BioTechniques Spotlight

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eBook: Imaging in neuroscience

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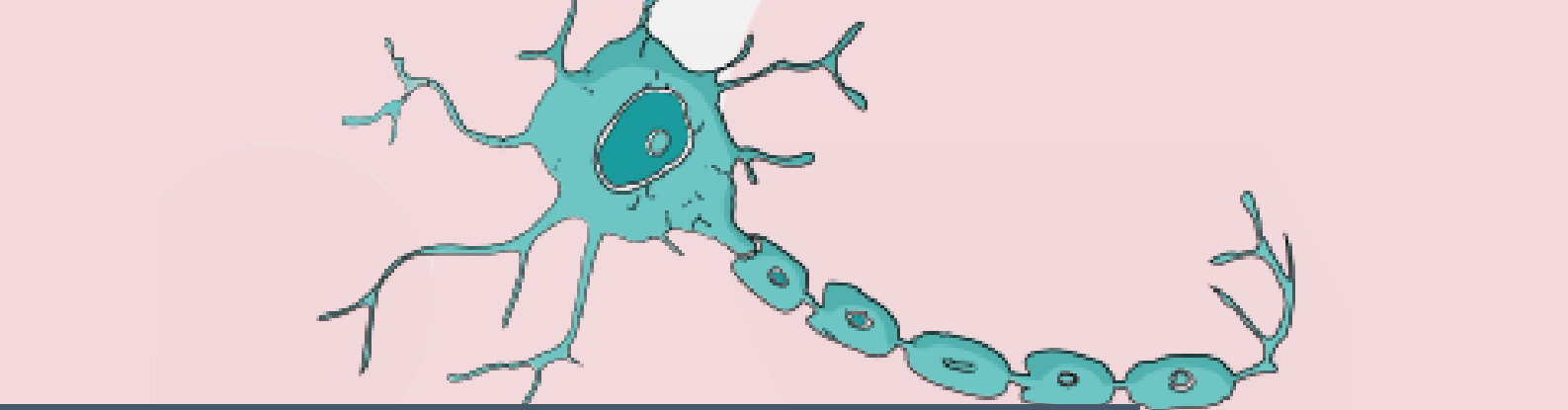

eBook: Imaging in neuroscience

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Introduction

Imaging methods help neuroscientists understand the structure and function of the central nervous system, from cell-to-cell interactions and signal transduction to regional brain connectivity and differences between varying functional areas. Understanding these gives us insight into various functions, such as speech and memory, and how these are impacted in neurological disorders, including dementia and Alzheimer's, which have become more common as the population ages.

There are a wide range of neuroimaging methods, from non-invasive methods that are utilized in clinical settings, such as magnetic resonance imaging (MRI) to basic research techniques that are carried out on model organisms, such as fluorescence microscopy. Over the past decade, the field has seen advancements and the development of new technologies that take imaging that step further. For example, spatial and single-cell tools allow researchers to build a complete picture of the brain in multiple dimensions, and deep learning models enable images to be analyzed to a level that was not possible before.

This eBook rounds up key features from our Spotlight on imaging in neuroscience, exploring both well-established and cutting-edge techniques, how they can be used to better understand the brain and advances in the field.

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One scan is all it takes: diagnosing Alzheimer's disease

Researchers have trained a machine-learning algorithm to recognize signs of Alzheimer's disease in magnetic resonance imaging of the brain.

Beatrice Bowlby, Assistant Editor, *BioTechniques*

Marianna Inglese, Eric Aboagye, and fellow researchers at Imperial College London (UK) have utilized magnetic resonance imaging (MRI) and machine learning to recognize features, such as the texture, shape and size, of different areas of the brain to assist with the early diagnosis of Alzheimer's disease (AD). Although no treatment currently exists, the early detection of AD following a single MRI scan allows those affected to seek help with their symptoms and prepare for their future.

AD is massively prevalent in the UK and has quickly become a focus of research groups across the globe due to its devastating impact on an individual and their loved ones. Current diagnostic tests, such as memory and cognitive tests and brain scans, take a long time to plan, administer and analyze. However, training a machine-learning algorithm to automatically analyze a single MRI scan and pick out areas that are exhibiting similarities to an AD brain in just moments, accelerates the diagnostic process.

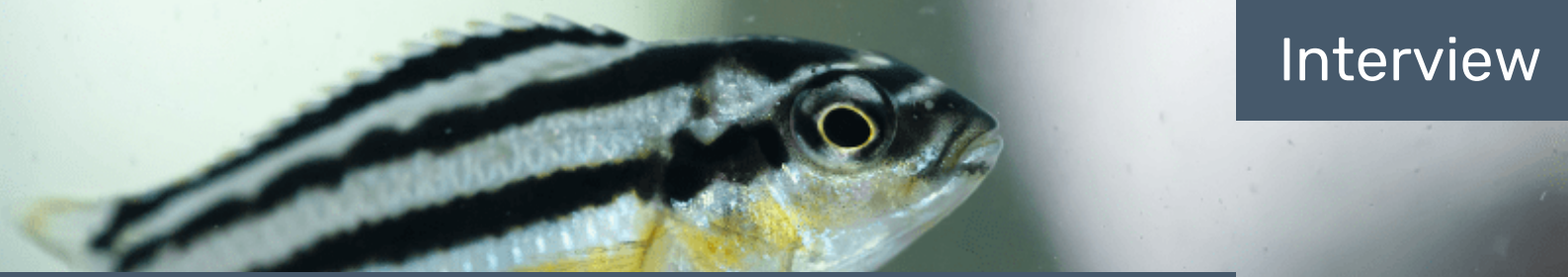
Researchers sectioned the brain into 115 areas, each of which was associated with 660 features. The algorithm was then trained to recognize when these brain features resembled AD characteristics from a single MRI scan. Adapted from a cancerous tumor classification algorithm, their algorithm was tested using MRI scans provided by the Alzheimer's Disease Neuroimaging Initiative from more than 400 patients with early- and late-stage AD, patients with other neurological diseases and healthy brains. Additionally, current patients' brain scans were also tested.

This MRI and machine learning diagnostic tool successfully predicted whether the patient had AD in 98% of cases. In 79% of patients, it was also able to deduce whether the patient had early- or late-stage AD. Lead researcher Eric Aboagye commented, "Currently no other simple and widely available methods can predict Alzheimer's disease with this level of accuracy, so our research is an important step forward. Many patients who present with Alzheimer's at memory clinics do also have other neurological conditions, but even within this group our system could pick out those patients who had Alzheimer's from those who did not."

Combining this machine learning technology with commonly used brain scanning techniques has the potential to alert people with signs of AD to their options, such as clinical trials of novel drugs or lifestyle changes, earlier than current diagnostics can. The algorithm has also demonstrated that there are parts of the brain that seem to be affected by AD that previously were not recognized as affected regions; further research can investigate the role these regions play in AD. Overall, this new technique for combining machine learning and MRI scans may take us one step closer to understanding AD and eventually finding an effective treatment.

Source

Inglese M, Patel N, Linton-Reid, K *et al*. A predictive model using the mesoscopic architecture of the living brain to detect Alzheimer's disease. *Commun. Med*. 2(70) (2022).



Interview

Imaging the retina with DIY microscopes

Tom Baden is a professor in the field of systems neuroscience, based at the University of Sussex (UK). In this interview, Baden discusses the different imaging techniques used in his research, including two-photon microscopy, and the benefits of customizing lab equipment.

Please give an overview of your research and the projects carried out in your lab.

In general, our research aims to answer the following questions: how do neurons compute? How are they connected? Why are they connected in a certain way? We try to understand this at the level of single neurons and small networks, but also hundreds of neurons together. One place to do this very nicely is in the vertebrate retina, which in some respects is pretty much the same complexity as the cortex.

The retina has a very clear computational purpose. Retinal circuits have adjusted to different environments over evolutionary times, therefore by comparing how different vertebrate retinas function we might learn something about how nervous systems evolve from a computational perspective.

The lab primarily utilizes the zebrafish due to its adaptability to *in vivo* studies, as well as being a great model organism for genetic manipulation in general. More recently we have started to expand, utilizing chickens, frogs and even sharks. The big hope is that by looking very broadly, but at a large scale, we might identify general principles that have been missed because we've been looking too closely in one or two animals.

Our projects tend to focus on color vision, as its arguably one of the simpler things the retina does. You need to establish the wavelength of light, independent of the intensity of light, and as we can only see a certain range of wavelengths, which is defined by the cone photoreceptors that an animal has, it's a reasonably small dimension. From an evolutionary perspective, color vision is very old. As the vertebrate eye first evolved, it probably already had a basic capacity for color vision, and then all the spatial temporal processing that we take for granted today was built on top. In a way, color vision is like the soil of the retina; somehow, it's always there.

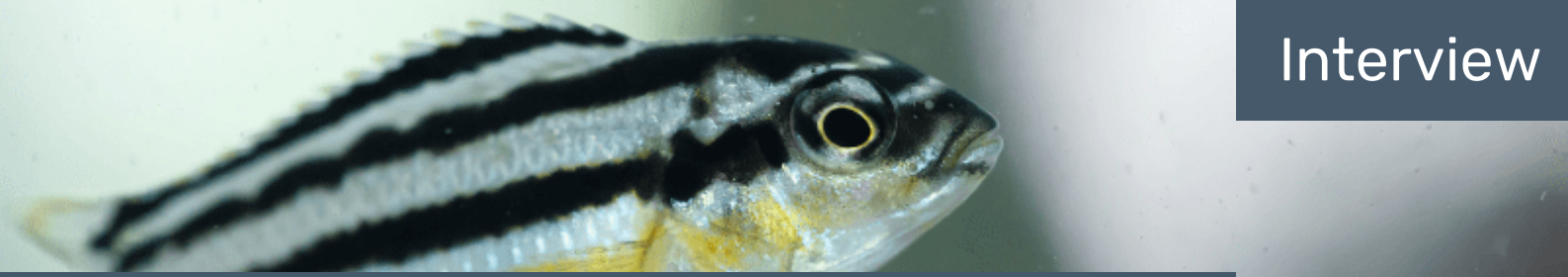
What techniques are you using in your research?

Two-photon microscopy is the main workhorse in the lab. This is because the retina does not see this microscope's infrared excitation light very well. Although you are using a light sensitive tissue, you still need excitation light to do anything.

It is possible to reach every neuron in the retina because it is transparent and thin. There is even enough depth penetration to do *in vivo* work in the retina of a live zebrafish. People have started to be able to do this with other species using adaptive optics, complex lens systems that lead up to the eye, but this is not yet a routine technique.

The downside of two-photon, and fluorescence microscopy in general, is that unless you've artificially introduced something that fluoresces, there's nothing to look at. Therefore, species whose genomes are relatively easily to manipulate – zebrafish, mice and even primates to an extent – are usually easier to use. The next big one is probably amphibians; however, people are also increasingly improving the genetic access of species of fish that aren't zebrafish.

For larger species, you probably want to be working with electrophysiological techniques. However, if you want to measure many cells you need many electrodes. This is known as electrical imaging, or a multi electrode array recording, and it is commonly



Imaging the retina with DIY microscopes

used with the retina as the sheet-like organization of cells means that it is possible to record from many cells in parallel. The problem with this technique is that you don't know what happens upstream, and you can't easily record from photoreceptors, as they don't generate action potentials.

You build and customize a lot of your lab equipment, are there any examples that have particularly helped with imaging?

Scientists have always had to build equipment. At some point, companies understood that if they could build standard tools, they could earn money. Nowadays, most of the tools in the average lab are bought; however, there is always going to be customization.

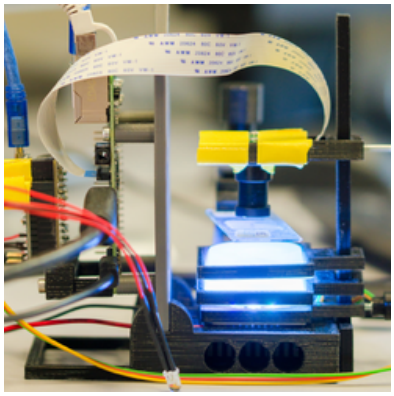
The level of customization varies between labs, but there is always something. In our case, the two-photon microscope is customized, but it is built starting from the blueprint of something that you purchase.

One piece of equipment we have built is a UV projector to maximize visuals when working on zebrafish. We bought a projector, replaced the lights, and optimized our optics for UV. However, this still presented a problem because the projector projected lots of visible light, swamping the detectors of the microscope. So, we then had to interface the microscope with the projector too, to time separate stimulation from recording.

These types of nuances are too niche to be solved by industry, meaning you can't really do the research unless you build them yourself. In the last 10 years, we've seen an increase in people using standard parts to build equipment, and publish this in a paper or online repository, so others can replicate. This is what we broadly refer to as open hardware, which is a relatively recently popularized term.

For us it started with 3D printing, making equipment such as pipettes. We found an example in an online repository, improved it slightly and put ours online, and then someone else improved it. If you look at 3D printable pipettes now, that have been improved over a number of years, some are arguably equivalent to pipettes you can buy. Often other people are interested in improving an open hardware device in a shared collaborative way.

Microcontrollers are becoming increasingly used in open hardware. They allow you to interface your computer with some electronics, enabling you to, for example, control an LED using your computer.



Fly Pi. Image courtesy of Tom Baden.

We used a similar but slightly more powerful device than a microcontroller, called a Raspberry Pi, to create a camera system and optical and thermal control circuits for monitoring groups of model organisms like zebrafish, which we called 'Fly Pi'. When we published the paper, it was fairly popular, not because it performed particularly well (it performed okay), but because it showed that it wasn't terribly difficult to create something like this, and for very little money.

I think we're starting to see the possibility of building your own microscope, rather than buying it, without sacrificing performance. It was less than 10 years ago that we started playing around with 3D printers, and the landscape has changed dramatically since then. I have no idea what the next 10 years are going to bring!

What are the main challenges that you face in your research?

The type of research carried out in this field doesn't always have a big hypothesis to test, which doesn't

Imaging the retina with DIY microscopes

necessarily conform to the way the traditional grant system is set up to work. It's therefore incredibly difficult to get a government funding agency to fund, for example, research into understanding how the chameleon retina works simply because it would be useful to know. So, the main problem I am facing is that I need to adjust the questions that I want to ask to make them fundable. As a consequence, I write a lot of grants, many of which don't get funded. I think there's a systemic failure in our system in the way that money for research gets allocated. That's the main challenge I see.

What is your main focus as you look to the future?

I want to get deeper into questions about evolution, although there isn't really a specific field that mixes paleontology and neuroscience at this point in time. I believe we're entering a time where by combining comparative neuroscience, molecular tools, and insights from classical paleontology, you can start to understand how the brains of extinct animals may have been organized.

Comparing the circuit motifs of extant species that are related at different distances from these extinct animals will help to place the extinct animal amongst its relatives. I guess we're trying to jump across disciplines a bit, and I think that's exciting.

BioTechniques

X MARKS THE SPOT: MAPPING THE BRAIN WITH EEG AND MRI

In this month's Technology News, we go back to the basics of two neuroimaging techniques and look at how these have been advanced in 2022.

Before the arrival of neuroimaging, the brain could only really be studied at autopsy or after accidents with a sort of 'spot-the-difference' approach to someone's behavior. For example, in 1953 we learned about the importance of the hippocampus for normal memory development from Patient HM following an experimental procedure to alleviate his seizures [1].

