CytoBuoy 浮游植物扫描成像
流式细胞监测系统
——海洋篇

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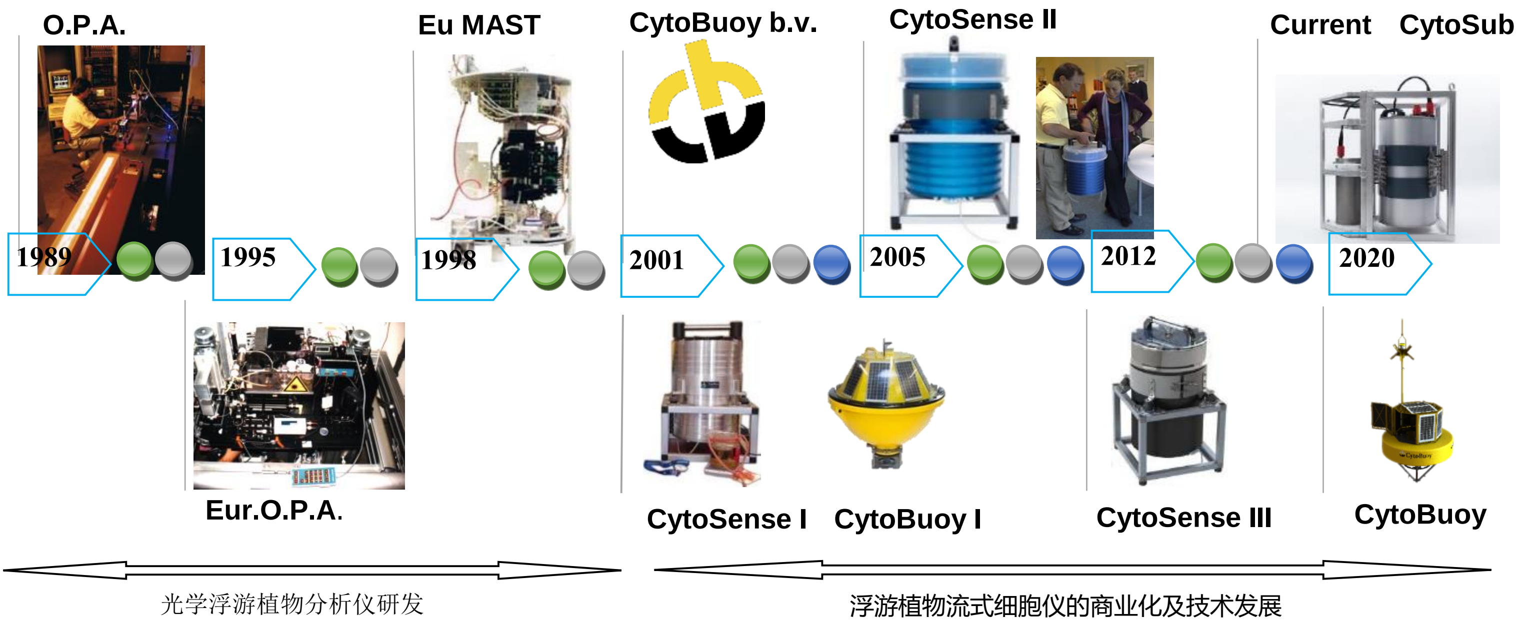
发展历程



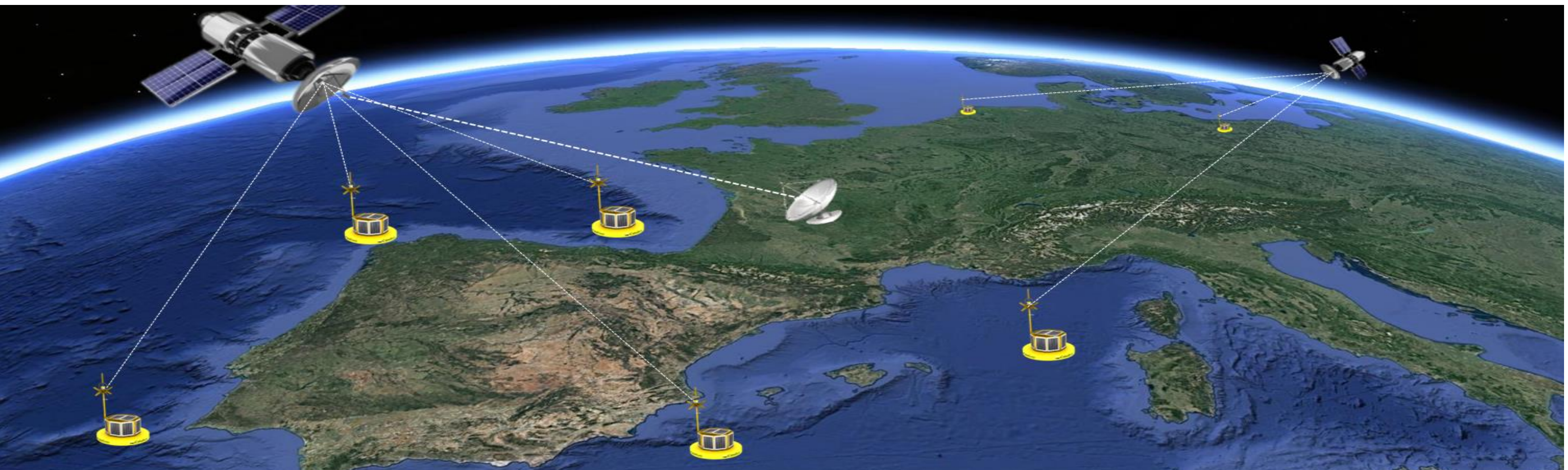
30 年前，第一台光学浮游植物分析仪问世，这一大型的流式细胞仪可测定单细胞、易碎细胞群体和丝状藻。最初设计便具有独特的颗粒分析的粒径范围

