Neuro3D: an interactive 3D reconstruction system of serial sections using automatic registration

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ABSTRACT

Three dimensional detailed visualization of neurons represents a source of valuable information when trying to understand integrative phenomena at cellular and circuital levels in neurobiology. We describe a low cost system built by an interdisciplinary team for 3D reconstruction of slices observed through a Transmission Electron Microscope (TEM). We have developed and tested a completely automatic registration method which combines local histogram equalization with correlation in multi-resolution. This method gives very good experimental results in real images without being very time consuming neither needing dedicated hardware. The different parts of the system are briefly presented: image acquisition, non uniform illumination compensation, manual segmentation, automatic registration, 3D visualization and Graphic User Interface (GUI). Experimental results are presented.

Keywords: Automatic registration, 3D reconstruction, TEM, neuroanatomy

1. INTRODUCTION

There is little doubt that three dimensional (3D) detailed visualization of neurons and processes represents a source of valuable information when trying to understand integrative phenomena at cellular and circuital levels. Moreover, it would be highly desirable to have at hand computational tools allowing the incorporation of different kinds of signals (electrical, chemical, etc) within the framework of the 3D images. To create such a powerful tool it seems mandatory to join forces of research groups with credits in at least two main fields: informatics and neurobiology. This article presents the firsts results of a joint work done by the Compared Neuroanatomy Division at the Instituto de Investigaciones Biológicas Clemente Estable (IIBCE) and the Image Processing Group (GTI) of the Instituto de Ingeniería Eléctrica (IIE) of the Facultad de Ingeniería, Montevideo, Uruguay

2. NEUROBIOLOGIC MOTIVATION

Owing largely to the monumental work of Santiago Ramón y Cajal[?] it is now a well established fact that the nervous system consists of numerous complex circuits connecting myriads of neuronal assemblies. The circuitry nature of the nervous system occurs all along the phylogenic tree reaching its maximal complexity in the so called cortex of the human brain. There are few doubts that the overall performance of any brain -human or non-human- is highly dependent of both the total number of nerve cells and the complexity of their connections. Understanding the rules commanding the operations in the nervous system is unquestionably linked to a precise knowledge of the neural circuits that ocurr at this level. Therefore, important amount of work has been devoted to explore the neuronal connections in different kinds of animals, including man. This is a laborious task that began one hundred years ago, defying the ingenuity and technical skills of numerous investigators. During several decades the main tools have been the silver impregnation methods to stain neurons and the light microscope to visualize them. With

the development of the electron microscope and its associated techniques a new frontier was reached; at present interneuron connections are studied with unexpected detail. However, the high resolution offered by the elctron microscope is based on the observation of ultra-thin sections. This represents a serious handicap since the range of thickness of an electron-lucid section is only 1000 Å. Consequently, approximately 30-40 serial sections are needed to reconstruct a single interneuronal contact. The biologists have methods to obtain ordered ultra-thin sections (800 Åto 1000 Å) and observe this slices with a TEM.[1] From this images it is possible to reconstruct the celular structurs. Obtaining this 3D models implies long and tedious manipulations:

- Taking a photo of the TEM observed section.

- Marking the signifactive elements of each slice in an appropiate material, plastic or wood, and cutting it.

- Building a 3D model piling up the solid slices.

The aim of this project is the development of reliable computational tools to facilitate the capture of the electron microscope images, the extraction of celular contours, the registration of succesive slices and finally the three-dimensional reconstruction of neurons and connections.

3. PREVIOUS WORK

For many years, 3D Reconstruction of neural tissue using electronmicroscope images were an entirely manual operation.[?] Recently, some efforts have being done to incorporate computer aided techniques to reduce this time consuming task. Carlbom et al.[2] have develloped an interactive system which includes a digital "blink comparator" to facilitate manual registration of slices and deformable active contours for semiautomated cell segmentation.

