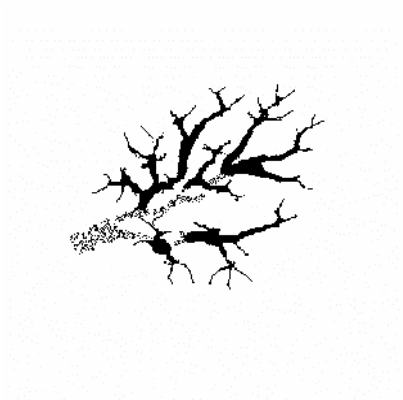
STEREOSCOPY OF *KRYPTOPTERUS BICIRRHIS* (VALENCIENNES 1840) ELECTRORECEPTOR ORGAN DENDRITIC TREES

Struik-ML* 2011



SDT Reference Series International 1, 12-18

This publication is supplementary to chapter 4 of the PhD-thesis of Mieke L. Struik, entitled 'A study on transduction and transmission in catfish ampullary electroreceptor organs (2001)'. It presents stereo images of the dendritic trees of ampullary electroreceptor organs of *Kryptopterus bicirrhis*, the transparent catfish, omitted from the original thesis for economy reasons. The original thesis is available through Igitur, Utrecht University's digital archive.

Received 25 July 2011, Accepted 01 August 2011, Published 26 August 2011,
Correction of printed errors 18 March 2012

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Abstract

1. Afferent nerve fibers of *Kryptopterus bicirrhis* (Valenciennes 1840) electroreceptor organs were stained immunocytochemically for neurotubulin. The staining showed that the afferent nerves branche heavily, and that each receptor cell makes contact with several nerve terminals.
2. Two-dimensional scans and the corresponding stereo images[1] were subsequently made by means of confocal scanning laser microscopy. The morphology of the afferent nerve fiber obtained from one of these images (Fig 1) was numerically reconstructed with a semi-automatic image analysis system, allowing the formulation of a mathematical model involving passive membrane properties and Hogkin and Huxley type kinetics.
3. Mathematical simulations then showed that the functional properties of the electroreceptor organ can be described relatively accurate with a rather simple model, and that apparently the shape of the tree is a crucial factor in the receptor organ's functioning.
4. In the following, stereo images are presented of the dendritic trees of different ampullary organs, provided with a brief description of the method used.

Keywords

Transparent catfish, Kryptopterus bicirrhis, dendrite, dendritic tree, stereo, confocal scan microscopy, 3D images, red-green anaglyphs, ampullary electroreceptor organ

1 Red-Green Anaglyphs

Materials and Methods

Staining procedure

A tropical fresh water catfish *Kryptopterus bicirrhis* (Valenciennes 1840) was kept in Utrecht copper free tap water at 25°C. It was sacrificed with an overdose Saffan (Glaxo, Harefield, UK). The anal fin was removed and fixated overnight in 4% paraformaldehyde. After rinsing with phosphate-buffered saline with 0.5% bovine serum albumin (PBS/BSA)(Sigma, St Louis, USA), the fin was incubated overnight in goat anti-mouse monoclonal anti-acetylated tubulin T-6793 (Sigma, St Louis, USA). The fin was rinsed with PBS/BSA and incubated overnight with anti-goat antibody labeled with Cy3 (Sigma, St Louis, USA). Finally, the fin was rinsed with PBS/BSA again, dehydrated in an alcohol series and embedded in Depex (British Drug Houses, Poole, England).

Confocal microscopy

Data was gathered using a confocal laser scanning microscope (CLSM) (Carl Zeiss Vision, Germany). Of each organ, a stack of 32 scans of 512*512*31 voxels was made. Spatial resolution of the scans was 0.195 µm/voxel for x and y directions, for z direction 0.78 (± 0.173) µm/voxel. In this way, the data of each ampulla is represented by a stack of 32 images, each image being about 100*100 μm^2,

Image processing

The stacks were analyzed semi-automatically with an IBAS imaging system (Carl Zeiss Vision, Germany). Of each segment, the start and end-points were marked. The distance between those markings was calculated using the Pythagorean theorem, which resulted in the length of the segments. The stack was transformed into a single layer projection image (Fig 1). In this projection image the length and area of each projected segment were determined. This area, divided by the length, provides an approximation of the average diameter of each segment.

