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Biophotonics and machine learning model for the diagnosis and treatment of HIV

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ABSTRACT

All over the world the scientists working in biophotons and biophotonic therapy gave the hope to those who have struggled with a disease that have not been treated. This method is inexpensive and shows no side effects prove better in diagnosis and treatment such as HIV transmission. Some biomedical techniques of HIV detection need photons as source of light. These results have been obtained by CCD cameras or highly modified digital systems. The noisy background in these pictures gave the idea of implementing machine learning models. They can be extremely fast, offer high degree of picture quality and differentiation or classification of molecules of interest. In this paper libSVM (Support Vector Machines) models are applied to classify CD4+ cells from whole blood cells with great accuracy.

KEY WORDS: BIOPHOTONIC THERAPY, CCD CAMERAS, CD4+ CELLS, MACHINE LEARNING, SUPPORT VECTOR MACHINES

INTRODUCTION

Fritz-Albert Popp proposed the theory of Biophotons which is a single quantum that is transmitted by living systems in a continuous and repeated cycle Popp (1996). The scientists and experts that are working in this area referred the radiation biophotons and systematic fields as Biophotonics. Biophotonics allows scientists to view and detect diseases such as HIV (Human Immunodeficiency Virus) transmission. This has led to a revival of interest in BioPhotonic Therapy (BPT) among medical practitioners worldwide regarding the ability of using ultraviolet (UV) light rays in the treatment of such disorders. BioPhotonic Therapy (BPT) is a process of exposing blood to ultraviolet (UV) light rays to stimulate the immune system to destroy any and all pathogens, whether they are viral, bacterial or fungal. BioPhotonic Therapy (BPT) is also known by other names such as Ultraviolet Blood Irradiation (UBI), Photoluminescence, Photopheresis, Photodynamic Therapy and Hematologic Oxidative Therapy. There have been over 1 million BPT treatments. BPT has proved to be highly effective in treating bacterial infections, including septicaemias, pneumonia, wound infections, peritonitis and typhoid.

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BPT presents an interesting and relatively low cost alternative for patients willing to try this therapeutic modality. This is close to nature therapy which gives hope to those who have struggled with a disease Dillon et al (2003) and Scientia Press (2015).

Recent research in the field of HIV-AIDS diagnosis or treatment by Biophotonics threw light in some of the important work does is described as: Kufa (2017) presented his research findings on laser-driven label-free approaches of detecting HIV-1, which is the most widespread type worldwide, in living biological cells. While laser technology is used in the detection and treatment of cancer cells, it is rarely used as a technological tool to investigate HIV-infected cells also used for diagnosis. Lemboumba and Kufa (2017), have focussed on the novel tag-free detection of HIV-1 infected and uninfected cells via Raman and transmission spectroscopy coupled with optical trapping, which are techniques used to improve the reliability of data when cells are being analysed. Compared to gold-standard HIV-1 diagnostics such as the enzyme-linked immunosorbent assay and nucleic acid based tests that require the use of labels and substrates respectively for the first time in HIV research, coupling of laser trapping with Raman spectroscopy allows a non-invasive immobilisation and label-free analysis of single infected cells was also studied. The optical system, essentially allows grabbing single cells at will and analysing their chemical fingerprint by merely shining laser light of varying wavelengths and carefully structured beams on them are also for diagnosis for diseases like HIV/AIDS," says Ombinda-Lemboumba (2017).

According to him, this detection technique, compared to the labelled detection technique, is more likely to provide accurate results within a short period of time at a cost-effective rate. "Addressing issues of accuracy, cost and time were important for the purpose of the biophotonics research. In light of HIV/Aids being a serious disease claiming the lives of many in developing countries, particularly in Sub-Saharan Africa, it is important that all the research presently going on addresses the challenges of the day," Mthunzi-Kufa (2017). Present HIV detection or ART response techniques are [A] Flowcytometry where lasers are source of light in terms of photon. [B] Fluorochromes, and [C] Photonic crystal biosensors which have been described as:

{A}FLOWCYTOMETRY: HIV infected person go through flow cytometry for detection of HIV infection. Throughout the progression of disease, T-cell subsets are followed as the best surrogate marker for immune status Stein (1992). An AIDS definition was established based on a CD4 T-cell count of less than 200 cells/ml centers for disease control (1993). As T- cell count decreases from 350 to 200, millions of viruses have started reproducing into the body, which gives indication of start of antiretroviral therapy. So accurate and reproducible measurement of T-cell subset during therapy is an important part of managing treatment for HIV infected patients BergeronM(1998). Current therapies add years of quality life to patients living with HIV. Monitoring through FC is as follows: Fresh whole blood with anticoagulant is required by immunophenotyping of T-cell subsets (viral load is also important tool for HIV detection). Fluorescein isothiocynate (FITC) is the most universal fluorochrome for phenotyping and R-phycoerythrin (PE) are the dyes excited using 488 nm light.

