请注意,目镜下观察荧光信号较差的样品无法通过共聚焦扫描获得高质量图像。

Tra	cks	Channels			
	Track 1	A647		0.	
	Track 2	mChe		1	
	Track 3	EGFP			
	Track 4	DAPI		- 1	
~	A + 1	D	Expand	All Collapse A	All
Track C Track Lasers	onfiguration 1 - LSIM 405 458	not defined 488 514	→ 543 633		Ū
Track C Track Lasers	1 - LSM 405 458	not defined 488 514	543 633	2.9	Đ
Track C Track Lasers A 6 Pinhole	onfiguration 1 - LSM 405 458 33 nm	not defined 488 514 9	543 633	2.9 - 89.9	
Track C Track Lasers A 6 Pinhole 1.8	onfiguration 1 - LSM 405 458 33 nm 3 Airy Units	not defined 488 514 5 0 ≜ 25.1 μm sect	■ ■ 543 633	2.9 1 AU m	
Track C Track Lasers A Pinhole 1.8 A647	onfiguration 1 - LSM 405 458 33 nm 3 Airy Units Gain (Ma	not defined 488 514 ! 0 ≜ 25.1 μm sect ister)	543 633	► ► 0 2.9 89.9 1 AU m 638	
Track C Track Lasers A Pinhole 1.8 A647	onfiguration 1 - LSM 405 458 33 nm 3 Airy Units Gain (Ma Digital O	not defined 488 514 9 0 ≜ 25.1 µm sect ister)	543 633	2.9 89.9 1 AU m - 638	

点击"Channels"按钮,调节下列参数: Pinhole Gain (Master) Digital Offset Digital Gain 激光强度

Trac	ks	Channels			
	Track 1	A647		Π.	
	Track 2	mChe			
	Track 3	FGFP			
	Track 4	DAPI			
	∧ + m		Expand A	UI Collapse /	All.
Track Co	of muration	tdofined		e	-
Track Lasers	1 - LSM	88 514 54	3 633		
Track	1 - LSM 405 458 4	88 514 54	3 633	2.9	
Track Lasers	1 - LSM 405 458 4 33 nm	88 514 54	3 633	2.9	
Track Lasers A 63 Pinhole 1.83	1 - LSM 405 458 4 33 nm Airy Units ≜	88 514 54 25.1 μm sectio	3 633	2.9 - 89.9 1 AU m	w Tax
Track Lasers A 60 Pinhole 1.83 A647	1 - LSM 405 458 4 33 nm Airy Units ≜ Gain (Maste	88 514 54 	3 633 m	2.9 - 89.9 1 AU m - 638	m P Nax
Track Lasers 63 Pinhole 1.83 A647	1 - LSM 405 458 4 33 nm Airy Units ≜ Gain (Maste Digital O <u>ffse</u>	25.1 μm sectio r)	3 633	2.9 - 89.9 (1 AU m - 638 - 0	ax
Track Lasers 65 Pinhole 1.83 A647	1 - LSM 405 458 4 33 nm ——— Airy Units ≜ Gain (Maste Digital Offse Digital G <u>ain</u>	25.1 μm sectio r)	3 633	2.9 - 89.9 (1 AU m - 638 - 0 - 1.0	

点击"Channels"按钮,调节下列参数: Pinhole ——该值增加,光通量增加, 图像亮度上升、信噪比变好。通常该值 为1AU,再最大程度上屏蔽非焦平面的信 号。

