

Team Members:

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Introduction:

ELISA (Enzyme-linked immuno-sorbent assay) is one of immunoassay method used to detection of,

1. Antibodies
2. Proteins
3. Biomolecules
4. Peptides

Immunoassay:

The term “immunoassay” is a combined term of “immuno”(immunological, practically immunochemical antigen-antibody-reaction) and “assay” (determination of the purity of a substance or the amount of any constituent of a mixture).

History of Elisa:

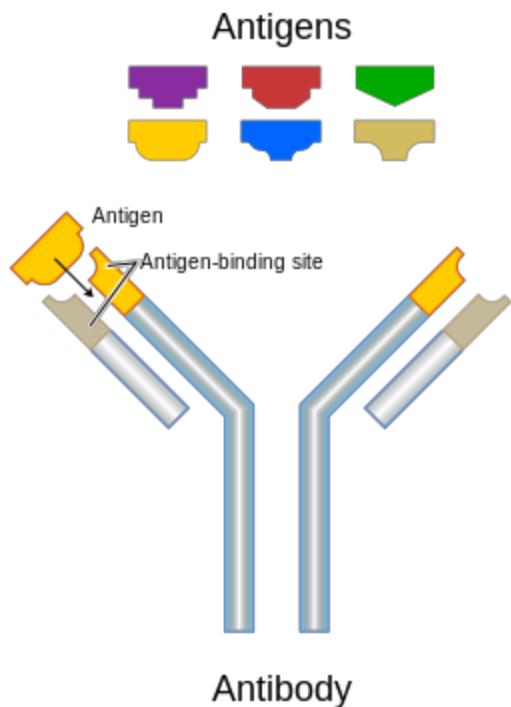
Radioimmunoassay was first described in a scientific paper by Rosalyn Sussman Yalow and Solomon Berson published in 1960. In 1971, Peter Perlmann and Eva Engvall at Stockholm University in Sweden, and Anton Schuurs and Bauke van Weemen in the Netherlands independently published papers that synthesized this knowledge into methods to perform EIA/ELISA.

Components:

Three important components of Immunoassay are,

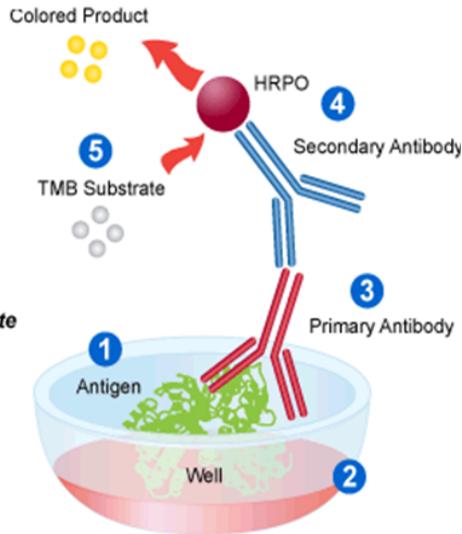
1. Antigen
2. Antibody
3. Labelling Material

- Antigen: In [immunology](#), an **antigen** (Ag), or **antibody generator**, is any substance which provokes an adaptive immune response.
- Antibody: An **antibody** (Ab), also known as an **immunoglobulin** (Ig), is a large Y-shape [protein](#) produced by [plasma cells](#) that is used by the [immune system](#) to identify and neutralize foreign objects such as [bacteria](#) and [viruses](#).



Steps involved in Testing:

- ① Antigen/sample is added to plate.
- ② Blocking buffer is added to block remaining protein-binding sites.
- ③ Next a suitable **primary antibody** is added.
- ④ A suitable **secondary antibody – HRPO conjugate** is then added which recognizes and binds to the primary antibody.
- ⑤ TMB substrate (*Leinco Prod. No. T118*) is added and is converted by HRPO to detectable form.



Advantages of ELISA:

- Reagents are relatively cheap & have a long shelf life.
- ELISA is highly specific and sensitive.
- No radiation hazards occur during labelling or disposal of waste.
- Easy to perform and quick procedures.
- Equipment can be inexpensive and widely available.
- ELISA can be used to a variety of infections.

Disadvantages:

- Measurement of enzyme activity can be more complex than measurement of activity of some type of radioisotopes.
- Enzyme activity may be affected by plasma constituents.
- Kits are commercially available, but not cheap.
- Very specific to a particular antigen. Won't recognize any other antigen.
- False positives/negatives possible, especially with mutated/altered antigen.

Types of ELISA:

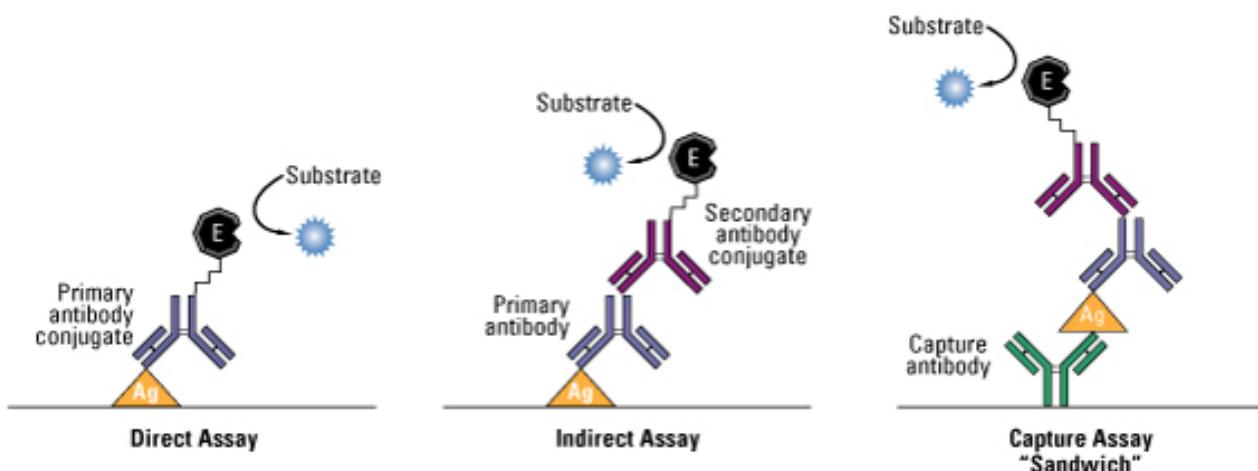
- Direct ELISA
- In-direct ELISA
- Sandwiched ELISA
- Competitive ELISA

Direct: Direct ELISAs involve attachment of the antigen to the solid phase, followed by an enzyme-labeled antibody. This type of assay generally makes measurement of crude samples difficult, since contaminating proteins compete for plastic binding sites.

