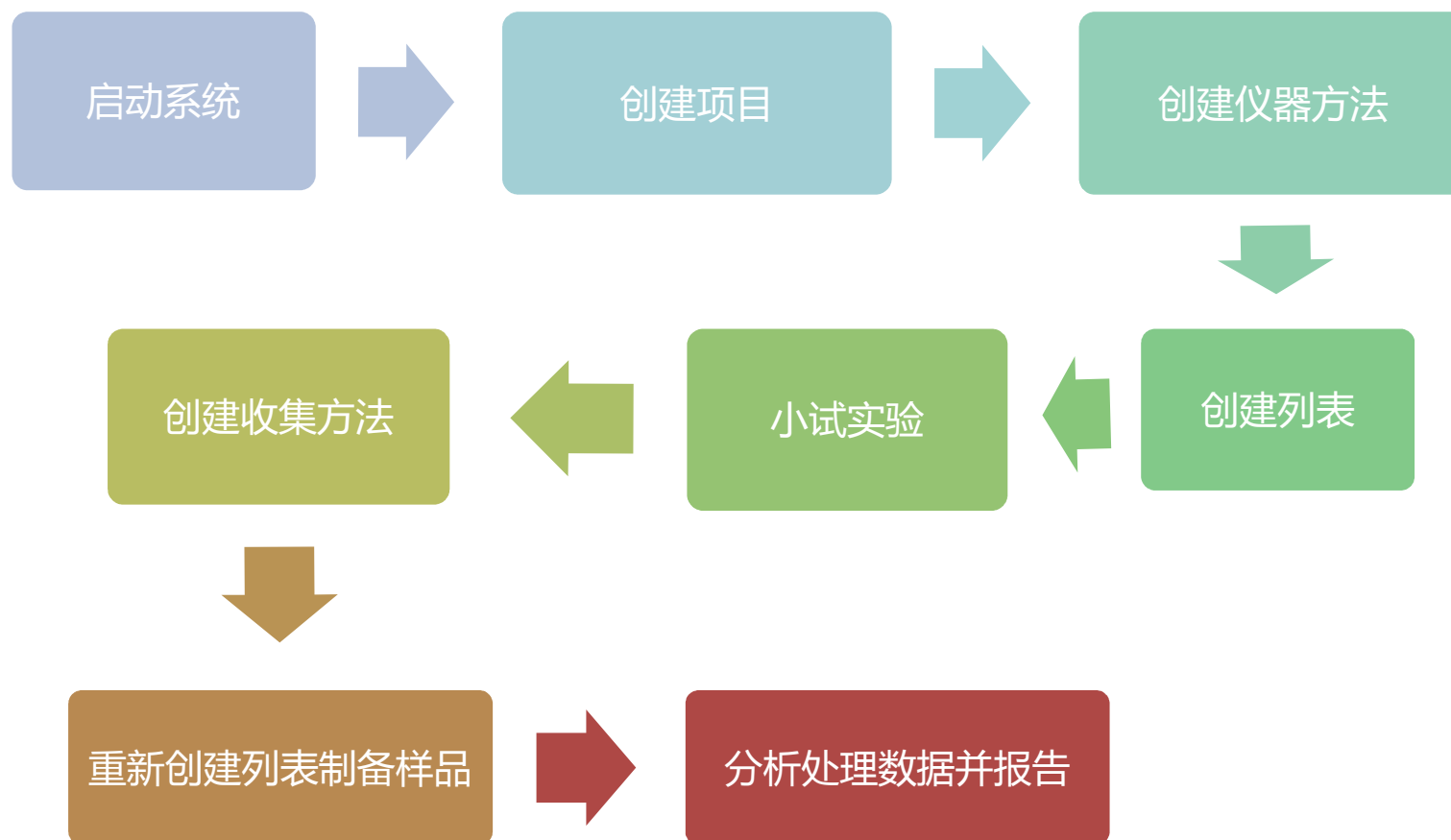




Prep 150 LC 操作指南



■ 开机顺序

1. 打开交换机电源
2. 启动计算机进入Windows系统
3. 开启仪器系统各个组件的开关
4. 待自检结束，启动软件

■ 实验前准备

— 流动相

- 数量充足
- 水相新鲜配制
- 符合液相色谱的级别要求

— 硬件

- 2545 在面板上启动Prime按钮 (或使用 Launch Console)
- 手动进样器：清洗进样口
- 自动进样器：检查洗针液
- WFCIII：确认已正确放置合适的样品盘以及接收管
- 检查废液系统通畅并已清空

登录操作界面



2. 输入用户名和密码

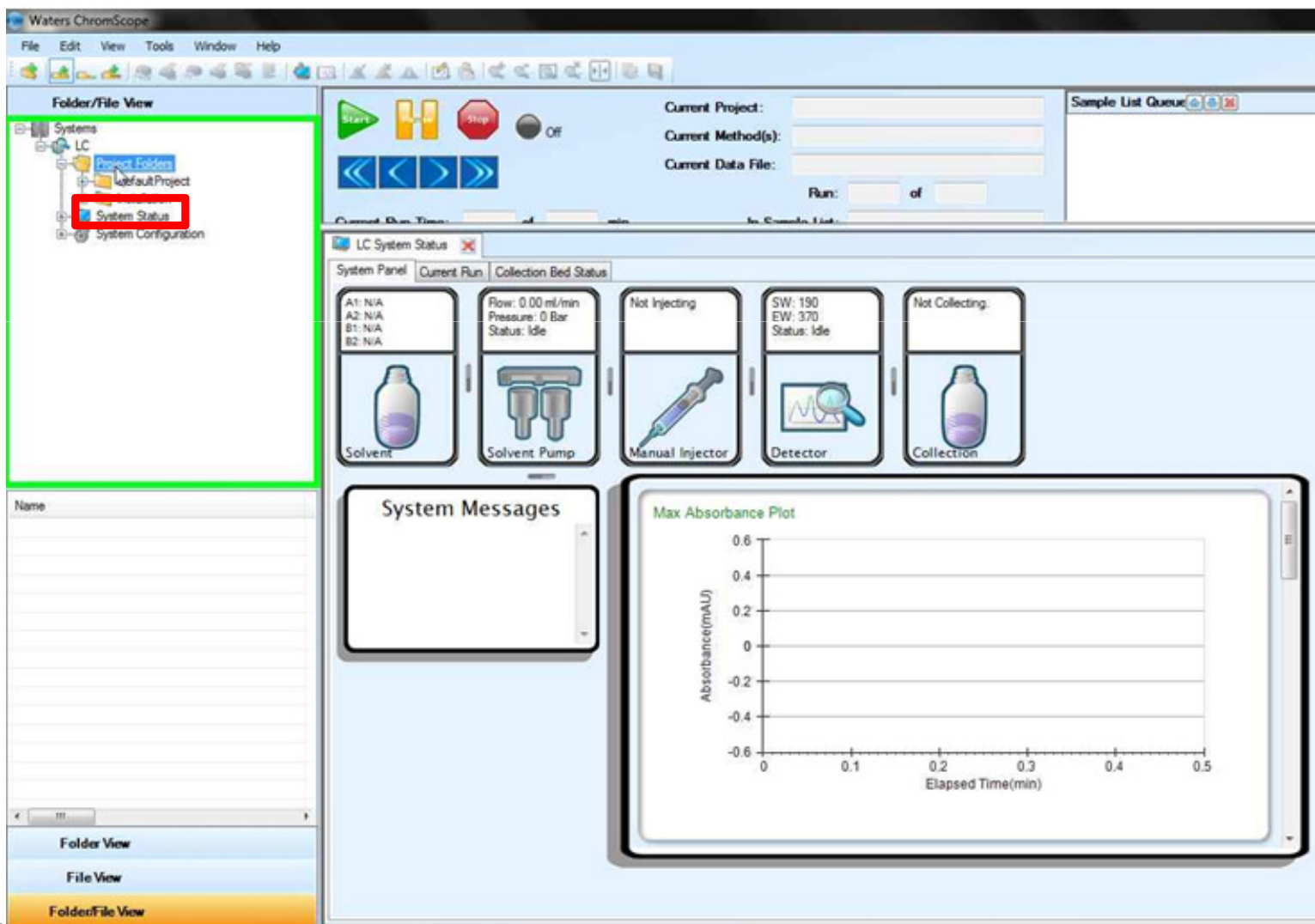
用户名：Administrator

密 码：waters



软件操作

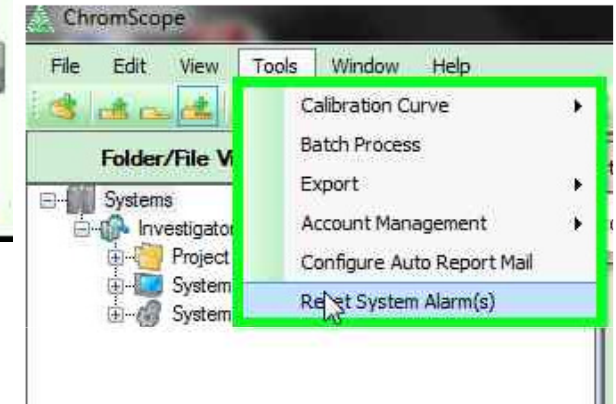
仪器状态界面



■ 所有组件应如图所示：黑色外框

— 红色框

- 并伴有“Alarm”的警告提示，说明仪器未连接
- 解决办法：重启组件，重新打开软件，并Reset System Alarm



✓ 黄色框

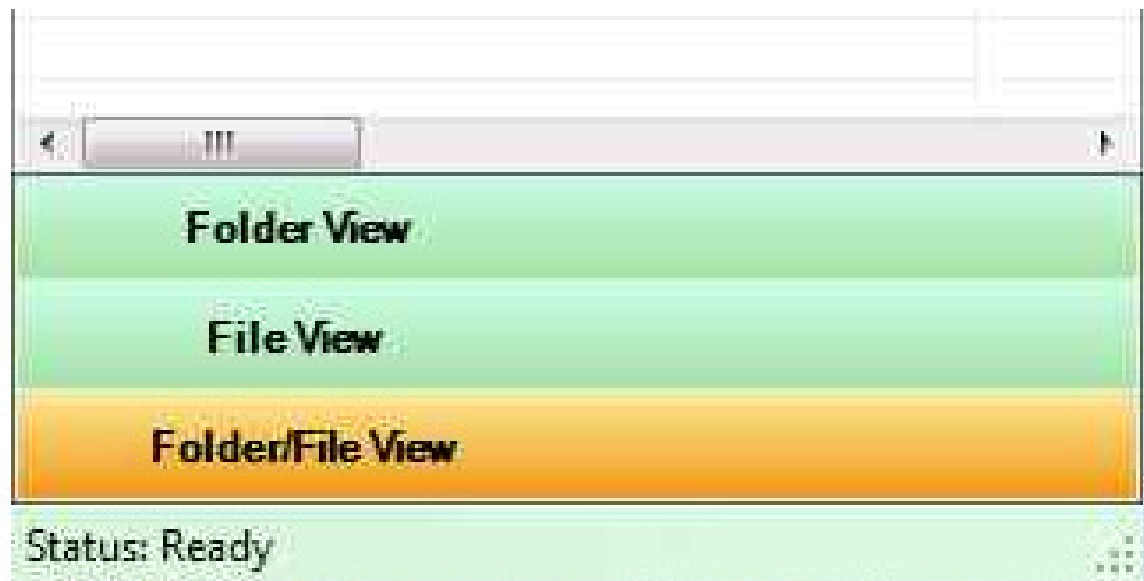
- 该组件工作中
- 例如：2998正在自检

Chromscope 三种界面

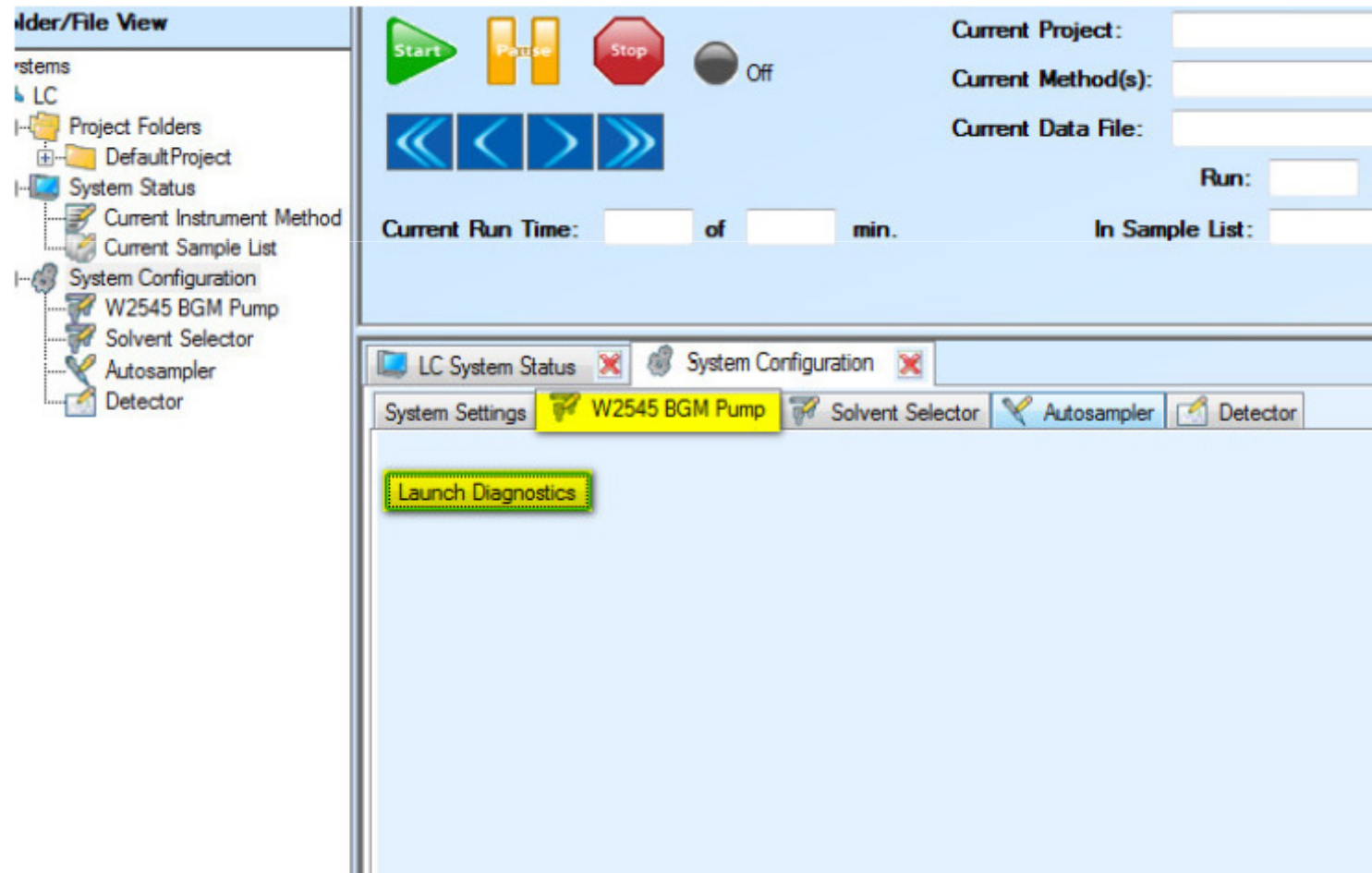
- 1. Folder only 2. File only 3. Fold/File View

The image displays three side-by-side screenshots of the Chromscope software interface, illustrating different views of the project structure. Each screenshot shows a tree view of the project hierarchy, including folders like 'Systems', 'Investigator', 'Project Folders', 'Default Project', 'Investigator ChromScope IE', 'Sequence Files', 'Method Files', 'Instrument Methods', 'Integration Methods', 'Collection Methods', 'Data Files', 'Report Templates', 'System Status', 'Current Instrument Method', 'Current Sequence', and 'System Configuration'. The 'Data Files' folder is expanded in all three views, showing sub-folders for '7_12_2011' and '7_13_2011'. The 'File View' screenshot shows a list of files with names like 'Unknown Sample_7-12-2011 9-46-20 AM.tta' and 'Steroid Mix Repro_7-12-2011 10-06-48 AM.tta'. Below each screenshot is a control bar with three buttons: 'Folder View', 'File View', and 'Folder/File View'. In the first screenshot, 'Folder/File View' is selected. In the second, 'Folder View' is selected. In the third, 'File View' is selected.

