Three-Dimensional Reconstruction of the Florid Plaque in variant Creutzfeldt-Jakob Diseases

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ABSTRACT

The paper presents the possibility of 3-D reconstruction of the florid plaques obtained in Transmission Electron-Microscopy. The TEM images registration process is quite different from Nuclear Magnetic Resonance and Computed Tomography on account to increasing amount of the image size and resolution together. The normalisation of image brightness and contrast, produced by differences in slices thickness and differences in time of photograph developing, have also been proposed and discussed. Finally the florid plaque segmentation and reconstruction based on the k-means clustering algorithm will be demonstrated.

Keywords: neurodegenerative diseases, CJD, vCJD, image registration; clustering

1 INTRODUCTION

The Alzheimer, BSE[1], CJD[2] and vCJD[3] diseases belong to a group of neurodegenerative diseases characterised by an accumulation of extraneuronal filamentous material consisting of β-sheet proteins of various biochemical properties [3][4][5]. The structurally modified protein izoform (*prion*) reduplicated its spatial form into surroundings proteins (S. B. Prusiner). The spatial characteristic of plaques (formed in the vCJD) is observed as a so-called florid plaque (see Figure 1). Clinically, the all of mentioned diseases are manifested as dementia i.e. permanent state of losing cognitive functions like: a memory, an orientation, a visual spatial abilities, etc [2].

The study presented in this paper is the integral subpart of the research programme concerning the investigation into the vCJD pathology by ultrastructural and image analysis techniques. It is foreseen that the results will make it possible to recognize the plaques nature, and will explain how the diseases evolution progress. The investigation results may also indicate the potential therapeutic strategies.

The immunofluorescent reconstruction of a plaque is very difficult [1]; therefore the undertaken study has been concentrated on 3-D plaque reconstruction using the serial sections of the human hipocamp sliced by microtome (typical slice thickness is ca. 300÷400Å). The series of plaque images have been obtained using a Transmission Electron-Microscope at the image magnification of ×5000÷9000. The proposed reconstruction procedure for 3-D recognition of slice structure will be presented in the following sections.

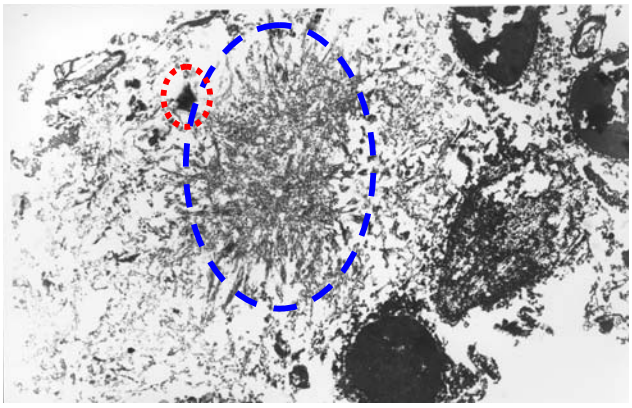

Figure 1: Florid plaque image surrounded by the blue dashed line (TEM photograph at a magnification of ×5000÷9000, specimen thickness ca. 300÷400Å). The artefact has been selected using the red dotted line.

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[1] BSE - Bovine Spongiform Encephalopathy, known as *MadeCow diseases* has now been transmitted to cattle, mice, sheep, and goats both orally and by inoculation, and to pigs, marmoset monkeys but not hamsters merely by inoculation.

[2] CJD - Creutzfeldt-Jakob Disease, was first described in 1920/21 when it was known as spastic pseudosclerosis or subacute spongiform encephalopathy.

[3] vCJD – The variant of Creutzfeldt-Jakob disease (discovered in 1996). This disease resulted from the BSE infection of humans is known as the *human BSE*.

