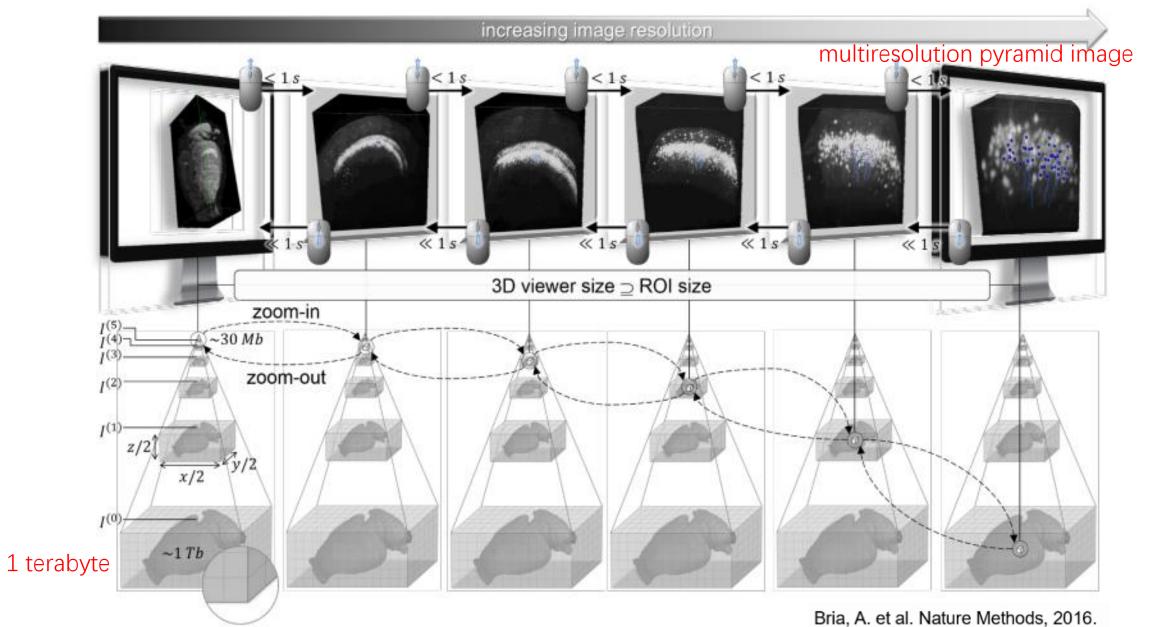
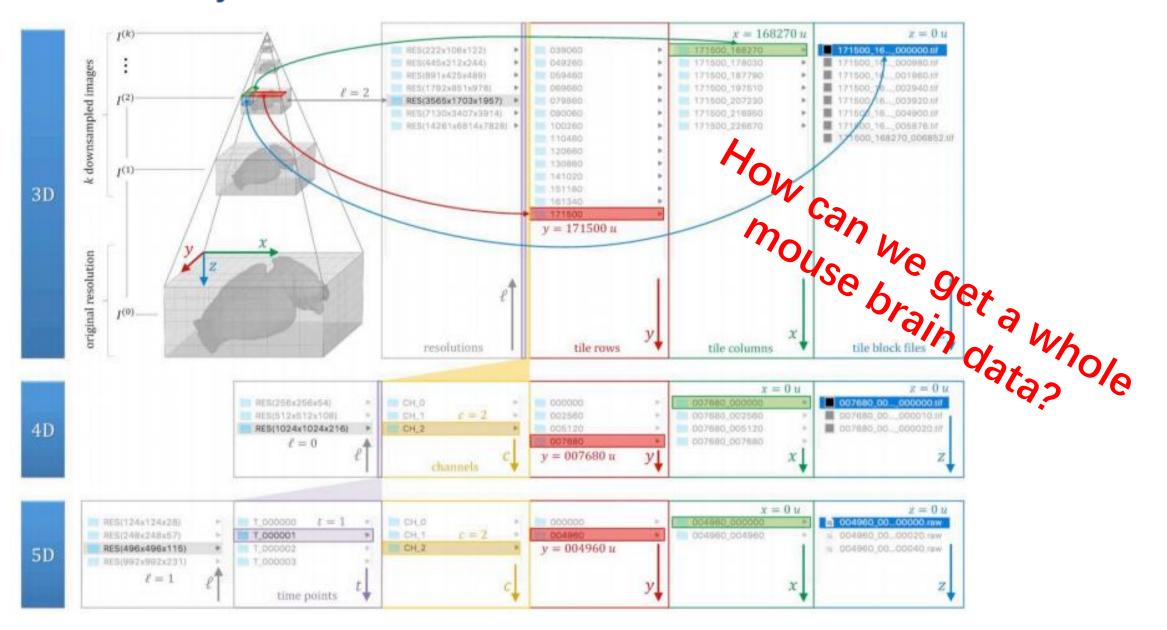
# How to use TeraStitcher and NeuronStitcher

