BIOCHIPS & BIOSENSORS

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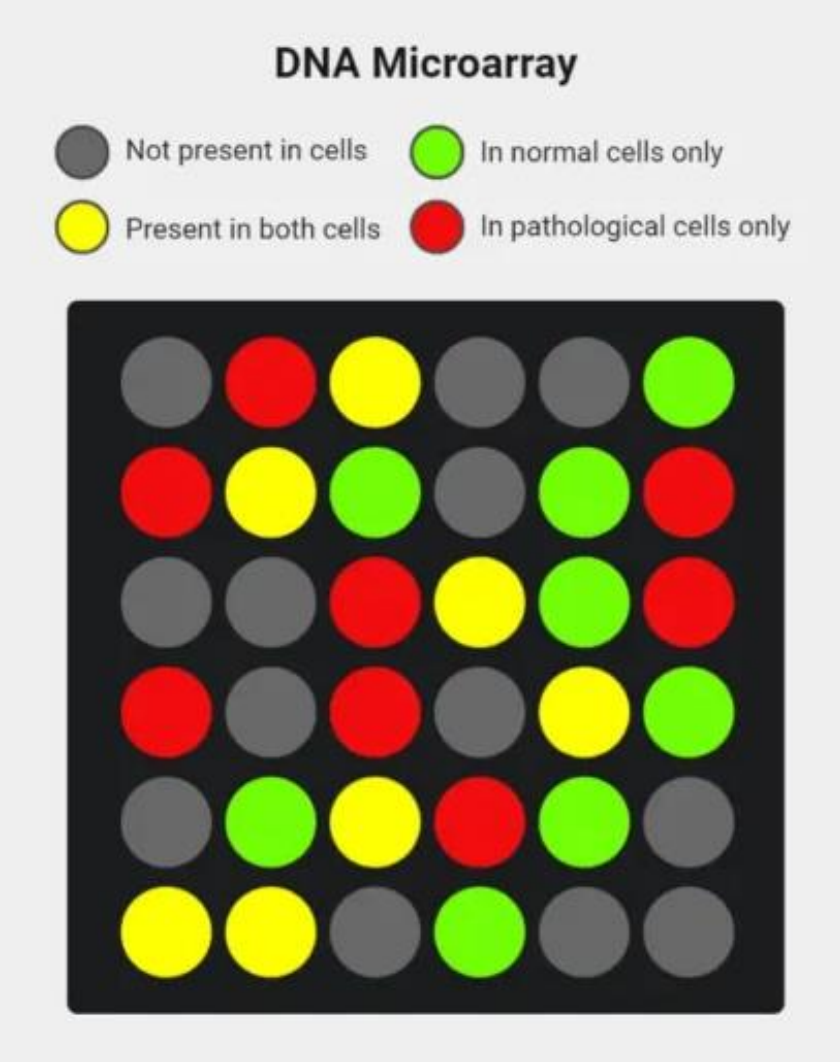
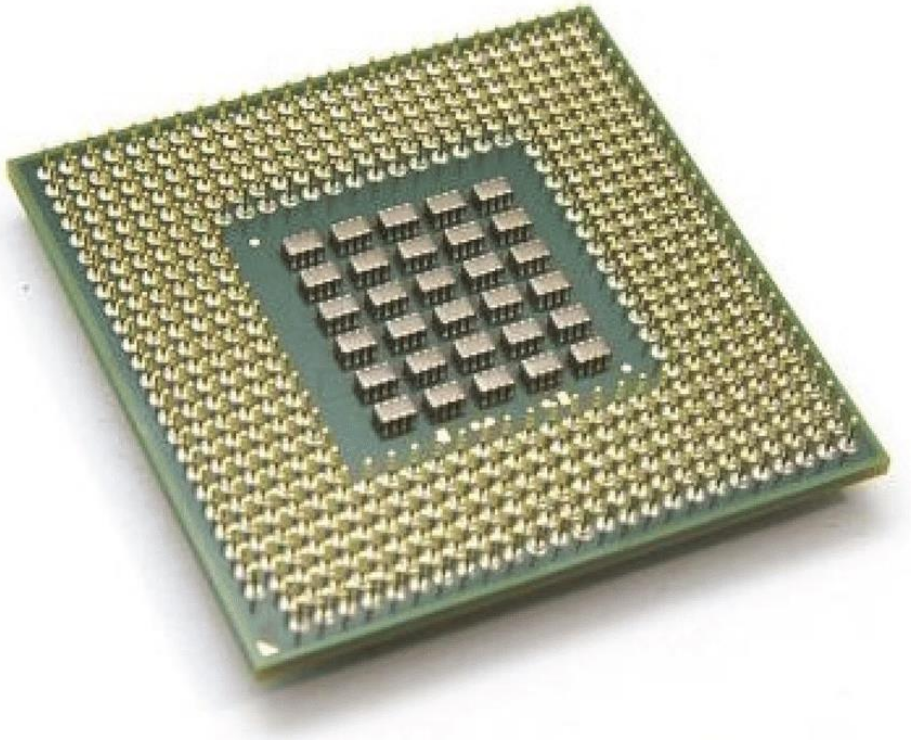
Biochips


DNA Microarray
Not present in cells
In normal cells only
Present in both cells
In pathological cells only

- ***What is a computer chip??***

- It is a miniature site which is designed to perform millions of mathematical calculations simultaneously.
- It performs these calculations in a very small time scale i.e. milliseconds to seconds.



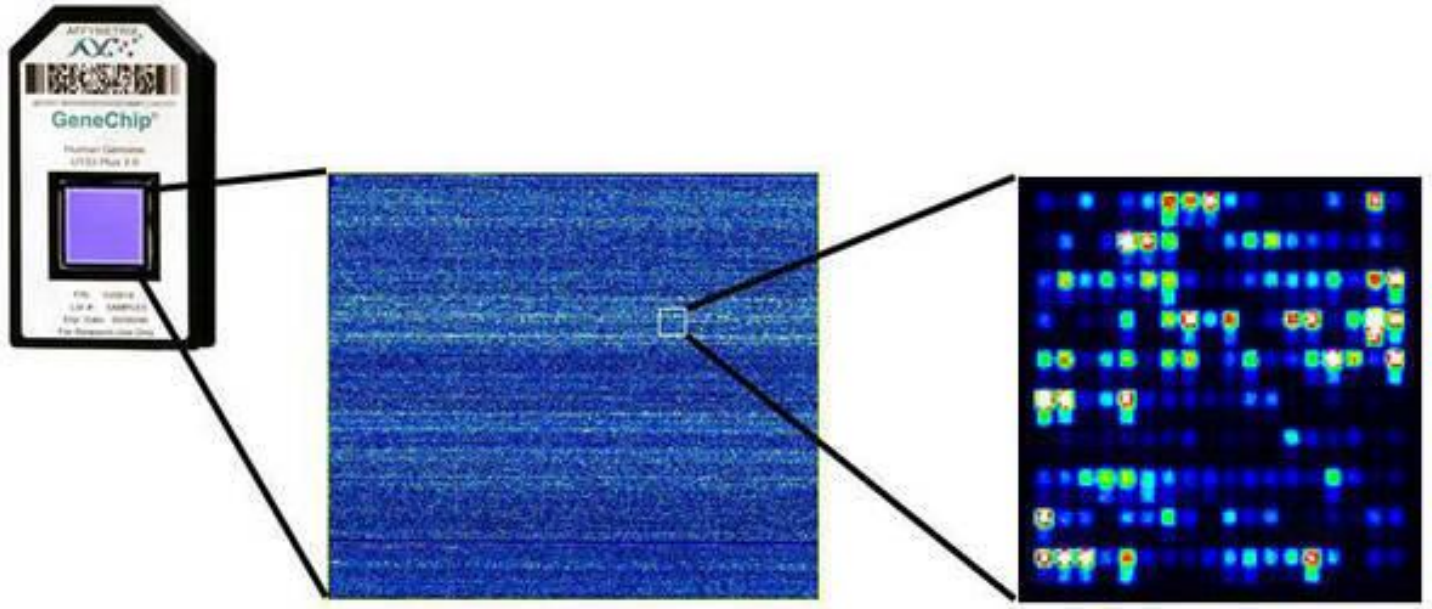
Can we do the same with biological reactions?