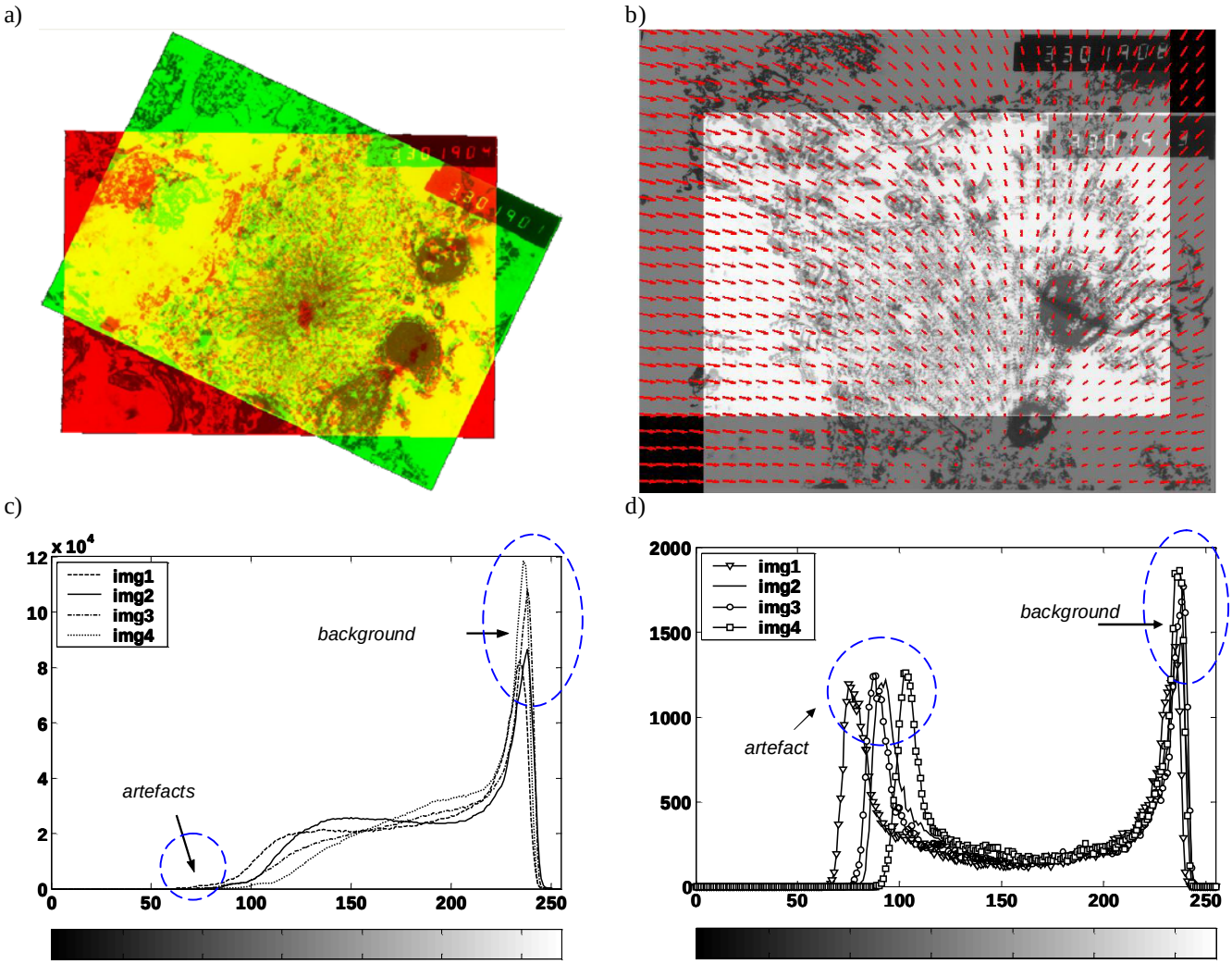

Figure 2: Distortions and preliminary registration of florid plaques: a) visualisation of distortion introduced by mounting and rough slices arrangement using rigid transformation; b) visualisation of distortion due to compression at cutting procedure (images before projective image transformation); c) exemplary intensity histogram of the images contained florid plaque and artefacts; d) intensity histogram of an artefact and a background region (see Figure 1).

2 IMAGE CONGRUENCING

The 3-D reconstruction of the florid plaque requires the congruence of photographed specimen slices. Unfortunately, the microtome introduces distortion caused by a tissue stretching and compression at cutting (Figure 2b) as well as the specimens mounting process (Figure 2a). Assuming the parallel plane of the slice cutting, these distortions can be successfully eliminated by registration using 2-D projective image transformation (8 DOF: rotation, translation, scaling, shears and projection).

Another problem arise from by the difference nature of TEM photograph from NMR[4]/CT[5] images on account to increasing amount of the image size and resolution together. The considered problem requires registration with the multi-resolution of photographed slices (typically 8100x5700 pixels/voxels per image). Fortunately this problem can be simply avoided performing following steps:

- preliminary image alignment using rigid registration at a reduced images resolution (e.g. ×12/×12, Figure 2a),
- execution the classical landmark-point based registration (Figure 2b),
- at the end of registration process the final tuning realignment using the cross correlation of uniformly distributed square blocks at the original image resolution.

