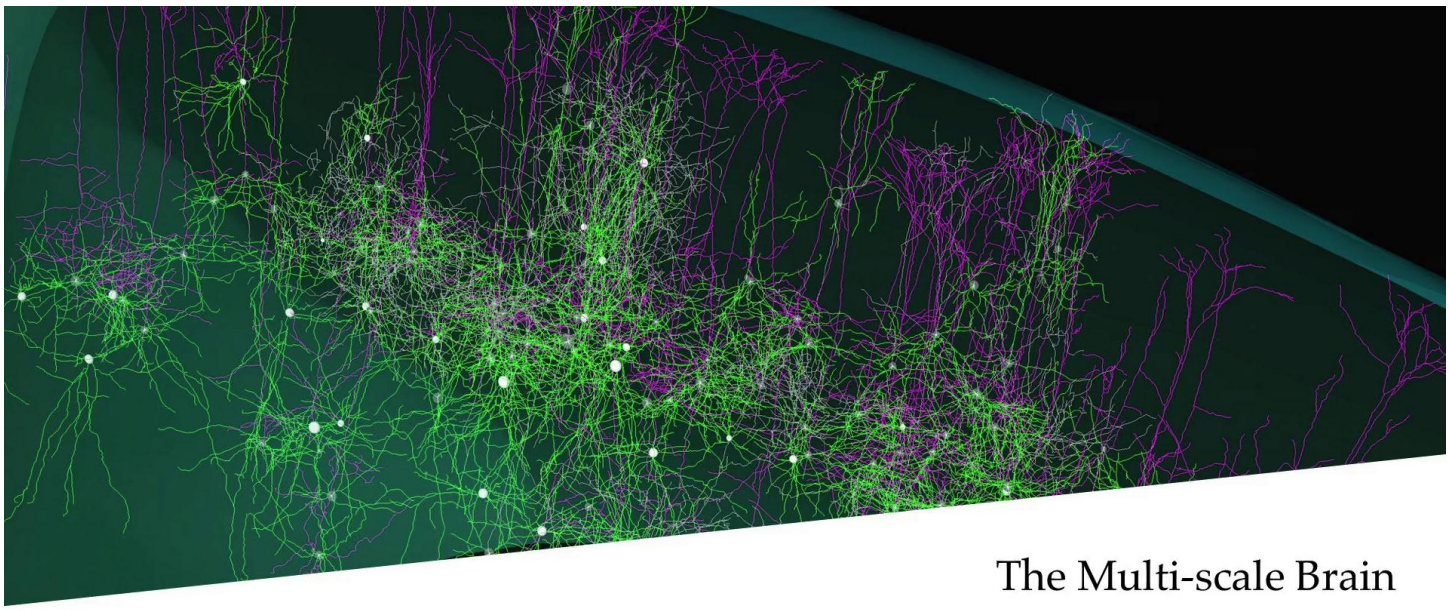
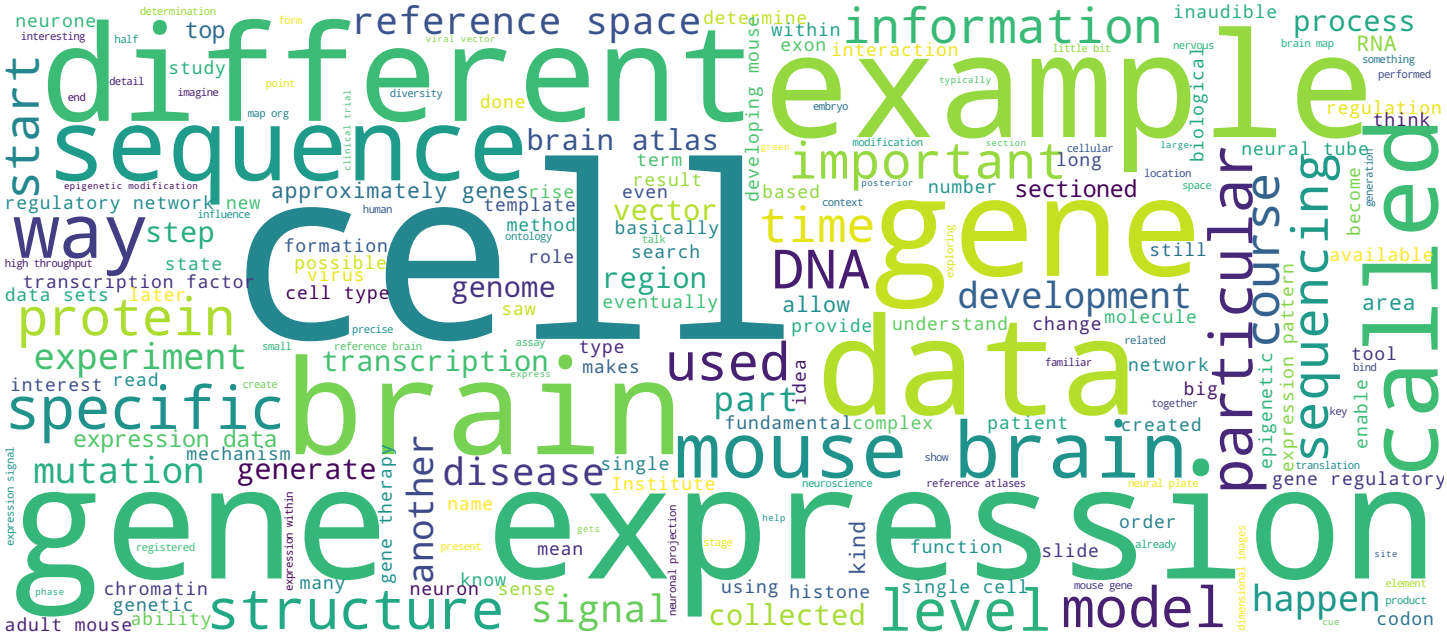


