

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

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University of Utah, Neurobiology & Anatomy
Scientific Computing Imaging Institute



Collaboration with Y. Wan & C. Hansen

Scientific Computing Imaging Institute



FluoRender

<http://fluorender.com>

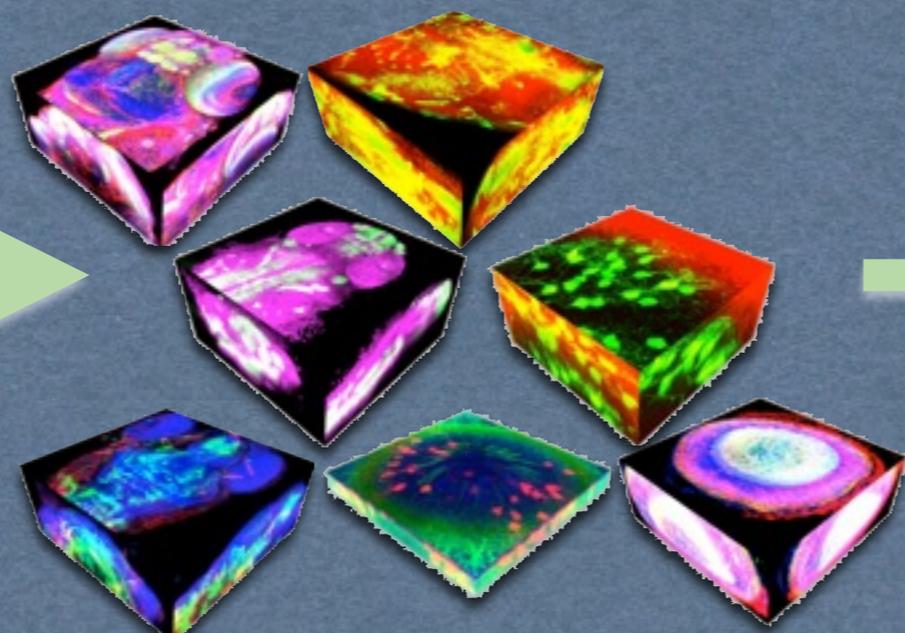
<http://www.sci.utah.edu>

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

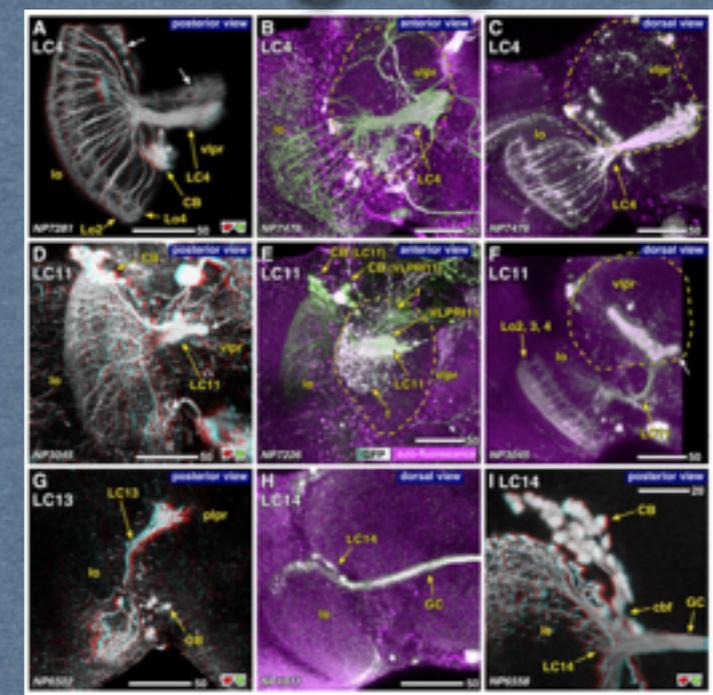
confocal microscope volume data 3D/4D reconstruction



need to be screen
for good sample

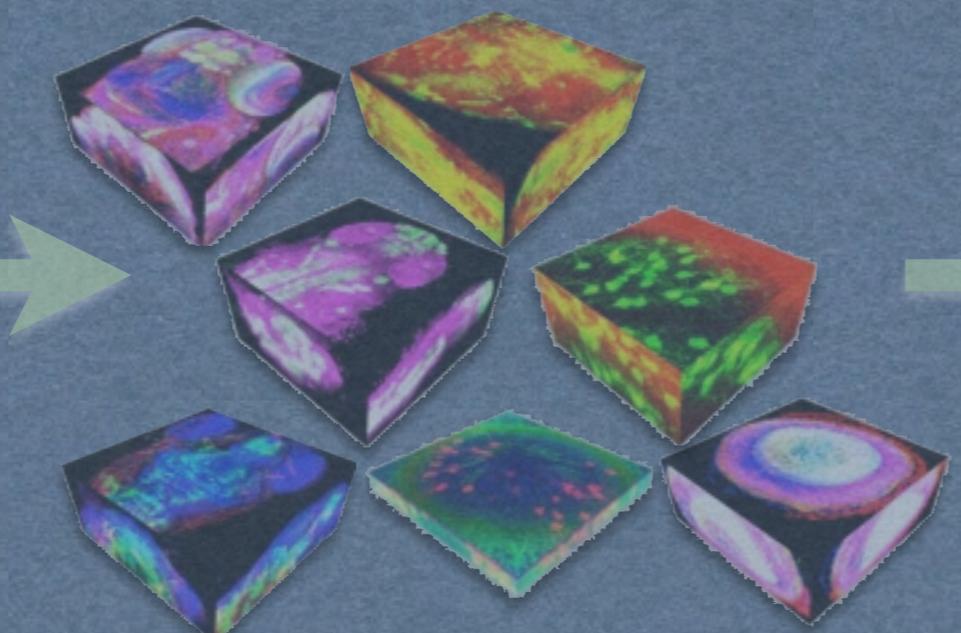


Making figures

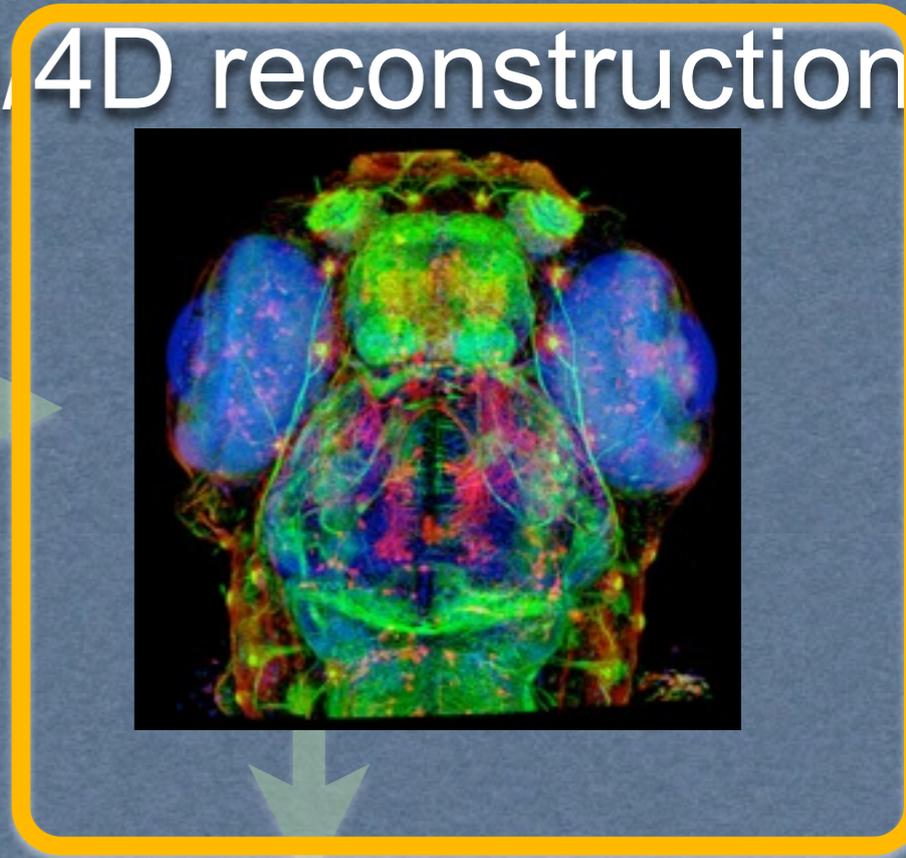


共焦点レーザー顕微鏡のデータから論文の図を作る

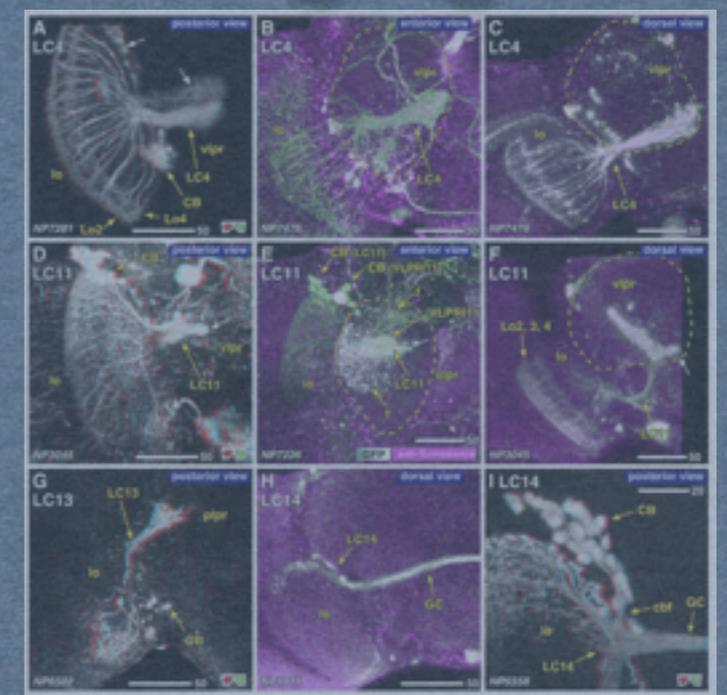
confocal microscope volume data 3D/4D reconstruction



need to be screen
for good sample



Making figures



FluoRender Graphical User Interface Ver2.12

open volume, open/save project

loaded data files

rendered volumes

FluoRender 64bit started normally.

FluoRender Graphical User Interface Ver2.12

The screenshot displays the FluoRender software interface. The central window shows a 3D rendering of a biological specimen, likely a zebrafish embryo, with various internal structures highlighted in different colors (blue, green, red, purple). The interface includes several panels:

- Top Bar:** Contains icons for Open Volume, Open Project, Save Project, New View, Show/Hide UI, Open Mesh, Edit, Settings, Check Updates, and social media links for Facebook and Twitter.
- Datasets Panel:** Lists datasets with columns for Type, Name, and Path. It shows three volume datasets: sep26no50p.oib_1, sep26no50p.oib_2, and sep26no50p.oib_3.
- Workspace Panel:** Shows the active datasets and their hierarchy, including Render View:1 and Group 28.
- Export Panel:** Allows users to capture the view in different modes (3D Rot, 3D Batch, 4D Seq, 4D Rot) and set rotation axes and frame rates.
- Output Adjust... Panel:** Provides sliders for Gamma, Saturation Point, Luminance, Alpha, and Shading, along with checkboxes for Link, Sync, and Depth.
- Render View:1 Panel:** Shows the current rendering mode (Layered, Depth, Composite, Capture, Persp, Ortho, FreeFly) and background color. It also includes a zoom slider and a table for clipping planes.
- Clipping Planes Panel:** Allows users to define clipping planes for X1, Y1, Z1, X2, Y2, and Z2, with options to link or sync them.
- Rotations Panel:** Provides controls for aligning the view and resetting rotations to zero.
- Properties Panel:** Shows detailed settings for the selected dataset, including Gamma (1.00), Saturation Point (4095), Luminance (4095), Alpha (2056), and Shading (checked).

A text box overlaid on the 3D view reads: "rendering view, rendering mode settings".

FluoRender 64bit started normally.

FluoRender Graphical User Interface Ver2.12

The screenshot displays the FluoRender software interface. At the top is a menu bar with icons for Open Volume, Open Project, Save Project, New View, Show/Hide UI, Open Mesh, Edit, Settings, Check Updates, and social media links for Facebook, Twitter, and About. Below the menu bar is a Datasets table with columns for Type, Name, and Path. The main workspace shows a 3D visualization of a biological specimen, overlaid with the ZEISS and OLYMPUS logos. The visualization is rendered in a composite mode, showing various channels in different colors (red, green, blue, yellow, magenta). A central text overlay reads: ".ism .oib, .oif (3D/4D files) 2D/3D/4D .tif". To the right of the main view is a Clipping Planes panel with sliders for X1, Y1, Z1, X2, Y2, Z2 and checkboxes for Link, Sync All Channels, and Rotations. Below the main view is a Properties panel for the selected dataset (sep26no50p.oib_2), showing various adjustment parameters such as Gamma, Saturation Point, Luminance, Alpha, Shading, and Color. A bottom-left panel shows rotation and time settings, including a rotation axis selector (X, Y, Z), rotation speed (30), and time range (from 1 to 1). A bottom-right panel shows a status bar with the text "FluoRender 64bit started normally."

ZEISS OLYMPUS

.ism .oib, .oif (3D/4D files) 2D/3D/4D .tif

XYZ clipping & Rotation

movie creation

14 image adjustment parameters

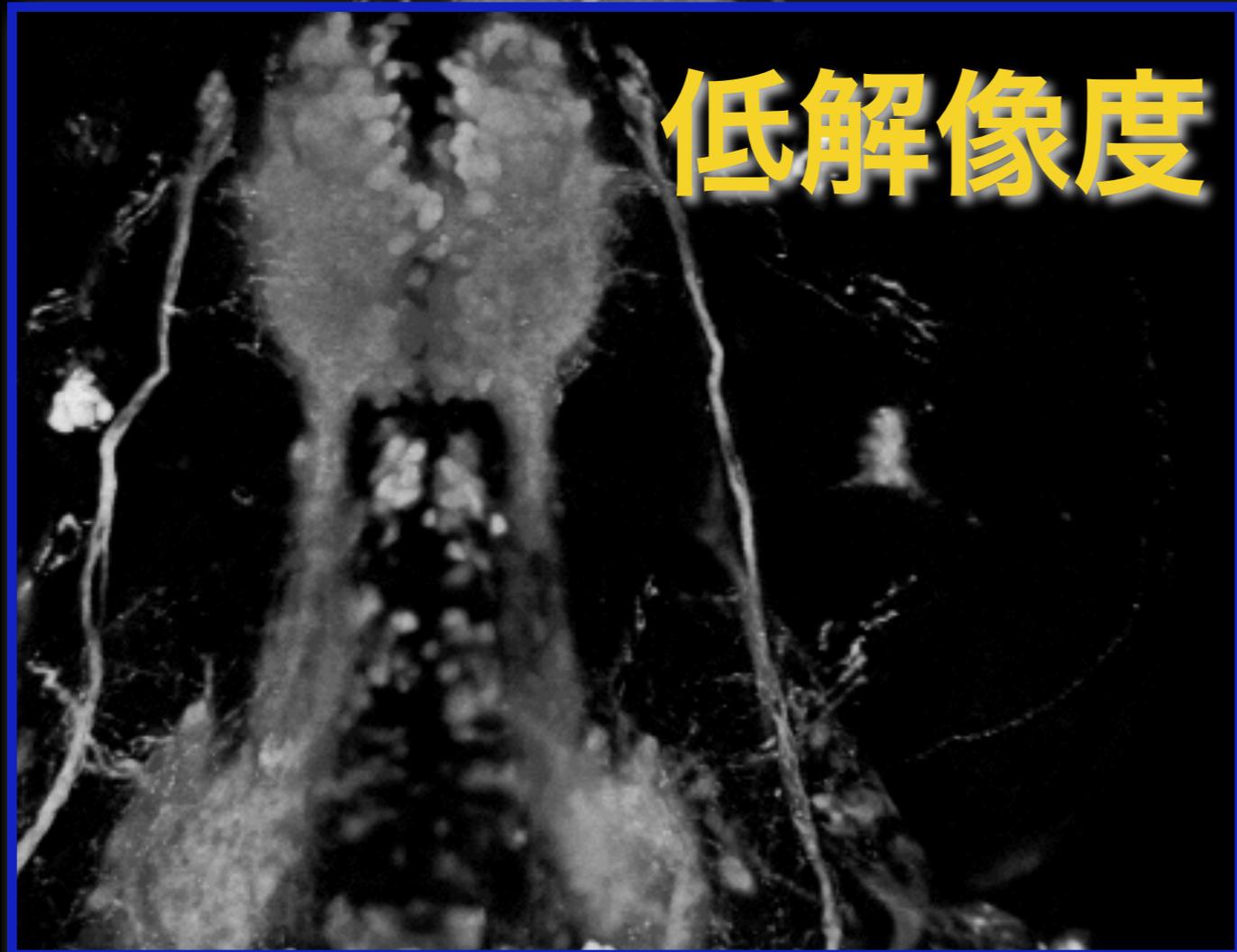
FluoRender 64bit started normally.

