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Three-dimensional strain fields in human brain resulting from formalin fixation

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ABSTRACT

Before investigating human brains post mortem, the first preparation step is often formalin fixation of the brain. As the brain consists of inhomogeneous tissues, the fixation leads to a three-dimensional strain field within the tissue. During the single case MR-based investigation of the brain, first, the starting point with the brain post mortem but still within the cranium, was examined. Then 13 MR data sets were acquired over a fixation period of 70 days and compared to the initial data set. Based on affine registration of the data sets, the global volume shrinkage was found to be 8.1%. By means of a non-rigid registration additional maximal local volume strains of 32% were determined.

1. Introduction

The current developments in neurosurgical approaches, like magnetic resonance (MR)-guided neurosurgery with high-intensity focused ultrasound (e.g., Kennedy et al., 2003; Jolesz and McDannold, 2008; Martin et al., 2009) require a profound knowledge of the morphology of the human brain down to the micrometer level. Currently, neurosurgeons use detailed stereotactic atlases of human brain (Schaltenbrand and Wahren, 1977; Morel, 2007) to plan the treatment. The generation of such a brain atlas involves several steps. First, the brain tissue has to be fixated in order to avoid its degradation. In most cases formalin fixation is used. After sectioning the brain in few several dozen micrometer thin slices, it is stained using different protocols. The histological slices enable the distinction between different nuclei and the fibre system. Unfortunately, the different preparation steps cause local deformations of the brain tissue compared to the in vivo situation. Such deformations were already investigated by Germann et al. (2008), where two-dimensional histological slices were corrected by the related computed tomography (CT) slices and by Schulz et al. (2010), where the histological data was stacked to a three-dimensional (3D) data set and corrected by the 3D CT data set. The present contribution examines the formalin fixation, namely the global shrinkage and related local deformations resulting from the inhomogeneity of the brain tissues.

The shrinkage of soft tissues during fixation results from several physico-chemical reactions (Burck, 1982; Romeis and Böck, 1989). The most important process is the fixation of proteins (see scheme in Fig. 1). The structure of native proteins is maintained by numerous chemical bonds like hydrogen, covalent, dative and hydrophobic bonds as well as electrostatic and Van der Waals forces. The classical way to cross-link proteins is by denaturation: bonds break and chemically active groups become available for cross-linking. Another procedure is the application of fixatives like formaldehyde where the cross-linking of the proteins takes place without the denaturation by formation of methylene bridges and Schiff bases (see scheme in Fig. 2).

Most examinations on shrinkage during formalin fixation were accomplished on tissues like liver and kidney (Wüstenfeld, 1955; Bloom and Friberg, 1956; Bahr et al., 1957) or on brain tissue of animals like rats (Leibnitz, 1967; Hillman and Deutsch, 1978) and dogs (Fox, 1965). Of course, it has to be disputed how far these studies can be compared with human brain tissue. Examinations of the human brain stem exist where the shrinkage is measured on the basis of 2D histological slices (Quester and Schröder, 1997). The present study however uses 3D magnetic resonance imaging (MRI) data sets of the whole brain post mortem. Data sets of the brain inside the skull, after extraction, and at different fixation times were investigated.

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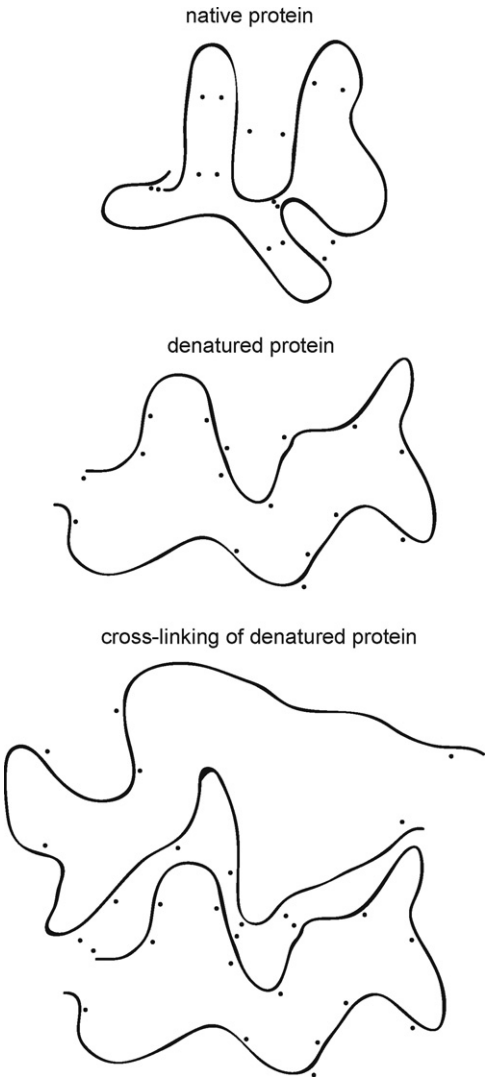

Fig. 1. Scheme of the cross-linking process of denatured proteins during fixation.

The evaluation of the deformations of the brain is based on 3D affine and non-rigid registration. This non-destructive technique allows examination of the influence of formalin on the brain tissue quantitatively. The usage of a 3D imaging technique combined with non-rigid registration permits the extraction of the local deformations, which are relevant for the inhomogeneous brain tissue, and the separate examination of expansion and shrinkage of regions of interest inside the brain.

2. Materials and methods

For the measurements, a human brain of a 68 year old male with no neuropathological signs at autopsy was used. All procedures were conducted in accordance with the Declaration of Helsinki and according to the ethical guidelines of the Canton of Basel. After the first MRI scan of the intact head (within 48 h after death), the following steps of preparation were carried out. First, the scalp was detached and the calvaria removed through a horizontal cut. After the transection of the tentorium cerebelli and a cut through vasculature, nerves and the medulla, the brain was extracted and put into 10% formalin for fixation. The brain was then measured again after diverse formalin fixation degrees. During the measurements it was in a container filled with formalin. Here, the brain was sinking to the ground of the container, but because of similar density to the formalin solution the touching area was restricted to a very small interface with negligible influence on the brain's shape.

All MRI scans were performed on a Verio 3T whole body scanner (Siemens Health Care, Erlangen, Germany). MPRAGE acquisitions with 0.7 mm isotropic resolution and a field of view (FOV) of $268.0 \times 268.0 \times 179.2\,mm^3$ were performed with an 8° flip angle, 2000 ms repetition time, 2.72 ms echo time, and 700 ms inversion time. Twelve averages were taken in order to reach sufficient signal to noise ratio (SNR) within a total scan time of 154 min. The determined SNR of the data sets had a value of 200. Four of the data sets were scanned using MPRAGE acquisitions with 1.0 mm isotropic resolution, a FOV of $256.0 \times 256.0 \times 256.0\,mm^3$, an 8° flip angle, 2000 ms repetition time, 2.41 ms echo time and 700 ms inversion time. Taking four averages to reach identical SNR level, the total scan duration was 34 min.

