无标记蛋白质芯片生物传感器及其应用

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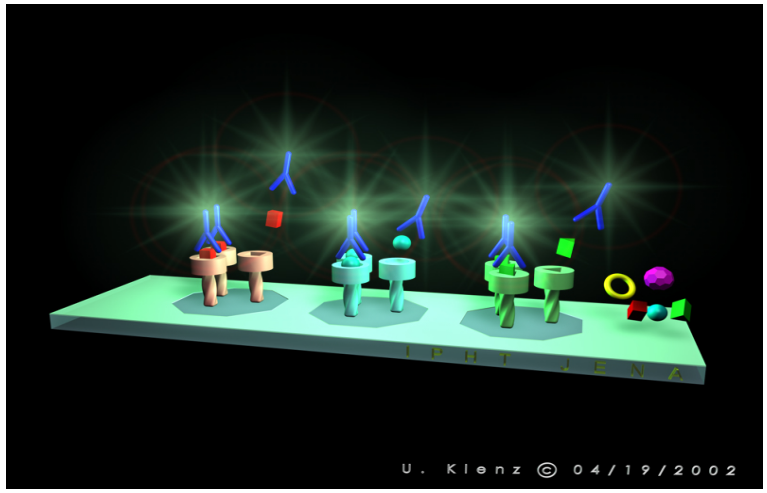
报告内容

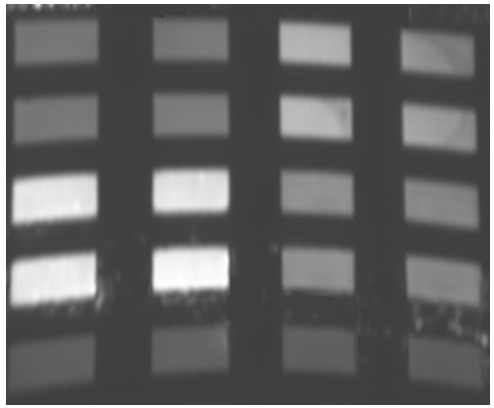
- 定义和背景
- 原理和技术
- 生物医学应用
- 展望

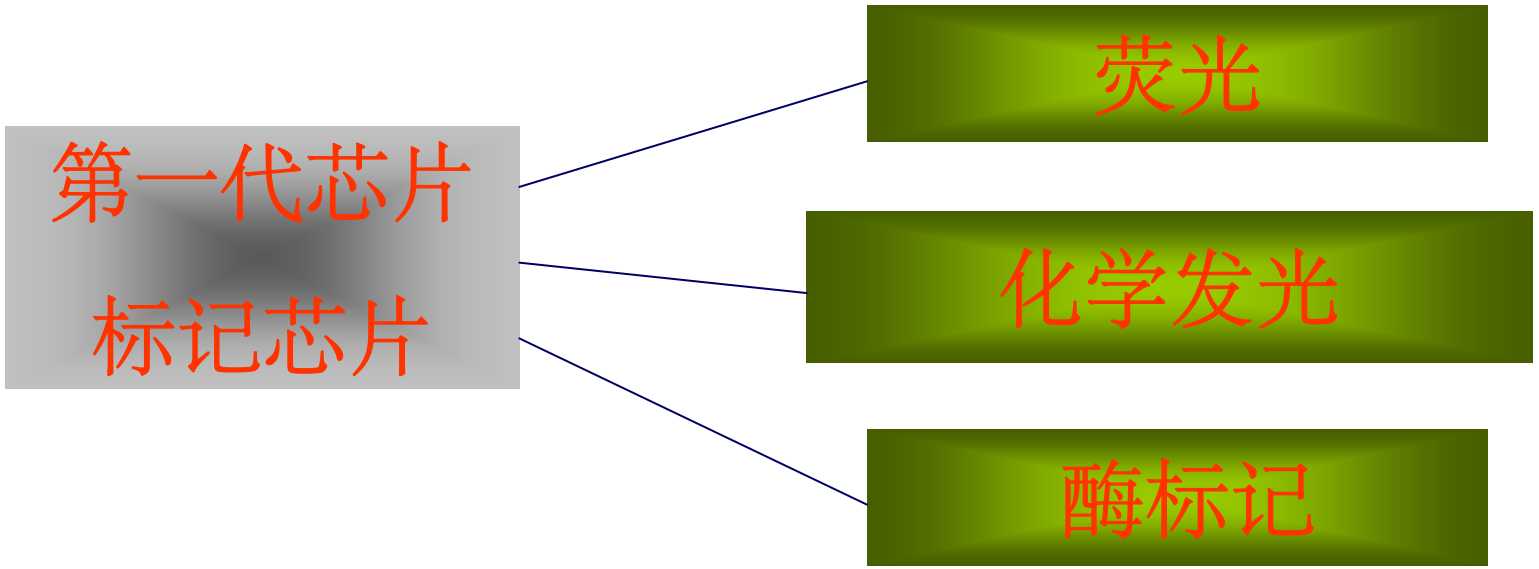
蛋白质芯片生物传感器

在很小几何尺度的表面积上，以阵列分布多种蛋白活性，仅用微量生理或生物采样，即可以同时分析不同的生物分子，相互作用，以获得生命活动的规律……



采样方法：光、色、电……




第一代芯片
标记芯片
荧光
化学发光
酶标记

第一代

标记蛋白质芯片

特征:

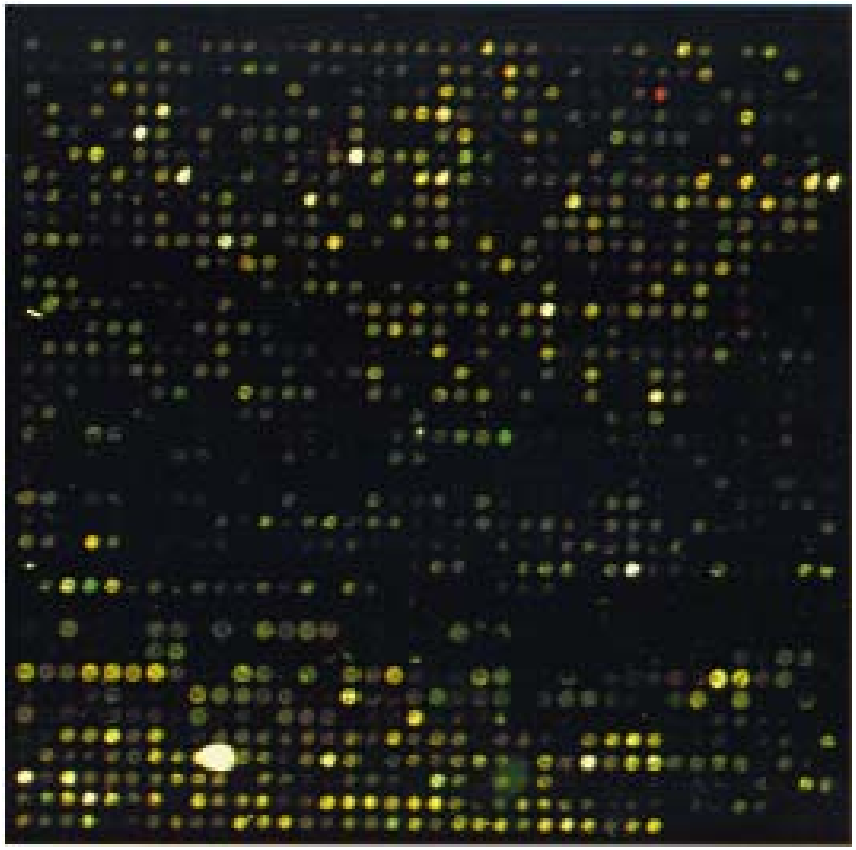
通过标记生物分子进行检测

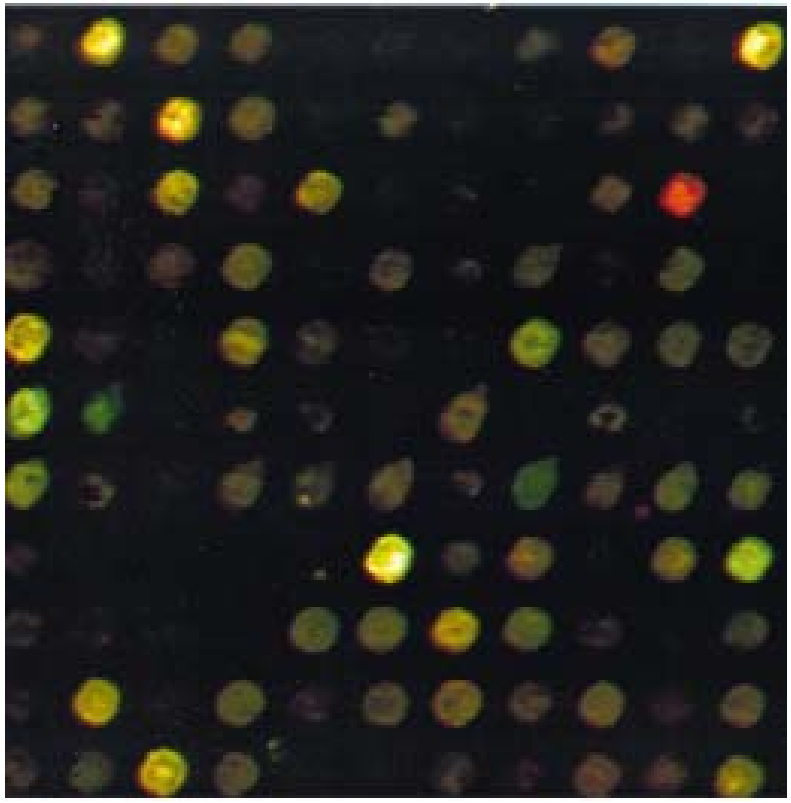
机械方式点样

基片材料通常是玻璃片

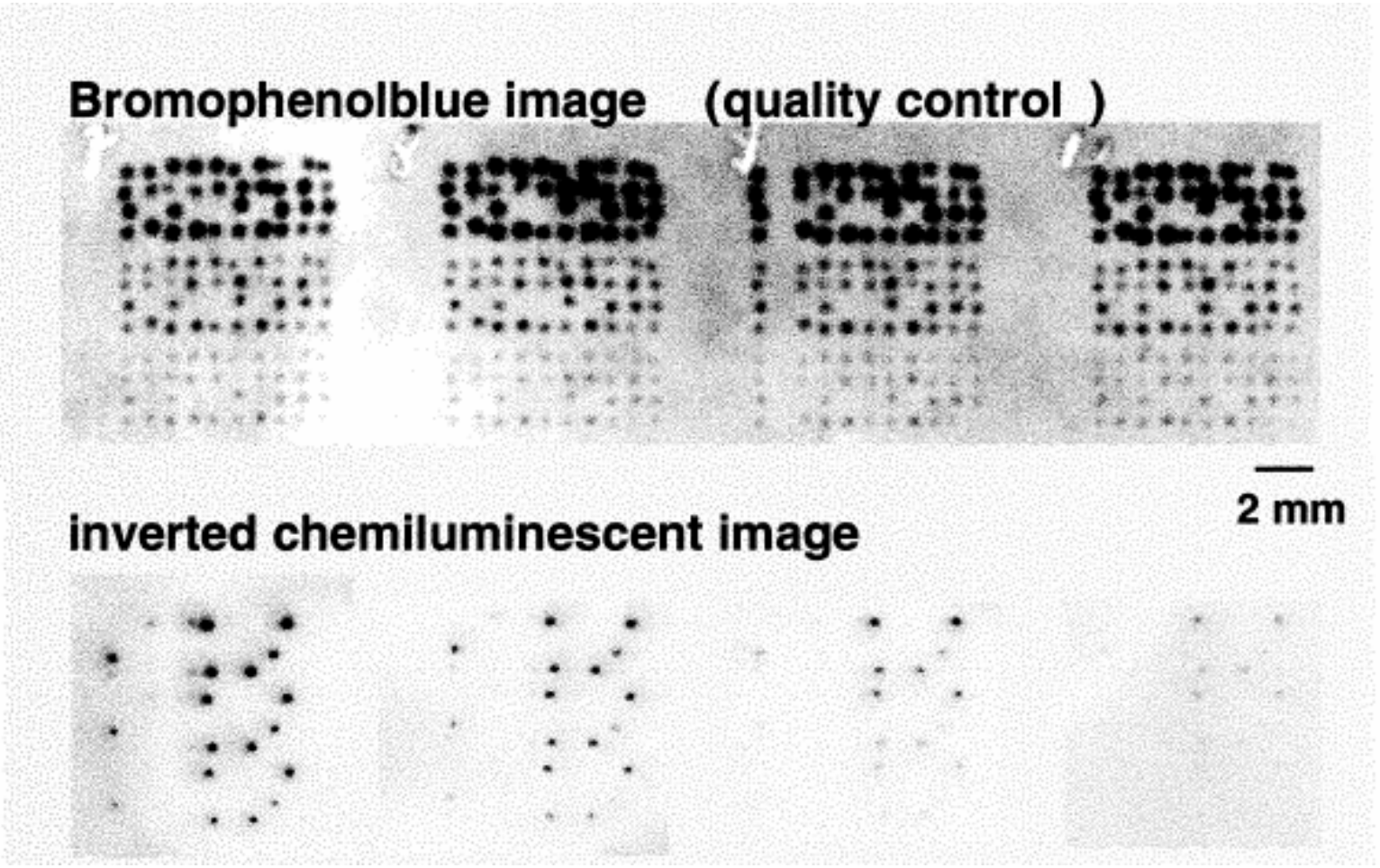
把点完样的芯片整个放入待测液中反应

扫描仪分析





荧光标记


Bromophenolblue image (quality control)
2 mm
inverted chemiluminescent image

酶 标

评价：标记蛋白质芯片

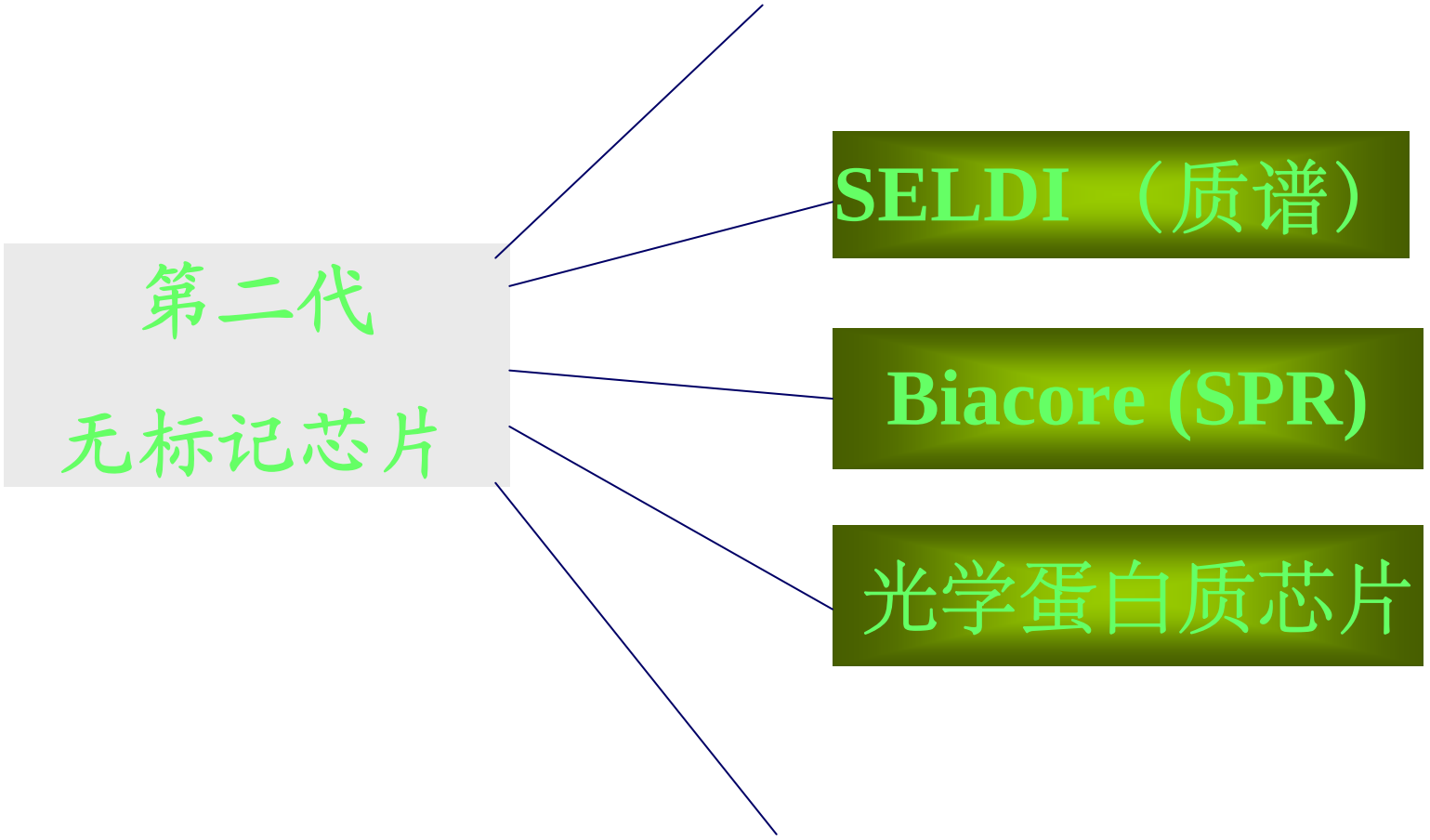
- 高通量
- 灵敏度高
- 不能准确定量
- 灵敏度太高，易出现交叉污染，导致假阳性
- 标记物的发光或显色较难处理，本底高
- 两种以上试剂、探针标记成本昂贵
- 被标记的生物分子活性易受影响
- 点样的均匀性，基底的均一性的影响很大