Some neuroimaging techniques date back almost a century and continue to be developed and tailored for specific applications in both research and clinical practice. Our Assistant Editor, Aisha Al-Janabi, takes a quick glance back at the origins of two commonly used neuroimaging techniques, electroencephalogram (EEG) and magnetic resonance imaging (MRI), and looks at some novel uses of them.

EEG

EEG is a non-invasive technique to measure electrical activity in the brain with electrodes placed on the scalp – up to 256 electrodes for a high-density scalp array [2]. EEGs have a high temporal resolution, meaning they can pick up frequencies quickly; however, they have a poor ability to pinpoint where exactly in the brain the electrical activity is taking place due to poor spatial resolution.

Hans Berger (University of Jena, Germany) is largely considered the father of the EEG. [2] In 1924, Berger first recorded brain activity by placing silver electrodes under the scalp of patients with skull defects. He continued to develop this method and in 1927 connected the electrodes to the scalp with a rubber bandage, to non-invasively record the electrical activity of a human brain in a similar way to current EEGs [3]. Using this technique, Berger studied how electrical activity changes during periods of mental activity and sleep, and how electrical waves differ around a tumor. Berger published the first paper on EEG in 1929 [4].

This technique continues to be used in both clinical practice and neuroscience research and is used in ways that in 1929 Berger probably could not have imagined – like monitoring brain activity more than 62 vertical miles away, up in space.

EEGS UP IN SPACE

Researchers at Aarhus University (Denmark) have developed a discrete in-ear EEG (ear-EEG), which looks like an earphone, to monitor astronauts' brain activity and study the differences between sleep patterns on earth and out in space (Figure 1) [5].

As sleep stages are related to brain states, sleep can be assessed from the electrical signals recorded by EEGs. The ear-EEG measures small changes in voltage on the surface of the skin inside the ear, which is caused by electrical activity from the brain's neurons.

"We've been working on developing ear-EEG technology for more than 15 years as a way of measuring electrical activity from the brain outside a laboratory," explained Preben Kidmose, the head of the Aarhus University's Centre for ear-EEG. *"This technology gives us a unique opportunity to conduct long-term measurements of brain activity. And that means we can begin to study things that we otherwise wouldn't be able to measure."*

Spending time in space means living with an artificial day-night cycle, leading to difficulties getting a good 'nights' sleep, something which astronauts often complain about. Kidmose explained: *"Sleep is a kind of biomarker for our health and well-being. In fact, a great many diseases also impact the way we sleep, including a wide range of psychiatric disorders and neurodegenerative diseases. In general, however, there is no clear correlation between experienced sleep quality and physiological sleep. But physiological sleep is what's crucial to our cognitive function."* Currently, there isn't much understanding about how space affects astronauts' sleep physiologically.

The discreet nature of the ear-EEG makes it ideal for long-term monitoring. It will be sent into space along with Danish astronaut Andreas Mogensen when he travels to the International Space Station, planned to launch in September 2023 [6], to get a better understanding of sleeping in space and ensure the best possible conditions for astronauts. ▶

Figure 1. Ear-EEG technology.
Photo: Lars Kruse, AU Photo.

MRI, BUT MAKE IT FUNCTIONAL

Unlike EEG, MRI has good spatial resolution; however, it has poor temporal resolution and responds to changes in the brain relatively slowly. Paul Lauterbur (University of Illinois at Urbana-Champaign, IL, USA) and Peter Mansfield (University of Nottingham, UK) were awarded a Nobel Prize for Medicine in 2003 for their contribution to MRI scanners in 1971, which was a culmination of the work from many researchers, scientists and doctors [7].

MRI continued to be improved and functional-MRI (fMRI) entered the scene in the 1990s, with groups led by Seiji Ogawa (University of Tokyo, Japan) [8] and Ken Kwong (University of California, CA, USA) [9] independently leading the research effort. This is a method that indirectly measures neural activity by detecting changes in blood flow and is a blood oxygenation level-dependent (BOLD) technique [10]. Blood flow can be used as a proxy for neuronal activity because as neuronal activity increases, there is a greater demand for oxygen in that part of the brain leading to increased blood flow. As oxygenated and deoxygenated hemoglobin respond differently to the applied magnetic field, different MRI signals arise.

HIJACKING FMRI PRINCIPLES FOR CALCIUM MONITORING

Now, researchers at MIT (MA, USA) have hijacked the principle of fMRI and instead of indirectly measuring neuronal activity through blood flow, they have developed a novel probe to do so by monitoring calcium [11,12]. This probe means that individual populations of neurons can be monitored to see how they interact with one another to perform specific tasks.

An influx of calcium is caused by neurons receiving neuronal signals and leads to the release of nitric oxide, which acts as a vasodilator to increase blood flow. Hence, calcium can be used to indirectly study brain activity. Alan Jasanoff, the senior author of the paper, compared individual neurons to gears in a clock and explained: *"With regular fMRI, we see the action of all the gears at once. But with our new technique, we can pick up individual gears that are defined by their relationship to other gears, and that's critical for building up a picture of the mechanism of the brain."*

Normally, measuring calcium signals requires fluorescent chemicals in an invasive procedure, so the researchers developed a non-invasive method using a genetically targeted MRI probe.

The researchers engineered a genetic probe from the nitric oxide synthase enzyme, which codes for a protein they call NOSTIC (nitric oxide synthase for targeting image contrast). This protein detects elevated levels of calcium and generates nitric oxide, resulting in artificial fMRI signals from cells that contain NOSTIC. Neurons without NOSTIC genetic modifications also generate fMRI signals, so this experiment is performed twice: once with the probe and once with a drug that inhibits the probe. This allows the researchers to determine the activity present in probe-containing cells specifically.

The genetic probe is encapsulated in a virus and injected into a specific population of cells, which then travels along the neurons' axons so every neural population that feeds into the injected location is labeled. *"When we use this virus to deliver our probe in this way, it causes the probe to be expressed in the cells that provide input to the location where we put the virus,"* said Jasanoff. *"Then, by performing functional imaging of those cells, we can start to measure what makes input to that region take place, or what types of input arrive at that region."*

STUDYING BRAIN NETWORKS

The researchers utilized this fMRI probe to label populations of neurons from the striatum, part of the brain involved in planning movement and responding to rewards. The researchers studied the neuronal response of deep brain stimulation, a method where electrodes are implanted in the brain and produce electrical impulses. They performed this in the lateral hypothalamus (involved in appetite and motivation) in rats and could identify the neural populations that send an input to the striatum either during or immediately after a reward stimulus. It was not known how wide-reaching the effects of deep brain stimulation in the lateral hypothalamus were, but with this technique, the researchers found that neural populations in the motor cortex and entorhinal cortex also send signals to the striatum. *"It's not simply input from the site of the deep brain stimulation or from the cells that carry dopamine,"* said Jasanoff. *"There are these other components, both distally and locally, that shape the response, and we can put our finger on them because of the use of this probe."*

The researchers call this method 'hemogenetics' and hope to study other networks in the brain with it, including identifying which regions receive input from the striatum after deep brain stimulation.

"One of the things that's exciting about the approach that we're introducing is that you can imagine applying the same tool to many sites of the brain and piecing together a network of interlocking gears, which consist of these input and output relationships," Jasanoff said. *"This can lead to a broad perspective on how the brain works as an integrated whole, at the level of neural populations."*

Neuroimaging has certainly come a long way in the past century and is opening up new avenues of what we can visualize in brains, and we're only just scratching the surface. The technology used made leaps and bounds since Patient HM and we can now non-invasively study the brain in many ways whilst people perform tasks or are asleep (even up in space!). Let's see where the next 70 years take the field.

Written by Aisha Al-Janabi

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Resolving brain architecture with comprehensive spatial gene expression

Introduction

Spatially resolved gene expression can provide a powerful complement to traditional histopathology methods, enabling a greater understanding of cellular heterogeneity and organization within the central nervous system. Here, we show how integrated sequencing and histological data from the Visium Spatial Gene Expression assay enabled unbiased clustering of cell types that reliably correlates with the neuroanatomy of both rodent and human specimens. We also demonstrate the addition of immunofluorescence for the simultaneous examination of protein and gene expression from the same tissue, which enables a deeper, more complete assessment of brain architecture in a single experiment.

Highlights

- Characterize discrete neuroanatomical brain regions
- Map expression of genes to specific brain regions or structures
- Visualize spatial patterns of gene expression together with protein detection by immunofluorescence (IF) on the same tissue section

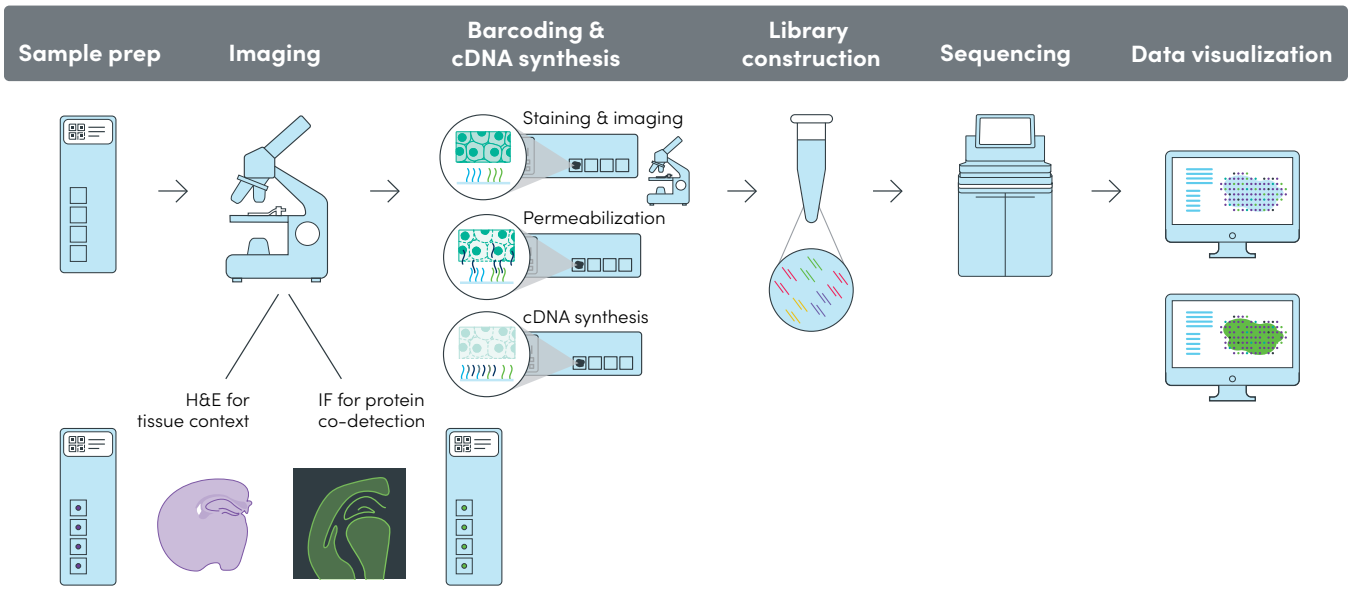

Figure 1. Experimental methods for Visium Spatial workflows. For Visium Spatial Gene Expression analyses, fresh-frozen tissues (mouse hippocampus, rat olfactory bulb, and human cerebellar tissue) were embedded in optimal cutting temperature (OCT) compound and cryosectioned. Sectioned tissues were then placed on the Capture Areas of a library preparation slide, fixed, and stained according to either the Demonstrated Protocol: Methanol Fixation, H&E Staining & Imaging for Visium Spatial Protocols (Document CG000160) or Demonstrated Protocol: Methanol Fixation, Immunofluorescence Staining & Imaging for Visium Spatial Protocols (Document CG000312). Stained tissues were imaged following Visium Spatial Gene Expression Imaging Guidelines (Document CG000241). After imaging, the tissues were permeabilized, cDNA was then synthesized from captured mRNA, and sequencing libraries were prepared following the Visium Spatial Gene Expression Reagent Kits User Guide (Document CG000239). The resulting libraries were then sequenced on a NovaSeq 6000, analyzed using the Space Ranger pipeline, and visualized on top of their respective tissue images in Loupe Browser.

Results

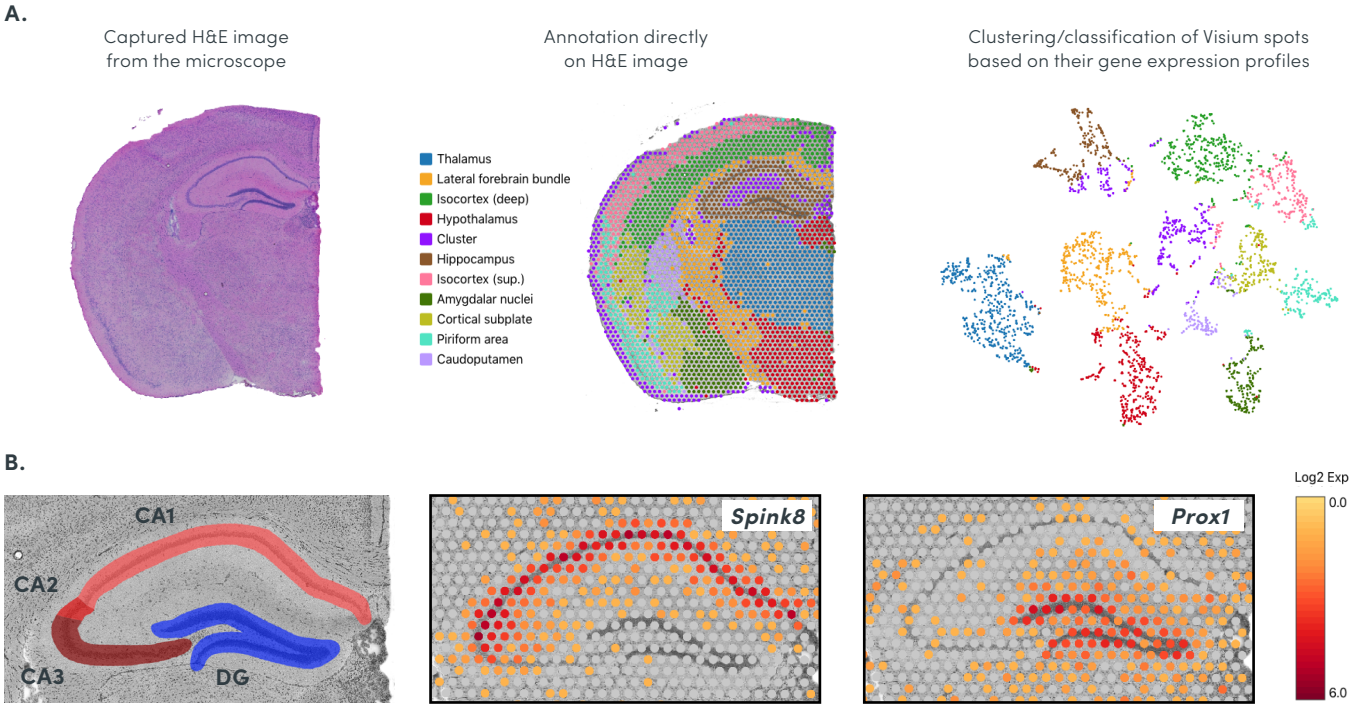

Figure 2. Spatial gene expression patterns recapitulate brain tissue architecture. **A.** Left: Brightfield H&E image. Center: Overlay of H&E staining and graph-based cluster assignment visualized in Loupe Browser comparing unbiased gene clustering of RNA transcripts (from Space Ranger) with neuroanatomy of the tissue section. Right: The t-SNE projection of the sequencing data illustrates cell-type clustering based on anatomical regions. **B.** Expression levels (Log2 fold change) of known hippocampal genes *Spink8 and Prox1*, which show specific localization in the cornu ammonis (CA1 and CA2) and dentate gyrus (DG), respectively.

