Saving Oneself

PROJECT DESIGN CHOICES

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December 1, 2015

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Cutting Technology

Overview

- **Inital Sectioning**
  - Necessary to cut down to size which final section methods can deal with, without causing significant ultrastructure damage.
- **Final Sectioning**
  - Sections must be thin enough to achieve sufficient Z-axis resolution.
  - Must have a practical sectioning speed.

Initial Sectioning

Options Considered for Initial Sectioning

- Tissue Choppers
    - Significant tissue damage.
- Vibratome
    - Significantly better tissue preservation.
    - No commercially avaliable device capable of required sample size.

Figure 1:

Tissue Chopper [4]

Vibratome

(Very) Approximate Time to Process

- $\sim 0.5 mms^(- 1)$ cutting rate.
- $\sim 0.5\ mms^- 1$ cutting rate.
- Aproximately 3 months.

(Very) Approximate Cost

- £$\sim$ 20,000 per machine

Final Sectioning

Options Considered for Final Sectioning

- Automatic Tape-collecting UltraMicrotome (ATUM) [13]
    - $30nm$ thick sections
    - $25mm^2$ section area
    - 1000 sections per day possible
- Focused Ion Beam (FIB) [16]
    - $15nm$ thick sections
    - $900\mu m^2$ section area
    - 75 seconds per section
    - $900\mu m^2$ section area
    - 4 minutes per section
- Laser
    - Focal point too large to achieve acceptable tissue loss between sections.

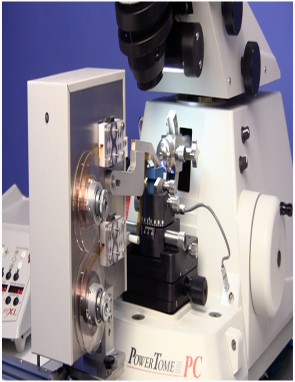

Figure 2:

ATUMtome machine [13]

Aproximate Processing Time

Method	Approximate Processing Time
ATUM	6000 years[13]
FIB	100 million years[16]

ATUM Aproximate Costing

- Commercially avaliable ATUMtome avaliable, estimate cost at £50,000 per machine.
- To achieve acceptable timescale at least 1000 ATUMtomes will be required, costing a total £50 million.
- Estimated that a single technican should be able to supervise about 50 machines.
- Therefore, at least 20 fulltime technicans required.
- Annual salary of £35,000, resulting in a wage cost of £4.2 million over 6 years

Recording Technology

Overview

Options Considered for Imaging

- MRI and fMRI
- LM/Fluorescent LM/Cubic
- MRFM
- Super FM
- TEM
- SEM

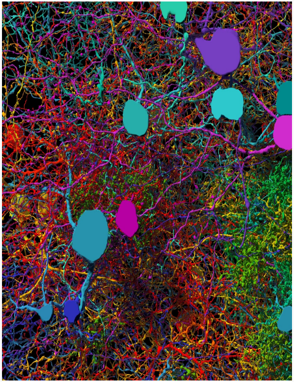

Figure 3:

Cell Carpet: formed from SEM Images via a game called Eyewire [6]

Resolution

- Algorithms can identify vesicles in order to locate synapses
- Therefore a resolution of around 10nm or better will be necessary

Method	Approximate Resolution
Magnetic Resonance Imaging	300nm [19]
Light Microscopy	250nm [20]
Fluorescent Light Microscopy	250nm [20]
Magnetic Resonance Force Microscopy	5nm [9]
Super Fluorescent Microscopy	20nm [20]
Transmission Electron Microscopy	1nm [16]
Scanning Electron Microscopy	2nm [16]

Other Issues

- MRFM
  - Time for scanning just 50x50nm's is 32 hours [9]
- Super FM
  - The process of getting fluorophores to mark specific locations is time consuming, labour intensive and difficult [17]
- TEM
  - The loading of samples onto TEM slides is very difficult and can lead to distortion

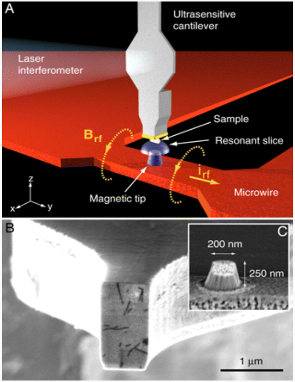

Figure 4:

MRFM tip used on a virus[9]

Decision

Scanning Electron Microscopy

✓ Good Resolution

✓ Easy to automate

✓ Works with ATUM

× Still quite slow

Which SEM

- An appropriate SEM microscope could be the Zeiss Merlin FEG
  - Works with WaferMapper and proven in use by Jeff Lichtman, Costing around $1 million
- Also available is the Zeiss MultiSEM 505/506, 61/91 Beam SEM
  - Costing around $2 million and $3 million respectively, with a resolution of 4nm and 8nm

All costings based on [21]: The cost for the single beam being $595,000 2nd hand

Time

We may wish to store the brain for as long as possible, but will the customer be convinced we'll keep the job going, even after a thousand years?

- An area of $1mm^2$ would require around 2.5 hours to image with a Zeiss Merlin SEM at a resolution of 4nm
- The Human Brain volume is approximately $1400cm^3$ [22]
- Scanning sections of 20nm at a time would give $2x10^{-8}cm^3$ sections every 2.5 hours
- Need $7x10^{10}$ sections so $1.75x10^{11}$ hours or $2x10^7$ years

Solution

- Parallelisation
  - Using multiple microscopes
- MultiBeam SEM
  - Our other two microscope options

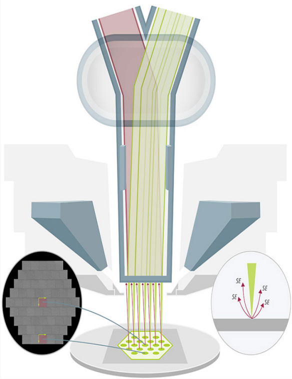

Figure 5:

How Multi Beam SEM Works[10]

All Imaging Times

- 61 Beam: 6.5 minutes for 1mm x 1mm with 4nm resolution
- 91 Beam: 3 minutes for 1mm x 1mm with 8nm resolution

Microscope	Full Brain Scan Time(years)	Cost (Millions$)
Zeiss Merlin	$2x10^7$	1
Zeiss MultiSEM 505	800 000	2
Zeiss MultiSEM 506	400 000	3

[10]

Recording Conclusion

- Chosen Microscope Zeiss MultiSEM 506 (91 Beam)
    - Will need 1000
- Time till completion minimum 400 years, Total cost of $3 Billion
- Bonus - Zeiss software support

Chemical Considerations

Fixation

ASC - Aldehyde-Stabilized Cryopreservation

Breakdown of the cost for Aldehyde-Stabilized Cryopreservation:

- Perfusion Machine £300 [12]
- Chemical Costs £1,500 [4] [2]
- Labour Costs £10,000

    Surgeon £6,000 [3]

    Assistant £4,000

Total £11,800

Total time taken is one day

Staining

Staining	Contrast	Uniformity	Preservation	**Total**
OTO	4	2	1	**7**
rOTO	4	3	2	**9**
BROPA	5	4	4	**13**

From the above table BROPA is clearly the best method for staining

BROPA

Breakdown of the cost for BROPA:

- Labour cost £3000
- Chemical £1000

Total cost £4000

Embedding

Options for embedding:

- Epoxy Resin
- Acrylic Resin [1]

Data and Software challenges

Data organization

We need a standardised model for storing our data in a meaningful way. The two solutions used in the past are:

- NeuroML - XML-based markup language storing metadata, membrane electrical properties and **anatomical structure and synaptic connectivity of neurons.** [11]
- CAJAL3D with RAMON - Reusable Annotation Markup for Open coNnectomes - **lightweight** framework containing a set of minimum annotation data necessary for capturing the biological information. [15]

Data organization - design choice

CAJAL3D

representation of a neuron in RAMON:

```
       segments: []
             id: []
     confidence: 1
dynamicMetadata: [0x1 containers.Map]
         status: [1x1 eRAMONAnnoStatus]
         author: 'unspecified'
```

Data storage

Image storage - requirements

Around **2 million petabytes** for one human brain [**?**]

Data access - empirical measurements

Best performance of 19MB/s for annotated data

$$\frac{2 \times 10^{15}}{19 \times 10^{6}} = 105 \times 10^{6} \text{seconds} = 1218 \text{days} = 3.3 \text{years} \tag{1}$$

only to access the data!