- 单击 “Fold/File View”

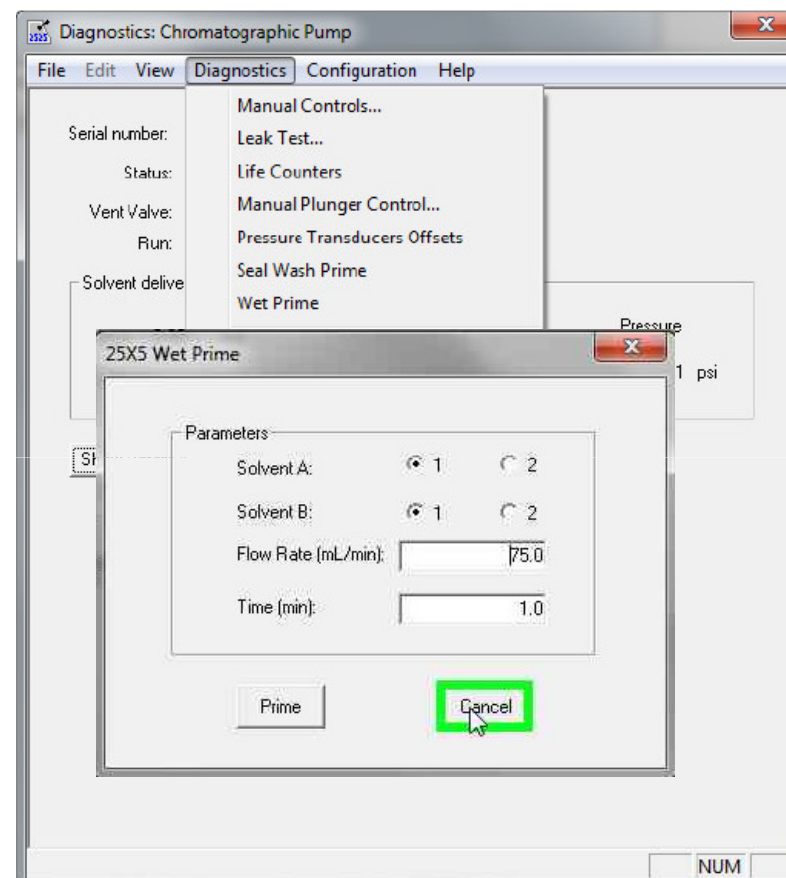


- I. System Configuration 项下会显示Investigator 系统中每个组 件的参数 (模块组成在软件安装时已预设)
- I. 仪器的手动操作可以在configuration项下完成



系统配置具体内容

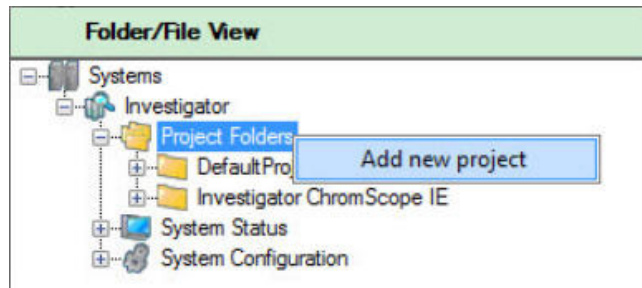
1. System setting
2. 2545 BGM 或QGM Pump
 - 25X5 诊断功能
 - 手动设置流速
 - Wet Prime 切换AB流路
3. Detector
 - 开关灯 控制



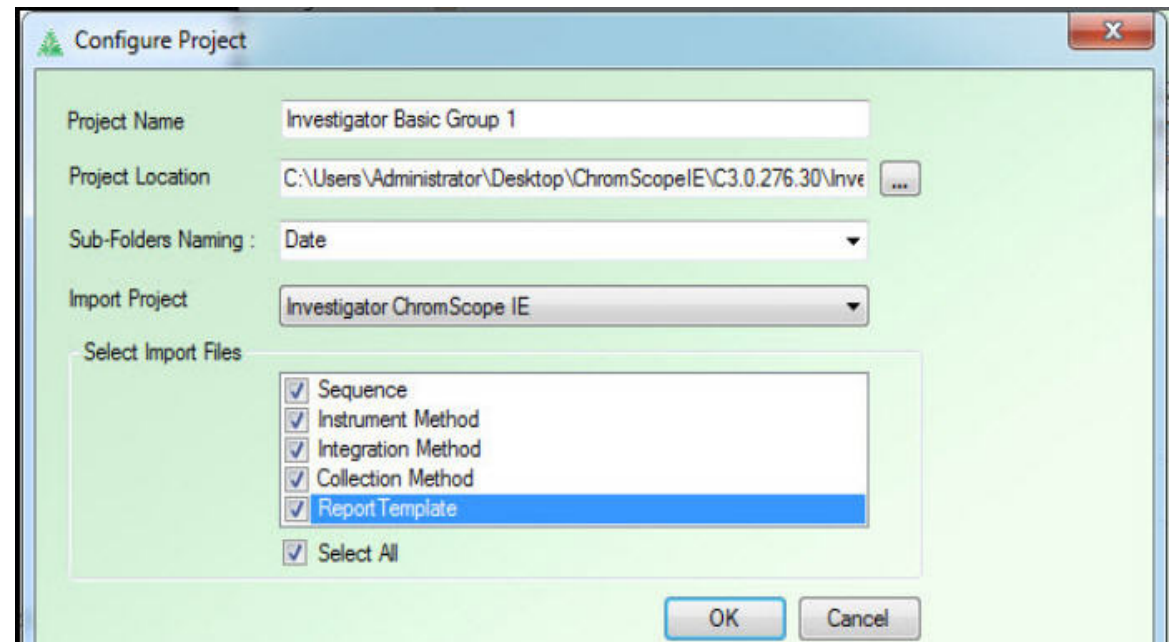
创建一个项目

新建一个项目文件夹

在Project Folders上右键
选择 “Add new project”



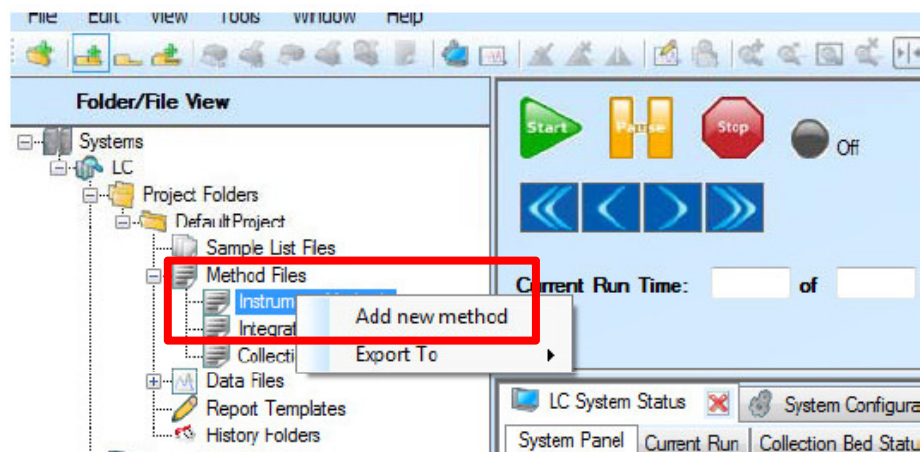
- 为项目文件夹设置名字.
- 选择 “Import Files” ，此功能可从其他项目文件夹复制已建好的方法
- 例如，从 “Investigator ChromScope IE” 项目中导入方法project, 选择 OK ，建立一个新的项目文件夹



创建一个采集方法

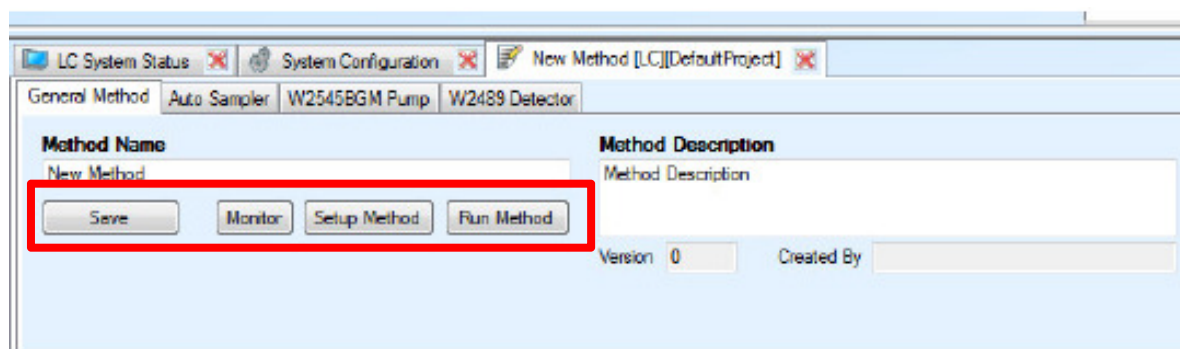
新建一个仪器方法

在Instrument Method上右键
选择“Add new method”



➤ General Method界面

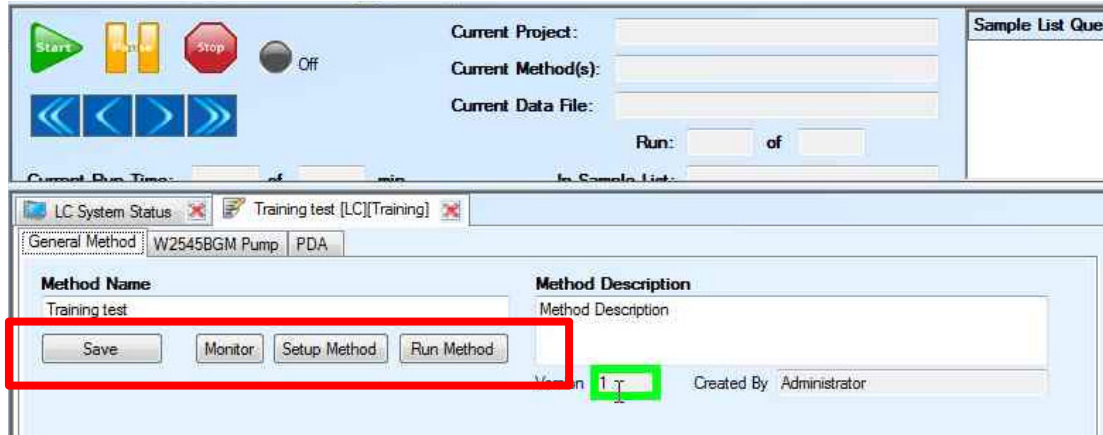
- 命名新的仪器方法
- Setup Method
- Monitor
- Run Method



新建一个仪器方法之 General Method

➤ General Method界面

- 命名新的仪器方法
- Setup Method
- Monitor
- Run Method



➤ Save

保存新建的方法 并生成相应的版本号。（仪器名相同会覆盖，但版本号不同）

➤ Monitor

- 采集监视数据并运行初始条件
- 在“Current Run”中显示图谱

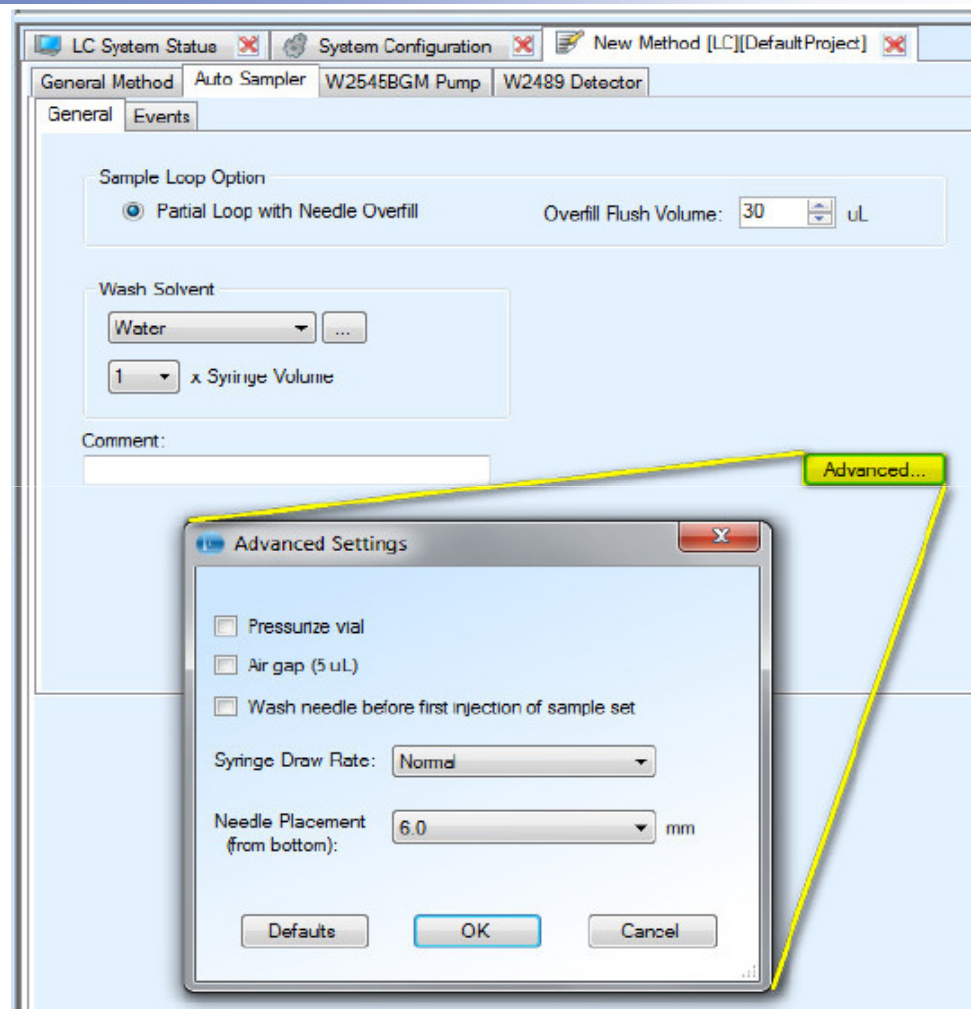
➤ Setup Method - 只运行初始条件 不采集数据

➤ Run Method - 运行梯度并采集数据

新建一个仪器方法之 自动进样器

➤ Auto Sampler界面

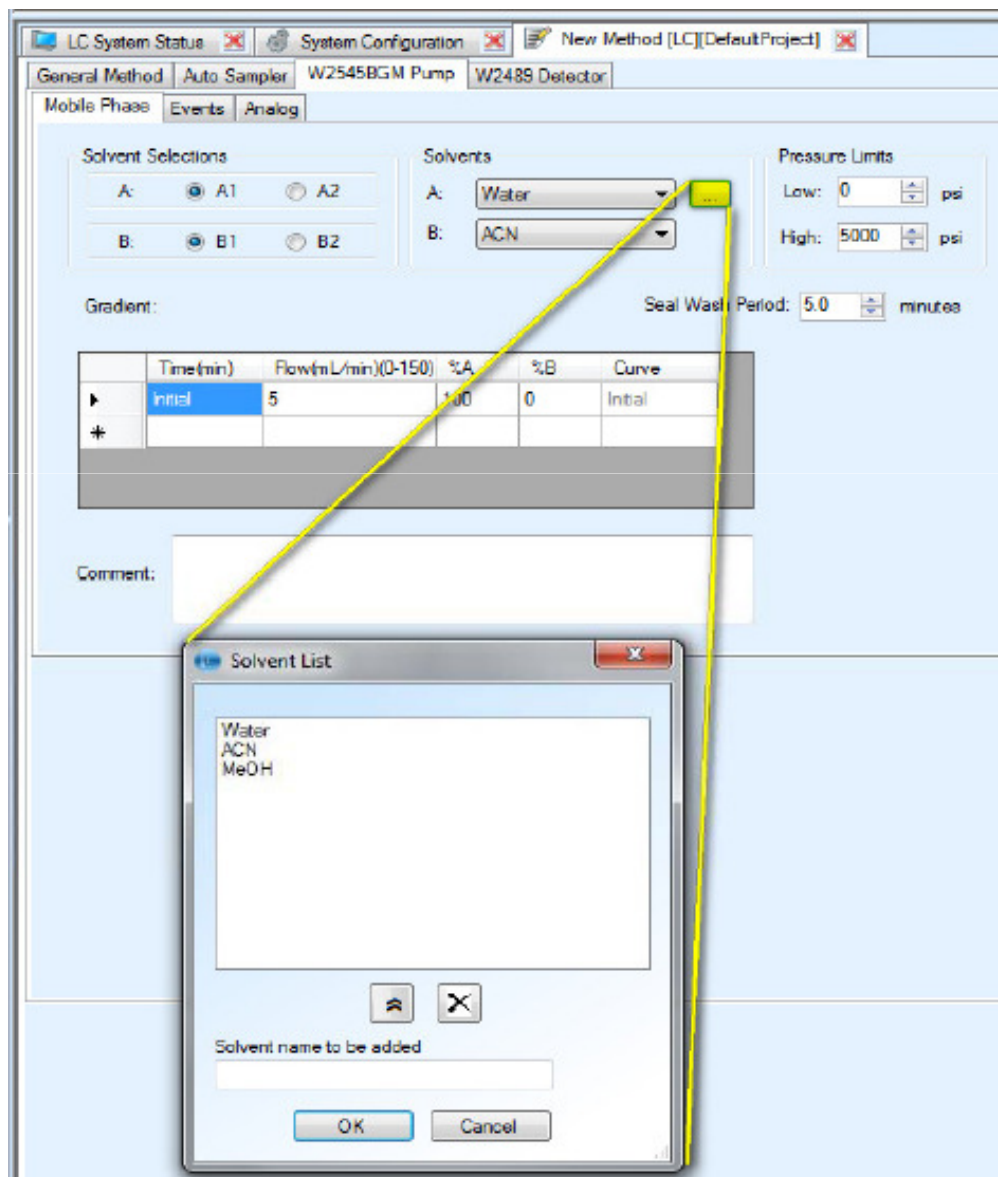
- 采集方式 Needle Overfill
- Advanced 选项



新建一个仪器方法之BGM

➤ 2545 BGM界面

- 选择溶剂
- 设置等度或梯度方法



新建一个仪器方法之BGM

➤ 梯度方法设置

- 注意流速要与色谱柱相匹配
- 注意平衡时间
- 注意最高压力

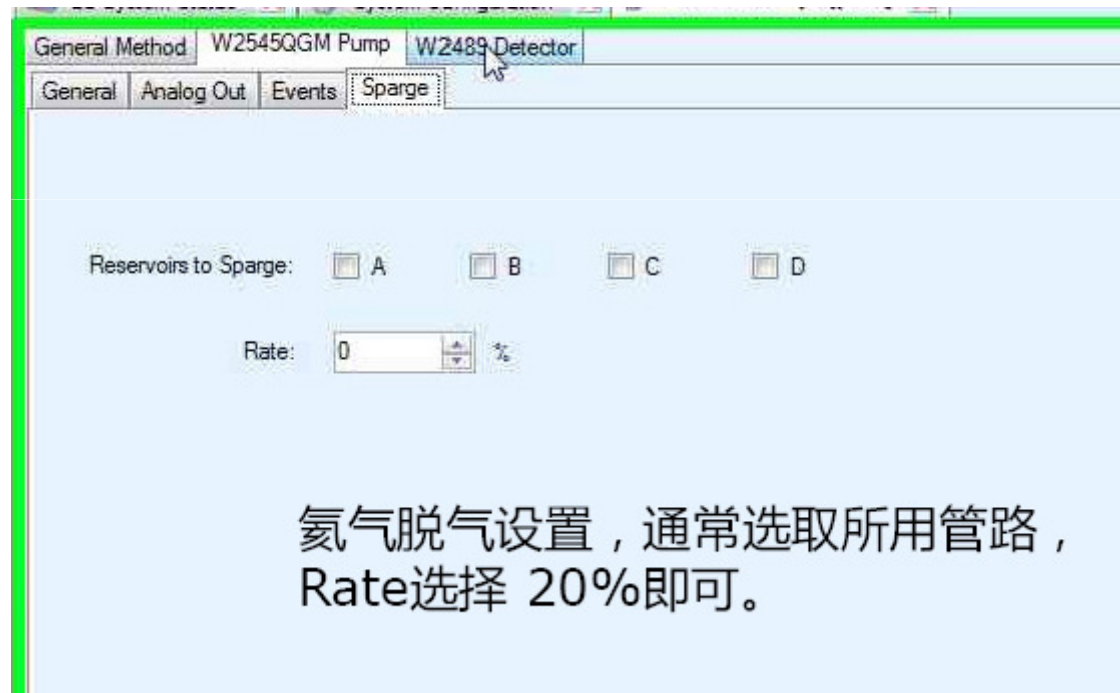
	Time(min)	Flow(mL/min)(0-150)	%A	%B	Curve
▶	Initial	10.00	95.00	5.00	Initial
	10.00	10.00	0.00	100.00	6
	13.00	10.00	0.00	100.00	6
	14.00	10.00	95.00	5.00	6

(此梯度未完成，还有最后一行 14-18分钟 95 : 5 初始条件平衡)

Length (mm)	Diameter (mm)				
	4.6	10	19	30	50
30	-	8	27	-	-
50	3	15	45	110	310
75	-	-	-	165	-
100	5	25	90	225	620
150	8	40	135	335	930
250	13	60	225	560	1550
Reasonable Flow Rate (mL/min)	1.4	6.6	24	60	164
Reasonable Injection Volume (μL)	20	100	350	880	2450