Montgomery et al.[3-5] at the NASA-Ames Biocomputation Center have build the Reconstruction Of Serial Section (ROSS) system which permits manual segmentation, registration and 3D reconstruction of slices.

Both groups chose interactive manual registration and have concentrate in semiautomatic segmentation and visualization. As Carlbom says "the automatic alignement of successive EM images is a very difficult problem".[2] More recently, Montgomery[6] have presented a non-fiducial, shape-based automatic registration method which is part of the ROSS system.

There are at least two methods to obtain the 3D models. One is to mark or detect the celular contours in each slice, register the images and then build the 3D models. A second method is to register the gray level images first, build the 3D model and use the complete set of 3D points to detect the celular boundaries. The second method must allow to use the (dis)continuity information in all directions facilitating a better detection of these complex surfaces.

Neuro3D is a complete interactive system, whith tools to facilitate manual 2D segmentation and registration, in order to permit to the biologist the use of the traditional technique. But the goal of our project is to build a complete automatic system. We have choose to automatize first the registration step and we work now in the automatization of the segmentation steps.[7,8]

This paper presents the system as it works today and specifically the automatic registration method.

4. METODOLOGY

The experimental procedure employed by the biologists, before Neuro3D, was the following:

1. A neuron's activity is registered in a certain region of the Central Nervous System. This neuron may be marked by a colorant substance injected in the tissue.

2. A portion of nervous tissue is extracted from the animal. This tissue is dehydrated and embedded in plastic epoxy for its hardening.

3. The hardened tissue is sliced into very thin sections (800 to 1000 Å). The ordered slices are colected and placed in a microscope support. In this process the relative position and orientation between slices are lost.

4. The slices are observed in a TEM and the image is captured by photografic means.

5. A biologist marks and transfers to an appropriate material the contours of the neuron and/or its processes.

6. The material is cutted and supperposed to build a scale-model.

7. Some measures are made in the 3D model.

The aim of the Neuro3D system is to automatize the last four steps.

4.1. Image Acquisition

The first step is the acquisition of the TEM images. Three possibilities appear:

1. To take a photo and scan it.

2. Use a special instrument to acquire a digital image directly from the electron microscope.

3. Acquire the image with a CCD camera through the binoculars used by the operator to observe the image and focus the TEM.

One basic criteria of the project is to adapt the available material rather than making expensive investments. The TEM used at the IIBCE is an old model without the capability to acquire digital images. Special instruments that allows direct digitalization of electron images are very expensive.

Direct acquisition avoids the costs and time associated to the photografic procedure. The acquired images, while being smaller and of poorer quality than those produced from the scanning of a photo taken from the TEM, have all the information needed for the registration and 3D reconstruction. This reasons led us to choose the third option for the acquisition stage. The implemented solution uses:

- A CCD camera mounted in the binoculars used for focus adjustments as shown in the figure 2.

- A Personal Computer with an acquisition board.

- A software, developed during the project, wich controls the acquisition board with several utilities to facilitate the acquisition of the sequences:

  - Graphic interface to mark and capture the region of interest (ROI).
  - Average of variable number of images to minimize electronic noise.
  - Real time presentation of the ROI's histogram in order to maximize the gray levels used in the acquired image.

The image formed in the electroluminiscent phosfor is observed by the camera through the protection window, a very thick cristal not built to produce a good quality image but to resist the high vacuum and to protect the TEM operator against the radiations. This acquisition method introduces some perturbations which must be compensated.[9] Some of this perturbations are:

- Non uniform illumination. Due to the camera sensibility, the electronic beam must be concentrated in order to obtain an intense enough image. This causes the illumination to be non uniform over the image. This non-uniform illumination is estimated and cancelled.

- Presence of a focus point. The biologist makes use of a black round spot that can be seen on the plate to adjust the TEM focus controls. This black point is located and eliminated (substituted by an average of its neighborhood) for a better presentation, minimizing also its influence during the registration step.