2021-11-09 Yanyan Guo

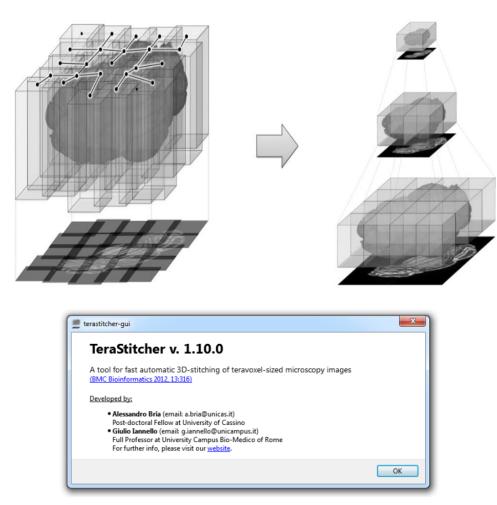
## Vaa3D-TeraFly: Architecture



### Vaa3D-TeraFly: Schema of 3D - 5D Formats



# **TeraStitcher: Introduction**

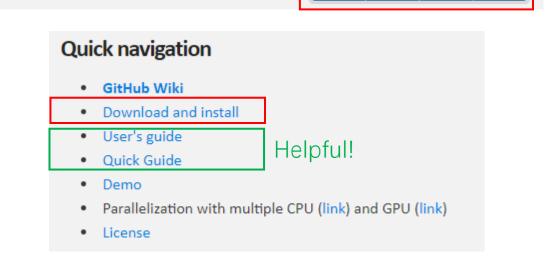


Bria A, Iannello G (2012) TeraStitcher—a tool for fast automatic 3D-stitching of teravoxel-sized microscopy images. BMC Bioinform 13:316

#### https://abria.github.io/TeraStitcher/

#### TeraStitcher

A tool for fast automatic 3D-stitching of teravoxel-sized microscopy images (BMC Bioinformatics 2012, 13:316)



**ZIP File** 

TAR Ball

GitHub

Binaries

**TeraStitcher** is a powerful software tool designed to **stitch** very large datasets corresponding to 3D volumes acquired with a wide range of acquisition systems.

# **Step 1: Import the RUI**

#### RUI: the Raw Unstitched Image

1.1 Direct import of RUI

1	<u>1</u>	TeraStitcher v1.10.0 (with Qt 5.2.1)						<	1		
	File	e Options Help									
		1 Import 2 Align 3 Merge				2	1		<b>An xml file</b> must		
L		Volume/XML path: C:/mydata/	_			I/O plugin:	be generated that				
h		Filenames regular expression:				🔽 Rescan files 🔲 S	parse data		is compliant with		
3	$\mathbb{H}$	Y - X	-	Z	•	j _	If tiles may have a		what TeraStitcher		
4	_	— voxel (µm): 1,00 🚔 voxel (µm): 1,0	)	voxel (µm): 1,00		5	different number of		expects.		
T		Volume's informations					slices or may even be empty				
L		Absolute path:									
L		Number of tiles:		1	×						
H		Tile dimensions (voxels):			×		×				
		Voxel's dims (µm):			×		×				
		Origin (mm):									
H		Tile overlap (voxels):		1	×				000000		
l		Stitch test:	S	lice 0/0		unselected 🔻	6 Preview		002000		
H									004000		
L							Stop		mdata.bin		
									xml_import		

