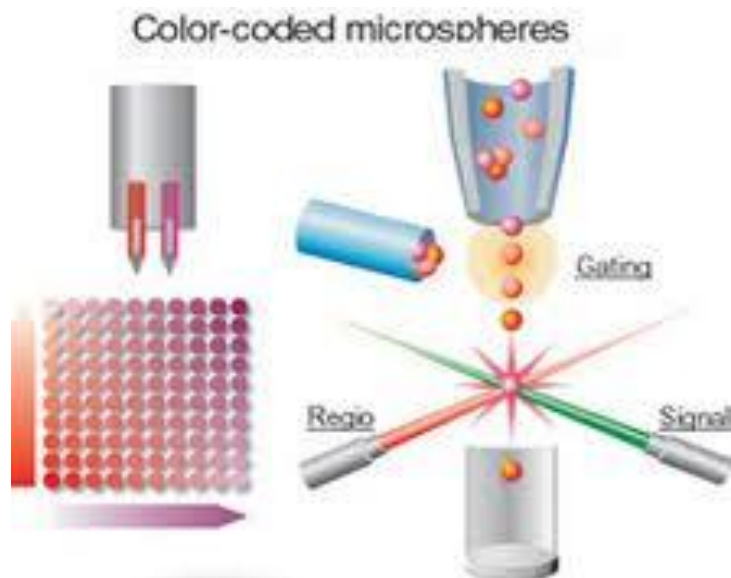


An expedition to modern optical bio-diagnostic tools

Advisor – Prof. James F. Rusling



Dhanuka Wasalathanthri
CHEM 5395



How sensitive it is ?

- Prostate Specific Antigen (PSA) in serum (normal) - 0.5 to 2 ng mL⁻¹
- Interleukin-6 in serum (normal) - <6 pg mL⁻¹

Amplification strategies



Target

Ex : Polymerase
chain reaction (PCR)

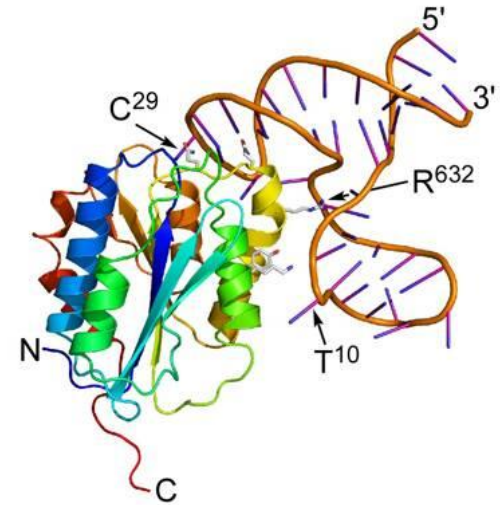
Signal

Ex. Enzyme-linked
immunosorbent assay (ELISA)

Microbead -
Nanotechnology

How selective it is ?

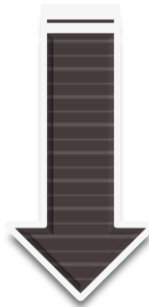
- Specificity
 - Cross reactivity
- Ex :- Immuno-PCR techniques
Aptamer based systems



www.ash.confex.com

Versatility and portability

Micro
arrays



Microfluidics

Future of bio-diagnosis

Outline

➤ **Absorbance**

Enzyme Linked Immuno-Sorbent Assay (ELISA)

