Department of Neurology
Presentation
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State of the Core: NUSeq in 2024

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The Mission of NUSeq

Wet Lab: Tarry 2-770 / Morton 4-625; Bioinformatics: Rubloff 11-144

NUSeq Team

NUSeq is committed to providing project support on:

Next-gen sequencing,

other genomics technologies,

with **integrated bioinformatics and data mining**

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Most Requested NGS Applications

- Bulk RNA-seq (from standard to ultra-low RNA input)
- Single cell sequencing
- Spatial transcriptomics
- ChIP-seq
- ATAC-seq
- DNA methylation sequencing
- Whole exome sequencing
- Whole genome sequencing

Other Genomics Services

- Illumina bead arrays: for genotyping and epigenomics (DNA methylation) studies
- NanoString nCounter: direct detection of up to 800 gene expression targets
- Digital droplet PCR and real-time qPCR: target DNA and RNA analysis, and validation of NGS results
- Cell line authentication
- DNA/RNA extraction
- Sanger sequencing (via ACGT)

Bioinformatics Services

- Support analysis of all data generated in the Core
- You can also bring your own data to us for analysis

NUSeq Fiscal Year 2023 Core Utilization Numbers

(2022-09-01 to 2023-08-31)

- 762 Direct Users (Service Requesters)
- 357 PIs/Groups, 288 NU + 69 External
- 82% of services completed for NU users, 18% for external users
- $3.7M: Users spent on NUSeq services

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2024 Sequencing Updates

Short Read Sequencing

- 2024 is a major transition year
- Focus will be on latest technologies
- Services on some of the old sequencers are being discontinued
  - NovaSeq 6000
  - HiSeq 4000

Short Read Sequencing

Latest technologies to focus on

1+ and 3+ billion reads per lane
$1,250 - $2,800 per lane

Illumina NovaSeq X Plus
Top data throughput
Most cost effective

250 - 500 million reads per lane
2-week turnaround

Element AVITI
Low to intermediate throughput
Quick turnaround time

100 - 450 million reads per lane
2-week turnaround

Complete Genomics DNBSEQ-G400
Low to intermediate throughput
More flexibility

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How to Select the Right Sequencer

- How many reads are needed
  - Over 1 billion reads: suggest NovaSeq X Plus (replaces NovaSeq 6000)
  - 250-500 million reads: Element AVITI (replaces HiSeq 4000)
  - As low as 100 million reads: Complete Genomics G400
- Turnaround time: AVITI and G400 have shorter TAT
- Cost efficiency:
  - All better than previous sequencers
  - Running 25B flow cell on NovaSeq X Plus is most cost effective for high volumes of data (e.g., high volumes of single cell or whole genome sequencing)

Long Read Sequencing

Oxford Nanopore MinION & PromethION (running)

PacBio Revio (upcoming soon this year)

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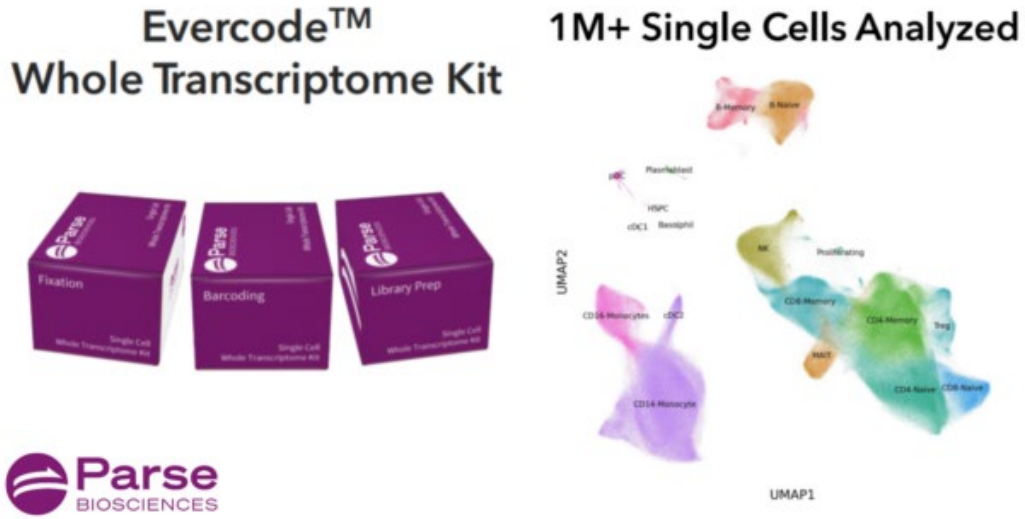
When to Use Long Read Sequencers