なぜ共焦点レーザー顕微鏡が必要なのか？

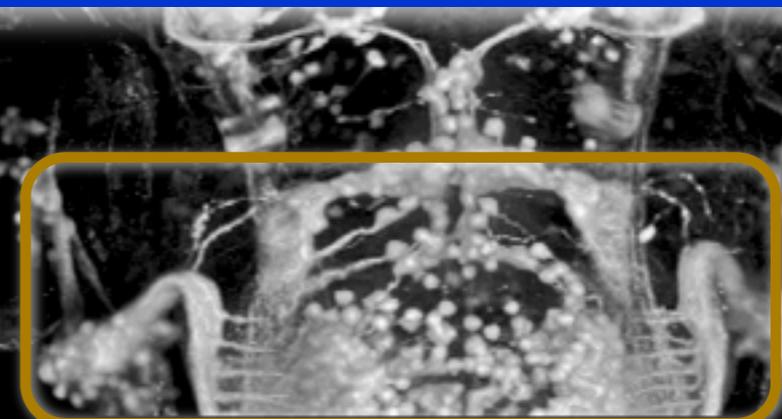
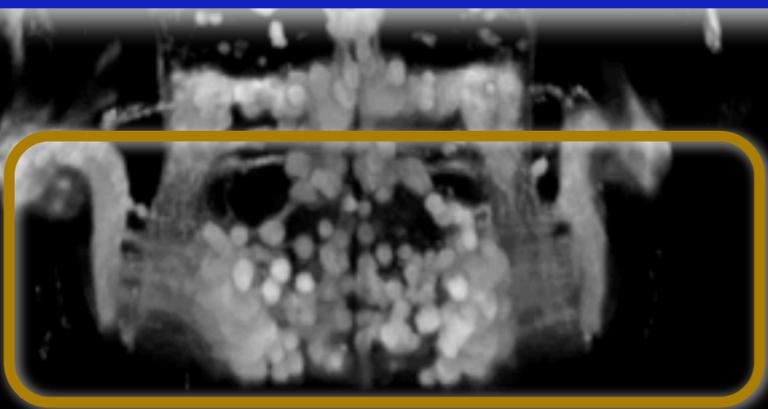
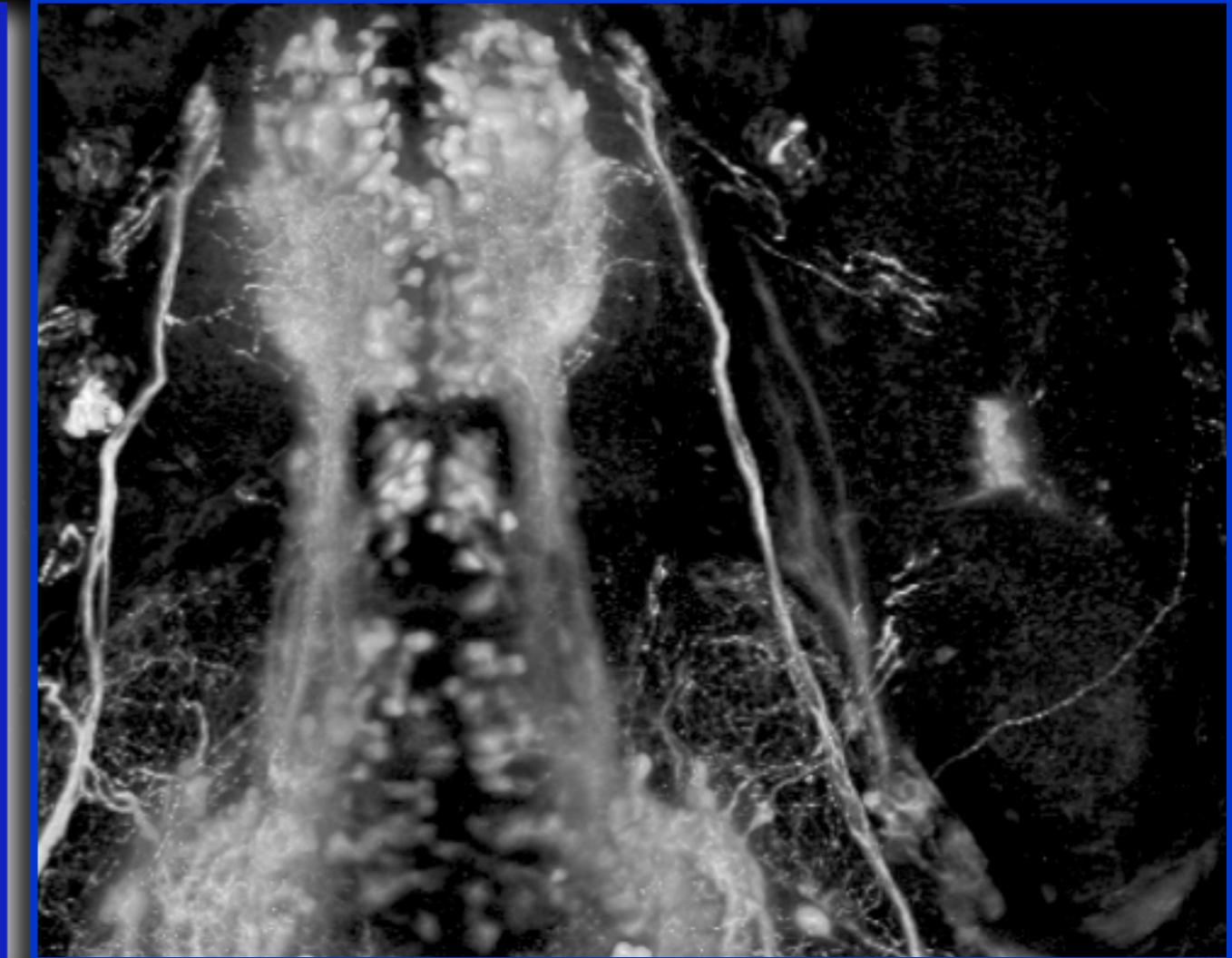
- ▶ 複式顕微鏡よりも高解像度 (x, y, z)
- ▶ 複式顕微鏡より高いS/N比
- ▶ 三次元の構造データを取得可
- ▶ 異なる生物組織を複数のチャンネルに分けてデータ取得可能

FluoRender does not lose any information from
your confocal data

Volocity



FluoRender



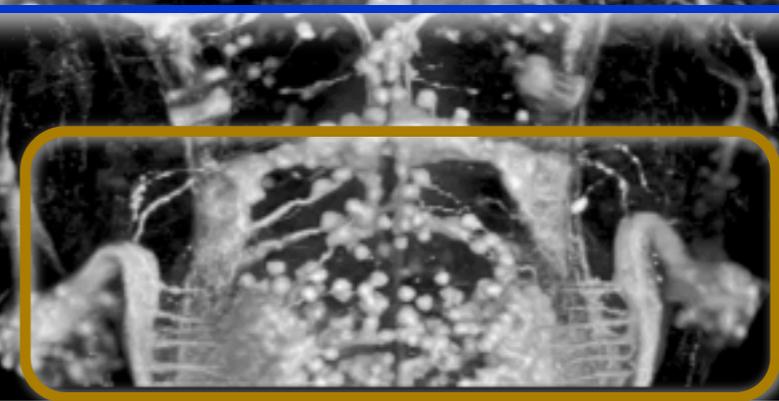
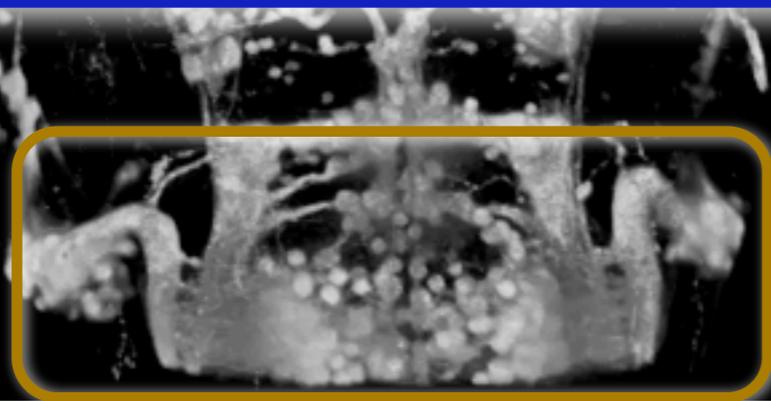
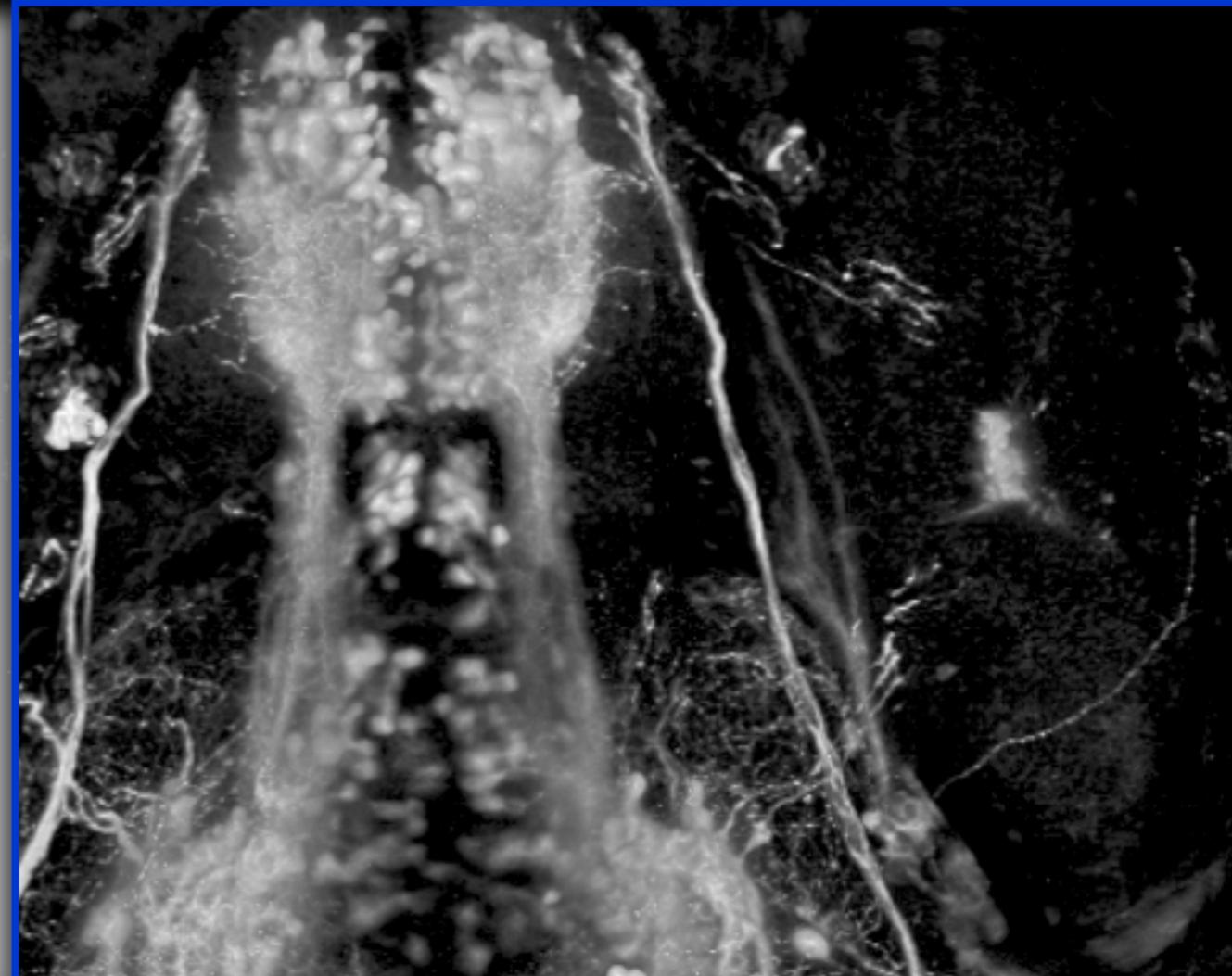
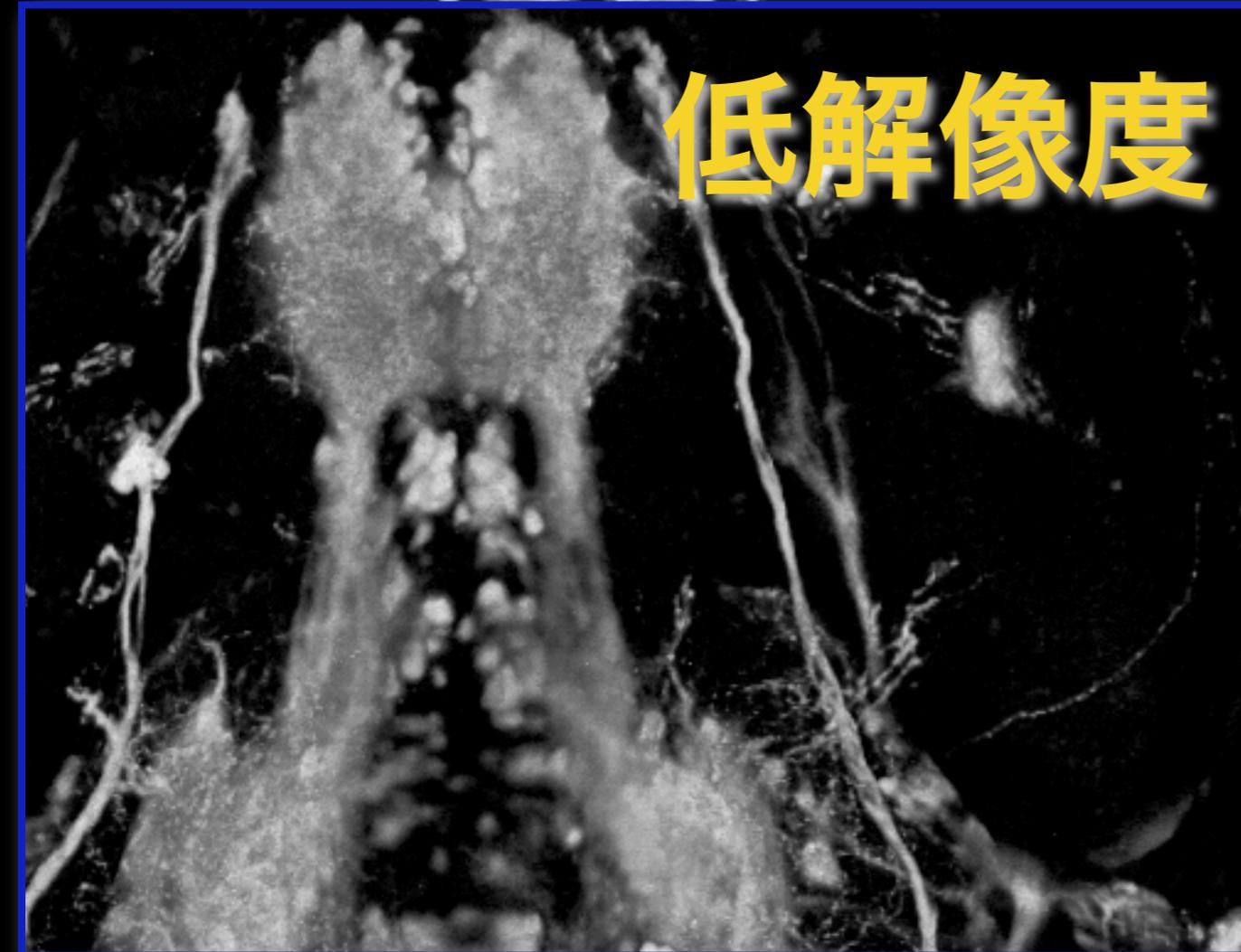
5dpf zebrafish, isl1:GFP

