MICRODX—POINT-OF-CARE MICROPARTICLE DETECTION SYSTEM

By
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A Thesis Submitted to The W.A. Franke Honors College

In Partial Fulfillment of the Bachelors degree
With Honors in

Biomedical Engineering

THE UNIVERSITY OF ARIZONA

M A Y 2 0 2 2

Approved by:

Dr. Larry Head
Department of Biomedical Engineering

i. Abstract

The following is the Final Report for the *MicroDx—Point-of-Care Microparticle Detection System*. This system was constructed under the sponsorship of the Arizona Center for Accelerated Biomedical Innovation (ACABI). ACABI is based at the University of Arizona and specializes in developing solutions for unmet medical needs. One such medical need stems from platelet-derived microparticles (PDMPs).

PDMPs are generated when blood is exposed to supraphysiological shear forces, which typically occurs as a result of blood-contacting medical devices. After exposure to these extreme shear forces, platelets will shred apart into PDMPs, which range in size from 100-1000 nm. Elevated levels of PDMPs have been linked to overall increased thrombotic risk. There are currently no available point-of-care devices that can detect PDMPs within a blood sample, and the MicroDx system will be the first to do this.

There are 3 phases of the MicroDx system that are discussed more in-depth within the project description. The goal for the 2021-2022 Senior Design Project Team was to complete Phase I, where the average size of synthetic microparticles within a size range of 500-1000 nm is calculated. In conjunction with Phase II and III, the MicroDx system will be a low cost device that can function on a clinical tabletop and detect PDMPs within the size range of 500-1000 nm. The MicroDx system will use the optical technique of dynamic light scattering to analyze patient blood samples. As a result, the system will determine the average size and concentration distribution of the PDMPs within these blood samples. A GUI will be used to control the system and display the size and concentration results. Lastly, the system will be capable of creating, saving, and exporting up to five data files.

ii. Group Members

Name	Major	Contribution
Alyssa Barney	Biomedical Engineering	Alyssa worked closely with Megan, the software lead, on the development of the GUI for the MicroDx system. Additionally, she assisted with data collection for the project.
Cody Wilcox	Mechanical Engineering	Cody served as the procurement lead for this Senior Design project. In this role, he was in charge of placing purchase orders for any equipment, materials, or services that were necessary for the project. As the only mechanical engineer on the team, he was the sole creator of the 3D SolidWorks drawings for the MicroDx system.
Ethan Ross	Biomedical Engineering	Ethan served as the team lead for this Senior Design project. In this role, he acted as the point-of-contact for the project sponsor and mentor, scheduled team meetings, divided up project roles, and scheduled consultatory meetings with faculty and professional engineers. Additionally, he performed the majority of the research on the optical technique of dynamic light scattering and dedicated a great deal of time to collecting experimental data and troubleshooting the system. He was also the sole producer and editor of the Design Day Video that the team submitted for the project.
Giang Nguyen	Biomedical Engineering	Giang led the team in completing many of the additional deliverables that were requested by our sponsor, Dr. Marvin J. Slepian. The major deliverables included the submission of an abstract and proposal to the ASAIO Student Design Competition. Additionally, he kept track of the team’s progress in an application called SmartSheets and assisted with data collection for the project.
Megan Mickey	Electrical and Computer Engineering	Megan served as the software lead for this Senior Design project. In this role, she was in charge of developing a working GUI and creating software that could properly process the raw data collected via the avalanche photodiode. She acted as the primary data collector for the project because she had the most background in using an oscilloscope.
Rainee Meuschke	Biomedical Engineering	Rainee greatly contributed to the creation of different microparticle concentrations for the project. Additionally, she assisted with data collection for the project.

Project 22050

Final Report

MicroDx—Point-of-Care Microparticle Detection System

Alyssa Barney, Rainee Meuschke, Megan Mickey, Giang Nguyen, Ethan Ross, and Cody Wilcox

Project #	Date	Revision
22050	5/3/2022	1

iii. Table of Contents

1.0 Scope

1.1 Background

The following is the Final Report for the *MicroDx—Point-of-Care Microparticle Detection System*, which has been constructed under the sponsorship of the Arizona Center for Accelerated Biomedical Innovation (ACABI). ACABI is based at the University of Arizona and specializes in developing solutions for unmet medical needs. One such medical need stems from platelet-derived microparticles (PDMPs). PDMPs are generated when blood is exposed to supraphysiological shear forces, which typically occurs as a result of blood-contacting medical devices, such as stents, replacement heart valves, and catheters. After exposure to these extreme shear forces, platelets will shred apart into PDMPs, which range in size from 100-1000 nm. Elevated levels of PDMPs have been linked to overall increased thrombotic risk, which can lead to occluded devices, heart attacks, strokes, and even death. There are currently no available point-of-care devices that can detect PDMPs within a blood sample, and the MicroDx system will be the first to do this.

1.2 Overview

There are 3 phases of the MicroDx system that are discussed more in-depth within the project description. The goal for the 2021-2022 Senior Design Project Team was to complete Phase I, where the average size of synthetic microparticles within a size range of 500-1000 nm is calculated. In conjunction with Phase II and III, the MicroDx system will be a low cost device that can function on a clinical tabletop and detect PDMPs within the size range of 500-1000 nm. The MicroDx system will use the optical technique of dynamic light scattering to analyze patient blood samples. As a result, the system will determine the average size and concentration distribution of the PDMPs within these blood samples. A GUI will be used to control the system and display the size and concentration results. Lastly, the system will be capable of creating, saving, and exporting up to five data files.

1.3 References

Arizona Center for Accelerated Biomedical Innovation (ACABI). (2022). Project Sponsor. The University of Arizona.

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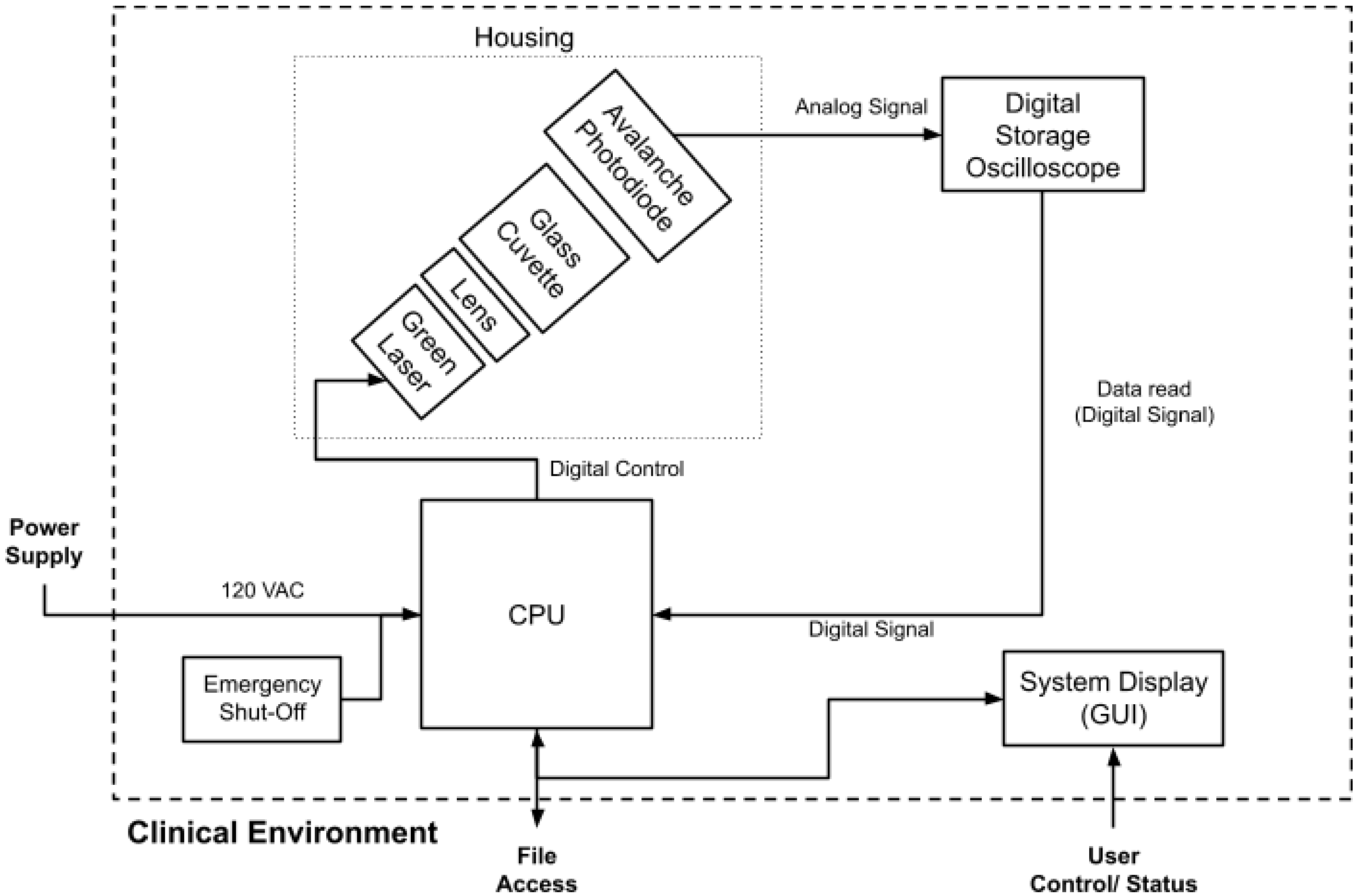
2.0 System Block Diagram

2.1 MicroDx System Block Diagram



2.2 Optical and Mechanical Design Concept

2.2.1 System Assembly

The overall system design is made up of a 520 nm compact laser module that shines through a plano convex lens with a focal point of 5 cm and then passes through an unfrosted cuvette containing the microparticle sample. The lens ensures that the light rays meet at the center of the cuvette to reduce the size of the beam before finally reaching the avalanche photodiode that reads the varying changes of light intensity over time with the help of an oscilloscope.

2.2.2 Optical Assembly

The four main optical components are held within a rigid assembly using 3 optical posts and pedestal holders, lens and kinematic mounts, and a 3D printed cuvette holder fixed with thru holes and a pedestal to provide the ability to accurately adjust the system to the necessary distance and height requirements. These parts are then bound to an aluminum breadboard with cap screws that can be adjusted if movement within the system has occurred.

2.2.3 Mechanical Assembly

This full assembly is then placed inside of an enclosure and closed off with a system access wall that has been 3D printed using PLA and painted inside and out with a matte black to ensure that outside light and reflections do not affect the performance of tests.

2.3 Computer/GUI Design Concept

The sample scatters light forming a speckle pattern. Dynamic light scattering reads the intensity of the speckle pattern over time and runs the data through an autocorrelation function which produces a graph that is averaged over many data samples to get more accurate results. Then a function that fits the curve is calculated and its coefficients are used to calculate the hydrodynamic size of the particles using the Stokes-Einstein equation. Smaller particles will become uncorrelated faster while larger particles will become uncorrelated more slowly. To accomplish this process, the Raspberry Pi is connected to the oscilloscope that reads this data and has the capabilities for file export to an exterior device. The software smooths the curve, runs it through an autocorrelation function, and calculates a curve to fit, which is where the diameter comes from. In conjunction, the GUI allows the user to run and save new samples as well as display the data and autocorrelation results. In addition, the user can power the system on/off using an emergency shut-off button on the GUI.

3.0 Technical Data Package

3.1 Project Description

As mentioned previously, the overall project has been divided into three phases: Phase I, Phase II, and Phase III. In Phase I, the system will be tested with polystyrene microsphere samples containing individual particle diameters of 500 nm, 720 nm, or 1000 nm. In this first phase, the system will only be required to determine the average size of the synthetic microparticles within these samples. In Phase II, the system will be tested with polystyrene microsphere samples containing mixed particle diameters of 500 nm, 720 nm, and 1000 nm. In this second phase, the system will be required to determine the average size and concentration distribution of the synthetic microparticles within these samples. In Phase III, the system will be tested with real patient blood samples containing PDMPs that range in diameter and shape. In this final phase, the system will be required to determine the average size and concentration distribution of the PDMPs within these samples. This was the first year of the MicroDx project. With the end of 2021-2022 academic school year, Phase I of the MicroDx system is now complete. The team has also completed research and proposed methods for the completion of future phases of the project.

NOTE: This Final Report contains all the information (requirements, models, verification tests, results, etc.) for the completed Phase I Model of the *MicroDx—Point-of-Care Microparticle Detection System*.

3.2 Functional Requirements

The *MicroDx—Point-of-Care Microparticle Detection System* shall meet the following functional requirements

1. The MicroDx system shall size platelet-derived microparticles in the range of 500-1000 nanometers with a tolerance of +/- 50nm and accuracy of 75%.
2. The MicroDx system shall be safe to operate and have a power shut off in case of emergency.
3. The MicroDx system shall store results from multiple samples.
4. The MicroDx system shall display the average size and autocorrelation graph on a GUI.
5. The MicroDx system shall be reusable in a clinical setting.
6. The MicroDx system shall accommodate synthetic microsphere samples.
7. The MicroDx system shall be more cost effective than flow cytometry.

3.3 System Verification Plan

3.3.1 Overview

This section will cover the plans made to verify that the system's components comply with the functional requirements agreed to with our sponsor and the current verification status of whether or not the system components comply with those requirements. Certain requirements only demand simple inspection or testing, while system requirements such as the testing and demonstrating the functionality of the optical system are much more important and require significantly more testing and research to meet.

3.3.2 Verification Flow Down Diagram

System Requirement	Subsystems			Limit/ Reference	Measured/ Predicted Value	Pass/Fail
	Optical	Computer/ GUI	Mechanical Frame			
1. Performance						
1.1 Size Accuracy (T/A): 75% accuracy	1. (T-Direct Flow) Particle sizes are distinguishable + or - 50nm	1. (T/A-Direct Flow) 75% accuracy		> 75% accuracy	1000 nm: 80% 720 nm: 78% 500 nm: 20%	**Pass/Fail**
2. Safety						
2.1 Emergency Shutoff (D): Completely shutdown system		3. (T-Allocated) Shuts off completely	1. (T-Allocated) Power switch on GUI	Drawing 100200	Button on GUI capable of performing this action	**Pass**
2.2 Safe Operation (I): Circuits grounded and covered		4. (I-Direct Flow) All circuits are grounded	2. (I-Direct Flow) All components are covered	Subassemblies will be inspected in final design	Safe operations inspected	**Pass**

System Requirements	Subsystems			Limit/ Reference	Measured/ Predicted Value	Pass/Fail
	Optical	Computer/ GUI	Mechanical Frame			
3. Data						
3.1 Data file (T): 5 files created	3. (T-Direct Flow) Send information to create files	5. (T-Direct Flow) Capable of creating 5 files		Drawing 100200	SW demonstrated	**Pass**
3.2 File Export (D): Export data files onto USB		6. (T-Direct Flow) Exportable files	3. (I-Direct Flow) USB port for flash drive access	Drawing 100200	SW demonstrated	**Pass**
3.3 Results (T/I): Display results		7. (T-Derived) Display results	4. (I-Direct Flow) House GUI	Drawing 100200	User interface displayed	**Pass**

System Requirements	Subsystems			Limit/ Reference	Measured/ Predicted Value	Pass/Fail
	Optical	Computer/ GUI	Mechanical Frame			
4. Design Features						
4.1 Size & Weight (A/I): < 20 pounds and no greater than 24” x 24”	4. (A/I-Allocated) < 5 lbs	8. (A/I-Allocated) < 5 lbs	5. (A/I-Allocated) < 10 lbs	Solidworks Models	15 lbs 14” x 10”	**Pass**
4.2 Cuvette (I): Hold cuvette sizes 12.5x12.5x45 mm	5. (I-Direct Flow) Mount does not interfere with laser		7. (I-Direct Flow) Accepts patient blood samples for system analysis	Drawing 100100	Cuvette standards met	**Pass**
4.3 Optical System (T/D): Create speckle patterns from microparticles	6. (T/D-Derived) Speckle created	9. (T/D-Derived) Calculate from optical data	8. (T/D-Derived) Does not interfere with laser	Drawing 100100	Speckle pattern created and analyzed using oscilloscope and autocorrelation curves.	**Pass**
4.4 Inexpensive (A): The MicroDx device shall cost less than $4000.	7. (I-Allocated) < $4000	10. (I-Allocated) < $4000	9. (I-Allocated) < $4000	Drawings 100100, 100200, 100300 and 100400	$2,644.30	**Pass**

3.3.3 System Requirement Verification Matrix						
Requirements	Verification Method				Verification Plan	
1 Performance	T	A	D	I	Description	Reference
1.1 Size Accuracy	X	X			(T) Run multiple samples and record the displayed average size and size distribution displayed for each sample. (A) Compare the displayed information to the known average size and size distribution of the microparticles in the sample and calculate if it is ≥75% accurate.	Test #: 2.4.1, 3.4.3 Data Sheet: 2.7.1, 3.7.3
2 Safety						
2.1 Emergency Shutoff			X		(D) Turn off power and verify that the system shutdown completely.	Test #: 2.4.2 Data Sheet: 2.7.2
2.2 Safe Operation				X	(I) Observe that all electrical and mechanical components are covered and protected from users. Ensure that all circuits are grounded.	Inspection Report: 1.1.1
3 Interfaces						
3.1 Data File	X				(T) Run a sample and check that a file is successfully created. Each file should contain the average size and autocorrelation curve.	Test #: 2.4.1 Data Sheet: 2.7.2
3.2 File Export			X		(D) Insert a flash drive into the USB port and export a specific file. Remove the flash drive and open the document on another device to check that it contains all of the sample information.	Test #: 2.4.2 Data Sheets: 2.7.2
3.3 Results	X			X	(T) Run the system and compare the results displayed on the GUI to the known values, checking that they are ≥75% accurate. (I) Observe if the GUI is displaying correctly.	Test #: 2.4.1, 3.4.1, 3.4.2, 3.4.3 Data Sheets: 2.7.1, 3.7.1, 3.7.2, 3.7.3 Inspection Report: 3.1.1

Requirements	Verification Method				Verification Plan	
4 Design Features	T	A	D	I	Description	Reference
4.1 Size & Weight		X		X	(I) Weigh and measure the system. Ensure that the system weighs no more than 20 pounds and that the system dimensions are no greater than 24” x 24”. (A) Calculate if it is under the limit.	Inspection Report 2.1.1
4.2 Cuvette			X		(D) Insert cuvette, confirm it lines up with the system, and remove the cuvette.	Part #: CV10Q35FA
4.3 Optical System	X				(T) Run the system and use software to display messages that the system is running correctly.	Test #: 1.4.1, 1.4.2, 1.4.3, 3.4.1, 3.4.2, 3.4.3 Data Sheets: 1.7.1, 1.7.2, 1.7.3, 3.7.1, 3.7.2, 3.7.3
4.4 Inexpensive		X			(A) Add the cost of each item in the system and compare it to the cost of current methods (a.k.a. flow cytometry).	Part #: 100100, 100200, 100300, 100400

3.4 Design Documentation

3.4.1 Indentured Document List

Part Number	Document		
100100	MicroDx System	100200	Computer Assembly
PL203	520nm Compact Laser Module with Bare Wire Leads	4296	Raspberry Pi 4 Model B
APD130A2	Si Avalanche Photodetector	2718	7” Touch Screen Display (GUI)
MK11F	Mini-Series Kinematic Mount for Ø11 mm	1294	SD/MicroSD Memory Card
LA1131	Plano Convex Lens	4377	Stand for Touchscreen Display
TR2	Optical Post, 2”	100210	DSI Ribbon Cable
TR3	Optical Post, 3”	MK1619-00B	Step-Up Power Module
UPH2	Universal Post Holder	100220	Jumper Wires
GDS-1054B	Instek GDS-1054B Digital Storage Oscilloscope	100230	Adapter Board
LMR1	Lens Mount	1995	Switching Power Supply
100100-ATP	MicroDx Acceptance Test Procedure	BS05T	Bitscope Micro

100300	Mechanical Assembly	100400	Prototype
100310	Cuvette Holder	B01N0L5HTF	5mW Green Laser Module
100320	Enclosure	532MD-1250-BL	10mW Green Laser Module
100330	System Access Wall	532MD-50-1348-CAB	50mW Green Laser Module
100340	Wall Supports	11051001	30mW Green Laser Module
100350	Wire Box Lid	PJ08	Raspberry Pi Camera, 5 Megapixel
MB8	Aluminum Breadboard	B07F1T97V4	Laser Module Stand
AP8E4E	Screw Adapter	FM-ALOX-120-8LBS	Aluminum Oxide
W252050	Washers	CS165CU	Zelux 1.6 MP Color CMOS Camera