Multiplex ELISA

➤ **Fluorescence**

Single fluorophore detection – singulex technology

Luminex technology

➤ **Electrochemiluminescence (ECL)**

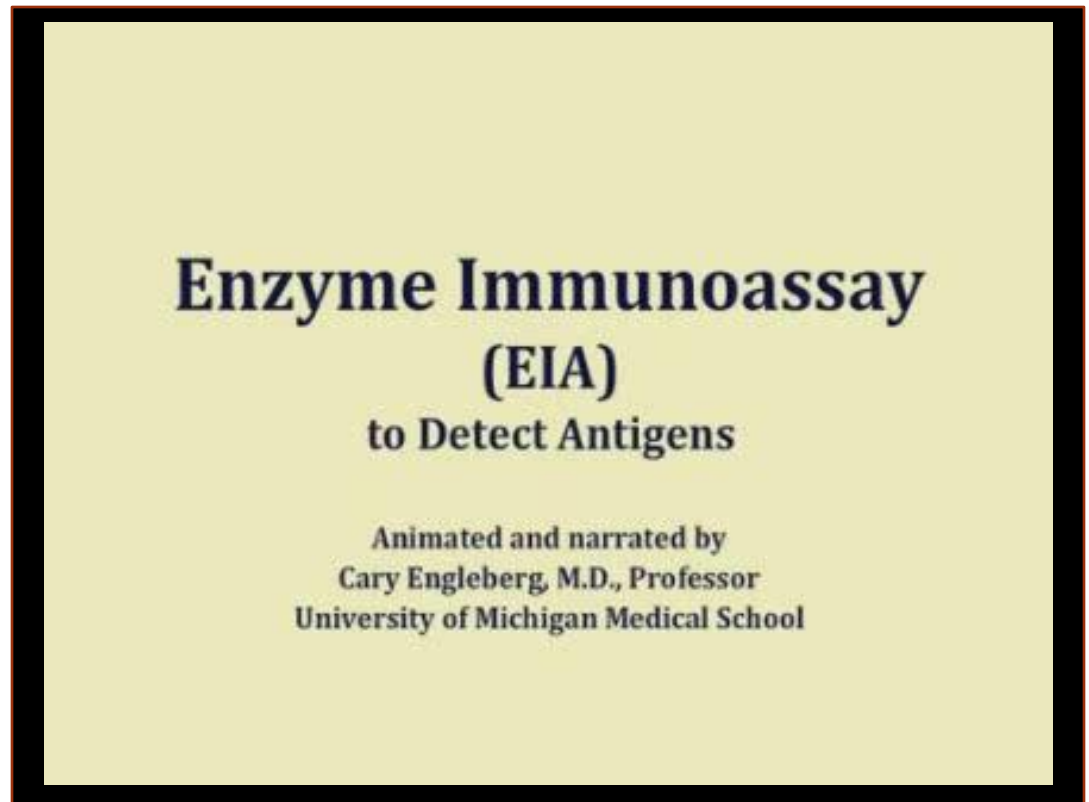
Magnetic bead conjugate assisted ECL assay

Multiplexing on ECL platform

Absorbance

Enzyme-linked immuno-sorbent assay (*ELISA*)

- A fundamental optical diagnostic tool in immunology
- ELISA kits – commercially available



Enzyme label – Horse raddish peroxidase (HRP)
Chromogenic substrate – 3,3'-5,5'-Tetramethylbenzidine (TMB)

Absorbance at 450 nm

Limitations

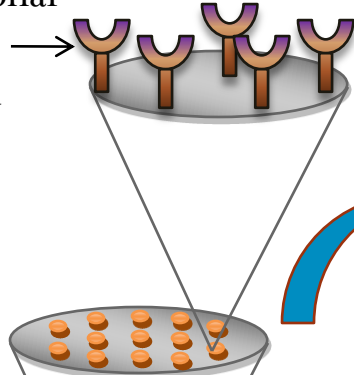
- Best DL ~ 3 pg/mL in serum
- Can detect one analyte a time
- Sample size
- Time consuming.

Multiplexing is a necessity in positive detection of diseases - cancer

Multiplex ELISA

By Quansys Biosciences

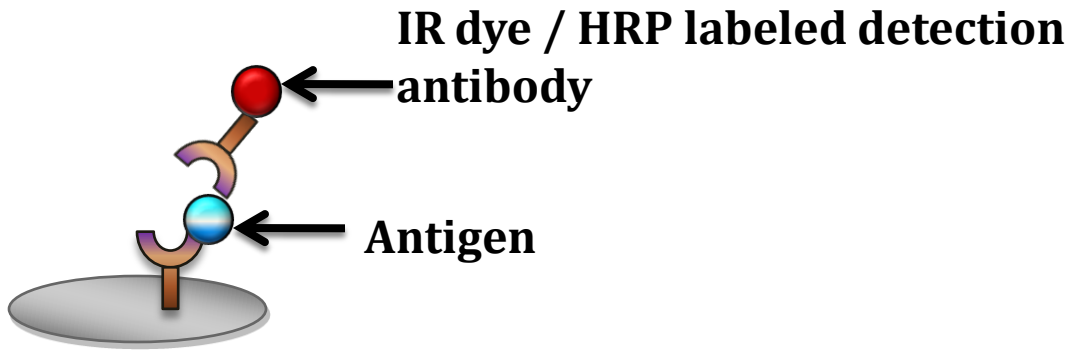
Monoclonal
primary
antibody



- ✓ Robotic liquid handlers print 20-50nl spots of capture antibody
- ✓ Size - 350-500 μm
- ✓ Each spot is an unique assay



