



EST. 1960 AS THE
TERATOLOGY SOCIETY

*Mini Course: Single-Cell RNA Sequencing in Understanding Normal
and Abnormal Development (July 29, 2021)*

Single-Cell Profiling for Advancing Birth Defects Research and Prevention

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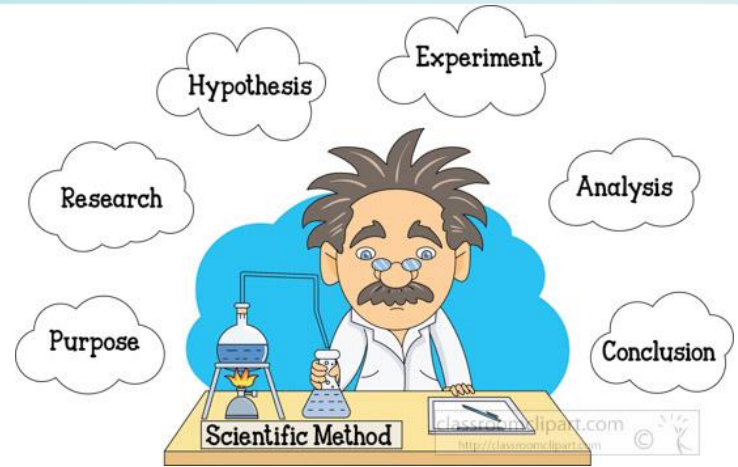
Society for Birth Defects Research and Prevention's **2021 Virtual 61st Annual Meeting**

Introduction to single-cell RNA-seq (scRNAseq)

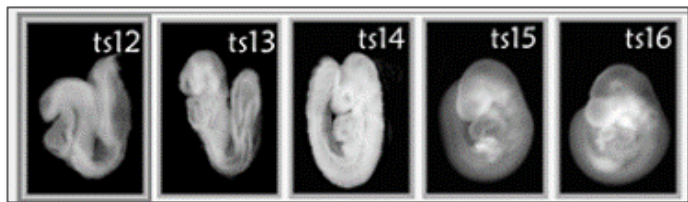
- The fundamental unit in developmental biology is ‘the cell’, each with its own unique history (lineage) and environment (microphysiology).
- NexGen sequencing (RNA-seq) averages the transcriptome across a composite system but scRNAseq can now map individual cell lineages and state dynamics.
- With methods to separate and tag individual cells, scRNAseq enables annotation of tens of thousands of cells in parallel from a composite sample.
- Computational methods are then used to unravel cellular complexity and reconstruct gene expression dynamics for quantitative lineage tracing.

Why profile transcriptomes at the single-cell level?

