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(71)出願人　501175605
シェモメテック・アクティーゼルスカブ
ＣＨＥＭＯＭＥＴＥＣ　Ａ／Ｓ
デンマーク、デーコー－３４５０アレレズ、ギューゼヴァング４３番
(74)代理人　100062144
弁理士　青山　葆
(74)代理人　100086405
弁理士　河宮　治
(74)代理人　100106231
弁理士　矢野　正樹
(72)発明者　マルティン・グレンスビェアウ
デンマーク、デーコー－２７００ブレンシェイ、４テル・ヴェンストレ、ネスビホルムヴェイ２番

最終頁に続く

(54)【発明の名称】空間解像型酵素結合アッセイ

(57)【要約】
本発明は、少なくとも１の分析物の少なくとも１の質的パラメータおよび／または少なくとも１の量的パラメータを調べる方法に関し、ここに該少なくとも１の分析物は基質を生成物に触媒することができる触媒に結合し、それによって該分析物を分析物の周りに生成した生成物の検出を介して調べる。

【特許請求の範囲】
【請求項１】
少なくとも１の壁部を有する試料ドメインを確立し、
試料ドメインの壁部（群）に対して（ｒｅｌａｔｉｖｅ ｔｏ）分析物が移動し得るように、少なくとも１種の分析物と少なくとも１の触媒との間の触媒－分析物複合体を配置し、
基質を試料ドメインに配置し、ここに該基質は該触媒による触媒作用を介して生成物に変換し得、
該基質と個々の分析物の触媒－分析物複合体とを接触させて検出可能な量の生成物を生成させ、
試料ドメイン中の個々の分析物に対して生成物の像を記録し、
少なくとも１種の分析物の少なくとも１の質的パラメータまたは少なくとも１の量的パラメータに対して像を関連付ける
工程を含む試料中の少なくとも１種の分析物の少なくとも１の質的パラメータまたは少なくとも１の量的パラメータを調べる方法。
【請求項２】
触媒－分析物複合体が種－選択的な結合を含む請求項１記載の方法。
【請求項３】
種－選択的な結合が抗原－抗体結合を含む請求項２記載の方法。
【請求項４】
種－選択的な結合がＤＮＡまたはＲＮＡ、またはＰＮＡまたはＬＮＡハイブリダイゼーションを含む請求項２記載の方法。
【請求項５】
触媒－分析物複合体の形成が触媒されたレポーターの沈澱を含む請求項１記載の方法。
【請求項６】
分析物が生物粒子のごとき粒子である請求項１記載の方法。
【請求項７】
分析物が固体支持体に結合し、好ましくは該固体支持体が懸濁液中のビーズである請求項１記載の方法。
【請求項８】
分析物が細胞、細胞壁、細菌、変形体（ｐｌａｓｍｏｄｉａ）、ウイルス、プリオン、巨大分子、タンパク質、ポリペプチド、ペプチド、遺伝子、ＤＮＡ、ＲＮＡ、またはそれらのフラグメントもしくはクラスターよりなる群から選択される請求項１記載の方法。
【請求項９】
少なくとも１種の分析物が疾患の医学マーカーである請求項１記載の方法。
【請求項１０】
マーカーが心臓の梗塞（ｃａｒｄｉａｌ ｉｎｆａｒｃｔ）に対するマーカーである請求項９記載の方法。
【請求項１１】
細胞が哺乳動物細胞、昆虫細胞、爬虫類細胞、魚類細胞、酵母細胞および真菌類細胞から選択される請求項８記載の方法。
【請求項１２】
細胞が血液細胞、精子細胞および骨髄細胞から選択される請求項８記載の方法。
【請求項１３】
試料が液体試料である請求項１ないし１２いずれか１項記載の方法。
【請求項１４】
試料が、ミルク、ミルク製品、尿、血液、精子、鼻分泌物、涙、糞便、廃水、プロセス用水、飲料水、髄液、胆汁もしくはえい瘤（ｇａｌｌ）、骨髄、食品、飼料、ならびにそれらの混合物、希釈物または抽出物よりなる群から選択される請求項１ないし１３いずれか１項記載の方法。

【請求項１５】
試料が、試料ドメインに配置する前に前－処理した固体試料である請求項１ないし１２いずれか１項記載の方法。
【請求項１６】
試料が筋肉、脳、腎臓、肝臓または脾臓のバイオプシーである請求項１５記載の方法。
【請求項１７】
基質が、ＡｔｔｏＰｈｏｓ、４－ＭＵＰ、ＨＮＰＰ、４－ＭＵＧ、ＣＤＰ－Ｓｔａｒ、ＣＳＰＤ、Ｓｕｐｅｒ Ｓｉｇｎａｌ Ｓｕｂｓｔｒａｔｅ（Ｐｉｅｒｃｅ，Ｒｏｃｋｆｏｒｄ，ＩＩＩ）、ルミノール／４－ヨードフェノール、Ｇａｌａｃｔｏｎ Ｐｌｕｓ、ＤＡＢ、ＯＰＤ、ＡＥＣ、５ＡＳ、２，２’－アジノ－ビス（３－エチルベンズチアゾリン－６－スルホン酸）、４Ｃ１Ｎ、ｏ－ジアニシジン、ＴＭＢ、ＡＢＴＳ、ＢＣＩＰ、Ｎａｐｈｔｈｏｌ ＡＳ－ＴＲ ｐｈｏｓｐｈａｔ、ｐＮＰＰ、ＰＭＰ、Ｘ－ＧａｌまたはＣＰＲＧである請求項１ないし１６いずれか１項記載の方法。
【請求項１８】
触媒が無機触媒である請求項１ないし１７いずれか１項記載の方法。
【請求項１９】
触媒が有機触媒である請求項１ないし１７いずれか１項記載の方法。
【請求項２０】
触媒が酵素である請求項１９記載の方法。
【請求項２１】
触媒が、アルカリホスファターゼのごときホスファターゼ、β－ガラクトシダーゼ、西洋ワサビペルオキシダーゼのごときペルオキシダーゼ、β－グルクロニダーゼ、β－グルコース－６－リン酸デヒドロゲナーゼ、グルコースオキシダーゼ、ウレアーゼ、ルシフェラーゼ、β－ラクタマーゼおよびβ－アミラーゼよりなる群から選択される請求項２０記載の方法。
【請求項２２】
少なくとも１の得られた生成物が形成の際に沈澱する請求項１ないし２１いずれか１項記載の方法。
【請求項２３】
少なくとも１の得られた生成物が試料コンパートメントの表面に沈澱する請求項２２記載の方法。
【請求項２４】
少なくとも１の得られた生成物が発色する請求項１ないし２３いずれか１項記載の方法。
【請求項２５】
少なくとも１の得られた生成物がフォトルミネセンスまたは化学ルミネセンス発光性である請求項１ないし２４いずれか１項記載の方法。
【請求項２６】
少なくとも１の得られた生成物が蛍光性である請求項１ないし２５いずれか１項記載の方法。
【請求項２７】
少なくとも１の得られた生成物が、２５０ｎｍないし６００ｎｍの範囲の電磁放射線に照射した場合に３００ｎｍないし１２００ｎｍの範囲の電磁放射線を発する請求項１ないし２６いずれか１項記載の方法。
【請求項２８】
少なくとも１の得られた生成物を、像を記録する前に励起光によって励起する請求項１ないし２７いずれか１項記載の方法。
【請求項２９】
励起光の光源が、発光ダイオード（ＬＥＤ）、気体レーザー、固体レーザー、レーザーダイオード、ガス灯、ハロゲン灯、またはキセノン灯よりなる群から選択される請求項２８記載の方法。

【請求項３０】
試料ドメインが三次元である請求項１ないし２９いずれか１項記載の方法。
【請求項３１】
試料ドメインがフロースルー・チャンバーである請求項１ないし３０いずれか１項記載の方法。
【請求項３２】
試料ドメインがディスポーザブル・カセットの一部分である請求項１ないし３１いずれか１項記載の方法。
【請求項３３】
試料ドメインの少なくとも１の壁部が透明である請求項１ないし３２いずれか１項記載の方法。
【請求項３４】
触媒と分析物との間の少なくとも１の結合が２またはそれを超える抗体を含む請求項１ないし３３いずれか１項記載の方法。
【請求項３５】
少なくとも１の触媒を、分析物の種上の抗原に免疫学的に結合する抗体にコンジュゲートする請求項１ないし３４いずれか１項記載の方法。
【請求項３６】
少なくとも１の触媒を、分析物の種上の抗原に免疫学的に結合する第２の抗体に免疫学的に結合する第１の抗体にコンジュゲートする請求項１ないし３５いずれか１項記載の方法。
【請求項３７】
少なくとも１の触媒をアビジンにコンジュゲートする請求項１ないし３６いずれか１項記載の方法。
【請求項３８】
少なくとも１の触媒をストレプトアビジンにコンジュゲートする請求項１ないし３７いずれか１項記載の方法。
【請求項３９】
少なくとも１の結合が、アビジンにコンジュゲートされた触媒、ビオチンにコンジュゲートされ、かつ、分析物の種上の抗原に免疫学的に結合する抗体、あるいはその反対のものを含む請求項１ないし３８いずれか１項記載の方法。
【請求項４０】
少なくとも１の結合が、アビジンにコンジュゲートされた触媒、ビオチンにコンジュゲートされた第１の抗体、および分析物の種上の抗原に免疫学的に結合している第２の抗体を含む請求項１ないし３９いずれか１項記載の方法。
【請求項４１】
結合が、試料を試料ドメインに移す前に形成される請求項１ないし４０いずれか１項記載の方法。
【請求項４２】
分析物がＤＮＡを含有する分析物であって、ＤＮＡまたはＤＮＡの画分をＤＮＡ染色化合物で染色する請求項１ないし４１いずれか１項記載の方法。
【請求項４３】
さらなる結合が、第２の分析物の種と第２の触媒との間に形成される請求項１ないし４２いずれか１項記載の方法。
【請求項４４】
２またはそれを超えるさらなる結合が、第２、第３および所望によりそれにつづく分析物の種と第２、第３および所望により第３の触媒との間に形成される請求項１ないし４３いずれか１項記載の方法。
【請求項４５】
さらに、分析物の種に結合していない過剰な触媒を除去する工程を含む請求項１ないし４

４いずれか１項記載の方法。
【請求項４６】
遠心を介して過剰な触媒を除去する請求項４５記載の方法。
【請求項４７】
濾過を介して過剰な触媒を除去する請求項４５記載の方法。
【請求項４８】
フラッシング（ｆｌｕｓｈｉｎｇ）を介して過剰な触媒を除去する請求項４５記載の方法。
【請求項４９】
過剰な触媒の除去が、分析物－触媒複合体を磁性ビーズに結合させることを含む請求項４５記載の方法。
【請求項５０】
さらに、補因子と触媒－分析物複合体とを接触させることを含む請求項１ないし４９いずれか１項記載の方法。
【請求項５１】
さらに、緩衝液と触媒－分析物複合体とを接触させることを含む請求項１ないし５０いずれか１項記載の方法。
【請求項５２】
少なくとも１の基質を試料ドメイン中の触媒－分析物複合体に添加する請求項１ないし５１いずれか１項記載の方法。
【請求項５３】
少なくとも１の基質を、触媒－分析物複合体を試料ドメインに移す前にそれに添加する請求項１ないし５２いずれか１項記載の方法。
【請求項５４】
触媒によって触媒される反応の開始を、温度変化によって制御する請求項１ないし５３いずれか１項記載の方法。
【請求項５５】
前基質を、触媒－分析物複合体を試料ドメインに移す前にそれに添加する請求項１ないし５４いずれか１項記載の方法。
【請求項５６】
基質への前基質の変換を外部的に制御し得る請求項５５記載の方法。
【請求項５７】
変換を照明によって制御する請求項５６記載の方法。
【請求項５８】
変換を温度変化によって制御する請求項５６記載の方法。
【請求項５９】
触媒によって触媒される反応を外部的に制御可能に終止し得る請求項１ないし５８いずれか１項記載の方法。
【請求項６０】
生成物を生成する工程を液体環境中で行う請求項１ないし５９いずれか１項記載の方法。
【請求項６１】
生成物を生成する工程を粘性環境中で行う請求項１ないし５９いずれか１項記載の方法。
【請求項６２】
生成物を生成する工程を半固体環境で行い、好ましくは半固体環境がゲルである請求項１ないし５９いずれか１項記載の方法。
【請求項６３】
半固体環境を、分析物を試料コンパートメントに導入した後に形成し、好ましくは半固体環境の形成を温度、光および振盪のごとき外部要因によって制御する請求項６２記載の方法。
【請求項６４】

生成物を生成する工程の時間が６０分未満である請求項１ないし６３いずれか１項記載の方法。
【請求項６５】
生成物を生成する工程の時間が１５分未満、好ましくは５分未満、より好ましくは１分未満、より好ましくは３０秒未満、より好ましくは１５秒未満、より好ましくは１０秒未満、より好ましくは５秒未満、より好ましくは２秒未満である請求項６４記載の方法。
【請求項６６】
像の記録が共焦点スキャナーの使用を含む請求項１ないし６５いずれか１項記載の方法。
【請求項６７】
生成物の像を検出デバイスのアレイを使用して記録する請求項１ないし６６いずれか１項記載の方法。
【請求項６８】
生成物の像を検出デバイスの一次元アレイを使用して記録する請求項６７記載の方法。
【請求項６９】
生成物の像を検出デバイスの二次元アレイを使用して記録する請求項６７記載の方法。
【請求項７０】
生成物の像をＣＣＤ、ＣＭＯＳ、ビデオカメラまたはフォトンカウンティングカメラを使用して記録する請求項６７記載の方法。
【請求項７１】
像を拡大することなく記録する請求項１ないし７０いずれか１項記載の方法。
【請求項７２】
像を２０未満、好ましくは１０未満、より好ましくは４のごとき５未満、より好ましくは２のごとき４未満、より好ましくは１のごとき２未満の拡大率で記録する請求項１ないし７１いずれか１項記載の方法。
【請求項７３】
像を１未満、好ましくは０．８のごとき０．９未満、より好ましくは０．６のごとき０．８未満、より好ましくは０．５のごとき０．６未満の拡大率で記録する請求項１ないし７２いずれか１項記載の方法。
【請求項７４】
像を１の照射で記録する請求項１ないし７３いずれか１項記載の方法。
【請求項７５】
像を２、３またはそれを超える照射で記録する請求項１ないし７３いずれか１項記載の方法。
【請求項７６】
少なくとも１の質的パラメータまたは少なくとも１の量的パラメータの調査を、１を超える像を少なくとも１の質的パラメータまたは少なくとも１の量的パラメータに関連付けることによって、好ましくは２の像を関連付けることによって、より好ましくは２を超える像を関連付けることによって、より好ましくは４を超える像を関連付けることによって行う請求項７５記載の方法。
【請求項７７】
経時的な像の変化に関する情報を、少なくとも１の質的パラメータまたは少なくとも１の量的パラメータの調査に用いる請求項７６記載の方法。
【請求項７８】
記録した像をプロセシングする請求項１ないし７７いずれか１項記載の方法。
【請求項７９】
記録した像をデータプロセシング手段を用いてプロセシングする請求項７８記載の方法。
【請求項８０】
データプロセシング手段が生成物の部分的に重複する領域を識別する請求項７９記載の方法。
【請求項８１】

関連付けが、像上のスポットの数の測定を含む請求項１ないし８０いずれか１項記載の方法。
【請求項８２】
関連付けが、像のスポットのサイズの測定を含む請求項１ないし８１いずれか１項記載の方法。
【請求項８３】
関連付けが、生成物の少なくとも２のスペクトル特性の間の識別を含む請求項１ないし８２いずれか１項記載の方法。
【請求項８４】
さらに、少なくとも１のさらなる質的パラメータまたは少なくとも１のさらなる量的パラメータの調査を含む請求項１ないし８３いずれか１項記載の方法。
【請求項８５】
少なくとも１のさらなる質的パラメータまたは少なくとも１のさらなる量的パラメータの調査が、分析物の種の蛍光、化学ルミネセンス、フォトルミネセンス、自律ルミネセンスの検出を含む請求項８４記載の方法。
【請求項８６】
少なくとも１の質的パラメータが、分析物の生存能力、サイズ、同一性、呼吸および存在よりなる群から選択される請求項１ないし８５いずれか１項記載の方法。
【請求項８７】
少なくとも１の量的パラメータが、試料の体積中の分析物の種の数、試料の体積中の分析物の種の濃度、試料の体積中の分析物の種の量よりなる群から選択される請求項１ないし８６いずれか１項記載の方法。
【請求項８８】
さらに、像の記録が、少なくとも第１の検出器を含む第１の検出手段に対する試料の第１の表面からの蛍光シグナルを検出する焦点合わせ手段を使用することによって、少なくとも第１の光源を有する第１の光手段からの励起光で試料の第１の表面を直接的に照射することを含む請求項１ないし８７いずれか１項記載の方法。
【請求項８９】
少なくとも第１の光手段が試料面に平行な第１の光面に位置し、該第１の光面が試料面と第１の検出手段との間に存在する請求項８８記載の方法。
【請求項９０】
励起光フィルターを少なくとも１の光源からの励起光路中に挿入する請求項８８または８９記載の方法。
【請求項９１】
励起光を支持材料上の光源として配置する請求項８９記載の方法。
【請求項９２】
実質的に同一のフィルターをすべての光源に使用する請求項８８ないし９１いずれか１項記載の方法。
【請求項９３】
第１の光源を第１のフィルターを通して濾過し、第２の光源を第２のフィルターを通して濾過し、該第１のフィルターと該第２のフィルターとが異なる請求項８８ないし９２いずれか１項記載の方法。
【請求項９４】
さらに、少なくとも１の光源を有する第２の光手段からの励起光で、試料の第２の表面を直接的に照射することを含む請求項８８ないし９３いずれか１項記載の方法。
【請求項９５】
第２の励起光手段を第２の光面に位置させ、該面が試料面と平行であって第１の光面よりも試料面の他の側に位置して、試料を２の向い合う表面上に暴露させる請求項９４記載の方法。
【請求項９６】

第２の光手段からの光路に挿入したフィルターが、第１の光手段の光路に挿入したフィルターと異なる請求項９４または９５記載の方法。
【請求項９７】
第２の検出手段を、試料コンパートメントが第１の検出手段と第２の検出手段との間に位置するように配置する請求項８８ないし９６いずれか１項記載の方法。
【請求項９８】
第１の検出手段が第２の検出手段と同一である請求項９７記載の方法。
【請求項９９】
発光フィルターを、少なくとも第１の検出器に対する発光路に挿入する請求項８８ないし９８いずれか１項記載の方法。
【請求項１００】
コリメーターレンズを発光路に配置する請求項８８ないし８９いずれか１項記載の方法。
【請求項１０１】
励起主光と検出－試料軸との間の角度が３５°ないし９０°、好ましくは４５°ないし８５°、より好ましくは５０°ないし８５°の範囲に存在する請求項８８ないし１００いずれか１項記載の方法。
【請求項１０２】
少なくとも第１の光手段を試料面に平行な第１の光面に位置させ、該第１の光面を検出器の背部の試料面から一定の距離に位置させる請求項８８記載の方法。
【請求項１０３】
検出器を、発せられたシグナルを検出器（群）に到達させる開口部を有するハウジング中に位置させる請求項１０２記載の方法。
【請求項１０４】
照射ドメインを含む試料ドメイン、分析物物質を表す一定体積の液体試料を導入することができる流入口、および一定体積の液体試料の少なくとも一部をデバイス内に流動させる少なくともチャネルを含む流動システムを含むデバイス、ここに、該デバイスは、さらに試料ドメイン中の触媒－分析物複合体の周囲の液体の流動を制御するための手段を含み；および
少なくとも空間像データを定量的に検出するための第１の検出器および検出した像の表示をプロセシングするためのプロセッサーを含む検出デバイス
を含む液体試料中の分析物の少なくとも１のパラメータを調べるためのシステムであって、
ここに該デバイスおよび該検出デバイスが、該デバイスの照射ドメイン中の試料からの電磁シグナルを検出デバイスを通過させて、検出デバイス中で照射ドメインの空間像表示を形成するように、該検出デバイスに対して該デバイスを配置するための手段を有する該システム。
【請求項１０５】
さらに、流動システムが、コンパートメントまたは流動チャネル部の中でまたはそこから、最初にコンパートメントまたは流動チャネル部に負荷された１またはそれを超える反応成分の少なくとも一部を、試料を表す一定体積の液体の少なくとも一部に添加するコンパートメントまたは流動チャネル部を含む請求項１０４記載のシステム。
【請求項１０６】
さらに、少なくとも第１の光源を含む請求項１０４記載のシステム。
【請求項１０７】
第１の光源が励起光源を含む請求項１０６記載のシステム。
【請求項１０８】
第１の光源および検出器が、照射ドメインの同じ側に位置する請求項１０７記載のシステム。
【請求項１０９】
第１の光源および第１の検出手段から反対の側に位置する第２の光源および第２の検出器

を含む請求項１０８記載のシステム。
【請求項１１０】
さらに、励起光路に挿入された励起光フィルターを含む請求項１０６または１０８記載のシステム。
【請求項１１１】
励起光フィルターが実質的に環のごとき円形に形成された請求項１１０記載のシステム。
【請求項１１２】
個体における症状を診断するための請求項１０４ないし１１１いずれか１項記載のシステムの使用。
【請求項１１３】
個体がヒトである請求項１１２記載の使用。
【請求項１１４】
個体がウシ、ブタ、ウマ、家禽類、ヒツジおよびヤギのごときヒト以外の動物である請求項１１２記載の使用。
【請求項１１５】
症状が心臓の梗塞（ｃａｒｄｉａｌ　ｉｎｆａｒｃｔ）または心臓の梗塞に罹る危険性である請求項１１２記載の使用。
【発明の詳細な説明】
【０００１】
本発明は、少なくとも１の分析物の少なくとも１の質的パラメータおよび／または少なくとも１の量的パラメータを調べる方法に関し、ここに該少なくとも１の分析物は基質を生成物に触媒することができる触媒に結合し、それによって該分析物を分析物の周りに生成した生成物の検出を介して調べる。
【０００２】
発明の背景
検出を支援するために物質または粒子の染色を用いた物質または粒子の検出が広く使用されている。しかしながら、多くの物質および粒子は小さすぎて、染色することはできるが、非常に高い拡大を用いずにはそれらを検出することが困難であり、使用する器具の要件を増している。
【０００３】
古典的な増幅技術は酵素結合アッセイのものである。分析物と特異的に反応するリガンドを酵素に結合し、過剰なリガンド－酵素複合体を除去した後に、分析物－リガンド複合体を、酵素によって発色生成物が発色生成物が形成する際に作用される無色の物質である発色性基質と反応させることによって分析物－リガンド－酵素複合体を検出する。その大きな増幅率のため、酵素結合アッセイは高い感度を供し、少量の抗原を検出するのに特に有用である。
【０００４】
伝統的な酵素結合アッセイ（ＥＬＩＳＡ）においては、増幅が精度を犠牲にして感度を上昇させている。これは増幅率が正確でないからである。多数の分子の弱い反応から数個の分子の強力な反応を区別することは不可能である。
【０００５】
不正確な検出に関連する幾つかの問題点を克服する試行は、ＥＬＩＳＰＯＴまたはイン・サイチュＥＬＩＳＡまたはｓｐｏｔ－ＥＬＩＳＡを使用することであり、それは名称とは独立して、固体支持体上の局在する発色スポットを検出するためにＥＬＩＳＡ技術を使用することに関係する。
【０００６】
ＥＬＩ－ＳＰＯＴ分析により、ウイルスまたはポリペプチド産生細胞または抗原提示細胞のごとき粒子の検出および計数が可能である。生成したスポットは、顕微鏡を使用することによって、またはビデオ－イメージングを使用することによって計数し得る。ＥＬＩ－ＳＰＯＴの原理は、例えばＳｅｄｇｗｉｃｋ，　Ｊ．　Ｄ．　およびＨｏｌｔ，　Ｐ．　Ｇ．

”Ａ Ｓｏｌｉｄ－Ｐｈａｓｅ Ｉｍｍｕｎｏｅｎｚｙｍａｔｉｃ Ｔｅｃｈｎｉｑｕｅ ｆｏｒ ｔｈｅ Ｅｎｕｍｅｒａｔｉｏｎ ｏｆ Ｓｐｅｃｉｆｉｃ Ａｎｔｉｂｏｄｙ－Ｓｅｃｒｅｔｉｎｇ Ｃｅｌｌｓ”, Ｊｏｕｒｎａｌ ｏｆ Ｉｍｍｕｎｏｌｏｇｉｃａｌ Ｍｅｔｈｏｄｓ, ５７ （１９８３） ３０１－３０９に記載されており、そこでは、検出すべき分泌抗体を固定した固体支持体に結合した抗原によって捕捉している。分泌抗体は、抗体－酵素複合体を添加することによって検出し、該抗体は分泌抗体に対して特異性を有する。基質は加温したアガロースゲルに添加し、それを放置して固化させ、不溶性生成物のスポットを顕微鏡を用いて固化したアガロースゲル上で検出し得る。

【０００７】

発明の概要

本発明は、

少なくとも１の壁部を有する試料ドメインを確立し、

試料ドメインの壁部（群）に対して分析物が移動し得るように、少なくとも１種の分析物と少なくとも１の触媒との間の触媒－分析物複合体を試料ドメインに配置し、

基質を試料ドメインに配置し、ここに該基質は該触媒による触媒作用を介して生成物に変換し得、

該基質と個々の分析物の触媒－分析物複合体とを接触させて検出可能な量の生成物を生成させ、

試料ドメイン中の個々の分析物に関係する生成物の像を記録し、

少なくとも１種の分析物の少なくとも１の質的パラメータまたは少なくとも１の量的パラメータに対して像を関連付ける

工程を含む試料中の少なくとも１種の分析物の少なくとも１の質的パラメータまたは少なくとも１の量的パラメータを調べる方法に関する。

【０００８】

生成物の検出可能な量は、検出デバイスによって検出可能なスポットに通じる各分析物または分析物群の周囲の生成物の実質的に球形の量の形成として理解される。生成物から生成したスポットは１または幾つかの分析物に関係し、分析物に関するパラメータの調査を行うことができる。生成物の三次元形成はバックグラウンドに対する分析物のより明確な同定につながる。かかるスポットの像は、多くの具体例において、多くの他の適用に遭遇する粒子の像と同様である。したがって、実質的に同一の技術および方法を、粒子に対して使用されるであろうスポットの像を記録し、処理し、および分析するのに使用し得ることは理解される。

【０００９】

伝統的なＥＬＩ－ＳＰＯＴ技術とは反対に、検出すべき分析物は、基質と分析物－触媒－複合体との間の接触の間に、試料ドメインに固定した固体支持体にカップリングせず、試料ドメイン中の何処かに位置させ得る。分析物は、少なくとも基質が試料ドメインに導入されるまで、試料ドメインの壁部（群）に対して移動することができる。それによって、分析物－触媒－複合体と基質との間の接触がより容易に行われる。

【００１０】

分析物と触媒との間の複合体は、幾つかの方法のうちの１またはそれを超える方法で形成され得る。これらの方法には、例えば：

結合を介することが含まれ、ここに結合なる語は分析物と触媒とが互いに結合することを意味する。

【００１１】

例えば、分析物－触媒複合体は、免疫学的結合、すなわち抗体とその抗原との間で形成される結合のごとき種－選択的結合を介して形成し得る。

【００１２】

また、結合は、コンジュゲーション、すなわち、２の化合物間、例えば酵素と抗体との間、酵素とアビジンとの間、抗体とビオチンとの間の共有結合を介してもよい。

【００１３】

もう１の具体例において、分析物と触媒との間の複合体は、細胞からの酵素の発現のごとき、分析物の中またはそれに近接する触媒の生成を介して形成される。酵素は、発現後は細胞に近接して位置し、分析物－触媒複合体が形成される。

【００１４】

試料中の少なくとも１種の分析物の少なくとも１の質的パラメータまたは少なくとも１の量的パラメータに対する像の関連付けには、好ましくは、像上のスポットの数の測定および／または像上のスポットのサイズの測定が含まれる。

【００１５】

第二の具体例において、本発明は、

照射ドメインを含む試料ドメイン、分析物物質を表す一定体積の液体試料を導入することができる流入口、および一定体積の液体試料の少なくとも一部をデバイス内に流動させる少なくともチャネルを含む流動システムを含むデバイス、ここに、該デバイスは、さらに、試料ドメイン中の触媒－分析物複合体の周囲の液体の流動を制御するための手段を含み；および

少なくとも、空間像データを定量的に検出するための第１の検出器および検出した像の像を処理するためのプロセッサーを含む検出デバイスを含む液体試料中の分析物の少なくとも１のパラメータを調べるためのシステムであって、

ここに該デバイスおよび該検出デバイスは、該デバイスの照射ドメイン中の試料からの電磁シグナルを検出デバイスを通過させて、検出デバイス中で照射ドメインの空間像の表示を形成するように、検出デバイスに対してデバイスを配置させるための手段を有する該システムに関する。

【００１６】

詳細には、流動システムは、さらに、その中でまたはそれから、コンパートメントまたは流動チャネル部に最初に負荷した１またはそれを超える反応成分の少なくとも一部分を、試料を表す一定体積の液体の少なくとも一部分に添加するコンパートメントまたは流動チャネル部を含む。

【００１７】

さらに、システムは、少なくとも、励起光を都合よく発する第１の光源を含み得る。試料から発せられた蛍光を検出するために適合した検出器は、光源と同じ照射ドメインの側に都合よく位置する。

【００１８】

第三の具体例において、本発明は、心筋梗塞（ｃａｄｉａｌ　ｉｎｆａｒｃｔ）の診断のごとき個体における症状を診断するためのシステムの使用に関する。

【００１９】

図１は、片側型励起システムを示す。

図２は、試料面に平行な面の励起光フィルターの断面図を示す。

図３は、集光角Ｃおよび励起主光路と検出－試料軸との間の角度Ｅを示す。

図４は、両側型励起／検出システムを示す。

図５は、両側型励起システムを示す。

図６は、両側型検出システムを示す。

【００２０】

発明の詳細な説明

シグナルの増幅

分析物は、分析物の周囲に形成される生成物の量によって検出可能である。試料ドメイン中の物理学的束縛に依存して、生成物は分析物から拡散して分析物または分析物のクラスターの周囲に局在する実質的に球形のスポットを形成するであろう。本発明のもう１の具体例において、生成物は試料コンパートメントに供される条件下で実質的に不溶性であり、したがって、生成物は沈澱またはコロイド体を形成するであろう。スポットは、一般的には拡散を介して媒体中の生成物の運搬によって経時的に大きくなるであろう。したがって、強度およびサイズの両方に関して、経時的に成長するスポットの形成をモニターする

ことが可能である。

【００２１】

パラメータ

本発明による量的パラメータは、試料中に存在する分析物の数および／または試料中の分析物の濃度であり、一方、質的パラメータは、生存能力、アポトーシスを含む死亡および／または死亡しつつある生物、サイズ、同一性、呼吸、存在および形態に関する情報である。

【００２２】

動力学

生成物の形成速度およびしたがって記録された像における変化の速度は、試料コンパートメント中の媒体の化学的および／または物理学的性質に依存する。流動が制限される状況では、このことは、分析部位に向けての基質の拡散速度および／または分析部位から離れる生成物の拡散速度によって決まる場合があるであろう。

【００２３】

検出可能な量の生成物が生成した後であって種々のスポットが融合する前に、生成物のスポットの像記録を記録しなければならず、それによって像の記録が阻害される。当該像は、分析物の少なくとも１の質的パラメータまたは少なくとも１の量的パラメータと関連付け得る。

【００２４】

分析物－触媒－複合体を基質と接触させてからスポットを記録するまでの時間は、主として、基質の拡散速度および生成物の拡散速度ならびにプロセスの動力学に依存する。拡散速度およびプロセスの動力学は、以下に論じるようにして制御し得る。

【００２５】

検出可能なスポットの発生は、好ましくは、分析物－触媒－複合体と基質とを接触させてから６０分以内に観察する。より好ましい具体例においては、生成物を生成する工程が１５分未満、好ましくは５分未満、より好ましくは１分未満、より好ましくは３０秒未満、より好ましくは１５秒未満、より好ましくは１０秒未満、より好ましくは５秒未満、より好ましくは２秒未満である。

【００２６】

好ましい具体例において、試料ドメインは検出可能なスポットのいずれかの発生の前に検出手段に暴露し、発生したスポットを得るのに十分な時間に達した場合に少なくとも１の他の暴露を行う。

【００２７】

試料ドメイン中の環境の粘度を変化させることによって、生成物を生成する時間を制御することも可能である。生成物を生成する工程は、液体環境または粘性環境において行い得る。後者の場合においては、粘度を使用する基質および触媒に調節し得る。また、生成物を生成する工程は、半固体環境中でも行い、好ましくは半固体環境はゲルである。半固体環境は、好ましくは、分析物を試料コンパートメントに導入した後に形成し、好ましくは、半固体環境の形成は温度、光および攪拌のごとき外部因子によって制御する。

【００２８】

分析物

分析物は、分析物－触媒複合体を形成することができるいずれの分析物であってもよい。前記に論じたごとく、分析物は、抗原－抗体結合のごとき種－特異的結合を介して触媒によって結合され得る。

【００２９】

分析物は、好ましくは生物粒子のごとき粒子である。詳細には、生物粒子は、細胞、細胞壁、細菌、変形体（ｐｌａｓｍｏｄｉａ）、ウイルス、プリオン、巨大分子、タンパク質、ポリペプチド、ペプチド、遺伝子、ＤＮＡ、ＲＮＡ、またはそれらのフラグメントもしくはクラスターよりなる群から選択される。

【００３０】

細胞は、好ましくは、哺乳動物細胞、昆虫細胞、爬虫類細胞、魚類細胞、酵母細胞、および真菌類細胞、より好ましくは血液細胞、精子細胞および骨髄細胞から選択する。

【００３１】

分析物は、ビーズのごとき固体支持体にカップリングし得、該ビーズを試料ドメインに懸濁させることができる。ビーズはポリマービーズとし得る。ポリマービーズが、常磁性ビーズのごとく、分析物を取り扱うのを補助し得る物理学的および／化学的特性を有する場合もあり得る。

【００３２】

ウイルスまたは他の小さい分析物の調査を含む本発明の１の具体例は、ビーズに分析物を結合させることに基づく。このことにより、遠心分離、濾過または磁性分離を用いるような前処理の間に、分析物および／または分析物－触媒複合体をより単純な方法で処理することが可能となる。

【００３３】

ビーズは、それ自体を標識して、１またはそれを超える分析物の同定における精度を改善し得る。本発明の１の具体例は、２またはそれを超える異なる分析物を結合するアフィニティーを有し得る２またはそれを超える型のビーズを用いる。分析物が含まれるかにかかわらず、分析物－触媒複合体が同一の生成物を生成する場合には、ポリマービーズの同定を用いて、どの分析物－触媒複合体がシグナルを検出する生成物を生成するかを区別し得る。ポリマービーズのかかる標識は、発色、蛍光、サイズまたはいずれか他の物理学的または化学的特性に基づき得る。

