DeltaVision Ultra 32-Bit Deconvolutions



By default, softWoRx converts deconvoluted images to 16 bit. This feature is problematic with the 16-bit images from the sCMOS camera, because deconvolution increases a signal, which can, hence, exceed the 16-bit range. Some images within a series of samples (with some high signal) may be rescaled to fit 16 bits, while others (with lower signal) may be left unchanged, making it impossible to compare them side-by-side or to stitch them. The following instructions describe workflows for deconvolutions in a 32-bit range, circumventing this problem.

1. Manual deconvolutions

In softWoRx, setup a deconvolution as usual. In the "Deconvolve" dialog, click "More Options...". In the dialog popping up

- uncheck "Save intermediate Results as 2-byte Integer"
- check "Save Final Results as Floating-Point"

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Output	y				
Select Re	gion Reset Details				
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Deconvo	olution Options				
OTF F	ile				
1	vlethod Ratio (conservative)				
Number of	Cycles 10				
Noise F	Noise Filtering Medium (200 nm)				
Apply Correction					
Deconvolve Projections					
Run Options More Options 🗹 Show image when finished					
Done	Do It	Help			

	More	e Deconvoluti	on Options	 S
- Deconvo	lution Opt	ions ———		
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	Sn	noothing (0-1)	0.150	
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	Intensity	Scale Factor	[1.000	_
– Correcti	on Option:	s ———	,	
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😽 Replace	e Z-Lines	😿 Smooth Z	-Lines	
Camera In	tensity Offsi	et In		
Pass Wav	es l'Innroce	ssed: II I		1
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Wave 2	Subtract	10:000		
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Wave 4	Subtract	[0.000		
Wave 5	Subtract	[0.000		
Save In	termediate	Results as 2-b	iyte Integer ┥	
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🔽 Automa	tically Term	inate		
Close				Help



2. Deconvolutions as background task

Acquire Ultra allows to perform deconvolutions as background task while acquiring images. The standard Task Builder chain can be used, but the Task Builder GUI exposes only a reduced set of optional parameters. To perform 32-bit deconvolution, select "Save 4 byte floating point" in the "Deconvolution Task Options" dialog:

Deco	\odot	\otimes	
	Auto-Select OTF		
OTF File	I		
Deconvolution Method	Ratio (conservative)		
Number of Cycles	<u>]</u> 10		
Deconvolve Projections			
	Save 4 byte floating point		
	 Apply Correction 		
	✓ Normalize Intensity		
	🔽 Use Photosensor		
Camera Intensity Offset	Įo		
Done			

If you want to have full control on all parameters, use the following procedure instead of the Task Builder:

a) Acquire Ultra

• In the "Processing" tab, *uncheck* "Enable Post-Acquisition Processing"



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- In the "Run Protocol" tab, uncheck "Deconvolve during time lapse"
- Acquire the first image

 Experiment Channels * Z Sectioning * Time Series * Slide/Plate Paneling Processing 	Experiment Channels * Z Sectioning * Time Series * Slide/Plate Paneling Processing					
Run Protocol	Deconvolve during time lapse	Deconvolution Options				
	Change Time Lapse					
	Add Note to Log File	Note text				
	Run Stop					

b) softWoRx

- The first deconvolution has to be done manually, including the adjustments for 32bit deconvolutions as outlined in chapter 1 (Manual deconvolutions)
- Once all parameters are set, click "Run Options". Check "Run at Low Priority". In the "Run Options" combo box, select "Add To Queue", then "Close". In the "Deconvolve" Dialog, click "Do It"

Deconvolve	\odot	\otimes			
Input					
Output					
Select Region Reset Details					
Wavelengths 🔟 🔟 🔟 🔟					
- Deconvolution Options					
OTF File					
Method Ratio (conservative) -					
Number of Cycles 10					
Noise Filtering Medium (200 nm)					
pply Correction					
L Deconvolve Projections					
Run Options More Options 🔽 Show image when finishe	d				
Done Do It	_	Help			

	Deconvolution Run Options 🕑 🛞					
Log File	ome/facility/Data/TEST/Convallaria_01_R3D_D3D_log.txt					
Command File	me/facility/Data/TEST/Convallaria_01_R3D_D3D_cmd.sh					
Run Options	Add To Queue 💷					
🗸 Run at Low Priority 🔽 Show Output Log						
Maximum Number of CPUs to use 4						
Close						



• The "Queue Manager" dialog should pop up and display the job submitted, including the name of the command file (ending with "_R3D_D3D_cmd.sh"), which is required in the next step.

softWoRx Queue Manager (Host: dv-c02.deltavision.internal.mfpl.ac.at) 🕑 🔗 🛛 🛞					
Current Job: <a>local queue not running > Cancel Job Pause After Job					
0% Done					
Queued Loca	al Jobs:				
Job ID	Owner	Status	Command	Delete	
Job ID Owner Status Command Delete 1 facility Queued Convallaria_01_R3D_D3D_cmd.sh X Start Now					
Quit Delete My Queued Jobs Help					

c) Ultra-Monitor helper program

- On the desktop, click on the "Ultra-Monitor" icon
- Click "Create...". First select the command file from the previous step and then choose a file name for the executable to be created. The file name will end with "_R3D_D3D_exe.sh". By clicking "Executable...", you can reuse a previously created executable without creation of a new one (as long as your acquisition protocol remains unchanged)
- Click "Directory" and select the folder in which Acquire Ultra saves your images

	-	l	Ultra Monitor	\odot \odot \otimes
	Executable	No executab	le set	Create
	Directory	No directory set		
Ultra-Monitor	Time	Status	File	
		No	content in table	
	4	CPU Threads	✓ Notifications	Start

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- Click "Start" to monitor the selected directory. If the "Notifications" check box is checked, a notification will appear upon completed deconvolutions (in the lower right corner of the screen)
- The control "CPU Threads" allows to adjust the number of CPU kernels used (a high value interferes with fast acquisitions). The maximum is 8, the default is 4

d) Limitations

The helper program tolerates different slice numbers or time points in time series, as well as changes in exposure time or intensity. All other changes of the acquisition protocol (in particular addition or removal of channels) may require creation of a new executable from a softWoRx deconvolution command file. Deconvolutions of running time series is not possible (they will be processed automatically once a series has ended). Multi-image acquisitions ("Paneling") have not yet been tested.