- Acquisition noise. Modelling the electronic noise as gaussian, the averaging of several images reduces its variance.
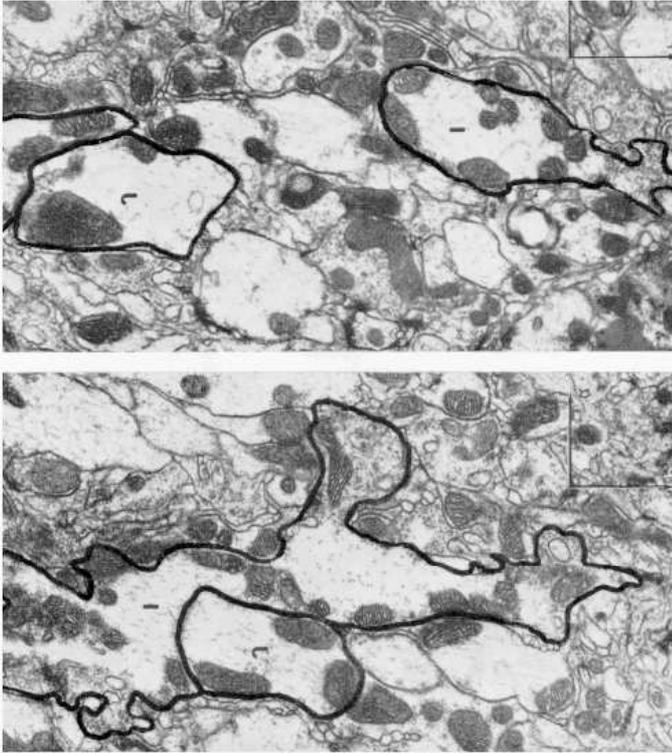
Figure 1. Cellular contours marked by a biologist. Note the difficulty to determine the borders of interest.

4.2. Graphic interface

The 3D reconstruction of a synaptic button implies the registration of dozens and eventually hundreds of slices (typically, hundreds of megabytes). In order to work fast enough with this amount of data we have choosen a UNIX workstation for the Neuro3D system: a DEC Alpha workstation with OSF MOTIF.

In a project like this one it is essential to have a friendly GUI wich enables the user to interact with the data all along the process. The principal characteristics of the Neuro3D GUI are:

- All the functions (registration, contour extraction, verification) can be done manually. The registration is implemented also with automated algorithms and the results can be verified and corrected manually.

- All the manual functions reproduce the methods used by the biologists.

4.3. Celular contour extraction.

Extracting the significative cellular contour is a difficult task. In general, the observed tissue is formed by numerous similar structures, but only one or two are of interest for the biologist. Figure 1 illustrates the problem.

Neuro3D uses the GUI to extract the significant structures manually, in a slice-by-slice basis. The user can mark points along cellular borders, being the contour interpolated by means of B-splines. This is a very fast and user-friendly way of contour drawing, that allows easy editing of the curves and also requires very little memory for the storing of each curve (since only the nodes on each curve are stored). Other facilities, like associating each curve with a name and color (that matches the one used in the 3D visualization) have also been developed.

