生物学者のワークフローにフィットさせた可視化解析ソフトの開発と応用

大綱　英生

University of Utah, Neurobiology & Anatomy
Scientific Computing Imaging Institute

FluoRender

http://fluorender.com

Collaboration with Y. Wan & C. Hansen

Scientific Computing Imaging Institute

FluoRender

http://fluorender.com

Y. Wan; programmer

H Otsuna; GUI, function design, parameter adjustment

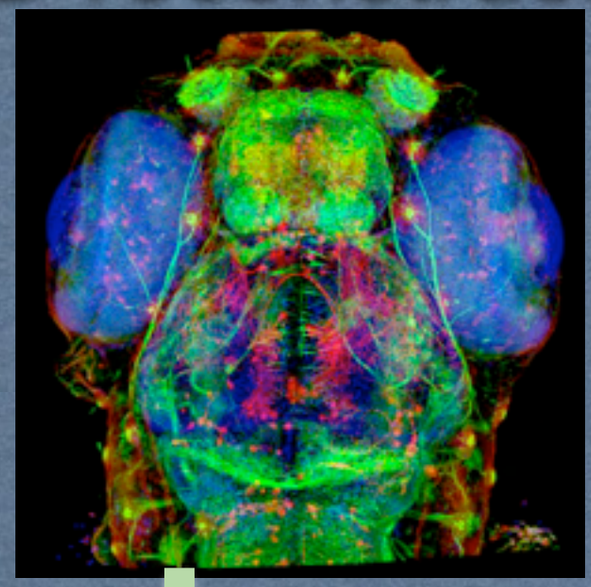
共焦点レーザー顕微鏡のデータから論文の図を作る

confocal microscope

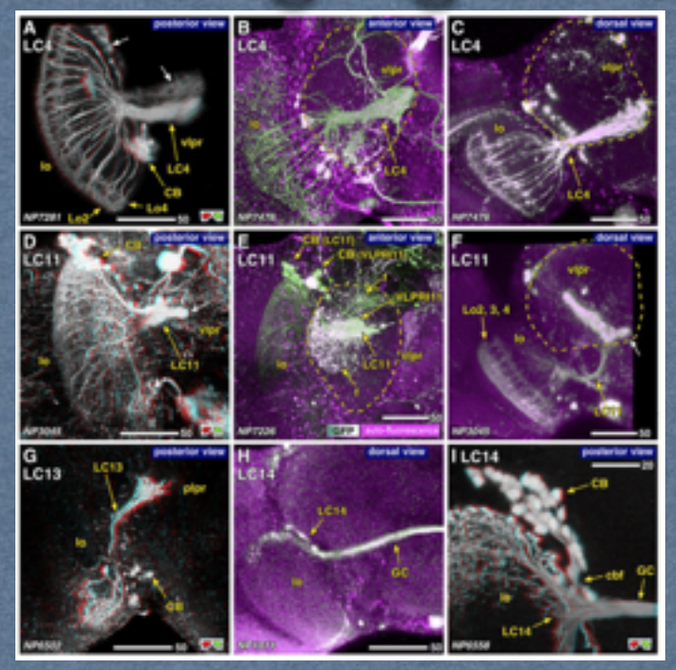
volume data

need to be screen
for good sample

3D/4D reconstruction

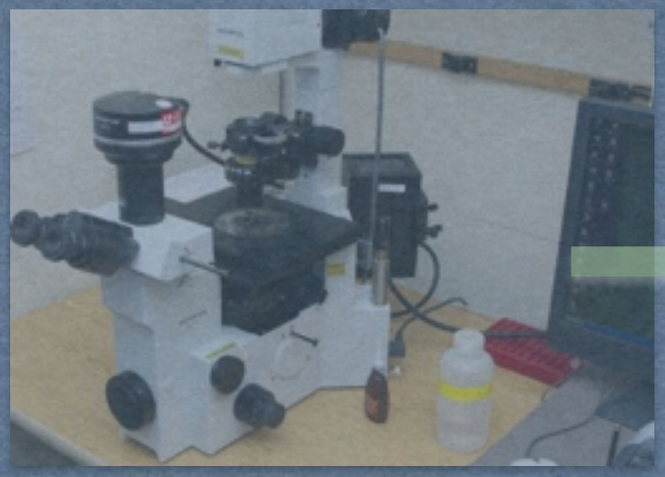


Making figures

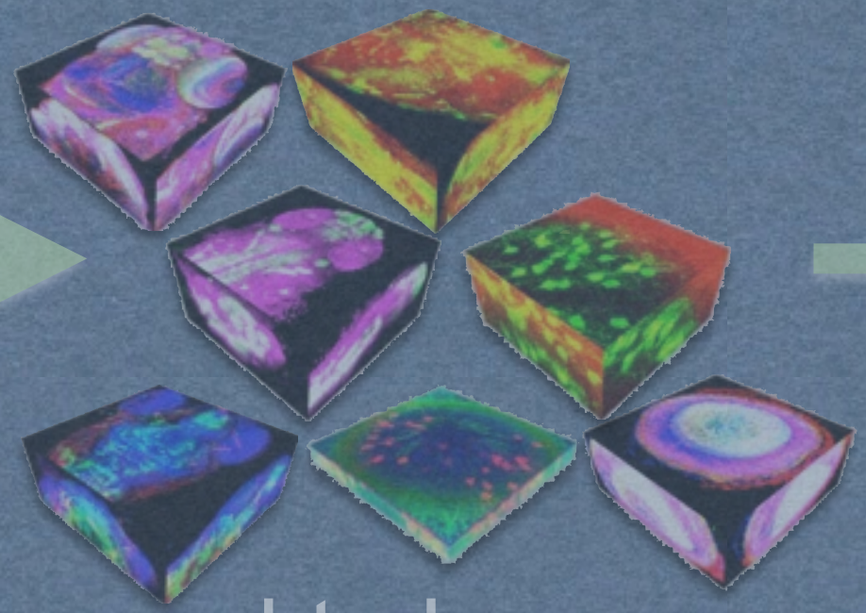


共焦点レーザー顕微鏡のデータから論文の図を作る

confocal microscope

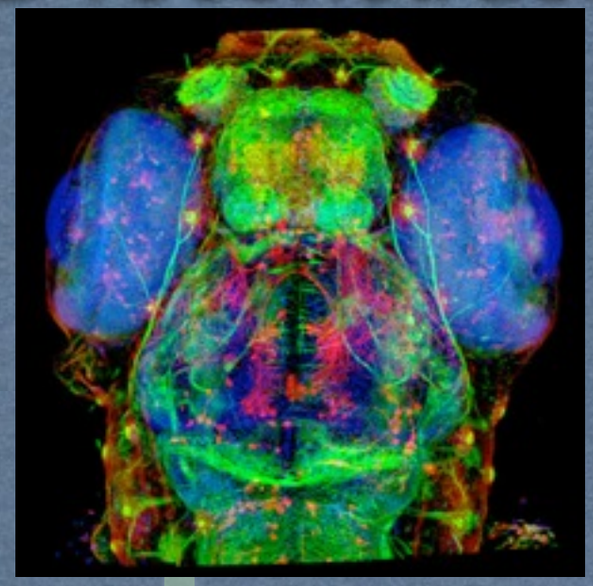


volume data

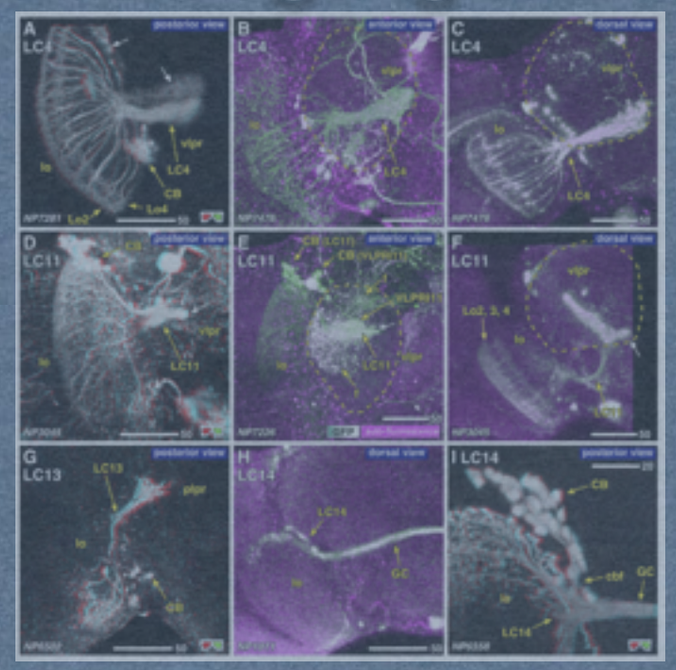


need to be screen
for good sample

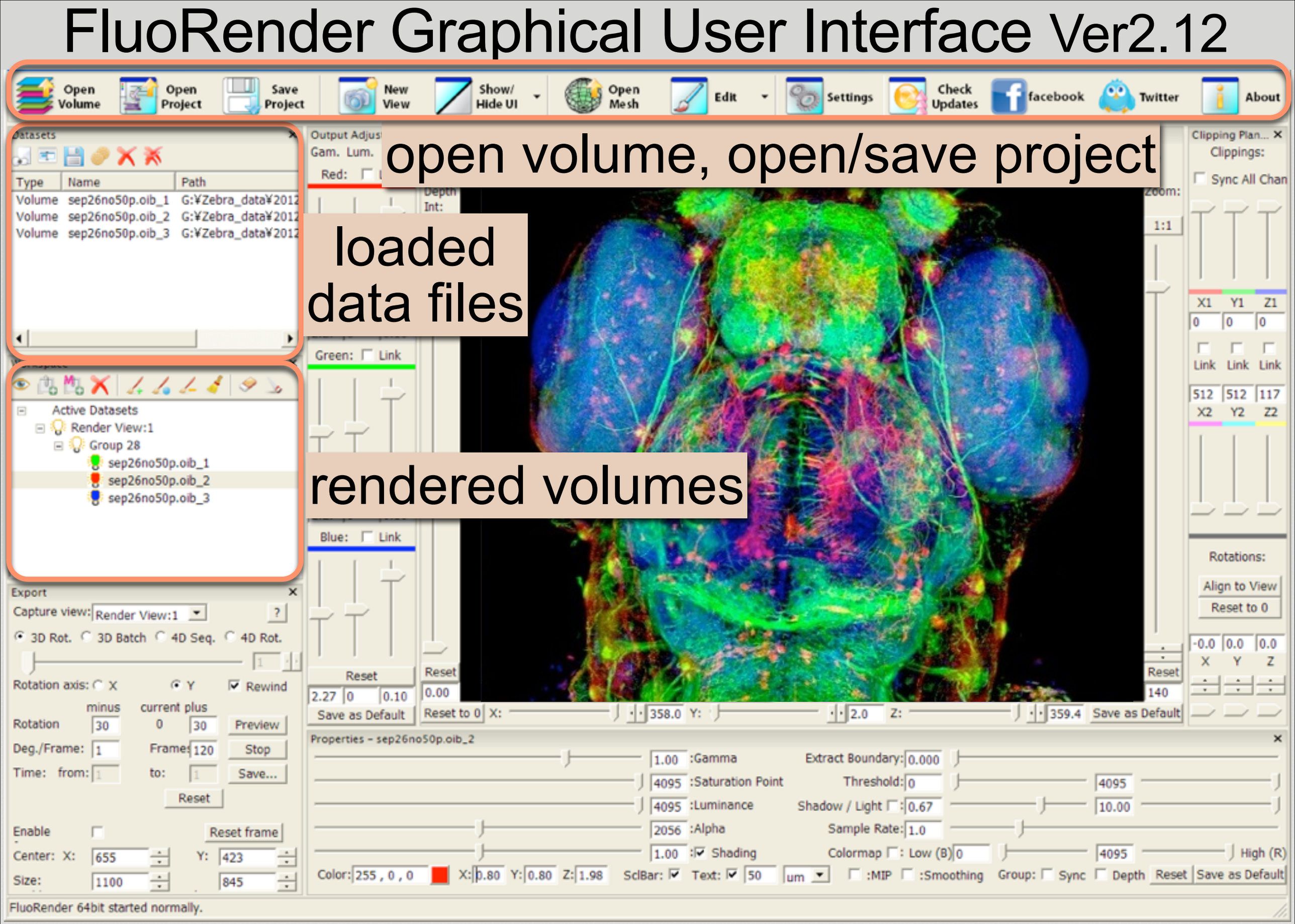
3D/4D reconstruction



Making figures



FluoRender Graphical User Interface Ver2.12



FluoRender Graphical User Interface Ver2.12

Open Volume | Open Project | Save Project | New View | Show/ Hide UI | Open Mesh | Edit | Settings | Check Updates | facebook | Twitter | About

Datasets

Type	Name	Path
Volume	sep26no50p.oib_1	G:¥Zebra_data¥2012
Volume	sep26no50p.oib_2	G:¥Zebra_data¥2012
Volume	sep26no50p.oib_3	G:¥Zebra_data¥2012

Workspace

- Active Datasets
  - Render View:1
    - Group 28
      - sep26no50p.oib_1
      - sep26no50p.oib_2
      - sep26no50p.oib_3

Output Adjust... Gam. Lum. Eql.

Red: Link — Reset — 2.27 | 0 | 0.10

Green: Link — Reset — 2.27 | 0 | 0.10

Blue: Link — Reset — 2.27 | 0 | 0.10

Save as Default

Render View:1

Layered | Depth | Composite | Capture | Persp | Ortho | FreeFly | Background: | Center | FPS | Name

Depth Int: — Reset — 0.00

Zoom: 1:1 — Reset — 140

Reset to 0 | X: 358.0 | Y: 2.0 | Z: 359.4 | Save as Default

rendering view,
rendering mode settings

Clipping Plan... Clippings:

Sync All Chan

X1	Y1	Z1
0	0	0
Link	Link	Link
512	512	117
X2	Y2	Z2

Rotations:

Align to View | Reset to 0

-0.0	0.0	0.0
X	Y	Z

Export

Capture view: Render View:1 ?

3D Rot. | 3D Batch | 4D Seq. | 4D Rot.

Rotation axis: X | Y | Rewind

	minus	current	plus	
Rotation	30	0	30	Preview
Deg./Frame:	1	Frames:	120	Stop
Time: from:	1	to:	1	Save...

