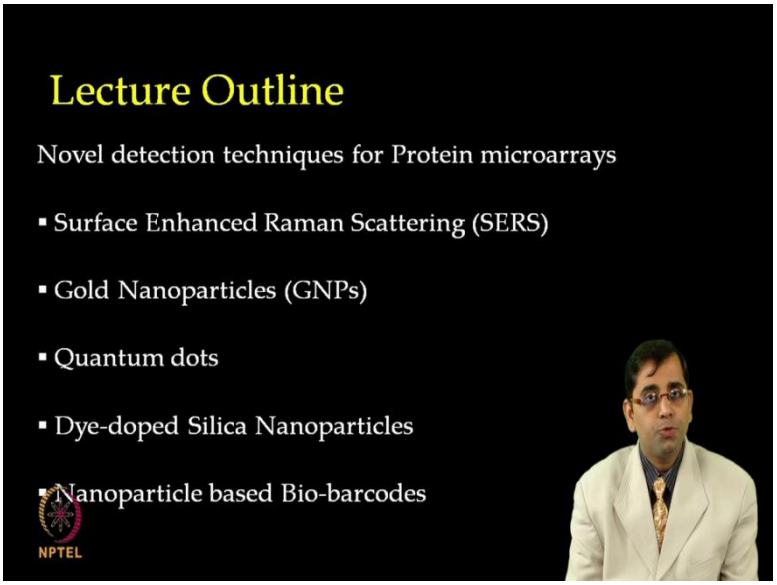
Interactomics Protein Arrays and Label-Free Biosensors
Professor Sanjeeva Srivastava
Department of Biosciences and Bioengineering
Indian Institute of Technology Bombay
Module 05
Lecture 24
Novel Detection techniques for Proein Microarrays

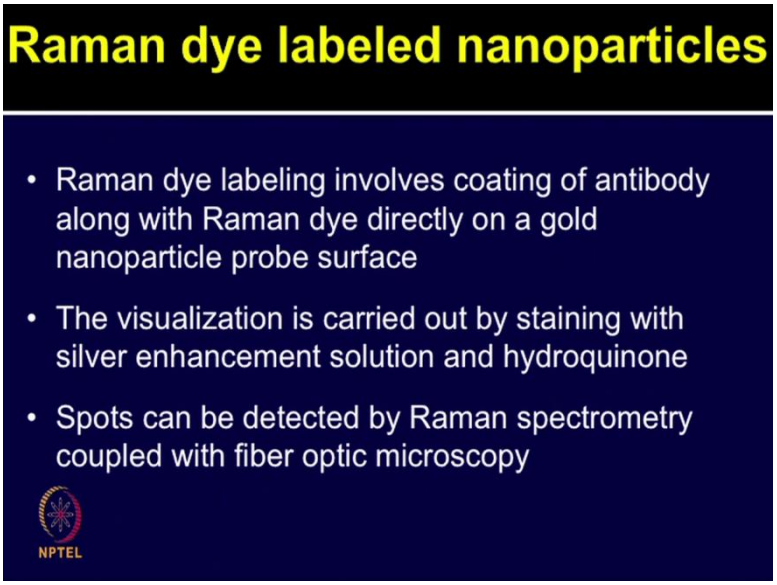
(Refer Slide Time: 0:39)



Welcome to the MOOC course on interactomics. In last lectures, we have discussed various conventional detection methods employed for protein microarrays. In today's lecture, we will discuss about various advanced detection methods. We have to talk about few methods in more detail such as SERS based Nanoparticles, quantum dots, dye-doped Nanoparticles and bio-barcodes.

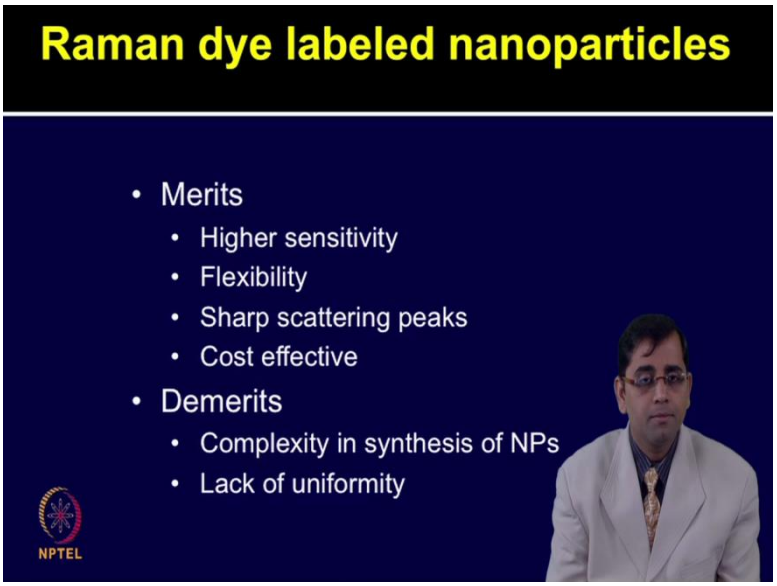
We will first start with surface enhanced Raman scattering or SERS based methods. Light which is incident on an atom or molecule, it is scattered back the same energy and wavelength. This is a general phenomenon; however Raman Effect prevails in a small fraction of photons, where energy of scattered photo is different from the incident photons. Therefore, the improved optical properties are obtained because of enhanced electromagnetic field at the surface of the particle which is detected by using spectroscopic methods such as SERS. The surface enhancing agents include gold and silver as well as functionalization with target molecules, which enhances the sensitivity of Raman spectroscopy. Let us now look at how this system has been employed for the detection methods for microarrays.

(Refer Slide Time: 2:16)



The Raman dye labeling involves coating of antibodies on the array surface like gold by using the Raman dye directly on the gold surface which are in nanoparticles probes. The visualization can be carried out by staining with silver enhancement solution as well as hydroquinom. Spot can be detected by Raman spectrometry coupled with fiber optic microscopy.

