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APPENDIX 3

SUPPLEMENTARY INFORMATION

Comparing Analytical vs. AI-based Image Analysis for Micron-sized Particle Detection and Measurement

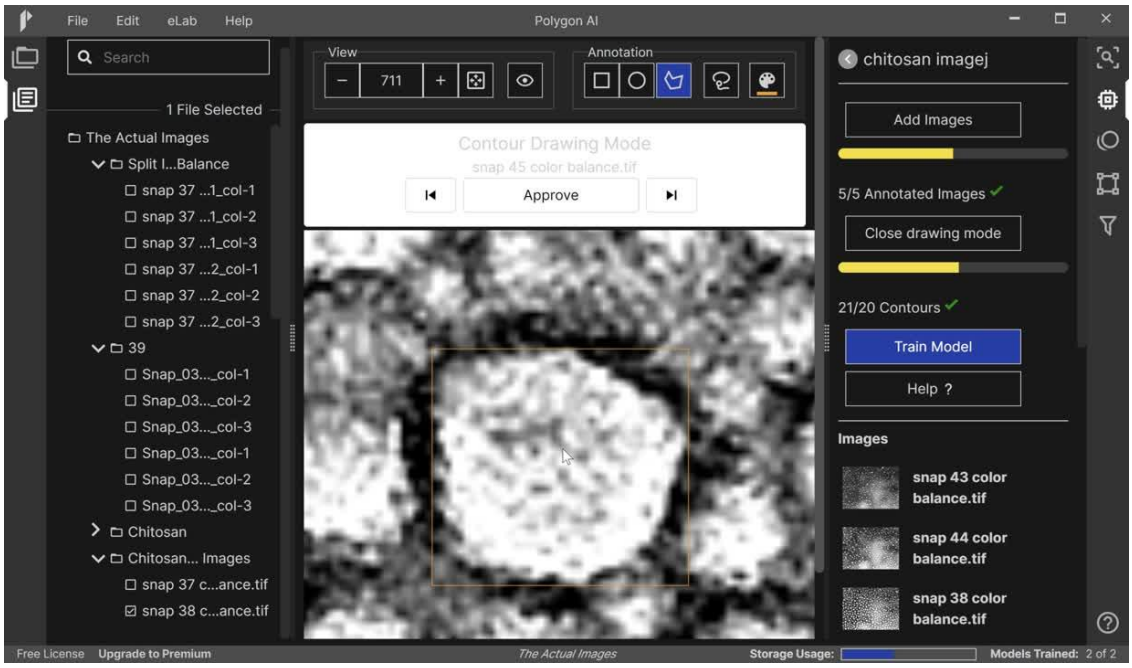

Figure S1: The video shows the user drawing the contour outline of the cell, where the contours will be sent to the Rewire AI server to train and analyze all the contours sent from the user. Link of video on the procedure for custom model training: https://drive.google.com/file/d/1qIK0ZHAqhWOtGKxUU3WdTfuYT3VH5kDs/view?usp=drive_link