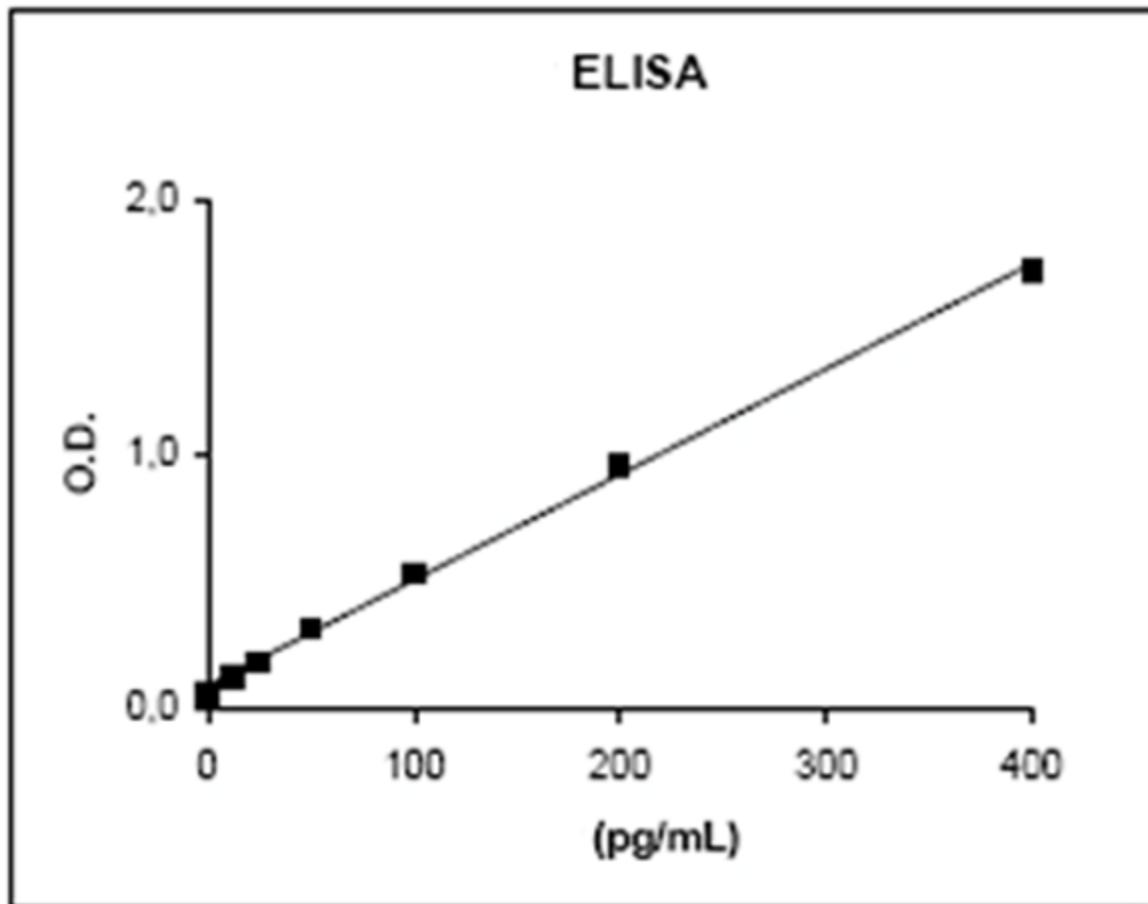
In-direct: Indirect ELISAs also involve attachment of the antigen to a solid phase, but in this case, the primary antibody is not labeled. An enzyme-conjugated secondary antibody, directed at the first antibody, is then added. This format is used most often to detect specific antibodies in sera.

Sandwiched: Sandwich ELISAs involve attachment of a capture antibody to a solid phase support. Samples containing known or unknown antigen are then added in a matrix or buffer that will minimize attachment to the solid phase. An enzyme-labeled antibody is then added for detection.

Competitive: Last type is Competition Assay, which involves the simultaneous addition of 'competing' antibodies or proteins. The decrease in signal of samples where the second antibody or protein is added gives a highly specific result.



Standard results:



References:

1. <http://www.elisa-antibody.com/ELISA-Introduction/ELISA-types>
2. <http://www.slideshare.net/ParasuramanParasuraman/principles-and-applications-of-elisa>
3. <http://www.slideshare.net/sehamfawzy14/elisa-from-a-to-z>
4. http://www.slideshare.net/science_lablinks/official-elisa-powerpoint
5. <http://www.mdbioproducts.com/resources/protocols/standard-curve>
6. <http://www.labanimal.co.kr/product/TechInform/ELISAbYEXCELE.pdf>
7. [http://www.abcam.com/ps/products/140/ab140363/documents/ab140363_Cell%20Cycle%20In-Cell%20ELISA%20Kit%20\(Fluorescent\) Booklet djs 09-Nov-2012%20\(website\).pdf](http://www.abcam.com/ps/products/140/ab140363/documents/ab140363_Cell%20Cycle%20In-Cell%20ELISA%20Kit%20(Fluorescent) Booklet djs 09-Nov-2012%20(website).pdf)

8. <http://www.jdvandenberge.com/>
9. http://infoscience.epfl.ch/record/175067/files/SadeghipoorEI12_1.pdf

Previous Work:

This document links to the work carried out by a team who worked on mELISA during jan 27th to Feb 1st 2014.

<https://docs.google.com/document/d/1P21rUWZQtapsq80BVgWsQwuDizwX6hxoYY4WdeZj96Y/edit>

Day wise progress update:

Day 1 (31 May 2014):

1. Setting up the lab.
2. creating dark areas for our Spectrometer analysis.
3. Buying Components.



Day 2 (1st June 2014):

