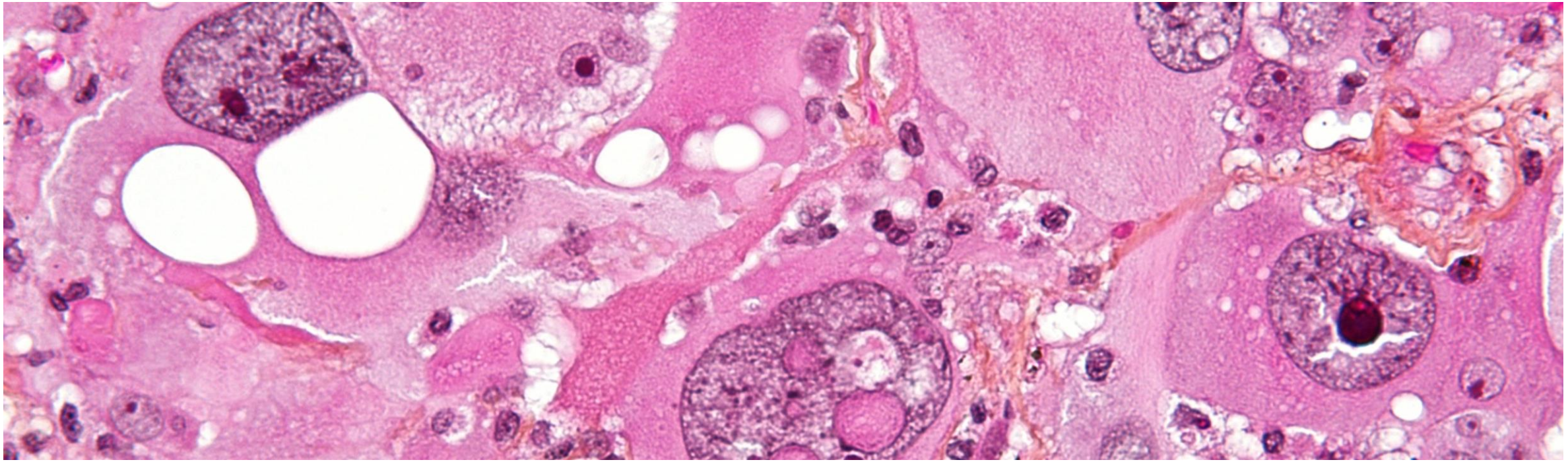
PREDICTING GLIOBLASTOMA BEHAVIOUR

RNA Velocoraptors

Alice Eddershaw, Ester Paolocci, Ashwin Jainarayanan, Jakke Neiro, Szymon Stodolak

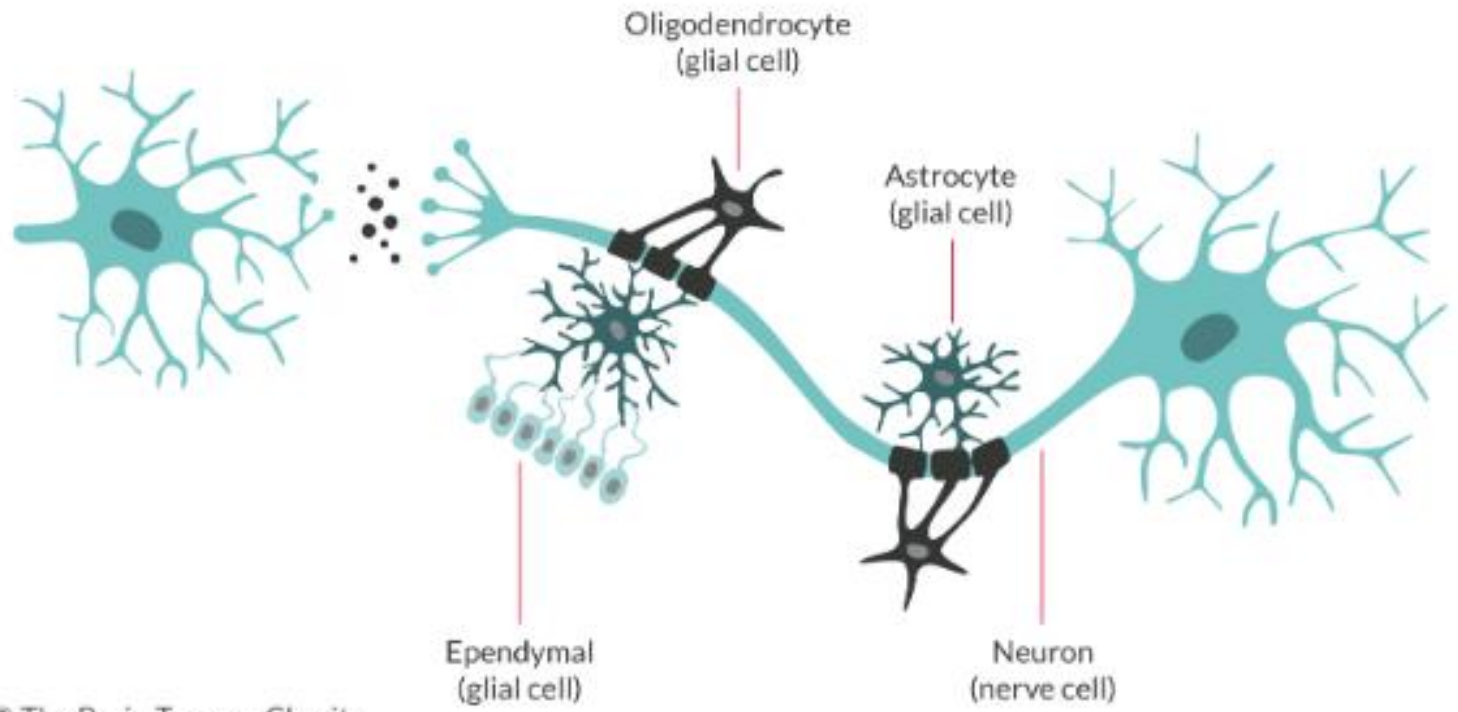


SUMMARY

- Glioblastoma biology
- Intro to RNA Velocity
- Methodology
- Results
- Discussion
- Future Applicaions

