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# Automatic Hippocampus Localization in Histological Images using Differential Evolution-Based Deformable Models

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## Abstract

In this paper, the localization of structures in biomedical images is considered as a multimodal global continuous optimization problem and solved by means of soft computing techniques. We have developed an automatic method aimed at localizing the hippocampus in histological images, after discoveries indicating the relevance of structural changes of this region as early biomarkers for Alzheimer's disease and epilepsy. The localization is achieved by searching the parameters of an empirically-derived deformable model of the hippocampus which maximize its overlap with the corresponding anatomical structure in histological brain images. The comparison between six real-parameter optimization techniques (Levenberg-Marquardt, Differential Evolution, Simulated Annealing, Genetic Algorithms, Particle Swarm Optimization and Scatter Search) shows that Differential Evolution significantly outperforms the other techniques in this task, providing successful localizations in 90.9% and 93.0% of two test sets of real and synthetic im-

24 ages, respectively.

25 *Keywords:* Hippocampus, Deformable Models, Automatic Localization,  
26 Histological Images, Global Continuous Optimization, Differential Evolution

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## 27 **1. Introduction**

28 Among the different anatomical structures which make up the mammalian  
29 brain, the hippocampal formation (HPF) (see Figure 1) is particularly inter-  
30 esting. From an anatomical viewpoint, the HPF, composed by the Hip-  
31 pocampus and the Subiculum (SUB), is located within the medial temporal  
32 lobe. In turn, the Hippocampus is composed by the Dentate Gyrus (DG) and  
33 Ammon's Horn (CA), which is further composed by three different regions  
34 (CA1, CA2, and CA3).

35 The hippocampus has long been known for its crucial role in learning  
36 and memory processes [1]. Moreover, it has recently been demonstrated that  
37 the volume of the hippocampus is an early biomarker for Alzheimer's dis-  
38 ease. Therefore, there is a great interest in understanding the cellular and  
39 molecular events that take place in this structure, under both normal and  
40 abnormal conditions. From this point of view, a precise gene expression map  
41 at the cellular and subcellular level within this region can provide crucial  
42 information for understanding such biological mechanisms. A very promis-  
43 ing data source to derive this map has recently been provided by the Allen  
44 Brain Atlas (ABA), a huge, publicly available database that contains high-  
45 resolution images mapping the expression patterns of most genes contained in

46 the genomes of the analyzed organisms. The first release of the ABA was fo-  
47 cused on mouse, and contained the expression patterns of about 20,000 genes  
48 obtained by In Situ Hybridization (ISH) of mouse brain sections [2]. In a  
49 more recent release, a similar resource based on spatially mapped microarray  
50 data has also been provided for the human brain [3]. There is wide avail-  
51 ability of brain images containing morphological and functional information  
52 on the hippocampus in different organisms. Thus, it has become extremely  
53 important to design image analysis methods that accurately, robustly, and  
54 reproducibly identify the hippocampus region, to automatize any relevant  
55 analytic procedure. In the ABA, for each gene under consideration, several  
56 images are provided, corresponding to different sections of the brain. Each  
57 image is labelled according to the corresponding images of a reference atlas  
58 comprising 132 coronal and 21 sagittal sections spaced at 100  $\mu\text{m}$  and 200  
59  $\mu\text{m}$  intervals, respectively.

60 In this paper, we describe a general model-based method for the auto-  
61 matic localization of anatomical districts in histological images, which could  
62 be also applied to other structures, as well as to different imaging modalities.  
63 In particular, we describe an application in which such a method is used to  
64 locate the hippocampus in ISH images from the ABA. This method allows  
65 us to automatically extract image parameters from corresponding regions of  
66 a huge number of images, to cluster genes with similar expression patterns  
67 and subcellular mRNA distribution. It can be hypothesized that genes whose  
68 expression is mapped in images with similar visual features are likely to be

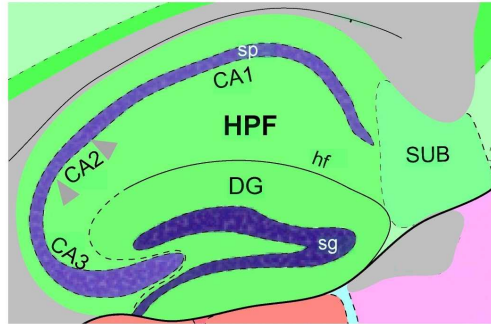


Figure 1: Regions in Hippocampal Formation (HPF)

69 also functionally similar.

70 In our method, the hippocampus is located by detecting, as landmarks,  
 71 two regions which are usually well distinguishable within the structure (see  
 72 Figure 1): the pyramidal (*sp*) and granule (*sg*) cell layers, which belong to  
 73 the CA and DG regions, respectively.

74 As in most medical imaging applications, the problems we have to solve  
 75 are mainly related with the characteristics of the images involved in the  
 76 process. The main problematic features of these images in general, and of  
 77 the hippocampus region in particular, related with biological features or with  
 78 the image acquisition process, can be summarized as follows:

- 79 • natural variability of brain structure shapes in different subjects;
- 80 • fuzziness of the hippocampus boundary;
- 81 • limited relevance of color for detecting anatomical structures: regions  
 82 with similar colors may represent different structures and vice versa,  
 83 depending on the dye used as well as on local image acquisition settings;

- 84 • contrast variability between structures: different genes are not ex-  
85 pressed equally in the same anatomical region, making it difficult to  
86 construct a consistent model for each landmark in all images. More-  
87 over, grained patterns with many irregularities hamper the classifica-  
88 tion of individual pixels as belonging to the anatomical structures under  
89 consideration;
- 90 • orientation issues: the imaged structures may be rotated or displaced  
91 on the slice with respect to a “standard” alignment;
- 92 • lighting issues: within the same set of images, some are much brighter  
93 than others.
- 94 • variable resolution even within the same image: high-resolution regions  
95 coexist with low-resolution ones;
- 96 • presence of artefacts: tears, scraps, bubbles, streaks in tissues, partial  
97 cut-off of regions;
- 98 • large image size (the typical resolution of ABA images is about 15,000  
99  $\times$  7,000 pixels).

