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Statistical physics and Alzheimer's disease

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Abstract

We discuss the possible utility of statistical physics in elucidating some of the puzzling phenomena that seem to occur in the brains of patients affected with Alzheimer's disease. Further, we report three specific results from this approach: (i) The size distribution of senile plaques appears to be log-normal, (ii) We develop a model for growth of senile plaques that is characterized by both *aggregation and disaggregation*, and (iii) We quantify neuron architecture and find quantitative evidence for the existence of microcolumns positioned at right angles to the known lamina.

1. Introduction

The challenge of biology for those of us who work in statistical physics is that biology has no metronome in time and no evident architecture – crystalline or otherwise. As if by magic, out of *randomness* we find remarkably fine-tuned biological processes in time and biological forms and structures in space. To understand this "miracle", we should put aside the human tendency (almost universal, it seems) to see the universe as a machine. Our task is to find out how, through pure (albeit, as we shall see, strongly correlated) randomness, we can arrive at the structures in biology we all know exist.

My talk today is not about biology in general, but about one specific and very terrible disease. I am assuming that not everyone in this room is familiar with Alzheimer's disease and so I will organize this presentation around three basic and obvious questions: (i) What is the puzzle or problem? (ii) Why do we physicists care? and

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(iii) What do we actually do? The "we" in that last question includes not only my collaborators who kindly consented to allow their names to appear on this talk, but also many others working in this vast field.

2. What is the problem?

The first question – "*What is Alzheimer's disease?*" – presents a special problem: unlike many diseases (e.g., heart disease, stroke, cancer), we don't even know what the disease is! The operational answer to the problem is that the disease is a killer.

Contrary to what many think, if we make a graph of the probability that a person will die as a function of the time after diagnosis, it will monotonically increase but not rapidly approach one. It is an S-shaped curve that shows that the probability of our dying of AD before age 60 is about 10%. Between ages 60 and 80 this rises very rapidly to 50%. Many of us choose to do science that funding agencies are interested in. It goes without saying that many who donate funds are in that 60 to 80 age group, and presumably are quite interested!

In fact, the actual death of a person from this disease often comes about simply because of the inability of caregivers to continue to give adequate care, and this is the case because the decay of mental activity from the disease is very rapid.

In contrast to other killer diseases, AD is very debilitating. You can teach until the moment of your heart attack or stroke. It's a common joke that that's the way we want to go – because we'll be ourselves until the very last moment. Even in the case of cancer this is often true. Richard Feynman continued to teach until just before he died. In contrast, Alzheimer patients have ten, even fifteen years during which time they progressively lose their functions. We all know people who have suffered in this way. Edward Purcell, one of my professors in graduate school, passed away a few days ago from this awful disease. Ronald Reagan continues to suffer.

3. Why do we statistical physicists care?

The second question – why do we physicists care? The scientific answer is that this new phenomenon contains some interesting physics. That may sound absurd, probably the same as when someone in the early days of polymer science – a very "dirty" science – said that *it* contained some interesting physics. To look for interesting physics in a more or less mundane and "dirty" phenomenon takes a certain amount of optimism! And, of course, many who have looked for interesting physics in such places have failed to find it.

As we shall see in the next section, AD is associated with clustering of aggregates in the brain. To understand how these aggregates are generated may help to understand the origin of the disease. Since aggregation models are part of statistical physics, we will apply statistical physics to study these aggregates.

4. What do we actually do?

The third question is what do we do? The first thing we need to do is, like Sherlock Holmes, look for clues. Depending on who we are, we have access to different clues.

Clue Set #1: If you are a practicing physician, you can actually interact with the AD patient. You can diagnose the progress of the disease by the patient's performance in certain tests designed to measure mental function. In early stages of the disease, the patient typically loses short-term memory. In later stages the long-term memory is lost. Physicians know of no drug therapy that will stop the progress of the disease.

