



MASARYKOVA
UNIVERZITA

Detekce rakovinných markerů prostřednictvím proteinových čipů

C4700 – Vybrané biochemické metody – cvičení

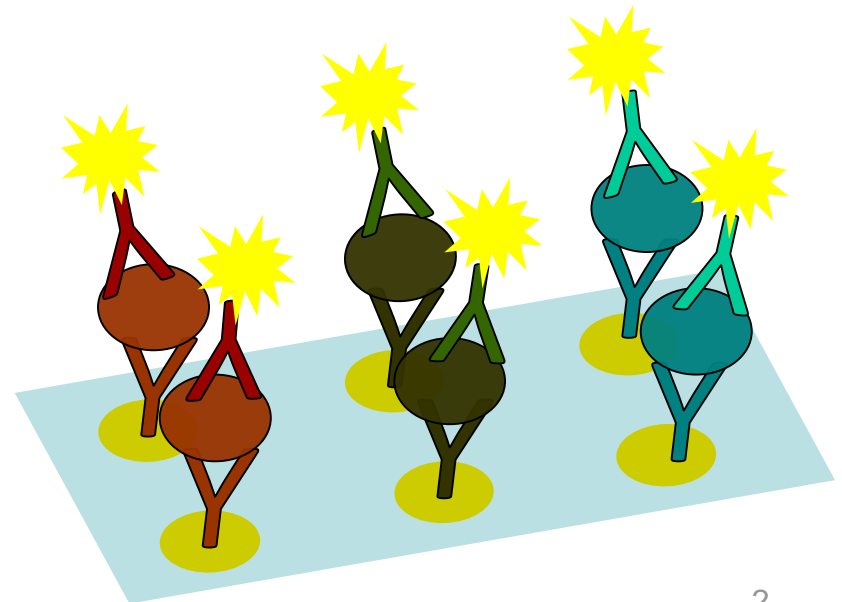
Matěj Pastucha

Jaro 2019

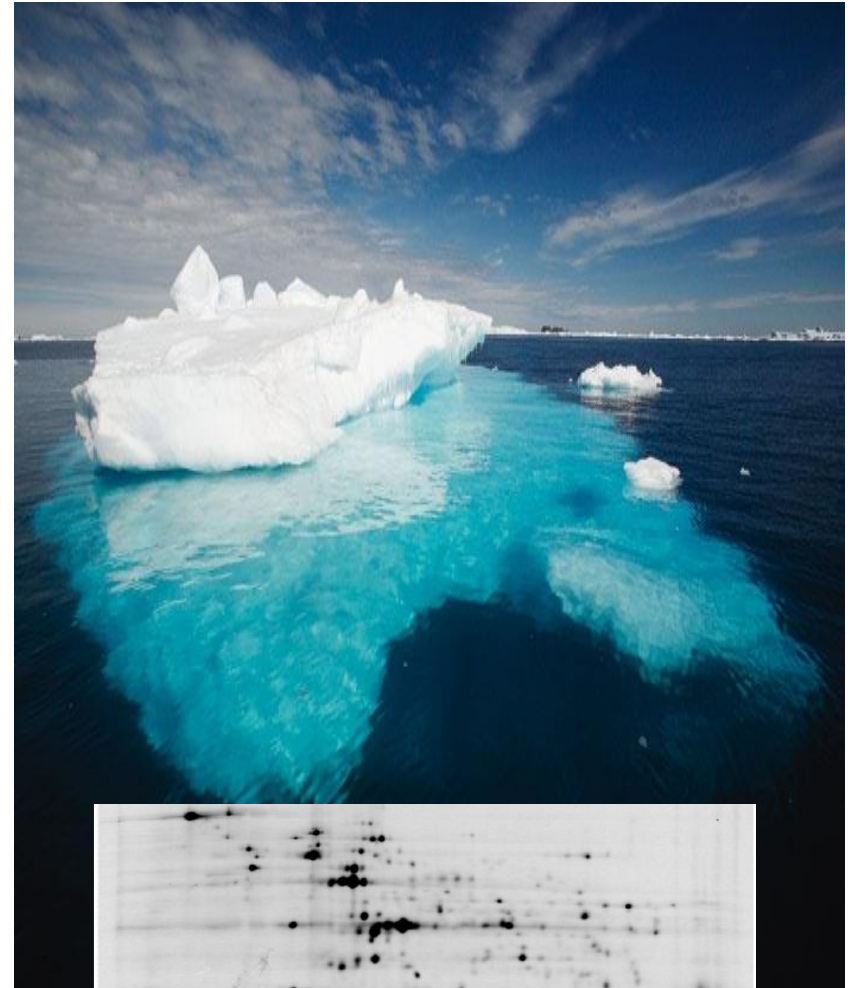
Proteinové čipy (microarrays)

- Multiplexní analýza proteinů – proteomika
- Mapování proteomu, profilování exprese
- Screening interakčních partnerů/inhibitorů apod.
- Detekce diagnostických markerů
- Miniaturizace
- High-throughput
- Nízká spotřeba vzorku
- Selektivita a citlivost

- IVD (In-vitro diagnostika)

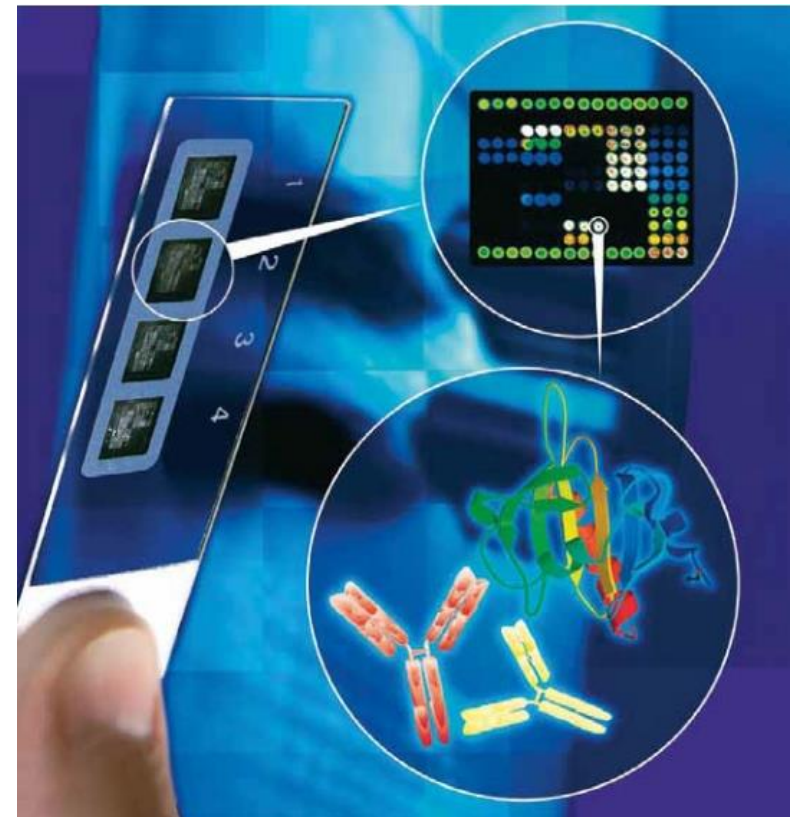


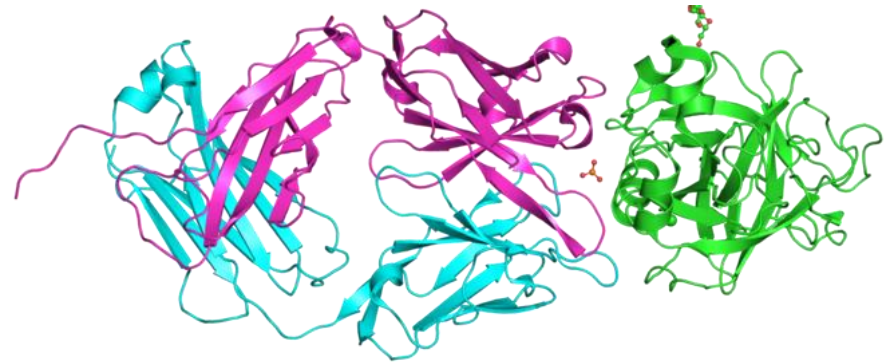
- Genom - DNA
 - Transkripce
 - Modifikace
 - Sestřih
- Transkriptom - mRNA
 - Translace
 - Modifikace
- Proteom
 - Korelace?
 - Funkce
 - Dynamický systém



Aplikace - motivace: ImmunoCAP® ISAC

- Měření specifických IgE
- 112 alergenních komponent z 51 zdrojů
- 30 μ l krevního séra, 3 h
- 5 000 Kč





PDB crystal structure 2zcl

PSA – diagnostický marker

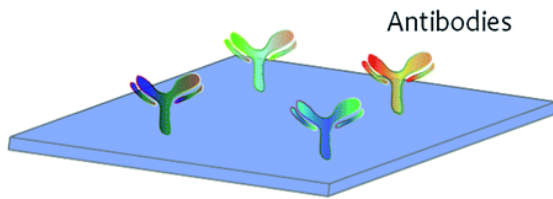
- Human kallikrein 3
- <http://www.uniprot.org/uniprot/P07288>
- Serinová proteasa, v seminální tekutině
- 261 AK; 28,7 kDa; pI 7,61 (teor.)
- Marker nádoru prostaty
- Norm. hladina < 4 ng/mL (diskutabilní)
- Monitorování léčby
- Další markery - kallikrein-related peptidase 2, prostate cancer antigen 3 (PCA3), fúzní gen TMPRSS2-ERG

Typy proteinových čipů

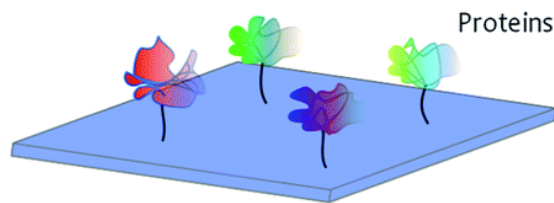
- **Protilátkové – „analytické“, „capture“**
 - Expresní profily; hladiny proteinů
 - Diagnostika – detekce biomarkerů, stanovení cytokinů
- **Funkční**
 - Hledání interakčních partnerů
 - Srovnání vazebných vlastností např. Ab
 - Posttranslační modifikace
 - Diagnostika – detekce autoprotilátek
- **Reverzní – RPMA – „lyzátové“**
 - Expresní profily, hladiny proteinů
 - Diagnostika – detekce biomarkerů
 - Klinické testy léčiv

Typy proteinových čipů

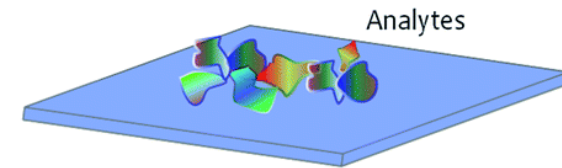
Analytical



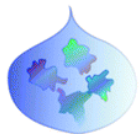
Functional



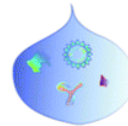
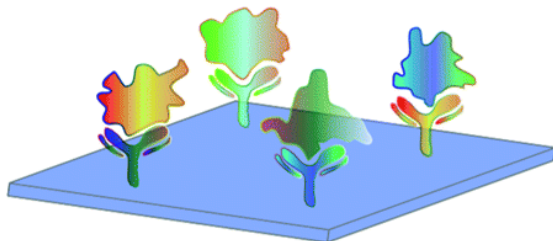
Reverse Phase