The Multi-scale Brain

Tutorial: The Allen Institute Mouse Brain data

Terri Gilbert, PhD

ALLEN INSTITUTE for BRAIN SCIENCE



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Video

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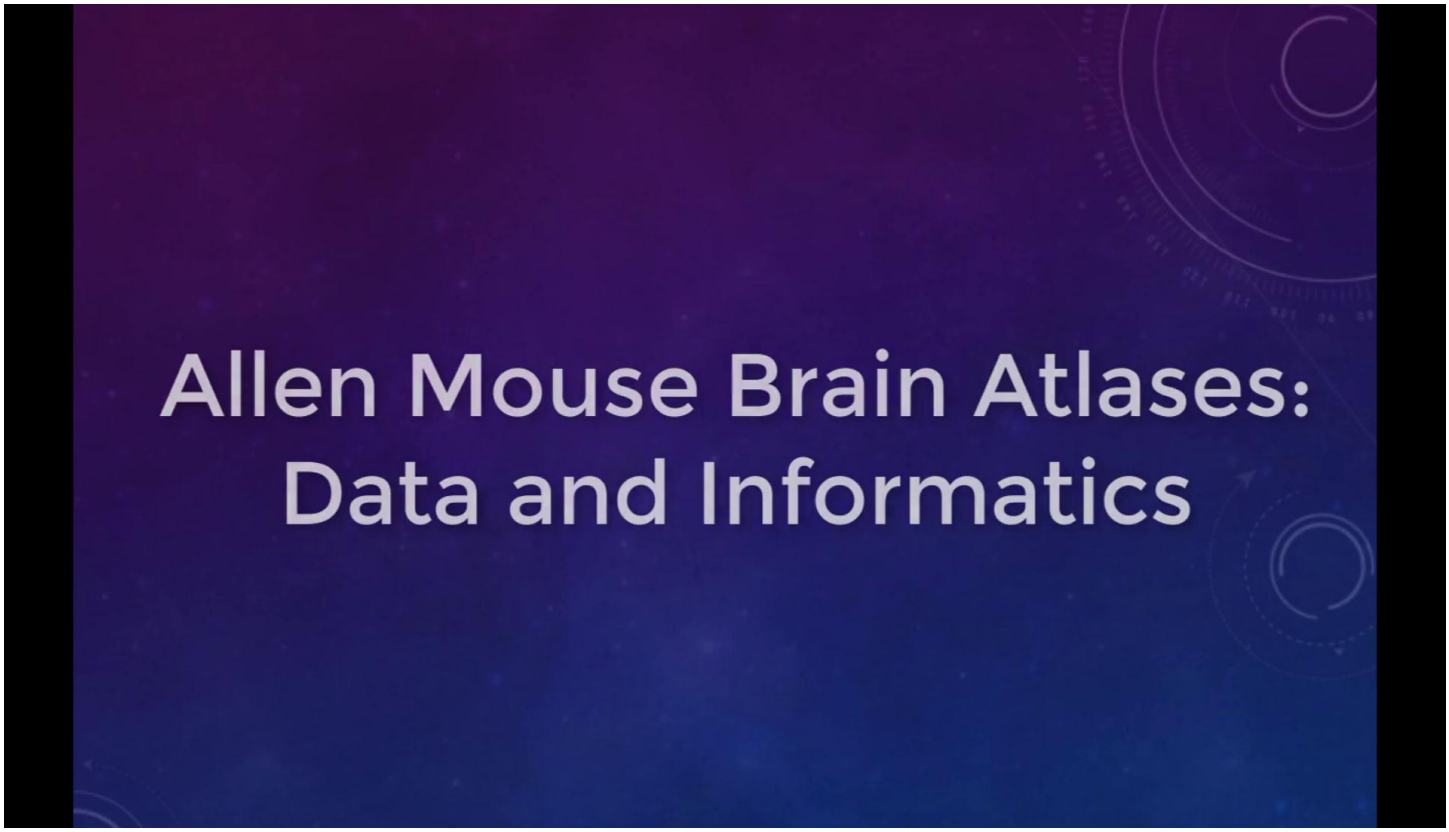
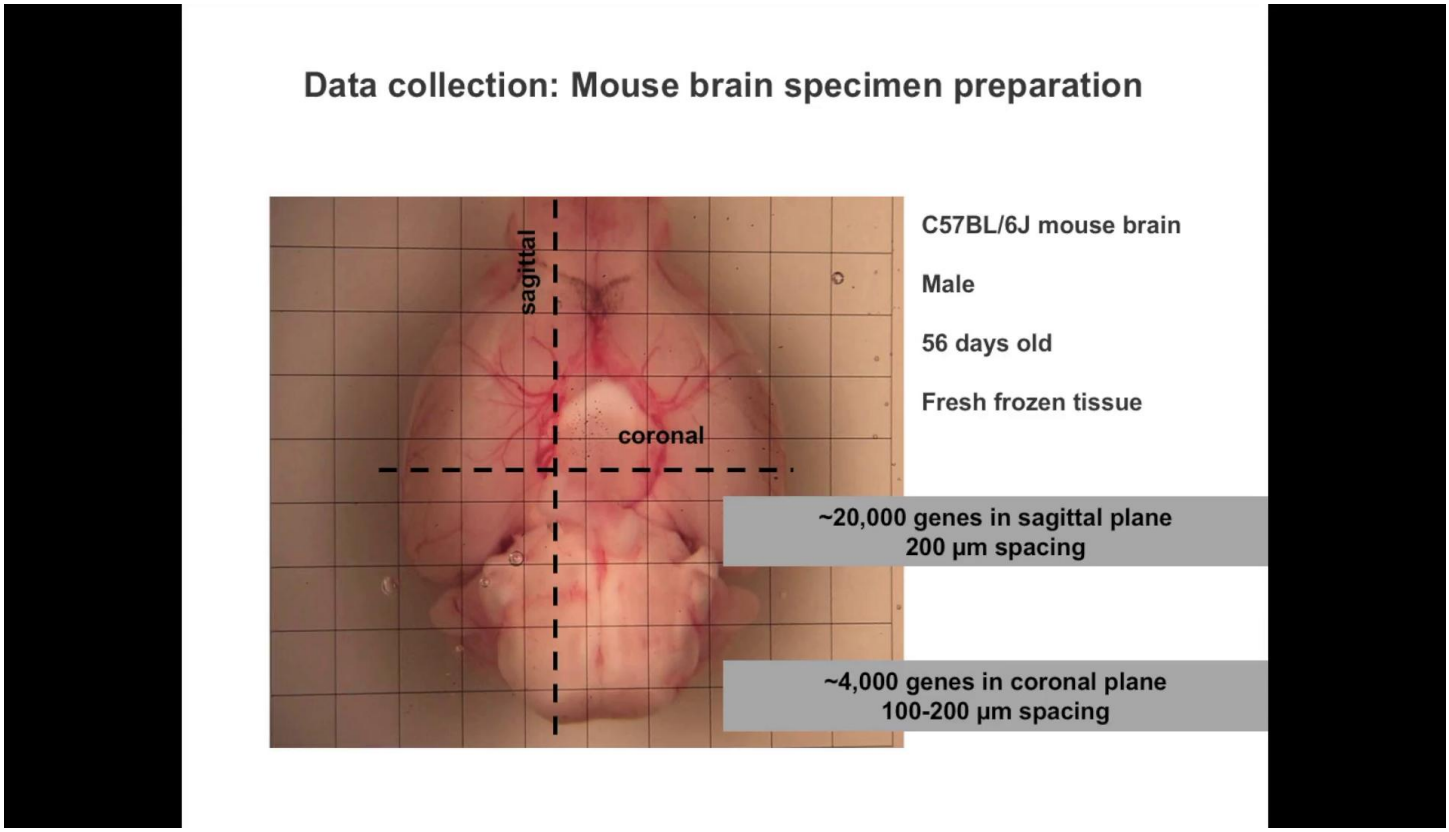


Understanding the kinds of data that were collected in the Allen Brain Atlas data sets will enable you to most effectively use the Allen Institute for brain science data discovery tools.

