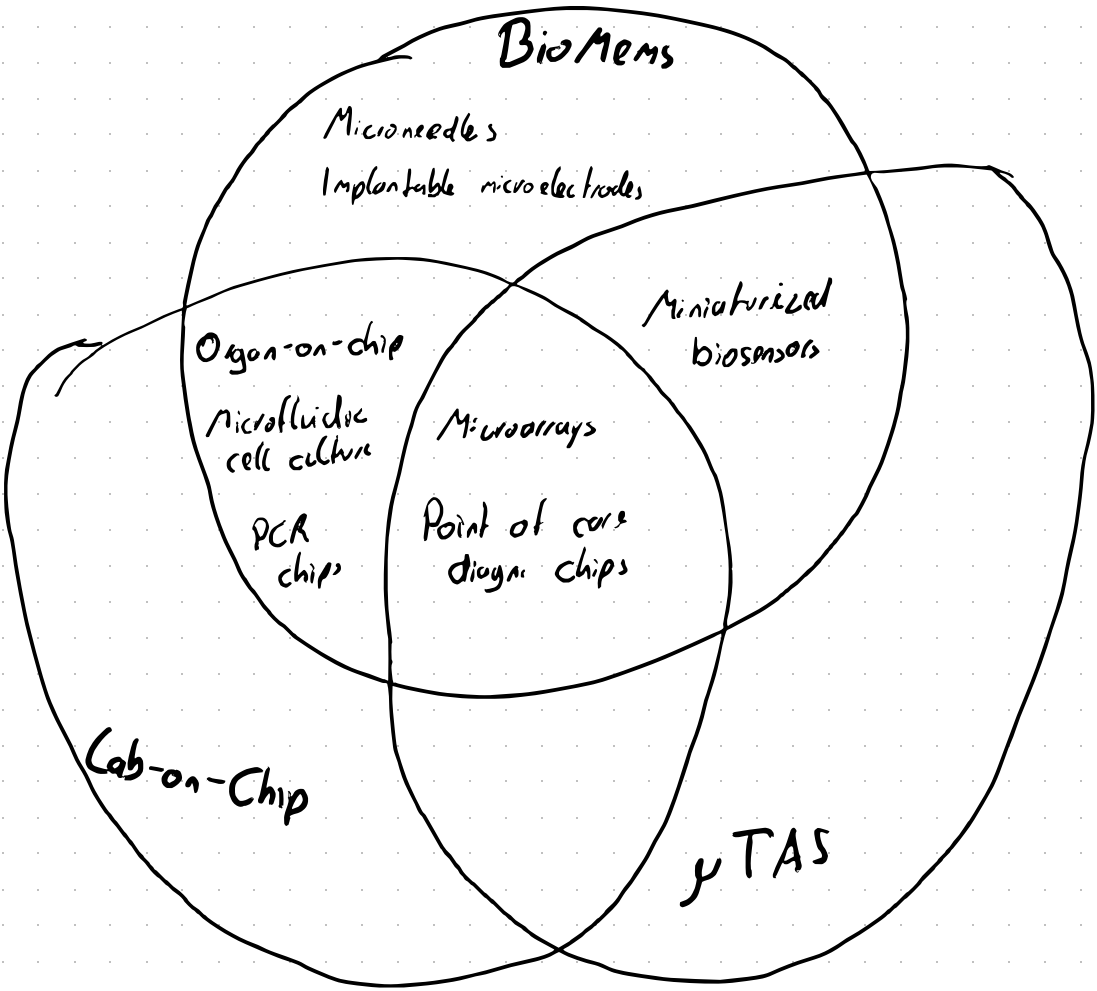
Kategorien biochip (Exam Q...)

biochips are "miniturized Labs" that can host large numbers of biochemical reactions (source: Wiki)

↳ sampling / washing / detection is performed seperately

Broad overview of biochips



USP (Exam Quest)

- parallel
- miniaturization
- automation
- integration of functions

Microarray types (Exam Qu)

"in body"