Clue Set #2: If you are an anatomist, at autopsy you can study the brain of a person who has succumbed to AD. In order to describe the clues available at this stage, we need a definition: senile plaque [1–3] (Fig. 1). If you take a small specimen of brain tissue, say $1\,\text{mm} \times 3\,\text{mm} \times 50\,\mu\text{m}$, and stain it – i.e., add some chemical that highlights some feature – you can then observe that the brain tissue of those who have died of AD has more *senile plaques* than that of those who died of other causes. Senile plaques are formed by the aggregation of some peptide (which we will discuss in greater detail). Statistically, we can say that the brain tissue of those who have died of AD has more senile plaques than that of those who died of other causes, but there are caveats: *some* healthy old people have senile plaques, but *very few* AD patients are without senile plaques. Basically, if you have senile plaques in your brain, it could be bad news.

In order to develop this picture, we also need another fact, one that may strike you as counterintuitive. To do this we need to measure the number of senile plaques in the brain of a person who has succumbed to AD *as a function of time*. Obviously in this case we mean the time between diagnosis and death – for some a much longer period of time than for others – and not the ruthless extraction of brain tissue at regular intervals from a living person with AD! There is a caveat here – people are first diagnosed at different times, some early on in the progress of the disease and some not – so this unit of time is somewhat ambiguous. But the important thing is that the number of senile plaques does not keep on increasing, but seems to plateau. Also the "coverage" (as we would call it in solid state physics), i.e., the fraction of the area covered by the senile plaques, also seems to plateau.

Clue Set #3: If you are a neurologist, you will be measuring numbers of nerve cells with a stain to highlight certain features – again, as a function of time after diagnosis – and will discover that the number of neurons decreases. Typically, the fraction of neurons decreases until it is one-half the number in a healthy brain. Of course, it has been pointed out that we have a *lot* of neurons – that we lose one-half of 10^{11}, we still have 10^{11}, more or less! There is also some shrinkage of the brain such that, although the number of neurons decreases, the *density* of neurons (and hence their connectivity, perhaps) does not change as drastically.

Clue Set #4: If you are a biochemist, you may notice that there is a particular protein, called τ protein, that aggregates and forms a kind of skeleton inside the nerves of individuals who succumb to AD. This is a fairly recent discovery and not much is known about it, but it appears that this skeleton keeps on growing inside neurons as

Fig. 1. A typical cross-section of the Alzheimer brain, stained in such a way as to display the morphology of a senile plaque (red).

the disease progresses, eventually filling – and killing – them. Again, they don't grow forever, but may saturate at some stage.

Clue Set #5: If you are a geneticist, you may well believe that all diseases are due to bad genes. That also seems to be statistically true of AD. This particular senile plaque is formed by the aggregate of a little peptide of only 40 amino acids in length. This peptide, in turn, breaks off from some trans-membrane protein. And this trans-membrane protein appears to be associated with some other protein ("apolipoprotein E"), which comes in three principal forms: E2 (which is good), E3 (which is a little less good), and E4 (which is not good).

You will recall that the disease called "sickle-cell hemoglobin" is caused by one base pair in an individual's DNA being wrong. Because of that one wrong base pair there is one wrong amino acid, and because of that one wrong amino acid there is a

