Updated Aug. 5, 2019

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（Abroad・Domestic）Internship report form（Student） 2020/9/12 (Year/Month/Day)

Name	LEE Ming-Liang
Laboratory	Biochemistry
Year (Grade)	D4
Internship institution	Division of Biophotonics, Exploratory Research Center on Life and Living Systems
Internship period	Internship period: 08/18/2020 - 08/28/2020 (Departure Date from Sapporo: 08/17/2020, Arrival Date in Sapporo: 08/29/2020)
Purpose	In vivo and ex vivo imaging of brain with two-photon microscopy

- The reason why you chose this institute

Brain is the most mysterious organ in our body. Neuroscience is a discipline to study function of the brain. The couple decades, neuroscience research is florescent thanks to appearance of many novel techniques includes two-photon microscopy. Two-photon microscopy can image deep area in brain slices which means we can use thick sample to preserve the structure of axons and dendrites. Moreover, because of its property, invasive *in vivo* imaging of neuronal activity is available to study neuronal functions in live animals. Therefore, two-photon microscopy has been a powerful tool to decipher brain functions and neuronal circuits.

Prof. Nemoto's lab is professional in building two-photon microscopy. They have developed several imaging techniques, for example, they can image neurons at as deep as ~1.6mm from skull surface of live mice. He has a good reputation in the field of bio-optics. Therefore, I can learn a lot about techniques to image neurons in his lab.

- Result of the activity （about 800 words、provide photos, tables and figures that clearly show the activities during the period）

During this internship, I tried to image dendritic spines of deep brain regions ex vivo and in vivo by using two-photon microscopy. Before the trip to Okazaki, I prepared several samples of brain slices for ex vivo imaging. I labeled neurons with expression of intracellular tdTomato. After I arrive Prof. Nemoto's lab, I discussed with lab members to make a strategy for imaging neuronal soma and dendritic spines in deep region (>150um in my case). To clearly image the tiny structures such as dendritic spines, we transparentized the brain slices to reduced optical scattering. I tried several different clearing strategy and reagent such as

SeeDB2[Ke et al., 2016, Cell reports] 2,2'-Thiodiethanol(TDE)[Aoyagi et al., 2015, PLOS ONE]. I found that I can't image deep regions with treatment of SeeDB2 due to short working distance of the objective lens (oil lens). Then I tried to use TDE to transparentize samples and use water lens to image. First, I use 60% of TDE which was reported to provide the best transparency on the brain slice. However, because of the reflect index of 60%TDE (~1.46) is too far from the numerical aperture (NA) value of the water lens I used (~1.3), the image was not clear, and the dendritic spines can't be imaged (Figure1A). I was then changed concentration of TDE from 60% to 40%, then I successfully image dendritic spines by using two-photon microscopy. Therefore, I would use 40% TDE to image dendritic spines in deep regions of brain slices in following experiments. Before imaging dendritic spines, I made a column imaging to see if deep region can be caught with this microscopy system. Expectedly, this system can catch high quality images as deep as 450um from slice surface (Figure2). I think it can catch much deeper than 450um, but my slice sample are prepared only with thickness of 450um. After understood that this system is able to image deep regions, I next tried to image tiny neuronal structure such as dendritic spines. Again, I treated the brain slices with 40% TDE and imaged with two-photon microscopy. In the shallow regions (Z=50um), the dendritic spines could be clearly imaged (Figure3 upper panels). To understand how deep the dendritic spines can be imaged by the system, I imaged them in the same slice sample with different depth. At Z=150um, the dendritic

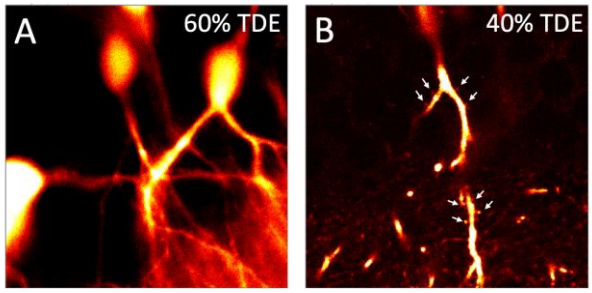

Figure 1. Dendrites images in brain slices treated with different concentration of TDE. A. 60% TDE treated slice. B. 40% TDE treated slice.

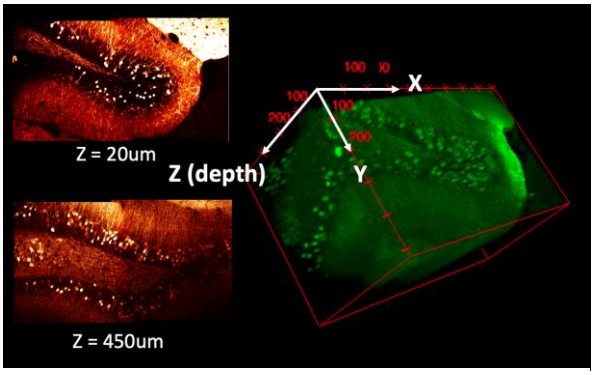

Figure 2. Column imaging on 450um thickness of brain slice by two-photon microscopy.

