Multimodal imaging of human cerebellum - merging X-ray phase microtomography, magnetic resonance microscopy and histology

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Imaging modalities including magnetic resonance imaging and X-ray computed tomography are established methods in daily clinical diagnosis of human brain. Clinical equipment does not provide sufficient spatial resolution to obtain morphological information on the cellular level, essential for applying minimally or non-invasive surgical interventions. Therefore, generic data with lateral sub-micrometer resolution have been generated from histological slices post mortem. Sub-cellular spatial resolution, lost in the third dimension as a result of sectioning, is obtained using magnetic resonance microscopy and micro computed tomography. We demonstrate that for human cerebellum grating-based X-ray phase tomography shows complementary contrast to magnetic resonance microscopy and histology. In this study, the contrast-to-noise values of magnetic resonance microscopy and phase tomography were comparable whereas the spatial resolution in phase tomography is an order of magnitude better. The registered data with their complementary information permit the distinct segmentation of tissues within the human cerebellum.

Minimally or non-invasive surgical interventions on the brain are generally based on less detailed patient-specific imaging (pre- and intra-operative) and high-resolution generic anatomical data. The generic knowledge of brain microstructure currently relies on two-dimensional (2D) evaluation of histological slices, which requires time-consuming sectioning, staining, and (manual, expert-based) image analysis. The advantages of histology are sub-micrometer resolution and excellent tunable contrast depending on the staining protocol¹². However, the technique does not provide isotropic three-dimensional (3D) information of the soft and hard tissues, as is regularly obtained using magnetic resonance imaging (MRI) and X-ray computed tomography (CT). Furthermore, it is hard to imagine image-guided neurosurgery without MR guidance. Conventional MRI is a well-established technique for brain imaging, and yields superb contrast between white and grey matter but only limited spatial resolution. At present, medical MR systems produce images with typically sub-millimetre voxel lengths. Small animal MR microscopy (μMRI) scanners equipped with stronger gradient systems and operating at higher magnetic field strengths have been used to visualize mouse brains in vivo¹⁶ and pieces of human brain post-mortem with voxel sizes of a few tens of micrometers. Conventional CT also provides fully quantitative 3D data ultimately reaching higher spatial resolution than MRI. The CT contrast for brain tissue, however, is weak. Despite low inherent brain tissue contrast, micro-CT (μCT) as a non-destructive technique has already been used to correct deformations induced in data sets obtained by histological sectioning during the preparation of histology. In order to obtain a better contrast than in these absorption-based studies, synchrotron radiation-based phase-contrast μCT (PC-μCT)¹⁰ was recently applied to human brain¹¹. X-ray phase-contrast methods are based on the phase shifts of X-ray waves penetrating the specimen which is related to the decrement δ(x, y, z) of the real part of the refractive index distribution. A variety of PC-μCT methods based on crystal interferometry¹², propagation-based (or inline) contrast¹³¹⁴ or analyzer-based imaging (ABI)¹⁵¹⁶ are available today. More recently, grating interferometry⁷⁹⁻⁸¹ (also known as Talbot interferometry)
Figure 1 | Cerebellum block extraction. The photograph (right) and MR-slice acquired using a Verio 3T whole body scanner (Siemens Health Care, Erlangen, Germany) illustrate the location and size of the cerebellum within the donated body.

was developed, which reaches the especially high contrast required for identifying structures with small differences in electron density as is the case in brain13,20,21.

In the light of these inherent advantages and disadvantages of MR- and CT-based techniques and histology, the aim of the present communication is to elucidate how far the combination of the complementary methods μMRI, grating-based PC-μCT and histology yields additional information on the microanatomy of the human cerebellum.

Results

3D imaging. A specimen taken from the human cerebellum (Figure 1) was visualised in 3D using grating-based PC-μCT and μMRI. Figure 2 shows virtual cuts through identical regions of the brain tissue obtained with the two imaging techniques after non-rigid 3D registration22. In the mixed T1/T2*-weighted μMRI images white matter structures appear darker than grey matter structures. Grey matter consists of two components: the stratum granulosum and the stratum moleculare. Because of the weak MR-contrast between these layers, however, it is impossible to segment these structures with an intensity-based algorithm. PC-μCT, on the contrary, provides high contrast between these grey matter layers: with the stratum granulosum appearing as a bright feature and the stratum moleculare as the dark outer structure. The white matter (interior dark region in PC-μCT) exhibits δ-values very similar to the stratum moleculare and can only be segmented because the stratum granulosum is located between the two tissues. Therefore, an intensity-based segmentation is also impossible in this case. This can be deduced from the related histograms of the 3D data in Figure 2. The histogram of the μMRI data (green coloured triangles in the figure) shows a shoulder (left) associated with white matter, and a peak associated with grey matter (right). The histogram of the PC-μCT data (red coloured squares) contains two peaks and a shoulder. The first peak corresponds to the stratum moleculare, which was in direct contact with formalin during the whole fixation period of the brain. The second peak is a superposition of the stratum moleculare that was in contact with formalin only for restricted periods of time and the white matter. The shoulder on the right originates from the stratum granulosum.

