BIOPHOTONICS AND BONE BIOLOGY

Gregory Zimmerli and David Fischer
NASA Glenn Research Center, Cleveland, OH

Marius Asipauskas, Chirag Chauhan, Nicole Compitello, and Jamie Burke
National Center for Microgravity Research, Cleveland, OH

Melissa Knothe Tate
Cleveland Clinic Foundation, Lerner Research Institute, Cleveland, OH

One of the more-serious side effects of extended space flight is an accelerated bone loss [Bioastronautics Critical Path Roadmap, http://research.hq.nasa.gov/code_u/bcpr/index.cfm]. Rates of bone loss are highest in the weight-bearing bones of the hip and spine regions, and the average rate of bone loss as measured by bone mineral density measurements is around 1.2% per month for persons in a microgravity environment [T. Lang et al., *JBMR* 2004]. Figure 1 shows that an extrapolation of the microgravity-induced bone loss rates to longer time scales, such as a 2.5 year round-trip to Mars (6 months out at 0 g, 1.5 year stay on Mars at 0.38 g, 6 months back at 0 g), could severely compromise the skeletal system of such a person.

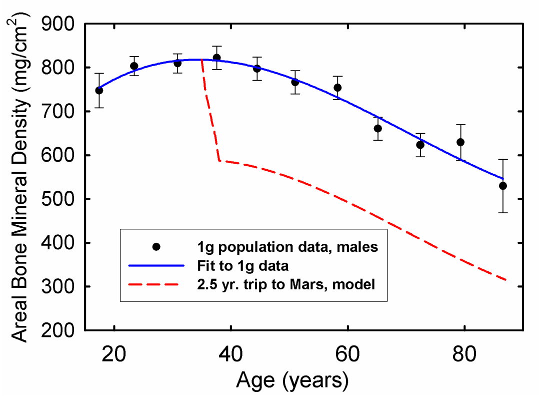


Figure 1. Age-related bone loss in a 1g population of males (data from Atlas of Clinical Endocrinology: Osteoporosis, 2003) compared to a hypothetical person exposed to microgravity and partial gravity during a 2.5 year Mars trip. The model assumes a linear response of bone loss with g-level, and does not account for the possibility of new bone growth upon returning to 1 g, as no data yet exists for such an effect.

It is well known that bone remodeling responds to mechanical forces. We are developing two-photon microscopy techniques to study bone tissue and bone cell cultures to better understand the fundamental response mechanism in bone remodeling. Osteoblast and osteoclast cell cultures are being studied, and the goal is to use molecular biology techniques in conjunction with Fluorescence Lifetime Imaging Microscopy (FLIM) to study the physiology of in-vitro cell cultures in response to various stimuli, such as fluid flow induced shear stress and mechanical stress. We have constructed a two-photon fluorescence microscope for these studies, and are currently incorporating FLIM detection. Current progress will be reviewed. This work is supported by the NASA John Glenn Biomedical Engineering Consortium.

Contact: Gregory.A.Zimmerli@nasa.gov
NASA GRC, 21000 Brookpark Road, MS 110-3, Cleveland, OH 44135

Biophotonics and Bone Biology

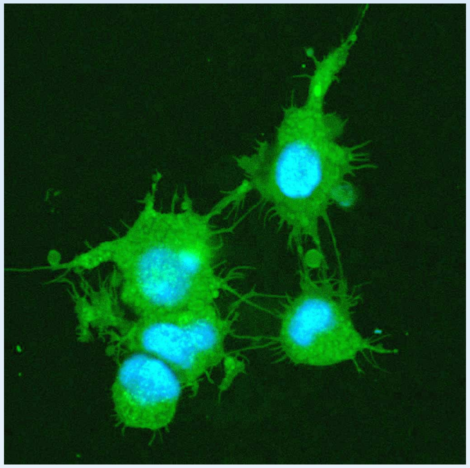
- Greg Zimmerli, Ph.D.
NASA Glenn Research Center

Colleagues:

David Fischer[1], Ph.D.; Marius Asipauskas[2]; Melissa Knothe Tate[3], Ph.D.; Chirag Chauhan[2,3], Jamie Burke[2], Nicole Compitello[2]

[1] NASA Glenn Research Center
[2] National Center for Microgravity Research
[3] Cleveland Clinic Foundation

<u>Goal</u>: Develop advanced fluorescence microscopy techniques to study bone cell physiology



<u>Motivation:</u>

- Cells cultured in microgravity exhibit different gene expression profiles.
- Cytoskeleton in space-based osteoblast cell cultures is less well-developed.
- T-cell lymphocyte (immune cells) activation is suppressed in microgravity

Microgravity has a harmful effect on human physiology

- Bone loss in hips and spine, 1% per month
- Immunodeficiency
- Loss of blood plasma, anemia
- Cardiac dysrhythmia

Like an accelerated osteoporosis

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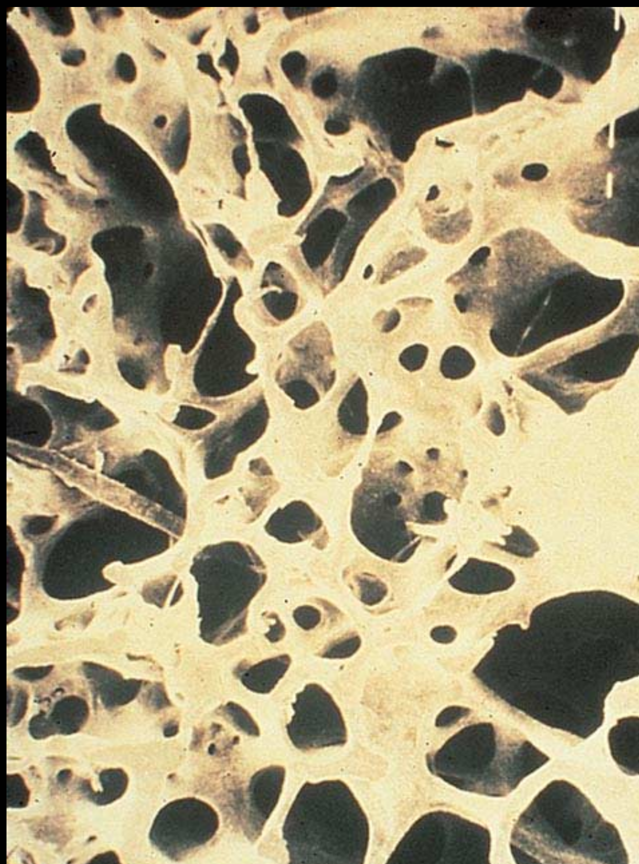
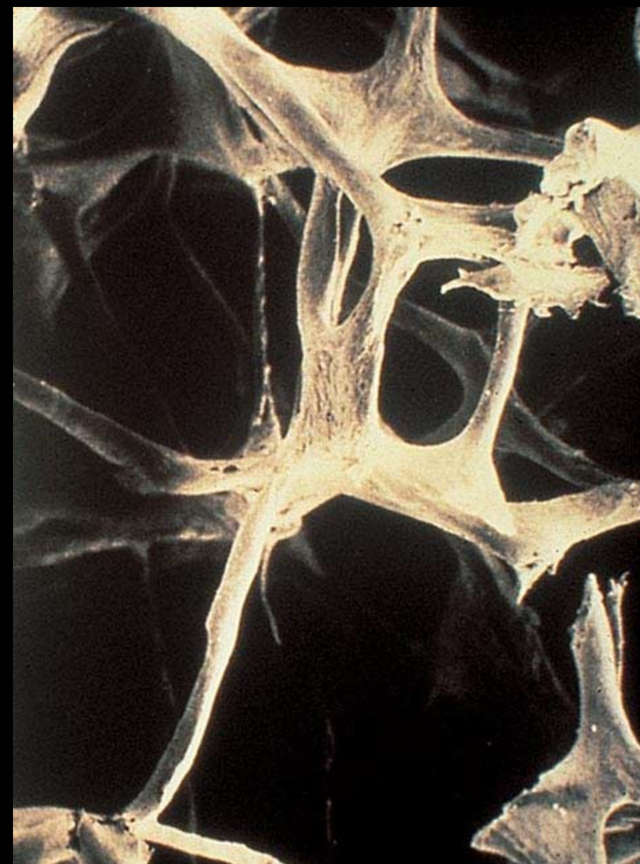
•
•

