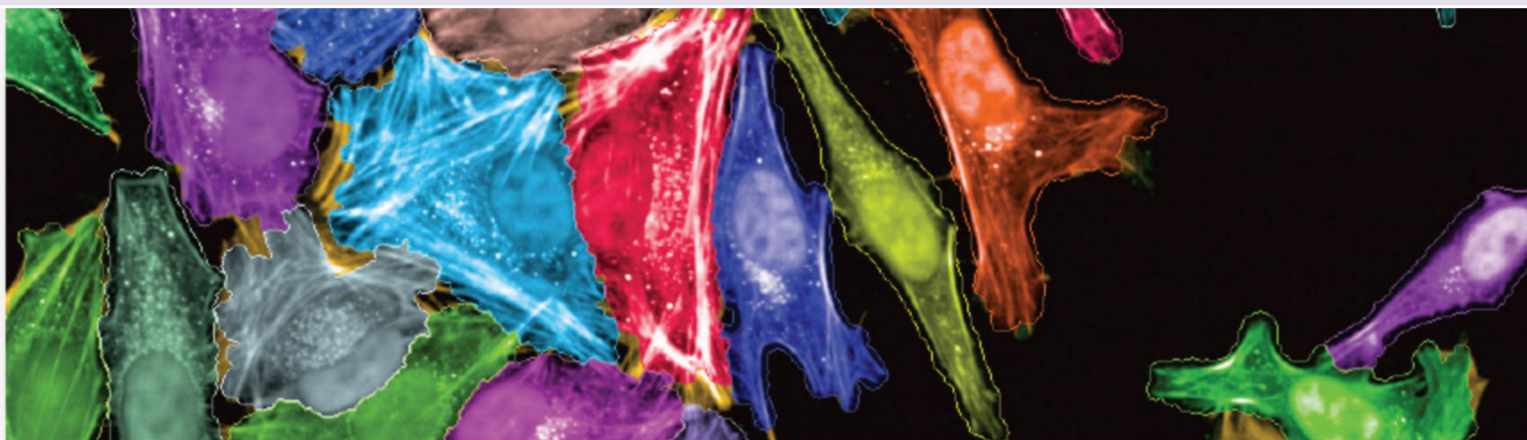


revvity

Opera Phenix Plus 操作指南



For Research Use Only.
Not for Use in Diagnostic Procedures.

操作指南

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Chapter 1: 图像采集

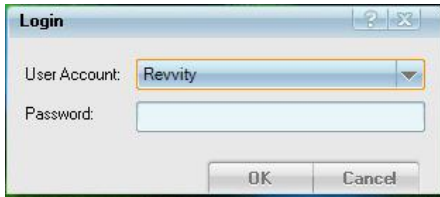
1. 开启 Opera 与 Harmony

1.1 打开电源开关：依次为电脑、Opera 主机；

1.2 进入 Window, 用户名: Harmony, 密码: Harmony;

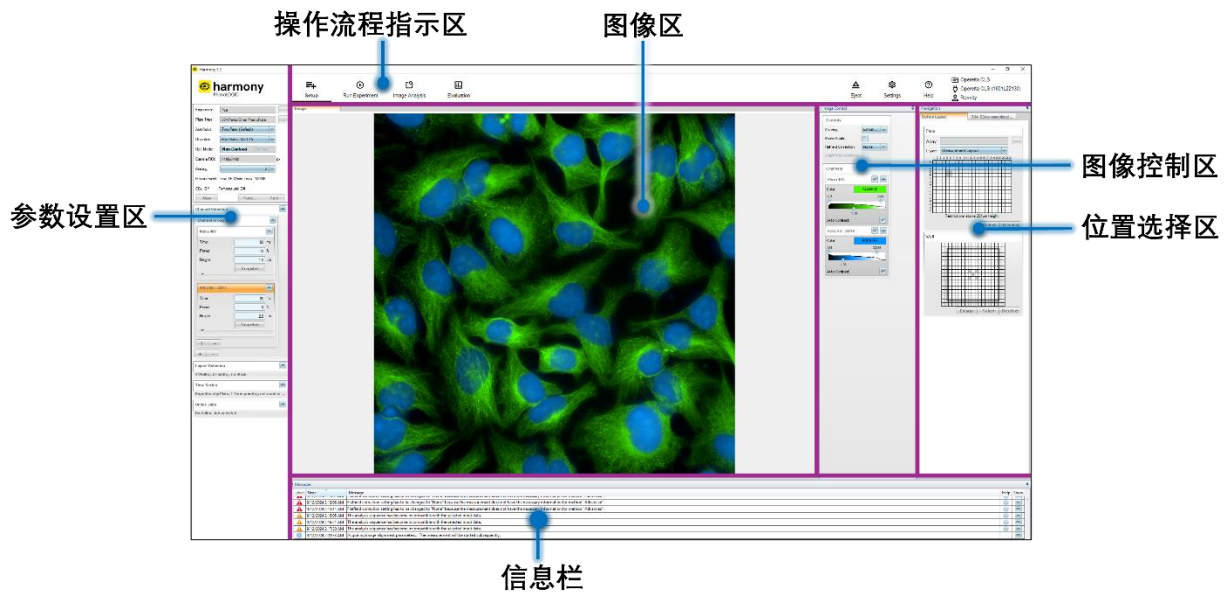


1.3 双击桌面上的 Harmony 图标，

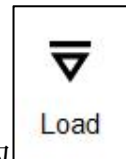
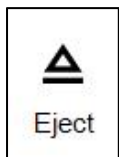


1.4

密码：与用户名一致（大小写也要一致）

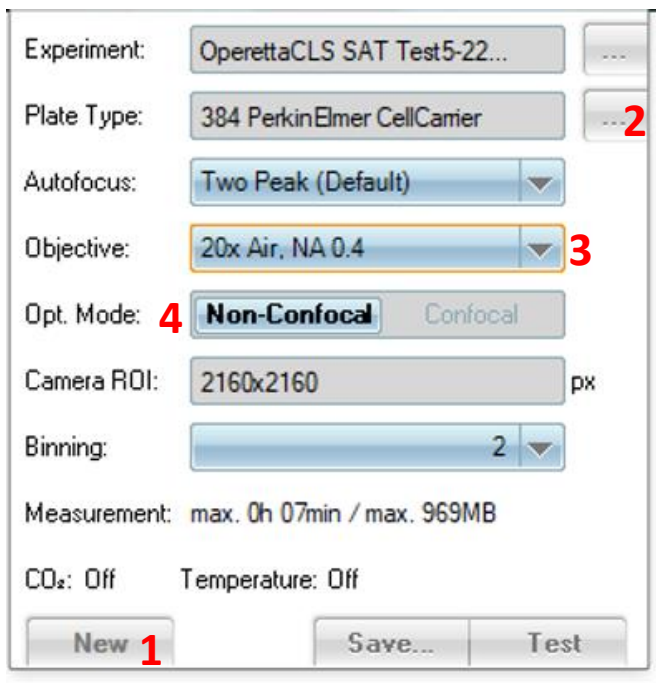


2. 设置拍摄条件



2.1 点击 Eject，等待主机载物台升起，图标变为 Load，放入待测细胞板或玻片，点击 Load，载物台载入样品；

2.2 点击操作流程指示区的 ，设置图像采集条件：



步骤 1: 点击按钮“new”，消除上一次实验的信息；

步骤 2: 点击“...”，选择测试细胞板的品牌规格；

步骤 3: 点击下拉箭头，选择物镜倍数；

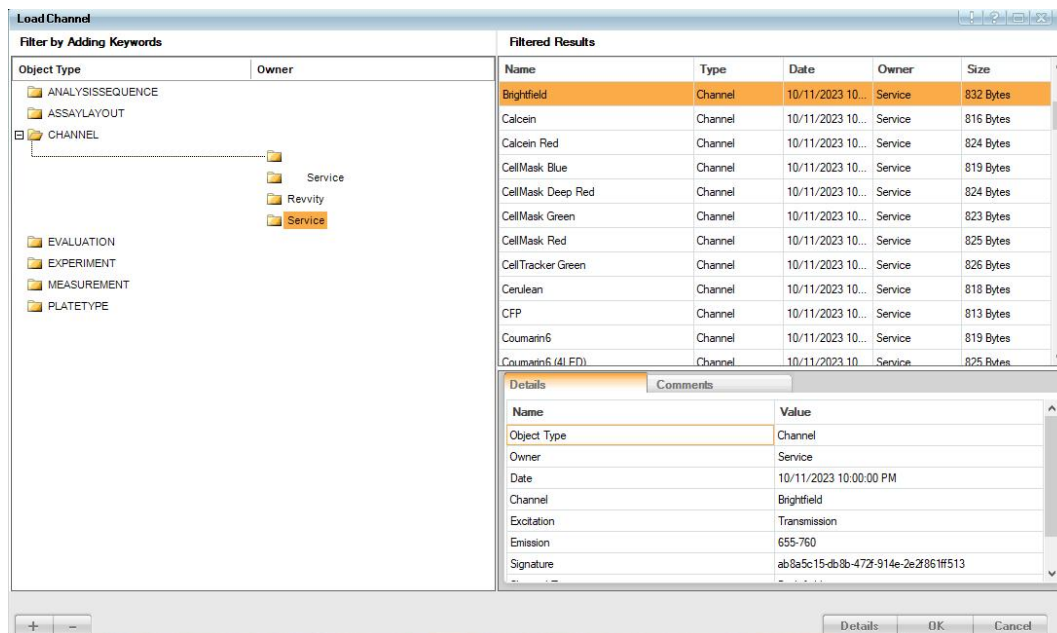
步骤 4: 选择是否 confocal 模式。

2.3 设置图像荧光/明场通道

2.3.1 点击“Channel Seletion”右侧下拉箭头，在“Channel Group1”右侧点下拉箭头，再点击“+”

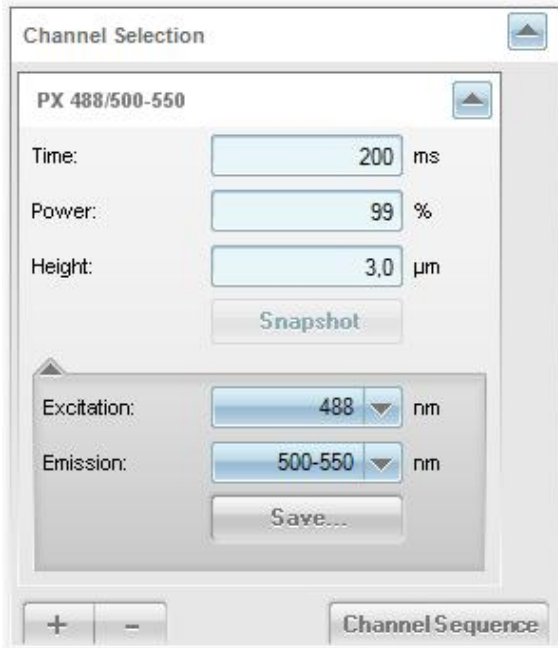


2.3.2 弹出 Load Channel 窗口，选择待测板中的荧光通道；注：明场为 Brightfield.