GLIOBLASTOMA

WHO classification "central nervous system tumour of grade IV histological malignancy"



- Primary gliomas, arise from normal glial cells
- Secondary gliomas originate from tumours of lower grade
- Deregulation of checkpoint G1/S of a cell cycle and occurrence of multiple genetic abnormalities
- Infiltration is common in the brain
- Incidence of extracranial metastasis of GBM is as low as 0.5%

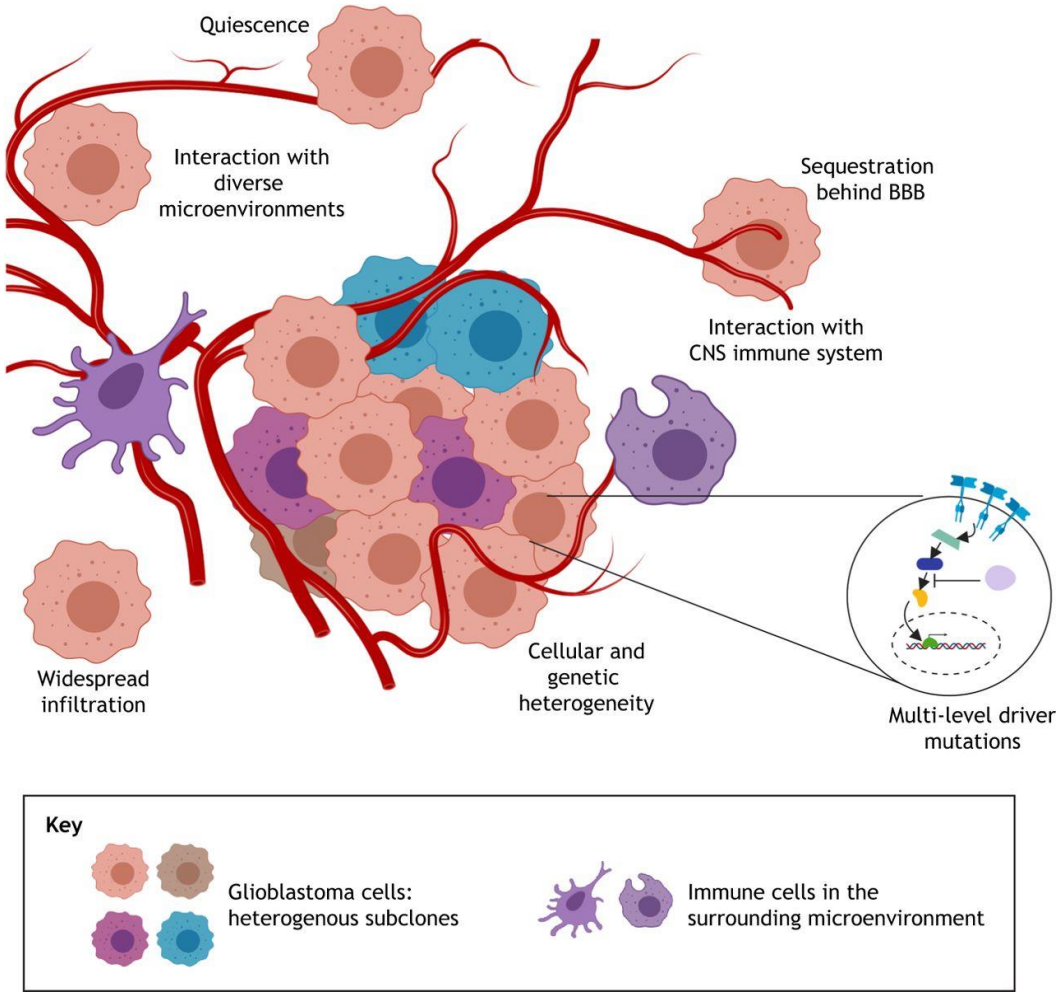
GLIOBLASTOMA

"Intricate ecosystems composed of diverse cells, including malignant, immune, and stromal subsets"

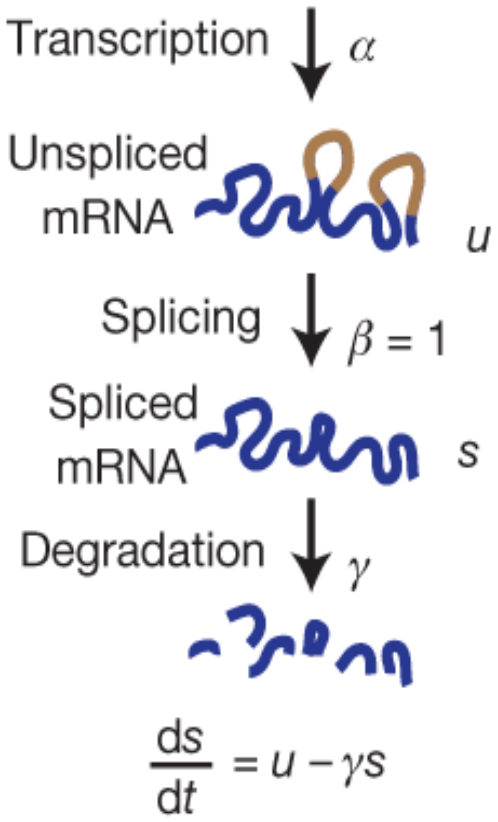
Cell types available for analysis:

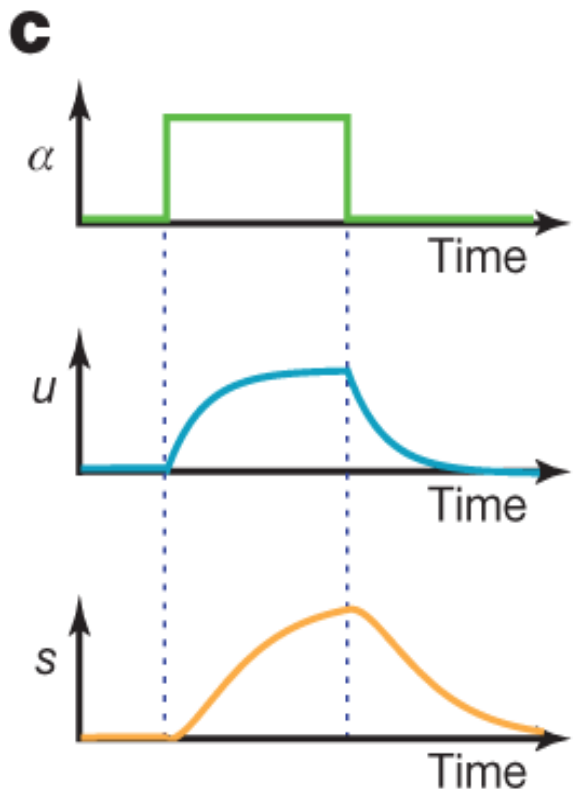
- Neoplastic
- OPC (Oligodendrocyte precursor cells)
- Immune cells
- Oligodendrocytes
- Astocytes
- Vascular
- Neuron

Origin likely from OPC



RNA VELOCITY

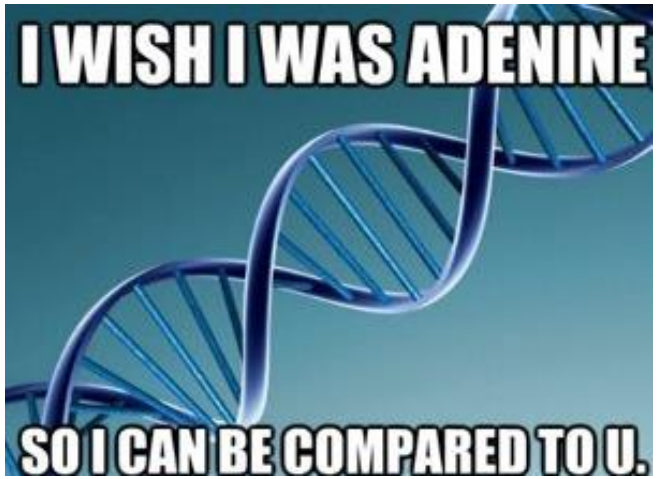




- Package for analysis of expression dynamics in single cell RNA seq data
- Enables estimations of RNA velocities of single cells by distinguishing unspliced and spliced mRNAs

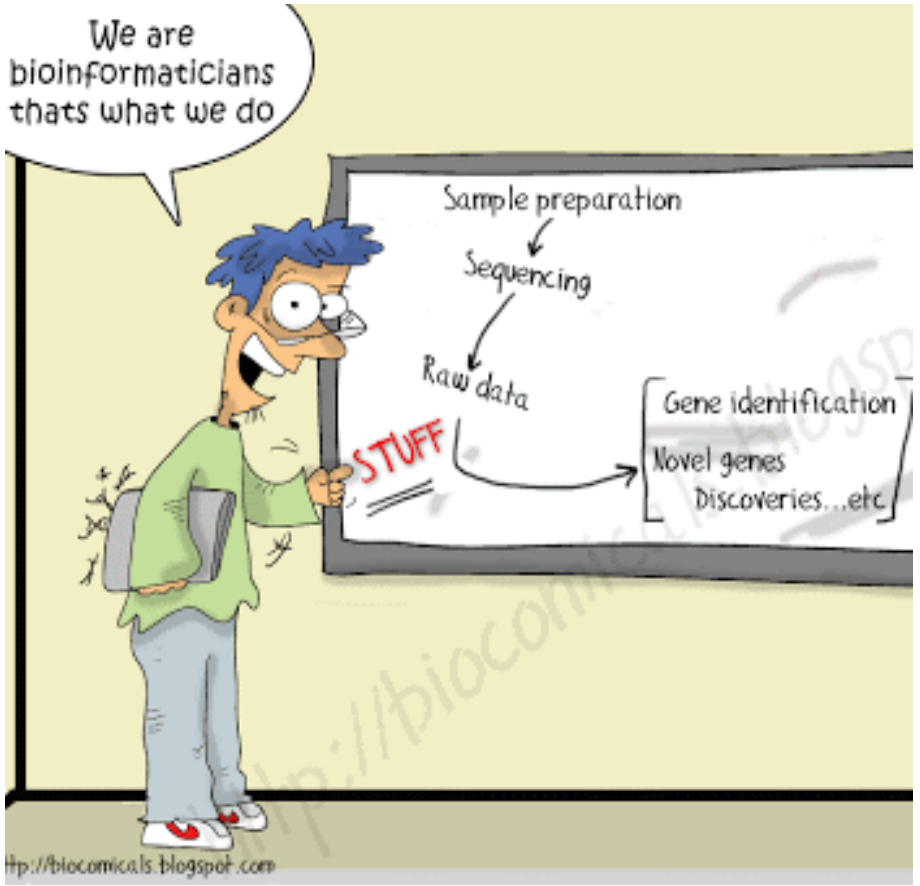
AIM OF OUR HACKATHON

- Could we use RNA velocity to understand differences between Glioblastoma tumour layers?
- Would RNA Velocity help identify origin of Glioblastoma?
- How could we use RNA Velocity to predict/detect tumour migration?
- Could RNA Velocity help identify new markers of glioblastoma progression?



