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A LOW-COST, TWO-AXIS, PRECISION ROBOT FOR AUTOMATED FLUORESCENCE IN-SITU HYBRIDIZATION ASSAYS

M.J. KULIK, D.S. SHENODA, C.R. FOREST

Department of Mechanical Engineering, Georgia Institute of Technology
Atlanta, GA, USA

ABSTRACT

Genetics research often relies on experiments that require repetitive, time-consuming handling of small volumes of liquid (1 mL) and biomass (10-20 μ L) such as fluorescence in-situ hybridization (FISH), β -galactosidase staining, immunohisto chemistry, skeletal and tunel assays. Often manual, these experiments are time intensive and error-prone. We report on the design, fabrication, and testing of a low-cost, two-axis, precision robot for FISH assays on whole mice embryos. The robot can complete 20 successive embryo immersions in unique isothermal solutions in minutes for 6 samples. Repeatability of the orthogonal axes is 66 and 214 μ m, near the measurement uncertainty limit and sufficient for operation. Accuracy is achieved by systematic error compensation. Low-cost and precision are obtained using design and manufacturing techniques and processes, resulting in a cost of 15% of comparable instruments (e.g., InsituStain, Intavis Bioanalytical Instruments). This design demonstrates a simple, automated platform to perform a typically manual experimental genetics technique.

INTRODUCTION

Genetics research is replete with experiments that require repetitive, time-consuming handling of small volumes of liquid (1 mL) and biomass (10-20 μ L). Manual labor in handling these volumes leads to increased errors in accuracy and repeatability, increased costs and time. For example, fluorescence in-situ hybridization (FISH) is a three day experimental procedure that primarily involves washing samples such as embryos in different solution baths for different periods of time, ranging from 5-120 min. Each of these three days involves an eight hour protocol to physically transport samples within 12 mm diameter baskets between solution baths.

Several automation solutions have been developed specifically for FISH including the InsituStain [1] and the Automated Immunohistochemical and In Situ Hybridization Assay Formulations Instrument [2]. In both cases, these instruments are prohibitively expensive and complex for many laboratories and experiments, approaching \$60,000. Further, the samples are held fixed while solutions are washed over them, which can damage tiny samples such as mouse embryos (14 μ L) of interest in this work. More generally, a variety of flexible, multi-axis robots have been developed to automate chemical and biological laboratory practices (e.g., [3-5]). In this work, we focus on low-cost and precision for a set of laboratory tasks that involve delicate samples being repeatedly immersed in solutions without damage, an unmet laboratory automation need. There is a need to deliver automated, precision controlled (100 μ m), low-cost (<\$8,000), low-volume (μ L-mL) instrumentation.

DESIGN

Mechanical

We report on the design and fabrication of a low-cost, two-axis, precision robot for automated fluorescence in-situ hybridization assays, shown in Fig. 1. We designed the instrument to be capable of translating and immersing up to six baskets containing embryos between a total of 20 rows of wells amongst five well plates (2 mL/well, 24 wells/plate, Corning Inc.) at room temperature.

As shown in Fig. 1, both axes are driven by stepper motors with 800 micro-counts/revolution (Danaher Motion, CT Series, CTP10ELF10MMA00). The horizontal (x-axis) utilizes a lead screw transmission (M6, 1 mm pitch) resulting in 1.25 μ m resolution, 500 mm range. The vertical assembly (see Fig. 1, Fig. 2) utilizes a rack and pinion drive with 100 μ m resolution, 20 mm range.

To reduce cost, we have attempted to minimize part count, keep the form factor compact and rectilinear, use uniform fasteners (M3), use off-the-shelf parts (e.g., lead screw all-thread), use the same motors, bearings (nylon bushings), and ground steel guide rods for both axes, and mass align the well trays with a pair of orthogonal, spring-loaded constraint plates rather than individual positioners. Further, the structure of the instrument is entirely comprised of extruded aluminum sections (80/20 Inc., 25-2525) with associated fasteners and brackets. Low cost, versatile manufacturing processes such as waterjet cutting, milling, and turning were used throughout to reduce fabrication costs.

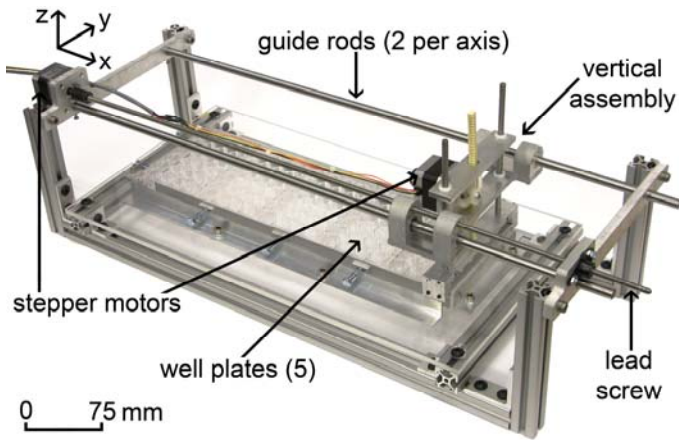


Figure 1. Photograph of a low-cost, two-axis, precision robot for automated fluorescence in-situ hybridization assays.