2001 年，CytoBuoy 公司创立，专注原位应用，并将便携式远程操作的流式细胞仪集成于浮标内。

新一代流式细胞仪扩展了颗粒检测和识别的动态范围，使得 CytoSense,CytoSub,和 CytoBuoy 自动监测的时间更长。

Current

- 单细胞逐一分析，可产生兆字节的信息量
- 浮游植物群落结构快速分析与追踪调查
- 光学扫描成像，产生定量及定性的高效数据
- 卫星遥感可测定大的水体表面颜色和反射信息，几种形式和不同阶段的信息巧妙结合则可建立一种全面的观测方法

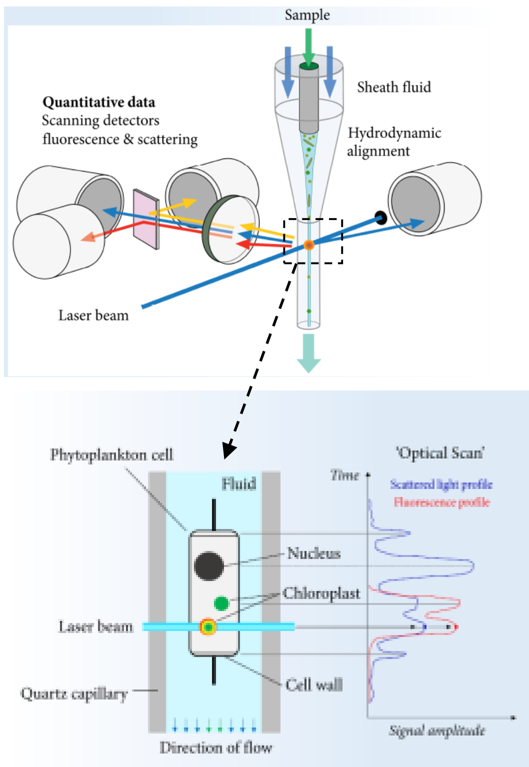


1. 简介

随着人类文明的不断进步，流式细胞仪逐渐成为研究微观生物最先进的科研工具。但是传统的为临床医学开发的流式细胞仪因为分析管径较小，容易堵塞而无法得到更好的应用。因此，结合长期的监测经验和当前用户需求，荷兰 CytoBuoy 系列扫描成像浮游植物流式细胞仪，针对传统流式细胞仪分析水环境样品的诸多不足进行了技术突破，使得流式细胞仪技术从实验室走出到野外，可现场原位进行更为快捷的藻类检测分析和自动识别。是目前在浮游植物监测领域中技术最先进的仪器之一。

- ✧ 独创的脉冲信号指纹图谱技术
- ✧ 高质量高速流动成像技术
- ✧ 超大的分析流动池（粒径范围）
- ✧ 内置循环鞘液系统
- ✧ 可定制模块化的系统设计
- ✧ 稳健的野外适用性
- ✧ 原位自动监测

2. 测量原理



样品中的细胞和颗粒物在鞘液的流体力学聚焦作用下高速列队经过狭窄的喷嘴，在测量区液流和激光垂直相交。颗粒经过激光时产生的前向、侧向散射和叶绿素等色素荧光通过检测器收集而进一步分析。

基于激光扫描的人工或自动粒子数据聚类，扫描记录各种光学信号（散射、荧光）的动态变化，包括多达 8 个光散射和荧光散射信号通道 每个信号有 10 个形状和大小参数，包涵了丰富的细胞形态学信息，因此每个粒子有 80 个相关变量可用于聚类，可以将不同特征（大小，形态，色素等）藻细胞聚类区分开来，利用这些形态学信息结合流动成像可以建立浮游植物特征信息数据库，进而利用 CytoSense 对浮游植物详细分类，了解浮游植物的种群变化和水华预警。

在自然水体中，我们需要关注的是优势藻种，正是这些藻种在生态中其主导作用。CytoSense 快速聚类分析，对于定点水域，同样参数的散点图可以以时间序列来反映水体优势种类的变化，对未来的藻华预警。藻类的自动识别是目前藻类监测领域最为复杂和最具有挑战性的工作，我们正在和相关单位、用户合作完成的藻类建库和自动识别功能，使该仪器得到更好地应用。

在实际应用中，CyoSense 可以在种群之间进行良好区分。除浮游植物外，纤毛虫、无节幼体、线虫、卵等也容易被鉴别。这些颗粒 100%可以被检测和表征，因此浓度的测定非常可靠。散射和荧光是定

