

## Three-dimensional *in situ* measurements of *Mycobacterium tuberculosis* infection by optical Mesoscopy and novel acid-fast staining

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Tuberculosis (TB) is the world's deadliest infectious disease, claiming ~1.7million lives / year. TB vaccine testing relies on *in vivo* models. The only method of determining the amount of infection in a tissue *in situ* is two-dimensional (2D) histopathology. 2D measurements consider heterogeneity within a single observable section but not above and below, which could contain critical information. To overcome this TB infected lung was optically cleared using CUBIC protocol (1), stained using a novel acid-fast procedure, and then refractive index matched (Figure 1). The resulting tissue was then imaged with either CLSM or Mesoscopy (2), and isosurface rendered (Figure 2). Using this, we show the volume of TB infection can be quantified within tissue, to individual TB colonies by Mesoscopy. We now hope to use these techniques to compare between vaccinated and non-vaccinated mice.

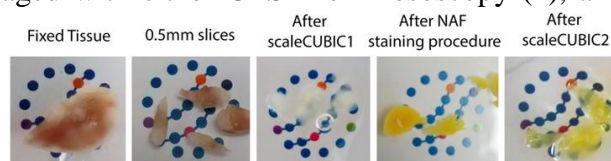


Figure 1: Photographs of the stages of tissue clearing and staining. Logo = 13mm in diameter

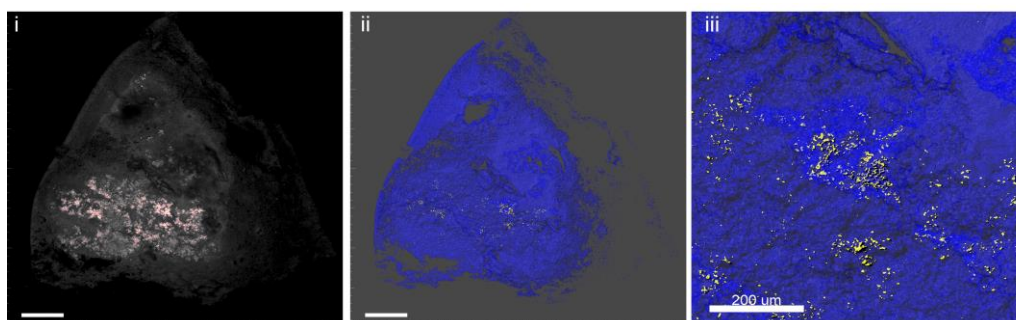


Figure 2: Mesoscopy of TB infected lung. (i) Max intensity projection of mesoscopic Z stack. (ii) Isosurface rendering of (i). (iii) Zoomed in image of (ii). Scale = 500 $\mu$ m unless otherwise stated.

[1] Susaki EA, Tainaka K, Perrin D, Kishino F, Tawara T, Watanabe TM, et al. "Whole-brain imaging with single-cell resolution using chemical cocktails and computational analysis." *Cell*, **157**(3):726-39 (2014)

[2] McConnell G, Tragardh J, Amor R, Dempster J, Reid E, Amos WB. "A novel optical microscope for imaging large embryos and tissue volumes with sub-cellular resolution throughout." *Elife*, **5** (2016).