Portable Optical Nanoshell-based Whole Blood Immunoassay

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Healthcare in Long-term Spaceflight

- In long-term space flight, robust and reliable medical diagnostics are needed to maintain health of astronauts

- **Our goal is to implement a versatile and reliable diagnostic system for long-term space flight**
  - Versatile: ability to monitor a wide range of medical conditions, including bone loss and cardiac conditions
  - Reliable: limited false positives and negatives

Background on Immunoassays

- Immunoassays are highly specific and accurate diagnostic tools
  - Can monitor and detect bone loss, risk of heart attack, and the presence of many diseases
  - Challenging to implement in space where lab space is limited
  - Requires a lot of technical expertise and specialized equipment

Traditional	Nanoshell
• Poor whole-blood detection	• Accurate analyte detection in whole-blood
• Time-consuming	• Fast, quantitative results
• Extensive sample preparation	• Minimal sample preparation
• Technical expertise required	• Easy to run
• Expensive equipment	• Inexpensive equipment

The Proposed Solution

- Build a portable, low cost optical device for use with the nanoshell-based immunoassay
  - Maintains advantages of traditional immunoassays while eliminating key disadvantages

- DESIGN GOAL: Construct a monochromatic optical device for the measurement of nanoshell aggregation in the presence of analyte in whole blood
  - Choose appropriate light source: Nanoshell peak extinction within a well-defined range
  - Construct robust signal conditioning circuitry: Success of device relies on ability to detect aggregation of nanoshells

Design Objective	Target Criterion
Accurate and Precise	Error ≤ 10%; variation ≤ 10%
Immunoassay sensitivity	≤ 5 ng/mL
Immunoassay specificity	≥ 97%
Device cost	≤ $200
Immunoassay cost	≤ $10/use
Device portability	weight ≤ 7 lbs. dimensions ≤ 3in x 5in x 7in
Low power usage	≤ 9V
Rapid assay run time	≤ 15-30 min

Nanoshell-based Immunoassay Schematic

- Silica-gold nanoshells can be fine-tuned to specifications
  - Functionalizable: Ability to immobilize antibodies that recognize specific analytes in blood

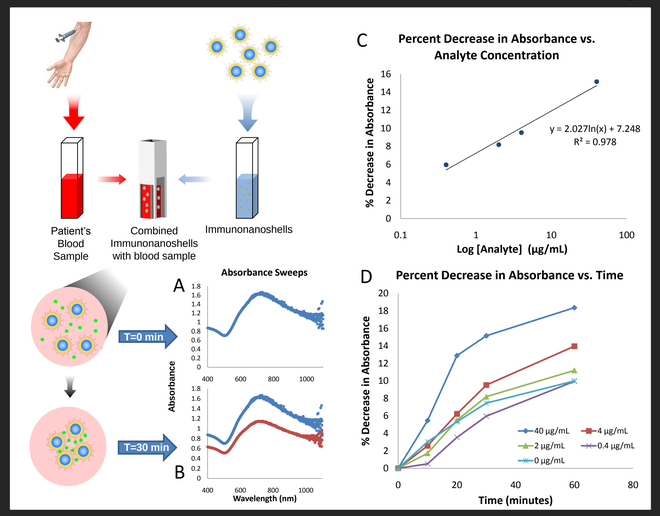

Figure 1. (A) Absorbance sweep before analyte addition. Peak absorbance is estimated at 725 nm. (B) Absorbance sweep 30 minutes after analyte addition. (C) Percent decrease in absorbance dependence on analyte concentration, fitted to a log-linear relationship. The equation shown corresponds to the "correlation factor" that can be used in place of a standard curve. (D) Percent decrease in absorbance measured for various analyte concentrations over 60 minutes.

- For proof-of-concept, we targeted the rabbit IgG protein in buffered solution. For practical use, antibodies specific to disease pathogens or biomarkers can be used to detect for infections or health conditions
  - Nanoshells conjugated with anti-rabbit IgG aggregate in the presence of rabbit IgG
  - Aggregation detected as the decrease in the peak extinction of the sample
  - Quantification of analyte concentration can be performed

Nanolyte Prototype

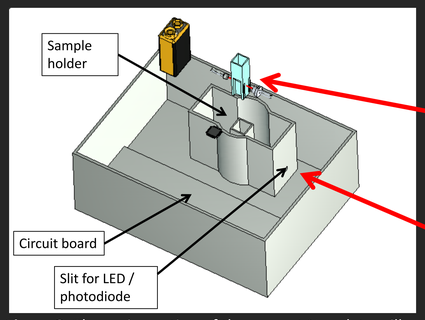

Figure 2. The main portion of the current Nanolyte will contain circuitry and systems for taking optical measurements from the sample.

