

Reliable identification and quantification of neural cells in microscopic images of neurospheres



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Abstract

Neurosphere cultures consisting of primary human neural stem / progenitor cells (**hNPC**) are used for studying the effects of substances on early neurodevelopmental processes in vitro. Differentiating hNPCs migrate and differentiate into radial glia, neurons, astrocytes, and oligodendrocytes (oligos) upon plating on a suitable extracellular matrix and thus model processes of early neural development. In order to characterize alterations in hNPC development, it is thus an essential task to reliably identify the cell type of each migrated cell in the migration area of a neurosphere. To this end, we introduce and validate a deep learning approach for identifying and quantifying cell types in microscopic images of differentiated hNPC. As we demonstrate, our approach performs with high accuracy and is robust against typical potential confounders. We demonstrate that our deep learning approach reproduces the dose responses of well-established developmental neurotoxic (**DNT**) compounds and controls, indicating its potential in medium or high throughput in vitro screening studies. Hence, our approach can be used for studying compound effects on neural differentiation processes in an automated and unbiased process.

Data & Methods

Plate Ind.	Stainings		DNT		#Wells distributed		
	Neurons	Oligos	Compound ID	Effects	Train	Val	Test
I1	✓		3	Both	36	4	0
I1	✓		11		36	4	0
I1	✓		11		36	4	0
I1	✓	✓	3	Both	36	4	0
I1	✓	✓	13	Oligos	36	3	0
I1	✓	✓	4		36	4	0
I1	✓	✓	1	Both	36	3	0
I2		✓	1	Both	36	3	0
I3		✓	9	Both	36	4	0
I1	✓	✓	13	Oligos	36	4	0
I1		✓	6	Both	36	3	0
I2		✓	6	Both	36	3	0
I2		✓	12	Oligos	36	4	0
I4		✓	2	Oligos	36	4	0
I4		✓	5	Both	36	4	0
I1	✓	✓	8	Neurons	36	4	1
I2	✓	✓	7	Neurons	36	4	1
I1	✓	✓	10	Oligos	36	4	1
I4	✓	✓	11		36	4	1

Detailed Assay & Staining protocol published in [2].

Neurosphere data distribution:

- Can model DNT in vitro
- 19 Plates with 40 Neurospheres each
- One sphere per well
- Grown from hNPCs for 7-8 weeks
- Antibody stained, differentiated cells
- 8 different compound concentrations
- 5 replicates each

	Neuron Set		Oligo Set	
Plates	11	16		
Total Nuclei	222,423	309,753		
Label Training	28,548	19,486		
annotated Validation	3,866	2,443		
Test	1,114	718		
Label not Training	158,932	248,908		
annotated Validation	20,132	27,971		
Test	9,831	10,227		

Annotations & Data imbalance:

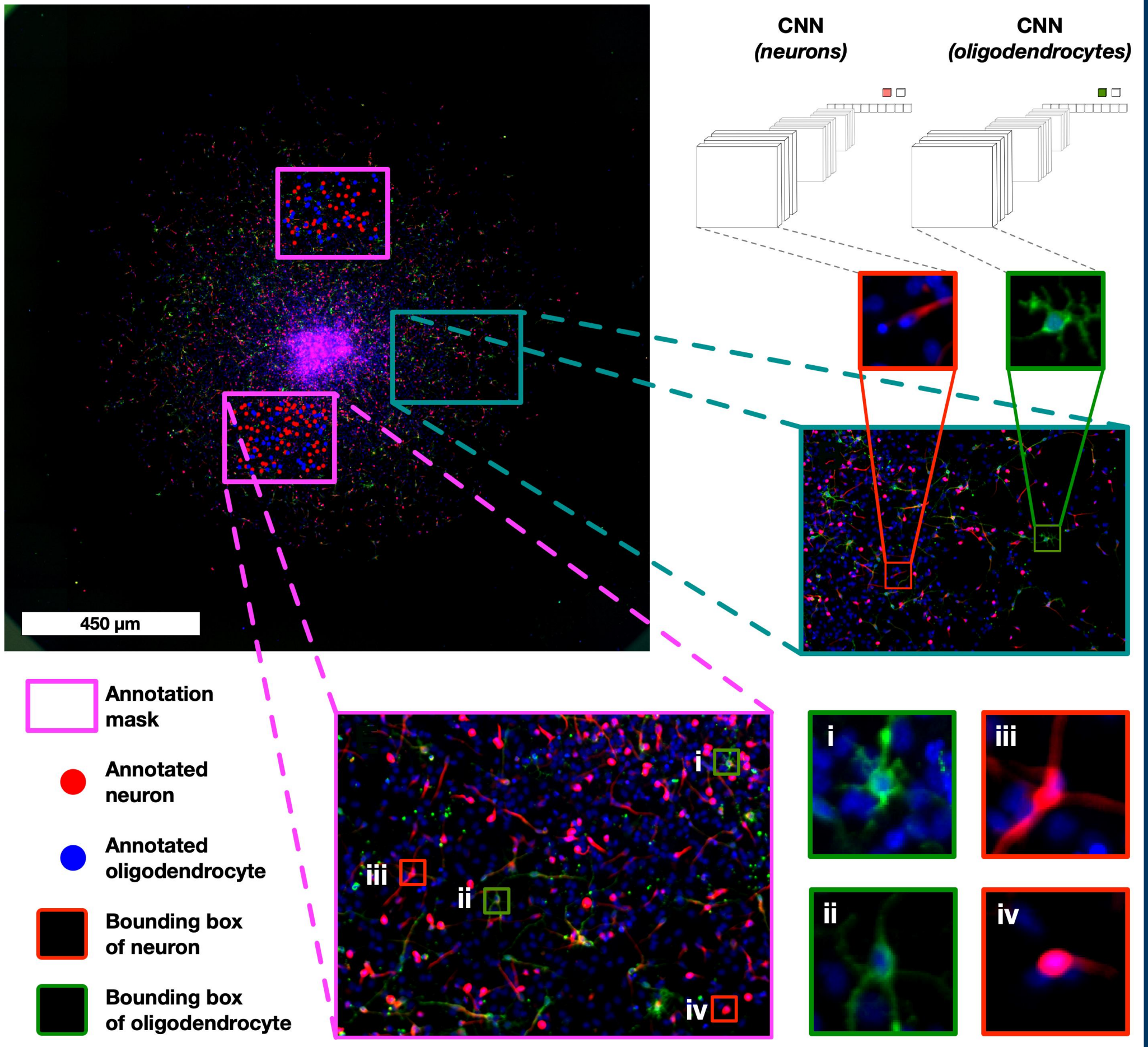
- Roughly 1 out of 5 nuclei is a neuron
- Roughly 1 out of 12 nuclei is an oligo