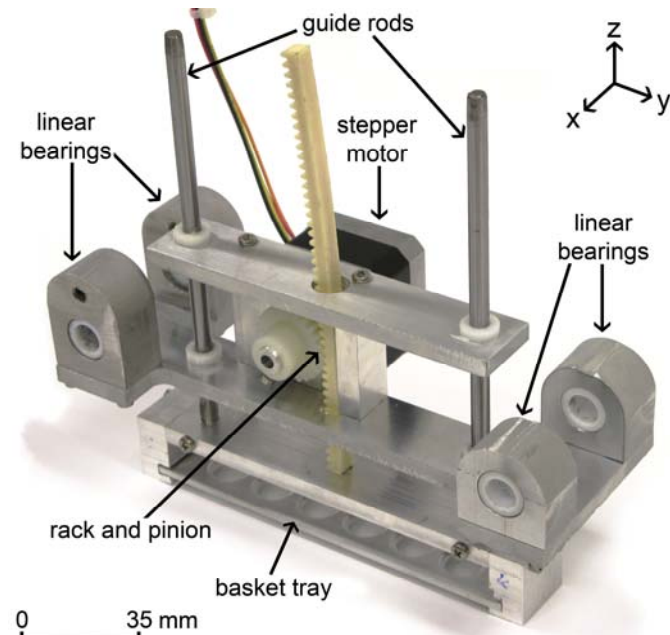


Figure 2. Photograph of the vertical assembly within the robot instrument for raising and lowering baskets into wells for hybridization assays.

Baskets (*not shown*) are held in the basket tray which is constrained by spring-loaded ball-nose plungers within the vertical assembly. These 14 mm diameter baskets are porous with a 100 μm mesh screen. Embryos are loaded into the basket manually.

The wells within the well plates are filled with solutions using pipettes. Thus the volume accuracy and precision are controlled by the pipetting technique, typically 1%. The volumes required are less than comparable instruments in which solutions are washed over the samples.

Software/Electronics

Automated control is performed using LabView to easily control the stepper motors through a programming interface and hardware data I/O boards. The simple graphical user interface (GUI), shown in Fig. 3, requires the user to specify the amount of time that the baskets dwell at each row of solution baths. The axes' acceleration, deceleration and velocity are also programmable.

The motors are controlled by individual drivers (Copley Controls, STP-075-07) via a PCI card (Copley Controls, CAN-PCI-02) installed in a computer. The control algorithm was calibrated to the spacing between adjacent wells and adjacent well plates.

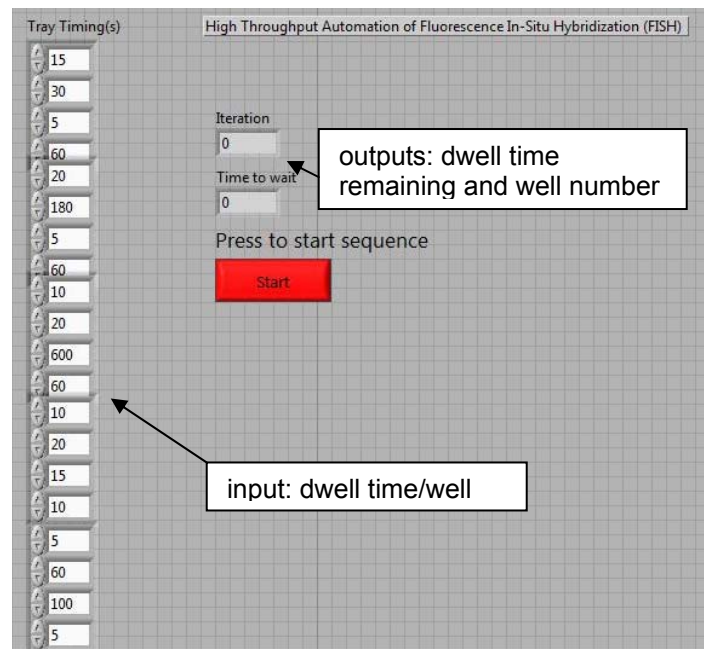


Figure 3. LabView interface, or GUI, for robot control.

RESULTS AND DISCUSSION

We fabricated the instrument as shown in Fig. 1 and performed a series of experiments to characterize its performance. The x-axis and z-axis were programmed to travel at 5 mm/sec and 10 mm/sec respectively across the range of motion. The instrument moves between successive wells as intended, requiring a few minutes for a full run with 1 sec dwell time/well. The positioning accuracy required is determined in the x-axis by the difference in the radii of the well and basket, 2 mm, and in the z-axis by the depth of the solution and height of the embryo. For full immersion, this z-axis accuracy requirement is 1 mm. These accuracies were achieved by calibration of the well and basket positions prior to the start of an experiment. The repeatability of positioning in the axes was determined by repeatedly (20 trials) moving to a well position from a "home" position and measuring the actual basket location. Uncertainties in this repeatability measurement were determined by repeatedly measuring basket position without moving it between measurements. The stated repeatability and uncertainty is the standard deviation of these measurements. The uncertainty is attributable to errors in positioning of a hand-held digital caliper with 10 μm resolution. Table 1 shows the measured repeatabilities and uncertainties for both axes.

Table 1. Repeatability and uncertainty of measurement for the instrument axes

	x-axis (horizontal) (μm)	z-axis (vertical) (μm)
repeatability	66	214
uncertainty	65	89

Measurement of the repeatability in the x-axis of the instrument is limited by the uncertainty—it is, at worst, 66 μm , which is sufficient for the operations required. In the z-axis, measurement of the repeatability is not limited by the uncertainty. The repeatability in this axis is 214 μm , largely due to the low-cost rack and pinion transmission.

The total instrument cost is 15% of comparable instruments (e.g., InsituPro VSi, Intavis Bioanalytical Instruments). Thus this design demonstrates a simple, automated platform to perform a typically manual, time intensive, error-prone, experimental genetics technique.

Future work will include independent well plate temperature control for more experimental freedom as

well as a web-accessible interface to further reduce cost and footprint by eliminating the need for a display. The current design meets the accuracy and repeatability requirements, while automating the process in a low-volume, low-cost design.

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