Results

Fig 1 shows the projection image of an afferent nerve fiber innervating an ampullary electroreceptor organ. This particular electroreceptor organ contains 16 receptor cells, and is derived from the scans of the electroreceptor organ of Fig 2. The afferent dendrite of the electroreceptor organ branches strongly. All branches originate from one afferent myelinated fiber only, called the single parent afferent. This single parent afferent is indicated with a freckled structure. The staining is less intense, most likely because the myelin sheath prevented the antibodies to access the neural membrane. However, with normal light microscopy, it can be seen that the myelinated branches converge to form the single parent afferent (cf. Bretschneider, 1991).

The length of the segments varies between 7 and 82 µm. The branches vary in thickness from 1.75-13.36 µm, they taper and are thinnest at the terminals. The afferent has 45 terminals, connecting to 16 cells. In other words, each receptor cell forms more than one synapse with the afferent nerve. This implies that the convergence is considerably stronger than we initially thought: not 16 cells to 1 nerve fiber per organ, but 45 synapses to 1 nerve separated by 0.78 µm.

Two more examples of the dendritic arborization of the nerve fiber innervating ampullary electroreceptor organs are presented in Figs 3 and 4. The length of the passive dendritic membrane shows considerable variation. According to the mathematical model the amount of dendritic membrane is not the limiting factor of the electroreceptor's sensitivity; on the other hand the convergence of the receptor cells is dominant in sensitivity enhancement.

Figs 5 and 6 give overviews of ampullary organs in the anal fin at lower magnifications. Fig 7 (appendix) presents 18 sample of stereo images of ampullary electroreceptor organs, which were not further analyzed due to budget constraints.

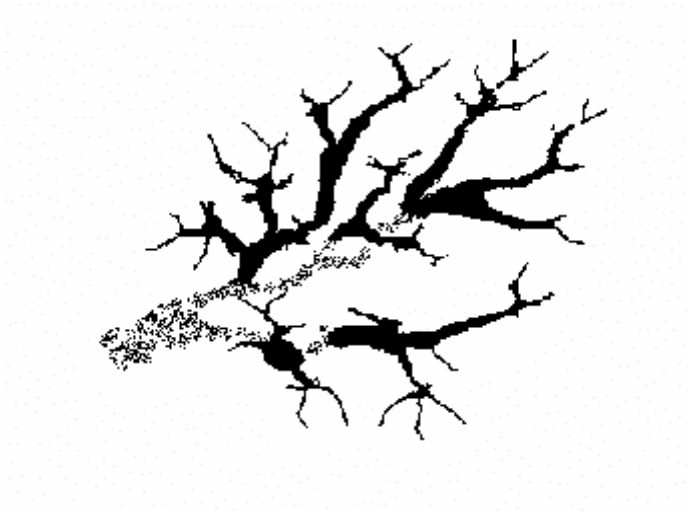

Fig 1. Projection image of an afferent nerve fiber. The average size of an ampullary organ is about 0.1 mm (cf Fig 5). The length of the segments varies between 7 and 82 µm. This particular projection is based on the scans of the electroreceptor organ shown in Fig 2.