第二代

无标记蛋白质芯片

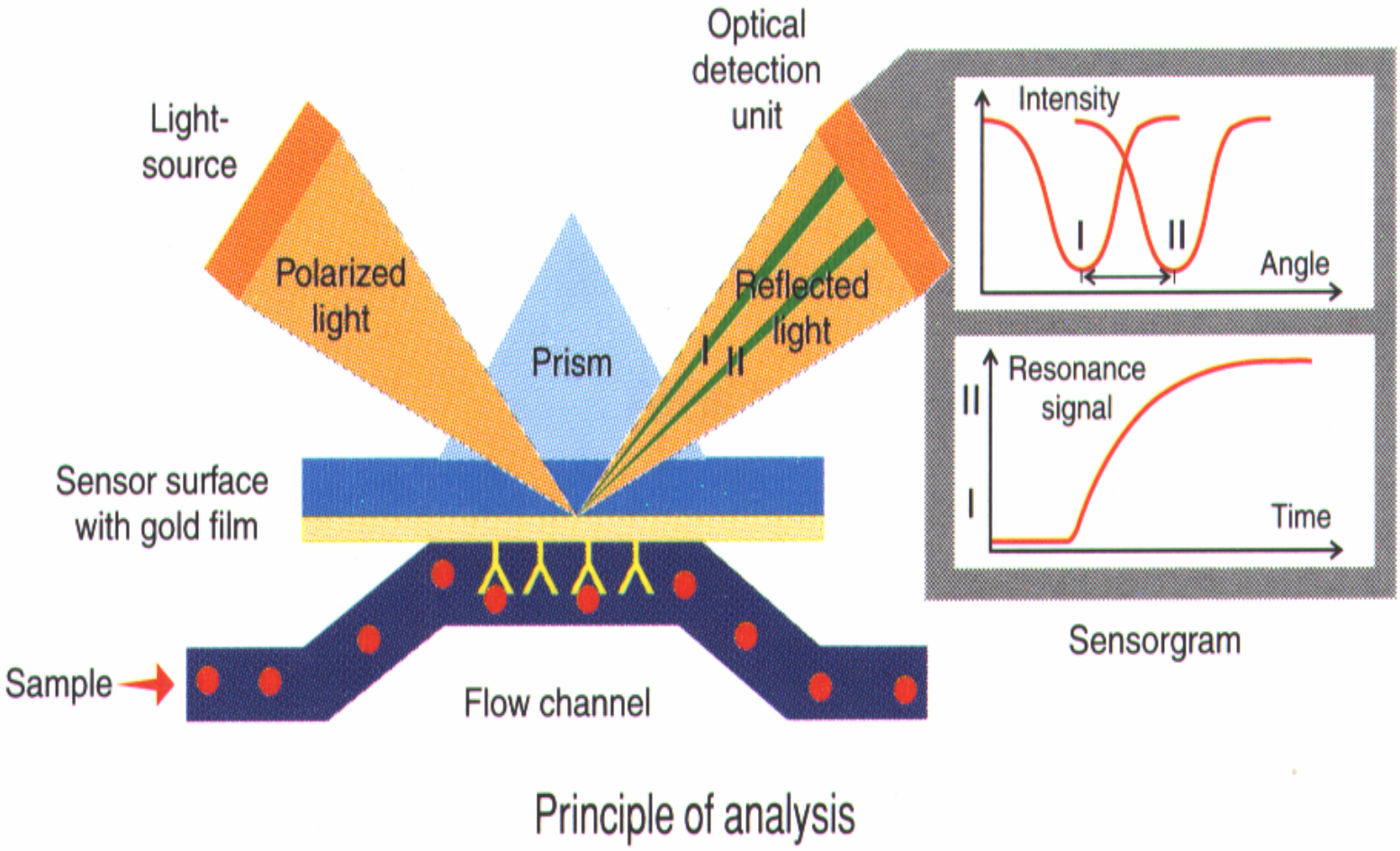
特征:

- 无标记检测
- 自动流动系统
- 强大的数据处理软件

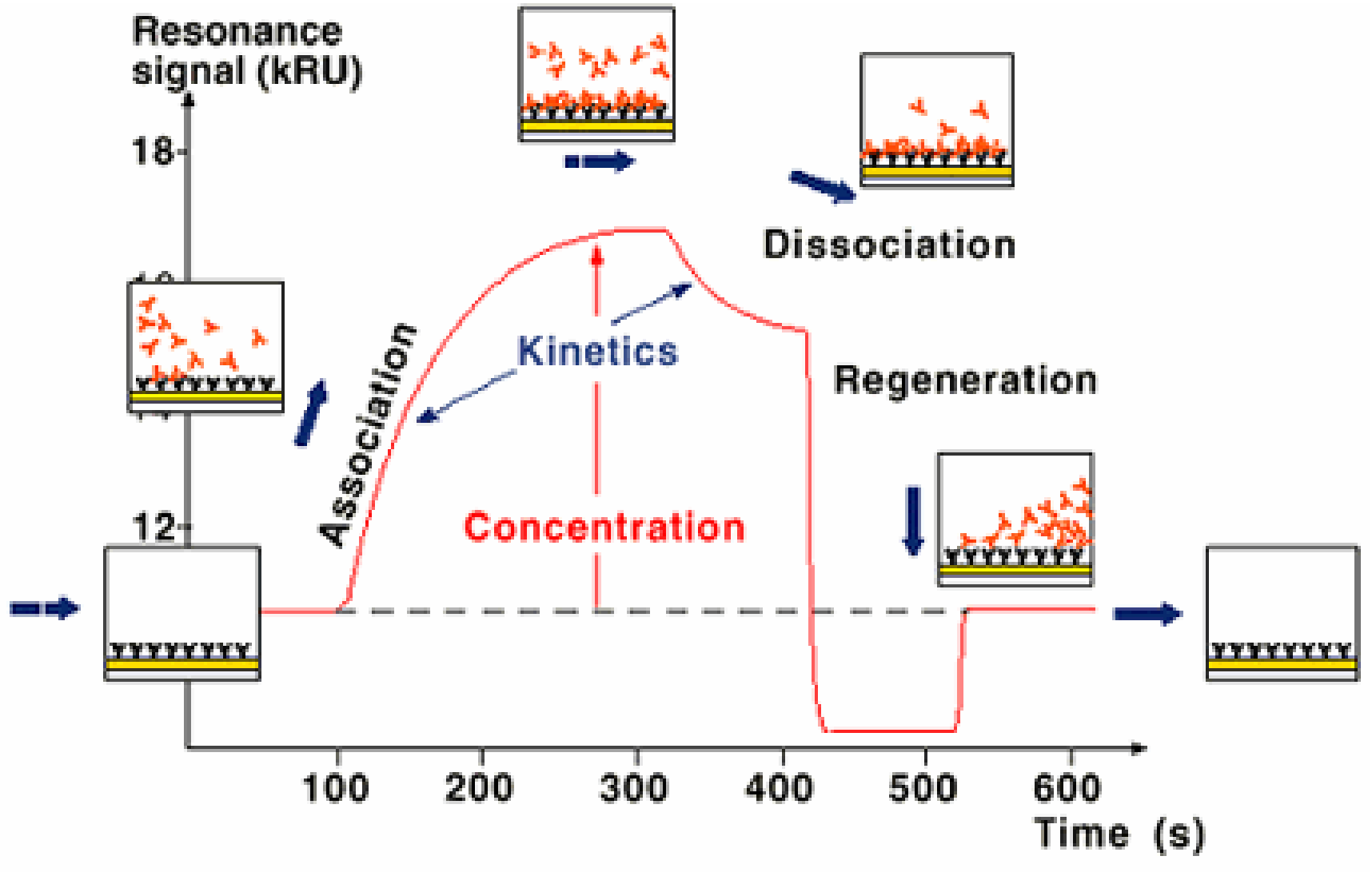

第二代
无标记芯片
SELDI （质谱）
Biacore (SPR)
光学蛋白质芯片

BIACORE

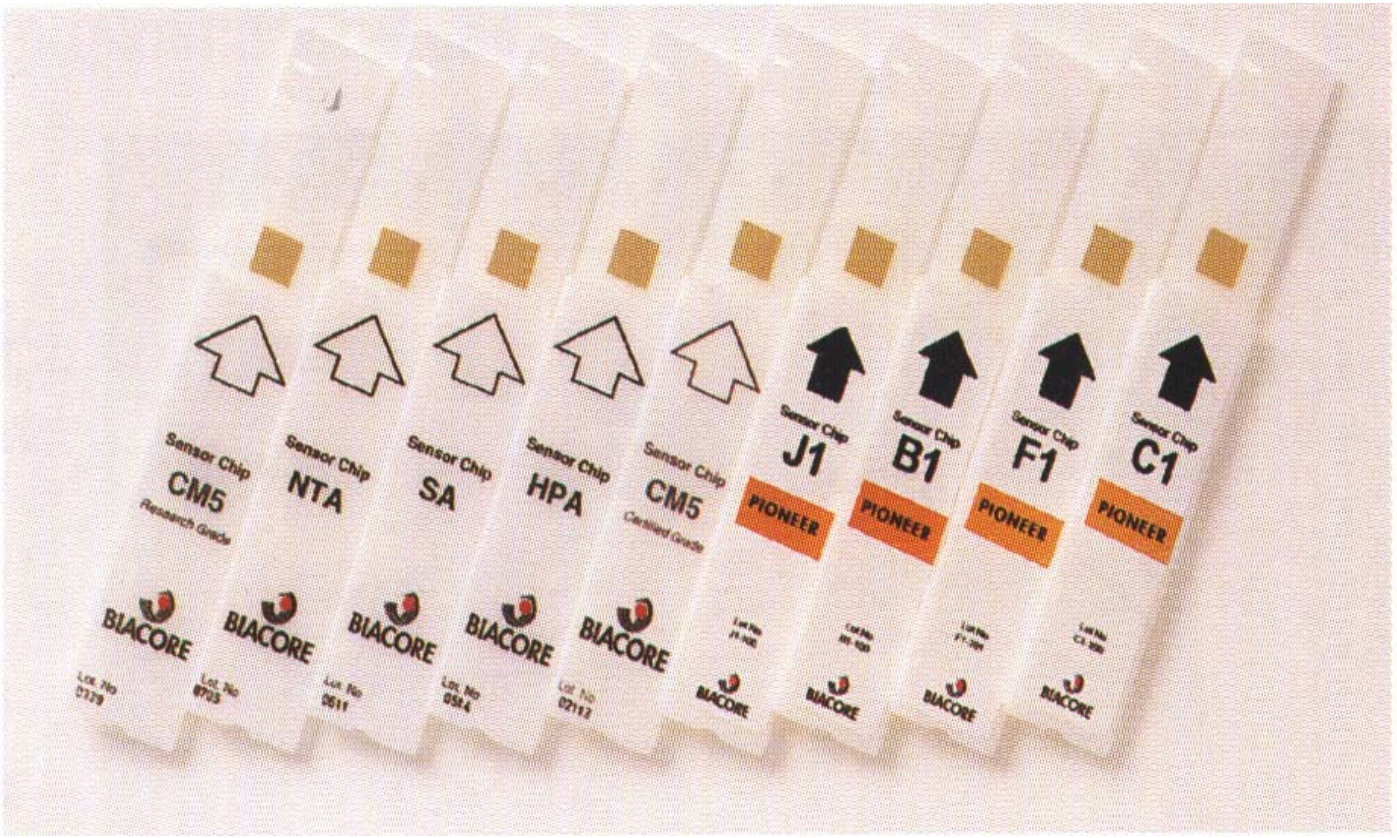
BIACORE生物传感器



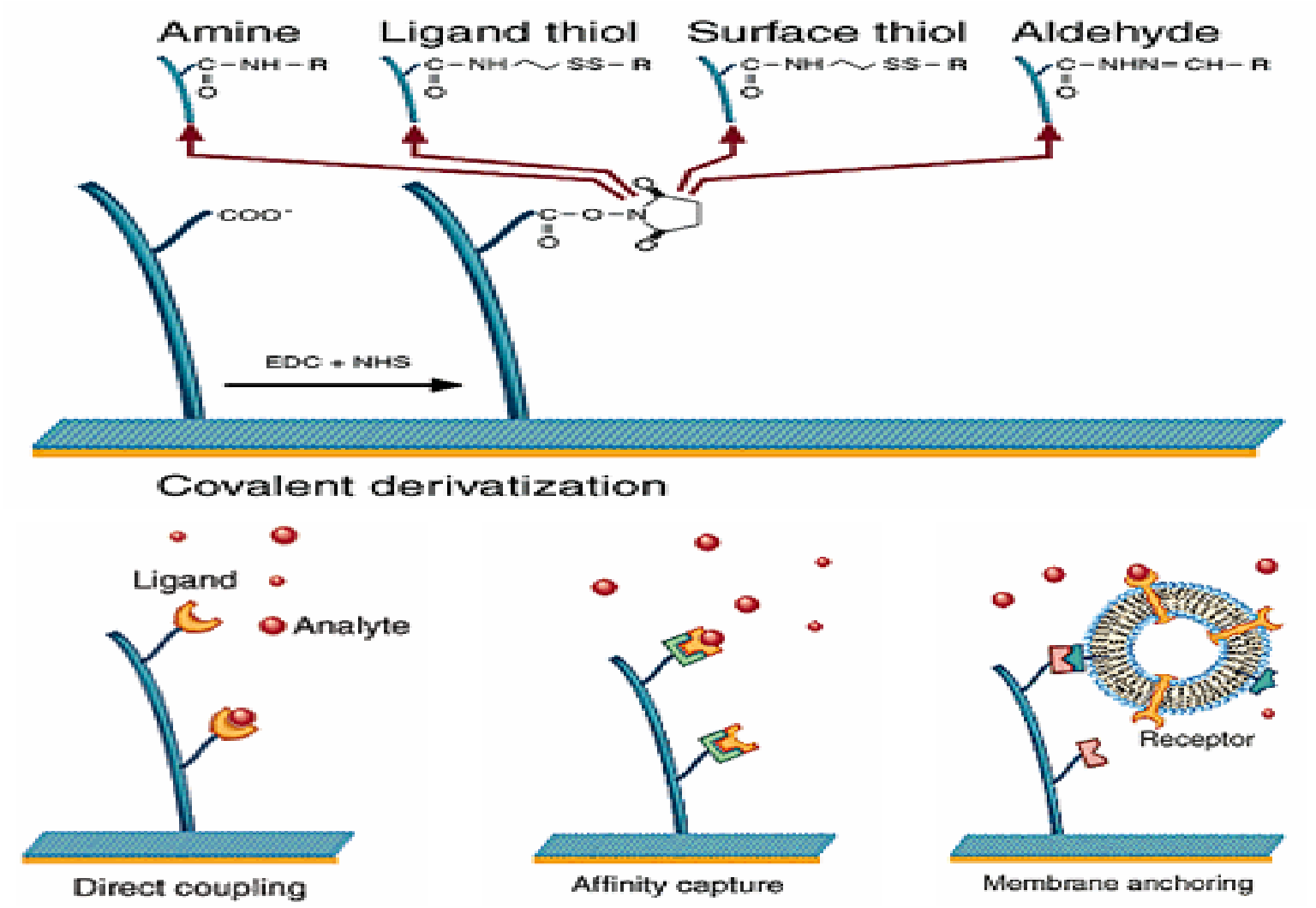
BIACORE 分析原理


Resonance signal (kRU)
18
12
Association
Kinetics
Concentration
Dissociation
Regeneration
100
200
300
400
500
600
Time (s)