## 1.2 Import using an externally generated xml import file

TeraStitcher v1.10.0 (with Qt 5.2.1)				
File Options Help				
Import Align Import form		2	]	1
Volume/XML path: C:/mydata/xml_import.xml		tiff2	<b>-</b> C	Select folder Select XML
Filenames regular expression:		Rescan files	Sparse da	ita
Volume's informations			3	
Absolute path:				
Number of tiles:		×		
Tile dimensions (voxels):		×	>	<
Voxel's dims (µm):		×	>	<
Origin (mm):				
Tile overlap (voxels):		×		
Stitch test:	Slice 0/0	unselected	V	4 Preview
				Start Stop
Ready.				mdata.bin

## 1.3 Generation of a preview image

Import form							
Volume/XML path: C:/mydata/xml_i	mport.xml		tiff2D	* Select	folder Select XML		
Filenames regular expression:			Rescan files	oarse data			
Volume's informations							
Absolute path:	C:/Users/Roberto/Deskto	p/Test_Data/u	ta/unstitched_2D_init_2				
Number of tiles:	3 (rows)	×	3 (columns)				
Tile dimensions (voxels):	300 (X)	×	300 (Y)	×	500 (Z)		
Voxel's dims (µm):	1 (X)	×	1 (Y)	×	1 (Z)		
Origin (mm):	2		1		3		
Tile overlap (voxels):	100 (X)	×	100 (1)		L'		
Stitch test:	Slice 250/500		unselected - A channel		Q Preview		

Volume/XML path:			Import fro	om dir	Open XML
Image name regex:			I/O plug	jin: 🔻	(Re-)scan all files
			Volume fo	ormat 🔻	Sparse data
Volume's informations					
Absolute path:					
Number of tiles:		×			
Tile dimensions (voxels):		×		) × [	
Voxel's dims (µm):		×		) × [	
Origin (mm):					
Tile overlap (voxels):		×			
Stitch test:	Slice 0/0		all channels	-	Q. Preview

Ready.

