Functionally Diagram Human Brains using Ganged Confocal Scanning UV Fluorescence Microscopes with 3-D Substage Micromanipulator and Cryostat Microtome

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Abstract - *This is a proposal to construct practical automated neurological diagramming machines.*

This is a new class of devices that will be able to functionally diagram complex biological systems, eventually including human brains. Development of these devices should quickly lead to a greatly improved understanding of human cognition sufficient to assure the success of ongoing AI/AGI development efforts, and provide key information needed to develop treatments for various neurological conditions.

These microscopes will look into bulk tissue, focusing UV spots and recovering scattered UV and visible-light fluorescence from the same side, but along different light paths. This will work to a depth of ~10μ, even on living tissue. Computed tomography and image processing will transform the information from observed construction into a wiring diagram, including component values.

In a whole-brain diagramming version, brains can then be diagrammed by analyzing their surface ~10μ, then microtoming away ~4μ of the surface to analyze deeper in, and continuing this process one slice at a time until the entire brain has been diagrammed.

Keywords: diagramming, confocal, scanning, ultraviolet, fluorescence, microscope

1 Introduction

If only AI researchers had full wiring diagrams of brains, if only pharmacology researchers could watch chemical messengers move about within living cells, if only doctors could analyze the functional differences between diseased and healthy tissue; then both computer science and medical science would be suddenly propelled forward by decades.

Scientific research utilizes existing or easily fabricated equipment. Equipment development is a business built on prior scientific research. This "loop" sometimes leaves "islands of opportunity" where some advanced product development could produce equipment to greatly advance science, yet no equipment manufacturer is able to address the market. These islands exist in areas like microscopy, where equipment manufacturers, operating on thin profit margins in highly competitive markets, lack the financial resources and multidisciplinary expertise to develop radically new equipment. This proposal addresses the largest known island of opportunity.

Much of biological research, neuroscience research, artificial general intelligence research, and numerous other smaller areas are now substantially "hung up" on the lack of a particular equipment capability, namely, the ability to "functionally diagram" tissue, especially brain tissue. When available, functional diagramming will probably be more transforming to these and several other areas than were computers.

functional diagramming : (aka neuromorphic diagramming or computational diagramming) is the process of identifying the functional interrelationships of the components of cells and their quantitative interrelationships with other cells, and then filing this information into a database without regard for the physical structure and dimensions being represented.

To date, the most ambitious functional diagramming project was done manually, to diagram the 302 neurons in the nematode (roundworm) Caenorhabditis elegans, and this database is now on-line. Unfortunately, without the capabilities of the SUVFM described herein, that database does not include component values. Without component values, numerous researchers have been unable to understand its operation, or even label the neurons beyond "sensory", "interneuron", and "motor"[9]. Cognition is primarily concerned with interneuron functionality, which is determined by component values like synaptic efficacy. Note that the same neuron in different subjects may have different functions, as their operation is probably the result of self-organization. Hence, a useful analysis would have to be completed on a single subject, which would preclude all but fully automated methods

Note that each synapse probably has several quantitative component values. Aside from efficacy, there may be a variety of statistical accumulators that control changes (learning), nonlinearities that may be needed for certain computations, synaptic integration and/or differentiation, and other as-yet unknown characteristics.

2 Obama's B.R.A.I.N. Initiative

On April 2, 2013, President Obama announced the B.R.A.I.N. Initiative, supposedly to understand the operation of the brain. However, the requested $100M funding for this "initiative" is less than 2% of the present $5.5B NIH neurosciences budget. Careful examination of the proposal shows no funding at all for the present roadblocks of diagramming, identifying component values, or reaching a detailed understanding of the computations involved.

Washington insiders seem to think that this is politics as usual. This new "initiative" is being used as a foil to fight the present economic "sequester", which fails to provide funding for new projects. It is also possible that Obama may want to start something really big, in order to be long remembered, akin to President Kennedy starting the Apollo moon missions. Either way, much more fame and funding could be in the future for the B.R.A.I.N. initiative.

It seems obvious to nearly everyone that a major breakthrough in research methodology is needed before a major breakthrough in brain research can occur. We believe that a "Big Iron" approach, such as that described herein, will produce the information needed to sidestep the present roadblocks.

