

# Scientific Volume Imaging (SVI)

## About us



Paul Bloembergen, Msc.  
Developer  
paul@svi.nl

Frans van der Have, PhD  
Developer  
frans@svi.nl



Scientific Volume Imaging

Deconvolution – Visualization - Analysis

Huygens Software

# Light Microscopy



# Super-resolution light-microscopy



4.00  $\mu\text{m}$



# Huygens

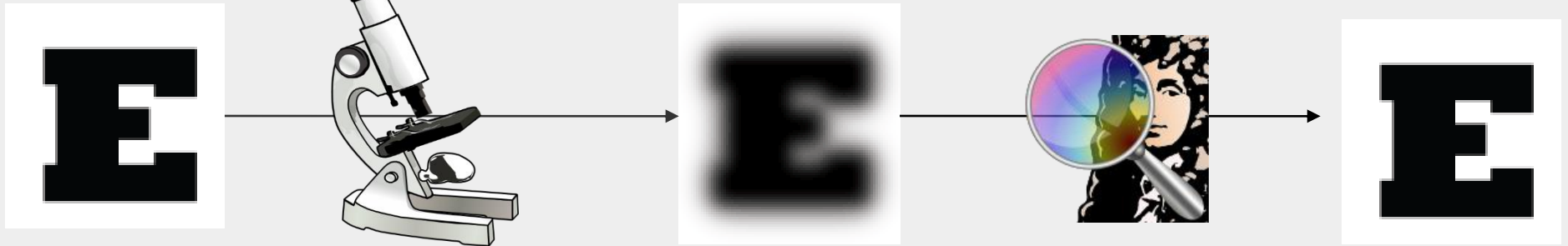
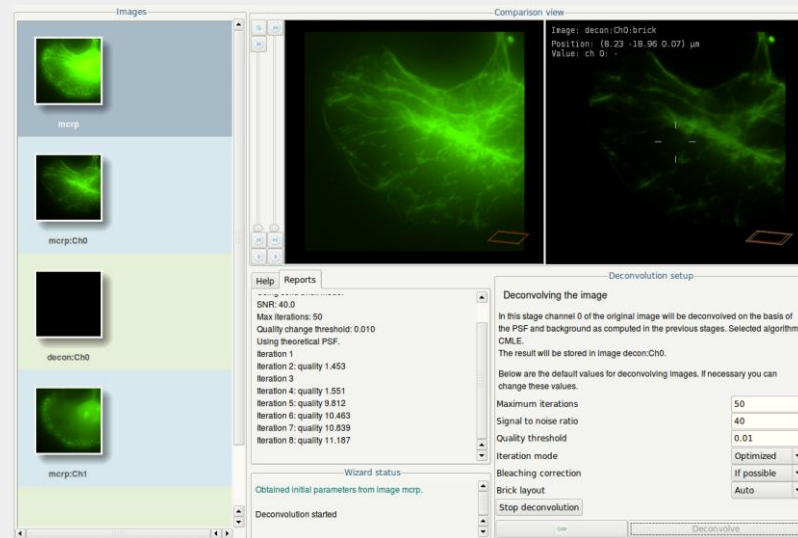
Isaac Newton (1642-1725)



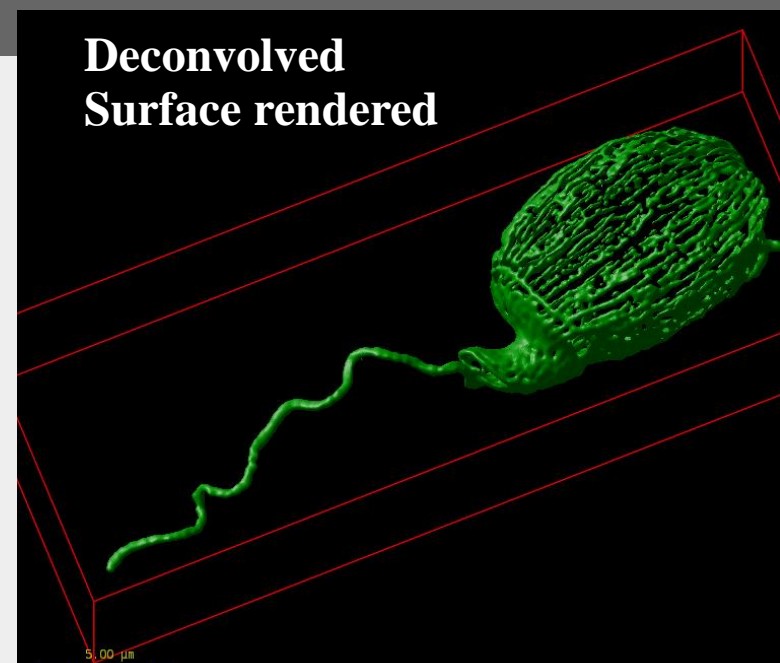
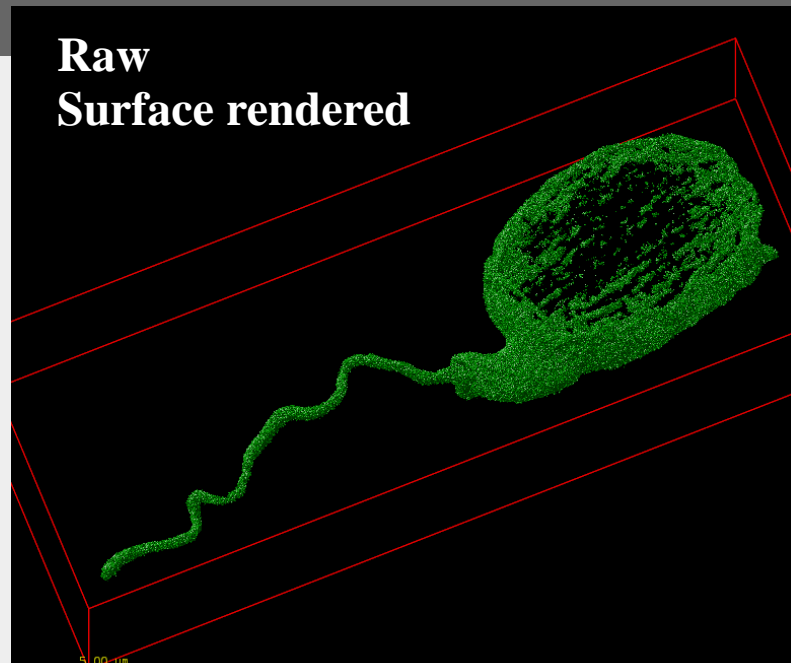
Christiaan Huygens  
(1629-1695)

