

Outline

- **Multiplexing Fluorescence Western Blot**
- **ChemiDoc™ MP Image System**
- **BioRad Total Solution on Western Blot**
 - TPN with StainFree Work flow
- **Data Analysis**
 - ImageLab software



BIO RAD

BIORAD MP 優勢及特性

應用廣泛

操作介面友善直觀

Admin 權限方便管理
- 獨立 Account

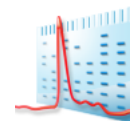
Smart Tray Technology

USB 存取 / 網路線 Output
- Linux 系統不擔心中毒

移動式鏡頭
- 獲得最佳 resolution 影像

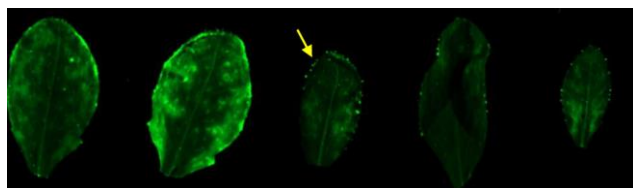
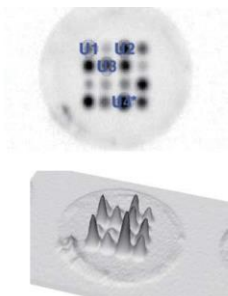
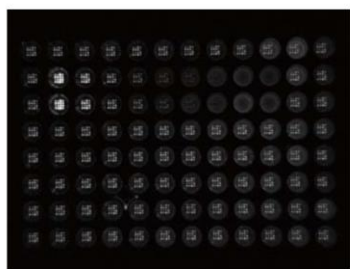
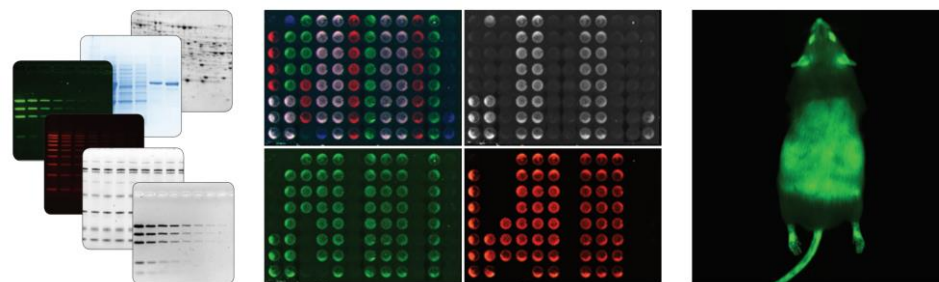
平場校正專利
- 影像不扭曲變形

搭配 Image Lab 軟體快速且準確分析數據



All-In-One Flexible Imager

What we can do with ChemiDoc MP?



Application List

- ✓ Nucleic Gel
- ✓ Protein Gel
- ✓ Chemiluminescence
- ✓ Colorimetric
- ✓ Fluorescence: RGB, FR, NIR
- ✓ *in vivo* fluorescence imaging

螢光優勢

■ 多通道拍攝 Multiplexing

- ◇ 磷酸化蛋白+ Total蛋白
- ◇ Target + Loading Control

■ 不須裁膜、Stripping

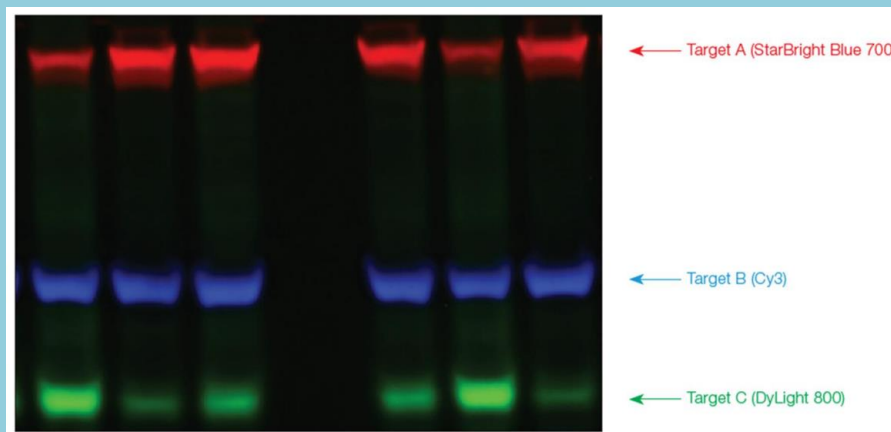
■ 步驟簡便，僅需更換二抗

■ 精準定量

- ◇ 寬廣的動態範圍 (Dynamic Range)
- ◇ 數據更為線性

■ 媲美冷光靈敏度的BioRad StarBright螢光二抗

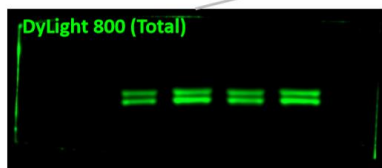
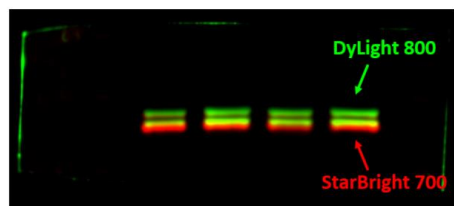
■ Blots膜能被久放儲存 — 好的保存條件甚至能超過十年



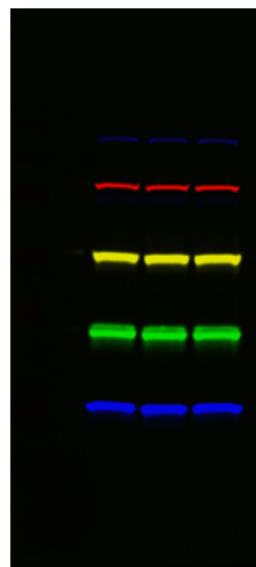
螢光應用—磷酸化蛋白

※註：螢光技術使以往操作不易的磷酸化蛋白變得更簡單

Total/ Phospho Protein



(From北醫呼治)



ATG7, StarBright Blue 700
Tubulin, Rhodamine
AKR1C2, StarBright Blue 520
TPI, DyLight 800

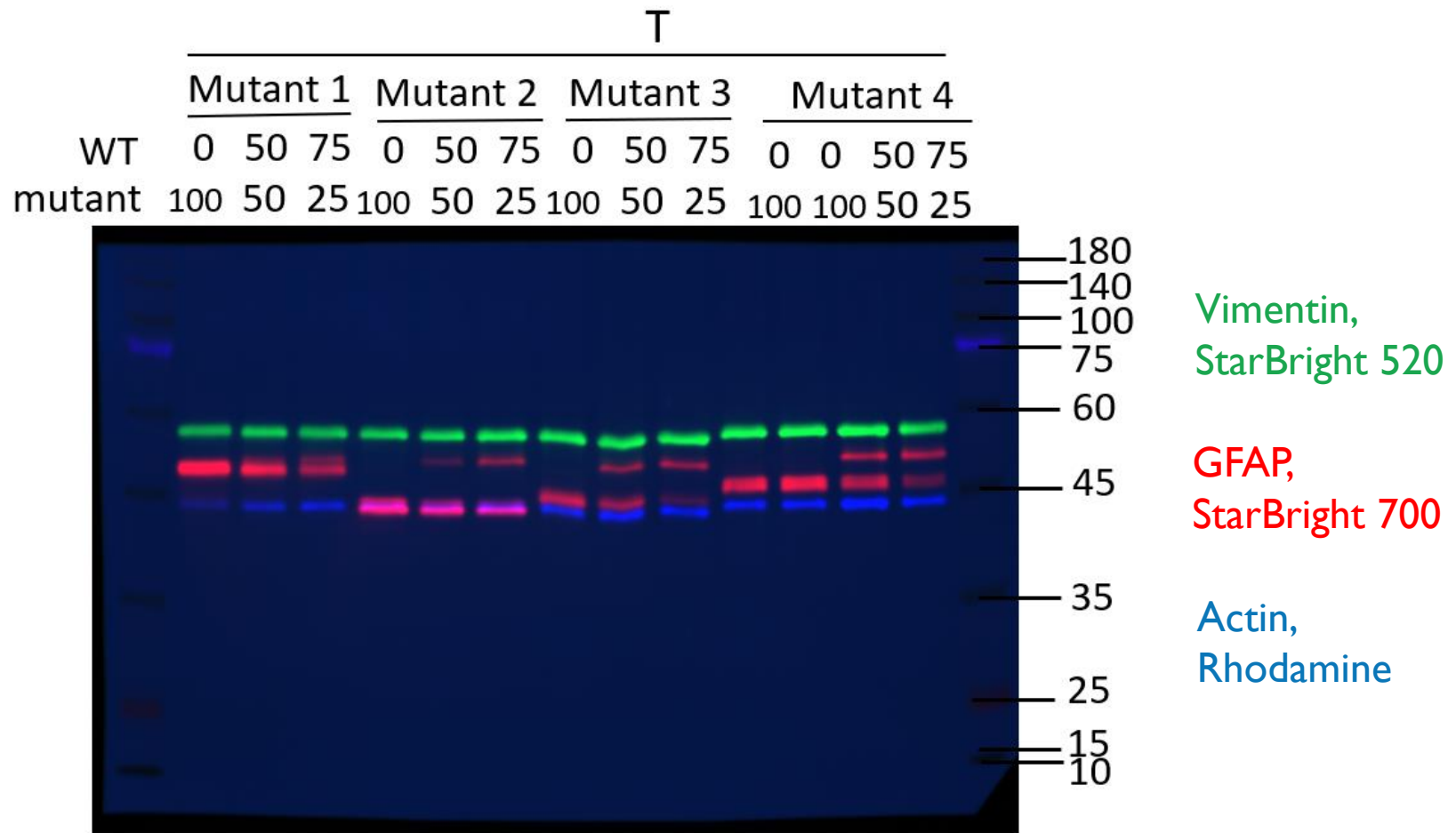
(From BioRad)

