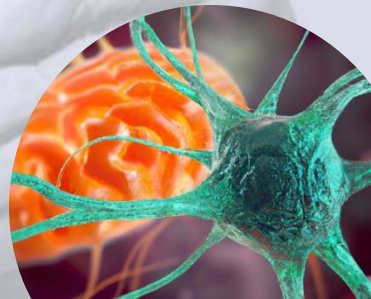
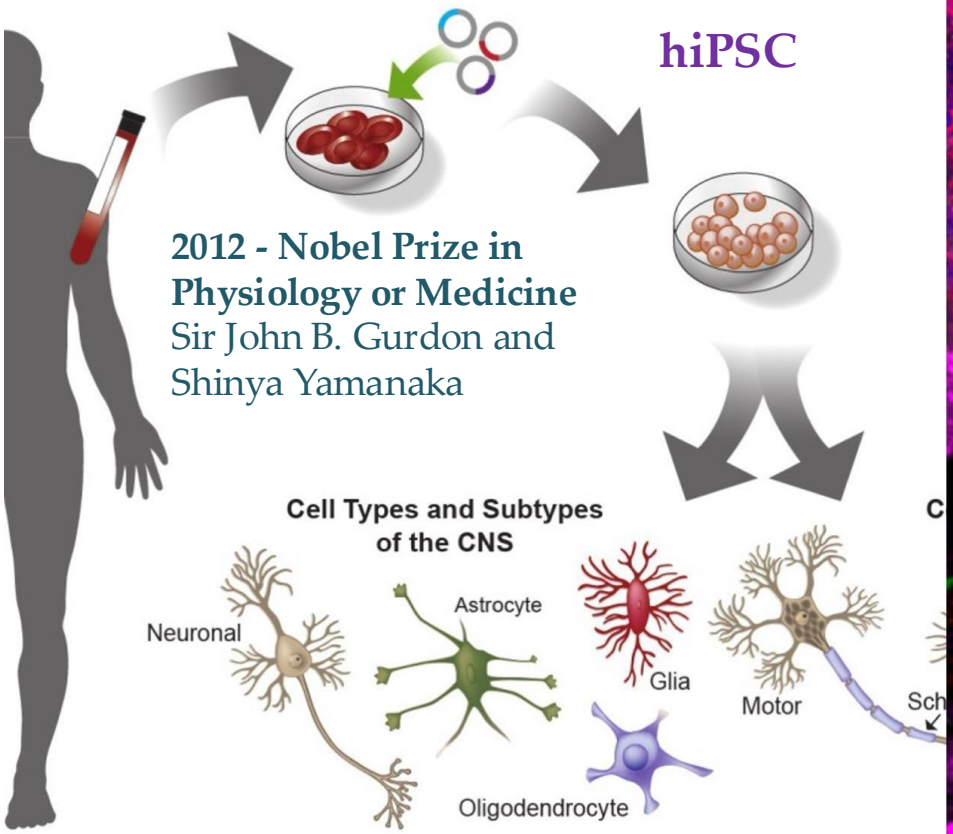
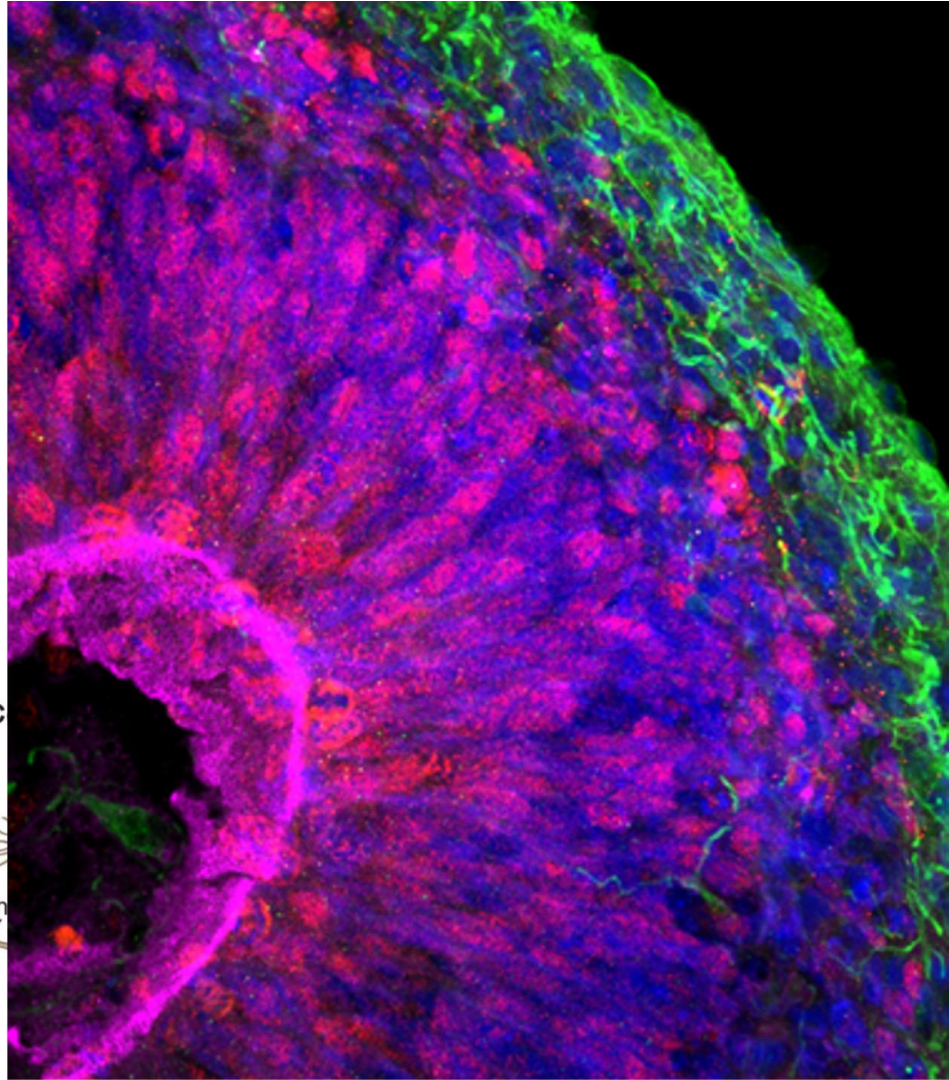
Multiscale Approaches with 3D Brain Organoids for the Study of Brain Disorders Across Development and Degeneration



Biological twins





Shifting paradigms

2012 - Nobel Prize in Physiology or Medicine

Sir John B. Gurdon and Shinya Yamanaka

"for the discovery that mature cells can be reprogrammed to become pluripotent."

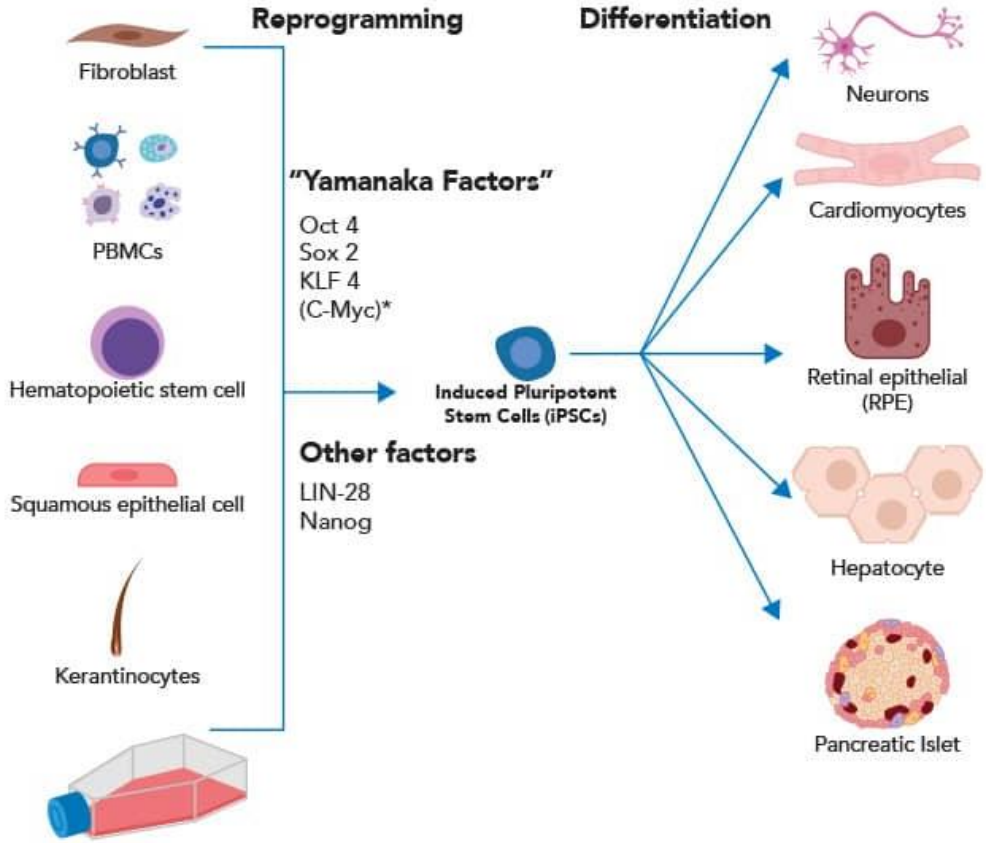
2020 - Nobel Prize in Chemistry

Emmanuelle Charpentier and Jennifer A. Doudna

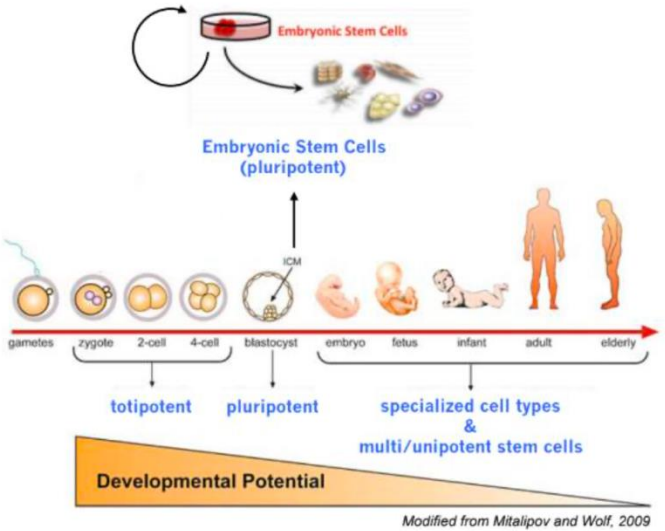
"for the development of a method for genome editing."

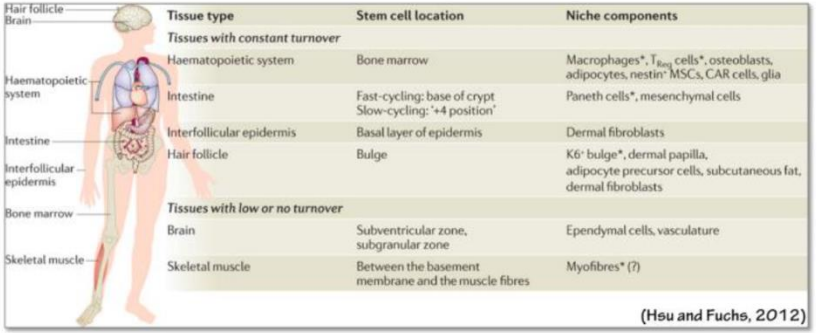
Induced Pluripotent Stem Cells (iPSCs)

- **Definition**: Somatic (adult) cells reprogrammed back to a pluripotent stat
- **Reprogramming Factors**: *OCT4*, *SOX2*, *KLF4*, *c-MYC* ("Yamanaka factors").
- **Properties**:
- Self-renew indefinitely in vitro
- Differentiate into all three germ layers (endoderm, mesoderm, ectoderm)
- **Applications**:
- Disease modeling (*patient-derived organoids*)
- Drug screening & toxicity testing
- Personalized & regenerative medicine



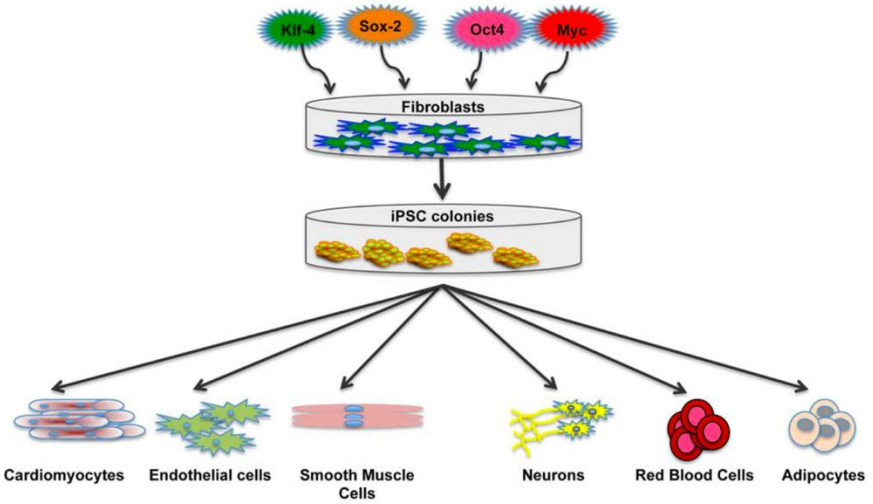
Induced Pluripotent Stem Cells (iPSCs)





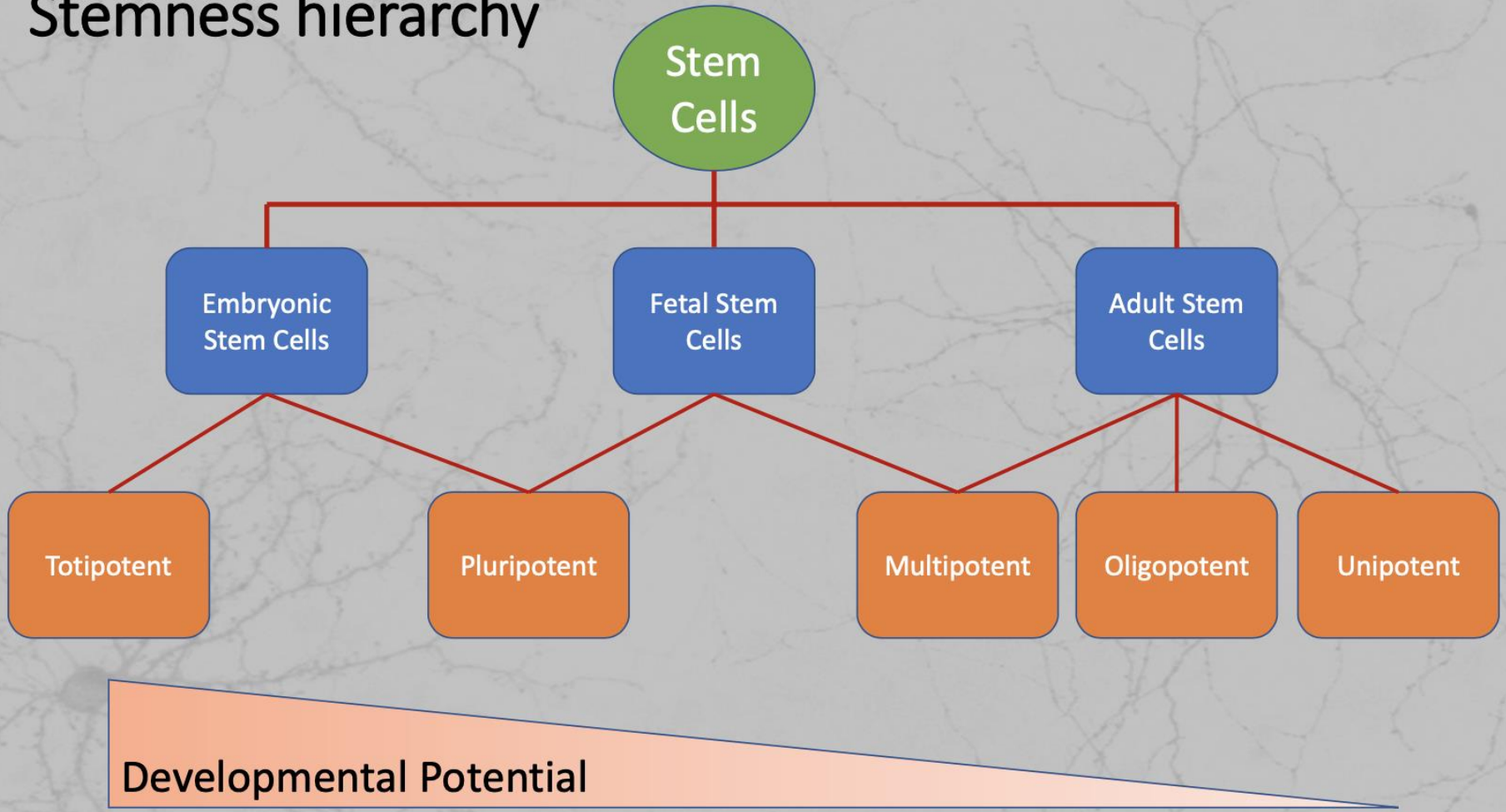
Tissue type	Stem cell location	Niche components
Tissues with constant turnover		
Haematopoietic system	Bone marrow	Macrophages*, T_{Reg} cells*, osteoblasts, adipocytes, nestin$^+$ MSCs, CAR cells, glia
Intestine	Fast-cycling: base of crypt Slow-cycling: '+4 position'	Paneth cells*, mesenchymal cells
Interfollicular epidermis	Basal layer of epidermis	Dermal fibroblasts
Hair follicle	Bulge	K6$^+$ bulge*, dermal papilla, adipocyte precursor cells, subcutaneous fat, dermal fibroblasts
Tissues with low or no turnover		
Brain	Subventricular zone, subgranular zone	Ependymal cells, vasculature
Skeletal muscle	Between the basement membrane and the muscle fibres	Myofibres* (?)

(Hsu and Fuchs, 2012)

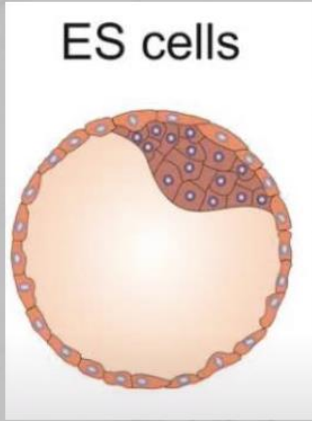


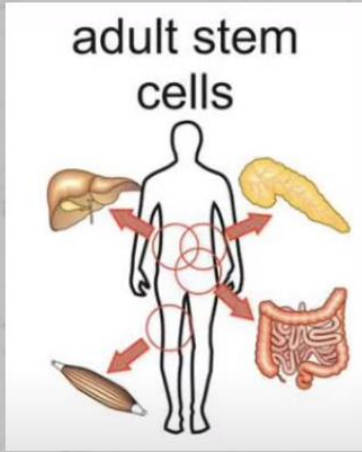
The human induced pluripotent stem cells represent an easy accessable, convenient and valuable alternative to embryonic stem cells and other in situ stem cell populations.

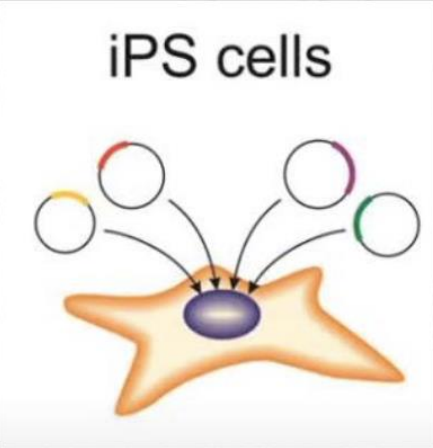
Stemness hierarchy



Stem cell types







Pros:
- Highly expandable
- Pluripotent

Cons:
- Tumorrisk
- Geneticinstability
- Ethicalissue

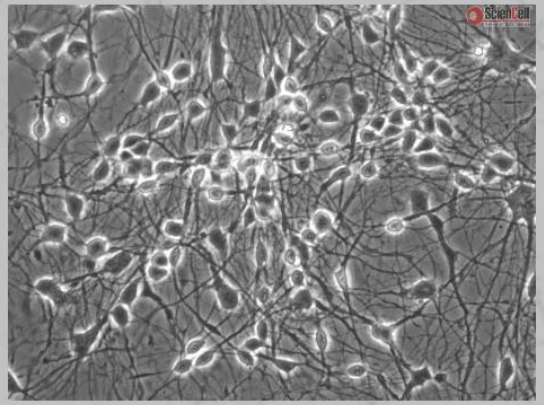
Pros:
- Multipotent
- Low tumor risk
- Tissue specification

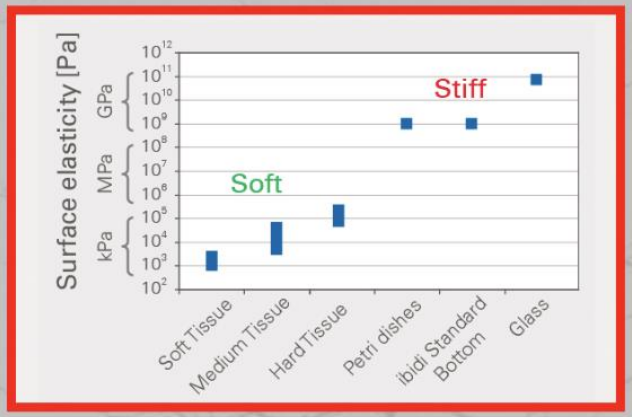
Cons:
- Invasiveness
- Inefficient in vitro expansion

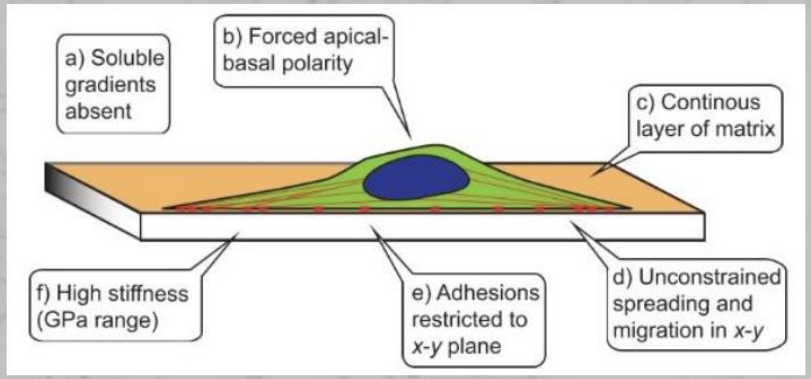
Pros:
- Highly expandable Pluripotent
- Reprogrammed

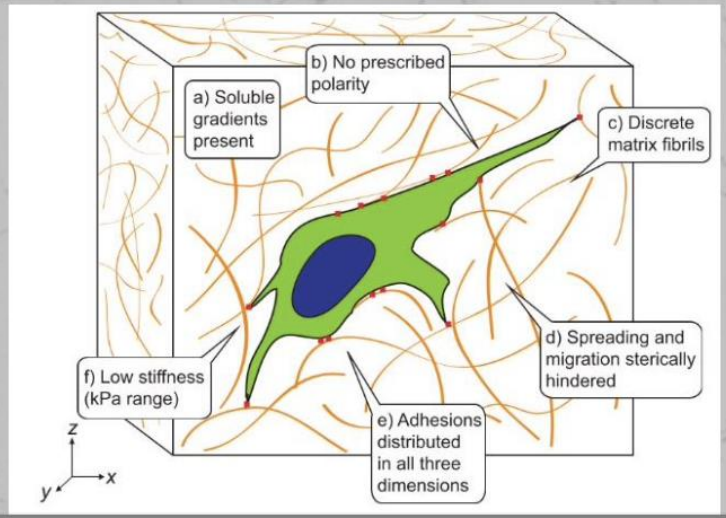
Cons:
- Tumor risk
- Genetic instability
- Reprogramming approach

Culturing system shift

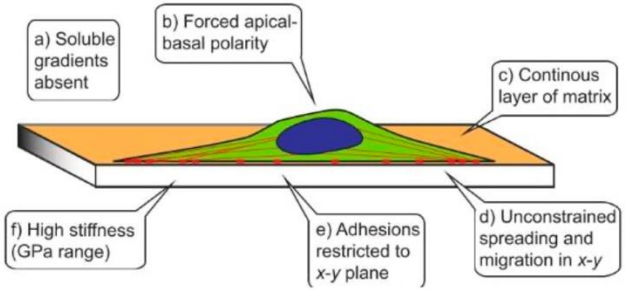


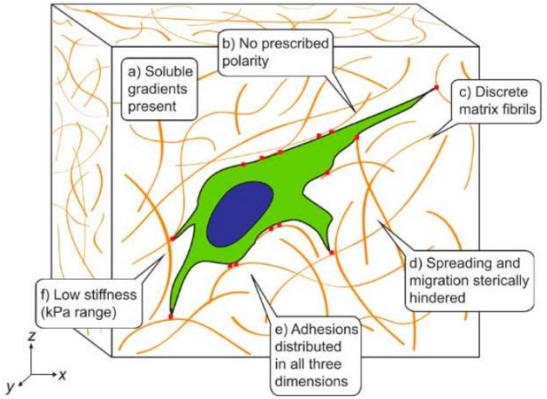


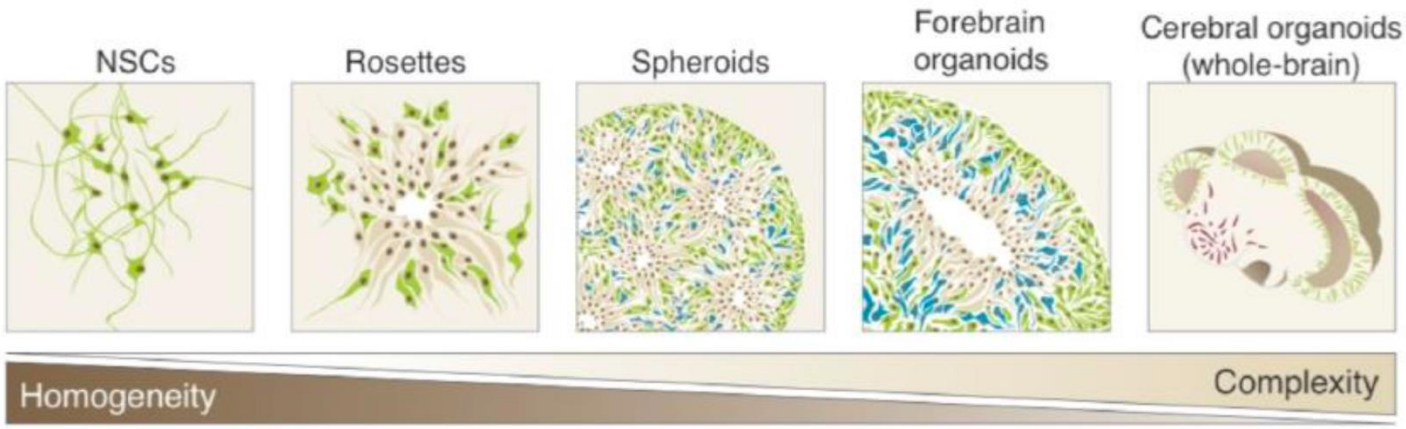




From 2D to 3D cell cultures





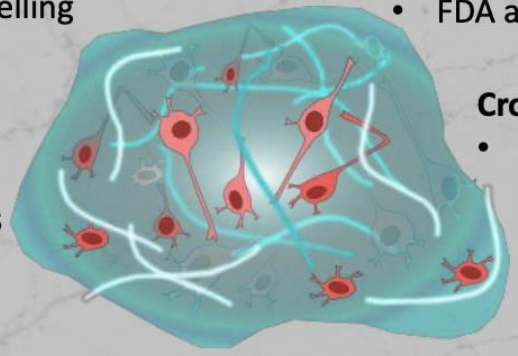


The importance of the 3D matrix

Mechanical properties
- Tunable to reach the elasticity of the desired tissue
- Mesh size, porosity, crosslinking density, swelling

Biocompatibility
- No or negligible toxic effects
- Sterilization
- FDA approval

Mass transport
Continuous exchange of nutrients, proteins, gases and waste products



Crosslinking in presence of cells
- Limited noxious effects on cells

Mimicking microenvironment
- Mimicking the native extracellular matrix (ECM)
- Allowing the cells to produce their own ECM

Degradability
- Control of degradation kinetics/stability

What are cerebral organoids?

- A cerebral organoid describes artificially grown, *in vitro*, miniature organs resembling the brain.

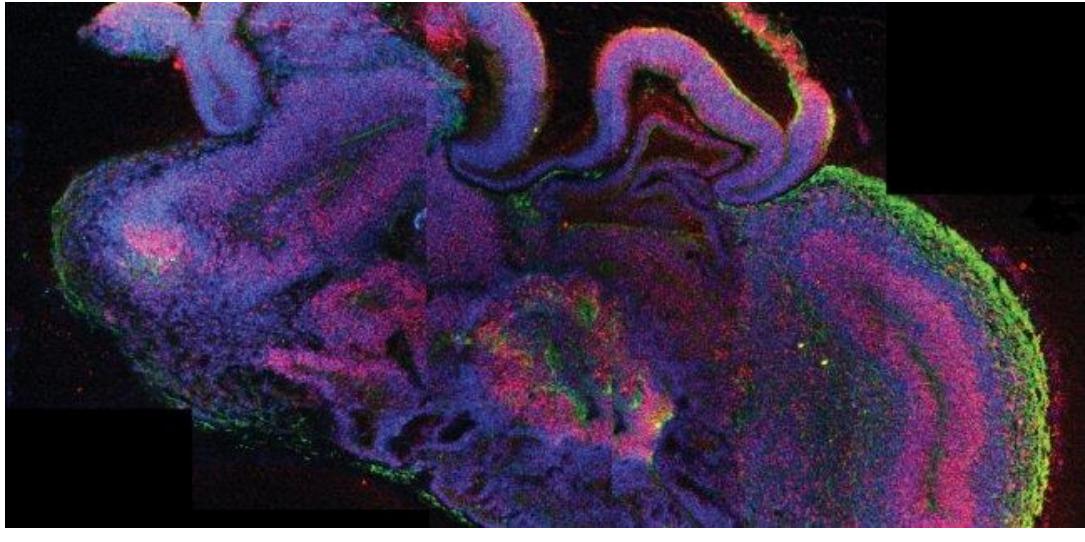
- They are created by culturing human pluripotent stem cells in a three-dimensional rotational bioreactor and develop over a course of months .

ARTICLE

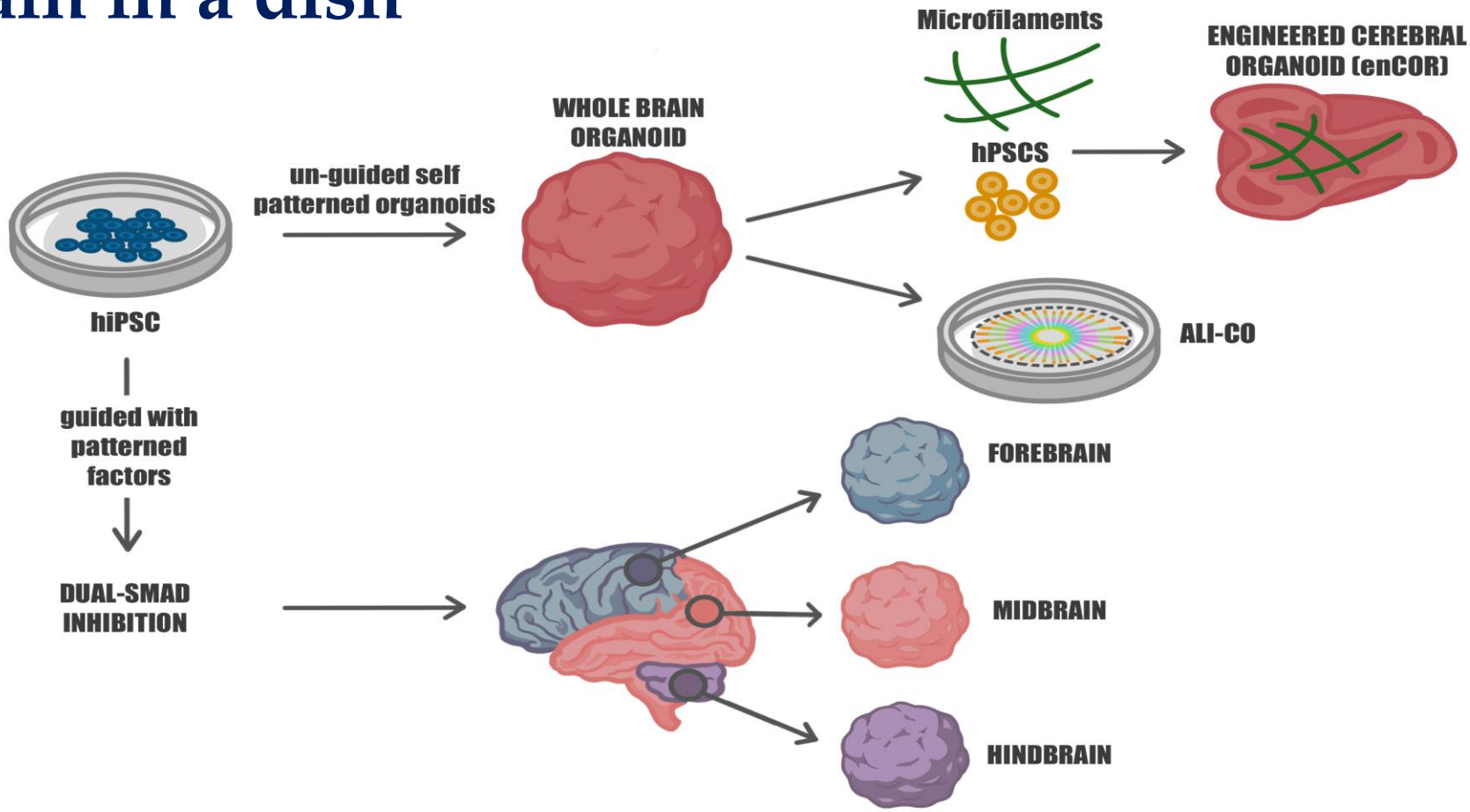
doi:10.1038/nature12517

Cerebral organoids model human brain development and microcephaly

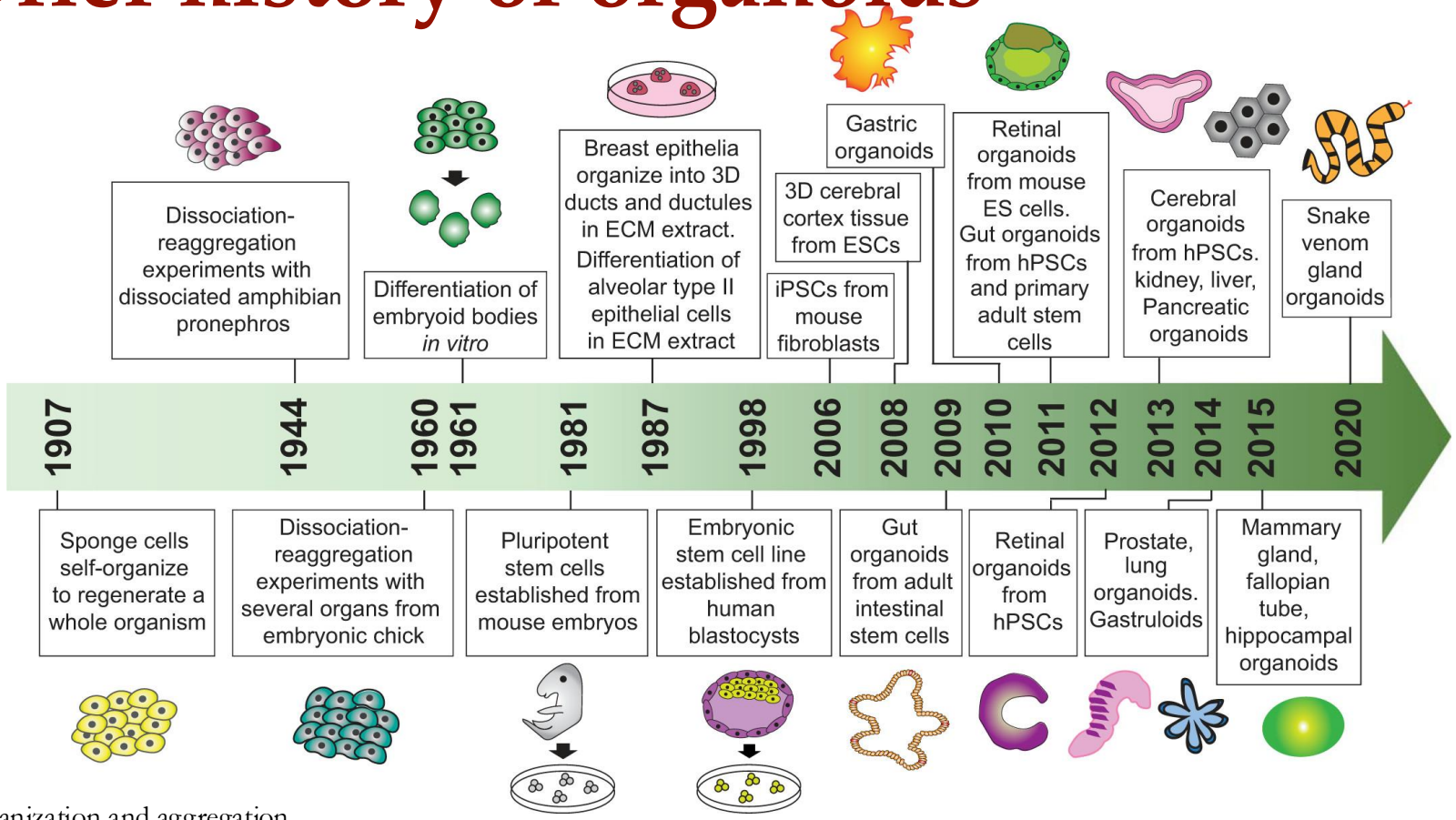
Madeline A. Lancaster[1], Magdalena Renner[1], Carol-Anne Martin[2], Daniel Wenzel[1], Louise S. Bicknell[2], Matthew E. Hurles[3], Tessa Homfray[4], Josef M. Penninger[1], Andrew P. Jackson[2] & Juergen A. Knoblich[1]



Increasing complexity to mimic the human brain in a dish



A brief history of organoids



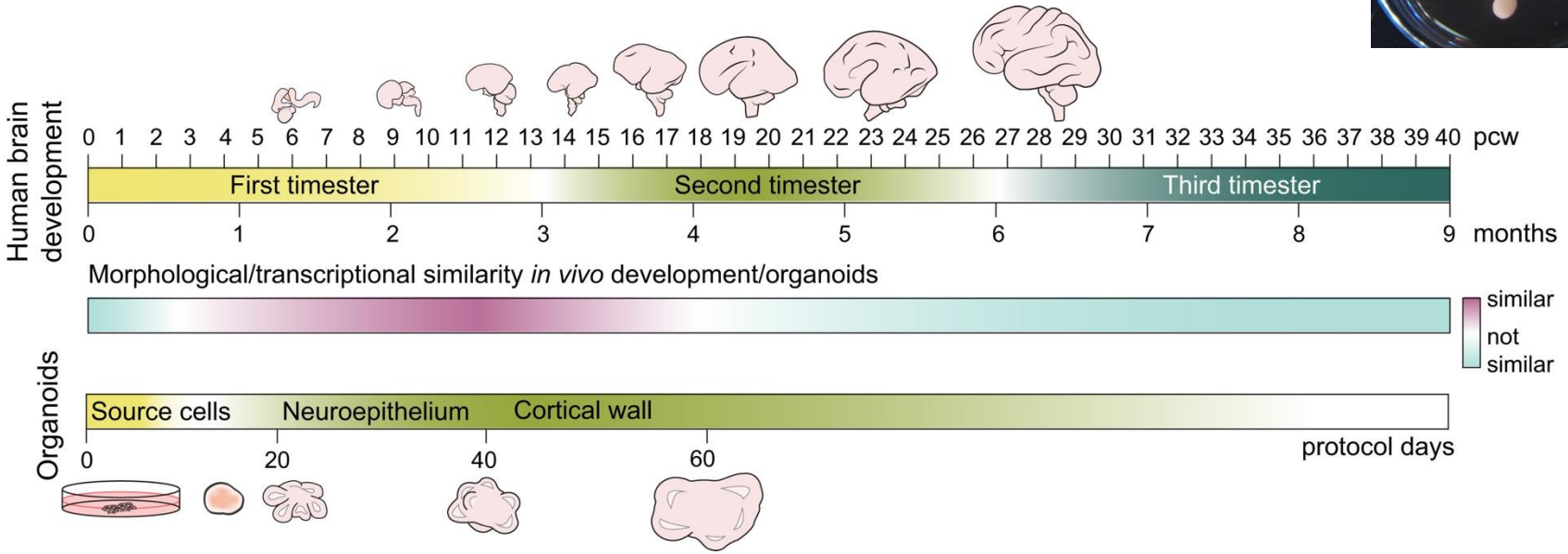
Self-organization and aggregation properties in vitro

(Corrò et al, 2020)

Similarities between human brain and organoids development: the concept of mini-brains

Mini-brains in a dish

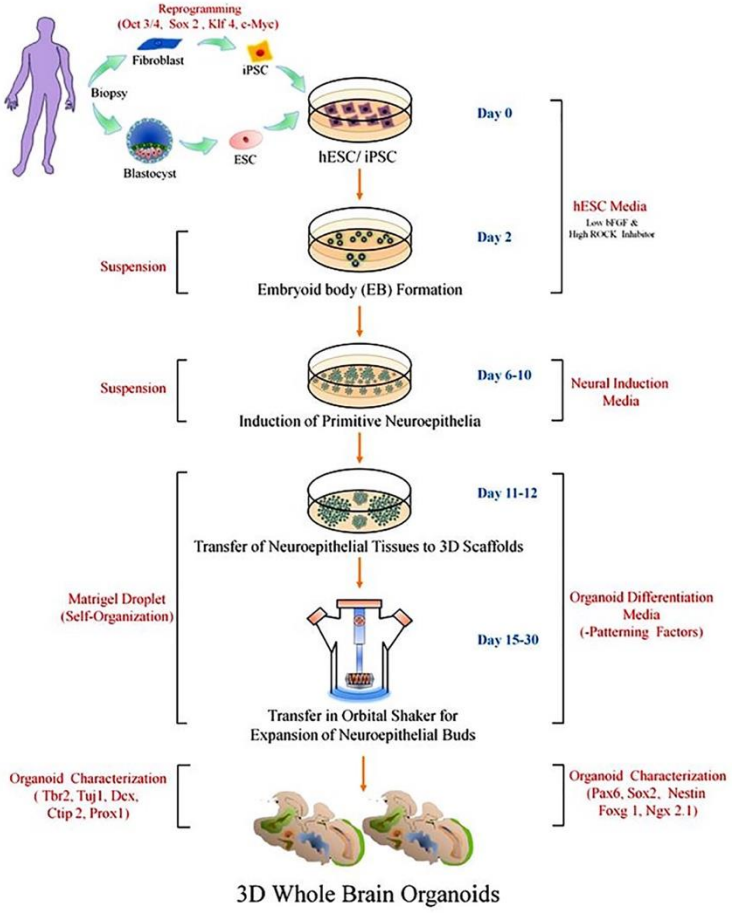
We are currently able to model early human neocortical development accurately



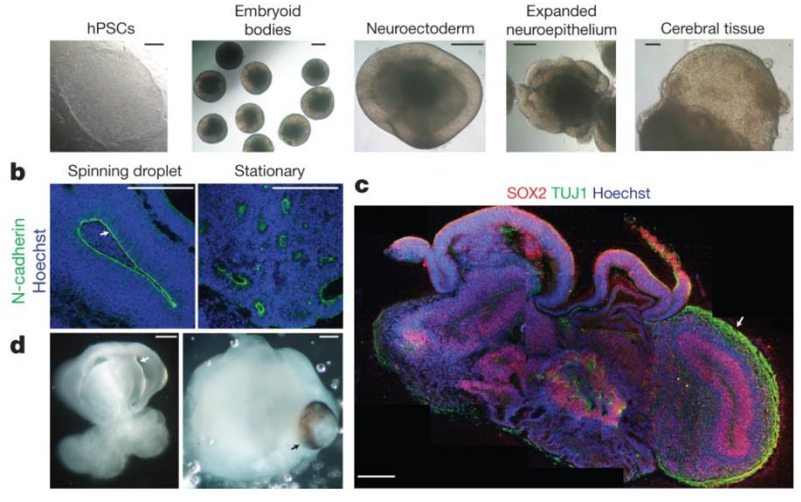
Similarities are based on cell biology and transcriptional (RNA-seq and single-cell RNA-seq) features.
However, there are methods' limitations, such as the inability to vascularize the cultures and the possible lack of some intrinsic and extrinsic cues, which are not replicated with the current protocols.