Other dyes are also used & multicolour applications make better understanding of distinguished components. And laser light is passed through. Various components of T-cell subsets can be identified with lymphocytes CD4/CD8 T-cells have been reported as % of lymphocytes. The antigens most often monitored on CD8 T-cells are CD57, CD 28 and CD38. NK cells (CD16) decreases with HIV infection, particularly in later phase of disease Hardy (1992) & Lucia et al 1995. Additional uses of FC in the detection of HIV infection includes evaluation of cell function such as activation, in vitro proliferation,cytotoxic T-lymphocytes responses, detection of various cytokine producing cells and measurement of in-situ HIV antigens and apoptosis. All these results have been monitored through fully automated computer system linked with FACS (Fully Activated Cell Sorter).

{B}.Fluorochromes like GFP (green fluorescent protein): This is obtained from jellyfish is of much importance as a probe to track recombinant proteins movements, location and expression in living cells, The recent successful creation of a replication competent clone of the HIV-1 containing GFP (HIV Gag-Igfp) has enabled, for the first time the direct visualization of viral spread between live cells Hubner et al (2007) and Hubner et al (2009). When a HIV-1 infected T cell and an uninfected CD4+ T-cell engage each other through interactions of HIV Env and CD4 they can form a virological synapse Chen (2007), Jolly (2004) Jolly and Sattentau (2005 and 2007), Fackler (2007).

HIV-1 viral proteins are then observed to rapidly and focally assemble virus particles at the point of cell-cell contact. Virus is subsequently transferred to target cell, leading to productive infection. This process is highly efficient and very rapid. Manipulating microscopic objects by optical tweezers utilizes changes in the momentum of photons to drive a target with a high index of refraction to the centre of tightly focused laser beam Ashkin (1970 & 1986). Once the cell has trapped in this fashion it will follow the light focus when moved. The use of near infra-red laser sources for optical tweezers enables the manipulation of non-adherent cells such as germ cells, red blood cells, and T-lymphocytes because

cells have no to little absorption at these wavelengths and the process is virtually non-invasive Chen (2008), Ozkan (2003), Nilsson (2009) Anveri (2004), Rao (2009), Shi (2009). Another advantage is that optical tweezers do not require open access to the sample or the use of needle aspirators, both of which significantly escalate the risk of accidental exposure when handling infections like HIV-1.

{C}Photonic Crystal biosensors: Flow cytometry and reverse transcription quantitative polymerase chain reaction (RT-Qpcr) are sensitive standards methods for CD4 cell count and viral load measurements to monitor ART, but they require complex laboratory infrastructure, expensive reagents, and skilled operators Wang (2010), Fiscus et al (2006). Several technologies have been developed for virus detection utilizing optical, electrical, and acoustic sensing methods such as surface Plasmon resonance (SPR), localized surface Plasmon resonance etc. A nanoplasmonic-based platform was developed to detect intact HIV-1 using self-assembled gold nanoparticles conjugated with biotinylated anti-gp120 antibodies through impedance spectroscopy of viral lysate samples Shafiee et al (2013). Among these approaches, photonic crystal (PC) biosensors offer a rapid and sensitive optical detection method for biomolecules, cells, and viruses by monitoring the dielectric permittivity changes at the interface of a transducer substrate and liquid media Shamah (2011). A PC biosensing platform was also developed that captures and detects intact viruses (HIV-1) as well as bio molecules and antibodies. Multiple HIV-1 subtypes (A, B and D) were detected in spiked samples with viral loads ranging from 10 4 to 10 8 copies/ml and validated with gold standard method (i.e. RT-q PCR) Shafiee et al (2014).

These techniques are used as they are described but researches would improve the way of diagnosis. Hence machine learning techniques are in advance position to enhance the quality of these photonics methods by reducing the time, manpower and accurately predict the results. In view of this here a method of machine learning is presented which surely threw a light on biophotonics research of diagnosis of diseases.