BIACORE 传感图



BIACORE 芯片系列

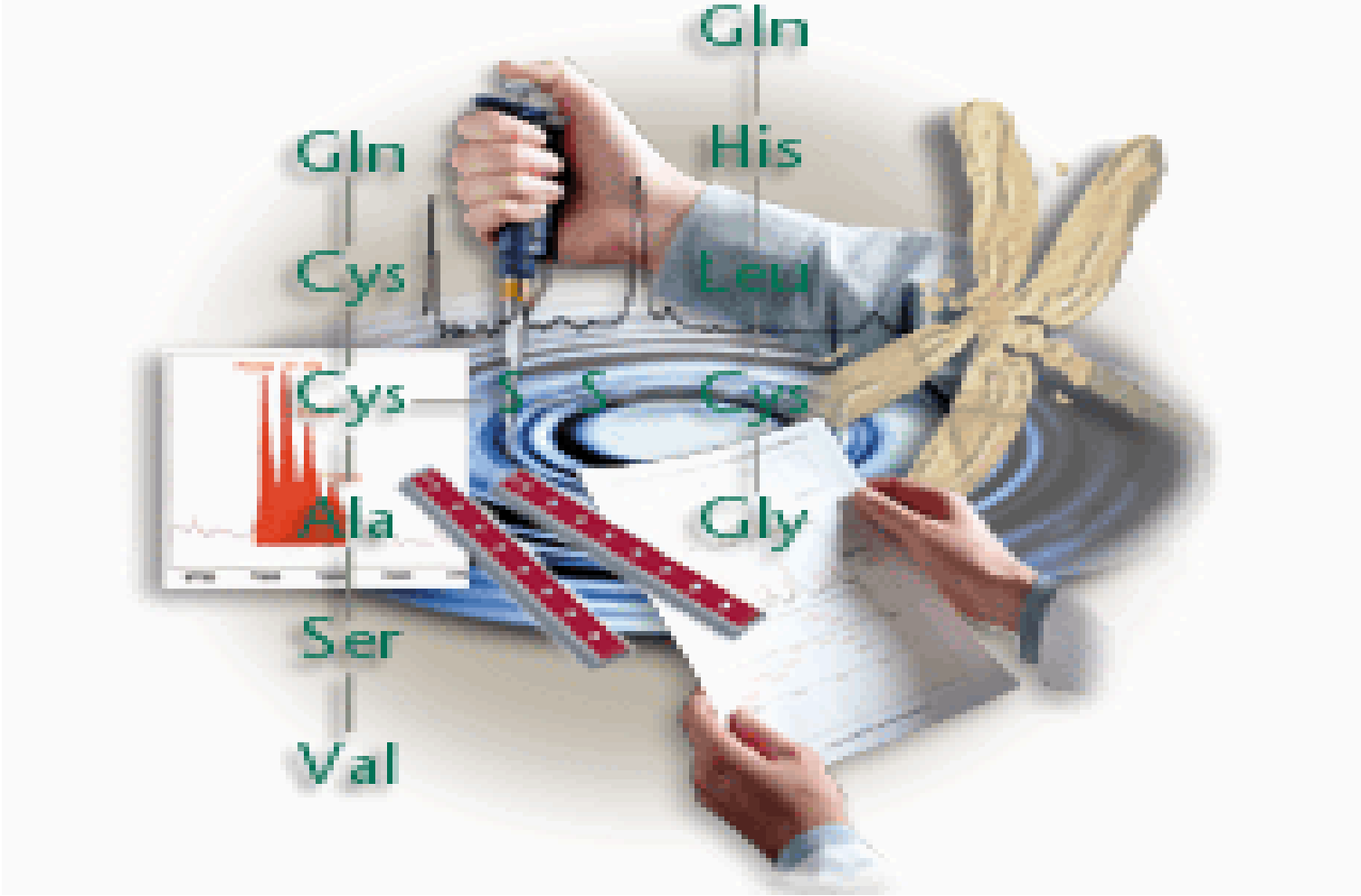


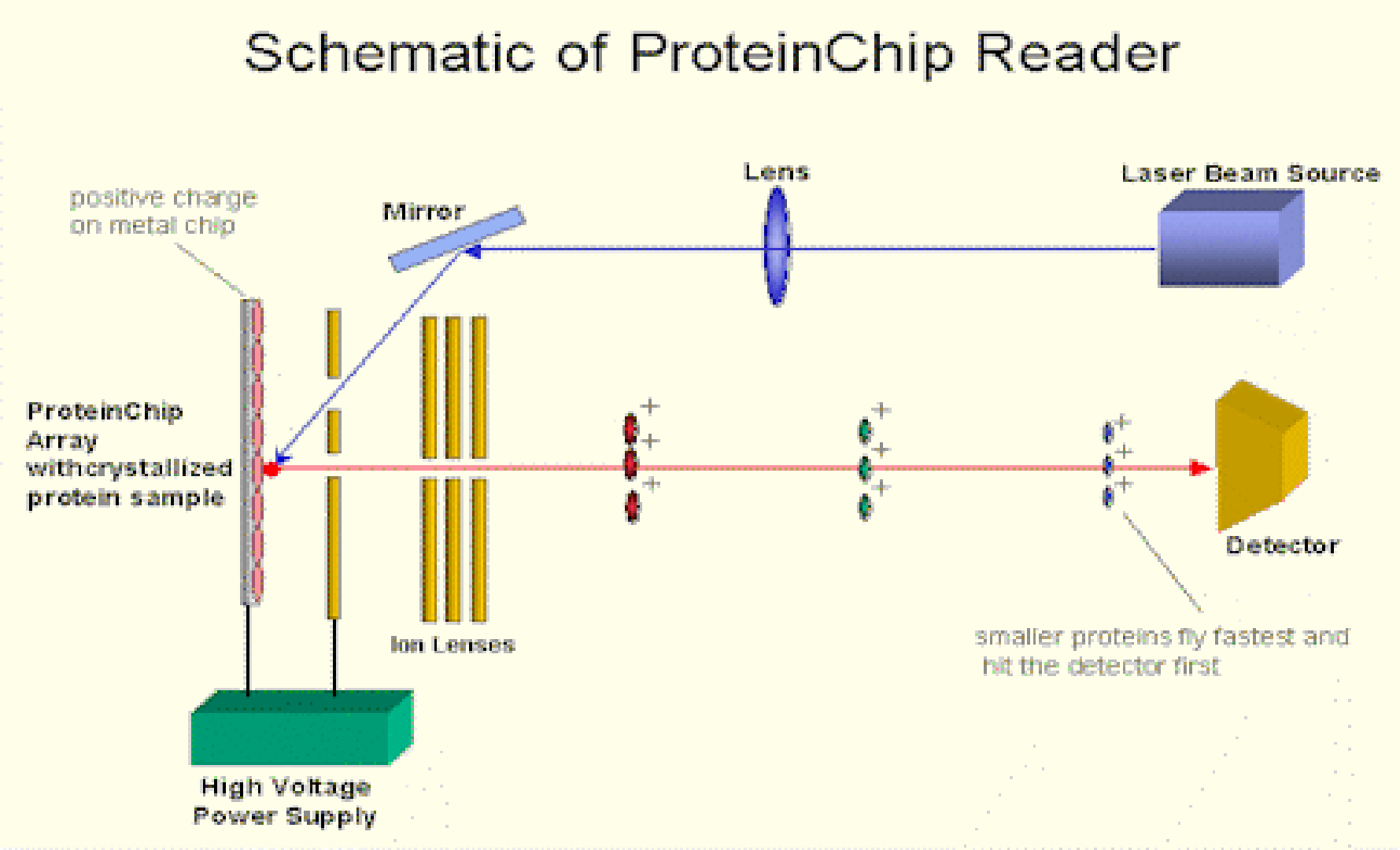
Sensor Chip CM5

应用领域:

- 特异性结合
- 多分子聚合物
- 免疫调节机理
- 药物开发
- 分子生物学
- 层析工艺开发
- 细胞信号传导
- 免疫检测
- 配体垂钓
- 疫苗开发
- 复制与转录调控
- 食品工业

CIPHERGEN




Schematic of ProteinChip Reader
positive charge
on metal chip
Mirror
Lens
Laser Beam Source
ProteinChip
Array
withcrystallized
protein sample
Ion Lenses
Detector
smaller proteins fly fastest and
hit the detector first
High Voltage
Power Supply

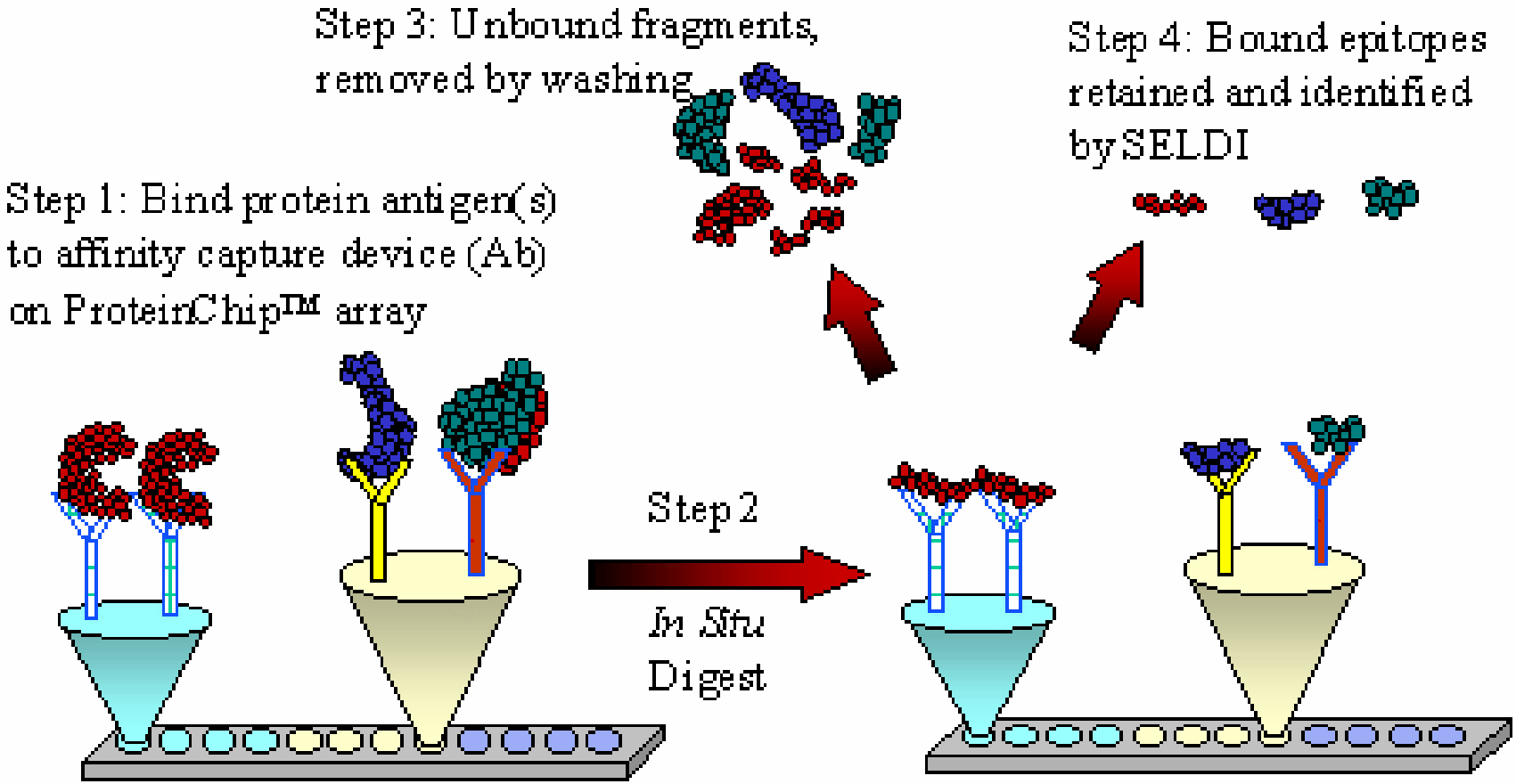
检测原理

芯片系列

- Hydrophobic ProteinChip Arrays
- Hydrophilic ProteinChip Arrays
- Anion Exchange ProteinChip Arrays
- Cation Exchange ProteinChip Arrays
- Immobilized Metal Affinity ProteinChip Arrays
- Preactivated ProteinChip Arrays

SELDI ProteinChip™ Arrays:

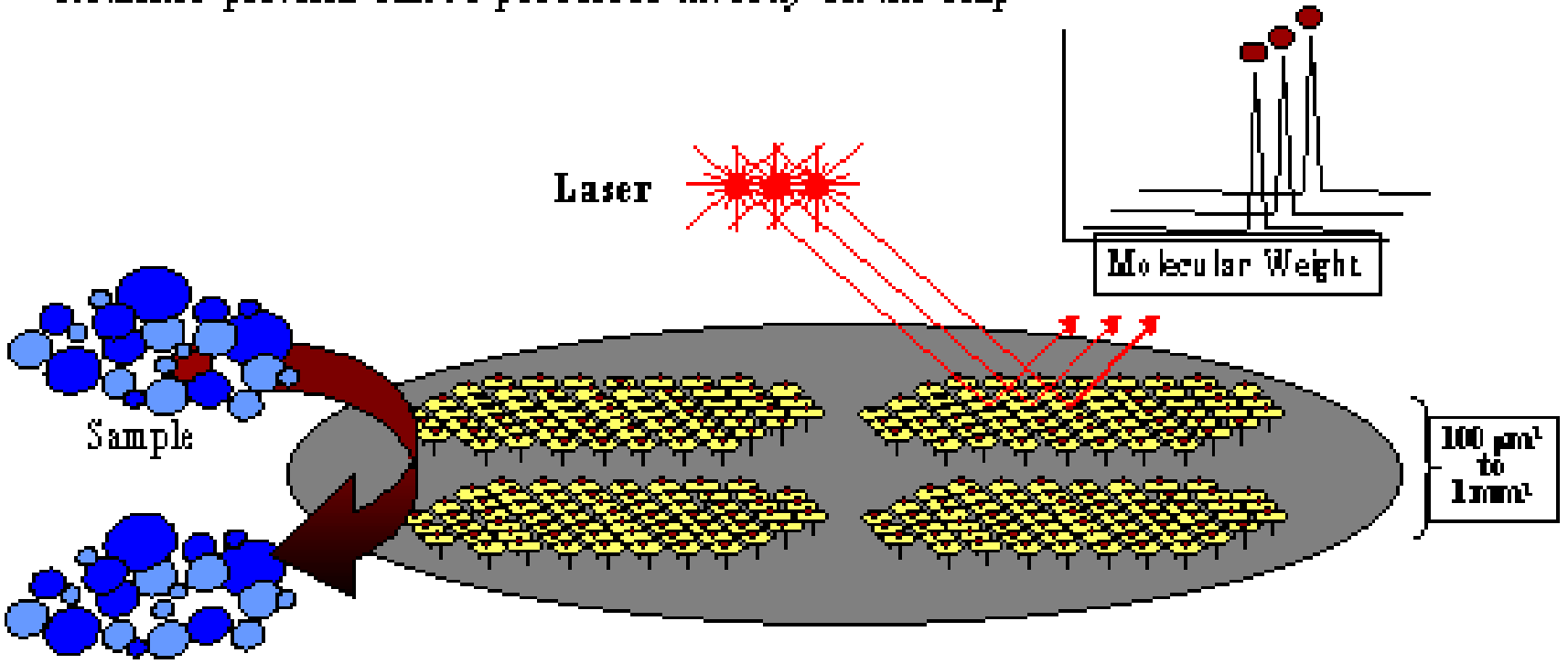
----- for Epitope Mapping



Definition of Antigenic Determinants: Epitope Mapping

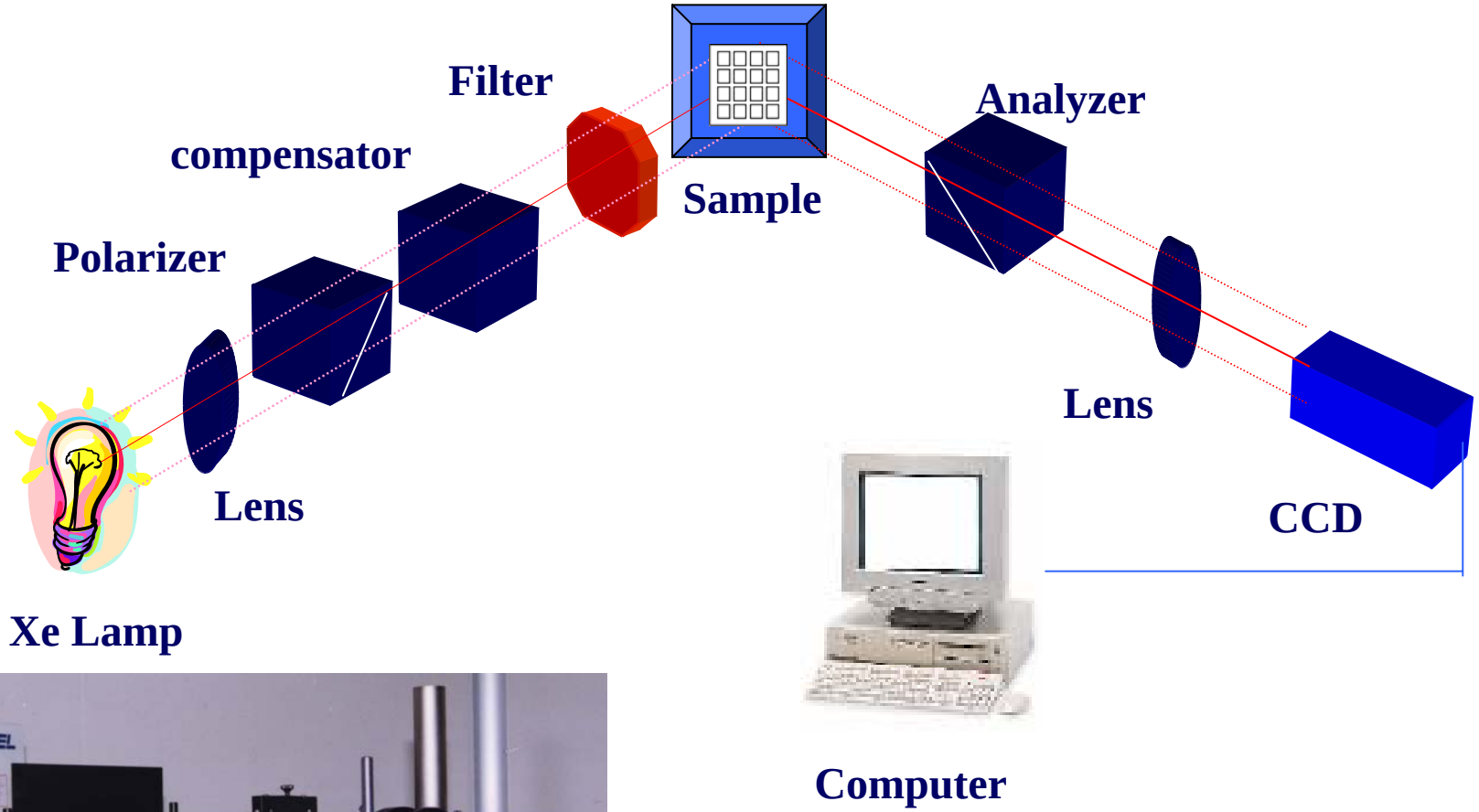
The SELDI Process and ProteinChip™ Arrays

