Multiple Desorption Electrospray Ionization (DESI) mass spectrometry imaging at different pixel resolutions on the same tissue section.

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Over the years Desorption Electrospray Ionization (DESI), a surface analysis technique incorporating an electrospray probe, can be utilized as a spatially resolved imaging technique by rastering a surface under the spray using a high precision XY stage. As the electrospray droplets impacts upon the surface, chemical constituents are desorbed and carried towards the atmospheric inlet of the instrument. Ionization occurs due to the charge imparted onto the droplets; therefore no modifications to the sample such as matrix addition are required. The profile of the spray point on the surface affects the spatial resolution of the imaging experiment. Modification of the spray profile can be achieved using different gas and solvent flow rates and this affects the spatial resolution. Moreover using them appropriately along with the right voltages, the DESI technique does not destruct the tissue surface and the same tissue section can be re-analyzed with different experimental conditions or techniques. Here we describe the workflow that allows one experiment to be acquired with a SYNAPT G2-Si at a low spatial resolution, followed by the analysis of a specific region of interest at a higher spatial resolution and finally the same tissue section being histologically stained.

In the first DESI imaging experiment, a raster pattern was defined over the whole tissue with a pixel size of 150 µm for the porcine liver and 200 µm for the human liver sample. The second experiment was carried out on a specific region of the same tissues, both at 50 µm (workflow in figure 1,A).

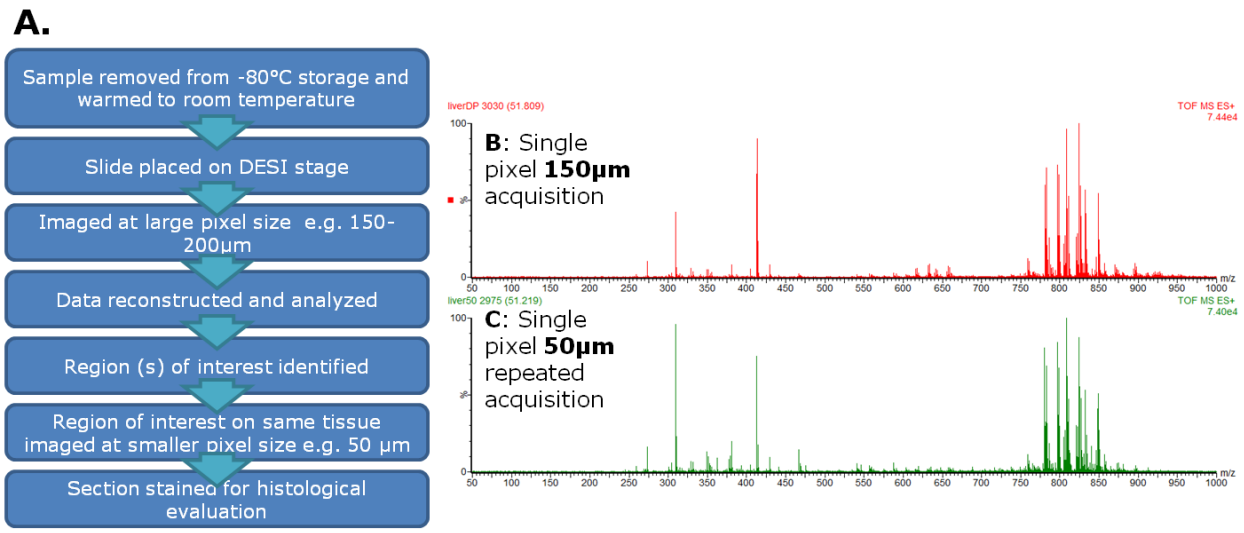

Figure 1: A) Workflow for the multiple DESI imaging experiment using different spatial resolutions, B) MS spectrum from a single pixel of 150 µm on a porcine liver pristine surface, C) MS spectrum from a single pixel of 50 µm on a altered surface (same porcine liver after the first pass of the DESI spray).

Figure 1,B and C), the mass spectra show that endogenous lipid and metabolite signals are plentiful from the DESI analysis. Each spectrum was obtained from a single pixel acquired on the porcine tissue section at different spatial resolutions (150 µm followed by 50 µm) from the same tissue. Interestingly the relative intensities of the lipid signals are comparable, showing that the surface analytes are not depleted by the DESI analysis. Examples ion images are presented in figure 2. Figures 2,A and B display the ion images of PG lipid at m/z 927.7 showing a pixelated ion image of 150µm spatial resolution from the pristine surface, followed by the DESI imaging from the altered surface at 50µm spatial resolution. It can be clearly seen from the pixilated ion images the improvement of the ion image quality in the higher spatially resolved experiment, without delocalization of the molecules on the tissue surface from the first passage of the DESI spray. Figure 2,C and D are Red/Green (RG) overlay of ion images from m/z 848.55 lipid PC (38:4)K+ (red ion image) with PG lipid at m/z 927.7 (green ion image). Figures 2,C and D) display the ion images of the same lipid species acquired during the second experiment acquired at 50 µm.

