

ISSN 1742-6596

Journal of Physics
Conference Series

The 11th Biennial Conference on
Classical and Quantum Relativistic
Dynamics of Particles and Fields

1239

VOLUME 1239 – 2019

4–7 June 2018
Mérida, Yucatán, Mexico

EDITOR
Markus Lind

The open access journal for conference proceedings
iopscience.org/jpcos

IOP Publishing

Table of contents

Volume 853

May 2017

◀ Previous issue Next issue ▶

**International Conference on Physical Instrumentation and Advanced Materials 27 October 2016,
Hotel Santika Premiere, Surabaya, Indonesia**

Accepted papers received: 22 May 2017

Published online: 07 June 2017

Open all abstracts

Preface

OPEN ACCESS 011001

International Conference on Physical Instrumentation and Advanced Materials

+ Open abstract  View article  PDF

OPEN ACCESS 011002

Peer review statement

+ Open abstract  View article  PDF

Instrumentation Physics

OPEN ACCESS 012001

Modeling of electrical impedance tomography to detect breast cancer by finite volume methods

K. Ain, R A Wibowo and S Soelistiono

+ Open abstract  View article  PDF

OPEN ACCESS 012002

Dual frequency electrical impedance tomography to obtain functional image

Imam Sapuan, Khusnul Ain and Alif Suryanto

+ Open abstract  View article  PDF


OPEN ACCESS 012003

IoT real time data acquisition using MQTT protocol

This site uses cookies. By continuing to use this site you agree to our use of cookies. To find out more, see our Privacy and Cookies policy.

+ Open abstract  View article  PDF



-
- OPEN ACCESS** 012004
Simulation of leakage current measurement on medical devices using helmholtz coil configuration with different current flow
E Sutanto, F Chandra and R Dinata
[+](#) [Open abstract](#) [View article](#) [PDF](#)
-
- OPEN ACCESS** 012005
A simulation of surface plasmon resonance-based tapered fiber and sensing
Nina Siti Aminah, Siti Chalimah, Hendro, R Hidayat and M Djamal
[+](#) [Open abstract](#) [View article](#) [PDF](#)
-
- OPEN ACCESS** 012006
Development of TGS2611 methane sensor and SHT11 humidity and temperature sensor for measuring greenhouse gas on peatlands in south kalimantan, indonesia
I Sugriwan and O Soesanto
[+](#) [Open abstract](#) [View article](#) [PDF](#)
-
- OPEN ACCESS** 012007
Development of ozone sterilization system based microcontroller for *E. Coli* bacteria sterilization
Arfan Eko Fahrudin, Endarko, Amar Vijai Nasrulloh and Nurma Sari
[+](#) [Open abstract](#) [View article](#) [PDF](#)
-
- OPEN ACCESS** 012008
Cholesterol detection using optical fiber sensor based on intensity modulation
Moh Budiyanto, Suhariningsih and Moh Yasin
[+](#) [Open abstract](#) [View article](#) [PDF](#)
-
- OPEN ACCESS** 012009
IIR digital filter design for powerline noise cancellation of ECG signal using arduino platform
Akif Rahmatillah and Ataulkarim
[+](#) [Open abstract](#) [View article](#) [PDF](#)
-
- OPEN ACCESS** 012010
Development of wireless sensor network for landslide monitoring system
Suryadi, Prabowo Puranto, Hendra Adinanta, Adrin Tohari and Purnomo S Priambodo
[+](#) [Open abstract](#) [View article](#) [PDF](#)
-
- OPEN ACCESS** 012011
This site uses cookies. By continuing to use this site you agree to our use of cookies. To find out more, see our Privacy and Cookies policy. 

Detection of acute lymphocyte leukemia using k-nearest neighbor algorithm based on shape and histogram features

Endah Purwanti and Evelyn Calista

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012012

Colour segmentation of multi variants tuberculosis sputum images using self organizing map

Riries Rulaningtyas, Andriyan B Suksmono, Tati L R Mengko and Putri Saptawati

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012013

Dose evaluation of organs at risk (OAR) cervical cancer using dose volume histogram (DVH) on brachytherapy

R Arif Wibowo, Bambang Haris and dan Inganatul Islamiyah

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012014

Effect of window length on performance of the elbow-joint angle prediction based on electromyography

Triwiyanto, Oyas Wahyunggoro, Hanung Adi Nugroho and Herianto

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012015

Confocal microscope is able to detect calcium metabolic in neuronal infection by *toxoplasma gondii*

A D Sensusiati, T K S Priya and Y P Dachlan

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012016

Performance weight sensor using graded index optical fiber on static test with UTM

R Khamimatul Ula and Dwi Hanto

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012017

Investigation of michelson interferometer for volatile organic compound sensor

Marzuarman, Muhammad Rivai, Tri Arief Sardjono and Djoko Purwanto

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012018

The transformation of equation of the characteristic curves to widen the working area of the hall effect sensor

This site uses cookies. By continuing to use this site you agree to our use of cookies. To find out more, see our Privacy and Cookies policy.