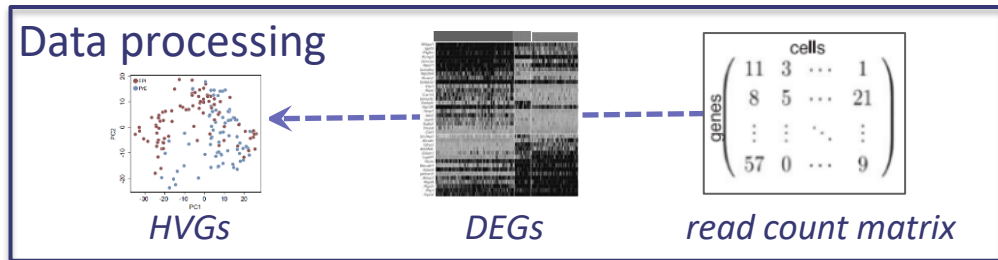
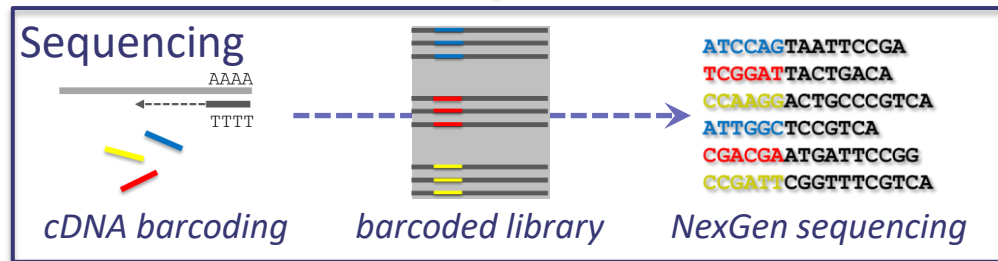
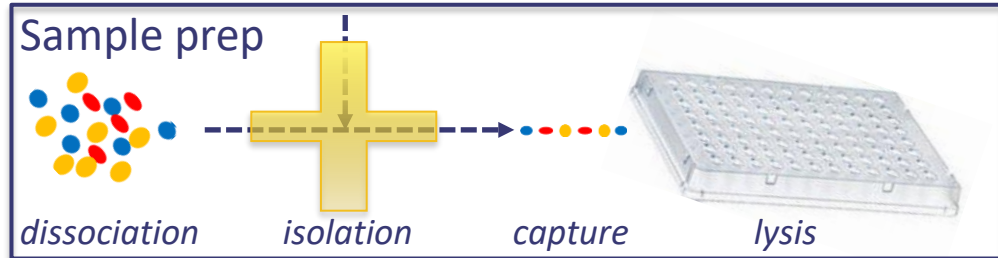
- Fulfills the need for greater detail:
 - molecular progression of all cell lineages, from pluripotency to adulthood
 - how cell-cell signaling pathways control progression at every step.
- Translational opportunities for the science and technology:
 - elucidating pathogenesis of genetic birth defects with cellular precision
 - improvements for predictive toxicology of chemical teratogenesis.



scRNAseq Workflow

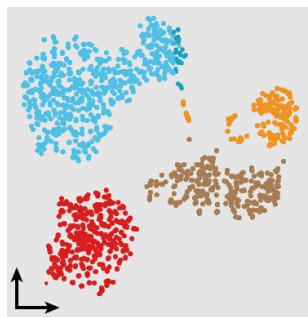


<https://www.emouseatlas.org/emap/ema/home.php>

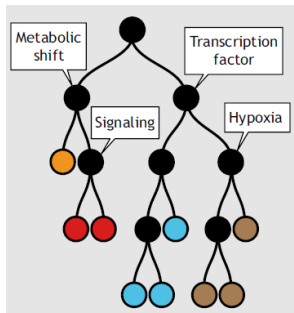


Deconvolution

t-SNE: *t*-distributed stochastic neighbor embedding



t-SNE plot



lineage/state

McKenna and Gagnon (2019) Development 146

Technical Challenges

- Cell separation tools: how well does the scRNA-ome survive preparation steps to generate isolated cells from a composite system?
- Bioinformatics: how to retain individual cell provenance while deep-sequencing thousands of cells in parallel?
- Analysis: how to distinguish highly-variable genes (HVGs) from technical variation (eg, PCR artifacts and over-sequencing)?
- Computational: how to validate transitional cell states, map developmental trajectories, and spatially reconstruct the composite system in pseudotime?

David Gallegos (USEPA) will delve into technical considerations for scRNAseq workflows.

Biological Coverage

- PubMed query for 'scRNAseq + embryo' returned 98 records:
 - pre 2018 = 7
 - 2018/19 = 31
 - 2020/21 = 60
- Developmental systems covered (to date, abridged):

Cellular development

pluripotent stem cells
preimplantation embryo
epiblast-gastrulation
neural crest cells
cell atlases (MCA, HCA)
morphogenetic fusion
epith/mes transformation

