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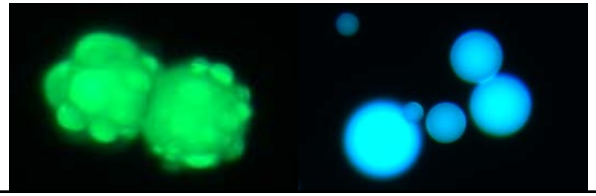
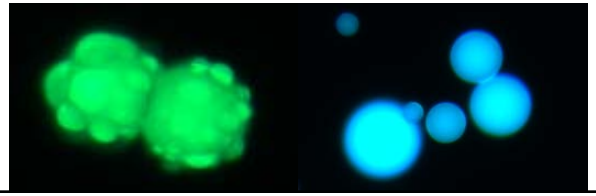


INNOPROFILE UNTERNEHMEN REGION

Bead-based multiplex assays for autoimmunity screening

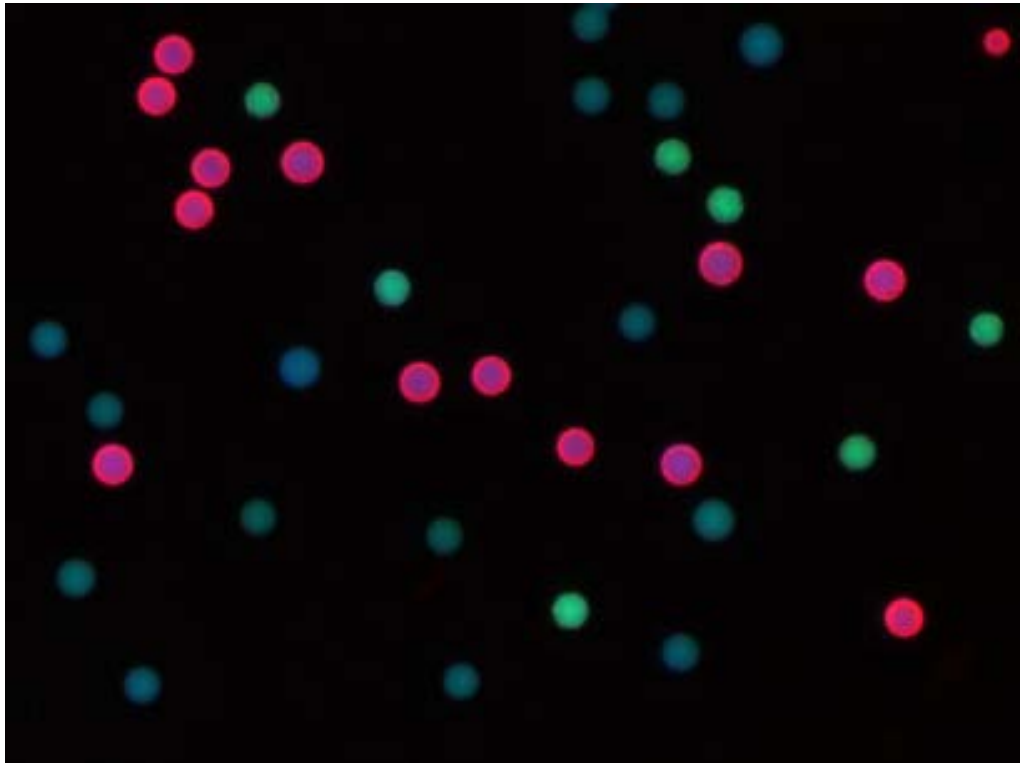
Rico Hiemann, Doctor of Theoretical Medicine
Department of Biology, Chemistry and Process Technology
Lausitz University of Applied Sciences, Senftenberg, Germany
October, 2009

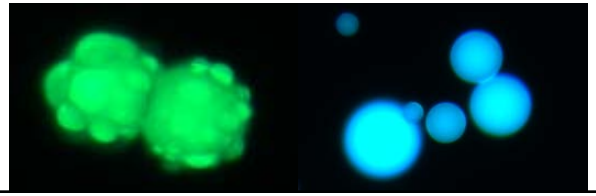


Micro- and Nanoparticles, Microspheres, Beads – definitions

- Beads are defined as spheric, non-porous particles
- Diameter of <100 µm
- Different geometrical forms
- Beads and particles can be dyed (e.g. fluorescent) or colourless or show magnetic behaviour

Beautiful beads

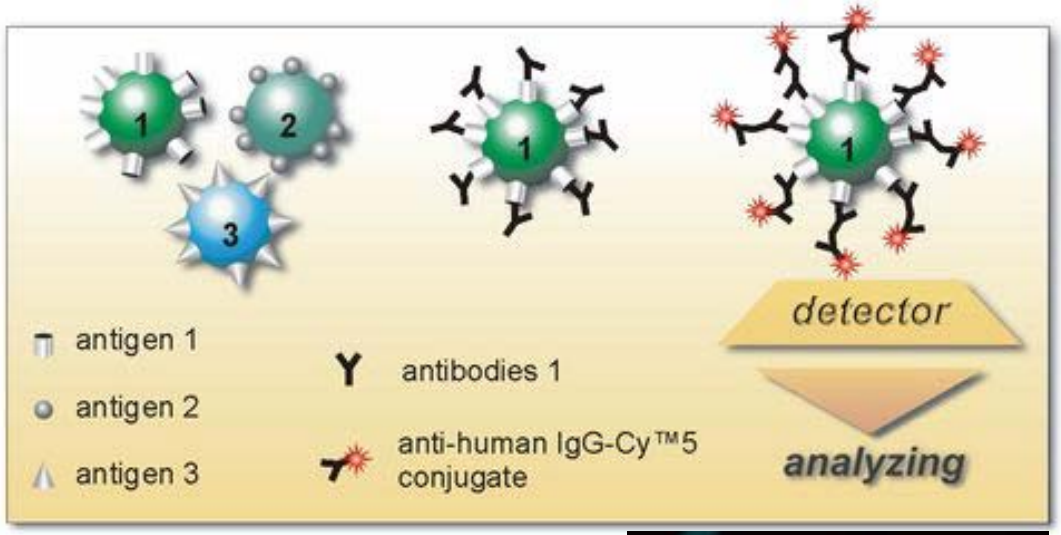




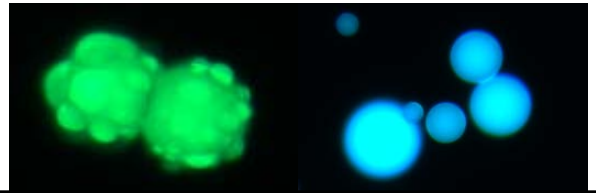
Routine diagnostic

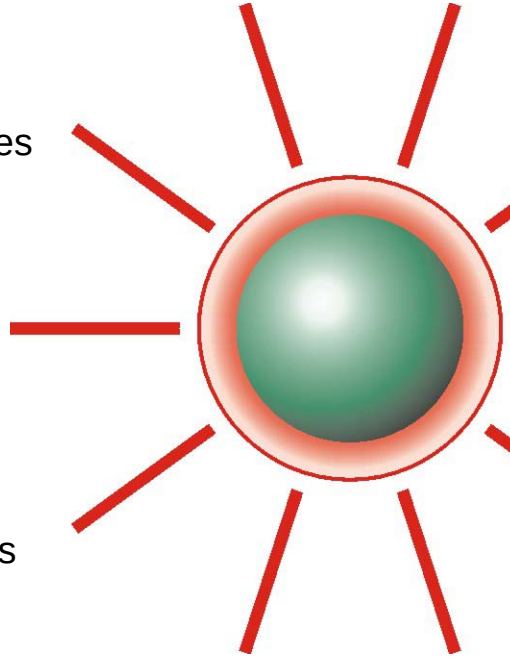
The requirements of assays in routine diagnostic are permanently rising.

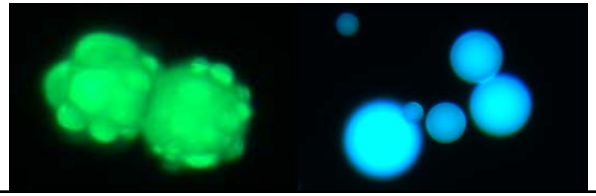
- Low costs
- More cost effectiveness
- Highest quality
- Full automated
- Standardization
- Quantification
- Multitasking platforms
- Multiplex immunoassays

