<u>**Section 1**</u>**: Principles of biochemistry, cell biology, physics and microelectronics.**
<u>**Lecture 1.1**</u>**: Cell biology principles (2hours, Dr Dimitris Mastellos)**
- Introduction to the cell and its organization (from prokaryotes to eukaryotic cells, distinct cellular compartments, main organelles and related functions)
- Cell membranes - structure and topography
- Protein biosynthesis and turnover-regulation and degradation
- Cell signaling: relaying the signal from the cell membrane to the nucleus
- The cell cycle – deregulated cell division and cancer
- The immune system-General overview
    - Innate and acquired immunity, target cells and main immune mediators
    - Antibody structure and function - B and T- cell receptors
    - Monoclonal antibodies- applications
- Towards model biological systems-Divergent signal integration within the cell

<u>**Lecture 1.2**</u>**: Structure of biological macromolecules (2hours, Dr Metaxia Vlassi)**
- Introduction to building blocks of biological macromolecules
- Principles of protein structure
- Principles of DNA structure
- Sequence/Structure relationships. Structural motifs. Examples
- Introduction to in silico prediction of 3D-structure
- Structure/function relationships. Examples

<u>**Lecture 1.3**</u>**: Microelectronic Materials and Device Technology (2 hours, Dr Spyros Gardelis)**
- Semiconductors (What is a semiconductor? energy gap, conduction and valence band, electrons and holes, p-type, n-type)
- Basic Devices (p-n junction, bipolar transistor, MOS transistor, MOSFET, CMOS technology)
- Optical properties of semiconductors (direct and indirect band gap semiconductors, laser, photodetector)

  Applications of microelectronics in every day life
- Integration – miniaturization revolutionize the way we live (Moore's law)
- Examples of devices used in every day life (logic devices, memories, lasers, displays, detectors, sensors (chemical, gas, bio-sensors)

<u>**Lecture 1.4**</u>**: Introduction to nanobiotechnology (Invited speaker from N2L: Dr Yoshi Shacham-Diamand, Tel-Aviv University, 2 hours)**

<u>**Section 2**</u>**: Core Nanobiotechnology methods and practices**
Unit 2.1: Micro and Nano-fabrication science and technology (Lecturers: Drs E. Gogolides, A. Tserepi, P. Argitis, N.Glezos, IMEL)
<u>**Lecture 2.1.1**</u>**: Conventional patterning schemes for hard substrates for bioanalytic microdevices (Instructor Evangelos Gogolides 1.5 hours)**
- The top-down approach in nano fabrication. History of microfluidics, and motivation for going micro and nano for life sciences and chemistry
- Lithography fundamentals: Light Sources, Lithography Systems, Photoresists, Lithographic Processes
- Wet etching and Silicon micromachining
- Plasma etching and its mechanisms. Isotropic and Anisotropic etching
- Deep etching processes for Si bioanalytic microdevices
- Glass micromachining
- Novel Deep etching processes for polymer bioanalytic microdevices

<u>**Lecture 2.1.2**</u>**: Patterning technologies for plastic substrates: fabrication of (bio) analytical microdevices (Instructor Angeliki Tserepi 1.5 hours)**
- Why use plastic substrates?
- Master Fabrication for plastic patterning
- Injection molding,

- Hot embossing,
- Casting-replica molding-soft lithography. Variations of soft lithography
- Other novel methods, such as Deep Polymer Etching
- Surface modification and sealing
- Examples

Lecture 2.1.3: Patterning of biomolecules and other biological substances (Instructor P. Argitis 1.5 hours)
- The necessity for the patterning of biomolecules and other biological substances on solid surfaces
- Chemical/physical binding of biomolecules on surfaces
- Patterning methods :
  - microspotting, mechanical methods for delivery and synthesis, dip-pen lithography
  - microcontact printing methods (soft lithography related methods)
  - photochemical modification of SAMs, light guided DNA and protein synthesis,
  - photochemical modification of polymeric films, photoresist-based methods
  - other / emerging methodologies

Potential applications and comparison.