For the determination of the volume changes of the brain one day after extraction and during formalin fixation a 3D affine registration algorithm was used (Fierz et al., 2008). The registrations were performed using the classical maximization of mutual information (MI) principle (Maes et al., 1996; Viola and Wells, 1995). In order to determine local deformations of the brain caused by extraction and formalin fixation, the related 3D data sets were registered by means of a non-rigid registration algorithm

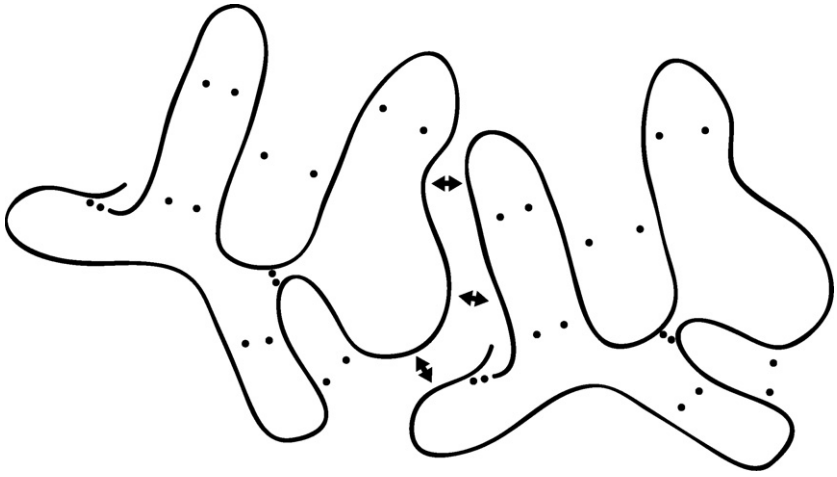

Fig. 2. Scheme of the cross-linking process of non-denatured or minimally denatured proteins during fixation using a fixative.

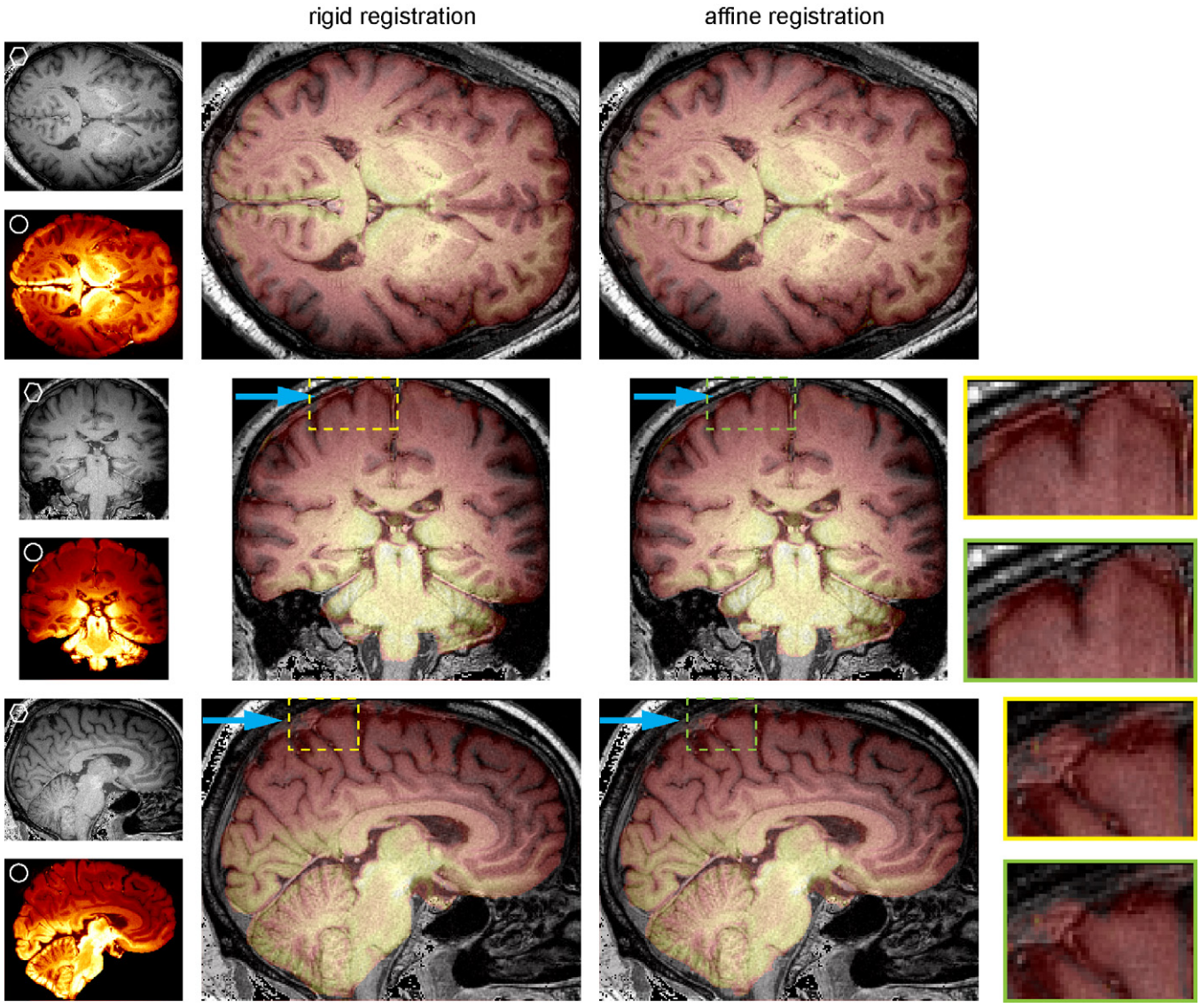

Fig. 3. Three-dimensional rigid and affine registration of the data sets of the brain inside the cranium (gray scale, hexagon) and one day after extraction (orange colored, circle). On the right side the images before and after registration are overlaid with the colored image being semi-transparent. The arrowheads demonstrate the deformations caused by extraction and one day of formalin fixation and the correction of them using affine registration. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

developed at Computer Vision Lab, ETH Zurich. The chosen registration algorithm is based on the adaptive hierarchical image subdivision strategy, which decomposes the non-rigid matching problem into numerous local affine registrations of sub-images of decreasing size (Andronache et al., 2008). The local registrations were again performed using the classical maximization of MI principle. The local registration parameters were found using the Powell multi-dimensional search algorithm (Press et al., 1988) such that the MI between the reference and the floating sub-images was maximized. The hierarchical image splitting strategy was proposed by Likar and Pernus (2001) and was recently extended to 3D with several improvements (Andronache et al., 2008). The hierarchical splitting was governed by a sub-image information consistency test in the form of the Moran spatial autocorrelation coefficient. At each level of the hierarchy, the consistency of the information contained in each of the further subdivided images was tested, and all those sub-images failing this test were no longer subdivided or registered at the successive levels. This consistency test was also used as the stopping criterion for the entire registration algorithm. The hierarchical image subdivision was complete when no structural information was found in any of the currently partitioned sub-images, and therefore, their local registration was meaningless. As a consequence of the use of the information consistency test, at the last hierarchical level, the size of the sub-images may differ from one another. A typical minimum size is around $8 \times 8 \times 8$ voxels depending on the level of details and noise in the original image. The final deformation field was estimated from all registration parameters of all sub-images at the last hierarchical level by thin plate spline (TPS) interpolation. As the deformation field induced by formalin fixation has been unknown so far, it was impossible to provide a validation of the registration result for the particular case. The error bars were deduced using this registration method validated for the liver (Andronache, 2006). More precisely, using a MR scanner, T1 and T2 weighted images of the liver were acquired simultaneously at different stages of the respiratory cycle. The non-rigid registration was then used to recover the deformation fields, and the statistics led to an accuracy of 1.07 ± 0.75 in voxel dimensions.