Pre-Processing

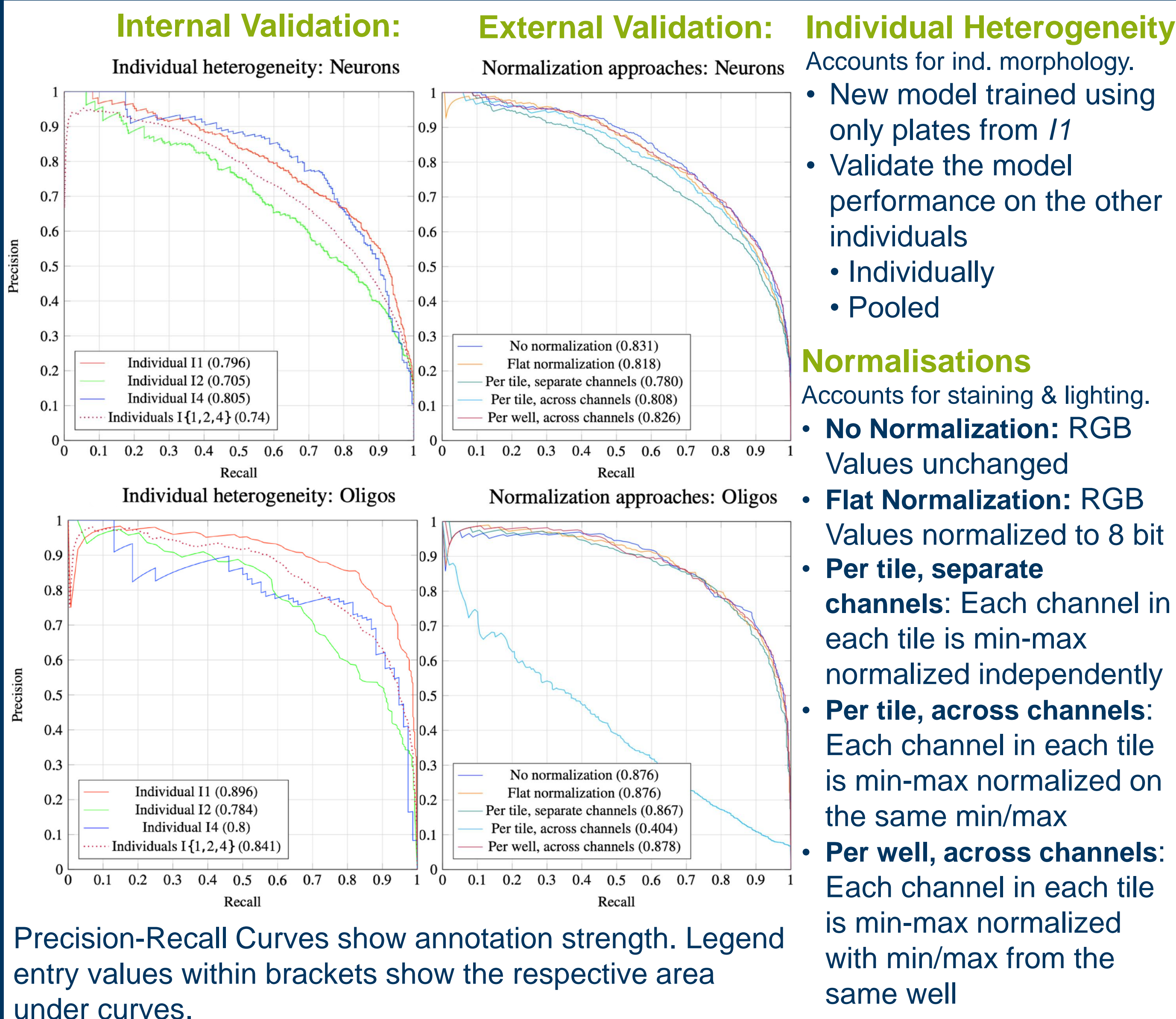
- Capture whole well images via fluorescence microscopy
- Image resolution 4858×4858 μm → 5520×5520 px
- Annotate every nucleus in 2 ROIs (purple) per neurosphere
- Capture 64×64 px 8-Bit RGB images centered on every nucleus
- Train on ROIs to predict sphere migration area

