

An Automated Microscope for Screening *Ciona intestinalis* Embryos for Growth and Staining

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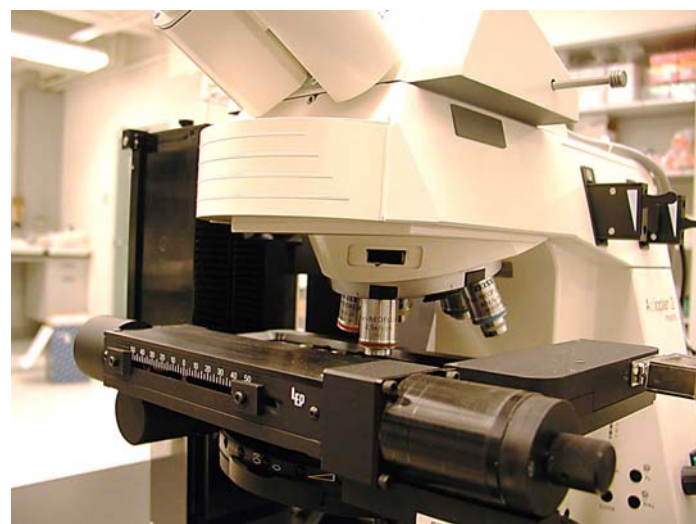
Introduction

The ascidian *Ciona intestinalis* is an experimentally accessible primitive chordate. The larval stage has the appearance of a tadpole with a tail containing a prominent notochord and dorsal tubular nerve cord. The tadpole is composed of 2500 cells with a well studied lineage. Embryogenesis is a rapid 18 hours. Transgenic DNA can easily be introduced with simple electroporation techniques. The Joint Genome Institute recently sequenced and published the 160 million base pair draft genome (Science, vol 298, 2002). Further experiments are ongoing to screen *Ciona intestinalis* embryos for development and staining patterns as part of a program to investigate gene regulatory networks.



Project Definition

The goal of this instrumentation project was to develop an automated microscope platform capable of digital image acquisition, detection, and screening of stained embryos. The system was designed to be easily operated by lab technicians. The target process throughput is 100 slides per day. Each slide has a cover slip/image area of 1" x 1.8" containing approximately 100-300 image objects. These embryo image objects have a distinct tadpole shape with an oval head of approximately 50-100 um in diameter and a total body length of approximately 300-400 um. The automated stage moves slides from the slide cassettes onto the XY stage. The microscope then scans each slide across the cover slip area collecting multiple images which are then stitched together for digital analysis. The image analysis software analyzes the complete image and locates well developed embryos that exhibit blue staining due to a LacZ reporter gene. The software selectively identifies stained tadpole image objects in the midst of undeveloped embryos and other image debris. The location of each positive identification is recorded and a report is generated to display positive hits. Slides can be reloaded onto the microscope and moved to pre-recorded positions of selected embryos for further study.



System Components

The microscope platform is a Zeiss Axioplan 2 IE motorized research microscope. This microscope platform was chosen based on previous positive experience with this model microscope as well as compatibility with other microscopes in the lab. It is configured with 10x oculars and Neo-Fluar 2.5x, 5x, 10x, 20x, and 40x objectives. Objects are illuminated with a tungsten lamp. The system is capable of fluorescence microscopy but this configuration has not yet been tested.

The automated slide stage is a Ludl Electronics Slide Handler System which consists of: Two 25 capacity slide cassettes mounted on a motorized vertical indexer, an XY stage, and a slide transfer mechanism to move the slides from the cassettes to the stage. The slide transfer mechanism is a horizontally moving "tongue" that moves beneath each slide and lifts the slide off of its cassette shelf. The vertical motion is through the cassette indexer. The slide is securely and positively located on the by stage with several cam actuated pusher levers. The system is controlled with a MAC 5000 controller. At the time of development this was the only commercially available slide loader/by stage available with this continuous run capacity. The stage is equipped with a bar code reader.

The digital camera is a Nikon DXM-1200 Digital Camera with 3840 x 3072 pixels. This camera was chosen because of it's similar performance specs to the Zeiss AxioCam HRc digital microscope camera. However the Zeiss camera could not be controlled with the ImagePro software package.

The microscope system is controlled by a Dell OptiPlex GX400 1.8 GHz computer with 2 Gb of memory with a Windows 2002 operating system.

Software Components

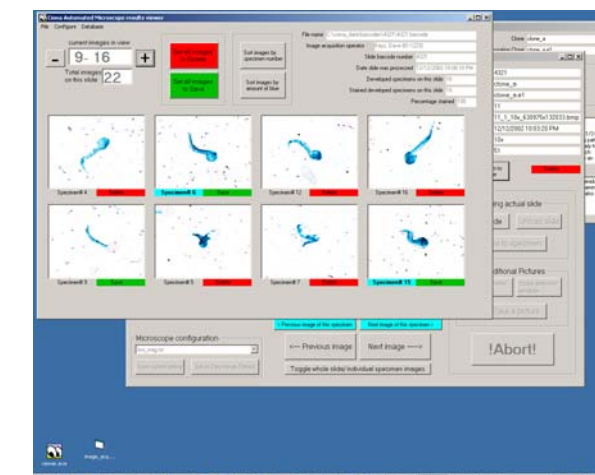
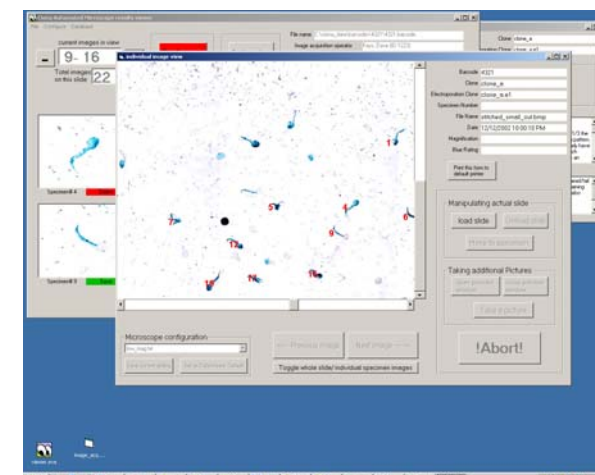
The image analysis software package is MediaCybernetics ImagePro Plus with the Scope-Pro Plug-in to control the camera image acquisition and transfer. ImagePro plus has similar image processing, measurement, and analysis functions as other commercial image analysis software packages but it also has extensive customization capabilities. The software development kit provides the functions for customization of ImagePro Plus as well as integration into a Visual Basic user interface and control environment.

Initial entry, initialization, and software configuration is through a Visual Basic user interface. In operation, the VB control program calls ImagePro Plus to execute slide handling, image acquisition, and digital analysis routines. File handling and database connectivity is handled through the VB control environment.

The image analysis problem is to detect tadpole shaped image objects (i.e fully developed embryos) and distinguish them from the surrounding background and associated objects such as unfertilized eggs, undeveloped embryos, dust, and debris. The screening detection standards are fairly relaxed. The goal is to find as many positively stained fully developed embryos as possible but 100% detection is not required. Thumbnail results are displayed and manually edited and sorted.

Test slides were developed which contained a representative training set of image objects and varying density of objects. The full set of objects was analyzed with the ImagePro measurement functions and the range of measurement results were analyzed to identify functions and ranges that would selectively detect the tadpole images. The functions currently in use are: polygon area, minor axis, convex perimeter, perimeter, radius ratio, roundness, length, and width.

Status: The system is currently assembled, installed in the laboratory, and is undergoing final testing



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