(Kelava and Lancaster, 2016)

Generation of brain organoids



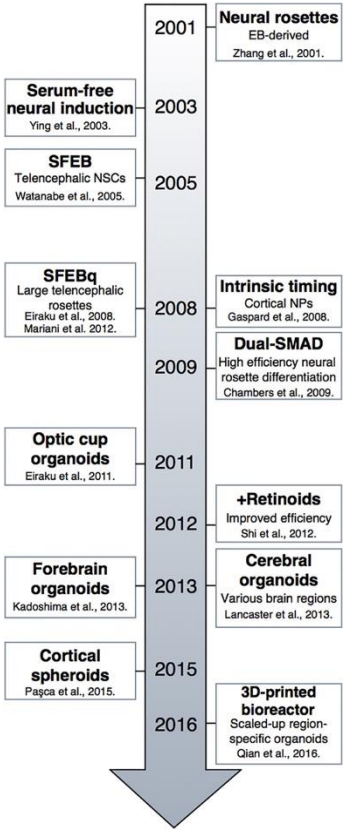
- Organoids evolve from 3D aggregates of stem cells known as EBs, which partially self-organize to mimic the developing organs.
- Various signals (internal and external) guide the acquisition of apicobasal polarity and generates functional spherical structures showing resemblance to the architecture of human tissues.
- Establishing organoid culture is a bit challenging, as organoids do not develop in the same manner as regular organs do. But, the addition of tissue-specific components and modulators for the resident stem cell populations can result in a successful organoid generation.

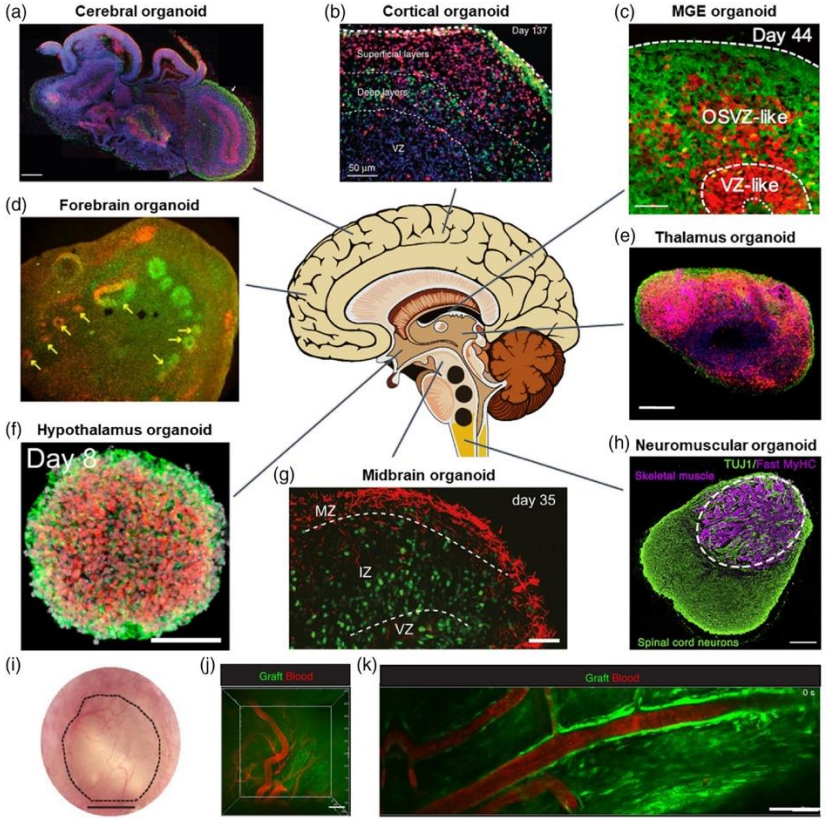


Neural progenitors (SOX2, red) and neurons (TUJ1, green)

(Lancaster et al, 2013; Yadav et al, 2021)

Multiple in vitro methods of neural differentiation



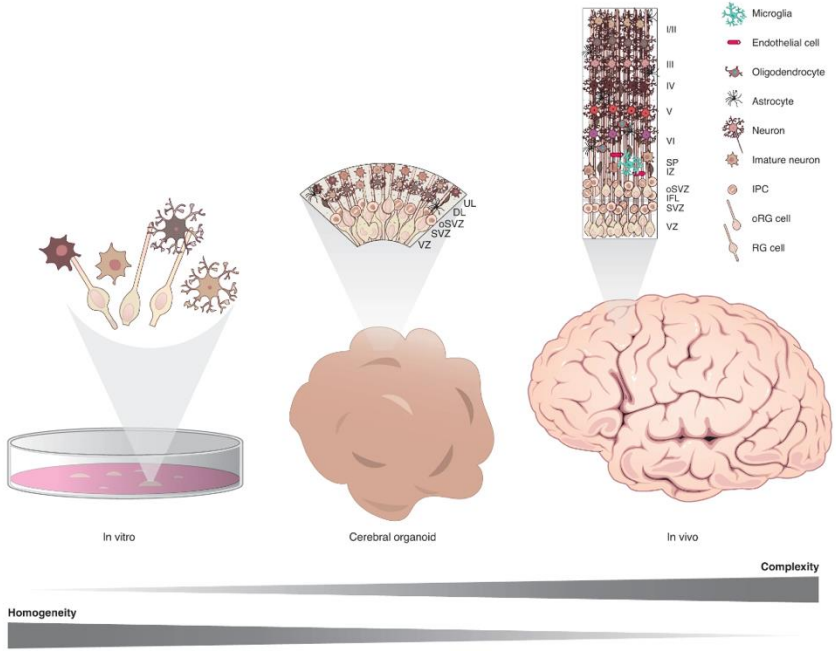


(Kelava and Lancaster, 2016; Chung et al, 2022)

Brain organoid technology for human brain research

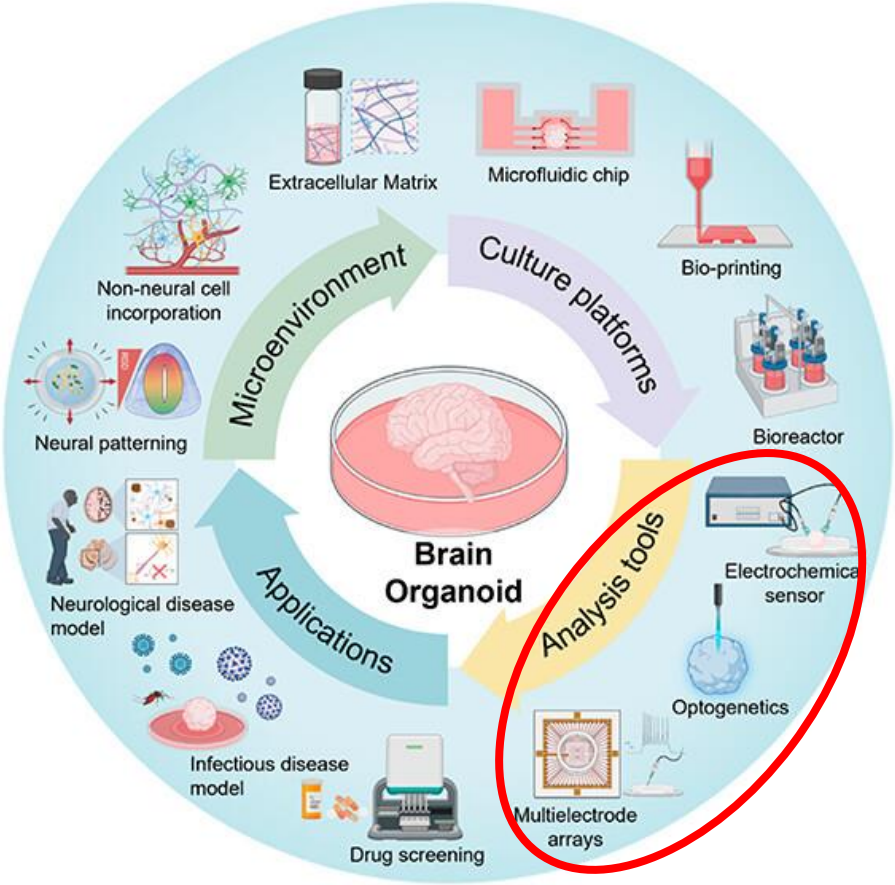
Advantages of using 3D vs 2D cultures

- Brain organoids mimic the development of human brain
- The heterogenous cell polarities and phenotypes present are more representative of native tissue architecture
- Brain organoids mimic the three-dimensional organization of neuronal networks (e.g., cell-cell and cell-environment interactions)



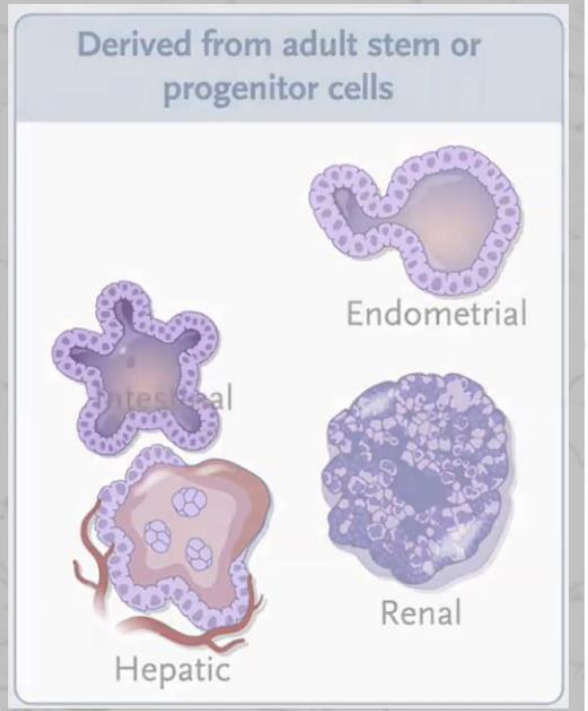
(Chiaradia and Lancaster, 2020)

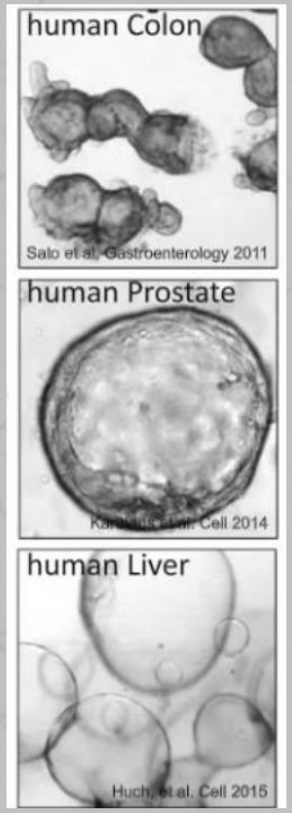
Brain organoid technology for human brain research

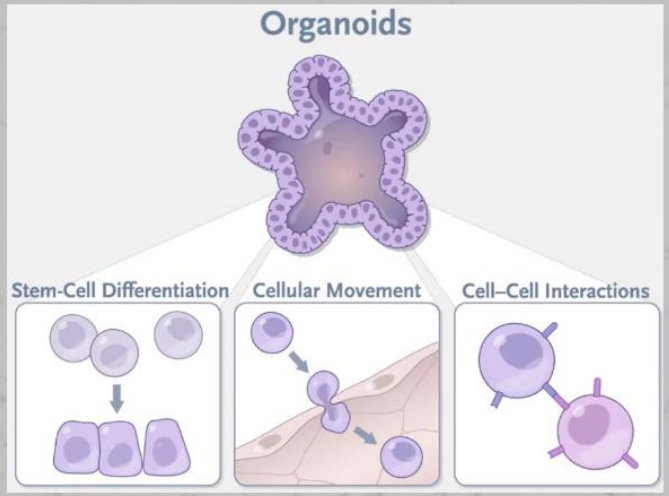


(Jeong et al, 2022)

Organoid technology







Organoid features:

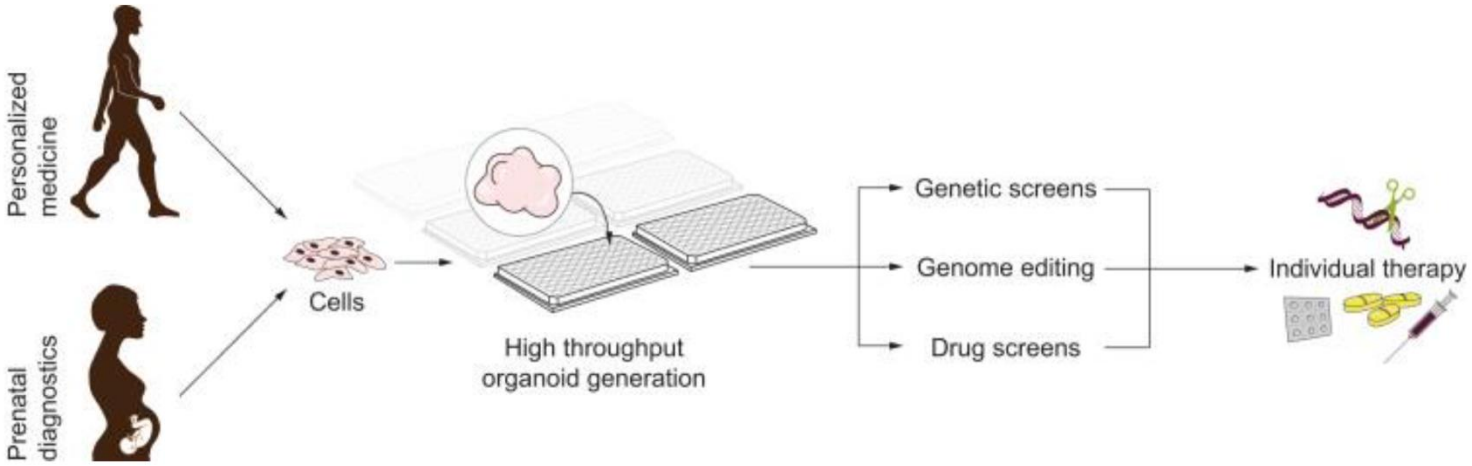
- untransformed
- 3 dimensional with realistic micro-anatomy
- highly proliferative and expandable
- recapitulate functions of their parent tissue
- very high genetic stability

https://www.youtube.com/watch?v=2SG5ivm6jkw

Modeling human brain development using iPSCs

- The human brain is one of the most complex organs in animal kindom, both structurally and functionally.

- hiPSCs can be used to have access to a physiologically relevant human model for drug discovery, cell therapy validation and neurological disease research.



What are cerebral organoids?

- A cerebral organoid describes artificially grown, *in vitro*, miniature organs resembling the brain.

- They are created by culturing human pluripotent stem cells in a three-dimensional rotational bioreactor and develop over a course of months .

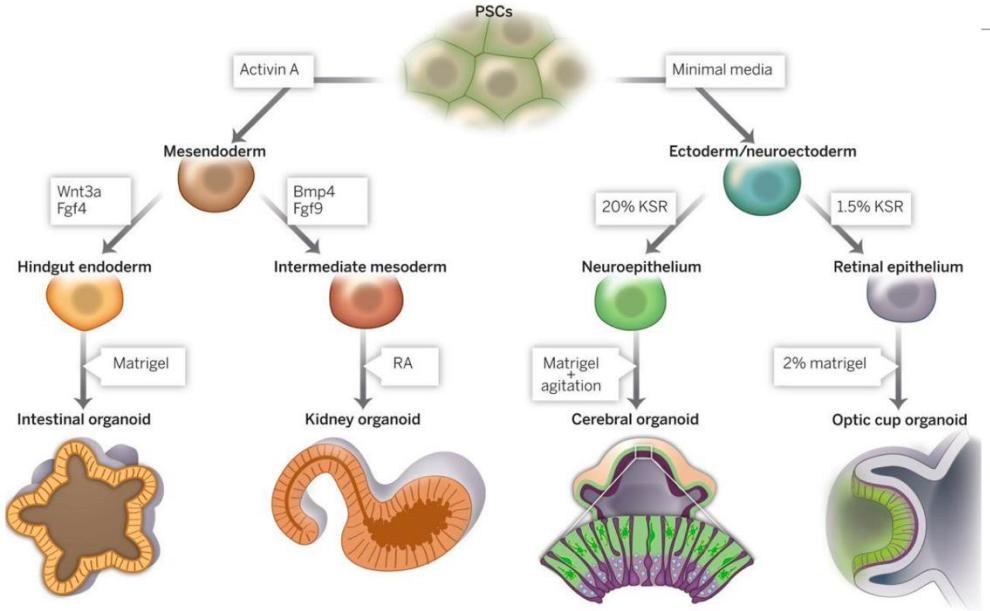
Review article

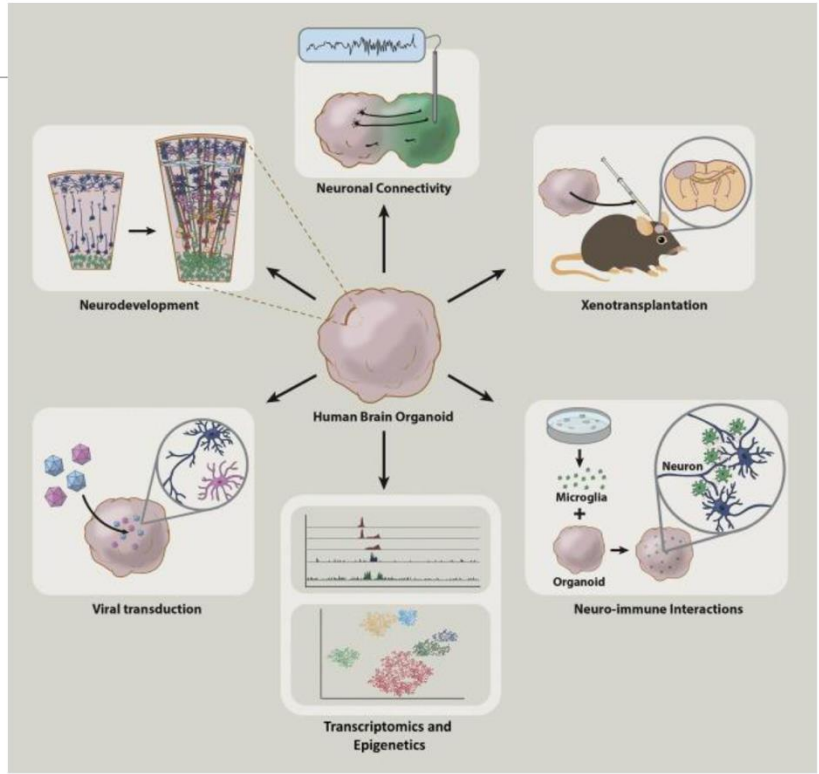
Dishing out mini-brains: Current progress and future prospects in brain organoid research

Iva Kelava, Madeline A. Lancaster*

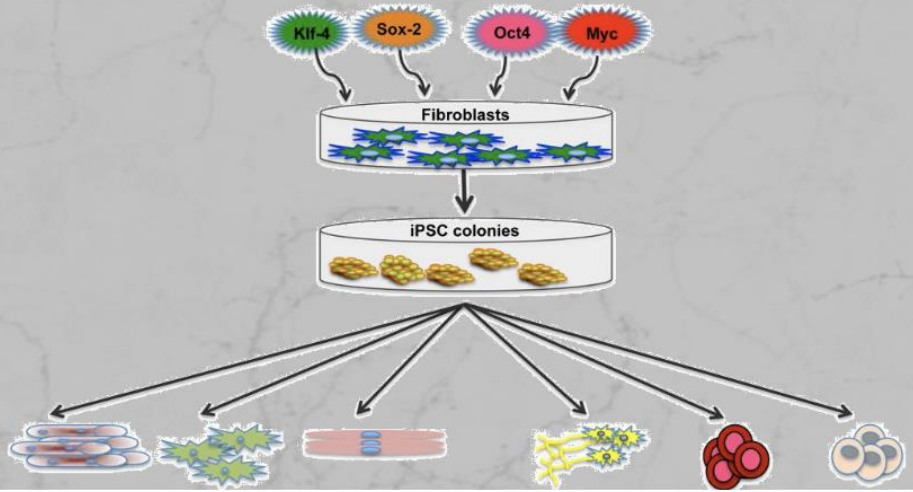
MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus, Francis Crick Avenue, CB2 0QH Cambridge, United Kingdom

What are cerebral organoids?



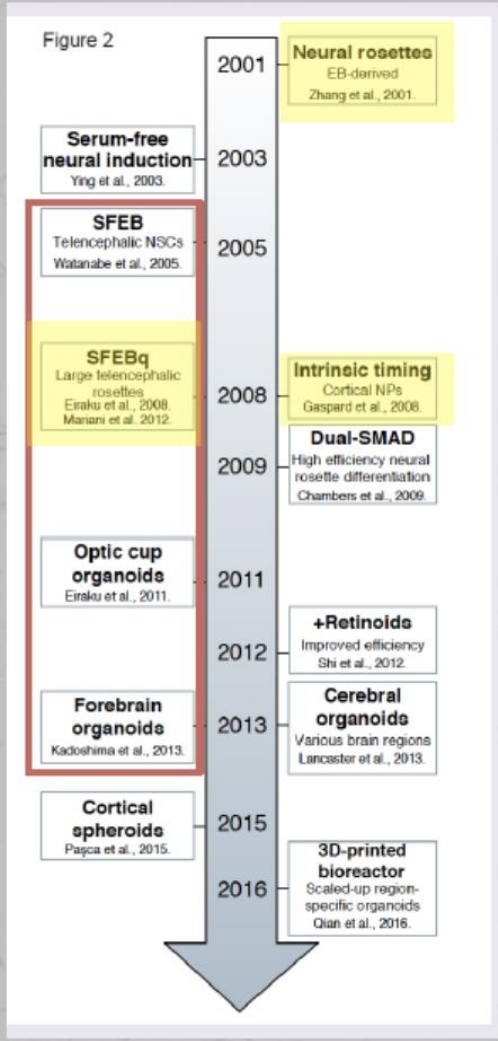


Cerebral organoid

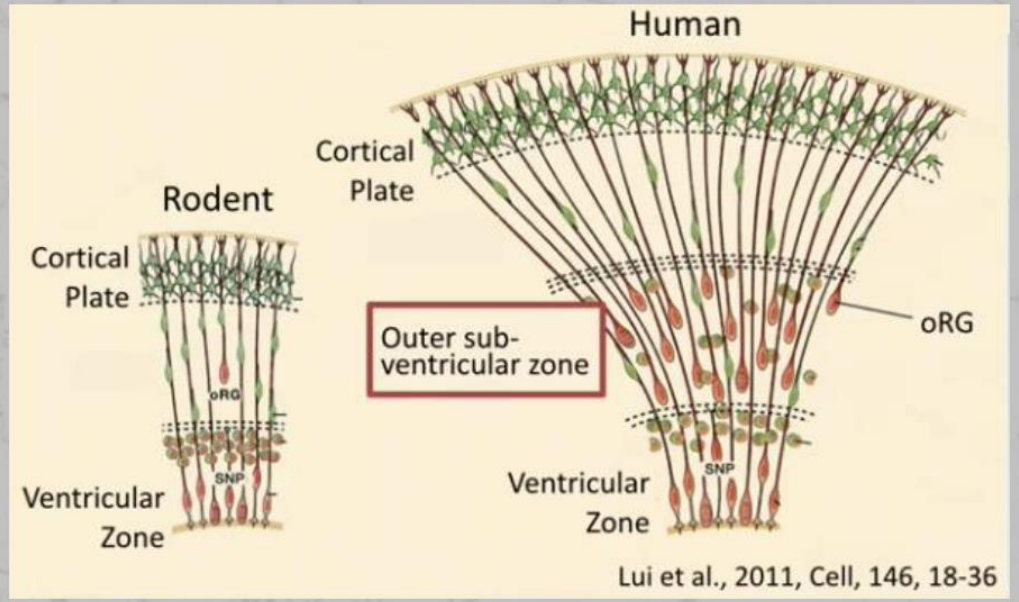


Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

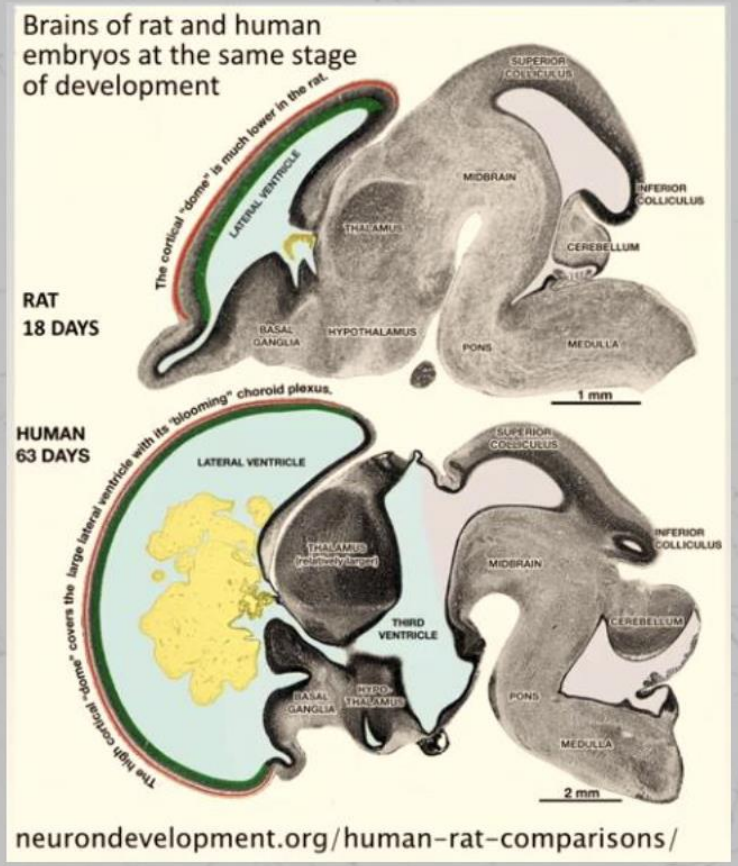
Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1]Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2]CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024



Cortical plate development

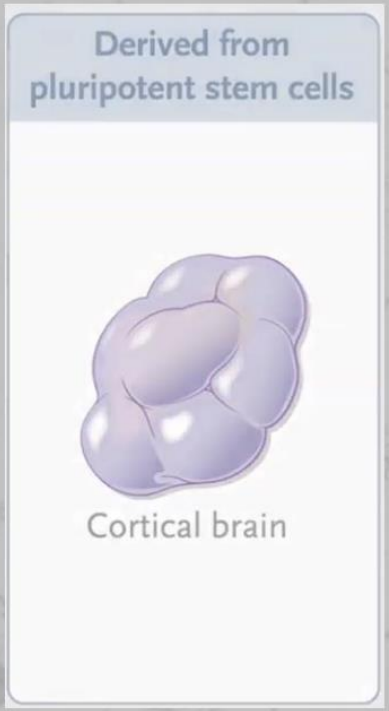


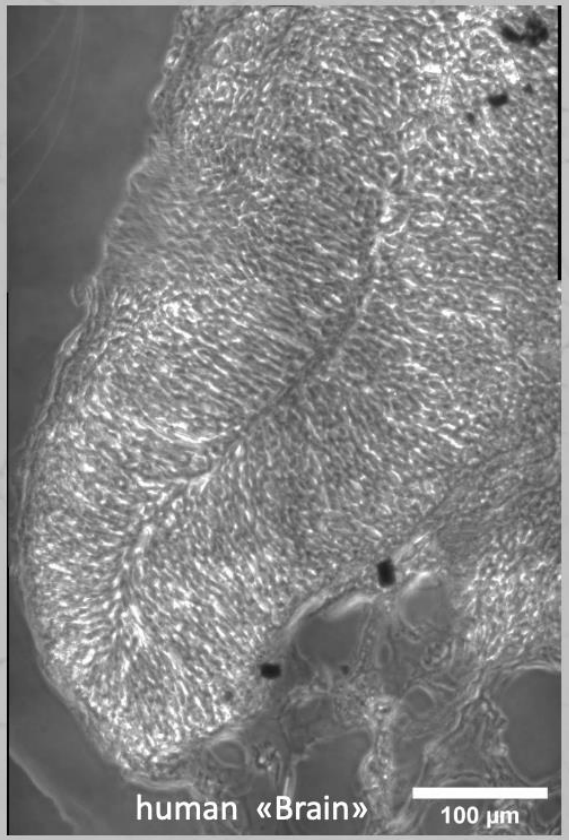
Lui et al., 2011, Cell, 146, 18-36



neurondevelopment.org/human-rat-comparisons/

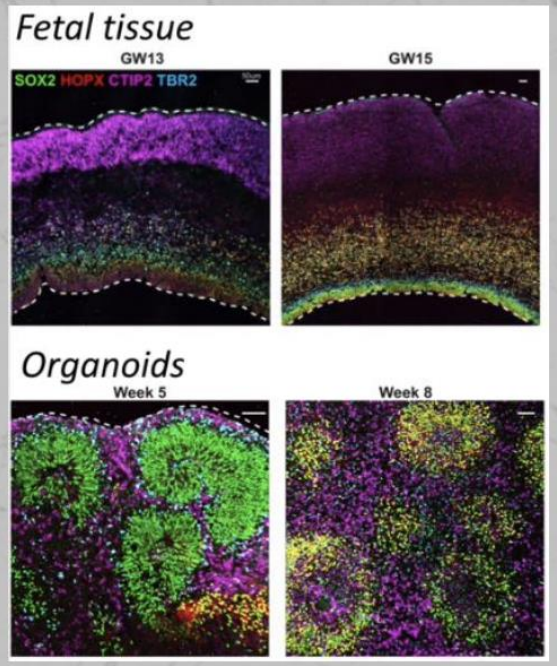
Cerebral organoid



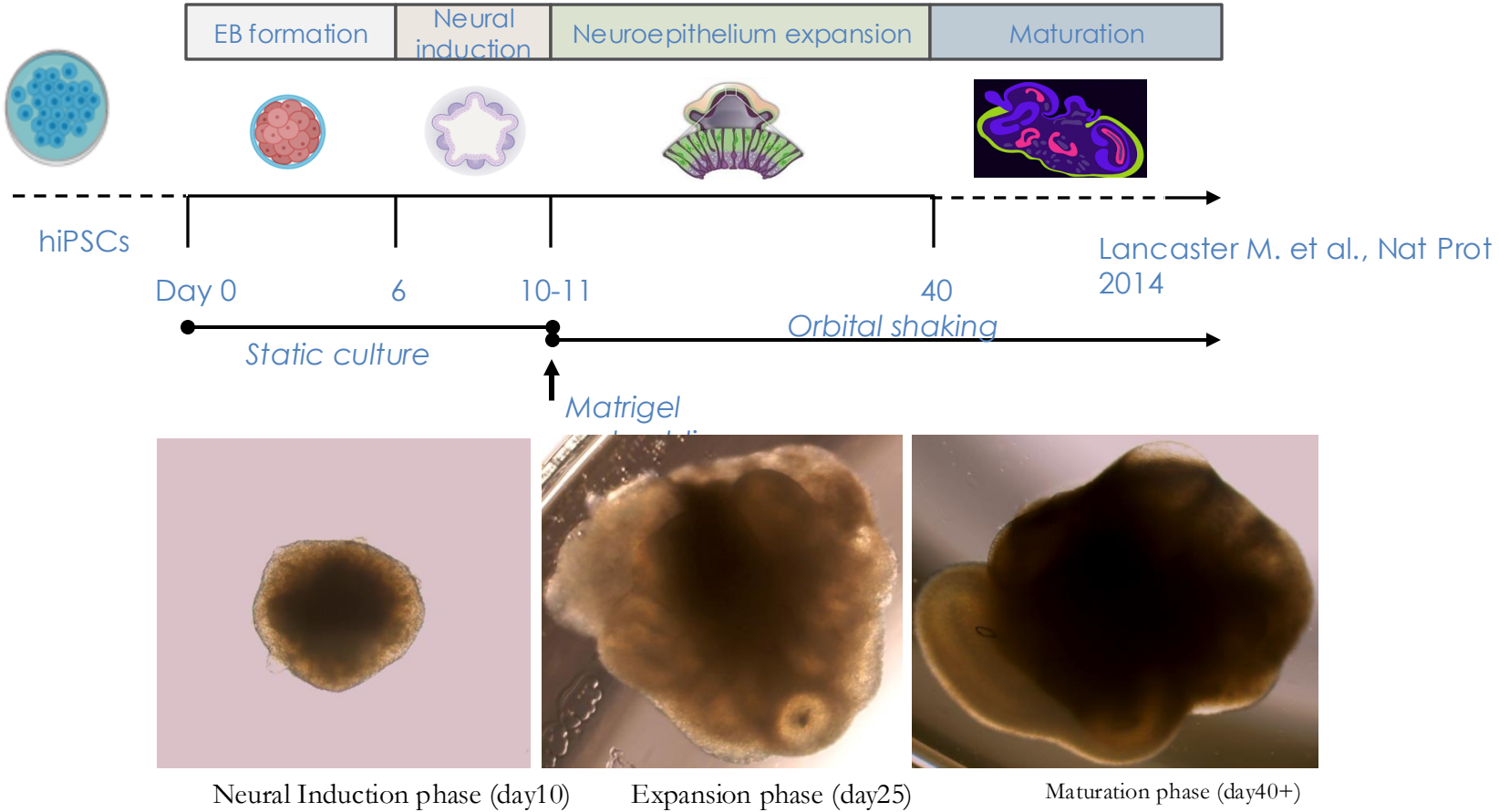


Advantages:

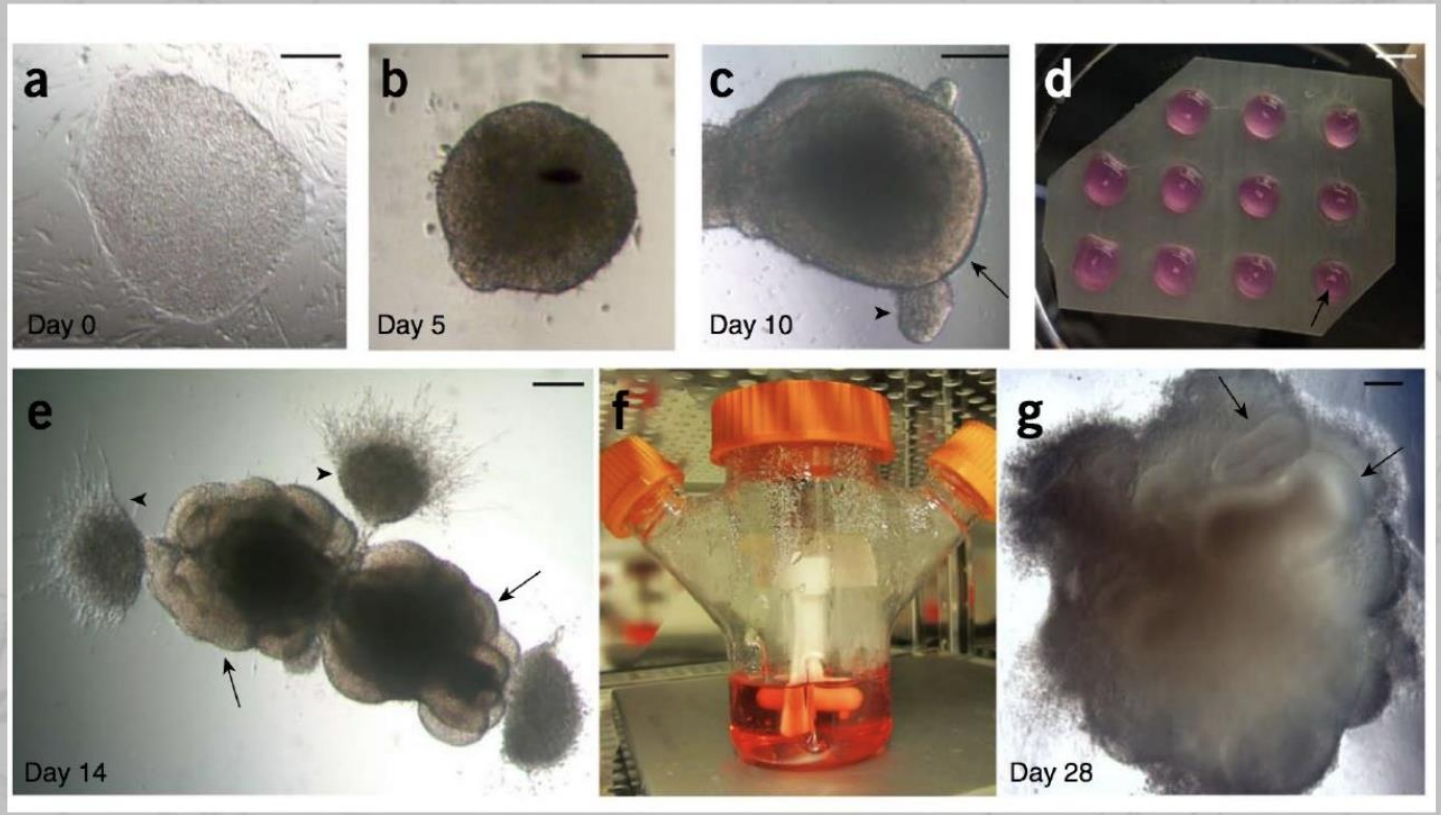
- Reduced experimental complexity and costs
- Suitable for live imaging exps
- More accurate model of human brain development and disease



Generation of human cortical organoids

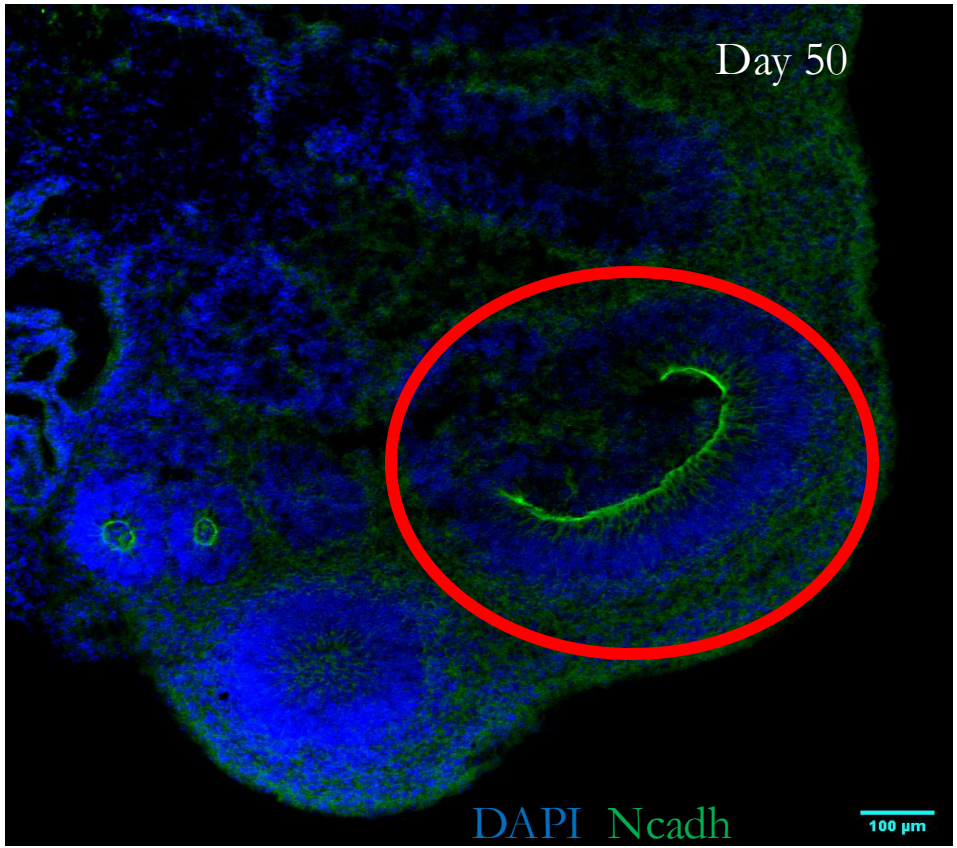


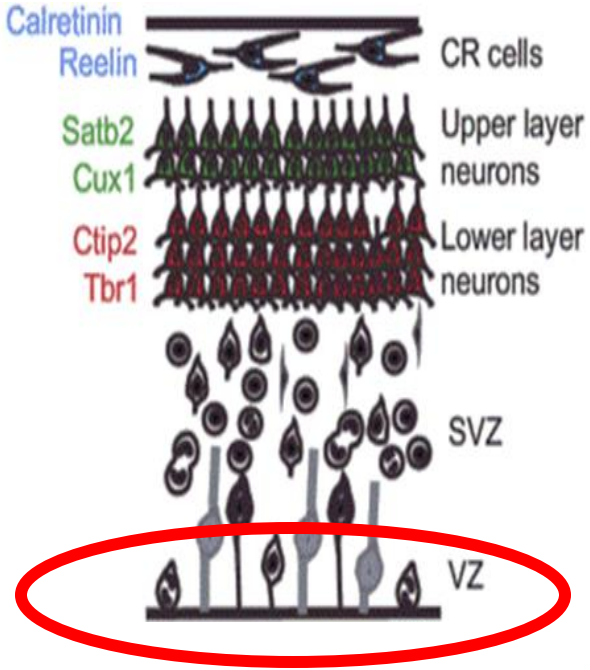
Whole brain organoids



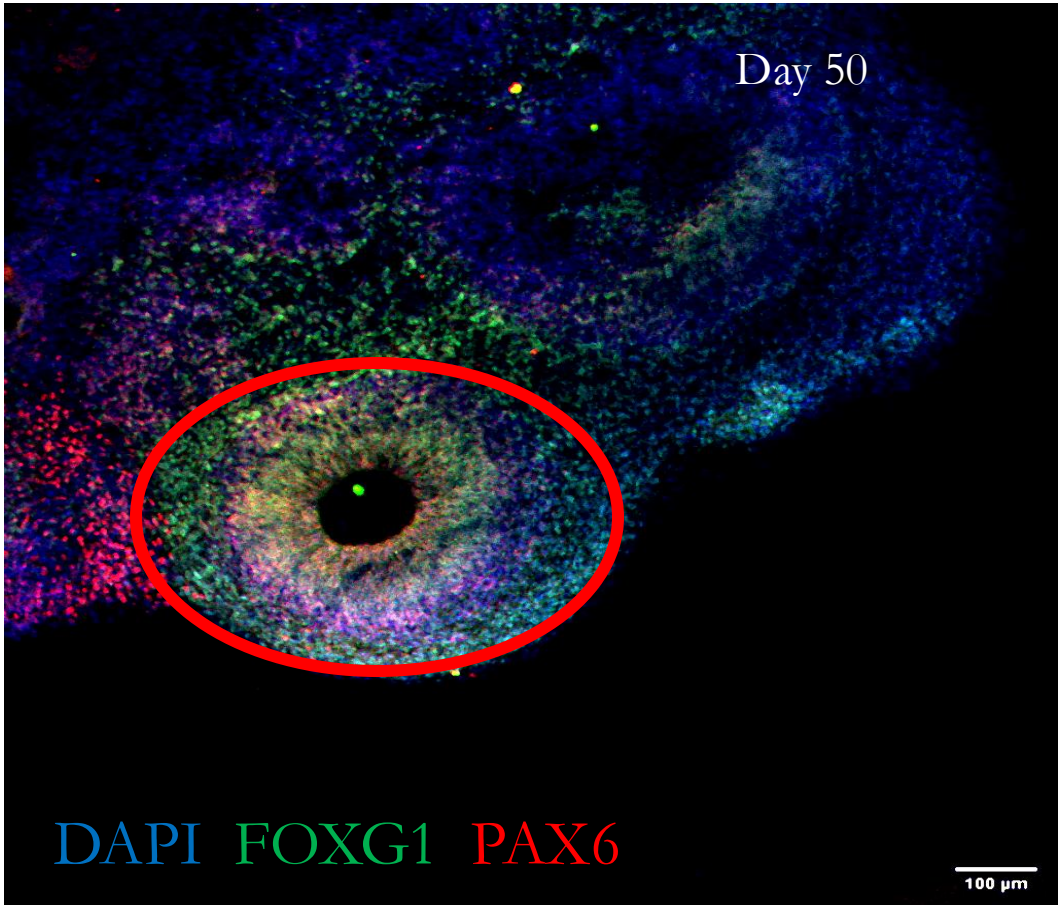
doi: 10.1038/nprot.2014.158

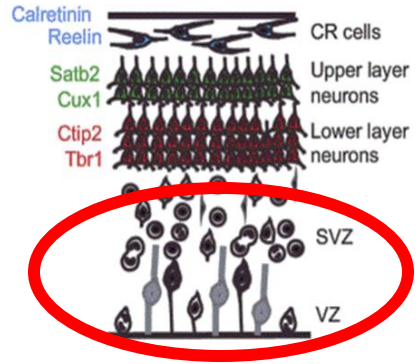
Generation of human cortical organoids

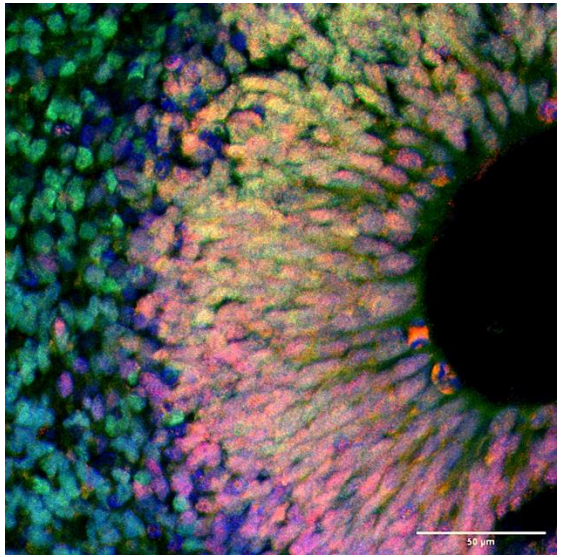




Generation of human cortical organoids

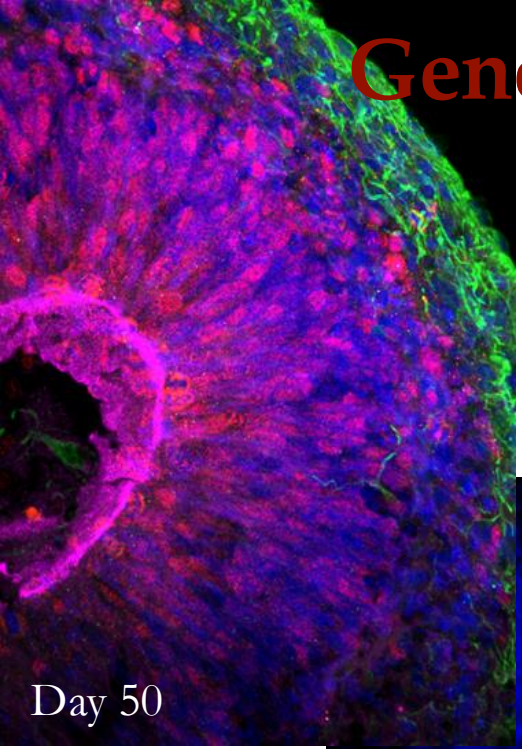


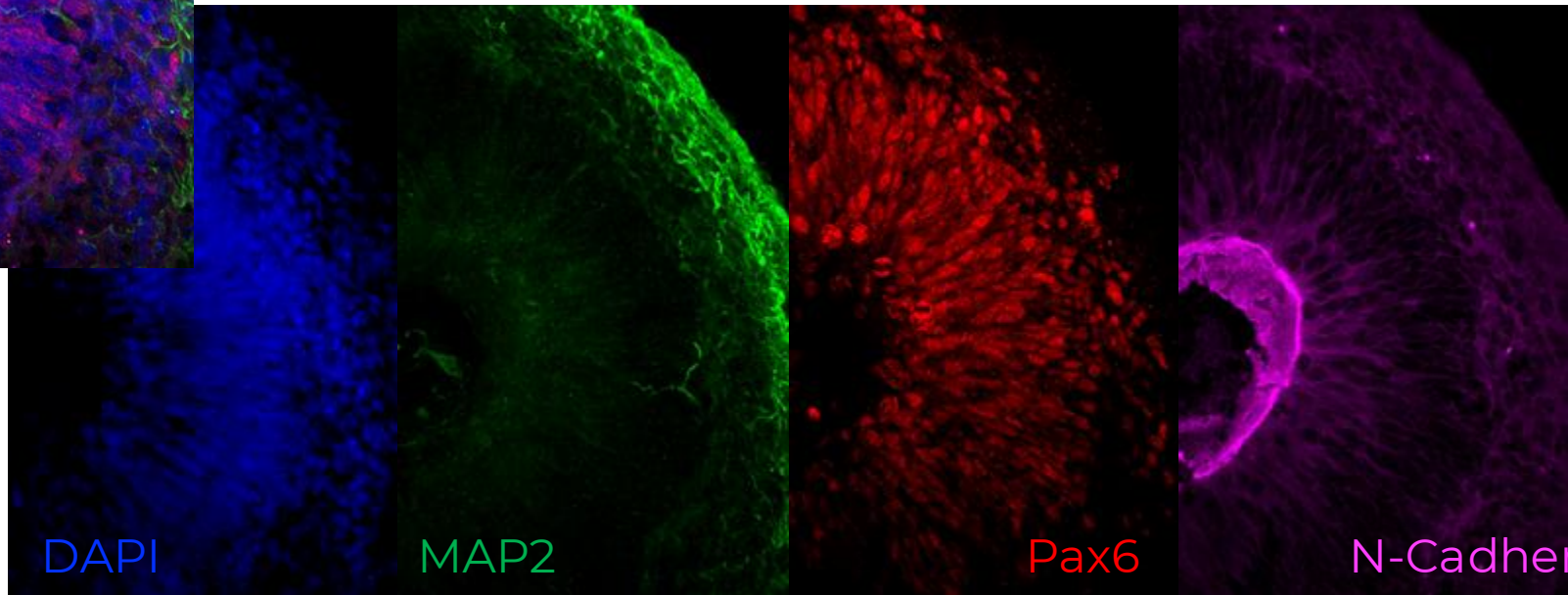




Generation of human cortical organoids

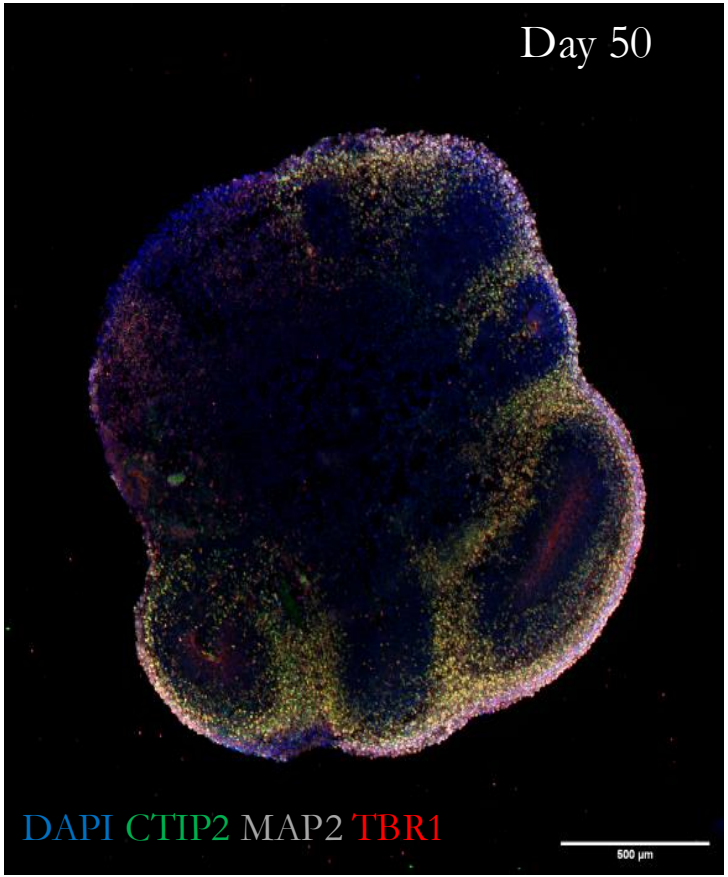
ventricle-like structure - ventricular and subventricular-like regions

