数据非依赖采集

DIA 解决方案

Thermo
SCIENTIFIC

目 录

编写：张伟

校对：李静

封面设计：贾伟

数据非依赖采集（DIA）

随着生命科学的快速发展，蛋白质组学的关注焦点和研究趋势已经逐渐从定性转向定量。定量蛋白质组学是对细胞、组织乃至完整生物体的蛋白质表达量及差异进行分析，对于生物过程机理的探索和临床诊断标志物的发现与验证具有重要意义。

基于静电场轨道阱 Orbitrap 的数据非依赖采集（Data Independent Acquisition, DIA）是赛默飞为用户带来的一项全新的、全息式的质谱技术。DIA 将质谱整个全扫描范围分为若干个窗口，高速、循环地对每个窗口中的所有离子进行选择、碎裂、检测，从而无遗漏、无差异地获得样本中所有离子的全部碎片信息（图 1）。

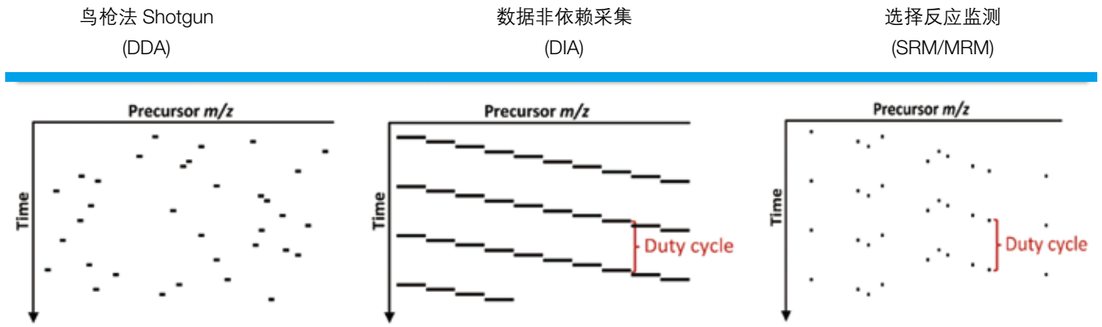


图 1. DIA 与经典 Shotgun、SRM/MRM 的原理比较

与传统蛋白质组学“鸟枪法”（Shotgun）相比，DIA：(1) 无歧视地获得所有肽段的信息，不会造成低丰度蛋白信息的丢失，(2) 循环时间固定，扫描点数均匀，定量准确度高，(3) 肽段的选择没有随机性，数据可以回溯，对于复杂蛋白样本，特别是低丰度蛋白具有更优异的重现性。与传统质谱定量“金标准”选择反应监测 / 多反应监测（SRM/MRM）相比，DIA：(1) 无需提前指定目标肽段，适用于未知蛋白分析；(2) 无需优化方法，获得数据后再基于谱图库实现定性确证和定量离子筛选；(3) 通量无上限，适合大规模蛋白定量分析。

形象地说，Shotgun 就像机枪扫射，高效率地打击尽可能多的目标；SRM/MRM 就像精准狙击，准确无误地打击特定目标；而 DIA 就像地毯式轰炸，无遗漏地打击全部目标（图 2）。

图 2. Shotgun、DIA 和 SRM/MRM 的特点比较

DIA 有效结合了 Shotgun 和 SRM/MRM 的优势和特点，为用户带来全新的质谱分析体验，和强大的蛋白质组学定量策略。在临床研究中，高度的复杂性、庞大的样本量、样本的不稳定性是分析的难点，而 DIA 提供条件统一、无差别的质谱采集方法，能够在样本信息“完全未知”的情况下，对样本进行高通量、高速度采集，获得数据之后再进行深入解析和挖掘，是临床蛋白质组学实验的利器。在生物学研究中，多个时间点或多种条件下蛋白表达量的变化趋势是分析的重点，而 DIA 的灵敏度、精确度和重现性为获得准确、可靠的定量结果提供了有力保障。

1. DIA

DIA 通常将 *m/z* 400–1200 的全扫描范围分割为 25 *m/z* 的窗口（共 32 个窗口），依次（*m/z* 400-425、*m/z* 425-450、……、*m/z* 1175-1200）将窗口内所有的离子选择、碎裂，并检测该窗口内离子产生的所有碎片，一次循环获得 32 张二级谱图，并不断循环往复地进行采集（图 3）。

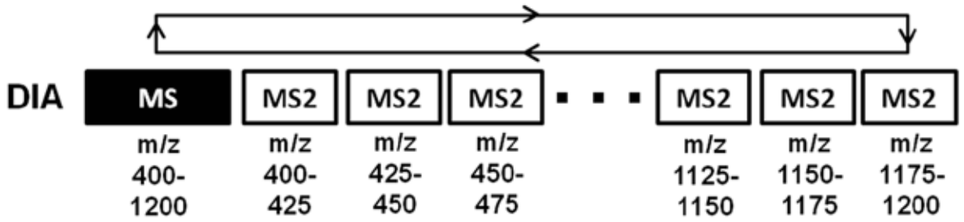


图 3. DIA 采集原理

Orbitrap 所具有的超高分辨率（≥ 140,000）、超快扫描速度（32–64 ms）、超高灵敏度（amol 级），使 DIA 相比目前其他类似技术，如 SWATH 等，能够为用户带来更出色的性能体验。此外，Orbitrap 灵活多样的采集方式，还能为用户带来更灵活的方法设置：(1) 根据样本的复杂程度使用更窄的选择窗口（如 20 *m/z*）或不对称窗口（在出峰密集的范围使用更窄的选择窗口），实现更好的选择性；(2) 根据肽段的信号强度调整离子注入时间及分辨率，达到更高的灵敏度；(3) 针对大规模样本使用更快的扫描速度和更短的梯度，进一步提高分析效率；(4) 加入一级全扫描，采集碎片的同时获得母离子信息，实现更准确的定性定量。

2. msxDIA

Orbitrap 超高分辨质谱除了为用户带来超高分辨率、超快扫描速度和超高灵敏度的分析性能外，先进的设计理念使其具有其他任何质谱都无法实现的功能——多重累积技术（Multiplexing, msx）。将多重累积技术与 DIA 相结合，形成独一无二的 msxDIA，进一步缩小 DIA 的选择窗口，提高 DIA 的选择性。

msxDIA 随机将 5 个 4 *m/z* 窗口（或 2 个 10 *m/z* 窗口）依次选择、碎片、并累积在一起，最后同时注入 Orbitrap 进行扫描，获得 5 个 4 *m/z* 窗口（或 2 个 10 *m/z* 窗口）中全部碎片信息的谱图，再随机选择另外 5 个 4 *m/z* 窗口（或 2 个 10 *m/z* 窗口）分析并依次进行下去，直至覆盖整个全扫描范围。msxDIA 总步长为 20 *m/z*，不影响扫描速度，而这 20 *m/z* 由 5 段（或 2 段）独立的窗口组成，客观上使选择窗口缩小到仅 4 *m/z*（或 10 *m/z* 窗口）。图 4 以 2 x 10 *m/z* 模式为例，展示了 msxDIA 的原理。

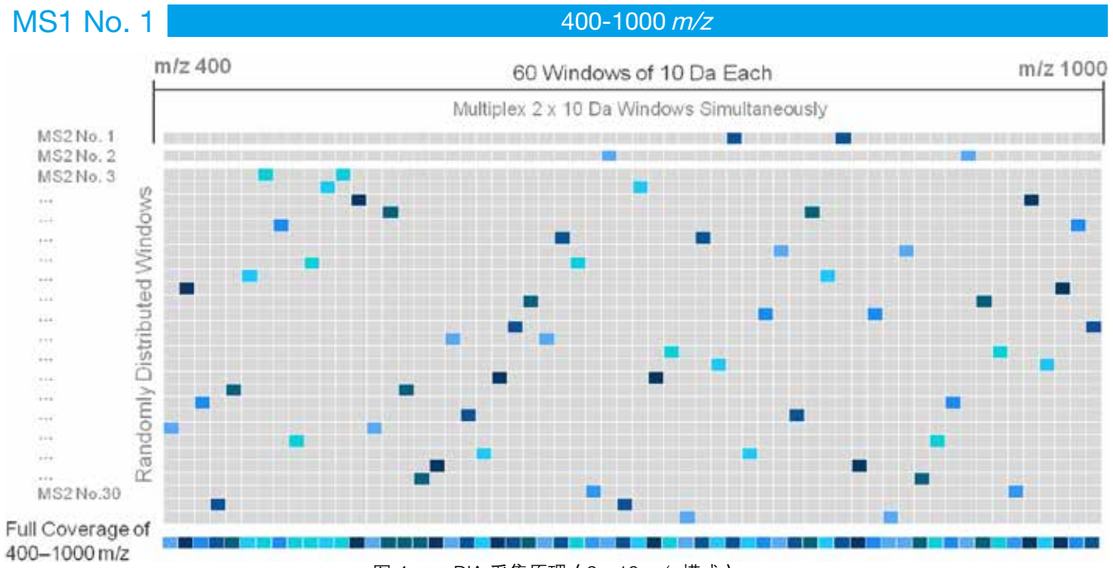


图 4. msxDIA 采集原理（2 x 10 *m/z* 模式）

msxDIA 采集得到的数据再利用 Skyline 软件去多重累积（de-multiplexing），即可将每个碎片及响应强度归属到特定窗口，实现与 Shotgun、SRM/MRM 相当的选择性，最大程度地减少共流出肽段和杂质的干扰（图 5）。msxDIA 是 Orbitrap 独一无二的全新扫描技术，其成果最先由 Skyline 的开发者、华盛顿大学 MacCoss 教授团队提出，并发表在《Nature Method》杂志上（Nat. Methods, 2013, 10(8): 744-746）。

