

## Supplementary figures 18 through 21

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for

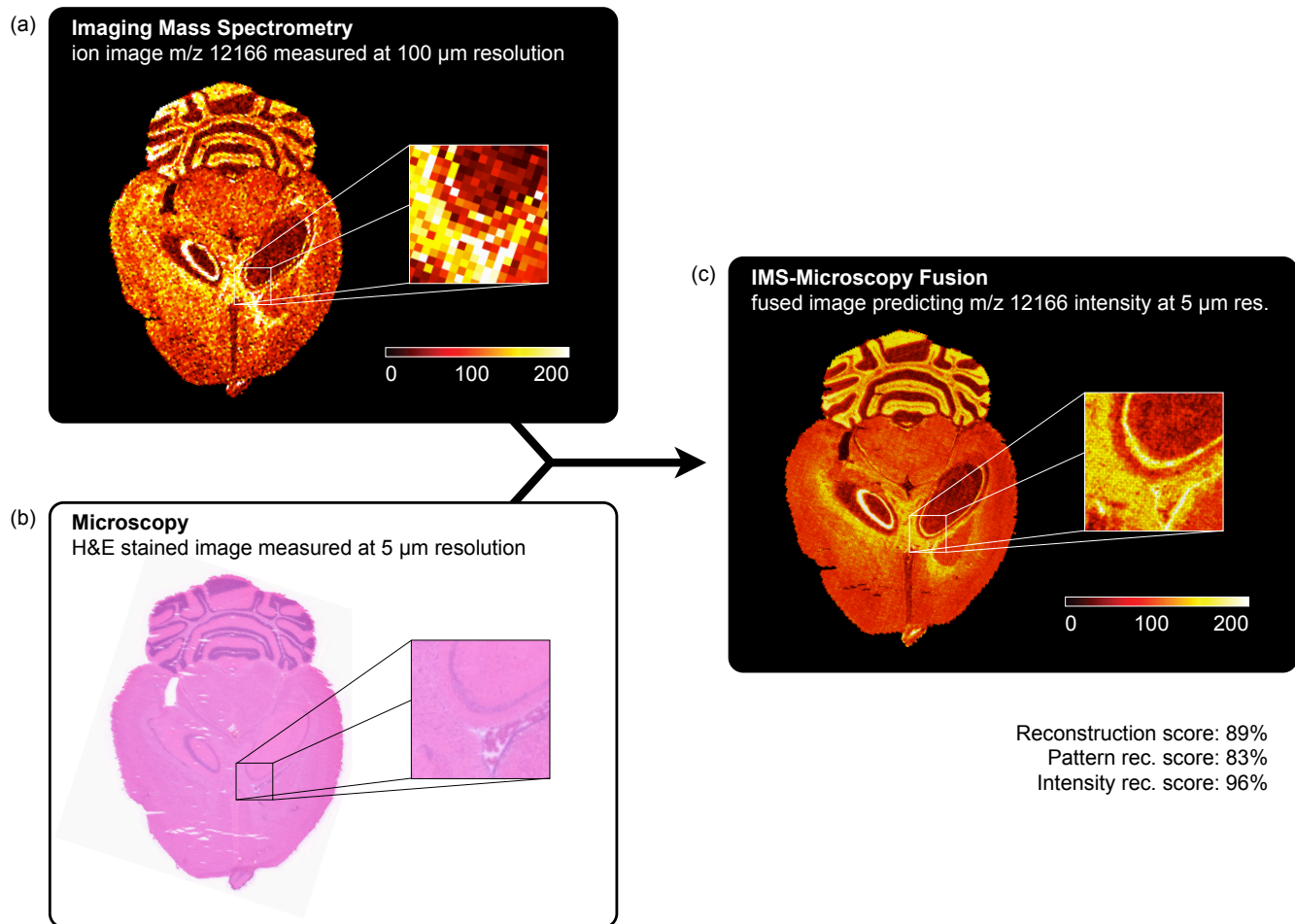
### **Image fusion of mass spectrometry and microscopy: a new multi-modality paradigm for molecular mapping of tissue**