- Genome assembly
- Structural variant detection
- Full length transcript sequencing for splicing isoform detection
- Epigenomics: direct calling of DNA/RNA modifications without derivatization
- Any applications that need long reads

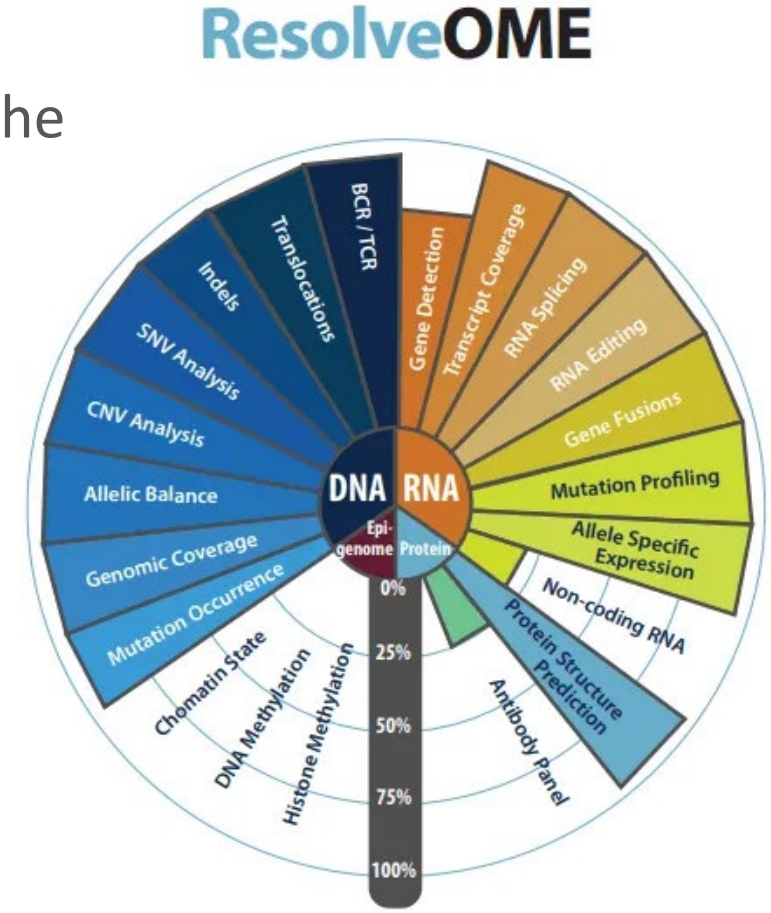
Single Cell Sequencing Updates

Single Cell Sequencing

- 10x Chromium X based single cell sequencing is still the most requested
- New technologies are also available



Parse analyzes 1 million cells in a single sample (running)



Bioskryb scDNA+RNA (coming soon)

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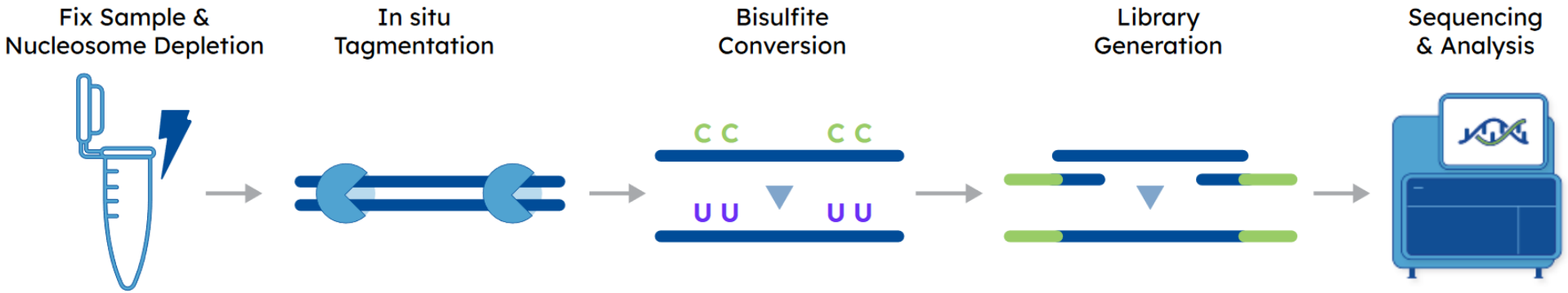
Single Cell Sequencing