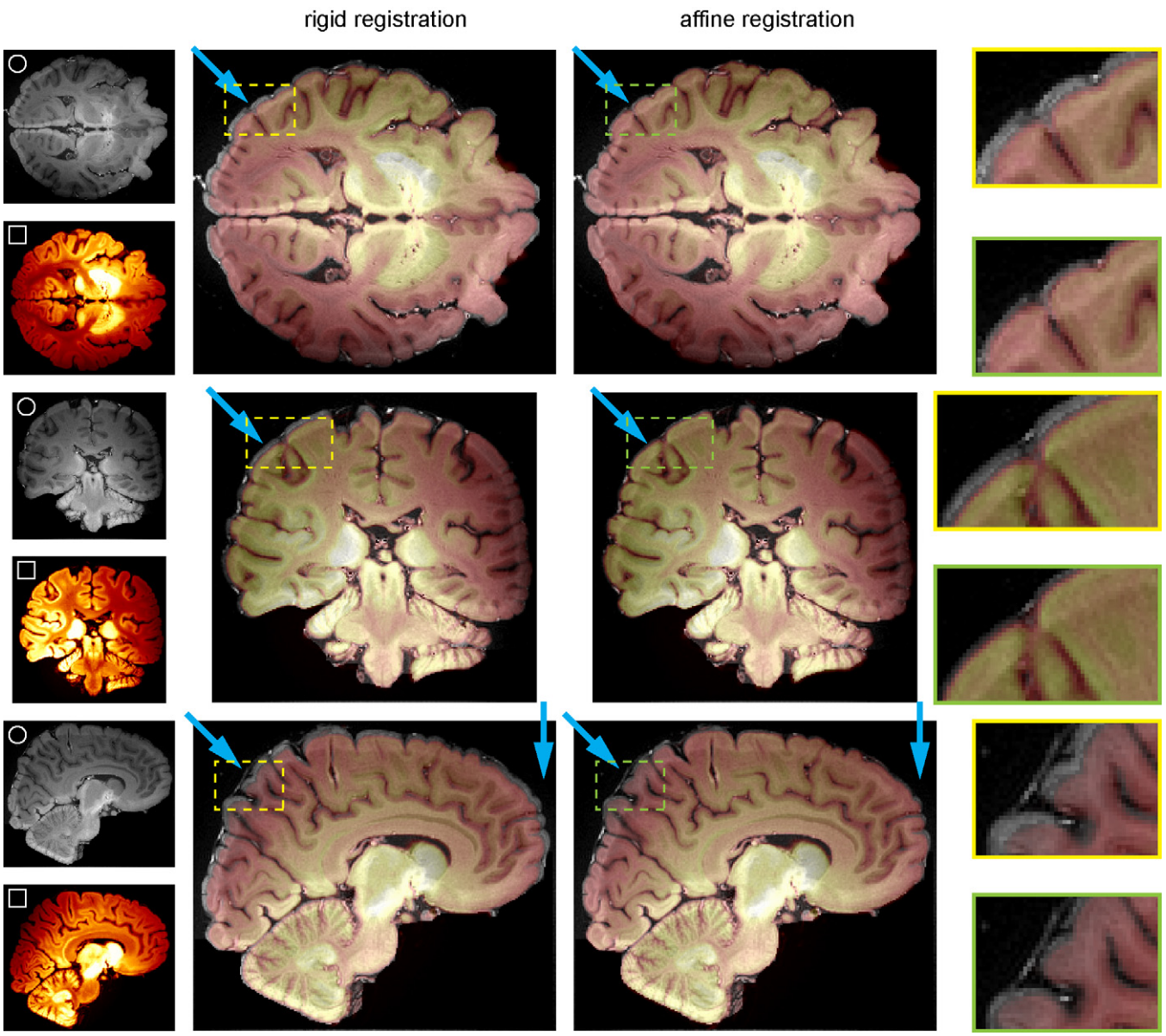

Fig. 4. Three-dimensional rigid and affine registration of the data sets of the brain one day after extraction (gray scale, circle) and after 70 days of formalin fixation (orange colored, square). Here, as well, the images before and after registration are overlaid with the colored image being semi-transparent. The arrowheads demonstrate the deformations arisen during formalin fixation and the correction of them using affine registration. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3. Results

3.1. Global volume changes due to extraction and formalin fixation

Fig. 3 illustrates the deformations caused during the extraction and after one day of formalin fixation. For this purpose the MRI data set of the brain inside the cranium (hexagon) was registered with the data set one day after extraction (circle) using a rigid and affine registration algorithm. Having six degrees of freedom, i.e. three degrees of translation and three degrees of rotation, the two data sets were rigidly registered. Affine registration also includes scaling in the three orthogonal directions. The virtual cuts are given according to the data acquisition to avoid potential artifacts as the result of resampling. The data set scanned one day after extraction (colored orange) is matched to the data set before extraction and is made semi-transparent in order to indicate the differences. Regarding the rigid registration, the blue arrowheads hint that the volume of the data set of the brain one day after extraction is larger than that of the data set inside the skull. After the affine registration, where additionally a scaling factor is used for the registration, the boarders of the brain fit much better. The scaling factor of 1.052 ± 0.003 means an expansion of the brain of $5.2\% \pm 0.3\%$ resulting from extraction and one day of formalin fixation. Furthermore the registered data set of the brain one day after extraction was compared to the data sets at further steps of fixation. Fig. 4 shows the comparison of the brain one day after extraction (circle) with the one after 70 days of fixation (square). Here, the data set after 70 days of fixation is colored orange, made semi-transparent and laid over the data set one day after extraction. Again the arrowheads illustrate the differences between the data sets. The matching indicates that the volume of the data set after 70 days of fixation is smaller than that one day after extraction. This assumption can be proved by the affine registration of the data sets resulting in a

scaling factor of 0.934 ± 0.003. The modification of the brain volume at different steps of formalin fixation can be seen in Fig. 5. During a fixation period of 70 days, nine scans with 0.7 mm isotropic voxel size (blue data sets) and four scans with 1.0 mm isotropic voxel size (orange data sets) were acquired. After the increase of the volume one day after extraction, a decrease of the volume can be observed during the fixation period. A fit using an exponential function provides the value for the volume shrinkage of the brain after infinite time of fixation. The fit parameter y_0 in Fig. 5 represents a total volume shrinkage value (compared to the volume inside the skull) of 96.7% ± 0.5%. This value contains both effects, namely the expansion during the first day after extraction and shrinkage during fixation. Consequently, the net shrinkage value during fixation without the effect of extraction and one day of formalin fixation is 91.9% ± 0.6%.

