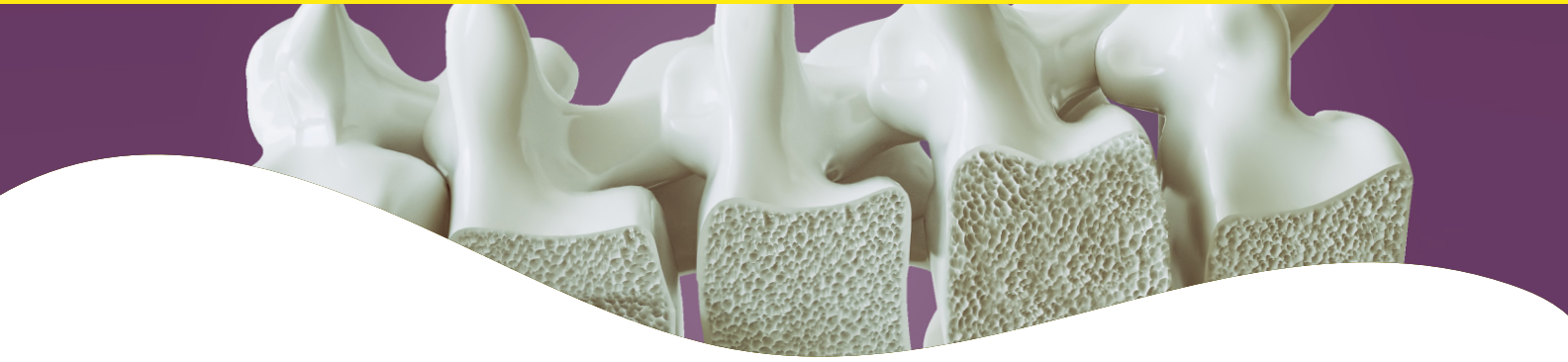
Optimize bone imaging data without risking safety of your animal models.



In preclinical *in vivo* studies of bone disease, including osteoporosis, arthritis, and cancer metastases, computed tomography (CT) and X-ray imaging can provide bone density measures as definitive indications of bone turnover and bone loss. However, such measurements not only come at the cost of repeated exposure to hazardous X-ray irradiation, but they give little information about the biology of bone remodeling - the complex cellular mechanism by which bone turnover is achieved.

Bone remodeling is the result of coordinated activity between two cell types; osteoblasts, necessary for the formation of bone, and osteoclasts, essential for bone resorption. When in balance, bone formation and resorption happen throughout the life span for vertebral skeleton development and maintenance, and in response to environmental or biological factors such as loading or metabolism. A change in the balance between osteoblasts and osteoclasts is characteristic of numerous disease states.

Though CT and x-ray imaging can track the volume of bone formed or resorbed over time, they raise the risk of harmful irradiation exposure, and are less useful in applications where tracking and measuring the extent of osteoblast activation (or lack thereof) is important.

In contrast, fluorescent *in vivo* imaging offers no irradiation exposure concerns, and when performed with optimized and validated hydroxyapatite-targeting probes, can image and quantify bone growth and elucidate the biology of bone remodeling.

What is Hdroxyapatite?

Hydroxyapatite (HA) is a mineral form of calcium apatite and is the major mineral product of osteoblasts for bone formation. Therefore, HA levels are a useful biomarker for osteoblast activity, where abnormal accumulation or reduction of HA can be indicative of a disease state, such as:

- Arthritis
- Osteoporosis
- Tumor damage to bone (in metastasis)
- Fracture repair
- Tissue calcification (atherosclerosis and Vitamin D toxicity)