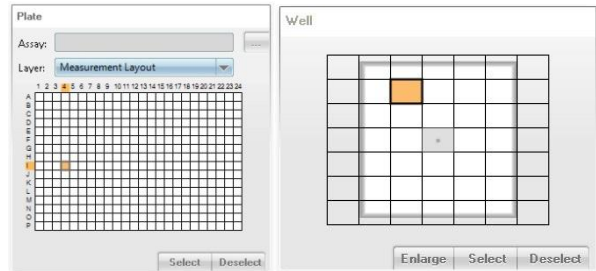


2.3.3 点击 **OK**，重复以上步骤，选取所有荧光通道；

2.3.4 所选荧光通道显示在参数设置区，

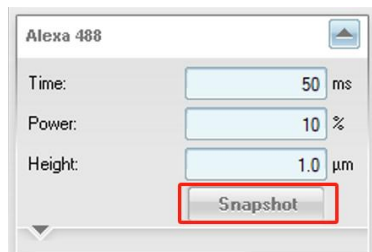


2.3.5 在屏幕中 plate 窗口点击选择一个孔(显示橙色), 在 well 窗口点击



选择一个视野 (显示橙色),

2.3.6 在 “ Channel Selection ” 窗口点击某一通道的 “ Snapshot ”



, 所采集的预览图像出现在屏幕中心位置。



2.3.7 打开图像控制区 channels 的下拉箭头，该曲线提示上述抓取图片的荧光信号强度分布。实际荧光强度可以在图片上任意位置右键选中 **show intensity**，后左键点选需要查看的像素点，可以看到真实强度。

2.3.8 调整 **time**(曝光时间), **power**(光源强度) 及 **height**(聚焦高度, 见 2.5), 保证一定好的信噪比同时没有过曝 (图片 **show intensity** 不超过



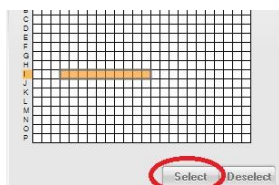
65535)

以获取清晰图像;

2.3.9 在 **well** 窗口选择另外的孔, 点击 **Snapshot** 采集另一张图片, 共采集 3-5 个代表性的孔, 确定整板最佳曝光时间, 光源强度与聚焦高度, 手动输入到 **time**, **power** 和 **Height** 后面的框中。

2.4 选择要拍摄的孔与视野

2.4.1 在屏幕右侧的 “plate” 窗口用鼠标选中要拍摄的孔,

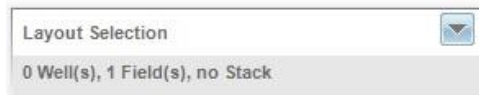


点击 “select”, 被选中的孔变灰色

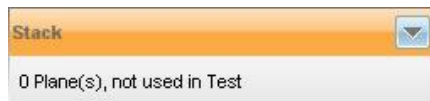
2.4.2 在“**Well**”窗口用鼠标选中要拍摄的视野，点击“**select**”，被选中的视野变灰色

2.5 设置多层拍摄(如需要三维图像)，注：可使用此功能寻找合适聚焦高度
(Height)

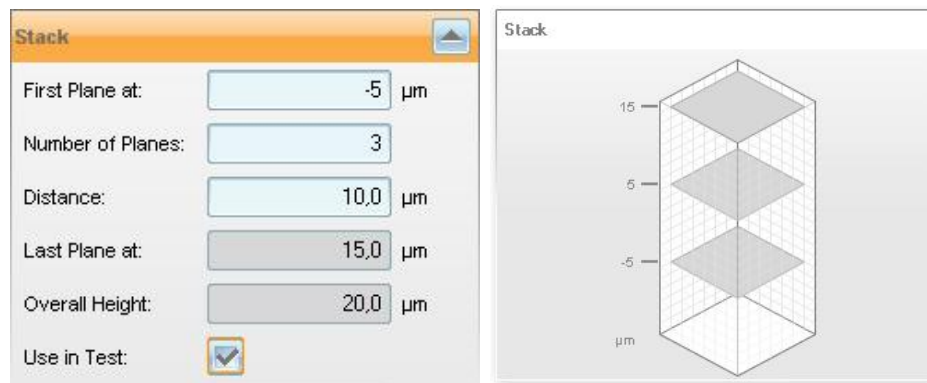
2.5.1 点击“**layout selection**”右侧下拉箭头



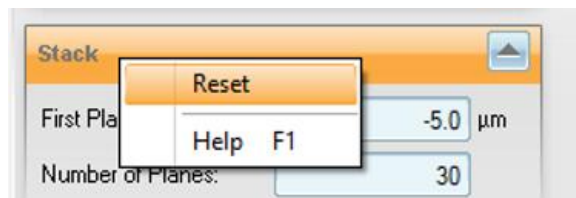
2.5.2 点击“**stack**”右侧下拉箭头




2.5.3 设定层扫范围(first plane)、层数(number of stack)及层高(distance)
(该步骤如果用于找焦设置 height，推荐参数为，第一层-10um，层数 20，距离 1um。注意找完焦面后填入 2.3.8 中的 height 参数处。)



如果只是找焦面，则在 **Stack** 橘色窗口处点击右键，再点击 **reset** 对多层设置进行归零



2.5.4 点击“Test” ，在“test image”区域，用鼠标点击不同层高，查看图像质量。

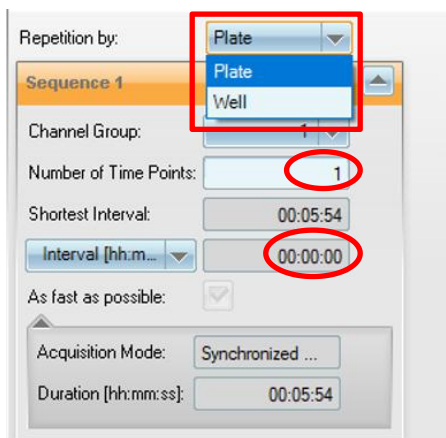
2.6 设置时间系列参数（如需要活细胞长时间拍摄，无需要可跳过）


2.6.1 点击下拉箭头



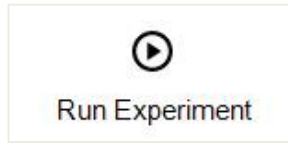
2.6.2 选择以 plate 为单位重复，还是以 well 为单位重复。然后分别填写拍摄的时间点与拍摄间隔

