Supplementary figures 5 through 9

for

Image fusion of mass spectrometry and microscopy: a new multi-modality paradigm for molecular mapping of tissue

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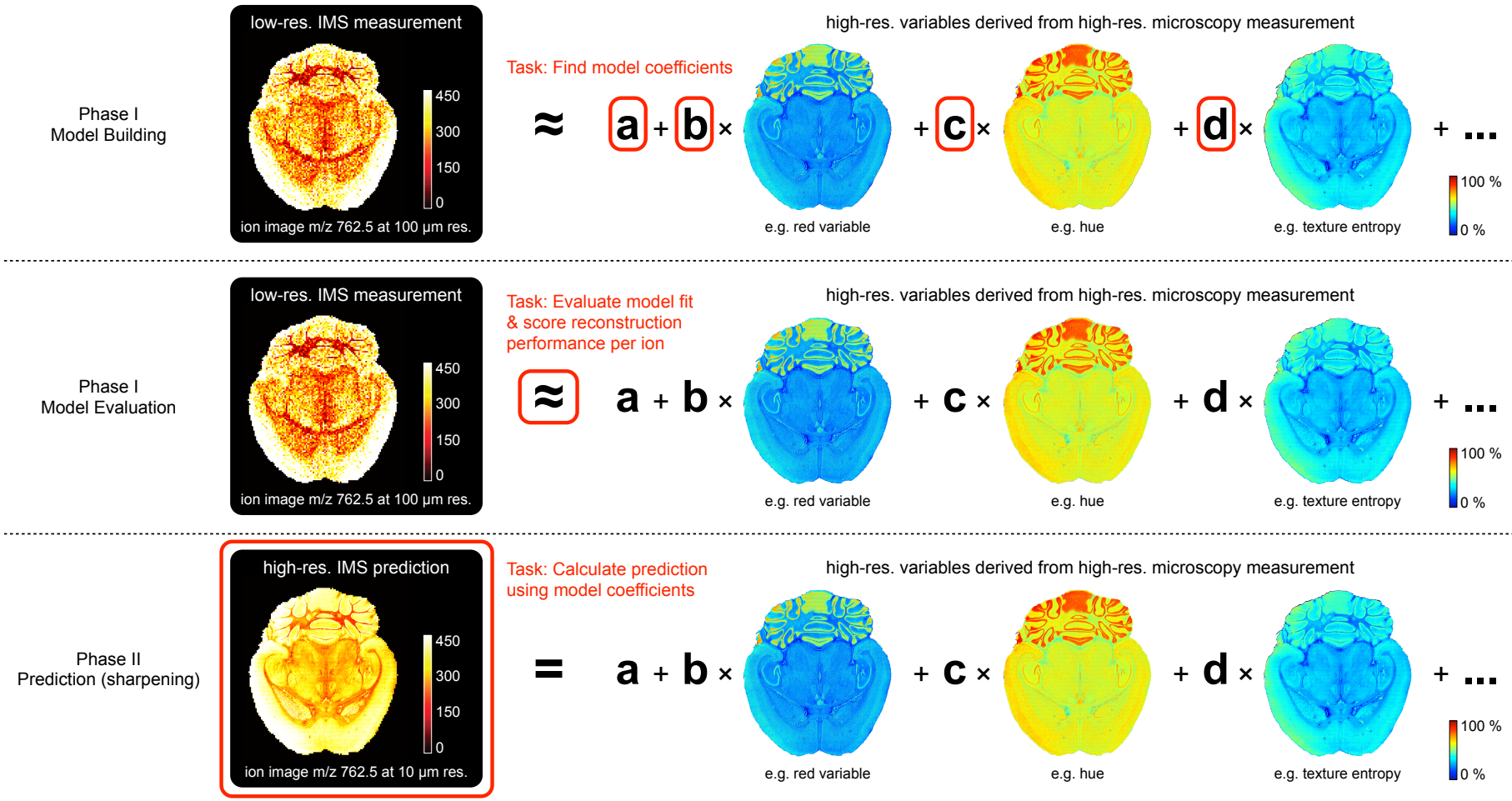
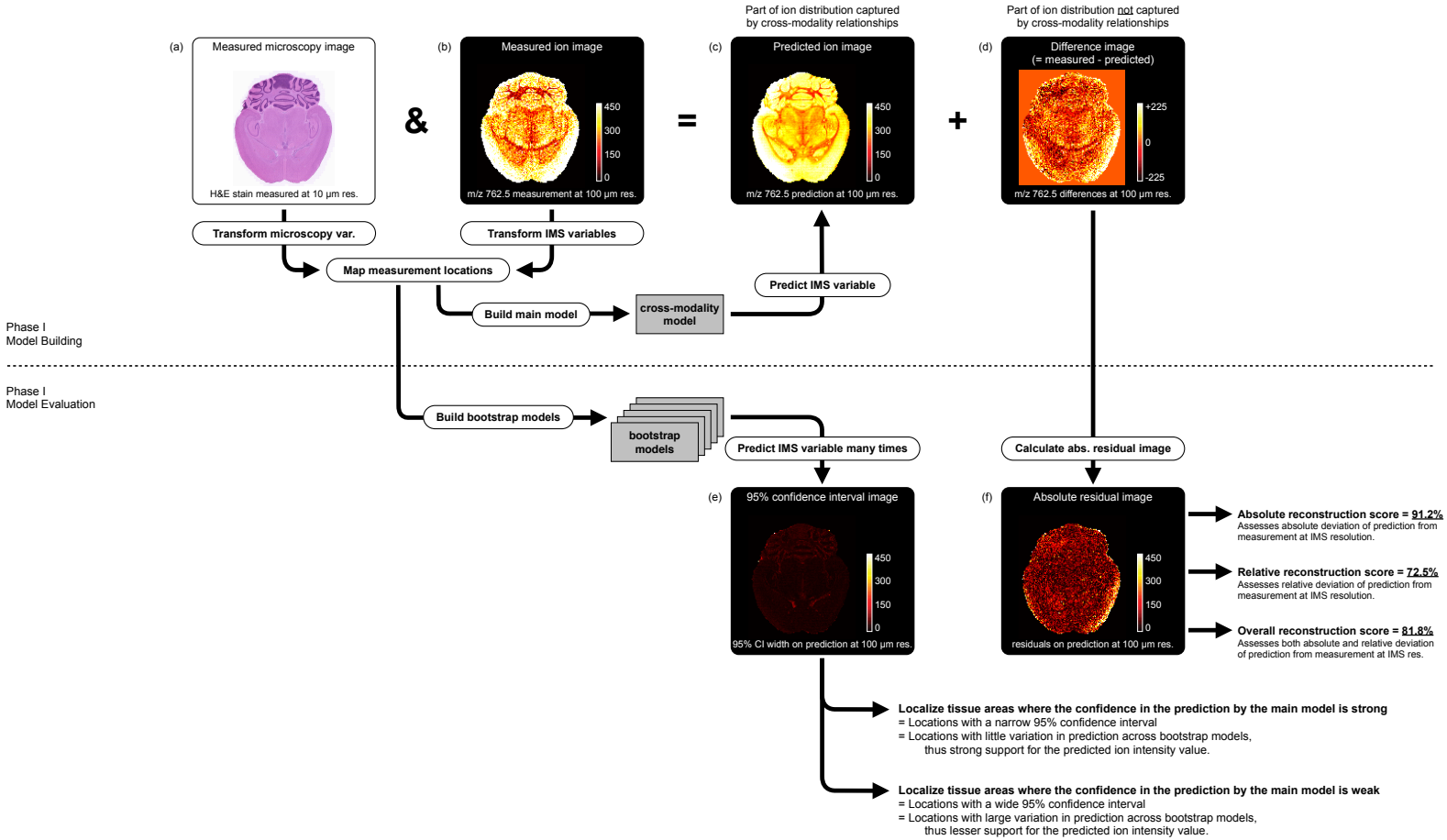
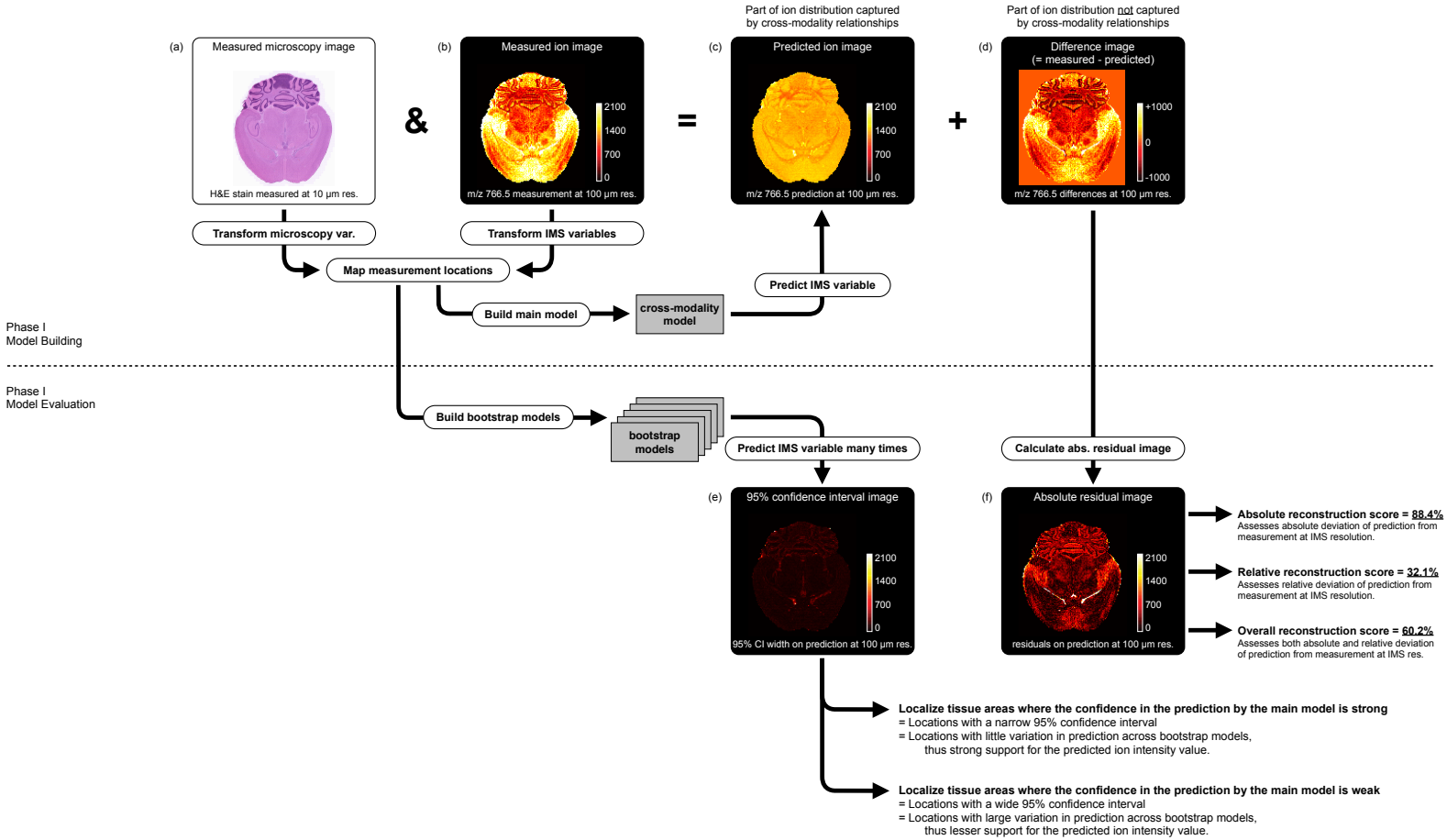


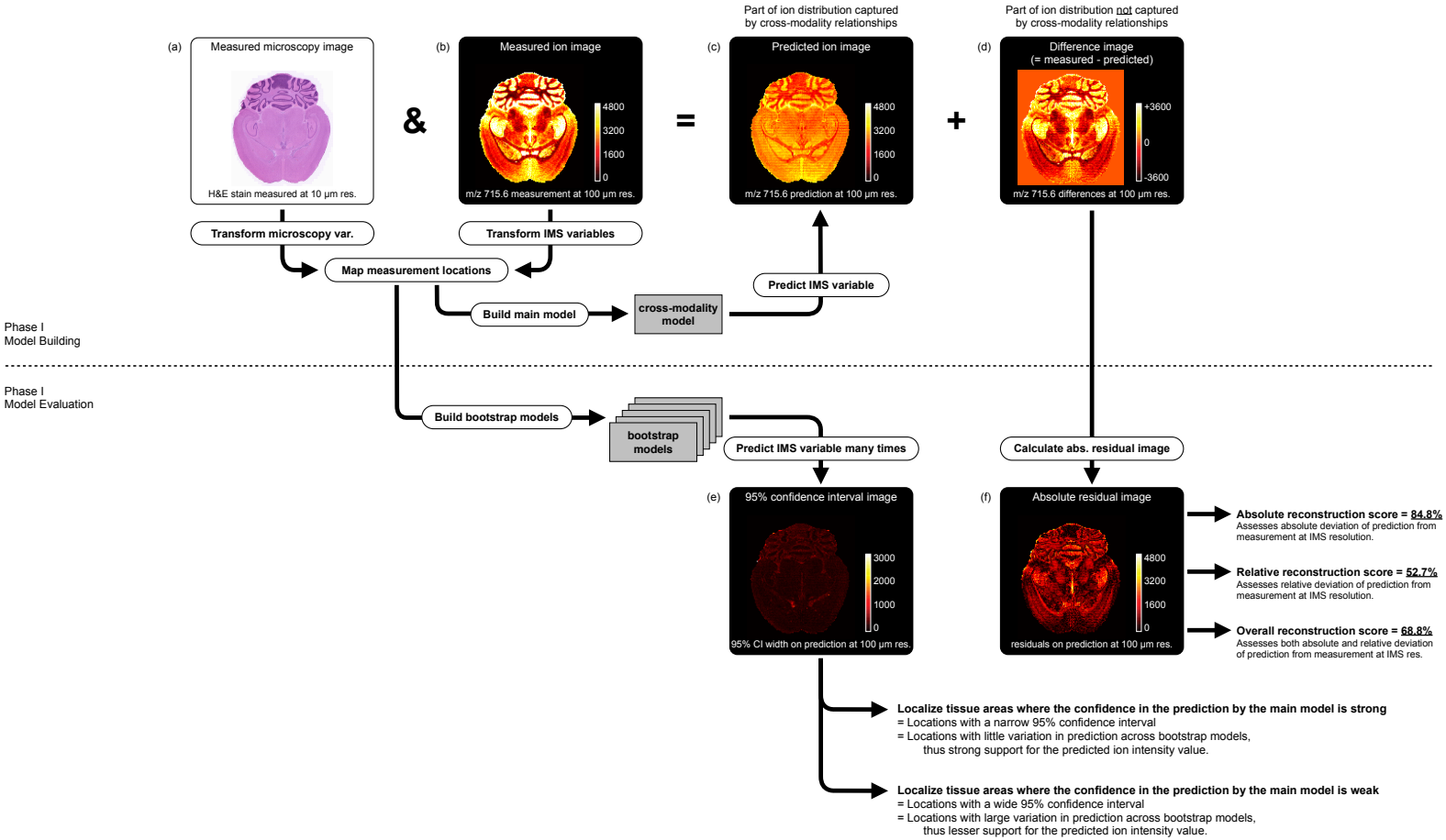
Supplementary Figure 5 Modeling details - Each IMS variable is approximated by a linear combination of microscopy-derived variables. (**top**) Model building step: the best linear equation coefficients are calculated. (**middle**) Model evaluation step: the fit of the linear sub-model to the measurements is determined and summarized as a reconstruction score. (**bottom**) Sharpening-specific prediction step: For those IMS variables with a high reconstruction score, apply the