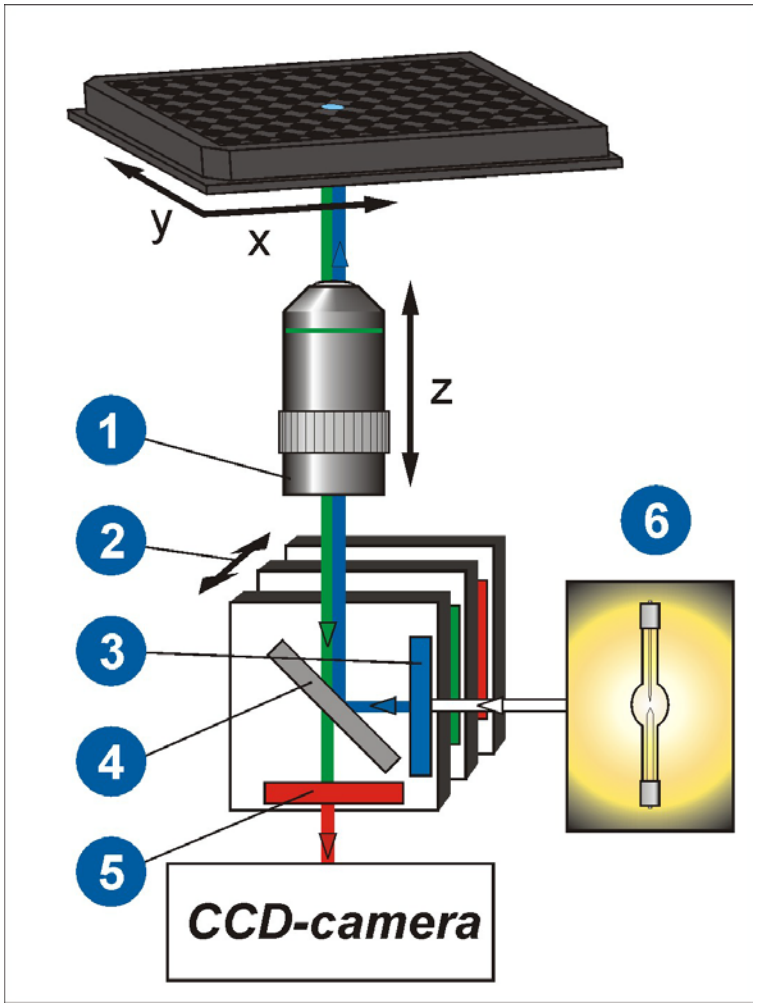


Detection of multiple specific autoantibodies as separate entities in parallel at the same time.

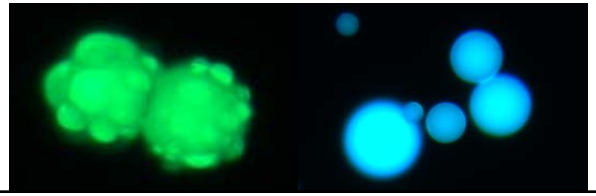


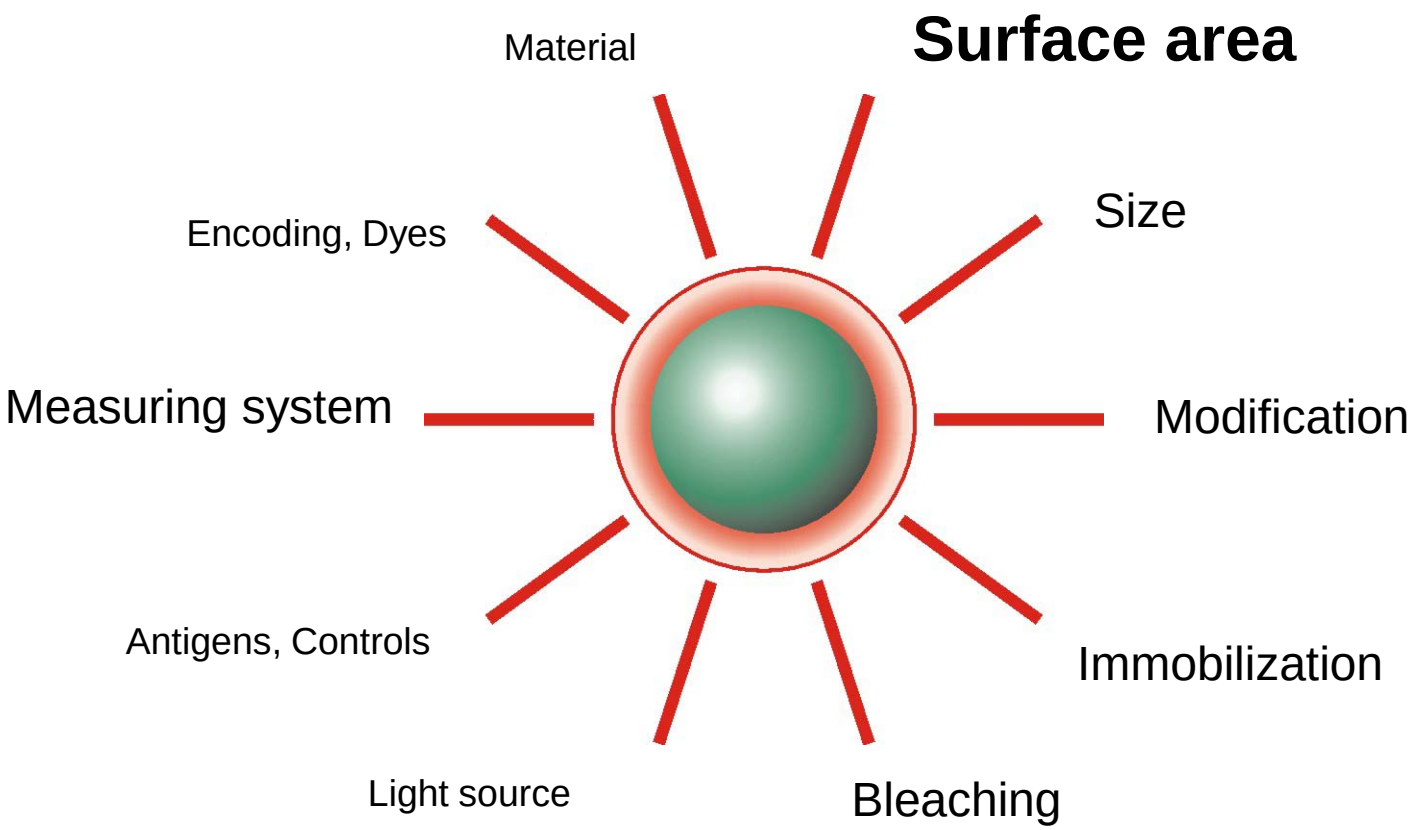

Material
Surface area
Encoding, Dyes
Size
Measuring system
Modification
Antigens, Controls
Immobilization
Light source
Bleaching





Measuring principle
1= objective
2= filter turret
3= excitation filter
4= dichroic mirror
5= emission filter
6= light source (LED)

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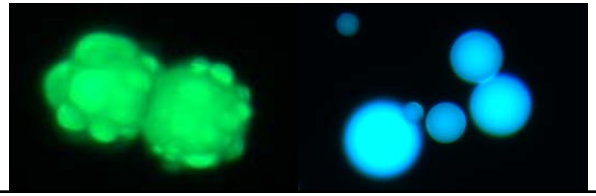
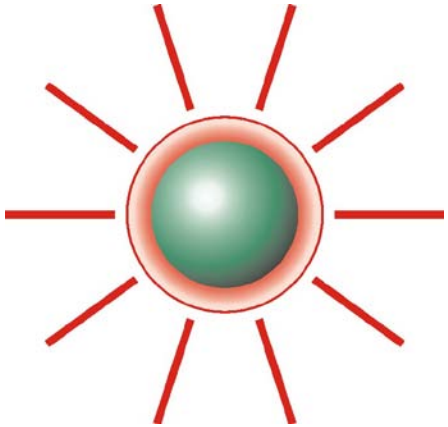
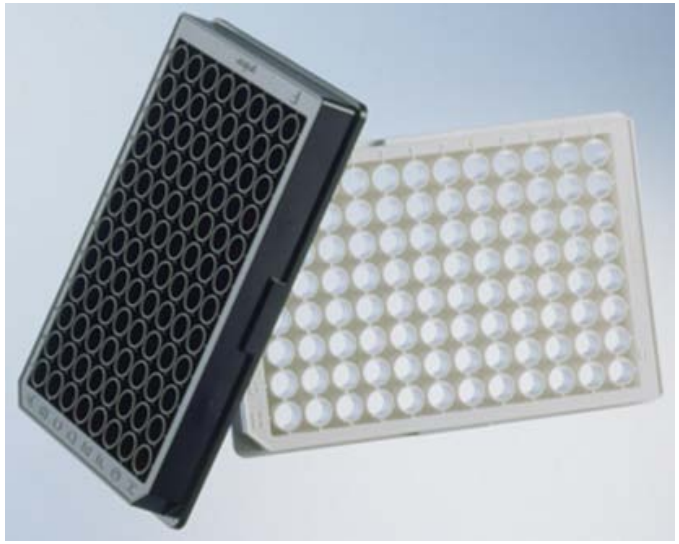


