nanomaterials

introduction
**Nanomaterials**
- Top-down Science
- Bottom-up Science

**What are nanomaterials?**

Nanomaterials are materials possessing grain sizes on the order of a billionth of a meter. \((10^{-9} \text{m})\)

A material in which at least one side is between 1 and 1000 nm.

Nanomaterial research literally exploded in mid-1980's

Some slides by Maya Bhatt
Single-walled Carbon Nanotube: 1 nanometer diameter
Strand of Hair: 100 micrometers diameter
House: 10 meters wide
history

• Big bang
• Fires
• 1950- fused silica
• Why such a buzz word today?
Examples

• Several biological materials are nanomaterials- bone, hair, wing scales on a butterfly etc.
• Viruses are nanomaterials
• Clays
• Pigments
• Volcanic ash
Engineered nanomaterials (ENM or EN)

Materials manufactured for their properties

• Sunscreens
• Paints
• Cosmetics
• Fillers
• Catalysts

INCIDENTAL: ultrafine airborne particles
NATURAL
• Conventional materials have grain size anywhere from 100 µm to 1mm and more

• Particles with size between 1-100(0) nm are normally regarded as Nanomaterials

• The average size of an atom is in the order of 1-2 Angstroms in radius.

• 1 nanometer = 10 Angstroms

• 1 nm there may be 3-5 atoms
Two principal factors cause the properties of nanomaterials to differ significantly from Bulk materials:

- **Increased relative surface area**: a greater amount of the material comes into contact with surrounding materials and increases reactivity.

- **Quantum effects**.
  
  These factors can change or enhance properties such as reactivity, strength and electrical characteristics.
Surface Effects

- As a particle decreases in size, a greater proportion of atoms are found at the surface compared to those inside. For example, a particle of
  - Size-30 nm -> 5% of its atoms on its surface
  - Size-10 nm -> 20% of its atoms on its surface
  - Size-3 nm -> 50% of its atoms on its surface
- Nanoparticles are more reactive than large particles (Catalyst)
Quantum Effects

Quantum confinement
The quantum confinement effect can be observed once the diameter of the particle is of the same magnitude as the wavelength of the electron Wave function. Quantum confinement is responsible for the increase of energy difference between energy states and band gap. A phenomenon tightly related with the optical and electronic properties of the materials.

When materials are this small, their electronic, mechanical and optical properties deviate substantially from those of bulk materials.(GOLD)
Fig. (12): Fluorescence emission of (CdSe) ZnS quantum dots of various sizes and absorption spectra of various sizes and shapes of gold nanoparticles (Chem. Soc. Rev., 2006, 35, 209–217).
Smaller particles have bigger band gaps
Nanomaterials have unique properties

<table>
<thead>
<tr>
<th>Properties</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalytic</td>
<td>Better catalytic efficiency through higher surface-to-volume ratio</td>
</tr>
<tr>
<td>Electrical</td>
<td>Increased electrical conductivity in ceramics and magnetic nanocomposites, increased electric resistance in metals</td>
</tr>
<tr>
<td>Magnetic</td>
<td>Increased magnetic coercivity up to a critical grain size, superparamagnetic behaviour</td>
</tr>
<tr>
<td>Mechanical</td>
<td>Improved hardness and toughness of metals and alloys, ductility and superplasticity of ceramic</td>
</tr>
<tr>
<td>Optical</td>
<td>Spectral shift of optical absorption and fluorescence properties, increased quantum efficiency of semiconductor crystals</td>
</tr>
<tr>
<td>Sterical</td>
<td>Increased selectivity, hollow spheres for specific drug transportation and controlled release</td>
</tr>
<tr>
<td>Biological</td>
<td>Increased permeability through biological barriers (membranes, blood-brain barrier, etc.), improved biocompatibility</td>
</tr>
</tbody>
</table>