🐼 Stop

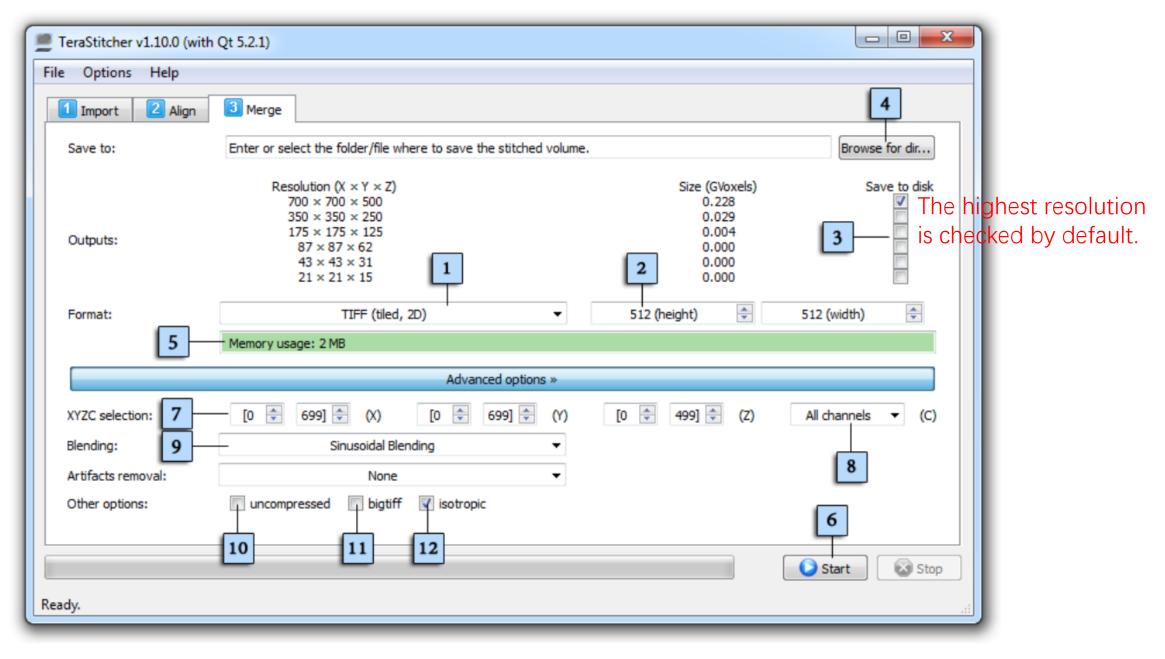
Start

## **Step 2: Compute the corrected alignments**

TeraStitcher v1.10.0 (with Qt 5.2.1)					Ľ		
File Options Help							
1 Import 2 Align 3 Merge			1				
Save project XML to:	C:/mydata/xml_displcon	np.xml					
Algorithm:	MIP-NCC	•					
Number of slices per layer: 2	100	-					
Channel selection:	unselected	•					
Estimated memory usage:	137.329 MB		5				
		Advanced op	tions »				
4							
Data subset selection (rows):	0	🔹 to	2	-			
Data subset selection (columns):	0	🗢 to	2	· · · · · · · · · · · · · · · · · · ·			
Data subset selection (slices):	0	🗢 to	499	•			
Search Region (voxels):	25 (X)	×	25 (Y)	÷ ×	25 (Z)	· · · · · · · · · · · · · · · · · · ·	
Overlap (voxels):	100 (X)	×	100 (Y)	-			
SPIM artifacts removal:	Compute stacks prof	iles to be used	l in the Merging tiles ste	ep	6		
					Start	Stop	
Ready.							

