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**THE DEVELOPMENT OF OPTICAL STIFFNESS  
CALIBRATION SOFTWARE BASED ON  
EQUIPARTITION THEOREM,  
BOLTZMANN STATISTICS  
AND POWER SPECTRUM  
DENSITY**



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**MUHAMMAD YUNUS BIN HAMID**

**UNIVERSITI PENDIDIKAN SULTAN IDRIS**

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**THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENT FOR THE  
DEGREE OF MASTER OF SCIENCE  
(MASTER BY RESEARCH)**

**FACULTY OF SCIENCE AND MATHEMATICS  
UNIVERSITY PENDIDIKAN SULTAN IDRIS**

**2018**



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## ACKNOWLEDGEMENT

First and foremost, thanks to Allah SWT for His blessing and approval in the completion of this thesis.

The utmost dedication of appreciation toward my supervisor, Profesor Madya Dr. Shahrul Kadri Bin Ayop for his guidance and brilliant insight throughout my journey of completing this thesis. His constant supervision and motivational supports helped me to get through this challenging journey.

My appreciation also goes to Dr. Mohd Ikhwan Hadi Bin Yaacob, Dr. Nurul Syafiqah Yap Abdullah and Dr. Mohd Norzaidi Mat Nawawi for sharing of their knowledge and gave constructive comments that helped this research to be a success.

I would also like to acknowledge all the staffs of Faculty of Science and Mathematics that involved directly or indirectly in the making of this research. Without their help, this thesis would not be completed as it is today.

I am also grateful for my friends which I have crossed path during my study, namely Wan Nor Suhaila Binti Wan Aziz, Muhammad Azrin Bin Azman, Nor Shaida Binti Mohd Saufi, Muhamad Safuan Bin Mat Yeng @ Mat Zin, Muhammad Rashidi Bin Ab Razak, Nurul Husna Binti Osman and Mohd Farid Bin Mohamad Yusof. Thanks for all the memories.

Last but not least important, sincere gratitude toward my parents Hamid Bin Yub Mahidin and Hafizah Binti Aiyub and also to the rest of my other siblings. During this quest, we were tested with some difficulties but every single one of them showed their responsibility and support. I am very touched. Thank You.





## ABSTRACT

This study aimed to develop an optical stiffness calibration system using three methods. The used methods were Boltzmann statistics (BS), equipartition theorem (ET) and power spectrum density (PSD) analysis. This study consisted of two phases; system development phase and testing phase. System development phase involved hardware and software components. Testing phase involved actual experimental optical stiffness calibration. The finding of the study was a calibration system consisting hardware and software parts. The hardware part was an optical tweezers that was equipped with quadrant photodiode (QPD) and piezostage. The software part was a custom made software which was constructed on LabVIEW 2012 platform, namely OSCal. This study showed the conversion factor (used in ET and BS methods) and optical stiffness depends on trapping laser power. In conclusion, this study successfully developed the aimed system with comparable optical stiffness between each method and other reference studies. The implication of this study is that the time spent for calibration for optical tweezers procedure can be shortened so that other researcher can focus more on the intended applications of the optical tweezers.





## **PEMBANGUNAN PERISIAN PENENTUKURAN KEKAKUAN OPTIK BERASASKAN TEOREM PEMETAKAN, ANALISIS STATISTIK BOLTZMANN DAN KETUMPATAN SPEKTRUM KUASA**

### **ABSTRAK**

Kajian ini bertujuan membangunkan sistem penentukuran kekakuan optik menggunakan tiga kaedah. Kaedah yang digunakan ialah analisis statistik Boltzmann (BS), teorem pemetakan (ET) dan ketumpatan spektrum kuasa (PSD). Kajian ini terdiri daripada dua fasa iaitu fasa pembangunan sistem dan fasa pengujian. Fasa pembangunan sistem melibatkan komponen perkakasan dan perisian. Fasa pengujian melibatkan penentukuran sebenar kekakuan optik secara eksperimen. Dapatan kajian adalah satu sistem penentukuran yang terdiri daripada bahagian perkakasan dan perisian. Bahagian perkakasan ialah penyepit optik yang dilengkapi dengan fotodiod kuadran (QPD) dan piezopentas. Bahagian perisian merupakan perisian khusus yang dibangunkan atas platform LabVIEW 2012 yang dinamakan OSCal. Kajian ini menunjukkan faktor penukaran (digunakan dalam kaedah ET dan BS) dan kekakuan optik bergantung kepada kuasa laser pemerangkap yang digunakan. Sebagai kesimpulan, kajian ini berjaya membangunkan sistem yang disasarkan dengan kekakuan optik yang setanding antara setiap kaedah dan kajian rujukan lain. Kajian ini memberi implikasi terhadap masa yang digunakan untuk prosedur penentukuran penyepit optik yang boleh disingkatkan supaya penyelidik lain boleh memfokuskan kepada kegunaan penyepit optik yang diinginkan.