D Darmawan, F Rahmawati, Suprayogi and A Suhendi

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012019

Design of vein finder with multi tuning wavelength using RGB LED

Franky Chandra, Aries Wahyudianto and M Yasin

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012020

Optimization image of magnetic resonance imaging (MRI) T2 fast spin echo (FSE) with variation echo train length (ETL) on the rupture tendon achilles case

Akhmad Muzamil and Achmad Haries Firmansyah

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012021

Fabrication of prototype for measuring the exhaled breath temperature (EBT) to support detection of asthma

A A Harnawan, Mariati, A Fahrudin and A Assegaf

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012022

Characterization of transimpedance amplifier as optical to electrical converter on designing optical instrumentation

D Hanto and R K Ula

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012023

Density characterization of radiochromic film through source axis distance (SAD) technique in linac with slab phantom for radiotherapy applications

Yousida Hariani and Bambang Haris

[+ Open abstract](#) [View article](#) [PDF](#)

Advanced Materials

OPEN ACCESS

012024

Permanent magnetic field, direct electric field, and infrared to reduce blood glucose level and hepatic function in *mus musculus* with diabetic mellitus

Suhariningsih, Hari Basuki Notobroto, Dwi Winarni, Saikhu Achmad Hussein and Tri Anggono Prijo

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS cookies. By continuing to use this site you agree to our use of cookies. To find out more, see our Privacy and Cookies policy.

012025

Hydroxyapatite coating on cobalt alloys using electrophoretic deposition method for bone implant application

Aminatun, Shovita M, Chintya K I, Dyah H and Dwi W

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012026

The effectiveness of laser diode induction to *Carica Papaya L.* chlorophyll extract to be ROS generating in the photodynamic inactivation mechanisms for *C.albicans* biofilms

S Dewi Astuty and A Baktir

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012027

Preparation of creating active carbon from cigarette filter waste using microwave-induced KOH activation

Yanuar Hamzah and Lazuardi Umar

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012028

Synthesis and characterization of breast-phantom-based gelatine-glutaraldehyde-TiO₂ as a test material for the application of breast cancer diagnosis

Nuril Ukhrowiyah, Novi Setyaningsih, Dyah Hikmawati and Moh Yasin

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012029

Collagen-chitosan-glycerol bio-composite as artificial tympanic membrane for ruptured inner ear organ

Prihartini Widiyanti, Rara Setya Angtika, Brillyana Githanadi, Ditya Hanif Kharisma, Tarikh Omar Asyraf and Adita Wardani

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012030

Effects of hyperbaric oxygen therapy in enhancing expressions of e-NOS, TNF- α and VEGF in wound healing

Imam Susilo, Anita Devi, Azham Purwandhono and Sunaryo Hadi Warsito

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012031

The first-principle study on the stability of trans-HCOH in various solvents

Rizka Nur Fadilla, Nufida Dwi Aisyah, Hermawan K Dipojono and Febdian Rusydi

[+ Open abstract](#) [View article](#) [PDF](#)

This site uses cookies. By continuing to use this site you agree to our use of cookies. To find out more,

[View our Privacy and Cookies policy.](#)

012032

Analysis of thin layer optical properties of A-Si:H P-Type doping CH₄ and P-Type without CH₄ is deposited PECVD systems

Soni Prayogi, Ayunis, Kresna, Yoyok Cahyono, Akidah and Darminto

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012033

The detection of organic solvent vapor by using polymer coated chemocapacitor sensor

Apik Rusdiarna Indrapraja, Muhammad Rivai, Achmad Arifin and Djoko Purwanto

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012034

The dosage optimization of He-Ne laser energy as a candidate for photodynamic therapy of cancer cells with exogenous photosensitizer variations

R Apsari, I Maghfiroh, H Setyawati, Winarno, D Arifianto and A Zaidan

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012035

The effect of TiO₂ thin film thickness on self-cleaning glass properties

Nandang Mufti, Ifa K R Laila, Hartatiek and Abdulloh Fuad

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012036

Hyaluronic Acid (HA)-Polyethylene glycol (PEG) as injectable hydrogel for intervertebral disc degeneration patients therapy

Cityta Putri Kwarta, Prihartini Widiyanti and Siswanto

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012037

Preparation of molecular sieve from natural pyrophyllite and characterization of its Al/Si ratio, crystal structure, and Porosity

Riris Idiawati, Abdulloh Fuad, Nandang Mufti, Hartatiek, Syamsul Bahtiar, Subakti and Ahmad Taufiq

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012038

An in vivo photodynamic therapy with diode laser to cell activation of kidney dysfunction

Suryani Dyah Astuti, Brahma Indra Prasaja and Tri Anggono Prijo

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012039

Antimicrobial effect of *pleomeleangustifolia* pheophytin A activation with diode laser to *steptococcus mutans*

This site uses cookies. By continuing to use this site you agree to our use of cookies. To find out more, see our privacy and cookies policy.