Single cell methylation sequencing (Scale Bioscience, coming soon)

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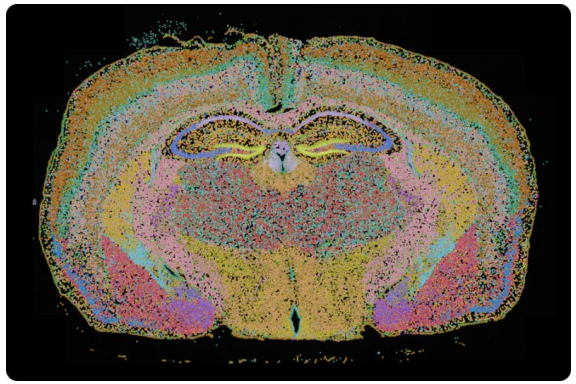
Spatial Transcriptomics Updates

Spatial Transcriptomics

Latest Acquisition



- Subcellular resolution: 200 nm optical resolution
- Fresh Frozen (FF) and FFPE samples compatible
- Section thickness: 10 um for FF, 5 um for FFPE
- Sample imageable area: 10.45 x 22.45 mm
- High sensitivity achieved using hybridization-based in situ sequencing
- High specificity from the use of barcoded padlock probes
- Targeted analysis of hundreds to thousands of genes, using predesigned human/mouse brain panels among others
- Allows IF and H&E staining, or Visium whole transcriptome analysis on the same tissue section

10x Xenium In Situ System (running, certified service provider)

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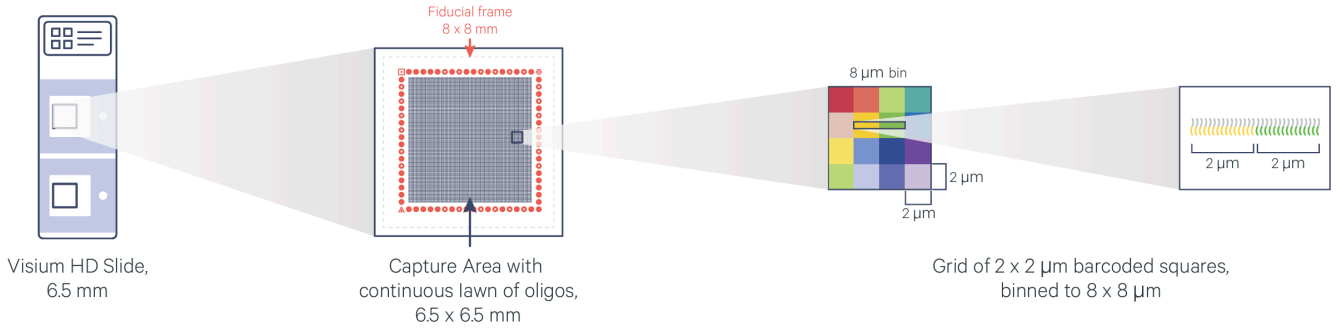
Spatial Transcriptomics

Existing Platforms

Visium System with CytAssist (running)



Visium HD to be released by 10x this year

- Spatial whole transcriptome analysis
- Resolution: 50 um (2 um with upcoming Visium HD)
- Fresh Frozen (FF) and FFPE samples compatible, FFPE preferred
- Tissue capture area: 6.5 x 6.5 mm (x4, v1), 11 x 11 mm (x2, v2)
- Protein panel and IF staining doable on the same tissue section