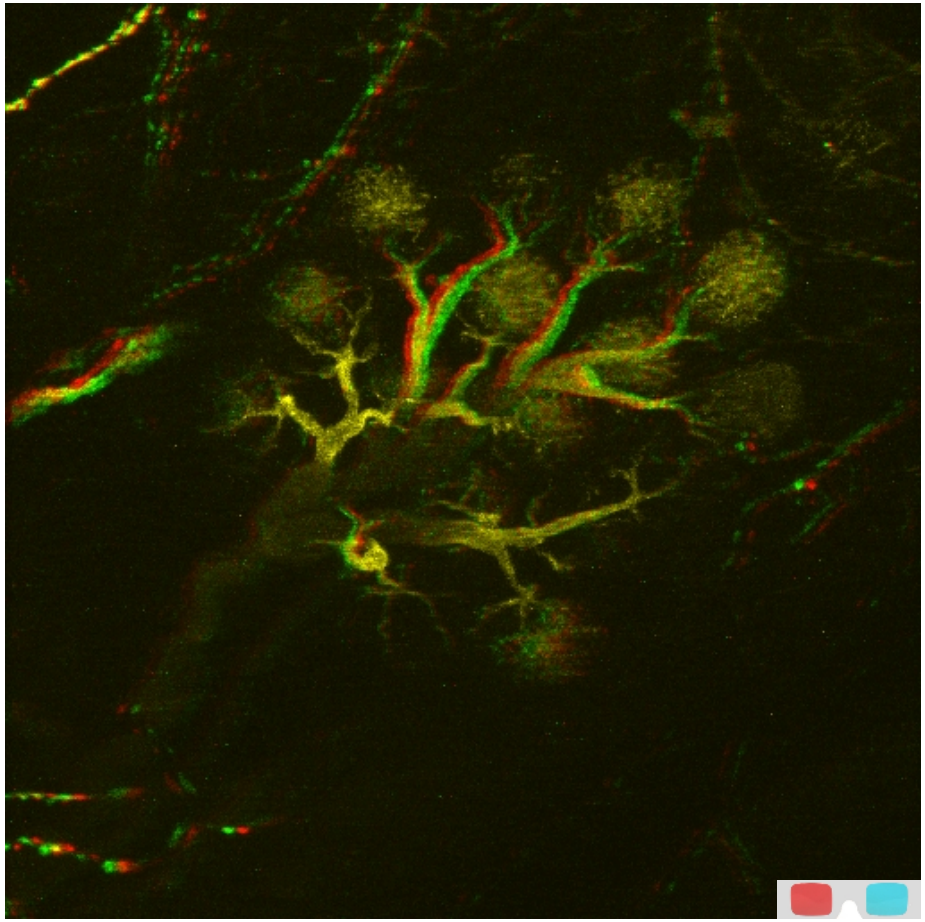

Fig 2. Stereo image of the dendritic tree of a Kryptopterus *ampullary electroreceptor organ. There is electrophysiological evidence that the convergence of the dendritic terminals on to a single afferent nerve fiber serves to raise the sensitivity of the organ to stimuli (Peters & van Ieperen, 1989; Peters et al., 1997).*