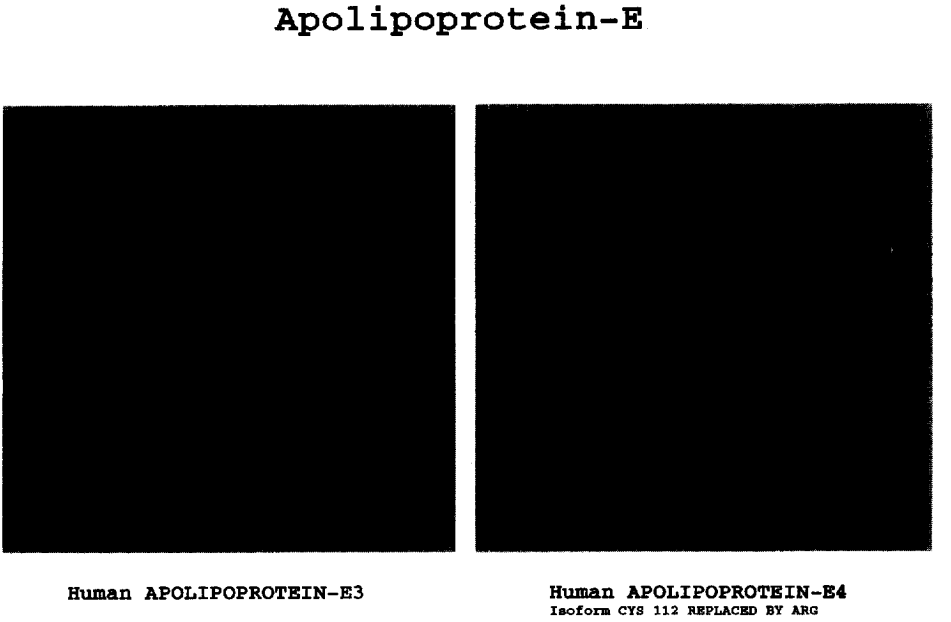

Fig. 2. Comparison between the structures of the "good" protein E3 and the "not good" protein E4 – which differs from E3 in that one amino acid is replaced by another.

different conformation of the hemoglobin protein in such individuals. The same is true in AD. In apolipoprotein E4 there is a single mutation in position 112 (Fig. 2). One amino acid is replaced by another amino acid, giving the protein a slightly different shape. About 15% of the general population has this mutation. Among those with AD, roughly 50% have this mutation – so this is beyond any statistical fluctuation, and is definitely associated with AD.

AD is also associated with Down Syndrome. For over twenty years, we have been able to do prenatal testing for Down Syndrome. This terrible disease has many features in common with AD. Senile plaques occur, perhaps even more than in the case of AD. The tremendous loss of mental function is similar. In fact, the gene for the amyloid precursor protein associated with AD resides on the same chromosome (#21) that is associated with Down Syndrome.

Some researchers have claimed that susceptibility to AD can be predicted by studying a young person's handwriting. If true, this would be consistent with the genetic component: many people's handwriting is remarkably similar to that of their parents.

Our conclusion is that if AD is genetic in origin, many of us have reason to be concerned; almost everyone has some relative in their family or in their spouse's family who has AD. At the very least, this puts our children at risk. This is one more reason that many want to find some way to cure, or at least some way to slow the progress of AD, before our children are old enough to be affected.

5. Working hypothesis

We now come to our main point: our working hypothesis. It is not my working hypothesis. I did not invent it, nor did my co-authors. Many researchers working on AD share this working hypothesis, though certainly not all. The hypothesis is that *Alzheimer's disease is somehow a function of the senile plaques*, $AD = f(SP)$. This function f is not defined exactly. It is a working hypothesis in the sense that we will assume it and see what we can do with it. We are not alone in this. Many of you know from the media about the "$2 billion mouse" that has been genetically engineered so that it can get AD. This "animal model" does not get *all* the features of AD, but it does get senile plaques. This is terribly important because, unlike human sufferers, this mouse can be sacrificed at any stage in the progress of the disease, and the time-development of these plaques studied. Our collaboration is one of a handful in the world that has access to this mouse, and hence to the raw data. We are also one of only a very few groups studying AD using 3-dimensional microscopy. And we are the only group in the world studying AD using statistical mechanics (the final utility of this fact I'll leave for you to judge!).

And now to consider the firm results. There are not a lot of them, but anything may be potentially useful in the fight against AD.