Trac	:ks	Channels		
	Track 1	A647		
	Track 2	mChe		
	Track 3	EGFP		
	Track 4	DAPI		
×			Evened A	II Callenas All
	A	w.	Expano A	II Collapse All
Track Co Track Lasers	nfiguration 1 - LSM 405 458	mot defined 488 514 543	€Xpanu A 633	in Conlapse All
Track Co Track Lasers	1 - LSM 405 458	not defined 488 514 54:	633	2.9
Track Co Track Lasers A 63 Pinhole	1 - LSM 405 458 33 nm	not defined 488 514 543	633	2.9 \$
Track Co Track Lasers A 65 Pinhole 1.83	1 - LSM 405 458 33 nm	mot defined 488 514 543 0 0 ≙ 25.1 μm section	633	2.9 ÷ 89.9 ÷ 1 AU max
Track Co Track Lasers Action 1.83	1 - LSM 405 458 33 nm 3 Airy Units Gain (Ma	mot defined 488 514 543 0 ≙ 25.1 μm section aster)	633	2.9 \$ 89.9 \$ 1 AU max
Track Co Track Lasers A647	anfiguration 1 - LSM 405 458 33 nm 34 Airy Units Gain (Ma Digital O	mot defined 488 514 54: 0 0 ≙ 25.1 µm section ister)	633	2.9 \$ 89.9 \$ 1 AU max 638 \$ 0 \$

点击"Channels"按钮,调节下列参数: Gain(Master) ——该值增加,图像 对比度上升,过高则信噪比下降,图像 粗糙。通常Gain值在500~700之间。

Trac	ks	Channels		
	frack 1	A647		0.
	Frack 2	mChe		
	Frack 3	EGFP		
	frack 4	DAPI		
~	∧ +	ŵ	Expand All Co	ollapse All
-				
Track Co Track Lasers	nfiguration 1 - LSM 405 458	not defined 488 514 54	€ 633	8
Track Co Track Lasers	nfiguration 1 - LSM 405 458 3 nm	not defined 488 514 543	€ 633	2.9
Track Co Track Lasers A 63 Pinhole	nfiguration 1 - LSM 405 458 3 nm —	not defined 488 514 543	633 633	⊣ ⊤ 2.9 ‡ 9.9 ‡
Track Co Track Lasers A 63 Pinhole 1.83	nfiguration 1 - LSM 405 458 3 nm	not defined 488 514 543 0 0 ≜ 25.1 μm sectio		EI ⊕ 2.9 ‡ 9.9 ‡
Track Co Track Lasers A Pinhole 1.83	nfiguration 1 - LSM 405 458 3 nm Airy Units Gain (Ma	not defined 488 514 54 0 ≜ 25.1 μm sectio aster)		El ⊕ 2.9 ‡ 3.9 ‡ .AU ma) 88 ‡
Track Co Track Lasers 63 Pinhole 1.83 A647	nfiguration 1 - LSM 405 458 3 nm Airy Units Gain (Ma Digital O	not defined 488 514 54: 0 ≜ 25.1 µm section aster)		2.9 ‡ 2.9 ‡ .AU max 8 ‡

点击"Channels"按钮,调节下列参数: Digital Offset ——背景扣除,但标 本信号也有一定程度的扣除,需要调节 其它参数来弥补。背景过分消除会导致 图像失真。

Trac	:ks	Channels		
	Track 1	A647		
	Track 2	mChe		
	Track 3	EGFP		
	Track 4	DAPI		. •
~	~ + 0	Ū.	Expand	All Collapse All
Track Co Track Lasers	nfiguration 1 1 - LSM 405 458	not defined 488 514 54	■ ▼ 13 633	► ► ■
Track Co Track Lasers	1 - LSM 405 458 33 nm	488 514 54	3 633	Provide 10 minutes
Track Co Track Lasers A 6: Pinhole	1 - LSM 405 458 33 nm	488 514 54	3 633	 2.9 ₹89.9
Track Co Track Lasers A 6: Pinhole 1.8:	onfiguration 1 - LSM 405 458 33 nm	not defined 488 514 54 0 25.1 µm sectio	3 633 m	2.9 ÷ 89.9 ÷ 1 AU max
Track Co Track Lasers (A 6: Pinhole 1.8: A647	onfiguration 1 - LSM 405 458 33 nm 3 Airy Units ≜ Gain (Mas	488 514 54 0 25.1 μm sections ter)	3 633	 ₽ ₽ ₽ 2.9 2.9 2.9 2.9 2.9 3 4 <
Track Co Track Lasers A647	anfiguration r 1 - LSM 405 458 33 nm Airy Units ≜ Gain (Mas Digital Off	not defined 488 514 54 0 25.1 µm section ter) set	3 633 m	2.9 ↓ 89.9 ↓ 1 AU max 638 ↓