Table 3: Adjustable properties of nanomaterials
• Nanomaterial properties can be ‘tuned’ by varying the size of the particle (e.g. changing the fluorescence colour so a particle can be identified)
• Their complexity offers a variety of functions to products
Classification of Nanomaterials

- Nanomaterials as those which have structured components with at least one dimension less than 100nm.
- One dimension in nanoscale (Other two dimensions are extended)
  - Thin films
  - Surface Coatings
  - Computer chips
- Two dimensions in nanoscale (Other one dimension is extended)
  - Nanowires
  - Nanotubes
- Three dimensions in nanoscale
  - Nanoparticles
  - Precipitates
  - Colloids
  - Quantum dots (tiny particles of semiconductor material)
  - Nanocrystalline materials
- Nanocomposites, Nanophase materials
3D nanomaterials are also known as nanophase materials
There are also nanocomposites.

Fig (3): Classification of Nanomaterials (a) 0D spheres and clusters; (b) 1D nanofibers, nanowires, and nanorods; (c) 2D nanofilms, nanoplates, and networks; (d) 3D nanomaterials.
Characterizing Nanomaterials

**Origin**
- natural
- unintentionally released
- manufactured ("old", "new")

**Chem. composition**
- metals/ metal oxides
- polymers, carbon
- semiconductors
- biomolecules
- compounds...

**Dispersion in**
- gases (aerosols)
- liquids (e.g. gels, ferrofluids)
- solids (e.g. matrix materials)

**Shape/Structure**
- spheres
- needles
- platelets
- tubes

**Aggregation state**
- single particles
- aggregates
- agglomerates

**Surface modification**
- untreated (as obtained in production process)
- coated (e.g. conjugates, polymeric films)
- core/shell particles (e.g. spheres, capsules)

**Nanoparticulate Materials**
- Quantum dots
- Nanoparticles
- Nanotubes
- Ultrafine Aerosols
- Nanocapsules

Figure 1: Characterisation parameters of nanoparticulate materials (source: VDI-TZ)
## Applications of Nanotechnology

<table>
<thead>
<tr>
<th>Automotive industry</th>
<th>Chemical industry</th>
<th>Engineering</th>
</tr>
</thead>
<tbody>
<tr>
<td>lightweight</td>
<td>fillers for paint systems</td>
<td>wear protection for tools and machines (anti blocking coatings, scratch resistant coatings on plastic parts, etc.)</td>
</tr>
<tr>
<td>construction</td>
<td>coating systems based on nanocomposites</td>
<td>lubricant-free bearings</td>
</tr>
<tr>
<td>painting (fillers, base coat, clear coat)</td>
<td>impregnation of papers</td>
<td></td>
</tr>
<tr>
<td>catalysts</td>
<td>switchable adhesives</td>
<td></td>
</tr>
<tr>
<td>tires (fillers)</td>
<td>magnetic fluids</td>
<td></td>
</tr>
<tr>
<td>sensors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coatings for wind-screen and car bodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electronic industry</td>
<td>Construction</td>
<td>Medicine</td>
</tr>
<tr>
<td>data memory (MRAM, GMR-HD)</td>
<td>construction materials</td>
<td>drug delivery systems</td>
</tr>
<tr>
<td>displays (OLED, FED)</td>
<td>thermal insulation</td>
<td>active agents</td>
</tr>
<tr>
<td>laser diodes</td>
<td>flame retardants</td>
<td>contrast medium</td>
</tr>
<tr>
<td>glass fibres</td>
<td>surface-functionalised building materials for wood, floors, stone, facades, tiles, roof tiles, etc.</td>
<td>medical rapid tests</td>
</tr>
<tr>
<td>optical switches</td>
<td>facade coatings</td>
<td>prostheses and implants</td>
</tr>
<tr>
<td>filters (IR-blocking)</td>
<td>groove mortar</td>
<td>antimicrobial agents and coatings</td>
</tr>
<tr>
<td>conductive, antistatic coatings</td>
<td></td>
<td>agents in cancer therapy</td>
</tr>
</tbody>
</table>
## Applications of Nanotechnology