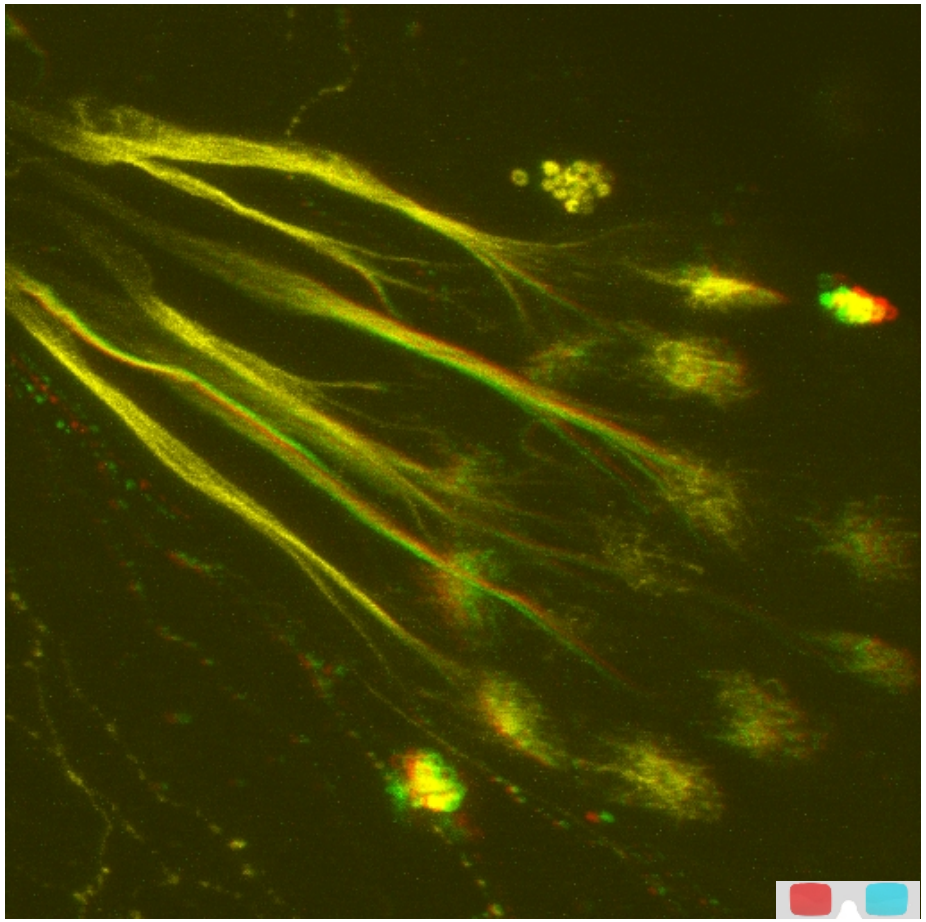

Fig 3. Stereo image of the dendritic tree of a Kryptopterus *ampullary electroreceptor organ, with relatively long, parallel, dendritic branches.*

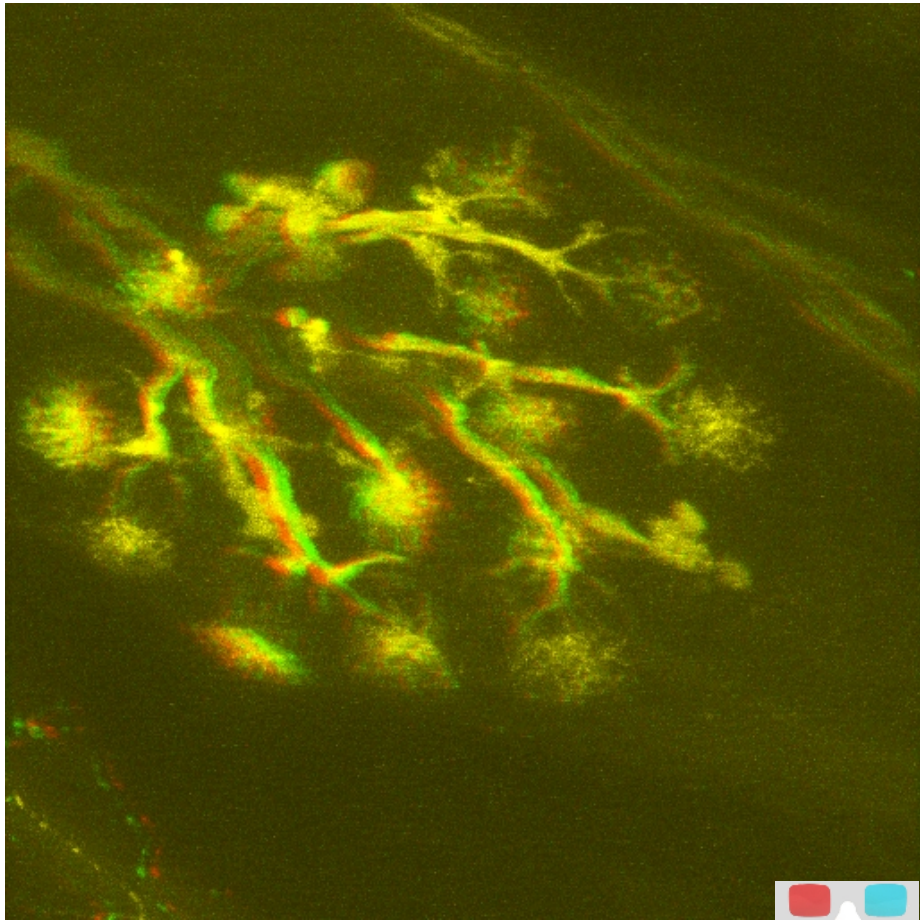

Fig 4. Stereo image of the dendritic tree of a Kryptopterus *ampullary electroreceptor organ, where the dendritic branches show a more compact build.*