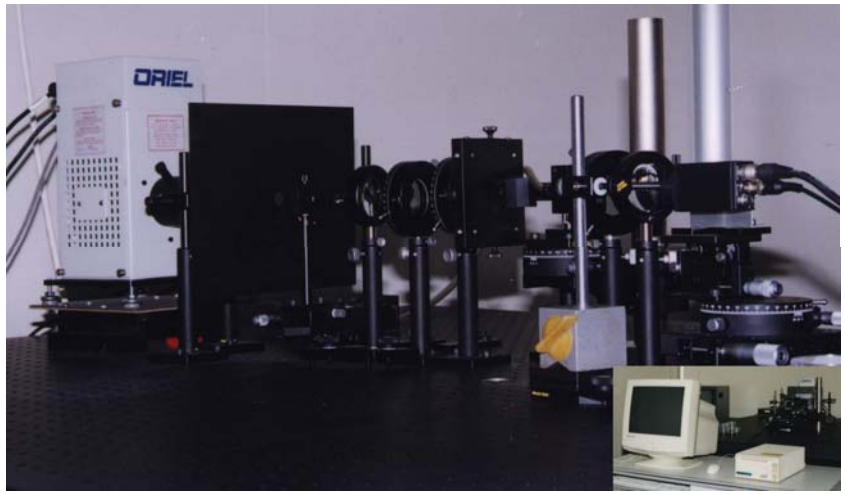
- Sample goes *directly* onto the ProteinChip™ Array
- Proteins are captured, retained and purified directly on the chip (affinity capture
- Retentate Map™ is "read" by Surface-Enhanced Laser Desorption/Ionization (SELDI)
- Retained proteins can be processed *directly* on the chip



ProteinChip™ Array

Chemical, Biochemical or Biological Affinity Capture Surface

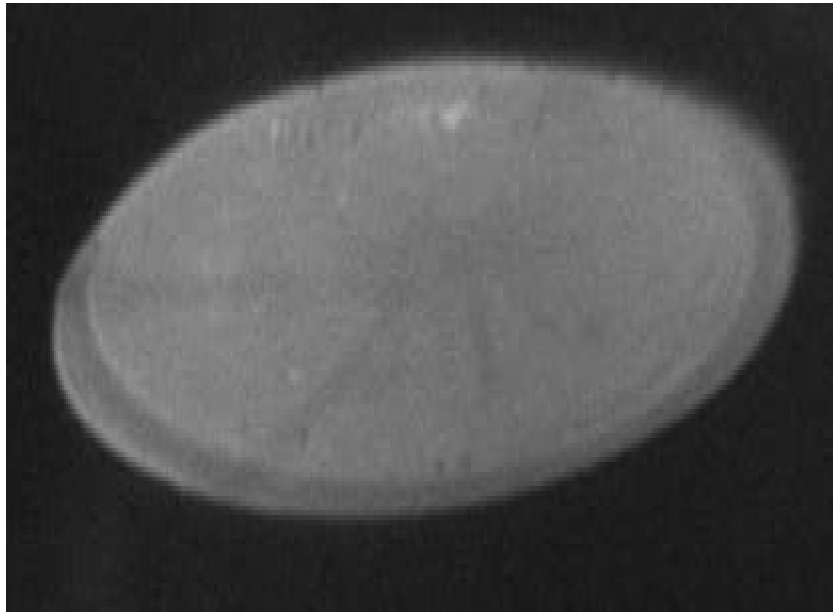


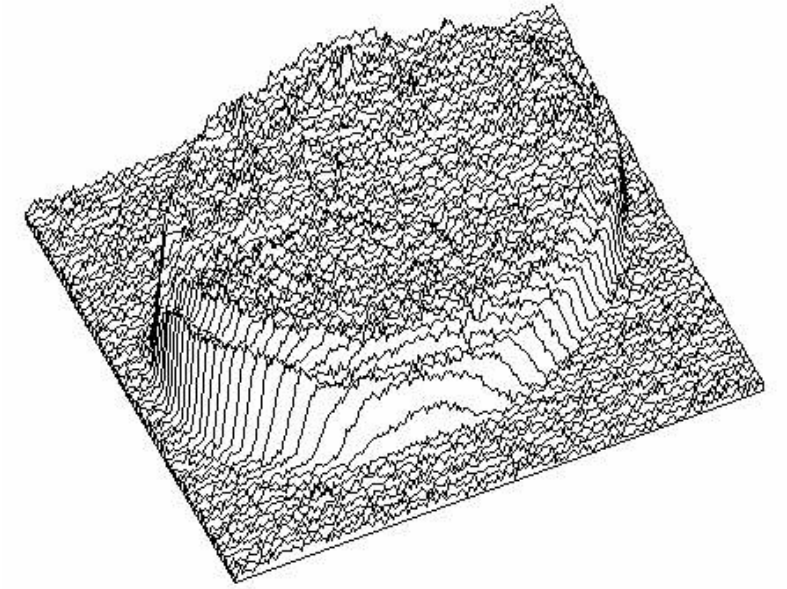


Optical detection system

G. JIN, et al, Rev. Sci. Instrum., 67, 2930-2936 (1996).

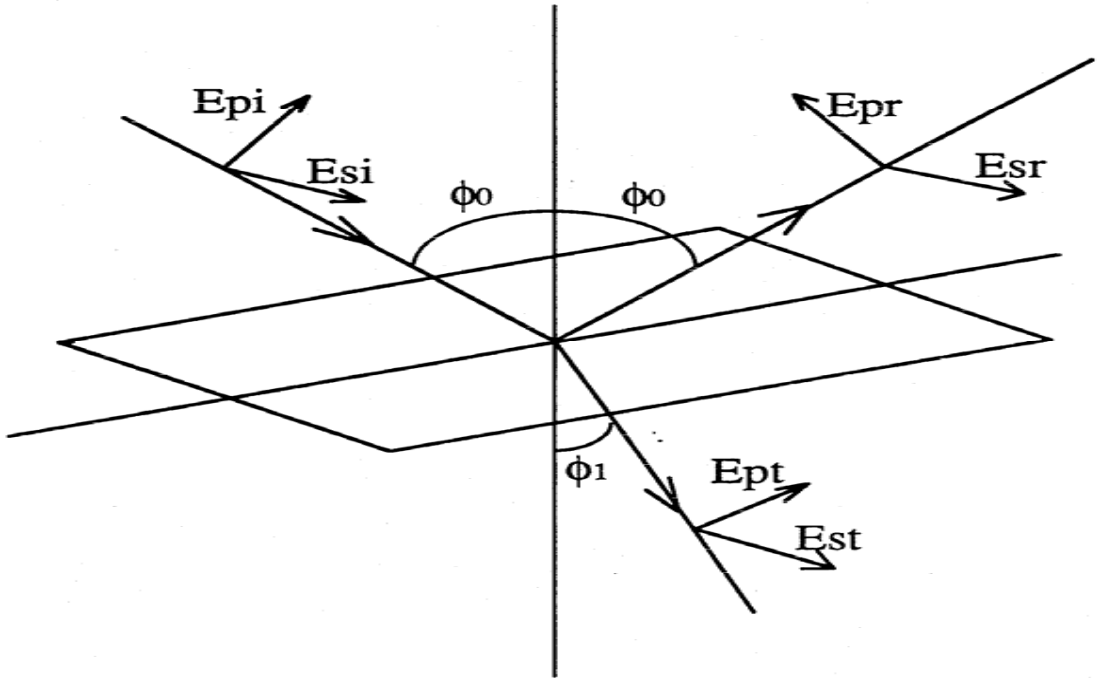
Immunoglobulin G monolayer





G. JIN, et al., ANALYTICAL BIOCHEMISTRY, 232, 69-72 (1995).

DEFINITION OF ELLIPSOMETRY PARAMETER



The reflection coefficient in the plane of incidence is

$$r_p = E_{pr} / E_{pi} = |r_p| \cdot e^{i\delta_p}$$

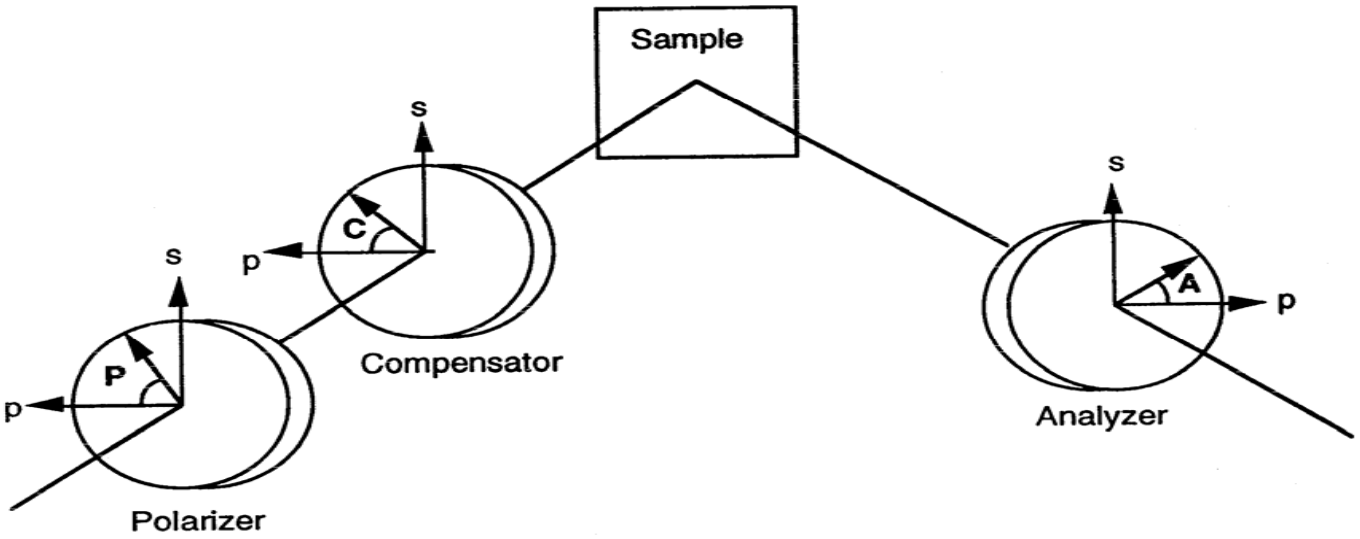
The reflection coefficient in the plane perpendicular to the plane of incidence is

$$r_s = E_{sr} / E_{si} = |r_s| \cdot e^{i\delta_s}$$

The ratio is defined as ellipsometry parameter

$$\rho = r_p / r_s = \mathrm{Tg}\psi \cdot e^{i\Delta}$$

Basic principle for PCSA system



$$I = K\frac{|R_s|^2}{8\cos^2 A_n}\left[\sin^2(A-A_n)+\sin 2A\sin 2A_n\sin^2(P-P_n)\right]$$

for protein adsorption on silicon wafer

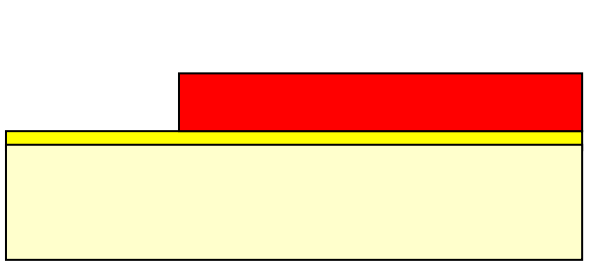
$$I = K\frac{|R_s|^2\sin^2 A_{no}}{8}\sin^2(P_{no}-P_n)$$

