

Abstract

Nanomaterial use continues to rise in biomedical applications, and the need for rapid, accurate, and non-invasive cytotoxicity measurements has become increasingly evident. Existing approaches for evaluating nanomaterial cytotoxicity are often indirect and typically depend on well-plate, salt-based fluorescence assays or complicated microscopy methods. To address these limitations, we introduce FluoAI, a two-stage neural network workflow that directly determines live/dead cells from the nanomaterial's fluorescence, eliminating the need for additional labeling. The workflow uses two consecutive convolutional neural networks: Mask R-CNN first performs instance segmentation of individual cells from grayscale, single-channel fluorescence images, followed by a DenseNet-121 classifier that assigns alive or dead labels to each segmented cell, achieving performance values of up to 92.0%. In addition to viability classification, FluoAI also performs expert-level analyses of corrected total cell fluorescence (CTCF), mean fluorescence, and cell area, with results showing minimal to no significant differences compared with human measurements of Graphene Quantum Dot (GQD)- and fluorescein dye-treated model cells. Because the entire pipeline is automated, these quantitative fluorescence metrics are generated faster than manual analysis while maintaining comparable accuracy. Overall, this AI pipeline enables non-invasive cytotoxicity assessment and automated in vitro analysis using a conventional fluorescence microscopy setup. As its dataset continues to expand, FluoAI provides a strong foundation for reliable, high-throughput nano-cytotoxicity assays and automated data analysis, ultimately supporting the development of novel and safer nanomedicines.

Introduction

Fallbacks of current cytotoxicity methods

- Often rely on additional stains or reagents, which can interfere with nanomaterials and misrepresent viability
- Many methods can not provide direct single cell level assessment

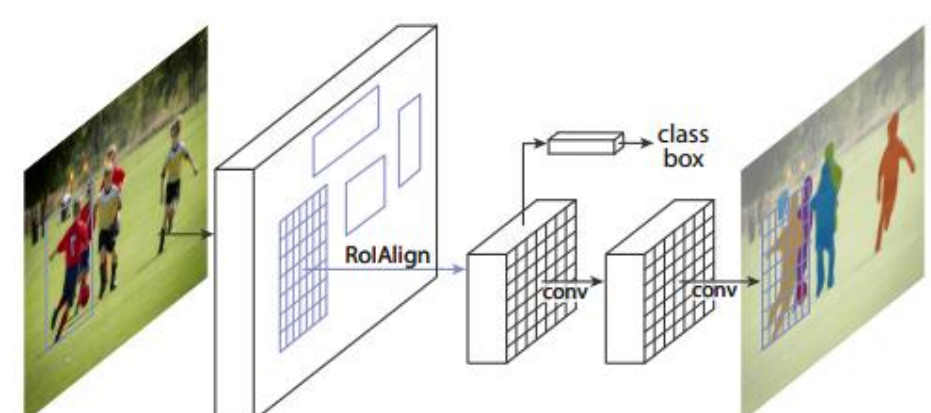
We can use AI to outline cells in microscopy but...

- Segmentation-only pipelines do not provide **live/dead classification**
- Many AI methods use **specialized fluorescence imaging techniques and costly instrumentation**
- **No prior pipeline combines instance segmentation + live/dead classification on nanomaterial-only grayscale fluorescence images**