Sinari Alfat Sunarko, Wiwied Ekasari and Suryani Dyah Astuti

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012040

Influences of the calcination temperature and polymethyl methacrylate templates to characteristic pore membrane of bioceramic titanium dioxide

Jan Ady, Eka Viandari and Dewi W Hasanah

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012041

Fabrication and characterization of Zinc Oxide (ZnO) nanoparticle by sol-gel method

Siswanto, Nurul T. Rochman and Putri Riski Akwalia

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012042

The synthesis and characterization of hydrogel chitosan-alginate with the addition of plasticizer lauric acid for wound dressing application

Djony Izak Rudyardjo and Setiawan Wijayanto

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012043

Implementation of density functional theory method on object-oriented programming (C++) to calculate energy band structure using the projector augmented wave (PAW)

E Alfianto, F Rusydi, N D Aisyah, R N Fadilla, H K Dipojono and M A Martoprawiro

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012044

Study on conventional carbon characteristics as counter electrode for dye sensitized solar cells

Muhammad Noer Fajar and Endarko

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012045

The effect of Cr₂O₃ doping on structures and dielectric constants of SiO₂-Bi₂O₃-B₂O₃-Na₂CO₃ glass based on silica gel of natural sand

M Diantoro, M B Zaini, Z Muniroh, Nasikhudin and A Hidayat

[+ Open abstract](#) [View article](#) [PDF](#)

OPEN ACCESS

012046

Effect of temperature on the corrosion inhibition of iron in liquid lead using oxygen

inhibitor: studied by MD simulation

This site uses cookies: By continuing to use this site you agree to our use of cookies. To find out more, see our Privacy and Cookies policy

Author: Prayudha, Fauzan, Fauzan and Zaki Su'ud



[+ Open abstract](#)[View article](#)[PDF](#)**OPEN ACCESS**

012047

Microstructure and dielectric properties of cellulose acetate-ZnO/ITO composite films based on water hyacinth

M Diantoro, A A Mustikasari, N Wijayanti, C Yogihati and A Taufiq

[+ Open abstract](#)[View article](#)[PDF](#)**OPEN ACCESS**

012048

Folate receptor mediated in vivo targeted delivery of human serum albumin coated manganese ferrite magnetic nanoparticles to cancer cells

A Zaidan, F Ilhami, M Z Fahmi, B Purwanto and R Z Kharisma

[+ Open abstract](#)[View article](#)[PDF](#)

JOURNAL LINKS

[Journal home](#)[Journal Scope](#)[Information for organizers](#)[Information for authors](#)[Contact us](#)[Reprint services from Curran Associates](#)



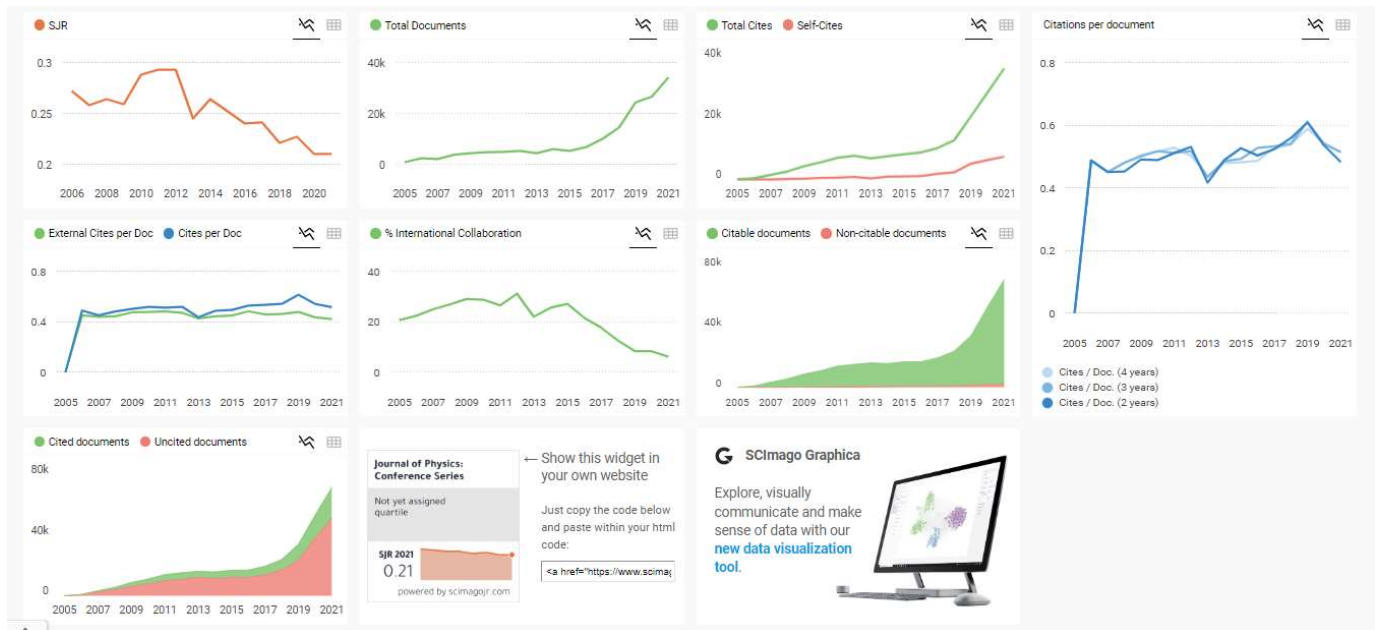
This site uses cookies. By continuing to use this site you agree to our use of cookies. To find out more, see our [Privacy and Cookies policy](#).