【００３４】

また、分析物は、ＤＮＡまたはＲＮＡを含有する分析物とし得、それによってＤＮＡ／ＲＮＡまたはそれらのフラクションをＤＮＡ染色化合物を用いて染色し得る。生存能力の調査のごとき分析物特異性の分析物特性をさらに確認または調べる場合には、上記のことが好ましい場合がある。

【００３５】

酵素増幅（Ｅｎｚｙｍｅ Ａｍｐｌｉｆｉｃａｔｉｏｎ Ｓｙｓｔｅｍｓ，ＥＡＳ）に基づく方法とは別に、本発明の多くの好ましい具体例には他のシステムも含まれる。かかる特異的増幅システムの中には以下のものが含まれる：

ＰＡＰ：ペルオキシダーゼ・抗－ペルオキシダーゼ複合体

ＡＰＡＡＰ：アルカリホスファターゼ・抗－アルカリホスファターゼ複合体

ＢＧＡＢＧ：β－ガラクトシダーゼ・抗－β－ガラクトシダーゼ複合体

特にＮＡＤＨの形成がＮＡＤＰＨおよびアルカリホスファターゼを含む場合のＮＡＤへのＮＡＤＨのサイクリック変換。

【００３６】

試料

試料は、ミルク、ミルク製品、尿、血液、精子、鼻分泌物、涙、糞便、廃水、プロセス用水、飲料水、髄液、胆汁もしくはえい瘤（ｇａｌｌ）、骨髄、食品、飼料、ならびにそれらの混合物、希釈物または抽出物よりなる群から選択される試料のごとき液体試料とし得る。

【００３７】

試料は、飲料水の制御、廃水または水精製工場もしくはスイミングプールからの水の制御のごとき、水中の粒子の調査に関し得る。すべての適用において、該制御は、細菌計数のごとき全粒子の計数に関し得、より詳細には、それは病原性細菌のごとき特定の細菌に対するモニター方法に関し得る。

【００３８】

さらに、醗酵制御、すなわち醗酵槽における細胞増殖および生存可能な細胞の制御も本発明によって行い得る。これは、ペプチドまたはタンパク質組成物を製造する医薬産業のごとき、醗酵を用いるすべての技術分野および産業分野に関連する。

【００３９】

液体試料は、遠心分離、沈降分離、濾過、抽出、希釈、放射、振盪、化学物質の添加、クロマトグラフィー分離のごときいずれかの好適な処理によって前処理し得る。

【００４０】

もう１の具体例において、試料は、試料ドメインに配置する前に前処理した固体試料である。前処理の一例は混合であり、所望によりそれにつづいて液体試料について言及したいずれの処理を行ってもよい。

【００４１】

試料は、筋肉、脳、腎臓、肝臓または脾臓のバイオプシーのような組織のバイオプシーのごときいずれの生物試料ともし得る。

【００４２】

また、試料は、細菌コンタミネーションのごときコンタミネーションについて試験すべき食品または飼料の試料ともし得る。本発明は、サルモネラ種（Ｓａｌｍｏｎｅｌｌａ ｓｐｅｃｉｅｓ）を検出する方法のごとき、食品または飼料中の細菌を検出および計数する非常に迅速な方法を提供する。

【００４３】

試料の形態とは独立して、分析物は、基質に接触させる前に媒体に懸濁することが必要である。該媒体は、分析物用の天然媒体または検出に好適ないずれかの液体とし得る。１の具体例において、分析物は前処理した後に媒体に懸濁する。媒体は適当な場合には触媒を含み得る。

【００４４】

触媒

触媒なる語は、検出手段によって検出可能な生成物に基質を変換することができるかまたは変換するのを支援することができるいずれの化合物をも意味する。したがって、触媒は、無機触媒ならびに有機触媒とし得る。１の具体例において、触媒は酵素であり、その場合には酵素なる語はその通常の意味で用いる。酵素を標識することについて使用する場合、単一酵素、酵素のオリゴマー形態、または酵素／抗－酵素複合体のいずれかを使用し得る。

【００４５】

酵素は、アルカリホスファターゼのごときホスファターゼ、β－ガラクトシダーゼ、例えば西洋ワサビペルオキシダーゼのごときペルオキシダーゼ、β－グルクロニダーゼ、β－グルコース－６－リン酸デヒドロゲナーゼ、グルコースオキシダーゼ、ウレアーゼ、ルシフェラーゼ、β－ラクタマーゼおよびβ－アミラーゼよりなる群から選択される酵素のごとき、ＥＬＩＳＡ技術に有用ないずれの酵素ともし得る。

【００４６】

１の回転で２、３またはそれを超える異なる型の分析物を検出する場合、異なる酵素はそれらが互いに干渉しないように選択しなければならない。例えば、酵素は、ほぼ同じｐＨを必要とする酵素を有利に選択する。有利には、基質は、本発明の２またはそれを超える酵素のうちの１によってのみ生成物に変換される基質を選択すべきである。同様に、基質は、好ましくは、基質もその生成物も試料コンパートメントに存在するいずれの酵素も阻害しないように選択し得る。

【００４７】

さらに、酵素は、増幅システムのごとき、アビジン－ビオチン・抗－ペルオキシダーゼ技術（ＡＢＡＰ）のごとき別の検出システムにカップリングし得る。標識はビオチンとすることもでき、それによって、酵素標識した標識アビジンまたはストレプトアビジンを用いてビオチン化抗体を検出する。好ましい具体例において、アビジンおよびストレプトアビジンは、前記に論じた酵素のうちの１で標識する。もう１の具体例において、標識はアビジンまたはストレプトアビジンであり、それによって、抗体は酵素標識したビオチンを用いて検出する。ビオチン／アビジンまたはストレプトアビジンで標識することによって、酵素で直接標識することと比較してシグナルを増強することが可能であり、より好ましくは酵素は西洋ワサビペルオキシダーゼである。

【００４８】
幾つかの具体例には、分析物がＤＮＡまたはＲＮＡ物質である場合やそれらを含む場合、ＬＮＡおよびＰＮＡを使用することが含まれる。
【００４９】
１の具体例において、触媒反応は、触媒反応の動力学が制御されるように、温度または照明もしくは光照射によって制御するごとく制御し得る。例えば、触媒反応は、触媒の局部温度を変化させ、または基質もしくは補因子を除去するための照明、または化合物を基質もしくは補因子に変換させるための照明のごとき、光に触媒を暴露することによって、開始する前に始らないことを制御し得る。もう１の具体例において、触媒反応は、ｐＨにおける変化が触媒反応を増大させ得るように、ｐＨによって制御し得る。また、好ましい具体例において、温度シフトなどを制御することによくごとく、外部的に触媒によって触媒する反応を終結させることが可能である。
【００５０】
種－特異的結合
種－選択的結合なる語は、種－特異的結合なる語、すなわちパラメータを調べるべき分析物に特異的である結合と同義に用いる。１の具体例において、種－選択的結合は、分析物の１のエピトープに指向された、モノクローナル抗体のごとき抗体を用いる抗原－抗体結合である。
【００５１】
モノクローナル抗体は、直接的または間接的に標識し得る。直接標識には、典型的に、酵素のごとき触媒およびビオチンが含まれる。間接標識には、モノクローナル抗体に対する抗体が含まれ、該抗体を酵素標識のごとき触媒標識またはビオチンで標識する。
【００５２】
間接標識においては、分析物のエピトープまたはヌクレオチド配列に対して指向した一次抗体またはヌクレオチド・プローブを、ビオチン、ストレプトアビジン、アビジン、ハプテン、ジゴキシゲニン、ジニトロフェニルまたはフルオレセインのごとき化合物に共有結合し得る。ついで、分析物を、一次抗体またはプローブに結合した化合物に特異的に結合する第二の標識によって視覚化し得る。かかる間接標識の例には、限定されるものではないが：ハプテン・抗－ハプテン複合体；ビオチン・ストレプトアビジン複合体；ビオチン・アビジン複合体；ジゴキシゲニン・抗－ジゴキシゲニン複合体；ジニトロフェニル・抗－ジニトロフェニル複合体；フルオレセイン・抗－フルオレセイン複合体が含まれる。
【００５３】
一次抗体またはプローブをビオチンに結合する場合、ビオチンにストレプトアビジンを結合させ、さらにビオチン－酵素－ビオチン複合体をストレプトアビジンに結合させることによってシグナル増幅を得ることができる。さらにストレプトアビジンおよびビオチン－酵素－ビオチン複合体を結合させることによって、さらに仕上がった増幅を得ることができる。その結果、わずか１の酵素の代わりに、幾つかの酵素が複合体リンケージを介してエピトープまたはヌクレオチド配列に結合する。
【００５４】
もう１の具体例において、種－選択的結合は、分析物に関連するＤＮＡおよび／またはＲＮＡ、および／またはＤＮＡおよび／またはＲＮＡに対して選択的なＰＮＡおよび／またはＬＮＡプローブを用いて提供し得る。プローブは、抗体の標識に関して前記したさらなるプローブを介して直接的または間接的に標識し得る。
【００５５】
本発明は、さらなる結合を第２の種の分析物と第２の触媒との間に形成する特徴をも含む。
【００５６】
結合は、分析物および触媒を試料ドメインに配置して分析物－触媒複合体を形成させることによって試料ドメイン中で形成される。もう１の具体例において、分析物－触媒複合体は、試料または分析物を試料ドメインに移す前に形成される。

【００５７】
分析物－触媒複合体が形成された後に、分析物の種に結合されなかった過剰な触媒は、好ましくは分析物－触媒複合体から除去する。過剰な触媒は、遠心分離、濾過および／またはフラッシング（ｆｌｕｓｈｉｎｇ）のごときいずれかの好適な手段を介して除去し得る。
【００５８】
過剰な触媒を除去する工程には、分析物－触媒複合体を磁性ビーズに結合させることも含まれ得る。
【００５９】
本発明の他の具体例においては、過剰な触媒は分析物－触媒複合体から実質的に除去しない。これは、シグナルが分析物の中、そこにおいてまたはその付近のみならず、分析物を取り囲む培地中にも生成するため、いずれかの像を記録するより複雑な条件を明らかに意味する。分析物の中、そこにおいてまたはその付近に生成した生成物を起源とするシグナルを検出し得る場合の例は、分析物－触媒複合体における触媒の濃度または効力が分析物を取り囲む培地中よりも大きい場合である。
【００６０】
触媒－分析物複合体は、さらに、複合体を基質と接触させる前に補因子または緩衝液と接触させることもできる。
【００６１】
分析物および触媒の間に形成される種－特異的結合の例の以下の非限定的なリストにおいて、アスタリスク＊は、抗体と抗原との間の結合またはアビジンとビオチンとの間の結合のごときアフィニティー結合を示す。ダッシュ－は共有結合を示す。
【００６２】
分析物＊抗体－触媒
分析物＊抗体＊抗体－触媒
分析物＊抗体－ビオチン＊アビジン－触媒
分析物＊抗体－ビオチン＊ストレプトアビジン－触媒
分析物＊抗体－アビジン＊ビオチン－触媒
分析物＊抗体－ストレプトアビジン＊ビオチン－触媒
分析物＊抗体＊抗体－アビジン＊ビオチン－触媒
分析物＊抗体＊抗体－ストレプトアビジン＊ビオチン－触媒
分析物＊抗体＊抗体－アビジン＊ビオチン－触媒
分析物＊抗体＊抗体－ストレプトアビジン＊ビオチン－触媒
分析物＊抗体－ビオチン＊ストレプトアビジン＊（ビオチン－触媒－ビオチン）ｎ＊ストレプトアビジンｎほか
分析物＊抗体－ジゴキシゲニン＊抗ジゴキシゲニン－触媒
分析物＊抗体－ジニトロフェニル＊抗ジニトロフェニル－触媒
分析物＊抗体－ハプテン＊抗ハプテン－触媒
分析物＊抗体－フルオロセイン＊抗フルオロセイン－触媒
【００６３】
分析物＊ＤＮＡフラグメント－触媒
分析物＊ＤＮＡフラグメント＊ＤＮＡフラグメント－触媒
分析物＊ＲＮＡフラグメント－触媒
分析物＊ＲＮＡフラグメント＊ＲＮＡフラグメント－触媒
分析物＊ＰＮＡフラグメント－触媒
分析物＊ＰＮＡフラグメント＊ＰＮＡフラグメント－触媒
分析物＊ＬＮＡフラグメント－触媒
分析物＊ＬＮＡフラグメント＊ＬＮＡフラグメント－触媒
分析物＊ＤＮＡフラグメント＊ＲＮＡフラグメント－触媒
分析物＊ＤＮＡフラグメント＊ＬＮＡフラグメント－触媒

分析物＊ＤＮＡフラグメント＊ＰＮＡフラグメント－触媒
分析物＊ＲＮＡフラグメント＊ＬＮＡフラグメント－触媒
分析物＊ＲＮＡフラグメント＊ＰＮＡフラグメント－触媒
分析物＊ＲＮＡフラグメント＊ＬＮＡフラグメント－触媒
分析物＊ヌクレオチドプローブ－ビオチン＊ストレプトアビジン＊（ビオチン－触媒－ビオチン）ｎ＊ストレプトアビジンｎほか
分析物＊ヌクレオチドプローブ－ジゴキシゲニン＊抗ジゴキシゲニン－触媒
分析物＊ヌクレオチドプローブ－ジニトロフェニル＊抗ジニトロフェニル－触媒
分析物＊ヌクレオチドプローブ－ハプテン＊抗ハプテン－触媒
分析物＊ヌクレオチドプローブ－フルオロセイン＊抗フルオロセイン－触媒

【００６４】
アビジン＊ビオチンまたはＲＮＡ＊ＰＮＡほかの順序を逆転させるごとき、アフィニティー結合に関与する２のコンポーネントの位置を逆転させることによって種－特異的結合を形成し得ることも同様に考えられる。

【００６５】
基質
基質は、典型的に、試料ドメインのいずれかの像を記録する前に分析物－触媒複合体に添加する。
基質は、試料ドメインに混合物を配置する前に分析物－触媒複合体と混合し得る。混合物が適所に存在する前に実質的ないずれかの触媒反応が起こることを避けるために、触媒に関して前記したごとく触媒反応を制御し得る。
もう１の具体例において、分析物－触媒複合体は、基質を添加する前に試料ドメインに配置し、その場合、基質および分析物－触媒の混合物が試料ドメインに形成する。

【００６６】
触媒されたレポーター沈澱
本発明による触媒－分析物複合体は、触媒されたレポーター沈澱の使用を介して増幅し得る。触媒されたレポーター沈澱とは、多くのレポーター分子の分析物に対する、共有結合のごとき沈澱を意味する。レポーター分子のこの沈澱により、一種の分析物に結合する触媒の数を増加することができ、それによって、単一種の分析物から発生するシグナルを増加させることができる。レポーター分子が、例えば種－特異的結合を介して、本発明による触媒が結合し得るいずれの分子をも包含することは理解される。

【００６７】
典型的には、レポーター分子は２の成分、分析物上の受容体に結合を形成し、アフィニティー結合ペアの１の部分に対して基質成分を共有結合するであろう基質を含む。

【００６８】
レポーターの以下の原理において、活性化沈澱を例としてチラミン－ビオチンを用いて説明する。チラミン－ビオチンを説明目的のみで使用し、触媒されたレポーター沈澱に使用し得るレポーターの他の例が当業者に利用可能であることは理解される。

【００６９】
この方法の第一の工程には、種－特異的結合を介して、例えば分析物の表面のエピトープに対して指向された一次抗体を介する結合を介して、ペルオキシダーゼを分析物に結合することが含まれる。

【００７０】
一次抗体を結合させた後に、チラミン－ビオチンおよびH_2O_2を添加する。ペルオキシダーゼによる酸化を介して、レポーター分子のチラミン成分が活性化され、ペルオキシダーゼの近距離範囲内の分析物の表面に、チロシンまたはトリプトファン残基のごとき電子に富む基に対する共有結合を形成する。最終的に、最初にペルオキシダーゼが結合した分析物に多数のビオチン分子が共有結合する。第二ラウンドの標識においては、アビジンまたはストレプトアビジンに結合したさらなる触媒を分析物に添加して、分析物に結合したビオチンの間に結合を形成する。したがって、最終的に、最初に分析物に結合したすべて

の一次抗体に対して、各分析物に多数の触媒が結合する。
【００７１】
レポーター分子の基質コンポーネントの多くの例は、出典明示して本明細書の一部とみなす米国特許第５，１９６，３０６号に開示されている。
【００７２】
クロマトグラフィー／ルミネセンス
基質は発色性基質または発光性基質、例えば蛍光発光性基質のごときフォトルミネセンス、自律ルミネセンス、化学ルミネセンス発光性の基質とし得る。
【００７３】
発色性基質は、吸光度を測定することによって検出可能な発色生成物に通じる。伝統的なＥＬＩＳＡに用いられているいずれの好適な発色性基質も、スポットの色彩をバックグラウンドから識別し得る限り、本発明において使用することができる。
【００７４】
発光性基質は、励起した場合またはそれ自体でフォトンを放出することができる生成物に通じる。
【００７５】
本発明の多くの具体例において使用することができる基質システムの例は以下のとおりである：
ＡｔｔｏＰｈｏｓ（ＴＭ ａｓｓｏｃｉａｔｅｄ ｔｏ ＪＢＬ Ｓｃｉｅｎｔｉｆｉｃ Ｉｎｃ．（Ｓａｎ Ｌｏｕｉｓ Ｏｂｉｓｐｏ，ＣＡ）．ＡｔｔｏＰｈｏｓの化学式は、２の参考文献によって別々に報告された：Ｚａｈｒａ Ｐ．ら；Ｉｎ Ｖｉｔｒｏ Ｃｅｌｌ．Ｂｉｏｌ．－Ａｎｉｍａｌ ３４：７７２－７７６，１９９８；Ａ ｆｌｕｏｒｏｍｅｔｒｉｃ ａｓｓａｙ ｆｏｒ ｔｈｅ ｍｅａｓｕｒｅｍｅｎｔ ｏｆ ｅｎｄｏｔｈｅｌｉａｌ ｃｅｌｌ ｄｅｎｓｉｔｙ ｉｎ ｖｉｔｒｏ：リン酸２’－［２－ベンズチアゾイル］－６’－ヒドロキシ－ベンズチアゾール、またはＹｕ Ｈ．ら；Ａｎａｌｙｔｉｃａｌ Ｂｉｏｃｈｅｍｉｓｔｒｙ ２６１：１－７，１９９８；Ｄｅｖｅｌｏｐｍｅｎｔ ｏｆ ａ ｍａｇｎｅｔｉｃ ｍｉｃｒｏｐｌａｔｅ ｃｈｅｍｉｆｌｕｏｒｉｍｍｕｎｏａｓｓａｙ ｆｏｒ ｒａｐｉｄ ｄｅｔｅｃｔｉｏｎ ｏｆ ｂａｃｔｅｒｉａ ａｎｄ ｔｏｘｉｎ ｉｎ ｂｌｏｏｄ：（リン酸２’－［２－ベンズチアゾイル］－６’－ヒドロキシ－ベンズチアゾール＝ビス－［２－アミノ－２－メチル－１，３－プロパンジオール］）
４－ＭＵＰ（リン酸４－メチルウンベリフェリル）
ＨＮＰＰ（ロッシュ番号１ ７５８ ８８８）
４－ＭＵＧ（４－メチルウンベリフェリル＝β－ガラクトシド）
ＣＤＰ－Ｓｔａｒ（ロッシュ番号１ ６８５ ６２７）
ＣＳＰＤ（ロッシュ番号１ ６５５ ８８４）
Ｓｕｐｅｒ Ｓｉｇｎａｌ Ｓｕｂｓｔｒａｔｅ（Ｐｉｅｒｃｅ，Ｒｏｃｋｆｏｒｄ，ＩＩＩ）．化学式は提供されていない。
参考文献：Ｔｒｅｖａｎｉｃｈ Ｓら，Ｊｏｕｒｎａｌ ｏｆ Ｆｏｏｄ Ｐｒｏｔｅｃｔｉｏｎ ６３：５３４－５３８，２０００；Ｒａｐｉｄ Ｄｅｔｅｃｔｉｏｎ ｏｆ Ｅｎｔｅｒｏｔｏｘｉｇｅｎｉｃ Ｅｓｃｈｅｒｉｃｈｉａ ｃｏｌｉ ０６ ｉｎ Ｗａｔｅｒ ｂｙ Ｕｓｉｎｇ Ｍｏｎｏｃｌｏｎａｌ Ａｎｔｉｂｏｄｙ ａｎｄ Ｐｈｏｔｏｎ－Ｃｏｕｎｔｉｎｇ Ｔｅｌｅｖｉｓｉｏｎ Ｃａｍｅｒａ
【００７６】
ルミノール／４－インドフェノール（例えば、ＢＭ Ｃｈｅｍｉｌｕｍｉｎｅｓｃｅｎｃｅ ＥＬＩＳＡ Ｓｕｂｓｔｒａｔｅ（ＰＯＤ）ロッシュ番号１ ５８２ ９５０）
エンハンサーを含むまたは含まないＧａｌａｃｔｏｎ Ｐｌｕｓ（例えば、ＢＭ Ｃｈｅｍｉｌｕｍｉｎｅｓｃｅｎｃｅ ＥＬＩＳＡ Ｓｕｂｓｔｒａｔｅ－ロッシュ番号１ ７５９ ７８７）

金属エンハンサーを含むまたは含まないＤＡＢ （３，３’－ジアミノベンジジン 四塩酸塩）
ＯＰＤ （ｏ－フェニレンジアミン二塩酸塩）または遊離塩基
ＡＥＣ （３－アミノ－９－エチルカルバゾール）
５ＡＳ （５－アミノサリチル酸）
２，２’－アジド－ビス（３－エチルベンズチアゾリン－６－スルホン酸）
４Ｃ１Ｎ（４－クロロ－１－ナフトール）
ｏ－ジアニシジン（３，３’－ジメトキシベンジジン）
ＴＭＢ （３，３’， ５，５’－テトラメチルベンジジン）遊離塩基または二塩酸塩
ＡＢＴＳ
ＮＢＴ（ニトロブルーテトラゾリウムまたは塩化２－（インドフェニル）－５－（４－ニトロフェニル）－３－フェニルテトラゾリウム）を含むまたは含まないＢＣＩＰ （５－ブロモ－４－クロロ－３－インドイルホスフェート）
Ｆａｓｔ Ｒｅｄ／Ｎａｐｈｔｈｏｌ ＡＳ－ＭＸ （Ｓｉｇｍａ Ｆ４６４８）
Ｎａｐｈｔｈｏｌ ＡＳ－ＴＲ ｐｈｏｓｐｈａｔ （Ｓｉｇｍａ Ｎ８５１８） ｗｉｔｈ ａｎ ｄｗｉｔｈｏｕｔ Ｆａｓｔ Ｒｅｄ ＲＣ （Ｓｉｇｍａ Ｆ５１４６） ｐＮＰＰ （リン酸ｐ－ニトロフェニル）
ＰＭＰ （フェノールフタレイン一リン酸塩－Ｓｉｇｍａ Ａ３３４４）
Ｘ－Ｇａｌ （５－ブロモ－４－クロロ－３－インドリル－ベーター－Ｄ－ガラクトピラノシド）
ＣＰＲＧ （クロロフェノール－ベーター－Ｄ－ガラクトピラノシド） Ｒｏｃｈｅ Ｄｉａｇｎｏｓｔｉｃｓ ＧｍｂＨが所有するＥＰ特許０１４６８６６

【００７７】
発色性基質
好ましい具体例によれば、基質は発色体基質であり、それはその吸光度を測定することによって検出し得る。特に好ましい具体例によれば、発色体基質から形成される発色生成物は形成の際に沈澱する。このようにして、検出可能な生成物は検出すべき分析物の付近に留まることが確保される。試料コンパートメントが検出に対して水平に維持されている場合、形成した生成物は試料コンパートメントの低部表面に単純に沈澱し、そこに不溶性の沈澱物を形成するであろう。この型の検出に特に好適な基質は、低水溶性を有するものである。

【００７８】
ある場合においては、検出デバイスにフィルターを加えることが有利となり得る場合がある。好ましくは、フィルターは、検出すべき生成物に対して選択的であるべきである。フィルターの利点は、それが、関連する生成物から生じていないいずれのバックグラウンドからも効率的に濾別し得ることである。システムは、２、３またはそれを超える異なって発色する生成物を検出するための幾つかのフィルターも備えることができる。

【００７９】
検出器がカラー感受性ＣＣＤである場合には、カラー特異的なフィルターの必要性はない。

【００８０】
蛍光
好ましい具体例において、基質は触媒によって蛍光生成物に変換されることができる蛍光発色性である。蛍光に基づくシステムは、一般的に発色性よりもより感受性である。試料ドメインからの像を記録するためにより少ない生成物分子しか必要でないからである。したがって、一般的には、生成される十分量の生成物分子に対してより短いインキュベーション時間しか必要とせず、発色体基質を用いる場合よりも迅速に像を記録し得る。

【００８１】
蛍光性基質は、好ましくは、励起光によって励起した場合に３００ないし１２００ｎｍの波長域のシグナルを発する蛍光生成物に通じる。１の好ましい蛍光方法は、蛍光偏光法で

ある。
【００８２】
励起光源は、発光ダイオード（ＬＥＤ）、気体レーザー、固体レーザー、レーザーダイオード、ガス灯、ハロゲン灯、またはキセノン灯のごとき２５０ｎｍないし６００ｎｍの範囲の励起光を発することができるいずれかの好適な光源である。
コスト的に有効な様式で、できるだけ大きな面積の試料を励起光に照射するためにには、発光ダイオードのごとき発散する励起光を使用することが好ましい。
【００８３】
試料に対する光束を増加させる目的で、例えば２またはそれを超える発光ダイオードを用いるように、１を超える光源を使用することが好ましい。また、光源のうちの幾つかが異なる電磁気特性を有する場合には１を超える光源を使用することも可能である。
【００８４】
幾つかのＬＥＤを用いることによって、試料を幾つかの角度からの励起光に曝して実質的に最適の試料の励起につなげ、光源は好ましくは全てが実質的に同時に伝達されるように操作する。
【００８５】
しかしながら、第１の光源が第２の光源とは異なる波長域を有する少なくとも第１および第２の光源を第１の励起光手段に配置するある種の適用については、光源は交互の様式で伝達し得る。２の異なる光源を使用することによって、試料から２の異なる蛍光シグナルを得ることが可能である。使用するＬＥＤの数に上限は存在しないが、２０にのぼるＬＥＤのごとき、３０ほども多いＬＥＤを配する場合がある。
【００８６】
あまり発散しない光源を用いる場合には、発散光学手段を励起光路に配置して、励起光を適当に発散させることができる。
励起光としてレーザーダイオードを用いる場合、所望により発散手段が配されていてもよい少なくとも４のレーザーダイオードを配置することによって適当な発散を行い得る。
【００８７】
試料の適当な励起を得るための励起光の入射角は、５０°ないし８５°のごとき、好ましくは３０°ないし９０°、より好ましくは４５°ないし８５°の範囲である。
【００８８】
励起光は、試料に対して直接伝達し得る、すなわちビームスプリッターなどによって反射させることなく伝達してもよく、それによってシステムおよび装置をよりコンパクトに構成することが可能である。
【００８９】
二重および多重色
好ましい具体例において、関連付けには生成物の少なくとも２のスペクトル特性間を識別することが含まれる。したがって、本発明により、少なくとも２の異なる型の分析物を同時に検出することが可能である。このことは、２の異なる分析物に指向された少なくとも２の抗体を用い、２の異なる酵素を有する２の抗体を直接的または間接的に準備し、ついでさらに、２の異なるスポットの評価により２の異なる分析物を同時または実質的に同時に検出するための対応する基質を準備することによって達成される。
【００９０】
分析物から発生するスポットは、２の異なる色彩を有するか、または１が発色し、もう１が蛍光性で、または両方が異なる波長領域で蛍光を発するものとすることができる。
【００９１】
２の異なる型の分析物は、異なる細胞、例えば特異的ＩｇＧおよびＩｇＡ－分泌細胞、または死滅および生存細胞の間の識別のごとき、２の異なる状態の同一細胞とすることができる。
【００９２】
また、後者の状況においては、二重標識は分析物に対する１の標識抗体を用いることによ

って行い、ついで慣用的な生存染色のごとき生存細胞から死滅細胞を区別するための分析物の標識のほかの型によって行うことが可能である。
例えば３の分析物のごとき、２を超える異なる分析物をそれによって調べることができることは理解される。
【００９３】
試料ドメイン
本発明により確立した試料ドメインは、三次元試料ドメインのごとき記録する間に試料がその中に位置する、コンパートメントまたはその等価物とすることができる。
【００９４】
試料ドメインは、各試料が一連の試料の一部分であるフロースルー・システムの一部分であってもよく、それによって１の試料が試料ドメイン中の先の試料と置換わる。
【００９５】
もう１の具体例において、試料ドメインは、ＰＣＴ／ＤＫ９９／００６０５号に記載されているディスポーザブル・カセットのごときカセットの一部である。
【００９６】
試料は試料コンパートメントの内部に含まれ、それは２０μｍないし２０００μｍの、普通２０μｍないし１０００μｍの、および多くの実施態様においては２０μｍないし２００μｍの平均厚さを通常有する。
【００９７】
通常、試料コンパートメントは、１ｍｍ×１ｍｍないし１０ｍｍ×１０ｍｍの範囲の、照射ウィンドウの壁部に対して実質的に平行の向きで寸法を有するが、設計に依存して、それをより大きくすることも、または場合によってはより小さくすることも可能であることは理解される。
【００９８】
シグナルを検出することができる試料ドメインの一部分は照射ウインドウといい、それは０．０１ｍｍ^{2}以上ほども小さいもの、好ましくは０．１ｍｍ^{2}以上の面積、より好ましくは１ｍｍ^{2}以上の面積、好ましくは２ｍ^{2}以上の面積、好ましくは４ｍｍ^{2}以上の面積、好ましくは１０ｍｍ^{2}以上の面積、好ましくは２０ｍｍ^{2}以上の面積、好ましくは４０ｍｍ^{2}以上の面積、より好ましくは１００ｍｍ^{2}以上の面積、好ましくは２００ｍｍ^{2}以上の面積、好ましくは４００ｍｍ^{2}以上の面積、好ましくは１０００ｍｍ^{2}以上の面積、好ましくは２０００ｍｍ^{2}以上の面積、好ましくは４０００ｍｍ^{2}以上の面積、好ましくは１００００ｍｍ^{2}以上の面積を有する。同様にして、照射ウインドウの面積を広げるために試料からのシグナルを外部に照射するいずれのウインドウの面に対して平行である方向で試料コンパートメントのウインドウを広げ、したがって外部に照射する試料の体積を増加させることが有利である。
【００９９】
試料ドメインまたはシグナルを検出デバイスに照射するウインドウの領域の形態およびサイズの空間的規定に関しては、この領域のサイズおよび形成の実質的に信頼し得る定義のための少なくとも２の実行可能な方法が存在する。多くの具体例の中で第一の好ましい方法は、例えば検出デバイスのいずれかの焦点合わせ手段を適合することによって、照射ウインドウの実質的に規定された領域からの照射されたシグナルに対して検出手段を感受性に適合することである。検出デバイスの検知領域に適合することが困難である場合に特に好ましい第二の方法は、例えば、照射領域を規定する試料コンパートメントの寸法を制御するか、またはディスポーザブルデバイス中もしくはその上に存在するかまたは検出デバイスと結合する、照射領域を規定するマスクまたは有効ウインドウを形成することによって、試料コンパートメントのかかる照射領域の境界を規定することである。
【０１００】
試料コンパートメントの壁部の要件は、特に、壁部がいずれの重要な制限なしにシグナルを通過させることである。実際には、コストおよび設計によって決まるものから離れて壁部の厚さについて上限は与えられない。壁部は、好ましくは実質的に安定な壁部であり、

それは用いる各材料についての厚さの下限につながる。好ましくは、壁部は０．５ｍｍないし１．５ｍｍ、より好ましくは０．７５ｍｍないし１．２５ｍｍのごとき、０．１ｍｍないし２ｍｍである。

【０１０１】

幾つかの具体例においては、可撓性のある壁部が有用であるが、量的な測定に関しては、これには、調査を行う前に照射した試料の体積を測定することが必要であろう。

【０１０２】

試料体積

１ｍｌ以下および０．０２ｍｌほども小さい試料体積を用いることができる。必要な試料の最適体積は、試料中に存在する分析物の数および求められる所定の統計学的質的パラメータに大きく依存する。

【０１０３】

本発明の他の好ましい具体例により、かなり大きい体積の試料からの分析物を調べることが可能となる。これにより、試料の体積当りのほとんど存在しない目的の分析物しか含んでいない試料を測定することが可能となる。１ｍｌより大きいおよび１００ｍｌよりも大きな試料体積でさえ分析に使用することができ、体積は、試料の測定の前にデバイスに結合したいずれかの流動システムに導入したいずれかの液体試料の全体積として定義される。

【０１０４】

試料コンパートメントまたは試料の設計は、液体試料の体積のサイズが少なくとも１の量的パラメータまたは少なくとも１の質的パラメータの調査を許容して、１の照射で像を記録するように、実質的に１の照射に基づく調査の統計学的な質に対する所定の要件を実行するために少なくとも１の量的パラメータまたは少なくとも１の質的パラメータの調査を許容するほど十分に大きなものであるようなものである場合がある。もう１の具体例において。少なくとも１の質的パラメータまたは少なくとも１の量的パラメータの調査は、１を超える像、好ましくは２の像、より好ましくは２を超える像、より好ましくは４を超える像を、少なくとも１の質的パラメータまたは少なくとも１の量的パラメータに関連付けることによって行う。これらの場合には、像は２、３またはそれを超える照射を通して記録する。

【０１０５】

また、経時的な像の変化についての情報も少なくとも１の質的パラメータまたは少なくとも１の量的パラメータの調査に用い、かかる場合においては１を超える照射を行う。

【０１０６】

分析物の多くの調査において、実質的に大きな体積の試料からのシグナルを照射させることが重要である。電磁放射線のごときシグナルが検出システムに照射される液体試料の体積は、通常０．０１μｌないし２０μｌの範囲である。一般的に、分析する試料の体積は可能な限り大きいものとすべきである。これにより、より多数の分析物の同時の調査が可能となるが、最適体積は検出システムおよび分析する試料の１またはそれを超える状況によって決まる場合もある。したがって、試料コンパートメント中の試料の体積は０．１μｌ未満とすることができるが、０．１μｌ、１．０μｌまたは１０μｌを超える体積を用いる場合さえある。いまだ他の適用において、１００μｌ以上ほども大きな試料コンパートメントの体積を用いることができる。

【０１０７】

大きな体積の試料は、好ましくはフィルター、電場、磁場、重力場、のごとき分析物保持手段に試料の体積を通過させることによって測定し、かかる手段は好ましくはデバイス中に含め、あるいはデバイス内のいずれかの試料と相互作用するように配置し得る。分析物保持手段は、好ましくは試料中に存在する実質的に全ての分析物または試料中に存在する少なくとも１の型の分析物の少なくとも実質的に代表的な画分を保持し得るべきである。