<table>
<thead>
<tr>
<th>Textile/fabrics/non-wovens</th>
<th>Energy</th>
<th>Cosmetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>• surface-processed textiles</td>
<td>• fuel cells</td>
<td>• sun protection</td>
</tr>
<tr>
<td>• smart clothes</td>
<td>• solar cells</td>
<td>• lipsticks</td>
</tr>
<tr>
<td></td>
<td>• batteries</td>
<td>• skin creams</td>
</tr>
<tr>
<td></td>
<td>• capacitors</td>
<td>• tooth paste</td>
</tr>
<tr>
<td><strong>Food and drinks</strong></td>
<td><strong>Household</strong></td>
<td><strong>Sports /outdoor</strong></td>
</tr>
<tr>
<td>• package materials</td>
<td>• ceramic coatings for irons</td>
<td>• ski wax</td>
</tr>
<tr>
<td>• storage life sensors</td>
<td>• odors catalyst</td>
<td>• antifogging of glasses/goggles</td>
</tr>
<tr>
<td>• additives</td>
<td>• cleaner for glass, ceramic, floor,</td>
<td>• antifouling coatings for ships/boats</td>
</tr>
<tr>
<td>• clarification of fruit juices</td>
<td>windows</td>
<td>• reinforced tennis rackets and balls</td>
</tr>
</tbody>
</table>

Table 5: Overview on applications of nanomaterial based products in different areas
• Examples:
  • Amorphous silica fume (nano-silica) in **Ultra High Performance Concrete** – this silica is normally thought to have the same human risk factors as non-nano non-toxic silica dust
  • Nano platinum or palladium in **vehicle catalytic converters** - higher surface area to volume of particle gives increased reactivity and therefore increased efficiency
  • Crystalline silica fume is used as an **additive in paints or coatings**, giving e.g. self-cleaning characteristics – it has a needle-like structure and sharp edges so is very toxic and is known to cause silicosis upon occupational exposure

References:
http://www.efbww.org/pdfs/Nano.pdf
http://www.nano.gov/nanotech-101/special
http://old.vscht.cz/monolith/
http://www.efbww.org/pdfs/Nano.pdf
Nanoparticle synthesis

Nanotechnologies can be:

- Top-down
  - Etching a block of material down to the desired shape
  - Chips and processors

- Bottom-up
  - Building materials atom by atom - like lego
  - Nanoparticles such as C_{60}, carbon nanotubes, quantum dots
Gas phase synthesis
Sol: colloid, molecules suspended in a liquid
Gel: the molecules form a cross linked polymer
Aerogel: gas phase
Eg. metal chlorides, silica
• In 2004, a scientist working in the US proposed 4 generations of nanotechnologies, with the 1st generation already existing: nanomaterials

• 1st generation generally combines a nanomaterial with another material to introduce a new functionality or enhance performance/behaviour

• Increasing complexity and ethical issues

• Generational timeline was optimistic
**Graphite**

It is also a crystalline form of carbon.

**STRUCTURE OF GRAPHITE**

In graphite each carbon atom is covalently bonded to three carbon atoms to give trigonal geometry. Bond angle in graphite is 120°. Each carbon atom in graphite is sp² hybridized. Three out of four valence electrons of each carbon atom are used in bond formation with three other carbon atoms while the fourth electron is free to move in the structure of graphite. Basic trigonal units unite together to give basic hexagonal ring. In hexagonal ring C-C bond length is 1.42Å. In graphite these rings form flat layers. These layers are arranged in parallel, one above the other. These layers are 3.35Å apart and are held together by weak van der waals forces only. These layers can slide over one another. Thus it is very soft. Fourth electron of each carbon atom forms delocalized p-bonds which spreads uniformly over all carbon atoms. Due to this reason graphite conducts electricity parallel to the of its plane.