- ***So what is a biochip?***

- A biochip is a collection of miniaturized test sites arranged on a solid substrate that permits many tests to be performed at the same time.

- These biochips are called as microarray as well and are made using a technology which is used in semiconductor manufacturing.

- It is a high throughput technology.

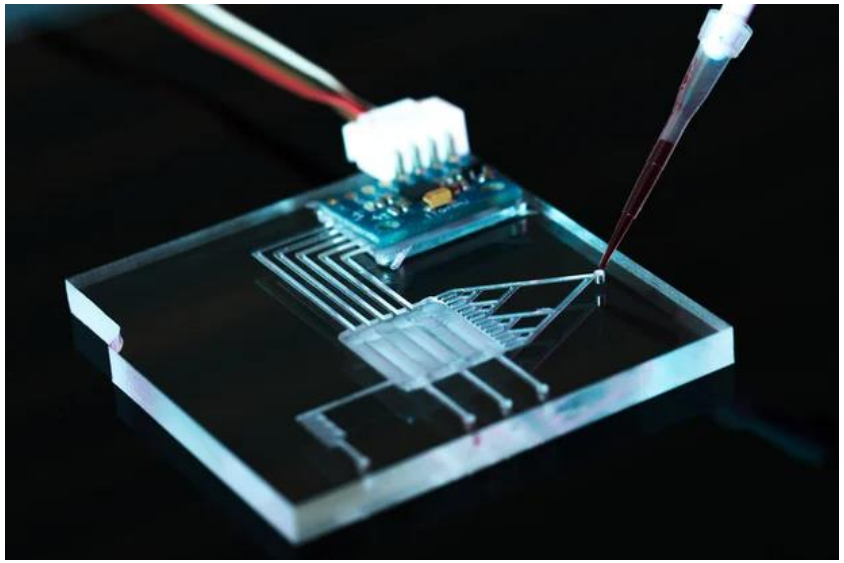


- ***Evolution of biochips***

- 1st biochip was made by an American company Affymetrix (now Applied Biosystems) in 1981.
- With development of technology 1st GeneChip for HIV genotyping was launched in 1994.
- Now, biochips have extensive uses.

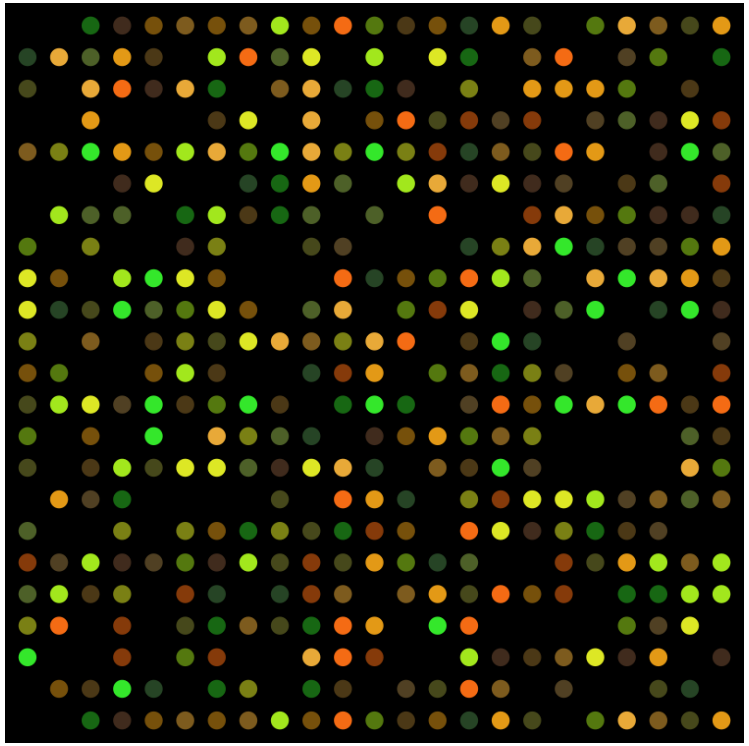
- We generally come across the following types:

1. DNA microarray.
2. Protein Microarray.
3. Microfluidic chip.



Microfluidic Chip

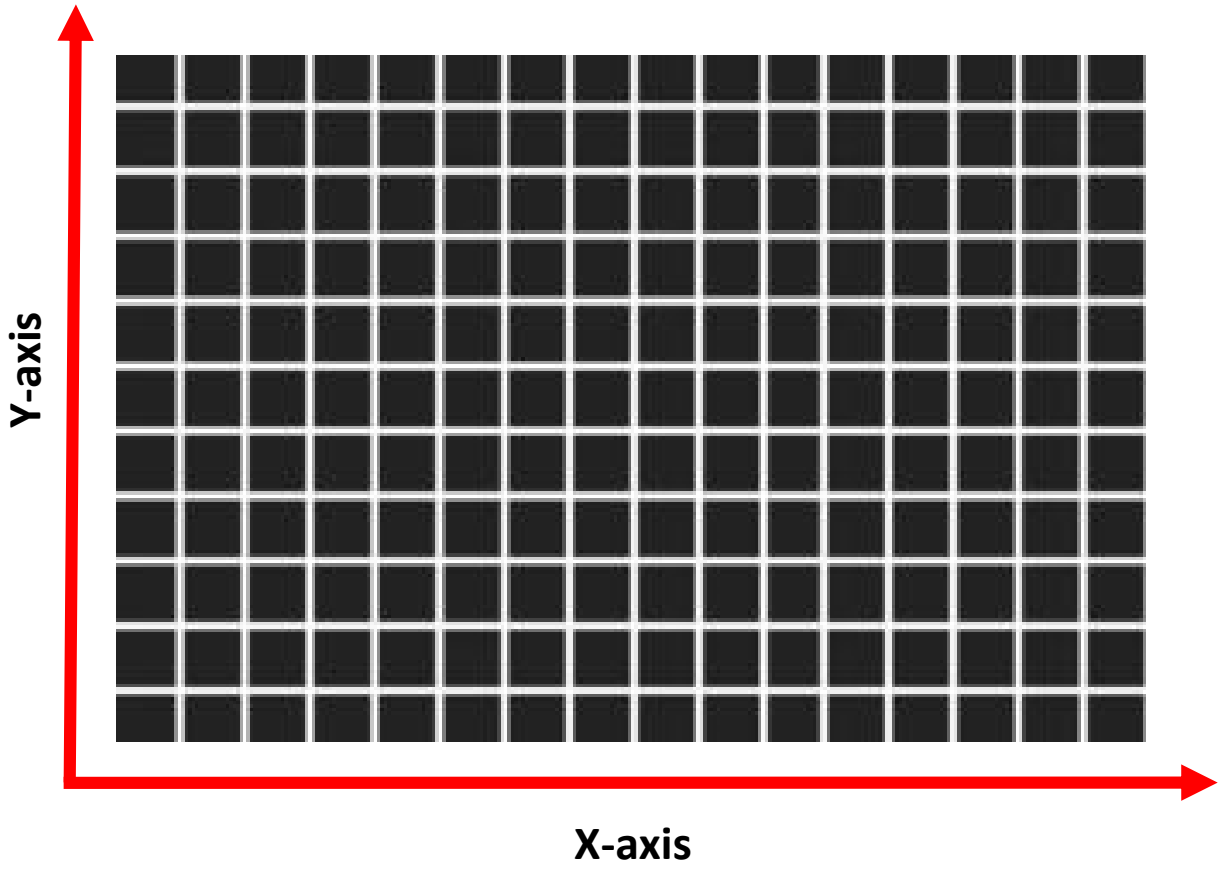
- ***DNA microarray:***
- It comprises of a large number of tiny DNA spots which are fixed to a strong surface.
- Each and every DNA mark comprises of probes (the single stranded, picomoles of a particular gene).
- Generally, probe-target hybridization is observed and calculated by recognition of fluorophore labeled targets.
- The signals received are used to decide the relative quantity of nucleic acid series in the target.



