3D Whole Brain Atlases

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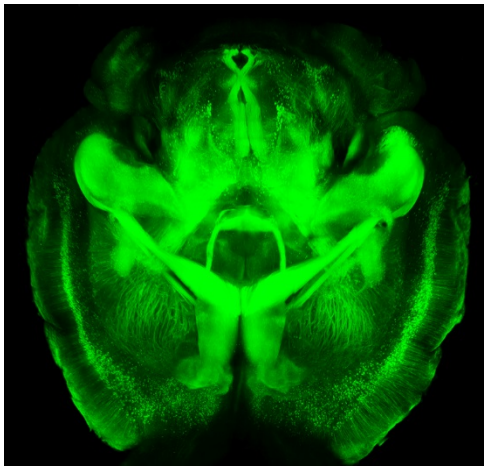
Technical Journal Club 20180410

Clearing technologies enabling whole organ analyses

- From 2D to 3D datasets

- Clearing technologies: CLARITY, DISCO, CUBIC …



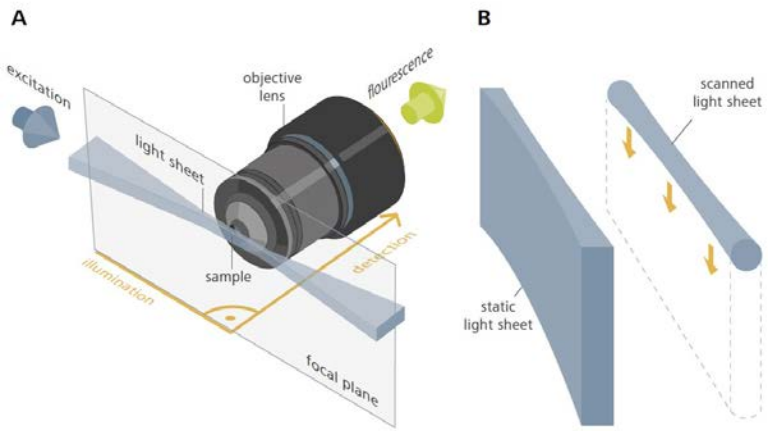
Chung & Deisseroth, 2013. *Nature methods*

Light sheet fluorescence microscopy: imaging of whole organs

- the detection beam path is placed perpendicular to the illumination beam path

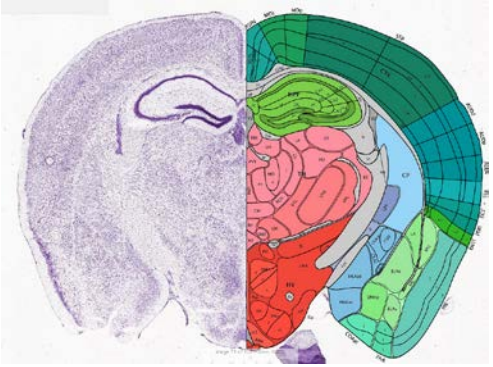
- laser light is formed into a thin sheet of light, exciting only fluorophores within the focal plane of the detection objective

- Advantages:
    - Fast
    - Good resolution
    - Imaging of large volumes are possible



http://blogs.zeiss.com/microscopy/news/en/light-sheet-microscopy-with-zeiss-lightsheet-z-1/

Atlases



Allen brain atlas

- Current mouse brain atlases
  - Mouse brain architecture
  - Allen brain atlas
  - Mouse connectome project
  - Waxholm space atlas
  - The mouse brain in stereotactic co-ordinates (Paxinos)

- Limitations:
  - A standarized system to collect, analyze, visualize and share whole brain data
  - Lack of standarized and accessible computational tools

Interactive whole brain atlases

- Paper 1 (Fürth et al., 2018):
  - Generated open source software to support whole-brain mapping efforts
  - Quantify and spatially map multidimensional data
  - Compare results obtained from different experiments using different modalities
  - Sharing data via a web-based framework

- Paper 2 (Murakami et al., 2018):
  - Establish a new clearing method
  - Create an editable, point-based mouse brain atlas
  - Single-cell resolution
  - Deconstructing images to cellular points reduces data size
  - Automatic detection of cells

nature neuroscience

TECHNICAL REPORT

https://doi.org/10.1038/s41593-017-0027-7

An interactive framework for whole-brain maps at cellular resolution

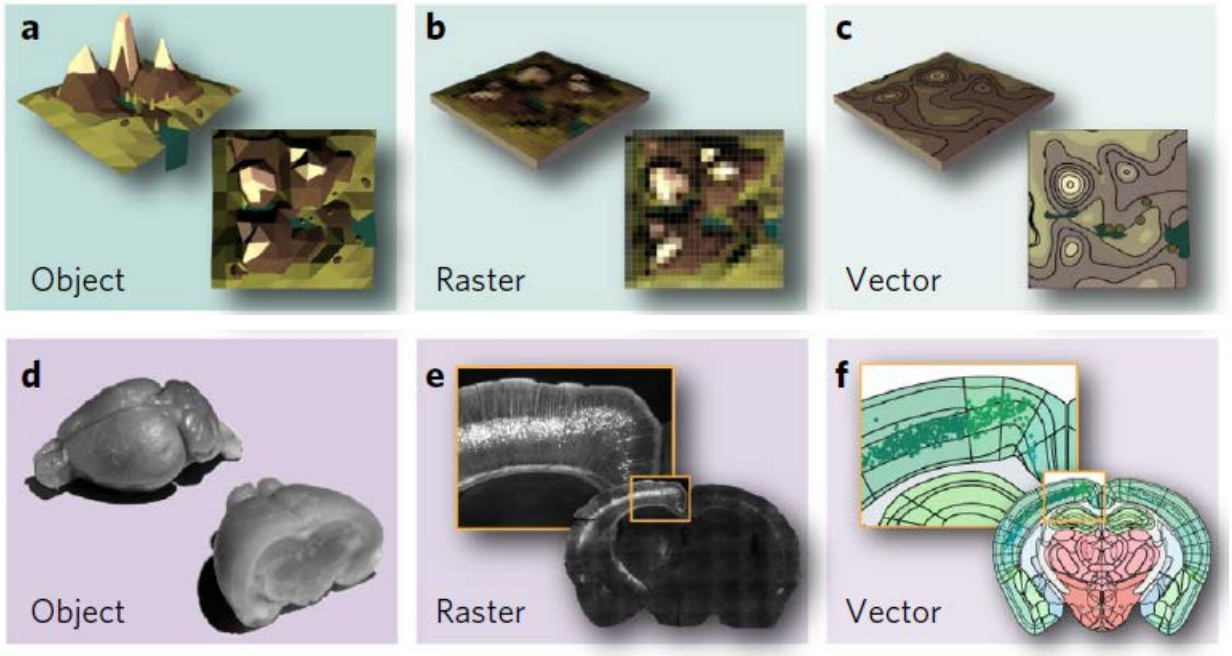
Daniel Fürth[1], Thomas Vaissière[2], Ourania Tzortzi[1], Yang Xuan[1], Antje Märtin[1], Iakovos Lazaridis[1], Giada Spigolon[1], Gilberto Fisone[1], Raju Tomer[3], Karl Deisseroth[3], Marie Carlén[1], Courtney A. Miller[2,4], Gavin Rumbaugh[2] and Konstantinos Meletis[1*]

January 2018

Raster and Vector representation in neuroanatomy

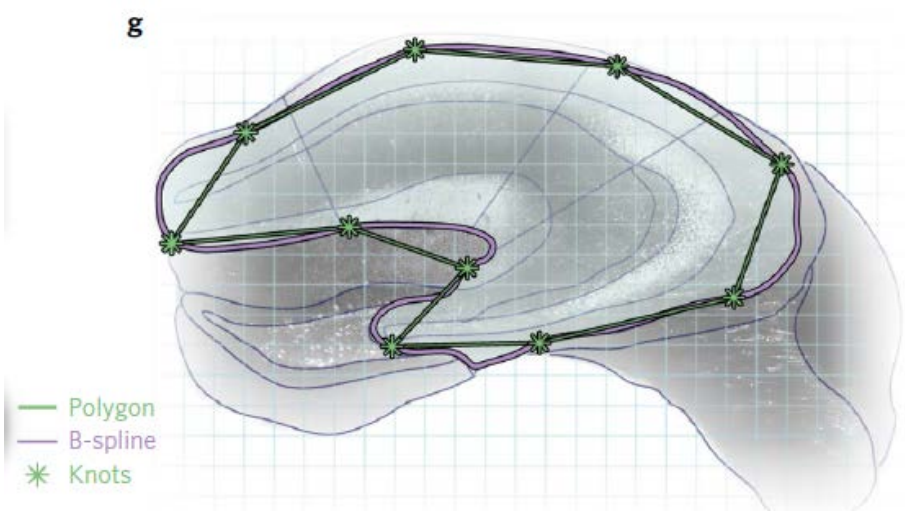
Cartography

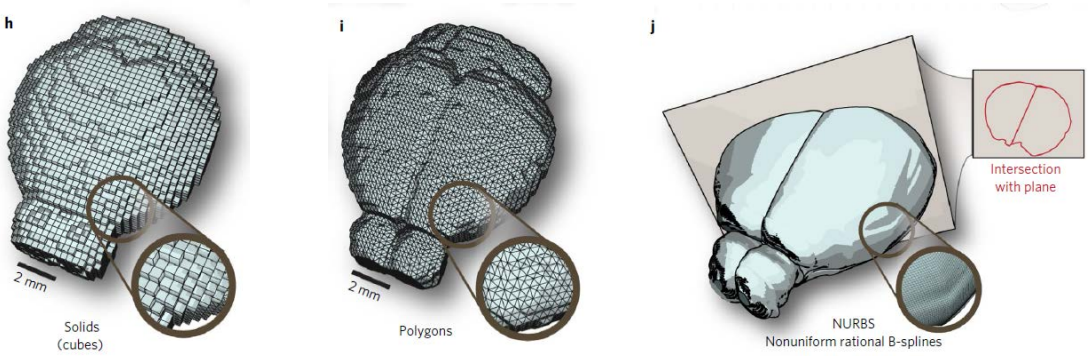
Neuroscience



- Raster graphics represent data in a grid cell structure (pixel/voxel) comprising of a matrix
- A comprehensive framework to represent data at the cellular level
- Allows the mapping of features through the use of points and curves
- Data can be represented simultaneously in multiple coordinate reference systems
    - Image pixel
    - Stereotactic co-ordinates
    - Actual tissue dimensions