No more membrane cutting, stripping and reprobing

磷酸化的樣品以多通道螢光應用顯示在同一張膜上

BIO RAD

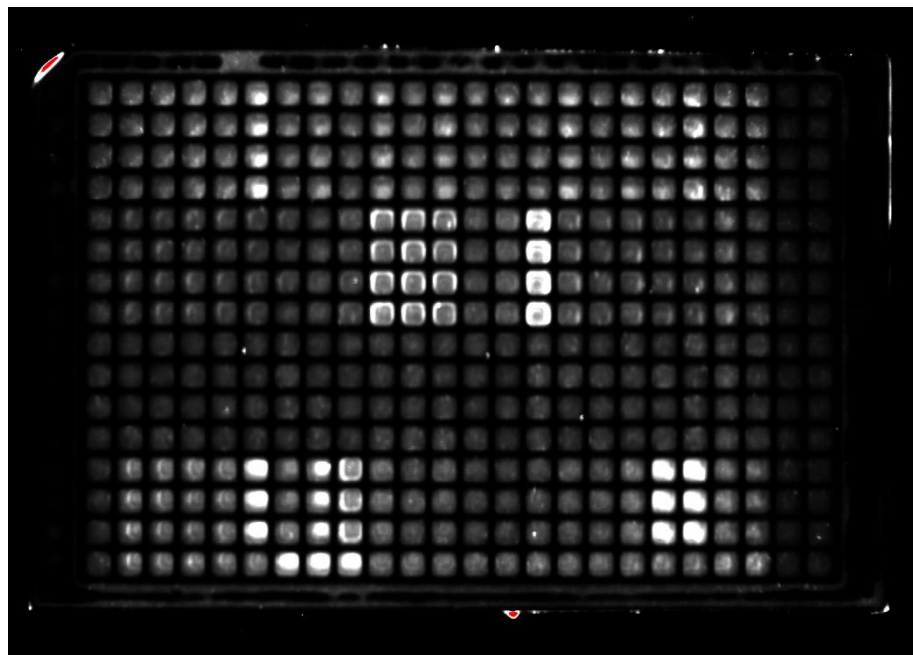
螢光+冷光應用—多目標呈現



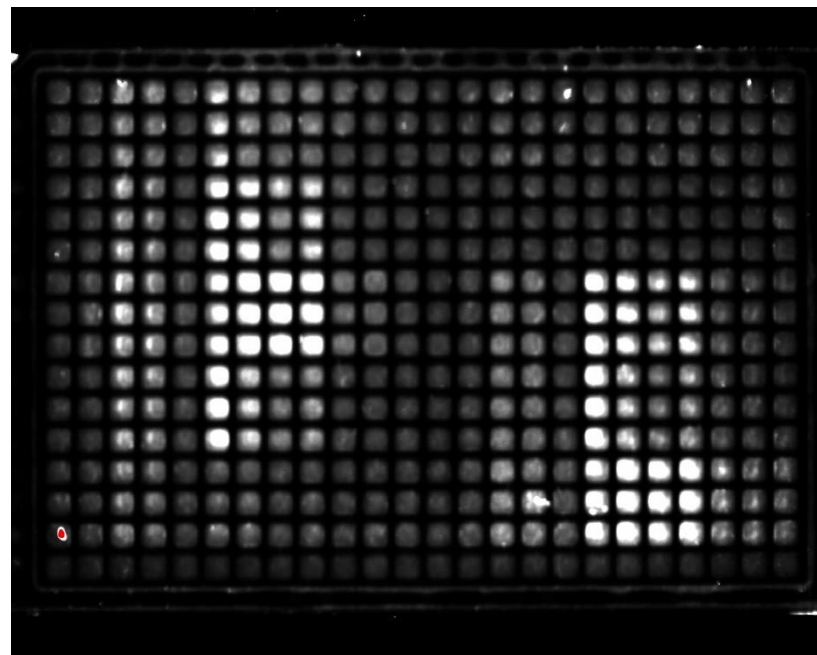
(From 清大生科)