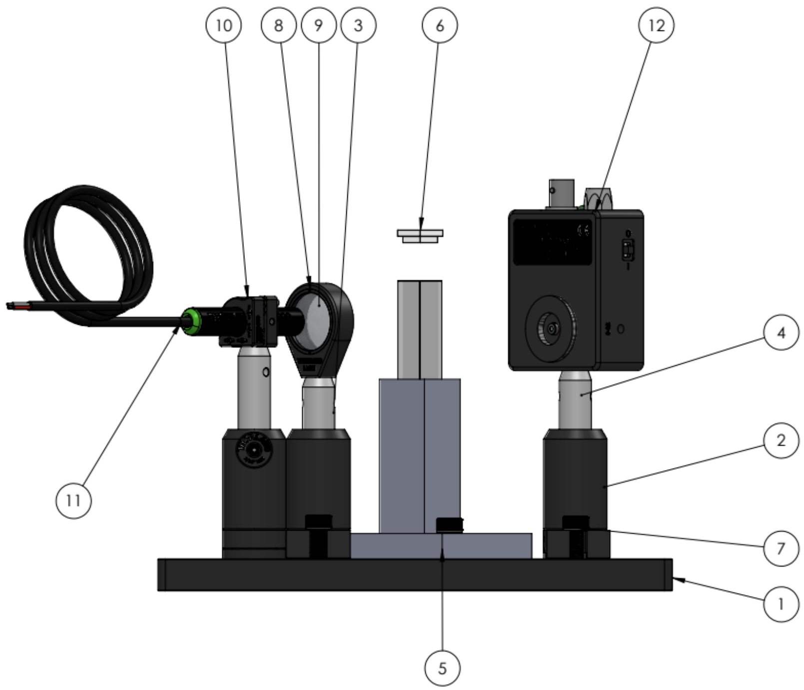
SH25S075	1/4"-20 Stainless Steel Cap Screw, 3⁄4” Long
SS252050	1/4"-20 Stainless Steel Setscrew, 1⁄2” Long

ER18	Cage Assembly Rod 18"
CP44F	30 mm Removable Cage Plate
CP36	30 mm Cage Plate, Double Bore
CPVMP	Vertical Cage Mounting Plate
PH2E	Pedestal Post Holder

100500	Testing Materials
3G010	Glass Cuvettes
PS004UM	Polystyrene Latex Particles, 4um
PS001UM	Polystyrene Latex Particles, 1um
PS720NM	Polystyrene Latex Particles, 720nm
PS500NM	Polystyrene Latex Particles, 500nm
PS100NM	Polystyrene Latex Particles, 100nm

100600	System Models
100610	Dynamic Light Scatter
100620	Autocorrelation
100630	Cost

3.4.2 MicroDx System (100100)



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1	MB8	ALUMINUM BREADBOARD	1
2	UPH2	UNIVERSAL POST HOLDER	3
3	TR2	2" PILLAR POST	1
4	TR3	3" PILLAR POST	2
5	100310	CUVETTE HOLDER	1
6	CV10Q35	CUVETTE	1
7	SS25S075	CAP SCREW	5
8	LMR1	LENS MOUNT	1
9	LA1131	PLANO CONVEX LENS	1
10	MK11F	KINEMATIC MOUNT	1
11	PL203	COMPACT LASER	1
12	APD130A2	AVALANCHE PHOTODIODE	1

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THREE PLACE DECIMAL ±0.001
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NEXT ASSY | USED ON
APPLICATION

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ENG APPR.		
MFG APPR.		
Q.A.		

COMMENTS:

PROJECT 22050

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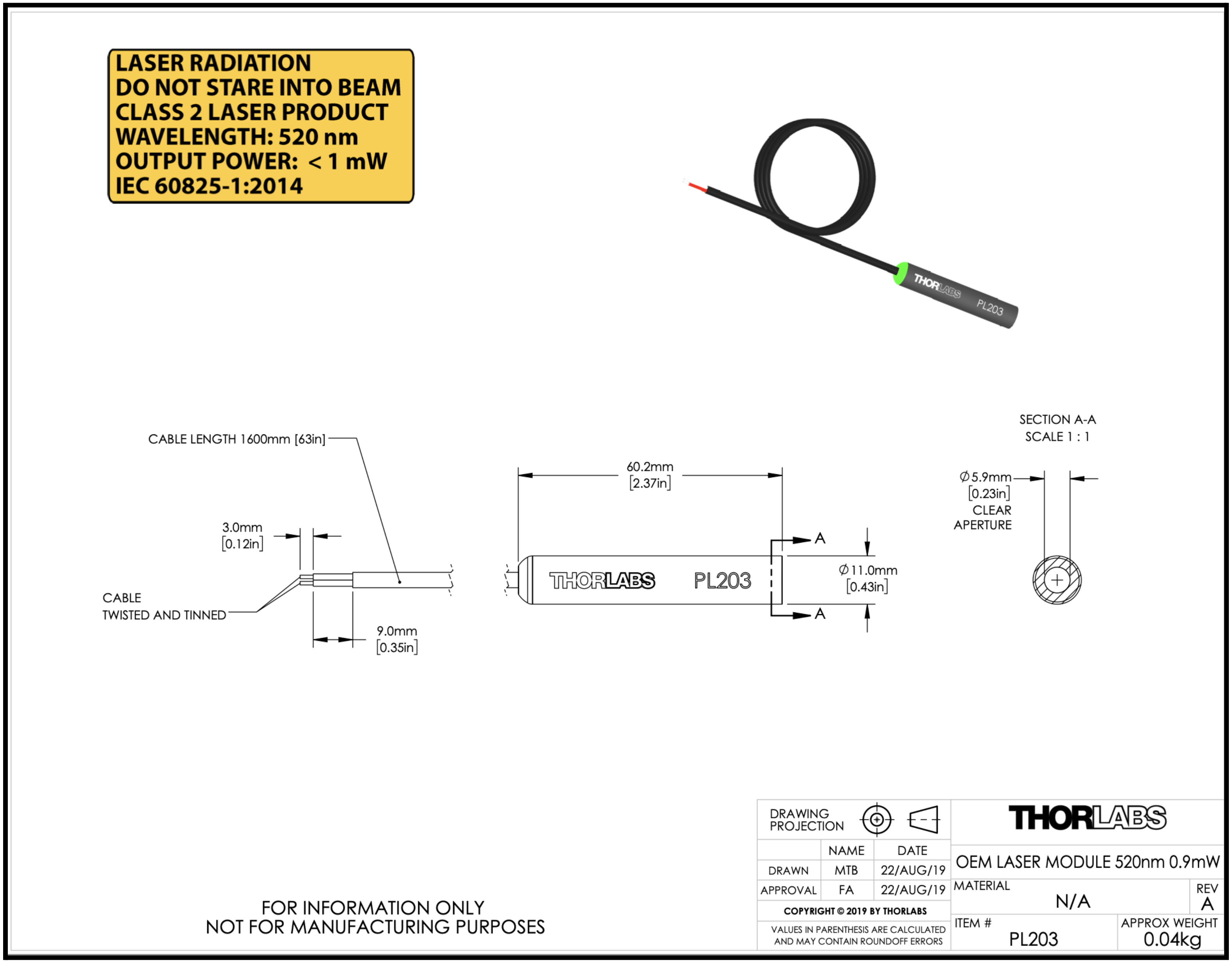
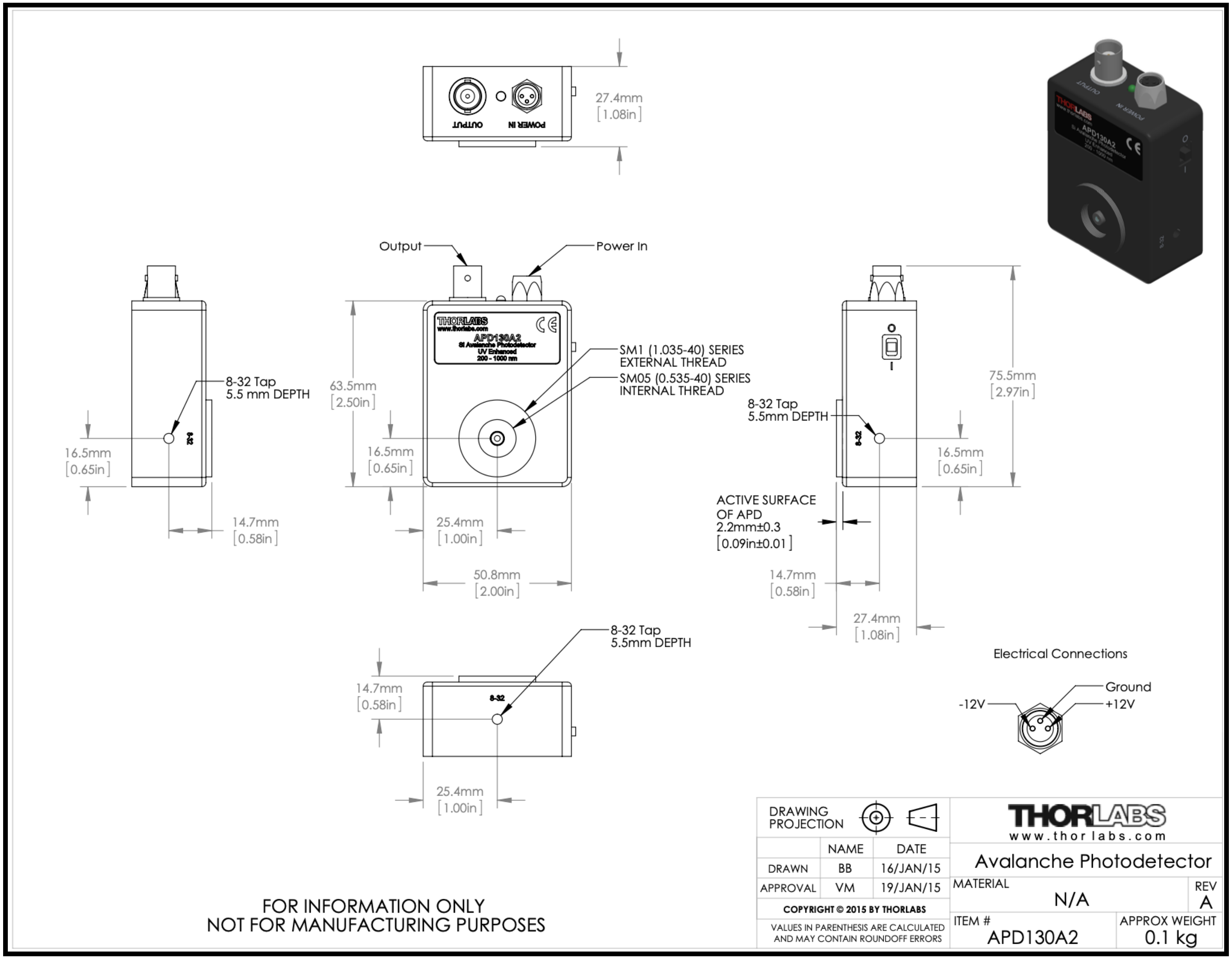
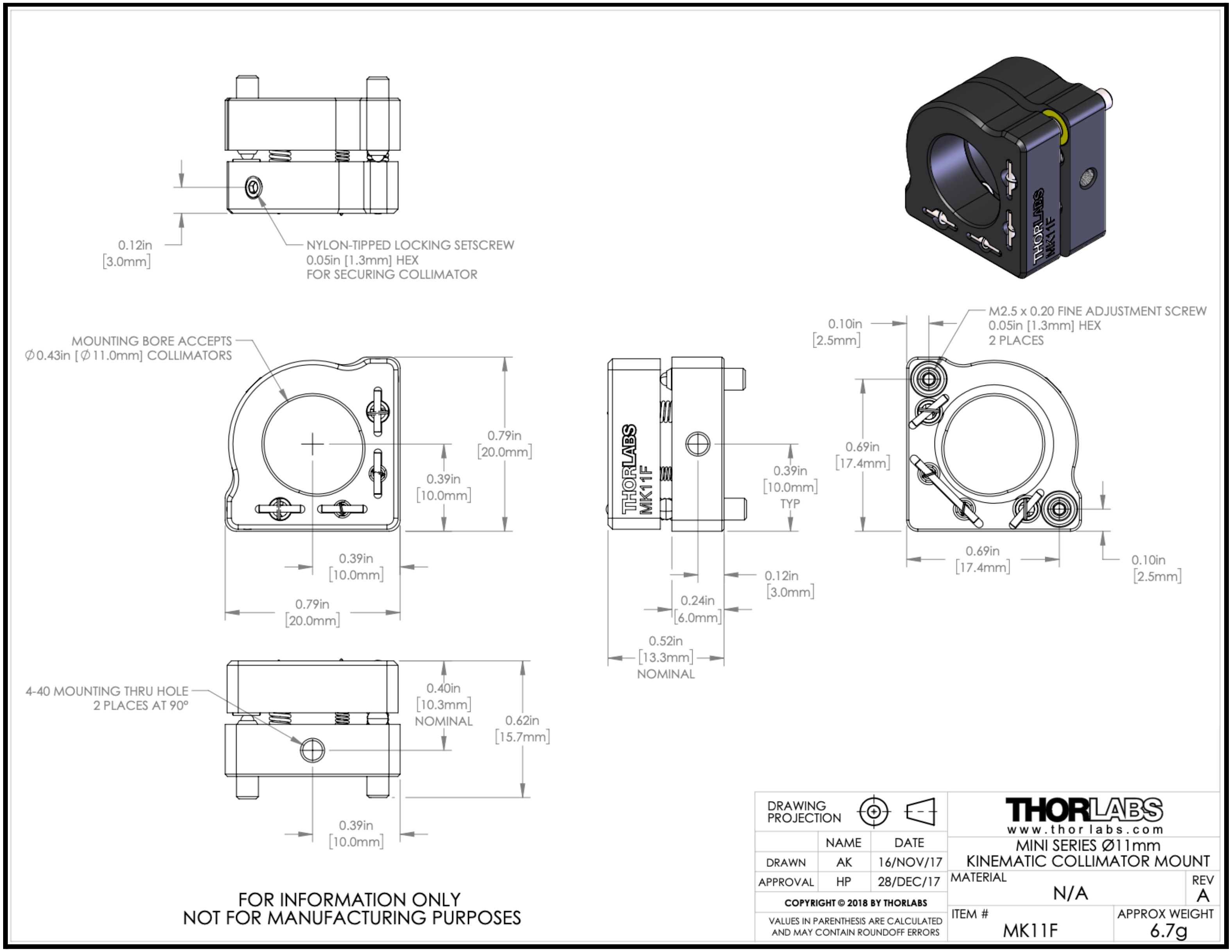
3.4.3 Compact Laser Module



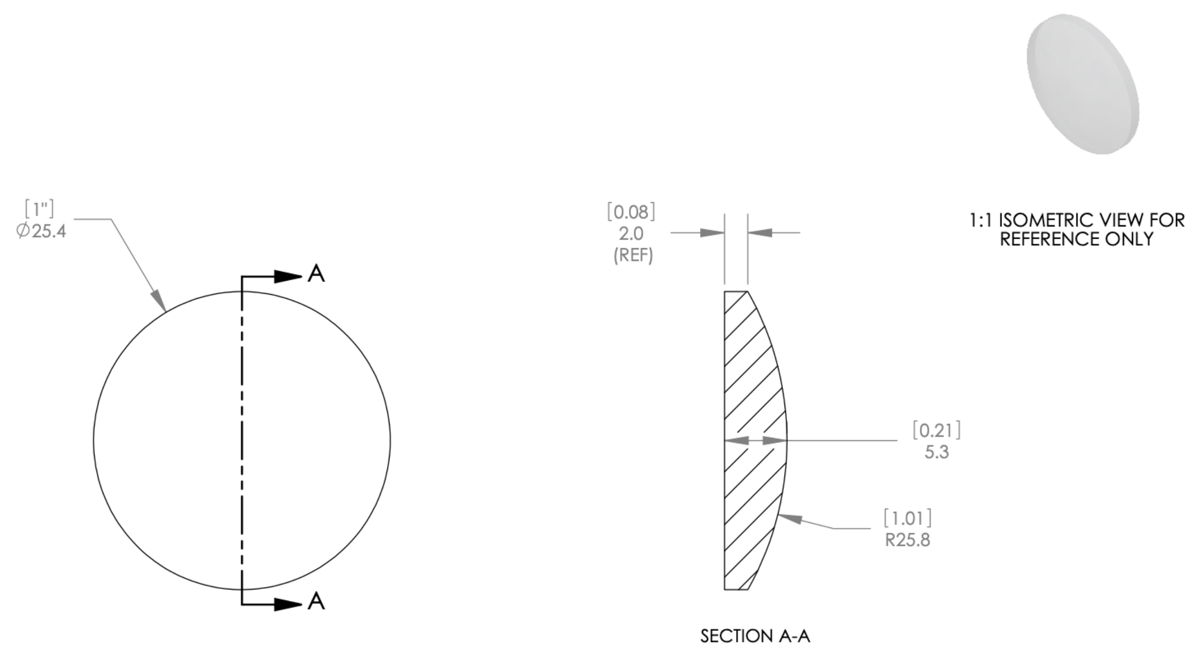
3.4.4 Avalanche Photodetector



3.4.5 Kinematic Mount



3.4.6 Plano Convex Lens



NOTES/SPECIFICATIONS:

1. DESIGN WAVELENGTH: 587.6nm
2. FOCAL LENGTH: f=49.8mm ± 1%
3. BACK FOCAL LENGTH(REF): bf=46.3mm
4. CLEAR APERTURE: >90%
5. SURFACE QUALITY: 40-20 SCRATCH-DIG
6. CENTRATION: <3arcmin
7. DIAMETER TOLERANCE: +0.0/-0.1mm
8. THICKNESS TOLERANCE: ±0.1mm

ALL DIMENSIONS ARE IN THE FORM [in] / mm.

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	NAME	DATE
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ENG APPR.	BW	11/24/2009
MFG APPR.	DD	12/09/2009