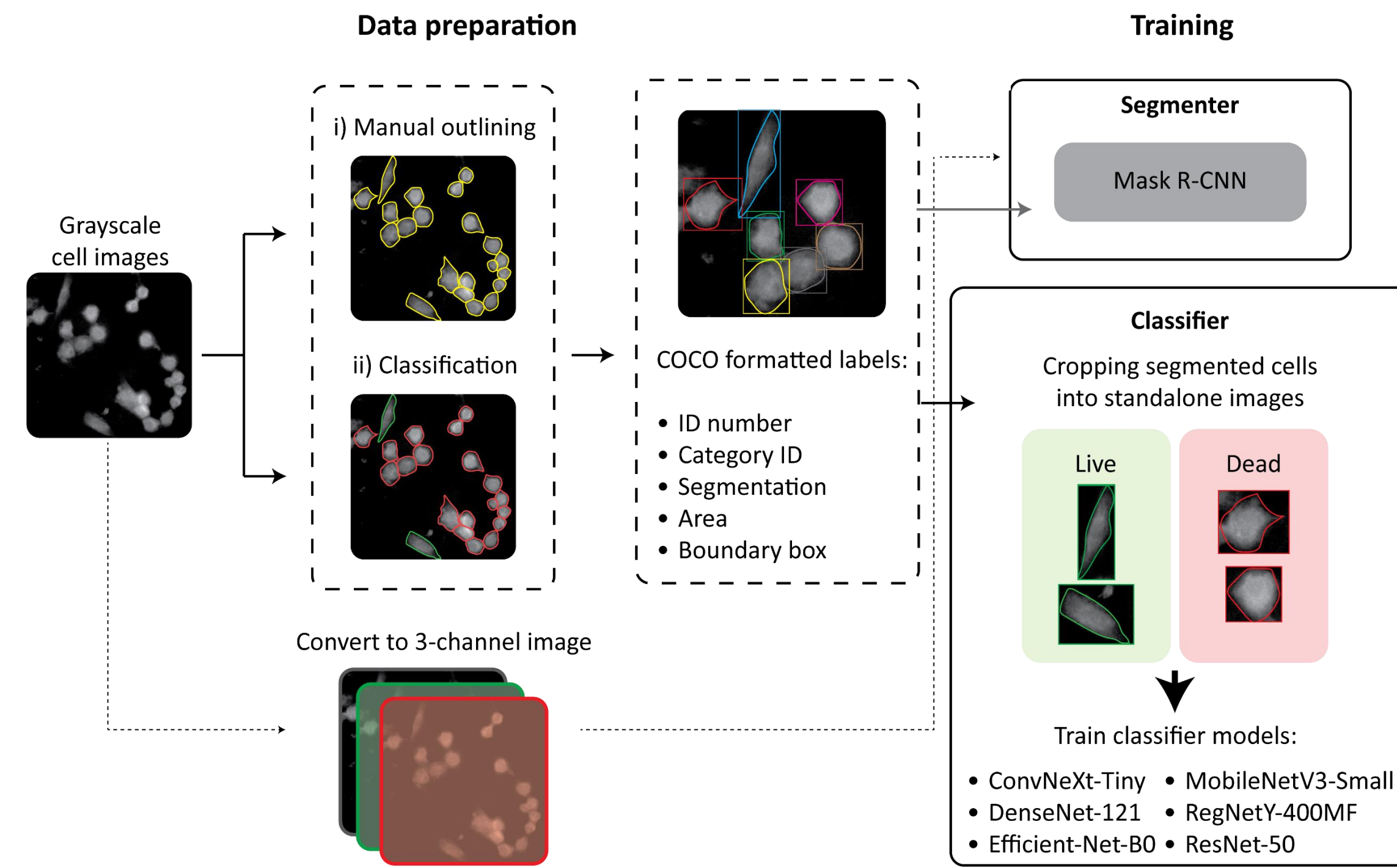


Questions asked?