FluoRender does not lose any information from
your confocal data

Imaris

FluoRender

低解像度



5dpf zebrafish, isl1:GFP

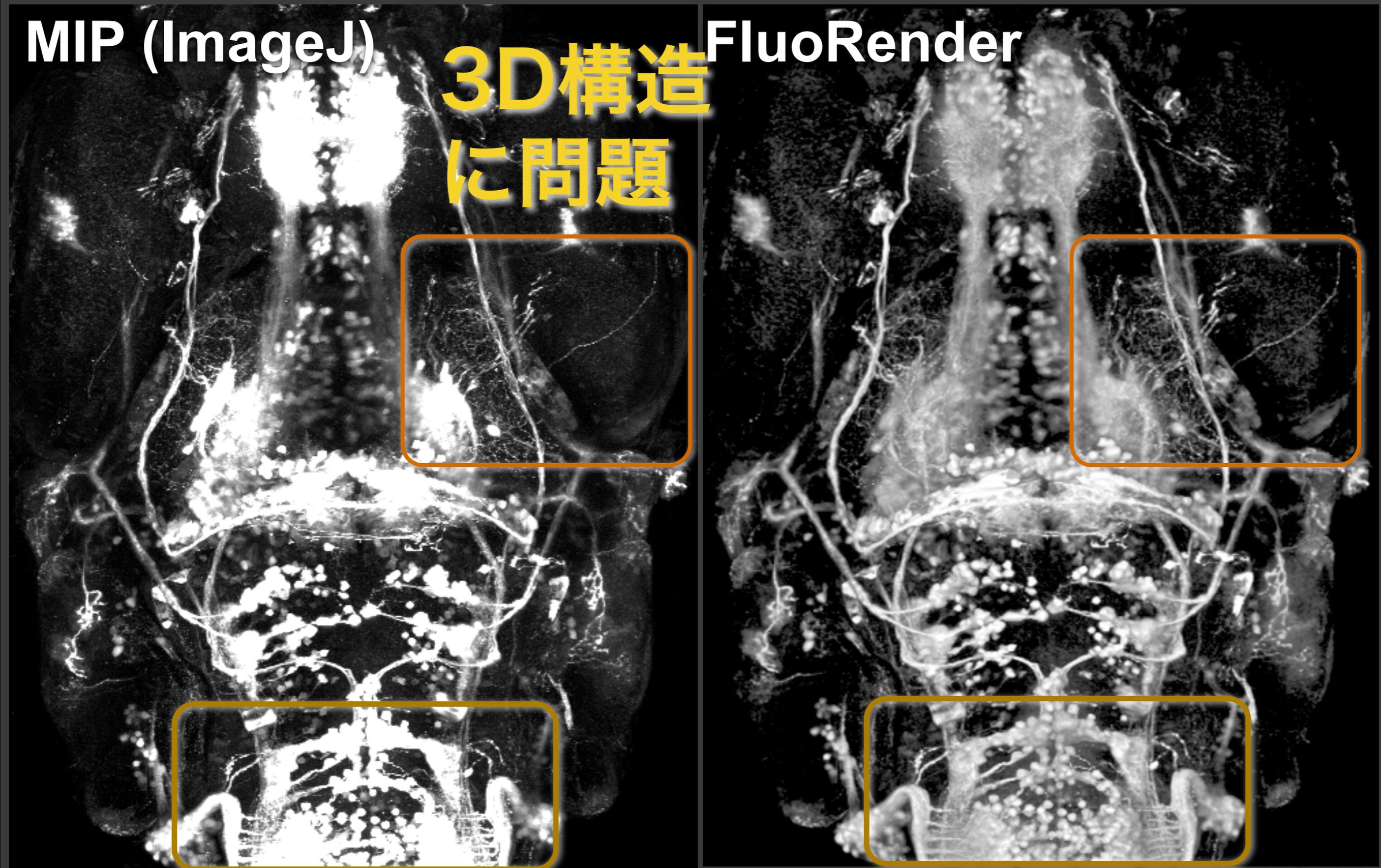
MIP vs FluoRender (direct volume rendering)

MIP (ImageJ)

3D構造
に問題

FluoRender

5dpf zebrafish, isl1:GFP

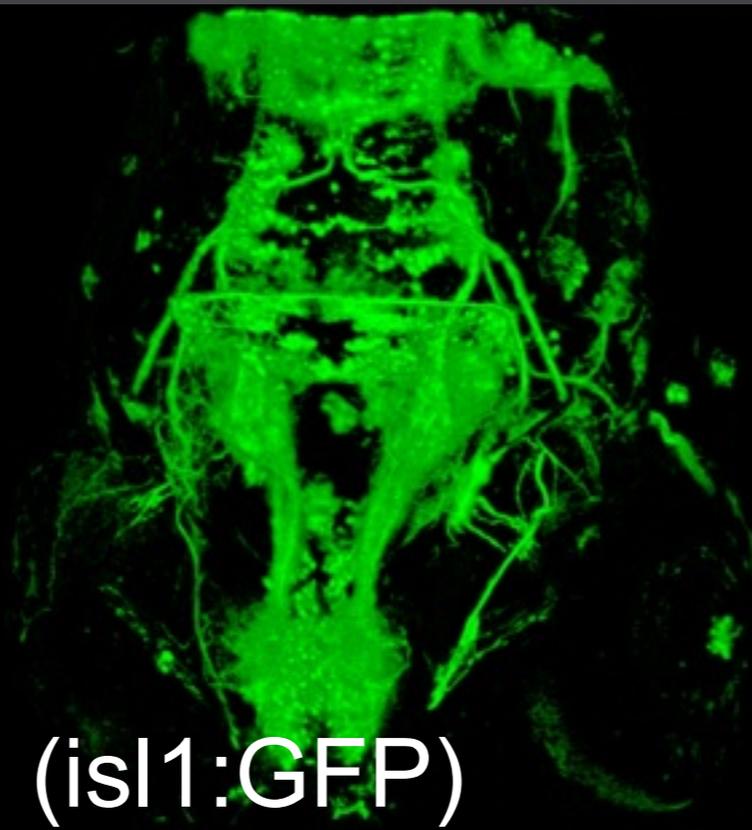


多チャンネルデータをどのようにレンダリングするのか？

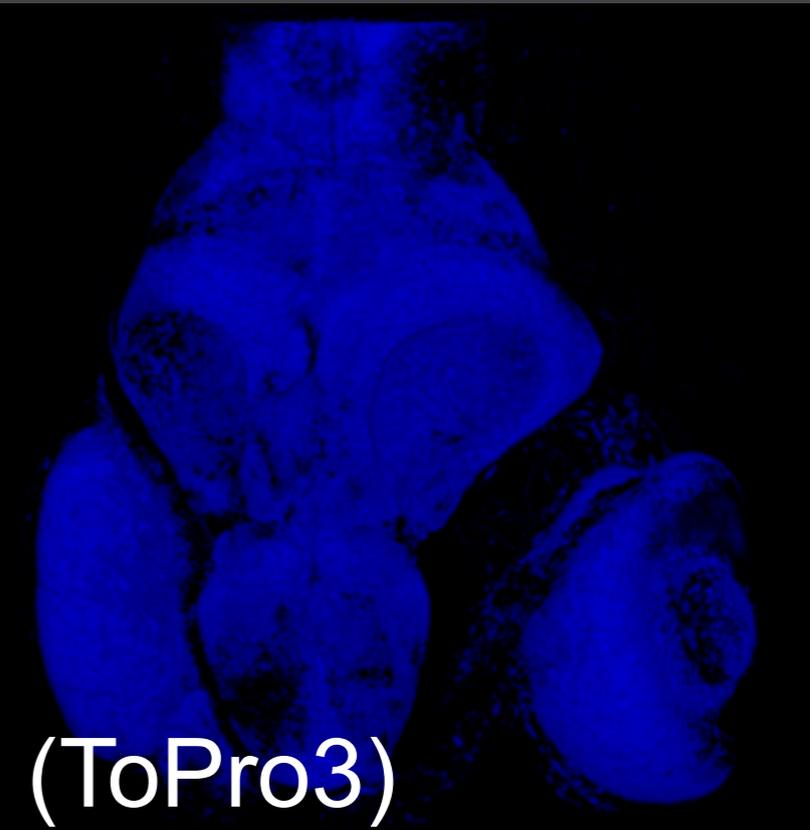
Muscle



Neurons



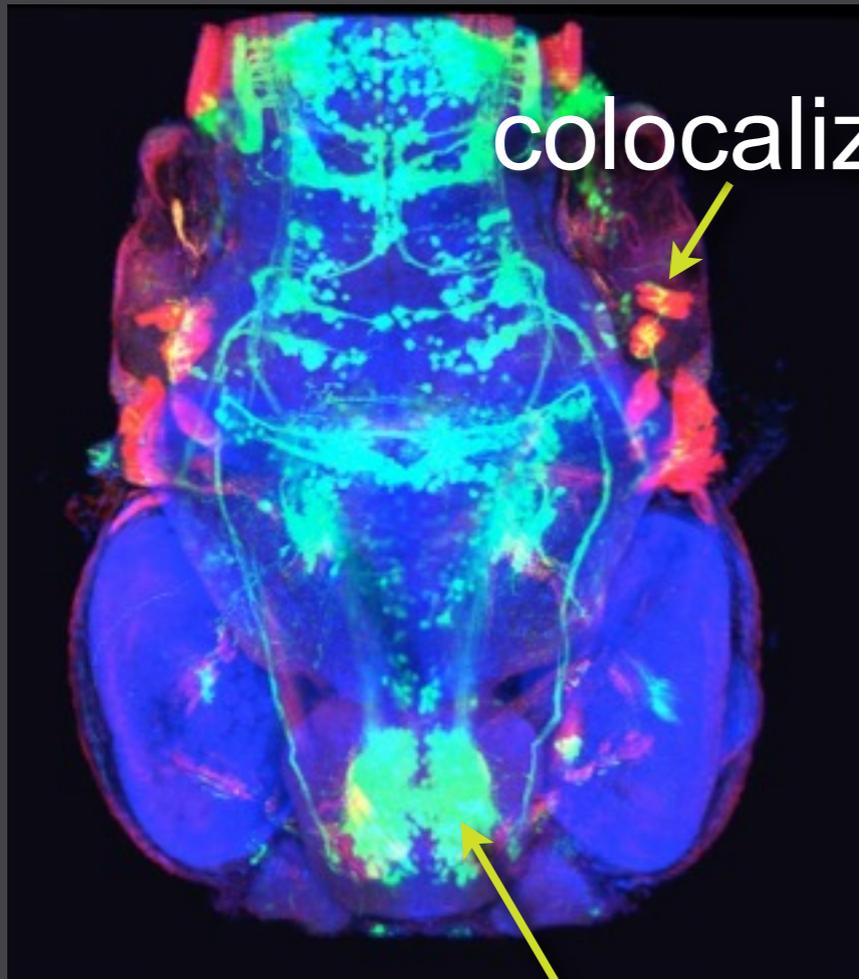
Whole tissue



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？

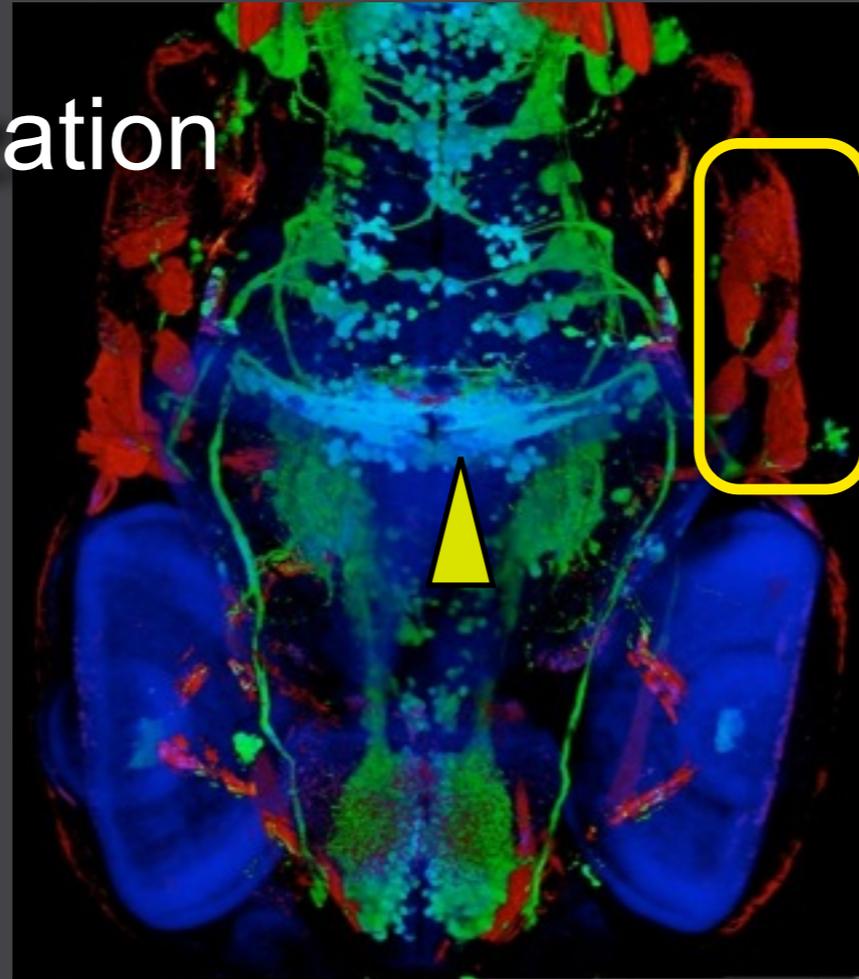
Amira5.2 (MIP)