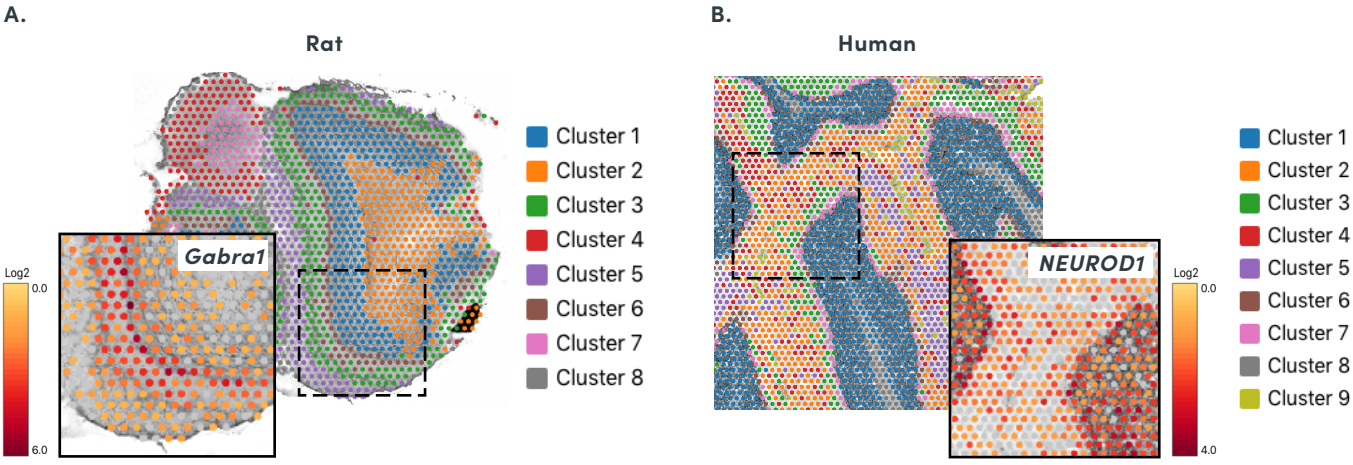

Figure 3. Visium Spatial Gene Expression is compatible with rat and human neural tissue. **A.** Spatially resolved clustering of the Sprague-Daley rat olfactory bulb with a demonstration of *Gabra1* expression (Log2 fold change) corresponding to the external plexiform layer (inset). **B.** Spatially resolved clustering of human cerebellar tissue from a female patient (BioIVT: Asterand) with a demonstration of *NEUROD1* expression (Log2 fold change) corresponding to the granule cell layer (inset).

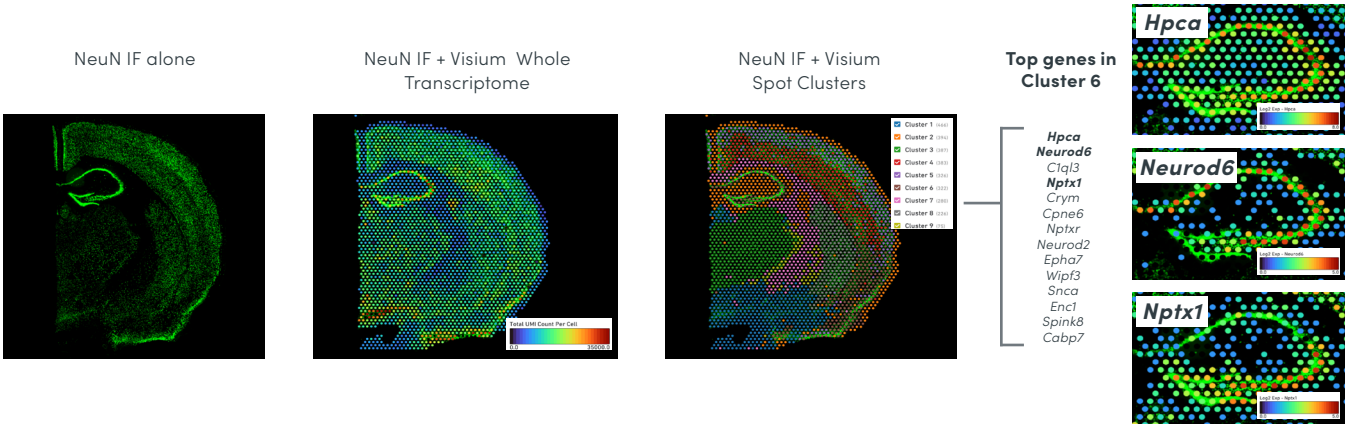

Figure 4. Spatially resolved clustering based on simultaneous gene expression and protein detection in the mouse brain. A mouse brain section was immunofluorescently labeled to visualize NeuN, and then processed through the Visium Spatial Gene Expression workflow. Shown are an immunofluorescence-only image (IF), an IF image overlaid with Visium data containing total UMI counts, and an IF image overlaid with Visium data for spatially naïve clustering based on total differentially expressed genes. The top 10 genes that are more highly expressed in Cluster 6 are shown to the right along with example images.

What to look for

Each Capture Area on a Visium Spatial Gene Expression slide contains 5,000 barcoded spots, and each spot, containing millions of capture oligonucleotides with spatial barcodes unique to that spot, captures mRNA from the cells in the tissue section above it. Graph-based clustering is performed on the sequencing data via Space Ranger to segregate each spot based on gene expression. Spots in the same gene expression–based cluster are the same color. Clusters can be visualized spatially by overlaying the gene expression data on the histological image of the tissue section (Figures 2–4). The Visium Spatial Gene Expression assay has diverse sample compatibility with mouse (Figures 2 & 4), rat (Figure 3), and human (Figure 3) tissues. A full list of compatible tissue types can be found on our support site.

As shown in Figure 2, the brown cluster annotated as hippocampus overlaps with the distinctive hippocampal structures identified in H&E. Alternatively, the whole transcriptome data can also be represented as a t-SNE plot showing the distance between gene expression–based clusters (Figure 2, Panel B). While a traditional single cell t-SNE plot is made up of dots representing single cells, with Visium Spatial Gene Expression, each dot represents a tissue-covered spot that may include mRNA from one to ten cells on average. In addition to spatial localization, Visium Spatial Gene Expression provides a quantitative measurement of gene expression, letting you compare and differentiate across samples, specific brain structures (Figure 2, Panel C), and treatments. When using Visium Spatial Gene Expression with Immunofluorescence (Figure 4), the spatial patterns of gene expression can be visualized together with cell type specificity, providing a new perspective on tissue complexity.

See how researchers used spatial gene expression to build a molecular atlas of the adult mouse brain.

Explore what you can do

Gaining insights into the normal, developing, or diseased brain requires a comprehensive understanding at both the cellular and molecular levels. More critically, this information needs to be understood within the biological context in which it occurs.

Visium Spatial Gene Expression combines traditional histopathology with unbiased, high-throughput gene expression analysis from the same tissue section at high resolution and sensitivity. This allows you to generate spatial clustering that reliably correlates with the neuroanatomy of intact tissue, across different mammalian brain regions. The addition of immunofluorescence staining enables the simultaneous examination of protein and gene expression from the same tissue, providing additional insights. With Visium's whole transcriptome and protein co-detection approach, you can:

- Gain a new perspective on cell-to-cell interactions with spatial context
- Characterize cellular sub-types and functional states
- Discover regional cell heterogeneity throughout the brain
- Uncover new insights into neural disease and neuroinflammation

Resources

To explore these and other Visium mouse data further, download the following annotated datasets:

Adult mouse brain with H&E
coronal; sagittal-posterior; sagittal-anterior

Adult mouse brain with IF
GFAP and NeuN antibodies

Contact us
10xgenomics.com | info@10xgenomics.com

LIT000111 - Rev A - Data Spotlight - Resolving brain architecture with comprehensive spatial gene expression

Alzheimer's fabrication controversy

In July 2022, Science revealed that an Alzheimer's research paper from 2006, detailing the toxicity of an amyloid fragment, may have contained fabricated images (read more about it here).

We asked Becky Carlyle (University of Oxford, UK), Mark Dallas (University of Reading, UK) and John Hardy (University College London, UK) to provide insight into what this allegation means for Alzheimer's disease (AD) research, drug development, scientific publication and the public.

Where did you stand on the amyloid-beta (Aβ) hypothesis of Alzheimer's before this allegation was publicized?

Becky Carlyle

As a relatively late entrant to the AD research field, really immersing myself in it when I joined Massachusetts General Hospital (MA, USA) in 2017, I've tried to take a nuanced view of the importance of the Aβ hypothesis. There was strong initial evidence for this hypothesis from cell biology and genetics, and it makes common sense that having large deposits of insoluble protein hanging around in your cortex is probably not great for cortical function.

However, the presence of a very significant number of resilient individuals in the wider population (people with a high load of amyloid plaques but little to no cognitive impairment) certainly suggests that amyloid is not the only factor contributing to cognitive decline in the aging population. The reality is likely to be a subtle mix of genetic susceptibility, lifestyle risk factors and environmental exposures, accumulated across a long lifespan.

Mark Dallas

Prior to these revelations, our research has looked at the toxicity of the Aβ peptide in driving neurodegeneration evident in AD. I do not believe it is the sole contributing factor to the disease, but it has a role to play. Here, more research is key to understanding its role and what impact targeting it can have on those living with AD. Our research has moved to examine common pathways across brain diseases and those that occur prior to amyloid accumulation. These might prove more fruitful in our efforts to combat AD and understand the changes that take place in our brains prior to diagnosis.

John Hardy

When I started working on AD, there were very few people in the field, and there were a number of different ideas. I was a neurochemist, and I was looking to see exactly which nerve cells were lost for transmitter replacement therapies. Inspired by Gusella's 1983 paper, which investigated where and how Huntington's

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disease starts, I realized we should try and understand how AD starts.

When I came to London to work at St. Mary's (UK), with the clinician Martin Rossor, we started to collect families with AD. In one of those families, we found the amyloid gene mutations. I wrote two very simple reviews. One with David Allsop, reporting that amyloid was how the disease started, and one with Gerald Higgins, which detailed the amyloid cascade hypothesis.

The amyloid theory is based on the observation of the pathology in the brain, but it's mainly based on the genetics. Lesné's paper suggests a particular form, which is 12 molecules of amyloid stuck together, is the toxic species that leads to disease. I think the hypothesis has a lot of truth to it, and I think that the evidence says that amyloid is where the disease starts.

How did these revelations change your perception of the hypothesis?

Becky Carlyle

While not wishing to minimize the severity of the likely fabrication of data in this particular case, which is appalling, I believe the reporting of these revelations has also been extremely irresponsible. In all honesty, as someone who joined the field 5 years ago, I had not heard of the Aβ*56 peptide. Since these revelations, I've read statements from a number of people who were in the field around the release of this data who had tried and failed to replicate this work and then moved on. These failures to replicate were not published, which is a large problem that all researchers face; it's difficult to publish negative results and isn't hugely beneficial to your career progression. I don't believe, therefore, that the existence of this particular peptide really swayed the amyloid hypothesis one way or another and certainly this potential data fabrication does not change the strong original evidence for the amyloid hypothesis.

Mark Dallas

These allegations do not change my perceptions of the hypothesis, merely that an author had misled others through their deceit.

John Hardy

I have to say when that paper came out, I was not convinced by it. I don't think I've ever cited it. I don't think it's particularly important. I don't think it says anything about the amyloid hypothesis. It didn't really fit with what my view of the disease was, and it's strictly irrelevant to my research.

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What does this allegation mean for amyloid-based Alzheimer's research conducted between 2006 and now?

Becky Carlyle

In reference to my answer to the previous question, I believe the field would have looked mostly the same. The vast majority of funding awarded that relied on the amyloid hypothesis was not awarded as a result of this work, and the hypothesis that removal or reduction of amyloid from the brain may help disease progression was worthy of pursuit. The failure of Aduhelm and secretase inhibitors – the enzymes that produce Aβ peptides and many other peptides, too – to contribute meaningfully to protection against cognitive decline was of far greater impact to this field than the fabrication of these papers.

Mark Dallas

I don't think these allegations have a massive impact on the amyloid field. It certainly has not tainted other research efforts which have led to clinical trials to modulate Aβ production. The studies in question focused on the Aβ*56 species, suggesting it was pivotal in the cognitive decline observed in animal models. Other researchers have not been able to replicate these finding or detect the specific form of Aβ in question.

John Hardy

The paper containing potentially fabricated images has been cited 3,000 times. So, although it hasn't influenced my work at all, it's influenced other people. I'm sure that there have been multiple grant applications and multiple labs trying to replicate it. I'm sure it had influence. And if it was fraudulent, then that influence has been toxic; people have wasted time and money chasing it down.

What is the impact on funding and drug development moving forward?

Becky Carlyle

My hope is that these revelations, and the failure of Aduhelm, may cause funding agencies and pharmaceutical companies to lessen their mono-focus on amyloid as the sole cause of AD. In my experience, this is already happening and is strongly reflected in the *Requests for Applications* currently being issued by the National Institutes of Health in the USA and a noticeable shift in the pharma landscape towards immunomodulation and endosomal/lysosomal biology.

I had some really interesting discussions at a recent conference with staff from Alzheimer's

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Research UK, where they mentioned that with other complex disorders associated with aging that we understand much better, such as cardiovascular disease, renal disease and cancer, there is no single causative pathway, no single diagnostic test and no single miracle treatment. Yet for some reason, in brain disorders (I found the same in molecular psychiatry), we have remained relatively mono-focused.

Is the cause amyloid, or it is tau? Why not both, with a healthy dose of inflammatory dysregulation and metabolic changes alongside? I believe this is where the field must progress, and there's tons of fantastic academic and commercial work already heading in this direction.

Mark Dallas

This will have a minimal impact going forward. Drug development programs are well established and providing valuable information to the research community. Even those that do not make clinical trials provide pieces of the jigsaw that we must complete to unravel the full complexities of AD.

John Hardy

This allegation is bad for the field. One of the things that worries me is that heads of pharmaceutical companies might be reluctant to invest in AD and amyloid treatments if they read about this potential fabrication. The same sort of thing happens on grant review bodies. You get to a grant review body, let's say there's 30 people on the grant review committee. Of that 30, maybe 25 of them will not be AD researchers. Your Alzheimer's grant will be competing with all the other grants in neuroscience. The AD reviewers might think this is a good grant, but then the other reviewers, who really don't know the field completely, won't want to give more money to researchers in AD because of this potential fabrication. It's harm by reputation rather than harm by science. So that summarizes what I think really. I'm sure it stopped people investing in amyloid. I'm sure it's done some harm.

What are the lessons taken from this situation and how can it be avoided in future?

Becky Carlyle

A lot of lessons have already been learned between 2006 and now; screening software to look for image manipulation, the provision in the supplement of non-cropped raw western blot images and improvements in the level of antibody validation required have all been adopted by the larger journals. The biggest thing I've been thinking a lot about recently is having paid peer reviewers, employed by the journals. We have a huge employment problem in science, with a tiny number of

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professor positions available and a huge swath of incredibly skilled post-docs with nowhere to go.

