

芯片实验室的最终目的就是要将核酸分析所有的操作步骤，包括样品预处理、PCR 扩增、除盐、荧光标记、筛分、检测等集成在同一个芯片上完成，其中最重要的是 PCR 扩增反应和产物分离检测^[117,118]这两步的集成。集成式 PCR 微流控芯片可以直接对血或尿等临床样品进行分析检测，得到与疾病有关的基因信息。PCR 扩增反应是将含有 DNA 样品、核苷酸、引物和 DNA 聚合酶的反应溶液，在三个不同温度之间进行循环反应，使微量的核酸片段数量成指数倍增加。最初研究人员是将 PCR 扩增反应溶液加入到样品池内^[119]，微通道内充满筛分介质，所有的缓冲液池都用矿物油封口，将整个芯片同时进行温度循环，经过一定的循环次数后，向样品池内添加 DNA 标准片段和染料，进行 PCR 产物的电泳分离检测。这种芯片并不是严格意义上的功能单元集成式微流控芯片。后来人们通过将珀耳帖电热元件（Peltier thermoelectric element）放置在芯片的加热区域附近，就可以将热循环过程控制在样品池内进行^[120,121]，将热电耦埋在电热元件表面进行温度控制和监测。

与加热样品池不同，Burns 等人^[122]将加热器和温度传感器通过微加工技术都集成在微流控芯片上，该芯片上设置了三个温度区域，这些区域通过蛇形微通道相连，每个区域温度固定，蛇形微通道依次通过每个温度区域，经过的次数由 PCR 扩增所需要的循环次数决定。因为微通道的长度一定，因此反应的时间将由微通道内液体的流速来决定^[117,118,123]。这种芯片被称为连续流动型 PCR 微流控芯片。如果在该芯片上再集成一个出口，则可以在循环反应中间（循环 20、25、30、35、40 次时）将样品移走^[123]。当然也可以在连续流动型微流控芯片上实现 RNA 的逆转录扩增，因为该逆转录扩增是在连续流动的模式下进行的（流速为 $2-3 \text{ nL}\cdot\text{s}^{-1}$ ，整个反应时间小于 30min），所以可以通过连续进样（每次反应混合溶液 $0.7 \mu\text{L}$ ）的方式，来提高该微流控装置的单位产量。每次进样中间用 $0.7 \mu\text{L}$ 的水将样品隔开，这就可以避免不同样品之间的相互干扰。但经过一段时间的操作后，高温区域因为蛋白质的变性沉淀而容易被堵塞。目前该微流控装置尚未实现与电泳分离或流体疏运的集成化。

最近 Vahedi 等人首先将双链 PCR 产物变性解链、荧光标记、最后通过两种不同的分析方法（单链构象多态性(single stranded conformation polymorphism

(SSCP); 和异源性双链分析 heteroduplex analysis) 对 PCR 产物的突变基因进行了检测, 整个分析过程被集成在同一个芯片上, 在几分钟内即可完成^[124]。

1.6.2 微流控芯片中蛋白质分离分析

随着微流控芯片技术在蛋白质组学中应用的不断发展, 目前在玻璃和石英微流控芯片, 以及 PDMS、PMMA、聚碳酸酯等聚合物微流控芯片上, 通过区带 (CZE)、凝胶 (CGC)、电色谱 (CEC)、等电聚焦 (IEF) 等电泳模式, 已成功实现了微流控芯片上蛋白质的分析^[125,126,127,128]。这一节将主要就蛋白质在微流控芯片上的分离作一简单介绍, 关于微流控芯片上蛋白质其它方面的研究, 如样品预处理、富集和浓缩、免疫分析、酶反应等, 可以参考文献综述^[129,130,131]。

Bousse 等人^[132]研制了一个集成式微型凝胶电泳芯片装置, 可以在芯片上完成样品预处理、标记、灌胶、分离和检测等操作。目前该装置已成功完成商品化 (Bioanalyzer, Caliper Technologies 和 Agilent 公司出品)。它可以在一个分离微通道内连续进样、分离 8 个不同的蛋白样品和 1 个蛋白标准样品, 准确度在 5% 左右, 可以检测到 30 nM 的碳脱水酶。最近 Wang 等人^[133]同样采用凝胶电泳模式, 但以反向散射干涉法代替常用的 LIF 法进行检测, 避免了荧光标记, 分离效率在 7000—11000 塔板数之间, 塔板高度为 1—4 mm。