Surface area





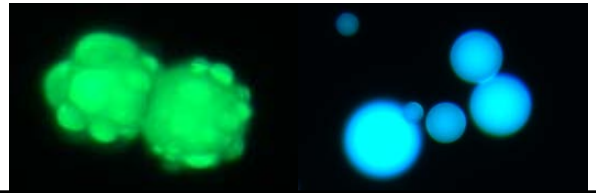
Microbeads
Diameter: 10.00 µm

Surface area: 314.16 μm^2

96 Well cavity
Diameter: 6.50 mm

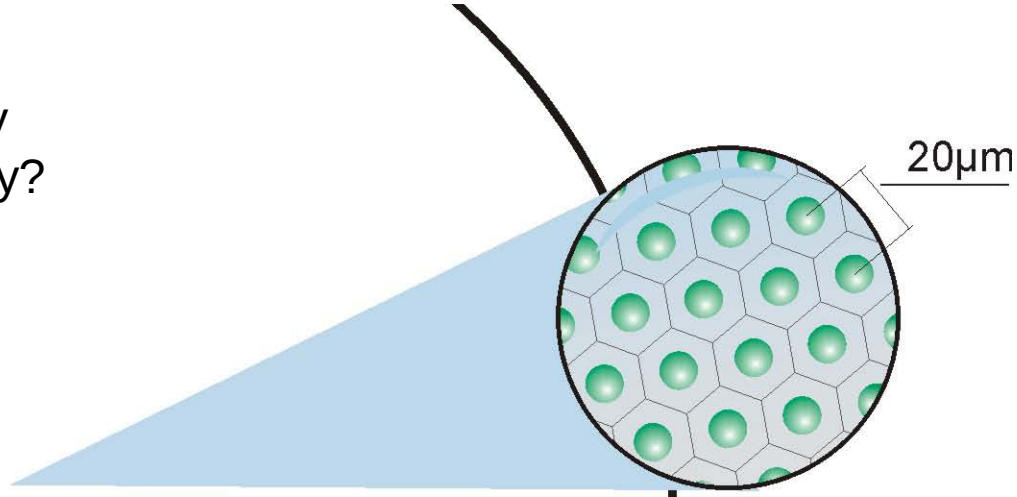
Surface area: 33 180 000.00 μm^2

- **Amount of biomolecules for surface saturation can differ**
- **Microbeads have a small reaction surface**
- **Special equipment for analyzing of microbeads is necessary**

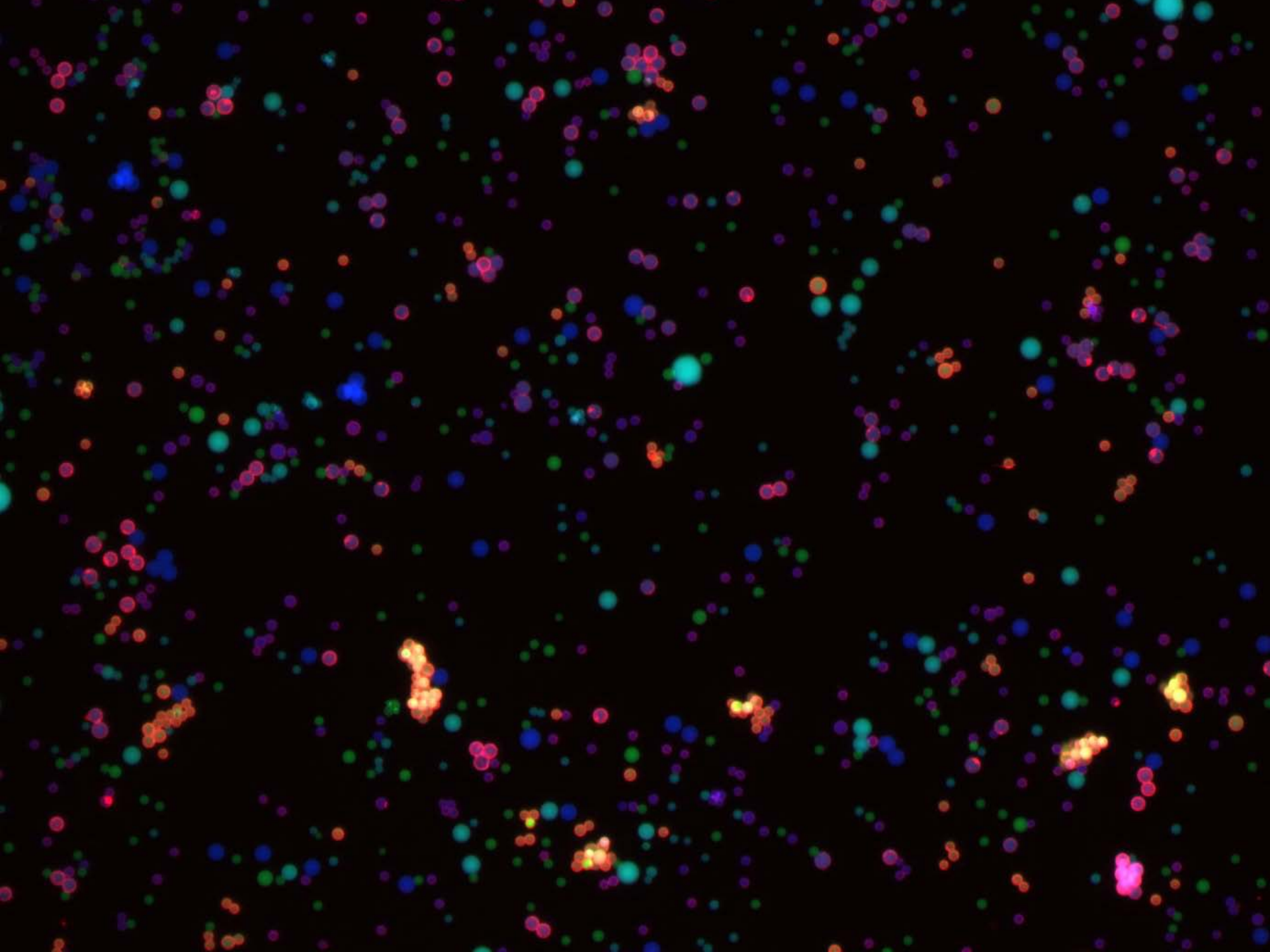


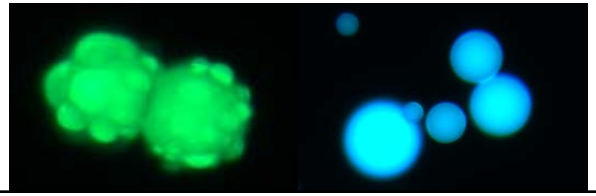
Surface area

How many microbeads are necessary to cover the surface area of one cavity?

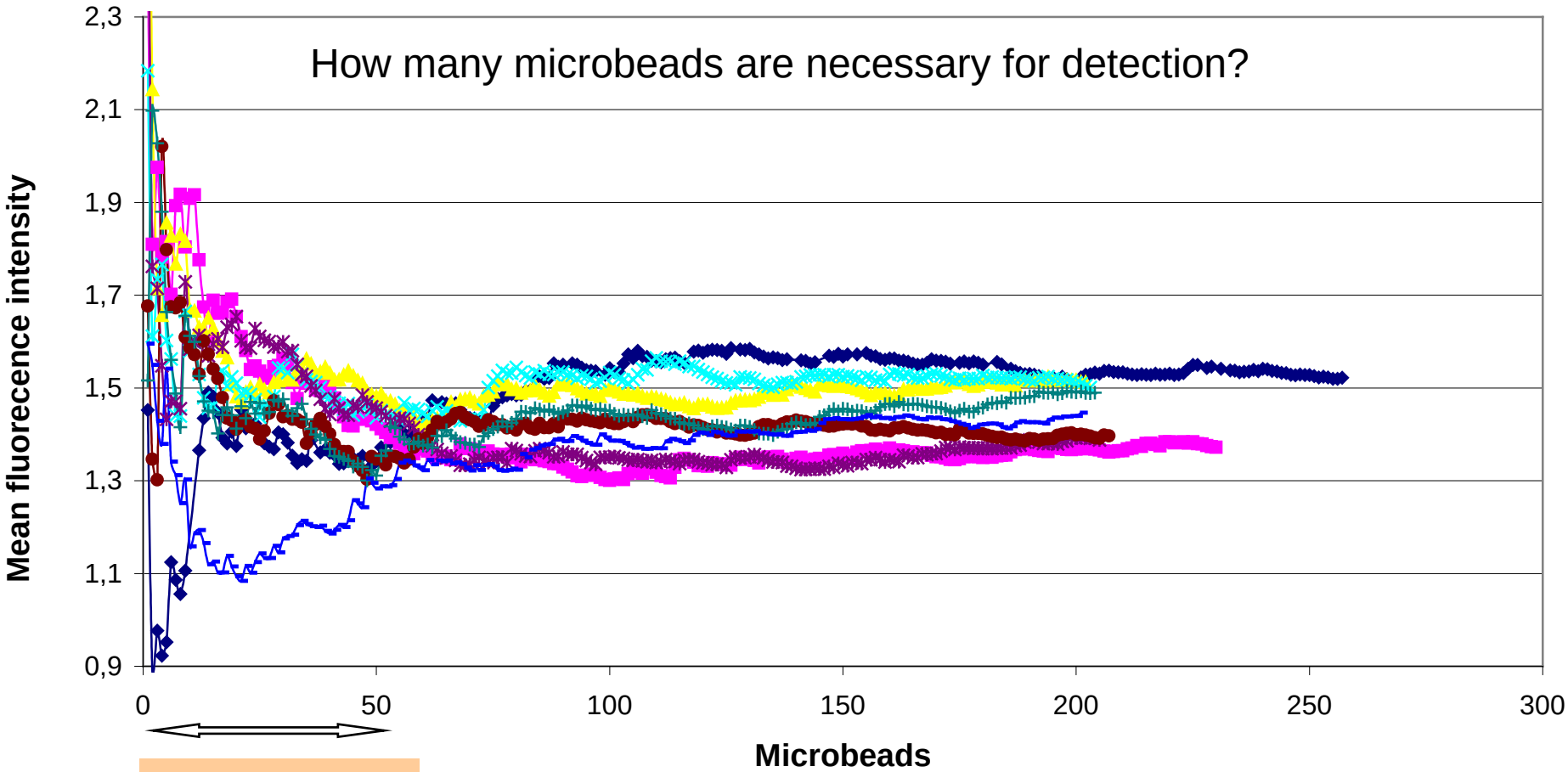


Theoretical:	20x objective
Microbeads per well :	**95 383**
Microbeads per image :	**5 436**