3 Prospective Diagramming Methods

Some formal proposals and many informal proposals for methods of diagramming have emerged. Each method images neurons differently, so that understanding of the operation of living tissue gained with one method cannot then be transferred to other methods for diagramming. This is the present roadblock. When the functionality of neurons and synapses is better understood, diagramming methods that can't work on living tissue (e.g. scanning electron microscopy) might become applicable and produce superior diagramming results.

Diagrams are needed to understand neurons, and neuronal understanding is needed to produce better diagrams. This particular proposal is seen as the next logical step on the long path to complete neuronal understanding and the production of accurate functional diagrams.

The fundamental limitation in resolution is ~1/3 of the wavelength used for illumination or observation. This limitation applies to all methods from MRI to electron microscopes. Brains are transparent to radiation of nearly all wavelengths up into the near UV region, whereupon they become opaque. You can probably see the veins in your wrist because of transparency to visible light. Opacity to shorter wavelengths is the basis of Lasik eye surgery, as its use of short wavelength UV only affects the surface cells.

Either observation must be made at near-UV wavelengths to utilize transparency at maximum possible resolution, or alternatively, methods not relying on transparency must be employed. Unfortunately, we don't know enough to understand what can be seen at higher resolutions. Without transparency, there is presently no known way to observe detail in living neurons, a necessary requirement to close our present gap between form and function.

Near-UV just happens to have another wonderful feature for this application, namely, that in addition to being able to see near-UV light scattered by transparent structures, complex molecules fluoresce when exposed to near-UV. Their fluorescence provides for limited chemical analysis of complex molecules – a feature not available with other methods. Conventional subtractive staining provides fluorescence, but hides structures that are beneath the stained details, making it unusable for diagramming.

Through a process of elimination, there seems to be little choice but to diagram utilizing near UV scattered light and fluorescent microscopy techniques, at least until the relationships between form and function has been discovered. However, there is a residual problem. Present confocal microscopy methods fail to produce images from bulk tissue of sufficient quality for use in automated diagramming. This proposal advances a method of utilizing separated point scanning and UV computed tomography (UV CT) to overcome those shortcomings.

Once neuronal and synaptic operation is much better understood, other methods will probably supersede the ones presented herein to provide more accurate diagrams.

4 Overview

Cytometry : an analytical method capable of precisely quantifying the functional states of individual cells by measuring their optical characteristics based on fluorescence or scattered light.

This proposal leverages on several well known physical characteristics of brain and other neural tissue:

1. Chemical components of brain tissue fluoresce richly when exposed to blue or near-UV light.
2. Brain tissue is transparent at microscopic scales.
3. The boundaries between transparent structures having differing indexes of refraction are made visible because the change in index of refraction reflects light when flat like a window, but scatters light from rough biological structures.
4. Brain tissue can be accurately sectioned away in 4μ slices when held at -4°C.

The SUVFM achieves UV resolution in 3-D while reading out chemical composition, and comes in two forms:

1. A laboratory instrument to identify what physical structures in <u>living</u> tissue, identified by their time-dependent fluorescence spectra, perform what computational processes.
2. An automated tissue diagramming machine, which incorporates the information gained from the laboratory version, and diagrams the surface ~10μ of surface volume in <u>frozen</u> tissue, removes ~4μ, and repeats this process, one slice at a time until the entire brain is completely diagrammed. Since

the slices are immediately discarded prior problems of preserving, processing, and analyzing them are eliminated.

5 Background

The scanning ultraviolet fluorescence microscope (SUVFM) comes at the end of a half-century of advancements in microscopes of various sorts. While the SUVFM would have far less resolution than electron microscopes, it has other crucial characteristics that are now needed to move cognitive computing forward, including the ability to examine living tissue and the ability to perform limited chemical analysis on individual 3-D pixels.