Fréchet 小组^[134,135]对光聚合甲基丙烯酸酯类整体柱进行了大量的研究工作, 在芯片上进行了标准肽的 CEC 分离。Shediac 等人^[136]和 Throckmorton 等人^[137]采用同样的方法合成了丙烯酸酯整体柱, 对 6 种 CZE 模式分不开的肽进行 CEC 模式分离, 样品得到了基线分离, 塔板高度为 1.7 mm。

IEF 是根据蛋白质等电点的不同来对样品进行分离的, 微通道内的 pH 梯度通常也是由两性电解质产生, 各种常规毛细管电泳所用的冲洗方法, 在微流控芯片中也都有应用, 如化学法^[138]、疏水法^[125]、残余电渗法^[139]等都被用来将微通道内聚焦后的蛋白质冲出。Hofman 等人^[138]报道了玻璃芯片上不同的 IEF 分离模式, 可以达到 83,000 塔板数, 400 塔板数/s, 峰容量约为 70。与之相对比, Tsai 等人^[140]在 3 分钟内所得到的分离塔板数为 19,600, 玻璃芯片内壁通过等离子体聚合一层硅氧烷薄膜来抑制电渗。Macounova 小组^[141,142]新开发出一种在微通道内产生 pH 梯度的方法。通过电解水, 在阳极和阴极分别产生 H_3O^+ 和 OH^- , 这两种

离子通过扩散,在通道内产生一个可控的pH梯度,在pH 3.0—7.0的梯度范围内,实现了牛血清白蛋白的聚焦。Huang等人^[143]根据Tris-HCl溶液pKa随热量的变化在微通道内产生pH梯度。当在锥形微通道末端施加电压时,所产生的焦耳热与微通道横截面成反比,因此就会得到一个pH梯度。

除了微通道内合成整体柱外,还有人将聚合物膜集成在芯片上。Jiang等人^[144]将一个PVDF薄膜夹在两个0.5 cm长,直径为100 μm的微通道内。细胞色素C的酶切产物被注到膜上,用酸化的乙睛逐步冲洗,然后通过一个微型化的透析界面进入ESI-MS中,完成整个分析需要80分钟,峰容量比微型LC稍低。

尽管各种分离模式都已被用于蛋白质的微流控芯片分离,但单一分离模式所能得到的最大峰容量仅为150/次^[145],远远不能满足蛋白质组学研究的需要,因此多维微流控芯片中分离就逐渐应运而生。Ramsey小组是较早开展这一方面研究工作的,在芯片上实现了CEC或MEKC与CZE的联用。其中CEC-CZE芯片^[146]结构十分简单,1条内壁用十八烷基三甲基硅氧烷处理(C₁₈固定相),长25 cm的蛇形微通道与另1条长0.8 cm的微通道正交。如果将第二维分离进样频率控制适当,以酪蛋白酶切产物为分析对象,在13分钟内,峰容量为150。MEKC-CZE芯片^[147]与CEC-CZE芯片的操作原理基本相同,19.6 cm长的MEKC微通道与1.3 cm长的CZE微通道正交。通过提高CZE进样频率,每个MEKC峰被进样6次,这就使峰容量得到显著提高,在15分钟内,峰容量为4200。Herr等人^[148]在塑料芯片上实现了IEF-CZE的联用,首先通过IEF将样品等电聚焦,然后再将样品逐一进行第二维分离。以绿荧光蛋白、卵白蛋白和右旋糖苷混合物为检测对象,在5分钟内,峰容量为1300。

在进行肽谱解析和蛋白质定性分析时,MS是必不可少的检测手段。将MS与芯片联用时,要解决的一个关键因素就是二者的接口,关于蛋白质的MS分析,可以参照前面1.5.3章节中部分内容。将微流控芯片通道阵列化^[149],或者将MS串联^[150],然后再将二者联用,则可以进一步提高单位信息量。将串联MS用于肽的分析,首先通过化学反应将胰岛素固定在微通道内的凝胶床上,然后通过固定化的胰岛素酶对蛋白质进行在线降解,肽的分离和MS分析可以集成在同一个微装置中完成^[151]。