Notes

Summary

0m 05s

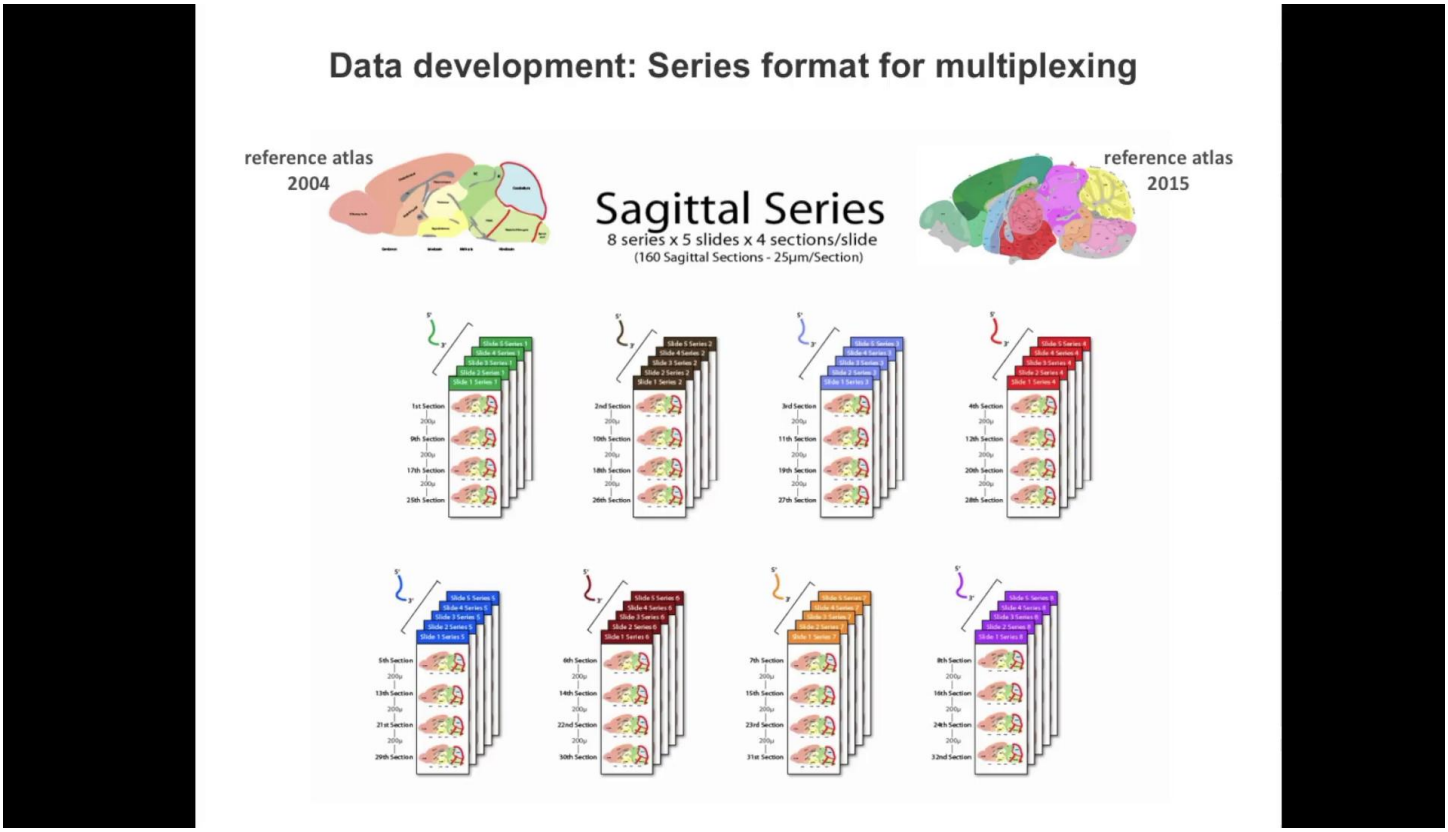


In this video, I will be describing how the data and the mouse gene expression data sets were collected, as well as give you a brief intro into the informatics tools overlaid on these data. For the mouse adult gene expression data set available from brain mouse.brain-map.org, the model system used was a male C57 Black6 mouse at postnatal day 56. Each mouse used in this study was housed and treated identically to ensure a baseline gene expression pattern. The initial survey looked at approximately 20,000 genes in the sagittal plane, starting from just past the midline through most of the brain. For a non-random set of approximately 4,000 genes, data were collected in the coronal plane, resulting in bilateral expression signal through almost the full extent of the brain.

Notes

Summary

0m 14s

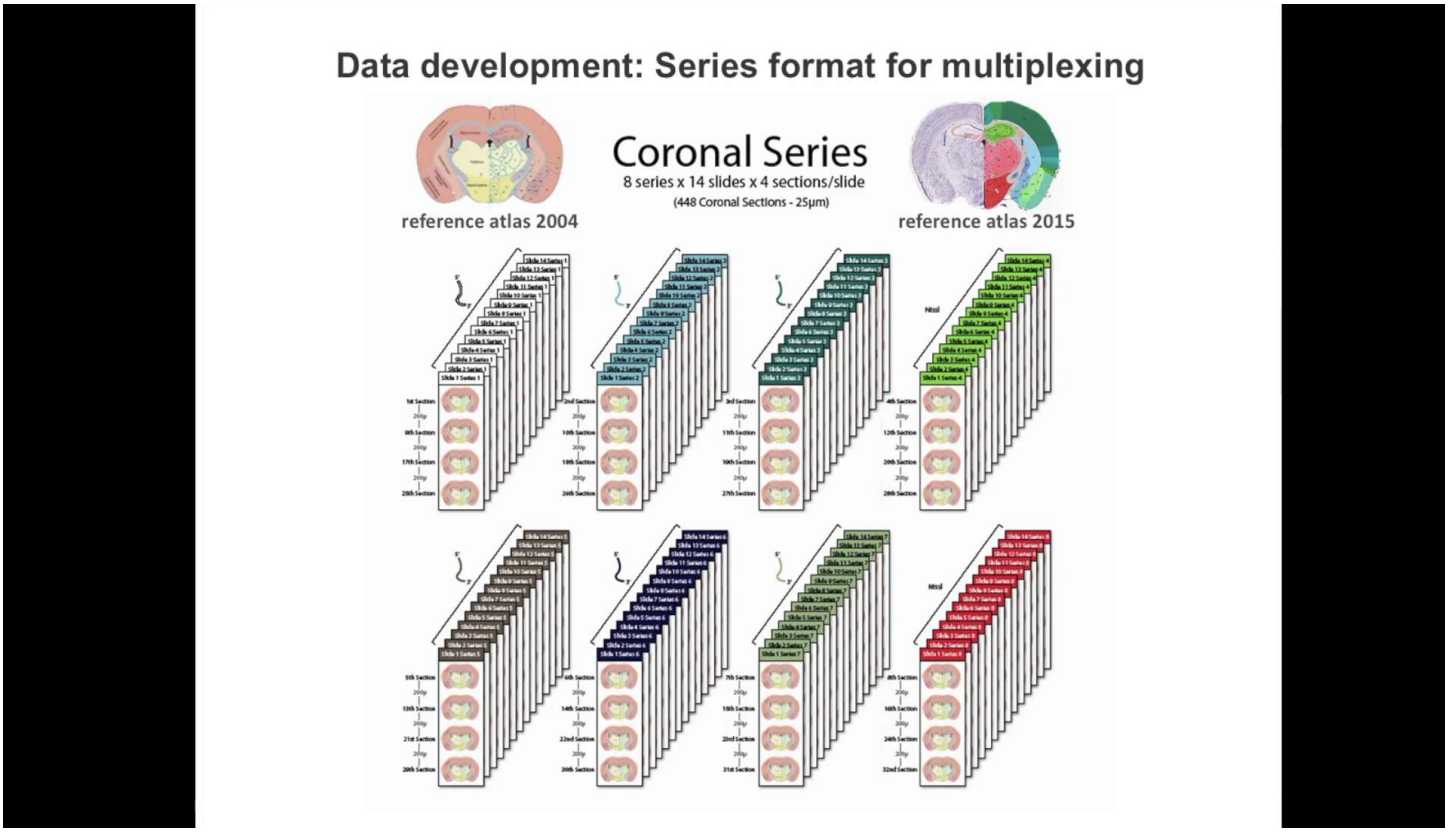


Each brain was sectioned at 25 microns as laid out in this diagram, with the first section, second section etc. which results in 200 microns spacing between each section within each experiment. Each brain was sectioned into eight experiments which means eight separate assays were performed on each brain. Therefore, in the sagittal sections each experiment contains approximately 20 images spanning about half of the brain.

Notes

Summary

1m 03s



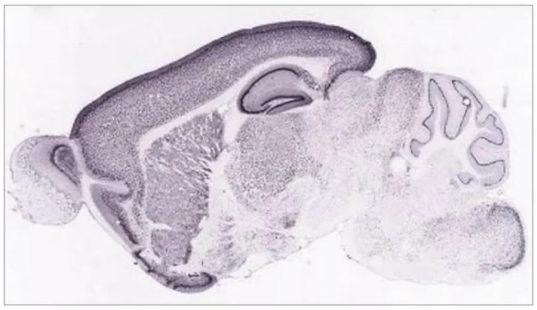
The brains used in the coronal experiments were sectioned in a similar fashion, resulting in 50 to 60 slides per experiment in this plane. There were eight experimental assays per brain, which includes probes for six genes and two reference data sets which were not sustained.

Notes

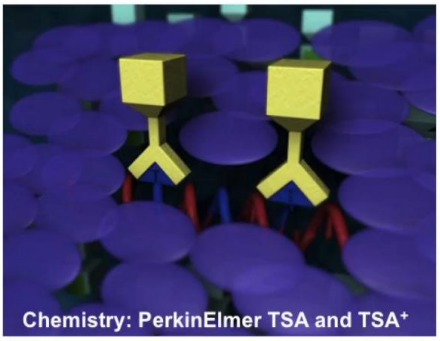
Summary

1m 29s

Data development: Survey of gene expression in mouse brain



Gene expression detection method:
Colorimetric, non-radioactive *in situ* hybridisation (ISH)



1. Fixed and acetylated tissue with mRNA
2. DIG-labeled riboprobe hybridizes
3. Anti-DIG antibody-peroxidase binds
4. Signal amplification (tyramide-biotin)
5. Detection with neutravidin-alkaline phosphatase
6. BCIP/NBT substrate visualized as purple precipitate

Gene expression was detected using a Colorimetric, in situ hybridization or ISH assay. In this assay a tagged gene specific probe was used to detect existing mRNA in each section. Antibody to the tag binds to any probe present in the cells and enables any detectable signal to be amplified. After a succession of amplification steps, the resulting sections were labelled with a purple precipitate, primarily in the cells that contain the gene being probed for.