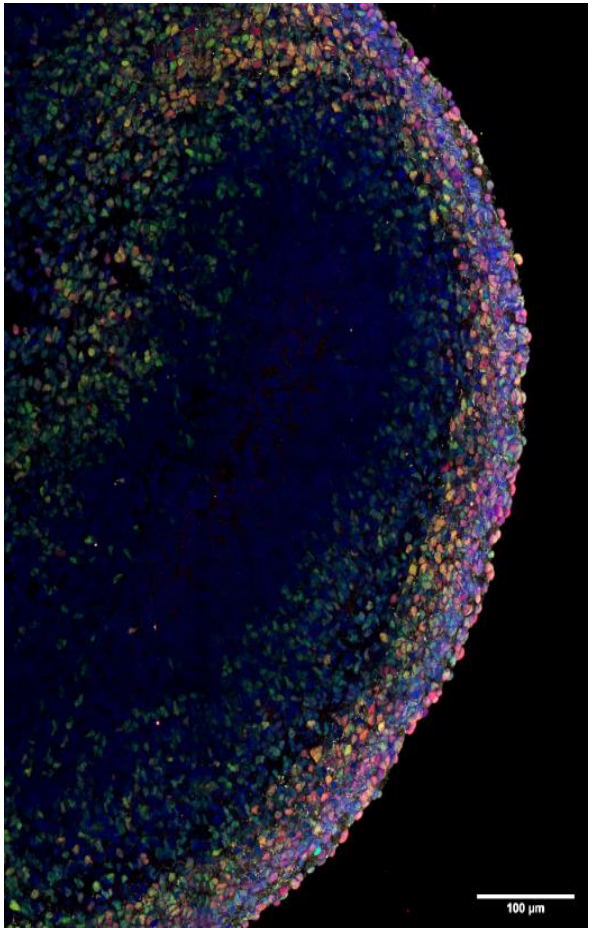


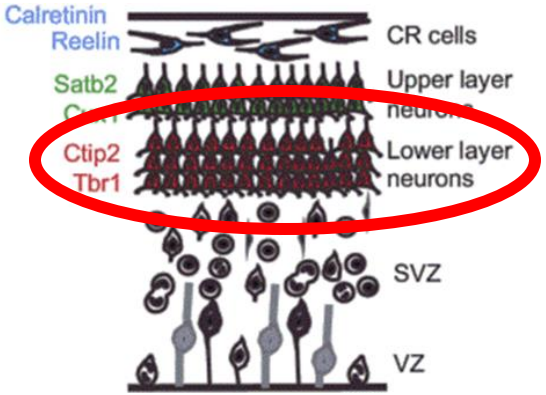


40x

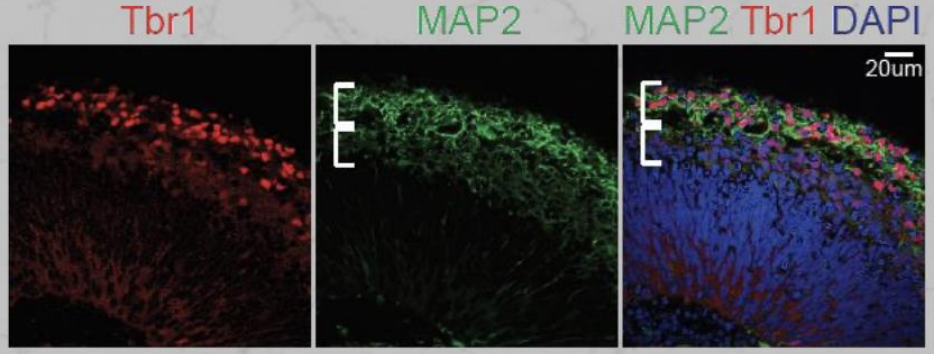
Generation of human cortical organoids

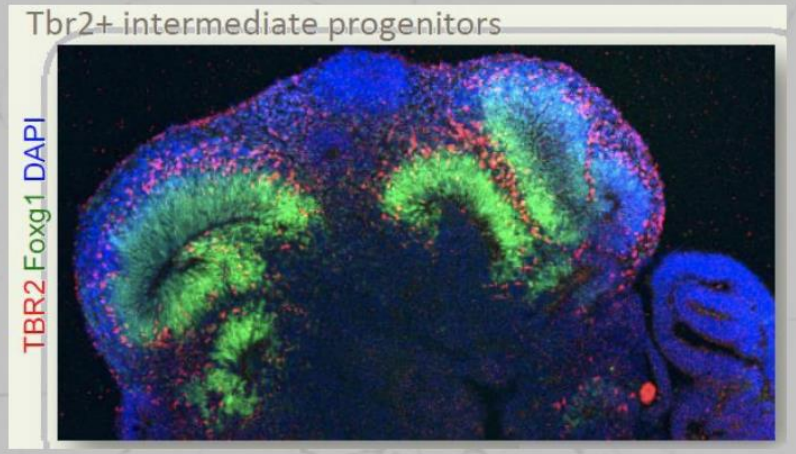


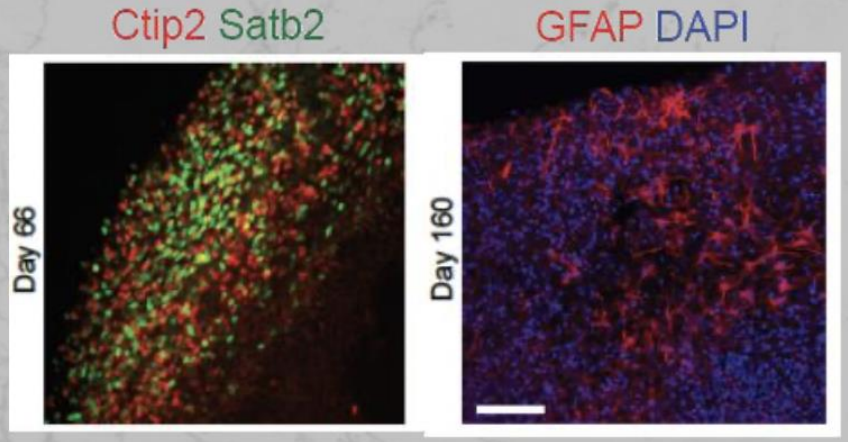


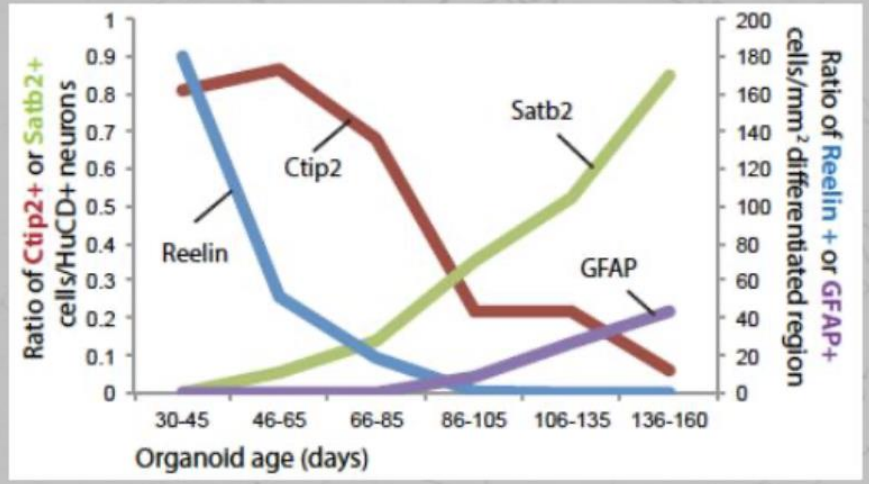


Whole brain organoids





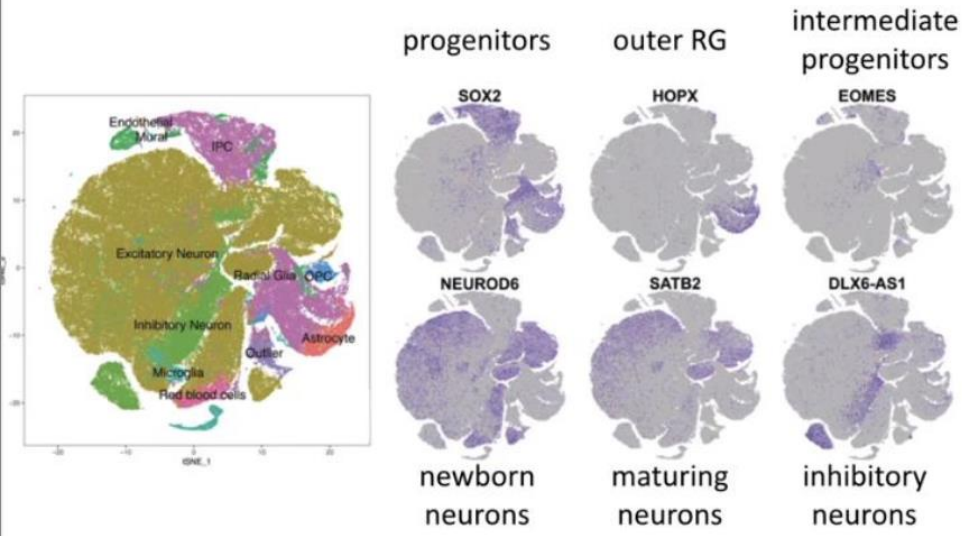




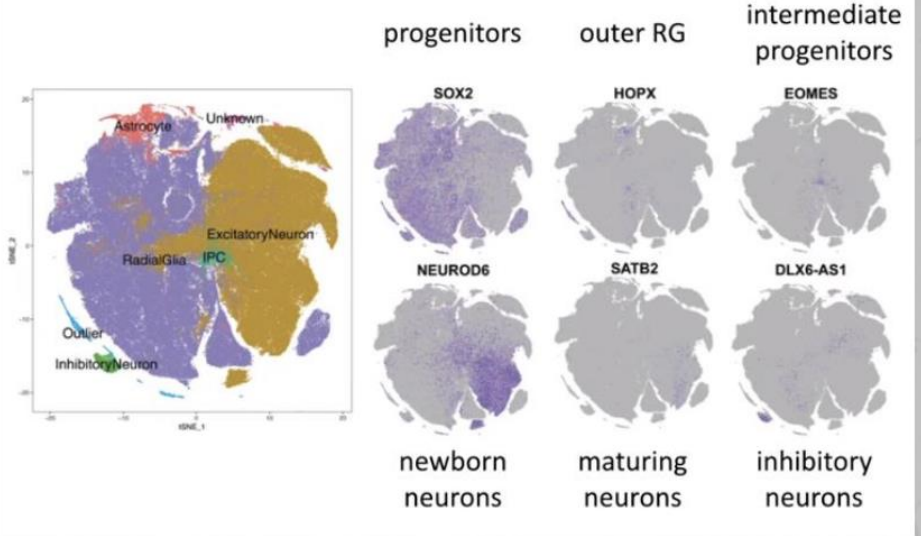
doi: 10.1038/nprot.2014.158

Whole brain organoids

GW6-22, 5 individuals, 7 cortical areas, 189.000 cells

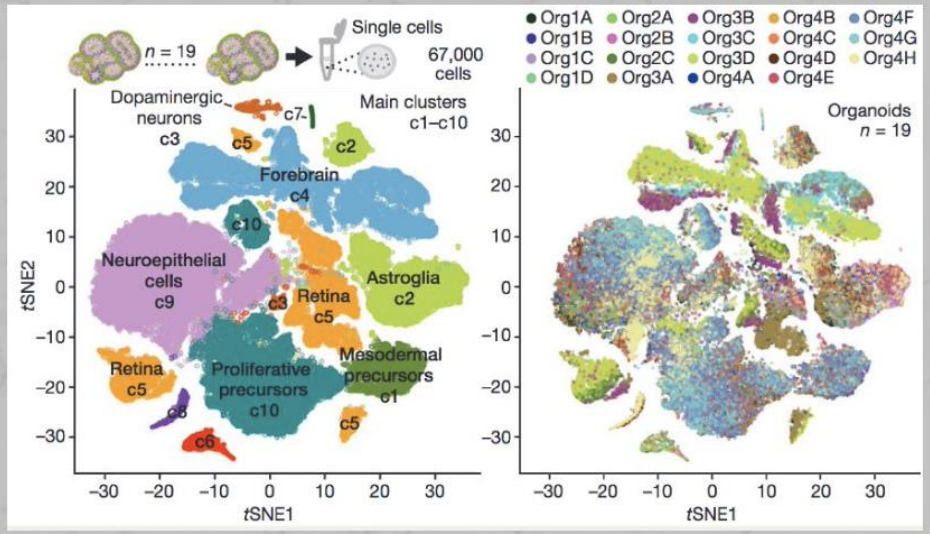


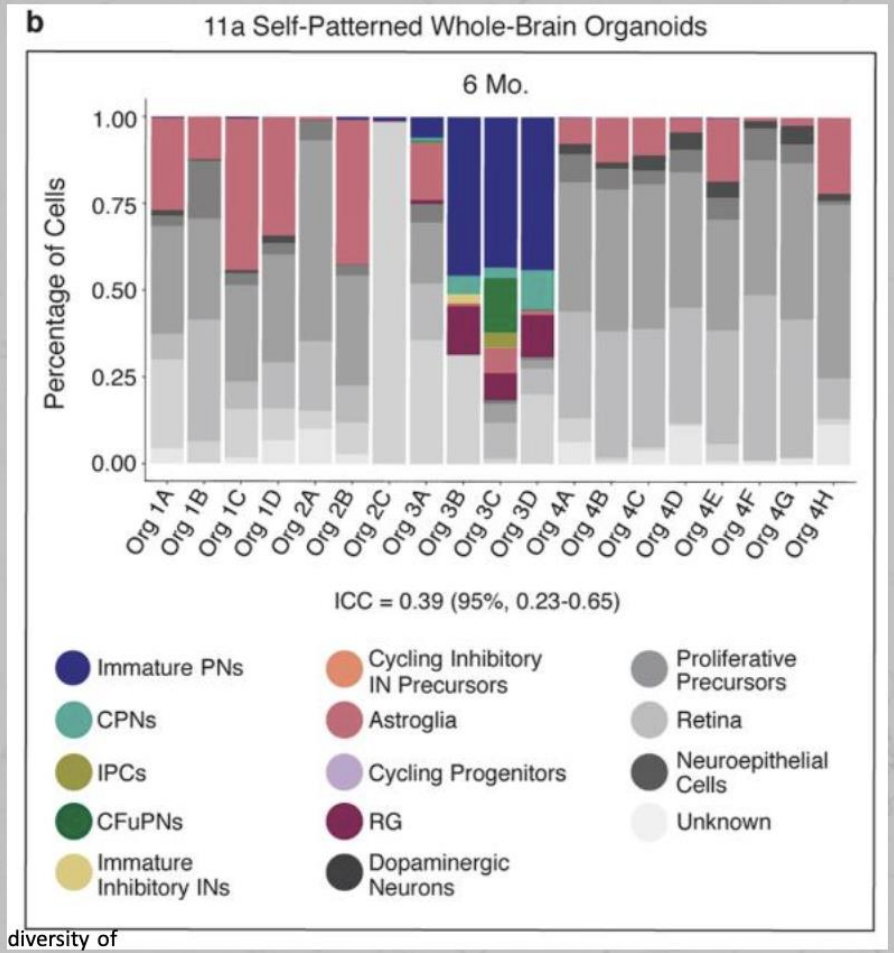
Weeks 3-10, 4 cell lines, 3 protocols,, 109.000 cells



Bhaduri, A., Andrews, M.G., Mancia Leon, W. *et al.* Cell stress in cortical organoids impairs molecular subtype specification. *Nature* 578, 142–148 (2020). https://doi.org/10.1038/s41586-020-1962-0

Whole brain organoids

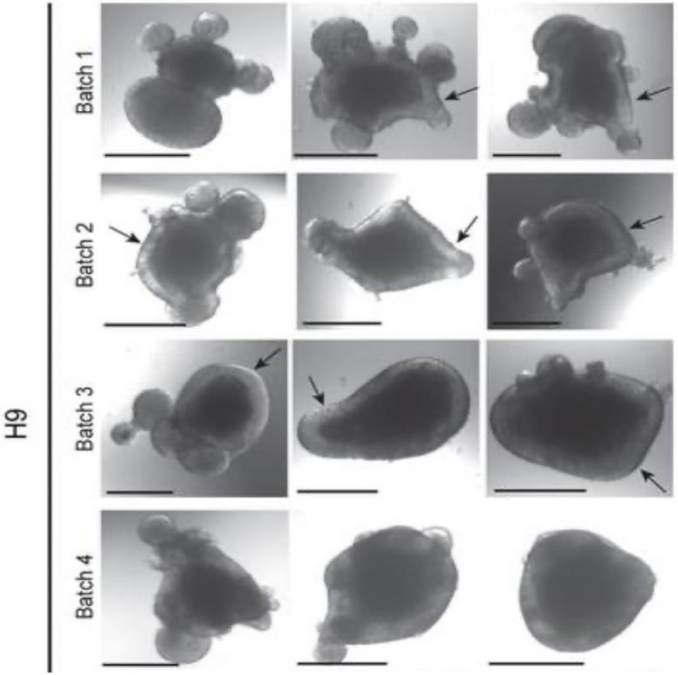


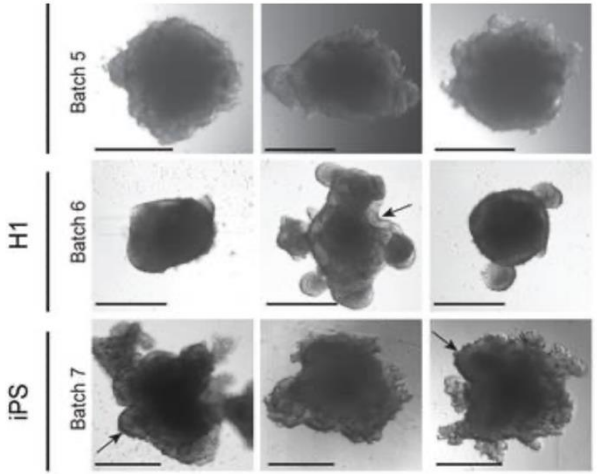


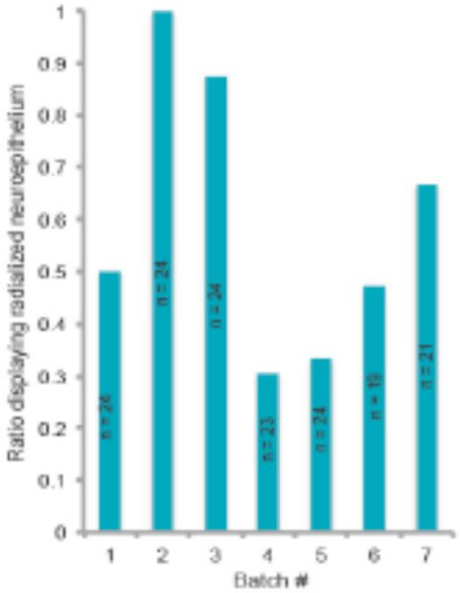
Velasco, S., Kedaigle, A.J., Simmons, S.K. *et al.* Individual brain organoids reproducibly form cell diversity of the human cerebral cortex. *Nature* 570, 523–527 (2019). https://doi.org/10.1038/s41586-019-1289-x

ARE HUMAN CORTICAL ORGANOIDS A RELIABLE MODEL?

The Batch Syndrome







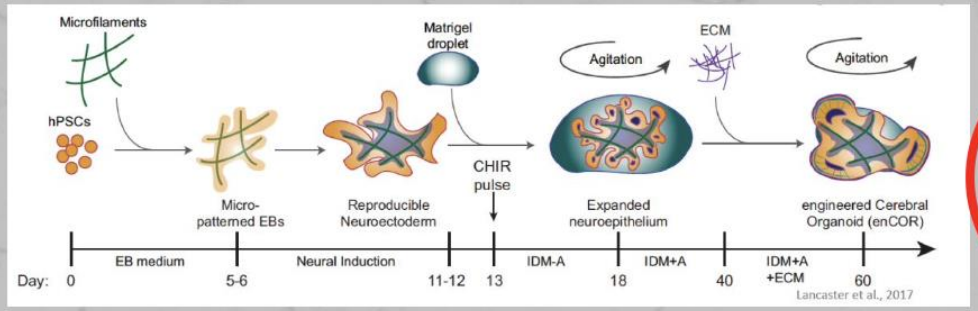
Variable efficiency of neural ectoderm formation.

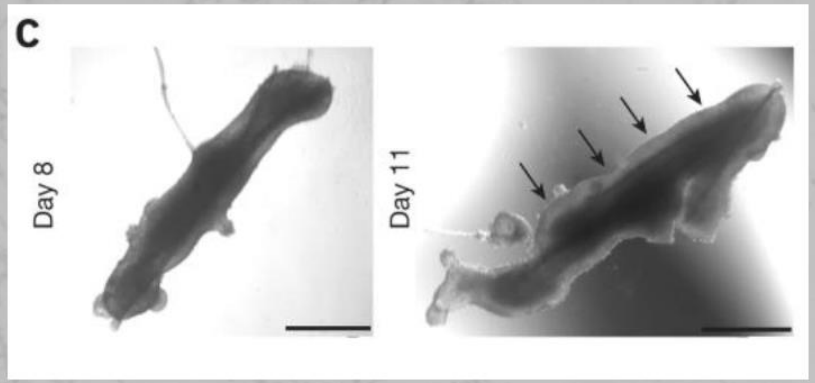
Possible Solutions:

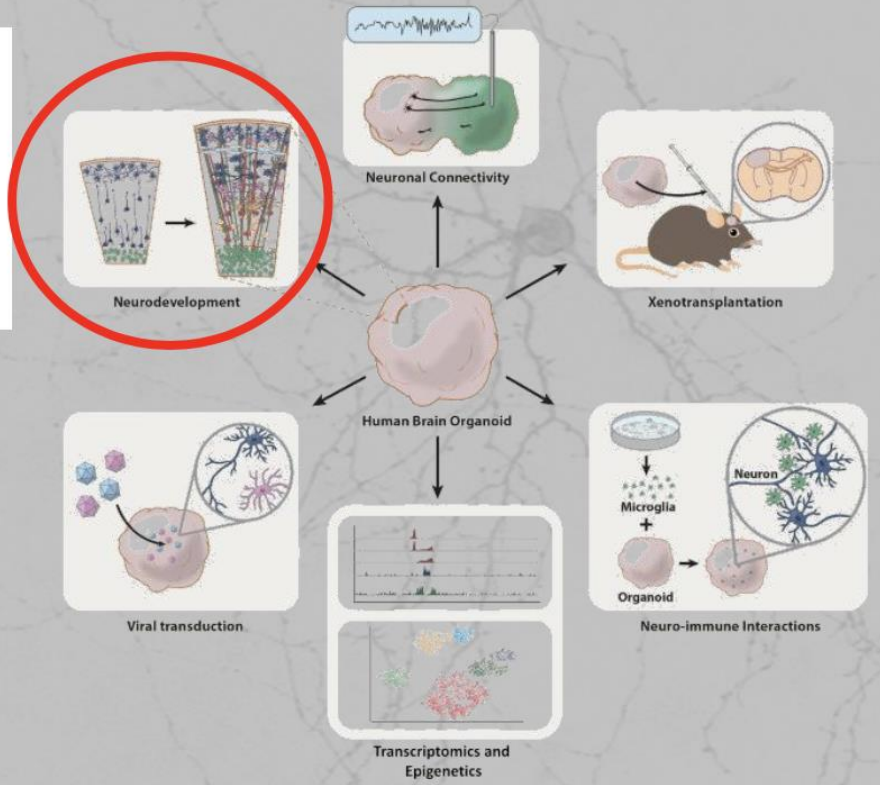
Engineered Cerebral Organoids (enCORs)

3D Bioprinted Constructs

Further improvements

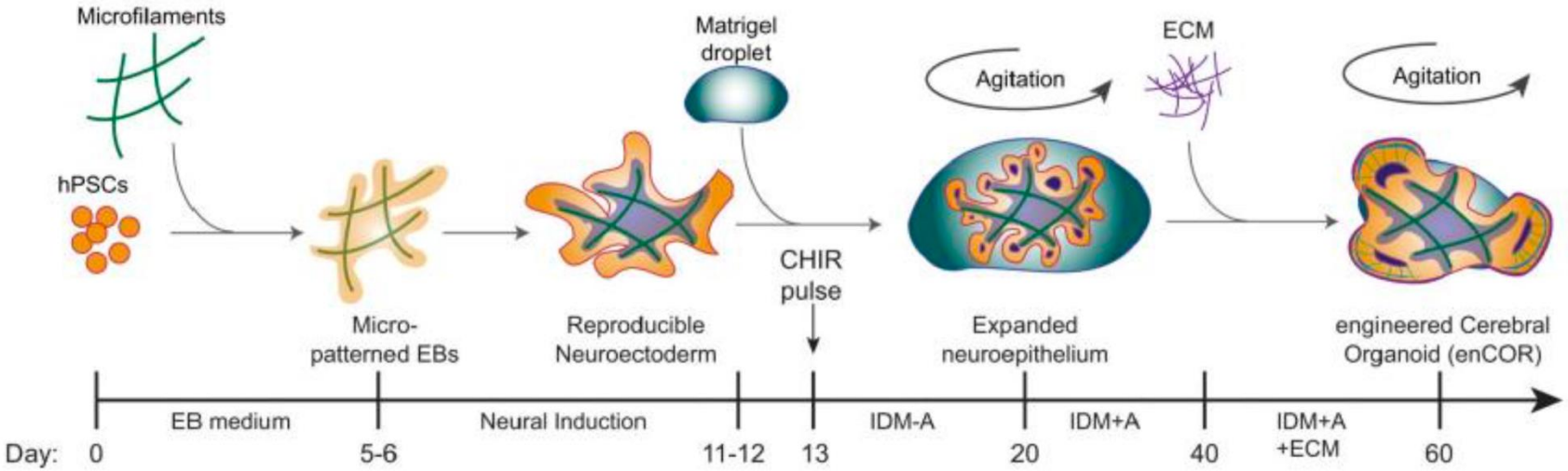




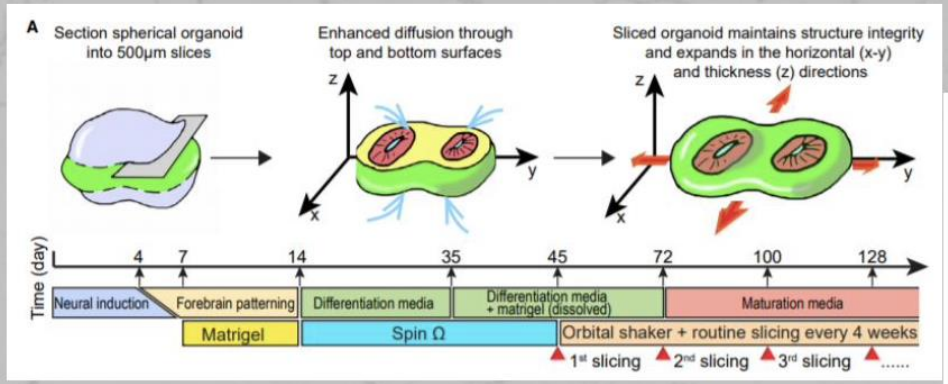


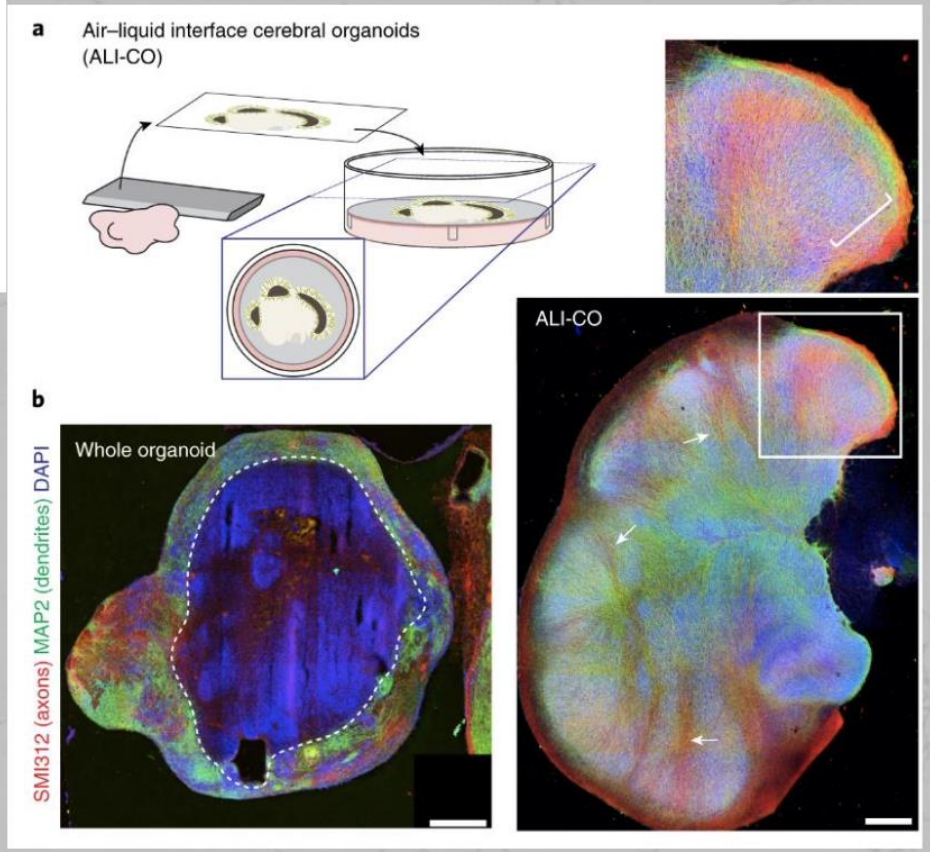
doi: 10.1038/nbt.3906

Engineered Cerebral Organoids (enCORs)

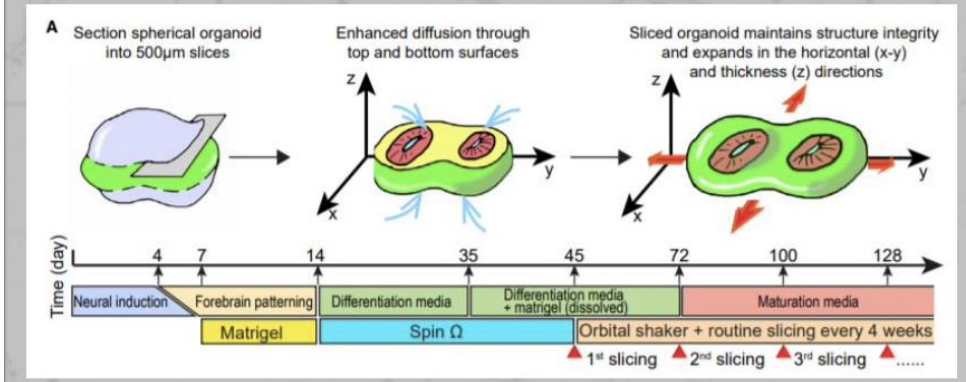


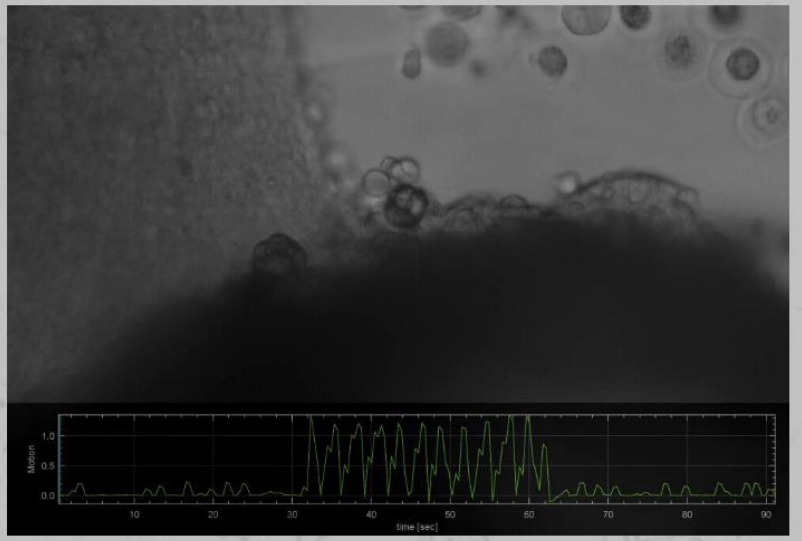
This would allow for the formation of larger tissues with increased surface area to volume ratio.

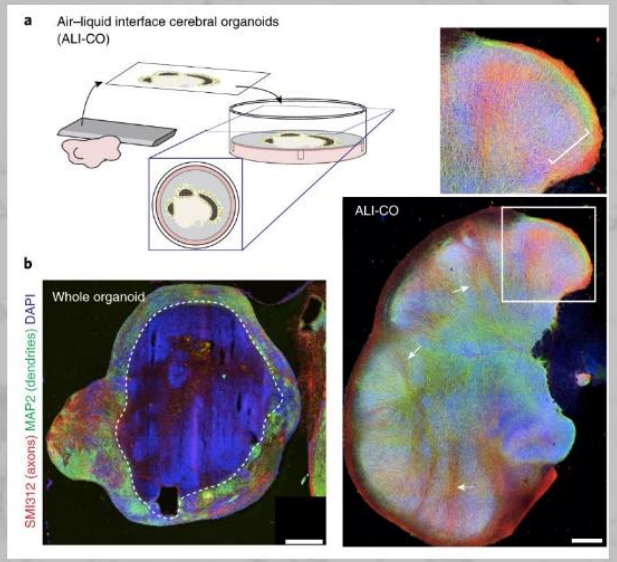


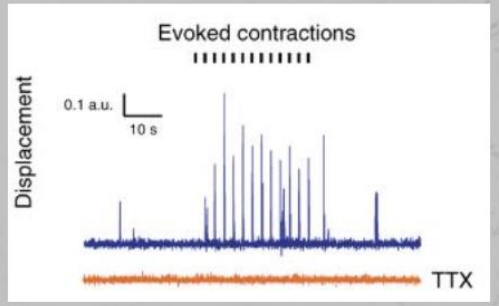


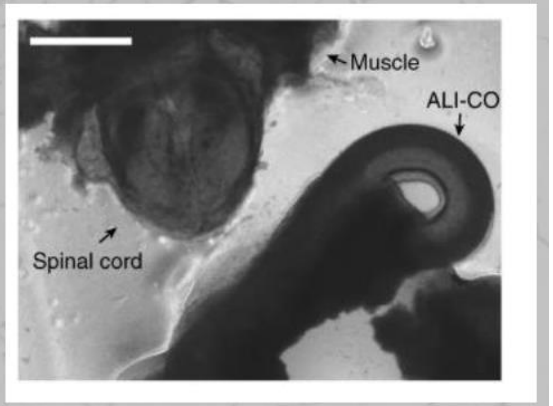
Nat Neurosci. 2019 April ; 22(4): 669–679. doi:10.1038/s41593-019-0350-2





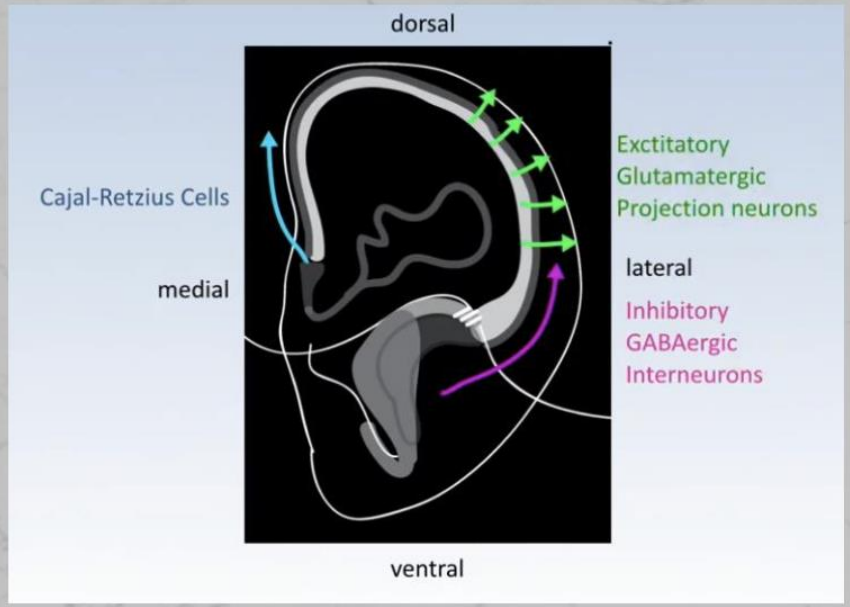


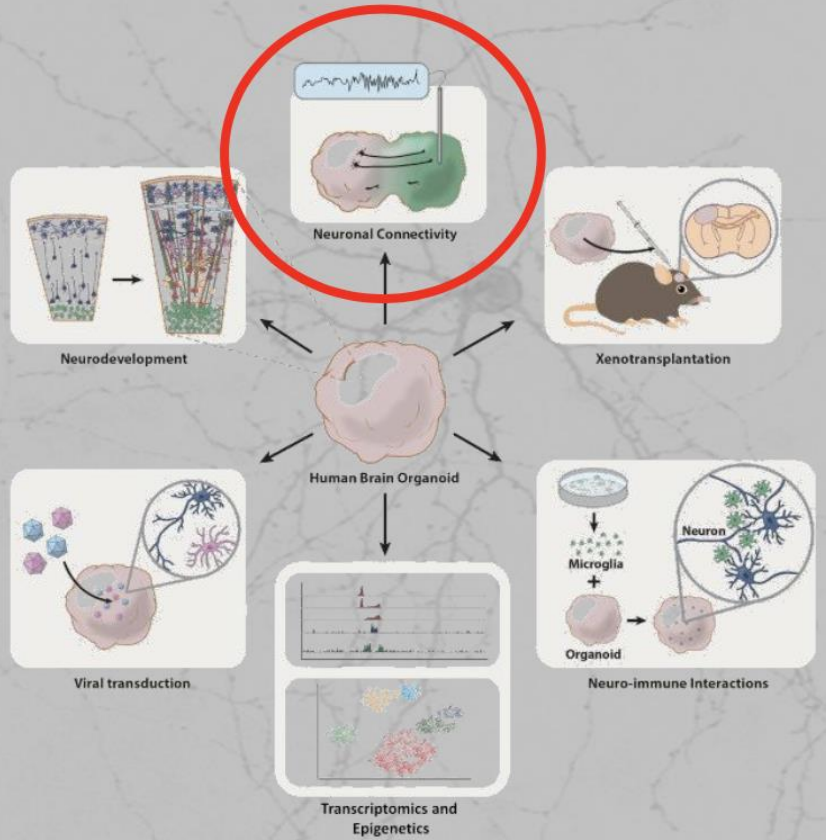




Nat Neurosci. 2019 April ; 22(4): 669–679. doi:10.1038/s41593-019-0350-2

Further improvements

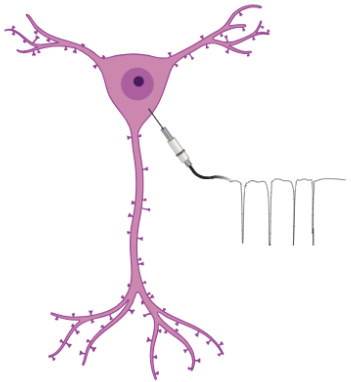




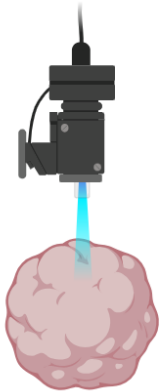
How to study single-cell and network activity in human brain organoids?