PROPRIETARY AND CONFIDENTIAL

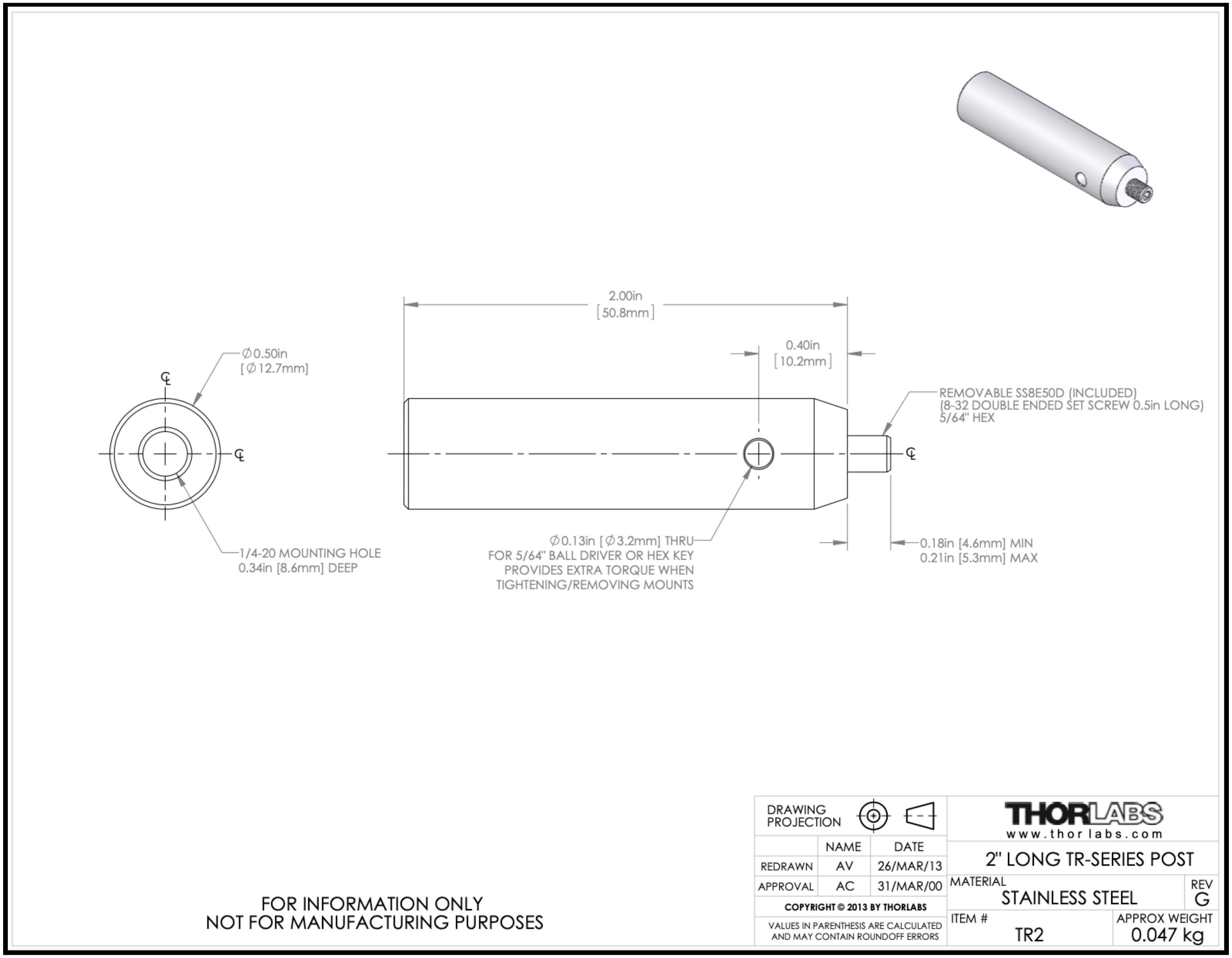
THORLABS INC. PO BOX 366 NEWTON NJ

TITLE: D=25.4 F=50.0 N-BK7 PLANO CONVEX LENS

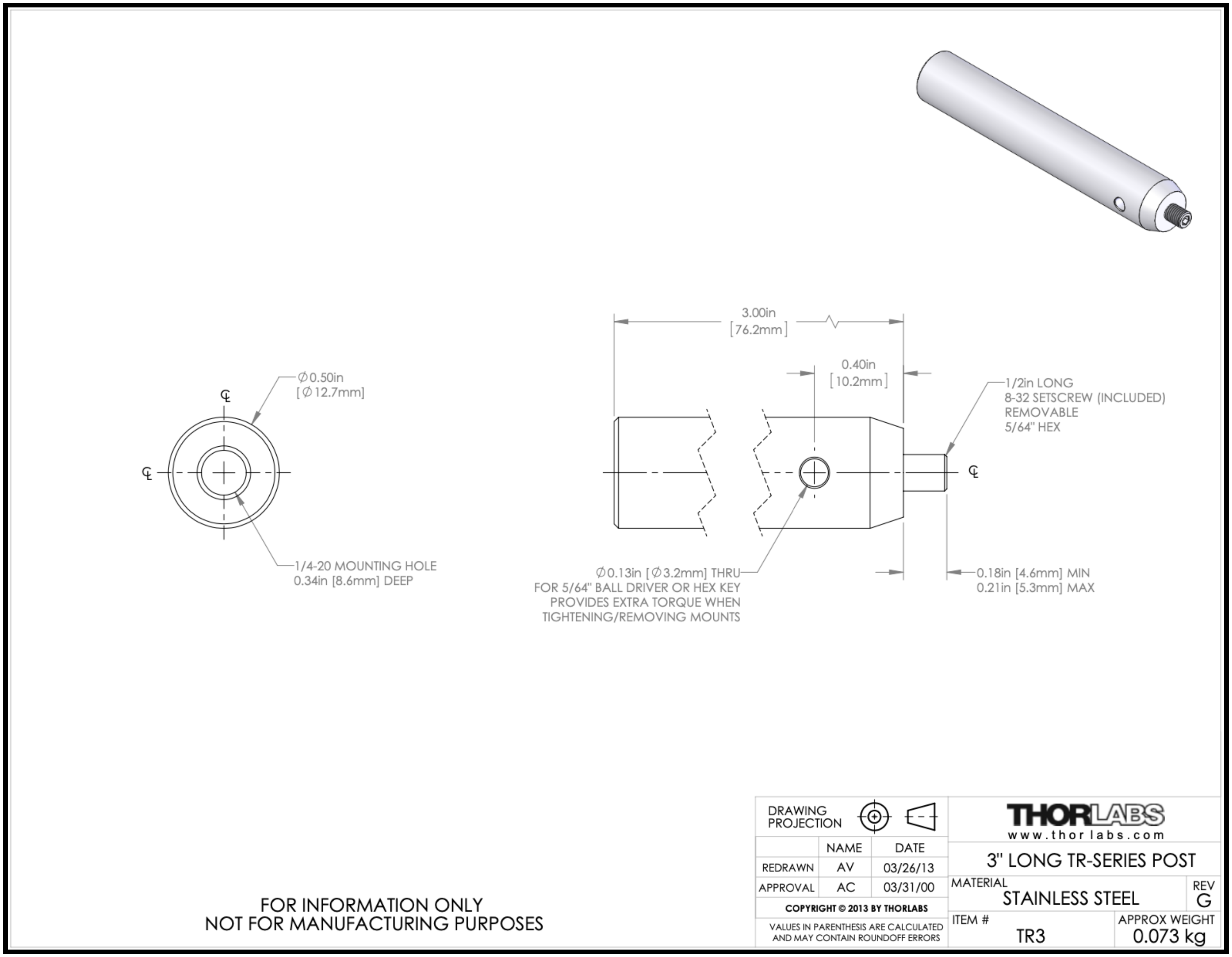
MATERIAL: N-BK7	SIZE A	REV. B
SCALE: 2:1	SHEET 1 OF 1	
DWG. NO. 3249-E01	PART NO. LA1131	

5 4 3 2 1

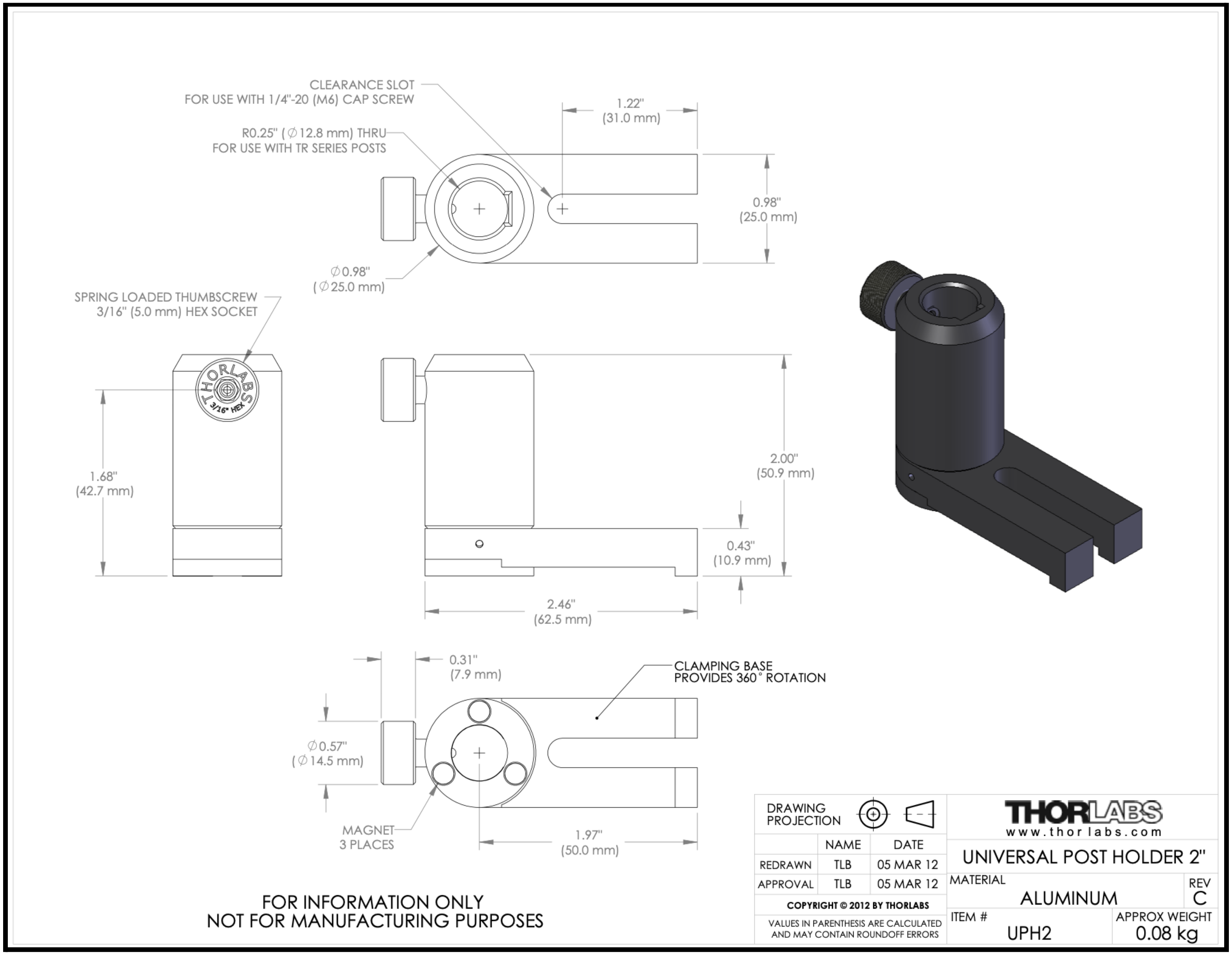
3.4.7 Pillar Post, 2”



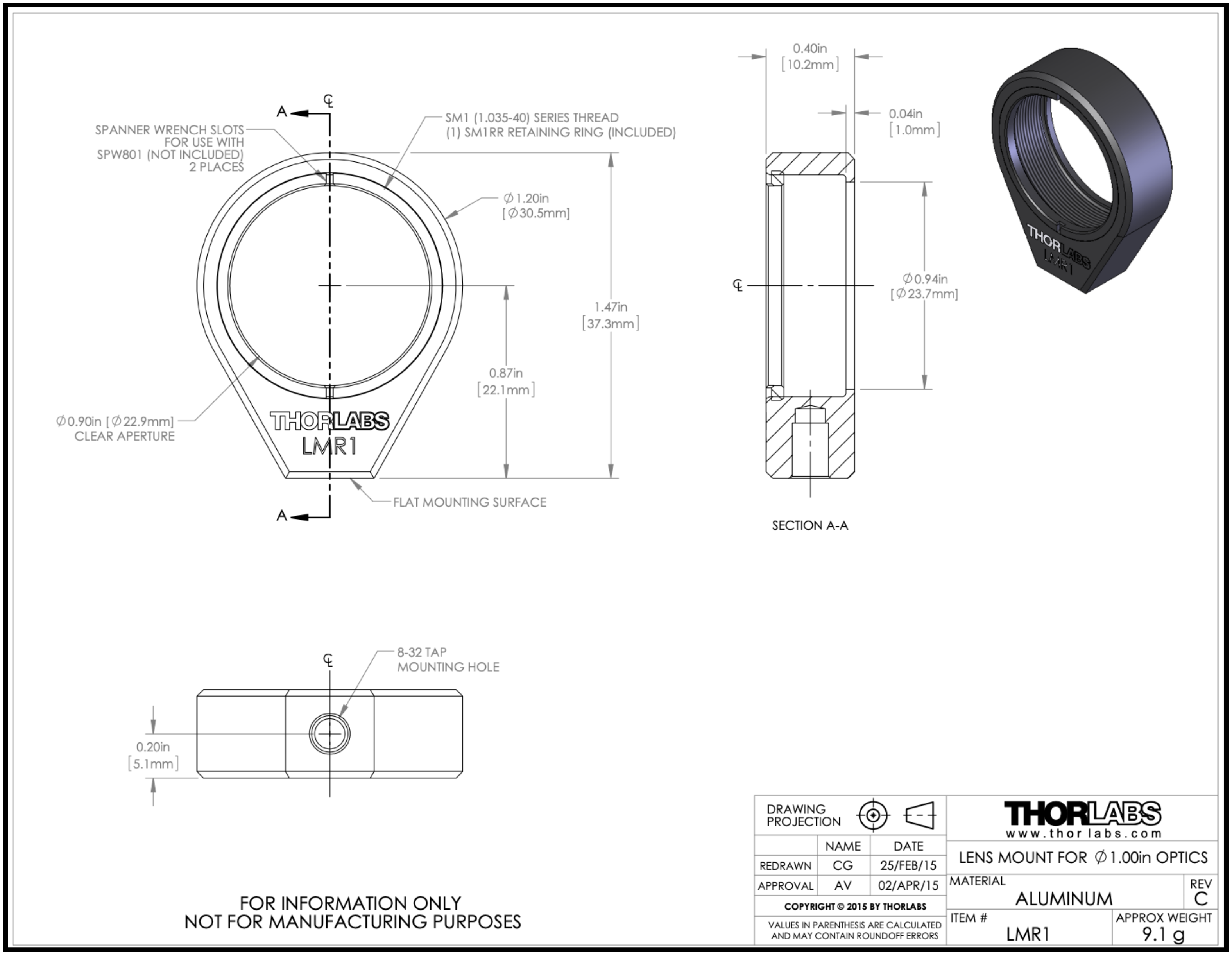
3.4.8 Pillar Post, 3”



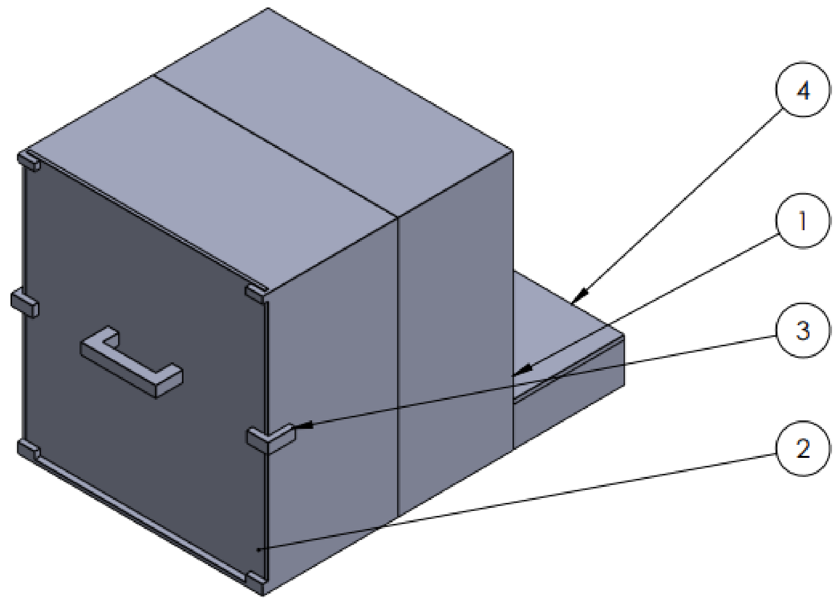
3.4.9 Universal Post Holder



3.4.10 Lens Mount



3.4.11 Mechanical Assembly (100300)



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2	100330	SYSTEM ACCESS WALL	1
3	100340	WALL SUPPORTS	4
4	100350	WIRE BOX LID	1

PROPRIETARY AND CONFIDENTIAL

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APPLICATION	

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TOLERANCES:
FRACTIONAL ±0.5
TWO PLACE DECIMAL ±0.01
THREE PLACE DECIMAL ±0.001

INTERPRET GEOMETRIC TOLERANCING PER:

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FINISH: MATTE BLACK

DO NOT SCALE DRAWING

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CHECKED		
ENG APPR.		
MFG APPR.		
Q.A.		

COMMENTS:

PROJECT 22050

TITLE: MECHANICAL ASSEMBLY

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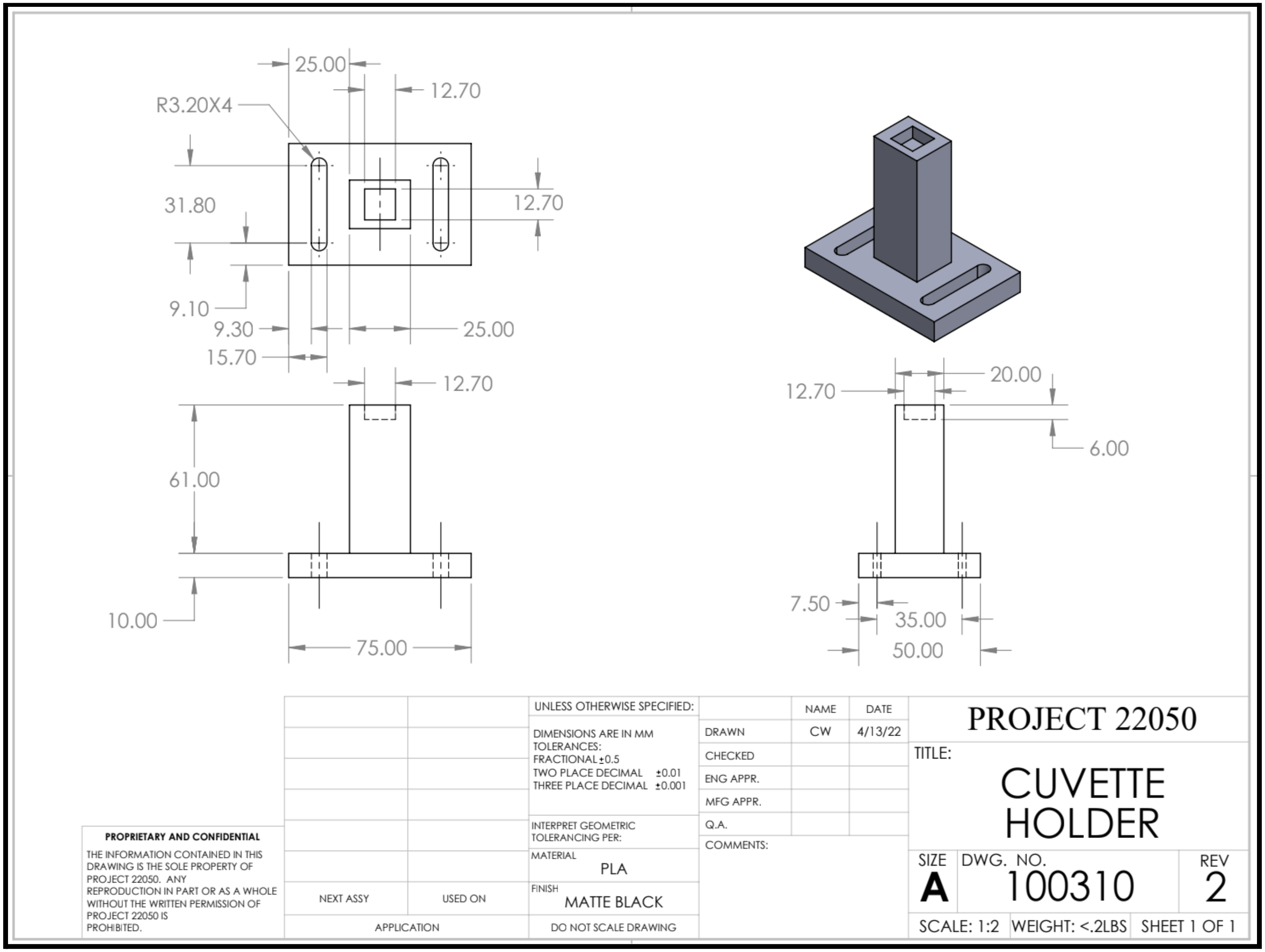
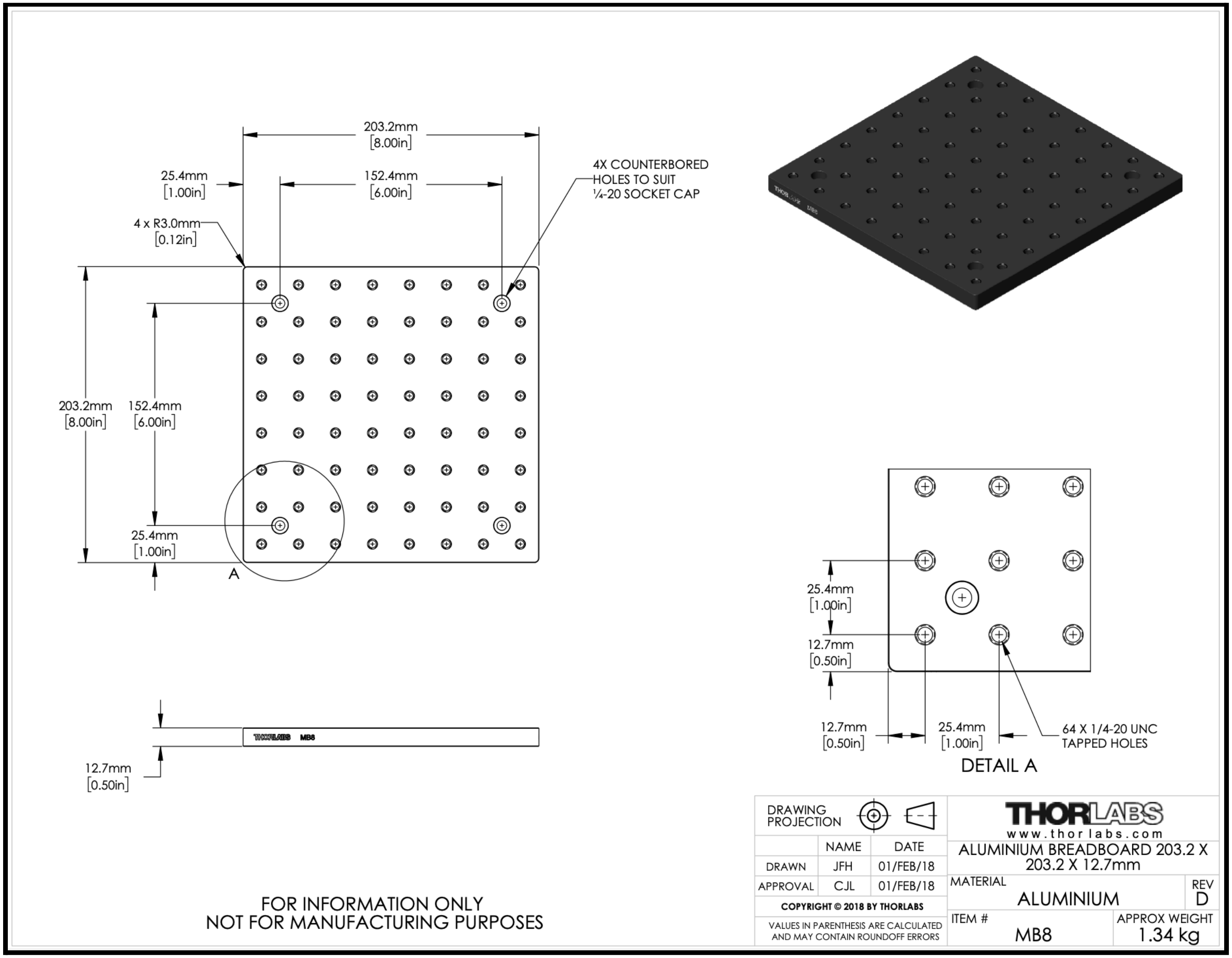
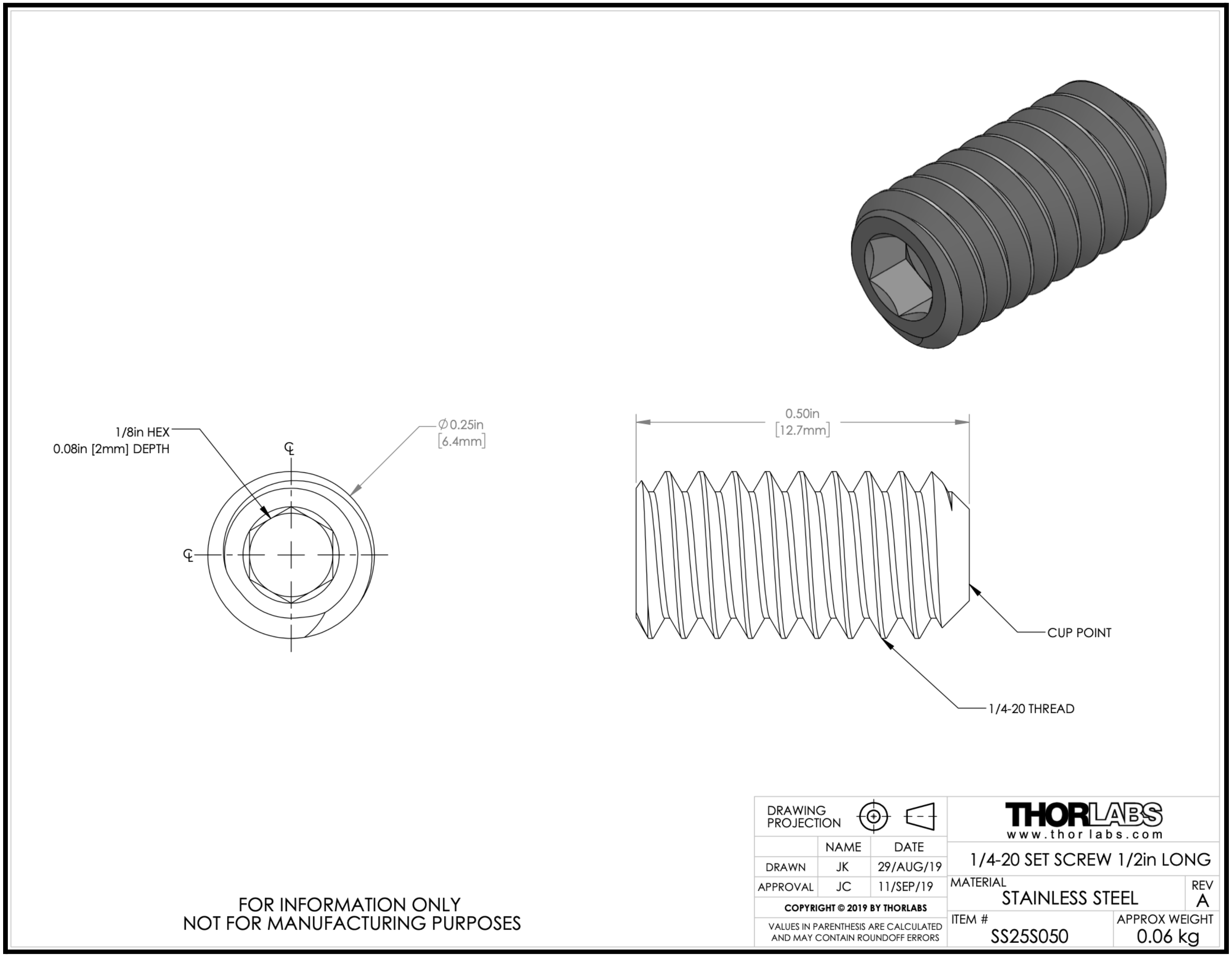
3.4.12 Cuvette Holder (100310)