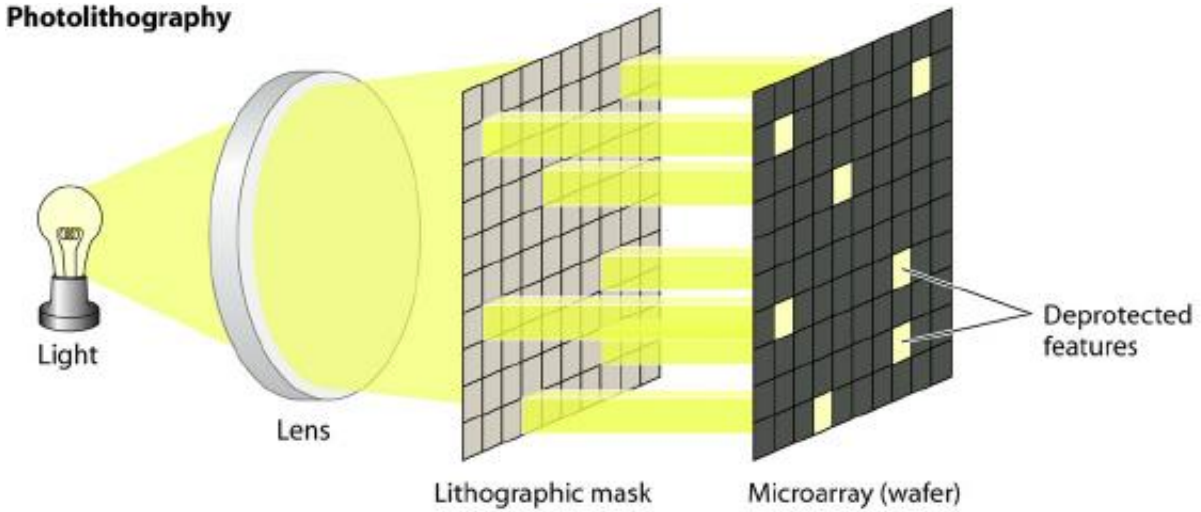
DNA microarray visual signals.

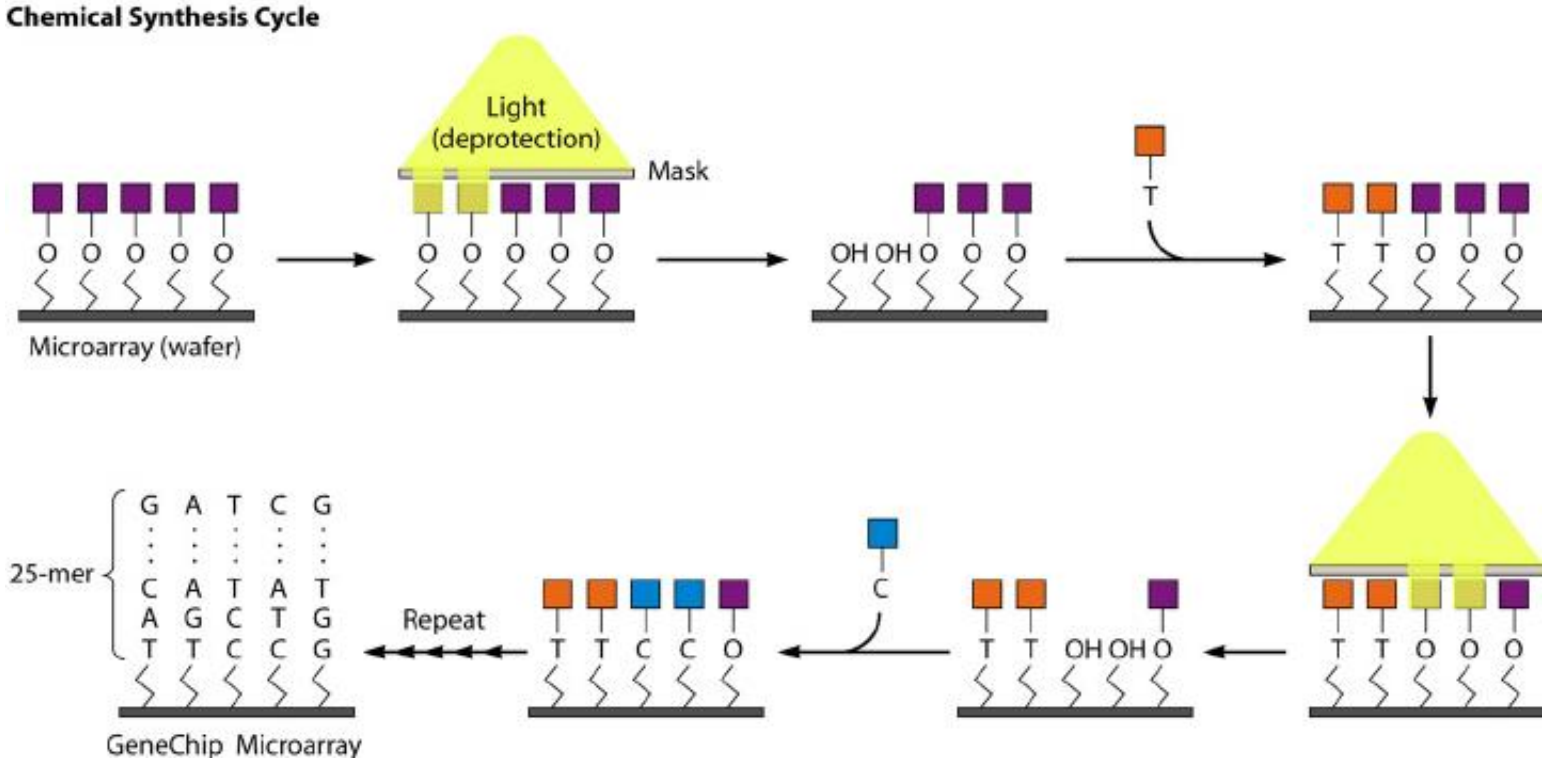
So, how do we synthesize the DNA microarrays?

