Cluster optical coding – from biochips to counterfeit security

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Abstract

Spatially tuned resonant nano-clusters allow high local field enhancement when excited by electromagnetic radiation. A number of phenomena has been described and subsequently applied for the construction of novel nano- and bionano-devices.
Easy to manufacture, cost efficient and high throughput transducers using metal cluster resonance technology are based on surface-enhancement of metal cluster light absorption (SEA). The optical phenomenon driving SEA in metal cluster films is the so-called anomalous absorption. At a well defined nanometric distance of a cluster to a mirror the reflected electromagnetic field has the same phase at the position of the absorbing cluster as the incident fields. This feedback mechanism strongly enhances the effective cluster absorption coefficient. Such a system is characterized by a narrow reflection minimum.
Based on this SEA-phenomenon (licensed to and further developed by november AG) a number of commercial products has been constructed. *Brandsealing*[R] uses the patented SEA cluster technology to produce optical codings. Cluster SEA thin film systems show a characteristic color-flip effect and are extremely robust. Both properties are vital for application as a unique security feature. The specific narrow band multi-resonance of the cluster layers allow easy authentication of the optical code. This can be achieved with a hand-held reader being developed by november AG and Siemens AG. SEA features are machine-readable which makes them superior to comparable technologies. Cluster labels are available in two formats: as a label for tamper-proof product packaging, and as a direct label, where label and logo are permanently applied directly and unremovable to the product surface. Together with Infineon Technologies and HUECK FOLIEN, the SEA technology is currently developed as a direct label for e.g. SmartCards.

Keywords: counterfeit, surface enhanced absorption, nano-cluster, optical sensor, biochip

1. Introduction

Nano clusters, nano-particles or nano islands are assemblies of up to 10^8 atoms with unique physical and chemical properties. Clusters are not really a fifth state of matter but combine rather specific features of metals and semiconductors including unique optical properties of Plasmons and Phonones. They are either nano-crystals or amorphous lumps bound together by crystal energy. While the first generation of cluster particles was based on pseudo-spherical clusters, within the last decade rod-shaped metal particles, tubes, prisms and cubes with specific optical properties have been designed. Clusters can be formed out of a wide variety of materials only limited by their chemical stability in air or humid environment. For application in nano-optical resonators particles are preferably composed out of a conducting metal with high electron density and mobility such as e.g. gold, silver, copper or platinum. To achieve efficient collective behavior noble metal clusters of high homogeneity in size and shape are required. Asymmetric particles have unique properties but require a deep understanding to predict and tune the excitation of various electric, magnetic, dipolar and multipolar modes (Fig.1, 2).

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Photon Processing in Microelectronics and Photonics III, edited by P. R. Herman, J. Fieret, A. Piqué, T. Okada, F. G. Bachmann, W. Hoving, K. Washio, X. Xu, J. J. Dubowski, D. B. Geohegan, F. Träger, Proc. of SPIE Vol. 5339 (SPIE, Bellingham, WA, 2004) · 0277-786X/04/$15 · doi: 10.1117/12.537429

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Particles up to a few nanometers in size exhibit no typical cluster behavior (Fig.1). Assemblies composed out of a few atoms are best described as molecular structures with well defined excitation frequencies. Large metal particles of 300nm or more show no well defined dipolar modes but extended plasmons resulting in metallic luster and bulk conductivity.
Thus, cluster particles useful for optical devices cover an extremely wide range in size from around 3 to around 100nm[1,2,3]. Cluster structures set up of a plurality of clusters are often referred to as cluster matter or island films exhibiting bulk behavior. Contrary to this, well defined nano-assemblies of clusters are precise elements of novel nano-optical devices for defined plasmon guiding and switching.
To understand the cluster state of matter it is necessary to describe the behavior of isolated clusters. Based on the solution of simplified single cluster model systems more complex cluster-cluster, cluster-molecule and cluster–resonator assemblies can be described. Clusters as a base for nanotechnology, nano-engineering or nano-devices gained a central role in the upswing caused by tunneling and scanning techniques which was followed by the nano-boom.
Although it is possible to gain cluster-matter by a break down and milling process, for nano-optics clusters are preferably synthesized either freely in solution or via lithography. For application clusters are deposited or bound to a surface, embedded within a material or coated from nano-powder. Electrical and optical properties are primarily determined by material and size but are also modified by shape, by surface bound molecules, and the embedding medium. The energy scheme of electron-levels is determined by the material, by cluster size and cluster shape. Simplified models can be set up for ideal clusters such as sodium or potassium since these are not disturbed by interband interference. The behavior of isolated and assembled metal nanoparticles larger than 3nm in size is deduced from classic electromagnetic theory without any consideration of quantum effects or statistics. Contrary to this, ultra-small or semi-conductor clusters are not readily described by bulk phenomena. When reducing the size of the cluster particle a fundamental change in behavior is observed. At a well defined size a transition from metal to semi-conductor-type clusters and further on in between 2 and 100 atoms to atom assemblies and isolated atoms is obtained. In any metal cluster the boundary region reaches deep beyond the surface. The high surface to volume ratio offers to use otherwise inefficient surface-confined energy transduction. Even clusters with several thousand atoms still contain up to 25% of their atoms in the surface confined layer. Only at a size of more than 5nm the behavior is primarily described by bulk electron resonance (< 1% of atoms are near the surface).

