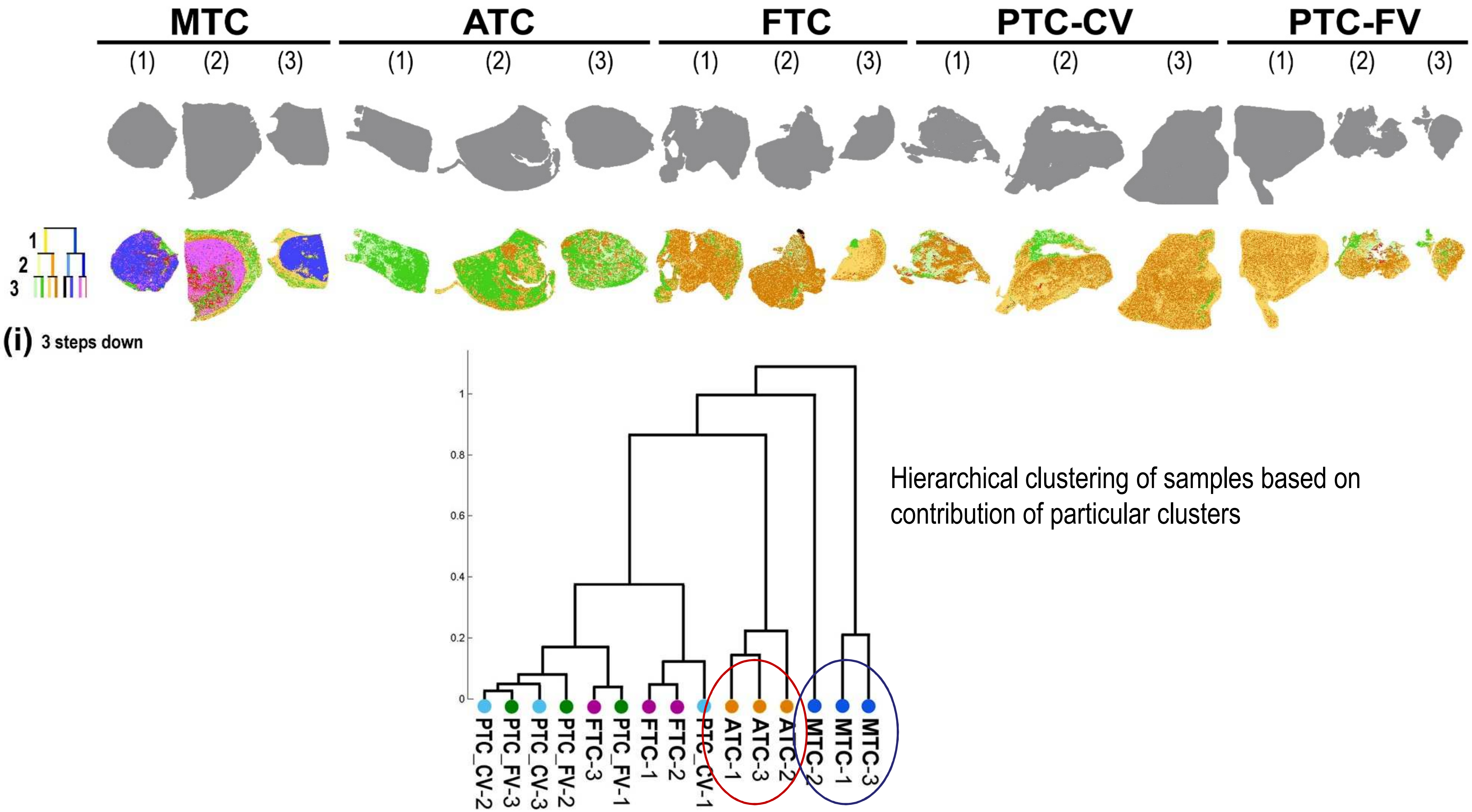
Therefore, both cancer proteome and lipidome are promising sources of biomarkers of oral malignancies

Example 2 – Thyroid Cancer

Aim 1:

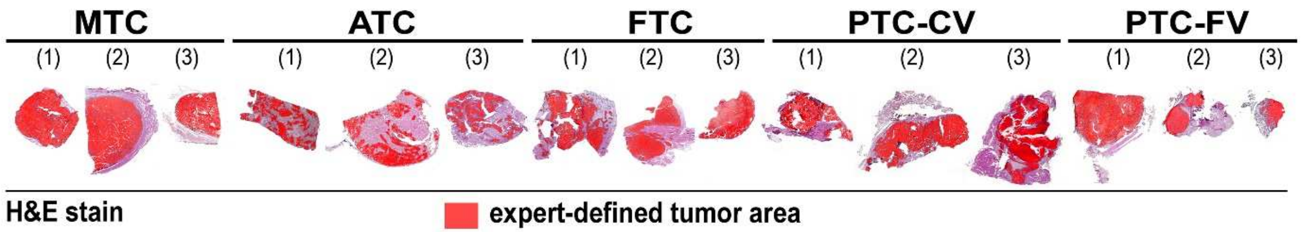
- to validate a potential of MALDI-MSI in the classification of different types of thyroid cancers
  - medullary thyroid cancer – MTC
  - anaplastic thyroid cancer – ATC
  - follicular thyroid cancer – FTC
  - classical variant of papillary thyroid cancer – PTC-CV
  - follicular variant of papillary thyroid cancer – PTV-FV

Unsupervised approach based on general distribution of clusters detected during global deglomerative segmentation of MALDI-MSI maps

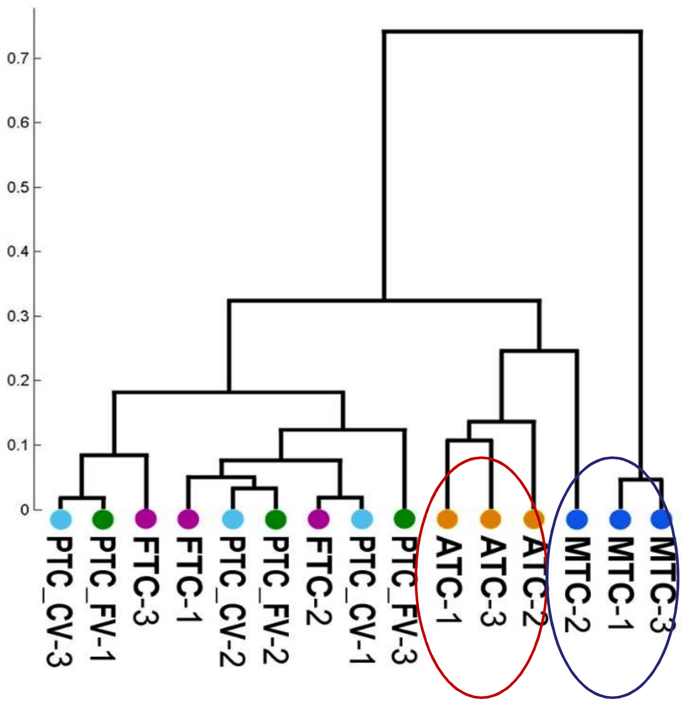


Hierarchical clustering of samples based on contribution of particular clusters

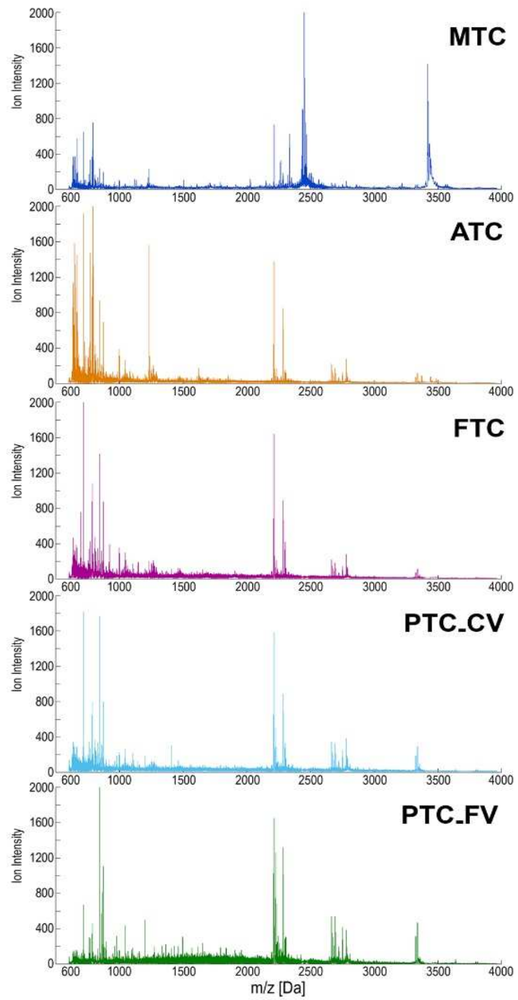
Supervised approach based on spectra „extracted" from tissue regions defined by a pathologist (expert-defined tumor areas)