δP_n is proportional to the film thickness d for thin film

$$d = k_d\sqrt{I}$$

<u>thickness is proportional to the square root of I</u>

活性探针

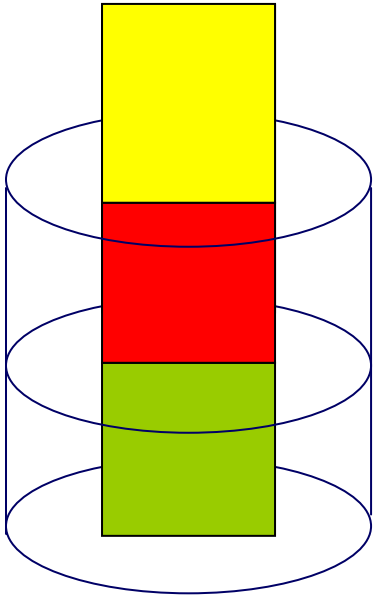


感应层

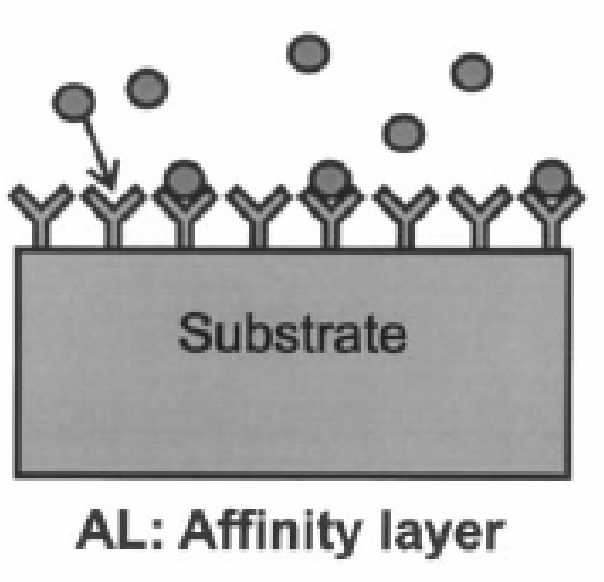
改性层

基底

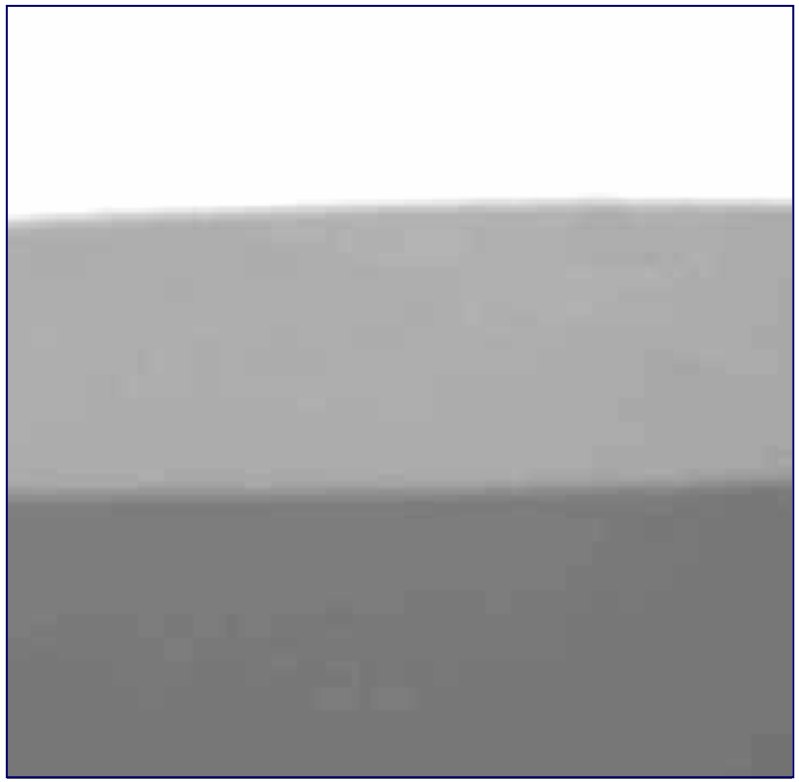
反应

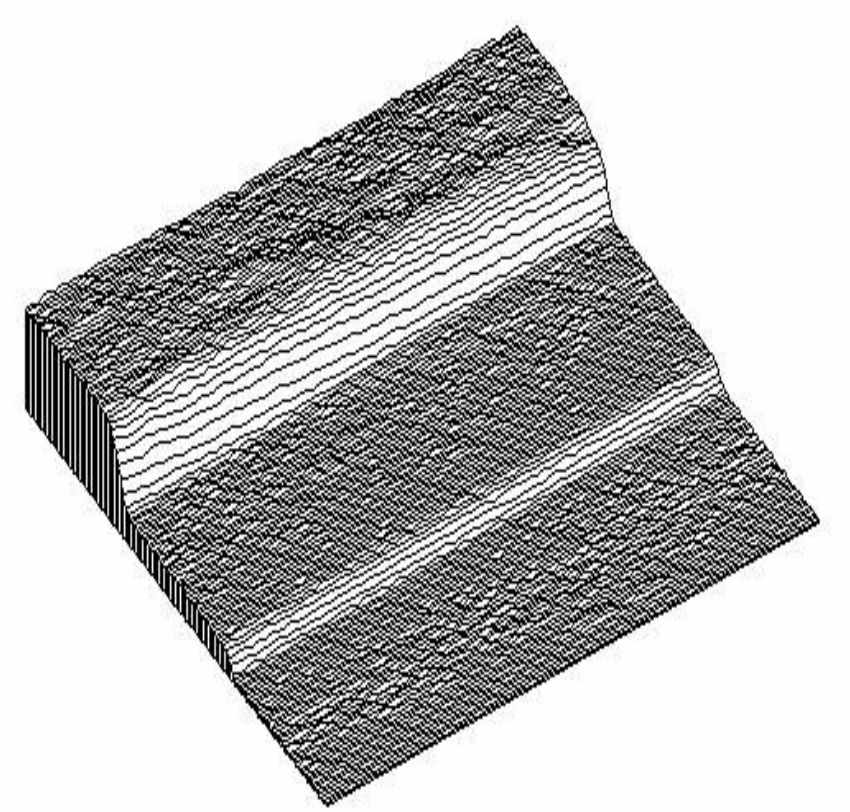


样品

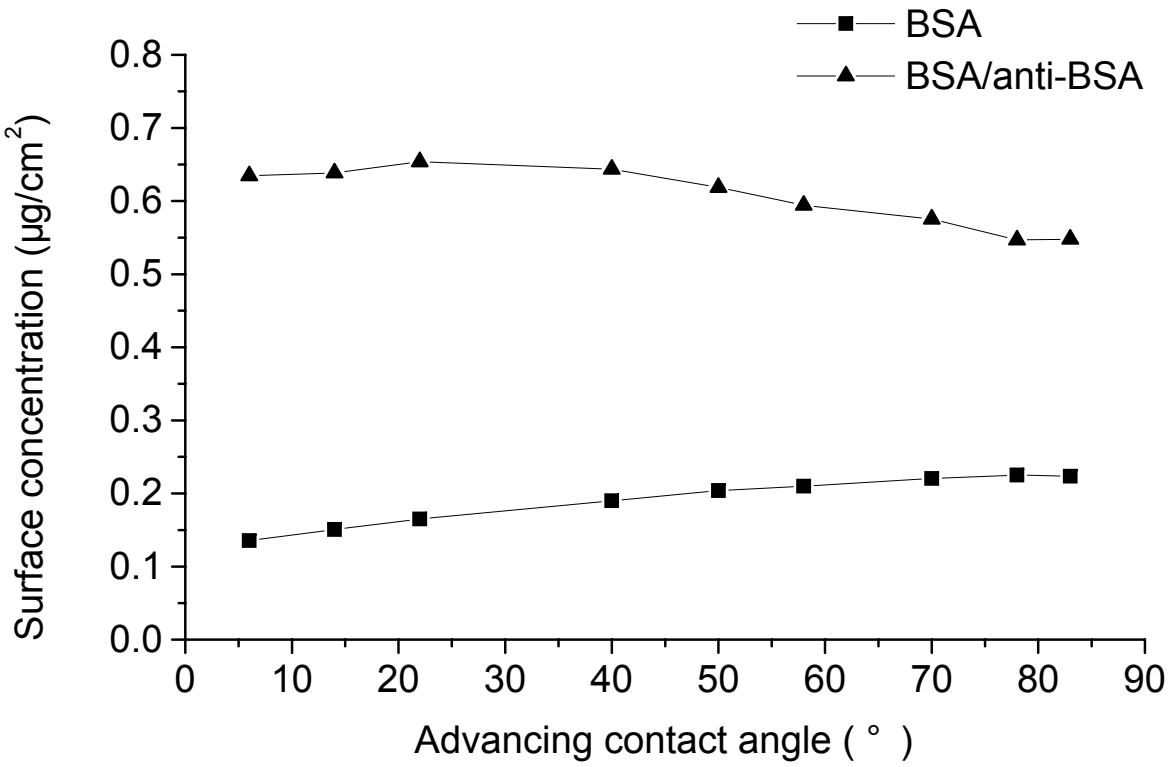


HSA和anti-HSA/HSA膜层





BSA 在不同亲疏水性表面的吸附



BSA（1 mg/ml）adsorption and interaction with its antibody

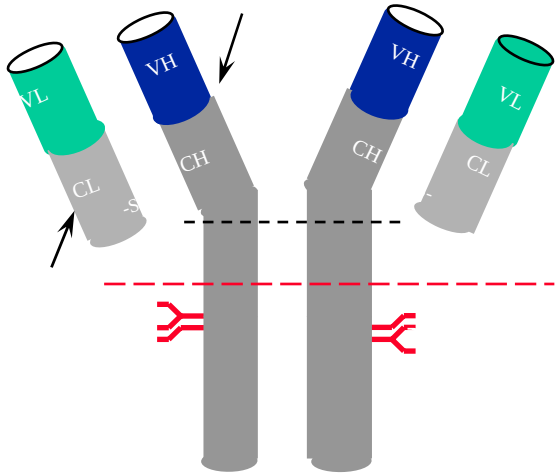
（BSAadsorption time:2 h, anti-BSA and BSA binding time: 1 h）

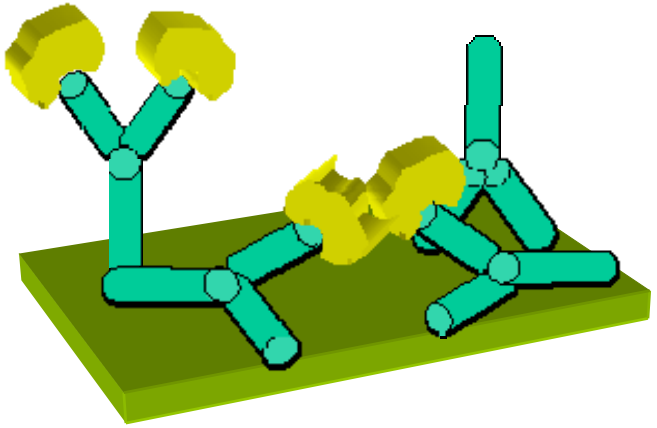
P.Q. Ying, et al., COLLOIDS AND SURFACES B: BIOINTERFACES, Vol 32/1 pp 1-10, 2003.

Questions for chip preparation

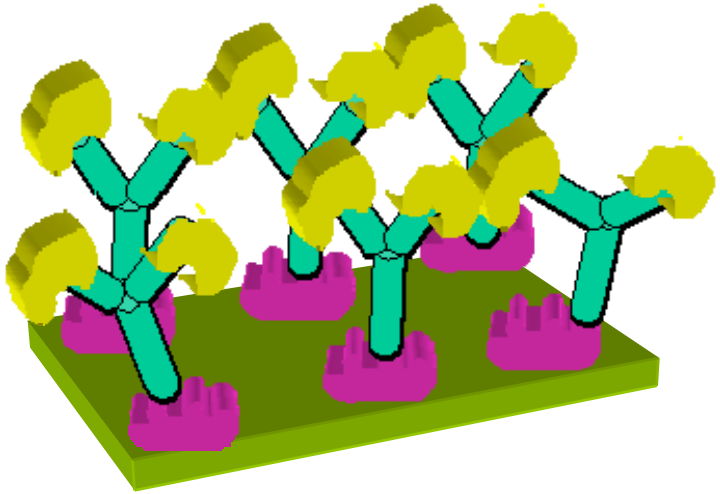
- How to immobilize ligands ?
- What the optimized surface concentration of ligand is?
- How to minimize the steric hindrance
- How to decrease the unspecific binding……

Protein A modification for Oriented antibody immobilization



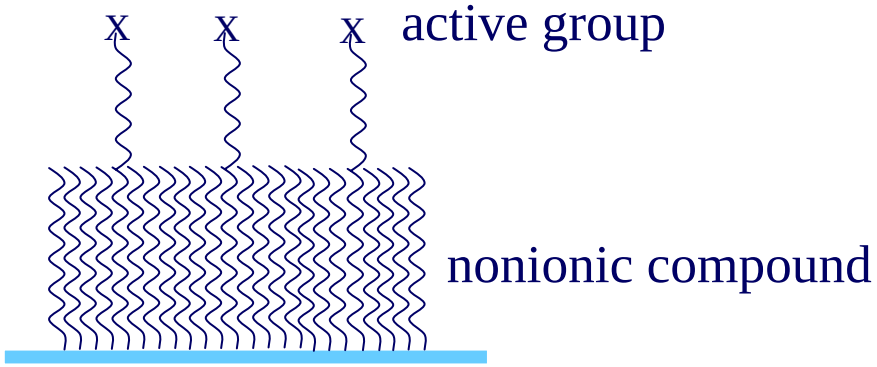


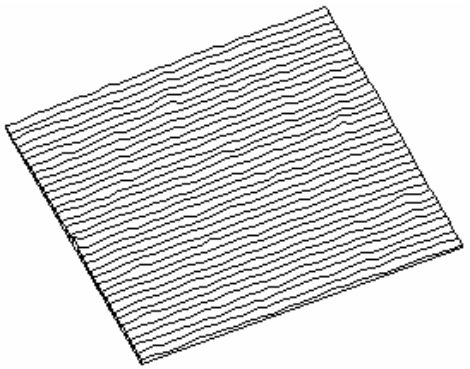
Before modification



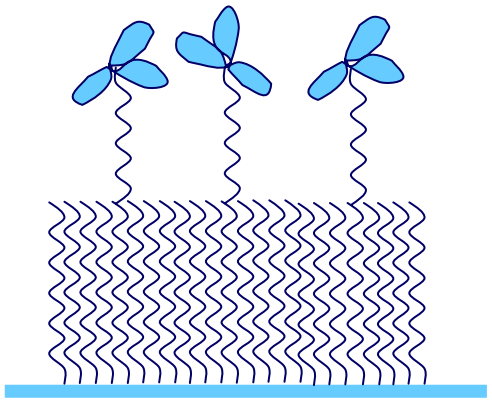
After modification

Zhanhui Wang et al, Journal of Biochemical and Biophysical Methods, Vol.57。(2003) p.203-211

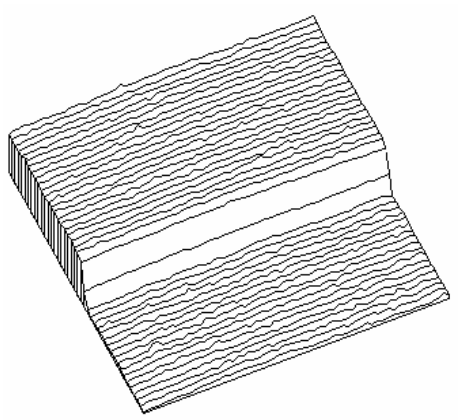




No physical adsorption



Protein covalently immobilized



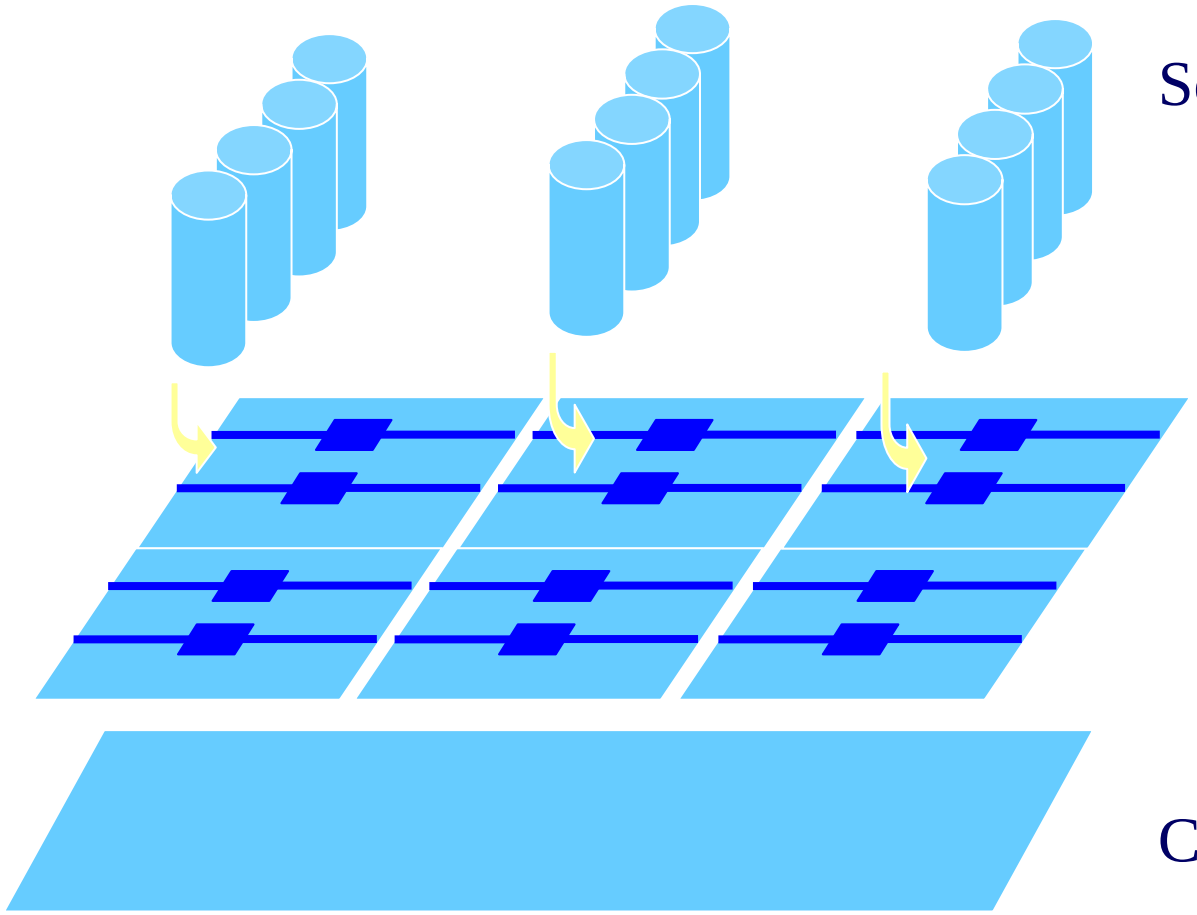
IgG covalently immobilized

Silicon Surface modified with mixed SAMs

Zhan-Hui Wang, et al COLLOIDS AND SURFACES B: BIOINTERFACES, 34 (2004), 173-177

Questions for protein detections

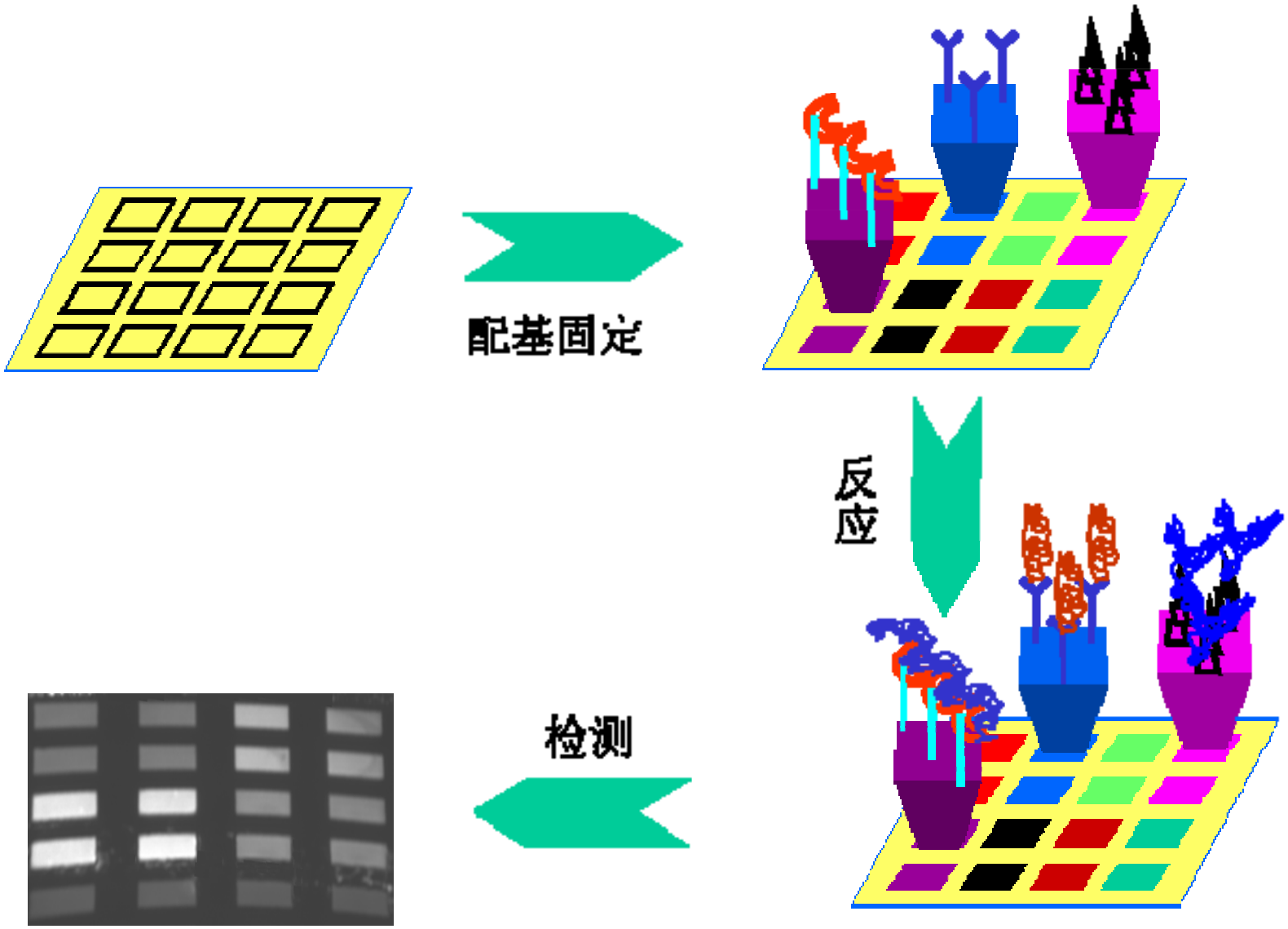
- How to increase the sensitivity?
- How to minimize ligand and sample consumption?
- How to save the test time?
- How to decrease the cost?
- …….