（number of Time Points 代表拍摄多少次重复，设置大于 1 的重复之后，将 As fast as possible 取消勾选）



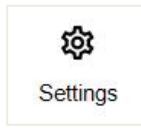
2.7 点击“save”存储实验条件 ，命名实验名称，点击“OK”，保存成功。

3. 图像采集与图像保存

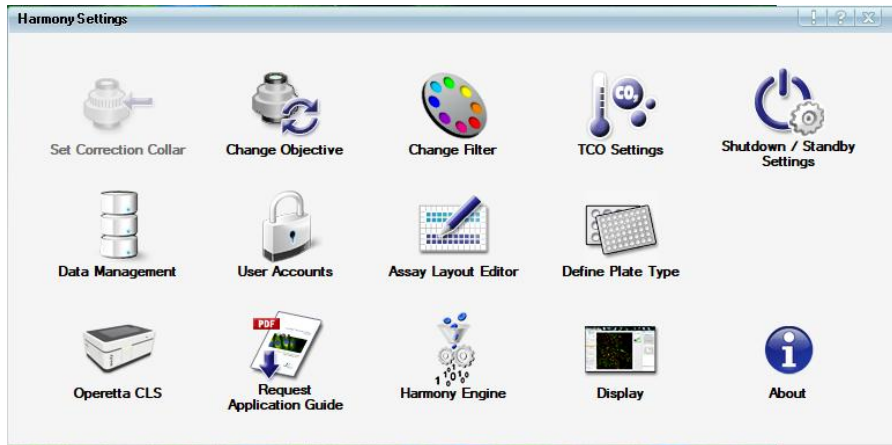


3.1 点击操作流程指示区的

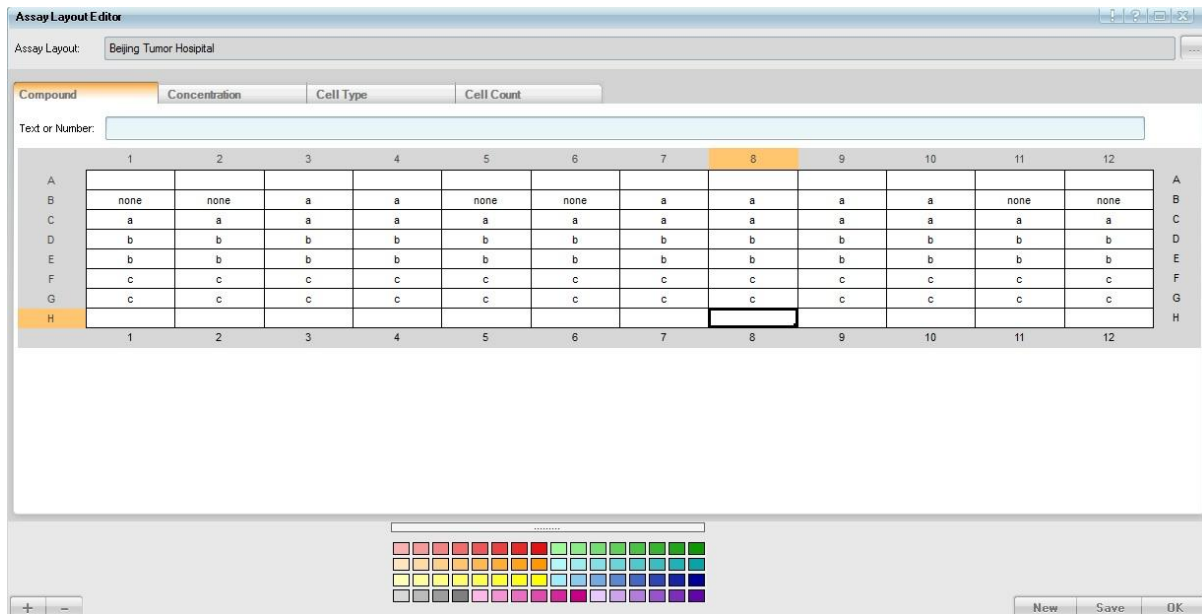
3.2 (可忽略步骤) 输入细胞板的药物浓度或细胞类型等信息



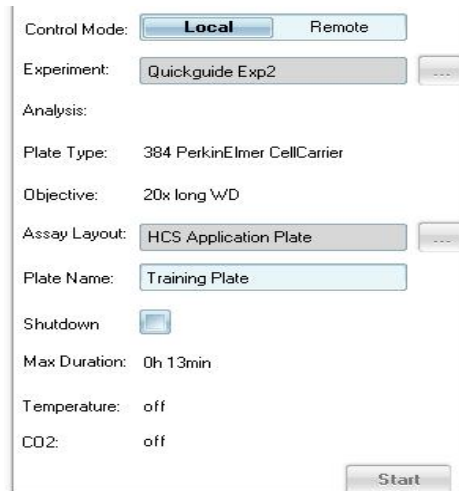
3.2.1 点击 **Settings**，在弹出窗口中选择“Assay Layout Editor”



3.2.2 在弹出窗口中输入实验信息，点击“save”，命名并保存。



3.2.3 点击 “ assay layout” 右侧的 ，选取刚才保存的板位信息。

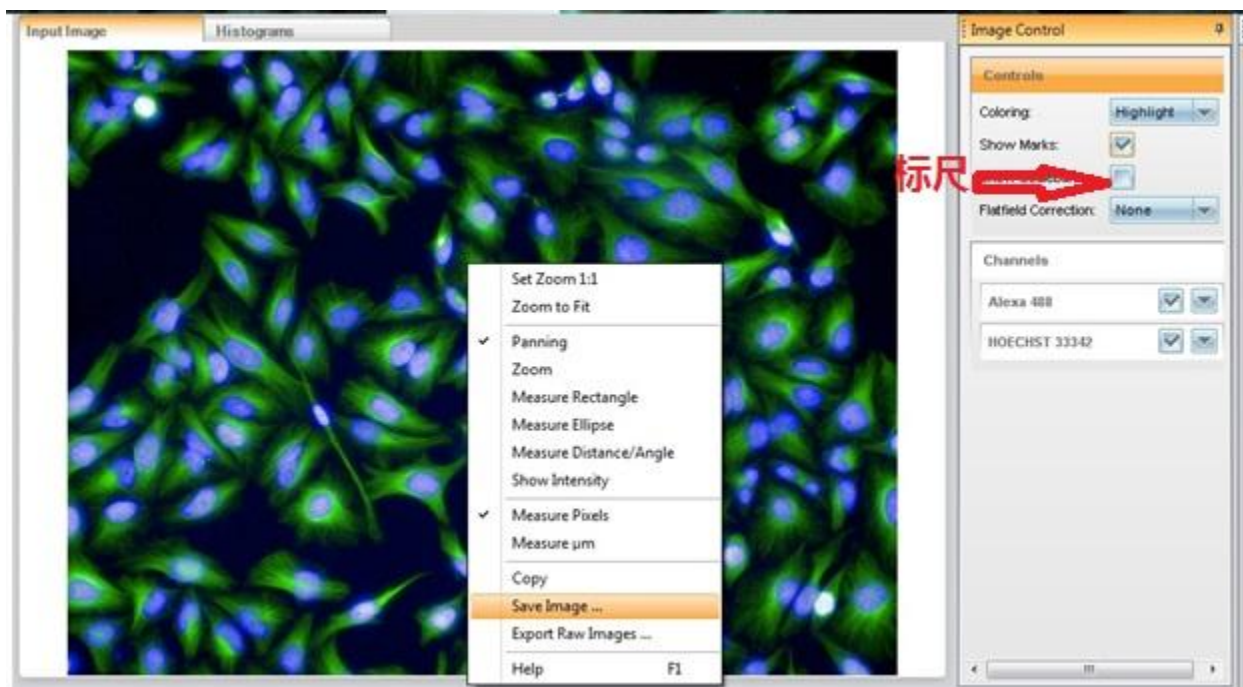


Control Mode:	<input checked="" type="radio"/> Local	<input type="radio"/> Remote
Experiment:	Quickguide Exp2	
Analysis:		
Plate Type:	384 PerkinElmer CellCarrier	
Objective:	20x long WD	
Assay Layout:	HCS Application Plate	
Plate Name:	Training Plate	
Shutdown	<input checked="" type="checkbox"/>	
Max Duration:	0h 13min	
Temperature:	off	
CO2:	off	

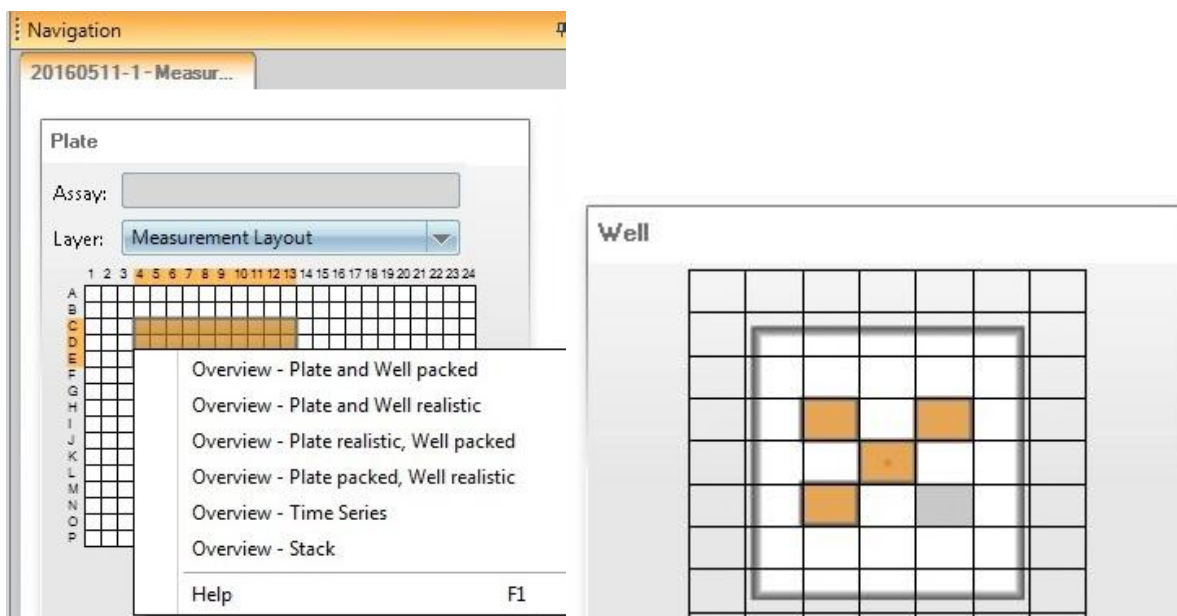
3.3 手动键入 Plate name ，
始拍摄。

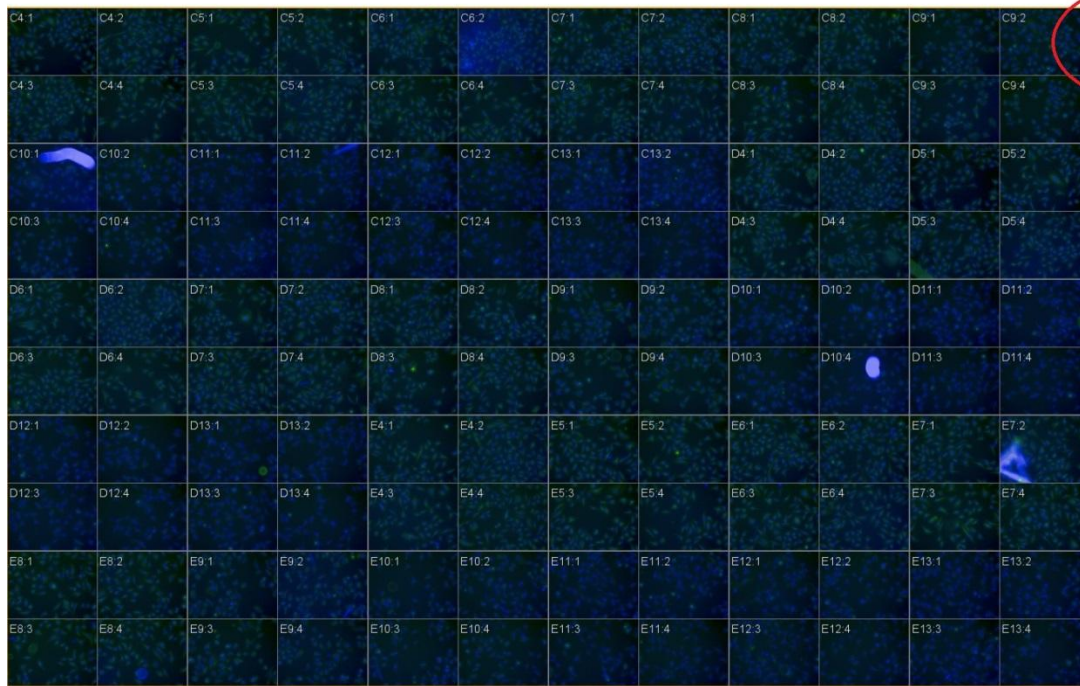
3.4 拍摄完毕 tiff 格式会自动保存在数据库 Measurement。

3.5 在图片上点鼠标右键，“Save Image” 可保存图片为 JPG 或 PNG 格式



3.6 多图显示：在位置选择区，鼠标选取要显示的孔和视野(蓝色变橙色)，点击右键，选择六中显示方式的任意一种。





Coloring: Highlight
Labels: Small
Show Plate: None
Show Grid: Small
Show Scale: Watermarked
Filled condition: None