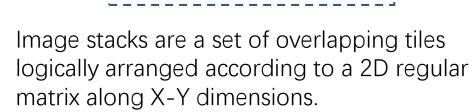
Image name regex: opencv2D • (Re-)scan all   TiledXY[2Dseries • Sparse data   Volume's informations   Absolute path:   C:\Campus BioMedico\Stitching\Data\TeraStitcher_TESTSET\tomo300511_subv2.sparse   Number of tiles: 2 (rows) × 2 (columns)   Tile dimensions (voxels): 512 (X) × 512 (Y) × 600 (Z)   Voxel's dims (µm): -0.8 (X) × 0.8 (Y) × 1 (Z)	Importing Aligning	3 Merging						
Image name regex: opencv2D • (Re-)scan all   TiledXY[2Dseries • Sparse data   Volume's informations   Absolute path: C:\Campus BioMedico\Stitching\Data\TeraStitcher_TESTSET\tomo300511_subv2.sparse   Number of tiles: 2 (rows) × 2 (columns)   Tile dimensions (voxels): 512 (X) × 512 (Y) × 600 (Z)   Voxel's dims (µm): -0.8 (X) × 0.8 (Y) × 1 (Z)	port form							
TiledXY[2Dseries Sparse data   Volume's informations C:\Campus BioMedico\Stitching\Data\TeraStitcher_TESTSET\tomo300511_subv2.sparse   Absolute path: C:\Campus BioMedico\Stitching\Data\TeraStitcher_TESTSET\tomo300511_subv2.sparse   Number of tiles: 2 (rows) × 2 (columns)   Tile dimensions (voxels): 512 (X) × 512 (Y) × 600 (Z)   Voxel's dims (µm): -0.8 (X) × 0.8 (Y) × 1 (Z)	ta/TeraStitcher_TESTSET/tor	no300511_subv2.sparse3	D/xml_in	nport.xml	Import	from o	lir	Open XML
Volume's informations   Absolute path:   C:\Campus BioMedico\Stitching\Data\TeraStitcher_TESTSET\tomo300511_subv2.sparse   Number of tiles: 2 (rows) × 2 (columns)   Tile dimensions (voxels): 512 (X) × 512 (Y) × 600 (Z)   Voxel's dims (µm): -0.8 (X) × 0.8 (Y) × 1 (Z)	mage name regex:				open	cv2D	٣	(Re-)scan all file:
Absolute path: C:\Campus BioMedico\Stitching\Data\TeraStitcher_TESTSET\tomo300511_subv2.sparse   Number of tiles: 2 (rows) × 2 (columns)   Tile dimensions (voxels): 512 (X) × 512 (Y) × 600 (Z)   Voxel's dims (µm): -0.8 (X) × 0.8 (Y) × 1 (Z)				[	TiledXY	2Dseri	es 🔻	Sparse data
Absolute path: C:\Campus BioMedico\Stitching\Data\TeraStitcher_TESTSET\tomo300511_subv2.sparse   Number of tiles: 2 (rows) × 2 (columns)   Tile dimensions (voxels): 512 (X) × 512 (Y) × 600 (Z)   Voxel's dims (µm): -0.8 (X) × 0.8 (Y) × 1 (Z)								
Number of tiles:   2 (rows)   ×   2 (columns)     Tile dimensions (voxels):   512 (X)   ×   512 (Y)   ×   600 (Z)     Voxel's dims (µm):   -0.8 (X)   ×   0.8 (Y)   ×   1 (Z)	ume's informations							
Tile dimensions (voxels):   512 (X)   ×   512 (Y)   ×   600 (Z)     Voxel's dims (µm):   -0.8 (X)   ×   0.8 (Y)   ×   1 (Z)	solute path:	C:\Campus BioMedic	co\Stitchi	ng\Data\Ter	aStitcher_T	ESTSE	T\tomo300	511_subv2.sparse3D
Voxel's dims (µm): -0.8 (X) × 0.8 (Y) × 1 (Z)	umber of tiles:	2 (rows)	×	2	(columns)			
	le dimensions (voxels):	512 (X)	×	1	512 (Y)		×	600 (Z)
Octobe (mm): 15 0000 (V) 7.2 (V) 12 000 (7)	ixel's dims (µm):	-0.8 (X)	×		0.8 (Y)		×	1 (Z)
Origin (mm): 15.8088 (X) 7.2 (Y) 13.899 (Z)	rigin (mm):	15.8088 (X)			7.2 (Y)			13.899 (Z)
Tile overlap (voxels): 137 (X) × 137 (Y)	le overlap (voxels):	137 (X)	×	£ [	137 (Y)			
Stitch test: Slice 300/600 🖨 all channels 👻 Q Preview	itch test:	Slice 300/600		all	channels	-	ſ	Q Preview

