Quantitative Cell and Tissue Analysis

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1 Block I

Term	Definition
AFM	Atomic force microscopy
DIC	Differential interference microscopy
STED	Stimulated emission depletion microscopy (doughnut)
TIRF	Total internal reflection interference microscopy
PALM	Photo-activated localisation microscopy
FACS	Fluorescence activated cell sorting (using positive and negative charges for binning)
FRAP	Fluorescence recovery after photo bleaching (diffusion of fluorophores)
FCS	Fluorescence correlation spectroscopy
FRET	Fluorescence resonance energy transfer
SEM	Scanning electron microscopy
TEM	Transmission electron microscopy (preparation of sample!)
NRM	Nuclear magnetic resonance
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
PCR	Polymerase chain reaction
PMT	Photomultiplier
CCD	Charge coupled device
LED	Light emitting diode
GFP	Green fluorescence proteïn
SE	Secondary electron
BSE	Back-scattered electron
SRRF	Super resolution radial fluctuations (software)
DHM	Digital holographic microscopy (increase z resolution using ϕ, λ, and h(height))
MSI	Mass spectrometry imaging
LC	Liquid chromatography
FCM	Flow cytometry

2 Block II

2.1 Abbreviations

Term	Definition
EV	Extracellular vesicle
RBC	Red blood cell
WBC	White blood cell
LPP	Lipoproteïn particles
SEC	Size exclusion chromatography
UF	Ultrafiltration
ODG	OptiPrep density gradient (Iodixanol)
THP	Tamm - Horsfall proteïn
rEV	Recombinant extra cellular vesicle (it was modified to express a protein more)
TRPS	Tunable resistive pulse sensing
NTA	Nano praticle tracking analysis
AF-MALS	Assymetric flow field flow fractionation coupled with multi-angled light scattering
AF4	Assymetric flow field flow fractionation
MVE	Multi vesicular endosome
PBS	phosphate buffered saline
ECM	Extracellullar matrix
BM	Basement membrane

2.2 Technologies

- TRPS uses a tunable voltage, pore size and pressure to filter the particles in a more exact way adn detect their size and concentration. (-) Clotting of the pores is possible. (+) Measures the ζ potential at once. Which is related to the electrophoretic mobility.

- ExoView: technology that uses an antibody coated chip to capture EV's on the plate. After that stained antibodies detect the used antibody on these EV's. We could also use a mild detergent (SDS) to visualise the RNA molecules that are in the EV. (+) No complicated separation needed assessment is directly possible of the bio fluid. Immediately biological markers... (-) less quantitative (cost).

- Nano-FCM: low pressure sheath fluid and a reduced flow rate (+) estimation on the size, concentration also biological information. Multiple labels can be visualised at once. (-) The fluorescent technology needs to be applied in a fluid and so will also need to be removed again so there is no background signal...

- AF4: First the smaller and then the larger particles will elude. here the labels wont need to be removed because these are very small particles and will so elude the first. (why do small particles elude first? because these have a higher diffusion coefficient and will be able to get higher in the downwards applied stream and so be in the more center of the horizontal flow that is larger in the middle (parabolic pattern)) (+) The fluorescent particles dont need to be removed since these will elude first.
- EXODUS: Isolation technique. Exosome detection via ultra fast isolation system. Based on negative pressure oscillation and membrane vibration.
- Anion exchange chromatography: Uses the negative surface charge of EV's to select them (determined by the ζ potential)
- ELISA bead-base flow cytometry: ELISA or Enzyme-Linked Immunosorbent Assay is an immuno assay technique utilized to detect diseases. The principle of ELISA is antigen-antibody interaction.
- SDS-Page used to determine protein amount (see later)
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2.3 Cell culturing

37°C + 5% CO2 cadherins (cell-cell adhesion)(Na2+) integrins(cell-ECM adhesion) 3 steps in sub culturing:

- cell collection
- cell counting
- cell seeding

PH is maintained using a buffer system. Mycoplasms contamination is not visible

But also limitations a cell culture reall doesnt simulate real body environment. E.g.: 2d situations most of the time only 1 cell type at the time. cell lines have been cultured for multiple generations resulting into a drift of their characteristics. But also limitations due to the culture conditions. see slide 33!!!