Organ-specific

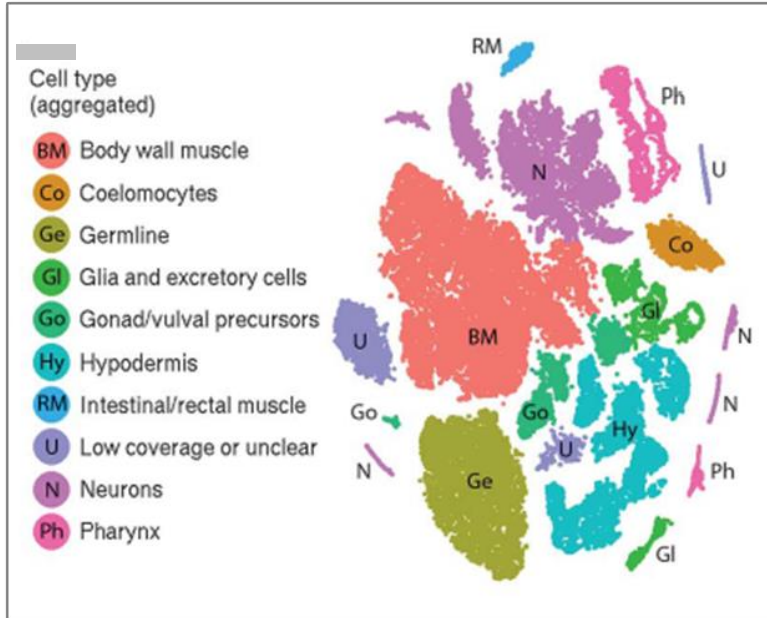
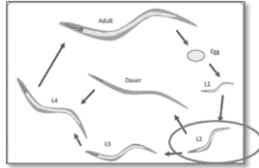
brain and spinal cord
eye and ear
cardiovascular
respiratory
urogenital
musculoskeletal
gastrointestinal

Pregnancy

gametogenesis
maternal/fetal interface
Reproductive immunology
microglial activation
COVID-19 infection

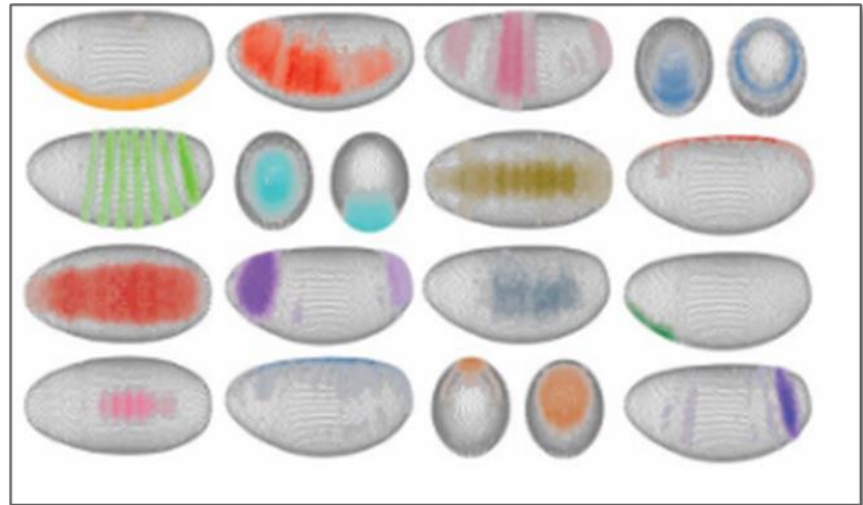
Simple Model Organisms (SMOs)

C elegans: *t*-SNE
decomposing L2 larva
into 29 cell clusters.