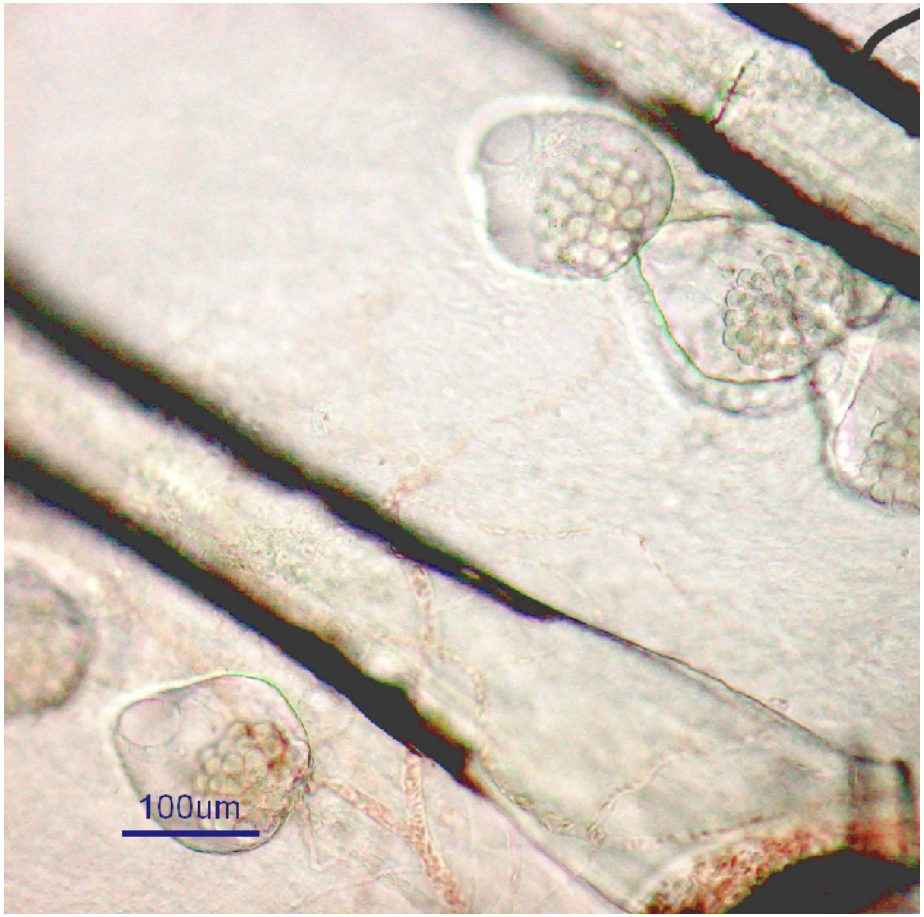

Fig 5. Photomicrograph of Kryptopterus *ampullary electroreceptor organs. Note the fin rays, the openings of the ampullary lumina, the receptor cells and some blood vessels. Note that the ampullary organs are at both sides of the - very thin - anal fin (Courtesy of Rob C. Peters and Ronald Leito).*