Tissue contrast. The images in Figure 2 acquired with PC-μCT and μMRI show the characteristic morphology of the human cerebellum. The contrast-to-noise ratio \( c \) between two anatomical structures can be defined as

\[
c = \frac{|x_1 - x_2|}{FWHM_{back}}
\]

where \( x_1 \) are the centre positions of the related peaks in the histograms and \( FWHM_{back} \) is the full-width-at-half-maximum of the background signal, here formaldehyde solution and perfluoropolyether, respectively. This definition of the denominator was chosen for practical reasons (see supplementary material). Using Equation 1, six \( c \)-values between the four anatomical structures were determined for PC-μCT and μMRI (Figure 3). The highest contrast in the PC-μCT images is the contrast between stratum granulosum and tissue in contact with formalin (E) and has the value of \( c_{PC-\mu CT\text{-max}} = 6.4 \pm 0.2 \). Using μMRI a maximum value of \( c_{\mu MRI\text{-max}} = 9.5 \pm 0.1 \) was determined, corresponding to the contrast between white matter and tissue in contact with formalin (C).

Joint histogram. The superposition of peaks in the individual histograms prevents the segmentation of the anatomical features. In particular, it is impossible to differentiate between stratum moleculare and stratum granulosum in the μMRI data, and between white matter and stratum moleculare in the PC-μCT data. Combining μMRI and PC-μCT using a joint histogram, however, allows the distinct discrimination between the characteristic soft tissues (Figure 4). To do so, the first step was the identification of the 2D maxima in the joint histogram corresponding to the tissues of interest using a multi-Lorentzian fit. Subsequently, the intersections of the Lorentzians were calculated. These intersections characterize the interfaces between the anatomical structures and allow a distinct differentiation between white matter, given in red, stratum granulosum in blue, and stratum moleculare in yellow. In addition, the green-coloured peak indicates regions of stratum moleculare, which were in direct contact with formalin during the whole fixation period.
Spatial resolution. In order to evaluate the spatial resolution of μMRI, PC-μCT, and histology, firstly line plots through the registered data of the human cerebellum (see Figure 5) were inspected. The spatial resolution of the imaging techniques can be deduced by comparing the roughness of the line plots. Histology has the best spatial resolution followed by PC-μCT then μMRI. To quantify this observation, the spatial resolution $l_{SR}$ was calculated by Fourier analysis\textsuperscript{11,23}. The radial spectral power ($rSP$) of a structure of interest in a $100 \times 100$ pixel region of a selected slice ($rSP_{struc}$) was calculated and divided by the $rSP$ of a background region of same size ($rSP_{back}$) (i.e. of formalin for PC-μCT, perfluoropolyether for μMRI and glass without brain tissue for histology). This ratio was plotted against the spatial frequency. The peak-to-peak distance of the first frequency larger than twice the mean value of the baseline (noise) was divided by two to determine the spatial resolution\textsuperscript{24}. The $l_{SR}$ values corresponded to (6 ± 1) μm for histology, to (20 ± 5) μm for PC-μCT, and to (210 ± 40) μm for μMRI.

Multimodality. The morphology of the cerebellum can be properly visualized combining μMRI, PC-μCT, and histology. Figure 6 illustrates one possible way of combining the 2D images of the three techniques used in this study. Each technique is represented by one of the colour channels red/green/blue (RGB) (see Figure 6, left image). Such a colour representation directly illustrates the contributions of the individual techniques. For example the yellow colour, which indicates the stratum granulosum, is mainly the superposition of contrast from the PC-μCT and μMRI, whereas the purple colour (white matter) primarily arises from the PC-μCT and the histology signals.

Discussion
Grating interferometry is based on the detection of the deflection angles of the X-rays passing through the specimen. After reconstruction, a 3D distribution of the decrement $\delta$ of the real part of the refractive index of the specimen can be obtained, which is directly related to the electron density distribution

$$\delta(x,y,z) = \frac{r_e \lambda^2}{2\pi} \rho_e(x,y,z)$$

with the classical electron radius $r_e$ and the X-ray wavelength $\lambda$\textsuperscript{25}.

In MRI, contrast between different tissues is determined by their relative spin densities $\rho_s$, their characteristic relaxation properties at the purported magnetic field strength, i.e. their distinguished $T_1$ (longitudinal) and $T_2$ resp. $T_2^*$ (transversal) relaxation times, and the imaging sequence and parameters used (in particular the echo time $TE$, the repetition time $TR$ and the flip angle $\alpha$). In a very general manner, the measured amplitude of the signal for a gradient echo sequence can be described as
Figure 4 | Segmentation by means of a joint histogram. From the joint histogram of the phase tomography and MR microscopy data it is possible to distinctly segment the stratum granulosum, stratum moleculare, white matter, and brain tissue that was in direct contact with formalin for a longer period of time than other parts. The white arrows indicate the positions of the related line plots. The colours of the virtual cuts of PC-μCT and μMRI on the right side correspond to the colours assigned to the four peaks in the joint histogram.