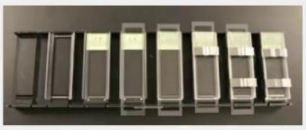
Notes

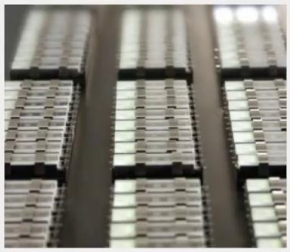
Summary

1m 45s

Data collection: Histology and mRNA detection at scale

Slide assembly





Scale for mouse brain atlas data production:

4,000 slides/week

16,000 brain sections/week

760 genes/week

Automated ISH

Tecan liquid-handling robots permit output for:

38 genes/ISH run

192 slides/ISH run

190 genes/day

Coverslipping

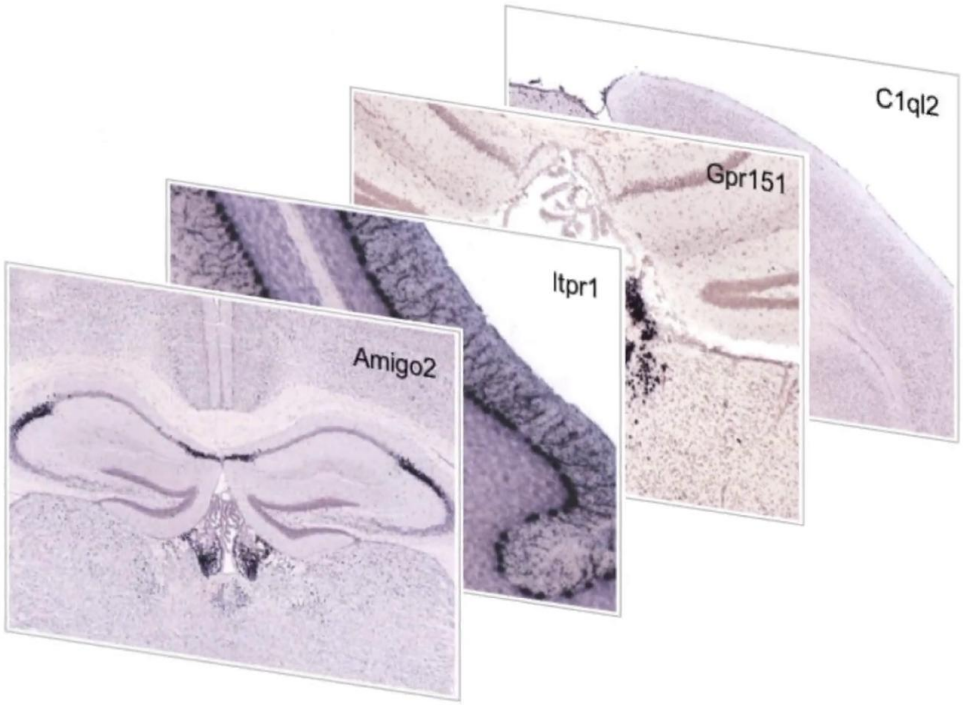
Leica coverslippers: 5 slides per minute

Each slide was assembled into a cassette, which created a reservoir for facilitating the automated ISH process created at the Allen Institute. The hybridization process was completely automated, allowing for a high throughput procedure that expedited the assaying of approximately 20,000 genes within three years. Even the cover slipping was automated to ensure reproducibility.

Notes

Summary

2m 15s

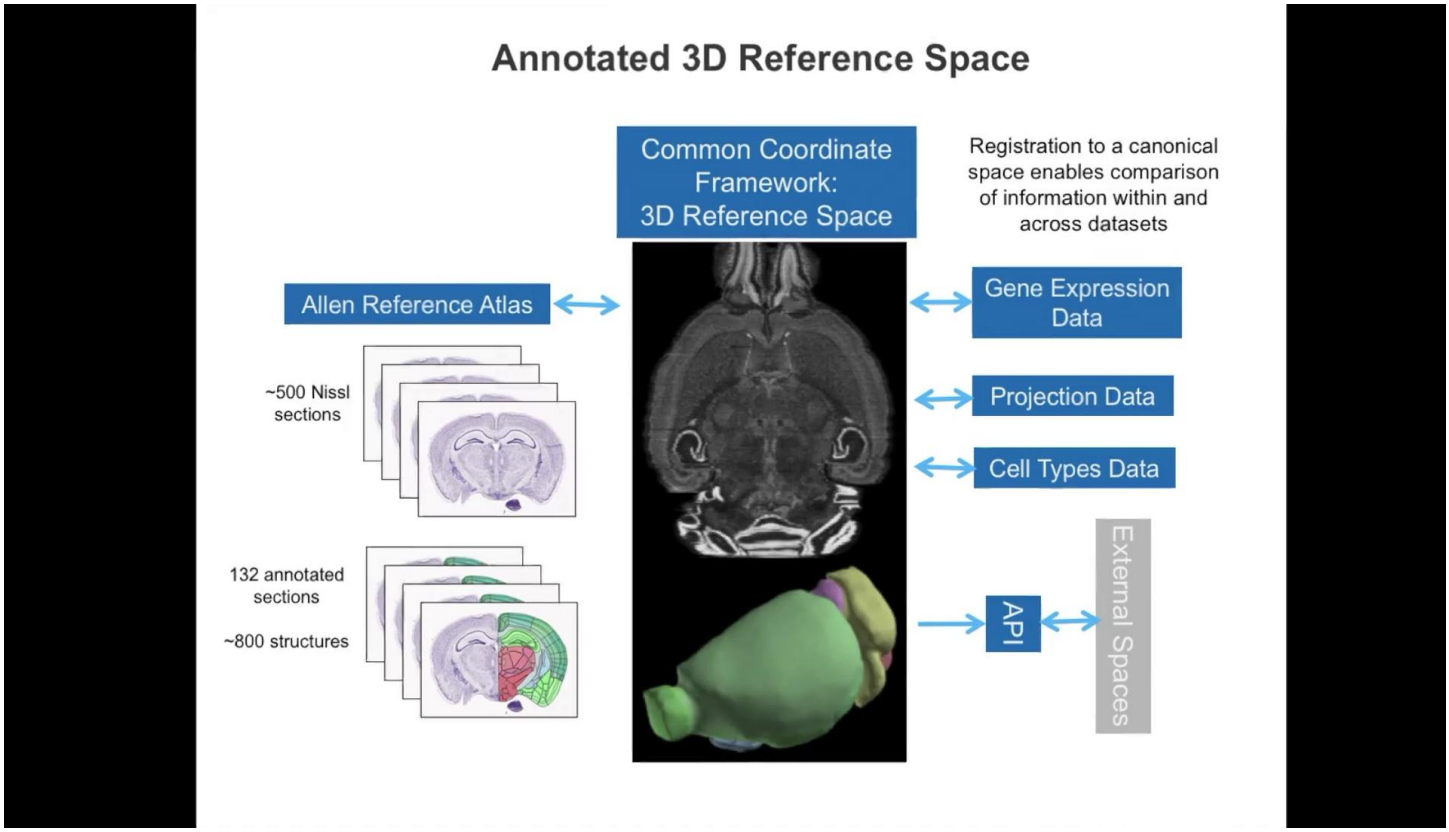


The result of this high-throughput methodology is vast numbers of two-dimensional images, that look like these select examples. Collecting these images in the precise and reproducible fashion is only half of what makes the tools in the brain atlas, so remarkable.