384-WELL PLATE 成像

GFP 螢光



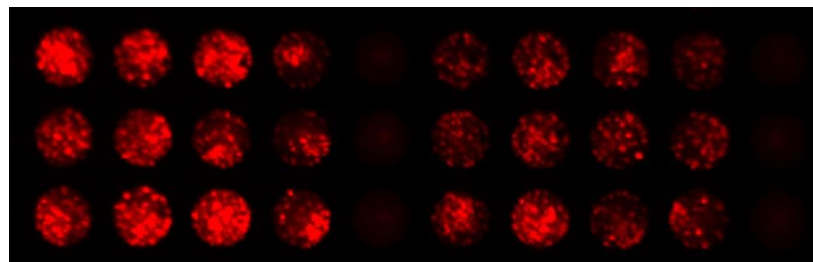
GFP 螢光



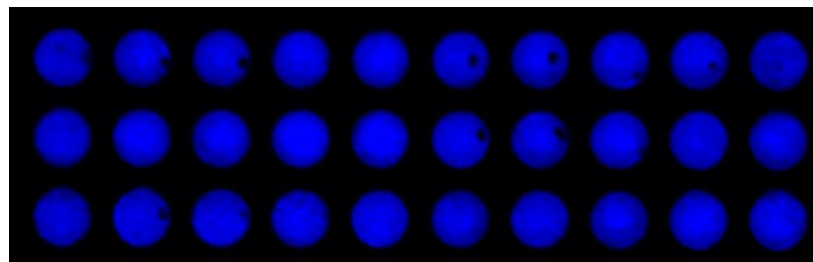
※註: 客戶以384 plate養細菌後以不同條件處理，用GFP蛋白確認結果

螢光應用— In-cell Western

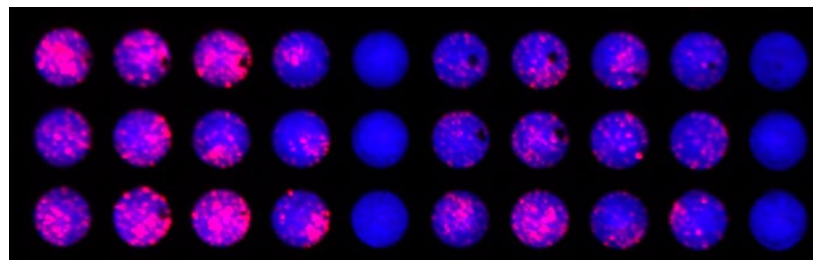
Anti-FLAG
SIRT6



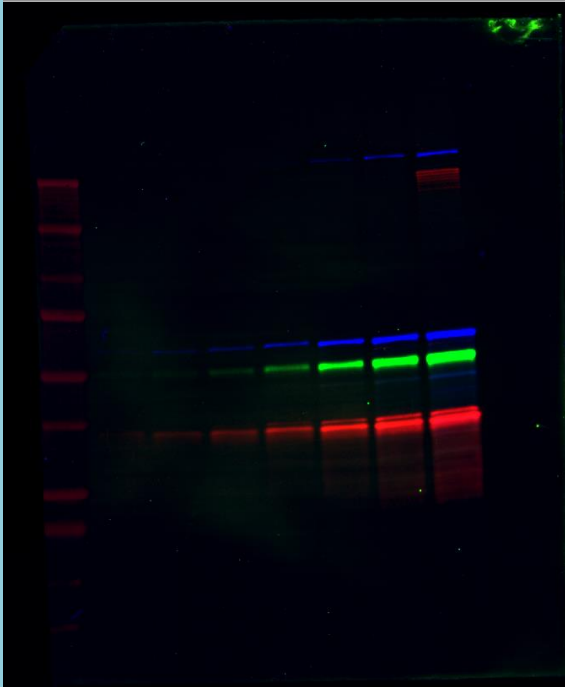
DRAQ5



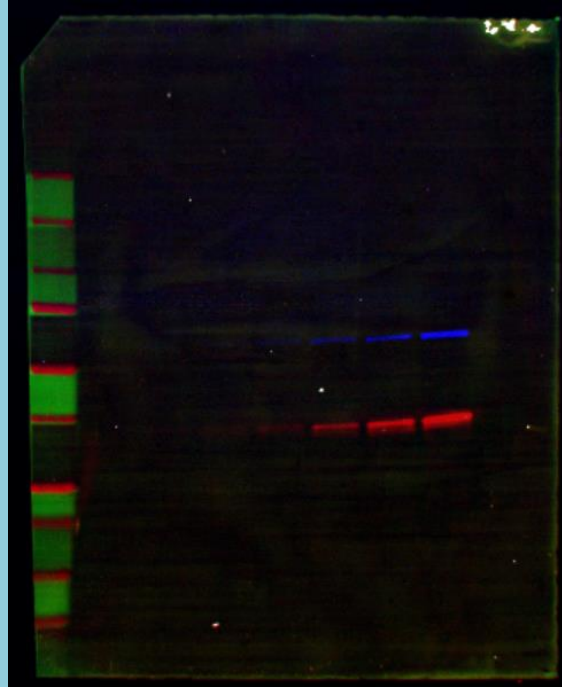
Merge



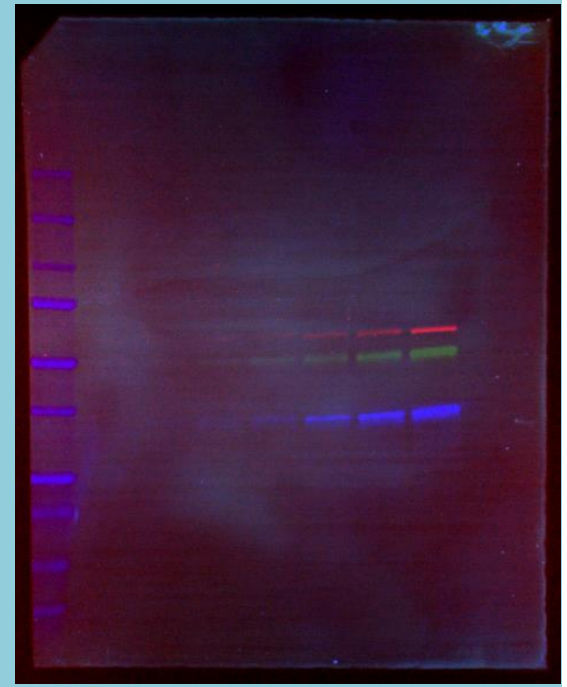
BIORAD MP 螢光成像品質佳



Biorad
ChemiDoc MP



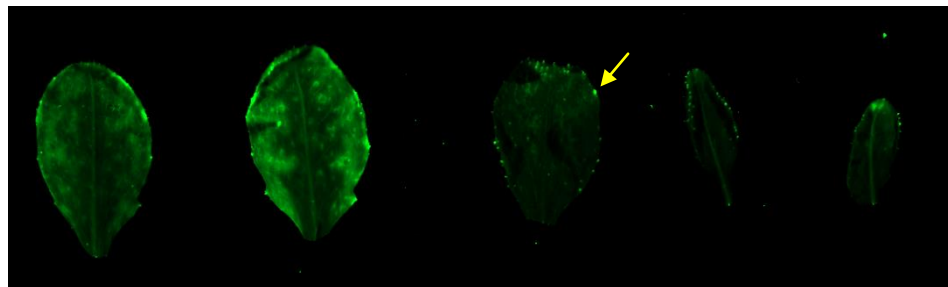
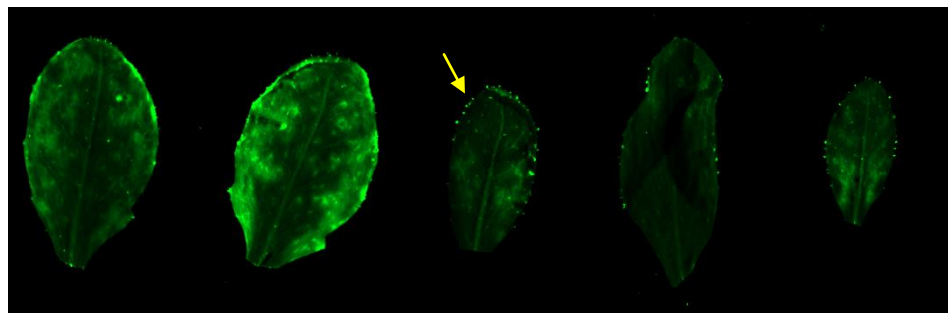
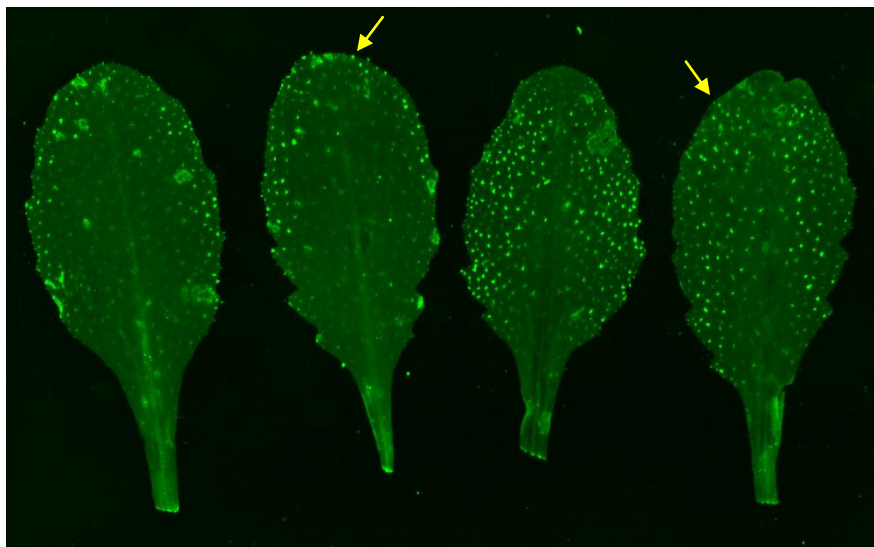
T Brand