## TABLE OF CONTENTS

	Page
<b>DECLARATION OF ORIGINAL WORK</b>	ii
<b>DECLARATION OF THESIS</b>	iii
<b>ACKNOWLEDGEMENT</b>	iv
<b>ABSTRACT</b>	v
<b>ABSTRAK</b>	vi
<b>TABLE OF CONTENT</b>	vii
<b>LIST OF TABLES</b>	xi
<b>LIST OF FIGURES</b>	xii
<b>LIST OF SYMBOLS</b>	xv
<b>LIST OF CONSTANTS</b>	xvii
<b>LIST OF ABBREVIATIONS</b>	xviii
<b>LIST OF APPENDICES</b>	xix
<b>CHAPTER 1 INTRODUCTION</b>	
1.1 Introduction	1
1.2 Background of the Study	2
1.3 Problem Statement	4
1.4 Objectives of the Study	5
1.5 Significance of the Study	5
1.6 Scope and Limitation of Study	6



1.7 Thesis Summary	6
--------------------	---

## CHAPTER 2 LITERATURE REVIEW

2.1 Introduction	8
2.2 Optical Tweezers Principle	8
2.3 Optical Stiffness Calibration Methods	14
2.3.1 Boltzmann Statistics (BS) Analysis Method	15
2.3.2 Equipartition Theorem (ET) Analysis Method	18
2.3.3 Power Spectrum Density (PSD) Analysis Method	19
2.3.4 Lorentzian Fitting	21
2.3.4.1 Lorentzian as a line profile	22
2.3.4.2 General Concept of Lorentzian Fitting in PSD	25
2.3.4.3 Lorentzian Fitting on LabVIEW	27

2.4 Calibration Computation	29
2.5 Quadrant Photodiode (QPD) Voltage-to-Position Calibration	30
2.6 Reported Optical Stiffness	33
2.7 Summary	34

## CHAPTER 3 RESEARCH METHODOLOGY

3.1 Introduction	37
3.2 Sample Preparation	38
3.3 Development of Optical Stiffness Calibration System	40
3.4 System Hardware	42
3.4.1 Trapping Process	46
3.5 Quadrant Photodiodes Voltage-to-Position Calibration	48
3.6 System Software	56
3.6.1 Data Upload	56

3.6.2	ET and BS Methods	59
3.6.2.1	Equipartition Theorem Display	59
3.6.2.2	Equipartition Theorem Block Design	60
3.6.2.3	Boltzmann Statistics Display	61
3.6.2.4	Boltzmann Statistics Block Design	62
3.6.3	PSD Methods	65
3.6.3.1	Power Spectrum Density Calculation	66
3.6.3.2	Program Lorentzian Fitting	67
3.6.3.3	Plotted Graph	68
3.6.4	Program Flowchart	69
3.7	Summary	70

## CHAPTER 4 RESULT AND DISCUSSION

4.1	Introduction	72
4.2	Performance of the System	73
4.2.1	Laser Power	74
4.2.2	Conversion Factor	75
4.2.3	Stiffness Determination for PSD, ET and BS	77
4.3	System Validation	80
4.4	Summary	81

## CHAPTER 5 CONCLUSION AND RECOMMENDATIONS

5.1	Introduction	82
5.2	Conclusion	83
5.2.1	Objective 1	83
5.2.2	Objective 2	84
5.3	Unique Characteristic of the System	85



5.4 Limitation of the Developed System 86

5.5 Significance of the Study 87

5.6 Recommendation for Further Study 88

**REFERENCES** 89





## LIST OF TABLES

Table No.		Page
2.1	Literature Review Stiffness	34
2.2	Comparison Between BS, ET and PSD methods	35
3.1	List of Hardware for Specific Module	41
4.1	The Current Supply Need for The Laser	75
4.2	The Stiffness Obtained With Three Methods Of Determination	77