100 These problems significantly hamper tasks like localization and segmen-  
101 tation of structures in such images.

102 The fully automatic 2D localization method we propose is based on atlas-  
103 based registration and on the optimization of the parameters of a parametric  
104 deformable model. Our method can be divided in two stages: (i) selection

105 of the corresponding slice in the reference atlas based on a two-step affine  
106 registration, and (ii) proper localization of the hippocampus.

107 This technique was initially presented in [4], where the stochastic search  
108 of the hippocampal region was performed using Particle Swarm Optimiza-  
109 tion (PSO) [5]. In this paper, we give more details about the second stage  
110 of the method, we delve into the rationale of its operation and we widen its  
111 experimental evaluation performing more extensive tests. Moreover, we com-  
112 pare six real-parameter stochastic optimization techniques on the task under  
113 consideration; in particular, we show that Differential Evolution (DE) signif-  
114 icantly outperforms the other methods taken into consideration: Levenberg-  
115 Marquardt (LM) [6], Simulated Annealing (SA) [7], Scatter Search (SS) [8],  
116 Genetic Algorithms (GA) [9] and PSO. In our tests, we evaluate the lo-  
117 calization of the hippocampus in real and synthetic sagittal images, but this  
118 method could as well be applied to other subcortical structures, image modal-  
119 ities or anatomical planes.

120 The paper is organized as follows: in section 2 we provide the theoretical  
121 foundations of our work, as well as an overview of previous related work. In  
122 section 3, a general overview of the method is presented, providing details  
123 about the implementation of the localization of the hippocampus. Finally,  
124 section 4 presents results on both synthetic and real images with appropriate  
125 statistical tests, followed, in section 5, by some final remarks and a discussion  
126 about possible future developments.

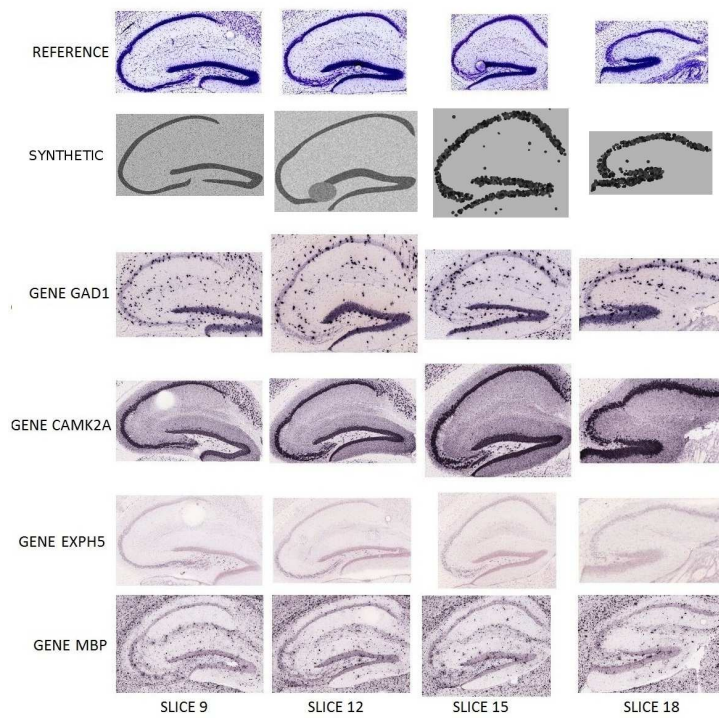


Figure 2: Hippocampus variability. Horizontal-wise: hippocampus sections taken at different levels look different, and different genes can as well produce very different visual features. Vertical-wise: corresponding sections from different brains maintain some shape similarity.



## 127 **2. Theoretical background and related work**

### 128 *2.1. Deformable Models*

129 Deformable models are curves or surfaces, defined within the image do-  
130 main, that are deformed under the influence of “internal” forces, related with  
131 the curve features, and “external” forces, related with the surrounding im-  
132 age. Internal forces enforce regularity constraints and keep the model smooth  
133 during deformation, while external forces are defined such that the model is  
134 attracted toward an object or other features of interest within the image.  
135 The term “deformable models” was first used in the late eighties [10, 11],  
136 and one of the first examples, called “snakes” or Active Contour Models, was  
137 presented shortly after in [12].

138 Active Shape Models (ASMs) [13] add more prior knowledge to deformable  
139 models. These shape models derive a “point distribution model” from sets of  
140 labelled points (landmarks) selected by an expert in a training set of images.  
141 Thus, the final model of the object of interest can be derived by examining  
142 the distribution of the positions of the labelled points. The model considers  
143 the points’ average positions and the main modes of variation observed in the  
144 training set. It is important to notice that an instance of the model can only  
145 take into account deformations which appear in the training set: because of  
146 this, the model may have problems with unexpected shapes, but it is robust  
147 with respect to noise and image artefacts, like missing or damaged parts.