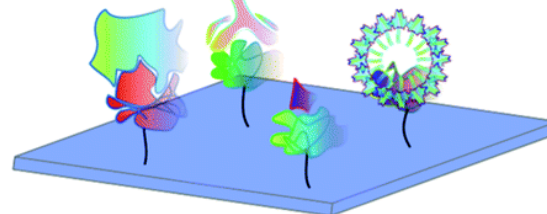
Incubation



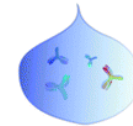
Proteins



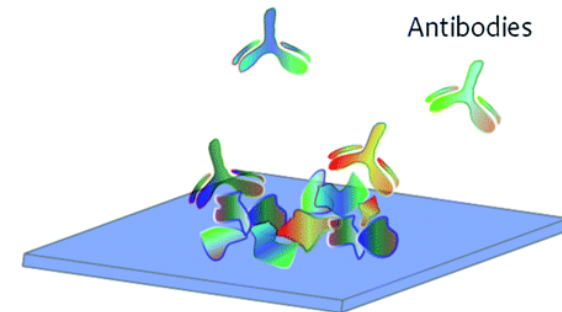
Protein Antibody Phospholipid



Small Molecule

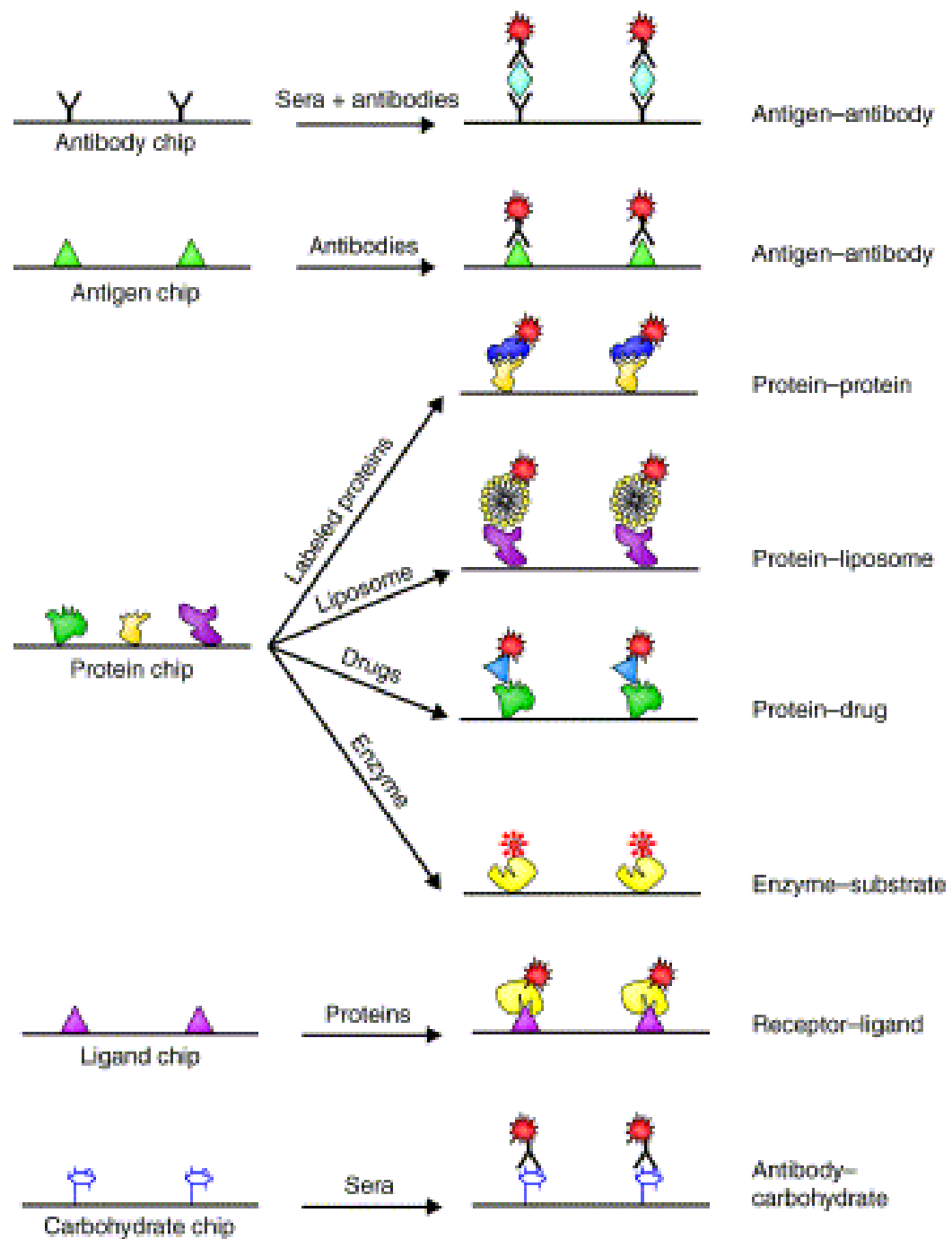


Antibodies



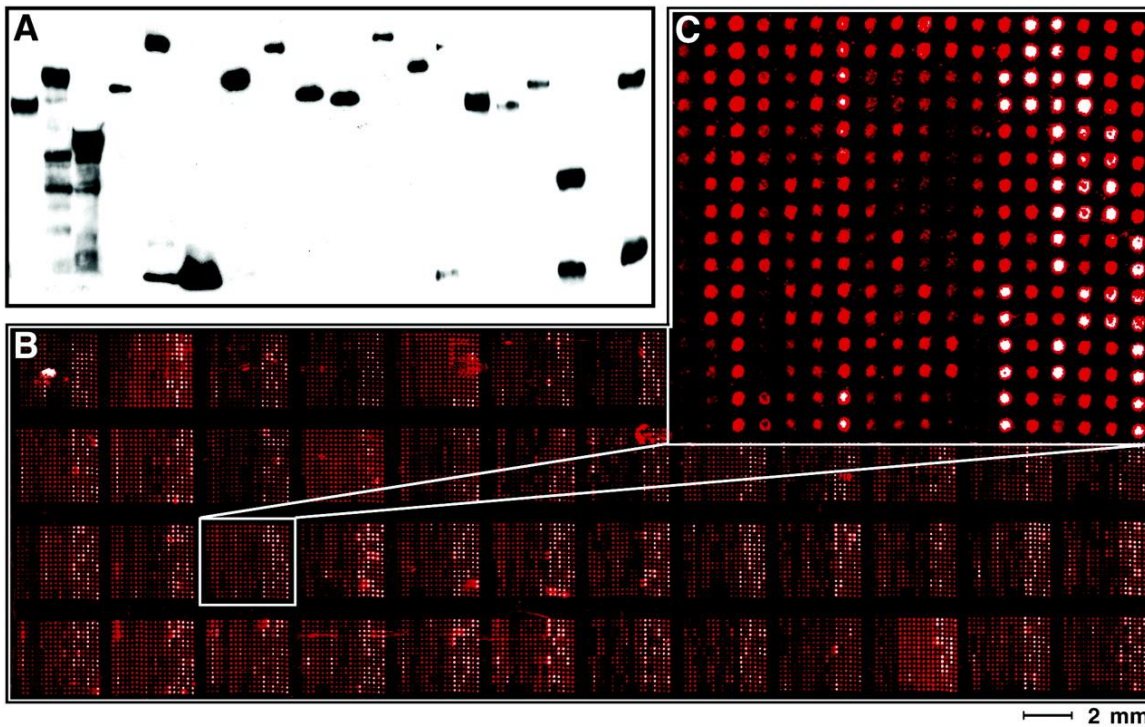
Romanov et al., Analyst 2014

Funkční čipy

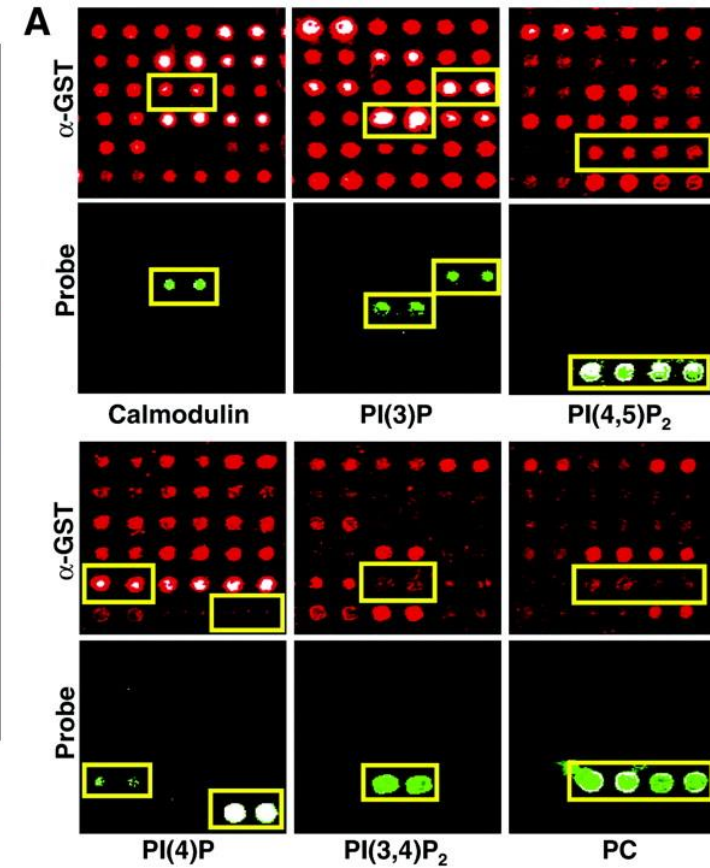


Zhu, Snyder;
Curr Opin Chem Biol. 2003

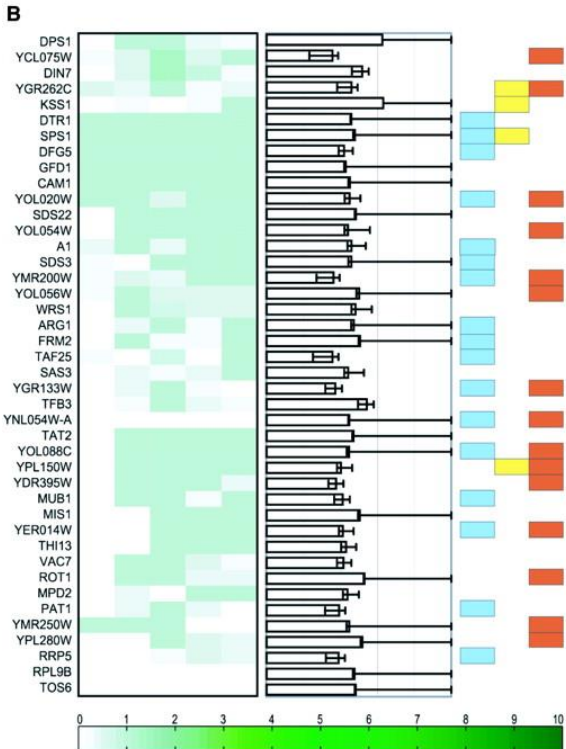
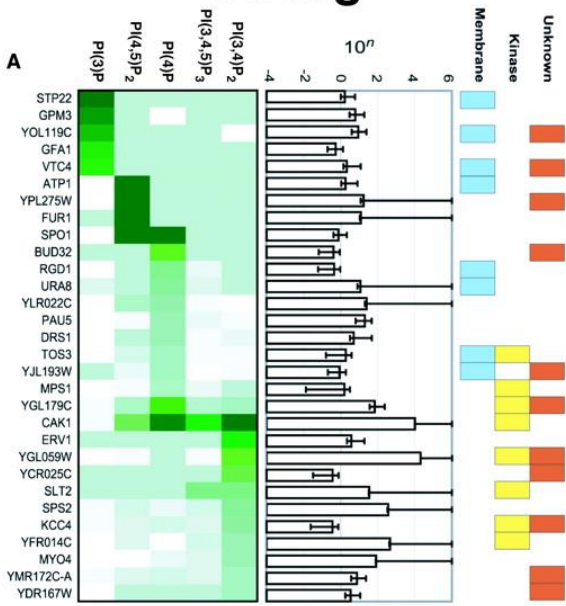
Kvasinkový čip – hledání interakcí



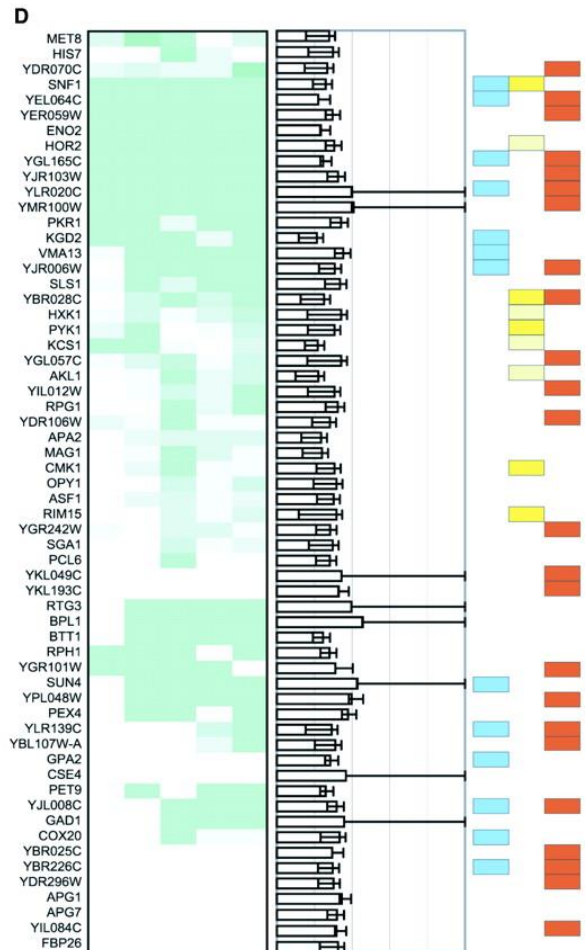
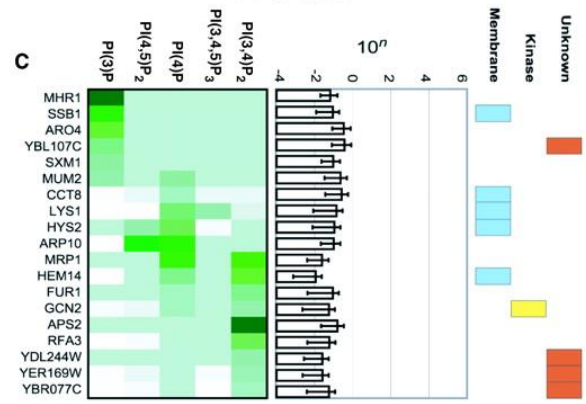
Zhu et al., Science 2001



Strong

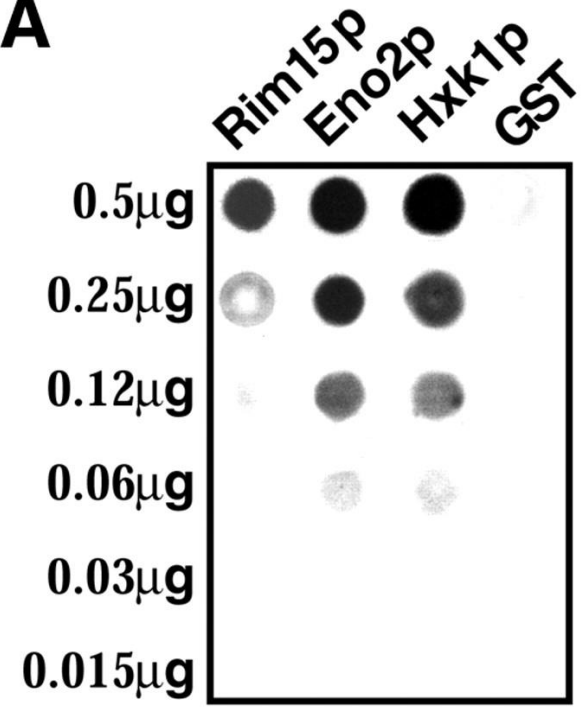


Weak

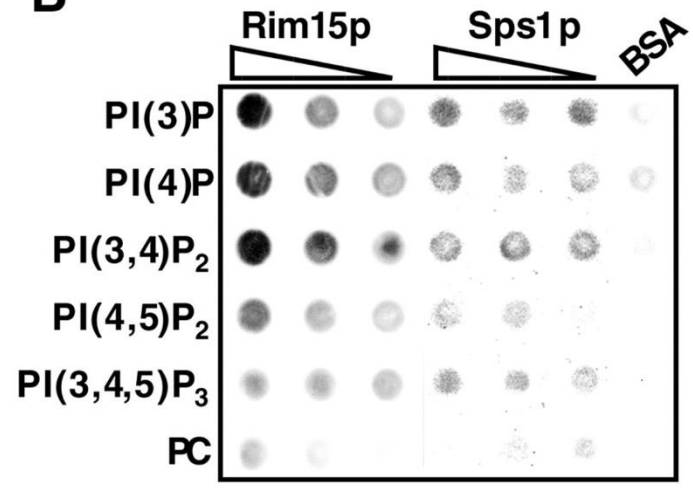


Ověření výsledků!