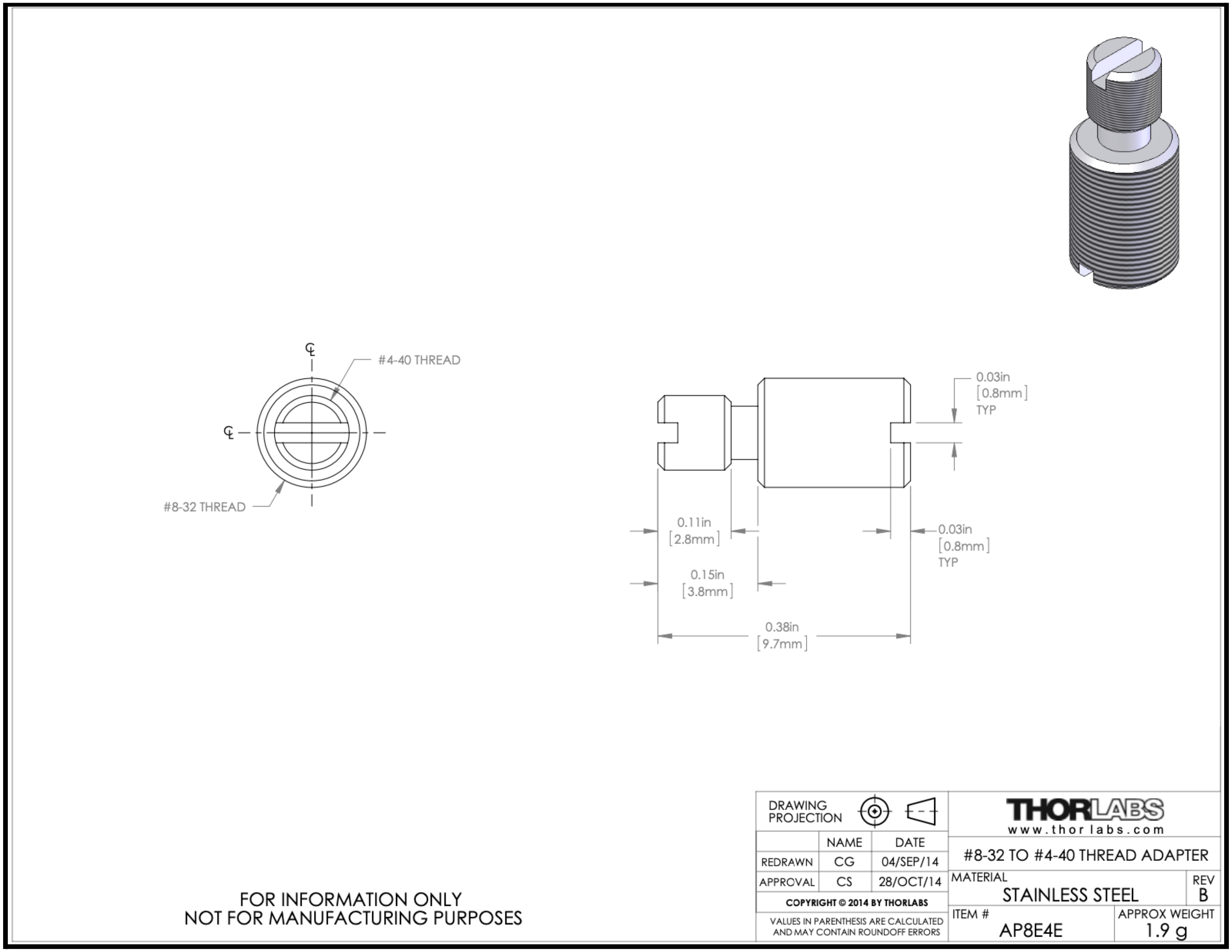
3.4.13 Aluminum Breadboard



3.4.14 Set Screw

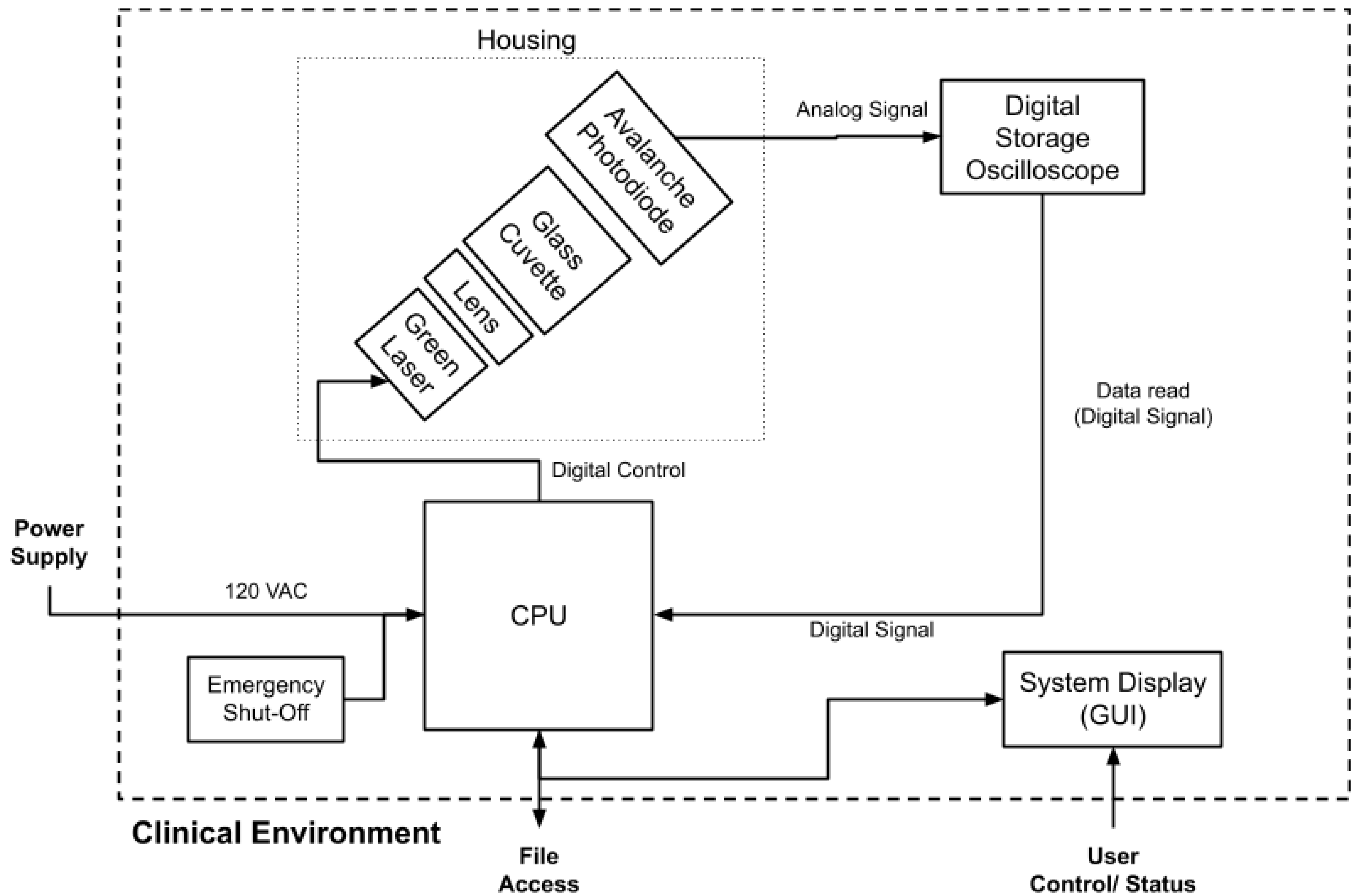


3.4.15 Adapter

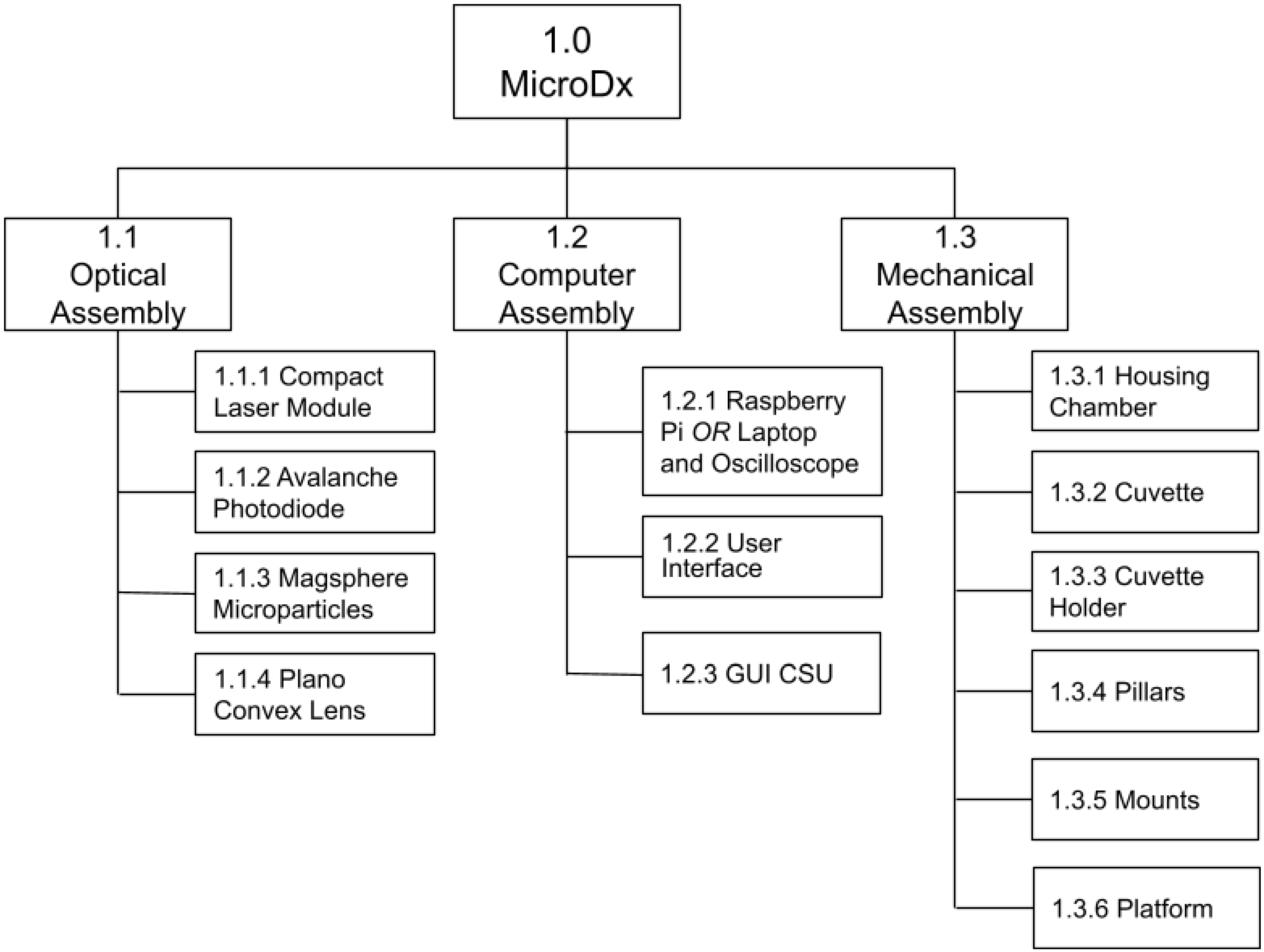


3.5 System Requirements Documentation

3.5.1 System Block Diagram



3.5.2 System Architecture



3.5.3 System Requirements

4.1. Performance Requirements

4.1.1. Size Accuracy: The MicroDx system shall detect synthetic microspheres in the range of 500 nm -1000 nm within buckets of + or - 50nm with 75% accuracy.

4.2. Safety Requirements

4.2.1. Emergency Shutoff: The MicroDx system shall have the capability to shut off all power in the event of an emergency.

4.2.2. Safe Operation: The MicroDx system shall have all electrical and mechanical components covered and protected from users and have all circuits grounded.

4.3. Interface Requirements

4.3.1. Data File: The MicroDx system shall have the capability of creating and saving a file for 5 samples providing its size and autocorrelation graph.

4.3.2. File Export: The MicroDx system will have a USB port to export the data.

4.4. Design Feature Requirements

4.4.1. Size & Weight: The MicroDx system shall weigh no more than 20 pounds and not exceed a footprint of 24” x 24”.

4.4.2. Cuvette: The MicroDx system shall accommodate a cuvette for holding synthetic microspheres.

4.4.3. Optical System: The MicroDx system shall have the capability of utilizing dynamic light scattering to determine average particle sizes.

4.4.4. Inexpensive: The MicroDx system shall cost less than $4,000.

3.5.4 Verification Requirements

5.1. Performance Requirements

5.1.1. Size Accuracy: Using synthetic microsphere samples of a known size, an analysis shall be performed to compare the sizing results of the MicroDx with the known sizes. This will allow us to determine if the system is working within the desired ≥75% accuracy.

5.2. Safety Requirements

5.2.1. Emergency Shutoff: It shall be demonstrated that the device will have an emergency shutoff that will halt all commands and power down safely when the button is pushed on the GUI.

5.2.2. Safe Operation: The MicroDx device shall be inspected to ensure that all electrical circuits are grounded, and each mechanical and electrical component has been properly covered and protected to allow for safe operation.

5.3. Interface Requirements

5.3.1. Data File: The device shall be tested to ensure that a file can be created to hold up to 5 tests that contain the size and autocorrelation graph.

5.3.2. File Export: It will be demonstrated to contain a USB port in which a flash drive can be inserted to transfer stored samples from the device onto said flash drive.

5.4. Design Feature Requirements

5.4.1. Size & Weight: The MicroDx device shall be analyzed using measurements and a scale to ensure that the device does not exceed the footprint and weight specifications. It shall then be inspected that the device can fit on a tabletop in a clinical setting.

5.4.2. Cuvette: The MicroDx device shall be inspected to determine if it can accommodate a cuvette of the dimensions 12.5 x 12.5 x 45 mm.

5.4.3. Optical System: The device shall be tested to determine that it can create speckle patterns from dynamic light scattering and will demonstrate that the pattern can be analyzed through the utilization of oscilloscope and autocorrelation curves.

5.4.4. Inexpensive: Through analyzing receipts and purchase orders, the device and its components shall cost less than the given budget of $4000.

3.6 Software Design Document

3.6.1 User Interface Software

This consists of a GUI created using the TKinter module in Python. There is a function to create the display with all its buttons and their functions. There is a separate class called gui that implements the tkinter functions with easily callable functions for adding new components to the display. All of the buttons' functions are implemented to control the correct files and Raspberry Pi pins. There are buttons for running the system, stopping the run, displaying the data, and shutting down the entire system. Additionally, a drop-down menu is implemented to allow the user to select which run (of the 5 that can be saved at a time) they would like to display information about. This software will also handle creating text files containing all the data about a single sample that can be exported to an external drive.

3.6.2 Data Analysis Software

The design for this portion of the software will include reading, running it through an autocorrelation function, and applying the Stokes-Einstein equation to calculate an actual diameter value.

Reading the Data

We are using an ADC (analog to digital converter) device connected to the raspberry pi to work as an oscilloscope to read from the detector. This uses something called serial communication to interact, so the code imports the Adafruit_ADS1x15 library to handle that. There will also be code to set up the ADC and read from it. As it periodically reads the values from the ADC, it will add them to an array of a predetermined length. This length corresponds to how long we want to gather data for, and will be controlled by a global variable.

