

Super-fast elastic registration of histologic images of a whole rat brain for three-dimensional reconstruction

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ABSTRACT

We present a super-fast and parameter-free algorithm for non-rigid elastic registration of images of a serially sectioned whole rat brain. The purpose is to produce a three-dimensional high-resolution reconstruction. The registration is modelled as a minimization problem of a functional consisting of a distance measure and a regularizer based on the elastic potential of the displacement field. The minimization of the functional leads to a system of non-linear partial differential equations, the so-called Navier-Lamé equations (NLE). Discretization of the NLE and a fixed point type iteration method lead to a linear system of equations, which has to be solved at each iteration step. We not only present a super-fast solution technique for this system, but also come up with sound strategies for accelerating the outer iteration. This does include a multi-scale approach based on a Gaussian pyramid as well as a clever estimation of the material constants for the elastic potential. The results of the registration process were controlled by an expert who was able to recognize histological details like laminations which was not possible before. Therefore, it is essential to apply elastic registration to this kind of imaging problem. Finally, the visually pleasing results were quantified by a distance measure leading to an improvement of about 79% after just 35 iteration steps.

Keywords: super-fast elastic registration, whole brain sectioning, 3D-reconstruction, neuroimaging, alignment, multi-scale

1. INTRODUCTION

In neuroscience it is essential to obtain the exact spatial localization of single neurons resp. structures and functions of interest. If the data of the biologic material contains disturbances like distortions it must be processed adequately in order to visualize structures of interest. In order to obtain structural information, the brain must be serially sectioned (in our case into $20\ \mu\text{m}$ thin slices) and structures of interest need to be stained by a histological technique (see Fig. 1). Thereafter, the stained sections can be digitized by a light microscope or a high resolution transparent flat-bed scanner. This leads to a series of consecutive and unaligned images (see Fig. 2). Deformations coming from the sectioning and the staining process and artifacts like wrinkles, cracks and loss of tissue make a superposition of all consecutive slices looking fuzzy and coherent areas do not fit anymore. Therefore, the brain slices have to be registered in order to recognize relevant three-dimensional structure.¹

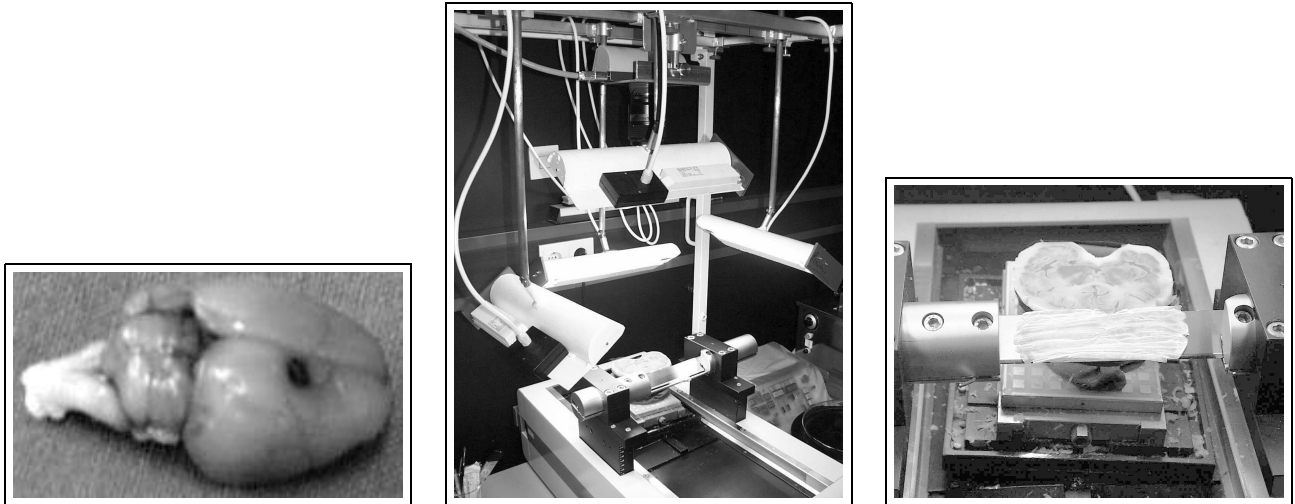
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(a) Rat brain before sectioning.

(b) Sectioning of a brain.

(c) Detail of the cutting unit.