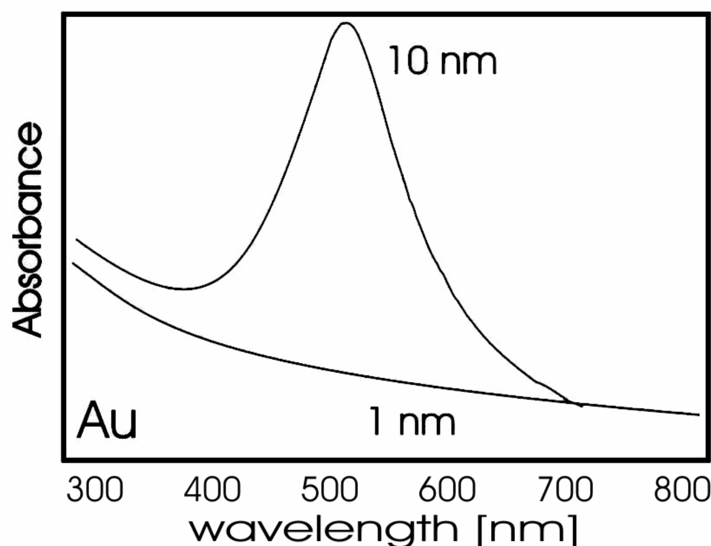

Figure 1: Optical spectrum versus size (gold - cluster). Note: for Au a distinct plasmon band is only obtained at a size of > 5nm.

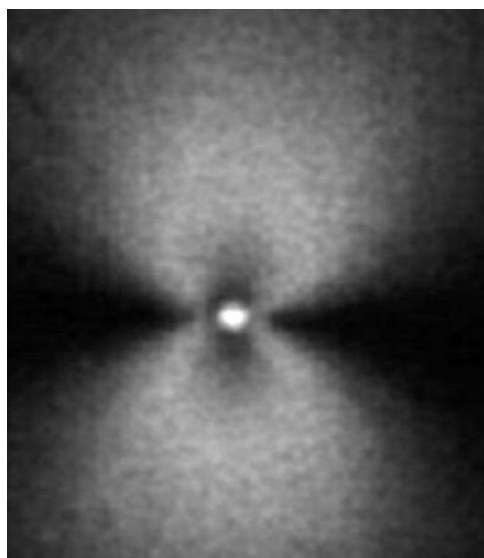

Figure 2: Local field of an aspherical Ag-cluster deposited at a surface

Various techniques are applied to study fundamental properties of nano clusters including optical spectra,

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photo-ionization, magnetic moment, or polarizability. To image clusters electron microscopy (EM), surface tunneling microscopy (STM), atomic force microscopy (AFM), dynamic light scattering (DLS) or optical near field techniques (e.g. SNOM) directly access size, shape and electro-optical properties of individual cluster particles. SNOM proved to be particularly suitable for detection and characterization of the optical response of resonant nano-cluster devices and to investigate details as e.g. shape - resonance relation in nano-clusters.

The first introduction of gold and silver cluster staining methods dates back into the mid 20th century. Rapid and simple one pot chemical synthesis, a narrow size distribution and efficient coating by thiols, phosphines and bio-ligands enabled the application of cluster particles as transducers of biorecognitive binding and molecular structure. Fundamental properties and key-techniques are high extinction coefficients, electron dense core, highly resonant particle plasmons, direct visualization of single nano clusters by scattering of light, catalytic size enhancement by deposition of e.g. nickel or silver and no photo-bleaching under illumination.

The strong coupling and excitation of metal nano clusters with an electromagnetic field via radiation is among the most exciting properties of metal clusters. In a macroscopic metal sheet its electrons move unconfined through the whole piece resulting in a strong and broad reflectivity, well known as metallic luster. Contrary to that, optical properties of metal nano clusters are primarily determined by the strongest oscillation process of the electron gas – the particle plasmon. This resonance of 5 - 30nm clusters is dominated by the collective dipolar oscillation of the electrons within the cluster. To describe the behavior of the plasmon oscillation it is appropriate to apply a quasi-static regime only for clusters of around 10 - 20nm. Spectra of clusters larger than 30nm are determined by finite penetration depth and multi-polar modes of the electromagnetic resonance. The static regime assumes that the phase shift in the cluster particle is small enough to be neglected reducing the cluster oscillation to a simple dipole. In most experiments and assemblies the optical spectra of millions of individual clusters are combined. Thus, a broad and smeared out resonance due to the cluster-size and shape-distribution is observed. Resonant optical phenomena are found in the visible and infra red part of the spectrum. The UV and blue range of the spectrum is heavily deteriorated by interband energy transfer.

Metals with a high number of free electrons and minimal damping such as silver, gold or copper exhibit the strongest optical effects. These metals have partially filled conduction bands but completely filled valence bands. Noble or semi-noble metals exhibit both free electron and interband transition behavior. Often the IR and red part of the spectrum exhibit ideal behavior while the spectra of higher energy are dominated by interband excitation. In an ideal cluster the visible region of the spectrum is dominated by the dipolar plasmon and damping is small.

Only silver has a well-defined resonance peak near 300 - 400nm. Gold exhibits a broad peak at around 520nm with an interband shoulder to higher energy. Resonance in silver or gold is no longer a free electron resonance but a cooperative effect based on conduction and mostly d electrons.

Gold clusters are yellow to red depending on their size. Yellow-orange indicates the lack of a plasmon band and is a direct indicator of ultra-small gold clusters up to a few nm in size. A red gold sol indicates clusters with a developed plasmon resonance. Violet indicates formation of at least dimeric particles. A grey color indicates a non-crystalline core, thus an amorphous state of the cluster.