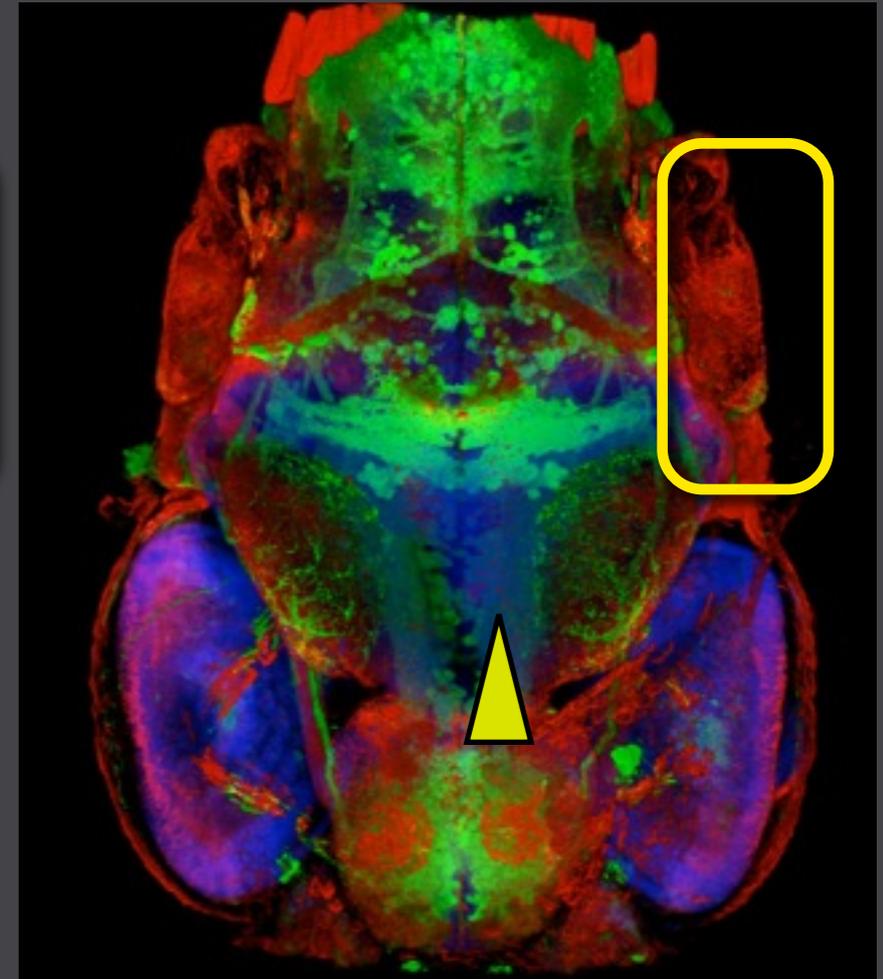
colocalization

saturated signals

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)
Volocity, Imaris etc..
- ▶ 複式顕微鏡より高いS/N比

- ▶ **MIP**の構造データを取得可

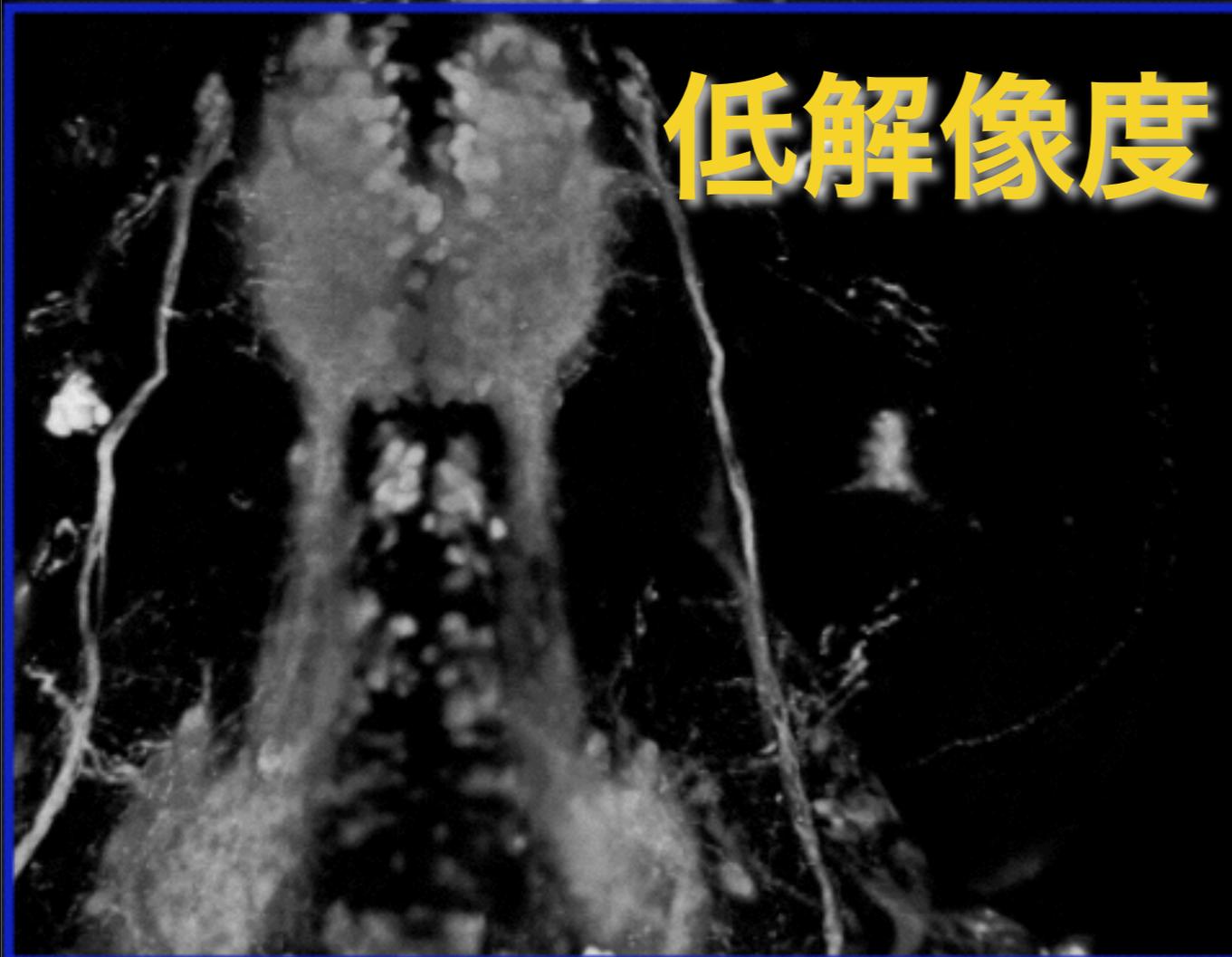
Jerold Wallis 1988

- ▶ 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..

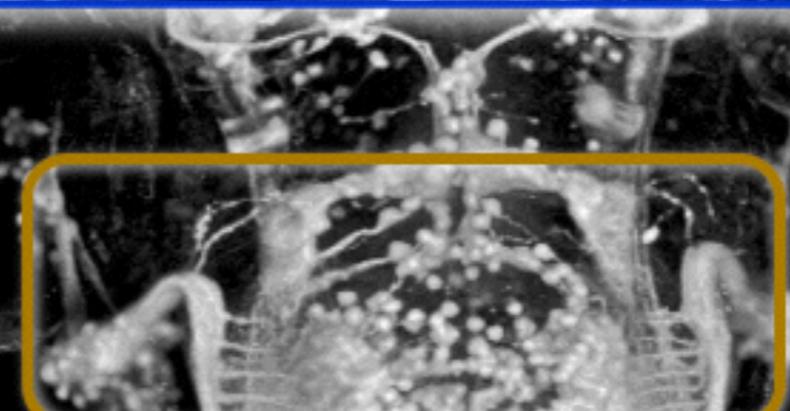
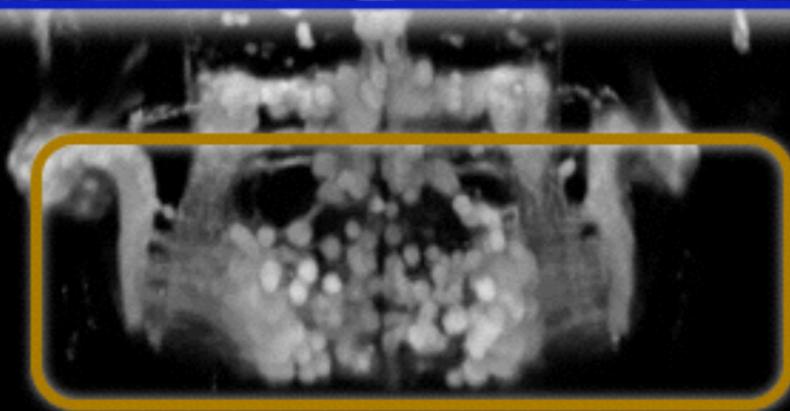
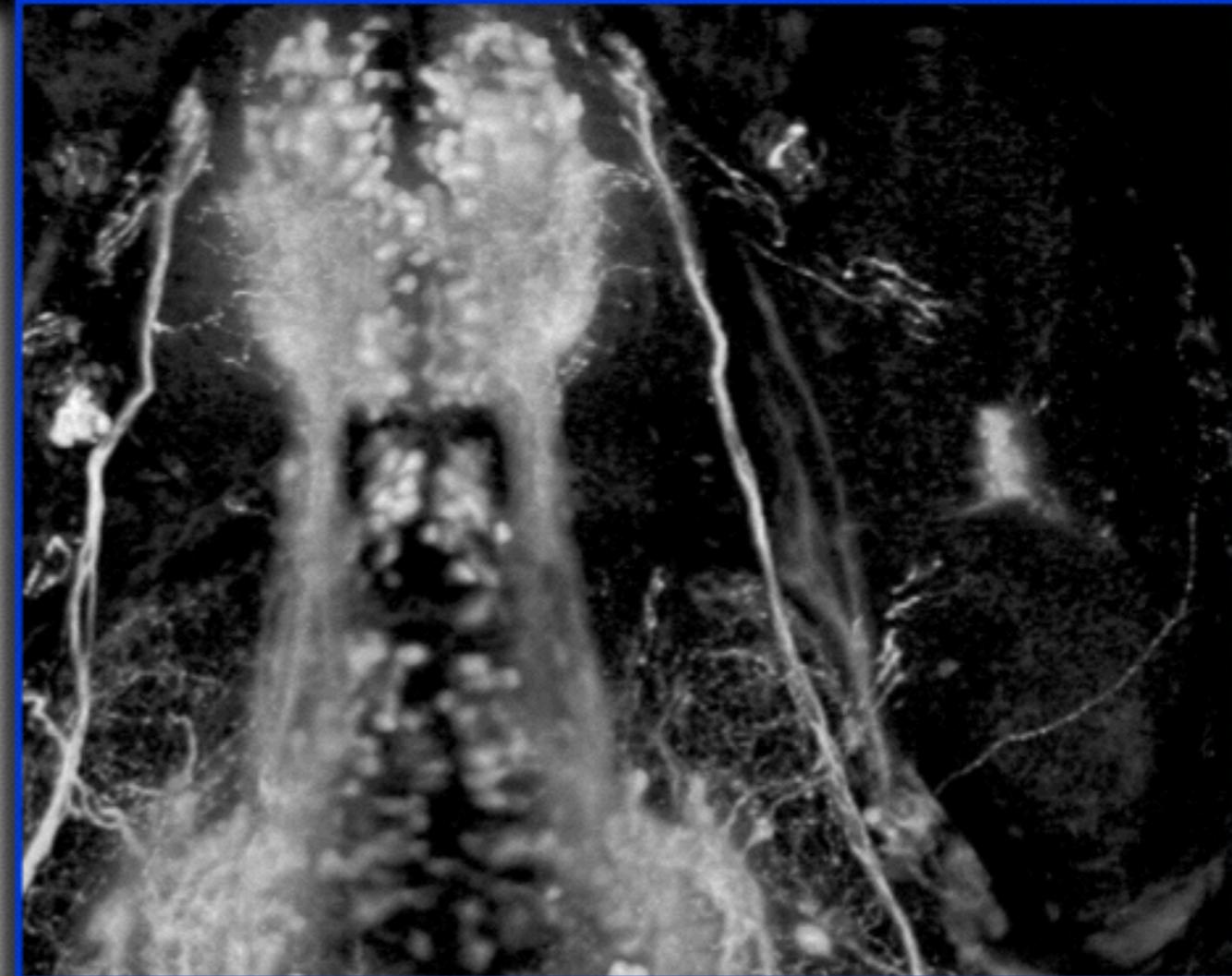
既存の3Dレンダリング法・ソフトウェアは生物学の研究で要求される条件を満たしていない