Cao et al. (2017) Science

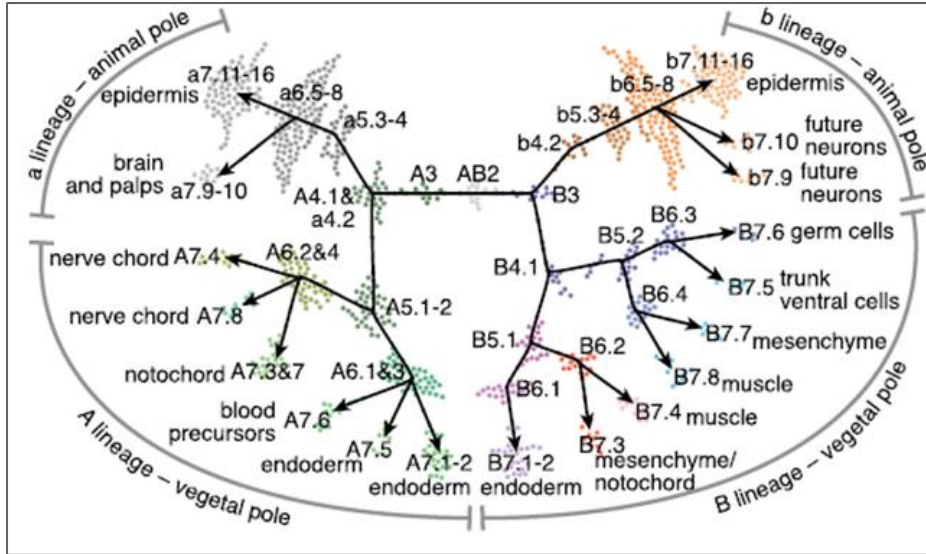
Drosophila: embryo decomposed by
scRNAseq into 84 annotated cell clusters
that can reconstruct developmental
trajectories linked to transcription factors
and signal gradients (virtual ISH).



Karaikos et al. (2017) Science

Ascidian

Lineage tree: 1,042 cells by gene expression history (scRNAseq) and physical position (DLSM) from the 4- to 64-cell stage.



Sladitschek et al. (2020) Cell

vISH: expression dynamics for two genes (chordin, nodal) selected from their online digital library.

02728|chordin

<http://digitalembryo.org>



0 : 00

vegetal pole

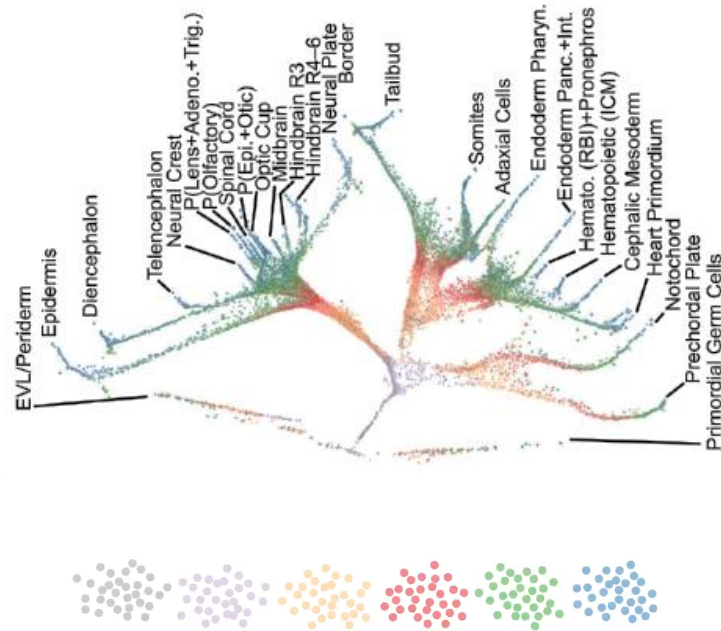
animal pole

02320|nodal



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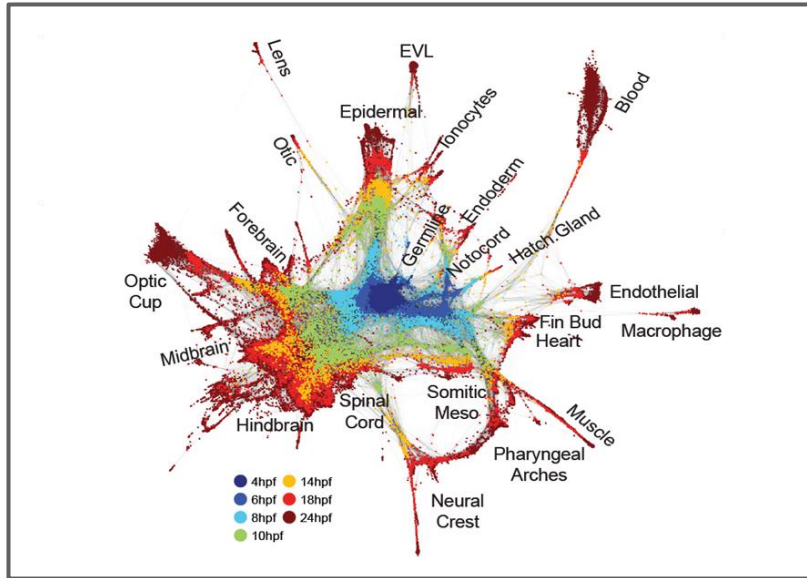
Zebrafish