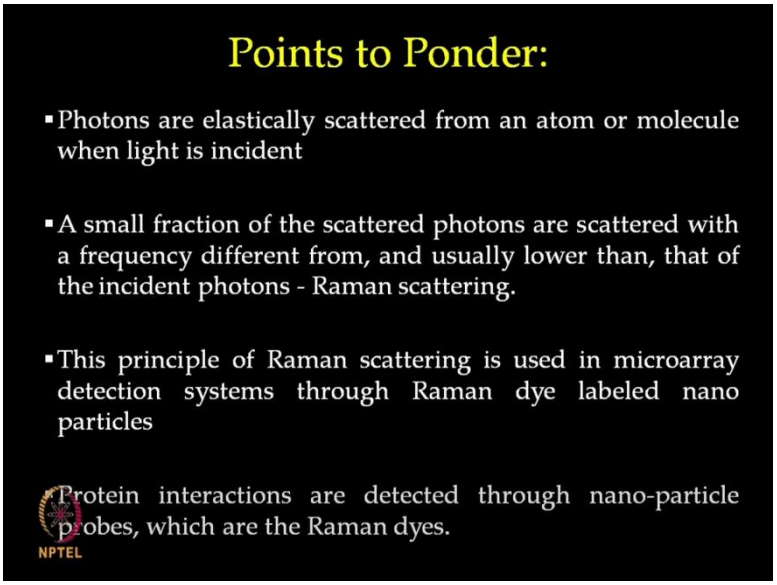
(Refer Slide Time: 3:03)

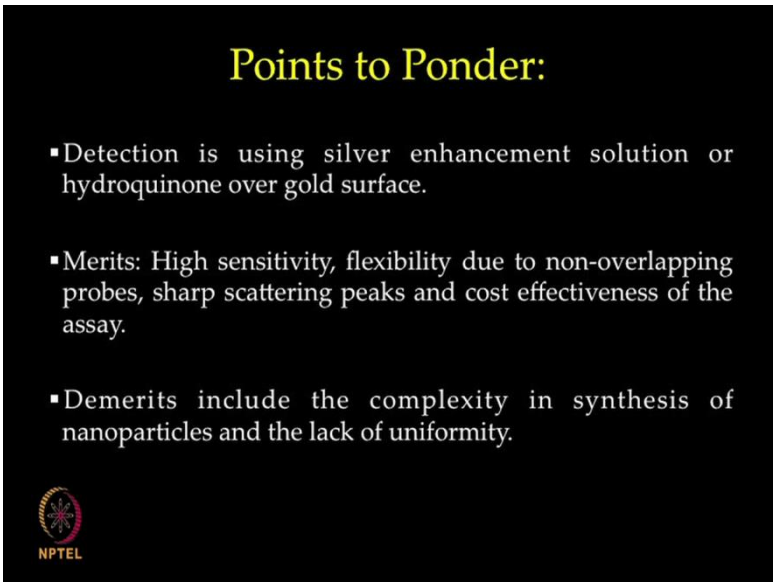


As compared to the fluorescence based detection methods the Raman dye labeled, gold Nano probes offers several advantages which include high sensitivity, flexibility due to non-overlapping probes, sharp scattering peaks and cost effectiveness of the assays. However, the

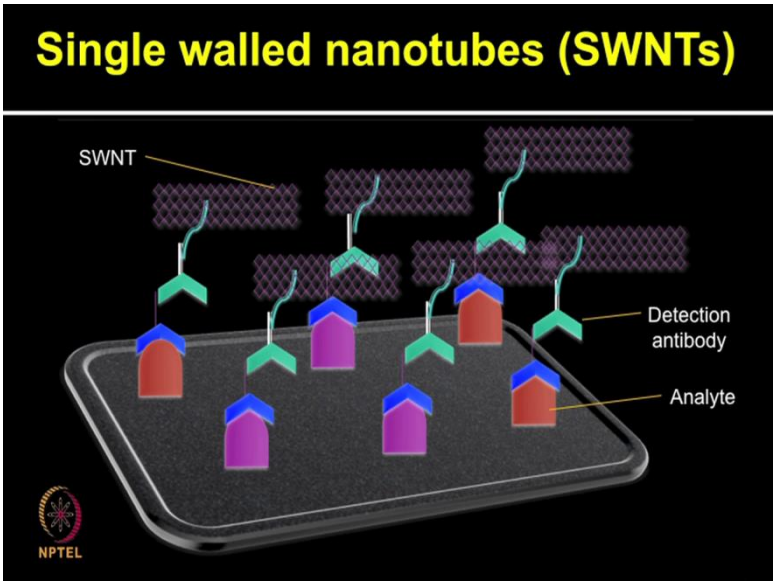
certain demerits of using Raman dye labeled nanoparticles, these drawbacks include the complexity in synthesis of nanoparticles and the lack of uniformity.

(Refer Slide Time: 3:46)



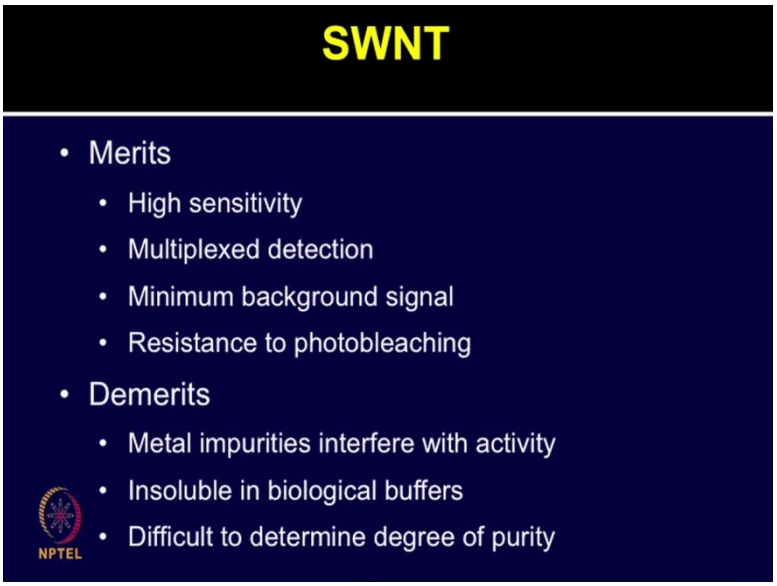


(Refer Slide Time: 4:36)



The macromolecular single walled Nano tubes SWNTs functionalized with a specific Raman dye neighbor antibodies are used for multiplexed detection target proteins bound on the microarrays slide, which is gold coated. As you can see this slide SWNT functionalized with Raman dye labeled antibodies are used for multiplexed detection of target proteins which are bound on the gold coated microarray surface.