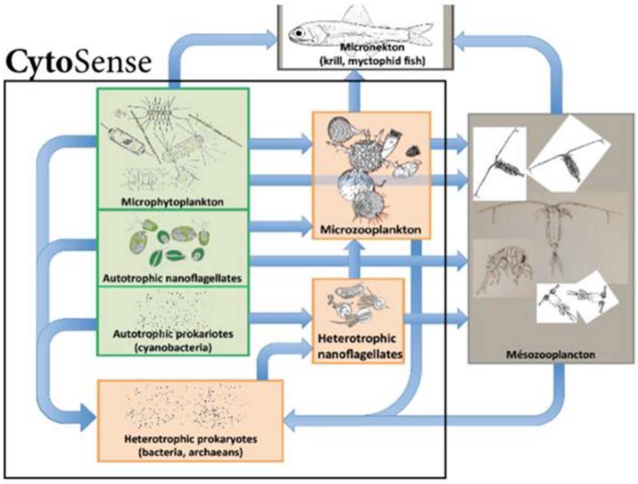
量的，可很好的表征生物量和体积，较易生成分布，并且单细胞表达的生物量是线性的，因此比图像分析*更可靠。

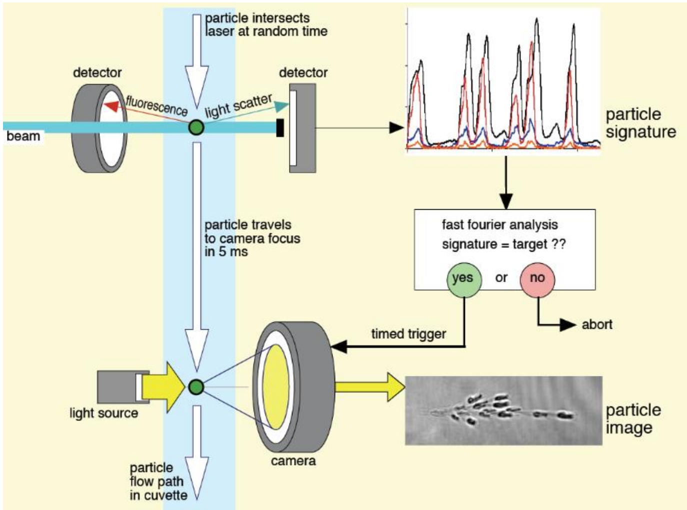
**二维图像的生物量/体积分析取决于粒子的方向（对于所有非球形形状）。CytoSense 进行的激光扫描与粒子的方向无关，颗粒通过薄层激光束，发射光被实时采集并数字化，从而对每种光学特性进行稳健的体积测定。*

3. CytoSense 扫描成像藻类分析仪在浮游植物监测中的优势

3.1 全粒级分析范围，样本无需过滤分级

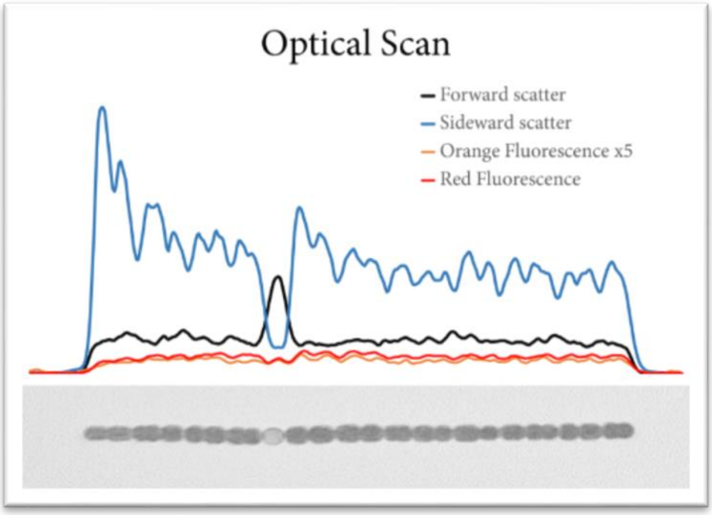
- ❖ 可测定粒径范围 0.1-700um 的浮游植物细胞或其他颗粒（细菌、浮游动物及沉积物等）。
- ❖ 浓度范围广，无需对样品浓缩、过滤或分级处理 (10^2-10^{10}Particles/L)
- ❖ 可直接测定大粒径藻细胞和藻群体（如常见的微囊藻、棕囊藻群体等）
- ❖ 低剪切力设计可测定易碎颗粒和丝状藻。（最长 4mm）





3.3 定量光学检测，大数据实现藻类识别

- ❖ 前向散射（FWS）表示颗粒大小；
- ❖ 侧向散射（SWS）对颗粒的结构很敏感，可用于对形态特征相对较显著的颗粒进行分类，例如颗石藻和可形成气囊的鱼腥藻等物种；
- ❖ 在不同波段产生荧光与各种细胞色素的含量成正比。这些色素可以是藻类自带的天然色素或对细胞进行荧光标记的人工染料。
- ❖ 激光扫描提供所有信号的一维形状信息。



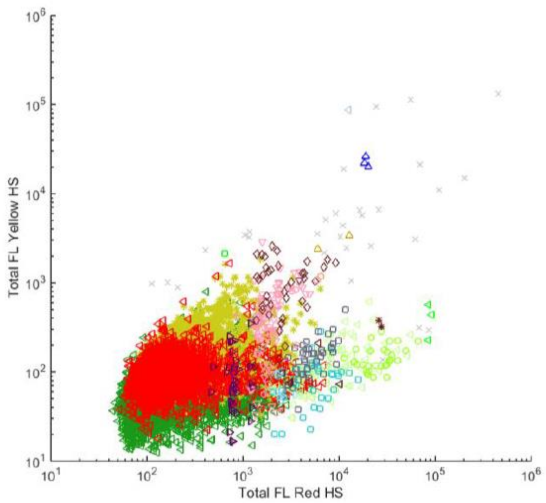
3.2 高速流动成像技术，藻类识别更直观

- 流动成像功能，可在颗粒通过系统时对其进行实时拍照，对应脉冲图谱，对藻类进行初步鉴定。
- 靶向成像功能，可实现对感兴趣的某一类群单独拍照
- 特定的光学元件，可获得高质量的图像。可拍照粒径范围 1-500μm (减少过滤对样品的破坏)