The SUVFM would rapidly flash weak focused spots of near-ultraviolet light into biological samples at various places and depths and observe the fluorescence spectra and decay rates at those places. A computer would analyze the decay spectra and profiles, and reconstruct the 3-D structure. This would be capable of structural and some chemical imaging in 3-D with considerably better than visible light resolution. Further, by scanning to a sufficient depth that the top layer could then be sectioned off and the process continued through an entire brain, there could be enough redundant overlap to ensure that there would be no errors, even if there was a problem removing one of the ~4μ sections. With this device it should eventually become possible to automatically reconstruct the complete functional diagram of a brain, including individual synapse characteristics and other similar details.

In the late 1960s, Marvin Minsky of MIT's AI lab developed the first working machine vision system that successfully parsed visual scenes, thereby paving the way for brain diagramming as now contemplated. Marvin Minsky also invented the confocal microscope[14].

Soon after, there was an early effort at Carnegie Mellon University to diagram insect brains using a computer program written by Michael Everest. Researchers attempted to microtome off slices and stain them to microscopically scan using 2-D visible light methods. This effort failed because some slices were inadvertently destroyed, and staining is a subtractive process (whereas fluorescence is additive) so that it was impossible to see what was behind a stained detail. Further, large microtomed slices must be >4μ thick to withstand handling, whereas some important parts of neurons (like their axons) may only be 1μ or less wide. The scanning ultraviolet fluorescence microscope easily avoids all of these prior problems by immediately discarding the slices and looking into the unsliced brain. It provides more than an order of magnitude more real-world resolution than prior methods by working in 3-D with ultraviolet, and using UV CT to extract more detail than visual methods can extract.

So far there have been no successful automated brain diagramming projects, and there won't be until scanning UV fluorescence microscopes similar to those described herein are constructed. To diagram brains, such a microscope will require the largest supercomputers now available to deal with the horrendous computational load and produce diagrams in months, rather than centuries.

A scanning UV fluorescence microscope could also non-destructively observe the operation of living cells in far more detail than is currently possible with direct visual observation. Researchers now routinely observe living neurons in operation under UV fluorescence because their fluorescence changes as they operate. The addition of scanning to improve resolution and provide depth separation and real-time logging should make it possible to characterize synapses by their appearance under fluorescent conditions as their electrical operation is simultaneously observed.

6 Basic Physics

Complex molecules often fluoresce. A higher-energy photon (or simultaneous lower-energy photons[13]) activates them, and results in the sometimes delayed release of lower-energy photon(s)[3]. Chemicals can be identified by the energy needed to activate them, the energy of the released photon(s), the delay between activation and fluorescence, the recovery time, and the response to photobleaching. Where several chemical constituents are present their fluorescence is combined, leaving the computer to unravel the combined fluorescences.

A point within tissue can be chemically analyzed by flashing a point of UV or blue light through the tissue and focused at the point and observing the visible-light fluorescent decay. The visible light will be a combination of the fluorescent decay of everything at that point, plus far more light from everything before and after the point that is illuminated by the UV or blue light. However, clever optical design must limit the extraneous field of view. The defocused extraneous light will be very nearly constant from point to point so it can be subtracted off to yield just the spectral characteristics at the targeted point. Computerized image enhancement will clean up any remaining problems.

The basis of successful diagramming is 3-D chemical mapping. This image, instead of being in color, will be a 3-D map of the spectral and decay characteristics at the many points in the tissue. A computer will then analyze the image and form a map of the chemicals present. From the chemical map the computer can infer the structure, and from the structure the computer can infer function, and then relate each functional element to its neighbors by examining the chemical and electrical interfaces.

7 Optics

A new principle of microscopy is employed here, where a single near-UV LED in an array of LEDs illuminates a point within the sample via a thin hollow cone of focused light. A coaxial central cone of receptivity then focuses an image of that point within the sample onto an imaging array, where a computer then looks at the same pixel position as the LED illuminated. Only a tiny micron-wide volume where the two coaxial cones intersect is visible to the microscope at any instant in time.

Image enhancement methods applied to the point observations will provide for ~0.1μ near-UV limited resolution. Physically moving the LED and imaging arrays facilitates the processing of virtual slices, interpolation between pixels, and working

around any dead pixels. Beam splitters will provide for any number of color-sensitive imaging arrays, as may be needed for adequate chemical analysis.

