INTRODUCTION
Breast cancer is a complex and heterogeneous disease that has distinct biological features and clinical phenotypes. The biochemical understanding of breast cancer, which is the most frequently diagnosed form of cancer and the second leading cause of death in Western women, has greatly profited from research using genetically modified mouse models.

Mass spectrometry imaging (MSI) is an established analytical tool for biomolecular research which can accurately determine the spatial location of molecules in a tissue section. MALDI has been a widely used tool for biomolecular research which can accurately determine the spatial location of molecules in a tissue section. However, MALDI-MSI has not been applied to breast cancer and is not as widely known as MALDI-MS.

METHODS
Tissue sample preparation
Experiments were conducted on normal (control) and tumour samples from mouse mammary tissue using the polyoma middle T oncoprotein (PyMT) mouse model of breast cancer, which were dissected and stored at -80°C until analysis by mass spectrometry. For MALDI experiments, a SunCollect nebulizing spray device was used to evenly apply the different matrices in several coats: 1) o-quinon-4-hydroxycinnamic acid (CHCA) 5 mg/mL solution in acetonitrile/water (70/30 v/v), 2) CHCA 5 mg/mL solution in methanol/water (70/30 v/v), and 3) 2,5-Dihydroxybenzoic acid (DHB) acid in 5 mg/mL solution in methanol/water (70/30 v/v). DESI imaging experiments require no sample preparation as desorption and ionization are initiated by charged droplets (95% MeOH, 5% water) impacting directly on the surface.

Mass spectrometry
All experiments were carried out on a MALDI SYNAPT HDMS G2 -Si mass spectrometer in positive ionisation mode, with a mass range of m/z 100–2,000. When the intermediate vacuum MALDI source was in operation, a solid-state diode-pumped Nd:YAG laser with a repetition rate of 1 KHz was used. The 2D DESI stage was mounted, the MALDI source was uncoupled and the electrospray inlet block was installed along with an inlet capillary. When the Prosolia 2D DESI stage was mounted, the MALDI source was uncoupled and the electrospray inlet block was installed along with an inlet capillary.

MALDI-MS
Laser: Nd:YAG laser (355 nm)
Pulse rate: 1,000 Hz
Spatial resolution: 45 µm (lateral)
DESI-MS
Flow rate: 1.5 µl/min
Capillary voltage: 4.5 kV
Nebulising gas: 5 bar
Spatial resolution: 50 µm (lateral)
Stage speed: 100 µm/sec
Data management
Slides were scanned using a flatbed scanner and regions to be imaged were defined in High Definition Imaging Software (HDI) v1.4 (Waters). DESI and MALDI imaging datasets were mined using MassLynx and DriftScope as well as processed and visualized using HDI 1.4 where the ion mobility dimension is fully utilized and integrated. Extensive statistical analyses were carried out using EZ Info (Umetrics). Regions of Interest (ROIs) defined in HDI 1.4 and associated intensities were averaged and TIC normalised in the form of a .csv file which was loaded directly into EZInfo.

RESULTS
Control breast tissue analysis
Initial experiments were carried out using the control tissue consecutive tissue sections, prepared with the three sample preparation methods for the MALDI experiment and also analyzed using DESI. Furthermore an additional tissue was H&E stained. The control breast tissue contained mostly adipose tissue, surrounding the remaining lymph node tissue for two of the tissue sections (DESI and MALDI CHCA MeCN/Water). The TG which are delocalized are the peaks highlighted in grey on the MS spectra in figure 1. The TG localized within the tissue are highlighted in yellow. To understand the difference between the two series, MSI/MS experiments were carried out (see figure 3) and identification was possible using the fragment information from Lipid Maps. From the statistical PCA results (figure 4) and database search by pathologist annotation from the consecutive H&E stained image.

CONCLUSION
- Control and tumour mouse breast tissue sections were analyzed by DESI and MALDI.
- Three sample preparation methods for the MALDI imaging experiments were tested: CHCA in MeCN, CHCA in MeOH and DHB in MeOH.
- From the control tissues which contain a high level of adipose tissue, phosphatidylcholine and triglyceride class of lipids were detected.
- Depending on the type of TGs the protonated ones were detected outside of the tissue whereas potassiated species were detected within the tissue, mainly by DESI.
- Tumour breast tissue analysis
Tumor breast sections contained 95% of tumor tissue according to pathologist annotation from the consecutive H&E stained image. From the statistical PCA results (figure 4) and database search by mass accuracy from Lipid Maps, it can be seen that most of the lipids present in the data were phosphatidylcholine and phosphatidyl acid: PC and PA. From the Total Ion Current (TIC) normalized ion images, PA either sodiated and potassiated were detected using MALDI, with slightly more intense ion images generated with CHCA in MeCN.
- Potassiated PC’s were better ionized using MALDI prepared using the DHB matrix. Potassiated PC’s were identified preferentially using DESI whereas sodiated PC’s were less abundant from the MALDI CHCA in MeOH dataset.
- TGs were also detected on the edge of the tumor tissue, especially from the DESI and CHCA in MeOH datasets.