点击"Channels"按钮,调节下列参数: Digital Gain-一对所有信号进行数码 放大,包括来自样品的真实信号和环境 背景噪音。通常设置为1。

Trac	ks	Channels		
	Track 1	A647		
	Track 2	mChe		
	Track 3	EGFP		
	Track 4	DAPI		II +
~	~ + 🛈		Expand /	All Collapse All
Track Co Track Lasers	nfiguration m 1 - LSM 405 458 4	ot defined 188 514 54	I3 633	
Track Co Track Lasers	nfiguration n 1 - LSM 405 458 4	ot defined 188 514 54	I3 633	₽ 1.1 1.1 1.1 1.2 1.2 1.1 1.2 1.1 <
Track Co Track Lasers A 65 Pinhole	nfiguration nd 1 - LSM 405 458 4 13 nm	ot defined 188 514 54	I3 633	 Image: Big (1) Image: Big (2) Image: Big (2)<!--</td-->
Track Co Track Lasers A 6: Pinhole 1.83	nfiguration nd 1 - LSM 405 458 4 13 nm	ot defined 188 514 54 25.1 μm sectio	I3 633	In the second secon
Track Co Track Lasers A Pinhole 1.83	nfiguration nd 1 - LSM 405 458 4 13 nm	ot defined 88 514 54 0 25.1 μm sectio	I	 ➡ ➡ ■ 2.9 2.9 39.9 1 AU max 538
Track Co Track Lasers A Pinhole 1.83 A647	nfiguration nd 1 - LSM 405 458 4 13 nm i Airy Units ≜ Gain (Master Digital Offse	ot defined 88 514 54 25.1 μm sectio er)	u √ 13 633	 ₽ ₽ ₽ 2.9 <l< td=""></l<>
Track Co Track Lasers A Pinhole 1.83 A647	nfiguration nd 1 - LSM 405 458 4 33 nm Airy Units = Gain (Master Digital Offser	ot defined	I3 633	2.9 ↓ 89.9 ↓ 1 AU max 638 ↓ 0 ↓

点击"Channels"按钮,调节下列参数: <u>激光强度</u>——激光调大,图像整体亮度 上升,信噪比变好,但是容易淬灭。在 保证图像质量的前提下,激光强度越低 越好。

扫描图像参数设置

1024

2048

💌 🛥 Acquisition	Mode 🗸	Show all
Objective	EC Plan-Neofluar 10x/0.30 M27	128 x 256 x 2
Scan Mode	Frame	→ 512 x 3
Frame Size	X 512 🗘 X*Y Y	512 🗘 1024 x
Line Step		Optimal 2048 x
Speed	9\$	Max 6144
Pixel Dwell	1.58 µsec Scan Time 2.90 sec	01447
Averaging		
Number	Bit Depth 8 B	it 🔽 1
Mode	Line Direction >	2
Method	Mean	8
HDR		16
👻 Scan Area		
	Image Size: 848.5 µm x 8	48.5 um
	Pixel Size: 1.66 um	
		Ç C
		\$ 0
	• Zoom [] 1.0	1
·		Reset All

1、从"Frame Size"栏中选择图像的大小 (e.g : 512x512 or 1024x1024) 2、从"Scan speed"栏中选择扫描速度(一 般情况下,快速扫描速度数值设定为9,正式 出图时设定为4~7)。 3、从 "Mode" 栏中选择 "Line", 从 "Method"栏中选择"Mean".从"Number" 栏中选择扫描时采用几次平均(一般情况下, 快速扫描采用一次平均;正式出图时,4次平 均的图像质量已经可以接受)。 4、扫描时间和速度:时间长、速度慢,图像

信噪比好,清晰度高。但是耗时,易淬灭。