148 Although originally developed for computer vision applications to natu-  
149 ral scenes and computer graphics problems, the applicability of deformable

150 models in medical image analysis has already been proven [14, 15].

## 151 *2.2. Differential Evolution*

152 In the last two decades, research on global optimization has been very ac-  
153 tive [16, 17, 18], and many different deterministic and stochastic algorithms  
154 for continuous optimization have been developed. Among the stochastic  
155 approaches, Evolutionary Algorithms (EAs) [19, 20] offer a number of ad-  
156 vantages that make them attractive: implicit parallelism, robust and reliable  
157 performance, global search capability, no need of specific information about  
158 the problem to solve, easy implementation, good insensitivity to noise, and  
159 no requirements for differentiable or continuous objective functions. DE,  
160 first introduced by Storn and Price [21], has recently been one of the most  
161 successful evolutionary algorithms. Unlike traditional EAs, DE perturbs the  
162 current population members with the scaled differences of randomly selected  
163 and distinct individuals [22]. This way, in the first iterations, the elements  
164 are widely scattered in the search space and have a great exploration ability.  
165 During optimization, the individuals tend to concentrate in the regions of  
166 the search space with the best fitness values [23].

167 In DE, new individuals that will be part of the next generation are created  
168 by combining members of the current population. Every individual acts as  
169 a parent and is associated to a donor vector. In the basic version of DE,  
170 the donor vector  $V_i$  for the  $i^{th}$  parent ( $X_i$ ) is generated, by combining three  
171 random and distinct population members ( $X_{r1}$ ,  $X_{r2}$ ) and  $X_{r3}$ , as follows:

$$V_i = X_{r1} + F \cdot (X_{r2} - X_{r3})$$

172 where  $F$  (scale factor) is a real-valued parameter that strongly influences  
173 DE's performances and typically lies in the interval  $[0.4, 1]$ . Other mutation  
174 strategies have been applied to DE, experimenting with different base vectors  
175 and different numbers of vectors for perturbation.

176 After mutation, every parent-donor pair generates an offspring  $O_i$  by  
177 means of a crossover operation. The newly-generated offspring is evaluated  
178 and its fitness and its parent's are compared. The better survives and will  
179 be part of the next generation.

### 180 *2.3. Related Work*

181 We shortly review some of the most relevant and recent approaches which  
182 combine deformable models and evolutionary computation techniques. In  
183 [24], "Genetic snakes" are active contour models with an optimization pro-  
184 cedure based on genetic algorithms. In [25] a GA evolves a population of  
185 medial-based shapes, using prior shape knowledge to produce feasible defor-  
186 mations while also controlling the scale and localization of these defor-  
187 mations. In [26] a GA is used to perform level set curve evolution using texture  
188 and shape information to automatically segment the prostate in CT and MRI  
189 pelvic images. Finally, in [27], the authors describe a GA-based method that  
190 minimizes the Topological Active Nets energy, evaluating the segmentation  
191 results of a greedy, a genetic, and a memetic algorithm over seven synthetic

192 images and three CT scans of human bones.

193 Recently, PSO has been successfully used in conjunction with various  
194 types of deformable models. Asl and Seyedin [28] apply the technique pro-  
195 posed in [29] using PSO instead of a GA, obtaining similar results in terms  
196 of precision but in shorter time. In [30] the initial segmentation based on the  
197 level set method is refined using swarms of intelligent agents. Finally, in [31],  
198 a customized PSO algorithm overcomes the drawbacks of snakes, including  
199 initialization, concave boundaries, sensitivity to noise and local minima.

200 To the best of our knowledge, one of the few works that uses DE and  
201 deformable models for solving medical imaging problems is presented in [32],  
202 where DE, in combination with a greedy search algorithm, is used to evolve  
203 Topological Active Nets, a discrete implementation of an elastic mesh, for  
204 CT image segmentation.

### 205 **3. DE-based hippocampus localization**

206 Our accurate and automatic structure localization method consists of two  
207 phases:

- 208 1. *Best Reference Slice Selection*, implemented as a two-step affine regis-  
209 tration method which: (i) determines the position of the section dis-  
210 played in the target image according to a reference atlas, and (ii) ex-  
211 tracts the region of interest (ROI) where the hippocampus is more likely  
212 to be located. Hence, it represents the initialization of the deformable

213 model: it determines which template should be used and where it  
214 should be applied.

215 2. *Structure Localization*, in which the point distribution model selected  
216 in the previous step is adapted by a stochastic optimization procedure  
217 based on DE, to fit the image region where the structure of interest (if  
218 any) is located.

### 219 3.1. *Best Reference Slice Selection*

220 The initialization of the Deformable Model, in terms of choosing a model  
221 and its starting position, is obtained using an atlas-based affine registration  
222 with the reference images of the ABA. The main idea is to find the sagittal  
223 reference slice of the atlas which best matches the target image. This  
224 phase produces two results: firstly, based on the information contained in  
225 the corresponding reference atlas image, it allows one to extract the Region  
226 of Interest (ROI) where the hippocampus is expected to be located. Sec-  
227 ondly, it makes it possible to determine the position, within the brain, of  
228 the section represented in the target image. Consequently, one can select the  
229 corresponding point distribution model of the hippocampus (derived empir-  
230 ically as described for the ASMs), which is to be applied in the following  
231 step within the selected ROI. This can be achieved by the two-step matching  
232 method presented in [4].

