Interactomics Protein Arrays and Label-Free Biosensors
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Module 04
Lecture 21
Nanotechniques in Proteomics-2

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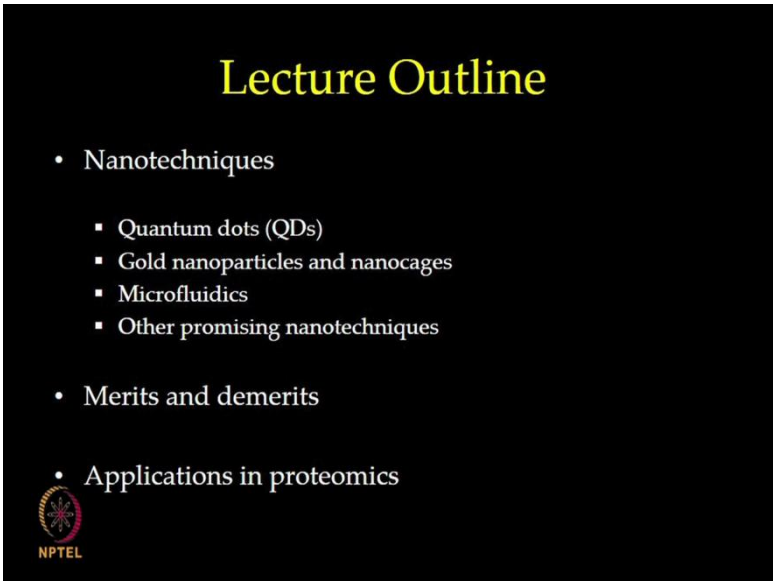

Welcome to NOOC interactomics course. In our last lecture, we talked about carbon Nanotubes, CNTs and nanowires CNWs, Carbon Nanotubes field effect transistors CNT FETs and silicon nanowires field effect transistors. We discussed about their principle, some of the associated advantages and disadvantages and how they can be used for various type of proteomics applications. In today's lecture, we will continue our discussion about Nano-techniques in proteomics; we will discuss few nano-techniques such as quantum dots, gold nanoparticles and nanocages and microfluidics.

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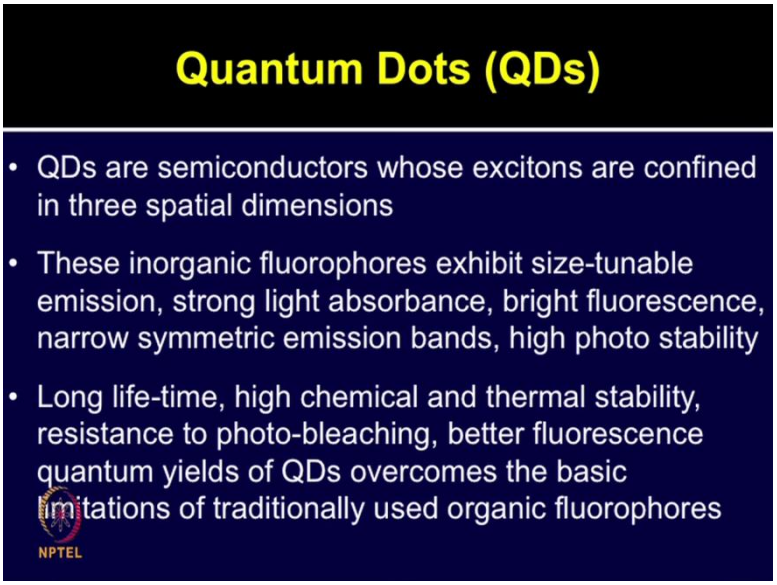

Let us first discuss about Quantum dots or QDs. So, what are these QDs? QDs are Nano crystals or nanoparticles in size range of 1 to 10 nanometer displaying unique photochemical and photo-physical properties. They are semiconductors whose excitons are confined in three spatial dimensions. These are inorganic fluorophores that exhibit size-tunable emission, a strong light absorbance, bright fluorescence, narrow and symmetric emission bands and high photo stability. The long life, high chemical and thermal stability, resistance to photo-bleaching and ability to provide better fluorescence quantum yields of quantum dots have overcome several basic limitations of traditionally used organic fluorophores and therefore, made them the fluorophore of choice to be used in various applications in different biological fields.

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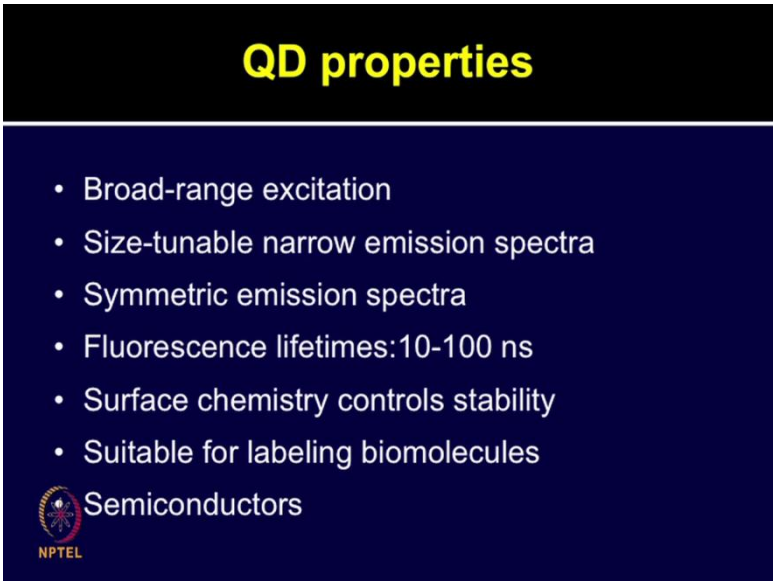

So, what are the different properties of quantum dots? They have broad-range excitation, size-tunable narrow as well as symmetric emission spectra. The fluorescence lifetime ranges between 10 to 100 nanoseconds and the surface chemistry controls their stability. QDs offer great potential in biological studies due to their remarkable photo-stability, which allows long term observation of biomolecules. They are suitable for labeling a variety of bio-molecules and can be applied as semiconductors.