1. The day started off with lecture on Spectrometers and about various spectroscopic analysis ie.

- Emission
- Transmission

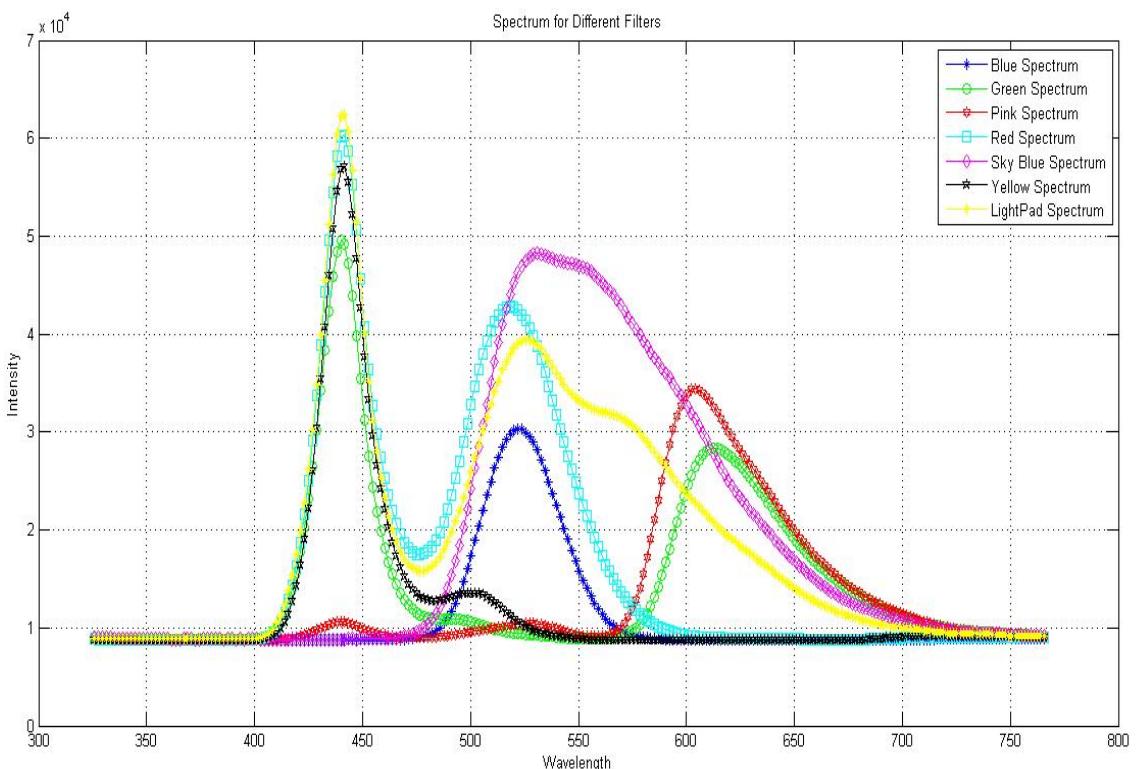
- Reflective
- Fluorescence



2. The later half of the day we characterized our Light Pads and Filters with Hamamatsu Mini-Spectrometer MS Series C10988MA-01.
3. The Light Pad is basically lit by Led lights, because we got a typical white led Spectrum.



Spectroscopic analysis of Filters and plotting:



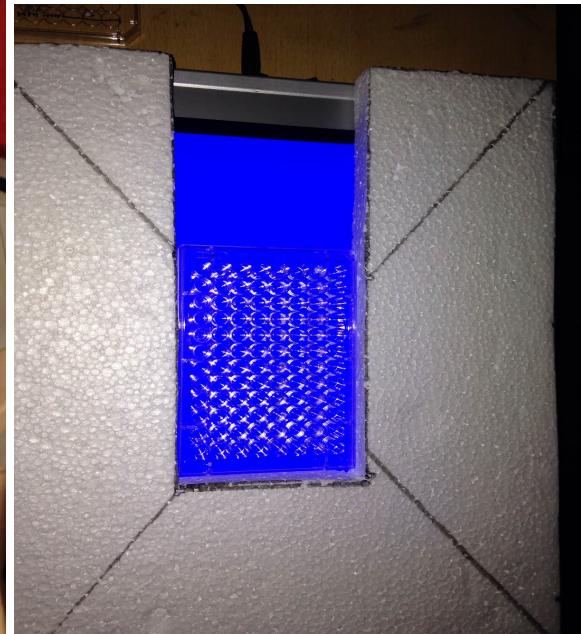
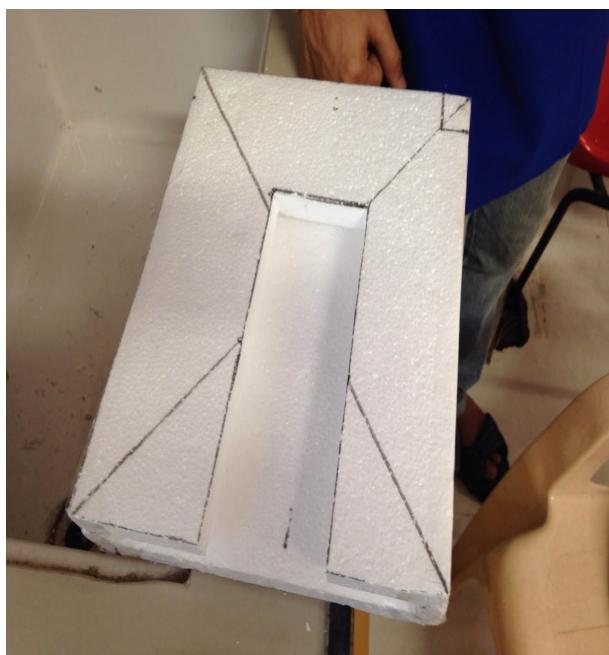
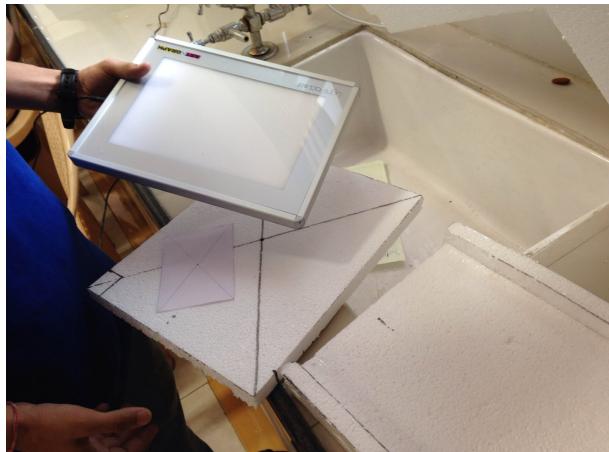
Day 3 (2nd June 2014) :

1. We mounted the camera on the Table enclosure we made and figuring out camera distance from the Light Pad.
2. We made a complete literature survey and studies about the ELISA test procedures.
3. We dedicated some time setting up deliverables and decided on timelines.
4. Waiting for microplates to start of testing and analysis.



Day 4 (3rd June 2014):

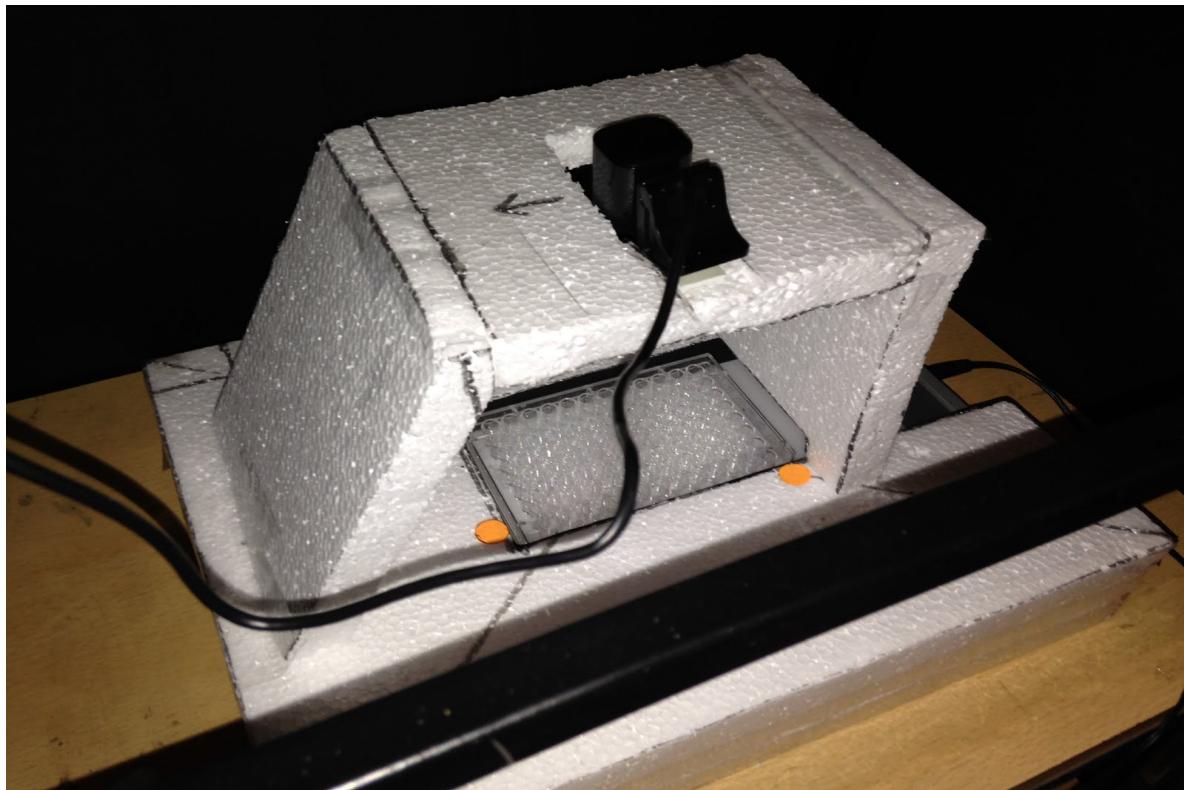
1. Day started off with a briefing about hardware methods and the components required to do that.
2. We started off building a thermocol structure for housing the LightPad, ELISA plate and Camera mount.
3. We acquired few images of microplate.
4. Had few issues with camera, which was giving irregular focus.





