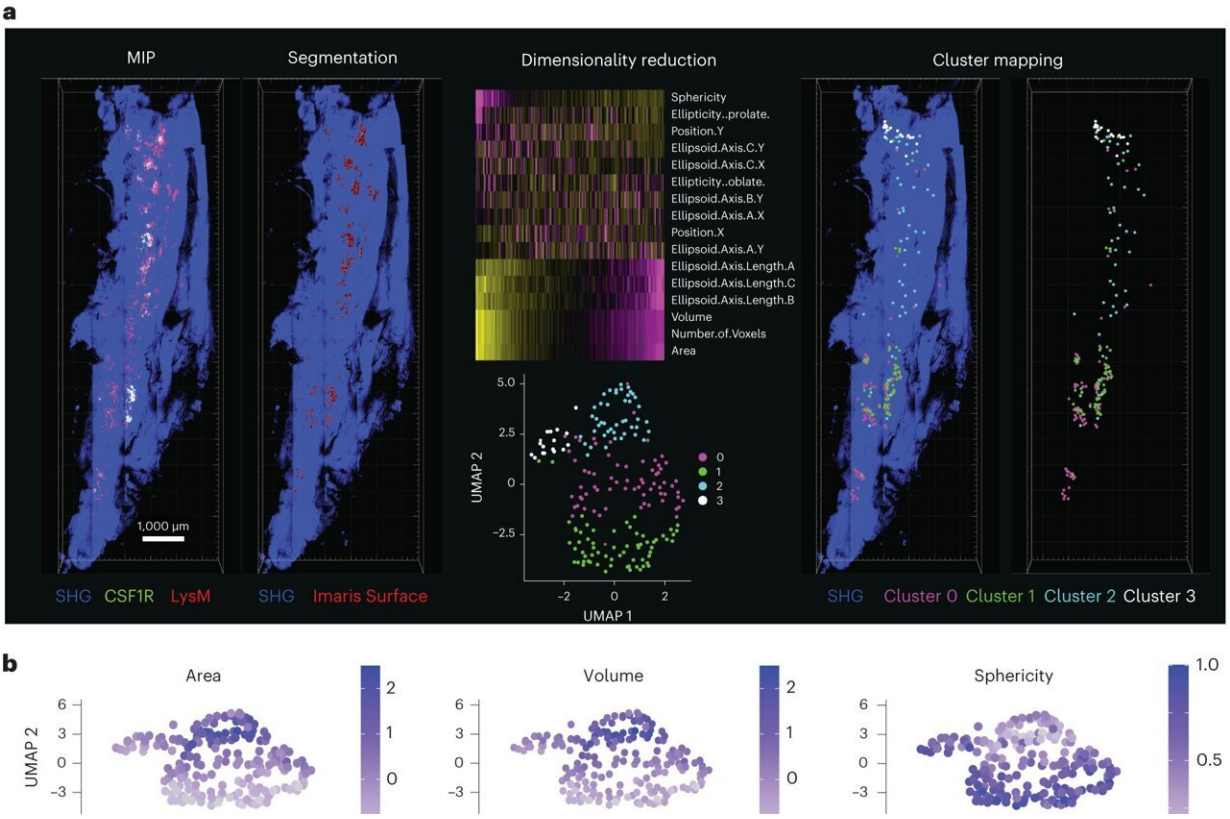
New imaging method reveals activity of cells that break down bone

October 26 2023



Mapping osteoclastic activity to different regions in the tibia. **a**, MIP of a tile scan of the entire exposed tibia. SHG (blue), CSF1R (green), LysM (red). $LysM^+$ and $CSF1R^+$ cells were segmented in Imaris. Dimensionality reduction was performed on exported data and used to identify cluster 0 (magenta), cluster 1 (green), cluster 2 (blue) and cluster 3 (white). Clusters were manually mapped back to the bone surface using the cell segment IDs. **b**, Relative values for area, volume, sphericity, prolate ellipticity and *xy* position in the identified clusters. Values for area and volume are plotted in a logarithmic scale, with all other

values plotted in a linear scale. **c**, Pseudocolor density plot of volume and sphericity for cells in clusters 0–4. Credit: *Nature Protocols* (2023). DOI: 10.1038/s41596-023-00894-9

Bone may seem as if it's a hard, lifeless structure, but now the cells living within have been imaged in unprecedented detail, thanks to an innovative imaging method developed at the Garvan Institute of Medical Research.

The new method lets researchers study cells inside the bones of mice, to visualize not just isolated sections, but the entire length of a bone. With a new level of visual detail, the researchers discovered that osteoclasts, cells that break down bone tissue, are more active in some parts of the bone compared to others. This knowledge could be used to develop new treatments for osteoporosis, and for dormant cancer cells, which can stay hidden in bone for years until they are reactivated by osteoclasts.

"Our method has given us an unprecedented window into how cells go about breaking down bone, giving us a new way to investigate osteoporosis and cancer relapse in bone," says Professor Tri Phan, Head of the Intravital Microscopy Lab and Gene Expression (IMAGE) Lab, immunologist at St Vincent's Hospital Sydney, Co-Director of the Precision Immunology Program at Garvan and senior author of the paper , published in *Nature Protocols*.

"We can finally image processes inside bone that we thought were happening, but which were until now beyond the limits of conventional microscopy techniques. We are only beginning to understand the implications of this exciting technology."

Giving disease-causing cells no place to hide

Osteoclasts are crucial to the normal maintenance and repair processes of bone, but when they are overly active, they can cause excessive breakdown, known as osteoporosis.

"The inside of living bone is a 'dark space' that is difficult to study, because of its hard, mineralized structure," says co-first author Dr. Nayan Deger Bhattacharyya, post-doctoral researcher in the IMAGE Lab. "In order to understand diseases such as osteoporosis and cancer recurrence, we've needed to develop the technology to look inside bone tissue."

The new technique developed at Garvan's ACRF INCITe Center can be used to image other dynamic cellular processes until now hidden in bone.

"Our new imaging method is minimally invasive and lets us map out localized populations of cells along the length of an entire bone in our mouse models, instead of just in small sections," says co-first author Wunna Kyaw, Ph.D. student in the IMAGE Lab.

The researchers tracked down distinct pockets of bone resorption activity as the cells "morph" between actively resorbing osteoclasts and an intermediate cell state called osteomorphs, in real time.

"We suspect these osteomorphs are dangerous as they can accumulate while osteoporosis treatment is administered but can rapidly reform activated osteoclasts to supercharge bone breakdown as soon as treatment is stopped. This would explain an observation in the clinic, that many osteoporosis patients taking the medication denosumab, which blocks osteoclasts from resorbing bone, experience rebound vertebral fractures after they stop using the drug. We will use our imaging method to study how this withdrawal effect could be prevented," says co-author Professor Peter Croucher, Head of the Bone Biology Lab at Garvan.

The researchers say their method could also be used to investigate cancer cells that can migrate to bone during cancer treatment and lie dormant there for years, only to be reactivated by osteoclasts breaking down the bone tissue that surrounds them.

"Being able to see cells and molecules interact in the bone—and one day target them—could be a critical new tool for diseases relating to bone," says Professor Phan.

More information: Nayan Deger Bhattacharyya et al, Minimally invasive longitudinal intravital imaging of cellular dynamics in intact long bone, *Nature Protocols* (2023). DOI: 10.1038/s41596-023-00894-9

Provided by Garvan Institute of Medical Research