1.7 本论文所涉及的微流控芯片基础研究工作

芯片实验室的早期形式是微流控芯片电泳, 芯片电泳分析至今仍是芯片实验室中分离部分的主体。与常规毛细管电泳相似, 二者均是以电场力为主要驱动力, 借助于样品中带电粒子(离子或分子)在迁移或分配行为上的差异, 来对其进行快速分离分析。不同之处在于, 微流控芯片电泳分析是在芯片的微通道内进行的, 分离平台由线(毛细管)变成了面(芯片), 由此产生了一系列新的理论和技术。

本论文的工作主要着眼于芯片实验室中电泳分析的基础研究, 将围绕微流控芯片电泳分析的三个重要组成部分: 微流控芯片检测装置、微流控芯片以及包含有实现芯片功能化方法和材料的试剂盒这三方面来进行。其中论文的第 2-4 章主要是微流控芯片装置的搭建和微流控芯片电泳中样品迁移行为特征的研究; 第 5、6 章主要是关于玻璃微流控芯片及其筛分介质的研制; 第 7、8 章则主要研究 PMMA 微流控芯片中动态和静态修饰方法。通过以上几个方面的基础研究, 我们试图得到一些关于微流控芯片电泳分析的基本规律, 并在此基础上, 研制微流控芯片电泳分析专用装置和试剂盒, 促进微流控芯片电泳分析和芯片实验室研究的发展。

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第二章 四色荧光共聚焦激光诱导荧光微流控芯片装置

2.1 引言

按照目前的一般理解，一个微流控芯片实验室系统大体包括三个部分：一是芯片；二是芯片工作站，包括微流体控制装置及信号检测装置，当应用集中于分析领域时，又称为芯片分析仪；三是包含有实现芯片功能化方法和材料的试剂盒。本论文的前半部分工作主要着眼于微流控芯片装置的研制和开发。为了满足微流控芯片实验研究的需要，我们自行研制和开发了一系列微流控芯片装置，主要包括：一台四色荧光和两台单色荧光共聚焦激光诱导荧光微流控芯片装置，根据所用激光光源不同，单色荧光微流控芯片装置又分为脉冲式和连续式两种。

随着微流控芯片体系微型化的不断发展，对检测器尺寸和灵敏度也提出了更为苛刻的要求。目前在微流控芯片体系中常用的检测方法主要包括激光诱导荧光法（LIF）、电化学法、质谱法和化学发光法等^[1]。其中 LIF 因其灵敏度比较高，在微流控装置中应用最为广泛。LIF 通常需要对所分析的样品进行荧光标记，而且检测器体积也较大，这些都在某种程度上限制了 LIF 的应用。目前已有报道将体积较小的发光二极管（light-emitting diodes, LED）通过光纤或原位聚合的方法集成在塑料芯片上，以减小整个微流控装置的体积^[2,3]。为了提高检测窗口的峰容量，增大单位信息量，Paegel 等人搭建了一个四色荧光共聚焦旋转扫描检测体系，目前已可以同时检测同一芯片上的 384 分离通道，该体系已成功的用于 DNA 片段的分离研究^[4,5,6]。

我们实验室与上海冶金所合作，共同设计玻璃微流控芯片结构，研制搭建了四色荧光共聚焦激光诱导荧光微流控芯片装置，并对其基本性能进行了测试，取得了初步的结果。在该实验中，以国产的 488 nm 氩离子（Ar⁺）激光器为激发光源，以自制的玻璃芯片为平台，通过手动高压装置控制进样和分离，检测光路分为四路，不同波长的荧光通过不同的光路收集后由 PMT 检测，然

后由计算机进行数据采集与处理，最后将处理后的图像实时显示出来。通过初步实验验证，以测序用的四色荧光能量转移染料 F (10F, 10T, 10J, 10R) 为分析对象，该装置的质量检测限约为 10^{-19} mol。在此基础上，初步尝试了荧光标记 DNA 片段在玻璃芯片中的分离分析研究。

2.2 装置搭建

2.2.1 元件与材料

高压电源(电压 0—3 kV),购自上海原子核所;氩离子激光器,波长 488nm,功率为 4mW,购自南京电子管厂;半反半透镜(505DRLP, 545 DRLP, 570 DRLP, 590 DRLP);单色滤光片(525DF30, 555DF30, 580DF24, 605DF30);显微物镜(20×0.5N.A);光电倍增管 PMT (HC120-01)