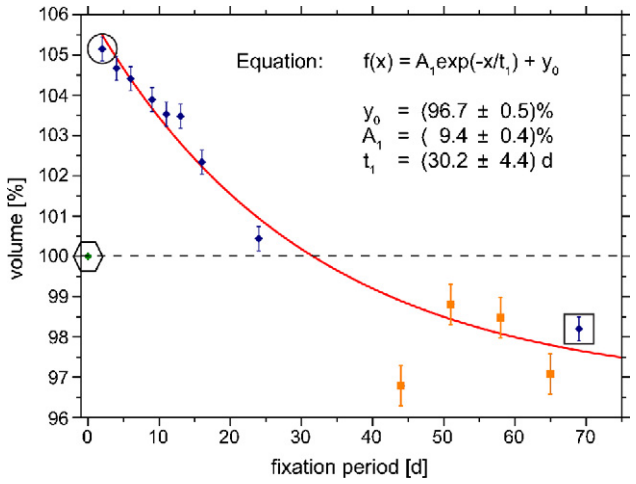

Fig. 5. Time-dependent progress of the whole brain volume one day after extraction and further formalin fixation. Highlighted are the moments of the brain inside the cranium (hexagon), one day after extraction (circle) and after 70 days of fixation (square) which are illustrated in Figs. 3 and 4. The blue data sets were acquired with an isotropic voxel size of 0.7 mm, the orange data sets with 1.0 mm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3.2. *Local deformations caused by extraction and formalin fixation*

After the affine registration, the data sets were registered using the non-rigid registration algorithm in order to determine additional local deformations inside the brain induced by extraction and formalin fixation. Figs. 6 and 7 compare the results of the affine and

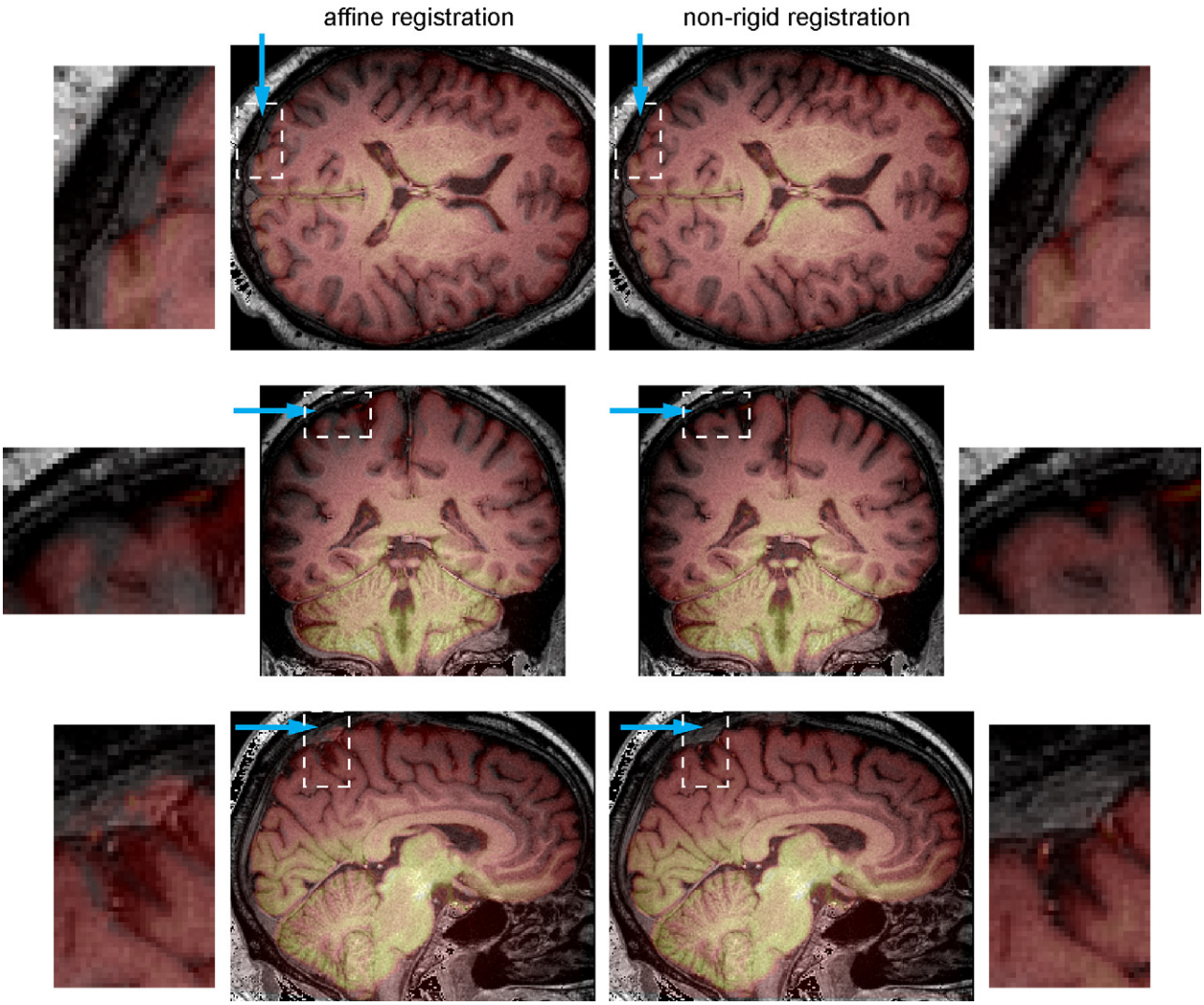

Fig. 6. Comparison between three-dimensional affine and non-rigid registration of the data set of the brain inside the cranium with the data set one day after extraction. The arrowheads illustrate the benefits of the non-rigid registration algorithm.