不同规格色谱柱 参考流速以及进样体积

若配置为2545QGM 且无在线脱气机 须设置氮气脱气参数



General Method W2545QGM Pump W2489 Detector

General Analog Out Events Sparge

Reservoirs to Sparge: A B C D

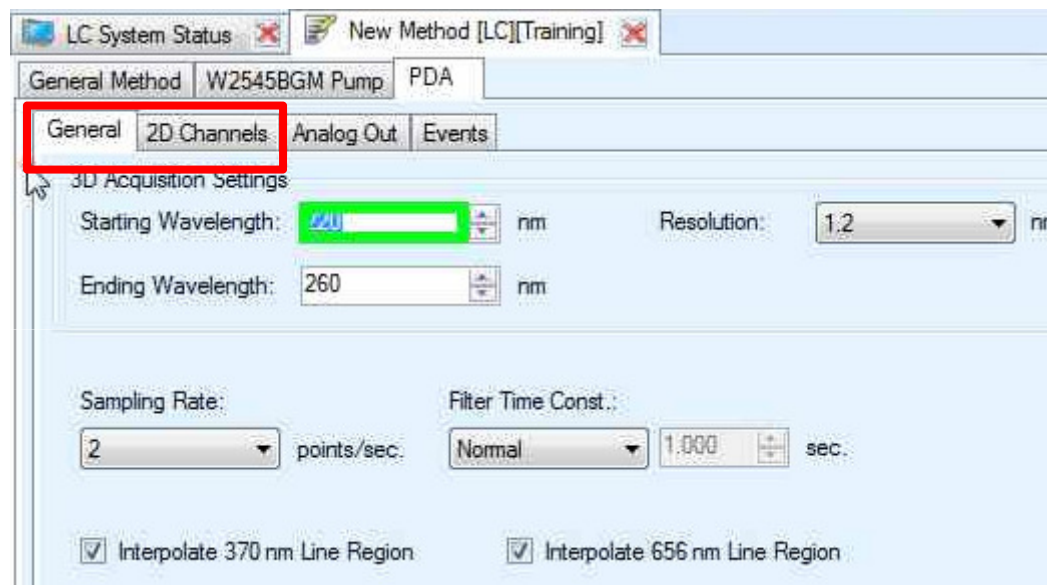
Rate: 0 %

氮气脱气设置，通常选取所用管路，
Rate选择 20%即可。

新建一个仪器方法之检测器

➤ 2998 PDA界面

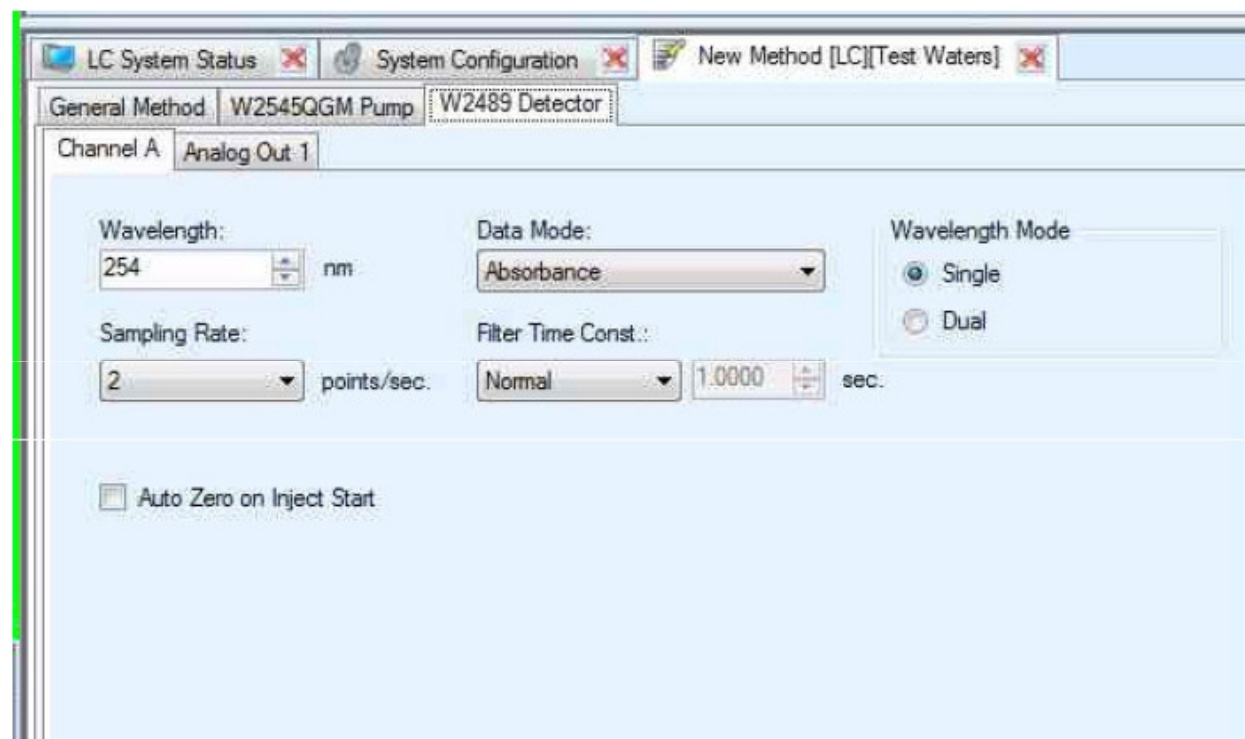
- 设置3D 波长范围
 - 3D数据可查看光谱
- 设置2D 指定波长
 - 8通道可选
 - 波长范围190-800nm



新建一个仪器方法之检测器

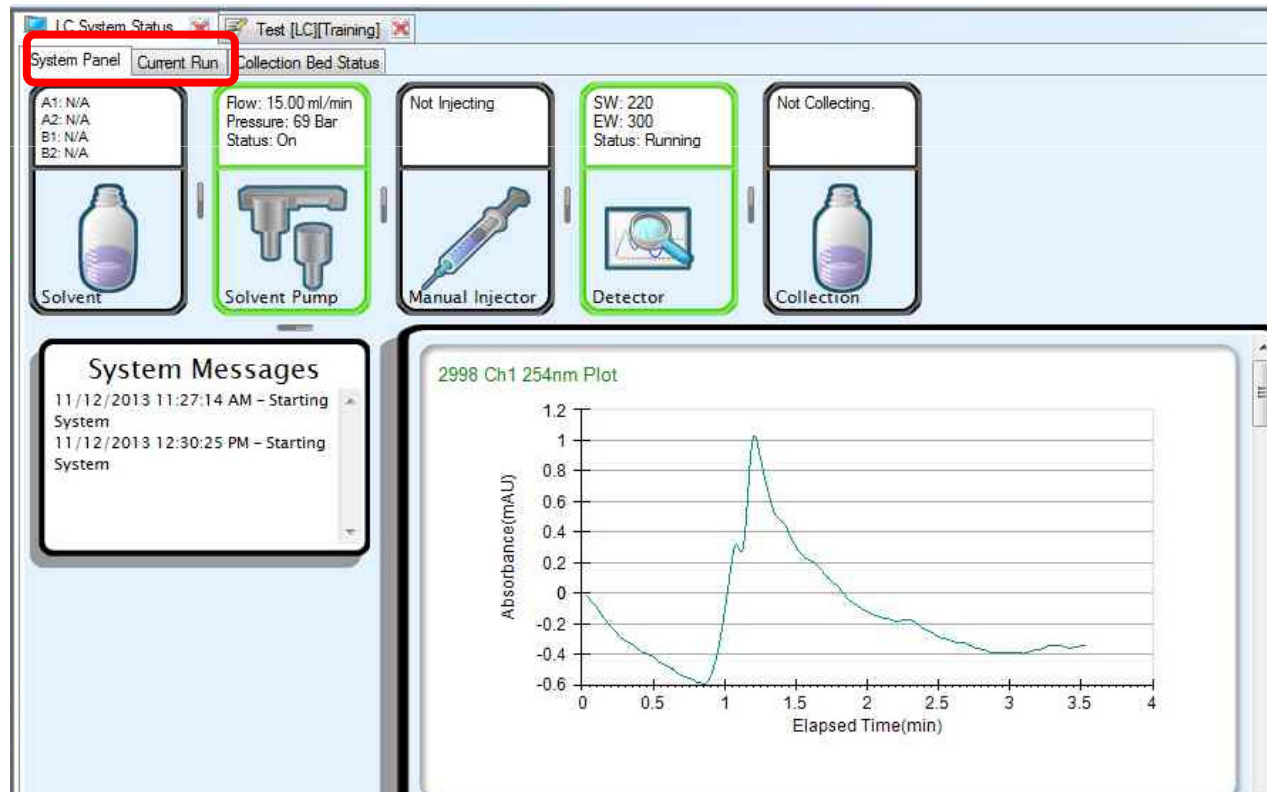
➤ 2489 紫外检测器界面

- 设置 指定波长
 - 双通道可选
 - 波长范围190-800nm
- 自动归零功能
- 单进样勾选
- 连续进样不选，防止漏收馏分



新建一个仪器方法之检测器

- 设置完所有参数 返回 General Method 界面，并点击 “Save”
- 点击 “Monitor” 返回至 “Current Run” 查看基线
系统将在0.17min后采集基线数据，数据将在 “XXX project” - “Data” - “Monitor” 项下显示

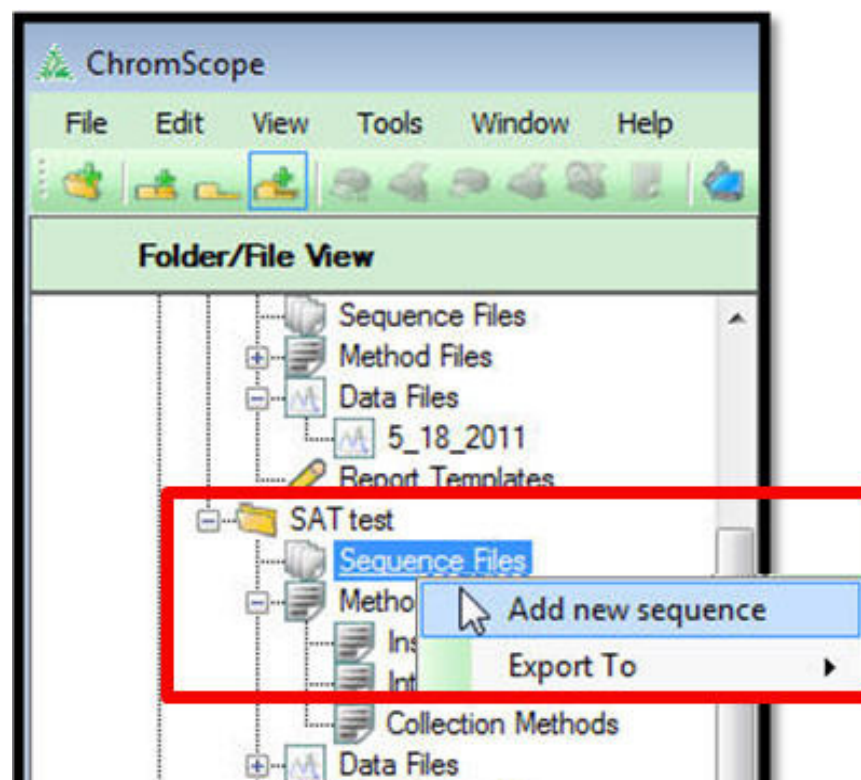


采集数据

- 对于新制备的样品，需要进行一次试验性进样，已测试分离效果，并得到相关的制备参数
- 根据试验性进样的结果，调整最佳的色谱条件，并确定收集方法

新建一个样品列表

在Sample List Files上右键 选择 “Add new sample List”



新建一个样品列表

依次填好 红色方框内的各项信息

进样前延迟

数据文件

进样体积

Inj. #	Run. Dur. (min.)	Inj. Delay (min.)	Instrument Method *	Data File *	Sample Name *	Inj. Vol. (uL) *	Collection Method
1	10	0	New Method	File Name And Date/Time	Unknown Sample	1	

运行时间

仪器方法

样品名称

新建一个样品列表

- 设置完所有参数 点击 “Save” 并点击 “Add to Queue”
- 若出现图示的提示，请点击 “Yes”

Sample List Name: Test Sample Version: 2

Make a single acquisition for entire sample list Created By: Administrator Total Duration (min): 20

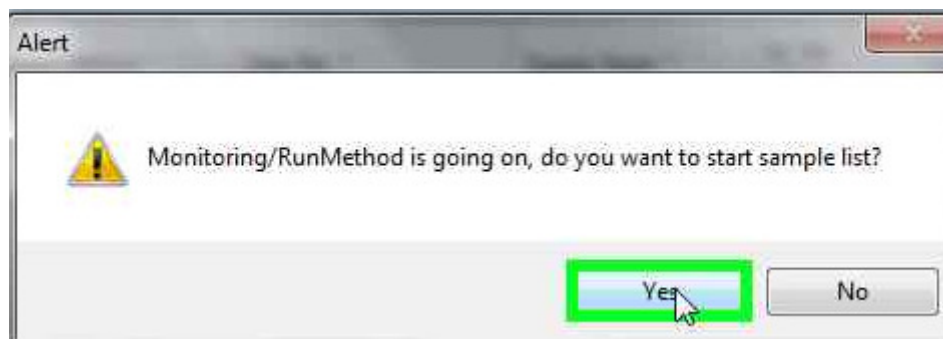
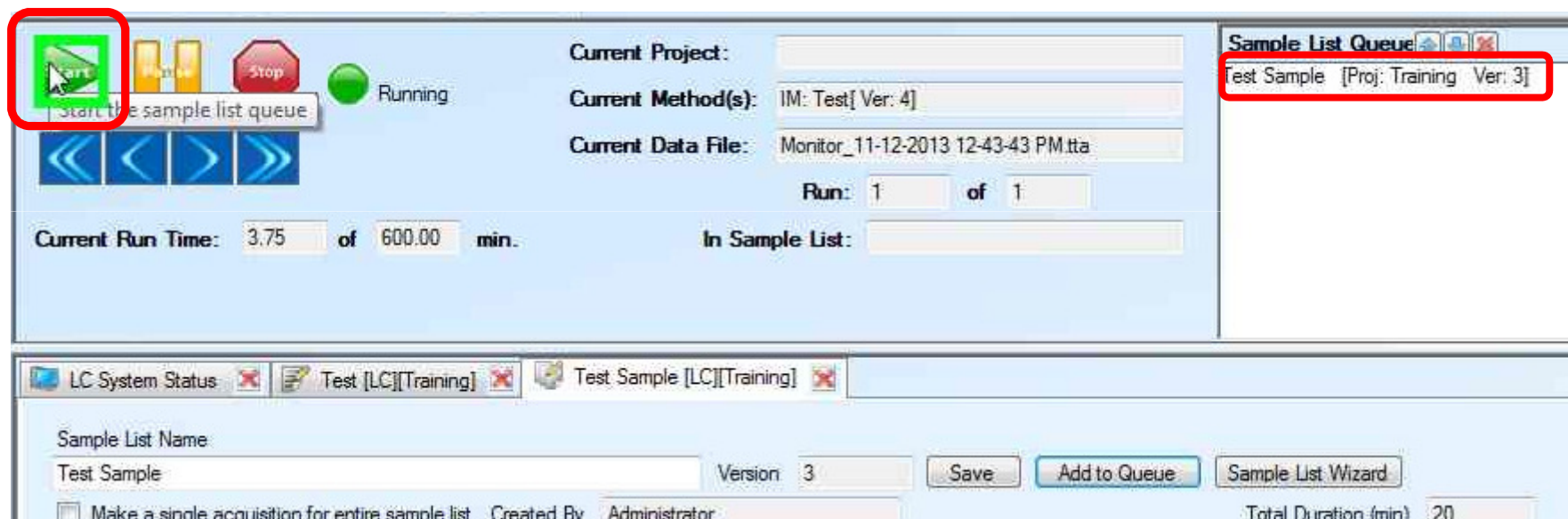
Inj. # *	Run. Dur. (min.)	Inj. Delay (min.)	Instrument Method *	Data File *	Sample Name *	Inj. Vol. (μL) *	Collection Method
1	20	0	Test	Sample Name And D...	Parabenze	200	
*							

Confirm Save

There are changes made to method(s) after last sample list saved version.
Do you want to save these changes to this Sample List File?
Press Yes to save the changes and add sample list with latest method(s) version to the queue
Press No to add the last sample list saved method(s) version to the queue.

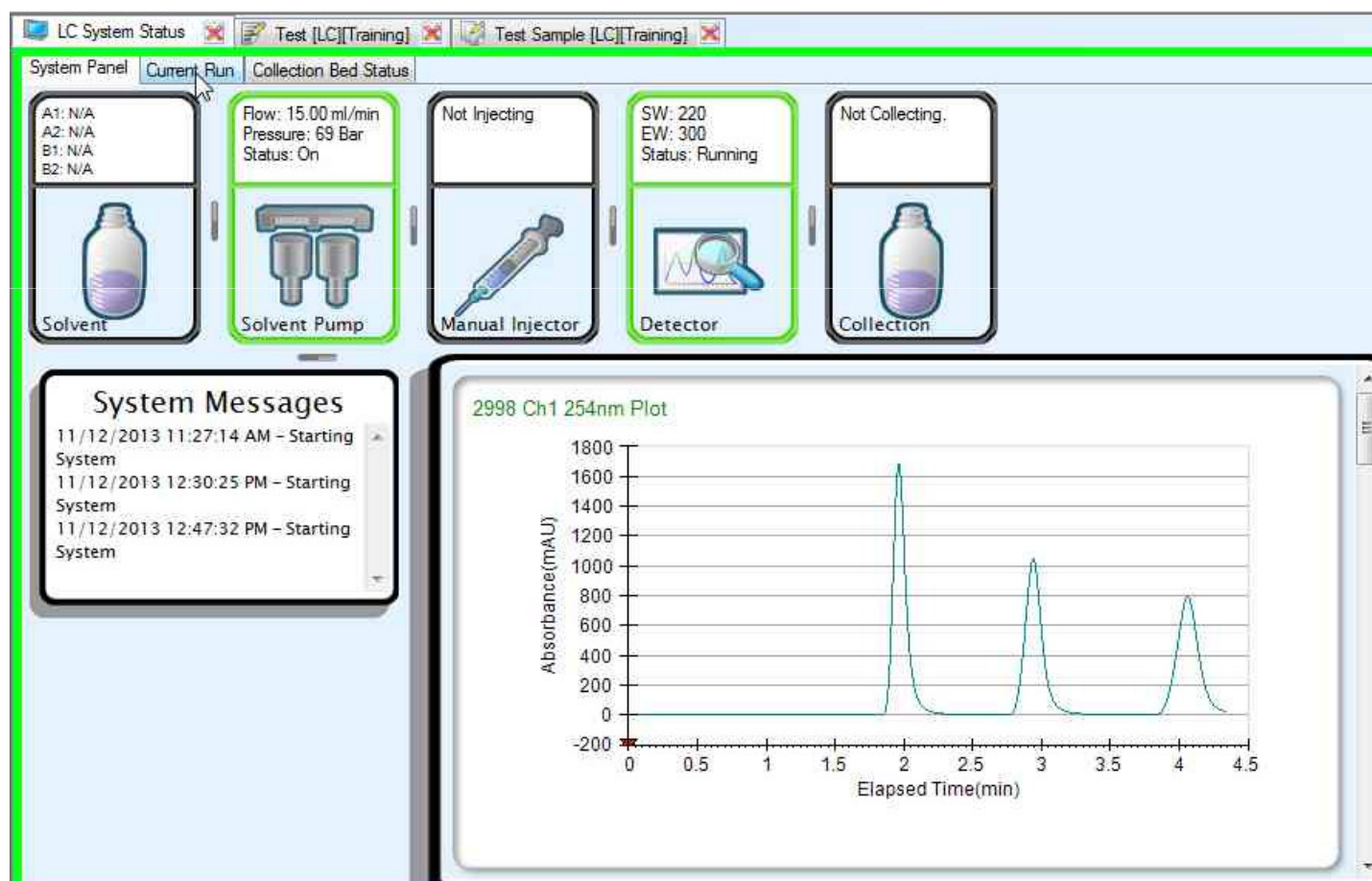
Yes No

- 样品序列将在Sample List Queue中出现
- 点击“Start”，系统将在短暂平衡后进行进样
- 若出现图示的提示，请点击“ Yes ”，先退出监视界面再采集数据