Most noble metal clusters are prepared by reducing metal salts with various reagents in aqueous or organic solution. The most common reagents are sodium borohydride, citrate, phosphorus or tannic acid. Depending on the method and the exact reagent composition of the reaction mixture, metal cluster preparations vary in particle size and size distribution.

Simplified models describe clusters of spherical shape. Clusters of increasing eccentricities exhibit a splitting and shifting of the plasmon peak. The oblate cluster

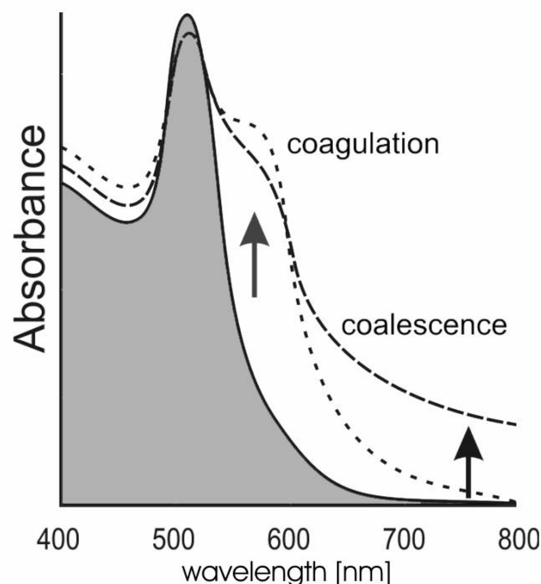

Figure 3: Spectral change induced by cluster aggregation and coalescence shifting color from red

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shape induces a defined splitting of plasmon modes and allows directed excitation of dipolar modes based on the wavelength and polarization angle of the exciting beam. Aspherical clusters randomly oriented in a medium result in a flat and broad peak[4]. Aspherical clusters oriented on a flat substrate result in a well defined aspherical local field (Fig. 2) which might be precisely tuned by cluster geometry and size. The combination of two nano-clusters with non identical band gaps is an optical analogue to a diode (unidirectional light pipe). Amorphous metal clusters are broadened in resonance towards the long-wave-absorption of metal-cluster-films. In small clusters even sub-monomolecular ad-layers of sulfides, iodides, oxides or borates lead to a significant modification of the plasmon resonance[5]. Cluster approaching each other in solution will exhibit a red-shift of the plasmon peak (Fig. 3) being rather broad and distorted.

2. Nanocluster-technology

While clusters deposited by sputter coating or vacuum deposition are neither round nor free of crystal defects, chemically synthesized clusters are closer to the ideal model. Design and synthesis of ideally resonant clusters is essential for resonant nano assemblies.

To produce layers of metal nano clusters from silver, gold, palladium, copper, tin and indium slow thermal evaporation or sputter coating at elevated substrate temperature are employed. Films of a wide variety of materials are deposited within seconds or minutes. Sputter coating is usually done in an inert argon plasma at 10^{-2} to 10^{-1} mbar. The thickness of the metal layer is adjusted via sputter time, pressure and current.

At high interaction energy a *layer-by-layer growth mode* is observed, resulting in a film instead of clusters. If the interaction between neighboring metal atoms exceeds the interaction with the surface, an *island -cluster growth* is the result.

Sputter-coated clusters are small, flat and asymmetric. Upon heating to 100 to 350°C the clusters as well as layers melt, resulting in defined nano-cluster films of round shape (NOTE: Even at 100°C Au-atoms are mobile and recrystallize within a few minutes in solution). Melting gold on chromium coated adhesion layers results in a terrace re-crystallization of the gold film without cluster formation.

To achieve well-defined cluster layers by a coating process chemically synthesized clusters are coated to a substrate via adsorption or (bio) chemical bonding or printing.

Cluster solutions[6,7] are inherently unstable. Aqueous dispersions are stabilized only by ion repulsion, often by adsorbed citrate ions. Without repulsive stabilization dispersed cluster particles will aggregate within seconds. The strong tendency of coagulation and coalescence is caused by attractive van der Waals forces (Fig. 3). Two nano clusters colliding by Brownian motion will adhere and precipitate. The first van der Waals minimum for cluster particles is much deeper than the thermal energy. A dispersion of charged nano-clusters is usually stable at low salt concentrations. At a high concentration of salt ions the particle charge is shielded and the repulsive electrostatic force is outperformed by the attractive van der Waals force. This leads to precipitation at a given concentration of the salt. To avoid aggregation metal nano clusters are stabilized by phosphines, alkanethiols, cyclic dithiols, complex molecules, polymer shells or simultaneous coating with a set of ligands[8,9,10]. Synthesis is usually performed via citrate reduction and stabilization follows as an independent step. Various compounds have been applied to stabilize gold clusters. One of the first has been thiol-linked DNA[13]. Since monothiolated agents are affected by place exchange reactions, multiple thiol anchors[11] or cyclic disulfides[12] are applied to overcome that limitation.

Another way of protecting nano-clusters are hydrophilic polymers[14] attached to the surface by various linker groups.

A general pathway to thiolate gold clusters for application in SEA-devices can be given as follows:

1.) Synthesize clusters
2.) Adjust pH (only if required by stabilizing agent)
3.) Since about 3nmol of Au-sites per ml (in a standard cluster solution of 17nm) are capable of coupling to a thiol group, a concentration equal or higher to that amount has to be used for complete coverage[15].
4.) Perform flocculation assay: incubate different amounts of agent with same amount (e.g. 0,5ml) of cluster solution over night and then add NaCl (1M) in increasing concentration. The required amount for stabilization is found by monitoring color changes. The concentration with the highest NaCl stability is the ideal one. Note that sometimes addition of an excess of stabilizer *reduces* NaCl stability. This is due to the ionic character of some agents.