Day 5 (4th June 2014):

- Complete hardware for the microplate capture was completed.



- Cameras had some irregularities, the entire image plane was not focused. Only the center was in focus and sides weren't.

- We shifted to new Microsoft LifeCAM HD 6000 cameras, and these cameras gave really good picture clarity and was entirely in focus.
- With new Camera issues of irregular focus was removed and also the height for mounting the camera for full coverage of the plate, reduced.
- As a learning exercise on infrared filters, Anshuman took out the infrared filter from the old camera and we plotted its characteristics using Spectrometer.

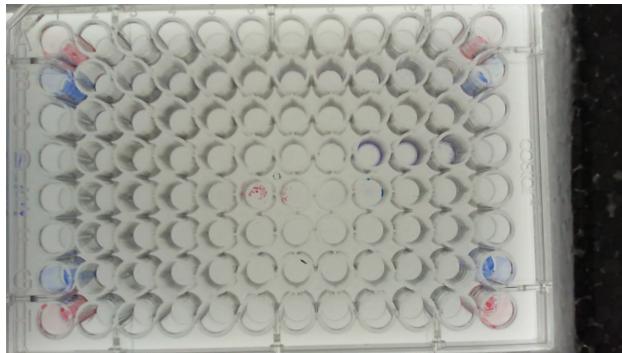


Image Without any Filter

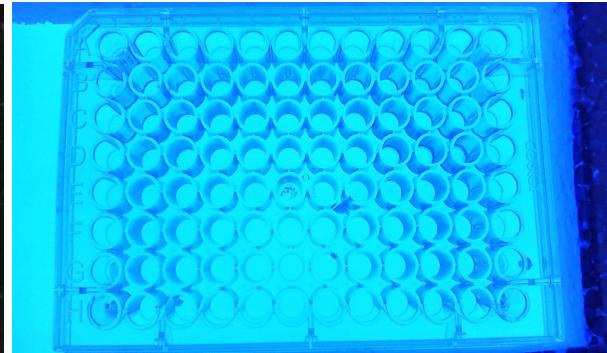
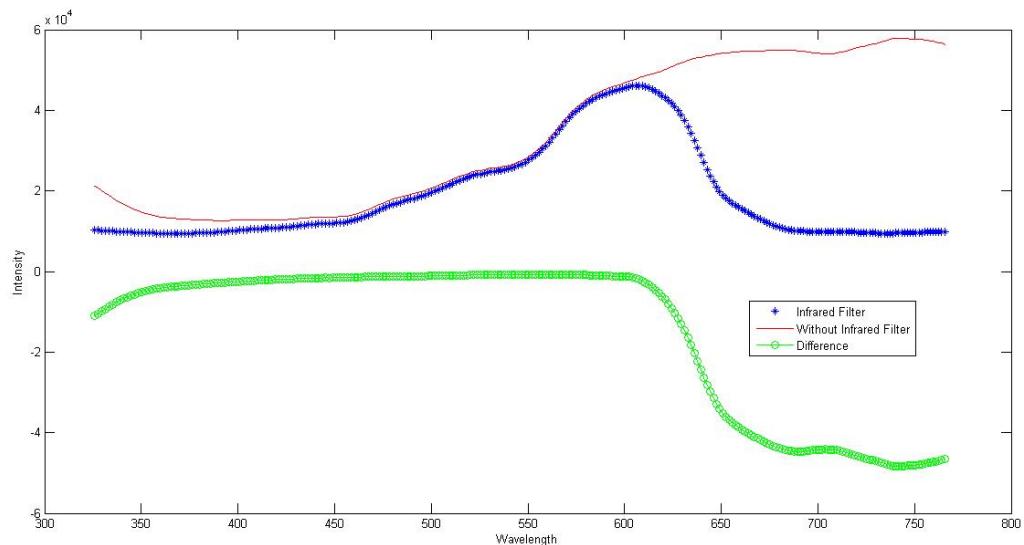
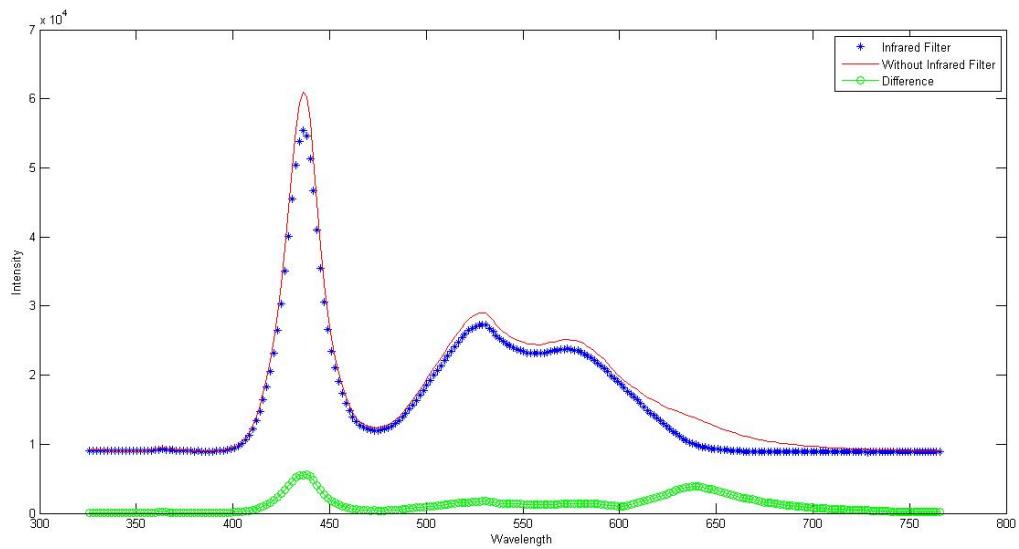