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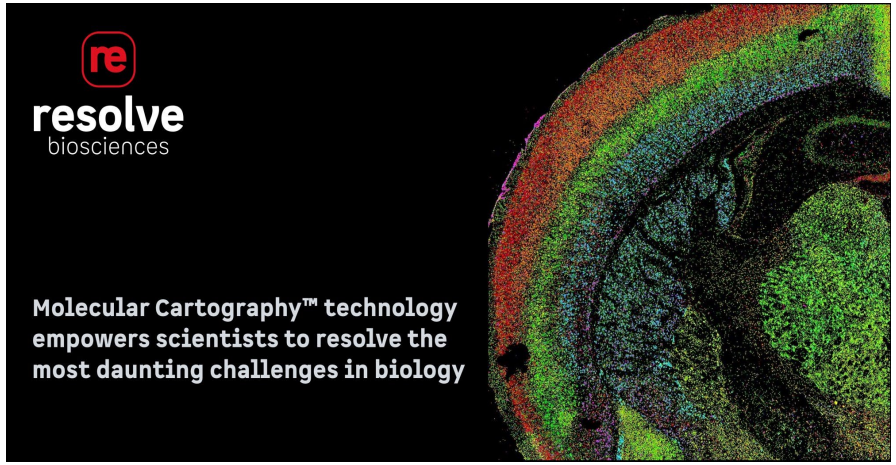
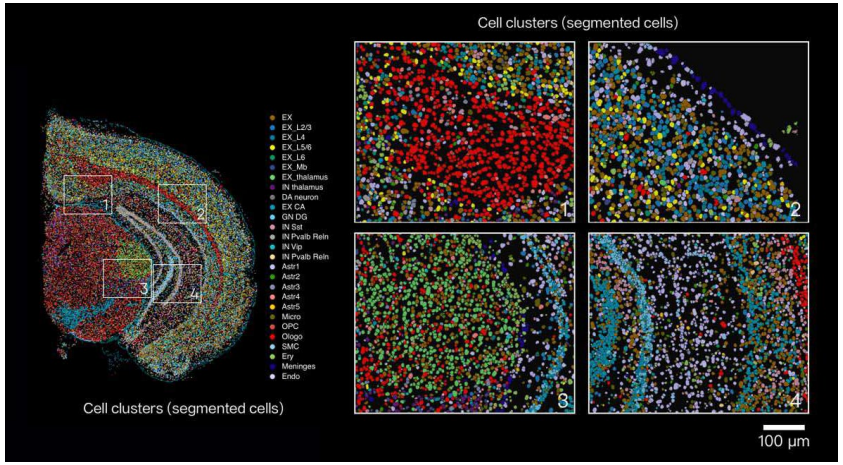
Spatial Transcriptomics

Other Available Platforms



Molecular Cartography (partnership, running)



Stereo-Seq (coming soon)