Data storage - cost and design choice

Local

3TB SATA drive: £100

Total cost: £100 billion

Cloud

Amazon AWS S3 storage: $0.0275 per GB

Total cost: £**37 billion**

our choice - use the cloud for data storage

Data quering

WaferMapper (2014)

Raw image manipulation [13]

TrakEM2 (2012)

Raw images and reconstructed sections [7]

ConnectomeExplorer (2013)

Reconstructed sections [5]

Data quering - WaferMapper

Designed for ATUM

High throughput automated imaging

Image mapping and annotation

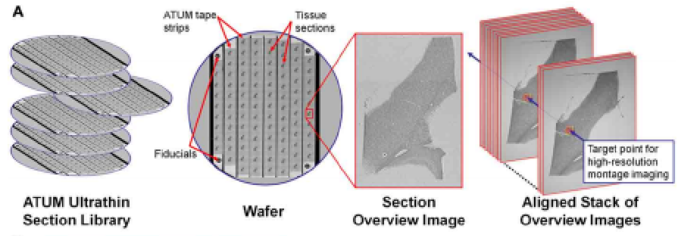

Figure 6: Key features

[13]

Data quering - TrakEM2

Rapid entry, organization and navigation through **EM images**
Manipulating, visualizing, reconstructing, annotating and measuring **neural components**

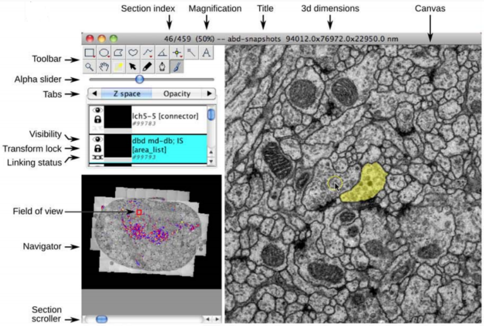

Figure 7: GUI

[7]

Data quering - ConnectomeExplorer

Statistical querying of the segmented data

Step by step **visual query builder**

Topological and spatial queries

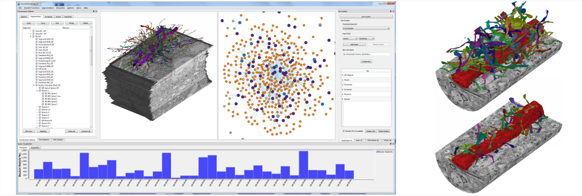

Figure 8: GUI and queried sections

[5]

Data quering - design choice

use the three in conjunction

...

at different stages of the project!

Manual segmentation

VAST

✓ Computer assisted manual segmentation

✓ 99% accuracy

✓ Used as the training data

$\times$ Not at all scalable - 2.8×10^{15} years for a human brain

[14]

Automatic segmentation

The need for speed!

(2008) 3.2×10^{10} years [18]

(2010) 4.3×10^{9} years [8]

... for (sort of) automatic reconstruction of a human brain

The need for accuracy!

Time bottleneck - proofreading

(2015) 87.6% of sections correctly segmented [14]

10x increase every 2 years $\Rightarrow$ manageable timescales in around 20 years

Automatic segmentation - design choice

RhoANA

✓ Maxout deep-learning **CNN**

✓ **Open source** - community collaboration

✓ Increasing **accuracy**

! **need to give back to the community** !

[14]

Labour costs

Even though small compared to equipment cost, labour salaries for the best data scientists, developers and scientists in the world isn't insignificant!

- Data scientists - £70,000/year x 10
- Biologists and chemists - £50,000/year x 5
- Lab technicians - £40,000/year x 10
- Management - £100,000/year x 4

Total: £**1,750,000/year**

Conclusions

Cost summary

Table 1: Saving oneself - money

Name	Price
Microscopes	£3 billion
ATUMtomes	£50 million
Servers	£37 billion
Labour	£1.75 million/year

BUT highest **Kickstarter** project raised $ 100,000,000... and we will make it scale!

Questions?

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- Ketamine.
- Surgeon cost.
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