Lecture 2.1.4: Molecular bioelectronics (Instructor Nikos Glezos, 1.5 hours)
- Principles of molecular electronics
- Memory and switching devices
- Discussion of electronic transport mechanisms in molecular systems
- Specific applications of DNA and metalloproteins.
- DNA as a patterning template for molecular devices

Unit 2.2: Nanomaterials for bio-applications, Characterization, Imaging (Lecturers: Drs C. Paleos, I. M. Mavridis, K. Yannakopoulou, D. Vourloumis, D. Tsiourvas, M. Sanopoulou, K. Papadocostaki, IPC ; V. Constantoudis, IMEL; Drs G. Papavassiliou, I. Rabias, IMS; A. Mitraki, FORTH)

A. DRUG DELIVERY METHODS/ MATERIALS

Lecture 2.2.1: Targeting RNA with small molecules; a Pharmaceutical Industry Study (Instructor D. Vourloumis, 1 hour)
- Pharmaceutical requirements for project initiation
- Multidisciplinarity of the approach
- RNA and Aminoglycosides
- The Bacterial ribosome
- Evaluating advantages and disadvantages
- Development of specific biological assays based on crystallographic information
- Chemical simplifications of natural binders
- Successful bridging of different disciplines

Lecture 2.2.2 and 2.2.3: Drug Release and Delivery Systems-Methods: Nanoparticles, Dendrimers, Polymers, Cyclodextrins, Liposomes, Artificial Membranes.
  Why controlled release. Advantages of controlled drug release
  Polymers in controlled drug release (1h, Kyriaki Papadokostaki, IPC)
- Mechanism-based classification of polymeric controlled release devices
- Diffusion in polymers. Kinetics of drug release. Membrane controlled drug delivery systems- Matrix systems- Swelling controlled systems
- Modeling for the optimization of matrix-controlled release systems
- Polymers used in drug release. Environmentally sensitive hydrogels
- Examples of pharmaceutical controlled release devices and classification according to administration site
  Dendrimers as Drug Delivery Systems (0,5h, Constantinos Paleos, IPC)
- Preparation of PEGylated dendrimers functionalized with targeting ligands and transporting agents.
- Application of functional dendrimers for the development of targeted and controlled release drug delivery systems.

- Complexes of cationic dendrimers with DNA for application in gene therapy

Liposomes as Drug Delivery Systems (0,5 Constantinos Paleos, IPC, IPC)
- Preparation of PEGylated liposomes incorporating at their surface targeting ligands and transporting agents.
- Application of functional liposomes in cancer treatment and other deceases
- Complexes of cationic liposomes with DNA for gene therapy applications

Cyclodextrins as molecular carriers (1h I. M. Mavridis, K. Yannakopoulou)
- The basis of cyclodextrins as pharmaceutical excipients
- Inclusion complexes of cyclodextrins with drugs - Pharmaceutical applications
- Basics in NMR spectroscopy and X-ray crystallography as methods to study
- Cyclodextrin inclusion complexes.

B. NANOSTRUCTURED MATERIALS FOR BIOAPPLICATIONS
Lecture 2.2.4: **Bioengineered nanomaterials (Instructor: Anna Mitraki, University of Crete, 2 hours)**
- Why use Nature as a "model for new nanotechnology-based processes"?
- Principles of structure and self-assembly of natural biomaterials
- Natural composite materials and interfacing role of proteins
- How natural building blocks can be used for the rational design of artificial nanoscale objects
- Design of biological nanofibers, nanotubes, and closed-caged nanoassemblies
- Application fields and "interfacing" with other disciplines

Lecture 2.2.5: **Magnetic Nanoparticles for Bioapplications (1,5 hours, I. Rabias)**
- **A study in ferrofluids and their uses in nanotechnology**
  - ❖ A review on what is a ferrofluid?
  - ❖ The origin of their magnetism, their stability as magnetic colloids.
  - ❖ Synthetic methods and applications.
  - ❖ Nature, properties and their high technological value.
- **Synthetic Routes for Hydrosol and Organosol colloidal fluids**
  - ❖ differences and similarities in these two approaches
  - ❖ applications

C. IMAGING OF BIOMATERIALS
Lecture 2.2.6: **Imaging with Scanning Probes (AFM, STM, SNOM) (Invited from N2L: Dr Martin Bennick, Twente University, 2hours)**