Ref. document: Bioastronautics Critical Path Roadmap

Normal trabecular
(spongy bone) structures

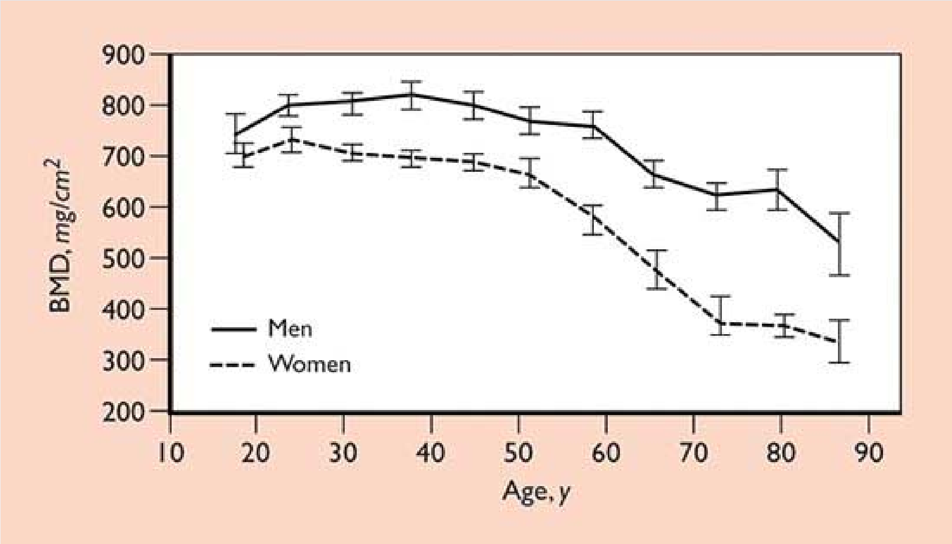


Osteoporotic trabecular structures



Source: Atlas of Clinical Endocrinology: Osteoporosis (2003)

Bone mineral density (BMD) loss: Effect of aging



Source:
Atlas of Clinical Endocrinology:
Osteoporosis (2003)

Microgravity BMD loss: 1.2% per month (Lang et al., *JBMR* 2004)

Model a trip to Mars: $\frac{d}{dt}BMD = \frac{d}{dt}BMD_{aging} + \frac{d}{dt}BMD_{g-level}$

$$\boxed{\frac{d}{dt}BMD_{g-level} = \frac{0.012 \cdot BMD}{mo.}(g^* - 1) \quad \text{Linear response model}}$$

