Supplementary figures 1 through 4

for

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

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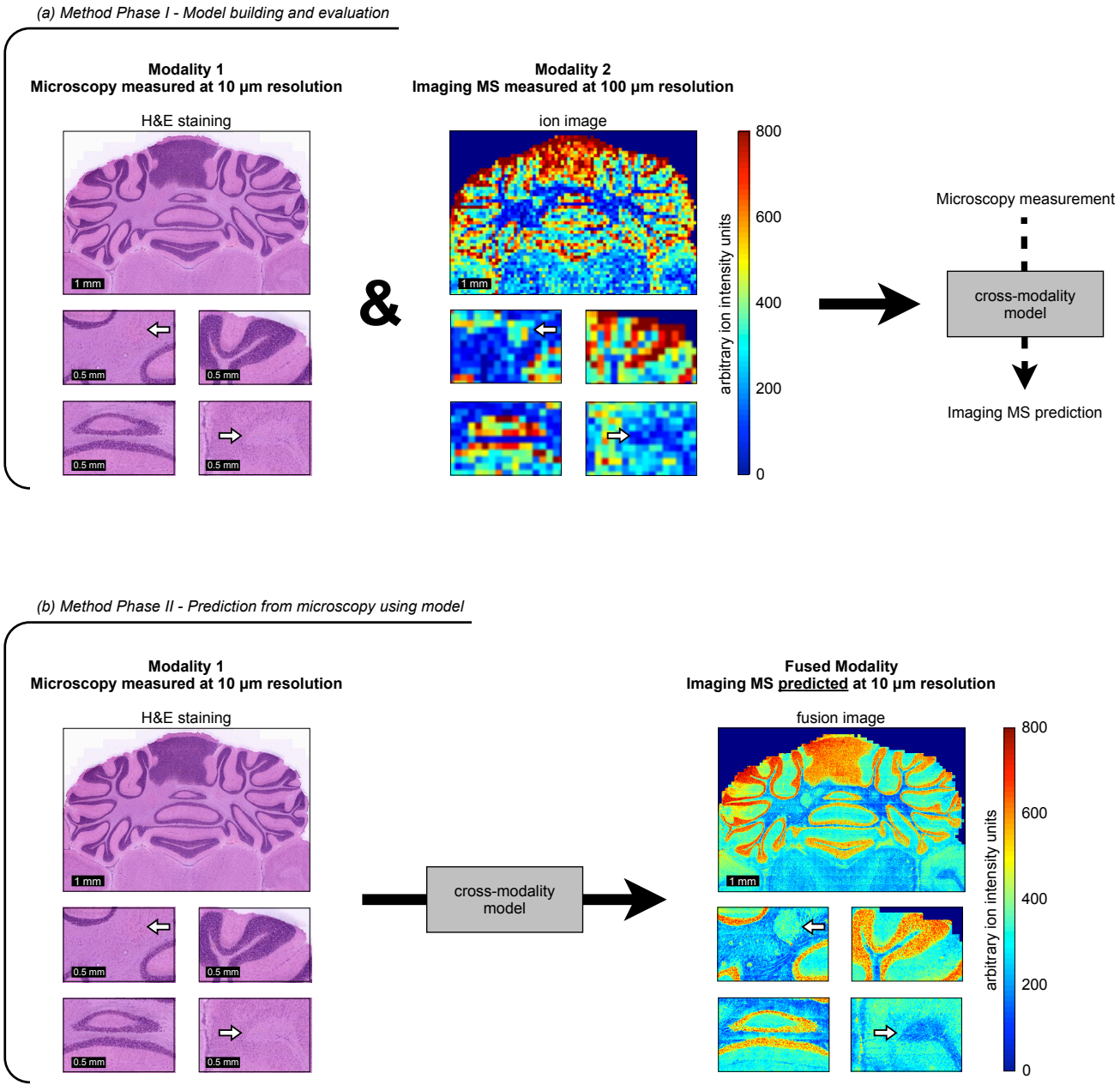
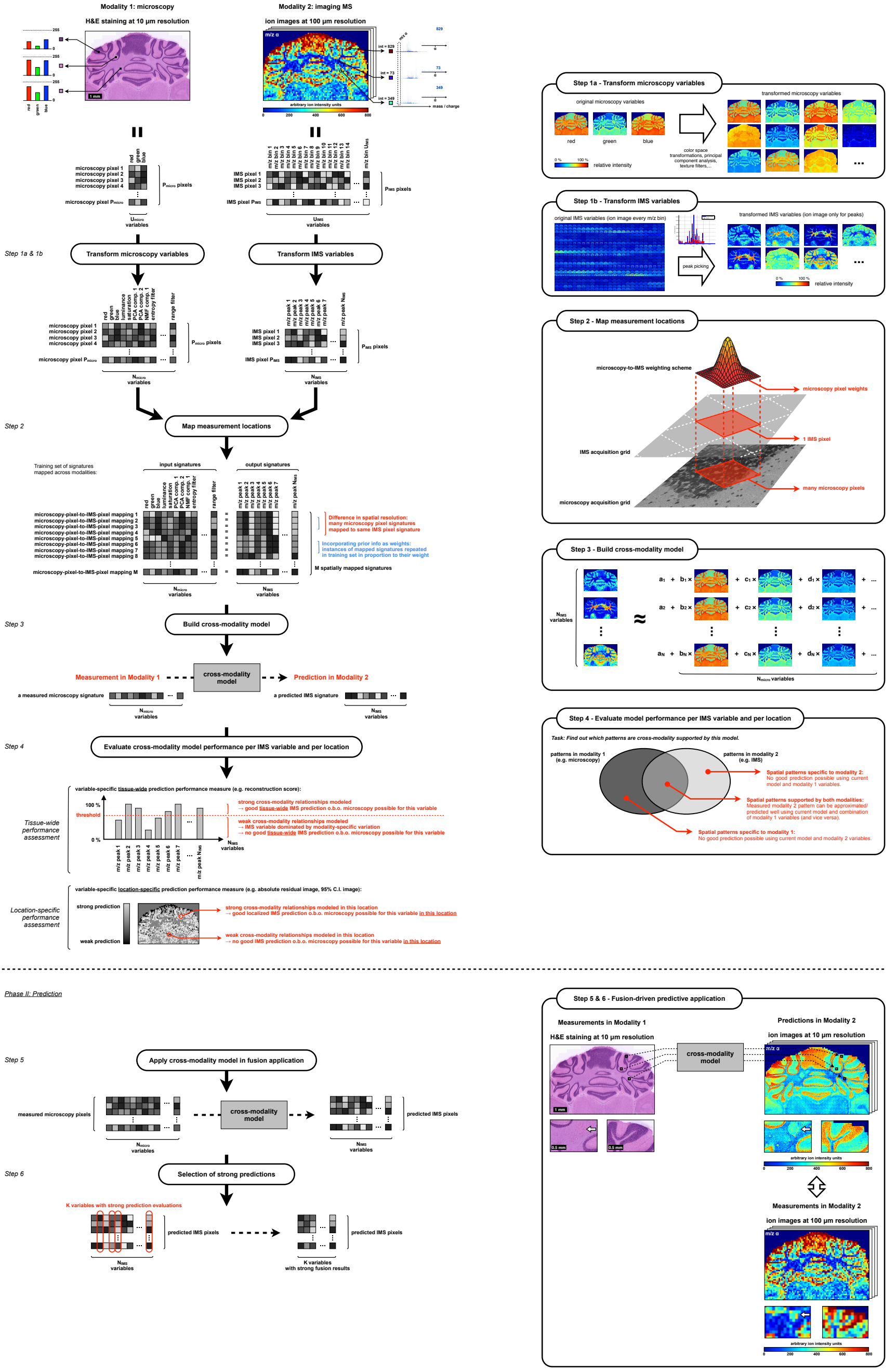
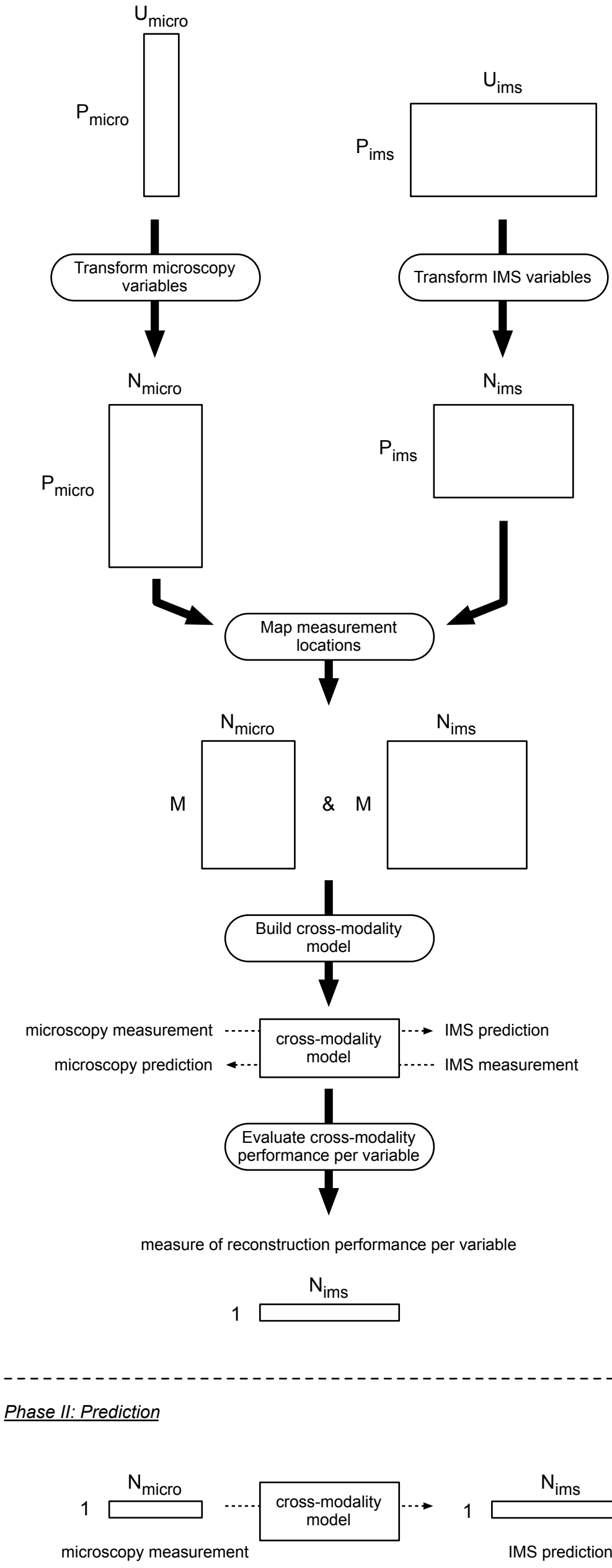