- DNA Microarray = can also be used in situ
  = ressources amount / type mRNA / genes
- Peptide — " —
- Protein — " —
- MM Chips
- Tissue Microarray
- Cellular Microarray
- Antibody — " —
- Carbohydrate — " —
- μ CCA - µcell culture analogues → replace animal tests
  → simulate human physiology on micro fluidic platform
- Organ on a chip (OOAC) - 20/30 cell culture chips
  → Aim Body / Human on a chip

focus on dna-microarray

Why use microarrays? / Advantages

- determine active genes in a cell / level of gene in cell
  - ↳ comparison of gene expression control vs treatment
  - ↳ conclusion which genes increase / reduce
  - ↳ conclusion which gene have biological significance
    - ↳ hard to show
    - import. for alzheimer, parkinson.
- discover new genes / pathways
- can be cheap and flexible
  - ↳ maybe not all microarrays

Limitations / things to keep in mind / Disadvantages

→ Technology
- measures only relative level of expression 2 samples
- features / probes can come off surface / poor adhesion
- labor intensive
- data can be variable
- needs sequence verification
- spotting efficiency
- image segmentation

→ Biology
- tissue contamination
- RNA degradation
- hybridisation efficiency and specificity

6 steps DNA microarray experiment (Exam Qu)

↳ name 3 of 6

1) manufactor microarray

2) experimental design. What to compare against

3) Target Prep and hybridization

4) Image aquisition (Readout)

5) Database building, filtering and normalisation

6) Bioinformatics

↳ Statistical analysis / data mining / pathway analysis

(Exam Question)

Categories Microarrays

and don't bind homogenously → donut shape

1) "classical" 2D microarray

→ most common

→ small features (~5 µm)

→ flourescene marker → Flourescene marker bleach
↳ cannot be excited multiple times

Two dyes
↳ dye-Cy5 Red
↳ dye-Cy3 Green

unsure about that

→ immobilisation on array surface

→ special reader + software (Bioinformatics) correlate readout
↳ 3 colours have to be detected
green / red / yellow

→ examples

- spotted glass microarray
  ↳ printed on flat transparent surface (glass, nylon, plastic)
  ↳ thousands of analytes on 1 chip
  ↳ can analyse cDNA, oligonucleotides, protein, antibody
- cartridge based microarrays
  ↳ high density arrays in plastic housing ???
  ↳ less spots than spotted glass possible

2) Suspension bead array

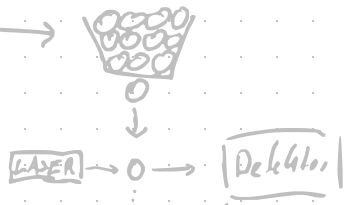
Flowing Farbstoff

→ features are microspheres (~ 5,6 µm diameter)

→ spheres are flourochrome coded → jedes sphere eigene Farbe

→ target immobilized on microsphere

→ readout via flow-cytometry →



→ limited to ~ 100 targets

provides info on measured analyte and concentration

Odds in VO

3) high density bead array

vorgestellte Dinge nur für Allele erkennung

→ features are microspheres (~ 3 µm)

↳ located in wells → well is optical fibre and used for laser detection (see youtube video from illumina)

← only detects allele

→ target immobilized on microsphere

↳ binds to complementary seq

↳ flourescent marked nucleotide added

↳ can be detected

→ limited to ~ 1500 targets

4) electronic microarray

→ features are spots / electrodes

→ flouresent or ferroscene labels
↳ visual ↳ electric (through electron transfer)

→ electric field used for binding and surface modification
↓
+ charge attracts negative DNA

→ less spots than 2D micro arrays

Fabrication

↳ techniques:
- contact
- non-contact } next page
- in-situ

↳ Materials desired properties
- chemically stable
- biocompat. - DNA should not denature on contact
- optical properties
- thermally stable
- machining
- cheap

⇓

either glass or plastic have most/all these properties

glass: only transparent for infrared
- silica (SiO_2) , cheap
- Sodium carbonate (Na_2CO_3)
- Limestone

plastic: - haven't been used historically because it suffers from auto fluorescent
↓
modern plastic only at high frequencies
- PMMA
- Polystyrene
- COC
- Polycarbonate

(Exam Quest)

Impact. Parameter Fabrication (partly solved by reader)
- Spot uniformity
  - spot size
  - spot to spot distance
  - shape repeatability
- Influencing factors
  - sample viscosity
  - substrate surface properties
  - fabrication device
- environmental factors
  - humidity
  - temperature
  - contamination

Fabrication techniques (Exam Qu.)