These post-docs are the ones looking at raw data every day, who actually run the experiments that require a critical eye in review and who know which common errors and issues to look out for. Academics are increasingly pressed for time and being asked to review for free on top of the huge workloads we already have is an enormous pressure and will lead to important flaws in papers being missed.

In the last year I have reviewed a couple of papers that have taken me well over two days to review properly, with large errors in their analysis, and when I've received the other reviewer comments post-submission, they've been a couple of sentences long. The system is broken. Professional post-doctoral level reviewers would be a really great addition to the peer review process.

There's the argument that once you leave the lab, you're too detached from the experiments to critically review them, but let's be honest, this applies to professors reviewing papers too. How many of them have actually been at the bench running the type of experiments they're now reviewing? And there's always the option to bring in an academic reviewer with a particular skill set if this is not covered by your professional staff.

Mark Dallas
I think there will need to be a greater effort at screening images submitted to journals for publication. It can be added to the workflow to act as a triage for incoming manuscripts prior to sending out for peer review.

John Hardy
This is difficult because fraud is the issue, and fraud is very difficult to prove. Universities are not set up to deal with fraud. Of course, you have to remember that people are innocent until proven guilty. Even if you see an image used twice, people could say that it was a mistake and it's not important. You can't prove that they're guilty of anything nefarious because you can't reach that standard. Universities are not really well set up to deal with that. I mean, if you found out that one of your staff members was producing fraudulent papers, you'd like to fire them. However, they've got legal rights, which include employment rights. So, it's just very difficult to deal with.

What areas of Alzheimer's research do you think are particularly promising?

Becky Carlyle
It sounds like a cliché at this point, but I think big data can be really helpful here. I think 'omics

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studies of carefully selected tissues and biofluids from individuals with AD and those who are resilient to the effects of the pathology will be very useful. Large population studies like Generation Scotland, UK Biobank and the ROSMAP projects have already revealed novel risk factors and phenomena that we'd never have seen otherwise and will continue to do so as these populations age. But we need to make sure we join the dots: the epidemiologists need to talk to the 'omics specialists, who have to be in touch with the cell biologists and clinicians, if these findings are to be translated to the clinic.

Mark Dallas
I have long been an advocate of the non-neuronal cells and think they will offer real potential to combat a range of brain diseases. The question remains when and how we should be targeting these cells to offer a real therapeutic difference to those living with AD.

John Hardy
Polypharmacy is an approach taken in nearly all complicated diseases. So, I think that eventually, for AD, we'll get to polypharmacy involving an anti-amyloid drug, an anti-tau drug and a microglial drug, perhaps. That's where we're going to end up. All of the genetics to do with late-onset risk have really been pointing at microglial biology, which almost every group is now trying to modulate.

Will this change the way we teach about imaging, analysis and critical thinking in science?

Becky Carlyle
I think there's already a shift towards an improvement. Most graduate-level students can now code and many labs are supplying analytical code with paper submissions to ensure that others can reproduce their analysis. Of course, many of the analytical packages used can be black boxes, which may also be subject to misuse or incorrect usage, so it's important to include strong teaching of basic statistical principles for all students.

Teaching the importance of sharing "raw" data, such as intact blots, and making sure that peer reviewers look at them is going to be important. We're going to be discussing these papers in our next journal club to identify red flags and features that may help lead a reviewer to uncover these practices. And ultimately, teaching people to stay sceptical as a basic tenet of scientific thinking is going to continue to be very important.

Mark Dallas
Most scientific publications reflect true observation, and these have given so much to society, but

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we should not believe all that we read or see. It is important that the next generation of scientists can critique both the science and the presentation of data in an open forum that leads to action, such as the retraction of published papers or editors voicing their concerns about an article.

John Hardy
We should always look at papers critically. This is the purpose of journal clubs. And just because a paper is in a “big” journal does not mean we should believe it.

Becky Carlyle
Alzheimer’s Research UK Senior Research Fellow at Oxford University (UK)
Dr Becky Carlyle is an Alzheimer’s Research UK Senior Research Fellow at Oxford University where she researches how molecular changes in the brain lead to neurodegenerative disease and their biomarkers. She uses induced pluripotent stem cell derived models as well as post-mortem human brain tissue.

Mark Dallas
Associate Professor in Cellular Neuroscience, School of Pharmacy at the University of Reading (UK)
Dr Mark Dallas is an Associate Professor in Cellular Neuroscience at the University of Reading. His research investigates the regulation of ion channels and transporters, with special interest in glial cells within the central nervous system. This has opened an exciting area of research pointing to their use as therapeutic targets to tackle brain disease.

John Hardy
Chair of Molecular Biology of Neurological Disease at University College London (UK)
Sir John Hardy is a geneticist and molecular biologist working at the Reta Lila Weston Institute of Neurological Studies at University College London. His research focuses on the genetic basis of neurological diseases, such as Alzheimer’s, Parkinson’s and motor neuron disease.

Click here to read the full article.

10x GENOMICS

BioTechniques Spotlight

Key trends in neuroimaging

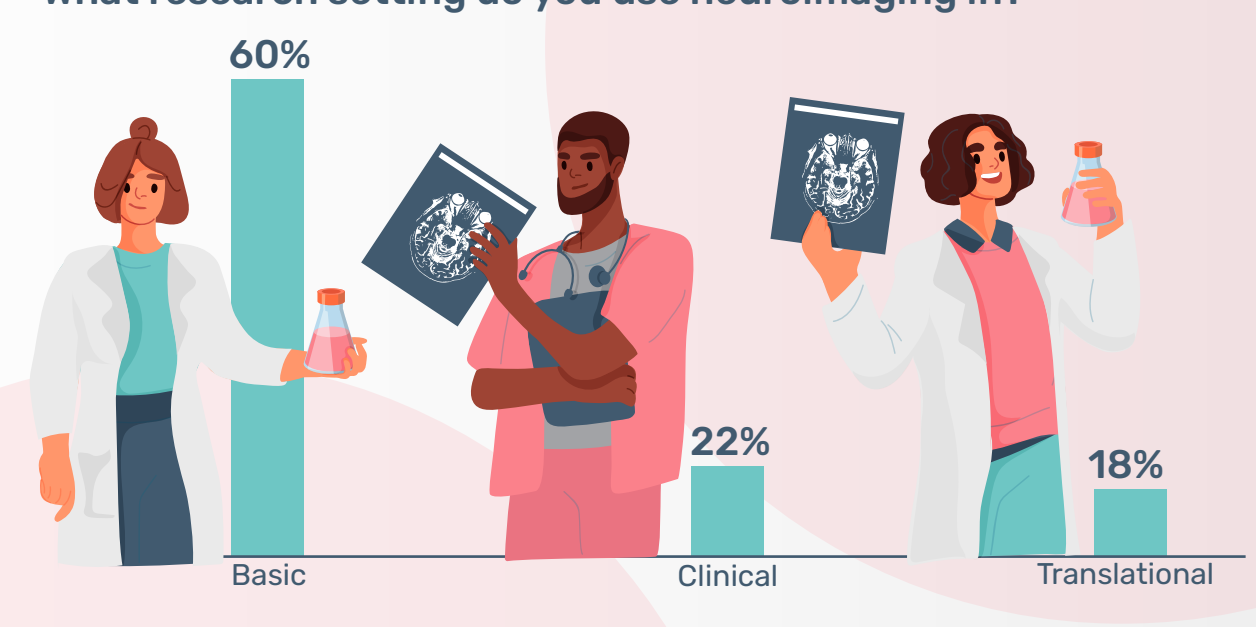
For our **Spotlight on imaging in neuroscience,** we surveyed our audience to find out the latest trends, opinions and behaviors in the field.

Imaging techniques

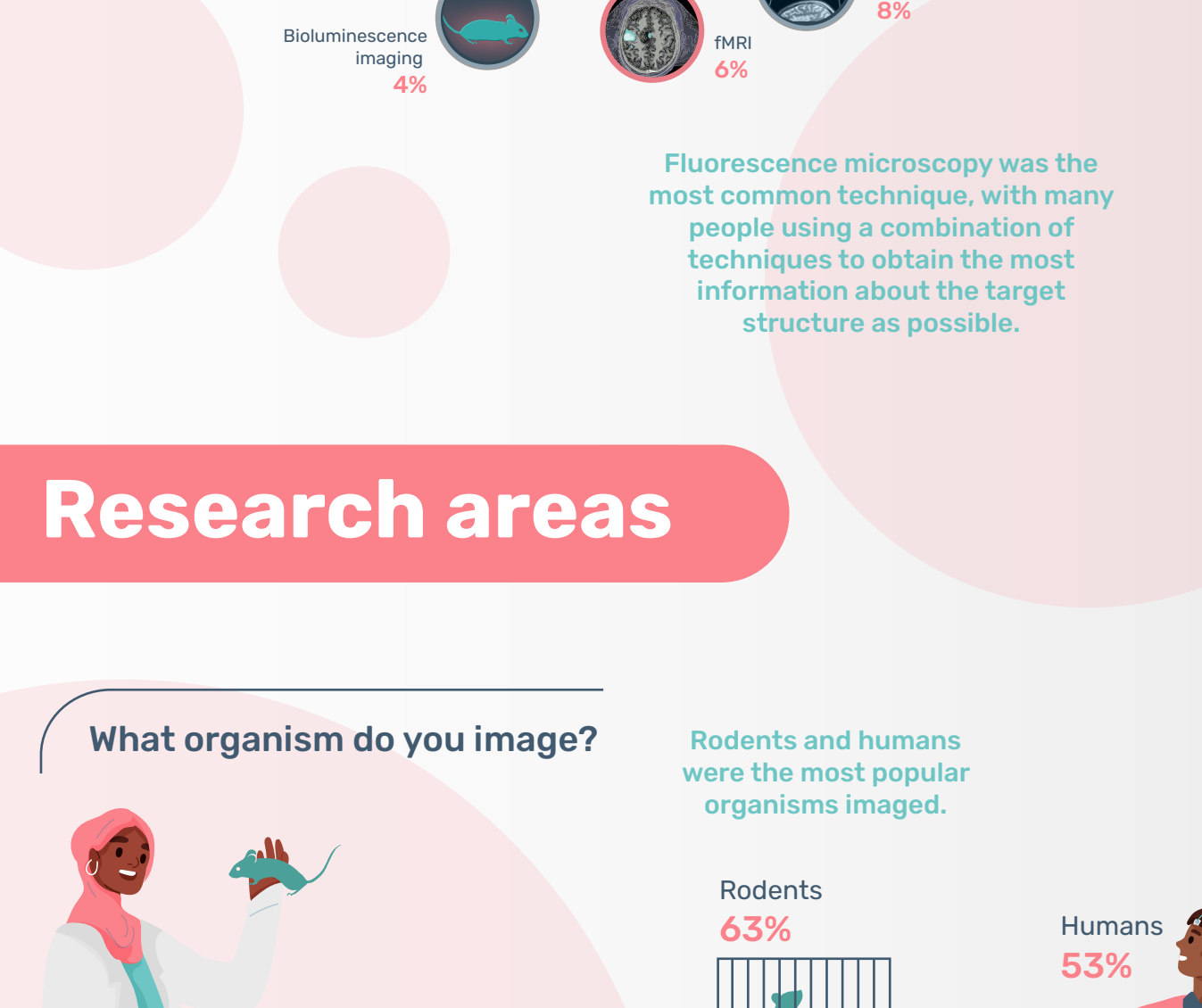
Which category does your imaging fall under?

- Structural 53%
- Functional 47%

What research setting do you use neuroimaging in?



What imaging technique(s) do you use?

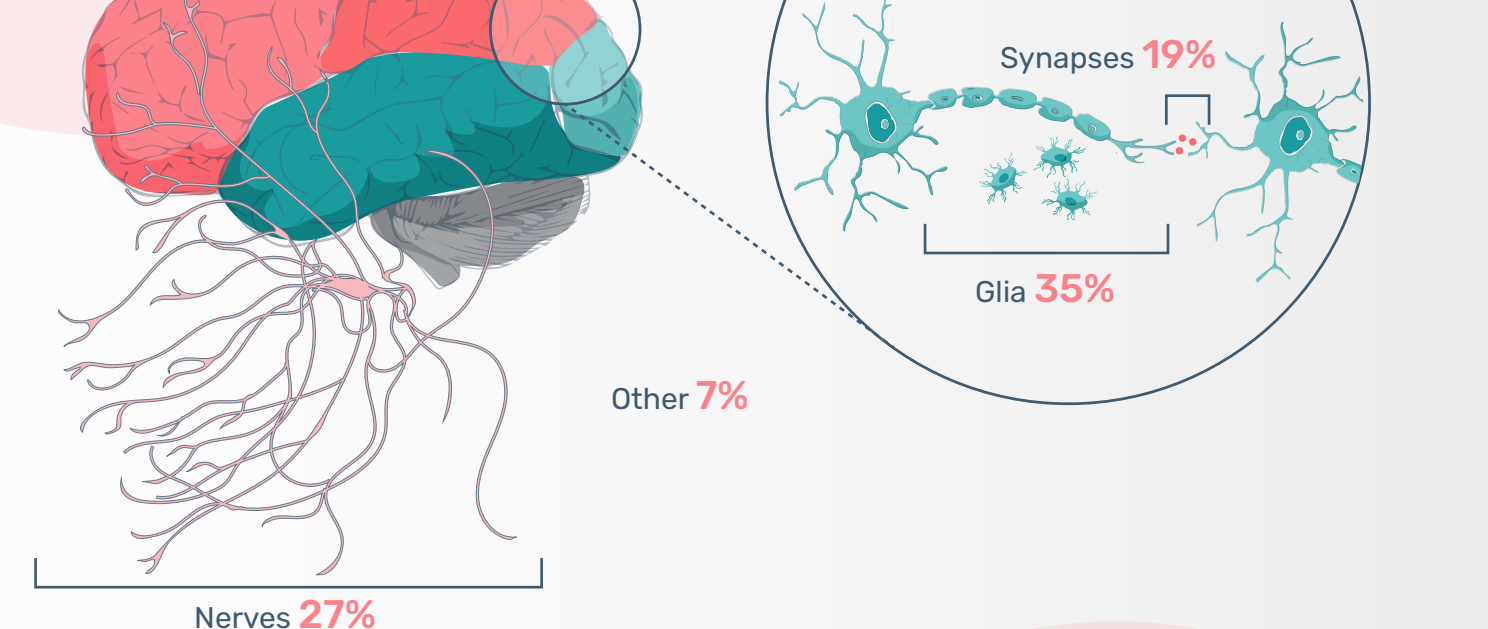


Fluorescence microscopy was the most common technique, with many people using a combination of techniques to obtain the most information about the target structure as possible.

