

Computer vision and machine learning algorithms for controlling an image-guided robotic microinjector

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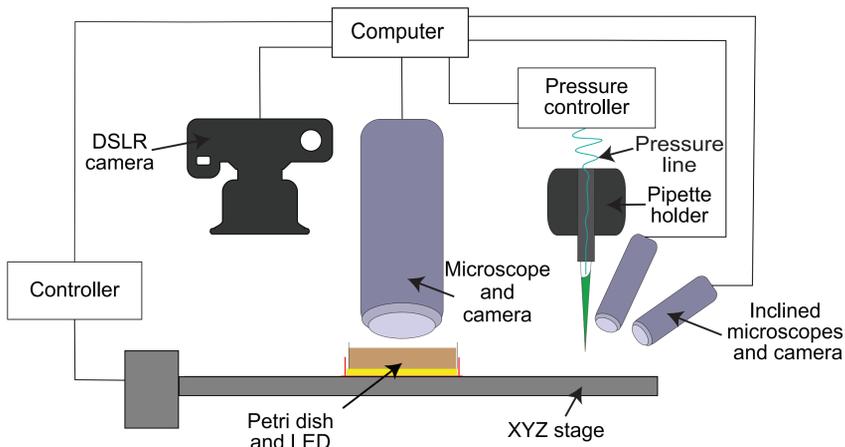


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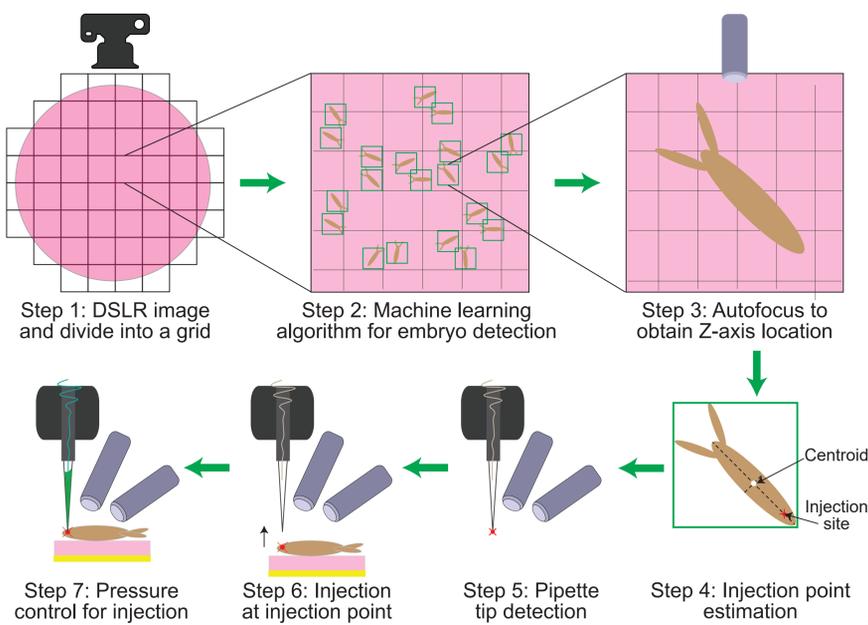
Abstract

The fruit fly, *Drosophila melanogaster*, is an extremely important model organism in biological research and has led to seminal discoveries in genetics. Microinjection is a powerful technique employed by *Drosophila* biologists to generate transgenic flies and perform gene editing and silencing. However, microinjection is an extremely tedious manual procedure, which makes it a critical bottleneck in the field and thus ripe for automation. Here, we present a computer-guided robot that automates microinjection into single *Drosophila* embryos. The robot uses a camera to capture a high resolution image of the entire petri dish containing embryos. This image is then divided into smaller images using an image processing algorithm. A machine learning algorithm is used to detect single isolated embryos in each image. Morphological algorithms are used to detect key anatomical features and ultimately injection points at the posterior of each embryo. This information allows us to determine the exact X and Y axis location of each injection. To determine the exact Z axis location of the embryo, we use computer vision algorithms to get the embryo in focus at a specific Z value. At this point, a series of transformation matrices is used to calculate the correct X, Y, and Z coordinates for the XYZ stage to guide each embryo to the micropipette for injection. Once the micropipette penetrates the embryo, a pressure controller is used to deliver femtoliters of DNA solution to each embryo. This process is repeated for every detected embryo on the petri dish.