A



B



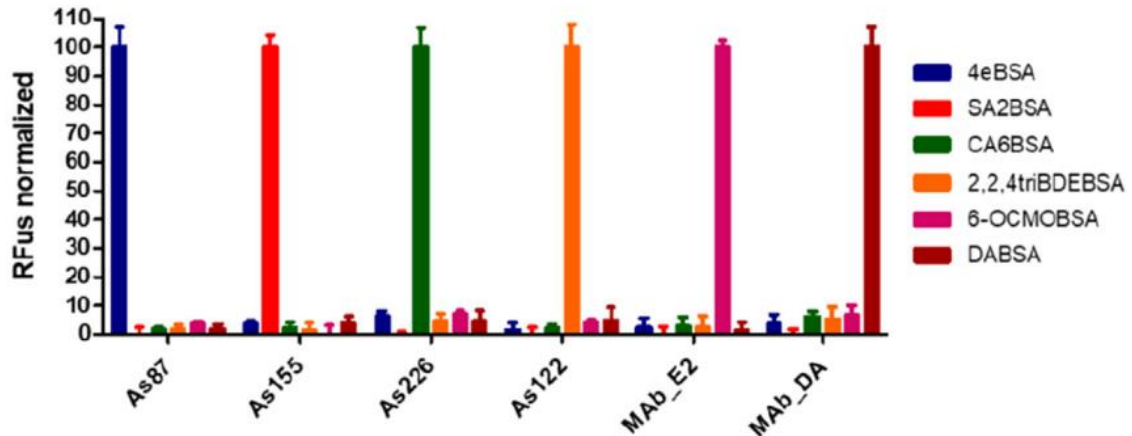
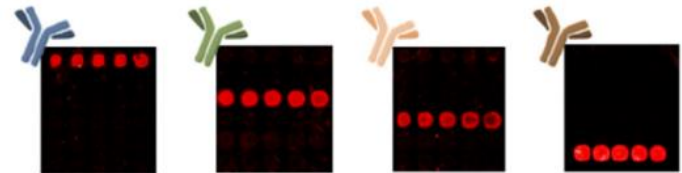
Multiplexní detekce kontaminantů životního prostřední

- Mořská voda
- Není nutná
příprava
vzorku
- Kompetitivní
stanovení
- 1h 30min

Sanchis et al., Talanta 2018

Analyte	Contaminant type	EQS ^a	ACD/ LogP	Levels reported ($\mu\text{g L}^{-1}$)
Irgarol 1051 [®]	Herbicide	2.5 ng L ⁻¹	3.27	0.013–2 [41,42]
Sulfapyridine	Antibiotic	–	0.03	0.05–0.3 [43,44]
Chloramphenicol	Antibiotic	–	1.02	0.001–0.2 [45,46]
Polybrominated diphenyl ether: BDE-47	POP ^b	2.4 fg L ⁻¹	7.39	0.004–0.11 [38,47]
17 β - Estradiol	Hormone	80 pg L ⁻¹	4.13	0.004–0.016 [48,49]
Domoic acid	Algal toxin	–	0.61	0.02–13 [50,51]

Matrix of spots





Komerčně dostupné proteinové čipy

*Sutandy et al., Curr Protoc
Protein Sci 2013*

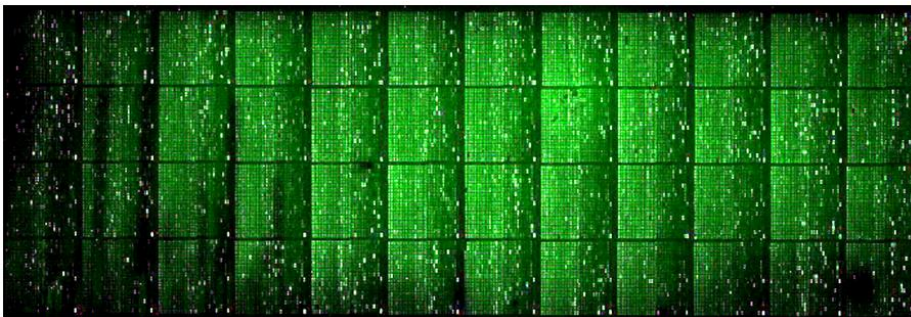
C4700 - Proteinové čipy

Product Type	Product Name	Company	Type of Array	Protein Content
Human protein	ProtoArray®	Invitrogen	Functional	9000 human proteins
Kinase	Kinex™	Kinexus Bioinformatics	Functional	200 human kinase proteins
Pathogen	Arrayit Pathogen Antigen Microarray	Arrayit Corporation	Functional	Essential proteins of different pathogens
Antibody for specific group of proteins	RayBio® Human RTK Phosphorylation Antibody Array	RayBiotech, Inc	Analytical	Antibodies against 71 human kinases
	RayBio® Human Cytokine Antibody Array	RayBiotech, Inc	Analytical	Antibodies against various human cytokines
	PlasmaScan™ 380 Antibody Microarray	Arrayit Corporation	Analytical	Antibodies for human plasma detection
	Cytokine Antibody Microarray	Full Moon BioSystems, Inc	Analytical	Antibodies against 77 human cytokines
	Kinase Antibody Microarray	Full Moon BioSystems, Inc	Analytical	Antibodies against 276 human kinases
Antibody for pathway detection	MAPK Pathway Phospho Antibody Array	Creative Bioarray	Analytical	185 antibodies against phospho-proteins in the MAPK pathways
	Signaling Explorer Antibody Microarray	Full Moon BioSystems, Inc	Analytical	1358 antibodies for multiple pathways
	Wnt Signaling Phospho Antibody Microarray	Full Moon BioSystems, Inc	Analytical	227 phospho-antibodies for cell growth, movement and development pathways
Cell lysate	SomaPlex™	Protein Biotechnologies	Reverse-phase	A variety of human cancer cell lysates

Komerčně dostupné proteinové čipy

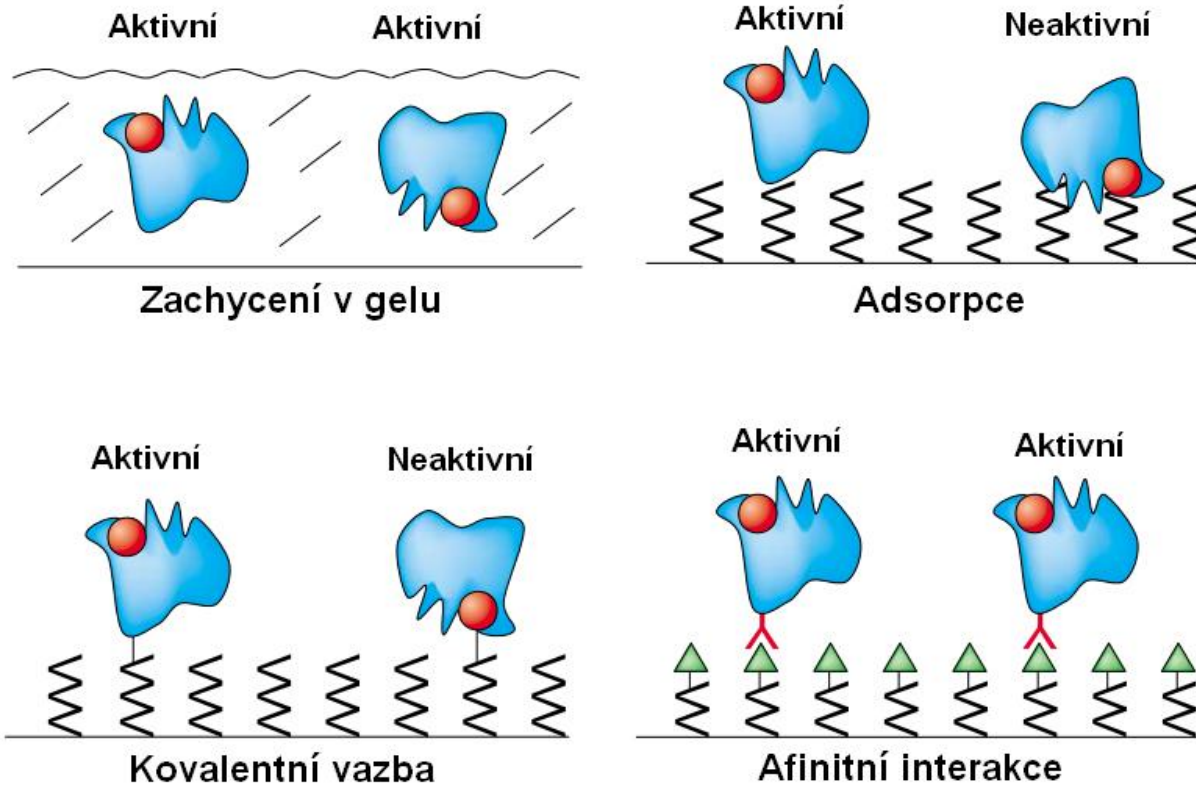
- [Abcam protein arrays](#)
- [ProtoArray® Human Protein Microarray v5.0](#)
 - > 9 000 lidských proteinů
- [Arrayit HuProt™ v3.1 24K](#)
- Příprava čipů na zakázku

Class	No. on array
Protein kinases (unique)	268
Protein kinases (including domains, splice variants, and mutants)	776
Transcription factors	328
Membrane proteins	2,635
Nuclear proteins	2,252
Signal transduction	1,526
Secreted proteins	192
Cell communication	1,687
Metabolism	3,862
Cell death	505
Protease/peptidase activity	219



C4700 - Proteinové čipy

Způsoby imobilizace



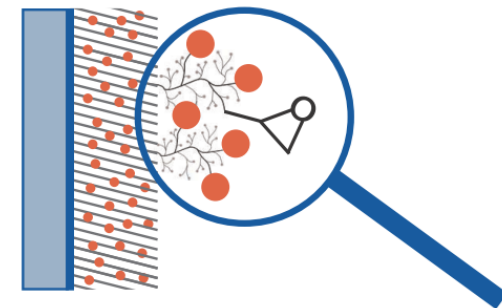
Zhu, Snyder, Curr Opin Chem Biol. 2003

Způsoby imobilizace

- Kovalentní – Aldehyd-, Amino-, Epoxy-, NHS-
- Adsorpce – PVDF / nitrocellululosové membrány, polystyren
- Difuze, trapping – agarózové, polyakrylamidové gely
- Afinitní záchyt – Ni-NTA, Streptavidin, GSH
- 2D vs. 3D povrchy
- Polymerní kartáče „Brushes“
- „Antifouling“



