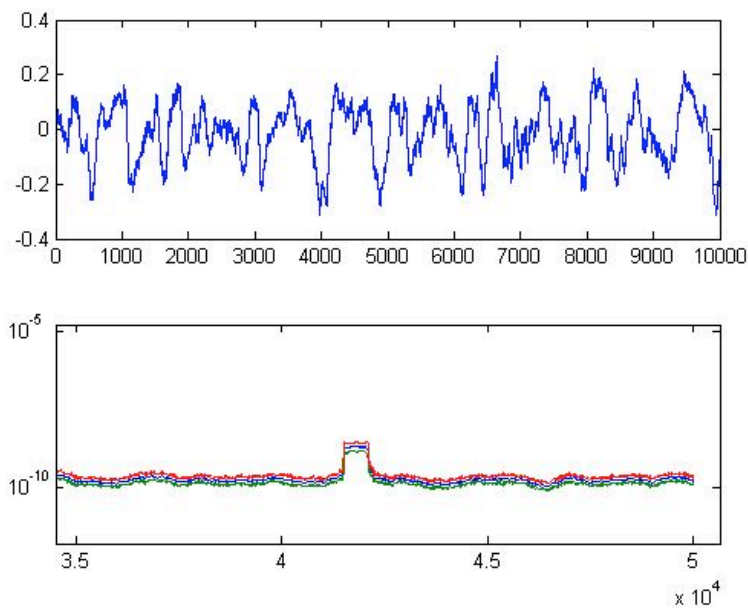


Laser Doppler Velocimetry

Lucas Keenan¹ and Katie Osterday Chapin²



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Performed under the supervision of Professor David Kleinfeld

Physics Department
University of California, San Diego
La Jolla, CA 92093-0411

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¹ lkeenan@ucsd.edu

² kosterda@ucsd.edu

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- A.1 Summary of recorded measurements
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A laser Doppler velocimetry apparatus was constructed complete with signal detection software in order to record accurate measurements for the frequency of the signals detected when micron-sized particles traveled through millimeter diameter glass tubes. A MATLAB file was used to process the recorded signals in order to calculate the mean velocity of the particles. This apparatus is successful in recording and analyzing signals for micron sized glass beads suspended in water and colloidal suspensions, such as milk.

1. Introduction

Laser Doppler Velocimetry is a well-understood and accurate method for detecting and calculating the velocity of small particle droplets or small particles suspended in a liquid medium. This method has many biology related applications. One such possible application is for the study of the velocity of red blood cells suspended in a fluid medium. This is a heavily researched area of interest because certain diseases affecting the stiffness of the red blood cell membrane often affects the velocity at which they travel through capillary tubes. Laser Doppler Velocimetry allows for the ability to easily detect changes in mean velocity of particles, and therefore is a very valuable method for which to study this and similar problems.

By splitting a laser beam into two beams of equal intensity, and focusing them onto a common focal point, we are able to create a region with an elliptical shaped cross sectional area where the diffraction of the laser beam light causes a predictable and uniform fringing pattern of light and dark regions.

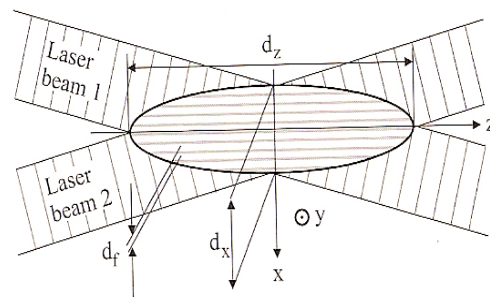


Figure 1 Fringing pattern observed from the two incident laser beams.

As particles with diameter sizes of the same order of magnitude as the fringe spacing pass through this elliptical cross sectional area, they scatter light as they pass through each individual fringe. Because the particles are traveling with a constant velocity, the scattered light obtains a Doppler shift. The frequencies of the corresponding Doppler shifts are too large to be detected and measured alone, so instead the beat frequency is analyzed. Because the two laser beams are incident at different angles, the frequencies of their relative Doppler shifts are different. As a result, the superposition of the two Doppler shifted scattered light beams create a beat frequency which we can detect on a

photodiode, and use to calculate the velocity of the particle.