4.4. Registration

Figure 2 ilustrates the registration procedure. Let us name the image of the nth slice I_n and $T_{n,n+1}$ the $3x3$ matrix wich takes into account the relative position and orientation between I_n and I_{n+1}. The aim of the registration
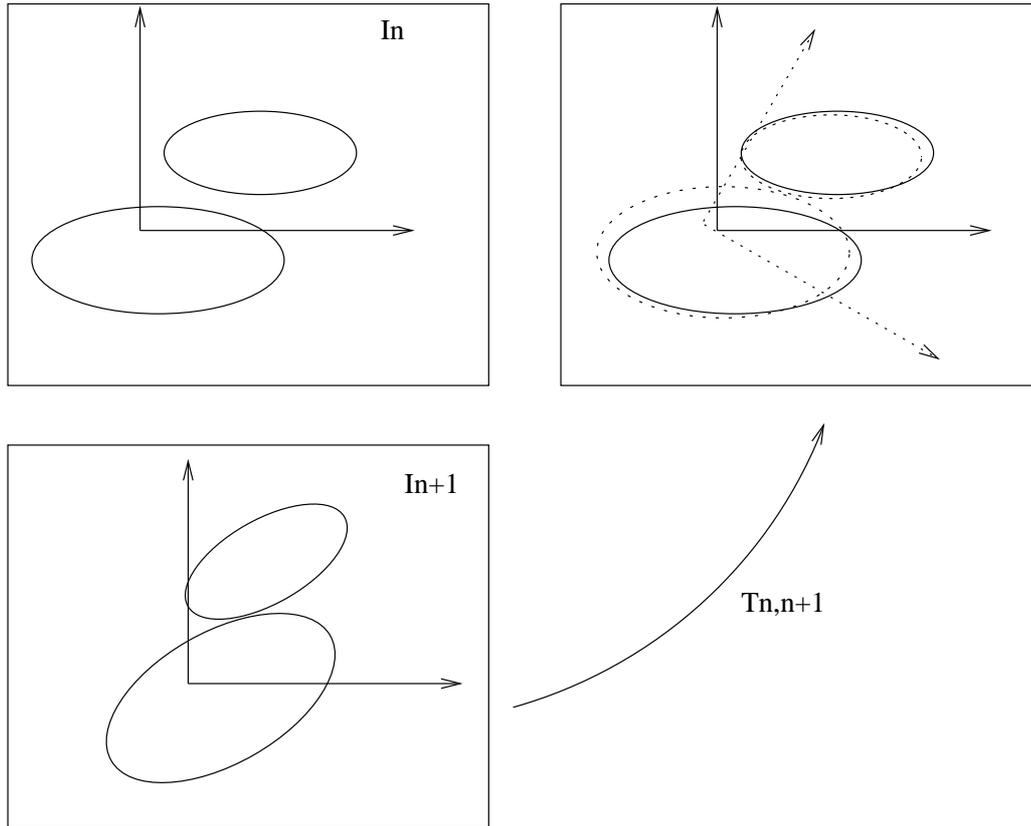
Figure 2. The aim of the registration step is to find the matrix $T_{n,n+1}$ which best place the slice I_n over I_{n+1}

step is to find all the matrices $T_{n,n+1}$ for the n slices of a sequence. This step is essential for the subsequent 3D reconstruction.

Often, the experimental procedure gives the data needed for an easy registration: the relative position and orientation between slices are known. This is not our case, the ultra thin slices are transported floating in a fluid. Moreover, the region of interest is a very little portion of the slice, unknown before observation through the TEM. It is imposible to use any landmarks on the slices, since this landmarks could fall far away from the structures of interest for the biologists. Use of implicit landmarks or computing the inertia momentum have also proven to be fruitles, due to the significant change of our images from one slice to the following both in shape and luminance (being the objects of interest of a size in the order of the depth-resolution).

To solve the registration step, we have implemented two methods: one manual and one automatic.

4.4.1. Manual registration

Once the user has marked the cellular borders in each slice, the system allows to manually position the marked border of one slice onto the next one's, using the GUI. This method, which imitates the one traditionally used by the biologists, can be used also to correct the results of the automatic procedure.

