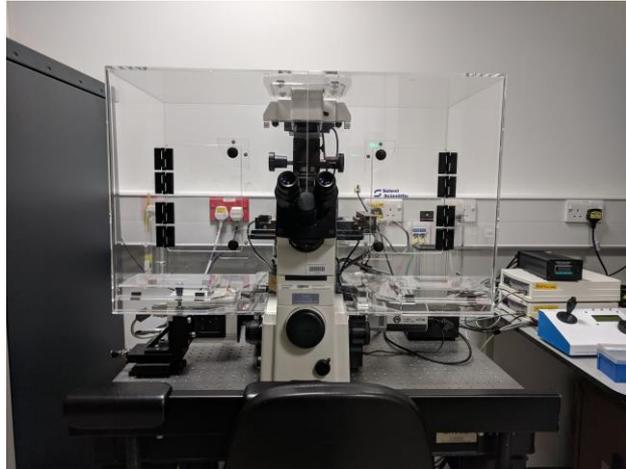


High throughput image analysis for Cardiomyocyte

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High-throughput screening (HTS) is a method for scientific experimentation especially used in drug discovery and relevant to the fields of biology and chemistry. High-throughput screening allows a researcher to quickly conduct millions of chemical, genetic, or pharmacological tests. Through this process one can rapidly identify active compounds, antibodies, or genes that modulate a particular biomolecular pathway.



Nikon TE2000

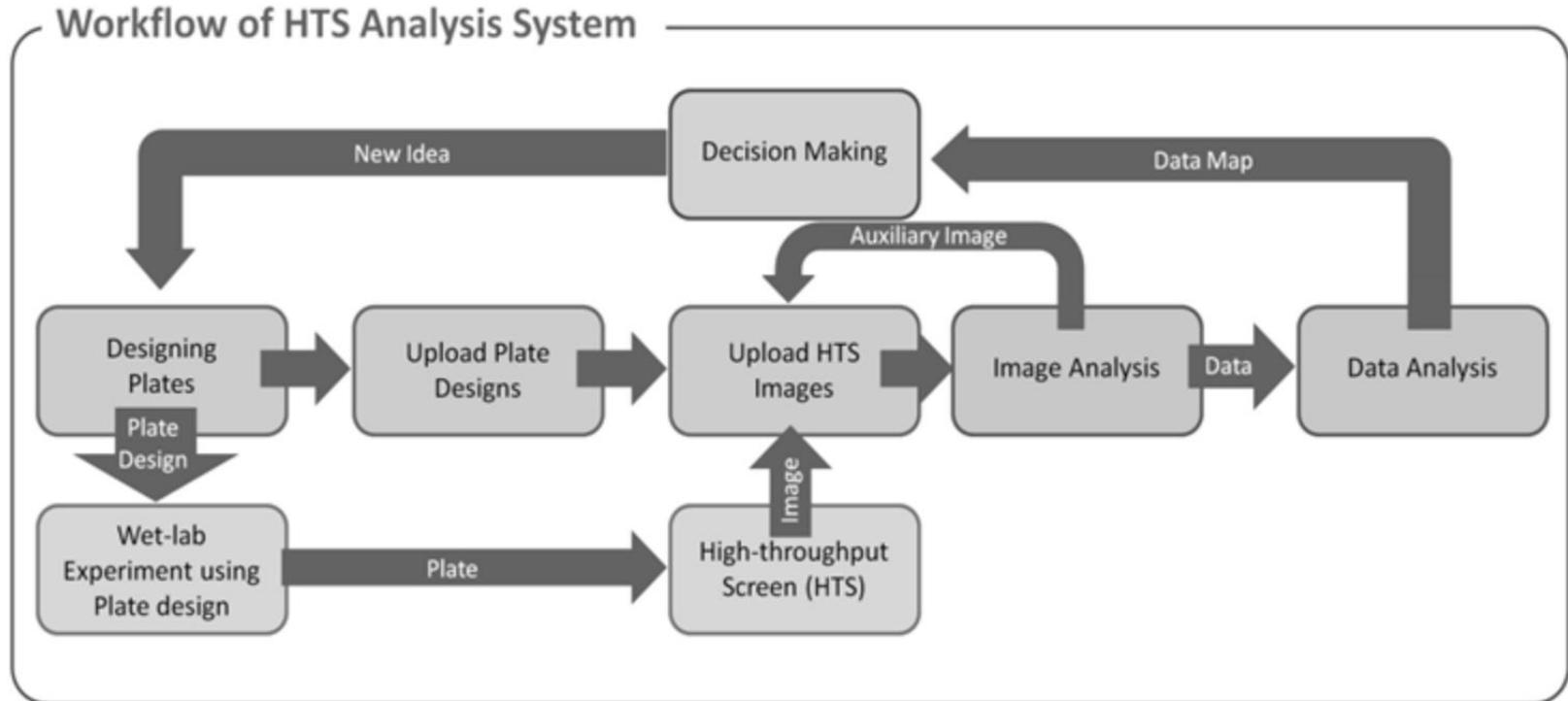


BD Pathway 855



Perkin Elmer Opera

Each high throughput screening (HTS) experiment starts with the design of the experimental scheme, followed by the wet-lab experiment and high throughput microscopy- based imaging. Both experimental schemes and image data are organized and stored in a database. Subsequently, **image analysis is used to extract phenotype measurements. Machine learning technologies are further applied in the Data Analysis step.** Finally, comprehensive conclusions are drawn.