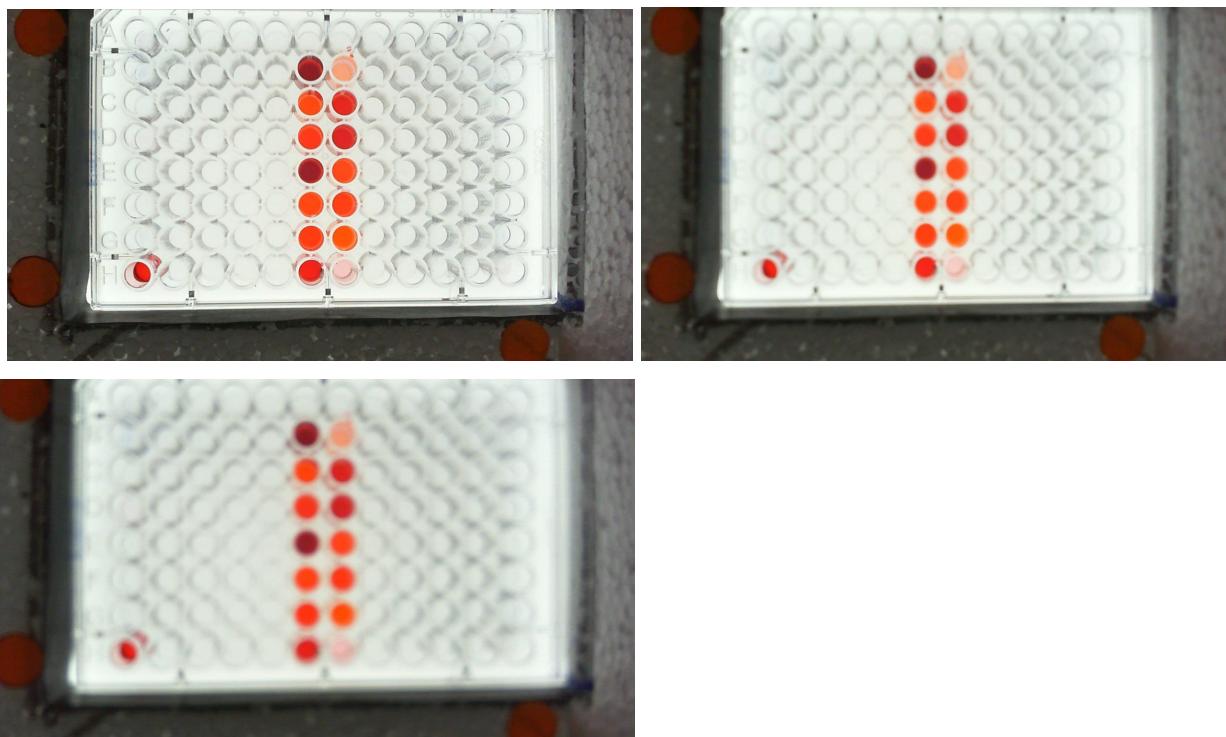
Image With BLUE Filter





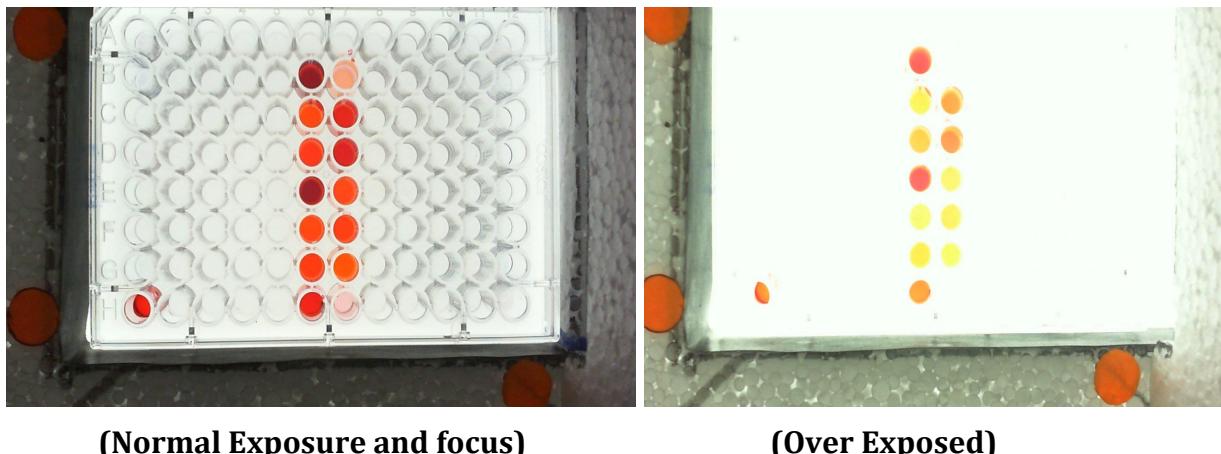
Day 6 (5th June 2014):

- Anshuman had carried few dyes from Boston. We used the Eosin dye with different concentrations and captured images to study them.
- We used filters to characterize the response for various wavelengths.
- We played around with exposure and focus and found out that over exposed images seem to have better results.



Day 7 (6th June 2014):

- The captured images on previous day was analyzed and we found that the over exposed images gave better results for the fact that the reflections from microplate were averaged out.



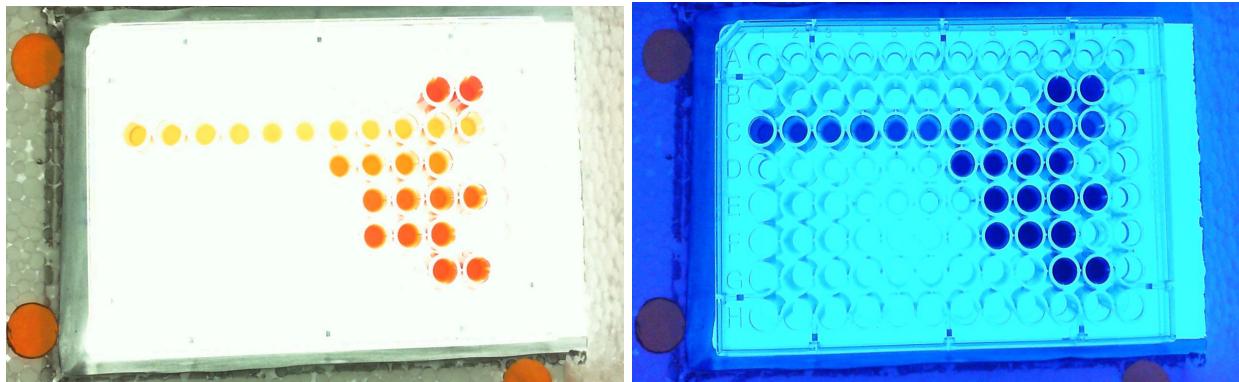
- Only the wells were prominently visible and all the other artifacts were taken out from the image.
- We used three orange dots to create a mask and track the required wells using them as a standard endpoints.