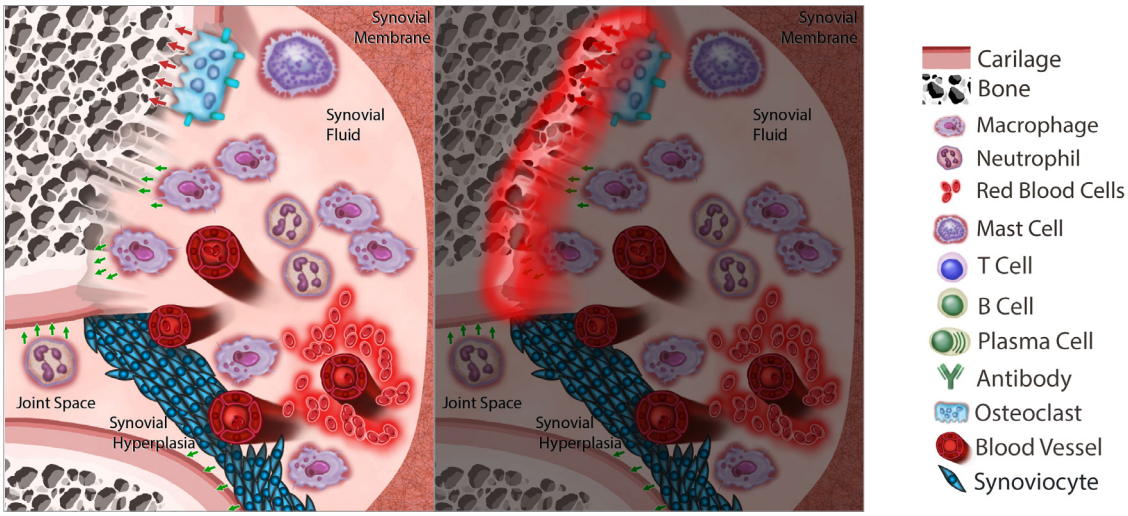


Figure 1. Hydroxyapatite as a biomarker for osteoblast activity indicates bone remodeling/bone formation (or the lack there of). IVISense Osteo fluorescent imaging of bone remodeling is represented in the figure on the right.

What is IVISense™ Osteo?

IVISense Osteo NIR fluorescent bisphosphonate imaging agents bind with high affinity to hydroxyapatite (HA) both *in vivo* and *in vitro*. Since HA is known to bind pyrophosphonates and phosphonates as well as synthetic bisphosphonates with high affinity, IVISense Osteo probes offer a safe and easy means to assess the subtle bone mineral changes that occur in bone growth, bone loss, and in diseases that damage bone, using subtherapeutic doses for imaging. Specifically, IVISense Osteo targeted imaging agents can be used to image areas of microcalcifications and bone remodeling, and can be used to measure the effects of therapeutic stimulation of bone growth, enabling *in vivo* detection, measurement, and monitoring of skeletal changes in a wide range of disease states.

How is IVISense Osteo probe used?

Here we show two case studies demonstrating possible applications of IVISense Osteo fluorescent probes.

Case study 1

Vitamin D3 toxicity

Vitamin D3, at excessive doses, is known to cause bone loss by increasing calcium mobilization and reabsorption. In this case study, 0.06 µg of Vitamin D3 was injected daily for four days into SKH-1 mice. On day three, a 2-5-fold increased signal was detected in the proximal tibial regions of the mice treated with high-doses of vitamin D as compared to control. A constitutively fluorescent, un-targeted control agent was used to confirm imaging specificity. This signal increase was supported by the significantly increased plasma levels of calcium in these mice. Figure 2A shows mice exhibiting clear changes in the knees from increased bone turnover as detected by IVISense Osteo fluorescent probe.

Figure 2B shows soft tissue calcification detected in the kidneys by calcium microplate assay, with higher levels than in plasma. A 5-8-fold fluorescent increase was detected within the kidneys of treated mice, correlating with both *ex vivo* imaging of kidneys, fluorescence microscopy of kidney tissue sections, and assays for tissue calcium. Imaging with IVISense Osteo probe confirmed biological alterations in the kidneys consistent with calcification.

The data highlights the utility of IVISense Osteo fluorescent probe in models of bone resorption as well as in models known to develop soft tissue calcification and allowed robust quantification of tissue changes in living animals