C Brand

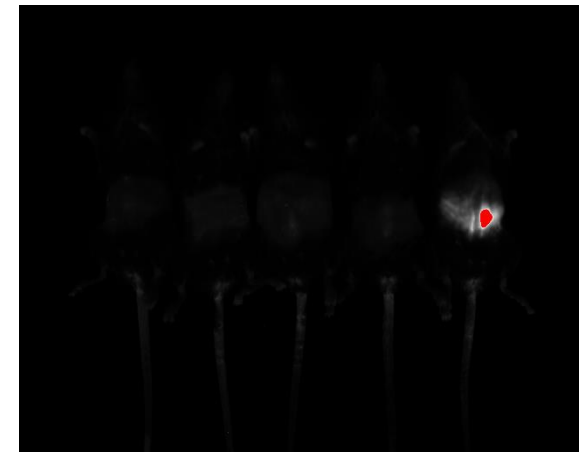
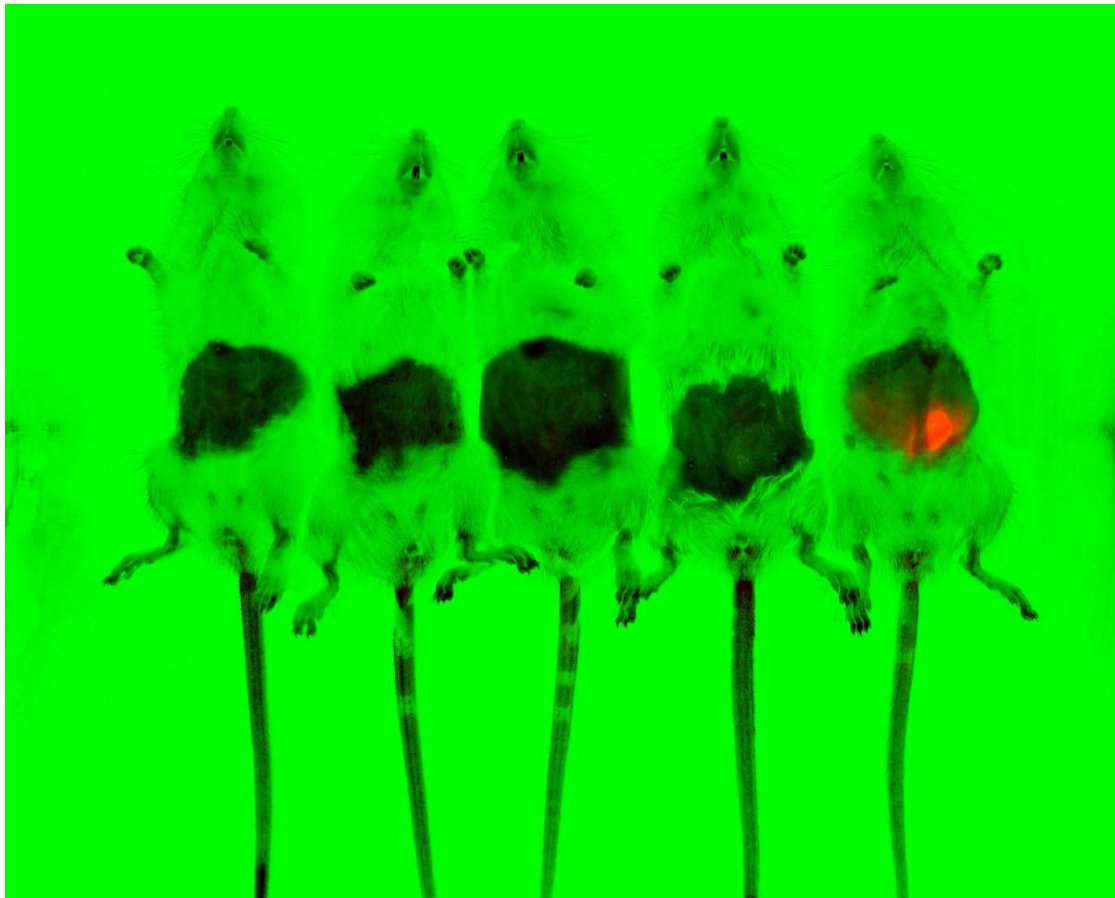
螢光應用— 螢光葉片

GFP-labeled (如黃色箭頭所示)



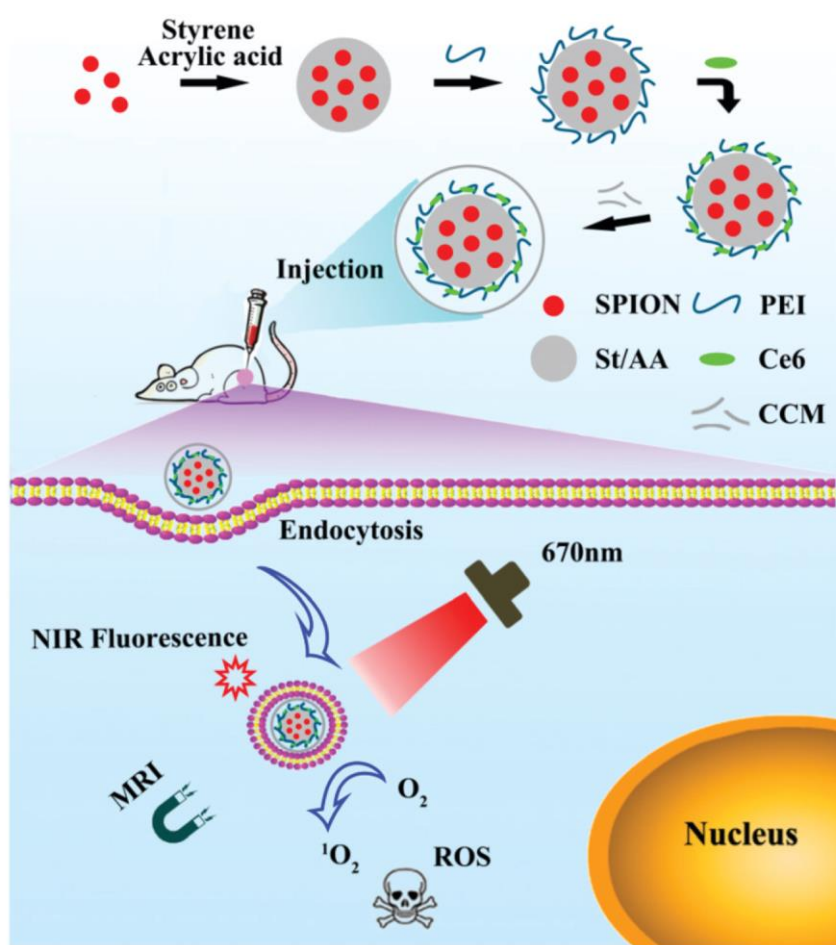
(From 台大生科)

螢光應用—小鼠活體造影



(From 義守大學)

螢光應用—小鼠活體造影

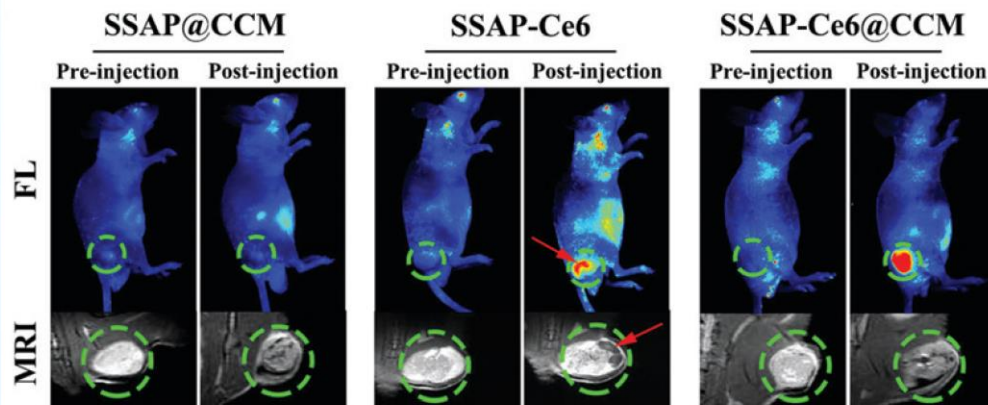


Biomaterials Science

Check for updates

Cite this: *Biomater. Sci.*, 2018, 6, 1834

Cancer cell membrane-coated magnetic nanoparticles for MR/NIR fluorescence dual-modal imaging and photodynamic therapy†



mg mL⁻¹). After 12 h of injection, the mouse was imaged by the ChemiDoc™ MP imaging system (BIO-RAD) and a 7.0 T MRI scanner.