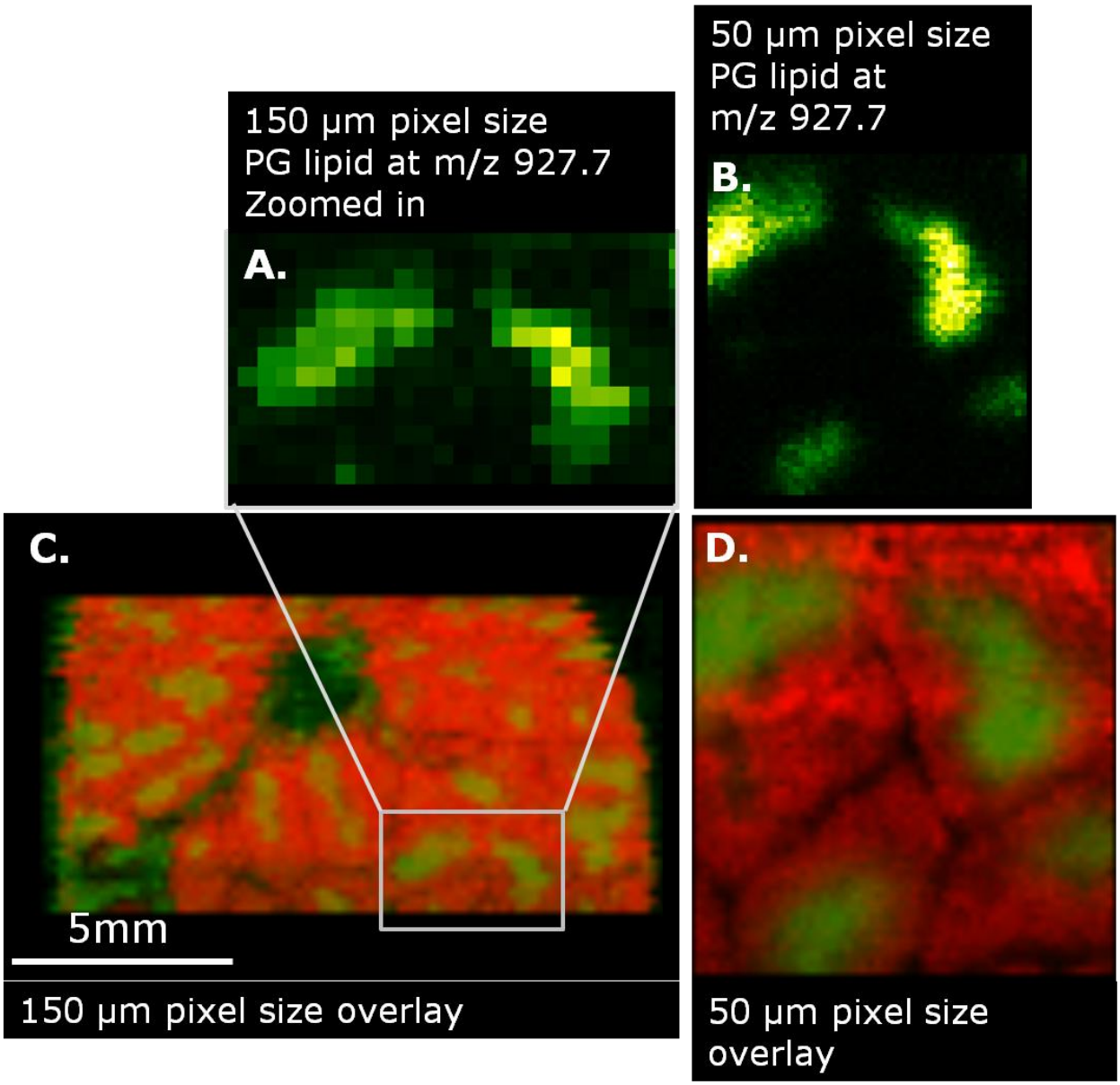

Figure 2: DESI imaging analyses of a porcine liver section in positive ion mode. A & B) Ion images of m/z 927.7 at A) at 150µm spatial resolution on pristine surface and B) at 50µm spatial resolution on the previously analyzed surface. C&D) Red and green overlay of m/z 848.55 (PC (38:4) K^+) (red) and m/z 927.7 (green) in interpolate mode with C) at 150µm spatial resolution on pristine surface and D) at 50µm spatial resolution on altered surface

Analysis of a clinically relevant human liver sample containing both healthy cells and a secondary tumor was performed. Imaging the whole tissue DESI imaging at 200 µm optimized the speed of analysis and allows regions of interest to be investigated further. Figure 3 shows

images from the 200 um acquisition with lipids m/z 771.52 PG (36:3)$^-$ (red ion image) and m/z 750.55 PE (O-38:5)$^-$ (green ion image) specifically differentiating the tissue type (3,A). Focusing in on the healthy region of the tissue with the 50 µm DESI imaging experiment, improved image clarity is obtained and it can be seen that some tumor cells may have already penetrated the healthy region of the tissue. The tissue is in a sufficiently unaltered condition that it can be subsequently stained following the Hematoxylin and Eosin (H&E) protocol (figure 3,D) for accurate characterization of the tissue regions.