IMPLEMENTATION OF MACHINE LEARNING

The result of experiments done in FC and other techniques are analyzed by CCD Cameras or by automated computer system. These digital imaging technologies have the advantage of regularly capture high-content images that may contain the location of thousands of moving particles or molecules in a single cell. Due to low contrast and noisy background of the images, sometimes the molecules appear more like a shapeless blob than a distinct feature. This traditional method shows the failure of automated detection system. Thus researchers have sorted this problem by implementing machine learning. Computers are trained by algorithms that are capable to distinguish a molecule from what is not a molecule through training data. Haar features are used o make the distinction. This method is time consuming to extract such a molecular data. Haar features were developed in part to ease the computational burden arising from image analysis using such as pixel intensity alone. Haar methods look at rectangular regions in an image and sum up the number of pixels per region.

The resulting value then is used to classify the image and to categorize an area as either having a particle (molecule) or not, for example: in the case of particles within a cell, Haar features are a combination of the intensity, shape and size of the objects. To get around fluorescence intensity fluctuations in classifying objects, the researchers relied upon signal-to-noise ratios to decide which automatically identified particles were valid. They discarded those too low ratios and kept those with a ratio above a threshold. Support vector machines with kernel functions are able to distinguish molecules of interest with whole cells. Here our interest of molecule is CD4+ cells in whole blood cells. Flow cytometry and other techniques are applied while using fluorochromes to clearly distinguish CD4+ cells to understand its morphology, interactions with other cells and the image is taken by different intensities of cameras. The combination of SVM (machine learning) algorithms with above discussed methods leads to classify our interest molecule in better intensity images. For making this analysis possible, the training set consisting of 10pixel subwindows. Some are selected because they did not contain molecule of interest, i.e. negative sample. Others are selected as positive samples, i.e. images that contain molecules of interest. Using these training and testing samples (sets) support vector machine based classifier is developed to finally find the molecule of interest without noise, i.e. molecule is being categorized as a molecule.

Datasets and Methods: The protein data of CD4+ cells and whole blood protein have been taken from Uniprot/Swissprot database Uniprot 2016.

Dataset: For support vector machines dataset is needed. Here in our study two datasets are required. Dataset (1): This consists of CD4+cells which is our interested molecule i.e. the positive sample, to be separated from whole blood (whole sequence), the other sample which is marked as negative.

Dataset(2): This consist of 100 whole blood sequences which marked as positive and other sequences of any animal marked as negative..

Amino acid compositions of these two datasets have been taken. The amino acid composition is the fraction of each amino acid type within a protein.

The fractions of all 20 natural amino acids were calculated by using Equation 1,

$$= \frac{Total\ Number\ of\ amino\ acid\ i}{Total\ number\ of\ amino\ acids\ in\ a\ protein}$$

Support Vector Machine (SVM) a machine learning technique that has a potential for learning separating functions in pattern recognition (classification) tasks and in performing functional estimation in regression problems. It originated from the statistical learning theory (Vapnik 1995) and represents the novel learning techniques that were introduced in the framework of structural risk minimization and in the theory of Vapnik Chervonenkis dimension (VC) (1995). SVMs are a set of related supervised learning methods used for classification and regression. A classification task usually involves separating data into training and testing sets. Each instance in the training test contains one "target value" (i.e. class labels) and several "attributes" (i.e. the features or observed variables). The goal of SVM is to produce a model (based on the training data) which predicts the target values of the test data given only the test data attributes. SVM is a supervised machine learning method which is based on the statistical learning theory Vapnik (1995) and Wang (2004). When used as a binary classifier, an SVM will construct a hyperplane, which acts as the decision surface between the two classes. This is achieved by maximizing the margin of separation between the hyperplane and those points nearest to it. The SVMs were implemented using freely downloadable software, lib SVM, (Cheng 2001). In this software there is a facility to define parameters and choose among various inbuilt kernels. They can be radial basis function (RBF) or a polynomial kernel (of given degree), linear, sigmoid. Simulations were performed using LIBSVM version 2.89 (a freely available software package). For our study RBF Kernel was found to be the best. The SVM training was carried out by the optimization of the value of the regularization parameter and the value of RBF kernel parameter.

RESULTS AND DISCUSSION

The values of these two datasets are fed into LibSVM and analysis done for linearly separable data. The present work describes the use of LibSVM models for the classification of CD4+ cells of HIV than other cells present in blood. The performance of classifier is checked by following:

$$Accuracy = \frac{tp + tn}{tp + tn + fp + fn}$$

True positives [TP] and True negatives [TN] were identified as the positive and negative samples, respectively. False positives [FP] were negative samples identified as positive. False negatives [FN] were positive samples identified as negative. The prediction performance was tested with sensitivity [TP/ [TP+FN]], specificity [TN/ [TN+FP]], and overall accuracy.

