Machine Learning Automation of Supernatant Removal in Cellular Centrifugation Applications

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OVERVIEW

- **Purpose:** Automated supernatant removal in liquid handler's on-deck centrifuge.
- **Methods:** Compact centrifuge and image collection setup allows for AI/ML feedback to assist in cell pellet detection and inform supernatant removal.
- **Results:** 99.47% accuracy in ML detection of cell pellets within 0.008 seconds.

INTRODUCTION

On-Deck Centrifuges: Centrifugation compatible with automated liquid handling is desirable in bioprocessing to reduce cumbersome/time consuming manual processing.

Drawbacks with current approaches: Commercialized systems are unable to recapitulate visual feedback and careful pipette positioning that a trained practitioner can achieve. This could lead to device unintentionally aspirating precious cell samples. Even human operators can struggle to accurately identify cell pellets in low density samples.

Artificial Intelligence (AI) / Machine Learning (ML): Deep learning models excel at recovering complex information from biological datasets/images, showing excellent promise for this application [1-3].

METHODS: DEVICE

Device Design:

- **Two axis liquid handling system**, XZ axis linear motion, four pipette tips are able to transfer samples from a linear (well plate) to a square (centrifuge) format.
- **Compact Centrifuge** holds 4 tubes in a square with a spacing of 57 mm.
  - A DC motor with low cogging torque powers the centrifuge and enables stopping at particular positions (Maxon). Separate PIDs control the speed and angle of the centrifuge.
  - AS5600 magnetic absolute encoder was used to measure rotational speed and angle (AMS).
- **Microscope** integrated with AI/ML software.

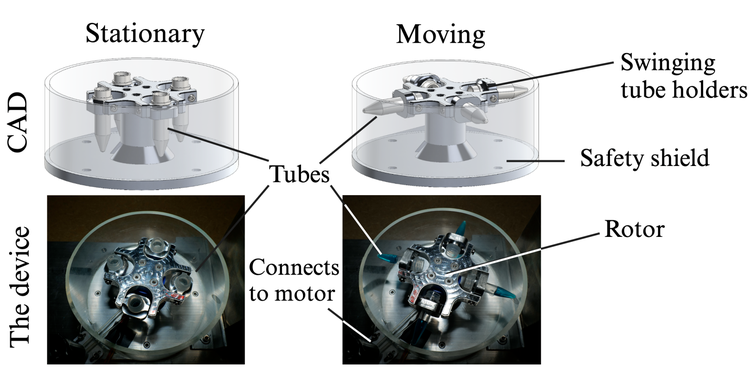

Figure 1. Schematic of the centrifuge.

Mechanical Testing of the Centrifuge:

- Java programs scripted using Processing IDE. The program controlled the centrifuge through a USB cable, and recorded performance data.
- **Position Convergence:** programmed to spin, rapidly stop, & turn to a specific angle; actual performance was measured over time.
- **Speed validation:** programmed to send a specific amount of power to the motor, stabilize for 15 s, and record a speed reading with the encoder. A tachometer was aimed at the centrifuge to validate the encoder.

RESULTS: DEVICE

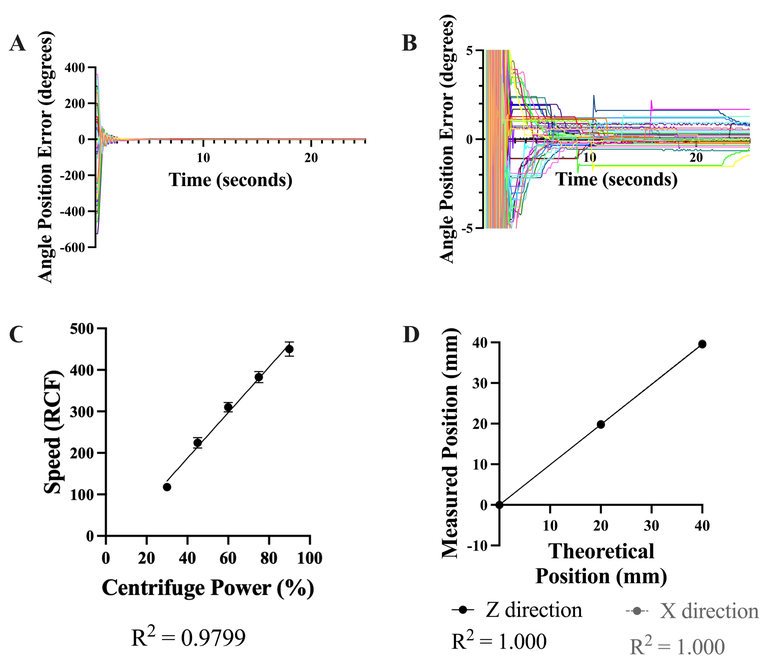

Figure 2. Quantification of centrifuge (A-C) and liquid handler (D-E) performance. A) and B) show target angle vs. measured angle of the centrifuge with N=43. C) Centrifugal speed vs. power. D) Positioning accuracy and E) error.

METHODS: AI/ML

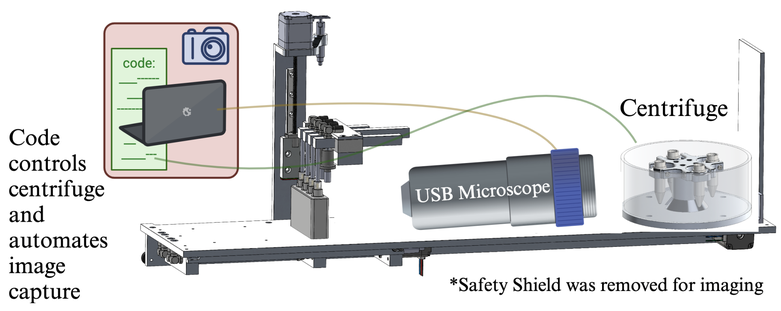

Figure 3. Schematic of the Image Collection Setup.

