Supplementary figures 10 through 12

for

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

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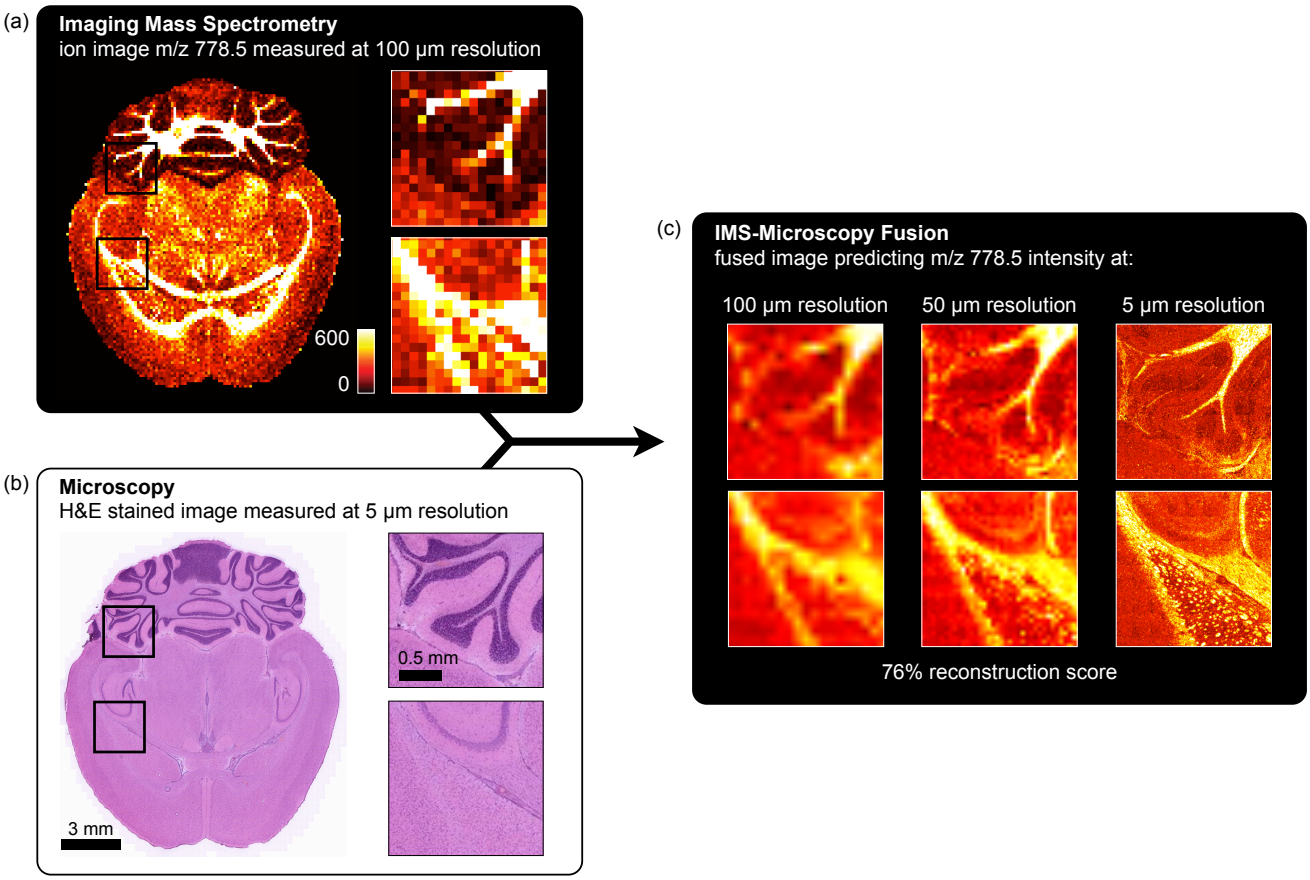
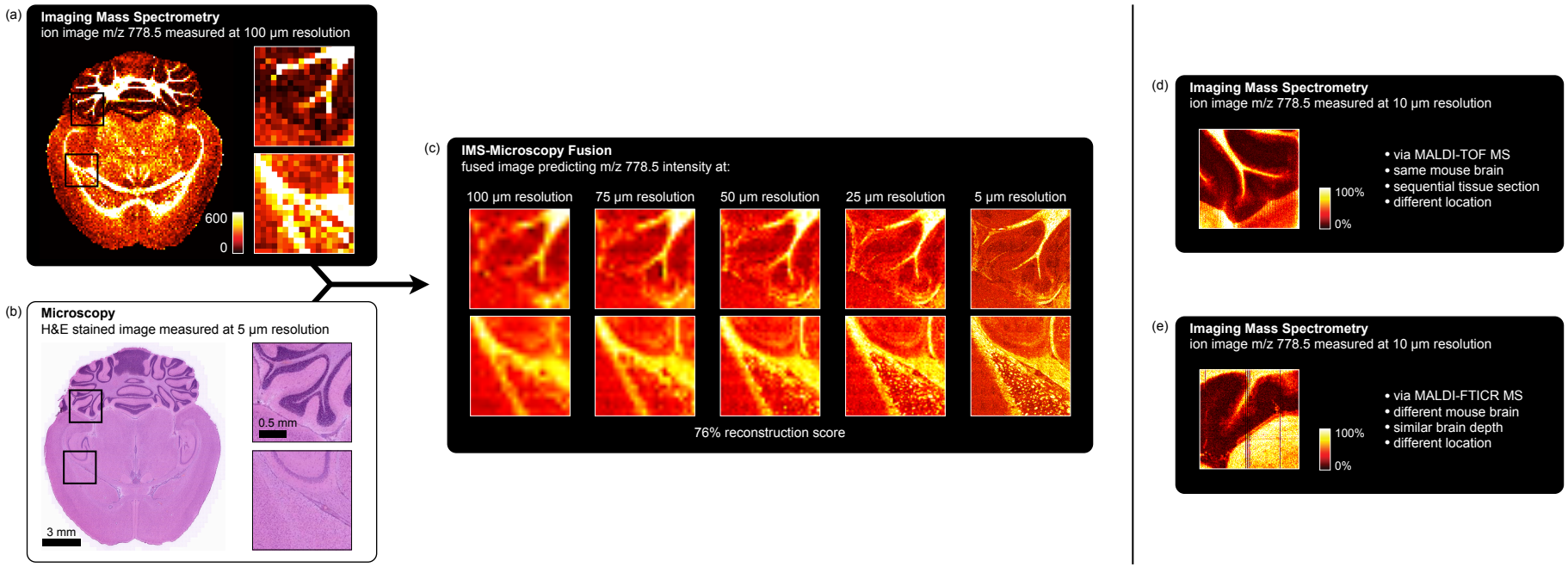
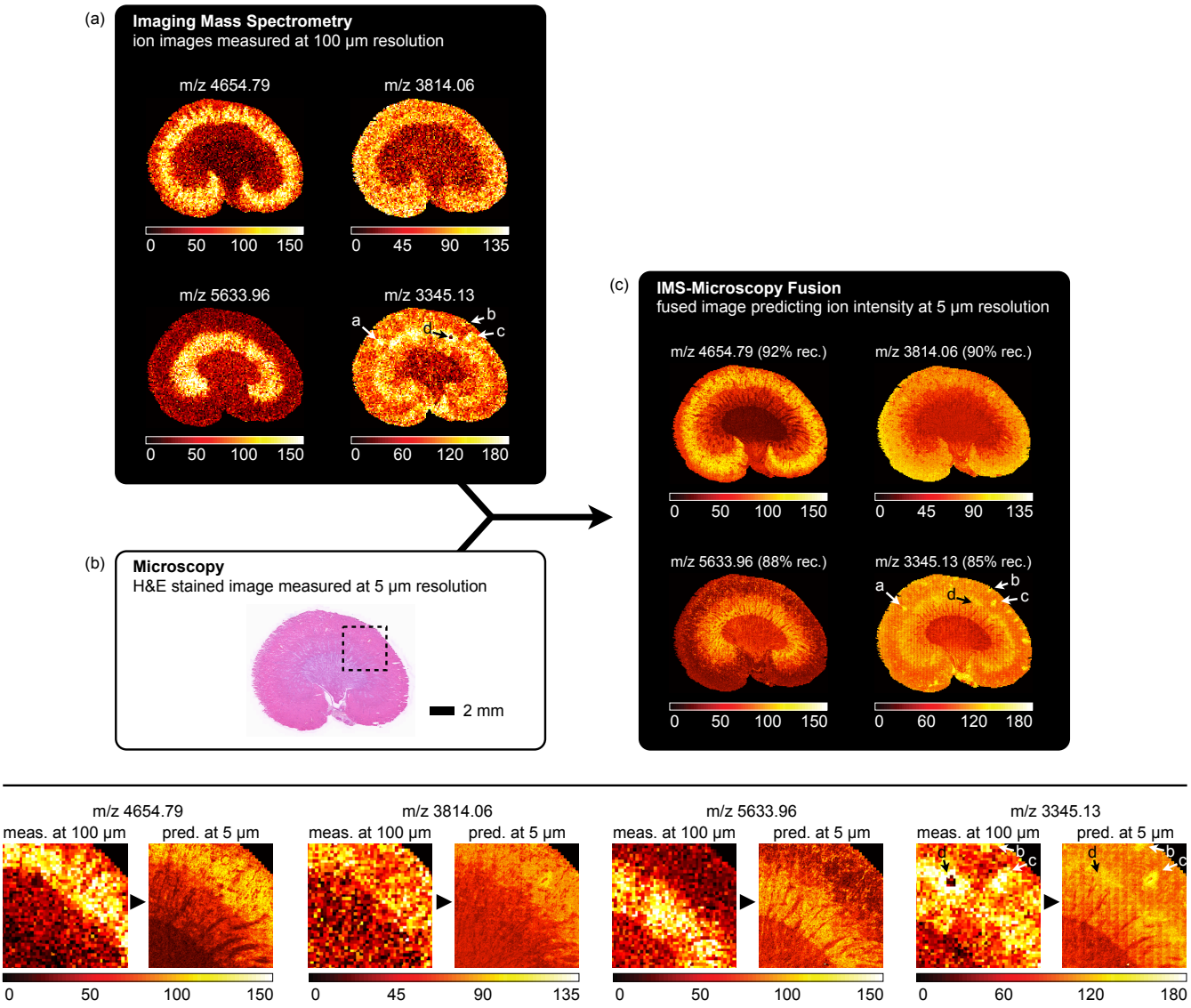