2D-Slide
with functional groups, e.g. Epoxy



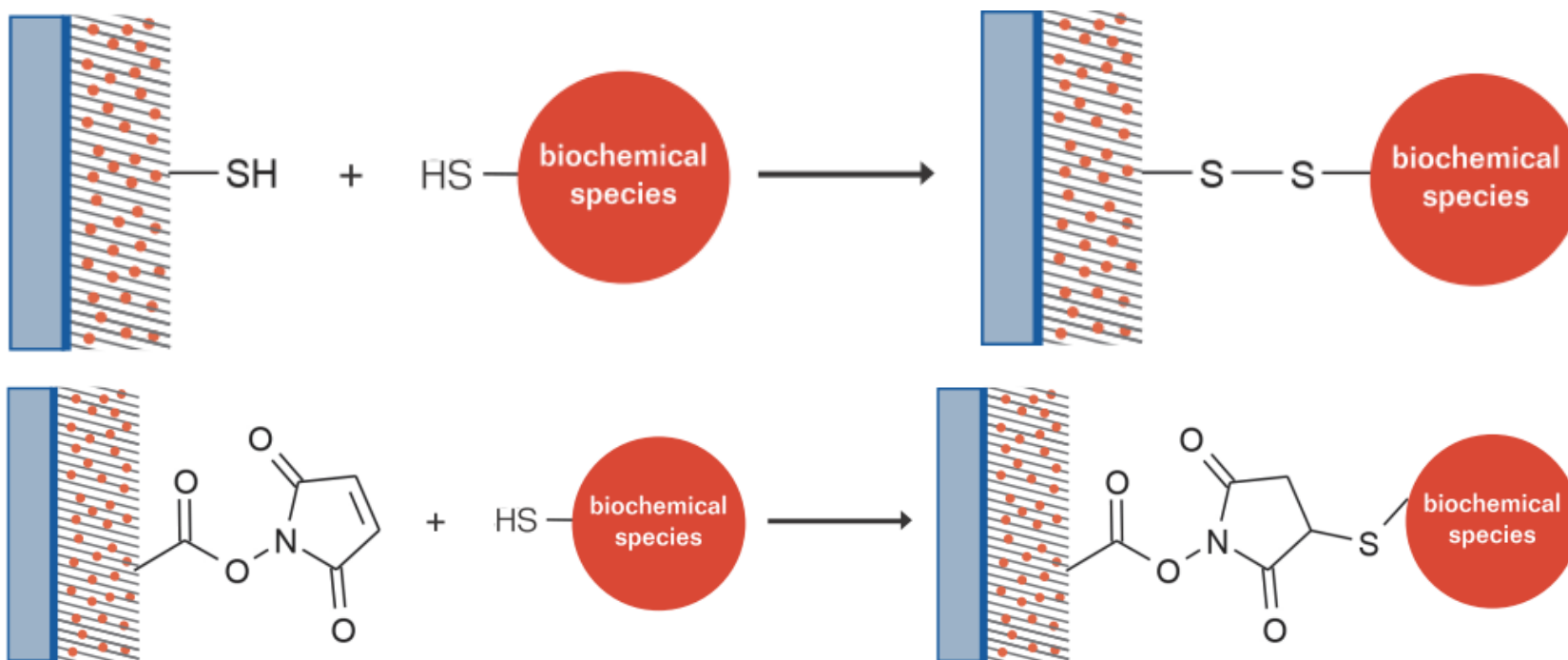
3D-Slide with Antifouling Matrix
and functional groups, e.g. Epoxy

Kovalentní imobilizace

*Li et al., Microarray
Technology 2013*

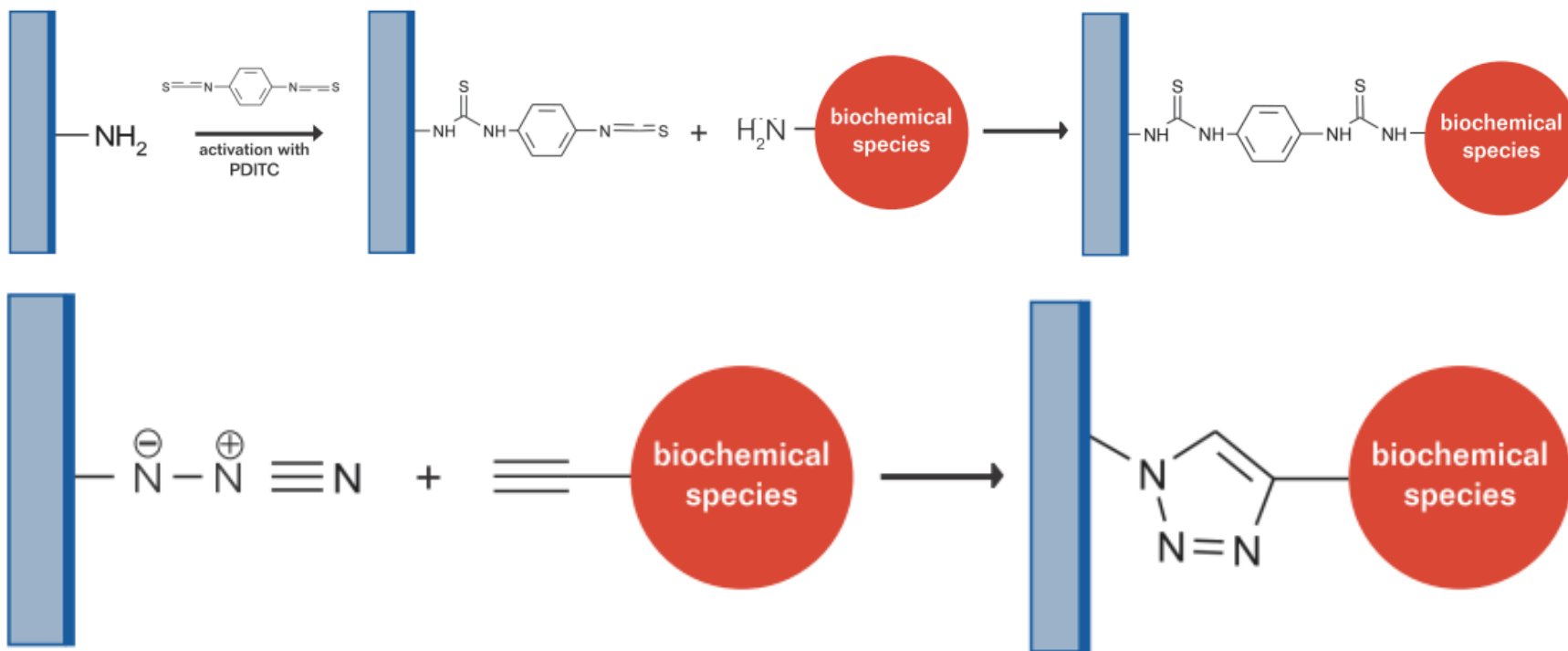
Surface functional groups	Protein functional groups	Covalent bond formed	
NHS ester 	Amine (H_2NR)		Amide
Aldehyde 	Amine (H_2NR)		Imine
Isothiocyanate 	Amine (H_2NR)		Thiourea
Epoxide 	Amine (H_2NR)		Amino-alcohol
Amine 	Carboxylic acid $HO(O)CCH_2R$		Amide

Kovalentní immobilizace - thioskupiny



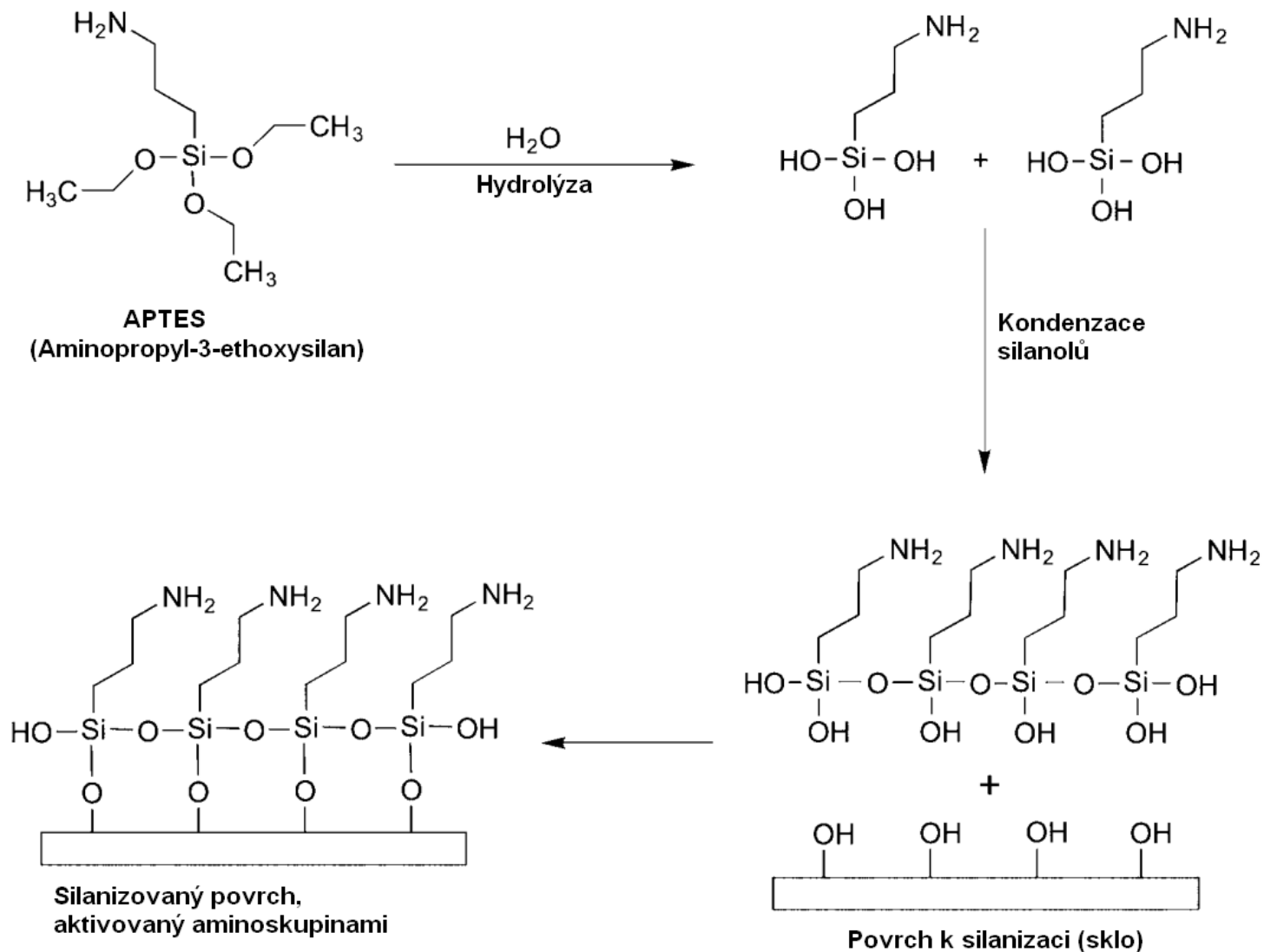
*PolyAn Functionalised Microarray Slides Handbook;
www.poly-an.de*

Další kovalentní



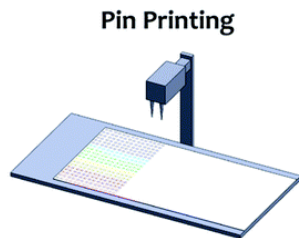
PolyAn Functionalised Microarray Slides Handbook;
www.poly-an.de