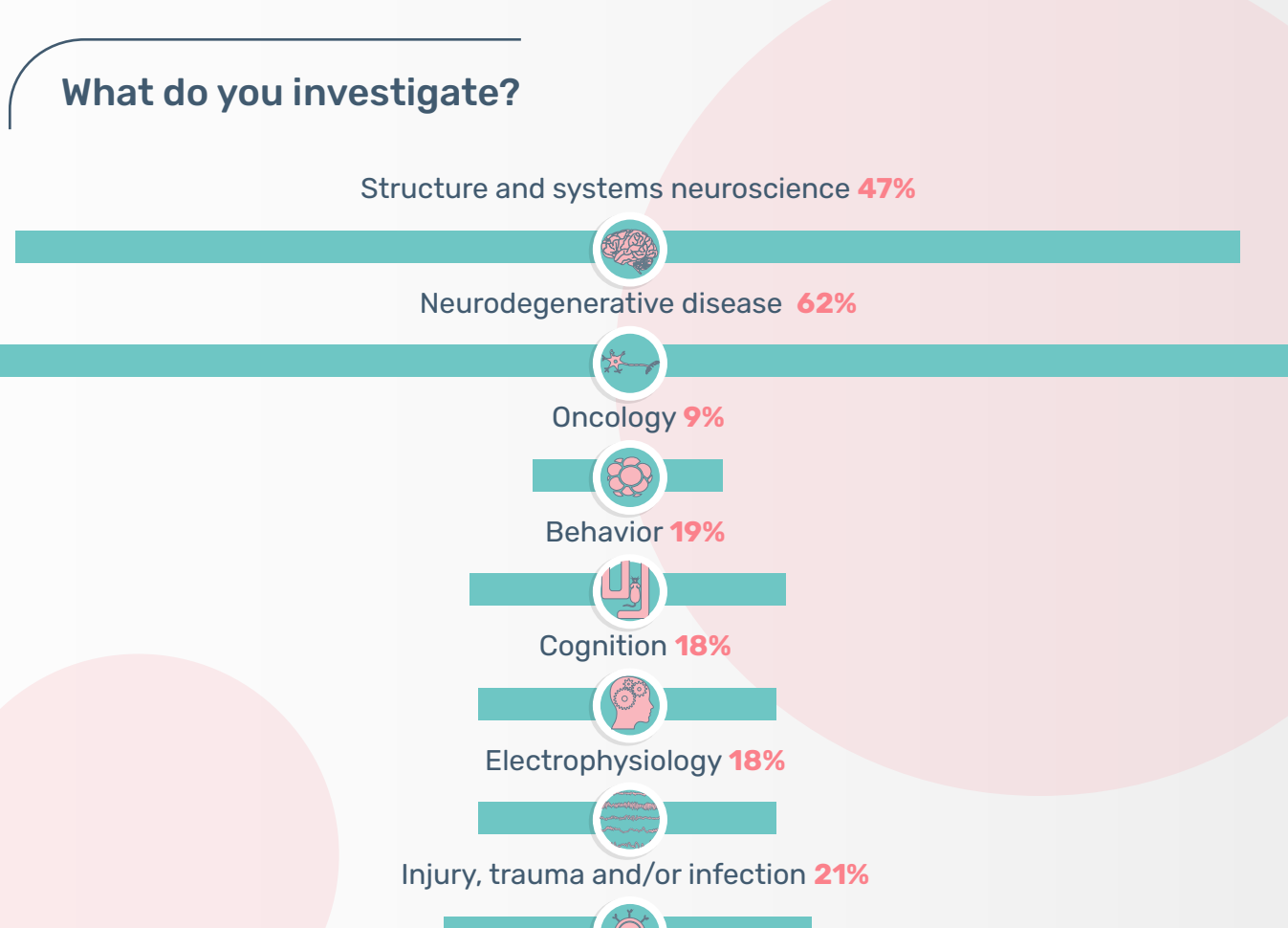
Research areas

What organism do you image?

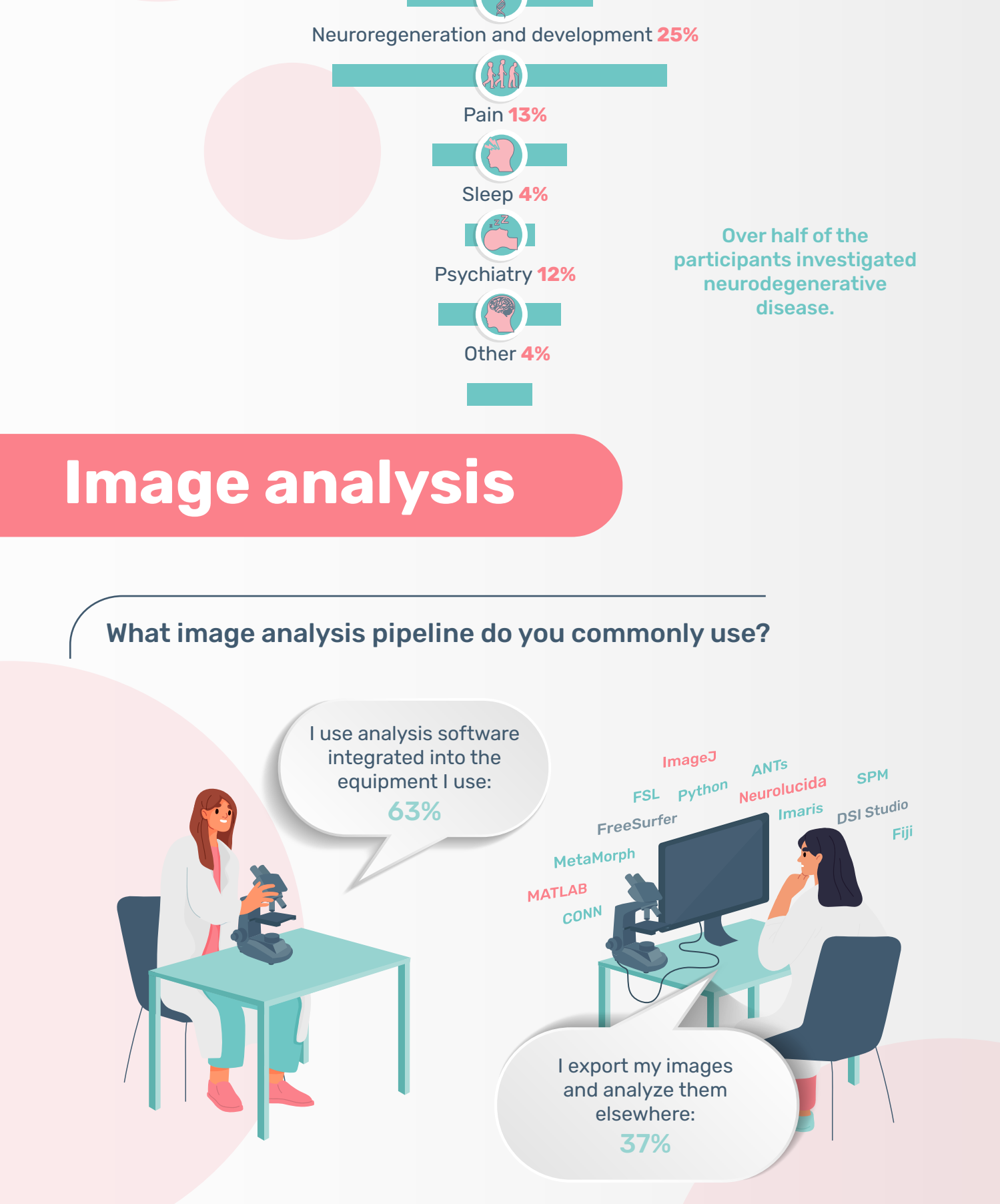
Rodents and humans were the most popular organisms imaged.



What part of the nervous system do you image?



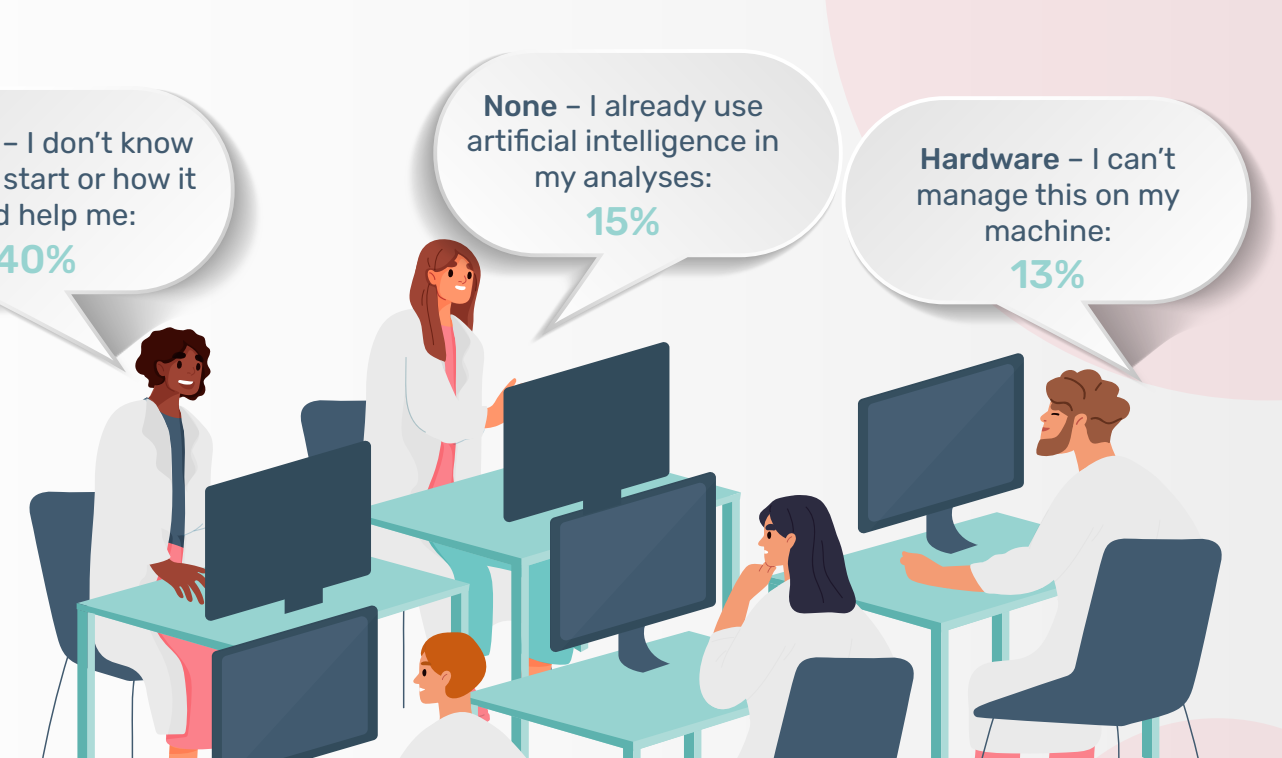
What do you investigate?



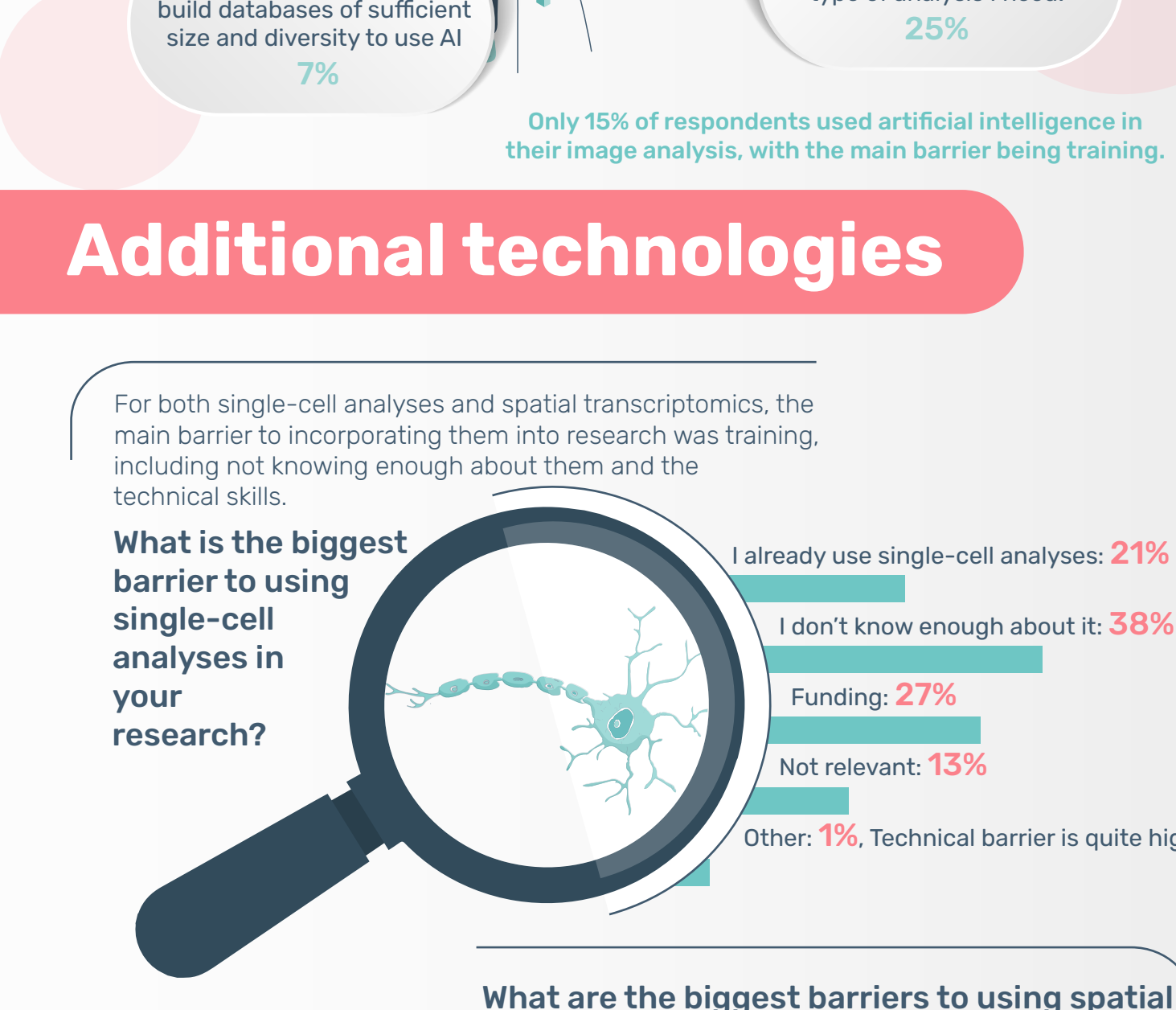
Over half of the participants investigated neurodegenerative disease.

Image analysis

What image analysis pipeline do you commonly use?



What are the main barriers to incorporating artificial intelligence into your image analysis?