- 剖面扫描信号的振幅、长度和形状由粒子的大小和形状决定。
- 可获得叶绿体等“内部结构”在颗粒长度方向上的分布信息，这对于大于 5μm 的藻细胞很有用。
- 对于硅藻形成的链状群体，扫描图中的单个细胞显示为“驼峰”。
- 此外， “曲率”检测器，可用于检测螺旋形状、平板类型、弯曲细胞和群体。
- 扫描的形状不受颗粒旋转方向的影响。可实现对不同物种的数据稳健聚类，而不受同一物种不同视角的干扰。

3.4 散点图聚类，可准确计数每个亚群

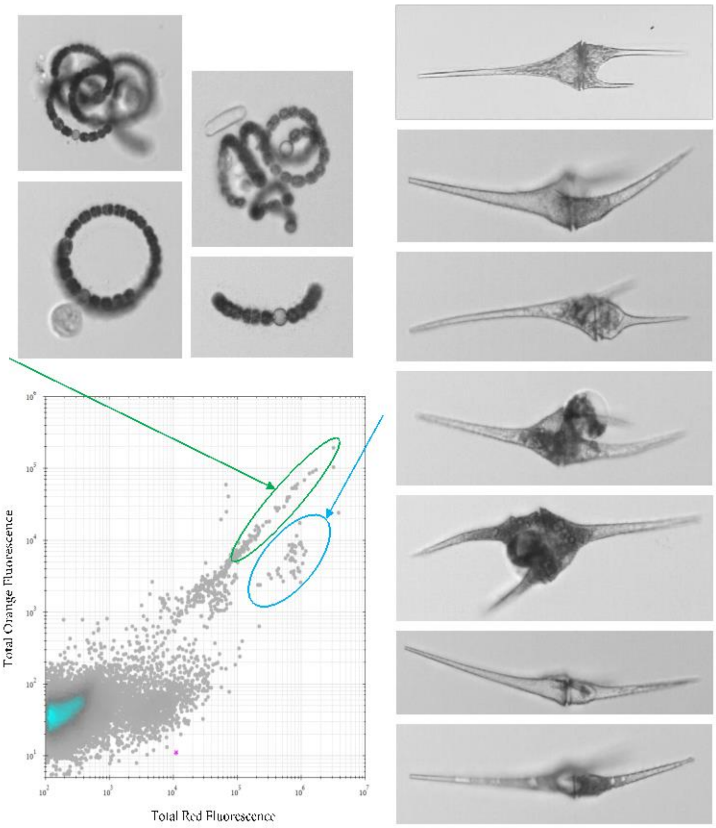
- 每个粒子的测量参数都绘制在细胞散点图中（每个点代表一个粒子）。
- 在混合培养物或现场自然水样中，显示为物理形态、光学特性相似的颗粒形成团簇，并且可以选择不同的横纵坐标以区分不同组并生成每组细胞生理特性的分布图。
- 粒子的独特性越强，分析的分辨力越强，出现的团簇越多。依此可直接识别群体和物种。



3.5 高速流动成像技术藻类识别更直观

流动成像模块可在颗粒通过系统时对其进行实时拍照，对应脉冲图谱，对藻类进行初步鉴定。

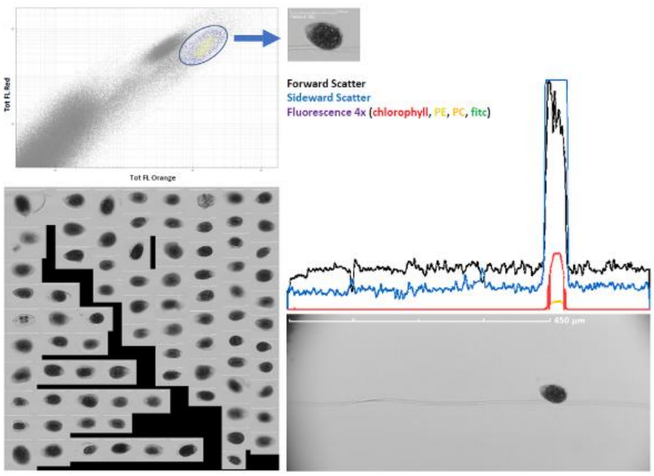
由于鞘液流体聚焦作用，粒子的拍摄方向与激光束扫描的方向相同。拍摄照片与激光扫描扫描图可相对应显示。



“smart grid”模式可以控制图像目标，作为数据分析和自主操作的识别辅助，自动从整个动态范围捕获等量的照片。它有助于在存在大量优势物种的情况下识别稀有物种和新兴物种。

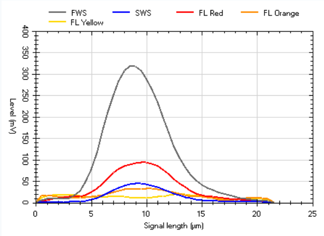
图像信息结合扫描信号，藻类识别更准确

CytoSense 将激光扫描和图像结合，快速激光扫描为高效地收集相关和有代表性的图像奠定了基础。成像可以以用户定义的粒子子集的为目标，也可以使用“smart grid”模式自主测量，该模式自动捕获代表整个动态范围的图像。