【０１０８】

大きな試料からの分析物を保持する場合、これらの分析物は分析物保持手段を通過した試

料の体積よりも小さい体積中に再懸濁することができる。
【０１０９】
１の具体例において、１の部分を超える同一の試料材料を、検出システムに暴露することによって分析に付することができる。このことは、試料コンパートメントを移動させ、したがって試料コンパートメントの異なる部分を暴露することによって行い得る。
【０１１０】
拡大率
好ましくは、当該方法は低い拡大率で行い、それによって１または数回の暴露で大きな体積中のスポットを検出することが可能となる。拡大率は、１０未満、５未満、４のごとき好ましくは２０未満、より好ましくは２のごとき４未満、より好ましくは１のごとき２未満である。かかる低拡大率の利点は、数ある中の幾つかであり、観察領域の増加および検出デバイスに暴露される体積の増加を含む焦点合わせ深度の増大である。
【０１１１】
問題のスポットが検出エレメントのサイズに匹敵する寸法を有する場合には、約１／１の拡大率を有し、したがって、いずれか１または数個のみの検出エレメント上の幾つかのスポットの像を焦点合わせすることが好ましい場合がある。このことは、いずれかのシグナルの好ましい検出を与えるある種の条件下で行うことができる。
【０１１２】
使用する検出エレメントに匹敵するかまたはそれよりも大きな寸法を有するスポットを分析する場合には、かかるスポットの像のサイズを、像のサイズが検出エレメントのサイズに匹敵する程度まで減少させることが有利な場合もある。したがって、１の具体例において、拡大率は、好ましくは０．８のごとき０．９未満、より好ましくは０．６のごとき０．８未満、より好ましくは０．５のごとき０．６未満とすることが好ましい。
【０１１３】
これらの場合においては、検出エレメントのアレイ上の粒子の像のサイズに対するスポットのサイズの比は、１／１以下、好ましくは１／１未満であって１／１００よりも高く、より好ましくは１／１未満であって１／４０よりも高く、より好ましくは１／１未満であって１／１０よりも高く、より好ましくは１／１未満であって１／４よりも高く、より好ましくは１／１未満であって１／２よりも高い。
【０１１４】
したがって、検出エレメントのアレイ上に暴露された空間表示は、線形拡大が、試料ドメイン中の元の線形寸法に対する検出エレメントのアレイ上の線形寸法の像の比が４０：１より小さい、通常せいぜい２０：１、好ましくは１０：１よりも小さい、多くの場合においてはせいぜい６：１または４：１よりも小さいように付すことが好ましい場合もある。
【０１１５】
像の横縦比は、分析物の調査に対して重大なマイナスの影響をそれが有することなく、検出エレメントのアレイ上にかなり歪ませ得る。かかる場合においては、検出エレメントのアレイ上の粒子の像の２の寸法のうちの長い方に対する短い方の比は、粒子の対応する寸法の比に対して、実質的に１以下、好ましくは１／２以下、より好ましくは１／４以下、より好ましくは１／１０以下、より好ましくは１／５０以下、よりこのましくは１／１００以下、より好ましくは１／２００以下が好ましい。かかる場合においては、検出エレメントのアレイ上の粒子の像の２の寸法のうちの長い方に対する短い方の比は、ある種の状況下において、検出エレメントのアレイによって広がった領域内とは実質的に同一でない。
【０１１６】
パラメータまたはパラメータ群を調べる個々の分析物からの生成物の像は、最大で２５の検出エレメント、詳細には最大で１６の検出エレメントおよびより好ましくは最大で９の検出エレメント上にイメージ化することが好ましい場合がある。パラメータまたはパラメータ群を調べる個々の分析物からの生成物の像は、最大で５の検出エレメント、または最大で１の検出エレメントの上にさえイメージ化することがなおより好ましい。分析物当り

のエレメント数がより多ければ個々の分析物に対するより多くの情報が得られ、一方、分析物当りのエレメント数がより少なければ暴露中で行い得る合計計数が増加するであろう。

【０１１７】

前記したごとく、体積のサイズは判定の所望の統計学的な質に好適に適合する。したがって、判定が一定体積中の分析物の数の判定または分析物のサイズおよび／もしくは形態の判定である場合、好ましくは液体試料の体積はその中における少なくとも２の分析物の同定を許容するのに十分に大きい。より好ましくは、液体試料の体積のサイズは、その中における少なくとも４の分析物の同定を許容するのに十分に大きい。この場合は、ほぼ５０％の再現性誤差に相当するであろう。いまだより好ましくは、液体試料の体積のサイズは、その中における少なくとも１０の分析物の同定を許容するのに十分に大きい。この場合は、ほぼ３３％の再現性誤差に相当する。なおより好ましくは、液体試料の体積のサイズは、その中における少なくとも５０の分析物の同定を許容するのに十分に大きい。この場合は、ほぼ１４％の再現性誤差に相当する。明らかに、可能である場合には、体積のサイズがより多数の分析物の同定を許容する場合の条件を目標とすることが好ましい。したがって、液体試料の体積のサイズがその中の少なくとも１００の分析物の同定を許容するのに十分に大きい場合にはほぼ１０％の再現性誤差に相当し、液体試料の体積のサイズがその中の少なくとも１０００の分析物の同定を許容するのに十分に大きい場合にはほぼ３％ほども低い再現性誤差に相当するであろう。

【０１１８】

本発明の好ましい具体例において、調査すべき分析物は分析の間に実質的に静置し、したがって、いずれかの信号対雑音比条件を改善するために測定時間を最適に使用し得る。この配置によって、特に特性の調査が試料の体積中の分析物の計数のごとき体積に関連する特性である場合には、流動条件における変動によって引起こされる分析物の調査に固有であるかもしれないいずれの誤差も排除される。

【０１１９】

分析物、触媒および基質の試料ドメインへの導入は、流動システムによって提供する。流動システムは試料に対して行う幾つかの操作の少なくとも１を提供し得、該操作は、限定されるものではないが、輸送、試薬との混合、試料および所望により試薬のホモジネート、熱処理、冷却、音波処理、超音波処理、光処理および濾過から選択される。

【０１２０】

試料ドメインの中へおよびそこから外へ試料を流動させるためには、当該システムに少なくとも１の推進手段を準備させることが好ましい。

【０１２１】

好ましくは、流動調整手段は、試料および／または試薬成分がシステム全体にわたって段階的に流動し得るように段階的に作用するように配置する。

【０１２２】

デバイス中の試料は、ポンプまたは圧縮ガス、好ましくは空気によって、または、流入口の外部に対する圧力がシステムの少なくとも一部分の内側の圧力よりもより高く、その結果として試料が流入口を通して流動するように力が加わるような圧力差を引起こすことによって駆動し得る、流動手段によって流動し得る。本発明の多くの具体例において、該流動システムにおける流動は、試料の流動速度を調節し得る１またはそれを超えるバルブによって制御する。多くの好ましい状況において、デバイス中の液体の流動は真空によって引起こされ、該真空は、好ましくはデバイス内に含まれるリザーバーから適用する。真空は、試料の導入または移動と実質的に同時に真空を引起こす機械的または物理的作用によって確立し得る。これらの機械的または物理的作用は：ペリスタポンプ、往復動ポンプ（ｐｉｓｔｏｎ　ｐｕｍｐ）、膜ポンプ、遠心往復動ポンプおよび皮下注射器とし得る。

【０１２３】

試料コンパートメントからの流出は、ガスのみの通過を許容するバルブのごとき流動制御手段を通過させ得る。好ましい場合もある１のかかる型のバルブは、ガスおよび空気の通

過は許容するが、バルブが液体と接触した場合には不可逆的に閉口し得るものである。かかるバルブの効果は、分析の間の試料コンパートメント内のいずれの試料の移動も最小限化することである。

【０１２４】

本発明の好ましい具体例において、システムには少なくとも１のコンパートメントが含まれ、その中で試料物質と触媒および／または媒体との混合が可能である。

【０１２５】

本発明のシステムの１の利点は、液体に分散または溶解した液体医薬および分析物のみを用いて分析を行うことである。このレイアウトにより、操作および取扱い簡便性が保証される。驚くべきことに、いずれの固体支持体に対して結合させなくても、分析物を特異的に検出することが可能であることが判明している。

【０１２６】

検出デバイス

デバイスのウインドウから検出し得る画像は、例えば、検出エレメントのアレイによって検出し得、検出エレメントのアレイには個別のエレメントが含まれ、その各々は試料ウインドウ領域の一部分からのシグナルを感知することができ、アレイはそれ全体で実質的に全ての試料ウインドウ領域からのシグナルまたは少なくとも試料ウインドウ領域のよく決められた部分からのシグナルを感知することができる。例えば、検出デバイスのアレイは、一次元アレイまたは二次元アレイとし得る。分析物の調査を容易にするために、検出エレメントのアレイによって検出する強度を、分析物からの電磁気シグナルの表示が電磁気バックグラウンドのシグナルの表示とは異なって同定されるようにプロセシングする。

【０１２７】

検出手段は、蛍光シグナルのごとき試料から発せされるシグナルを感知または検出することができるいずれかの検出器を含み得る。

好ましい具体例において、検出手段は電荷結合素子（ＣＣＤ）のごとき検出素子または検出エレメントのアレイとして存在する検出器を含み、該ＣＣＤはフルフレームＣＣＤ、フレームトランスファＣＣＤ、インターライン・トランスファＣＣＤ、ラインスキャンＣＣＤ、ｅｇ波長強化型ＣＣＤアレイ、フォーカルプレーン・アレイ、ＣＭＯＳのごときフォトダイオード・アレイまたはフォト検出器・アレイとし得る。ＣＭＯＳは、好ましくはチップ上に集積されたシグナル条件および／またはシグナル処理を有するＣＭＯＳイメージセンサーである。上記のいずれの検出デバイスを選択するかにかかわりなく、検出手段にはさらに白黒またはカラーＣＣＤもしくはＣＭＯＳも含み得る。

【０１２８】

共焦点光学顕微鏡は当該技術分野でよく知られており、典型的な光学顕微鏡よりも優れた多くの利点を与える。共焦点走査型顕微鏡の１の主たる利点は、それが焦点面に存在しない光を減衰させるために試料の光学的分割が得られることである。したがって、焦点面に存在する光のみが最終的な像に寄与する。

【０１２９】

走査型共焦点顕微鏡においては、ビームを試料の表面にわたって掃引する。試料から放出された光（例えば、それから反射した光、それから発せられた光またはそれを通して発せられた光）はピンホールに向けて指向される。焦点面に存在する光はピンホールを通過し、光学検出器に到達する。ビームは試料の表面にわたって掃引するため、光学検出器からの出力を蓄積し、走査した表面の像を形成し得る。

【０１３０】

試料ドメインに形成した生成物からのシグナルを検出するための共焦点走査型顕微鏡、特に共焦点レーザー走査型顕微鏡を使用することが、検出する像がよりシャープであることに起因して有利である。

【０１３１】

検出エレメントのサイズはある程度その感度を決定する。したがって、適用によっては、約１μｍ^2 以下のサイズの検出エレメントを有することが重要となる場合がある。ある種

の状況下においては、検出エレメントのアレイの検出エレメントのサイズは２０μｍ^{2}未満、好ましくは１０μｍ^{2}未満、より好ましくは５μｍ^{2}未満、より好ましくは２μｍ^{2}未満、より好ましくは１μｍ^{2}以下となる場合がある。他の状況下においては、検出エレメントのアレイの検出エレメントのサイズは、２０００μｍ^{2}以上のごとき５０００μｍ^{2}以上、より好ましくは５００μ^{2}以上のごとき１０００μｍ^{2}以上、または２００μｍ^{2}以上でさえ、より好ましくは１００μｍ^{2}以上であって２００μｍ^{2}未満、より好ましくは５０μｍ^{2}以上であって１００μｍ^{2}未満、より好ましくは２０μｍ^{2}以上であって５０μｍ^{2}未満となる場合がある。

【０１３２】

検出エレメントのアレイは、好ましくは以下の領域のうちの１または幾つかの波長の電磁放射線に感度が高い：１００ｎｍないし２００ｎｍ，２００ｎｍないし６００ｎｍ、３００ｎｍないし７００ｎｍ、４００ｎｍないし８００ｎｍ、６００ｎｍないし１μｍ、８００ｎｍないし２μｍ、２μｍないし１０μｍ、５μｍないし１０μｍ、１０μｍないし２０μｍ、２０μｍないし４０μｍ。

【０１３３】

その中でシグナルを検出する全面角度として規定される集光角を最大化するように検出エレメント上に試料からのシグナルを焦点合せするために焦点合せデバイスを含めることは、多くの状況下において、改善された条件を調査に提供することが判明している。驚くべきことには、かかる広い集光角を、焦点合せに使用する対物レンズが検出エレメントを設置する面を別々に横切るいずれの分析物の像の画像比を歪ませるか、または分析する試料を通過する焦点合せに変動を生じるか、または焦点合せの質を低下させる程度に及ぶ場合でさえ、例えば試料中の分析物の数の調査に使用し得ることが判明した。

【０１３４】

検出エレメントの画像比は、分析物を調べるためのシグナルの集光において重要となり得る。約１／１の比が好ましい場合もあるが、ある種の条件においては１／１とは異なる比を使用することが好ましい場合もあり得る。特に、このことによっていずれかの大きな体積の試料からのシグナルの検出を簡便化する場合には、例えばより多くの分析物の同時の調査が許容される。これらの状況下では、検出エレメントのアレイの検出エレメントの縦または横のより長い方に対する縦または横のより短い方に対する比が実質的に１以下、好ましくは１／２未満、より好ましくは１／４未満、より好ましくは１／１０未満、より好ましくは１／５０、より好ましくは１／１００、より好ましくは１／２００未満である。

【０１３５】

焦点合せ－レンズ

試料の少なくとも一部分からのシグナルは、焦点合せ手段を使用することによって、好ましくは１のレンズを使用することによって検出エレメントのアレイに焦点合せするが、しかしながら、２のレンズまたは２を超えるレンズを使用することも可能である。焦点合せシステムに使用するレンズの数は、いずれかの測定システムの複雑度に影響し得る。

【０１３６】

いずれかの検出器に対する試料からのシグナルの焦点合せは、いずれかの検出器に対する試料の位置に依存する。測定システムの構造が試料およびいずれかの検出器の相対的位置が変動し得るような場合にはシステムの焦点合せを調節し得ることに利点がある。このことは、最初に試料からのいずれかのシグナルの少なくとも１の測定値を採取し、ついでこれに基づいてシステムの焦点合せを調節することによって達成し得る場合がある。このことは、許容し得る焦点合せを得るために多くの回数繰返し得る。好ましくは調節の程度は試料からのシグナルの少なくとも１の測定値によって決定する場合には、同様にして、試料または試料材料からのシグナルの焦点合せを調節する。

【０１３７】

使用する焦点合せ配置の集光角は、検出エレメントのアレイに収集されるいずれかのシグナルの強度に影響を有し得る。したがって、高感度が必要な場合には、集光角を増大させることが実用的である。集光角の好ましいサイズは、焦点深度のごときシステムに生じる

他の要件によって決まる場合もある。これらの状況下において、焦点合せ手段の集光角は好ましくは少なくとも２°、好ましくは５°よりも大きい、より好ましくは１５°よりも大きい、より好ましくは２０°よりも大きい、より好ましくは５０°よりも大きい、より好ましくは１２０°よりも大きい、より好ましくは１５０°よりも大きい。

【０１３８】

シグナル

該検出エレメントのアレイの１またはそれを超える検出エレメントについて測定した各シグナルが１またはそれを超える既定値（群）を有する場合、より好ましくは各既定値を１またはそれを超えるいずれかの以前の測定値に基づいて決定する場合には、１またはそれを超える検出エレメントから測定するシグナルは、計算手段を使用することによって系統的または変化するバイアス（ｓｙｓｔｍａｔｉｃ ｏｒ ｖａｒｙｉｎｇ ｂｉａｓ）に補正し得、そのバイアス補正は１またはそれを超える既定値（群）を使用することによって行う。

【０１３９】

バイアス補正は、測定したシグナルから１または幾つかの他の測定で得られた結果を差し引くことによって行い得る。この場合、他の測定が同一の試料または試料材料の１または幾つかの測定である場合に好ましく、他の測定が同一試料または試料材料の以前に採取した測定である場合により好ましい。

【０１４０】

１またはそれを超える検出エレメントからのシグナルは、計算手段を使用することによって強度を補正することができ、当該補正は、該検出エレメントのアレイの１またはそれを超える検出エレメントについて測定された各シグナルが１またはそれを超える既定値（群）を有する場合に好ましく、１またはそれを超えるいずれかの以前の測定に基づいて各既定値を決定する場合により好ましい。

【０１４１】

ある状況下においては、例えば、アナログ－デジタル変換においては、１、好ましくは１を超える出力チャネルが他の出力チャネル（群）とは実質的に異なるレベルを有するように、２、好ましくは３、より好ましくは４、より好ましくは５、より好ましくは６、より好ましくは７、より好ましくは８、より好ましくは８を超えるレベルの異なる出力チャネルを調節することが重要である場合もある。この場合、出力チャネルまたはその組合せのその同定は、実質的に異なる出力レベルを有し、該シグナルの強度に補正する。

【０１４２】

いずれかの測定したシグナルを分析することに関しては、いずれかのシグナルの得られた強度をデジタル表示に変換するようにシグナルをデジタル化することが必要な場合もある。これは、これらのチャネルが他のチャネルとは異なるシグナルを有することが強度を決定することについての情報を有させることによって、または１を超えるこのチャネルに好ましくは二進法表示と同様に組合せを形成させることによって行い得る。

【０１４３】

プロセッサー

検出手段にとって検出されたシグナルの情報は、情報をプロセシング、表示および所望により保存するためのプロセッサーに入力される。

シグナル情報は、プロセッサーに接続されたディスプレー上に表示しおよび／またはプリントし得る。表示される情報は、分析物の数、サイズ分布、形態、分類、励起波長、発光波長、拡大率のごとき測定したシグナルおよび／または使用したシステムに関連するいずれの種類の情報であってもよい。詳細には、データプロセシング手段は、生成物の部分的に重複する領域を識別することができる。

【０１４４】

例えば検出エレメントから測定したシグナルについての情報を保存するために用いる保存容量は、製造のコストにかなりの影響を有するコンポーネントのうちの１である場合もある。したがって、用いる実質的にいずれかの保存容量手段を使用して検出エレメントのア

レイの検出エレメントからの測定したシグナルを保存することなく試料中の分析物の調査を行うように、かかる保存用量の実質的ないずれかの使用なしにパラメータの調査を行い得ることは重要である。

【０１４５】

一方、いずれの保存容量も使用しないで調査を行うことが困難である場合もあるが、好ましくはかかる保存容量の量はすべての測定した検出エレメンからの好ましくは情報のフラクションのみを保存し得る場合の情報を保存するのに必要な容量を超えるべきである。

【０１４６】

幾つかの状況下においては、検出エレメントのアレイの検出エレメントから測定したシグナルは保存容量によって保存し、該保存容量は検出エレメントの数と等しいかまたはそれよりも少ない測定の数、好ましくは検出エレメントのアレイの検出エレメントの数の１／２未満、より好ましくは検出エレメントの数の１／４未満、より好ましくは検出エレメントの数の１／８未満、より好ましくは検出エレメントの１／１６未満、より好ましくは検出エレメントの数の１／３２未満、よりこのましくは検出エレメントの数の１／６４未満、より好ましくは検出エレメントの数の１／１２８未満、より好ましくは検出エレメントの数の１／２５６未満、より好ましくは検出エレメントの数の１／５１２、より好ましくは検出エレメントの数の１／１０２４未満を保存することができる。

【０１４７】

他のある種の状況下においては、検出エレメントのアレイの検出エレメントから測定したシグナルは、保存手段によって保存することが有利であり、保存容量は検出エレメントのアレイの検出エレメントの数よりも大きい測定数、好ましくは検出エレメントのアレイの検出エレメントの数の２倍以上、より好ましくは検出エレメントの数の４倍以上、より好ましくは検出エレメントの数の８倍以上、より好ましくは検出エレメントの１６倍以上、より好ましくは検出エレメントの数の３２倍以上、より好ましくは検出エレメントの数の６４倍以上、より好ましくは検出エレメントの１２８倍以上、より好ましくは検出エレメントの数の２５６倍以上、より好ましくは検出エレメントの数の５１２倍以上、より好ましくは検出エレメントの数の１０２４倍以上の測定数を保存することができる。

【０１４８】

パラメータの調査のほかのより複雑な具体例では、かなりの量の保存容量の使用が必要となる場合がある。したがって、この具体例においては、使用する検出エレメントの１の測定で収集されるよりもより多い情報を保存し得る保存容量を有することが必要となり得る。

【０１４９】

計算手段、好ましくは検出手段の総数の小さい画分に実質的に等しい量の情報のみを保存し得る保存容量を備えたＡｎａｌｏｇｕｅ Ｄｅｖｉｃｅｓ（ＡＤＳＰ ２１０１）から市販されているデジタルコンピュータを用いることによって試料のパラメータの相関および調査を作成し、ついで好ましくは各検出エレメントまたは検出エレメントの列、または検出エレメントの２またはそれを超える列からの測定した情報を測定した情報を保存することによって生じる遅延のごとき実質的にいずれの遅延なしに調査に使用するように、データの実質的にリアルタイムのプロセシングに基づいて対象物の数の調査を作成することが可能である。

【０１５０】

しかしながら、第２の計算手段、好ましくはデジタルコンピュータによって情報をプロセシングする前に第１の計算手段、好ましくはデジタルコンピュータを使用することによって実質的に全ての測定した情報を保存し、したがってそれを得たのと実質的に同一の速度で測定した情報をプロセシングできるがいずれかの情報の測定と同一の情報のプロセシングとの間に実質的な時間の遅延を有することが好ましい場合もあり；これは、タスクを実行するために十分なリソースを備えた１の計算手段のみ、好ましくは１のデジタルコンピュータのみを使用することによって行い得る。

【０１５１】

医学マーカー
本発明のシステムおよび方法は、ヒトまたは動物における症状の臨床マーカーの検出に使用し得る。該方法およびシステムの１の好ましい使用は、心筋梗塞または心臓の梗塞の早期診断用である。心臓の細胞が罹病または死亡している場合、それは多数の酵素を血中に漏出する。これらの酵素の血中レベルは、患者が心臓の梗塞の急性病徴を示す数時間前に正常なレベルを超えて上昇する。したがって、心臓に関連する酵素の血中レベルを測定することによって、信頼し得る早期の診断を行い得る。
【０１５２】
先行技術によれば、研究室において血液試料をＥＬＩＳＡに付することによって酵素を測定する。このＥＬＩＳＡ検出の時間は数時間である。この時間の間に、患者が心臓の梗塞のいずれかの急性の病徴を示すことはない。診断の準備ができたのと同時に心臓の梗塞が患者の生命を助けるには遅すぎるレベルまで発展するかもしれない。
【０１５３】
医学または臨床マーカーであるこれらの特定の酵素または他の酵素の血中レベルを、本発明の検出技術を用いて迅速に測定し得ることは予想される。
【０１５４】
当該システムは、実質的に全ての動物、詳細には、ヒト、ウシ、ウマ、ブタ、ヒツジ、ヤギのごとき哺乳動物における臨床または医学マーカーの検出に使用し得る。
【０１５５】
片側および両側システム
検出デバイスは、片側デバイス、すなわちシグナルを検出する側と同じ試料の側から試料に対して光が指向されるデバイスとしてレイアウトすることもできる。
検出デバイスは、試料から発せられたシグナルを検出する側と同じ試料の側から試料に対して励起光が指向される片側デバイスとしてもレイアウトすることもできる。
【０１５６】
この装置によって、従来の蛍光顕微鏡と比較して種々の利点が達成された。まず最初に、調べる試料を検出器と励起光との間の試料面に滑動させる代わりにそれを試料面に直接的に配置することが可能である。さらに、透明でない試料の表面蛍光を検出することが可能となった。
【０１５７】
検出器を危険に曝すことなく励起光の強度を増大させることも可能である。
また、それによって通常は顕微鏡に試料を配置することが不可能な性質を有する試料を、顕微鏡を試料上に直接設置し、それによって試料の表面が単純に試料面を構成するという点において本発明のシステムを使用することによって調べることができる。
【０１５８】
最後に、励起光手段を検出器と同じ試料面の側に配置し、したがって従来の装置と比較して少なくとも２５％装置の軸が短くなることにおいて、よりコンパクトであってそれによって取り扱うのがより簡単な装置を作成することが可能である。
【０１５９】
本発明により、フローサイトメトリー装置を使用することによってのみ今まで信頼し得る調査が可能であった試料のパラメータを調べることが可能である。１の照射において大きな試料のパラメータを調べることが可能であり、したがって照射当りに大きな試料の一部分のみを調べることによって大きな試料を調べる場合に通常考慮する統計誤差を低下させることが可能である。
【０１６０】
さらに、１の照射で試料から１を超える蛍光シグナルを得、それによって、試料の粒子の分類をそれらの異なる蛍光シグナルに起因して容易にすることができる。
【０１６１】
したがって、本発明による片側装置は、広範な種々の組み合わせで構成し得、それらはすべて本発明の範囲に入る。詳細には、以下に論じる原理組合せが予想される。

【０１６２】
装置は、光源と励起光フィルターとが同一である単一蛍光装置として構成し得る。
【０１６３】
少なくとも２の異なる蛍光シグナルを供する装置のごときマルチ蛍光装置は、以下のうちの少なくとも１によって提供し得る：
・第１および第２の光源、該光源は異なる波長の光を発する
・第１および第２のフィルターが異なり、それによって少なくとも２の異なる波長の励起光が試料に照射される
・デュアル波長域のフィルターのごとき、第１および第２の発光フィルターが異なり、それによって少なくとも２の異なる蛍光シグナルが検出器（群）に発せられる
【０１６４】
しかしながら、本発明の装置を両側装置として構成し得、それによって試料の両側から試料に対して励起光を指向し得、または検出手段を試料の両側からのシグナルを検出するために配置するか、あるいはその双方をなし得ることはさらなる利点である。
【０１６５】
かくして、両側装置とは、さらに以下のことが配された本発明による装置を意味する：
－第２の励起光手段が第２の光面に位置し、該第２の光面は試料面と平行して存在し、かつ、第１の光面と向い合う試料面の他の面上に位置する。それによって、試料は試料の両側から励起光を受け、試料に照射されるエネルギーがかなり増大する、および／または
－第２の検出手段は、試料が第１の検出手段と第２の検出手段との間に位置するように配置する。それによって、１の照射の検出によって試料からのシグナルに関して異なる情報を調べることが可能である。例えば、第１の検出手段は試料の粒子の数を記録するように適合し得、一方、第２の検出手段は試料中の粒子の形態を記録するように適合し得る。
【０１６６】
好ましい具体例において、両側装置には両側励起システムと両側検出システムとが含まれる。
第２の励起光手段は第１の光手段に関して論じたいずれの光手段とすることもできる。蛍光顕微鏡の目的に依存して、光手段は異なるかまたは同一とし得る。
【０１６７】
さらに、励起光が異なる波長域を構成し、それによって異なる波長を有する発光を達成されることは重要となり得る。
【０１６８】
第２の検出手段は第１の検出手段に関して論じたいずれの検出手段とすることもできる。光源、フィルター、拡大率および検出器のいずれの好適な組合せも本発明によって予想される。以下の好ましい具体例においては両側システムを論じる。
【０１６９】
装置は、実質的に同じ波長の励起光を両側から試料に対して照射する単一蛍光システムとし得る。それによって、励起光を強め得る。
両側励起光装置においては、第１の励起光手段が試料の片側から試料に１の波長を照射し、第２の励起光手段が試料の片側から試料にもう１の波長を照射する。もちろん、第１の励起光および第２の励起光が各々異なる光源および／またはフィルターを含み得、それによって前記に論じごとくなお多い波長を試料に照射し得ることは本明細書において理解される。
【０１７０】
両側励起光装置は１の検出器を含み得、それによって装置は部分透過システムとして機能する。
もう１の具体例において、両側励起光装置は２の検出器を含む。それによって、大きな量の情報を試料から得ることができる。１の具体例において、２の検出手段は、情報の妥当性を提供する試料に関する等しい（鏡像であるが（２の検出器の像は互いに鏡像である））情報を得ることができる。

【０１７１】
本発明による装置は、片側励起光手段を用いる両側検出装置ともなり得る。それによって、１の検出器が試料を透過したシグナルを検出する。
【０１７２】
励起光の配置から独立して、両側検出システムは受ける情報の量を増加することができる。例えば、２の検出器によって異なる波長を受けることができ、および／または異なる感度を有する異なる検出器を使用し得る。さらに、例えば２の検出器に異なる拡大率を用いることによって、試料に関する情報を増加することができる。例えば、システムの片側が例えば低拡大率によって、大面積の試料中の粒子の数を調査し、システムの他の側がより大きな拡大率を用いることによって、粒子の形態を調査し得る。拡大率の組合せは、例えば、１：１および１：４、１：１および１：１０、１：２および１：４、１：２および１：１０とし得る。２の検出器から移されたシグナル情報は、好ましくは同一のプロセッサーに伝達され、それによって情報を別々に表示することも、結合して、位置および数を他の検出器によって検出した特定の粒子に関する特定の形態的情報を提供することもできる。
【０１７３】
他の側が従来の光学顕微鏡またはいずれか他の型の顕微鏡である両側装置として本発明による装置を用いることも可能である。システムの他の側を非蛍光顕微鏡として使用する場合、顕微鏡用の照射光は顕微鏡に対して試料のいずれかの側に好適に配置し得る。
【０１７４】
１の側に従来の顕微鏡を含む両側装置は、システムの蛍光部分について片側または両側励起光システムを含み得る。
【０１７５】
両側検出システムを用いる場合、第１の検出手段のプロセッサーは装置を単純化するために同様にして第２の検出器からのシグナルデータも受け得る。しかしながら、各検出手段について別々のプロセッサーを備え付けることも可能である。
【０１７６】
片側および両側励起および検出システムの実施例
以下に検出システムの１の具体例を図面と関連付けてより詳細に論じる。
図１において、照射および検出システム１の例を概略形態で示す。試料は試料コンパートメント２試料面に配置する。励起光手段３の光源４ａ、４ｂからの励起光は、主光路５ａ、５ｂを通って試料にあてられる。
【０１７７】
試料からの蛍光シグナルは、少なくとも１の検出器７を含む検出手段６に発せされる。発せられたシグナルの光路は試料と検出器との間の軸、すなわち検出－試料軸８をたどる。
【０１７８】
シグナルデータは、検出手段６に結合されたプロセッサーに伝達される。試料からの蛍光シグナルは発光フィルター１４によって濾過され、焦点合せレンズ１０によって検出手段９に焦点合せされる。
【０１７９】
光源４ａ、４ｂは光ハウジング１１中に配置され、それによって、検出手段に対する励起光の直接的な伝達が回避される。さらに、励起光フィルター１２ａ、１２ｂが励起光線中に位置する。
【０１８０】
図２は励起光フィルターの円形支持材料１３の断面図を示し、ここでは、光源の位置を破線の円によって示している。
【０１８１】
図３では光路およびシグナル路をより詳細に示している。光路において主光路を５として示す。さらに、検出－試料軸を破線８によって示す。システムの集光角を２の矢印の間に示すＣとして示し、主光路と検出－試料軸との間の角度をＥとして示す。

【０１８２】
図４では両側励起／検出システム１を示し、ここでは、試料の片側のシステム同士は同一であって、図１の片側システムについて説明したものと同じである。
【０１８３】
図５は両側励起システムを示し、ここでは、第１の励起光手段３ａの光源４ａ、４ｂからの励起光および第２の励起光手段３ｂの光源４ｃ、４ｄからの励起光が試料２の両側から試料２にあてられる。前記に論じたごとく、光源は調査すべき情報に依存して同一であっても異なっていてもよい。さらに、各光源に使用したフィルターは同一であっても異なっていてもよい。
【０１８４】
蛍光シグナルは励起光の配置に起因して試料を通過しおよびそれから反射して、検出手段６に発せされる。発せられたシグナルの通路は試料と検出器との間の軸、すなわち検出－試料軸８をたどる。
【０１８５】
シグナルデータは、前記したごとく検出手段に連結されたプロセッサーに伝達される。
【０１８６】
図６は片側励起システムを用いた両側検出システムを示し、ここでは、試料２からの反射した蛍光シグナルを検出器７ａを含む検出手段６ａによって検出する。反射した蛍光シグナルはフィルター１４ａを透過し、レンズ１０ａによって焦点合わせされる。
【０１８７】
さらに、試料２からの透過した蛍光シグナルは、検出器７ｂを含む検出手段６ｂによって検出される。反射した蛍光シグナルはフィルター１４ｂを通過し、レンズ１０ｂによって焦点合わせされる。
【０１８８】
図１４ａは好ましくはフィルター１４ｂとは異なり、それによって、少なくとも２の異なる蛍光シグナルに関する情報を得ることができる。
【０１８９】
また、２の検出システムの拡大率は、例えばレンズ１０ａをレンズ１０ｂと異なったものとすることによって異なるものとし得る。
【０１９０】
実施例
試料型　　クエン酸ナトリウムで抗凝固化したヒト全血。
分析物　　表面に位置するＣＤ４５光源を有するヒトＣＤ４５＋血液細胞。
抗体－コンジュゲート　　ＣＤ４５抗原に対して特異的なモノクローナル・マウスＩｇＧ（Ｆａｂ）。ＩｇＧはビオチンにコンジュゲートされている（ｐＨ７．２の生理食塩水（ＰＢＳ）１ｍｌ当り１００μｇの抗体コンジュゲート）。
アビジン－ＡＰコンジュゲート　　アルカリホスファターゼにコンジュゲートしたストレプトアビジン（ＰＢＳ　１ｍｌ当り１０μｇのアビジン・コンジュゲート）。
酵素基質　　ＡｔｔｏＰｈｏｓ™ Ｓｕｂｓｔｒａｔｅ Ｓｅｔ（Ｂｏｅｈｒｉｎｇｅｒ Ｍａｎｎｈｅｉｍ番号１６８１ ９８２）。アルカリホスファターゼはＡｔｔｏＰｈｏｓ Ｓｕｂｓｔｒａｔｅを４３５ｎｍに最大励起を、５６０ｎｍに最大発光を有する蛍光色素（生成物）に変換するであろう。
溶解緩衝液Ａ＋Ｂ　　Ｕｔｉ－Ｌｙｓｅ（Ｄａｋｏ番号Ｓ－３３５０）を、赤血球を溶解するが表面マーカーに基づく細胞分析を行うために白血球の細胞膜は十分に無傷のまま残すために用いる。
顕微鏡計数　　ＣＤ４５＋標的細胞に由来するスポットの計数は、好適な光源（例えば、キセノン灯）、生成物に対して設定された好適なフィルター、４×対物レンズ、ＣＣＤカメラおよびＢｕｒｋｅｒ－Ｔｕｒｋカウンティングチャンバー（深さ０．１ｍｍ）を備えたＥＰＩ－蛍光顕微鏡を用いて行い得る。

【０１９１】
１０μｌの抗体コンジュゲートの部分を１００μｌの試料に添加する。ゆっくりと混合し、室温（ＲＴ）にて３０分間インキュベートした後に、２ｍｌのＰＢＡの部分を懸濁液に添加する。ゆっくりと混合した後に、その懸濁液を３００ｇにて５分間遠心する。ついで、上清を除去し、ペレットを２ｍｌのＰＢＳに再懸濁する。ゆっくりと混合した後に、その懸濁液を３００ｇにて５分間遠心する。その上清を除去し（ほぼ１００μｌの流体を残しつつ）、１００μｌの溶解緩衝液Ａの部分を添加する。ゆっくりと混合した後に、その懸濁液をＲＴにて１０分間インキュベートする。１ｍｌの溶解緩衝液Ｂの部分を懸濁液に添加し、ＲＴにて１０分間インキュベートする。ついで、その懸濁液を３００ｇにて５分間遠心する。ついで、その上清を除去し、ペレットを２ｍｌのＰＢＳに再懸濁する。ゆっくりと混合した後に、その懸濁液を３００ｇにて５分間遠心する。ついで、上清を除去し、ペレットを２ｍｌのＰＢＳに再懸濁する。ゆっくりと混合した後に、その上清を３００ｇにて５分間遠心する。

【０１９２】
ついで、上清を除去し、１００μｌのアビジン－ＡＰコンジュゲートの部分をペレットに添加する。ゆっくりと混合し、ＲＴにて１５分間インキュベートした後に、２ｍｌのＰＢＳの部分をその懸濁液に添加する。ゆっくりと混合した後に、懸濁液を３００ｇにて５分間遠心する。ついで、上清を除去し、ペレットを２ｍｌのＰＢＳ中に再懸濁し、ゆっくりと混合した後にその懸濁液を３００ｇにて５分間遠心する。上清を除去した後に、ペレットを２ｍｌのＰＢＳに再懸濁し、ゆっくりと混合した後に、懸濁液を３００ｇにて５分間遠心する。上清を除去した後に、製造業者の指示書に従って調製したＡｔｔｏＰｈｏｓ^{TM} Ｓｕｂｓｔｒａｔｅ溶液５００μｌにペレットを再懸濁する。直ちに、少量部分の懸濁液をＢｕｒｋｅｒ－Ｔｕｒｋ計数チャンバーに加え、ついでＥＰＩ－蛍光顕微鏡を用いて像を生成する。１秒の短い期間でもって、さらなる像が生成され、ドットの形成およびこれらのドットの成長を観察する。ドットは、生成物の拡散に起因して直径が増大するであろう。

【０１９３】
ＣＤ４５＋細胞は、測定する体積内の蛍光ドットとして観察し得る。ドットの数および像によって表されるＢｕｒｋｅｒ－Ｔｕｒｋ計数チャンバー中の混合物の体積に基づいて、Ｂｕｒｋｅｒ－Ｔｕｒｋ計数チャンバー中のＣＤ４５＋細胞の濃度を算出し得る。その際、希釈率がわかっているため、血液試料中のＣＤ４５＋細胞の濃度を算出し得る。

【図面の簡単な説明】
【図１】図１は片側型励起システムを示す。
【図２】図２は試料面に平行な面の励起光フィルターの断面図である。
【図３】図３は集光角Ｃおよび励起主光路と検出－試料軸との間の角度Ｅを示す。
【図４】図４は両側型励起／検出システムを示す。
【図５】図５は両側型励起システムを示す。
【図６】図６は両側型検出システムを示す。

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(71) Applicant *(for all designated States except US)*: **CHEMOMETEC A/S** [DK/DK]; Gydevang 43, DK-3450 Allerød (DK).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **GLENSBJERG, Martin** [DK/DK]; Næsbyholmvej 2, 4.tv., DK-2700 Brønshøj (DK).

