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研究課題名	Analysis of tissue tropism of Toxoplasma gondii using CUBIC tissue-clearing system	
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(Form 2-2)

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IMSUT International Joint Usage/Research Center Project <Domestic>

Joint Research Report (Annual/Project Completion)

Annual Report

Report

The goal of our joint project is to detect the spatial distribution of infected *Toxoplasma gondii* in tissues at high resolution using the CUBIC tissue-clearing system. In the first year (FY2022), we tested the detection of GFP from the CUBIC-treated mouse tissues infected with GFP-expressing *Toxoplasma* to demonstrate the technology of our research proposal. Since *Toxoplasma* is almost certainly detected in the brain of *Toxoplasma*-infected mice, the brain was selected as the first tissue to be examined for CUBIC treatment. For this first investigation, we used a *Toxoplasma* strain that constitutively expresses GFP, and determined sampling conditions from infected mice. *Toxoplasma*-infected brains were treated with CUBIC reagents and 3D imaging was performed using a light-sheet microscope. Several small GFP signals, which appeared to be *Toxoplasma* cells, were detected near the brain surface.

Results and Discussion

Determination of sampling conditions

We have never used the RH-GFP strain for mouse infection experiments. Sampling conditions were tested based on several references using this strain, and determined the condition (number of *Toxoplasma* cells, infection method, and sampling schedule; infection for more than 7 days increases the risk of death). Then we confirmed the GFP signal of *Toxoplasma* by the fixed-brain section samples. We detected the GFP signals from the samples, indicating that the signal is not lost until sampling from the mouse infection (**Figure 1**). This shows that our sampling condition would be available for the CUBIC treatment and imaging.

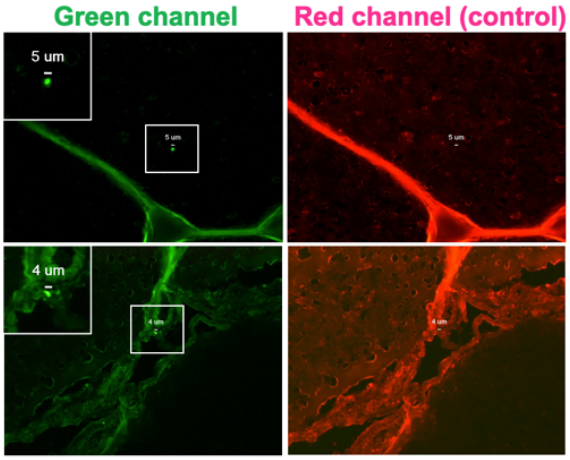

Figure 1. The GFP signals of the RH-GFP cell were detected from the brain section

GFP signals were detected from CUBIC-treated brain samples

We sampled *Toxoplasma*-infected brains from four mice, and treated them with the CUBIC reagents. 3D imaging was performed using the treated brains by a light-sheet microscope. Several small GFP signals, which appeared to be *Toxoplasma* cells, were detected in the brain tissue, near the brain surface. These signals were to be *Toxoplasma* cells that have invaded brain tissue because the samples were perfused. In addition, infection of the brain surface is consistent with a previous report (Olivera et al, eLife, 2021).

The data is the first data of *Toxoplasma* detection in brains transparented by the CUBIC system, indicating that the CUBIC system is available to detect the spatial distribution of *Toxoplasma* in tissues. Therefore, we decided to conduct a validation using type II *Toxoplasma* that can form cysts, which are more similar to clinical conditions.