Scale-invariant reference atlas



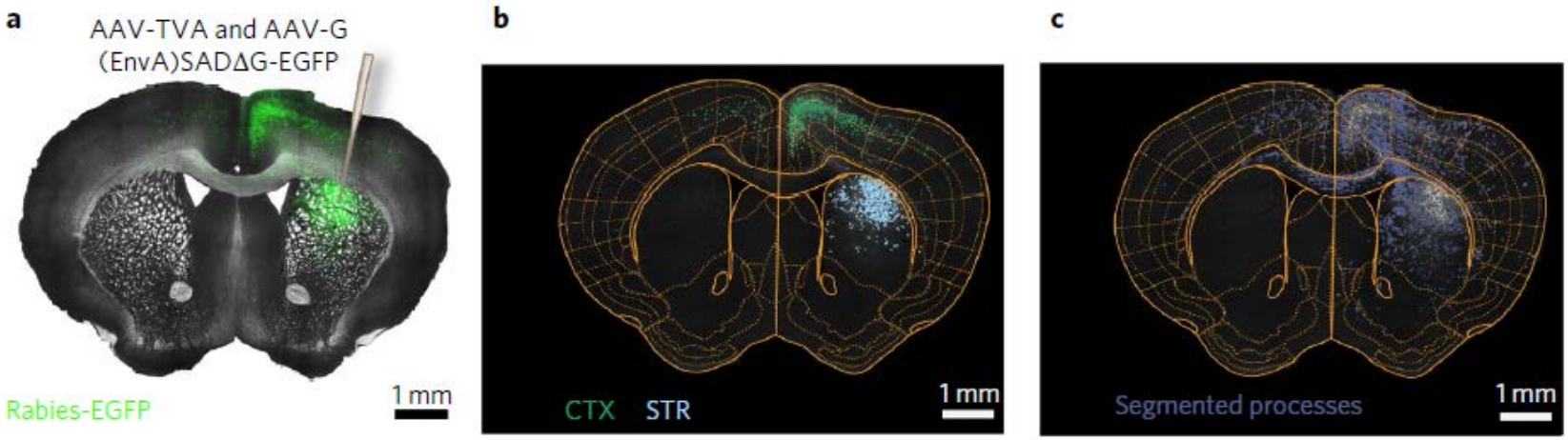


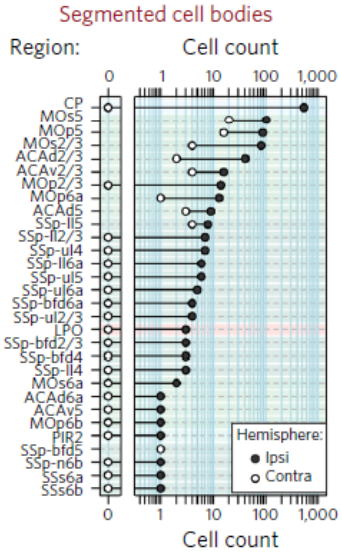
- Scale-invariant: can be easily applied on images of any pixel resolution
  - Enables comparison of image data across microscope systems
- Atlas intersections can be computed at arbitrary angles
  - Visaulization of regions better acheived than the canonical
- Data-efficient
  - Shared in small format
- Non-uniform format enables editing of the brain atlas
  - Customization and atlas improvements

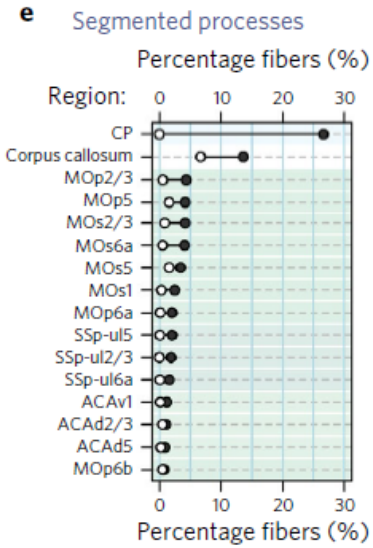
Interactive web interface: A framework of standardizing neuroanatomical data

- To allow investigators
  - to map results in a standardized format
  - Share data with collaborators
- Detected features: cell bodies, fibre tracts
- Overlaid layer of vector graphics on the original raster image
  - Fit of reference atlas to the tissue

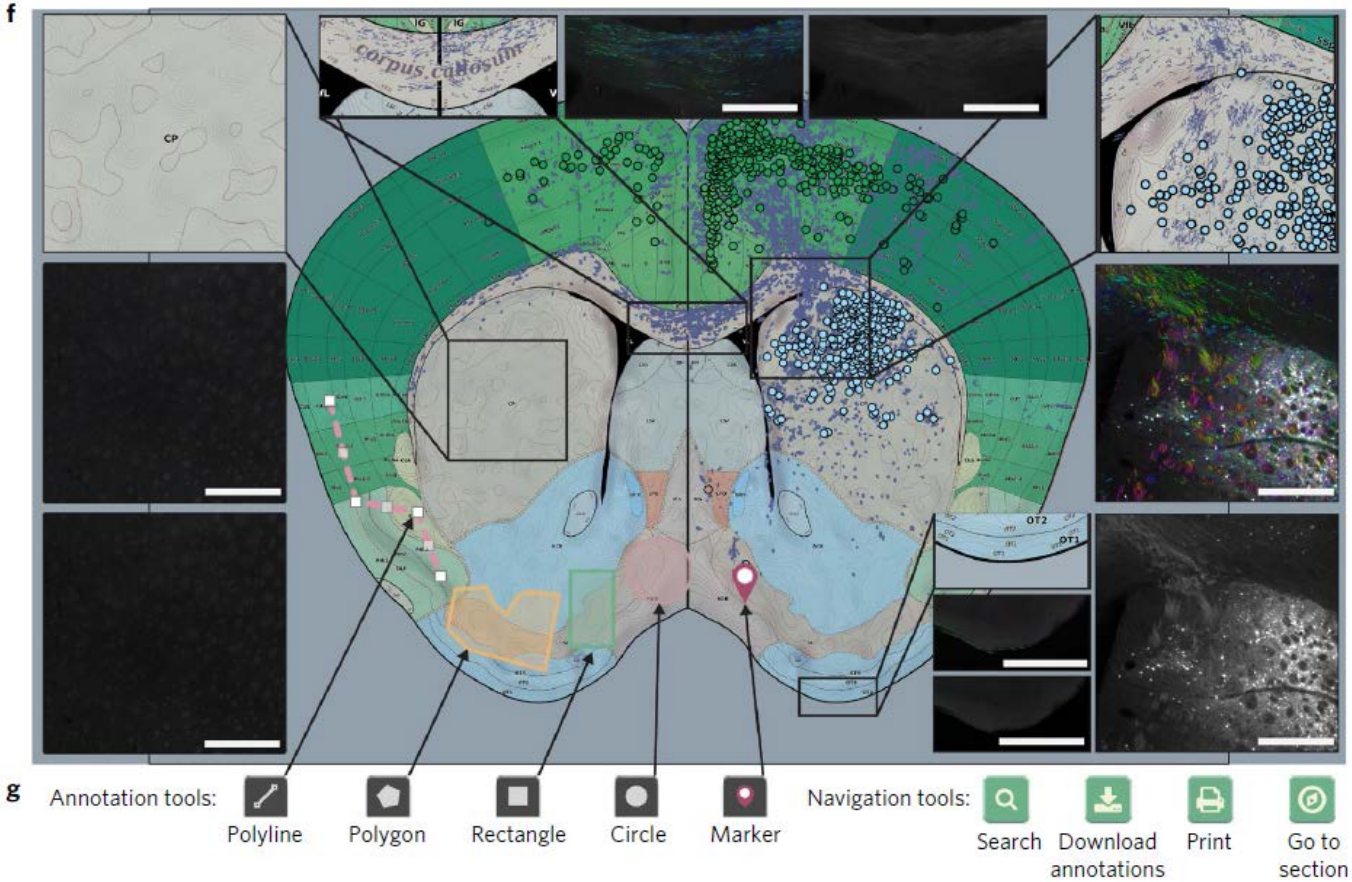
Interactive web interface: A framework of standardizing neuroanatomical data







Interactive web interface: A framework for sharing data



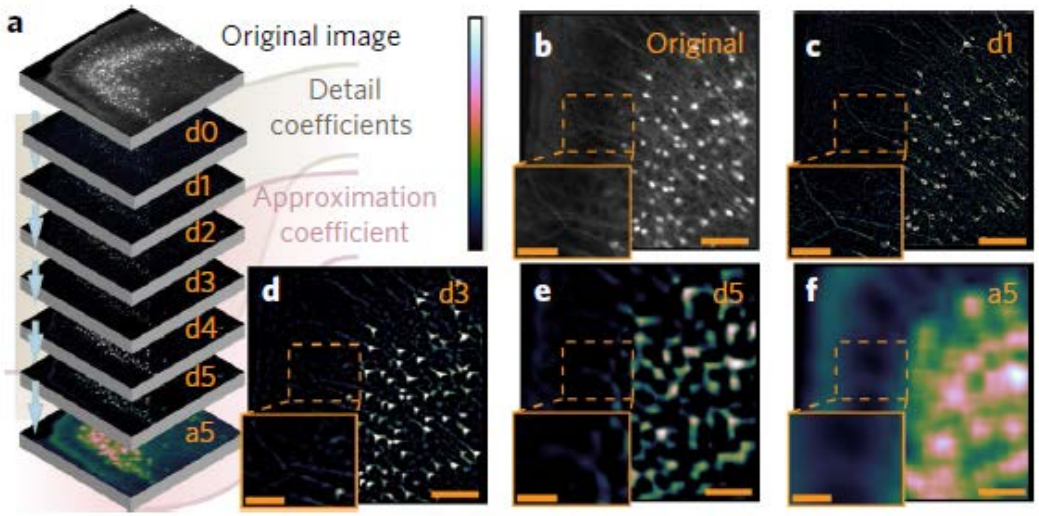
- Rendered within the stereotactic co-ordinates
- Annotation tools enable to further draw, edit or define ROIs.