Lecture 2.2.7: **Experimental techniques for magnetic characterization of ferrofluids and Applications of ferrofluids in medicine (1,5 hours, M. Fardis)**
- **Experimental techniques for magnetic characterization of ferrofluids**
  - ❖ Short review for the most common experimental techniques for the study of ferrofluids and other magnetic nanoparticles
  - ❖ X-ray diffraction
  - ❖ Transmission Electron Microscopy
  - ❖ Magnetization measurements
  - ❖ Nuclear Magnetic Resonance
  - ❖ Mossbauer spectroscopy
- **Applications of ferrofluids in medicine. Two case studies: 1) Hyperthermia and 2) Contrast Agents for Magnetic Resonance Imaging (MRI)**
  Two applications of ferrofluids in medicine will be discussed:
  - ❖ Hyperthermia covers a wide variety of techniques in which elevation of temperature in ferrofluids is achieved using low-frequency electromagnetic radiation. In this way, hyperthermia is a promising approach for cancer therapy, by locally heating a tumor without damaging the healthy tissues in the tumor surrounding.
  - ❖ Magnetic Resonance Imaging (MRI) is one of the most powerful diagnostic techniques in medicine. The use of superparamagnetic contrast agents based on ferrofluids, which enhance image contrast, will be discussed.

Lecture 2.2.8: **Fluoresence imaging and 3D image visualization using confocal microscopy (Stamatis Pagakis, duration 1 h)**

- Principles of fluorescence
- Most microscopy samples are 3-dimensional – Examples.
- Commonly used fluorescent probes
- Basic optical design of a confocal microscope
- Different types of Confocal microscopes
- Application Examples

Unit 2.3: Molecular and Cellular biology and Applications (Drs A. Vlahou, Garbis, Bioacademy; Drs S. Kakabakos, P. Petrou, IRRP).
A. PROTEOMICS AND ANALYSIS
Lecture 2.3.1.- 2.3.2: Proteomic technologies
2.3.1 Gel-based protein analysis methods (Instructor Antonia Vlahou 1.5hrs)
- Definition and significance of proteomics research
- Introduction to protein separation methodologies: Principle of two-dimensional electrophoresis (2DE) and liquid chromatography
- Steps of analysis by 2DE : isoelectric focusing , vertical polyacrylamide gel electrophoresis, protein spot detection and quantification
- Principles of matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS)
- Identification by peptide mass fingerprinting
- Coupling of 2DE with MALDI
- Protein profiling with MALDI MS analysis
- Applications of 2DE-MALDI MS in biomedical research
- Limitations-Avenues for improvement

2.3.2 Non-gel based protein analysis methods (Instructor Spiros D. Garbis 1,5 h)
- Separation of proteins and peptides with high performance liquid chromatography (HPLC)
- Combining two or more chromatographic chemistries for peptide/protein separation (multi dimensional HPLC approaches)
- Introduction of principles of electrospray ionization (ESI)
- Coupling micro- and nano-bore LC with mass spectrometry ionization sources (MALDI; ESI; nano-ESI)
- Introduction to the principles of tandem mass spectrometry using ion-trap, time-of-flight, quadrupolar mass analyzer designs and their variants.
- Protein identification by tandem mass spectrometry of their peptide products
- Protein quantification by LC-MS analysis of isotope-labeled peptide products
- Applications of LC-MS to biomedical research
- Limitations-Avenues for improvement
B. ASSAYS AND ARRAYS
Lecture 2.3.3: Binding Assays and Immunosensors (Duration 2 hours)
Part A: Binding assays (Instructor Sotirios Kakabakos)
- Binding assay principles
- Binder molecules
- Antibodies: structure and production
- Labels and labelling procedures
- Standard solutions for quantities determinations
- Surface immobilization of biomolecules
- Configurations of binding assays
- Signal amplification systems
Part B: Immunosensors (Instructor Christos Mastichiadis)
- What is a (immuno)sensor?
- Main immunosensor configurations
- Principles of optical immunosensors
- Examples of optical immunosensors developed at NCSR "Demokritos"

Lecture 2.3.4: DNA and Protein arrays: fabrication, detection and applications. (Dr P.Petrou-Duration: 2 hours).