+ limitations

- in situ synthesis
  - photolithography
  - maskless synth.
- contact fabrication
  - pin printing
  - microstamping
  - flow printing
- non-contact fabrication
  - spotting
  - inkjet printing

} printing by either
- thermal
- piezo
- valve

Contact-Printing — also possible with non aqueous solutions

(Exam Que)

PIN PRINTING

- ↳ old technique
- ↳ mostly used non-commercial
- ↳ temp. and humidity control important (limitation)
- ↳ printing down to nano-level
- ↳ pin metal or silicon
  - ↳ either solid or split

spot quality highly depends on that

spot diam. 75 µm–450 µm vs 25 µm – 215 µm

MICROSTAMPING

- ↳ fast and rapid
- ↳ mostly in research
- ↳ stamp produced by photolithography and PDMS molding
- ↳ types
  - direct stamping
    - sample directly applied via stamp
  - indirect stamping
    - ligand applied via stamp

look at pictures in slides

FLOW PRINTING

- ↳ substrate flows through printhead over surface

Non Contact (exam quest)

name 3 non-contact printing method

SPOTTING / INK JET
↳ similar technique to desktop inkjet
↳ 3 activation types of nozzle
- thermal
- piezo
- valve

Limitations (Exam Quest)

- droplet stacking
- "donal" structuring — can be improved with additives (e.g. glycerol)

How to analyze Dot Morphology

→ AFM
→ Flourescent Micros
→ Raster electron Microscope

depend on
- surface properties
- print solution
- analyte concentration

In-Situ

PHOTOLITHOGRAPHY

↳ only with DNA-Microarrays

↳ Ablauf

1) 
2) 
3) 

repeat and build wanted sequence on surface  ← result

MASKLESS

↳ same as bit with mirrors instead of mask

↳ higher flexible

LABEL AND Detection (Exam Que)

Name 2 label techn.

- Flouresceне (Cyanine 3 (Cy3) and Cy5 mostly used)

green / red

→ Label added via ligation / reverse transcription / PCR

\+ efficient quantum yield and unique excitation / emission spectra

\- moderate photostability (bleaches after each excitation)

→ Detection with separate scanner

- Enhanced Flouresceне

→ two types DNA dendrimer and Quantum Dot

one sample binds to dendrimer which contains up to 250 flourophore

↳ nanosized particle with optical properties (brighter fluorophore)

↳ stable against photo bleaching

- Electrochemical detection

\+ cheap / portable / robust / useful when optical readout not possible

\- lower sensitivity

→ amount of nucleic acids can be read via current

- Metal Nanoparticle Labels

→ detection electrical or optical

→ direct labels or indirect labels

gold nanopart. directly bound to nucleotide

↘ nucleotides labeled with hapten (disadvantage)

labeled with Antibody + Nanoparticles

DNA Microarray

↳ DNA revision: - sugar phosphate backbone → negative charge
↓
polymers are mostly positively charged → good polymer
Glass: mostly negative

- 4 dist. Bases attached
  - Adenine + Thymine
  - Guanine + Cytosine — strong binding ↳ defines melting point dep: on amount for PCR
- 5' end and 3' end
- RNA Uracil instead of Thymine

↳ Application DNA Microarray

- gene expression profile
- Protein - DNA interaction
- single nucleotide polymorphism (SNP) - detection
- viral pathogen detection
- microbial infection detection

↳ How is it done?