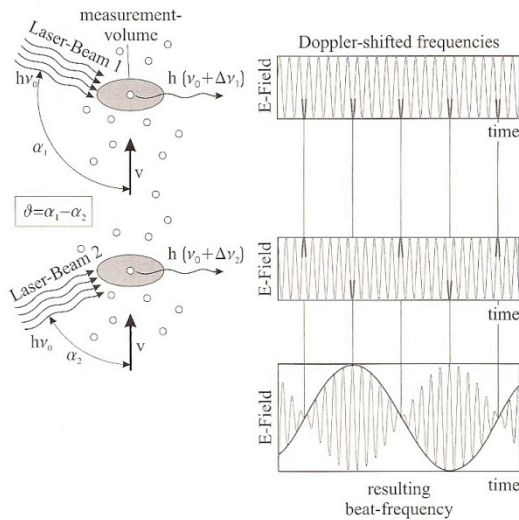


Figure 2 Beat frequency of the two Doppler shifted laser beams

The photodiode transforms the incident light into a current and then, through an internal current to voltage converter, transforms this current into a voltage. The relative voltages are then passed onto a computer where the signal is recorded for later processing. Using a personalized MATLAB file originally downloaded from Cronux.org, the Fourier analysis of the signals tell us the peak beat frequency found within the sample. Using the equation:

$$v = \frac{f_{beat} \cdot \lambda}{2 \cdot \sin\left(\frac{\theta}{2}\right)}$$

with λ equal to 632.8 nm, and half of the angle between the incident beams equal to 14.25 degrees, the mean velocity of the particles is calculated.

2. Laser Doppler Velocimetry

2.1 Experimental setup: Optics

A 2 mW Helium-Neon laser was used in this experiment with $\lambda = 632.8 \text{ nm}$. The laser passed through a beam splitter that yielded two equal-intensity parallel beams that were passed through a series of lenses and other optical components. The first lens focused the two beams onto the measuring volume. Because the curvature of the glass tube used to flow the measuring volume through was very small, the glass acted as a lens and deformed the incident beams. To correct this issue, we used a 3 mm glass tube glued between two glass slides. The glue used was an optical glue specifically designed to have an index of refraction close to that of glass. This allowed us to use a glass tube with a small radius of curvature while having the lasers be incident on a flat plane. As the goal was to collect only the scattered light from the particles moving through the measuring volume, an iris was placed after the measuring volume, to block out the incident beams. A collected lens collimated the scattered light and passed it through a final focusing lens that focused the light onto the photodiode. A laser line filter for $632.8 \pm 10 \text{ nm}$ was utilized in order to filter out environmental white light contributions.

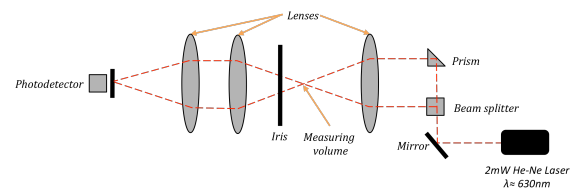


Figure 3 Laser Doppler schematic showing the optical components.

2.2 Experimental setup: Flow system

In order to provide a constant flow, a constant pressure in the flow system was desired. This was achieved by utilizing a large-diameter upper reservoir such that the height of the water remained relatively constant during the trials where data was recorded. A syringe was used to create a siphon that allowed for the fluid to flow continuously while a flow restrictor was placed in line in order to regulate the rate of flow. A second syringe was used to allow for the injection of various fluids of interest without the necessity of filling the large upper reservoir. As mentioned, the measuring volume consisted of a 3 mm OD glass tube that was glued in between two glass slides using an optical glue, the measuring volume was interchangeable via Fleur connectors various sized tubes could be tested.

2.3 Data recording and electronics

As mentioned, the photodiode recorded the varying intensities of the Doppler-shifted scattered light. This signal was converted, by the diode, to a voltage and passed to an Ithalco amplifier. The signal was amplified by 40 dB and then passed successively to an oscilloscope and to a computer for recording. Data recording software^[3] was used to record the signal and format it into a matrix for analysis by MATLAB.

2.4 Data processing and analysis

The recorded data was analyzed using the Chronux^[4] software package that utilized the MATLAB statistics toolbox. The Fourier transform of the recorded signal was taken to provide a semi-log plot in frequency

space where the signal could be analyzed for the beat frequency. Clear peaks could be read from the data corresponding to the expected beat frequencies corresponding to the flow rates being used. 95% expectation curves were also graphed on the log scale along with the data that was taken.

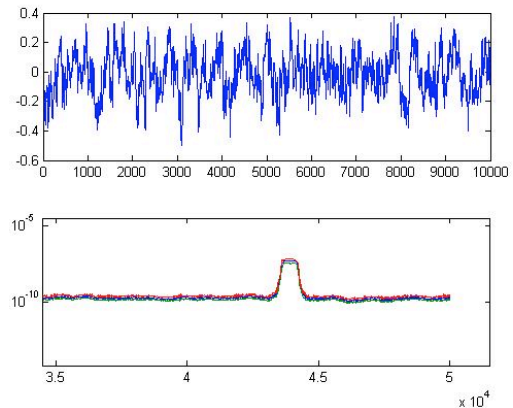


Figure 4 Plot showing the time-space signal versus voltage on the top with the Fourier transformed frequency-space (Hz) signal on the bottom showing a frequency spike at 44kHz corresponding to a particle velocity of 1.4 cm/s.