- ***Photolithography:***
- This process is used for printing of DNA probes on the glass surface.
- This process is superior to inkjet printing of probes as it reduces the risk of cross contamination.



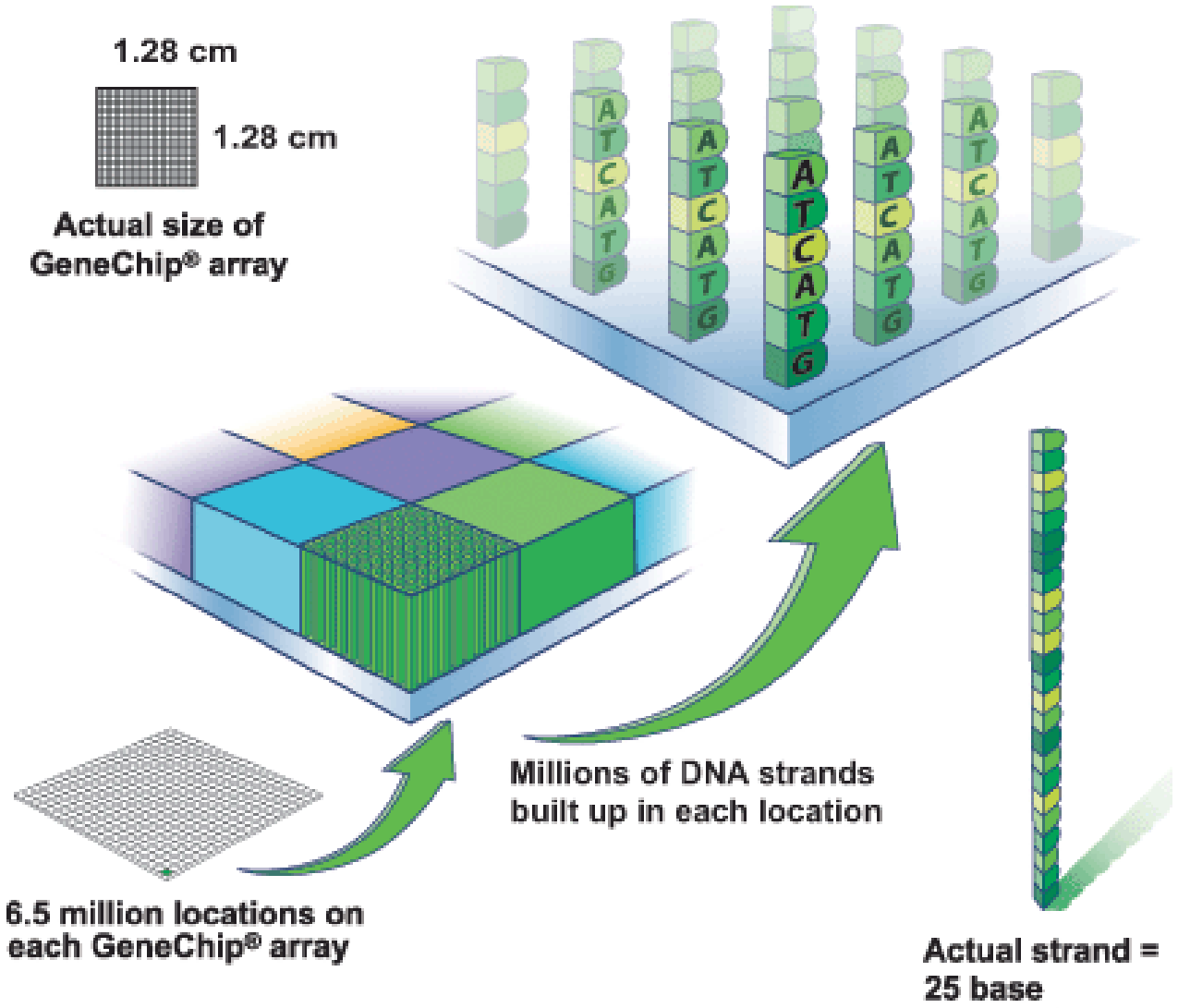
- Each square is a spot for probe printing.
- In one square, one unique probe is printed.
- There are millions of such squares on a DNA microarray.
- Here, printing means synthesis on a well defined square.



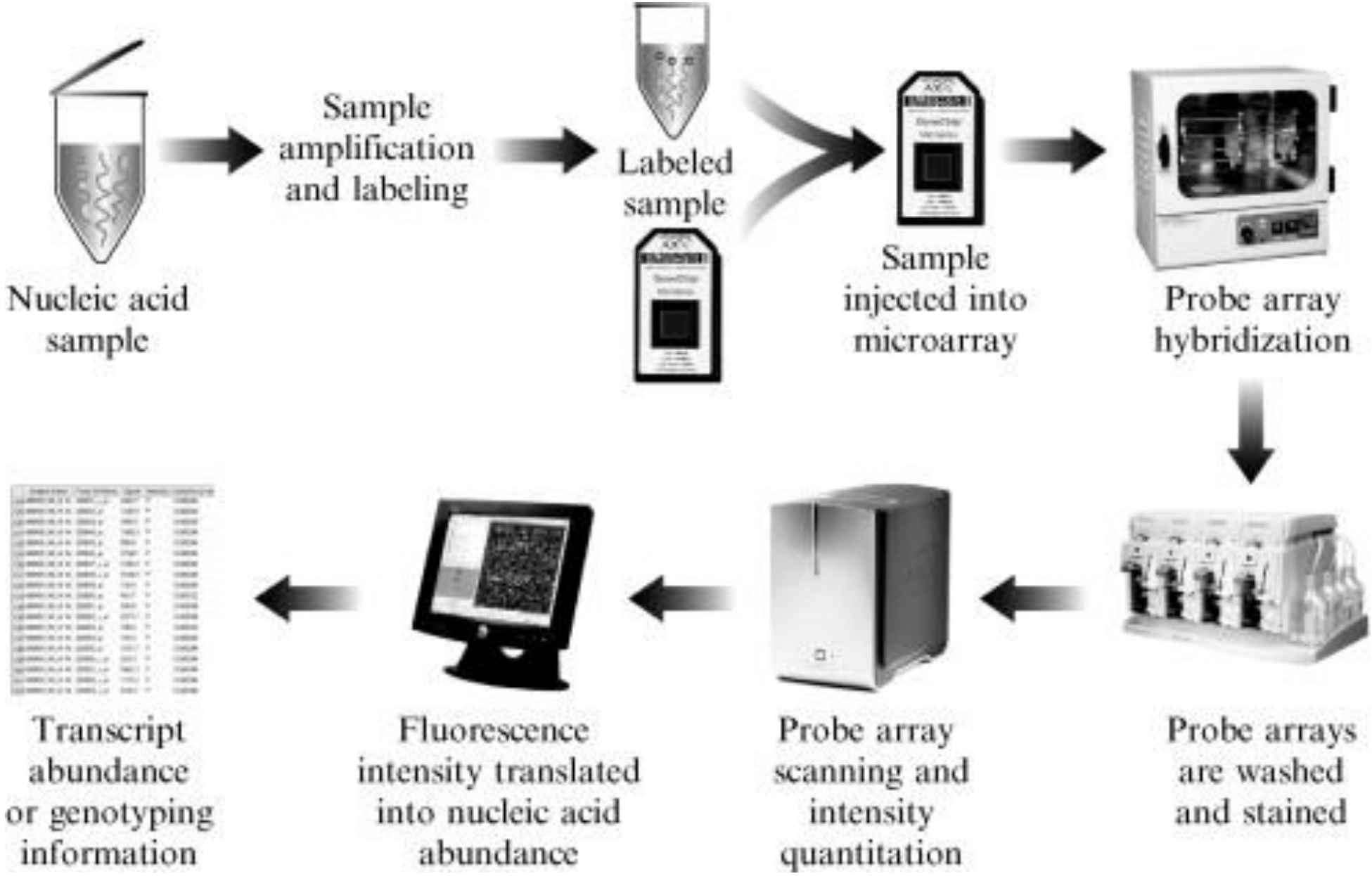


- Originally all sites are masked.
- Defined squares are deprotected.
- UV light falls and removes the protecting groups which leads to 3’-OH being available.
- A defined nucleotide is added in the square.
- The added nucleotide also has a protecting group which is used in the next cycle.
- Synthesis moves to new squares and same cycle is repeated.
- After all rounds, we have defined single stranded oligonucleotide sequences in each square with some of the nucleotides being labelled with fluorophores.