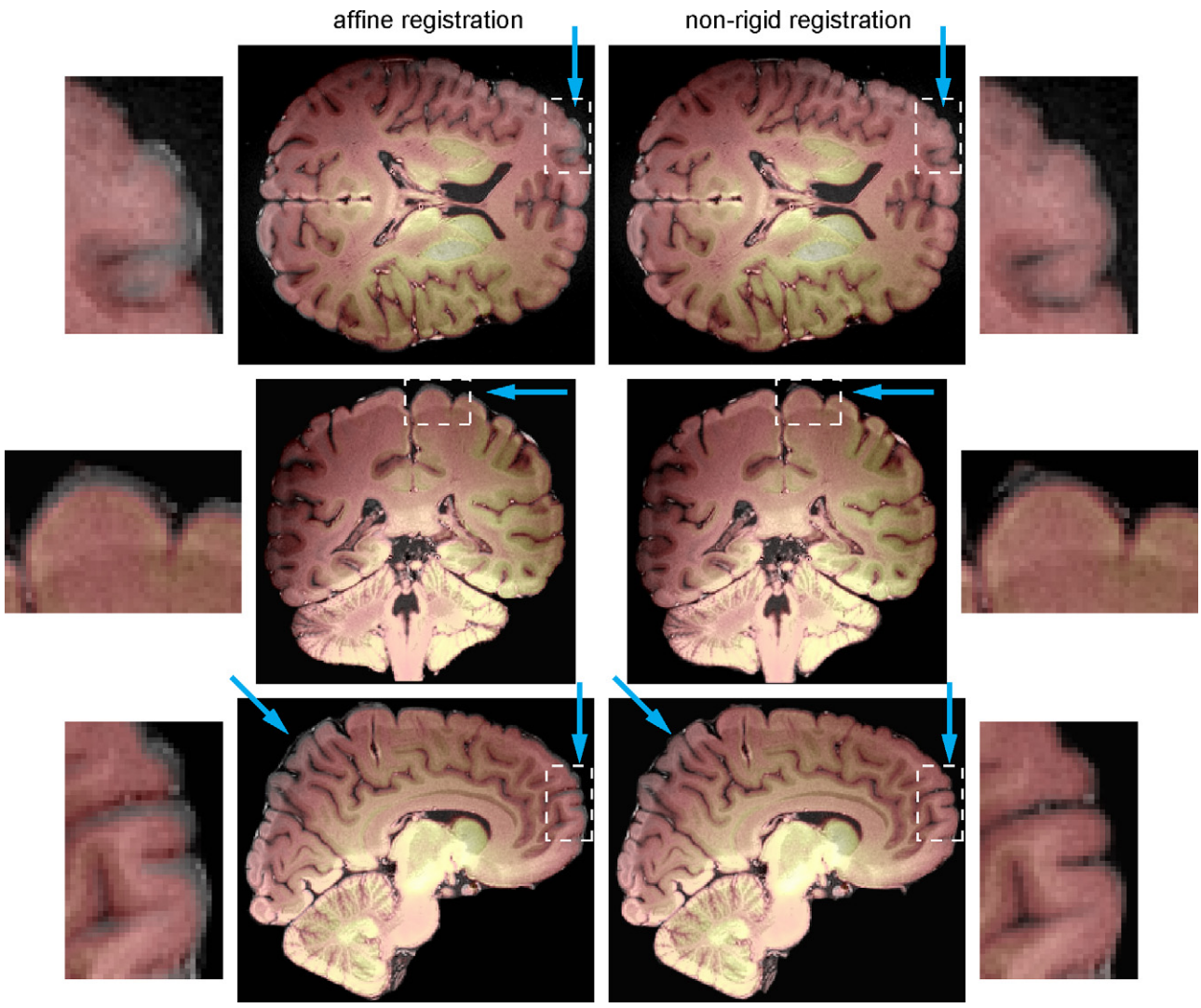

Fig. 7. Comparison between three-dimensional affine and non-rigid registration of the data set one day after extraction with that after 70 days of formalin fixation. The arrowheads illustrate the benefits of the non-rigid registration algorithm.

the non-rigid registration. The arrowheads indicate the differences of the registration results.

Performing the non-rigid registration, the individual voxels of the deformed data set are shifted to the related 3D positions of the initial data set of the intracranial state. The resulting 3D deformation vector field caused by the extraction and one day formalin fixation of the brain is illustrated in Fig. 8. First, the magnitude of the voxel displacement vectors is shown by means of three orthogonal slices on the left side of the figure. The average strain value, which is equal to the average magnitude value of the 3D displacement vectors, is 0.6 mm. The more interesting value is the maximal magnitude of the displacements which amounts to 3.7 mm. In order to demonstrate the orientations of the displacement vectors, a more detailed 2D vector field was shown for several interesting areas (insets at the right side of the figure). The mean absolute pixel displacements (arrow length) of the whole data set in *x*-, *y*- and *z*-direction amount to 0.34 mm, 0.25 mm and 0.30 mm. The maximal pixel displacements amount to 3.62 mm, 2.30 mm and 2.81 mm in the directions of the *x*-, *y*- and *z*-axis. The 3D deformation field induced by formalin fixation is shown in Fig. 9. Again the values of the averaged extension, amounting to 0.15 mm, 0.11 mm and 0.19 mm, and the maximal strains, amounting to 1.88 mm, 1.30 mm and 2.65 mm, were determined for *x*-, *y*- and *z*-direction. The maximal magnitude of the displacement vector field due to formalin fixation amounts to 2.7 mm.

3.3. Determination of the local volume strain field

The deformation field shown in Section 3.2 corresponds to a voxel displacement field. In order to investigate differences in the shrinkage values of interested regions of the brain, a local volume strain field was determined.

Having the 3D displacement field $\overline{D}$, a 3D local strain field in *x*-direction $\overline{\varepsilon}_x$ can be calculated by

$$\overline{\varepsilon}_x = \overline{D}_x * \overline{k}_x, \tag{1}$$

the convolution of the *x*-coordinate of the displacement field $\overline{D}_x$ with the 3D kernel

$$\overline{k}_x(:,1,:) = \overline{k}_x(:,2,:) = \overline{k}_x(:,3,:) = \frac{1}{18 \cdot l_x} \begin{pmatrix} -1 & 0 & 1 \\ -1 & 0 & 1 \\ -1 & 0 & 1 \end{pmatrix}, \tag{2}$$

l_x being the voxel length of the data set in *x*-direction. Eq. (1) is a matrix which contains local shrinkage values (negative signs) and local expansion values (positive sign) of data set sub-volumes having the size of $3 \times 3 \times 3$ voxels. Values equal to zero represent