2.2.2 装置设计

图 2-1 为微流控芯片体系结构示意图,主要包括微流控芯片、高压部分、光学部分和数据采集处理部分。其中,图中 a: 波长 488nm 氩离子激光器; b: 半反半透镜; c: 20×0.45 N.A 显微物镜; d: 电泳芯片(1: 加样池; 2: 缓冲池; 3、4: 分离缓冲池); e: 低压电源; f: 高压电源; g: 光学检测系统(详见图 2); h: 检测窗口。

2.2.2.1 光学部分设计

如图 2-2 所示,氩离子激光器 a 发出波长为 488nm 的激光束,经半反半透镜 b 反射至物镜 c,通过物镜 c 将激光聚焦在分离微通道的某一点上,当微通道内已标记过的样品经过该检测点时,所激发出来的荧光由同一物镜 c 收集,通过半反半透镜 b 与激光区别开来,然后进入四路光学系统中,根据荧光波长的不同在不同的光路中加以检出。四路光学系统的具体光路如图 2-2 所示。荧光在进入每一单光路前,首先经过一个半反半透镜,其中一部分荧光被反射到较短波长的单光路中进行收集检测,而其余的荧光在经过多次分配后,逐级进入到不同波长的光路中,分别得到收集检测。在每一单光路中,反射进来的荧光首先经过一个单色滤光片(4 路滤光片中心波长分别为 525nm, 555nm, 580nm, 605nm)滤去杂散光,然后由透镜聚焦,再由空间滤波器进行空间滤波后,进

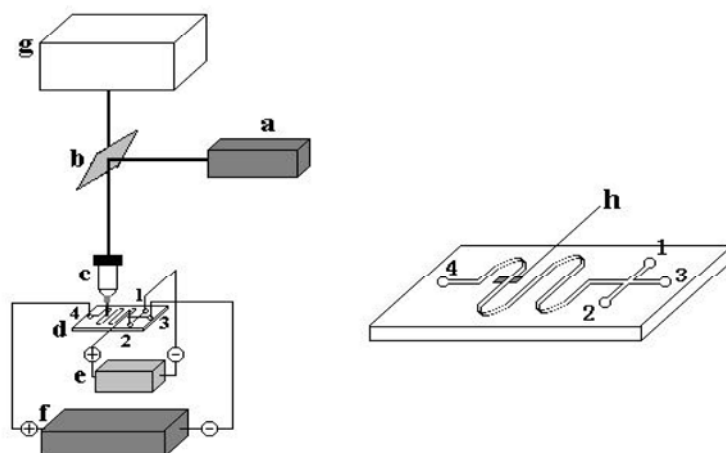


图 2-1 微流控芯片及其系统示意图

Fig 2-1 The layout of total microfluidic device

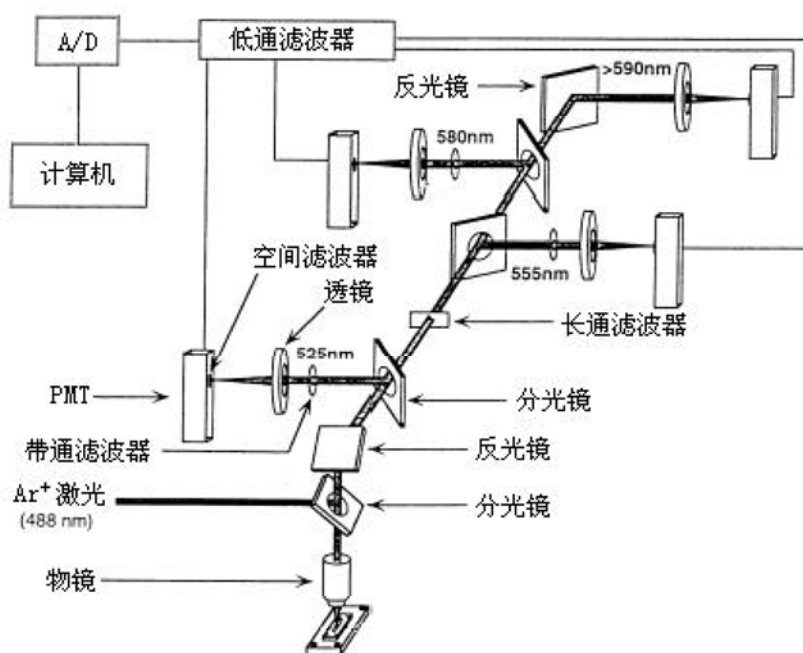


图 2-2 微流控芯片检测系统中四路光通道示意图

Fig 2-2 Four detection channels with different fluorescence wavelength in microfluidic device.