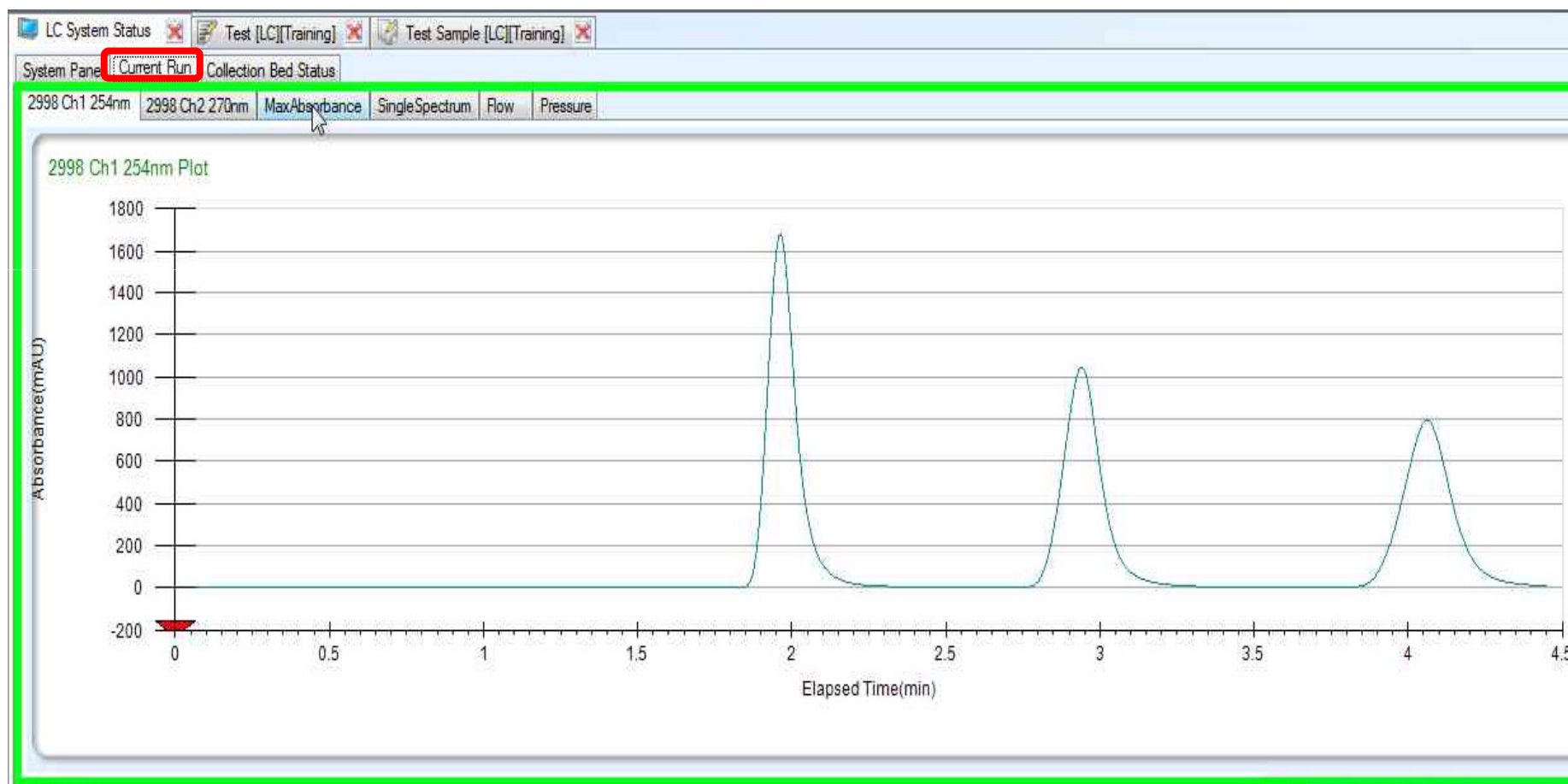


- 若仪器配置为自动进样器，在退出监视界面后，自动进样器会自动进行采样。
- 若仪器配置为手动进样器，在退出监视界面后，会弹出一个准备进样的窗口，请将手动进样阀搬至Load位，推进样品，并迅速再搬至Inject位。之前的弹出窗口会自动消失，数据采集开始。

- 样品运行后，在 System Panel 界面查看仪器的运行状态



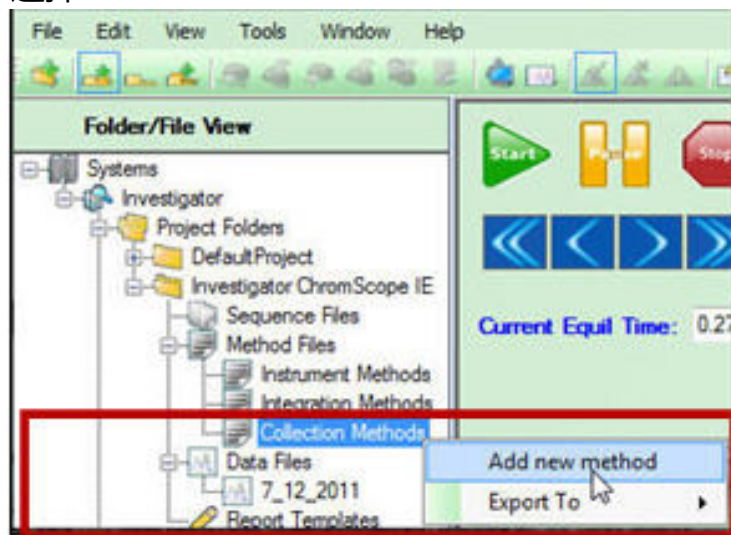
- 样品运行后，在 Current Run 界面查看样品的数据状态



创建一个收集方法

新建一个收集方法

在Collect Method上右键
选择“Add new method”



Collection Method界面

The screenshot shows the 'New Collection Method' configuration window. The window has tabs for 'Method Name' and 'Method Description'. The 'Method Name' tab is active, showing fields for 'New Collection Method', 'Save', 'Version: 0', and 'Created By:'. Below are checkboxes for 'Collect waste in between time window', 'Maximum Tubes per Injection: 1', and 'Maximum Fractions per Injection: 1'. A 'Volume Delay: 0.00' field is also present. The 'Fraction Collection Parameters' section contains a table with columns: Window #, Single Fraction, Collection Type, Select Detector Signal, Time Window Start (min.), Time Window Stop (min.), and Pool Fraction. The table has two rows: one for window 1 and one for a new window marked with an asterisk.

Window #	Single Fraction	Collection Type	Select Detector Signal	Time Window Start (min.)	Time Window Stop (min.)	Pool Fraction
1	<input checked="" type="checkbox"/>	Specified Time	(W2998) MaxAbsorbance	1.700	2.2	<input type="checkbox"/>
*	<input type="checkbox"/>					<input type="checkbox"/>

新建一个收集方法

- Select Scout Run --选择已分离好的样品图谱，进行模拟收集
- 设置新的收集方法参数
 - Collection Type：根据需求选择 Threshold、Slope以及Time收集等方式
 - Select Detector Signal：选择收集波长或是通道
 - Threshold Start and Stop：若Collection type 选择了“Signal Threshold”，则须设置收集起始和截至阈值参数
 - Time window start and stop：若Collection type选择了“Specified Time”则须设置收集的起始时间和截至时间
 - Pool Fraction—选择此功能，用户会在同一管内重复收集同一组分。

Method Name: New Collection Method

Method Description: Collection Method Description

Save Version: 0 Created By:

Select Scout Run

Collect waste in between time window

Maximum Tubes per Injection: 1

Volume Delay: 0.00 ml

Maximum Fractions per Injection: 1

Fraction Collection Parameters

Current selected window: 1

Window #	Single Fraction	Collection Type	Select Detector Signal	Time Window Start (min.)	Time Window Stop (min.)	Pool Fraction
1	<input checked="" type="checkbox"/>	Specified Time	(W2998) MaxAbsorbance	1.700	2.2	<input type="checkbox"/>
*	<input type="checkbox"/>					<input type="checkbox"/>

新建一个收集方法

- 参数的具体意义请参考Help以及工程师的现场讲解。
- 注意 “Volume Delay” 参数设置（参照工程师现场测试结果）
- 设置好收集参数以后，为此方法命名，并点击 “Save”

至此，收集方法建立完毕。

样品收集

收集样品

- 在样品列表中加入收集方法 “Collection Method”
- “Save” 序列列表并 “Add to Queue”

The screenshot shows the Waters software interface for creating a new sample list. The window title is "New Sample List [LC][Test]". The interface includes a "Sample List Name" field with the value "New Sample List", a "Version" field with the value "1", and buttons for "Save", "Add to Queue", and "Sample List Wizard". There is also a checkbox for "Make a single acquisition for entire sample list" and a "Created By" field with the value "Administrator". The "Total Duration (min)" is set to "10".

	Inj. # *	Run. Dur. (min.)	Inj. Delay (min.)	Instrument Method *	Data File *	Sample Name *	Inj. Vol. (uL) *	Collection Method
▶	1	10	0	New Method	Sample Name And D...	Dyetest	1	
*								New Collection Methc

收集样品

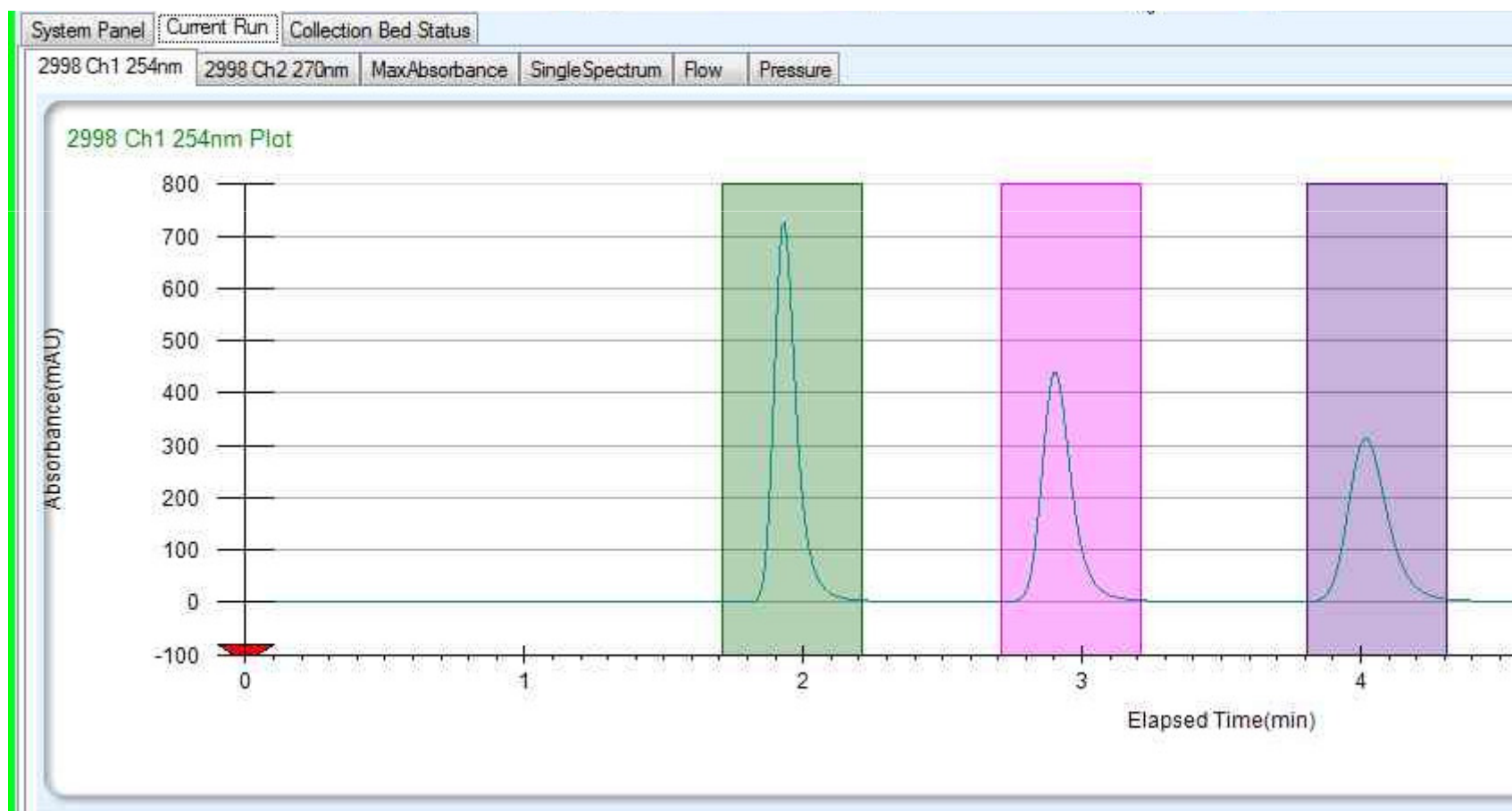
- 在点击“Start”之前，请先确认收集管的位置。
- 点击“Reset Bed”，并点击“Apply”，收集位置会从1号开始。否则默认为上一次收集后的位置。

The screenshot shows the 'Collection Bed Status' window in the Waters software. The window title bar includes 'LC System Status' and several open windows. The main area displays a grid of collection tubes, with rows labeled 1, 21, 41, 61, 81, 101, 11, 31, 51, 71, 91, and 111. The tubes are color-coded: green, magenta, purple, and red. The control panel on the right includes a 'Max. Tube Volume' dropdown set to 10 ml, a 'Reset Bed' button, and an 'Apply' button. A legend below the control panel identifies collection types: Manual Collection (yellow), Waste collected within/between time window (grey), and Fractions 1-4 (green, magenta, purple, red). A table at the bottom shows injection details.

Injection	Sample	Fraction Location
1	para	4, 5, 6, 7
1	Para	8, 9, 10, 11
2	Para	9, 12, 10, 13, 11, 14

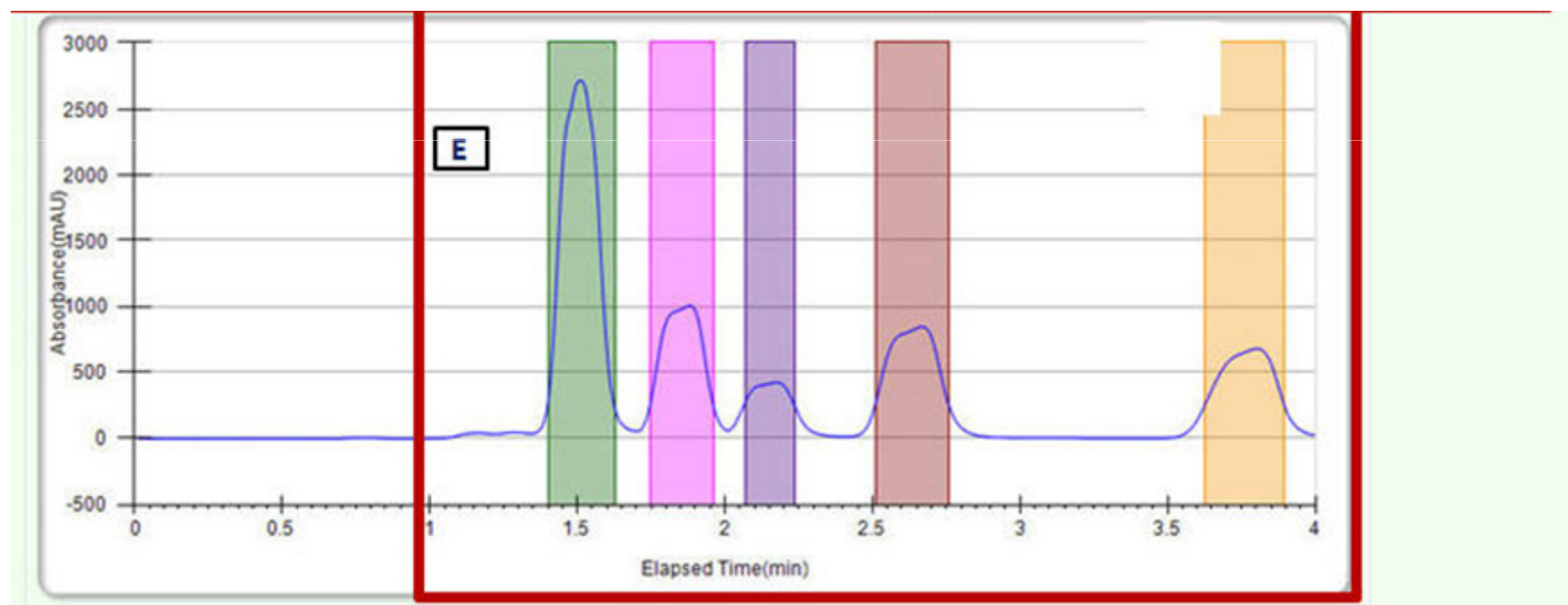
收集样品

- 确认完收集管的位置以及状态，点击“Start”
- 在“Current Run”界面会显示收集状态。



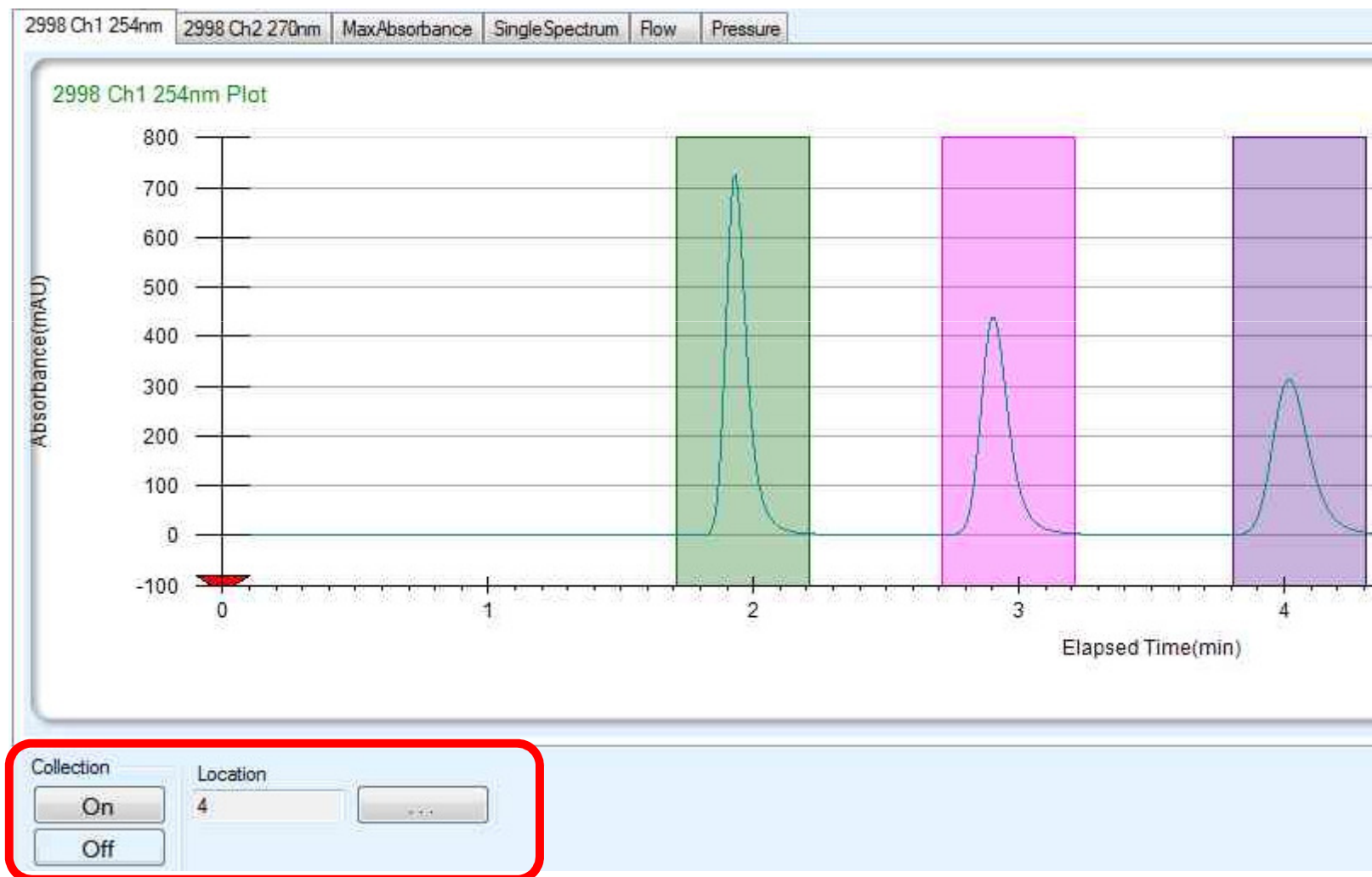
制备的色谱峰往往不会像分析型LC峰型出色，更注重收集的效率

典型的制备型色谱峰：



紧急收集

- 若遇到出峰异常或收集异常，需挽救样品的情况，可使用紧急收集



Collection

On

Off

Location

41

...