Notes

Summary

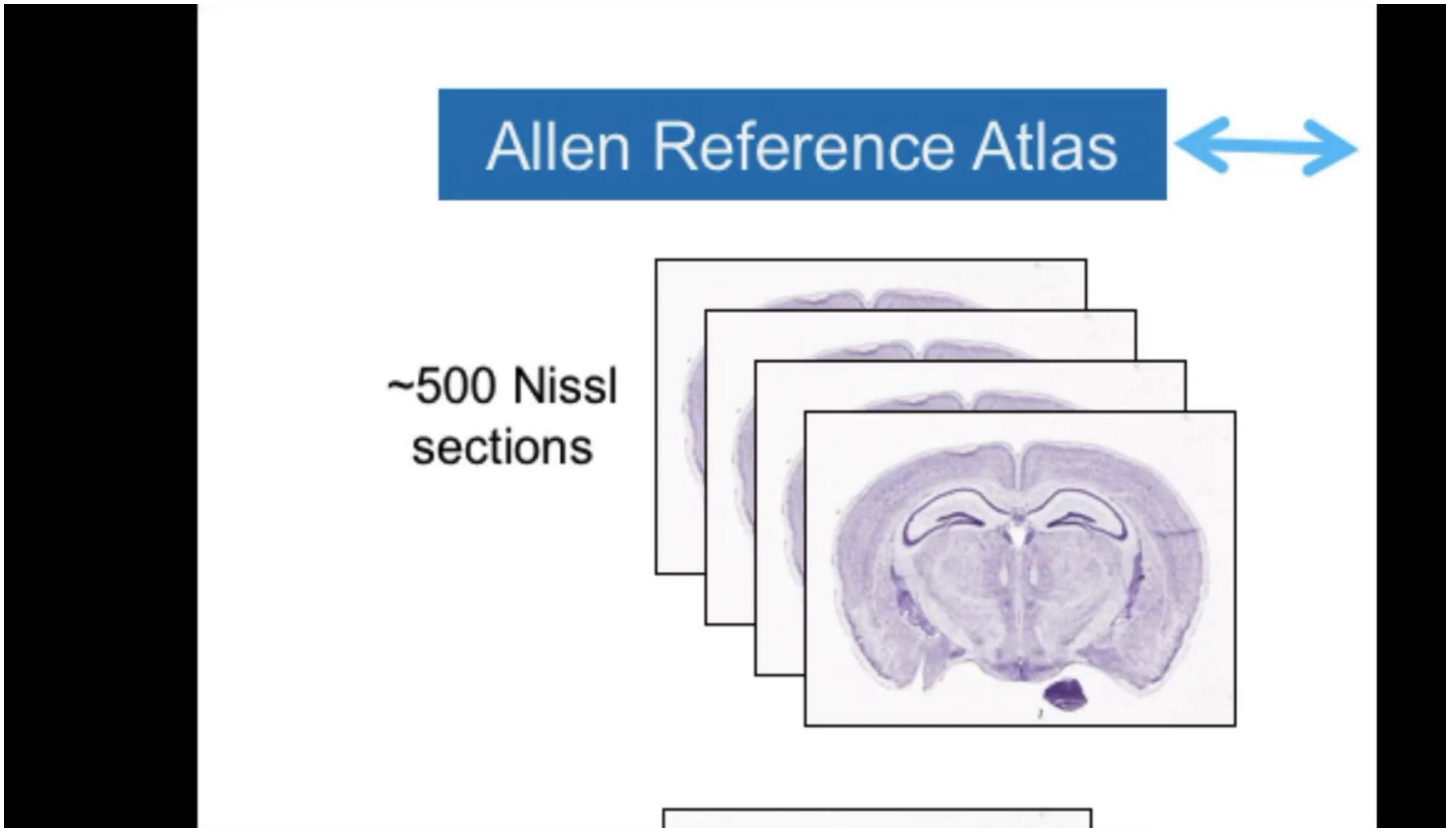
2m 38s



Notes

Summary

2m 53s

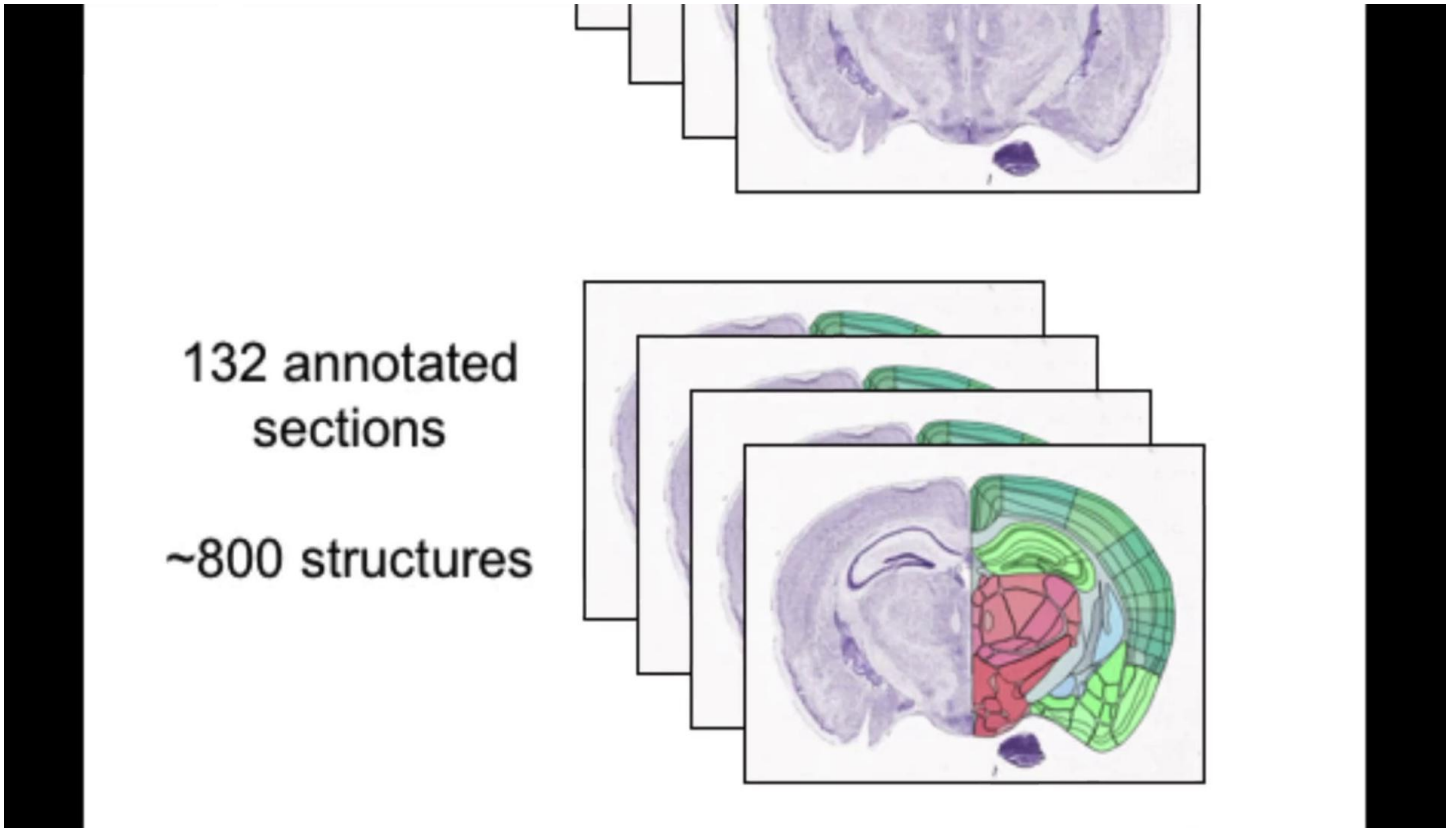


The Allen Institute created their first reference space from a single mouse brain, which was sectioned into about 525-micron sections.