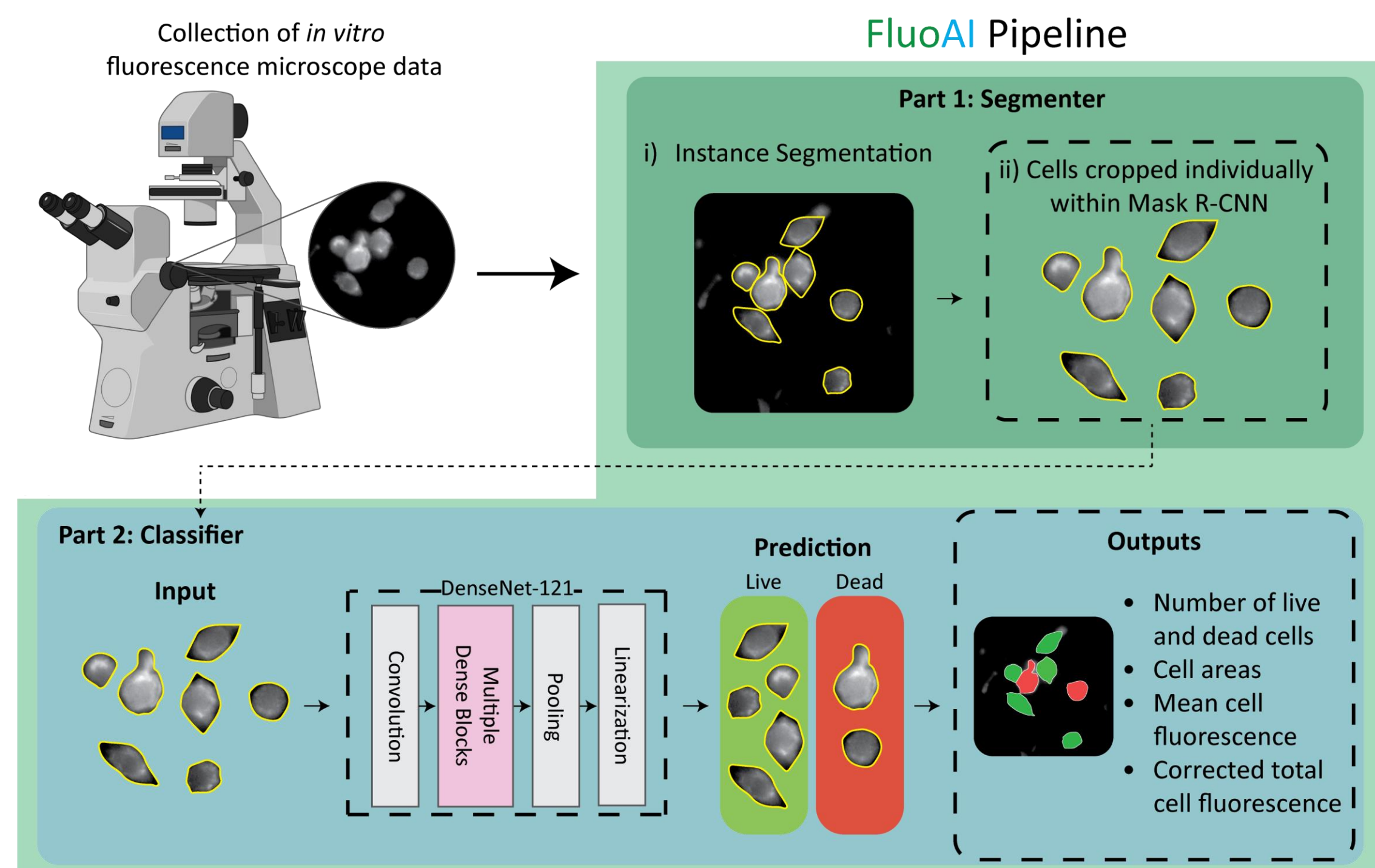
- Which endocytosis pathways contribute to GQD uptake in mammalian cells?
- Do these mechanisms differ between cancerous and non-cancerous cell lines?
- How does surface charge and surface functional groups effect GQD endocytosis?