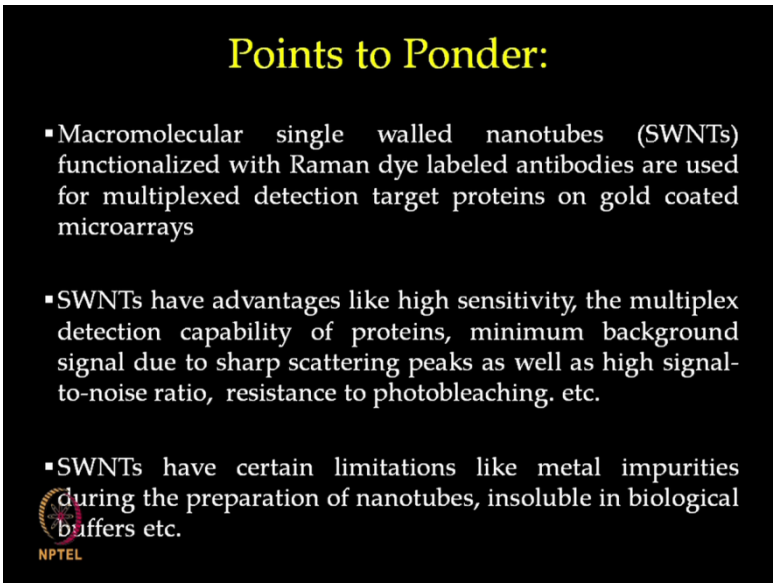
(Refer Slide Time: 4:53)



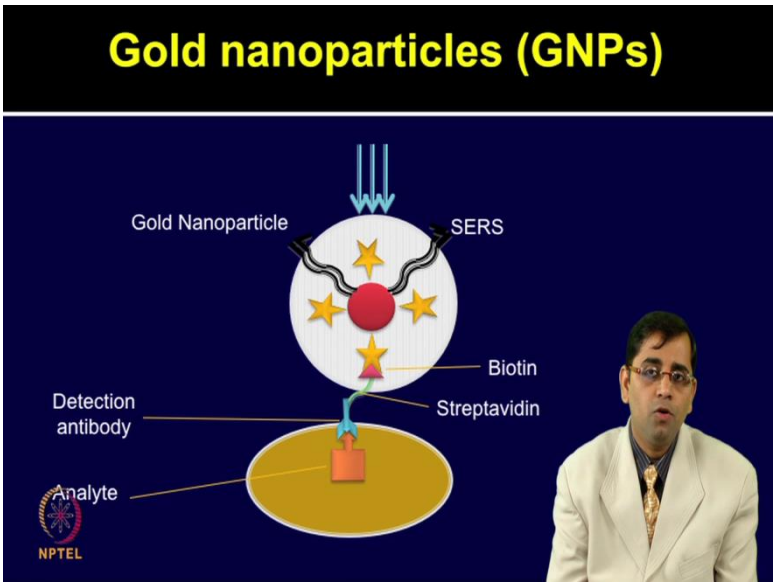
The SWNTs offer several advantages such as high sensitivity, the multiplex detection capability of proteins, minimum background signal due to sharp scattering peaks and high signal to noise ratio, you also offer resistance to photobleaching therefore, SWNTs have several advantages. However, they also possess some limitations such as metal impurities

during preparation of these nanotubes that can be interfered with the activity. They are insoluble in biological buffers and there is also difficulty in determining how pure your preparation is? The degree of purity is one of the measure limitations here.

(Refer Slide Time: 5:58)



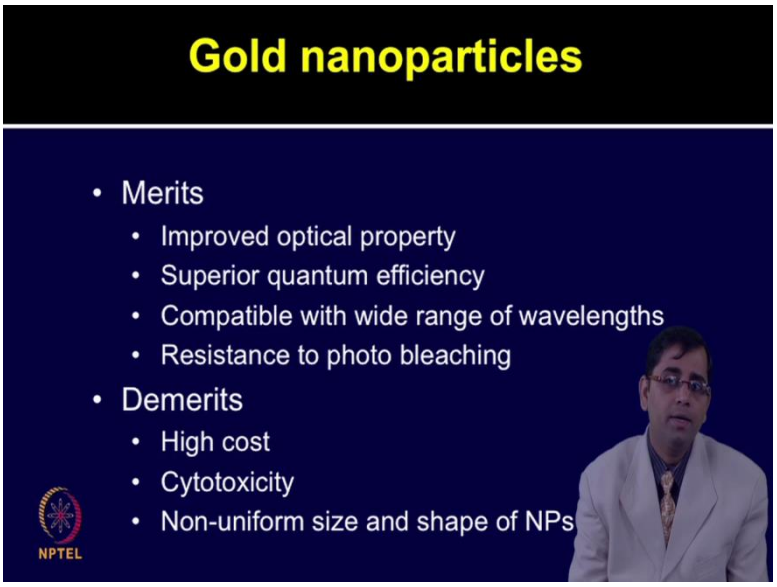
(Refer Slide Time: 7:00)



Let us discuss, gold Nanoparticles GNPs. The excitation of coherent electron oscillations that exist on interface of 2 materials is known as surface plasmon resonance, which forms the basis for the use of gold nanoparticles as detection system. The proportion of light absorption to a scattering depends on the size of the nanoparticle. Enlarge nanoparticles can be used for biological imaging due to the need for high scattering cross section. The GNP labeled with a suitable capture molecules exhibit change in the emission spectrum of scattered light upon