Schematic of DNA microarray after photolithography



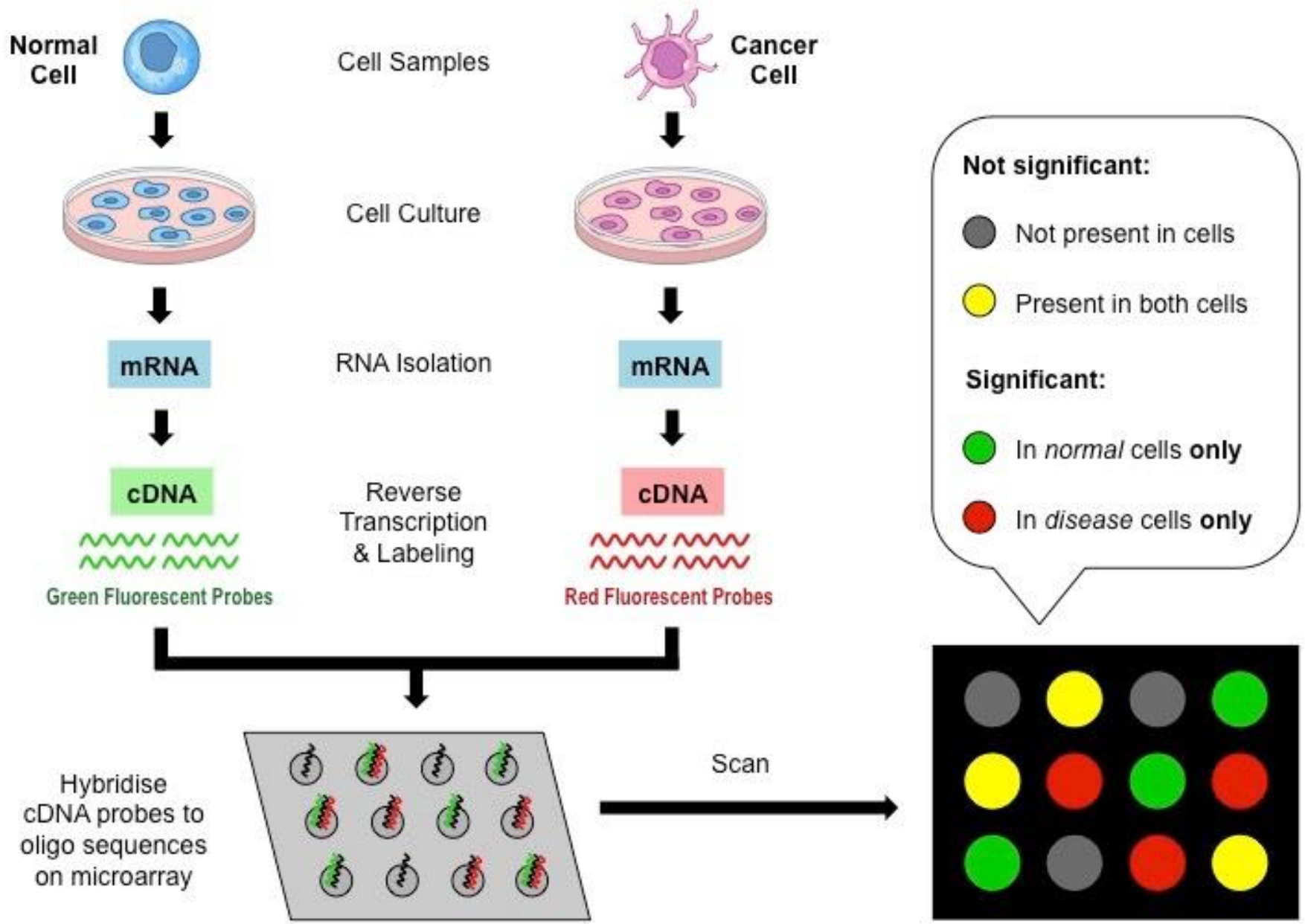
Workflow of the DNA microarray



- **Applications of DNA microarray technology:**

1. Gene expression analysis.
2. Detection of cancer cells and their mutations.
3. Study of natural variations in the DNA.
4. Study of pathogenic variations.
5. Drug screening assays for pharmacogenomics.
6. Tissue microarrays.
7. Study of microbial ecology.
8. Population genetics studies.

- **Gene expression analysis using DNA microarray:**

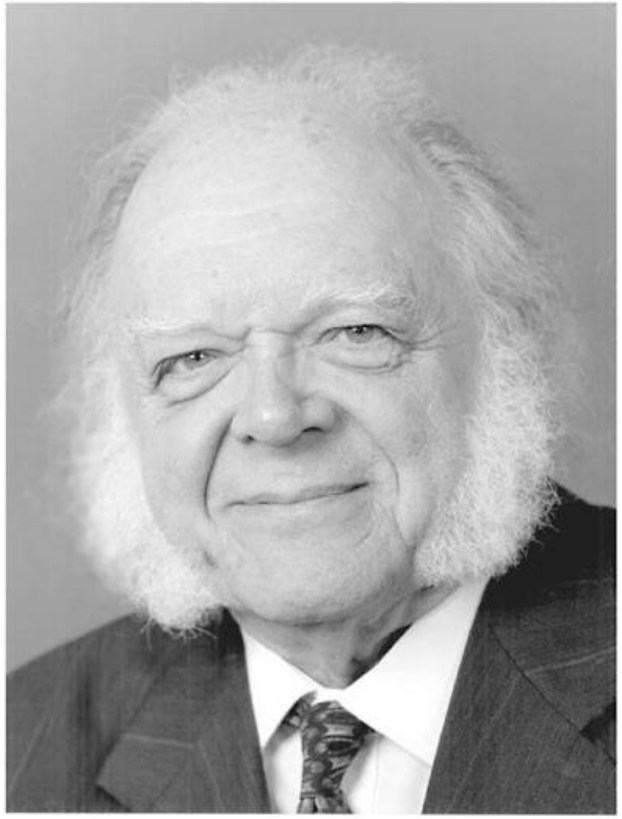


Biosensors


ACE
AVG.
MEM
DAY MONTH YEAR
12-12-12
HOUR
9:38
235
mg/dl
AFTER MEAL
blood glucose meter

- **History of Biosensors**

- 1st true biosensor was developed by Leland C. Clark, Jr in 1956.
- This biosensor was used for oxygen detection via electrodes.
- He is called as father of biosensors.
- The electrode he invented is called as Clark electrode.
- He invented amperometric enzyme electrode for glucose detection in 1962.
- In 1969, 1st potentiometric biosensor to detect urea was developed.