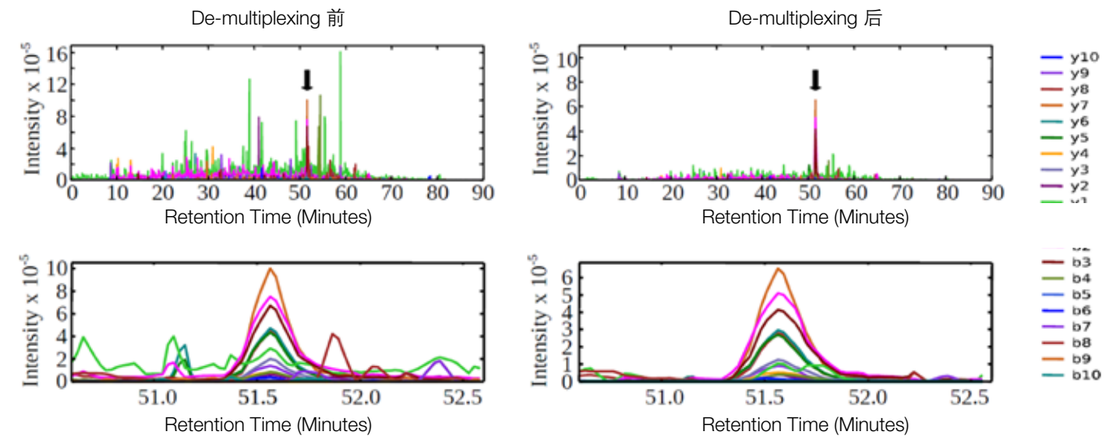


图 5. msxDIA 去多重累积前后比较

特点与优势

数据非依赖采集（DIA）：

- **采集所有离子及碎片谱图，不丢失任何信息，重现性好；**
- **数据易于追溯，即使目前的水平无法发现某些蛋白 / 化合物，未来可以回溯；**
- **无需优化方法，对于易降解样品可以即刻采集，获得数据后再深入挖掘；**
- **基于碎片离子（即母子离子对）定量，选择性好，与 SRM/MRM 相当；**
- **循环时间固定，扫描点数均匀，定量准确度高；**
- **通量无上限，同时监测所有目标蛋白 / 化合物；**
- **相同肽段不同翻译后修饰位点的定量区分与辨别。**

基于 Orbitrap 的数据非依赖采集（DIA）：

- **通常 35,000–60,000 分辨率采集，对复杂样品定性定量准确度高；**
  - ◆ Q-TOF DIA* 采集分辨率为 20,000 左右
- **质量轴长期稳定地保持在 1 ppm，无需内标校正或频繁外标校正，数据更放心、通量更高；**
  - ◆ Q-TOF DIA* 采集需内标校正或频繁外标校正
- **Amol（10-18 mol）级的超高灵敏度，谱图质量高；**
  - ◆ Q-TOF DIA* 采集灵敏度低
- **12–20 Hz 的超快扫描速度，同时保持 amol 级的超高灵敏度；**
  - ◆ Q-TOF DIA* 采集提高扫描速度则丢失灵敏度
- **动态范围及线性范围达 5 个数量级以上；**
  - ◆ Q-TOF DIA* 采集动态范围在 3~4 个数量级
- **独一无二的多重累积 msxDIA，进一步提高选择性；**
  - ◆ Q-TOF DIA* 无此功能
- **灵活多样的扫描功能，实现 pSMART、WiSIM 等诸多独特 DIA 模式。**
  - ◆ Q-TOF DIA* 扫描模式单一

*: 此处 DIA 指 Ruedi Aebersold 发展的“Sequential Window Acquisition Trough High-resolution”技术（Mol. Cell. Proteomics, 2012, 11(6): O111.016717）

工作流程

Thermo Fisher Scientific 建立的专门针对基于 Orbitrap 的数据非依赖采集（DIA）解决方案，工作流程（图 6）统一、方法成熟、简单易用，适用于任何复杂生物学样本和临床样本的高通量蛋白质组学定量分析。

基于 Orbitrap 的 DIA 解决方案分为三步：首先，样本前处理与经典的 Shotgun 流程完全一致，样本经蛋白提取、酶解、除盐，再根据需要经过特定的分离富集、纯化后，即可直接进样分析，或加入混合标肽后再进样分析（保留时间校正）；然后，无需事先了解样本信息，直接使用统一的 DIA 模板，即可快速、高通量地进行 Orbitrap 质谱采集，获得高质量 DIA 数据；最后，DIA 数据使用权威 Skyline 软件与谱图库匹配打分，获得可信的鉴定结果，并自动挑选响应最高、选择性最好的若干子离子，进行离子对色谱峰提取，即可获得准确的定量信息。

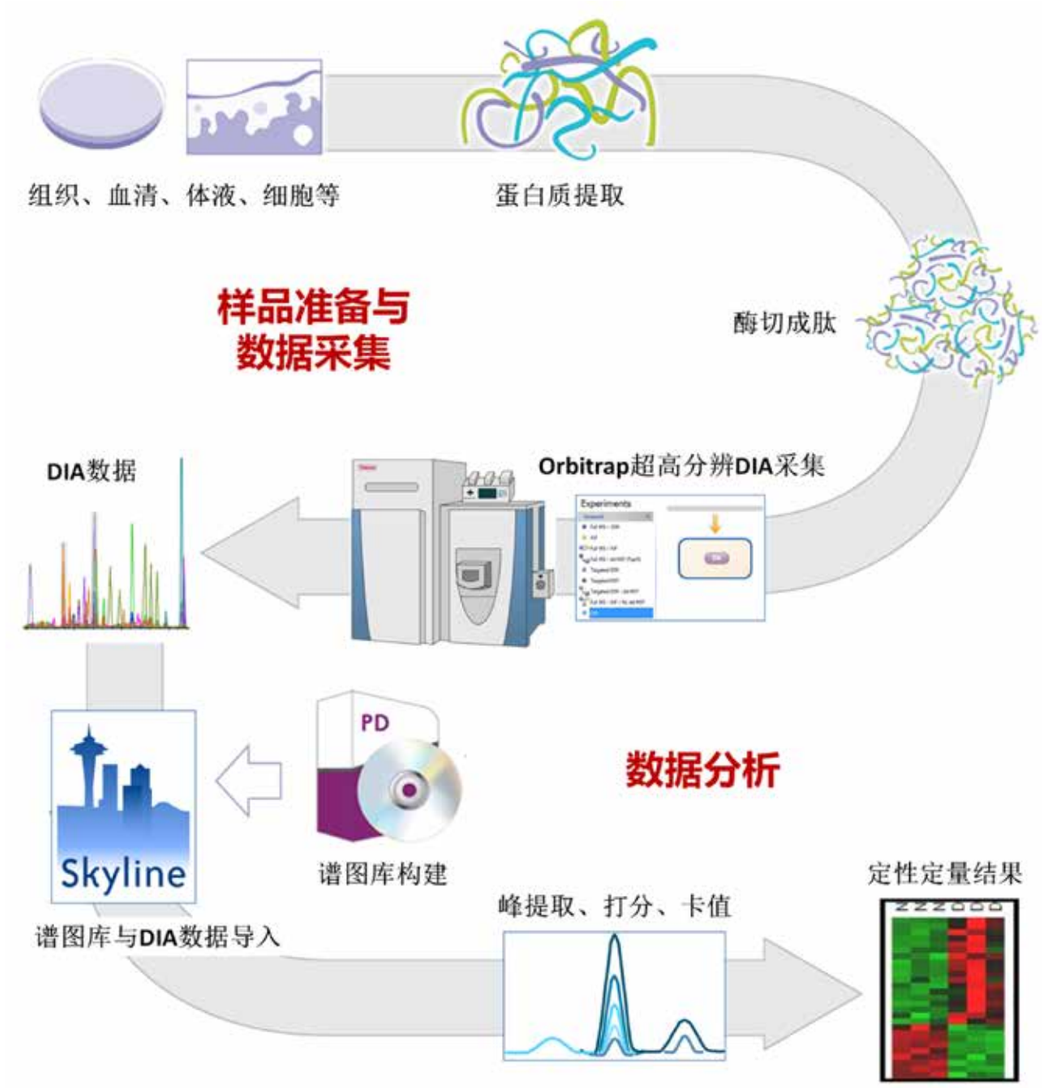


图 6. 基于 Orbitrap 的数据非依赖采集（DIA）解决方案

Q Exactive 系列 Orbitrap 质谱仪

静电场轨道阱 Orbitrap 超高分辨质量分析器

基于傅里叶变换原理的静电场轨道阱 Orbitrap 是 30 年来唯一基于全新理论的质量分析器，集超高分辨率、超高质量精度、超高灵敏度等卓越性能于一体，还具有质量轴极其稳定、操作维护简单、使用成本低廉等优势。从 2005 年第一台商品化的 Orbitrap 质谱上市以来，极大地推动了以蛋白质组学为代表的高水平科学研究的发展，迅速成为蛋白质组学研究的唯一金标准。据统计，近年来，使用 Orbitrap 在 Nature、Science、Cell 等高水平期刊上发表论文的数量，是使用所有其他高分辨质谱发表论文数量总和的三倍，成为前沿科学研究的利器（图 7）。

