Proteomics Course

LECTURE-27
Microarray workflow:
Label-based detection techniques

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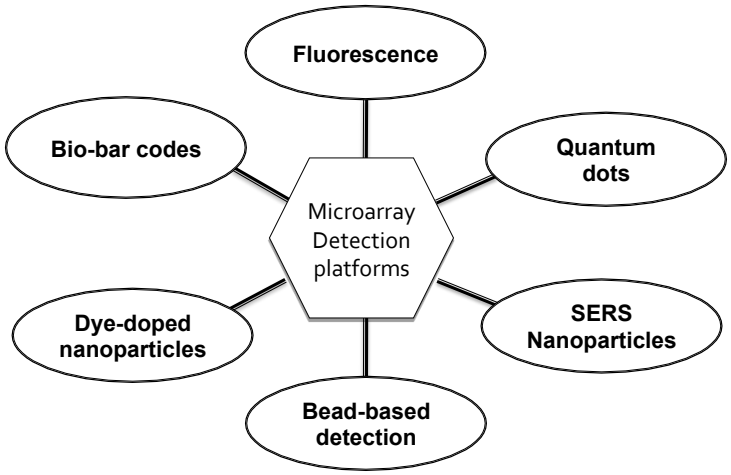
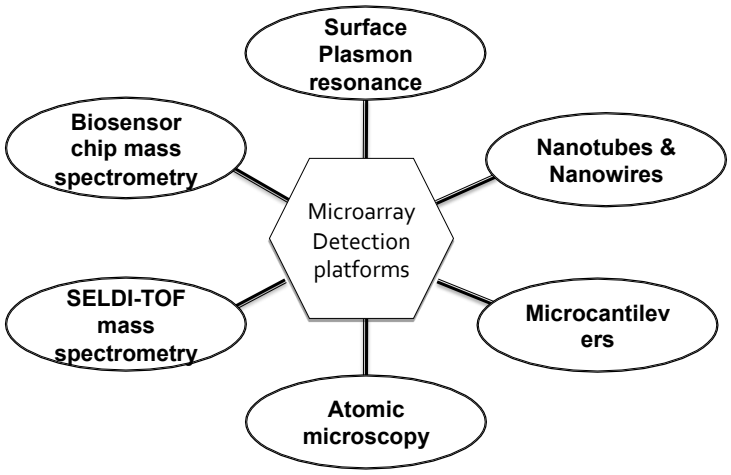
Lecture outline

- Different detection platforms
- Label-based detection
  - Conventionally used detection methods
    - Chromogenic, Chemilumniscence, Radioactivity, Fluorescence
  - New advanced detection methods
    - Quantum dots, SERS Nanoparticles, Dye-doped nanoparticles, Bio-bar codes