The hallmark of functional analysis for neural cells and tissues, including brain organoids, is electrophysiology. The ability to record neuronal function is essential for many brain organoid applications, especially disease modeling and drug development → functional readout

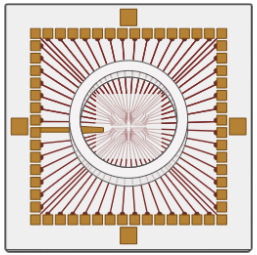
Patch clamp



Calcium imaging



Multi-electrode array



Critical points when performing functional analysis:

- Maturation state of the circuit
- Type of starting material

All these techniques are suitable for studying how neuronal/network activity changes in response to genetic or pharmacological manipulations.

Calcium imaging

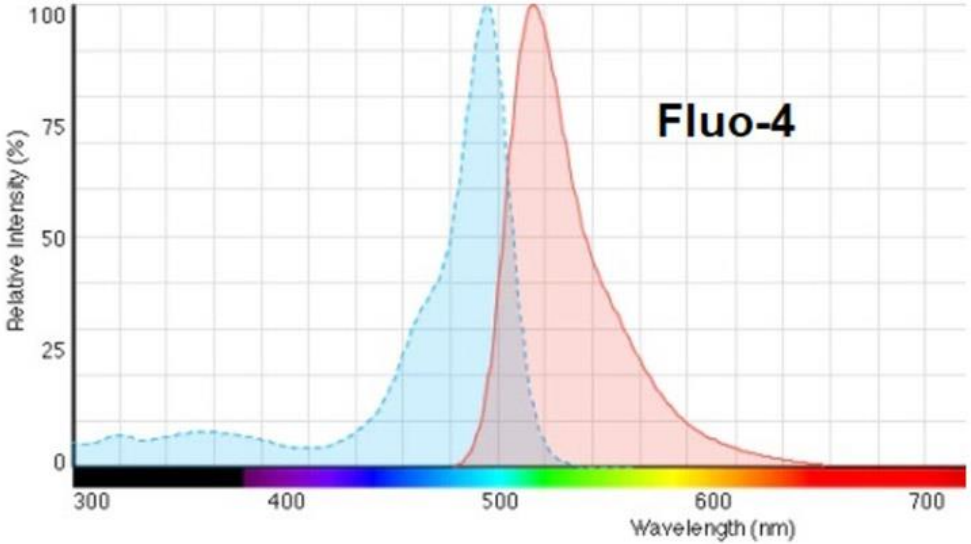
Use of calcium indicators, fluorescent molecules that respond to the binding of Ca2+ ions by fluorescence properties.

Chemical indicators

- Small molecules that bind calcium ions via chelation
- Often modified with acetoxymethyl esters (AM), in order to render the molecule lipophilic and to allow easy entrance into the cell

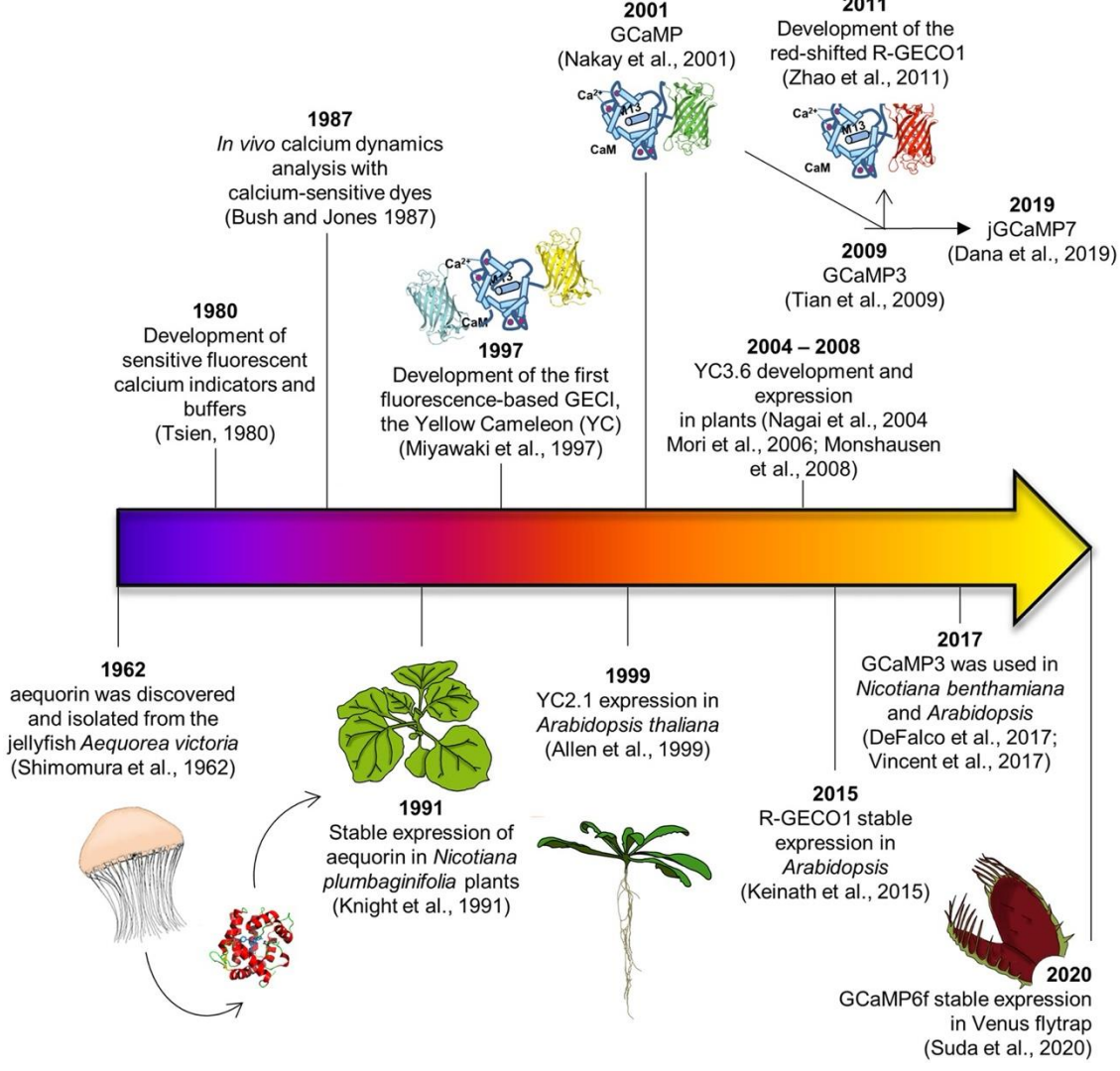
Critical point

- "Bulk" loading of cells
- Not suitable for in vivo or screening application



Indicator	K_d for Ca^{2+} (nM)	Excitation (nm), emission (nm)
Calcium Green-1	190	490 ex, 531 em
Fluo-3	325	506 ex, 526 em
Fluo-4	345	494 ex, 516 em
Fura-2	145	363/335 ex, 512 em
Indo-1	230	488 ex, 405/485 em
Oregon Green 488 Bapta-1	170	488 ex, 520 em
Fura-4F	0.77	336/366 ex, 511 em
Fura-5F	0.40	336/363 ex, 512 em
Calcium Crimson	185	590 ex, 615 em
X-Rhod-1	0.7	580 ex,602 em

Calcium imaging



Calcium imaging

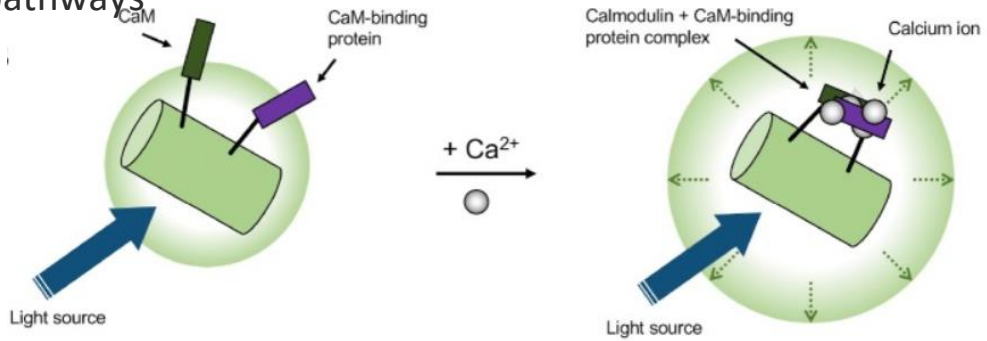
Use of calcium indicators, fluorescent molecules that respond to the binding of Ca2+ ions by fluorescence properties.

Genetically encoded calcium indicators

- Obtained from the fusion of voltage-sensing domains and fluorescent proteins (e.g., GFP, RFP,…)
- Monitoring of Ca2+ changes in the cytosol and subcellular compartments
- Selective expression in neuronal populations
- Potentially suitable for high-throughput screenings

Critical point

- Interference with intracellular C2+-dependent pathways
- Virus titer conditions

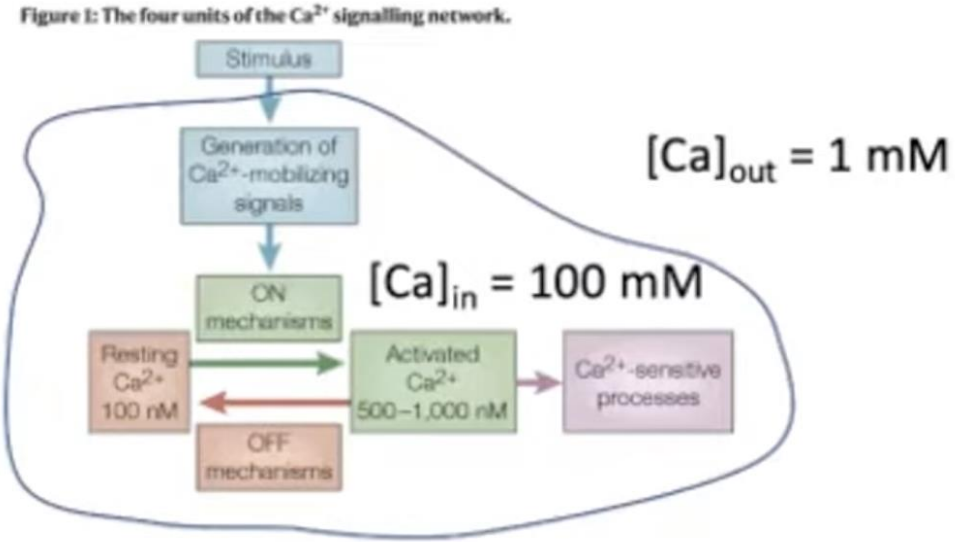


Calcium imaging to measure the activity of large populations of individual neurons

Calcium Signaling

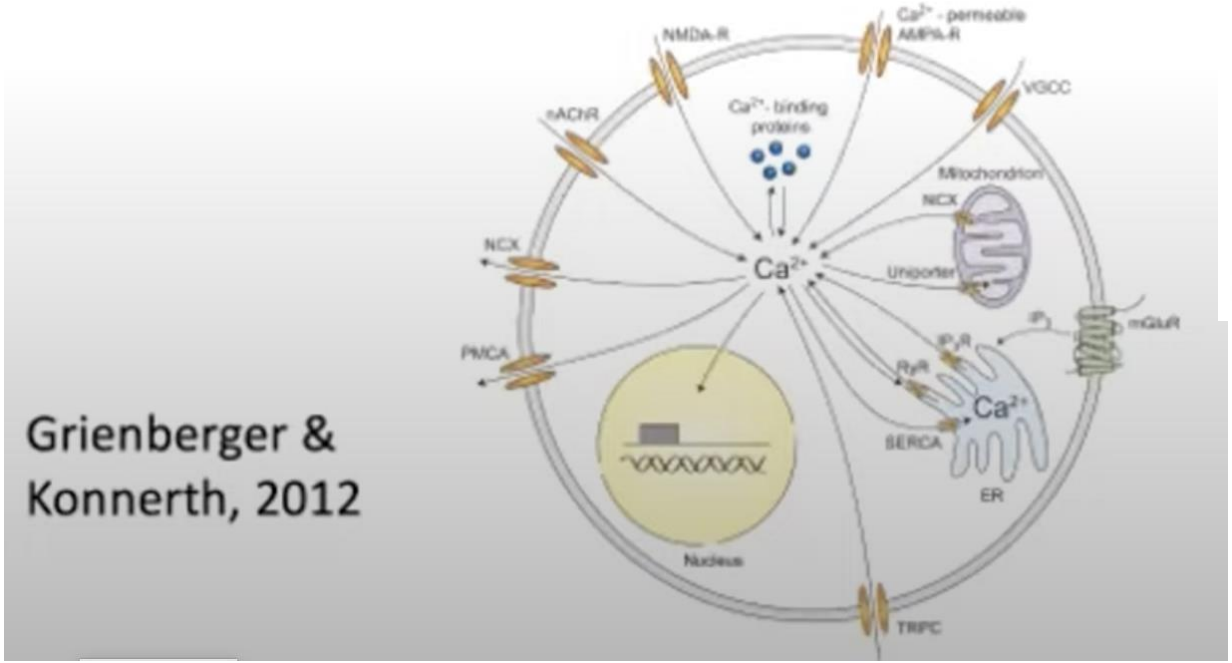
- Calcium is produced in helium shell detonation of sub-Chandrasekhar white dwarfs (JAP 2020)
- Calcium makes up about 4% of the earth's crust by weight (as limestone, gypsum, and fluorite)
- Calcium gives rigidity to bones
- Ca^{2+} is a a ubiquitous second messenger – a signal downstream of extracellular signals
- Intracellular calcium is low - likely because it precipitates phosphate – 10,000 x lower than extracellular calcium
- Cytoplasmic [Ca] can increase 10-100 fold during physiological signaling

Berridge, 2010

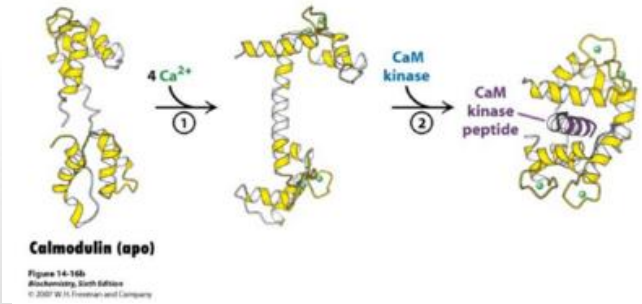


Calcium Signaling

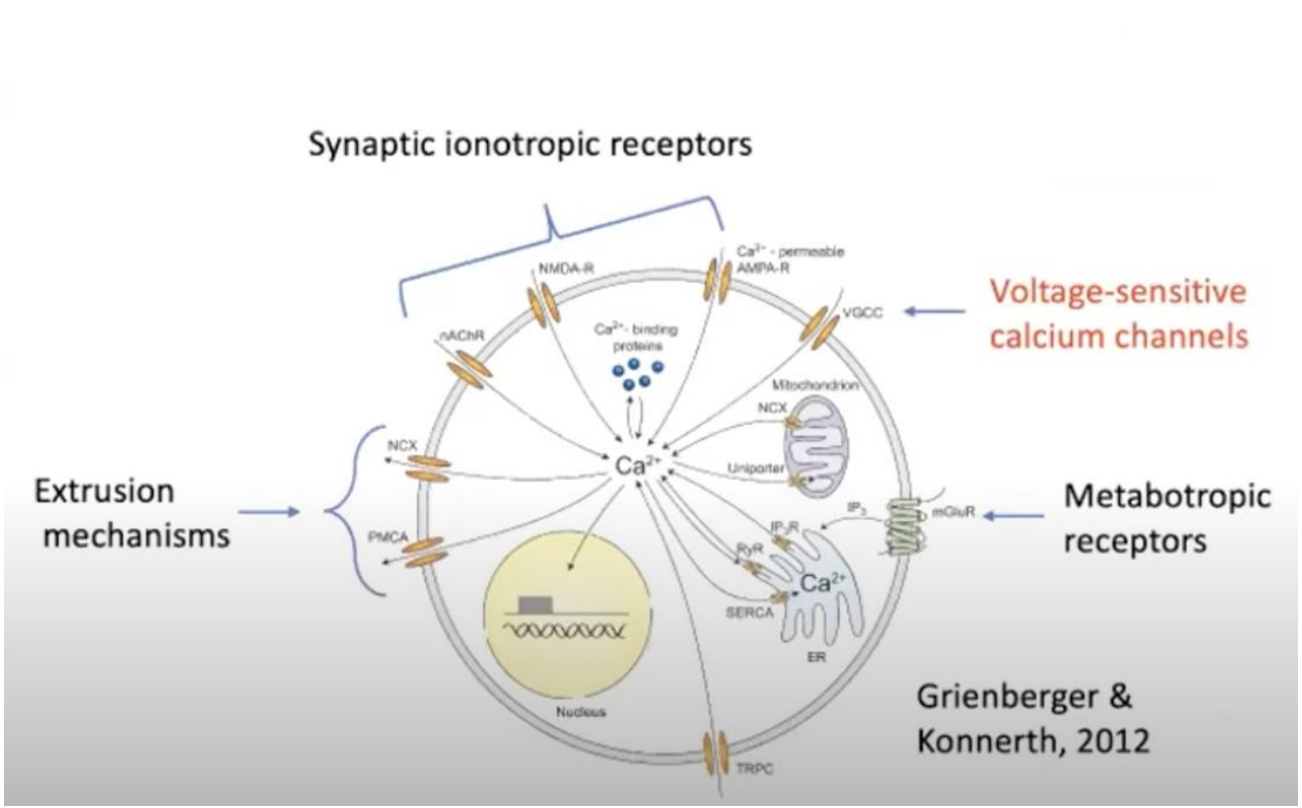
- Unlike more complex signaling molecules, Ca^{2+} cannot be chemically altered
- To exert control over Ca^{2+}, cells must chelate, compartmentalize, or extrude it
- Hundreds of cellular proteins bind Ca^{2+} over a million-fold range of affinities (nM to mM)
- Some buffer Ca^{2+}, others lower calcium (pumps) or trigger cellular processes
- Ca-binding to proteins changes the electrostatics and can cause large conformational changes to initiate signaling

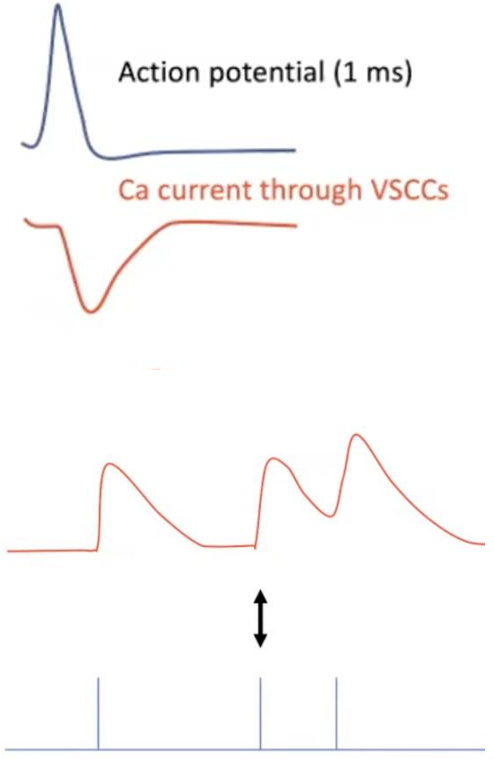


Grienberger & Konnerth, 2012



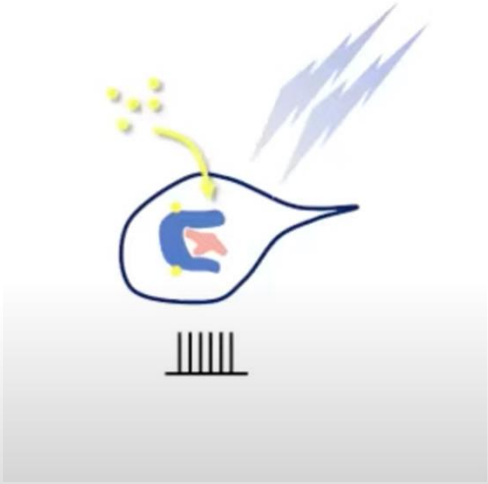
Calcium Signaling in Neurons

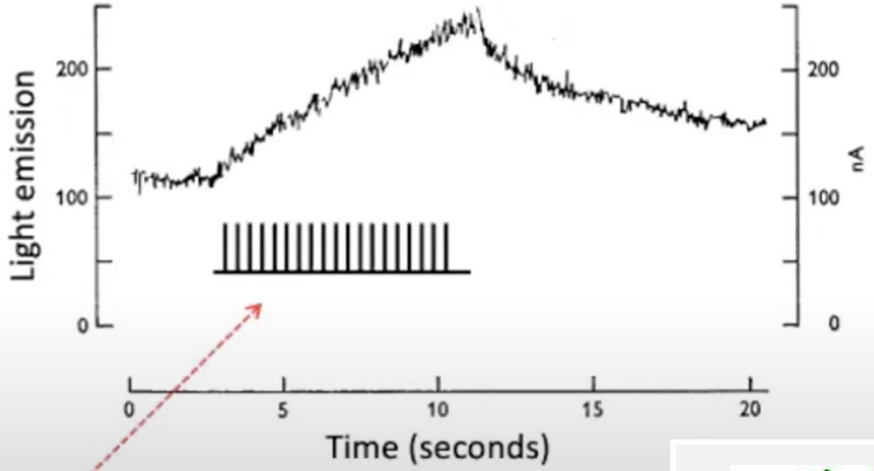




The fisrt optical measurement of neuronal calcium

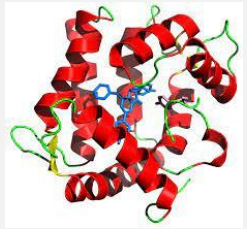
Blue light from aequorin





Action potentials

Baker, Hodkin, Ridgway, 1971



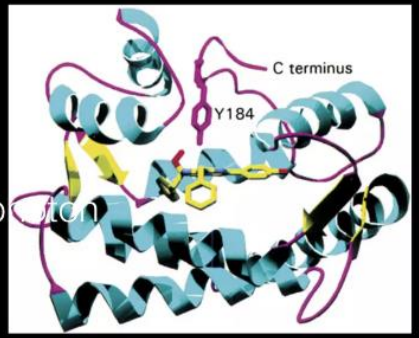
In the beginning, there were jellyfish

SHIMOMURA O, JOHNSON FH, SAIGA Y. Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea J Cell Comp Physiol. 1962 Jun;59:223-39.

- First calcium indicator was "genetically encoded" *in vivo*
- Calcium sensitive, bioluminescent Aequorin was purified from Aequorea victoria

1st GFP ref in footnote! "A protein giving solutions that look slightly greenish in sunlight through only yellowish under tungsten lights, and exhibiting a very bright, greenish fluorescence in the ultraviolet of a Mineralite, has also been isolated from squeezates."

Properties of Aequorin

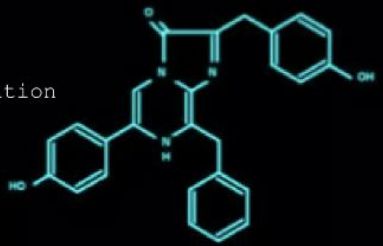


- Cobinding of 3 Ca^{++} sites gives blue fluorescence
- Required cofactor, coelenterzine, destroyed from p[illegible] emission
- Low light output. 12 Ca^{++} ions used and 6 aqueorins destroyed per photon
- Classically microinjected

Johnsn FH, Shimomura O.

Preparation and use of aequorin for rapid microdetermination of Ca 2+ inbiological systems.

Nat New Biol. 1972 Jun 28;237(78):287-8.



- Now cloned. Coelenterzine is commercially available, membrane permeant

Other techniques

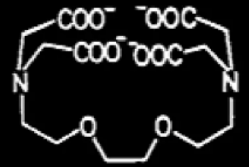
- Ion selective electrodes

  Rink TJ, Tsien RY, Warner AE.

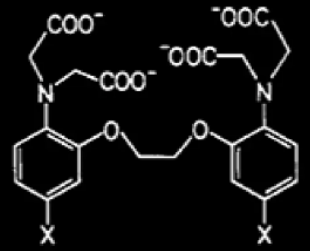
  Free calcium in Xenopus embryos measured with ion-selective microelectrodes. Nature. 1980 Feb 14;283(5748):658-60.

- Metallochromic dyes

- Fluorescent dyes
  - BAPTA, quin2 - 1980
  - fura2, indo - 1985 Tsien et. al
  - fluo, rhod - 1989
  - Molecular Probes derivatives
    - Kuhn 1993

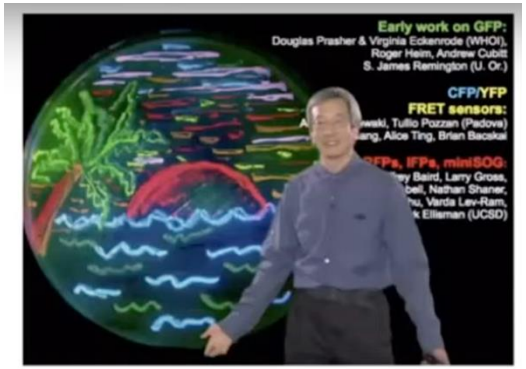


EGTA (1)



2a : X = H
2b : X = CH_3
2c : X = Br

Bapta, Fura, Fluo and all that



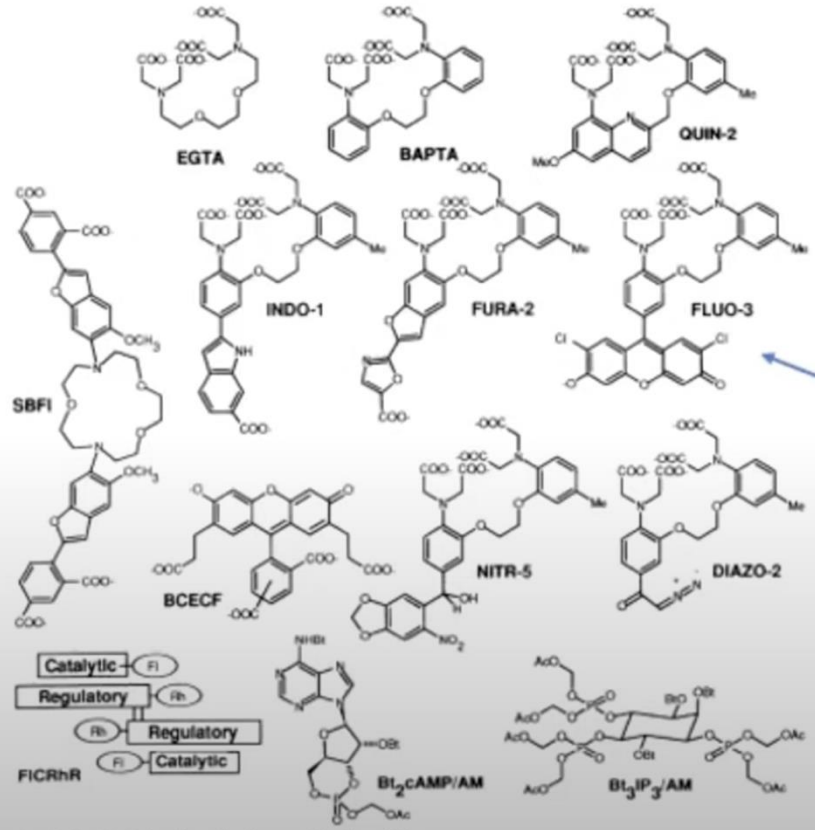
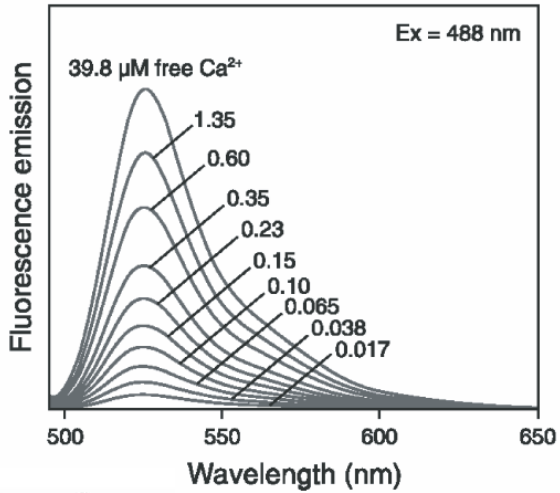


Fig. 1. Structures of physiological probe molecules named in this article.



40x increase in fluorescence upon Ca binding!

Calcium imaging

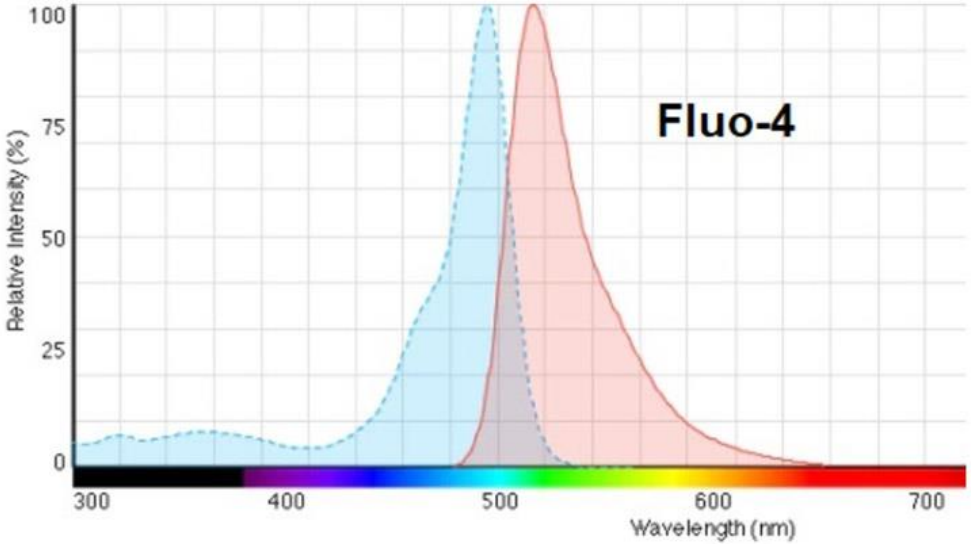
Use of calcium indicators, fluorescent molecules that respond to the binding of Ca2+ ions by fluorescence properties.

Chemical indicators

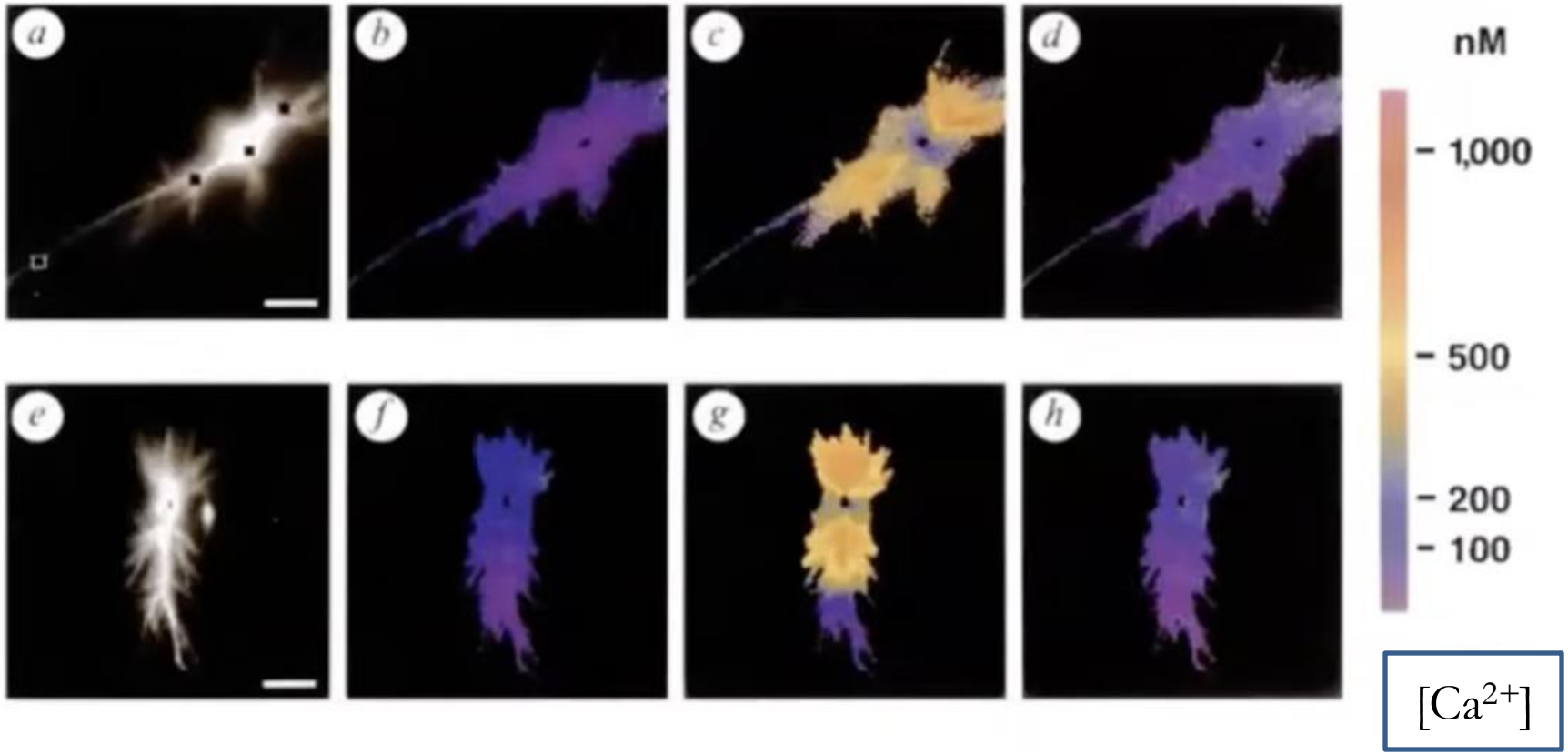
- Small molecules that bind calcium ions via chelation
- Often modified with acetoxymethyl esters (AM), in order to render the molecule lipophilic and to allow easy entrance into the cell

Critical point

- "Bulk" loading of cells
- Not suitable for in vivo or screening application



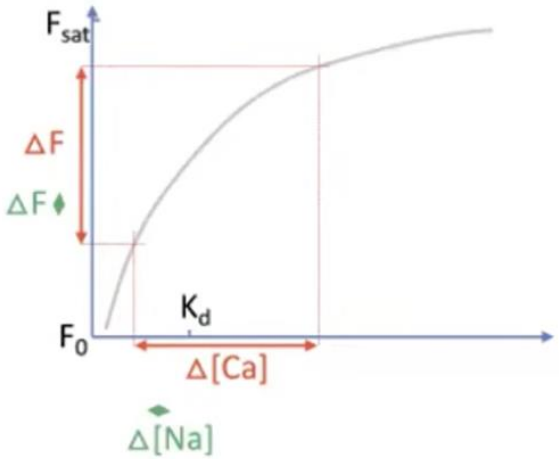
Indicator	K_d for Ca^{2+} (nM)	Excitation (nm), emission (nm)
Calcium Green-1	190	490 ex, 531 em
Fluo-3	325	506 ex, 526 em
Fluo-4	345	494 ex, 516 em
Fura-2	145	363/335 ex, 512 em
Indo-1	230	488 ex, 405/485 em
Oregon Green 488 Bapta-1	170	488 ex, 520 em
Fura-4F	0.77	336/366 ex, 511 em
Fura-5F	0.40	336/363 ex, 512 em
Calcium Crimson	185	590 ex, 615 em
X-Rhod-1	0.7	580 ex,602 em



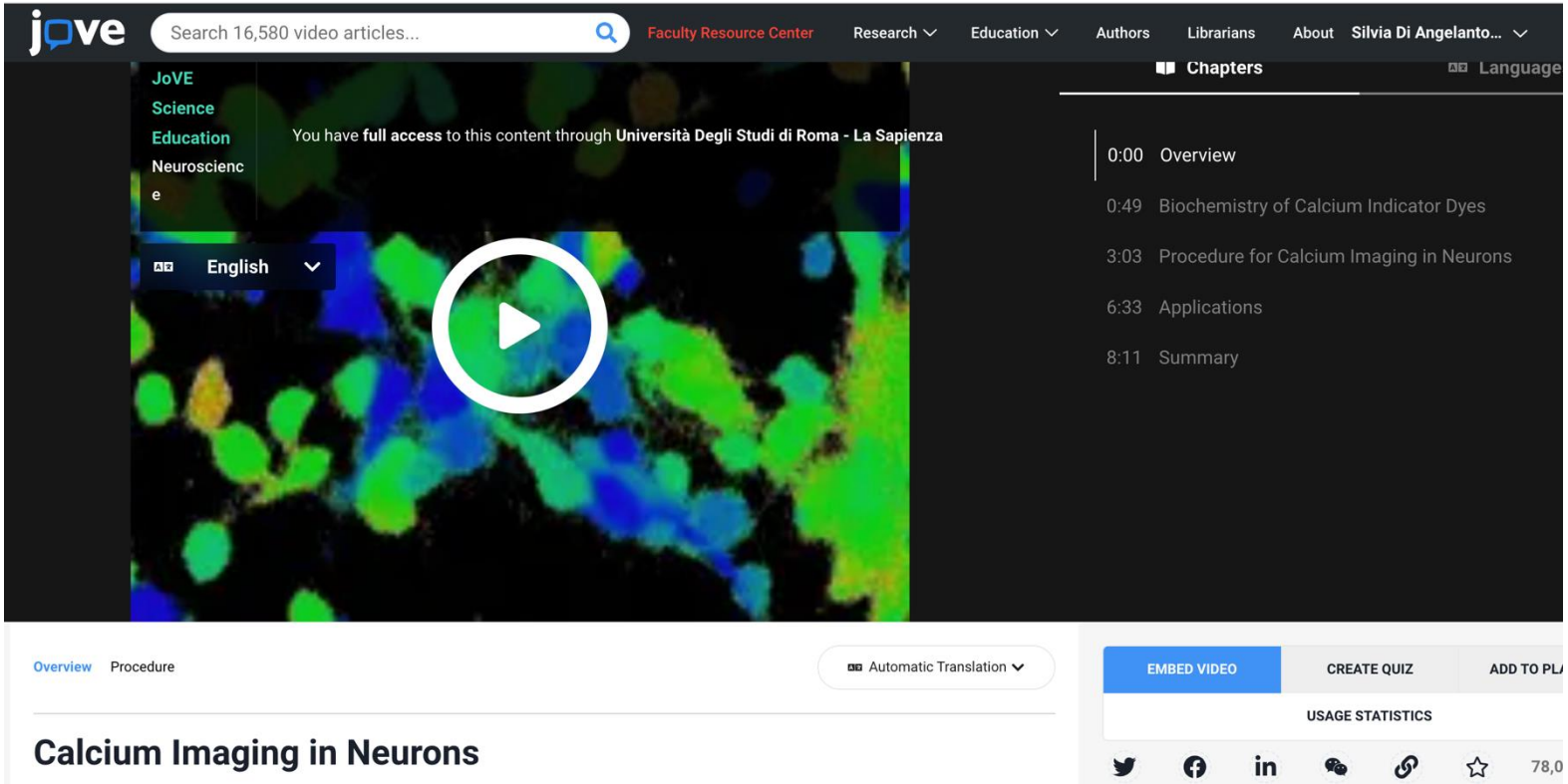
Cooled CCD

Why calcium (as opposed to Na, K, Cl, …)?