(74) Agent: **HØIBERG APS**; St. Kongensgade 59 B, DK-1264 Copenhagen K (DK).

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(54) Title: SPATIALLY RESOLVED ENZYME-LINKED ASSAY

(57) Abstract: The present invention relates to a method of assessing at least one quality parameter and/or at least one quantity parameter of at lest one analyte wherein said at least one analyte is connected to a catalyst capable of catalysing a substrate into a product, whereby the analyte is assessed through detection of product produced around the analyte. More particularly the present invention relates to a method of assessing at least one quality parameter or at least one quantity parameter of at least one species of analytes in a sample comprising the steps of establishing a sample domain having at least one wall, arranging in the sample domain catalyst-analyte complexes between the at lest one species of analytes and at least one catalyst in a manner allowing the analytes to move relative to the wall(s) of the sample domain, arranging a substrate in the sample domain, said substrate being capable of being converted into a product through catalysation by said catalyst, contacting the substrate with the catalyst-analyte complexes of individual analytes allowing a detectable amount of product to be produced, recording an image of the product related to individual analytes in the sample domain, correlating the image to the at least one quality parameter or the at least one quantity parameter of the at least one species of analytes.

Spatially Resolved Enzyme-linked Assay

The present invention relates to a method of assessing at least one quality parameter and/or at least one quantity parameter of at least one analyte wherein said at least one analyte is connected to a catalyst capable of catalysing a substrate into a product, whereby the analyte is assessed through detection of product produced around the analyte.

Background

Detection of a substance or a particle using staining of the substance or particle to aid detection is widely used. However, many substances and particles are so small that although stained it is difficult to detect them without using very high magnification increasing the requirements to the equipment used.

A classical amplification technique is that of enzyme-linked assay. A ligand reacting specifically with the analyte is bound to an enzyme, and after excess ligand-enzyme is removed, the analyte-ligand-enzyme complex is detected by reaction with a chromogenic substrate, a colourless material which is acted upon by the enzyme to form a coloured product. Because of its large amplification factor, enzyme-linked assays offer high sensitivity, and are particularly useful for detection of small amounts of antigens.

In traditional enzyme-linked assays (ELISA) the amplification increases sensitivity at the expense of precision, because amplification factors are not exact. It is not possible to distinguish a strong reaction from a few molecules from a weak reaction from a high number of molecules.

An attempt to overcome some of the problems related to preciseness detection is using ELISPOT or in situ ELISA or spot-ELISA, that independent of the name relates to the use of ELISA technique to detect localised coloured spots on a solid support.

ELI-SPOT analysis allows the detection and enumeration of particles, such as virus or polypeptide producing cells or antigen-presenting cells. The spots produced may be enumerated by the use of a microscope or by use of video-imaging. The principle

of ELI-SPOT is for example described in Sedgwick, J.D. and Holt, P.G. " A Solid – Phase Immunoenzymatic Technique for the Enumeration of Specific Antibody-Secreting Cells, Journal of Immunological Methods, 57 (1983) 301-309, wherein secreted antibodies to be detected are captured by antigens attached to a fixed solid support. The secreted antibodies are detected by adding antibody-enzyme complexes, said antibodies having specificity towards the secreted antibodies. The substrate is added in a warm agarose gel, that is allowed to hardened whereupon spots of insoluble product may be detected in the hardened agarose gel using a microscope.

Summary of the invention

The present invention relates to a method of assessing at least one quality parameter or at least one quantity parameter of at least one species of analytes in a sample comprising the steps of

establishing a sample domain having at least one wall,

arranging in the sample domain catalyst-analyte complexes between the at least one species of analytes and at least one catalyst in a manner allowing the analytes to move relative to the wall(s) of the sample domain,

arranging a substrate in the sample domain, said substrate being capable of being converted into a product through catalysation by said catalyst,

contacting the substrate with the catalyst-analyte complexes of individual analytes allowing a detectable amount of product to be produced,

recording an image of the product related to individual analytes in the sample domain,

correlating the image to the at least one quality parameter or the at least one quantity parameter of the at least one species of analytes.

A detectable amount of product is understood as the formation of a substantially spherical amount of product around each analyte or group of analytes, leading to a

spot, detectable by a detection device. The spot produced from the product relates to one or a few analytes, allowing an assessment of the parameter relating to the analytes to take place. The three-dimensional formation of product leads to a more distinct identification of the analytes relative to the background. The image of such a spot is in many aspects similar to an image of particles encountered in numerous other applications. It is therefore understood that substantially same techniques and methods can be used in the recording, processing and analysing an image of spots as would be used for particles.

As opposed to traditional ELI-SPOT technique the analytes to be detected are not coupled to a solid support fixed to the sample domain but may be positioned anywhere in the sample domain during the contact between the substrate and analyte-catalyst-complexes. The analytes are capable of moving relative to the wall(s) of the sample domain at least until the substrate is introduced into the sample domain. Thereby contact between the analyte-catalyst-complexes and the substrate is conducted more easily.

The complex between analyte and catalyst may be formed in one or more of several ways, such as:

Through a linkage, wherein the term linkage means that the analyte and catalyst are bound to each other.

For example, the analyte-catalyst complex may be formed via a species-selective linkage, such as an immunological binding, i.e. the linkage formed between an antibody and its antigen.

Also the linkage may be through conjugation, i.e. the covalent binding between two compounds, for example between an enzyme and an antibody, an enzyme and avidin, an antibody and biotin.

In another embodiment the complex between the analyte and the catalyst is formed through a production of catalyst in or adjacent the analyte, such as expression of an enzyme from a cell. The enzyme is located adjacent the cell after expression and an analyte-catalyst complex is formed.

The correlation of the image to the at least one quality parameter or at least one quantity parameter of at least one species of analytes in a sample preferably comprises estimation of the number of spots on the image and/or estimation of the size of spots on the image.

In a second aspect the invention relates to a system for conducting the method according to the invention comprising

a device comprising a sample domain comprising an exposing domain, an inlet through which a volume of a liquid sample representing the analyte material can been introduced, and a flow system comprising at least a channel allowing at least a portion of the volume of the liquid sample to flow within the device,

the device further comprising means to control the flow of liquid around a catalyst-analyte complex in the sample domain,

a detection device comprising at least a first detector for quantitatively detecting spatial image data and a processor for processing the detected image presentation,

the device and the detection device having means for arranging the device in relation to the detection device in a manner allowing electromagnetic signals from a sample in the exposing domain of the device to pass to the detection device and to form, in the detection device, a spatial image representation of the exposing domain.

In particular the flow system additionally comprises a compartment or a flow channel part in or from which at least part of one or more reaction components initially loaded in the compartment or flow channel part is added to at least a portion of the volume of the liquid representing the sample.

Furthermore, the system may comprise at least a first light source, which advantageously emits excitation light. The detector adapted to detect the fluorescence emitted from the sample is advantageously located on the same side of the exposing domain as the light source.

In a third aspect the invention relates to the use of a system for diagnosis of a condition in an individual, such as the diagnosis of cardial infarct.

Drawings

Fig. 1 shows a one sided excitation system.

Fig. 2 shows a cross-section of the excitation light filter in a plane parallel to the sample plane.

Fig. 3 shows the collection angle C and the angle E between the excitation main light path and the detection –sample axis.

Fig. 4 shows a double-sided excitation/detection system.

Fig. 5 shows a double-sided excitation system.

Fig. 6 shows a double-sided detection system.

Detailed description of the invention

Amplification of signal

The analyte is detectable due to the amount of product being formed around the analyte. Depending on the physical constraints in the sample domain the product will diffuse from the analytes to form localised substantially spherical spots around the analytes or clusters of analytes. In other embodiments of the present invention the product is substantially insoluble under the conditions provided in the sample compartment and the product will therefore form a deposit or colloid matter. The spot will increase with time due to the transport of product in the media generally through diffusion. Thus it is possible to monitor the formation of the spots growing with time both with regard to intensity and size.

Parameter

A quantity parameter according to the invention is the number of analytes present in the sample and/or the concentration of analytes in the sample, whereas quality parameter is information regarding viability, dead and/or dying organism including apopotosis, size, identity, respiration, presence, and morphology.

Kinetics

The rate of formation of the product and thus the rate of change in the recorded image is dependent on the chemical and/or physical properties of the media in the sample compartment. In situations where the flow is limited this will often be defined by the rate of diffusion of substrate towards the catalytic site and/or the rate of diffusion of the product away from the catalytic site.

The image recorded of the product spots has to be recorded after a detectable amount of product has been produced and before the various spots become confluent, thereby inhibiting recording of an image. The image may be correlated to the at least one quality parameter or the at least one quantity parameter of the analyte.

The time period from contacting the analyte-catalyst-complexes with the substrate to recording the spots is mainly depending on the diffusion rate of substrate as well as product and on the kinetics of the process. The diffusion rate and the kinetics of the process may be controlled as discussed below.

The development of detectable spots is preferably observed within 60 minutes from contacting the analyte-catalyst-complexes with the substrate. In more preferred embodiments the step of producing a product is below 15 minutes, preferably below 5 minutes, more preferably below 1 minute, more preferably below 30 seconds, more preferably below 15 seconds, more preferably below 10 seconds, more preferably below 5 seconds, more preferably below 2 seconds.

In a preferred embodiment the sample domain is exposed to the detection means before any development of detectable spots is commenced and at least one other

exposure is conducted when sufficient time to obtain the developed spots have been reached.

By changing the viscosity of the environment in the sample domain it is also possible to control the period of time of producing the product. The step of producing a product may be carried out in a liquid environment, or in a viscous environment. In the latter case, the viscosity may be adjusted to the substrate and catalyst used. Also, the step of producing a product is carried out in a semi-solid environment, preferably where the semi-solid environment is a gel. the semi-solid environment is preferably formed after the analytes have been introduced to the sample compartment, preferably where the forming of the semi-solid environment is controlled by external factors such as temperature, light and agitation.

Analytes

The analyte may be any analyte capable of forming an analyte-catalyst complex. As discussed above the analyte may be bound by the catalyst via a species-specific binding, such as via an antigen-antibody binding.

The analytes are preferably particles such as biological particles. Biological particles are in particular selected from the group consisting of cells, cell walls, bacteria, plasmodia, virus, prions, macromolecules, proteins, polypeptides, peptides, genes, DNA, RNA, or fragments or clusters thereof.

The cells are preferably selected from mammalian cells, insect cells, reptile cells, fish cells, yeast cells, and fungi cells, more preferably from blood cells, sperm cells, and bone marrow cells.

The analytes may be coupled to a solid support, such as beads, said beads being capable of being suspended in the sample domain. The beads may be polymer beads. Often the polymer beads can have physical and/or chemical properties which can assist in the handling of the analyte such as paramagnetic beads.

One embodiment of the present invention which involves the assessment of virus or other small analytes is based on binding of the analyte to a bead. This allows the

analyte and/ the analyte-catalyst complex to be treated in a more simple manner during pre-treatment such as with centrifugation, filtration or magnetic separation.

The beads may be labelled themselves to improve accuracy in the identification of one or more analytes. One embodiment of the present invention uses two or more types of beads which can have affinity to bind two or more different analytes. If the analyte-catalyst complex produces the same product regardless of which analyte is involved, the identification of the polymer bead can be used to distinguish between which analyte-catalyst complex produces the product the signal of which is detected. Such labelling of polymer beads can be based on colour, fluorescence, size or any other physical or chemical property.

Also, the analyte may be a DNA or RNA containing analyte whereby the DNA/RNA or a fraction thereof may be stained with a DNA staining compound. This is often preferred when a further confirmation or assessment of analyte property of analyte specificity is needed such as assessment of viability.

Apart from methods based on enzyme amplification (Enzyme Amplification Systems, EAS) other systems are also included in many preferred embodiments of the present invention. Among such specific amplification systems are the follows:

PAP: Peroxidase anti-peroxidase complex
APAAP: Alkaline phosphatase anti-alkaline phosphatase complex
BGABG: beta-galactosidase anti-beta-galactosidase complex
Cyclic conversion of NADH to NAD especially when the formation of NADH involves NADPH and Alkaline Phosphatase.

Sample

The sample may be a liquid sample such as a sample selected from the group consisting of milk, milk products, urine, blood, sperm, nasal secrete, tears, faeces, waste water, process water drinking water, cerebro-spinal fluid, gall, bone marrow, food, feed, and mixtures, dilutions, or extracts thereof.

The sample may also relate to assessment of particles in water, such as control of drinking water, control of waste water or water from a water purifying plant or

swimming pool. In all applications the control may be related to the total particle count, such as bacteria count or it may more particularly be related to a monitoring process for specific bacteria, such as pathological bacteria.

Furthermore, fermentation control, i.e. control of cell growth and viable cells in fermentation tanks may be conducted by the invention. This relates to all technical and industrial fields using fermentation, such as the pharmaceutical industry for producing peptide or protein composition.

The liquid sample may be pre-treated with any suitable treatment, such as centrifugation, sedimentation, filtration, extraction, dilution, irradiation, agitation, addition of chemicals, chromatographic separation.

In another embodiment the sample is a solid sample which is pre-treated prior to being arranged in the sample domain. An example of pre-treatment is blending optionally followed by any of the treatment mentioned for the liquid sample.

The sample may be any biological sample, such as a biopsy of tissue, such as a biopsy of muscle, brain, kidney, liver or spleen.

Also, the sample may be a sample of food or feed to be tested for contamination, such as bacterial contamination. The present invention offers a very fast method of detecting and enumerating bacteria in food or feed such as a method of detecting Salmonella species.

Independent of the form of sample it is required that the analyte is suspended in a medium before contacting the substrate. Said medium may be the natural medium for the analyte or any liquid suitable for the detection. In one embodiment the analyte is suspended in a medium after being pre-treated. The medium may comprise the catalyst if appropriate.

Catalyst

By the term catalyst is meant any compound capable of converting or aiding in the conversion of a substrate into a product being detectable by the detection means. The catalyst may thus be an inorganic catalyst as well as an organic catalyst. In one

embodiment the catalyst is an enzyme wherein the term enzyme is used in its normal meaning. Where enzymes are used for labelling either a single enzyme, an oligomeric form of the enzyme, or an enzyme/anti-enzyme complex may be used.

The enzyme may be any enzyme useful in an ELISA technique such as an enzyme selected from the group consisting of phosphatases such as alkaline phosphatase, β-galactosidase, peroxidases such as for example horseradish peroxidase, β-glucuronidase, β-glucose-6-phosphate dehydrogenase, glucose oxidase, urease, luciferase, β-lactamase and β-amylase.

When two, three or more different types of analytes are detected in one turn, the different enzymes must be selected so that they do not interfere with one another. For example, enzymes are advantageously chosen that require approximate the same pH. Advantageously, substrates should be selected that are only converted to product by one of the two or more present enzymes. Similarly, the substrates may preferably be chosen so that neither the substrates nor their products inhibit any of the enzymes present in the sample compartment.

Further, the enzyme may be coupled to an alternative detection system, such as an amplification system, such as a avidin-biotin anti-peroxidase technique (ABAP). The label may also be biotin, whereby the biotinylated antibody is detected using a labelled avidin or streptavidin, which is enzymelabelled. In a preferred embodiment the avidin or streptavidin is labelled with one of the enzymes discussed above. In another embodiment the label is avidin or streptavidin whereby the antibody is detected using a biotin, which is enzymelabelled. By labelling with biotin/avidin or streptavidin it is possible to enhance the signal as compared to labelling directly with enzyme and more preferred the enzyme is horse radish peroxidase.

Several embodiments involve the use LNA and PNA when the analyte is or contains DNA or RNA material.

In one embodiment the catalyst reaction may be controlled such as controlled by temperature or illumination or light exposure, so that the kinetics of the catalyst reaction is controlled. For example it may be controlled that the catalyst reaction does not commence before initiated, for example by changing the local temperature

of the catalyst, or by exposing the catalyst to light such as illumination to remove a substrate or co-factor, or illumination to convert a compound into a substrate or a co-factor. In another embodiment the catalyst reaction is controlled by pH, so that changes in the pH may increase the catalyst reaction. Also in a preferred embodiment it is possible to stop the reaction catalysed by the catalyst externally such as by controlling a temperature shift or the like.

Species-specific linkage

The term species-selective linkage is used synonymously with the term species-specific linkage, i.e. a linkage that is specific for the analyte the parameter of which is to be assessed. In one embodiment the species-selective linkage is antigen-antibody binding, using antibodies, such as monoclonal antibodies, directed to an epitope on the analyte.

The monoclonal antibodies may be labelled directly or indirectly. Direct labels typically include catalysts, such as enzymes, and biotin. Indirect labels include antibodies against the monoclonal antibody, said antibodies being labelled with catalyst labels, such as enzyme labels, or biotin.

In an indirect label the primary antibody or nucleotide probe directed against an epitope or nucleotide sequence on the analyte may be linked covalently to a compound such as biotin, streptavidin, avidin, a hapten, digoxigenin, dinitrophenyl or fluorescein. The analyte may then be visualised by a second label which specifically binds to the compound linked to the primary antibody or probe. Examples of such indirect labels include but are not limited to: hapten anti-hapten complex; biotin streptavidin complex; biotin avidin complex; digoxigenin anti-digoxigenin complex; dinitrophenyl anti-dinitrophenyl complex; fluorescein anti-fluorescein complex.

In the case where the primary antibody or probe is linked to biotin, signal amplification can be obtained by linking streptavidin to the biotin and further linking biotin-enzyme-biotin complexes to the streptavidin. Further rounds of amplification can be obtained by linking further streptavidin and biotin-enzyme-biotin complexes. The result is that several enzymes are linked via complex linkages to the epitope or nucleotide sequence instead of just one enzyme.

In another embodiment the species-selective linkage may be provided using DNA and/or RNA and/or PNA and/or LNA probes selective for DNA and/or RNA related to the analyte. The probes may be labelled directly or indirectly through additional probes as described above in relation to labelling of antibodies.

The invention also comprises the feature that an additional linkage is formed between a second species of analyte and a second catalyst.

The linkage may be formed in the sample domain by arranging the analytes and the catalyst in the sample domain and allowing the analyte-catalyst complex to form. In another embodiment the analyte-catalyst complex is formed before the sample or analytes are transferred to the sample domain.

After forming the analyte-catalyst complex excess catalyst not being linked to the species of analytes is preferably removed from the analyte-catalyst complexes. The excess catalyst may be removed through any suitable means, such as centrifugation, filtration and/or through flushing.

The steps of removing excess catalyst may also comprise binding the analyte-catalyst complex to a magnetic bead.

In other embodiments of the present invention the excess catalyst is substantially not removed from the analyte-catalyst complex. This obviously implies a more complex conditions under which any image is to be recorded since signals are generated not only in, at or in the vicinity of the analyte but also in the media surrounding the analyte. An example of where it could be possible to detect signals originating from products produced in, at or in the vicinity of the analyte is where the concentration or efficiency of the catalyst in the analyte-catalyst complex is greater than in the media surrounding the analyte.

The catalyst-analyte complex may further be contacted with co-factors or a buffer before contacting the complex with the substrate.

In the following non-exclusive list of examples of species specific linkages formed between the analyte and the catalyst, an asterisk * denotes an affinity binding such

as the binding between antibody and antigen or the binding between avidin and biotin. A dash - denotes a covalent bond.

analyte*antibody-catalyst
analyte*antibody*antibody-catalyst
analyte*antibody-biotin*avidin-catalyst
analyte*antibody-biotin*streptavidin-catalyst
analyte*antibody-avidin*biotin-catalyst
analyte*antibody-streptavidin*biotin-catalyst
analyte*antibody*antibody-avidin*biotin-catalyst
analyte*antibody*antibody-streptavidin*biotin-catalyst
analyte*antibody*antibody-avidin*biotin-catalyst
analyte*antibody* antibody-streptavidin*biotin-catalyst
analyte*antibody-biotin*streptavidin*(biotin-catalyst-biotin)$_n$*streptavidin$_n$ etc
analyte*antibody-digoxigenin*antidigoxigenin-catalyst
analyte*antibody-dinitrophenyl*antidinitrophenyl-catalyst
analyte*antibody-hapten*antihapten-catalyst
analyte*antibody-fluorescein*antifluorescein-catalyst

analyte*DNAfragment-catalyst
analyte*DNAfragment*DNAfragment-catalyst
analyte*RNAfragment-catalyst
analyte*RNAfragment*RNAfragment-catalyst
analyte*PNAfragment-catalyst
analyte*PNAfragment*PNAfragment-catalyst
analyte*LNAfragment-catalyst
analyte*LNAfragment*LNAfragment-catalyst
analyte*DNAfragment*RNAfragment-catalyst
analyte*DNAfragment*LNAfragment-catalyst
analyte*DNAfragment*PNAfragment-catalyst
analyte*RNAfragment*LNAfragment-catalyst
analyte*RNAfragment*PNAfragment-catalyst
analyte*RNAfragment*LNAfragment-catalyst
analyte*nucleotide probe-biotin*streptavidin*(biotin-catalyst-biotin)$_n$*streptavidin$_n$ etc
analyte* nucleotide probe -digoxigenin*antidigoxigenin-catalyst

analyte* nucleotide probe -dinitrophenyl*antidinitrophenyl-catalyst
analyte* nucleotide probe -hapten*antihapten-catalyst
analyte* nucleotide probe -fluorescein*antifluorescein-catalyst

It is likewise conceivable that the species specific linkage may be formed by reversing the position of two components participating in the affinity binding such as reversing the order of avidin*biotin or RNA*PNA etc.

Substrate

The substrate is typically added to the analyte-catalyst complex before recording any images of the sample domain.

The substrate may be mixed with analyte-catalyst complex before arranging the mixture in the sample domain. In order to avoid substantially any catalyst reaction to take place before the mixture is in place, the catalyst reaction may be controlled as described above in relation to the catalyst.

In another embodiment the analyte-catalyst complex is arranged in the sample domain before adding the substrate, where the mixture of substrate and analyte-catalyst is formed in the sample domain.

Catalysed reporter deposition

The catalyst analyte complex according to the invention may be amplified through the use of catalysed reporter deposition. By catalysed reporter deposition is meant the deposition, such as covalent bonding, onto the analyte of a number of reporter molecules. By this deposition of reporter molecules the number of catalysts linked to one species of analyte can be increased and thereby the signal originating from a single species of analyte can be increased. It is to be understood that reporter molecules encompass any molecule to which a catalyst according to the invention can be linked, e.g. through a species specific linkage.

Typically the reporter molecule comprises two components, a substrate, which will form the linkage to a receptor on the analyte, and covalently linked to the substrate component one part of an affinity binding pair.

In the following the principle of reporter activated deposition is explained using tyramin-biotin as an example. It is to be understood that tyramin-biotin is used for illustrative purposes only, and that other examples of reporters that can be used in catalysed reporter deposition are available to the skilled practitioner.

The first step in the procedure involves the binding of a peroxidase to the analyte through a species specific linkage, e.g. through linkage via a primary antibody directed against an epitope on the surface of the analyte.

After binding of the primary antibody, tyramin-biotin and H_2O_2 is added. Through oxidation by the peroxidase, the tyramin component of the reporter molecule is activated and forms a covalent linkage to electron rich moieties, such as to tyrosin or tryptophan residues, on the surface of the analyte within close distance of the peroxidase. The end result is that a number of biotin molecules are bound covalently to the analyte to which the peroxidase was first bound. In a second round of labelling, a further catalyst linked to avidin or streptavidin is added to the analyte to form linkages between the biotin linked to the analyte. The end result is thus that numerous catalysts are linked to each analyte for every primary antibody linked to the analyte initially.

A number of examples of the substrate component of the reporter molecule are disclosed in US 5,196,306 which is hereby incorporated by reference.

Chromography/luminescence

The substrate may be a chromogenic substrate or a luminogenic substrate, for example a photoluminogenic, an autoluminogenic or chemoluminogenic substrate such as a fluorogenic substrate.

A chromogenic substrate leads to a coloured product being detectable by measuring the absorbance. Any suitable chromogenic substrate used for conventional ELISA may be used herein, as long as the colour of the spot is distinguishable from the background.

A luminogenic substrate leads to a product capable of emitting photons, either when excited or by itself.

Examples of substrate systems which can be used in many embodiments of the present invention are:

AttoPhos (TM associated to JBL Scientific Inc. (San Louis Obispo, CA). The chemical formula of AttoPhos reported differently by the two references: **Zahra P. *et al.***; In Vitro Cell. Biol. - Animal 34: 772-776, 1998; A fluorometric assay for the measurement of endothelial cell density in vitro: 2′-[2-benzthiazoyl]-6′-hydroxy-benthiazole phosphate, or **Yu H. *et al.***; Analytical Biochemistry 261: 1-7, 1998; Development of a magnetic microplate chemifluorimmunoassay for rapid detection of bacteria and toxin in blood: (2′-[2-benzthiazoyl]-6′-hydroxy-benzthiazole phosphate bis-[2-amino-2-methyl-1, 3-propanediol])

4-MUP (4-methylumbelliferyl phosphate)

HNPP (Roche no. 1 758 888)

4-MUG (4-methylumbelliferyl beta-galactoside)

CDP-Star (Roche no. 1 685 627)

CSPD (Roche no. 1 655 884)

Super Signal Substrate (Pierce, Rockford, Ill). No chemical formula given. Reference: Trevanich S et al., Journal of Food Protection 63: 534-538, 2000; Rapid Detection of Enterotoxigenic Escherichia coli O6 in Water by Using Monoclonal Antibody and Photon-Counting Television Camera.

Luminol/4-iodophenol (e.g. BM Chemiluminescence ELISA Substrate (POD) - Roche no. 1 582 950).

Galacton Plus with and without enhancer (e.g. BM Chemiluminescence ELISA Substrate - Roche no. 1 759 787).

DAB (3,3′-diaminobenzidine tetrahydrochloride) with and without metal enhancer

OPD (o-phenylenediamine dihydrochloride) or free base

AEC (3-amino-9-ethylcarbazole)

5AS (5-aminosalicyclic acid)

2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)

4C1N (4-chloro-1-naphtol)

o-dianisidine (3,3′-dimethoxybenzidine)

TMB (3,3′,5,5′-tetramethylbenzidine) free base or dihydrochloride

ABTS

BCIP (5-bromo-4-chloro-3-indoyl phosphate) with and without NBT (nitro blue tetrazolium) or 2-(iodophenyl)-5-(4-nitro-phenyl)-3-phenyltetrazolium chloride)
Fast Red/Naphthol AS-MX (Sigma F4648)
Naphthol AS-TR phosphat (Sigma N8518) with an dwithout Fast Red RC (Sigma F5146)
pNPP (p-nitrophenyl phosphate)
PMP (phenolphthalein monophosphate - Sigma A3344)
X-Gal (5-bromo-4-chloro-3-indoyl-beta-D-galactopyranoside
CPRG (Chlorophenol-beta-D-galactopyranoside) EP patent 0146866 owned by Roche Diagnostics GmbH)

Chromogenic substrates

According to a preferred embodiment, the substrate is a chromogenic substrate, which can be detected by measuring it's absorbance. According to an especially preferred embodiment, the coloured product formed from the chromogenic substrate precipitates upon formation. In this way, it is ensured that the detectable product remains in the vicinity of the analyte to be detected. When the sample compartment is kept horizontal during detection, the product formed will simply precipitate on the lower surface of the sample compartment and form an insoluble precipitate there. Substrates that are especially suitable for this type of detection are those with a low water solubility.

In some cases it may be advantageous to add a filter to the detection device. Preferably the filter should be selective for the product to be determined. The advantage of the filters is that they can effectively filter away any background signals not coming from the relevant product. The system may also be equipped with several filters for the detection of two, three or more differently coloured products.

If the detector is a colour sensitive CCD there may be no need for colour specific filters.

Fluorescence

In a preferred embodiment the substrate is a fluorogenic capable of being converted into a fluorescent product by the catalyst. A system based on fluorescence is generally more sensitive than a chromogenic since fewer product molecules are necessary for recording an image from the sample domain. Therefore a shorter incubation time is generally necessary for a sufficient amount of product molecules to be produced and the image may be recorded faster than when using a chromogenic substrate.

A fluorogenic substrate preferably leads to a fluorescent product emitting signals in the wave length range of from 300 to 1200 nm when excited by excitation light. One preferred fluorescence method is the method of polarised fluorescence.

The excitation light source is any suitable light source capable of emitting excitation light in the range of from 250 nm to 600 nm, such as a light emitting diode (LED), a gas laser, a solid state laser, a laser diode, a gas lamp, a halogen lamp, or a xenon lamp.

It is preferred to use a diverging excitation light, such as light emitting diodes for in a cost-effective manner to expose as large area as possible of the sample to the excitation light.

It may be preferred to use more than one light source for the purpose of increasing the flux of light onto the sample, for instance by using two or more light emitting diodes. It is also possible to use more than one light source where some of the light sources have different electromagnetic properties.

By the use of several LEDs the sample is exposed to excitation light from several angles leading to a substantially optimal excitation of the sample, the light source are preferably operated in such a way that all transmit substantially simultaneously.

However for some application wherein at least a first and a second light sources are arranged in the first excitation light means, the first light source having a different wavelength band than the second light source, the light sources may transmit in an alternating manner. By the use of two different light sources it is possible to obtain

two different fluorescence signals from the sample. There is no upper limit to the number of LEDs used, but often as many as 30 LEDs are provided, such as up to 20 LEDs.

If a less diverging light source is used a diverging optical means may be arranged in the excitation light path to diverge the excitation light properly.

When using laser diodes as the excitation light the proper divergence may be accomplished by an arrangement of at least 4 laser diodes optionally provided with diverging means.

The incident angle of the excitation light is preferably in the range between 30° and 90°, more preferably between 45° and 85°, such as between 50° and 85° to provide a suitable excitation of the sample.

The excitation light may be transmitted directly to the sample, i.e. without being deflected by a beam splitter or the like whereby it is possible to construct the system and apparatus more compact.

Dual or multiple colour

In a preferred embodiment the correlation comprises distinction between at least two spectral properties of product. Thus, by the present invention it is possible to simultaneously detect at least two different types of analytes. This is achieved by using at least two antibodies directed towards two different analytes, and providing the two antibodies with two different enzymes, either directly or indirectly, and then further providing the relevant substrates for simultaneous or substantially simultaneous detection of the two different analytes due to evaluation of two different spots.

The spots arising from the analytes may either have two different colours, or one may be coloured and the other fluorescent, or both may be fluorescent emitting in two distinct wave length areas.

The two different types of analytes may be two different cells, for example specific IgG and IgA-secreting cells, or the two different states of the same cell, such as to distinguish between dead and living cells.

Also, in the latter situation the a dual labelling may be carried out by using one labelled antibody towards the analyte and then another type of labelling of the analyte to distinguish dead from living cells, such as conventional vitality staining.

It is understood, that more than two different analytes may be assessed hereby, such as three analytes for example.

Sample domain

The sample domain established according to the present invention may be a compartment or an equivalent thereof, wherein the sample is located during recording, such as a three-dimensional sample domain.

The sample domain may be a part of a flow-through system, wherein each sample is part of a series of samples, whereby one sample is replacing the previous sample in the sample domain.

In another embodiment the sample domain is part of a cassette, such as a disposable cassette as described in PCT/DK99/00605.

The sample is contained in the interior of the sample compartment, which normally has an average thickness of between 20 µm and 2000 µm, usually between 20 µm and 1000 µm and in many practical embodiments between 20 µm and 200 µm.

Normally, the sample compartment has dimensions, in a direction substantially parallel to a wall of an exposing window, in the range between 1 mm by 1 mm and 10 mm by 10 mm, but it will be understood that depending on the design, it may also be larger and, in some cases, smaller.

The part of the sample domain allowing signals to be detected is referred to as the exposing window that can be as little as 0.01 mm^2 or more, preferably with an area of 0.1 mm^2 or more, more preferably with an area of 1 mm^2 or more, preferably with

an area of 2 mm^2 or more, preferably with an area of 4 mm^2 or more, preferably with an area of 10 mm^2 or more, preferably with an area of 20 mm^2 or more, preferably with an area of 40 mm^2 or more, more preferably with an area of 100 mm^2 or more, preferably with an area of 200 mm^2 or more, preferably with an area of 400 mm^2 or more, preferably with an area of 1000 mm^2 or more, preferably with an area of 2000 mm^2 or more, preferably with an area of 4000 mm^2 or more, preferably with an area of 10000 mm^2 or more. Similarly, it is advantageous to extend the window of the sample compartment in a direction which is parallel to the plane of any window exposing signals from the sample to the exterior in order to extend the area of the exposing window and thus increase the volume of the sample which is exposed to the exterior.