4.4.2. Automatic registration

In a first approach we make the hypothesis that two succesive slices are very similar and that it is possible to find a rigid movement between them. Correlation is a known robust similarity measure between two images.[10–12] It is possible to calculate the correlation factor for a large range of relative position and orientation between I_n and I_{n+1}. The value of $T_{n,n+1}$ is then determined by the movement that maximizes the correlation factor between both images.
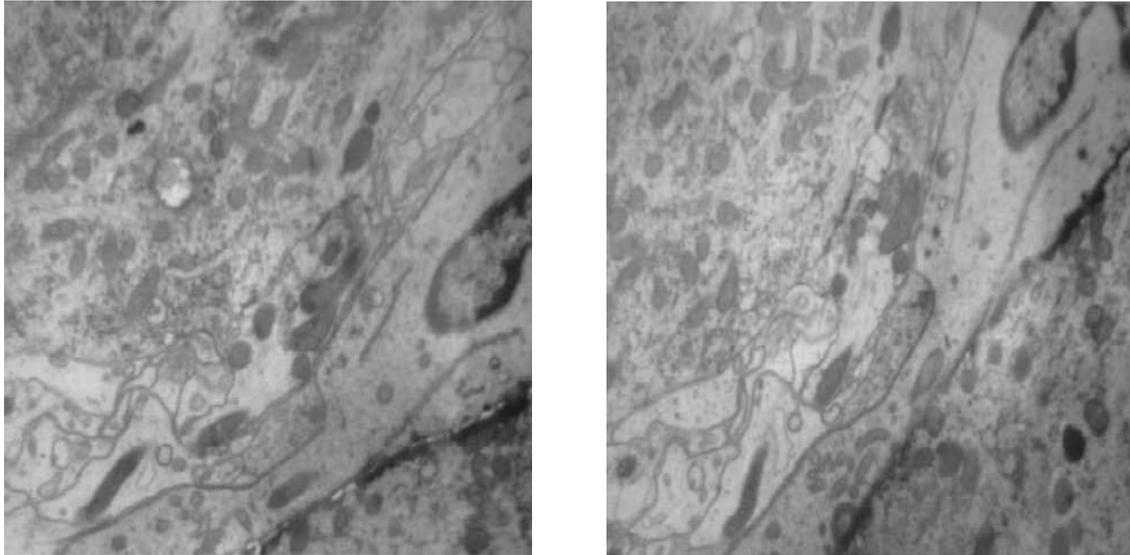
Figure 3. One couple of slices to be registered. Slices 14 and 15 of serie ref0-26

This method is very time consuming. To accelerate the computations we use multiresolution; at low resolution we search the best correlation factor for a big range of angular and translational displacements. Next the algorithm go to a higher resolution level and a search for a new maximum takes place in a limited zone around the maximum correlation point of the previous step.

The algorithm fails sometimes into a local minima. To minimize this posibility we preprocess the image with a local equalization operator which works in the following way; we define a support window, which is placed around each pixel and the pixel's gray level is substituted by the correspondent if we perform an histogram equalization within this window. Several tests have shown that the minimum dimension of the support window to have satisfacory results is 10 percent of the image.

Figures 3 and figure 4 ilustrates two couples of slices to be matched and figures 5 and 6 ilustrates the corresponding preprocessed images.

After this preprocessing step, the image of correlation factor shows a much more pronounced maximum and our experience have lead to very good results in terms of automatic registration as shown in 5.

Although the processes of interest for the bilogists change between slices, weakening the rigid motion hypothesis, there is a lot of other less important structures that remain almost inchanged from one slice to the next.

This fact can explain why this preprocess gives better results. As we can see in figures 5 and 6, in the preprocessed images all the structurs of the tissue appear with similar gray levels, this "texture" is then correlated, giving similar importance to all the present structurs. Most of this processes are present in both slices and strength the correlation between them.

The biologist can use the manual registration procedure to correct or adjust the automatic registration output.