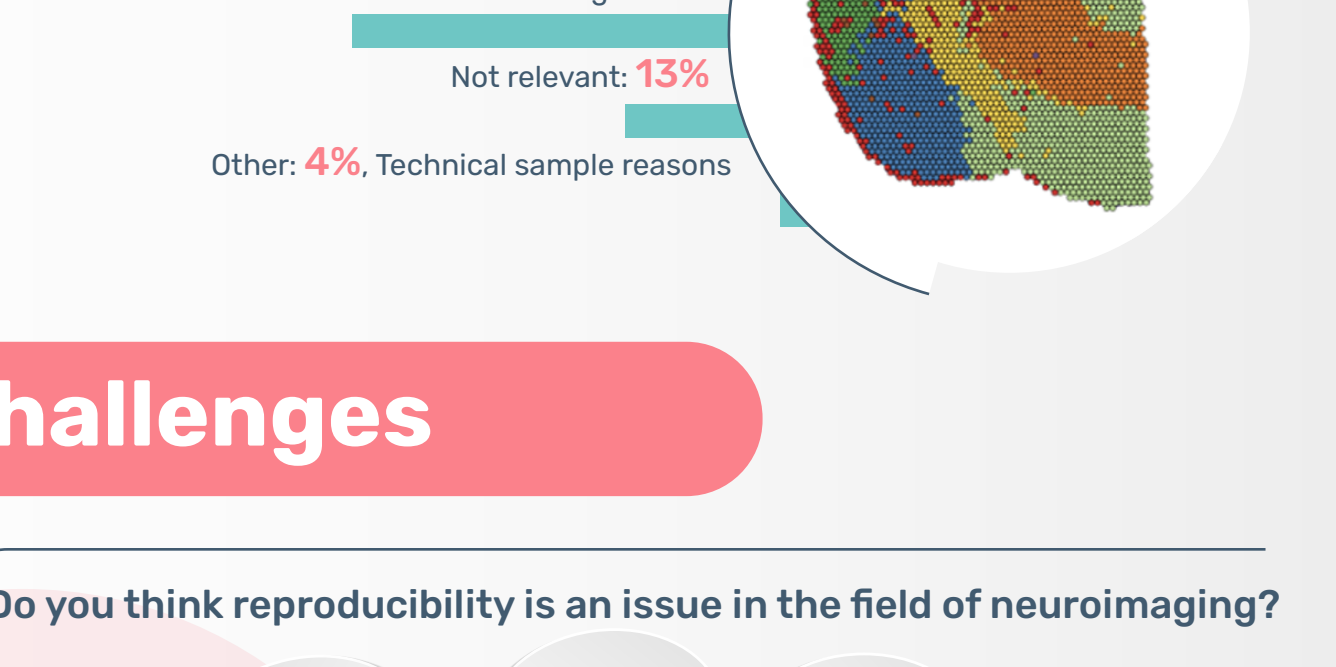


Only 15% of respondents used artificial intelligence in their image analysis, with the main barrier being training.

Additional technologies

For both single-cell analyses and spatial transcriptomics, the main barrier to incorporating them into research was training, including not knowing enough about them and the technical skills.

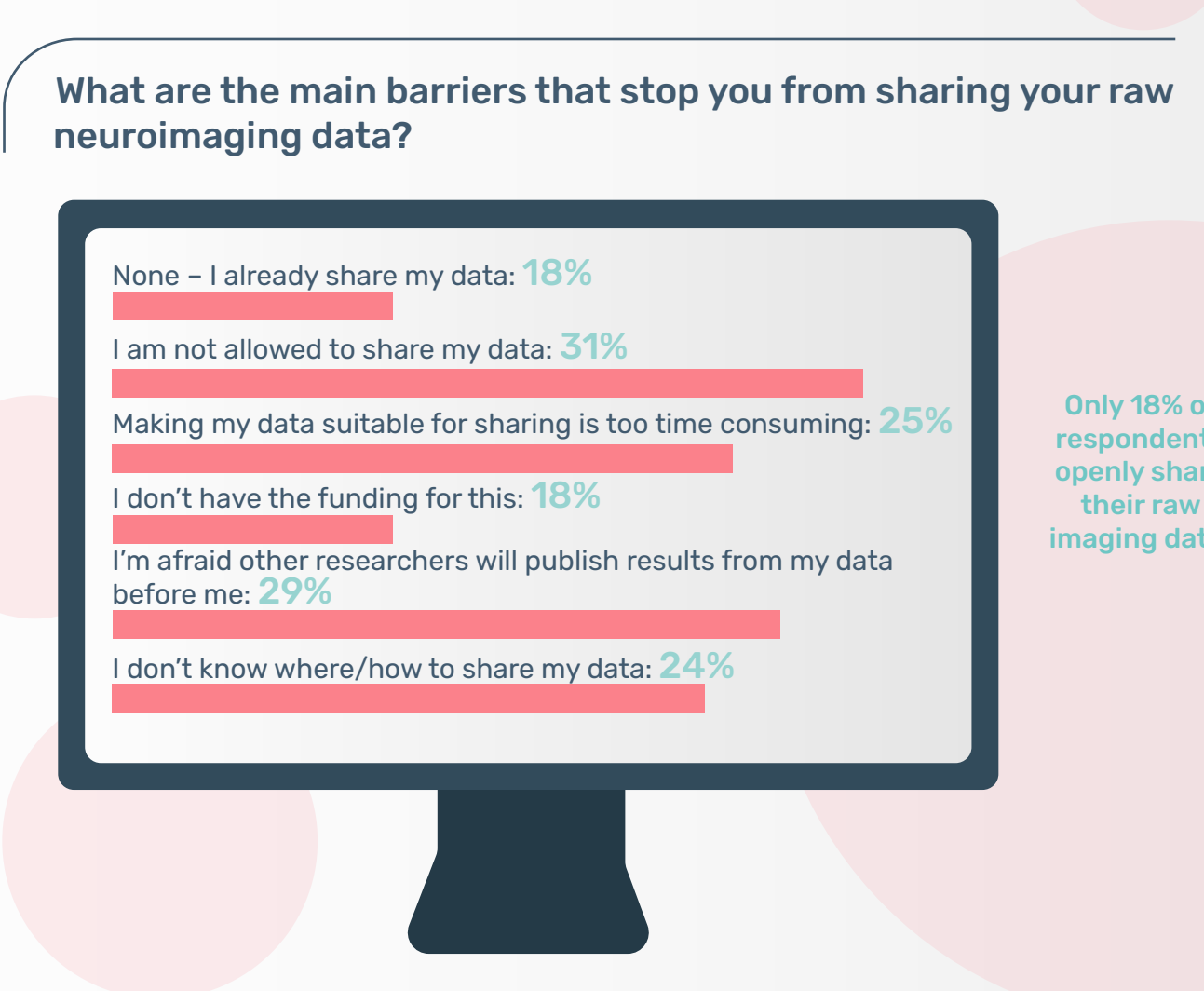
What is the biggest barrier to using single-cell analyses in your research?



What are the biggest barriers to using spatial transcriptomics in your research?

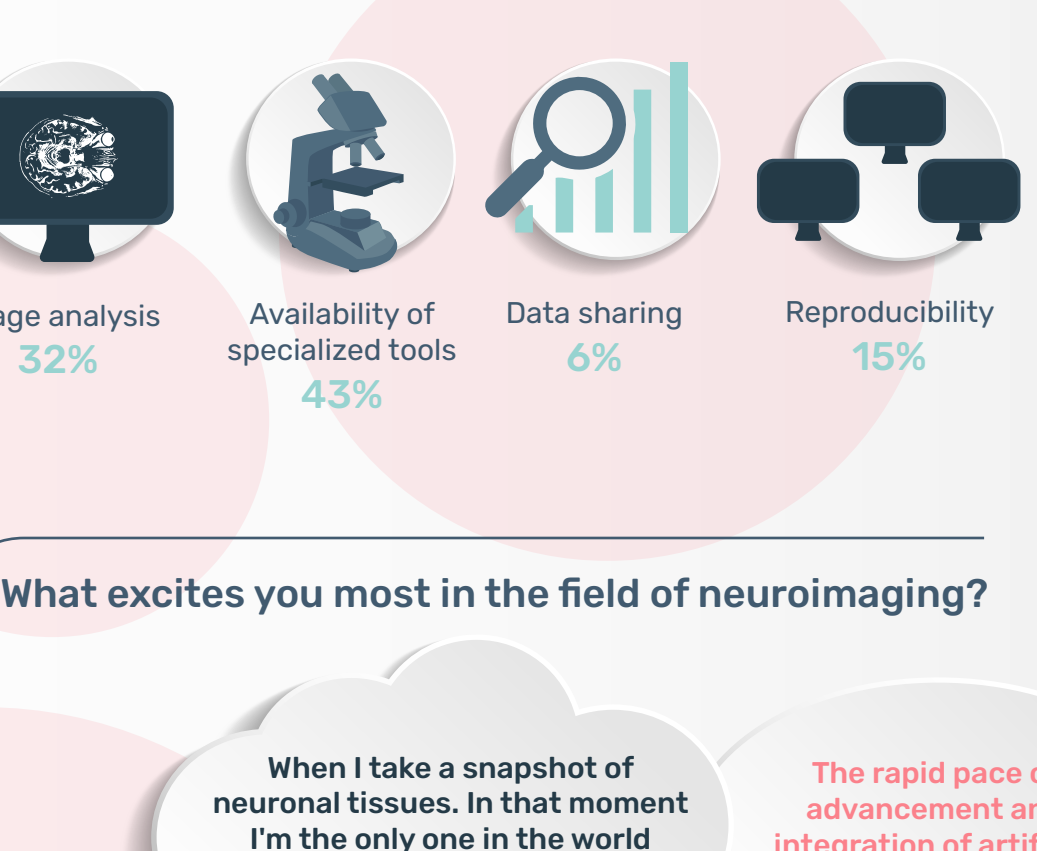
Challenges

Do you think reproducibility is an issue in the field of neuroimaging?



Nearly all the respondents thought that reproducibility was an issue in the field of neuroimaging.

What are the main barriers that stop you from sharing your raw neuroimaging data?



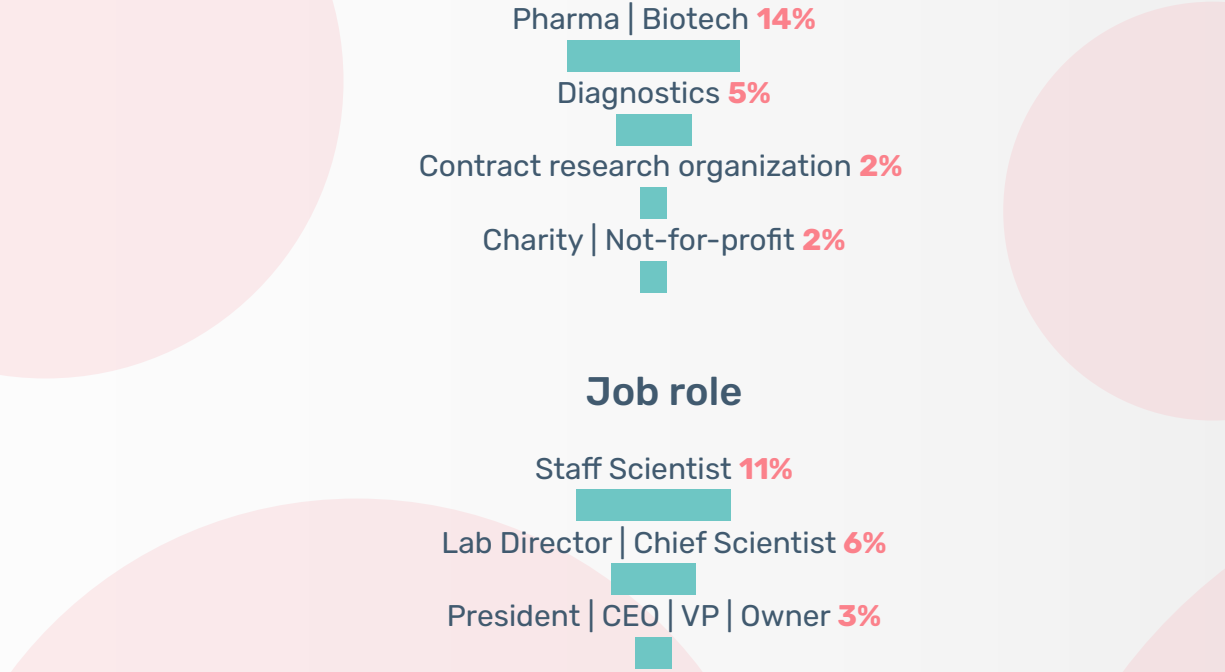
Only 18% of respondents openly share their raw imaging data.

What is the greatest challenge in the neuroimaging field?

What excites you most in the field of neuroimaging?

About the respondents

Location



Sector

- Academic 56%
- Hospital | Clinic 14%
- Private research Foundation 2%
- Government | NGO 6%
- Pharma | Biotech 14%
- Diagnostics 5%
- Contract research organization 2%
- Charity | Not-for-profit 2%

Job role

- Staff Scientist 11%
- Lab Director | Chief Scientist 6%
- President | CEO | VP | Owner 3%
- Postdoctoral fellow 11%
- Graduate Student 6%
- PhD 14%
- Department Head 5%
- Principal Investigator 10%
- Lecturer | Assistant | Associate Professor 10%
- Professor | Instructor 5%
- Medical Professional | Physician 6%
- Consultant 3%
- Technician | Research Assistant 6%
- Research Director | VP Research | CSO 2%
- Product Manager 2%

Single cell and spatial multiomics identifies Alzheimer's disease markers

Abstract

The pathogenesis of Alzheimer's disease (AD) is incompletely understood. Single cell and spatial solutions are powerful tools to improve our understanding of disease development and progression by offering insights into how chromatin accessibility and gene expression, specific to cell type and spatial localization, are associated with neuropathology. Here, we used a multiomics approach, combining Chromium Single Cell Multiome ATAC + Gene Expression and Visium Spatial Gene Expression for FFPE plus immunofluorescence protein detection, to resolve the relationship between progressive changes in cell type–specific differential gene expression and plaque burden in the TgCRND8 AD-like mouse model. We identified differences in gene expression and chromatin accessibility of well-known AD markers between transgenic and wild-type (WT) mice in concordance with plaque burden. We also uncovered the spatial organization of these changes across anatomical brain regions.

Highlights

- Several AD-related markers showed differential expression between transgenic and WT mice in microglia and oligodendrocytes at different time points
- Motif enrichment analysis of differentially accessible chromatin regions identified transcription factors (TFs) involved with amyloid plaque deposition
- Spatial analysis showed localized gene expression changes occurring in discrete anatomical brain regions
- Multiomic data analysis yielded new insights into single cell, chromatin, and spatial gene expression differences throughout the course of disease progression in an AD-like mouse model

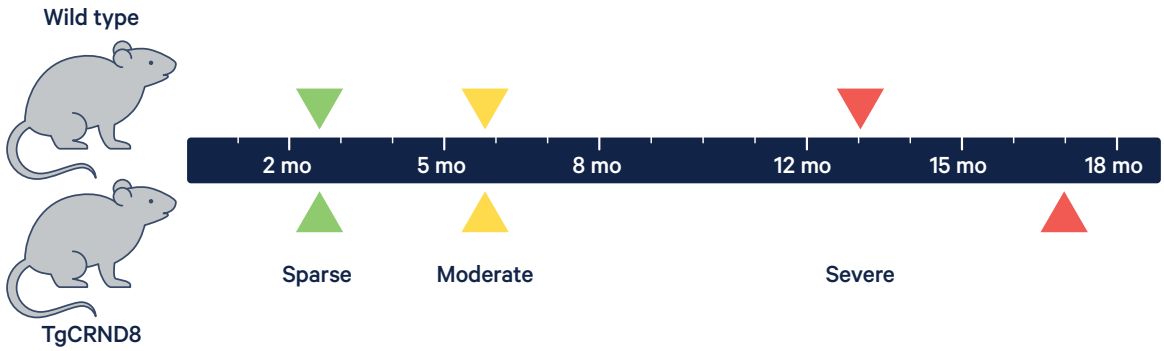


Figure 1. Experimental design. Twelve mouse brains were collected at timepoints spanning early, middle, and late life. Two replicates were collected per timepoint from TgCRND8 mice (n = 6) and WT littermates (n = 6). Selected timepoints were 2.5, 5.7, and 13.2 months of age for WT mice, and 2.5, 5.7, and 17.9 months of age for transgenic line. In TgCRND8 mice, plaque burden was sparse at 2.5 months, moderate at 5.7 months, and severe at 17.9 months, consistent with previous studies (3).