Příprava modifikovaných skel - silanizace

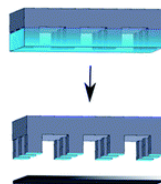


Způsoby tisku

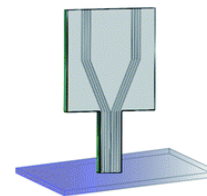
Contact



Microstamps

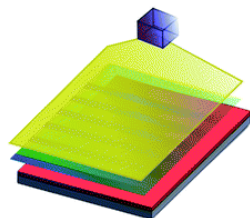


Flow Printing

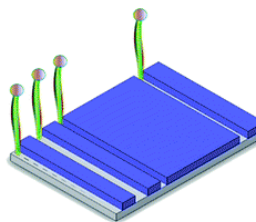


Lithography

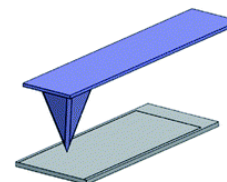
Photolithography



Electron-beam

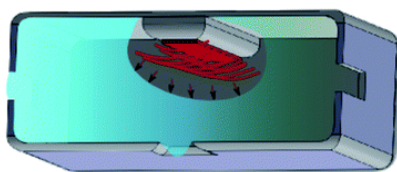


AFM Lithography

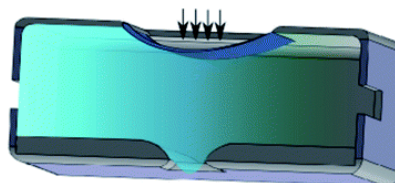


Non - Contact

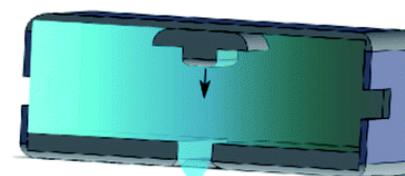
Thermal Inkjet



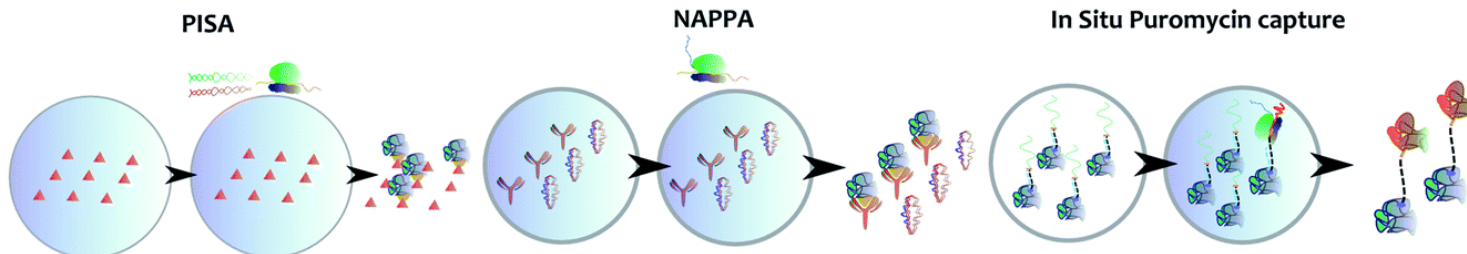
Piezo Actuation



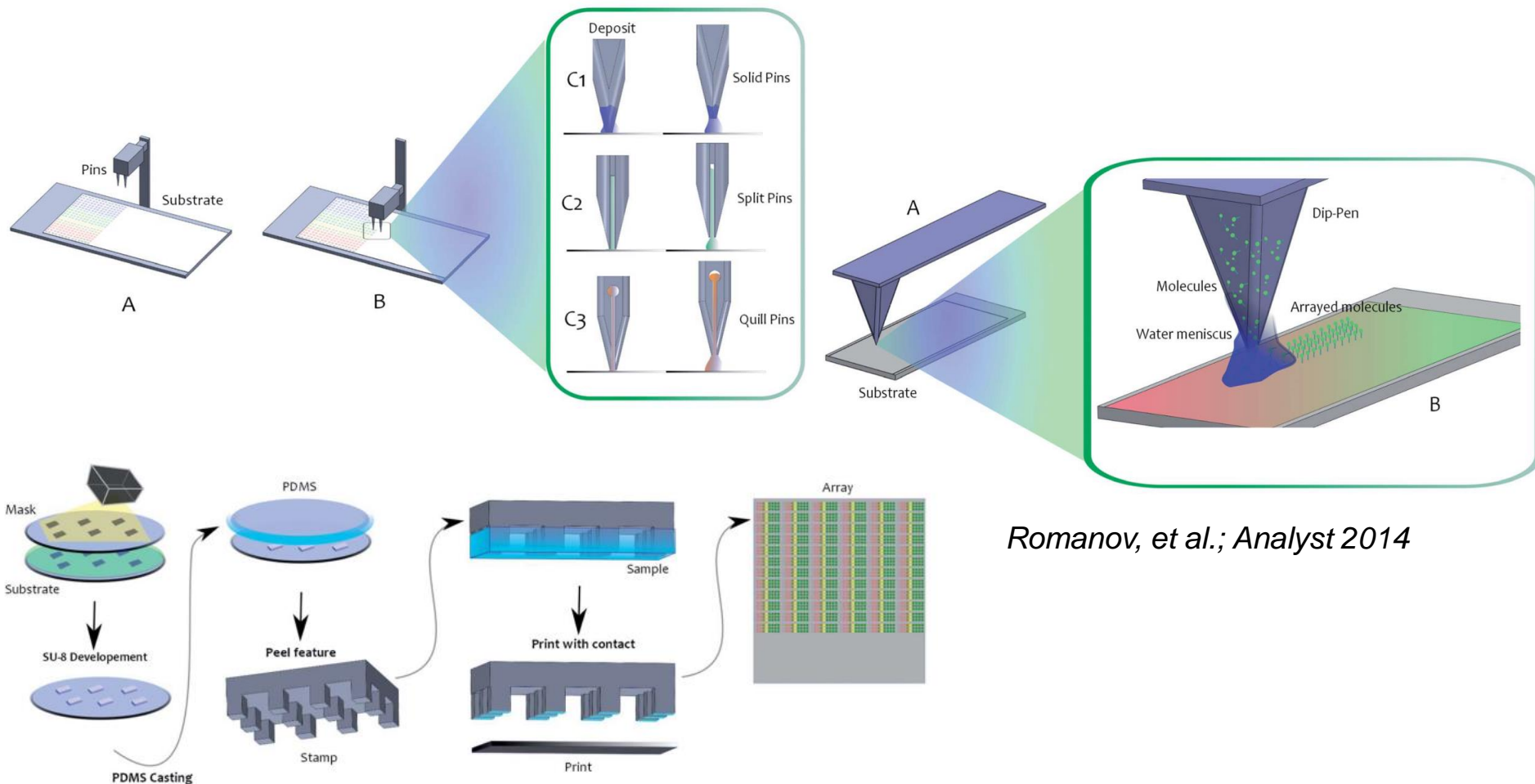
Valve Jet



Cell-Free

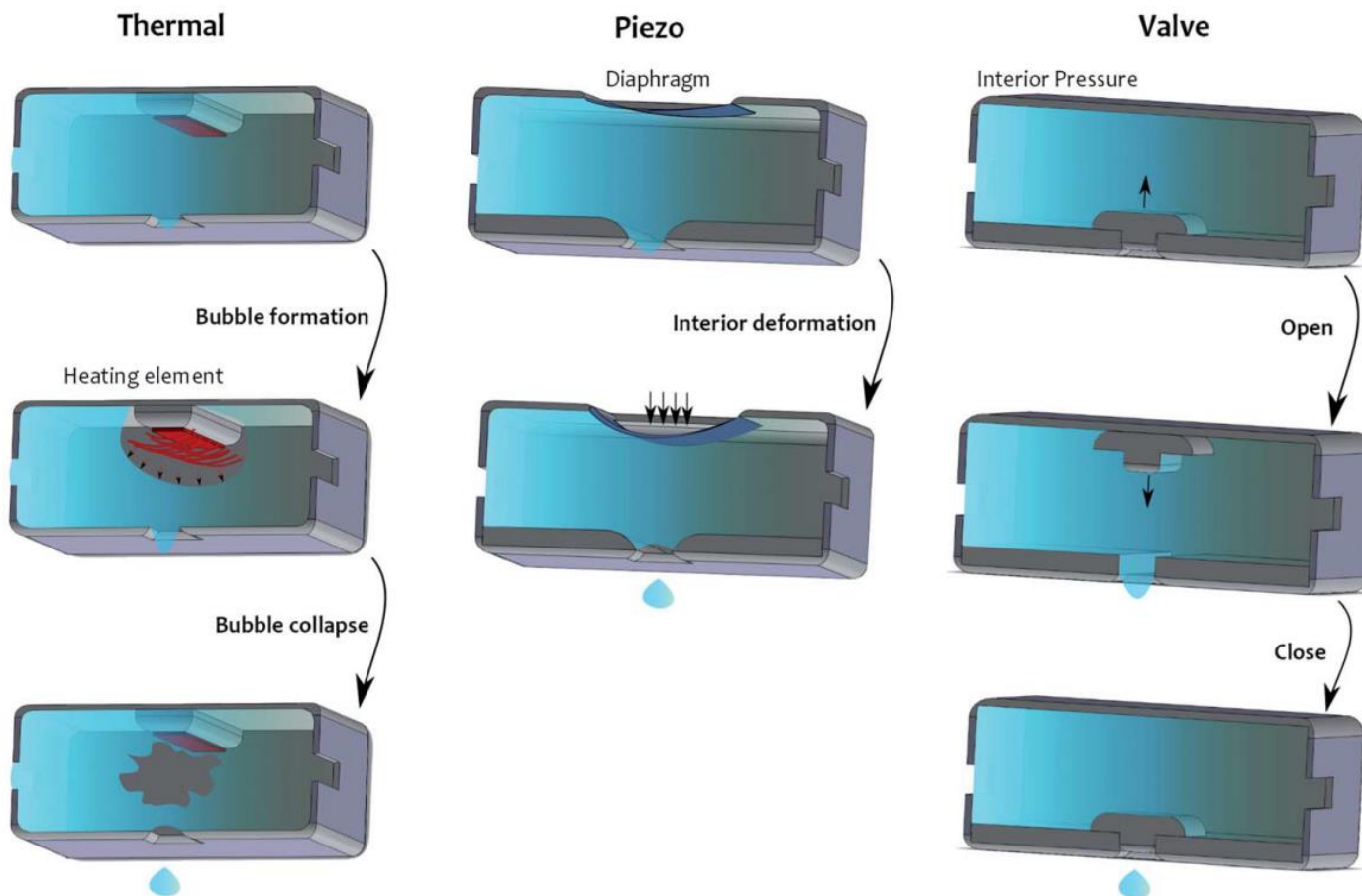


Kontaktní – pin printing, microstamping, dip-pen nanolitografie



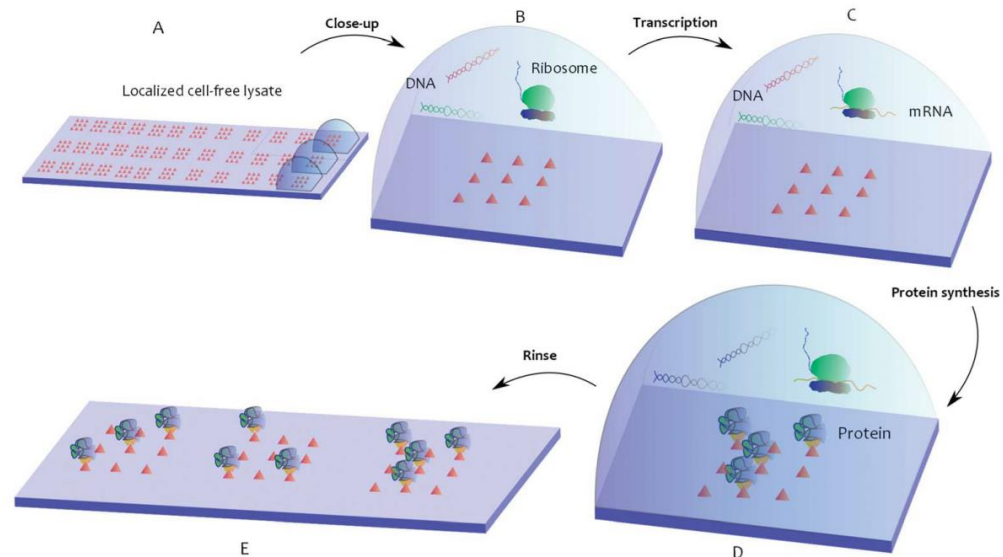
Romanov, et al.; Analyst 2014

Bezkontaktní



In-situ příprava prób přímo na čipu

- PISA - Protein In-Situ Array
- NAPPA – Nucleic Acid Programmable Protein Array
- DAPA – DNA Array to Protein Array





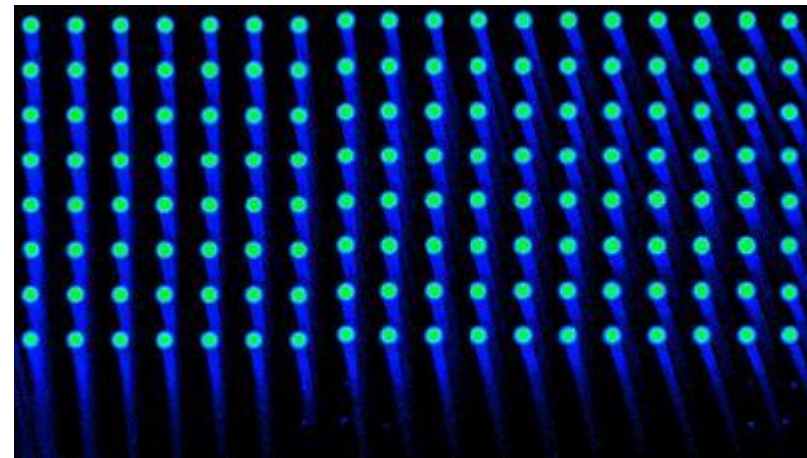
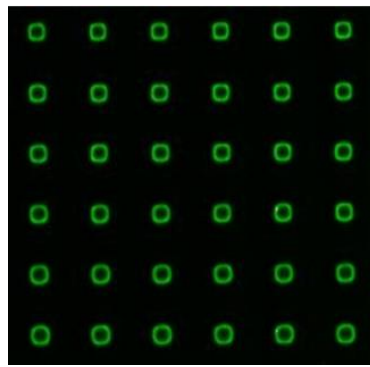
**zde plat'te
bezkontaktně**

Kontaktní vs. Bezkontaktní tisk

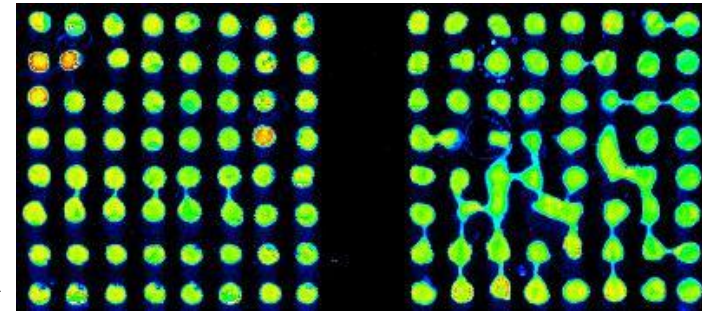
- Lepší kvalita a nižší variabilita spotů
- Šetrné k povrchům – nitrocelulóza, hydrogely
- CV 10-25 % vs. 3-7 %
- Přizpůsobitelné – široká využitelnost

- Horší throughput
- Vyžaduje optimalizaci
- Náchylné k tiskovým vadám – chybící a chybně umístěné spoty, satelitní kapky – kontaminace ústí kapiláry