入光电倍增管 (PMT) 中, 光信号在 PMT 中转化为电信号, 最后由计算机将所得到的信号记录储存下来。

在此光学系统中, 由于所检测到的四路荧光相邻波长只相差 30nm 左右, 因此每一光路中带通滤波器的带宽应近可能的小, 以降低相邻光路间的互相干扰; 此外, 由于四路荧光信号为依次分级检测, 所以在安装半反半透镜时要充分考虑每一级的透过率, 必须同时保证本路和后面几路荧光检测灵敏度的要求。

2.2.2.2 手动高压控制装置

如图 2-3 所示, 芯片毛细管电泳的进样和分离电压是由三刀三掷开关手动控制, 而非计算机控制。进样时, 将切换开关置于进样侧, +2kv 电源调到预设定值, +30kv 电源断开, 进样时间 30s; 分离时, 将切换开关切换到分离侧, 同时将 +30kv 电源电压升到预设定值, +2kv 电源也调到预设定值。由于该高压装置为手动操作, 因此会给实验带来一些人为干扰, 这将在一定程度上影响分析结果的重现性。

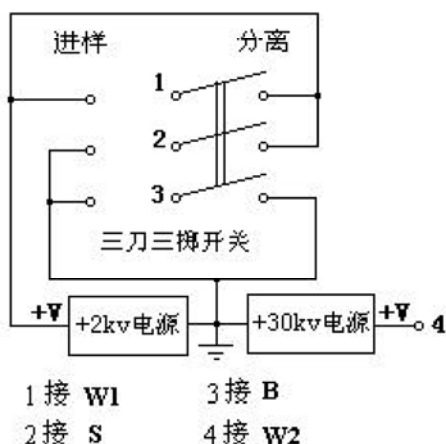


图 2-3 进样时和分离时的电源示意图。W1 为样品废液池; B 为缓冲液池; S 为样品池; W2 为缓冲液废液池。

Fig 2-3 The power supply used for injection and separation in microfluidic device.

2.2.2.3 快速采集与信号处理部分设计

光信号经光电倍增管 PMT 后转换为模拟信号, 经过低噪声放大器进行放大, 然后经过二阶低通滤波器滤除噪声, 经模数(A/D)转换成数字信号, 由计算

机进行数据采集与处理，最后将处理后的图像实时显示出来。信号采集与处理模块以及计算机处理与显示软件均由上海冶金所设计与开发。

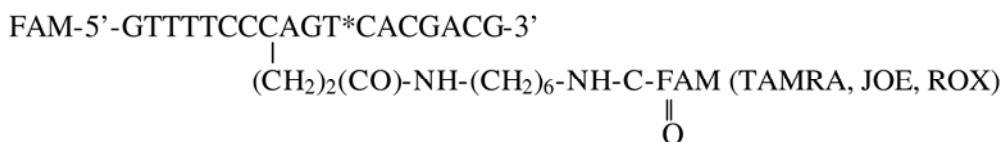
电泳芯片的检测时间只有几分钟，因此设计了快速的信号采集、处理与实时显示系统。同时由于分析的样品量只有几十皮升，要求很高的检测灵敏度，因此必须对信号进行放大与滤波，本系统中采用了低噪声放大器与二阶低通滤波器，有效地对信号进行了处理，提高了检测灵敏度。