- 收集设置
 - Location 设置收集位置
 - On 开始，Off 结束
 - 强制收集后仍会回到自动收集的最后一管

收集样品

- 完成收集后，在“LC System Status” - “Collection Bed Status” 查看收集状态
- 对应不同颜色和管号 收起全部样品

Max. Tube Volume: 10 ml

Reserve Tubes ...

Edit Labels

Reset Bed

Reset Tube(s)

Bed Layout Selection: Fraction 120 Test Tubes

Apply

Manual Collection

Waste collected within time window

Waste collected between time window

Fraction 1

Fraction 2

Fraction 3

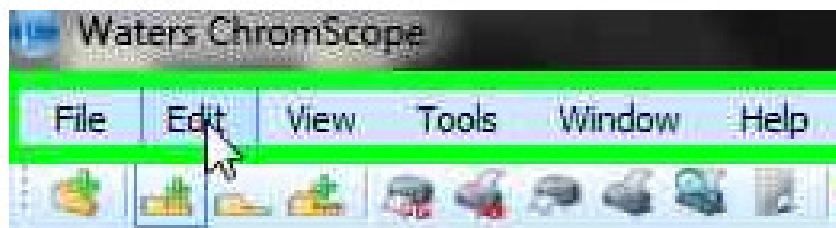
Fraction 4

Injection	Sample	Fraction Location
1	para	4, 5, 6, 7
1	Para	8, 9, 10, 11
2	Para	9, 12, 10, 13, 11, 14

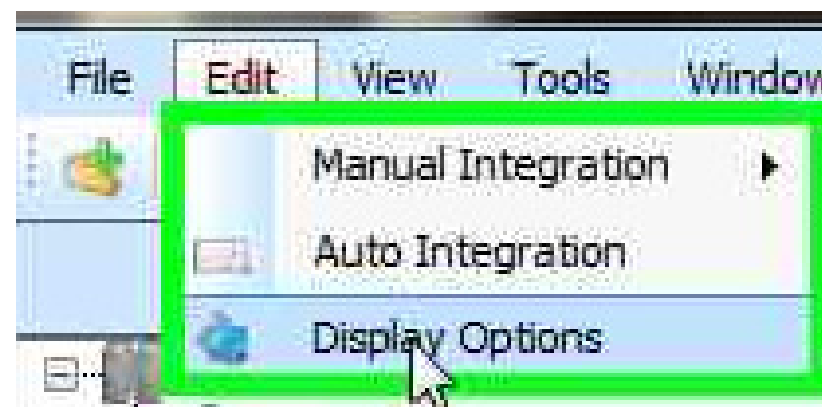
界面显示 数据分析 结果报告

界面显示

- 色谱界面显示设置 在菜单栏点击 “Edit”

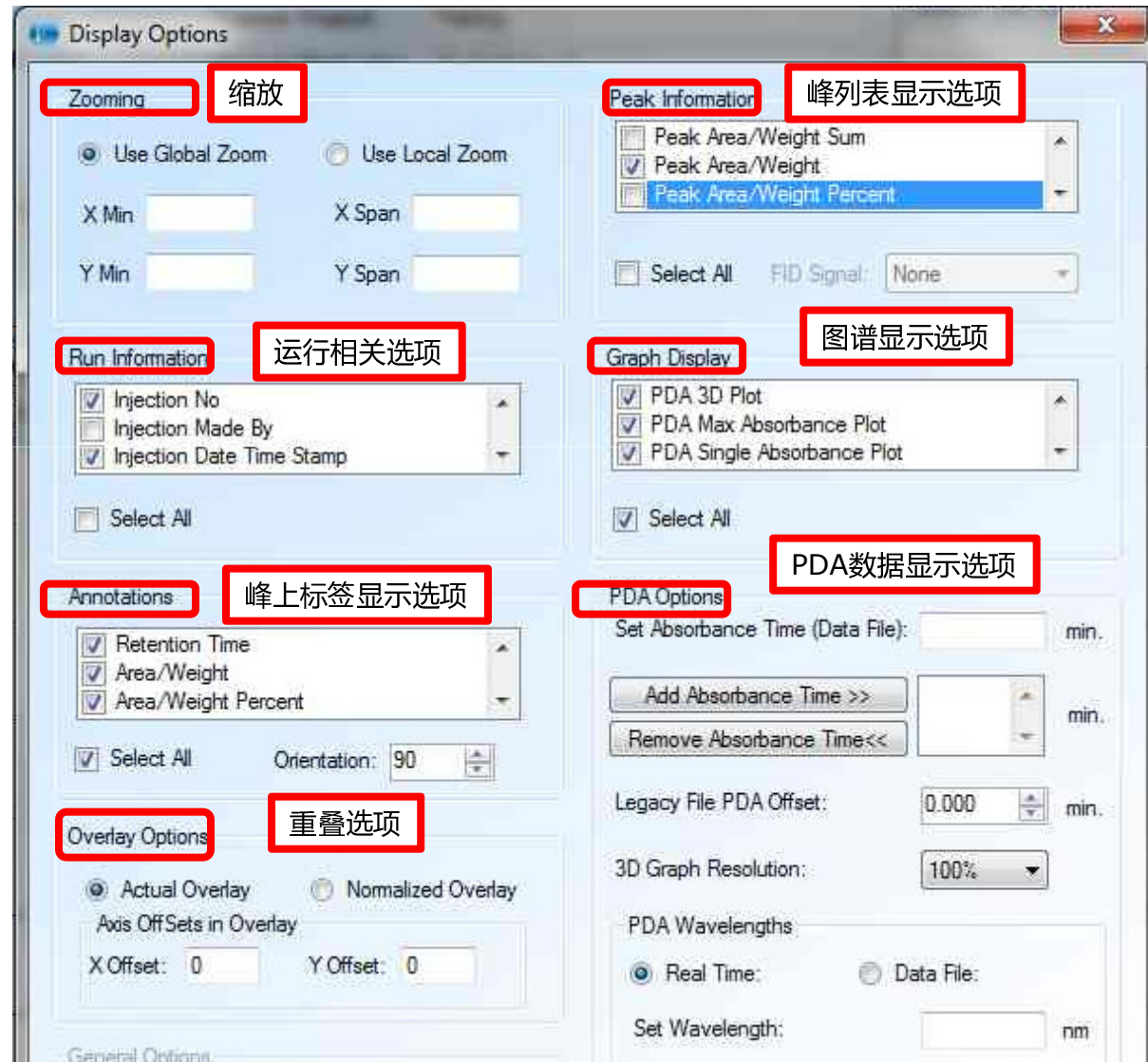


- 选择 “Display Options”



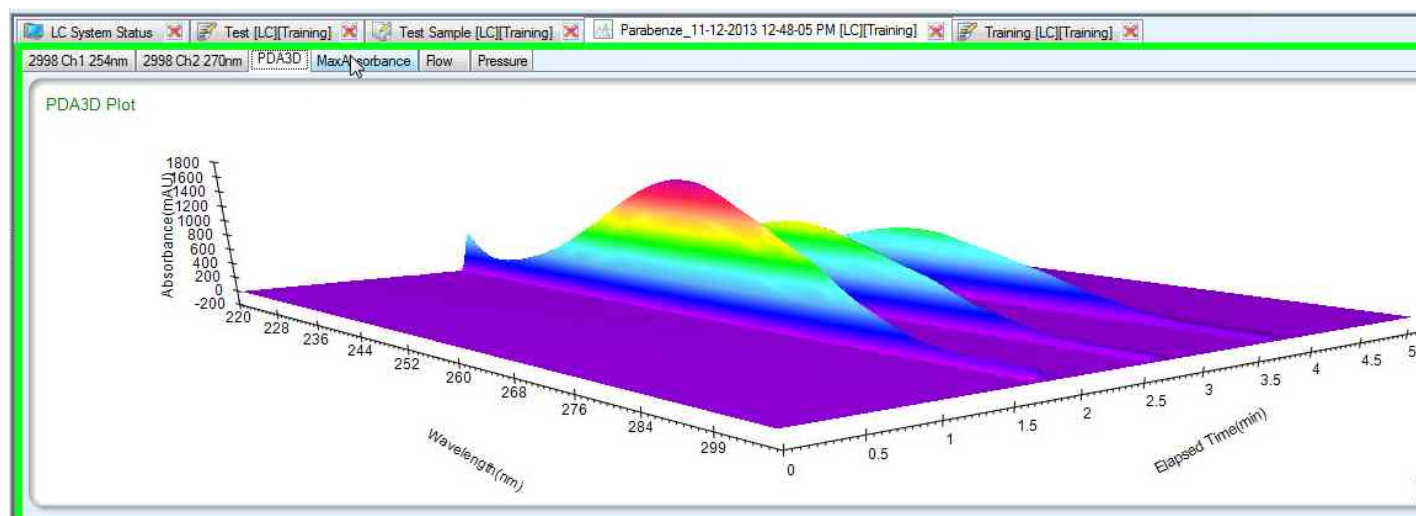
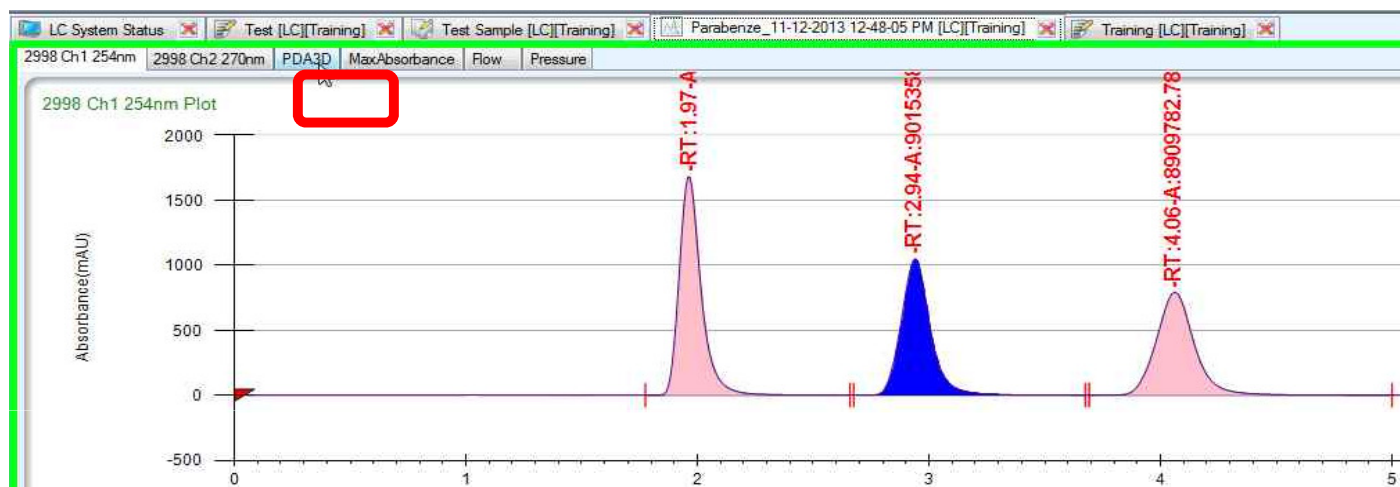
界面显示

- 可根据具体需求，选择不同的显示信息
- 注意色谱图界面的显示调整，不关联报告格式。
(即：报告模板须重新建立，与当前显示状态无关)



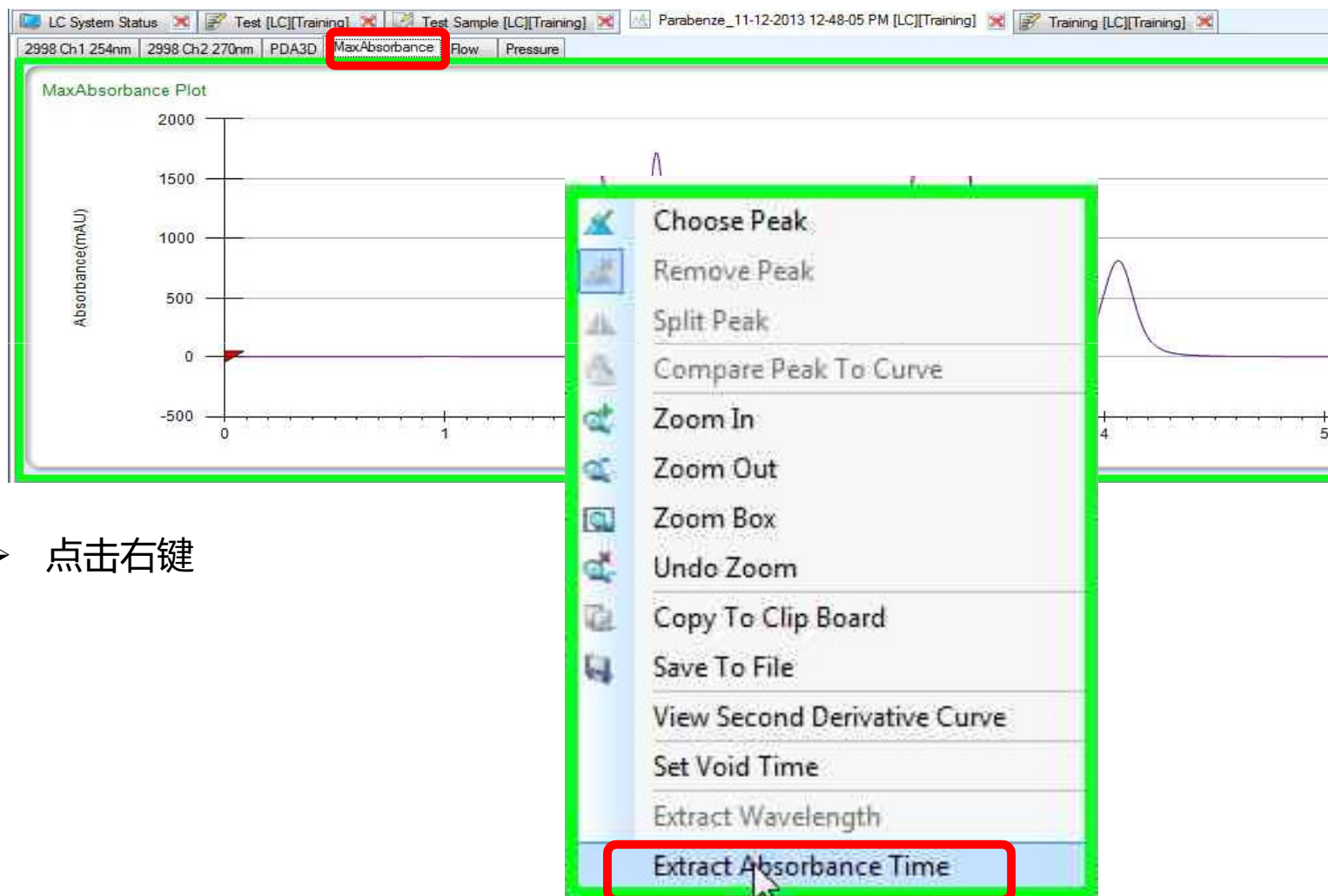
3D数据提取

- 在色谱图界面选择 “PDA 3D”