图 7. Orbitrap 结构图及发表高水平论文趋势比较

图 8. 定量分析的利器——Q Exactive 系列超高分辨 Orbitrap 质谱仪

方法设置与数据采集

1. DIA

Q Exactive 的 DIA 方法编辑简单、易上手，流程一目了然，无需任何 DIA 分析经验。仅三步，即可完成全部方法设置（图 9）：(1) 在模板中将 DIA 模块拖入方法流程（若加入一级扫描，则在 DIA 模块前拖入 Full Mass 模块）；(2) 根据需要修改 DIA 模块中的分辨率（通常设 35000）和窗口宽度（若实际窗口步长为 25 *m/z*，则设 26 *m/z*，多 1 *m/z* 为窗口之间 Overlap）；(3) Inclusion List 中生成每个窗口的中间值（*m/z*）作为 target。三步完成后保存方法，即刻开始样品数据采集。

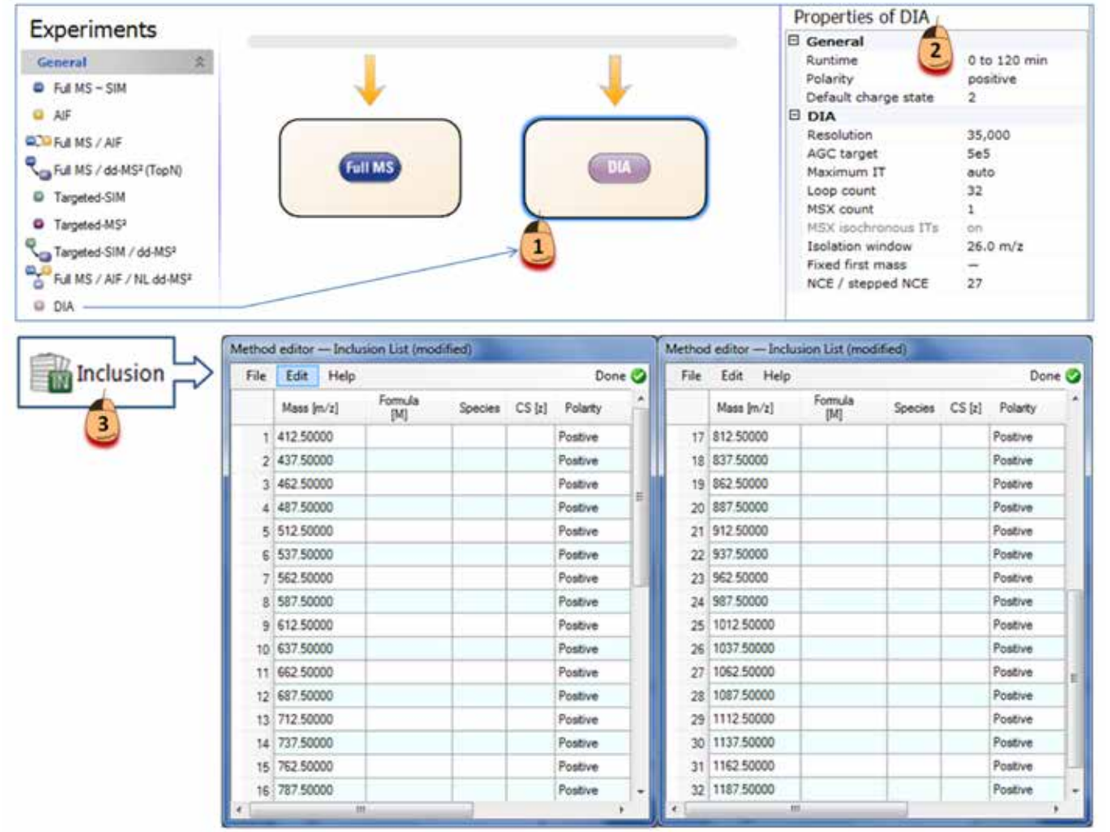


图 9. 三步完成 DIA 方法编辑

典型 DIA 数据如图 10 所示，从色谱图上看 DIA 数据与传统 Shotgun 数据没有明显区别，只是每个循环的二级谱图数量固定，因此扫描点分布均匀。

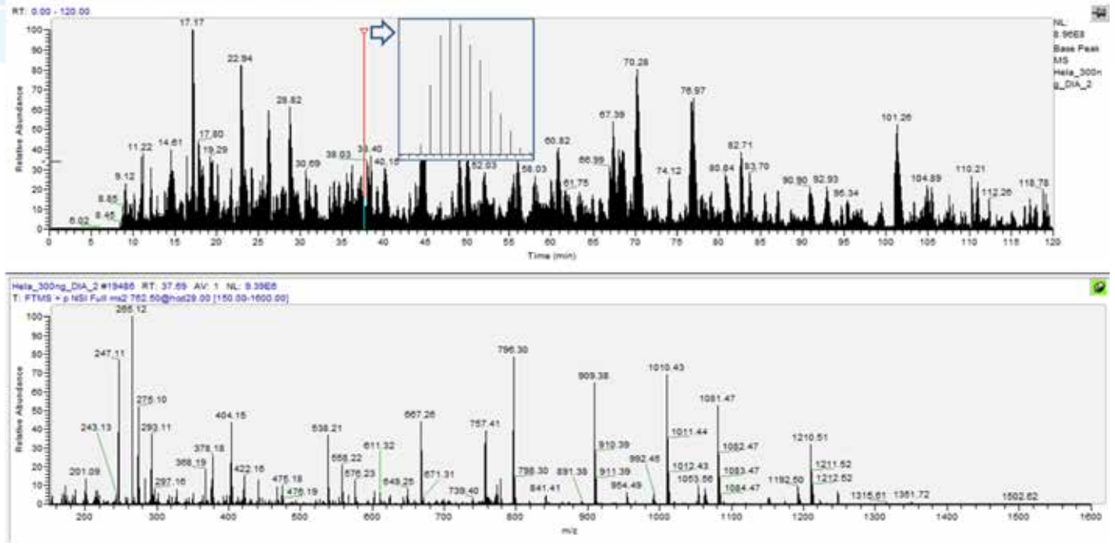


图 10. 典型的 DIA 数据

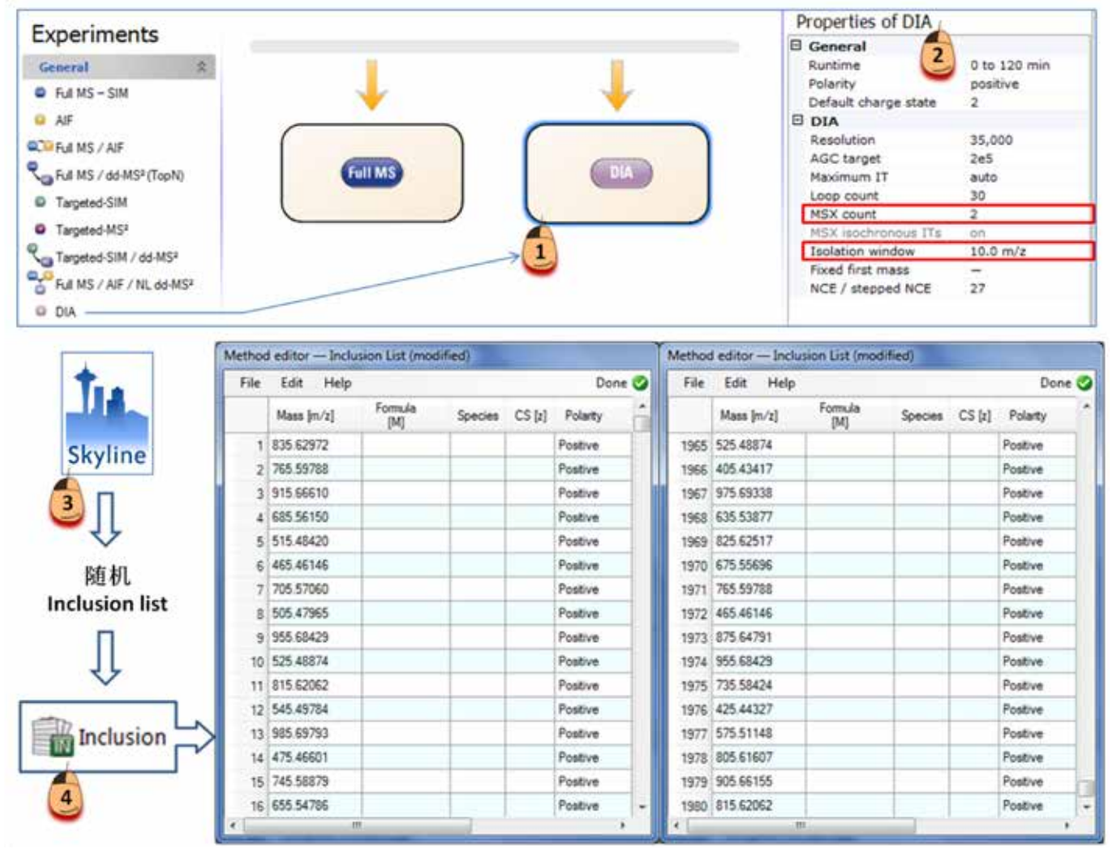


图 11. 四步完成 msxDIA 方法编辑

2. msxDIA

Orbitrap 具有的独一无二的 msxDIA 方法设置同样简单易用，只多一步随机 Inclusion list 生成（图 11）：(1) 在模板中将 DIA 模块拖入方法流程（若加入一级扫描，则在 DIA 模块前拖入 Full Mass 模块）；(2) 以 2 x 10 *m/z* 为例，DIA 模块中的 MSX count 设为 2，窗口宽度设为 10 *m/z*，即完成 2 x 10 *m/z* 模式参数设置；(3) 使用 Skyline 软件生成随机的 Inclusion list 表；(4) 将随机 Inclusion list 表导入方法的 Inclusion list 中，完成方法设置，即刻可以开始 msxDIA 数据采集。

