INTRODUCTION
Cancer incidence in Europe was recently estimated at 3.45 million cases, with 1.75 million deaths, and costing the EU 124 billion Euros every year. With such incidence rates, fundamental understanding of cancer biology is required to prevent (prognosis), identify (diagnosis) and treat cancer. Mass Spectrometry Imaging (MSI) is an emerging technology in digital pathology that generates hundreds of gigabytes of raw molecular data of potential diagnostic and prognostic importance. The current need for MS-based bioinformatics can be summarized as follows:

- Amount of data generated even for a single tissue due to the non-targeted nature of MSI
- High demand for precise and personalized molecular biomarkers for clinical research.
- Overall need for test automation and standardisation within budget constraints

While recent advances in MS technologies combined with the richness of molecular information, should ensure the widespread adoption of MSI technologies in the near- to midterm. The major impediment to this progress currently centres on the lack of a complete analytical solution(s) based on chemoinformatics strategies.

Here, we outline current roadblocks in translational MSI and introduce a comprehensive workflow designed to address current methodological limitations [1,2].

METHODS
An integrated bioinformatics solution is presented for clinical research, that allows intuitive histology-directed interrogation of MSI datasets for tissue specific biomarker recovery, automated tissue classification and tumour heterogeneity assessment [1]. The proposed bioinformatics pipeline includes a series of designated steps covering (i) robust pre-processing workflow that is capable of reducing data complexity while simultaneously retaining disease-relevant information; (ii) image-fusion algorithms for automated co-registration of biochemical and histological datasets;

(iii) putative molecular ion annotation tools; (iv) computational statistical learning approaches for amalgamation of MSI with other diagnostics techniques v) entropy driven tumour heterogeneity assessment.

RESULTS
Using the proposed bioinformatics solution, we have investigated region-specific lipid biochemistry in liver cancer tissue section generated from MSI data acquired on different Waters mass spectrometry platforms (Desorption Electrospray Ionization (DESI) mounted on a SYNAPT G2-Si and a Xevo G2-XS).

MSI profiles of the studied tissue sections consisted predominantly of complex lipids including phosphatidyl-inositol, phosphatidyl-serials, and phosphatidyl-ethanolamine plasmalogens, among others. As an example, the unsupervised image segmentation techniques are demonstrated to extract similarities and differences of biochemical patterns of tissue-realted pixels without prior knowledge of their origin (Figure 1).

Figure 1: Automated image co-registration, and unsupervised tissue segmentation using multivariate statistical approaches. Reconstruction of two distinct histological regions of hepatic liver tissue section (hepatic tumour and tumour adjacent muscle) based on molecular ion patterns extracted by means of principal component analysis.

Cancer is a heterogeneous disease characterised by a variety of molecular phenotypes influenced by a complex interplay of genomic and environmental factors. The quantitative metric based on entropic framework [2] is demonstrated to assess the heterogeneity of molecular phenotypes within the tumour and its surrounding tissues (Figure 2).

The use of supervised segmentation techniques and statistical correlation techniques for tissue-specific molecular feature recovery is shown on Figure 3. Unique lipid patterns were observed using this approach according to tissue type. The proposed strategy offers valuable research insights into tumour biochemistry, tumour induced heterogeneity of molecular phenotypes and should facilitate the compilation of a large-scale tissue morphology-specific MSI spectral database to research next-generation, fully automated histological approaches.

Figure 2: Entropy based strategy for tumour induced molecular heterogeneity assessment. A) PC scores of 1000 randomly selected spectra reveals larger scatter in tumour associated spectral profiles B) The simulated clustered and uniformly distributed sample points with equal multivariate variance but different entropic values show the advantage of entropy approach to capture the diffusiveness of metabolic phenotypes as opposed to the variability C) The entropy based approach shows that tumour associated molecular phenotypes are more heterogeneous that those in the surrounding tissues.

CONCLUSION

- Without a step-wise increase in the computational interpretation of the highly complex MSI data sets, it is unlikely that MSI technologies will be able to realize its significant potential in clinical research.
- The proposed bioinformatics solution will allow validation of Mass Spectrometry Imaging data in translational cancer research settings.

References:

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