Cancer cell membrane-coated magnetic nanoparticles for MR/NIR fluorescence dual-modal imaging and photodynamic therapy, 2018

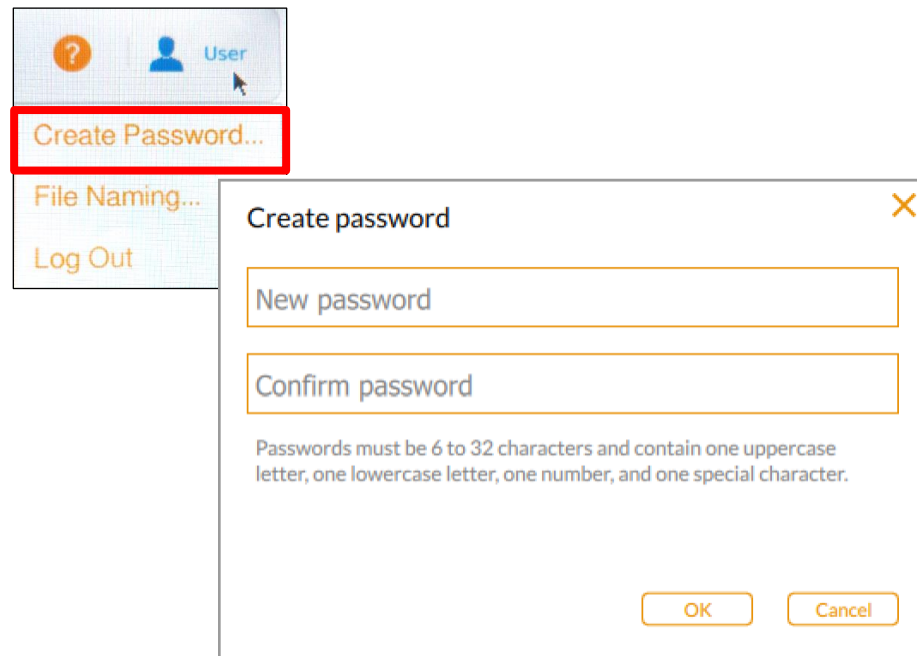
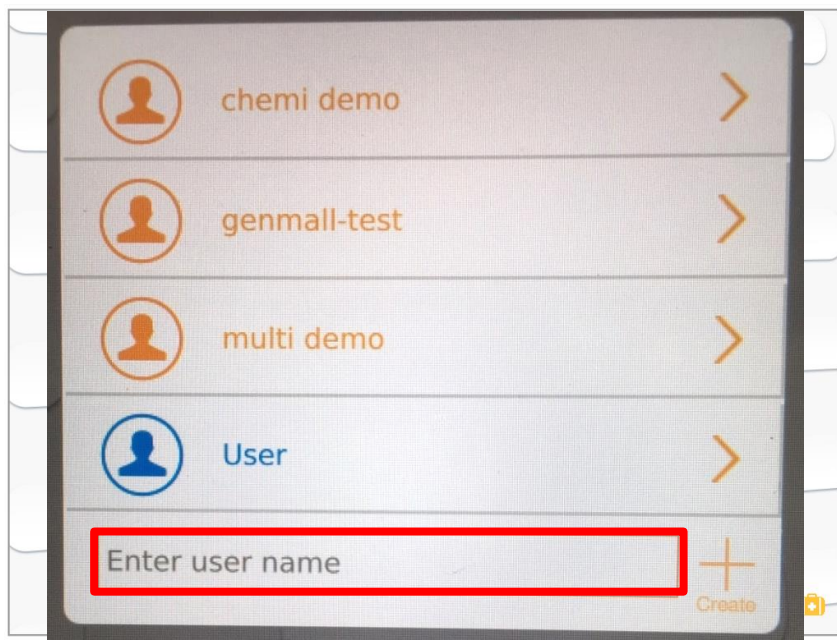
Fig. 1 Schematic of the formation, dual-modal imaging, and photodynamic therapy of SSAP-Ce6@CCM.

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**BIO RAD**

獨立User Accounts (可設密碼)



- ✓ 使用者可創建專屬資料夾，彼此數據不互相干擾

Admin 權限方便管理

Administrator Options ✕

Require Password
 Require Secure File Export

Require Approval for New Users
 Restrict Export to USB

Restrict Deletion of Images

Restrict Network Export Location

	Admin	Disabled	Must Change Password	Reset Password
User1	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="button" value="Reset"/>
User2	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="button" value="Reset"/>
User3	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="button" value="Reset"/>
User4	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="button" value="Reset"/>

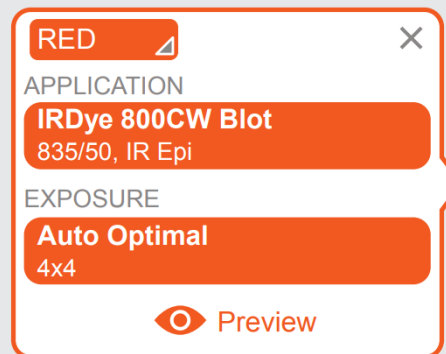
- 所有Data一覽
- 可限制使用者是否設置密碼
- 管理Data存取限制
- 封鎖黑名單使用者權限

介面友善易操作

Easy Workflow

Large
W 18.0 x L 14.4 cm

Define image size
(touch-pinch to zoom)