Day 8 (7th June 2014):

- Day started late and we tried few operations on images. The operations involved extracting the HSI components of the captured images, looking at different planes (RGB) etc and also mask creation.
- The complexity of operations increased with the mask creation, and was also prone to false positives. So we decided to use basic circle detection and averaging algorithms.
- Remaining half of the day we went around and exchanged what other teams are upto and helped them if they were stuck with some issues.

Day10 (9th June 2014):

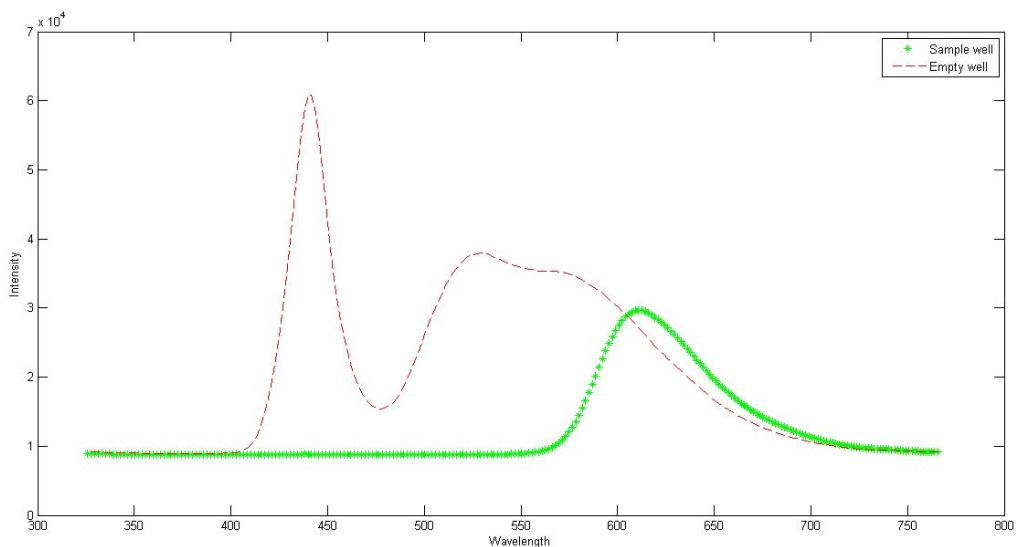
- Paid a visit to the Biosciences and Bioengineering lab and Anjali over there helped us with preparing a congo red dye microplate solution.
- With the plate set for testing, we captured few images for various exposure values and focus levels.



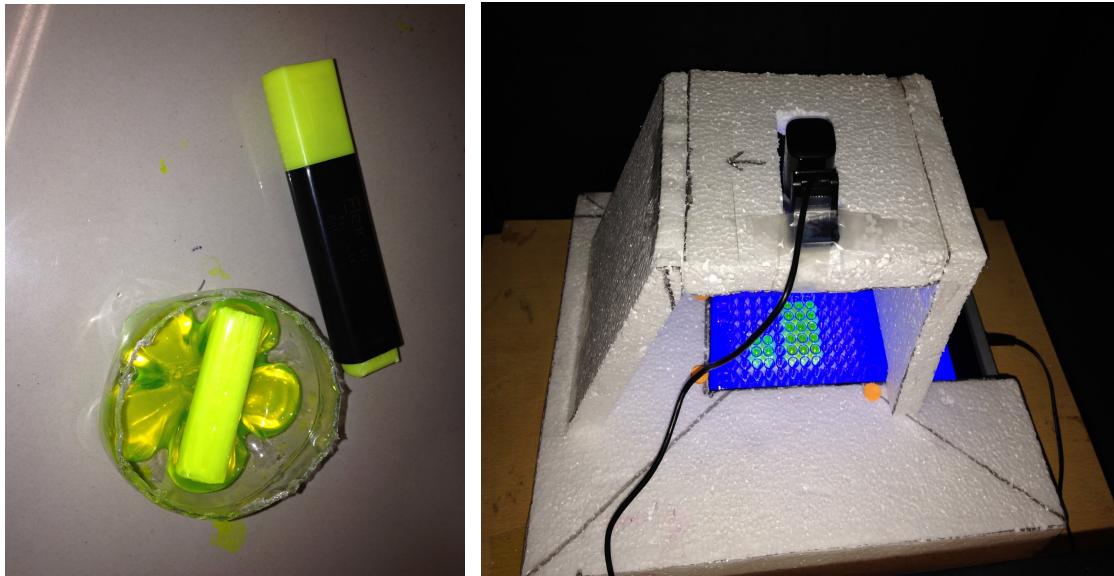
More sample images captured are available at:

<https://drive.google.com/?authuser=1#folders/0B7G0Cqqv4Mg5a28wVG5ORHhyRWs>

- We did the spectroscopic analysis of different wells and found that congo red was poor at fluorescence.



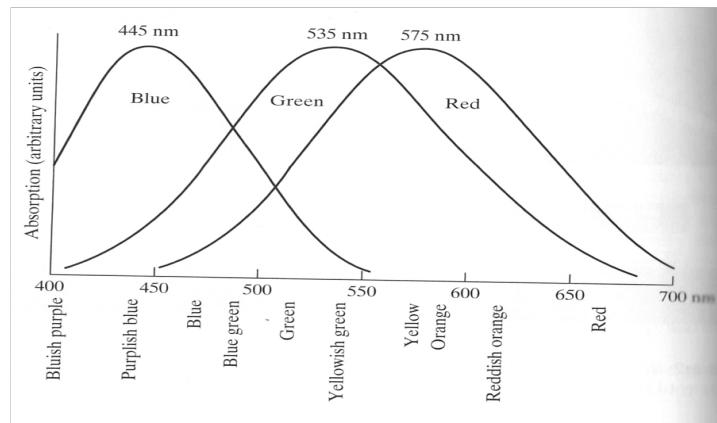
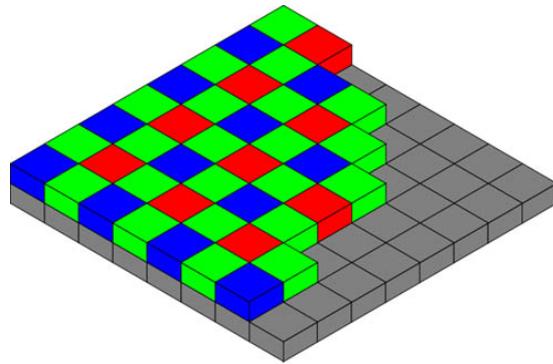
- The graph indicates that the congo red is absorbing blue and green part and letting red to pass through.
- Thus congo red is not ideal for our analysis as most of the ELISA devices use Fluorescence as a mode of measurement.
- So we dissolved a fluorescent highlighter pen in water to prepare our own dye! Here is how we did it,