FluoRender does not lose any information from your confocal data

Volocity

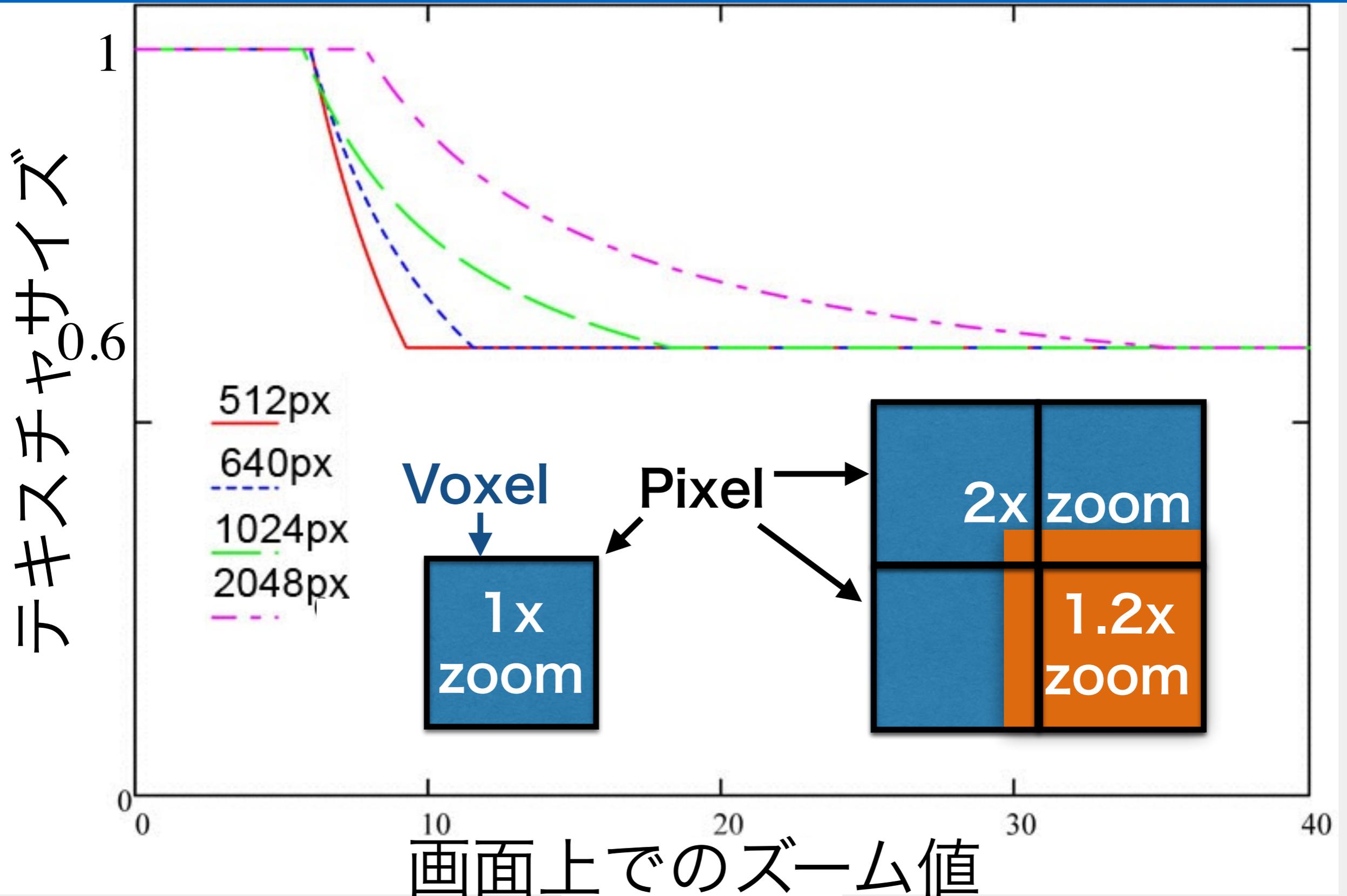


FluoRender



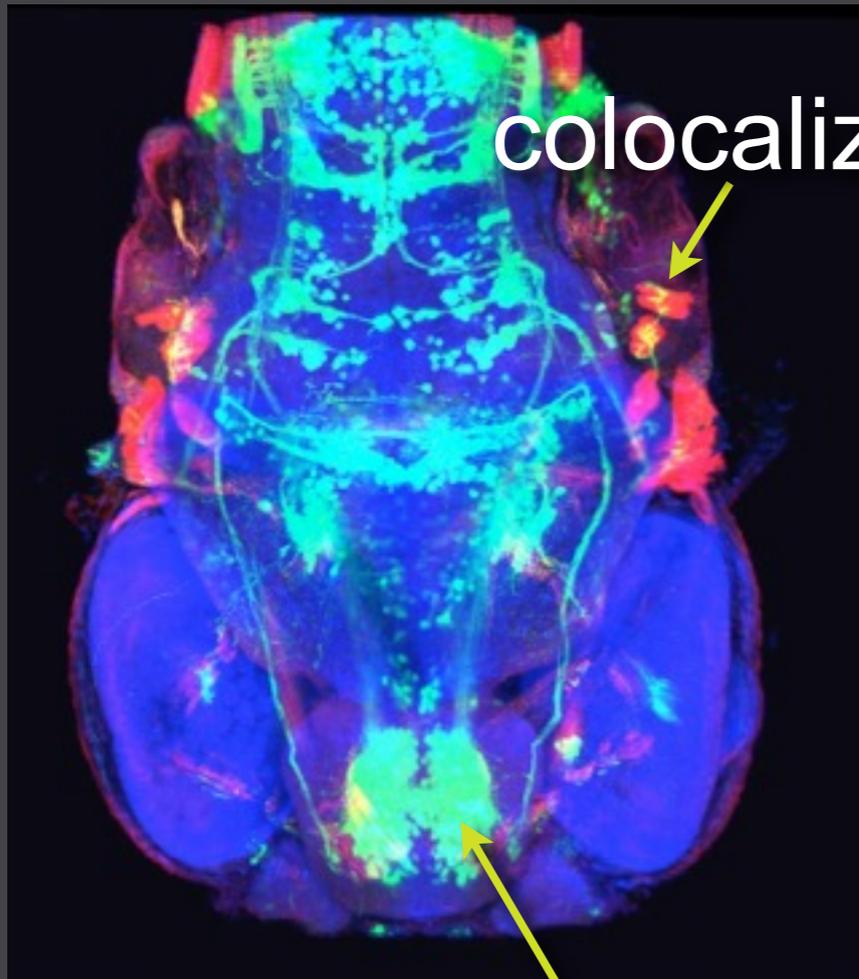
5dpf zebrafish, isl1:GFP

レンダリング時のテキスト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？

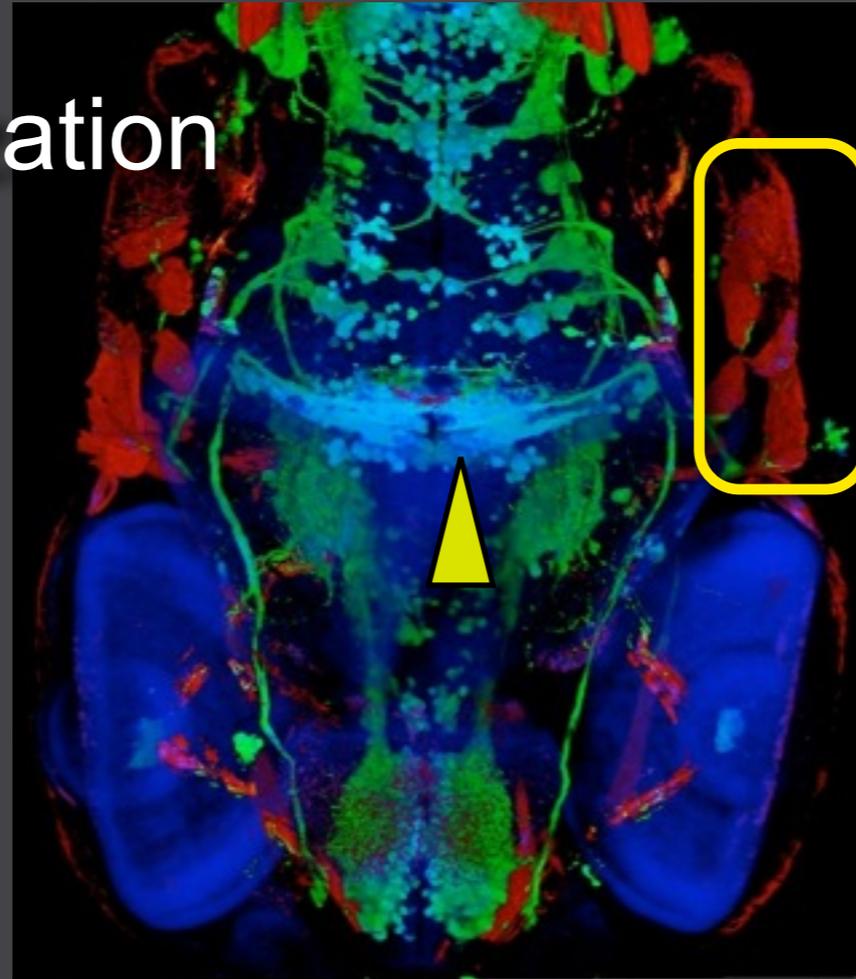
Amira5.2 (MIP)