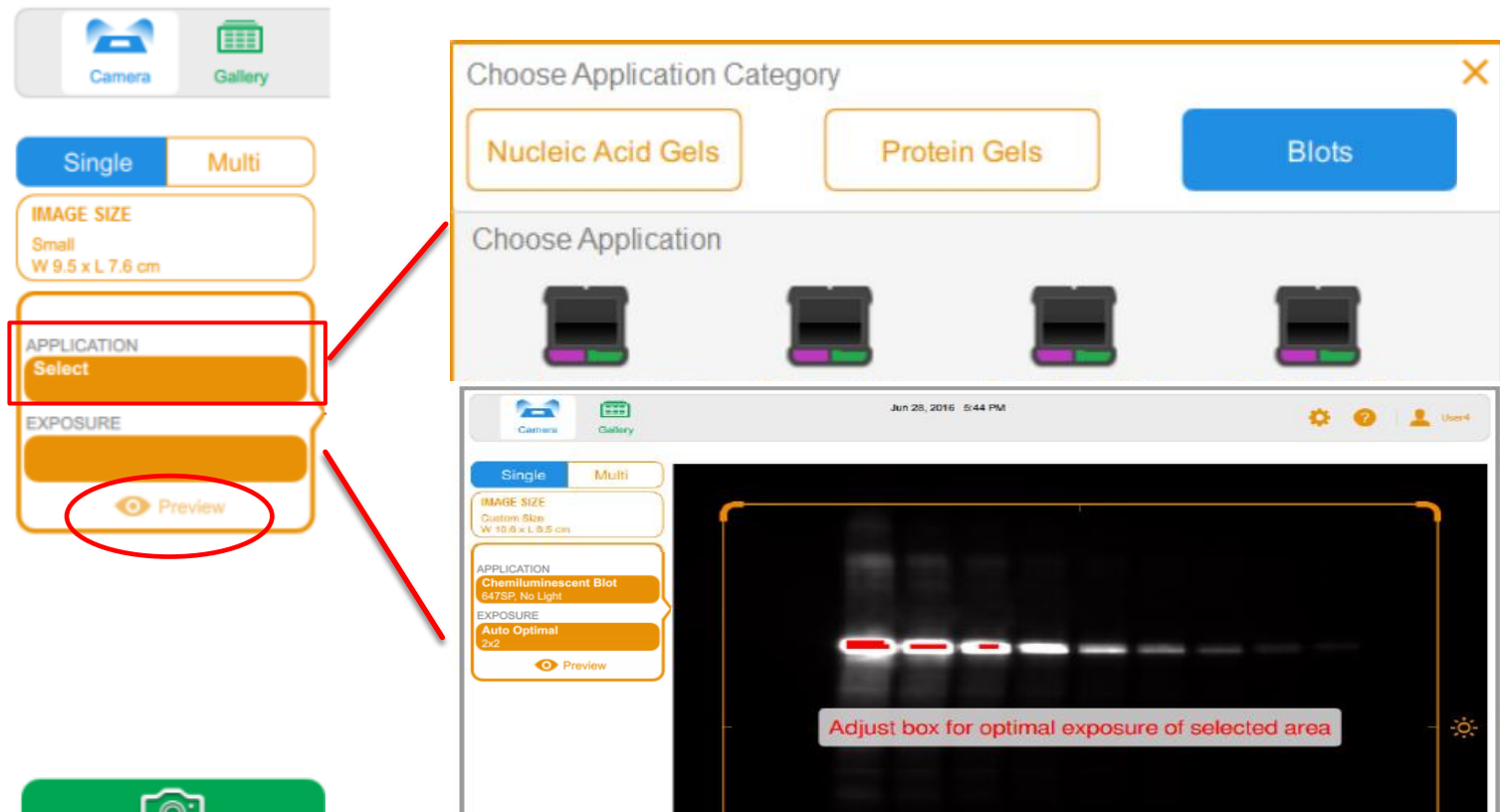


Select application
and exposure



Acquire image
Save or export via USB or Ethernet

指定偵測特定訊號



可手動選擇特定訊號區域，自動偵測最佳曝光時間

Image Information

The screenshot displays the BIO RAD software interface. At the top, there are icons for 'Camera' and 'Gallery', and the date 'Aug 11, 2016 3:34 PM'. Below this, a list of images is shown, with one selected: 'User1 2016-07-27 20h25m39s'. A yellow callout box highlights a red box on the image, which is linked to a 'Name' field containing 'User1 2016-08-09 19h32m45s' and a 'Notes' field. Below these fields is the 'Acquisition Information' section, which includes details about the imager (ChemiDoc™ MP), exposure settings, and acquisition time.

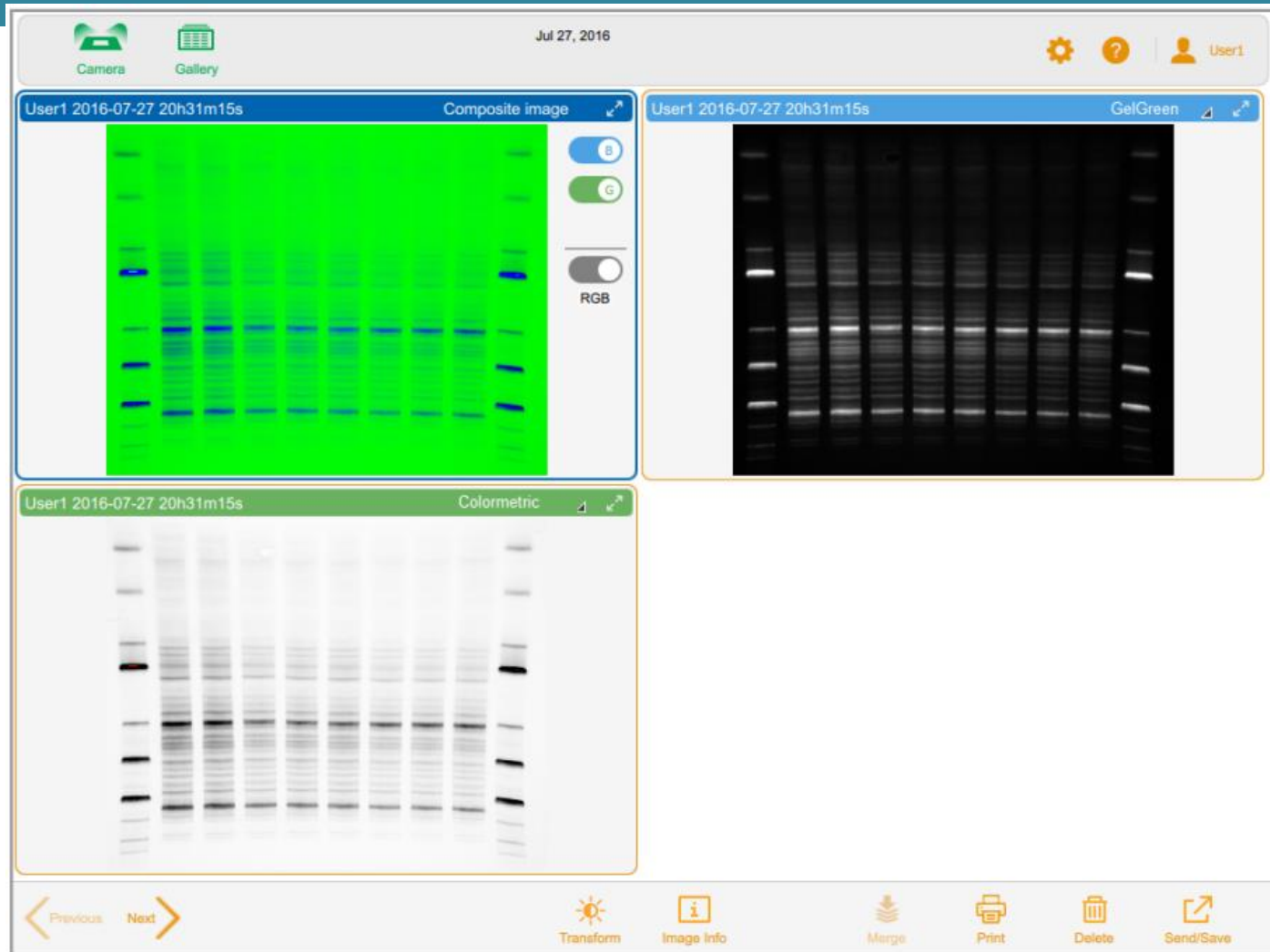
A green callout box highlights the histogram settings. The histogram shows a distribution of pixel intensities with a peak at 'H: 55518' and a minimum at 'L: 0'. The histogram is labeled 'G: 1.00' and has an 'Auto' button. The settings are:

- Log Histogram
- Invert Image Display
- Highlight Saturated

At the bottom of the interface, there is a navigation bar with icons for 'Previous', 'Next', 'Transform', 'Image Info', 'Merge', 'Print', 'Delete', and 'Send/Save'. The 'Transform' and 'Image Info' icons are highlighted with red boxes.

BIO RAD
 生物科技股份有限公司
 All Biotechnology Co., Ltd.

Merge Images



Export Images

The screenshot displays a software interface for managing a gallery of images. The interface includes a top navigation bar with 'Camera' and 'Gallery' tabs, a date and time display (Jul 27, 2016 7:01 PM), and user information (User1). Below the navigation bar, there are controls for 'Select All', 'Show All', and a search bar. The main area shows a grid of images, with four images selected (indicated by blue checkmarks in the top right corner of each image frame). The selected images are: 'User1 2016-07-27 18h51m07s', 'User1 2016-07-27 18h50m39s', 'User1 2016-07-27 18h49m25s', and 'User1 2016-07-27 18h46m42s'. An 'Export Options' dialog box is open, showing the following options:

- Image Lab
- Publication (tif)
- Analysis (raw.tif)
- Publication (jpg)