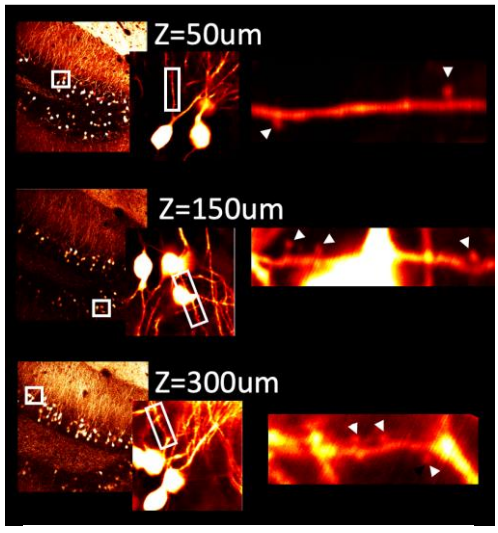

Figure 3. Imaging dendritic spines in different depth of brain

spines still can be imaged clearly and sharply (Figure3 meddle panel). At the deep region (Z=300um), the dendritic spines were still can be distinguished but the profile is blurred, and background became higher (Figure3 lower panel). Therefore, I successfully to image dendritic spines in thick slice sample. This experience can help me to profile distribution of dendritic spines in a neuron in future research.

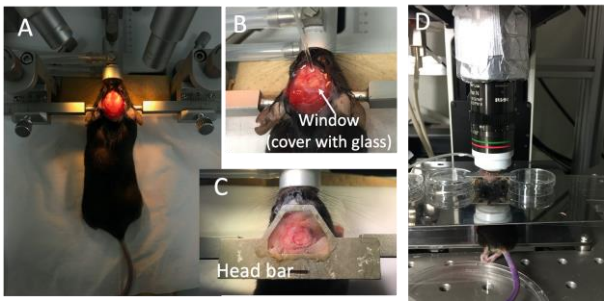

Figure 4. Steps (From A to D) to in vivo image brain cortex on live animals. A. Fix an anesthetized mouse on stereotaxic instrument and expose the skull. B. Make a hole with a dill and cover it with a glass slide. C. Glue a head bar on the skull and close the wound. D. Set the mouse under microscope after 3 days of recovery.

Next, I tried in vivo image. To perform in vivo image, we have to operate a stereotaxic surgery on a mouse to open a window on the skull. We can observe the cells or blood vessels through the window under a microscope. Briefly, an anesthetized mouse is fixed on a stereotaxic instrument and the skull is exposed(Figure 4A). Carefully remove a piece of skull bone with a drill and make a circle hole. Then put and glue a glass slide to cover the hole on the skull(Figure 4B). Finally, to fix the mouse head during imaging, a head bar is also glued on the skull and wound is closed with dental cement(Figure 4C). The mouse is recovered for 3 days before imaging. On the day of imaging, the mouse is set under the two-photon microscope after anesthetized (Figure 4D). To observe something in the brain, we planned to intravenous (i.v.) inject SR101 to stain blood vessels and astrocytes, and make a column image of brain cortex on live animals. Unfortunately, I failed to i.v. inject the dye into the mouse, and this time I am not able to see anything under microscope. Even through I failed on this experiments, I learned how to use this image cells in live animal. I will use this tool for my future research.

- What do you think the positive impact of the activity will have on your further career path?

Neurons interact with each other by their axons and dendrites, so the anatomic structure is important to decipher neuronal circuit and functions. Therefore, imaging on a bigger and thicker brain slices can provide more information about neuronal circuits because the axons are intact in bigger uncut samples. On the other hand, studies on neuronal function in vivo are more and more relied on in

vivo calcium imaging to observe neuronal real-time. It is my first time to use two-photon microscope, an important for current ex vivo and in vivo imaging. I understood how to choose a lens to fit the experiment what I would do. These knowledges are important to utilize microscope to image neurons ex vivo and in vivo. I learned how to apply this powerful technique on real research and understood how to choose and adjust strategy to achieve our goals. This internship extended my research field. During this internship, I understood how to cooperate with other researchers who are not in the similar field. I also understood how to get and apply knowledge on doing better research. With this experience, I am able to design experiments which need to use two-photon microscopy or in vivo imaging, moreover, I learned how to do interdisciplinary studies with other researchers.

- Advice for your junior fellows

Always be plastic on every plan, because you never know what will happen. This time, I planned to go Yale university, and this plan has been proposed when I just entered this school. In the early January, everything was well prepared, and I also submitted the application form to our office. However, the pandemic happened soon and destroyed everything, this disaster forced me to change my plan and to give up many opportunities to building connections. I have to make a decision to get the credit and to graduate. So please make yourself plastic and be clear what you should give up for more important things.

Approval of supervisor	Institution・Official title・Name Laboratory of Biochemistry, Graduate School of Veterinary Medicine, Professor Kazuhiro KIMURA

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