- [Ca] changes 10 x in response to one action potential (100 nM → 1 uM)
- [Na] changes by 10^{-4} (10 mM → 10 mM) (similar for K^+)
- Only [Ca] shows large and rapid changes, tighly coupled to normal neural activity



$$F = (F_{sat} - F_o)\, C /(K_d + C) + F_o$$



https://www.jove.com/embed/player?id=5203&t=1&chap=1&s=1&fpv=1

Imaging action potential evoked calcium

Ca^{2+} dynamics

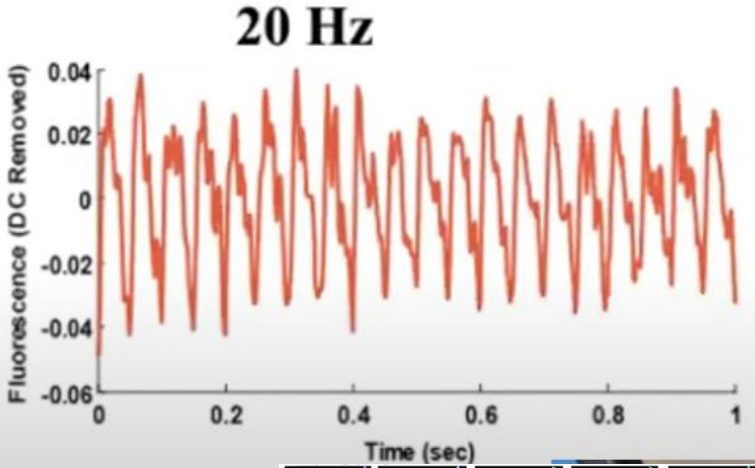
$I(t)$

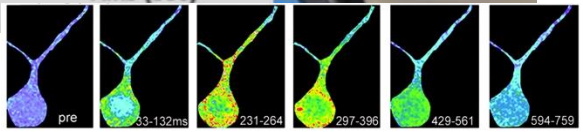
$G(t)$

20 Hz

$$I(t) = A * \sum_{i=0}^{N-1} \delta(t - (t_{delay} + \frac{i}{\omega}))$$

$$[Ca^{2+}] = I(t) \otimes G(t) + [Ca^{2+}]_o$$

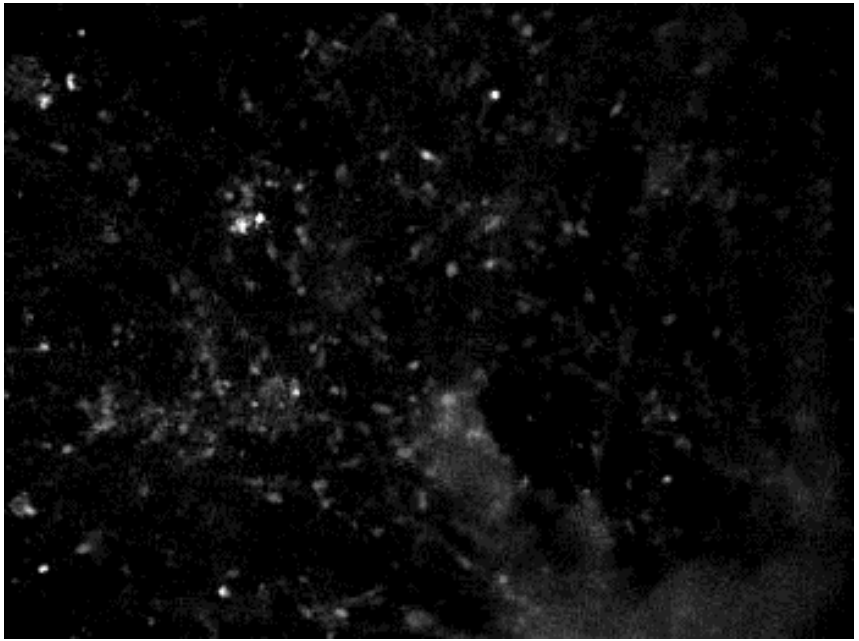




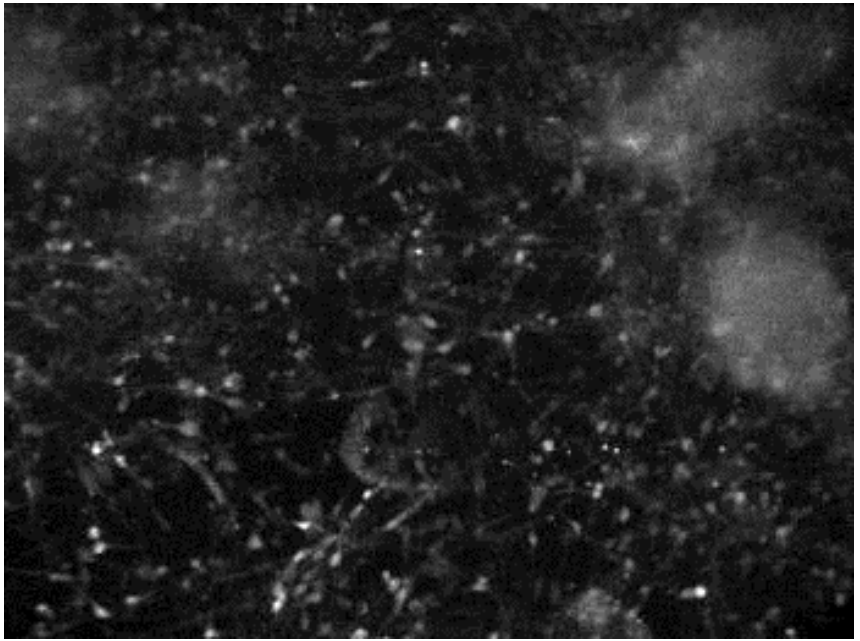
FMRP-KO cortical cultures display increased spontaneous and evoked calcium activity at day 70

Calcium Imaging

FMRP-WT day 70

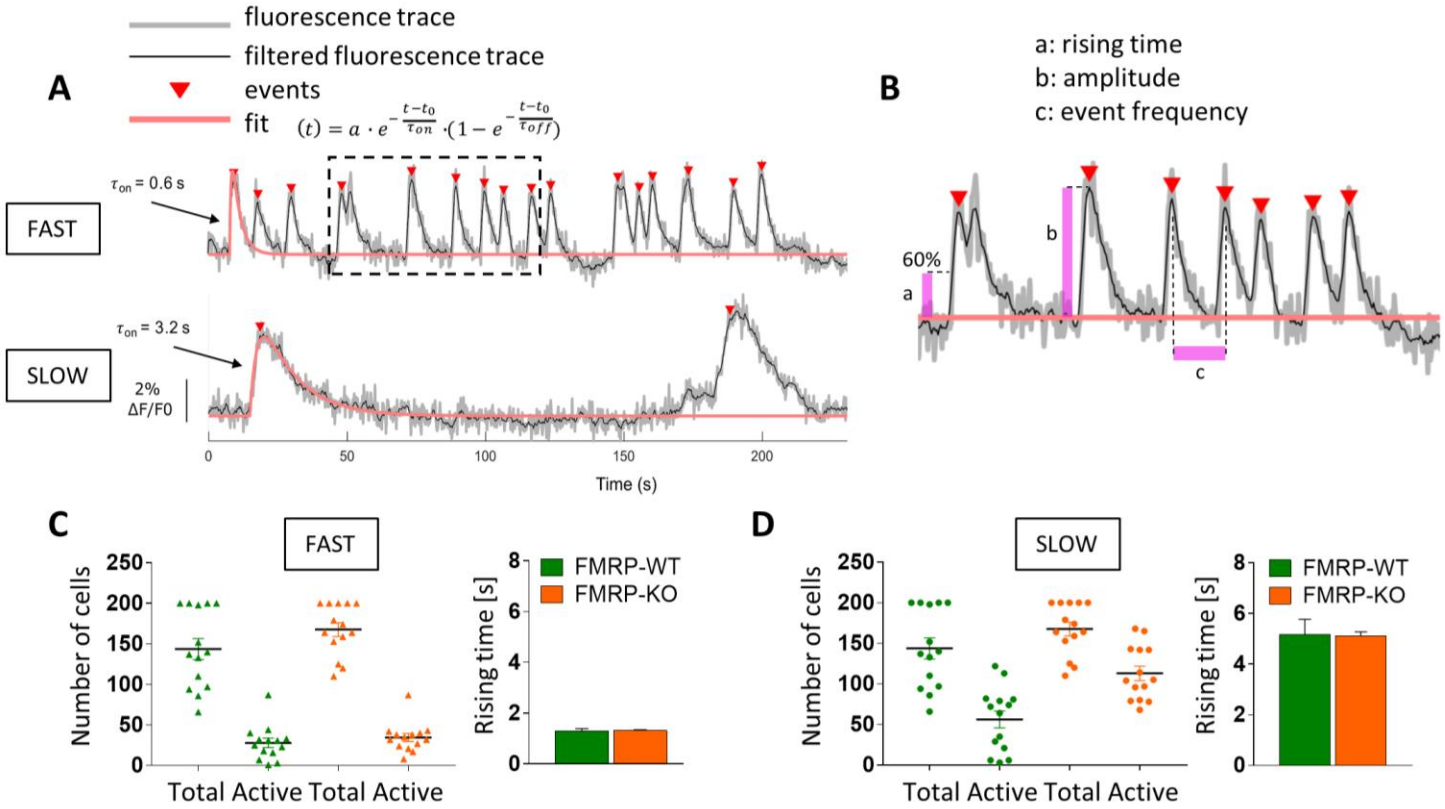


FMRP KO day 70

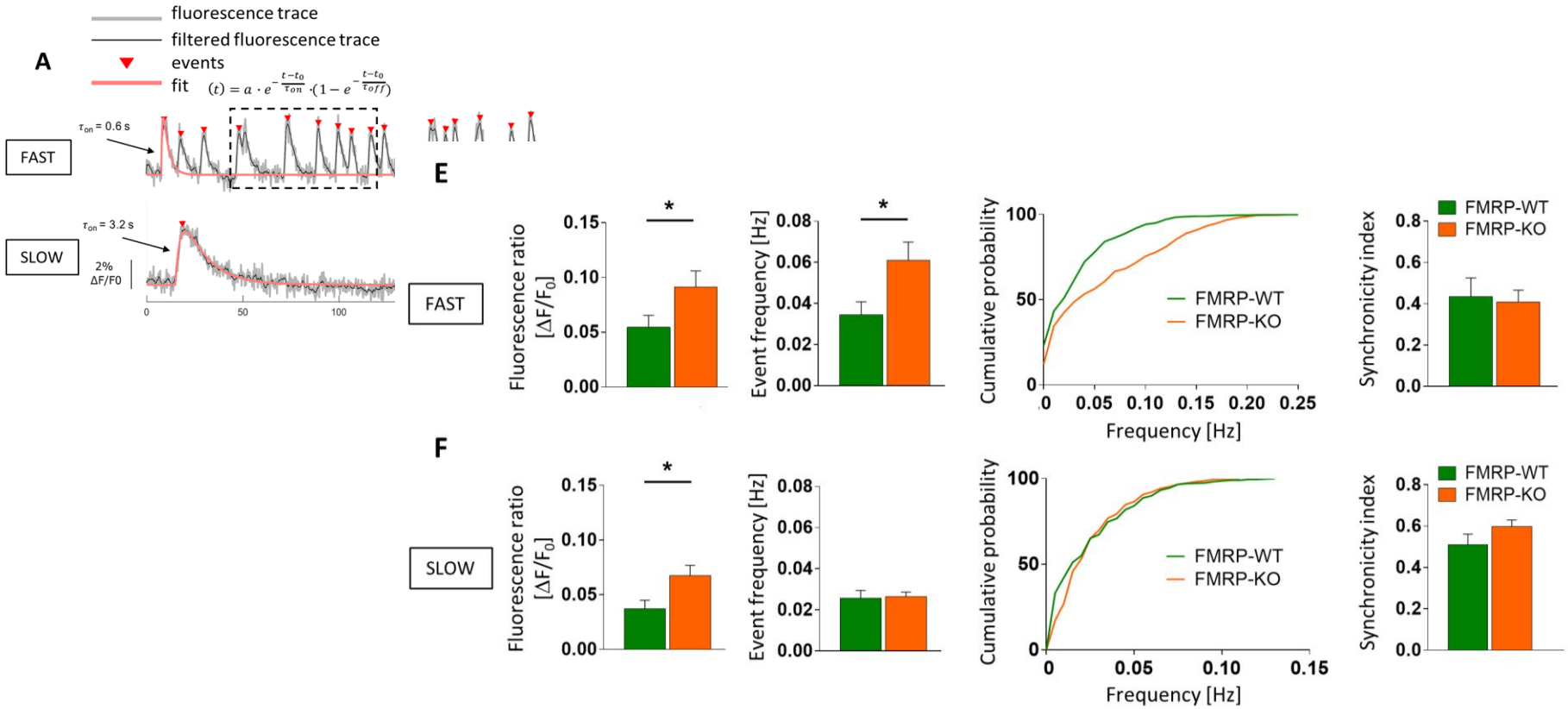


FLUO4 Calcium Indicator

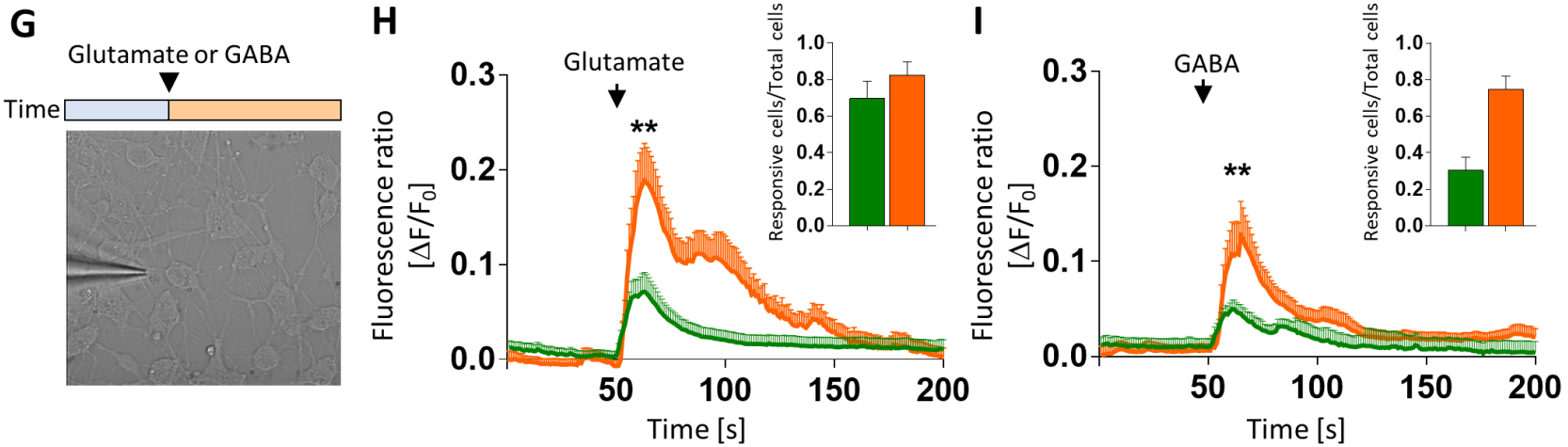
FMRP-KO cortical cultures display increased spontaneous and evoked calcium activity at day 70



FMRP-KO cortical cultures display increased spontaneous and evoked calcium activity at day 70



FMRP-KO cortical cultures display increased spontaneous and evoked calcium activity at day 70



Brighi et al, Cell Death and Disease (2021)

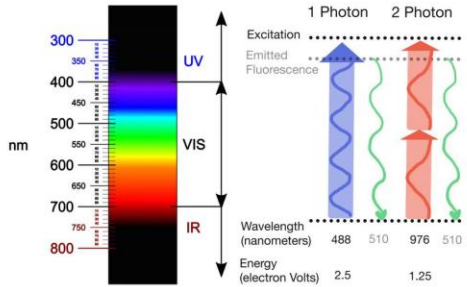
2-photon excitation of fluorescence

Maria Goeppert-Mayer

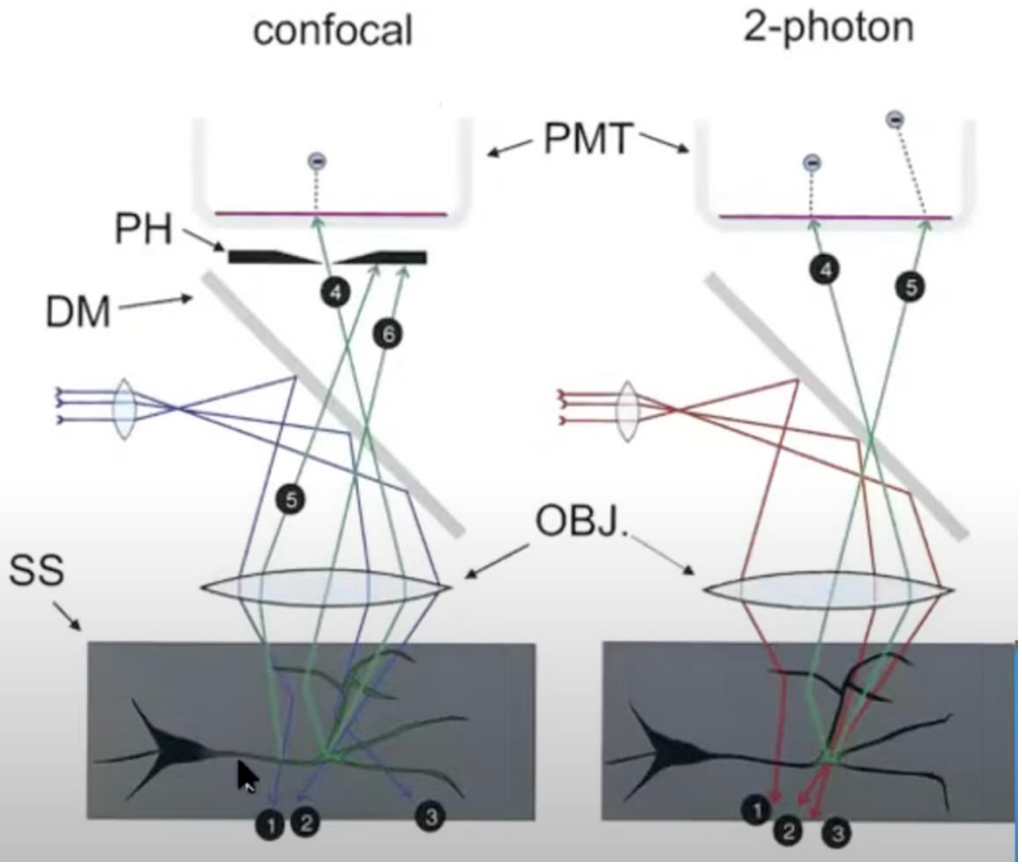
Drückt man noch die in der Formel vorkommenden Matrixelemente aus durch den Emissionskoeffizienten

$$A_{n\,E} = \frac{64\,\pi^4\,\nu_{E\,n}^3}{3\,c^3\,h}\,|P_{n\,E}|^2$$

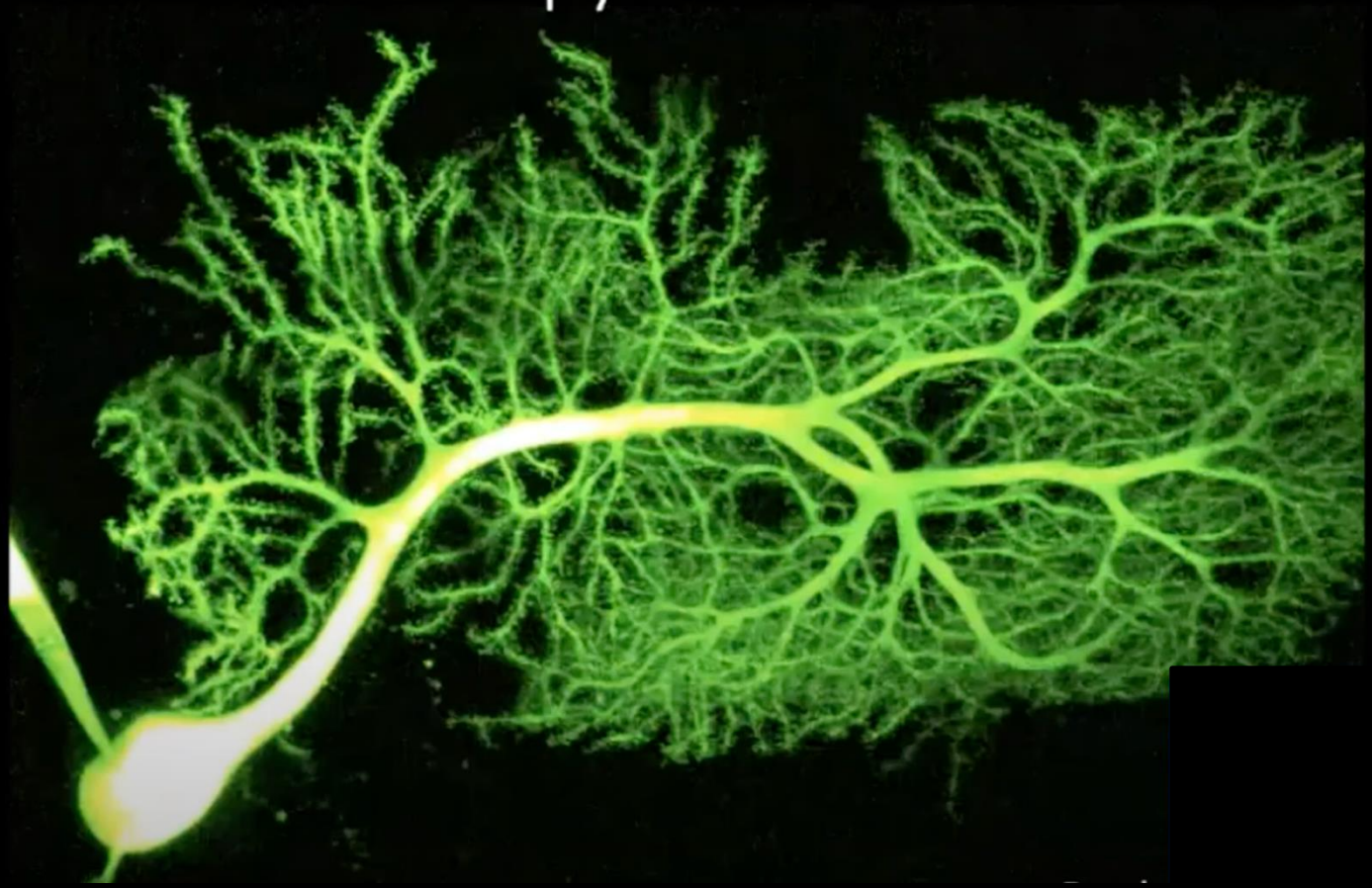
Two-photon fluorescence



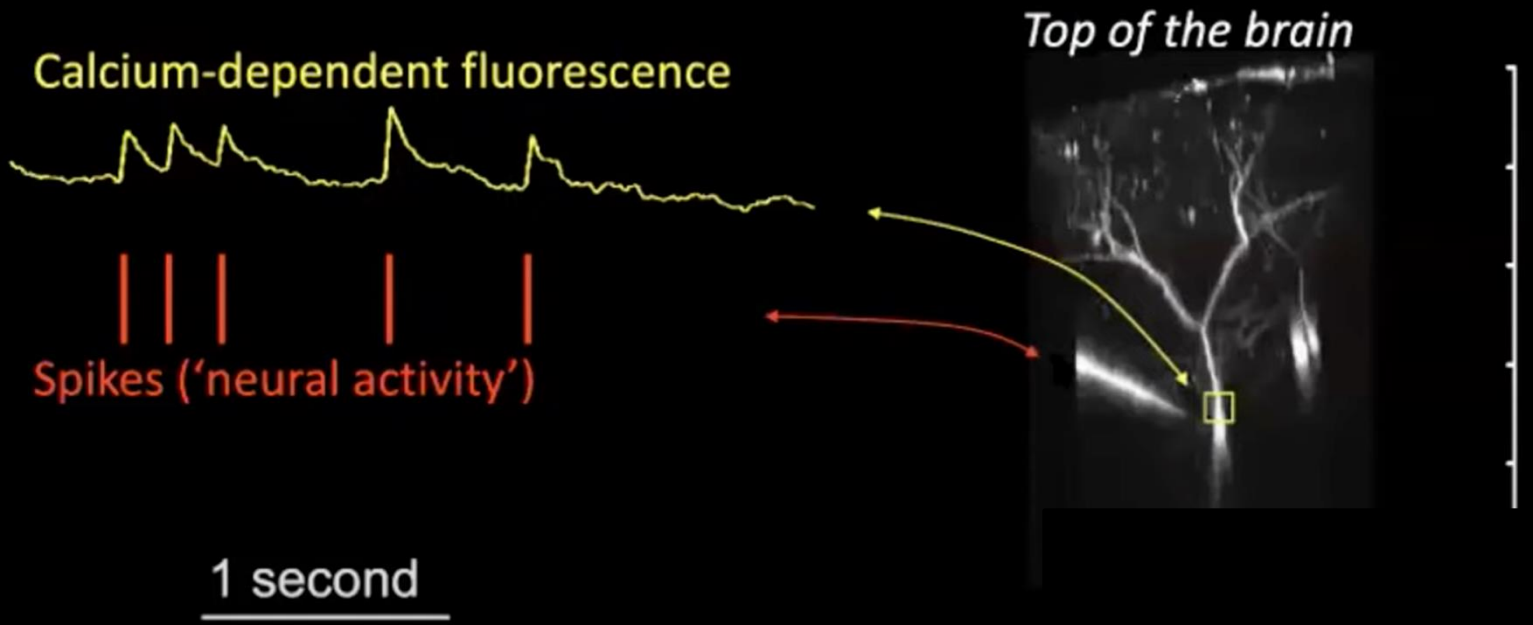
2-photon microscopy



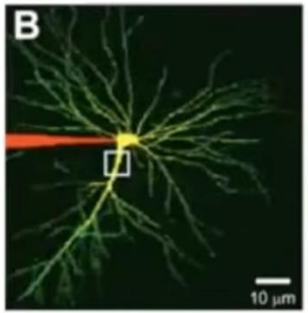
2-photon microscopy

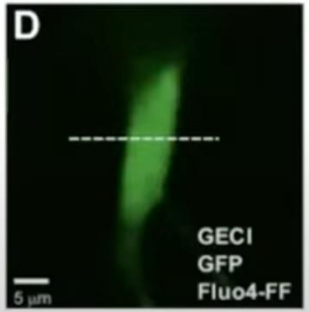


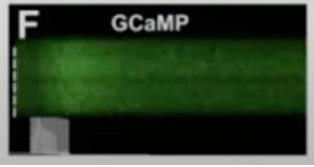
Calcium-dependent fluorescence changes reflect neural activity *in vivo*

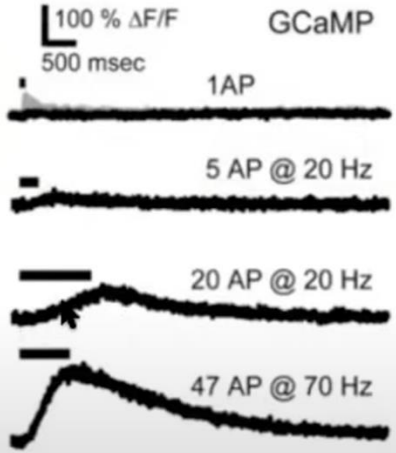


The need for protein engineering









Pologruto et al 2004

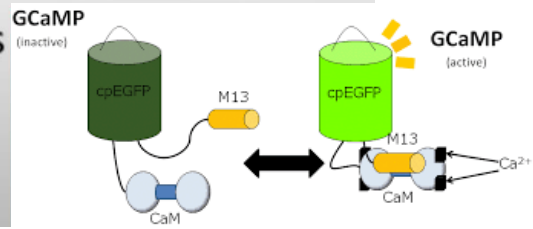
To use genetically encoded Ca sensors in vivo we need:

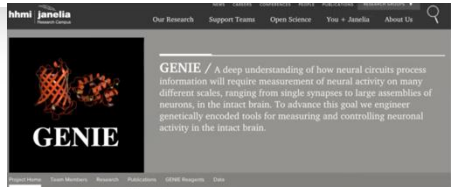
Brighter

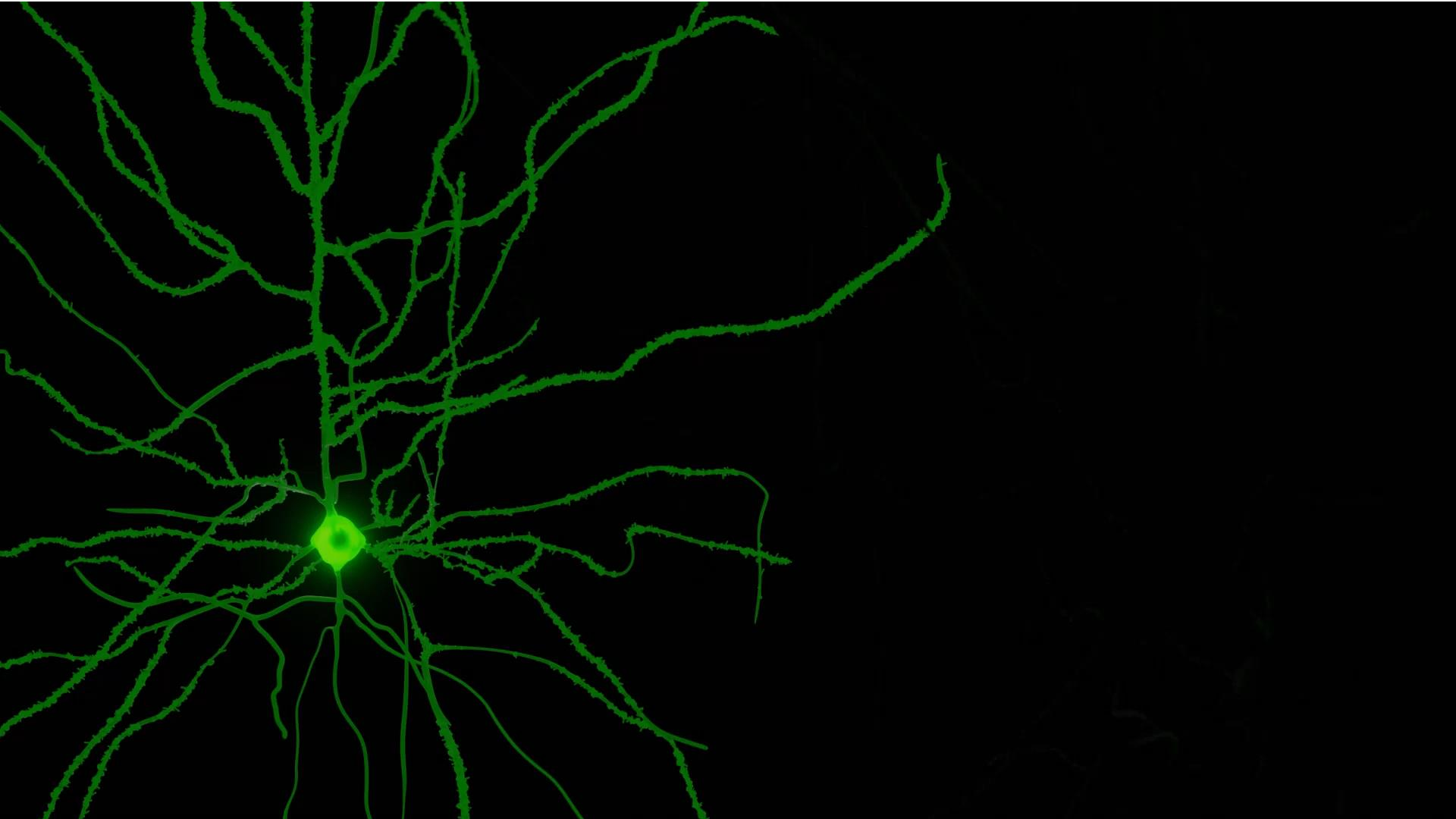
More sensitive ('higher affinity')

More stable at physiological temperatures

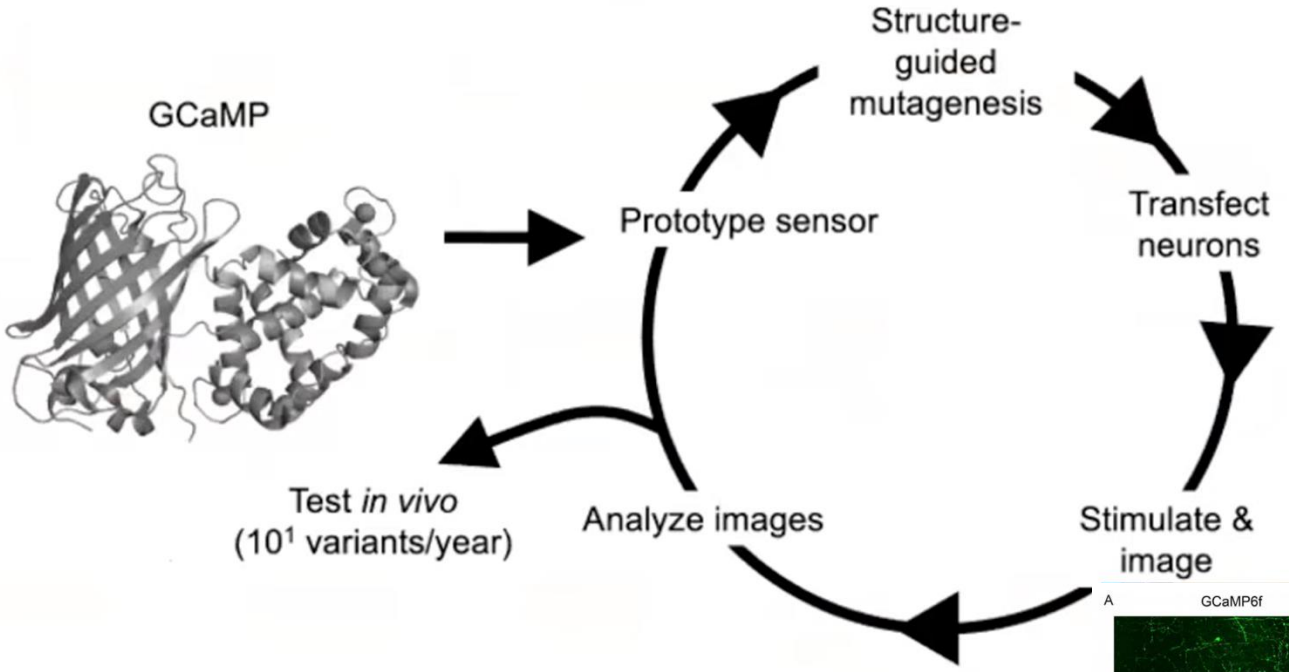
Faster kinetics

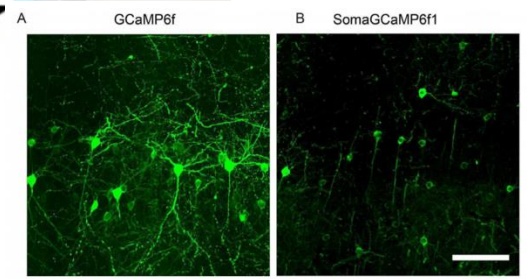






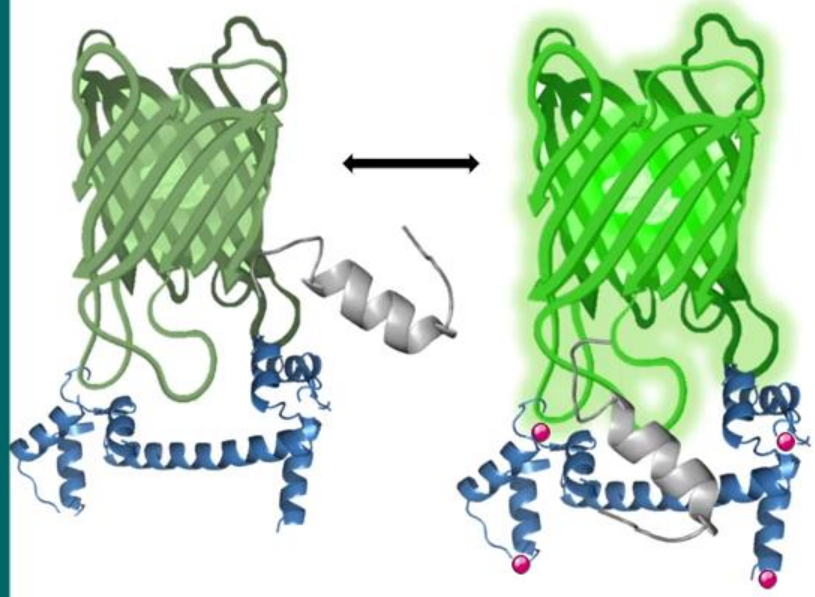
Calcium sensor screening pipeline
10^3 variants/year

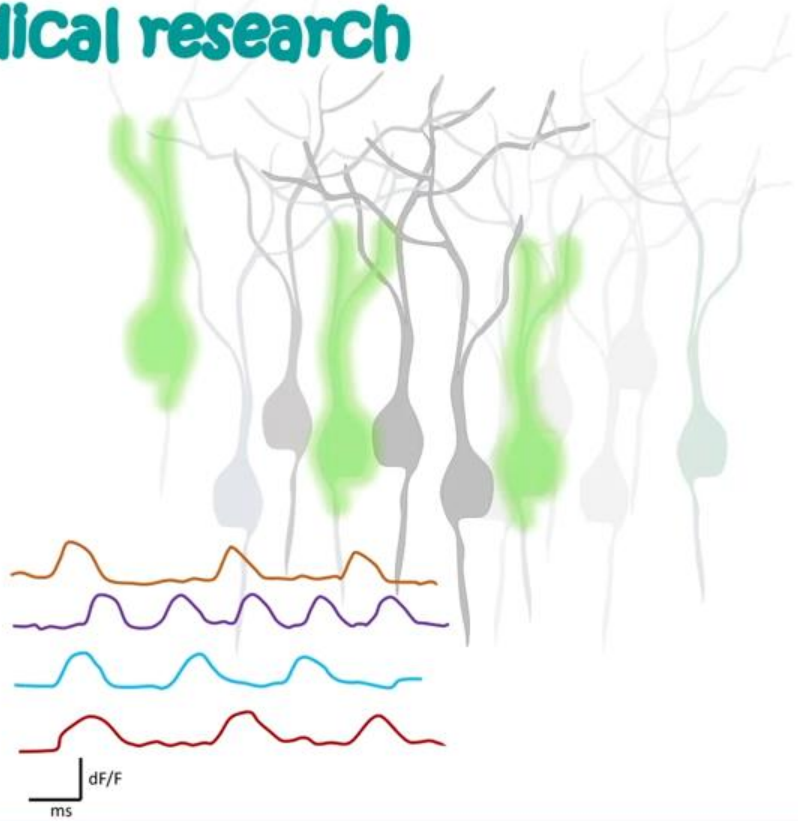


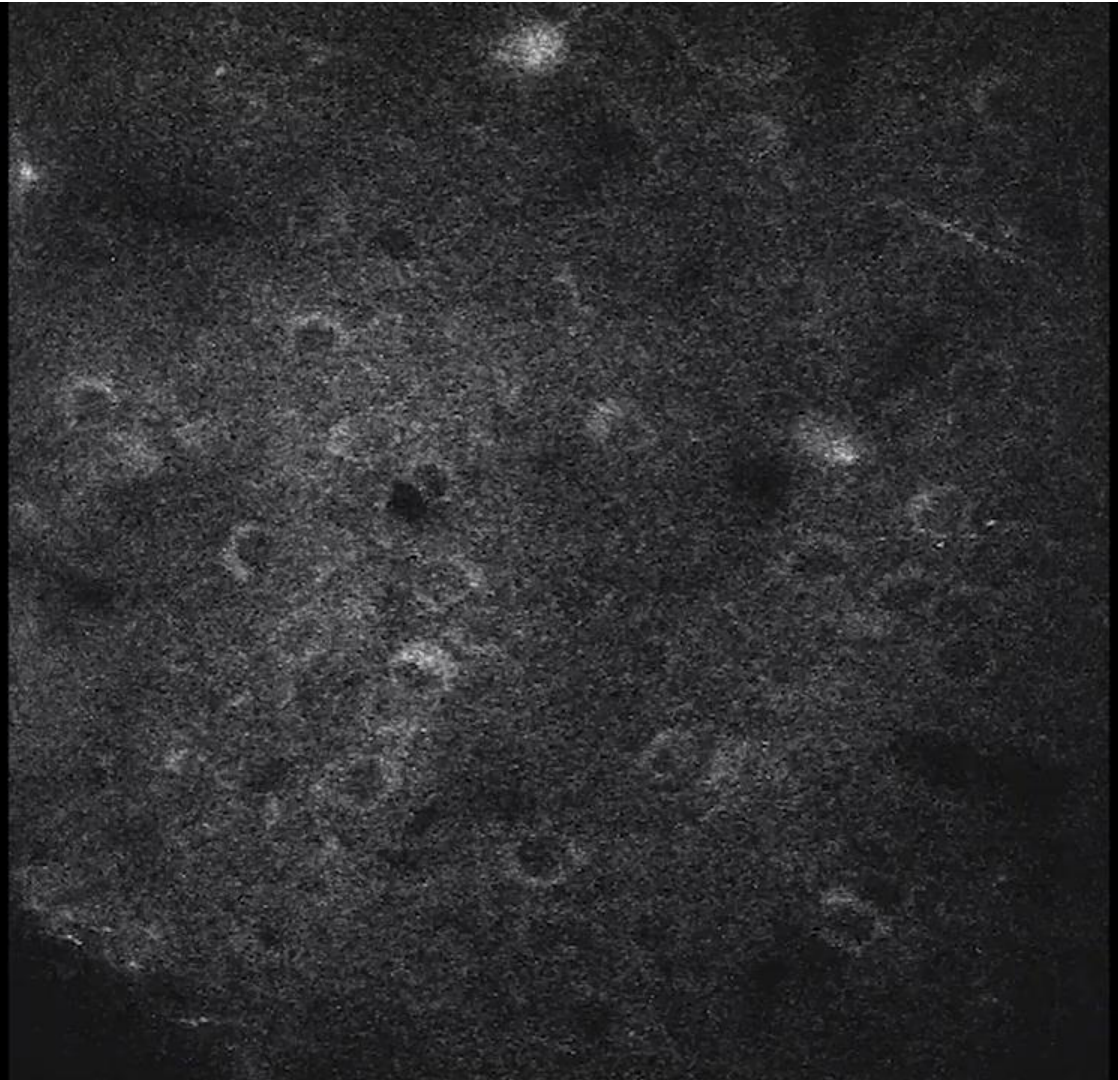


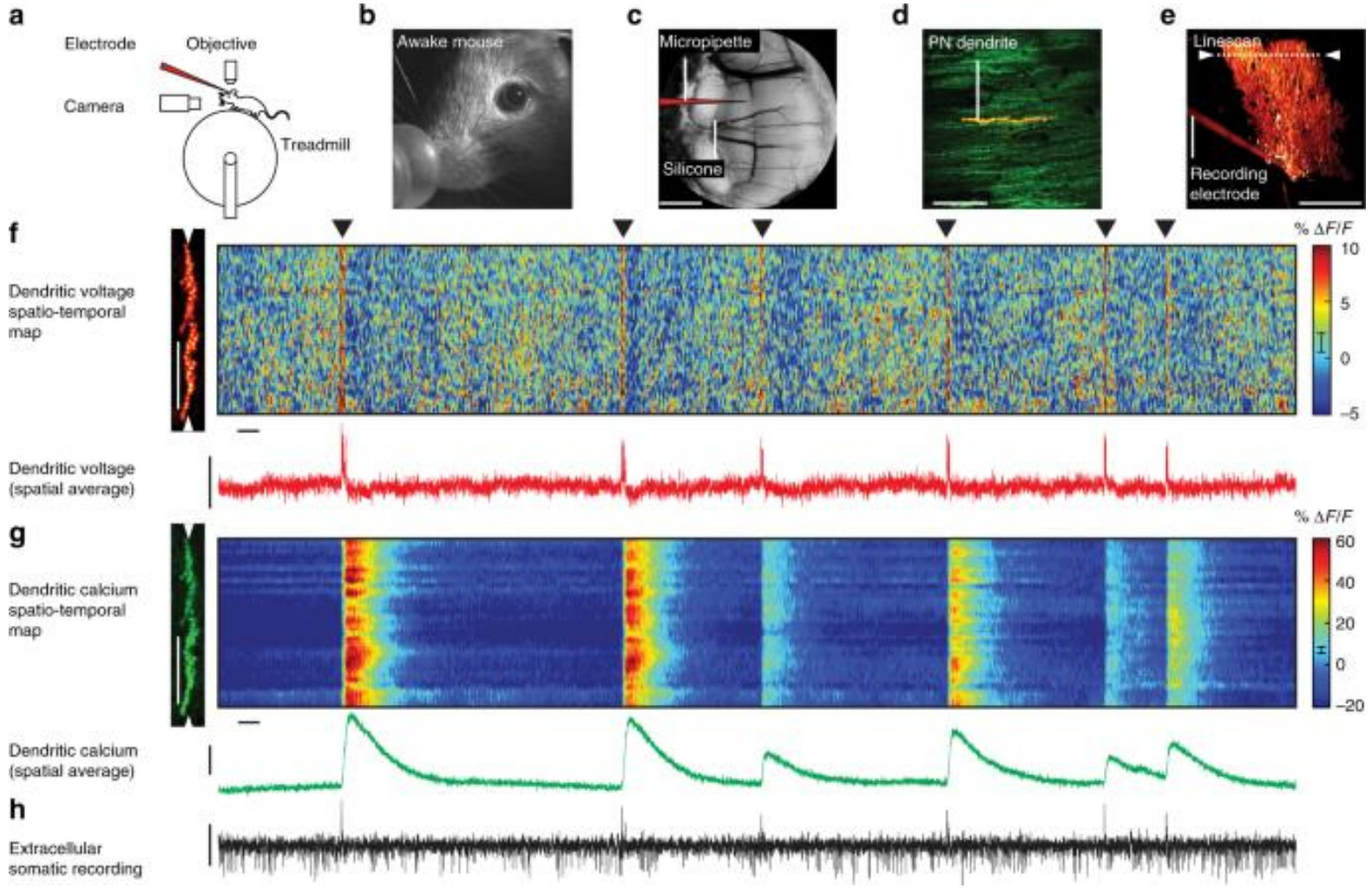
GCaMP (Calcium Sensor)

Application in biomedical research

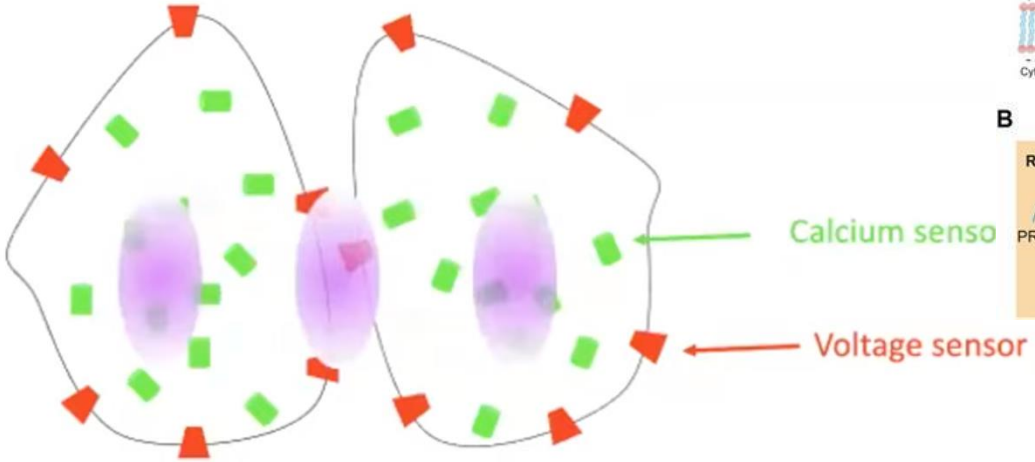


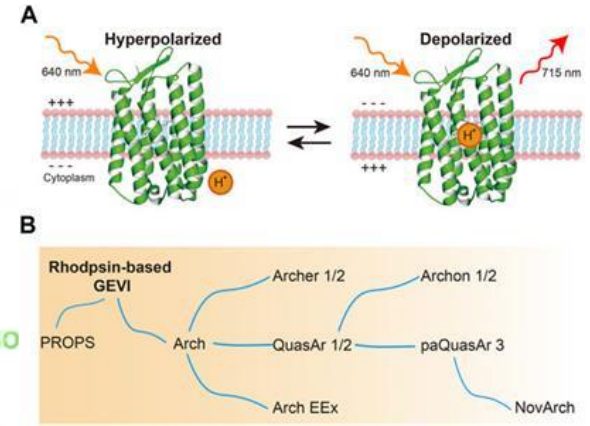




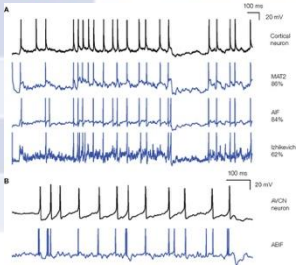

a
Electrode
Objective
Camera
Treadmill
b
Awake mouse
c
Micropipette
Silicone
d
PN dendrite
e
Linescan
Recording electrode
f
Dendritic voltage spatio-temporal map
% ΔF/F
10
5
0
−5
Dendritic voltage (spatial average)
g
Dendritic calcium spatio-temporal map
% ΔF/F
60
40
20
0
−20
Dendritic calcium (spatial average)
h
Extracellular somatic recording