Part A: DNA arrays
- What is a DNA array?
- The DNA molecule: structure and biological significance
- DNA analysis by conventional methods (Southern Blotting)
- DNA arrays versus DNA chip
- Fabrication of DNA arrays and chips
- Target DNA amplification and labelling by PCR
- Hybridisation and detection formats
- Main applications of DNA arrays
- Limitations of DNA arrays
- Perspectives

Part B: Protein arrays
- What is a protein array?
- Protein structure & sources
- Formats and surfaces for protein arraysImmobilization of proteins onto solid surfaces
- Fabricationof protein arraysDetection schemes for protein chipsAntibody arrays: specificity and cross-reactivity Areas of application
- Challenges and bottlenecks
- Perspectives

C. BIOINFORMATICS AND METABOLOMICS
Lecture 2.3.5: Metabolomics in the Post-Genomic Era (Instructor Maria I. Klapa) 2 hours
- High-Throughput vs. Conventional Biology
- The need for a metabolic fingerprint in the post-genomic era
- Metabolomic profile as a metabolic fingerprint
- Flow chart of metabolomic analysis
- Methods of metabolite extraction
- Technological Platforms for metabolomic measurements: advantages and limitations
- GC-MS metabolomics: the problem of derivatization, new strategy
- Metabolomic Data Normalization
- Multivariate Statistical Analysis of metabolic data / connection with the metabolic networks
- Metabolomic Analysis as part of integrated –omic studies

Lecture 2.3.6: Introduction into Bioinformatics (Dr S. Kossida, Bioacademy, 1h)
- Bioinfromatics – A Rapidly Maturing Science
- Biological Databases
- Genome Analysis
- Proteome Analysis
- The Bioinformatics Revolution in Medicine

Lecture 2.3.7: Applied Bioinformatics in BioNanoTechnology (1h, Dr Georgios Spyrou)
- Simulation methods as part of the applied Bioinformatics field
- Molecular Dynamics
- Simulations of biomolecular interactions with inorganic material
- Molecular Modelling in Bionanotechnology
- Bioinformatics insights for designing biomaterials
- Practical considerations in requirements for computing power – supercomputers and GRID architectures

Section 3: Towards Integrated Nanobiotechnology systems
Lecture 3.1: Principles of Integrated Biosensing Devices (2h, Dr K. Misiakos, IM)

Lecture 3.2: Acoustic wave sensors: from device fabrication to biological applications (Dr Gizeli-3 hours)
- Acoustic waves in solids: physical explanation and propagation characteristics
- Acoustic wave devices: description of two main device-types, i.e. quartz crystal microbalance (QCM) and surface acoustic wave (SAW)
- Fabrication of SAW devices supporting Rayleigh and shear horizontal waves

- Principle of operation of QCM sensors in dry and liquid samples
- Principle of operation of SAW sensors in dry and liquid samples
- Applications of acoustic biosensors to biology and biotechnology (detection of protein-protein, protein-cell and membrane-ligand interactions)
- Acoustic wave sensors advantages and limitations in comparison with optical techniques

Lecture 3.3: Lab on chip devices: Principles, applications, opportunities. (Invited from N2L.)

HANDS ON EXPERIENCE-LABORATORIES

SESSION 1:
Choose 4 out of 5 laboratories:
<u>**Laboratory 2.3.3:**</u> **Fabrication of protein microarrays using nanoplotter (Bioacademy……)**
- Microspoting methods for the fabrication of microarrays : presentation of nanoplotter and familiarization with its use

<u>**Laboratory 2.2.4**</u>**: State of the art confocal microscopy of biological samples (Instructor Stamatis Pagakis, Bioacademy, duration 2 hours/group)**
Aim of the Lab:
- Familiarize students with a fluorescence microscope
- Demonstrate the design differences which characterize a confocal microscope
- Demonstrate Image processing and 3D visualisation software.

Content:
- Two microscope workstations (one confocal) will be used.
- Observation of fluorescent proteins (e.g YFP, GFP) and other fluorophores common in fluorescence microscopy.
- Hands-on experience and data acquisition on a digital camera fluorescence microscope
- Demonstration of 3D data acquisition using a confocal microscope
- Imaging methods for image deconvonlution, 3D reconstruction and data analysis will be demonstrated on computer workstations with specialised software packages.