## LIST OF FIGURES

Figures No.		Page
1.1	Optical resultant force on the transparent dielectric sphere due to refraction of two rays of light.	3
2.1	Change of momentum of the bead when the centre of the bead (O) is below the laser focus.	10
2.2	Change of momentum of the bead when the centre of the bead (O) is above the laser focus.	11
2.3	Bead motion in $x$ direction due to gradient change of beam.	12
2.4	Restoring force acting on the bead when displace from the trap focus.	14
2.5	(a) The spatial data of trapped microparticle, (b) the sum histogram based on the spatial data and (c) the potential energy for the trapped microparticle.	16
2.6	Two signals A and B (above) superpose to form a new signal (below)	20
2.7	Comparison of Normalised Gaussian, Lorentzian and Voigt functions describing an ideal symmetrical absorption band.	23
2.8	(a) Shows that power spectrum obtained from QPD signal, while (b) shows that the power spectrum obtained from Video tracking method with both were fit by using Lorentzian function to obtain $f_c$ .	27
2.9	LabVIEW Lorentzian fitting for laser spectral.	28
2.10	The histogram of position distribution and Trap Potential embed in TweezPal	29
2.11	Schematic description of laser pointed on QPD sensor.	31
2.12	The output voltage of QPD against position of the particle in $y$ -axis.	33
3.1	A drop of manufactured solution being put in a sample vial	38

3.2	(a) Photo of two drops of solution on the microscope slide and (b) the schematic diagram of the sample chamber.	39
3.3	The concept of the optical tweezers stiffness determination system concept.	40
3.4	Optical Tweezers Hardware.	43
3.5	Piezostage set in optical tweezers setup to control stage position	44
3.6	Thorlabs Current Controller	45
3.7	Newport Optical Power Meter	46
3.8	Trapping setup for the prepared sample	47
3.9	Stage sample observed by CCD camera	48
3.10	Photo of Thorlabs QPD device	49
3.11	Bead settling onto the slide wall mechanism	50
3.12	ActiveX block diagram for QPD device.	50
3.13	(A) QPD position calibration display (B) QPD beam diagram for specific position in graph.	52
3.14	QPD Position Calibration System for Graph Analyser.	53
3.15	Digital Oscilloscope of Yokogawa DL6054	55
3.16	Modification of data for software uploading.	56
3.17	LabVIEW Read from Measurement File block diagram	57
3.18	Block Diagram for Button and Case Structure in LabVIEW	58
3.19	LabVIEW Split Signal block	58
3.20	Equipartition Theorem method front panel display.	60
3.21	Extraction Portion of Signal block diagram to select desired range of data	61
3.22	The block used to find Variance of the selected data range	61
3.23	Boltzmann Statistics for (a) Histogram display and (b) Potential Graph display.	62



3.24	Block Design for histogram calculation.	63
3.25	LabVIEW Curve fitting block for histogram spline fit.	63
3.26	Integration block for used for the program.	64
3.27	General polynomial fit block to perform parabolic fit.	64
3.28	PSD Calculation display for $x$ -dimension signal.	65
3.29	The extraction data from FFT and Power Spectrum Density block.	66
3.30	Lorentzian fitting by using Non-linear Curve Fit block.	67
3.31	Block Design for PSD graph plotting and its Fitting.	68
3.32	Graph display for PSD and Lorentzian fitting in one display	69
3.33	Software flowchart	70
3.34	Optical stiffness determination process	71
4.1	The front display of the software	73
4.2	Graph of power laser against current supply.	74
4.3	Behaviour of the conversion factor $b$ in different power laser for experiment setup.	76
4.4	Stiffness for the optical trapping obtained by the PSD, ET and BS methods with the changed of power laser for $x$ -dimension.	78
4.5	Stiffness for the optical trapping obtained by the PSD, ET and BS methods with the changed of power laser for $y$ -dimension.	79





## LIST OF SYMBOLS

Symbols	Meaning	Units
$w$	full width at half maximum	arbitrary
$L$	Lorentzian distribution	arbitrary
$G$	Gaussian distribution	arbitrary
$\sigma$	standard deviation	arbitrary
$\sigma^2$	variance	arbitrary
$x_o$	mean of a statistical distribution	arbitrary
$m$	mass	kg
$t$	time	s
$\gamma_0$	coefficient of friction	Ns/m
$k$	optical stiffness	pN/ $\mu\text{m}$
$R$	bead's radius	m
$\rho$	fluid's density	$\text{m}^3/\text{s}$
$\nu$	kinematic viscosity	$\text{m}^2/\text{s}$
$\eta$	fluid viscosity	$\text{kg}/\text{m}^3$
$T$	absolute temperature	K
$D$	coefficient of diffusion	$\text{V}^2/\text{s}$
$f_c$	corner frequency	Hz
$b$	conversion factor voltage-to-position	m/V
$A$	$D/2\pi^2b^2$	$\text{V}^2/\text{s}$