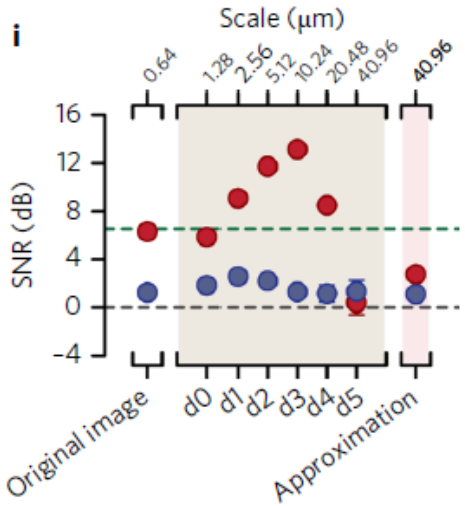
Segmentation by multiresolution decomposition

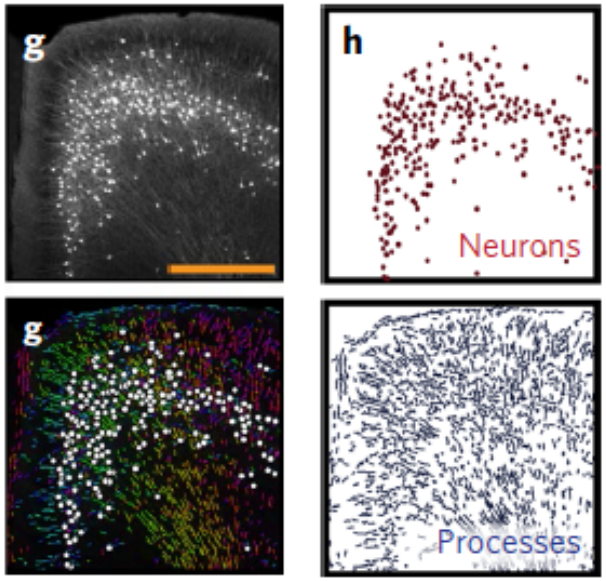
- Segmentation is the process of assigning labels to distinct parts of an image

- Multiresolution decomposition to automatically segment
  - Features of a different size are detected by mapping on a distinct spatial scale
  - Detail coefficients contain information on the correlation between a wave-like function at a specific scale and the fluorecent signal at a specific location in the image
  - Different resolution images can be directly compared

Segmentation by multiresolution decomposition



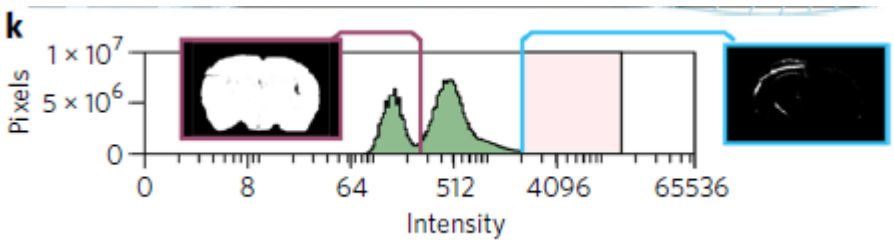


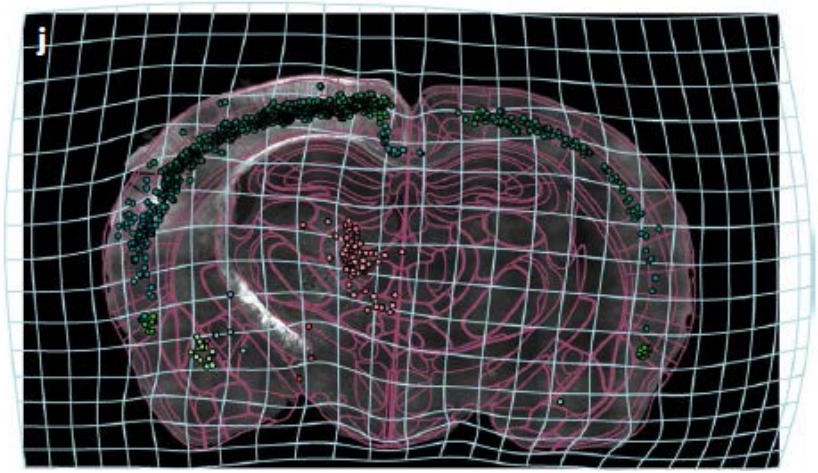


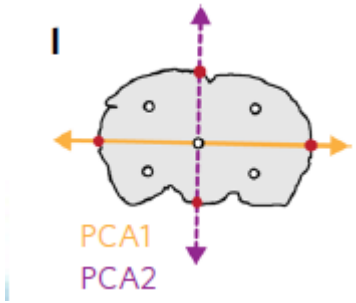
Registration to a standardized atlas

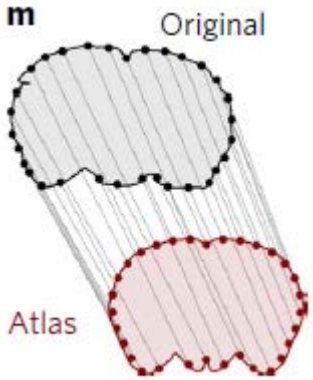
- The process by which sets of images are transformed into another co-ordinate system
- Current registration systems are limited to a voxel resolution
- To reduce computational time, usually imaged brain section is downsampled to match atlas resolution

