Cross-modality Correlation of Prostate Cancer Data from Multimodal Mass Spectrometry Imaging

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Background

Prostate cancer is one of the most common cancers diagnosed, and it is one of the main causes of cancer-related death in men in the United States and Europe. While surgery, radiation and other therapies have increased the chance of survival, the five-year survival rate is still about 30% for patients with the metastatic form of the disease. Using MALDI imaging mass spectrometry (IMS), we have been investigating cancer associated distribution patterns of lipids and proteins in mice prostate inoculated with human prostate cancer cells. Here were present novel strategies for multivariate analysis of MALDI IMS datasets of mice metastatic prostate cancer tissue for the identification of cancer-related lipid-peptide interplay.

Experimental

- Fresh frozen mouse prostate affected by highly metastatic Dunning R3327-MLL cancer was cryosectioned (12 µm).
- Matrices for MALDI imaging were applied using an HTX TM matrix sprayer: 1,5-diaminonaphthalene (for lipids), 2,5-dihydroxy acetophenone (for peptides).
- MALDI imaging data were acquired at 30µm using an MALDI TOF/TOF UltrafleXtreme mass spectrometer
- Spectral data processing, registration and combination of IMS modalities, and data visualization were done in MATLAB.
- Chemometrics analyses were done in SIMCA software (Sartorius Stedim Biotech)

Results and Discussion

MALDI imaging provides chemical information and localization from a sample surface. Image Principal Components Analysis (PCA) of IMS data is a multivariate analysis approach to analyze the image. In here, PCA resulted in enhanced clarity of anatomical features pertaining to prostate and prostate cancer (Figure 1). Thereby, PCA loadings provide the chemical explanation to the features' appearance on the scores images.

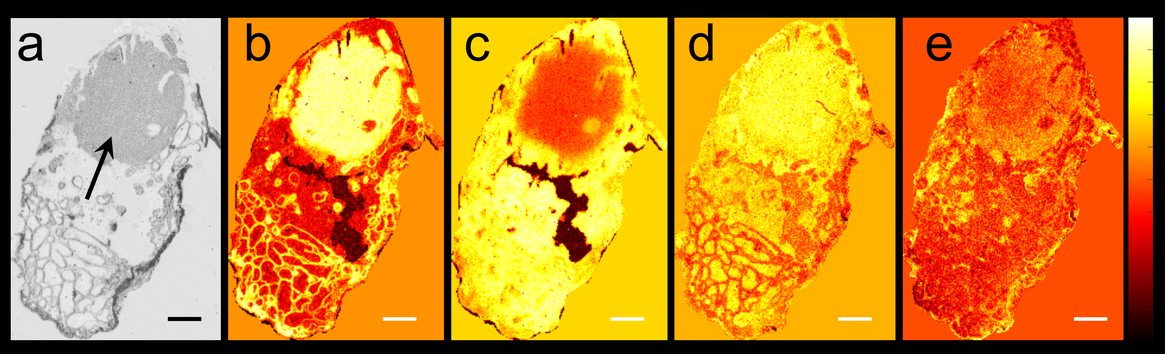

Figure 1: Mouse prostate with human prostate cancer. a) Bright-field microscopy image, arrow indicates cancer; b)+c) PCA scores images of negative ion mode lipid data; d+e) PCA scores images of positive ion mode lipid data; error bars = 800 µm

Multimodal acquisition adds complementary chemical information and thus allows a more comprehensive analysis. Yet, data from the different imaging modalities may be geometrically off-set or even distorted. Therefore, such datasets can not be combined directly for multivariate data analysis. As a solution, we applied an intensity-based automatic image registration approach in order to geometrically correct and precisely align the image data (Figure 2). The aligned data can then be combined and analyzed as one large dataset using multivariate methods such as PCA (Figure 3a) and Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) .

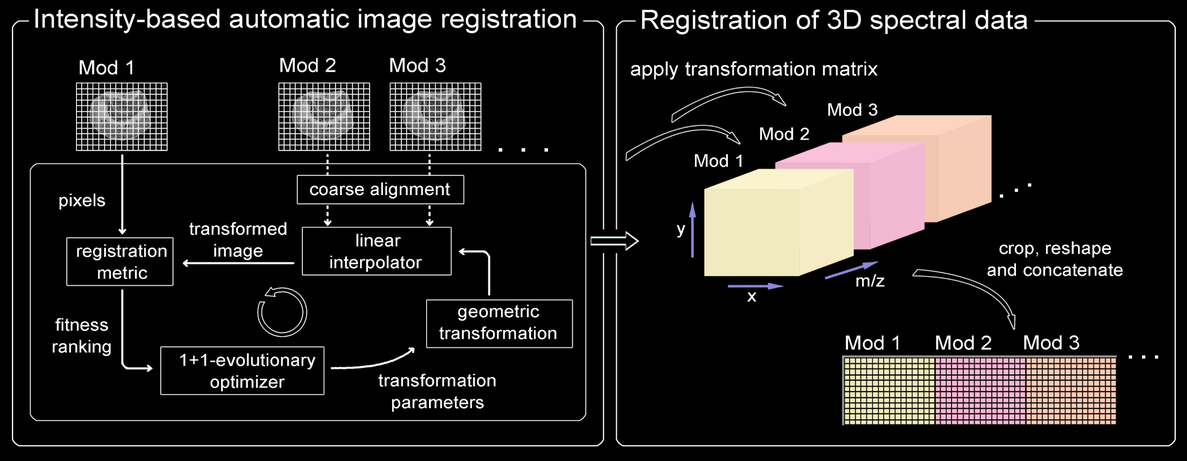

Figure 2: Image data registration workflow for the combination of IMS data from multiple imaging modalities. Automated image registration is performed using an intensity-based optimization approach particularly suited for multimodal applications. The appropriate transformation matrix is applied onto the IMS data cube for geometrically transforming the image data to match the reference modality (Mod1). The registered datasets are then combined into a multimodal dataset and prepared for multivariate analysis.