2.3 实验部分

2.3.1 试剂与样品

18 聚(mers)寡核苷酸 (5OD, FAM 修饰)、91 聚 DNA 片段 (0.7OD, TAMRA 修饰)、117 聚 DNA 片段 (2OD, FAM 修饰) 和 208 聚 DNA 片段 (2OD, FAM 修饰)，均由上海生工合成。能量转移荧光染料 F (10F, 10T, 10J, 10R) 及 TEMED 均购自 Amersham Pharmacia Biotech, Inc., USA。丙烯酰胺 (分析纯) 购自 Sigma 公司。Tris (分析纯) 与 EDTA (分析纯) 均购自华美生物工程公司。硼酸 (分析纯) 购自上海试剂一厂，其余所用试剂均为国产分析纯，所有溶液均使用二次水配置。

能量转移荧光染料 F (10F, 10T, 10J, 10R) 结构如下所示：



其中，6-FAM: 6-carboxyfluorescein;

TAMRA: N,N,N',N'-tetramethyl-6-carboxyrhodamine;

JOE: 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein;

ROX: 6-carboxy-X-rhodamine.

2.3.2 微流控芯片及电泳分析条件

微流控芯片以玻璃为材质，采用标准的湿法刻蚀技术加工制作而成，具体工艺可以参考 1.3.1 章节的内容^[7]。微流控芯片的设计由大连化学物理研究所和上海冶金所共同完成，上海冶金所承担了具体的制作工作，其结构如图 2-4 所

示。

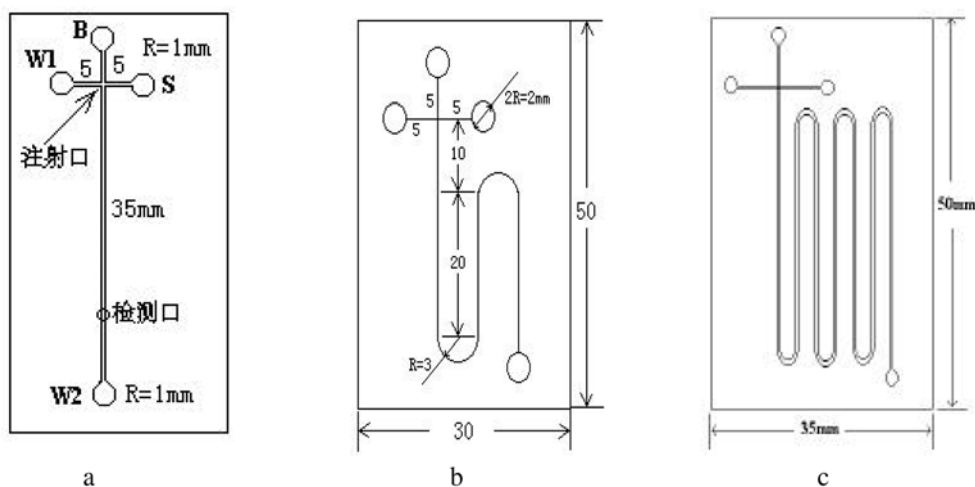


图 2-4 不同微流控芯片结构示意图。a 分离长度 4cm，泳道宽度 $50\mu\text{m}$ ；b 分离长度 9cm，泳道宽度 $30\mu\text{m}$ ；c 分离长度 20cm，泳道宽度 $30\mu\text{m}$ 。

Fig 2-4 Schematic diagram of different microfluidic chips.

对于四色荧光染料样品，所用芯片如图 2-4a 所示，有效分离距离为 3 cm。分析缓冲液为 TBE(45 mM Tris-Borate/1 mM EDTA, pH8.5)，采用正向电压，400 V，30 s 进样，分离场强为 150 V/cm，激发波长为 488 nm，样品的发射波长分别为 525，555，580，605 nm。

对于荧光标记的 DNA 片段样品，所用芯片如图 5c 所示，有效分离距离为 12 cm（分离通道为蛇行结构），内壁用聚丙烯酰胺涂层改性。采用负向电压，800 V，15 s 进样，分离电压为 6000 V，以 2% 的线性聚丙烯酰胺为筛分介质。

2.4 结果与讨论

2.4.1 四色荧光微流控芯片电泳装置的建立

加入样品和缓冲液的芯片放在倒置显微镜平台上，在倒置显微镜的目镜上加一滤光片，通过目镜观察激光是否对准狭缝。电极固定在一塑料平板上，平板由铁架台固定。激光光源先经过反射镜反射，再通过半反半透镜（488 nm 反射，510 nm 以上透过）反射后，进入 20X 物镜内，聚焦在芯片微通道的检测窗