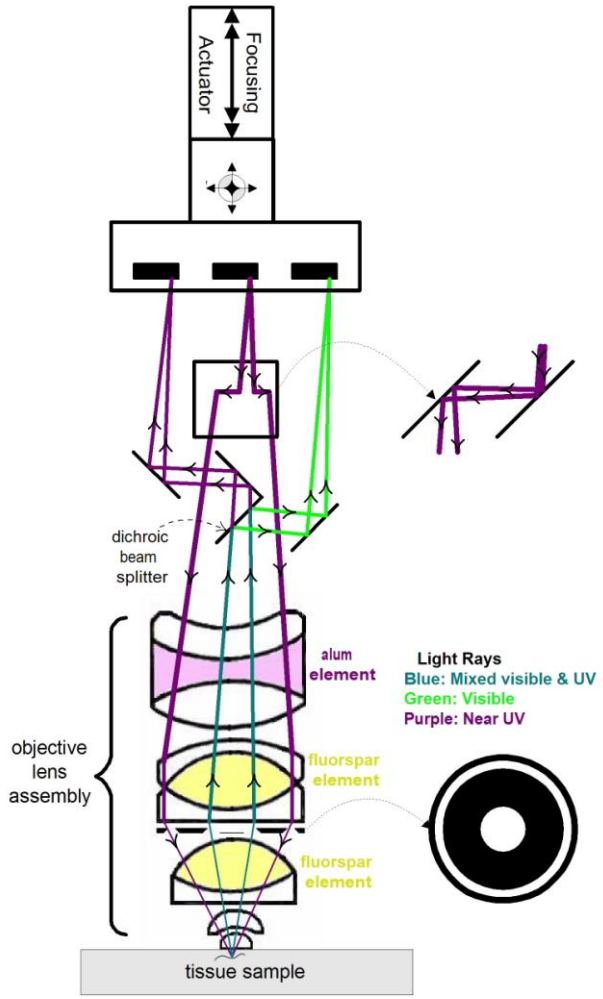

Fig. 1 Design of SUVFM Microscope

8 Computational Feasibility

There is an incredible amount of detail in a human brain. It will be analyzed in ~250K 4µ physical slices. UV analysis will be on ~0.1µ virtual slices, so that each physical slice is analyzed as ~40 virtual slices. That means that every additional minute of time spent analyzing each physical slice translates into another 6 months of total scanning and diagramming time.

Presuming that an array of objective lenses are on 1cm centers, presenting 2K x 2K images at 0.1µ pitch means that each objective would have to mechanically scan a 1cm x 1cm area on a 50 x 50 grid and stop at 2.5K separate points for each physical slice. At each one of these 2-D points it would then be necessary to explore the ~40 virtual slices.

However, miniaturization solves these scanning speed issues. For example, if objective lenses can be placed on a 1mm grid there can be 100 times as many microscopes, which would give the scanning system 100 times the speed.

The length of time a supercomputer barely able to simulate a brain in real time would require to scan and diagram a similar brain is nearly constant. This time is nearly invariant with brain complexity, from insect to human, because a more complex brain would require more supercomputer(s) to simulate it, and the additional supercomputer(s) would speed the scanning and diagramming process. Since many computers can be networked for scanning and diagramming, the actual time required will be reduced approximately in proportion to the number of networked computers. This time is not yet known, but is suspected to be sufficiently low so that a small number of supercomputers, each capable of simulating a human brain, perhaps just one such supercomputer, could keep up with scanning hardware to produce a complete diagram in about a month.

There are ~10^{14} 0.1µ spaced points in a 10µ thick piece of human brain. There are also ~10^{15} synapses in the human brain. This puts a tractable cloud-sized upper limit on the memory requirements. These numbers aren't so daunting when you think in terms of server containers – shipping containers that each holds ~2,000 servers, rather than in terms of individual CPUs and disk drives. Of course, Moore's Law will soon bring computer-related costs down to a much more affordable range

Diagramming will proceed by assigning tentative IDs to all 2-D areas separated by boundaries, or bounded by the field of view. As subsequent virtual slices are analyzed, IDs from prior slices will propagate. Where different ID'd volumes subsequently merge, showing those volumes to be from the same structure, the diagramming software will go back into the previously diagrammed database and re-mark all of the entries for one of the IDs to be the other ID. Hence, 3-D analysis can be performed on successive 2-D virtual slices, with no need to re-examine prior slices.