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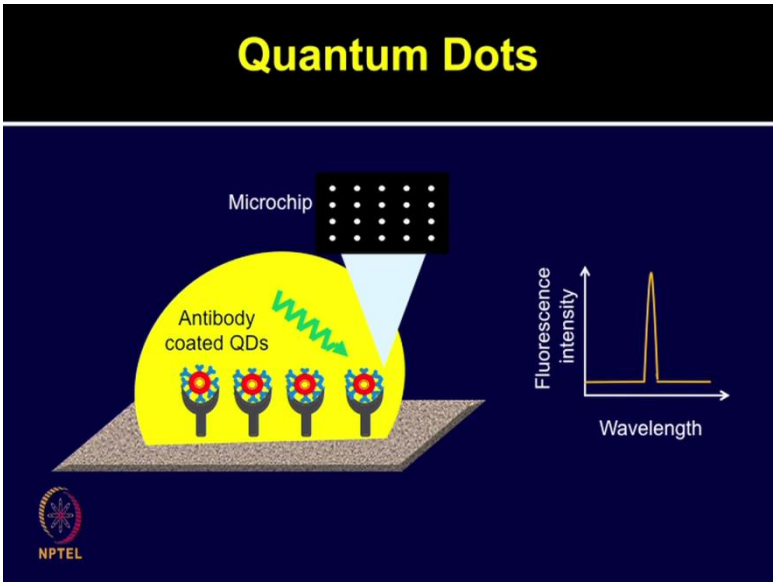

In this slide, the application of semiconductor, quantum dots as labeling agent for sensitive detection of target proteins in an array format is shown. The protein analytes are detected by monitoring the emission spectra of QDs. The excitation of QD with incident light leads to

formation of excitons due to the absorbance of a photon with higher energy than that of a band gap of component semiconductor and this fluorescence intensity is measured as shown on the right hand side of this graph. The smaller is the nano crystal the higher the energy level can be reached. QDs have shown wide variety of promising applications in different areas of biological sciences such as immunoassays, nucleic acid detection, analysis of genetic polymorphism, diagnostic imaging, single molecule detection, cellular localization studies, drug delivery as well as proteomics and genomics studies.

You may realize that it offers a very wide range of applications in different labels of bio-sensors. So, you may realize that it offers a very wide range of applications in different biological applications that superior optical properties and ability to conjugate with several biomolecules like peptides, nucleic acids and antibodies have established quantum dots as a sensitive detection tool in clinical proteomics research.

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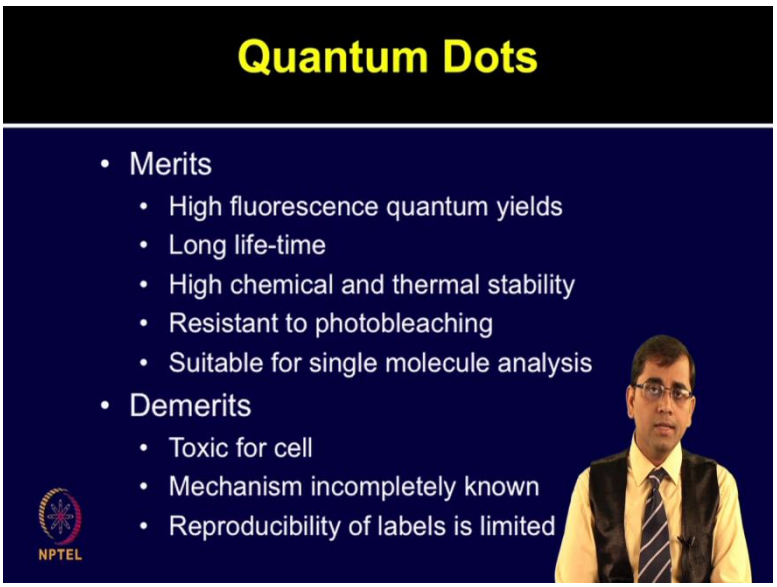

The different merits and demerits of using quantum dots, the merits of QDs include high fluorescence quantum yield, long life-time, high chemical and thermal stability, resistant to photo-bleaching over long period of time and its suitability for even single molecule analysis. The various chemistries available to conjugate biologically active molecules to the surface of nano-crystals. The demerits associated with QDs include toxicity of the cell, incomplete knowledge of the mechanism involved and the limited reproducibility of the labels. The cellular toxicity and biocompatibility are the major limitations of using quantum dots as there is limited knowledge on their (())(6:13) from the living system however, there is an ongoing research to make these quantum dots more bio-compatible by the surface modification

including hydrophobicity and encapsulating them within the phospholipid basis, future research will defiantly make them more widely applicable for different applications.

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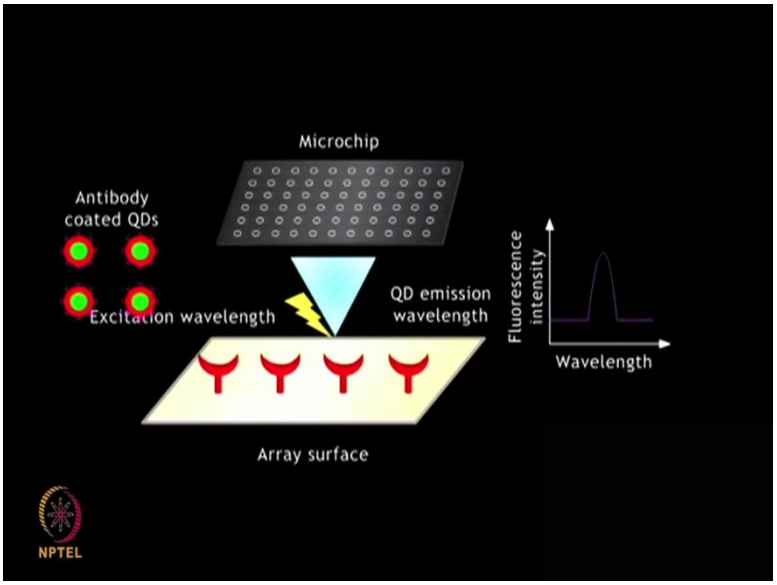