233 The first step performs a “global” affine registration between the target  
234 image and each image in the reference atlas, considering the shapes of the

235 whole sections and ignoring the internal structures. Then, using the atlas,  
236 the ROI where the hippocampus is more likely to be located is extracted, and  
237 a “local” affine registration is performed in order to match the hippocampus  
238 in the reference and the target image. After these two registration steps,  
239 two similarity measures between the target image and each reference image  
240 are computed. The reference image that achieves a better trade off between  
241 these two measures is selected as the best reference.

242 In order to evaluate the correctness of this two-step approach, we com-  
243 puted the distance (in slices) between the slice selected by our system and  
244 the one suggested by the ABA on a test set of 320 images. In 45% of cases  
245 the slice selected was exactly the same, while in 43%, 10% and 2% of cases  
246 there was a difference of one, two, and three or more slices, respectively. In  
247 those cases in which our system selected a reference slice different from the  
248 one proposed by the ABA, we also compared the fitness values obtained by  
249 the hippocampus localization algorithm. The results of these experiments  
250 showed that there are no statistically significant differences between the two  
251 choices. Therefore, these results demonstrate that our initialization method  
252 is effective and can be used also in problems where no ground truth is avail-  
253 able.

### 254 *3.2. Hippocampus Localization*

255 While in the standard ASMs external forces are driven by the contours  
256 in the image, in our approach we parametrically deform the model shape to

257 match as closely as possible the shape of the hippocampus in the region we  
258 want to locate. The model is moved and deformed, by altering its parametric  
259 representation using an optimization heuristic like DE or PSO, which max-  
260 imizes a function which measures the similarity between the model and the  
261 object itself. Since, in our problem, color and shapes are greatly variable, the  
262 only common information on which we can rely is that all hippocampi have  
263 two substructures (*sg* and *sp*) whose shapes have more consistent features,  
264 besides being usually characterized by lower intensity values and higher color  
265 saturation with respect to the surrounding structures.

266 To enhance the contrast between *sg*, *sp* and the surrounding parts, we  
267 pre-process the ROI containing the hippocampus by stretching its histogram.  
268 We invert pixel intensities, to consistently use the convention by which, as  
269 for binarized images, the most relevant regions in the image have higher  
270 intensities. These operations are not applied to the original images of the  
271 ABA we show, to avoid altering their actual appearance.

### 272 *3.2.1. Templates*

273 Obviously, our main concern is to adapt a model that permits an effi-  
274 cient localization, but we want, as well, to obtain this goal with the simplest  
275 possible model, i.e. having the least possible number of points in each tem-  
276 plate. In this work, for every slice of the reference atlas, two templates (one  
277 composed of 7 points for *sg*, one with 8 points for *sp*) have been created  
278 by manually selecting significant corresponding landmarks in all images in a

279 training set.

280 A template aims at taking into account all possible positions and defor-  
281 mations with respect to the prototypical shape of the structure it represents.  
282 The template does not refer to the absolute positions of the points, but de-  
283 scribes the relative positions (or shifts) between consecutive points in polar  
284 coordinates. This is uncommon for a DM, since the majority of the imple-  
285 mentations use cartesian coordinates. Every template is composed of two  
286 parts:

- 287 • an “inner set” of points that lies on the anatomical part we want to  
288 locate;
- 289 • an “outer set” of points that lies just outside it, obtained by rigidly  
290 shifting the previous set.

291 For a proper localization, the “outer set” is fundamental; otherwise, using  
292 only the “inner set”, a completely dark and large area would always achieve  
293 the highest values of the target function.

294 A template is fully described by four  $2 \times n$  matrices, where  $n$  is the number  
295 of points in the template:



$$\mathbf{S} = \begin{bmatrix} \rho_{m1} & \vartheta_{m1} \\ \rho_{m2} & \vartheta_{m2} \\ \vdots & \vdots \\ \rho_{mn} & \vartheta_{mn} \end{bmatrix} \quad \mathbf{\Delta} = \begin{bmatrix} \Delta_{\rho1} & \Delta_{\vartheta1} \\ \Delta_{\rho2} & \Delta_{\vartheta2} \\ \vdots & \vdots \\ \Delta_{\rho n} & \Delta_{\vartheta n} \end{bmatrix}$$
  

$$\mathbf{L} = \begin{bmatrix} \rho_{l1} & \vartheta_{l1} \\ \rho_{l2} & \vartheta_{l2} \\ \vdots & \vdots \\ \rho_{ln} & \vartheta_{ln} \end{bmatrix} \quad \mathbf{U} = \begin{bmatrix} \rho_{u1} & \vartheta_{u1} \\ \rho_{u2} & \vartheta_{u2} \\ \vdots & \vdots \\ \rho_{un} & \vartheta_{un} \end{bmatrix}$$

296  $\mathbf{S}$  is the “standard template” and represents the standard coordinates of  
297 the inner set,  $\mathbf{\Delta}$  is the displacement of the outer set with respect to the  
298 inner set,  $\mathbf{L}$  and  $\mathbf{U}$  are the minimum and maximum values allowed for every  
299 parameter that describes the inner set. It should be noticed that  $\rho_1$  and  
300  $\vartheta_1$  represent the positions of the first point with respect to the upper left  
301 corner of the image. After that point, proceeding row-wise, every  $(\rho, \vartheta)$   
302 pair represents the shifts of the subsequent point in terms of distance and  
303 angle with respect to the previous point, respectively. As a consequence, the  
304 number of parameters to optimize for each model is twice the number of the  
305 points in the template (14 parameters for *sg* and 16 for *sp*).