Channels: All 488
SAF: []

孔位标识

Chapter 2: Image Analysis 图像分析

4. 图像分析参数设定




4.1 点击操作流程指示区的

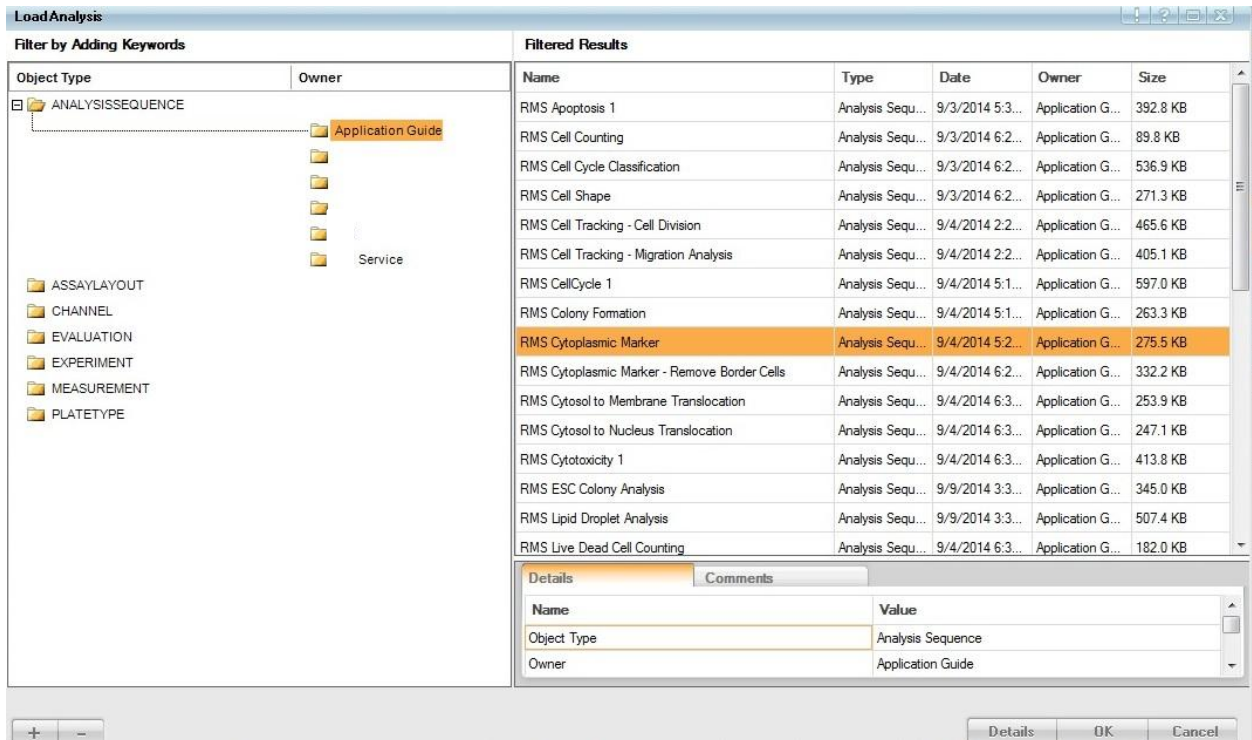
案例一：

4.2 通过已有分析程序进行修改，以胞浆蛋白表达定量为例：

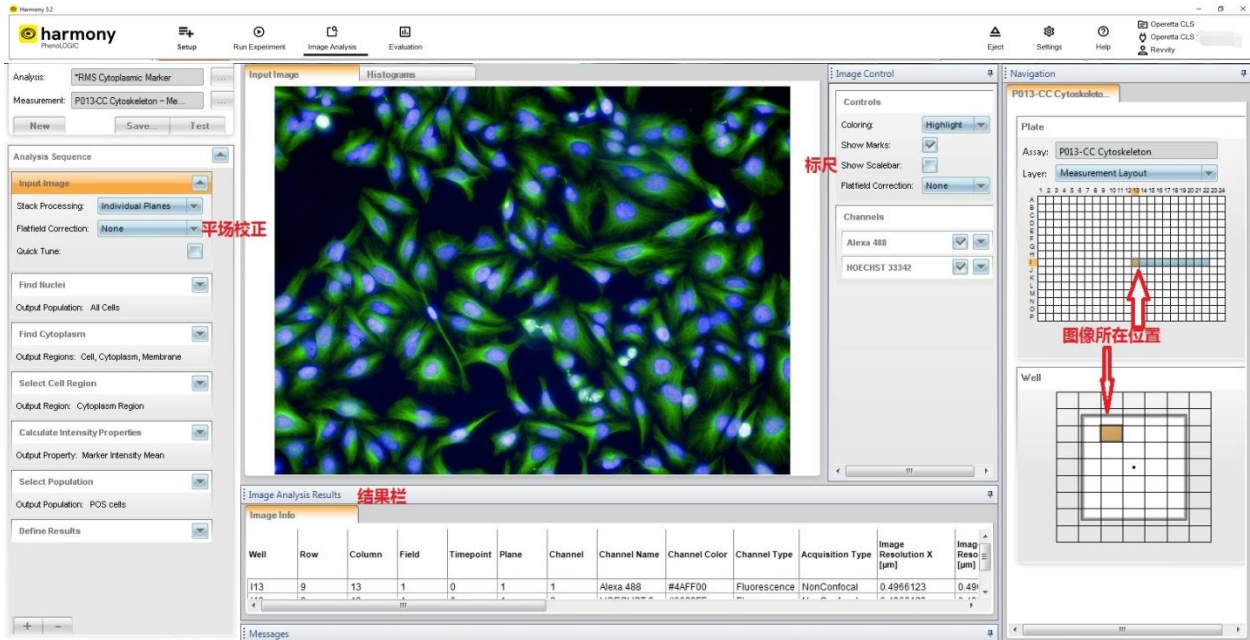


点击“analysis”右侧 ，弹出“load Analysis”窗口，选择预设分析程序。

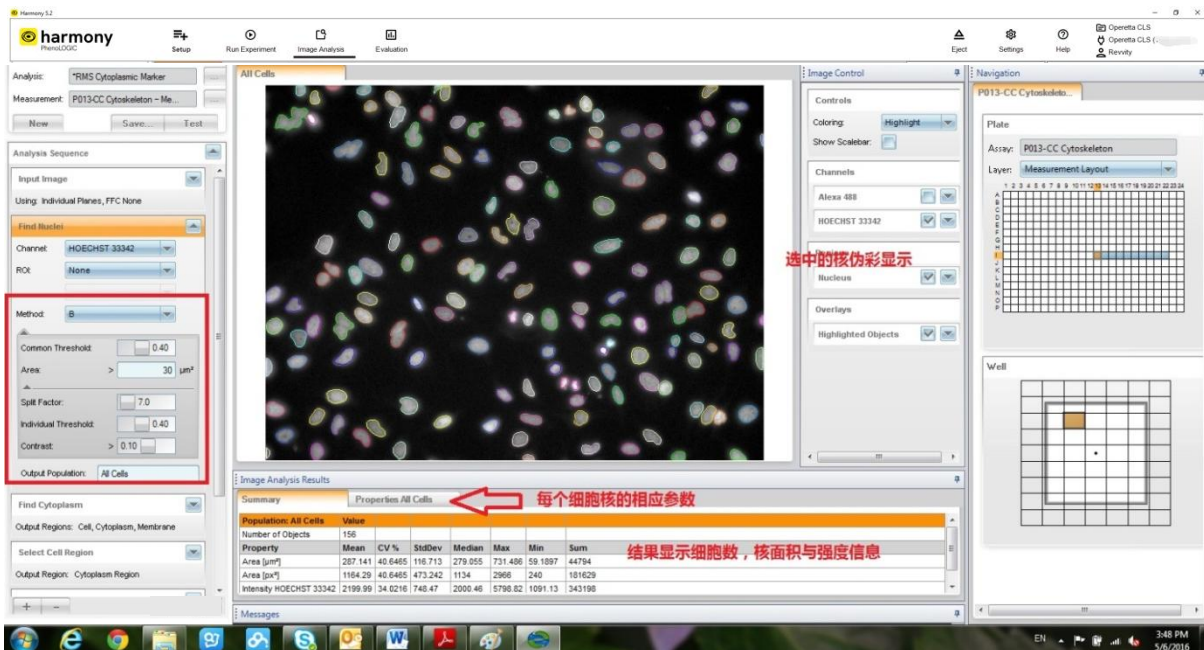
4.2.1 例如：分析某蛋白表达强度，选择“RMS cytoplasmic Marker”



4.2.2 选择一张图片作为分析例图



4.2.3 点击 Find Nuclei 右侧下拉箭头，调整红色方框内的方法与参数。各指标的意义可通过点鼠标右键，“help”获取。



4.2.4 点击 Find cytoplasm 右侧下拉箭头，调整红色方框内的方法与参数。

Image Analysis Results

Summary		Properties All Cells						
Population: All Cells		Value						
Property	Mean	CV %	StdDev	Median	Max	Min	Sum	
Number of Objects	156							
Cell Area [µm²]	5417.22	48.7043	2838.42	5180	13176	243	845086	
Cell Area [µm²]	1336.01	48.7043	850.696	1274.92	3249.51	59.9296	208418	
Cytoplasm Area [µm²]	4252.93	52.8995	2249.4	3962	10936	0	663457	

单个细胞参数
细胞数，细胞面积，强度信息

4.2.5 点击 calculate intensity properties 右侧下拉箭头，点击 region 右侧下拉箭头，选择荧光强度计算的范围，细胞核，膜或胞浆等。