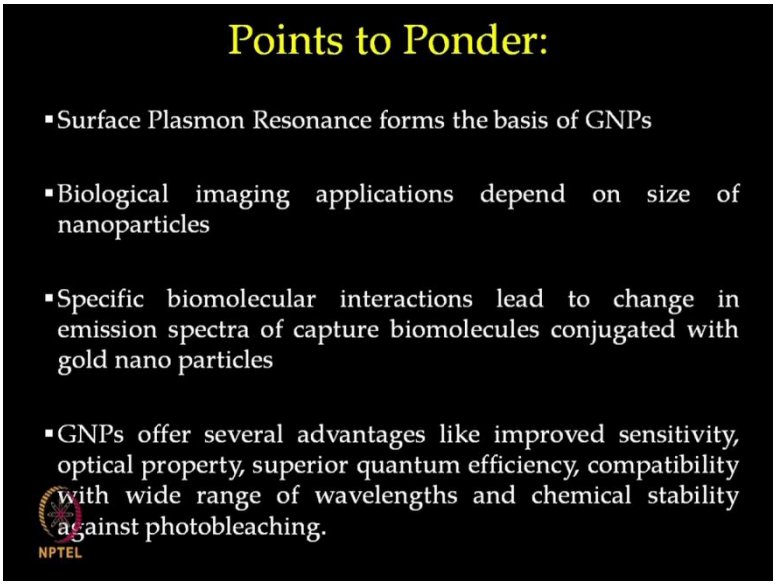
binding to the analyte of interest from a protein mixture due to specific bimolecular interactions. As we can see here in this slide, the change in the emission spectrum of scattered light directed upon binding of gold nanoparticle which is conjugated with antibody to the analyte of interest.

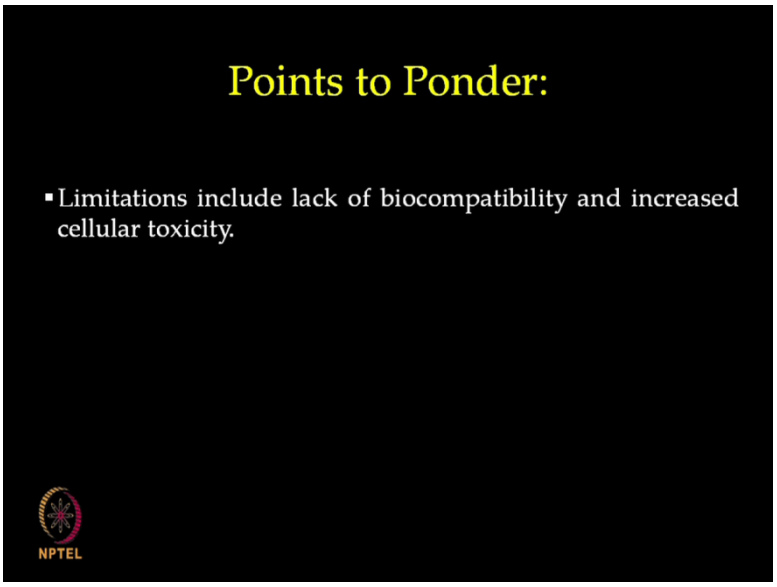
(Refer Slide Time: 7:34)



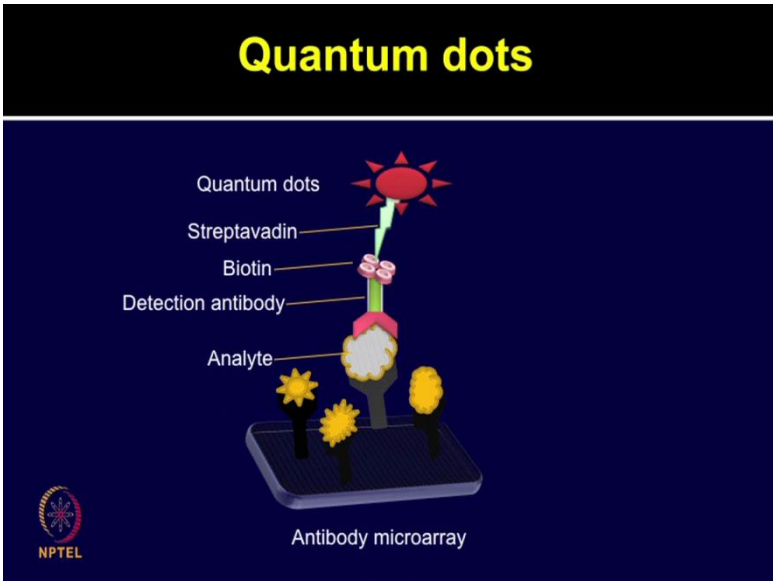
The gold nanoparticles offer several advantages, it has been used for several applications for sensitive detection of standard proteins, they provide improved optical property, superior quantum efficiency, so compatibility with wide range of wavelengths and chemical stability against photobleaching. However, there are certain limitations of using GNPs, which are similar to other Nano techniques, such as biocompatibility and no cellular toxicity. The systematic cytotoxicity study should be performed if you want use these GNPs for different protein microarray based applications. The high cost, cytotoxicity and non-uniform size and shape of the nanoparticles are some of the limitations of using GNPs as sensitive detection platform for microarray experiments.

(Refer Slide Time: 9:01)



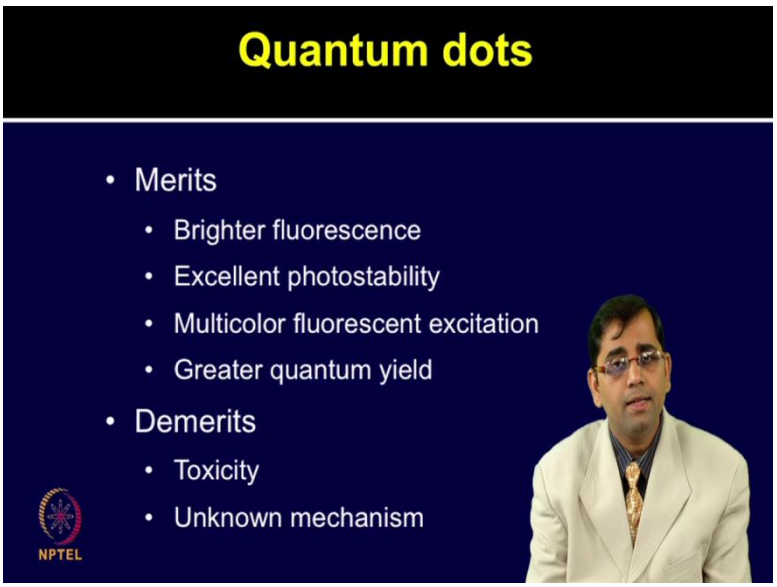


(Refer Slide Time: 9:45)