BUKTI SCOPUS

Journal of Physics: Conference Series

COUNTRY United Kingdom 	SUBJECT AREA AND CATEGORY Physics and Astronomy Physics and Astronomy (miscellaneous)	PUBLISHER IOP Publishing Ltd.	H-INDEX <h1 style="font-size: 2em; margin: 0;">85</h1>
PUBLICATION TYPE Conferences and Proceedings	ISSN 17426588, 17426596	COVERAGE 2005-2021	INFORMATION Homepage How to publish in this journal jpcs@iopublishing.org



PAPER • OPEN ACCESS

The dosage optimization of He-Ne laser energy as a candidate for photodynamic therapy of cancer cells with exogenous photosensitizer variations

To cite this article: R Apsari *et al* 2017 *J. Phys.: Conf. Ser.* **853** 012034

View the [article online](#) for updates and enhancements.

Related content

- [Antimicrobial Photodynamic Inactivation and Antitumor Photodynamic Therapy with Fullerenes: Photodynamic therapy](#)
L F de Freitas and M R Hamblin
- [A study of the photodynamic effect on cancerous cells](#)
M S AISalhi, M Atif, A A Alobiadi *et al.*
- [Erratum: Monte Carlo modelling of daylight activated photodynamic therapy \(2015 Phys. Med. Biol. 60 4059\)](#)
C L Campbell, K Wood, R M Valentine *et al.*



IOP | ebooks™

Bringing you innovative digital publishing with leading voices to create your essential collection of books in STEM research.

Start exploring the collection - download the first chapter of every title for free.

The dosage optimization of He-Ne laser energy as a candidate for photodynamic therapy of cancer cells with exogenous photosensitizer variations

R Apsari^{1,2,*}, I Maghfiroh¹, H Setyawati³, Winarno^{1,2}, D Arifianto^{1,2} and A Zaidan¹

¹Department of Physics, Faculty of Science and Technology, Airlangga University, Surabaya 60115, Indonesia

²Department of Engineering, Faculty of Vocational Studies, Airlangga University, Surabaya 60286, Indonesia

³Department of Chemistry, Faculty of Science and Technology, Airlangga University, Surabaya 60115, Indonesia

*Corresponding email address: apsari.unair@gmail.com

Abstract. The aim of this research was to find out the most effective energy dose of laser energy per unit area irradiated to the subject, used in T47D cancer cell photodynamic therapy. The experiment used three variations of energy doses and two exogenous photosensitizers. The laser used was the He-Ne laser with a wavelength of 632 nm. Laser exposure on the T47D cancer cells was conducted in vitro at a distance of 1 cm with energy doses of 30.4 J/cm², 18.8 J/cm², and 30.4 J/cm². The photosensitizers used in the experiment were chlorophylls isolated from sour-sop leaves and *Protoporphyrin IX* (*PpIX*). The concentration of photosensitizers which is not toxic to cells, based on the toxicity test, is 300 ppm for chlorophylls and 350 ppm for *PpIX*. At the energy dose of 30.4 J/cm², the percentage of cell death was 96.25% with chlorophyll addition, while with *PpIX* addition, the percentage was 81.62%. At the same energy dose, cell death in apoptosis was 95.78% with chlorophyll addition and 80.56% with *PpIX* addition. Based on the result of this research, it was revealed that the energy dose of He-Ne laser output of 30.4 J/cm² was the most effective energy dose between the energy dose variation that could yield T47D cell death in an apoptosis way.

1. Introduction

Cancer is a disease caused by abnormal and uncontrollable body tissue's cell growth. The prevalence of cancer in Indonesia is 1.4 per 1000 people and has become a major cause of death around the world [1]. At the moment, there are several alternative medications for cancer with their own downsides. As a consequence, alternative medication which is minimally invasive and selective against cancer cells is needed.

One type of medication that can be an alternative against cancer is photodynamic therapy [2]. Photodynamic therapy involves three main components: light, photosensitizer, and oxygen. Chlorophylls and Protoporphyrin IX (*PpIX*) have chromophore and auxochrome clusters, allowing them to be made as photosensitizers. Both of them have absorption spectrums within the red



wavelength [2]. In photodynamic therapy, there is a process where photosensitizers are localized on cancer tissues and when exposed with lasers the photosensitizers become active and produce Reactive Oxygen Species (ROS). ROS then induces apoptosis death. Apoptosis is an organized and programmable death, so pathologically only apoptosis death is allowed [3].

Li *et al.* (2010) have stated that the combination of C-phycoyanin and He-Ne laser irradiation is able to increase the activity effects of anti-tumor, body immune, and pro-apoptosis against cancer cells [4]. Similar research was also conducted by Al-Khafaji *et al.* (2010) who showed that the most damaging effect on cancer cells resulted from the combination of Hematoporphyrin derivative photosensitizers and He-Ne laser irradiation on low power with energy doses of 3.6 and 7.2 J/cm² [5]. Fadilla (2015) also conducted research using PpIX and chlorophylls with radiation from lasers with a wavelength of 650 nm, and a laser energy dose of 18.8 J/cm² could cause apoptosis death to cancer cells [6]. The weakness of this research is that the laser interaction effects on cells could not be achieved with an effective energy dose. Therefore, the aim of this research was to find out the most effective energy dose of lasers using non-toxic photosensitizer solutions between variation of energy doses similar to the previous studies.