Concerning the spatial definition of the shape and size of the area of an sample domain or a window exposing signals to the detection device there are at least two feasible methods for substantially reliable definition of the size and shape of this area. The first, and in many embodiments preferred method, is to adapt the detection device to be sensitive to exposed signals from a substantially defined area of the exposing window, e.g. by adapting any focusing means of the detection device. The second method, which is in particular preferred when it is difficult to adapt the sensing area of the detection device, is to define the boundaries of such exposing area of the sample compartment, e.g. either by controlling the dimensions of the sample compartment which define the exposing area, or by forming a mask or and effective window defining the exposing area, either in or on the disposable device or in connection with the detection device.

The requirements of the wall of the sample compartment are in particular that the wall allows the signals to pass without any significant limitations. In practice no upper limit is given for the wall thickness apart from what is defined by cost and design. The wall is preferably a substantially stable wall, which leads to a lower thickness limit for each material used. Preferably, the wall is from 0.1 mm to 2 mm, such as from 0.5 mm to 1.5 mm, more preferred from 0.75 mm to 1.25 mm.

In some embodiments, a flexible wall is useful, however, for quantitative measurements this will require measurement of the volume of the sample exposed before the assessment is carried out.

Sample volume

Sample volumes as small as 1 ml or less and even as small as 0.02 ml can be used. The optimal volume of the sample needed is highly dependent on the number of analytes present in the sample and the predetermined statistical quality parameter sought.

Other preferred embodiments of the present invention make it possible to assess analytes from a considerably large volumes of sample. This can allow the measurement of samples with only few analytes of interest per volume of sample. Sample volumes larger than 1 ml and even larger than 100 ml can be used for the analysis, the volume being defined as the total volume of any liquid sample introduced to any flow system connected to the device before the measurement of the sample.

Often the design of the sample compartment or the sample is such that the size of the volume of the liquid sample is sufficiently large to permit the assessment of the at least one quantity parameter or the at least one quality parameter to fulfil a predetermined requirement to the statistical quality of the assessment based on substantially one exposure, so that the image is recorded in one exposure. In another embodiment the assessment of at least one quality parameter or at least one quantity parameter is done by correlating more than one image to the at least one quality parameter or at least one quantity parameter, preferably by correlating two images, more preferably correlating more than two images, more preferably correlating more than four images. In these situations the images are recorded through two, three or more exposures.

Also, information about the changes in the image in course of time is used in the assessment of at least one quality parameter or at least one quantity parameter, and in such situations more than one exposure is made.

In many assessments of analytes it is of interest to allow exposure of signals from substantially large volumes of sample. The volume of the liquid sample from which signals such as electromagnetic radiation is exposed onto the detection system is normally in the range between 0.01 µl and 20 µl. Generally the volume of the

sample being analysed should be as large as possible. This allows the simultaneous assessment of a higher number of analytes, but the optimal volume is often defined by one or more aspects of the detection system and the sample being analysed. Thus the volume of the sample in the sample compartment can be less than 0.1 µl but often volume of more then 0.1 µl, 1.0 µl or even 10 µl is used. In still other application volume of the sample compartment as large as 100 µl or more can be used.

A large volume of the sample is preferably measured by passing the volume of sample through a analyte retaining means, such as a filter, electrical field, magnetic field, gravitational field, such means preferably being included in the device or can be arranged to interact with any sample within the device. The analyte retaining means should preferably be able to retain substantially all analytes present in a sample, or at least a substantially representative fraction of at least one type of analyte present in the sample.

When the analytes from a large sample are retained, those analytes can be resuspended in a volume which is less than the volume of sample passed through the analyte retaining means.

In one embodiment more than one portion of the same sample material can be subjected to analysis by exposure to the detection system. This can be done by allowing the sample compartment to be moved, thus exposing a different portion of the sample compartment.

Magnification

The method is preferably carried out at a low magnification whereby it is possible to detect spots in a large volume in one or a few exposures. The magnification factor is preferably below 20, such as below 10, such as below 5, such as 4, more preferably below 4, such as 2, more prefereably below 2, such as 1. The advantage of such low magnification are several, among other things increased area of observation and increased depth of focusing implying increased volume exposed to the detection device.

When the spots in question have dimensions which are comparable to the size of a detection element, it is often preferred to have magnification of about 1/1, thus focusing the image of any spot on any one or just few detection elements. This can under some condition give favourable detection of any signal.

When analysing spots which have dimensions which are comparable to, or bigger than the detection elements used, it is often advantageous to reduce the size of the image of such spot, to a degree where the size of the image is comparable to the size of a detection element. Thus in one embodiment it is preferred that the magnification factor below 1, preferably below 0.9, such as 0.8, more preferably below 0.8 such as 0.6, more preferably below 0.6 such as 0.5.

In these situations it is preferred that the ratio of the size of a spot, to the size of the image of the particle on the array of detection elements is 1/1 or less, preferably less than 1/1 and higher than 1/100, more preferably less than 1/1 and higher than 1/40, more preferably less than 1/1 and higher than 1/10, more preferably less than 1/1 and higher than 1/4, more preferably less than 1/1 and higher than 1/2.

Thus, it is often preferred that the spatial representation exposed onto the array of detection elements is subject to such a linear enlargement that the ratio of the image of a linear dimension on the array of detection elements to the original linear dimension in the sample domain is smaller than 40:1, normally at the most 20:1, preferably smaller than 10:1 and in many cases even at the most 6:1 or even smaller than 4:1.

The aspect ratio of an image can be considerably distorted on the array of detection elements, without that having considerable negative effect on the assessment of analytes. In such a situation it preferred that the ratio of the shorter to the longer of the two dimensions of the image of a particle on the array of detection elements is substantially 1 or less, preferably 1/2 or less, more preferably 1/4 or less, more preferably 1/10 or less, more preferably 1/50 or less, more preferably 1/100 or less, more preferably 1/200 or less, relative to the ratio of the corresponding dimensions of the particle. In such situation the ratio of the shorter to the longer of the two dimensions of the image of a particle on the array of detection elements is in certain

circumstances substantially not the same within the area spanned by the array of detection elements.

It is often preferred that the image of the product from the individual analytes the parameter or parameters of which is/are to be assessed are imaged on at the most 25 detection elements, in particular on at the most 16 detection elements and more preferred at the most 9 detection elements. It is even more preferred that the image of the product from the individual analytes the parameter or parameters of which is/are to be assessed are imaged on at the most 5 detection elements, or even on at the most 1 detection element. The larger number of elements per analyte will provide more information on the individual analytes, while the smaller number of elements per analyte will increase the total count that can be made in an exposure.

Statistics

As mentioned above, the size of the volume is suitably adapted to the desired statistical quality of the determination. Thus, where the determination is the determination of the number of analytes in a volume, or the determination of the size and/or shape of analytes, the size of the volume of the liquid sample is preferably sufficiently large to allow identification therein of at least two of the analytes. More preferably, the size of the volume of the liquid sample is sufficiently large to allow identification therein of at least four of the analytes. This will correspond to a repeatability error of approximately 50%. Still more preferably, the size of the volume of the liquid sample is sufficiently large to allow identification therein of at least 10 of the analytes. This will correspond to a repeatability error of approximately 33%. Even more preferably, the size of the volume of the liquid sample is sufficiently large to allow identification therein of at least 50 of the analytes. This will correspond to a repeatability error of approximately 14%. Evidently, where possible, it is preferred to aim at conditions where the size of the volume allows identification of even higher numbers. Thus, when the size of the volume of the liquid sample is sufficiently large to allow identification therein of at least 100 of the analytes, it will correspond to a repeatability error of approximately 10%, and when the size of the volume of the liquid sample is sufficiently large to allow identification therein of at least 1000 of the analytes, it will correspond to a repeatability error of as low as approximately 3%.

Stand still

In a preferred embodiment of the invention the analytes being assessed are substantially at stand-still during analysis, thus allowing the optimal use of measurement time in order to improve any signal to noise conditions. This arrangement also eliminates any error which could be inherent in the assessment of analytes caused by variation in flow conditions, particularly when an assessment of a property is a volume related property such as the counting of analytes in a volume of sample.

Flow system

The introduction of analyte, catalyst and substrate into the sample domain may be provided by means of a flow system. The flow system may provide at least one of several operations to be carried out on the samples, said operations being selected from but not limited to transport, mixing with reagent, homogenising of sample and optionally reagent, heat treatment, cooling, sound treatment, ultra sound treatment, light treatment and filtering.

In order to flow the sample into or within or out of the sample domain it is preferred to have at least one propelling means provided in the system.

Preferably the flow regulation means is arranged to function stepwise so that the sample and/or the reagent component may be flowed stepwise through the system.

The sample in the device can be flown by the means of a flow system, which can be driven by a pump or a pressurised gas, preferably air, or by causing a pressure difference such that the pressure on the exterior of the inlet is higher than the pressure within at least a part of the system thus forcing the sample to flow through the inlet. In many embodiments of the present invention the flow in said flow system is controlled by one or more valves which can adjust the flow speed of the sample.
In many preferred situations the flow of liquid in the device can be brought about by a vacuum, the vacuum being applied from a reservoir, preferably contained within the device. The vacuum can be established by a mechanical or physical action creating the vacuum substantially simultaneously with the introduction or the movement of the sample. These mechanical or physical actions can be: a peristaltic

pump, a piston pump, a membrane pump, a centrifugal pump and a hypodermic syringe.

The outlet from the sample compartment can be passed through a flow controlling means, such as a valve, which only allows gas to pass through. One such type of valves which often is preferred, is one which allows gas and air to pass but can close irreversibly when the valve comes in contact with liquid sample. The effect of such valve is to minimise the movement of any sample within the sample compartment during analysis.

In a preferred embodiment of the invention the system contains at least one compartment wherein the mixing of the sample material with catalyst and/or media is possible.

One advantage of the present system and method is that the analysis is carried out using only liquid reagents and analytes suspended or dissolved in liquid. This layout ensures ease of operation and handling. Surprisingly it has been determined that it is possible to detect analytes specifically in the absence of bonds to any solid support.

Detection device

The image which can be detected from the window of the device can for instance be detected by an array of detection elements, the array of detection elements comprising individual elements each of which is capable of sensing signals from a part of the sample window area, the array as a whole being capable of sensing signals from substantially all of the sample window area, or at least a well defined part of the sample window area. The array of detection devices may for example be a one-dimensional array or a two-dimensional array. In order to facilitate the assessment of analytes the intensities detected by the array of detection elements are processed in such a manner that representations of electromagnetic signals from the analytes are identified as distinct from representations of electromagnetic background signals.

The detection means may comprise any detectors capable of sensing or detecting the signal emitted from the sample such as a fluorescence signal.

In a preferred embodiment detection means comprises a detector being an array of detecting devices or detection elements, such as a charge coupled device (CCD) the CCD may be a full frame CCD, frame transfer CCD, interline transfer CCD, line scan CCD, an eg. wavelength intensified CCD array, a focal plane array, a photodiode array or a photodetector array, such as a CMOS. The CMOS is preferably a CMOS image sensor with on-chip integrated signal condition and/or signal processing. Independent of the choice of any of the above detection devices the detection means may further comprise a white/black or colour CCD or CMOS.

Confocal scanning optical microscopes are known in the art and offer a number of advantages over traditional optical microscopes. One main advantage of a confocal scanning microscope is that it provides optical sectioning of a sample because it attenuates light which is not in focus. Thus, only light which is in focus contributes to the final image.

In a scanning confocal microscope, a beam is swept across a surface of a sample. The light which emanates from the sample (e.g., reflected from, emitted from or transmitted through) is directed towards a pinhole. Light that is in focus passes through the pinhole and onto an optical detector. As the beam is scanned across the surface of the sample, the output from the optical detector can be accumulated and formed into an image of the scanned surface.

Use of a confocal scanning microscope especially a confocal laser scanning microscope for detecting the signals from the product formed in the sample domain is advantageous due to the greater sharpness of the detected image.

The size of the detection elements determines to some extend its sensitivity. In some applications it is therefore of interest to have detection elements of size of about 1 μm^2 or less. In certain situations the size of the detection elements in the array of detection elements is less than 20 μm^2, preferably less than 10 μm^2, more preferably less than 5 μm^2, more preferably less than 2 μm^2, more preferably less than or equal to 1 μm^2. In other situations the size of the detection elements in the array of detection elements is greater than or equal to 5000 μm^2, such as greater than or equal to 2000 μm^2, more preferably greater than or equal to 1000 μm^2, such

as greater than or equal to 500 μm^2, or even greater than or equal to 200 μm^2, more preferably greater than or equal to 100 and less than 200 μm^2, more preferably greater than or equal to 50 and less than 100 μm^2, more preferably greater than or equal to 20 and less than 50 μm^2.

The array of detection elements is preferably sensitive to electromagnetic radiation of wavelength in one or several of the following regions: 100 nm to 200 nm, 200 nm to 600 nm, 300 nm to 700 nm, 400 nm to 800 nm, 600 nm to 1 µm, 800 nm to 2 µm, 2 µm to 10 µm, 5 µm to 10 µm, 10 µm to 20 µm, 20 µm to 40 µm.

The inclusion of a focusing device for the focusing of a signal from the sample onto the detection elements in such a manner as to maximise the collection angle, the collection angle being defined as the full plane angle within which a signal is detected, has in many situations been found to give improved conditions for an assessment. Surprisingly it was found that such a wide collection angle, even to the extent that the objective used in the focusing distorted the aspect ratio of the image of any analyte differently across the plane in which the detection elements were placed, or produced variation in the focusing across the sample being analysed, or reduction of the focusing quality, could be used in the assessment of for example the number of analytes in the sample.

The aspect ratio of the detection elements can be important in the collection of signals for the assessment of analytes. A ratio of about 1/1 is some times preferred, but under some conditions it can be preferred to use ratio different from 1/1. In particular when this facilitates detection of signals from increased volume of any sample, thus allowing simultaneous assessment of for examples more analytes. In those circumstances the ratio of the shorter of the height or the width, to the longer of the height or the width of the detection elements in the array of detection elements is substantially equal or less than 1, preferably less than 1/2, more preferably less than 1/4, more preferably less than 1/10, more preferably less than 1/50, more preferably less than 1/100, more preferably less than 1/200.

Focusing - Lenses

Signals from at least a portion of the sample are focused onto the array of detection elements, by the use of a focusing means, preferably by the use of one lens, it is

however possible to use two lenses, or more than two lenses. The number of lenses used for the focusing system can affect the complexity of any measuring system.

The focusing of a signal from the sample onto any detector is dependent on the position of the sample relative to any detector. When the construction of measuring system is such, that the relative position of the sample and any detector can vary, then there is advantage in being able to adjust the focusing of the system. This can often be achieved by first taking at least one measurement of any signal from the sample and then on the bases of this, to adjust the focusing of the system. This procedure can be repeated a number of times in order to obtain acceptable focusing. In the same manner the focusing of signal from the sample or sample material is adjusted, preferably where the extend of the adjustment is determined by at least one measurement of a signal from the sample.

The collection angle of a focusing arrangement used can have effect on the intensity of any signal collected on the array of detection elements. When high sensitivity is needed it is therefore practical to increase the collection angle. The preferred size of the collection angle can also be determined by other requirements which are made to the system, such as focusing depth. In these situations the collection angle of the focusing means is preferably at least 2 degrees, preferably more than 5 degrees, more preferably more then 15 degrees, more preferably more than 20 degrees, more preferably more than 50 degrees, more preferably more than 120 degrees, more preferably more than 150 degrees.

Signal

The signals measured from one or more detection elements may be corrected for systematic or varying bias by the use of a calculating means, the bias correction being accomplished by the use of one or more pre-defined value(s), preferably where each measured signal for one or more detection elements in said array of detection elements has one or more pre-defined value(s), more preferably where each pre-defined value is determined on the bases of one or more of any previous measurements.

The bias correction may be performed by subtracting the results obtained in one or several of other measurements from the measured signal, preferably where the

other measurements are one or several of measurements of the same sample, or sample material, more preferably where the other measurement is the measurement taken previously of the same sample or sample material.

Also the signal from one or more detection elements may be corrected for intensity by the use of a calculating means, said correction being accomplished by the use of one or more pre-defined value(s), preferably where each measured signal for one or more detection elements in said array of detection elements has one or more pre-defined value(s), more preferably where each pre-defined value is determined on the bases of one or more of any previous measurements.

In some situations e.g. in an analogue-to-digital conversion it could also be of interest to adjust the level of 2, preferably 3, more preferably 4, more preferably 5, more preferably 6, more preferably 7, more preferably 8, more preferably more than 8, separate output channels in such a way that one, preferably more than one, of the output channels has/have substantially different level from the other output channel(s), where the identification of which of the output channels, or combination thereof, has substantially different output level, is correlated to the intensity of said signal.

For the analysis of any measured signal it is often necessary to digitalise the signal, in such a way that a given intensity of any signal is transformed into a digital representation. This can be done by having a series of channels, were the information about which of these channels has signal which differs from the other channels determines the intensity, or even by having more than one of this channels forming a combination, preferably in a way similar to binary representation.

Processor

Information of the signals detected by the detection means are input into a processor for processing, displaying and optionally storing the information.

The signal information may be displayed on a display connected to the processor and/or printed. The information displayed may be any kind of information relating to the signals measured and/or the system used, such as a number, size distribution, morphology, classification of analytes, excitation wavelength, emission wavelength,

magnification. In particular the data processing means is capable of distinguishing partially overlapping areas of product.

Storage capacity, for instance used for storing information about measured signals from the detection elements, is often one of those components which have considerable effect on the cost of production. It is therefore of interest to be able to perform the assessment of parameters without substantial any use of such storage capacity, such that the assessment of analytes in a sample is performed without the use of substantially any storage capacity means being used to store measured signals from the detection elements in the array of detection elements.

On the other hand, it is often difficult to accomplish assessment without the use of any storage capacity, but preferably the amount of such storage capacity should not be more than what is needed to store the information from all measured detection elements, preferably where only a fraction of the information can be stored.

In some situations measured signal from the detection elements in the array of detection elements is stored by means of storage capacity, the storage capacity being able to store a number of measurements equivalent to, or less than, the number of detection elements, preferably less than 1/2 the number of detection elements, more preferably less than 1/4 the number of detection elements, more preferably less than 1/8 the number of detection elements, more preferably less than 1/16 the number of detection elements, more preferably less than 1/32 the number of detection elements, more preferably less than 1/64 the number of detection elements, more preferably less than 1/128 the number of detection elements, more preferably less than 1/256 the number of detection elements, more preferably less than 1/512 the number of detection elements, more preferably less than 1/1024 the number of detection elements in the array of detection elements.

In other certain circumstances it is advantageous that the measured signal from the detection elements in the array of detection elements is stored by means of storage capacity, the storage capacity being able to store a number of measurements greater than the number of detection elements, preferably equivalent to, or greater than, 2 times the number of detection elements, more preferably equivalent to, or greater than, 4 times the number of detection elements, more preferably equivalent

to, or greater than, 8 times the number of detection elements, more preferably equivalent to, or greater than, 16 times the number of detection elements, more preferably equivalent to, or greater than, 32 times the number of detection elements, more preferably equivalent to, or greater than, 64 times the number of detection elements, more preferably equivalent to, or greater than, 128 times the number of detection elements, more preferably equivalent to, or greater than, 256 times the number of detection elements, more preferably equivalent to, or greater than, 512 times the number of detection elements, more preferably equivalent to, or greater than, 1024 times the number of detection elements in the array of detection elements.

Other, more complicated aspects of the assessment of parameters, can require the use of considerable amount of storage capacity. In this aspect it can therefore be necessary to have storage capacity which can store more information than is collected in one measurement of the detection elements used.

It is possible to make the correlation and the assessment of the parameters of the sample by using a calculation mean, preferably a digital computer, one commercially available from Analogue Devices (ADSP 2101), equipped with storage capacity which can only store information in amount substantially equivalent to a small fraction of the total number of detection elements, the assessment of the number of objects then being based on substantially real time processing of data, preferably in such a way that the measured information from each detection element, or a line of detection elements, or two or more lines of detection elements, is used for the assessment, substantially without any delay, such as a delay which would otherwise be caused by storing the measured information.

However, it is often preferred to store substantially all measured information by the use of a first calculation mean, preferably a digital computer, before the processing of the information by a second calculation mean, preferably a digital computer, and thus allowing the measured information to be processed at substantially the same rate it is obtained, but with a substantial time delay between the measurement of any information and the processing of the same information; preferably, this is accomplished by using only one calculating mean, preferably a digital computer, equipped with enough resources to accomplish the task.

Medical markers

The system and the method of the current invention may be used for detection of clinical markers of conditions in a human being or in an animal. One preferred use of the method and system is for the early diagnosis of myocardial infarction or cardial infarct. When cells in the heart suffer or die they leak a number of enzymes into the blood. The blood level of these enzymes raise above the normal levels hours before the patient show acute symptoms of cardial infarct. A reliable and early diagnosis can thus be made by measuring the blood level of cardial related enzymes.

According to prior art techniques the enzymes are measure by subjecting a blood sample to traditional ELISA in the laboratory. The duration of this ELISA detection is several hours. During this period the patient does not show any acute symptoms of cardial infarct. At the moment the diagnosis is ready, the cardial infarct may have developed to a level, where it is too late to save the patient's life.

It is envisaged that the blood levels of these particular enzymes and of other enzymes being medical or clinical markers can be measured rapidly using the detection technique of the present invention.

The system may be used for detection of clinical or medical markers in essentiallly all animals, particularly in mammals such as human beings, cows, horses, poultry, sheep, goat.

One-sided and two-sided systems

The detection device may be laid out as a one-sided device, i.e. a device for which the light is directed to the sample from the same side of the sample as the side for which the signals are detected.

The detection device may also be laid out as a one-sided device, in which the excitation light is directed to the sample from the same side of the sample as the side for which the signals emitted from the sample are detected.

By this apparatus a variety of advantages have been achieved as compared to conventional fluorescence microscopes. First of all it is possible to arrange the sample to be assessed directly in the sample plane instead of sliding it into the sample plane between the detector and the excitation light. Furthermore it has become possible to detect surface fluorescence of a sample not being transparent.

It is also possible to increase the intensity of the excitation light without compromising the detectors.

Also samples having a nature whereby it is normally not possible to arrange the sample in a microscope may be assessed by the use of the present system, in that the microscope may be placed directly on the sample whereby the surface of the sample simply constitutes the sample plane.

Finally it is possible to produce a more compact and thereby more easily handled apparatus, in that the excitation light means is arranged on the same side of the sample plane as the detector, thus shortening the axis of the apparatus by at least 25% as compared to conventional apparatuses.

By the present invention it is possible to assess parameters of a sample which has up to now only been reliably assessed by the use of flow cytometric equipment. It is possible to assess parameters of a large sample in one exposure thus reducing the statistical errors normally counted for when assessing large samples by assessing only parts thereof per exposure.

Furthermore, it is possible to obtain more than one fluorescence signal from the sample in one exposure thereby facilitating classification of particles of the sample, due to their different fluorescence signals.

Thus, the one-sided apparatus according to the invention may be constructed in a wide variety of combination, which are all within the scope of this invention. In particular the principal combination discussed below are envisaged.

The apparatus may be constructed as a single fluorescence apparatus wherein the light sources and the excitation light filters are identical.

A multiple fluorescence apparatus, such as an apparatus providing at least two different fluorescence signals, may be provided by at least one of the following:

- A first and a second light source, said light sources emitting light of different wavelengths
- A first and a second filter being different whereby the excitation light of at least two different wavelengths are exposed to the sample
- A first and a second emission filter being different, such as a dual band filter, whereby at least two different fluroescence signals are emitted to the detector(s)

It is however a further advantage that the present apparatus may be constructed as a double-sided apparatus, whereby excitation light may be directed onto the sample from both sides of the samples, or detection means are arranged to detect signals from both sides of the samples, or a combination of both.

Thus by a double-sided apparatus is meant an apparatus according to the invention further provided with:

- A second excitation light means located in a second light plane, said second light plane being parallel with the sample plane and located on the other side of the sample plane as opposed to the first light plane. Thereby the sample is receiving excitation light from both sides of the sample considerably increasing the energy exposed to the sample, and/or
- A second detection means arranged so that the sample is positioned between the first detection means and the second detection means. Hereby it is possible to assess different information regarding the signals from the sample by one exposure detection. For example the first detection means may be adapted to register the number of particles of the sample, whereas the second detection means is adapted to register the morphology of the particles in the sample.

In a preferred embodiment the double-sided apparatus comprises both double-sided excitation system and double-sided detection system.

The second excitation light means may be any of the light means discussed in relation to the first light means. Depending on the purpose of the fluorescence microscope the light means may be different or identical.

Furthermore, it may be of interest that the excitation light would constitute different wavelength bands whereby illumination with different wavelengths is achieved.

The second detection means may be any of the detection means discussed in relation to the first detection means.
Any suitable combination of light sources, filters, magnification and detectors are envisaged by the present invention. In the following preferred embodiments of the two-sided system is discussed.

The apparatus may be a single fluorescence system, wherein excitation light of substantially identical wavelength are exposed to the sample from two sides. Thereby the excitation light may be intensified.

In a double-sided excitation light apparatus a first excitation light means exposes the sample to one wavelength from one side of the sample, and the second excitation light exposes the sample to another wavelength from the other side of the sample. It is understood herein, that of course the first excitation light and the second excitation light respectively, may comprise different light source and/or filters, whereby the sample may be illuminated with even more wavelengths as discussed above.

The double-sided excitation light apparatus may comprise one detector, whereby the apparatus functions as a partly transmitting system.

In another embodiment the double-sided excitation light apparatus comprises two detecting means. Thereby an increased amount of information may be obtained from the sample. In one aspect the two detecting means may obtain equal, although

mirror images (the images on the two detectors are mirror images of each other), information relating to the sample providing a validation of the information.

The apparatus according to the invention may also be a double-sided detection apparatus using a one-sided excitation light means. Thereby one detector detects signals being transmitted through the sample.

Independent of the arrangement of excitation light, a double-sided detecting system is capable of increasing the amount of information received. For example different wavelength may be received by the two detectors, and or different detectors, having different sensibility may be used. Furthermore, by using for example different magnification for the two detectors the information relating to the sample may be increased. One side of the system may assess for example number of particles in a large area of the sample, for example by a low magnification, and the other side of the system may assess the morphology of the particles by using a larger magnification. Combinations of magnification may for example be 1:1 and 1:4, 1:1 and 1:10, 1:2 and 1:4, 1:2 and 1:10. The signal information transferred from the two detectors is preferably transmitted to the same processor, whereby the information may be displayed separately, as well as being combined providing for example specific morphology information related to specific particles the position and number of which are detected by the other detector.

It is also possible to use the apparatus according to the invention as a double-sided apparatus where the other side is a conventional light microscope or any other type of microscope. When using the other side of the system as a non-fluorescence microscope, the illumination light for the microscope may be suitably arranged on either side of the sample in relation to the microscope.

The double-sided apparatus comprising a conventional microscope on one side, may comprises a one-sided or a double-sided excitation light system for the fluorescence part of the system.

When using a double-sided detection system the processor of the first detection means may receive signal data from the second detection means as well in order to

simplify the apparatus. It is however possible to install a separate processor for each detection means.

Examples of one and double sided excitation and detection systems

In the following one embodiment of the detection system is discussed in more detail in relation to the drawings.

In Fig. 1 an example of the illumination and detection system 1 is shown in schematic form. The sample is arranged in a sample compartment 2 the sample plane. Excitation light from the light sources 4a, 4b in the excitation light means 3 is exposed onto the sample through a main light path 5a, 5b.

Fluorescence signals from the sample is emitted to the detection means 6 comprising at least one detector 7. The path of the emitted signals is following an axis between the sample and the detector, the detection-sample axis 8.

The signal data are transmitted to a processor 9 coupled to the detecting means 6. The fluorescence signals from the sample is filtered by means of emission filter 14 and focused to the detection means 9 by means of a focusing lens 10.

The light sources 4a, 4b are arranged in a light housing 11, whereby the transmission of excitation light directly to the detection means is avoided. Furthermore excitation light filters 12a, 12b are positioned in the excitation light beam.

Fig. 2 shows a cross-section of the circular supporting material 13 of the excitation light filters wherein the position of the light sources have been indicated by circles in broken lines.

In Fig. 3 the light path and signal path is shown in more detail. In the light path the main light path is shown as 5. Furthermore, the detection-sample axis is shown by broken lines 8. The collection angle of the system is denoted C shown between two arrows and the angle between the main light path and the detection–sample axis is denoted E.

In Fig. 4 a double-sided excitation/detection system 1 is shown wherein the systems on each side of the sample are identical and as described for the one-sided system of Fig. 1.

Fig. 5 shows a double-sided excitation system wherein excitation light from the light sources 4a, 4b in the first excitation light means 3a and excitation light from the light sources 4a, 4b in the second excitation light means 3b is exposed onto the sample 2 from both sides of the sample 2. As discussed above, the light sources may be identical or different depending on the information to be assessed. Furthermore, the filters used for each light source may be different or identical.

Fluorescence signals are transmitted through and reflected from the sample due to the excitation light arrangement and emitted to the detection means 6. The path of the emitted signals is following an axis between the sample and the detector, the detection-sample axis 8.

The signal data are transmitted to a processor coupled to the detecting means as described above.

Fig. 6 shows a double-sided detecting system, using a single-sided excitation system, wherein reflected fluorescence signals from the sample 2 are detected by detecting means 6a comprising detector 7a. The reflected fluorescence signals are transmitted though filter 14a and focused by lens 10 a.

Furthermore, transmitted fluorescence signals from the sample 2 are detected by detecting means 6b comprising detector 7b. The reflected fluorescence signals are transmitted though filter 14b and focused by lens 10b.

Filter 14a is preferably different from filter 14b, whereby information relating to at least two different fluorescence signals is obtainable.

Also the magnification in the two detecting systems may be different, for example by lens 10a being different from lens 10b.

Example

Sample type	Human whole blood anticoagulated with sodium citrate.
Analyte	Human CD45+ blood cells with CD45 antigen localised on the surface.
Antibody-conjugate	Monoclonal mouse IgG (Fab) specific for CD45 antigen. IgG is conjugated to biotin (100 µg antibody conjugate per ml of Phosphate Buffered Saline with pH 7.2 (PBS)).
Avidine-AP conjugate	Streptavidine conjugated to Alkaline Phosphatase (10 µg Avidine conjugate per ml PBS).
Enzyme substrate	AttoPhos™ Substrate Set (Boehringer Mannheim no. 1681 982). Alkaline Phosphatase will convert the AttoPhos Substrate to a fluorochrome (the Product) which has maximum exitation at 435 nm and maximum emission at 560 nm.
Lysis Buffer A + B	Uti-Lyse (Dako no. S-3350) is used for lysis of red blood cells but leaving the cell membrane of the white blood cells sufficiently intact in order to carry out a surface marker based cell analysis.
Microscopic counting	The counting of spots derived from the CD45+ target cells can be carried out using an EPI-fluorescence microscope equipped with a suitable light source (e.g. xenon lamp), a suitable filter set for the Product, a 4x objective, a CCD camera and a Bürker-Türk counting chamber (depth 0.1 mm).

A portion of 10 µl of antibody conjugate is added to 100 µl of sample. Following gentle mixing and incubation for 30 minutes at room temperature (RT) a portion of 2

ml PBS is added to the suspension. Following gentle mixing the suspension is centrifuged at 300g for 5 minutes. Then the supernatant is removed and the pellet is resuspended in 2 ml of PBS. Following gentle mixing the suspension is centrifuged at 300g for 5 minutes. Then the supernatant is removed and the pellet is resuspended in 2 ml of PBS. Following gentle mixing the suspension is centrifuged at 300g for 5 minutes. The supernatant is removed (leaving approximately 100 µl fluid) and a portion of 100 µl of Lysis Buffer A is added. Following gentle mixing the suspension is incubated for 10 minutes at RT. A portion of 1 ml of Lysis Buffer B is added to the suspension and incubated for 10 minutes at RT. Then the suspension is centrifuged at 300g for 5 minutes. Then the supernatant is removed and the pellet is resuspended in 2 ml of PBS. Following gentle mixing the suspension is centrifuged at 300g for 5 minutes. Then the supernatant is removed and the pellet is resuspended in 2 ml of PBS. Following gentle mixing the suspension is centrifuged at 300g for 5 minutes.

Then the supernatant is removed and a portion of 100 µl of Avidine-AP conjugate is added to the pellet. Following gentle mixing and incubation for 15 minutes at RT a portion of 2 ml PBS is added to the suspension. Following gentle mixing the suspension is centrifuged at 300g for 5 minutes. Then the supernatant is removed and the pellet is resuspended in 2 ml of PBS and after gentle mixing the suspension is centrifuged at 300g for 5 minutes. After removing the supernatant the pellet is resuspended in 2 ml of PBS and after gentle mixing the suspension is centrifuged at 300g for 5 minutes. After removing the supernatant the pellet is resuspended in 500 µl of AttoPhos™ Substrate solution prepared according to manufacturers instruction. Immediately, a small portion of the suspension is applied to a Bürker-Türk counting chamber and then an image is created using the EPI-fluorescence microscope. With short intervals of 1 second further images are created and the formation of dots and the growths of these dots is observed. The dots will increase in diameter due to diffusion of the product.

CD45+ cells can be observed as fluorescent dots within the measuring volume. Based on the number of dots and the volume of the mixture in Bürker-Türk counting chamber which is represented by the image a concentration of CD45+ cells in Bürker-Türk counting chamber can be calculated. The concentration of CD45+ cells in the the blood sample can then be calculated because the dilution factor is known.

Claims

1. A method for assessing at least one quality parameter or at least one quantity parameter of at least one species of analytes in a sample comprising the steps of

establishing a sample domain having at least one wall,

arranging catalyst-analyte complexes between the at least one species of analytes and at least one catalyst in a manner allowing the analytes to move relative to the wall(s) of the sample domain,

arranging a substrate in the sample domain, said substrate being capable of being converted into a product through catalysing by said catalyst,

contacting the substrate with the catalyst-analyte complexes of individual analytes allowing a detectable amount of product to be produced,

recording an image of the product related to individual analytes in the sample domain,

correlating the image to the at least one quality parameter or at least one quantity parameter of the at least one species of analytes.

2. The method according to claim 1, wherein the catalyst-analyte complex comprises a species-selective linkage.

3. The method according to claim 2, wherein the species-selective linkage comprises an antigen-antibody linkage.

4. The method according to claim 2, wherein the species-selective linkage comprises a DNA, or RNA, or PNA, or LNA hybridisation.

5. The method according to claim 1, wherein formation of the catalyst-analyte complex comprises catalysed reporter deposition.

6. The method according to claim 1, whereby analytes are particles, such as biological particles.

7. The method according to claim 1, wherein the analytes are bound to a solid support, preferably where such solid support are beads in suspension.

8. The method according to claim 1, whereby the analytes are selected from the group consisting of cells, cell walls, bacteria, plasmodia, virus, prions, macromolecules, proteins, polypeptides, peptides, genes, DNA, RNA, or fragments or clusters thereof.