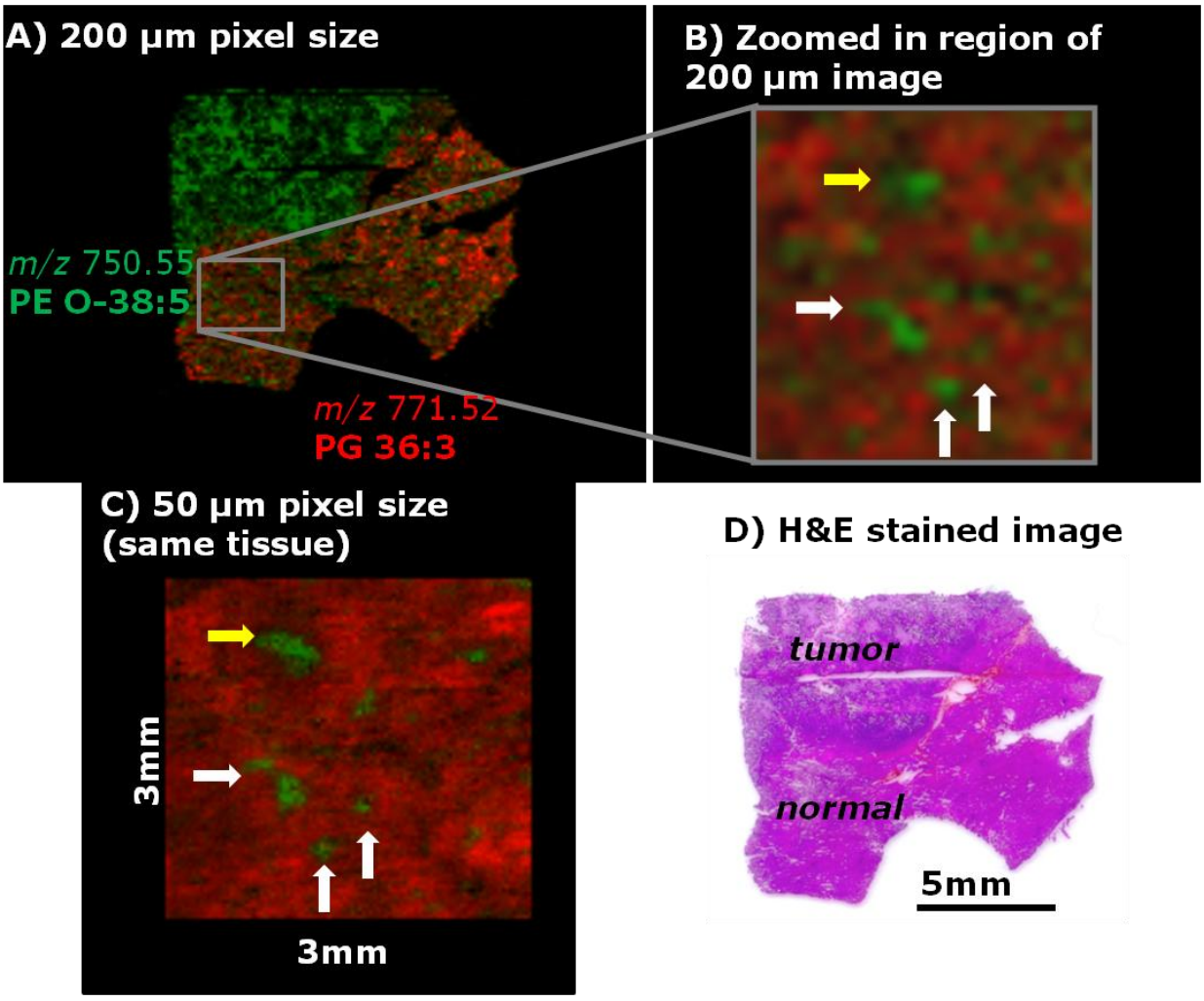

Figure 3: DESI Imaging analyses of a human clinical sample in negative ion mode, followed by H&E staining. A) Red & green overlay of m/z 771.52 (PG (36:3)$^-$ (red ion image)) and m/z 750.55 (PE (O-38:5)$^-$ (green ion image)) at 200 µm from a pristine surface, B) zoomed-in view of an area from the "normal" tissue section at 200 µm. C) Red & green overlay of the same ion

species imaged at 50 µm in a second experiment from the same tissue section. D) H&E staining of the tissue section after the two DESI experiments.

DESI Imaging provides metabolite and lipid information directly from a tissue section with no pre-treatment. With low flow rates the tissue section can be analyzed multiple times without significant degradation of signal or modification of chemical signature. A whole tissue can be scanned at a coarse spatial resolution then a region of interest can be interrogated at a smaller step size.

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