Method Overview

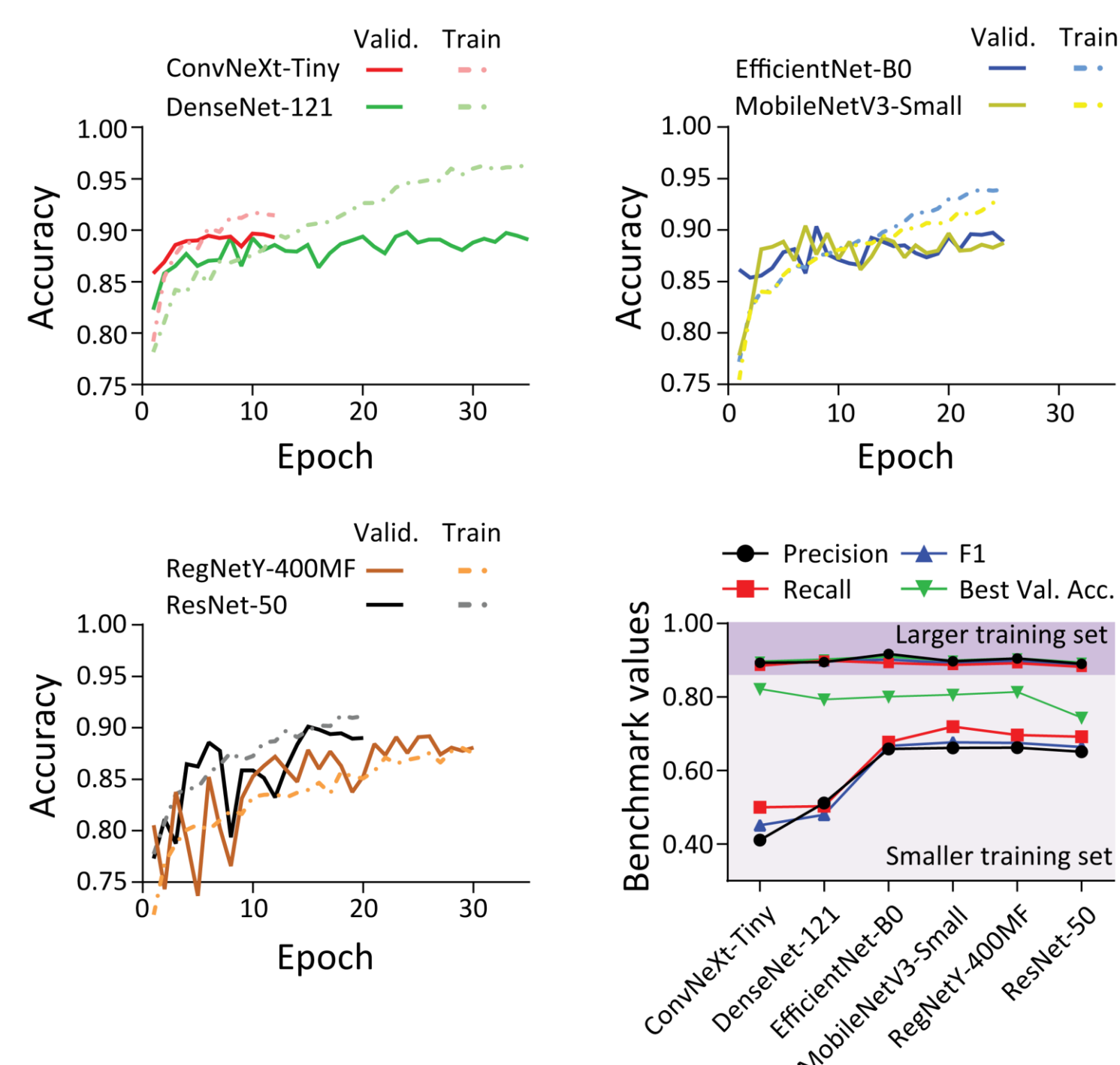


- Schematic explaining the processes of **training convolutional neural networks in our pipeline**