**Uses**

- Solid lubricants
- Moderator in nuclear reactors
- Electrodes
- Pencil lead
Fullerenes
The 1996 Nobel Prize for Chemistry was won by Harold W. Kroto, Robert F. Curl and Richard E. Smalley for their discovery in 1985 of a new allotrope of carbon, in which the atoms are arranged in closed shells. The new form was found to have the structure of a truncated icosahedron, and was named Buckminster fullerene, after the architect Buckminster Fuller who designed geodesic domes in the 1960's. In 1990 physicists W. Krätschmer and D.R. Huffman for the first time produced isolable quantities of C60 by causing an arc between two graphite rods to burn in a helium atmosphere and extracting the carbon condensate.

Professor Sir Harold W. Kroto

C 60

Football like structure
20 hexagones
12 pentagones

Geodesic dome
Applications
The fullerenes have synthetic pharmaceutical and industrial applications. Degenerative diseases and ordinary aging processes are caused by intracellular Oxygen free radicals with unpaired electrons. C60 fullerenes can react with radicals Thus halting the process of aging. It acts as a good MRI contrast agent. They can also be used in light emitting diodes in different electronic equipment and computing devices
Carbon nanotubes (CNTs) are allotropes of carbon with a cylindrical nanostructure. Nanotubes have been constructed with length-to-diameter ratio of up to 132,000,000:1, significantly larger than for any other material. These cylindrical carbon molecules have unusual properties, which are valuable for nanotechnology, electronics, optics and other fields of materials science and technology. In particular, owing to their extraordinary thermal conductivity and mechanical and electrical properties, carbon nanotubes may find applications as additives to various structural materials. Nanotubes are members of the fullerene structural family, which also includes the spherical buckyballs, and the ends of a nanotube may be capped with a hemisphere of the buckyball structure. Their name is derived from their long, hollow structure with the walls formed by one-atom-thick sheets of carbon, called graphene. These sheets are rolled at specific and discrete ("chiral") angles, and the combination of the rolling angle and radius decides the nanotube properties; for example, whether the individual nanotube shell is a metal or semiconductor. Nanotubes are categorized as single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs). Individual nanotubes naturally align themselves into "ropes" held together by van der Waals forces.
(a) Armchair, (b,c) zig-zag and (d) chiral tube; (a) metallic, (b) small gap semiconductor, and (c,d) semiconductor.
Properties and Applications:-
Nanotubes are extremely strong and stiff and relatively ductile. For single walled nanotubes tensile strengths range between 50 and 200 Gpa more than carbon fibres. This is strongest known material. Elastic modulus values are of the order of one tera (10^12) pascal with fracture strains between 5% to 20%. They have very low densities. Because of these properties carbon nano tube has been termed as ultimate fibre. It has been regarded as 100 times stronger Than steel at the same time six times lighter (weight wise). They find applications in filling material in composite material.

They can act as either conductors or semiconductors depending on their chirality so they find their applications in molecular electronics and Computers they are used as ultra sensitive electrochemical sensors. They are used in batteries and fuel cells.

Field emission results from the tunnelling of electrons from a metal tip into Vacuum under application of strong electric field. Small diameter and high aspect ratio (lower loading of CNTs is needed compare to other conductive additives to achieve same electrical conductivity .CNTs are very favourable for field emission.) Even for moderate voltages a strong electric field develops at free end of supported CNTs because of their sharpness. The large surface area and high absorbency of CNTs make them ideal candidates for use in air gas and water filtration.
Carbon nanocones

The open carbon cone can be modeled as a wrapped graphene sheet. In order to have strain-free, seamless wrapping, a sector has to be cut out of the sheet. That sector should have an angle of $n \times 60^\circ$, where $n = 1, \ldots, 5$