What physicists always do that anatomists may not always do is quantify. We want to quantify everything. We aren't satisfied with a statement such as "50 μm – ten red blood cells – is the *average* diameter of senile plaques." We want to do more, and we set out to make a histogram of sizes. We are not the first to make such a histogram. This histogram of sizes has been made and known for some time. It turns out to be a very skewed histogram. When we plot the number of plaques having a given area as a function of area we find something very skewed to the right. The maximum of this curve is of order 50 μm^2, but there is still some reasonable probability at 500 μm^2. How does a statistical physicist respond to such a histogram? If we replace the area with the logarithm of the area, we get our first firm result: *The size distribution of senile plaques is log-normal* (Fig. 3) [4,5]. Logarithms are not that common in medicine, so we actually measured the area in powers of 4, which has the same effect. We get a graph that looks more like a Gaussian distribution. We depend on the researchers at Massachusetts General Hospital in Boston, not only for their intellectual guidance, but also for the sheer "brawn" required to measure the areas of all these plaques – enough to get good statistics. The whole elaborate process was then repeated for Down Syndrome, and the results were remarkably similar (Fig. 4).

What is this result – that the size distribution of senile plaques is log-normal – telling us? If we make this histogram in volume, V, it is consistent with the idea that this quantity, $\log V$, is like a random walk, i.e., the time derivative of the $\log V$ is a noise term (η). This means that when volume doubles, the time derivative of the volume also doubles. This is surprising because normally we would expect that the time derivative of the volume would be proportional to the hull, the external surface. That's where the peptides are coming from; they're coming from outside and intuition

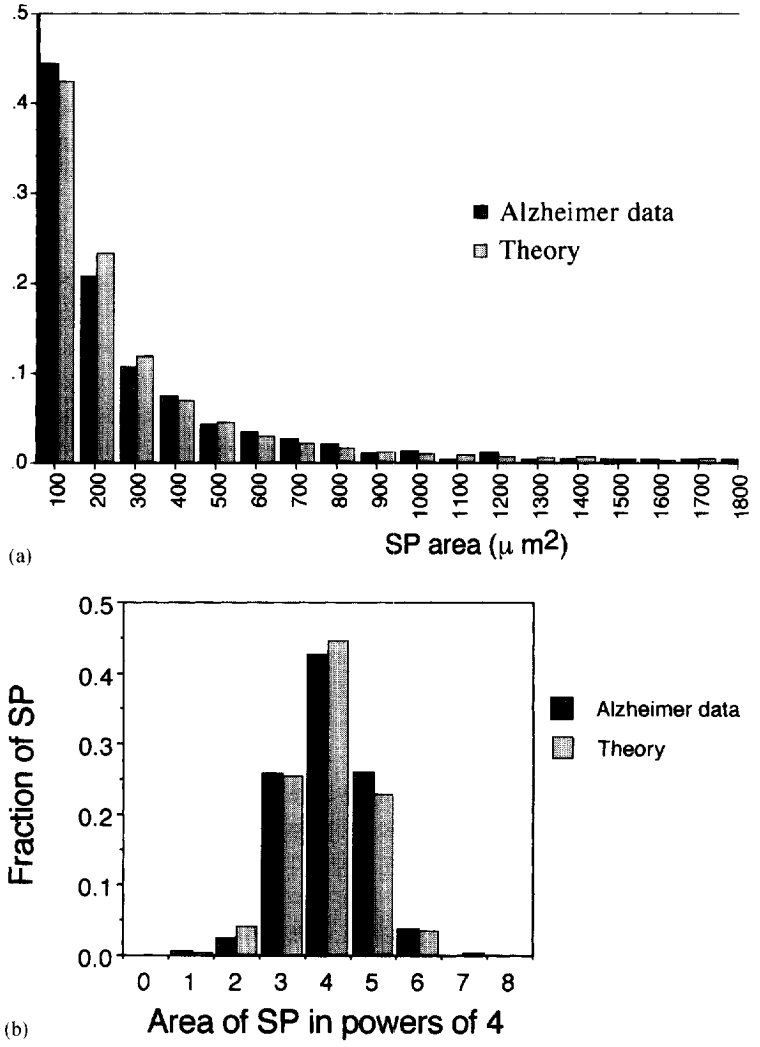

Fig. 3. Senile plaque size profile distribution histograms of an AD patient. A 50 μm thick section of the temporal lobe was immunostained with anti-A' monoclonal antibody 10D5. In this case, six 780 μm by the depth of the cortex traverses of the inferior bank of the superior temporal sulcus located approximately 1 cm from the crest of the middle temporal gyrus were analyzed. Plaque size profile (closed bars) is (a) plotted arithmetically in bins of 100 μm squared, or (b) as a log4 function of plaque size (in bins of powers of 4). A curve generated from a theoretical log-normal distribution is shown as open bars in (b) to illustrate how well this distribution fits the data.