Hierarchical clustering of samples based on average spectra (centroids)

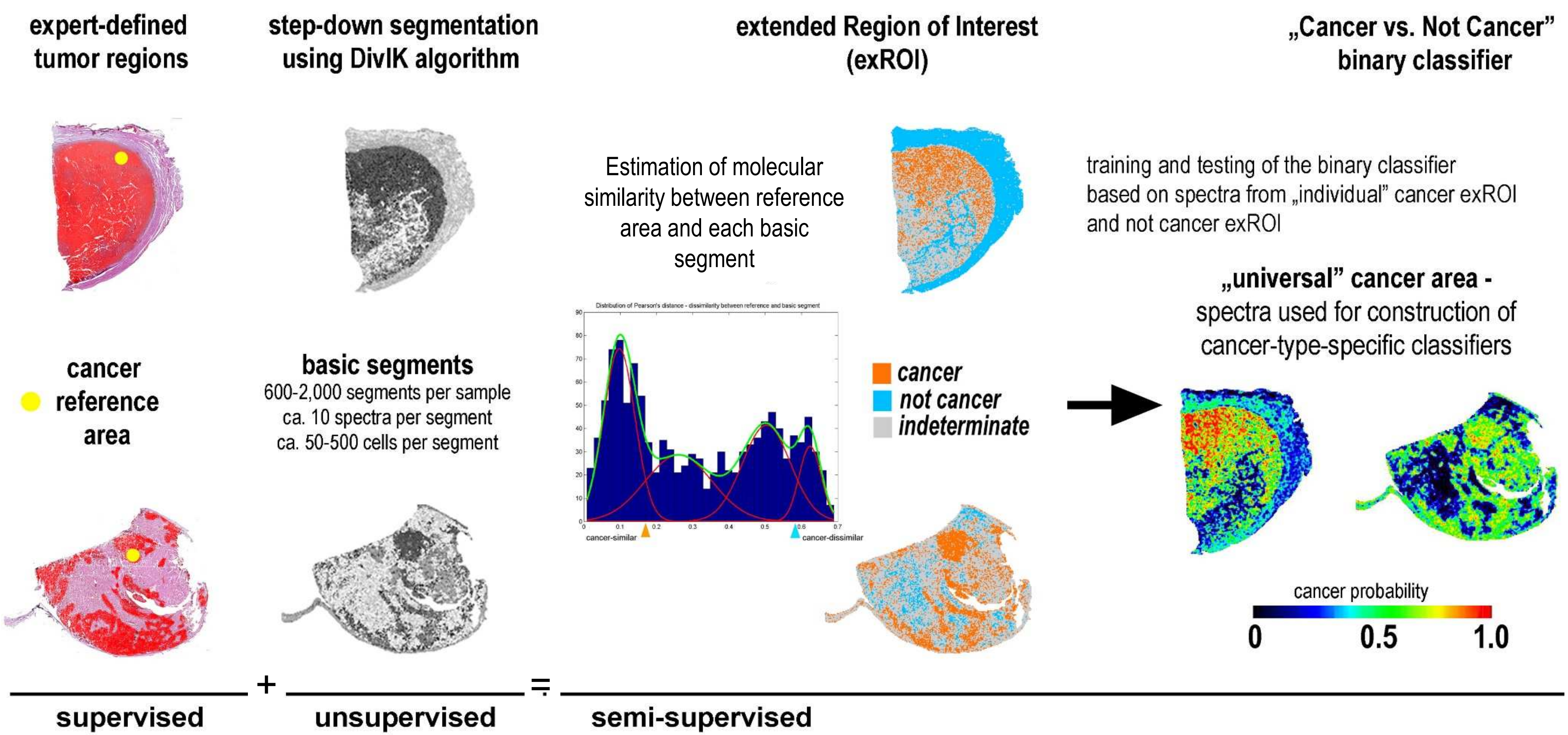


average „cancer" spectra



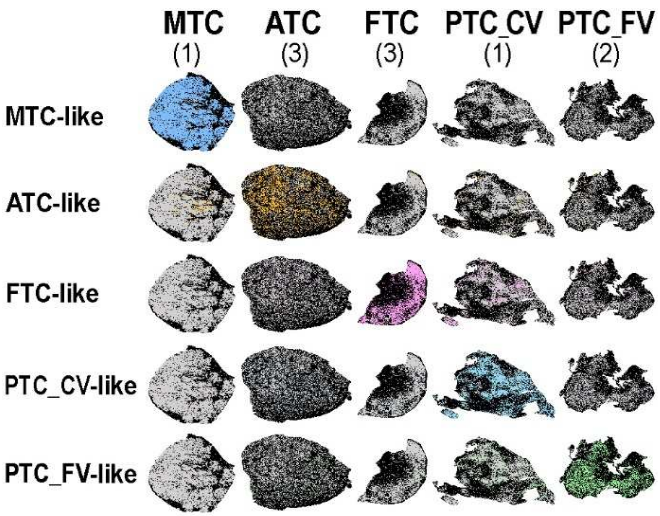
Semi-supervised approach based on combination of expert knowledge and unsupervised segmentation of MALDI-MSI maps:

automated detection of cancer exROI and binary classification „Cancer vs. Not Cancer”



Semi-supervised approach based on combination of expert knowledge and unsupervised segmentation of MALDI-MSI maps:

classification of cancer types based on annotation of individual „cancer” spectra using five „One (cancer type) vs. (all) Other (cancer types) classifiers



