Investigation of HIV-1 infected and uninfected cells using the optical trapping technique

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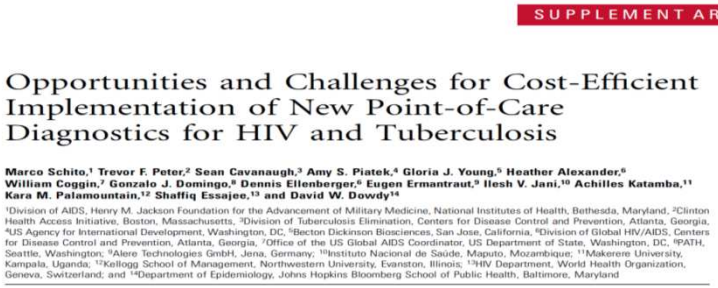
Outline

- Introduction
- Aim
- Background
  - Optical trapping of living cell
  - Transmission spectroscopy
- Experimental setup
- Results
- Conclusions

Introduction

- Current HIV diagnostics tests are:
  - Too expensive (CD4 count, PCR)
  - Require laboratory facilities and high skilled personnel
  - Long testing time (ELISA, P24 test, Reverse transcriptase test)
- New point-of-care diagnostics that are ASSURED*

Biophotonics group has embarked on developing a laser based point of care diagnostics tool.


SUPPLEMENT ARTICLE

Opportunities and Challenges for Cost-Efficient Implementation of New Point-of-Care Diagnostics for HIV and Tuberculosis

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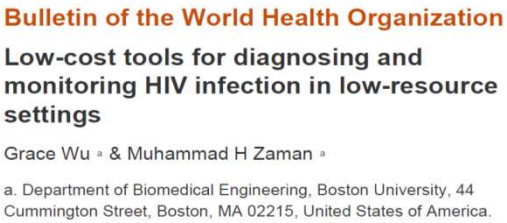
Bulletin of the World Health Organization

Low-cost tools for diagnosing and monitoring HIV infection in low-resource settings

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*ASSURED = Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment free, Delivered

Aim

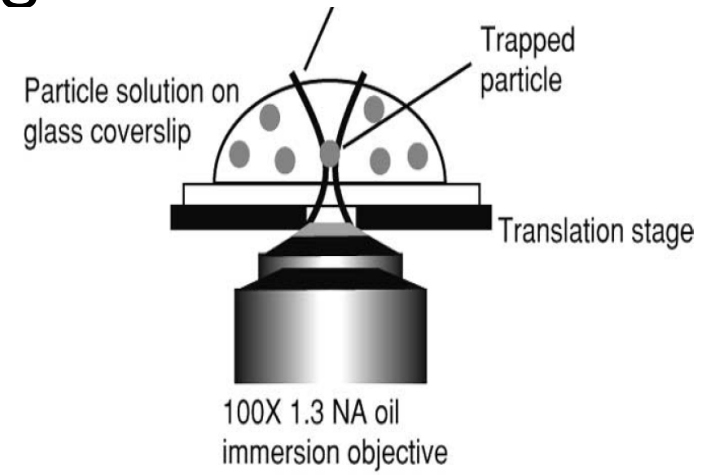
Develop an accurate, fast, label-free and non-invasive laser based diagnostic system for HIV-1 research to enhance and improve currently used HIV-1 diagnostics tools

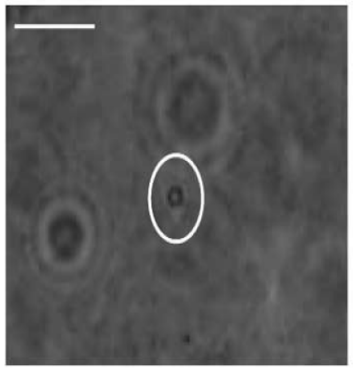
Research Focus

Label free investigation of HIV-1 infected and unifected single cells via transmission Spectroscopy combined with laser trapping system.

Optical trapping in living cells

- Cells can be grabbed and immobilized.
- This non-invasive immobilisation of the cell can allow accurate investigation of single cell.
- Tool for the manipulation of viruses, bacteria and cells suspended in liquid media.