Challenges for imaging voltage





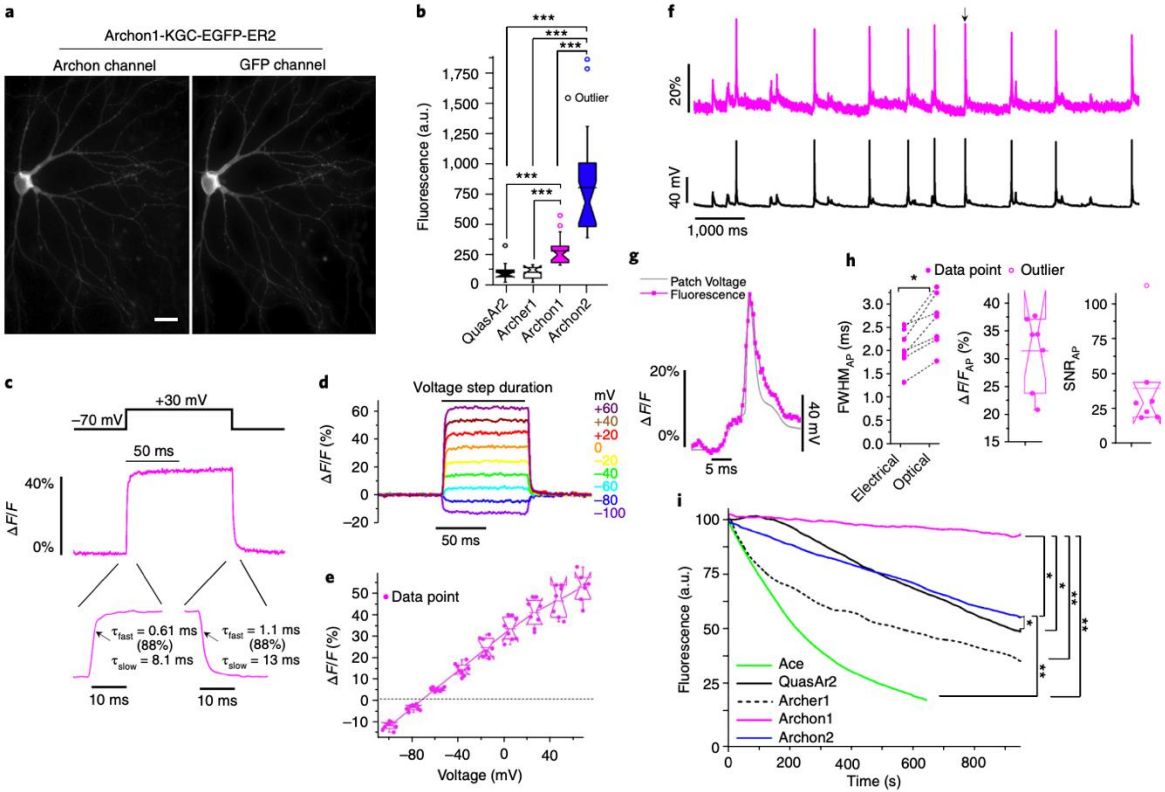
	GEVI	GECI
Fluorescence change	40 %	1000 %
Molecules per resel	100	10,000
Time per AP	2 ms	100 ms
Sensor distribution	Membrane	Cytosol



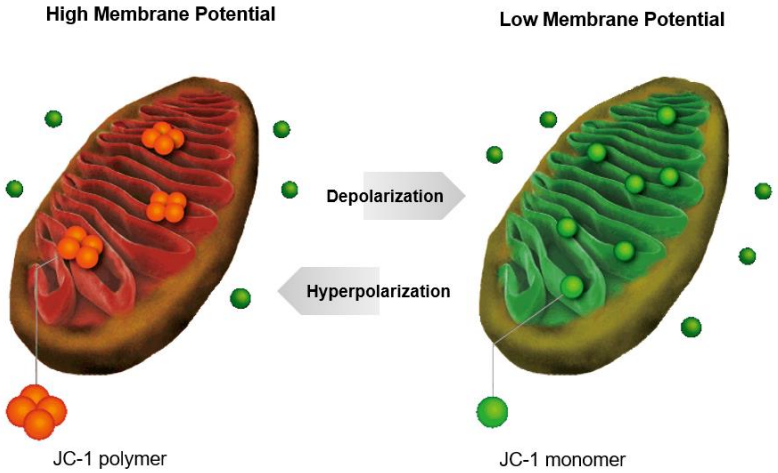
ARTICLES
https://doi.org/10.1038/s41589-018-0004-9
nature chemical biology

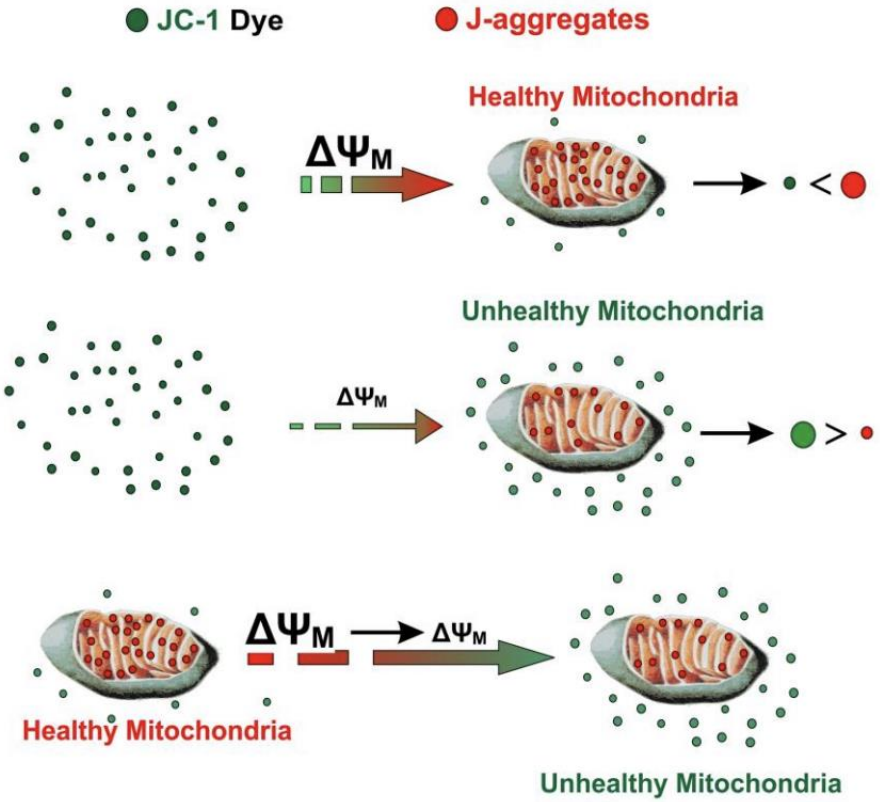
A robotic multidimensional directed evolution approach applied to fluorescent voltage reporters

Kiryl D. Piatkevich[1,17], Erica E. Jung[1,17], Christoph Straub[2], Changyang Linghu[1,3], De
Ho-Jun Suk[1,4], Daniel R. Hochbaum[2], Daniel Goodwin[1], Eftychios Pnevmatikakis
Takashi Kawashima[7], Chao-Tsung Yang[7], Jeffrey L. Rhoades[8], Or Shemesh[1], Sh
Young-Gyu Yoon[1,3], Limor Freifeld[1], Jessica L. Saulnier[2], Clemens Riegler[9,10], F
Thom Hughes[11], Mikhail Drobizhev[11], Balint Szabo[12], Misha B. Ahrens[7], Steven V
Bernardo L. Sabatini[2] and Edward S. Boyden[1,13,14,15,16]*



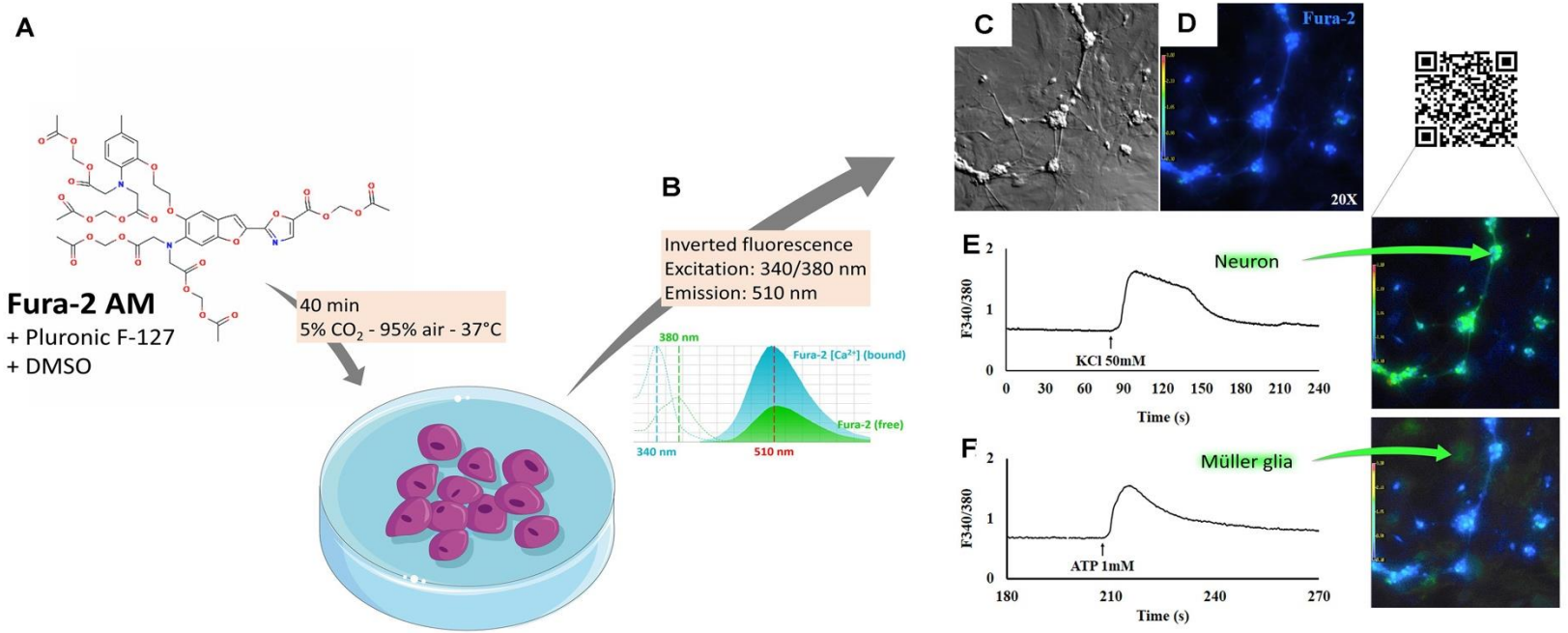
Imaging of mitochondrial potential





Calcium imaging in 3D constructs

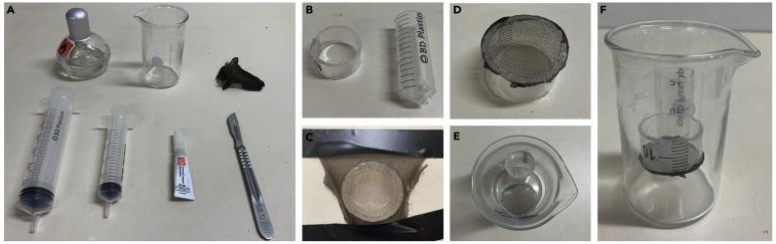
- From whole organoid or slices (see slicing procedure)
- Loading with calcium dye OR viral labelling (i.e., AAV, lentivirus) for genetic indicators
- Imaging equipment: fluorescent microscope or confocal for high resolution



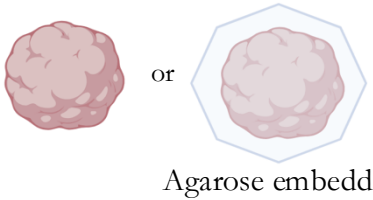
(de Melo Reis et al, 2020)

Slicing organoids

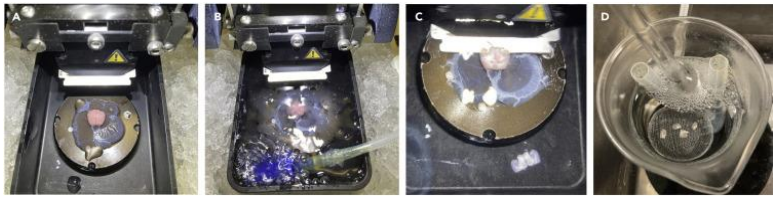
Custom made holding chamber for slice recovery



Vibratome for slicing



Agarose embedding



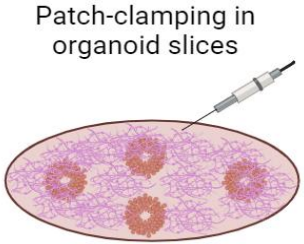
Experimental settings:

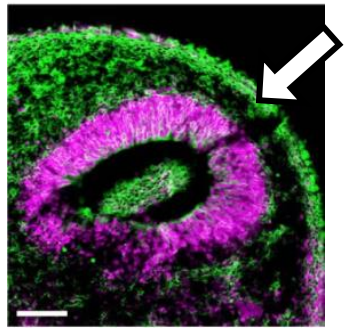
- Slice thickness (200-300 µm)
- Slice recovery (ACSF @ 32°-37°C) for at least 30 min
- Recordings in controlled temperature

Critical points:

- Slicing
- Orientation into the slice – the cytoarchitecture is not easy to decipher
- Residual matrigel on the slice – the pipette cannot reach the surface

Where to patch?

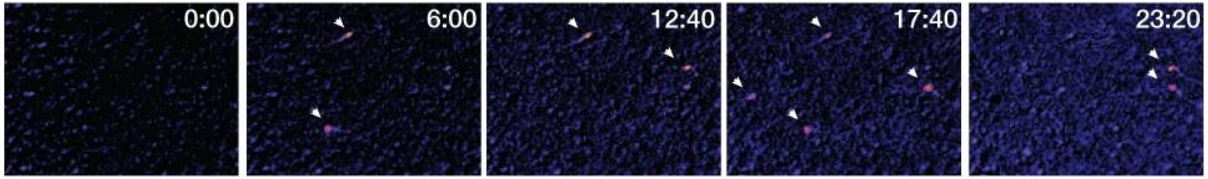


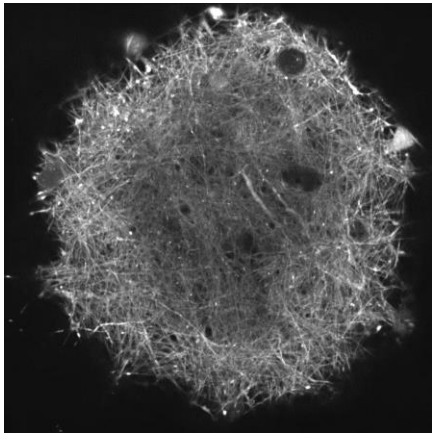


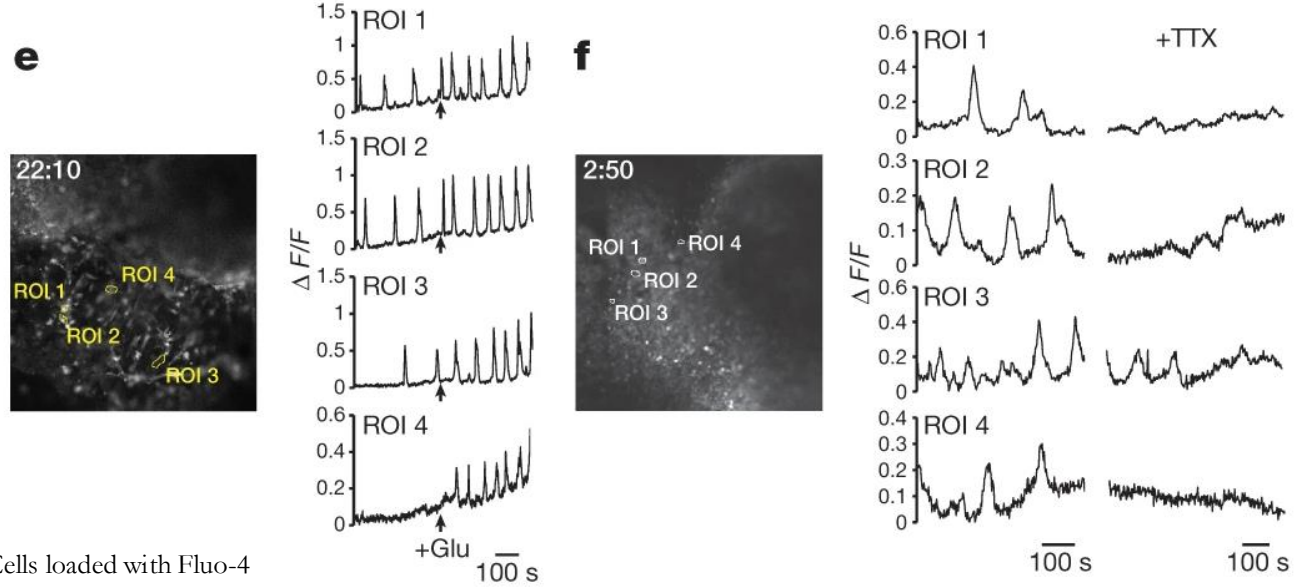
Monitoring spontaneous and evoked network activity in cerebral organoids

(Chemical indicator)

Time-lapse experiment



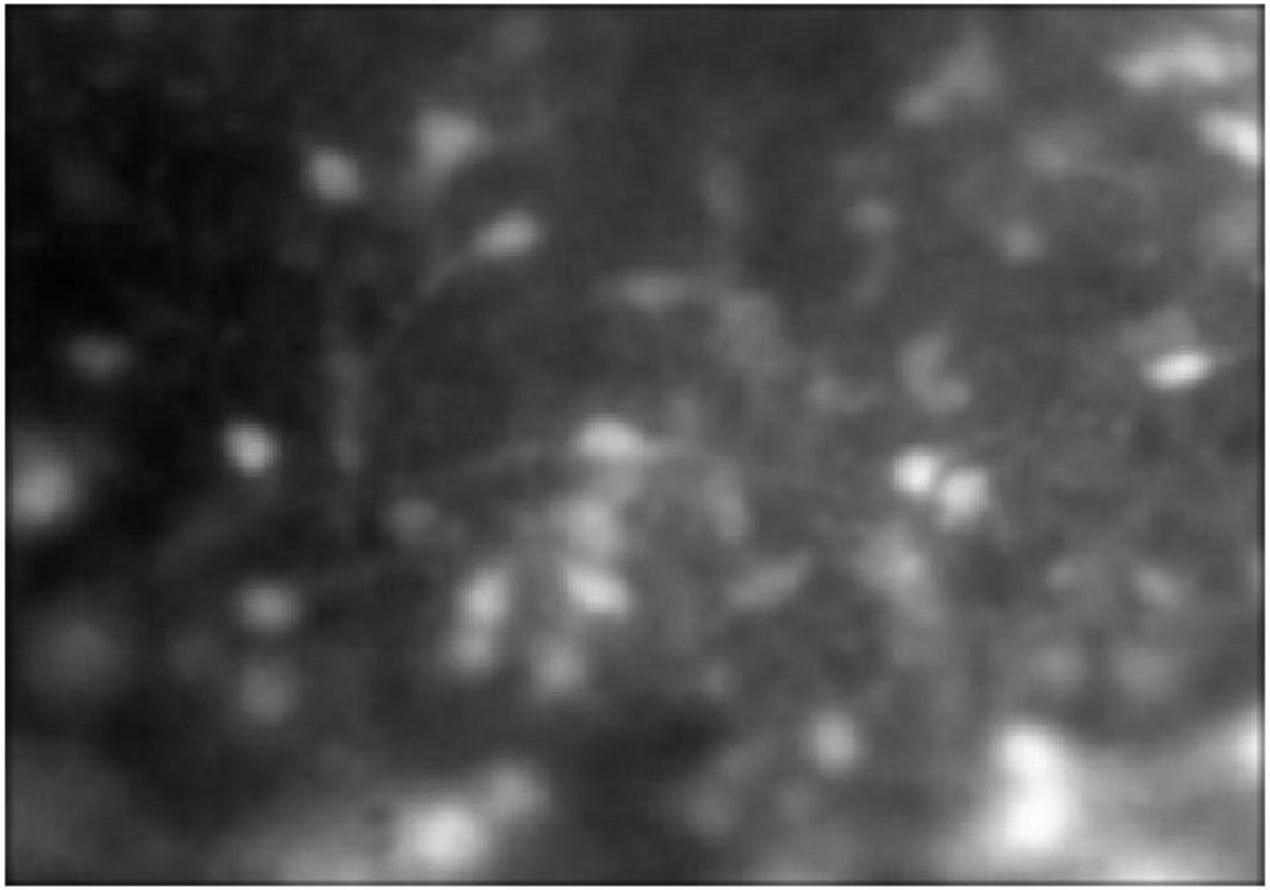




*Cells loaded with Fluo-4

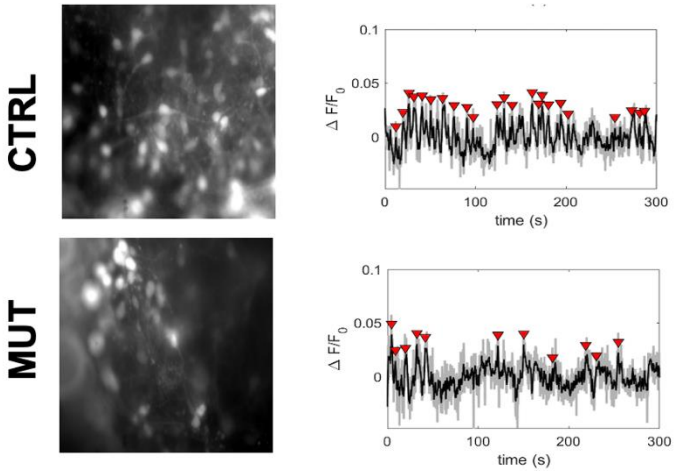
** Evoked activity with bath application of glutamate

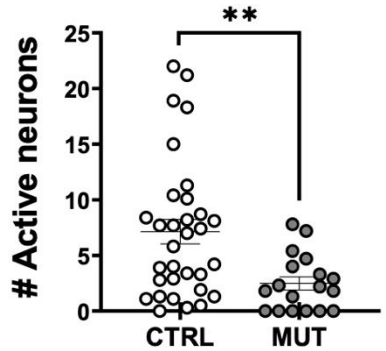
(Lancaster et al, 2013; Renner et al, 2020)

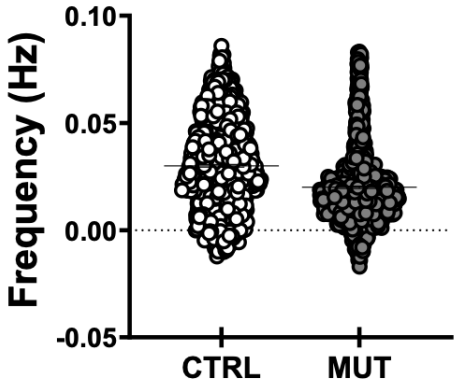


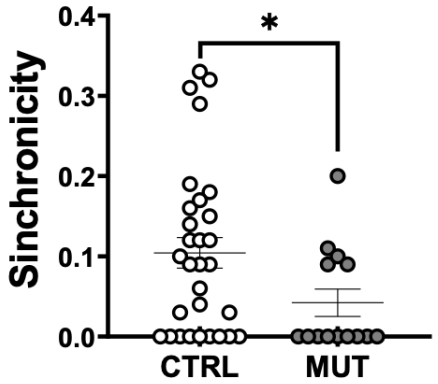
Monitoring spontaneous and evoked network activity in cerebral organoids

(Chemical indicator)



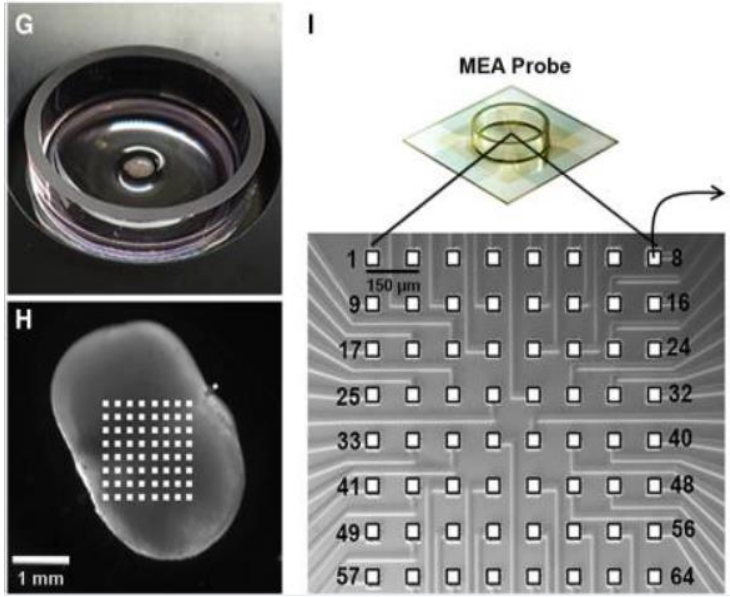






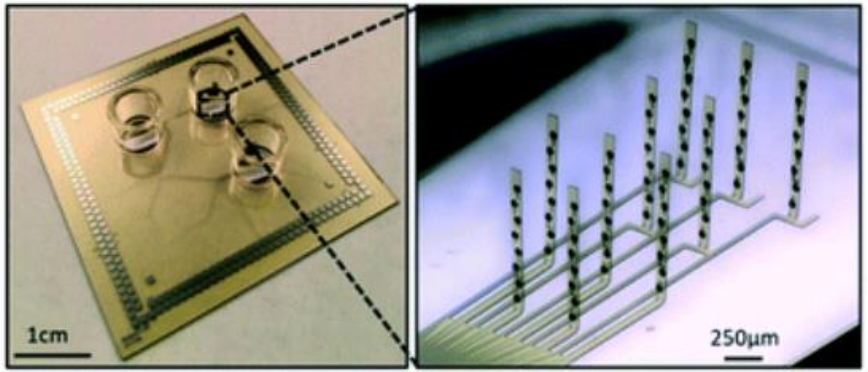
Multi-electrode array

Increasingly adopted for screening applications and other studies due to the ability to **combine** the **temporal resolution** of patch clamping with the **network resolution** of calcium imaging.
MEA provides similar connectivity data as calcium imaging but on a much larger scale, allowing for entire region analysis or potential analysis of several organoid regions.



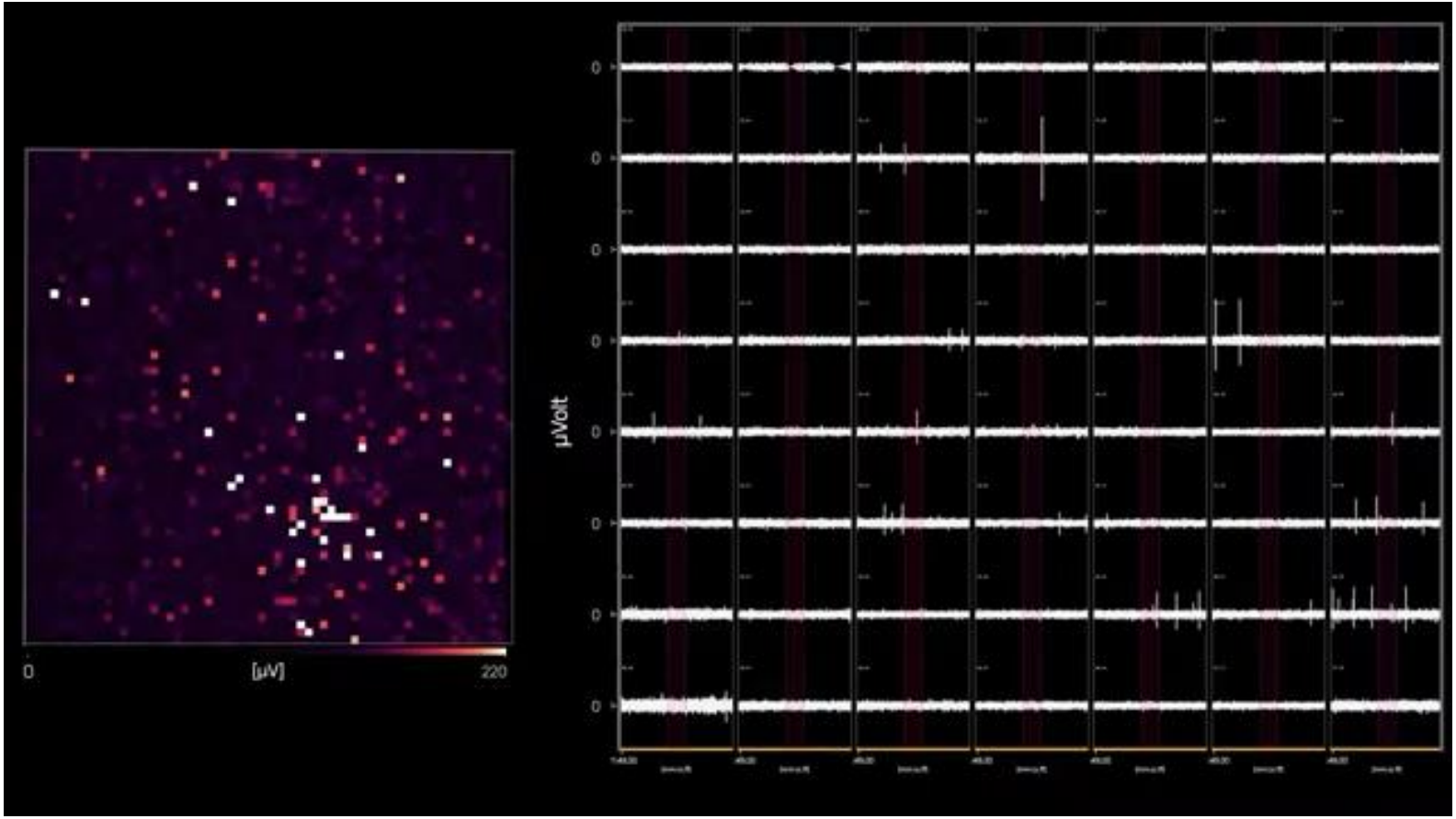
* Planar probes

Recordings from whole organoid or slices (see slicing procedure)
To note: the same organoid can be reused for several recordings over time

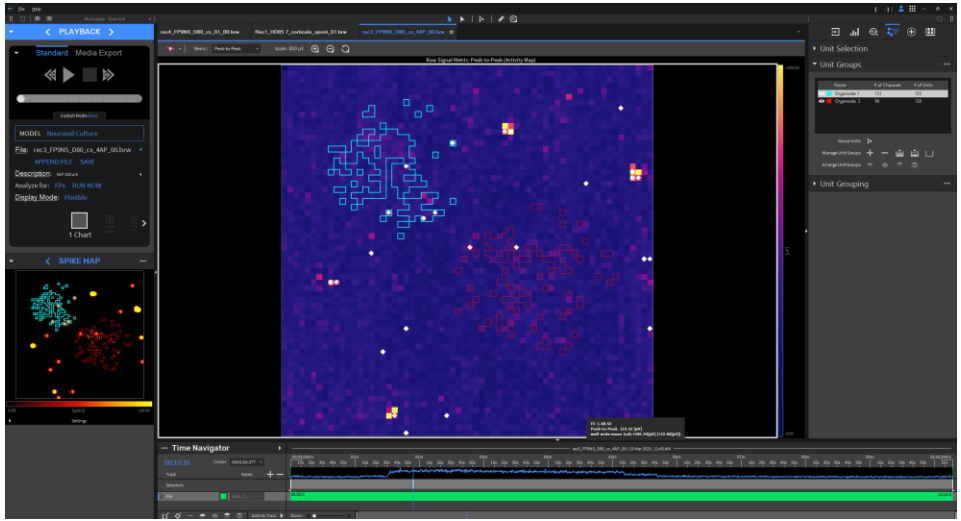


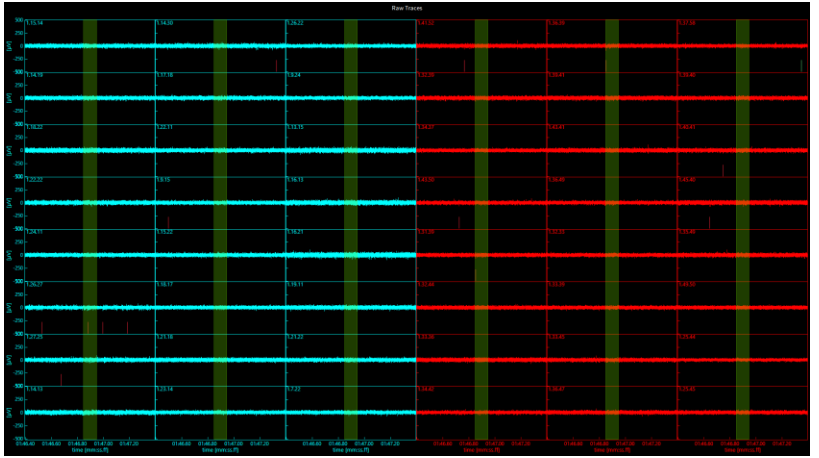
* 3D flexible probes

(Fair et al, 2020; Soscia et al, 2020)

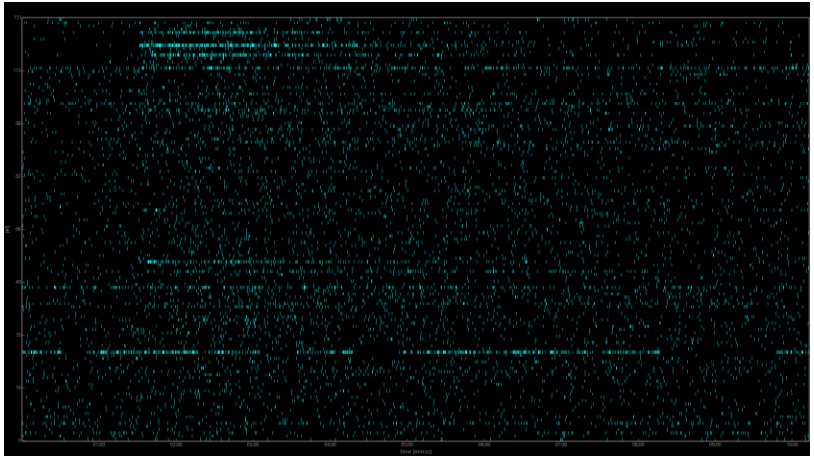

0
[µV]
220
µVolt
0
0
0
0
0
0
0
0

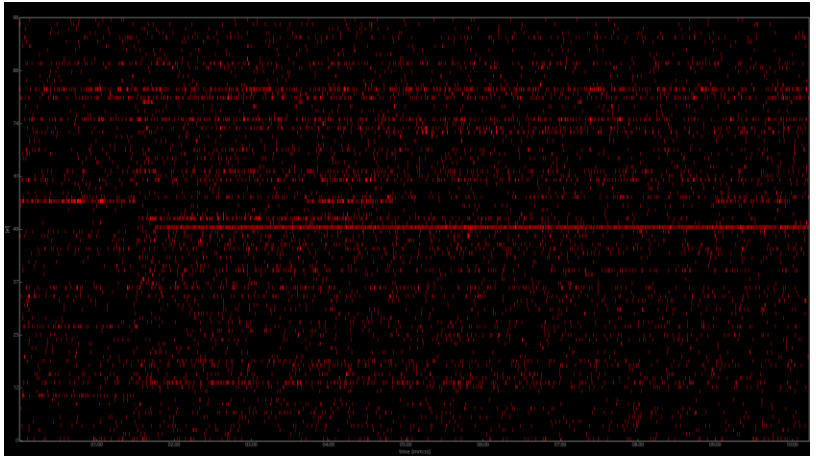
Raw Traces





Raster Plots





	Advantage	Disadvantage
Patch clamp	▪ High cellular resolution (single-cell) ▪ High temporal resolution	▪ Little-to-no information on network connectivity or dynamics (low spatial resolution) ▪ Patch clamp in organoids is hard and time consuming → do not indicate for large screening
Calcium imaging	▪ Medium spatial resolution (imaging of small groups of neurons) ▪ Suitable for screening	▪ Loss of temporal resolution ▪ The 3D configuration of organoids limits imaging of the entire tissue
Multi-electrode array	▪ High temporal resolution ▪ High spatial resolution (inter-regional connectivity) ▪ Suitable for screening	▪ Loss of cellular resolution ▪ The 3D configuration of organoids limits recordings to the outer edges

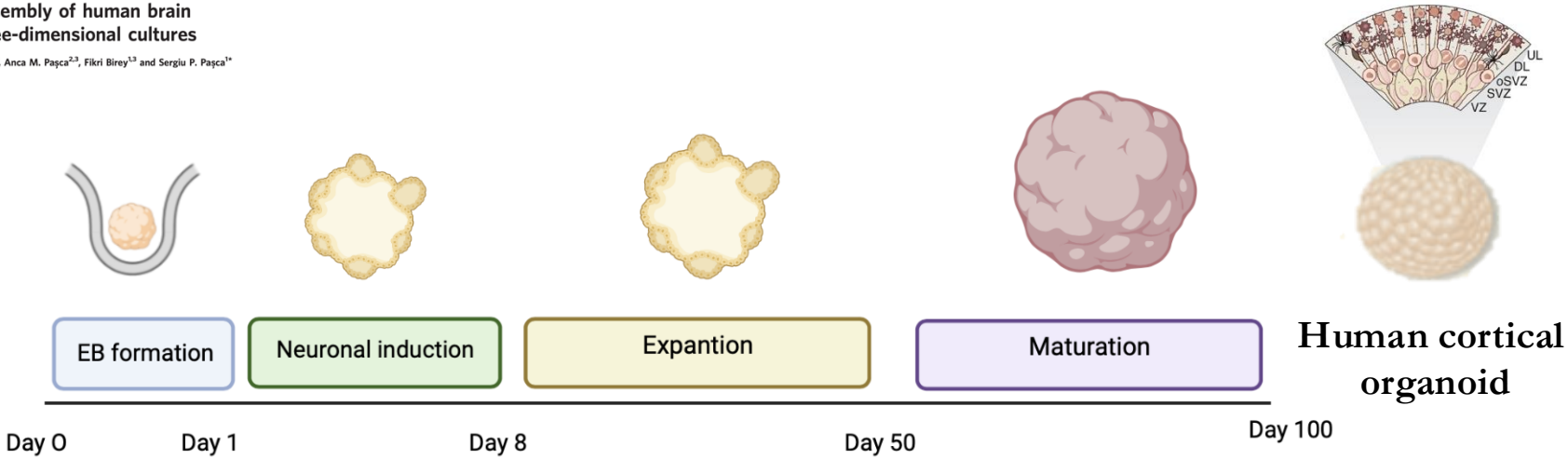
Transparency Matters: Tissue Clearing for Enhanced Cerebral Organoid Research

Generation protocol for human cortical organoids

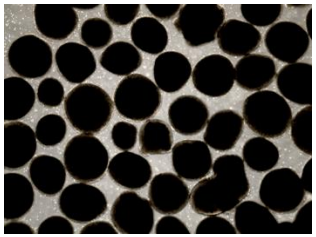
nature protocols PROTOCOL
https://doi.org/10.1038/s41596-018-0032-7

Generation and assembly of human brain region–specific three-dimensional cultures

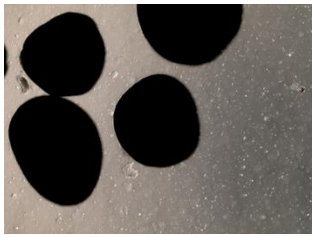
Steven A. Sloan[1,3], Jimena Andersen[1,3], Anca M. Paşca[2,3], Fikri Birey[1,3] and Sergiu P. Paşca[1*]



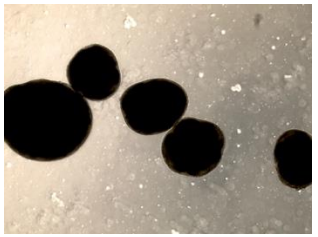
Human cortical organoid



Day 30

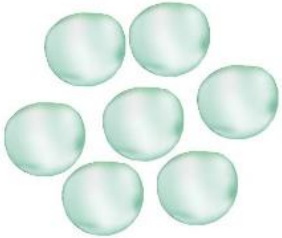


Day 50

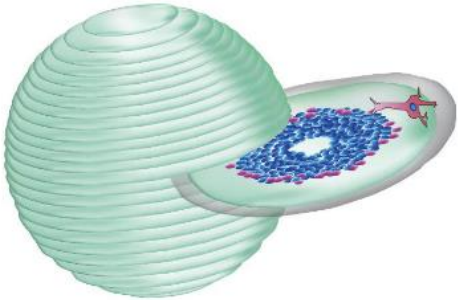


Day 100

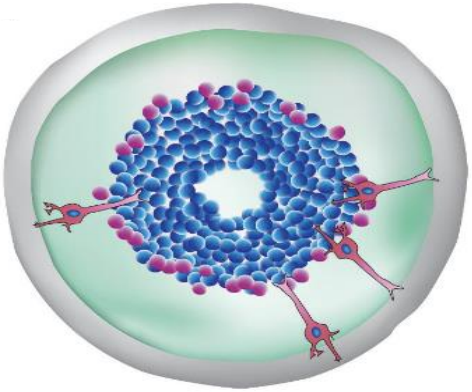
Counting cortical plates… on a slice…



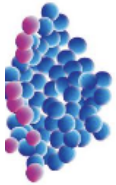
3D human brain organoids



Slicing of a human brain organoids



A single slice showing cytoarchitecture



Progenitor cells in a ventricular zone

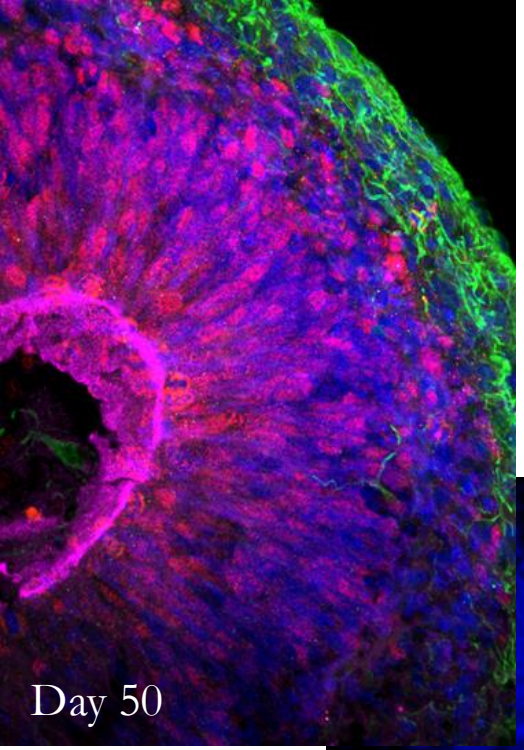
Inner sub-ventricular zone progenitors

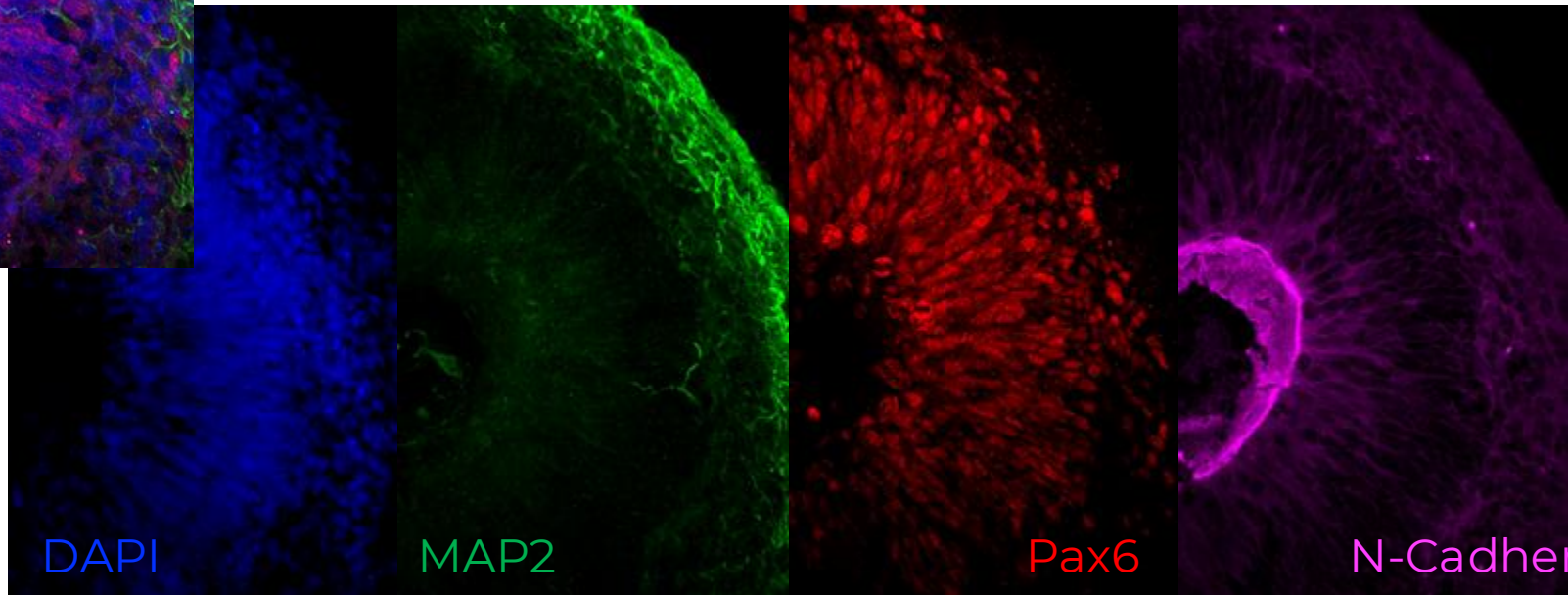
Outer sub-ventricular zone progenitors

Primitive cortical plate

Neurons

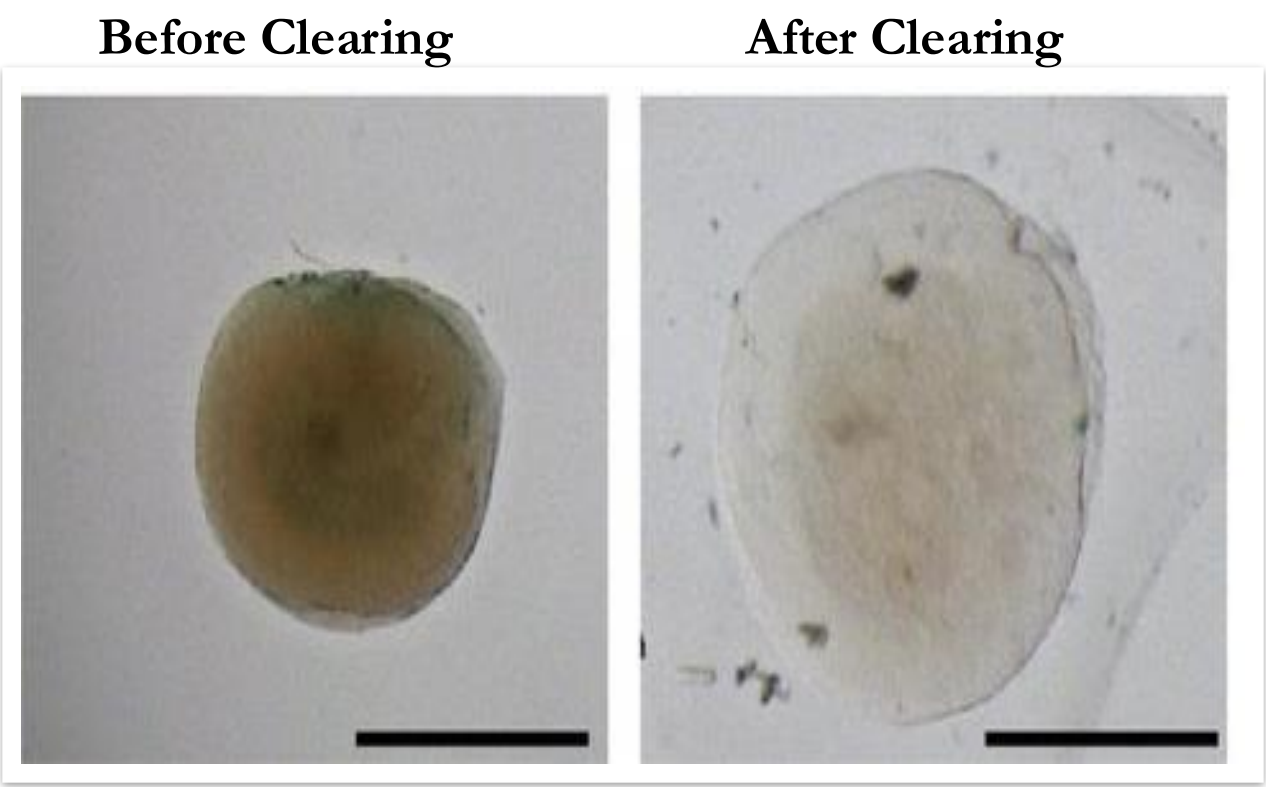
Counting cortical plates… on a slice…





40x

Counting cortical plates… on the entire organoid



Transparency = homogenizing the refractive index (RI)

Imaging the whole brain organoids while keeping them intact with their details.

http://dx.doi.org/10.3390/cells8050391

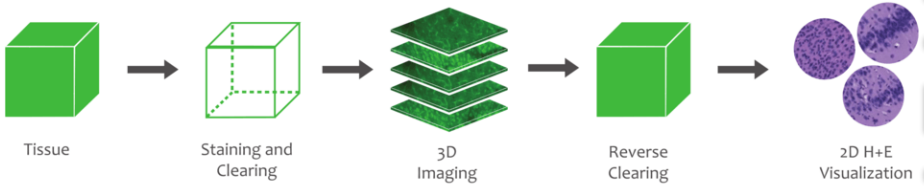
Clearing Approaches

Visikol

RI 1.48

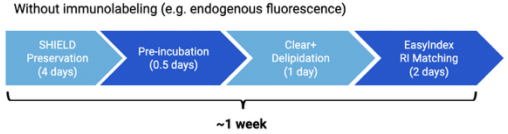
Getting the most out of Visikol HISTO depends on your labeling method.