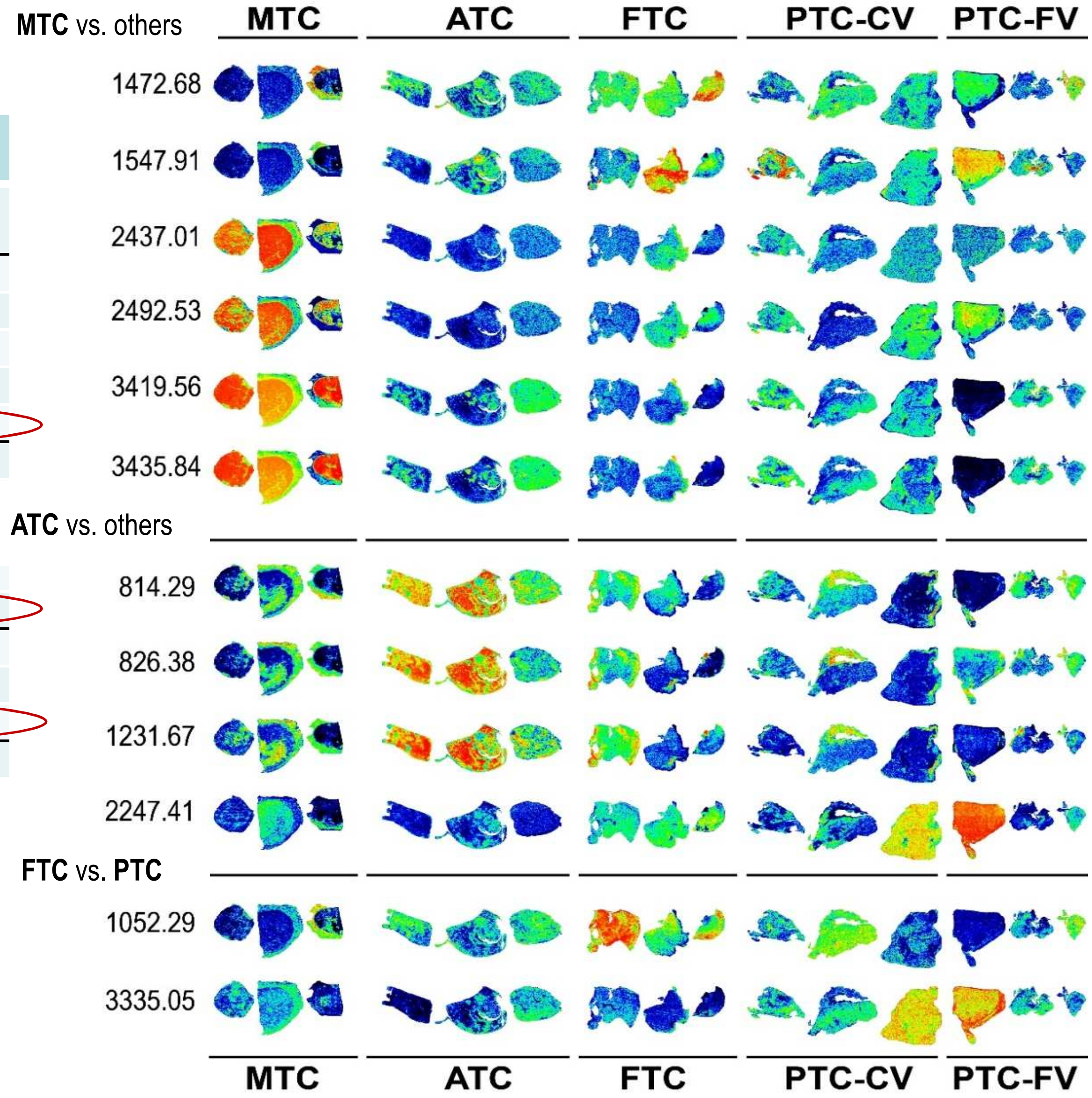
	Sample	MTC-like	ATC-like	FTC-like	PTC_CV-like	PTC_FV-like	Result	Actual Type
Training Set	MTC-1	**93.1%**	4.2%	0.2%	0.2%	0.5%	MTC	MTC
	MTC-2	**89.3%**	4.5%	1.6%	2.1%	3.2%	MTC	MTC
	ATC-1	<0.1%	**98.1%**	5.8%	1.4%	1.6%	ATC	ATC
	ATC-3	<0.1%	**62.1%**	5.6%	11.1%	6.9%	ATC	ATC
	FTC-2	0.4%	4.2%	**81.3%**	10.9%	2.5%	FTC	FTC
	FTC-3	0%	1.9%	**94.2%**	4.4%	3.5%	FTC	FTC
	PTC_CV-1	<0.1%	5.1%	14.4%	**78.4%**	15.7%	PTC_CV	PTC_CV
	PTC_CV-2	<0.1%	5.6%	14.9%	**66.2%**	25.8%	PTC_CV	PTC_CV
	PTC_FV-2	<0.1%	4.1%	6.1%	4.1%	**86.8%**	PTC_FV	PTC_FV
	PTC_FV-3	0%	15.4%	2.3%	11.4%	**71.6%**	PTC_FV	PTC_FV
Testing Set	MTC-3	**56.2%**	6.1%	11.6%	0.4%	3.8%	MTC	MTC
	ATC-2	<0.1%	**57.1%**	33.4%	14.8%	12.9%	ATC	ATC
	FTC-1	0%	11.2%	16.3%	28.3%	38.4%	undetermined	FTC
	PTC_CV-3	<0.1%	11.3%	28.7%	33.3%	**66.9%**	PTC_FV	PTC_CV
	PTC_FV-1	<0.1%	8.8%	74.4%	3.6%	**81.1%**	PTC_FV	PTC_FV

The proposed classification approach was further positively validated with <u>independent</u> samples of anaplastic (ATC) and medullary (MTC) cancers (not shown)

Detection of components with different abundances between types of thyroid cancers

comparison of „cancer” spectra selected in the supervised and semi-supervised approach