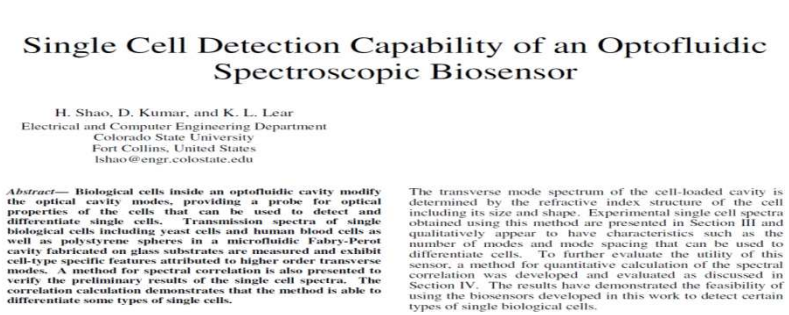




Chan et al 2005, IEEE Journal of selected topics in quantum electronics

Transmission Spectroscopy in biological material

- Transmission spectroscopy is a real time, label free detection, non-destructive technique that allows analysis of biological material.
- A simple, quick and highly effective technique to interrogate individual cells.
- Differentiate red from white blood cells
- Differentiate cancerous from non-cancerous cell species.



Single Cell Detection Capability of an Optofluidic Spectroscopic Biosensor

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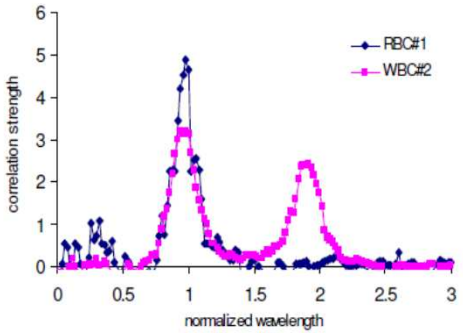
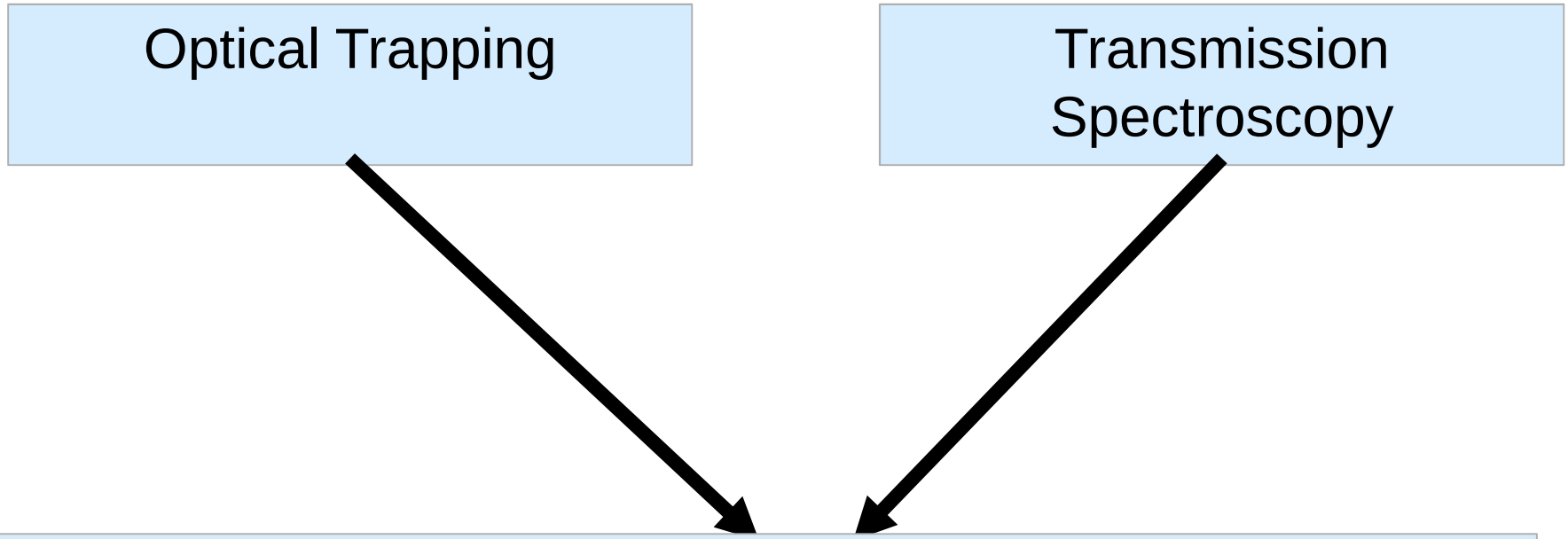
Abstract— **Biological cells inside an optofluidic cavity modify the optical cavity modes, providing a probe for optical properties of the cells that can be used to detect and differentiate single cells. Transmission spectra of single biological cells including yeast cells and human blood cells as well as polystyrene spheres in a microfluidic Fabry-Perot cavity fabricated on glass substrates are measured and exhibit cell-type specific features attributed to higher order transverse modes. A method for spectral correlation is also presented to verify the preliminary results of the single cell spectra. The correlation calculation demonstrates that the method is able to differentiate some types of single cells.**