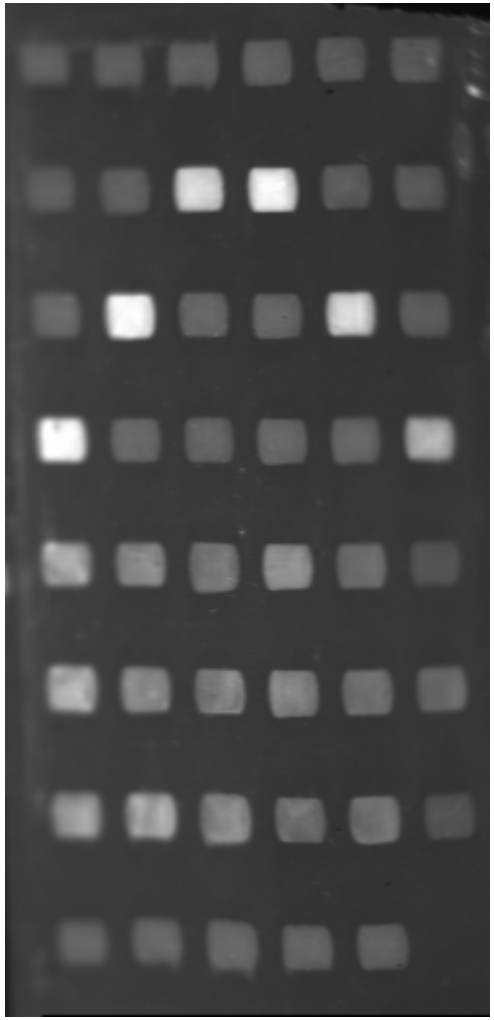
microfluidic system and micro-reactor

Patented by Z-H. Wang and G.Jin, et al. 2003

G. Jin et al, EMBEC'05, 3rd EMBEC, Prague, Czech Republic, Nov. 20 - 25, 2005



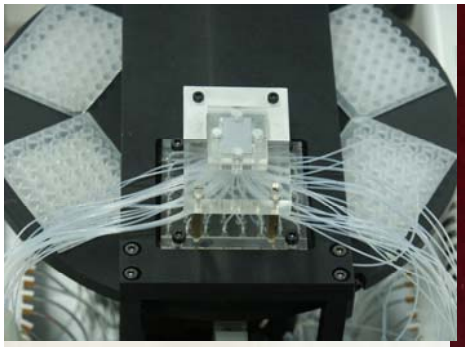
蛋白质芯片模型



IgG	IgG	IgG	IgG	IgG	IgG
IgG	IgG	IgG/anti-IgG	Fib/antiFib	Fib	Fib
IgG	IgG/anti-IgG	IgG	Fib	Fib/antiFib	Fib
IgG /anti-IgG	IgG	IgG	Fib	Fib	Fib/antiFib
HBsAb	HBsAg	HBeAb	HBeAg	HBcAb	AA98/serum
HBsAb /positive serum	HBsAg /positive serum	HBeAb /positive serum	HBeAg /positive serum	HBcAb /positive serum	AA98/serum
HBsAb /negative serum	HBsAg /negative serum	HBeAb /negative serum	HBeAg /negative serum	HBcAb /negative serum	AA98
IgG	IgG	IgG	IgG	IgG	

Protein array with 48 units

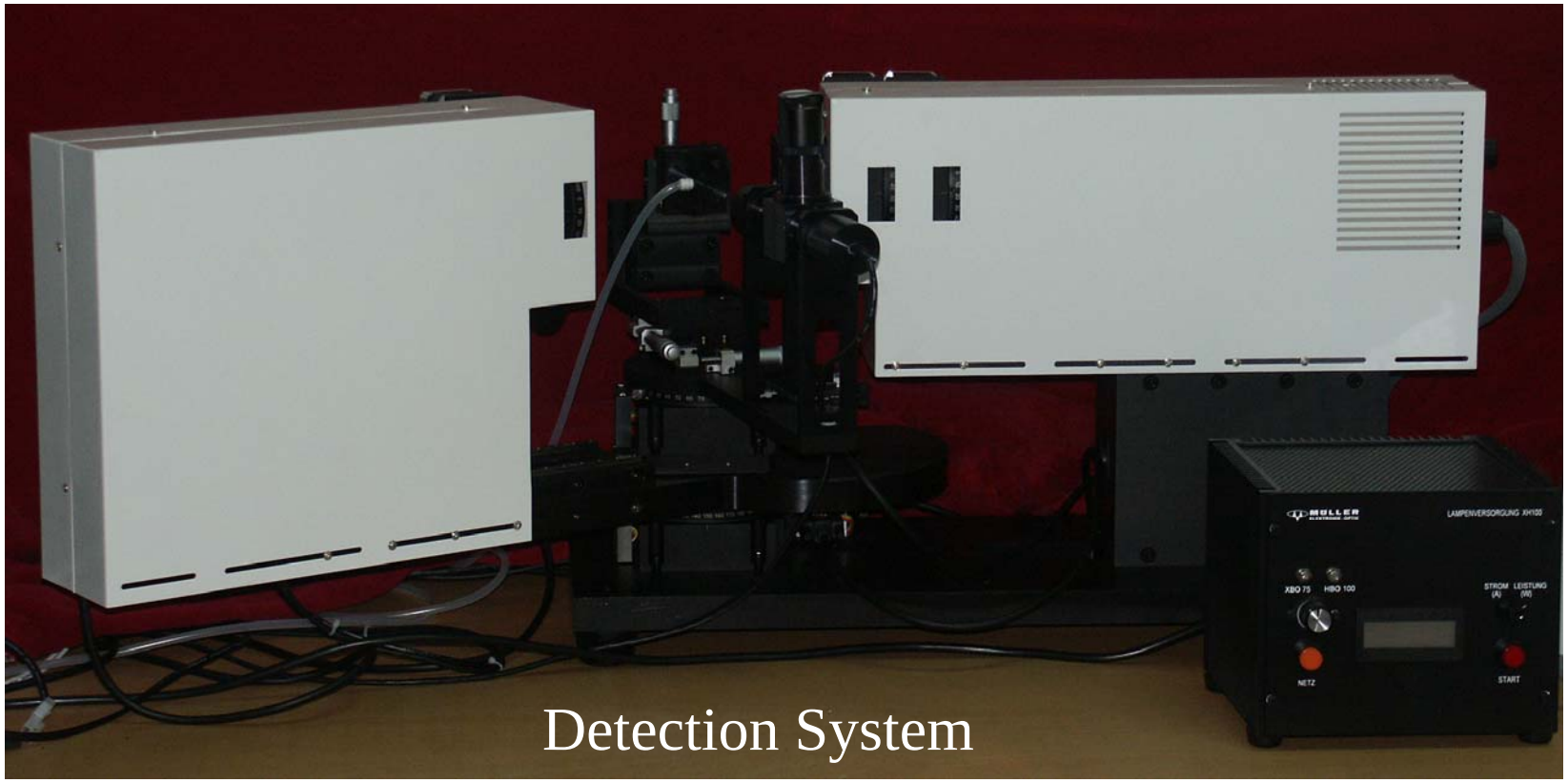
MAReactor F1000



Nov. 2005, Fabricated in I mech. CAS

Specifications:
channel：48
Flux：auto1-1000 μl/min
Probe：1 mm^2 x 24
Sample Amount：15 μl/unit

MAReader – IE1000



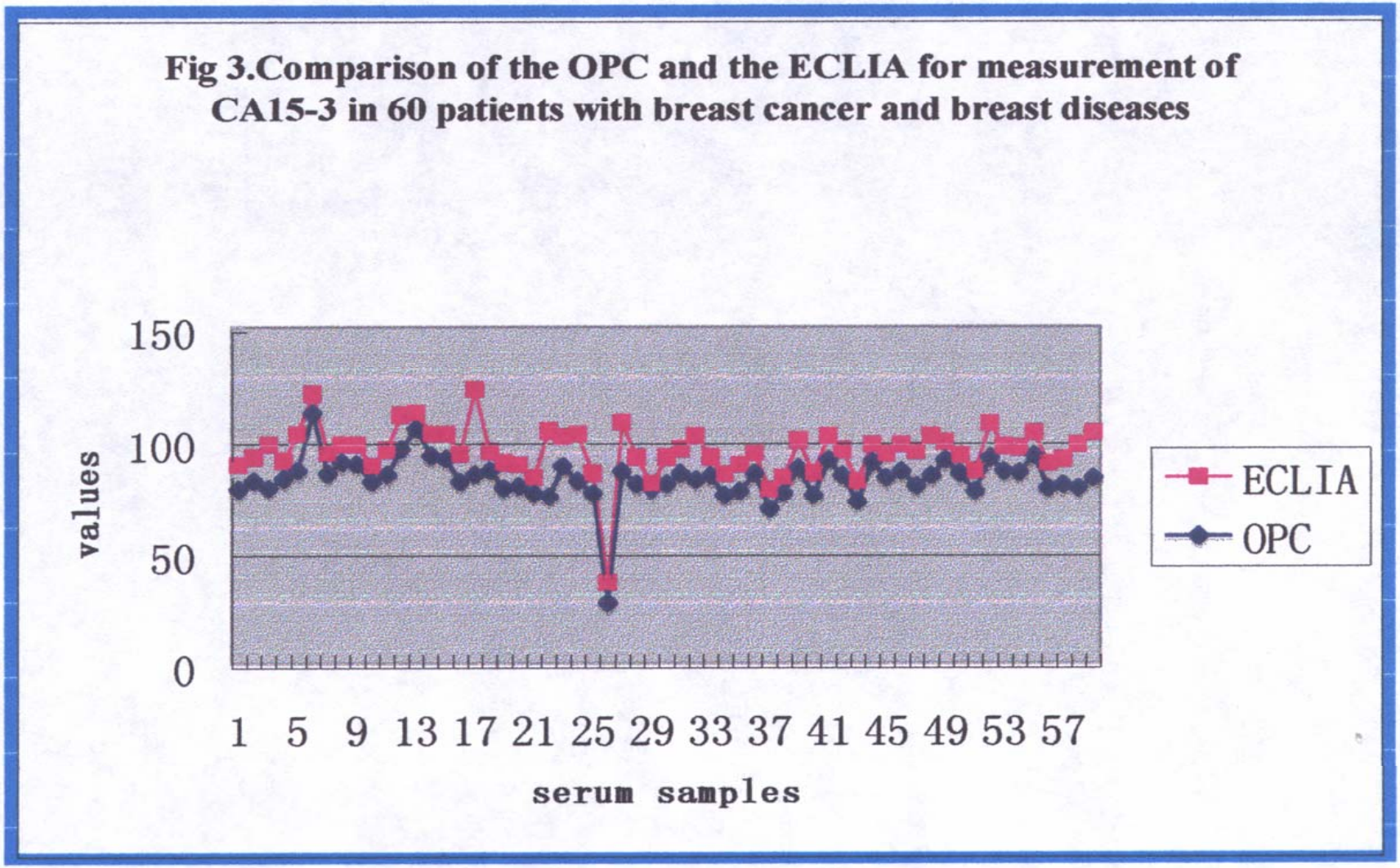
Detection System

Specifications:
Test area：12x36 mm^2
Th resolution：<0.5 nm
Lateral Res：< 3 μm
Sampling time：0.1 S/image

Nov. 2005, Fabricated in I mech. CAS

Control System

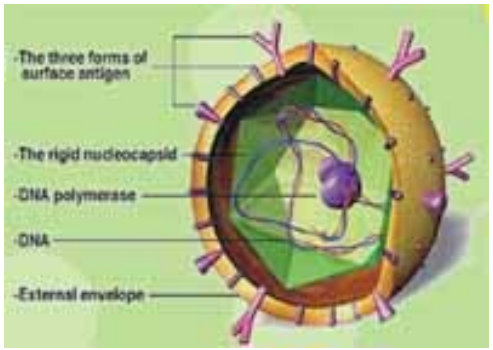
乳腺癌标志物CA15-3定量检测

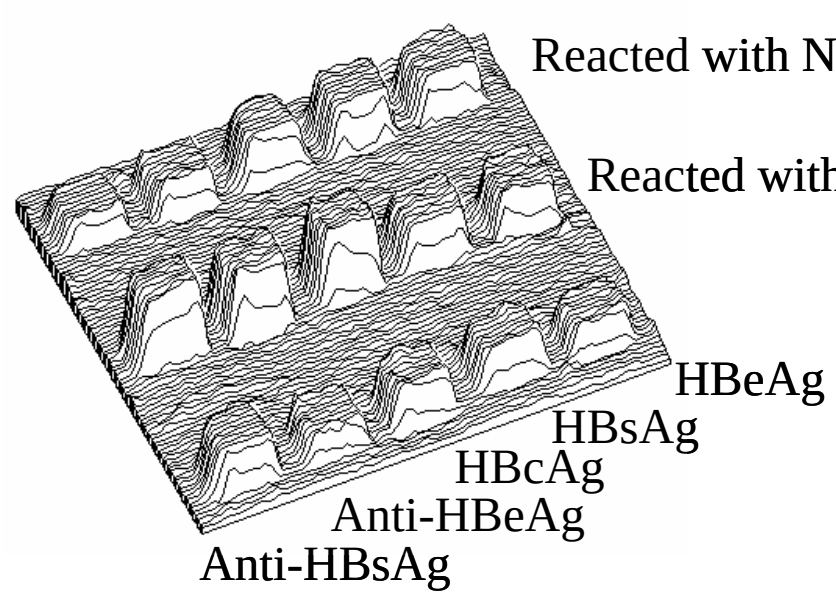


大量样品定量检测

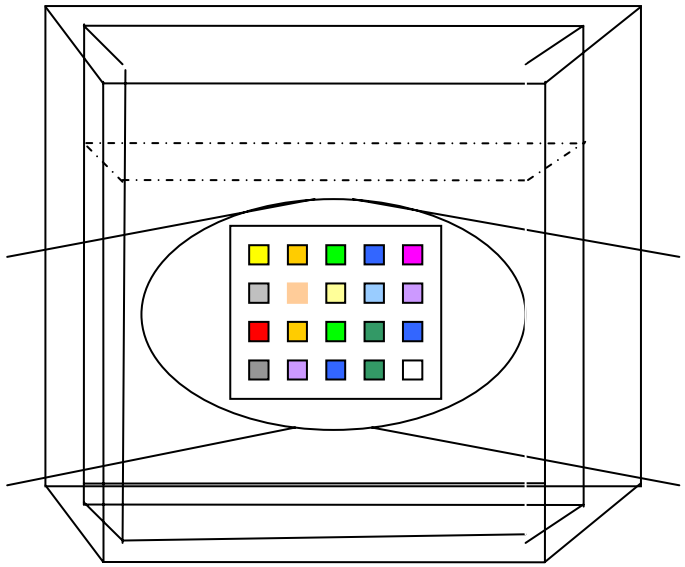
- *H-G. Zhang, Cai Qi, et al. CLINICAL CHEMISTRY 2005, Vol. 51(6),1038-1040*

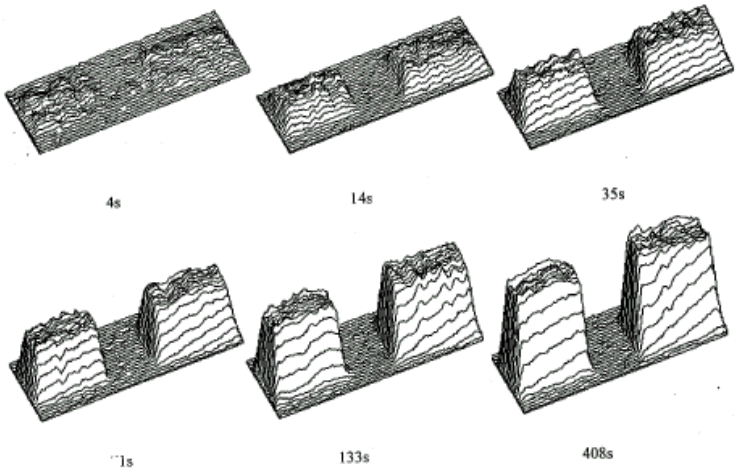
Detection of Hepatitis B



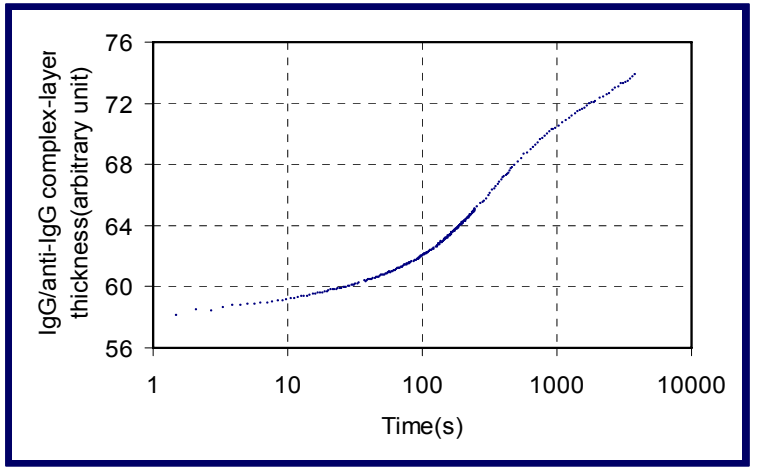


Detection of a Hepatitis B patient serum with protein chip: Anti-HBsAg, Anti-HBeAg, HBcAg, HBsAg and HBeAg were first immobilized covalently on protein chip in triple by microfluidic system. Result showed three makers were positive, which were HBsAg, HBeAg, and Anti-HBcAg, respectively, which was in an agreement with ELISA





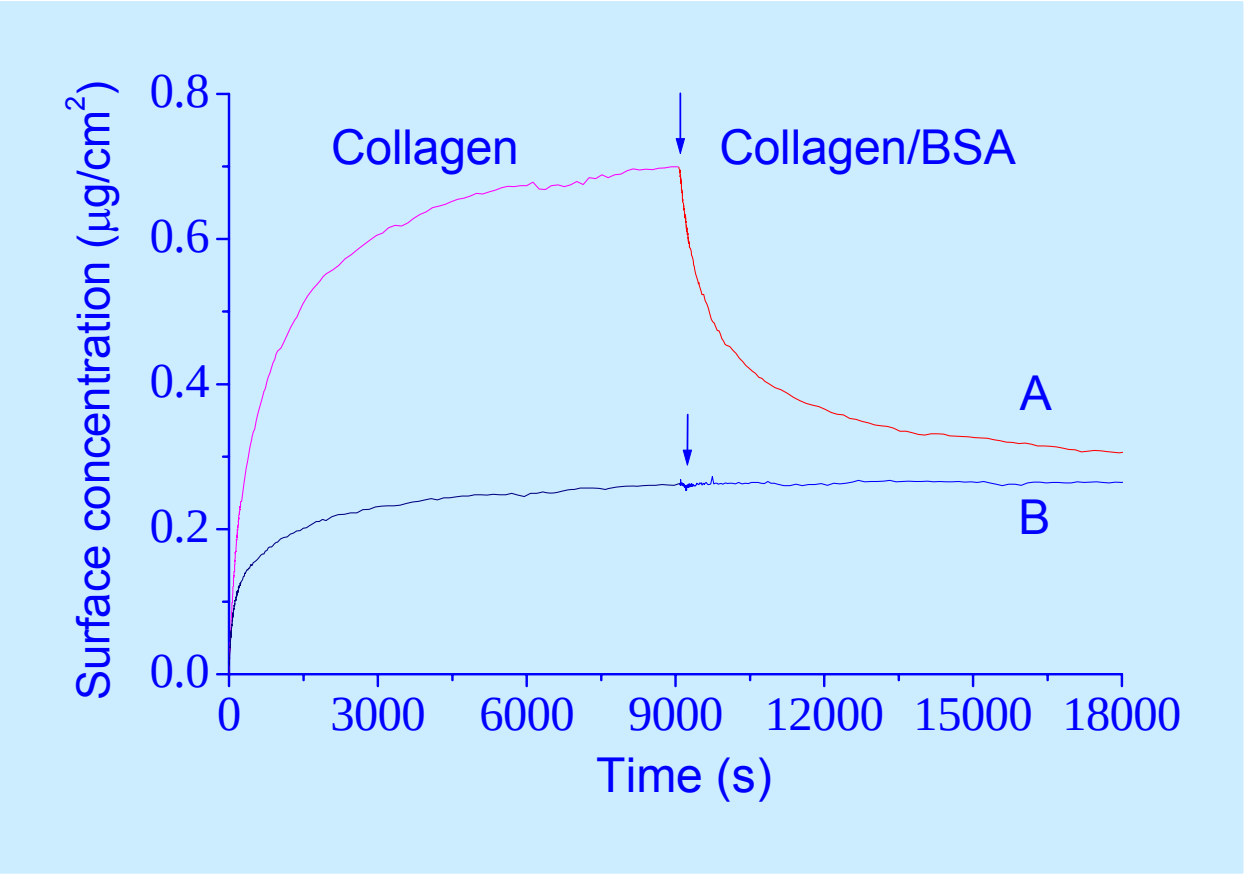
Multi-kinetic detection



The result of immunoglobulin G (IgG) reacted with its antibody (anti-IgG) during the binding process.