ELISA plate 96/384 wells

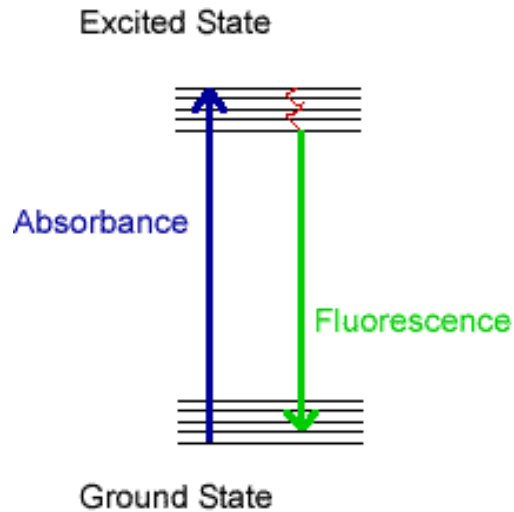


Detection

- If antigen is present the spot emits light
 - If no antigen is present the spot is not visible
-
- Small volume requirement - 5-30 μl / test
 - Easy to handle- Similar to ELISA
 - Low cost \$ 1.00 per data point

Detection limit – pg /mL

Fluorescence



- Excitation energy – monochromatic radiation – Laser
- Various fluorophores are commercially available.