<u>**Laboratory 2.3.1**</u>**: Protein separation by two-dimensional electrophoresis (Instructor Antonia Vlahou, duration 2 hours/group)**
Aim of the lab:
- Familiarize attendees with the isoelectric focusing technology and vertical polyacrylamide gel electrophoretic systems
- Familiarize attendees with automated technologies for spot picking and processing for peptide mass fingerpriniting
- Demonstrate the processing of a cell extract by the 2DE methodology

Content
- Processing of cell extract on IPG strips for first dimensional separation
- Application of IPG strips on PAGE gels
- Gel staining, scanning and processing for spot excision
- Tryptic digestion of excised spots

<u>**Laboratory 2.3.2:**</u> **Mass spectrometry (Instructor Spiros D. Garbis, duration 2 hours/group)**
Aim of the lab:
- Familiarize attendees with mass spectrometry technologies (MALDI; ESI QqTOF; ESI QIT)
- Demonstrate tryptic peptide analysis by MALDI-MS; LC-ESI-QqTOF MS; LC-ESI-QIT)
- Familiarize attendees with database searches for protein identification
- Familiarize attendees with the custom preparation of capillary LC columns to be used for the LC-ESI-MS based methods

Content
- Tuning and calibration of mass spectrometers (MALDI; ESI QqTOF; ESI QIT)
- Application of tryptic peptide extracts and proteins and matrix on MALDI targets
- MALDI MS analysis of peptide and protein samples
- Sample preparation for LC–ESI-MS
- LC-ESI-MS-MS analysis of peptide extracts
- Database search methods

<u>**Laboratory 2.3.6:**</u> **Bioinformatics laboratory (Dr S. Kossida, Bioacademy, duration 2 hours/group)**
Aim of the lab:
- Familiarize attendants with biological databases
- Familiarize attendees with software for genome analysis
- Familiarize attendees with software for proteome analysis

Content:
- Query and retrieve info from biological databases
- Analyze genes/ genomes with software packages
- Analyze peptides/ proteins with software packages

SESSION 2:
Choose 4 out of 6 laboratories:
Laboratory 2.1.1: Fabrication of microfluidic devices on plastic substrates by lithographic techniques (Instructor Angeliki Tserepi, duration 3 hours)
Aim of the lab:
- Familiarize attendants with both conventional photolithography
- Familiarize attendees with (replica molding/soft lithography)
- Demonstrate the fabrication and sealing of a PDMS microfluidic device.

Content
- Fabricate mold on SU8
- Fabricate microfluidic channels by PDMS replica molding on SU8 mold
- Device Sealing

Laboratory 2.1.2: Fabrication of plastic microfluidic Devices by Lithography and deep polymer plasma etching techniques. (Instructor E. Gogolides, duration 3 hours)
Aim of the lab
- Familiarize attendees with the ICP plasma reactor, its operation and diagnostics, as a tool for microfluidics and surface modification
- Perform plasma etching of PMMA microchannels and observe results
- Show surface analysis methods, ellispometry, contact angle (wetting)

Content
- Lithography on a PMMA sheet
- Demonstration of the plasma reactor (ICP) and diagnostics
- Patterning of microchannels on PMMA using the ICP
- Measurement of the wetting properties of PMMA before and after etch
- Sealing of microfluidic channels and measurement of flow properties
- Spectroscopic ellipsometry of polymer films

Laboratory 2.1.3: Electrical characterization of tunneling devices based on organic molecules or biomolecules (Instructor Nikos Glezos, duration 3 hours):
Aim of the lab
- Familiarize attendants with electrical characterisation methods of molecular systems
- Discuss how the electrical transport properties of the measured devices can be used in future nanoelectronic devices

Content
- Demonstration of electrical measurements of hybrid Si/molecular devices containing organic molecules and biomolecules with different geometries (planar nanodistant electrodes, vertical structures, structures containing gold nanoparticles).
- Analysis of experimental data and extraction of information for the molecular systems

Laboratory 2.3.4: Fabrication of protein microarrays using lithography(Drs M.Chatzichristidi, A.Douvas)
- Photoresist processing fundamentals : processing of chemically amplified photoresists
- Lift-off process : use of lift off process for the patterning of SAMs or proteins,   and **(optional)**
- Patterning of different types of proteins on the same substrate by successive lithographic steps under biocompatible conditions

Laboratory 2.3.5: Fluorescence detection of protein arrays (Instructor Panagiota Petrou, duration 2 hours).
Aim of the lab:
- Familiarize the attendees with optical detection of protein arrays.
- Demonstration of the epifluorescence microscope.
- Image processing for quantitative fluorescence measurements.