Autocorrelation

Autocorrelation is simply a mathematical relationship between the data points and can be handled by imported mathematical functions. The software will import and utilize the fft, rfft(), irfft(), and conj() functions from the numpy library. It will take the array created in the first step and insert it into a combination of the functions above to create a new series which will be used in the next step. There will also be a separate function to plot this new series. This isn't required for the final product but will be used for the testing and verification steps.

Size Calculation (Stokes-Einstein equation)

Once a new series has been created by these functions, a best-fitting curve will be created. This will have to import the curve_fit function from the scipy.optimize library. First the scattering vector will be created from inputs given to the software. The variables needed include the wavelength of incident light, solvent refractive index, and angle at which the signal is read. Next a variable called τ needs to be calculated, and this is simply the time of the sample multiplied by 10^{-6}. The code starts with a function with initial coefficients as a guess. The initial coefficients are guessed based on the initial and ending values of the data in addition to a few more constants given. The required constants are k (Botzman's constant), T (temperature in Kelvin), and viscosity of the solvent (in our case DI water). Then the curve_fit function calculates the best fitting curve and returns the coefficients that describe that curve. Based on the curve that was created, we can use the Stokes-Einstein equation to calculate the hydrodynamic radius of the particles. The code also calculates the error of this result to show how accurate the result is. There will also be a function to plot this fitted curve as a function of τ which was calculated earlier. This plot function will be utilized by the user interface software to display it on the GUI.

3.7 Models

3.7.1 Analysis Prediction Summary

Requirement Title	System/Subsystem Requirements				Model	Analysis/ Prediction	Margin
	MicroDx	Optical	Computer/ GUI	Mechanical Frame			
Size Accuracy	> or equal to 75% accuracy	> or equal to 75% accuracy	+ or - 50 nm particle size distinction		Intensity Measurements	+ or - 50 nm particle size distinction	0 nm
		> or equal to 75% accuracy	+ or - 50 nm particle size distinction		Autocorrelation Function	+ or - 50 nm particle size distinction	0 nm
				180° of angular adjustment	Angular Geometry	170° of angular adjustment	~ 10°
Size and Weight	576 in^2 < 20 lbs	< 5 lbs	< 5 lbs	< 10 lbs	Size and Weight Solidworks	174.79 in^2 12.18 lbs	401.21 in^2 7.82 lbs
Inexpensive	< $4,000	< $2,000	< $500	< $1,000	Cost Excel	$2,644.30 tot	$1,355.70 tot

3.7.2 Dynamic Light Scatter (100610)

Dynamic light scattering (DLS) is used to determine the size distribution of a specified particle within a suspension. DLS uses an oscilloscope that is wired to a photodiode that measures the change in intensity of light as a laser beam is passed through the desired particulate solution. This difference allows the user to determine the particle sizes within the solution using an autocorrelation curve from the intensity over time data collected by the oscilloscope. In general, the intensity reading for larger particles varies less frequently over time, whereas the intensity reading for smaller particles varies much more frequently over time.

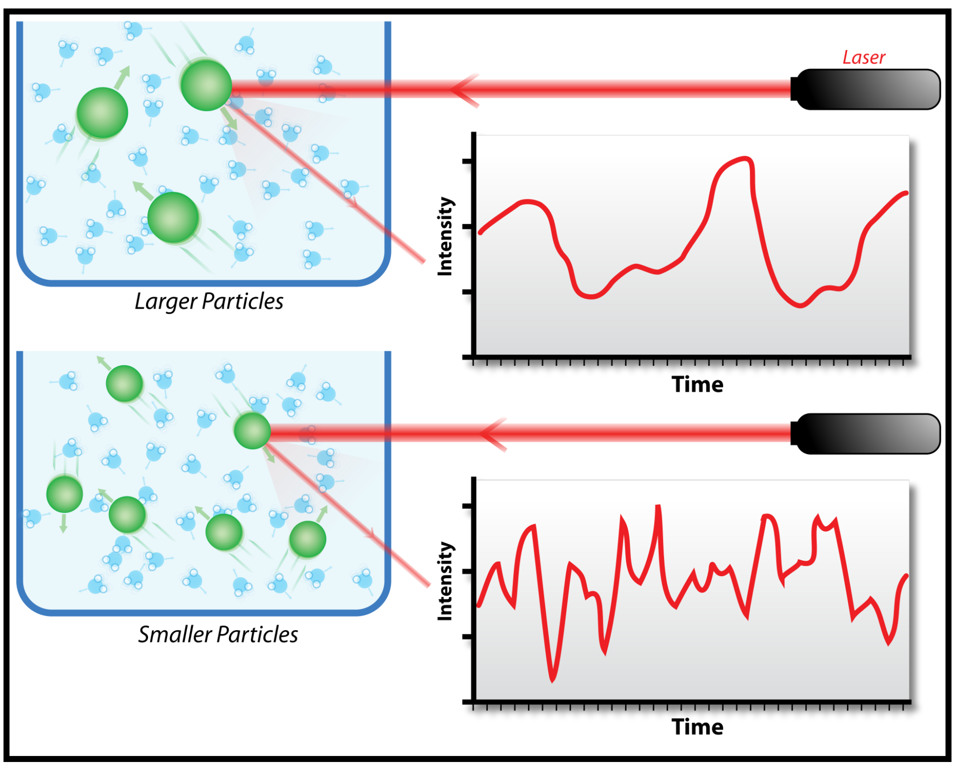


Figure 1: Dynamic Light Scattering Intensity vs Time Plots

3.7.3 Autocorrelation (100620)

The autocorrelation is the correlation of the intensity signal acquired by the oscilloscope with a delayed copy of itself as a function of delay. Using this autocorrelation curve, the team is able to understand how each specific curve is correlated to the differing particle sizes. With this correlation, using a concentration of unknown particle sizes, the autocorrelation curve will express a specific trend in data that will trace back to a known curve. Using these previously acquired curves, along with the curve on hand, the team is able to determine the sizes of particles within the unknown concentration.

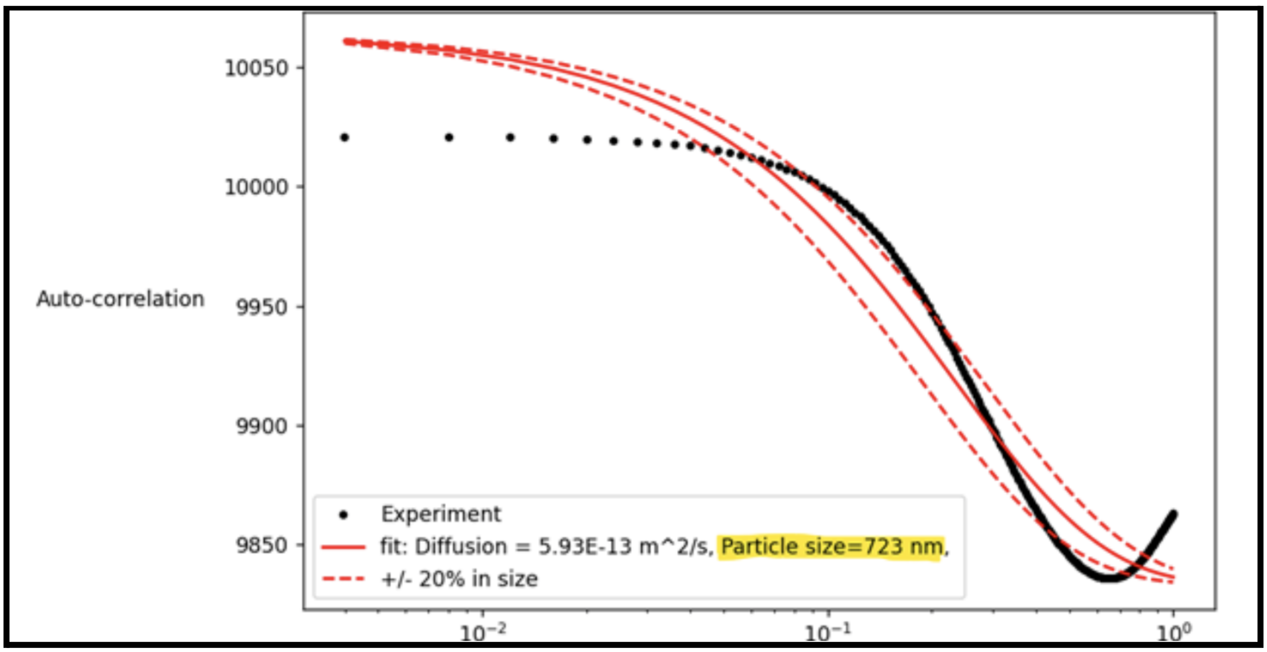


Figure 2: Autocorrelation Function Plot

3.8 Verification Procedures

3.8.1 Optical Acceptance Tests

Test #	Test	Requirement
1.4.1	Create Speckle Pattern	Speckle pattern created Proper spacing Proper alignment
1.4.2	Detect Intensity Signal	Intensity function detected MPs within range Detector/laser compatibility
1.4.3	Size	Intensity function analyzed Size output

1.5.1 Create Speckle Pattern

a. Power up MicroDx system.

b. Place the sample inside the prototype system.

c. Power the laser and look for speckle patterns. Mark Pass/Fail on the datasheet.

d. Remove the sample from the prototype system.

e. Compare the new light pattern to ensure that there is no speckle pattern. Mark Pass/Fail on the datasheet.

1.5.2 Detect Intensity Signal

a. Power up MicroDx system.

b. Load the microparticle sample into the optical subsystem.

c. Select “Run” on the GUI display.

d. Verify the system detects a sample. Mark Pass/Fail on the datasheet.

e. When the system has completed the run, verify that the results are displayed in the GUI window. Mark Pass/Fail on the datasheet.

f. Remove the sample from the optical subsystem.

g. Repeat steps a-f with an empty cuvette.

1.5.3 Size

a. Power up MicroDx system.

b. Load microparticle sample of known concentration and size into the cuvette and into the optical subsystem

c. Select “Run” on the GUI display.

d. When the system has completed the run, verify that the correct size of sample is displayed on GUI. Mark Pass/Fail on the datasheet.

e. Repeat a-e for a total of 3 different microparticle and size samples.

3.8.2 Computer Data File Acceptance Tests

Test #	Test	Requirement
2.4.1	Create and Save Data File	Particle distribution plot created Average particle size displayed
2.4.2	Export File and System Shutdown	Export file function External computer can read data System shutdown completely

2.5.1 Create and Save Data File

a. Power up the MicroDx system and prepare a sample for testing.

b. Select “Run” on the GUI display.

c. Verify the system prompts the user for a filename. Mark Pass/Fail on the datasheet.

d. Enter file name for patient (for the sake of the test, enter “Test 1” as the file name).

e. When the system has completed the run, verify that the average value is shown. Mark Pass/Fail on the datasheet.

f. Select both the load data and load curve buttons, and verify that these buttons display the proper items. Mark Pass/Fail on the datasheet.

2.5.2 Export and Emergency Power Off

a. Power up the MicroDx system.

b. Plug external hard drive (or USB) into the USB port on the Raspberry Pi.

c. Select “Export File” on the GUI display, and save “Test 1” file onto the external hard drive.

d. Select “Shutdown” and verify that the system completely turns off. Mark Pass/Fail on the datasheet.

e. Remove external hard drive from the USB port of the Raspberry Pi.

f. Plug the external hard drive into another device (laptop) and load the data file.

g. Verify that all information including the curve and average size value are shown in the file. Mark Pass/Fail on the datasheet.

3.8.3 Analysis Software Acceptance Tests

Test #	Test	Requirement
3.4.1	Retrieve Data from Sensor	Data file created Data file accessible
3.4.2	Apply Autocorrelation Function	Light from blank subtracted Calibration curve created
3.4.3	Calculate Particle Size	Calculate particle diameter

3.5.1 Retrieve Data from Sensor

a. Power up the MicroDx system and prepare a sample for testing.

b. Select “Run” on the GUI display.

c. From the software, verify that the intensity function was recorded. Mark pass/fail on the data sheet

d. Choose Sample number and select the “Load Data” button on the GUI display.

e. Verify the data just taken is displayed on the GUI. Mark pass/fail on the data sheet

3.5.2 Apply Autocorrelation Function

a. Power up the MicroDx system and prepare a sample for testing.

b. Select “Run” on the GUI display.

c. In software, check that the light signal from the blank cuvette run is subtracted from the sample’s signal. Mark Pass/Fail on data sheet.

d. In software, check that the autocorrelation function created has the correct form. Mark Pass/Fail on data sheet.

3.5.3 Calculate Particle Size

a. Power up the MicroDx system and prepare a sample for testing.

b. Select “Run” on the GUI display.

c. Check the calculated diameter of the particles in the sample. Record diameter in data sheet.

d. Compare calculated diameter to known diameter and verify it is within 50 nm of the actual diameter. Mark pass/fail in data sheet.

e. Repeat steps a-d for 2 more sets of samples with different diameters. In total 3 samples should have been run and verified.

4.0 Lessons Learned & Next Steps

4.1 Lessons Learned

4.1.1 Small Errors Have Enormous Impacts

Through the completion of this project, the team learned a number of different lessons, some stemming from our lack of knowledge of optics and others from learning how to effectively work in a team of individuals from different disciplines. Working with an optical system taught the team that even small errors would have large scale effects on our system. When we first set up our final prototype, we struggled to get the results we wanted. After many small adjustments, we were able to get it to run properly. However, we learned that even small elements such as reflections, dust, and extraneous light had major impacts on the way our system ran.

4.1.2 Optics Requires Deep Understanding

Since optics was a new discipline for every member of the team, we gained extensive knowledge on how to build an optical system, as well as the specific optical technique we used. We each gained a deep understanding of the theory behind dynamic light scatter as well as how to use it in our own system. We were able to gain this knowledge from countless meetings with different professionals in the optical industry. This included individuals on LinkedIn, professors, and masters students at the university. We discovered the importance of talking to these individuals as the project continued, as it ultimately was very important towards our success.

4.1.3 Starting Early is Important

A lesson that the team will carry with us as we continue with our education and careers is the importance of starting early. With the limited time available for this project, we found that acquiring materials became difficult due to shipping delays and waiting for products to be restocked. By ordering these materials before we needed them, the team was able to complete multiple prototypes of the system. However, each of these prototypes used up significant portions of our budget. We learned that while prototyping and looking at different approaches is beneficial in finding what works best, it can also be very costly and ultimately lead to limitations in final purchases.

4.1.4 Teamwork is Critical

The most important thing that the team learned was how to work as a team and hold each other accountable. Throughout the process, we each learned how to work together in order to create the most efficient team possible. This included each of us focusing on areas that we were best suited for and having good communication that allowed us to successfully complete the project. Each of these lessons learned made the team grow stronger as engineers and as collaborators.

4.1.5 Troubleshooting is Challenging

Over the course of the project, the team came up with four different data processing methods, which were necessary to achieve accurate results. These four methods were curve-smoothing, adding autocorrelation curves as a way of averaging them, eliminating outliers within each data set, and cutting off the tails of the autocorrelation curves. None of these methods were obvious or explained to us. We simply discovered them as a result of experimentation with our system.

Curve-Smoothing

The following figure shows two intensity vs time plots for the same set of data that was collected using the oscilloscope. In this specific test, we were testing our system's ability to accurately determine the size of a 1000 nm sized sample of synthetic microparticles. The top plot is the data being processed by the curve-smoothing function, and the bottom plot is the data after being processed by the curve-smoothing function. The purpose of this function was to get rid of noise that was diminishing the accuracy of the fitting of the autocorrelation function and size prediction. The accuracy of the MicroDx system improved greatly after the implementation of this function.

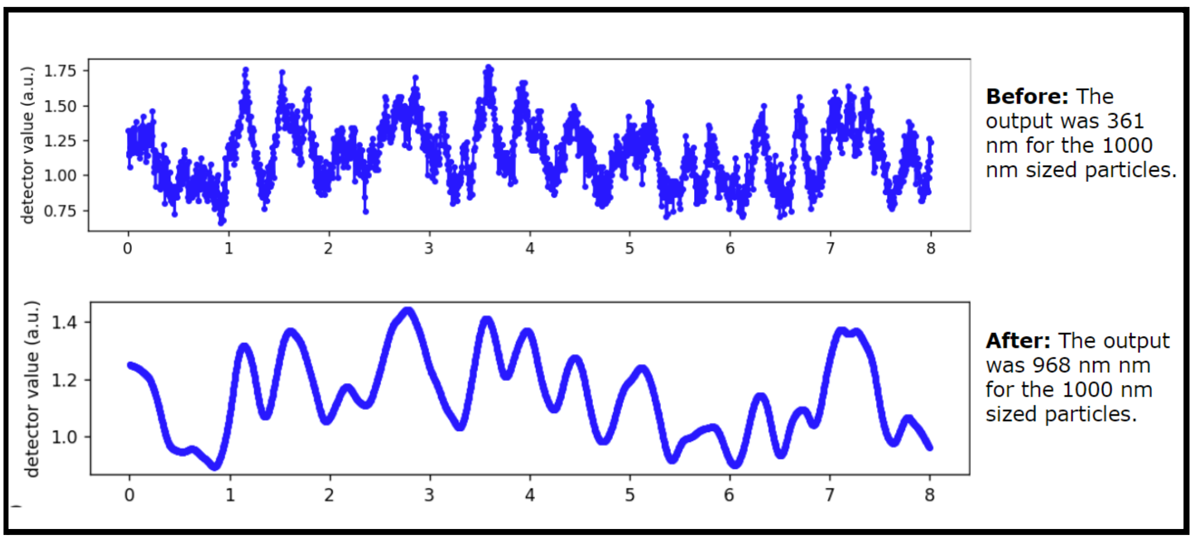


Figure 3: Curve-Smoothing Function

Adding Many Autocorrelation Curves as a Way of Averaging

During the troubleshooting of the MicroDx system, we found that adding the autocorrelation curves was a better alternative to averaging the final calculated sizes because it created a more consistent curve for the software to analyze.

This ultimately resulted in a more well-fit curve, which gave more accurate size results.

Eliminating Outliers

Sometimes a run would have a large peak that didn't match the rest of the data. This would not only throw off the individual run's curve, but it would greatly skew the cumulative curve. We found that by eliminating these extreme outliers gave much more accurate results.

Cutting the "Tail" Off the Autocorrelation Function

Our autocorrelation curves would often produce "tails" that would greatly throw off our size results. For visualization of this, consider the figure below.

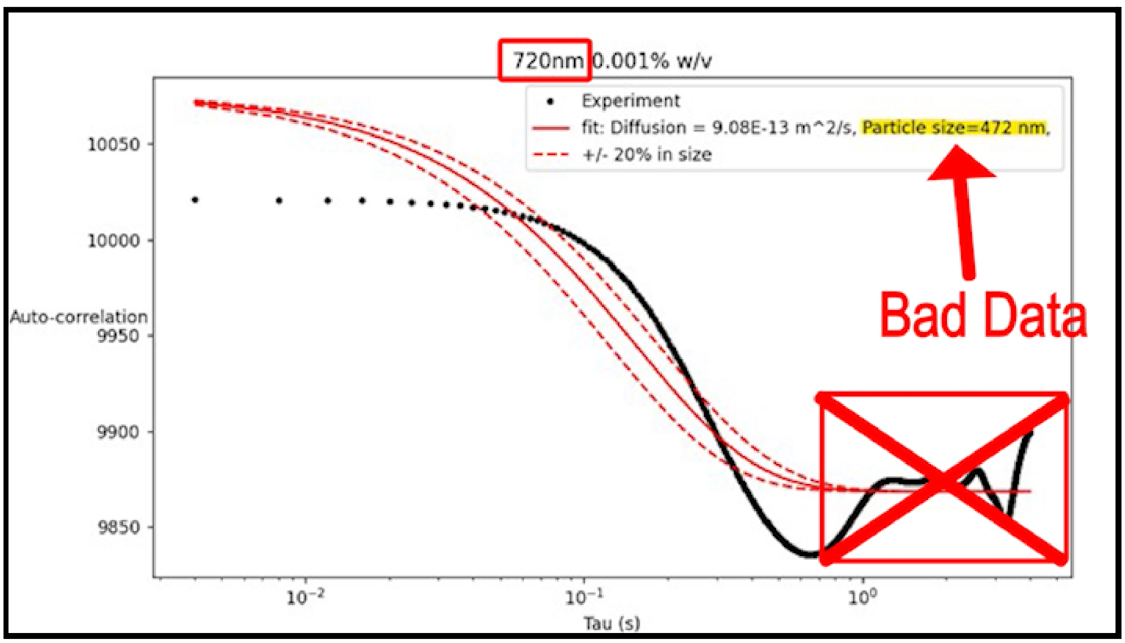


Figure 4: Autocorrelation Plot With "Tail"

For this particular data set, we were testing our system's ability to accurately calculate the size of a 0.001% w/v sample of 720 nm sized particles. Notice how the experimental curve (black line) has a greatly variable end portion that has been crossed out with a red X. This variable portion is representative of what we refer to as a "tail" on our autocorrelation plot. Also, notice how the fitted curve (solid red line) does not actually fit the experimental curve very well. This particular run estimated the size of this sample to be 472 nm (highlighted in yellow). To improve our size estimation results for this same data set, we simply cut out the "tail" portion of the experimental curve and recalculated the fitted curve. This can be visualized in the figure below.