Difference between cancer types	Number of discriminating components (% of all components)		
	expert-defined cancer areas	expanded cancer ROI	both criteria
MTC vs. ATC	49%	58%	48%
MTC vs. FTC	62%	64%	59%
MTC vs. PTC-CV	51%	55%	49%
MTC vs. PTC-FV	56%	55%	50%
MTC vs. all no-MTC	**25%**	**34%**	**24%**
ATC vs. PTC-CV	9%	8.6%	6.7%
ATC vs. PTC-FV	59%	21%	20%
ATC vs. all PTC	8.0%	7.5%	5.6%
ATC vs. FTC	5.3%	2.5%	2.3%
ATC vs. all no-ATC	**2.3%**	**1.7%**	**1.2%**
FTC vs. PTC-CV	2.6%	3.2%	1.9%
FTC vs. PTC-FV	42%	13%	12%
FTC vs. all PTC	**1.7%**	**2.9%**	**1.6%**
PTC-CV vs. PTC-FV	37%	2.4%	2.2%



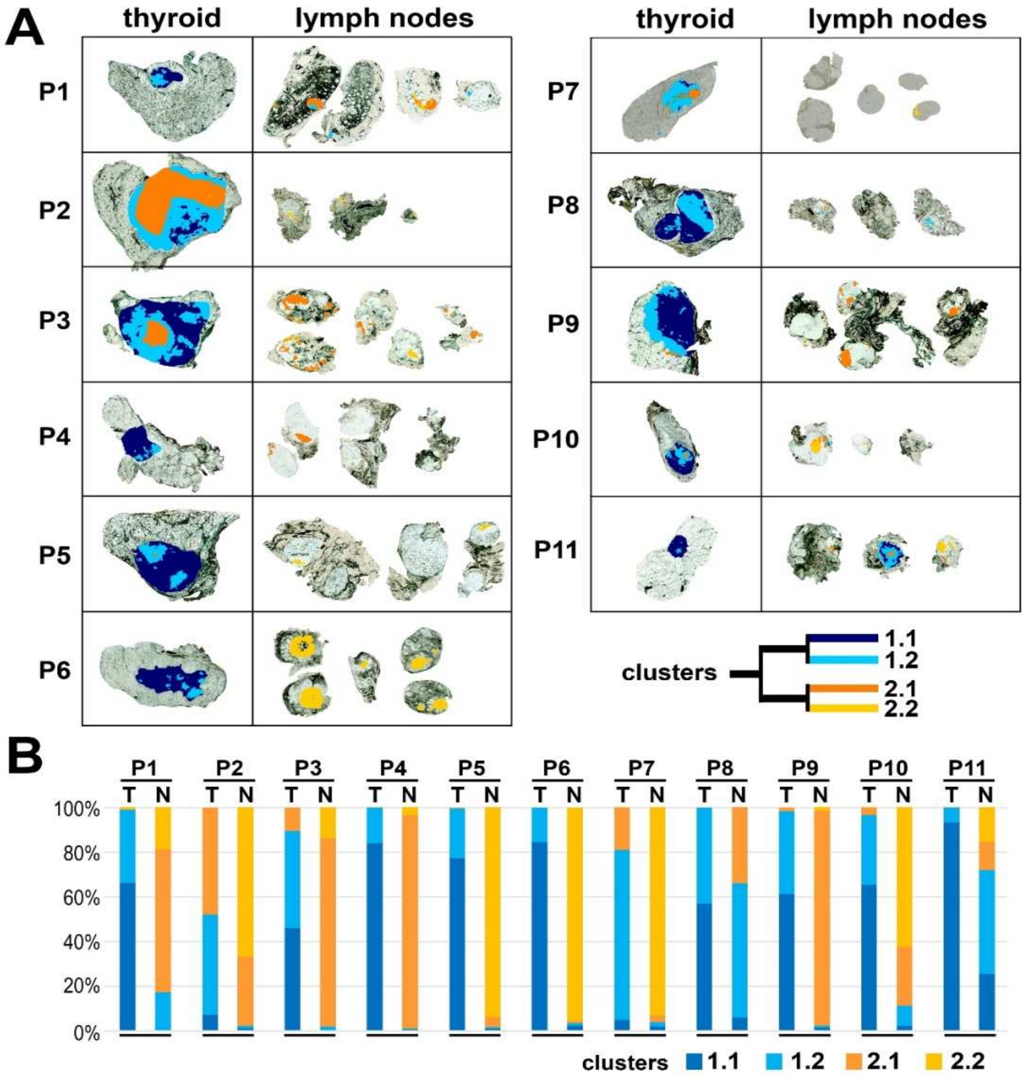
Conclusions (1)