The transverse mode spectrum of the cell-loaded cavity is determined by the refractive index structure of the cell including its size and shape. Experimental single cell spectra obtained using this method are presented in Section III and qualitatively appear to have characteristics such as the number of modes and mode spacing that can be used to differentiate cells. To further evaluate the utility of this sensor, a method for quantitative calculation of the spectral correlation was developed and evaluated as discussed in Section IV. The results have demonstrated the feasibility of using the biosensors developed in this work to detect certain types of single biological cells.



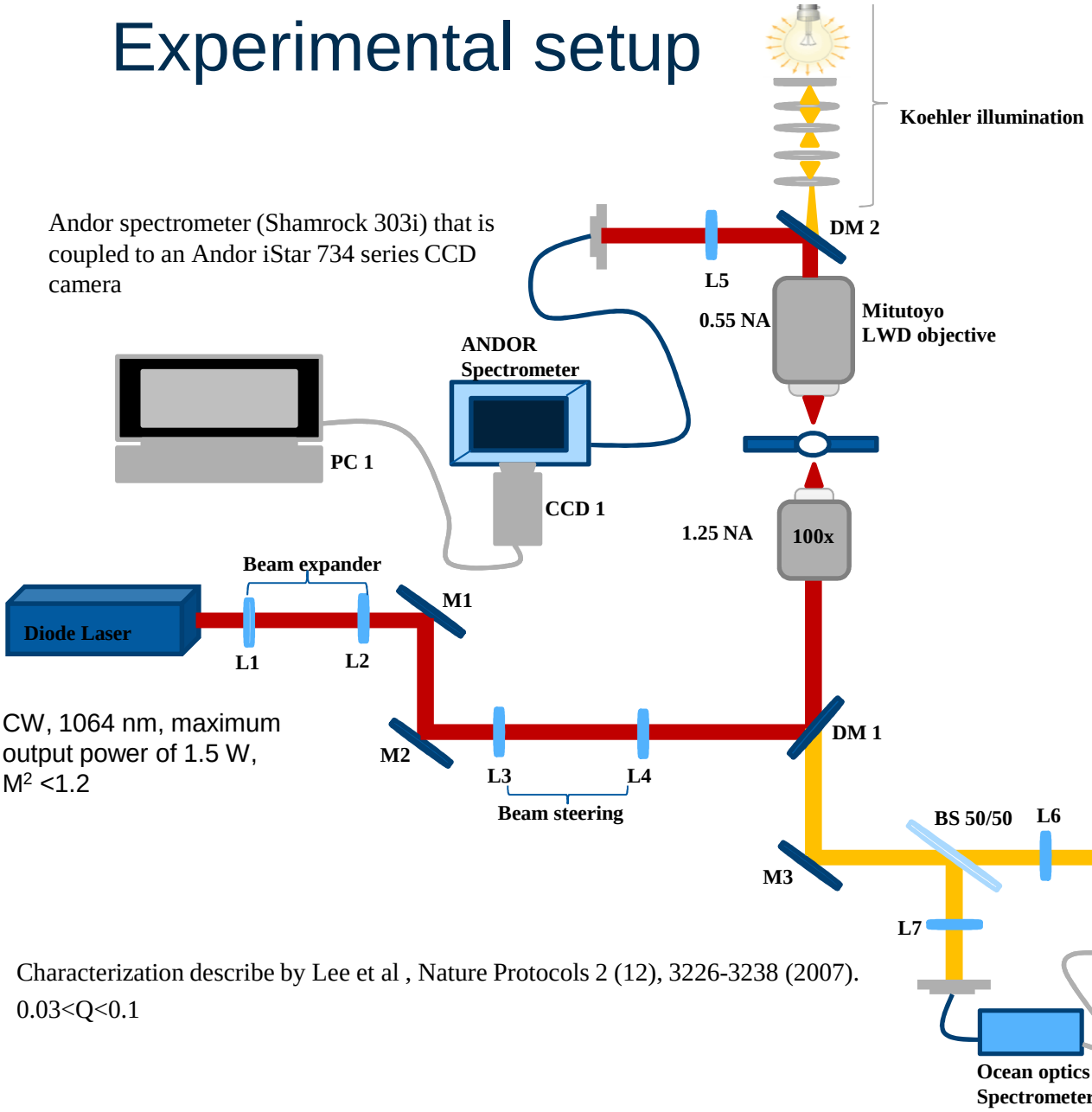
Optical trapping transmission Spectroscopy