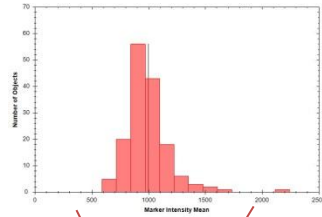
Image Analysis Results

Summary		Properties All Cells						
Population: All Cells		Value						
Property	Mean	CV %	StdDev	Median	Max	Min	Sum	
Number of Objects	156							
Marker Intensity Mean	991.207	20.2511	200.731	960.244	2242.42	587.587	153637	

细胞数，平均荧光强度等参数

4.2.6 通过 select population 功能，在“filter by property”方法下，假设筛选 intensity 值大于 1000 的细胞为阳性细胞（图中显示绿色）。点击“histograms” (红圈所示)，看到本图中各细胞平均荧光强度分布图，可帮助

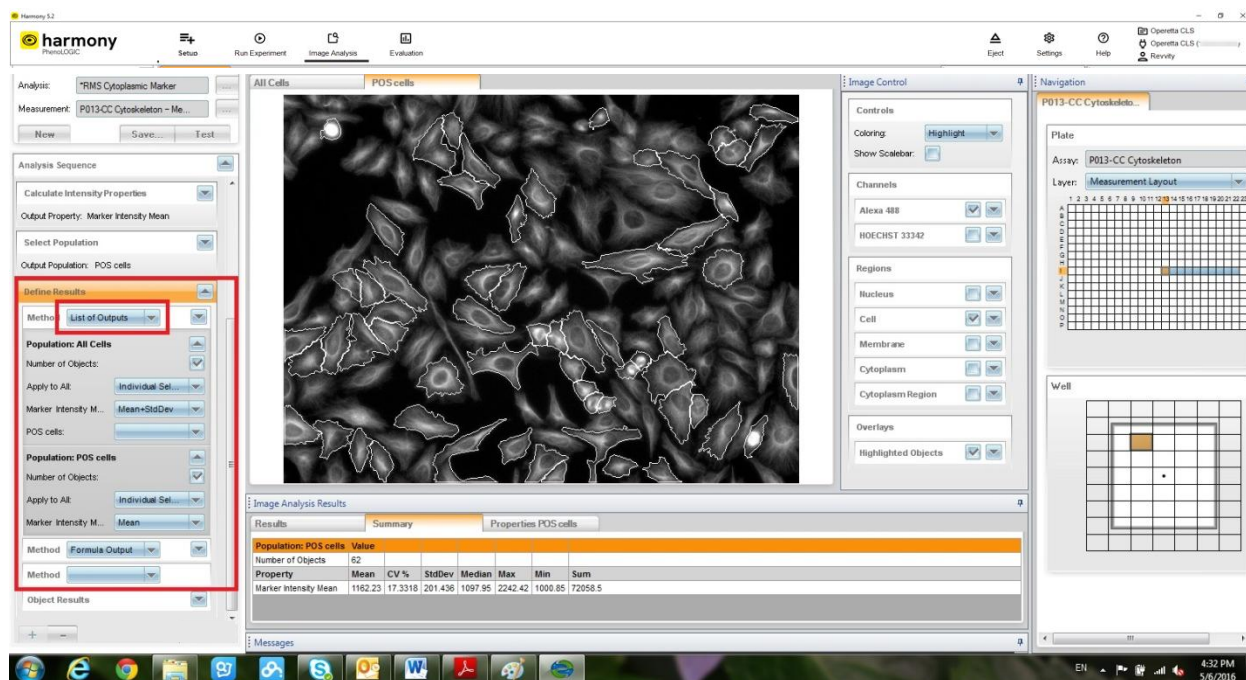
界定阈值。



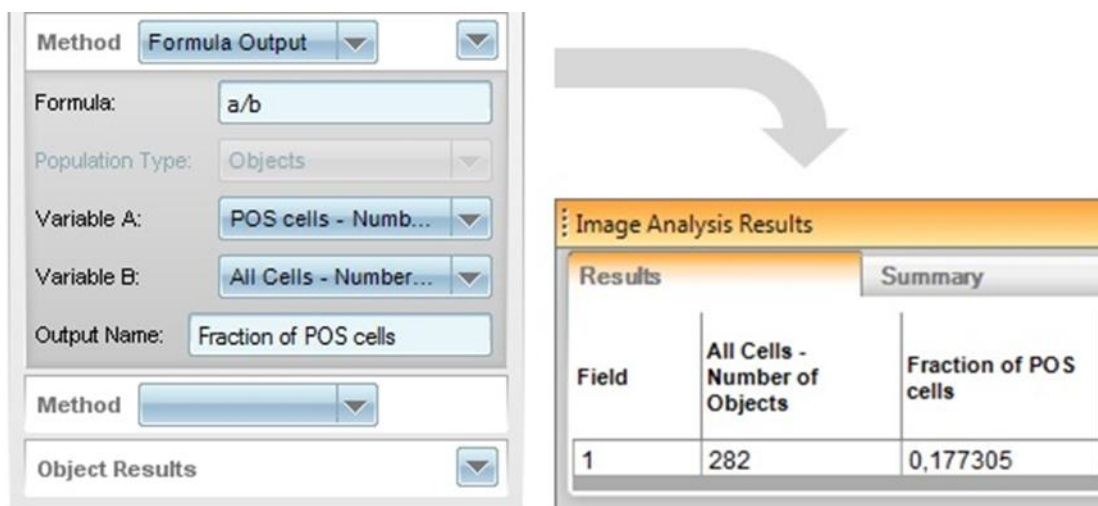
The screenshot shows the Harmony 5.2 software interface. The 'Select Population' panel is active, showing 'Population: All Cells' and 'Method: Filter by Property'. The 'Marker Intensity Mean' is set to 1000. The 'Image Control' panel shows 'Selected Objects' checked, with a red arrow pointing to it and the label '阳性细胞显示'. The 'Image Analysis Results' table is visible at the bottom.

Population: All Cells		Value					
Number of Objects	156						
Property	Mean	CV %	StdDev	Median	Max	Min	Sum
Marker Intensity Mean	991.207	20.2511	200.731	960.244	2242.42	587.587	153637
Marker Intensity StdDev	342.137	37.607	126.603	320.683	1277.73	120.348	53031.2
Marker Intensity CV [%]	33.9984	20.1614	6.86134	33.0168	59.1346	19.423	5289.75

4.2.7 输出结果设定。在“list of output”中勾选需要输出的参数



4.2.8 输出结果设定，在“formula output”中输出阳性率。公式可用符号包括“+”，“-”，“*”，“/”“()”



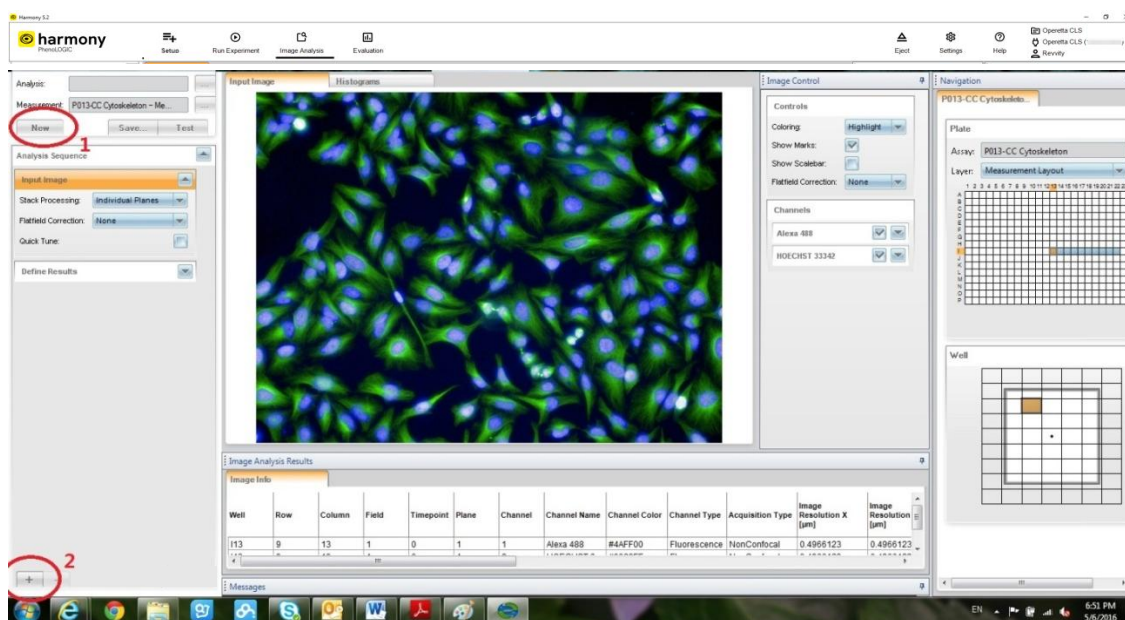
4.2.9 点击“Test”或“apply”输出单张图片的结果，点击“save”保存分析程序；