306 The matrices  $\mathbf{S}$ ,  $\mathbf{L}$  and  $\mathbf{U}$  have been computed by manually selecting  
307 the reference points in a training set. The first one is the median of the  
308 selected shifts and the other two are the minimum and the maximum values

309 observed in the training set, respectively. To improve the templates, a manual  
 310 refinement of the parameters has been performed. The matrix  $\mathbf{\Delta}$  has been  
 311 manually built based on the observation of several hippocampi.

312 From a computational point of view, a model is a vector of  $2 \times n$  elements  
 313 that ranges within the “boundary templates”  $\mathbf{L}$  and  $\mathbf{U}$ ; its elements (coor-  
 314 dinates) are evolved by a metaheuristic which optimizes the target function  
 315 described in section 3.3. The aims with which the target function was defined  
 316 are to match the model with the target while keeping it as close as possible  
 317 to the shape of the standard template  $\mathbf{S}$ .

### 318 3.3. Target Function

319 As in classical deformable models, we consider the model to be subjected  
 320 to external forces (driven by the image features) and internal forces (driven  
 321 by the model itself).

322 The target function  $F$ , which is to be maximized, has three components:  
 323 external energy  $E$ , internal energy  $I$ , and contraction factor  $C$ :

$$F = E - (I + C)$$

324 The external forces move (and deform) the model to maximize the in-  
 325 tensity of pixels in the inner set, while minimizing the intensity of pixels in  
 326 the outer set. For both sets, we evaluate the intensity of the image within a  
 327  $3 \times 3$  neighborhood  $N_3$  of all points in the model (Punctual Energy,  $PE$ ) and  
 328 in  $p$  intermediate points along the segment between two consecutive points

329 (Continuous Energy,  $CE$ ), i.e.

$$PE = \sum_{i=1}^n [T(N_3(I_i)) - T(N_3(O_i))]$$

330 where  $n$  is the number of points in the model,  $I_i = \{x_i, y_i\}$  is the  $i^{th}$  point  
 331 of the inner set (in cartesian coordinates),  $O_i = \{x_i + \Delta x_i, y_i + \Delta y_i\}$  is the  
 332  $i^{th}$  point of the outer set,  $T(P)$  is the intensity of the image in  $P$ , if  $P$  is  
 333 a point, or the average intensity, if  $P$  is a neighborhood;  $\Delta x_i, \Delta y_i$  are the  
 334 elements of  $\Delta$  in cartesian coordinates. As well,

$$CE = \sum_{i=2}^n \sum_{j=1}^p T(I_{i-1} + \frac{j}{p+1}(I_i - I_{i-1})) \\ - \sum_{i=2}^n \sum_{j=1}^p T(O_{i-1} + \frac{j}{p+1}(O_i - O_{i-1}))$$

335 where  $p$  is the number of points to evaluate in every segment. In our case  
 336 we set  $p = 20$ . The final external energy is computed as

$$E = \gamma_P \cdot PE + \gamma_C \cdot CE$$

337 where the weights  $\gamma_P$  and  $\gamma_C$  have been empirically set to 5 and 1 for all  
 338 tests, respectively (see Figure 3).

339 The internal energy  $I$ , related with the forces that oppose the deformation  
 340 of the model, is computed as:

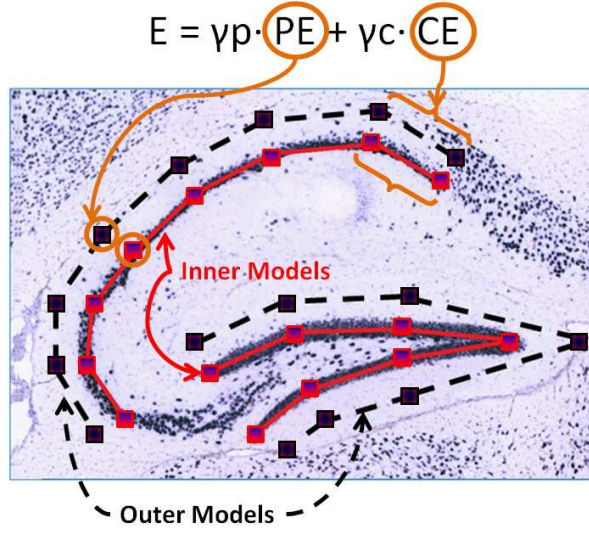


Figure 3: Inner and outer models superimposed to a hippocampus image, showing the components of the external energy.

$$I = \xi_\rho \cdot \sqrt{\sum_{i=2}^n (\rho_i - \rho_{mi})^2} + \xi_\vartheta \cdot \sqrt{\sum_{i=2}^n (\vartheta_i - \vartheta_{mi})^2}$$

341 where  $\xi_\rho$  and  $\xi_\vartheta$  are two positive weights that regulate the deformability  
 342 of the model. The higher their values, the less the model deforms and the  
 343 more it keeps the shape of the standard template  $\mathbf{S}$ . Please notice that the  
 344 index  $i$  starts from 2. This way, the first shift (which actually represents the  
 345 starting position of the model) is not taken into account and the model is  
 346 independent of its position in the image.

347 Finally, the contraction factor  $C$  also regulates the model's deformability  
 348 to avoid unfeasible situations (e.g. having the extremes of  $sg$  too close to  
 349 each other), and is defined as follows:

$$C = \xi_c \cdot \|I_n - I_1\|$$

350 If  $\xi_c < 0$  the two extremes of the model repel each other, if  $\xi_c > 0$  they  
351 attract each other. In our case we set  $\xi_c > 0$  for the *sp* models and  $\xi_c < 0$   
352 for the *sg* models.