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Many stabilizers have been tested this way. Small binding moieties (e.g. thiomalic acid, mercaptoacetic acid) are directly coupled to various stabilizing oligo- and polymers (e.g. Gly-gly-gly or Methoxypolyethylene glycol amine) via EDC chemistry. After purification significant stabilization is observed. While unstabilized clusters precipitate at less than 10mM of sodium chloride - stabilized clusters will tolerate at least 200mM.

Passivation of metal-clusters by silica shells around the metal core is another viable technique: 10ml of an aqueous gold cluster sol are diluted to 50ml with ethanol. 2 - 5µl Tetraethyl orthosilicate (TEOS) are added. The reaction is started by addition of 1480µl aqueous ammonia (~25%) over a 5min time period. Incubation takes place over night at 30°C. Even if a precipitation is observed resuspension of the clusters is possible due to the fact that no core to core aggregation is possible due to the outer protection shell. Coupling of biomolecules to the outer shell is achieved by activation of the silica matrix with aminosilanes (e.g. Aminopropyltriethoxysilane 1mg / 20ml cluster solution).

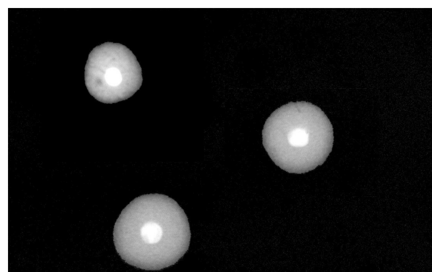

Figure 4: TEM of core-shell cluster (core: gold, shell: SiO_2)

3. Cluster and biomolecules

All reactive reagents used in metal-salt reduction coat the surface via physisorption or chemisorption (e.g. citrate or chloride ions used in gold cluster synthesis). As described above salts added to a gold-cluster sol turn the color to blue, indicating cluster precipitation. However, if polymers such as proteins or DNA are added, these molecules bind via adsorption (or covalent attachment) to the metal cluster and stabilize the sols. In a simple approach even the addition of soluble polyvinyl-pyrrolidone (PVP) significantly increases the stability of colloidal preparations.

Binding of proteins to metal clusters is more or less irreversible due to multiple attachment sites including cysteine, aspartic- and glutamic acid, lysine, arginine or histidine moieties. Most of the proteins retain their biological activities, at least in part. The degree to which biological activity is lost depends on the structural integrity and will vary from protein to protein.

The ease of preparation and the chemical as well as optical robustness make protein and DNA coated clusters useful tools for biochip technology. Due to the high number of protein molecules bound to each cluster particle (e.g. 50 proteins / 20nm gold particle) even a poor immobilization yield or low biological activity results in quite useful cluster reagent. For Proteomics or if only very tiny amounts of the proteins are available it is vital to determine the minimal amount of protein needed to stabilize the cluster. Expensive proteins are coated in sub-equimolar ratio to the cluster surface. In addition to the functional protein in a second step a non-reactive protein such as BSA is applied for further stabilization of clusters.

While protein-coating can often be done within 20 minutes, coating with protein in a 2-3 fold excess for several hours may help if a low binding affinity is observed. Excess protein has to be removed.

Coating of gold clusters with proteins is done as follows: 1. Adjust 50ml cluster suspension (OD540 ~ 1) to pH 8.5 with 0.2M potassium carbonate. 2. Coating is performed by gently swirling the purified protein for 20min at ambient temperature 3. While adding the protein, the sol should be stirred vigorously 4. After coating, the sol is further stabilized by adding 5ml 1% (w/v) skimmed milk powder that has been adjusted to pH 8.5 with 0.2M potassium carbonate. 5. The mixture is gently swirled for another 60min.

Coating of gold clusters with DNA is done by: 1. A gold cluster solution (15-20nM in particles) is reacted with a 200 fold access of a thio-functionalized oligonucleotide (3,5µM) in water. 2. The solution is allowed to stand for 24 hours at room temperature. 3. The excess of reagent is removed by centrifugation or dialysis. 4. Up to 100 DNA-molecules are immobilized to the surface of a single 15nm cluster resulting in a nano-brush-like surface.

Isolation and purification of protein coated gold clusters is achieved using the following protocol: 1. To purify the protein coated cluster the obtained mixture is centrifuged through a 50% (v/v) glycerol layer that is mounted onto an 80% (v/v) glycerol cushion. 2. The centrifugal speed and/or running time is adjusted to the point where coated antibody clusters of desired size are found almost entirely in the 50% glycerol layer. 3. After centrifugation, antibodies and/or blocking proteins that are not bound to the nanoclusters will be found in the upper water layer, while the large, purple colored cluster aggregates will be present in the 80% glycerol layer and on the bottom of the

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centrifuge tube. 4. In order to avoid contamination of the detector reagent with free protein and/or large antibody nanocluster complexes, the coated cluster is removed sideways out of the 50% glycerol layer with a syringe. 5. Centriprep-30 concentrators are used for purification and washing steps

A well-defined plasmon peak is typical for stable and isolated metal clusters. Standard plasmon behavior can only be observed as long as no significant coagulation of the clusters takes place. A gold sol will turn from red to violet on aggregation and further on to blue on coalescence and precipitation (Fig. 3). Based on the detection of an optical peak induced by cluster aggregation a number of analytical devices were developed by Mirkin and coworkers[16,17,18]. All these assays have a unique advantage over ELISA being quick and simple single step assays but suffer from a lower sensitivity.