Notes

Summary

2m 56s

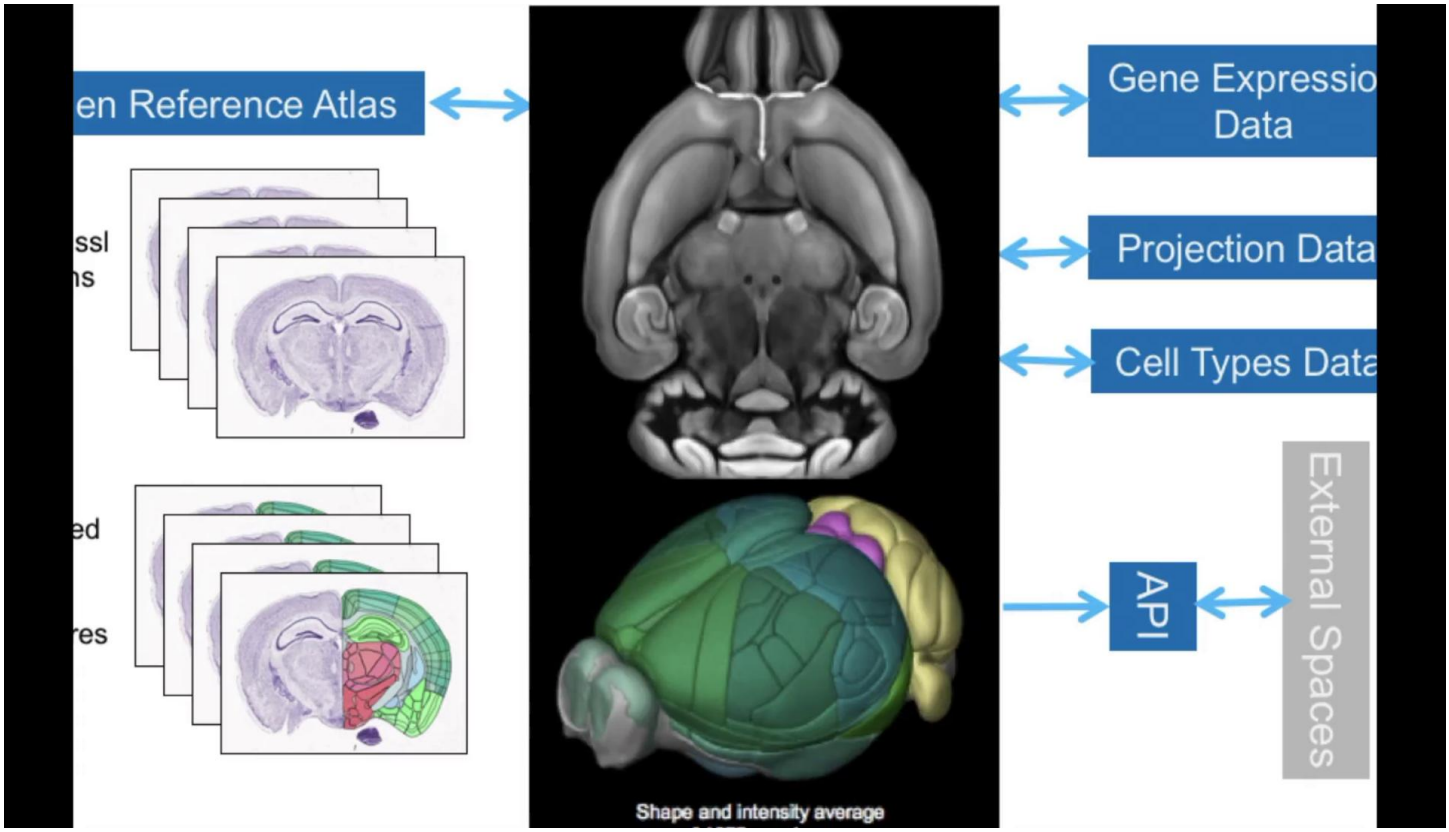


On every fourth section, anatomist drew structures on one hemisphere based on the Cytoarchitecture.

Notes

Summary

3m 03s

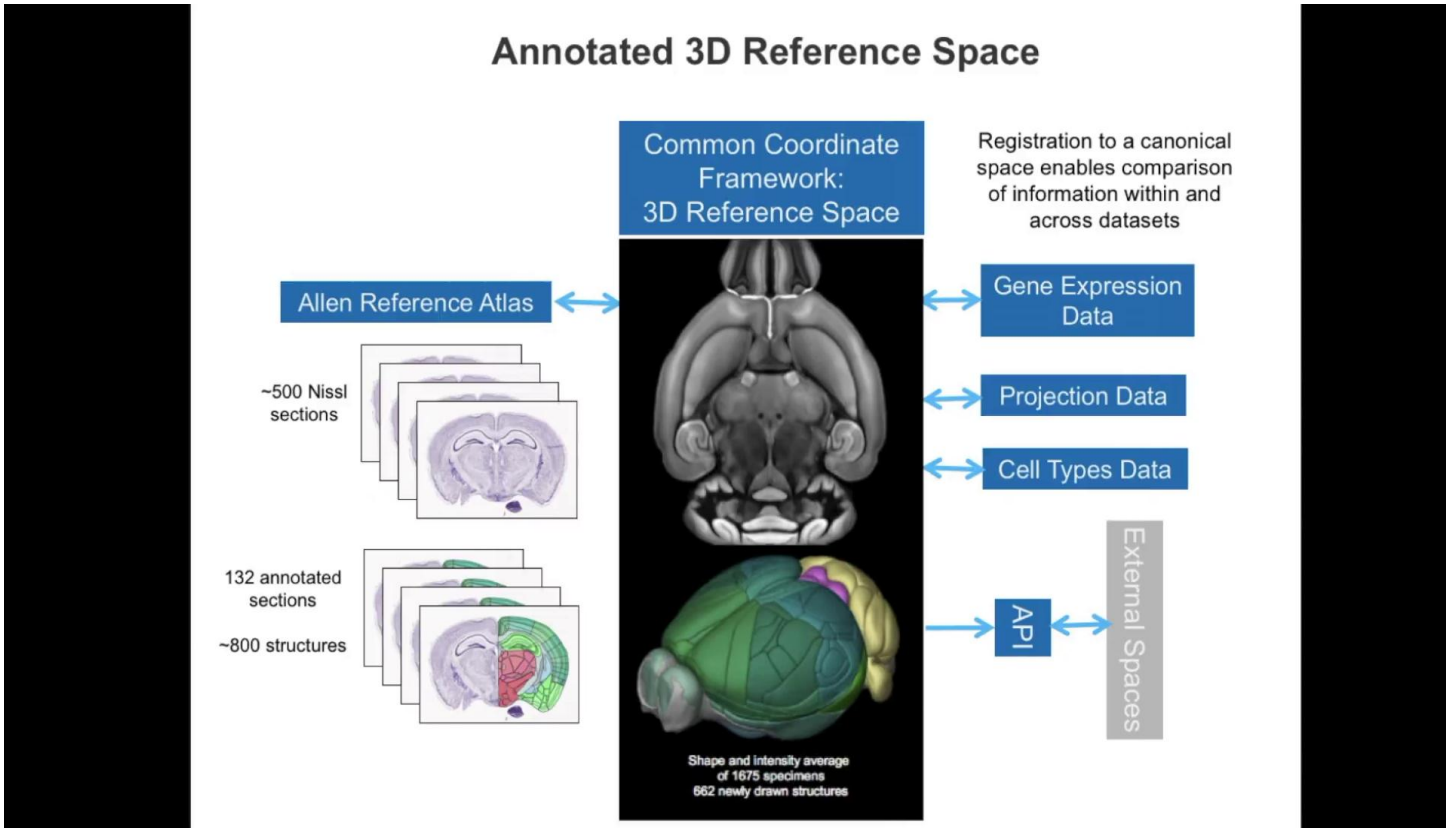


Those two dimensional images were warped into a three dimensional space. And in 2005 this static atlas was displayed alongside the gene expression images, allowing for the breakthroughs in Neuroanatomy and Gene expression to be married. In 2011 the reference atlas was digitally upgraded, more deeply annotated and made symmetrical so as to better locate gene expression with structure. With the advent of more precisely located data from the neuronal projection and cell typing projects, the reference space was again upgraded.