METHODS OVERIEW

- 1. Single-cell RNA data from glioblastoma-associated cells
- 2. Alignment to human genome
- 3. Counting spliced/unspliced RNA
- 4. RNA velocity estimation and PCA



METHODS 1: RAW DATA

PRELIMINARY SET : 4 cells

- Neoplastic tumour
- Neoplastic periphery
- Immune tumour
- Immune periphery

FINAL SET: 100 cells

- Neoplastic tumour (41)
- Neoplastic periphery (29)
- Immune tumour (15)
- Immune periphery (15)

- Data dimensions 600 Mb/cell (FASTQ)
- Darmanis et al. 2018. *Single-Cell RNA-Seq Analysis of Infiltrating Neoplastic Cells at the Migrating Front of Human Glioblastoma*
- Paired-end reads Smart-seq2 protocol
- Two analysis runs: preliminary set and final set
- Sequences retrieved with SRA toolkit and the paired reads were split into two files

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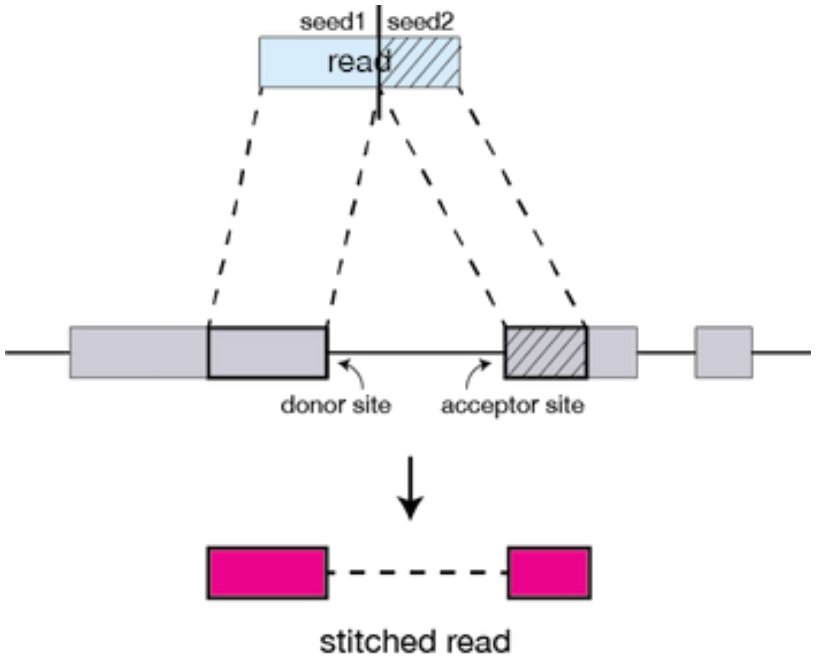
METHODS 2: STAR INDEX AND ALIGNMENT



- The RNA-seq readas were aligned to the human genome (hg19) using STAR
- The STAR genome index was generated with SLURM Workload Manager (1 Node, 32 cores, 300GB RAM)
- STAR aligmnet was performed as a long-running screen process (~3h)
- Measurement of RNA splicing dynamics was performed with velocyto (run-smartseq2) as a long running screen process (~7h)