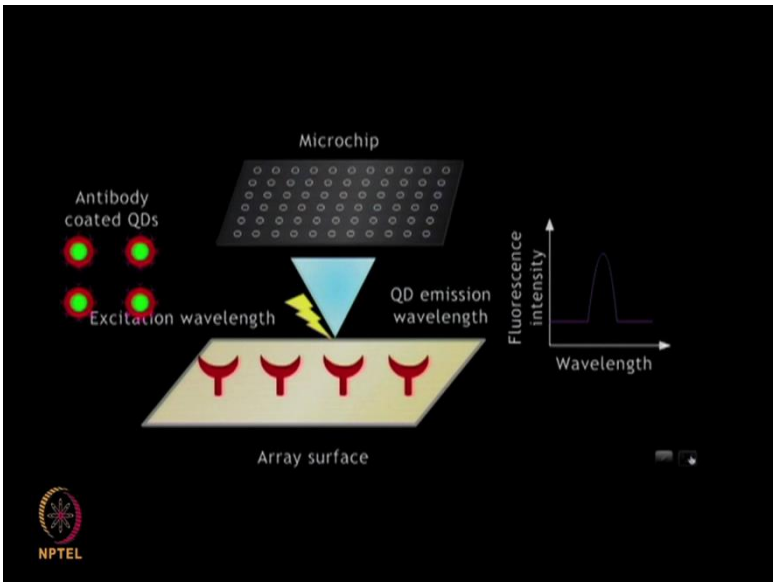
Let us now discuss Quantum dots, Quantum dots are nanometer size crystals composed of semiconductor, fluorescence core coated with another semiconductor shell having large spectral band gap, which is the stable light scattering or emitting properties. In quantum dots, the formation of excitons takes place when light of higher energy in that of the band gap of composite semiconductor is incident on the quantum dots. When these excitons return to their energy level, in fact low energy level, then emission of narrow symmetric energy takes place as we can see in the slide, the change in the optical properties because of the formation of a excitons upon binding of quantum dot conjugated antibody to the target analyte can be used as a method for detection of microarray based method.

(Refer Slide Time: 10:37)



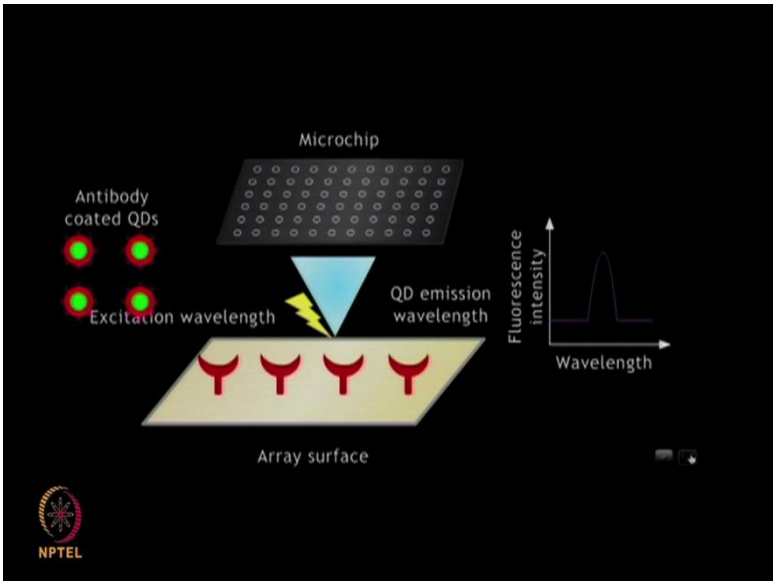
Key advantages of quantum dots compare to the organic dyes include its brighter fluorescence, excellent photostability, multicolour fluorescent excitation and higher quantum yield. Despite of several benefits and applications in a variety of biological sample and demerits includes toxicity. Therefore, quantum dots have shown various applications but the still they have certain limitations. We will talk some of these applications during the course of interactomics molecules.

(Refer Slide Time: 11:47)



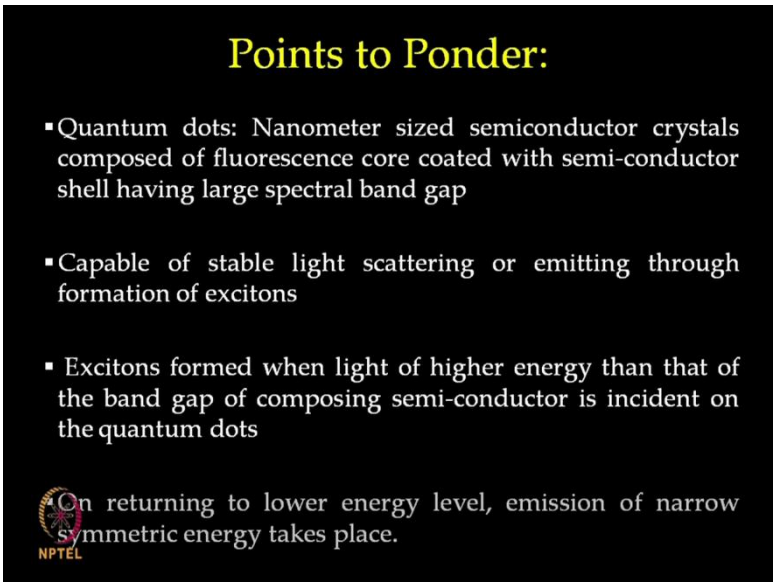
Changes in the emission wavelength upon binding to the antibody conjugated quantum dot are recorded by the microchip and used for detection of various biomolecules. Quantum dots are capable of detecting molecules down to femtomolar levels and provide significant advantages over conventionally used organic fluorophores. In this interaction, we will see how quantum dots work? The inorganic fluorophores known as quantum dots have been developed that can conjugate with several biomolecules and they use for protein microarrays signal detection. They are made up of semiconductor devices which form excitons up on absorption of light. There is emission of a narrow energy band, when these excitons are return to their lower energy level. Let us click on these quantum dots to view how they work?