Reset

Enable — Reset frame

Center: X: 655 Y: 423

Size: 1100 845

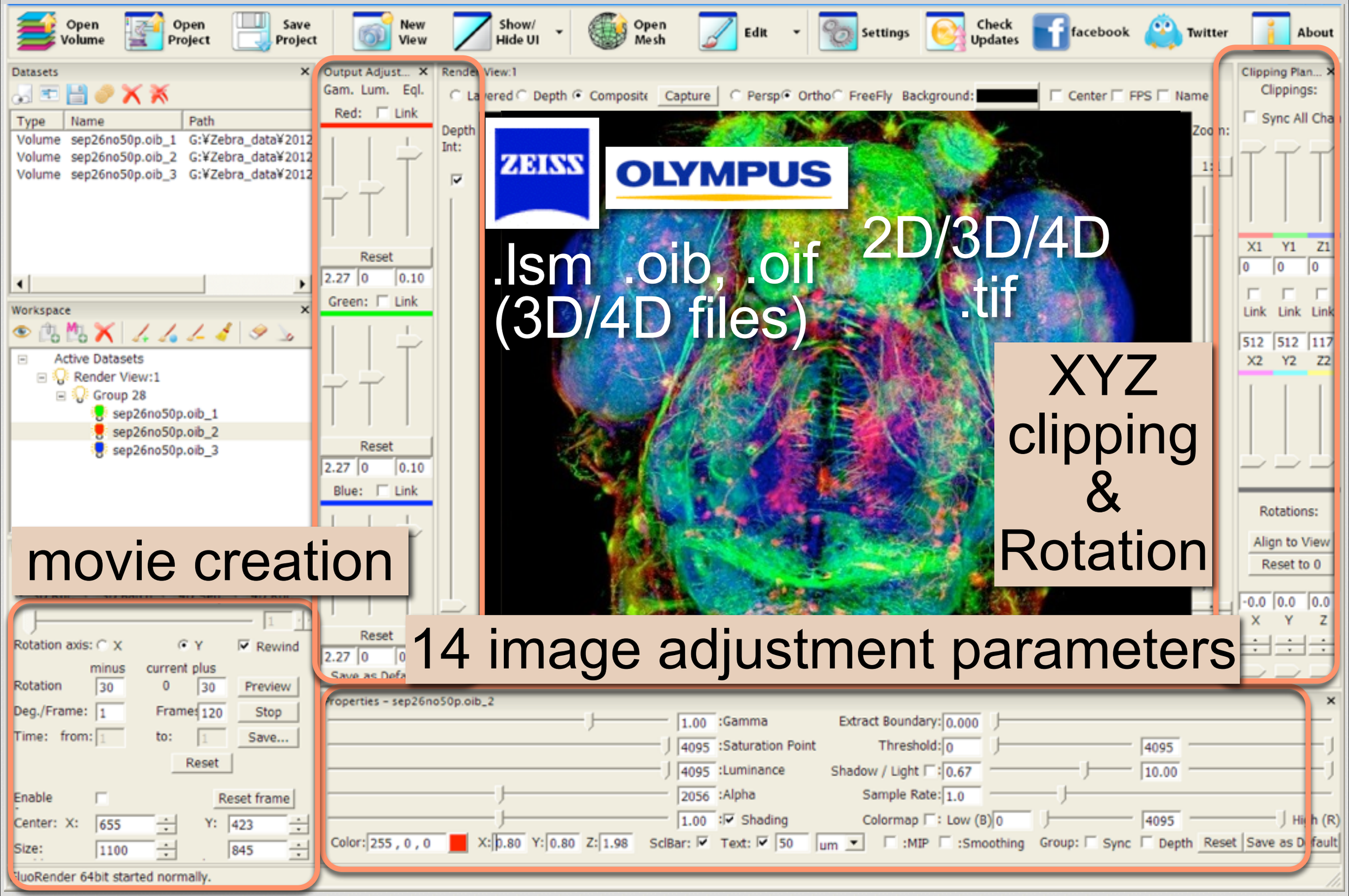
Properties – sep26no50p.oib_2

1.00 :Gamma		Extract Boundary: 0.000	
4095 :Saturation Point		Threshold: 0	4095
4095 :Luminance		Shadow / Light: 0.67	10.00
2056 :Alpha		Sample Rate: 1.0	
1.00 :Shading		Colormap: Low (B) 0	4095 High (R)

Color: 255 , 0 , 0 X: 0.80 Y: 0.80 Z: 1.98 SclBar: Text: 50 um :MIP :Smoothing Group: Sync Depth Reset Save as Default

FluoRender 64bit started normally.

FluoRender Graphical User Interface Ver2.12



なぜ共焦点レーザー顕微鏡が必要なのか？

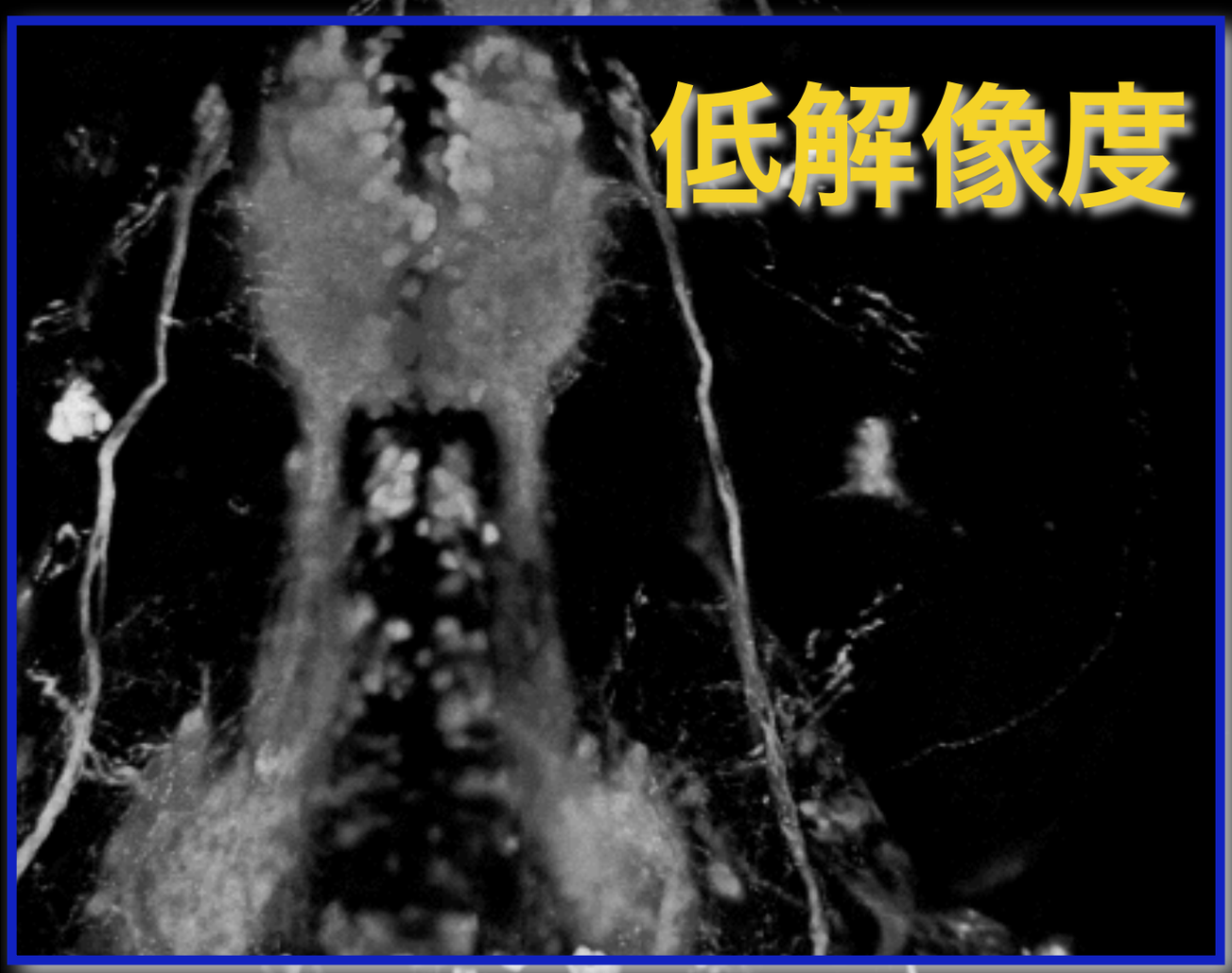
- 複式顕微鏡よりも高解像度 (x, y, z)
- 複式顕微鏡より高いS/N比
- 三次元の構造データを取得可
- 異なる生物組織を複数のチャンネルに分けてデータ取得可能

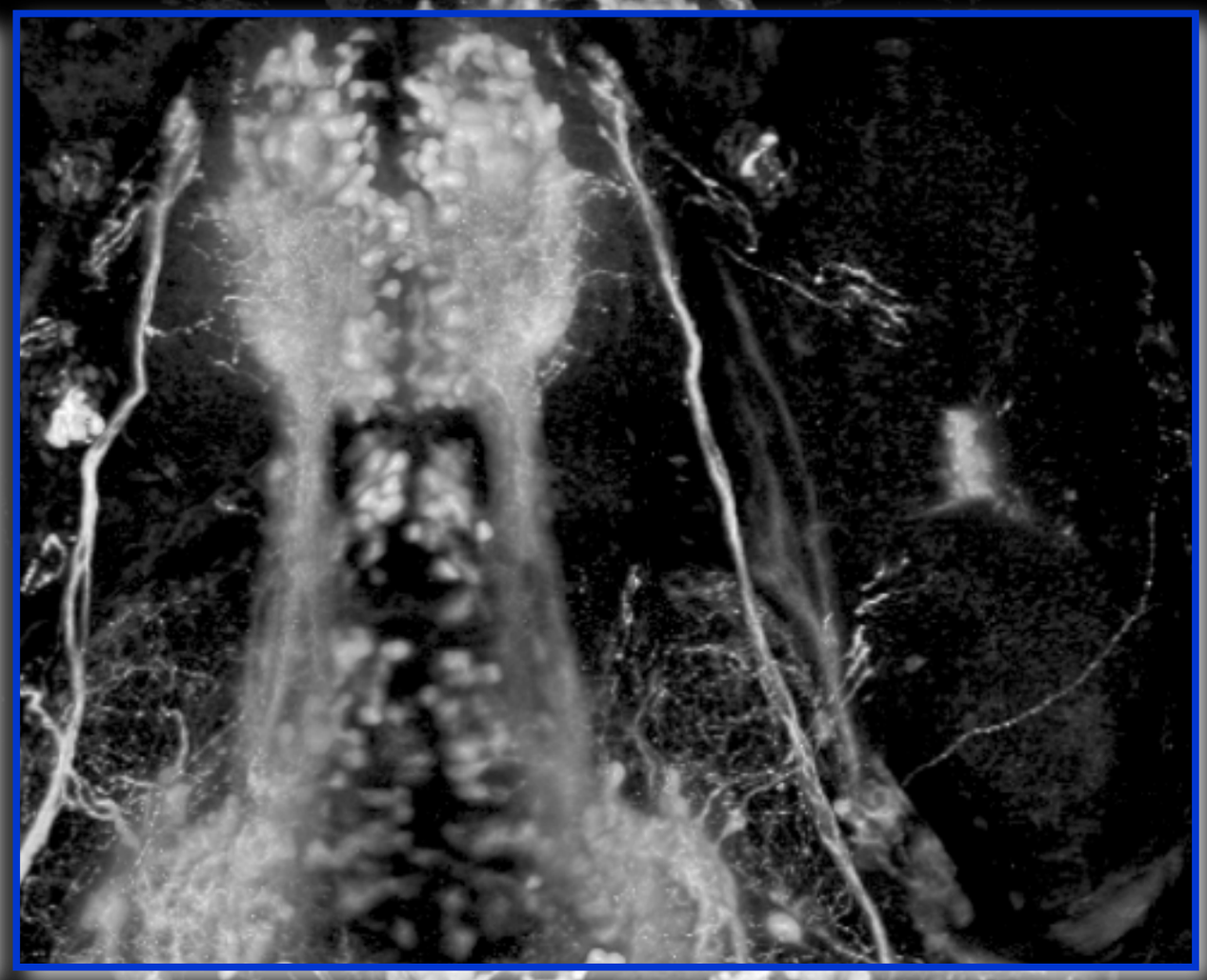
FluoRender does not lose any information from your confocal data