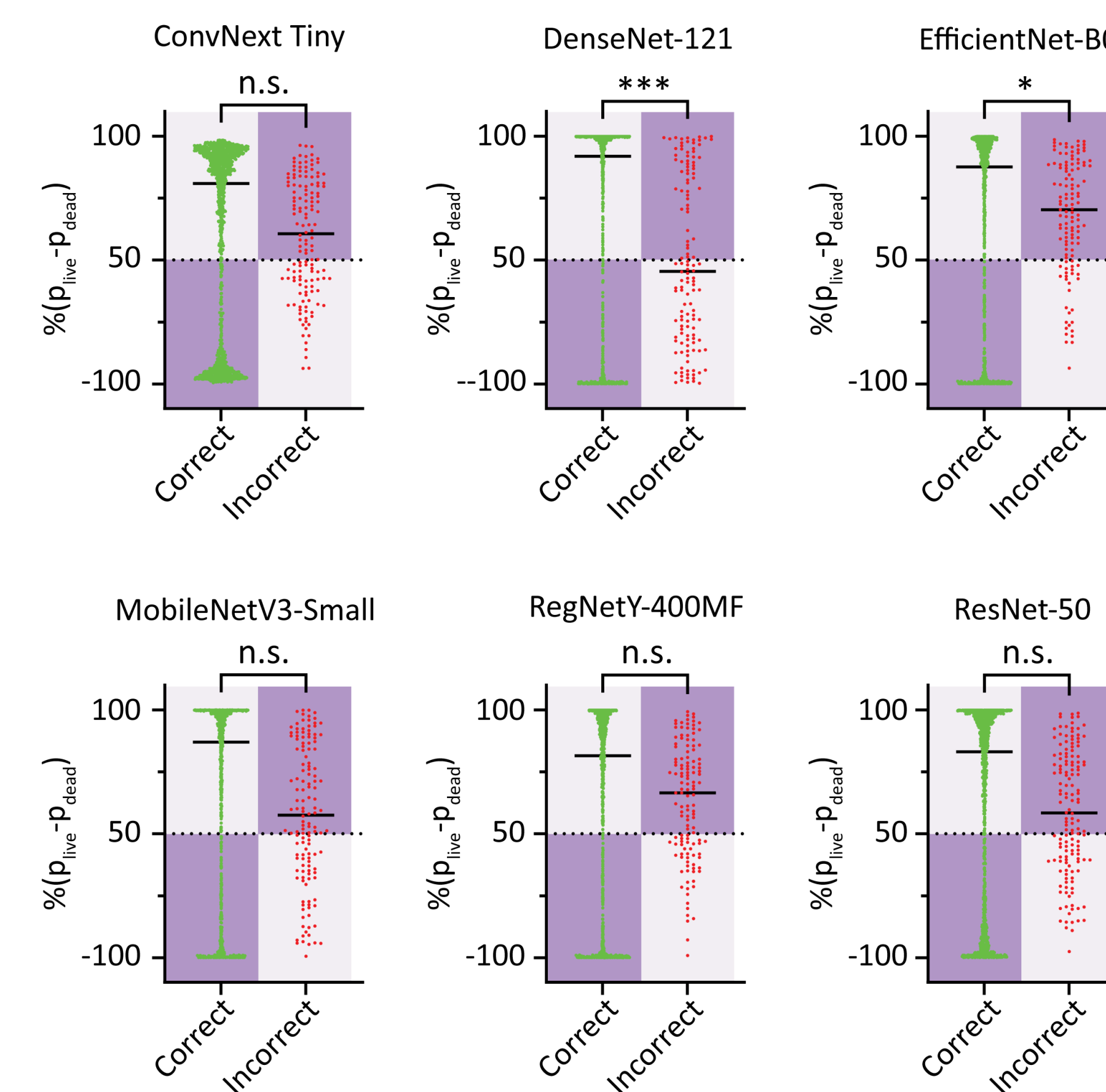
- Overview of the **FluoAI pipeline workflow**

