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## Crossing the Styx: From In Vivo MRI to Post Mortem Histology in Human Brain Atlasing

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(Immuno-)histological and magnetic resonance imaging (MRI) research both provide information on the functional neuroanatomy of the human brain. Microscopy techniques provide an unmatched level of anatomical detail but are usually limited by low numbers of observations. MRI research does not achieve the same level of detail but provides us with insight in interindividual variation through larger numbers of observations. Integrated approaches allow the bridging between these complementary imaging modalities.

In our research we combine techniques. Detailed information is acquired from 7 individual post mortem brains which undergo quantitative 7 Tesla MRI data at 400  $\mu\text{m}$  isotropic resolution. After serial coronal cutting, we create integrated full 3D reconstructions based on blockface images, and the immunoreactivity of calcium binding proteins (parvalbumin, calretinin, calbindin), Alzheimer related neuropathology (amyloid beta, pTau), vascular markers (CD31 and smooth muscle actin) and/or Bielschowsky and Nissl staining. Coregistration of the microscopy and MRI data at a 200  $\mu\text{m}$  resolution in blockface space allows the subsequent transfer of the data to MNI-space (1,2). The resulting datasets can be used for MRI-validation, as well as for brain atlasing purposes.

The acquired post mortem datasets are currently being used to further advance our atlasing efforts, and to bring the data together with the previously published in vivo Amsterdam Ultra-high field adult lifespan database (AHEAD). The AHEAD dataset consists of 105 7 Tesla whole-brain datasets at 0.7 mm isotropic resolution, and was recently extended with slab quantitative MRI contrasts covering the subcortex at a 0.5 mm isotropic resolution (3,4). Our in vivo atlasing efforts have been funneled into the MASSP 2.0 algorithm that now allows the automated parcellation of 35 individual structures in both cerebral hemispheres (3,5). Finally, our post mortem resources now allow the retraining and expansion of the algorithm using post mortem delineations.

The resulting brain models provide us with the best of both worlds, and can be applied to create advanced atlasing tools for application in neuroimaging research and clinical applications (Fig 1). Given the labour intensive nature of whole-brain histological approaches, individual research initiatives can only provide a limited number of donor brains. Open access publication and sharing of the datasets and derived algorithms and atlases will strongly benefit the progress of the research field.

1. Alkemade, A. et al. 7 Tesla MRI Followed by Histological 3D Reconstructions in Whole-Brain Specimens. *Front Neuroanat* 14, (2020).
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3. Bazin, P.-L. et al. Automated parcellation and atlasing of the human subcortex with ultra-high resolution quantitative MRI. *Imaging Neuroscience* (2025) doi:10.1162/IMAG\_A\_00560.
4. Alkemade, A. et al. The Amsterdam Ultra-high field adult lifespan database (AHEAD): A freely available multimodal 7 Tesla submillimeter magnetic resonance imaging database. *Neuroimage* 221, 117200 (2020).
5. Bazin, P.-L., Alkemade, A., Mulder, M. J., Henry, A. G. & Forstmann, B. U. Multi-contrast anatomical sub-cortical structures parcellation. *Elife* 9, (2020).

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