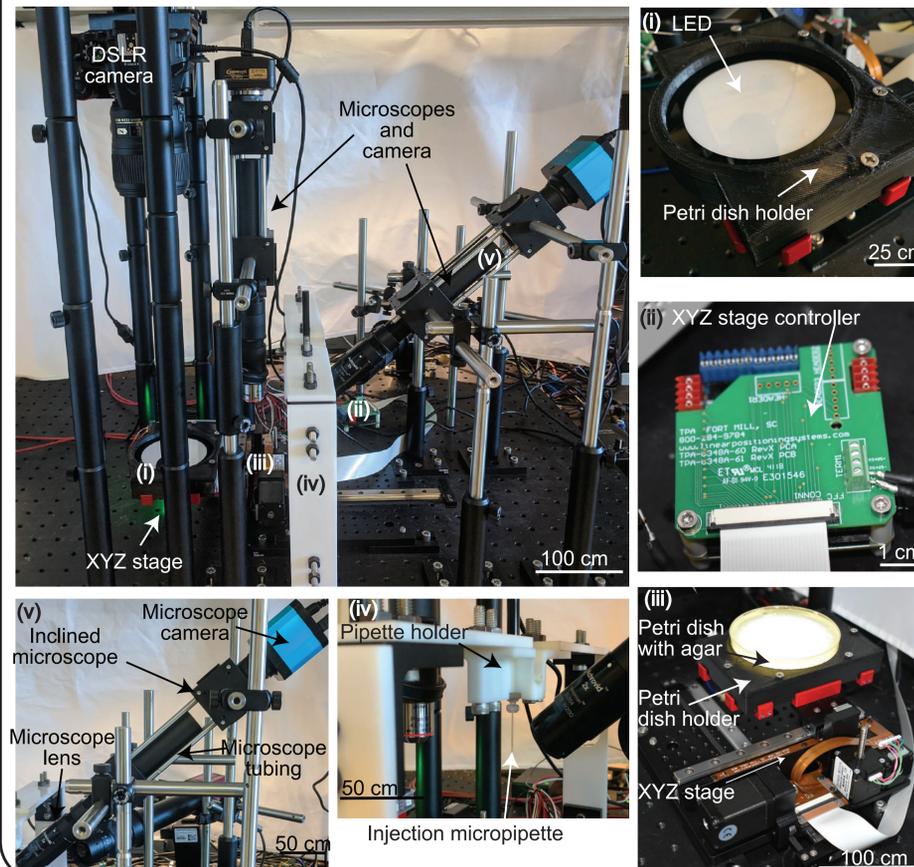
Schematic diagram: Hardware



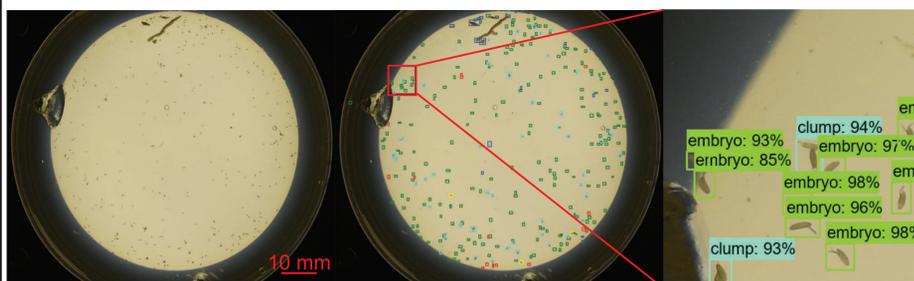
Schematic diagram: Operation workflow



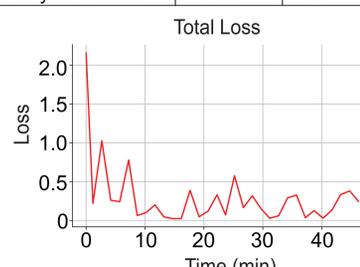
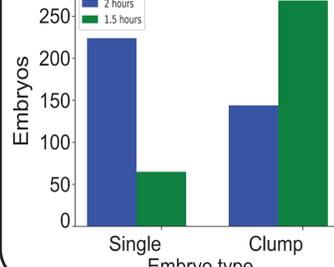
Hardware