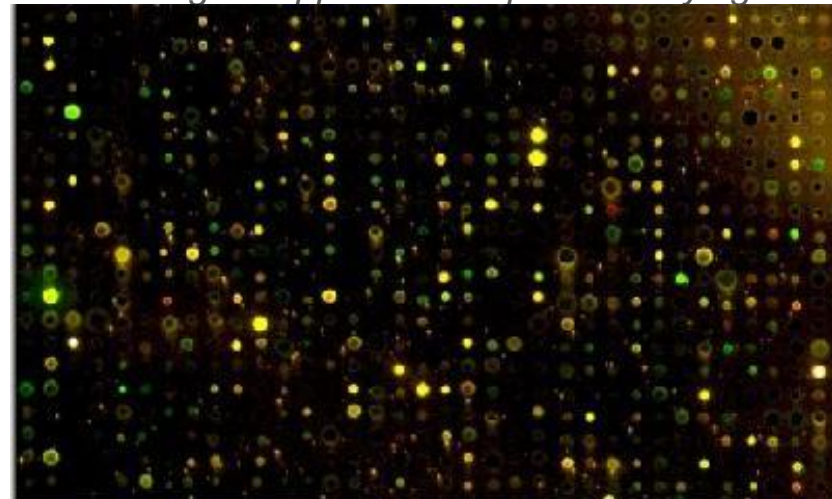
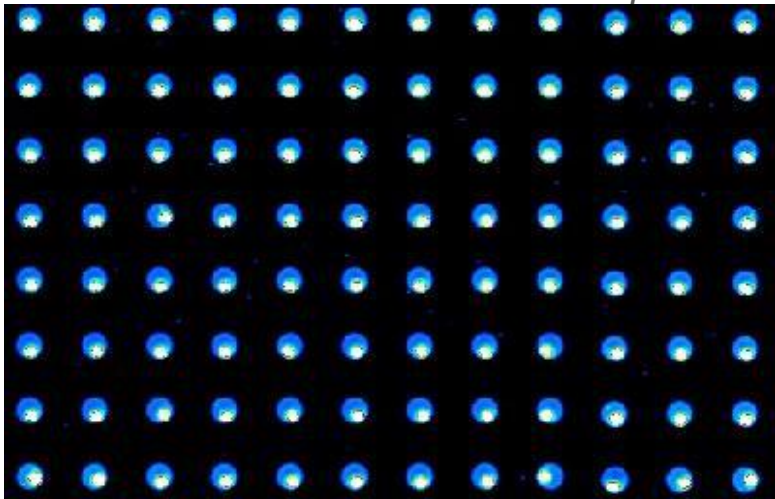
Defekty spotů



- Nehomogenní spoty, pozadí
- Coffee ring / doughnut – zasychání
- Fried egg – zasychání, fáze
- Comet tail – deaktivace povrchu
- Chybné spoty – tiskem / (bio)chemií



<https://www.schott.com/nexterion/english/application/faq/microarraying.html>

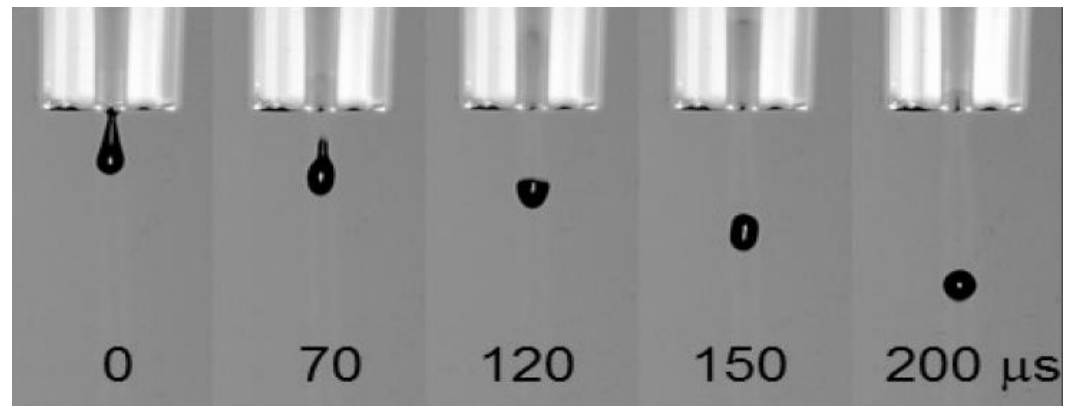


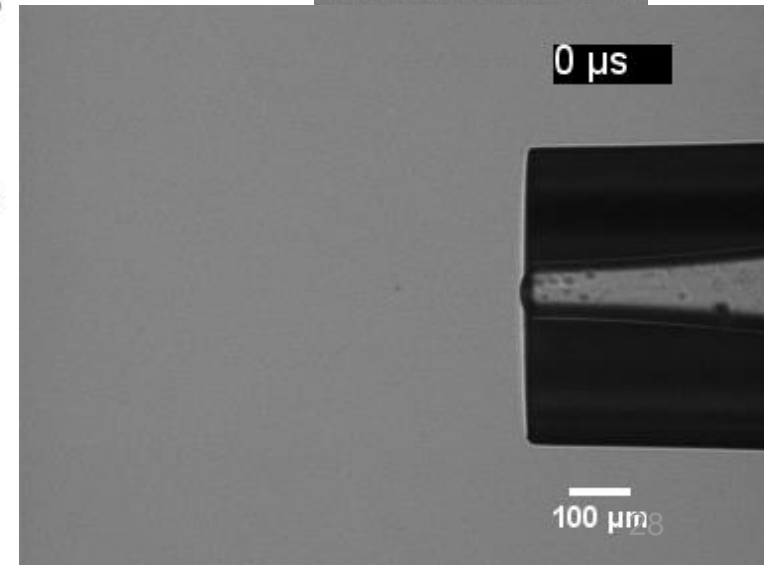
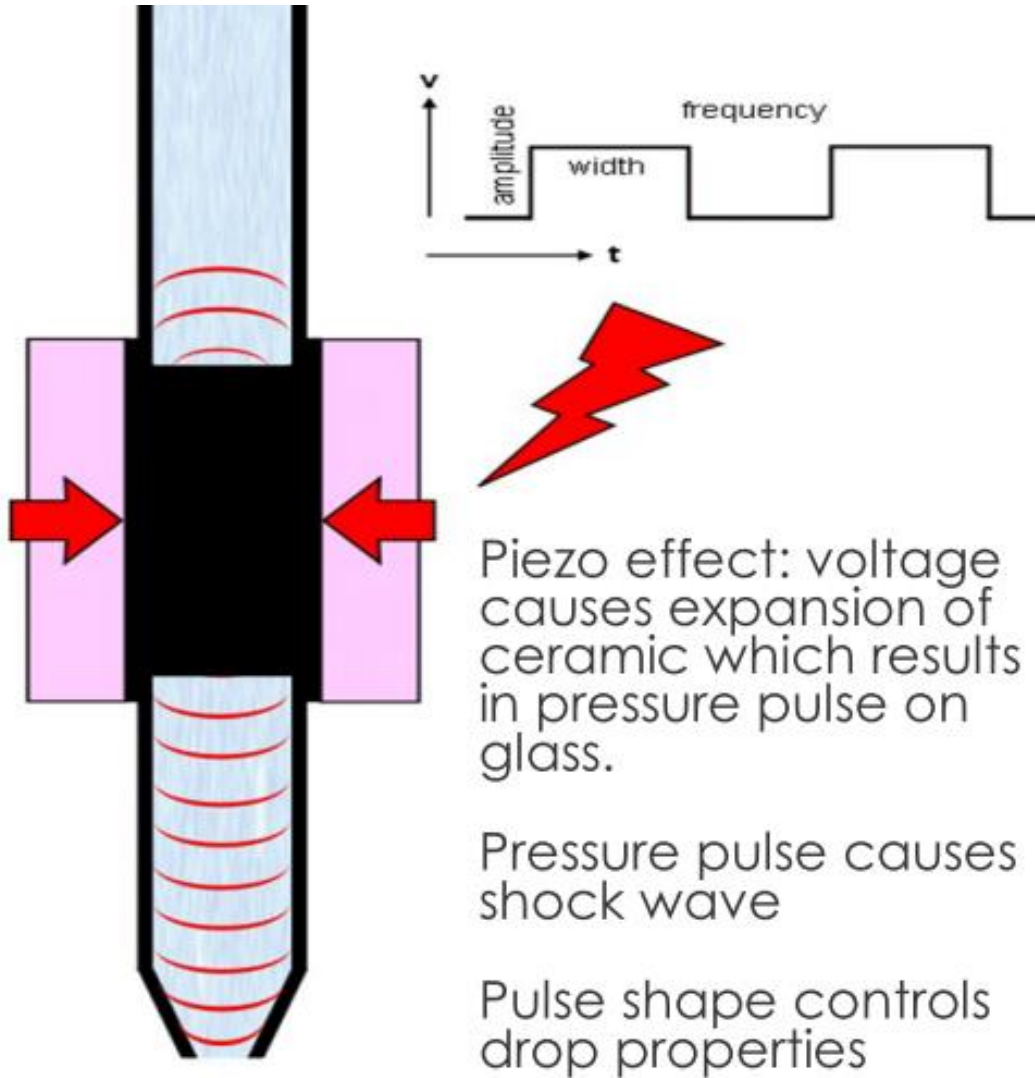
Aditiva pro zlepšení tisku

- Detergenty – Triton X-100, Tween-20
 - Cukry – trehalosa, glukosa, sacharosa
 - Polyhydroxyly – glycerol, PVA, PEG, ethylenglykol, butandiol
 - Jiné – DMSO, betain, $MgSO_4$
-
- Lepší homogenita spotu
 - Snížení CV
 - Může snížit efektivitu imobilizace

Proč piezo ink-jet?

- Dávkování malých objemů (pl – nl)
- Přesná depozice
- Zacházení s biomolekulami – jemná metoda
- Přizpůsobitelná – různé kapaliny
- Opakovatelnost
- Automatizace

