Saving Oneself

Team One

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Chemical Preparation

- **Chemical Decomposition**
  - Decomposition of cells via autolysis and putrefaction causing significant damage to the sample
- **Physical Deformation**
  - Due to the untreated brain being not rigid enough, damage would occur if sectioned untreated
- **Poor Image Definition**
  - Due to the poor contrasting data, the images produced will not contain enough contrast to allow for synapse identification

Method Outline

1. Fixation and Cryopreservation
2. Post-fixation and Staining
3. Dehydration
4. Embedding

Method: [12]

1. Euthanasia
2. Bilateral Carotid Cannulation
3. Blood Washout Solution
4. Perfusion of Fixative
5. Perfusion of Cryoprotectants
6. Vitrification
7. Brain Extraction

Chemicals:

- 3% w/v Glutaraldehyde Solution
- 65% w/v Ethylene Glycol

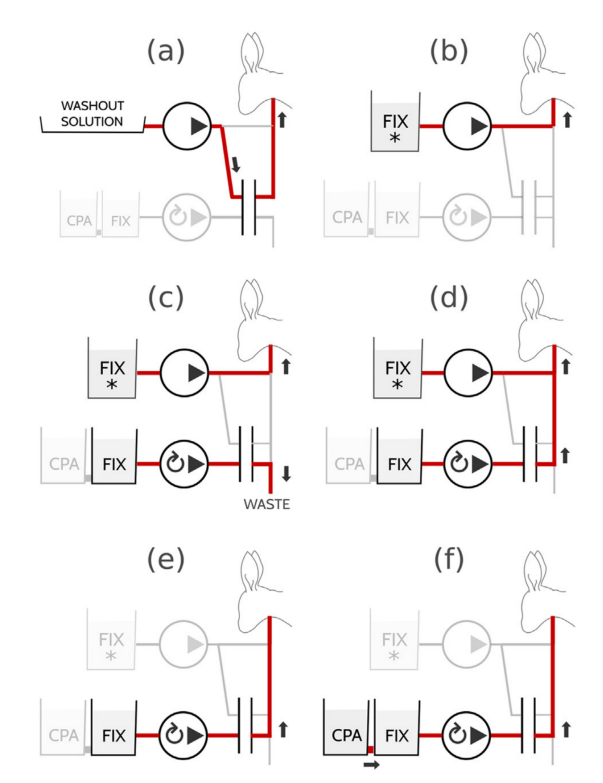


Figure 1:

Perfusion Transfer Method [12]

BROPA

Method: [13]

1. Initial Sectioning
2. Intermediate Sectioning
3. Final Sectioning
4. Removal of Cryoprotectants
5. Diffusion of BROPA Chemicals

Chemicals:

- Reduced Osmium and Formamide
- Osmium Tetroxide
- Pyrogallol

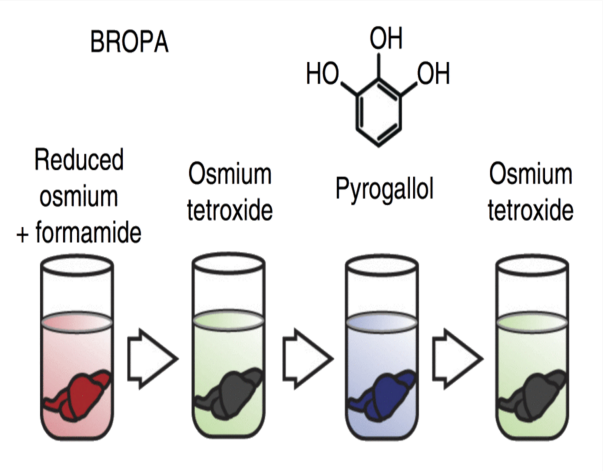


Figure 2:

BROPA Method [13]

Solvent Dehydration

Method: [8]

1. Rinse sectioned sample for 20 minutes in double distilled water
2. Transfer the sections of tissue into increasingly strong concentrations of ethanol
3. Finish with a second 100% ethanol solution for 1 hour

Chemicals:

- Ethanol

Unicryl

Method: [18]

1. Infiltrate with 100% resin for 2 hours while agitating gently on a shaker or rotating wheel
2. Infiltrate with fresh resin for 10 hours to allow full penetration
3. Heat sample at 60°C for 48 hours

Chemicals:

- UNICRYL

- By automating the chemical process, greater uniformity, efficiency and precision can be achieved
- By using 170 Lynx II for Microscopy machines, the process for chemically preparing the brain is completed in under a year
- Each Lynx II machines costs £13,750 [16] therefore the total cost for automation is £2,454,375.00 (this cost includes maintenance)

