UNIQUE APPLICATION OF MACHINE VISION IN FUTURE AUTOMATED IMMUNOASSAY SYSTEMS

Amit Sawhney, Marie Willette, Nicole Sovde, Favio Arita, Taka Mizutani
Beckman Coulter, Inc., Chaska, MN USA

BACKGROUND

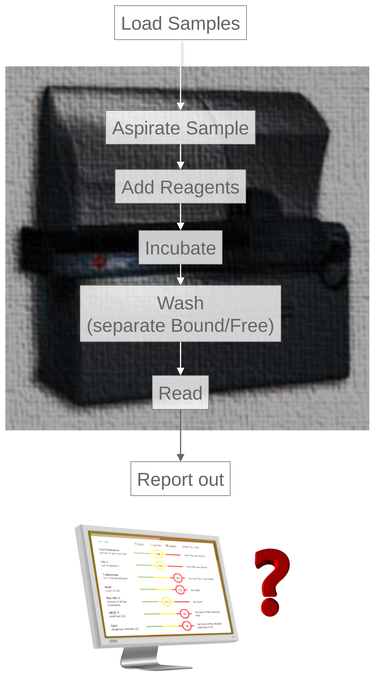
Conventional automated clinical diagnostic testing systems have various process monitoring functions (e.g. optical sensors, pressure sensors, thermistors, etc). These monitors check the integrity of instrument function, but most of those tools are limited to indirect sensing and do not directly monitor the critical elements of correct assay processing. This study examines the use of a new tool, machine vision, to directly monitor critical assay processing steps.

The purpose of this poster is to describe the methods and results of the following machine vision applications:

1. **Sample volume monitoring**: image and software algorithms measure the distance from bottom of tip to sample meniscus, using pixels, then convert measurement to volume
2. **Total reaction volume monitoring**: image and software algorithms measure the distance from bottom of vessel to reaction meniscus, using pixels, then convert measurement to volume
3. **Particle retention monitoring**: image and software algorithms execute measurement of gray-scale gradient and convert to particle concentration
4. **Residual volume monitoring**: image and software algorithms execute pattern matching and convert to residual volume

PROBLEM STATMENT

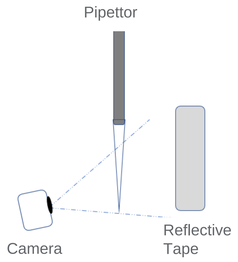
- When we see erroneous results, the system doesn't really provide information on each process inside instrument (= black box)



SAMPLE VOLUME MONITORING METHODS

Purpose:
- Camera at precise pipettor is used to detect tip presence/absence through all positions of the instrument and measure sample volume aspirated and dispensed
- Camera can tell software to flag samples out of volume specification (kinks in tubing, pump/valve failures, tip alignment) or if tip presence/absence is in an incorrect state (fail to discard tip, fail to pick up, drops)

Technology Used:

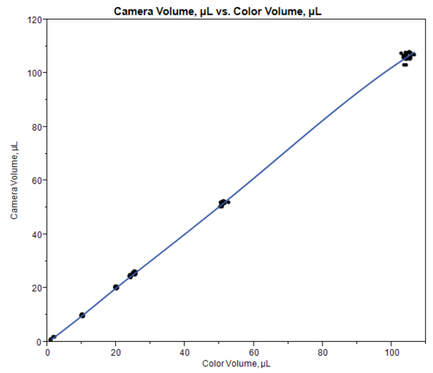


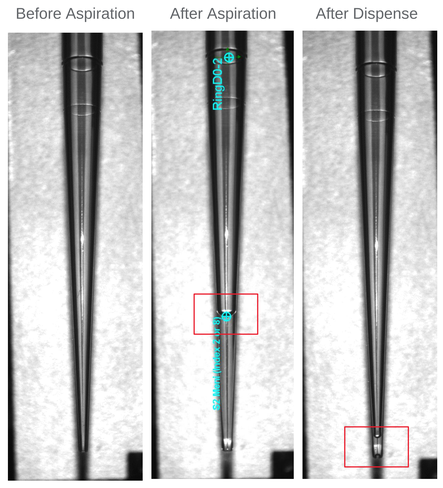
- Monochrome Camera - 1280x960, 130in CMOS, 40 FPS with a 6.2mm lens
- Reflective Tape Background