Benchmarking of different classifiers

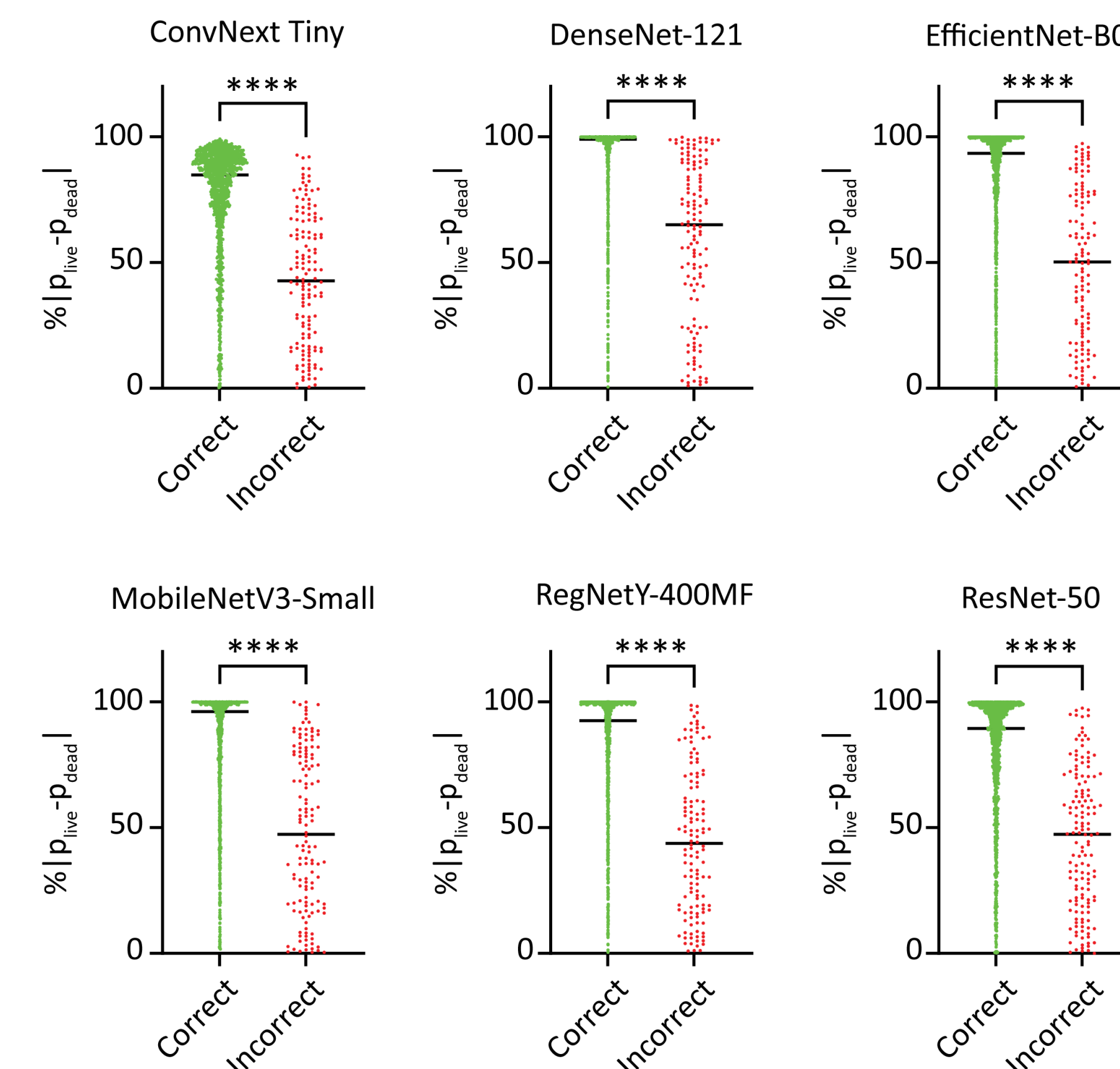


- **Accuracy curves and Precision, Recall, F1 and Best Value Accuracy scores** plotted for each classifier architecture trained with a smaller and a larger dataset

CNN benchmarks display robust live/dead classifying performance



- **Directional prediction confidence** outlines the confidence of correct and incorrect cell label predictions across CNN classifiers