- Discovery of ion-sensitive field-effect transistor by Bergvald in 1970. This later led to development of i-STAT sensors.
- Since this discovery the field of biosensors got revolutionized and led to rapid increase in the new biosensors such as for blood glucose, pregnancy, i-STAT blood biosensor for different parameters of blood.

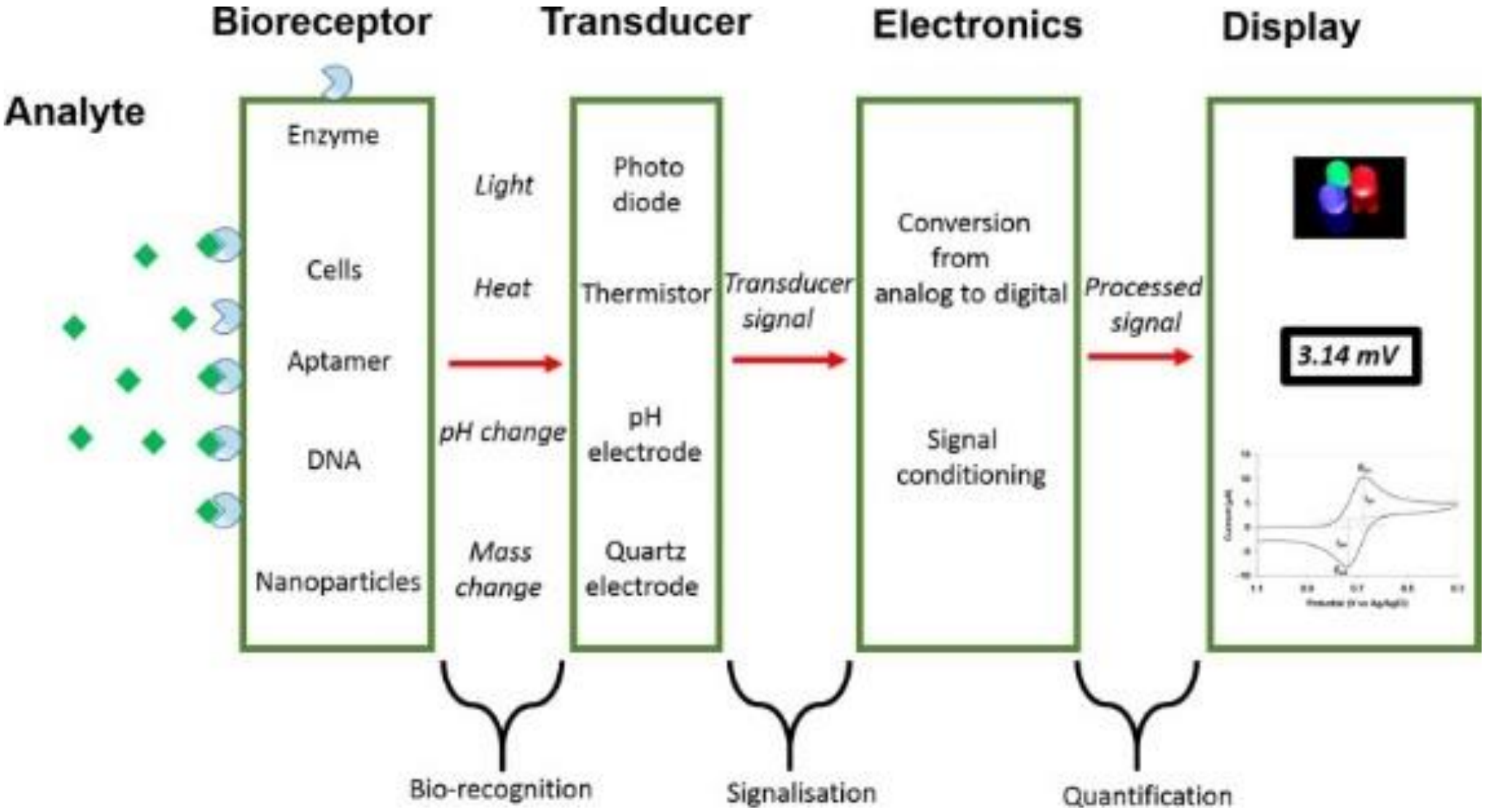
- **Biosensor**
- It is a device that measures biological or chemical reactions by generating signals proportional to the concentration of an analyte in the reaction.

- A typical biosensor has following components:

1. Analyte
2. Bioreceptor
3. Transducers
4. Electronics
5. Display

- These components work in a sequential pattern.

- **Biosensor sequence of components**



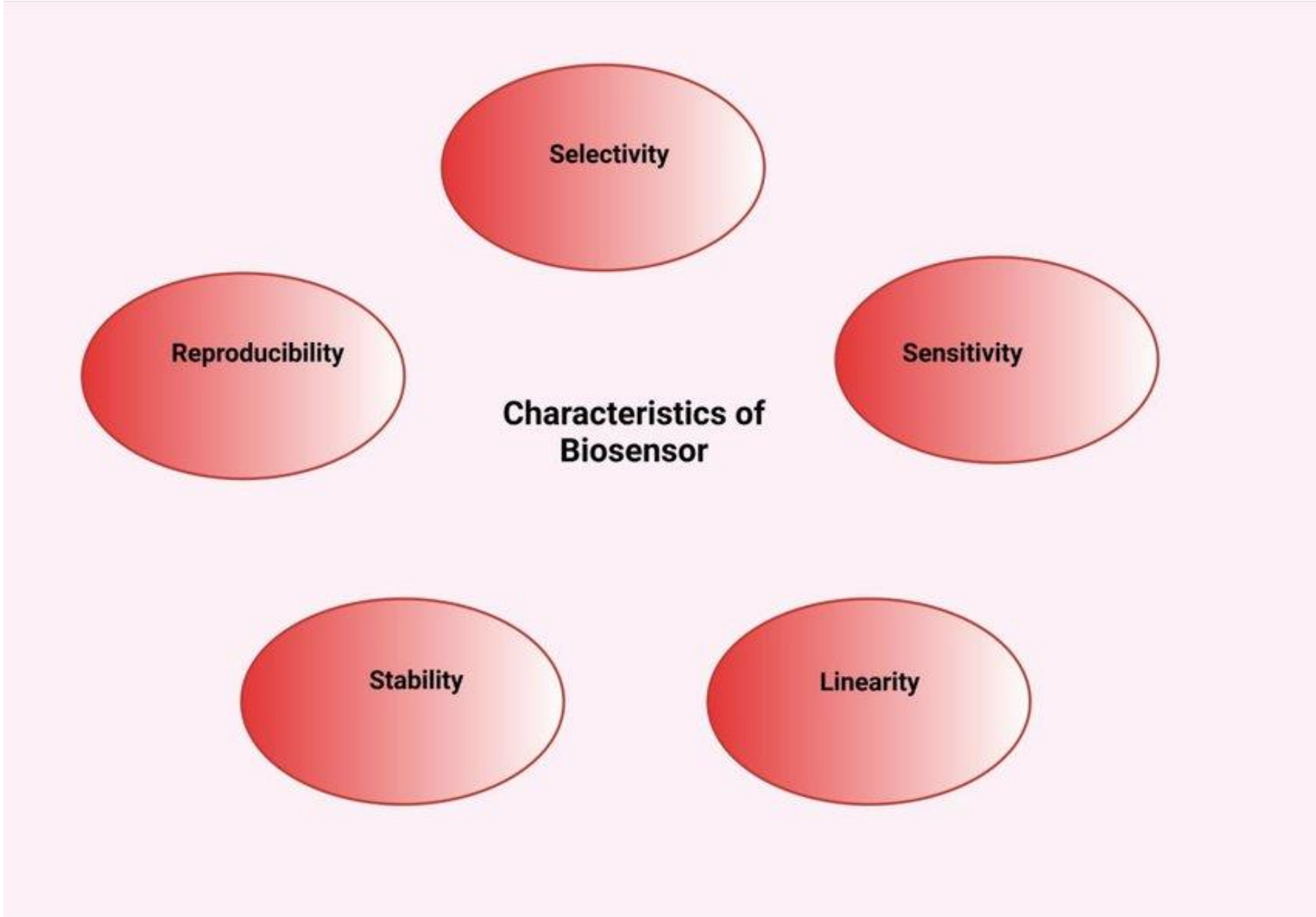
- **Components of Biosensor:**
- **Analyte:** Molecule of interest which needs detection.