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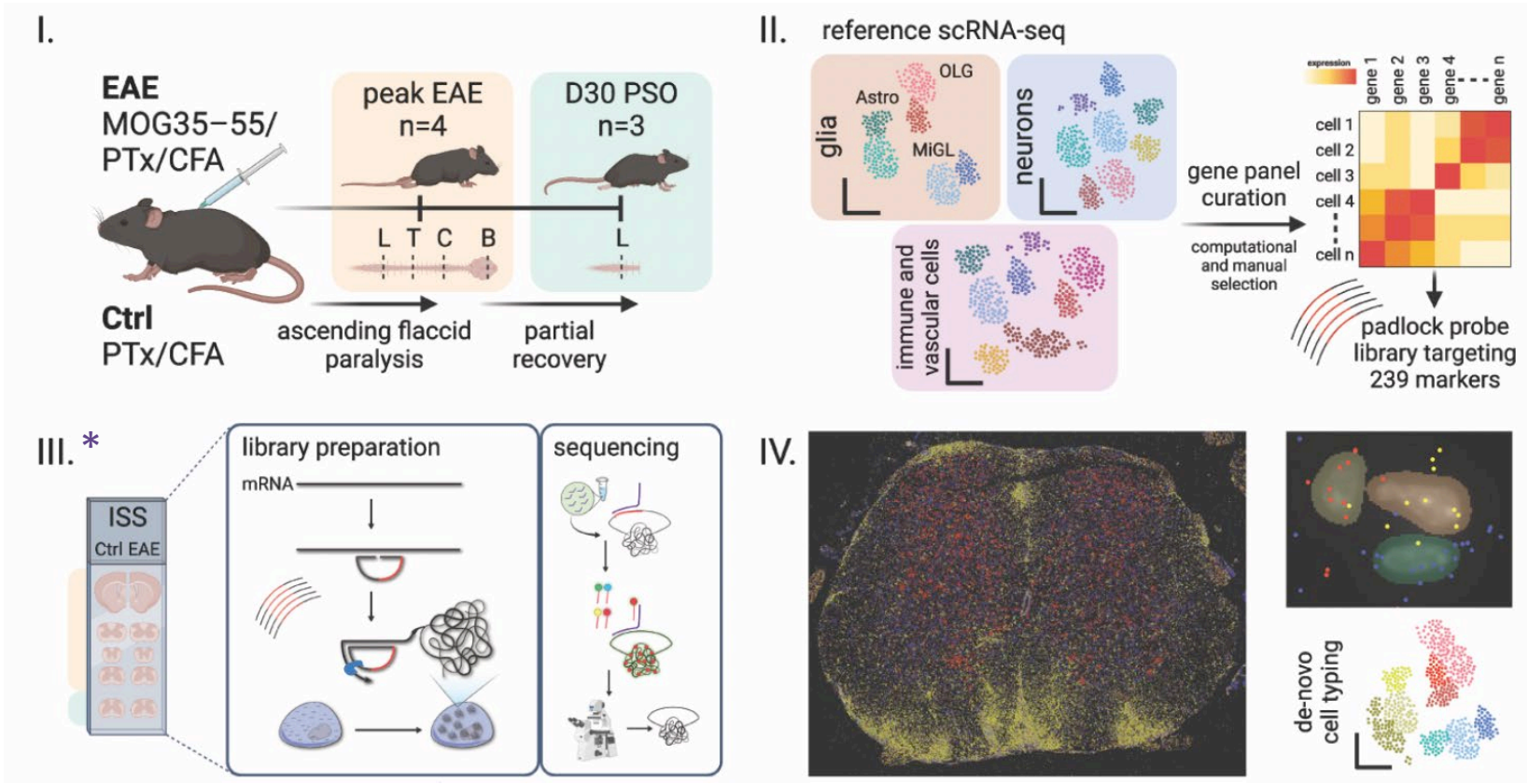
Use Case of Single Cell Sequencing Coupled with Latest Spatial Transcriptomics

Single-Cell Resolution Spatial Analysis of Multiple Sclerosis Development



*Acquired by 10x and developed into Xenium

Langseth, Christoffer Mattsson, et al. "Single cell-resolution in situ sequencing elucidates spatial dynamics of multiple sclerosis lesion and disease evolution." *bioRxiv* (2023): 2023-06.

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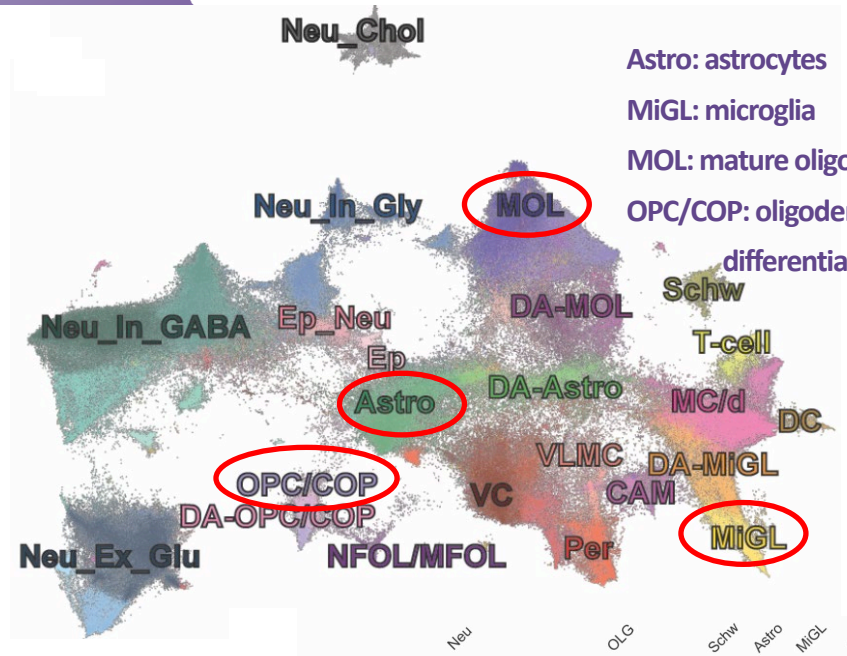
Spatial Organization of Various Cell Types