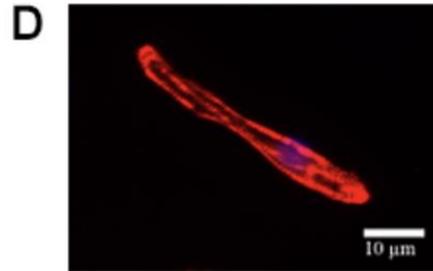
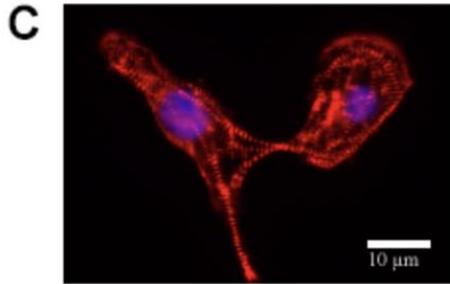
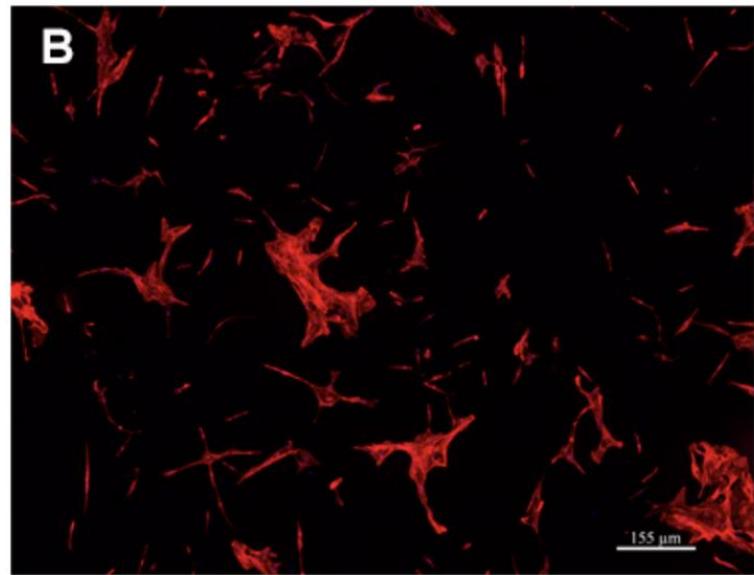
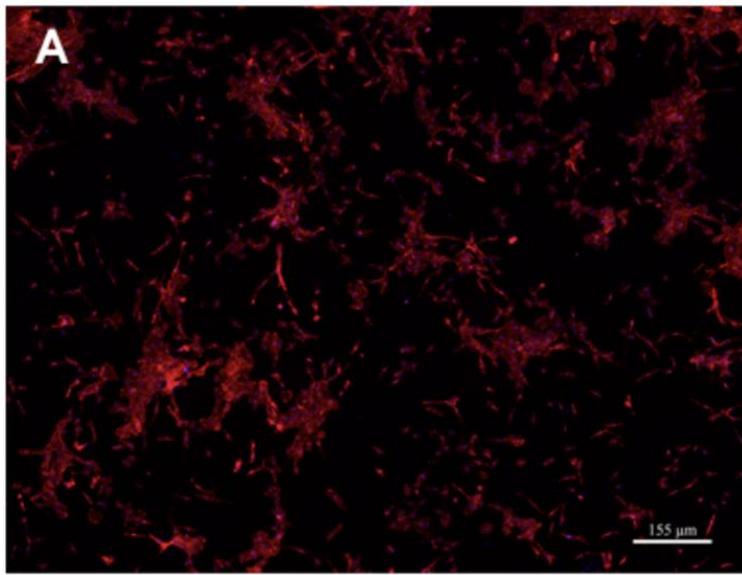


Cardiotoxicity Study

Cardiotoxicity, characterized by severe cardiac dysfunction, is a major problem in patients treated with different classes of anticancer drugs.

human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) show great potential for disease modelling and drug-induced toxicity screenings.

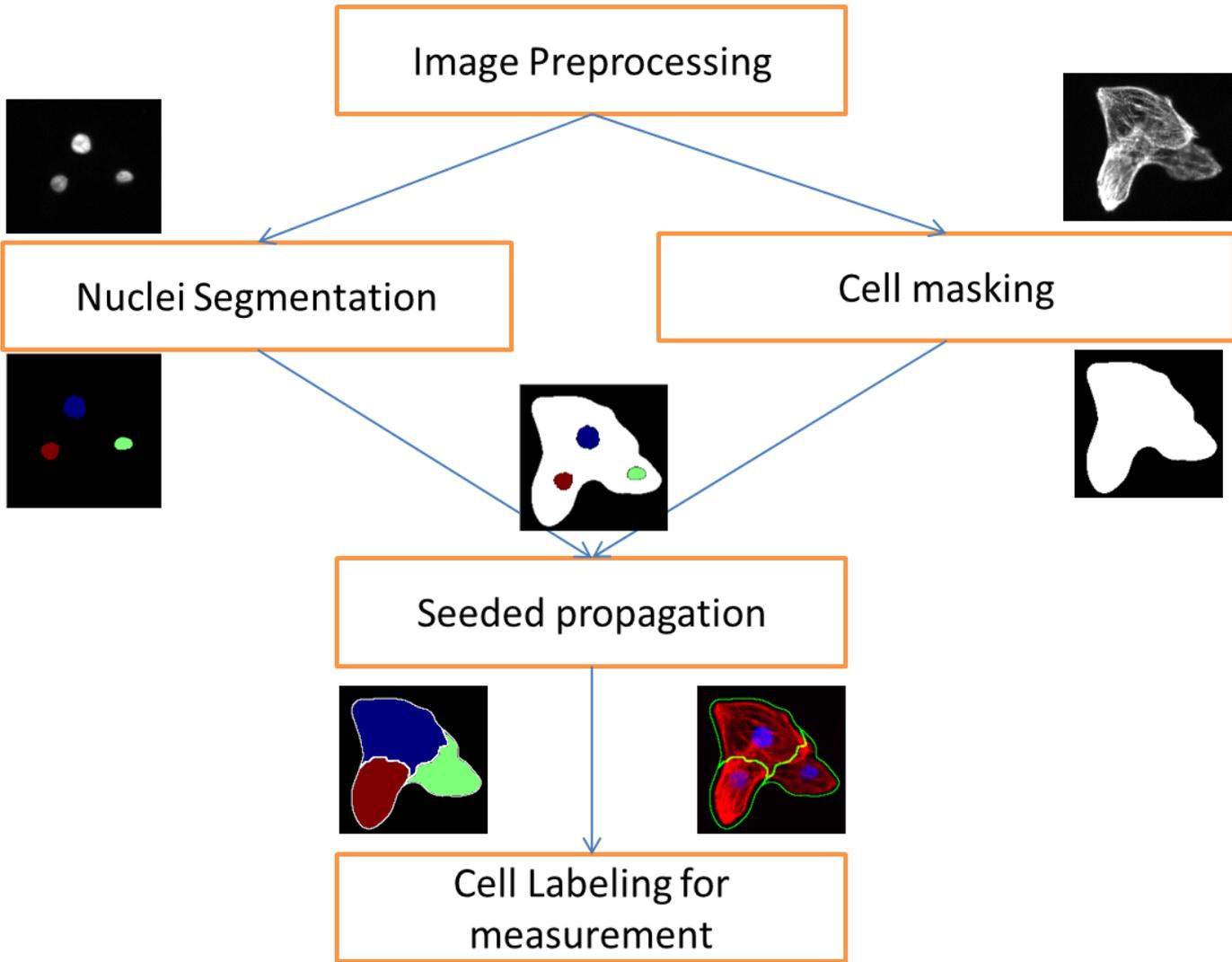
However, fully automated high-throughput screening of drug toxicity on hiPSC-CMs by fluorescence image analysis is very challenging, due to **clustered cell growth patterns** and **strong intracellular and intercellular variation in the expression of fluorescent markers**.