- **Bioreceptor:** A molecule which will specifically recognize the analyte.
- E.g. Enzymes, cells, aptamers, DNA, antibodies.
- When bioreceptor interacts with analyte, it generates a signal. The signal could be light, heat, pH, charge or mass change etc.
- This process of signal generation is called as bio-recognition.

- **Transducers:** It converts one form of energy into another. In biosensors, its role is to convert the bio-recognition event into a measurable signal. This process of energy conversion is called as signalisation.
- The signals produced are generally optical or electrical and usually proportional to amount of analyte-bioreceptor interactions.

- **Electronics:** It is complex electronic circuit & prepares the signal for display by various processes such as amplification and conversion of analog into digital signal.

- **Display:** It shows output signal in a user friendly form and format.


Selectivity
Reproducibility
Sensitivity
Characteristics of Biosensor
Stability
Linearity

- **Characteristics of Biosensors:**

1. **Selectivity:** It is the ability of a bioreceptor to detect a specific analyte in a sample containing other admixtures & contaminants.
2. **Reproducibility:** It is the ability of the biosensor to generate identical responses for a duplicated experimental set-up. It depends on precision of transducer & electronics used in the biosensor.

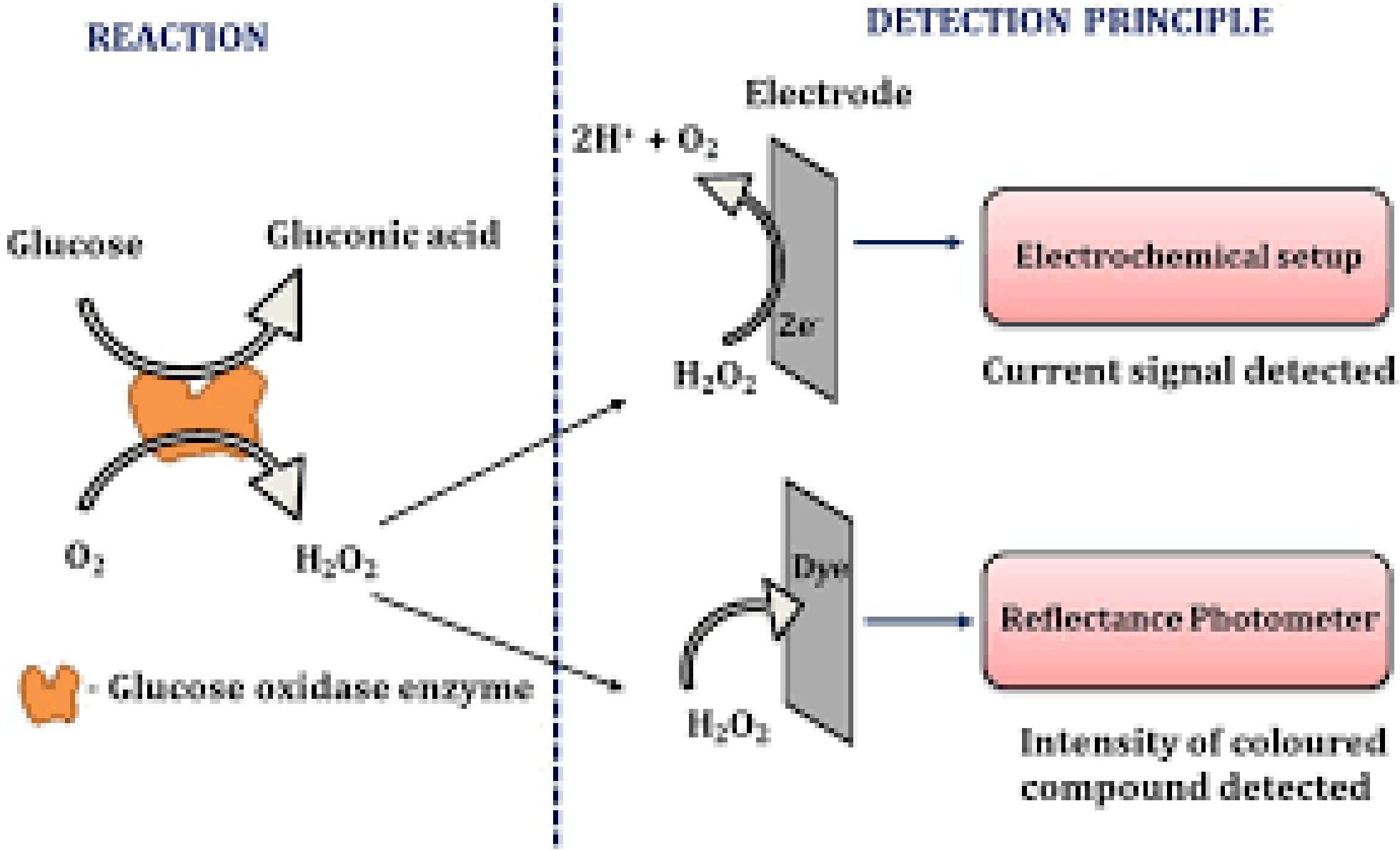
3. **Stability:** It is the degree of susceptibility to disturbances in and around biosensor. If biosensors are susceptible to disturbances then it could lead to errors in measurements with direct effects on accuracy of the biosensor.
4. **Sensitivity:** It refers to the minimum amount of analyte that can be detected by a biosensor. Lesser the amount of analyte that can be detected, better is the limit of detection or sensitivity.

5. **Linearity:** It is the attribute which shows the accuracy of the measured responses. These responses are measured with different concentrations of analyte.