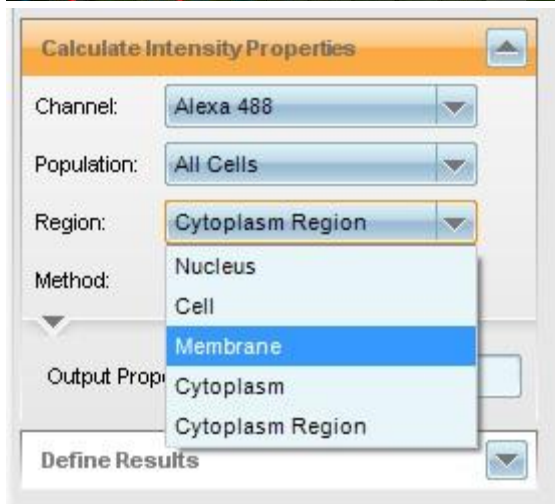
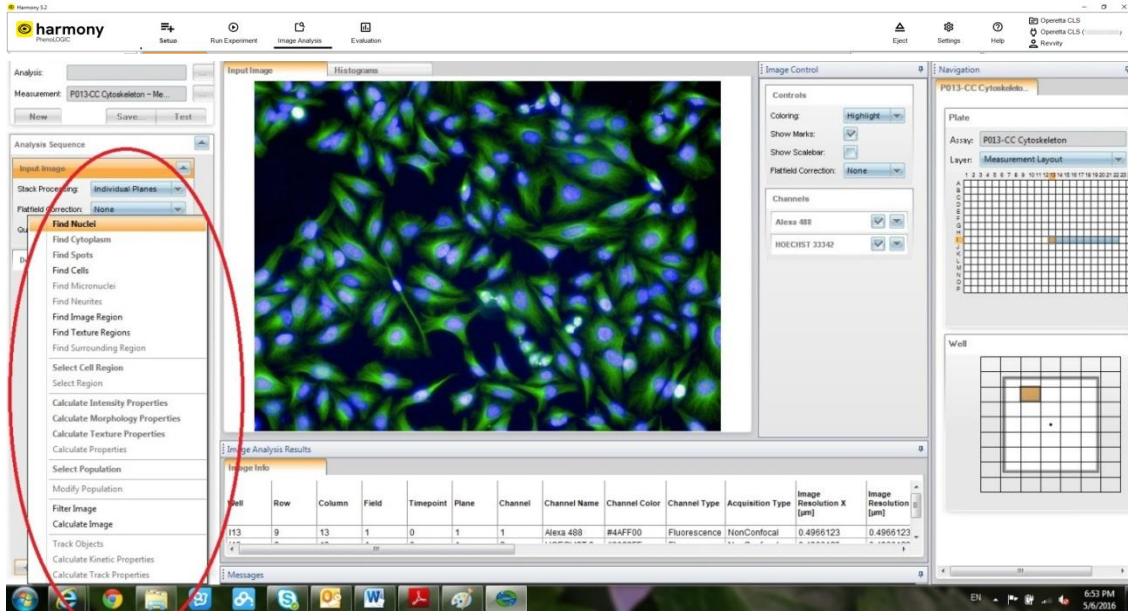
案例二：

4.3 手动编辑新的分析程序：以细胞膜蛋白表达定量为例

4.3.1 点击“New”，消除上一次实验的分析程序，点击“+”，

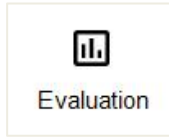


4.3.2 在弹出的分析模块中依次选择“Find Nucleus”，“Find Cytoplasm”，“calculate intensity properties”(此项内 region 下拉菜单选择 membrane)， “select population”；



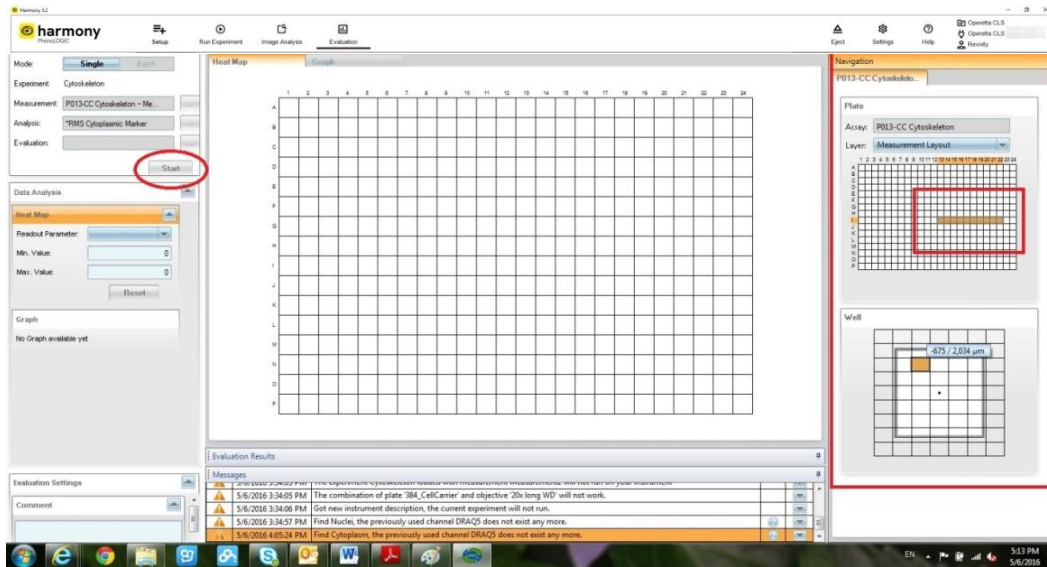
4.3.3 调整参数，结果设定以及分析程序保存与 4.2 所述相同。

5. 结果评估及数据输出

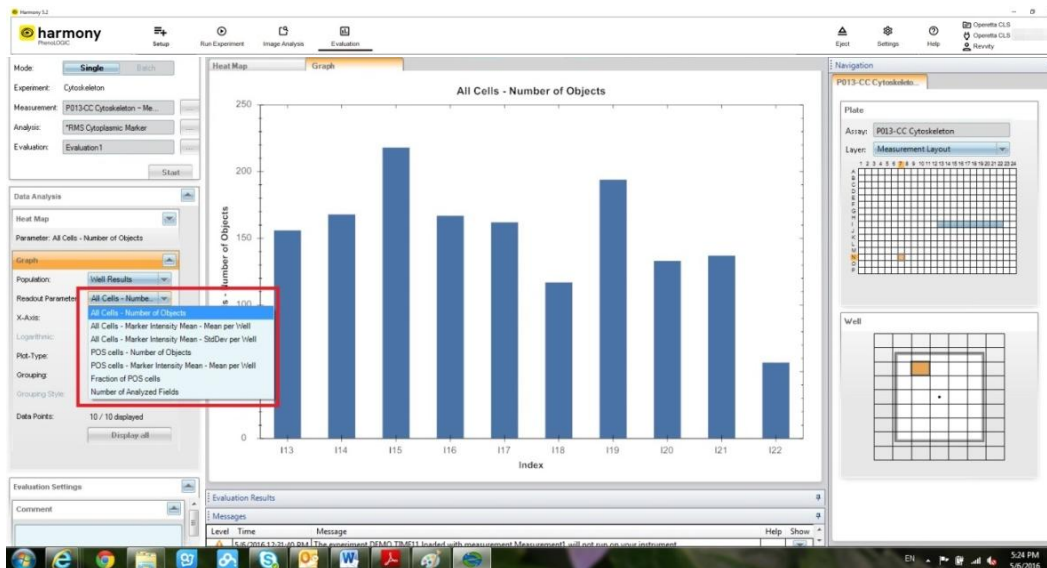


5.1 点击操作流程指示区的

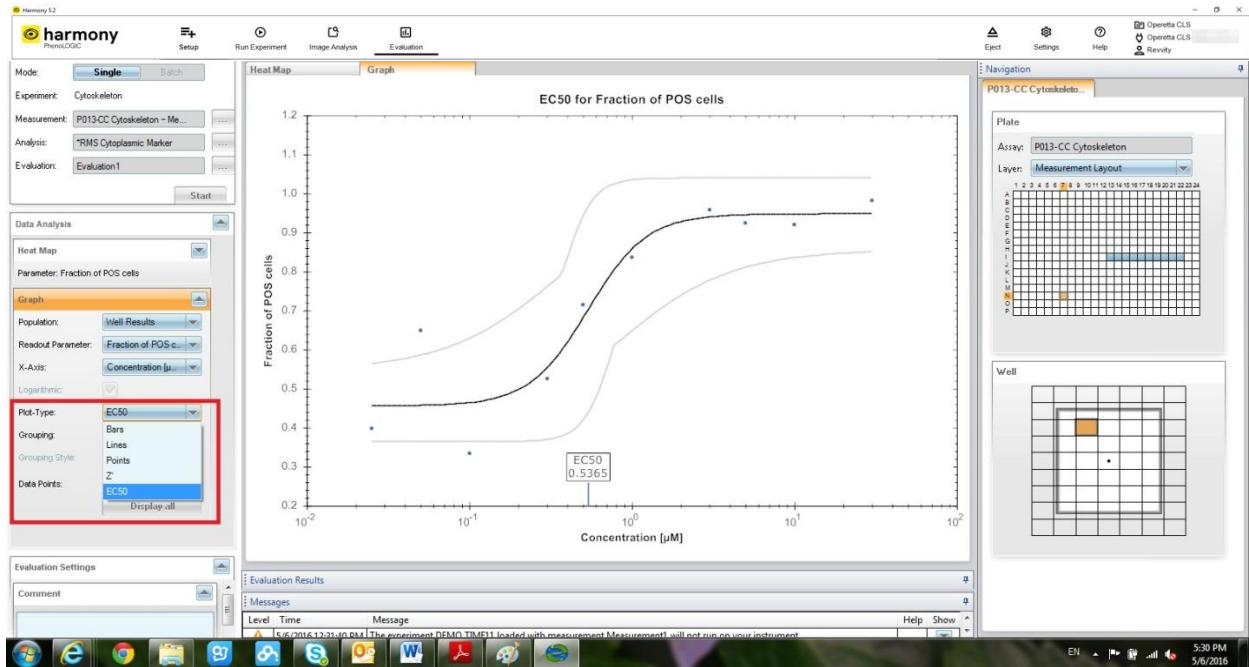
5.2 鼠标左键拖拉选择要分析的区域（显示为橙色），点击“start”开始多孔分析。



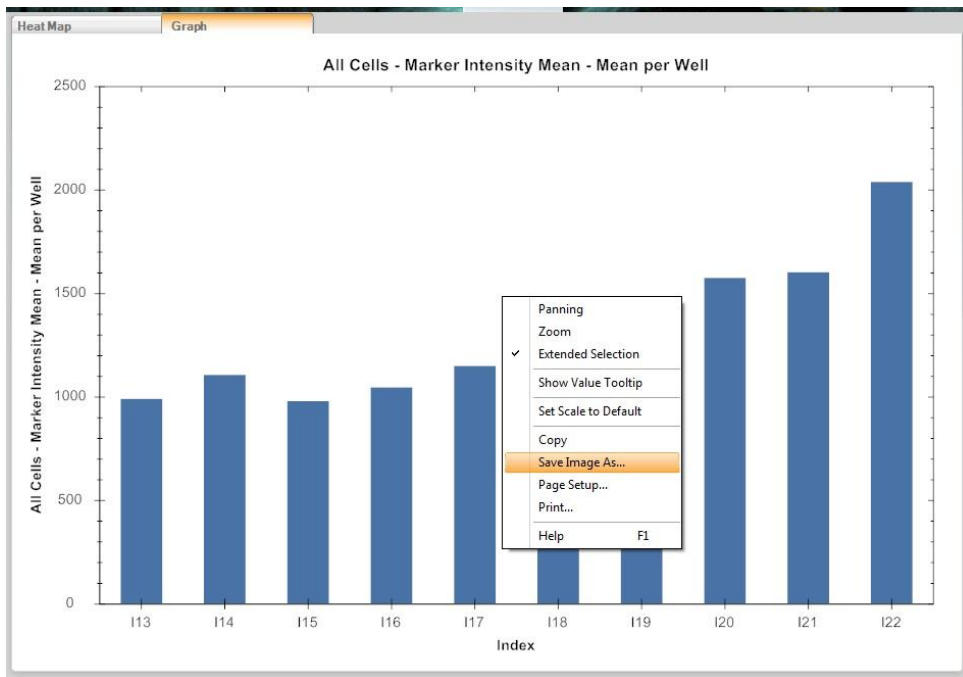
5.3 分析结束后，通过 readout parameter 右侧下拉菜单，选择显示的参数