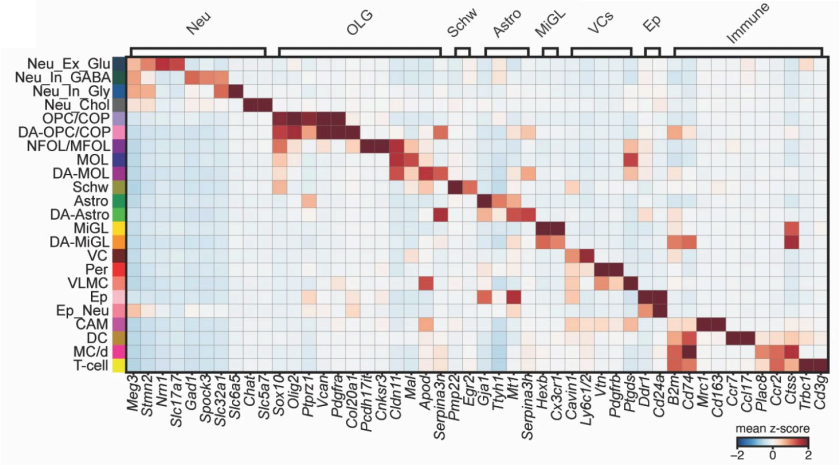


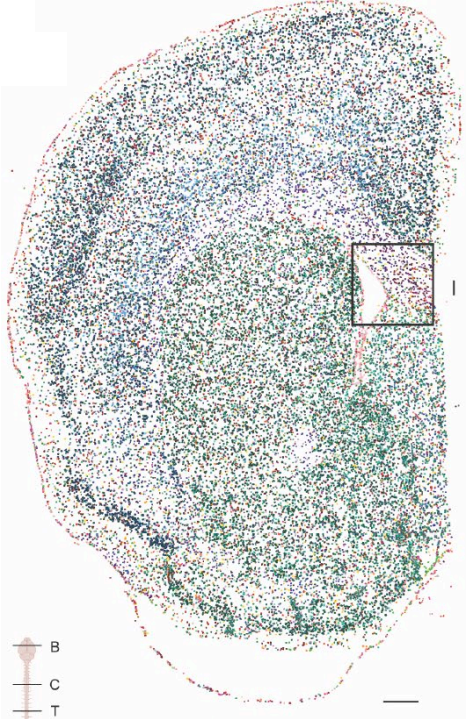
Astro: astrocytes

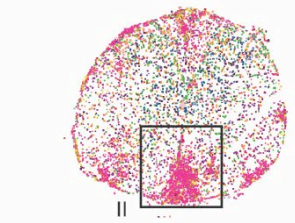
MiGL: microglia

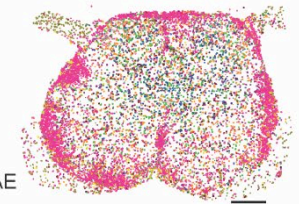
MOL: mature oligodendrocytes

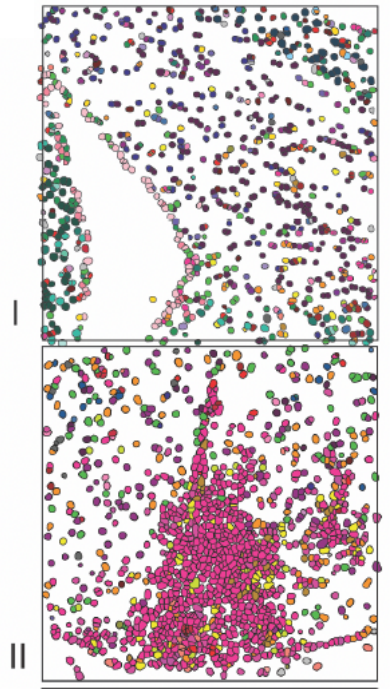
OPC/COP: oligodendrocyte precursor cells and differentiation-committed precursors











Interactive dataset at:

https://tissuumaps.scilifelab.se/web/2023_spinal_brain.html

Langseth, Christoffer Mattsson, et al. "Single cell-resolution in situ sequencing elucidates spatial dynamics of multiple sclerosis lesion and disease evolution." *bioRxiv* (2023): 2023-06.