Note that there are ~18 3-D serial section visualization software packages now available, but diagramming is very different from visualization – easier in some ways, but harder in others. Diagramming need provide no graphics beyond those needed for debugging, but must have some limited image "understanding" abilities.

The only significant image storage requirements are for holding information in the top ~10µ of depth, in case a subsequent problem develops with the cryostat microtome. The 3-D images within each physical slice are fused with the previous slice, the IDs are propagated, and the previous physical slice's image can then be discarded.

9 Improvements over Prior Methods

The basic microscopic principles of flashing UV spots into neurological tissue and observing the responding fluorescence is not at all new. Therefore, there should be no unfortunate technical surprises. However, the following logical extensions of this basic technique are new:

1. Automatically moving samples around in 3-D, so that volumes can be automatically analyzed that far exceed the field of view of the microscope's optics.

2. Ganging many microscopes together, to provide a >100X improvement in speed for large samples.
3. Incorporating a cryostat microtome to automatically remove scanned tissue, so that scanning can continue automatically throughout a thick sample without human intervention.
4. The design for an optical "head" to provide 3-D pixels of sufficient resolution and quality for diagramming.

None of these improvements involves new physics or way-out engineering. Just some plain old product development is needed to build an SUVFM.

The total is much greater than the sum of the parts, because all of these parts are needed for diagramming, diagramming is needed for understanding, and understanding is needed to advance the several fields that are currently "hung up" on the lack of this understanding. Research has gone about as far as possible with present equipment. These new capabilities will open up whole fields of research, especially in AI by answering most of the questions now vexing AI developers, like how apparently unorganized infant brains seemingly self-organize as they grow to adulthood.

10 Why now?

We are right now at the coming-of-age of the two critical technologies:

1. UV and confocal microscopy are ~50 years old. SUVFMs were first proposed ~30 years ago. However, real working SUVFMs are just now being demonstrated, albeit without any of the improvements necessary to support diagramming.
2. These analyses are computationally intensive. A cloud of networked computers, each with GPU and/or FPGA arrays, could now provide the needed computational power, as none of their several present weaknesses appears to affect this particular application, wherein the problem arrives literally chopped into small pieces.

11 Important Details

Fortunately, only certain points need be fully decay-analyzed to measure component values. While this won't identify all chemical constituents, it should identify enough to separate most structures. There will be cases where unobservable structural details must be inferred from what can be seen. It is expected that future simulation results and mathematical breakthroughs will fully fill in any such gaps.

Note that to be able to calibrate this process, functional diagramming must be initially usable on living tissue to be able to understand what does what on a physical level never before seen. Later, once it is possible to reliably relate fluorescence to chemistry to structure to function to diagram, living tissue capability may no longer be needed.

12 Other Issues

There are a variety of miscellaneous issues, such as avoiding opaque objects like red blood cells, adding additional fluorescent markers to identify things that may not otherwise fluoresce uniquely, preserving tissue during months of analysis, etc. These are addressed by perfusing a carefully designed fluid to replace the blood, akin to the way that the blood is replaced with a cryoprotectant fluid before freezing organs for later transplantation. Present cryoprotectant fluids are not suitable for this use because their physical properties aren't compatible with microtoming, but engineering a more suitable fluid is not seen as being a major engineering challenge, as even blood plasma performs marginally.

Microscopy would all be performed under oil immersion, which would perform the dual purposes of preservation and facilitate the use of high numerical aperture (NA) objectives.

13 Uncertainties

Given the wealth of available UV-based techniques, applied to the wealth of neurological unknowns, it is not yet possible to accurately judge the prospects of eventual 100% success. All that can be done is to address the issues now known, and discover what, if anything, remains. Nonetheless, if the SUVFM only does what is clearly possible without significant problems, it will still transform the biological sciences.