9. The method according to claim 1, whereby the at least one species of analyte is a medical marker of a disease.

10. The method according to claim 9, whereby the marker is a marker for cardial infarct.

11. The method according to claim 8, wherein the cells are selected from mammalian cells, insect cells, reptile cells, fish cells, yeast cells, and fungi cells.

12. The method according to claim 8, wherein the cells are selected from blood cells, sperm cells, and bone marrow cells.

13. The method according to any of the preceding claims, whereby the sample is a liquid sample.

14. The method according to any of the preceding claims, whereby the sample is selected from the group consisting of milk, milk products, urine, blood, sperm, nasal secrete, tears, faeces, waste water, process water drinking water, cerebro-spinal fluid, gall, bone marrow, food, feed, and mixtures, dilutions, or extracts thereof

15. The method according to any of claims 1-12, whereby the sample is a solid sample, which is pre-treated prior being arranged in the sample domain.

16. The method according to claim 15, whereby the sample is a biopsy of a muscle, a brain, a kidney, a liver, a spleen.

17. The method according to any of the preceding claims, whereby the substrate is AttoPhos, 4-MUP, HNPP, 4-MUG, CDP-Star, CSPD, Super Signal Substrate (Pierce, Rockford, Ill), Luminol/4-iodophenol, Galacton Plus, DAB, OPD, AEC, 5AS, 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid), 4C1N, o-dianisidine, TMB, ABTS, BCIP, Naphthol AS-TR phosphat, pNPP, PMP, X-Gal, CPRG.

18. The method according to any of the preceding claims whereby the catalyst is an inorganic catalyst.

19. The method according to any of the claims 1-17, whereby the catalyst is an organic catalyst.

20. The method according to claim 19, whereby the catalyst is an enzyme.

21. The method according to claim 20, whereby the catalyst is selected from the group consisting of phosphatase such as alkaline phosphatase, β-galactosidase, peroxidase such as for example horseradish peroxidase, β-glucuronidase, β-glucose-6-phosphate dehydrogenase, glucose oxidase, urease, luciferase, β-lactamase and β-amylase.

22. The method according to any of the preceding claims, whereby at least one obtained product precipitates upon formation.

23. The method according to claim 22, whereby at least one obtained product precipitates on a surface of the sample compartment.

24. The method according to any of the preceding claims, whereby at least one obtained product is coloured.

25. The method according to any of the preceding claims, whereby at least one obtained product is photoluminescent or chemiluminescent.

26. The method according to any of the preceding claims, whereby at least one obtained product is fluorescent.

27. The method according to any of the preceding claims, whereby at least one obtained product emits electromagnetic radiation in the range of 300 nm to 1200 nm when exposed to electromagnetic radiation in the range of 250 nm to 600 nm.

28. The method according to any of the preceding claims, wherein at least one product obtained is excited by excitation light prior to recording an image.

29. The method according to claim 28, wherein the excitation light is a light source selected from the group of, light emitting diode (LED), gas laser, solid state laser, laser diode, gas lamp, halogen lamp, xenon lamp.

30. The method according to any of the preceding claims, whereby the sample domain is three-dimensional.

31. The method according to any of the preceding claims, whereby the sample domain is a flow through chamber.

32. The method according to any of the preceding claims, whereby the sample domain is part of a disposable cassette.

33. The method according to any of the preceding claims, whereby at least one wall of the sample domain is transparent.

34. The method according to any of the preceding claims, whereby at least one linkage between the catalyst and the analyte comprises two or more antibodies.

35. The method according to any of the preceding claims, whereby at least one catalyst is conjugated to an antibody being immunologically bound to an antigen on the species of analyte.

36. The method according to any of the preceding claims, whereby at least one catalyst is conjugated to a first antibody being immunologically bound to second antibody, being immunologically bound to an antigen on the species of analyte.

37. The method according to any of the preceding claims, whereby at least one catalyst is conjugated to avidin.

38. The method according to any of the preceding claims, whereby at least one catalyst is conjugated to streptavidin.

39. The method according to any of the preceding claims, whereby at least one linkage comprises a catalyst conjugated to avidin, an antibody conjugated to biotin and being immunologically bound to an antigen on the species of analyte or vice versa.

40. The method according to any of the preceding claims, whereby at least one linkage comprises a catalyst conjugated to avidin, a first antibody conjugated to biotin, and a second antibody being immunologically bound to an antigen on the species of analyte.

41. The method according to any of the preceding claims, whereby the linkage is formed before the sample is transferred to the sample domain.

42. The method according to any of the preceding claims, wherein the analyte is a DNA containing analyte and the DNA or fractions of the DNA are stained with a DNA staining compound.

43. The method according to any of the preceding claims, whereby an additional linkage is formed between a second species of analyte and a second catalyst.

44. The method according to any of the preceding claims, whereby two or more additional linkages are formed between a second, third and optionally subsequent species of analyte and a second, third, and optionally third catalyst.

45. The method according to any of the preceding claims, further comprising the step of removing excess catalyst not being linked to the species of analytes.

46. The method according to claim 45, whereby excess catalyst is removed through centrifugation.

47. The method according to claim 45, whereby excess catalyst is removed through filtration.

48. The method according to claim 45, whereby excess catalyst is removed through flushing.

49. The method according to claim 45, whereby removal of excess catalyst comprises binding the analyte-catalyst complex to a magnetic bead.

50. The method according to any of the preceding claims, further comprising the contacting of co-factors with the catalyst-analyte complex.

51. The method according to any of the preceding claims, further comprising the contacting of a buffer with the catalyst-analyte complex.

52. The method according to any of the preceding claims, whereby at least one substrate is added to the catalyst-analyte complex in the sample domain.

53. The method according to any of the preceding claims, whereby at least one substrate is added to the catalyst-analyte complex before transferring it to the sample domain.

54. The method according to any of the preceding claims, whereby the initiation of the reaction catalysed by the catalyst is controlled by temperature changes.

55. The method according to any of the preceding claims, whereby a pre-substrate is added to the catalyst-analyte complex before transferring it to the sample domain.

56. The method according to claim 55, whereby a conversion of the pre-substrate into the substrate can be controlled externally.

57. The method according to claim 56, whereby the conversion is controlled by illumination.

58. The method according to claim 56, whereby the conversion is controlled by a change in temperature.

59. The method according to any of the preceding claims, whereby the reaction catalysed by the catalyst can be controllably stopped externally.

60. The method according to any of the preceding claims, whereby the step of producing a product is carried out in a liquid environment.

61. The method according to any of the claims 1-59, whereby the step of producing a product is carried out in a viscous environment.

62. The method according to any of the claims 1-59, whereby the step of producing a product is carried out in a semi-solid environment, preferably where the semi-solid environment is a gel.

63. The method according to claim 62, wherein the semi-solid environment is formed after the analytes have been introduced to the sample compartment, preferably where the forming of the semi-solid environment is controlled by external factors such as temperature, light and agitation.

64. The method according to any of the preceding claims, whereby the duration of the step of producing a product is below 60 minutes.

65. The method according to claim 64, whereby the duration of the step of producing a product is below 15 minutes, preferably below 5 minutes, more preferably below 1 minute, more preferably below 30 seconds, more preferably below 15 seconds, more preferably below 10 seconds, more preferably below 5 seconds, more preferably below 2 seconds.

66. The method according to any of the preceding claims, whereby the recording of image comprises the use of a confocal scanner.

67. The method according to any of the preceding claims, whereby the image of product is recorded using an array of detection devices.

68. The method according to claim 67, wherein the image of product is recorded using a one-dimensional array of detection devices.

69. The method according to claim 67, wherein the image of product is recorded using a two-dimensional array of detection devices.

70. The method according to claim 67, wherein the image of product is recorded using a CCD, a CMOS, a video camera or a photon counting camera.

71. The method according to any of the preceding claims, whereby the image is recorded without magnification.

72. The method according to any of the preceding claims, whereby the image is recorded with a magnification factor below 20, preferably below 10, more preferably below 5, such as 4, more preferably below 4 such as 2, more preferably below 2 such as 1.

73. The method according to any of the preceding claims, whereby the image is recorded with a magnification factor below 1, preferably below 0.9, such as 0.8, more preferably below 0.8 such as 0.6, more preferably below 0.6 such as 0.5.

74. The method according to any of the preceding claims whereby the image is recorded in one exposure.

75. The method according to any of the claims 1-73 whereby the image is recorded in two, three or more exposures.

76. The method according to claim 75, wherein the assessment of at least one quality parameter or at least one quantity parameter is done by correlating more than one image to the at least one quality parameter or at least one quantity parameter, preferably by correlating two images, more preferably correlating more than two images, more preferably correlating more than four images.

77. The method according to claim 76, where information about the changes in the image in course of time is used in the assessment of at least one quality parameter or at least one quantity parameter.

78. The method according to any of the preceding claims, whereby the recorded image is processed.

79. The method according to claim 78, whereby the recorded image is processed using data processing means.

80. The method according to claim 79, whereby the data processing means distinguish partially overlapping areas of product.

81. The method according to any of the preceding claims, whereby the correlation comprises estimation of the number of spots on the image.

82. The method according to any of the preceding claims, whereby the correlation comprises estimation of the size of spots on the image.

83. The method according to any of the preceding claims, whereby the correlation comprises distinction between at least two spectral properties of product.

84. The method according to any of the preceding claims, further comprising the assessment of at least one additional quality parameter or at least one additional quantity parameter.

85. The method according to claim 84, whereby the assessment of the at least one additional quality parameter or at least one additional quantity parameter

comprises detection of fluorescence, chemiluminescence, photoluminescence, autoluminescence of a species of analyte.

86. The method according to any of the preceding claims, whereby the at least one quality parameter is selected from the group consisting of viability, size, identity, respiration, and presence of an analyte.

87. The method according to any of the preceding claims, whereby the at least one quantity parameter is selected from the group consisting of number of species of analyte in a volume of sample, concentration of species of analyte in a volume of sample, amount of species of analyte in a volume of sample.

88. The method according to any of the preceding claims, whereby the recording of an image further comprises exposing a first surface of the sample directly with excitation light from a first light means having at least a first light source, by use of focusing means detecting a fluorescence signal from the first surface of the sample onto a first detection means comprising at least a first detector.

89. The method according to claim 88, wherein at least the first light means is located in a first light plane parallel to the sample plane, said first light plane being between the sample plane and the first detection means.

90. The method according to any of the preceding claims 88-89, wherein an excitation light filter is inserted in the excitation light path from at least one light source.

91. The method according to claim 89, wherein the excitation light is arranged as light sources on a supporting material.

92. The method according to any of the preceding claims 88-91, wherein substantially identical filters are used for all the light sources.

93. The method according to any of claims 88-92, wherein a first light source is filtered through a first filter, and a second light source is filtered through a second filter, the first filter and the second filter being different.

94. The method according to any of the preceding claims 88-93, further comprising exposing a second surface of the sample directly with excitation light from a second light means having at least one light source.

95. The method according to claim 94, wherein the second excitation light means is located in a second light plane said plane being parallel with the sample plane and located on the other side of the sample plane than the first light plane allowing the sample to be exposed on two opposite surfaces.

96. The method according to claim 94 or 95, wherein a filter inserted in the light path from the second light means is different from a filter inserted in the light path of the first light means.

97. The method according to any of claims 88-96, wherein a second detection means is arranged so that the sample compartment is positioned between the first detection means and the second detection means.

98. The method according to claim 97, wherein the first detection means is identical with the second detection means.

99. The method according to any of the preceding claims 88-98, wherein an emission light filter is inserted in the emission light path to at least the first detector.

The method according to any of the preceding claims 88-99, wherein a collimating lens is arranged in the emission light path.

100. The method according to any of the preceding claims 88-100, wherein the angle between the excitation main light and the detection-sample axis is in a range between 35° and 90°, preferably between 45° and 85°, more preferably between 50° and 85°.

101. The method according to claim 88, wherein at least the first light means is located in a first light plane parallel to the sample plane, said first light plane being positioned at a distance from the sample plane behind the detector.

102. The method according to claim 102, wherein the detector is positioned in a housing having an opening allowing the emitted signals to reach the detector(s).

103. A system for the assessment of at least one parameter of analytes in a liquid sample, comprising

a device comprising a sample domain comprising an exposing domain, an inlet through which a volume of a liquid sample representing the analyte material can been introduced, and a flow system comprising at least a channel allowing at least a portion of the volume of the liquid sample to flow within the device,

the device further comprising means to control the flow of liquid around a catalyst-analyte complex in the sample domain,

a detection device comprising at least a first detector for quantitatively detecting spatial image data and a processor for processing the detected image presentation,

the device and the detection device having means for arranging the device in relation to the detection device in a manner allowing electromagnetic signals from a sample in the exposing domain of the device to pass to the detection device and to form, in the detection device, a spatial image representation of the exposing domain.

104. A system according to claim 104, wherein the flow system additionally comprises a compartment or a flow channel part in or from which at least part of one or more reaction components initially loaded in the compartment or flow channel part is added to at least a portion of the volume of the liquid representing the sample.

105. A system according to claim 104, further comprising at least a first light source.

106. A system according to claim 106, wherein the first light source comprises an excitation light source.

107. A system according to claim 107, wherein the first light source and the detector are located on the same side of the exposing domain.

108. A system according to claim 108, comprising a second light source and a second detector, located on the opposite side from the first light source and first detection means.

109. The system according to claim 106 or 108, further comprising an excitation light filter inserted into the excitation light path.

110. The system according to claim 110, wherein the excitation light filter is essentially circular, such as essentially ring formed.

111. Use of a system according to claim 104-111 for diagnosis of a condition in an individual.

112. The use according to claim 112, wherein the individual is a human being.

113. The use according to claim 112, wherein the individual is an animal other than humans, such as cow, pig, horse, poultry, sheep, goat.

114. The use according to claim 112, wherein the condition is cardial infarct or a risk for suffering form a cardial infarct.

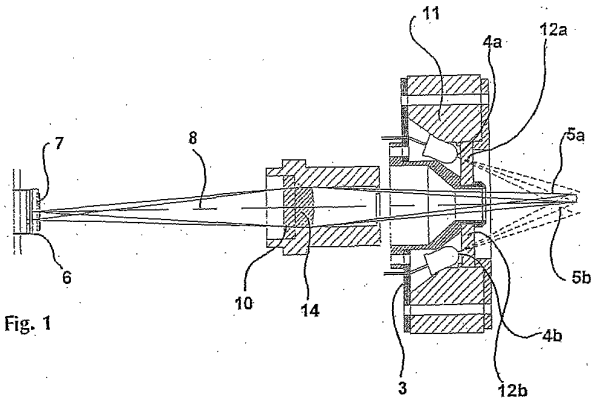

Fig. 1

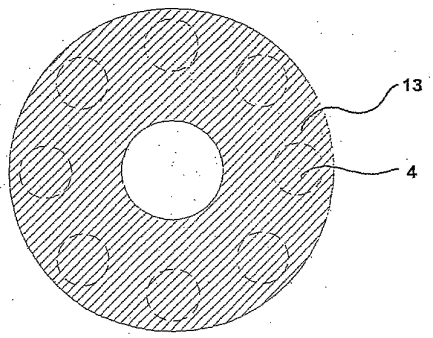

Fig. 2

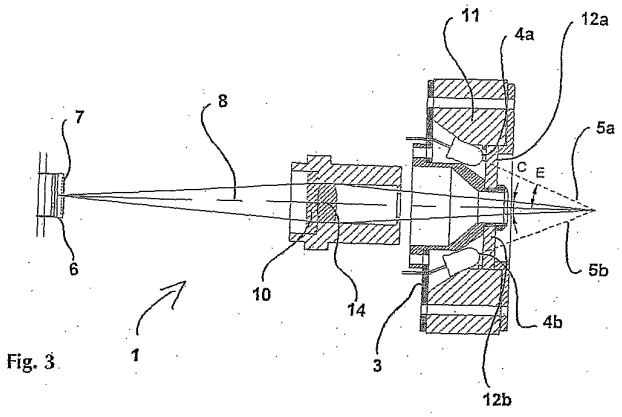
11
4a
12a
7
8
5a
C
E
6
10
14
4b
5b
3
12b
1

Fig. 3

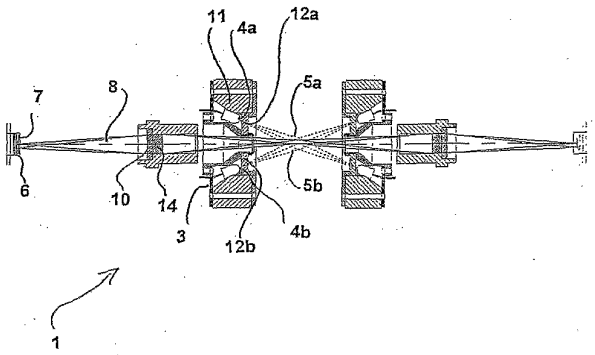
11
4a
12a
7
8
5a
6
10
14
3
12b
4b
5b
1

Fig. 4

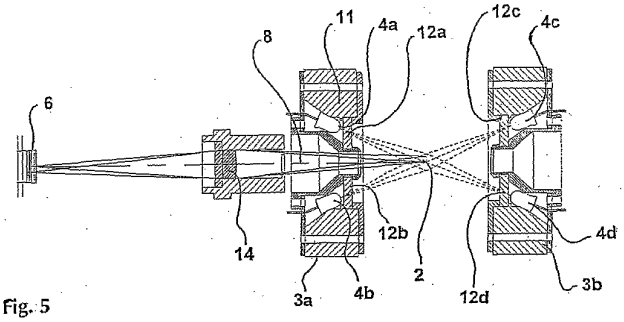
6
8
11
4a
12a
12c
4c
14
12b
2
12d
4d
3b
3a
4b

Fig. 5

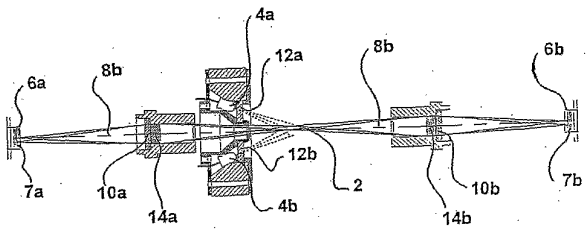
4a
12a
8b
6b
6a
8b
12b
2
10b
7b
7a
10a
14a
4b
14b

Fig. 6

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(71) Applicant *(for all designated States except US)*: **CHEMOMETEC A/S** [DK/DK]; Gydevang 43, DK-3450 Allerød (DK).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **GLENSBJERG, Martin** [DK/DK]; Næsbyholmvej 2, 4.tv., DK-2700 Brønshøj (DK).

(74) Agent: **HØIBERG APS**; St. Kongensgade 59 B, DK-1264 Copenhagen K (DK).

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(54) Title: SPATIALLY RESOLVED ENZYME-LINKED ASSAY

(57) Abstract: The present invention relates to a method of assessing at least one quality parameter and/or at least one quantity parameter of at lest one analyte wherein said at least one analyte is connected to a catalyst capable of catalysing a substrate into a product, whereby the analyte is assessed through detection of product produced around the analyte. More particularly the present invention relates to a method of assessing at least one quality parameter or at least one quantity parameter of at least one species of analytes in a sample comprising the steps of establishing a sample domain having at least one wall, arranging in the sample domain catalyst-analyte complexes between the at lest one species of analytes and at least one catalyst in a manner allowing the analytes to move relative to the wall(s) of the sample domain, arranging a substrate in the sample domain, said substrate being capable of being converted into a product through catalysation by said catalyst, contacting the substrate with the catalyst-analyte complexes of individual analytes allowing a detectable amount of product to be produced, recording an image of the product related to individual analytes in the sample domain, correlating the image to the at least one quality parameter or the at least one quantity parameter of the at least one species of analytes.

Spatially Resolved Enzyme-linked Assay

The present invention relates to a method of assessing at least one quality parameter and/or at least one quantity parameter of at least one analyte wherein said at least one analyte is connected to a catalyst capable of catalysing a substrate into a product, whereby the analyte is assessed through detection of product produced around the analyte.

Background

Detection of a substance or a particle using staining of the substance or particle to aid detection is widely used. However, many substances and particles are so small that although stained it is difficult to detect them without using very high magnification increasing the requirements to the equipment used.

A classical amplification technique is that of enzyme-linked assay. A ligand reacting specifically with the analyte is bound to an enzyme, and after excess ligand-enzyme is removed, the analyte-ligand-enzyme complex is detected by reaction with a chromogenic substrate, a colourless material which is acted upon by the enzyme to form a coloured product. Because of its large amplification factor, enzyme-linked assays offer high sensitivity, and are particularly useful for detection of small amounts of antigens.

In traditional enzyme-linked assays (ELISA) the amplification increases sensitivity at the expense of precision, because amplification factors are not exact. It is not possible to distinguish a strong reaction from a few molecules from a weak reaction from a high number of molecules.

An attempt to overcome some of the problems related to preciseness detection is using ELISPOT or in situ ELISA or spot-ELISA, that independent of the name relates to the use of ELISA technique to detect localised coloured spots on a solid support.

ELI-SPOT analysis allows the detection and enumeration of particles, such as virus or polypeptide producing cells or antigen-presenting cells. The spots produced may be enumerated by the use of a microscope or by use of video-imaging. The principle

of ELI-SPOT is for example described in Sedgwick, J.D. and Holt, P.G. " A Solid – Phase Immunoenzymatic Technique for the Enumeration of Specific Antibody-Secreting Cells, Journal of Immunological Methods, 57 (1983) 301-309, wherein secreted antibodies to be detected are captured by antigens attached to a fixed solid support. The secreted antibodies are detected by adding antibody-enzyme complexes, said antibodies having specificity towards the secreted antibodies. The substrate is added in a warm agarose gel, that is allowed to hardened whereupon spots of insoluble product may be detected in the hardened agarose gel using a microscope.

Summary of the invention

The present invention relates to a method of assessing at least one quality parameter or at least one quantity parameter of at least one species of analytes in a sample comprising the steps of

establishing a sample domain having at least one wall,

arranging in the sample domain catalyst-analyte complexes between the at least one species of analytes and at least one catalyst in a manner allowing the analytes to move relative to the wall(s) of the sample domain,

arranging a substrate in the sample domain, said substrate being capable of being converted into a product through catalysation by said catalyst,

contacting the substrate with the catalyst-analyte complexes of individual analytes allowing a detectable amount of product to be produced,

recording an image of the product related to individual analytes in the sample domain,

correlating the image to the at least one quality parameter or the at least one quantity parameter of the at least one species of analytes.

A detectable amount of product is understood as the formation of a substantially spherical amount of product around each analyte or group of analytes, leading to a

spot, detectable by a detection device. The spot produced from the product relates to one or a few analytes, allowing an assessment of the parameter relating to the analytes to take place. The three-dimensional formation of product leads to a more distinct identification of the analytes relative to the background. The image of such a spot is in many aspects similar to an image of particles encountered in numerous other applications. It is therefore understood that substantially same techniques and methods can be used in the recording, processing and analysing an image of spots as would be used for particles.

As opposed to traditional ELI-SPOT technique the analytes to be detected are not coupled to a solid support fixed to the sample domain but may be positioned anywhere in the sample domain during the contact between the substrate and analyte-catalyst-complexes. The analytes are capable of moving relative to the wall(s) of the sample domain at least until the substrate is introduced into the sample domain. Thereby contact between the analyte-catalyst-complexes and the substrate is conducted more easily.

The complex between analyte and catalyst may be formed in one or more of several ways, such as:

Through a linkage, wherein the term linkage means that the analyte and catalyst are bound to each other.

For example, the analyte-catalyst complex may be formed via a species-selective linkage, such as an immunological binding, i.e. the linkage formed between an antibody and its antigen.

Also the linkage may be through conjugation, i.e. the covalent binding between two compounds, for example between an enzyme and an antibody, an enzyme and avidin, an antibody and biotin.

In another embodiment the complex between the analyte and the catalyst is formed through a production of catalyst in or adjacent the analyte, such as expression of an enzyme from a cell. The enzyme is located adjacent the cell after expression and an analyte-catalyst complex is formed.

The correlation of the image to the at least one quality parameter or at least one quantity parameter of at least one species of analytes in a sample preferably comprises estimation of the number of spots on the image and/or estimation of the size of spots on the image.

In a second aspect the invention relates to a system for conducting the method according to the invention comprising

a device comprising a sample domain comprising an exposing domain, an inlet through which a volume of a liquid sample representing the analyte material can been introduced, and a flow system comprising at least a channel allowing at least a portion of the volume of the liquid sample to flow within the device,

the device further comprising means to control the flow of liquid around a catalyst-analyte complex in the sample domain,

a detection device comprising at least a first detector for quantitatively detecting spatial image data and a processor for processing the detected image presentation,

the device and the detection device having means for arranging the device in relation to the detection device in a manner allowing electromagnetic signals from a sample in the exposing domain of the device to pass to the detection device and to form, in the detection device, a spatial image representation of the exposing domain.

In particular the flow system additionally comprises a compartment or a flow channel part in or from which at least part of one or more reaction components initially loaded in the compartment or flow channel part is added to at least a portion of the volume of the liquid representing the sample.

Furthermore, the system may comprise at least a first light source, which advantageously emits excitation light. The detector adapted to detect the fluorescence emitted from the sample is advantageously located on the same side of the exposing domain as the light source.

In a third aspect the invention relates to the use of a system for diagnosis of a condition in an individual, such as the diagnosis of cardial infarct.

Drawings

Fig. 1 shows a one sided excitation system.

Fig. 2 shows a cross-section of the excitation light filter in a plane parallel to the sample plane.

Fig. 3 shows the collection angle C and the angle E between the excitation main light path and the detection –sample axis.

Fig. 4 shows a double-sided excitation/detection system.

Fig. 5 shows a double-sided excitation system.

Fig. 6 shows a double-sided detection system.

Detailed description of the invention

Amplification of signal

The analyte is detectable due to the amount of product being formed around the analyte. Depending on the physical constraints in the sample domain the product will diffuse from the analytes to form localised substantially spherical spots around the analytes or clusters of analytes. In other embodiments of the present invention the product is substantially insoluble under the conditions provided in the sample compartment and the product will therefore form a deposit or colloid matter. The spot will increase with time due to the transport of product in the media generally through diffusion. Thus it is possible to monitor the formation of the spots growing with time both with regard to intensity and size.

Parameter

A quantity parameter according to the invention is the number of analytes present in the sample and/or the concentration of analytes in the sample, whereas quality parameter is information regarding viability, dead and/or dying organism including apopotosis, size, identity, respiration, presence, and morphology.

Kinetics

The rate of formation of the product and thus the rate of change in the recorded image is dependent on the chemical and/or physical properties of the media in the sample compartment. In situations where the flow is limited this will often be defined by the rate of diffusion of substrate towards the catalytic site and/or the rate of diffusion of the product away from the catalytic site.

The image recorded of the product spots has to be recorded after a detectable amount of product has been produced and before the various spots become confluent, thereby inhibiting recording of an image. The image may be correlated to the at least one quality parameter or the at least one quantity parameter of the analyte.

The time period from contacting the analyte-catalyst-complexes with the substrate to recording the spots is mainly depending on the diffusion rate of substrate as well as product and on the kinetics of the process. The diffusion rate and the kinetics of the process may be controlled as discussed below.

The development of detectable spots is preferably observed within 60 minutes from contacting the analyte-catalyst-complexes with the substrate. In more preferred embodiments the step of producing a product is below 15 minutes, preferably below 5 minutes, more preferably below 1 minute, more preferably below 30 seconds, more preferably below 15 seconds, more preferably below 10 seconds, more preferably below 5 seconds, more preferably below 2 seconds.

In a preferred embodiment the sample domain is exposed to the detection means before any development of detectable spots is commenced and at least one other

exposure is conducted when sufficient time to obtain the developed spots have been reached.

By changing the viscosity of the environment in the sample domain it is also possible to control the period of time of producing the product. The step of producing a product may be carried out in a liquid environment, or in a viscous environment. In the latter case, the viscosity may be adjusted to the substrate and catalyst used. Also, the step of producing a product is carried out in a semi-solid environment, preferably where the semi-solid environment is a gel. the semi-solid environment is preferably formed after the analytes have been introduced to the sample compartment, preferably where the forming of the semi-solid environment is controlled by external factors such as temperature, light and agitation.

Analytes

The analyte may be any analyte capable of forming an analyte-catalyst complex. As discussed above the analyte may be bound by the catalyst via a species-specific binding, such as via an antigen-antibody binding.

The analytes are preferably particles such as biological particles. Biological particles are in particular selected from the group consisting of cells, cell walls, bacteria, plasmodia, virus, prions, macromolecules, proteins, polypeptides, peptides, genes, DNA, RNA, or fragments or clusters thereof.

The cells are preferably selected from mammalian cells, insect cells, reptile cells, fish cells, yeast cells, and fungi cells, more preferably from blood cells, sperm cells, and bone marrow cells.

The analytes may be coupled to a solid support, such as beads, said beads being capable of being suspended in the sample domain. The beads may be polymer beads. Often the polymer beads can have physical and/or chemical properties which can assist in the handling of the analyte such as paramagnetic beads.

One embodiment of the present invention which involves the assessment of virus or other small analytes is based on binding of the analyte to a bead. This allows the

analyte and/ the analyte-catalyst complex to be treated in a more simple manner during pre-treatment such as with centrifugation, filtration or magnetic separation.

The beads may be labelled themselves to improve accuracy in the identification of one or more analytes. One embodiment of the present invention uses two or more types of beads which can have affinity to bind two or more different analytes. If the analyte-catalyst complex produces the same product regardless of which analyte is involved, the identification of the polymer bead can be used to distinguish between which analyte-catalyst complex produces the product the signal of which is detected. Such labelling of polymer beads can be based on colour, fluorescence, size or any other physical or chemical property.

Also, the analyte may be a DNA or RNA containing analyte whereby the DNA/RNA or a fraction thereof may be stained with a DNA staining compound. This is often preferred when a further confirmation or assessment of analyte property of analyte specificity is needed such as assessment of viability.

Apart from methods based on enzyme amplification (Enzyme Amplification Systems, EAS) other systems are also included in many preferred embodiments of the present invention. Among such specific amplification systems are the follows:

PAP: Peroxidase anti-peroxidase complex
APAAP: Alkaline phosphatase anti-alkaline phosphatase complex
BGABG: beta-galactosidase anti-beta-galactosidase complex
Cyclic conversion of NADH to NAD especially when the formation of NADH involves NADPH and Alkaline Phosphatase.

Sample

The sample may be a liquid sample such as a sample selected from the group consisting of milk, milk products, urine, blood, sperm, nasal secrete, tears, faeces, waste water, process water drinking water, cerebro-spinal fluid, gall, bone marrow, food, feed, and mixtures, dilutions, or extracts thereof.

The sample may also relate to assessment of particles in water, such as control of drinking water, control of waste water or water from a water purifying plant or

swimming pool. In all applications the control may be related to the total particle count, such as bacteria count or it may more particularly be related to a monitoring process for specific bacteria, such as pathological bacteria.

Furthermore, fermentation control, i.e. control of cell growth and viable cells in fermentation tanks may be conducted by the invention. This relates to all technical and industrial fields using fermentation, such as the pharmaceutical industry for producing peptide or protein composition.

The liquid sample may be pre-treated with any suitable treatment, such as centrifugation, sedimentation, filtration, extraction, dilution, irradiation, agitation, addition of chemicals, chromatographic separation.

In another embodiment the sample is a solid sample which is pre-treated prior to being arranged in the sample domain. An example of pre-treatment is blending optionally followed by any of the treatment mentioned for the liquid sample.

The sample may be any biological sample, such as a biopsy of tissue, such as a biopsy of muscle, brain, kidney, liver or spleen.

Also, the sample may be a sample of food or feed to be tested for contamination, such as bacterial contamination. The present invention offers a very fast method of detecting and enumerating bacteria in food or feed such as a method of detecting Salmonella species.

Independent of the form of sample it is required that the analyte is suspended in a medium before contacting the substrate. Said medium may be the natural medium for the analyte or any liquid suitable for the detection. In one embodiment the analyte is suspended in a medium after being pre-treated. The medium may comprise the catalyst if appropriate.

Catalyst

By the term catalyst is meant any compound capable of converting or aiding in the conversion of a substrate into a product being detectable by the detection means. The catalyst may thus be an inorganic catalyst as well as an organic catalyst. In one

embodiment the catalyst is an enzyme wherein the term enzyme is used in its normal meaning. Where enzymes are used for labelling either a single enzyme, an oligomeric form of the enzyme, or an enzyme/anti-enzyme complex may be used.

The enzyme may be any enzyme useful in an ELISA technique such as an enzyme selected from the group consisting of phosphatases such as alkaline phosphatase, β-galactosidase, peroxidases such as for example horseradish peroxidase, β-glucuronidase, β-glucose-6-phosphate dehydrogenase, glucose oxidase, urease, luciferase, β-lactamase and β-amylase.

When two, three or more different types of analytes are detected in one turn, the different enzymes must be selected so that they do not interfere with one another. For example, enzymes are advantageously chosen that require approximate the same pH. Advantageously, substrates should be selected that are only converted to product by one of the two or more present enzymes. Similarly, the substrates may preferably be chosen so that neither the substrates nor their products inhibit any of the enzymes present in the sample compartment.

Further, the enzyme may be coupled to an alternative detection system, such as an amplification system, such as a avidin-biotin anti-peroxidase technique (ABAP). The label may also be biotin, whereby the biotinylated antibody is detected using a labelled avidin or streptavidin, which is enzymelabelled. In a preferred embodiment the avidin or streptavidin is labelled with one of the enzymes discussed above. In another embodiment the label is avidin or streptavidin whereby the antibody is detected using a biotin, which is enzymelabelled. By labelling with biotin/avidin or streptavidin it is possible to enhance the signal as compared to labelling directly with enzyme and more preferred the enzyme is horse radish peroxidase.

Several embodiments involve the use LNA and PNA when the analyte is or contains DNA or RNA material.

In one embodiment the catalyst reaction may be controlled such as controlled by temperature or illumination or light exposure, so that the kinetics of the catalyst reaction is controlled. For example it may be controlled that the catalyst reaction does not commence before initiated, for example by changing the local temperature

of the catalyst, or by exposing the catalyst to light such as illumination to remove a substrate or co-factor, or illumination to convert a compound into a substrate or a co-factor. In another embodiment the catalyst reaction is controlled by pH, so that changes in the pH may increase the catalyst reaction. Also in a preferred embodiment it is possible to stop the reaction catalysed by the catalyst externally such as by controlling a temperature shift or the like.

Species-specific linkage

The term species-selective linkage is used synonymously with the term species-specific linkage, i.e. a linkage that is specific for the analyte the parameter of which is to be assessed. In one embodiment the species-selective linkage is antigen-antibody binding, using antibodies, such as monoclonal antibodies, directed to an epitope on the analyte.

The monoclonal antibodies may be labelled directly or indirectly. Direct labels typically include catalysts, such as enzymes, and biotin. Indirect labels include antibodies against the monoclonal antibody, said antibodies being labelled with catalyst labels, such as enzyme labels, or biotin.