As cited above thiol bonds are meta-stable at room temperature and exchange within minutes to days of incubation with free thiols. Stability at 70-100°C is not given. To increase the chemical stability multivalent thiols, polymer- or silane-shells are applied. All new strategies clearly target at multiple attachment sites between oligonucleotide and the cluster surface and a further crosslinking to stabilize the outer shell. Using silane coated and cross-linked clusters the DNA is bound covalently to amino groups of the coating.

Assembly of nano-clusters via bio-templating is among the most promising strategies for the fabrication of nano-optical devices[19,20,21]. Protein recognition and hybridization of DNA are tools to directly and precisely assemble nanometer-sized elements. These biotool-kits allow the fabrication of materials with defined nano-properties. Well-defined protein layers (e.g. bacterial surface S-layer proteins) deposited at the chip surface are used as templates for the build-up of organic and inorganic nano-assemblies. The periodicity arises from the self-organization of biomolecules[22].

Sequence properties of RNA or DNA can be employed in template synthesis of supra-molecular assemblies to construct coupled plasmon nano waveguides. Either clusters are coupled via site-specific DNA hybridization or the negatively charged phosphate backbone of the DNA-double helix enables to accumulate multivalent metal ions. Subsequently metal ions are used as a catalyst for gold, silver or nickel deposition. Based on electrostatic attraction the assembly of poly-L-lysine coated cluster particles has been reported.

Figure 5: Surface enhanced absorption - setup

4. SEA-chips

SEA needs a number of elements to allow the construction of optical and opto-analytical devices including a substrate, a mirror, a resonance layer and topmost the cluster layer (Fig. 5).

Anomalous absorption is the basis of surface enhanced optical absorption. The positioning of an absorbing cluster layer or a single cluster in a well defined nano-distance to a metal mirror is required for this effect. For a defined cluster - mirror distance only a well defined subset of wavelengths can be in phase, therefore the output of the system is a spectrum with strong and narrow spectral reflection minima or absorption maxima. Variations of the optical path (cluster – mirror – cluster) result in a spectral shift of these reflection minima[23,24] (Fig. 6, 9). These variations are generated by either changing the cluster layer - mirror distance or the angle of the incident light. Both approaches result in a change of the color impression of the surface enabling various applications of the effect.

The options to use SEA for identification tags as well as analytical devices are:

- Induced distance changes of the cluster layer to the mirror
- induced changes in the packing density of the cluster layer
- Induced local variation of ε_m

The induced changes due to e.g. binding or catalytic activity of the analyte are transduced via changes of the sensor surface's optical appearance, either via

- a spectral shift of an absorption maximum or
- a change in absorption at a defined wavelength

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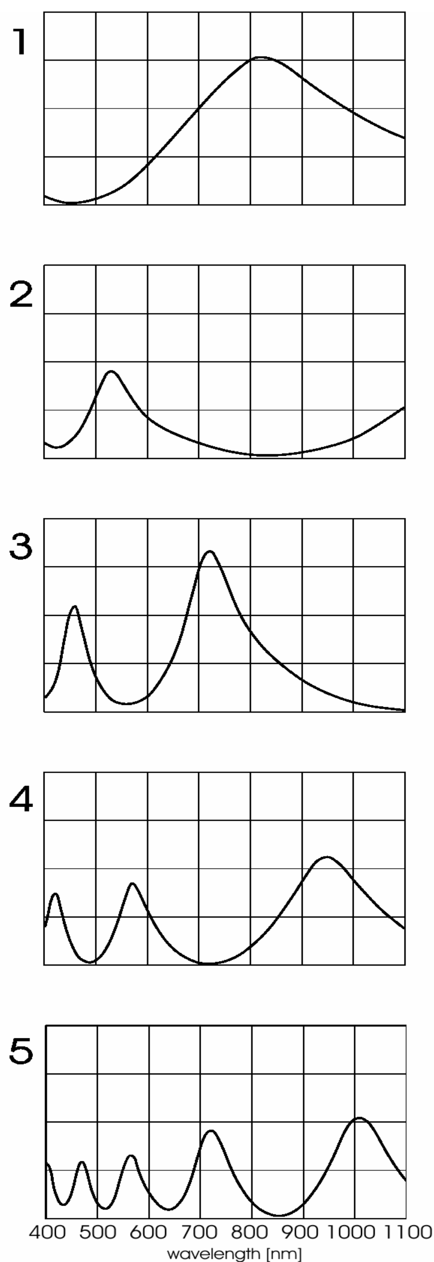

Figure 6: Spectral response of a SEA - chip with increasing distance layer thickness (1-5)

The most obvious approach is binding the analyte (preferably a bio-component) to the cluster and making the clusters presence or absence in the SEA setup dependent on a bio-recognition process. The result of these binding or dissociation events are changes of the color response, which in an optimal setup and a proper concentration range can be observed with the naked eye.

Due to the fact that the absorption is directly proportional to the number of interacting clusters, in principle quantitative measurements can be applied. A reduction or increase of the number of clusters leads to a quantitatively recordable decrease or increase of the absorption. However, most of the described SEA systems focus on semi-quantitative measurements ("present / not present").

Another SEA approach makes use of a permanently attached cluster layer. Influencing the height of the distance layer (the "optical" length of the linker) results in a color shift of the setup. So the presence of an analyte (e.g. a substrate reacting with an enzyme) is transduced into an optical signal.

Clusters in a broad range of sizes can be manufactured, the choice of cluster size depends on the application. Considering diffusion speed (small clusters diffuse faster) and contribution to the SEA effect (bigger clusters give better resonance) a compromise has to be found. Usually clusters of 12 to 40nm are chosen.