2. Research Methods

The laser used in this research is the He-Ne laser LSW-10 from Jianxi Liansheng Technology Co., Ltd with a wavelength of 632 nm. The stability of the laser was characterized using an experimental setup shown in Figure 1.

Chlorophylls as photosensitizers in this research were extracted from the leaves of sour-sop. Sour-sop leaves were chosen because they can grow abundantly only from one tree which will not disturb the ecosystem by endangering the existence of sour-sop plants due to exploitation. They were also chosen because they are not a food commodity so they can be used for medical purposes as photosensitizers to cure cancer due to their chlorophyll content. The chlorophyll isolation was conducted by drying the sour-sop leaves that had been cut into small pieces in the open air but without direct sunlight. The process was then continued with maceration, evaporation, partition, and VLC (Vacuum Liquid Chromatography) to obtain 11 fractions of sour-sop leaf extract as shown in Figure 3. The green bluish fraction is the chlorophylls [8]. Then, a thin layer chromatography was conducted and the fractions were photographed with 366 nm UV light to know the fraction node patterns and to determine fractions with the most chlorophylls. The chosen fractions were then dried further in a 40°C oven to gain thick chlorophyll extracts as shown in Figure 4.



Figure 1. Checking of He-Ne Laser Power with A Digital Powermeter.

Another photosensitizer also used in this research was the commercial Protoporphyrin IX (PpIX) from Sigma-Aldrich. Absorption wavelength characterization was then conducted on both photosensitizers using a UV-Vis spectrophotometer.

Simultaneously, the T47D cancer cell culture reproduction in a petri dish using a set of cell culture tools such as a micropipette (Thermo), a centrifuge, a CO₂ incubator (Thermo), a 96-well plate, a conical tube (Biologix), a vortex, a light microscope (Nikon), ELISA Reader (Bio-Rad iMark), air flow laminar (Dalton), a fluorescence microscope (Olympus). The growth medium used in the culture reproduction of T47D cancer cells is DMEM medium with PBS (Phosphate Buffered Saline) 10%, Penicillin-streptomycin 1%, and fungison 0.5%.

Before the treatment to expose the laser to cancer cells with and without the photosensitizer, there was a photosensitizer toxicity test toward cancer cells with the MTT Assay method to find out the concentration of photosensitizers which was not toxic (safe) to cells. In the toxicity test, the photosensitizers (chlorophylls and PpIX) were prepared, with varied concentrations: 100 ppm, 150 ppm, 200 ppm, 250 ppm, 300 ppm, and 350 ppm. The photosensitizers were solved with DMSO 0.1% solvent in DMEM cancer cell growth medium. DMSO 0.1 % was chosen because it was safe to cells [7]. A safe to cell photosensitizer solvent was chosen to avoid the influence on the result of the photosensitizer toxicity test.

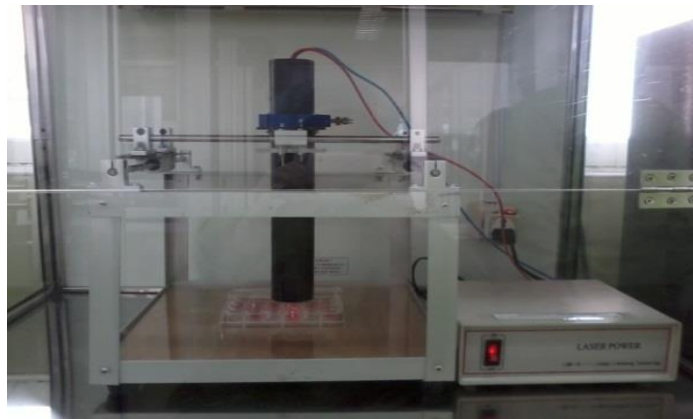


Figure 2. Setting Up the T47D Cancer Cell Exposure with Red He-Ne Laser inside Laminar Air Flow (LAF).



Figure 3. Eleven fractions produced from the VLC process.

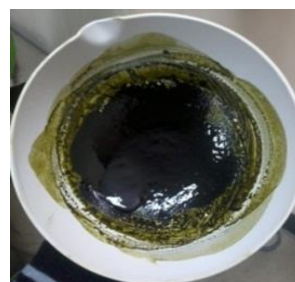


Figure 4. Thick chlorophyll extract after being in an oven.

T47D cancer cells which had been grown to reach enough population for the test, were then divided into sample groups. There were three treatment groups, namely a laser without photosensitizer addition (L), a laser with chlorophyll addition (LK), and a laser with PpIX addition (LP). Cells were exposed with lasers inside Laminar Air Flow (LAF) so that they were still in sterile condition, with mountings and a slider that had been arranged in such a way that the lasers could slide along the X and Y axis (Figure 2). Before the exposure began, the laser mounting tools were cleaned first with alcohol.