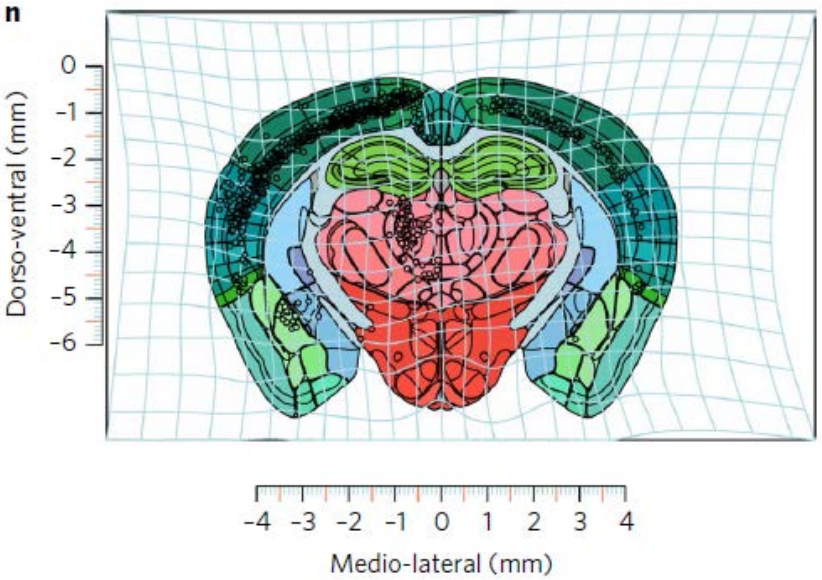
Registration to a standardized atlas



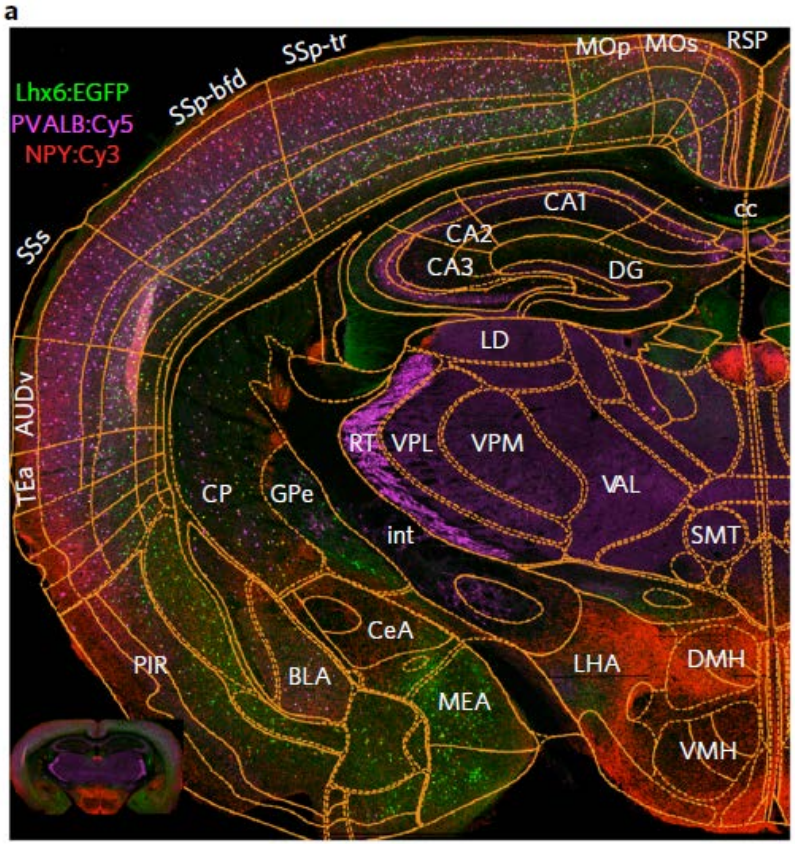


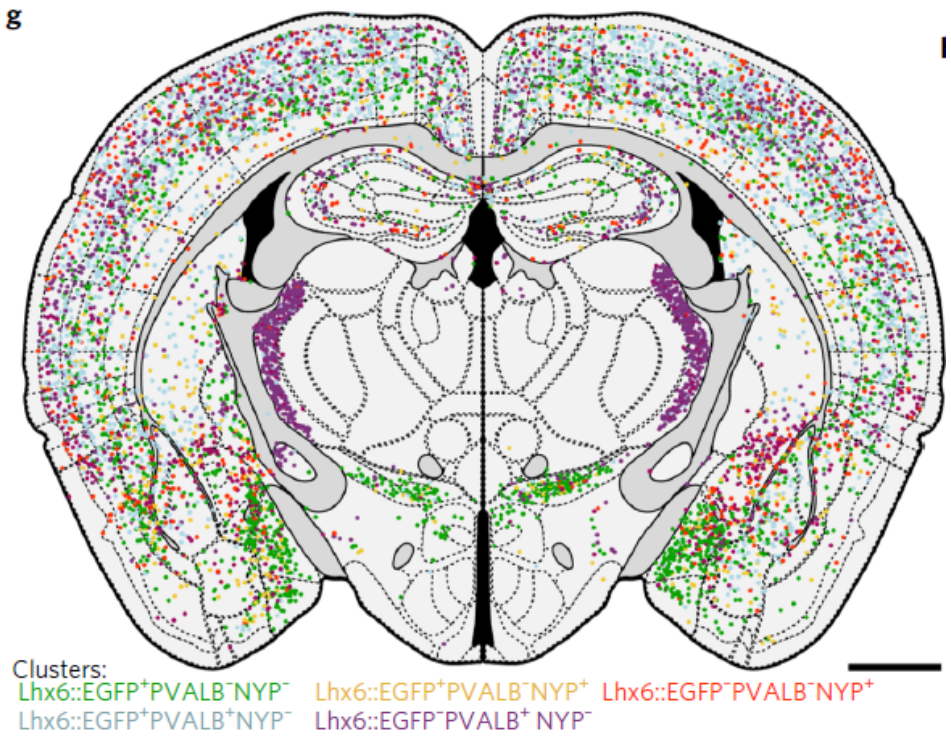


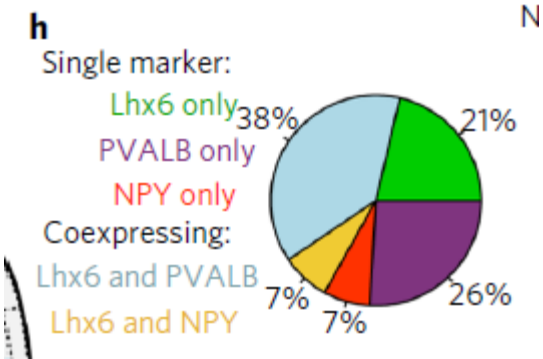




Mapping of single cell co-expression

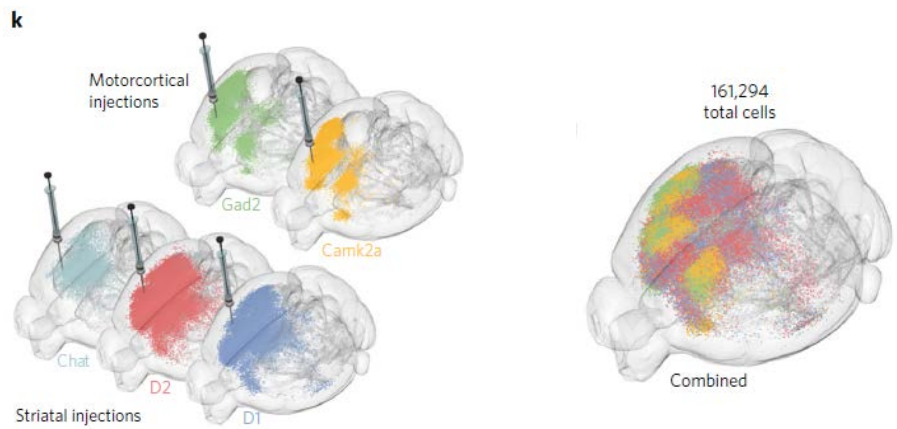


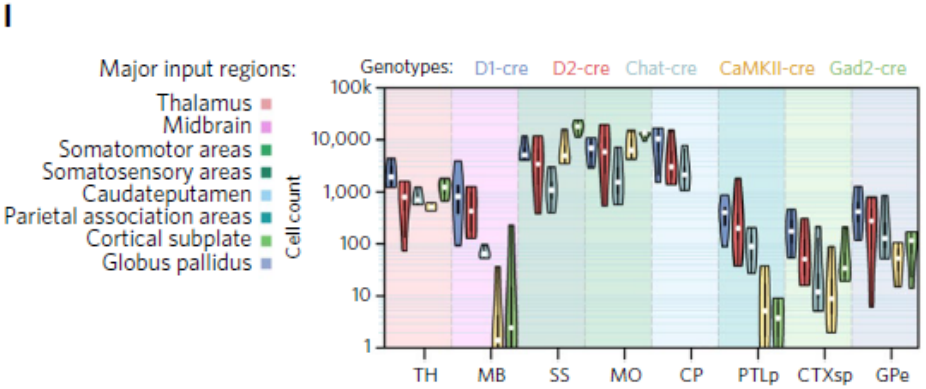




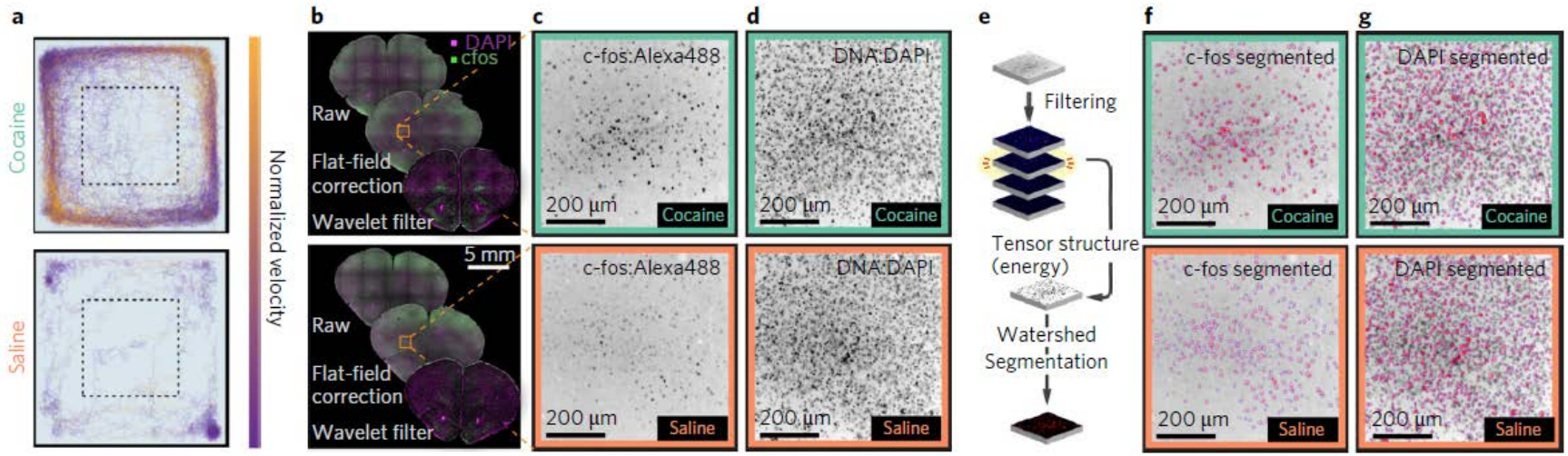
Tracing connectivity

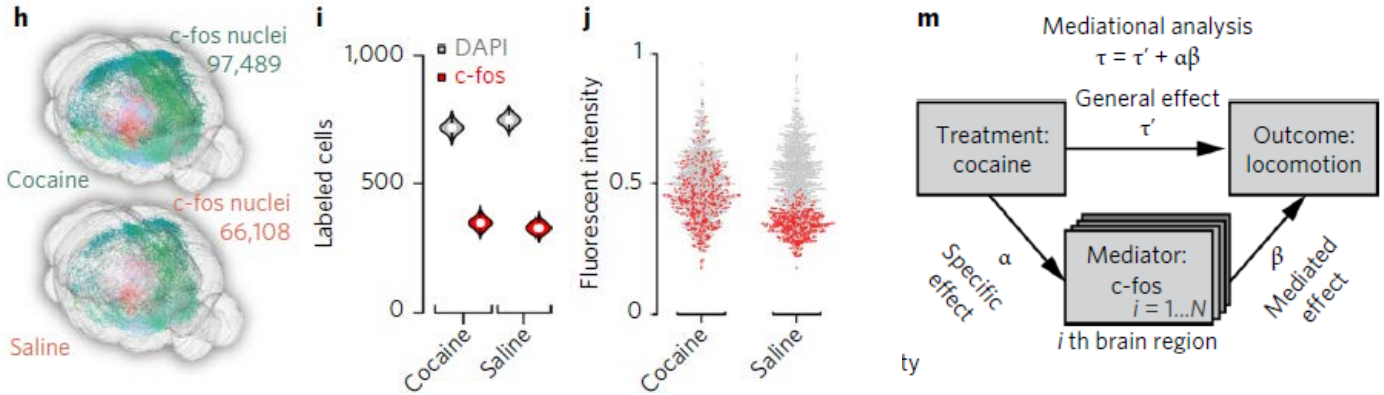
- Rabies virus tracing
  - To identify the presynaptic partners of defined neuronal subtypes in the corticostriatal pathway
- Cre-mediated genetic targeting to limit the uptake of rabies virus
- Map the precise neuroanatomical distribution of the labelled presynaptic neurons at a whole brain scale





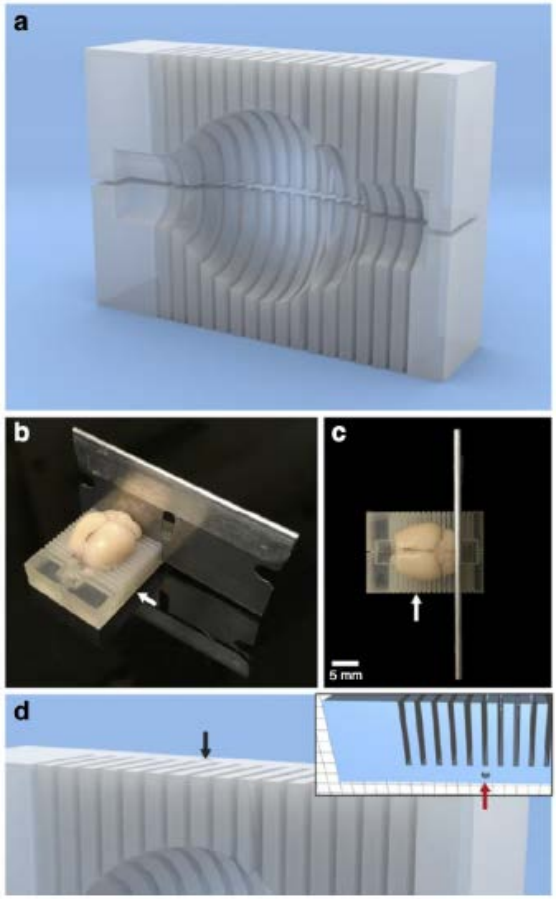
Decoding motor behaviour



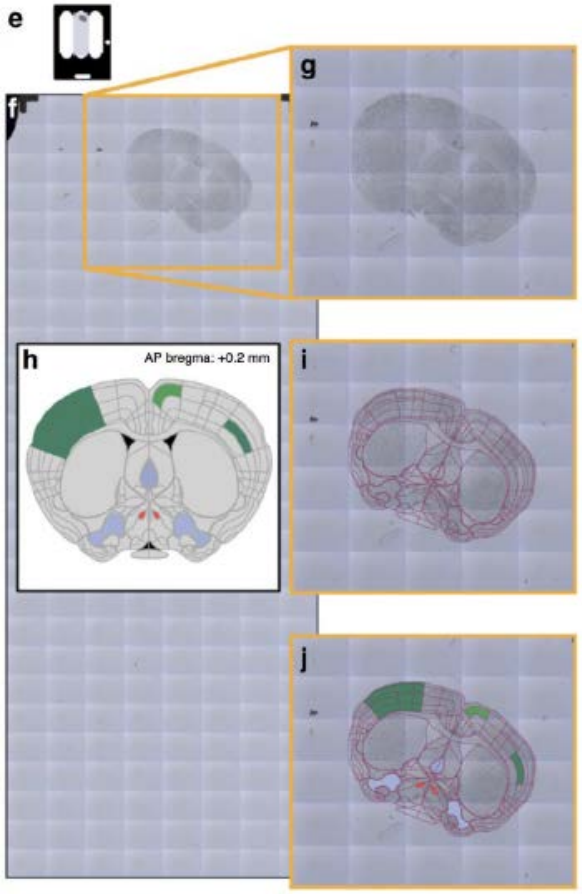


Special features

Atlas-based mechanical dissection



Atlas-based laser dissection



Conclusion

- Computational framework
  - Facilitate mouse brain circuits at the cellular level
  - Automated segmentation
  - Independent of the imaging modality
  - Rapid to visualize and share whole-brain data
- Provide a website with video tutorials
- Reference atlas is based on neuroanatomical definitions found in the Allen Institute mouse reference atlas
  - Integrating all the neuroanatomical and molecular data

nature neuroscience

RESOURCE

https://doi.org/10.1038/s41593-018-0109-1

A three-dimensional single-cell-resolution whole-brain atlas using CUBIC-X expansion microscopy and tissue clearing