Spherical metal clusters enhance absorption approximately 8 fold at the peak maximum, relative absorption enhancement is above 100 fold. Below a wavelength of 600nm the enhancement is independent of the way of application of the cluster layers, but depends strongly on the attachment of clusters under given conditions[25-30]. At any wavelength above 600nm the optical properties of the clusters become dependent on the manufacturing process, due to differences in asymmetry and the degree of crystallinity of the nano-structure. Ideal nano-crystalline gold or silver clusters are not the best choice at a wavelength of >600nm and plasmon tuning is required to achieve the best resonance effect.

As support and as mirror a variety of materials can be used, among them highly polished aluminum or stainless steel foils as well as metallized plastic foils (titanium or aluminum coated). Other substrates including glass slides, silicon wafers or a variety of polymer sheet materials such as e.g. polycarbonate or polyethylene-terephthalate. Due to poor surface properties most plastic based substrates need an oxygen plasma hydrophilization (passivated aluminum sputter targets, low power settings) to achieve sufficient adhesion of a sputter-coated metal mirror.

Reflecting surfaces like silver, aluminum, gold or gold-palladium mirrors are deposited by DC- or RF-sputter-coating using argon as sputter gas. For analytical SEA-devices a high reflectivity is needed, therefore only a few metals are useful. In case of materials with poor attachment to the support (e.g. silver on glass) it may be necessary to introduce adhesion layers, e.g. chromium. Using such materials or the chemically unstable silver or aluminum a protecting distance layer may be inevitable. To gain optimal chip stability under high temperature conditions a complex multilayer setup is required.

Adhesion of coatings can be improved by adjusting several sputter parameters. Higher substrate temperature during deposition (~ 300°C) enhances the surface diffusion on the substrate. A negative voltage (-25 V BIAS) on the substrates increases the kinetic energy of the incoming ions and enhances the formation of chemical

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bonds with atoms of the substrate.

As explained in the introduction all interference-like phenomena are strongly dependent on the thickness of the resonance layer. In practice surface adhesion and material compatibility strongly contribute to the applicability of a specific set of layers.

Correlation of real thickness with the required optical thickness is achieved by AFM measurements.

Unspecific background absorption is the major limit for bio-analytical SEA-assays. The problem can be overcome by using two angles of observation. Due to the fact that the SEA signal shifts with the angle of observation while absorption of chromophores does not, subtraction of both signals eliminates the background absorption.

In principle any conventional ELISA or DNA hybridization assay can be adjusted to a SEA assay. SEA chips are applicable in direct as well as sandwich assays. In the direct assay the analyte is incubated (and subsequently bound) to the clusters, forming e.g. protein or DNA coated clusters. At incubation on chip this coating interacts with the biorecognitive molecules on the chip surface and in case of biorecognition leads to a binding of the cluster to the surface. The sandwich assay starts with the incubation of the analyte directly to the biochip, which leads to a binding of the analyte to the biorecognitive molecules on the chip surface. In a second incubation step pre-synthesized clusters coated with a second biorecognitive molecule fix the cluster in the required distance to the mirror. The binding of the cluster to the protein dots is followed either visually or via a CCD camera (Fig. 7, 8),

Spin coating and further on screen-printing enables a very cost- and time-efficient application of distance layers. For large batches of chips of high quality and homogeneity metallic glass distance layers are the better choice.

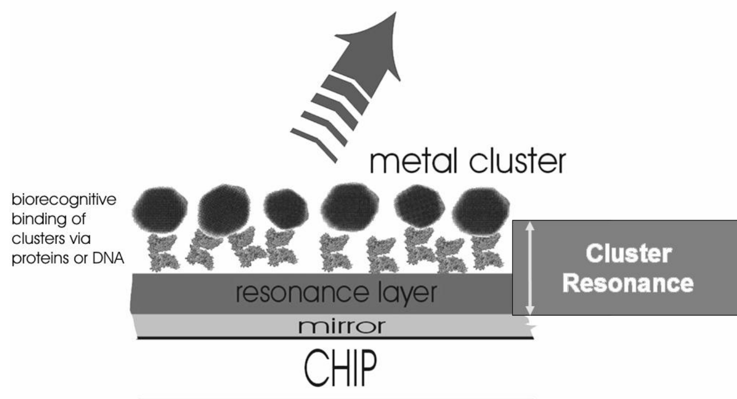

Figure 7: Setup of a SEA-biochip

The printing of the biorecognitive components is done with standard commercial printers or via microarrayers such as pin ring dotting robots or inkjet nozzles. Arrays are scanned down to 1 µm resolution with rather cheap and simple devices based on commercial CCD-camera technology.

Resonance layers can be activated via oxygen plasma sputtering or via silane based chemistry. A variety of silane derivatives can be used to form a stable monolayer introducing chemically reactive groups to bind ligands such as DNA or proteins.