Uniform Manifold Approximation and Projection (UMAP): scRNAseq lineage tree from 25 cell types built on 38,731 cells captured 3.3 hpf (pluripotent blastula) to 12 hpf (6-somite pharyngula).

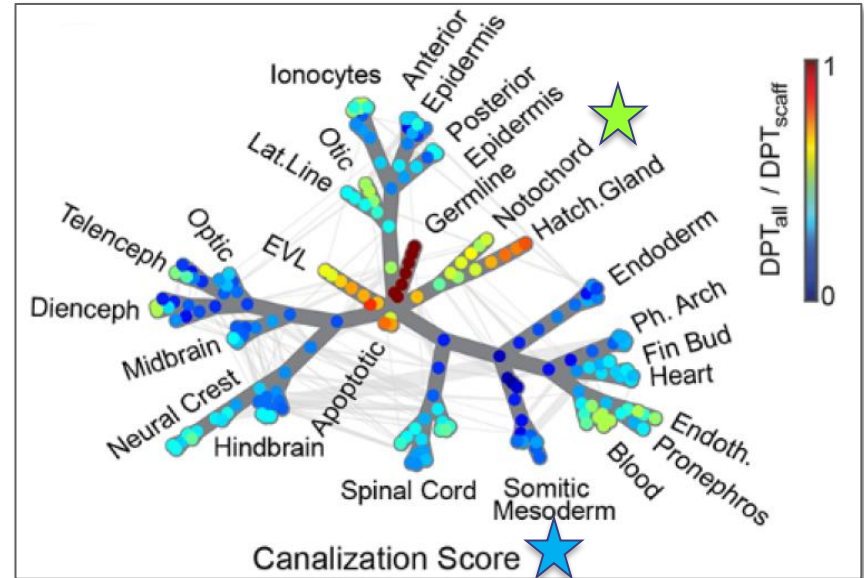
Zebrafish

Comprehensive manifold of lineage and state diversification trajectories for 10^5 individual cells (4- 24 hpf).



Wagner et al. (2018) Science

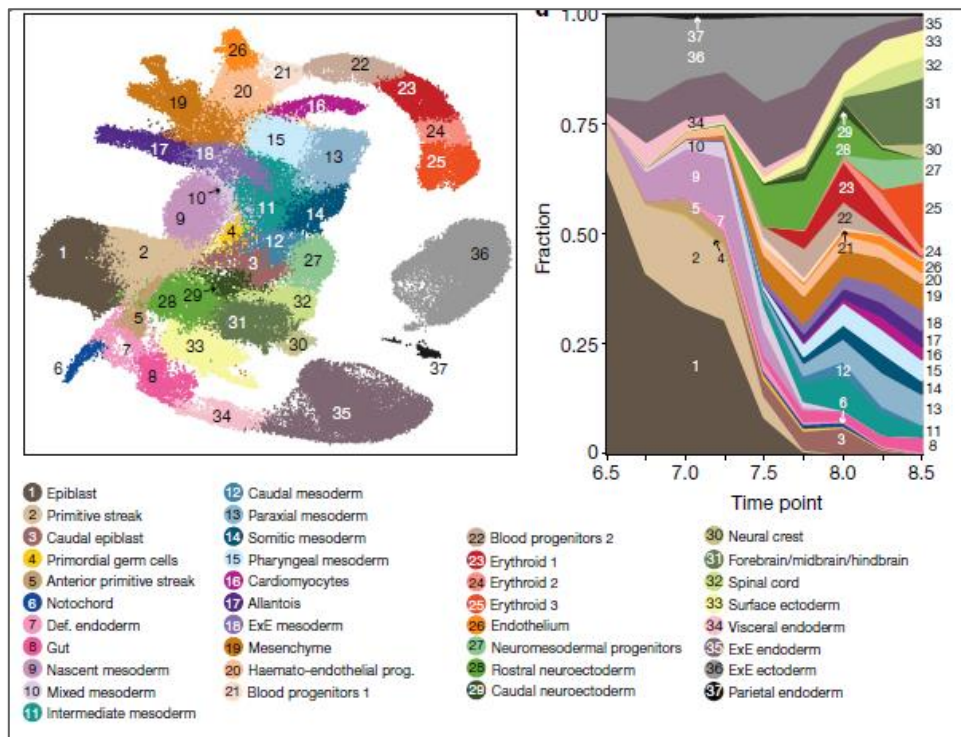
Canalization tree built on 92,000 cells 4 hpf through organogenesis (24 hpf); note buffering notochord > somitic lineages.



