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

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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 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.

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LIST OF SYMBOLS

Symbols	**Meaning**	**Units**
w	full width at half maximum	arbitrary
L	Lorentzian distribution	arbitrary
G	Gaussian distribution	arbitrary
σ	standard deviation	arbitrary
σ^2	variance	arbitrary
x_o	mean of a statistical distribution	arbitrary
m	mass	kg
t	time	s
γ_0	coefficient of friction	$\mathrm{Ns/m}$
k	optical stiffness	$\mathrm{pN/\mu m}$
R	bead's radius	m
ρ	fluid's density	$\mathrm{m^3/s}$
v	kinematic viscosity	$\mathrm{m^2/s}$
η	fluid viscosity	$\mathrm{kg/m^3}$
T	absolute temperature	K
D	coefficient of diffusion	$\mathrm{V^2/s}$
f_c	corner frequency	Hz
b	conversion factor voltage-to-position	m/V
A	$D/2\pi^2 b^2$	$\mathrm{V^2/s}$

$a1, a2$	changeable parameters on LabVIEW fitting	arbitrary
ρ'	probability density	---
ρ	particle position	---
U	trap potential energy	J
C	normalisation constant	---

LIST OF CONSTANTS

Symbols	**Meaning**	**Units**
k_B	Boltzmann constant	J/K
π	pie constant	---
λ	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

CHAPTER 1

INTRODUCTION

1.1 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).

In OT, the manipulated samples are generally very small, about micron-sized 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_{scat} arised from the radiation pressure. These three forces, F_1, F_2 and F_{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.

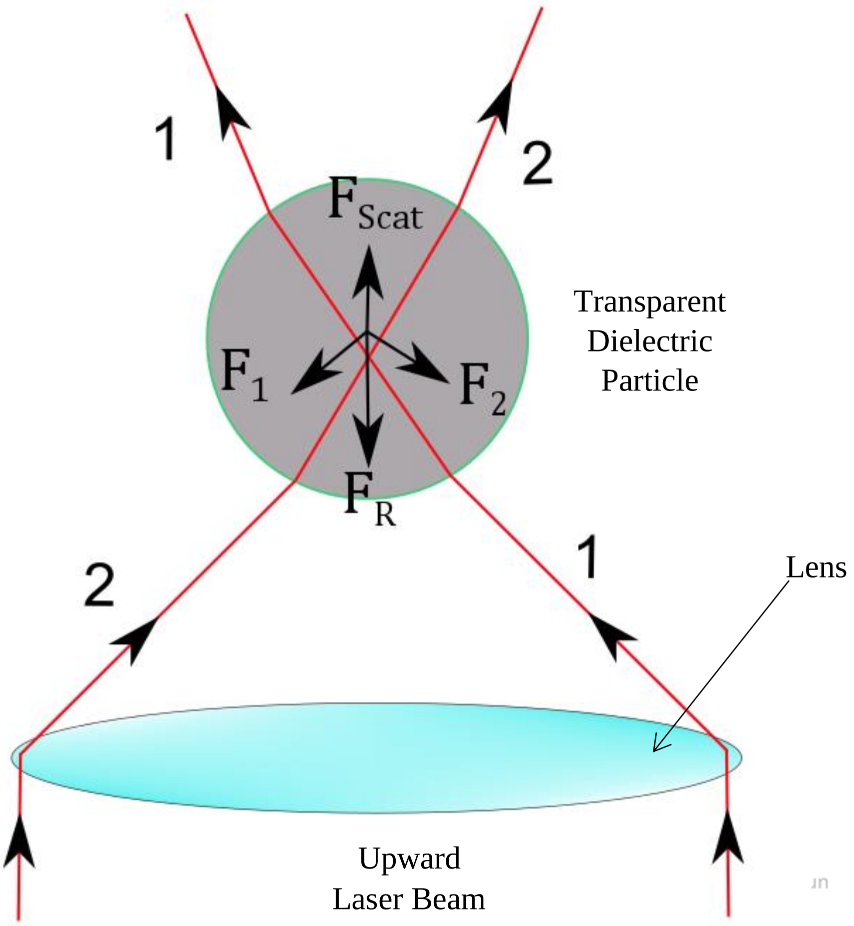

Figure 1.1. Optical resultant force on the transparent dielectric sphere due to refraction of two rays of light.

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 (Friese 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 it reliability due to the conventional use of Brownian motion theory by Einstein-Orntein-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

The objectives of the study are:

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 inexperience researches. The developed software also provides a platform for the next upcoming researches 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 ± 0.007 µm 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 researches.

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.

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