Label-based platforms for microarrays



Label-free platforms

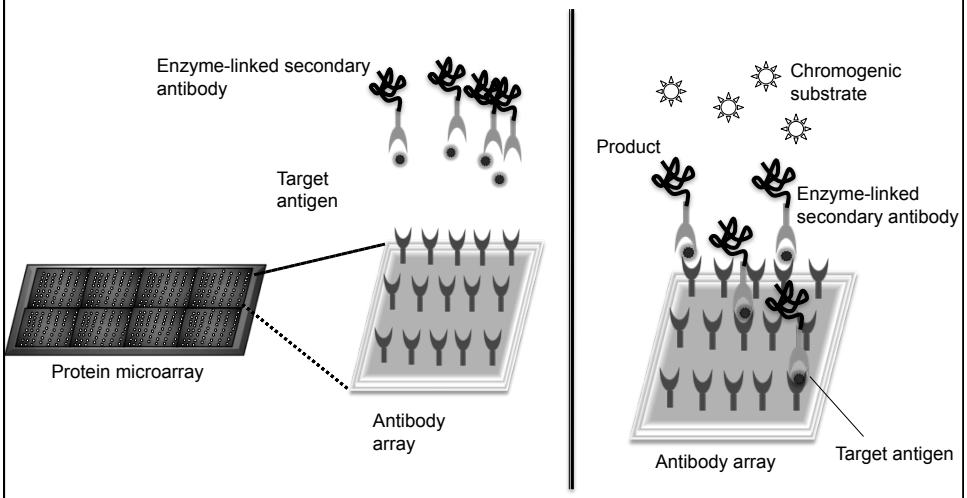


Conventional detection methods

- Chromogenic
- Chemilumniscence
- Radioactivity
- Fluorescence

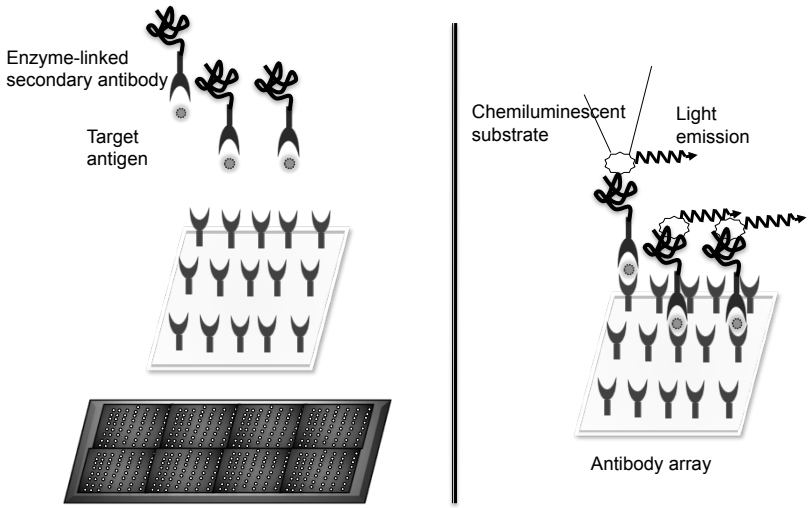
Chromogenic detection

Chromogenic detection



Chemiluminescence detection

Chemiluminescence detection



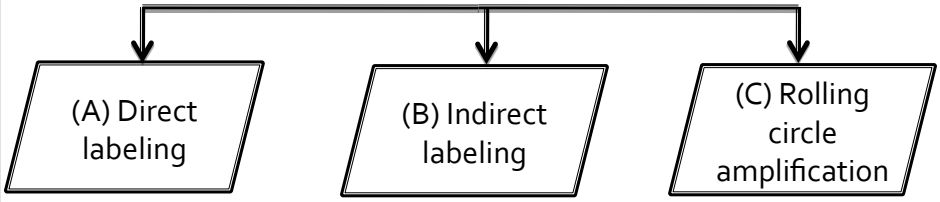
Radioactivity labeling

Radioactivity labeling

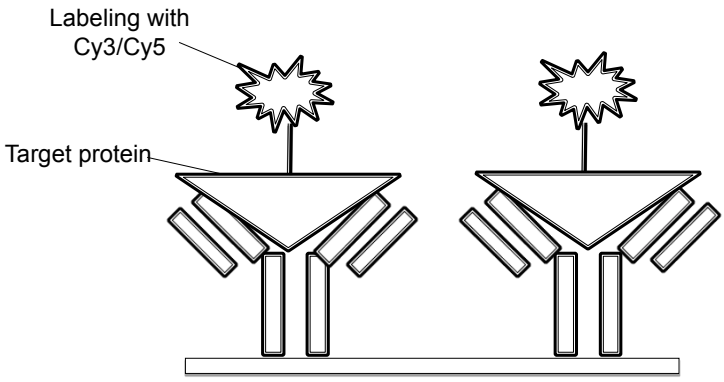
- Radiolabeling is commonly accomplished by incorporating ^{3}H, ^{14}C, ^{35}S, ^{32}P, ^{125}I into proteins
- Signal detection by
  - Direct autoradiography – γ - emitting isotopes
  - Fluorography – β - emitting isotopes
- Radiolabels are used to assess protein synthesis rate, kinase substrate identification etc.
- Radiolabeling is hazardous and expensive