# Deconvolution

Lens	TECNIS® Lens	AcrySof**IQ IOL	B&L LI61A0 IOL	Spherical IOL
Point Spread Function**				
20/20*	<b>E</b>	<b>E</b>	<b>E</b>	<b>E</b>
Average Corneal SA	+0.27	+0.27	+0.27	+0.27
Lens SA**	-0.27	-0.17	0.0	+0.15
Total Residual SA	<b>0.0</b>	<b>+0.10</b>	<b>+0.27</b>	<b>+0.42</b>

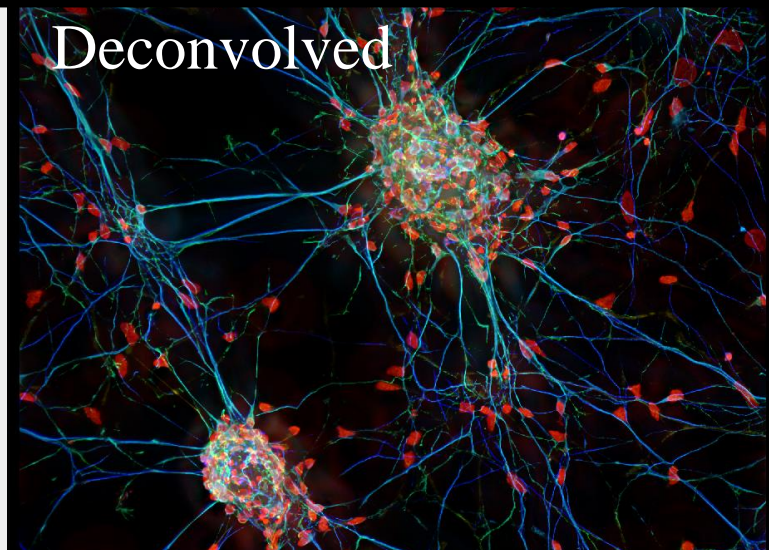


# Deconvolution



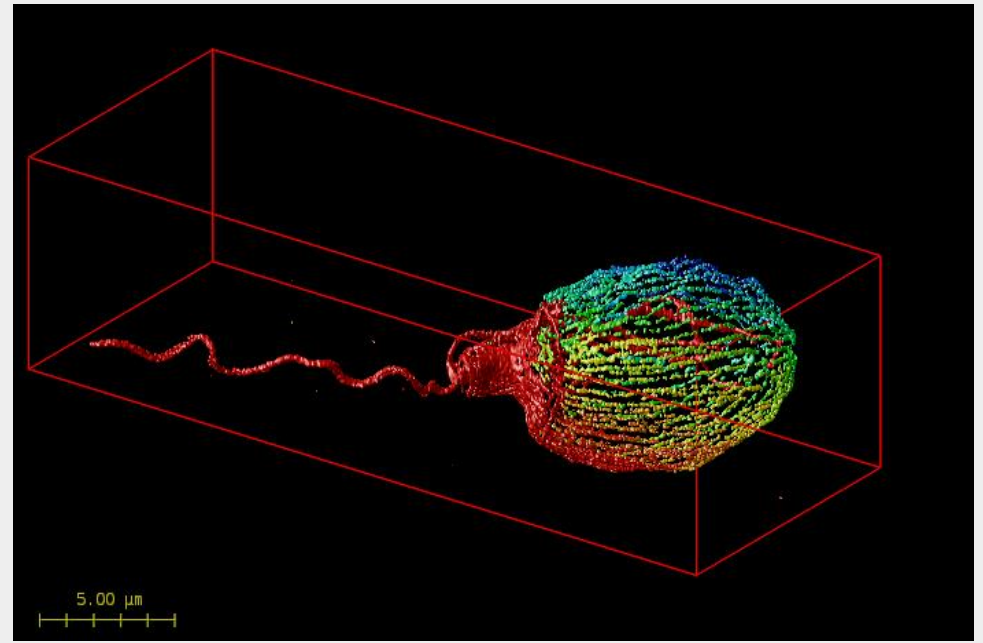
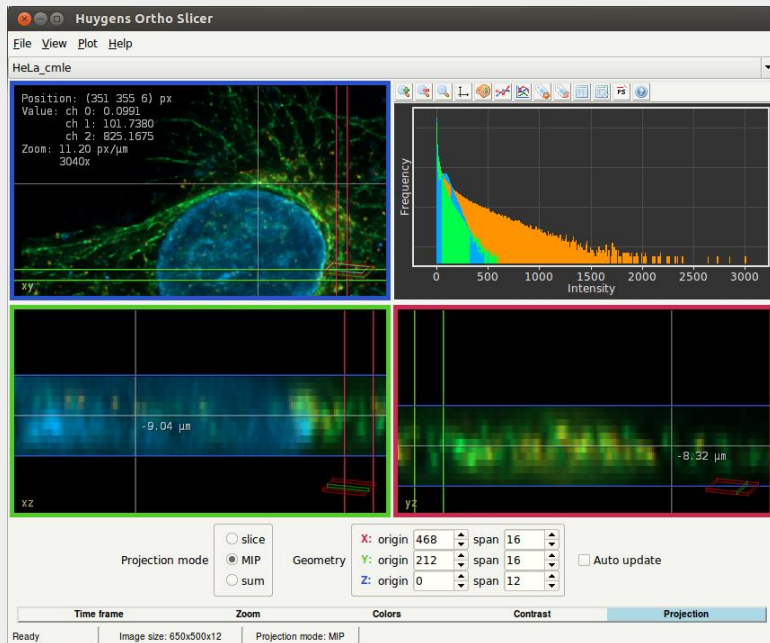
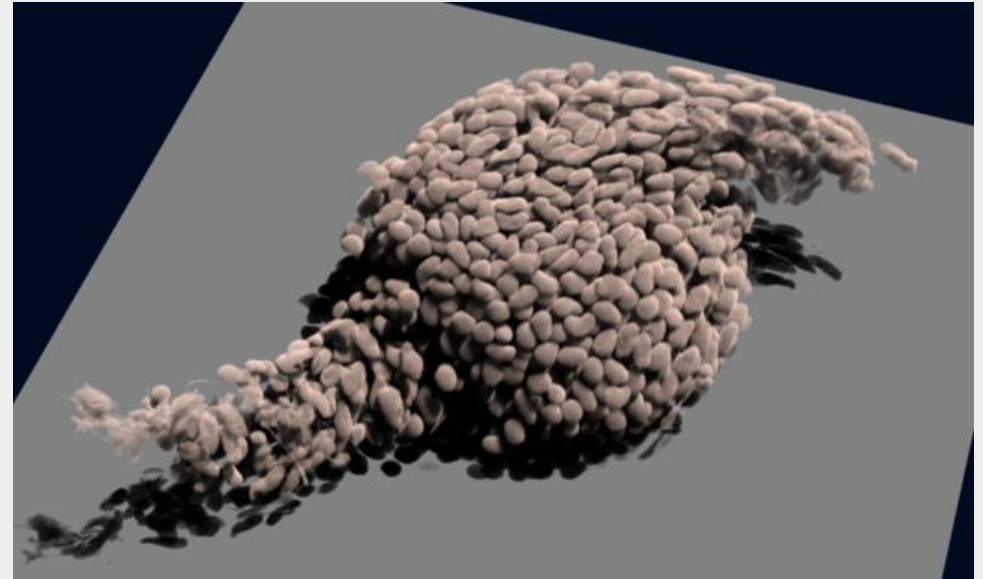
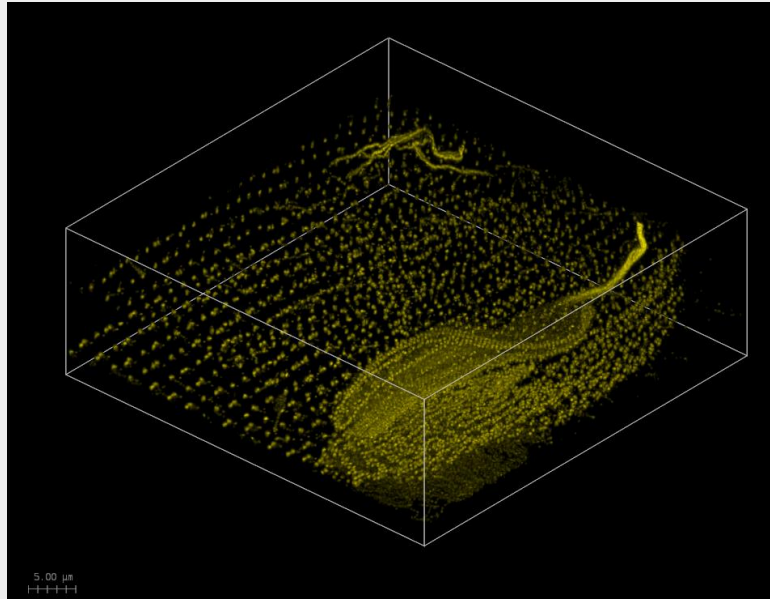
Microtubular staining after methanol fixation using a primary antibody against a Tubulin and an Oregon Green 488 conjugated secondary antibody.