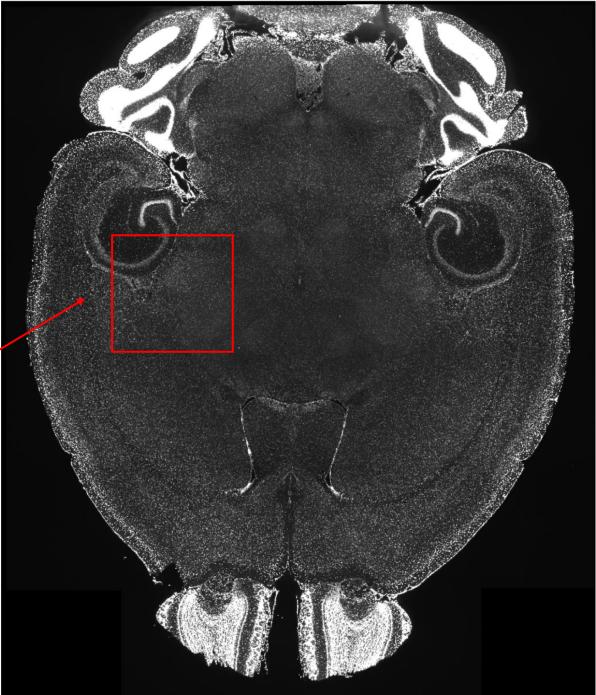
# **Step 3: Generate a stitched image**

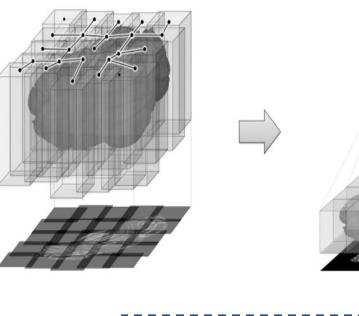


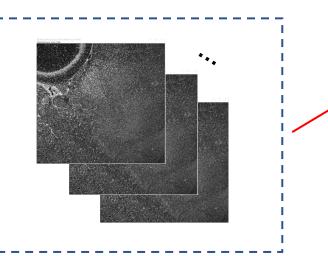
le Options	Help								
Importing	Alignin	g 🚺 Pr	ojecting	Threshol	ding 🚺	Placing	Merging		
Save to:	Enter or selec	t the directo	where to	o save the stitch	ed volume.				
Outputs:	889 × 444 × 222 × 111 > 55 > 27 > 13	on (X × Y × 7 896 × 594 448 × 297 224 × 148 < 112 × 74 < 56 × 37 < 28 × 18 × 14 × 9 × 7 × 4	, , ,			Size (GVo: 0.441 0.055 0.007 0.001 0.000 0.000 0.000 0.000		Save to disk	Open
Format:	Volume fo	rmat 🔻		olugin: 🔻	512 (he Memory	ight) 🚔	512 (width) 9 MB	256 (de	pth) 🌲
				Advanced of					
Selection:	[0 🗘 1]	(rows)	[0 🗘	1] 🗘 (cols)	[3	\$ 596]	(slices)	stitchab	les only
Blending:	-	usoidal Blen		•	remove	SPIM arti	acts:	None	•
substi	tuted with a bl	ended versi	on of them		step has N	OT been per	formed, nomin	erlapping region al stage coordir nough.	