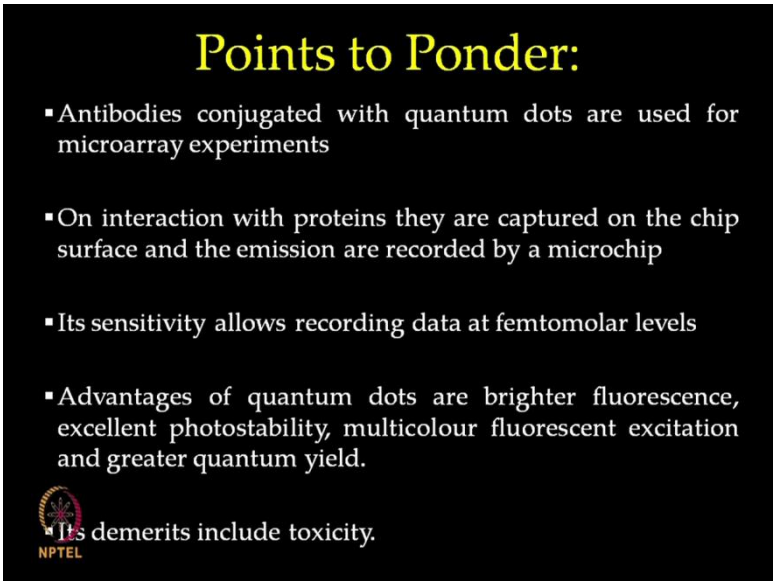
(Refer Slide Time: 13:10)



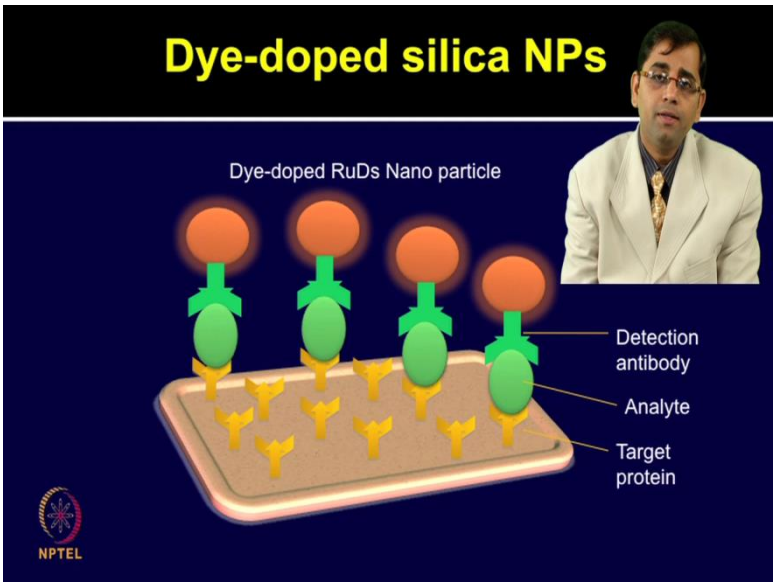
So, as you can see upon binding of the target protein to the antibody. Now, these changes are plotted on the wavelength verses florescence intensity graph. So these quantum dots can detect molecules with very high sensitivity in femtomolar range.

(Refer Slide Time: 13:52)



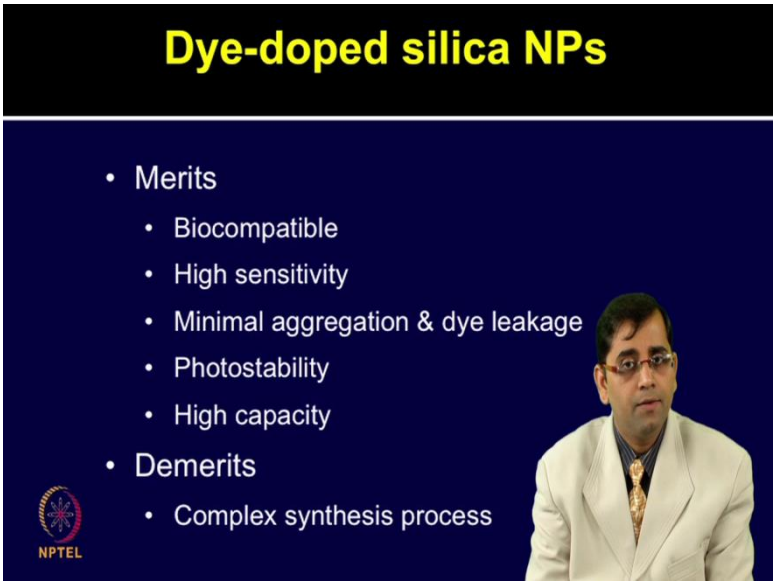


(Refer Slide Time: 15:00)



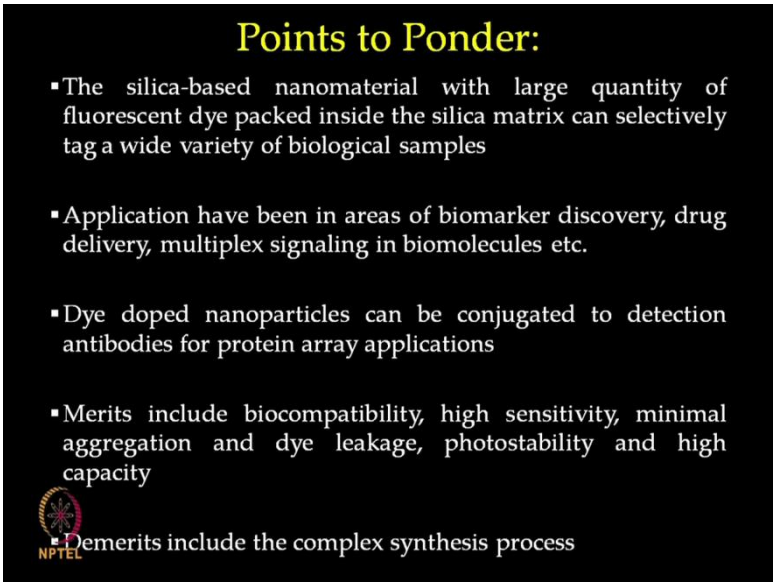
Now, let discuss about Dye-doped silica nanoparticles. The silica based nanomaterial have large quantity of fluorescent dye packed inside the silica matrix which possess ability to selectively tag a wide variety of biological important targets such as cancer cells, bacteria and many other biological samples as shown in this in this slide here, the silica based nanoparticles have large quantity of fluorescent dye packed inside the matrix and it can be used for selectively labeling of protein molecules for detection of bimolecular interactions.