Total Number of Instances in dataset: CD4+cells are 94. Which are correctly identified as positive out of 100.

SVM has the ability to separate between positives and negative instances. The table 1 shows FP is 0 then TP is 0.933 and if FP is 0.067 then TP is 1. The ROC is found to be 0.942 which correctly predicts the good quality of classifier. As it is inbuilt that if ROC > 0.5 then good classification as of FP or TP values lies between 0 and 1 respectively. Here ROC depicts the classification of CD4+ cells with an average of 0.942. Statistical analysis showed that blue points are marked as CD4+ cells as they are separated by other cells present in blood lymphocytes. The false positive rate was 4.44%. This technique is cost effective automated solution to detecting molecules of interest within living cells.

ROC curves depict the performance of a classifier without regard to class distribution or error costs. They plot the number of positives included in the samples on the vertical axis, expressed as a percentage of the total number of positives, against the total number of negatives on the horizontal axis. For each fold of a 10 fold cross validation ,weight the instances for a selection of different cost ratios train the scheme on each weighted set ,count the true positives and false positives in the test set, and plot the resulting point on the ROC axes. In diagnostic "accuracy" as a measure of decision performance require introduction of the concepts of the "sensitivity" and "specificity" of a diagnostic test. These measures and the related indices, "true positive fraction" and "false positive fraction," are more meaningful than "accuracy," yet do not provide a unique description of diagnostic performance because they depend on the arbitrary selection of a decision threshold. As a final

Table 1. Detailed Accuracy by Class

TP Rate	FP Rate	Precision	Recall	F-Measure	ROC Area	Class
0.933	0	1	0.933	0.966	0.942	Whole sequence
1	0.067	0.988	1	0.994	0.942	CD4+cells
0.989	0.056	0.989	0.989	0.989	0.942	Weighted avg.

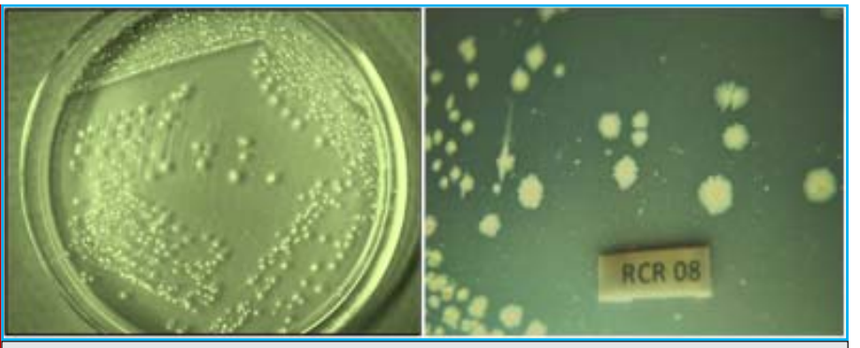

FIGURE 1. ROC for CD4+cells from other lymphocytes by LivSVM (WEKA3.7)

step, after molecule of interest (CD4+ cells) detection the data is further processed to extract the boundary and area of the particles for accurate tracking. Now this picture is compared with the results of manual approach to determine the true positive and false positive rates. Researchers found that the true positive rate averaged more than 98 %. Thus, more than 98 out of 100 times the algorithm successfully identified a molecule (CD4+cells) as a molecule.

CD4 cells are white blood cells that play an important role in the immune system. The CD4 cell count gives us an indication of the health of immune system. This is our body's natural defence system against pathogens, infections and illnesses. Several cohort studies and clinical trials have shown that the CD4 count is the strongest predictor of subsequent disease progression and survival Egger et al (2002) & Mellors et al (1997). The use of the CD4 count as an independent and reliable marker for treatment outcome is attractive from various aspects. First, CD4 counts are already the most important factor in deciding whether to initiate antiretroviral therapy and opportunistic prophylaxis. All HIV-positive patients in high-income countries and an increasing number of patients in low-income countries have a baseline CD4 count at entry into care Panel of Antiretroviral guidelines for Adult and Adolescent (2008). Second, the CD4 count is a relatively objective and simple marker to follow. Finally, the cost of CD4 counts has become more affordable, including in developing countries Lutwama (2008) & Mac Lennan (2007). This article further evaluates the use of the CD4 count in assessing the clinical status of HIV-infected individuals, in making informed decisions regarding the initiation of antiretroviral therapy and in monitoring by the use of machine learning in jointly working with biophotonics