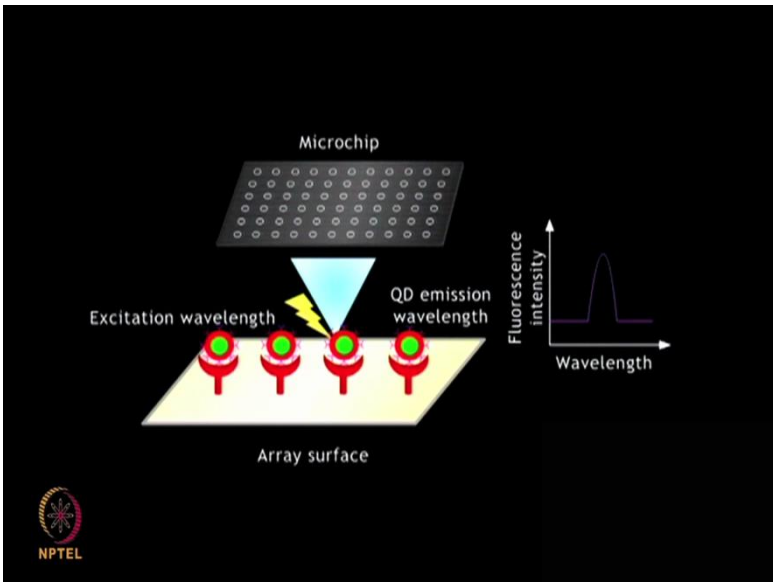

Let us now look at this animation of quantum dots. The changes in the emission wavelength upon target binding antibody conjugated quantum dots are recorded by the microchip and used for detection of various biomolecules. Quantum dots are capable of detecting molecules down to femtomolar levels and provides significant advantages over conventionally used organic fluorophores. As you can see on the right hand side, the wavelength verses fluorescence intensity is plotted and change in the fluorescence intensity is observed when the molecule is binding on the antibody surface which is labeled with the quantum dots.

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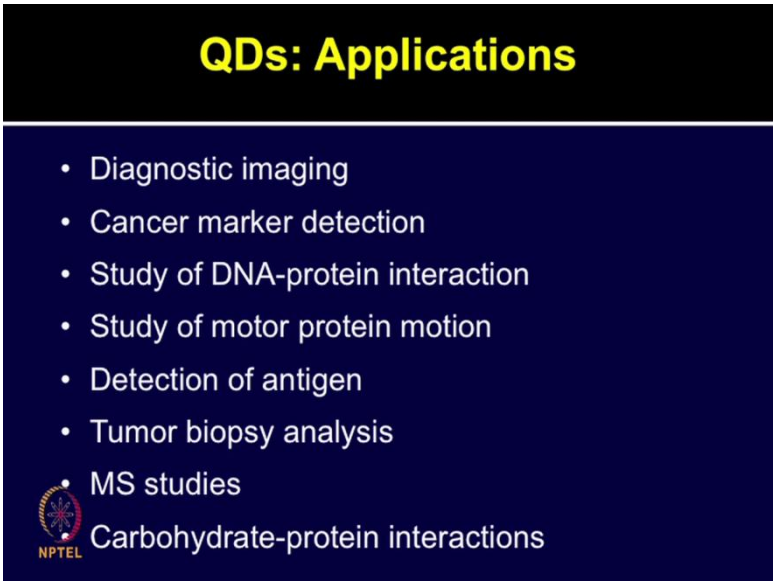

The usuality of quantum dots has been exhibited in variety of biological and clinical applications including immunohistochemical detection, drug delivery and therapeutic, diagnostic imaging, biomarker detection in cancer, a studying DNA protein interactions, tracking extra and intra-cellular targets, detection of antigens and different type of tumor biopsy analysis. The mass spectrometry based studies have also started exploring quantum dots and it has also been used for carbohydrate and protein interactions. These are some of the published report that shows the potential of using quantum dots for various proteomic applications.

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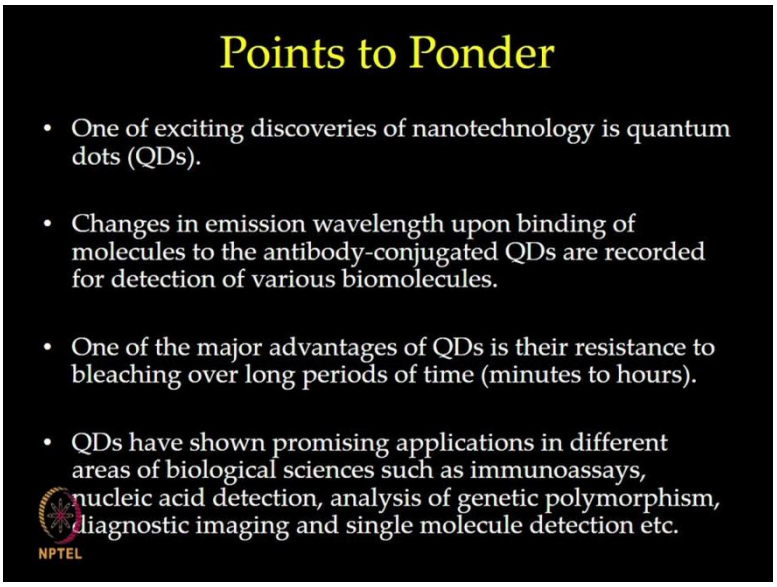

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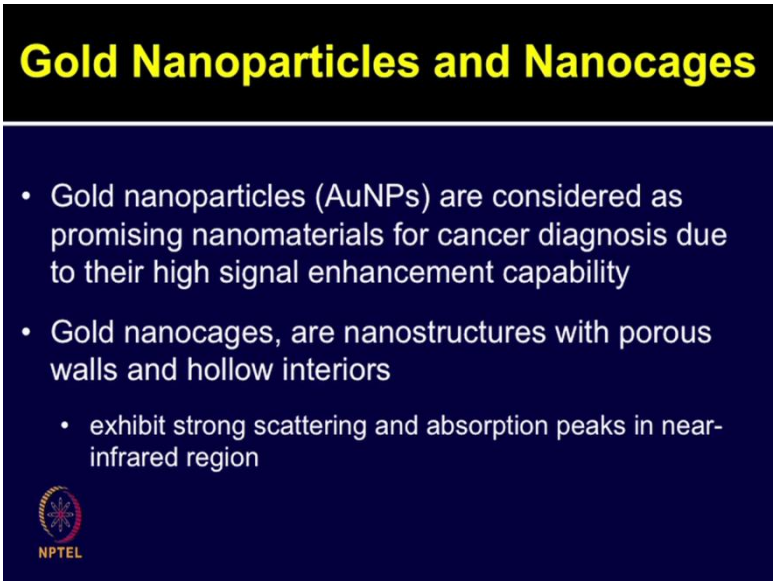

Let us now discuss other platform which is gold nanoparticles and nanocages. The gold nano material show versatile bio-medical applications due to their attractive structural and physicochemical properties. The size of the gold nanoparticles determines the proportion of absorption to scattering with biological imaging and it requires a very high scattering cross section which is achieved by increasing the size of nanoparticles. These gold nanoparticles and nanocages are considered as promising nano materials for cancer diagnosis because of their high signal enhancement capability. Gold nanocages which have been developed more recently are nanostructures with porous walls and hollow interiors. They exhibit a strong scattering and absorption peaks in the near infrared region.