$a_1, a_2$	changeable parameters on LabVIEW fitting	arbitrary
$\rho'$	probability density	---
$\rho$	particle position	---
$U$	trap potential energy	J
$C$	normalisation constant	---





## LIST OF CONSTANTS

Symbols	Meaning	Units
$k_B$	Boltzmann constant	J/K
$\pi$	pie constant	---
$\lambda$	wavelength	m





## LIST OF ABBREVIATIONS

CCD            Charged Coupled Device

ET             Equipartition Theorem

BS             Boltzmann Statistics

PSD           Power Spectrum Density

OT             Optical Tweezers

QPD           Quadrant Photodiode

NA             Numerical Aperture

WD             Working Distance



ASCII           American Standard Code for Information  
Interchange

USB           Universal Serial Bus

OSCal          Optical Stiffness Calculator

GUI             Graphical User Interface





## LIST OF APPENDICES

- A Knowledge Dissemination
- B System Specification
- C Software Interfaces





## CHAPTER 1

### INTRODUCTION



This chapter presents an introduction to an optical tweezers (OT) study and its applications which lay the foundation of this thesis. This chapter composes of five parts which are background of the study, the problem statement, objectives of the research, significance of the study, scope and limitation of the study, and the summary of the thesis.





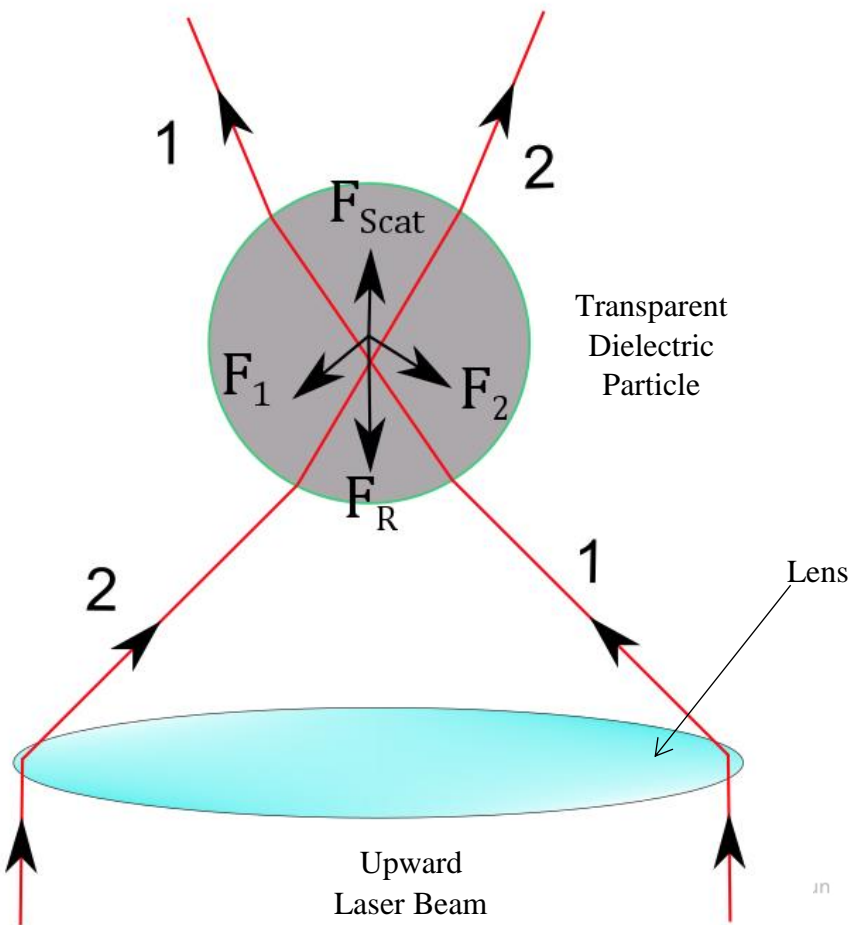
## 1.2 Background of the Study

Force of radiation pressure was demonstrated to be able to push microparticle suspended in water by Arthur Ashkin in 1970 (Arthur Ashkin, 1970). By using visible light, the particles were accelerated from the light source. The experiment was then extended with the use of two light sources side by side shone toward one another. The particles located between the light sources were hold in position due to the radiation pressure from both directions. It was not until 1986, it was found out that a single highly tight focused laser alone was sufficient to optically trap microparticle. The experimental setup for this type of trapping was later called as OT (Arthur Ashkin et al., 1986).



depending on the used laser wavelength. Figure 1.1 illustrates the basic principle of OT. Suppose a light beam enters a lens is being focused in a certain point inside a transparent microparticle. Line 1 and 2 represent the outmost end of the beam ray. The optical forces,  $F_1$  and  $F_2$  are resulted from the conservation of light momentum. Upward scattering force,  $F_{\text{scat}}$  arised from the radiation pressure. These three forces,  $F_1$ ,  $F_2$  and  $F_{\text{scat}}$  produce the resultant force,  $F_R$ .  $F_R$  pull the microparticle toward the laser focus thus locking the movement of the microparticle. Further explanation on the trapping principle will be discussed in the next chapter.