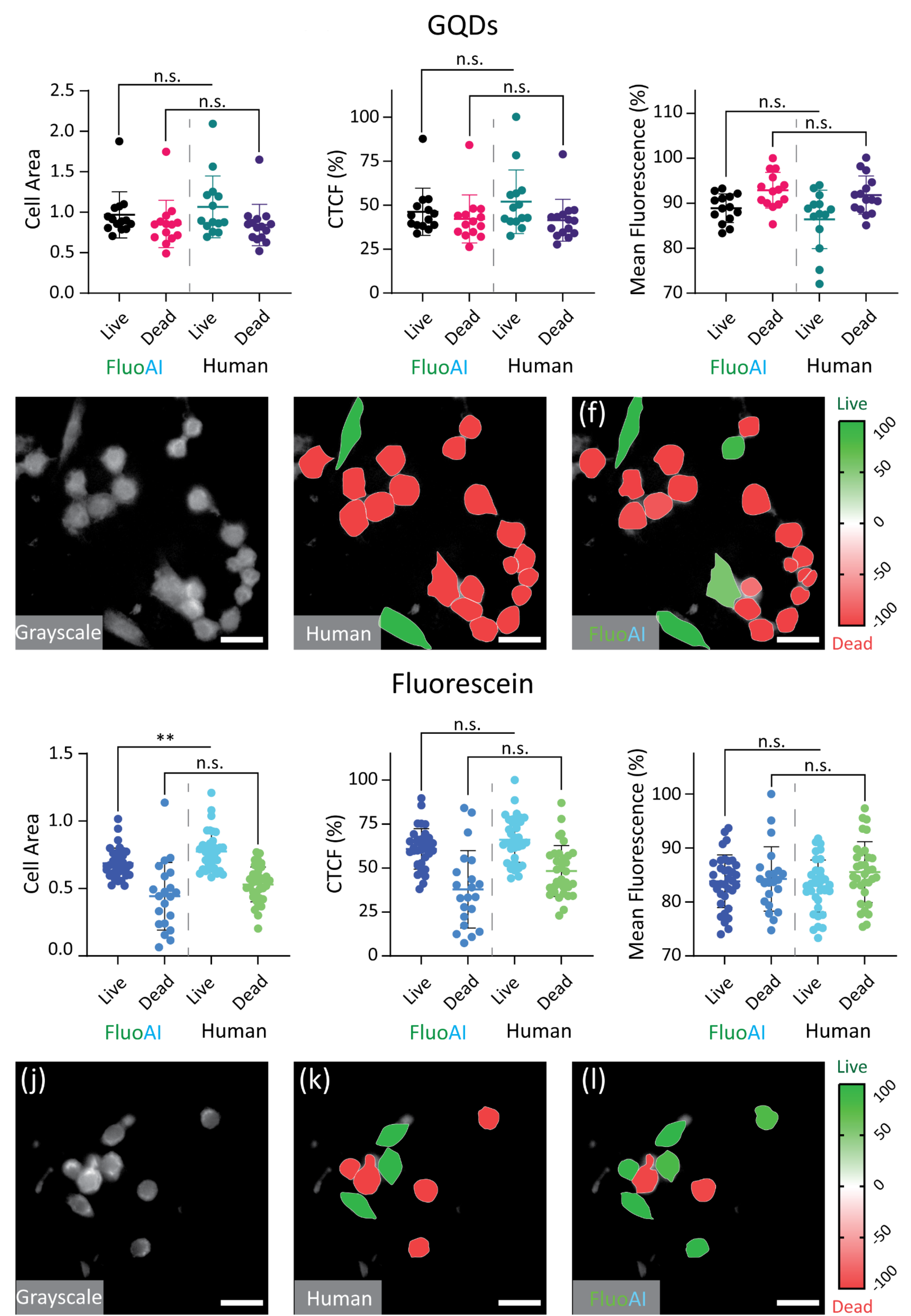


- **Prediction confidence** on the validation set distinguishes correct from incorrect cell labels across CNN classifiers

Conclusion

- FluoAI enables direct, high-throughput nano-cytotoxicity assessment from grayscale fluorescence images without additional staining, specialized instrumentation, or extra experimental steps
- **The pipeline achieves human-expert-level performance, reaching above 90% confidence**

FluoAI pipeline performs image analysis at human level



- **FluoAI outputs are compared to human expert live/dead classifications for cells treated with either GQDs or fluorescein**

References

- He, Kaiming, et al. "Mask r-cnn." Proceedings of the IEEE international conference on computer vision. 2017.
- Wu, Y.K., Alexander; Massa, Francisco; Lo, Wan-Yen; Girshick, Ross, Detectron2. 2019, Meta AI / Facebook AI Research.
- Our paper is in late stage review at *Advanced Intelligent Systems***