- Put gene sequence on array / surface — DNA immobilisation
  - ~20-70 bases long
  - each spot different sequence
  - "probes"
    - probe set ↳ multiple oligonucleotides with similar hybridizations
- add labeled dna "target"
- readout DNA - / imaging
  - fragmented using heat and salt
  - cDNA ← Abschrift von mRNA and more stable

Influencing factors / problems

- length and GC content
  influences melting point
  positions close surface are less accessible / important
- end nucleotides bind less
- secondary structure probe / target
- mismatches
- overhanging ends of target
- unknown effects

with - O_2 Plasma
- NH_4OH/H_2O_2

↑ cleaning input and creation OH group

DNA Immobilization

1) ↳ use amino modified DNA which binds to aldehyd surface
   ↳ spacers can also be used

modified DNA binds to this

2) ↳ use crosslinker

3) ↳ activate metal surface to create self assembled monolayer (SAM)

takeaway points:

→ immobilisation has to
- ↳ create good accessibility target
- ↳ has low non-specific binding
- ↳ stable link
- ↳ surface functionality (make bindable)

DNA - Imaging

↳ flourescene needs expensive filters
  ↳ works by exitation, emission principle

↳ based on assumption:

mRNA correlates with expression associated gene
↓ is measured

↳ We measure:
- gene expression
  ↳ mRNA level in cell
  ↳ mRNA level over population cell
- error propagation
- relative mRNA hybridization

↳ We miss:
- post-translation modification
- transcription genes to mRNA
- translation mRNA to proteins

→ Difficulties
- finding spots — cookie cutter algorithm → uses gaps / manual
  Problems: 1) irregular size / shape 4) saturation
  2) irregular placement 5) spot variance
  3) low intensity 6) Background variance
- converting image into numerical data
- image normalisation — via IQR
  ↳ remove background → some spots empty to determine background
  ↳ corrects:
  → different Labeling / detection efficiencies
  → different Quality mRNA

Quality Control

1) Probe Level

quality of expression on 1 spot on 1 array

Sources:
- faulty printing
- uneven distribution
- contamination

Inspection: Visual
- ↳ hairs
- ↳ Air bubbles
- ↳ scratches
- ↳ dust

Quality measures
- ↳ brightness (foreground/background)
- ↳ uniformity
- ↳ morphology
- ↳ spot size

2) Array Level

quality of expression on 1 array

Sources:
- fabrication defect
- problem RNA extraction
- failed labeling
- faulty scanner

Quality measures:
- ↳ % spots no signal
- ↳ range intensities
- ↳ distribution spot signal area
- ↳ have signals substantially changed during runs??

3) Gene Level

quality of expression of probe across all arrays

Source:
- some probes don't hybridize
- printing problem
  - ↳ bad wells

Validation:

→ quantitative results (we only measure relative difference control/treated) have to be measured via other method
- ↳ e.g. Quantitative real-time PCR

Types validation:
1 – validate instrument with same RNA
2 – validate mechanism/phenomenon with diff. experimental conditions

Examples DNA-Microarray

1) Diagnostic Microarray
   ↳ fast parallel detection and identific. of microorganism
   ↓
   needs molecular marker
   ↓
   phylogenetic marker
   give identity of bad thing
   ↓
   other markers evaluate their function

Example:
Mamma Print
↳ Breast cancer patients overtreated
   ↳ detect marker to prevent this → prevents unnecessary treatment
   ↳ result 46% patient at high risk no overtreatment necessary

2) cDNA array
   ↳ determine which genes active at which level.
      ↳ compare control / treated

3) gene discovery
   ↳ gene expression in cancer cells before / after electric pulse

4) ancient DNA
   ↳ learn about evolution / diseases / migration / roots
   ↳ Problems: degradation, age limit, contamination

Protein Microarray

↳ True Protein microarrays are evolving very slow
  ↳ only few exist
  ↳ most designed to detect AB or enzymes
↳ evolved from DNA-Microarrays

⇓ vs

Target	Protein	DNA
Binding	3D affinity	2D affinity
Stability	low	high
Surface	glass	glass
Printing	arrayer	arrayer
Amplification	Cloning	PCR

↳ Protein basic knowledge
  ↳ coded in DNA
  ↳ consist of 20 amino acids
  ↳ ≈ 2000 000 proteins
  ↳ functions: - immune response
    - signaling
    - transcription regulation
    - vital functions

Types Protein-Array

- expression array
  - ↳ probes (Antibody) on surface to recognize target protein
  - ↳ identifies expressed proteins
  - ↳ quantification method for large # (amount) of expressed protein