The nanocones are produced by carbon condensation on a Graphite substrates and pyrolysis of heavy oil. The essence of the method is heating the graphite surface with intensive short laser pulse, which evaporates some number of atoms from role as the cut and glue procedure. On the graphene sheet, and other atoms rearrange into the conical surface described above. There is also one special class of nanocones, called nanohorns with exact five defects. These structures with good electron emission properties are easy to get and stable enough. Nano horns show metallic behaviour.
A **nanowire** is a nanostructure, with the diameter of the order of a nanometer ($10^{-9}$ meters). Alternatively, nanowires can be defined as structures that have a thickness or diameter constrained to tens of nanometers or less and an unconstrained length. At these scales, quantum mechanical effects are important — which coined the term "quantum wires". Many different types of nanowires exist, including metallic (e.g., Ni, Pt, Au), semiconducting (e.g., Si, InP, GaN, etc.), and insulating (e.g., SiO$_2$, TiO$_2$). **Molecular nanowires** are composed of repeating molecular units either organic (e.g. DNA) or inorganic (e.g. Mo$_6$S$_{9-x}$I$_x$). The nanowires could be used, in the near future, to link tiny **components** into extremely small **circuits** using **nanotechnology**, such components could be created out of **chemical compounds**.
Typical nanowires exhibit aspect ratios (length-to-width ratio) of 1000 or more. As such they are often referred to as one-dimensional (1-D) materials. Nanowires have many interesting properties that are not seen in bulk or 3-D materials. This is because electrons in nanowires are quantum confined laterally and thus occupy energy levels that are different from the traditional continuum of energy levels or bands found in bulk materials.

Nano wires are synthesized in laboratory from elements. There are many applications where nanowires may become important in electronic, opto-electronic and nanoelectromechanical devices, as additives in advanced composites, for metallic interconnects in nanoscale quantum devices, as field-emitters and as leads for biomolecular nanosensors.
Haeckelites
If we try to bend graphene sheet it will straighten up. A new hypothetical type of graphene sheet which admits pentagons heptagons and hexagons. It has been proposed, noting that the no. of heptagons and pentagons should be same, in order to compensate for the negative curvature of the heptagons and positive curvature of pentagon. These arrangements are now called haeckelites in honour of Ernst Haeckel a German zoologist who produced a beautiful drawing of radiolaria, (Organism) in which heptagonal hexagonal and pentagonal rings are observed. Carbon haekalite structures are metallic. It is possible to roll up the haekelite sheets to form nanotubes which will be conductors independent of diameter and chirality.

Haeckelites are three-fold coordinated networks of carbon atoms generated by a periodic arrangement of pentagons, hexagons and heptagons. They were first proposed by Humberto and Mauricio Terrones and their colleagues in 2000. They were named in honour of Ernst Haeckel, whose diagrams of radiolaria contained similar structural features. They have not yet been synthesised in the laboratory, but have been the subject of a considerable amount of theoretical work.
Applications of nanocarbon

- Production is several 1000 tons/year
- Motor brushes
- Water filters
- Sport goods
- Boat hulls
- Automotive parts
- Nanotubes can improve the mechanical performance of biomedical polymers, but their toxicity is a concern.
Quantum dots

• Quantum dots (QD) are very small semiconductor particles, only several nanometres in size, so small that their optical and electronic properties differ from those of larger particles.

• In semiconductors, light absorption generally leads to an electron being excited from the valence to the conduction band, leaving behind a hole. The electron and the hole can bind to each other to form an exciton. When this exciton recombines (i.e. the electron resumes its ground state), the exciton's energy can be emitted as light (fluorescence). In a simplified model, the energy of the emitted photon can be understood as the sum of the band gap energy between the highest occupied level and the lowest unoccupied energy level, the confinement energies of the hole and the excited electron, and the bound energy of the exciton (the electron-hole pair).