Introduction

Numerous studies have used single cell transcriptomic profiling to characterize gene expression differences in discrete cell types in AD, but fewer studies have combined that analysis with assessment of transcriptional regulation and spatially localized cell-type changes to more completely understand disease pathogenesis and progression. Studies using single cell chromatin accessibility profiling have begun to define the regulatory landscape of AD, underscoring the role of non-neuronal cells in the pathology of the disease (1). Recently, one study took a spatial approach to characterize gene expression networks around plaque deposits (2). Despite these advances, a cohesive demonstration of how gene expression is regulated within discrete cell types and specific anatomical regions of the brain during the early stages of AD is still unavailable, though it is crucial to identifying novel therapeutic targets.

To gain a holistic view of cell type–specific contributions to pathogenesis, map anatomical protein accumulation in the brain over time, and understand the relationship between abnormal protein accumulation and cellular phenotypes, we utilized a multiomics approach. Using Chromium Single Cell Multiome ATAC (Assay for Transposase-Accessible Chromatin) + Gene Expression (the multiome assay), which profiles open chromatin and gene expression from the same cell, and Visium Spatial Gene Expression for FFPE (Visium for FFPE) plus immunofluorescence (IF), which combines whole transcriptome spatial analysis with immunofluorescence protein detection in the same tissue section, we evaluated the open chromatin landscape and gene expression profiles in brains of TgCRND8 transgenic AD-like mice (3) and WT mice ranging from 2 to 13+ months old (early- to late-stage plaque deposition).

In this Application Note, we demonstrate how a multiomic approach provides a more complete assessment for furthering our understanding of mechanisms of disease pathogenesis. Integrating data from single cell gene expression, chromatin state, spatial transcriptomics, and IF protein detection, we analyzed amyloid beta (Aβ)-associated neuroinflammation across several anatomical regions of the brain and correlated these data with regulatory programs identified based on single cell multiomic and spatial transcriptomic data. We confirm the predicted spatial distribution of plaque burden over the course of pathology progression, identify specific neuroinflammatory markers differentially expressed with increasing amyloid accumulation and observed in discrete cell types, and localize these cells to specific brain regions.

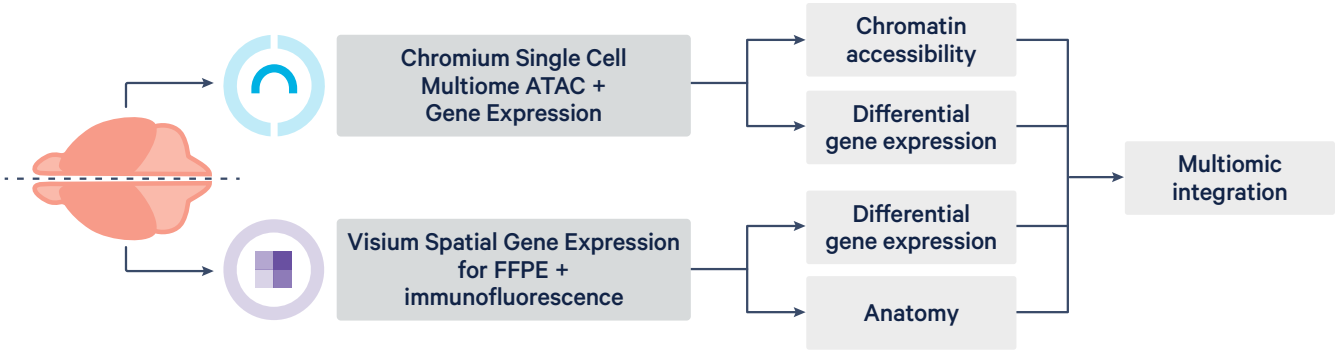


Figure 2. Multiomic integration from a single mouse brain. Each brain was divided by hemisphere, resulting in 24 separate samples for each timepoint. One hemisphere was flash frozen and used in the multiome assay. The other hemisphere was formalin-fixed, paraffin-embedded and used in the Visium for FFPE assay. These complementary data types were ultimately integrated in downstream analyses (Seurat and Signac).

Methods

Sample procurement and preparation. We analyzed 12 brains from male TgCRND8 mice (n = 6) and WT littermates (n = 6). For each genotype, two replicates at three different timepoints were analyzed (Figure 1). Selected timepoints were 2.5, 5.7, and 13.2 months of age for the WT mice, and 2.5, 5.7, and 17.9 months of age for TgCRND8 mice, corresponding to sparse, moderate, and severe Aβ plaque burden, respectively (3). Each brain was separated by hemisphere, where one was flash frozen (FF) and the other formalin-fixed, paraffin-embedded (FFPE) (Figure 2).

Sample and library preparation. Nuclei of FF hemispheres were isolated following the 10x Genomics Demonstrated Protocol (CG000375 Rev B). Paired multiome (Single Cell ATAC [scATAC] and Single Cell Gene Expression) libraries were prepared using the 10x Genomics User Guide (CG000338 Rev D).

For FFPE hemispheres, 5-µm sections were prepared and IF performed following Demonstrated Protocols (CG000408 Rev B and CG000410 Rev B, respectively). Amyloid precursor proteins were immunostained at 1:100 antibody dilutions. Additionally, nuclei were counterstained with DAPI. Imaging was performed on a Nikon Ti2 wide-field fluorescence microscope following Visium for FFPE Imaging Guidelines (CG000436 Rev A). Visium for FFPE libraries were prepared following the User Guide (CG000407 Rev C).

Sequencing. All libraries were sequenced on an Illumina NovaSeq 6000. Multiome libraries were sequenced at a depth of ~20,000 read pairs per cell. Visium for FFPE libraries were sequenced at a depth of ~25,000 read pairs per spot.

Primary data processing. Cell Ranger ARC 2.0 was used to process the multiome assay data, while Space Ranger 1.3 was used to analyze the Visium for FFPE data. After demultiplexing, a separate instance of `cellranger-arc count` was run on each of 12 paired multiome libraries, and `spaceranger count` for each of 12 Visium libraries. The `cellranger-arc aggr` pipeline was used to combine all multiome libraries into a single matrix, while the `spaceranger aggr` pipeline was likewise used to aggregate Visium for FFPE libraries.

Secondary analysis. Quality control was performed using Seurat for gene expression and Visium for FFPE data (4), and Signac for ATAC data (5). Metrics for both assays (number of unique molecular identifiers, number of genes) and percentage of mitochondrial reads were considered to define quality control thresholds.

Dimensionality reduction and visualization. Uniform Manifold Approximation and Projection (UMAP) projections were used for dimensionality reduction and visualization. We used the Weighted Nearest Neighbor (WNN) algorithm to obtain UMAP cell embeddings that consider information from both gene expression and chromatin accessibility assays (6).

Cell-type annotation. Loupe Browser 6.0 was used to perform manual annotation on two multiome and two Visium datasets using a predefined list of 35 marker genes for oligodendrocytes, oligodendrocyte precursor cells, microglia, pericytes/endothelial cells, inhibitory neurons, and astrocytes (Figure 3). An anchor-based algorithm was used to perform automated annotation of spatial and multiome datasets, assigning a probability for each barcode to belong to a specific cell type.

Differential expression. The Wilcoxon test was used to identify differentially expressed genes between transgenic and WT mice in each cell type. A logistic regression was used to quantify differential chromatin accessibility for the multiome data. Peaks and genes were linked using Signac. The differentially accessible regions were used to perform motif enrichment and identify transcription factors with a high probability of binding to those regions.

Anatomical classification and multiomic integration. To deconvolute Visium spots into cell types, we used spacexr in multi-mode, which allows discovery of more than two cell types per spot (7). The aggregated multiome data were used as a reference. Each Visium slide was individually processed to perform cellular deconvolution. Multiomic integration was performed by leveraging trimodal dataset integration in Seurat and assigning cell types to spots and vice versa, while layering on epigenetic information.

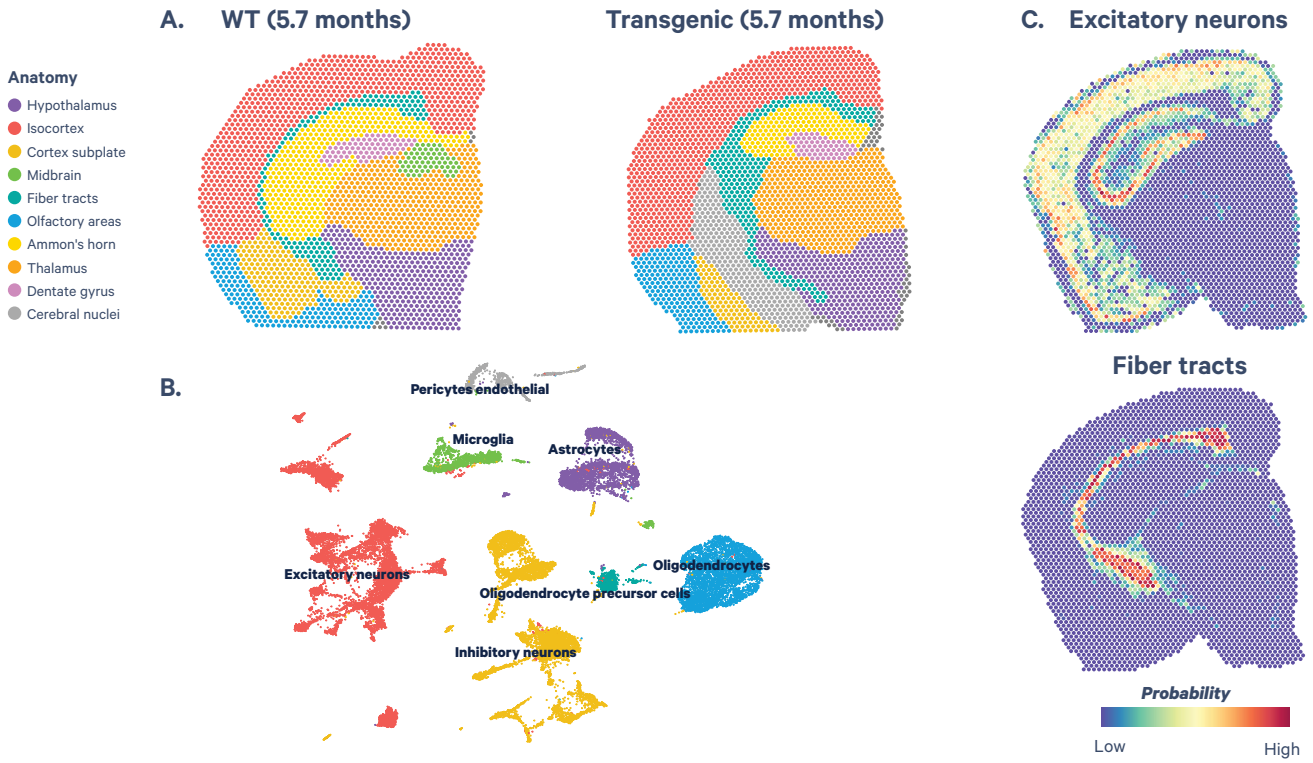


Figure 3. Manual annotation using marker genes. A. Visium sections with anatomical regions manually annotated. **B.** WNN UMAP of manually annotated barcodes. **C.** Results of anchor-based approach to predict anatomical identities and cell-type deconvolution.

Results

Spatial transcriptomics + IF enables localization of plaque-induced gene expression changes

While Visium alone provides a measurement of transcriptome-wide expression in the context of its geographical localization, adding protein detection via IF can identify and localize proteins on the same slide. For example, scientists recently used spatial transcriptomics to reveal multicellular gene co-expression networks in the vicinity of plaque deposits, one of which is a plaque-induced gene (PIG) network mainly involving microglia and astrocytes (2). In the context of AD, Visium with IF enables the identification of gene expression changes in specific cell types that can be associated with nearby features, including intracellular or extracellular inclusions or degenerating neurons, offering a new perspective on disease progression.

Our Visium for FFPE + IF data demonstrated that Aβ plaque burden increases with anatomic specificity over time (Figure 4A), with greater relative abundance in the cortex and dentate gyrus, populated primarily by excitatory neurons, as previously reported. Further analysis of Visium spots showed gene expression patterns dependent on proximity to plaques. Aggregating expression of the PIG set showed gradually decreasing expression from regions at the plaque, toward regions adjacent and distal to it (Figure 4B–D).

Exploring microglia with single cell multiomics

Previous single cell research identified a unique microglial phenotype associated with neurodegenerative diseases (8), which further substantiates these cells as key players in disease pathogenesis and warrants deeper investigation into their precise role.

Solute carriers (SLCs) are a family of transmembrane transporters of nutrients, ions, metabolites, and drugs. Evidence suggests that dysfunction of some SLCs are related to brain disorders, including neurodegenerative diseases (9). We identified *Slc1a3* as differentially expressed in microglia when comparing transgenic and WT mice (Figure 5A). The biggest difference was seen at early timepoints when plaque deposition is sparse.

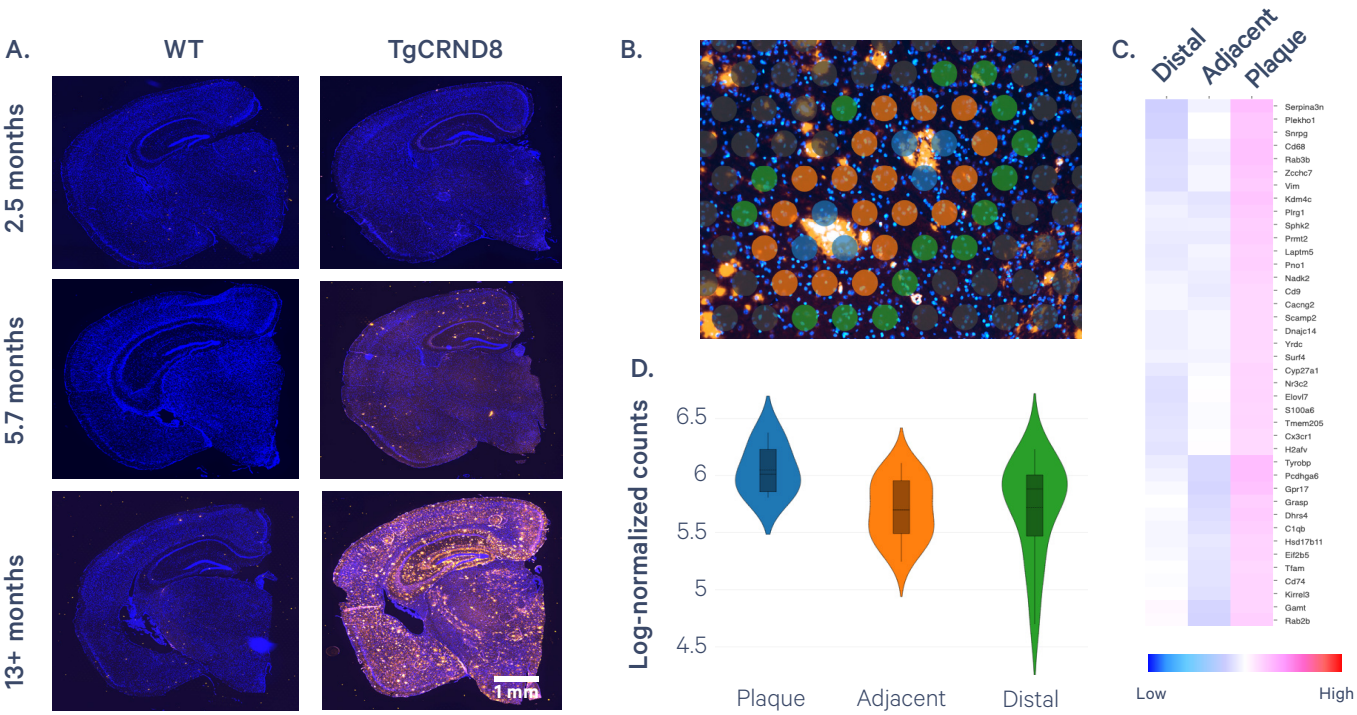


Figure 4. Aβ plaque burden. A. IF assessment of regional plaque burden (blue = DAPI, orange = human amyloid precursor protein). **B.** Close-up of a region within the thalamus of a 13+ month TgCRND8 mouse, with plaque in orange, grouping Visium spots based on the proximity to the plaque as "plaque" (blue), "adjacent" (orange), or "distal" (green). **C.** Heatmap of differentially expressed genes across the three plaque proximity regions. **D.** Violin plots of aggregated expression of the plaque-induced gene (PIG) network.