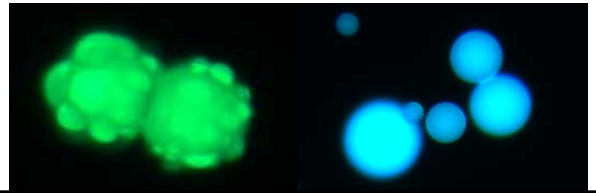


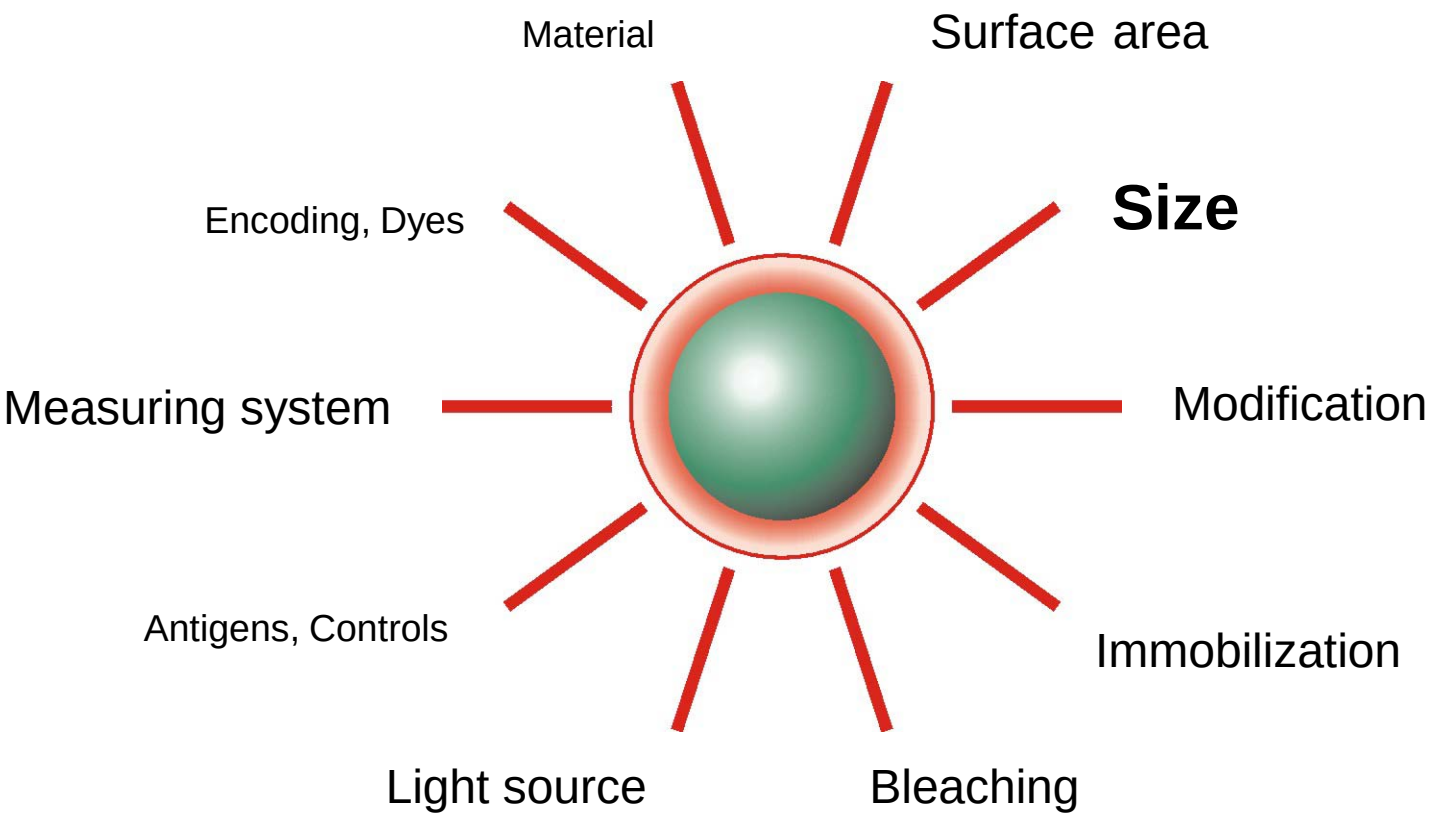


Mean fluorescence intensity of anti-IgG Cy5 conjugated antibody in relation to analyzed microbeads



random noise




Material
Surface area
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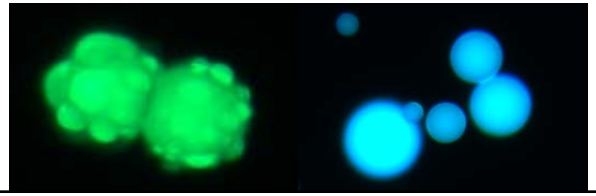
Encoding, Dyes
Measuring system
Modification
Antigens, Controls
Immobilization
Light source
Bleaching