Volocity

FluoRender

低解像度



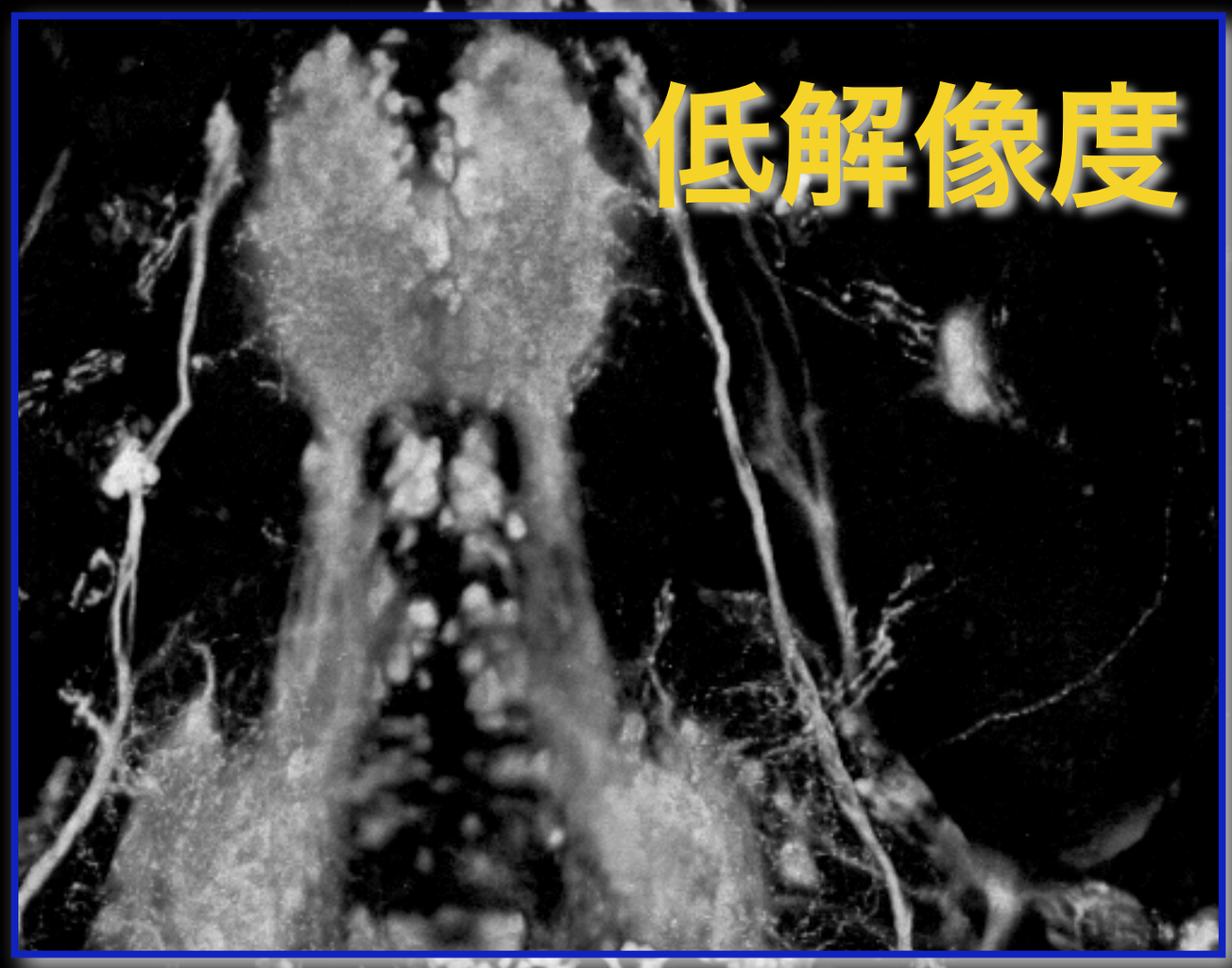


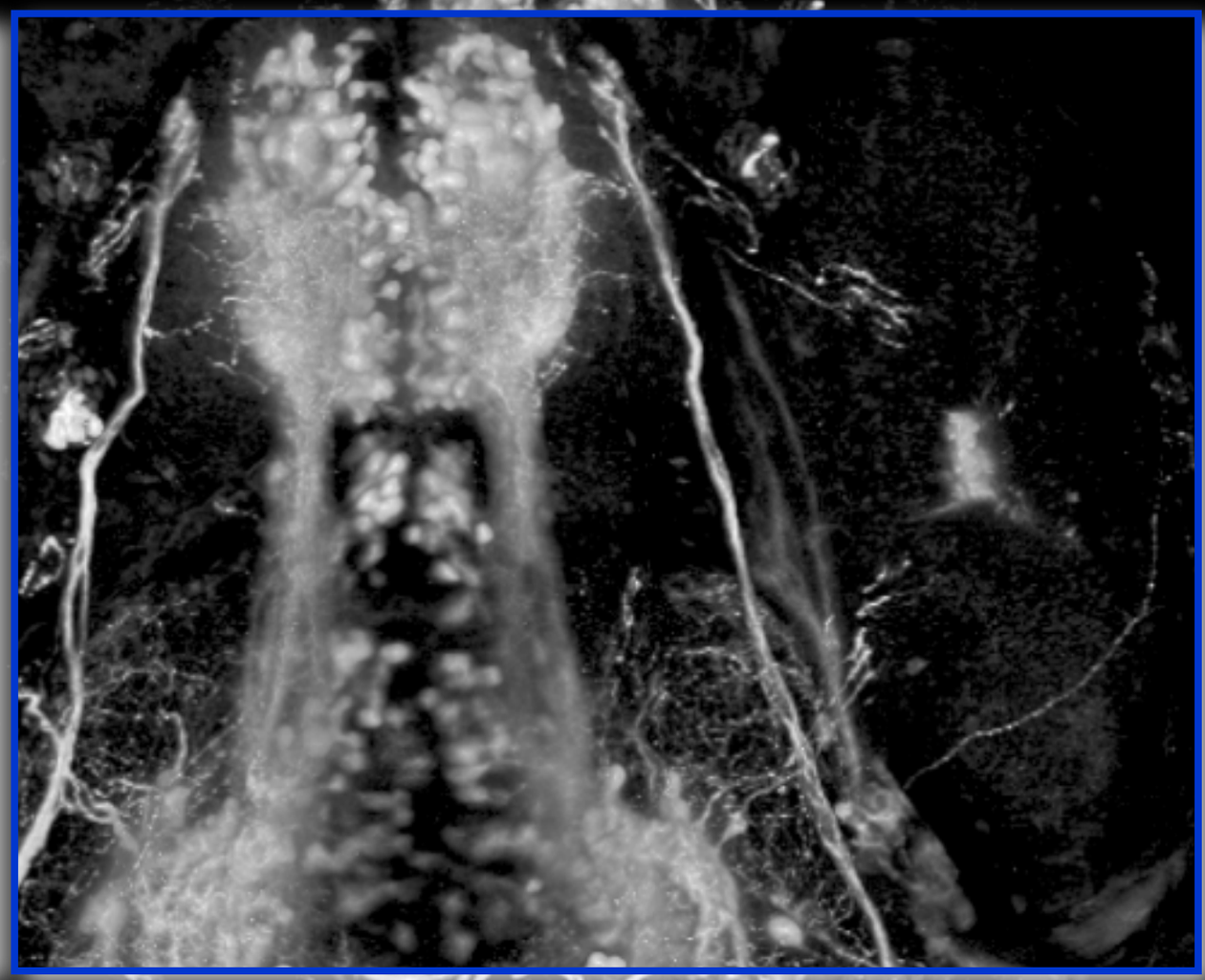
5dpf zebrafish, isl1:GFP

FluoRender does not lose any information from your confocal data

Imaris

FluoRender





5dpf zebrafish, isl1:GFP

MIP vs FluoRender (direct volume rendering)

MIP (ImageJ)