Cells that had been incubated with chlorophyll and PpIX photosensitizers were put on a sample plate. Then, cells in each well were exposed with the He-Ne laser one by one with a variation of energy doses of 7.2 J/cm², 18.8 J/cm², and 30.4 J/cm². These energy doses are used due to an effective dose for the goals of the therapy. Each treatment was repeated three times. After the exposure finished, the cells were observed with a fluorescent microscope with an addition of coloring reagents, Acridine Orange (AO) and Ethidium Bromide (EB), to know the number of living and dead cells, based on the different colors produced. The living cells percentage was calculated using equation (1).

$$\% \text{ living cells} = \frac{\text{OD Treatment} - \text{OD Media}}{\text{OD Cell Control} - \text{OD Media}} \times 100\% \quad (1)$$

3. Results and Discussion

3.1. Checking of the He-Ne laser power

Checking the power of the He-Ne laser was conducted by varying the laser distances from the samples, which were 1.0 cm up to 3.0 cm. Power measurements were conducted repeatedly up to three times, and an optimum average power of (0.603 ± 0.010) mW from the distance of 1 cm was obtained. Laser power changes due to variations of exposure distances were proven to be insignificant, which had a significant value of 0.128 (.05), so the laser power was stated as stable against changes in exposure distances.

In checking the stability of the He-Ne laser power against the exposure time, it was revealed that the He-Ne laser in the distance of 1 cm started to be stable 30 minutes after turning on the laser. Besides power, the calculation of laser energy doses also considered the laser area that could be measured if the diameter was known. Laser has a Gaussian file in which the Gaussian file diameter depends on the laser exposure distance. In this research, the laser file diameter was adjusted with the data sheet of LSW-10 He-Ne laser which was 1.2 mm.

3.2. Photosensitizer characterization (chlorophyll and protoporphyrin IX)

Based on a photosensitizer absorbency test, it was as expected that chlorophyll had the highest absorbent peaks at the red and blue wavelengths (Figure 5), which was in line with the statement of Lichtenthaler and Claus in 2009 [9]. This result indicates that our isolation method is appropriate for this current work. As seen in Figure 6, in PpIX photosensitizer there were several absorbent peaks in the wavelengths of 462 nm, 542 nm, 598 nm, and 646 nm (red area wavelength). This is the same with research conducted by Rossetti *et al* in 2010 who found that the absorbent spectrum of PpIX showed a strong absorbency in the wavelength of 406 nm, and four weak peaks in the wavelengths of 516 nm, 554 nm, 600 nm, and 626 nm (red area wavelength) [10]. The results of photosensitizer characterizations of both chlorophylls and PpIX were found to have the absorbency peaks in the red wavelength that were the same with the wavelength of the laser used in this research. The chlorophyll absorbency at the red wavelength ($\lambda = 671$ nm) is 0.129 while for the PpIX, the absorbency at the red wavelength ($\lambda = 646$ nm) is 0.088.

3.3. Photosensitizer toxicity test

The reading of toxicity tests was shown by Optical Density (OD) values which represent the number of living cells. Overall, with concentration variations from 100 ppm until 350 ppm, the toxicity tests showed that the percentage of living cells was more than 50%, so it could be concluded that with those concentrations, both photosensitizers (chlorophylls and PpIX) were not toxic. The concentrations of each photosensitizer with cell percentages are 300 ppm for the chlorophylls with the percentage of living cells of 97.6% and 350 ppm for PpIX with the percentage of living cells of 75.9% were chosen for the treatments with sample cells.

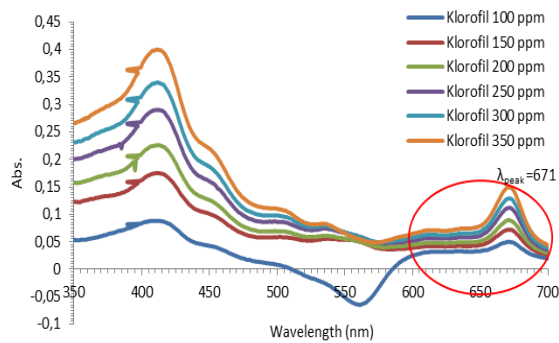


Figure 5. Chlorophyll Absorbance with Various Concentrations via UV-Vis Spectrophotometer.

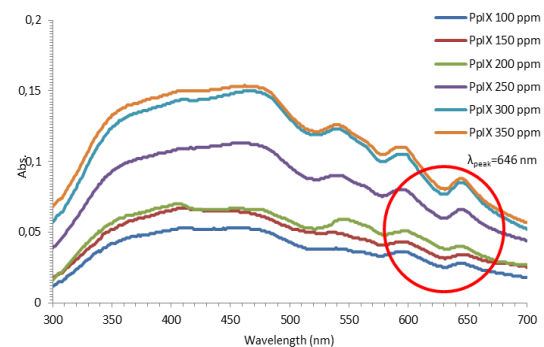


Figure 6. Absorbance of Protoporphyrin IX with Concentration Variations through UV-Vis Spectrophotometer.