linear equation on the high-resolution microscopy-derived variables and calculate a high-resolution ion intensity prediction.



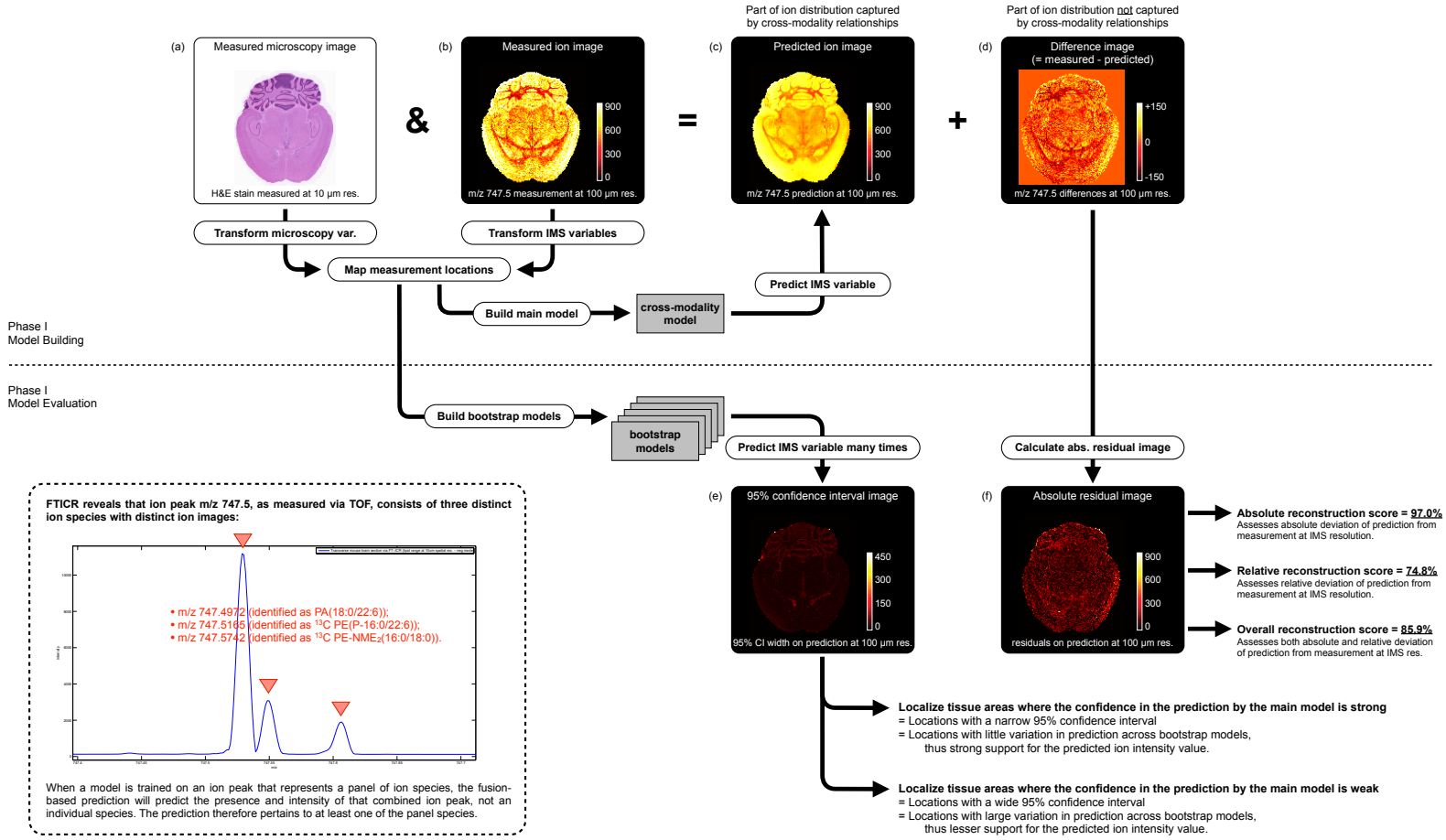
Supplementary Figure 6 Model building and evaluation for m/z 762.5. (**top**) Model building delivers for each IMS variable the linear model coefficients shown in Supplementary Figure 5. The linear equation pertaining to m/z 762.5, its 'sub-model', separates the measured ion image into two images: a microscopy-predicted approximation (the cross-modally supported part of the m/z 762.5 measurement) and a difference image, which encodes the size and location of ion variation not captured by the sub-model (the IMS-specific part of the m/z 762.5 measurement). (**bottom**) Model evaluation assesses the sub-model strength for m/z 762.5 across the entire tissue section by summarizing