FIND THE HUMOUR IN THE TUMOUR…

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46414235      saikon   csc     imap1-c2ureaPh      None 2019-12-19T1 2019-12-20T1   17:18:31     1 24        681
46357312      yuzitong csc             python      None 2019-12-17T0 2019-12-20T0   17:03:40     1 2         792
46356308      yuzitong csc             python      None 2019-12-17T0 2019-12-20T0   17:01:24     1 2         796
46356304      yuzitong csc             python      None 2019-12-17T0 2019-12-20T0   17:01:18     1 2         796
46243168      toropai3 csc     SYS_10Na_10Cl_      None 2019-12-13T1 2019-12-20T0   17:00:13     1 8        1168
46418184      keshavar csc           TSBN11b       None 2019-12-19T1 2019-12-20T0   16:56:21     1 4         668
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46326913      asalmiva csc              bash       None 2019-12-13T0 2019-12-20T0   16:55:57     1 1         743
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46417181      keshavar csc           TSPAH2a       None 2019-12-19T1 2019-12-20T0   16:39:30     1 4         668
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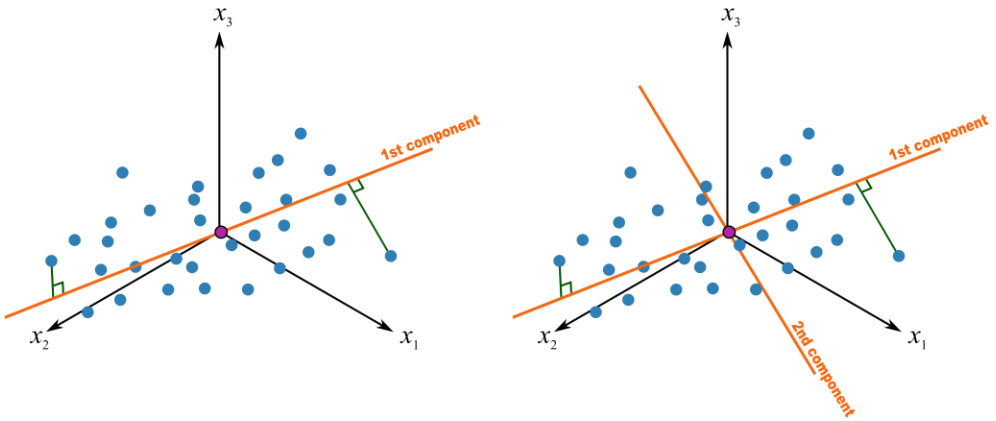
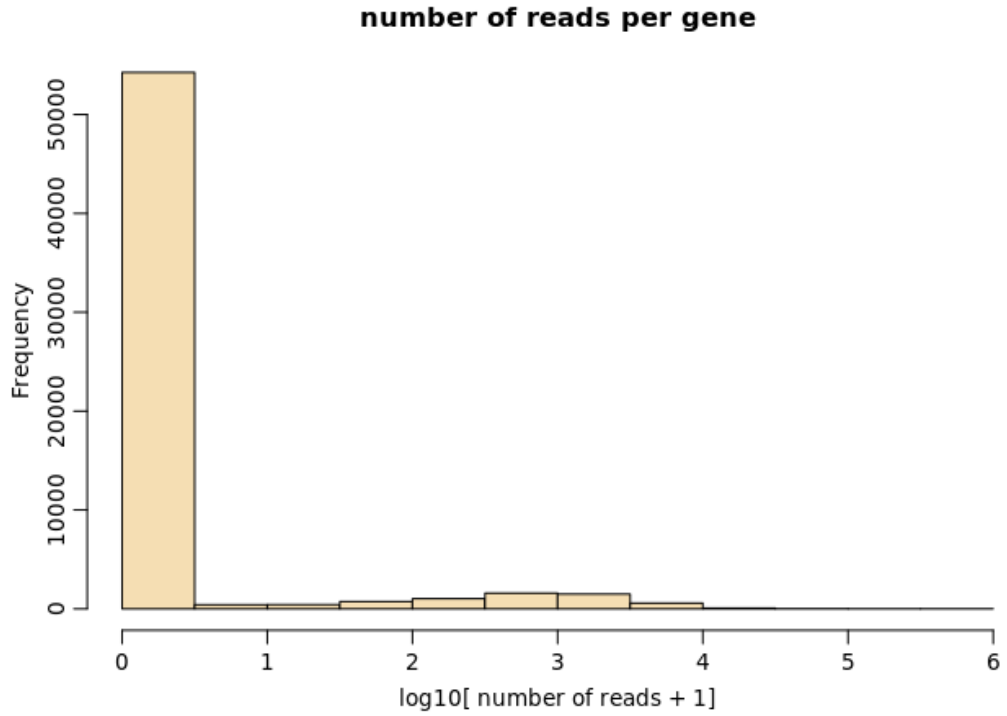
METHODS 3: RNA VELOCITY ANALYSIS

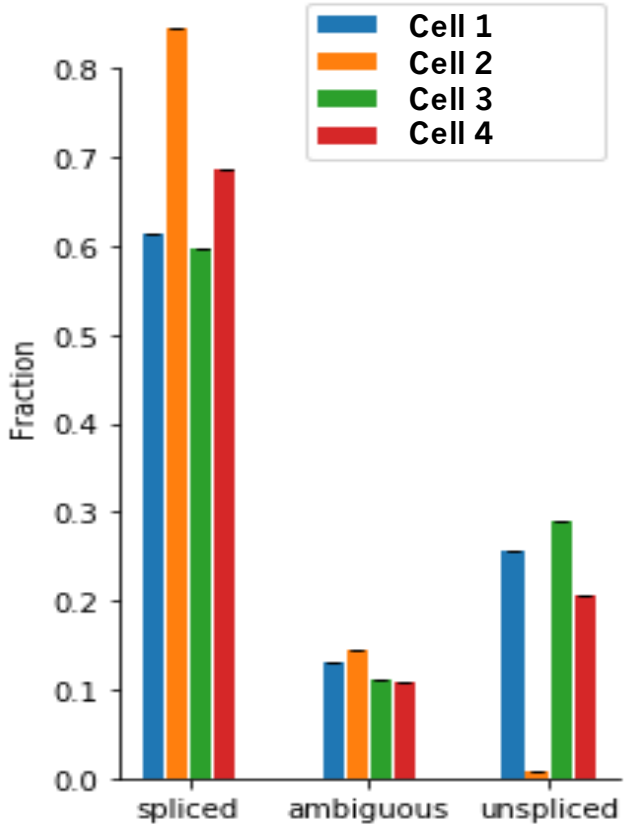


- Velocity estimates calculated with velocyto.R
- PCA: Clustering and visualizing the transcriptomic profile and RNA velocity of all the cells
- PCA: Clustering and visualizing the transcriptomic dynamics of individual genes