*Figure 1.1.* Optical resultant force on the transparent dielectric sphere due to refraction of two rays of light.

OT was used in many biological applications because of its wide advantages. OT are feasible tools that applicable to handle single cells, organelles and molecules without contacting the sample physically to minimize damaging and contaminating the sample. Due to the heat absorption, biological samples are in a risk to be damaged when the trapping laser is exerted onto them. However with fair parameter setting in power intensity, wavelength of the laser and duration of the trapping, biological sample can be trapped with minimal risk (Pan, 2012; Pilát et al., 2013). By using OT, researchers could determine the values of the small exert forces with nanometre precision (Dieckmann et al., 1998).





OT applications were applied in various science field such as in microfluidics, dynamics molecular motor, alignment in microscopic, and sorting colloidal (Frieze et al., 1998; Gier, 1997; MacDonald et al., 2003; Maier, 2005). The questions of physical fundamental can be answered in OT research which include the direct observation of angular momentum, and the interaction of light-matter (Dholakia & Zemánek, 2010; He et al., 1995).

This almost non-damaging technique requires calibration process to obtain optical stiffness value that could be used in experiments (Osterman, 2010). Boltzmann statistics (BS), equipartition theorem (ET) and power spectrum density analysis (PSD) are the available methods for the calibration purpose. Previous researchers showed that the PSD was the most reliable technique known up until now (Berg-Sørensen & Flyvbjerg, 2004; Soler, 2008). This method gained its reliability due to the conventional use of Brownian motion theory by Einstein-Ornstein-Uhlenbeck. Even though BS and ET not as decisive as PSD analysis, the outcome stiffness for all methods are crucial for comparison as they complement each other in a precise measurement.

### 1.3 Problem Statement

Even after the OT have been setup up and a microparticle is managed to be optically trapped, the OT is still not ideally ready to be used for force related experiments. The OT calibration is essential for each OT setup and unique to individual experiment.







Unfortunately the optical trapping calibration analysis required tremendous amount of time for all methods mentioned especially if it done manually (Osterman, 2010).

For the study of comparison among these methods, a special approach for the calibration analysis is needed to keep up with the time consuming calibration procedure. However, fast computation and user-friendly calibration design for these three methods is not publicly available. To resolve this issue, a custom build calibration software with decent calculation procedures need to be developed.

#### 1.4 Objectives of the Study



1. To develop graphical user interface (GUI) software for optical tweezers calibration based on Boltzmann statistics, equipartition theorem and power spectrum density analysis.
2. To compare the optical stiffness calculated using these methods of analysis.

#### 1.5 Significance of the Study

Usually the stiffness determination procedure is conducted by experts. With the help if the developed calibration software, OT calibration is easy to be done even by





novice users such as undergraduate students and inexperienced researchers. The developed software also provides a platform for the next upcoming researchers to proceed with the force spectroscopy using OT in wide applications. In the future, researchers can focus more on the application of OT rather than tedious analysis calibration steps.

## 1.6 Scope and Limitation of Study

The Modular OT OTKB (/M) from Thorlabs was used in this study to develop data acquisition module that receive signal from quadrant photodiode from the OT. The essential specification is listed in the Appendix (B). The sample for the performance check used was polystyrene beads with the diameter of  $3.004 \pm 0.007 \mu\text{m}$  in deionised water.

## 1.7 Thesis Summary

The thesis consists of five chapters. CHAPTER 1 which is the current chapter of the thesis covers background of the study, statement of problem, objectives of the study, research questions, significance of the study and scope and limitation of study.

The second chapter entitled CHAPTER 2 describes the theory about OT, its applications and stiffness calibrations procedures done by previous researchers.





CHAPTER 3 is the third chapter of the thesis that tells the modus operandi of the study. It consists of the methodology for sample preparation, data acquiring procedure and the system calibration development.

CHAPTER 4 is describing about the system calibration performance and its validation.

And the final chapter, CHAPTER 5 mentions the conclusion of the thesis and propose recommendation for future study.