#### 353 4. Experimental Results

354 To test the system we used both real and synthetic images. As for the  
355 former, we randomly selected 320 images (corresponding to 320 different  
356 genes) from the ABA within subsets of representative samples of all possible  
357 hippocampi, featuring high and low levels of gene expression, good-quality  
358 and low-quality images, and so on. We also created 20 synthetic images that  
359 represent simplified versions of the real ones. In these images, representing all  
360 reference slices, the hippocampus is made up of small circles having random  
361 radius and color; small and big ellipses were also added trying to simulate  
362 cells and, finally, gaussian ( $\mu \in [0.0, 0.2]$  and  $\sigma \in [0.01, 0.15]$ ) and salt and  
363 pepper noise (density  $\in [0.05, 0.25]$ ) were introduced to add fuzziness to the  
364 images.

365 Tests were run on a computer equipped with a 64-bit Intel® Core™i7  
366 CPU running at 2.67 GHz with 4 Gb of RAM.

367 We compared the results obtained by DE with those obtained by LM, a  
368 classical combination of the gradient descent and the Gauss-Newton methods,  
369 and the following stochastic optimization techniques: a variation of PSO,

370 used in [4], where it was shown to give better results than the standard PSO  
 371 on this task; SS with local search based on the line search algorithm and the  
 372 blx- $\alpha$  crossover, as described in [33]; GA and SA. In LM and SA we used, as  
 373 initial value, the best solution in a randomly generated set of the same size  
 374 as the other methods' populations.

375 The parameters (as well as the population topologies and the mutation  
 376 and crossover operators) set in the tests have been initially chosen based on  
 377 the most commonly used, and then refined during the development of the  
 378 system. The values chosen for the most relevant parameters are shown in  
 379 Table 1. For all techniques, a limit of 20000 function evaluations has been  
 380 set as the only termination criterion, and the limits for the deformation were  
 381 used as constraints in creating new solutions by crossover and mutation.

Table 1: Parameters used in testing different optimization techniques.

DE	PSO	SS	GA	SA	LM
$C_r = 0.9$	$c_1 = 2.05$	$b_1 = 7$	$P_{crossover} = 0.6$	$T_{start} = 1.5$	$\lambda_0 = 0.01$
$F = 0.7$	$c_2 = 1.75$	$b_2 = 8$	$P_{mutation} = 0.09$	$T_{end} = 1E-9$	
Uniform Crossover	$w_{max} = 1.0$	$\alpha = 0.5$	Population Size = 80	exponential cooling	
Mutation:	$w_{min} = 0.2$		Iterations = 250	schedule: $T_{k+1} = 0.8 \cdot T_k$	
DE/target-to-best/1	Population Size = 80		Tournament of size 4		
Population Size = 80	Iterations = 250		Uniform Crossover		
Iterations = 250					

382 For every image, we first performed the extraction of the ROI and the  
 383 selection of the best model, as described in Section 3.1. After this, for each  
 384 optimization technique, we ran 25 tests on every image, for a total of 8000  
 385 experiments on real images and 500 on synthetic images.

Table 2: Comparative results of *sp* and *sg* localization for synthetic and real images (20000 function evaluations per experiment; the higher the values, the better the localization).

<b>Synthetic Images</b>						
<i>sp</i> localization						
Method	Average	StdDev	Avg. Worst	Avg. Best	Avg. Median	Wilcoxon test
DE	120.41	5.49	106.30	126.37	121.68	-
PSO	105.86	8.67	84.86	118.61	107.71	<1.00E-16
SS	105.70	6.16	91.66	115.69	106.37	<1.00E-16
GA	105.93	15.64	54.93	120.40	110.11	<1.00E-16
SA	101.80	8.19	80.87	110.64	103.76	<1.00E-16
LM	35.43	30.86	19.96	94.24	34.11	<1.00E-16
<i>sg</i> localization						
Method	Average	StdDev	Avg. Worst	Avg. Best	Avg. Median	Wilcoxon test
DE	128.91	2.92	121.18	132.99	129.49	-
PSO	118.29	5.85	105.55	127.63	119.05	<1.00E-16
SS	115.56	6.00	101.37	124.81	116.08	<1.00E-16
GA	115.03	9.67	87.86	127.20	116.49	<1.00E-16
SA	111.23	3.87	102.49	117.91	111.40	<1.00E-16
LM	64.76	19.58	33.79	103.34	61.42	<1.00E-16
<b>Real Images</b>						
<i>sp</i> localization						
Method	Average	StdDev	Avg. Worst	Avg. Best	Avg. Median	Wilcoxon test
DE	141.47	5.59	124.36	146.06	145.21	-
PSO	133.56	8.24	110.94	143.89	138.64	<1.00E-16
SS	131.06	6.04	118.54	141.66	135.81	<1.00E-16
GA	132.00	14.49	86.07	143.70	138.58	<1.00E-16
SA	131.00	3.05	123.83	137.71	134.91	<1.00E-16
LM	79.32	28.26	9.60	126.11	85.02	<1.00E-16
<i>sg</i> localization						
Method	Average	StdDev	Avg. Worst	Avg. Best	Avg. Median	Wilcoxon test
DE	148.24	1.54	143.58	149.73	149.30	-
PSO	144.65	3.69	135.37	148.70	145.52	<1.00E-16
SS	140.07	4.27	129.10	146.23	140.69	<1.00E-16
GA	140.89	6.83	122.56	148.50	143.06	<1.00E-16
SA	138.31	1.70	134.60	141.51	139.28	<1.00E-16
LM	105.58	20.01	65.54	141.61	110.52	<1.00E-16

386 *4.1. Algorithm Comparison*

387 Table 2 summarizes the results of our tests. In the upper part of the table  
388 we report the results for the 20 synthetic images and, in the lower part, the  
389 ones related to the 320 real images. The second column reports the average  
390 fitness for all tests, while the third reports the average standard deviation of  
391 fitness in the 25 experiments performed on each image. The fourth and fifth  
392 columns report the average of the worst and best results obtained for each  
393 image. Similarly, the sixth column reports the average of the medians.