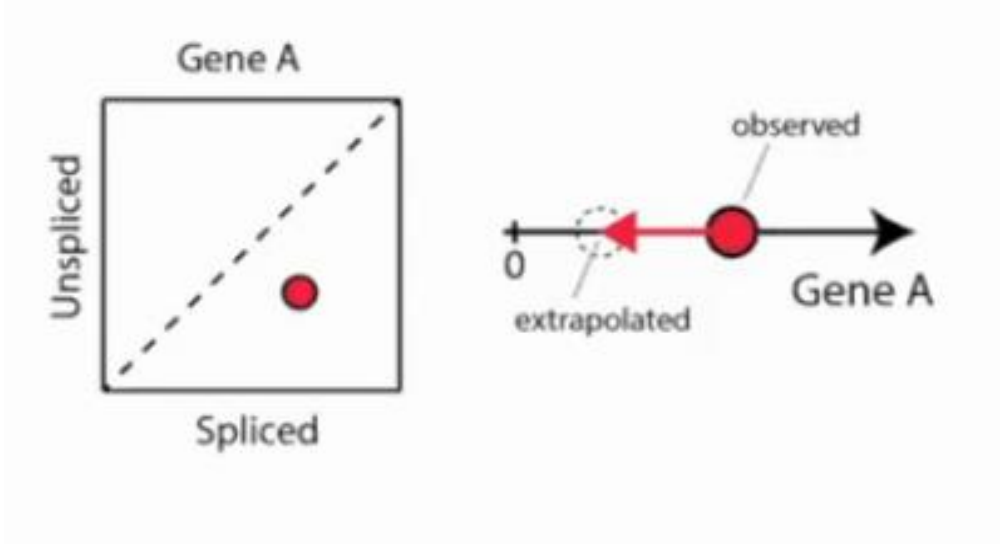
RESULTS

Primilinary Analysis

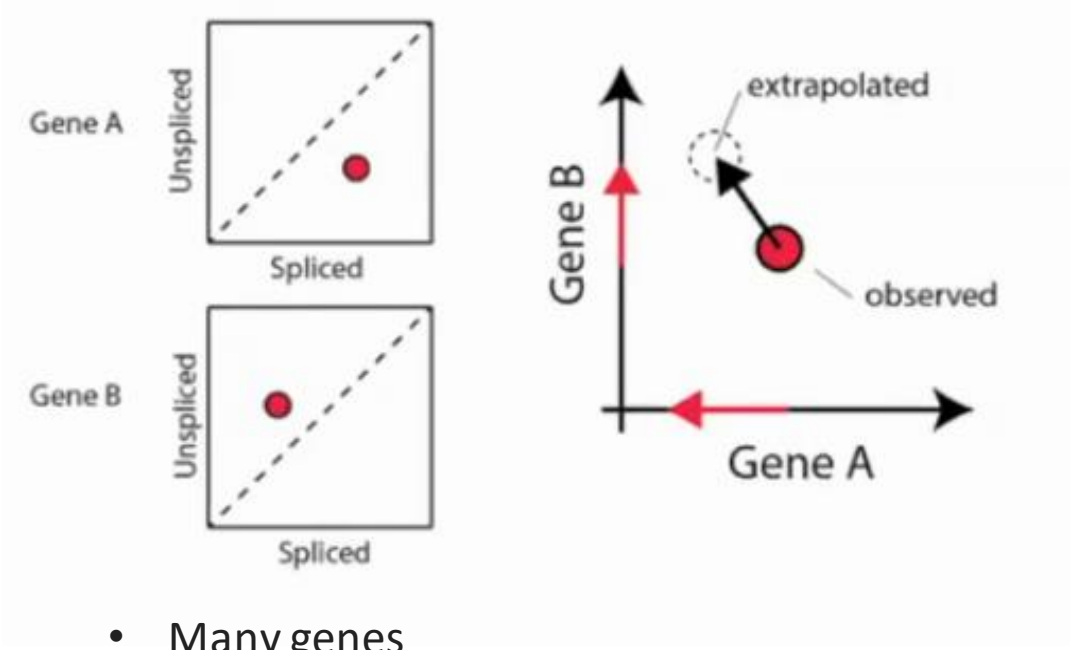




RNA velocity dynamics to predict the future expression state of cell

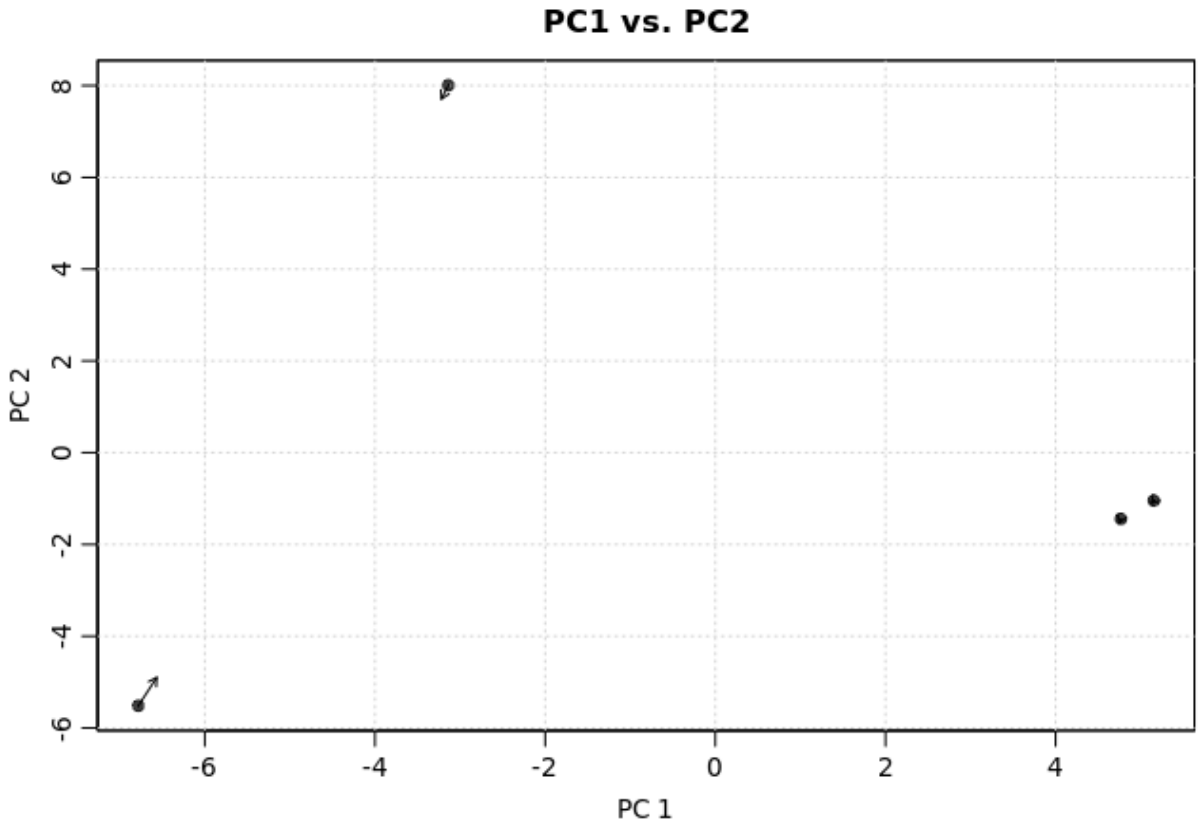