Figure 1. Photos of the Sectioning Process.

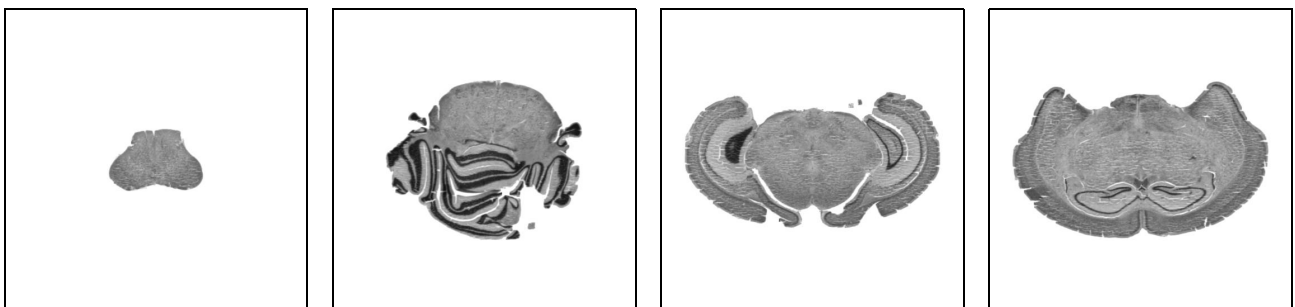
The digitization of the brain slices with a high-resolution transparent flat-bed scanner at a resolution of $12.5 \mu\text{m}$ per pixel leads to 503 images with a size of (1900×1900) pixels each with gray values between 0 and 255. This huge data amount (1.7 GB) necessitates a super-fast and efficient algorithm for registration.

Due to the nature of distortions we have chosen a registration scheme, the so-called elastic registration,²⁻⁵ which mimics the elastic behavior of the histological sections.

2. METHODS

Before elastic registration, a preprocessing followed by a linear preregistration, based on the principal axis transformation, was performed to compensate for rotational and translational artifacts and to have a better starting situation for the non-linear process.

For the non-linear part of the registration process, we use a variational formulation in order to come up with a fast implementation.⁶ As mentioned before, elastic registration appears to be the most meaningful. Each



(a) Slice no. 45.

(b) Slice no. 155.

(c) Slice no. 250.

(d) Slice no. 335.

Figure 2. Typical images of the series of slices.

digitized slice may be represented by a mapping

$$R^{(\nu)} : \Omega \subset \mathbb{R}^2 \rightarrow \mathbb{R}. \quad (1)$$

Let M denote the number of slices, then the goal of registration of image series with slices

$$R := \left(R^{(1)}, \dots, R^{(M)} \right), \quad (2)$$

is to find transformations $\varphi^{(\nu)} := \varphi^{(\nu)}(x) = x - u^{(\nu)}(x)$ based on the displacement fields

$$u := \left(u^{(1)}(x), \dots, u^{(M)}(x) \right), \quad u^{(\nu)}(x) : \mathbb{R}^2 \rightarrow \mathbb{R}^2, \nu = 1, \dots, M, \quad (3)$$

one for each slice, such that neighbored regions are coherent again. In a variational setting this amounts in the minimization of a functional

$$\mathcal{J}[R; u] := \mathcal{D}[R; u] + \alpha \cdot \mathcal{S}[u], \quad (4)$$

consisting of a distance measure \mathcal{D} and a regularizer (also called smoother)

$$\mathcal{S}[u] := \sum_{\nu=1}^M \int_{\Omega} \frac{\mu}{4} \sum_{j,k=1}^2 \left(\partial_{x_j} u_k^{(\nu)} + \partial_{x_k} u_j^{(\nu)} \right)^2 + \frac{\lambda}{2} \left(\operatorname{div} u^{(\nu)} \right)^2 dx, \quad (5)$$

based on the elastic potential, weighted by a parameter $\alpha \in \mathbb{R}^+$. The scalars λ and μ are material constants.⁷

For the distance measure, we used the *sum of squared differences* extended for image series

$$\mathcal{D}[R; u] := \frac{1}{2} \sum_{\nu=2}^M \int_{\Omega} \left(R^{(\nu)} \circ \varphi^{(\nu)} - R^{(\nu-1)} \circ \varphi^{(\nu-1)} \right)^2 dx. \quad (6)$$

This distance measure is also used as an error function to rate the results.