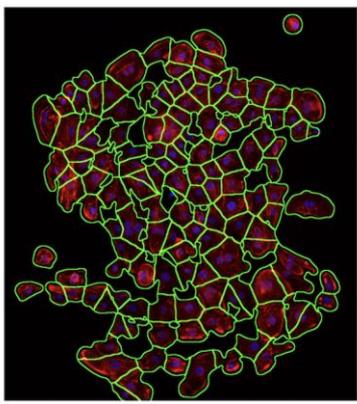
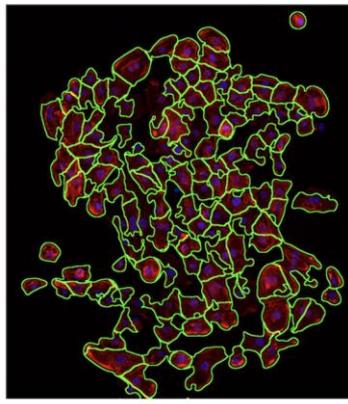
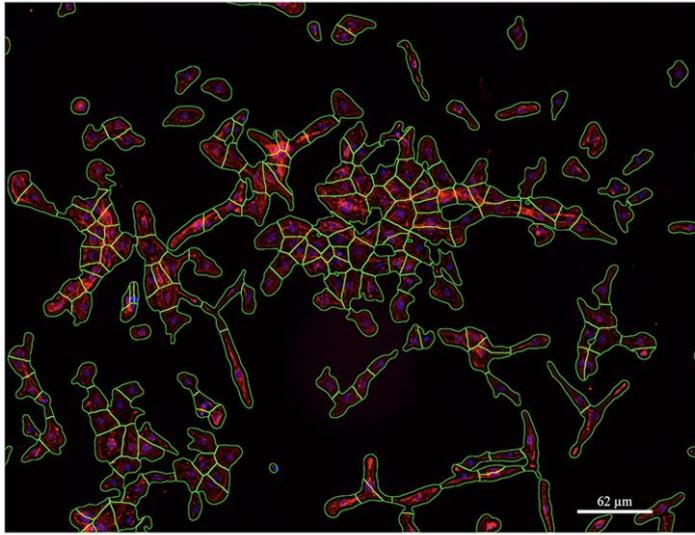
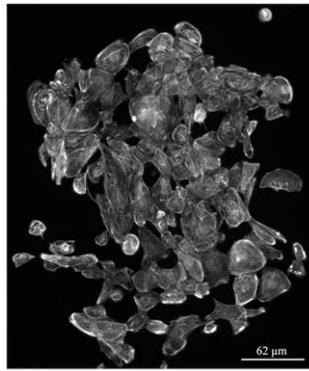
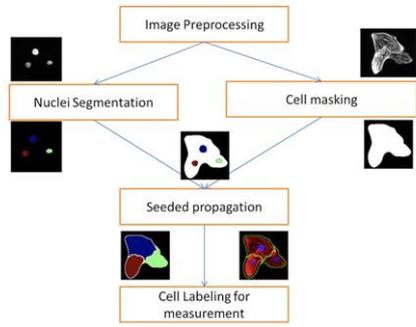


Sample images acquired from BD pathway 855 microscope with 7x7 montage setup. (A) Sample image in the control condition (DMSO). (B) Sample image with 1 μM doxorubicin treatment. (C) A close-up sample image from control condition (DMSO). (D) A close-up sample image with 1 μM doxorubicin treatment.

Our Contribution

- We report on the development of a fully automated image analysis system for quantification of cardiotoxic phenotypes from hiPSC-CMs that are treated with various concentrations of anticancer drugs doxorubicin or crizotinib.
- This high throughput system relies on single-cell segmentation by nuclear signal extraction, fuzzy C-mean clustering of cardiac α -actinin signal, and finally nuclear signal propagation.