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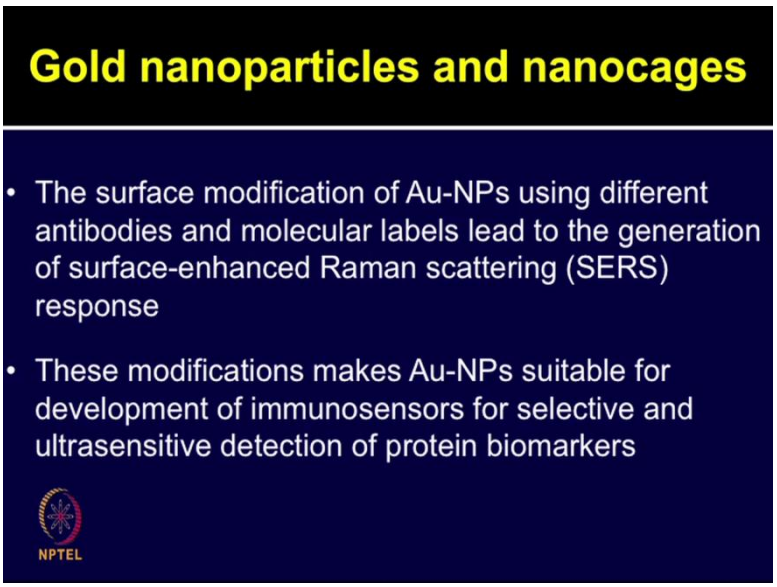

Various types of surface modifications have been tried for gold nanoparticles by using different type of antibodies as well as molecular labels which can lead to the generation of surface enhanced Raman scattering or SERS response. These modifications have made gold nanoparticles suitable for the development of immunosensors for selective and ultrasensitive detection of protein biomarkers.

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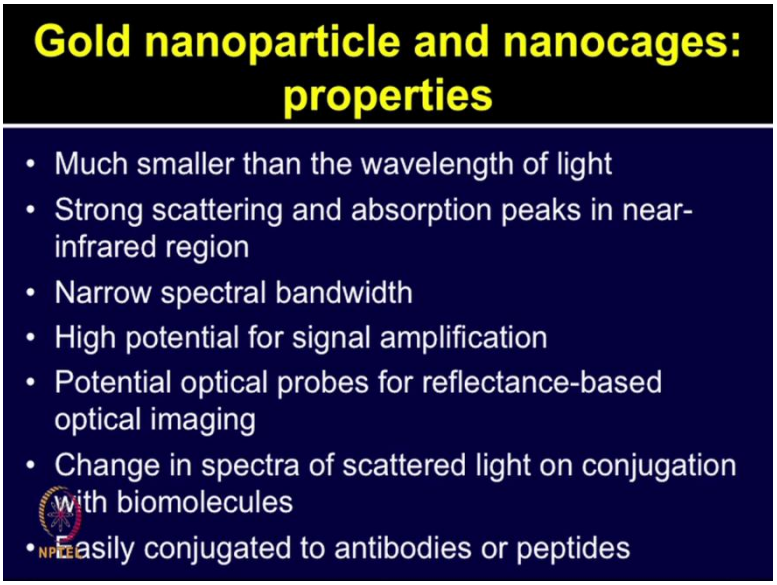

Let us now discuss some of the properties of gold nanoparticles and nanocages. These are much a smaller than the wavelength of light, they offer strong scattering and absorption peaks in the near infrared region. They have very narrow spectral bandwidth, they are high potential for signal amplification and they can act as optical probes for reflectance based optical imaging. They can be easily conjugated to antibodies or peptides and the change in the spectra of scattered light or conjugation with biomolecules is observed by using these gold nanoparticles and nanocages.

Some of the studies have also shown that gold nanocages can be conveniently functionalized with tumor specific antibodies and it serves as contrast agent for optical coherent tomography. These gold nanoparticles exhibit unique optical electronic and catalytic properties and signal enhancement capabilities which make them suitable for selective and ultra-sensitive detection of various biomarkers. The utilization of gold nanoparticles in electronic bio sensing process leads to signal amplification.

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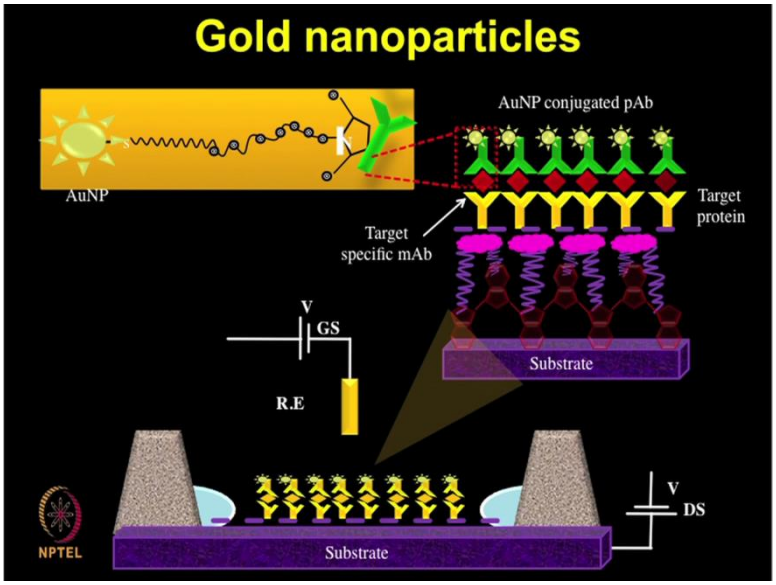

As shown in this slide, there are monoclonal antibodies which are immobilized on these gold nanoparticle surfaces which enables the specific detection of target proteins. Gold nanocages can also be functionalized in bio-molecules to target cancer cells for early stage cancer detection and thermal therapy of tumors.