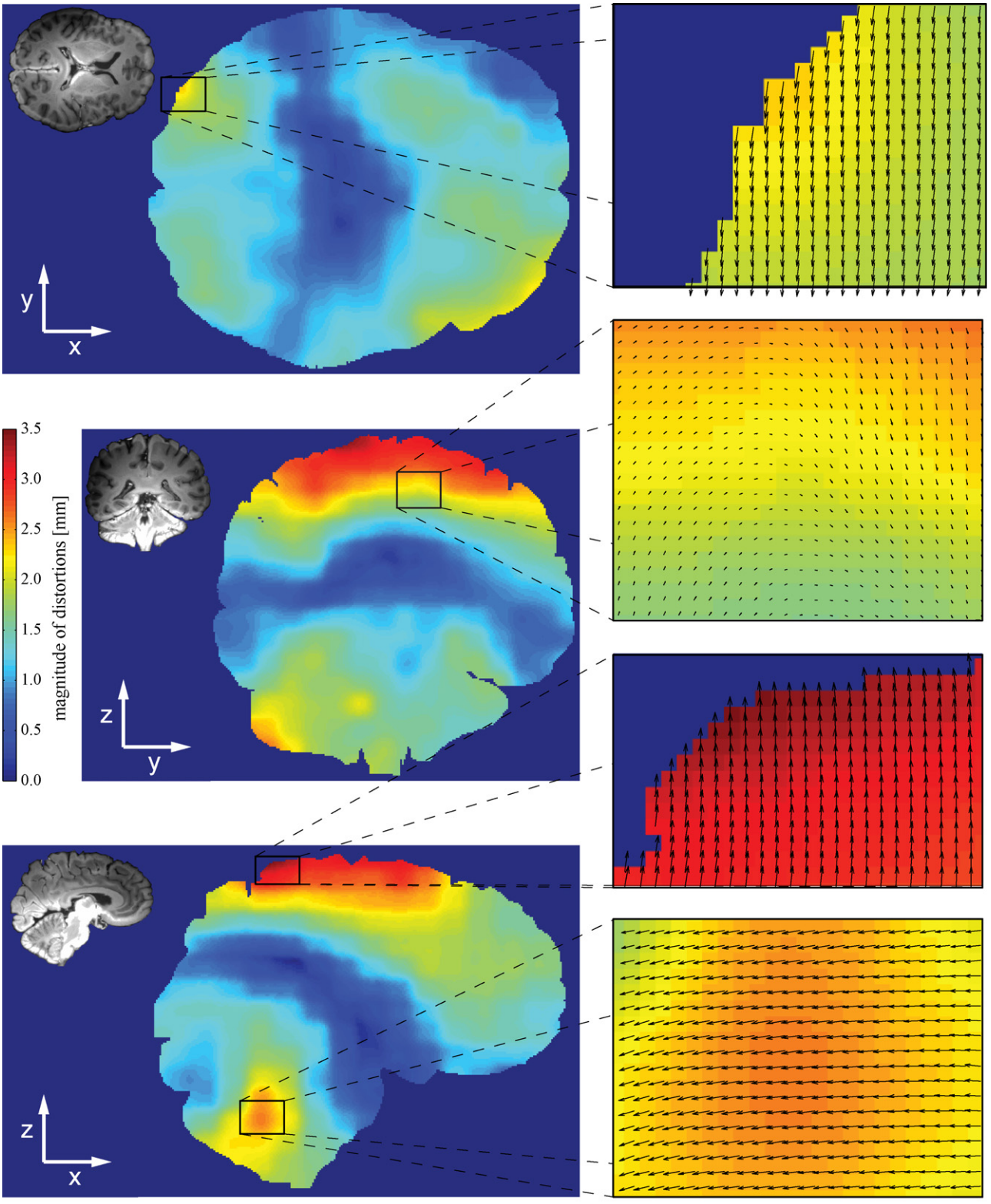

Fig. 8. Three-dimensional deformation vector field of the brain due to extraction and one day of formalin fixation, exemplary shown by means of the magnitude of the voxel displacement vectors for the three orthogonal slices. Zooms of selected regions illustrate the orientation of the vectors projected on the corresponding plane.

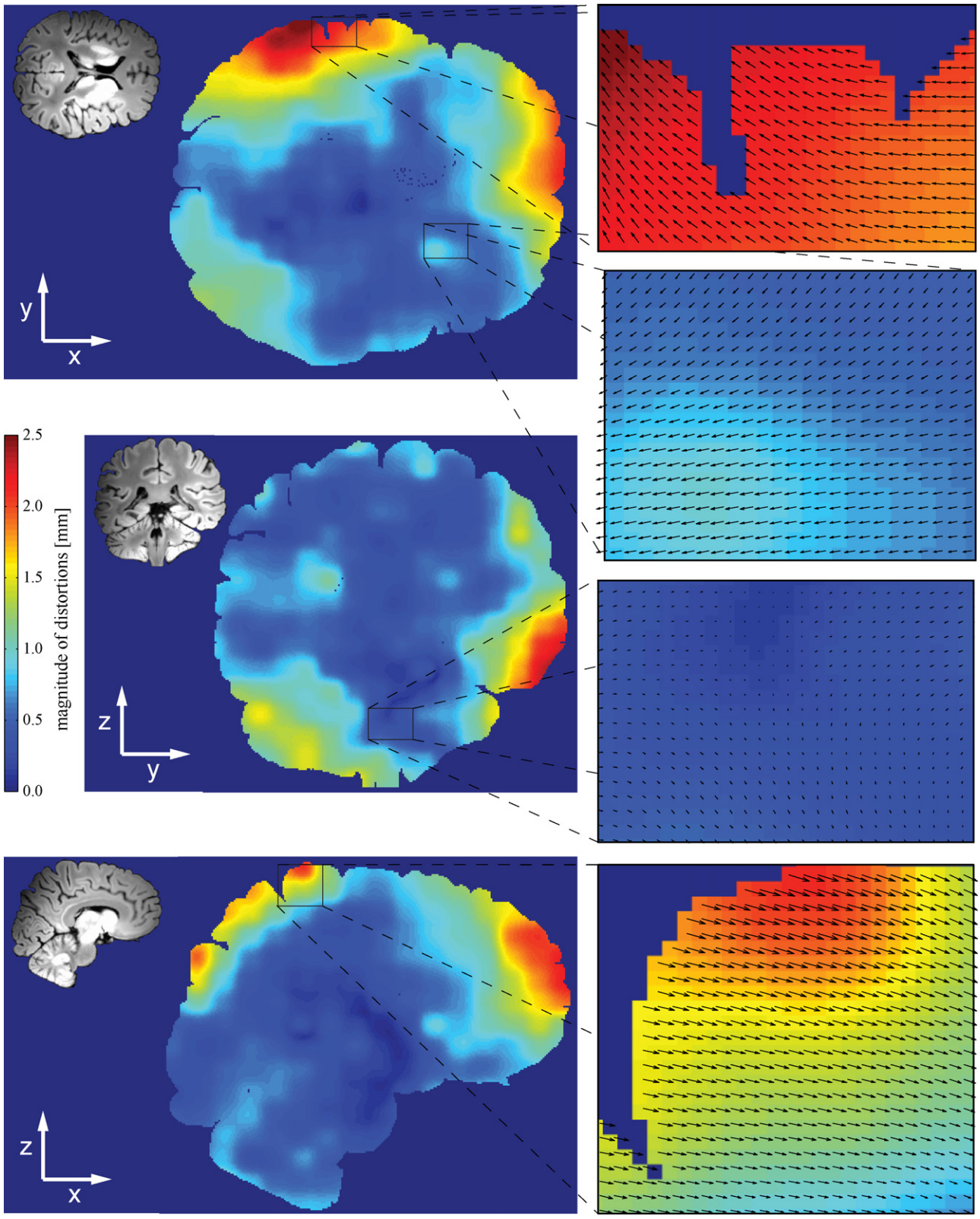

Fig. 9. Deformation vector field induced by formalin fixation shown by maps of the magnitude of the voxel displacement vectors and two-dimensional vector fields of selected regions which demonstrate the orientations of the deformations.