To minimize $\mathcal{J}[R; u]$, we simply compute a stationary point u of its Gâteaux-derivative, i.e. $d\mathcal{J}[R; u] = 0$. This results in a system of non-linear partial differential equations, the Navier-Lamé equations (NLE) for serially connected slices

$$\sum_{\nu=1}^M \left(f^{(\nu)} - \alpha \cdot (\mu \Delta u^{(\nu)} + (\lambda + \mu) \nabla \operatorname{div} u^{(\nu)}) \right) = 0. \quad (7)$$

Equation (7) describes the elastic deformation of an object subject to the force f . Here, f is nothing but the derivative of the chosen distance measure \mathcal{D} ,

$$f^{(\nu)} := \left(R^{(\nu-1)} \circ \varphi^{(\nu-1)} - 2R^{(\nu)} \circ \varphi^{(\nu)} + R^{(\nu+1)} \circ \varphi^{(\nu+1)} \right) \nabla R^{(\nu)} \circ \varphi^{(\nu)}, \quad (8)$$

with appropriately chosen $f^{(1)}$ and $f^{(M)}$. Except for a different calculation of the force f and a summation this system of equations is the same as the system for a pure two-dimensional registration. So the solution scheme from Fischer & Modersitzki⁶ can be used.

Due to an approximation of the NLE by finite differences and a fixpoint-type iteration method the non-linear partial differential equations turn into a linear system of equations with $2N$ unknowns, where N denotes the number of pixels of a single slice. This linear system has to be solved in each iteration step for every slice. The extremely huge matrix of the linear system (in our case, the matrix is of size $(7.220.000 \times 7.220.000)$, i.e. $5.2 \cdot 10^{13}$ entries) has not to be stored, because of a skilled rearrangement of coordinates the matrix breaks up into well structured, so-called circulant matrices which can be diagonalized by Fourier-matrices.⁸ Therefore, only the diagonal elements have to be kept in memory. This and applying a non-linear Gauss-Seidel iteration method leads to a super-fast algorithm for registration. Instead of cubic complexity and squared memory usage needed by most standard direct solvers, our algorithm has an asymptotic complexity of $\mathcal{O}(MN \log N)$ by linear (in N) memory usage. For additional speed-up and in order to handle large deformations we used on top a multi-scale

Table 1. Principle algorithm for elastic registration of image series.

1:	Initialize $u^{(\nu,0)} = 0$ for $\nu = 1, \dots, M$.
2:	Estimate material constants.
3:	for $k = 1, \dots, \text{maxIter}$ do
4:	for $\nu = 1, \dots, M$ do
5:	Calculate
	$f^{(\nu,k)} := (R^{(\nu-1)} \circ \varphi^{(\nu-1,k-1)} - 2R^{(\nu)} \circ \varphi^{(\nu,k-1)} + R^{(\nu+1)} \circ \varphi^{(\nu+1,k-1)}) \nabla R^{(\nu)} \circ \varphi^{(\nu,k-1)}$.
6:	Solve
	$\alpha (\mu \Delta \tilde{u} + (\lambda + \mu) \nabla \text{div} \tilde{u}) = f^{(\nu,k)}$.
7:	Update
	$u^{(\nu,k)} := u^{(\nu,k-1)} + \tilde{u}$.
8:	end for
9:	Calculate $\mathcal{D}[R; u^{(k)}]$.
10:	Check stopping criteria.
11:	end for

approach based on a Gaussian pyramid.^{9,10} For the lower levels of the Gaussian pyramid we restricted the number of iteration steps to a maximum of ten. If a step in a lower level does not decrease the error the scheme switches to the next higher level. On the highest level, i.e. the finest resolution, every step is monitored. If the error increases, the current step is dropped and the parameters are modified to force smaller gradient steps.

By applying elastic registration it is always difficult to correctly choose optimal values for material constants (λ, μ) . Varying image sizes and gray-value distributions demand for a different choice of the material constants to get morphologically meaningful registrations. A wrong choice may cause a failure of the registration. To receive a good-natured registration process, we estimated the constants automatically in an initial step.⁷ If $\lambda = 0$, the system is linear in μ . So we start with $\mu = 1$, calculate the forces for $u = 0$ and solve equation (7) to obtain the displacement fields. Afterwards, we set μ such that the new calculated displacement field is normalized, i.e. the maximum displacement of a pixel is one. As a byproduct of this setting the initial force decreases during the iteration, in the startup phase large deformations are computed which become smaller and smaller towards the end of the iteration. Altogether, we arrive a continuous registration process. In particular, no time-consuming oscillation phases during the iteration course are observed.