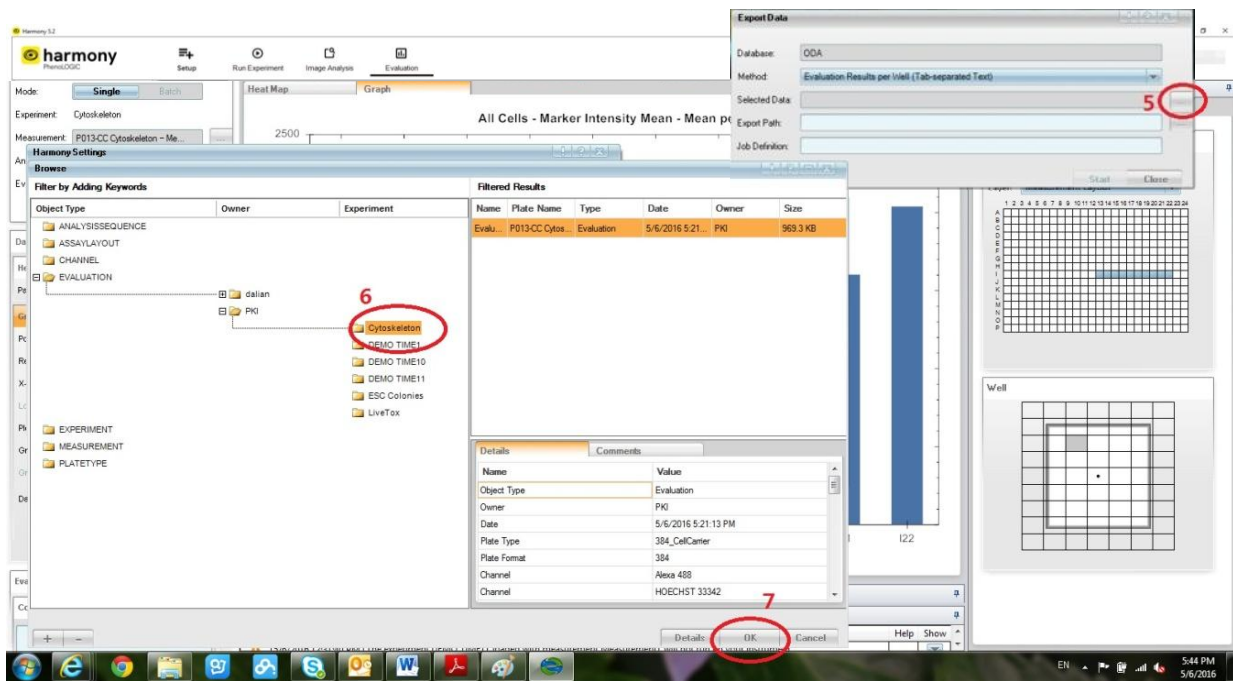
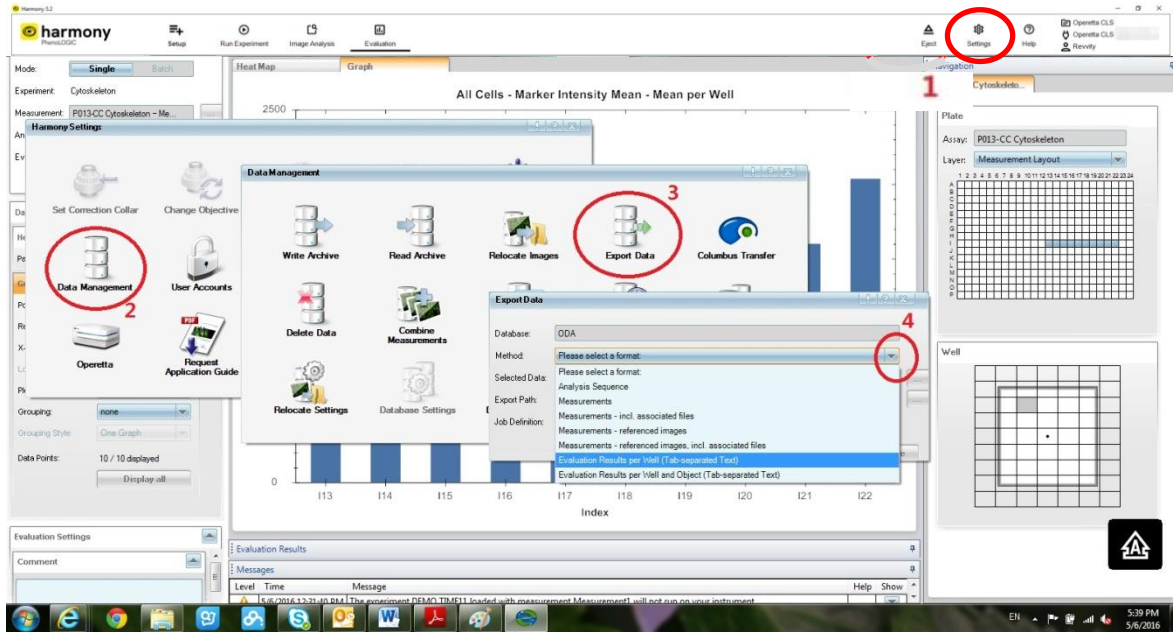
5.4 浓度曲线，EC50 显示