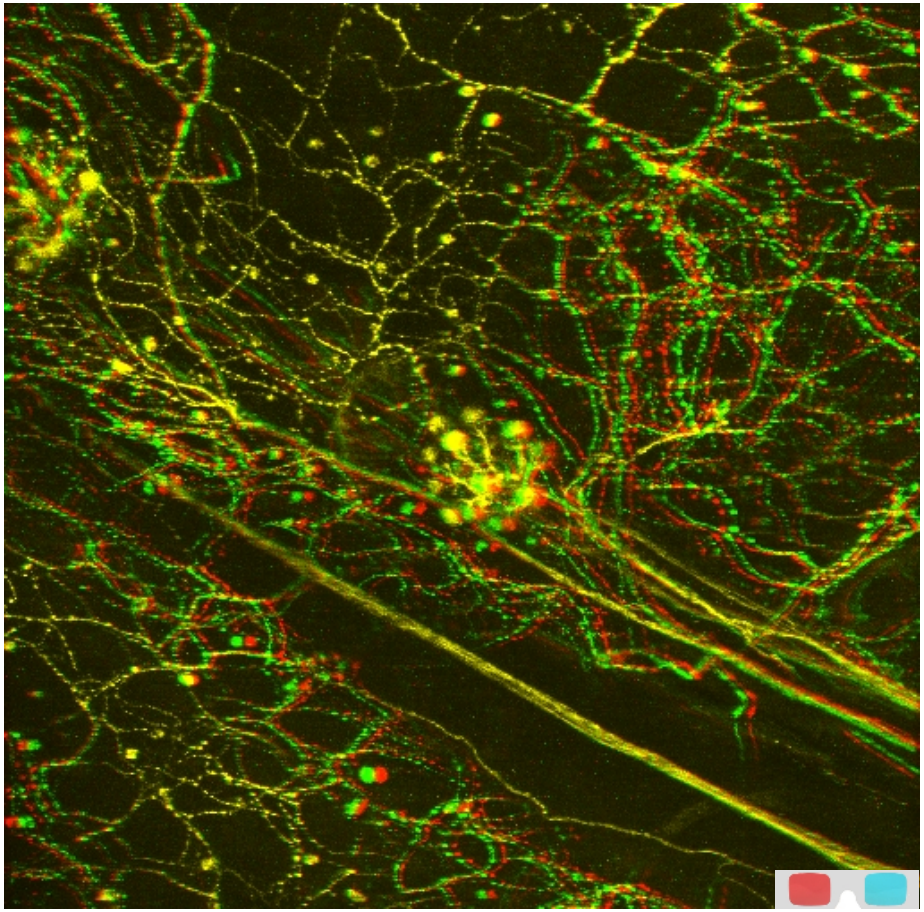

Fig 6. Stereo image of the dendritic tree of a Kryptopterus *ampullary electroreceptor organ; overview. Magnification as in Fig 5. Different location.*

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Acknowledgements

The author wishes to thank Willem Hage and Marian Pijnenburg of the Hubrecht Institute of the Royal Netherlands Academy of Arts and Sciences for their invaluable support in the present study. The support of *Stichting De Traditie* is gratefully acknowledged.

Appendix

The total number of scanned dendritic trees of the ampullary organs of *Kryptopterus bicirrhis* was 21, of which only three are presented in this publication. The scans of the remaining 18 ampullary ogans were never analysed. Sample stereo images of these ampullary organs are presented on the following page (Fig 7).

The complete set of scans plus the corresponding stereo images is available on CD ROM for further analysis, and may be used for new publications. Please contact Dr M.L. Struik through *Stichting De Traditie* for more information about copyright and co-authorship.