Overcome the use of substrate (label free), Brownian motion
Single cell investigation
Non-destructive, fast, real time detection

Experimental setup

Andor spectrometer (Shamrock 303i) that is coupled to an Andor iStar 734 series CCD camera



CW, 1064 nm, maximum output power of 1.5 W, M^2 <1.2

TZM-bl Cells were cultured in two groups: HIV-1 infected and uninfected cells

A total volume of 500 µl of 2.10^4 TZM-bl cells in 35 mm diameter glass bottom petri dishes with a 23 mm diameter type 1 glass coverslip

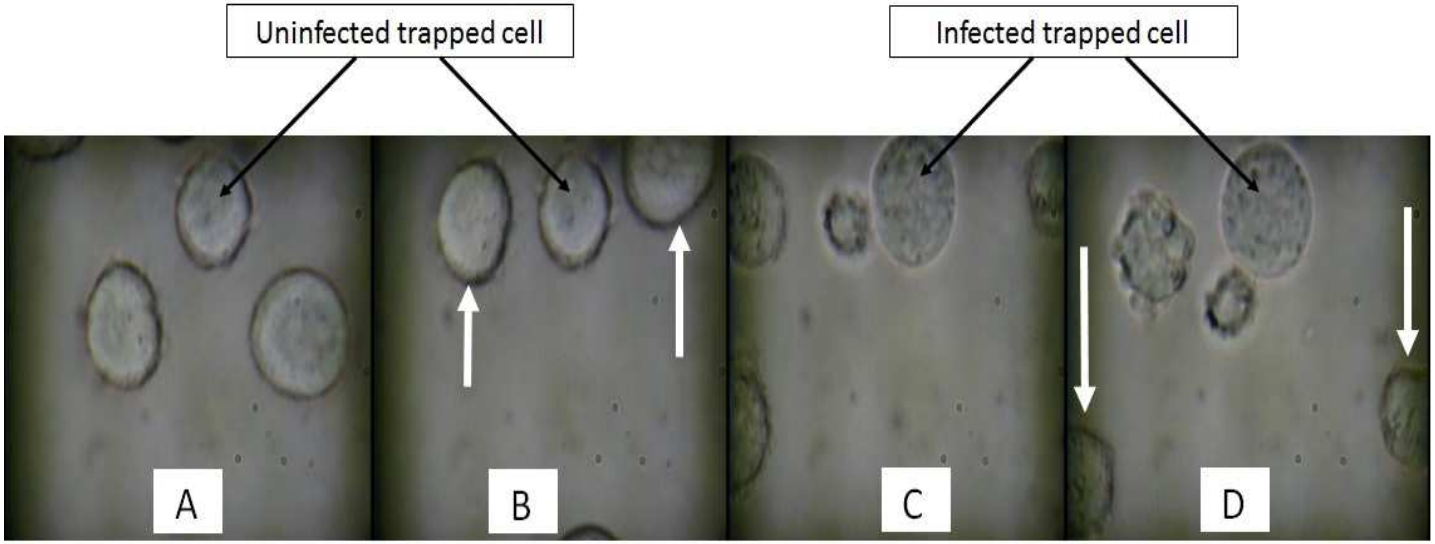
- Characterization describe by Lee et al , Nature Protocols 2 (12), 3226-3238 (2007).
- 0.03<Q<0.1