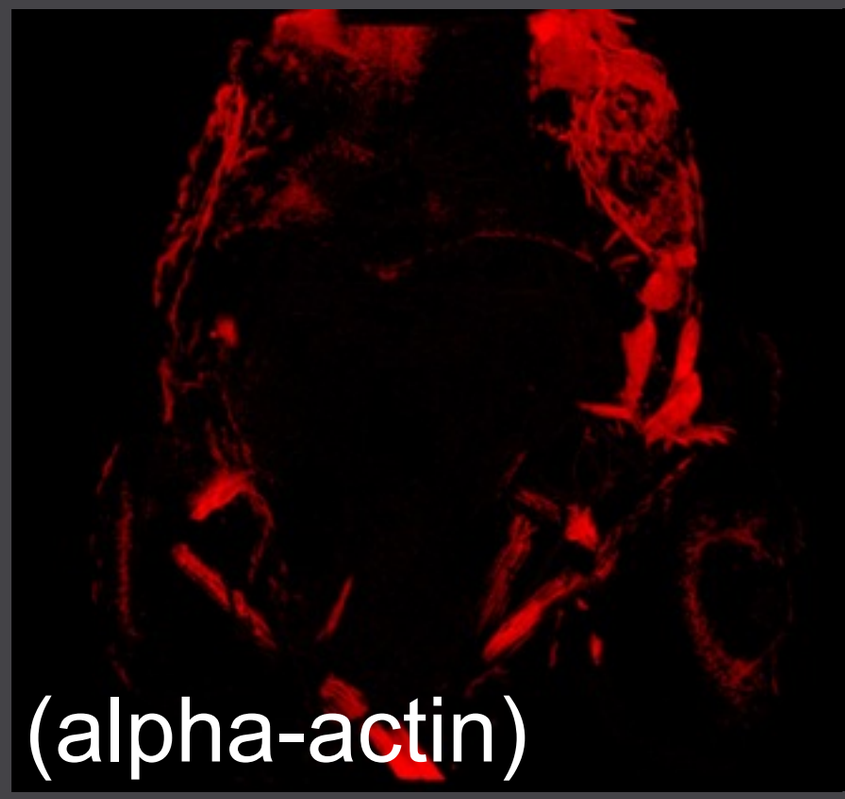
3D構造に問題

FluoRender

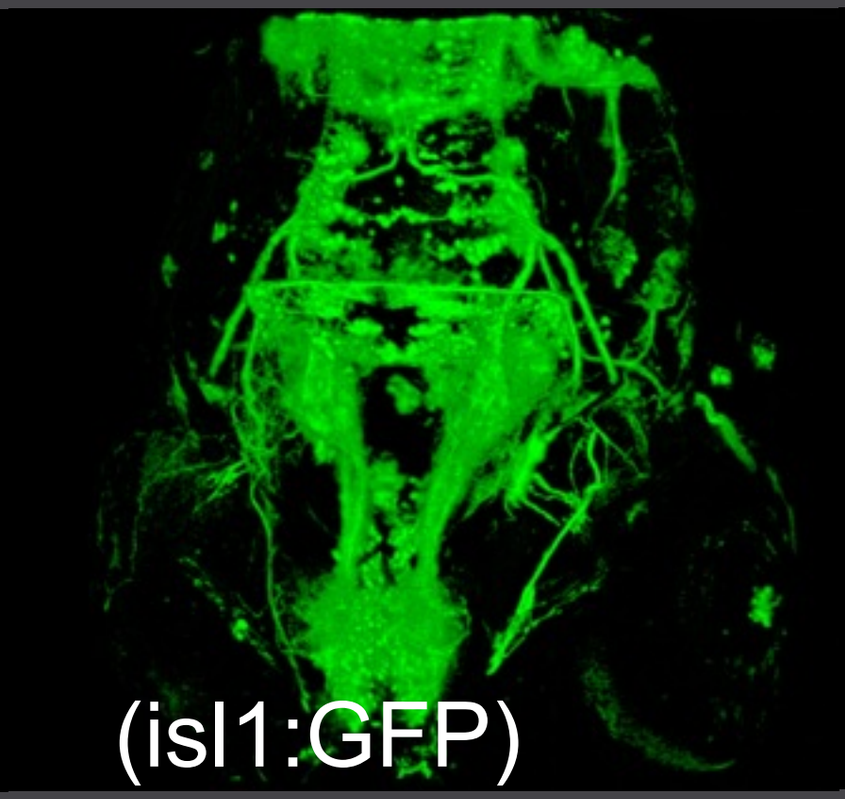
5dpf zebrafish, isl1:GFP

多チャンネルデータをどのようにレンダリングするのか？

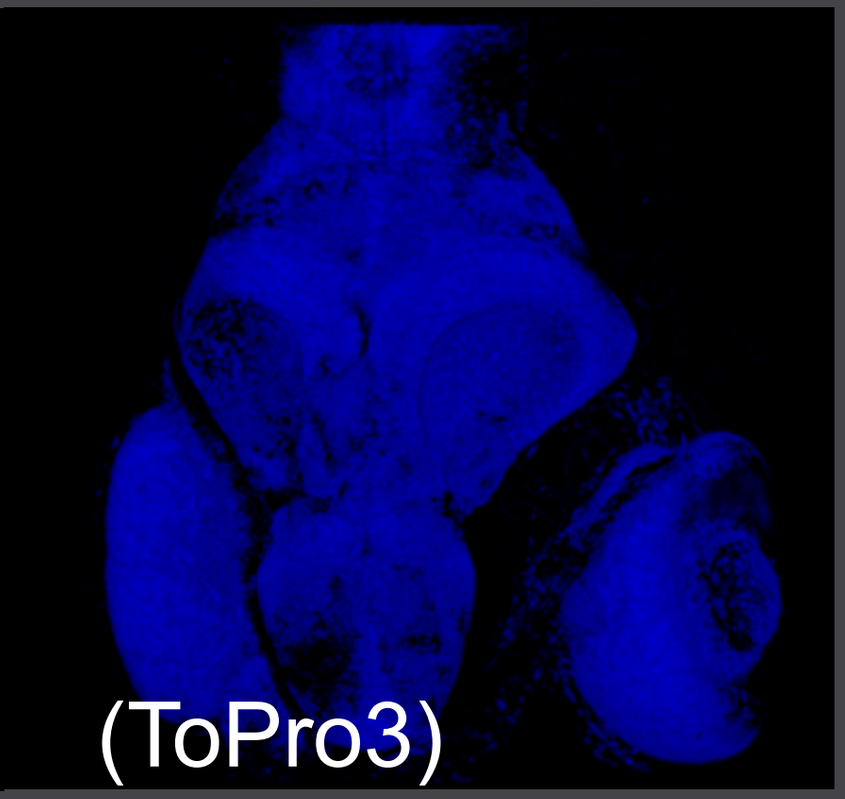
Muscle



Neurons



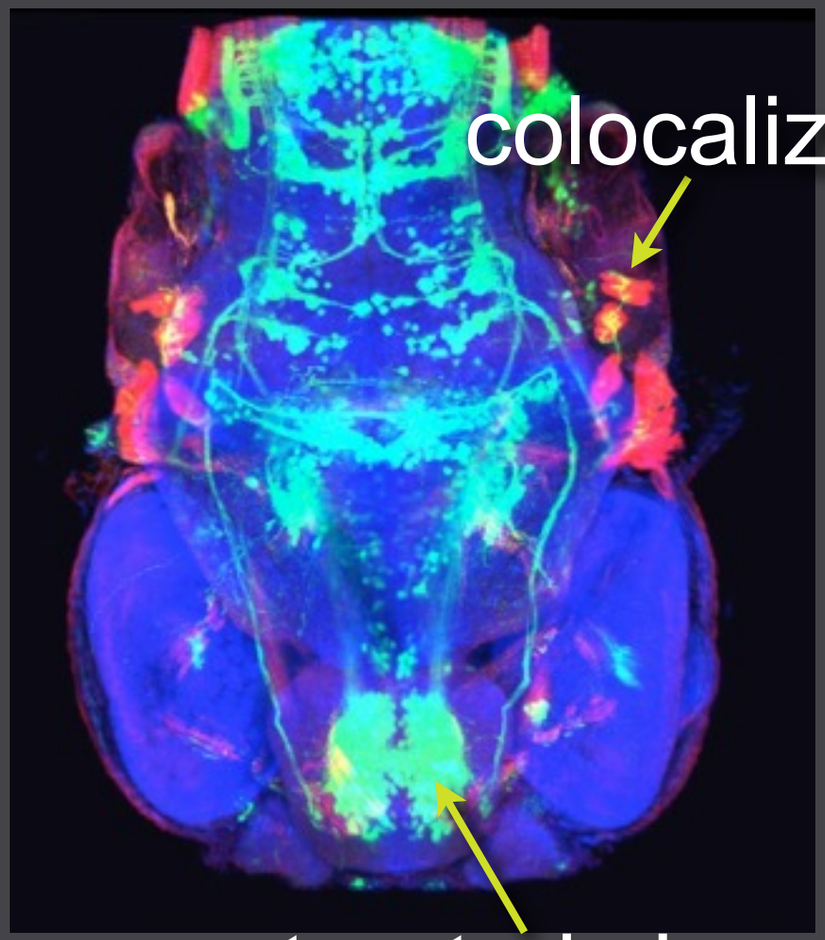
Whole tissue



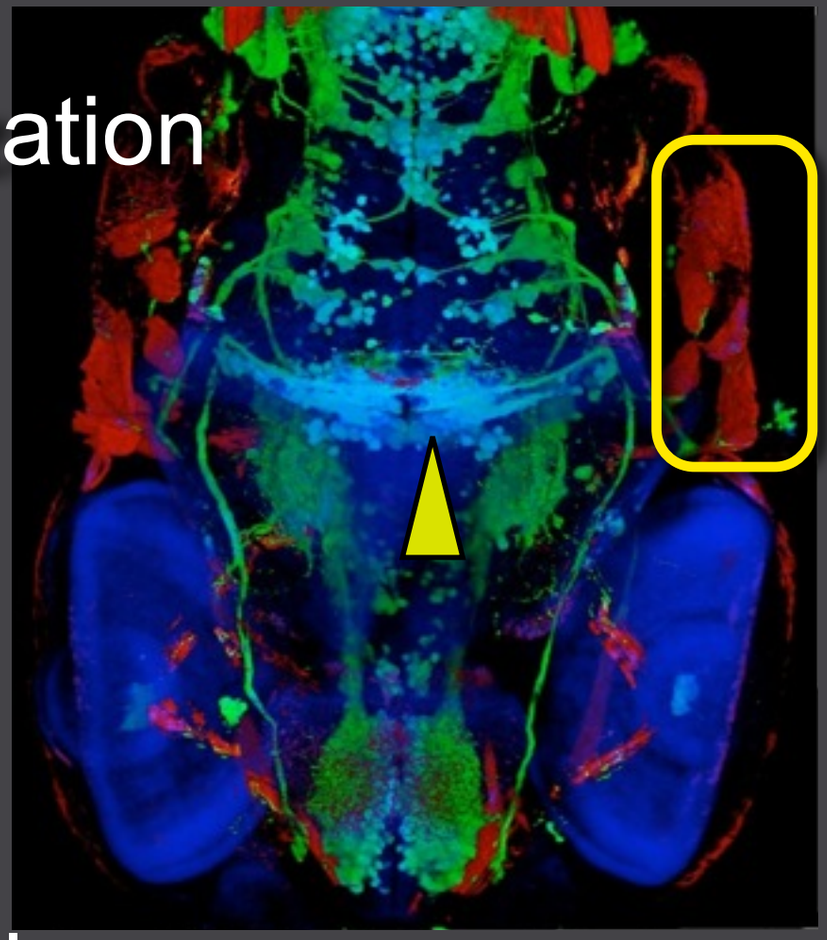
5dpf zebrafish

多チャンネルデータをどのようにレンダリングするのか？

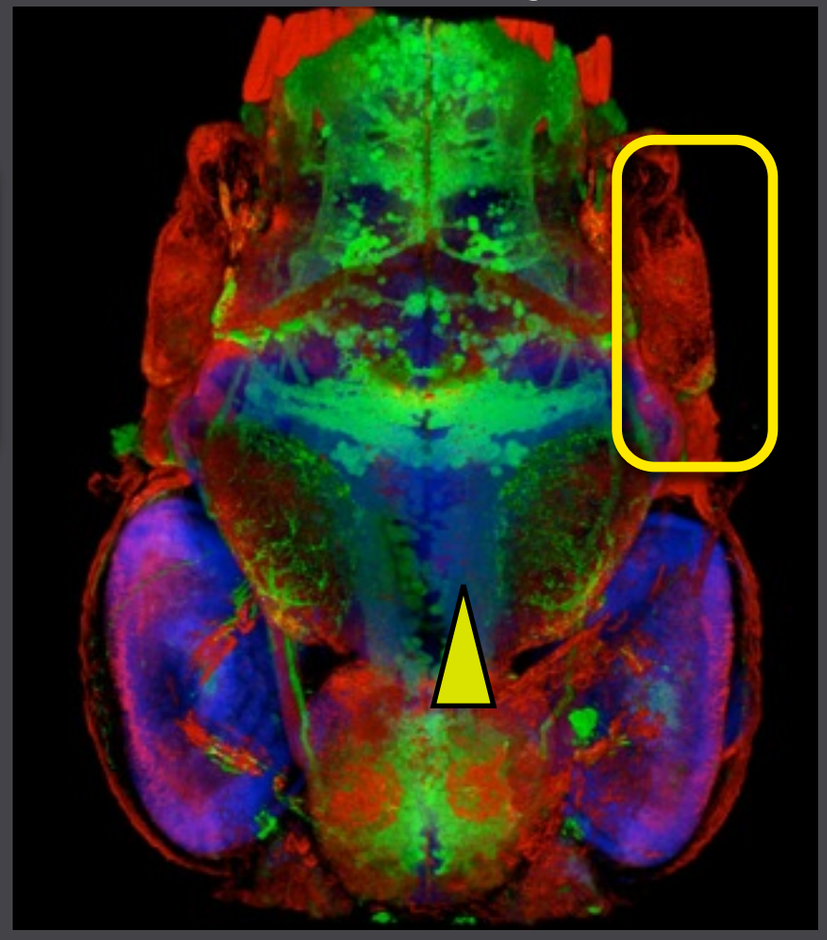
Amira5.2 (MIP)



Imaris6.3



Volocity5.2



三次元構造の表現に問題がある

なぜ共焦点レーザー顕微鏡が必要なのか？

- 複式顕微鏡よりも高解像度 (x, y, z)
- 複式顕微鏡より高いS/N比

Volocity, Imaris etc..

- 三次元の構造データを取得可

MIP

Jerold Wallis 1988

- 異なる生物組織を複数のチャンネルに分けてデータ取得可能

Volocity, Imaris etc..