would suggest that the time derivative of volume would be proportional to its external surface.

Perhaps the external perimeter (the "hull") is in fact proportional to the volume. To test this, we look at the 50 μm plaque through our 3-D microscope. What do we see? We see holes, a deeply invaginated structure (Fig. 5). It's not implausible that the

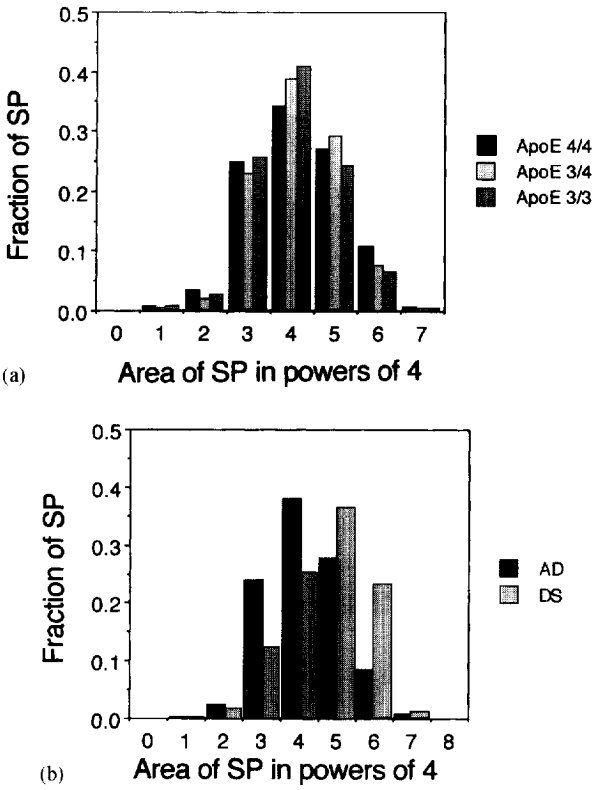

Fig. 4. (a) same as Fig. 3(b) except for all 3 genotypes of the apolipoprotein. (b) Comparison of AD with Down syndrome (DS), showing the same log-normal size distribution but with a mean roughly four times larger.

external perimeter of such a deeply invaginated shape might not scale as its volume. Why? Because we know other models for which the hull does scale as its volume: the total surface of a percolation cluster scales as its volume, and in DLA that is also true. Obviously this does not look like DLA; it is not a fractal, much less a fractal of dimension 2.5. It is also not a compact Eden cluster.

The model our group has come up with (Fig. 6) Ref. [6] moves outside statistical physics into biology and involves little organisms called microglia that "eat" senile plaque. If the right bonds are eaten then the senile plaque will come apart, dissolve. This, of course, is very important in the effort to find some way of slowing down the progress of AD. If we want to slow AD, then we need to find some way of stimulating the growth of these microglia.

The volume of senile plaque saturates in AD; it doesn't just grow forever. One might be tempted to speculate that the holes in the senile plaque are filled with neurons that have penetrated the senile plaque – or perhaps the neurons are there from the beginning and the senile plaque grows around them, enveloping them, and allowing the growth

Fig. 5. Three-dimensional reconstruction of an senile plaque (of diameter $\sim 60\,\mu m$) from 18 images ($\times 100$ oil immersion objective) separated by 0.3 μm. Each cross sectional image represents the average of three scans combined with a Kalman filter. All images were obtained from the multimodal superior temporal sulcus neocortex of six Alzheimer cases from the Massachusetts Alzheimer Disease Research Center Brain Bank. Photomicrograph made with a BioRad 1024 confocal microscope (BioRad, Hercules, CA) of a section of cerebral tissue of dimensions $600 \times 600\,\mu m$ in area.

of a "dead skeleton" of τ protein, a protein that is of no use to brain function – and that's why we lose half our neurons when we have AD (Fig. 7).