Experimental Methods:
1. Camera was calibrated on three instruments to convert pixel to µL. Spectrophotometer as measurement comparison was calibrated for 2 – 105 µL delivery with dilutions of Orange G solution.
2. Instruments were programmed to aspirate and dispense Orange G solution at 2 – 105 µL delivery.
3. After each aspiration and dispense, images were taken by the camera and vessels were transferred to area for collection
4. Upon collection, vessel concentration was measured on spectrophotometer

SAMPLE VOLUME MONITORING RESULTS

- Linear correlation plot across 4 instruments at different volumes (n = 24; 6 reps per volume) for non dilution and dilutions



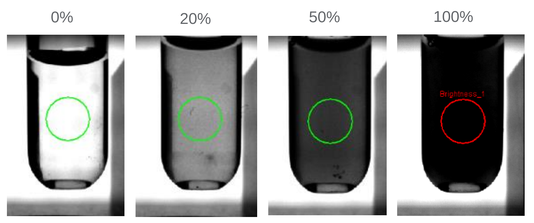


PARTICLE RETENTION MONITORING METHODS

Purpose:
- Camera at Wash Wheel is used to measure particle presence/absence and can be used as a service test to measure concentration.
- Particle concentration is a function of both pack fill and instrument (damaged magnetization, too much aspiration, misalignment)

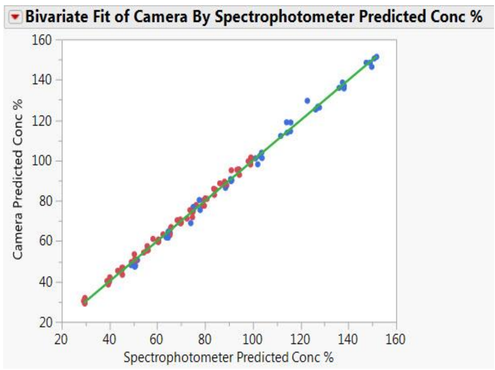
Technology Used:
- Same camera and setup as used in Reaction Volume Monitoring

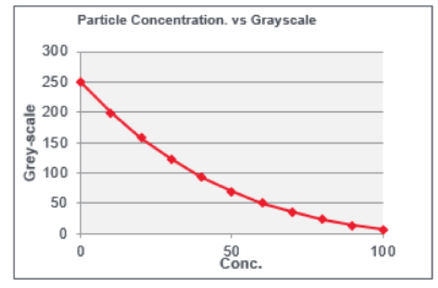
Experimental Methods:
1. The camera was calibrated by measuring known concentrations 0.1 mg/mL particles ranging from 0 – 250% to establish a histogram curve. Spectrophotometer as measurement comparison was also calibrated to the same concentrations.
2. Instrument was programmed to deliver known amount of fluid for 30 – 150% concentrated packs.
3. After each dispense and mix, images were taken by the camera and vessels were transferred to area for collection
4. Upon collection, vessel concentration was measured on spectrophotometer



PARTICLE RETENTION MONITORING RESULTS

- The bivariate fit between camera predicted %Conc and spectrophotometer predicted %Conc is linear with a strong correlation
- The mean variance across the range is 2.2 %CV for the camera and 1.5%CV for the spectrophotometer.

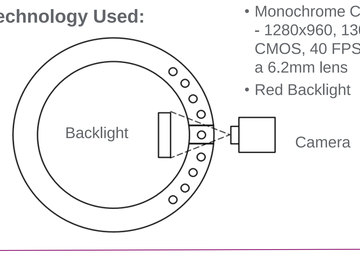




TOTAL REACTION VOLUME MONITORING METHODS

Purpose:
- Camera at Wash Wheel is used to measure substrate dispense volume before incubation and also assist with service and manufacturing for volume checks of pumps on the instrument
- If substrate volume is too low, test can report too low. Volume delivery of reagent and wash pumps out of specification (kinked tubing, pump/valve failure, obstruction) can also impact test results

Technology Used:

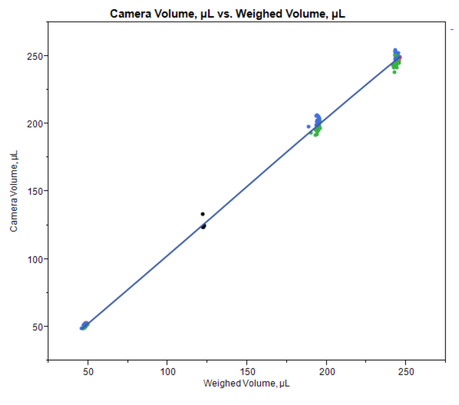


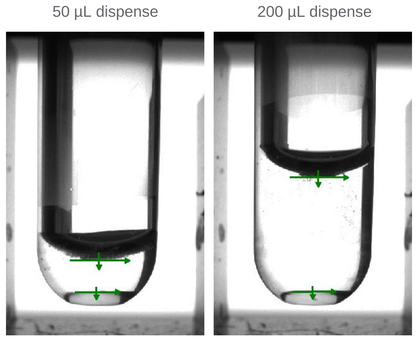
- Monochrome Camera - 1280x960, 130in CMOS, 40 FPS with a 6.2mm lens
- Red Backlight

Experimental Methods:
1. Camera was calibrated on three instruments to convert pixel to µL
2. Instruments were programmed to deliver 50 – 250 µL of Wash Buffer II solution and loaded with pre-weighed vessels
3. After each dispense and mix, images were taken by the camera and vessels were transferred to area for collection
4. Upon collection, vessel weight after dispense was measured
5. Actual volume ((post-fluid weight – pre-fluid weight)/density) was determined after measurements

TOTAL REACTION VOLUME MONITORING RESULTS

Linear correlation plot of camera volume vs weighed volume 50 – 250 µL (n=20 per volume)



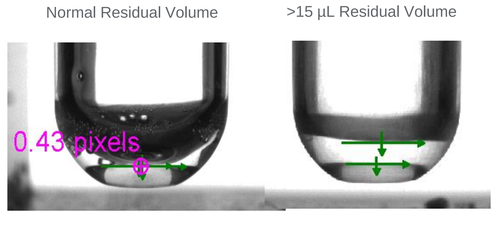


RESDUAL VOLUME MONITORING METHODS

Purpose:
- Camera at Wash Wheel is used to measure residual volume left in vessel after reaction build, wash of particles, and aspiration of excess fluid
- If residual volume is too high (aspiration probe misalignment, obstructed tubing, vacuum failure), tests can report too low

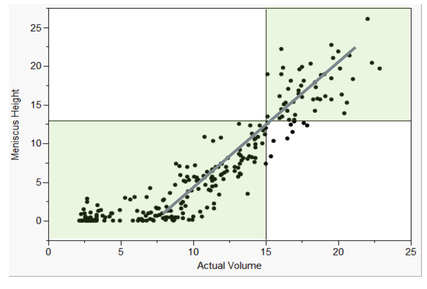
Technology Used:
- Same camera and setup as used in Reaction Volume Monitoring

Experimental Methods:
1. Instruments were programmed to inducing an aspiration failure mode by misalignment of aspiration probes at different heights and loaded with pre-weighted vessels
2. At each aspiration height, images were taken by the camera and vessels were transferred to area for collection
3. Upon collection, vessel weight after dispense was measured
4. Actual volume ((post-fluid weight – pre-fluid weight)/density) was determined after measurements



RESIDUAL VOLUME MONITORING RESULTS

- This study showed that at greater than 15 µL, the camera was able to correctly determine too high volume with 95% accuracy.



CONCLUSION

Summary of accuracy and capability of the four applications:

1. Sample volume detection range was demonstrated to be 2 to 100 µL with ± 10% accuracy capability
2. Reaction volume detection range was demonstrated to be 50 to 250 µL with ± 10% accuracy capability
3. Residual volume detection was demonstrated with a minimum volume of 15 µL capability
4. Particle retention range of 40-100% retention was demonstrated with ± 5% accuracy capability

This study confirms the performance of machine vision for direct measurement of various sample reaction volumes. Proactive and direct assessment will potentially permit future immunoassay systems to notify users of processing errors, permitting earlier detection and resolution, and lowering risk that erroneous but believable results will be reported.