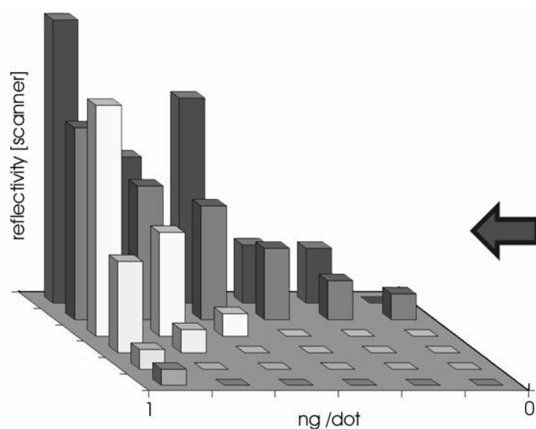

Figure 8: Optical scan of a cluster biochip and calibration graph for a protein - protein interaction panel

4.1 Typical SEA setup

Tungsten is sputtered onto a isopropanol cleaned glass substrates to serve as adhesion layer. On top of the adhesion layer a silver mirror is applied. Polyhexyl methacrylate is spin coated onto the silver using a 6% solution. The surface of the thin-film is oxygen plasma treated. The surface is activated via EDC (20mg/ml in 0.1M phosphate buffer pH 6 (freshly

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prepared) and subsequently washed twice with water. Proteins are micro-dotted (100µm) onto the chip, the EDC coupling is done in a moist chamber (2h, RT), subsequently the chip is washed several times with 0.1M phosphate buffer pH 7.
Antibodies are dissolved in dH_2O (1mg/ml; 200µl). Serial 100µl dilutions (1:5 to 1:10) are prepared. 500µl of the gold sol are added to each dilution, and after 10 minutes 100µl of 10% NaCl. The second dilution containing more protein than the last one whose color changed to blue is chosen. 10ml of the appropriate protein-cluster dilution are prepared, incubation over night. The product is centrifuged. The pelleted clusters are re-suspended in 500µl of water. 0.1% Tween 20 is added (to suppress unspecific binding). The solution is spread on the chip and incubated for 30 minutes (incubation time dependent on protein concentration). Subsequently the chip is rinsed with water, air dried and finally scanned in direct reflection mode.
The detection limit of this setup is in the range of fmol/mm^2 for a CCD-camera. Using an optical scanner single cluster resolution might be obtained.

4.2 Nano-distance transduction biochips

The 'metal island coated on reactive interlayer system' (MICORIS) is a SEA setup with a permanently linked metal cluster film. It responds to the presence or absence of an analyte by a thickness-change of the distance layer, thereby changing the cluster-mirror distance and therefore the color of the chip (Fig. 9). The MICORIS setup uses distance layers with an optical thickness in the range of 15nm up to 500nm with a resolution in the nanometer range.
The analyte can either change the thickness of the distance layer directly or an enzyme will perform this task reacting with the analyte. A product of the enzymatic reaction is used to change the thickness of the layer by e.g. a change of local pH. Such setups can use cycling assays boosting the signal, an additional advantage is the fact that the reactive molecule is produced inside the gel without the need of diffusion to reach its point of action.
Preferably the top cluster layer is sputtered onto the polymer surface. However, due to the limited stability of bio-distance layer materials this technique may not always be applicable. In such cases the cluster layer must be applied via adsorptive coupling of clusters to the polymer surface.
One of the polymers known to shrink and swell ion dependently is PVP crosslinked with sulfonated bisazidostilbenes. This was confirmed in a MICORIS setup. It was shown that the response of the biochip depended on the charge, concentration and type of ions. This setup proved fully reversible (> 500 cycles) and was used to monitor changes in the concentrations of different ions, pH, organic solvents and polyphenols[31-36].

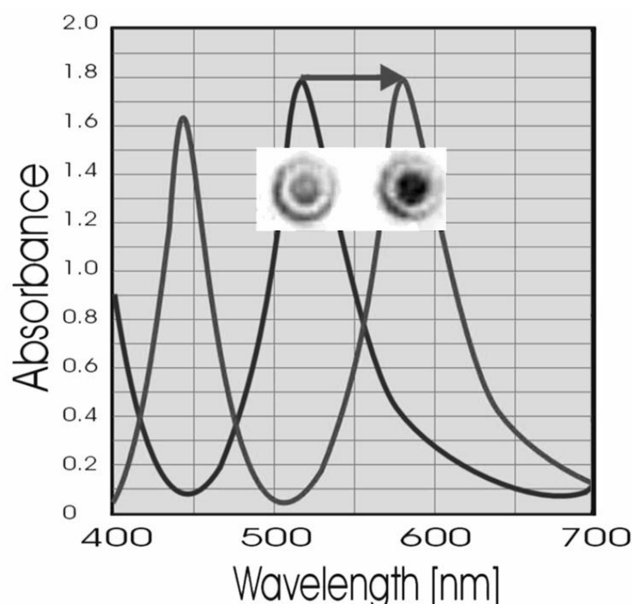

Figure 9: SEA response of a reactive interlayer (with a cluster top layer) and SEA-response of nano-protein gel dots

Any protein film can be used as a sensor distance layer[37,38] (Fig. 9). However, the protein layer must stay functional and the protein should not dissolve. This is achieved by cross-linking the proteins forming a thin-film gel pad. The linking procedure for proteins is exposure to UV-light or cross-linking with DIAS: 5% protein stock solution is mixed with a solution of 3% DIAS in a ratio of 10:1, spin- or arrayer-coated onto the chip, activated with a monolayer of aminosilane and finally UV-cross-linked.