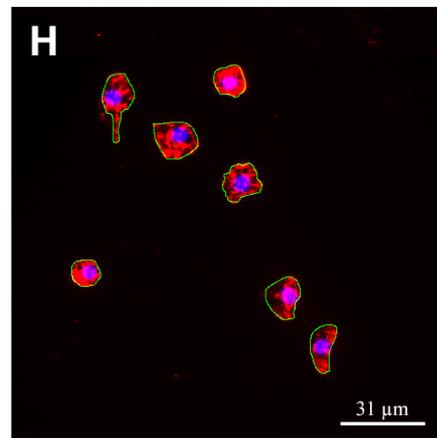
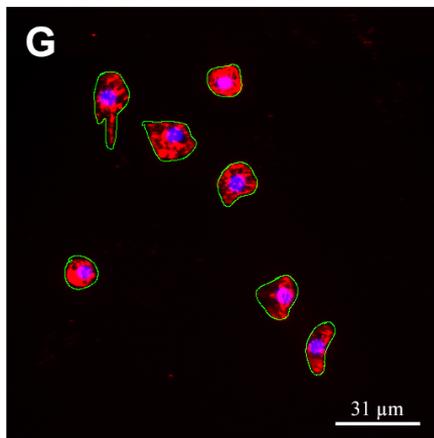
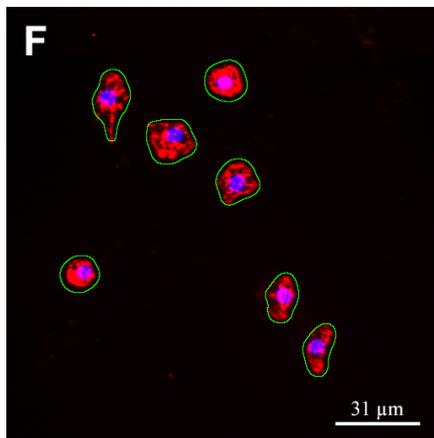
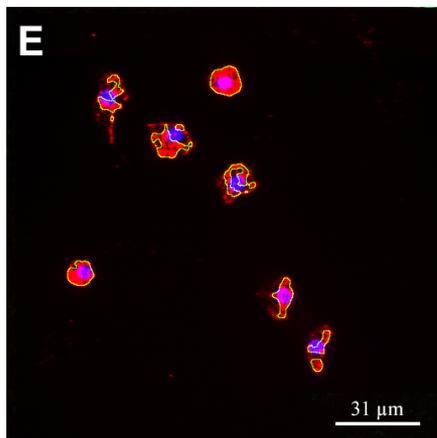
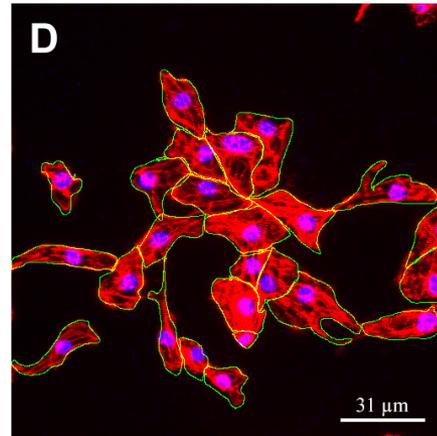
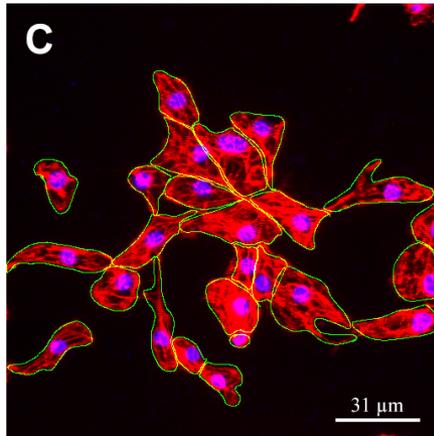
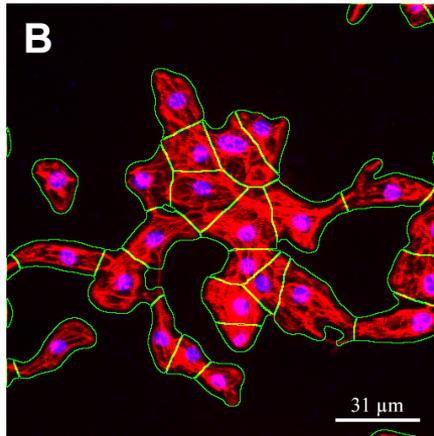
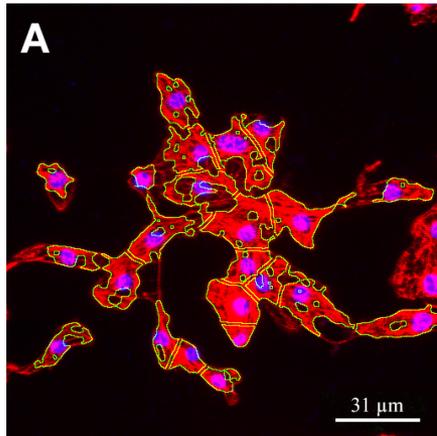