典型 msxDIA 二级质谱图如图 12 所示，每张谱图包含 2 个（2 x 10 *m/z* 模式）或 5 个（5 x 4 *m/z* 模式）窗口的碎片信息，即一次扫描采集 2 个或 5 个窗口，分析效率显著提高，选择性也因窗口缩小而明显改善。

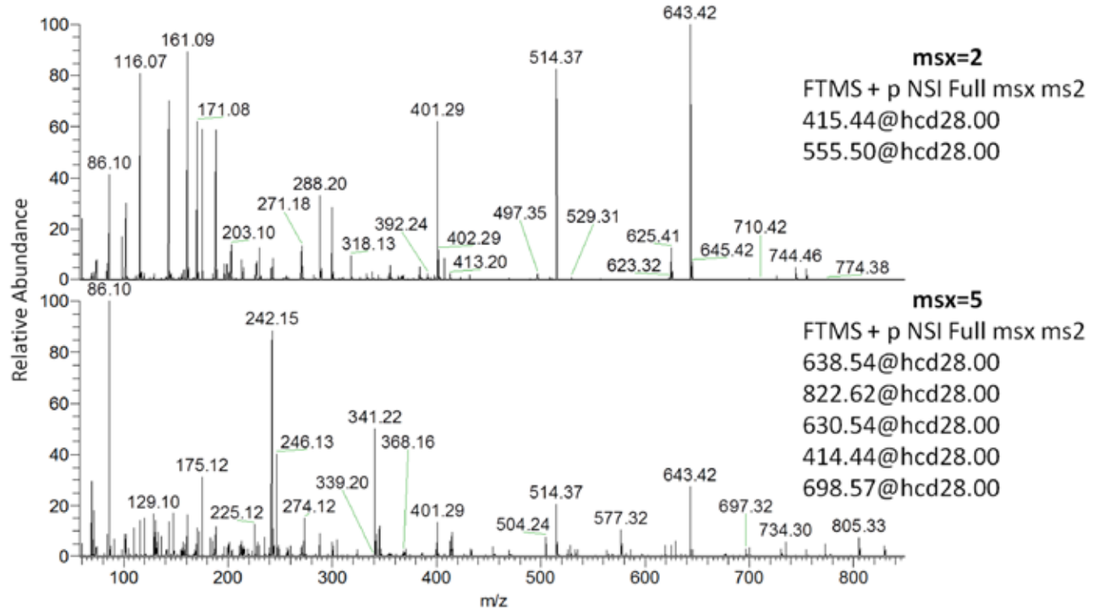


图 12. 典型的 msxDIA 二级质谱图

数据分析

DIA/SWATH 数据复杂、信息量庞大，因此，高效、可靠的数据解析和信息挖掘是 DIA/SWATH 分析的瓶颈。

Skyline 软件

Skyline 软件是华盛顿大学 MacCoss 教授实验室开发的目标蛋白质组学分析软件（https://skyline.gs.washington.edu），是 DIA/SWATH 数据处理的权威工具，由于功能全面、性能强大而受到普遍认可，是目前使用最广泛的 DIA/SWATH 解析软件。通过与 Thermo Fisher Scientific 的紧密合作，MacCoss 教授实验室有针对性地优化了 Skyline 对 Orbitrap 数据的处理，一步实现从原始数据到最终结果的完整 DIA 解析流程，使 Orbitrap DIA 如虎添翼。

以 Proteome Discoverer 软件（或 MaxQuant、Mascot 等）搜库鉴定结果为谱图库，导入 Skyline 软件；设置肽段、离子对挑选规则；将符合条件的肽段及子离子从谱图中挑选出来，生成 Target List；导入 DIA/msxDIA 数据，根据 Target List 提取离子对色谱峰，进行子离子匹配和峰面积计算，实现对肽段的同时定性和定量（图 13）。若样品中加入标准肽段，则可利用标准肽段进行保留时间校正。