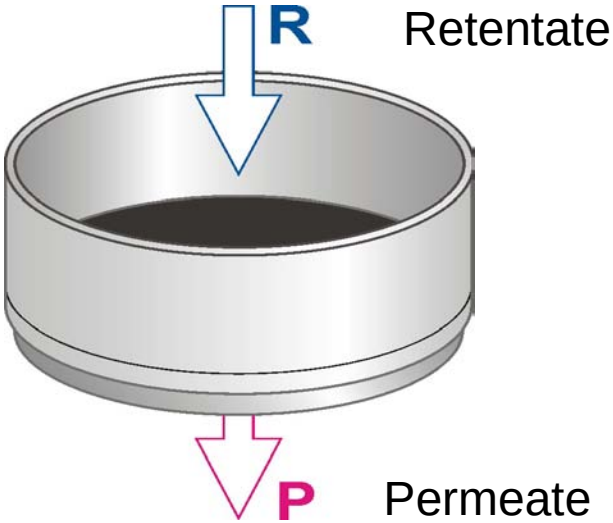


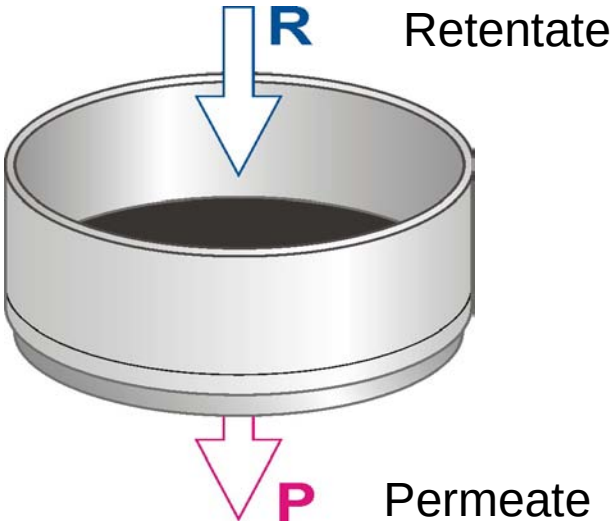
Microbead size

Important for

- Handling
- Analyzing
- Immobilization

- Additional encoding parameter of beads





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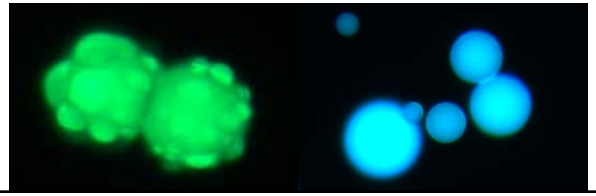
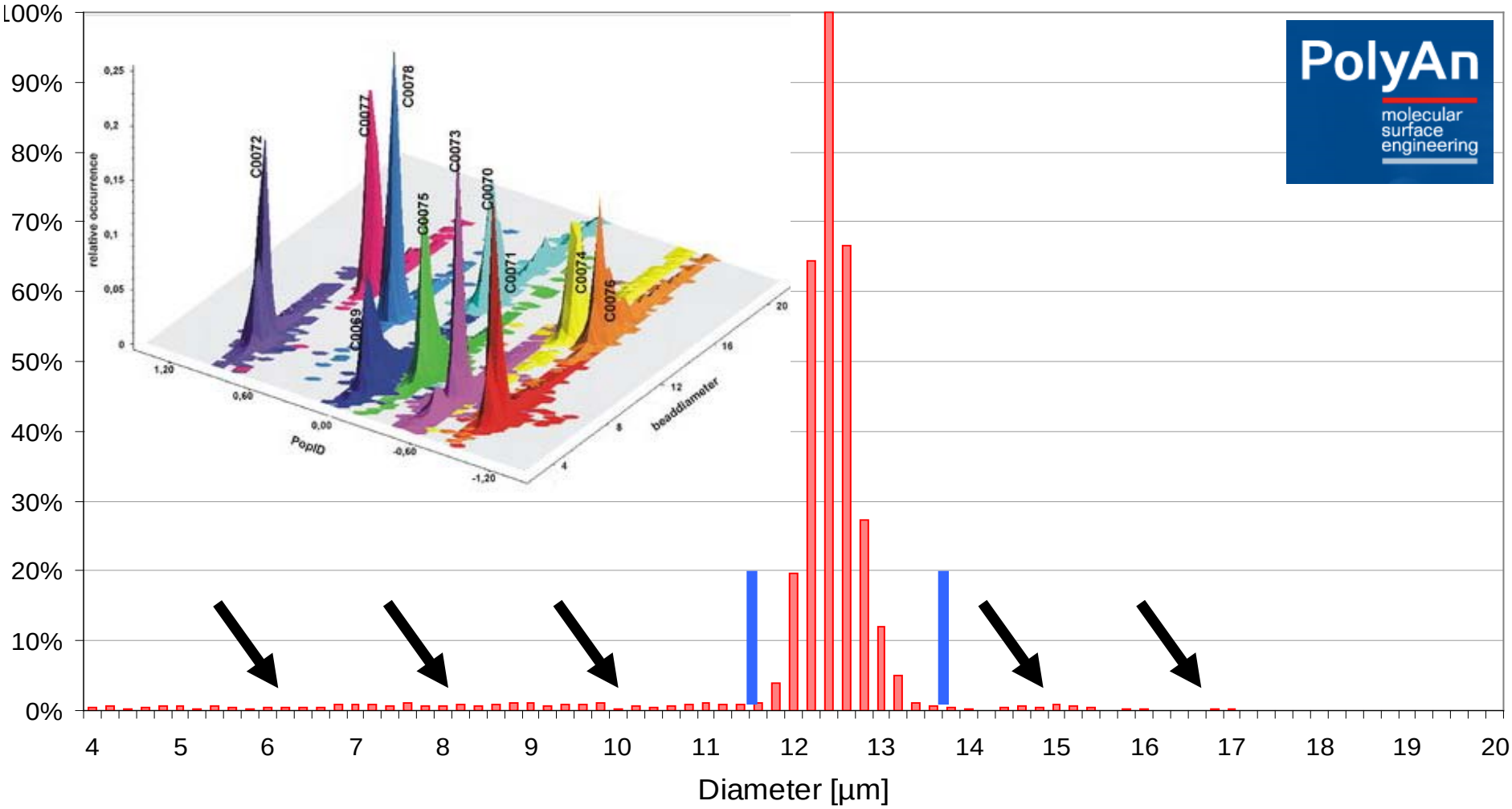
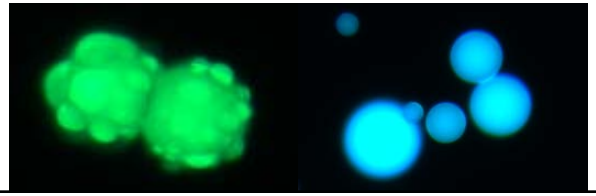
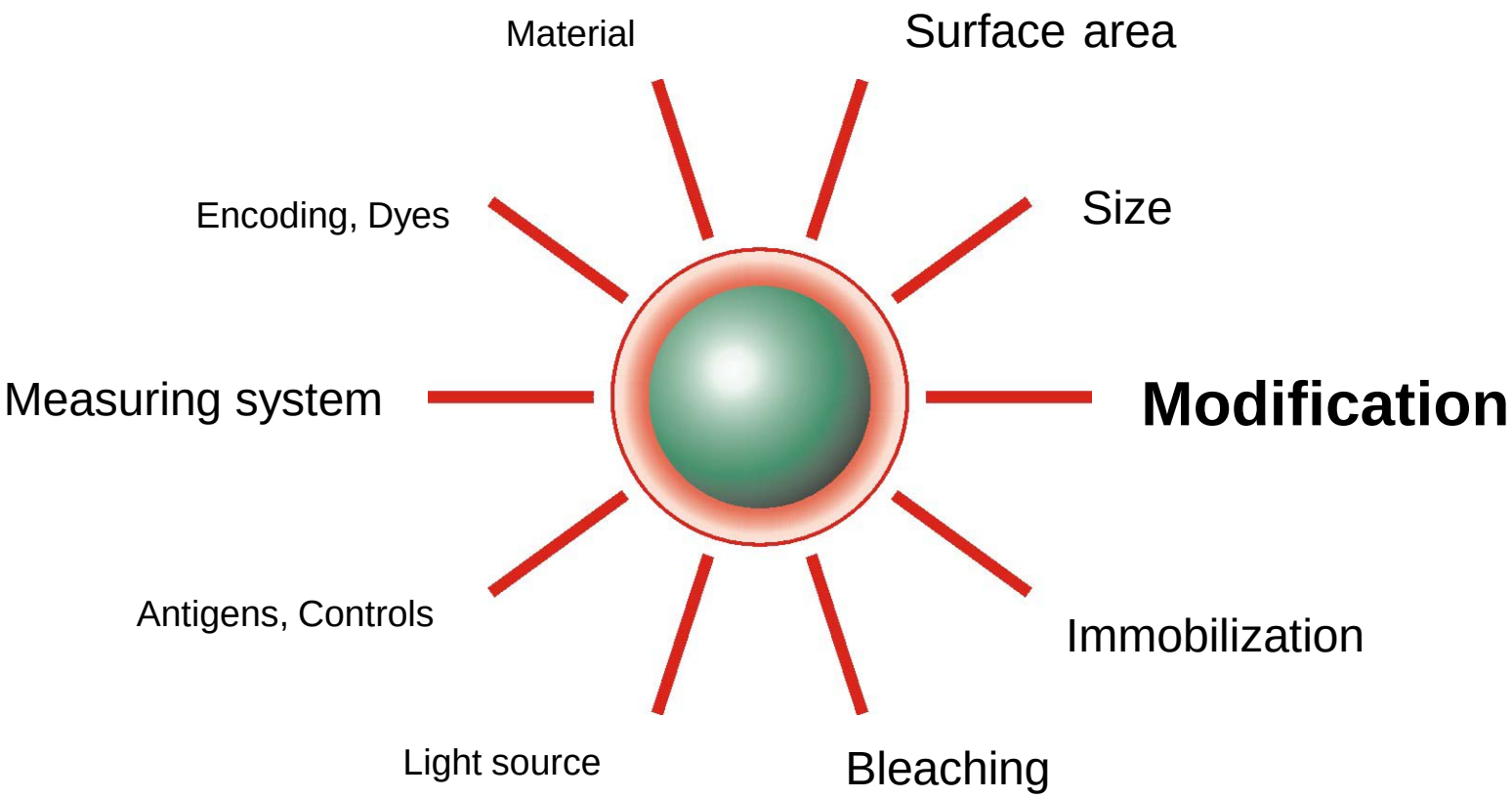


INNOPROFILE UNTERNEHMEN REGION

Microbead population before sieving






Material
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Size
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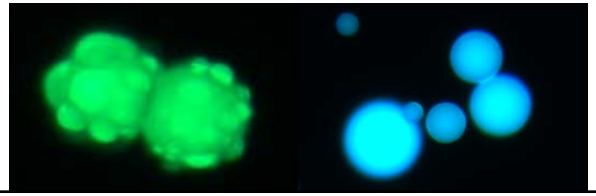
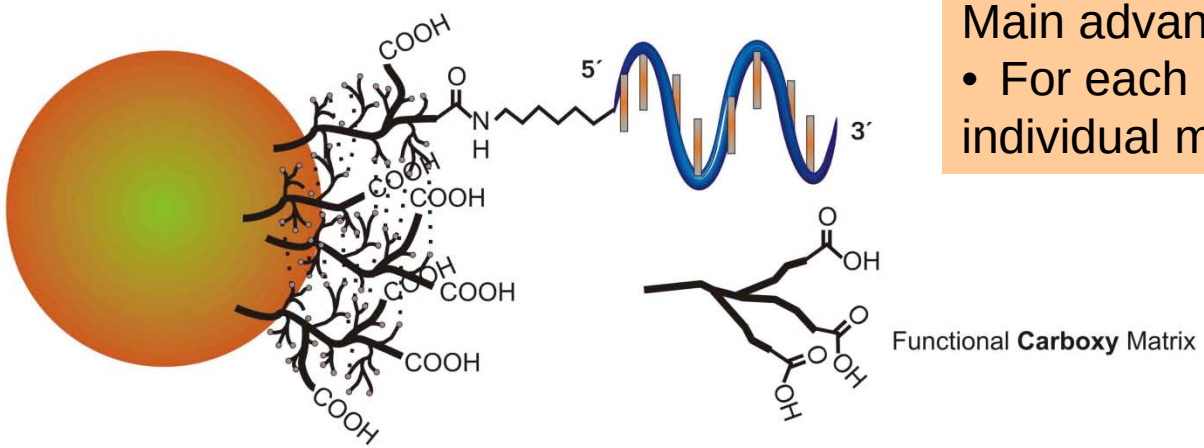
Immobilization
Light source
Bleaching