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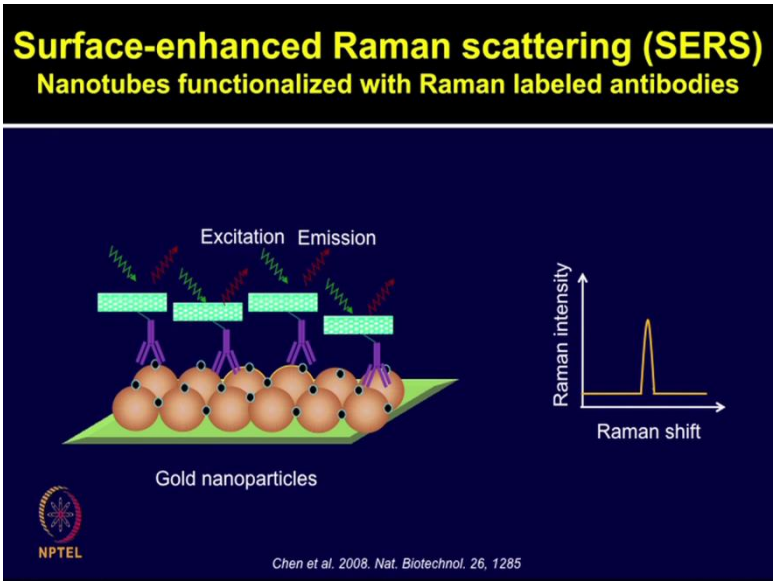

Let us now discuss surface enhanced Raman scattering or SERS. The SERS effect is achieved when an analyte is adsorbed on to or in the close proximity to a prepared metal surface. The Raman excitation laser produces surface plasmons on surface of the metal. These plasmons interact with analyte to greatly enhance the Raman emission. The surface enhanced Raman scattering or Raman spectroscopy lacks the sensitivity, but the silver and

gold nanoparticles can be used to enhance this scattering effect. The signal intensity of Raman active molecules is enhanced due to an increased local electric field at the nanoparticle surface and by electronic coupling between the absorbed molecule and the gold nanoparticle surface. As shown in the slide, these are gold nanoparticles which are functionalized with Raman labeled antibodies and the graph on the right shows changes in the Raman intensity due to the binding effect.

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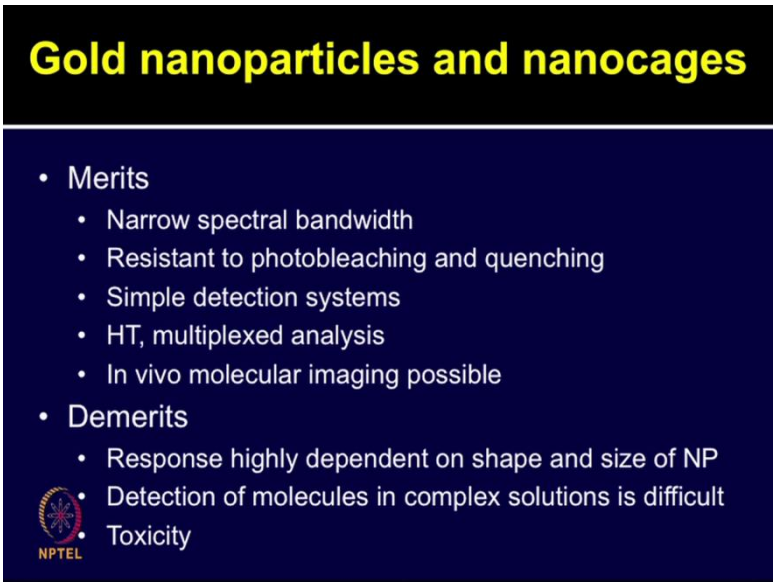

There are various merits and demerits of using gold nanoparticles and nanocages. The merits include its narrow spectral bandwidth, resistance to photo-bleaching and quenching, simple detection system and high throughput and multiplexing capabilities. Additionally, the bio-conjugated gold nanocages can act as molecular probe to target the desired cell which is in vivo molecular imaging. Discussing about its demerit, the response obtained is highly dependent on the shape and size of nanoparticles and detection of molecules in complex solution is also difficult. The toxicity associated with these nanoparticles is another major limitation of this technology.

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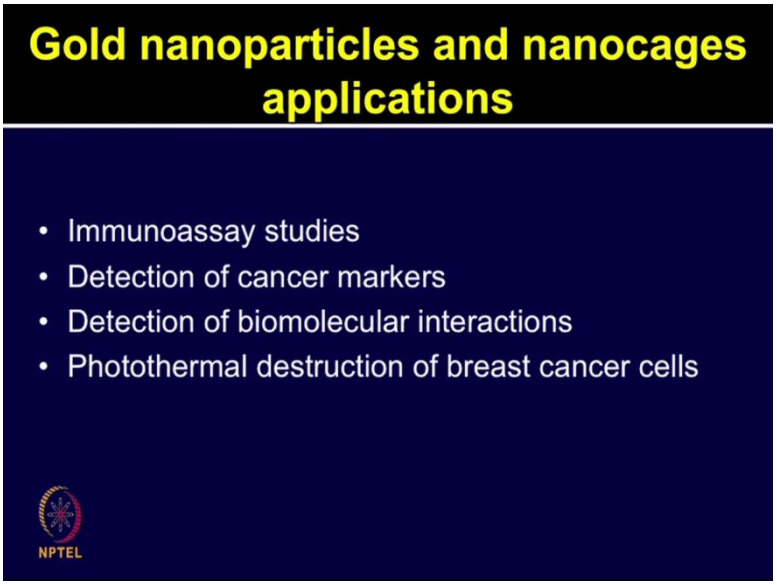

The gold nanoparticle and nanocages have shown applications in variety of fields specifically in proteomics. They have been used in immunoassay studies, detection of cancer biomarkers and detection of various bimolecular interactions as well as photo thermal destruction of breast cancer cells. These applications have been demonstrated in some of the published studies which have shown the potential of using gold nanoparticles and nanocages for various proteomic applications. Let us now discuss the principle of gold nanoparticles and nanocages in this interactive animation.