Method for Sectioning

- **Inital Sectioning**
  - Reduce size of sections from complete brain without causing significant ultrastructure damage
- **Intermediate Sectioning**
  - Further reduce size of sections to a size where final sectioning is possible
- **Final Sectioning**
  - Sections must be thin enough to achieve sufficient Z-axis resolution
  - Must have a practical sectioning speed

Precision Instruments VF-900 Compresstome

- Brain embedded in agarose
- Sliced coronally with thickness 0.5 mm
- Approximately 340 slices produced from a single brain

- Only one machine required
- Quoted at £32,000 [9]

Figure 3:

VF-900 Compresstome [17]

Custom Vibratome

- Coronal slices received from compresstome
- Slice divided up into blocks 3.5 mm x 2.5 mm x 0.5 mm
- Approximately 1400 blocks per slice
- Approximately 6.3hrs per slice

- Only one machine required
- Estimated cost £20,0000

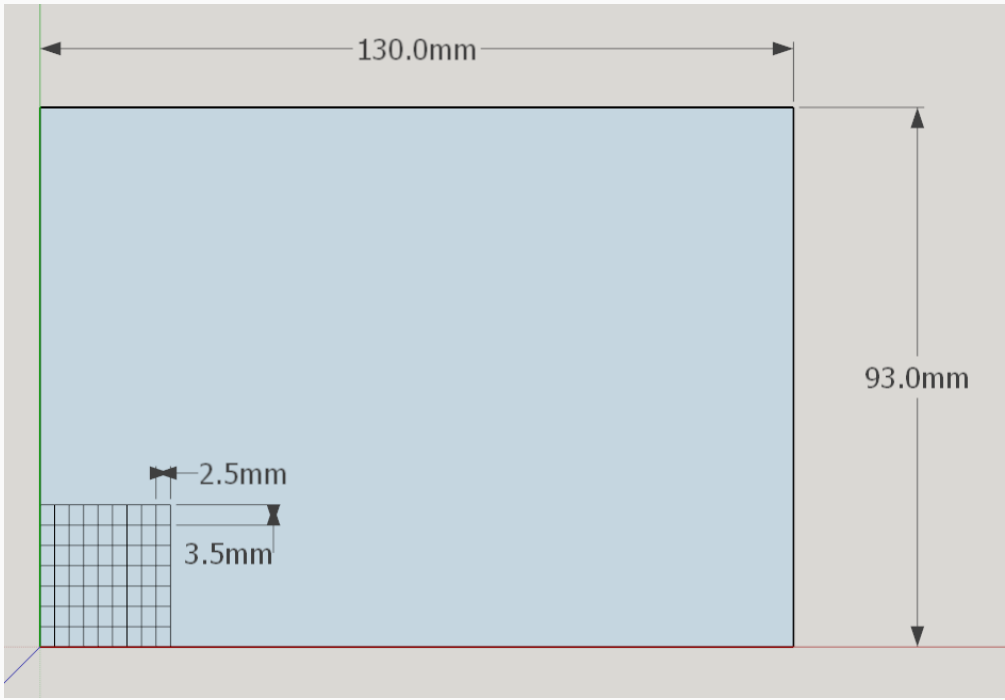


Figure 4:

Coronal brain slice modelled as a cuboid

Custom Vibratome

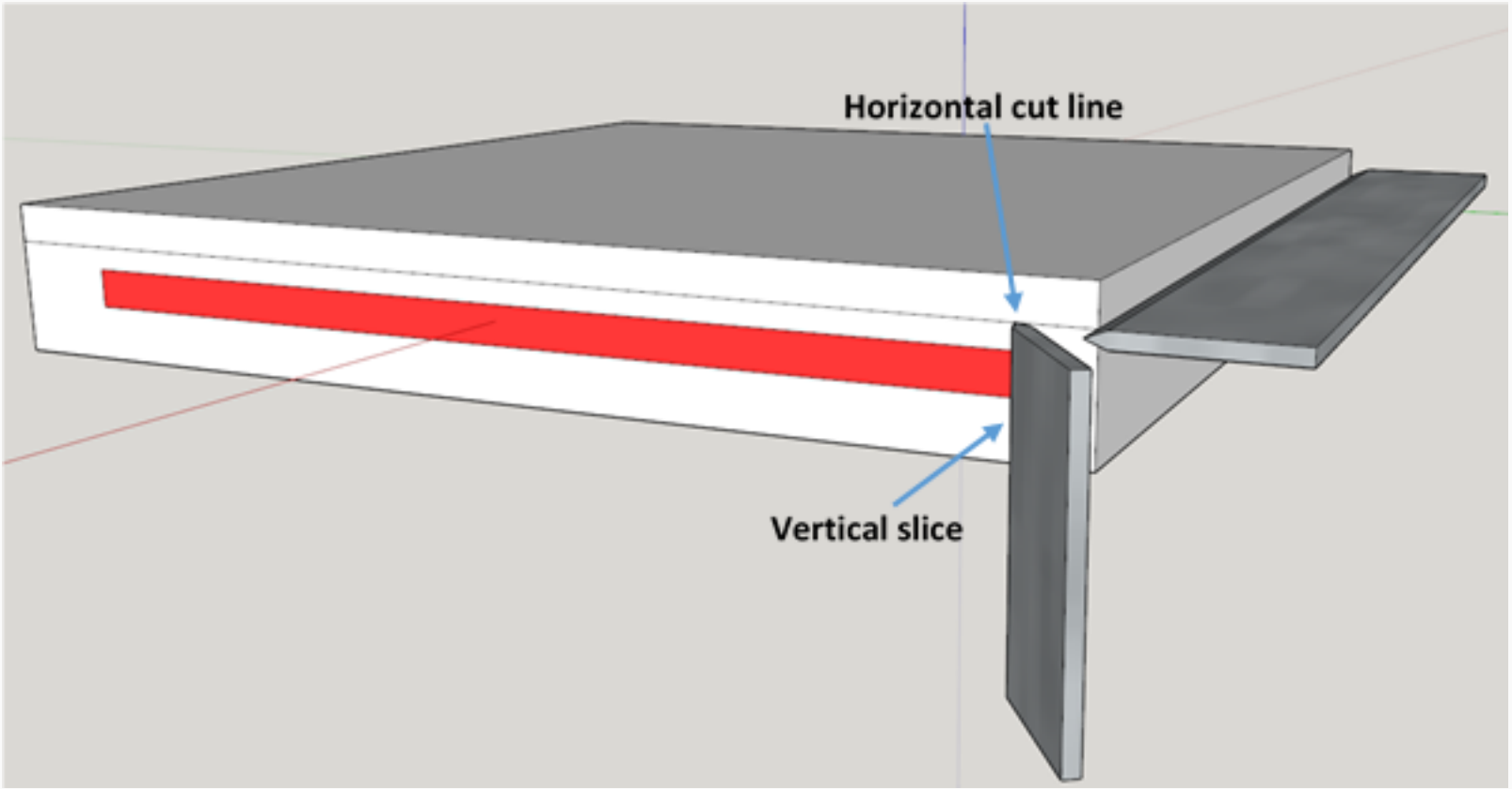


Figure 5:

Design concept of custom vibratome

RMC Boeckeler ATUMtome

- Developed in conjunction with the Lichtman Lab at Harvard
- Mount 3.5 mm x 2.5 mm x 0.5 mm blocks
- 30*nm* thick sections
- Up to 10,000 sections per day possible [7]

- One ATUMtome able to provide material for up to 40 microscopes
- 300 ATUMtomes are required, costing £50,000 each

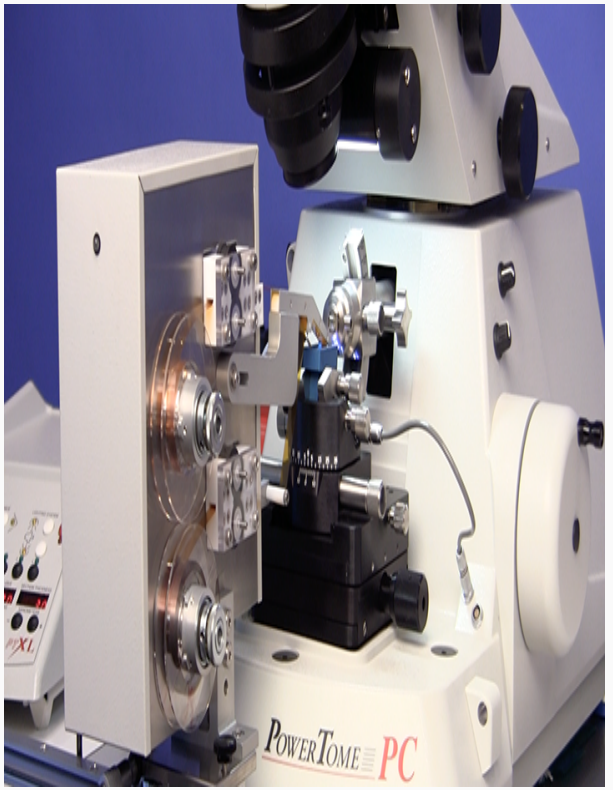


Figure 6:

ATUMtome machine [2]

Machine	Number	Total Cost
VF-900	1	£32,000
Custom Vibratome	1	£20,000
ATUMtome	300	£15,000,000
Total		£15,052,000

Recording Technology

Sectioning to Imaging Intermediary

- Wafers received from the ATUMtome systems
- Each holds $\approx 5\times 10^{-11}\text{m}^3$ of brain tissue [7]
- Distributed among 12,000 microscopes
- Require approximately 4×10^7 uses

- Will re-use around 120,000
- Cost per wafer $\sim$ £10 [19]

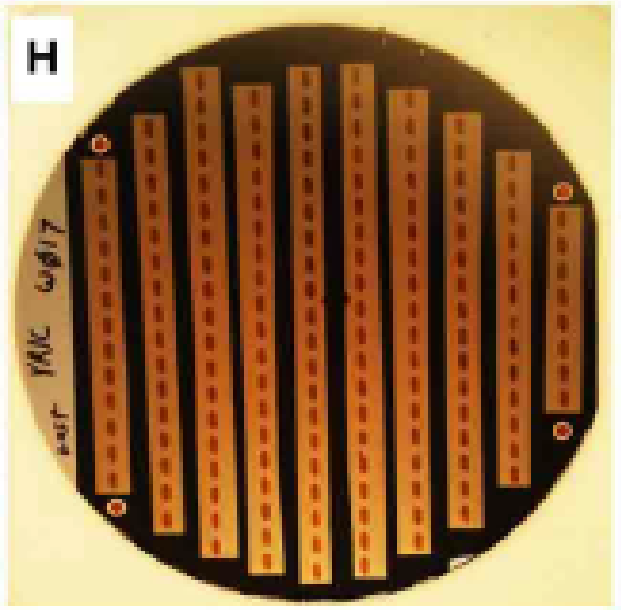


Figure 7:

A 100mm silicon wafer with 162 ultrathin sections.[7]

- **Transport**
  - Wafers enter onto a conveyor system which is the beginning of the imaging pipeline
- **Inital Imaging**
  - First the sections go through a very rapid light based imaging section (Around £20,000 per light microscope e.g. the ZEISS Axio Imager A2 Vario [20])
  - No delays are introduced as the whole process is occurring in parallel with the second stage
- **Loading**
  - The wafers are removed from the belt by a robotic arm
  - 12,000 robotic arms will be required, costing £10,000 per unit [15]

The MultiSEM 506

- Wafers are loaded into its vacuum chamber which automatically compresses
- Fiducial markers are identified via the ZEN software included[5]
- Automated imaging begins
- An area of approximately $2.55 \times 10^{-8}\text{m}^2$ is imaged in around 1.4s [21]

- Require around 12,000 machines for a 10 year imaging time
- Cost of £2.7 million per unit after speaking to Dr. Eberle at ZEISS

Figure 8:

The MultiSEM 506 [21]

Machine Costs

Machine	Number	Total Cost
Wafers	120,000	£1,200,000
Robo-Arm	12,000	£120,000,000
Light Microscope	12,000	£240,000,000
MultiSEM	12,000	£32,400,000,000
Total		£32,761,200,000

Data and Software Challenges

To decide on a standardisation model for storing our data two options were evaluated

- NeuroML - XML-based markup language storing metadata, membrane electrical properties and **anatomical structure and synaptic connectivity of neurons** [6]
- CAJAL3D with RAMON - Reusable Annotation Markup for Open coNnectomes - **lightweight** framework containing a set of minimum annotation data necessary for capturing the biological information [11]

Design choice - CALAJ3D with RAMON

↓

Sufficiently robust, widely used, highly compressible

WaferMapper (2014)

Image handling, mapping and alignment [7]

TrakEM2 (2012)

Computer-assisted identification and labelling of neurons [3]

ConnectomeExplorer (2013)

Global statistical querying [1]

Design choice - USE THE THREE IN CONJUNCTION

↓

Complimentary applications, open source

Image Storage - Requirements

Around **2M PB** (images) or **750 PB** (RAMON) for one human brain.