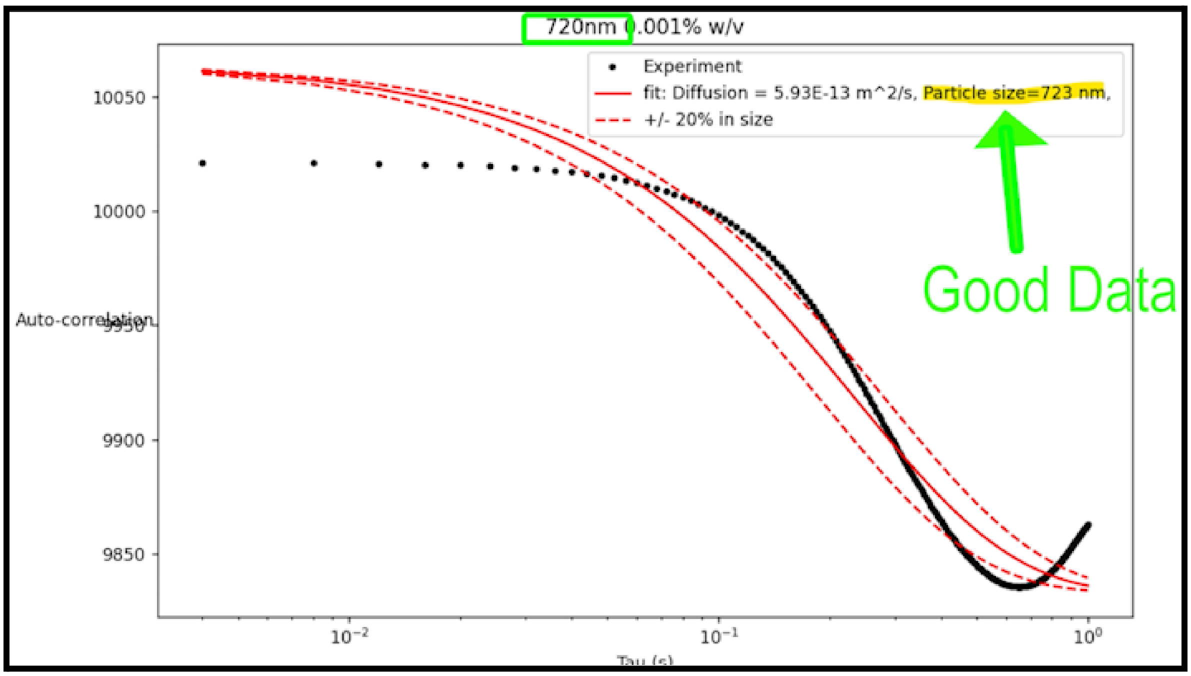


Figure 5: Autocorrelation Plot Without "Tail"

This resulted in much more accurate size estimation of 723 nm (highlighted in yellow) and a more precise fitted curve (solid red line).

4.2 Recommended Next Steps

4.2.1 Overview

As discussed previously, the MicroDx system is only at the completion of Phase 1, with three total phases to be completed. In order for future projects to be successful they should follow these recommended next steps:

1. Continue to develop the system to ensure that particles can be sized within +/- 50 nm per trial.
2. Begin acquiring concentration (particle count) data for each sample.
3. Demonstrate a capability for measuring sizes accurately (+/- 50 nm) for a mixture of 3 particle sizes.
4. Substitute blood samples into the testing process when previous steps are completed.

The team has also suggested methodologies for future teams that we believe will be beneficial in successfully completing the MicroDx system.

4.2.2 Proposed Methodology: Concentration Functionality

Something we observed during testing was that different concentrations of particles produced speckle patterns with different average intensities. The same happened for different sized particles at the same concentration. After some research into this phenomenon, our proposed solution to produce a concentration result is to create a database of the average intensities of the particles at different sizes at different concentrations. Then we can reference this in a similar fashion to a lookup table once the size or size distribution of the particles has been calculated. Consequently, this will heavily rely upon accurate size calculations. Therefore, the size calculation functionality will have to be perfected before using this method for calculating particle concentration. There may also be better ways to accomplish this functionality, but this method was proposed as it was seen as simple and feasible for this project.

4.2.3 Proposed Methodology: Mixed Particle Sizes

From our research on DLS, there is a functionality you can add where you provide a distribution curve of particle sizes. This would be done by transforming the autocorrelation function into an electric field autocorrelation function. Then use the relationship of that function to the average light intensity for each possible delay constant to get information about the size distribution. With this solution, the system would still output an average size, but a distribution curve showing common sizes would be added.

5.0 Final Budget

5.1 Subsystem Costs

The following is a series of charts that shows the breakdown of the purchases made for this project. The table below shows the purchase costs split up by each subsystem.

Subsystem Costs			
Part Number	Part Description	Supplier	Cost
100100	Optical System	thorlabs	$ 469.40
100200	Computer Assembly	adafruit	$ 342.47
100300	Mechanical Assembly	thorlabs	$ 177.00
100400	Prototype	Various	$ 840.23
100500	Testing Materials	Magsphere/Amazon	$ 939.93
Miscellaneous	Polos, Poster, and Tax/Shipping	Various	$ 715.23
		Total	$ 3,484.26

5.2 Borrowed Part Costs

Below are the borrowed materials that must be purchased to complete the system design. Due to limitations in our budget, we were unable to purchase these items outright. Fortunately, we were able to borrow these parts from the optical lab space to perform the necessary tests and system verifications.

Borrowed Part Costs			
Part Number	Part Description	Supplier	Cost
APD130A2	Si Avalanche Photodetector	UA	$ 1,304.39
GDS-1054B	Instek GDS-1054B Digital Storage Oscilloscope	UA	$ 389.16
		Total	$ 1,693.55

5.3 Purchased and Borrowed Material Costs

This table displays the breakdown of the purchased and borrowed materials. The net total is all costs combined, while the UA budget total is the overall costs within the $4,000 budget.

Purchased and Borrowed Material Costs					
Item		Supplier	Lead Time	Date Needed	Cost
MicroDx: Point-of-Care Microparticle Detection System					
	MicroDx Purchased Items	(See Purchased Items)	Acquired*	4/21/22	$ 3,284.26
	MicroDx Borrowed Items	UA	Acquired	4/1/22	$ 1,693.55
	Contingencies	Various	1 week	4/25/22	$ 200
				Net Total	$ 5,177.81
				UA Budget Total	$ 3,484.26
				Remaining UA Budget	$ 515.74

* Bitscope has yet to be received

5.4 MicroDx System Cost

This table represents the parts, descriptions, supplier, and costs of each component used within the MicroDx system.

MicroDx System Cost			
Part Number	Part Description	Supplier	Cost
APD130A2	Si Avalanche Photodetector	thorlabs	$ 1,304.39
TR3	Ø1/2" Pillar Post, 3” Length	thorlabs	$ 11.72
TR2	Ø1/2" Pillar Post, 2” Length	thorlabs	$ 5.62
UPH2	Ø1/2" Universal Post Holder	thorlabs	$ 101.49
4296	Raspberry Pi 4 Model B - 4 GB RAM	adafruit	$ 389.16
2718	Touchscreen Display for Raspberry Pi	adafruit	$ 79.95
1294	SD/MicroSD Memory Card	adafruit	$ 9.95
4377	Stand for Touchscreen Display	adafruit	$ 27.99
MK1619-00B	Step-Up Power Module	Walmart	$ 7.08
PL203	520nm Compact Laser Module	thorlabs	$ 132.00
MK11F	Kinematic Mount	thorlabs	$ 97.87
BS05T	Bitscope Micro	bitscope	$ 155.00
LA1131	Plano Convex lens	thorlabs	$ 23.49
LMR1	Lens Mount	thorlabs	$ 15.69
MB8	Aluminum Breadboard 8"x8"	thorlabs	$ 81.52
100300	Housing Assembly (Frame and Screws)	PLA/Stainless Steel	$ 193.88
1995	5V 2.5A Switching Power Supply	adafruit	$ 7.50
Total			$ 2,644.30

Appendix I: Acceptance Test Data Sheets

MicroDx Verification Test Sheet			
1.7.1 Optical Acceptance Test Data Sheet			
Referenced ATP Paragraph Number: **1.0**			
Name of Test: Create **Speckle Pattern**			
Unit Under Test (UUT): Name: **Optical Assembly** Part Number: Serial Number: n/a			

Results (Pass / Fail):
- Sample Present
  - Visible Speckle Pattern **pass** / fail
- No Sample Present
  - No Visible Speckle Pattern **pass** / fail
		Date of Test: **4/5/22**	

Recording of Test Measurements: **N/A**	Requirement (SR, with Tolerances) **N/A**	Test Equipment Error: **N/A**	Adjusted Test Limit: **N/A**
Computations, (Include Analyses Results, if any): **N/A**			
Signatures: Tester ____________**Ethan Ross and Giang Nguyen**_____________ Customer______________________________________			

<table>
<tr><th colspan="4">MicroDx Verification Test Sheet</th></tr>
<tr><td colspan="4">1.7.2 Optical Acceptance Test Data Sheet</td></tr>
<tr><td colspan="4">Referenced ATP Paragraph Number: 1.0</td></tr>
<tr><td colspan="4">Name of Test: Detect Intensity Signal</td></tr>
<tr><td colspan="4">Unit Under Test (UUT):
Name: Optical Assembly
Part Number:
Serial Number: n/a</td></tr>
<tr><td colspan="2">Results (Pass / Fail):
- Detects a sample pass / fail
- Results on GUI pass / fail</td><td colspan="2">Date of Test:
4/5/22</td></tr>
<tr><td>Recording of Test Measurements:
N/A</td><td>Requirement (SR, with Tolerances)
N/A</td><td>Test Equipment Error:
N/A</td><td>Adjusted Test Limit:
N/A</td></tr>
<tr><td colspan="4">Computations, (Include Analyses Results, if any):
N/A</td></tr>
<tr><td colspan="4">Signatures:
Tester ____________________Ethan Ross and Megan Mickey__________
Customer____</td></tr>
</table>

1.7.3 Particle Analysis Acceptance Test Data Sheet			
Referenced ATP Paragraph Number: **1.0**			
Name of Test: **Size Particles**			
Unit Under Test (UUT): Name: **Optical Assembly** Part Number: Serial Number: n/a			
Results (Pass / Fail): Sample 1: **500 nm** - Correct size pass / **fail** Sample 2: **720nm** - Correct size **pass** / fail Sample 3: **1000 nm** - Correct size **pass** / fail		Date of Test: **4/13/22**	
Recording of Test Measurements: Sample 1 (**500** nm): - Particle concentration: **N/A** - Particle size: **657 nm** Sample 2 (**720** nm): - Particle concentration: **N/A** - Particle size: **711 nm** Sample 3 (**1000** nm): - Particle concentration: **N/A** - Particle size: **968 nm**	Requirement (SR, with Tolerances): **Results need to be within +/- 50 nm of the size that the particles have listed on there standard bottles.**	Test Equipment Error: **The results for the 500 nm particle sample varied greatly. The team has several theories as to what is causing this error.**	Adjusted Test Limit:
Computations, (Include Analyses Results, if any): **N/A**			
Signatures: Tester **Rainee Meuschke and Megan Mickey** Customer____________________			

MicroDx Verification Test Sheet			
2.7.1 Create and Save Data File Acceptance Test Data Sheet			
Referenced ATP Paragraph Number: **1.0**			
Name of Test: **Create and Detect Speckle Pattern & Intensity**			
Unit Under Test (UUT): Name: **Computer/GUI Assembly** Part Number: Serial Number: n/a			
Results (Pass / Fail): - Filename prompted **pass** / fail - Average particle size value displayed **pass** / fail - Data displayed **pass** / fail		Date of Test: **4/5/22**	
Recording of Test Measurements: **N/A**	Requirement (SR, with Tolerances) **N/A**	Test Equipment Error: **N/A**	Adjusted Test Limit: **N/A**
Computations, (Include Analyses Results, if any): **N/A**			
Signatures: Tester ____________**Ethan Ross**____________________________ Customer_______________________________________			

MicroDx Verification Test Sheet			
2.7.2 File Export and Emergency Shutdown Test Data Sheet			
Referenced ATP Paragraph Number: **1.0**			
Name of Test: **Create and Detect Speckle Pattern & Intensity**			
Unit Under Test (UUT): Name: **Computer/GUI Assembly** Part Number: Serial Number: n/a			
Results (Pass / Fail): - System completely shuts down **pass** / fail - File exported **pass** / fail - Data accessed on external laptop **pass** / fail		Date of Test: **4/5/22**	
Recording of Test Measurements:	Requirement (SR, with Tolerances): **N/A**	Test Equipment Error: **N/A**	Adjusted Test Limit:
Computations, (Include Analyses Results, if any): **N/A**			
Signatures: Tester ______________**Alyssa Barney and Megan Mickey**____________ Customer___________________________________			

MicroDx Verification Test Sheet			
3.7.1 Retrieve Data from Sensor Acceptance Test Data Sheet			
Referenced ATP Paragraph Number: 1.0			
Name of Test: **Retrieve Data from Sensor**			
Unit Under Test (UUT): Name: **Computer/GUI Assembly** Part Number: Serial Number: n/a			
Results (Pass / Fail): - Intensity function recorded **pass** / fail - Data saved **pass** / fail		Date of Test: **4/5/22**	
Recording of Test Measurements: **N/A**	Requirement (SR, with Tolerances) **N/A**	Test Equipment Error **N/A**:	Adjusted Test Limit: **N/A**
Computations, (Include Analyses Results, if any): **N/A**			
Signatures: Tester ___________**Alyssa Barney and Giang Nguyen**____________ Customer___________________________________			

<table>
<tr><th colspan="4">MicroDx Verification Test Sheet</th></tr>
<tr><td colspan="4">3.7.2 Apply Autocorrelation Function Test Data Sheet</td></tr>
<tr><td colspan="4">Referenced ATP Paragraph Number: 1.0</td></tr>
<tr><td colspan="4">Name of Test: Apply Autocorrelation Test</td></tr>
<tr><td colspan="4">Unit Under Test (UUT):
Name: Computer/GUI Assembly
Part Number:
Serial Number: n/a</td></tr>
<tr><td colspan="2">Results (Pass / Fail):
- Autocorrelation function has correct form pass / fail</td><td colspan="2">Date of Test:
4/8/22</td></tr>
<tr><td>Recording of Test Measurements:
N/A</td><td>Requirement (SR, with Tolerances) N/A</td><td>Test Equipment Error:
N/A</td><td>Adjusted Test Limit:
N/A</td></tr>
<tr><td colspan="4">Computations, (Include Analyses Results, if any): N/A</td></tr>
<tr><td colspan="4">Signatures:
Tester ______________<u>Megan Mickey</u>_____________
Customer_____________________________________</td></tr>
</table>

Appendix II: Inspection Reports

MicroDx Inspection Report	
1.1.1 Safe Operation	
Referenced ATP Paragraph Number: **1.0**	
Name of Test: **Safe Operation**	
Unit Under Test (UUT): Name: **MicroDx** Part Number: Serial Number: n/a	
Results (Pass / Fail): - Is a user able to safely operate the MicroDx device? - **pass** / fail	Date of Inspection: **4/13/22**
Notes from Inspection: - **All wires have been grounded and covered** - **Laser light is safely contained within the enclosure** - **GUI button to shut off laser light works properly**	Test Equipment Error: **N/A**
Signatures: Tester ______**Rainee Meuschke and Megan Mickey**______________ Customer___________________________________	

MicroDx Inspection Report	
2.1.1 Size and Weight	
Referenced ATP Paragraph Number: **1.0**	
Name of Test: **Size and Weight**	
Unit Under Test (UUT): Name: **MicroDx** Part Number: Serial Number: n/a	
Results (Pass / Fail): - Can the MicroDx system easily fit on a tabletop in a clinical setting? - **pass** / fail	Date of Inspection: **4/13/22**
Notes from Inspection: - **Size:** 174.79 in^2 - **Weight:** 12.18 lbs -	Test Equipment Error: **N/A**
Signatures: Tester **Cody Wilcox** Customer	

MicroDx Inspection Report	
3.1.1 Results Displayed on GUI	
Referenced ATP Paragraph Number: 1.0	
Name of Test: **GUI Display Results**	
Unit Under Test (UUT): Name: MicroDx Part Number: Serial Number: n/a	
Results (Pass / Fail): - Are the results from the run completed with the MicroDx displayed on the GUI? - **pass** / fail	Date of Inspection: **4/5/22**
Notes from Inspection: - **Results are properly displayed on the GUI including the autocorrelation graph and the particle size estimation**	Test Equipment Error: **N/A**
Signatures: Tester ______________**Alyssa Barney and Megan Mickey**_____________ Customer___________________________________	

Appendix III: Software Design Document

[SDD: Software Design Document]

Section 1.0: Scope

The software in the MicroDx system will be responsible for processing the data measured by the optical system and displaying the results in a helpful way. The code will be downloaded to the processor of a RaspberryPi. The software will be required to control the laser, control power to the system, do mathematical analysis of the output of the optical system, accept and process user inputs, and build a GUI representing the results to send to a display. This document will contain our design and test plan for this software, including the architecture, interfaces, and procedures.

Section 2.0: Referenced Documents

MicroDx System Requirement Flowdown

Section 3.0: Software Specific Requirements and Traceability

SSR #	Requirement Description
4.1.1	Calculate MP average size with greater than 75% accuracy
4.2.1	Shuts off power in event of an emergency
4.3.1	Creates a file with data, autocorrelation curve, and size for the MPs.
4.3.2	Displays calculated particle size, raw data, and autocorrelation curve with its best-fit curve
4.4.6	Exports files
SSR #	Requirement Description

Section 4.0: Software Design

4.1. Software Wide Design Decisions

GUI Design Layout

Figure 1 shows the initial GUI layout that will be presented to the user once the system is on. This includes various interactive buttons and the displayed results. The GUI functions so that the user is able to run the system and interact with the results from the sample as well as export the data from specific samples to an external device.

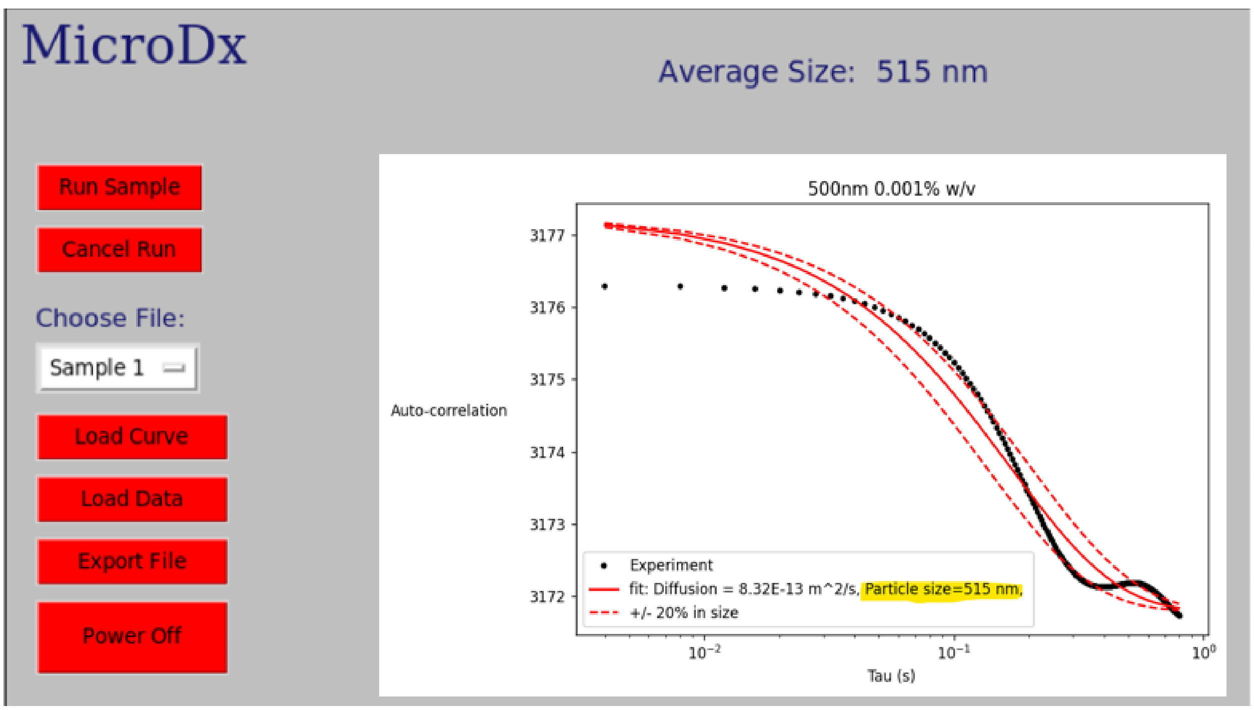


Figure 1: GUI Layout

Overview

The input to the entire software package mainly consists of the varying speckle pattern intensity detected by the optical system. This data is analyzed and translated into displayable results. The user will be able to give commands via the GUI touchscreen to run the optical system, display results, export files, and power off the entire system.