Otsu-based segmentation

EnFCM-based segmentation

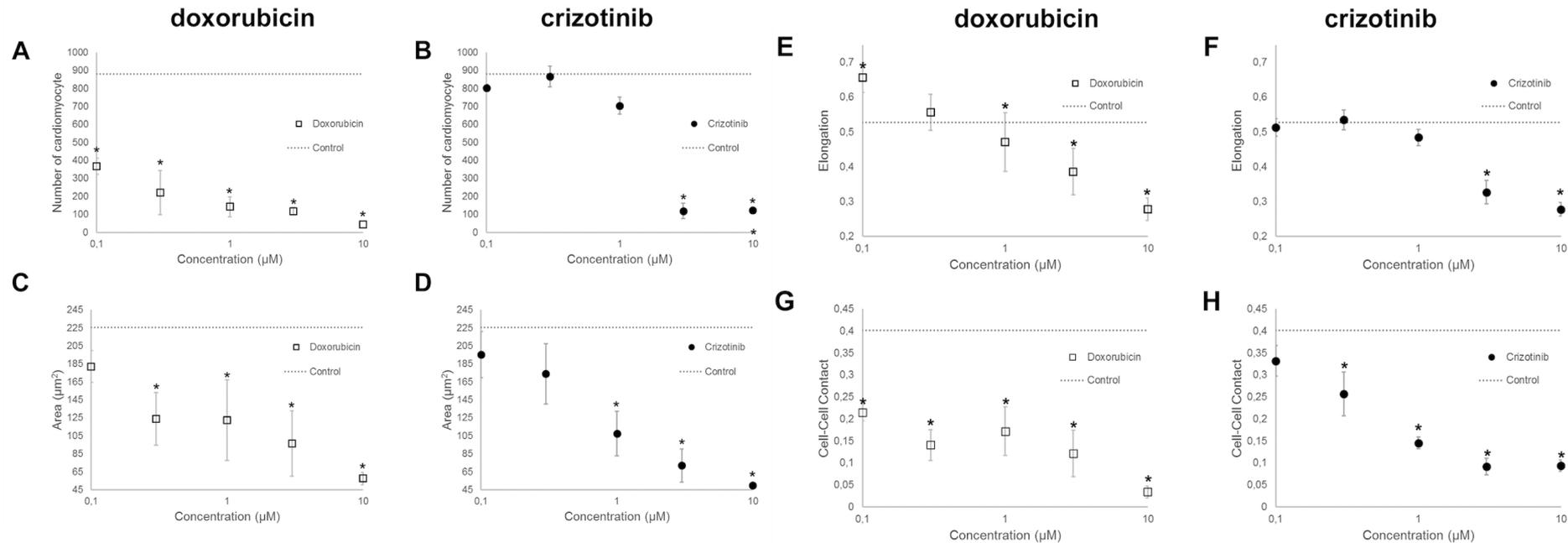
Manual segmentation 1

Manual segmentation 2



		Automated vs Manual 1			Automated vs Manual 2		
		Precision	Recall	F-Score	Precision	Recall	F-Score
Gregory's method	Mean	0.9728	0.5529	0.6945	0.9725	0.6123	0.7432
	SEM	0.0055	0.0361	0.0296	0.0047	0.0342	0.0263
Our method	Mean	0.8428	0.9197	0.8775	0.7849	0.9384	0.8533
	SEM	0.0122	0.0130	0.0054	0.0116	0.0102	0.0055

- When compared to manual segmentation, it generates precision and recall scores of 0.81 and 0.93, respectively.
- Our results show that our fully automated image analysis system can reliably segment cardiomyocytes even with heterogeneous α -actinin signals and that it can be used to determine cardiotoxicity based on phenotypic changes in hiPSC-CMs.



Representative results of phenotype measurements on single cell level. (A) The effects of doxorubicin treatment on cell viability (depicted as number of cardiomyocyte). (B) The effects of crizotinib treatment on cell viability (depicted as number of cardiomyocyte). (C) The effects of doxorubicin treatment on cell area. (D) The effects of crizotinib treatment on cell area. (E) The effects of doxorubicin treatment on cell shape (Elongation). (F) The effects of crizotinib treatment on cell shape (Elongation). (G) The effects of doxorubicin treatment on cell-cell contact. (H) The effects of crizotinib treatment on cell-cell contact. In general, data are represented as mean \pm s.e.m. * $p < 0.05$ by Two-sample Kolmogorov-Smirnov test.

High throughput system for adult cardiomyocyte analysis

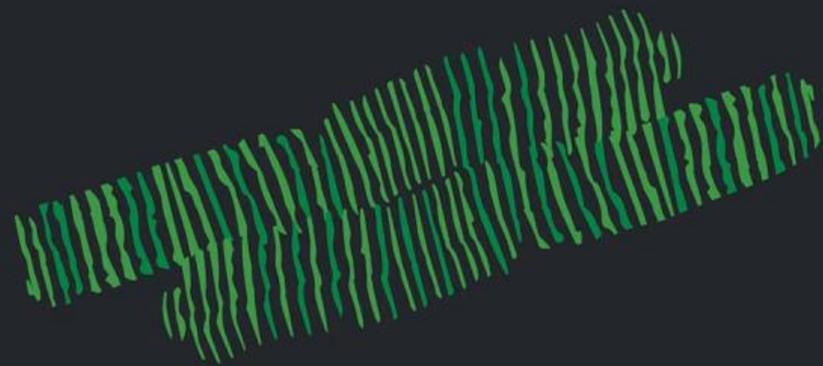
- Isolated adult cardiomyocytes are a well established model for drug or genetic screening.
- IonOptix developed a dedicated Myocyte Calcium and Contractility System which has been widely used in academic and Pharmaceutical laboratories worldwide.
- These contractility and Ca^{2+} handling measurements in vitro have been used as a platform for determining the physiological consequences of various genetic manipulations and identifying potential therapeutic targets for the treatment of heart failure.
- However, the throughput of this system is low which limits the number of compounds that can be tested per animal.



Our Contribution

- We present a pipeline which is able to find the best sarcomere region in an automatically found adult cardiomyocyte.
- This drastically increases throughput and provides a higher statistical reliability of our output dataset.