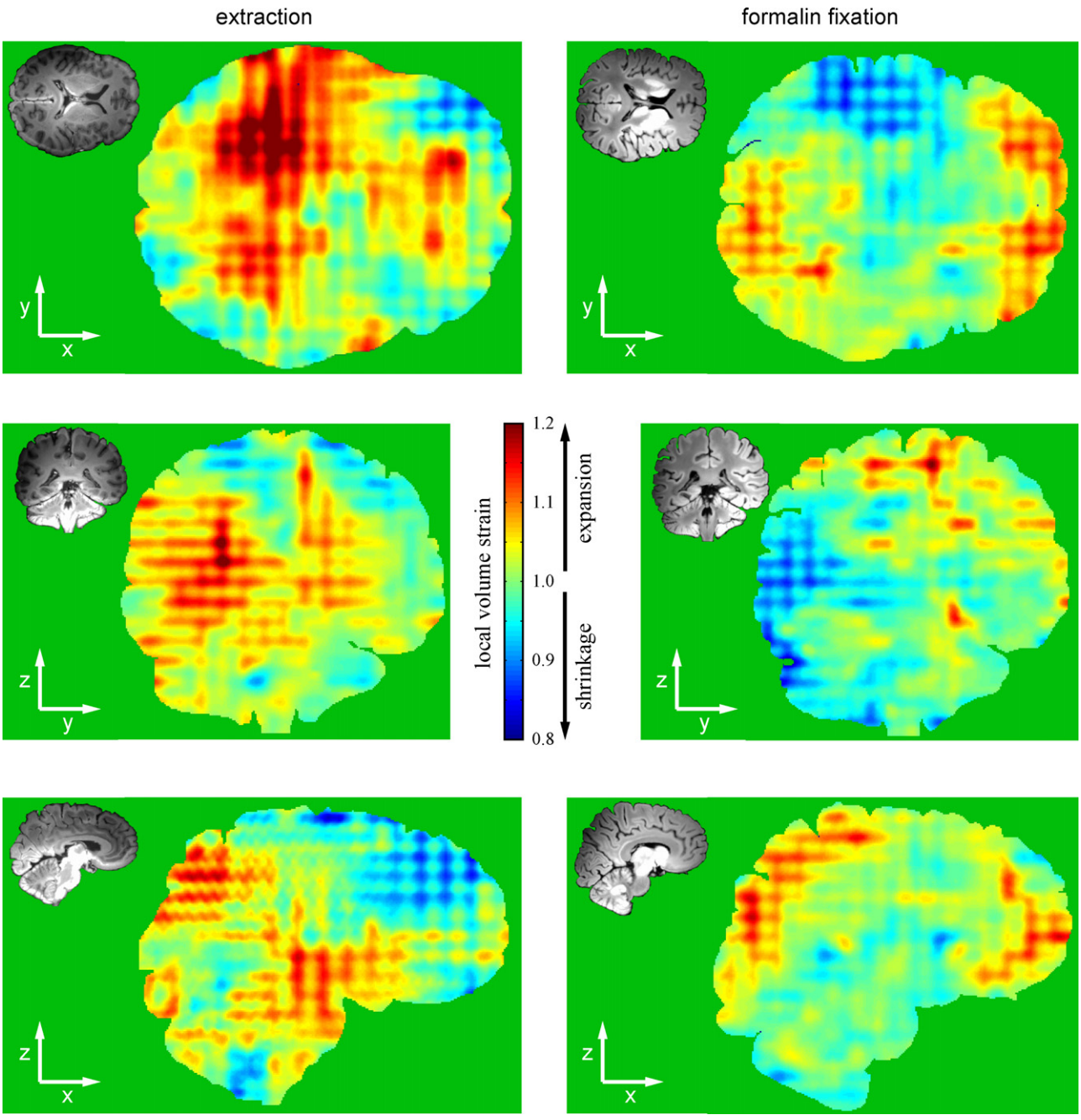

Fig. 10. Local volume strain field induced by extraction and formalin fixation shown by maps of the local scaling factors.

volumes with neither shrinkage nor expansion. An other way to define strains is the use of scaling factors. The scaling factors in x-direction can be determined by

$$\bar{S}_x = \bar{J} + \bar{\varepsilon}_x, \tag{3}$$

where $\bar{J}$ is a matrix of ones having the same dimensions as $\bar{\varepsilon}_x$. Using the kernels

$$\bar{k}_y(:,:,1) = \bar{k}_y(:,:,2) = \bar{k}_y(:,:,3) = \frac{1}{18 \cdot l_y} \begin{pmatrix} 1 & 1 & 1 \\ 0 & 0 & 0 \\ -1 & -1 & -1 \end{pmatrix} \tag{4}$$

and

$$\bar{k}_z(1,:,:) = \bar{k}_z(2,:,:) = \bar{k}_z(3,:,:) = \frac{1}{18 \cdot l_z} \begin{pmatrix} 1 & 1 & 1 \\ 0 & 0 & 0 \\ -1 & -1 & -1 \end{pmatrix}, \tag{5}$$

the local strains in y- and z-direction can then be determined by

$$\bar{S}_y = \bar{J} + \bar{D}_y * \bar{k}_y \quad \text{and} \quad \bar{S}_z = \bar{J} + \bar{D}_z * \bar{k}_z. \tag{6}$$

The determination of the local volume strains is a componentwise multiplication of the calculated scaling matrices

$$\bar{S}_{vol} = \bar{S}_x \cdot \bar{S}_y \cdot \bar{S}_z. \tag{7}$$

Fig. 10 demonstrates the local volume strain field induced by extraction plus one day of fixation and as a result of formalin fixation over a period of 70 days. The local strain field after one day after extraction has maximal volume strains of 1.32 and minimal values of 0.73. During the fixation period, maximal strains of 1.22 and minimal strains of 0.76 occurred. In order to affirm the global scaling factors determined by the affine registration in Section 3.1, the mean value of the local strains should amount to one being in

balance between shrinkage and expansion which is the case with the mean strain value of 1.008 for the data set one day after extraction and 1.001 for the data set after formalin fixation.

4. Discussion

Because of its high lateral spatial resolution, microscopy of histological sections is generally used for the characterization of brain tissue down to the cellular level. One of the first preparation steps is the formalin fixation of the whole brain after the extraction. Firstly, one expects a global volume change due to the extraction from the cranium and, inside the brain, local deformations caused by the handling procedure. Secondly, the fixation process should induce a shrinkage of the whole volume (Lang, 2006). Because of the inhomogeneity of the brain tissue, that can be explained by different chemical composition for instance of cerebral white and gray matter (Brooks et al., 1980), we also can expect a local strain field, which was not investigated until now. So far, the determination of the shrinkage of human brain during formalin fixation is based on the measurement of linear shrinkage values of brain slices (Mouritzen Dam, 1979; Quester and Schröder, 1997). During their study concerning Huntington's disease, Halliday et al. (1998) describe the determination of the volume shrinkage induced by formalin fixation measuring the volumes by fluid displacements. Unfortunately, using this technique, it is impossible to determine the volume changes induced by extraction of the brain or the local deformations due to the inhomogeneity of the brain tissue. Therefore, it is important to find a technique which enables us to determine the whole volume changes as well as the local strains in order to allow the correction of the artifacts induced by extraction and formalin fixation of the human brain.

MRI permits the non-destructive 3D visualization of the extracted human brain as well as inside the cranium and is known for its superb contrast between white and gray matter which makes registration between the data sets easy. The comparison of the data sets, intracranial and after extraction at different fixation steps can be arranged by means of an affine registration (for the determination of the volume change) and by means of a non-rigid registration (for the determination of the local strains). Rigid registration relates to solid states of identical size. Affine registration consequently belongs to the non-rigid approaches. Therefore, one might better term the non-rigid approach used in this study as elastic registration.

After the extraction of the brain and one day of formalin fixation, a volume expansion of the whole brain of $5.2\% \pm 0.3\%$ was determined. This first measurement after extraction is a superposition of the initial expansion related to extraction from cranium and the initiated early stages of fixation. The effect of expansion can be explained by lower pressure values outside the cranium and the swelling effect caused by osmotic pressure of the formalin solution (Hrdlicka, 1966; Blinkow and Glezer, 1968). Subsequently, the formalin treatment of the soft tissue induces brain shrinkage. The pure formalin fixation (without the effect caused after one day of extraction) of human brain tissue during a period of 70 days resulted in a volume shrinkage of $8.1\% \pm 0.6\%$. Mouritzen Dam (1979) indicates a volume shrinkage of gray matter of 33%. This value was determined by the measurement of slice thickness changes during formalin fixation with different concentrations and extrapolating the linear shrinkage value on the volume shrinkage. The assumption that the shrinkage of the different parts of the brain is isotropic is debatable, as shown by Quester and Schröder (1997). On the basis of an investigation of the formalin effect on human brain stem, they showed that the shrinkage is anisotropic, resulting in a shrinkage value of 8.3% along the longitudinal direction and almost no shrinkage along the transversal directions which results in a volume shrinkage of around 8.3%. The volume shrinkage of the human brain stem is in the range of the value we obtained for the volume shrinkage of the whole brain. Values between 5% and 9% volume shrinkage during formalin fixation with concentration of 5–10% and pH values between 7.1 and 7.4 were determined by Burck (1982), measuring area changes of slices of different human soft tissues. These values also conform well with our result. The listed results are linear (Mouritzen Dam, 1979; Quester and Schröder, 1997) or area (Burck, 1982) measurements of specific parts of the brain which then are extrapolated to the volume shrinkage value. Furthermore, these techniques cannot reveal the effect of the brain extraction. The technique used here allows, besides the real 3D determination of the brain shrinkage during formalin fixation, the evaluation of the effect of extraction on the brain volume expansion. The two counteractive effects result in a net volume shrinkage of the brain of $3.3\% \pm 0.5\%$ compared to the post mortem situation of the brain inside the cranium.