3D数据提取

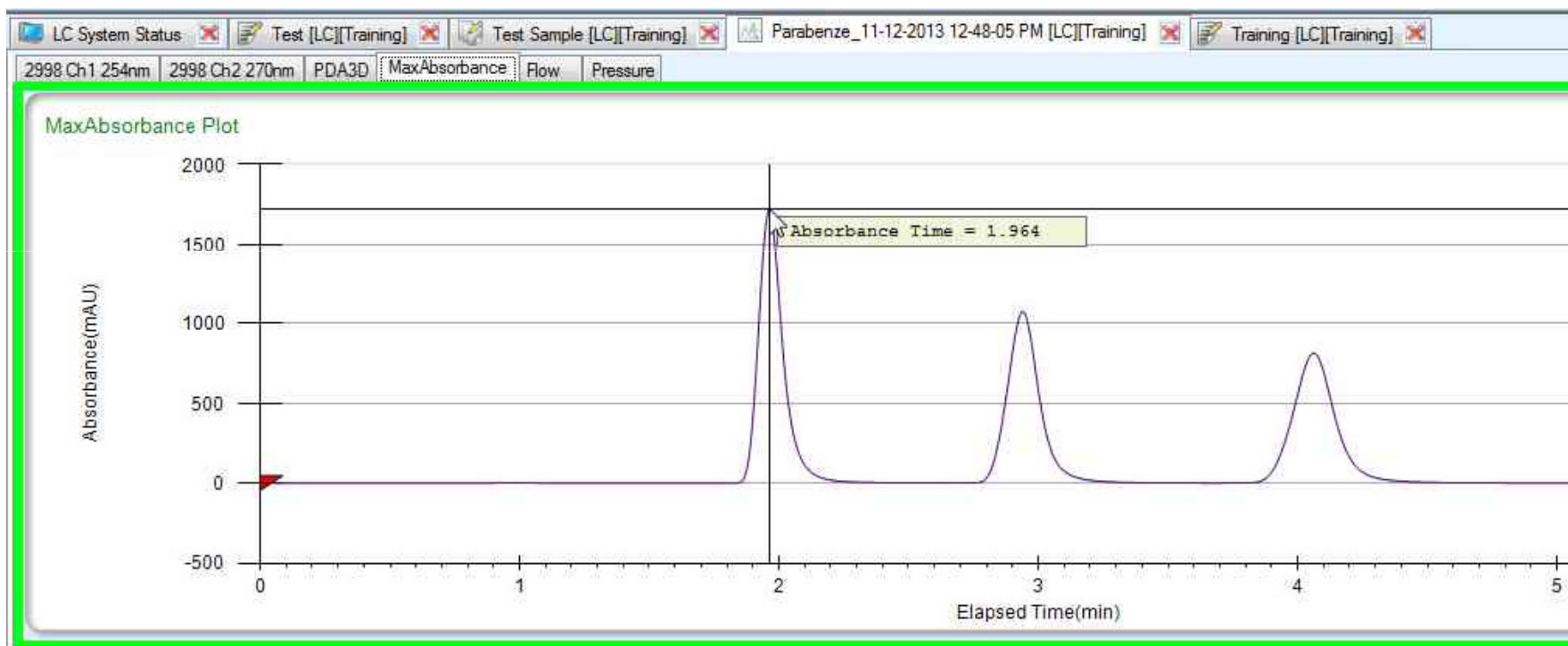
- 在色谱图界面选择 “Max Absorbance”



- 点击右键

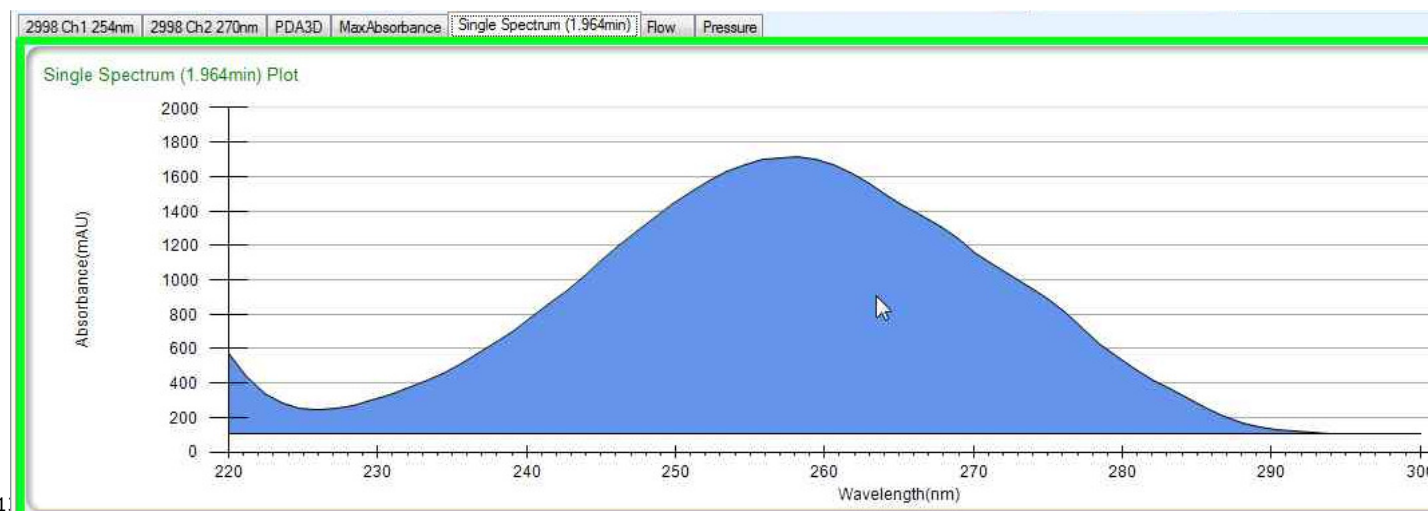
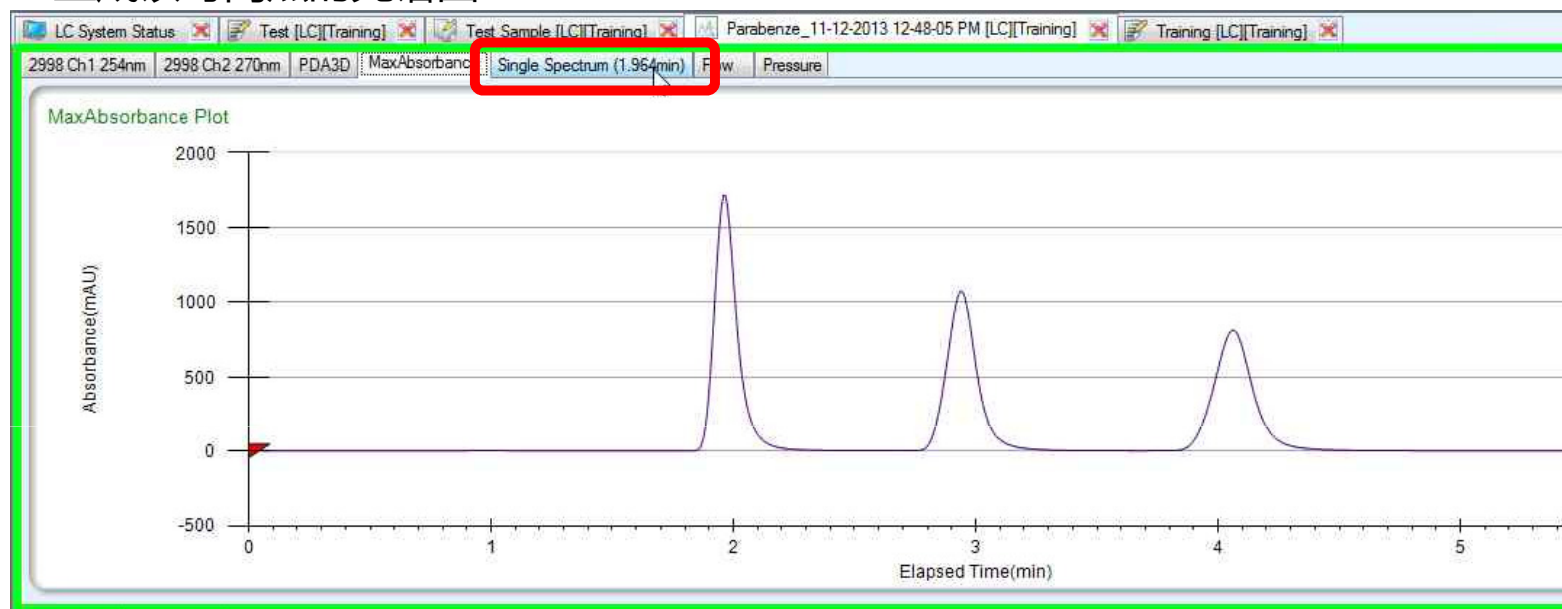
3D数据提取

- 选择色谱峰顶点保留时间



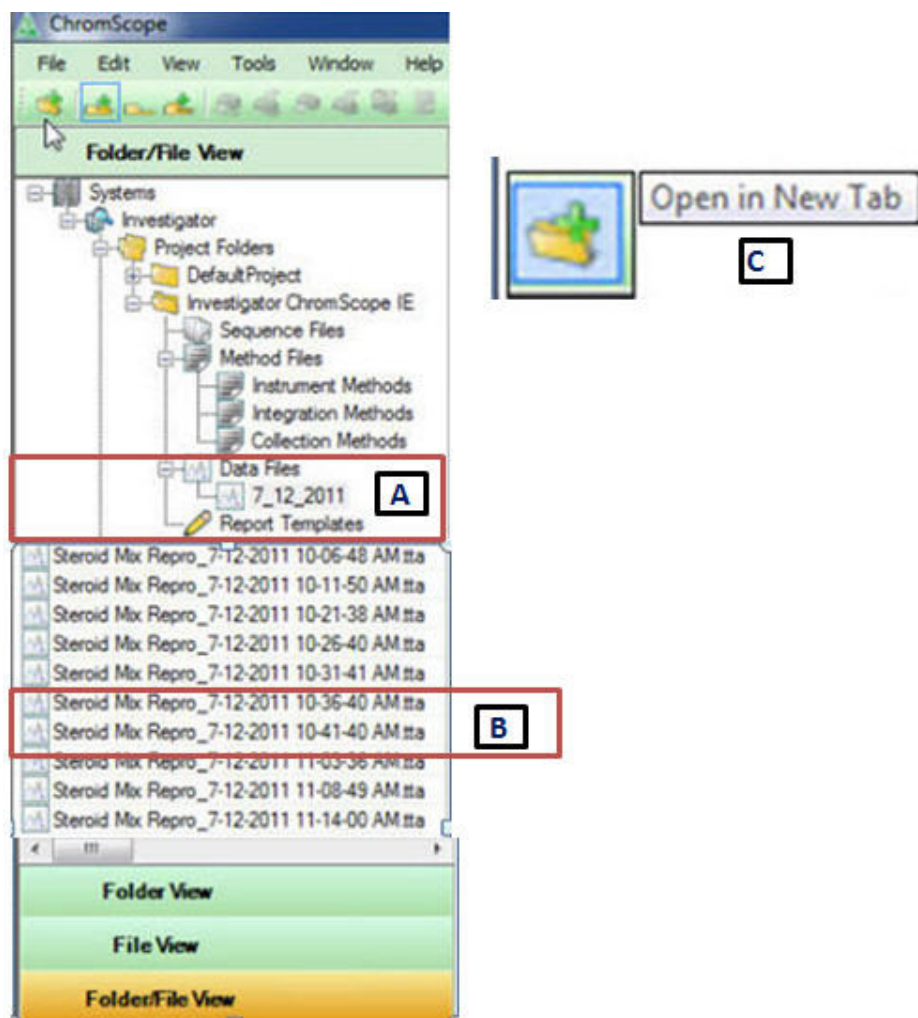
3D数据提取

➤ 生成该时间点的光谱图



积分方法

- 首先，选择待处理的数据，并选择合适的显示方式（请按照A-B-C 三步走）



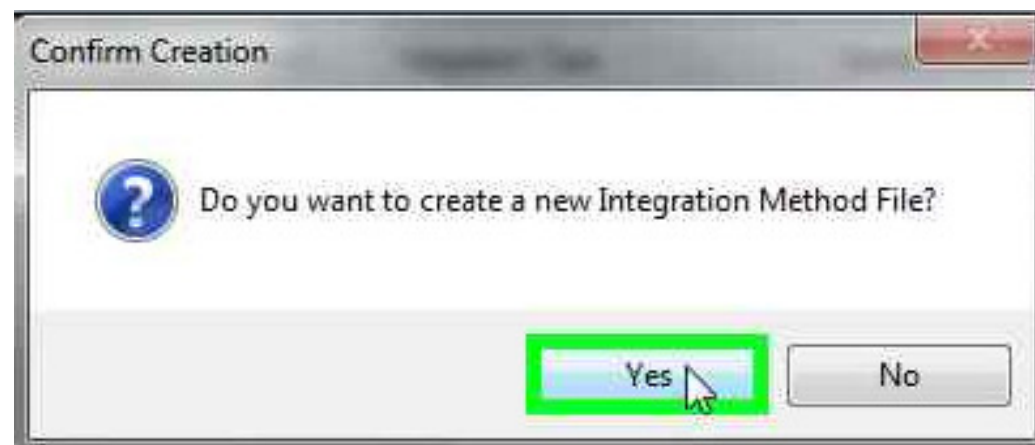
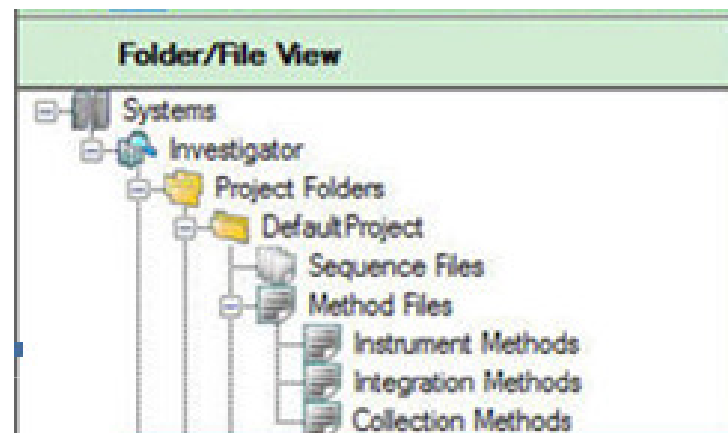
积分方法

- 手动积分：选中手动积分图标，在峰的起点和终点分别点击左键。即可得到峰积分结果



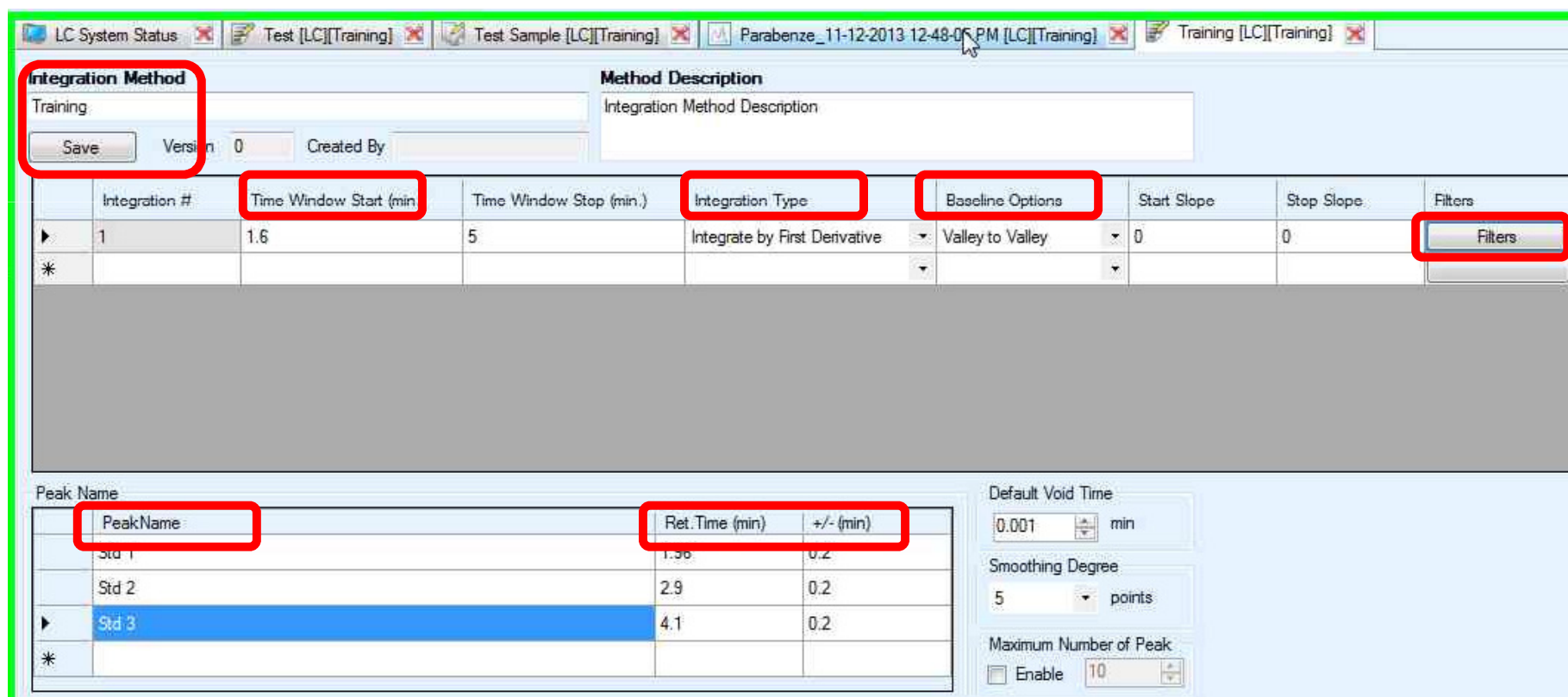
- 自动积分：

- 先在 "Integrate Method" 上点击右键
"Add New Method" ，并点击 "Yes"



积分方法

- 设置合适的积分参数（与其他LC软件类似，具体可参照工程师现场讲解以及Help）
- 命名并保存该方法



The screenshot displays the 'Integration Method' configuration window. The 'Integration Method' section shows the name 'Training' and a 'Save' button. Below this is a table with the following data:

Integration #	Time Window Start (min)	Time Window Stop (min.)	Integration Type	Baseline Options	Start Slope	Stop Slope	Filters
1	1.6	5	Integrate by First Derivative	Valley to Valley	0	0	Filters
*							

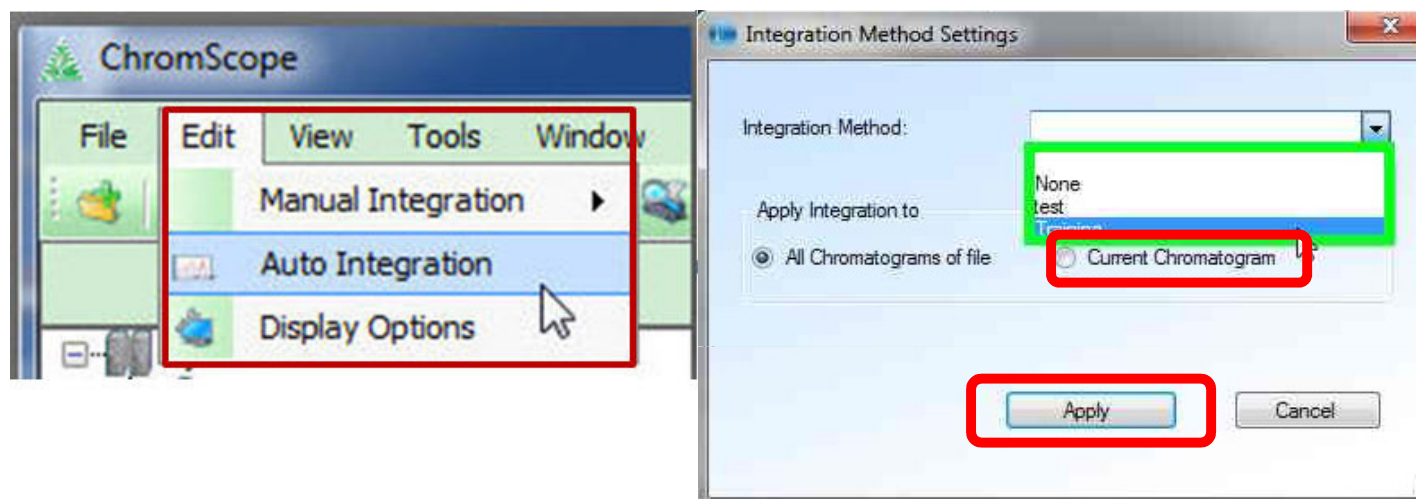
The 'Peak Name' section contains a table with the following data:

PeakName	Ret. Time (min)	+/- (min)
Std 1	1.36	0.2
Std 2	2.9	0.2
Std 3	4.1	0.2
*		

Additional settings on the right include: Default Void Time (0.001 min), Smoothing Degree (5 points), and Maximum Number of Peak (10).