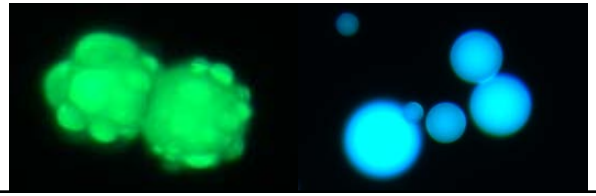
Modification

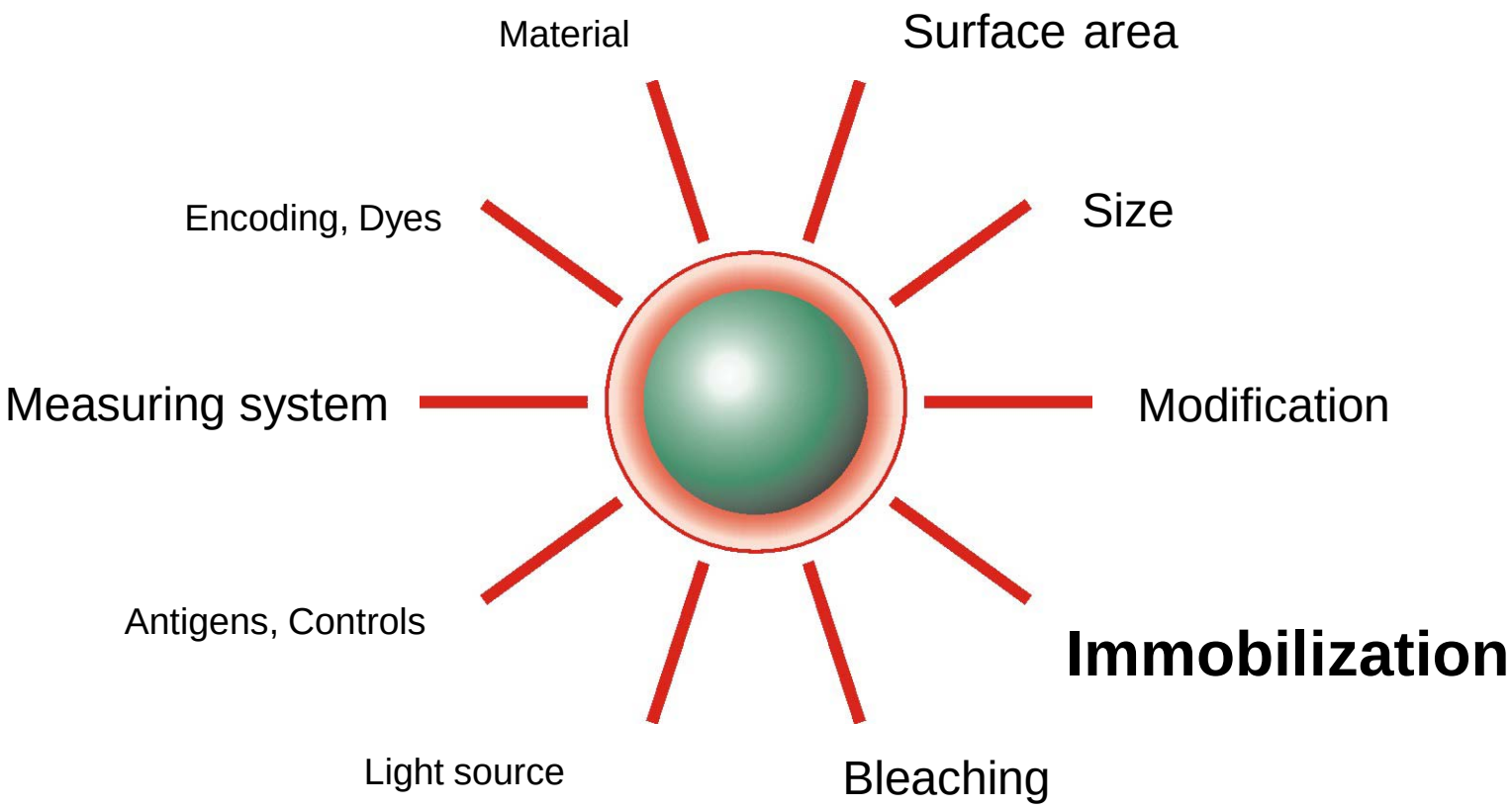


Main advantage:
- For each protein or DNA an individual modification is possible

Widely used:
covalent coupling of amino residues of proteins or amino-modified oligonucleotide with activated carboxy surface on microbeads

Other examples are:
- Epoxy surface → amino residues
- Aldehyd surface → amino residues
- Amino surface → carboxy residues
- Hydroxyl surface → amino residues
- Chloromethyl surface → amino residues

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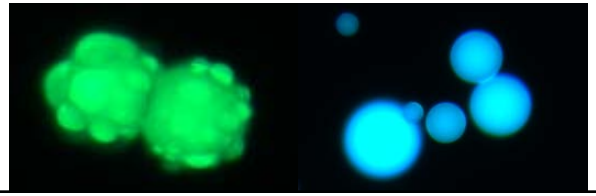
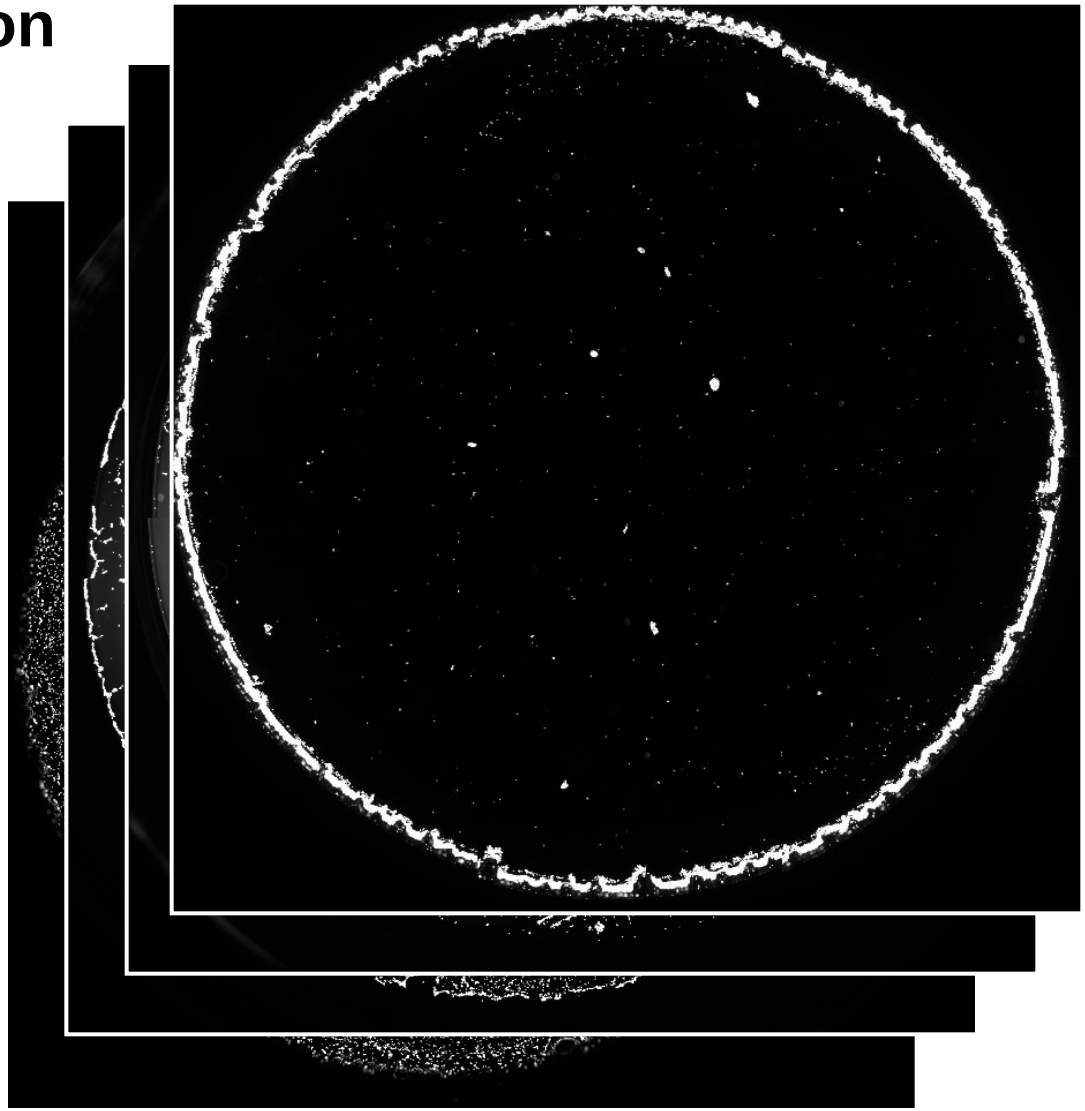
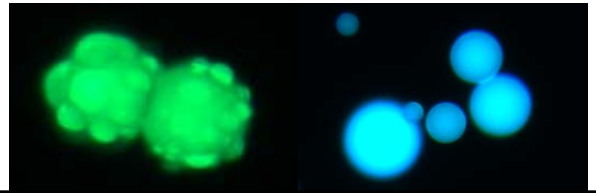