Notes

Summary

3m 10s

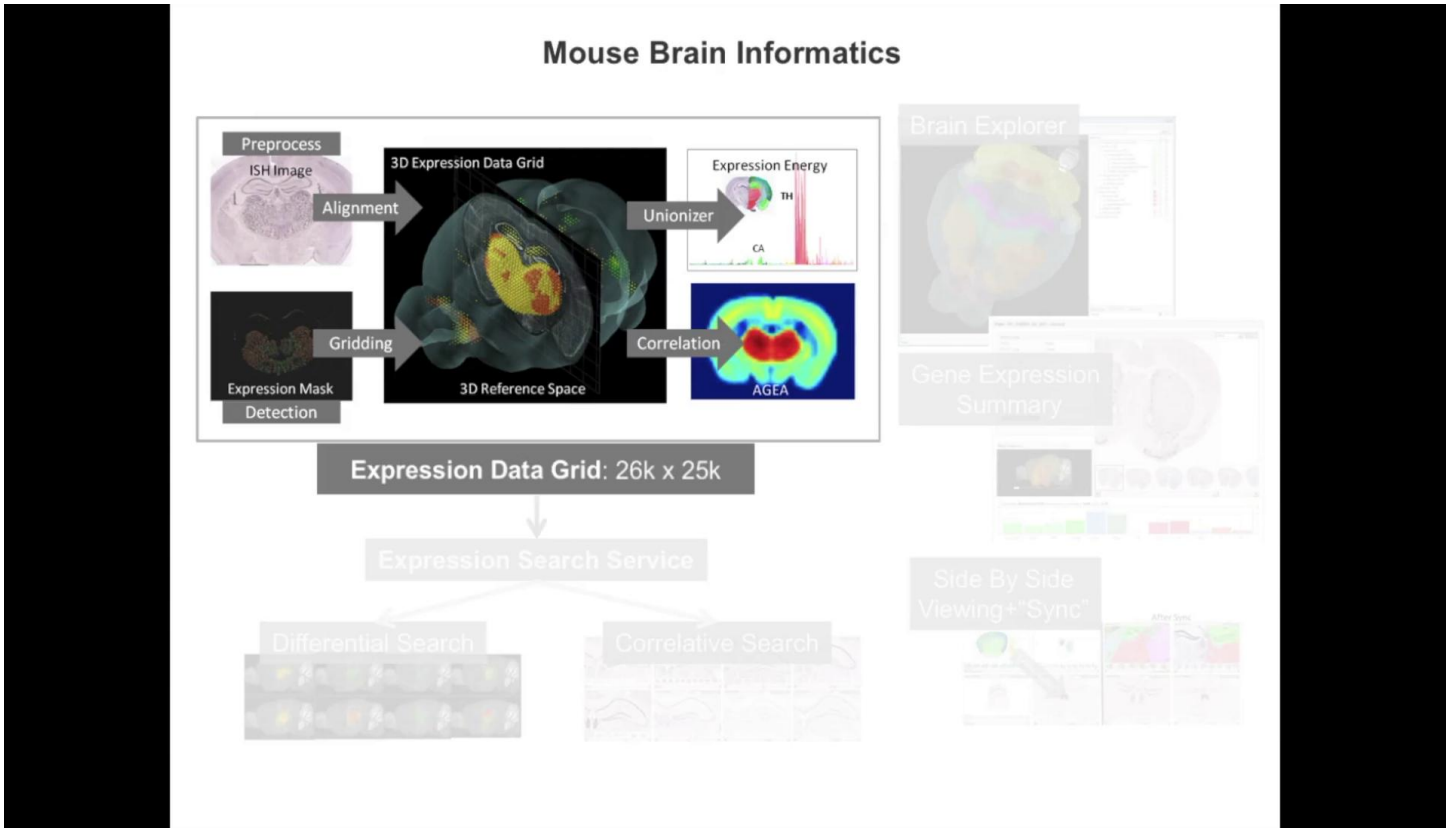


In 2015 the Ellen Institute published a new reference space that was created from an average of almost 1,700 imaged brains. In 2017 anatomist completed drawing almost 700 structures in the three-dimensional plains of this reference brain. This digital reference brain is available for download from the Allen Institute API or the Allen software development kit. Most of the adult mouse brain data including Gene expression, Neuronal projections and Single cell morphology has been registered into this reference space.

Notes

Summary

3m 42s

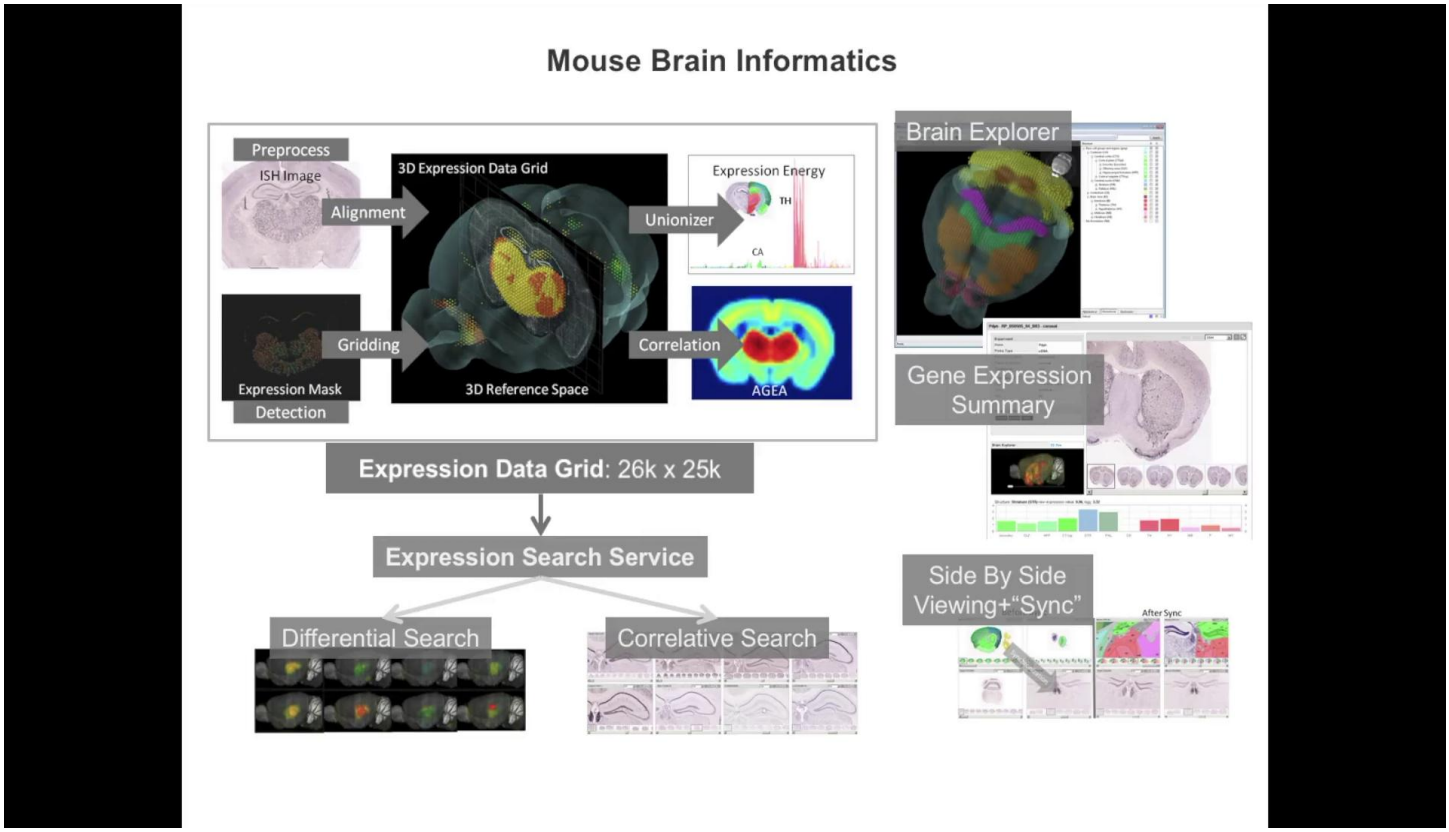


The informatics overlaying the gene expression data, allows for exploring the data in a way that doesn't require expertise in neuro-anatomy or brain function. Several of the informatics modules are illustrated here.

Notes

Summary

4m 13s



To enable the determination of possible positive signal in the gene expression data, an adaptive threshold algorithm subtracts signal and colorizes the positive gene expression signal. This visualization reveals high expression in the yellow and red colors, with the greens and blues representing lower gene expression. These false color images were registered into the three-dimensional brain space, which makes the expression and structures easier to comprehend. Further informatics such as the Unioniser and correlation modules allow for a better understanding of gene expression within an experiment and overall the gene expression combined. Visualization tools were created from these informatics algorithms such as a three-dimensional viewer, gene expression summaries and the ability to sync gene expression to a location in the reference space. This also allows the user to search for enhanced gene expression within a structure or find other genes that show a similar expression pattern to a gene of interest.