既存の3Dレンダリング法・ソフトウェアは生物学の研究で要求される条件を満たしていない

FluoRender does not lose any information from your confocal data

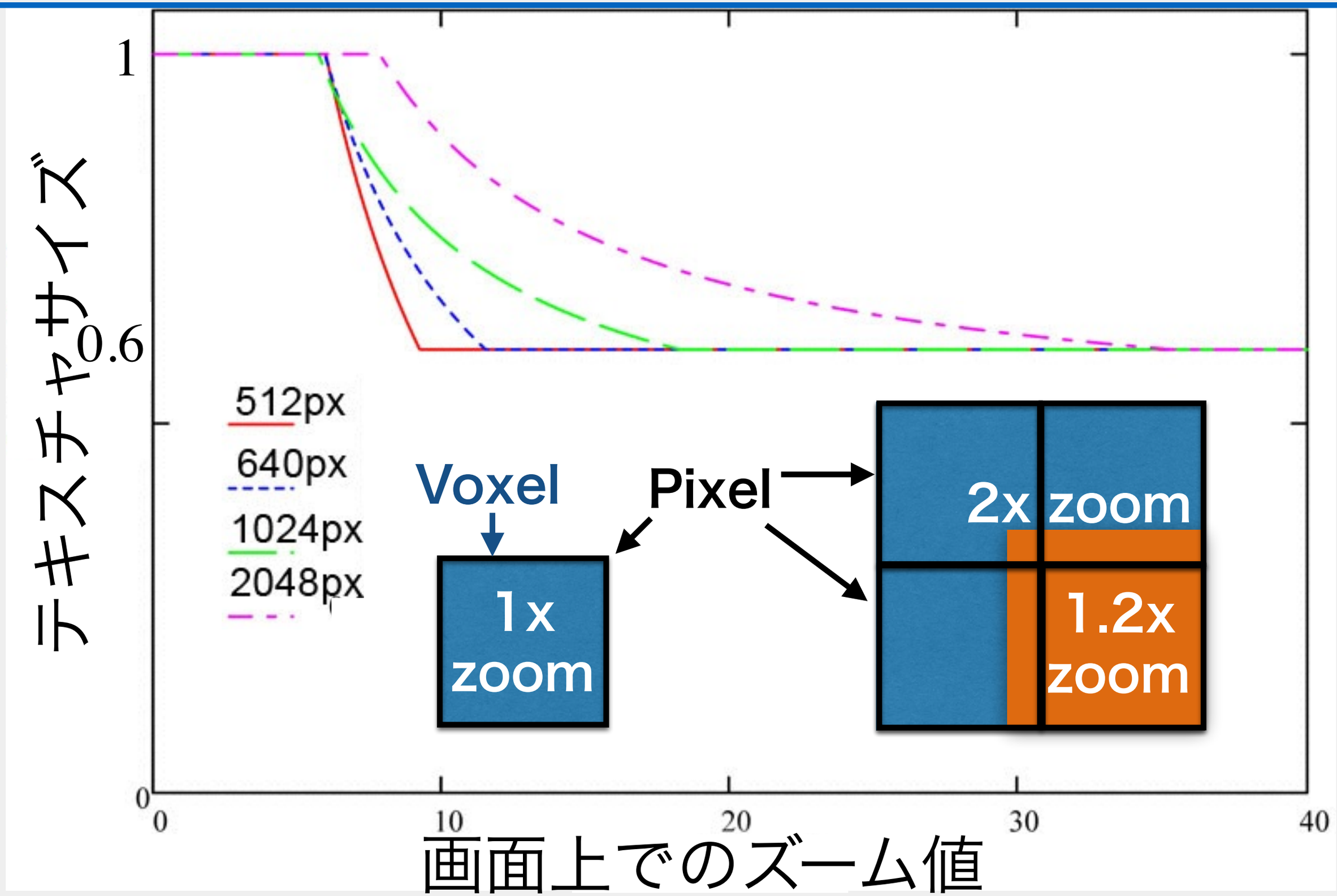
Volocity

FluoRender

低解像度

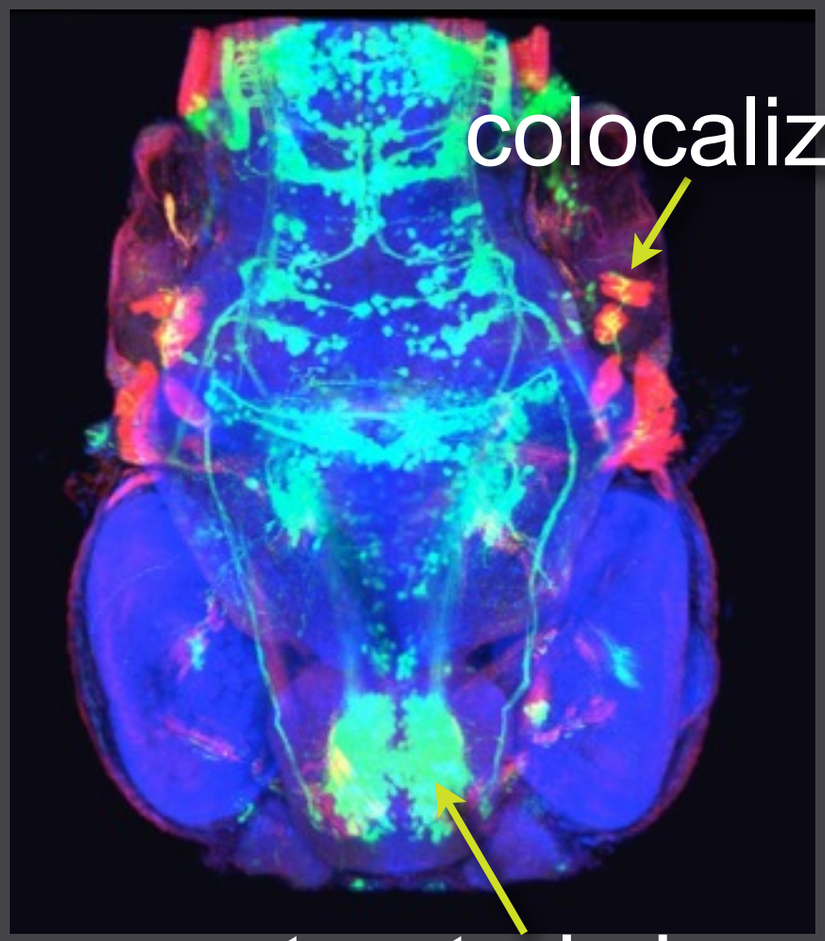
5dpf zebrafish, isl1:GFP

レンダリング時のテキスチャサイズと画面ズームサイズ --1:1レンダリング--

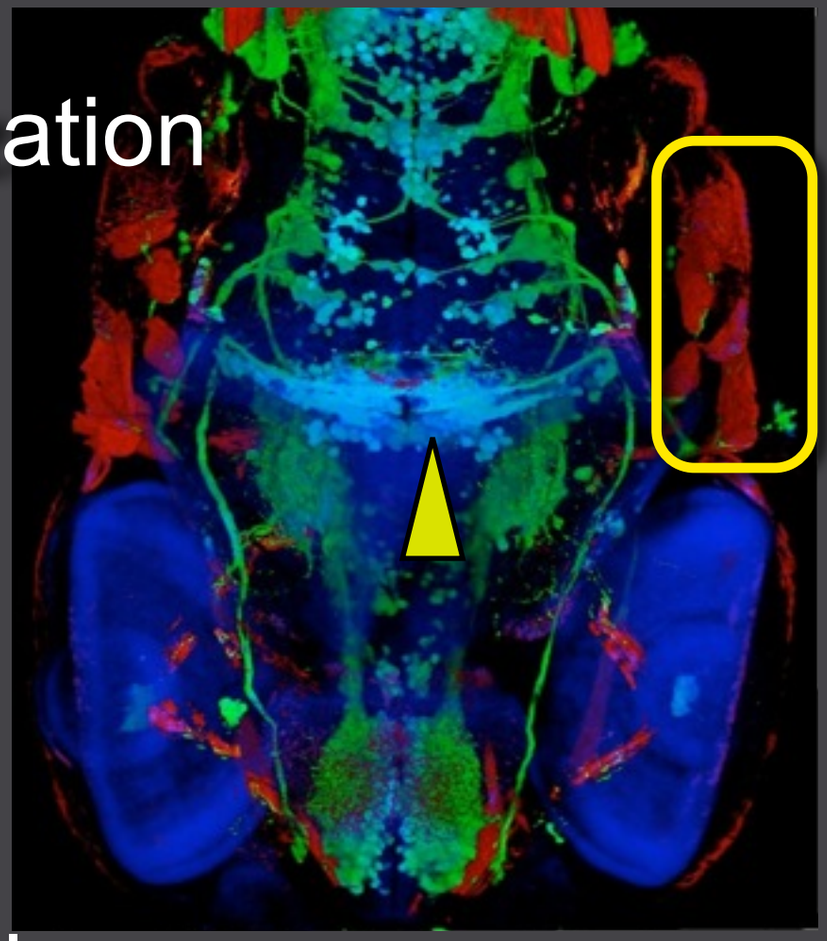


多チャンネルデータをどのようにレンダリングするのか？

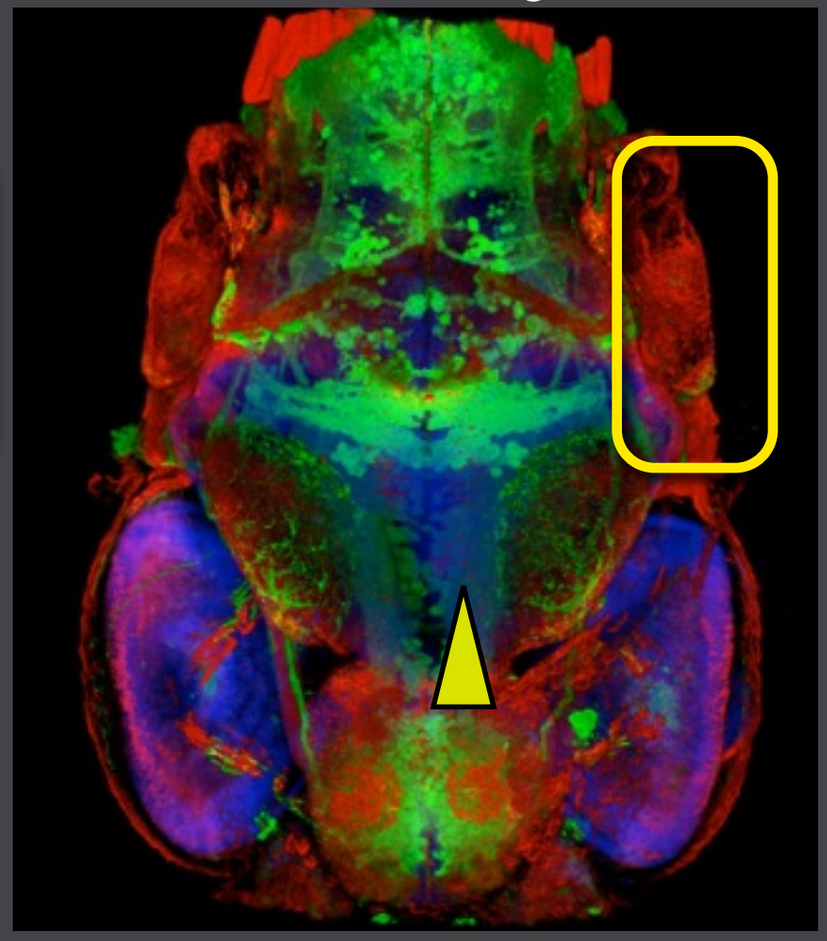
Amira5.2 (MIP)



Imaris6.3



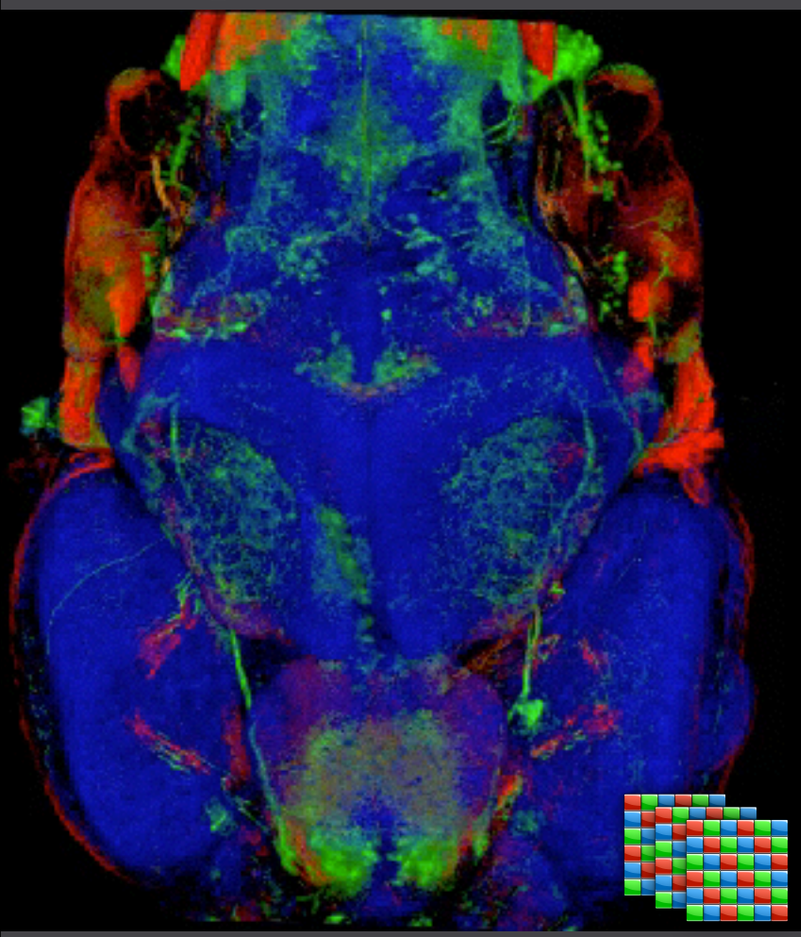
Volocity5.2



> FluoRender has 3 different rendering modes for multi-channel data

FluoRenderは３つの異なるレンダリングモードを持つ

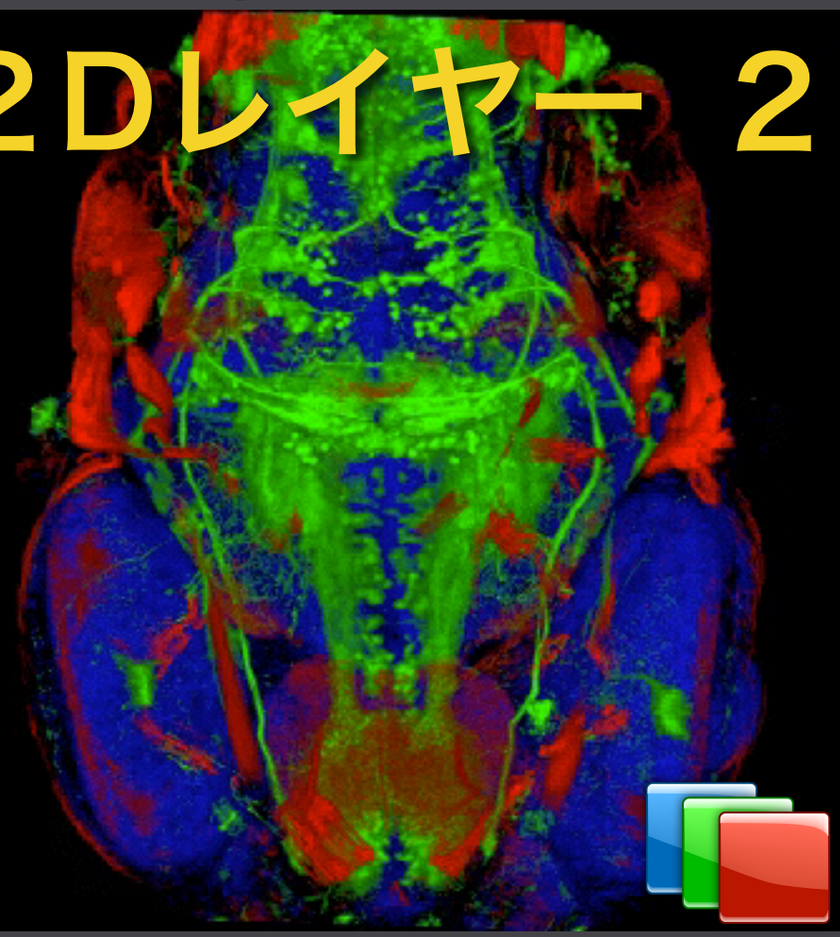
Depth mode



Channels are rendered according to their depth values; geometrically correct

Layer mode

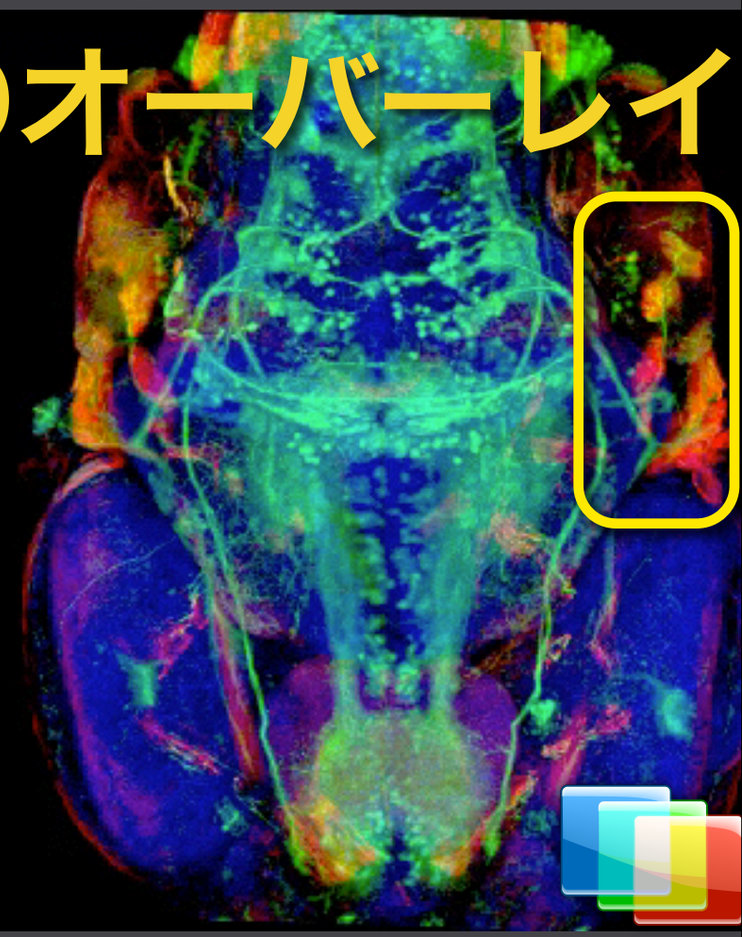
２Dレイヤー



Channels are layered separately on top of one another; can display most important first

Composite mode

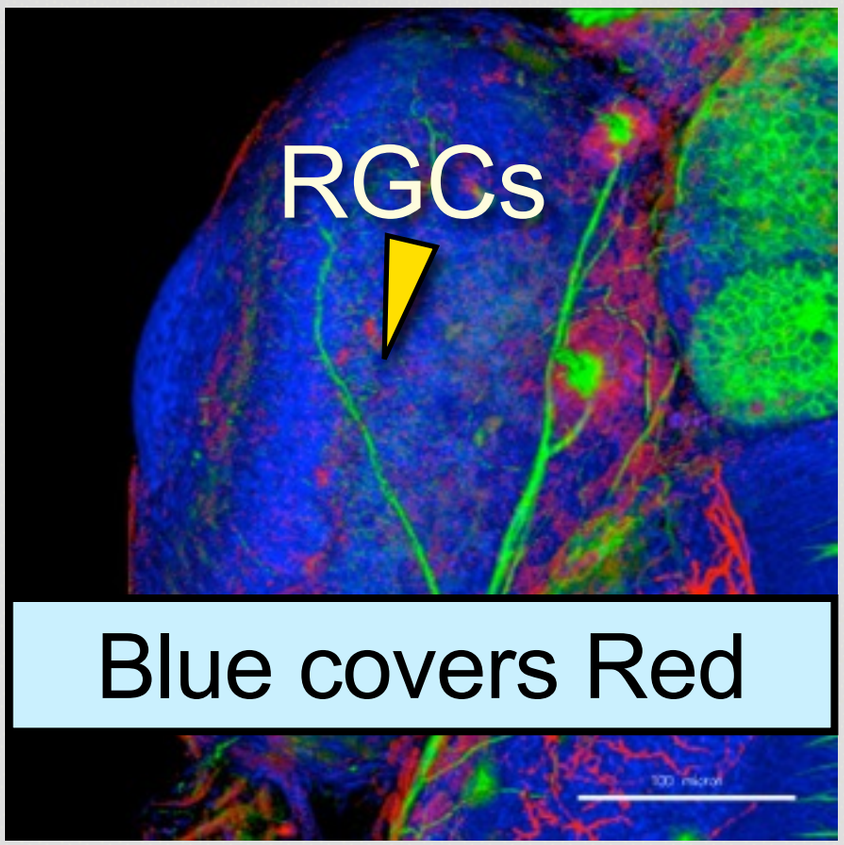
２Dオーバーレイ



Channels are rendered individually and then blended, so deeper features can be seen

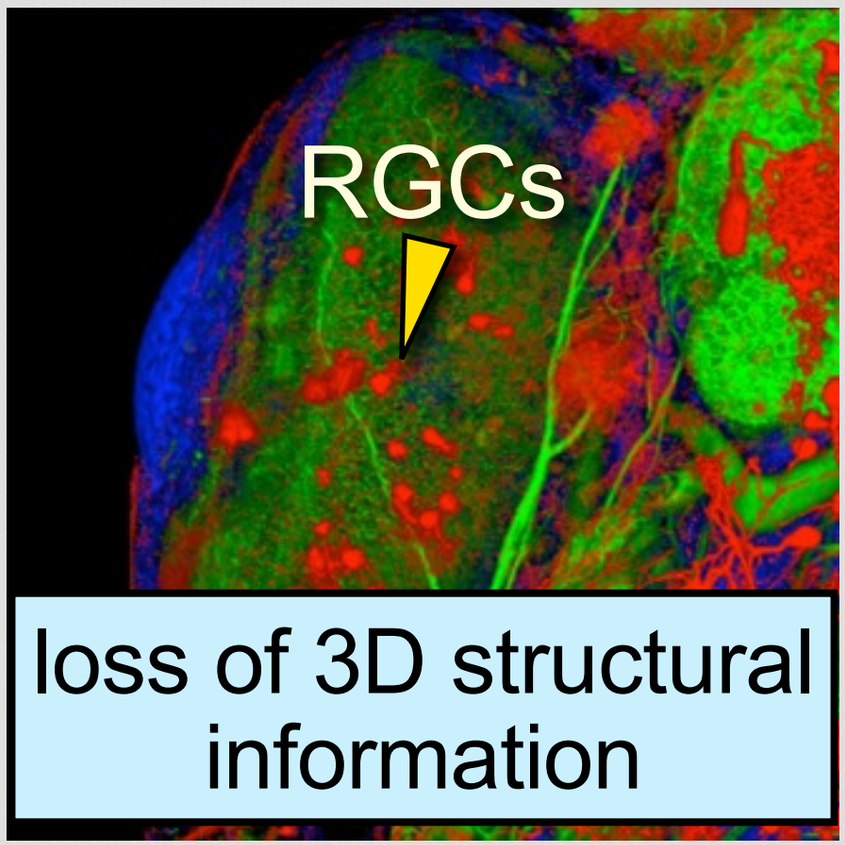
Multiple channels can cause information loss