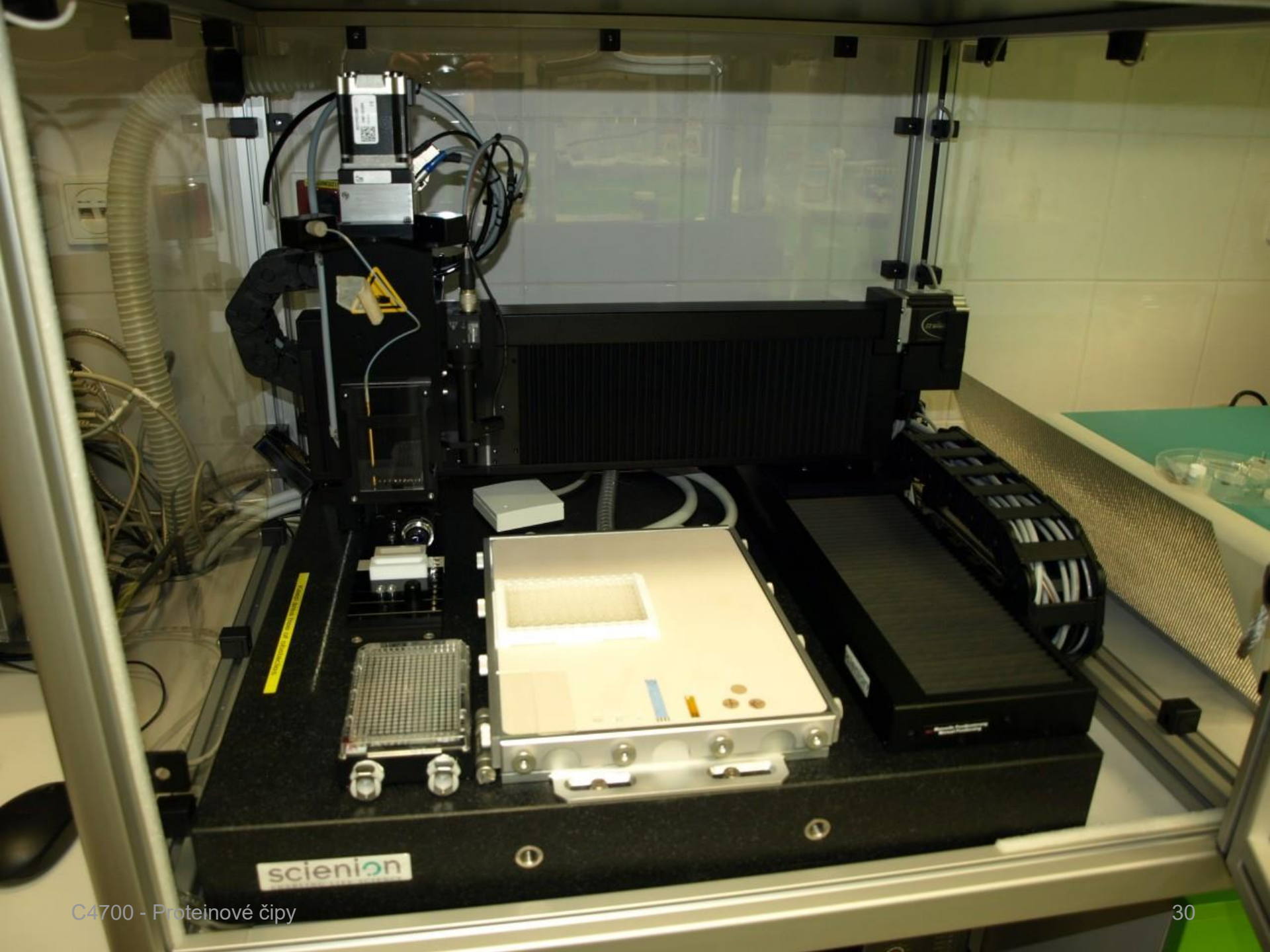




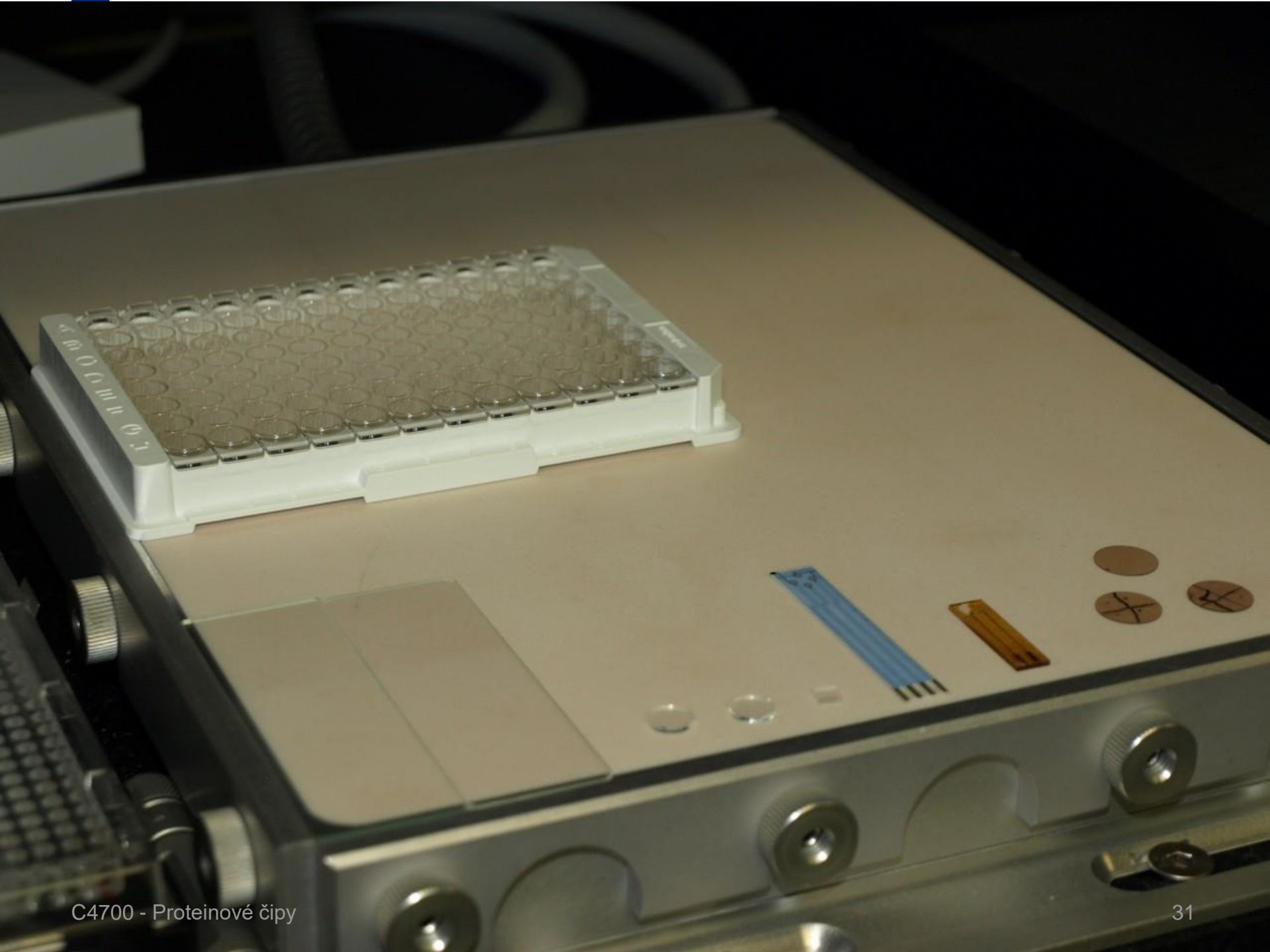
Scienion SciFLEXARRAYER S3

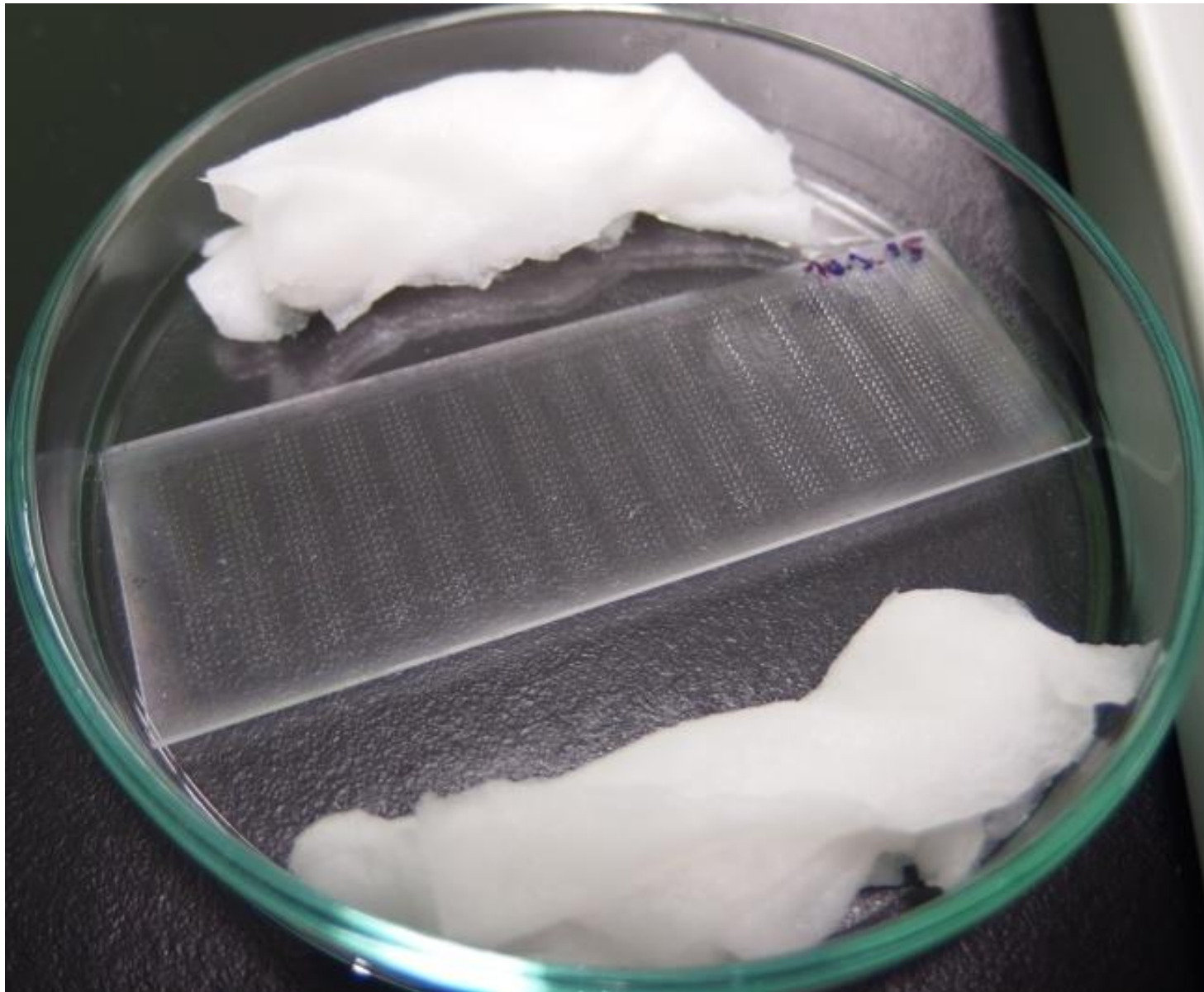
- Skleněná kapilára (až 8)
- Průměr ústí: 40 – 110 μm
- Objem kapky: 100 – 800 pl
- Průměr spotu: 80 – 250 μm
- Přesnost $\sim 5 \mu\text{m}$
- Různé vzorky a substráty





C4700 - Proteinové čipy

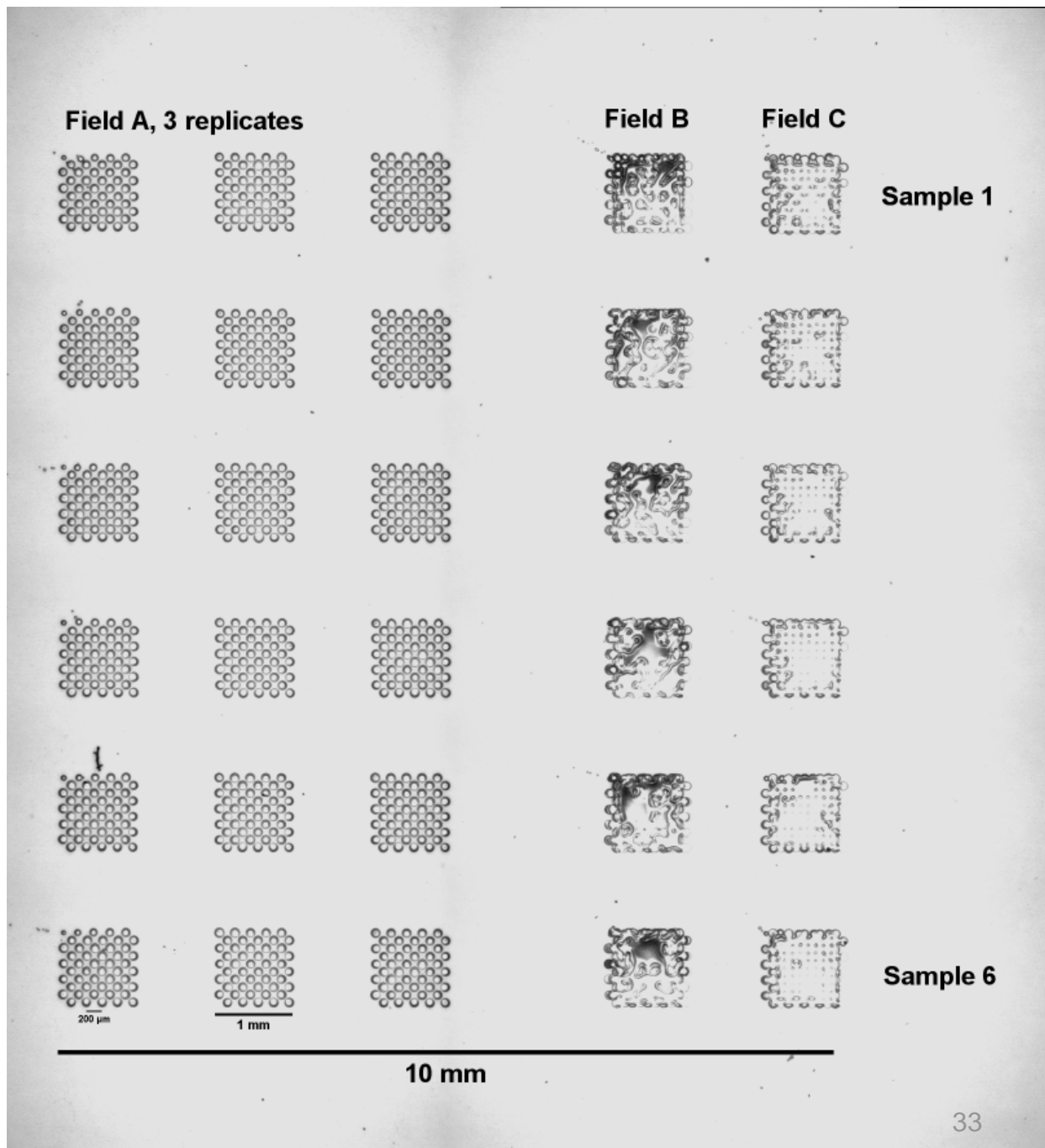
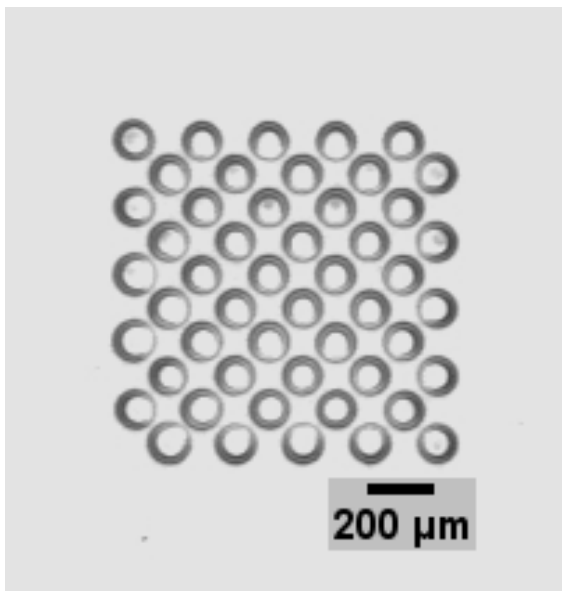