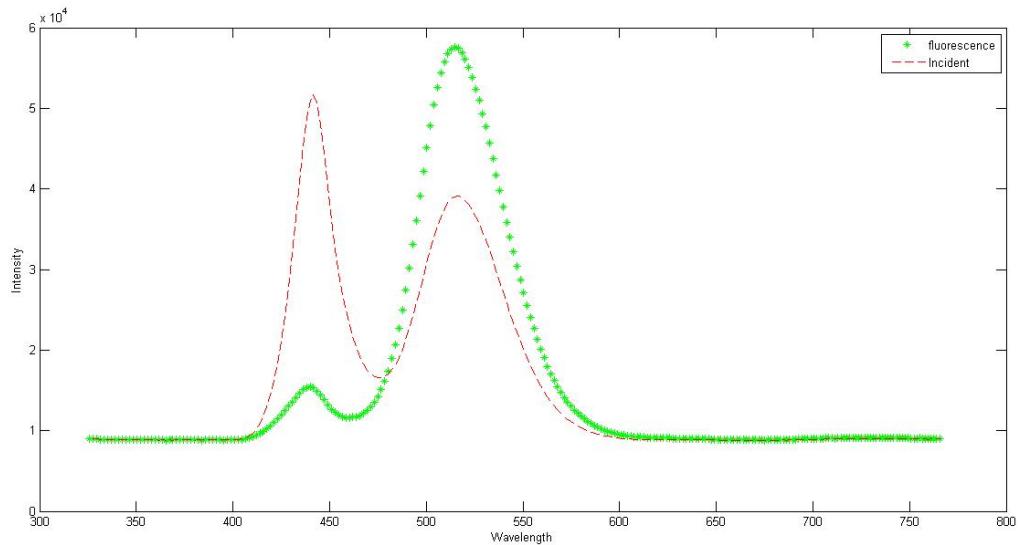
- We got the results as we were expecting with beautiful green tint!!

Day11 (10th June 2014):

- Characterized the Camera filter and the fluorescence dye (which we are using) and found the region of interest in the spectrum. (**Bayer Filter**)

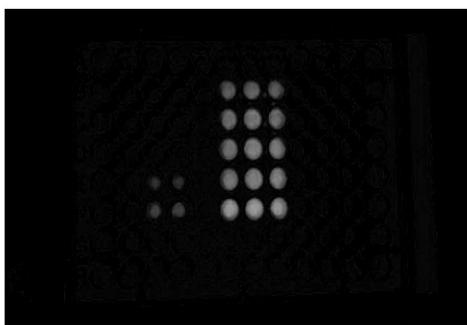


Spectral Sensitivity of Bayer Filter

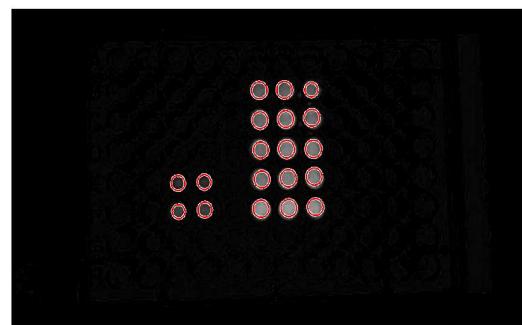


'--' Spectrum of light coming from blue filter
'*' Spectrum of Fluorescent Die (From Highlighter)

- So our concerned spectrum lies in the Green region and after observing the bayer filter spectrum, its clear we can simply ignore the blue channel for further experiments on fluorescence.
- This will simply remove the backlight from all the wells under consideration.
- We achieved some great images as a result of the uniform illumination and over exposure,

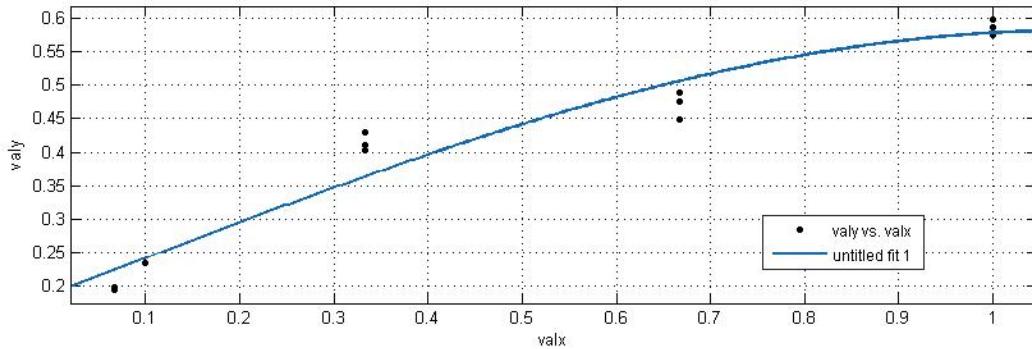


(Processed Image)

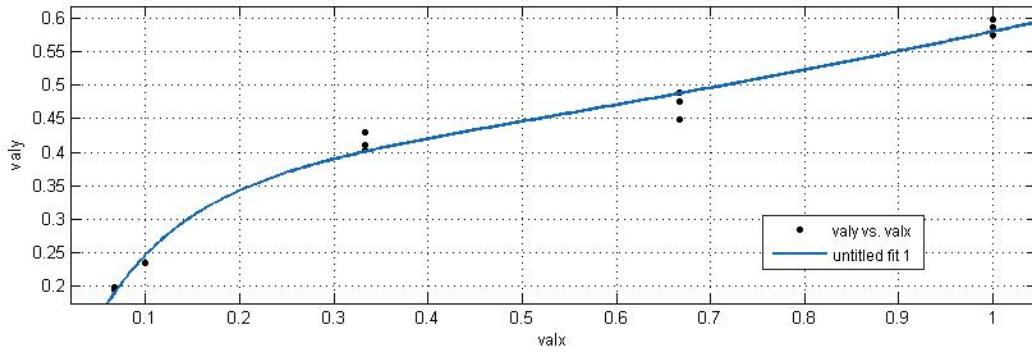


(Detected Circles)

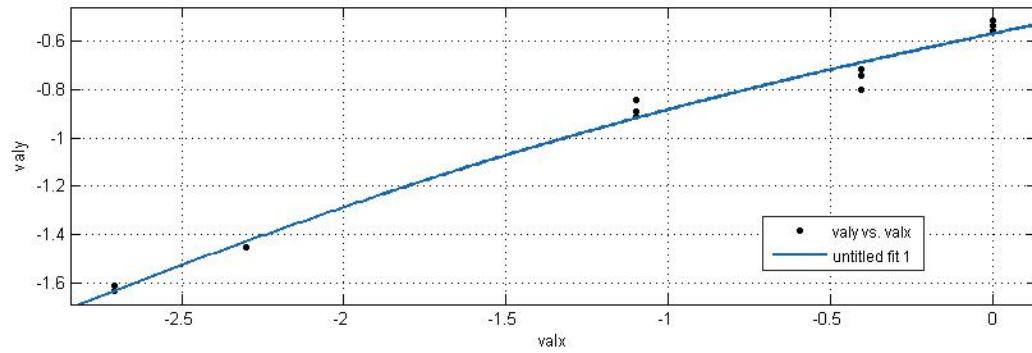
- We took the mean of Intensities of circular wells to plot the calibration curve and find the best fit.
- We found that for normal values, exponential fit gave better results over linear curve fitting.