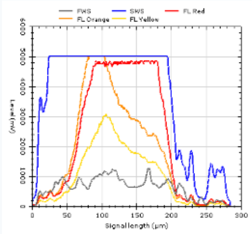


3.6 独创的脉冲信号指纹图谱技术获取更多特征信息

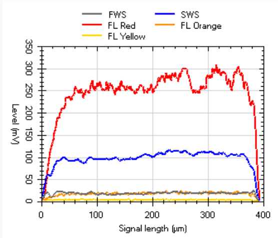
藻类独特的脉冲信号



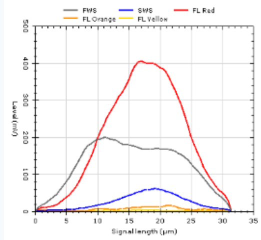
蓝藻 905 单细胞



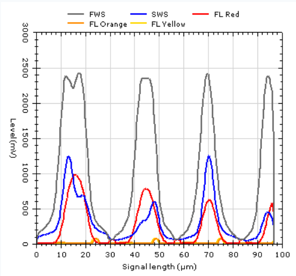
太湖微囊藻群体



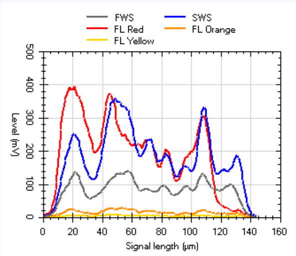
太湖直链藻



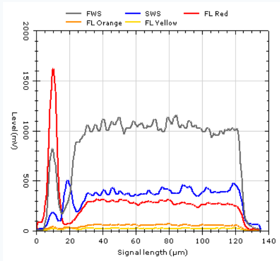
淡水甲藻



中肋骨条藻，每一个峰代表一个细胞

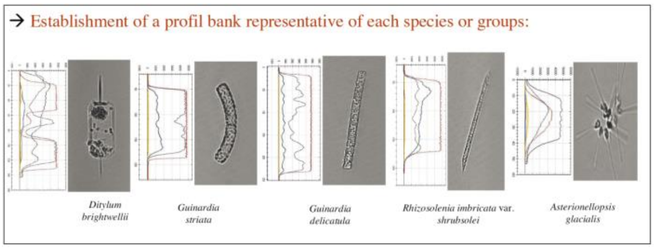


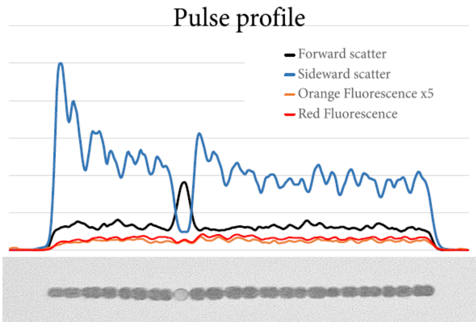
太湖星杆藻



鱼腥藻，链的开始部分有明显的异形胞

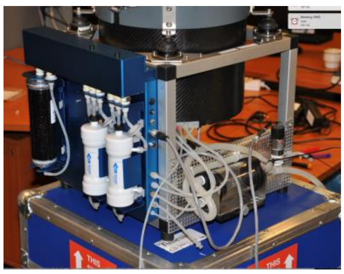
Silico-imaging 详细记录细胞的全部光学信息，逐个扫描，不放过自然水体中的稀有藻种。





3.7 循环鞘液系统

- 纯净的再循环鞘液：循环鞘液系统可提高数据质量，保证浓度计数可靠。防污染功能（样品不会污染流通池），可减少维护频率。
- 自清洗校准系统：自清洗系统含 2 个方便更换的外置滤器，可自动添加生物抑制剂和自动测定标准小球。



3.8 模块化的系统配置

通讯控制模块：接受外部控制指令并对外发送检测分析数据。

细菌染色模块：水体异养微生物自动染色、藻类、细菌、浮游动物及沉积物等在线检测。

水下测量模块：可升级为 CytoBuoy 和 CytoSub，最大工作水深 200 m，适合于水体剖面分层分析和水下原位分析。

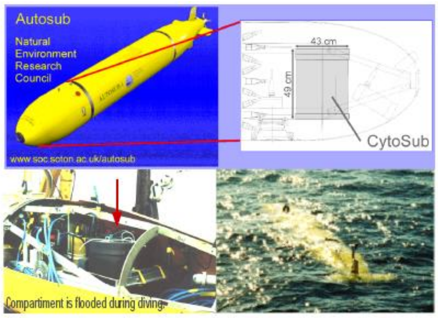



4 CytoSub 水下版应用

主机：台式机 CytoSense 是防溅水设计，可以在野外使用，但不能水下使用。CytoSense 加上一个水下模块（SUB MODULE）就组成了水下式流式细胞仪 CytoSub。