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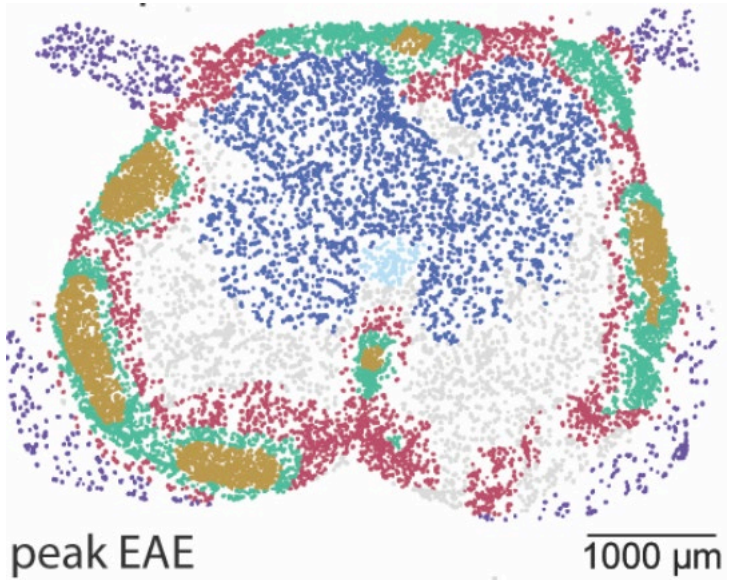
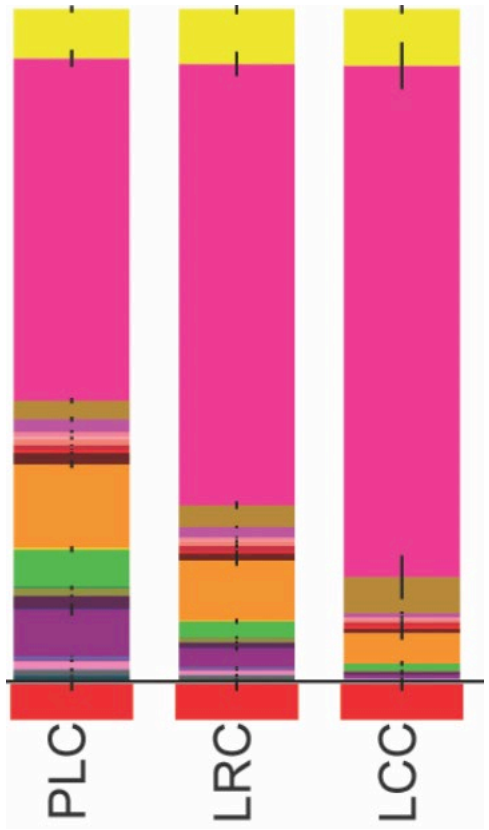
Cell Compartmentalization Analysis





Relative cellular composition of the different compartments.