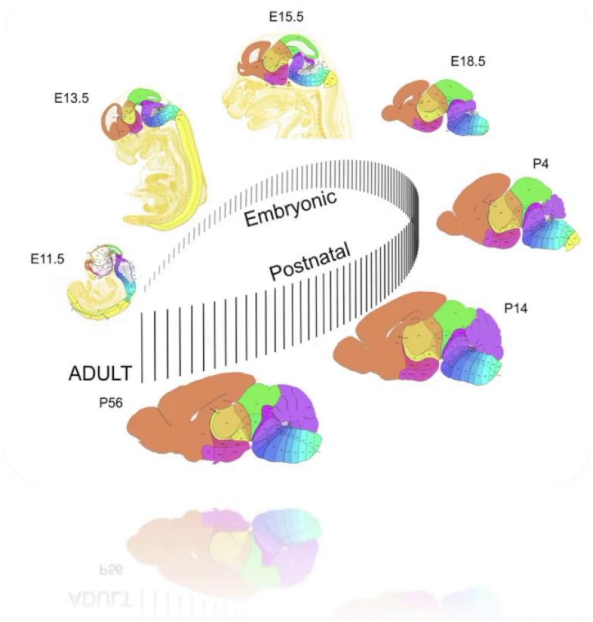
Notes

Summary

4m 24s

Allen Developing Mouse Brain Atlas

- ISH for ~2,000 genes
  - Sagittal ISH data for ~1830 genes
  - Coronal ISH data for ~60 genes
- 7 developmental stages
- Embryonic to postnatal
- Reference atlases by Luis Puelles



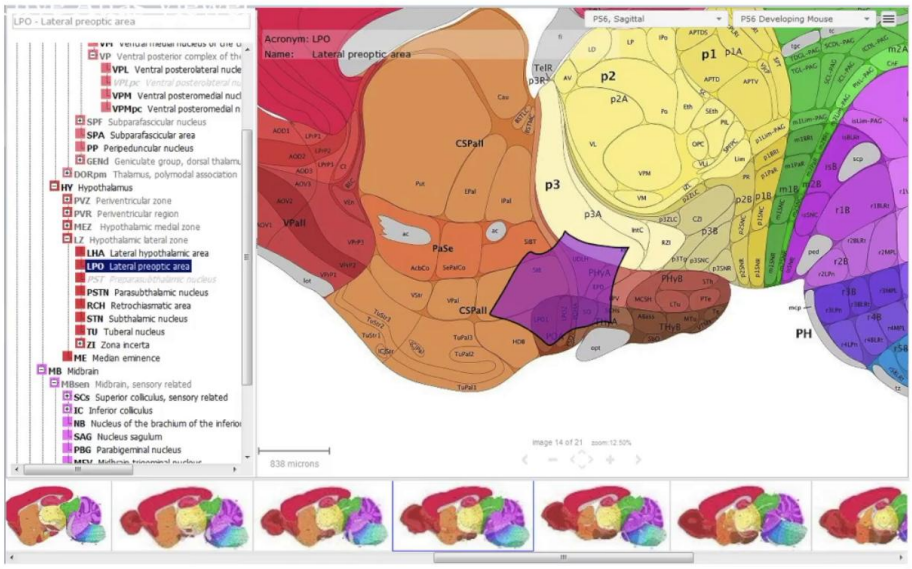
The data collected for the developing mouse brain project was done with an identical methodology, however the number of genes assayed, was limited to approximately 2,000 genes that were selected for their known relevance at the time. The gene expression pattern for these genes was collected over seven developmental stages, four embryonic stages and three postnatal stages.

Notes

Summary

5m 21s

Allen Reference Atlas



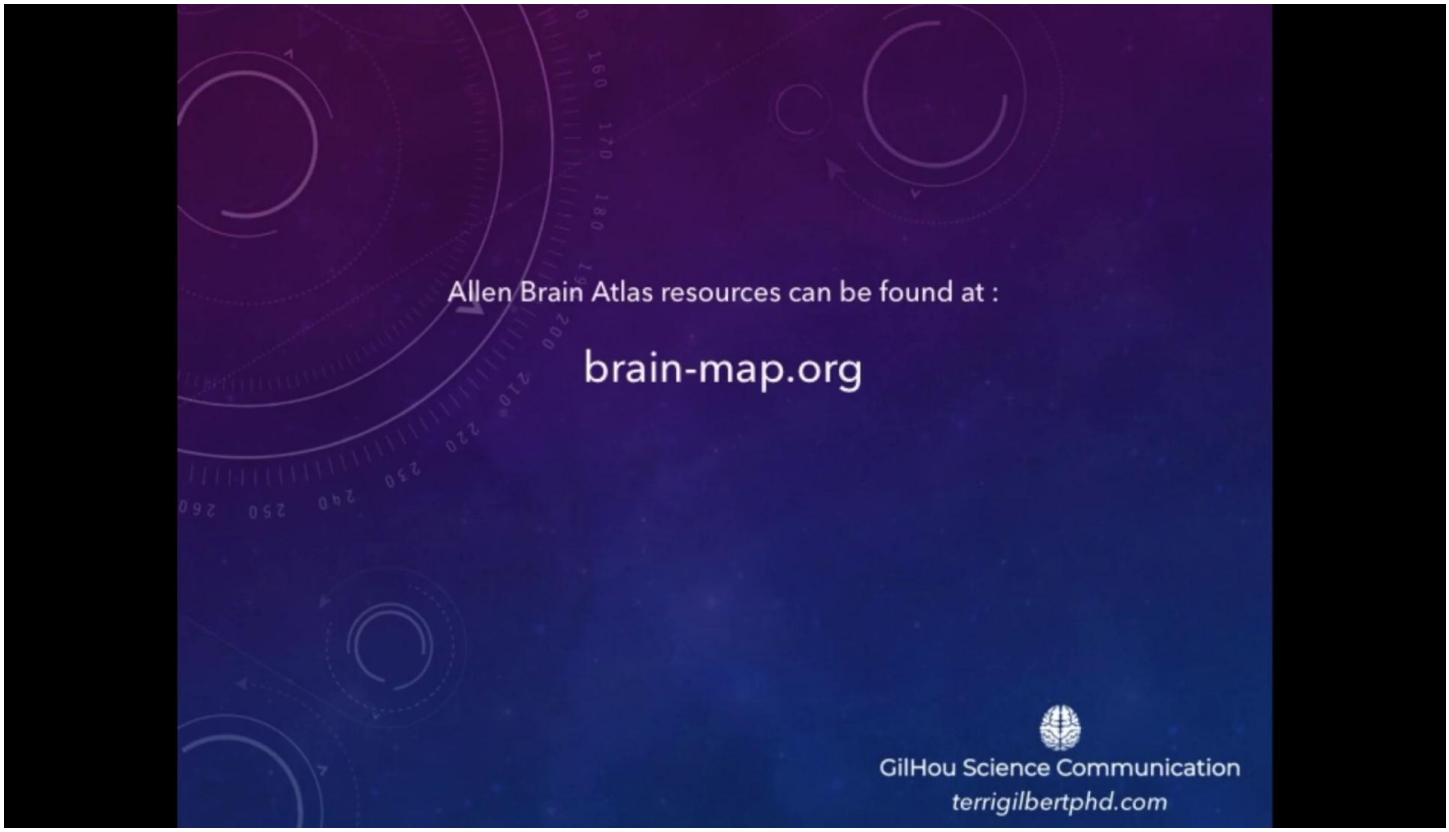
- Reference Atlas provides translation

Reference atlases for each of these stages were drawn to differing levels of complexity by an Allen Institute collaborator professor Lewis Puelles from the University of Murcia in Spain. This is a list of the kinds of genes that were determined by a scientific advisory board to be relevant genes to assay at the time. If you are at all familiar with neuroanatomic nomenclatures, getting neuroanatomist to standardize their naming strategies is problematic. So you'll likely be familiar with the same area of the brain sometimes being called by more than one name. The brain structure nomenclature or ontology problem also exists within the mouse brain reference atlases of the Allen Institute. The adult mouse brain Atlas uses terms that are structurally based and the Atlases created for the developing mouse brain were determined using gene expression during development. By registering the two ontologies into the same reference space however you do have the ability to translate between the different structure names. A structure in the adult mouse brain, for example, the lateral preoptic area or LPO can be overlaid on top of the developing mouse brain atlas, showing which developmental structures it aligns with.

Notes

Summary

5m 47s



This brief review of the data and the mouse gene expression atlases should be sufficient to give you a jumpstart to exploring the data and tools at brain-map.org.

Notes

Summary

6m 53s