In an indirect label the primary antibody or nucleotide probe directed against an epitope or nucleotide sequence on the analyte may be linked covalently to a compound such as biotin, streptavidin, avidin, a hapten, digoxigenin, dinitrophenyl or fluorescein. The analyte may then be visualised by a second label which specifically binds to the compound linked to the primary antibody or probe. Examples of such indirect labels include but are not limited to: hapten anti-hapten complex; biotin streptavidin complex; biotin avidin complex; digoxigenin anti-digoxigenin complex; dinitrophenyl anti-dinitrophenyl complex; fluorescein anti-fluorescein complex.

In the case where the primary antibody or probe is linked to biotin, signal amplification can be obtained by linking streptavidin to the biotin and further linking biotin-enzyme-biotin complexes to the streptavidin. Further rounds of amplification can be obtained by linking further streptavidin and biotin-enzyme-biotin complexes. The result is that several enzymes are linked via complex linkages to the epitope or nucleotide sequence instead of just one enzyme.

In another embodiment the species-selective linkage may be provided using DNA and/or RNA and/or PNA and/or LNA probes selective for DNA and/or RNA related to the analyte. The probes may be labelled directly or indirectly through additional probes as described above in relation to labelling of antibodies.

The invention also comprises the feature that an additional linkage is formed between a second species of analyte and a second catalyst.

The linkage may be formed in the sample domain by arranging the analytes and the catalyst in the sample domain and allowing the analyte-catalyst complex to form. In another embodiment the analyte-catalyst complex is formed before the sample or analytes are transferred to the sample domain.

After forming the analyte-catalyst complex excess catalyst not being linked to the species of analytes is preferably removed from the analyte-catalyst complexes. The excess catalyst may be removed through any suitable means, such as centrifugation, filtration and/or through flushing.

The steps of removing excess catalyst may also comprise binding the analyte-catalyst complex to a magnetic bead.

In other embodiments of the present invention the excess catalyst is substantially not removed from the analyte-catalyst complex. This obviously implies a more complex conditions under which any image is to be recorded since signals are generated not only in, at or in the vicinity of the analyte but also in the media surrounding the analyte. An example of where it could be possible to detect signals originating from products produced in, at or in the vicinity of the analyte is where the concentration or efficiency of the catalyst in the analyte-catalyst complex is greater than in the media surrounding the analyte.

The catalyst-analyte complex may further be contacted with co-factors or a buffer before contacting the complex with the substrate.

In the following non-exclusive list of examples of species specific linkages formed between the analyte and the catalyst, an asterisk * denotes an affinity binding such

as the binding between antibody and antigen or the binding between avidin and biotin. A dash - denotes a covalent bond.

analyte*antibody-catalyst
analyte*antibody*antibody-catalyst
analyte*antibody-biotin*avidin-catalyst
analyte*antibody-biotin*streptavidin-catalyst
analyte*antibody-avidin*biotin-catalyst
analyte*antibody-streptavidin*biotin-catalyst
analyte*antibody*antibody-avidin*biotin-catalyst
analyte*antibody*antibody-streptavidin*biotin-catalyst
analyte*antibody*antibody-avidin*biotin-catalyst
analyte*antibody* antibody-streptavidin*biotin-catalyst
analyte*antibody-biotin*streptavidin*(biotin-catalyst-biotin)$_n$*streptavidin$_n$ etc
analyte*antibody-digoxigenin*antidigoxigenin-catalyst
analyte*antibody-dinitrophenyl*antidinitrophenyl-catalyst
analyte*antibody-hapten*antihapten-catalyst
analyte*antibody-fluorescein*antifluorescein-catalyst

analyte*DNAfragment-catalyst
analyte*DNAfragment*DNAfragment-catalyst
analyte*RNAfragment-catalyst
analyte*RNAfragment*RNAfragment-catalyst
analyte*PNAfragment-catalyst
analyte*PNAfragment*PNAfragment-catalyst
analyte*LNAfragment-catalyst
analyte*LNAfragment*LNAfragment-catalyst
analyte*DNAfragment*RNAfragment-catalyst
analyte*DNAfragment*LNAfragment-catalyst
analyte*DNAfragment*PNAfragment-catalyst
analyte*RNAfragment*LNAfragment-catalyst
analyte*RNAfragment*PNAfragment-catalyst
analyte*RNAfragment*LNAfragment-catalyst
analyte*nucleotide probe-biotin*streptavidin*(biotin-catalyst-biotin)$_n$*streptavidin$_n$ etc
analyte* nucleotide probe -digoxigenin*antidigoxigenin-catalyst

analyte* nucleotide probe -dinitrophenyl*antidinitrophenyl-catalyst
analyte* nucleotide probe -hapten*antihapten-catalyst
analyte* nucleotide probe -fluorescein*antifluorescein-catalyst

It is likewise conceivable that the species specific linkage may be formed by reversing the position of two components participating in the affinity binding such as reversing the order of avidin*biotin or RNA*PNA etc.

Substrate

The substrate is typically added to the analyte-catalyst complex before recording any images of the sample domain.

The substrate may be mixed with analyte-catalyst complex before arranging the mixture in the sample domain. In order to avoid substantially any catalyst reaction to take place before the mixture is in place, the catalyst reaction may be controlled as described above in relation to the catalyst.

In another embodiment the analyte-catalyst complex is arranged in the sample domain before adding the substrate, where the mixture of substrate and analyte-catalyst is formed in the sample domain.

Catalysed reporter deposition

The catalyst analyte complex according to the invention may be amplified through the use of catalysed reporter deposition. By catalysed reporter deposition is meant the deposition, such as covalent bonding, onto the analyte of a number of reporter molecules. By this deposition of reporter molecules the number of catalysts linked to one species of analyte can be increased and thereby the signal originating from a single species of analyte can be increased. It is to be understood that reporter molecules encompass any molecule to which a catalyst according to the invention can be linked, e.g. through a species specific linkage.

Typically the reporter molecule comprises two components, a substrate, which will form the linkage to a receptor on the analyte, and covalently linked to the substrate component one part of an affinity binding pair.

In the following the principle of reporter activated deposition is explained using tyramin-biotin as an example. It is to be understood that tyramin-biotin is used for illustrative purposes only, and that other examples of reporters that can be used in catalysed reporter deposition are available to the skilled practitioner.

The first step in the procedure involves the binding of a peroxidase to the analyte through a species specific linkage, e.g. through linkage via a primary antibody directed against an epitope on the surface of the analyte.

After binding of the primary antibody, tyramin-biotin and H_2O_2 is added. Through oxidation by the peroxidase, the tyramin component of the reporter molecule is activated and forms a covalent linkage to electron rich moieties, such as to tyrosin or tryptophan residues, on the surface of the analyte within close distance of the peroxidase. The end result is that a number of biotin molecules are bound covalently to the analyte to which the peroxidase was first bound. In a second round of labelling, a further catalyst linked to avidin or streptavidin is added to the analyte to form linkages between the biotin linked to the analyte. The end result is thus that numerous catalysts are linked to each analyte for every primary antibody linked to the analyte initially.

A number of examples of the substrate component of the reporter molecule are disclosed in US 5,196,306 which is hereby incorporated by reference.

Chromography/luminescence

The substrate may be a chromogenic substrate or a luminogenic substrate, for example a photoluminogenic, an autoluminogenic or chemoluminogenic substrate such as a fluorogenic substrate.

A chromogenic substrate leads to a coloured product being detectable by measuring the absorbance. Any suitable chromogenic substrate used for conventional ELISA may be used herein, as long as the colour of the spot is distinguishable from the background.

A luminogenic substrate leads to a product capable of emitting photons, either when excited or by itself.

Examples of substrate systems which can be used in many embodiments of the present invention are:

AttoPhos (TM associated to JBL Scientific Inc. (San Louis Obispo, CA). The chemical formula of AttoPhos reported differently by the two references: **Zahra P. *et al.***; In Vitro Cell. Biol. - Animal 34: 772-776, 1998; A fluorometric assay for the measurement of endothelial cell density in vitro: 2′-[2-benzthiazoyl]-6′-hydroxy-benthiazole phosphate, or **Yu H. *et al.***; Analytical Biochemistry 261: 1-7, 1998; Development of a magnetic microplate chemifluorimmunoassay for rapid detection of bacteria and toxin in blood: (2′-[2-benzthiazoyl]-6′-hydroxy-benzthiazole phosphate bis-[2-amino-2-methyl-1, 3-propanediol])

4-MUP (4-methylumbelliferyl phosphate)

HNPP (Roche no. 1 758 888)

4-MUG (4-methylumbelliferyl beta-galactoside)

CDP-Star (Roche no. 1 685 627)

CSPD (Roche no. 1 655 884)

Super Signal Substrate (Pierce, Rockford, Ill). No chemical formula given. Reference: Trevanich S et al., Journal of Food Protection 63: 534-538, 2000; Rapid Detection of Enterotoxigenic Escherichia coli O6 in Water by Using Monoclonal Antibody and Photon-Counting Television Camera.

Luminol/4-iodophenol (e.g. BM Chemiluminescence ELISA Substrate (POD) - Roche no. 1 582 950).

Galacton Plus with and without enhancer (e.g. BM Chemiluminescence ELISA Substrate - Roche no. 1 759 787).

DAB (3,3′-diaminobenzidine tetrahydrochloride) with and without metal enhancer

OPD (o-phenylenediamine dihydrochloride) or free base

AEC (3-amino-9-ethylcarbazole)

5AS (5-aminosalicyclic acid)

2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)

4C1N (4-chloro-1-naphtol)

o-dianisidine (3,3′-dimethoxybenzidine)

TMB (3,3′,5,5′-tetramethylbenzidine) free base or dihydrochloride

ABTS

BCIP (5-bromo-4-chloro-3-indoyl phosphate) with and without NBT (nitro blue tetrazolium) or 2-(iodophenyl)-5-(4-nitro-phenyl)-3-phenyltetrazolium chloride)
Fast Red/Naphthol AS-MX (Sigma F4648)
Naphthol AS-TR phosphat (Sigma N8518) with an dwithout Fast Red RC (Sigma F5146)
pNPP (p-nitrophenyl phosphate)
PMP (phenolphthalein monophosphate - Sigma A3344)
X-Gal (5-bromo-4-chloro-3-indoyl-beta-D-galactopyranoside
CPRG (Chlorophenol-beta-D-galactopyranoside) EP patent 0146866 owned by Roche Diagnostics GmbH)

Chromogenic substrates

According to a preferred embodiment, the substrate is a chromogenic substrate, which can be detected by measuring it's absorbance. According to an especially preferred embodiment, the coloured product formed from the chromogenic substrate precipitates upon formation. In this way, it is ensured that the detectable product remains in the vicinity of the analyte to be detected. When the sample compartment is kept horizontal during detection, the product formed will simply precipitate on the lower surface of the sample compartment and form an insoluble precipitate there. Substrates that are especially suitable for this type of detection are those with a low water solubility.

In some cases it may be advantageous to add a filter to the detection device. Preferably the filter should be selective for the product to be determined. The advantage of the filters is that they can effectively filter away any background signals not coming from the relevant product. The system may also be equipped with several filters for the detection of two, three or more differently coloured products.

If the detector is a colour sensitive CCD there may be no need for colour specific filters.

Fluorescence

In a preferred embodiment the substrate is a fluorogenic capable of being converted into a fluorescent product by the catalyst. A system based on fluorescence is generally more sensitive than a chromogenic since fewer product molecules are necessary for recording an image from the sample domain. Therefore a shorter incubation time is generally necessary for a sufficient amount of product molecules to be produced and the image may be recorded faster than when using a chromogenic substrate.

A fluorogenic substrate preferably leads to a fluorescent product emitting signals in the wave length range of from 300 to 1200 nm when excited by excitation light. One preferred fluorescence method is the method of polarised fluorescence.

The excitation light source is any suitable light source capable of emitting excitation light in the range of from 250 nm to 600 nm, such as a light emitting diode (LED), a gas laser, a solid state laser, a laser diode, a gas lamp, a halogen lamp, or a xenon lamp.

It is preferred to use a diverging excitation light, such as light emitting diodes for in a cost-effective manner to expose as large area as possible of the sample to the excitation light.

It may be preferred to use more than one light source for the purpose of increasing the flux of light onto the sample, for instance by using two or more light emitting diodes. It is also possible to use more than one light source where some of the light sources have different electromagnetic properties.

By the use of several LEDs the sample is exposed to excitation light from several angles leading to a substantially optimal excitation of the sample, the light source are preferably operated in such a way that all transmit substantially simultaneously.

However for some application wherein at least a first and a second light sources are arranged in the first excitation light means, the first light source having a different wavelength band than the second light source, the light sources may transmit in an alternating manner. By the use of two different light sources it is possible to obtain

two different fluorescence signals from the sample. There is no upper limit to the number of LEDs used, but often as many as 30 LEDs are provided, such as up to 20 LEDs.

If a less diverging light source is used a diverging optical means may be arranged in the excitation light path to diverge the excitation light properly.

When using laser diodes as the excitation light the proper divergence may be accomplished by an arrangement of at least 4 laser diodes optionally provided with diverging means.

The incident angle of the excitation light is preferably in the range between 30° and 90°, more preferably between 45° and 85°, such as between 50° and 85° to provide a suitable excitation of the sample.

The excitation light may be transmitted directly to the sample, i.e. without being deflected by a beam splitter or the like whereby it is possible to construct the system and apparatus more compact.

Dual or multiple colour

In a preferred embodiment the correlation comprises distinction between at least two spectral properties of product. Thus, by the present invention it is possible to simultaneously detect at least two different types of analytes. This is achieved by using at least two antibodies directed towards two different analytes, and providing the two antibodies with two different enzymes, either directly or indirectly, and then further providing the relevant substrates for simultaneous or substantially simultaneous detection of the two different analytes due to evaluation of two different spots.

The spots arising from the analytes may either have two different colours, or one may be coloured and the other fluorescent, or both may be fluorescent emitting in two distinct wave length areas.

The two different types of analytes may be two different cells, for example specific IgG and IgA-secreting cells, or the two different states of the same cell, such as to distinguish between dead and living cells.

Also, in the latter situation the a dual labelling may be carried out by using one labelled antibody towards the analyte and then another type of labelling of the analyte to distinguish dead from living cells, such as conventional vitality staining.

It is understood, that more than two different analytes may be assessed hereby, such as three analytes for example.

Sample domain

The sample domain established according to the present invention may be a compartment or an equivalent thereof, wherein the sample is located during recording, such as a three-dimensional sample domain.

The sample domain may be a part of a flow-through system, wherein each sample is part of a series of samples, whereby one sample is replacing the previous sample in the sample domain.

In another embodiment the sample domain is part of a cassette, such as a disposable cassette as described in PCT/DK99/00605.

The sample is contained in the interior of the sample compartment, which normally has an average thickness of between 20 µm and 2000 µm, usually between 20 µm and 1000 µm and in many practical embodiments between 20 µm and 200 µm.

Normally, the sample compartment has dimensions, in a direction substantially parallel to a wall of an exposing window, in the range between 1 mm by 1 mm and 10 mm by 10 mm, but it will be understood that depending on the design, it may also be larger and, in some cases, smaller.

The part of the sample domain allowing signals to be detected is referred to as the exposing window that can be as little as 0.01 mm^2 or more, preferably with an area of 0.1 mm^2 or more, more preferably with an area of 1 mm^2 or more, preferably with

an area of 2 mm^2 or more, preferably with an area of 4 mm^2 or more, preferably with an area of 10 mm^2 or more, preferably with an area of 20 mm^2 or more, preferably with an area of 40 mm^2 or more, more preferably with an area of 100 mm^2 or more, preferably with an area of 200 mm^2 or more, preferably with an area of 400 mm^2 or more, preferably with an area of 1000 mm^2 or more, preferably with an area of 2000 mm^2 or more, preferably with an area of 4000 mm^2 or more, preferably with an area of 10000 mm^2 or more. Similarly, it is advantageous to extend the window of the sample compartment in a direction which is parallel to the plane of any window exposing signals from the sample to the exterior in order to extend the area of the exposing window and thus increase the volume of the sample which is exposed to the exterior.

Concerning the spatial definition of the shape and size of the area of an sample domain or a window exposing signals to the detection device there are at least two feasible methods for substantially reliable definition of the size and shape of this area. The first, and in many embodiments preferred method, is to adapt the detection device to be sensitive to exposed signals from a substantially defined area of the exposing window, e.g. by adapting any focusing means of the detection device. The second method, which is in particular preferred when it is difficult to adapt the sensing area of the detection device, is to define the boundaries of such exposing area of the sample compartment, e.g. either by controlling the dimensions of the sample compartment which define the exposing area, or by forming a mask or and effective window defining the exposing area, either in or on the disposable device or in connection with the detection device.

The requirements of the wall of the sample compartment are in particular that the wall allows the signals to pass without any significant limitations. In practice no upper limit is given for the wall thickness apart from what is defined by cost and design. The wall is preferably a substantially stable wall, which leads to a lower thickness limit for each material used. Preferably, the wall is from 0.1 mm to 2 mm, such as from 0.5 mm to 1.5 mm, more preferred from 0.75 mm to 1.25 mm.

In some embodiments, a flexible wall is useful, however, for quantitative measurements this will require measurement of the volume of the sample exposed before the assessment is carried out.

Sample volume

Sample volumes as small as 1 ml or less and even as small as 0.02 ml can be used. The optimal volume of the sample needed is highly dependent on the number of analytes present in the sample and the predetermined statistical quality parameter sought.

Other preferred embodiments of the present invention make it possible to assess analytes from a considerably large volumes of sample. This can allow the measurement of samples with only few analytes of interest per volume of sample. Sample volumes larger than 1 ml and even larger than 100 ml can be used for the analysis, the volume being defined as the total volume of any liquid sample introduced to any flow system connected to the device before the measurement of the sample.

Often the design of the sample compartment or the sample is such that the size of the volume of the liquid sample is sufficiently large to permit the assessment of the at least one quantity parameter or the at least one quality parameter to fulfil a predetermined requirement to the statistical quality of the assessment based on substantially one exposure, so that the image is recorded in one exposure. In another embodiment the assessment of at least one quality parameter or at least one quantity parameter is done by correlating more than one image to the at least one quality parameter or at least one quantity parameter, preferably by correlating two images, more preferably correlating more than two images, more preferably correlating more than four images. In these situations the images are recorded through two, three or more exposures.

Also, information about the changes in the image in course of time is used in the assessment of at least one quality parameter or at least one quantity parameter, and in such situations more than one exposure is made.

In many assessments of analytes it is of interest to allow exposure of signals from substantially large volumes of sample. The volume of the liquid sample from which signals such as electromagnetic radiation is exposed onto the detection system is normally in the range between 0.01 µl and 20 µl. Generally the volume of the

sample being analysed should be as large as possible. This allows the simultaneous assessment of a higher number of analytes, but the optimal volume is often defined by one or more aspects of the detection system and the sample being analysed. Thus the volume of the sample in the sample compartment can be less than 0.1 µl but often volume of more then 0.1 µl, 1.0 µl or even 10 µl is used. In still other application volume of the sample compartment as large as 100 µl or more can be used.

A large volume of the sample is preferably measured by passing the volume of sample through a analyte retaining means, such as a filter, electrical field, magnetic field, gravitational field, such means preferably being included in the device or can be arranged to interact with any sample within the device. The analyte retaining means should preferably be able to retain substantially all analytes present in a sample, or at least a substantially representative fraction of at least one type of analyte present in the sample.

When the analytes from a large sample are retained, those analytes can be resuspended in a volume which is less than the volume of sample passed through the analyte retaining means.

In one embodiment more than one portion of the same sample material can be subjected to analysis by exposure to the detection system. This can be done by allowing the sample compartment to be moved, thus exposing a different portion of the sample compartment.

Magnification

The method is preferably carried out at a low magnification whereby it is possible to detect spots in a large volume in one or a few exposures. The magnification factor is preferably below 20, such as below 10, such as below 5, such as 4, more preferably below 4, such as 2, more prefereably below 2, such as 1. The advantage of such low magnification are several, among other things increased area of observation and increased depth of focusing implying increased volume exposed to the detection device.

When the spots in question have dimensions which are comparable to the size of a detection element, it is often preferred to have magnification of about 1/1, thus focusing the image of any spot on any one or just few detection elements. This can under some condition give favourable detection of any signal.

When analysing spots which have dimensions which are comparable to, or bigger than the detection elements used, it is often advantageous to reduce the size of the image of such spot, to a degree where the size of the image is comparable to the size of a detection element. Thus in one embodiment it is preferred that the magnification factor below 1, preferably below 0.9, such as 0.8, more preferably below 0.8 such as 0.6, more preferably below 0.6 such as 0.5.

In these situations it is preferred that the ratio of the size of a spot, to the size of the image of the particle on the array of detection elements is 1/1 or less, preferably less than 1/1 and higher than 1/100, more preferably less than 1/1 and higher than 1/40, more preferably less than 1/1 and higher than 1/10, more preferably less than 1/1 and higher than 1/4, more preferably less than 1/1 and higher than 1/2.

Thus, it is often preferred that the spatial representation exposed onto the array of detection elements is subject to such a linear enlargement that the ratio of the image of a linear dimension on the array of detection elements to the original linear dimension in the sample domain is smaller than 40:1, normally at the most 20:1, preferably smaller than 10:1 and in many cases even at the most 6:1 or even smaller than 4:1.

The aspect ratio of an image can be considerably distorted on the array of detection elements, without that having considerable negative effect on the assessment of analytes. In such a situation it preferred that the ratio of the shorter to the longer of the two dimensions of the image of a particle on the array of detection elements is substantially 1 or less, preferably 1/2 or less, more preferably 1/4 or less, more preferably 1/10 or less, more preferably 1/50 or less, more preferably 1/100 or less, more preferably 1/200 or less, relative to the ratio of the corresponding dimensions of the particle. In such situation the ratio of the shorter to the longer of the two dimensions of the image of a particle on the array of detection elements is in certain

circumstances substantially not the same within the area spanned by the array of detection elements.

It is often preferred that the image of the product from the individual analytes the parameter or parameters of which is/are to be assessed are imaged on at the most 25 detection elements, in particular on at the most 16 detection elements and more preferred at the most 9 detection elements. It is even more preferred that the image of the product from the individual analytes the parameter or parameters of which is/are to be assessed are imaged on at the most 5 detection elements, or even on at the most 1 detection element. The larger number of elements per analyte will provide more information on the individual analytes, while the smaller number of elements per analyte will increase the total count that can be made in an exposure.

Statistics

As mentioned above, the size of the volume is suitably adapted to the desired statistical quality of the determination. Thus, where the determination is the determination of the number of analytes in a volume, or the determination of the size and/or shape of analytes, the size of the volume of the liquid sample is preferably sufficiently large to allow identification therein of at least two of the analytes. More preferably, the size of the volume of the liquid sample is sufficiently large to allow identification therein of at least four of the analytes. This will correspond to a repeatability error of approximately 50%. Still more preferably, the size of the volume of the liquid sample is sufficiently large to allow identification therein of at least 10 of the analytes. This will correspond to a repeatability error of approximately 33%. Even more preferably, the size of the volume of the liquid sample is sufficiently large to allow identification therein of at least 50 of the analytes. This will correspond to a repeatability error of approximately 14%. Evidently, where possible, it is preferred to aim at conditions where the size of the volume allows identification of even higher numbers. Thus, when the size of the volume of the liquid sample is sufficiently large to allow identification therein of at least 100 of the analytes, it will correspond to a repeatability error of approximately 10%, and when the size of the volume of the liquid sample is sufficiently large to allow identification therein of at least 1000 of the analytes, it will correspond to a repeatability error of as low as approximately 3%.

Stand still

In a preferred embodiment of the invention the analytes being assessed are substantially at stand-still during analysis, thus allowing the optimal use of measurement time in order to improve any signal to noise conditions. This arrangement also eliminates any error which could be inherent in the assessment of analytes caused by variation in flow conditions, particularly when an assessment of a property is a volume related property such as the counting of analytes in a volume of sample.

Flow system

The introduction of analyte, catalyst and substrate into the sample domain may be provided by means of a flow system. The flow system may provide at least one of several operations to be carried out on the samples, said operations being selected from but not limited to transport, mixing with reagent, homogenising of sample and optionally reagent, heat treatment, cooling, sound treatment, ultra sound treatment, light treatment and filtering.

In order to flow the sample into or within or out of the sample domain it is preferred to have at least one propelling means provided in the system.

Preferably the flow regulation means is arranged to function stepwise so that the sample and/or the reagent component may be flowed stepwise through the system.

The sample in the device can be flown by the means of a flow system, which can be driven by a pump or a pressurised gas, preferably air, or by causing a pressure difference such that the pressure on the exterior of the inlet is higher than the pressure within at least a part of the system thus forcing the sample to flow through the inlet. In many embodiments of the present invention the flow in said flow system is controlled by one or more valves which can adjust the flow speed of the sample.
In many preferred situations the flow of liquid in the device can be brought about by a vacuum, the vacuum being applied from a reservoir, preferably contained within the device. The vacuum can be established by a mechanical or physical action creating the vacuum substantially simultaneously with the introduction or the movement of the sample. These mechanical or physical actions can be: a peristaltic

pump, a piston pump, a membrane pump, a centrifugal pump and a hypodermic syringe.

The outlet from the sample compartment can be passed through a flow controlling means, such as a valve, which only allows gas to pass through. One such type of valves which often is preferred, is one which allows gas and air to pass but can close irreversibly when the valve comes in contact with liquid sample. The effect of such valve is to minimise the movement of any sample within the sample compartment during analysis.

In a preferred embodiment of the invention the system contains at least one compartment wherein the mixing of the sample material with catalyst and/or media is possible.

One advantage of the present system and method is that the analysis is carried out using only liquid reagents and analytes suspended or dissolved in liquid. This layout ensures ease of operation and handling. Surprisingly it has been determined that it is possible to detect analytes specifically in the absence of bonds to any solid support.

Detection device

The image which can be detected from the window of the device can for instance be detected by an array of detection elements, the array of detection elements comprising individual elements each of which is capable of sensing signals from a part of the sample window area, the array as a whole being capable of sensing signals from substantially all of the sample window area, or at least a well defined part of the sample window area. The array of detection devices may for example be a one-dimensional array or a two-dimensional array. In order to facilitate the assessment of analytes the intensities detected by the array of detection elements are processed in such a manner that representations of electromagnetic signals from the analytes are identified as distinct from representations of electromagnetic background signals.

The detection means may comprise any detectors capable of sensing or detecting the signal emitted from the sample such as a fluorescence signal.

In a preferred embodiment detection means comprises a detector being an array of detecting devices or detection elements, such as a charge coupled device (CCD) the CCD may be a full frame CCD, frame transfer CCD, interline transfer CCD, line scan CCD, an eg. wavelength intensified CCD array, a focal plane array, a photodiode array or a photodetector array, such as a CMOS. The CMOS is preferably a CMOS image sensor with on-chip integrated signal condition and/or signal processing. Independent of the choice of any of the above detection devices the detection means may further comprise a white/black or colour CCD or CMOS.

Confocal scanning optical microscopes are known in the art and offer a number of advantages over traditional optical microscopes. One main advantage of a confocal scanning microscope is that it provides optical sectioning of a sample because it attenuates light which is not in focus. Thus, only light which is in focus contributes to the final image.

In a scanning confocal microscope, a beam is swept across a surface of a sample. The light which emanates from the sample (e.g., reflected from, emitted from or transmitted through) is directed towards a pinhole. Light that is in focus passes through the pinhole and onto an optical detector. As the beam is scanned across the surface of the sample, the output from the optical detector can be accumulated and formed into an image of the scanned surface.

Use of a confocal scanning microscope especially a confocal laser scanning microscope for detecting the signals from the product formed in the sample domain is advantageous due to the greater sharpness of the detected image.

The size of the detection elements determines to some extend its sensitivity. In some applications it is therefore of interest to have detection elements of size of about 1 μm^2 or less. In certain situations the size of the detection elements in the array of detection elements is less than 20 μm^2, preferably less than 10 μm^2, more preferably less than 5 μm^2, more preferably less than 2 μm^2, more preferably less than or equal to 1 μm^2. In other situations the size of the detection elements in the array of detection elements is greater than or equal to 5000 μm^2, such as greater than or equal to 2000 μm^2, more preferably greater than or equal to 1000 μm^2, such

as greater than or equal to 500 μm^2, or even greater than or equal to 200 μm^2, more preferably greater than or equal to 100 and less than 200 μm^2, more preferably greater than or equal to 50 and less than 100 μm^2, more preferably greater than or equal to 20 and less than 50 μm^2.

The array of detection elements is preferably sensitive to electromagnetic radiation of wavelength in one or several of the following regions: 100 nm to 200 nm, 200 nm to 600 nm, 300 nm to 700 nm, 400 nm to 800 nm, 600 nm to 1 µm, 800 nm to 2 µm, 2 µm to 10 µm, 5 µm to 10 µm, 10 µm to 20 µm, 20 µm to 40 µm.

The inclusion of a focusing device for the focusing of a signal from the sample onto the detection elements in such a manner as to maximise the collection angle, the collection angle being defined as the full plane angle within which a signal is detected, has in many situations been found to give improved conditions for an assessment. Surprisingly it was found that such a wide collection angle, even to the extent that the objective used in the focusing distorted the aspect ratio of the image of any analyte differently across the plane in which the detection elements were placed, or produced variation in the focusing across the sample being analysed, or reduction of the focusing quality, could be used in the assessment of for example the number of analytes in the sample.

The aspect ratio of the detection elements can be important in the collection of signals for the assessment of analytes. A ratio of about 1/1 is some times preferred, but under some conditions it can be preferred to use ratio different from 1/1. In particular when this facilitates detection of signals from increased volume of any sample, thus allowing simultaneous assessment of for examples more analytes. In those circumstances the ratio of the shorter of the height or the width, to the longer of the height or the width of the detection elements in the array of detection elements is substantially equal or less than 1, preferably less than 1/2, more preferably less than 1/4, more preferably less than 1/10, more preferably less than 1/50, more preferably less than 1/100, more preferably less than 1/200.

Focusing - Lenses

Signals from at least a portion of the sample are focused onto the array of detection elements, by the use of a focusing means, preferably by the use of one lens, it is

however possible to use two lenses, or more than two lenses. The number of lenses used for the focusing system can affect the complexity of any measuring system.

The focusing of a signal from the sample onto any detector is dependent on the position of the sample relative to any detector. When the construction of measuring system is such, that the relative position of the sample and any detector can vary, then there is advantage in being able to adjust the focusing of the system. This can often be achieved by first taking at least one measurement of any signal from the sample and then on the bases of this, to adjust the focusing of the system. This procedure can be repeated a number of times in order to obtain acceptable focusing. In the same manner the focusing of signal from the sample or sample material is adjusted, preferably where the extend of the adjustment is determined by at least one measurement of a signal from the sample.

The collection angle of a focusing arrangement used can have effect on the intensity of any signal collected on the array of detection elements. When high sensitivity is needed it is therefore practical to increase the collection angle. The preferred size of the collection angle can also be determined by other requirements which are made to the system, such as focusing depth. In these situations the collection angle of the focusing means is preferably at least 2 degrees, preferably more than 5 degrees, more preferably more then 15 degrees, more preferably more than 20 degrees, more preferably more than 50 degrees, more preferably more than 120 degrees, more preferably more than 150 degrees.

Signal

The signals measured from one or more detection elements may be corrected for systematic or varying bias by the use of a calculating means, the bias correction being accomplished by the use of one or more pre-defined value(s), preferably where each measured signal for one or more detection elements in said array of detection elements has one or more pre-defined value(s), more preferably where each pre-defined value is determined on the bases of one or more of any previous measurements.

The bias correction may be performed by subtracting the results obtained in one or several of other measurements from the measured signal, preferably where the

other measurements are one or several of measurements of the same sample, or sample material, more preferably where the other measurement is the measurement taken previously of the same sample or sample material.

Also the signal from one or more detection elements may be corrected for intensity by the use of a calculating means, said correction being accomplished by the use of one or more pre-defined value(s), preferably where each measured signal for one or more detection elements in said array of detection elements has one or more pre-defined value(s), more preferably where each pre-defined value is determined on the bases of one or more of any previous measurements.

In some situations e.g. in an analogue-to-digital conversion it could also be of interest to adjust the level of 2, preferably 3, more preferably 4, more preferably 5, more preferably 6, more preferably 7, more preferably 8, more preferably more than 8, separate output channels in such a way that one, preferably more than one, of the output channels has/have substantially different level from the other output channel(s), where the identification of which of the output channels, or combination thereof, has substantially different output level, is correlated to the intensity of said signal.

For the analysis of any measured signal it is often necessary to digitalise the signal, in such a way that a given intensity of any signal is transformed into a digital representation. This can be done by having a series of channels, were the information about which of these channels has signal which differs from the other channels determines the intensity, or even by having more than one of this channels forming a combination, preferably in a way similar to binary representation.

Processor

Information of the signals detected by the detection means are input into a processor for processing, displaying and optionally storing the information.

The signal information may be displayed on a display connected to the processor and/or printed. The information displayed may be any kind of information relating to the signals measured and/or the system used, such as a number, size distribution, morphology, classification of analytes, excitation wavelength, emission wavelength,

magnification. In particular the data processing means is capable of distinguishing partially overlapping areas of product.

Storage capacity, for instance used for storing information about measured signals from the detection elements, is often one of those components which have considerable effect on the cost of production. It is therefore of interest to be able to perform the assessment of parameters without substantial any use of such storage capacity, such that the assessment of analytes in a sample is performed without the use of substantially any storage capacity means being used to store measured signals from the detection elements in the array of detection elements.

On the other hand, it is often difficult to accomplish assessment without the use of any storage capacity, but preferably the amount of such storage capacity should not be more than what is needed to store the information from all measured detection elements, preferably where only a fraction of the information can be stored.

In some situations measured signal from the detection elements in the array of detection elements is stored by means of storage capacity, the storage capacity being able to store a number of measurements equivalent to, or less than, the number of detection elements, preferably less than 1/2 the number of detection elements, more preferably less than 1/4 the number of detection elements, more preferably less than 1/8 the number of detection elements, more preferably less than 1/16 the number of detection elements, more preferably less than 1/32 the number of detection elements, more preferably less than 1/64 the number of detection elements, more preferably less than 1/128 the number of detection elements, more preferably less than 1/256 the number of detection elements, more preferably less than 1/512 the number of detection elements, more preferably less than 1/1024 the number of detection elements in the array of detection elements.

In other certain circumstances it is advantageous that the measured signal from the detection elements in the array of detection elements is stored by means of storage capacity, the storage capacity being able to store a number of measurements greater than the number of detection elements, preferably equivalent to, or greater than, 2 times the number of detection elements, more preferably equivalent to, or greater than, 4 times the number of detection elements, more preferably equivalent

to, or greater than, 8 times the number of detection elements, more preferably equivalent to, or greater than, 16 times the number of detection elements, more preferably equivalent to, or greater than, 32 times the number of detection elements, more preferably equivalent to, or greater than, 64 times the number of detection elements, more preferably equivalent to, or greater than, 128 times the number of detection elements, more preferably equivalent to, or greater than, 256 times the number of detection elements, more preferably equivalent to, or greater than, 512 times the number of detection elements, more preferably equivalent to, or greater than, 1024 times the number of detection elements in the array of detection elements.

Other, more complicated aspects of the assessment of parameters, can require the use of considerable amount of storage capacity. In this aspect it can therefore be necessary to have storage capacity which can store more information than is collected in one measurement of the detection elements used.