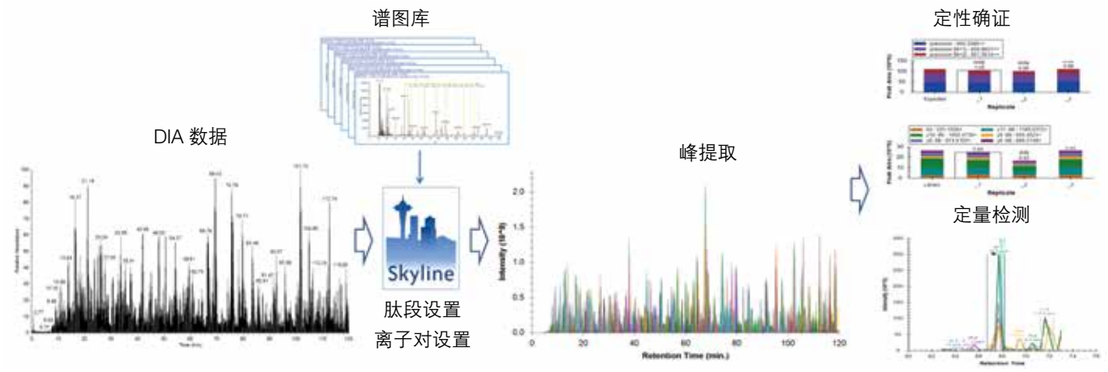


图 13. Skyline 处理 DIA 数据完整流程

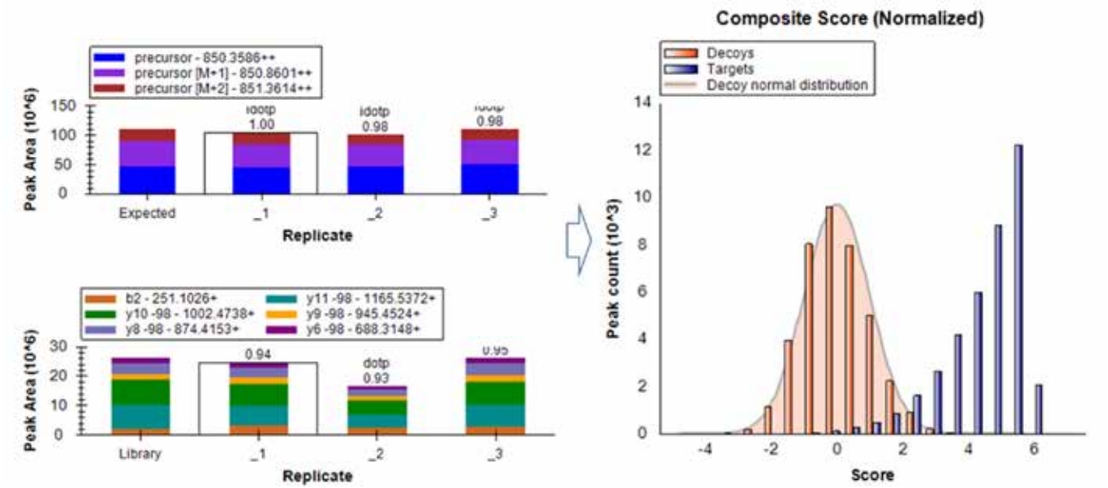


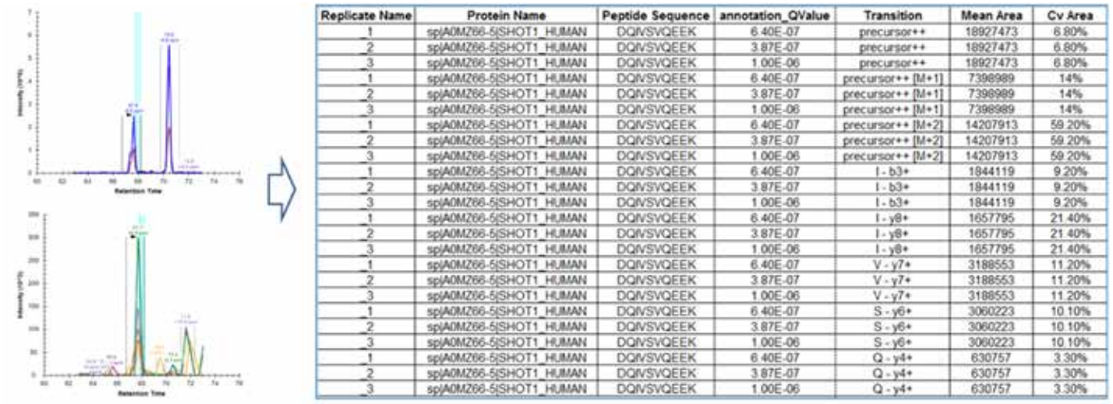
图 14. 使用 mProphet 进行统计学分析并卡值过滤

定性

使用 Skyline 内嵌的 mProphet 卡值工具对谱图匹配结果进行统计学分析，综合考虑 dotp 分数、信号响应、保留时间、质量偏差等因素计算 q 值，过滤结果，获得高度可信的肽段定性结果（$q < 0.01$）（图 14）。

定量

经定性卡值（$q < 0.01$）后，最终获得所有可靠离子对的色谱峰面积和 CV 值，将结果以 Excel 表格式导出，使用 CV < 20% 的离子对峰面积进行定量分析，完成 DIA 分析工作（图 15）。



Replicate Name	Protein Name	Peptide Sequence	annotation_QValue	Transition	Mean Area	Cv Area
1	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	6.40E-07	precursor++	18927473	6.80%
2	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	3.87E-07	precursor++	18927473	6.80%
3	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	1.00E-06	precursor++	18927473	6.80%
1	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	6.40E-07	precursor++ [M+1]	7398989	14%
2	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	3.87E-07	precursor++ [M+1]	7398989	14%
3	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	1.00E-06	precursor++ [M+1]	7398989	14%
1	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	6.40E-07	precursor++ [M+2]	14207913	59.20%
2	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	3.87E-07	precursor++ [M+2]	14207913	59.20%
3	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	1.00E-06	precursor++ [M+2]	14207913	59.20%
1	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	6.40E-07	I - b3+	1844119	9.20%
2	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	3.87E-07	I - b3+	1844119	9.20%
3	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	1.00E-06	I - b3+	1844119	9.20%
1	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	6.40E-07	I - y8+	1657795	21.40%
2	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	3.87E-07	I - y8+	1657795	21.40%
3	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	1.00E-06	I - y8+	1657795	21.40%
1	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	6.40E-07	V - y7+	3188553	11.20%
2	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	3.87E-07	V - y7+	3188553	11.20%
3	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	1.00E-06	V - y7+	3188553	11.20%
1	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	6.40E-07	S - y6+	3060223	10.10%
2	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	3.87E-07	S - y6+	3060223	10.10%
3	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	1.00E-06	S - y6+	3060223	10.10%
1	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	6.40E-07	Q - y4+	630757	3.30%
2	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	3.87E-07	Q - y4+	630757	3.30%
3	sp|A0MZ66-5|SHOT1_HUMAN	DQIVSVQEEK	1.00E-06	Q - y4+	630757	3.30%

图 15. 获得离子对峰面积、CV 值，计算定量结果

应用实例

该应用实例为采用 DIA 方法完成的大规模蛋白质组学及磷酸化蛋白质组学定量实验。在 120 分钟的有效梯度内，以 q < 0.01 和 CV < 20% 为过滤条件，在 200 ng Hela 全细胞裂解液中同时定量 3597 个高可信度蛋白质（21710 肽段）；并在同样的实验条件下，同时定量了 11341 条高可信度的大鼠磷酸化肽段。实验证实，无论是常规的定量蛋白质组学实验还是翻译后修饰定量研究，DIA 方法都具有无可比拟的定量性能。

Large-Scale Proteome and Phosphoproteome Quantification by Data Independent Acquisition on Ultra-High Field Orbitrap Mass Spectrometers

Wei Zhang, Jing Li

Thermo Fisher Scientific, Shanghai, China

Overview

1) Purpose: Data Independent Acquisition (DIA) has been applied for large-scale protein and PTM quantification on the Thermo Scientific ™ Q Exactive HF and Orbitrap Fusion ™ Tribrid ™ instruments, to evaluate performance of the ultra-high field Orbitrap ™ based DIA strategy for complicated sample analysis.

2) Methods: HeLa protein digest and enriched rat phosphorylated protein digest were analyzed on a Q Exactive HF MS and a Orbitrap Fusion MS by DDA and DIA methods, respectively. Data processing was performed on Skyline (version 3.1) using DDA database searching result (Proteome Discoverer version 1.4) as spectra library.

3) Results: A total of 21710 peptides corresponding to 3597 protein groups were extracted with high confidence (Q value ≤ 0.01) as well as good reproducibility from only 200 ng HeLa protein digest within 2-hour LC gradient. Moreover, A total of 9990 phosphorylated peptides were characterized and quantified with the same criterion. The results indicate the strengths of DIA on ultra high-field Orbitrap MS.

Introduction

Orbitrap platforms based Data Independent Acquisition (DIA) is becoming increasingly popular, owing to its high sensitivity, selectivity and reproducibility compared to classic Data Dependent Acquisition (DDA) as well as other DIA techniques. Meanwhile, the flexibility of Orbitrap MS permits the development of novel acquisition strategies as well as solving current puzzles in proteomics such as PTM quantification.

The latest ultra-high field Orbitrap technology combines the smaller size Orbitrap cell (20 mm ID) with 5 kV of the central electrode voltage, and realizes 1.8-fold scan rate increase at the same resolution or 1.8-fold resolution increase at the same frequency. State-of-the-Art performance and flexibility are thus accessible on ultra-high field Orbitrap based Q Exactive HF and Orbitrap Fusion mass spectrometers.

Herein, we utilized the ultra-high field Orbitrap MS for large-scale proteomic and phosphoproteomic DIA analysis, to evaluate performance and strengths of the ultra-high field Orbitrap based DIA strategy. The strategy shows outstanding quantification ability compare to current DIA techniques and provides a powerful tool for high-throughput proteomic sample application.

Methods

1) Materials

Commercial HeLa protein digest was purchased from Pierce ™ (PN: 88328), with a loading amount of 200 ng for each run. Phosphoprotein sample was enriched from rat tissue and digested by trypsin, with a final loading amount of 700 ng for each run.

2) Liquid Chromatography

Each sample was analyzed on a Thermo Scientific ™ EASY-nLC ™ 1000 nanoflow LC coupled to a Thermo Scientific ™ Nanospray Flex ™ ion source. The sample was loaded onto a nano-C18 column and separated at a flow rate of 300 nL/min with following gradient: 0–3 min, 3–7% buffer B (0.1% fomic acid in acetonitrile); 3–95 min, 7–22% B; 95–113 min, 22–35% B; 113–116 min, 35–90% B; 116–120 min, 90% B.

3) Mass Spectrometry

DDA performed 120K resolution MS scan and then triggered top 15 precursors (QE HF) or acquired under 3-second top-speed mode for 60K resolution MS/MS scans. The MS/MS AGC target value was set at 1e6 (QE HF) or 5e4 (OT Fusion) with 100-110 ms of max injection time (Figure 1A).

DIA collected high-resolution MS/MS spectra by sequential 25 amu step length (26 amu isolation window, 0.5 amu overlapping between windows) from *m/z* 400 to 1200 for HCD fragmentation (28%). MS/MS ions were detected using 30K/60K of Orbitrap resolution, 1e6 (QE HF) or 1e5 (OT Fusion) of AGC target value and 85–100 ms of max injection time. MS scan was also performed before each DIA cycle (Figure 1B).

4) Data Analysis

Database searching was performed on Thermo Scientific ™ Proteome Discoverer ™ software (version 1.4) with Uniprot human or rat protein database. Mass tolerance of precursor and fragment were set at 10 ppm and 0.02 Da, respectively. Results were validated by Percolator with q < 0.01 and then used as spectral library for DIA analysis.