This is a model with both aggregation and *dis*aggregation, due to the microglia. The qualitative test of this model is just to make a picture of the model and a picture of the real senile plaque and compare them. An anatomist, with an extremely well-trained eye for minute detail, would stop here. We can also compare the experimental log-normal distribution with the model (utilizing adjustable parameters) and see that the agreement is rather good.

Finally, we can measure quantitatively various correlation functions. This is the familiar $g(r)$ that we have measured in statistical physics since Van Hove's time. Simply put, we find a straight line for a compact disk with two deviations: one at the invaginations and one at the diffuse rim or outer surface of this structure. A histogram

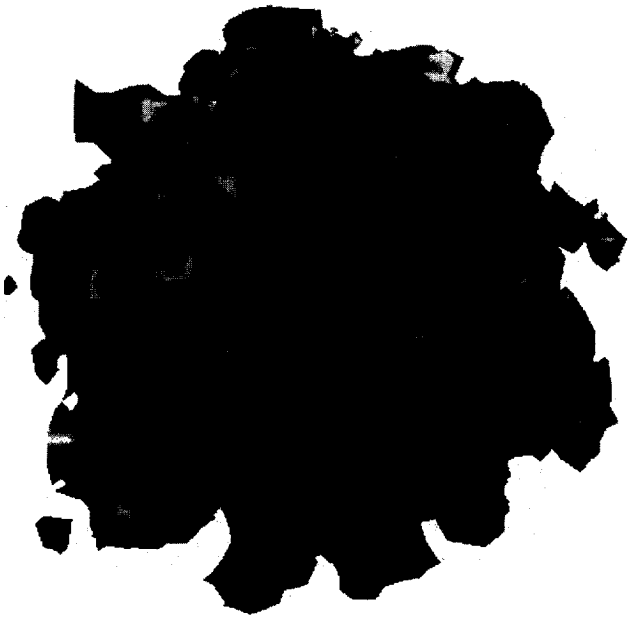

Fig. 6. Three-dimensional reconstruction of the model plaque. The dynamical model is defined on a discrete three-dimensional lattice with lattice sites which can be either empty or occupied. At each time step in the simulation each occupied site either grows with probability P_g, or is cleared with probability P_c. Depending on their relative values, a system may be predisposed to create plaques or to dissolve them. Nearest neighbor rules are incorporated such that aggregation at a site is more likely if its neighboring sites are empty, and less likely if they are occupied. On the other hand, an occupied site is more likely to be dissolved as the number of empty nearest sites increases. These rules follow from considering that in real SP, the more exposed sites have a greater probability of being surrounded by $A\beta$. At the same time, these exposed sites are more likely to be disaggregated by external agents. In order to avoid the final state where either all sites are occupied or empty (inevitable under the given rules), it is necessary to incorporate a dynamic feedback that allows the system to evolve into a steady state characterized by a burden that is on average conserved in time. The feedback modifies P_c by an amount that is proportional to the rate of change in the total burden. In addition, the model allows for a diffusion of aggregated particles on the model plaque. This diffusion permits a given occupied site to explore its immediate neighborhood and choose to change its position only if it ends up surrounded by more neighboring sites. This selective diffusive process allows for the system to relax so that the overall surface is smooth. The initial values of the disaggregation and aggregation probabilities are $P_c = P_g = 0.8$. The surface diffusion is set to allow sites to move up to 10 steps around its initial position at every time step.

of characteristic pore sizes indicates a number on the order of 5 μm, approximately 10% of the 50 μm diameter.