The outputs of the software package include the final calculated size, graphs of the data and autocorrelation curves, and an exportable file. The GUI will display the average size of the particles and graphs of both the raw data and calculated autocorrelation curve.

GUI Specific Responses

On the left of the GUI are the “Load Curve”, “Load Data”, and “Export File” buttons with a drop down menu above them. These will be used for samples that have already been analyzed and saved. The system will be able to save up to 5 samples, so the list of samples 1-5 will be options in the drop down menu. When a user runs a sample using the “Run” button, a pop up window will appear asking them which sample they would like to save this run under. Therefore, the drop down menu will contain options for the numbers the user has chosen to save samples under so far. The “Load Curve” button will display the autocorrelation curve calculated for the selected sample similar to the one in *Figure 1*. The data above it (Average size) will also

```
Date: 4/19/2022
Sample #: 2

Autocorrelation Curve Function:
3998, 25174.3
7996, 25173.8
11994, 25173.1
15992, 25172.25
23988, 25171.2
27986, 25169.9
...

Curve-fit Coefficients:
a = 24791.36
b = 402.9
c = 3.14

Diffusion Coefficient (m/s):
D = 4.35e-13

Hydrodynamic diameter (nm):
968.2
```

Figure 2. Exportable File

be displayed for that sample number. When the “Load Data” button is pressed, the intensity variation graph read from the detector will be displayed on the right side of the screen instead of the autocorrelation curve. However, the calculated values above it will remain the same. Finally, for the “Export File” button, a text file containing the data from the selected sample will be written and exported to the device plugged into the USB port on the raspberry pi. A shortened sample of what the file will display is shown in *Figure 2.* The “Power off” button will simply turn off the Pi, thus powering off the entire system. Any software running at that time will stop and the screen will turn off.

Analysis Specific Responses

When the “Run” button is pressed, the system will turn on the laser and begin recording data from the detector. It will record the light intensity using the detector for about 3 minutes and save that data in the location the user specifies based on the sample number they selected. Then it will immediately begin to analyze the signal to determine the average size of the particles. It will create the autocorrelation curve and calculate the size, saving both of these in the same sample number folder as the data.

\The method for actually calculating the particle size uses an autocorrelation function and the Stokes-Einstein equation. This will be accomplished by putting the array of intensity values through an autocorrelation function, which basically expresses how similar a point is to the previous point. A new array of values will be created by that function, which will look like a down-ward sloped curve. Next, a best-fit polynomial curve will be found for the autocorrelation curve. The coefficients from this best-fit curve will then be used in the Stokes-Einstein equation to calculate the scattering vector, diffusion coefficient, and finally hydrodynamic diameter of the particles. An error will also be calculated to see how accurate the value is.

Safety, Security, and Privacy Requirements

No private information will be needed.

4.2. Software Architecture Design Decisions

Component Identification

The software will mainly consist of two sections, the user interface software and analysis software. The user interface portion uses a class called gui to implement many of the buttons and displays so that portion is not handled in main. The analysis software calculates size and can be called by main when the “Run” button is pressed.

Software Libraries

Python TKinter library

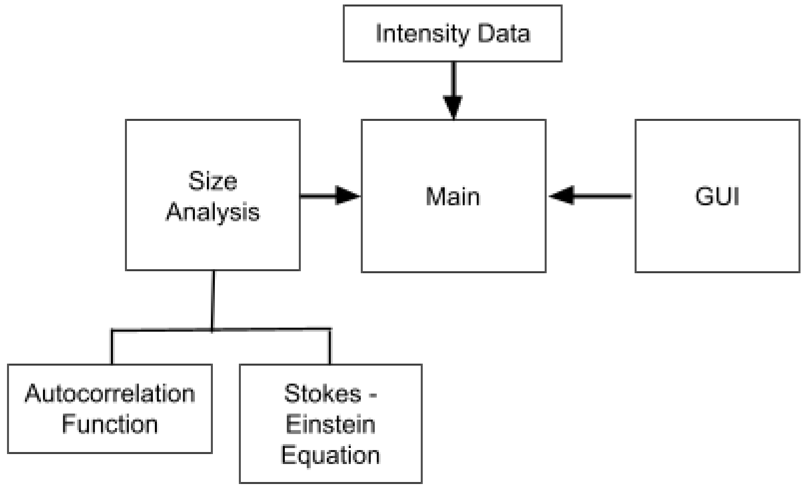
Numpy library

Matplotlib library

Scipy library

Statsmodels library

Concept of Execution



4.3. Software Detailed Design

User Interface Software

The GUI will be created using the TKinter module in Python. There is a function to create the display with all its buttons and their functions. There is a separate class called gui that implements the tkinter functions with easily callable functions for adding new components to the display. This simply has a function for creating buttons and drop down menus and text boxes wherever they are desired. All of the buttons' functions are implemented to control the correct files and Raspberry Pi pins. They are separate functions in the same file as main() that main() calls. There are buttons for running the optical system (which includes turning on the laser and detector for a specific period of time and calling the analysis functions), stopping the run (which cancels the data collection for the current sample), displaying the data (which includes intensity signal and the created autocorrelation curve), and shutting down the entire system. Additionally, a drop-down menu is implemented to allow the user to select which run (of the 5 that can be saved at a time) they would like to display information about. The option they chose is saved as a variable which is then used to access any data corresponding to that number. This software also creates text files containing all the data about a single sample that can be exported to an external drive. It saves it in a format with the sample number at the top, then the data points from the intensity, then the data points from the autocorrelation curve, then the 3 coefficients of the best-fit curve, diffusion coefficient, and finally the size of the sample.

Data Analysis Software

The design for this portion of the software will include reading, running it through an autocorrelation function, and applying the Stokes-Einstein equation to calculate an actual diameter value.

Reading the Data: We are currently reading csv files from an oscilloscope containing the intensity data. However, in future phases of the project the plan is to use a Bitscope device connected to the raspberry pi to work as an oscilloscope to read from the detector directly. Therefore some basic code and comments for how that would be implemented have been included in the final code. This uses something called serial communication to interact, so the code imports the Adafruit_ADS1x15 library to handle that. There will also be code to set up the Bitscope and read from it. As data is periodically read from the values from the Bitscope, it will add them to an array of a predetermined length. This length corresponds to how long we want to gather data for, and will be controlled by a global variable. Before sending this data through the autocorrelation function, it is put through a smoothing function to eliminate the noise in the signal. This is done using the smoothers.lowess function in the python statsmodels library.

Autocorrelation; Autocorrelation is simply a mathematical relationship between the data points and can be handled by imported mathematical functions. The software will import and utilize the fft, rfft(), irfft(), and conj() functions from the numpy library. It will take the array created in the first step and insert it into a combination of the functions above to create a new series which will be used in the next step. There will also be a separate function to plot this new series. This isn't required for the final product but will be used for the testing and verification steps.

Size Calculation (Stokes-Einstein equation): Once a new series has been created by these functions, a best-fitting curve will be created. This will have to import the curve_fit function from the scipy.optimize library. First the scattering vector will be created from inputs given to the software. The variables needed include the wavelength of incident light, solvent refractive index, and angle at which the signal is read. Next a variable called τ needs to be calculated, and this is simply the time of the sample multiplied by 10^{-6}. The code starts with a curve-fit function guessing the initial coefficients. The initial coefficients are guessed based on the initial and ending values of the data in addition to a few more constants given. The required constants are k (Botzman's constant), T (temperature in

Kelvin), and viscosity of the solvent (in our case DI water). Then the curve_fit function calculates the best fitting curve and returns the coefficients that describe that curve. Based on the curve that was created, we can use the Stokes-Einstein equation to calculate the hydrodynamic radius of the particles. The code also calculates the error of this result to show how accurate the result is. There will also be a function to plot this fitted curve as a function of τ which was calculated earlier. This plot function will be utilized by the user interface software to display it on the GUI.

Section 5.0: Software Test Plan and Description

5.1 Computer Data File Acceptance Tests

Test #	Test	Requirement
2.4.1	Create and Save Data File	Particle distribution plot created Average particle size displayed
2.4.2	Export File and System Shutdown	Export file function External computer can read data System shutdown completely

2.5.1 Create and Save Data File

a. Power up the MicroDx system and prepare a sample for testing.

b. Select "Run" on the GUI display.

c. Verify the system prompts the user for a filename. Mark Pass/Fail on the datasheet.

d. Enter file name for patient (for the sake of the test, enter "Test 1" as the file name).

e. When the system has completed the run, verify that the average value is shown. Mark Pass/Fail on the datasheet.

f. Select both the load data and load curve buttons, and verify that these buttons display the proper items. Mark Pass/Fail on the datasheet.

2.5.2 Export and Emergency Power Off

a. Power up the MicroDx system.

b. Plug external hard drive (or USB) into the USB port on the Raspberry Pi.

c. Select "Export File" on the GUI display, and save "Test 1" file onto external hard drive.

d. Select “Shutdown” and verify that the system completely turns off. Mark Pass/Fail on the datasheet.
e. Remove external hard drive from the USB port of the Raspberry Pi.
f. Plug external hard drive into another device (laptop) and load the data file.
g. Verify that all information including the curve and average size value are shown in the file. Mark Pass/Fail on the datasheet.

5.2 Analysis Software Acceptance Tests

Test #	Test	Requirement
3.4.1	Retrieve Data from Sensor	Data file created Data file accessible
3.4.2	Apply Autocorrelation Function	Light from blank subtracted Calibration curve created
3.4.3	Calculate Particle Size	Calculate particle diameter

3.5.1 Retrieve Data from Sensor

a. Power up the MicroDx system and prepare a sample for testing.
b. Select “Run” on the GUI display.
c. From the software, verify that the intensity function was recorded. Mark pass/fail on the data sheet
d. Choose Sample number and select “Load Data” button on GUI display.
e. Verify the data just taken is displayed on the GUI. Mark pass/fail on the data sheet

3.5.2 Apply Autocorrelation Function

a. Power up the MicroDx system and prepare a sample for testing.
b. Select “Run” on the GUI display.
c. In software, check that the light signal from the blank cuvette run is subtracted from the sample’s signal. Mark Pass/Fail on data sheet.
d. In software, check that the autocorrelation function created has the correct form. Mark Pass/Fail on data sheet.

3.5.3 Calculate Particle Size

a. Power up the MicroDx system and prepare a sample for testing.
b. Select “Run” on the GUI display.
c. Check the calculated diameter of the particles in the sample. Record diameter in data sheet.
d. Compare calculated diameter to known diameter and verify it is within 50 nm of the actual diameter. Mark pass/fail in data sheet.
e. Repeat steps a-d for 2 more sets of samples with different diameters. In total 3 samples should have been run and verified.

5.3 Computer Data File Acceptance Data Sheets

MicroDx Verification Test Sheet			
2.7.1 Create and Save Data File Acceptance Test Data Sheet			
Referenced ATP Paragraph Number: **1.0**			
Name of Test: **Create and Detect Speckle Pattern & Intensity**			
Unit Under Test (UUT): Name: **Computer/GUI Assembly** Part Number: Serial Number: n/a			
Results (Pass / Fail): - Filename prompted **pass** / fail - Average particle size value displayed **pass** / fail - Data displayed **pass** / fail		Date of Test: **4/5/22**	
Recording of Test Measurements: **N/A**	Requirement (SR, with Tolerances) **N/A**	Test Equipment Error: **N/A**	Adjusted Test Limit: **N/A**
Computations, (Include Analyses Results, if any): **N/A**			
Signatures: Tester ___________**Ethan Ross**__________________________ Customer____________________________________			

MicroDx Verification Test Sheet			
2.7.2 File Export and Emergency Shutdown Test Data Sheet			
Referenced ATP Paragraph Number: **1.0**			
Name of Test: **Create and Detect Speckle Pattern & Intensity**			
Unit Under Test (UUT): Name: **Computer/GUI Assembly** Part Number: Serial Number: n/a			
Results (Pass / Fail): - System completely shuts down **pass** / fail - File exported **pass** / fail - Data accessed on external laptop **pass** / fail		Date of Test: **4/5/22**	
Recording of Test Measurements:	Requirement (SR, with Tolerances): **N/A**	Test Equipment Error: **N/A**	Adjusted Test Limit:
Computations, (Include Analyses Results, if any): **N/A**			
Signatures: Tester ______________**Alyssa Barney and Megan Mickey**_____________ Customer___________________________________			

5.4 Analysis Software Acceptance Data Sheets

<table>
<tr><th colspan="4">MicroDx Verification Test Sheet</th></tr>
<tr><td colspan="4">3.7.1 Retrieve Data from Sensor Acceptance Test Data Sheet</td></tr>
<tr><td colspan="4">Referenced ATP Paragraph Number: 1.0</td></tr>
<tr><td colspan="4">Name of Test: Retrieve Data from Sensor</td></tr>
<tr><td colspan="4">Unit Under Test (UUT):
Name: Computer/GUI Assembly
Part Number:
Serial Number: n/a</td></tr>
<tr><td colspan="2">Results (Pass / Fail):
- Intensity function recorded pass / fail
- Data saved pass / fail</td><td colspan="2">Date of Test:
4/5/22</td></tr>
<tr><td>Recording of Test Measurements:
N/A</td><td>Requirement (SR, with Tolerances)
N/A</td><td>Test Equipment Error
N/A:</td><td>Adjusted Test Limit:
N/A</td></tr>
<tr><td colspan="4">Computations, (Include Analyses Results, if any): N/A</td></tr>
<tr><td colspan="4">Signatures:
Tester Alyssa Barney and Giang Nguyen
Customer________________________</td></tr>
</table>

<table>
<tr><th colspan="4">MicroDx Verification Test Sheet</th></tr>
<tr><td colspan="4">3.7.2 Apply Autocorrelation Function Test Data Sheet</td></tr>
<tr><td colspan="4">Referenced ATP Paragraph Number: 1.0</td></tr>
<tr><td colspan="4">Name of Test: Apply Autocorrelation Test</td></tr>
<tr><td colspan="4">Unit Under Test (UUT):
Name: Computer/GUI Assembly
Part Number:
Serial Number: n/a</td></tr>
<tr><td colspan="2">Results (Pass / Fail):
- Autocorrelation function has correct form pass / fail</td><td colspan="2">Date of Test:
4/8/22</td></tr>
<tr><td>Recording of Test Measurements:
N/A</td><td>Requirement (SR, with Tolerances) N/A</td><td>Test Equipment Error:
N/A</td><td>Adjusted Test Limit:
N/A</td></tr>
<tr><td colspan="4">Computations, (Include Analyses Results, if any): N/A</td></tr>
<tr><td colspan="4">Signatures:
Tester ______________Megan Mickey_____________
Customer_____________________________________</td></tr>
</table>

1.7.3 Particle Analysis Acceptance Test Data Sheet			
Referenced ATP Paragraph Number: **1.0**			
Name of Test: **Size Particles**			
Unit Under Test (UUT): Name: **Optical Assembly** Part Number: Serial Number: n/a			
Results (Pass / Fail): Sample 1: **500 nm** - Correct size pass / **fail** Sample 2: **720nm** - Correct size **pass** / fail Sample 3: **1000 nm** - Correct size **pass** / fail		Date of Test: **4/13/22**	
Recording of Test Measurements: Sample 1 (**500** nm): - Particle size: **657 nm** Sample 2 (**720** nm): - Particle size: **711 nm** Sample 3 (**1000** nm): - Particle size: **968 nm**	Requirement (SR, with Tolerances): **Results need to be within +/- 50 nm of the size that the particles have listed on there standard bottles.**	Test Equipment Error: **The results for the 500 nm particle sample varied greatly. The team has several theories as to what is causing this error.**	Adjusted Test Limit:
Computations, (Include Analyses Results, if any): **N/A**			
Signatures: Tester **Rainee Meuschke and Megan Mickey** Customer________________			

Section 6.0: Software Version Description

There is currently only one version of the software, completed on May 1, 2022.

Section 7.0: SW User's Manual

7.1 Software Instructions

This manual will cover how to use the analysis software with the oscilloscope. This is intended to be for the testing of the system. Another note is that the following may not apply for the future once the bitscope is integrated into the system.

Before beginning, make sure that all libraries listed in Section 4.2 are installed.

1. Run the system and record 10 trials, each 8-10 seconds in length.
2. Open the analysis software, "dls_modified_analysis.py".
3. Set the number of measurements to the number of trials that were run in line 18 of the code.
4. Verify that the file path is correct in line 77. This should be where the data is stored on the computer.
5. Set the plot title to output the correct size and concentration.
6. (Optional) To change the amount of tail that is cut off from the autocorrelation curve, change the cut value in line 136. The higher the cut value, the more tail that is cut off.
7. Run the software.

Another processing method is to identify and remove outlier trials. To identify outliers, each trial run can be looked at individually. To do so, change the number of measurements in line 18 to one. Then correct the file path in line 77 to read the selected trial run instead of looping through all of them. Run the software and determine whether the output is an outlier. If this is the case, delete the trial and rename the files so that they remain in order. Then follow the above steps, making sure the number of measurements is updated.

7.2 GUI Instructions

The following manual will cover how to operate the GUI for the MicroDx system. This might be updated if more functionalities are added to the MicroDx system.

Starting:

Once the system has been started, the GUI will display the starting screen, which has no graph and no samples saved. The selection dropdown menu will say “No Samples”. If a sample that has not been saved is selected and the “Load Curve”, “Load Data”, or “Export File” buttons are pressed, an error message will appear in the top right corner warning that no sample has been saved under that number. Only sample numbers that have samples saved under them will be displayed in the dropdown menu.

Running a sample:

In order to run a new sample, select the “Run Sample" button. A window will appear and ask which sample number to save the new sample under. Any number 1-5 can be selected, but if a sample is already saved under that number, the new sample will replace it. Once a sample number has been selected, press the save button. The window will close and a message that says, “Running…” will appear in the top right corner until the sample has finished. Once it has finished, the “Running…” message will disappear and the autocorrelation curve for the sample that was just run will appear on the GUI. Additionally, the average size for that sample will be displayed above the graph. The “Cancel Run” button will stop a run if there is currently one being processed and delete any data/files that have already been saved for that sample.

Sample options:

Once a sample has been run and saved, the three buttons just below the sample selection dropdown menu can be used for that sample. The “Load Curve” button will display the graph of the autocorrelation curve for that sample. The “Load Data” will display a graph of a portion of the data that was taken for that sample. Additionally, the “Export File” button will allow the user to save a text file containing all the important information from a file to an external device such as a flash drive. If there is a flash drive plugged in, it will appear in the pop up window and the user can navigate to the folder they would like to save it under. The sample number, such as “sample2” will automatically show up as the name of the file. Once the user has navigated to the desired folder, they can press the save button.

Power off:

Finally, the “Power off” button in the bottom left corner of the screen will power off the system in case of an emergency.

Section 8.0: Notes

Some comments and code written are there for future work on the project. These include some code to interact with a bitscope, code to control the laser through the i/o pins of the Raspberry Pi, and the idea of a function to cut the tail off of autocorrelation curves. Some code is included and is commented out that was used for testing of the system. This mainly consists of code to read csv files and code to display graphs of the raw data and individual autocorrelation functions.

Appendix IV: User Manuals

Section 1.0: Microparticle Sample Preparation

This manual will cover how to prepare a sample of synthetic microparticles for analysis with the MicroDx system. The primary audience of this manual is for anyone seeking to use synthetic microparticle samples in the MicroDx system. The creation of both single-sized particle samples and mixed particle size samples will be covered here. Sample preparation will differ slightly between single-sized particle samples and mixed particle size samples, as well as for samples with a microparticle concentration 0.05% or lower. These procedures were developed to create samples of 3.5 mL, which is enough to fill a singular vial or a cuvette. If necessary, scaling these procedures is possible to create larger samples. Sample dilution is necessary in order for dynamic light scattering to perform properly.

1.1 Materials

The materials necessary for these procedures are: a solvent for dilution, micropipette(s) with a volume range of 0.5 μL to 1000 μL., appropriate micropipette tips, synthetic microparticles, and containers to hold the samples. The solvent is necessary for dilution and the choice of which solvent to use depends on the material of the synthetic microparticles. For our purposes we used polystyrene latex particles sized 1000 μm and smaller, which can be suitably suspended in deionized water or methanol.