$$g^* = \frac{local\ accel.}{9.8\ m/s^2}$$

Calculate $BMD(t) = BMD(t_0) + \int_{t_0}^{t} \frac{d}{dt} BMD\ dt$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Strategic Research to Enable
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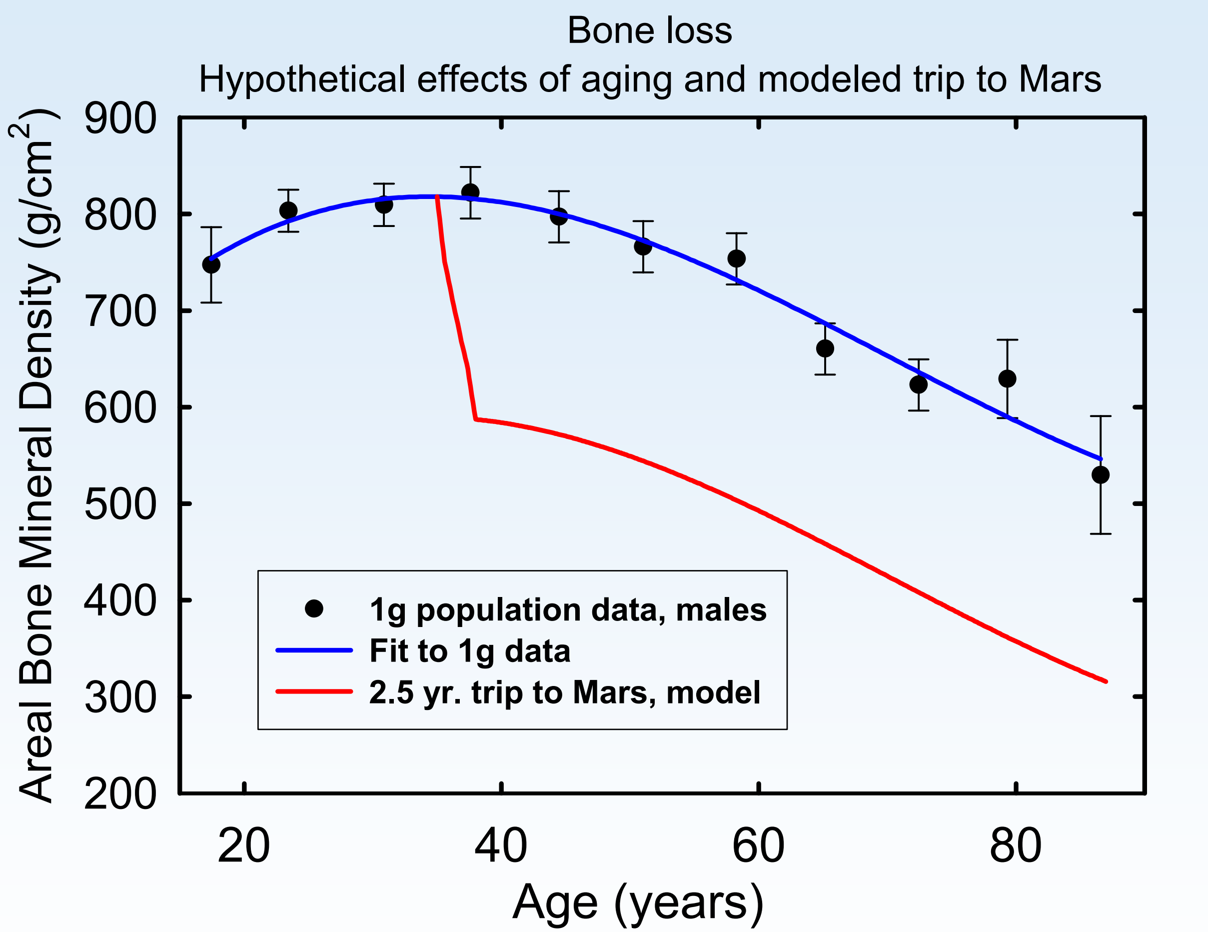
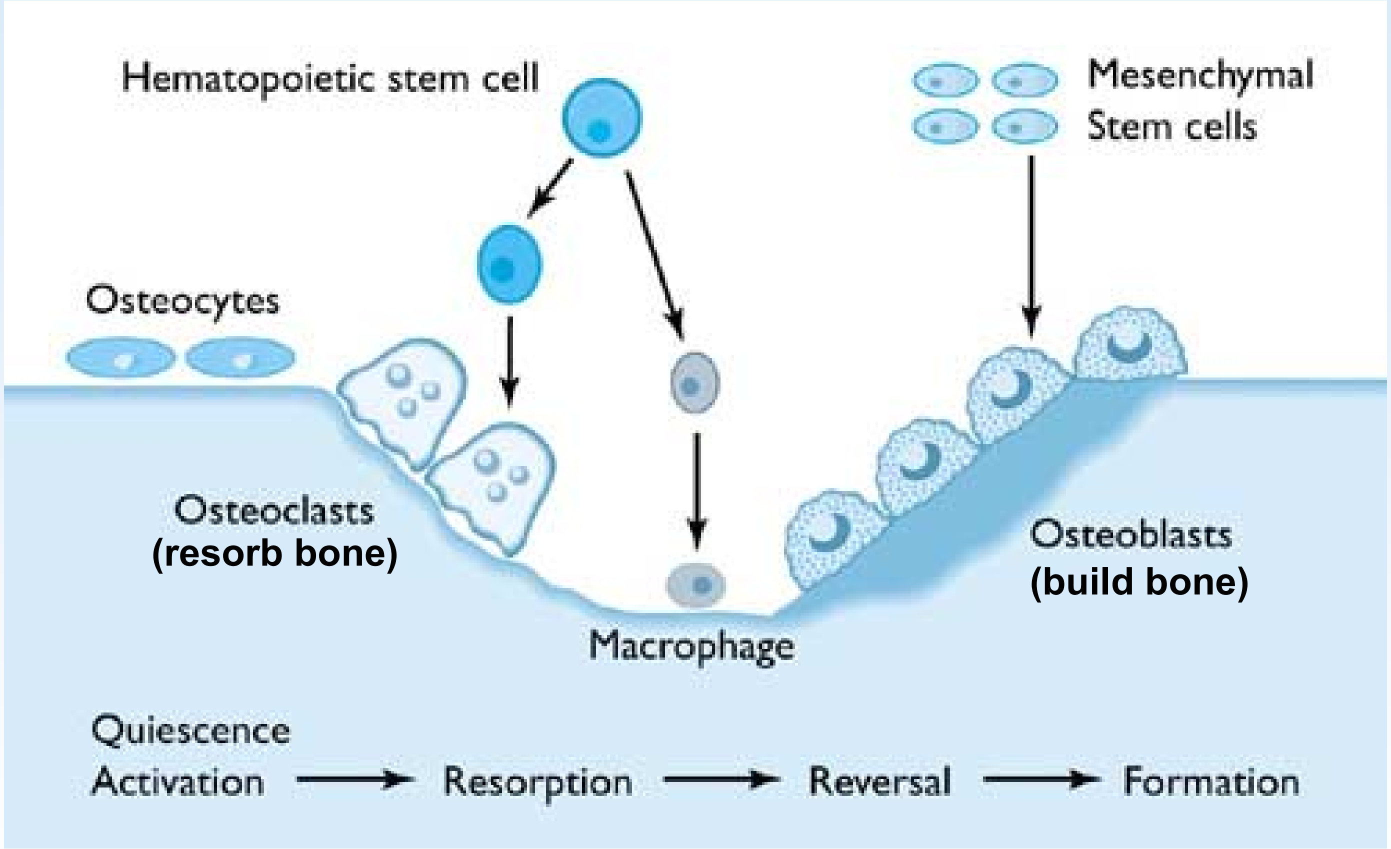
Conference 2004



Mars trip: 6 mo. out (0g), 18 mo. stay (0.38g), 6 mo. return (0g)

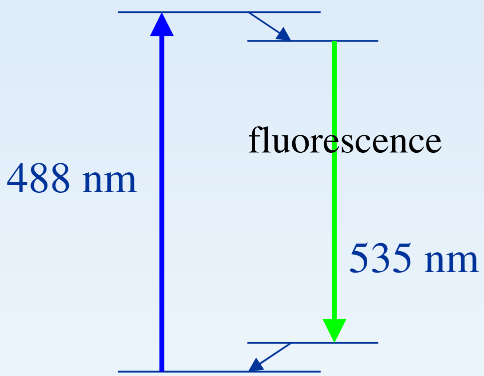
Bone Remodeling: Balance between osteoclasts and osteoblasts



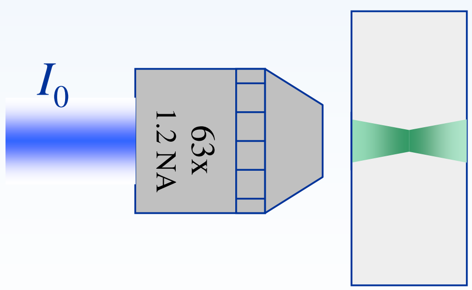
Use two-photon fluorescence microscopy to study macrophage, osteoclast and osteoblast cells

Background: Two-photon absorption

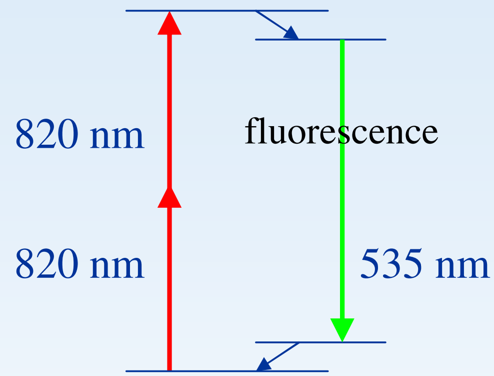
Single-photon absorption



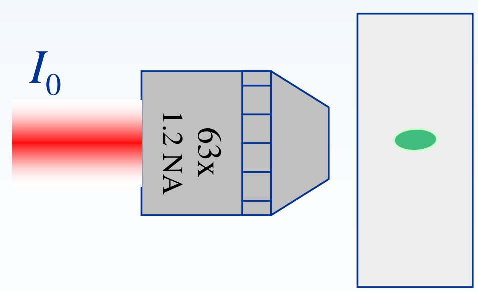
Fluorescence intensity ~ I_0



Two-photon absorption



Fluorescence intensity ~ I_0^2



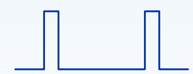
Excitation rate (photons/s), φ

1 - photon

$$\varphi_{1p} \approx 4 P_0 \eta_{1p} \sigma_{1p} \frac{(NA)^2}{hc\lambda}$$

2 - photon, pulsed laser

$$\phi_{2p} \approx 8 \langle P_0 \rangle^2 \frac{\eta_{2p} \sigma_{2p}}{\tau_p f_p} \frac{(NA)^4}{(hc\lambda)^2}$$