Tatsuya C. Murakami[1], Tomoyuki Mano[1,2], Shu Saikawa[3], Shuhei A. Horiguchi[4,5], Daichi Shigeta[1], Kousuke Baba[6,7], Hiroshi Sekiya[8], Yoshihiro Shimizu[9], Kenji F. Tanaka[10], Hiroshi Kiyonari[11], Masamitsu Iino[8,12], Hideki Mochizuki[6], Kazuki Tainaka[1,13] and Hiroki R. Ueda[1,2,4*]

April 2018

CUBIC and Expansion microscopy

CUBIC

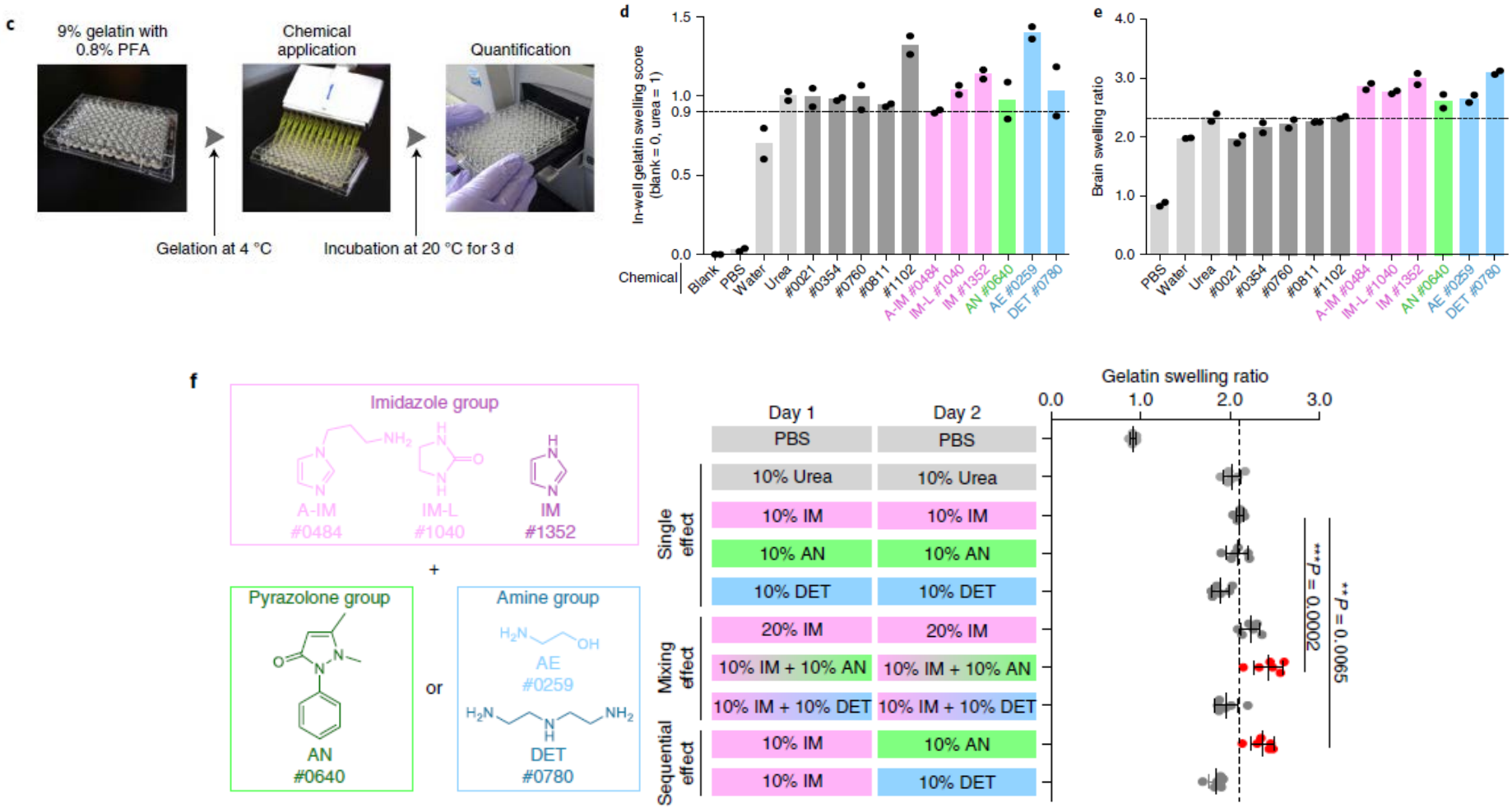
- *c*lear, *u*nobstructed *b*rain *i*maging *c*ocktails and *c*omputational analysis
- Relatively simple: requires the immersion of tissue in 2 solutions
- Antibody-compatible

Expansion microscopy

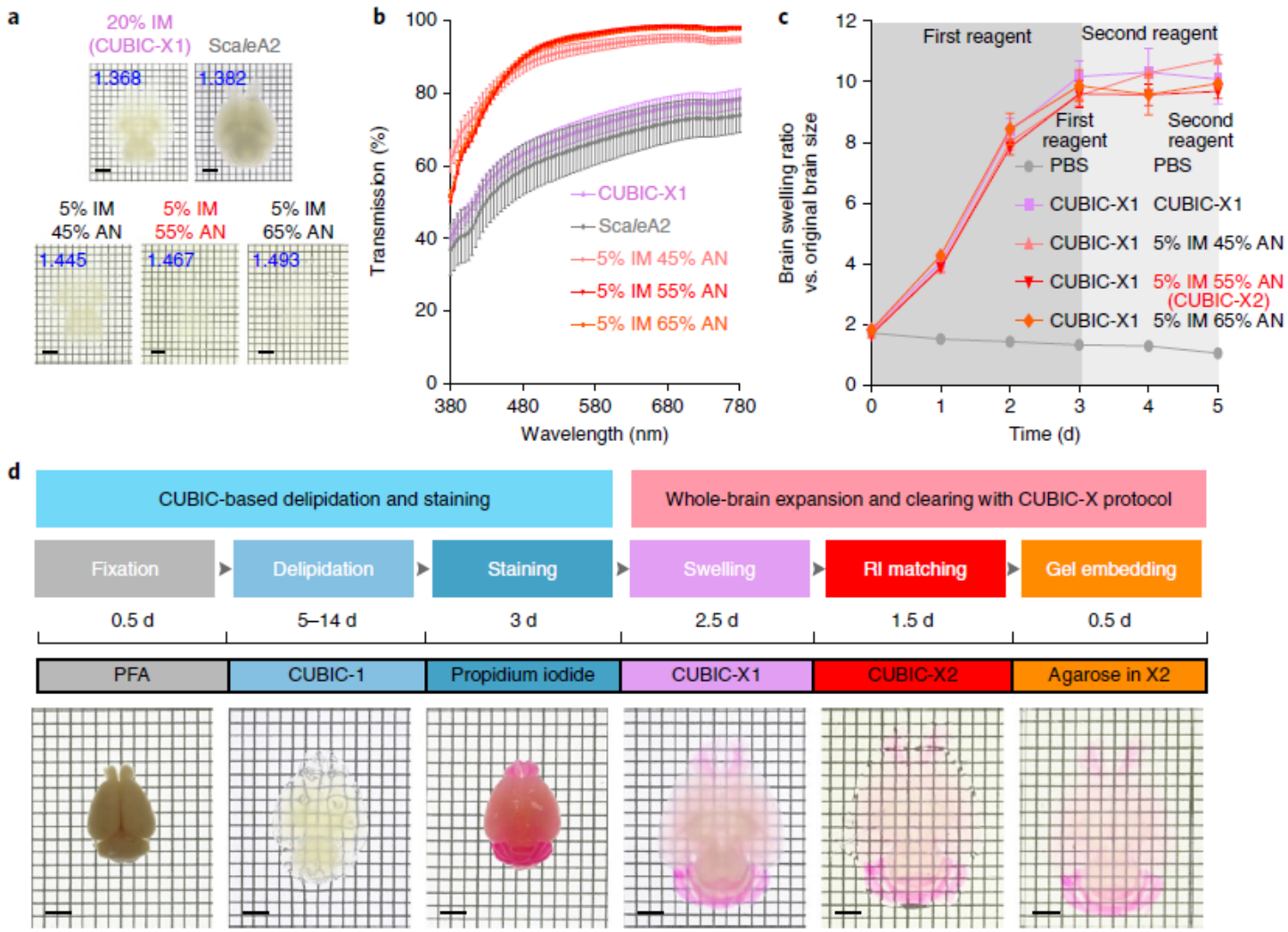
- Sample swelling resulting in a physical magnification
- Anchoring specific labels to a polymer network
- Can visualize structures closer than the diffraction limit
- Disadvantages:
  - Initially original proteins were lost
  - Lack of hyperhydrative RI reagent prevented whole brain scale coverage
  - Improvements in the methodology cannot preserve fluorescent proteins

Murakami et al. merged CUBIC and expansion microscopy in order to combine the advantages of both methodlogies while reducing the disadvantages.