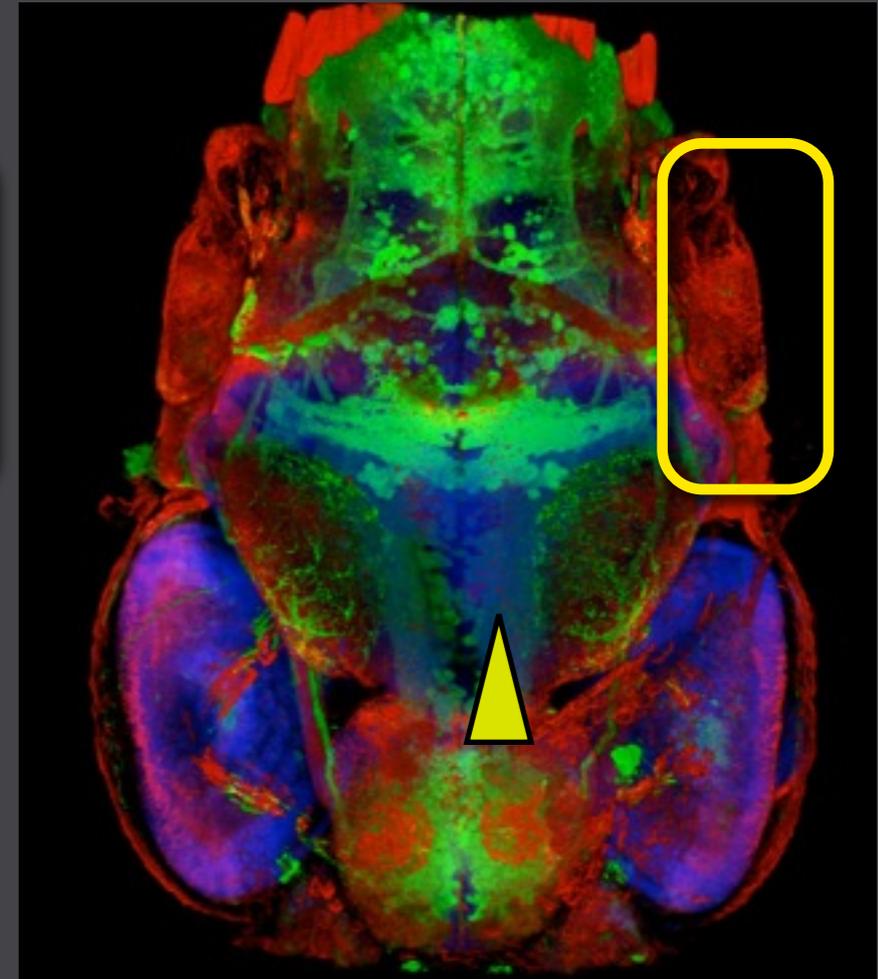
colocalization

saturated signals

Imaris6.3



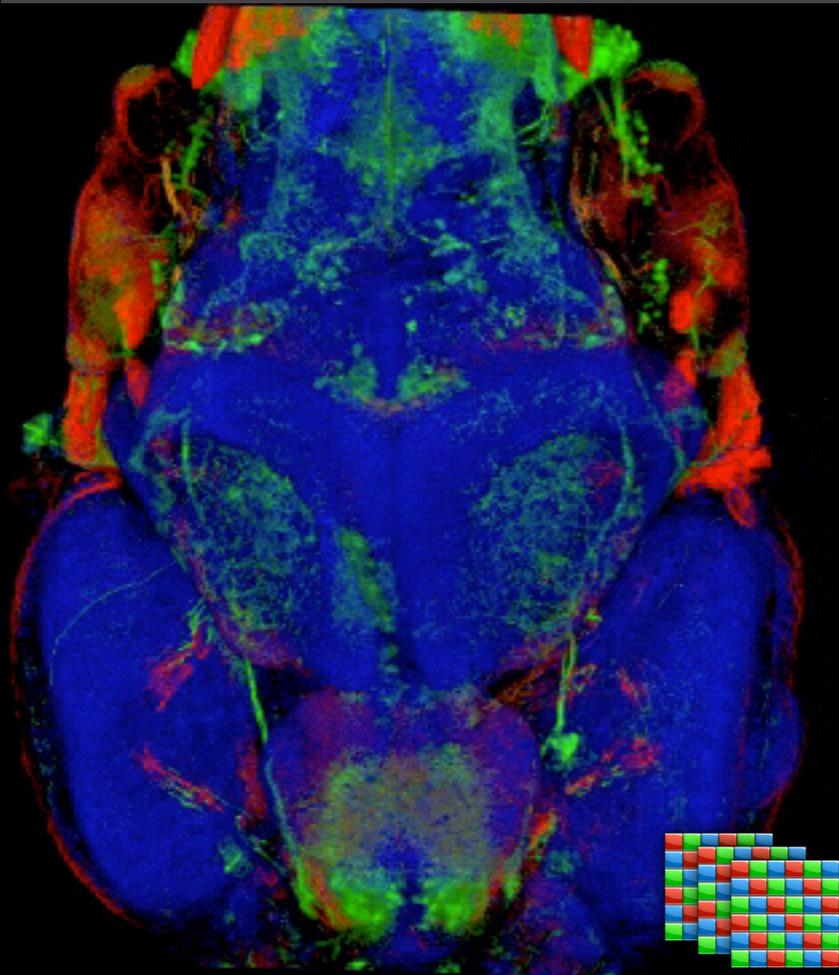
Volocity5.2



> 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

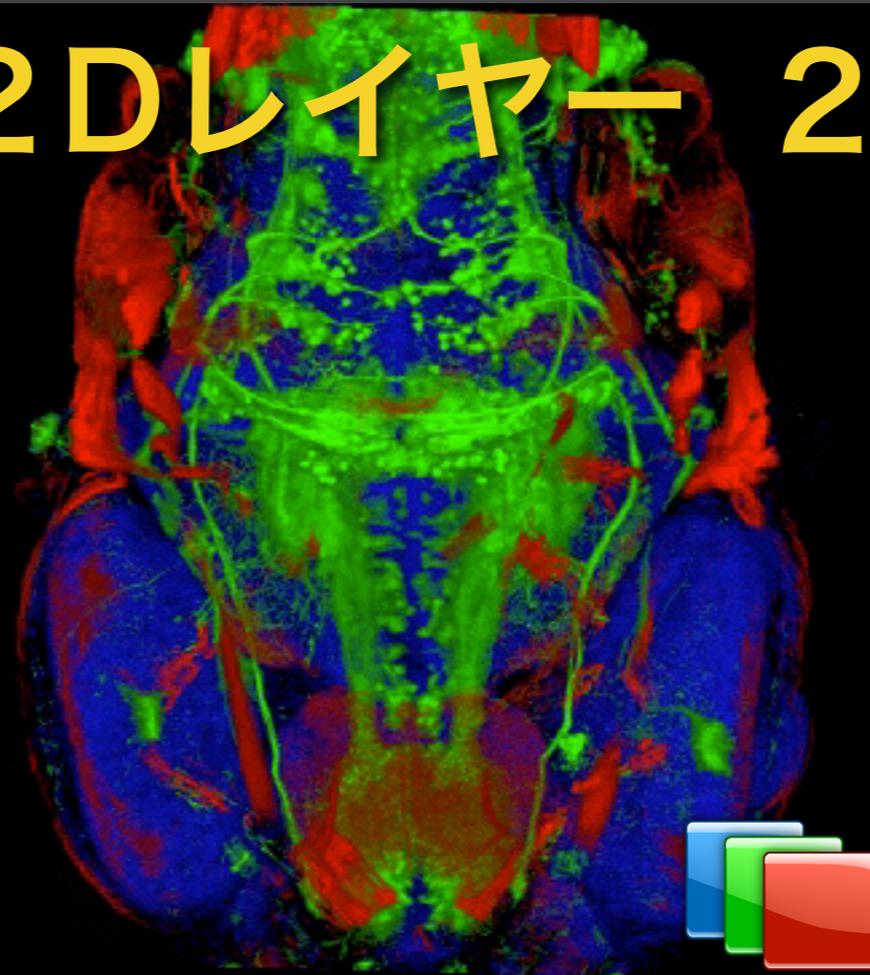
Depth mode



Channels are rendered according to their depth values; geometrically correct

Layer mode

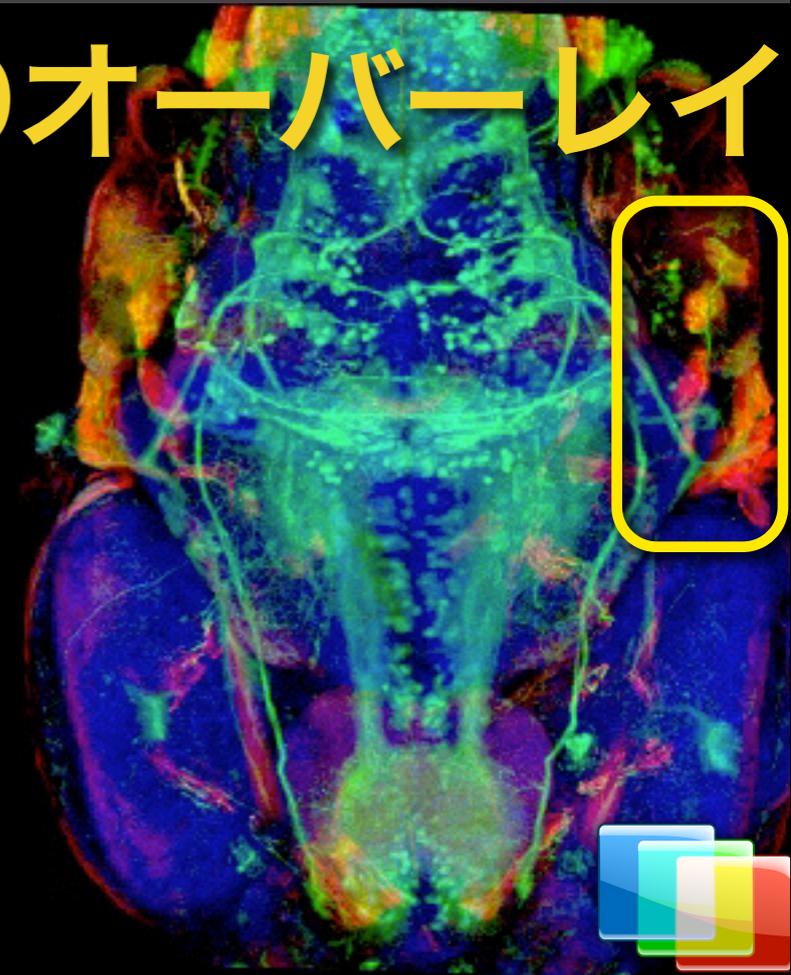
2Dレイヤー



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

Composite mode

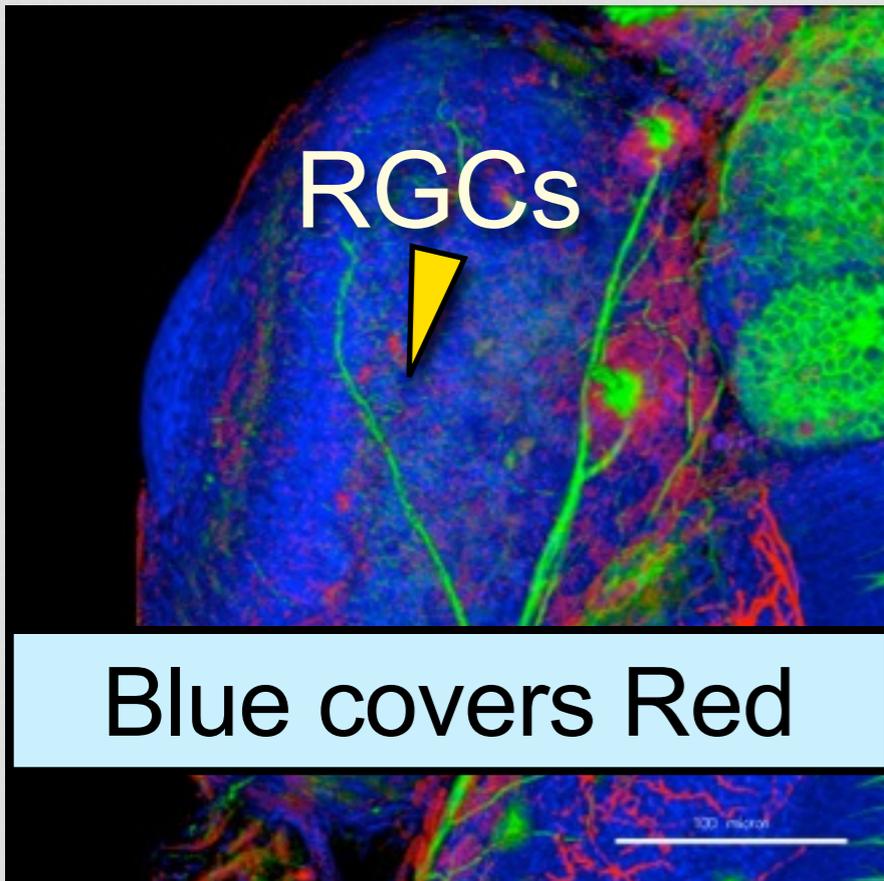
2Dオーバーレイ



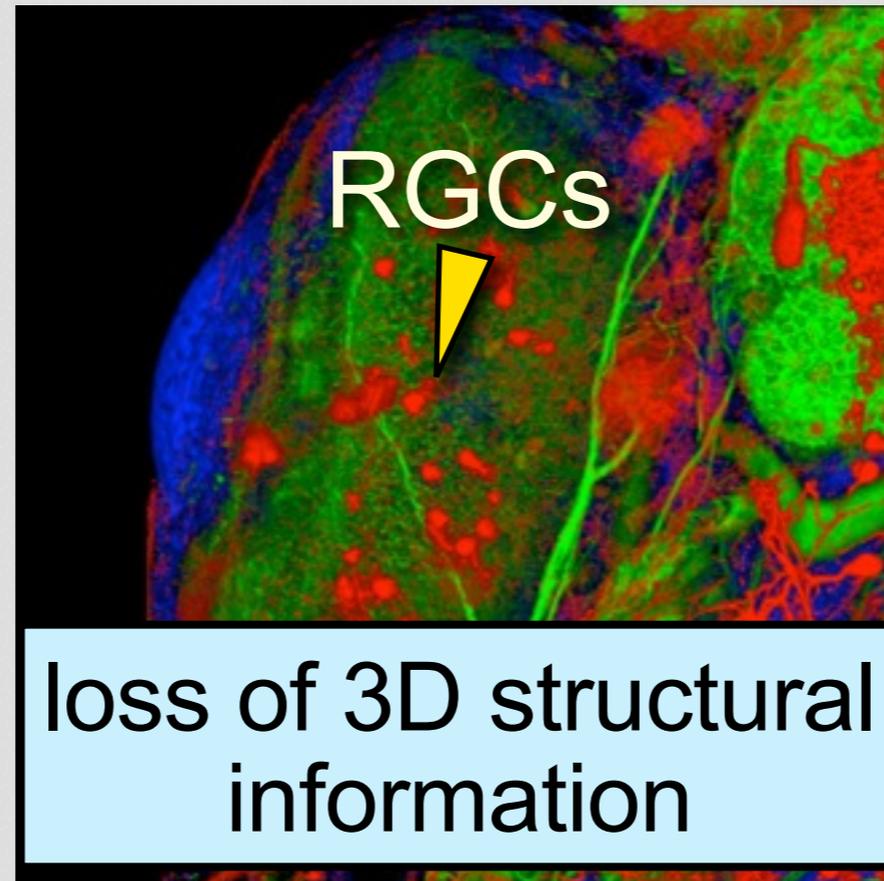
Channels are rendered individually and then blended, so deeper features can be seen

Multiple channels can cause information loss

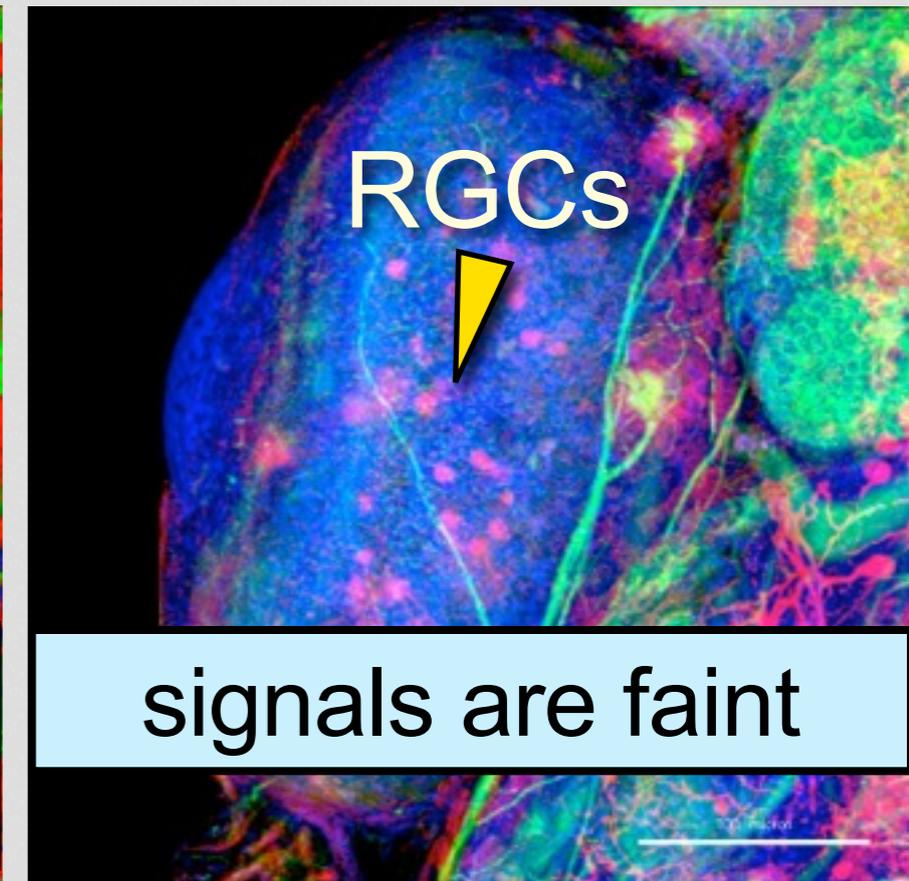
Depth mode



Layer mode



Composite mode



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

The screenshot displays a 3D rendering software interface. The central window shows a zebra head rendered with three channels: Red (Dec3no14He640px.tif(R)), Green (Dec3no14He640px.tif(G)), and Blue (Dec3no14He640px.tif(B)). The interface includes various control panels:

- Output Adjustments:** Sliders for Gamma, Luminance, and Depth Intensity for each channel (Red, Green, Blue).
- Render View:** Options for Layered, Depth, Composite, Persp., Ortho, and FreeFly views. The Ortho view is selected.
- Clipping Planes:** A panel on the right with a "Sync All Chan." checkbox and three sliders for X1, Y1, Z1 and X2, Y2, Z2. The values are X1: 0, Y1: 0, Z1: 0; X2: 640, Y2: 640, Z2: 110.
- Render View: 1 Group 1:** A list of channels with checkboxes for Dec3no14He640px.tif(R), Dec3no14He640px.tif(G), and Dec3no14He640px.tif(B).

An orange arrow points from the Clipping Planes panel to a text box in the bottom right corner.