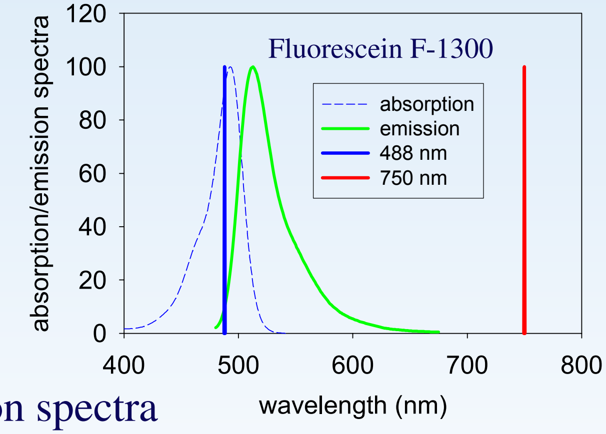
$$(\tau_p f_p)^{-1} \approx 10^5$$

$$\frac{\varphi_{2p}}{\varphi_{1p}} \approx 5 \cdot 10^{-4} / mW$$

Advantages of two-photon excitation:

Fluorescence excitation is limited to the focal volume

- confocal-like performance, but no need for pinhole in detection optics,
- less photobleaching
- improved contrast



Longer wavelength excitation

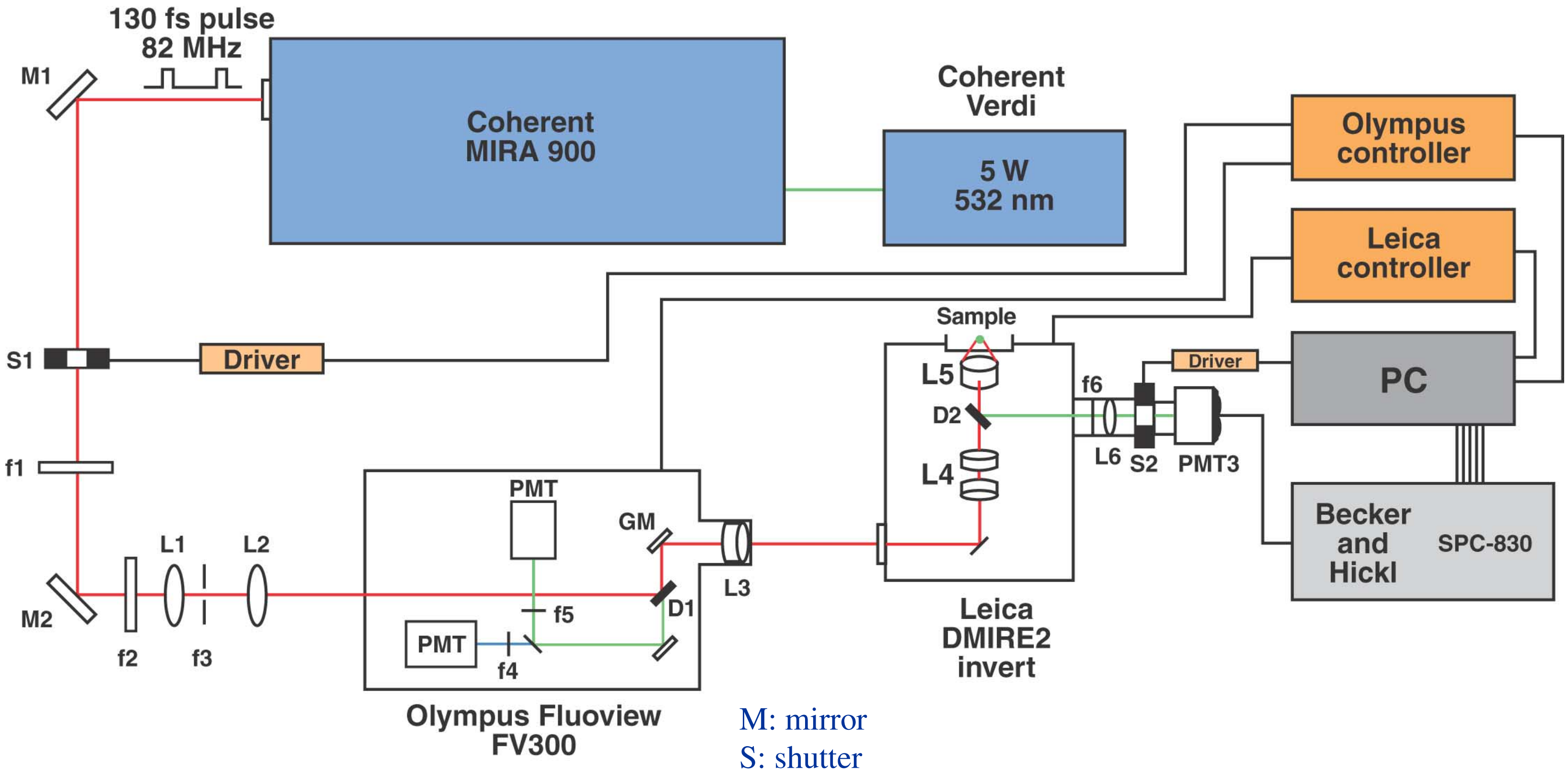
- reduced Rayleigh scattering ($1/\lambda^4$), better depth penetration
- less absorption/damage in tissue; biological "optical window"
- larger spectral gap in excitation/emission spectra

Disadvantages of two-photon:

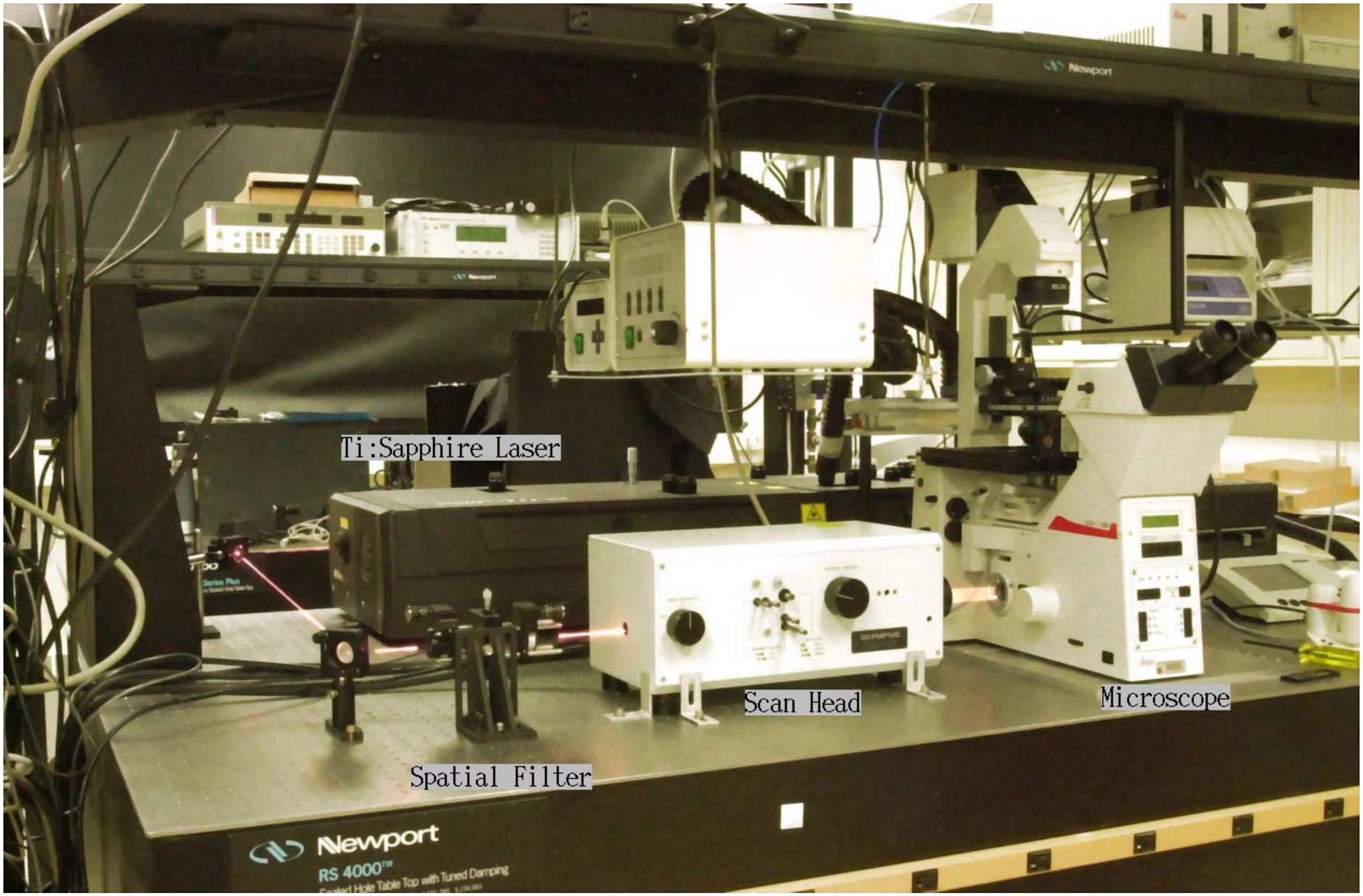
- Large, expensive laser:
  - complete two-photon systems available commercially for \$500k-\$700k
- Slightly lower resolution due to longer excitation wavelength

Glenn Research Center Biophotonics Lab

Two-photon microscopy layout

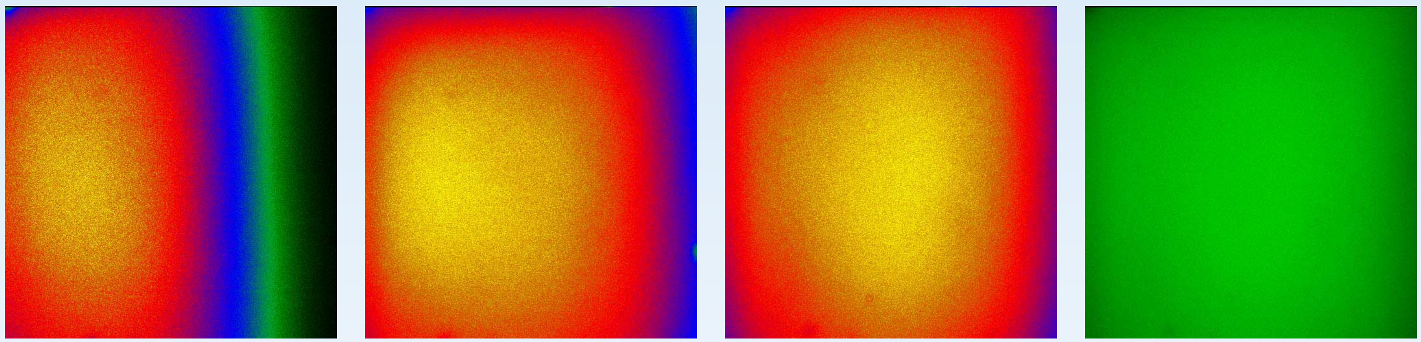


M: mirror
S: shutter
f: filter
L: lens
D: dichroic
GM: galvonmeter mirrors (2)
PMT: photomultiplier tube


Ti:Sapphire Laser
Scan Head
Microscope
Spatial Filter
Newport
RS 4000™
Sealed Hole Table Top with Tuned Damping

Fine tuning the optical alignment:

Scan a fluorescent lake sample (e.g., fluorescein in methanol), align scanhead, scope.

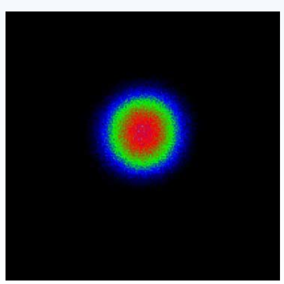


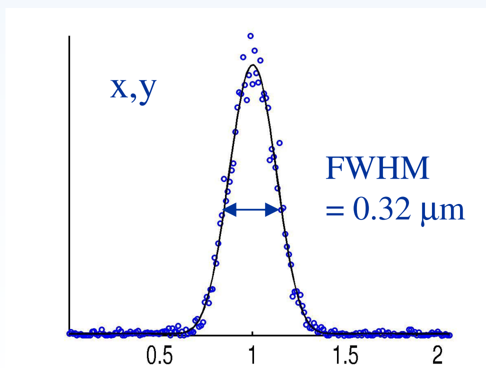
better alignment

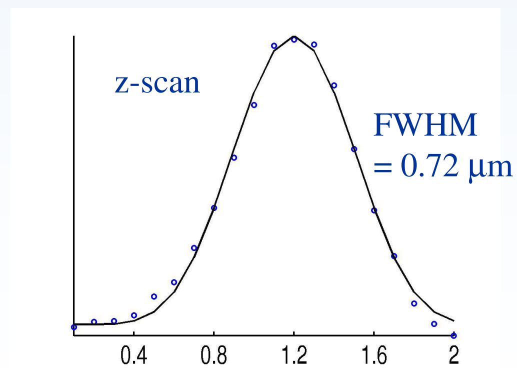
One-color representation

Point-spread-function measurement:

scan 0.093 µm diameter fluorescent microspheres in x,y,z







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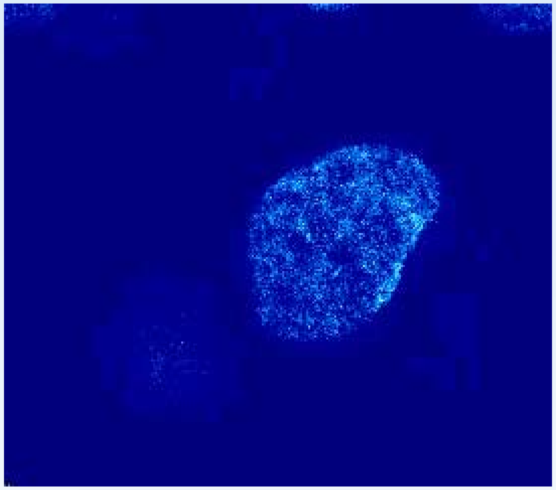
Biophotonics Lab