Fluorescence labeling

Fluorescence labeling



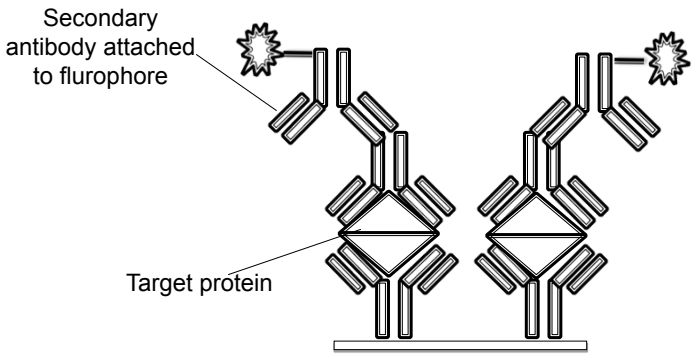
(A) Direct labeling



Direct labeling

- Merits
  - Only single antibody required
  - High reproducibility
  - Highly sensitive for abundant proteins
  - Multiple sample assay
- Demerits
  - Less sensitivity for low abundance proteins
  - Chemically modified sample
  - Cross reactivity

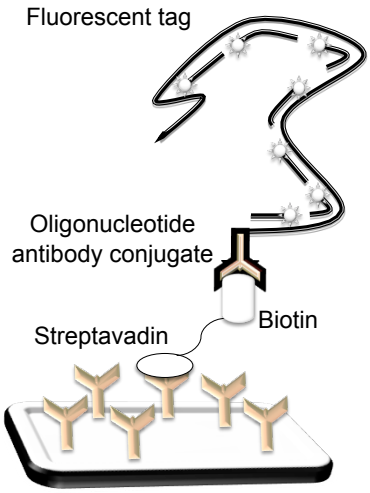
(B) Indirect labeling



Indirect labeling

- Merits
  - Higher specificity
  - Very sensitive
- Demerits
  - Cross reactivity
  - Multiplexed analysis not possible
  - High cost

(C) Rolling Circle Amplification



Rolling Circle Amplification

- Merits
  - High sensitivity
  - Reproducibility
  - Broad dynamic range
  - Multi-color detection
  - Detection of low-abundance proteins
- Demerits
  - Critical validation procedures
  - Higher variations due to different incubation times
  - Decrease in robustness

New advanced detection methods

- SERS Nanoparticles
- Quantum dots
- Dye-doped Nanoparticles
- Bio-bar codes

Surface enhanced Raman scattering based methods

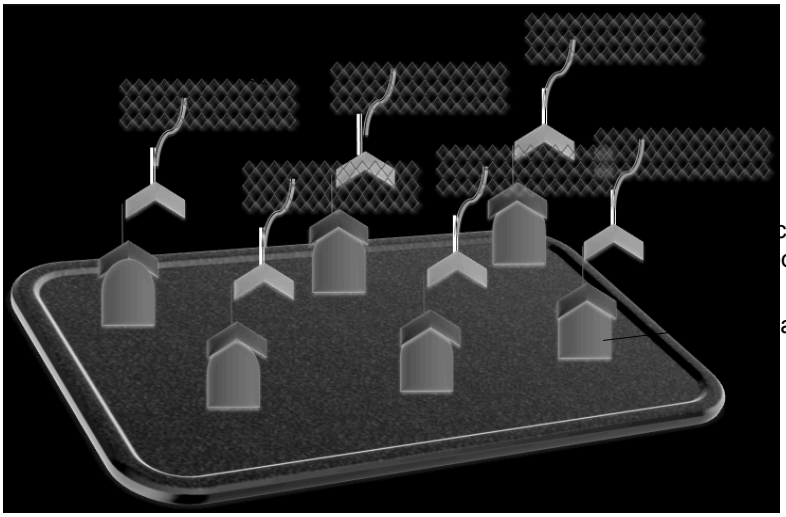
Raman dye labeled nanoparticles

- Raman dye labeling involves coating of antibody along with Raman dye directly on a gold nanoparticle probe surface
- The visualization is carried out by staining with silver enhancement solution and hydroquinone
- Spots can be detected by Raman spectrometry coupled with fiber optic microscopy