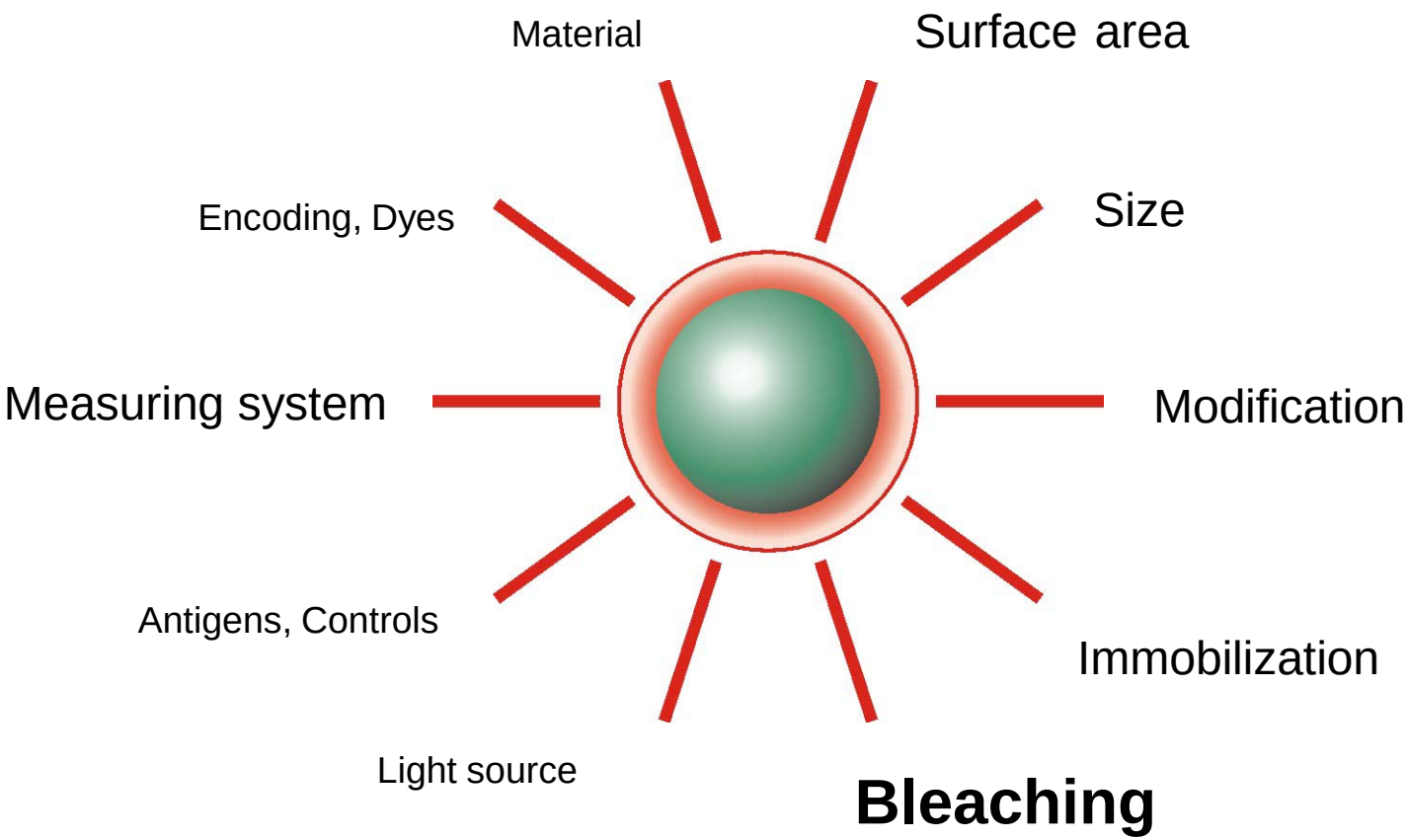
Microbead immobilization

Depends on:

- pH value
- Buffer conditions
- Ionic strength
- Material of microplates
- Material of microbeads
- Proteins and DNA used
- Dry process
- Surface tension



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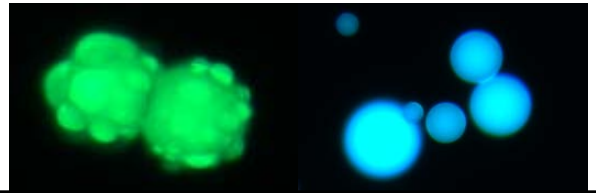
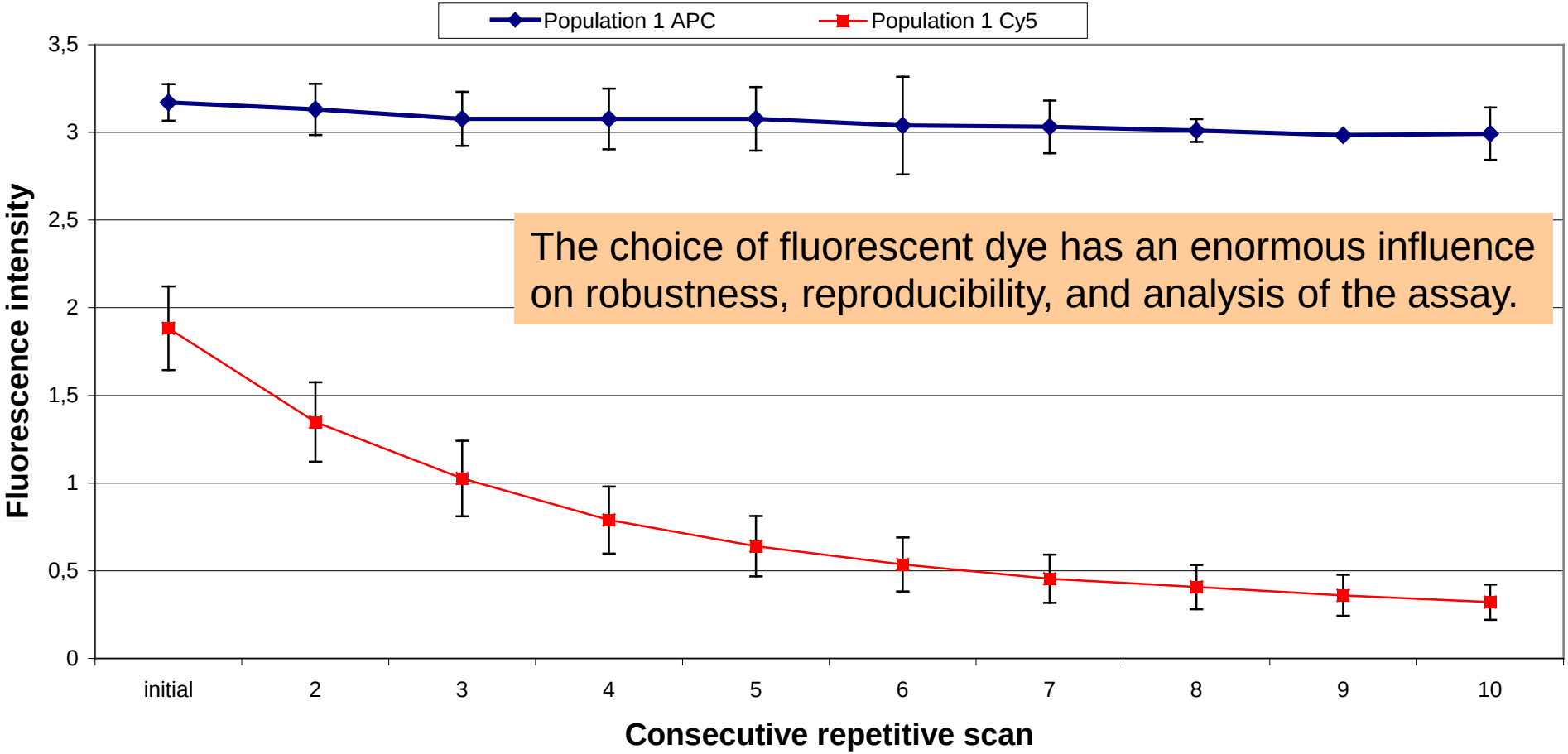
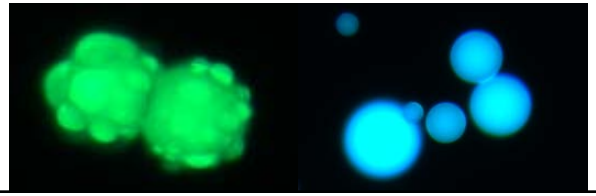
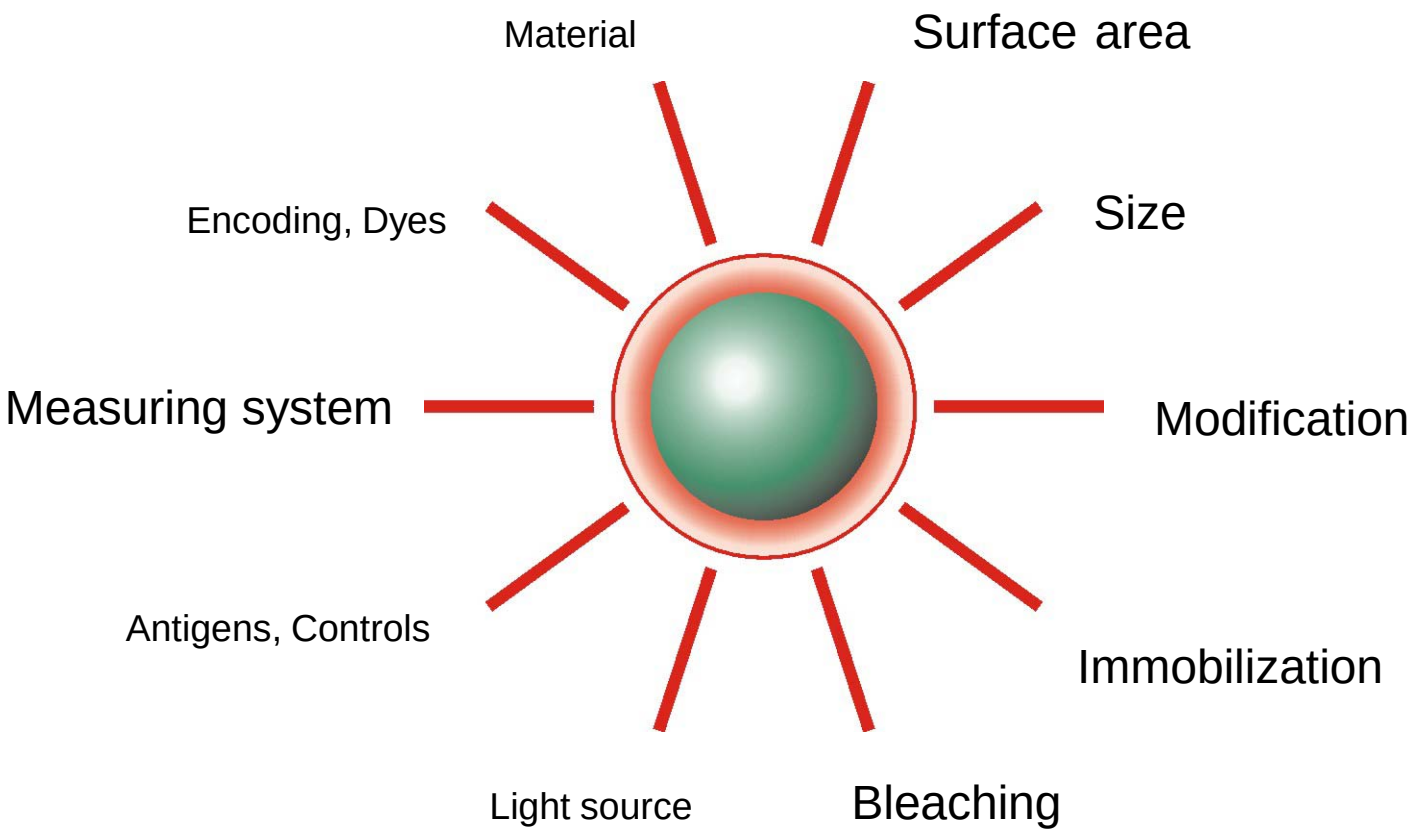