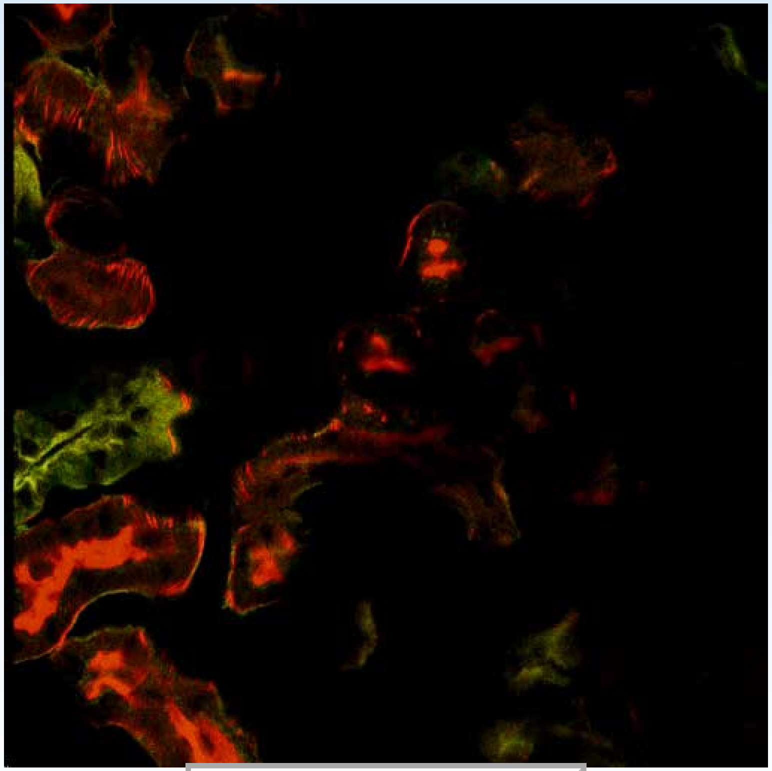
Add micro-incubator for 37 °C ,
5% CO_2 control:



Click here to play movie

CHO cells expressing YFP;
Time lapse: 2 minutes/frame

Cells provided by Prof. Gabor Forgacs, U. Missouri
and Dr. Rusty Lansford, CalTech

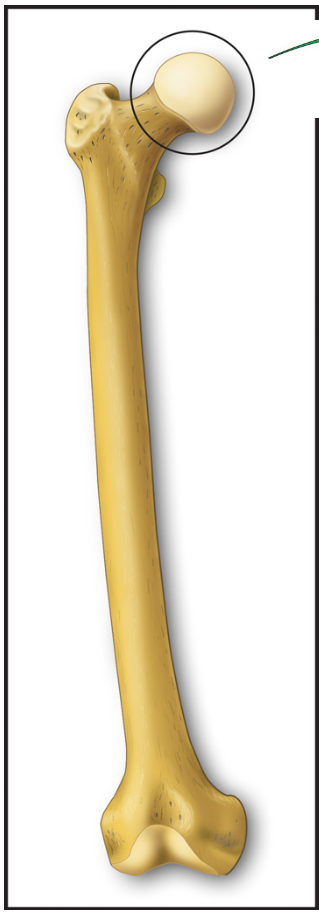


Click here to play movie

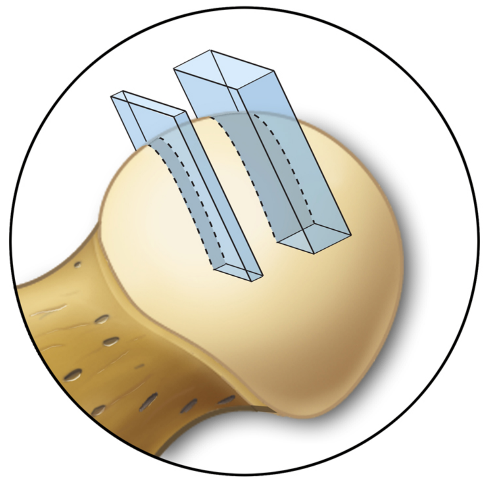
Mouse kidney section: z- scan

Application: Imaging bone tissue

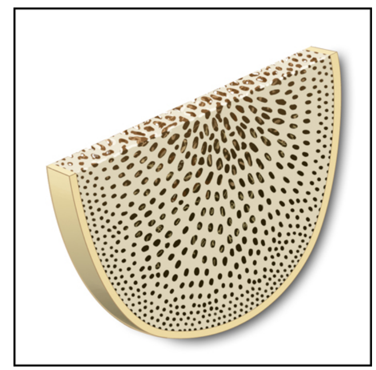
Sample provided by: Melissa Knothe Tate, Cleveland Clinic Foundation