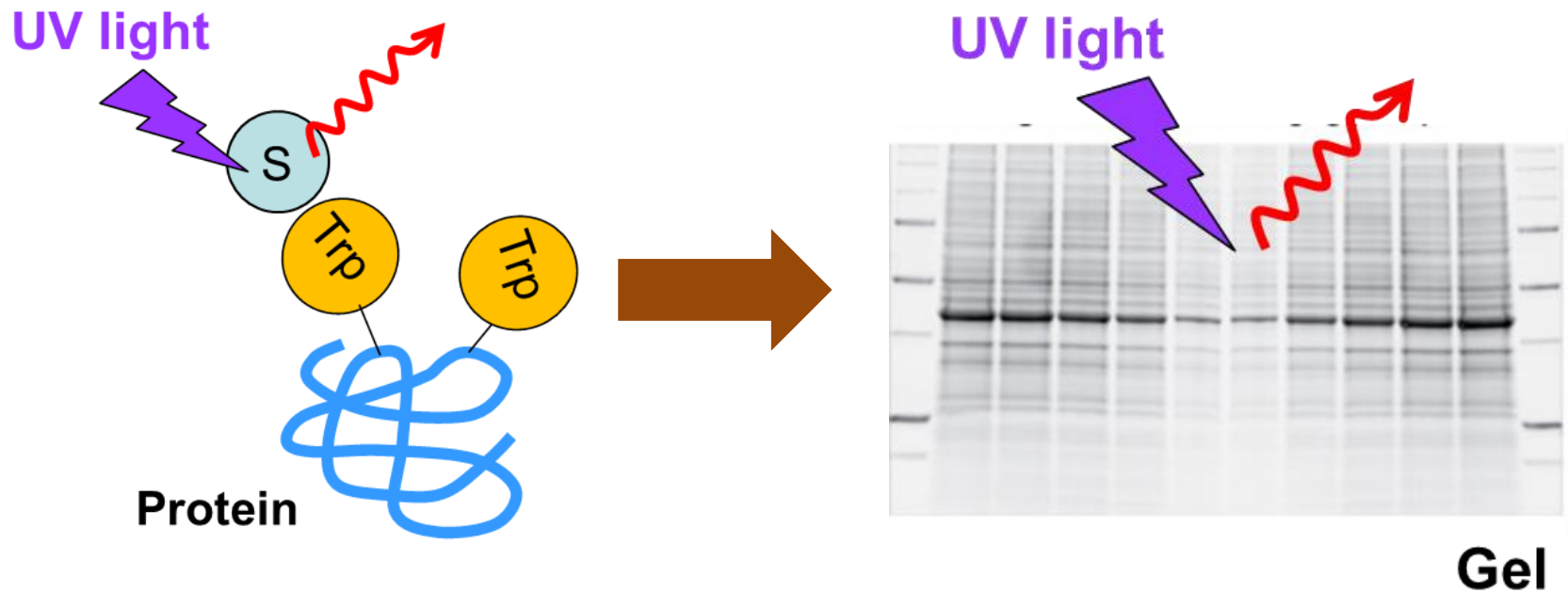
The dialog box is connected to the 'Export' button in the bottom right corner of the interface. The bottom status bar shows '4 of 28 Images Selected' and a 'View 4 Selected Images' button, along with icons for 'Merge', 'Rename', 'Delete', and 'Export/Save'.

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**BIO RAD**

STAIN-FREE TECHNOLOGY



BioRad專利技術，無須額外染色。

StainFree技術是將混在膠體內的特殊compound透過UV 激發後，和蛋白上的Tryptophan結合發出螢光，不需要額外染Coomassie blue就可以在電泳完觀察到total protein結果，增加WB workflow流程內check point點，並能獲得準確Total Protein Normalization定量結果。

BIO RAD

STAIN FREE WESTERN WORKFLOW

Traditional Workflow

Total Time: 16 hr

Step	Workflow	Time	Data
1	Gel Preparation 	>1 hr	—
2	Electrophoresis 	~1 hr	—
3	Transfer 	1-3 hr	—
4	Antibody Incubation 	~5 hr	—
5	Imaging and Analysis 	>30 min	 Target proteins
6	Strip and Reprobe Often need reprobing for actin/tubulin as loading control	~5 hr	 Loading control

Stain-Free Western Blotting

Total Time: 5 hr

Step	Workflow	Time	Data
1	Separate Proteins 	~15 min	 Protein separation at 300 V
2	Visualize Separation 	1 min	 Stain-Free image of pretransferred gel
3	Transfer Proteins 	3-7 min	—
4	Assess Transfer 	2 min	 Stain-Free image of blot
5	Analyze Western Blot by Total Protein Normalization 	~4.5 hr	 Chemiluminescence detection

→ 監控跑膠

→ 確認轉漬

→ 定量確效

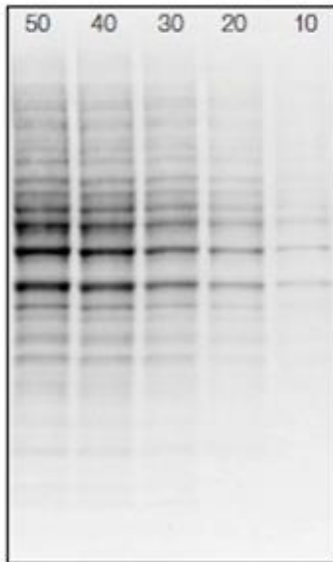
Confidential Result !

Total Protein Normalization

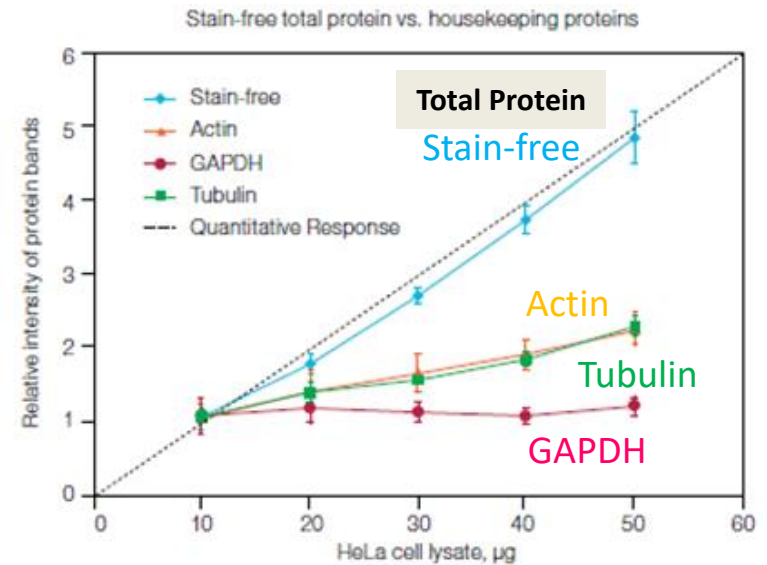
線性結果： Total Proteins vs. Housekeeping Proteins

Total Protein

Stain-free blot image



Housekeeping Protein



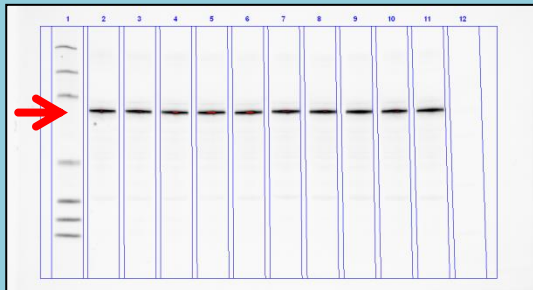
(Life Sciences, Available online 27 February 2013)

Total Proteins show nice excellent linearity!

STAINFREE優勢- TPN vs. HKP NORM.

Validated Housekeeping protein loading control (actin tubulin, GAPDH...)