CYT **O** CYP **H** ER

Deep learning: Instance segmentation

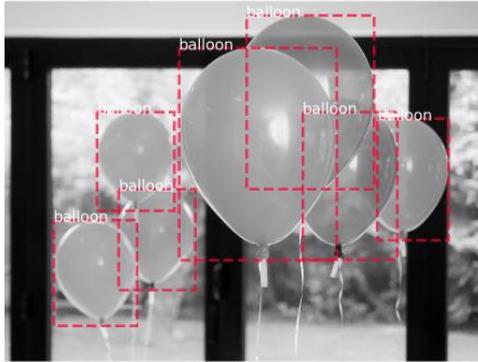
Classification



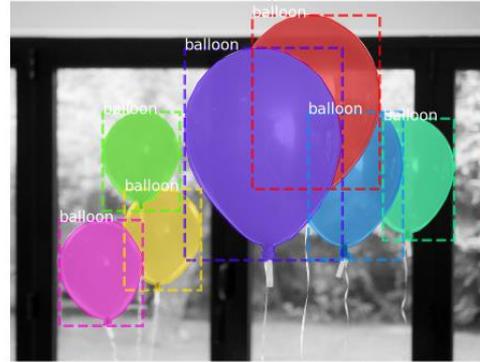
Semantic Segmentation



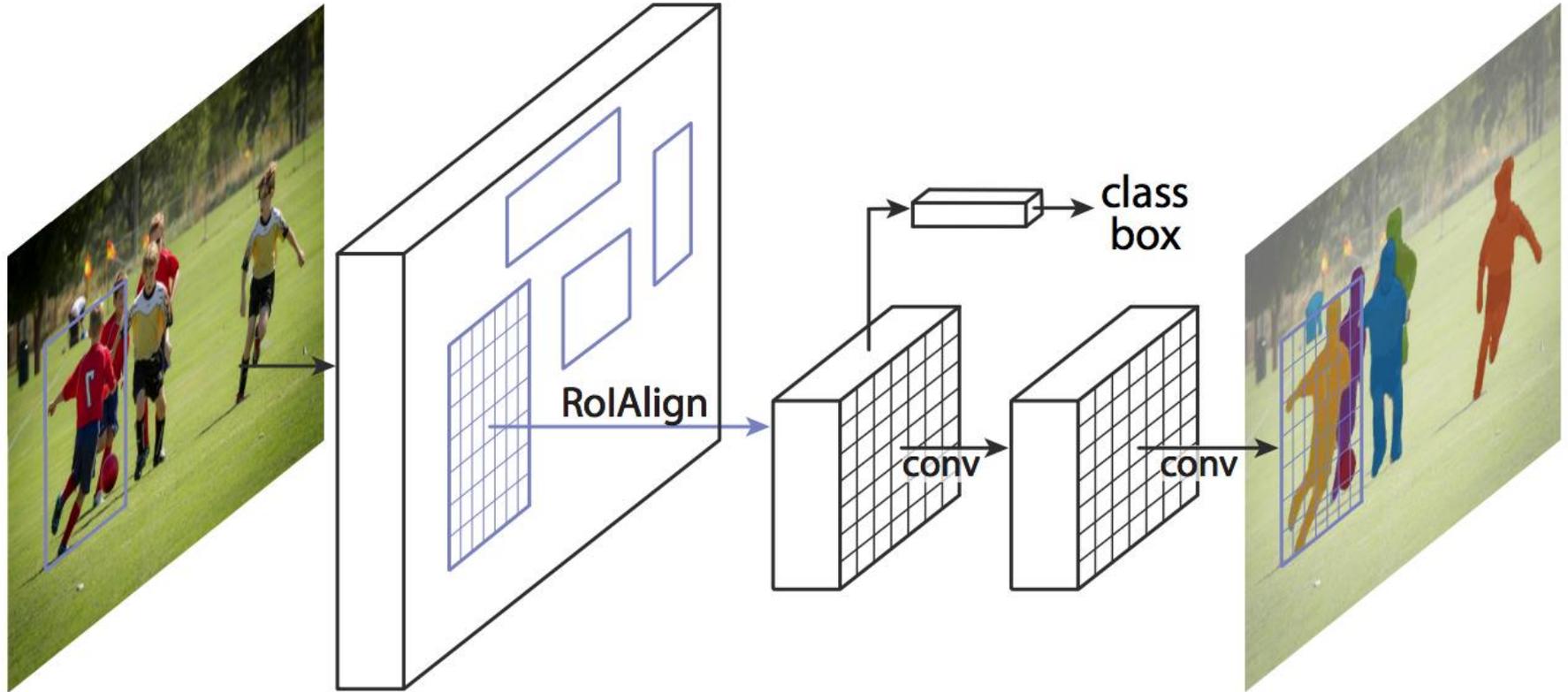
Object Detection



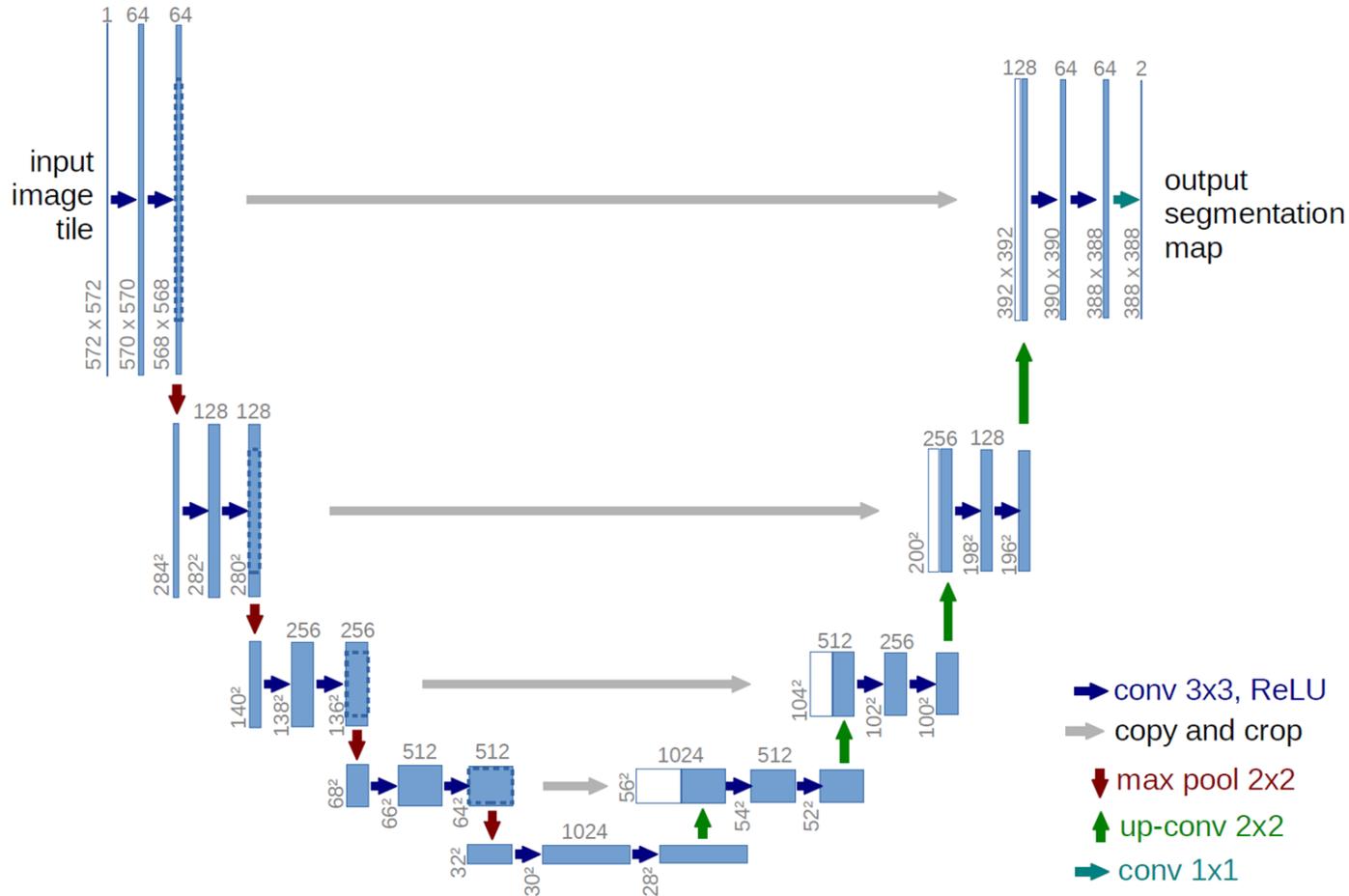