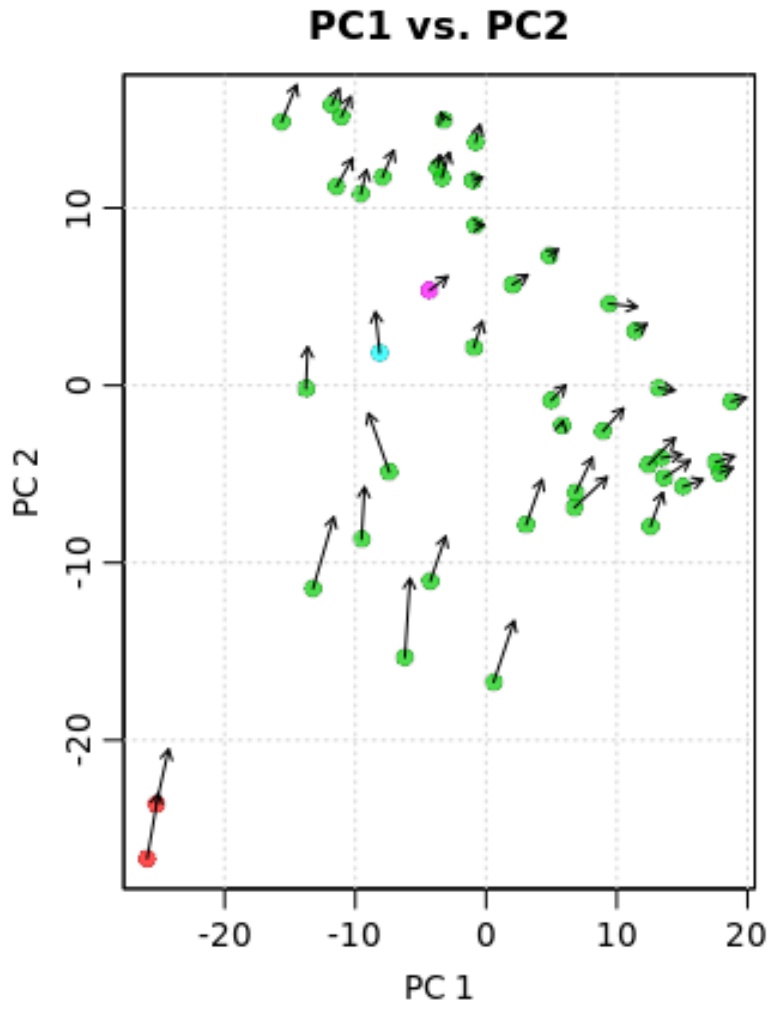
- Single gene
- Ratio of unspliced and spliced mRNAs



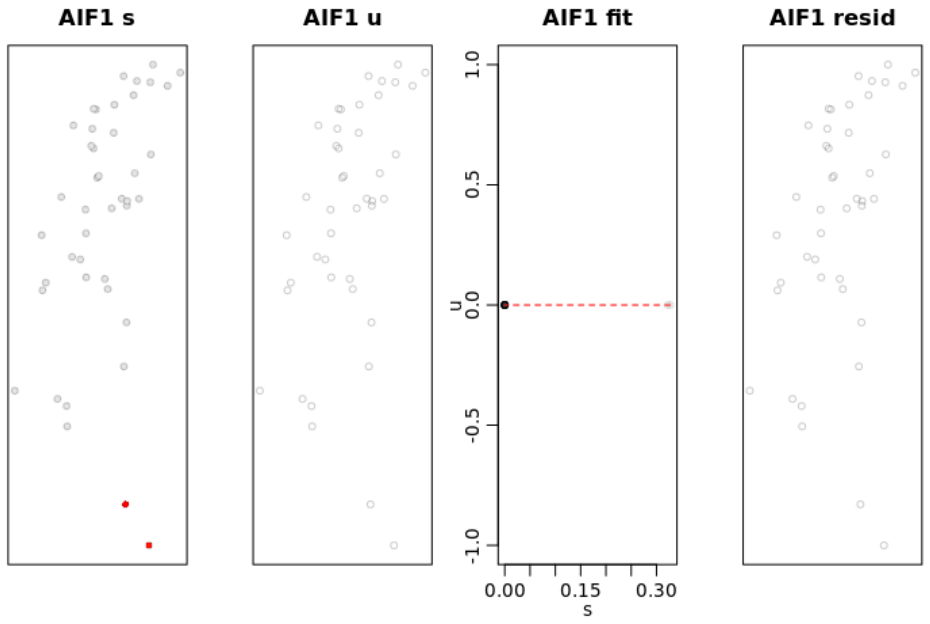
- Many genes
- Future expression state of the cell