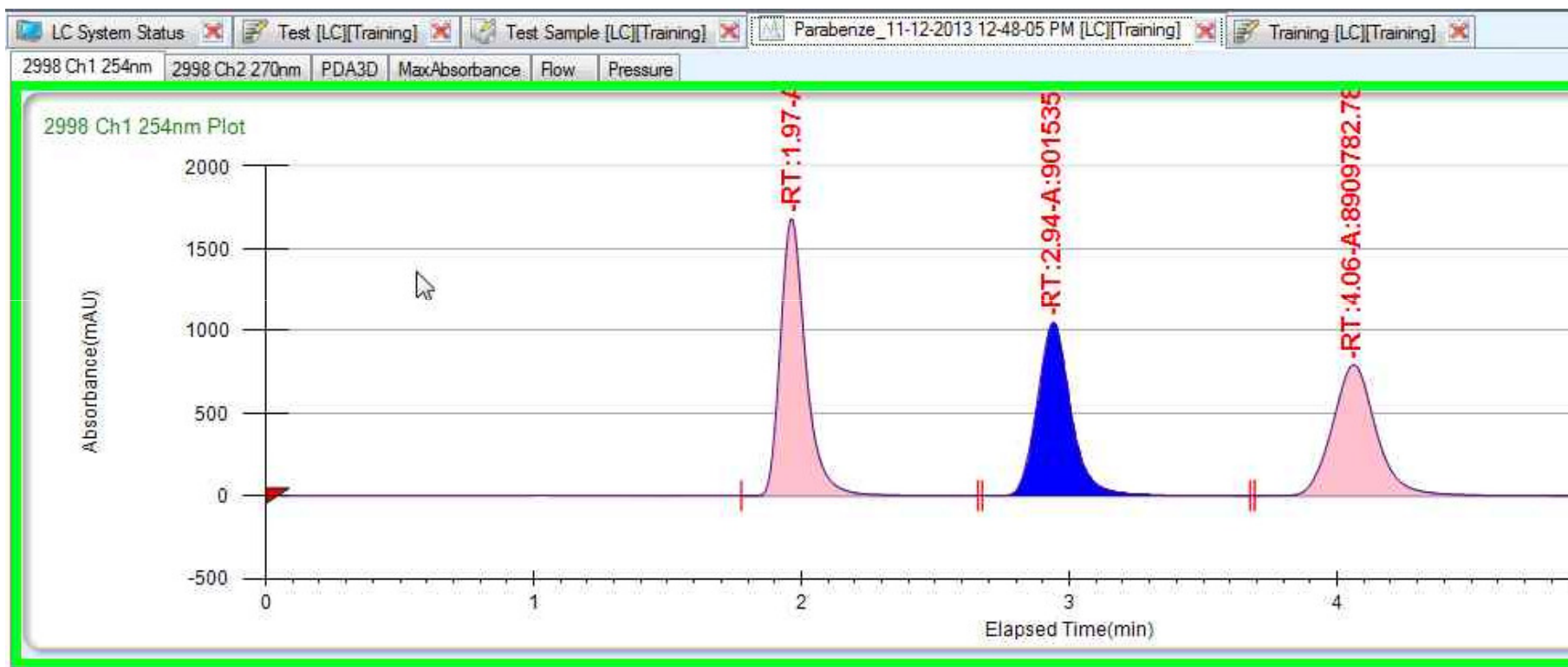
积分方法

- 选择 “Auto Integration”

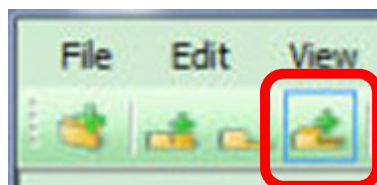


积分方法

- 积分结果会在色谱图以及图下方的表格内显示，具体显示内容由“Display Option”决定

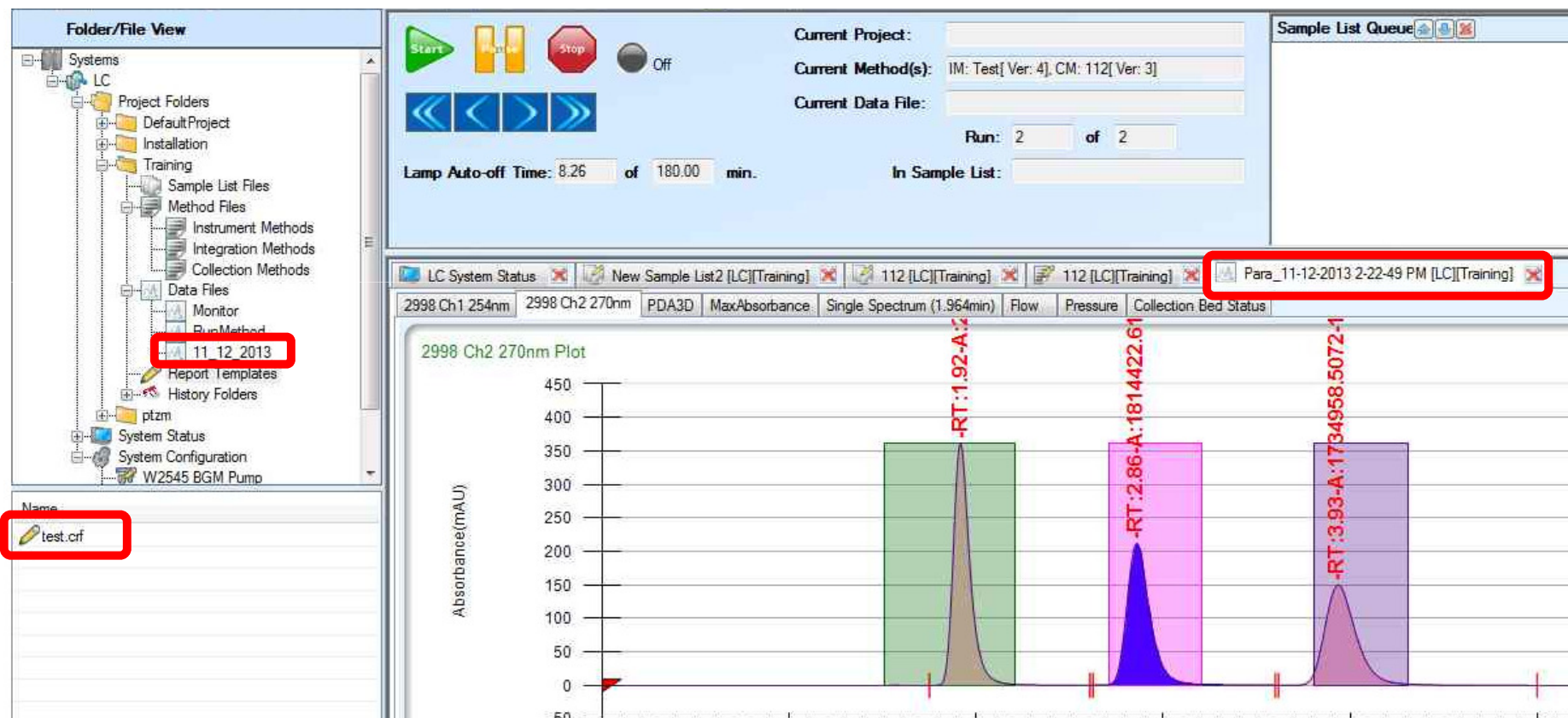


提示：请勿在选择“Overlay”

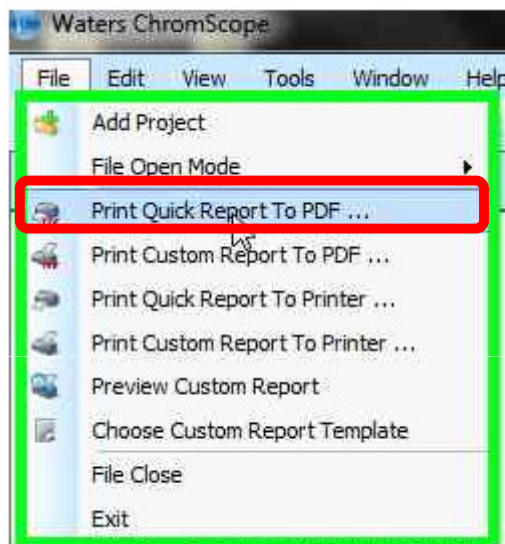


标签的同时积分，无法得到积分结果

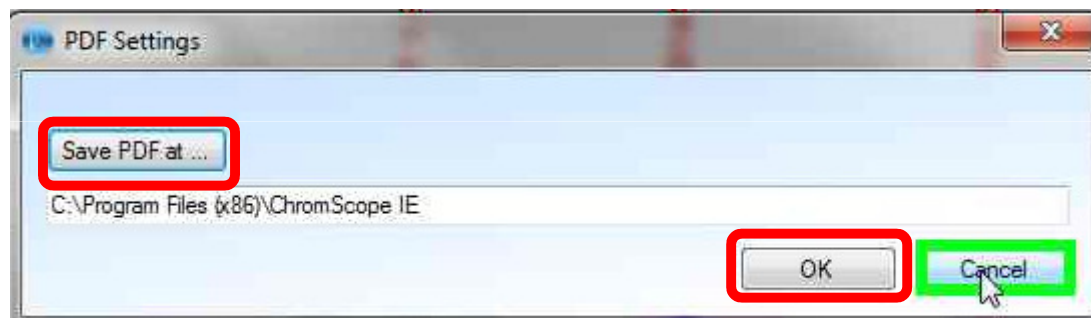
A. 选择所要打印的数据



A. 选择 “Print Quick Report to PDF”



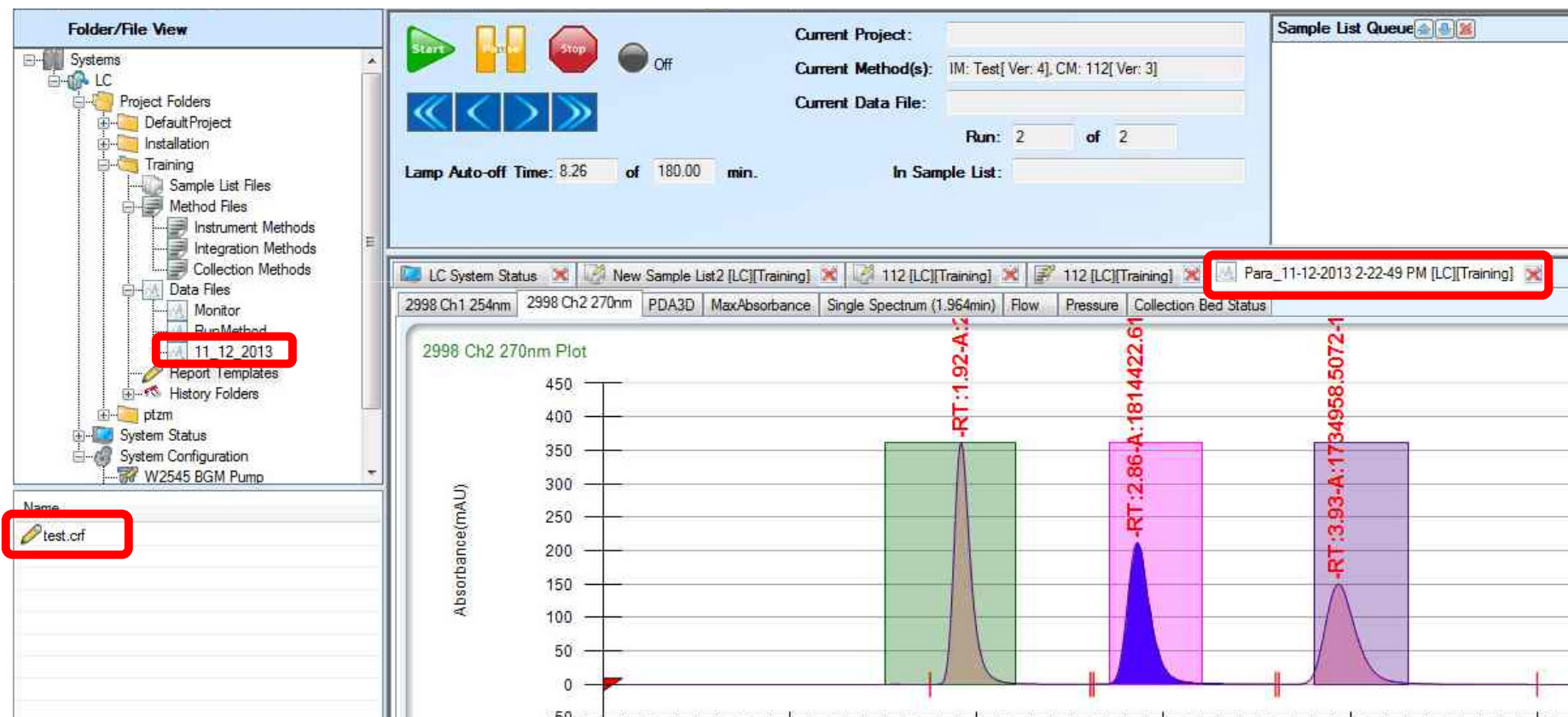
B. 选择存储默认路径或者“Save PDF at ...”



C. 所选数据将选择系统默认模板生成一个PDF文件

报告模板

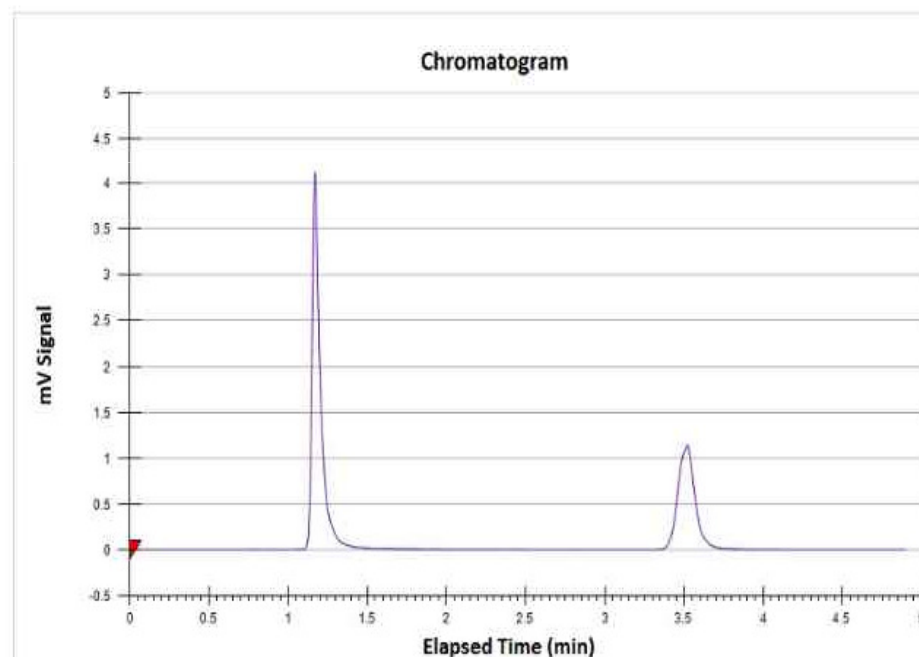
- 如需要自定义模板，请选择 “Report Templates” - “Add New report”



新建报告模板



如图所示：



Peak #	Peak Name	Area/Weight	Area/Weight %	Height	Theoretical Plates	Capacity Factor	Tailing Factor	SN Ratio
[Peak #]	[Peak Name]	[Area/Weight]	[Area/Weight %]	[Height]	[Theoretical Plates]	[Capacity Factor]	[Tailing Factor]	[SN Ratio]

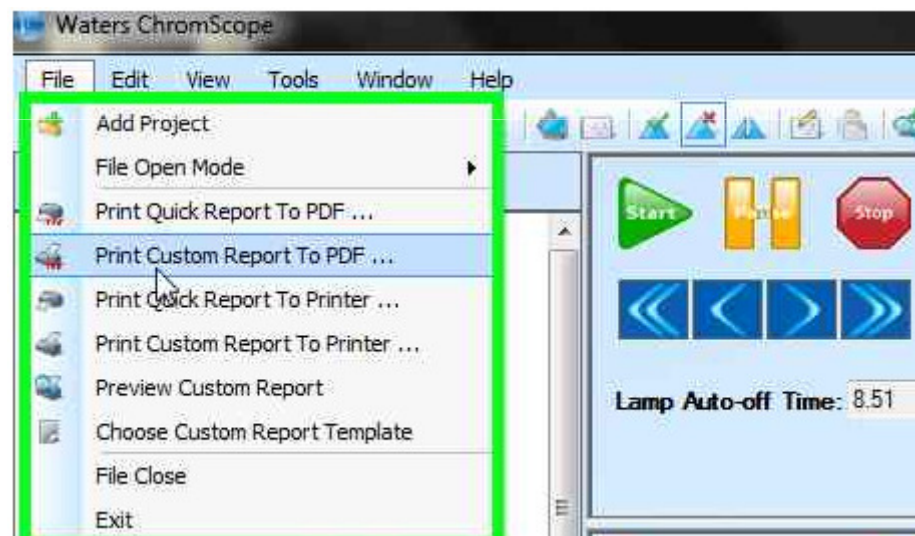
新建报告模板

- 表格组成可通过 鼠标右键 “Remove” 进行调整。内容不可改变

- 将建好的模板进行保存

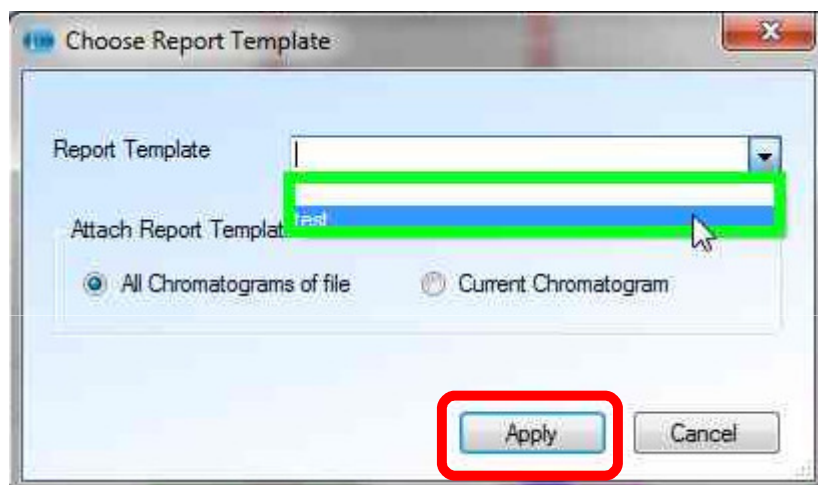


- 如图选择：



新建报告模板

- 在下拉菜单中选择新建立的模板，并点击“Apply”