the content of the difference image in a quality measure called the 'reconstruction score' (higher values mean better prediction). The location-specific prediction performance is reported by two evaluation images: the absolute residual image (low valued areas are well predicted using microscopy) and the 95% confidence interval image (low valued areas are predicted robustly). All model evaluation happens at the native IMS resolution (100 µm).



Supplementary Figure 7 Example of ion peak with low confidence cross-modality prediction, m/z 766.5 (identified as PE(18:0/20:4)). Although the average absolute peak intensity across the tissue is well approximated (absolute reconstruction score of 88%), the specific distribution pattern is not supported by the microscopy-derived patterns (relative reconstruction score of 32%). The overall reconstruction score therefore indicates a relatively low value of 60%, arguing against fusion-driven applications for this ion given the available microscopy measurements. See Supplementary Figure 6 for further diagram details.



Supplementary Figure 8 Example of ion peak with low confidence cross-modality prediction, m/z 715.6 (identified as PE-Cer(d16:1/22:0)). Although the average absolute peak intensity across the tissue is well approximated (absolute reconstruction score of 85%), the specific distribution pattern is not supported by the microscopy-derived patterns (relative reconstruction score of 53%). The overall reconstruction score therefore indicates a relatively low value of 69%, arguing against fusion-driven applications for this ion given the available microscopy measurements. See Supplementary Figure 6 for further diagram details.



Supplementary Figure 9 Example of ion peak with high confidence cross-modality prediction, m/z 747.5. The high overall reconstruction score for this ion peak indicates that its tissue presence and intensity can be predicted with high confidence using H&E microscopy. However, through the advanced mass resolution provided by MALDI Fourier transform ion cyclotron resonance (FTICR) mass spectrometry, we know that ion peak m/z 747.5 is actually a superposition of multiple ion species (**inset**): ^{13}C PE-NME$_2$(16:0/18:0) at m/z 747.5742, ^{13}C PE(P-16:0/22:6) at m/z 747.5165, and PA(18:0/22:6) at m/z 747.4972. This example demonstrates that our method not only predicts for individual molecular species, but also works for a panel of species without requiring them to be uniquely resolved. See Supplementary Figure 6 for further diagram details.