On the other hand, the different slices thickness and a time of photography development cause additional difficulty

[4] NMR - Nuclear Magnetic Resonance

[5] CT - Computed Tomography

observed in the image brightness and contrast variation (see Figure 2c). Fortunately, the artefacts and the background property can be used to eliminate mentioned mismatch in the following manner. It can be assumed that the background and the artefacts have intensity distribution similar to Gaussian process. Moreover it is assumed that they fill the whole lot of slices thickness (for $b_i \approx I$ for *i=artefact, background*). In this case, the colour intensity histogram around artefact can be approximated to the formula (1), after that estimated parameters (b_i for *i=artefact, background*) can be used in the each slice normalisation process to the common image brightness and contrast in according to equation (2).

$$histapprox(I) = \sum_{i=1}^{4}\left(a_i \exp\left(-\left((I-b_i)/c_i\right)^2\right)\right) \quad (1)$$

$$\hat{I} = \frac{\min\left(\max\left(I, b_{artifact}\right), b_{background}\right) - b_{artifact}}{b_{background} - b_{artifact}} \quad (2)$$

where $I = I(x,y), \hat{I} = \hat{I}(x,y)$ – source and normalised image intensity [0,255]; $histapprox(I)$ –intensity histogram approximation; a_i, b_i, c_i - estimated parameters (Table 1). The proposed intensity realignment procedure allows for obtaining the very good images congruence.

i	Img:	a_i	b_i	c_i
Background	1	1147(±80)	232.5(±0.3)	6.239(±0.548)
	2	1202(±71)	235.6(±0.3)	6.136(±0.467)
	3	1554(±163)	237.7(±0.2)	4.075(±0.438)
	4	1667(±146)	236.4(±0.15)	3.893(±0.335)
Artefacts	1	1038(±72)	77.96(±0.37)	7.518(±0.652)
	2	1084(±84)	92.36(±0.33)	6.958(±0.645)
	3	1107(±65)	89.25(±0.34)	7.275(±0.52)
	4	1121(±54)	103.9(±0.2)	7.397(±0.412)

Table 1: The estimated values of the artefacts and background parameters for Figure 2d (Degrees Of Freedom: 244; the Sum of Squares due to Error: img1=1.49E6, img2=1.33E6, img3=1.49E6, img4=0.98E6).

3 FLORID PLAQUE RECONSTRUCTION

The congruent images have been performed by pixel segregating to the corresponding tissue (e.g. plaque, background etc.), where a pixel intensity affiliation has been determined applied the k-means clustering algorithm to the vector contained the sum of normalised histograms $histogram\left(\hat{I}_{\text{Img1}}\right) + histogram\left(\hat{I}_{\text{Img2}}\right) + \ldots$. The results obtained in this way have been presented in the Table 2 and Figure 3a-d. It can be observed that the segmented images illustrate the infection progress in the analysed slices. It can be also noticed, that the images sortation cannot be determined using classical Euler distance of images, therefore slices have been arranged taking into account graphical similarity of the degenerated tissue (red colour in Img2-Img4-Img3-Img1). The final reconstruction of the florid plaque has been demonstrated in Figure 3e.

Partition	Avg. value	std	size
1 (black)	255.0	0.00	1
2 (black)	249.5	3.27	6
3 (blue)	195.3	43.00	45
4 (green)	137.6	55.90	109
5 (red)	74.7	64.40	95

Table 2: The pixel intensity affiliation determined using the k-mean clustering algorithm. Partitions: 1,2 – background; 3 – border between infected and not infected tissue; 4,5 – infected tissue in a typical and degenerated progress.

4 CONCLUSIONS

The 3-D reconstruction of florid plaque under electron-microscopic images is quite different to the techniques commonly applied to the reconstruction of the object observed in NMR/CT. It arise from a huge number of slice voxel (even more then ×1400), which considerable increase required resources and processing time. On the other hand, the analysed images geometry and intensity are changed by the microtome and photography developing time respectively. From the researcher point of view, it is also important conclusion that the final registration can be obtained by the perspective transformation and proposed intensity histogram normalisation.

The florid plaque segmentation has been simplified by a point operator application. As it can be seen, the segmented image can be used to analysis of the infection progress in vCJD. Presented approach will be applied in the future in the investigations concerning reconstruction of a plaque ultrastructural model in this novel prion disorder