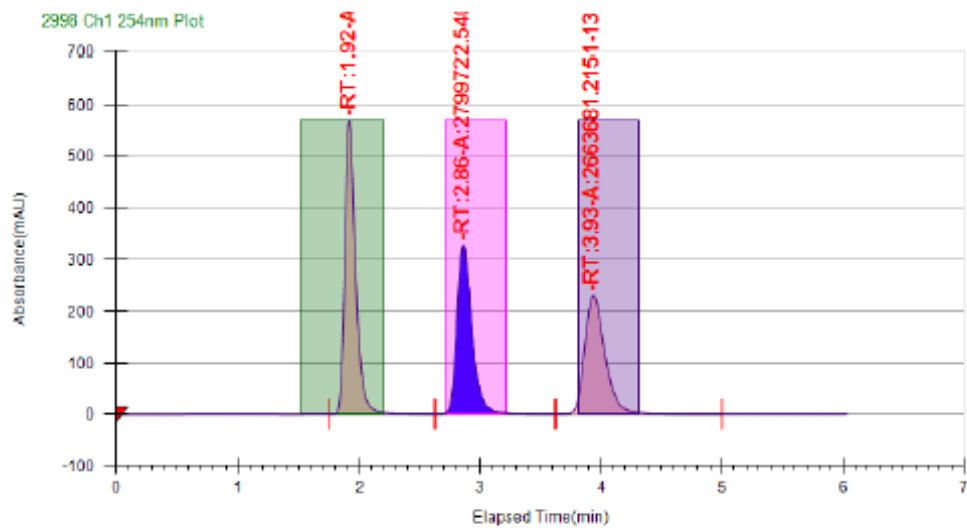
- 指定存储位置，并为PDF报告命名



报告实例



C:\Program Files (x86)\Micro Scope IELC\Projects\Training\Data Files\11_12_2013\Para_11-12-2013 2:22:49 P.M.mta



General Information

Log Author	Log Date	Report By	Report Date	Notes
Administrator	11/12/2013 2:22:49 PM	Administrator	11/12/2013	

Run Information

Instrument/Method	Inj. Vol.	Sample	Vial Location	Row	Pre course
Test1	100	Para		15	916

Peak Information

Peak No	%Area	Area	Ret Time	Height	Cap. Factor
1	38.1288	2512221.8596	1.92 min	569.3483	1915.6667
2	31.191	2156722.5403	2.86 min	327.7889	2367.3333
3	26.6754	2563881.2151	3.93 min	228.816	3332.3333

Waters Corporation



Method Screening Stacked Injection

注：以上两功能更适合于配置内含有自动进样器的用户

Method Screening

功能介绍 - 适用于方法开发，类似于正交实验。同一样品使用不同的柱子以及流动相进行分析分离

具体操作 - 使用 Sequence Wizard

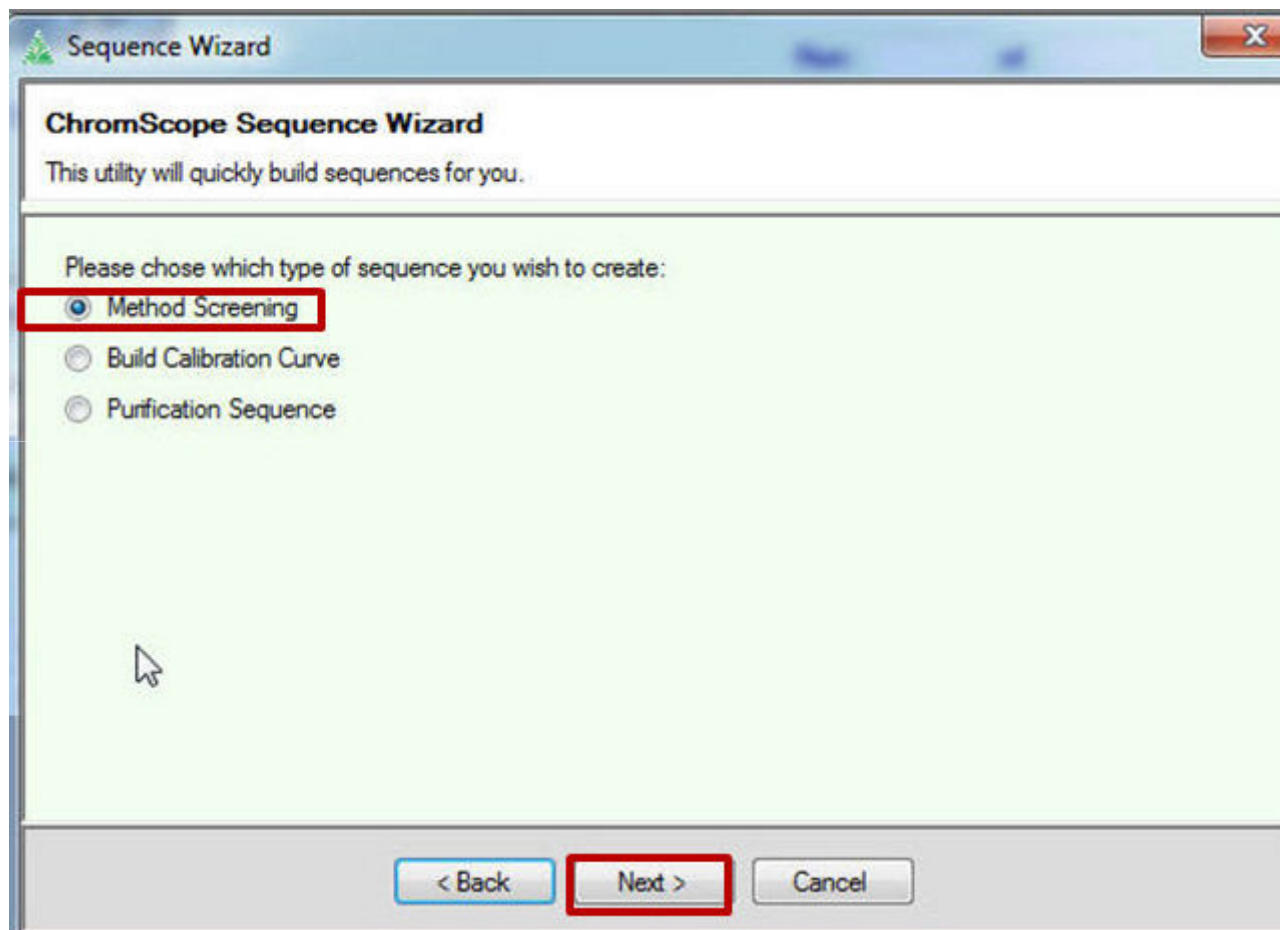


The screenshot shows the Waters software interface for method development. The top bar includes tabs for 'Method Station System Status', 'System Configuration', 'SAT SEQ [Method Station][SAT test]', and 'SAT test [Method Station][SAT test]'. Below the tabs, the 'Sequence Name' field contains 'SAT SEQ' and 'Version' is set to '1'. There are buttons for 'Save', 'Add to Queue', and 'Sequence Wizard'. The 'Sequence Wizard' button is highlighted with a red box and a red arrow. Below the buttons is a table with columns for 'Inj. #', 'Run. Dur. (min.)', 'Inj. Delay (min.)', 'Instrument Method', 'Data File', 'Inj. Loc.', 'Sample Name', 'Injection Volume (ml)', and 'Solvent'. The table contains two rows of data:

Inj. #	Run. Dur. (min.)	Inj. Delay (min.)	Instrument Method	Data File	Inj. Loc.	Sample Name	Injection Volume (ml)	Solvent
1	6	0	SAT test	Sample Name And D...	1,1.A	blank	10	methanol
2	6	1	SAT test	Sample Name And D...	1,1.B	steroid	10	Use Method Defa...



Method Screening



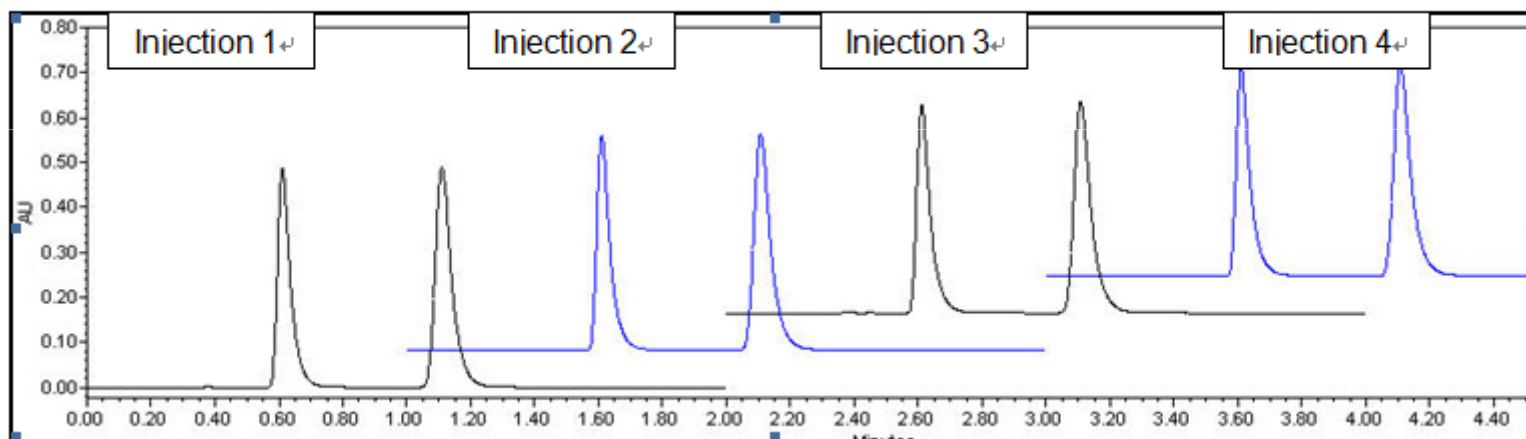
Method Screening

➤ 设置界面

- 依次选择样品、方法、溶剂以及色谱柱
- 设置其他共性参数

Stacked Injection

- 功能介绍 - 采用序列进样的方式，在第一次进样结束之前进行第二次进样，在第二次进样结束之前进行第三次进样，以此类推。从而大大缩短大制备量样品的采集时间，并有效地节约了溶剂。



Stacked Injection

具体操作 - 使用 Sequence Wizard

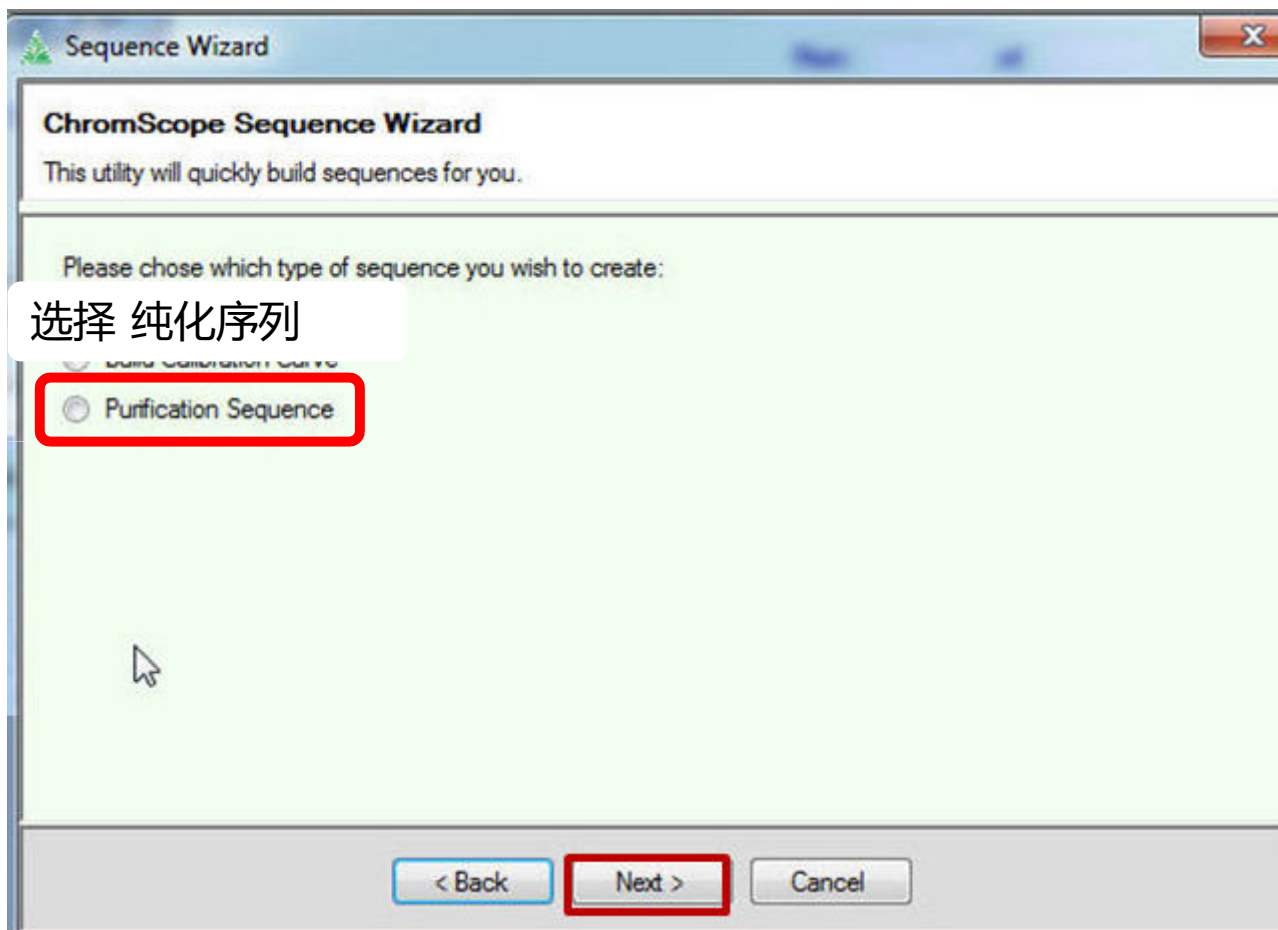


The screenshot shows the Waters software interface for configuring a sequence. The window title is "SAT SEQ [Method Station][SAT test]". The "Sequence Name" field contains "SAT SEQ" and the "Version" is "1". There are buttons for "Save", "Add to Queue", and "Sequence Wizard". The "Sequence Wizard" button is highlighted with a red box and a red arrow. Below the buttons is a table with columns: Inj. #, Run. Dur. (min.), Inj. Delay (min.), Instrument Method, Data File, Inj. Loc., Sample Name, Injection Volume (ml), and Solvent. The table contains two rows of data.

Inj. #	Run. Dur. (min.)	Inj. Delay (min.)	Instrument Method	Data File	Inj. Loc.	Sample Name	Injection Volume (ml)	Solvent
1	6	0	SAT test	Sample Name And D...	1,1.A	blank	10	methanol
2	6	1	SAT test	Sample Name And D...	1,1.B	steroid	10	Use Method Defa...



Stacked Injection



Stacked Injection

➤ 设置通用参数

➤ 设置特殊参数

- Cycle Time
- Spacing
- Total elution time

Sequence Wizard

ChromScope Sequence Wizard

This utility will quickly build sequences for you.

Purification Sequence Settings:

Instrument method: * Basic Start of Investigator w

Collection method: * Fraction Colelction Steroids

Number of injections: * 4

Injection volume: * 20 uL

Samples Locations: * 1.5.A

Column: * Column 4

Solvent: * Methanol

Cycle Time: * 4.00 min

Spacing: 0.00 min

Total elution time: * 4.00 min

Equil time before first injection: 1.00 min.

Data file name template: Sample Name And Date/Tin

Report template: Built in Quick Report format

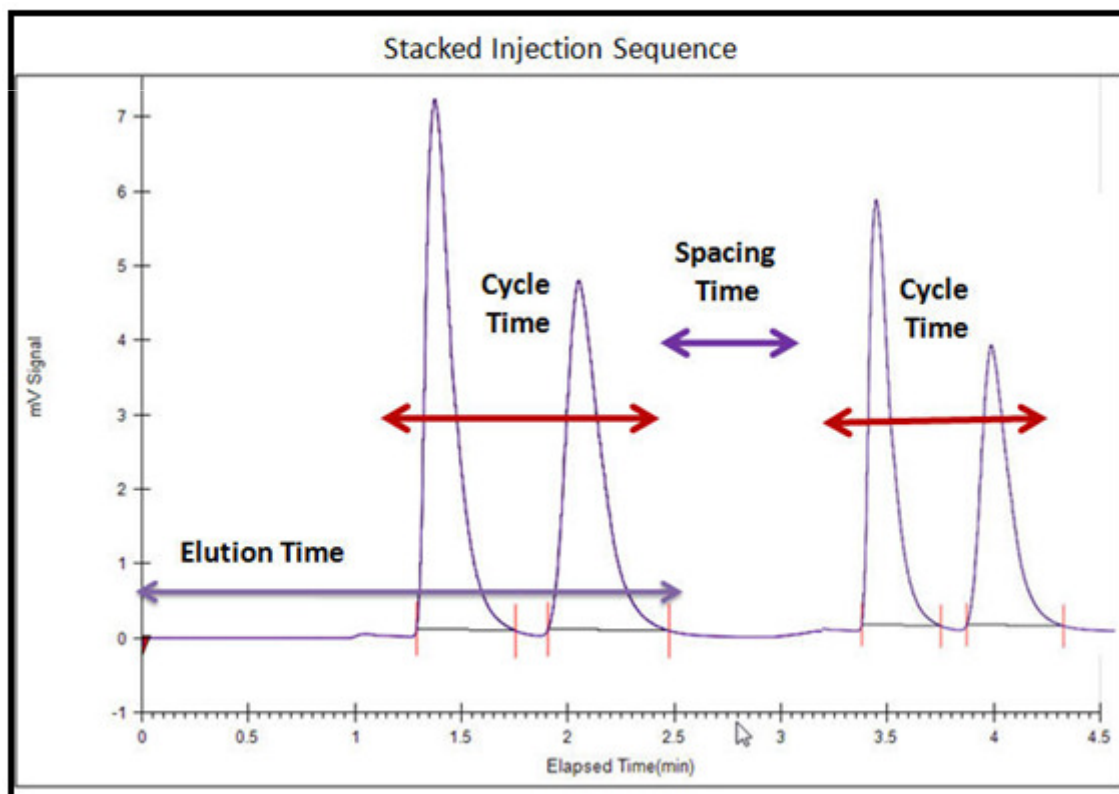
User notes:

< Back OK Cancel

Stacked Injection

➤特殊参数

- Cycle Time 被收集组分的起峰时间点至最后一个收集组分的落峰时间点之间的时间间隔
- Spacing Stacked 进样峰之间的时间间隔
- Total elution time 单次进样的运行时间





Thanks

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™