USING THE NANOLYTE:

Obtain blood sample & dose of nanoshells targeted for condition of interest.

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Mix blood sample & nanoshells and load into Nanolyte.

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Press "START" button on user interface.

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Nanolyte shines 780nm light through the sample.

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If condition of interest is present, Nanolyte detects decrease in sample absorbance within 15-30 minutes.

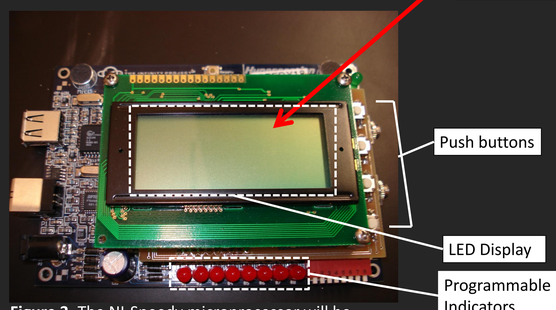


Figure 3. The NI-Speedy microprocessor will be programmed with the software used to run the Nanolyte.

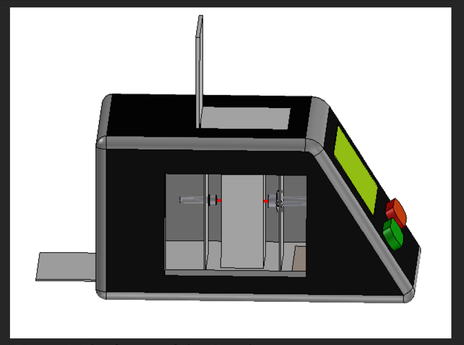
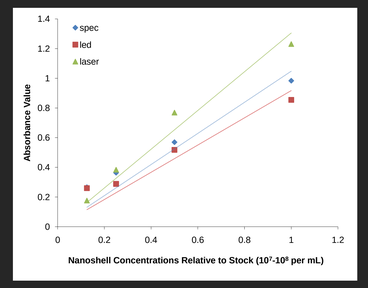


Figure 4. The future of the Nanolyte.

Testing & Results



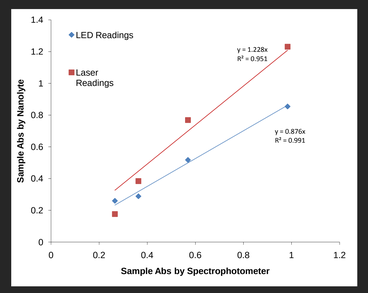


Figure 4. Comparing absorbance values from spectrophotometers, LEDs, and laser diodes. The LED shows the most similarity to the absorbance values from the spectrophotometer, which serves as the standard (student's t test, $p<0.1$). However, the laser diode and spectrophotometer and the laser diode showed significant difference in the absorbance readings ($p=0.3$).

Conclusions

- Aggregation of immunonanoshells with analyte causes decrease in absorbance proportional to analyte concentration
  - Log-linear relationship between the percent decrease in absorbance and analyte concentration
  - Detectable analyte range highly dependent on antibody concentration on nanoshell
  - Optimal assay run time is 30 min, but results may be visible within 10 min

- Optical device satisfactorily detects presence of an analyte

Future Work

- Long-term storage of nanoshells
- Continue to improve optical device performance
- Choose a smaller microcontroller to decrease device size

Acknowledgments

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References

1. Hirsch, L.R., et.al., *A Whole Blood Immunoassay Using Gold Nanoshells.* Anal. Chem., 2003. **75:** p. 2377-2381.
2. Hirsch, L.R., et.al., *Metal Nanoshells*. Ann. Biomed. Eng., 2006. **34:** p. 15-22.
3. Webster, J.G., *Medical Instrumentation: Application & Design (3rd ed.)*. New York, NY: John Wiley & Sons., 1997.