Results

Optical Trapping

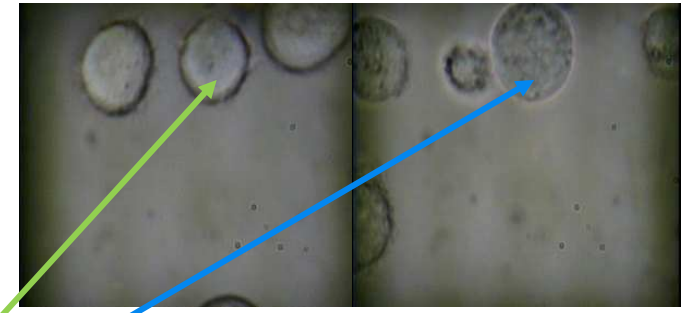
TZM-bl cells

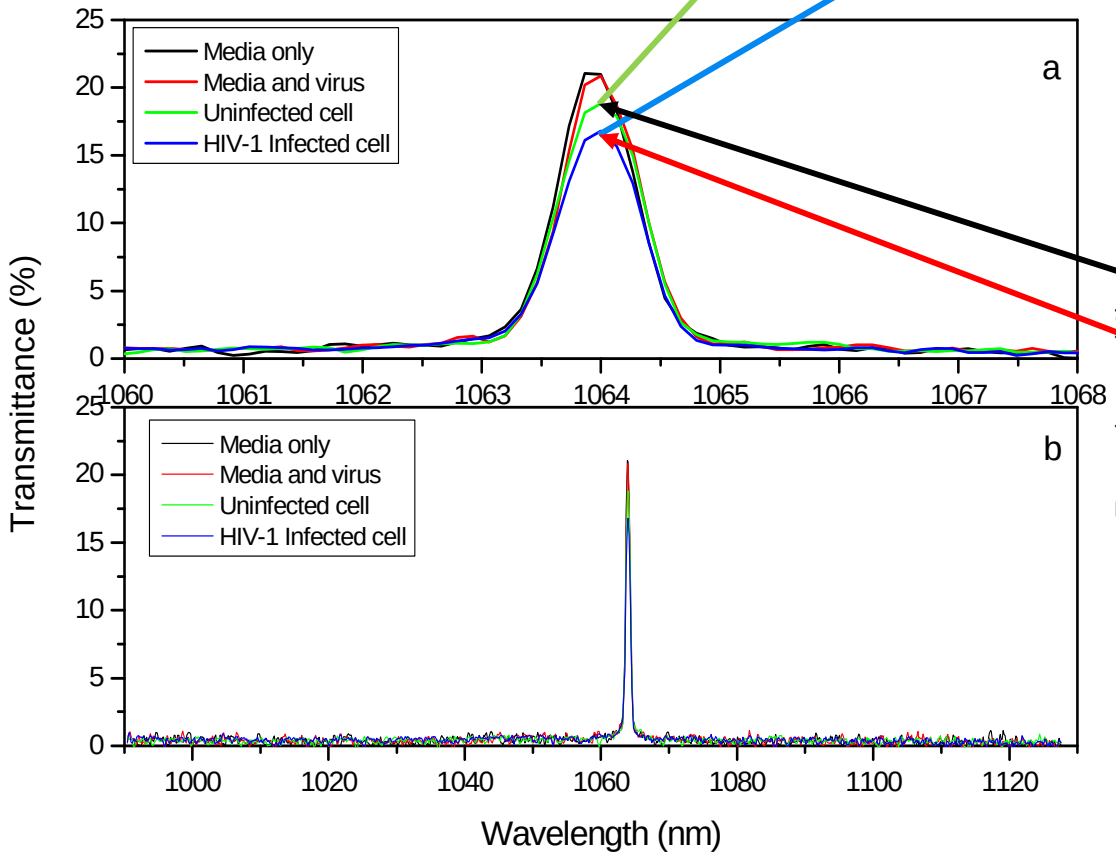
- 60 mW of 1064 nm CW laser light
- 100x objective lens
- $M^2 < 1.2$ and
- good power stability (variation <2%)