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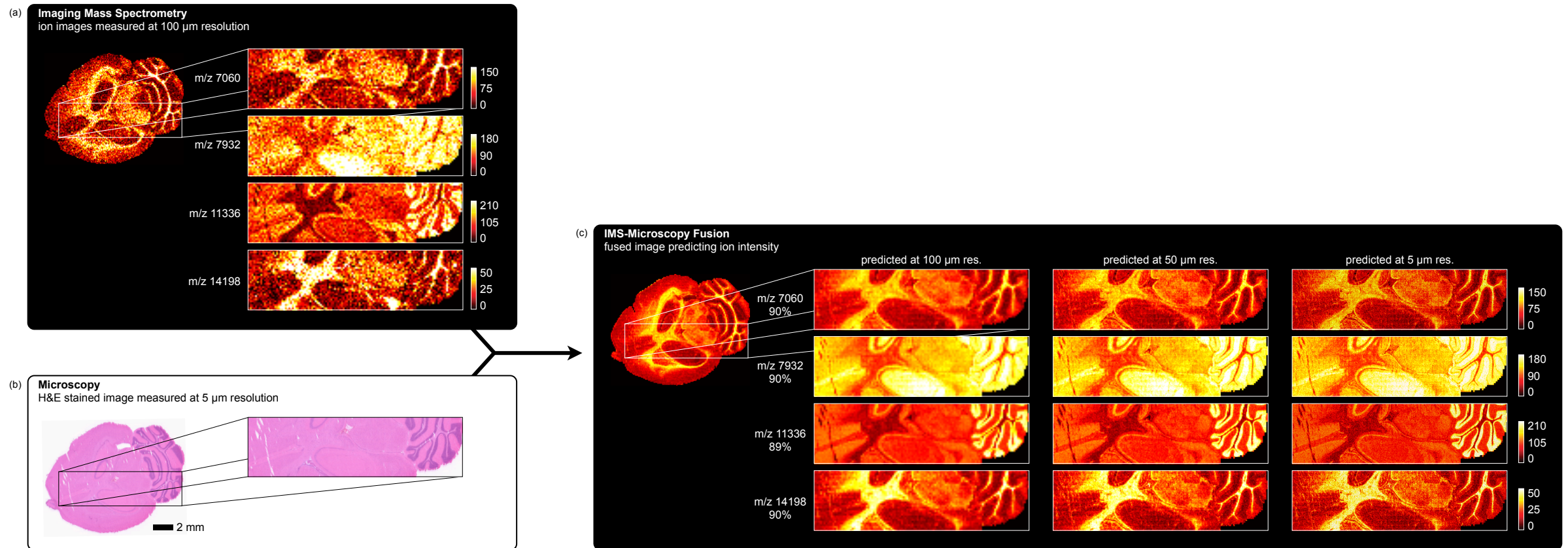
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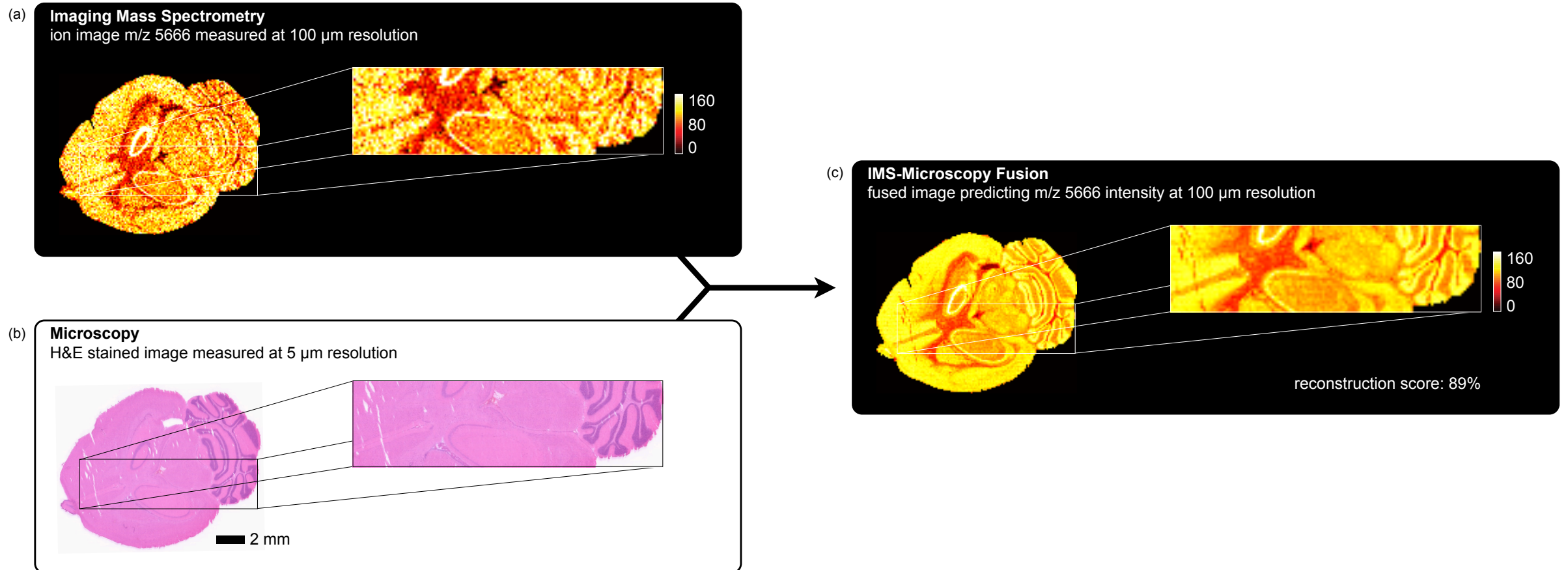
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**Supplementary Figure 18** Prediction of the ion distribution of m/z 12,166 in mouse brain at 5  $\mu\text{m}$  resolution from 100  $\mu\text{m}$  IMS and 5  $\mu\text{m}$  microscopy measurements (sharpening). This example in mouse brain fuses a measured ion image for m/z 12,166 at 100  $\mu\text{m}$  spatial resolution (a) with a measured H&E-stained microscopy image at 5  $\mu\text{m}$  resolution (b), predicting the ion distribution of m/z 12,166 at 5  $\mu\text{m}$  resolution (reconstr. score 89%) (c).