- interaction array
  - ↳ probes (proteins, peptides, Lipids) on surface interact with target protein
  - ↳ identifies protein interaction
  - ↳ high throughput discovery of interaction

- functional array
  - ↳ probes (proteins) on surface react with target molecule
  - ↳ reaction products are detected
  - ↳ main goal proteomics

Technical challenges

- ↳ poor control immobilized protein activity
- ↳ low yield immobilisation
- ↳ high non-specific adsorption
- ↳ fast denaturation protein
- ↳ limited number label → low multiplexing

multiple simultaneous performed tests

Proteomics

↳ study of proteins

↳ goal

↳ catalog, understand funct. and interaction of all proteins

↳ Methods

- protein micro array
- gel electrophoresis
- x-ray crystallography (freeze and analyse 3D structure)
- 2D- Mass Spect.
- SELDI-MS protein chips

Proteins are deposited on chip surface by robots

↓ glass / nanowells / gels

↓ attatched via (Exam Question)

1) Covalent
↳ statistically oriented

2) Electro static
↳ statistically oriented

3) Adsorbtion
↳ random

4) Affinity
↳ site specific
↳ best variant
↳ different ways
- biotin, protein A/G, DNA directed...

5) Diffusion
↳ random, but presum. active

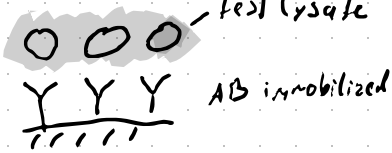
Random / statistical oriented lead to inactive proteins.

↳ example covalent

- real proteins can have multiple active sites

2 Strategies (Exam Question??)

↳ Forward Phase arrays

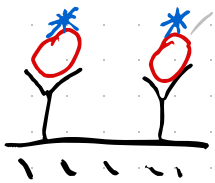


↳ Reverse Phase arrays

oder, dus.??

Protein detection

Direct Labeling



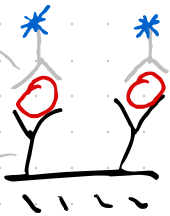
labeled target protein
↳ by flourescal or radioactive label

- proteins labeled
- can be detected after attatchment

immobilized AB on surface
↳ target specific

labeled secondary antibody
↳ target / diff. epitope as immobilized AB

Sandwich assay



- secondary AB detects different epitope and labels
- can be detected after attatchment secondary AB

Examples

1) extracellular vesicles in diagnostics

vary in size, are therefore probably important for cell function

2) peptide microarray

"small proteins" with up to 50 AA

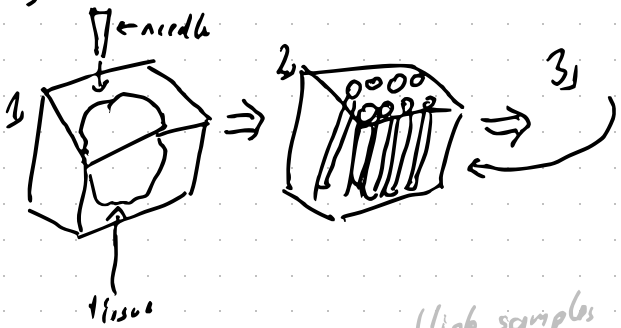
↳ new cancer diagnostic approach

Tissue Microarray (Exam Quest) ↳ Explain

1 ↳ tissue samples embedded paraffin

2 ↳ take out tissue samples with hollow needle → embedded in paraffin

3 ↳ thin sections are cut and immobilized on glass



via microtome
cutting of this block in thin slices
↓
put on glass

always use multiple samples from same tissue to be representative

Advantages:

- variety of 10000 tissue samples simultaneously stained and analysed under identical conditions

Applications (Exam Question)

- novel tumor biomarker detection
- tumor progression
- therapeutic outcome in cancer patients

Cell Microarray (CMA)

Application:
- toxicology studies
- drug discovery
- small molecular screening
- stem cell research
- gene funct. screening