14 Supporting Technologies

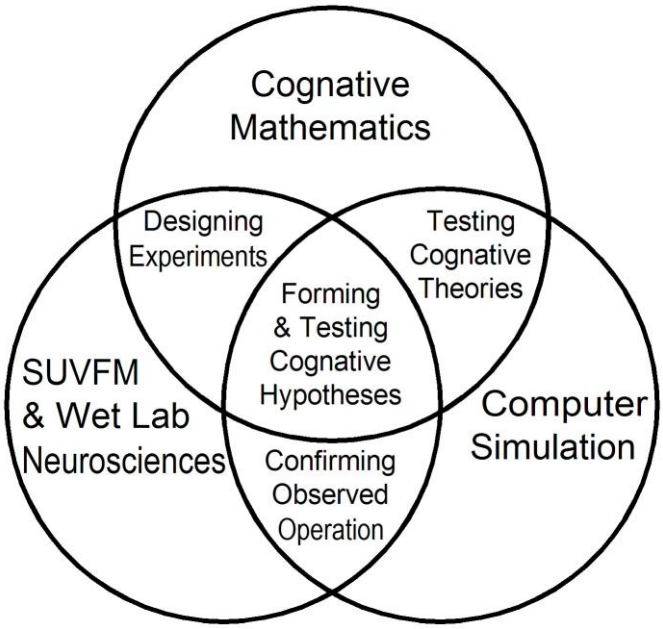

Fig. 2: Future Technology Triad

Initially, the SUVFM will simply do its part in this triad, and benefit from other areas doing their respective parts.

Ideally, wet lab research, cognitive mathematics, and computer simulation will all work together to get each other over the hard spots.

15 Other Advanced Methods

There is a rapidly evolving assortment of advanced methods not considered in this proposal. These have been omitted here both for brevity, and because they may not be needed to diagram things as "simple" as computational functionality. However, they remain "on the shelf" as engineering margin,

to "save the day" should unexpected problems emerge. The four front-running advanced methods are:

1. Instead of using UV, excite with low levels of photons that individually are insufficiently energetic to cause fluorescence. This results in a quadratic response to intensity that decreases the response away from the focal point. Two-photon Microscopy (TPM) has already been applied to study the neuron structure and location in intact brain slices, the role of calcium signaling in dendritic spine functions, neuronal plasticity and the associating cellular morphological changes, and hemodynamics in rat neocortex.
2. Identifying the precise locations of isolated fluorescent molecules (fluorophores) by precisely determining the centers of their diffraction-limited images. This can be done with ~0.002μ precision.
3. In addition to using near-ultraviolet as detailed in this proposal, far-ultraviolet could analyze the exposed surface in greater resolution, but without the ability to analyze it in sufficient depth for diagramming. This would provide some ability to analyze unfamiliar structures, albeit only at the random locations where why were sliced.
4. Instead of cutting off separate slices and discarding them, an alternative method is to turn the brain into a long tape of recovered sections, so that in effect the SUVFM becomes a just new type of tape drive[7]. While this runs the great risk of destroying a slice and thereby losing the entire diagram, it could nonetheless augment the methods presented here, so that a lost slice would only lose some additional detail in a region that has already been scanned.
5. Once brain operation is understood, multiple restarts on a simulator can be used to debug a diagram sufficiently for it to work, albeit suboptimaly due to errors in diagramming.
6. Once a brain diagram works suboptimaly, multiple restarts on a simulator can be used to allow the system to self-optimize.

16 High Risk?

Are we going to build this machine, turn it on, and immediately start diagramming brains? Of course not. The laboratory version of this machine will provide direct simultaneous observation of form and function. Once these relationships have been found and understood, programs can be written to identify and measure similar structures in brains and indicate their component values in the diagrams that are produced. Even when this machine has been constructed, useful diagrams will probably still be several years away.

Are we going to be able to fully characterize every component? Of course not. There is little need for perfection in component characterization, as once the functionality is well understood; most optimum component characteristics can be computed. Computed optimum component values are more valuable than imperfectly measured values for most uses.

After considerable research using the laboratory version, are we going to be able to fully diagram the brains of some small creatures that have large neurons? This seems like a safe bet. The first machines will find their limits somewhere in between the very easiest and the most difficult brains to diagram, and this limit will advance with each new generation of machine, hopefully to eventually include human brains.