(Refer Slide Time: 15:21)

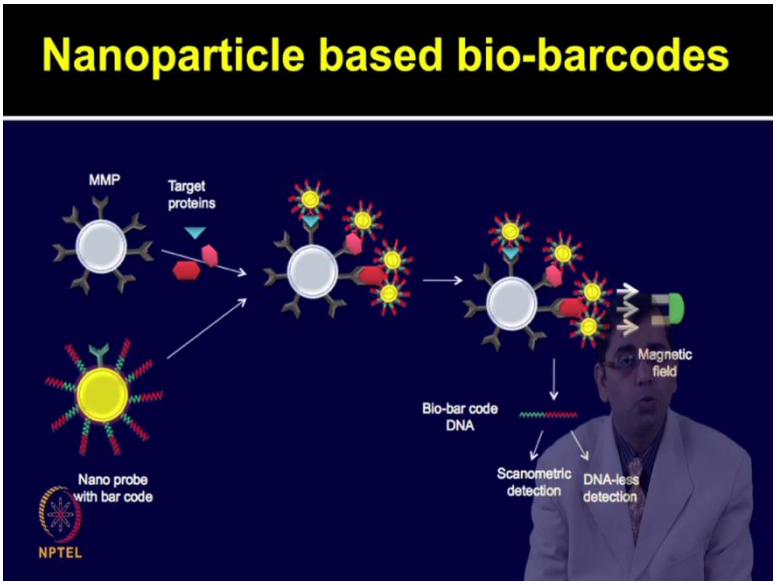


Dye-doped silica nanoparticles applications have been used for a variety of investigations. Application of various functionalized silica nanoparticles have been demonstrated in diversified fields (())(15:31) such as bimolecular discovery, drug delivery, multiplex signaling in biomolecules. Its various merits include biocompatibility, high sensitivity, minimal aggregation and dye leakage, photostability and high capacity. The demerits of dye-doped silica nanoparticles include it is complex synthesis process.

(Refer Slide Time: 16:19)

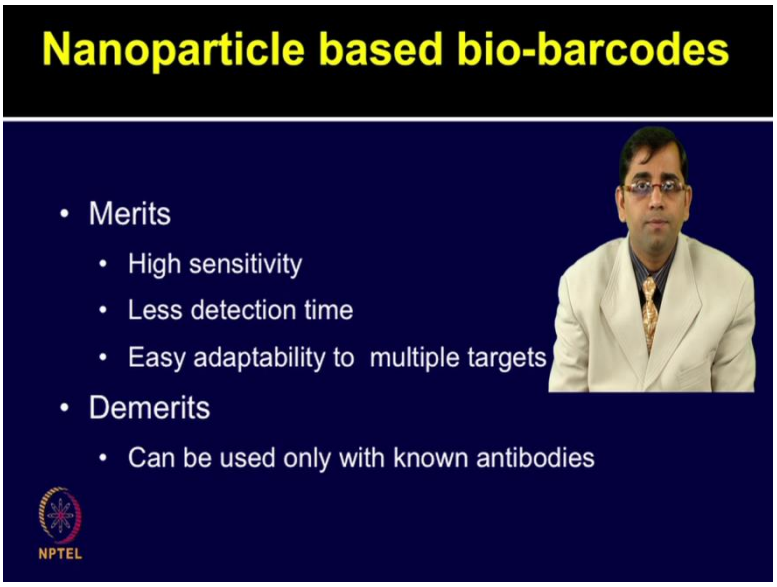


(Refer Slide Time: 16:32)



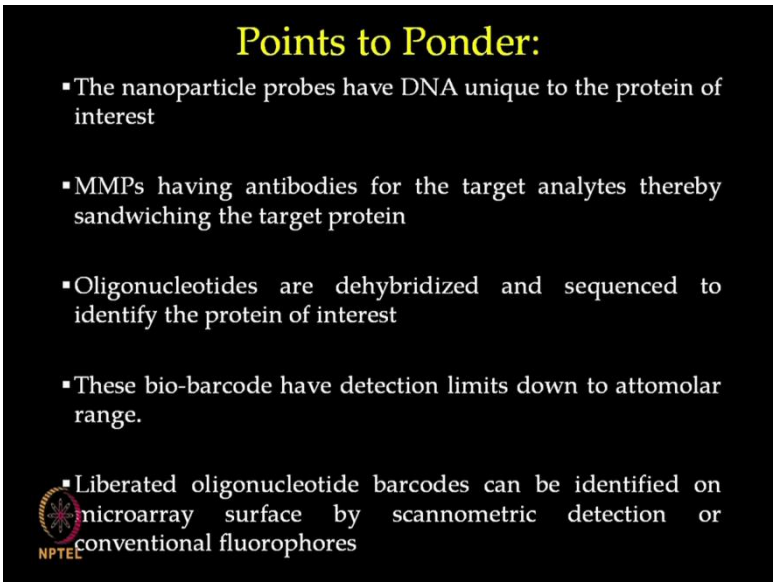
We will now talk about bio-barcodes. The nanoparticle probes encoded with DNA unique to the protein of interest and suitable antibodies capture the magnetic micro particle probes known as MMPs having antibodies for the target analytes thereby sandwiching the target proteins which is shown in this slide. These are magnetically separated oligonucleotides dehybridized and then sequenced to identify the protein of interest. The nanoparticle based bio-barcode have increase the detection limits down to attomolar range. The liberated oligonucleotide barcodes can be identified on microarray surface by scanometric detection as well as within conventional fluorophores.