Content:
- Immunoreaction of protein in spots created by photolithography with fluorescently labeled molecules
- Observation of protein spots with the epifluorescence microscope
- Capture of images of the arrays

- Image processing to receive quantitative results

Laboratory 3.1: Operation of a lab-on-a-chip optical device using model assays and real time measurements (Dr Constantinos Misiakos).
Aim of the lab:
Content:
- Integrated biochip wafer alignment on wafer prober
- Protein/DNA spotting on integrated waveguides
- Washing and microfluidic device application on biochip
- Protein/DNA real time assays through photocurrent monitoring

SESSION 3:
Choose 4 out of 6 laboratories:
Laboratory 3.2: Demonstration of a capillary fluoroimmunosensor. (Instructor Sotirios Kakabakos, duration 3 hours)
Aim of the lab:
- Familiarize attendees with optical immunosensors.
- Demonstration of multi-band capillary fluoroimmunosensor
- Real-time monitoring of the immunoreaction.

Content:
- Creation of distinct reaction bands in a single capillary.
- Performance of immunoreaction in the capillary.
- Demonstration of fluorescent bands detection using the prototype optoelectronic set-up in real time or after assay completion.
- Data processing and interpretation of the results.

Laboratory 2.2.1: Drug inclusion in cyclodextrins: monitoring in situ by NMR spectroscopy (Dr K. Yannakopoulou, IPC), X-ray diffraction characterisation of drug inclusion and 3-D visualisation. (Drs K. Yannakopoulou, and I. M. Mavridis, IPC).
- Determination of the formation of an inclusion complex of β-cyclodextrin and the drug piroxicam in situ by NMR spectroscopy.
- Determination of the formation of the same inclusion complex (β-CD/piroxicam) in the crystalline state by powder X-ray diffraction.
- Molecular docking of the drug inside the cyclodextrin cavity.

Laboratory 2.2.2: Liposomes: preparation and characterisation by dynamic light scattering and ζ-potential (Dr D. Tsiourvas, IPC).
Aim of the lab:
- Familiarize attendees with liposome preparation
- Perform standart liposomal characterization techniques

Content:
- Preparation of anionic and cationic liposomes of 50 and 100 nm diameters employing the extrusion method.
- Introduction to Dymanic Light Scattering (theory and applications).
- Dynamic light scattering experiments for the determination of the size and polydispersity of liposomal formulations.
- Introduction to ζ-potential (theory and applications).
- ζ-Potential measurements for the determination of the surface charge of liposomal formulations.

Laboratory 2.2.3: Video enhanced optical microscopy and Atomic Force Microscopy of Liposomes (Dr D. Tsiourvas, IPC).
Aim of the lab:
- Familiarize attendees with optical microscopy and Atomic Force Microscopy experimental set-up.
- Demonstrate the use of these techniques for the characterization of liposomal nanoparticles

Content:
- Optical microscopy – phase contrast optical microscopy.
- Phase contrast optical microscopy imaging of liposomal dispersions.
- Introducing the experimental set-up of Atomic Force Microscopy.
- Atomic Force Microscopy (tapping mode) imaging of liposomal dispersions

Laboratory 2.2.5: Magnetic nanomaterials for bio applications (Dr I. Rabias)
- Synthesis of colloidal ferrofluids of Fe-oxides (γ-Fe2O3, Fe3O4) magnetic nanoparticles (hydrosols) using the co-precipitation will be done.
- Coating of functional capping agent, such as dextran, on the iron oxide core for the conversion to stable ferrofluids by proper surface modification will be done.

Laboratory 2.2.6: MRI for Biomedical applications (Dr M. Fardis)

- Calculation of the specific absorption power, i.e. the power absorption in Watts per gram of Fe, which is given by the following formula, will be measured using RF-induction heater. For the above use of prepared ferrofluids in magnetic relaxitivity measurements to optimize the conditions for enhanced signal registration.

$$SAR = \frac{W}{m_{Fe}} = \frac{\Delta Q}{\Delta t\, m_{Fe}} = \frac{c m_f}{m_{Fe}} \frac{\Delta T}{\Delta t}, \quad \text{in W/gFe}$$

- Where c is the specific heat capacity of the ferrofluids, calculated as the mass weighted mean value of magnetite and water, mf is the mass of the ferrofluids, and mFe is the mass of the iron in the ferrofluids.