CONCLUSION & FUTURE ASPECTS

The study shows the handshake of biophotonics and machine learning techniques for diagnosis and treatment of diseases like HIV-AIDS in a better way with high speed and great accuracy. The future of biophotonics research and development is very bright. It will be necessary to bring together the wide spectrum possibilities offered by biophotonics and machine learning methods in conjunction with other biomedical techniques to develop solutions to existing clinical problems. Laser systems and fluorochromes are the possible form of light or photons that are implemented as light source for diagnosis or therapy. Nature always shows the path of light in constructive way, similarly if we will be able to utilize the constructive properties of light, the further progress is surely seen. The demand of todays is biophotonics with machine learning brings high speed, low cost and non-invasiveness in diagnosis and treatment of diseases. The advances in technology are being coupled with biology and medicine to revolutionize healthcare.

REFERENCES

Anveri B, Torres J, McIntyre B.J.(2004) Machines Software Biomed.Opt.,9(5), 2004,865-872.

Ashkin A, Dziedric J, Bjorkholm J, Chu S. (1986) Opt. Letter ; 11(5):288-290.

Bergeron,M., Farecher,S.Minkus,T., Lacroia, F.Ding, T.,Phanenf, S., Somorjai,R., Summers,R., and Mecndy, F., Impact of unified procedures as implemented in the Canadian Quality Assurance Program for T lymphocytes subset enumeration, Participating Flow cytometry laboratories of the Canadian clinical trial network for HIV –AIDS Therapies, Cytometry,1998, 33 (2),146

Centers for Disease Control, Revised Classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults, Morbidity, Mortality, Weekly Rep., 1993, 41, 1.

Chan JW, Taylor DS,Lane SM,Zwerdling T,Tuscano J,Huser TR. Anal.Chem.80 2008(6):2180-2187.

Chang CC and C.-J. Lin, LIBSVM: a library for support vector machines. Software," available at http://www.csie.ntu.edu.tw/~cjlin/libSVM, 2001

Charles Maphanga, Rudzani Malabi, Saturnin Ombinda-Lemboumba, Pros and cons of characterising an optical translocation setup (Poster presentation). The Society of Photo-optical Instrumentation Engineers (SPIE) West Conference February 2017 San Francisco, California, United States of America

ChenP, Huebner W, Spinelli MA, Chen BK.J.Virol.; 81(22), 2007, 12582-12595.

Dillon, Kenneth J: Close-to-Nature Medicine. Washington, D.C.: Scientia Press 2003

Egger M, May M, Chene G et al.: Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. Lancet 360(9327), 119–129 (2002).

Fackler O, Alcover A, Schwartz O, Nat.Rev.Immunol; 7(4), 2007, 310-317.. Fiscus, S.A.et al. HIV-1 viral load assays for resource-limited settings.Plos Med 3, E417, 2006.

Hardy, N.M. 1992 Lymphocyte subset changes in persons infected with immunodeficiency virus, Ann.Clin.lab.sci, 1992, 22, 286.

Hubner W, Chen P, Del Portillo A, Liu Y, Gorden RE, Chen BK 2007 . J.Virolgy;81:12596-12607,2007.

Hubner W,McNerney GP, ChenP, Dale BM, Gorden RE,Chuang FY, Li X-D,Asmuth DM,Huser TR, Chen DS, Lane SM, Zwerlding T, Tuscano J, Huser TR. 2009 Anal.Chem.; 80(6): 2009,2180-2187.

Jolly C, Kashefi K, Hollinshead M, Sattentau Q.J.Exp.Med.; 199(2), 2004,283-293.

Lebogang S et al, 2017 Real-time Raman spectroscopy for the characterization of biochemical changes in differentiated embryonic stem cells (Poster presentation),The Society of Photo-optical Instrumentation Engineers (SPIE) 2017 San Francisco, California, United States of America

Lebogang S. Thobakgale, et al 2017 ,Photo-transfection and differentiation of mouse embryonic stem cells using femtosecond laser pulses (Oral presentation)The Society of Photo-optical Instrumentation Engineers (SPIE) February 2017 in San Francisco, California, United States of America

Lucia, B.,Jennings, C.,Cauda,R Ortona,L.,&t Landey, A.L., 1995 Evidence of a selective depletion of a CD16+CD56+CD8+natural killer cell subset during HIV infection,Cytometry,22, 10,1995.