G. Jin, et al., World Congress on Medical Physics and Biomedical Engineering, 23-27 July, 2000, Chicago, U.S.A
Z-H. Wang, et al, Anal. Chem., Vol.75, No.22 (2003) p.6119-6123

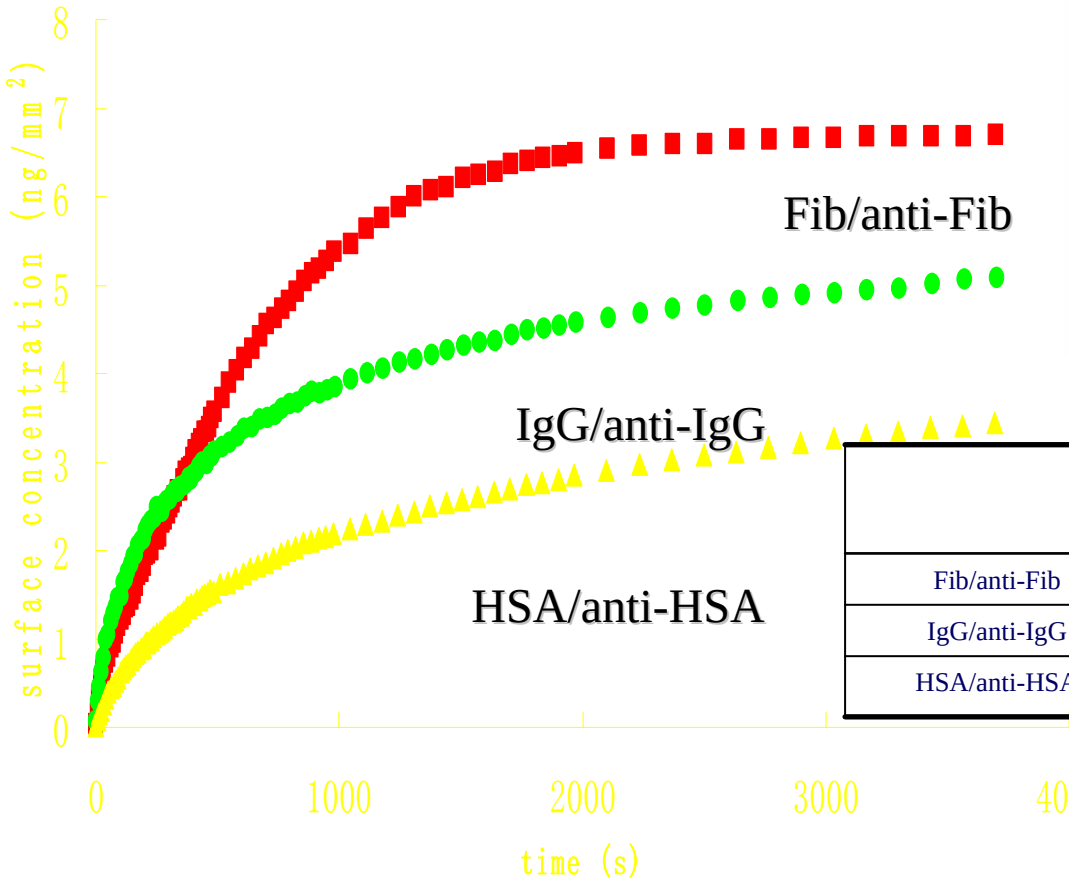
Protein competitive adsorption detection with the biosensor

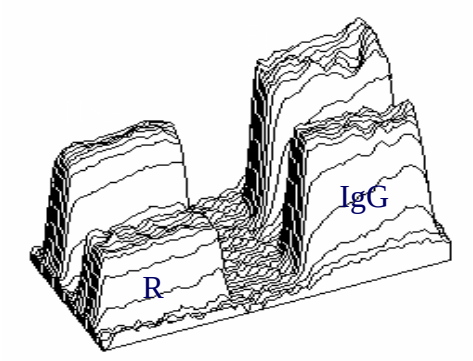


Adsorption of collagen (0.1 mg/ml) and then co-adsorption of collagen (0.1 mg/ml) and BSA (1.0 mg/ml) on hydrophobic (A) and hydrophilic (B) surfaces.

P.Q. Ying, et al, COLLOIDS and SURFACES B: BIOINTERFACES. 32(1). 1-10, 2003

Fib-antiFib, IgG-antiIgG, HSA-antiHSA in-situ detection





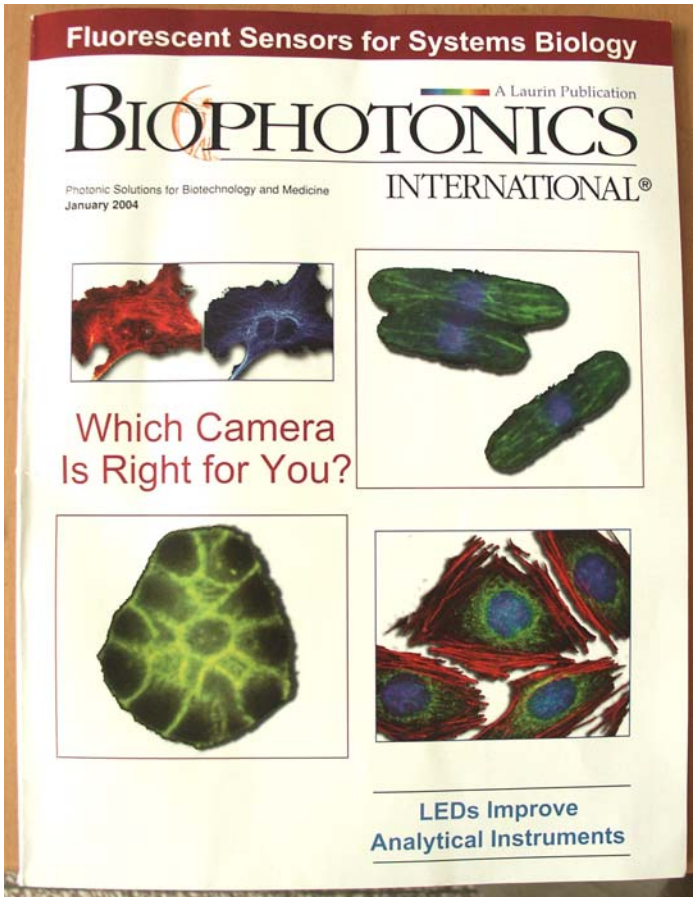
	$k_a(M^{-1} s^{-1})$	$k_d(s^{-1})$
Fib/anti-Fib	1.9×10^{4}	9.3×10^{-3}
IgG/anti-IgG	2.4×10^{4}	6.4×10^{-3}
HSA/anti-HSA	1.6×10^{4}	4.2×10^{-3}

Zhanhui Wang, et al., *Anal. Chem.* 2003.

已经应用方面:

1. 抗原-抗体相互作用
2. 内分泌激素检测
3. 细胞因子-受体相互作用
4. 肿瘤标志物检测
5. 单克隆抗体药物鉴定
6. 乙型肝炎五项检测
7. 分子间相互作用动态测量
8. 蛋白质竞争吸附
9. 病毒检测
10. 蛋白复性等……

国际报道
International News



Imaging ellipsometry applied to biological sensing

A group at the Chinese Academy of Sciences' Institute of Mechanics in Beijing has demonstrated that imaging ellipsometry, a technique employed in materials science and in the semiconductor industry, is suitable for use in immunoassays. The nondestructive technique offers users the ability to simultaneously detect multiple analytes without the need for the conjugated markers or radioactive labels that can limit the application of an immunosensor to particular biological systems.

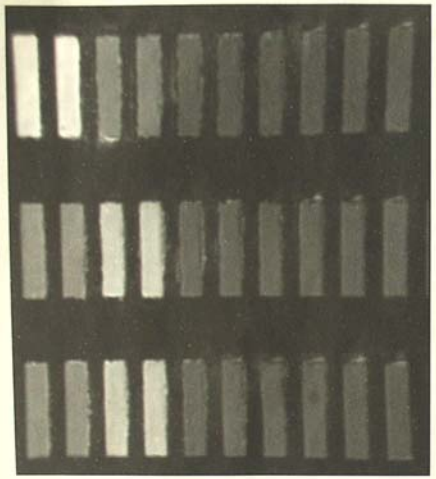
Gang Jin, a professor at the institute, explained that ellipsometry analyzes polarized light that is reflected or transmitted by a sample. "When a polarized light beam illuminates a sample at an angle of incidence, normally the polarized states of reflection or transmission are different from that of incident light, since the sample modulates the polarized state." These states carry information about the optical properties of the sample and can be used to deduce the presence and thickness of a film growing there.

Ellipsometry was used as early as 1945 to study the interaction of antigens and antibodies, the researchers note. Imaging ellipsometry, which incorporates microscopy techniques, parallel probe beams and CCD camera technology, offers enhanced lateral and vertical spatial resolutions and a larger field of view that enables the interrogation of thinner films over larger surfaces.

This is a boon for biological sensing, Jin said, because protein monolayers or complex layers on a surface can display average thicknesses on the order of a nanometer. Moreover, with a field of view of several centimeters and a lateral spatial resolution of a few microns, imaging ellipsometry can simultaneously interrogate multiple analytical areas on a lab-on-a-chip for high-throughput screening.

In their work, the researchers employed

BIOPHOTONICS NEWS



Scientists have demonstrated that imaging ellipsometry is suitable for use in immunoassays, including this protein chip with 30 analytical areas. The intensity of the gray-scale images of the areas indicates the thickness of the film that forms as antigens on the surface interact with antibodies introduced into the sample chamber. Courtesy of Gang Jin.

an imaging ellipsometer that they built in the lab. They prepared both a single-sample bioprobe and an eight-sample protein chip from silicon substrates, coating the hydrophobic site on the former with bovine serum albumin (BSA) and those on the latter with human fibrinogen (Fib), human immunoglobulin G (IgG), human serum albumin (HSA) and BSA. They designed a 1-ml reaction cell with optical windows for the probe and signal beams from the ellipsometer, into which antiserums could be added for the experiments.

In the case of the bioprobe, they collected gray-scale images of the surface, after they had exposed it to 0.1 to 30 µg/ml of anti-BSA and had incubated it for 30 minutes at room temperature. Measurements took approximately one second. The intensity of the signals was proportional to the square of the thickness of the layer that formed as the antigens and antibodies interacted to form complexes, and the scientists could deduce the surface concentrations of BSA/anti-BSA complexes from this calculated thickness.

The protein chip functioned similarly. With several types of antigens immobilized on the chip, however, they were able to introduce an antiserum mixture containing 0.1 mg/ml each of anti-IgG, anti-HSA and anti-Fib and to determine the presence of the antibodies by simultaneously resolving the films that formed at the 0.75 × 0.75-mm analytical areas with their respective antigens. The researchers note that the ellipsometer's lateral resolution of 3 µm enables it to measure films on much smaller spots than this, so the technique should be compatible with protein chips featuring many more analytical areas.

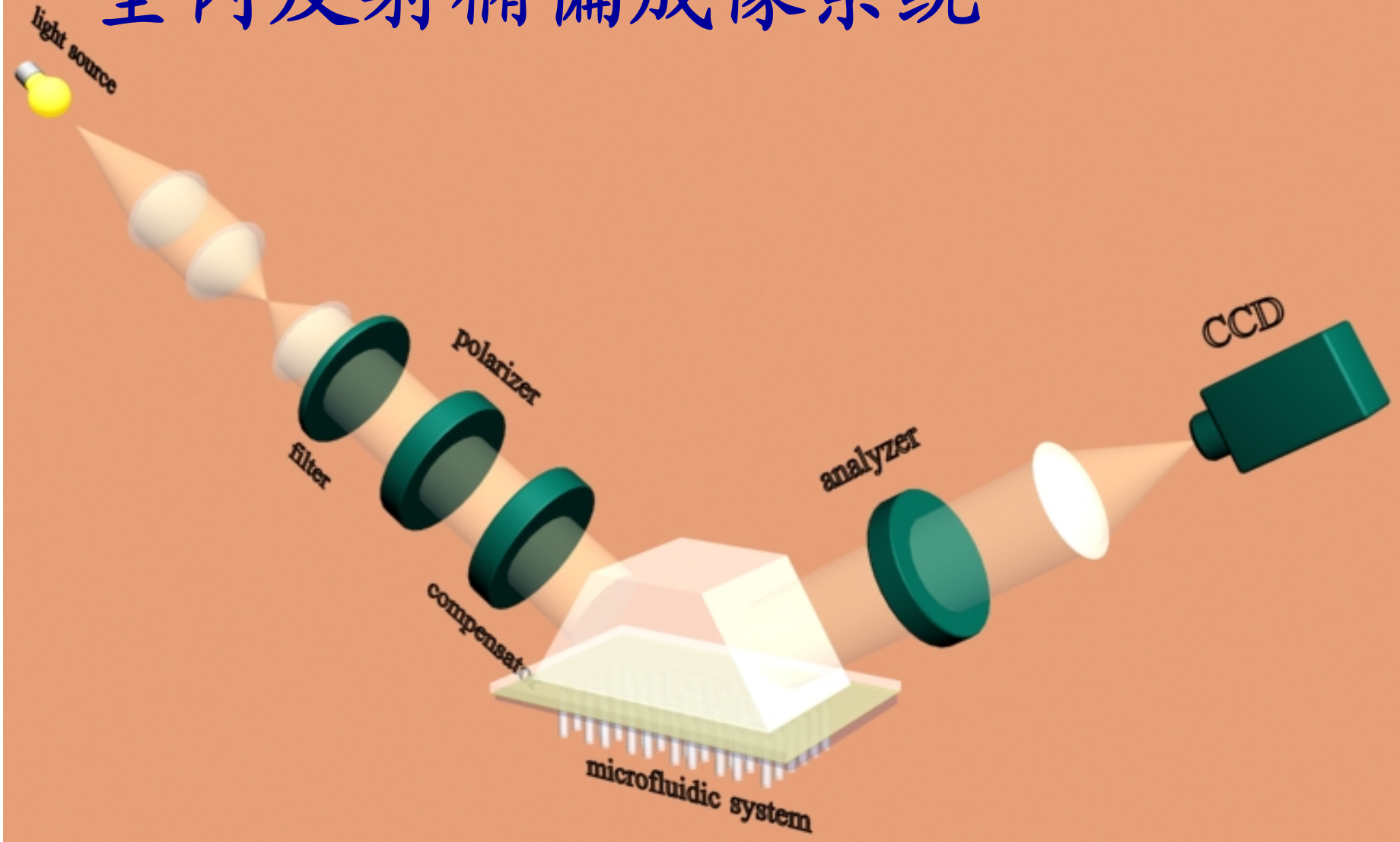
To establish the further functionality of imaging ellipsometry, they prepared another protein chip with Fib, HSA and IgG and recorded series of images of the chip as they added a mixture of antiserum to the reaction cell. Converting the real-time, gray-scale images into surface concentrations and plotting them as a function of time yielded binding curves for each of the proteins. Such information may be useful with multianalyte chips, in which the reactants are monitored for kinetics information, and with cross-reactive chips, in which different proteins bind to the same analyte but display different affinities.