↓ Local Storage

- OCZ Vertex 4 SSD 512GB
- £100 × 1.5 million = £**150M**
- Simple and quick access

↓ Cloud Storage

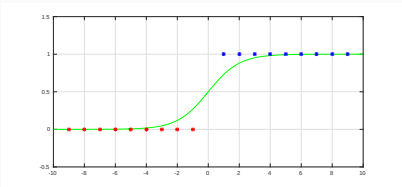
- Amazon Glacier (AWS)
- £0.005 / GB month × 10 years × 750PB = £**190M**
- Safe and gradually cheaper

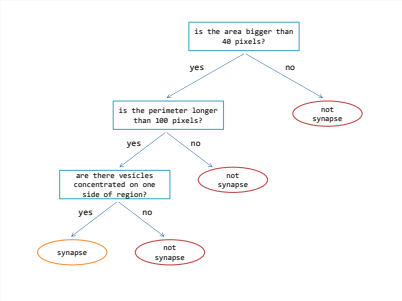
Use **Local Storage** - superior access speeds and price

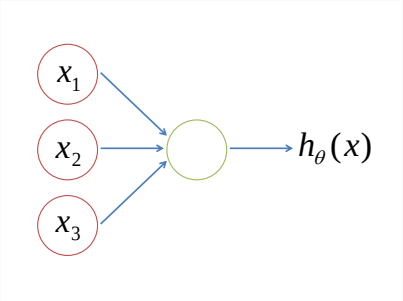
(1) - Use VAST (manual) to produce **training data** [10]

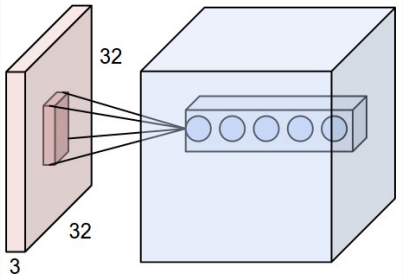
(2) - Use **machine learning** to segment automatically:

Logistic regression Random forests Neural networks Convolutional NN









After practical research - use RANDOM FORESTS

↓

Along with the hand-designed features

Pixel by pixel

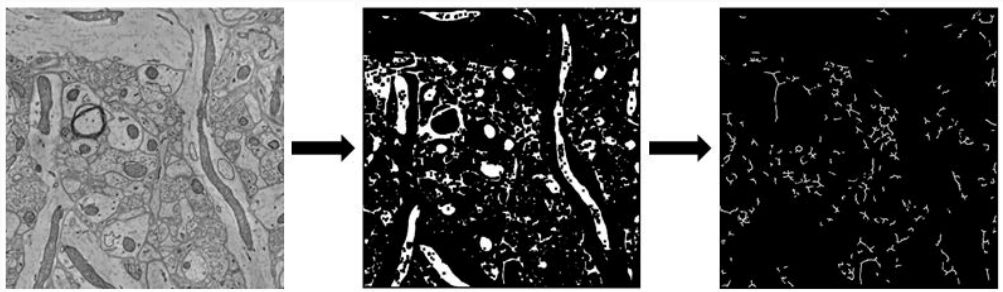
Colour profiles, Gray scale statistics, Vesicle number and Concentration of **regions around all pixels**

Regional

Preprocess the image to **generate false and true positives**
→ Extract **geometrical features** (area, perimeter, etc.) (bottom figure)

(... then run the classifier on the above features)

Regional Classification - most successful (12.5% accuracy)



The need for speed:

(2009) 3.2×10^{10} years [14]

(2010) 4.3×10^{9} years [4]

for automatic reconstruction of a human brain

10x increase every year $\Rightarrow$ **2 year segmentation in 2023**

Hardware $\rightarrow$ NVIDIA Tesla K80 with CUDA platform
Cost $\rightarrow$ 125,000 $\times$ £7000 = £875M

Conclusion

Process Summary

- First class amenities in Belgium
- Preliminary procedures for surgery and preservation
- Full brain cryo-storage
- Sectioning
- Imaging
- Synapse identification and Storage
- Future reanimation

Cost Summary

Pipeline	Approximate Total Cost
Chemical	£0.004 Billion
Sectioning	£0.098 Billion
Imaging	£33.031 Billion
Data	£1.705 Billion
Contingency/Misc	£5.000 Billion
Total	£39.840 Billion

- The cost for scanning the first brain is £5 Billion
- Will receive income from multiple interested sources, including human preservation societies, research grants and a secondary business of brain storage

Questions?

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