Image acquired by: Elisa Berdalet, CSIC Institute of Marine Sciences/Timo Zimmermann, Center for Genomic Regulation, Barcelona



Widefield imaging of Neurons. Image provided by Leica Microsystems

# 3D data visualization



# GUI

The screenshot displays the Huygens Professional software interface. At the top, the title bar reads "Huygens Professional" and the menu bar includes "File", "Edit", "Tools", "Deconvolution", "Visualization", "Analysis", and "Help". Below the menu bar is a toolbar with icons for various functions like "New", "Open", "Save As", "Decon", "Param", "Ops", "Batch", "Del", "Del. all", "Clear", "Copy", "Paste", "Repl.", "Crop", "Join", "Split", "Comb.X", "Comb.Z", "Comb.T", "Edit", "Params", and "Size".

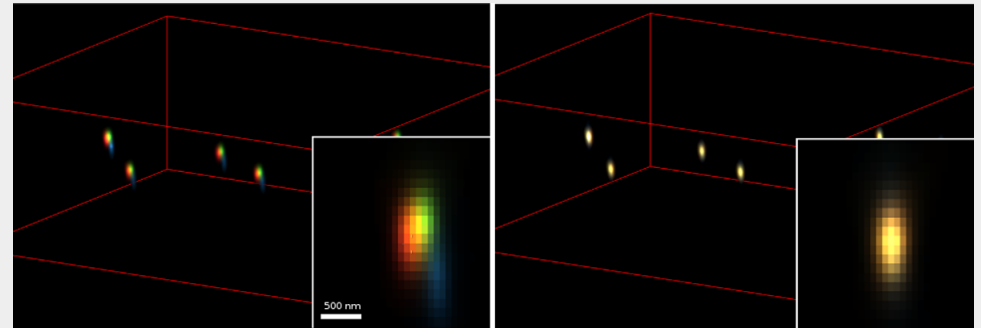
The main workspace is divided into several panels:

- Thumbnail overview:** A grid of image thumbnails. The thumbnail labeled "test\_image\_decon" is highlighted with a blue border. Other thumbnails include "Tetra\_125X\_001\_crop\_deconvExpPSF", "m110328\_4\_015\_LYStable\_deconvolved", "Crop40-original", "Crop40-deconvolved", and "Injected\_neuron".
- Huygens Icon Selector:** A central dialog box with a search bar and a grid of icons for various tools and functions, including "New", "Open", "Save As", "Decon", "Param", "Ops", "Batch", "Del", "Del. all", "Clear", "Tcl scr.", "Movie", "About", "Usage", "Analyzer", "Ortho", "Save rep", "Coloc", "Pref.", "Close W.", "Save hist", "Convert", "Adjust BL", "Help", "Features", "License", "Distiller", "Mip", "Open last", "Stats", "Hot pix", "Nyquist", "Histogram", "Sfp", "Slicer", "Track A", "Stabilizer", "FWHM", "Quit", "Plot flux", "Surf", "Tracker", "Twin", "Crosstalk", "Chromatic", "Brightf", and "Edit dec.". Buttons at the bottom include "Cancel", "Clear", "Set to default", "Revert", and "Accept".
- Histogram:** A panel on the right showing a histogram plot of "Frequency" vs. "Previous Image" (0 to 250). The plot is color-coded from green to blue. The "Histogram Info" section shows "Current channel: 0", "Current bin: -", and "Bin count: -".
- Huygens Tcl Scripting:** A console window at the bottom right containing a series of commands and their outputs, such as "img open", "img show", "img del", and "img open" for various image files.

The status bar at the bottom left shows "Ready" and "Memory usage 2079 MB". The system tray at the top right shows the time "14:05" and system icons.



# Chromatic Aberration



**Huygens Chromatic Aberration Corrector**

File View Plot Help

Value: ch 0: 0.2497  
 ch 1: 0.9463  
 ch 2: 0.8759  
 ch 3: 0.9591  
 Zoom: 15.38 px/μm  
 4175x

Active channels: All None Ch 0 Ch 1 Ch 2 Ch 3

Color scheme: Emission colors

Custom colors: 0

Time frame Colors Contrast Projection

Estimated chromatic aberration

**1. Estimate chromatic aberration**

Select reference channel: 0

Select correction method: Full correction (Correlation)

Estimate aberration

**2. Correct chromatic aberration**

	Chan 1	Chan 2	Chan 3
x (micron)	0.13	0.2	8.84
y (micron)	0.0	0.07	2.41
z (micron)	0.1	0.2	-0.4
i <sub>1</sub> (degrees)	0.0	0.0	0.0
scale (ratio)	1.0	1.0	1.0

Edit vector: Channel 1

Correct aberration

**Chromatic aberration templates**

Boris\_Airy  
nups

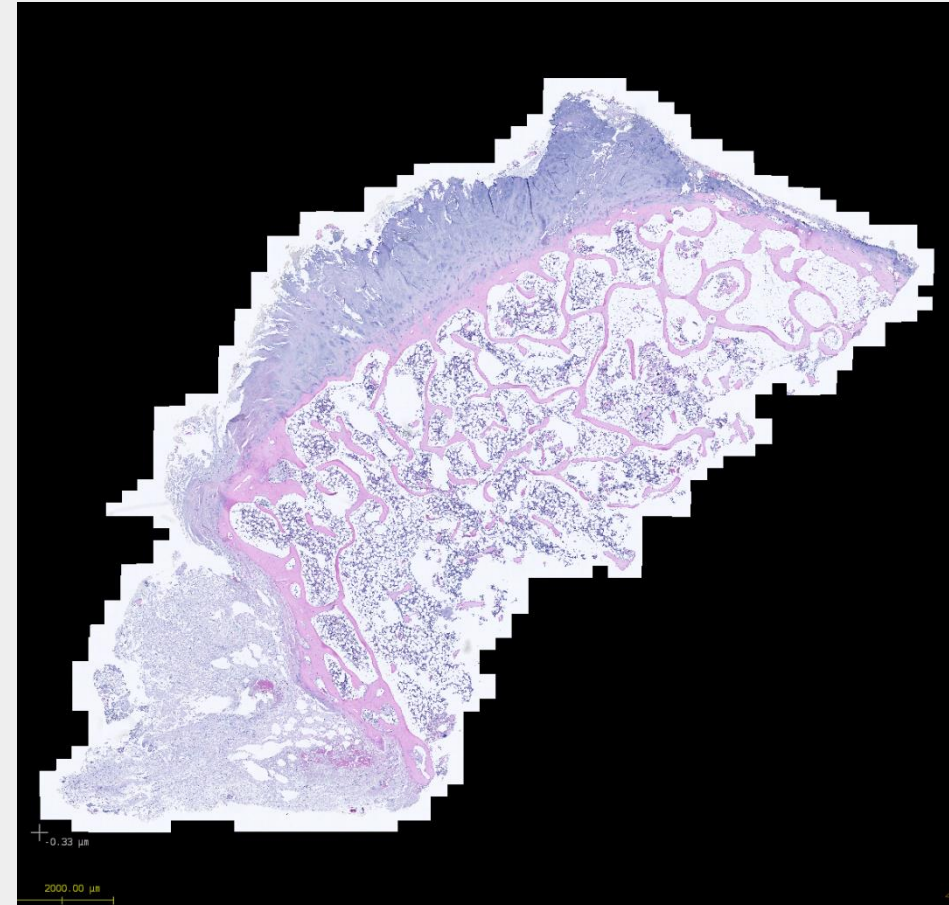
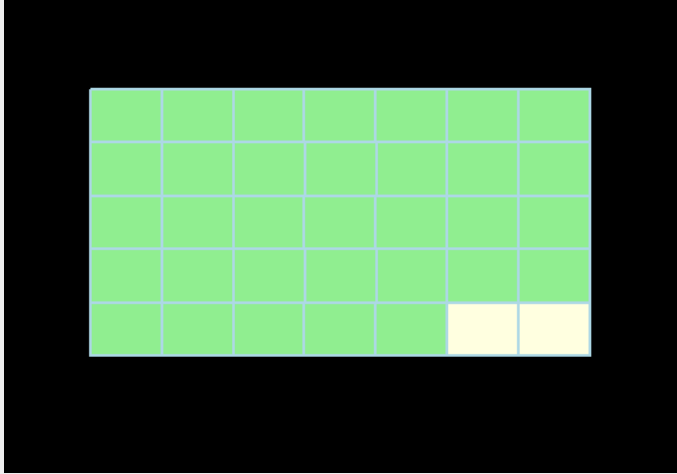
Save Load