Raman dye labeled nanoparticles

- Merits
  - Higher sensitivity
  - Flexibility
  - Sharp scattering peaks
  - Cost effective
- Demerits
  - Complexity in synthesis of NPs
  - Lack of uniformity

Single walled nanotubes (SWNTs)

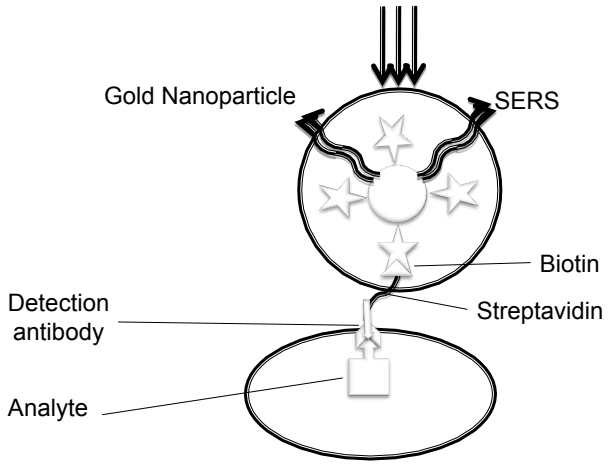


SWNT

- Merits
  - High sensitivity
  - Multiplexed detection
  - Minimum background signal
  - Resistance to photobleaching
- Demerits
  - Metal impurities interfere with activity
  - Insoluble in biological buffers
  - Difficult to determine degree of purity

Gold nanoparticles (GNPs)

Gold nanoparticles (GNPs)

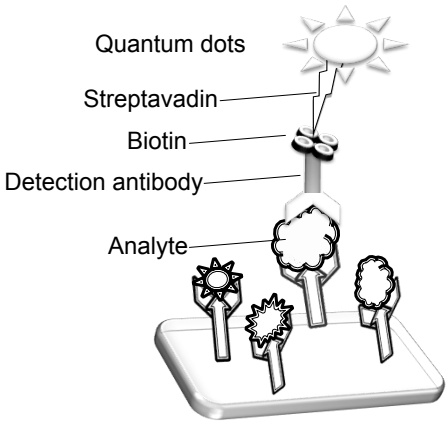


Gold nanoparticles

- Merits
  - Improved optical property
  - Superior quantum efficiency
  - Compatible with wide range of wavelengths
  - Resistance to photo bleaching
- Demerits
  - High cost
  - Cytotoxicity
  - Non-uniform size and shape of NPs

Quantum dots

Quantum dots



Antibody microarray

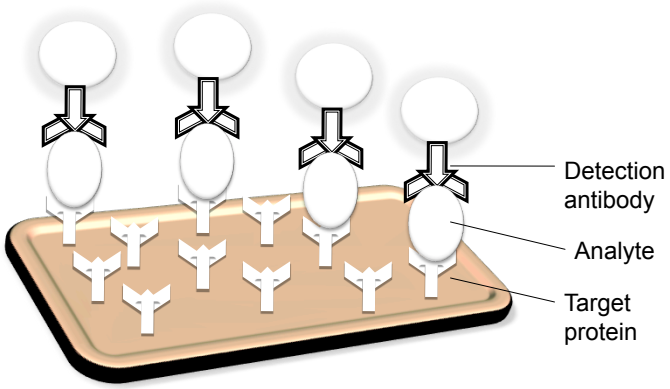
Quantum dots

- Merits
  - Brighter fluorescence
  - Excellent photostability
  - Multicolor fluorescent excitation
  - Greater quantum yield
- Demerits
  - Toxicity
  - Unknown mechanism