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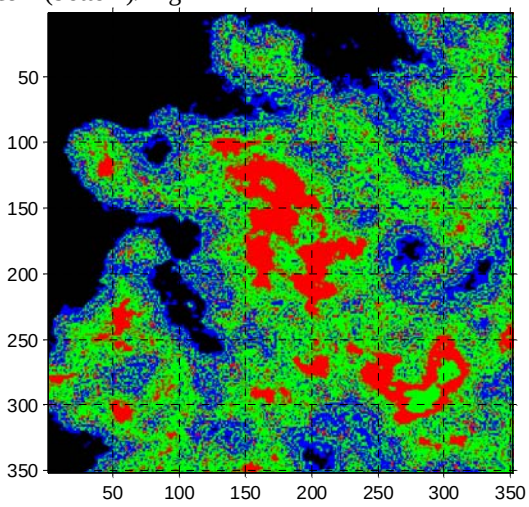
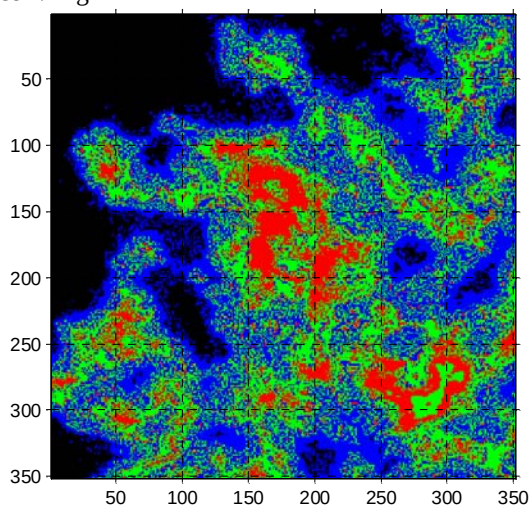
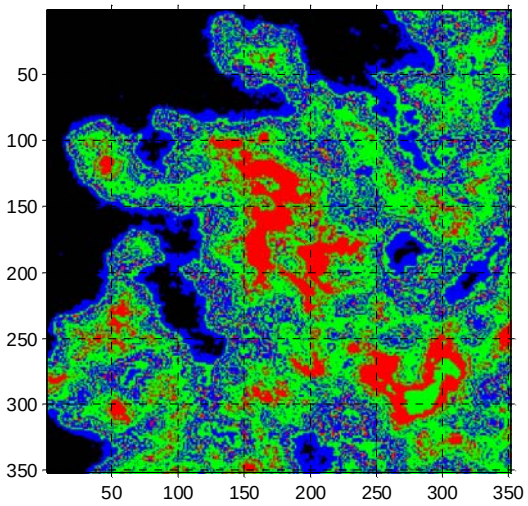
a) slice 1 (bottom)/img 2



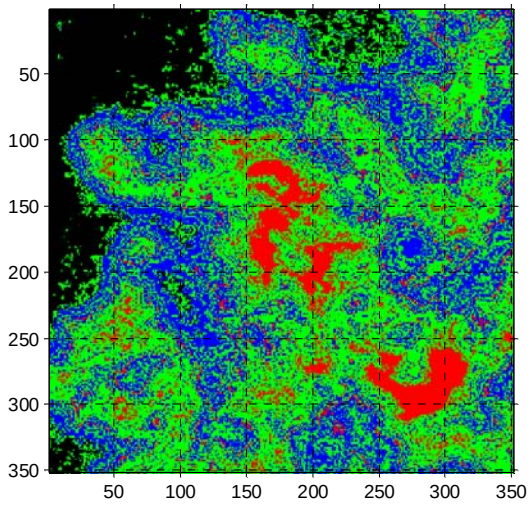
b) slice 2/img 4



c) slice 3/img 3



d) slice 4 (top)/img 1



e)

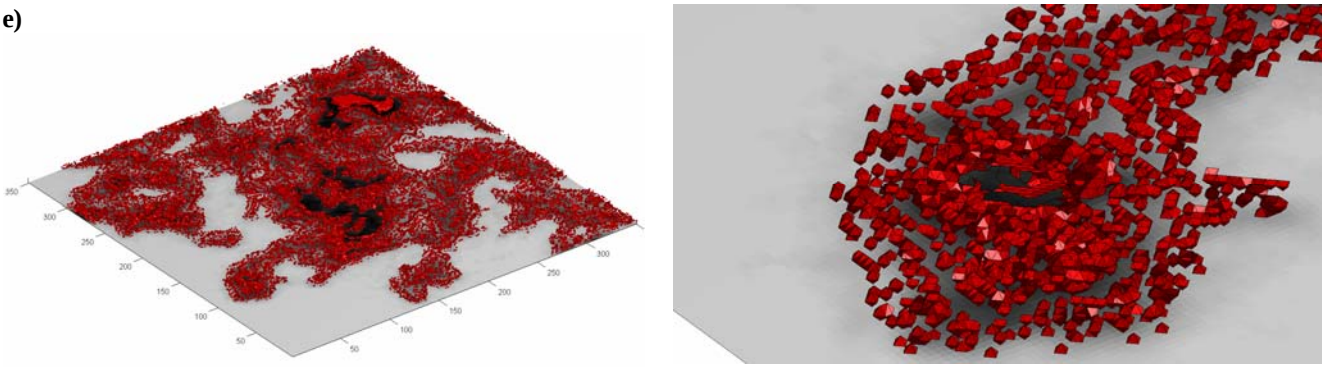


Figure 3: a-d) The sequence of segmented specimen slices using the k-means clustering algorithm, e) 3-D reconstruction of an infected (green) and degenerated (red) fibres using images: Img1-Img3-Img4-Img2 (The colour images have been published in the CDROM edition.)