5. EXPERIMENTAL RESULTS

To evaluate the capabilities of the registration algorithm we have used 140 neural tissue slices grouped in 6 different series. All the images was acquired with a TEM microscope GEOL ???. Table 1 resume the used series. Ref0-26 was photografied in the TEM and then acquired from the photos with a CCD camera. The other series were acquired with the acquisition system placed in the microscope and explained in.[9]
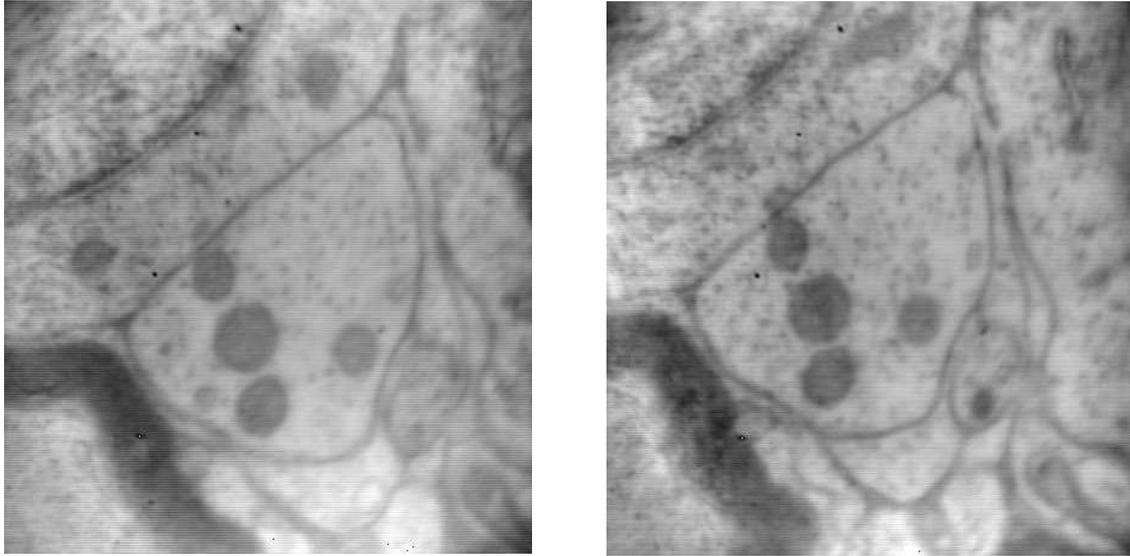
Figure 4. One couple of slices to be registered. Slices 20 and 21 of serie piram2r
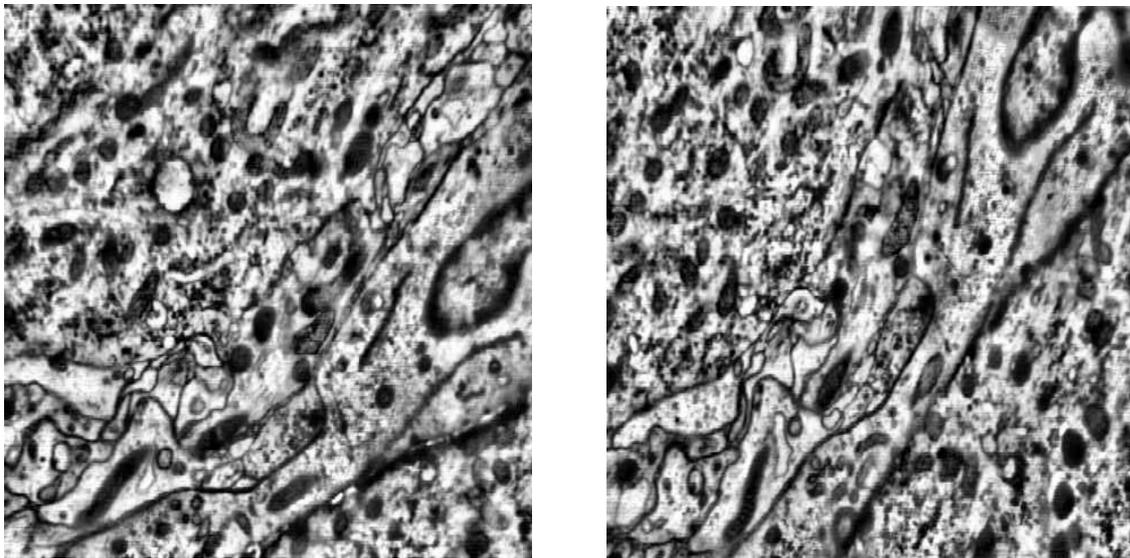