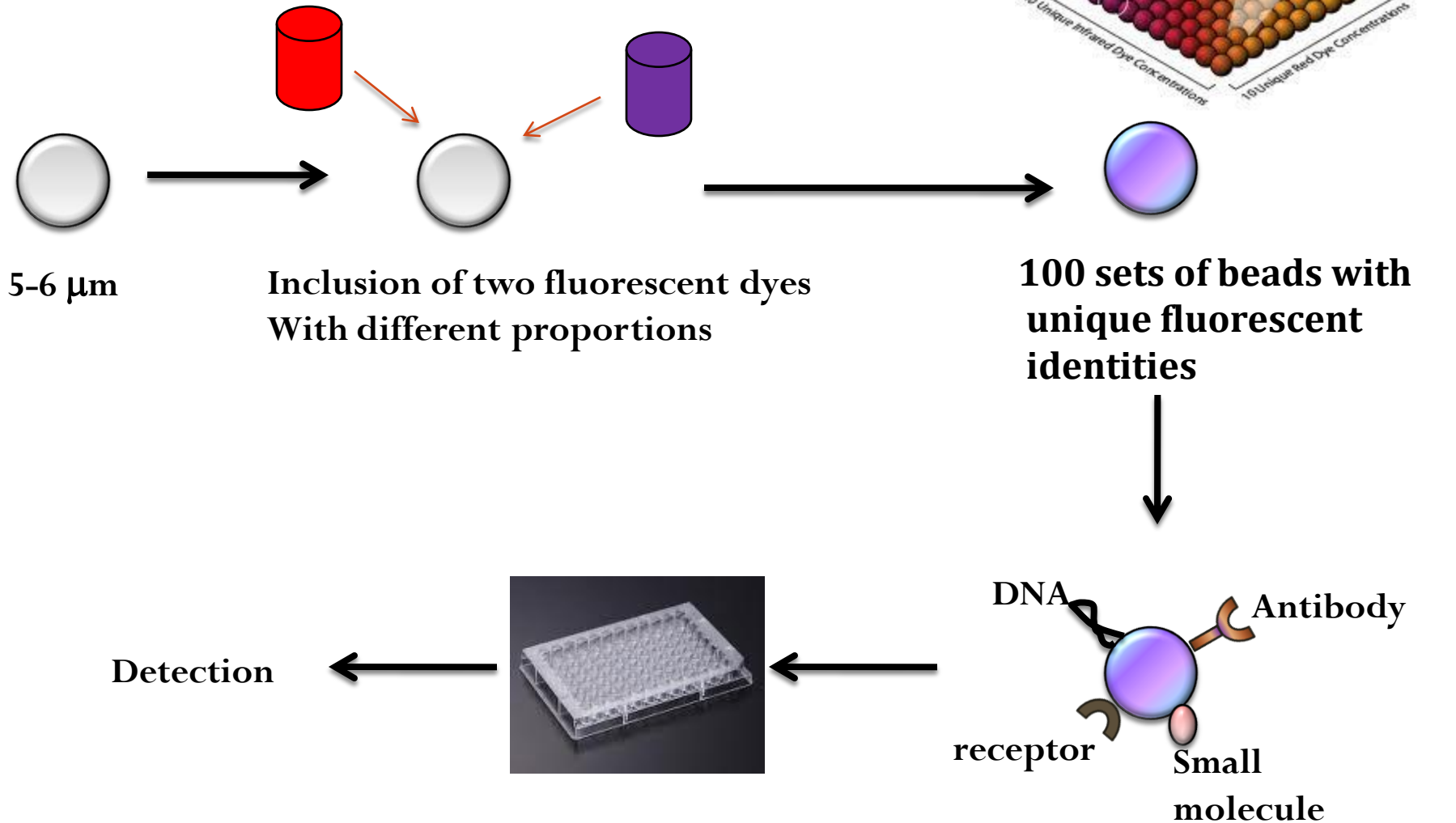
Low abundance biomarker assay

– single fluorophore detection

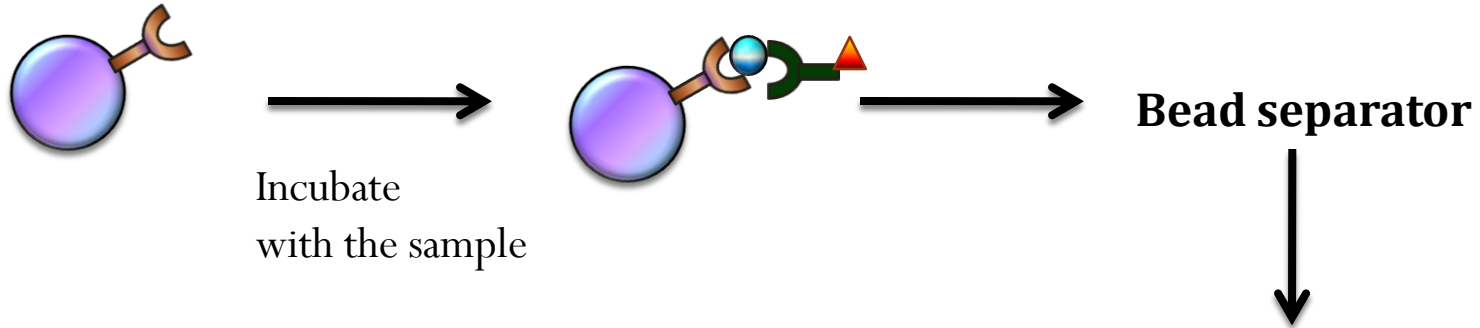
- Singulex Inc.

http://www.youtube.com/watch?v=3uWGL8Iw520&feature=player_embedded

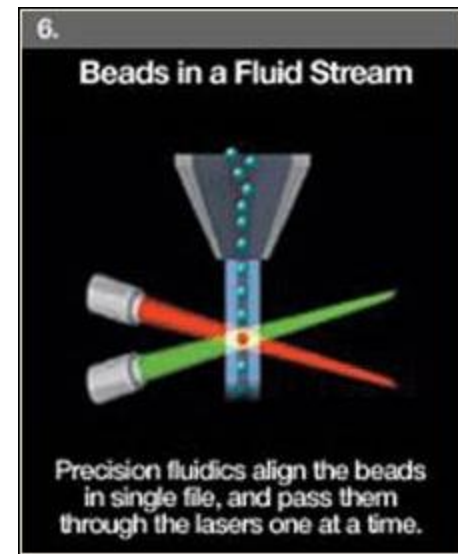
Luminex - xMAP technology



Detection based on the principle of flow cytometry



Detection based on laser excitation



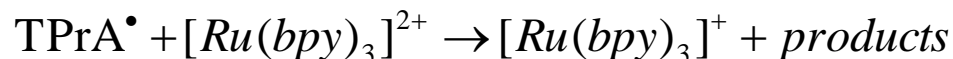
- ✓ Multiplexing enable
- ✓ fast,
- ✓ low sample volume
- ✓ flexibility