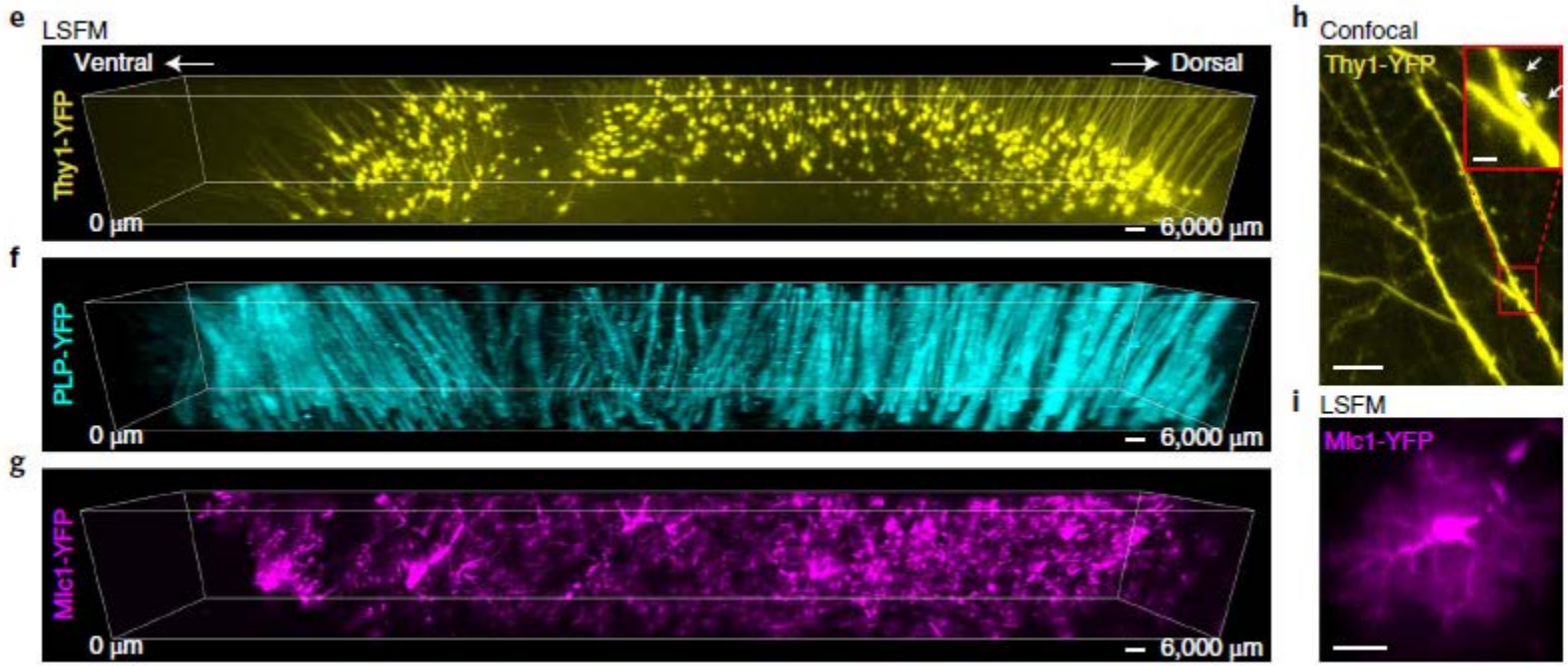
Identification of swelling reagents



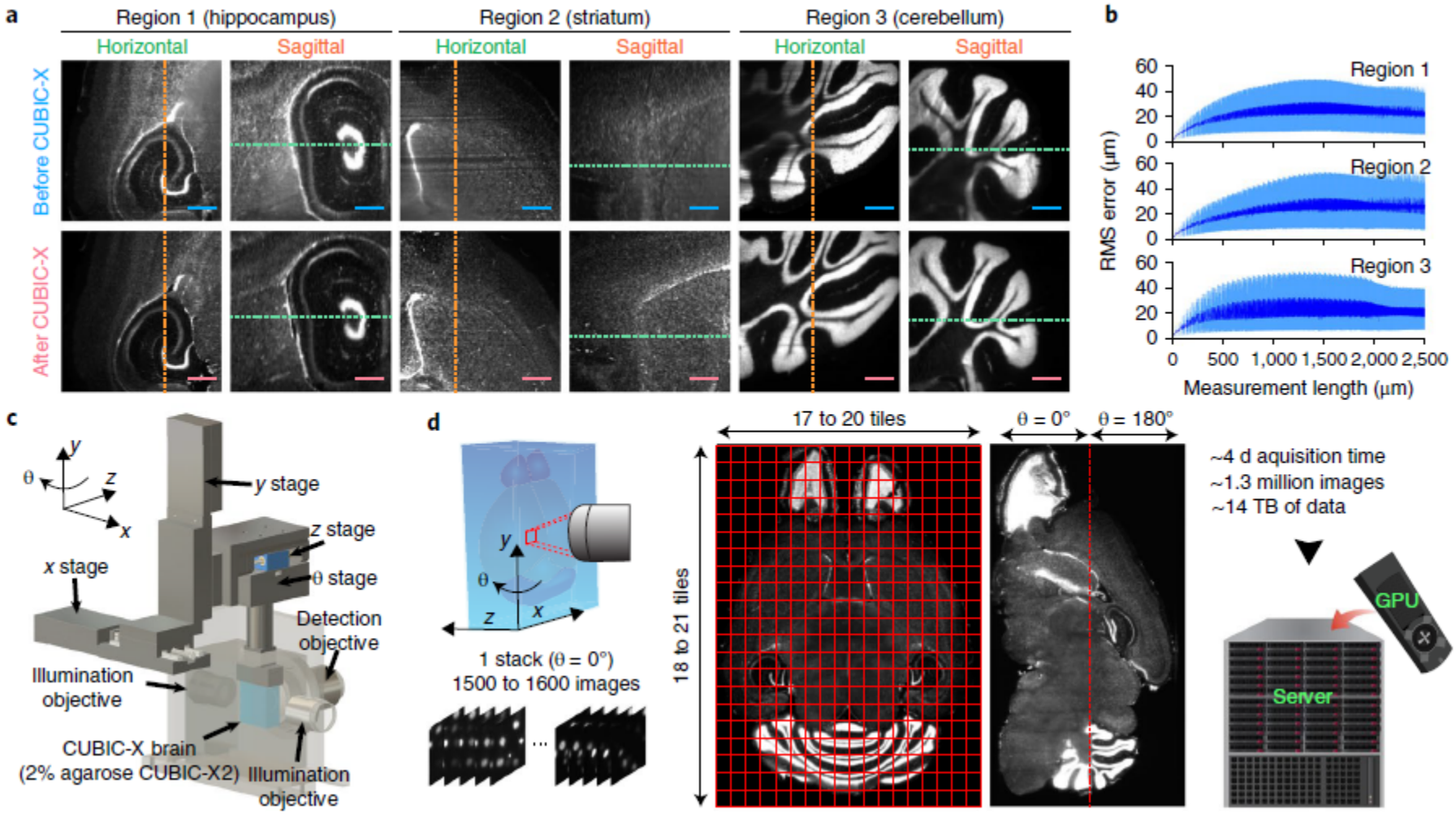
CUBIC-X for whole brain expansion



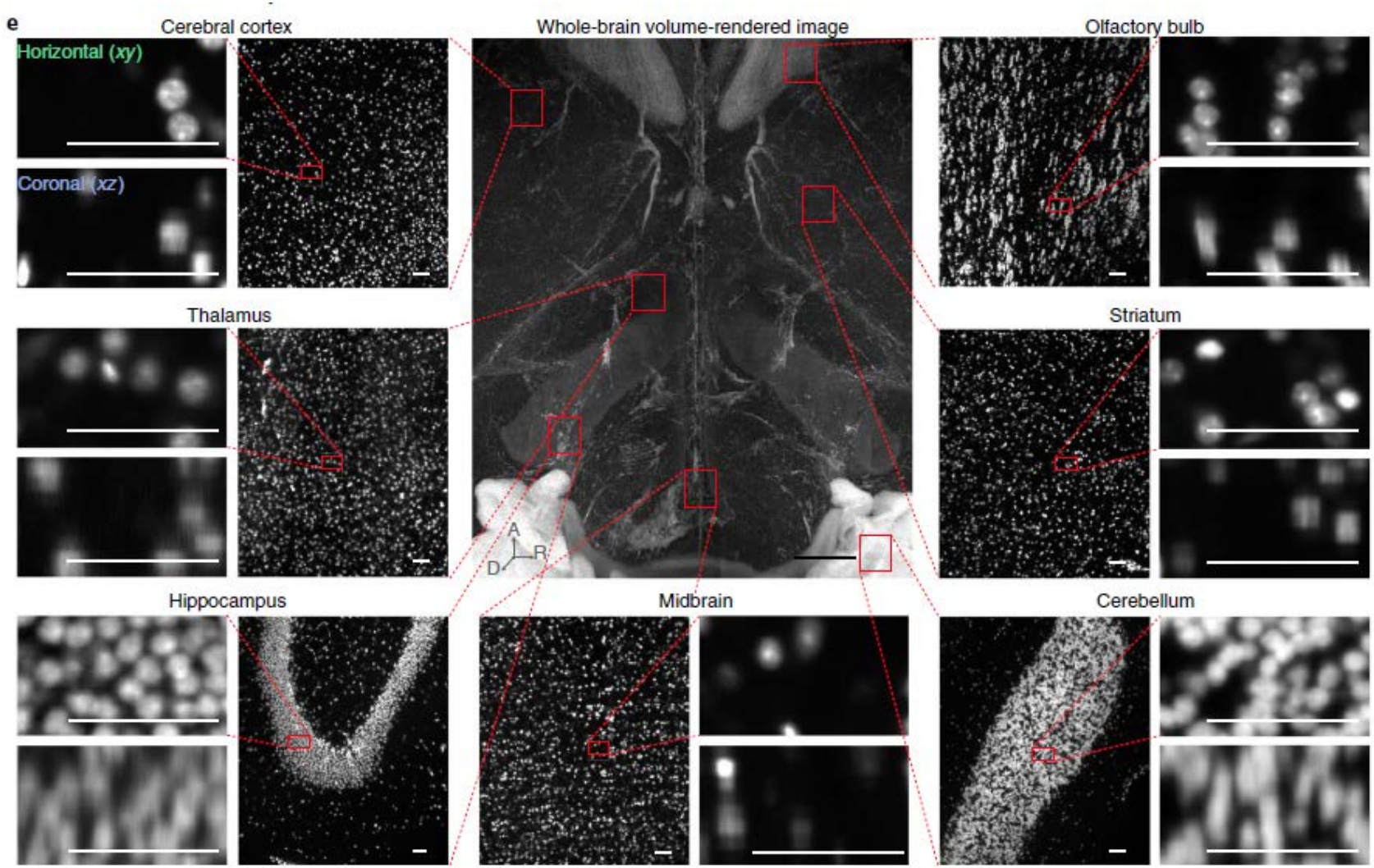
CUBIC-X with retention of native proteins



Whole brain nuclei imaging with CUBIC-X

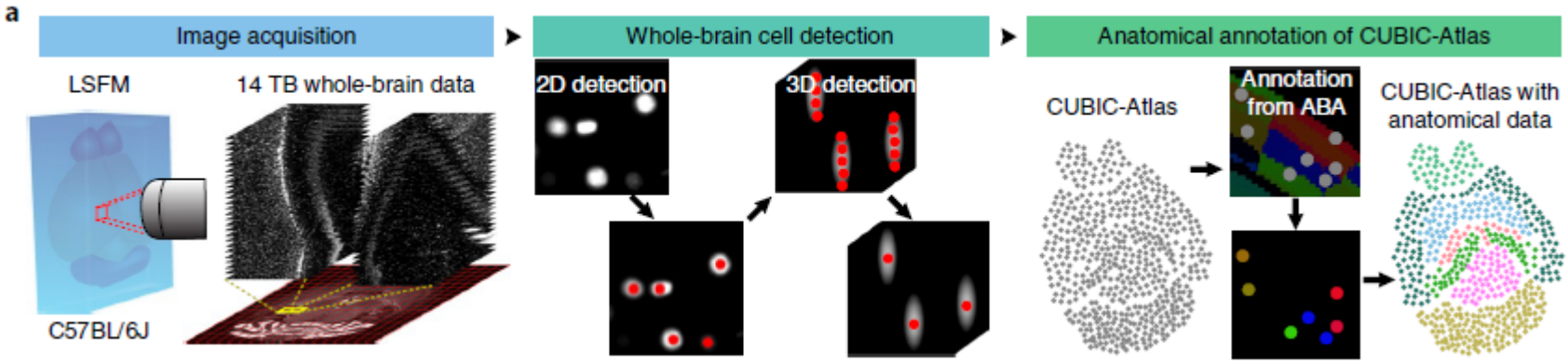


Whole brain nuclei imaging with CUBIC-X



Construction of a single-cell resolution mouse brain atlas

- Image acquisition
- Algorithm to detect nuclei
- Annotate against Allen brain atlas (ABA)