8444x10071 pixels; 16-bit; 162MB

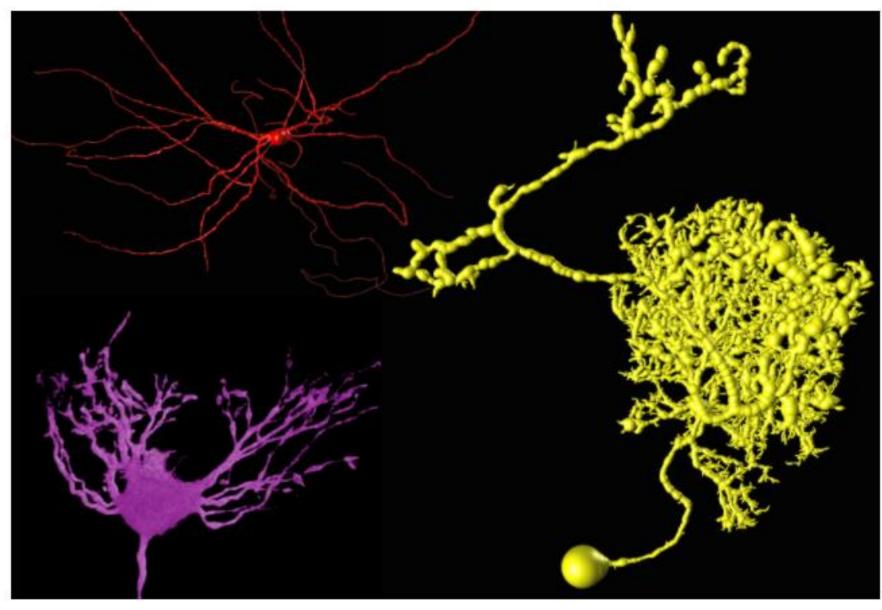








## NeuronStitcher



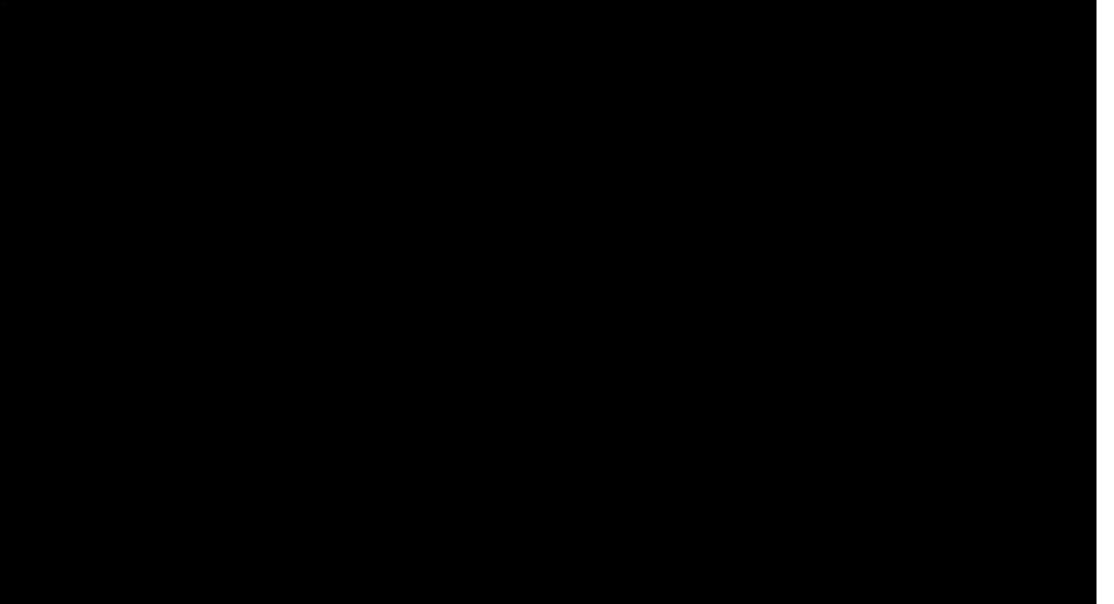
**Big picture:** NeuronStitcher can reconstruct entire neurons from multiple sections of brain tissue.