• In QDs the gap changes with QD size.
As the confinement energy depends on the quantum dot's size, both absorption onset and fluorescence emission can be tuned by changing the size of the quantum dot during its synthesis. The larger the dot, the redder (lower energy) its absorption onset and fluorescence spectrum. Conversely, smaller dots absorb and emit bluer (higher energy) light.
Can be purchased

CdSe Quantum Dots

$\text{Cd}_{43}\text{Se}_{44}$  $\text{Cd}_{152}\text{Se}_{141}$  $\text{Cd}_{484}\text{Se}_{495}$
Core shell nanomaterials and nanosensors

~3-15nm
Synthesis of magnetic cytosine-imprinted chitosan nanoparticles

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³Department of Chemical and Materials Engineering, National University of Kaohsiung, Kaohsiung 81148, Taiwan
⁴Department of Life Sciences, National University of Kaohsiung, Kaohsiung 81148, Taiwan
2.2. Formation of magnetic CIPs

The preparation of magnetic molecularly imprinted and non-imprinted chitosan nanoparticles (respectively CIPs and NIPs) is shown in scheme 1. MNPs [30] were added to the chitosan solution (chitosan/acetic acid solution = 0.0001–0.1 wt%) to a concentration of 0.2 mg ml\(^{-1}\). The interactions between chitosan segments and MNPs were the hydrogen bonds of hydroxyl groups, hydrophobic and carboxylic acid and amine with the oleic acid [30], which were used to enhanced the suspension of MNPs. The chitosan/magnetic particle solution was then mixed with 0–1.0 g ml\(^{-1}\) of cytosine. The chitosan/cytosine template/magnetic particle suspension was dispersed in 10 ml deionized water at 4 °C followed by removal of the cytosine template from magnetic chitosan nanoparticles by washing with 1 ml deionized (DI) water for 10 min and separating a magnetic plate for three times. All composite nanoparticles were equilibrated with deionized water overnight before use. The magnetic NIPs were prepared identically, except that the cytosine template was omitted.
Cytosine and chitosan in acetic solution + Magnetic Nanoparticles (MNP) → Phase Separation

Magnetic Cytosine-imprinted Chitosan Nanoparticles (CIPs) + pEGFP-C1 vector → Transfection

Template Removal
Figure 1. Optimization of the preparation of magnetic cytosine-imprinted chitosan nanoparticles. The cytosine adsorption of particles prepared with (a) various chitosan concentrations; (b) various cytosine imprinting concentrations and their mean particles size. The cytosine imprinting concentration in (a) and the chitosan concentration in (b) are 0.1 mg ml⁻¹ and 0.01 wt%, respectively, and rebound with 0.1 mg ml⁻¹ of cytosine for 30 min (n = 3 for each data point).

Figure 2. (a) Raman scattering of cytosine and magnetic cytosine-imprinted chitosan monocrystals before and after template cytosine
Nanosensors: site specific sensing at the nanoscale

Fluorescent nanosensors for intracellular measurements: synthesis, characterization, calibration, and measurement

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² Department of Chemical and Biomolecular Engineering, The University of Melbourne, Victoria, Australia