口上。激光光斑最初约为 1 mm，经物镜聚焦后，直径约为 50 μm 。荧光信号由同一物镜收集，通过半反半透镜后进入四色荧光检测系统（即图 2-5 中白色方盒部分）。在整个仪器的搭建过程中，光路的安装调制是最为关键的。由于四路信号为依次分级检测，因此在设计半反半透镜时要充分考虑每一级的透过率，以保证后面光路检测需求；另外由于相邻光路间的波长差仅为 30 nm 左右，因此每路中单色滤光片的带宽应近可能的小，以减小不同光路之间的相互干扰。在光路的实际调整过程中，前一级光路任何一微小的变动，都将会对后面几路光路的检测产生较大的影响。光电倍增管（PMT）所收集到的每一路的荧光信号经放大电路和 A/D 转换后输入计算机进行处理。



图 2-5 四色荧光微流控芯片装置外观图

Fig 2-5 The picture of home-made microfluidic device with four fluorescence detection channels.

2.4.2 芯片电泳装置的评价

以四色荧光能量转移染料 F (10F, 10T, 10J, 10R) 为样品，将其用二次水稀释（浓度约为 10^{-7} mol/L），进样电压 400V，进样时间 30s，此时进样量以芯片微通道十字交叉处的体积计算，约为 3.6×10^{-11} L，荧光染料的质量检测限约为 10^{-19} mol。该染料的芯片电泳分析图谱见图 2-6。

此时噪音水平约为 0.2mV，信噪比（S/N）约为 24。考虑到由于透镜、芯片通道多个界面的反射会引起光强的大量损失，加之一些如散射光、滤光片、

荧光收集等技术上的原因，可以认为，此组件的安装调试基本上可以满足设计的要求，能够用于微流控芯片电泳分析。

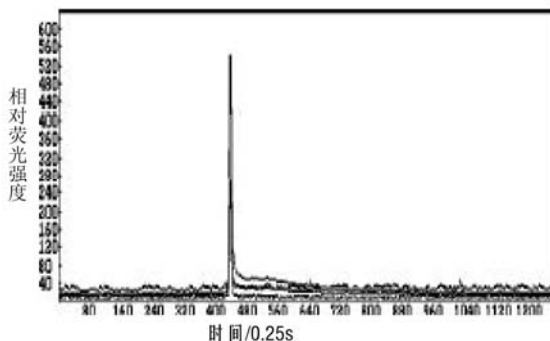


图 2-6 四色荧光染料的微流控芯片电泳分析谱图。

Fig 2-6 Electrophoregram of four energy-transferred fluorescence dyes obtained in glass microchip.

2.4.3 荧光标记 DNA 片段的芯片电泳分离分析

以 18 聚寡核苷酸，91 聚 DNA 片段，117 聚 DNA 片段，及 208 聚 DNA 片段的稀释后混合物为样品，负向进样，所得的芯片电泳图谱如下图所示。

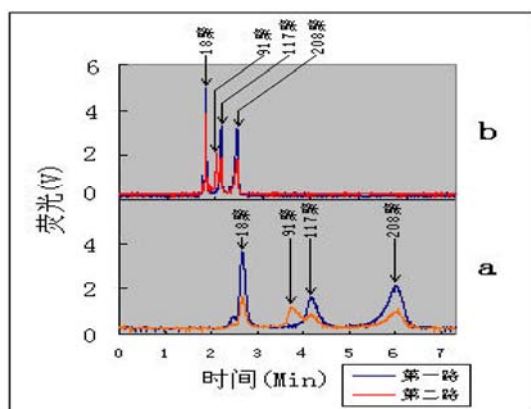


图 2-7 DNA 片断在 130 V/cm (a)和 300 V/cm (b)场强下的微流控芯片电泳分离谱图。

Fig 2-7 Electrophoregrams of DNA fragments in glass microchips under the separation electric field of 130 V/cm (a) and 300 V/cm (b), respectively.