水下模块：一个耐受 200 m 水深压力的防水外壳，阀门和进样环路部分（包括循环泵），电子控制单元，数采，水下连接器和支架。

工作模式一：AUV 搭载



利用英国国家海洋中心 AutoSub 型 AUV 搭载 CytoSub

工作模式二：水下垂直剖面分析



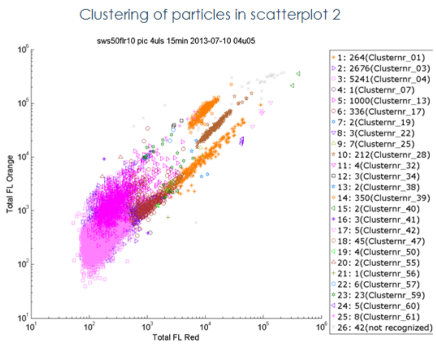
与 CTD 结合一起测量或自主剖面测量

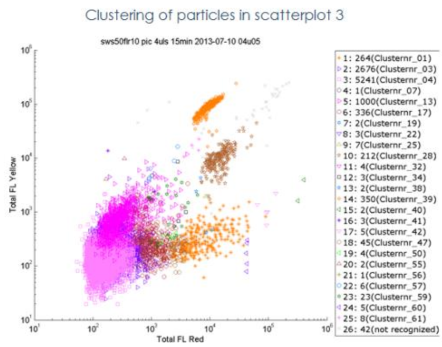
5 自动聚类分析软件：EasyClus

EasyClus 是针对浮游植物流式细胞仪 CytoSense/CytoBuoy/CytoSub 的数据分析编写的专业分析软件，可用于建立藻种专家库来对未知的环境水样中藻类信息进行自动聚类分析和比对。基于强大的 MATLAB 分析工具，Easyclus 不仅仅适用于 CytoSense 流式细胞仪数据，还可应用于其他流式细胞仪的数据处理。

EasyClus 软件采用多种逻辑运算方法，并经过多年的流式细胞仪数据分析检验，可根据人工预设的规则对流式细胞仪数据进行全自动的聚类分析，整个过程仅仅需要几分钟的时间，不仅减少了人工聚类分析带来的误差，而且大大提高流式细胞仪数据分析的效率。增加 Easyclus Live 功能，还可实现网站实时显示数据。

以下二维散点图由 EasyClus 自动在线聚类。每个 cluster 由特定的符号和颜色表示，代表一类散射和光学特征相似的颗粒。这些散点图可以清楚的表达不同浓度、大小及色素含量的藻类在水体中的分布（有时这些聚类只包含一种浮游植物，但有时也会由于极为相似的特性包含几种浮游生物）。

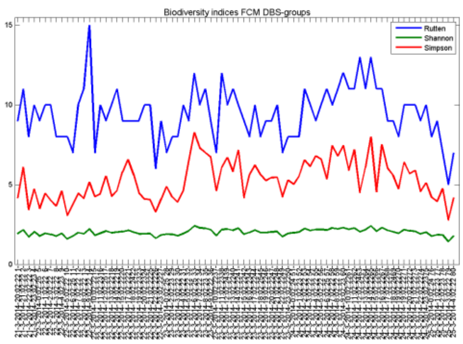


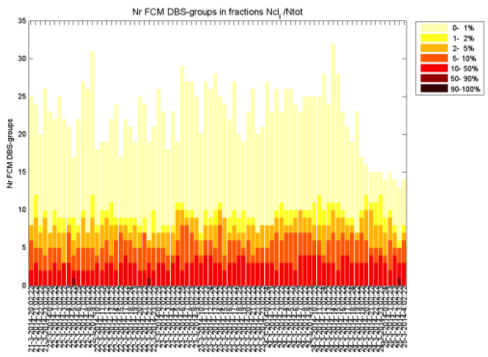


其中 Rutten-index 是由软件开发者 Thomas Rutten 根据前两个公式推算得到。一般该值越高表示生物多样性越高，越低表示生物多样性低。通过与已建立的数据库比对，可得到各相似度级别的聚类数量，从而确定优势种群。

5.6 Bio-indicators 生物多样性指数及优势种

光学特性相似的颗粒通过 EasyClus 聚类，如果将这些聚类视为一个“种”，那么根据这些聚类计算得到的生物指数可直观的表征类似生物多样性的特征。而如果对所测水域浮游植物种类有一定的了解，前期进行了已知种类的命名并建立数据库，则可根据已有的数据库比对后为聚类单位计算，更加准确的反应水体生物多样性。包括 Shannon-Wiener-index、 Simpson-index 及 Rutten-index。

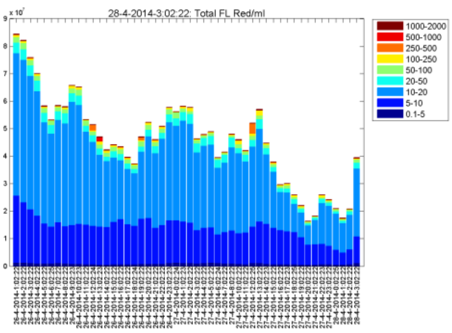


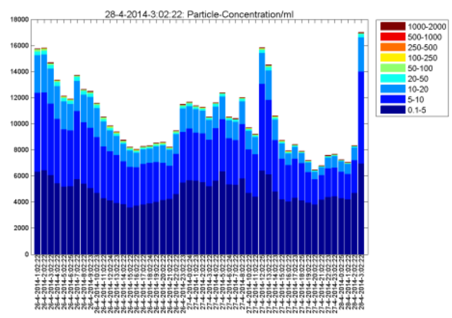


其中叶绿素 a-红色荧光强度与水体中叶绿素浓度 Chl-a/ml 高度正相关、藻胆蛋白-橙色荧光强度表征水体中含藻胆蛋白的藻类生物量、体积-散射光强度变化表征水体中藻类总体积变化，与含碳量高度相关。