Figure 5 | Spatial resolution of histology, X-ray phase tomography, and MR microscopy. Comparable line plots through selected slices demonstrate that the spatial resolution decreases from histology via PC-μCT to μMRI.
White matter (dark region) can easily be distinguished from the grey matter (bright region) in our mixed T1/T2*-weighted μMRI images. On the contrary, differentiation between layers of grey matter tissues, in particular between stratum granulosum and stratum moleculare, is hardly possible. Here, PC-μCT provides a clear distinction between these anatomical structures. White matter (inner dark region), however, has a comparable electron density to stratum moleculare (outer dark region). These observations are quantified using Equation 1 and summarized in Figure 3. With the definition of the contrast-to-noise ratio \( c \) presented in this manuscript, a value \( c > 1 \) is required to distinguish anatomical structures using intensity-based algorithms. Therefore, one can conclude that the PC-μCT contrast is sufficiently high to distinguish between the four tissues whereas μMRI contrast is restricted to white and grey matter. Additionally, the PC-μCT pixel size is a factor of nine smaller than μMRI, although the maximal \( c \)-value observed in the PC-μCT results is slightly lower than the maximal μMRI contrast-to-noise ratio. Binning the tomography data reduces the spatial resolution but improves the contrast\(^2\). Therefore, we can state that the contrast-to-noise between the two methods yields comparable values.

In addition to the contrast-to-noise ratio \( c \), we also consider the spatial resolution \( \lambda_{SR} \) to assess image quality. Following the work of Thurner et al.\(^2\), we combine these two values in the quality factor \( q \)

\[
q = \frac{c}{\lambda_{SR}}.
\]

Using the maximal contrast values of PC-μCT and μMRI and the corresponding spatial resolution values, one obtains quality factors of \((0.32 \pm 0.09) \mu m^{-1}\) for PC-μCT and \((0.05 \pm 0.01) \mu m^{-1}\) for μMRI. Based on this definition, PC-μCT yields six times better images than μMRI. More importantly, however, the complementary results of the two data sets can be derived using a joint histogram as presented in Figure 4. Three anatomical structures can be segmented, i.e. white matter (red), stratum granulosum (blue) and stratum moleculare (yellow). Furthermore, due to its high sensitivity, PC-μCT allows the detection of differences within the tissue induced by different periods of formalin fixation. The green regions in Figure 4 correspond to domains of stratum moleculare, which were in direct

\[
S(p, T_1, T_2, TE, TR) \propto p \sin \left( 1 - e^{-\frac{e^\gamma}{T_2}} \right) e^{-\frac{T_1}{1 - \cos \gamma e^{-\frac{e^\gamma}{T_2}}}}
\]

where

\[
\frac{1}{T_2} = \frac{1}{T_2} + \gamma \Delta B_0 \text{ with } \gamma \Delta B_0 \approx 60 \text{Hz}
\]

Figure 6 | Multimodal imaging of the cerebellum. The fusion of slices from PC-μCT, μMRI, and histology using the RGB channels directly visualizes the contributions from the three imaging techniques. The combination of the three techniques permits the distinction of otherwise indistinguishable anatomical features, such as the separation between white matter (violet), stratum granulosum (yellow), stratum moleculare (green) and blood vessels of different sizes (purple and white arrows). The enlarged regions (with different magnification) show the light blue coloured small capillaries.
contact with formalin for the whole fixation period of 120 days and not only for 20 days.

In summary, we demonstrate the advantages and disadvantages of PC-μCT, μMRI and histology and present a method to combine data from these complementary techniques in order to segment the anatomical layers of the human cerebellum post mortem. The clear segmentation of the three layers allows us to quantify the ratios between these volumes. Given that cell death in the cerebellum is a common cause of neurological disease, e.g. in cerebellar ataxias and that abnormalities in the cerebellum have more recently also been implicated in autism and neuropsychiatric disorders, methods for a reliable quantitative assessment of cerebellar morphology and pathology are certainly needed. Unfortunately, the currently available stereological methods for the cerebellum are very time consuming and often lack precision. The presented method has the potential to significantly reduce the effort and improve the quality of quantitative stereological assessments of the cerebellum.

Methods

Specimen preparation. The brain from the donated body of a 68-year-old male was extracted at the Institute of Anatomy (University of Basel, Switzerland) within 48 h after death. The whole brain was transferred to 10% formalin solution for fixation. All extracted at the Institute of Anatomy (University of Basel, Switzerland) within 48 h.

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