Figure 5. Slices from figure 3 processed with the local enhancement algorithm
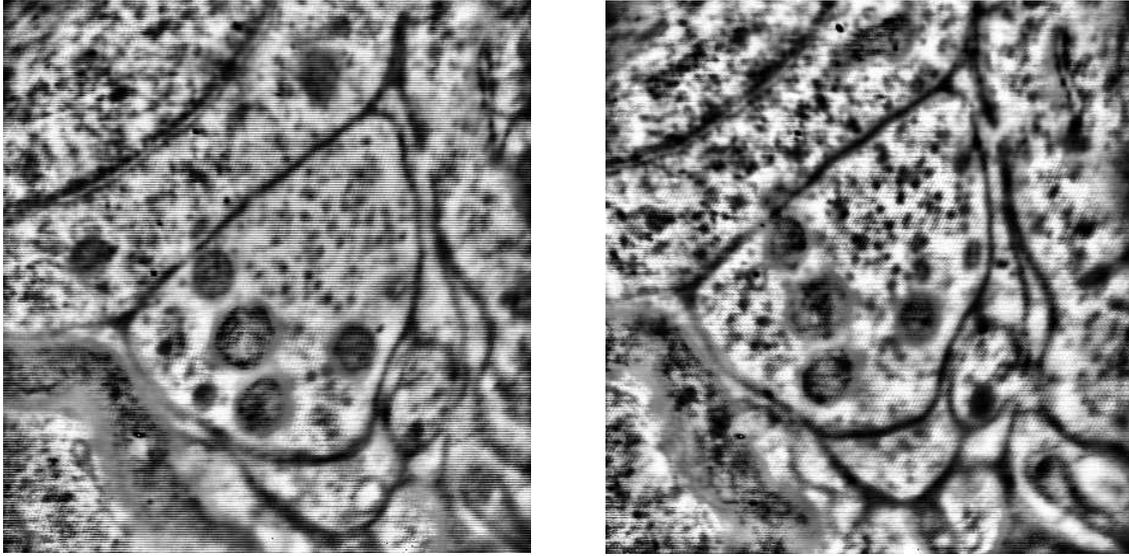
Figure 6. Slices from figure 4 processed with the local enhancement algorithm

Name of the serie	Nb. of slices	Nb. of manual registrations	Type of tissue	Acquisition method
ref0-26	27	4		CCD form photos
peroxdes	18	4		CCD from TEM
t2r	19	4		CCD from TEM
piram2r	14	4		CCD from TEM
lobuloel	26	2		CCD from TEM
piramidal	36	1		CCD from TEM

Table 1. Series of tissue used to test the automatic registration step. There are 140 slices grouped in 6 different series.

Each serie were manualy registered several times by one or more biologists. The idea was to define a reference positioning, accepted by an experimented human operator as a good one. We observed that this manual placement can vary in position and orientaion, even if all of them are considered "good" by the biologist. Define a truly reference positioning requires more experimentation in order to have statistical meaningful data sets. For the sake of this article we use one manual posing as the reference one.

To evaluate the automatic results we must compare the movement produced in the same image by automatic and manual registration. In order to measure the quality of the automatic registration step we have defined a distance between the automatic and the reference registration, taking into account both the traslation and the rotation movement, as follows:

$$d^2 = dx^2 + dy^2 + 2\rho^2(1 - \cos(d\phi)) \tag{1}$$

where:

- $dx = xt_a - xt_r$ is the difference between reference and automatic x displacements.

- $dy = yt_a - yt_r$ is the difference between reference and automatic y displacements.

- $d\phi = \phi_a - \phi_r$ is the difference between both angular movements.

- ρ is the mean distance between the pixels in the ROI and the center of rotation.

As ρ increases, the error introduced by a diference in the angular movements grouth. In order to estimate the registration error of table 2 we have use $\rho = 100$ corresponding to a typical ROI.