5.7 粒度分布

浮游植物的粒级结构能够反映浮游植物对环境的响应并影响海洋生态系统的物质循环和能量流动。近些年，关于浮游生物粒径结构的研究主要涉及粒级的划分及方法比较、粒级生物量及粒径谱分析等。一般情况下，水域的营养盐条件、温度、光照等环境因子是影响浮游植物粒度分布的主要因素。其次为悬浮物。与其他化学因子的胁迫作用不同，悬浮物对浮游生物的影响大多是间接和慢性的，更多地表现为其粒径结构方面的变化。

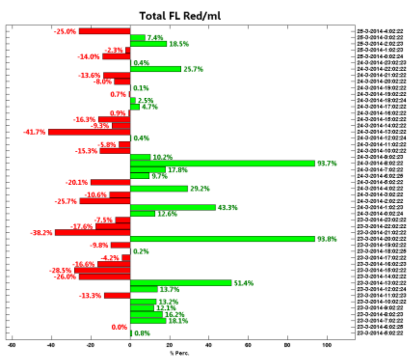




此外，水下型浮游植物流式细胞仪 CytoSub 可应用于浮标，Ferrybox 等监测平台，在垂直剖面不同层位获取浮游植物生物量信息，对研究微囊藻沉浮机制，浮游动物、水文、水质等因素对浮游植物生态位影响提供数据依据。

5.8 总生物量参数变化跟踪

叶绿素是通用的衡量浮游植物生物量的指标，将其作为藻类生物量的主要指标，在不同时间尺度上观测其变化趋势有利于了解浮游植物的生命周期及生物量变化。



EasyClus 根据 FCM 参数进行批量统计，可将测试样本中不同颗粒粒度进行分类计数。可得不同粒径颗粒的分布比例及浓度，选择 Red-FL 通道，可得到浮游植物粒级生物量分布特征。

6 CytoSense 海洋藻类监测系统案例

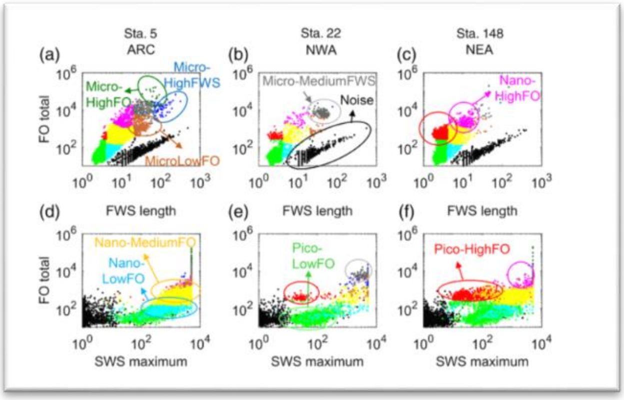
案例一： 北大西洋亚极地浮游植物和质体纤毛虫群落的特征分析

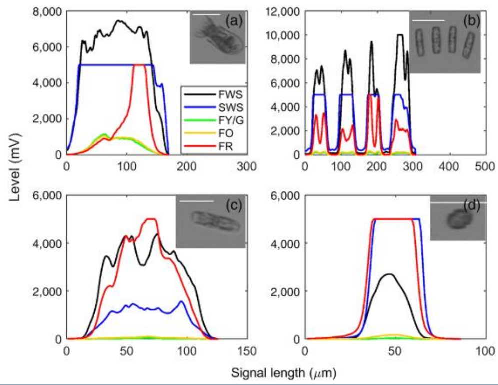
（Glaucia Moreira Fragoso 等，2019）

南安普敦大学和国家海洋中心的科研人员利用 CytoSense 的生物学参数，将其生态功能量化，通过随环境梯度的生态性状变异性，研究北极和大西洋亚极地北大西洋水域浮游植物和质体纤毛虫的特征变异。

细胞大小是本研究的主要特征，大的微型光合浮游生物（细胞直径>20μm）包括作为单细胞的链状硅藻，以及在北极水域发现的质体纤毛虫，而小的浮游植物群，如 picoeukaryotes（<4μm）和蓝藻聚球藻是大西洋水域的优势种。

并指出了 CytoSense 的指纹图谱和光散射参数具有量化形态和色素功能的特性。





CytoSense 脉冲形状和摄影图像的示例：

（a）含质体的纤毛虫（沙壳纤毛虫），

（b）海链藻（硅藻），

（c）蜉蝣（食硅藻），

（d）甲藻。

比例尺：80μm（a－c）；60μm（d）。

其中，光散射（正向/侧向）和荧光(红色（叶绿素 a）、黄/绿色（降解色素）和橙色（藻胆蛋白）.

案例二： 英国 Cefas Endeavour 科考船巡航过程中的在线监测棕囊藻水华

North Sea cruise on the Cefas Endeavour (UK) 21-28 June 2016

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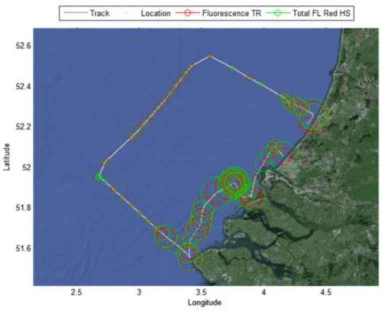
In the framework of WP3 and WP4 (JRAP#1), Machteld Rijkeboer from Rijkswaterstaat (NL) took part in a North Sea cruise on the Cefas Endeavour (UK) between 21st and 28th of June 2016. The aim of the work was to determine the diversity of the phytoplankton with a semi-automatic system including two Cytosense flow cytometers connected to a ferrybox. The on line analyses were programmed from every 20 minutes to every hour. The results can already be seen on http://fytoplankton.nl/CEFAS/Endeavour/phytoplankton_liveloc.shtml and http://fytoplankton.nl/RWS/Endeavour/phytoplankton_liveloc.shtml. A meeting in August will take place in VLIZ in Oostende (B) to finalise the outputs of the cruise.