1.2 Concentration Calculations

The polystyrene latex particles were labeled in $\%weight/volume$, which can be estimated in mg/mL by multiplying the provided $\%weight/volume$ by 10. This conversion turns $\%weight/volume$ into % concentration in mg/mL. This conversion will allow us to make our measurements mL, which is considerably easier than in $\%weight/volume$. The sample can now be easily prepared, only needing user determination the desired % concentration and whether or not the sample will be a single-sized particle sample and mixed particle size sample. For example: the synthetic microparticle that we ordered came as 10% $weight/volume$, meaning that we could do the following calculations to acquire a sample with 10% concentration for a 3.5 mL:

$$A = desired\ concentration\ (\frac{mg}{mL}),\ B = Solvent\ volume\ (mL)$$

$$S = synthetic\ particle\ volume\ (mL)$$

$$S = 0.1 \cdot A \cdot \frac{1}{10} \cdot 3.5 \quad \text{and} \quad B = 3.5 - S$$

$$S = 0.1 \cdot 0.10 \cdot \frac{1}{10} \cdot 3.5 = 0.0035\ mL$$

$$B = 3.5 - 0.0035 = 3.4965\ mL$$

The above equation means that to create a sample of 10% synthetic microparticle, then 0.0035 mL of microparticle solution and 3.4965 mL of solvent should be added. To use a micropipette, these values should be converted into μL: $3.5\ \mu L$ of microparticle solution and $3496.5\ \mu L$ of solvent.

It is important to note for this manual that the system tends to perform better with smaller concentration, but due to the limitations of the micropipettes, concentrations smaller than 0.05% can not be made directly. Further dilution by creating a seperate standard will be necessary here.

The following instructions of dilution are an example of how to create 0.0025%, 0.001%, and 0.0005% samples all at once:

1. Add $1798.2\ \mu L$ of solvent to a $3.5\ mL$ vial.
2. Add $1.8\ \mu L$ of 10% synthetic particle solution to the same vial. This will create a standard 0.01% solution to be used for dilution.
3. Create the 0.0025% sample:
   a. Add $2625\ \mu L$ of solvent and $875\ \mu L$ of the standard to a separate $3.5\ mL$ vial.
4. Create the 0.001% sample:
   a. Add $3150\ \mu L$ of solvent and $350\ \mu L$ of the standard to a separate $3.5\ mL$ vial.
5. Create the 0.0005% sample:
   a. Add $3325\ \mu L$ of solvent and $175\ \mu L$ of the standard to a separate $3.5\ mL$ vial.

These instructions can be adjusted to create different concentration samples. Using the 0.01% standard solution will allow for the creation of very small microparticle concentrations by varying the amount of solvent and standard, even with the limitations of the micropipettes. It is unlikely that a smaller concentration standard solution will be necessary, as the smallest concentration sample that can be made with the 0.01% standard solution would be 0.000001428571429%.

Preparation of mixed particle size samples is straightforward, in that the calculations are hardly any different. The actual concentration of total particles remains unchanged, but just split between different particle sizes based on the desire of the user. This means that the calculation instructions above can be used, just additionally dividing the volume of microparticle solution by however many sizes of synthetic particles are being used.

Then adding that amount of each particle size alongside the solvent will provide a mixed particle size sample at the correct concentration.

Section 2.0: Oscilloscope Instructions

The following instructions will cover how to use the oscilloscope for testing and data collection. Begin by pressing the power button on the top left (it might take a minute for it to turn on).

2.1 Getting Reading in Range

1. If you've completely lost the signal, press the "Default Setup" button in the top right corner.
2. Then, use volts/div knob for ch 1 to increase or decrease the vertical scale. If the reading then goes off the screen, use the vertical position knob to move up or down on the y-axis until you see the reading
3. Try to center the reading vertically in the screen with it taking up most of the screen but not going outside of it.
4. For the horizontal, turn the sec/div knob until it says 1s in the bottom middle of the graph on the screen, meaning 1 second per box on the graph.

2.2 Saving a Run

1. To clear the screen, press the run/stop button twice. Then cover the oscilloscope screen to prevent the light from affecting the reading and start a 2.5 minute timer.
2. Once the timer goes off, press the run/stop button to freeze the screen.
3. (Can be done before or after a run) Insert the flash drive into the oscilloscope. Wait for all the flashdrive setup screens to disappear.
4. Press the save/recall button to bring up the file saving menu.

5. Scroll on this menu using the general purpose knob until in the top right corner of the screen says “Action Save Waveform”. (You can also get there by pressing the button on the right of that box until you see that option)
6. On the box below that, press the button beside it until it says “Save To File”. Now on the bottom box on the menu it should say “Save to TEK000#.csv”. Make sure it is saving as a csv file!
7. To make sure you are saving the files to the correct folder, press the “Select Folder” button on the menu. Use the general purpose knob to scroll until the DLS folder is highlighted. Then select “Change Folder” to enter that folder. Scroll to the folder for the size of particle you are working with and “Change Folder” again to enter that folder.
8. Then press the “Save/Recall” button again to return to the screen with the data.
9. Once you have frozen the screen that has the data for that run, press the button next to “Save to TEK000#.csv”. Wait for the flashdrive to stop blinking before taking it out.

2.3 Renaming a File

1. The files created should be numbered 0-3 in the order you saved them. Then just go through them and verify that no numbers were skipped.
2. Save these files under a folder named with the size and concentration and test number. This is the folder you will input into the software.

Section 3.0: Testing Software Instructions

This manual will cover how to use the analysis software with the oscilloscope and is a copy of the SW user manual under the SDD. This is intended to be for the testing of the system. Another note is that the following may not apply for the future once the bitscope is integrated into the system.

3.1 Library Installation

Before beginning, make sure that all of the following libraries are installed and can be run: Python TKinter library, Numpy library, Matplotlib library, Scipy library, Statsmodels library.

1. Run the system and record 10 trials.
2. Open the analysis software, “dls_modified_analysis.py”.
3. Set the number of measurements to the number of trials that were run in line 18 of the code.
4. Verify that the file path is correct in line 77. This should be where the data is stored on the computer.
5. Set the plot title to output the correct size and concentration.
6. (Optional) To change the amount of tail that is cut off from the autocorrelation curve, change the cut value in line 136. The higher the cut value, the more tail that is cut off.
7. Run the software.

Another processing method is to identify and remove outlier trials. To identify outliers, each trial run can be looked at individually. To do so, change the number of measurements in line 18 to one. Then correct the file path in line 77 to read the selected trial run. Run the software and determine whether the output is an outlier. If this is the case, delete the trial and rename the files so that they remain in order. Then follow the above steps, making sure the number of measurements is updated.

Section 4.0: GUI Instructions

The following manual will cover how to operate the GUI for the MicroDx system. It is a copy of the GUI instructions in the SDD. This might be updated if more functionalities are added to the MicroDx system.

4.1 Turning GUI On

Once the system has been started, the GUI will display the starting screen, which has no graph and no samples saved. The selection dropdown menu will say “No Samples”. If a sample that has not been saved is selected and the “Load Curve”, “Load Data”, or “Export File” buttons are pressed, an error message will appear in the top right corner warning that no sample has been saved under that number. Only sample numbers that have samples saved under them will be displayed in the dropdown menu.

4.2 Running a Sample

In order to run a new sample, select the “Run Sample" button. A window will appear and ask which sample number to save the new sample under. Any number 1-5 can be selected, but if a sample is already saved under that number, the new sample will replace it. Once a sample number has been selected, press the save button. The window will close and a message that says, “Running…” will appear in the top right corner until the sample has finished. Once it has finished, the “Running…” message will disappear and the autocorrelation curve for the sample that was just run will appear on the GUI. Additionally, the average size for that sample will be displayed above the graph. The “Cancel Run” button will stop a run if there is currently one being processed and delete any data/files that have already been saved for that sample.

4.3 Sample Options

Once a sample has been run and saved, the three buttons just below the sample selection dropdown menu can be used for that sample. The “Load Curve” button will display the graph of the autocorrelation curve for that sample. The “Load Data” will display a graph of a portion of the data that was taken for that sample. Additionally, the “Export File” button will allow the user to save a text file containing all the important information from a file to an external device such as a flash drive. If there is a flash drive plugged in, it will appear in the pop up window and the user can navigate to the folder they would like to save it under. The sample number, such as “sample2” will automatically show up as the name of the file. Once the user has navigated to the desired folder, they can press the save button.

4.4 Power Off

Finally, the “Power off” button in the bottom left corner of the screen will power off the system in case of an emergency.

Appendix V: Test Results

Section 1.0: Particle Size Test Results

Below are all of the results and measurements we got throughout testing. Over time, we used different detector angles and concentrations to get more accurate results.

MicroDx: Particle Size Test Results					
Date of Test	Angle of Detector from Laser Beam (°)	Test Name	Actual Particle Size (nm)	Sample Concentration (% w/v)	Predicted Particle Size (nm)
4/7	6.75	Test 1	720	-	783
4/7	6.75	Test 2	1000	-	956
4/7	6.75	Test 3	1000	-	1034
4/7	6.75	Test 4	1000	-	802
4/8	6.75	Test 1	720	-	742.2
4/8	6.75	Test 2	720	-	815
4/8	6.75	Test 3	720	-	922
4/8	6.75	??	500	-	681
4/8	6.75	test1	500	-	692
4/8	6.75	test2	500	-	430
4/8	6.75	test3	500	-	562.6
4/8	6.75	test4	500	-	583.3
4/8	6.75	test5	500	-	628
4/13	6.75	test1	500	0.0025	-
4/13	6.75	test2	500	0.0025	2700
4/13	6.75	test3	500	0.0025	728

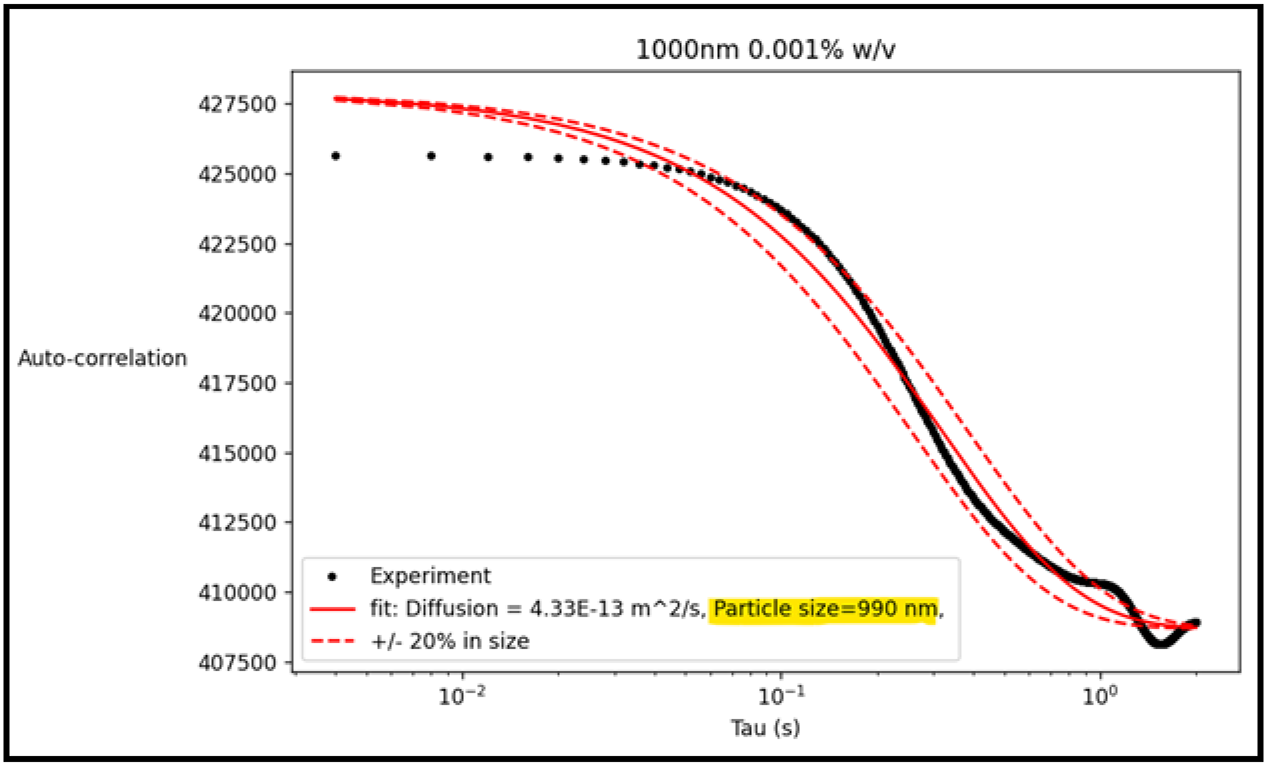
4/13	6.75	test4	500	0.0025	657
4/13	6.75	test5	500	0.0025	694
4/13	6.75	test1	720	0.0025	751
4/13	6.75	test2	720	0.0025	934
4/13	6.75	test3	720	0.0025	711
4/13	6.75	test4	720	0.0025	595
4/13	6.75	test5	720	0.0025	483
4/13	6.75	test1	1000	0.0025	499
4/13	6.75	test2	1000	0.0025	968
4/13	6.75	test3	1000	0.0025	742
4/13	6.75	test4	1000	0.0025	836
4/13	6.75	test5	1000	0.0025	1068
4/13	6.75	test6	1000	0.0025	570
4/18	6.75	test1	500&1000	0.0025	1299
4/18	6.75	test2	500&1000	0.001	986
4/18	6.75	test1	720	0.001	723
4/18	6.75	test2	720	0.001	794
4/18	6.75	test1	1000	0.001	990
4/18	6.75	test2	1000	0.001	1006
4/18	6.75	test1	500	0.001	515
4/18	6.75	test2	500	0.001	925

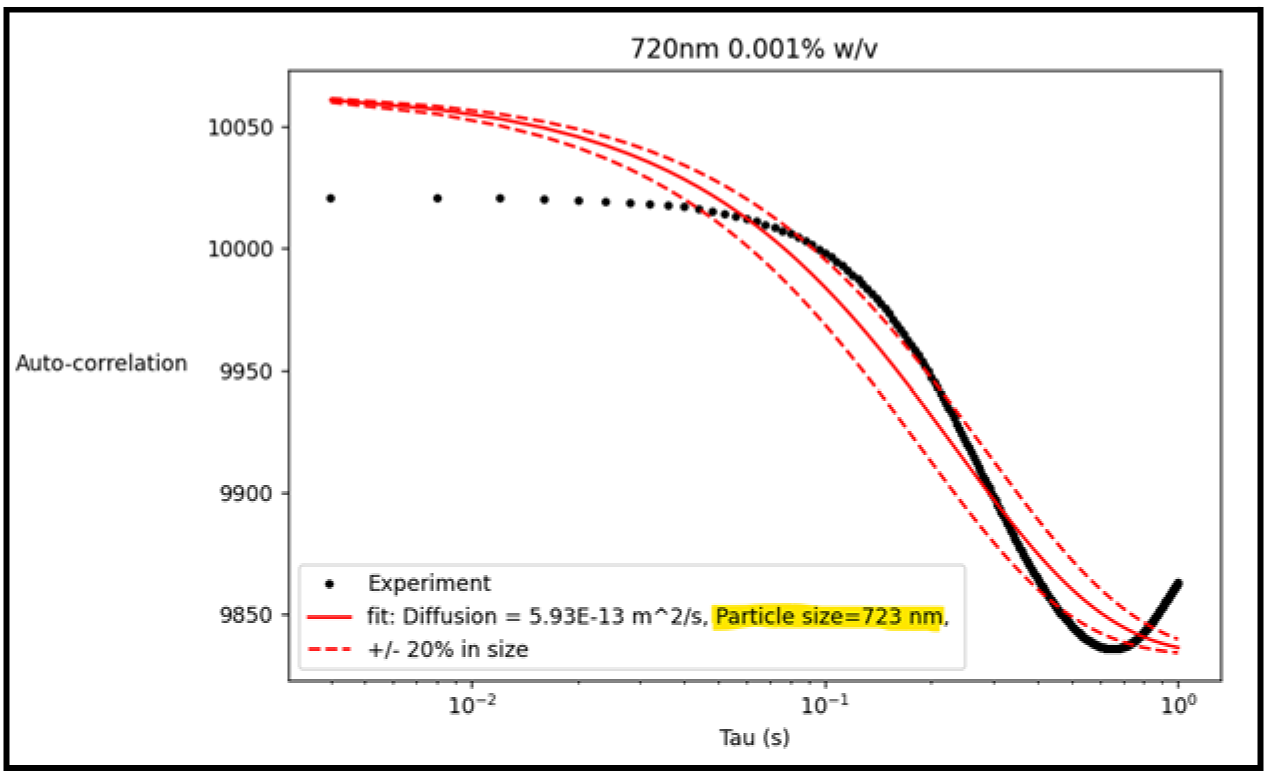
Section 2.0: Particle Size Accuracy Results

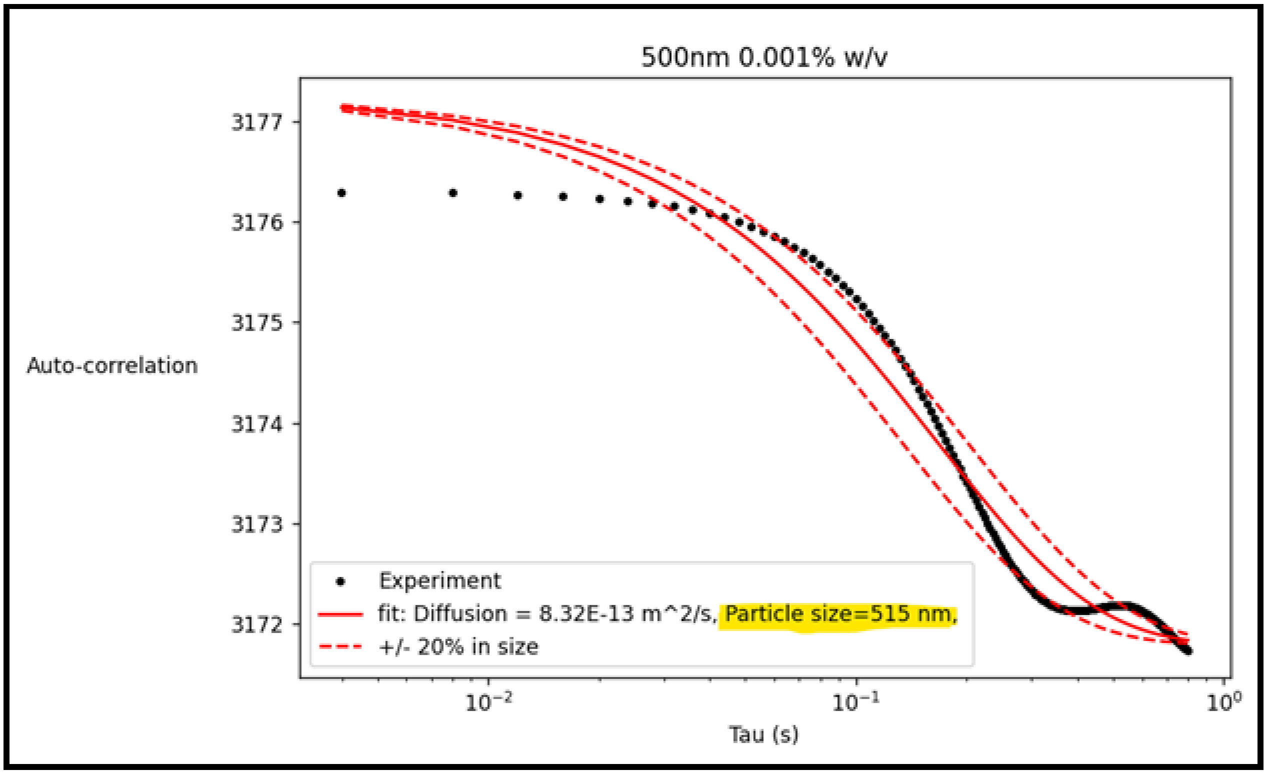
MicroDx: Particle Size Accuracy Results		
500 nm % accuracy	720 nm % accuracy	1000 nm % accuracy
20%	77.8%	80%

These specific accuracy calculations only account for the results from our latest tests. Much of our early April test results were prior to the implementation of our processing methods, such as curve-smoothing, adding autocorrelation functions, eliminating outliers, and “tail” cutting, which is why they were excluded from our system accuracy calculations.

Section 3.0: Autocorrelation Plots Samples






500nm 0.001% w/v
Auto-correlation
3177
3176
3175
3174
3173
3172
Experiment
fit: Diffusion = 8.32E-13 m^2/s, Particle size=515 nm,
+/- 20% in size
10^{-2}
10^{-1}
10^{0}
Tau (s)