DIA Data processing was performed on Skyline software (version 3.1). Three precursor (mono) isotopes and top six fragment ions in spectral library of each peptide were selected as transitions for peak extraction. Results were refined by mProphet using decoy sequences and then validated by Q value (< 0.01).

Results

1) Data Independent Acquisition for Large-Scale HeLa Proteome Analysis

Three replicates were performed for each DDA and DIA methods, with a loading amount of 200 ng Hela digest for each run. DDA and DIA were proceeded under identical sample and LC conditions to evaluate and compare results of the two strategy directly.

Figure 2 shows MS1 chromatograms of the DDA and DIA replicates. DIA limits cycle time at 3–3.5 seconds and thus maximizes sensitivity and accuracy by increasing AGC value, maximum injection time and resolution within the cycle time limitation. The zoomed-in chromatograms display scan point distributions of an example DDA and DIA peak. The cycle time of DIA is fixed and thus the chromatogram scan points are uniformly distributed, compared to that of DDA data.

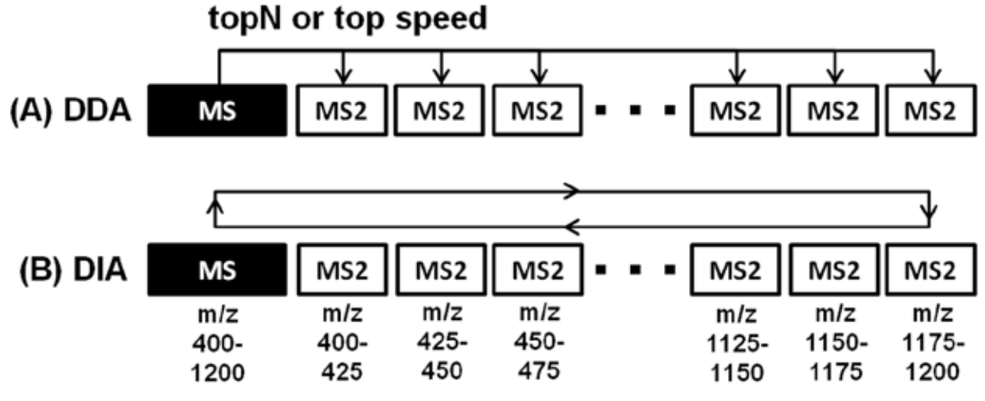


FIGURE 1. Schematic comparison of data dependent (A) and data independent (B) acquisition methods on ultra-high field Orbitrap platforms.

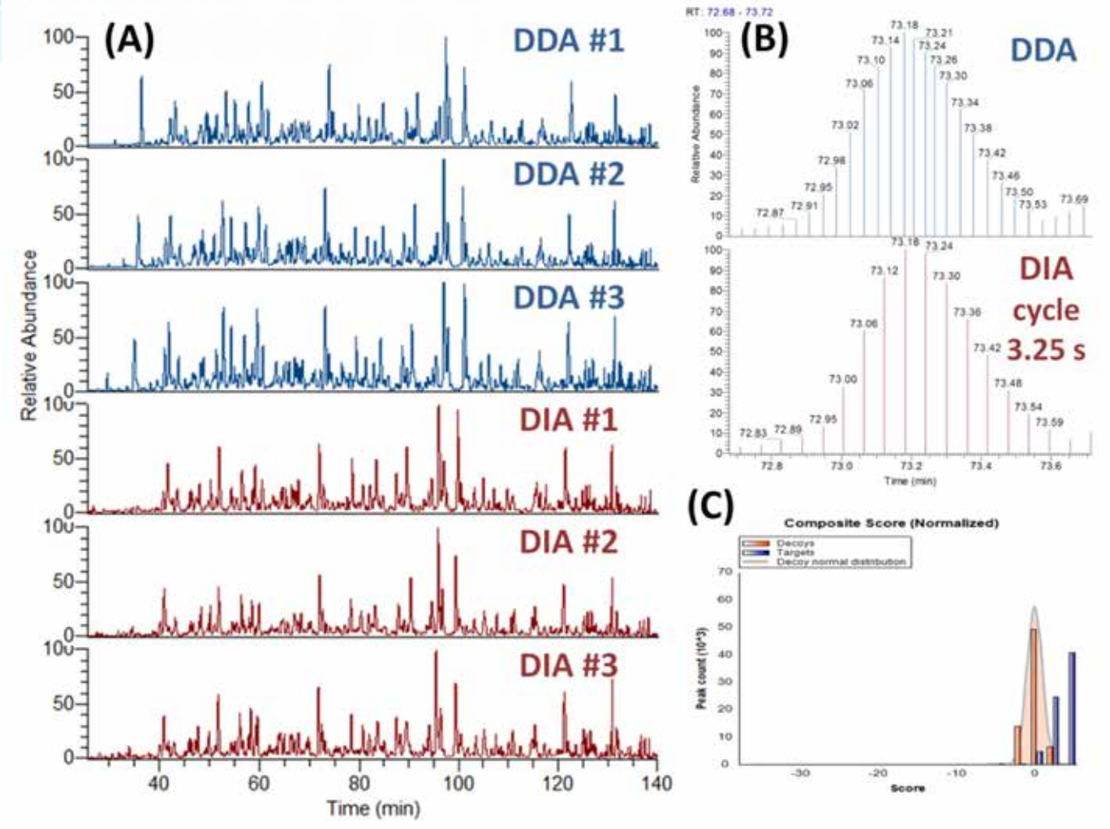


FIGURE 2. Comparison of DDA and DIA raw data. (A) Comparison of MS1 base peak chromatograms. (B) Comparison of zoomed-in chromatograms. (C) Composite score distribution of targets and decoys of DIA data.

DDA ID results were imported into Skyline as spectral library for DIA data processing. mProphet was used to perform statistical analysis of targets and decoys, and generated Q value for result refinement (Figure 2C). DDA IDs with q < 0.01 (Percolator) and DIA IDs with Q value < 0.01 were extracted as high confident results.

A total of 23072 peptides corresponding to 3794 proteins were identified from only 200 ng HeLa digest by DIA, showing good sensitivity of the ultra-high field Orbitrap. Moreover, 94.1% of the peptide IDs and 94.8% of the protein IDs were obtained from all three replicates of DIA, showing much higher reproducibility than DDA, as 57.0% of peptides and 72.8% of proteins identified from all replicates of DDA (Figure 3 & 4).

Conclusion

1) DIA has been applied and evaluated for large-scale protein and PTM quantification on the latest ultra-high field Orbitrap platforms.

2) A total of 21710 peptides from 3597 proteins were identified using DIA from only 200 ng HeLa digest, showing good sensitivity and reproducibility for quantification.

3) A total of 11341 phospho-peptides were identified using DIA and also displayed good sensitivity and reproducibility. Phospho-site ambiguity can be well distinguished, indicating the performance of DIA for PTM analysis.

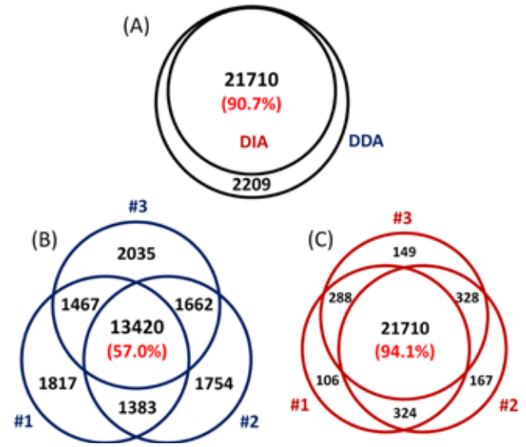


FIGURE 3. Sensitivity and reproducibility of peptide ID results of DDA and DIA. (A) More than 90% of library peptides (DDA result) can be identified from DIA with Q value of all three replicates <0.01. The ID reproducibility of DIA (C) is much better than DDA (B).

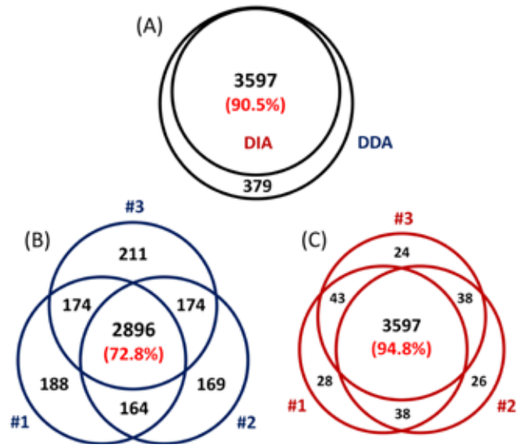


FIGURE 4. Sensitivity and reproducibility of protein ID results of DDA and DIA. (A) More than 90% of library proteins (DDA result) can be identified from all three replicates of DIA. The ID reproducibility of DIA (C) is also better than DDA (B).

The variations of transition/precursor peak areas were also calculated and compared to evaluate quantification ability of DDA and DIA. The CV of 95.2% DIA peptides are below or equal to 20%, while only 74.0% DDA peptides have a CV ≤ 20% (Figure 5).