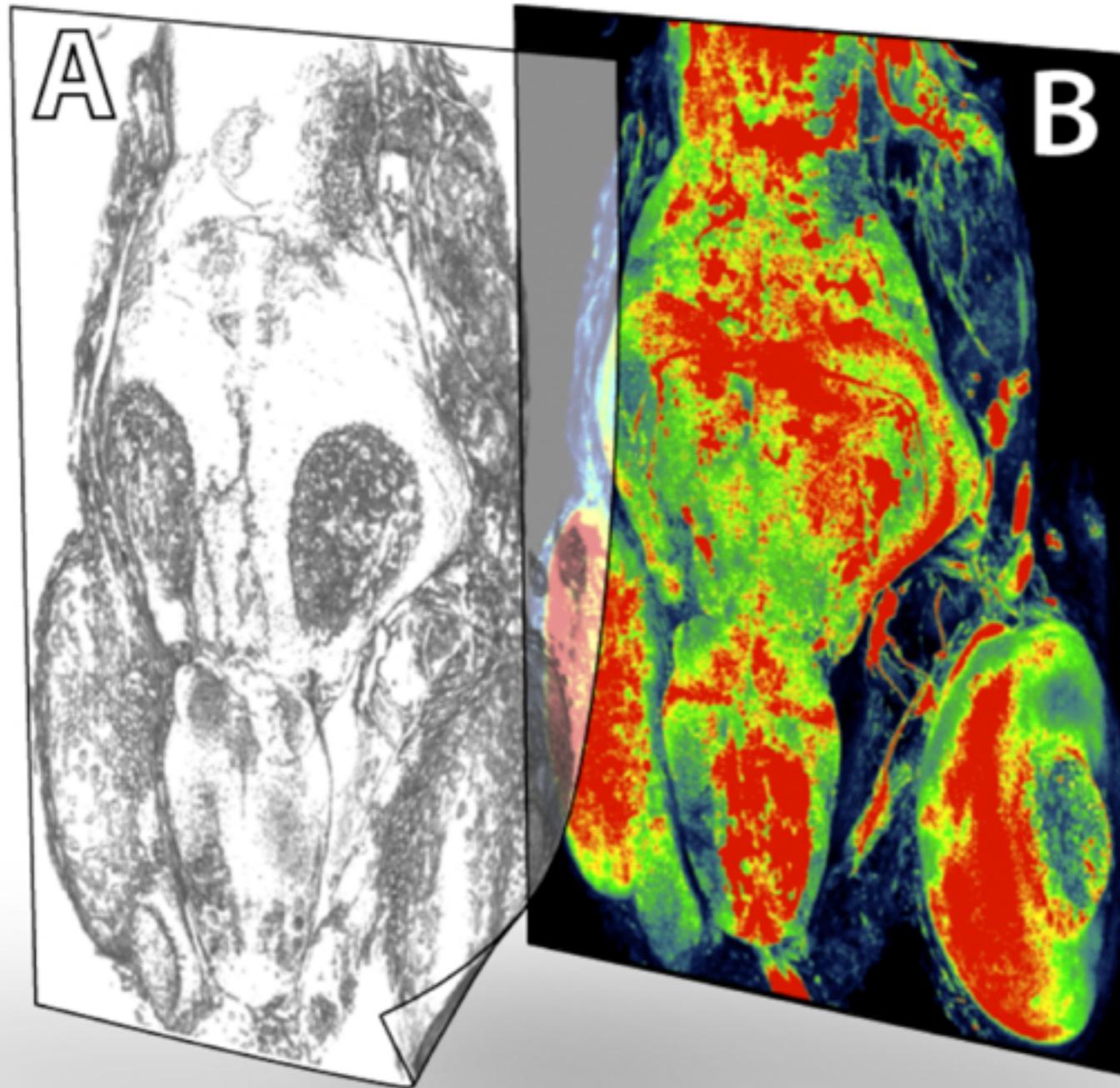
Clipping plane adjustment

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

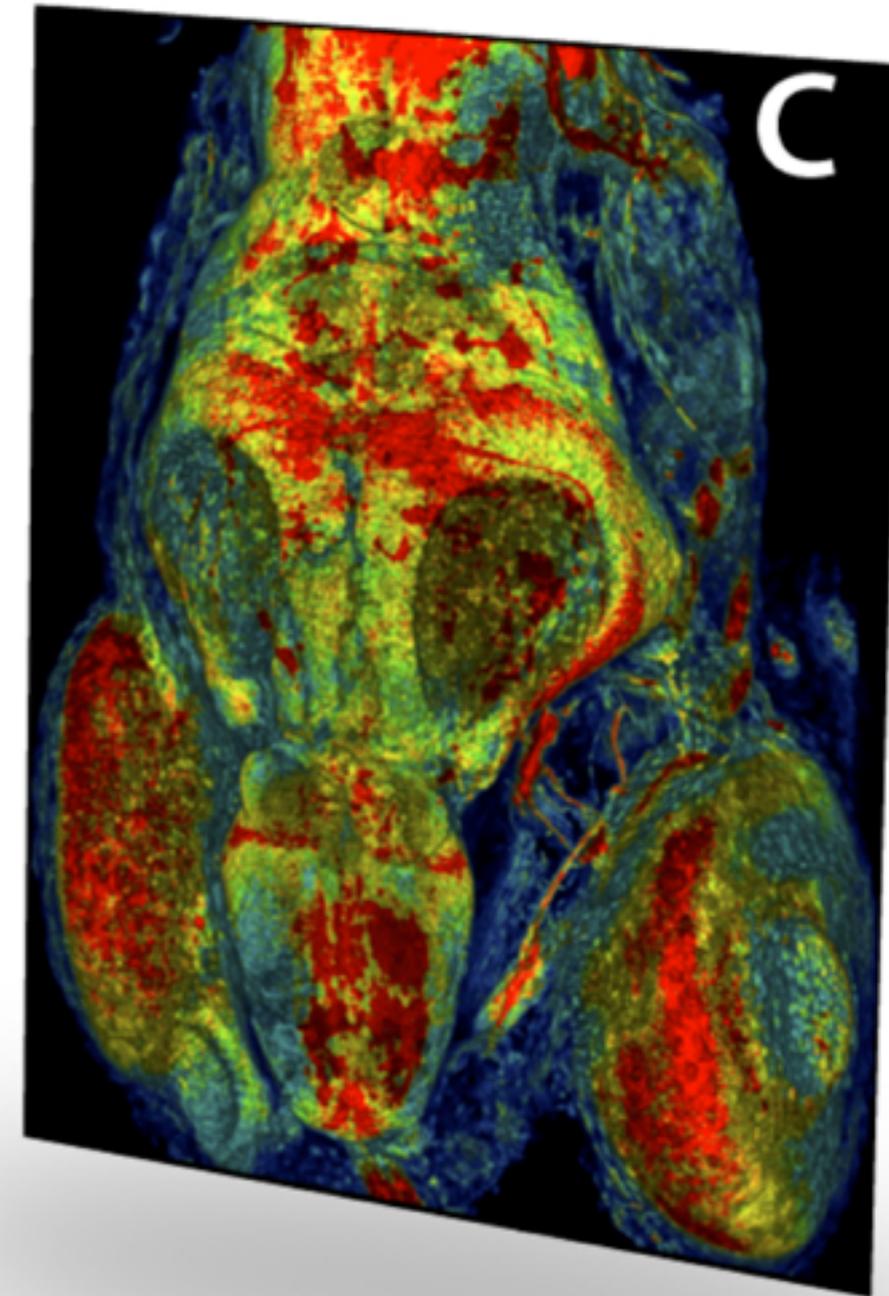
強いシグナルが重要

Maximum Intensity Projection + Shading



Brightness Modulation (X)

=

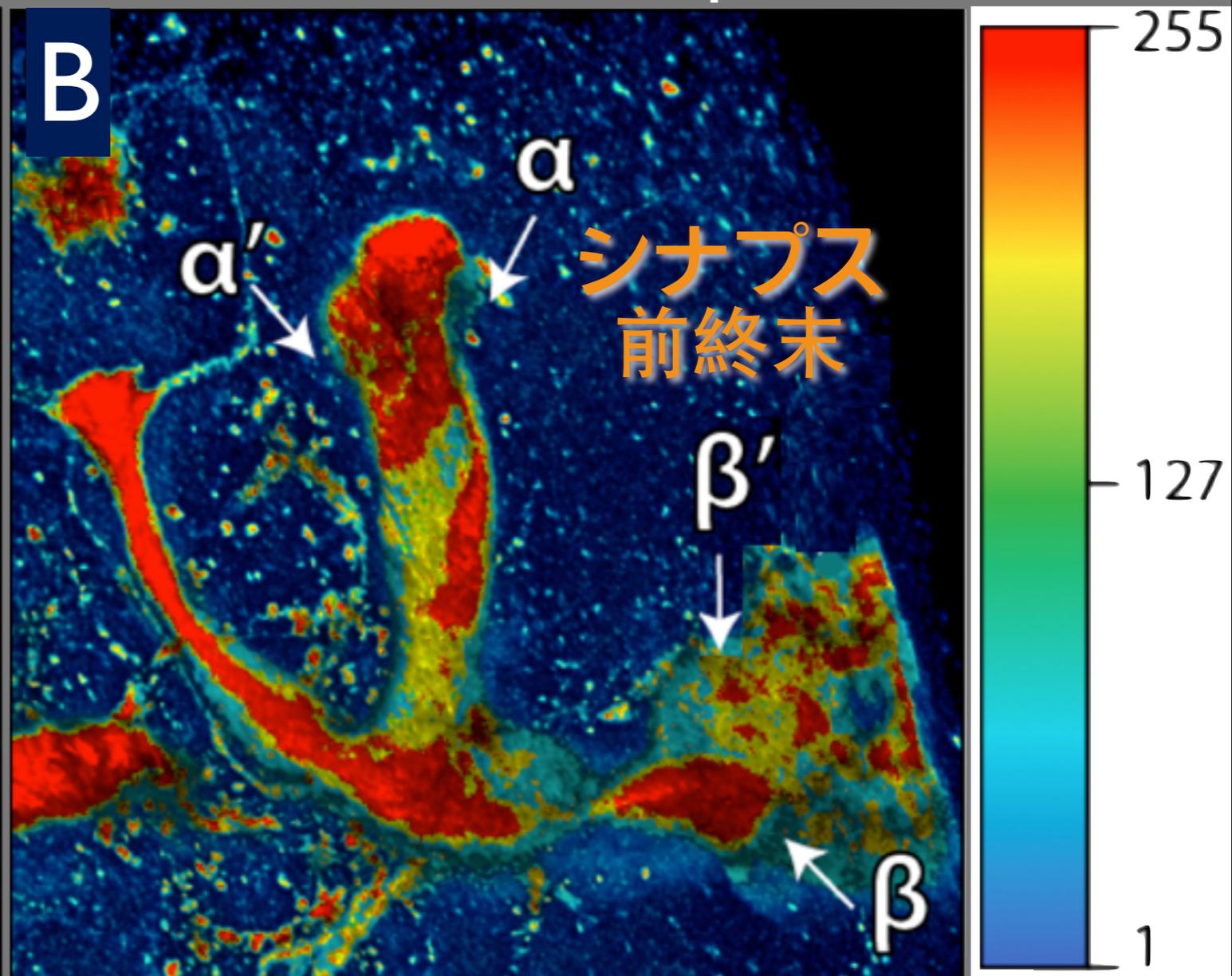
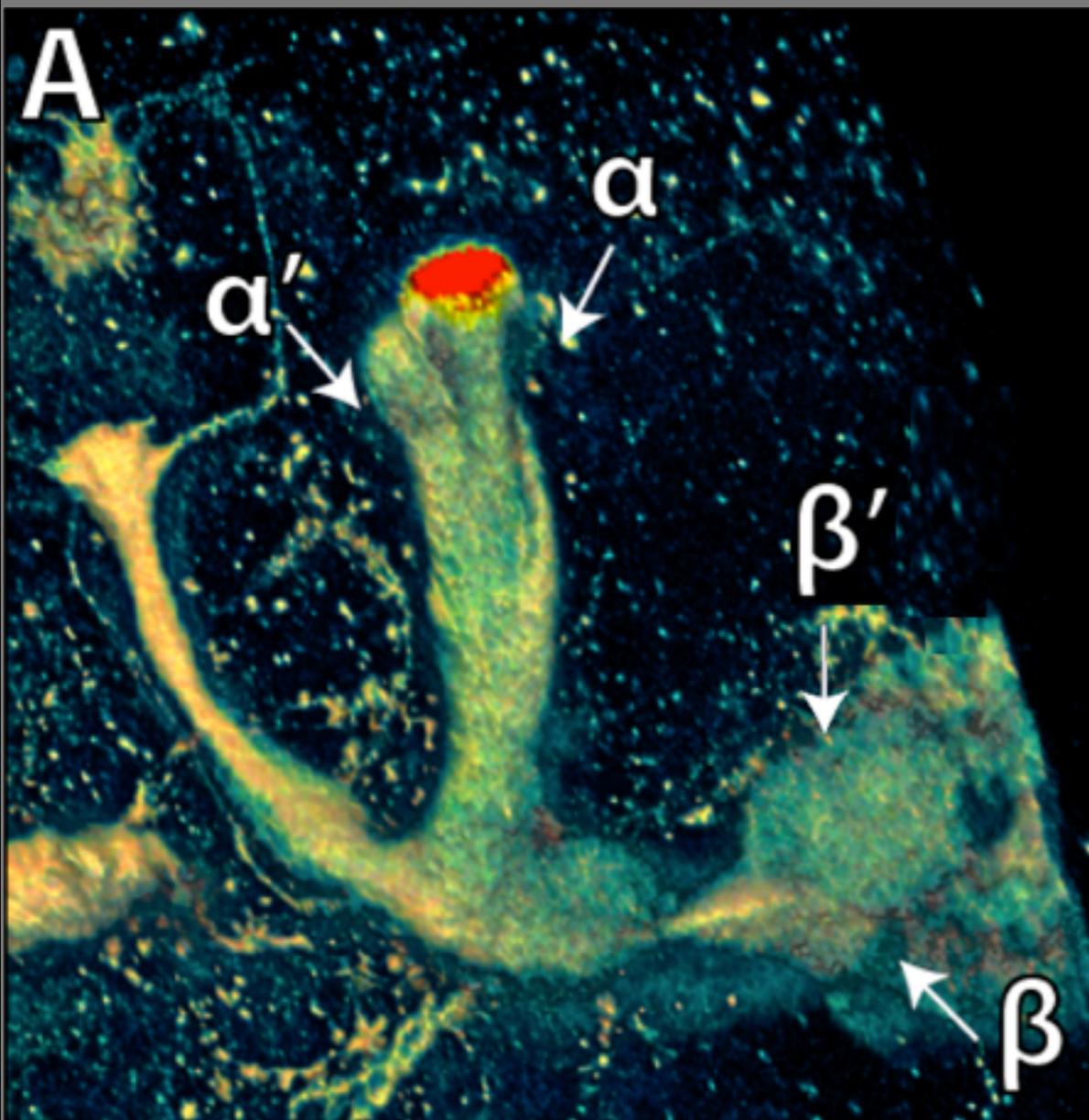