Our last topic is neuronal architecture (work being done primarily by Sergey Buldyrev) [7]. If we look at a picture of a 3 mm × 3 mm section of the brain, we see lamina about 300 μm in diameter [8,9]. By quantifying the correlations $g(x, y)$ in both x and y directions, we also discovered the existence of little columns (resembling little polymers of about ten monomers) positioned at right angles to the lamina, and we are studying these columns as they occur in both the healthy brain and the AD

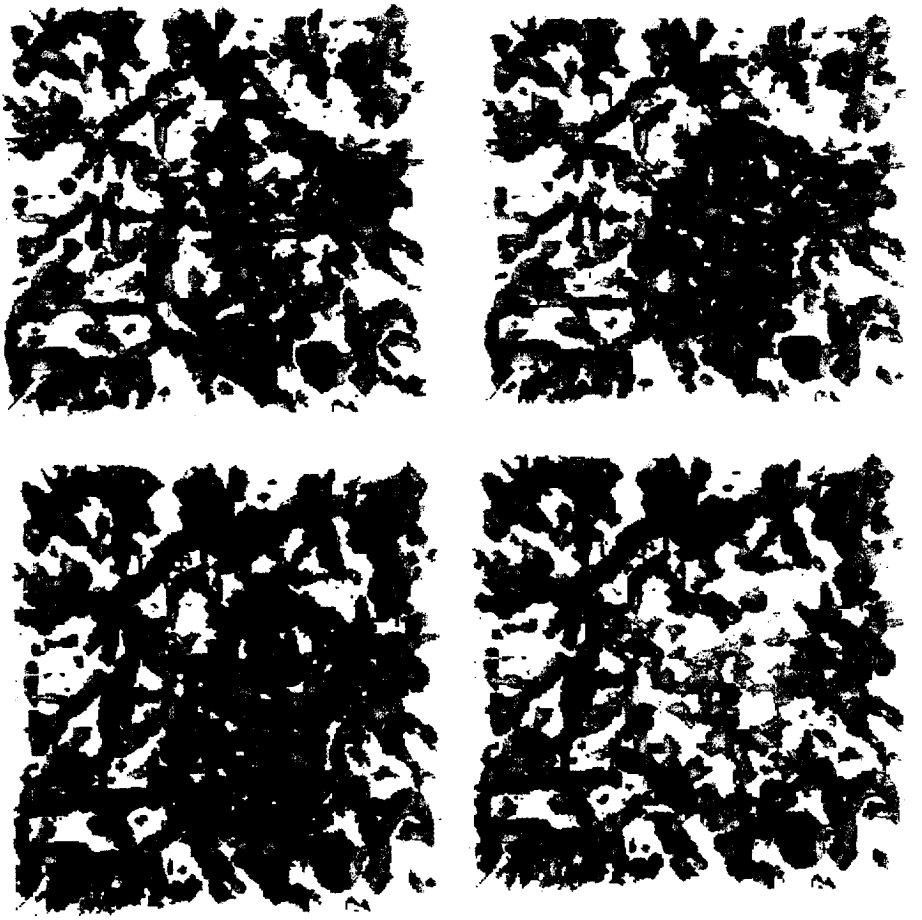

Fig. 7. Typical analysis of triple-staining experiment of a 3-dimensional image. The senile plaque is stained green, the unhealthy neuron red, and the healthy neuron blue. In the lower right corner, the senile plaque is "removed" by the computer, showing that nerves have passed through it.

brain [7]. The same sort of correlation analysis that we earlier applied to plaques can also be applied to neurons. We also find that the morphology of specific dendrites is disrupted in the AD brain [10].

6. Summary

In summary, we have three relatively firm results to report in our AD research thus far:

(1) The log-normal distribution of senile plaque size [4].

(2) A model characterized by both aggregation and *dis*aggregation [6].

(3) Quantification of neuron architecture that appears to give quantitative evidence for the existence of microcolumns positioned at right angles to the known lamina [7].

Acknowledgements

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