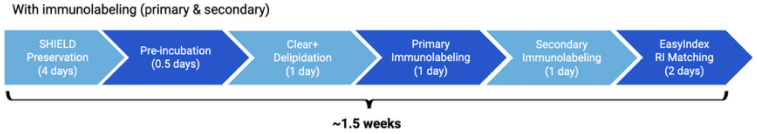
Fluorescent Protein	Nuclear & Viability Stains
Unless combined with immunolabeling, there is no need to perform permeabilitzation or labeling steps.	
Proceed directly to clearing and perform dehydration with ethanol at 4°C (see reverse side).	Apply stain as directed by manufacturer, fix tissue, and proceed directly to dehydration and clearing (see reverse side).

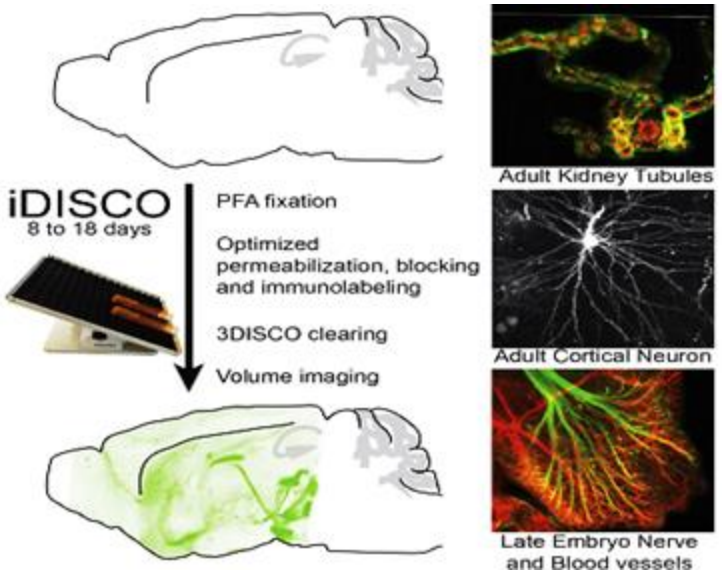


iDISCO

RI 1.55







VISIKOL

organic solvent-based tissue clearing techniques

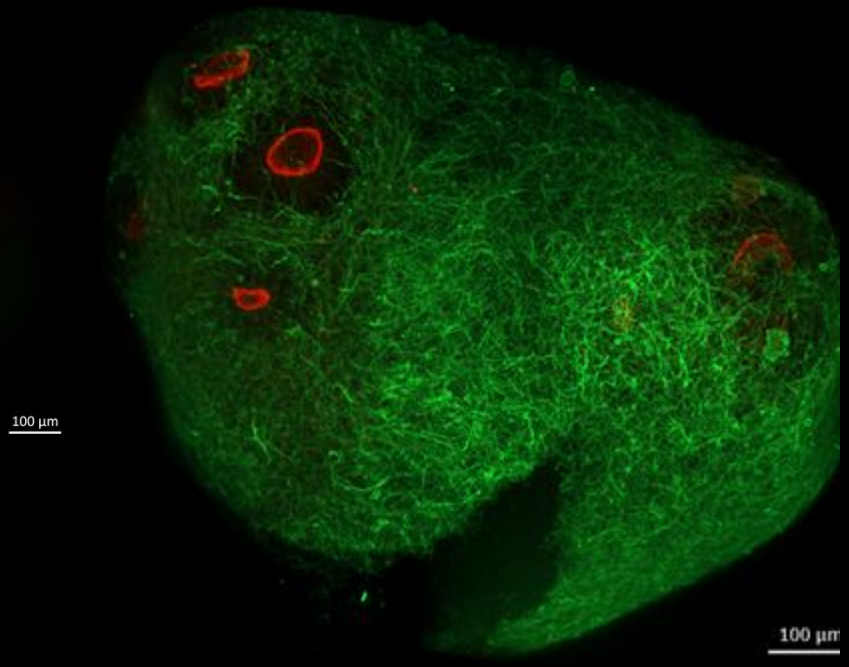
Cell Resource

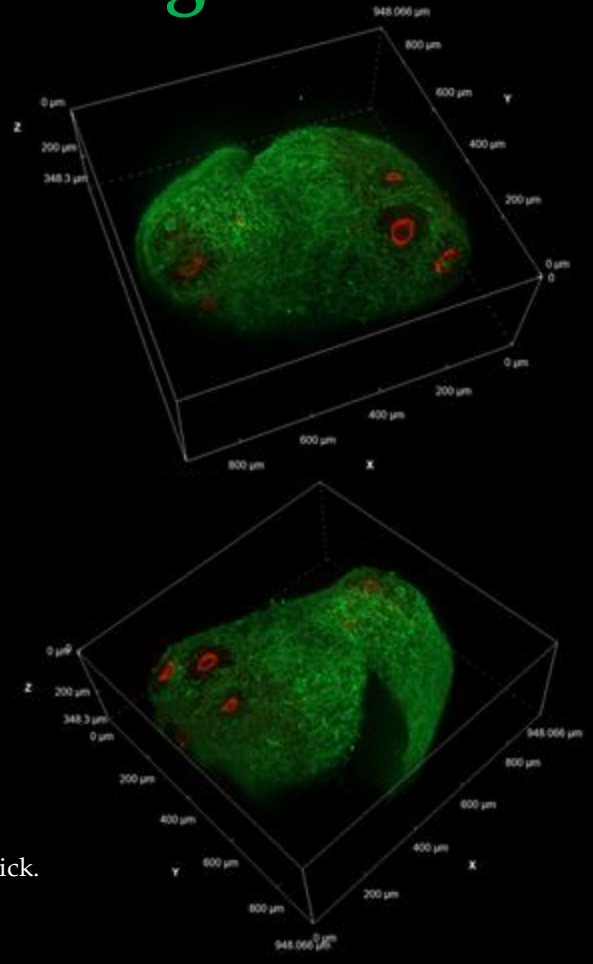
iDISCO: A Simple, Rapid Method to Immunolabel Large Tissue Samples for Volume Imaging

Counting cortical plates… on the whole organoid…

Visikol

MAP2 – N-cadherin

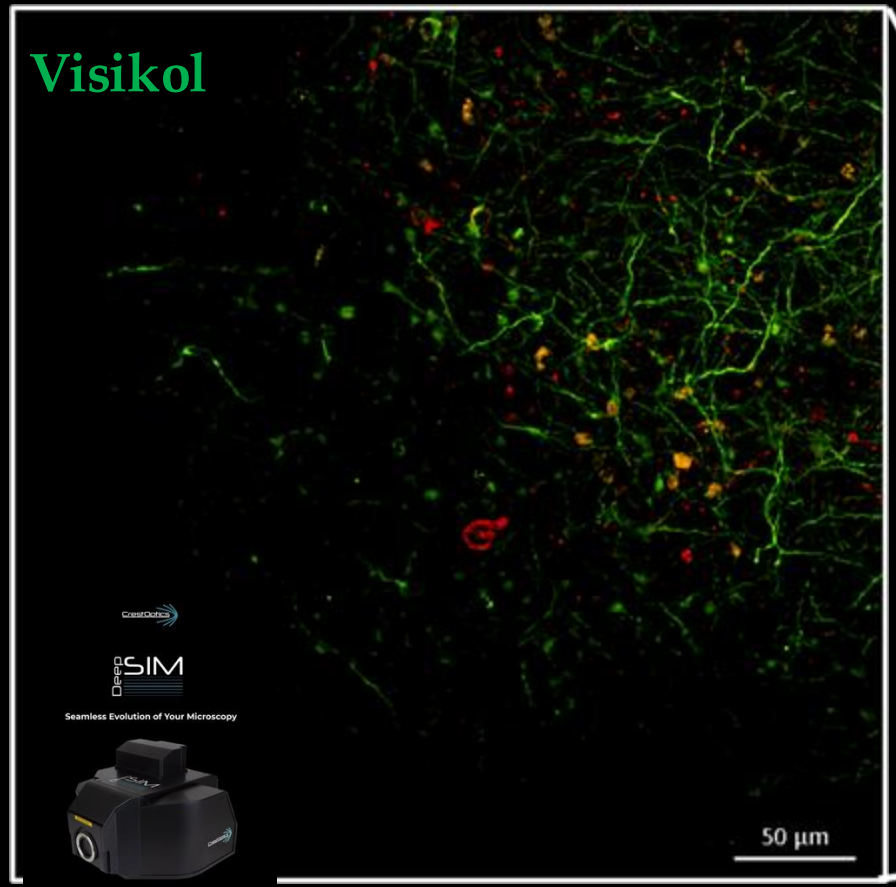




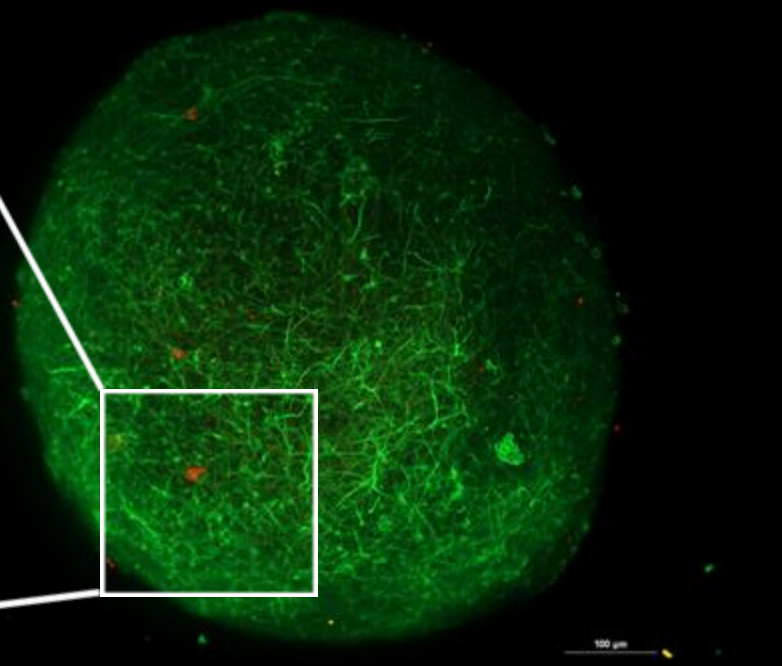
3D volume view 1mm thick.

PlanApo λD 20x NA 0.8; WD 800 µm

Analyzing neuronal morphology into the whole organoid…



MAP2 – PAX6 – N-cadherin

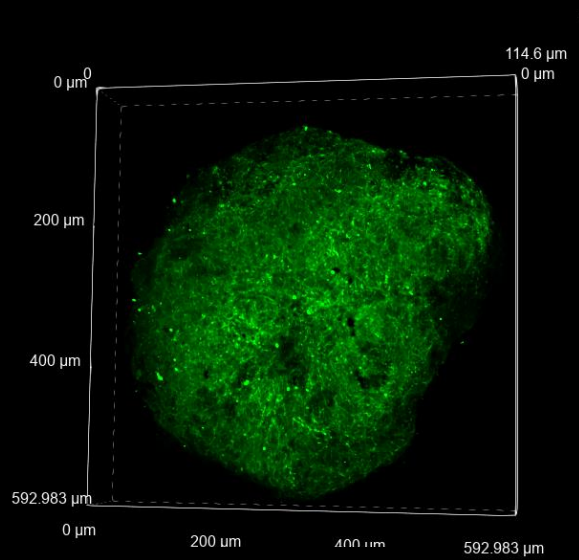


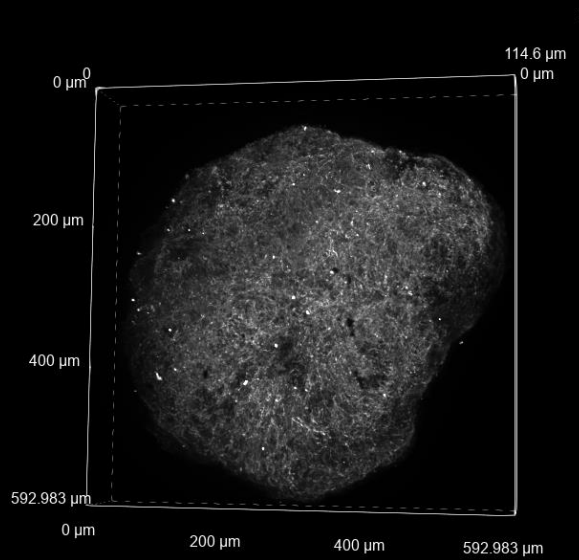
PlanApo λD 60x oil NA 1.42; WD 0.15 µm

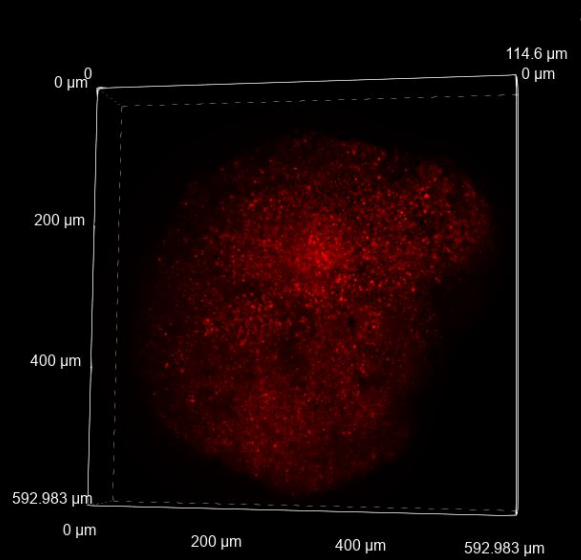
Looking at different cell types… on the whole organoid

iDISCO

HT-7 - GFAP - TUJI



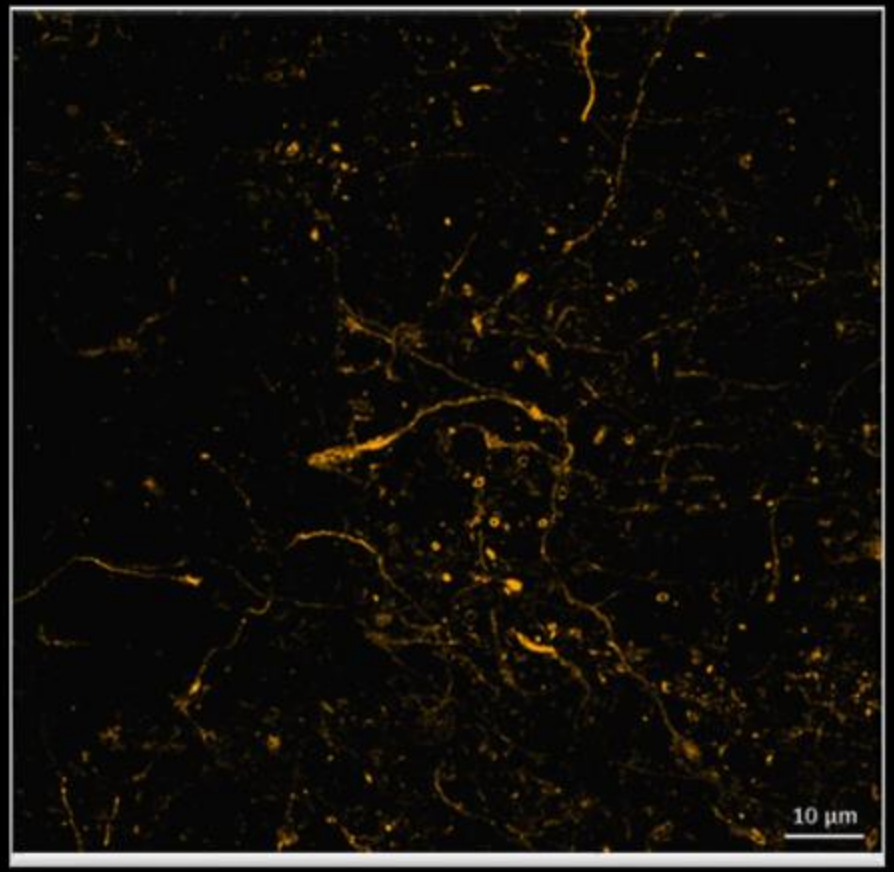




PlanApo λD 20x NA 0.8; WD 800 µm

Analyzing glial morphology into the whole organoid…

iDISCO



GFAP

PlanApo λD 60x oil NA 1.42; WD 0.15 µm

Pros and Cons of Brain Organoids Clearing

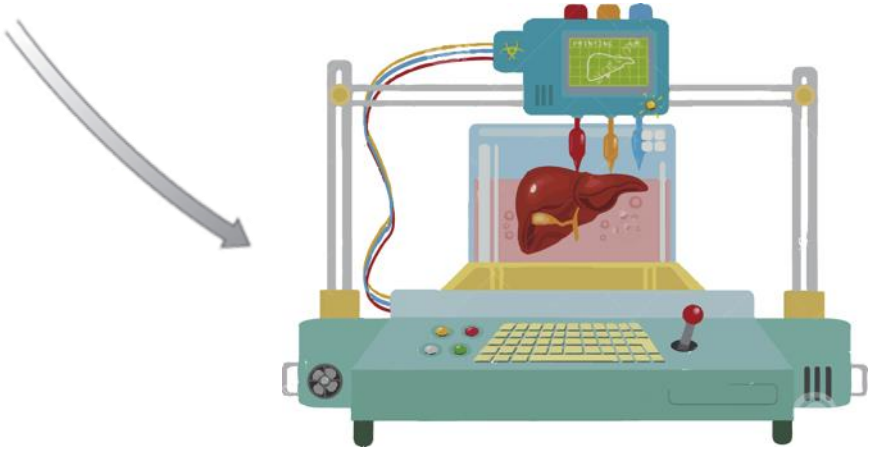
Pros	Cons
1.Improved Visualization	1.Complexity of Techniques
2.Preservation of 3D Architecture	2.Sample Size Limitations
3.Multi-Modal Imaging	3.Tissue Integrity
4.Quantitative Analysis	4.Costs
5.Reduced Sample Destruction	5. Data Handling

Take Home Message:

Incorporating tissue clearing techniques empowers organoid researchers to delve deeper, preserving intricate 3D structures and enabling enhanced visualization and analysis, although it requires careful consideration of technical challenges and limitations."

3D BIOPRINTED CONSTRUCTS

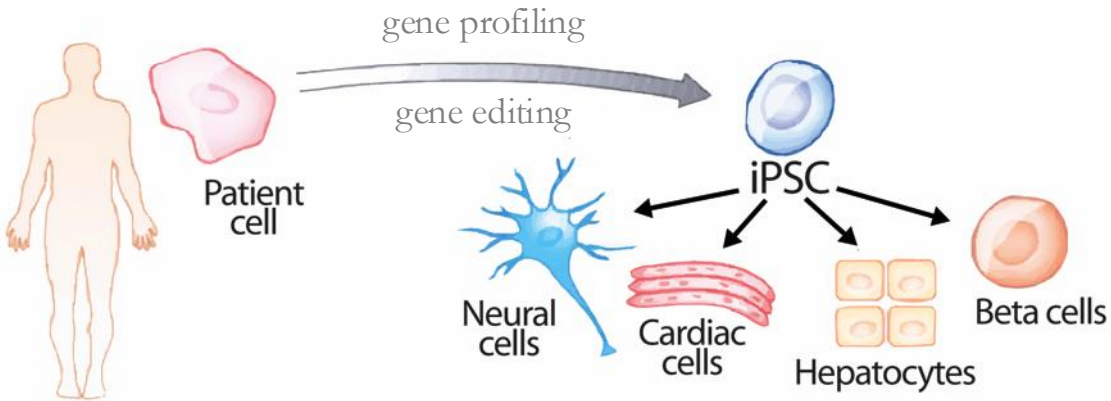
Induced Pluripotent
Stem Cells (iPSCs)



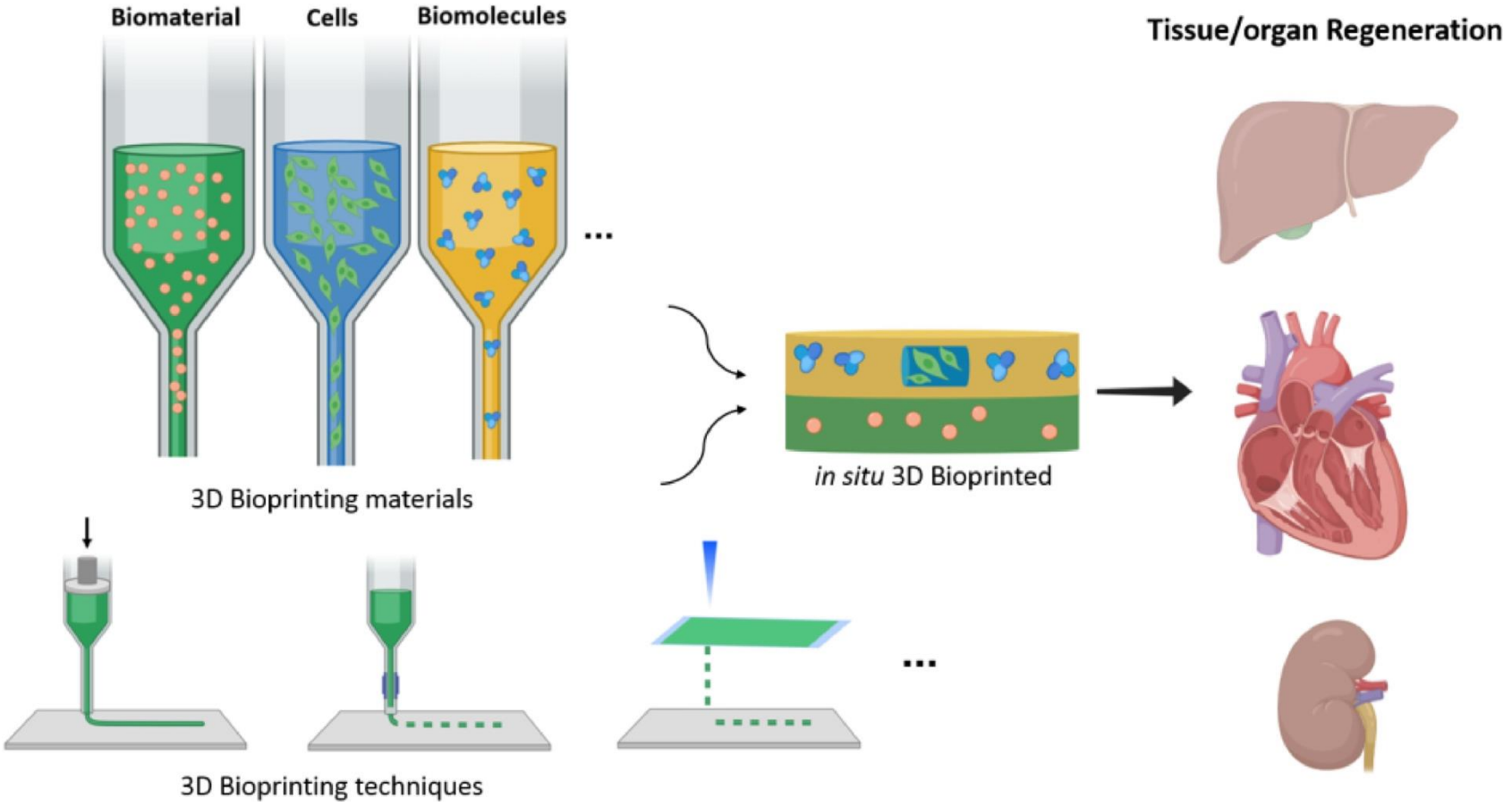
3D Bio-fabrication

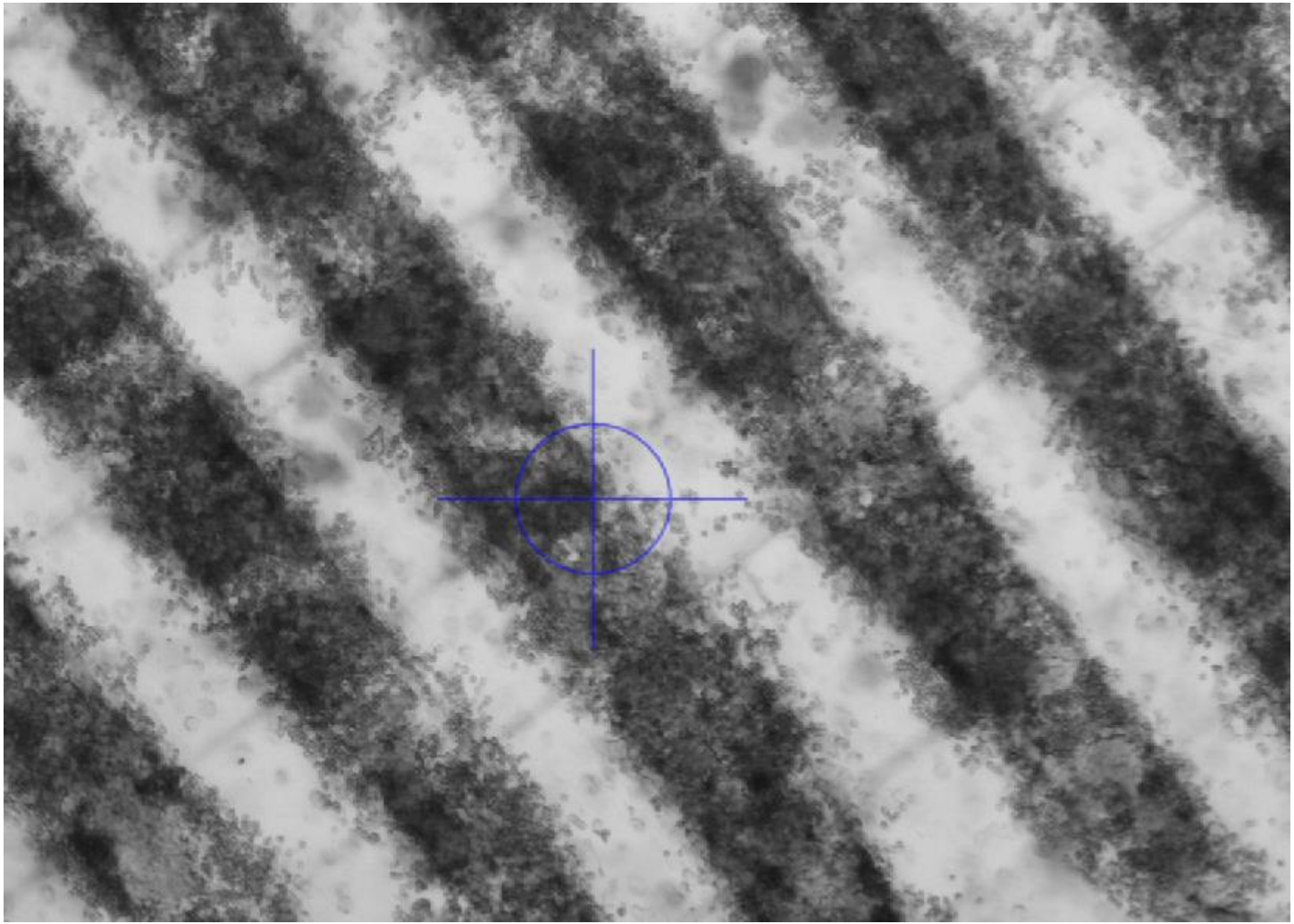
Bricks

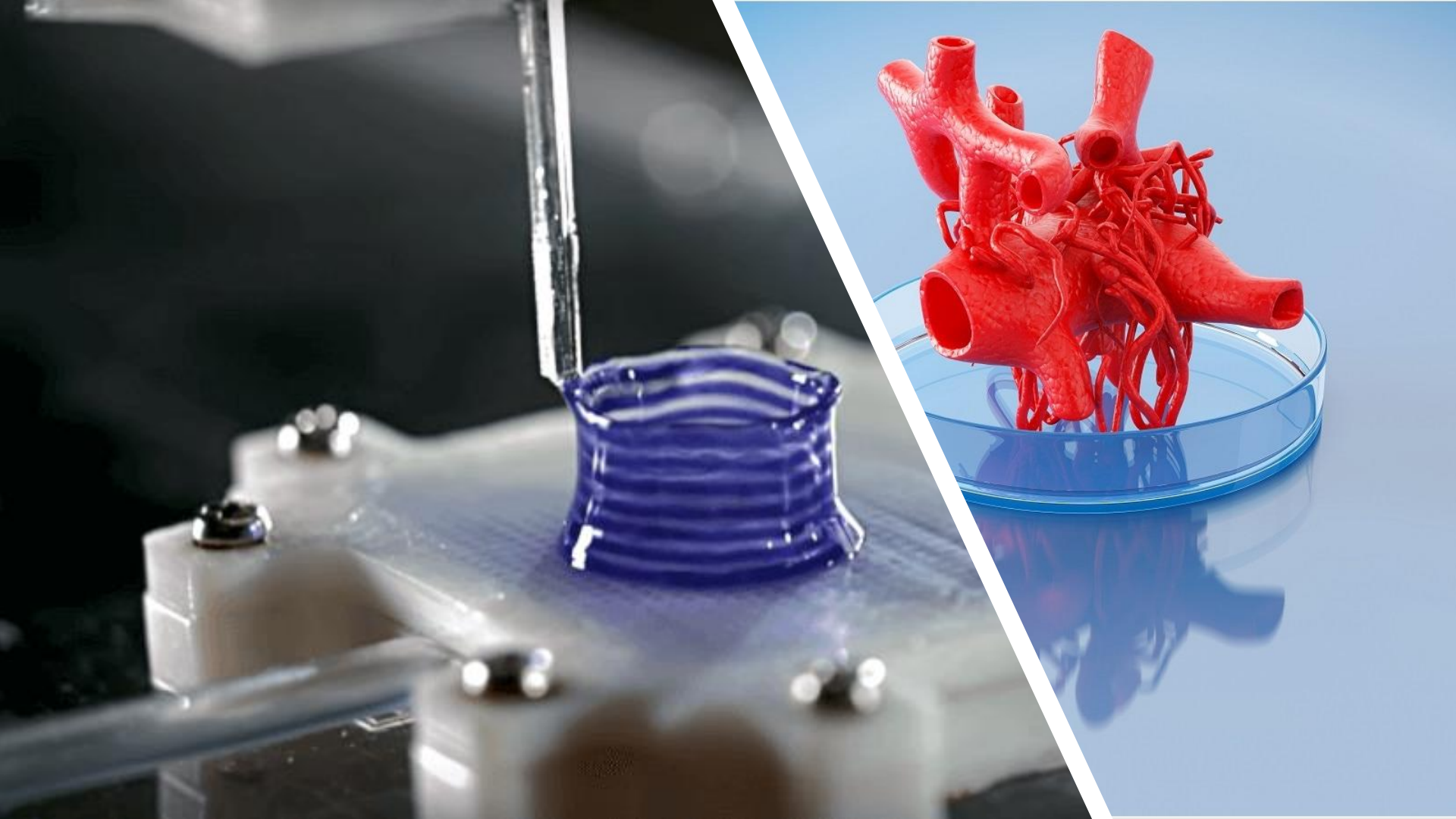
Induced Pluripotent Stem Cells (iPSCs)

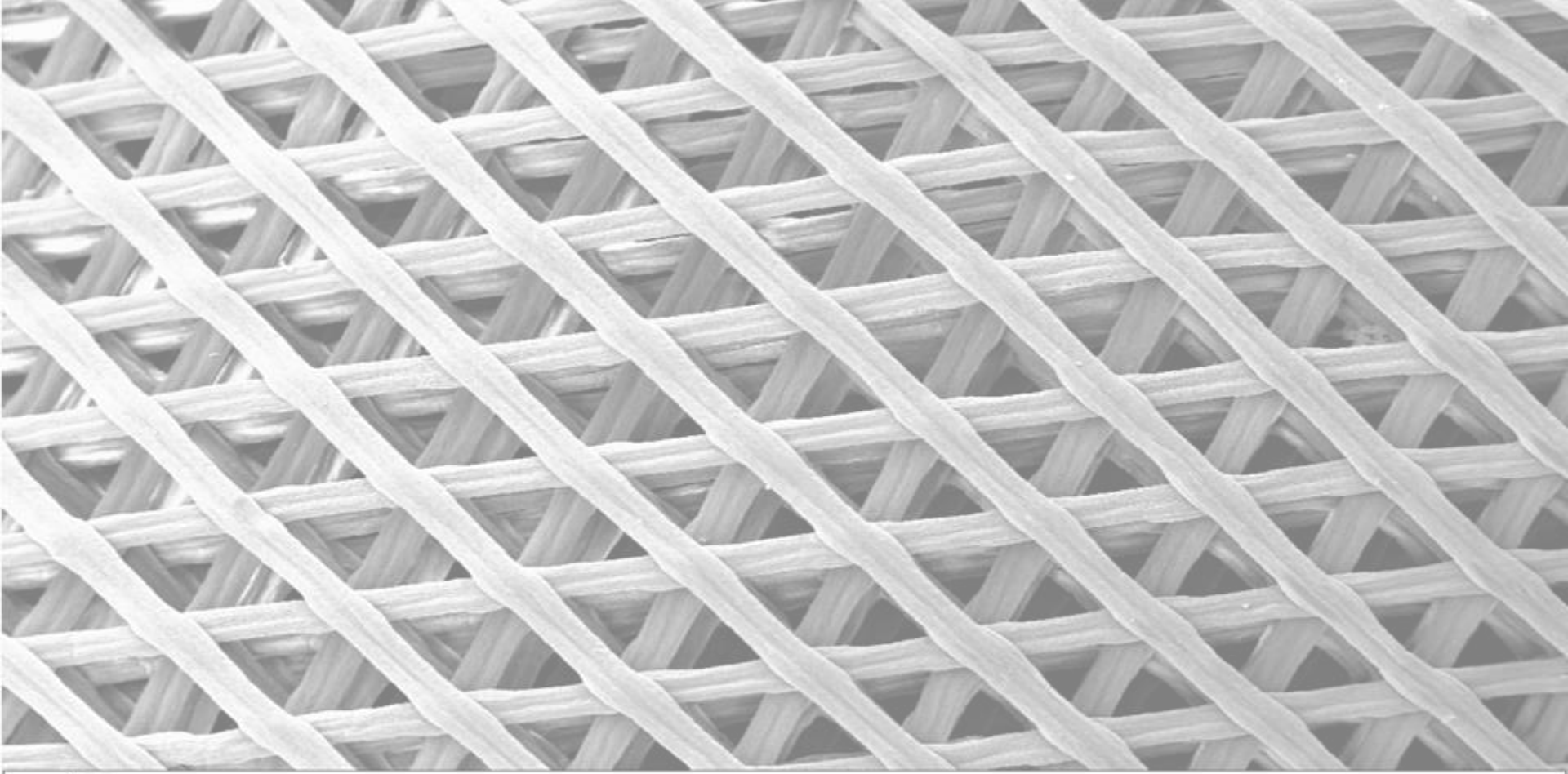


New tools- bricks



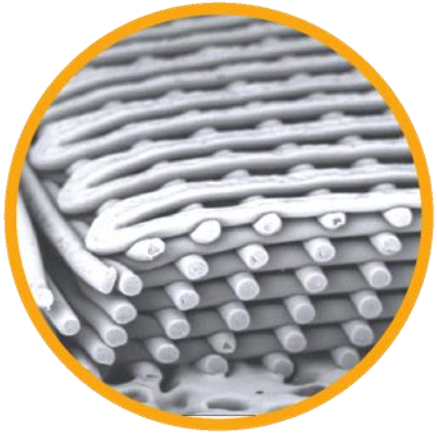




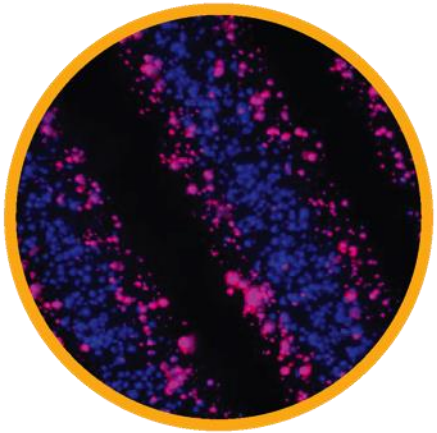

100µm
Mag = 50 X
WD = 12 mm
Spot Size = 279
Brightness = 49.5 %
Contrast = 36.2 %
EHT = 20.00 kV
Signal A = SE1