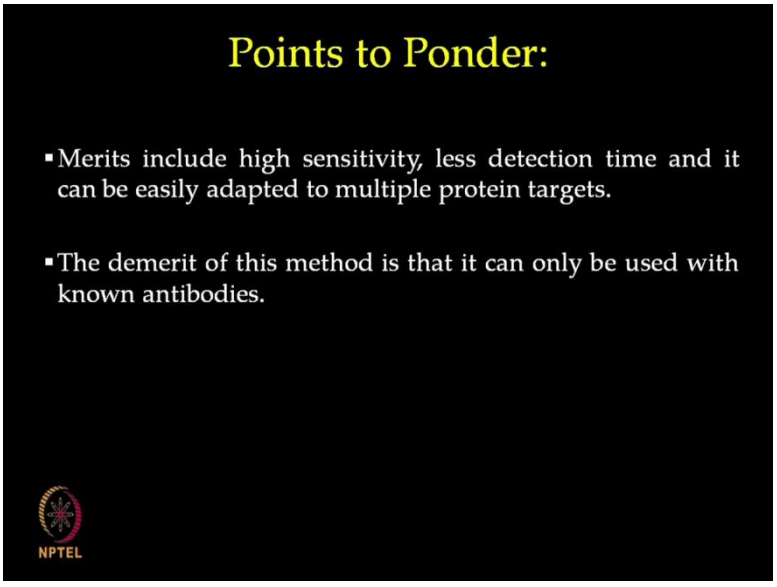
(Refer Slide Time: 17:42)



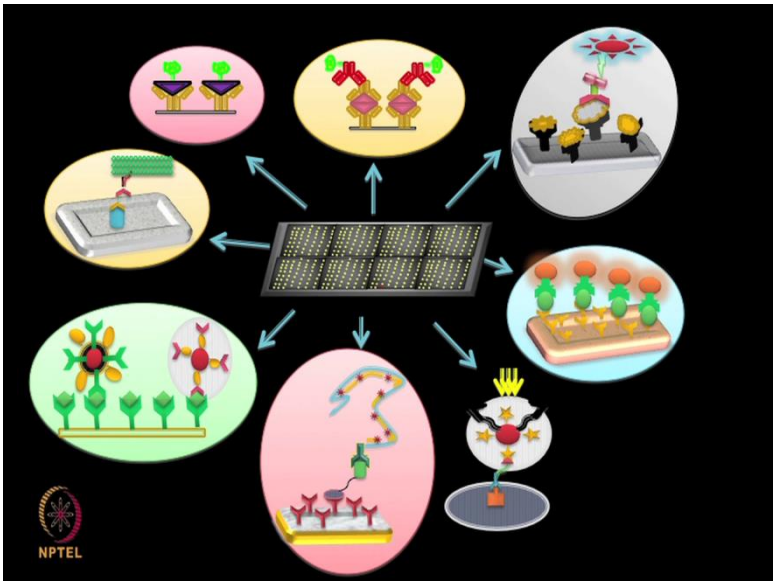
The nanoparticle based bio-barcodes offer various advantages. The merits include high sensitivity, less detection time and it can be easily adapted to multiple protein targets. The demerits of this method is that it can only be used with known antibodies, therefore a number of antibodies as well as good quality of antibodies is one of the limiting factor for performing the nanoparticle based bio-barcode assays. In fact, the same is also true for many applications in proteomics which also required antibodies.

(Refer Slide Time: 18:43)





(Refer Slide Time: 19:02)



To summarize all the various type of detection techniques which we have discussed today. The advancement in the microarray technology has let to the development of sensitive and reliable detection systems. There are different label-based detection techniques which have been employed to study high throughput ways of analyzing thousands of proteins as well as studying their interactions and function by using protein microarray platform. These various novel detection techniques which we have discussed today have facilitated sensitive, specific, high throughput as well as rapid analysis for many proteomics based applications.

The label based detection systems have been taken rapid strides to satisfy the demands of proteomic applications with significant improvement in sensitivity, multiplexing capability and reproducibility. So, we have discussed variety of label based methods although, fluorescence based method is one of the most commonly used method for various protein microarray based application but there is an increasing demand and need to try out new labels, so that one could achieve ideal system which can be applied for microarrays and also provide good detection system with high specificity, sensitivity and large dynamic range.

So, we have until now discussed various traditional and novel detection techniques used in protein microarrays. In next lecture, we would talk about recombination cloning and it is application in protein microarrays, Thank you.

(Refer Slide Time: 21:18)

Summary

- Various novel detection techniques which we have discussed today have facilitated sensitive, specific, high throughput as well as rapid analysis for many proteomics based applications.
- The label-based detection systems have taken rapid strides to satisfy the demands of proteomic applications with significant improvement in sensitivity, multiplexing capability and reproducibility
- Recently the nano-based system such as quantum dots, GNPs, Raman dye based systems, SERS-based dyes, silica nanoparticles have emerged successfully and are examples of the progress in various detection systems in microarray platform

NPTEL

References

- Chandra, H., and Srivastava, S. (2010). Cell-free synthesis-based protein microarrays and their applications. Proteomics 10, 717–730.
- Chandra, H., Reddy, P.J., and Srivastava, S. (2011). Protein microarrays and novel detection platforms. Expert Rev. Proteomics 8, 61–79.
- Gupta, S., Manubhai, K.P., Kulkarni, V., and Srivastava, S. (2016). An overview of innovations and industrial solutions in Protein Microarray Technology. PROTEOMICS 16, 1297–1308.
- Hu, S., Xie, Z., Qian, J., Blackshaw, S., and Zhu, H. (2011). Functional protein microarray technology. Wiley Interdiscip. Rev. Syst. Biol. Med. 3, 255–268.
- MacBeath, G. (2002). Protein microarrays and proteomics. Nat. Genet. 32, 526–532.

NPTEL

References

- Mitchell, P. (2002). A perspective on protein microarrays. Nat. Biotechnol. 20, 225–229.
- Ramachandran, N., Larson, D.N., Stark, P.R.H., Hainsworth, E., and LaBaer, J. (2005). Emerging tools for real-time label-free detection of interactions on functional protein microarrays. FEBS J. 272, 5412–5425.
- Ray, S., Mehta, G., and Srivastava, S. (2010). Label-free detection techniques for protein microarrays: Prospects, merits and challenges. PROTEOMICS 10, 731–748.
- Syed, P., Gupta, S., Choudhary, S., Pandala, N.G., Atak, A., Richharia, A., Kp, M., Zhu, H., Epari, S., Noronha, S.B., et al. (2015). Autoantibody Profiling of Glioma Serum Samples to Identify Biomarkers Using Human Proteome Arrays. Sci. Rep. 5, 13895.

NPTEL