The Model

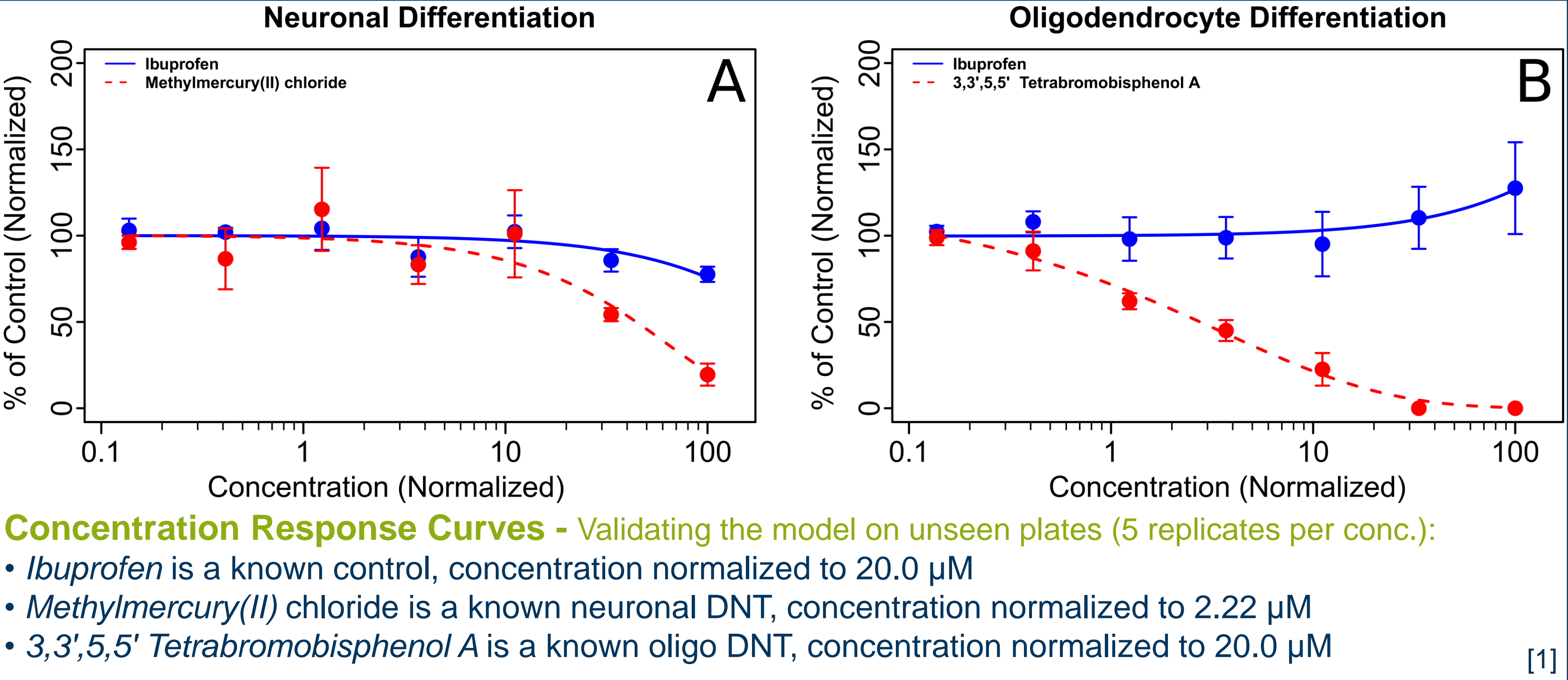
- Modified VGG architecture
- One model for Neurons and Oligodendrocytes each
- Trained with 100 batches
- Up to 5000 epochs & early stopping



Results & Discussion



Precision-Recall Curves show annotation strength. Legend entry values within brackets show the respective area under curves.



Concentration Response Curves - Validating the model on unseen plates (5 replicates per conc.):

- Ibuprofen* is a known control, concentration normalized to 20.0 μM
- Methylmercury(II) chloride* is a known neuronal DNT, concentration normalized to 2.22 μM
- 3,3',5,5' Tetrabromobisphenol A* is a known oligo DNT, concentration normalized to 20.0 μM

[1]

Conclusions

- Robust neuron & oligodendrocyte classifications
- Captures *dose response* relations
- Many confounders accounted for
- Model performance suitable for toxicology
- Regulators (US-EPA, EFSA - European Food Safety Authority) showing interest in assay and endpoints

Future Work

- Extended classifications for Radial Glia Cells and Astrocytes
- Weakly supervised learning to infer morphological endpoints

Contact

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Code & Example Data



Contact Information

References & Acknowledgements

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