3D Bioprinted Constructs

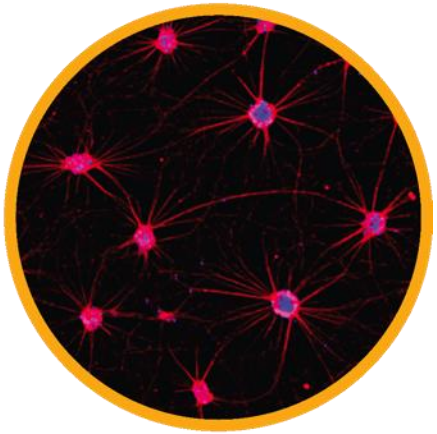
consistent



three-dimensional



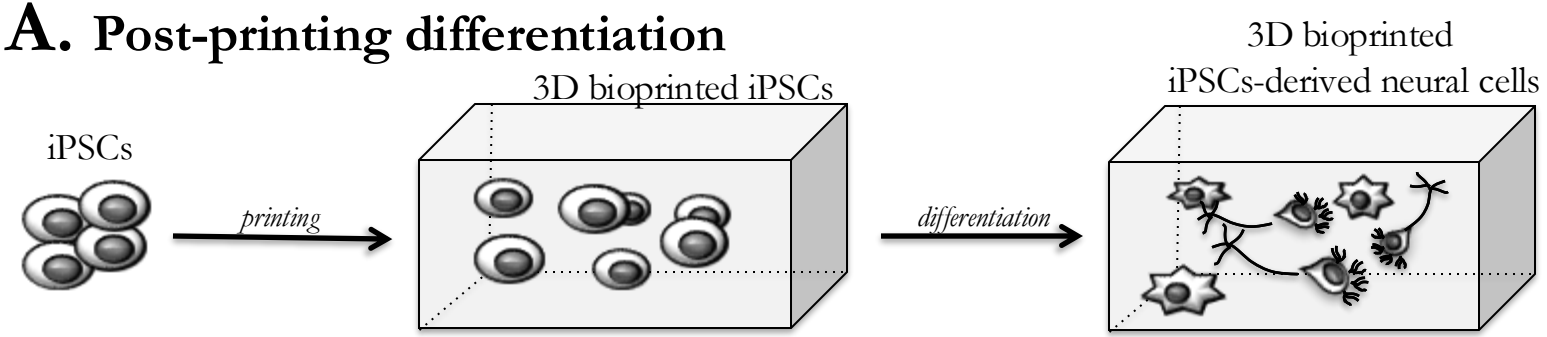
versatile



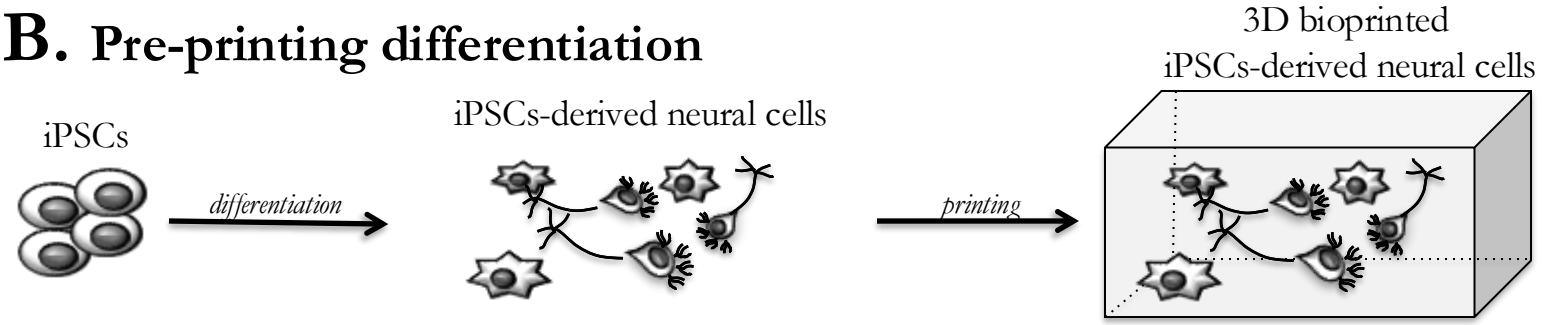
functional

iPSCs Printing Strategies

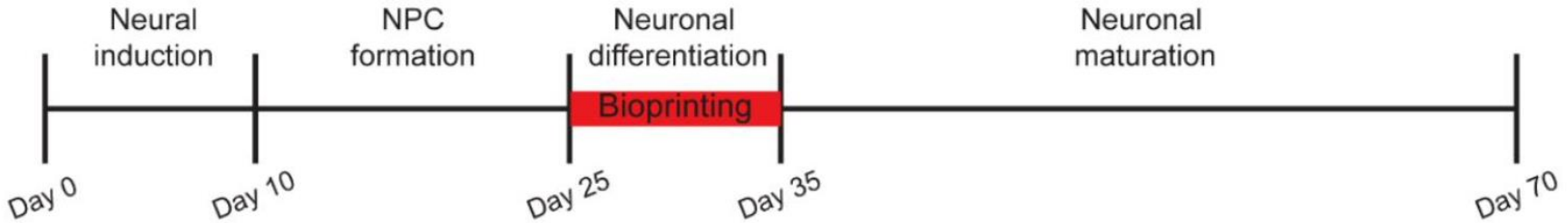
A. Post-printing differentiation

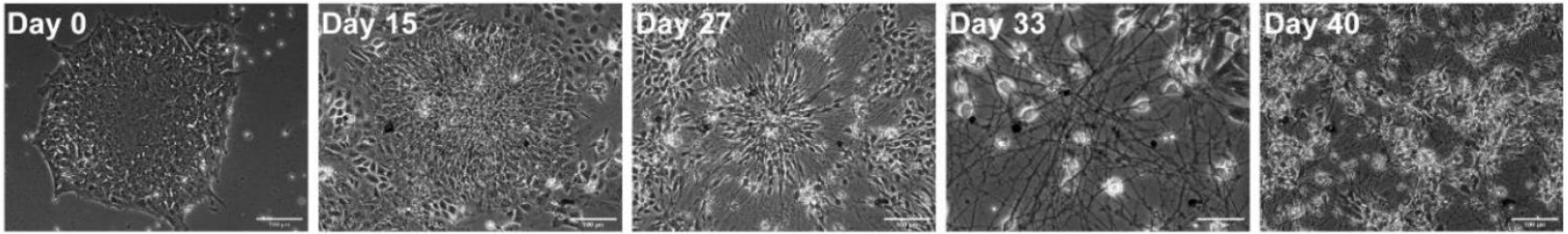


B. Pre-printing differentiation

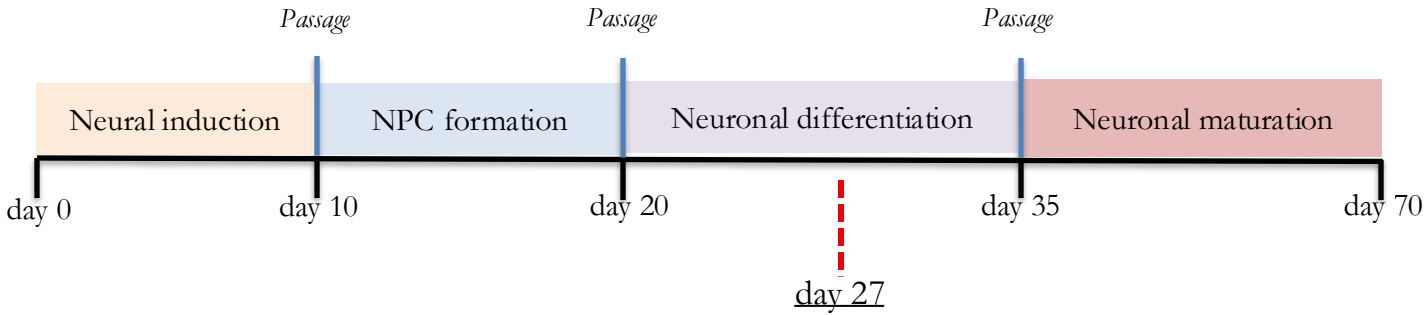


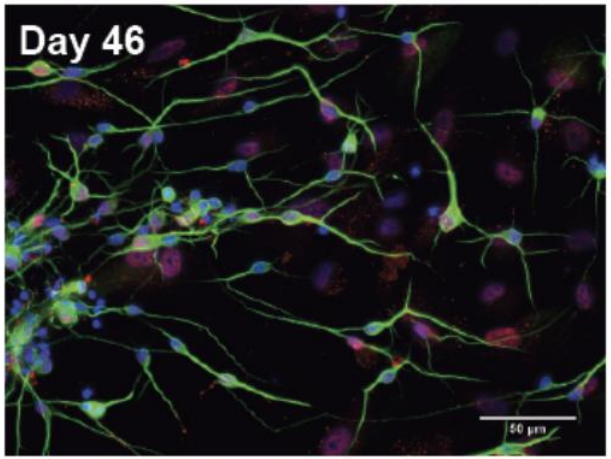
3D bioprinting of differentiating cortical neurons



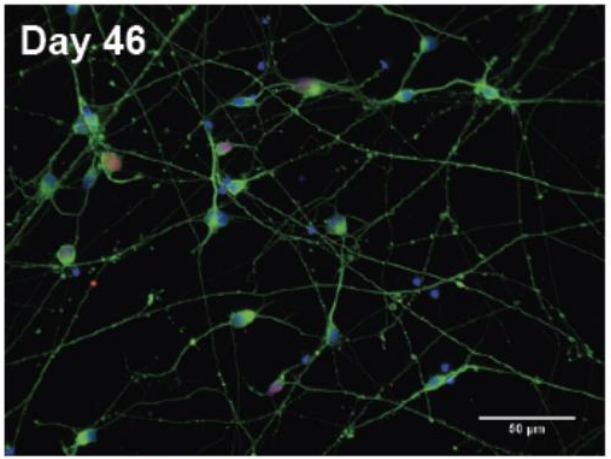


Imaging of differentiating cortical neurons

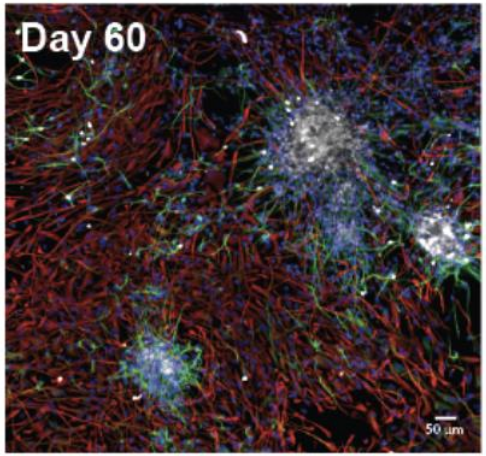




MAP2 NeuN DAPI

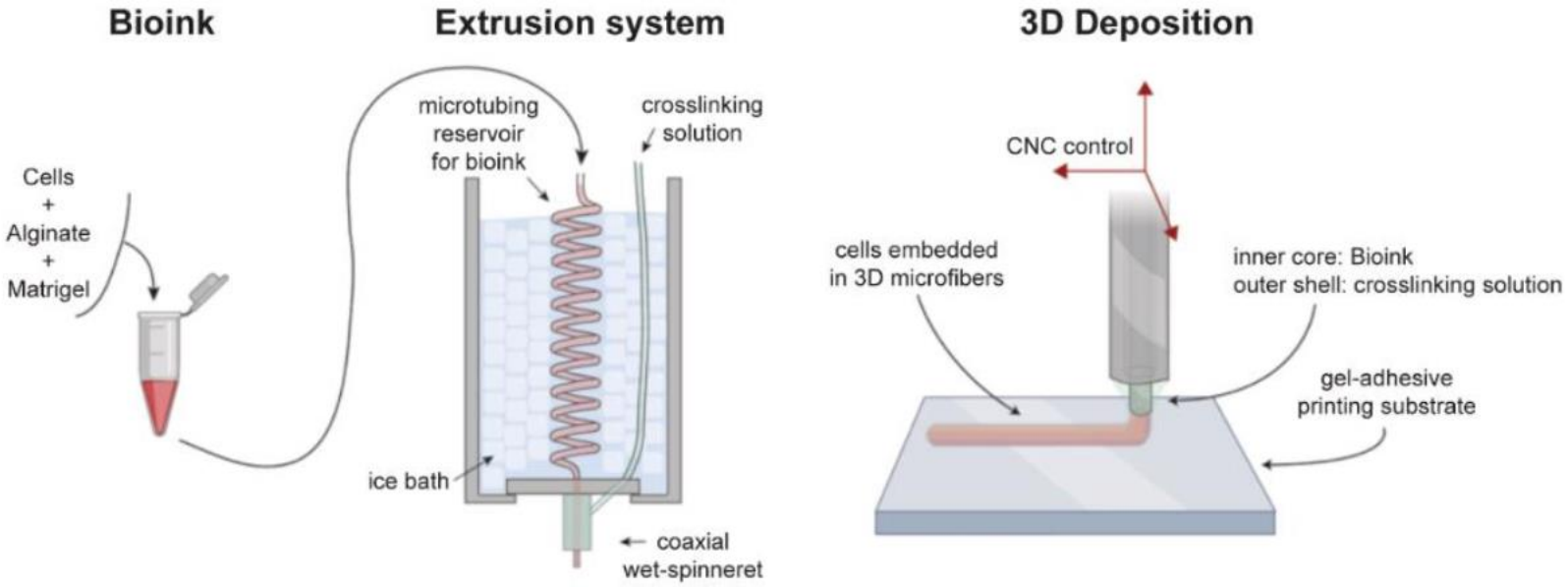


TUJ1 PAX6 DAPI

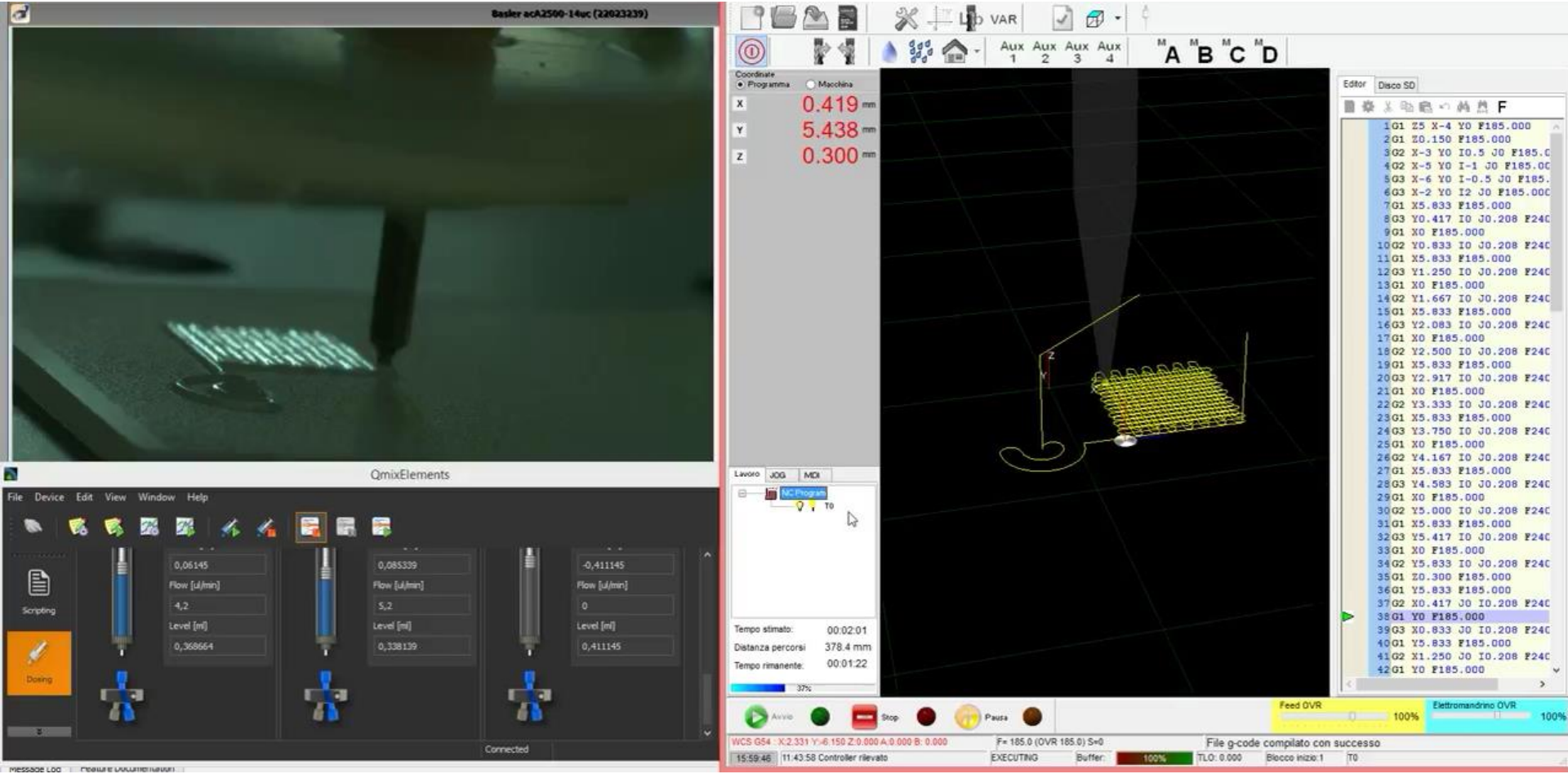


MAP2 GFAP TBR1 DAPI

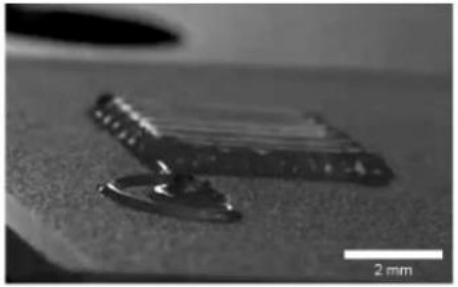
3D bioprinting of differentiating cortical neurons



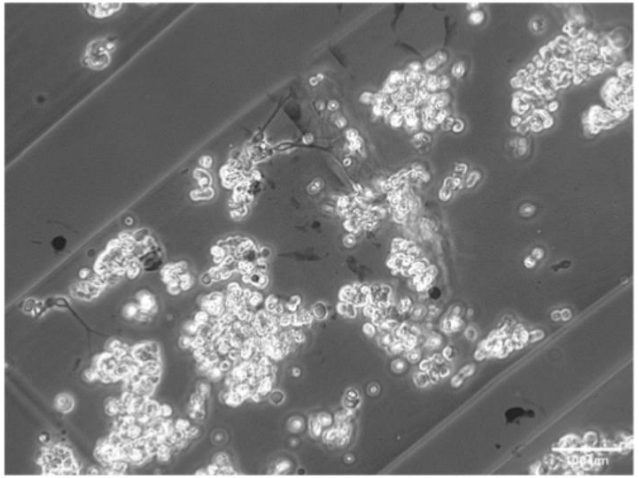
3D bioprinting of differentiating cortical neurons



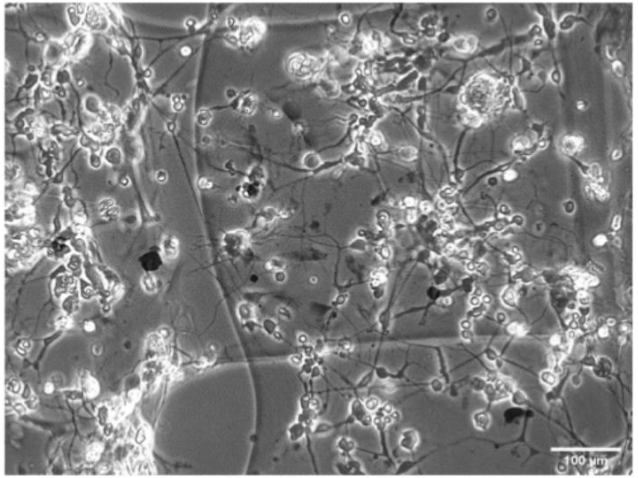
3D bioprinting of differentiating cortical neurons



- Alginate-lyase

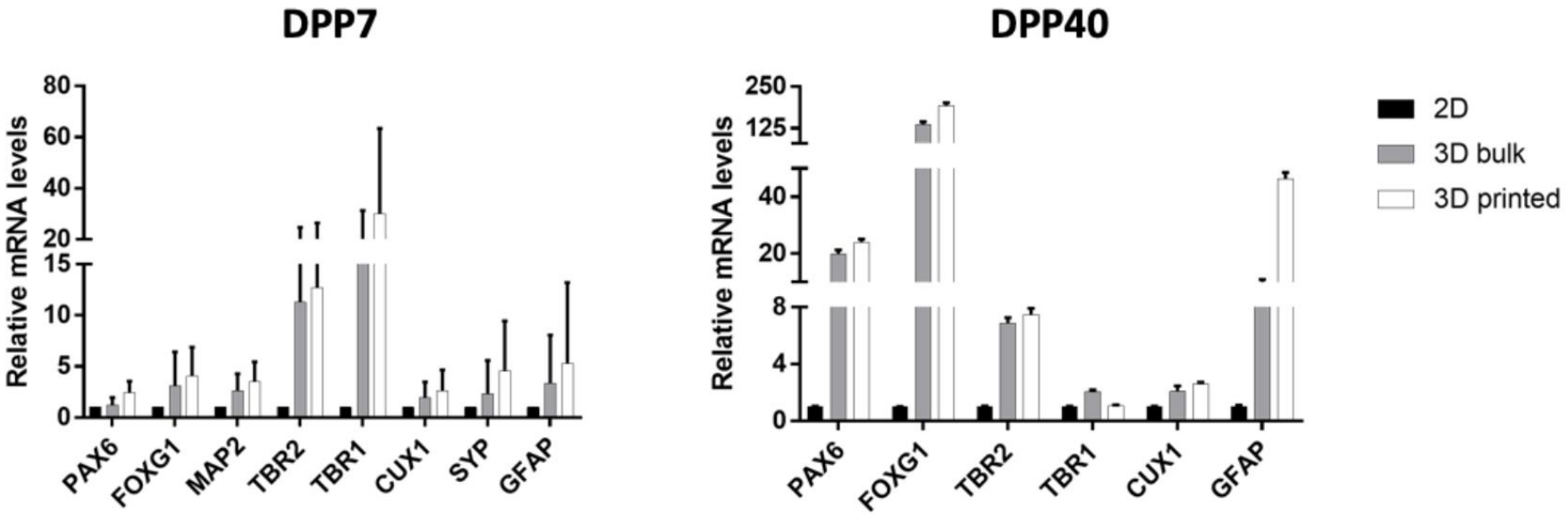


+ Alginate-lyase

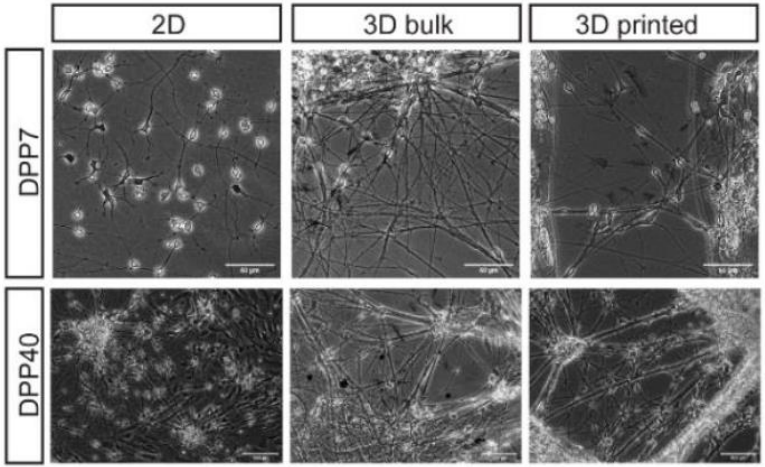


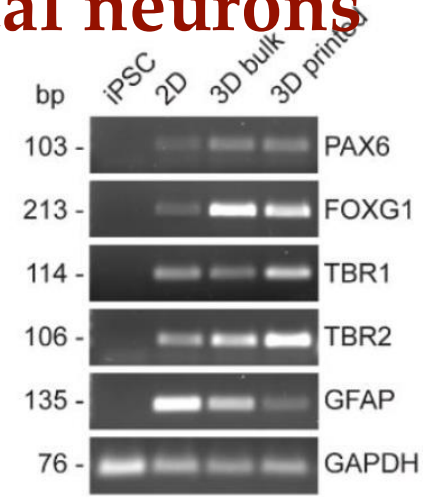
3D Neural Network Maturation

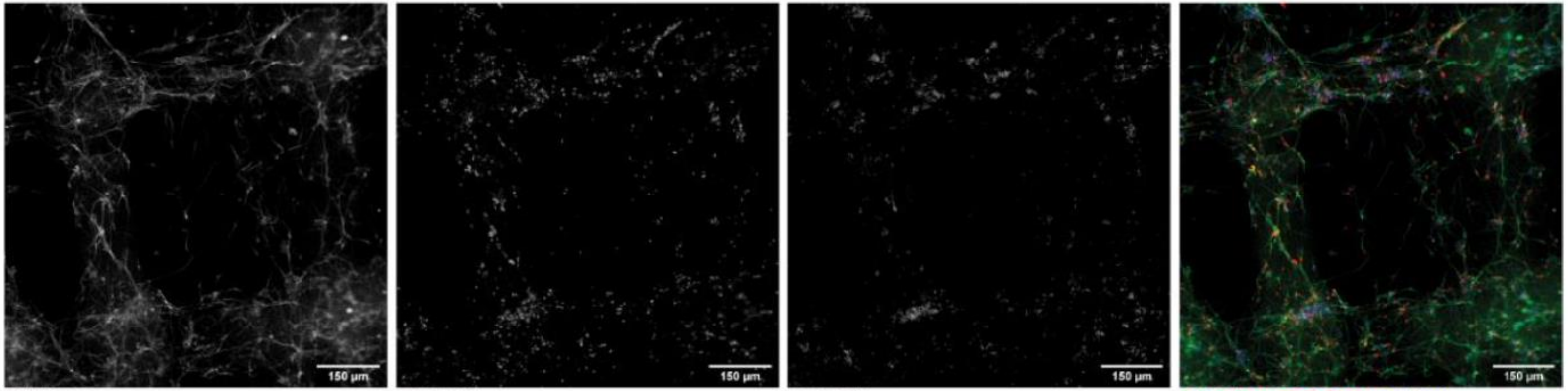
Gene expression



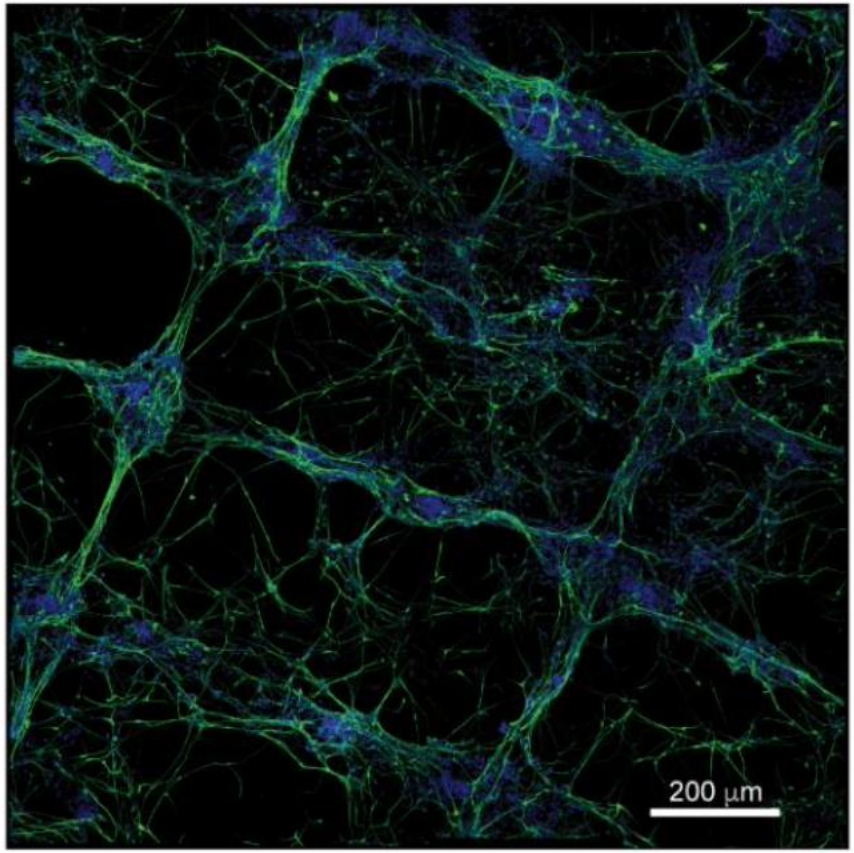
Imaging of 3D bioprinted cortical neurons



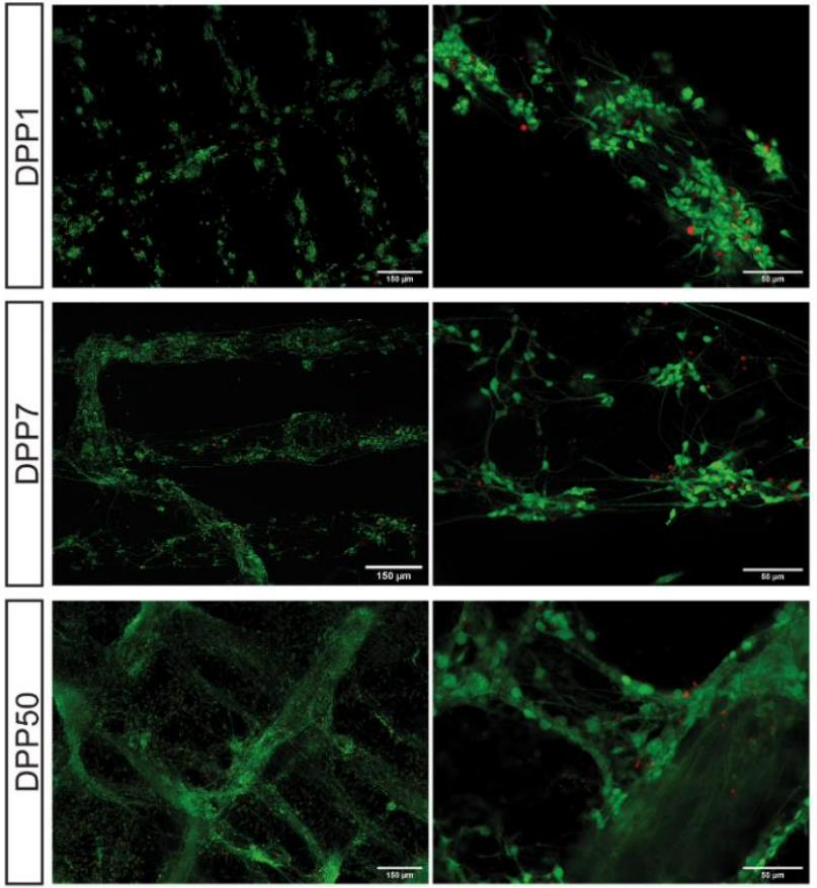




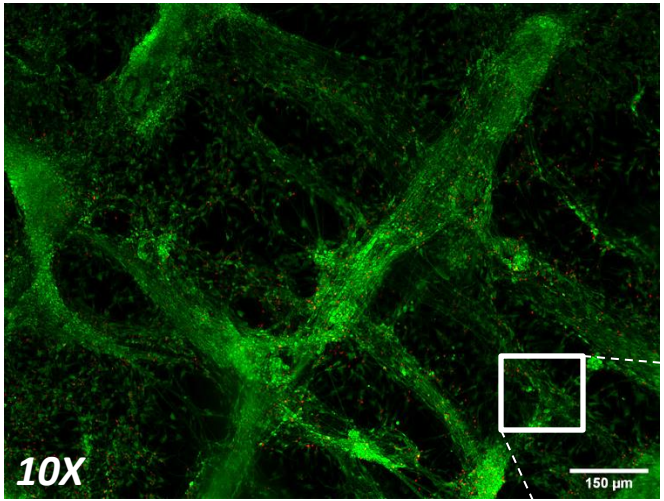
Imaging of 3D bioprinted cortical neurons



MAP2 DAPI

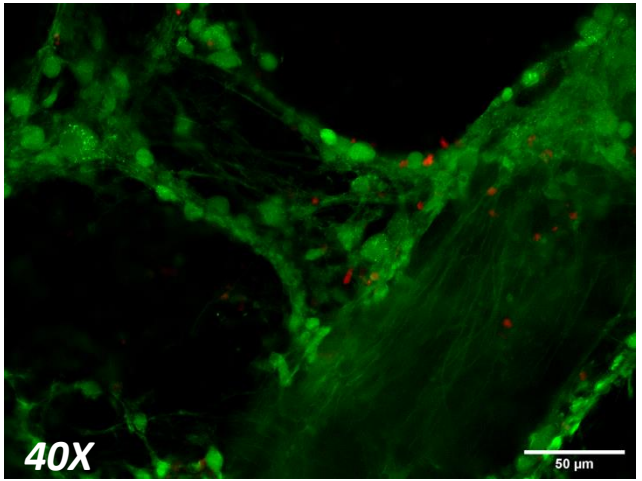


LIVE DEAD

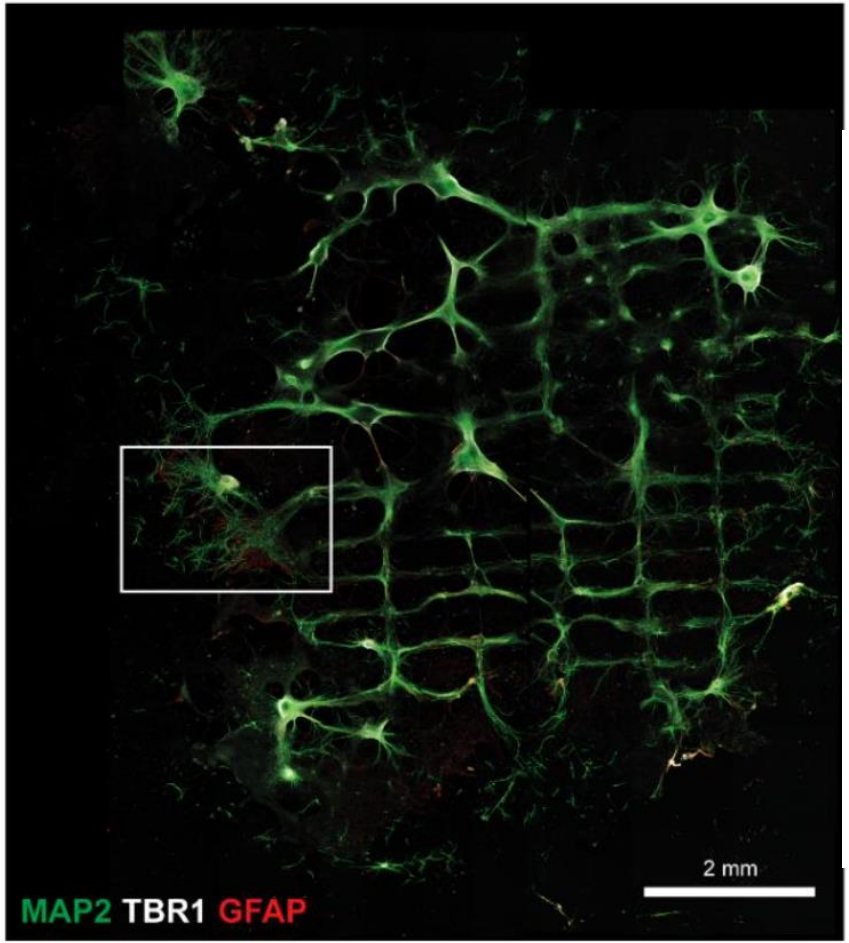


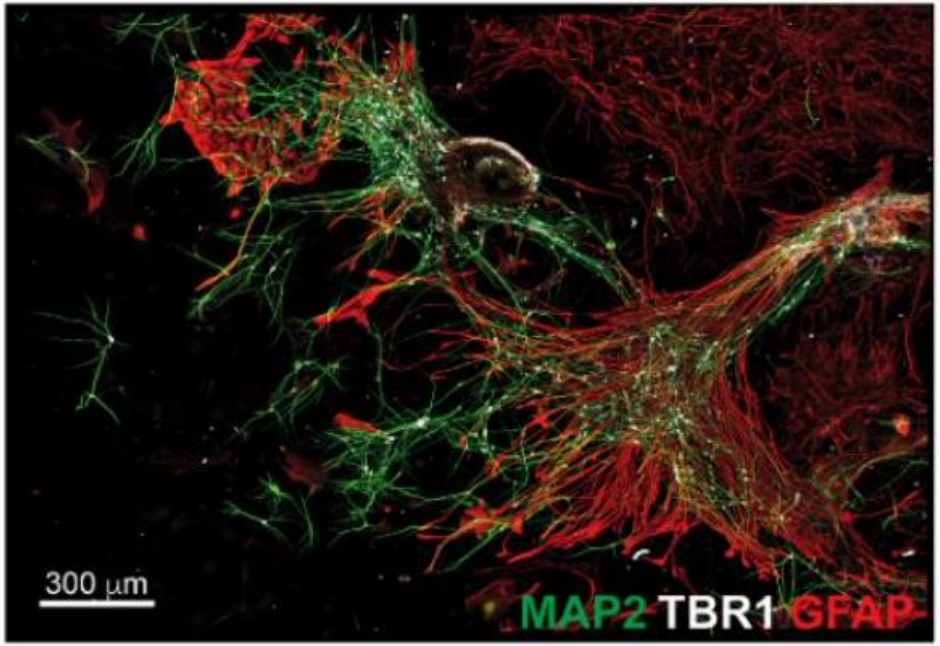
LIVE

DEAD



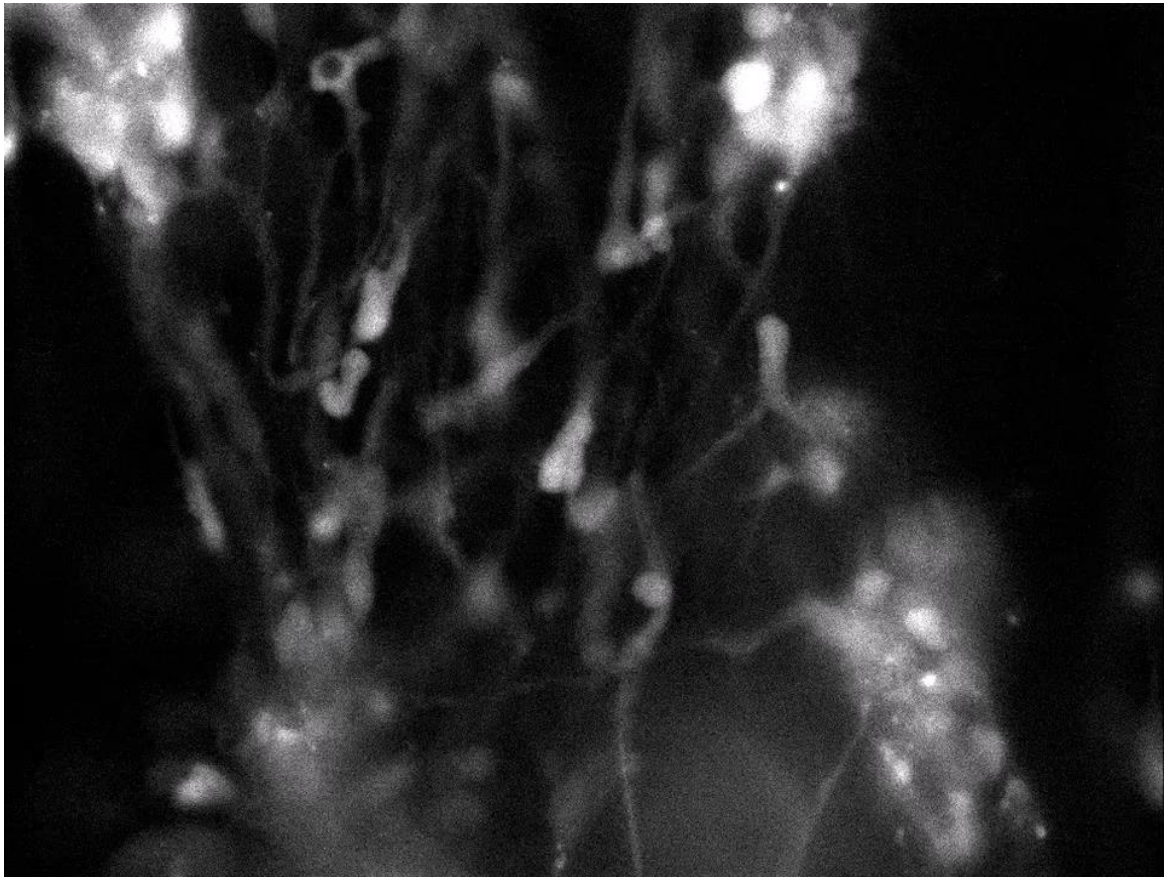
Imaging of 3D bioprinted cortical neurons

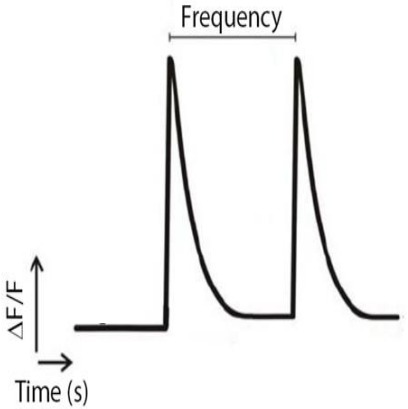




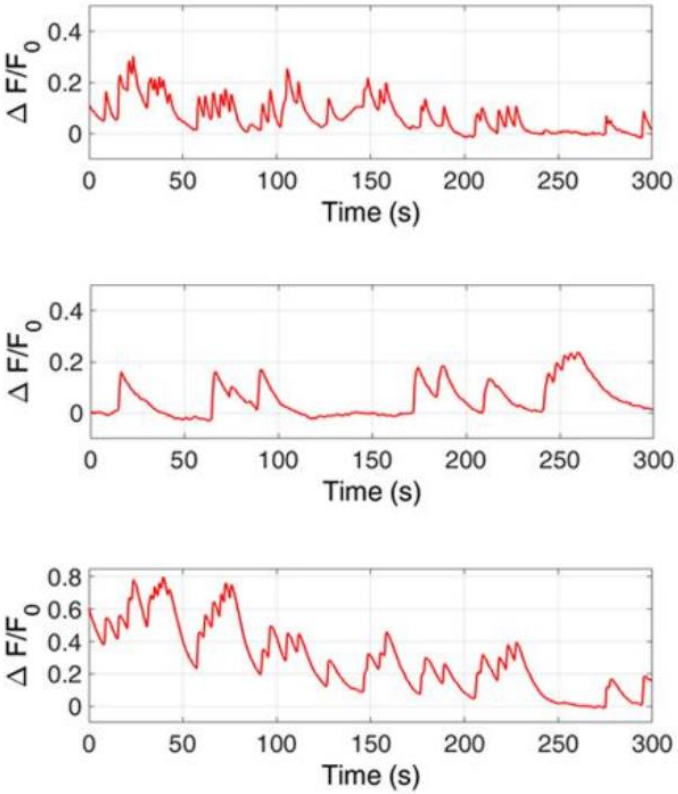
ASTROCYTES!

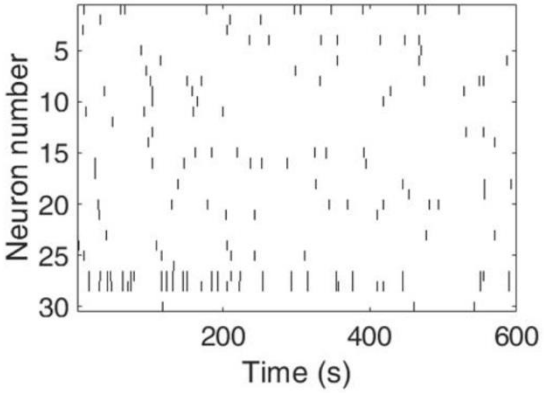
Calcium Imaging of 3D bioprinted cortical neurons

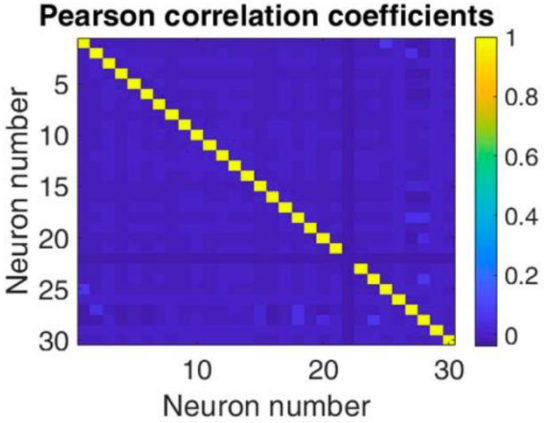




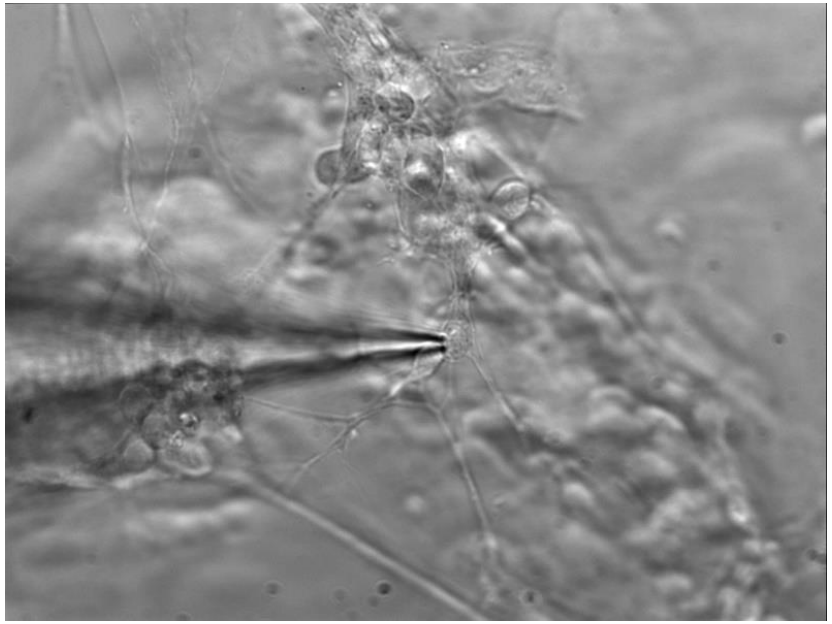
Calcium Imaging of 3D bioprinted cortical neurons

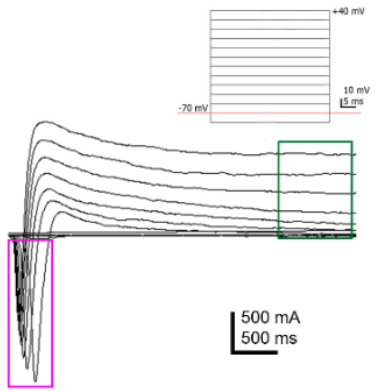


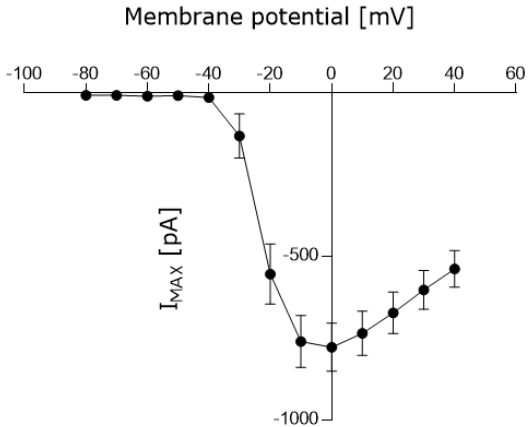


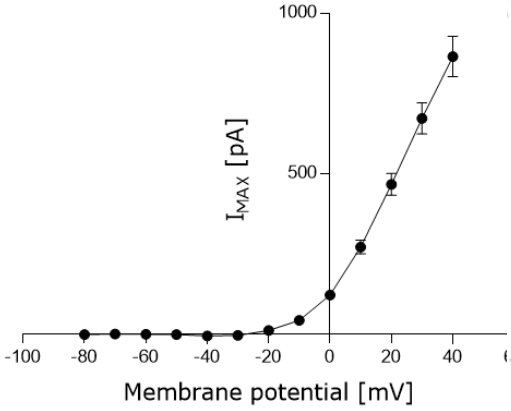


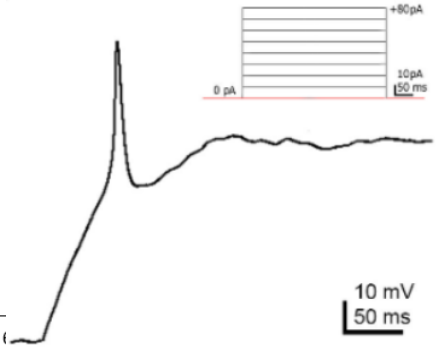
Patch clamp recording of 3D bioprinted cortical neurons











TheANGEL: The Amazing Neuro Glia Electrophysiology Lab

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