The most important advantage of the technique is the possibility, not only to determine the global shrinkage of the brain, but also to analyse local shrinkage values and deformations of different parts of the brain. After MRI of the brain at the different steps of the investigation, one can determine volume shrinkage values using an affine and local strain field using a non-rigid registration. The calculated maximal local volume strain values of 32% and −27% due to extraction and one day of formalin fixation and 22% and −24% due to formalin fixation in addition to the whole volume changes cannot be disregarded. The maximal local strains have been found at different parts of the brain depending on the fixation time, Fig. 10). After extraction and one day of formalin fixation, the maximal expansion values were mainly located at the ventricles what can be seen on top left of the figure. The enlargement of the ventricles due to the lower pressure outside the cranium explains this behavior in a natural way. During the earlier stages of formalin fixation, the maximal strain values were found in the outer region of the brain to which the formalin could diffuse rather fast.

The non-rigid registration algorithm not only determines the changes but also corrects them and thereby generating a 3D data set which, despite underlying different preparation steps, correlates well with the data set of the brain post mortem inside the cranium, although having endured different preparation steps.

5. Conclusions

In order to determine the volume change of the whole human brain during extraction and formalin fixation, an affine registration algorithm was applied on MRI data sets at diverse steps of the study. Furthermore, the contribution demonstrated the power of 3D non-rigid registration in order to correct local artifacts produced during the extraction and the formalin fixation. This technique generates a morphological condition of the brain that is very close to the post mortem situation inside the skull. This data set can then be used for the correction of the high resolution data set (e.g., synchrotron radiation-based microcomputed tomography) of a smaller part like the thalamus and finally for the correction of the micrographs of the histological slices and consequently the correction of stereotactic atlases.

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References

Andronache A. Multi-modal non-rigid registration of volumetric medical images. Information Technology and Electrical Engineering, ETH, Zürich, Zürich No. 16601; 2006.

Andronache A, von Siebenthal M, Székely G, Cattin P. Non-rigid registration of multi-modal images using both mutual information and cross-correlation. Med Image Anal 2008;12:3–15.

Bahr GF, Bloom G, Friberg U. Volume changes of tissues in physiological fluids during fixation in osmium tetroxide or formaldehyde and during subsequent treatment. Exp Cell Res 1957;12:342–55.

Blinkow SM, Glezer II. The human brain in figures and tables. New York: Plenum Press; 1968.

Bloom G, Friberg U. Shrinkage during fixation and embedding of histological specimens. Acta Morphol Neerl Scand 1956;1:12–20.

Brooks RA, Di Chiro G, Keller MR. Explanation of cerebral white–gray contrast in computed tomography. J Comput Assist Tomogr 1980;4:489–91.

Burck HC. Histologische Technik. 5th ed. Stuttgart: Thieme; 1982.

Fierz F, Beckmann F, Huser M, Irsen S, Leukers B, Witte F, et al. The morphology of anisotropic 3D-printed hydroxyapatite scaffolds. Biomaterials 2008;29:3799–806.

Fox MW. Uptake of formalin by brain tissue from dogs killed at various stages of development. Nature 1965;205:1221.

Germann M, Morel A, Beckmann F, Andronache A, Jeanmonod D, Müller B. Strain fields in histological slices of brain tissue determined by synchrotron radiation-based micro computed tomography. J Neurosci Methods 2008;170:149–55.

Halliday GM, McRitchie DA, Macdonald V, Double KL, Trent RJ, McCusker E. Regional specificity of brain atrophy in Huntington's disease. Exp Neurol 1998;154:663–72.

Hillman H, Deutsch K. Area changes in slices of rat-brain during preparation for histology or electron-microscopy. J Microsc 1978;114:77–84.

Hrdlicka A. Brain and brain preservatives. Proc US Nat Mus 1966;30:245–320.

Jolesz FA, McDannold NJ. Current status and future potential of MRI-guided focused ultrasound surgery. J Magn Reson Imaging 2008;27:391–9.

Kennedy JE, ter Haar GR, Cranston DW. High intensity focused ultrasound: surgery of the future? Br J Radiol 2003;76:590–9.

Lang G. Histotechnik: Praxislehrbuch für die Biomedizinische Analytik. Wien: Springerverlag; 2006.

Leibnitz L. Die Veränderung von Gewicht, Volumen und spezifischem Gewicht des Rattengehirnes nach Fixierung, Dehydrierung und Aufhellung. J Hirnforsch 1967;9:97–104.

Likar B, Pernus F. A hierarchical approach to elastic registration based on mutual information. Image Vis Comput 2001;19:33–44.

Maes F, Collignon A, Vandermeulen D, Marchal G, Suetens P. Multi-modality image registration by maximization of mutual information. In: Mathematical methods in biomedical image analysis. IEEE; 1996. p. 14–22.

Martin E, Jeanmonod D, Morel A, Zadicario E, Werner B. High intensity focused ultrasound for non-invasive functional neurosurgery. Ann Neurol 2009;66:858–61.

Morel A. Stereotactic atlas of the human thalamus and basal ganglia. New York: Informa Healthcare USA, Inc; 2007.

Mouritzen Dam A. Shrinkage of the brain during histological procedures with fixation in formaldehyde solutions of different concentrations. J Hirnforsch 1979;20:115–9.

Press WH, Flannery BP, Teukolsky SA, Vetterling WT. Numerical recipes in C – the art of scientific computing. Cambridge University Press; 1988.

Quester R, Schröder R. The shrinkage of the human brain stem during formalin fixation and embedding in paraffin. J Neurosci Methods 1997;75:81–9.

Romeis B, Böck P. Mikroskopische Technik. 17th ed. München: Urban & Fischer; 1989.

Schaltenbrand G, Wahren W. Atlas for stereotaxy of the human brain. Stuttgart: Thieme; 1977.

Schulz G, Morel A, Imholz MS, Deyhle H, Weitkamp T, Zanette I, et al. Evaluating the microstructure of human brain tissues using synchrotron radiation-based micro computed tomography. Proc SPIE 2010;78040:78040F.

Viola P, Wells WM. Alignment by maximization of mutual information. In: Proceedings of the fifth international conference on computer vision; 1995. p. 16–23.

Wüstenfeld E. Experimental contribution to the problem of volume changes and penetration time in histological technic. I. Effect of five fixatives in the volume of the liver, kidney, spleen and muscle at room temperature. Z Wiss Mikrosk 1955;62:241–7.