Supplementary Figure 10 Prediction of the ion distribution of m/z 778.5 in mouse brain at different target resolutions from 100 µm IMS and 5 µm microscopy measurements (sharpening). An IMS/microscopy model fuses information from an ion image for m/z 778.5 (identified as lipid PE(P-40:4)) measured at 100 µm spatial resolution (**a**), with that of an H&E-stained microscopy image measured at 5 µm resolution (**b**) (reconstr. score 76%). Combined with the microscopy measurements, the fusion model is then used to predict the ion distribution of m/z 778.5 at 100, 50, and 5 µm resolution (**c**).



Supplementary Figure 11 Prediction of the ion distribution of m/z 778.5 in mouse brain at different target resolutions, with comparison to measured TOF and FTICR ion images (sharpening). An IMS/microscopy model fuses information from an ion image for m/z 778.5 measured at 100 µm spatial resolution (**a**), with that of an H&E-stained microscopy image measured at 5 µm resolution (**b**) (reconstr. score 76%). Combined with the microscopy measurements, the fusion model is then used to predict the ion distribution of m/z 778.5 at 100, 75, 50, 25, and 5 µm resolution (**c**). For comparison: an ion image for m/z 778.5 measured at 10 µm resolution by TOF-based IMS from a neighboring tissue section (**d**), and an ion image for m/z 778.5 measured at 10 µm resolution by FTICR-based IMS from a different mouse brain at a similar brain depth (**e**).



Supplementary Figure 12 Prediction of the ion distributions in rat kidney for m/z 4,655, m/z 3,814, m/z 5,634, and m/z 3,345 at 5 µm resolution (sharpening & enrichment). This example in rat kidney fuses a measured ion images acquired via IMS at 100 µm spatial resolution (**a**) with a measured H&E-stained microscopy image at 5 µm resolution (**b**), predicting the ion distributions at 5 µm resolution (**c**). The IMS/microscopy model achieves for m/z 4,655, m/z 3,814, m/z 5,634, and m/z 3,345 an overall reconstruction score of respectively 92%, 90%, 88%, and 85% at the native IMS resolution (100 µm). (**bottom**) Enlarged views of the measured 100 µm and predicted 5 µm ion distributions. Annotations *a*, *b*, and *c* are examples of ion image features that could be considered matrix homogeneity artifacts if only IMS is considered, but the fusion procedure shows that they have a support base in the microscopy as well. These features are thus corroborated across different technologies, and are more likely to be of genuine tissue origin than apparent from IMS measurements alone. Annotation *d* is an example of an ion image feature that is not supported by microscopy, and thus appears to be a modality-specific feature (and potential IMS artifact).