**Help**

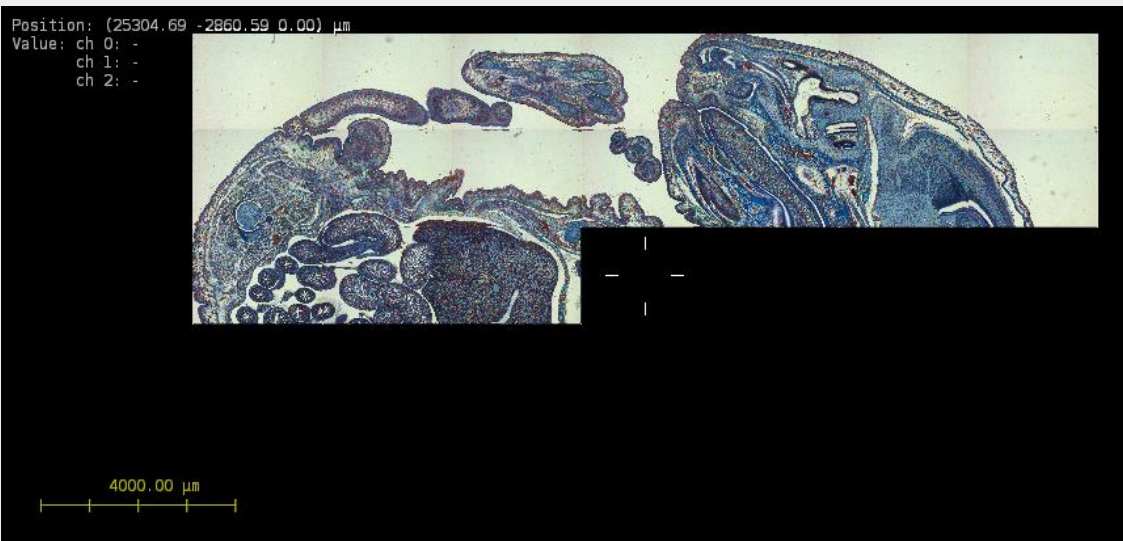
The plot shows a profile of a specific channel with the aberration from the table (dashed line) applied. Prior to correcting the image the aberrations can be modified for each channel with the "Edit vector" tool.

Click on the "Correct aberration" button to correct the image for chromatic aberration as defined by the aberration in the table. A new corrected image will be created.

# Huygens Stitcher



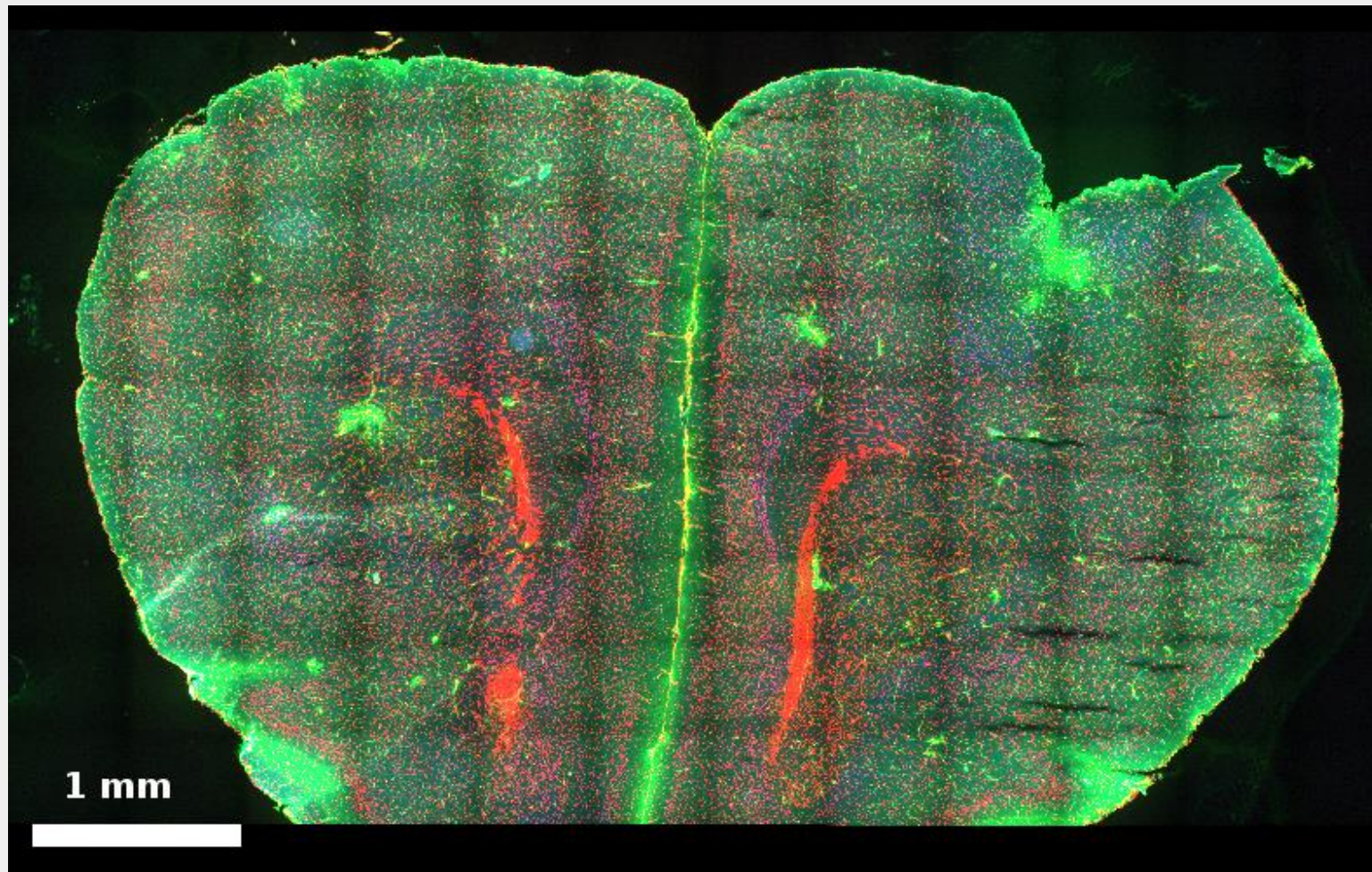
An impressive 1700+ tile compound merged by the Huygens Stitcher to a large 60GB dataset. Notice the scaling bar indicating 2 mm. Raw data by © Carl Zeiss Microscopy GmbH.