394 We focused our analysis on the comparison between the performance of  
395 DE and of the other methods. DE achieved the best average fitness as well  
396 as the lowest standard deviation, which indicates a more robust behavior,  
397 as it is able to produce more consistent results over different trials; the per-  
398 formance of the other algorithms is more dependent on their random initial-  
399 ization. To assess the significance of this result, we performed a statistical  
400 test with confidence level of 0.01. We used non-parametric tests because  
401 the assumption of normality was not met. The Kruskal-Wallis test [34] was  
402 statistically significant in all cases ( $p\text{-value} < 1.00\text{E-}16$ ) and proved the ex-  
403 istence of differences between the sets of results, where at least one sample  
404 median was significantly different from the others. After that, the paired  
405 Wilcoxon signed-rank test was performed [35], assuming as null hypothesis  
406 that the median of DE results is less than or equal to the median of the other  
407 methods. This is a one-tailed test in which the alternate hypothesis is that  
408 the median of any other method is less than the median of DE. The corre-



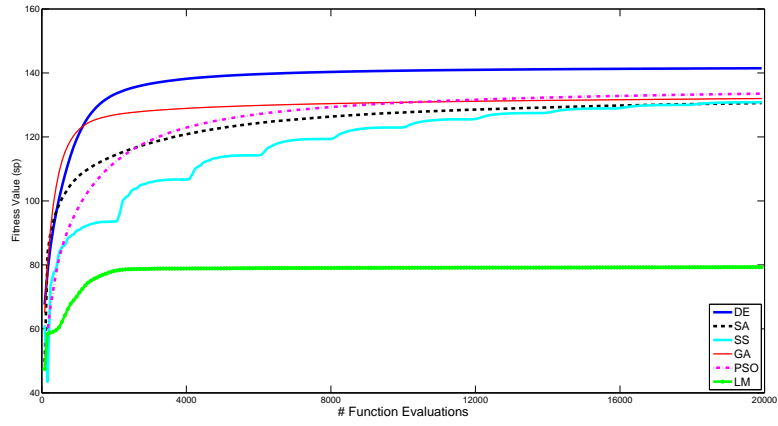
409 sponding p-values (shown in the last column of Table 2) reject, in all cases,  
410 the null hypothesis, showing that significant differences exist between the  
411 performance of DE and of the other methods. Moreover, the average worst  
412 values show that DE avoids local minima and is able to get good results more  
413 consistently than the other methods.

414 Figure 4 describes the behavior of the five metaheuristics and a gradient-  
415 based local search method by plotting the average fitness value versus the  
416 number of function evaluations. It can be seen how DE and GA start better  
417 than the other techniques but, while the former continues to improve its  
418 performance, the latter is not able to refine the solution found. At the  
419 same time, the evolution of SS is more discontinuous due to the premature  
420 convergence of the reference set, followed, after each reconstruction, by an  
421 abrupt improvement of the fitness values. The worst results were obtained  
422 by the gradient-based local search method (LM), due to the multimodality  
423 and non-differentiability of the function to optimize, providing a reason for  
424 using metaheuristics to solve this kind of problems.

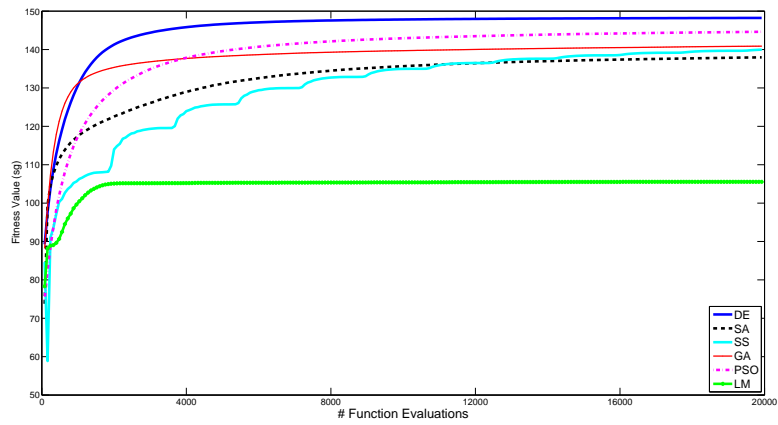
#### 425 *4.2. Overall System Performance*

426 In a second test we evaluated the results of the entire localization method  
427 by dividing the outcomes into three quality classes (see Figure 5):

- 428 1. Perfect Match: all points of the two models are over the corresponding  
429 parts and cover them almost entirely;
- 430 2. Good Localization: (i) all points of the two models belong to the regions



(a) *sp*



(b) *sg*

Figure 4: Evolution of the fitness function for the localization of *sp* (above) and *sg* (below).

431 which must be detected, but they do not cover them entirely or (ii) at  
 432 most two points are slightly outside of them;  
 433 3. Error: all other possibilities, from three or more misplaced points to  
 434 models which are located in a completely different position of the brain.