Mouse - gastrulation



- The vast majority of cell lineages derive from the 'epiblast' during gastrulation;
- body's fundamental genomic blueprint is also decoded during this period;
- t-SNE lineage staged from 116,312 cells epiblast (GD 6.5) to headfold (GD 8.5);
- Cell trajectories can be reconstructed quantitatively in 'pseudotime'.



Pijuan-Sala et al. (2019) Nature

Cell state manifold vs Cell lineage map



Video courtesy of M Spielmann and J Cao, University of Washington

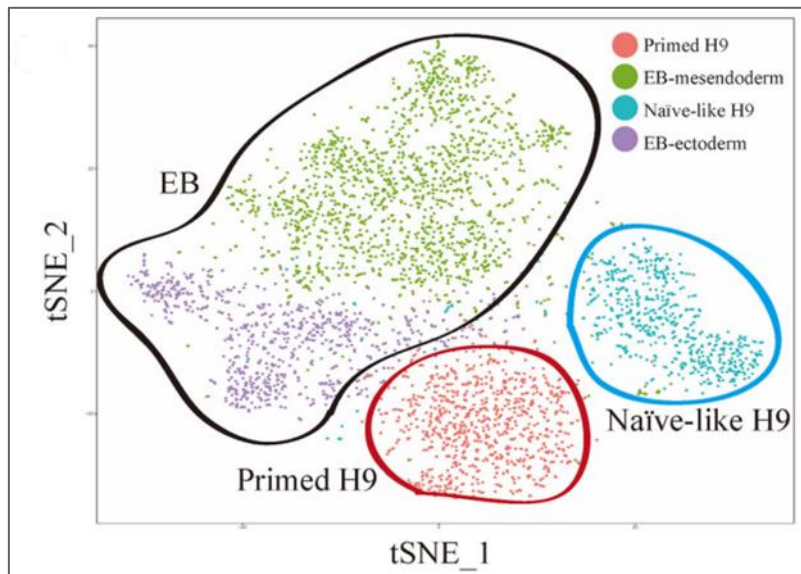
Cell state landscape has a higher dimensionality (655) than cell lineage (38).

Continuum gene expression manifolds reduce dimensionality of cell-cell distances to pseudotime state trajectories.

Yvanka de Soysa (Boston Children's Hospital) will show how this can be used to unravel developmental defects.