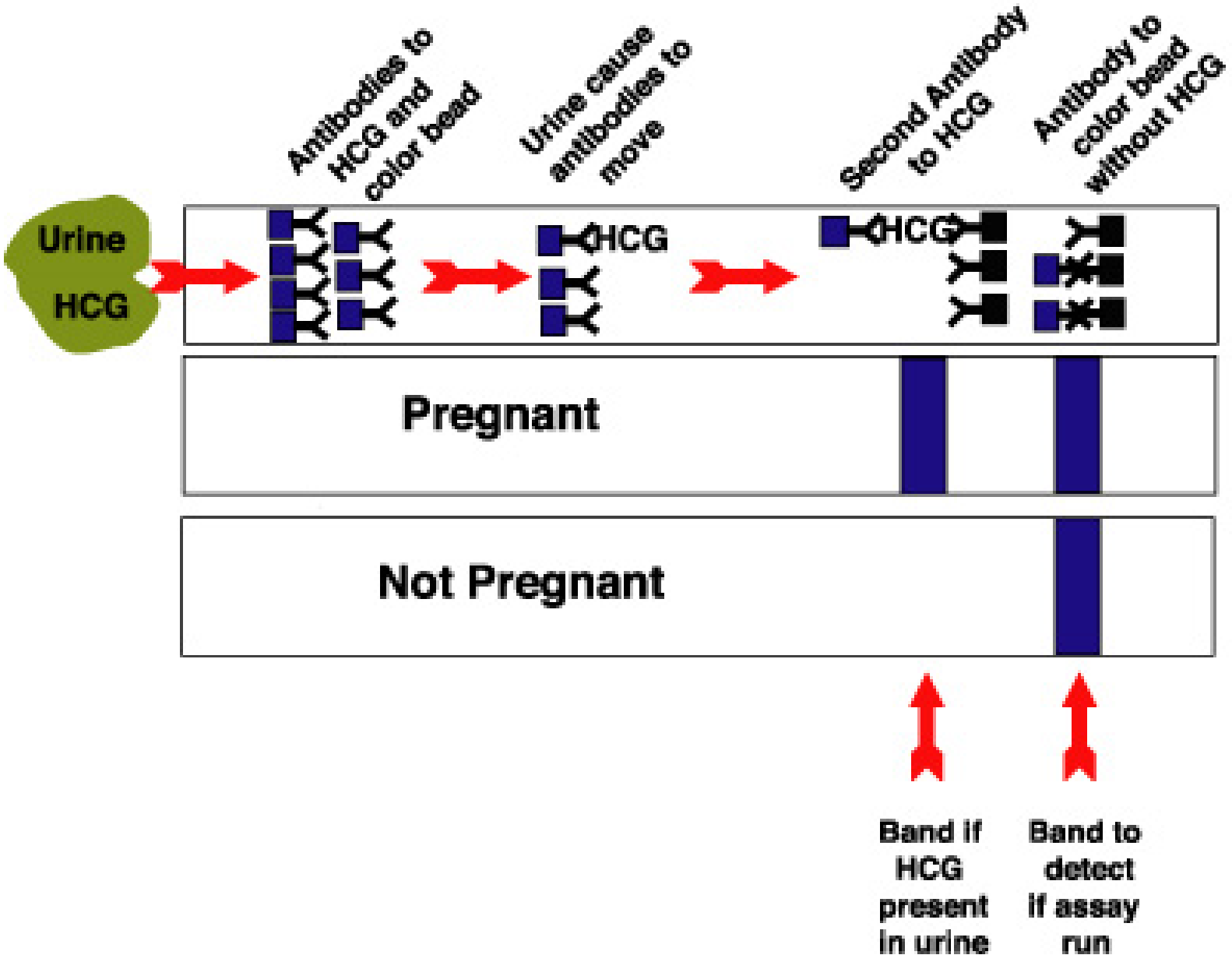
   Linearity of the biosensor is associated with resolution of the biosensor and its range of analyte.

Applications of Biosensors:

- **Glucometer as biosensor**



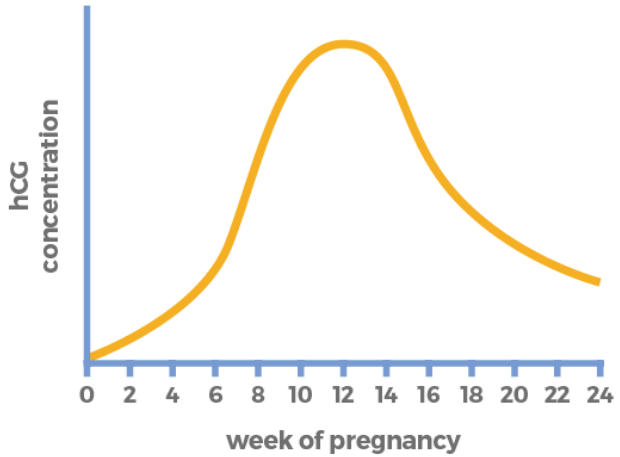
- **Lateral flow assay for pregnancy detection is an example of biosensor:**



HOW DO PREGNANCY TESTS WORK?

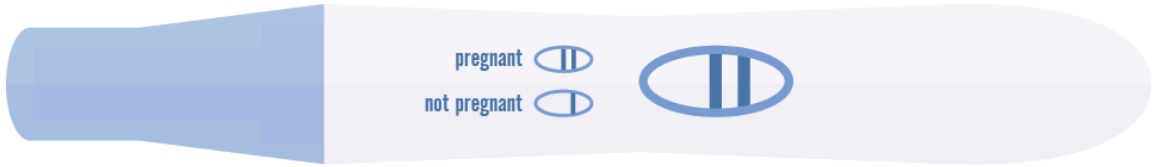
WHAT DO PREGNANCY TESTS DETECT?

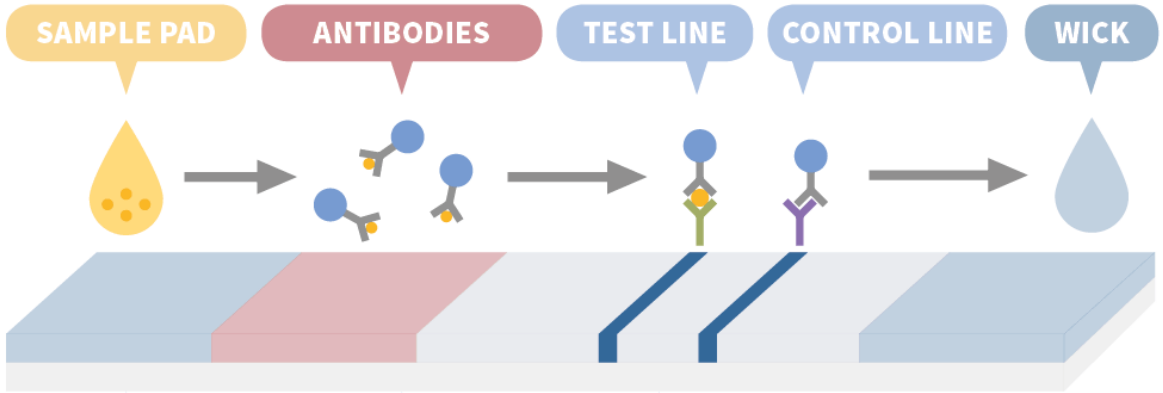
Pregnancy tests detect a hormone called human chorionic gonadotropin (hCG). This hormone is produced by the placenta from the time at which the embryo attaches to the uterus.



hCG is essential for the function of the corpus luteum, a temporary structure in the ovaries that produces the hormones progesterone and estrogen. It has also been linked to early pregnancy symptoms such as nausea and vomiting. hCG is eliminated in urine and can be detected by pregnancy tests around 9 days after fertilisation.

HOW DO PREGNANCY TESTS WORK?





Urine applied to the sample pad. If a woman is pregnant, urine contains hCG.

hCG binds to mobile antibodies. These antibodies also have an enzyme attached to them.

Immobilised antibodies in the test zone bind to hCG. The enzyme on the first antibody changes the test line colour. Excess antibodies bind to immobilised antibodies in the control zone to show the test worked correctly.


Thanks!