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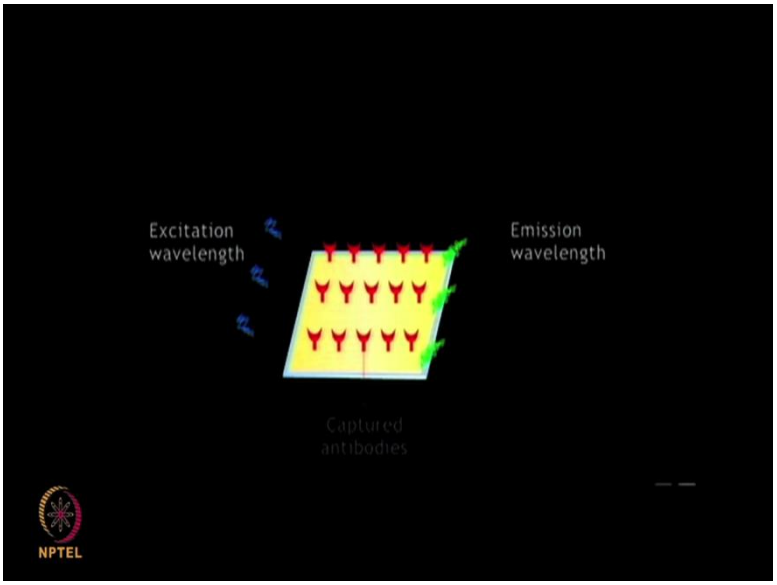

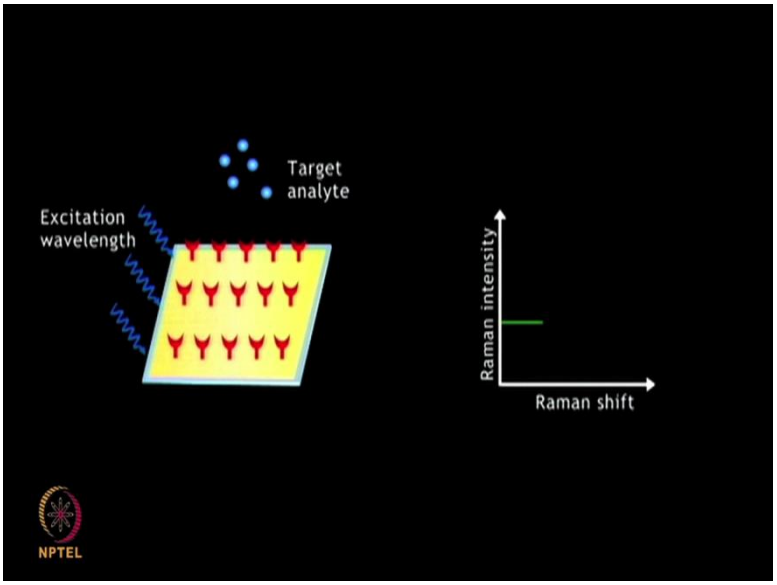

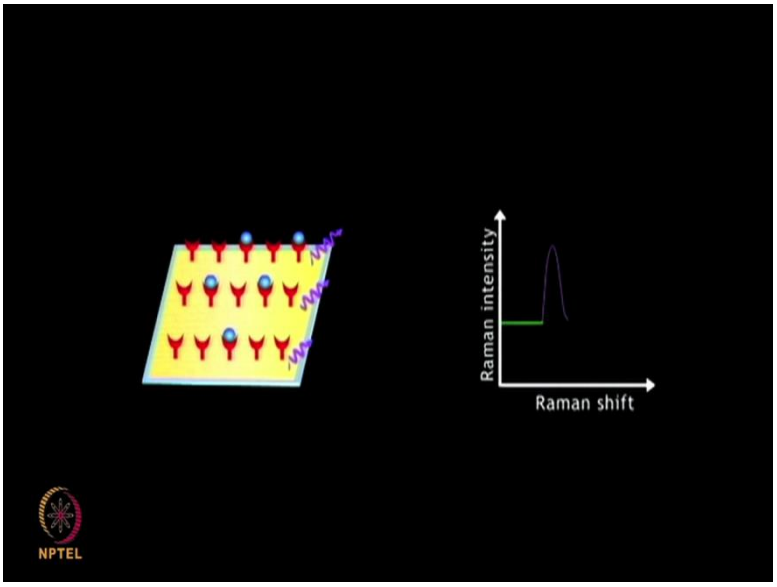

Points to Ponder

- The gold nanomaterial shows versatile biomedical applications due to their attractive structural and physicochemical properties.
- Gold nanoparticles and nanocages are considered as promising nanomaterials for cancer diagnosis because of their high signal enhancement capability.
- SERS nanoparticles are another gold-based nanoparticles, with excellent surface-enhanced Raman scattering properties.
- Some of the merits of gold nanoparticles include their narrow spectral band width, resistance to photobleaching and quenching and high throughput with multiplexing capabilities.

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The gold nanoparticle surface is functionalized suitably by antibodies which will bind to the analyte of interest. Any target binding is depicted by a change in the emission spectra as you can see on the right hand side, the Raman shift verses Raman intensity is plotted, after these gold nanoparticles are binding with the antibodies. A change in the Raman intensity is observed as shown in the plot on the right hand side.

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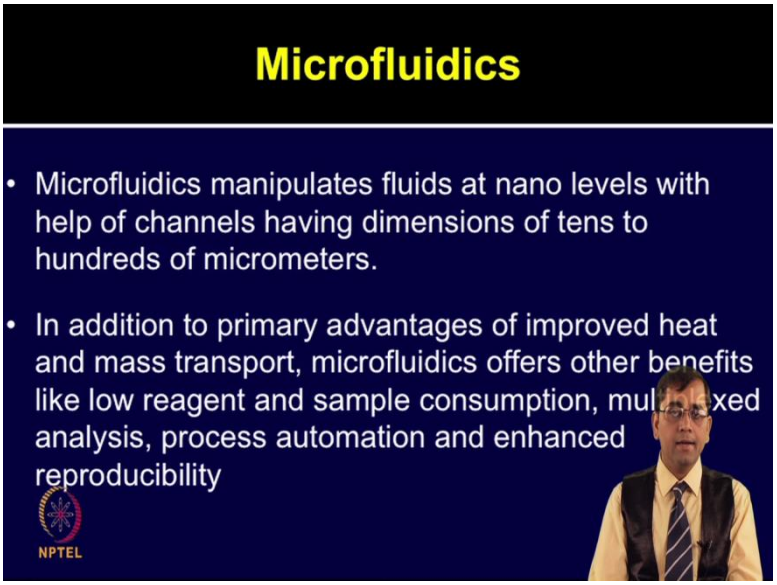