4.3 Forgery proof SEA-features

Counterfeit consumer goods, automotive parts, and drugs cause loss in revenue and put money in criminals' pockets. The International Anti-Counterfeiting Coalition came to the conclusion that around 7 % of all products

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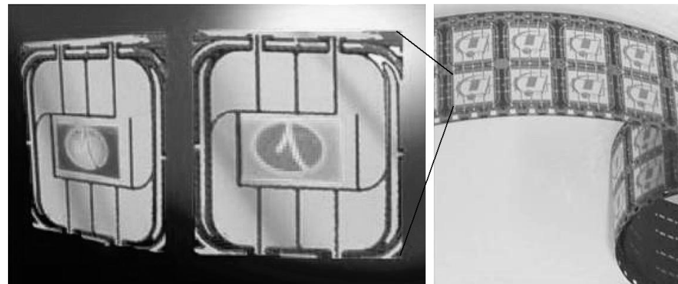
on the market are counterfeit. This means an annual loss of $300 billion. More important is the risk counterfeit products pose to the consumer. Based on SEA technology - invented by Schalkhammer et al. - November AG and Siemens developed the brandsealing product line, an authentication system like no other that offers unique options for the protection of products and brands. Brandsealing uses the patented cluster technology to produce optical codes. These optical codes can be customized to protect individual brands and products. Brandsealing is a product- and brand protection system that combines visible and invisible security features in one product.
The spectrum of counterfeited products reaches from consumer goods to safety documents and money. Even pharmaceuticals and spare parts are attacked by forgers. Because of the improved technological possibilities of product counterfeiting and forgery, there is a growing need of new, forgery proof labeling methods, which can be applied to a large variety of products. In most cases adhesive tags with different kinds of techniques (e.g. holograms, OVDs,..) are used to save products from being counterfeited.
Direct labeling of products or spare parts overcomes the limitations of adhesive tags concerning replacement as well as mechanical, chemical, and thermal stress. It is useful for higher priced products as the production is more costly as of adhesive tags but for some products it is the only possible way.

SEA techniques are a novel means for forgery-proof thin film setups which produces characteristic colors and special optical effects for direct labeling of different substrates. Nano- colors and effects - some with precise angle variation - are the basis of this technology (Fig. 10).
Based on the SEA-phenomenon a number of commercial products had been constructed. *Brandsealing*^{R} uses the patented SEA cluster technology to produce optical codings. Cluster SEA thin film systems show a characteristic color-flip effect and are extremely mechanically and thermally robust. This is the basis for its application as an unique security feature. The specific spectroscopic properties as e.g. narrow band multi-resonance of the cluster layers allow the authentication of the optical code which can be easily achieved with a mobile hand-held reader developed by november AG and Siemens AG. Thus, these features are machine-readable which makes them superior to comparable technologies.
The technology is available in two formats: as a label for tamper-proof product packaging (Fig. 11), and as "direct *brandsealing*^{R} ", where label and logo are permanently applied directly and unremovable to the product surface. Together with Infineon Technologies, the SEA technology is currently developed as a direct labeling of SmartCards (Fig. 10). For large scale production HUECK FOLIEN one of world's leaders in the field of technical films for security applications cooperate in the field of product security and brand protection. On the basis of the SEA cluster technology and the know-how of HUECK FOLIEN regarding engineered industrial films, new system solutions for security features had been



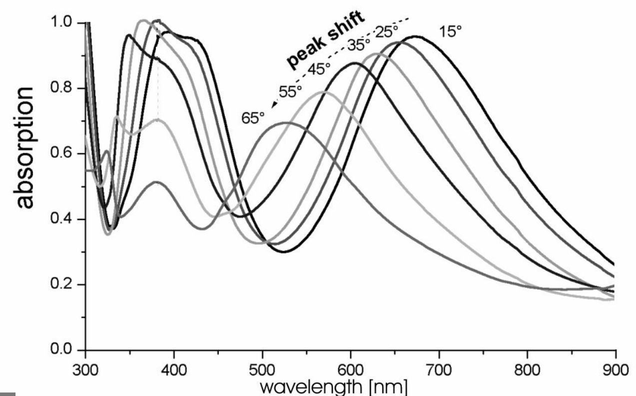


Figure 10: Chip surface at varying angle of incidence

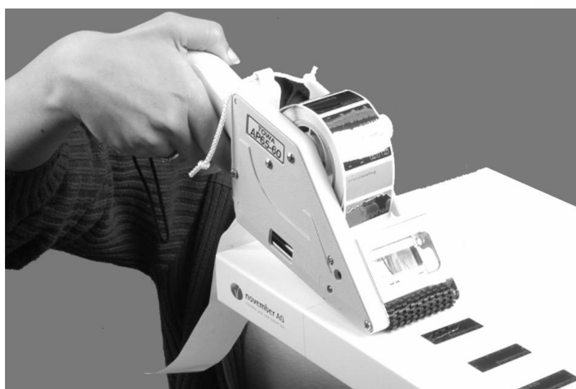


Figure 11: Product labeling

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developed. The banknote industry demands more and more sophisticated security features. As a new and unique feature SEA labels had been presented at the *Banknote 2003* international congress as a novel high quality security feature for banknotes.

HUECK FOLIEN GmbH purchases an exclusive license for the banknote sector to produce and sell *color-switch* security features based on the *brandsealing* technology. The jointly developed *color-switch* security features offer a unique combination of a brilliant color effect, extremely fast machine readability and highest forgery-proofness. They are far superior to existing technologies. The new feature significantly enhances security threads and can substitute the now commonly used holograms. First pilot projects are already being carried out. In contrast to other systems, this effect is machine readable. Every year, a dozen currencies are redesigned and specified. Forgery-proofness is an important factor to create trust into new currencies. With *color-switch,* everybody can identify real money at the first glance.

Summing up SEA color coded features are high security feature with enormous coding capacity, combine visible and invisible security feature in one product, are machine readable, allow mobile on-site testing, exhibit extreme thermal and physical robustness and allow direct label application onto the product.

5. Acknowledgement

Part of this work was supported by the LifeTech program at TUDELFT / The Netherlands.

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