3. Results

For detailed results please refer to Appendix A.

3.1 Baseline

Ultimately two different sized polystyrene beads were tested and three different flow rates were used. In each case a trial was run where no fluid was flowing in order to record a baseline for the noise that could be expected, and hence ignored, from the data trials.

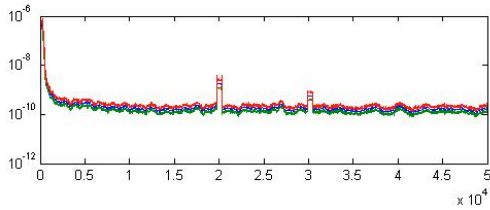


Figure 5 Plot showing two distinct peaks at 20 kHz and 30 kHz respectively which were present during all trials conducted with a sampling frequency of 100 kHz. These peaks were ignored during the data analysis.

3.2 Particles of different size

The first experiment conducted was to verify that polystyrene beads of different sizes could be detected via the Doppler setup. The larger beads, picked to be representative of the size of red blood cells, were 5.8 microns in diameter while the smaller beads were 2.0 microns in diameter. The flow rate of the system was set to be approximately 32 $\mu\text{L/s}$ with an expected corresponding frequency of 43 – 44 kHz. Both sizes of beads were passed along with a control trail of water and the signal from each trial was recorded. Since the bead size is independent of the velocity of the bead the expected result was that the frequency spike should appear in the same range for both trials, which was the result.

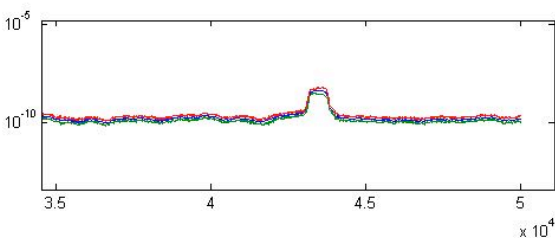


Figure 6 Frequency plots showing the expected beat frequency for polystyrene beads of 5.8 micron size traveling at the same flow rate.

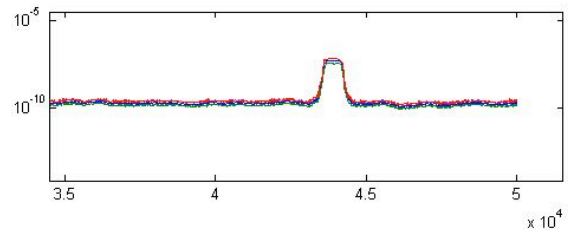


Figure 7 Frequency plots showing the expected beat frequency for polystyrene beads of 2.0 micron size traveling at the same flow rate.

3.3 Particles at different velocities

The second experiment determined whether the velocity of particles of the same size could be measured at different velocities. In order to accomplish this, the flow rate was first set to 1, 2, or 3 mL/min respectively. Control trials were taken at each speed as the sampling frequency was changed. The 5.8 micron bead was then injected into the flow system and the data was recorded. It should be noted that the data acquisition card used had a maximum sampling rate of 200 kHz limiting samples to a maximum beat frequency of 100 kHz. In addition, using extremely fast sampling frequencies resulted in very large data files on the order of several million data points. The software package used was unable to work with such large data sets and most of the data was attenuated in order to provide a smaller data set for computation. Unfortunately for flow rates much more than 2 mL/min no spikes were seen in the frequency plots. Measurement data was very accurate and reliable in the 30 – 50 kHz range, and showed promise at slower flow rates, however started to become confused with the baseline noise that was discussed above.

3.4 Doppler vs. dark field detection

The last experiment completed was designed to show that the measurements being taken were in fact Doppler measurements and not simply a result of the changing intensity expected as a solid bead passed between the incident beam and the photodiode. In order to measure the beat frequency of the two Doppler-shifted beams two beams were necessary, thus in this experiment one of the incident beams was blocked, data was recorded and then compared to the control test where two beams were used. The result was very clear that the measurements were in fact the Doppler-shifted beams as when one of the beams was blocked the system recorded no frequency peaks.