Depth mode



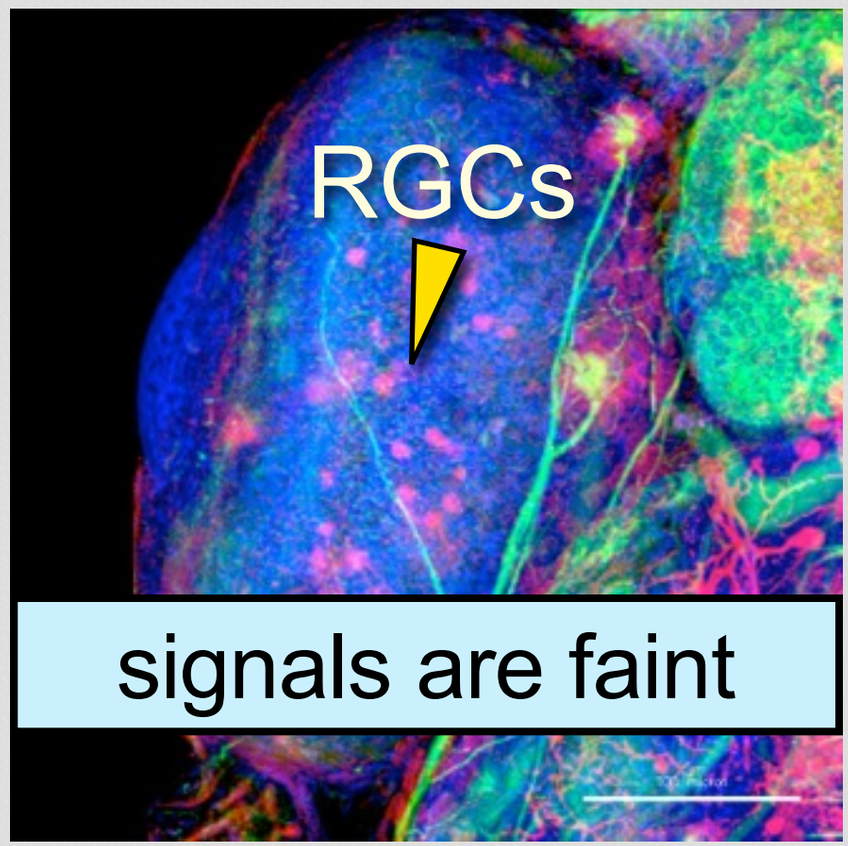
Blue covers Red

Layer mode



loss of 3D structural information

Composite mode

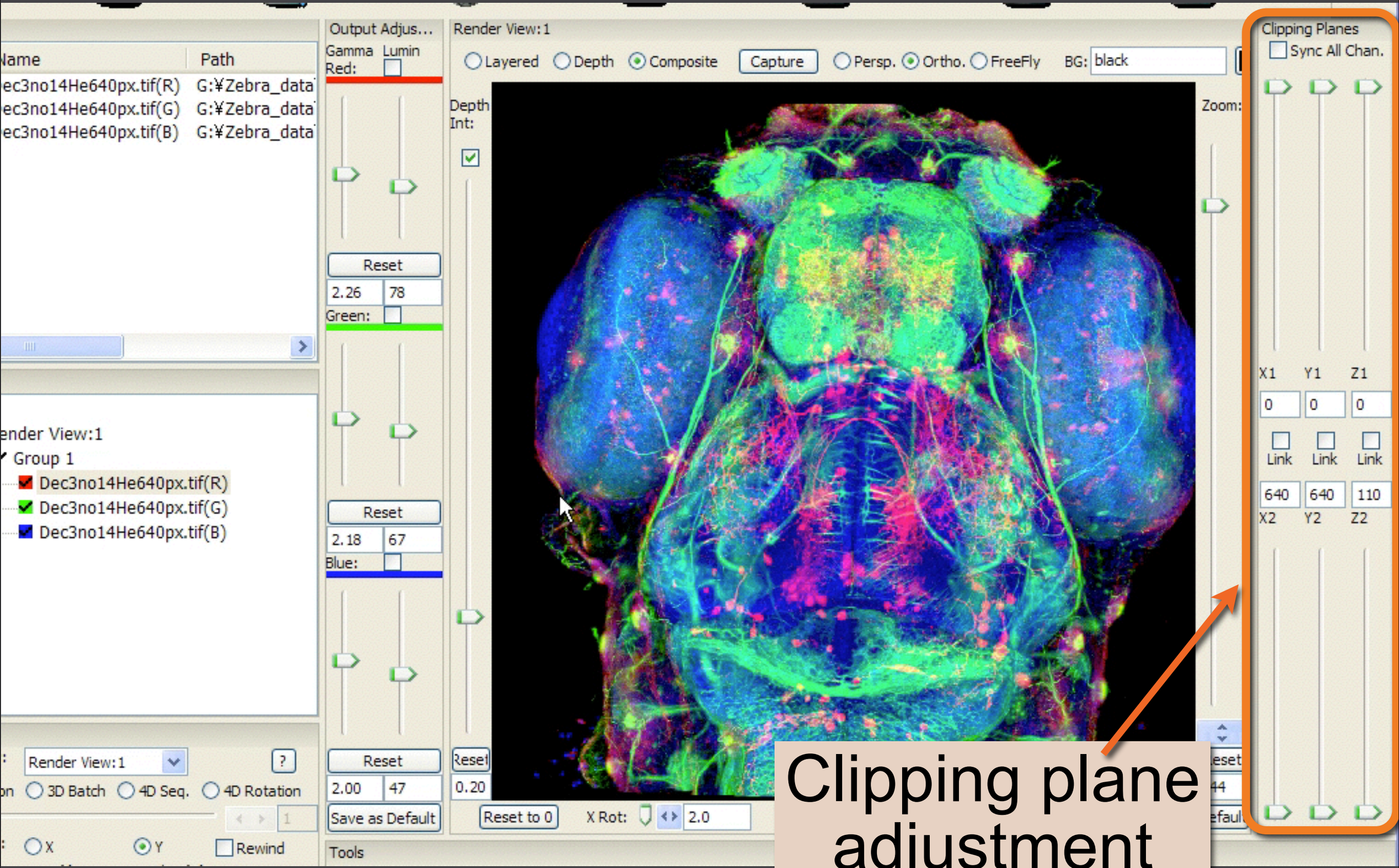


signals are faint

Green: Tubulin Red: UAS-NTRmcherry Blue: ToPro3

> それぞれのチャンネルを独立にクリッピングすることで上記の問題を解決

各チャンネルを独立にクリッピング



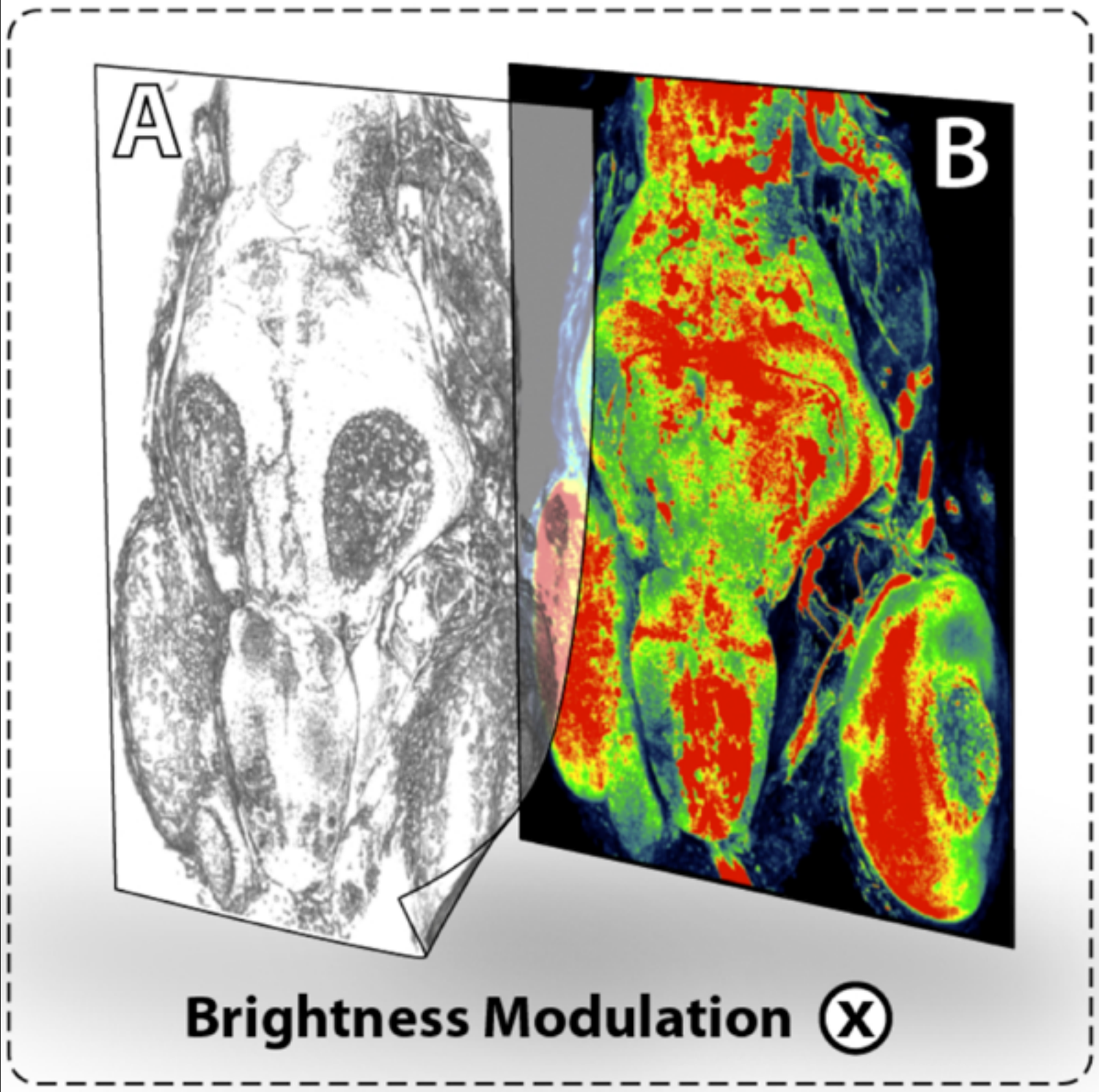
共焦点顕微鏡のデータに含まれるシグナル

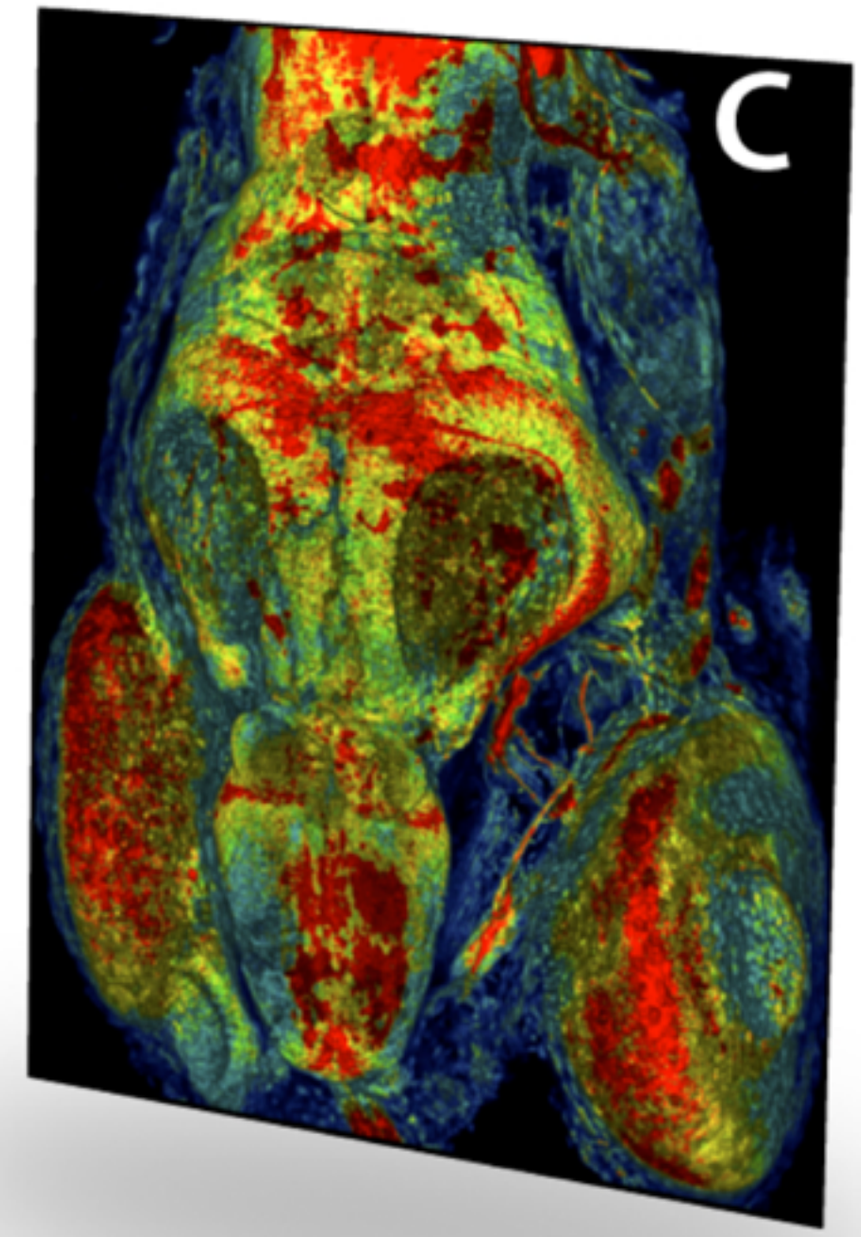
- 基本的に蛍光シグナル
- 蛍光シグナルには発現している遺伝子、タンパク質が含まれる