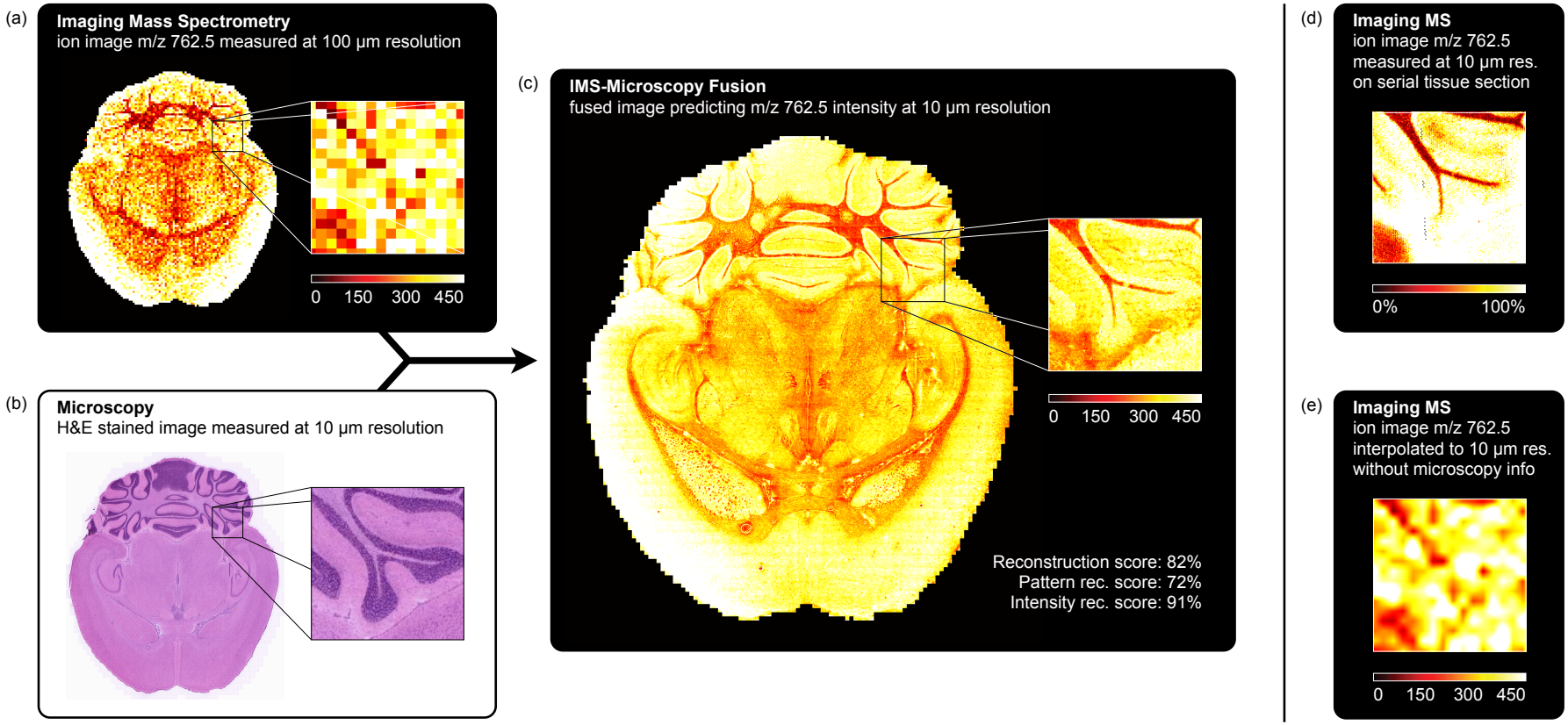
Supplementary Figure 1 Method overview. The fusion process consists of two phases. (**a**) Phase I builds a cross-modality model from the two measurement sources and evaluates for which ions good prediction is possible. (**b**) For those ions, phase II uses the model and the high-resolution microscopy measurements to predict the ion distribution at higher-than-IMS resolutions.