如图 2-7 所示, 在 130V/cm 场强(图中 a)下, 18 聚、91 聚、117 聚、208 聚寡核苷酸出峰时间分别为 2 分 40 秒、3 分 52 秒、4 分 08 秒、5 分 59 秒, 而在 300V/cm 场强(图中 b)下分别为 1 分 50 秒、2 分 02 秒、2 分 09 秒、2 分 27 秒; 每个峰出峰时间提前, 半峰宽也有明显的减小, 其中最小的半峰宽只有 1 秒, 同时分离效率有明显地提高。适当的提高场强, 可以使样品的出峰时间大大缩短, 并且半峰宽缩小。随着分离速度的提高, 检测时就必须采用更快的采样频率和更短的响应时间。另外, 从图上还可以看出, 不同的样品采用不同颜色的荧光标记, 可以增加峰容量。

选择 300V/cm 的场强为分离场强, 计算相邻峰之间的分离度, 结果如表 2-1 所示。该结果表明该体系已可成功用于 DNA 片段的分离分析, 随着分离条件的进一步优化, 该仪器的分离能力将会进一步得到提高。

表 2-1 DNA 样品不同峰间的分离度

出峰次序	1	2	3	4	5
对应样品	荧光染料	18 聚的寡核苷酸	91 聚的 DNA 片段	117 聚的 DNA 片段	208 聚的 DNA 片段
分离度 (R)	第 1,2 峰间 2.3	第 2,3 峰间 2.2	第 3,4 峰间 1.0	第 4,5 峰间 2.3	

2.5 结论

与上海冶金所合作, 自行设计、研制并搭建了四色荧光共聚焦激光诱导荧光微流控芯片装置。该装置不仅可以用于四色荧光检测, 还可以用于单色、双色荧光检测。此检测系统采用了自主开发的信号采集与处理模块, 大大提高了信号的采样频率与信噪比, 提高了检测灵敏度, 可用于荧光染料和 DNA 片段的多路荧光分离检测。四色荧光共聚焦激光诱导荧光微流控芯片装置在 PCR 产物分析、DNA 限制性片段、基因突变检测、以及测序等方面具有重要的应用价值, 已经在生命科学、环境科学、临床诊断等领域得到了广泛的应用, 今后还会在疾病诊断、病理研究、家庭医疗等方面发挥更加重要的作用。

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第三章 脉冲式单色激光诱导荧光微流控芯片装置及样品电泳迁移行为

3.1 引言

激光诱导荧光 (LIF) 检测法因其装置相对比较简单, 检测灵敏度较高而成为芯片实验室的主流检测技术之一^[1,2]。LIF 所采用的激光光源主要有两种, 连续式激光和脉冲式激光。连续式激光因其单色性和稳定性均较好, 噪声水平比较低, 因此在 LIF 中应用比较广泛。脉冲式激光虽然激光运行频率较低, 一般只有 10-100 Hz, 脉宽重复性较差, 但因其单脉冲峰值功率很高, 可以很容易的得到二倍频、三倍频和四倍频短波长的激光, 而且 Nd-YAG 泵浦的脉冲染料激光器可以通过选择不同的染料来改变激光波长, 因此脉冲式激光在毛细管电泳和微流控芯片中都有一定的应用^[3,4,5]。脉冲激光的脉宽一般只有 10 ns, 比荧光寿命要短得多, 因此可以采用门取样技术, 即取样平均器 (Boxcar) 技术, 通过控制取样延迟和门宽来降低噪声, 提高信噪比。

本章以 Nd-YAG 泵浦的脉冲染料激光器 (10 Hz, 10 ns 脉宽) 为激发光源, 所发出的激光中心波长为 480 nm, 采用 Boxcar 技术对弱信号进行处理, 自行搭建了一台脉冲式激光诱导荧光微流控芯片装置。微流控芯片电泳分析是芯片实验室的早期形式, 它与传统毛细管电泳分析之间存在一定的异同点, 尽管学术界已经注意到了微流控芯片电泳具有很多不同于传统毛细管电泳的个性特征, 但就整体而言, 关于这一方面的研究还很不完善^[6,7]。因此在本章中我们以玻璃微流控芯片为操作平台, 在白组装的脉冲式激光诱导荧光微流控芯片装置上, 以荧光染料和氨基酸为对象, 对微流控芯片电泳分析中组分的迁移特别是它的行为特征进行了探索和研究, 考察了微流控芯片中的电泳分析与传统毛细管电泳分析之间的异同点, 并在此基础上成功完成了氨基酸对映体在微流控芯片上的手性拆分。