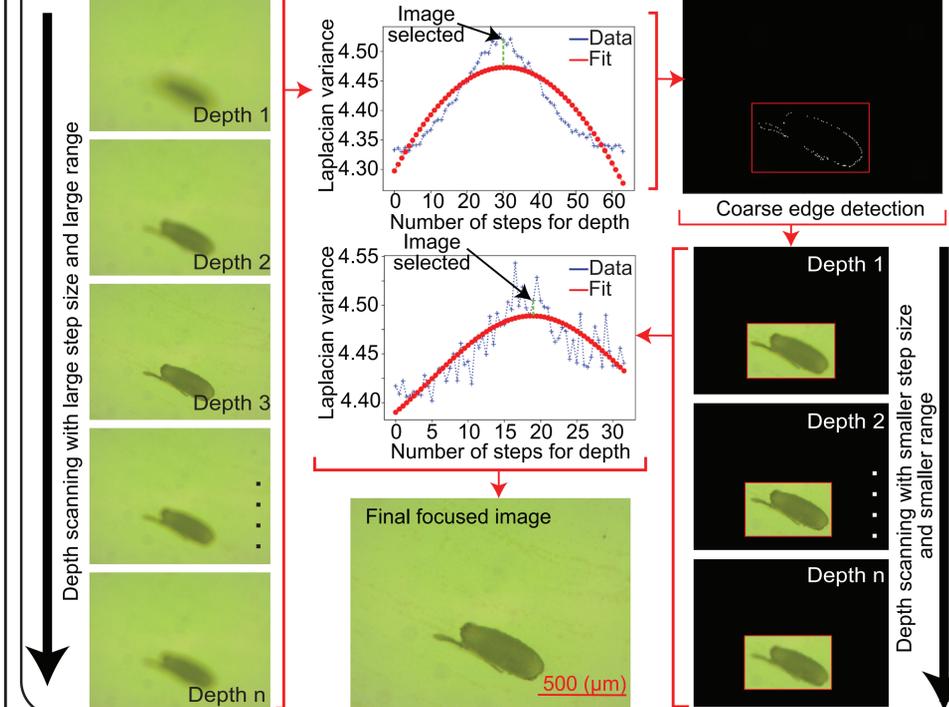
Machine learning algorithm



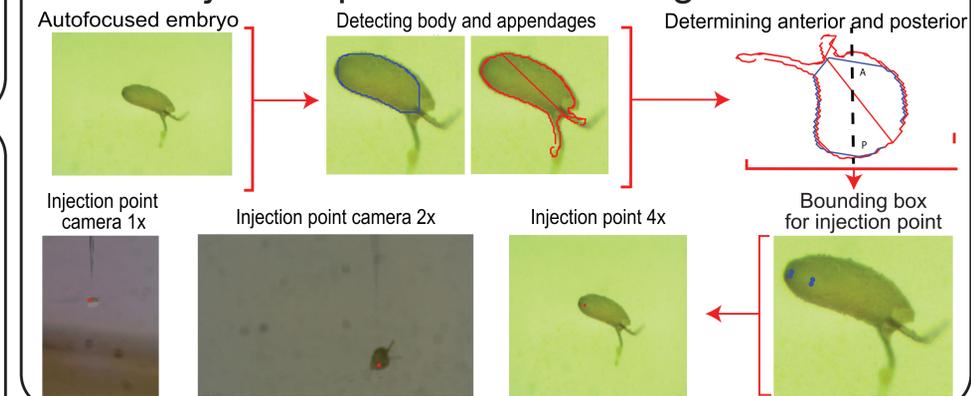
	Dish 1	Dish 2	Dish 3
% Success of ML algorithm	92%	95%	93%
% Single embryos detected	92%	97%	91%
% Clumps detected	94%	90%	96%
Total embryos estimate	368	71	334
Total embryos manual	362	59	365



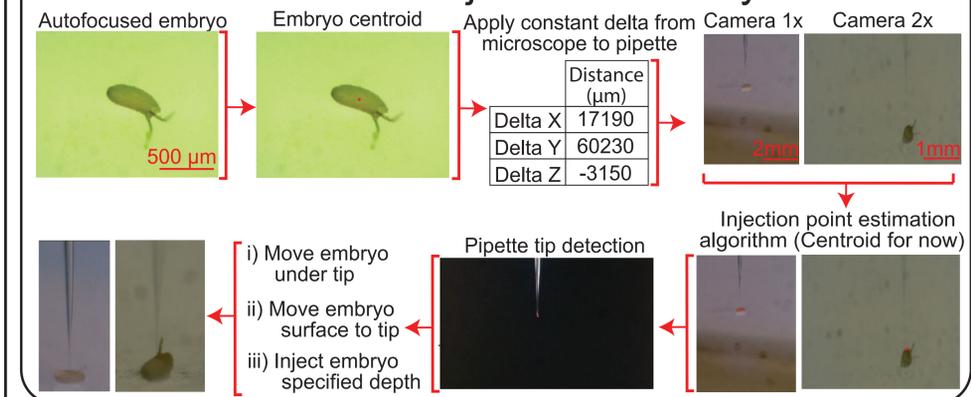
Autofocus algorithm



Injection point estimation algorithm



Automated injection of embryos



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