Dye-doped silica NPs

Dye-doped silica NPs

Dye-doped RuDs Nano particle

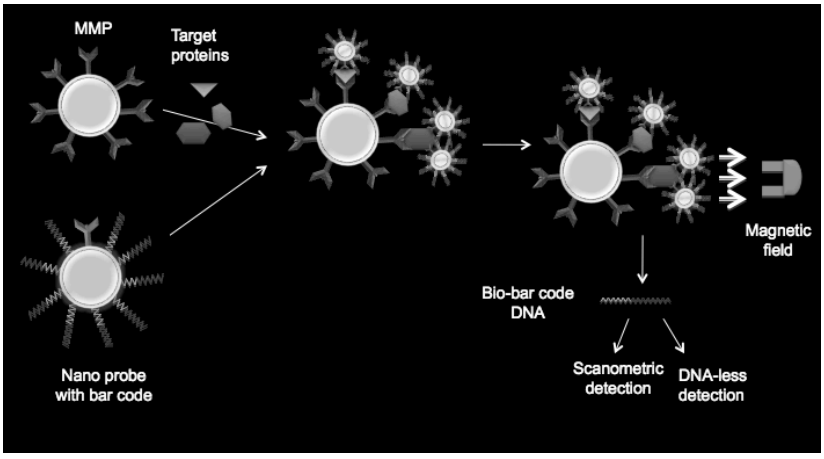


Dye-doped silica NPs

- Merits
  - Biocompatible
  - High sensitivity
  - Minimal aggregation & dye leakage
  - Photostability
  - High capacity
- Demerits
  - Complex synthesis process

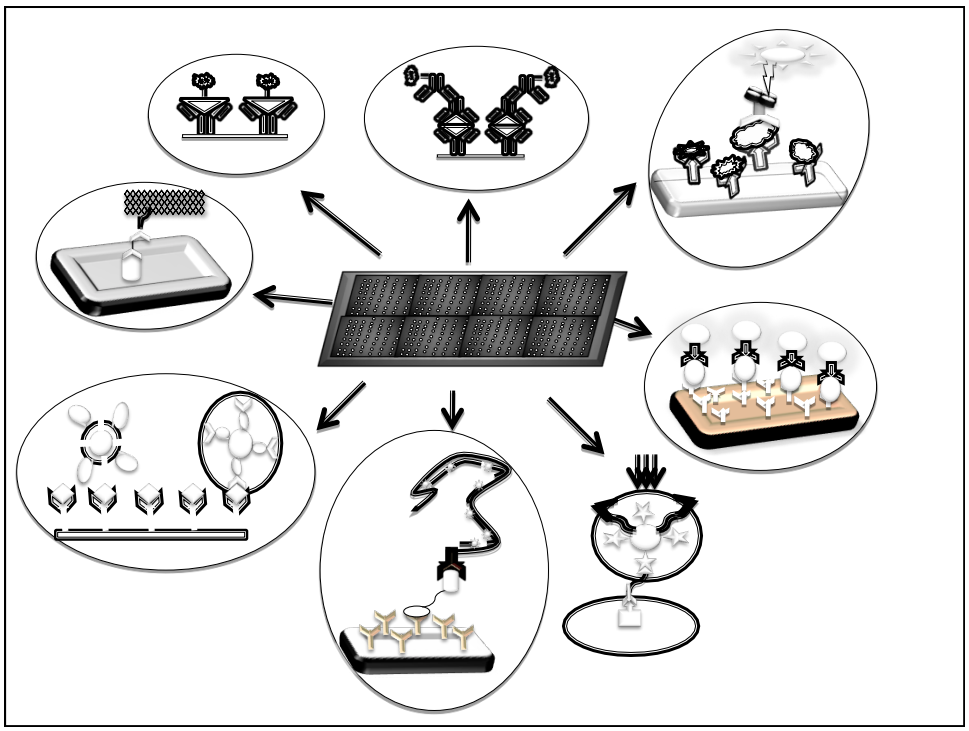
Nanoparticle based bio-barcodes

Nanoparticle based bio-barcodes



Nanoparticle based bio-barcodes

- Merits
  - High sensitivity
  - Less detection time
  - Easy adaptability to multiple targets
- Demerits
  - Can be used only with known antibodies



Summary

- For successful proteomics study, emerging protein microarray platforms should be coupled with sensitive and robust detection systems
- Label-based systems use a tag for query molecule
  - Conventionally, fluorescent dyes, radioisotopes
  - Recently, quantum dots, gold nanoparticles, Raman dye labeled carbon nanotubes, silica nanoparticles

References

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