Unique preparation technique and application for single neuron imaging with fine spine structure by Point Scanning Confocal Microscopy

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KEY WORDS: Numerical aperture (N.A.), Working distance (W.D.), Nyquist criteria, Stitching, Reflection Index (R.I.), Deconvolution

INTRODUCTION:

'Single neuron imaging with fine spine structure' is the starting point of 'Morphological and functional research of brain in synaptic-level'.

Here I'll show the unique sample preparation without 'Cover-slip', to extend the actual W.D of Objective-lens with high N.A. on 'Clearing brain slice'. Also I'll remark the issue of 'Z-PSF' parameters for deconvolution in different Index condition.

METODS, RESULTS AND CONCLUSION:

With 'Nyquist criteria', 60x/1.49 oil Objective-lens (W.D. is ~130um) will make 'high resolution images of fine spine structure' by Confocal Microscopy, and with stitching function, it will cover large area in XY. On the other hand, W.D. is fixed value by Objective-lens and we had no way to extend it.

After clearing brain slice by 'RapiClear 152', to fix slice on slide-glass, and to make membrane around slice to protect fragile surface of open-side, 'ProLong Glass (R.I.=1.52)' had good performance. Like many clearing medium, 'RapiClear' is prohibited to contact with another medium including immersion oil, so I user 'RapiClear 152' itself as immersion.

Finally I could skip cover-slip (thickness average 170um), and adjust R.I. of 'sample inside', 'membrane for protection' and 'immersion medium' as R.I. = 1.52, and broke the limitation of W.D. and reached up to 300um depth without loose resolution of fine spine .

On the other hand, still we should concern about 'Deconvolution' for 'Z-PSF' caused by complicated structure inside brain. I validated the suitable parameters of deconvolution using 'RapiClear beads gel' and actual brain sample including small fluorescence beads.

ACKNOWLEDGEMENTS:

New preparation trial and beads PSF measurement were done in collaboration with Dr. Daniel LIN (SUNJIN -Lab Co. Ltd, Taiwan). Shooting beads inside actual brain slice were done by Dr. Ryosuke KAWAKAMI, Dr. Kohei OTOMO, and Prof. Tomomi NEMOTO (Research Institute for Electronic Sciences, Hokkaido University.)