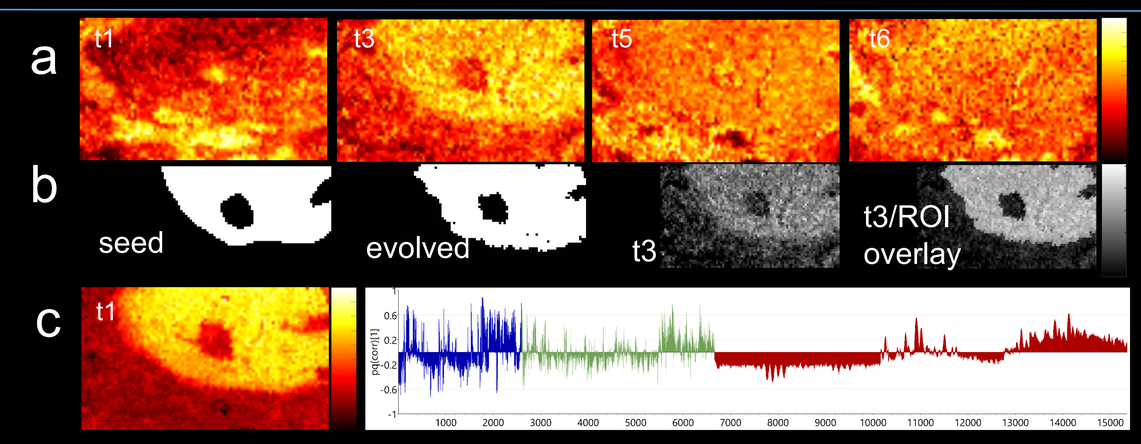

Figure 3: Region of interest analysis of trimodal MALDI IMS data from mouse prostate cancer. a) PCA scores of trimodal data: t3 scores appear to account for the cancerous tissue; b) region-based active contours segmentation based on the PCA scores matrix to assign pixels pertaining to cancerous tissue; c) OPLS-DA scores image and loadings differentiating cancer from prostate tissue. blue: lipid data negative ion mode, green: lipid data positive ion mode, red: peptide data.

For supervised multivariate analysis (OPLS-DA), the image data has to be segmented for the assignment of pixels to classes e.g. cancer vs non-cancerous tissue. In order to achieve this, we applied the strategy to compute region-based active contours segmentation based on the PCA scores matrix. The enhanced contrast of features in the PCA scores matrix finally allowed a precise segmentation of the image data (Figure 3b). With an accurate segmentation i.e. class assignment OPLS-DA computes a projection based on the differential chemical composition between the classes (Figure 3c). The OPLS-DA loadings provide the class specific mass spectral peaks which can be visualized in single ion images (Figure 4).

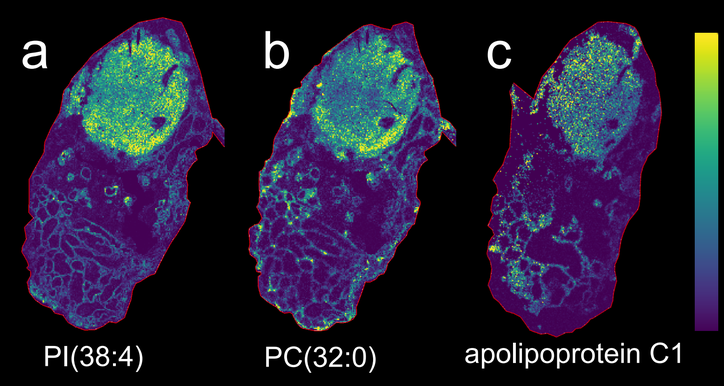

Figure 4: Ion species with high loadings for prostate cancer identified through OPLS-DA, visualized as single ion images in the original datasets. a) m/z 885.7 corresponding to phosphoinositol PI(38:4), b) m/z 734.8 corresponding to phosphatidyl-choline PC(32:0), and c) m/z 6640 corresponding to apolipoprotein C1.

Conclusions

The presented methodology allows multivariate analysis of combined IMS data from multiple modalities through accurate image registration. The presented segmentation workflow provides an unbiased strategy for sensitive annotation of regions of interest and quantitative comparison of processing procedures for multivariate analysis. These methods allow for unprecedented in-depth study of the chemical composition of cancer tissue which will hopefully allow for early discrimination between cancer types e.g. highly methastatic or less aggressive.

Terms explained

IMS: Mass spectrometry application in which the sample surface is analyzed spot-by-spot to afford an image in which each pixel is represented by a mass spectrum.

MALDI IMS: IMS technique which uses laser and a laser-absorbing matrix to desorb analytes from the sample surface. The sample preparation allows for the selection of target analytes e.g. lipids or peptides.

Registration: Alignment of different sets of image data by geometric transformation.

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