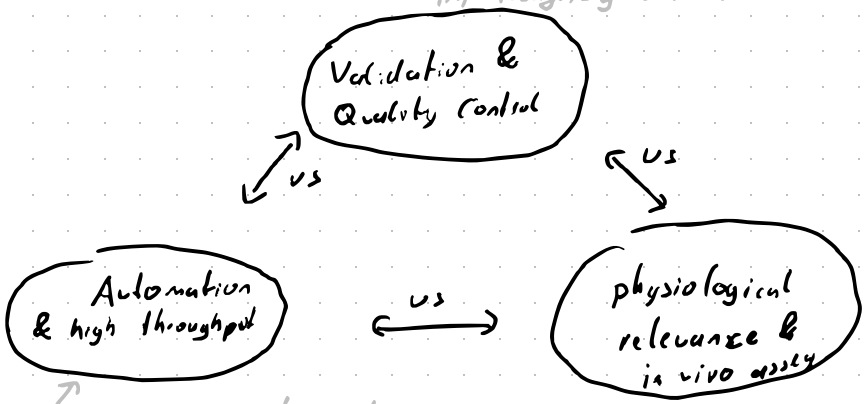
Two types

- fixed cell microarray
  - fixed in paraformaldehyde and then embedded in paraffin, agar, low-melting agarose
  - protein expression of WHOLE cell
    effect of drug treatment measureable
  - often cross linked cells
  - similar tissue Microarray
  - assay optimisation replacing expensive tissue
  - easy identification control for immunostaining

- living cell microarray / live-cell microarray
  - non destructive observation of cell response in real-time
  - monitor complex, functional and vital cellular responses
    - cell-cell interaction
    - cell interaction with micro environment
    - reactions to applied stimulus
    - molecular cellular mechanism
  - dyes normally kill cell after awhile
  - LABEL-FREE detection methods:
    - electrochemical detection - e.g. ion moving
    - surface plasmon resonance (SPR)
    - contrast (DIC) microscope
    - different. interference

Living cell - microarray

Problem / Challenge in Vitro assays
im Reagenzglas / im Labor



Approaches of cell adhesion to surface:

→ Hanging Drop technique (conventional)

+ embryonic body size can be controlled
- mass prep of embryonic bodys extremely troublesome

→ suspension culture method (conventional)

shake → cannot bind to floor
→ bind to each other

+ mass prep EB is easy
- EB size cannot be controlled → prob. not relevant at all

→ cell pulloning

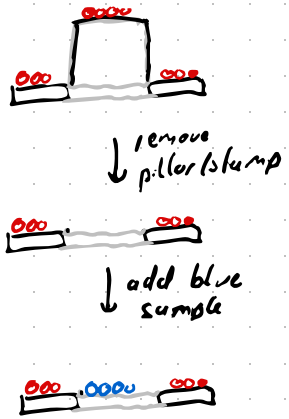
Cell patterning

↳ have pattern of cell → make analysis possible

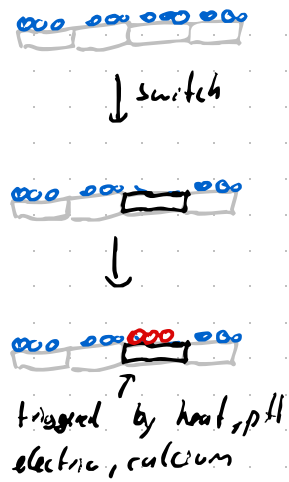
↳ 3 principles

1) selective adhesion

2) Physical limitation



3) switchable surface



Methods cell patterning → pictures in slides are good ~ Page 209

1) Photolithography
   - ↳ coat surface → rotate wafer → gets thinner and flat
   - ↳ UV exposure via mask
   - ↳ remove uncured photoresist with acetone
     this is where cells bind

⇒ can also use Parafilm → no UV + mask necessary, peel of puraform
↳ very hydrophobic ~ slide 208

2) Replica molding
   - ↳ use master mold fabricated by photolithography
   - ↳ cast polymer on surface structure

3) Microcontact / μContact Printing
   - ↳ use polymer from replica molding to contact print substance

4) Microfluidic patterning
   - ↳ use polymer from replica molding to build channel
   - ↳ use microfluidic flow pattern to place/bind substrate

5) Microwells ? unsure if separate method
   - ↳ put cells in wells
   - ↳ multi-cell patterning in wells also exist