Lutwama F, Serwadda R, Mayanja-Kizza H et al.2008 : Evaluation of Dynabeads and Cytospheres compared with flow cytometry to enumerate CD4+ T cells in HIV-infected Ugandans on antiretroviral therapy. J. Acquir. Immune Defic. Syndr. 48(3), 297–303 (2008).

M.Wang, J.Yang, G.P.Liu,Z.J.Xu, K.C.Chou, 2004 Weighted support vector machins for predicting membrane protein types based on pseudo amino acid composition, Protein Eng.Des. Sel, Vol.17, 2004,pp.509-516

MacLennan CA, Liu MK, White SA 2007 et al.: Diagnostic accuracy and clinical utility of a simplified low cost method of counting CD4 cells with flow cytometry in Malawi: diagnostic accuracy study. BMJ 335(7612), 190 .

Masixole Y, 2017 Could low level laser therapy and highly active antiretroviral therapy lead to complete eradication of HIV-1 in vitro? (Oral presentation).. The Society of Photo-optical Instrumentation Engineers (SPIE) 2017 San Francisco, California, United States of America

Mellors JW, Munoz A, Giorgi JV 1997 et al.: Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. Ann. Intern. Med. 126(12), 946–954 (1997).

Mthunzi-Kufa (2017) Could low level laser therapy and highly active antiretroviral therapy lead to complete eradication of HIV-1 in vitro? (Oral presentation).

Masixole Y. Lugongolo, Sello L. Manoto, Saturnin. Ombinda-Lemboumba (2017) The Society of Photo-optical instrumentation Engineers (SPIE) 2017 Meet San Francisco, California, United States of America

Mthunzi-Kufa (2017) Investigation of HIV-1 infected cells using laser trapping Raman spectroscopy technique (Oral Presentation), Saturnin Ombinda-Lemboumba, Masixole Y. Lugongolo, Lebogang S. Thobakgale, Sello L. Manoto. February 2017 San Francisco, California, United States of America

Nilsson J,Evander M,Hammarstrom B, Laurell T.Anal. Chim. Acta.; 649(2),2009,141-157

Ozkan M, Wang M, Ozkan C,Flynn R,Birkbeck A, Esener S.Biomed.Microdev.2003 Panel on Antiretroviral Guidelines for Adults and Adolescents: guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services, 1–139 (2008). www.aidsinfo.nih.gov/ContentFiles/ AdultandAdolescentGL.pdf

Mthunzi-Kufa and Tebello Nyokong et al 2017 Photodynamic activity of zinc monocarboxyphenoxy phthalocyanine (ZnM-CPPc) conjugated to gold silver (AuAg) nanoparticles in melanoma cancer cells (Poster presentation), The Society of Photo-optical Instrumentation Engineers (SPIE)

Rao S,Balint S,Cossins B,Guallar V,Petrov D. Biophys.J. 96(1), 2009, 209-216

Sello.L.Manoto, David Oluyinka Oluwole, Saturnin Ombinda-Lemboumba, Patience Mthunzi-Kufa and Tebello Nyokong. Phototoxic effects of free phthalocyanine and phthalocyanine conjugated to gold nanoparticles for targeted photodynamic therapy of melanoma cancer (Oral presentation).

Shafiee, H. 2013 et al. Acute On-Chip HIV Detection through Label Free Electrical Sensing of Viral Nano-Lysate. Small 9, 2013, 2553-2563.\

Shamah, S.M. &t Cunningham, B.T. Label free cell based assays using photonic crystal biosensors. Analyst 136, 12, 2011.

Shi L, Shao B, Chen T, Berns M.J. 2009 Biophoto. 2(3), 2009, 167-177 www.scientiapress.com/biophotonictherapy#sthash.2gQDOWbk.dpuf

Stein, D.S., Korwick, J.A., and Vermund, S.H., CD4+ Lymphocyte cell enumeration for prediction of clinical course of human immunodeficiency virus disease: a review, J.Infec.Dis., 1992,165,352.

Vapnik V. (1995) The nature of statistical learning theory, Springer, 1995

Wang, S. Xu, F.&t Demirci, U. Advances in developing HIV-1 viral load assays for resource limited settings. Biotechnol.Adv. 28, 2010, 770-781. www.Fritz-Albert-Popp. Wikipedia.org