Involved institutions: Cefas, Rijkswaterstaat, VLIZ/Ghent University, Thomas Rutten Projects

Cefas Endeavour 科考船上 CytoSense 与 FerryBox 联用。用于在海洋巡航中连续监测浮游植物。利用这种创新技术，可观察

到清晰片状分布的棕囊藻水华（*Phaeocystis spp*），并通过卫星通讯上传至互联网，而用户只需打开网站，便可以在家里，在另一个国家，随时观察巡航数据。



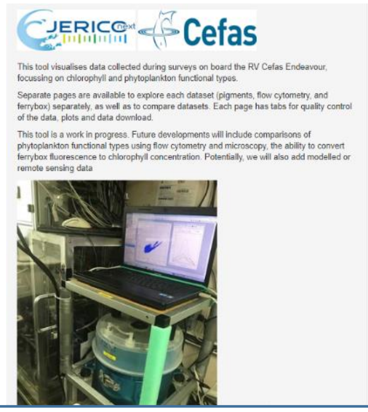
案例三：澳大利亚综合海洋观测网项目 IMOS——海洋浮游植物监测

澳大利亚的综合海洋观测系统（IMOS）于 2007 年根据国家合作研究基础设施战略（NCRIS）建立，旨在成为一个全面综合的国家系统，在海洋盆地和区域尺度上进行观测，涵盖物理、化学和生物变量。其中浮游植物数据由 CytoSense/CytoSub 收集

Splashdown at CSIRO (Australia)

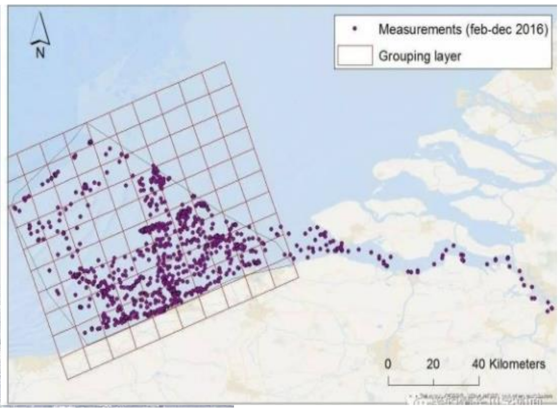
案例四：欧洲沿海观测网项目

2015 年开始，来自 15 个国家的 33 个专家合作伙伴，在 JERICO 内应用 8 台 CytoSense。测定了精细时空尺度（亚中尺度、微尺度）下浮游植物群落动态的信息。研究人员需要由此产生的高频数据来了解零星事件对浮游植物动态的影响。通过跟踪浮游植物的丰度、生物量和生理反应（例如，每个细胞的大小或荧光的变化）的变化，将这些生物数据与物理参数（例如，温度、营养素）相关联，进而在原位描述重要的生态过程，例如光适应、营养素变化的适应甚至人类造成的污染的适应。这种复杂的高频浮游植物监测是将全球变化与局部或零星变化分开的不可或缺的一步。



案例五：比利时海岸带硅藻水化周年观测

比利时根特大学的 Reinhoudde Blok 团队利用 CytoSense 对比利时海岸带有害藻进行了周年监测。采用半连续测试频率（30 分钟一次），Easyclus 软件分析浮游植物的时空组成。同时分析了硅藻藻华与水质要素氮磷之间的关系，为藻华预警的环境因子提供了有用数据。



案例七：厦门湾浮游植物群体细胞死亡的季节变化

浮游植物细胞死亡是一个不容忽视，对于了解浮游植物群落的动态和亚热带生态系统的功能具有重要意义，厦门大学用 CytoSense 对 6 组浮游植物（G1-G6）进行分类。分析了不同季节变化的浮游植物群落对环境约束的不同反应。

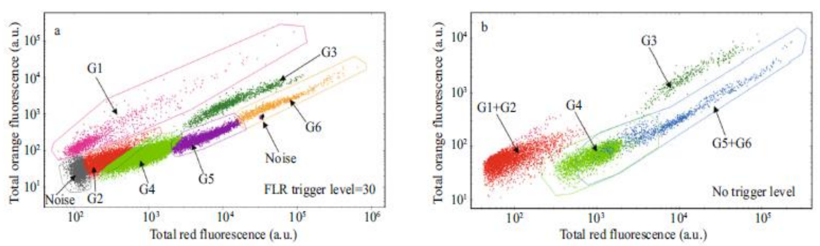


Fig.3 Red fluorescence (FLR) versus orange fluorescence (FLO) cytograms leading to the resolution of clusters (a.u.: arbitrary units)

a. a threshold on FLR recording was set at 30; b. no threshold on FLR recording.

低 FLR、FLO 和 FLO/FLR 比值、细胞体积小的 G1 和 G2 细胞被认为是蓝细菌；

其中，G2 细胞图谱与纯培养的聚球藻相似；

高 FLR、FLO 和 FLO/FLR 比值，G3 细胞被鉴定为隐藻。

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