Using scATAC, we looked for open chromatin regions that showed a strong relationship with gene expression. Paired gene expression and open chromatin signals from the multiome assay pave the way for gene regulatory network predictions by correlating, or linking, open chromatin regions to nearby genes. The nucleotide sequences of these open regions can then be compared against predicted binding motifs to identify transcription factors that may be acting upon that site. In this case, we identified a differentially accessible distal region to *Slc1a3* that may play a role in transcriptional regulation. Motif enrichment highlighted the transcription factors *Egr1, Wt1, Nfyb, Klf4*, and *Znf740* (Figure 5B).

Previous studies have shown that early growth response-1 (EGR1) may play a role in maintaining cholinergic function in the brain in the early stages of AD. In one study, scientists concluded that EGR1 can up-regulate acetylcholinesterase expression, which may contribute cholinergic changes in AD (10). In our Visium for FFPE data, *Egr1* was differentially expressed between transgenic and WT mice. Anatomical annotation of the slides also revealed that the change in expression is primarily localized to the isocortex (Figure 5C–D).

Single cell analysis uncovers oligodendrocyte changes

Oligodendrocytes are the myelinating cells of the central nervous system, and research has increasingly focused on elucidating the role that non-neuronal cells play in AD progression. A recent publication used single cell transcriptomics to evaluate 13 neural cell types, identifying two distinct oligodendrocyte transcriptional states among AD mouse models and noting differences in the effects of AD risk genes on microglia versus non-microglia cells, including oligodendrocytes (11).

Focusing on oligodendrocytes, we observed that *Mon2*, previously found to be differentially expressed in extracellular vesicles from an AD transgenic mouse model (12), was more highly expressed in transgenic mice at the late timepoint when plaque burden is severe (Figure 6A). scATAC analysis showed a weak correlation between gene expression profiles and chromatin accessibility. To perform the motif analysis, we focused on the list of differentially accessible peaks and identified a differentially accessible peak upstream to the transcription start site that could play a regulatory role. Motif enrichment analysis highlighted *Sox8, Rora, Nfya*, and *Sp3* (Figure 6B).

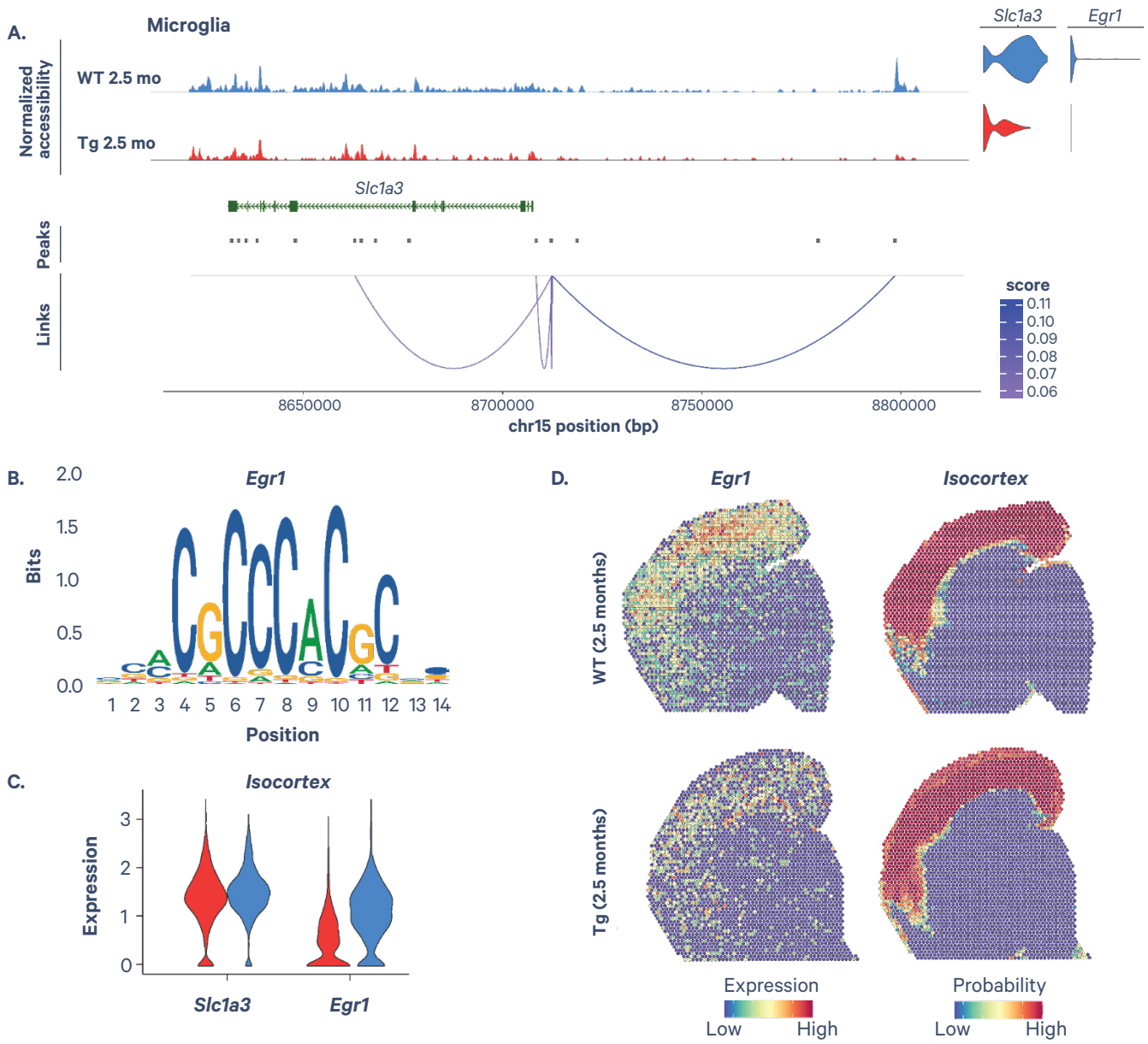


Figure 5. Microglia. Manual annotation using marker genes. A. Chromatin accessibility and expression profiles of regions linked to *Slc1a3* in microglia. **B.** *Egr1* motif enriched in regions upstream to *Slc1a3*. **C.** Violin plot of *Slc1a3* and *Egr1* differential expression in WT (blue) and transgenic (Tg) (red) mice in the isocortex as assessed by Visium for FFPE data. **D.** Spatial expression profile of *Egr1* and Visium spot probabilities belonging to the isocortex region.

It has been previously observed that *Sox8* activation (by notoginsenoside R2, a key saponin in the *Panax notoginseng* plant shown to improve AD symptoms) up-regulates β-catenin expression, helping prevent apoptosis and neuroinflammation in primary rat cortical neurons in an AD setting (13). *Sox8* was among those transcription factors that were found to be enriched using Visium for FFPE. The largest difference was observed in aged mice with advanced plaque burden (13+ months). As expected, anatomical localization of cell-type gene expression signatures revealed oligodendrocytes in the fiber tracts. Differential expression of *Sox8* was detected in overlapping regions, suggesting differential *Sox8* expression is specific to oligodendrocytes (Figure 6C–D).

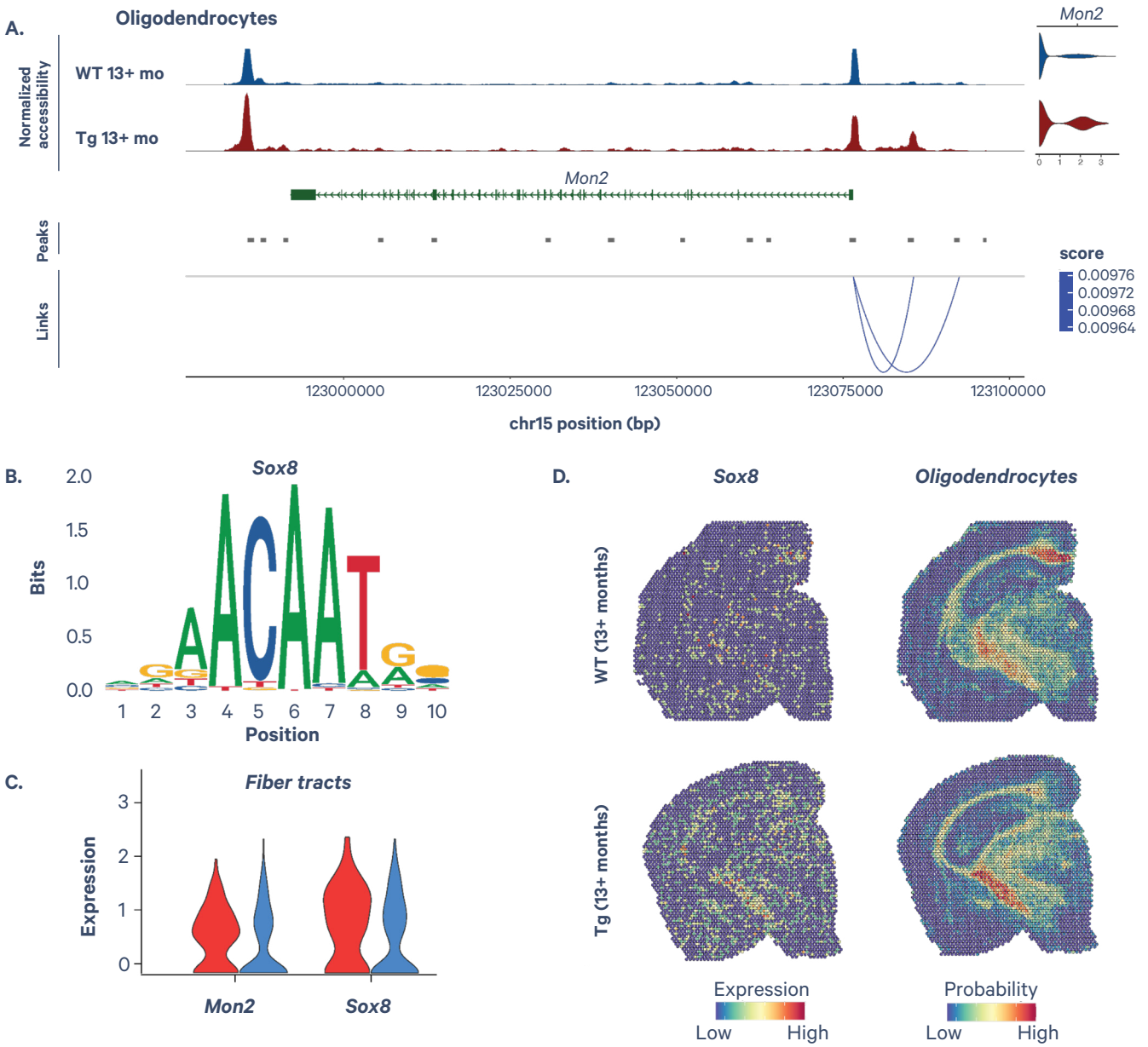


Figure 6. Oligodendrocytes. A. Chromatin accessibility and expression profiles of regions linked to *Mon2* in oligodendrocytes. **B.** *Sox8* motif enriched in regions upstream to *Mon2*. **C.** Violin plot of *Mon2* and *Sox8* differential expression in WT (blue) and Tg (red) mice in fiber tracts as assessed by Visium for FFPE data. **D.** Spatial expression profile of *Sox8* and Visium spot probabilities belonging to oligodendrocytes.

Conclusion

Integrating multiomic single cell gene expression, chromatin accessibility, and spatial transcriptomic data provides a comprehensive approach to detect changes in transcriptional expression and regulation. The data presented in this Application Note demonstrate identification of early- and late-stage markers associated with progressive amyloid deposition, showcasing changes in both gene expression and chromatin accessibility over time in specific cell types and across brain regions in association with neuropathology. Spatial transcriptomics additionally enabled the identification of anatomical regions undergoing significant age-dependent changes in this AD-like mouse model. Together, these data demonstrate the robust nature of a multiomic approach to identify disease markers and reveal dynamic processes occurring over time and across brain regions in association with neuropathology.

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Resources

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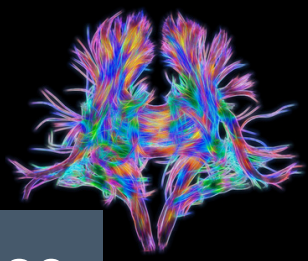

Panel Discussion

Imaging in neuroscience

In this Panel Discussion, experts from four areas of neuroscience discuss how they use imaging in their research. They also discuss key topics in the field, such as reproducibility, image analysis, new techniques such as single-cell and spatial biology and customizing equipment on a budget.

Panelists

Robert Prevedel

Robert Prevedel is a researcher at the European Molecular Biology Laboratory (EMBL; Heidelberg, Germany), whose lab focuses on developing light microscopy-based techniques for a variety of applications, including neuroimaging techniques that capture brain activity and structure in different model organisms, focusing especially on mouse models.

Harry Fu

Harry Fu is an Assistant Professor at The Ohio State University College of Medicine (OH, USA). Harry's research focuses on understanding tau pathology in early Alzheimer's disease and other tauopathies as well, as the molecular and cellular mechanisms underlying the selective neuronal vulnerability. He utilizes a multidisciplinary approach that combines neuropathology, genetics, neurobehavioral tests, confocal and light-sheet microscopy, molecular and cellular approaches together with single cell RNA-seq and spatial transcriptomic analysis.

Filip Janiak

Filip Janiak has a MSc and PhD in the field of solid-state physics, obtained from Wroclaw University of Science and Technology (Poland). After his PhD, Filip was at NeuroElectronics Research Flanders (Leuven, Belgium) for a short time, before moving to Kavli Institute for System Neuroscience (Trondheim, Norway), where he was a postdoc in Emre Yaksi's lab. Since 2017, Filip has been a Research Fellow in Tom Baden's lab at the University of Sussex (UK). His research focuses on *in-vivo* 2-photon imaging of neural activity in the eye and brain.

Myriam Chaumeil

Myriam Chaumeil is an Associate Professor at the University of California, San Francisco (CA, USA). Myriam's research focuses on developing and validating magnetic resonance-based imaging and spectroscopy methods for *in vivo* measurement of brain metabolism, in physiological and pathological conditions, in preclinical models and in patients. She has experience in studies of Huntington's disease, multiple sclerosis, Alzheimer's disease, traumatic brain injury, cerebral small vascular diseases and CNS lymphoma.

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