Osteoclasts are bone-resorbing giant polykaryons that differentiate from mononuclear macrophage/monocyte-lineage hematopoietic precursors. Upon the stimulation of essential factors such as M-CSF and RANKL, osteoclast precursor monocytes attach to the bone surface, fuse with each other to form giant cells and mediate bone resorption. To reveal the regulatory mechanism of osteoclast ‘function’ and ‘differentiation’, we generated various kinds of fluorescently-labeled mice.

First, we generated the mice expressing GFP under the promoter of vacuolar type H⁺-ATPase a3 subunit, that was abundantly expressed in differentiated osteoclasts. We succeeded in visualizing fluorescently-labeled mature osteoclasts in intact bone tissues, and identified two different populations of living mature osteoclasts, ‘static - bone-resorptive’ and ‘moving - non-resorptive’. We also developed a pH-sensing fluorescent chemical probe to detect the acidification by bone-resorbing osteoclasts in vivo. By means of this probe, we could visualize ‘bone-resorption’ by osteoclasts and found that the pH value in the resorption pit created by osteoclasts should be within the range of 4–6.

We next generated the double-fluorescently-labeled mice where Green and Red fluorescent proteins were expressed under the promoter of cell differentiation markers in different stages. We crossed CSF1R-EGFP mice where EGFP was expressed in CSF1R⁺ osteoclast precursor monocytes, with TRAP-tdTomato mice where tdTomato was expressed in TRAP⁺ mature osteoclasts. By intravital multiphoton imaging, we could chase the state of osteoclast ‘differentiation’ as a color change, in addition to 4-dimensional information such as x/y/z and temporal information. We named this strategy 5D imaging technology. Finally, we crossed TRAP-tdTomato mice with Col1a1-ECFP mice where ECFP was expressed in Col1a1⁺ osteoblasts. By intravital bone imaging of the mice, we succeeded in visualizing the interaction of bone-resorbing osteoclasts with bone-forming osteoblasts (‘coupling’) in living bones.

These approaches would be quite beneficial for studying the osteoclast dynamics in vivo and thus useful for evaluating novel anti-bone resorptive drugs currently developed in the world.