Tables 2 and 3 illustrates the experimental results for series Piram2r and Ref0-26. All the experimental results are available at the WEB address http://www.iie.edu.uy/gti/neuro3d/matchresults. Each serie was registered by the the automatic algorithm and compared with the orientation and position obtained by manual registration. Using the registration verifying tool of Neuro3D, which permits the observation of the contours marked in one slice over the next, blinking of both slices or a new image formed by the chez-like combination of both slices, the biologist considers 118 good matches out of 130 slices tested. The bad registrations have error measures $d > 50$ and correspond in general to images with big artifacts. We need more experimentation to have a finer interpretation of the significance of d as an error registration measure.

Slice	x_a	x_m	y_a	y_m	ϕ_a	ϕ_m	d
0	0	0	0	0	0,00	0,00	0,00
1	-5	-2	-15	-12	-1,66	-0,66	5,63
2	-12	-9	25	30	-2,71	-1,82	7,24
3	20	25	21	26	-1,63	-0,57	8,50
4	-42	-37	-13	-13	-1,63	-1,63	5,00
5	-11	-14	7	14	-3,65	-3,65	7,62
6	-19	-14	47	42	-3,66	-2,03	5,57
7	7	16	-23	-21	-1,66	-0,23	10,10
8	39	38	39	48	-1,29	-1,29	9,06
9	-74	-75	-49	-47	-2,34	-2,34	2,24
10	-20	-17	38	37	-2,37	-1,34	3,16
11	21	23	27	31	-1,63	-1,63	4,47
12	-18	-15	16	25	-3,37	-3,37	9,49
13	-6	0	-24	-27	-1,71	-0,01	6,04

Table 2. Manual and automatic registration results for serie Piram2r. Angle measures are in degres and x and y measures are in pixels. The last column gives the value of the error measure calculated using (1) with $\rho = 100$.

Figures 7 illustrates one view of the 3D reconstruction of serie Ref0-26 using automatic registration. Future work includes 3D soomthing, automatic segmentation and system calibration.

ACKNOWLEDGEMENTS

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Slice	x_a	x_m	y_a	y_m	ϕ_a	ϕ_m	d
1	51	60	-85	-91	8,61	9,41	9,92
2	-7	-8	62	66	9,08	9,83	5,43
3	-36	-38	14	13	-3,38	-3,38	2,24
4	4	4	2	4	-8,24	-6,27	5,44
5	54	53	-42	-39	14,24	12,22	0,48
6	28	23	-42	-37	-5,00	-5,50	6,55
7	-89	-100	42	55	-2,38	-4,14	14,96
8	-9	-12	24	25	2,24	2,24	3,16
9	105	109	-78	-79	8,00	8,00	4,12
10	-51	-53	45	42	2,80	2,80	3,61
11	6	6	2	2	-14,83	-13,21	2,84
12	-55	-50	56	53	-2,76	-2,76	5,83
13	-58	-64	-5	-5	1,44	1,44	6,00
14	30	23	0	6	0,42	-0,35	8,41
15	88	90	-19	-22	22,56	22,30	4,06
16	-88	-103	34	52	-9,61	-12,26	20,81
17	28	26	30	33	-7,71	-7,11	4,46
18	-3	-5	-28	-29	3,37	3,37	2,24
19	50	42	-40	-39	2,39	2,39	8,06
20	-6	-8	3	6	3,39	3,39	3,61
21	25	20	-10	-7	11,64	11,64	5,83
22	21	28	-1	-1	4,64	6,40	7,37
23	-61	-74	79	89	-10,85	-10,85	16,40
24	-287	-13	36	8	-44,07	0,67	305,14
25	201	-25	-212	-25	25,14	6,29	267,05
26	99	-8	46	-25	-31,56	-1,70	94,75

Table 3. Manual and automatic registration results for serie Ref0-26. Angle measures are in degres and x and y measures are in pixels. The last column gives the value of the error measure calculated using (1) with $\rho = 100$. Note that the automatic registration fails in the 3 last slices.

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