Tile image of a mouse Embryo being stitched.

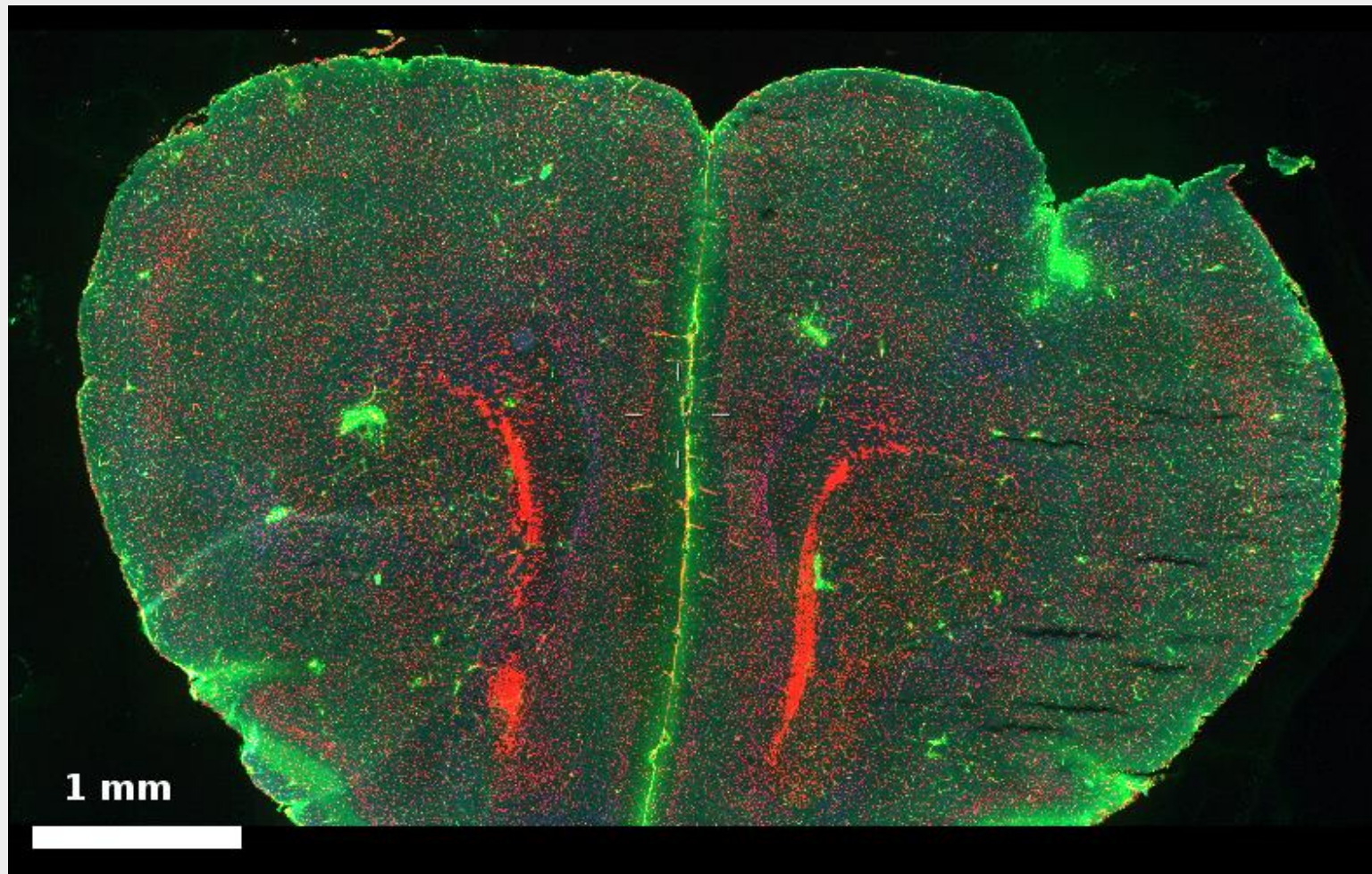
# Huygens Stitcher

Vignetting problem



# Huygens Stitcher

Automatic vignetting correction



# Titan: Indexing Microscopic Images

The screenshot displays the Huygens Titan software interface. On the left is a 'Library' tree view containing various folders such as 'Debug', 'Generator failed', 'Annotation', 'Project', and 'Huygens demo images'. The main area shows a grid of 48 image thumbnails, with 'decon2.lsm' highlighted in blue. The right panel shows 'Statistics' for the selected image 'decon2', including image statistics (size, frames, channels) and channel-specific statistics (scaling, max/min values, position of mass).

**Statistics** | Optical | Nyquist | Histogram | Annotations

Image name: decon2

Image statistics:

- X, Y, Z size (pixel): 583 576 20
- Number of time frames: 0
- Number of channels: 3
- Type of data: unsigned byte

Image statistics for channel: 0

- Scaling factor: 1
- Max value: 255
- Min value: 0
- Position of max (x,y,z,t): (208, 174, 5, 0)
- Position of min (x,y,z,t): (3, 0, 0, 0)
- Mean: 18.6
- Sum: 1.25e+08
- Standard Deviation: 21.5
- Norm: 7.36e+04
- Center of mass (x,y,z,t): (291.6, 262.7, 9.4, 0.0)

Image statistics for channel: 1

- Scaling factor: 1
- Max value: 187
- Min value: 0
- Position of max (x,y,z,t): (236, 279, 16, 0)
- Position of min (x,y,z,t): (2, 0, 0, 0)
- Mean: 12.1
- Sum: 8.15e+07
- Standard Deviation: 11.8
- Norm: 4.39e+04
- Center of mass (x,y,z,t): (281.8, 271.5, 9.9, 0.0)