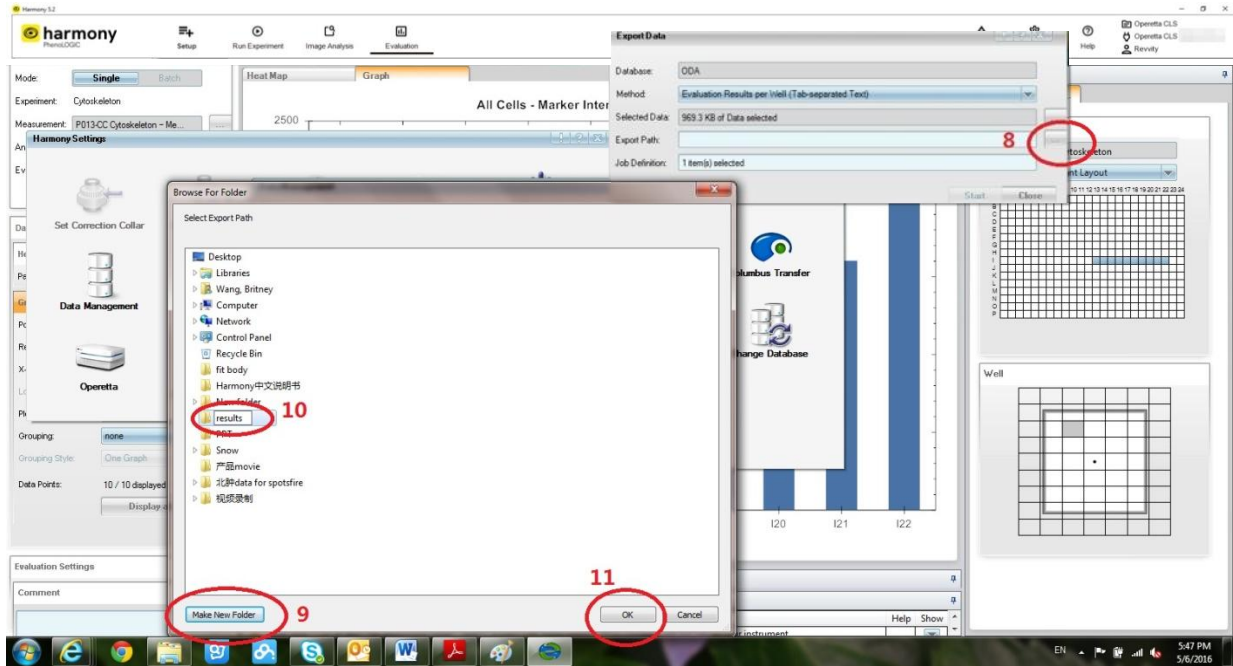


5.5 在图表上点鼠标右键，“Save Image as”可保存图片表为JPG、GIF或PNG等格式

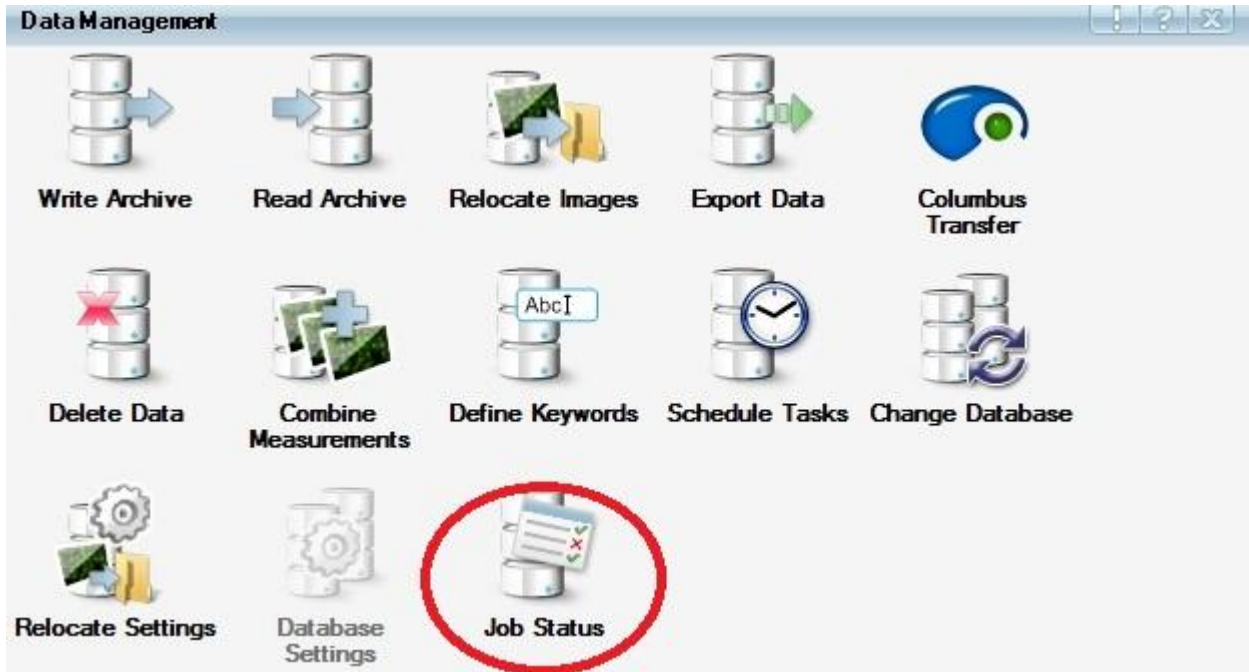


5.6 根据图示顺序，数据以 Excel 可读方式导出。注：本机没有安装 excel 软件。





通过点击“Job Status”查看传输进度



Job Type	Job Definition	Destination	User	Job Size	Progress [%]	Start Date	End Date	Status	Cancel	Job ID
Columbus Transfer	1 item(s) selected	http://192.168...	PKI	667.1 MB	48	11/05/2016 14:38		Running	Cancel	Job#1

Chapter 3: 预设程序

6. Ready Made Solution (RMS) 索引

6.1 通过“Load Analysis”窗口，可调用现有预设程序。

The screenshot displays the 'Load Analysis' software interface. It is divided into two main sections: a left-hand navigation pane and a right-hand data table.

Left Panel: Filter by Adding Keywords

- Object Type:** A tree view showing a hierarchy of folders. The 'ANALYSISSEQUENCE' folder is expanded, revealing an 'Application Guide' folder and several sub-folders. Other visible folders include 'ASSAYLAYOUT', 'CHANNEL', 'EVALUATION', 'EXPERIMENT', 'MEASUREMENT', and 'PLATETYPE'.
- Owner:** A column for the owner of the selected object type.

Right Panel: Filtered Results

Name	Type	Date	Owner	Size
RMS Apoptosis 1	Analysis Seque...	9/3/2014 5:3...	Application Gui...	392.8 KB
RMS Cell Counting	Analysis Seque...	9/3/2014 6:2...	Application Gui...	89.8 KB
RMS Cell Cycle Classification	Analysis Seque...	9/3/2014 6:2...	Application Gui...	536.9 KB
RMS Cell Shape	Analysis Seque...	9/3/2014 6:2...	Application Gui...	271.3 KB
RMS Cell Tracking - Cell Division	Analysis Seque...	9/4/2014 2:2...	Application Gui...	465.6 KB
RMS Cell Tracking - Migration Analysis	Analysis Seque...	9/4/2014 2:2...	Application Gui...	405.1 KB
RMS CellCycle 1	Analysis Seque...	9/4/2014 5:1...	Application Gui...	597.0 KB
RMS Colony Formation	Analysis Seque...	9/4/2014 5:1...	Application Gui...	263.3 KB
RMS Cytoplasmic Marker	Analysis Seque...	9/4/2014 5:2...	Application Gui...	275.5 KB
RMS Cytoplasmic Marker - Remove Border Cells	Analysis Seque...	9/4/2014 6:2...	Application Gui...	332.2 KB
RMS Cytosol to Membrane Translocation	Analysis Seque...	9/4/2014 6:3...	Application Gui...	253.9 KB
RMS Cytosol to Nucleus Translocation	Analysis Seque...	9/4/2014 6:3...	Application Gui...	247.1 KB
RMS Cytotoxicity 1	Analysis Seque...	9/4/2014 6:3...	Application Gui...	413.8 KB
RMS ESC Colony Analysis	Analysis Seque...	9/9/2014 3:3...	Application Gui...	345.0 KB
RMS Lipid Droplet Analysis	Analysis Seque...	9/9/2014 3:3...	Application Gui...	507.4 KB
RMS Live Dead Cell Counting	Analysis Seque...	9/4/2014 6:3...	Application Gui...	182.0 KB
RMS Live Dead Cell Counting - 2	Analysis Seque...	9/4/2014 6:3...	Application Gui...	342.5 KB
RMS Micronucleus Analysis	Analysis Seque...	9/4/2014 6:4...	Application Gui...	876.0 KB
RMS Microtissue Analysis - Brightfield	Analysis Seque...	9/3/2014 8:4...	Application Gui...	502.6 KB
RMS Microtissue Analysis - Fluorescence	Analysis Seque...	9/3/2014 8:4...	Application Gui...	343.5 KB
RMS Migration - Confluency	Analysis Seque...	9/4/2014 6:4...	Application Gui...	395.7 KB
RMS Migration - Hole in Cell Layer	Analysis Seque...	9/4/2014 6:4...	Application Gui...	273.6 KB

Bottom Panel: Filter by Adding Keywords

- Object Type:** Similar to the top panel, but with 'MEASUREMENT' instead of 'EXPERIMENT'.
- Owner:** A column for the owner of the selected object type.

Bottom Right Panel: Filtered Results

Name	Type	Date	Owner	Size
RMS Cytotoxicity 1	Analysis Seque...	9/4/2014 6:3...	Application Gui...	413.8 KB
RMS ESC Colony Analysis	Analysis Seque...	9/9/2014 3:3...	Application Gui...	345.0 KB
RMS Lipid Droplet Analysis	Analysis Seque...	9/9/2014 3:3...	Application Gui...	507.4 KB
RMS Live Dead Cell Counting	Analysis Seque...	9/4/2014 6:3...	Application Gui...	182.0 KB
RMS Live Dead Cell Counting - 2	Analysis Seque...	9/4/2014 6:3...	Application Gui...	342.5 KB
RMS Micronucleus Analysis	Analysis Seque...	9/4/2014 6:4...	Application Gui...	876.0 KB
RMS Microtissue Analysis - Brightfield	Analysis Seque...	9/3/2014 8:4...	Application Gui...	502.6 KB
RMS Microtissue Analysis - Fluorescence	Analysis Seque...	9/3/2014 8:4...	Application Gui...	343.5 KB
RMS Migration - Confluency	Analysis Seque...	9/4/2014 6:4...	Application Gui...	395.7 KB
RMS Migration - Hole in Cell Layer	Analysis Seque...	9/4/2014 6:4...	Application Gui...	273.6 KB
RMS Neurite Outgrowth Analysis 2	Analysis Seque...	9/9/2014 3:3...	Application Gui...	296.9 KB
RMS Neurite Outgrowth Analysis 2 - with Cell Body Refin...	Analysis Seque...	9/9/2014 3:4...	Application Gui...	444.1 KB
RMS Nuclear Analysis	Analysis Seque...	9/4/2014 6:4...	Application Gui...	207.6 KB
RMS Nuclear Classification	Analysis Seque...	9/4/2014 6:4...	Application Gui...	273.7 KB
RMS Nuclear Fragmentation	Analysis Seque...	9/4/2014 6:4...	Application Gui...	197.3 KB
RMS Online Quality Control	Analysis Seque...	9/4/2014 6:4...	Application Gui...	293.1 KB
RMS Phenotype Classification 2	Analysis Seque...	9/4/2014 6:4...	Application Gui...	690.0 KB
RMS Quantification of Marker in Nucleus	Analysis Seque...	9/4/2014 6:4...	Application Gui...	192.1 KB
RMS Receptor Internalization	Analysis Seque...	9/4/2014 6:4...	Application Gui...	258.5 KB
RMS Receptor Internalization - Remove Border Cells	Analysis Seque...	9/4/2014 6:5...	Application Gui...	316.1 KB
RMS Spot Analysis	Analysis Seque...	9/4/2014 6:5...	Application Gui...	244.3 KB
RMS Texture Analysis - Mitochondria Classification	Analysis Seque...	9/4/2014 6:5...	Application Gui...	306.4 KB

6.2 适用范围如下，但不仅限于。

RMS 3D Analysis	3D 分析, 用于胞囊, 微球等
RMS Apoptosis	凋亡检测
RMS Cell Counting	细胞计数
RMS Cell Cycle Classification	细胞周期分类
RMS Cell Shape	细胞形态
RMS Cell Tracking	细胞追踪—迁移、增殖
RMS Colony Formation	克隆计数
RMS Cytoplasmic Marker	胞浆标记物定量分析
RMS Cytosol to Membrane Translocation	信号胞浆到胞膜转位分析
RMS Cytosol to Nucleus Translocation	信号胞浆到胞核转位分析
RMS Cytotoxicity	细胞毒性
RMS ESC Colony Analysis	间充质干细胞克隆形成
RMS Lipid Droplet Analysis	脂滴形成
RMS Live Dead Cell Counting	活、死细胞计数
RMS Micronucleus Analysis	微核分析
RMS Microtissue Analysis	3D 微球分析
RMS Migration	迁移实验
RMS Neurite outgrowth Analysis	神经根生长
RMS Nuclear Analysis	细胞核分析
RMS Nuclear Classification	细胞核分类
RMS Nuclear Fragmentation	细胞核片段化分析
RMS Online Quality Control	细胞生长质控
RMS Phenotype Classification	细胞分类
RMS PreciScan Microtissue	微球预扫描
RMS PreciScan Mitotic Cells	有丝分裂细胞预扫描
RMS Quantification of Marker in Nucleus	核内蛋白表达分析
RMS Receptor Internalization	受体内化
RMS Spot Analysis	点分析, 用于自噬, DNA Damage, 蛋白表达等
RMS Texture Analysis	纹理分析, 用于细胞器分布-心肌跳动分析等