Cell Pellet Detection:

- **Mouse hepatocytes** (AML-12) seeded at 0.5 x10^6 cells per TC treated 25 cm^2 flask, were harvested after 3 days.
- **USB Digital microscope,** magnification: 1000X, resolution: 640x480p collected images.
- N = 504 images of cells (1.5 x10^6 cells/mL) were captured before and after centrifugation for 2.5 minutes at 300 x g.
- **Deep learning model VGG19** performed binary classification cell images, identifying pellets.
- 200 un-pelleted images, and 200 pelleted were used to train the model; it was tested on its ability to classify 50 images of pelleted and 50 of un-pelleted cells.
- **Manual Control:** Four human operators were tested for their ability to classify the same images on two occasions.

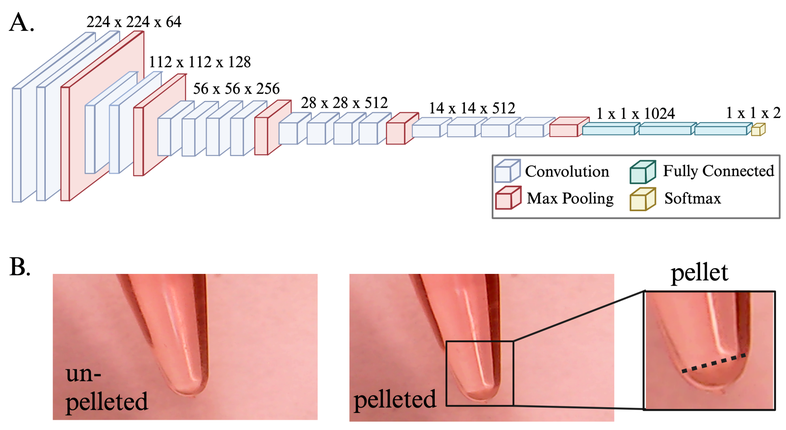

Figure 4. A) ML Model configuration, VGG19. B) Sample images for cell pellet detection: unpelleted on the left, and pelleted on the right, with a zoomed in section.

RESULTS: AI/ML

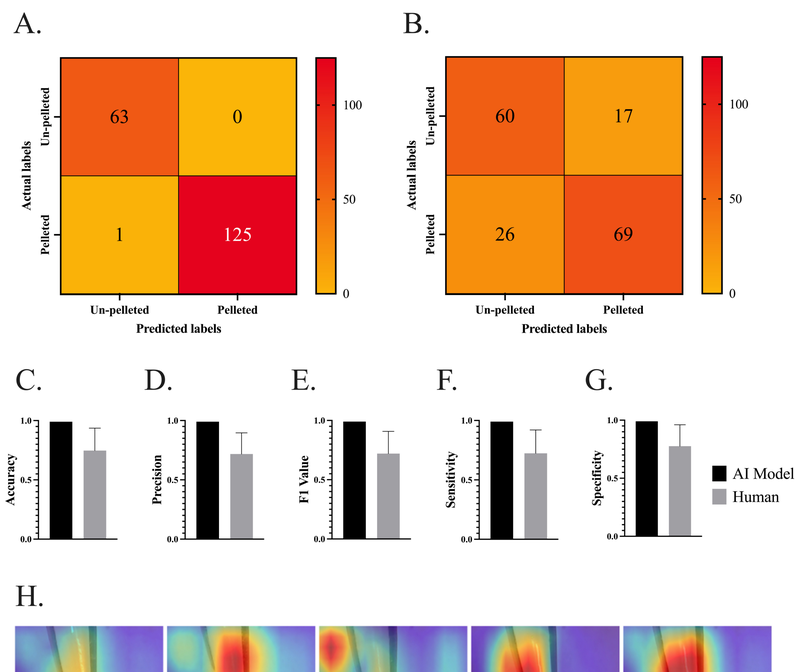

Figure 5. Pellet Recognition via machine learning: Confusion Matrix for the machine learning classifier (A) and human classification (B). Plots comparing Accuracy (C), Precision (D), F1 value (E), Sensitivity (F), Specificity (G) between the model and human classification. Heatmaps (H) showing the visual focus of the model.

CONCLUSIONS

Device calibration and validation:

- Centrifuge Position (angle): After 10 seconds settling, positioning error was <3 degrees across 43 tests, showing reliability.
- Centrifuge Speed: The centrifuge reached 500 rcf, well above the necessary speed for most cellular applications (300 rcf).
- XZ System movement: An R^2 = 1 was achieved for both axes when comparing actual movement to measured movement with 99% accuracy.

Deep Learning (AI/ML) model:

- Human operators had an average of 43 incorrectly classified images.
- The AI model only misclassified 1 image.
- Human classification took up to 10 seconds per image while the model classified each image within 0.008 seconds.
- Across all metrics, the model was observed to perform better than the human operators and with greater consistency.
- This demonstrates that automation offers benefits not only in time reduction for human operators, but that it can also reduce processing errors.
- In the future, our AI model may be improved to determine the height of the cell pellet to inform precision pipetting for supernatant removal.

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