This is a proposal for a process, rather than a proposal to build a specific model of equipment. As with everything from airplanes to computers, capabilities will dramatically grow with each new generation of equipment.

One thing seems certain: Even the very first machines will transform the biological sciences as they provide spectacular 3-D images with sub-micron resolution of the internal structures of living cells, displaying real-time views of cells' internal chemistry.

17 Conclusion

The SUVFM promises to transform neuroscience into an information technology, which is the fundamental criterion for applying Moore's Law and/or Ray Kurzweil's exponential growth curve to project future capabilities. Initial machines won't be capable of diagramming anything as big and complex as a human brain, and probably won't be able to provide all component values for any brains, but initial limitations will soon pass as this technology advances and computational substitution fills in for unreadable component values.

Try to imagine for a moment how different AI would now be, if for the last 20 years we were to have had substantially all of the answers to how human cognition works. Human-scale AI "research" would now be little more than deciding to turn it on.

Semiconductor manufacturers are now spending billions of dollars developing the fabrication facilities to produce better computers, as AI developers are investing millions of dollars, often in the form of their own "sweat equity", to develop better AI software. However, without closure of the technology triad that now appears to include building the SUVFM; this will all probably hit a "brick wall" before producing the hoped for singularity. The SUVFM is the only technology on the horizon to avoid that brick wall. The SUVFM can be built for only a few million dollars.

18 Distant Future

Eventually, in the distant future when cognition is sufficiently well understood so that AI experimentation can operate independently of mathematical developments and wet lab support, the SUVFM will take its place as the stepping stone to uploading and downloading, thereby allowing people's conscious minds to be transferred into perpetually maintainable computers.

This will work just like classical checkpoint/restart computing methods. A computer program can be abruptly stopped in one computer, its state read out, transferred to a second and potentially differently constructed computer, and the program continued right where it left off in the first computer. Your brain is the first computer, your neural diagram contains both the program and most of the state, and a computer simulating your neural diagram becomes the second computer that restarts your consciousness where your body left off.

This method cannot perfectly capture the state, because some of it is held electrically and would be lost. The effect of this loss would probably be about the equivalent of an electro-shock therapy treatment, probably resulting in the loss of several hours of memory.

Everlasting life on a mass production scale, implemented upon death via diagramming followed by uploading (into a virtual reality computer) or downloading (into an android body) for simulation, is probably worth more than the present value of the earth, because people would gladly mortgage their futures in order to have futures[18]. Regardless of whatever else develops on earth, a nation that makes this work on a large scale will eventually own everything, making this the world's most valuable technology and substantially more valuable than oil.

This line of economic thinking has already been real-world tested – in ancient Egypt. There, they sought resurrection by building gigantic pyramids. Despite being an apparent technical failure, this project drove their economy to propel their civilization to greatness.

Once this has become a reality, it will be a simple matter to run the simulating computers far faster than real-time speeds, and simulate far more than the original three pounds of brain, to eventually achieve limitless intellects. This will achieve the long-predicted "singularity", albeit by different means than has been widely predicted. Further, this approach deftly avoids most of the potential civilization-ending scenarios of past AGI (Artificial General Intelligence) based proposals because at its core, it will still be human.

With such a potentially valuable future, even modest advances toward that goal assume billion-dollar values that far exceed their cost by orders of magnitude.

This could easily ignite a technology race, akin to the race between the U.S. and the U.S.S.R. to develop thermonuclear weapons or space travel, where it is more important not to be left out than to be first.

This may be society's last chance to reclaim its future from the robber barons, by publicly funding, developing, owning, and controlling this technology.

There are substantial technical risks to achieving the goal of fully diagramming human brains, but those risks are tiny compared to the existential economic risks of letting another nation capture this technology. However, there seems to be little risk that the SUVFM would fail to revolutionize the biological sciences, regardless of any unforeseen technical limitations.

19 Special Thanks

This proposal would not have been possible without the considerable contributions of William Calvin, Michael Everest, Kathryn Graubard, Marvin Minsky, Les Westrum and other industry notables, hopefully soon to include you, for identifying weaknesses and/or suggesting improvements.

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