Fluorescence intensity of anti-IgG APC or Cy5 conjugated antibody using one microbead population



The choice of fluorescent dye has an enormous influence on robustness, reproducibility, and analysis of the assay.

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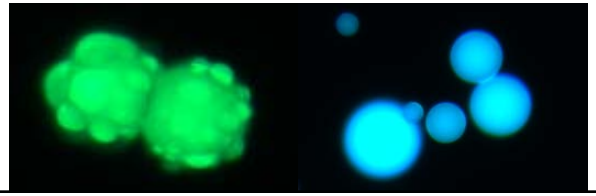
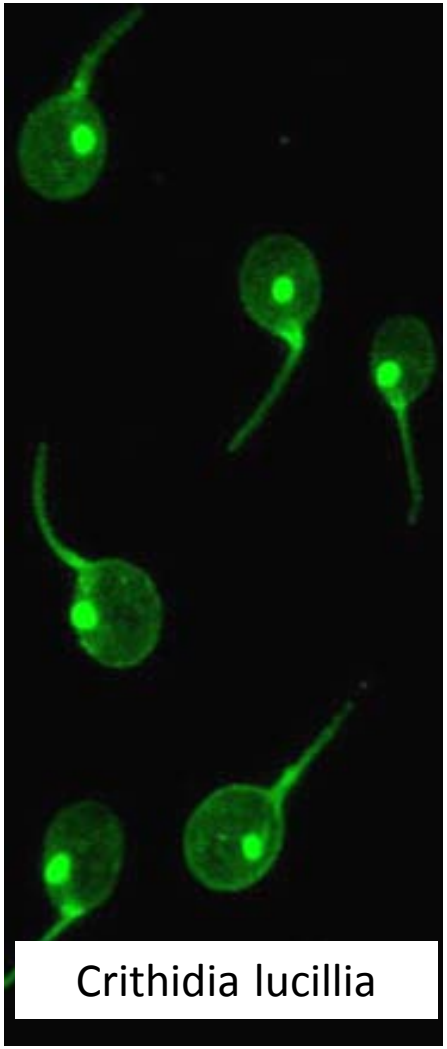
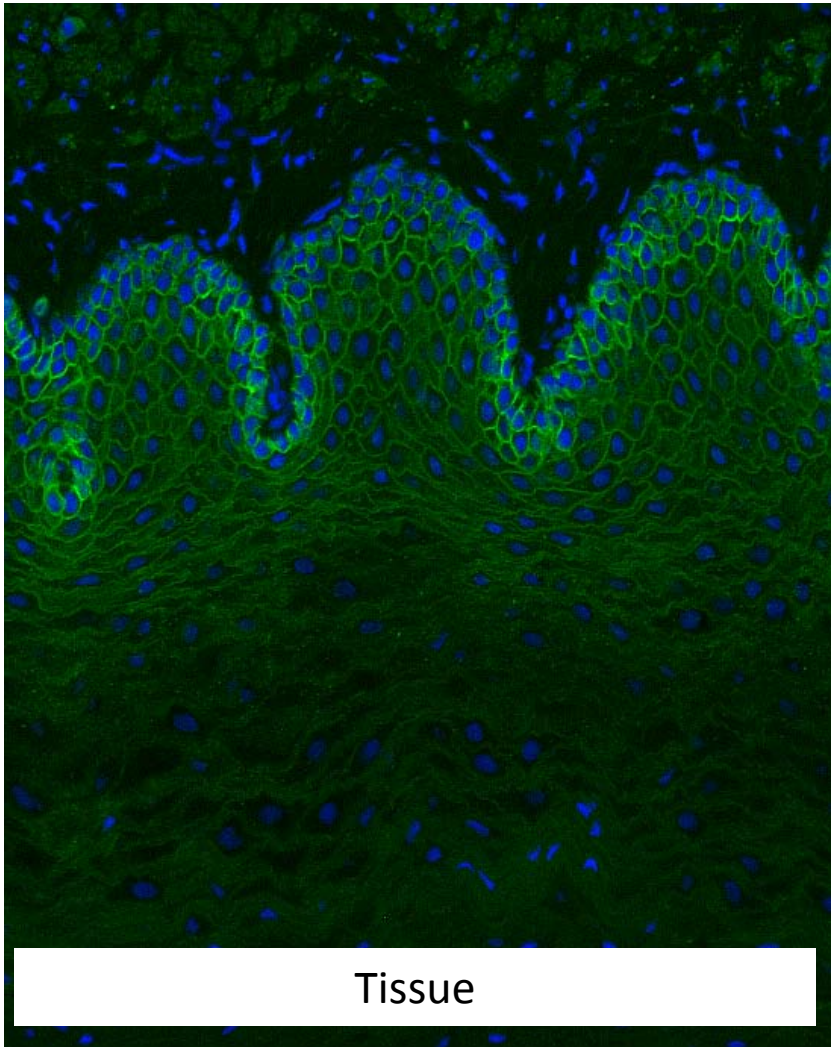


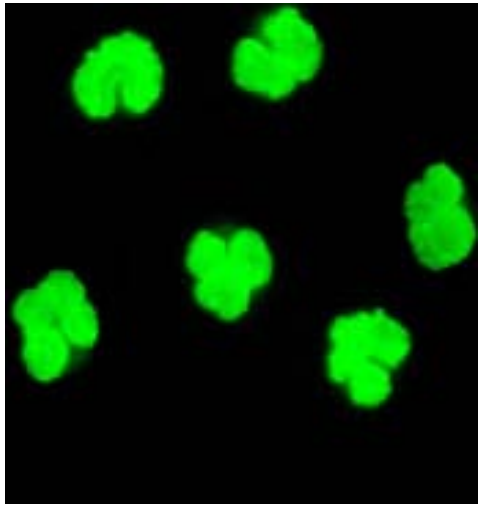
Combined with automated cell tests

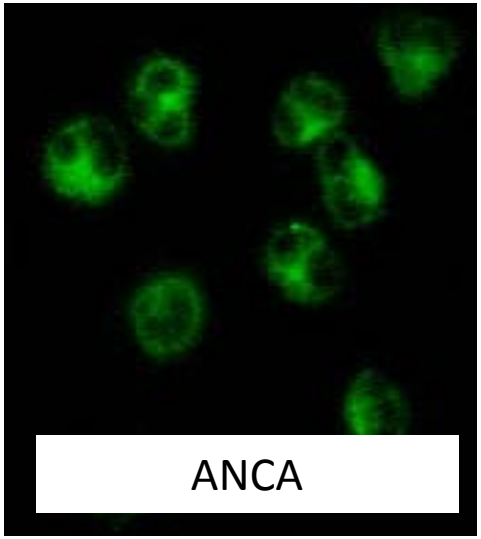


Crithidia lucillia



Tissue





ANCA

Thank you for your attention.