5dpf zebrafish, ToPro-3

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



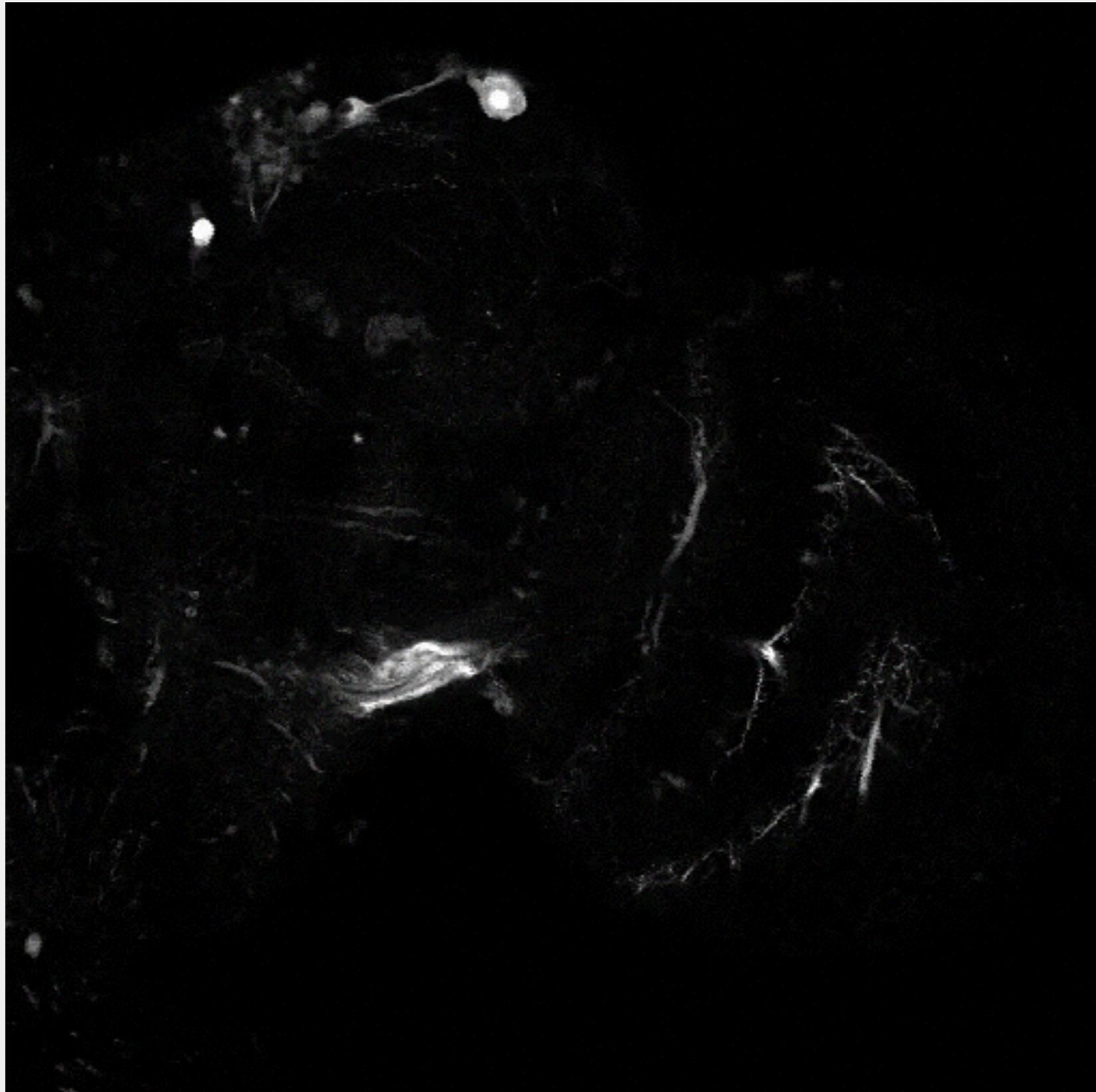
トランスジェニック技術や、抗体染色により、特定の細胞をラベルすることが可能



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

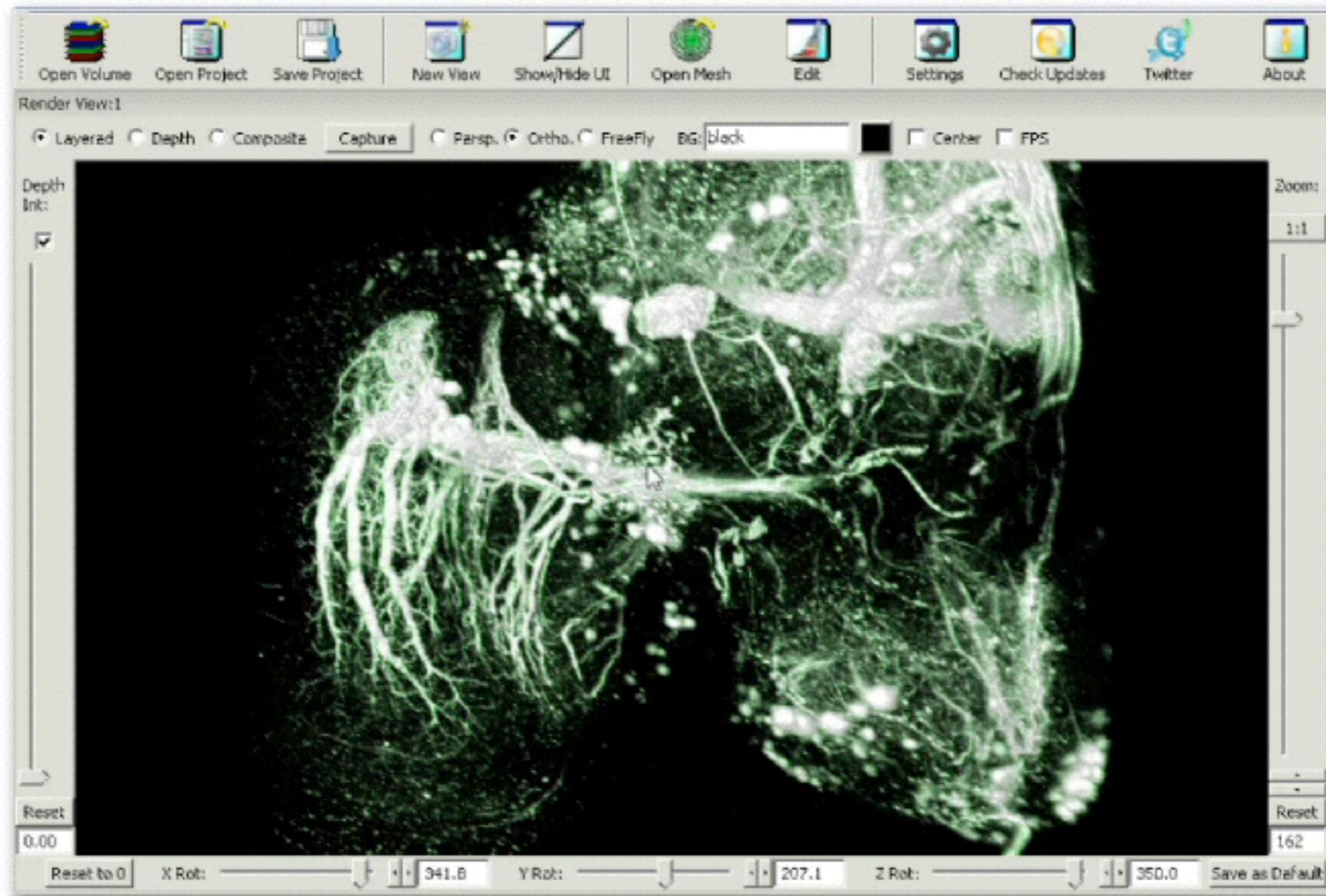
特定の組織を可視化・計測するため
に、セグメンテーションが必要

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



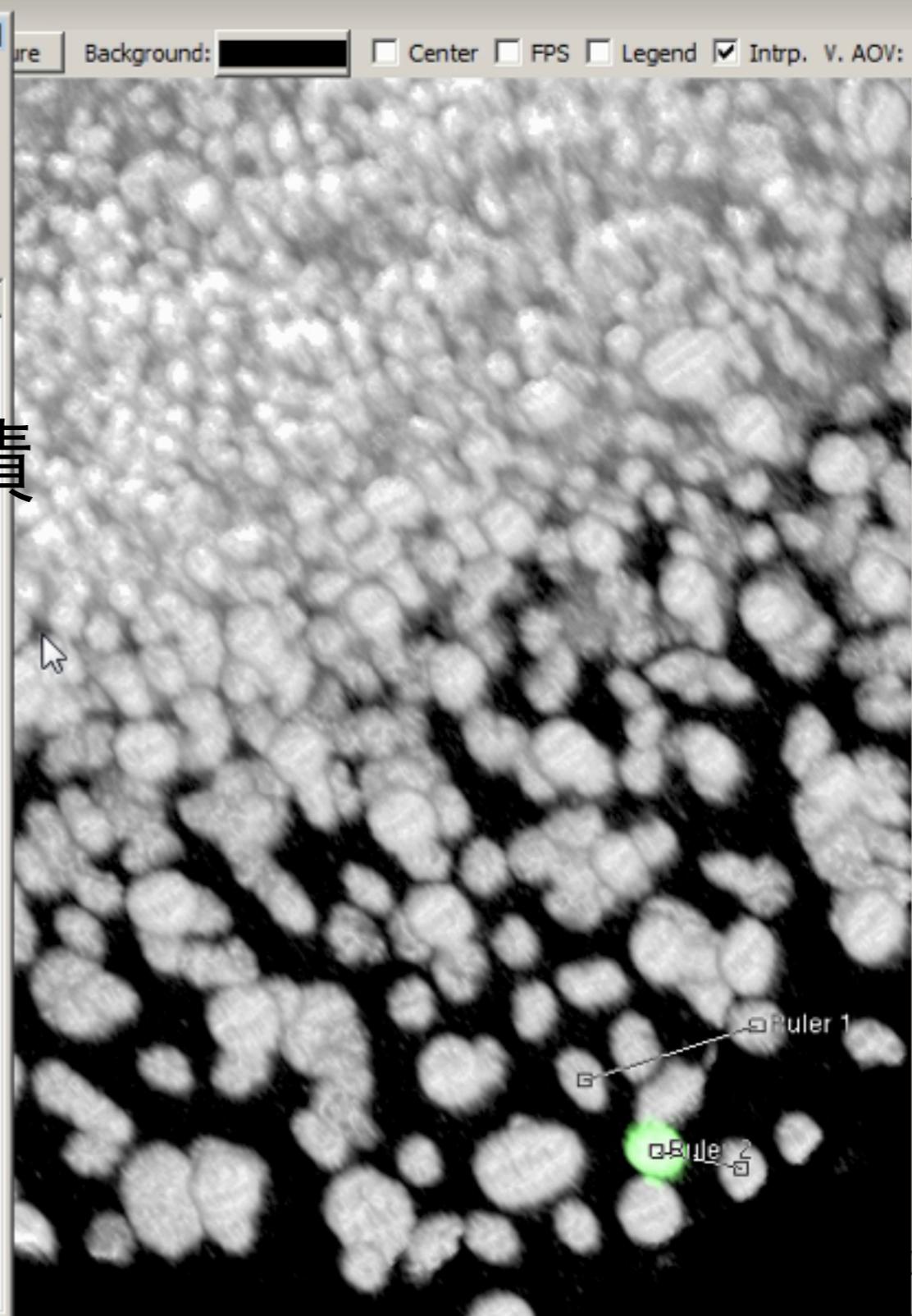
Measurement

2pt Ruler 2+pt Ruler Edit Delete Delete All Export

Maximum Intensity Accumulated Intensity

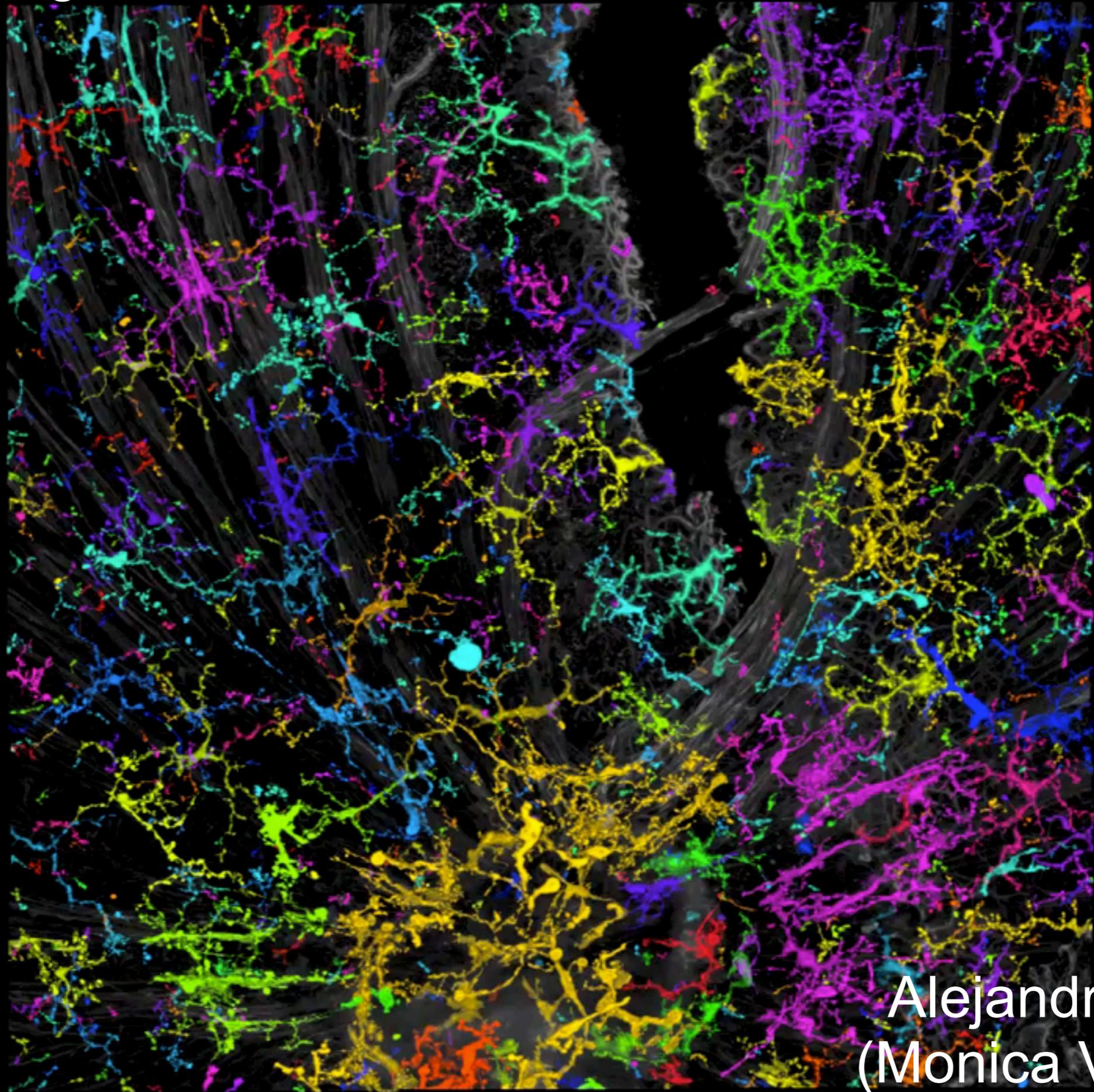
Transient Use Volume Properties

ID	Length	Angle	Start/End Points (X, Y, Z)	Time	Volumes
Ruler 1	33.42 μ m	154.2Deg	(99, 201, 18), (69, 186, 15)	0	899, 790
Ruler 2	15.89 μ m	178.7Deg	(70, 210, 4), (85, 211, 10)	0	526, 874



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

0	0	0	4	0	0
0	17	0	4	0	0
0	0	17	17	17	0
0	0	0	0	0	0
3	0	33	0	0	2
0	33	33	0	36	36

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



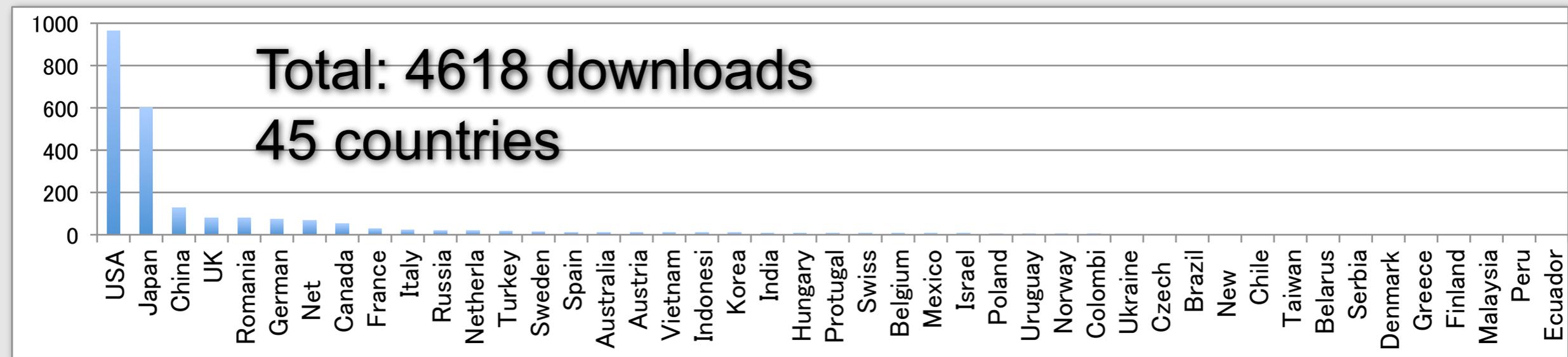
download
free!!

<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



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R01 GM098151-01

R01 MH092256-01