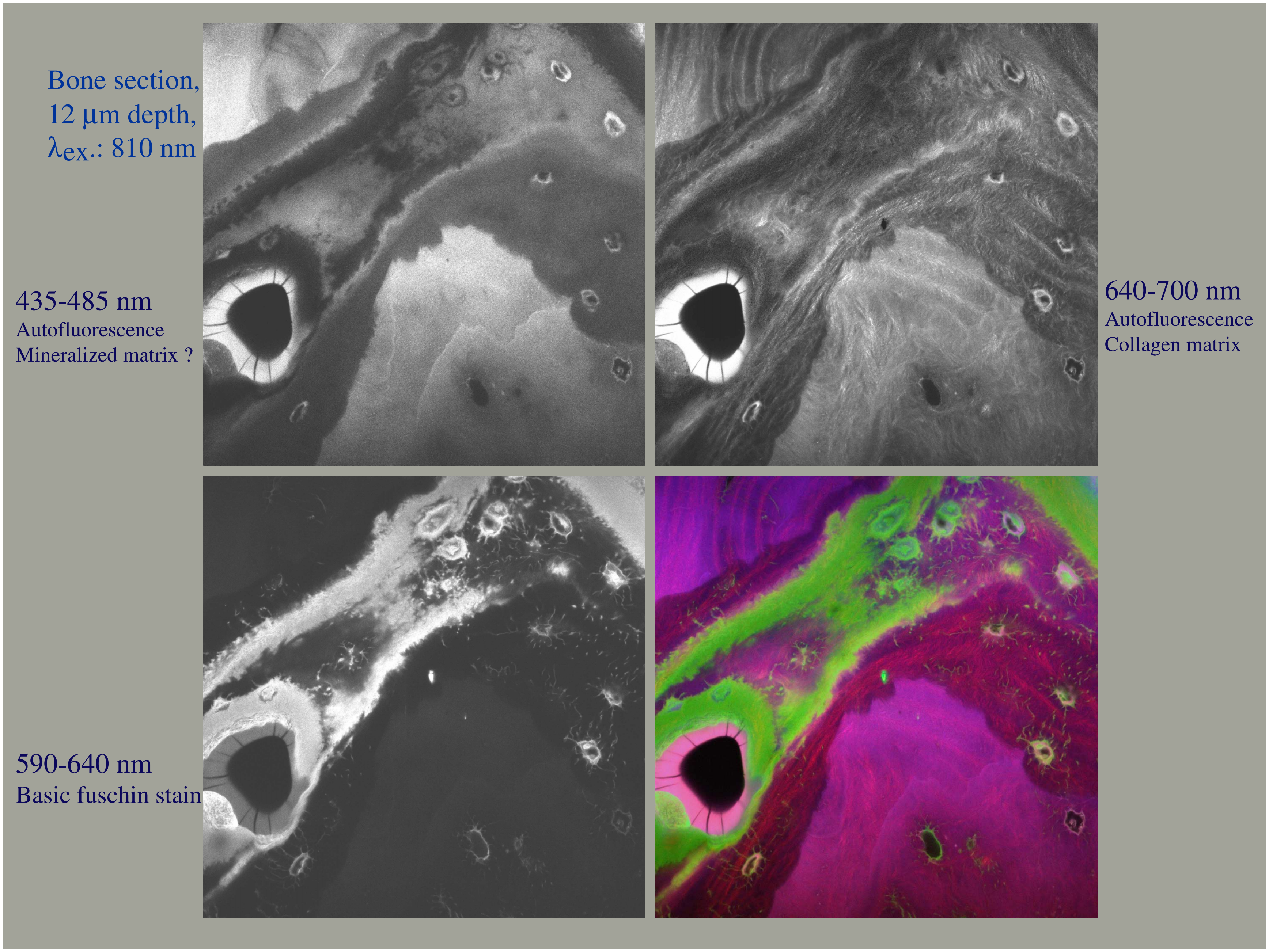
Human femur

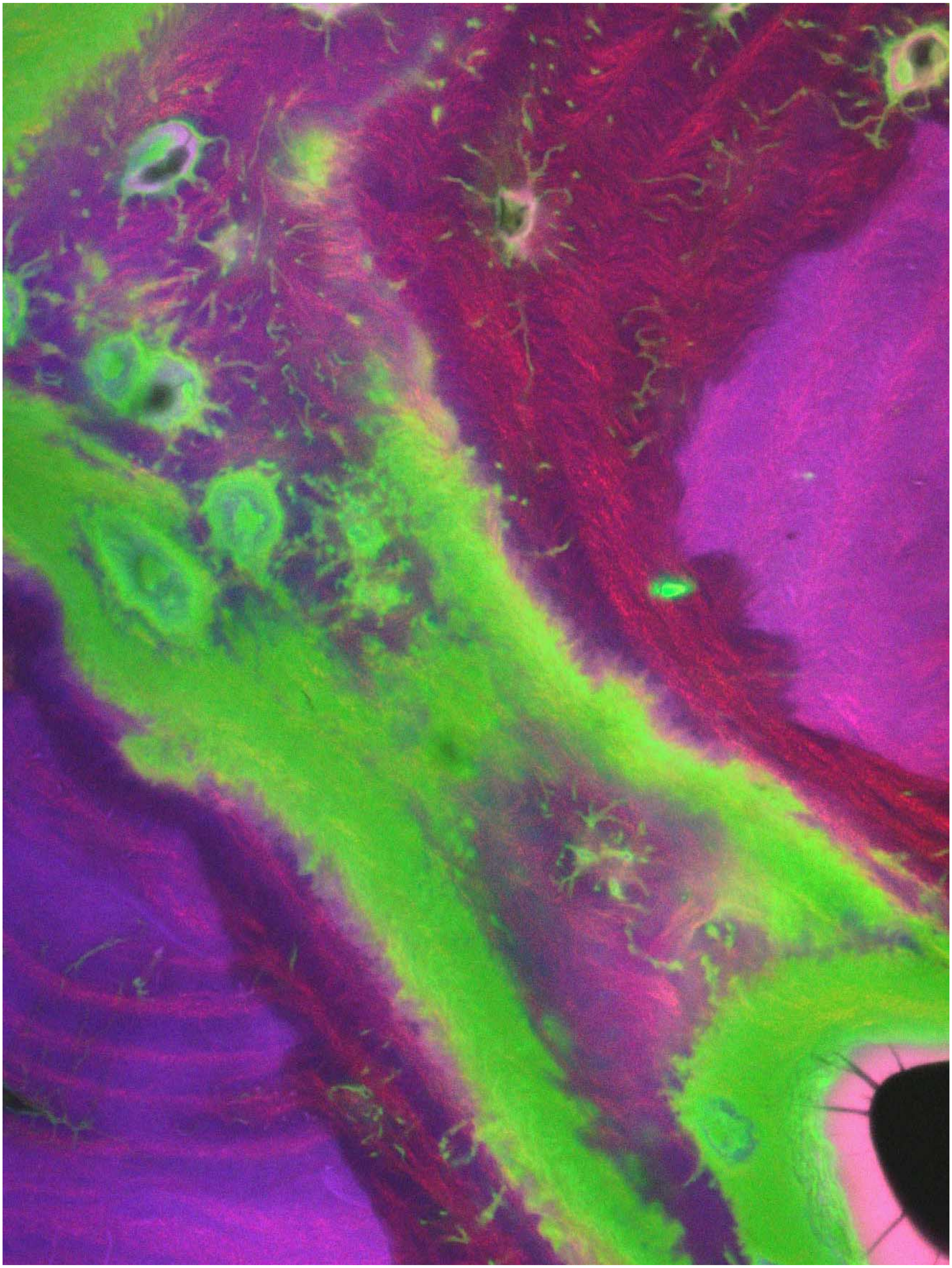