Let us now discuss next technology which is microfluidics. Microfluidics deals with processing of ultra-small fluid volumes with help of 10 to 100 micron channels which can provide sensitivity in the range of Pico molar to femtomolar. Microfluidics deal with fluids at nano level with help of channels having dimensions of 10 to 100s of micrometers. Microfluidic system helps to automate sequential sample processing as well as increasing sample throughput in parallel proteomic analysis. In addition to the primary advantage of improved heat and mass transport, Microfluidics also offer other benefits like low reagent requirement, low sample consumption, multiplexed analysis, process automation and enhanced reproducibility of assays. Microfluidic devices are now increasingly applied in proteomics and coupled with different type of proteomic technologies.

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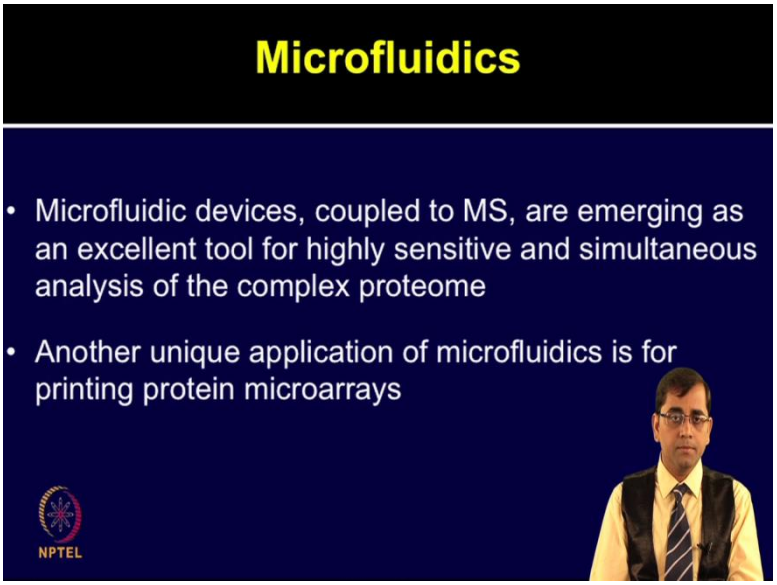

With intense research and increasing interest in using microfluidic devices and coupling them with mass spectrometry, it can provide an excellent platform for highly sensitive and simultaneous analysis of complex proteome. Another unique application in proteomics is application of microfluidics to print microarrays. Printing DNA, RNA and proteins on the chip surface is always challenging. A now different type of solutions are offered by microfluidic based system to enhance this spot to spot reproducibility and lower the sample consumption during the printing.

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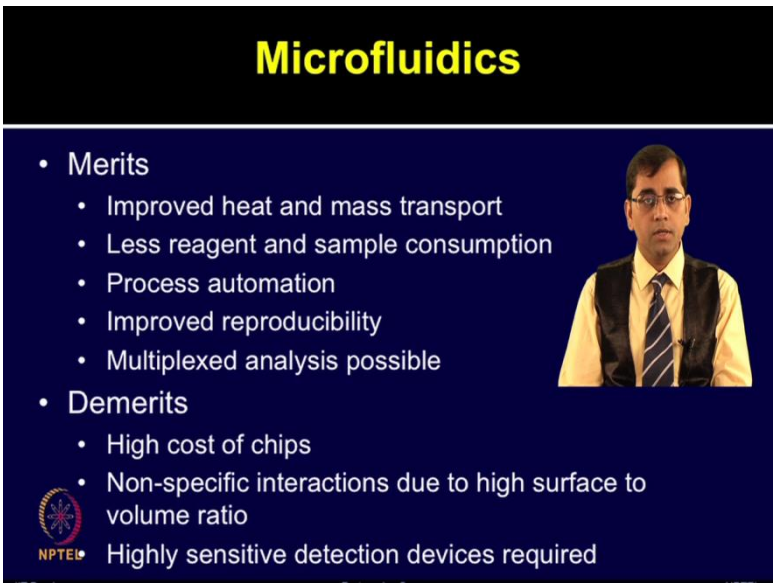

Microfluidic platforms offer many advantages such as improved heat and mass transports, lower reagents and sample consumption, automated process and it offers high reproducibility

and provides capability for multiplexing analysis, at the same time the limitations of microfluidics include the higher cost of chips, occurrence of non-specific interactions due to high surface to volume ratio and therefore, highly sensitive detection systems are required in such cases. Now, let us briefly discuss some of the major applications of microfluidics in proteomics.

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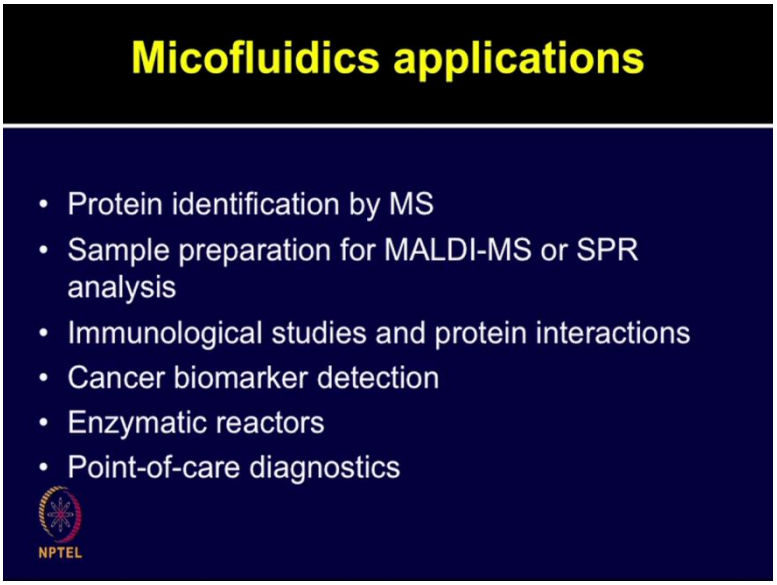

The protein identification is performed by mass spec. Now, there is increasing trend of using microfluidic based system with various proteomic platforms such as mass spectrometry and surface Plasmon resonance. The microfluidics being used in different applications such as immunological studies and bio-molecular interactions, biomarker discovery including cancer biomarker detection, enzymatic reactions and lastly, diagnostics as biochips mark the beginning of microfluidic applications in proteomics. These are just few of the microfluidic based applications mentioned here, it should be apparent that microfluidics have a significant potential to rapidly augment scientific discoveries in numerous fields including proteomics.