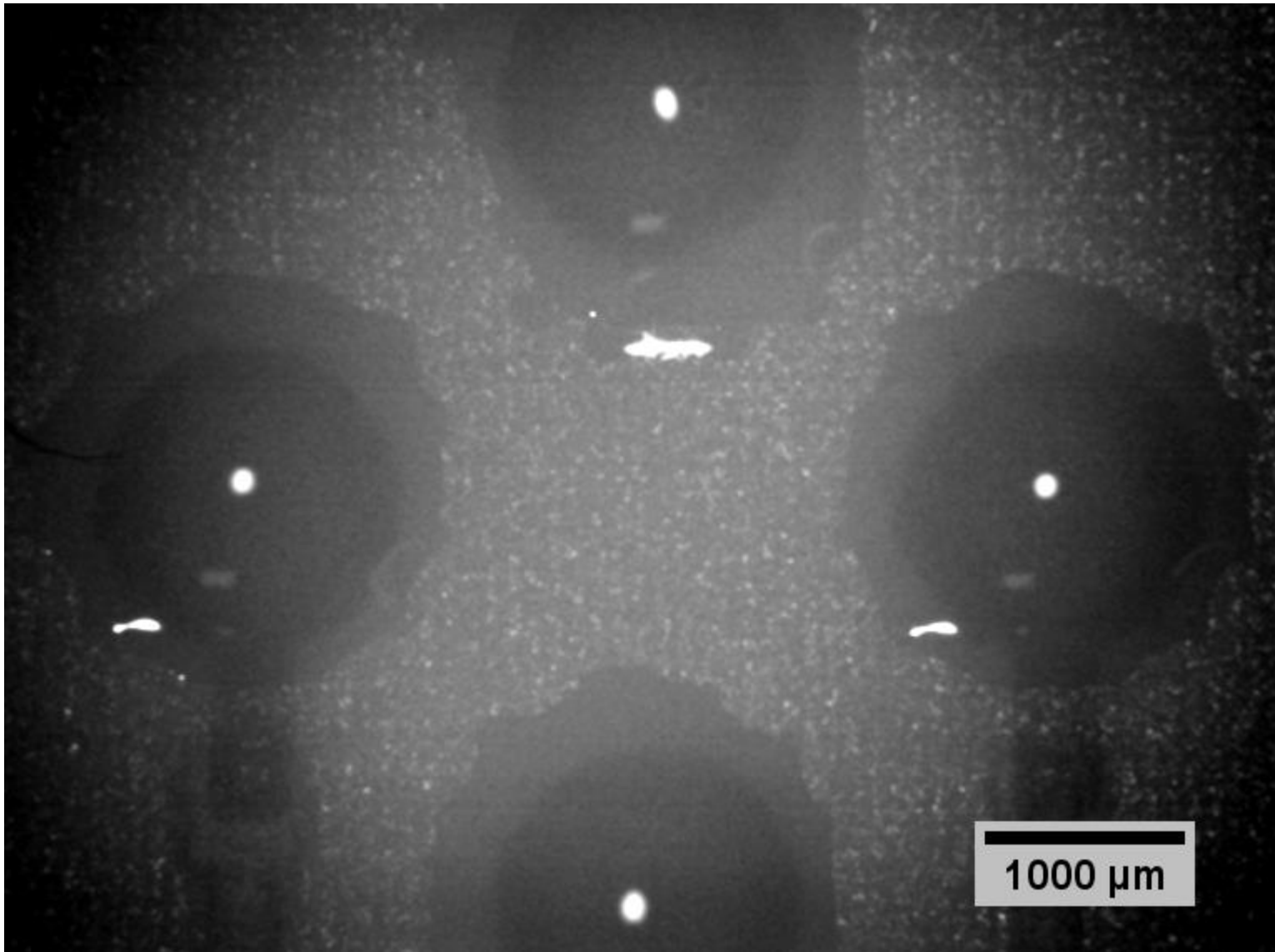
C4700 - Proteinové čipy

Různé podklady

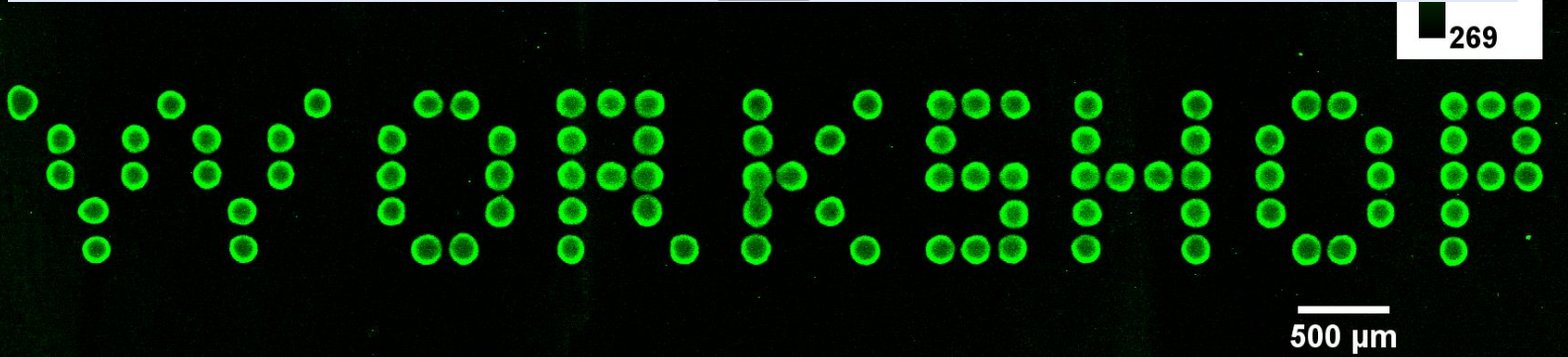
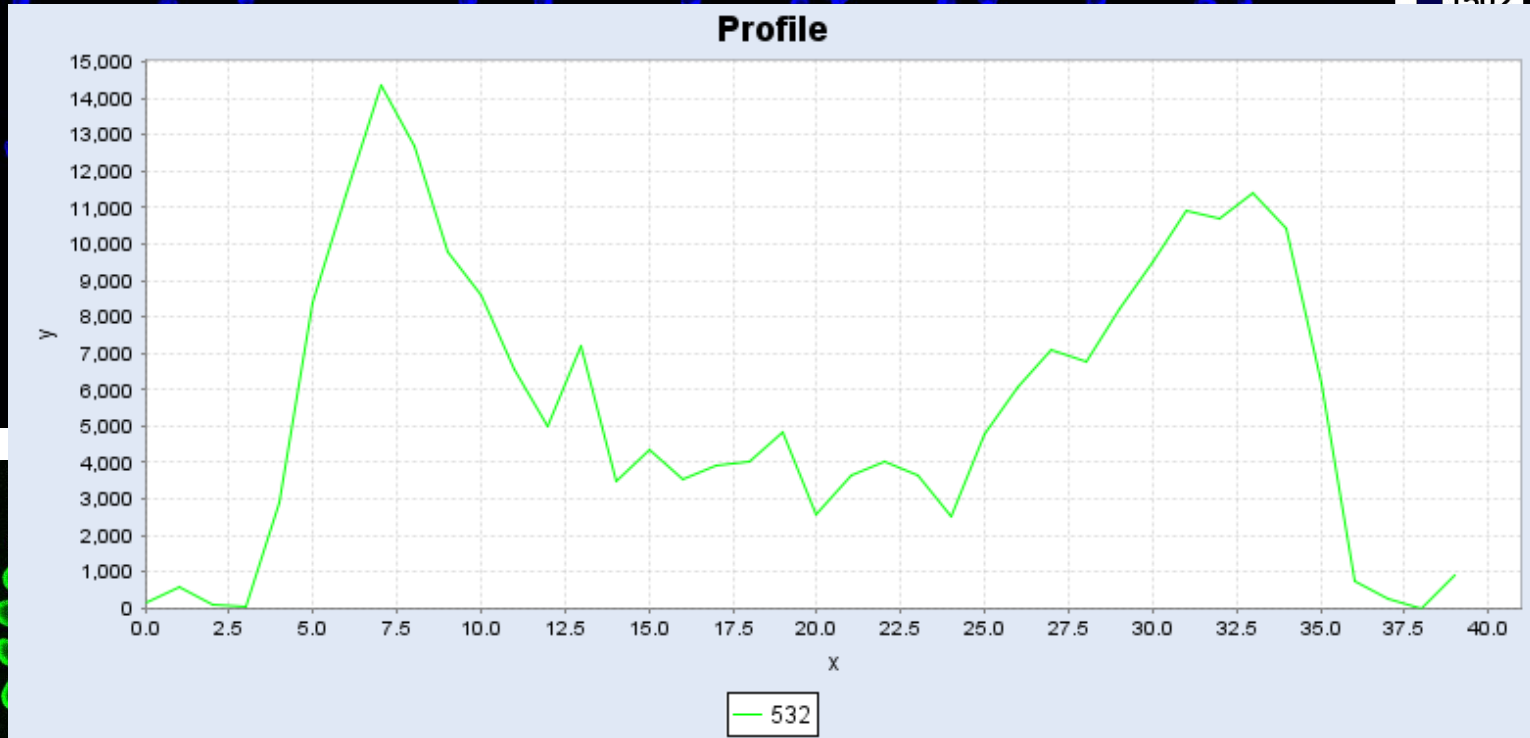
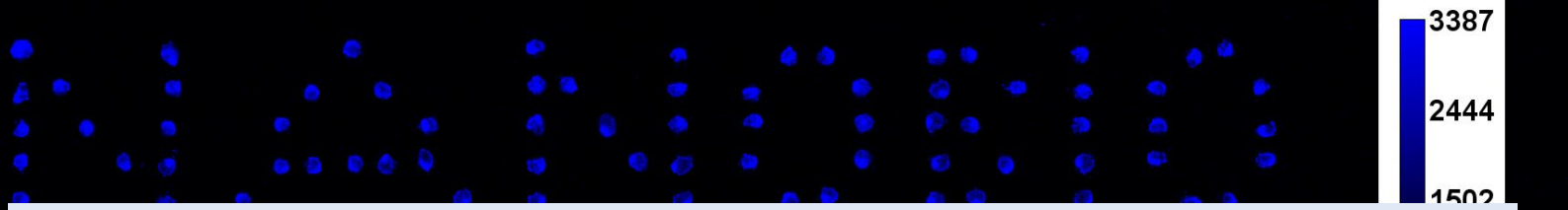
Nanášení vzorků



Biosensory



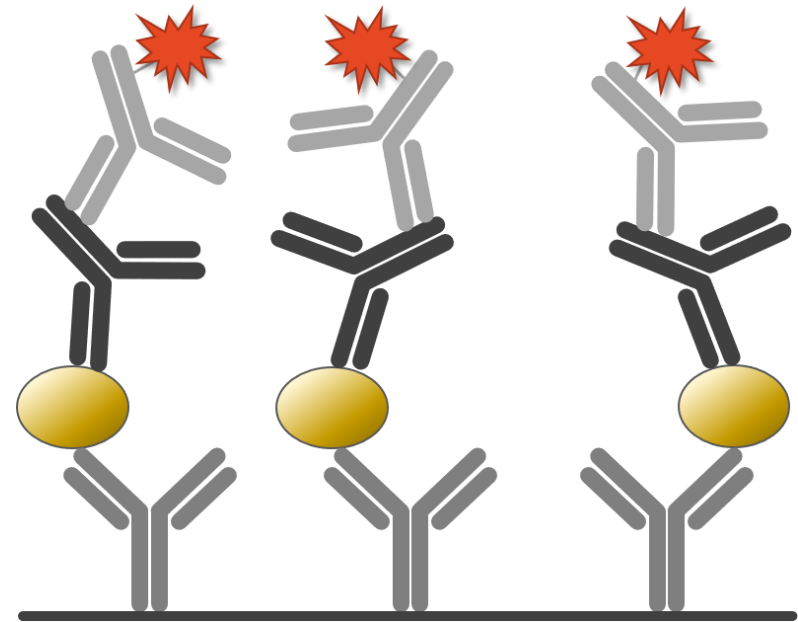
C4700 - Proteinové čipy



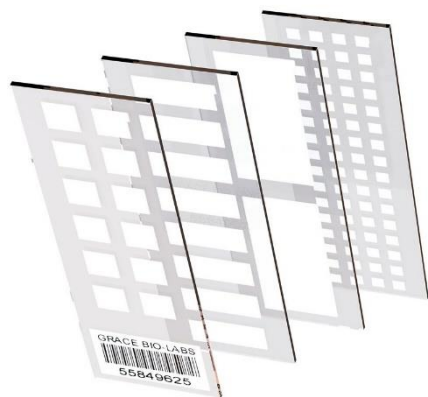
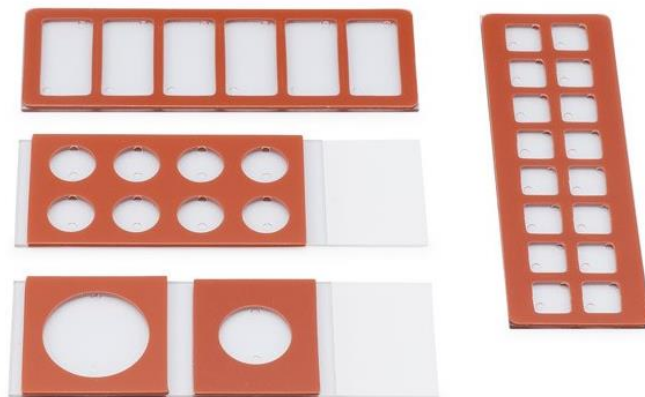
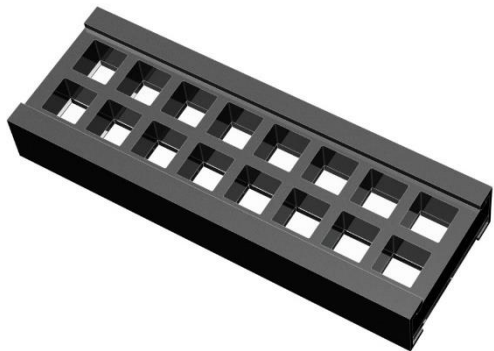
Protilátková array – sendvičové imunostanovení

- Tisk capture Ab
- Blokování povrchu
- Vzorek s antigenem
- Primární Ab
- Sekundární Ab
- Oplachy!

- Detekce
- Zpracování a analýza dat



Analýza více vzorků



Příště

- Vyhodnocení čipu – fluorescenční skener
- Zpracování dat

- Fluorescenční značení
- Jiné značky a možnosti pro vyhodnocení čipu
- Další formáty multiplexních analýz



Děkuji za pozornost

Financováno z projektu FRMU: Inovace výuky vytvořením laboratorní úlohy „Detekce rakovinných markerů prostřednictvím proteinových čipů“