3. RESULTS

A whole rat brain was reconstructed from a series of 503 high resolution images derived from adjacent histologic sections. Each image has a resolution of (1900×1900) pixels, i.e. a total amount of 1.7 GB of image data. The images no. 475 to no. 503 were discarded, so 474 images remained (1.6 GB). Fig. 3 depicts the three-dimensional reconstruction of the full rat brain before and after elastic registration. To make the morphologic effect of elastic

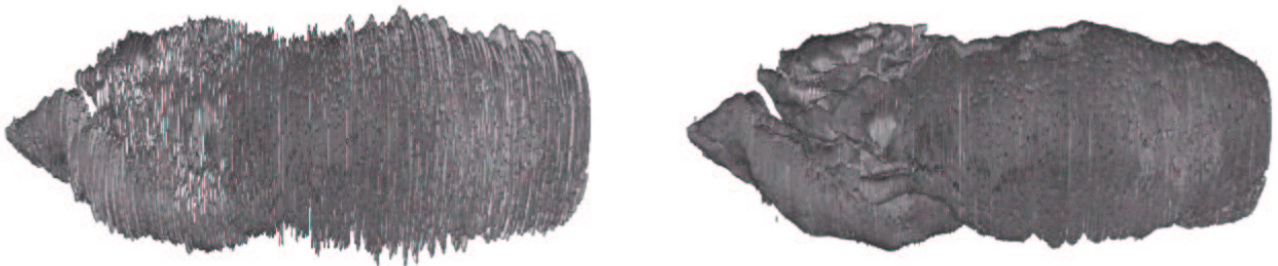


Figure 3. Lateral view of the three-dimensional reconstruction of the whole rat brain, left before, right after registration.

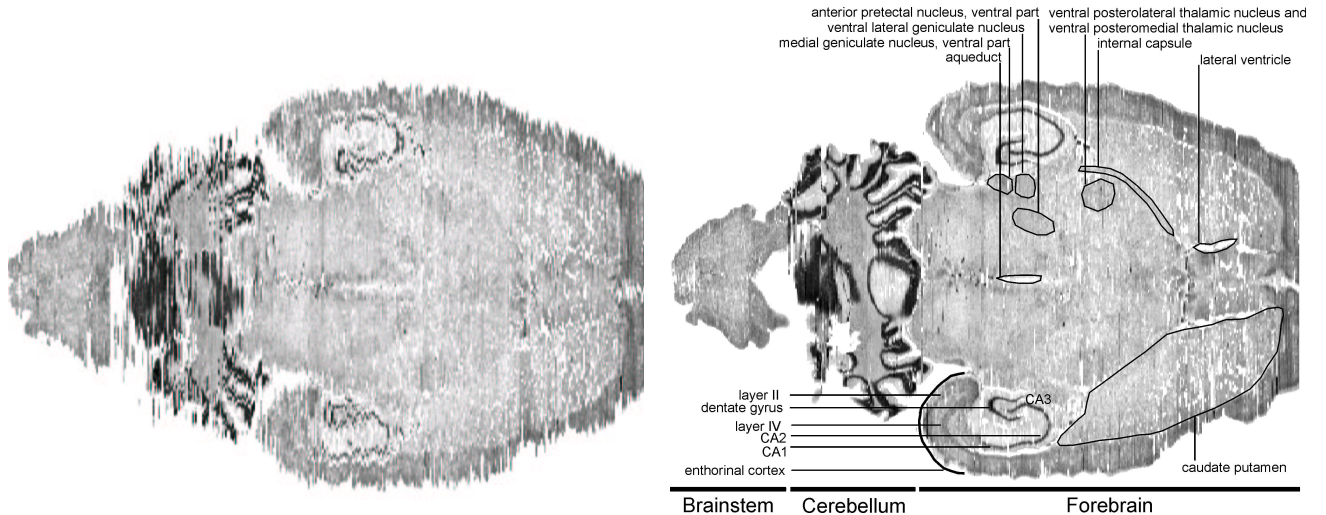


Figure 4. Orthogonally to the direction of sectioning resampled slice (Bregma -5.82 mm, Interaural 4.18 mm, (see Paxinos/Watson¹¹)). A column of this image represents a line of a slice. Left before, right after elastic registration. Note, the initial fuzzy looking areas are transformed into recognizable structures.