Image statistics for channel: 2

- Scaling factor: 1
- Max value: 236
- Min value: 0
- Position of max (x,y,z,t): (573, 277, 11, 0)

**Titan activity:**

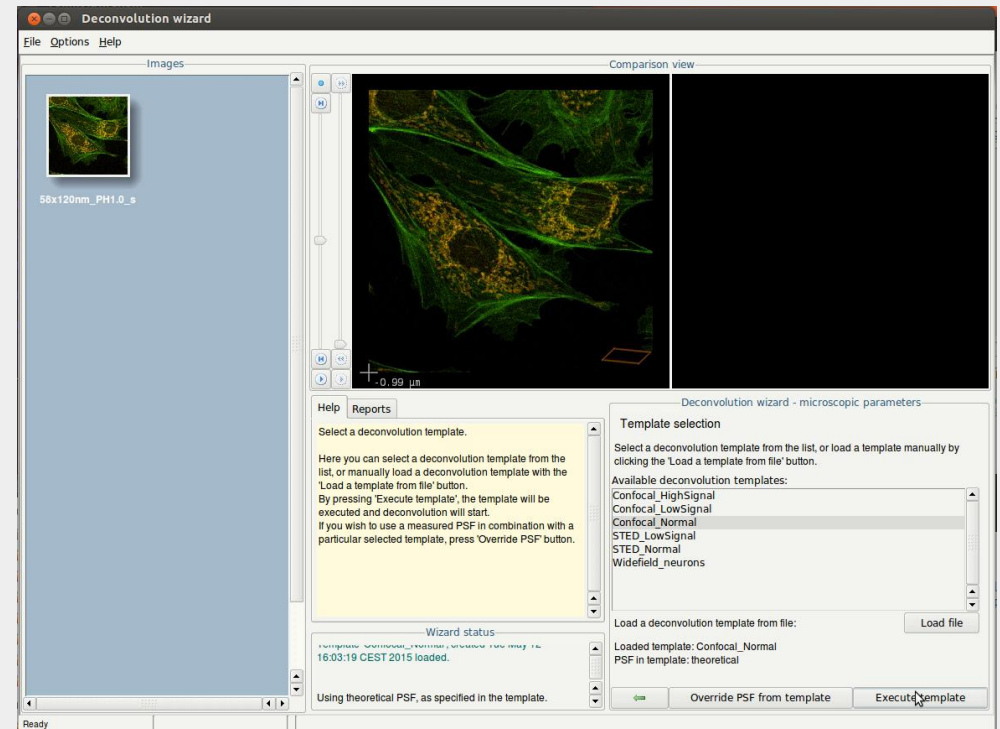
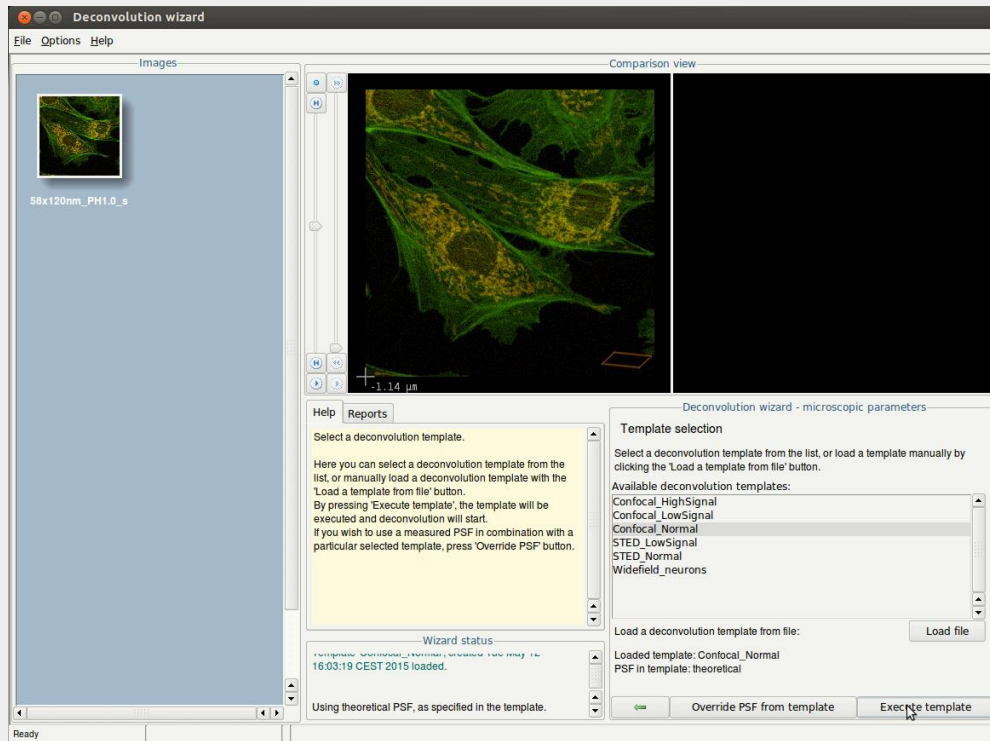
- Indexer: 99
- Thumbnail generator: 232
- 376
- 7.13 GB

Legend: up-to-date (green), refresh (blue), postponed (yellow), pending (orange), failed (red)

Pause indexing

# Huygens GPU acceleration

Confocal dataset: 2 channels, 1445 \* 1439 \* 18 (X\*Y\*Z) pixels

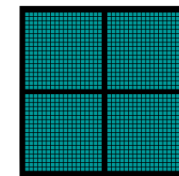


CPU  
Multiple Cores

Using CPU:  
Intel Xeon E5-2667 v3  
(4 cores @ 3.2 GHz)