Supplementary Figure 2 Extensive step-by-step details of the fusion process. In the two-phase fusion method, phase I focuses on building and evaluating a cross-modality model between the provided modalities. It entails a transformation of the microscopy variables (step 1a) and the IMS variables (step 1b), a spatial mapping of both measurement sets (step 2), building the model (step 3), and evaluating model performance both chemically and spatially (step 4). Phase II employs the cross-modality model in a predictive application, and entails a prediction for all IMS variables (step 5) followed by a pruning of IMS variables for which predictive performance is insufficient (step 6).



Supplementary Figure 3 Method phases and steps with algebraic details. Algebraic details on the structure and size of the input and output data of each method step throughout the fusion procedure. P_{ims} = number of pixels in the IMS data source; U_{ims} = number of native variables per pixel provided by the IMS data source; N_{ims} = number of variables per pixel provided by the IMS data source after transformation; P_{micro} = number of pixels in the microscopy data source; U_{micro} = number of native variables per pixel provided by the microscopy data source; N_{micro} = number of variables per pixel provided by the microscopy data source after transformation; and M = number of mapped IMS and microscopy signatures.



Supplementary Figure 4 Prediction of the ion distribution of m/z 762.5 in mouse brain at 10 µm resolution from 100 µm IMS and 10 µm microscopy measurements (sharpening). This example in mouse brain fuses a measured ion image for m/z 762.5 (identified as lipid PE(16:0/22:6)) at 100 µm spatial resolution (**a**) with a measured H&E-stained microscopy image at 10 µm resolution (**b**), predicting the ion distribution of m/z 762.5 at 10 µm resolution (reconstr. score 82%) (**c**). For comparison, (**d**) shows a measured ion image for m/z 762.5 at 10 µm spatial resolution, acquired from a neighboring tissue section. Additionally, (**e**) shows a 10 µm version of the m/z 762.5 ion image obtained through interpolation, a computational up-sampling method that does not employ information from another modality to guide its estimates.