[xTAG technology](#)

<http://www.youtube.com/watch?v=qPAOBij4Op4>

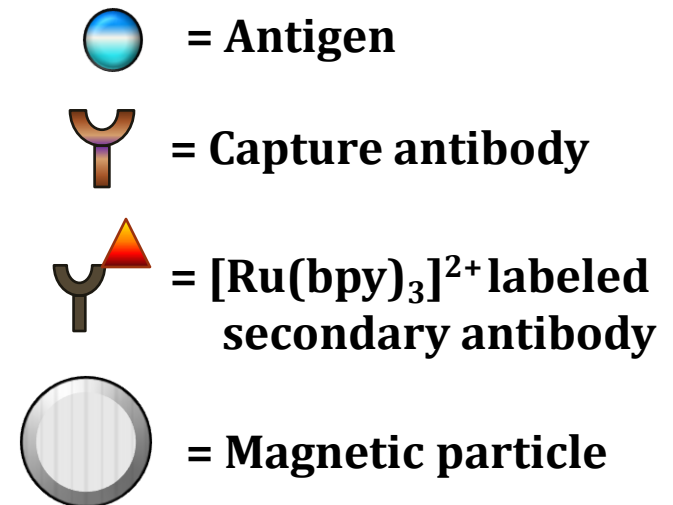
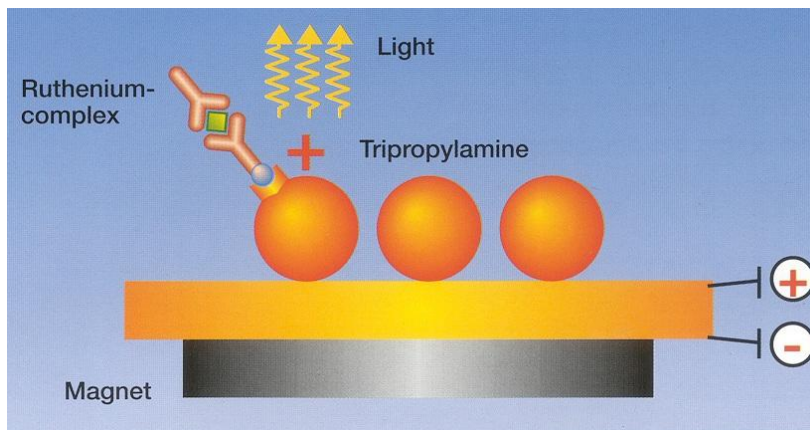
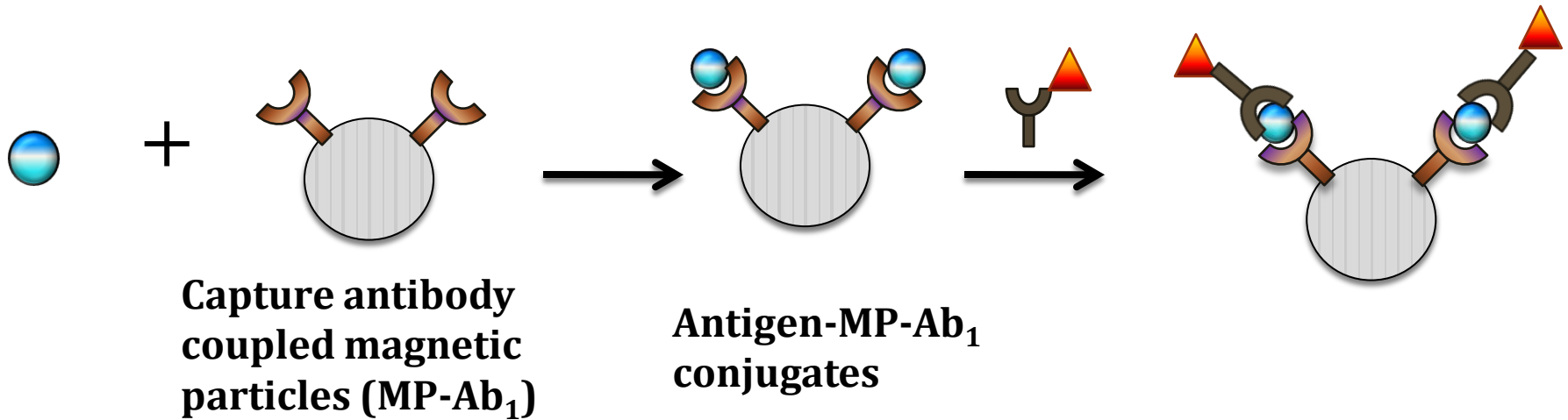
Electro-chemiluminescence (ECL)