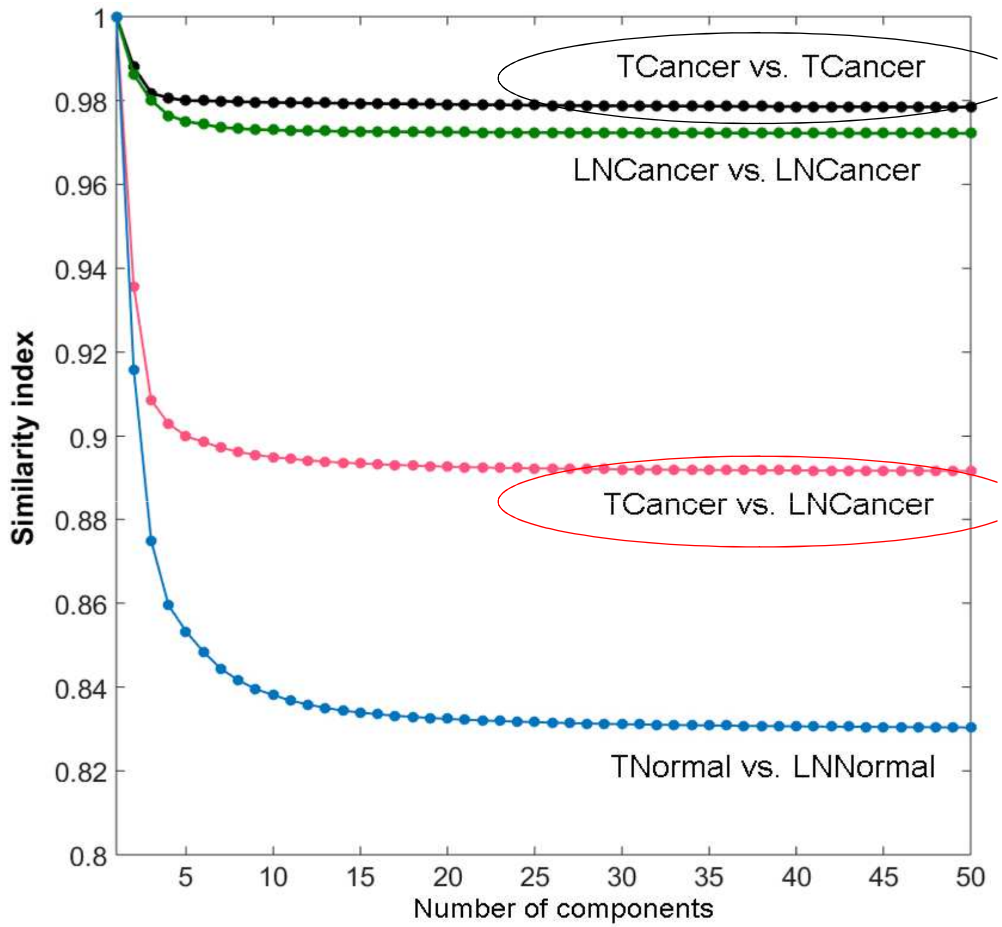
A strong separation of medullary cancer from malignancies derived from thyroid epithelium, and separation of anaplastic cancer from differentiated cancers was observed based on MALDI-MSI data.

„Extraction” of spectra from tumor areas allowed the detection of molecular components that differentiated follicular cancer and two variants of papillary cancer (classical and follicular).

Aim 2:

- to characterize molecular differences between cancer located in thyroid gland (primary location) and in local lymph nodes (cancer metastases) preliminary data





Conclusions (2)

At the phenotype level molecular inter-tumor heterogeneity (i.e., differences between cancers in different patients) could be lower than intra-tumor heterogeneity when cancer in primary location is compared to its metastasis to local lymph nodes.

Possible explanation – the influence of local microenvironment (MSI do not address the genotype of cancer cells).

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Thank you for your attention!

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Seventieth Anniversary