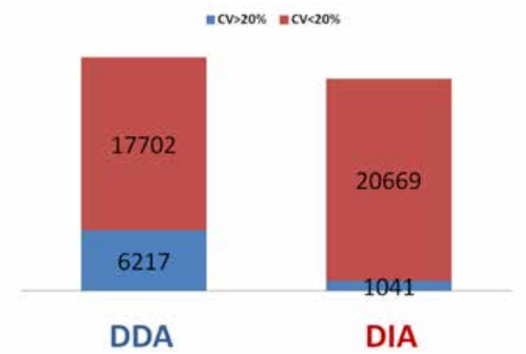


FIGURE 5. Comparison of peptide number with CV of transition/precursor peak area < 20% (red) or > 20% (blue). The quantification performance of DIA is much higher than DDA.

Data Independent Acquisition for Large-Scale Phosphorylation Analysis

The DIA strategy was further applied for phosphorylated protein sample to evaluate the performance of DIA for PTM analysis. A total of 11341 phospho-peptides were extracted with Q value <0.01 in all replicates. Figure 6 shows the DDA and DIA chromatograms as well as the DIA composite score distribution. Phospho-site ambiguity can be well distinguished due to high quality library spectra, good chromatogram separation and high sensitive DIA acquisition (Figure 7). DIA shows high ID reproducibility (Figure 8) and low peak area CV (Figure 9) as well in PTM analysis.

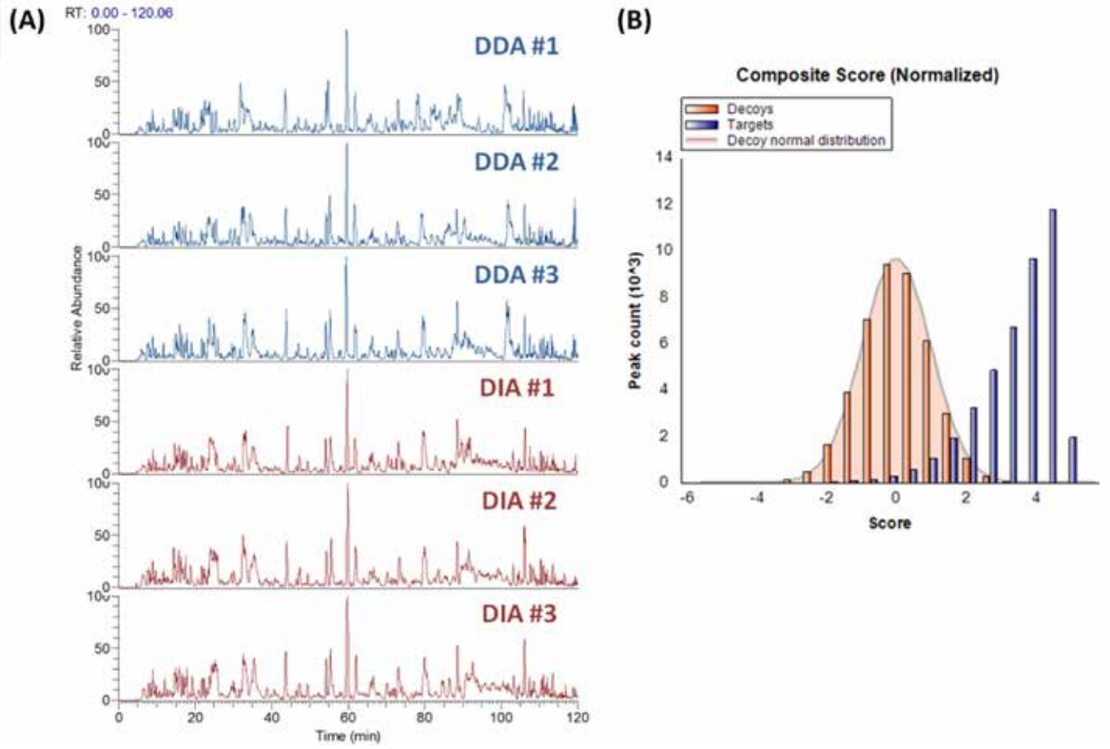


FIGURE 6. Comparison of phosphorylation sample data. (A) Comparison of MS1 base peak chromatogram. (B) Composite score distribution of targets and decoys of phosphorylation DIA data.

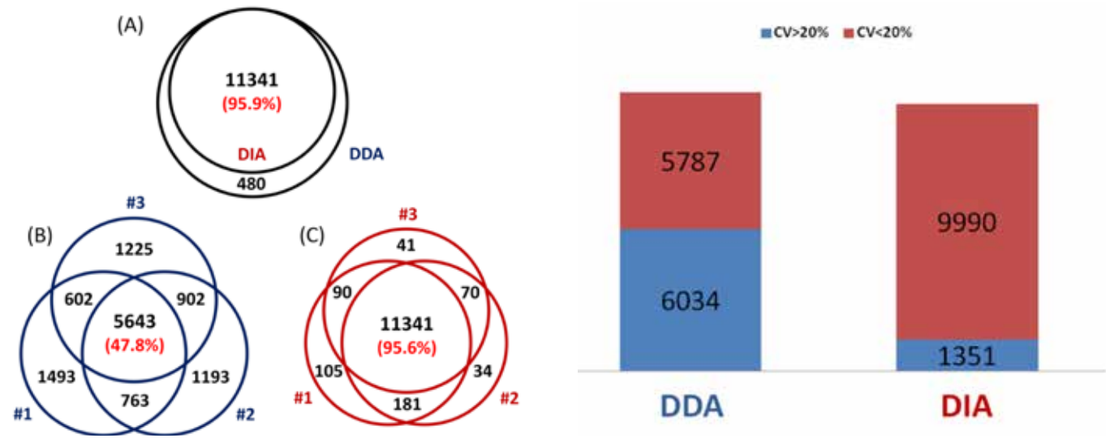


FIGURE 7. Sensitivity and reproducibility of phospho-peptide ID results. (A) More than 90% of library phospho-peptides (DDA result) can be identified from DIA with Q value of all three replicates < 0.01. The ID reproducibility of DIA (C) is much better than DDA (B).

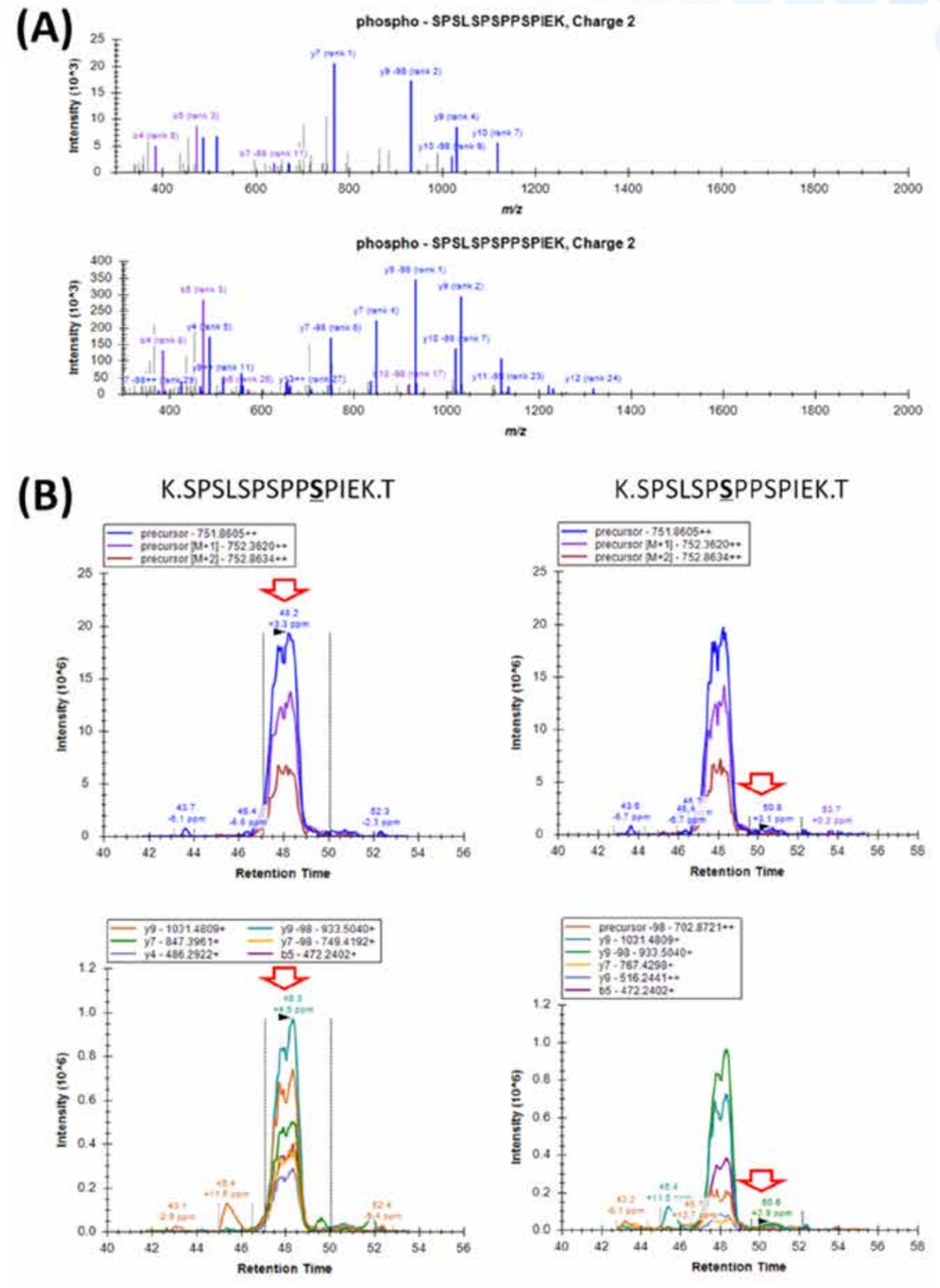


FIGURE 8. Example peptides with identical sequence and mass but different phosphorylation site. (A) Correct peak assignment was obtained due to high data quality.