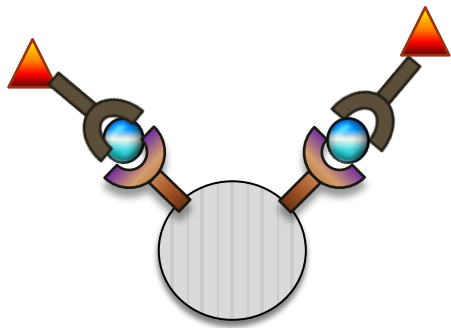
- Luminescent product is generated via electrical induction



Magnetic bead conjugate assisted ECL assay

Elecsys 2010 – roche diagnostics Inc.



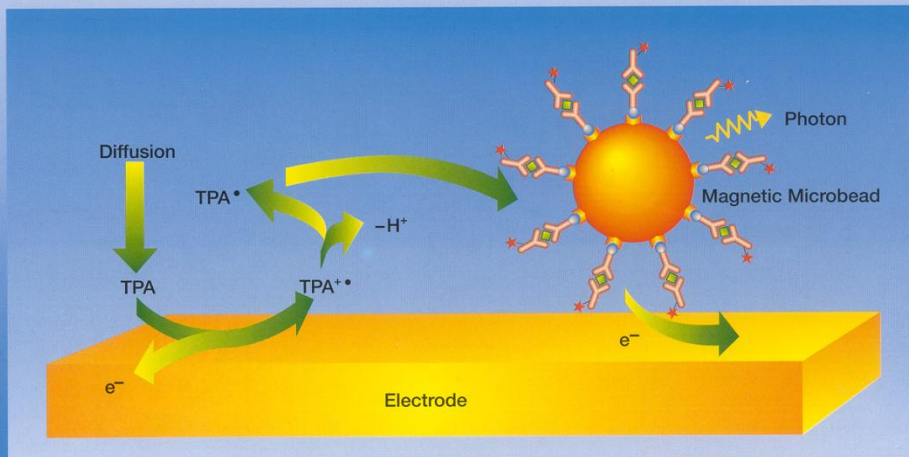


610 nm 

Electrode
Magnet

⊕
⊖

Detection of ruthenium labelled immune-complexes on a magnetic microbead



Single tag can emit multiple photons – enhanced sensitivity

- ✓ Fast turn around time
- ✓ Broad measuring ranges
- ✓ High sensitivity

Elecsys 2010 – Roche Diagnostics



- Fully automated immunoassay system.
- 15 different assays can be done simultaneously
- On board reagent storage facility.

https://www.mylabonline.com/products/flash_demos_v1_1/Elecsys2010/index.html

Limitation : Can detect only one protein at a time

Multiplexing on ECL platform

-Meso Scale Discovery Inc.

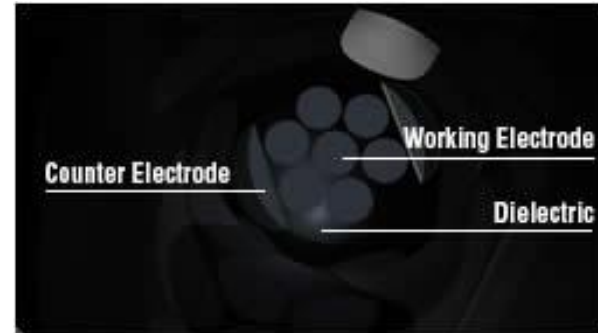
- Multi-Array and Multi-Spot technology



Multi-Array



Multi-Spot



C electrodes integrated into the bottom of the plate

Advantages

- Multi-multiplexing
- Real time detection technique



Real time visualization

Thank You !