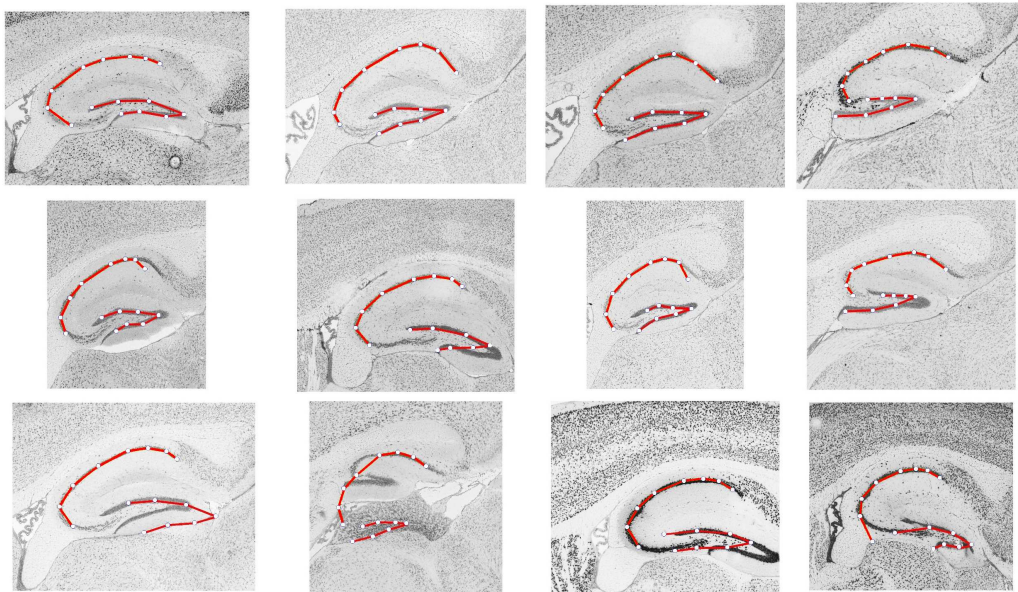


Figure 5: Upper row: perfect matches; middle row: good results; lower row: erroneous localizations.

435 If the results obtained by the model chosen in the Best Reference Slice  
 436 Selection phase were not good enough (using a threshold for the target func-  
 437 tion values), we repeated the procedure using the model which ranked second  
 438 in the previous phase, and took the best result.

439 Our method was able to perfectly localize the hippocampus in 58.0% of  
 440 synthetic images and in 47.8% of real images, and reached a good localization  
 441 in 35% and 43.1% of cases, respectively. This means that our method was

442 able to localize the hippocampus satisfactorily in 93.0% with synthetic images  
443 (20 images and 25 runs per image) and in 90.9% of cases with real images  
444 (320 images and 1 run per image).

445 The most problematic situations that appear to drive the system to bad  
446 localizations are substantially three:

- 447 • low quality of images (for instance, images that are damaged or have  
448 very low contrast): this can affect the results of ROI extraction or of  
449 the Best Reference Slice Selection phase;
- 450 • images where the hippocampus has low levels of gene expression and is  
451 surrounded by other anatomical parts with higher expression levels;
- 452 • hippocampi having shapes which differ substantially from the typical  
453 cases included in the training set.

454 Most of these errors could most probably be avoided by increasing the  
455 size of the training set for the point distribution models, or improving the  
456 preprocessing phase of the images.

457 On the other hand, the method we adopted shows very good performances  
458 when dealing with corrupted images of hippocampi.

459 With regard to time complexity, the localization task, implemented in  
460 C, employs on average 2.88 seconds per image, applying the Best Reference  
461 Slice Selection process on 10 reference slices.

## 462 5. Discussions and Conclusions

463 In this work we presented a two-step algorithm aimed at automatically  
464 localizing the hippocampus in histological images. The first phase roughly  
465 locates the anatomical structure by comparing the image under consideration  
466 with images taken from the ABA reference atlas. In the second phase, the  
467 structure is more precisely localized using a DE-based parametric deformable  
468 model that adapts its shape to match the anatomical structure of interest.  
469 The method is able to deal with imprecise and incomplete images, and, in  
470 our tests on actual images from the ABA, has been successful in 90.9% of  
471 cases.

472 Since classical gradient-based local search methods (like LM) are not able  
473 to solve this problem satisfactorily, due to its multimodality and high dimen-  
474 sionality, we have applied and studied different metaheuristics: DE, SA, GA,  
475 PSO, and SS. DE achieved the best results, for both average and standard  
476 deviation. This result can be explained by a better balance between explo-  
477 ration/diversification and exploitation/intensification and upholds the results  
478 obtained in other cases by DE on benchmarks containing multimodal non-  
479 separable functions [36]. Moreover, DE has shown greater robustness: it is  
480 able to reproduce good results more consistently over many trials, whereas  
481 the performance of other algorithms, like GA or SS, is more dependent on the  
482 stochastic initialization of individuals and parameters. DE is also, together  
483 with GA, the metaheuristic that converges faster to good solutions.

484 Possible future work includes automating the configuration step which

485 sets the algorithm parameters. Another interesting development could con-  
486 sider applying this method to other subcortical structures and anatomical  
487 planes in order to evaluate its general applicability. The automatic segmen-  
488 tation of the structure and the texture analysis of the segmented areas will  
489 lead, in a subsequent phase of the project within which this method was  
490 developed, to identifying sets of genes whose expression generates similar  
491 textural patterns in corresponding regions. We aim to cluster similar genes  
492 according to such visual features to detect correlations in their expression,  
493 since it can be argued that genes with similar expression patterns are also  
494 functionally correlated.

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