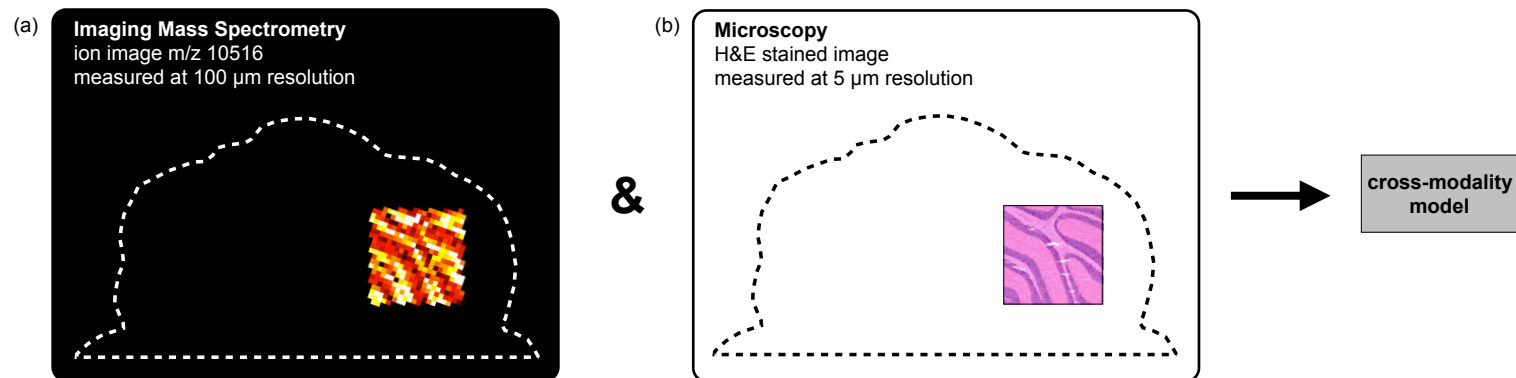


**Supplementary Figure 19** Prediction of the ion distributions of m/z 7,060, 7,932, 11,336, and 14,198 in mouse brain at different target resolutions from 100  $\mu\text{m}$  IMS and 5  $\mu\text{m}$  microscopy measurements (sharpening). An IMS/microscopy model fuses information from ion images for m/z 7,060, 7,932, 11,336, and 14,198, measured at 100  $\mu\text{m}$  spatial resolution (a), with that of an H&E-stained microscopy image measured at 5  $\mu\text{m}$  resolution (b). Combined with the microscopy measurements, the fusion model is then used to predict the ion distribution of m/z 7,060, 7,932, 11,336, and 14,198 at 100, 50, and 5  $\mu\text{m}$  resolution (c) (reconstr. score 90%, 90%, 89%, and 90% respectively).

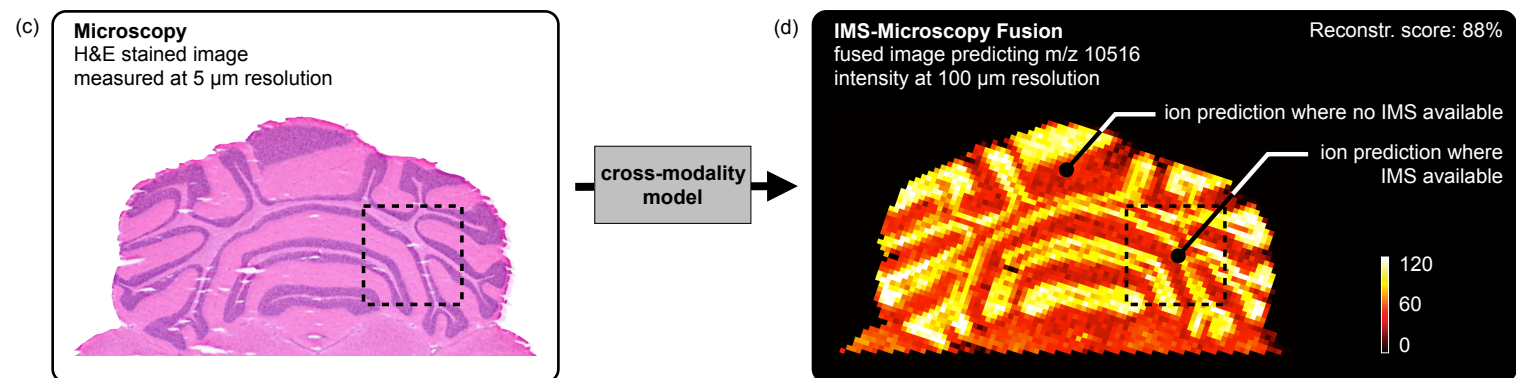


**Supplementary Figure 20** De-noising of the ion distribution of m/z 5,666 in mouse brain through round-trip prediction from 100  $\mu\text{m}$  IMS to 100  $\mu\text{m}$  fused resolution, using fusion to 5  $\mu\text{m}$  microscopy measurements as a filter (de-noising). This example in mouse brain fuses a measured ion image for m/z 5,666 at 100  $\mu\text{m}$  spatial resolution (a) with a measured H&E-stained microscopy image at 5  $\mu\text{m}$  resolution (b), predicting the ion distribution of m/z 5,666 at 100  $\mu\text{m}$  resolution (reconstr. score 89%) (c). No sharpening is pursued. The objective is to use fusion to employ microscopy measurements as a filter. This filtering application retains cross-modal (tissue) variation and removes modality-specific variation.

Phase I: Model Building & Evaluation



Phase II: Prediction



**Supplementary Figure 21** Prediction of m/z 10,516 distribution at native IMS resolution (without sharpening) in mouse brain areas not measured by IMS. An IMS/microscopy model is built on a tissue sub-area for which IMS is available at 100  $\mu\text{m}$  resolution (a) and H&E-stained microscopy is available at 5  $\mu\text{m}$  resolution (b). The model is then used to predict the distribution of m/z 10,516 at 100  $\mu\text{m}$  resolution in areas where no IMS was acquired and only microscopy is available (reconstr. score 88%) (d).