Jin said that the scientists will continue to optimize imaging ellipsometry for practical applications in the biosciences. They expect to collaborate with industry on its development, and they predict that the technique will find commercial uses. □

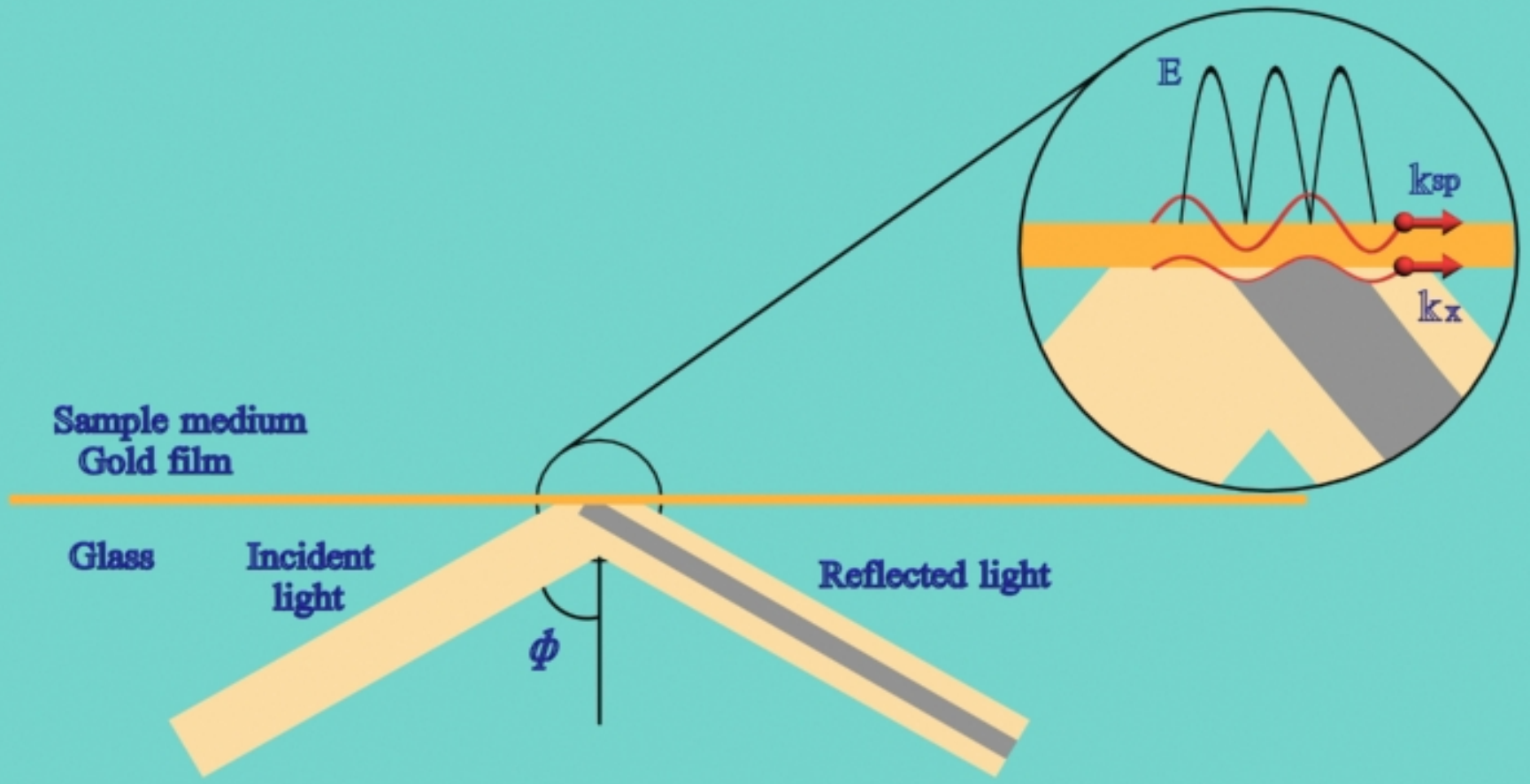
Daniel S. Burgess

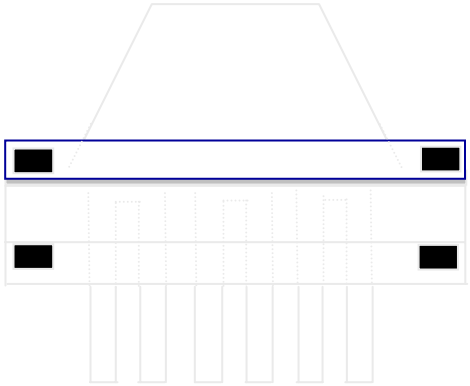
Analytical Chemistry, Nov. 15, 2003, pp. 6119-6123.

全内反射椭偏成像系统

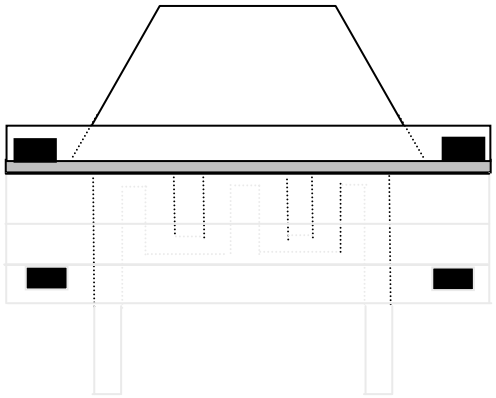

light source
filter
polarizer
compensator
analyzer
CCD
microfluidic system

TIRIE原理

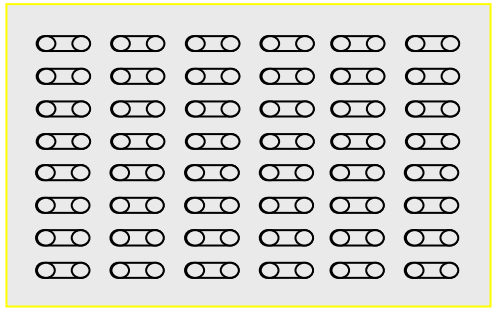

E
ksp
kx
Sample medium
Gold film
Glass
Incident
light
Reflected light
ϕ



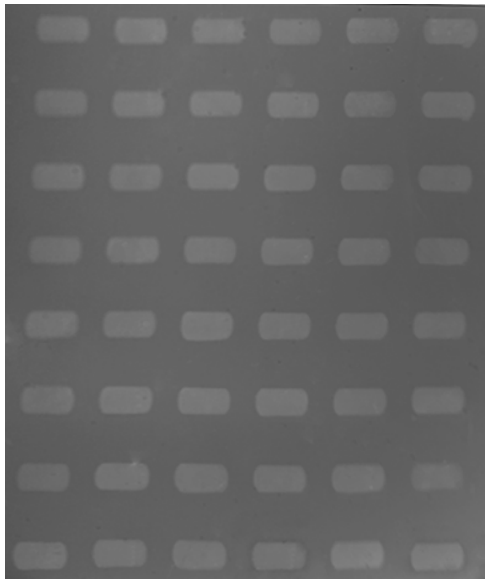
Parallel way



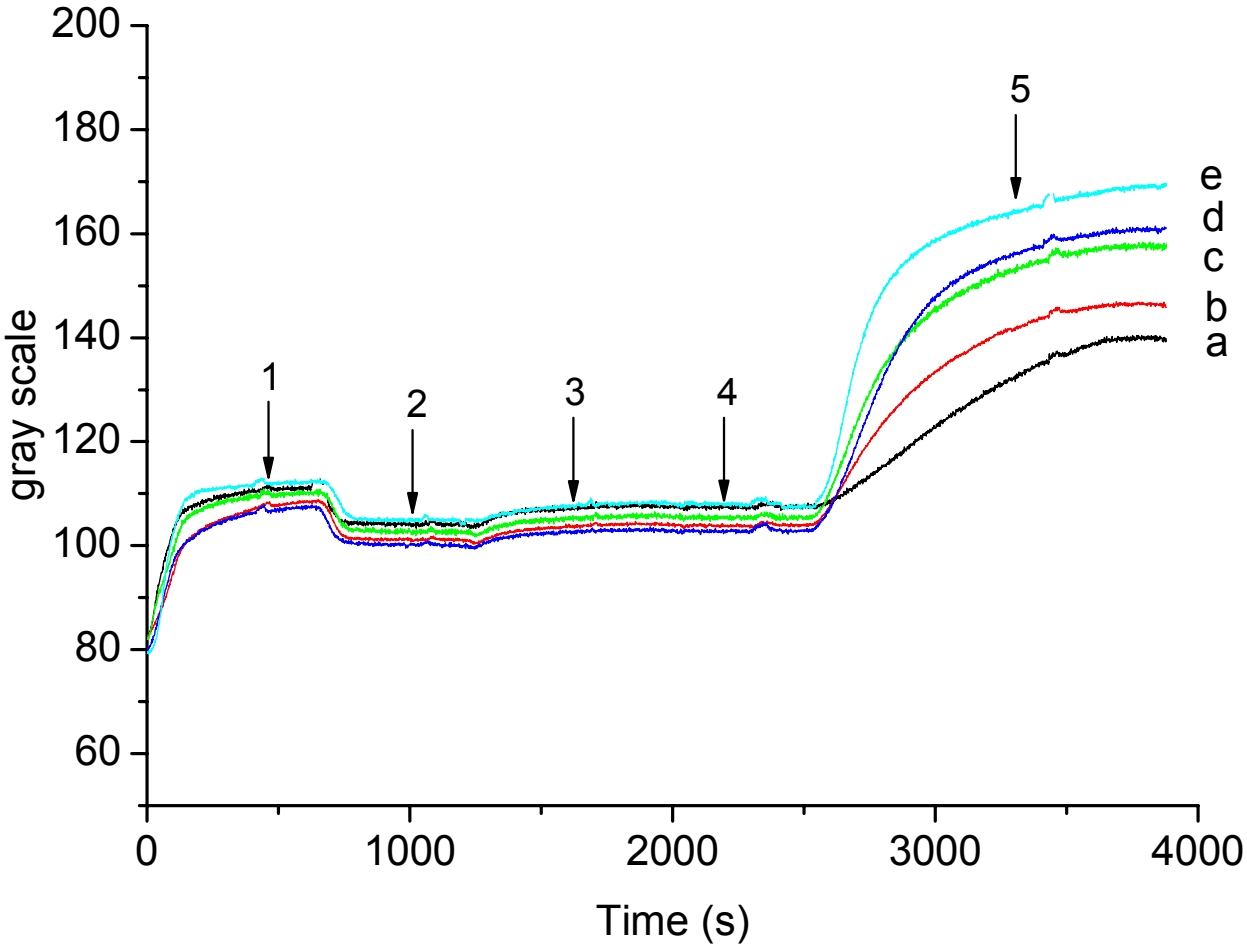
Serial way



Cell array



A gray scale image of spots array



5个连续的生物分子在芯片表面的动态过程的实时检测
包括：配基组装（ HBsAb ， 0.1mg/ml ）、清洗（PBS）、表面封闭（ BSA ） 和 HBsAg 与 HBsAb的相互作用, e–a 对应 HBsAg 浓度 80, 60, 36, 24 and 12 μ g/ml， 流速 10 μ l/min.

芯片式传感器的比较

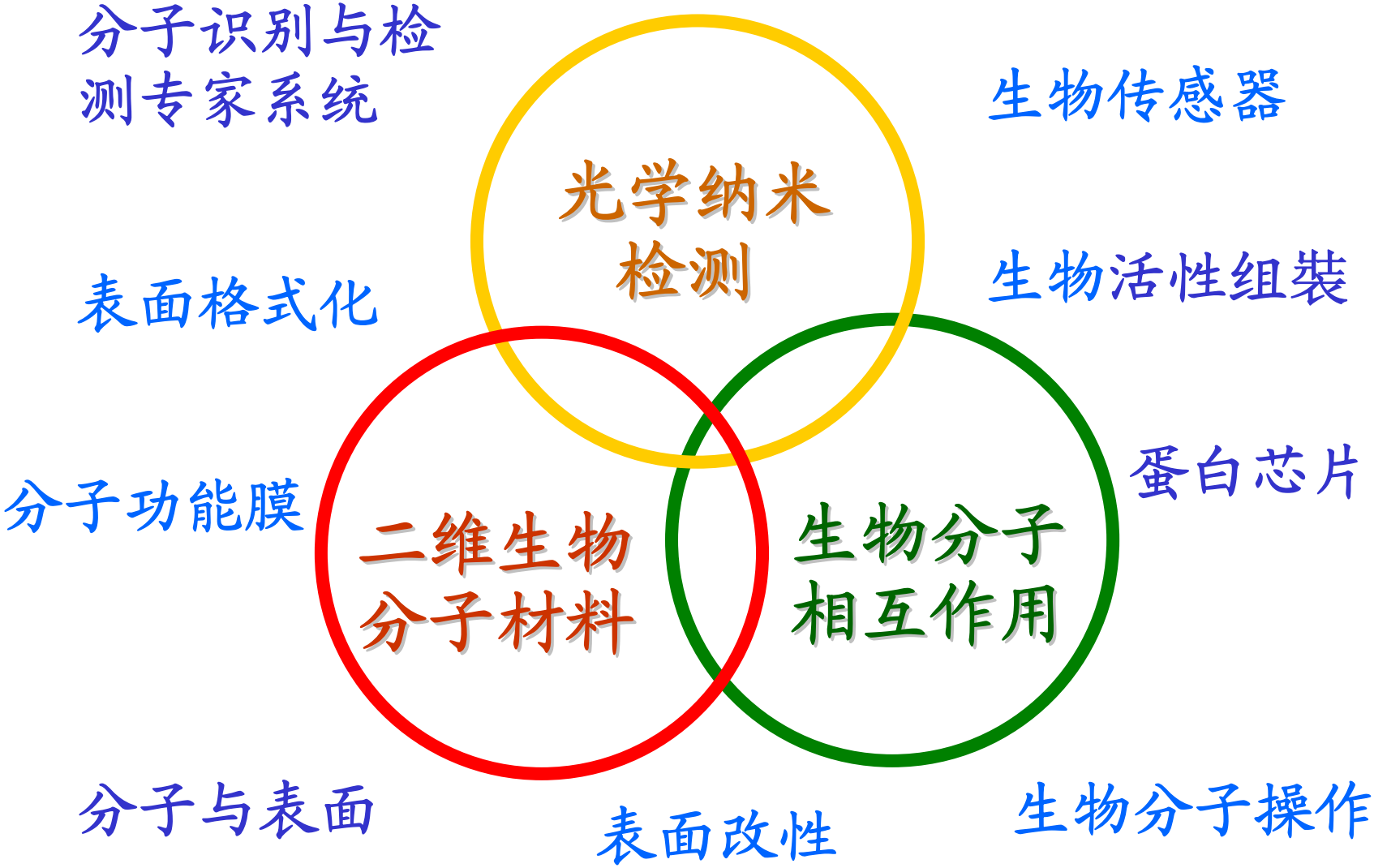
	BIACORE	SELDI	光学蛋白质芯片
通量	低	低	低—高
标记	不需标记	不需标记	不需标记
检测方式	非实时 / 实时	非实时	非实时 / 实时
结果输出	非图像显示 / 单点	非图像显示	图像显示
基底要求	导电	预处理	反射
灵敏度	< ng / ml	—	< ng / ml
样品用量	100 μl	—	＜ 15 μl

蛋白质芯片的用途

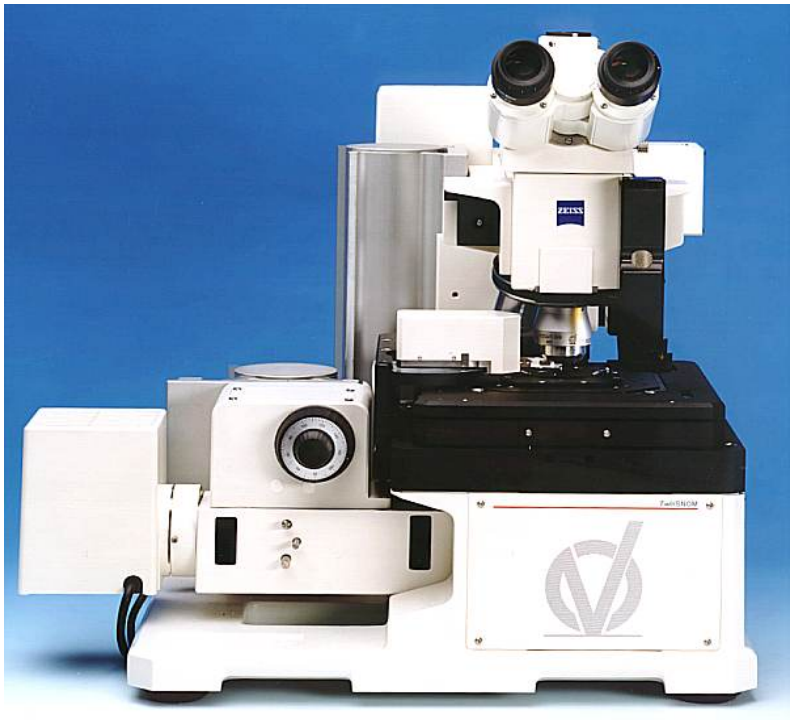
- 蛋白质功能研究、
- 疾病诊断和医疗、
- 新药开发、
- 生物工业等。

面对人体中数以十万计的蛋白质和可预想的尚未被认识的，以及自然生物链中广泛的物种所存在各种功能的大量蛋白质种类。今天发展的蛋白质芯片技术将会有怎样的应用前景？

纳米光学生物研究


分子识别与检测专家系统
生物传感器
光学纳米检测
表面格式化
生物活性组装
分子功能膜
蛋白芯片
二维生物分子材料
生物分子相互作用
分子与表面
表面改性
生物分子操作

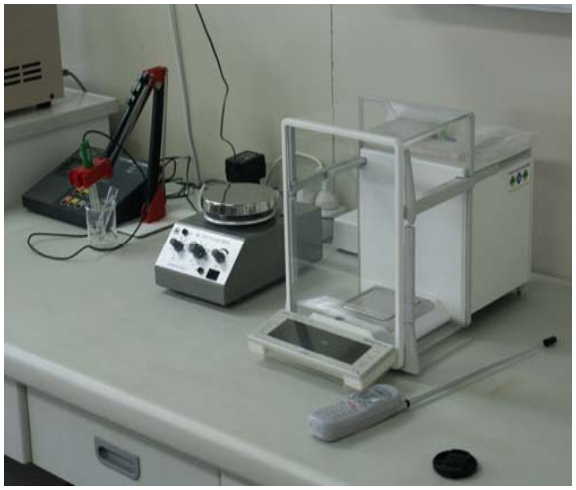
光学检测实验室



纳米光学检测系统

分子操作
实验室

蛋白质分离纯化



芯片制备实验室

谢谢各位