Instance Segmentation



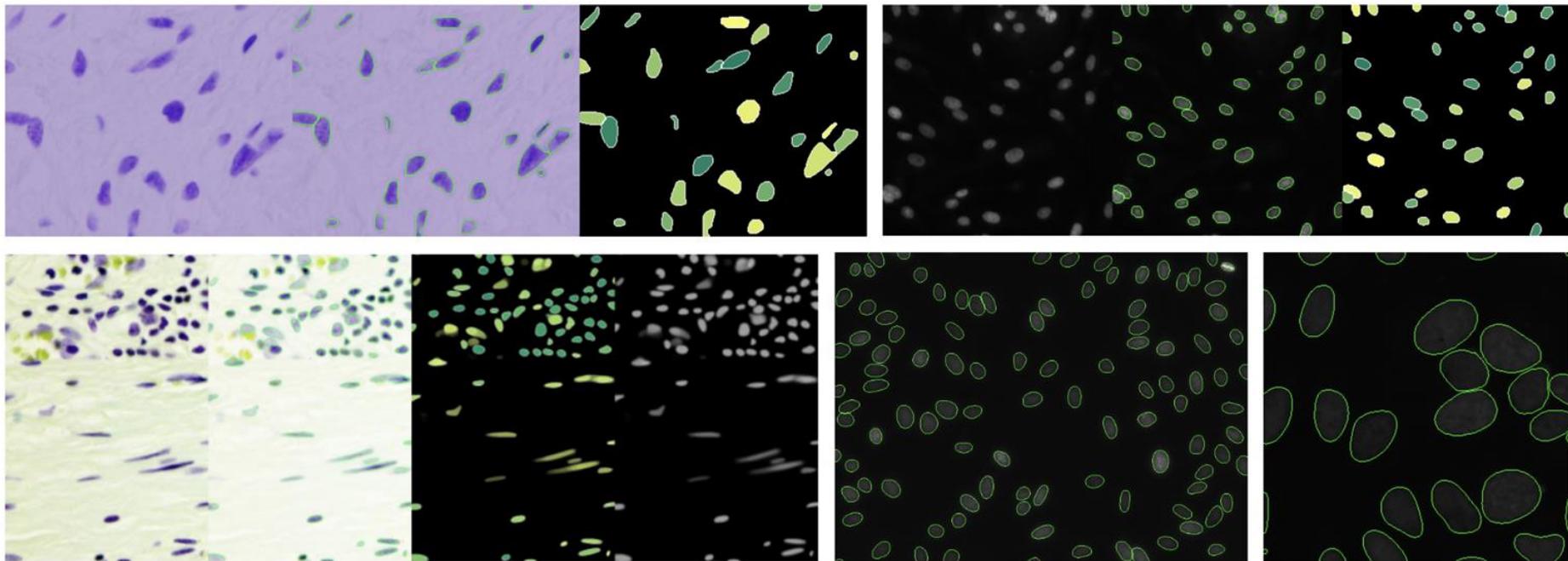
Model 1: Mask R-CNN -- region-based convolutional neural network



Model 2: Unet -- fully convolutional neural network

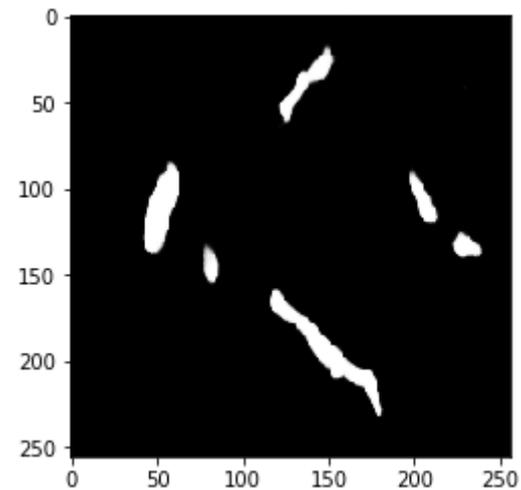
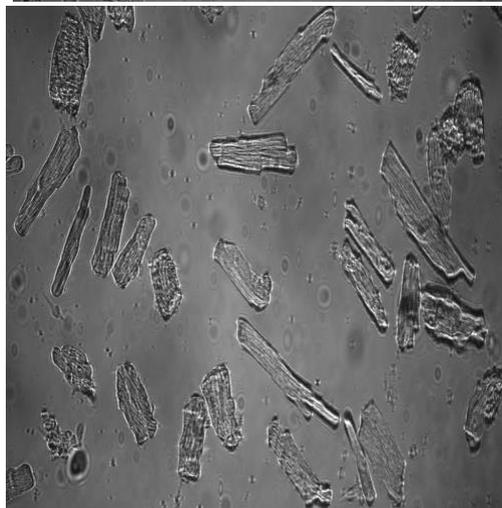
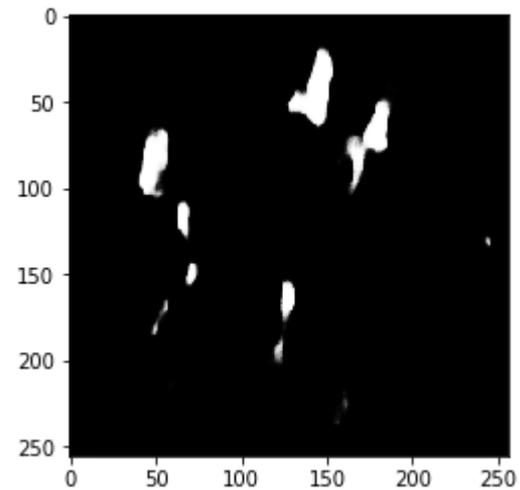