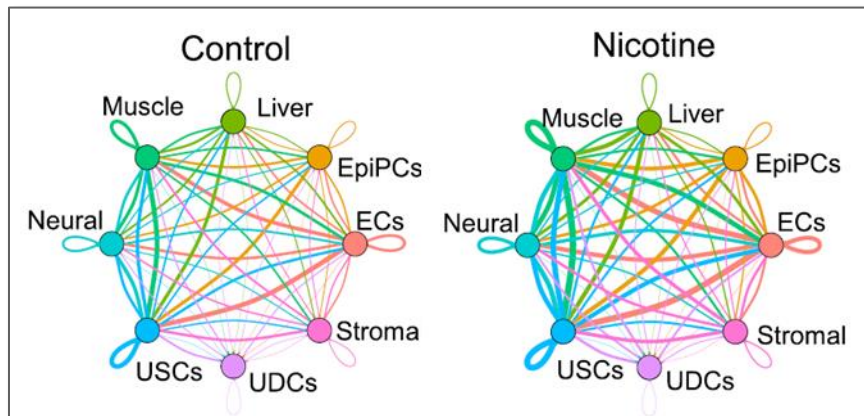
Embryonic stem cell (hESC) line culture

hESCs (0- 8 days in culture): 4822 barcoded progenitors at naïve, primed, and embryoid body stages.



Han et al. (2018) Genome Biol 19

Drug challenge: 21-day embryoid bodies barcoded for 10 μ M nicotine effects, inferring alteration of ligand-receptor interplay across specific cell types.



Guo et al. (2019) Stem Cell Reports 12

Developmental impairments

Recent studies have applied single-cell profiling to neuro-developmental outcomes, for example::

- cell-type-specific spatial organization of the brain [Cembrowski, 2019]
- cell-type-specific dysfunctions during impaired neurogenesis [Zywitz et al. 2018]
- transitional states during specification of neural-glial progenitors [Weng et al. 2019]
- transitional states in microglia activation during neuroinflammation [Sousa et al. 2018]
- tumor progression in patient-specific cerebral organoids [Krieger et al. 2020].
- ... and more.

Josh Robinson (UCSF) will address the vision of profiling developmental toxicity with single cell resolution.

Applications for birth defects research ...

Opportunities for unique data and information that can enhance understanding of birth defects and developmental disabilities:

- *defining trajectories of molecular processes driving development*
- *comparing cell types and functional states across species, tissues, and organs*
- *providing important information for human-relevant AOP frameworks*
- *cell atlases for anchoring new approach methodologies (in silico, in vitro)*
- *improving ability to identify early events and track pathogenesis.*



Knudsen, Spielmann, Megason and Faustman (2021) Birth Defects Res (BDRP 2020 symposium)

Now, let's hear from the experts ...

References for Images

- Slide 4 McKenna and Gagnon (2019) Recording development with single cell dynamic lineage tracing. Development 146, dev169730. doi:10.1242/dev.169730 (10 pages).
- Slide 7 Cao et al. (2017) Comprehensive single-cell transcriptional profiling of a multicellular organism. Science 357: 661–667.
- Slide 7 Karaiskos et al. (2017) The Drosophila embryo at single-cell transcriptome resolution. Science 358: 194-199.
- Slide 8 Sladitschek et al. (2020) MorphoSeq: full single-cell transcriptome dynamics up to gastrulation in a Chordate. Cell 181: 922–935.
- Slide 9 Farrell et al. (2018) Single-cell reconstruction of developmental trajectories during zebrafish embryogenesis. Science 360: doi:10.1126/science.aar3131.
- Slide 10 Wagner et al. (2018) Single-cell mapping of gene expression landscapes and lineage in the zebrafish embryo. Science 360: 981-987.
- Slide 11 Pijuan-Sala et al. (2019) A single-cell molecular map of mouse gastrulation and early organogenesis. Nature 566: doi.org/10.1038/s41586-019-0933-9.
- Slide 12 Cao et al. (2019) The single-cell transcriptional landscape of mammalian organogenesis. Nature 566: doi.org/10.1038/s41586-019-0969-x.
- Slide 13 Han et al. (2018) Mapping human pluripotent stem cell differentiation pathways using high throughput single-cell RNA-sequencing. Genome Biol. 19: 47. doi.org/10.1186/s13059-018-1426-0.
- Slide 13 Guo et al. (2019) Single-cell RNA sequencing of human embryonic stem cell differentiation delineates adverse effects of nicotine on embryonic development. Stem Cell Reports 12: 772–786.
- Slide 14 Knudsen TB, Spielmann, Megason SG and Faustman EM (2021) Single-cell profiling for advancing birth defects research and prevention. Birth Def Res 113: 546-559.