↳これらのデータを得るために生物学者は共焦点顕微鏡でサンプルを撮影する

強いシグナルが重要

Maximum Intensity Projection + Shading



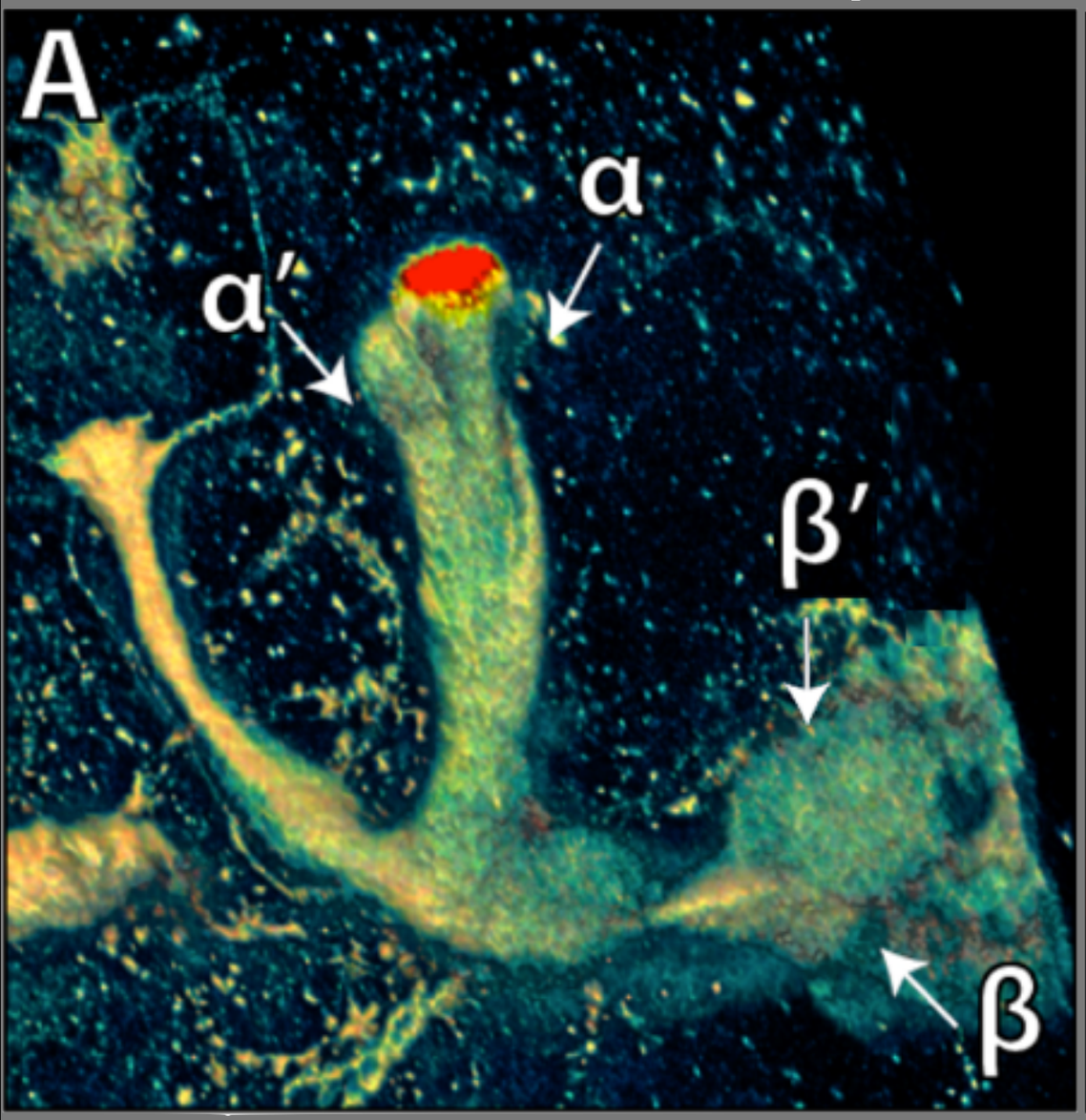


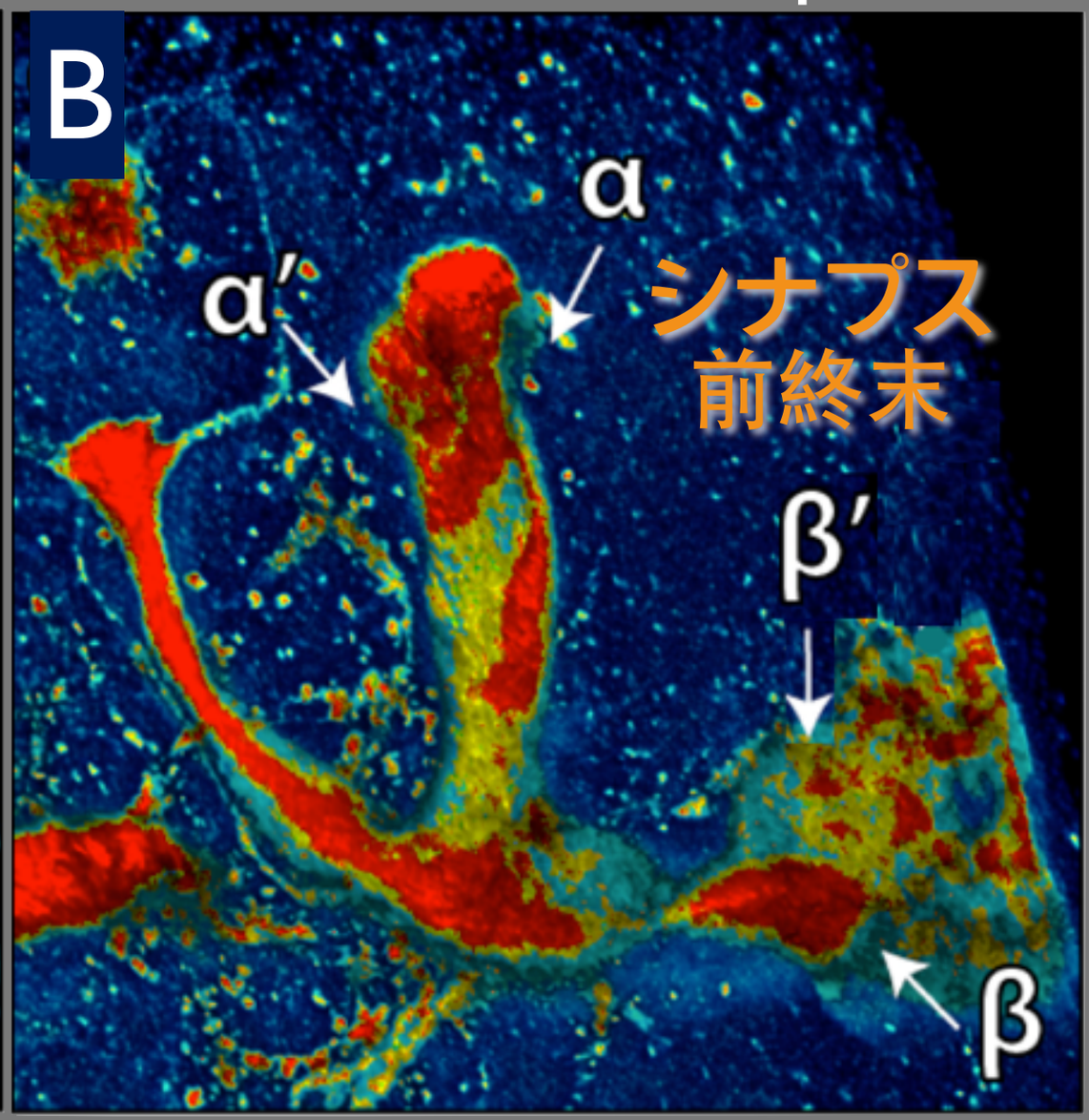
5dpf zebrafish, ToPro-3

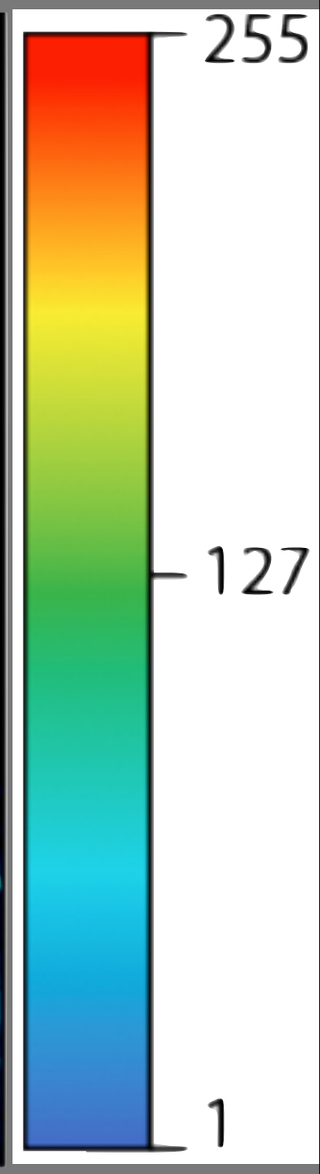
Drosophila mushroom body: higher intensity of UAS-n-syb::GFP expression on tips of lobes

DVR + color map

MIP + shading
+ color map







セグメンテーション
(目的の組織・細胞をデータから切り出す)