registration clear we resampled the data orthogonally to the direction of sectioning (see Fig. 4). Now structures like cerebellar fissures, molecular and granular layer and white substance of cerebellum are clearly recognizable. Note, that the initial fuzzy looking brain now offers morphological details and obviously dramatic increase of surface smoothness, overall, the displaced areas are coherent again. It should be noted, that the registration is an indispensable technique for recognition, discussion and three-dimensional measurement of internal and external morphologic entities.

Before elastic registration a linear preregistration based on the principle axis transformation was done. The error decreased by 27%, i.e.

$$\frac{\mathcal{D}[R; u_{\text{linear}}]}{\mathcal{D}[R; 0]} = 0.73. \quad (9)$$

It emerged that performing an elastic registration through five levels of a Gaussian pyramid leads to a satisfying convergence. The pure MATLAB implemented registration lasted approx. ten hours for the high-resolution images on a AMD Athlon XP 2700+, 1GB RAM, running Linux. Only 35 iteration steps were needed and the error decreased by 79%, i.e.

$$\frac{\mathcal{D}[R; u_{\text{elastic}}]}{\mathcal{D}[R; 0]} = 0.21. \quad (10)$$

In Tab. 2 the runtime results for the registration of images in different resolutions is assembled.

Beside measuring the registration results with the distance measure \mathcal{D} , we asked an expert for his opinion. As it turns out, a very important criterium for the expert for an examination of the registration results is an improvement of the representation of small structures (subcortical nuclei, cortical areas) and the smoothness of inner and

Table 2. Details of registration ("time per iteration" is the time of one iteration in the highest level).

data dimension	# levels	storage size	time per iteration	total time	# iterations
128 × 128 × 474	1	8 MB	0.5 min.	9 min.	19
256 × 256 × 474	2	30 MB	1.9 min.	32 min.	24
512 × 512 × 474	3	119 MB	10.7 min.	125 min.	28
1024 × 1024 × 474	4	474 MB	42.9 min.	240 min.	32
1900 × 1900 × 474	5	1632 MB	149.5 min.	547 min.	35

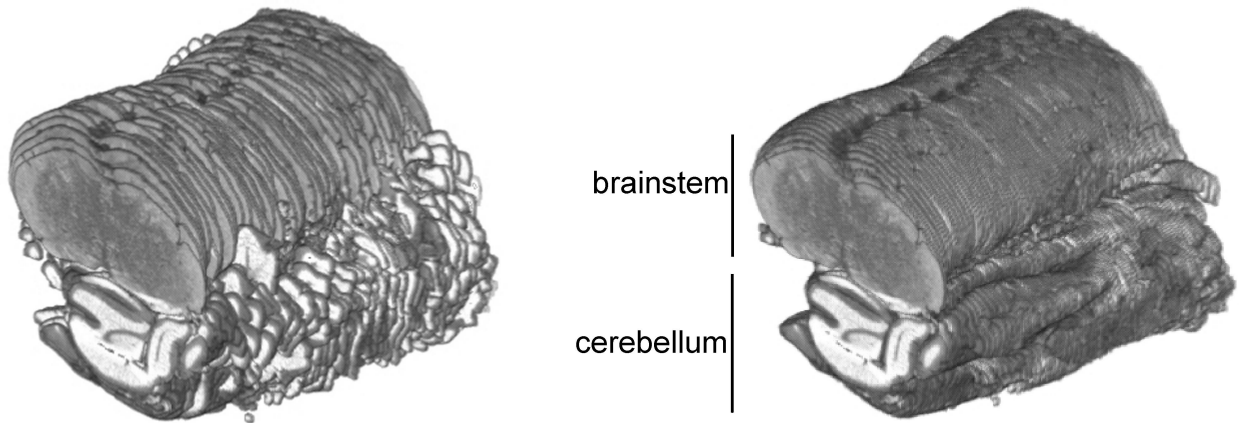


Figure 5. Three-dimensional lateral view of a part of the rat brain, left before, right after elastic registration. The volume of interest shows the brainstem at the top and the cerebellum with folia at the bottom.