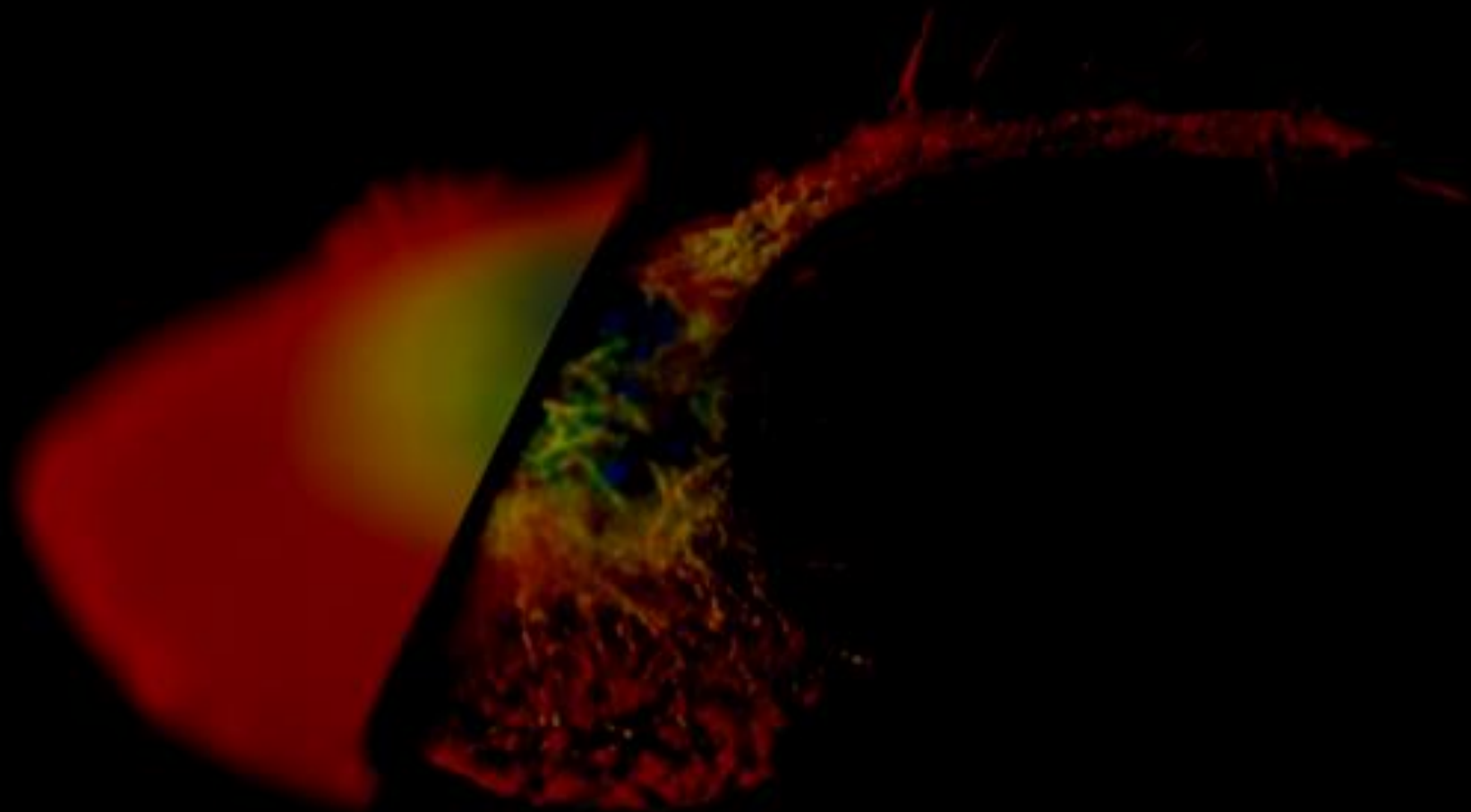


CPU  
Multiple Cores



GPU  
Thousands of Cores

Geforce GTX Titan-X  
3072 CUDA cores  
12 GB video-RAM



*Huygens Software*  
*by Scientific Volume Imaging*

# Languages used

Tcl / Tk	C / Cuda
GUI	Low level image processing (deconvolution, filters, etc)
Internal tcl shell	Renderings: (surface, volume slices, etc)



# Window Editor

The screenshot displays the Huygens Twin Slicer software interface in Advanced Mode. The main window shows a fluorescence microscopy image of cells with a blue channel (nuclei) and a green channel (cytoplasm). A 'Contrast Editor' window is open, showing a histogram of intensity values for the selected channels. The histogram has 'Intensity' on both the x and y axes, ranging from 0 to 800. A blue line represents the current contrast transfer function. The Contrast Editor window includes a 'Select Channel' section with three checked channels: 0: Confocal (blue), 1: Confocal (orange), and 2: Confocal (green). It also has buttons for 'All', 'None', and 'Inverse selection', and options for 'Plot mode' (Linear and Spline) and 'Compression' (Linear, Gamma: 1.0, Soft Threshold: 0.00). The main image view has a 20.00 μm scale bar and a position indicator showing (130 566 9) px. The bottom toolbar contains various controls for Contrast, Brightness, Gamma, and Contrast stretch, with 'Link channels' checked. The bottom status bar shows 'Time frame', 'Zoom', 'Orientation', 'Projection Mode', 'Channels & Colors', 'Contrast', 'Linking, View & Plot', 'Animate', and 'Shortcut Info'.

Left mouse button draws a ruler. Right mouse button zooms in on bright spots. Press <<c> to center the image.

# Tcl extensions

- Internal Tcl shell
- Register image names as Tcl commands

```
img1 save "foo1.png"
```

```
img1 + img2 -> img3
```

# Tk usage

- All of the GUI,
  - windows, widget
- Tkphoto  
from 2D pixel array -> photo
- Movie  
From multiple pixel array -> avi

# Our tcl approach

- Mainly namespace
  - 1000 lines each
  - 50 procedures
  - 1,2 or 3 array's to store namespace variables.
- Few objects
  - Widgets, window

# Typical use of tcl

```
namespace eval ::myTool {  
    variable vP  
  
    proc getTotalPixels { v } {  
        variable vP  
  
        set dimX $vP(dimx,$v)  
        set dimY $vP(dimy,$v)  
        set dimZ $vP(dimz,$v)  
        return [expr {$dimX * $dimY * $dimZ}]  
    }  
}
```

# Questions from our side

- openSceneGraph
- OpenGL
- Faster TkPhoto
- Advantages of using objects vs namespaces
- Font / widget scaling

# Font / Widget scaling