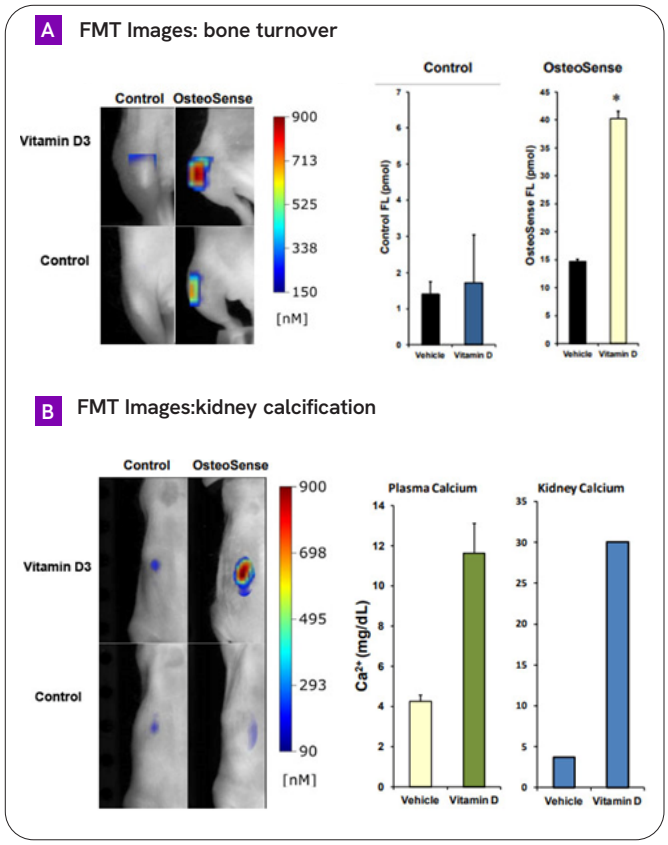


Figure 2A and 2B. Bone turnover and kidney calcification as detected by IVISense Osteo probe using a FMT® imaging system.

Case study 2

Collagen Antibody-Induced Arthritis (CAIA)

Collagen-specific antibodies can be used to trigger chronic arthritis in mice that occurs with peak inflammation by day eight and increased bone destruction evident by day 15. Standard metrics of disease assessment, including subjective clinical scoring and paw thickness measurements, do not provide a quantitative biological readout of bone changes. Rather, these standard metrics emphasize the edema, inflammation, and erythema that occur during the disease process. In contrast, IVISense Osteo fluorescent probe can be used in models of bone disease to detect HA that is either newly deposited (bone growth) or is exposed during bone destruction (bone loss).

The left panel of Figure 3 shows the disease incidence induced by collagen antibodies (assessed by clinical scoring) as compared to non-induced mice, and shows that prednisolone treatment effectively ablates the apparent disease throughout the treatment course. The right panel of Figure 3 shows that IVISense Osteo probe can be used to image disease-induced changes in bone turnover, as demonstrated by increased signal on Day 8 in CAIA mice, and no signal increase in mice treated with prednisolone compared to control. Interestingly, on day 15, there is a further increase in fluorescent signal in CAIA mice, but also an increase in prednisolone mice (blue arrow) despite the absence of arthritis (left panel). This late signal with treatment is attributed to steroid-induced osteoporosis.

As bone turnover is of critical importance in many diseases and conditions, IVISense Osteo fluorescent probe provides an important imaging tool to study bone biology *in vivo*, in this case detecting both arthritis-induced and prednisolone-induced bone loss.

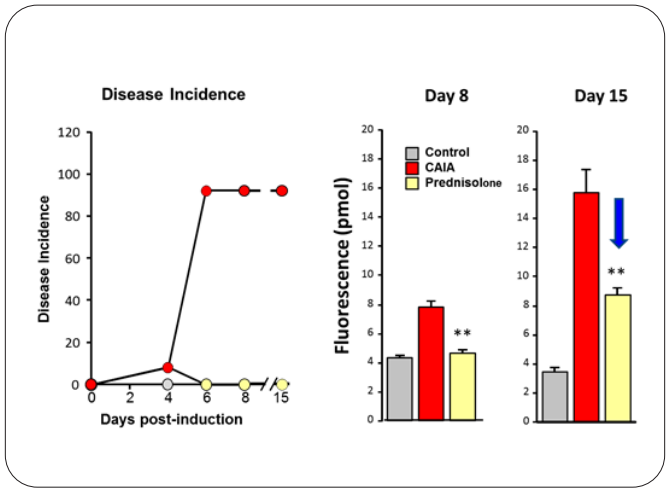


Figure 3. Left panel shows clinical scoring of disease incidence. Figure 3 right panel shows disease-induced changes as detected by IVISense Osteo probe.

Use IVISense Osteo fluorescent probe as part of a complete experimental solution package

Revvity provides complete *in **vivo*** imaging solutions including reagents, instrumentation and support expertise that can help you monitor and design experiments to understand the progression of diseases and their related processes, or to evaluate the potential therapeutic efficacy of drugs targeting the underlying mechanisms involved in disease.

IVISense Osteo fluorescent probes are available in three wavelengths; 680, 750, and 800, and are designed to target areas of bone remodeling and microcalcification *in vivo*.

Cat #	Product	
NEV10020EX	IVISense Osteo 680	Optimized in vitro and in *vivo* imaging of bone remodeling through binding of hydroxyapatite
NEV10053EX	IVISense Osteo 750	
NEV11105	IVISense Osteo 800	Further expands this agent family in enabling *in vitro* and in *vivo* detection, measurement, and monitoring of skeletal changes in a wide range of disease states, including arthritis, osteoporosis, and cancer metastasis

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