ショウジョウバエでは13,000個の遺伝子、10万個の細胞が脳の片半球に存在する

↓

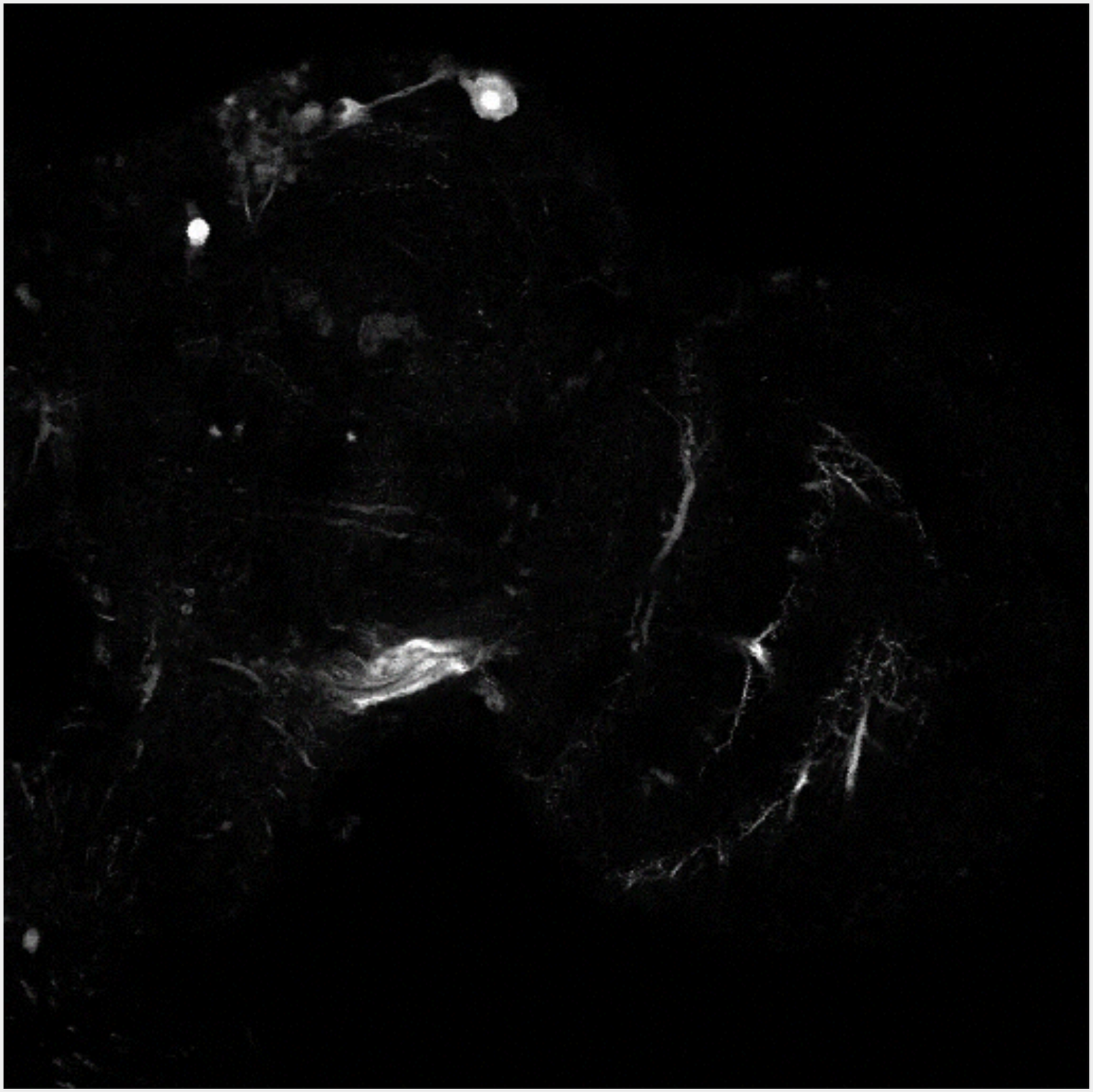
トランスジェニック技術や、抗体染色により、特定の細胞をラベルすることが可能

↓

しかしながら、一本の神経線維のみを可視化するには元の遺伝子数が少なすぎる

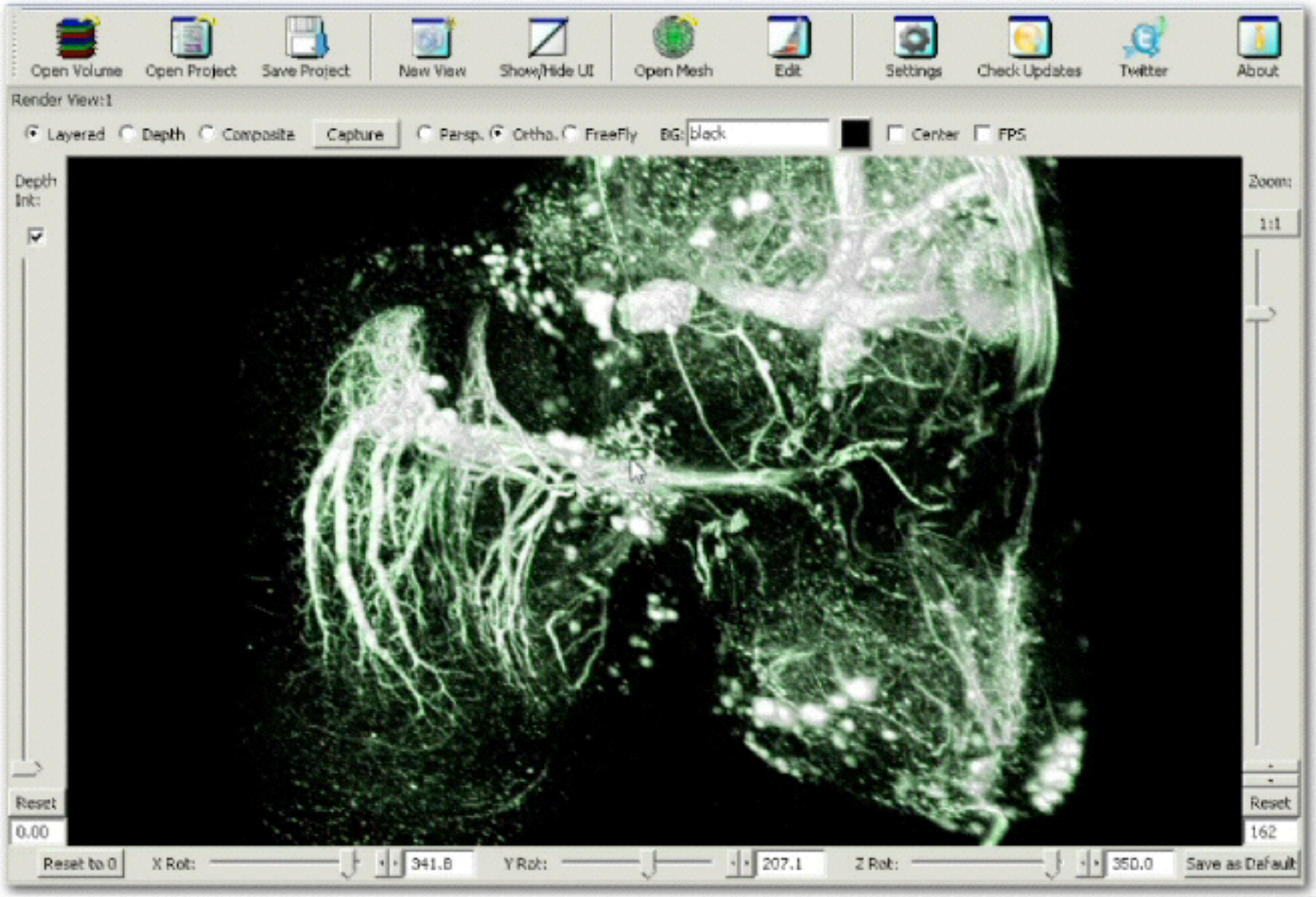
特定の組織を可視化・計測するために、セグメンテーションが必要

特異的に遺伝子が発現している
ショウジョウバエの神経線維



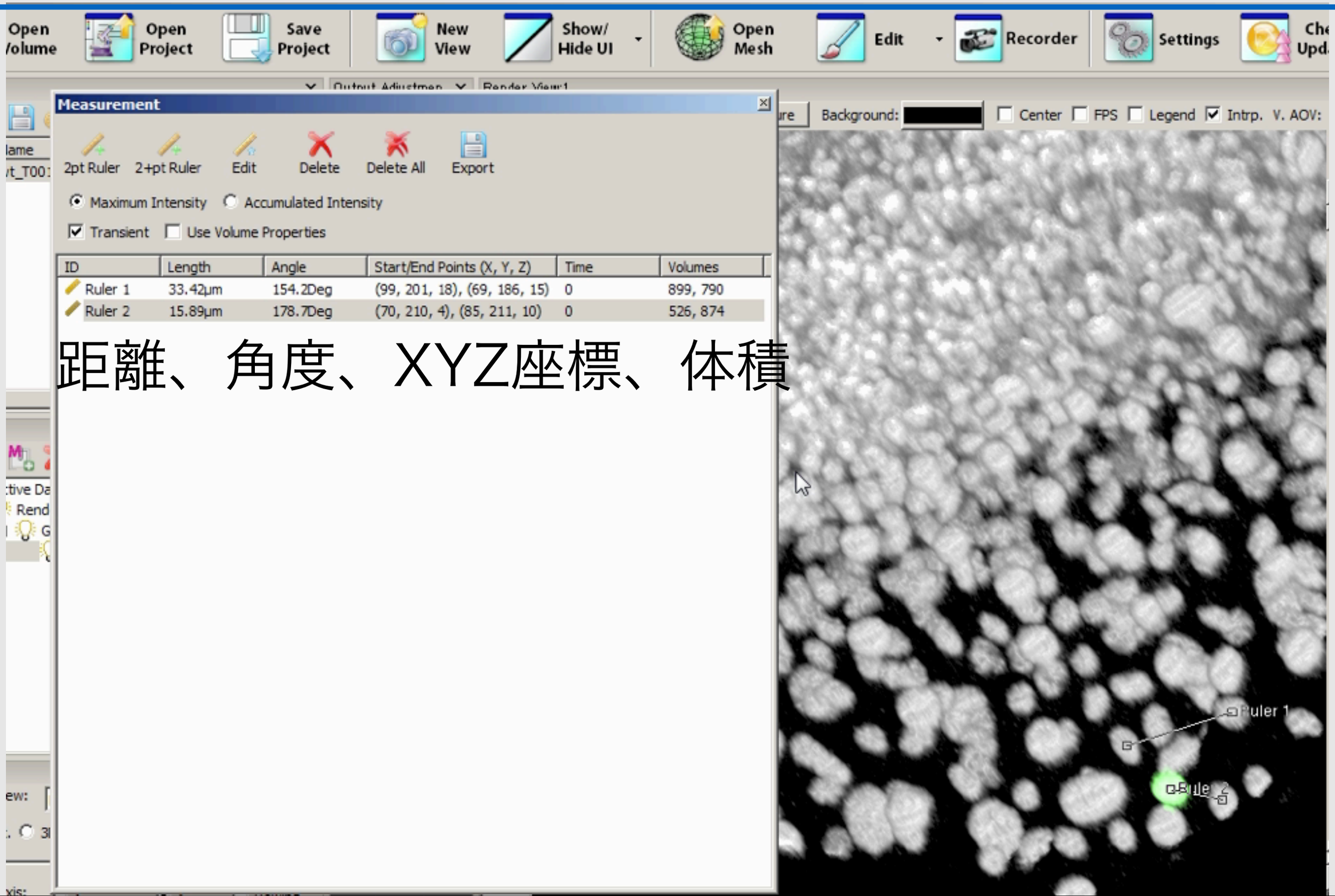
共焦点
連続画像
データ

インタラクティブ３Dセグメンテーション



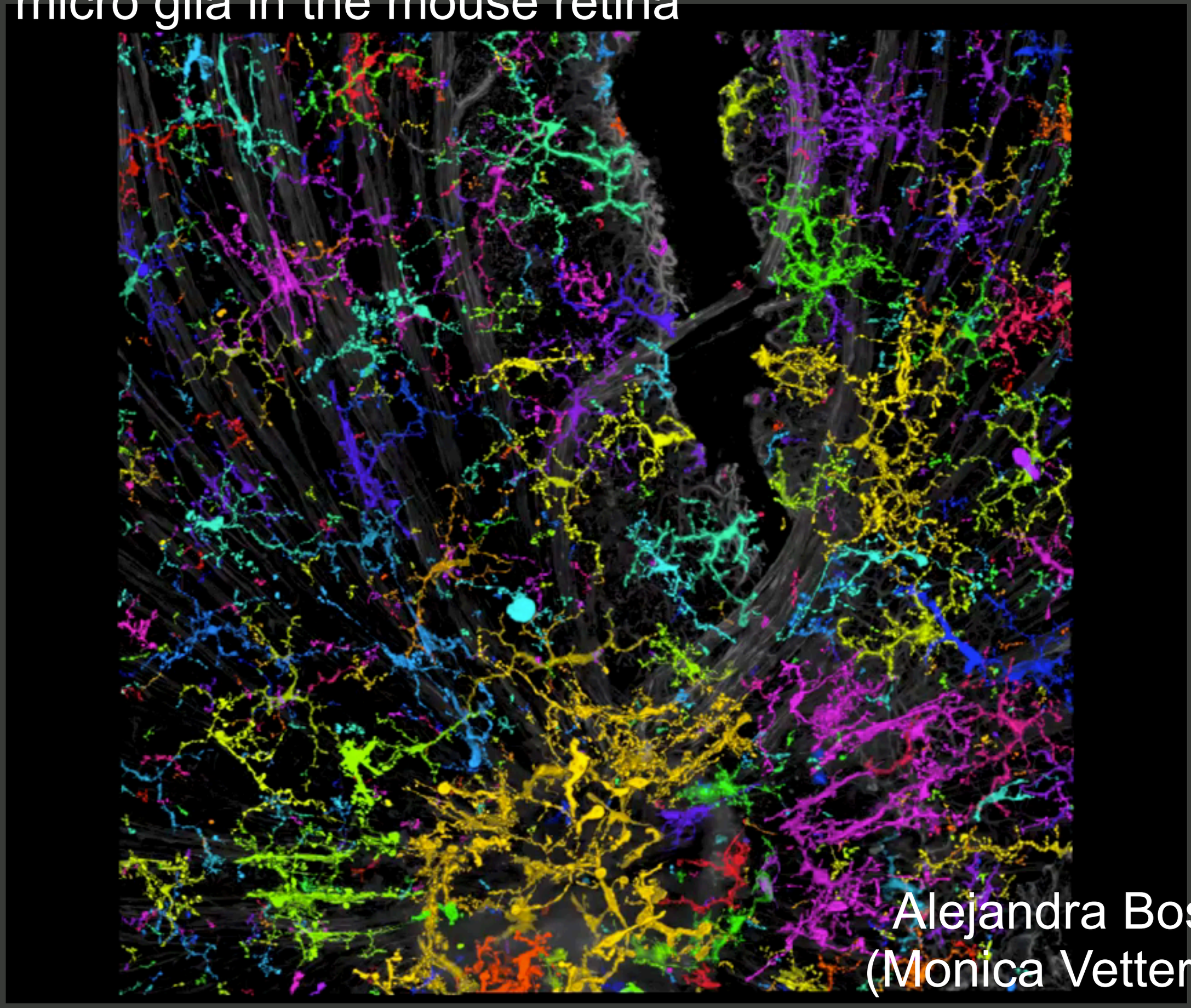
We reset the segmentation result and can use the diffusion brush to extract only connected structures.

セグメンテーション・インタラクティブGUIを用いた計測機能の例



距離、角度、XYZ座標、体積

micro glia in the mouse retina



Alejandra Bosco
(Monica Vetter lab)

3D Segmentation with connecting component

32bit addressing for each voxel

4,294,967,296 (~4.2bil ID)

1	2	3	4	5	6
7	8	9	10	11	12
13	14	15	16	17	18
19	20	21	22	23	24
25	26	27	28	29	30
31	32	33	34	35	36

in GPU

Connecting component

0	0	0	0	0	0
0	8	0	0	0	0
0	0	15	16	17	0
0	0	0	0	0	0
0	0	27	0	0	0
0	32	33	0	35	36

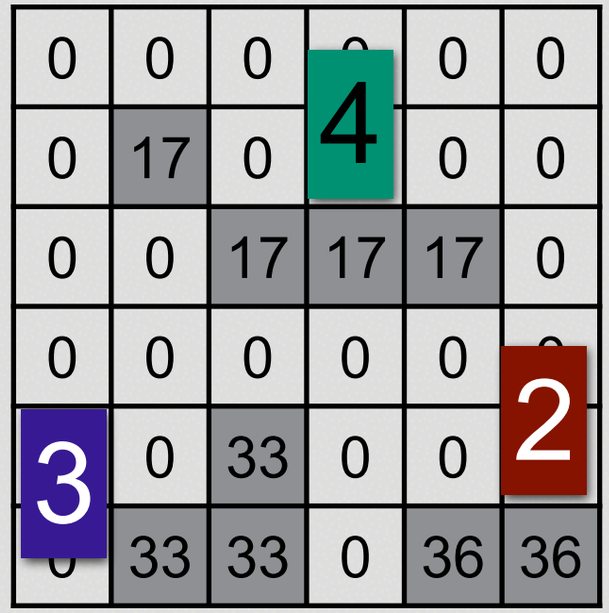
in GPU

Max filter (max value within the component)

0	0	0	0	0	0
0	17	0	0	0	0
0	0	17	17	17	0
0	0	0	0	0	0
0	0	33	0	0	30
0	33	33	0	36	36

in GPU

Counting voxel number for all components



in CPU

まとめ

- 解像度に関する問題
  - １：１テキスチャレンダリング
- マルチチャンネルのレンダリング法
  - ２つの異なる2Dドメインレンダリング
- 3Dシグナル強度値の表示法
  - MIP＋シェーディングオーバーレイ
- セグメンテーションと計測
  - インタラクティブ３D GUIと
    シグナル拡散アルゴリズム

download free!!

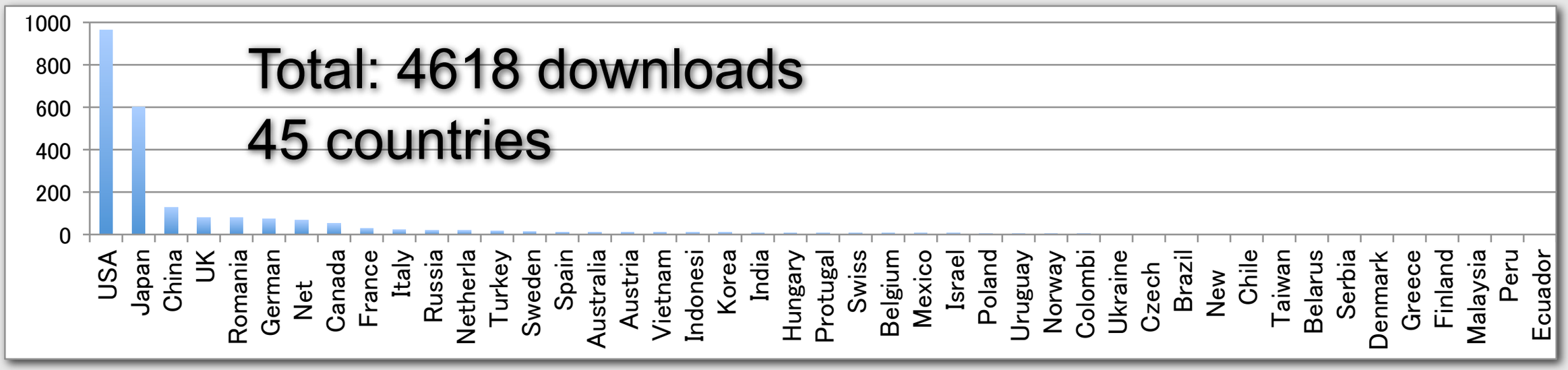
FluoRender

http://www.fluorender.com

Recommended system **(Windows only)**

Graphics card: **GTX Titan, 780, 770 (NVIDIA)**

System memory: 3GB~



Acknowledgment

- Chi-Bin Chien (Univ. of Utah)
- Richard Dorsky (Univ. of Utah)
- Charles Hansen (SCI) ”FluoRender”
- Yong Wan (SCI), “main programmer, FluoRender”
- Nisha Ramesh (SCI), “registration, FluoRender”
- Kei Ito (Univ of Tokyo), “cell counting software”
- Takeshi Shimada (Univ of Tokyo), “cell counting software”
- Cell Imaging Core facility
- Zebrafish Core facility

National Institutes of Health
NIH...Turning Discovery Into Health

R01 GM098151-01
R01 MH092256-01