HKP



1. **Need to Validate Consistency**

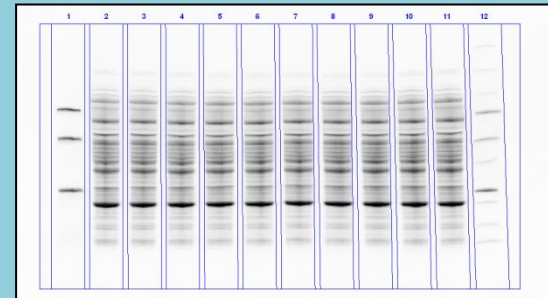
- Validate that HKP expression levels are unaffected by sample treatment

2. **Need Quantitative Detection**

- Protein load
- Antibody concentration
- Chemiluminescent substrate

Total protein loading control

TPN



1. **True measurement of protein load**

- Not dependent on the expression profile of a single protein

2. **Exhibits a broader linear dynamic range**

Total protein loading range for western blotting (10-50 μ g)

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BIO RAD

Bio-Rad Image Lab™ Software

- ✓ Update frequently
(latest: V6.1)

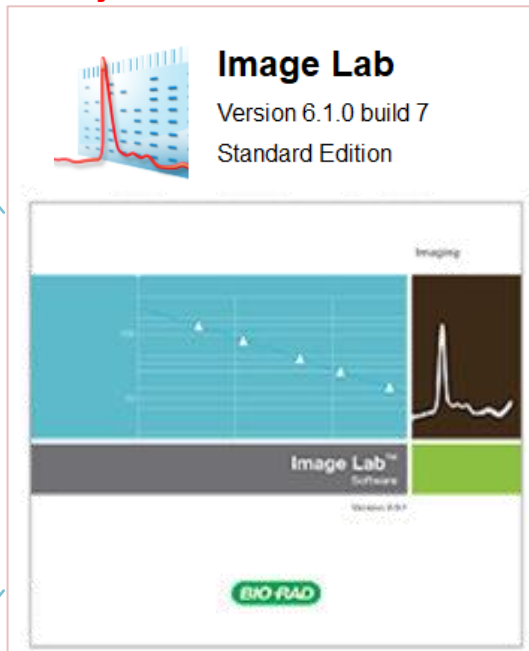
→ Support 16-bit TIF file

**Do everything with
one software**

- ✓ Multifunctional analysis
software

→ Image optimization,
Quantitative analysis,
Calculation, Annotation...

Easy to use



- ✓ License free **For Free**
→ Support (Windows/ Mac)
**Working on your own
computer!**

- ✓ Export the data
→ TIF, JPEG, Excel, Report
(PDF), Export Qualified
Image for Publish
(max: 1200 dpi)

BIO-RAD

Friendly Operator Interface

Image Lab 6.0.1

File Edit View Window Help

New Protocol Open Save Print Screenshot Undo Redo Analysis Table Lane Profile Standard Curve Report Start Page

系統工具列及主視窗工具列

分析工具列

- ← 影像形狀旋轉、裁切
- ← 自動偵測Lane & Band、定量分析、Total protein/ Housekeeping protein normalization
- ← 偵測分子量位置
- ← 相對定量/ 絕對定量計算
- ← 新增文字、箭頭註記
- ← 手動框選欲定量範圍

圖片顯示工具列

ChemiDoc™ XRS+ X Y: BIO-RAD

Multifunction in ImageLab

3D view

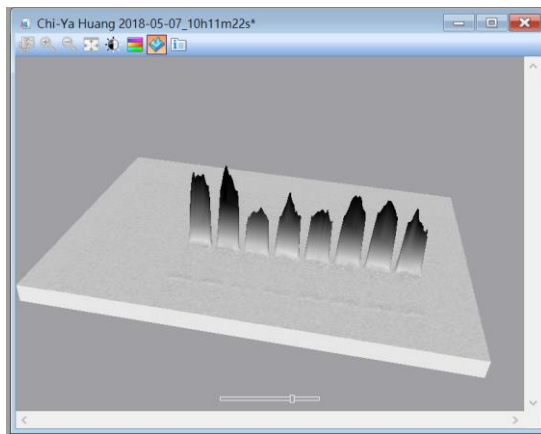


Image Information

Image Info - Donna Chi 2020-02-20_11h... X

Image Details Analysis Settings Notes

Acquisition Information

Imager ChemiDoc™ XRS+

Exposure Time (sec) 1.000 (Auto - Intense Bands)

Serial Number BR-SIMULATED

Software Version 6.0.1.34

Application Chemi

Excitation Source No Illumination

Emission Filter No Filter

Binning 3x3

Image Information

Acquisition Date 2020/2/20 11:06:30 上午

User Name Donna Chi

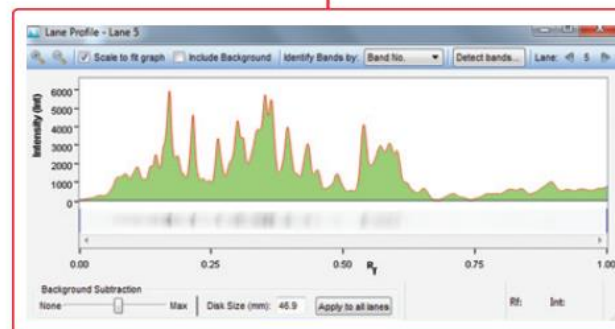
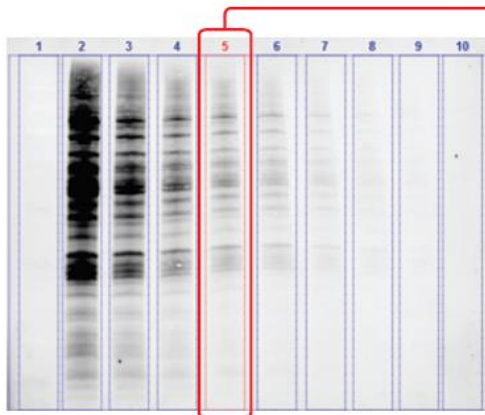
Image Area (mm) X: 139.2 Y: 104.0

Pixel Size (µm) X: 100.0 Y: 100.0

Data Range (Int) 0 - 52320

OK Cancel

Lane profile of protein signal



Total Protein Normalization by ImageLab

The screenshot displays the BIO-RAD ImageLab software interface. The main window shows a gel image with 8 lanes, labeled 'Norm' and 'Chemi'. The 'Norm' lane is highlighted in red, and the 'Chemi' lane is highlighted in green. The software interface includes a menu bar with options like 'New Protocol', 'Open', 'Save', 'Print', 'Screenshot', 'Undo', 'Redo', 'Analysis Table', 'Lane Profile', 'Standard Curve', 'Report', and 'Start Page'. The 'Analysis Table' is open, showing a table with columns: Channel, Lane, Band No., Relative Front, dj. Volume (Int), Volume (Int), Band %, Lane %, Norm. Factor, and Norm. Vol. (Int). The table shows data for 4 lanes, with the first lane being the 'Stain Free Biot' and the others being 'Chemi'.

Channel	Lane	Band No.	Relative Front	dj. Volume (Int)	Volume (Int)	Band %	Lane %	Norm. Factor	Norm. Vol. (Int)
Chemi	1	1	0.423	21,945,375	22,716,375	100.0	73.6	1.000	21,945,375
Lane 2									
Channel	Lane	Band No.	Relative Front	dj. Volume (Int)	Volume (Int)	Band %	Lane %	Norm. Factor	Norm. Vol. (Int)
Chemi	2	1	0.426	21,875,475	22,623,375	100.0	72.0	0.985	21,546,212
Lane 3									
Channel	Lane	Band No.	Relative Front	dj. Volume (Int)	Volume (Int)	Band %	Lane %	Norm. Factor	Norm. Vol. (Int)
Chemi	3	1	0.429	15,754,650	16,335,000	100.0	70.6	1.412	22,248,958
Lane 4									
Channel	Lane	Band No.	Relative Front	dj. Volume (Int)	Volume (Int)	Band %	Lane %	Norm. Factor	Norm. Vol. (Int)

Summary Table:

Channel	Lane No.	Adj. Total Band Vol. (Int)	Total Band Vol. (Int)	Adj. Total Lane Vol. (Int)	Total Lane Vol. (Int)	Bkgd. Vol. (Int)	Norm. Factor
Stain Free Biot	1	N/A	N/A	87,644,025	205,993,425	118,349,400	1.00
Stain Free Biot	2	N/A	N/A	88,983,375	207,426,900	118,443,525	0.98
Stain Free Biot	3	N/A	N/A	62,061,375	164,495,775	102,434,400	1.41
Stain Free Biot	4	N/A	N/A	64,682,625	169,146,975	104,464,350	1.35
Stain Free Biot	5	N/A	N/A	64,005,075	167,681,775	103,676,700	1.37
Stain Free Biot	6	N/A	N/A	49,960,200	143,635,350	93,675,150	1.75
Stain Free Biot	7	N/A	N/A	48,944,325	142,051,425	93,107,100	1.79



Thank you!

Product Specialist

Sunny Tu 杜尚修

Sunny.tu@genmall.com.tw

Mobile: 0905-259-537

BIO-RAD



正茂生物科技有限公司
Genmall Biotechnology Co., Ltd.