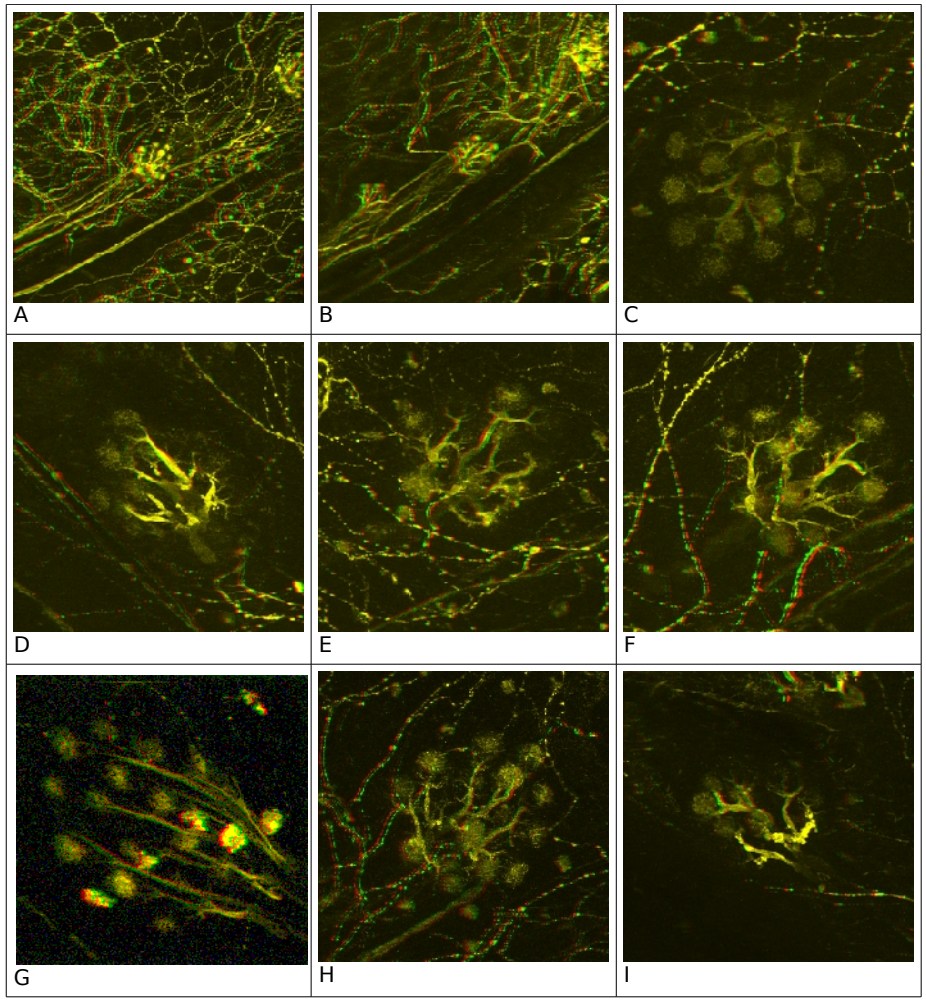

Fig 7 A-I. Sample stereo images of Kryptopterus *ampullary electroreceptor organ dendrites, waiting for further analysis.*

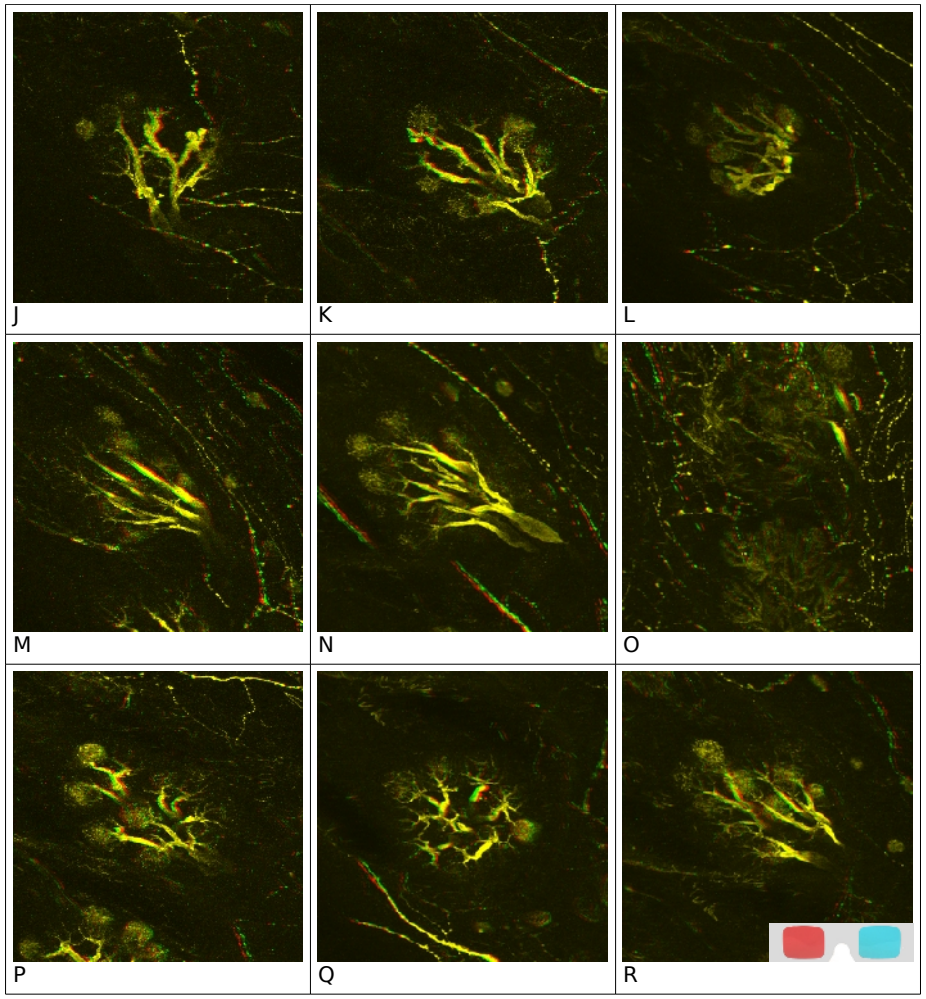

Fig 7 (continued) J-R.