It is possible to make the correlation and the assessment of the parameters of the sample by using a calculation mean, preferably a digital computer, one commercially available from Analogue Devices (ADSP 2101), equipped with storage capacity which can only store information in amount substantially equivalent to a small fraction of the total number of detection elements, the assessment of the number of objects then being based on substantially real time processing of data, preferably in such a way that the measured information from each detection element, or a line of detection elements, or two or more lines of detection elements, is used for the assessment, substantially without any delay, such as a delay which would otherwise be caused by storing the measured information.

However, it is often preferred to store substantially all measured information by the use of a first calculation mean, preferably a digital computer, before the processing of the information by a second calculation mean, preferably a digital computer, and thus allowing the measured information to be processed at substantially the same rate it is obtained, but with a substantial time delay between the measurement of any information and the processing of the same information; preferably, this is accomplished by using only one calculating mean, preferably a digital computer, equipped with enough resources to accomplish the task.

Medical markers

The system and the method of the current invention may be used for detection of clinical markers of conditions in a human being or in an animal. One preferred use of the method and system is for the early diagnosis of myocardial infarction or cardial infarct. When cells in the heart suffer or die they leak a number of enzymes into the blood. The blood level of these enzymes raise above the normal levels hours before the patient show acute symptoms of cardial infarct. A reliable and early diagnosis can thus be made by measuring the blood level of cardial related enzymes.

According to prior art techniques the enzymes are measure by subjecting a blood sample to traditional ELISA in the laboratory. The duration of this ELISA detection is several hours. During this period the patient does not show any acute symptoms of cardial infarct. At the moment the diagnosis is ready, the cardial infarct may have developed to a level, where it is too late to save the patient's life.

It is envisaged that the blood levels of these particular enzymes and of other enzymes being medical or clinical markers can be measured rapidly using the detection technique of the present invention.

The system may be used for detection of clinical or medical markers in essentiallly all animals, particularly in mammals such as human beings, cows, horses, poultry, sheep, goat.

One-sided and two-sided systems

The detection device may be laid out as a one-sided device, i.e. a device for which the light is directed to the sample from the same side of the sample as the side for which the signals are detected.

The detection device may also be laid out as a one-sided device, in which the excitation light is directed to the sample from the same side of the sample as the side for which the signals emitted from the sample are detected.

By this apparatus a variety of advantages have been achieved as compared to conventional fluorescence microscopes. First of all it is possible to arrange the sample to be assessed directly in the sample plane instead of sliding it into the sample plane between the detector and the excitation light. Furthermore it has become possible to detect surface fluorescence of a sample not being transparent.

It is also possible to increase the intensity of the excitation light without compromising the detectors.

Also samples having a nature whereby it is normally not possible to arrange the sample in a microscope may be assessed by the use of the present system, in that the microscope may be placed directly on the sample whereby the surface of the sample simply constitutes the sample plane.

Finally it is possible to produce a more compact and thereby more easily handled apparatus, in that the excitation light means is arranged on the same side of the sample plane as the detector, thus shortening the axis of the apparatus by at least 25% as compared to conventional apparatuses.

By the present invention it is possible to assess parameters of a sample which has up to now only been reliably assessed by the use of flow cytometric equipment. It is possible to assess parameters of a large sample in one exposure thus reducing the statistical errors normally counted for when assessing large samples by assessing only parts thereof per exposure.

Furthermore, it is possible to obtain more than one fluorescence signal from the sample in one exposure thereby facilitating classification of particles of the sample, due to their different fluorescence signals.

Thus, the one-sided apparatus according to the invention may be constructed in a wide variety of combination, which are all within the scope of this invention. In particular the principal combination discussed below are envisaged.

The apparatus may be constructed as a single fluorescence apparatus wherein the light sources and the excitation light filters are identical.

A multiple fluorescence apparatus, such as an apparatus providing at least two different fluorescence signals, may be provided by at least one of the following:

- A first and a second light source, said light sources emitting light of different wavelengths

- A first and a second filter being different whereby the excitation light of at least two different wavelengths are exposed to the sample

- A first and a second emission filter being different, such as a dual band filter, whereby at least two different fluroescence signals are emitted to the detector(s)

It is however a further advantage that the present apparatus may be constructed as a double-sided apparatus, whereby excitation light may be directed onto the sample from both sides of the samples, or detection means are arranged to detect signals from both sides of the samples, or a combination of both.

Thus by a double-sided apparatus is meant an apparatus according to the invention further provided with:

- A second excitation light means located in a second light plane, said second light plane being parallel with the sample plane and located on the other side of the sample plane as opposed to the first light plane. Thereby the sample is receiving excitation light from both sides of the sample considerably increasing the energy exposed to the sample, and/or

- A second detection means arranged so that the sample is positioned between the first detection means and the second detection means. Hereby it is possible to assess different information regarding the signals from the sample by one exposure detection. For example the first detection means may be adapted to register the number of particles of the sample, whereas the second detection means is adapted to register the morphology of the particles in the sample.

In a preferred embodiment the double-sided apparatus comprises both double-sided excitation system and double-sided detection system.

The second excitation light means may be any of the light means discussed in relation to the first light means. Depending on the purpose of the fluorescence microscope the light means may be different or identical.

Furthermore, it may be of interest that the excitation light would constitute different wavelength bands whereby illumination with different wavelengths is achieved.

The second detection means may be any of the detection means discussed in relation to the first detection means.
Any suitable combination of light sources, filters, magnification and detectors are envisaged by the present invention. In the following preferred embodiments of the two-sided system is discussed.

The apparatus may be a single fluorescence system, wherein excitation light of substantially identical wavelength are exposed to the sample from two sides. Thereby the excitation light may be intensified.

In a double-sided excitation light apparatus a first excitation light means exposes the sample to one wavelength from one side of the sample, and the second excitation light exposes the sample to another wavelength from the other side of the sample. It is understood herein, that of course the first excitation light and the second excitation light respectively, may comprise different light source and/or filters, whereby the sample may be illuminated with even more wavelengths as discussed above.

The double-sided excitation light apparatus may comprise one detector, whereby the apparatus functions as a partly transmitting system.

In another embodiment the double-sided excitation light apparatus comprises two detecting means. Thereby an increased amount of information may be obtained from the sample. In one aspect the two detecting means may obtain equal, although

mirror images (the images on the two detectors are mirror images of each other), information relating to the sample providing a validation of the information.

The apparatus according to the invention may also be a double-sided detection apparatus using a one-sided excitation light means. Thereby one detector detects signals being transmitted through the sample.

Independent of the arrangement of excitation light, a double-sided detecting system is capable of increasing the amount of information received. For example different wavelength may be received by the two detectors, and or different detectors, having different sensibility may be used. Furthermore, by using for example different magnification for the two detectors the information relating to the sample may be increased. One side of the system may assess for example number of particles in a large area of the sample, for example by a low magnification, and the other side of the system may assess the morphology of the particles by using a larger magnification. Combinations of magnification may for example be 1:1 and 1:4, 1:1 and 1:10, 1:2 and 1:4, 1:2 and 1:10. The signal information transferred from the two detectors is preferably transmitted to the same processor, whereby the information may be displayed separately, as well as being combined providing for example specific morphology information related to specific particles the position and number of which are detected by the other detector.

It is also possible to use the apparatus according to the invention as a double-sided apparatus where the other side is a conventional light microscope or any other type of microscope. When using the other side of the system as a non-fluorescence microscope, the illumination light for the microscope may be suitably arranged on either side of the sample in relation to the microscope.

The double-sided apparatus comprising a conventional microscope on one side, may comprises a one-sided or a double-sided excitation light system for the fluorescence part of the system.

When using a double-sided detection system the processor of the first detection means may receive signal data from the second detection means as well in order to

simplify the apparatus. It is however possible to install a separate processor for each detection means.

Examples of one and double sided excitation and detection systems

In the following one embodiment of the detection system is discussed in more detail in relation to the drawings.

In Fig. 1 an example of the illumination and detection system 1 is shown in schematic form. The sample is arranged in a sample compartment 2 the sample plane. Excitation light from the light sources 4a, 4b in the excitation light means 3 is exposed onto the sample through a main light path 5a, 5b.

Fluorescence signals from the sample is emitted to the detection means 6 comprising at least one detector 7. The path of the emitted signals is following an axis between the sample and the detector, the detection-sample axis 8.

The signal data are transmitted to a processor 9 coupled to the detecting means 6. The fluorescence signals from the sample is filtered by means of emission filter 14 and focused to the detection means 9 by means of a focusing lens 10.

The light sources 4a, 4b are arranged in a light housing 11, whereby the transmission of excitation light directly to the detection means is avoided. Furthermore excitation light filters 12a, 12b are positioned in the excitation light beam.

Fig. 2 shows a cross-section of the circular supporting material 13 of the excitation light filters wherein the position of the light sources have been indicated by circles in broken lines.

In Fig. 3 the light path and signal path is shown in more detail. In the light path the main light path is shown as 5. Furthermore, the detection-sample axis is shown by broken lines 8. The collection angle of the system is denoted C shown between two arrows and the angle between the main light path and the detection–sample axis is denoted E.

In Fig. 4 a double-sided excitation/detection system 1 is shown wherein the systems on each side of the sample are identical and as described for the one-sided system of Fig. 1.

Fig. 5 shows a double-sided excitation system wherein excitation light from the light sources 4a, 4b in the first excitation light means 3a and excitation light from the light sources 4a, 4b in the second excitation light means 3b is exposed onto the sample 2 from both sides of the sample 2. As discussed above, the light sources may be identical or different depending on the information to be assessed. Furthermore, the filters used for each light source may be different or identical.

Fluorescence signals are transmitted through and reflected from the sample due to the excitation light arrangement and emitted to the detection means 6. The path of the emitted signals is following an axis between the sample and the detector, the detection-sample axis 8.

The signal data are transmitted to a processor coupled to the detecting means as described above.

Fig. 6 shows a double-sided detecting system, using a single-sided excitation system, wherein reflected fluorescence signals from the sample 2 are detected by detecting means 6a comprising detector 7a. The reflected fluorescence signals are transmitted though filter 14a and focused by lens 10 a.

Furthermore, transmitted fluorescence signals from the sample 2 are detected by detecting means 6b comprising detector 7b. The reflected fluorescence signals are transmitted though filter 14b and focused by lens 10b.

Filter 14a is preferably different from filter 14b, whereby information relating to at least two different fluorescence signals is obtainable.

Also the magnification in the two detecting systems may be different, for example by lens 10a being different from lens 10b.

Example

Sample type	Human whole blood anticoagulated with sodium citrate.
Analyte	Human CD45+ blood cells with CD45 antigen localised on the surface.
Antibody-conjugate	Monoclonal mouse IgG (Fab) specific for CD45 antigen. IgG is conjugated to biotin (100 µg antibody conjugate per ml of Phosphate Buffered Saline with pH 7.2 (PBS)).
Avidine-AP conjugate	Streptavidine conjugated to Alkaline Phosphatase (10 µg Avidine conjugate per ml PBS).
Enzyme substrate	AttoPhos™ Substrate Set (Boehringer Mannheim no. 1681 982). Alkaline Phosphatase will convert the AttoPhos Substrate to a fluorochrome (the Product) which has maximum exitation at 435 nm and maximum emission at 560 nm.
Lysis Buffer A + B	Uti-Lyse (Dako no. S-3350) is used for lysis of red blood cells but leaving the cell membrane of the white blood cells sufficiently intact in order to carry out a surface marker based cell analysis.
Microscopic counting	The counting of spots derived from the CD45+ target cells can be carried out using an EPI-fluorescence microscope equipped with a suitable light source (e.g. xenon lamp), a suitable filter set for the Product, a 4x objective, a CCD camera and a Bürker-Türk counting chamber (depth 0.1 mm).

A portion of 10 µl of antibody conjugate is added to 100 µl of sample. Following gentle mixing and incubation for 30 minutes at room temperature (RT) a portion of 2

ml PBS is added to the suspension. Following gentle mixing the suspension is centrifuged at 300g for 5 minutes. Then the supernatant is removed and the pellet is resuspended in 2 ml of PBS. Following gentle mixing the suspension is centrifuged at 300g for 5 minutes. Then the supernatant is removed and the pellet is resuspended in 2 ml of PBS. Following gentle mixing the suspension is centrifuged at 300g for 5 minutes. The supernatant is removed (leaving approximately 100 µl fluid) and a portion of 100 µl of Lysis Buffer A is added. Following gentle mixing the suspension is incubated for 10 minutes at RT. A portion of 1 ml of Lysis Buffer B is added to the suspension and incubated for 10 minutes at RT. Then the suspension is centrifuged at 300g for 5 minutes. Then the supernatant is removed and the pellet is resuspended in 2 ml of PBS. Following gentle mixing the suspension is centrifuged at 300g for 5 minutes. Then the supernatant is removed and the pellet is resuspended in 2 ml of PBS. Following gentle mixing the suspension is centrifuged at 300g for 5 minutes.

Then the supernatant is removed and a portion of 100 µl of Avidine-AP conjugate is added to the pellet. Following gentle mixing and incubation for 15 minutes at RT a portion of 2 ml PBS is added to the suspension. Following gentle mixing the suspension is centrifuged at 300g for 5 minutes. Then the supernatant is removed and the pellet is resuspended in 2 ml of PBS and after gentle mixing the suspension is centrifuged at 300g for 5 minutes. After removing the supernatant the pellet is resuspended in 2 ml of PBS and after gentle mixing the suspension is centrifuged at 300g for 5 minutes. After removing the supernatant the pellet is resuspended in 500 µl of AttoPhos™ Substrate solution prepared according to manufacturers instruction. Immediately, a small portion of the suspension is applied to a Bürker-Türk counting chamber and then an image is created using the EPI-fluorescence microscope. With short intervals of 1 second further images are created and the formation of dots and the growths of these dots is observed. The dots will increase in diameter due to diffusion of the product.

CD45+ cells can be observed as fluorescent dots within the measuring volume. Based on the number of dots and the volume of the mixture in Bürker-Türk counting chamber which is represented by the image a concentration of CD45+ cells in Bürker-Türk counting chamber can be calculated. The concentration of CD45+ cells in the the blood sample can then be calculated because the dilution factor is known.

Claims

1. A method for assessing at least one quality parameter or at least one quantity parameter of at least one species of analytes in a sample comprising the steps of

establishing a sample domain having at least one wall,

arranging catalyst-analyte complexes between the at least one species of analytes and at least one catalyst in a manner allowing the analytes to move relative to the wall(s) of the sample domain,

arranging a substrate in the sample domain, said substrate being capable of being converted into a product through catalysing by said catalyst,

contacting the substrate with the catalyst-analyte complexes of individual analytes allowing a detectable amount of product to be produced,

recording an image of the product related to individual analytes in the sample domain,

correlating the image to the at least one quality parameter or at least one quantity parameter of the at least one species of analytes.

2. The method according to claim 1, wherein the catalyst-analyte complex comprises a species-selective linkage.

3. The method according to claim 2, wherein the species-selective linkage comprises an antigen-antibody linkage.

4. The method according to claim 2, wherein the species-selective linkage comprises a DNA, or RNA, or PNA, or LNA hybridisation.

5. The method according to claim 1, wherein formation of the catalyst-analyte complex comprises catalysed reporter deposition.

6. The method according to claim 1, whereby analytes are particles, such as biological particles.

7. The method according to claim 1, wherein the analytes are bound to a solid support, preferably where such solid support are beads in suspension.

8. The method according to claim 1, whereby the analytes are selected from the group consisting of cells, cell walls, bacteria, plasmodia, virus, prions, macromolecules, proteins, polypeptides, peptides, genes, DNA, RNA, or fragments or clusters thereof.

9. The method according to claim 1, whereby the at least one species of analyte is a medical marker of a disease.

10. The method according to claim 9, whereby the marker is a marker for cardial infarct.

11. The method according to claim 8, wherein the cells are selected from mammalian cells, insect cells, reptile cells, fish cells, yeast cells, and fungi cells.

12. The method according to claim 8, wherein the cells are selected from blood cells, sperm cells, and bone marrow cells.

13. The method according to any of the preceding claims, whereby the sample is a liquid sample.

14. The method according to any of the preceding claims, whereby the sample is selected from the group consisting of milk, milk products, urine, blood, sperm, nasal secrete, tears, faeces, waste water, process water drinking water, cerebro-spinal fluid, gall, bone marrow, food, feed, and mixtures, dilutions, or extracts thereof

15. The method according to any of claims 1-12, whereby the sample is a solid sample, which is pre-treated prior being arranged in the sample domain.

16. The method according to claim 15, whereby the sample is a biopsy of a muscle, a brain, a kidney, a liver, a spleen.

17. The method according to any of the preceding claims, whereby the substrate is AttoPhos, 4-MUP, HNPP, 4-MUG, CDP-Star, CSPD, Super Signal Substrate (Pierce, Rockford, Ill), Luminol/4-iodophenol, Galacton Plus, DAB, OPD, AEC, 5AS, 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid), 4C1N, o-dianisidine, TMB, ABTS, BCIP, Naphthol AS-TR phosphat, pNPP, PMP, X-Gal, CPRG.

18. The method according to any of the preceding claims whereby the catalyst is an inorganic catalyst.

19. The method according to any of the claims 1-17, whereby the catalyst is an organic catalyst.

20. The method according to claim 19, whereby the catalyst is an enzyme.

21. The method according to claim 20, whereby the catalyst is selected from the group consisting of phosphatase such as alkaline phosphatase, β-galactosidase, peroxidase such as for example horseradish peroxidase, β-glucuronidase, β-glucose-6-phosphate dehydrogenase, glucose oxidase, urease, luciferase, β-lactamase and β-amylase.

22. The method according to any of the preceding claims, whereby at least one obtained product precipitates upon formation.

23. The method according to claim 22, whereby at least one obtained product precipitates on a surface of the sample compartment.

24. The method according to any of the preceding claims, whereby at least one obtained product is coloured.

25. The method according to any of the preceding claims, whereby at least one obtained product is photoluminescent or chemiluminescent.

26. The method according to any of the preceding claims, whereby at least one obtained product is fluorescent.

27. The method according to any of the preceding claims, whereby at least one obtained product emits electromagnetic radiation in the range of 300 nm to 1200 nm when exposed to electromagnetic radiation in the range of 250 nm to 600 nm.

28. The method according to any of the preceding claims, wherein at least one product obtained is excited by excitation light prior to recording an image.

29. The method according to claim 28, wherein the excitation light is a light source selected from the group of, light emitting diode (LED), gas laser, solid state laser, laser diode, gas lamp, halogen lamp, xenon lamp.

30. The method according to any of the preceding claims, whereby the sample domain is three-dimensional.

31. The method according to any of the preceding claims, whereby the sample domain is a flow through chamber.

32. The method according to any of the preceding claims, whereby the sample domain is part of a disposable cassette.

33. The method according to any of the preceding claims, whereby at least one wall of the sample domain is transparent.

34. The method according to any of the preceding claims, whereby at least one linkage between the catalyst and the analyte comprises two or more antibodies.

35. The method according to any of the preceding claims, whereby at least one catalyst is conjugated to an antibody being immunologically bound to an antigen on the species of analyte.

36. The method according to any of the preceding claims, whereby at least one catalyst is conjugated to a first antibody being immunologically bound to second antibody, being immunologically bound to an antigen on the species of analyte.

37. The method according to any of the preceding claims, whereby at least one catalyst is conjugated to avidin.

38. The method according to any of the preceding claims, whereby at least one catalyst is conjugated to streptavidin.

39. The method according to any of the preceding claims, whereby at least one linkage comprises a catalyst conjugated to avidin, an antibody conjugated to biotin and being immunologically bound to an antigen on the species of analyte or vice versa.

40. The method according to any of the preceding claims, whereby at least one linkage comprises a catalyst conjugated to avidin, a first antibody conjugated to biotin, and a second antibody being immunologically bound to an antigen on the species of analyte.

41. The method according to any of the preceding claims, whereby the linkage is formed before the sample is transferred to the sample domain.

42. The method according to any of the preceding claims, wherein the analyte is a DNA containing analyte and the DNA or fractions of the DNA are stained with a DNA staining compound.

43. The method according to any of the preceding claims, whereby an additional linkage is formed between a second species of analyte and a second catalyst.

44. The method according to any of the preceding claims, whereby two or more additional linkages are formed between a second, third and optionally subsequent species of analyte and a second, third, and optionally third catalyst.

45. The method according to any of the preceding claims, further comprising the step of removing excess catalyst not being linked to the species of analytes.

46. The method according to claim 45, whereby excess catalyst is removed through centrifugation.

47. The method according to claim 45, whereby excess catalyst is removed through filtration.

48. The method according to claim 45, whereby excess catalyst is removed through flushing.

49. The method according to claim 45, whereby removal of excess catalyst comprises binding the analyte-catalyst complex to a magnetic bead.

50. The method according to any of the preceding claims, further comprising the contacting of co-factors with the catalyst-analyte complex.

51. The method according to any of the preceding claims, further comprising the contacting of a buffer with the catalyst-analyte complex.

52. The method according to any of the preceding claims, whereby at least one substrate is added to the catalyst-analyte complex in the sample domain.

53. The method according to any of the preceding claims, whereby at least one substrate is added to the catalyst-analyte complex before transferring it to the sample domain.

54. The method according to any of the preceding claims, whereby the initiation of the reaction catalysed by the catalyst is controlled by temperature changes.

55. The method according to any of the preceding claims, whereby a pre-substrate is added to the catalyst-analyte complex before transferring it to the sample domain.

56. The method according to claim 55, whereby a conversion of the pre-substrate into the substrate can be controlled externally.

57. The method according to claim 56, whereby the conversion is controlled by illumination.

58. The method according to claim 56, whereby the conversion is controlled by a change in temperature.

59. The method according to any of the preceding claims, whereby the reaction catalysed by the catalyst can be controllably stopped externally.

60. The method according to any of the preceding claims, whereby the step of producing a product is carried out in a liquid environment.

61. The method according to any of the claims 1-59, whereby the step of producing a product is carried out in a viscous environment.

62. The method according to any of the claims 1-59, whereby the step of producing a product is carried out in a semi-solid environment, preferably where the semi-solid environment is a gel.

63. The method according to claim 62, wherein the semi-solid environment is formed after the analytes have been introduced to the sample compartment, preferably where the forming of the semi-solid environment is controlled by external factors such as temperature, light and agitation.

64. The method according to any of the preceding claims, whereby the duration of the step of producing a product is below 60 minutes.

65. The method according to claim 64, whereby the duration of the step of producing a product is below 15 minutes, preferably below 5 minutes, more preferably below 1 minute, more preferably below 30 seconds, more preferably below 15 seconds, more preferably below 10 seconds, more preferably below 5 seconds, more preferably below 2 seconds.

66. The method according to any of the preceding claims, whereby the recording of image comprises the use of a confocal scanner.

67. The method according to any of the preceding claims, whereby the image of product is recorded using an array of detection devices.

68. The method according to claim 67, wherein the image of product is recorded using a one-dimensional array of detection devices.

69. The method according to claim 67, wherein the image of product is recorded using a two-dimensional array of detection devices.

70. The method according to claim 67, wherein the image of product is recorded using a CCD, a CMOS, a video camera or a photon counting camera.

71. The method according to any of the preceding claims, whereby the image is recorded without magnification.

72. The method according to any of the preceding claims, whereby the image is recorded with a magnification factor below 20, preferably below 10, more preferably below 5, such as 4, more preferably below 4 such as 2, more preferably below 2 such as 1.

73. The method according to any of the preceding claims, whereby the image is recorded with a magnification factor below 1, preferably below 0.9, such as 0.8, more preferably below 0.8 such as 0.6, more preferably below 0.6 such as 0.5.

74. The method according to any of the preceding claims whereby the image is recorded in one exposure.

75. The method according to any of the claims 1-73 whereby the image is recorded in two, three or more exposures.

76. The method according to claim 75, wherein the assessment of at least one quality parameter or at least one quantity parameter is done by correlating more than one image to the at least one quality parameter or at least one quantity parameter, preferably by correlating two images, more preferably correlating more than two images, more preferably correlating more than four images.

77. The method according to claim 76, where information about the changes in the image in course of time is used in the assessment of at least one quality parameter or at least one quantity parameter.

78. The method according to any of the preceding claims, whereby the recorded image is processed.

79. The method according to claim 78, whereby the recorded image is processed using data processing means.

80. The method according to claim 79, whereby the data processing means distinguish partially overlapping areas of product.

81. The method according to any of the preceding claims, whereby the correlation comprises estimation of the number of spots on the image.

82. The method according to any of the preceding claims, whereby the correlation comprises estimation of the size of spots on the image.

83. The method according to any of the preceding claims, whereby the correlation comprises distinction between at least two spectral properties of product.

84. The method according to any of the preceding claims, further comprising the assessment of at least one additional quality parameter or at least one additional quantity parameter.

85. The method according to claim 84, whereby the assessment of the at least one additional quality parameter or at least one additional quantity parameter

comprises detection of fluorescence, chemiluminescence, photoluminescence, autoluminescence of a species of analyte.

86. The method according to any of the preceding claims, whereby the at least one quality parameter is selected from the group consisting of viability, size, identity, respiration, and presence of an analyte.

87. The method according to any of the preceding claims, whereby the at least one quantity parameter is selected from the group consisting of number of species of analyte in a volume of sample, concentration of species of analyte in a volume of sample, amount of species of analyte in a volume of sample.

88. The method according to any of the preceding claims, whereby the recording of an image further comprises exposing a first surface of the sample directly with excitation light from a first light means having at least a first light source, by use of focusing means detecting a fluorescence signal from the first surface of the sample onto a first detection means comprising at least a first detector.

89. The method according to claim 88, wherein at least the first light means is located in a first light plane parallel to the sample plane, said first light plane being between the sample plane and the first detection means.

90. The method according to any of the preceding claims 88-89, wherein an excitation light filter is inserted in the excitation light path from at least one light source.

91. The method according to claim 89, wherein the excitation light is arranged as light sources on a supporting material.

92. The method according to any of the preceding claims 88-91, wherein substantially identical filters are used for all the light sources.

93. The method according to any of claims 88-92, wherein a first light source is filtered through a first filter, and a second light source is filtered through a second filter, the first filter and the second filter being different.

94. The method according to any of the preceding claims 88-93, further comprising exposing a second surface of the sample directly with excitation light from a second light means having at least one light source.

95. The method according to claim 94, wherein the second excitation light means is located in a second light plane said plane being parallel with the sample plane and located on the other side of the sample plane than the first light plane allowing the sample to be exposed on two opposite surfaces.

96. The method according to claim 94 or 95, wherein a filter inserted in the light path from the second light means is different from a filter inserted in the light path of the first light means.

97. The method according to any of claims 88-96, wherein a second detection means is arranged so that the sample compartment is positioned between the first detection means and the second detection means.

98. The method according to claim 97, wherein the first detection means is identical with the second detection means.

99. The method according to any of the preceding claims 88-98, wherein an emission light filter is inserted in the emission light path to at least the first detector.

The method according to any of the preceding claims 88-99, wherein a collimating lens is arranged in the emission light path.

100. The method according to any of the preceding claims 88-100, wherein the angle between the excitation main light and the detection-sample axis is in a range between 35° and 90°, preferably between 45° and 85°, more preferably between 50° and 85°.

101. The method according to claim 88, wherein at least the first light means is located in a first light plane parallel to the sample plane, said first light plane being positioned at a distance from the sample plane behind the detector.

102. The method according to claim 102, wherein the detector is positioned in a housing having an opening allowing the emitted signals to reach the detector(s).

103. A system for the assessment of at least one parameter of analytes in a liquid sample, comprising

a device comprising a sample domain comprising an exposing domain, an inlet through which a volume of a liquid sample representing the analyte material can been introduced, and a flow system comprising at least a channel allowing at least a portion of the volume of the liquid sample to flow within the device,

the device further comprising means to control the flow of liquid around a catalyst-analyte complex in the sample domain,

a detection device comprising at least a first detector for quantitatively detecting spatial image data and a processor for processing the detected image presentation,

the device and the detection device having means for arranging the device in relation to the detection device in a manner allowing electromagnetic signals from a sample in the exposing domain of the device to pass to the detection device and to form, in the detection device, a spatial image representation of the exposing domain.

104. A system according to claim 104, wherein the flow system additionally comprises a compartment or a flow channel part in or from which at least part of one or more reaction components initially loaded in the compartment or flow channel part is added to at least a portion of the volume of the liquid representing the sample.

105. A system according to claim 104, further comprising at least a first light source.

106. A system according to claim 106, wherein the first light source comprises an excitation light source.

107. A system according to claim 107, wherein the first light source and the detector are located on the same side of the exposing domain.

108. A system according to claim 108, comprising a second light source and a second detector, located on the opposite side from the first light source and first detection means.

109. The system according to claim 106 or 108, further comprising an excitation light filter inserted into the excitation light path.

110. The system according to claim 110, wherein the excitation light filter is essentially circular, such as essentially ring formed.

111. Use of a system according to claim 104-111 for diagnosis of a condition in an individual.

112. The use according to claim 112, wherein the individual is a human being.

113. The use according to claim 112, wherein the individual is an animal other than humans, such as cow, pig, horse, poultry, sheep, goat.

114. The use according to claim 112, wherein the condition is cardial infarct or a risk for suffering form a cardial infarct.

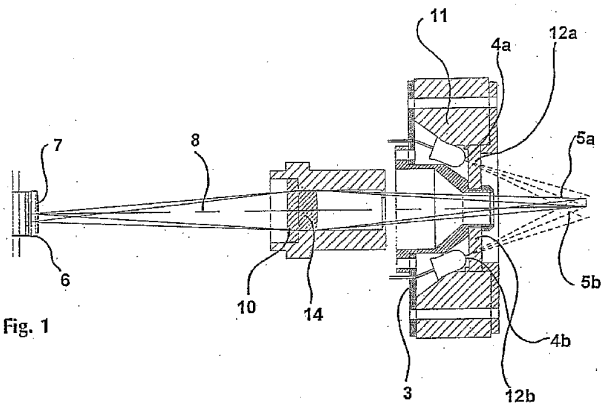

Fig. 1

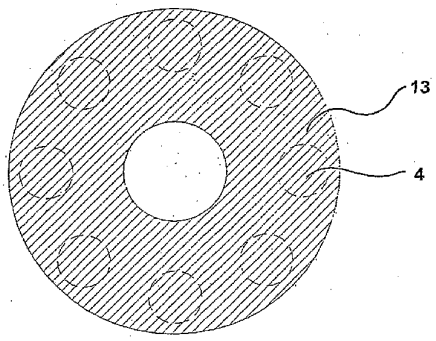

Fig. 2

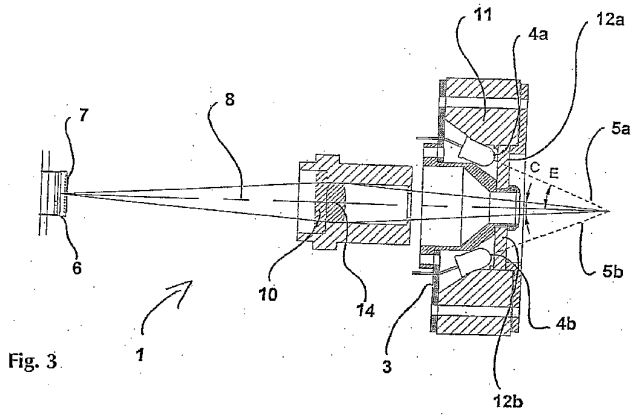
11
4a
12a
7
8
5a
C
E
6
10
14
5b
4b
1
3
12b

Fig. 3

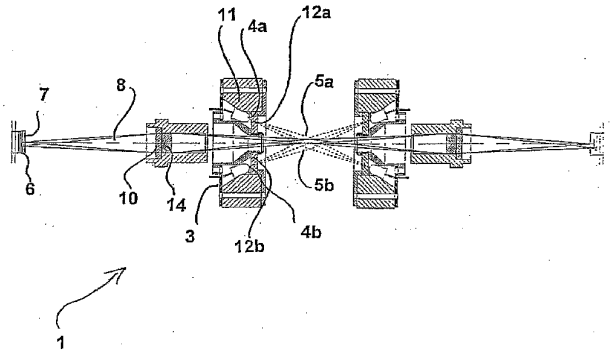
11
4a
12a
7
8
5a
6
10
14
5b
3
12b
4b
1

Fig. 4

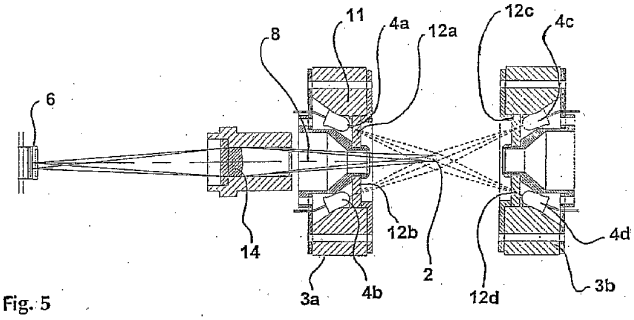
6
8
11
4a
12a
12c
4c
14
12b
2
4d
3a
4b
12d
3b

Fig. 5

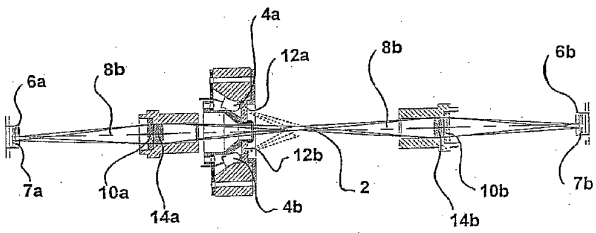
4a
6a
8b
12a
8b
6b
12b
7a
10a
14a
4b
2
10b
14b
7b

Fig. 6

【国際調査報告】

INTERNATIONAL SEARCH REPORT

International application no
PCT/DK 01/00490

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/53 C12Q1/68 G01N21/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 59233 A (LUMINEX CORP) 30 December 1998 (1998-12-30) page 21, line 54 -page 22, line 6; claim 1	1-114
X	WO 00 28297 A (CHEMOMETEC A S) 18 May 2000 (2000-05-18) page 10, line 11 - line 16 page 15, line 13 - line 20 page 31, line 9 - line 22	1-114
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☐ Further documents are listed in the continuation of box C. ☒ Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer
P. Andersson/BS

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Ｇ０１Ｎ 33/53	Ｇ０１Ｎ 33/53	Ｍ	
Ｇ０１Ｎ 33/543	Ｇ０１Ｎ 33/53	Ｓ	
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	Ｇ０１Ｎ 33/543	５４５Ａ	
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4B063 QA01 QA19 QQ02 QQ03 QQ05 QQ16 QQ18 QQ41 QQ79 QR02 QR04 QR10 QR13 QR15 QR41 QR55 QR57 QS11 QS34 QS39 QX02 QX07

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申请(专利权)人(译)	Shemometekku-ACTY洛杉矶萝卜		
[标]发明人	マルティングレンスビェアウ		
发明人	マルティン·グレンスビェアウ		
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代理人(译)	矢野正树		
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外部链接	Espacenet		

摘要(译)

本发明涉及检查至少一种分析物的至少一种定性参数和/或至少一种定量参数的方法，其中所述至少一种分析物是能够将底物催化到产物上的催化剂。因此，通过检测在分析物周围形成的产物来检查分析物。