3.4. T47D cancer cell death due to the red He-Ne laser exposure with or without photosensitizer additions

The percentages of cell death after the treatment in each group of L, LK, and LP with certain laser energy doses are presented in the form of a graph in Figure 7. As seen in the Figure 7, the highest cell death percentage occurred with the laser energy dose of 30.4 J/cm^2 and LK treatment was more effective in producing cell death compared to other groups of L and LP. It should be noted that the energy dose that became the independent variable in this research was the laser output energy dose (air dose), not the energy dose which was absorbed by the tissue of cancer cells (quantum yield).

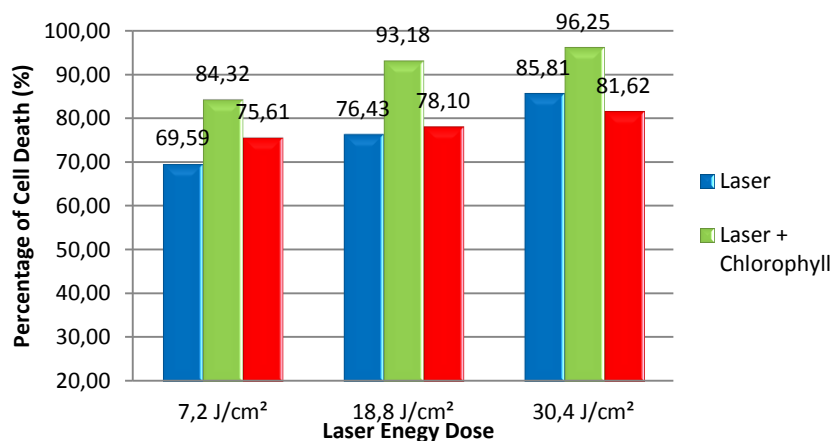


Figure 7. Graph of the Percentage of Cell Death After Treatment.

3.5. Observation of the number of apoptosis cells on T47D cancer cells after treatment

Coloring reagents (AO and EB) were given to differentiate the colors of the living cells and apoptosis ones. The difference in colors comes from the DNA fluorescent which is not same for each type of cells. Living cells with intact membranes have a uniform green color. When cells are in apoptosis stage and the membranes start to bleed, EB can enter into the cells and give an orange color. The picture of T47D cancer cells which was obtained from observation with a fluorescent microscope is divided into their treatment groups of control group, L, LK, and LP, respectively, as seen in Figure 8 and the percentage of apoptosis cell death due to treatments on each group is presented in the form of a graph as shown in Figure 9.

This graph shows that cancer cells exposed to the lasers with additions of photosensitizers with PpIX or chlorophylls were able to influence the cell death, and an addition of chlorophyll photosensitizer was found out to be more effective in affecting the cell death in an apoptosis way than addition of PpIX photosensitizer. This is because chlorophylls have more chromophore clusters than PpIX because chlorophylls experience more photophysical and photochemical interactions due to the more energy being absorbed from the given laser. Besides that, the peak absorption at the wavelength of 671 nm which falls into optical window range (650 nm – 850 nm) has quite high tissue penetration and enough triplet state energy to produce singlet oxygen. Meanwhile, PpIX has an absorption peak of a wavelength less than 650 nm (outside the optical window range for PDT, making it less effective in producing singlet oxygen). This is the reason why apoptosis cell death occurred more in the laser treatment group with chlorophyll addition (LK).

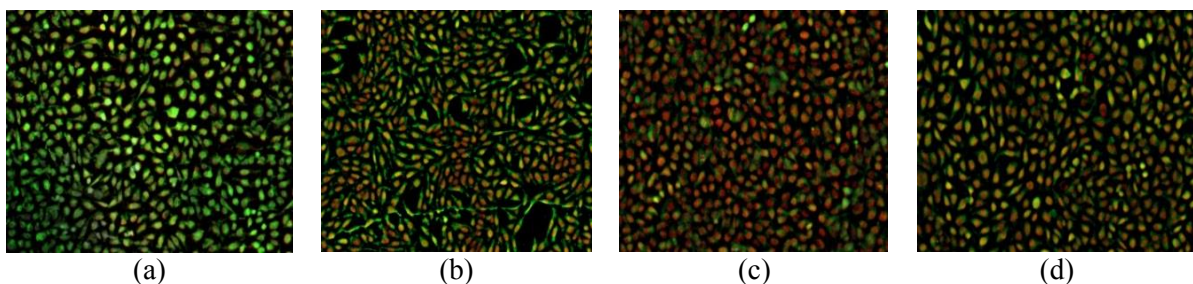


Figure 8. T47D Cancer Cells after Treatment; (a) Control group (b) L (c) LK (d) LP.

This cell death caused by laser exposure with a photosensitizer addition is due to the interaction between the laser and the tissue. In the photodynamic therapy on the cancer cells, there is a photophysical and photochemical interaction mechanism whereas when photosensitizers are activated by light there is a production of Reactive Oxygen Species (ROS). ROS, especially singlet oxygen, can easily react with many biological substrates such as amino acids, various unsaturated lipids, and basal DNAs and RNAs. Singlet oxygen will attach with basal DNAs which will change the structure of basal DNAs of cancer cells. This structure change will disturb the metabolism process of the cancer cells making them unable to grow well [11].