(Linear Fit for Normal Values)



(Exponential fit of second degree for Normal Values)

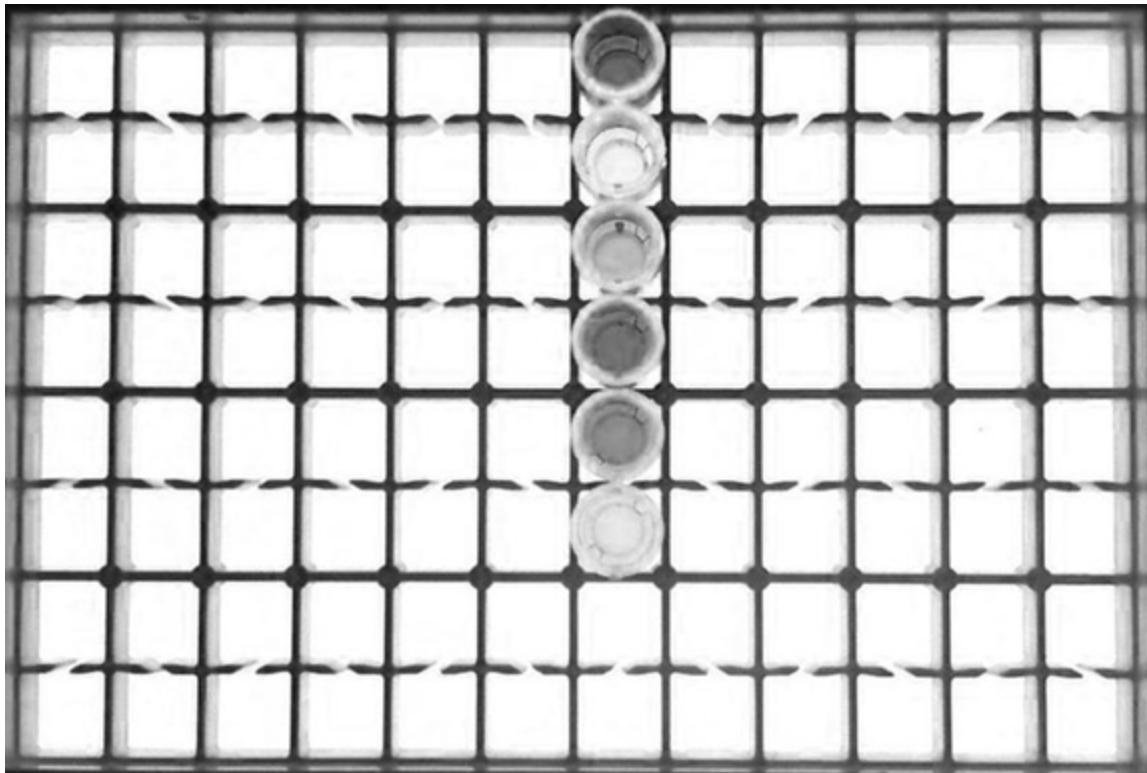


(Linear fit for Logarithmic Values)

- We interpolated the results and got closer values with a fitting factor R=0.98

Day 12 (11th June 2014) - Day 20 (19th June 2014):

- Paid Visits to P.D Hinduja Hospital, Pathology Lab to get some real time data.
 - Visits were dated on 13th, 16th and 17th of June.
- Took readings of actual infected samples and correlated our results with the standard ELISA devices.
- It was quite odd to

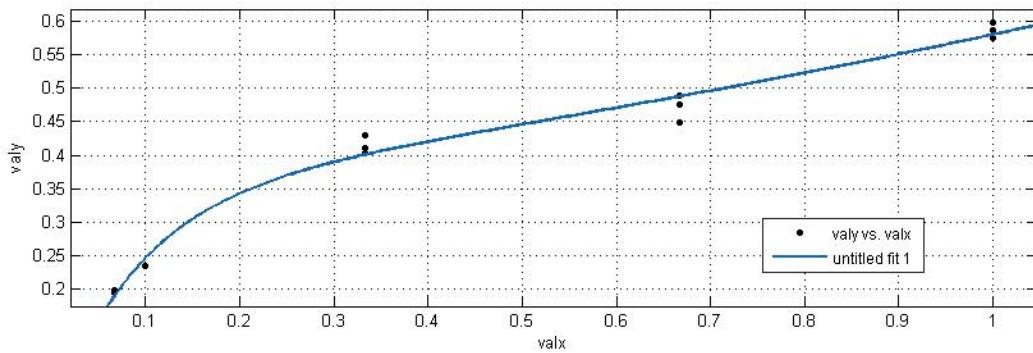
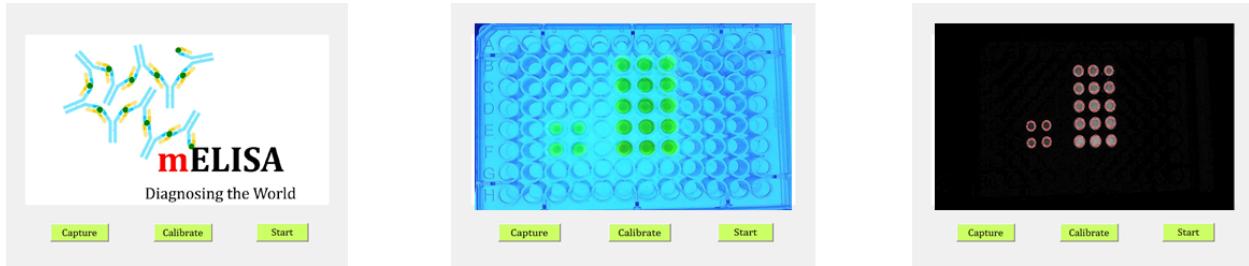


hear that every device, will be supplied with their own formulas and procedures for Thresholding and diagnosis.

(Processed image of a sample infected with Malaria. Courtesy: Microbiology lab, PD Hinduja Hospital, Mumbai, India)

- We had done Fluorescence based analysis at Lab, but most of the devices in Hospital used Absorbance. So we had to change over in technology.
- A single 450nm Filter couldn't suffice our purpose. So Anshuman suggested us to use two filters, i.e Blue and Green (One at a time). Operate on both the images and amplify the data within it. We also took a Blue- Green filter and tested the correlation. We got good results which can be visualised in the above picture.

- Wrote a MATLAB GUI, with 3 buttons, for easier interface.



- The main challenge that lies in front of us is to calibrate the device for accuracy and define an automation procedure.
- On 19th We presented to the other groups and the invited Doctors and delegates at Bio Sciences Dept. Seminar hall, and also bagged the **best Presentation award!**

To carry the work ahead, Vijay will be working at Media labs, with Anshuman and collect some data and refine the algorithms for faster analysis.

Signing off,
Team ELISA

PS: Here is the link to the Final presentation:

<https://docs.google.com/file/d/0B7G0Cqqv4Mg5bFhUbDJLZldZSms/edit>