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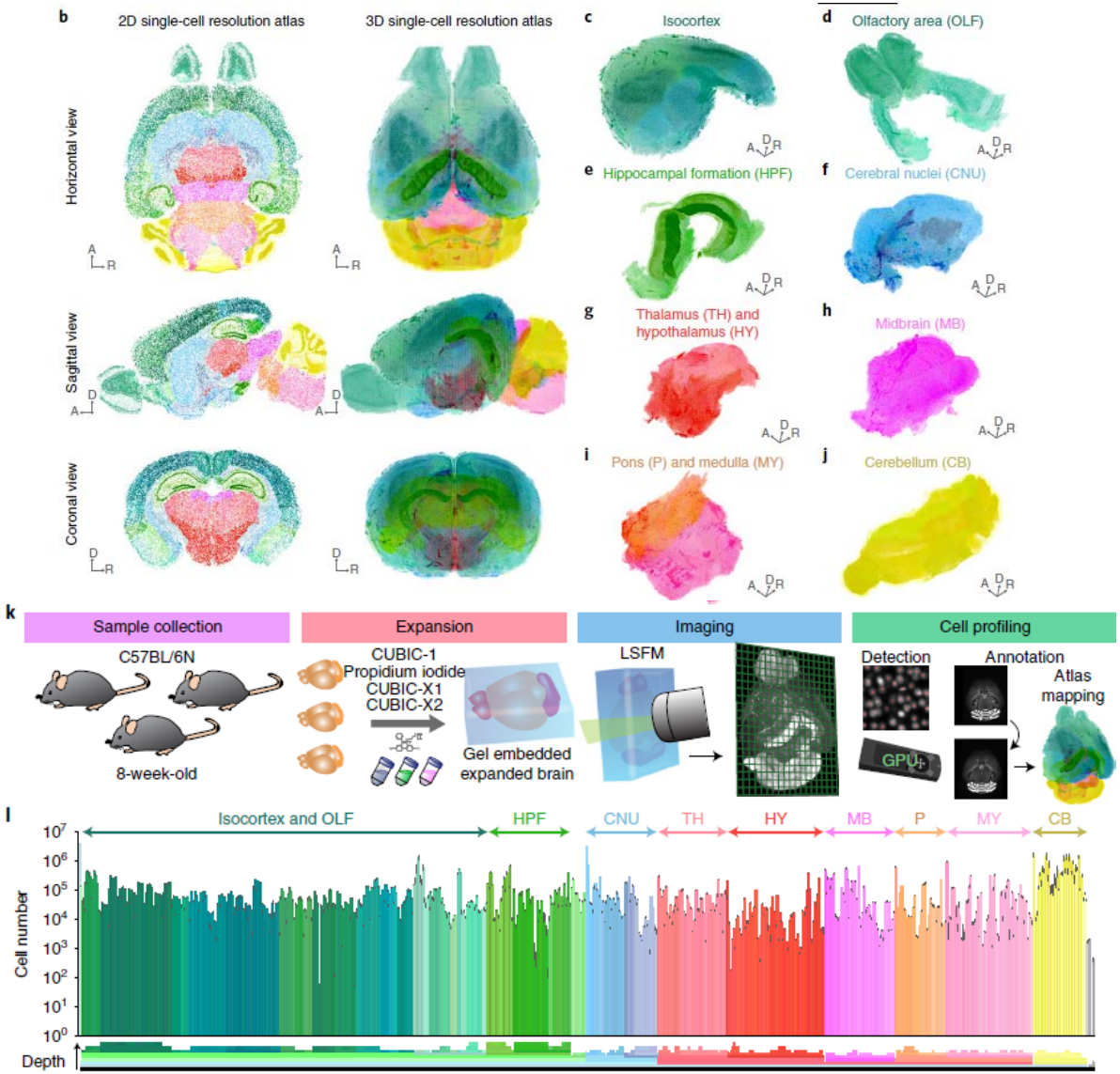
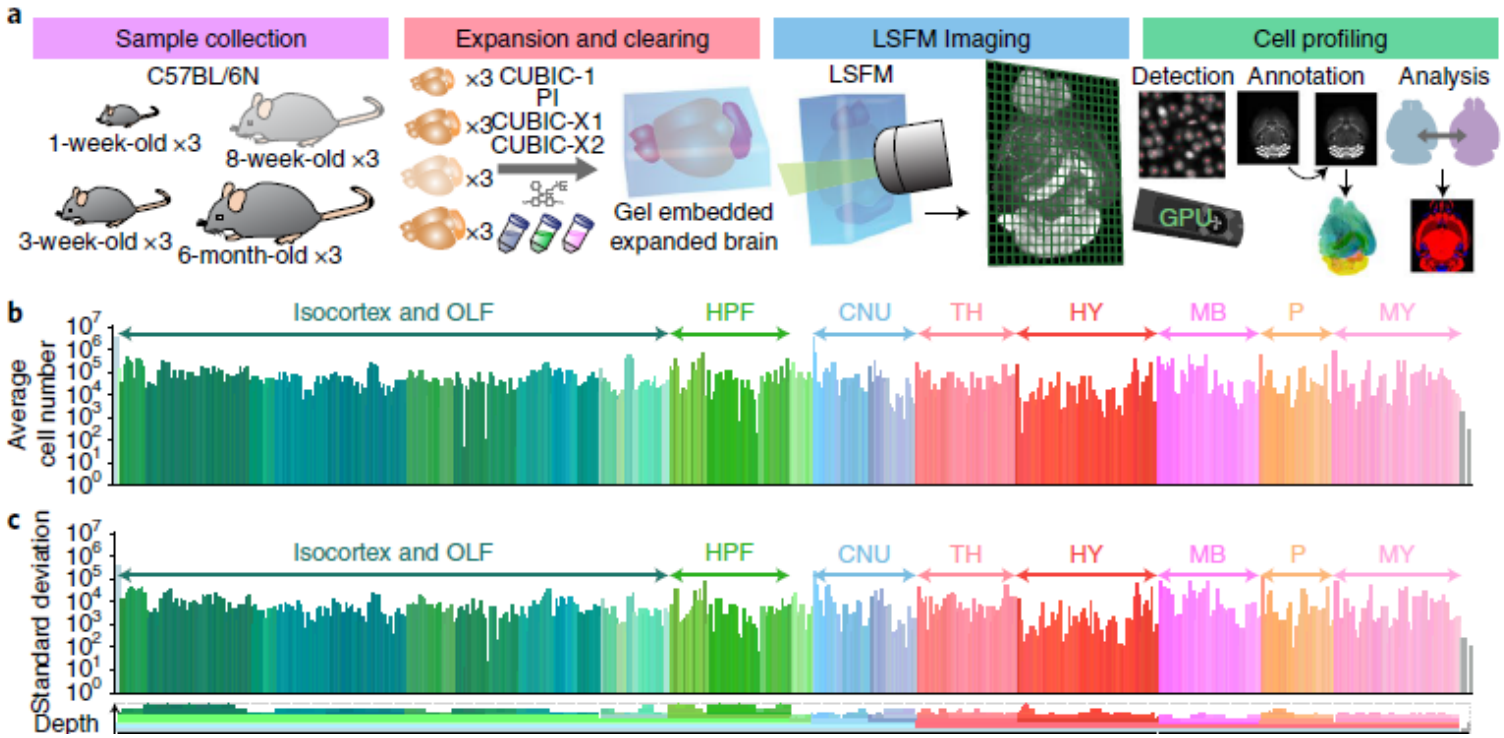
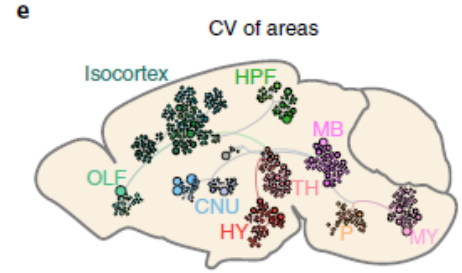