FIGURE 1 | Monomers (i–iv) and (v–viii) are used to synthesize polycrylamide and silica sol-gel nanoparticles, respectively: (i) acrylamide, (ii) \(N,N'\)-methylenebis(acrylamide), (iii) \(N\)-(3-aminopropyl) methacrylamide (APMA), (iv) \(3\)-acyramido\(propyl\)trimethylammonium (ACTA), (v) tetraethoxysilane (TEOS), (vi) methyltriethoxysilane (MTEOS), (vii) \(3\)-aminopropyl triethoxysilane, and (viii) trimethoxy silylpropyl \(N,N,N\)- trimethyl-aminium (TMAC). (A) Free radical polymerization of polycrylamide monomers. (B) Hydrolysis and (C) condensation of silica sol-gel monomers. (D) Diagrammatic representation of a functionalized nanoparticle.
<table>
<thead>
<tr>
<th>Analyte</th>
<th>Fluorophore</th>
<th>$\lambda_{\text{max,abs}}$ (nm)</th>
<th>$\lambda_{\text{max,em}}$ (nm)</th>
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</thead>
<tbody>
<tr>
<td>pH (near neutral)</td>
<td>BCECF</td>
<td>503</td>
<td>525</td>
</tr>
<tr>
<td></td>
<td>BCPCF</td>
<td>505</td>
<td>527</td>
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<tr>
<td></td>
<td>Carboxyfluorescein</td>
<td>492</td>
<td>516</td>
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<td></td>
<td>CarboxySNARF-1</td>
<td>544</td>
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<td>pH (acidic)</td>
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<tr>
<td></td>
<td>CDCF</td>
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<td>525</td>
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<tr>
<td></td>
<td>HPTS</td>
<td>405</td>
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<td></td>
<td>Acridine orange</td>
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<tr>
<td>Oxygen</td>
<td>Ru(II)-tris(4,7-diphenyl-1,10-phenanthroline) chloride</td>
<td>455</td>
<td>615</td>
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<tr>
<td>Reference</td>
<td>Alexa 488</td>
<td>495</td>
<td>519</td>
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<tr>
<td></td>
<td>TAMRA</td>
<td>555</td>
<td>580</td>
</tr>
</tbody>
</table>

The majority of fluorophores are available in an amine reactive form.

Abbreviations: BCECF, 2',7'-Bis-(2-carboxyethyl)-5-(and-6-)carboxyfluorescein; BCPCF, 20,70-bis-(2-carboxypropyl)-5-(and 6)carboxyfluorescein; CDCF, 5(6)carboxyfluorescein; HPTS, 8-hydroxyperene-1,3,6-trisulfonic acid; TAMRA, 6-carboxytetramethylrhodamine.
Image analysis for calibration

A
- Indicator channel
- Reference channel

B
- Remove background
- Remove background

C
- Mask/threshold

D
- Apply mask
- Apply mask

E
- Ratio images

F
- Weight measurement
- Measure mean ratio

G
- Present calibration curve
- Fit equation to curve

Repeat analysis in pH buffers
Nano-in-Micro Self-Reporting Hydrogel Constructs

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² University of Pisa, Research Centre “E. Piaggio”, Largo Lucio Lazzarino 1, 56126, Italy
³ School of Pharmacy, University of Nottingham, University Park, Nottingham, NG7 2RD, United Kingdom
Figure 1. Microbead fabrication: (A) The Sphyga layout; (B) The working principle, whereby multiple parameters are controlled to obtain microspheres with well-defined and controlled shape and size; (C) Brightfield microscope images of microbeads (scale bar 100 μm).
Figure 2. Calibration and time response of self-reporting Nano-in-Micro hydrogel beads. (A) Nano-in-Micro beads from ink ANs immersed in different pH buffers, showing maximum projections from confocal acquisitions. Scale bar 100 µm. (B) Reversibility of pH-sensitive microbeads with spectrofluorimetric tests: at the “start” samples were immersed in pH = 5.5 buffer solution, then different samples were exposed to “calibration” buffers [pH 5.5:0.5:7.5], finally they were placed in pH = 7.5 buffer (“end”). (C) Confocal analysis of microbeads immersed in cell culture medium: acquisitions were performed: immediately after fabrication (t = 0) and after 1, 5, 30 and 60 minutes of immersion in cell culture medium, reporting the measured pH value in the graph for both ACNs (gray squares) and ADNs (black circles). Reported values are obtained averaging several confocal sections of at least n = 5 beads.
Figure 5. Confocal acquisitions of DAPI stained HepG2 in alginate/protein/pH-Ns microbead (ACHNs, day 10): (A) Sequence of z-projections showing (clockwise from top left) the bead with respectively merged, TAMRA (red), DAPI (blue) and FAM (green) acquisition channels; (B) orthogonal views and (C) volume view. Scale bars 100 μm.