outer borders. The registered slices do fulfill this requirement. Generally, three classes of neuroanatomical structures are recognizable after registration: 1) subcortical nuclei, 2) ventricles, 3) certain cerebral and cerebellar cytoarchitectonic layers. More precisely, subcortical nuclei like the caudate putamen complex, medial geniculate nucleus - ventral part, anterior pretectal nucleus - ventral part, ventral posterolateral thalamic nucleus, ventral posteromedial thalamic nucleus among other things can be localized. Furthermore, the lateral ventricle and the aqueduct become visible. Finally, cytoarchitectonic layering at certain parts of the cerebral and the cerebellar cortex can be detected. In the forebrain we can observe hippocampal substructures like the CA1, CA2, CA3 regions (CA: cornu amonis) and the dentate gyrus, see Fig. 4. Moreover, in the entorhinal region the layer II (external granular layer) and IV (internal granular layer) are distinguishable.

In Fig. 5 and Fig. 6 the results of the registration process are visualized in detail for a subset of the whole rat brain. Fig. 5 depicts the three-dimensional reconstruction of 68 slices before (left) and after (right) registration. The massively shifted images yield to an blurred reconstruction without recognizable fine anatomic details.

Fig. 6 illustrates the variation of the internal structures before (left) and after (right) registration by means of orthogonally resampled slices. Note, that the registration result allows for a detailed discussion of the internal structures.

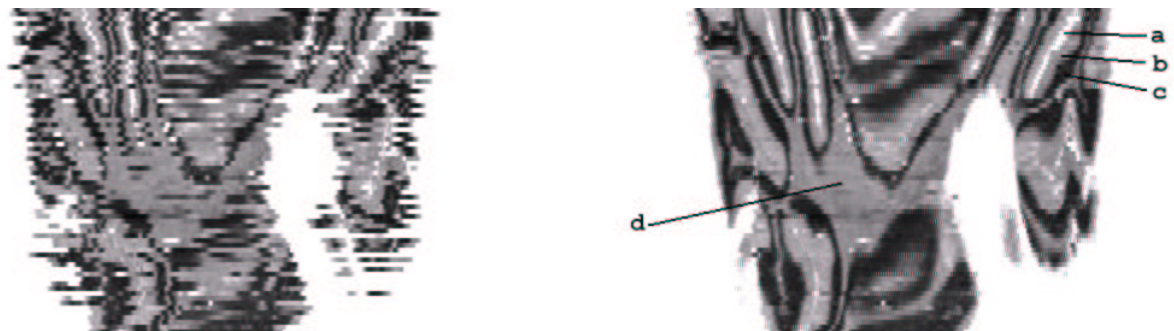


Figure 6. Orthogonally resampled slice to illustrate the morphologic effect of registration, left before, right after elastic registration. Recognizable structures: a cerebellar fissures, b molecular layer, c granular layer, d white substance of cerebellum.

4. CONCLUSION

To our best knowledge we have presented in this paper the first fully reconstructed rat brain at a resolution at level of the micrometer scale. The huge amount of data as well as the required quality demand for a special registration technique. Only the use of a specific variational technique accompanied by strategies to incorporate special properties of the underlying tissue enables us to match the high demands of an expert.

The backbone of the scheme is a super-fast solution technique for the inner linear system. This technique is accompanied by sound strategies for accelerating the outer iteration. This includes a multi-scale approach based on a Gaussian pyramid as well as a sophisticated estimation of the material constants for the elastic potential.

The results of the registration process were controlled by an expert who could recognize histological details that pertain to three distinct groups of neuroanatomical structures: subcortical nuclei, ventricles and cerebellar or cerebral cytoarchitectonic layers. Before registration the detection of these structures was impossible. Therefore, it is essential to apply elastic registration to this kind of non-linear problem. Furthermore, now it is feasible to develop strategies for three-dimensional morphometric analysis of specific areas in registered stacks of images derived from normal and pathologic brains for experimental studies. Finally, the obvious advantages were quantified by a distance measure leading to an improvement of about 79% after just 35 iteration steps.

Currently we are working at a full reconstruction of a human brain. Here the task is to align about 6000 slices of dimension (12000×7000) pixels (resolution: $31.75 \mu\text{m}$ per pixel)! Preliminary results look very promising and indicate that the outlined approach is capable of dealing with such an amount of data on a PC from the shelf.

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