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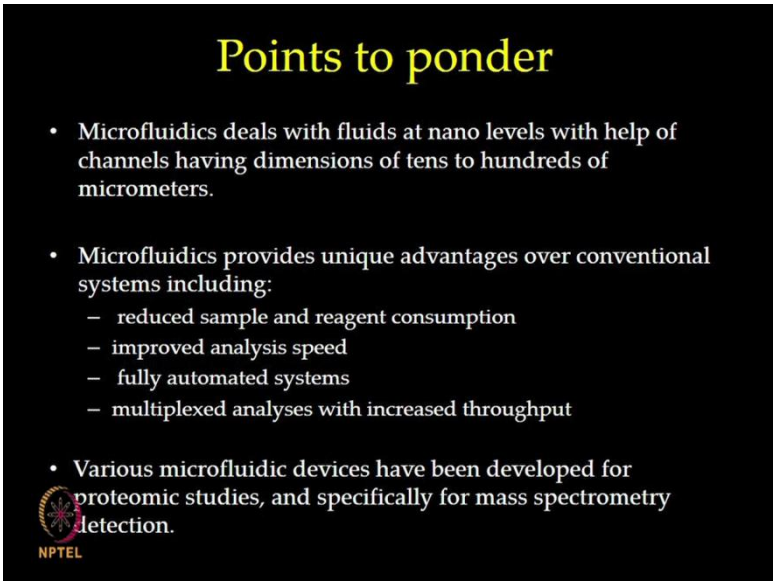

Other than these techniques which we have discussed in some detail, there additional promising nano techniques which are also being applied for proteomics applications. All of these techniques are at various stage proof of concept and development but have received increased attention due to their potential for proteomics based investigations.

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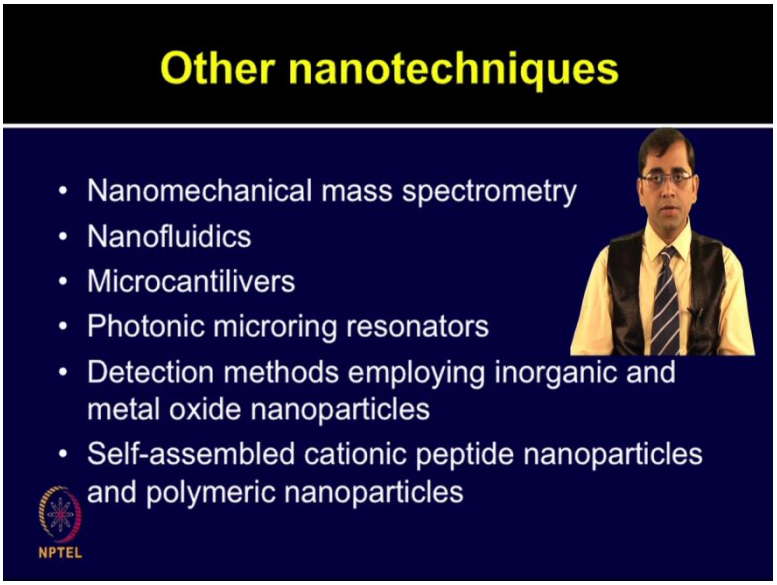

Some of these nano techniques involve nano mechanical mass spectrometry, Nano fluidics, Microcantilivers and photonic micro ring resonators. Various type of detection methods also employ in organic and metal oxide nanoparticles and self-assembled cationic peptide nanoparticles and polymeric nanoparticles. The study of proteomics which promises to provide solutions to several pathological conditions requires new potential techniques to

study the complex proteome. This has brought together the diverse field of proteomics and nanotechnology offering a new arena of nano proteomics over the last few years, nano proteomics have made significant progress from proof of concept to studies to applications in well-established and complex biological applications.

They have been used for several biological applications such as biomarker discovery and label free measurement of various biomolecular interactions; Nano proteomics hold great promise to become a technically robust and user-friendly platform for clinical and diagnostically studies. The advantages offered by various nano techniques have along them to be successfully coupled with rapidly growing field of proteomics. In summary, today we discussed about different type of nano techniques such as quantum dots, gold nanoparticles, nanocages and microfluidics. We discussed the merits and demerits of each of these techniques as well as some of their applications however, there still remains many challenges associated with this field, by looking at some of these applications, it can be concluded that nano techniques can offer significant advancement in proteomics research. Thank you.

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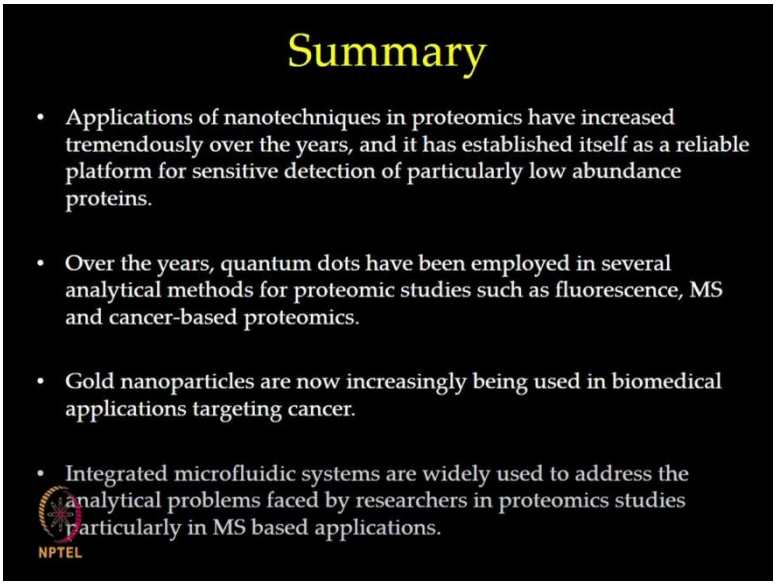

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