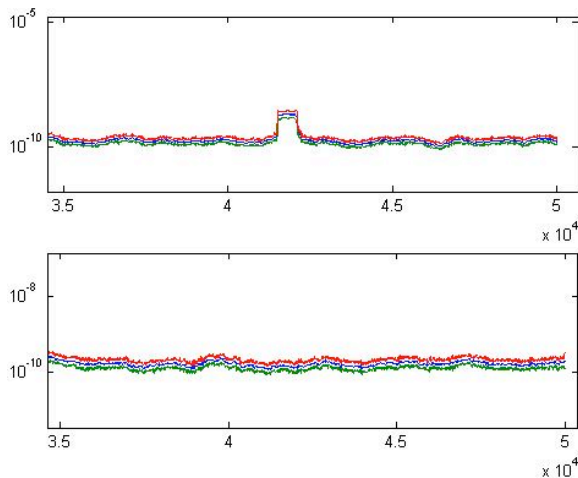


Figure 8 Frequency plots of the 5.8 micron bead traveling at 13.3 mm/s. Plot (a) corresponds to the measurement with two beams, which when Doppler-shifted formed a beat frequency that could be detected. Plot (b) corresponds to the control measurement with one blocked laser beam; no beat frequency was detected.

4. Discussion

4.1 Observed and measured results

This experiment recorded very successful preliminary data and showed proof of concept that a laser Doppler velocimetry apparatus could be built to successfully measure the velocity of particles of size on the order of red blood cells traveling in a tube representative of a medium sized vein at a comparable velocity. The apparatus was successful in using dual Doppler-shifted beams and the resulting beat frequency to make measurements in a low-intensity noninvasive way.

The size of the beads tested showed no appreciable difference in results displaying frequency spikes in the same range. The diameter of the measuring volume, however, did seem to affect results as capillary tubes with an inner diameter of 0.5 mm was unable to register results. Additionally, as flow rates caused the velocity of the particle to increase, the sampling frequency was increased. As a result, this created larger data files that were harder to process. As a second concern, ensuring that the electronics involved could operate on the order of hundreds of kilohertz was nontrivial. Ultimately, fast flow rates were not detected with this setup. Finally, the observation it was observed that higher density solutions yielded larger, broader peaks, while very dilute solutions yielded no results. The frequency dependence of the signal, however, was very obviously seen on the oscilloscope with the bead solutions and homogenous solutions, such as whole milk.

4.2 Future improvements

The largest obstacle in building the apparatus was aligning the optical axis and components where the use of a pinhole was found to be extremely helpful. Also, the use of a three-dimensional stage to properly position the measuring volume was critical.

The software package used to analyze the signals had memory deficiencies when attempting to analyze data sets larger than about 600,000 data points. A more efficient algorithm could be used in the future to allow for the processing of more of the data.

Furthermore, the measuring volumes used in these experiments needed to be larger than the size of true capillary tubes. It is believed that with the proper optical setup, velocities in very small tubes could be measured. Additionally, with this setup, the electronics used in this experiment could be updated to allow for velocity measurements up to 3.2 cm/s. With updated equipment, we believe that recording measurements at slower velocities is possible.

One exciting future direction of this project would be to pursue the measurement of more homogenous fluids and biological fluids to further test the reliability of this method.

4.3 Acknowledgements

Special thanks is given to Phil Tsai who was extremely helpful during all phases of this project and testing needed to show that the measurements were indeed real Doppler measurements. Thanks to Jeff Moore for

providing the data recording software, as well as to Ilya Valmianski for help with the Chronux software package during the signal analysis phase. Finally, thanks to David Kleinfeld for providing the opportunity to work on this most interesting experiment and encouraging our experimental curiosity.

4.4 References

- [1] Albrecht, H. –E. (2003). *Laser Doppler and Phase Doppler Measurement Techniques* (Springer, New York).
- [2] Mayinger, F. (1994). *Optical Measurements: Techniques and Applications* (Springer, New York).
- [3] Moore, Jeff. Data_recorder MATLAB utility.
- [4] Chronux 2.0 Signal Processing Software Package (www.chronux.org)

A.2 MATLAB code used for signal analysis

```
%% load data sets
clear;
close all;
    load trial_06.mat %File name of data file

%% parameters for spectral estimation

Nmax=10 e3;
if length (data)>Nmax,
    data=data (1:Nmax);
end
Fs=100 e3;
T=Nmax/Fs;

df=300;% frequency resolution/bandwidth
NW = round (df*T);% t*bandwidth

params.tapers = [NW (2*NW)-1];
params.pad    =    2;
params.Fs     = Fs;
params.fpass = [0 params.Fs/2];% displaying from 0 to nyquist
freq.
    params.err = [2 .05];
params.trialave = 0;

%% exercise 1 -- calculate power spectrum
%
[S,f,Serr]=mtspectrumc (data,params);
figure (1)
subplot (2,1,1)
plot (data)
subplot (2,1,2)
semilogy (f,(S),f,(Serr))
```