文献资料

类别	题目	作者	来源
Paper	Multiplexed MS/MS for improved data-independent acquisition	Egertson J D, Kuehn A, Merrihew G E, et al	Nat. Methods, 2013, 10: 744-746
Paper	Multiplexed peptide analysis using data-independent acquisition and Skyline	Egertson J D, MacLean B, Johnson R, et al	Nat. Protoc., 2015, 10: 887-903
Paper	DIA-Umpire: comprehensive computational framework for data-independent acquisition proteomics	Tsou C C, Avtonomov D, Larsen B et al	Nat. Methods, 2015, 12: 258-264
Paper	Extending the limits of quantitative proteome profiling with data-independent acquisition and application to acetaminophen treated 3D liver microtissues	Roland B, Oliver M, Bernhardt T G, et al	Mol. Cell. Proteomics, 2015, M114.044305
Paper	Multiplexed quantification for data-independent acquisition	Catherine E M, Alexander S H, Jarred W R, et al	Anal. Chem., 2015, DOI: 10.1021/ac503593d
Paper	Hybrid Data Acquisition and Processing Strategies with Increased Throughput and Selectivity: pSMART Analysis for Global Qualitative and Quantitative Analysis	Prakash A, Peterman S, Ahmad S, et al	J. Proteome Res., 2014, 13: 5415-5430
Paper	Quantitative Analysis of Targeted Proteins in Complex Sample Using Novel Data Independent Acquisition	Zhang W, Kiyonami R, Jiang Z, et al	Chin. J. Anal. Chem., 2014, 42: 1750-1758
Paper	Progress in Mass Spectrometry Acquisition Approach for Quantitative Proteomics	Zhang W	Chin. J. Anal. Chem., 2014, 42: 1859-1868
Poster	Characterizing Qualitative and Quantitative Global Changes in the Aging Heart Using a Novel HybridDA Method	Vogelsang M S, Prakash A, Sarracino D, et al	PN013 (USHUPO 2014) www.thermofisher.com
Poster	Large Scale Targeted Protein Quantification Using HR/AM Selected Ion Monitoring with MS/MS Confirmation on the Orbitrap Fusion Tribrid MS	Kiyonami R, Senko M, Zabrouskov V, et al	PN099 (USHUPO 2014) www.thermofisher.com
Poster	Large Scale Targeted Protein Quantification Using WiSIM-DIA Workflow on a Orbitrap Fusion Tribrid Mass Spectrometer	Kiyonami R, Patel B, Senko M, et al	PN 64085 (ASMS 2014) www.thermofisher.com

类别	题目	作者	来源
Poster	Evaluation of Data-independent Acquisition (DIA) Approaches for Spiked Peptides in HeLa Digest on Q-OT-qIT Mass Spectrometer	Zhang W, Kiyonami R, Jiang Z, et al	PN 64122 (ASMS 2014) www.thermofisher.com
Poster	Real-Time Qualitative and Quantitative Global Proteomics Profiling Using a Hybrid Data Acquisition Scheme	Schroeder T, Prakash A, Peterman S, et al	PN 64149 (ASMS 2014) www.thermofisher.com
Poster	Characterizing Qualitative and Quantitative Global Changes in the Aging Heart Using pSMART, a Novel Acquisition Method	Vogelsang M S, Prakash A, Sarracino D, et al	PN 64144 (ASMS 2014) www.thermofisher.com
Application Note	Large-Scale Targeted Protein Quantification Using WiSIM-DIA on an Orbitrap Fusion Tribrid Mass Spectrometer	Kiyonami R, Senko M, Zabrouskov V, et al	AN 600 www.thermofisher.com
Poster	Large Scale Targeted Protein Quantification Using HR/AM Selected Ion Monitoring with MS/MS Confirmation on a Novel Hybrid, Q-OT-qIT Mass Spectrometer	Kiyonami R, Senko M, Zabrouskov V, et al	PN W490 (ASMS 2013) www.thermofisher.com
Poster	Data-Independent Acquisition (DIA) Analysis on the Q Exactive Mass Spectrometer	Xuan Y, Kiyonami R, Egertson J, et al	PN Th229 (ASMS 2013) www.thermofisher.com
Poster	Proteomic Analysis of Acetaminophen Toxicity in 3D Human Liver Microtissues	Escher C, Ossola R, Gunness P, et al	http://cdn.f1000.com/posters/docs/299630429

相关产品

Q Exactive 系列 Orbitrap 超高分辨质谱仪
（Q Exactive、Q Exactive Plus 和 Q Exactive HF）

UltiMate ™ 3000 RSLCnano 纳升超高效液相色谱仪

Orbitrap Fusion“三合一”系列 Orbitrap 超高分辨质谱仪

Easy-nLC 纳升超高效液相色谱仪

Pierce ™ Peptide Retention Time Calibration Mixture 标准肽段
混合物（Catalog No. 88320 & 88321）

相关软件

Proteome Discoverer 蛋白质组学分析软件
（http://portal.thermo-brims.com/）

Skylines 数据非依赖采集分析软件
（免费，https://skyline.gs.washington.edu）

更多信息

更多 DIA 相关信息资源：

- 请访问 Orbitrap 官方信息网站：
Planet Orbitrap (http://planetorbitrap.com/)

- 请关注 Orbitrap 蛋白质组学微信公众号：
Orbitrap 组学俱乐部（MSOmics）

关于赛默飞世尔科技

赛默飞世尔科技（纽约证交所代码：TMO）是科学服务领域的世界领导者。公司年销售额 170 亿美元，在 50 个国家拥有约 50,000 名员工。我们的使命是帮助客户使世界更健康、更清洁、更安全。我们的产品和服务帮助客户加速生命科学领域的研究、解决在分析领域所遇到的复杂问题与挑战，促进医疗诊断发展、提高实验室生产力。借助于首要品牌 Thermo Scientific、Applied Biosystems、Invitrogen、Fisher Scientific 和 Unity Lab Services，我们将创新技术、便捷采购方案和实验室运营管理的整体解决方案相结合，为客户、股东和员工创造价值。欲了解更多信息，请浏览公司网站：www.thermofisher.com

赛默飞世尔科技中国

赛默飞世尔科技进入中国发展已有 30 多年，在中国的总部设于上海，并在北京、广州、香港、台湾、成都、沈阳、西安、南京、武汉等地设立了分公司，员工人数超过 3800 名。我们的产品主要包括分析仪器、实验室设备、试剂、耗材和软件等，提供实验室综合解决方案，为各行各业的客户服务。为了满足中国市场的需求，现有 8 家工厂分别在上海、北京和苏州运营。我们在全国共设立了 6 个应用开发中心，将世界级的前沿技术和产品带给国内客户，并提供应用开发与培训等多项服务；位于上海的中国创新中心结合国内市场的需求和国外先进技术，研发适合中国的技术和产品；我们拥有遍布全国的维修服务网点和特别成立的中国技术培训团队，在全国有超过 2000 名专业人员直接为客户提供服务。我们致力于帮助客户使世界更健康、更清洁、更安全。欲了解更多信息，请登录网站 www.thermofisher.cn

赛默飞官方微信　赛默飞官方网站　Orbitrap 组学俱乐部

赛默飞世尔科技（中国）有限公司

上海（中国总部）
上海浦东新金桥路27号7号楼

广州
广州东风中路410-412号
时代地产中心2405-2406，3001-3004

沈阳
沈阳市沈河区惠工街10号
卓越大厦3109室

武汉
武汉东湖高新技术开发区
高新大道858号A7楼

北京
北京市安定门东大街28号
雍和大厦西楼7层

成都
成都市武侯区临江西路1号
锦江国际大厦1406

香港
香港新界沙田，沙田乡事会路138号
新城市中央广场第一座九楼911-915室

全国服务热线：800 810 5118　400 650 5118（支持手机用户）

BR 1050_C_LCMSMS_201506Y

ThermoFisher SCIENTIFIC