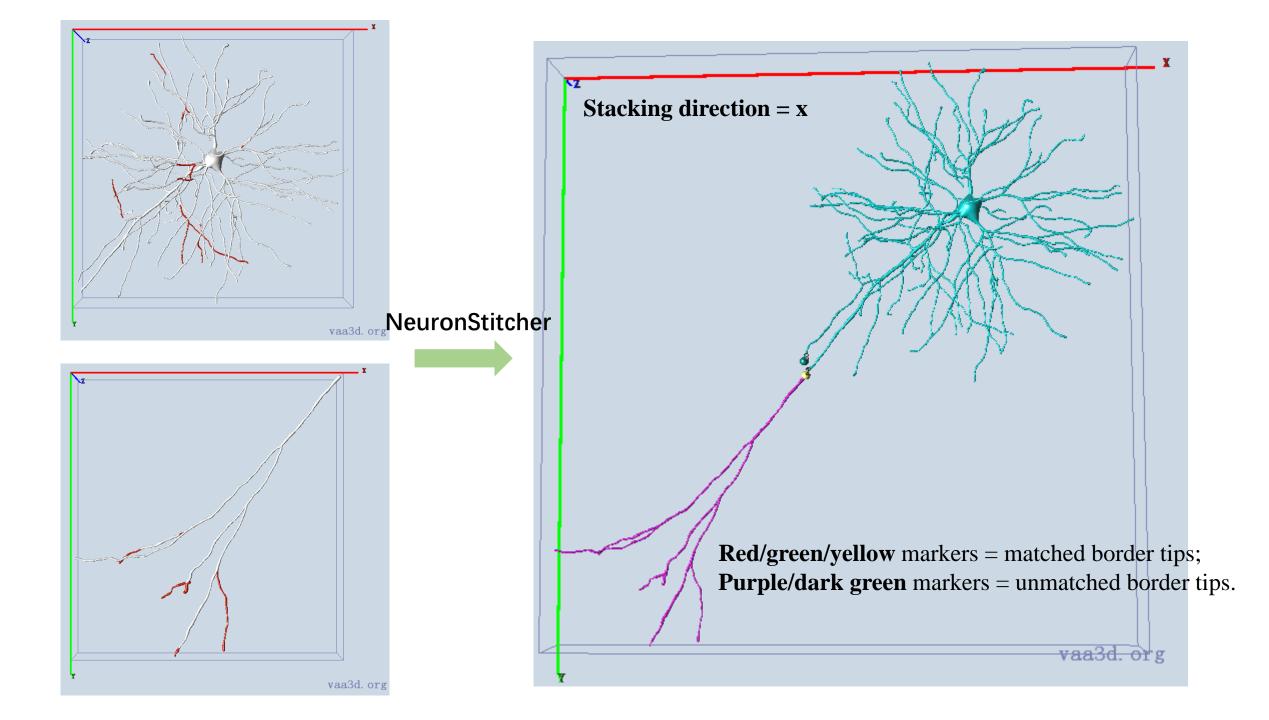
The framework matches separated dendritic or axonal **branches** 

H. Chen, D. M. Iascone, N. M. da Costa, E. S. Lein, T. Liu, and H. Peng, "Fast Assembling of Neuron Fragments in Serial 3D Sections," *Nat. Methods*.

# live\_stitch\_neuron\_SWC **Interface**:

neuron_stitch	•	1_stitchers	•	auto_stit	ch_neuron_SWC	2	
neuron_toolbox	•	2_search_border	r_tips →	live_stitc	h_neuron_SWC		
neuron_tracing	•	3_file_transform	•	_	 /_affine_neuron	SWC	
neuron_utilities	•	4_file_combine	+	about		-	
neuronQC	· · · · · · · · · · · · · · · · · · ·						
🔍 vaa3d_msvc 1. Au	itomati	ically matc	h and	align <b>ne</b>	euron se	gmei	nts ×
Step 1: match and affine	]						itch
stacking	direction:	Ζ		resacle stack	ing direction:	1.00	-
Max angular to match point	s (0~180):	91.00	] r	lax distance to	match points:	100.00	\$
Max distance to match	triangles:	100.00	Мах г	umber of trian	gles to match:	1000	-
mat	ch by type d	lefined in SWC file					
🗹 search for border tips	, otherwise	use existing ones:					
match candidates sear	ching span:	20.00	] 5	small gap filte	r (gap size):	0.00	-
fragment filter (O	=keep all):	0.00	-				
🗌 filter spines when mat	ching:						
segment point #: 5	* *	turning angle	: 30.00	* *	radius:	3.00	* *
Step 2: stitch paired poin	ts 2. N	<b>lanually</b> m			•		
	•	Manually Add	SI	kip	Stitch	Stite	ch All
zoom-in view the pair to							
Caution: zoom in view is	only for vis	sual inspection. Fo	r manual e	diting, please	operate in the	original :	
🗹 Auto Launch Local View		Launch I	Local View	Wind	ow margin: 50		-
Load the 3D image of the	section to d	lisplay: Bottom	Section	Top Sec	ction		
Color neuron by: stitch	result 💌				Save	Qı	uit





Thank you!