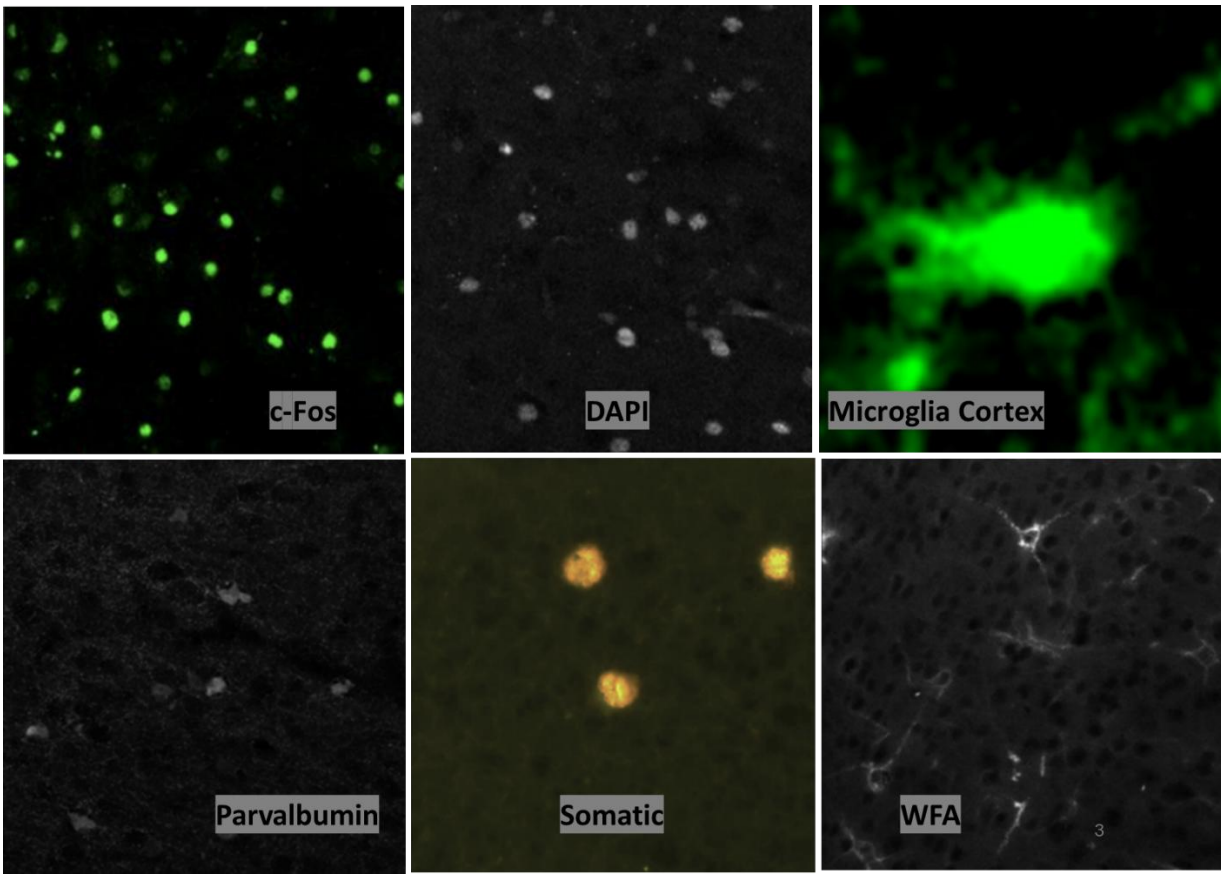

Figure S2. Polygon AI pre-trained models based on different cells and staining processes.

a. c-Fos

The c-Fos protein is an immediate early gene (IEG) that form heterodimers that is essential in regulating neuronal cells during excitability events (e.g. seizures). In this model, the imaged objects can be neurons with c-Fos proteins.

1. Zhang, J., Zhang, D., McQuade, J. *et al.* c-*fos* regulates neuronal excitability and survival. *Nat Genet* **30**, 416–420 (2002). https://doi.org/10.1038/ng859
2. Osanai, Hisayuki, et al. "Automated cell detection for immediate early gene-expressing neurons using inhomogeneous background subtraction in fluorescent images." *bioRxiv* (2024): 2024-11.

b. DAPI

DAPI (4′,6-diamidino-2-phenylin-dole) is a fluorescent molecule that binds to A-T regions in the DNA. It is widely used as a DNA-specific probe. As an example, due to its permeability differences in live and dead cells, it can be used to determine cellular health. In the case of the DAPI model, the imaged object is the DNA or the entire cell with DNA.

1. Kapuscinski, Jan. "DAPI: a DNA-specific fluorescent probe." *Biotechnic & histochemistry* 70.5 (1995): 220-233.

2. Estandarte, Ana Katrina, et al. "The use of DAPI fluorescence lifetime imaging for investigating chromatin condensation in human chromosomes." *Scientific reports* 6.1 (2016): 31417.
3. Johnson, M. Brittany, and Alison K. Criss. "Fluorescence microscopy methods for determining the viability of bacteria in association with mammalian cells." *Journal of visualized experiments: JoVE* 79 (2013): 50729.

c. Microglia cortex

Microglia is present in the central nervous system and is considered essential in maintaining homeostasis in its microenvironments. They clean-up microbes, dead cells, redundant synapses, protein aggregates, other particulate and soluble antigens that could affect the health of the CNS. In the software, the model looks for the characteristics of the microglia.

1. Colonna, Marco, and Oleg Butovsky. "Microglia function in the central nervous system during health and neurodegeneration." *Annual review of immunology* 35.1 (2017): 441-468.
2. Gao, Chao, et al. "Microglia in neurodegenerative diseases: mechanism and potential therapeutic targets." *Signal transduction and targeted therapy* 8.1 (2023): 359.

d. Parvalbumin

Parvalbumin is a Ca+2 binding protein that is present in muscles and the brain. In mucsles, it is responsible for fast contracting muscles. They are also present in the CNS neurones, so they are also used as neuronal marker and related to behavioral changes. In the model images the objects selected are cells containing parvalbumin.

1. Celio, M. R., and C. W. Heizmann. "Calcium-binding protein parvalbumin as a neuronal marker." *Nature* 293.5830 (1981): 300-302.
2. Staiger, Jochen F., et al. "Local circuits targeting parvalbumin-containing interneurons in layer IV of rat barrel cortex." *Brain Structure and Function* 214 (2009): 1-

e. Somatic

Somatic cells refer to all the different cells in the body except for germ cells (sperm and egg cells). They vary in size and shapes. One application of imaging ssomatic cells are in determining presence of somatic cells in milk due to the presence of white blood cells in mammary glands. Based on the type of somatic cells, other applications such as monitoring cell processes and counting are important studies in this field.

1. Kim, Byeongyeon, et al. "A portable somatic cell counter based on a multi-functional counting chamber and a miniaturized fluorescence microscope." *Talanta* 170 (2017): 238-243.
2. Reuter, Karin, et al. "Reassembly of somatic cells and testicular organogenesis in vitro." *Tissue and Cell* 46.1 (2014): 86-96.
3. Molugu, Kaivalya, et al. "Label-free imaging to track reprogramming of human somatic cells." *GEN biotechnology* 1.2 (2022): 176-191.

f. WFA

Wisteria floribunda agglutinin (WFA) is a lectin that is best suited for staining cardiac fibrotic tissues for fluorescence microscopy. It is used to detect cardiac fibrosis, a cause for poor dilatation and contractile functions of the heart. Because WFA is a stain for fibrosis, the regions identified in the software are fibrotic tissues.

1. Nagai-Okatani, Chiaki, et al. "Wisteria floribunda agglutinin staining for the quantitative assessment of cardiac fibrogenic activity in a mouse model of dilated cardiomyopathy." *Laboratory Investigation* 99.11 (2019): 1749-1765.
2. Slaker, Megan L., John H. Harkness, and Barbara A. Sorg. "A standardized and automated method of perineuronal net analysis using Wisteria floribunda agglutinin staining intensity." *IBRO reports* 1 (2016): 54-60.