CUBIC-atlas for whole brain cell profiling





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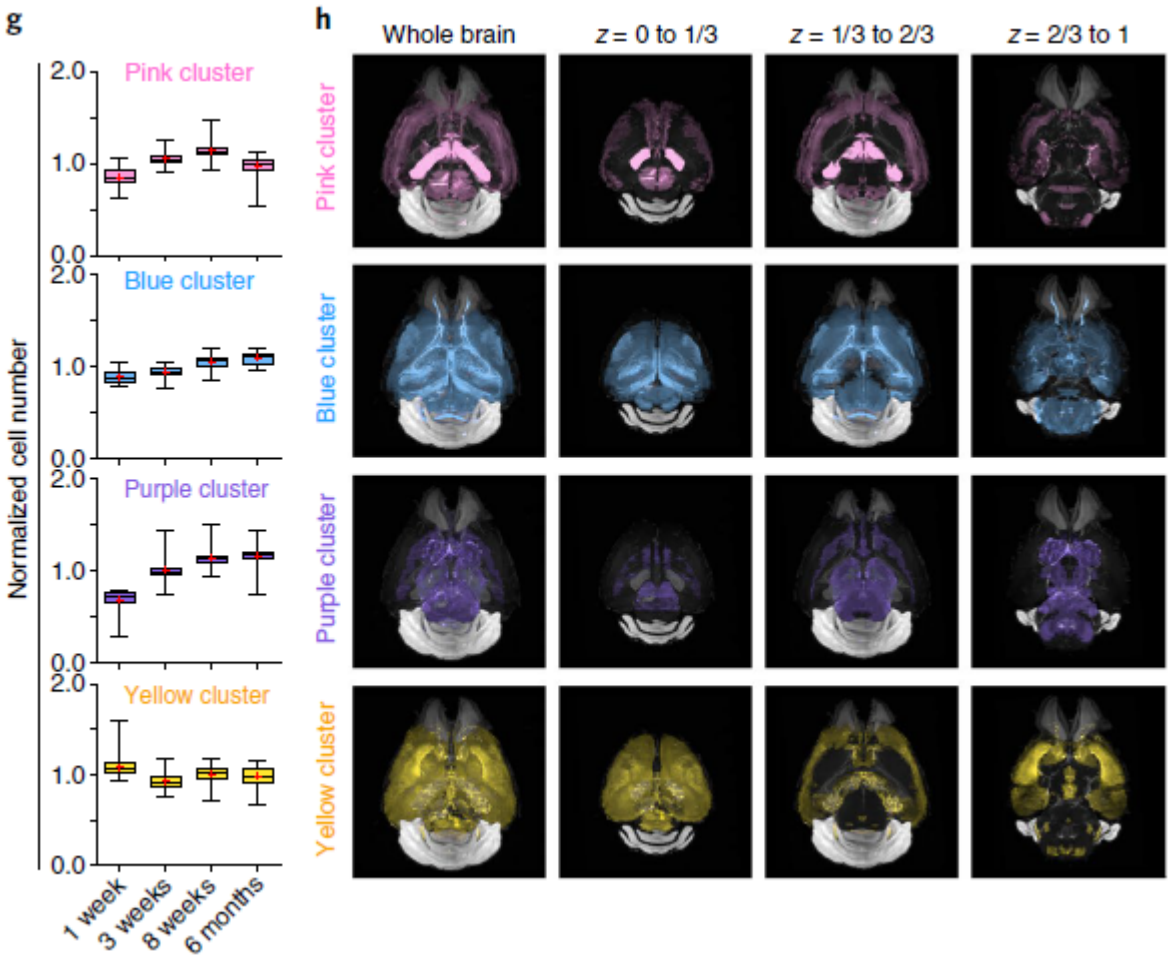
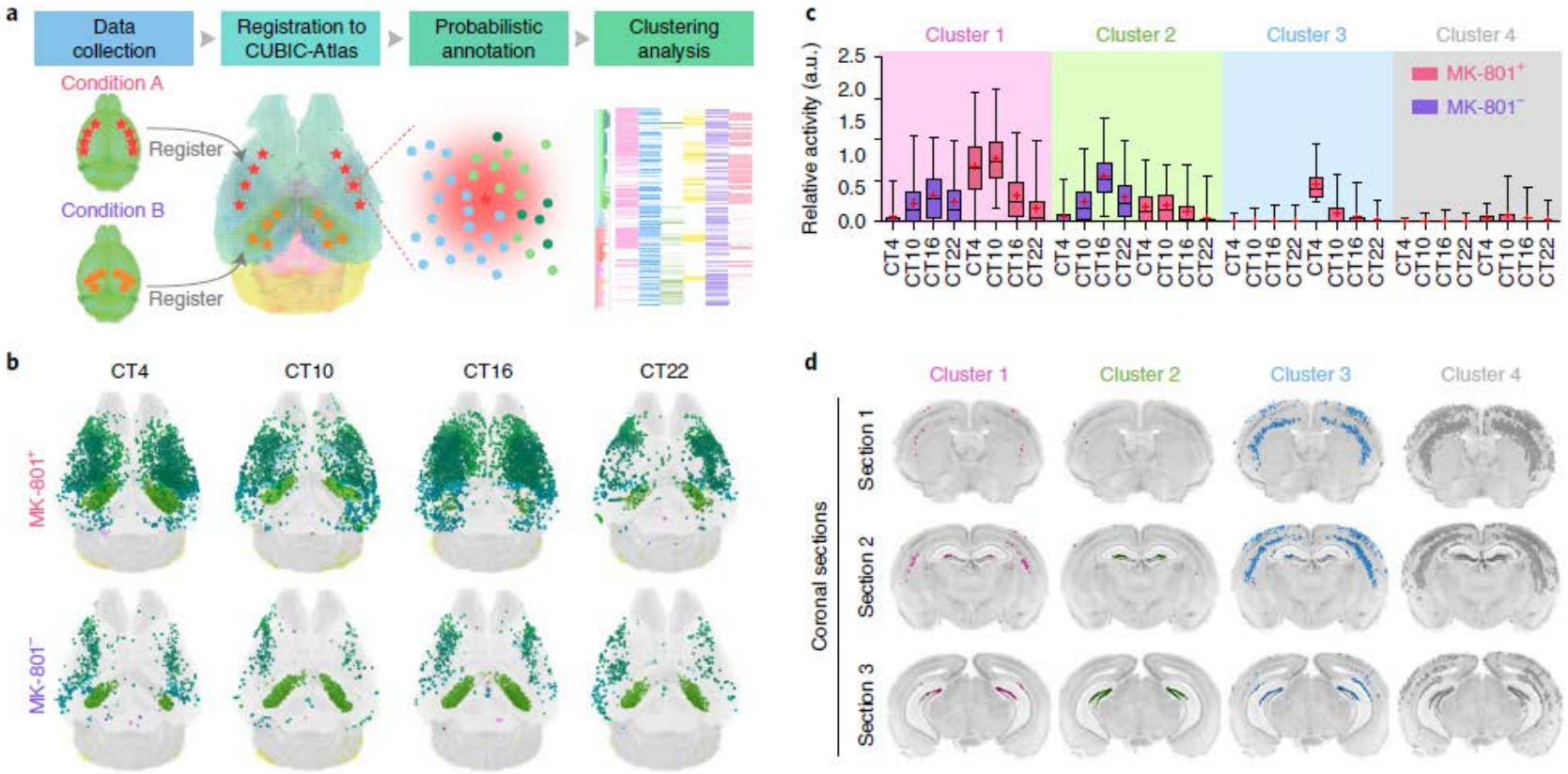
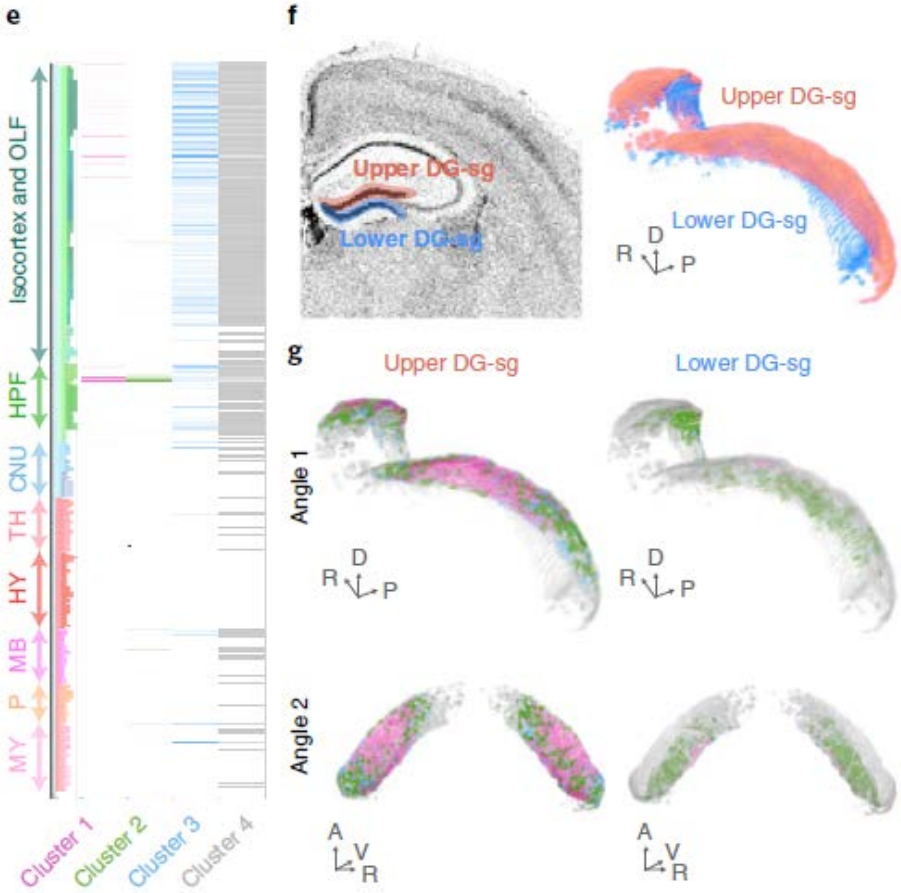


Probabilistic annotation revealed a functionally distinct structure

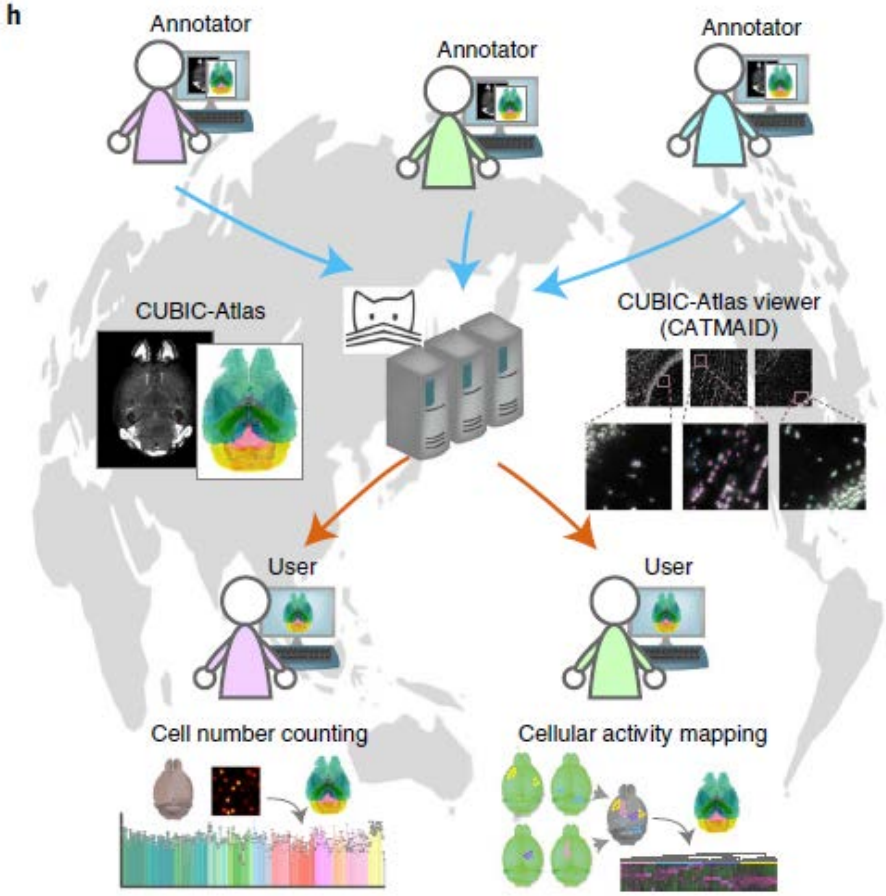


Probabilistic annotation revealed a functionally distinct structure

- Cells of cluster 1 + 2 localized to the dentate gyrus
- Mapping clusters revealed functional differences between the upper and lower DG-sg
- Indicating previously uncharaterized functionally distinct areas



Collaborative Annotation Toolkit for Massive Amounts of Image Data (CATMAID)



Conclusion

- Reference atlas is based on neuroanatomical definitions found in the Allen Institute mouse reference atlas
- Enables seamless imaging of the whole mouse brain at sub-cellular resolution
- CUBIC-atlas reduces huge data sets to portable data size
- Present a new clearing method
- Establish a universal data-sharing platform for CUBIC-atlas

Thanks!

Murakami et al., 2018. *Nature Neuroscience*