Intra-tumor heterogeneity

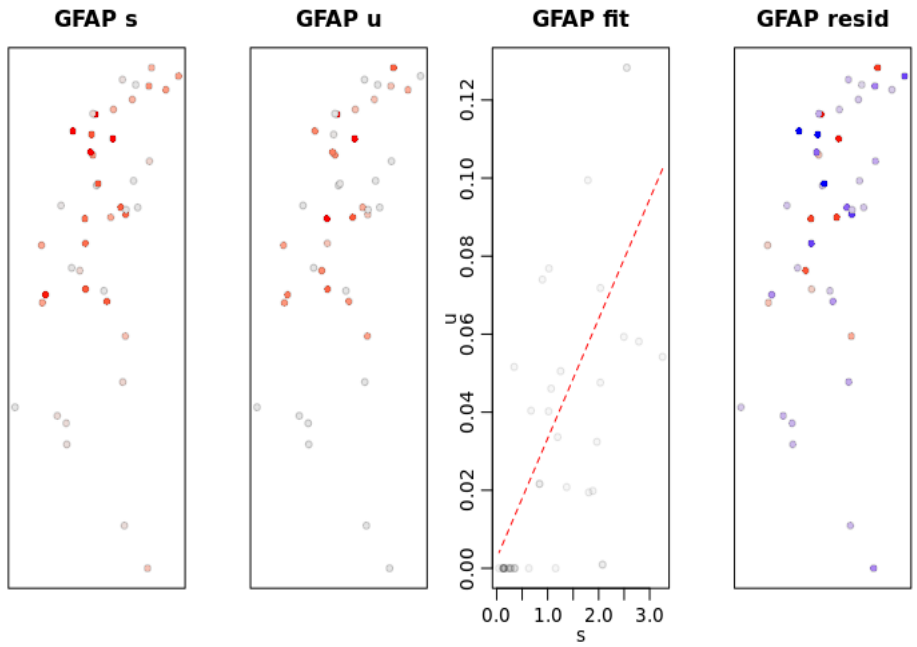




Primilinary validation

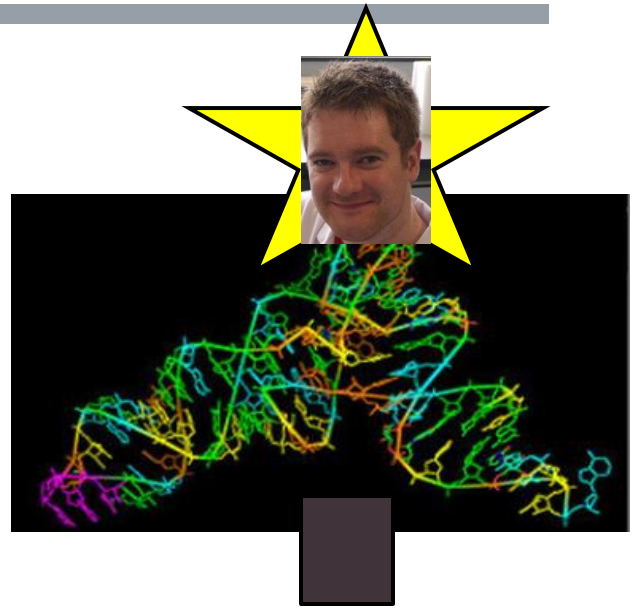


AIF1 – Gene marker for immune cells



GFAP – Gene marker for astrocytes

DISCUSSION



- LIMITED WITH THE DATA: I.E. USING SINGLE PATIENT DATA, mainly looked at neoplastic and immune cells
- Could compare tumour cells to OPC to discover info about the origin or state of the tumour cells
- Could apply our expression profiles to everything in a cancer gene database (not just specific hand-picked genes)
- LIMITED BY THE FACT WE DIDN'T DO THE PRIMARY RESEARCH AND SO DON'T KNOW THE EXACT CONDITIONS (I.e. didn't know the layer from the cell...what does periphery mean (I.e. problem with annotations)

FUTURE APPLICAITONS

- To validate the origin of gliobalstoma
- Use as a tool for predicting prognosis for a patient (predict whether a tumour has potential/has metastasised)
- Used to look at how a tumour is reacting drug treatment
- Can be applied to other cancers

THANKS FOR LISTENING – ANY QUESTIONS?

- **Sorry!**

REFERENCES

https://www.nature.com/articles/s41586-018-0414-6/figures/2

https://actaneurocomms.biomedcentral.com/articles/10.1186/s40478-019-0819-y

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6180049/

https://iopscience.iop.org/article/10.1088/2057-1739/aa9e6e

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4466750/#d35e296

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5810554/

https://www.nature.com/articles/s41598-019-45934-1

https://www.cell.com/molecular-cell/pdf/S1097-2765(19)30356-9.pdf