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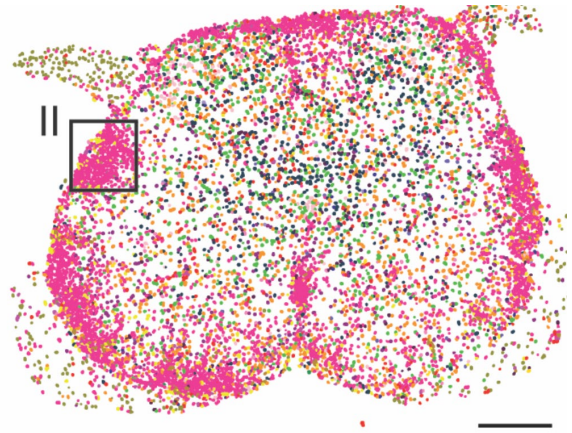
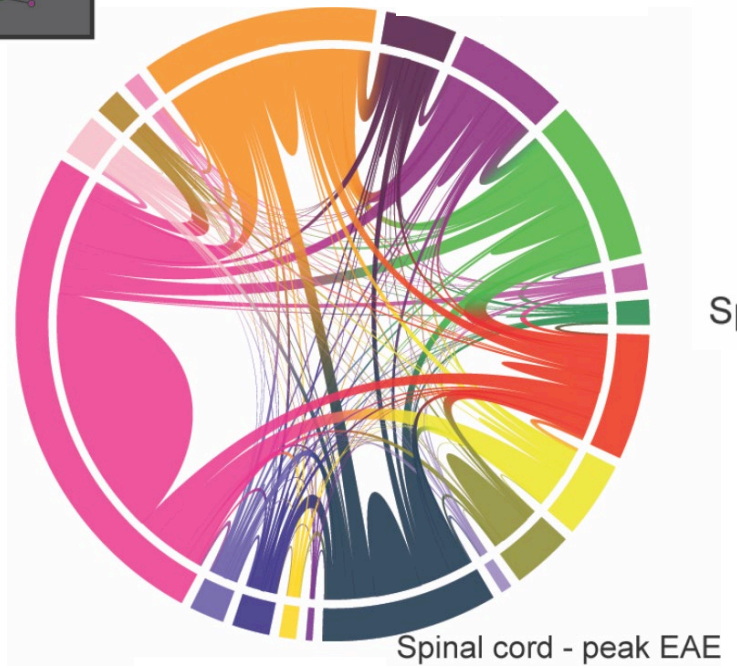
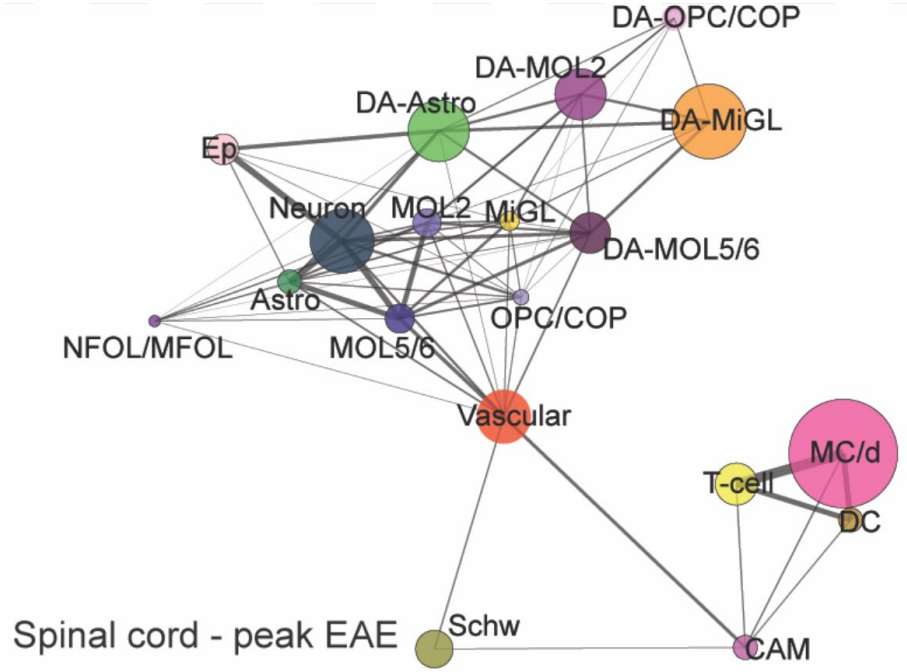
Langseth, Christoffer Mattsson, et al. "Single cell-resolution in situ sequencing elucidates spatial dynamics of multiple sclerosis lesion and disease evolution." *bioRxiv* (2023): 2023-06.

Connections between Cell Types from Spatial Neighborhood Analysis







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Summarization of 2024 NUSeq Updates

- Short read sequencing focuses on latest technologies
- PacBio Revio HiFi long read sequencing will greatly strengthen LRS
- More single cell sequencing options are available, not only on RNA, but also DNA and methylation sequencing
- More spatial transcriptomics platforms established, with 10x Xenium In Situ System being the latest offering
- Integrated single cell sequencing and spatial characterization creates unprecedented opportunities

Thank you

Questions?

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