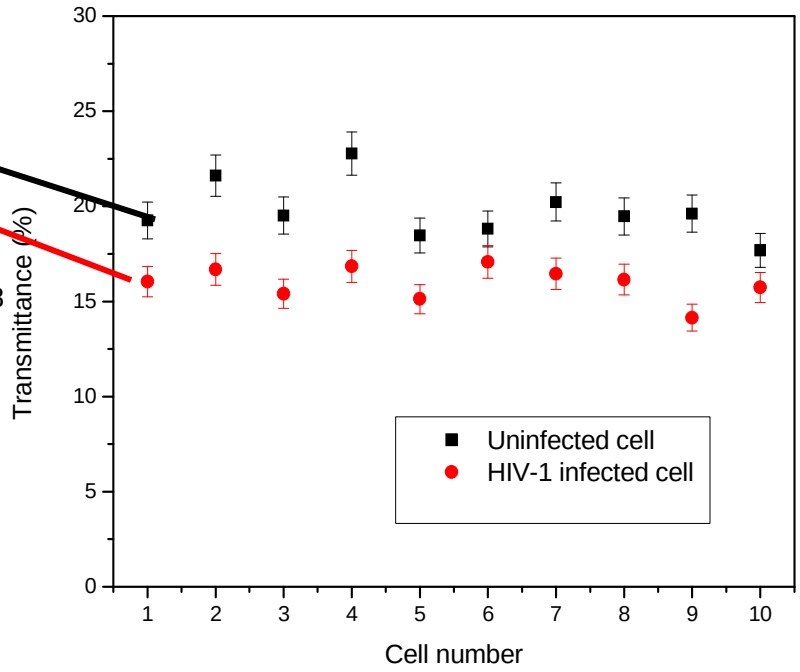


Results

Transmission spectroscopy





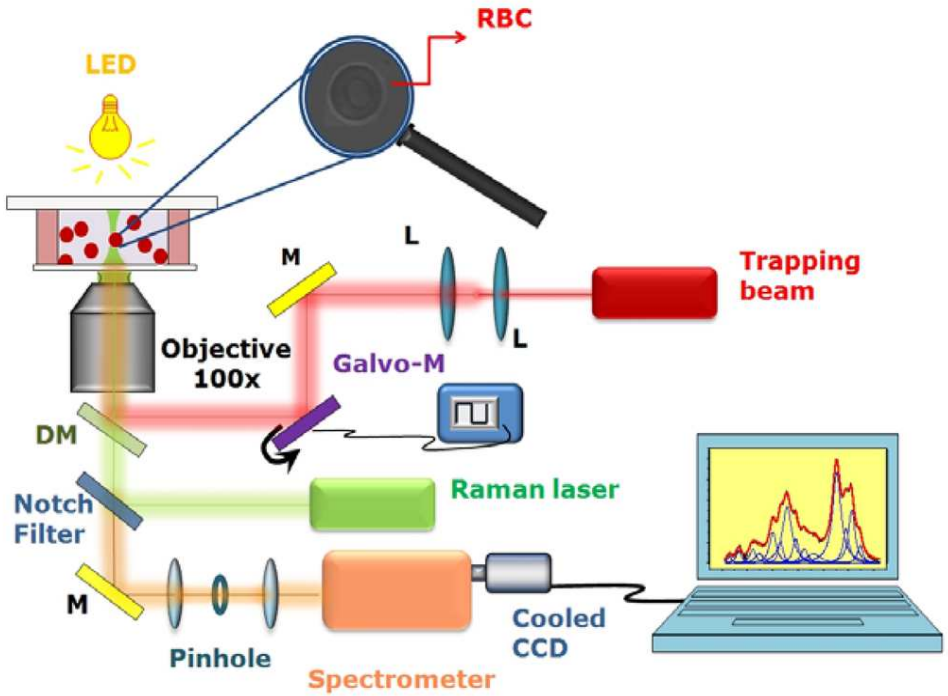


Conclusions

- Develop near infrared laser optical trapping to investigate single infected and uninfected TZM-bl cells
- Successful combine optical trapping and transmittance spectroscopy
- label-free trapping and transmittance spectra of infected and uninfected TZM-bl cells and without any phototoxic and/or thermal damage.
- Differences between the transmittance spectra of the HIV-1 infected and uninfected cells.
- Possibility to differentiate between infected and uninfected optically.

Future work

- Additional measurement and analysis to determine molecular species in infected and uninfected cells.
- Combine a Raman spectroscopy system with optical trapping system.



Giulia Rusciano et al Sensors 2008 Dec; 8(12): 7818–7832

Acknowledgement

CSIR
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Thank you

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