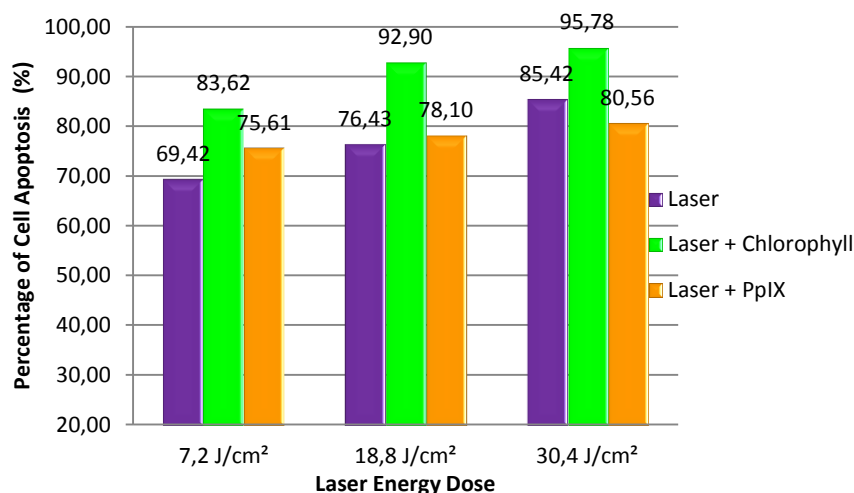


Figure 9. The Graph of the Percentage of Cell Apoptosis after Treatment.

4. Conclusions

Based on the toxicity test, the concentration of chlorophyll photosensitizers which is not toxic to cancer cells is 300 ppm with a living cell percentage of 97.6% and 350 ppm for the PpIX

photosensitizers with the percentage of living cells reaching 75.9%. The percentage cell death is the biggest on the red He-Ne laser output energy dose of 30.4 J/cm² which is 85.81% (without a photosensitizer addition), 96.25% (with a chlorophyll addition), and 81.62% (with the Protoporphyrin IX photosensitizer addition). The optimum energy dose of the red He-Ne laser to obtain apoptosis cell death is 30.4 J/cm² with cell apoptosis reaching 95.78% (chlorophyll addition) and 80.56% (with the Protoporphyrin IX photosensitizer addition).

Acknowledgment

We would like to express our gratitude to the Government of Indonesia through the Directorate General of Higher Education (PUPT Research) for financial support in this research.

References

- [1] Basic Health Research (Riskesdas) Jakarta Research Agency of Ministry of Health Republic of Indonesia 2013
- [2] Agostinis P et al 2011 *Photodynamic Therapy of Cancer: An Update*. CA: A Cancer J. for Clinicians. **61(4)** 250-81
- [3] Rode H J 2008 *Apoptosis, Cytotoxicity and Cell Proliferation Mannheim: Roche Diagnostics GmbH* p 178 (Doctoral Thesis)
- [4] Li B, Xianming C, Meihua G and Wuxiu L 2010 *Apoptotic Mechanism of MCF-7 Breast Cells In Vivo and In Vitro Induced by Photodynamic Therapy with C-Phycocyanin*. *Acta Biochim Biophys Sin.* **42** (1) 80-9
- [5] Al-Khafaji A S, Ahmed M H A and Firas S 2010 Photodynamic Action of Low Power He-Ne Laser on Photosensitized Human Hep-2 and AMN3 Cell Lines with Hematoporphyrin derivative In Vitro *Iraqi J of Canc and Med Gen* **3**(1) 54-60
- [6] Fadilla N R, *The Dosage Optimization of Red Laser Energy as The Candidate for Photodynamic Therapy of Cancer Cells with Exogenous Photosensitizer* [thesis] Surabaya: Universitas Airlangga 2015
- [7] Wang G H, Tzung H C, Rong J L, Jyh H S, Shih H W and Chia H L 2009 Cytotoxic Effect of the Genus *Sinularia* Extracts on Human SCC25 and HaCaT Cells *J Toxicology*. 634868 1-8
- [8] Budiyanto A W, Soenarto N and Leenawaty L 2008 *Effect of Acidification against Photodegradation of Chlorophyll a*. *J. Math. Sci.* **13** (3) 66-75
- [9] Lichtenthaler H K and Claus B 2001 Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy *Current Protocols in Food Analytical Chemistry* (CPFA) Wiley, New York, F4.3.1-F4.3.8
- [10] Rossetti F C, Livia V D, Antônio C T and Maria V L B B 2010 *Fluorometric Quantification of Protoporphyrin IX in Biological Skin Samples from in Vitro Penetration/Permeation Studies* *Brazilian J Pharm Sci.* **46**(4) 753-60
- [11] Wiseman H, Barry H 1996 *Damage to DNA by Reactive Oxygen and Nitrogen Species: Role in Inflammatory Disease and Progression to Cancer* *Biochem J.* **313** 17-2