Application: Nuclei Segmentation



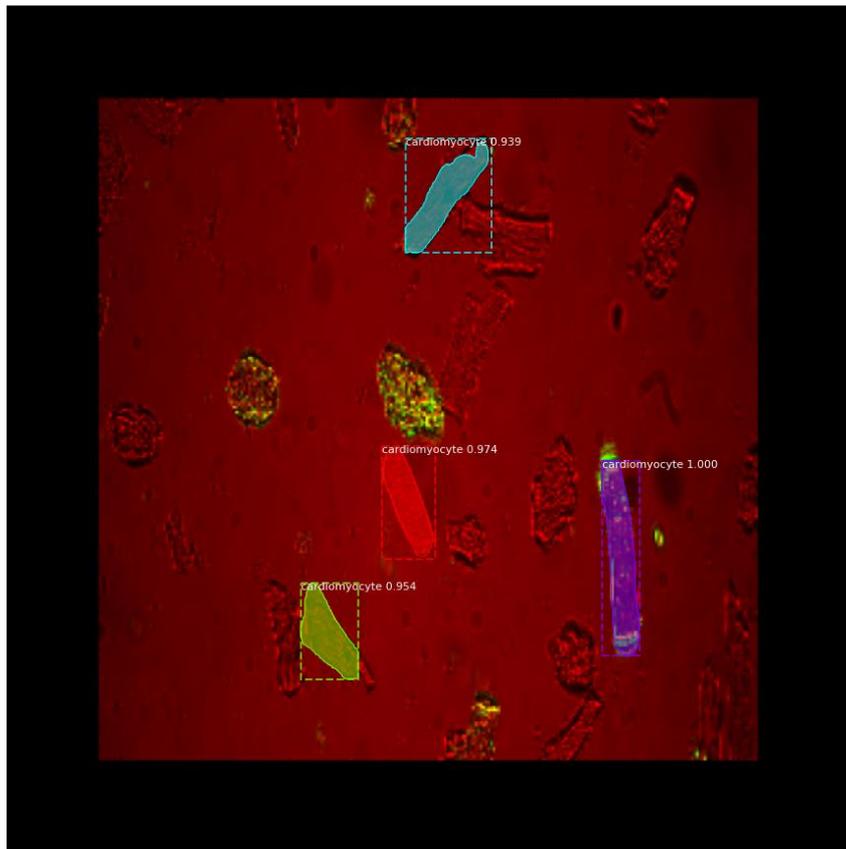
Ref: <https://towardsdatascience.com/instance-segmentation-automatic-nucleus-detection-a169b3a99477>

Unet

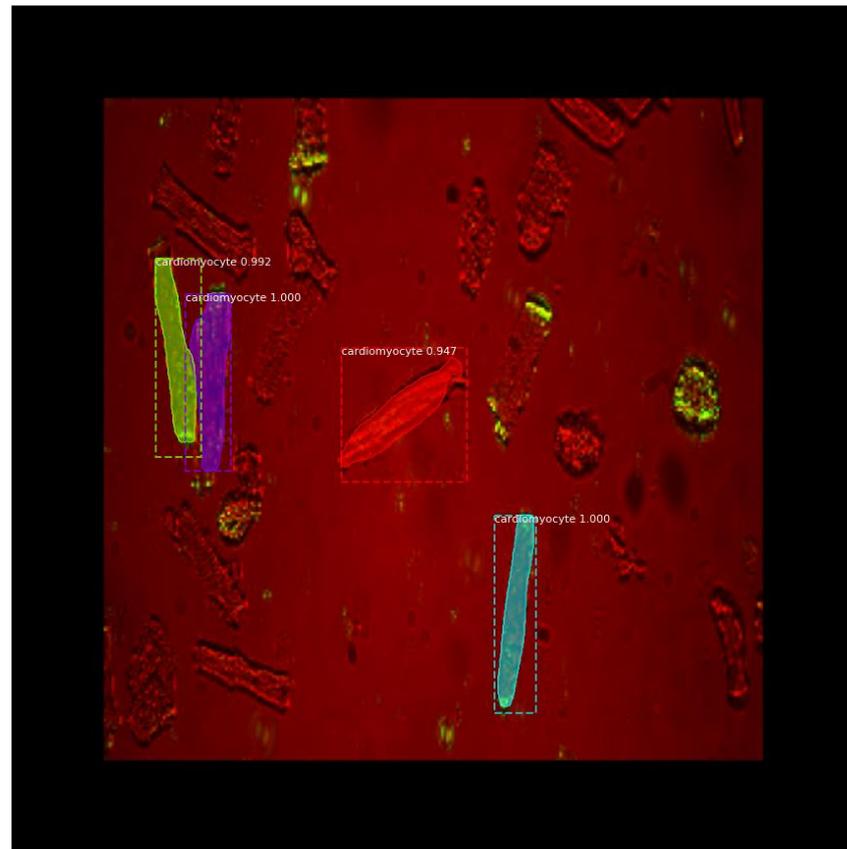


Mask R-CNN

Predictions



Predictions



Benefits of deep learning model:

Extract meaning from complicated data

Detect trends and identify patterns too complex for humans to notice

Learn by example

Speed advantages

Conclusion:

High throughput screening provides a higher statistical reliability of our output dataset.

With a good image analysis design, we can improve the quality of the results.

In the end, we could speed up the process of particular biological mechanism understanding, drug screening and disease modelling, etc.

Thank you for your attention!

Questions?