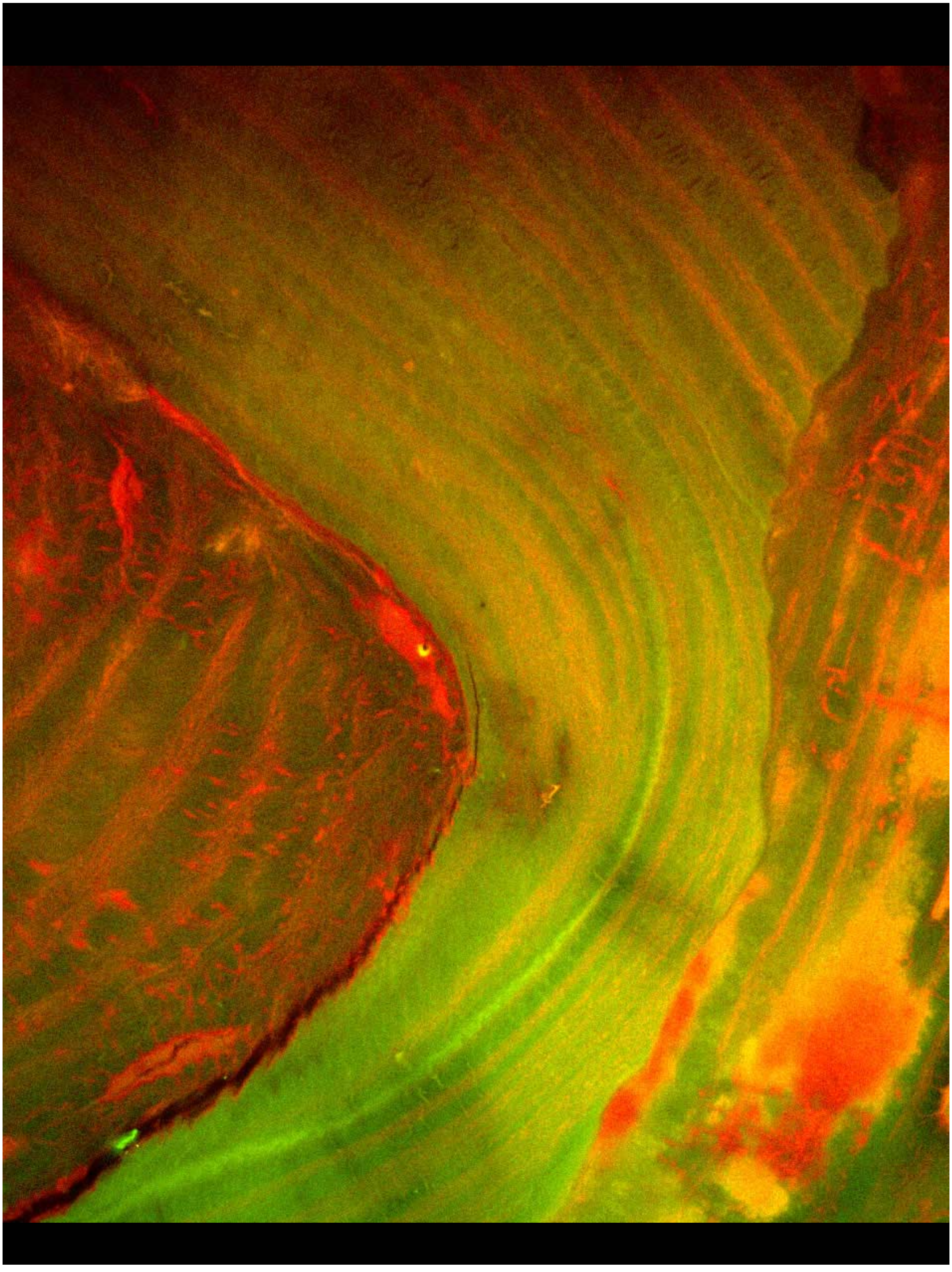
Femoral head

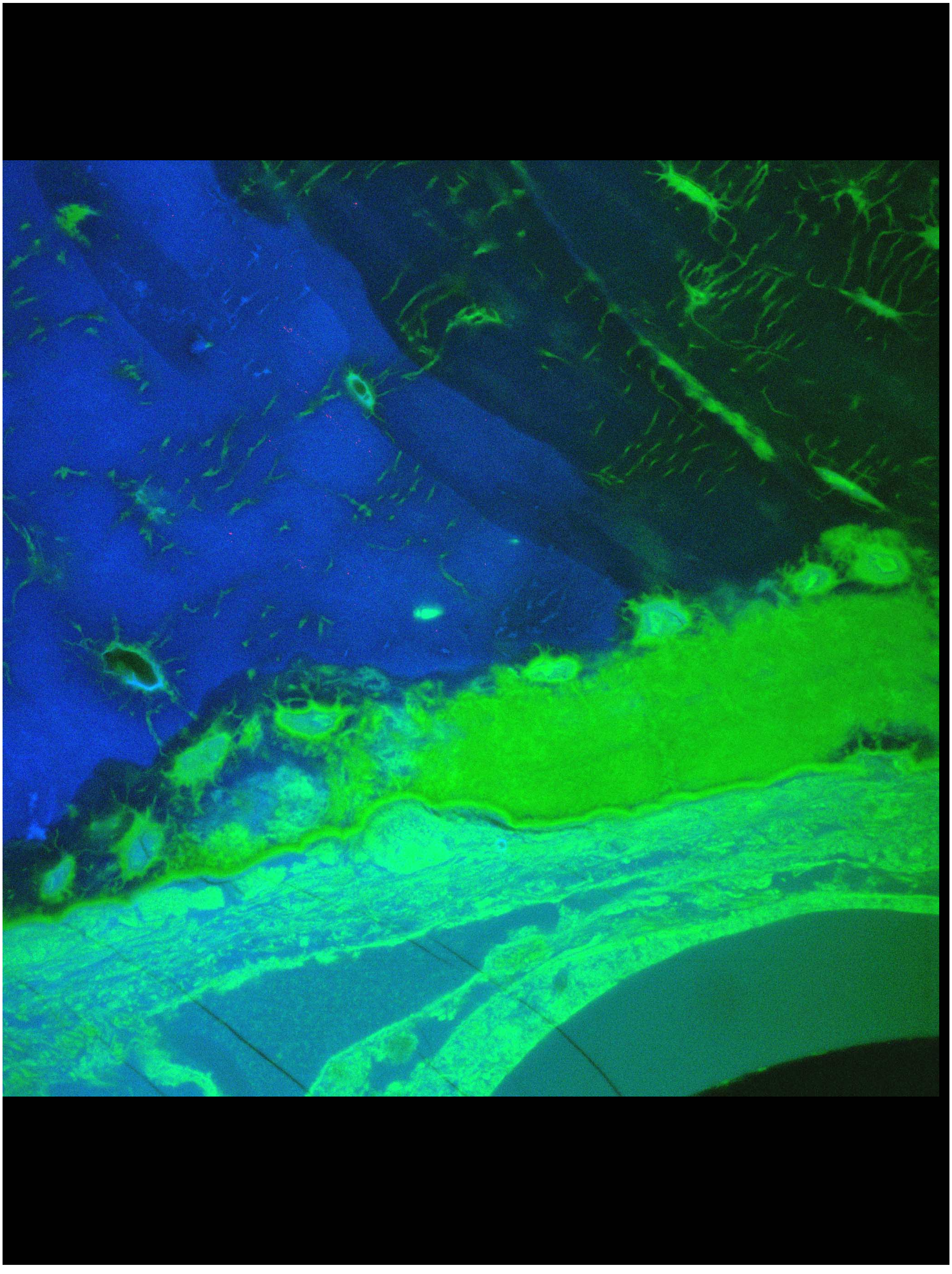


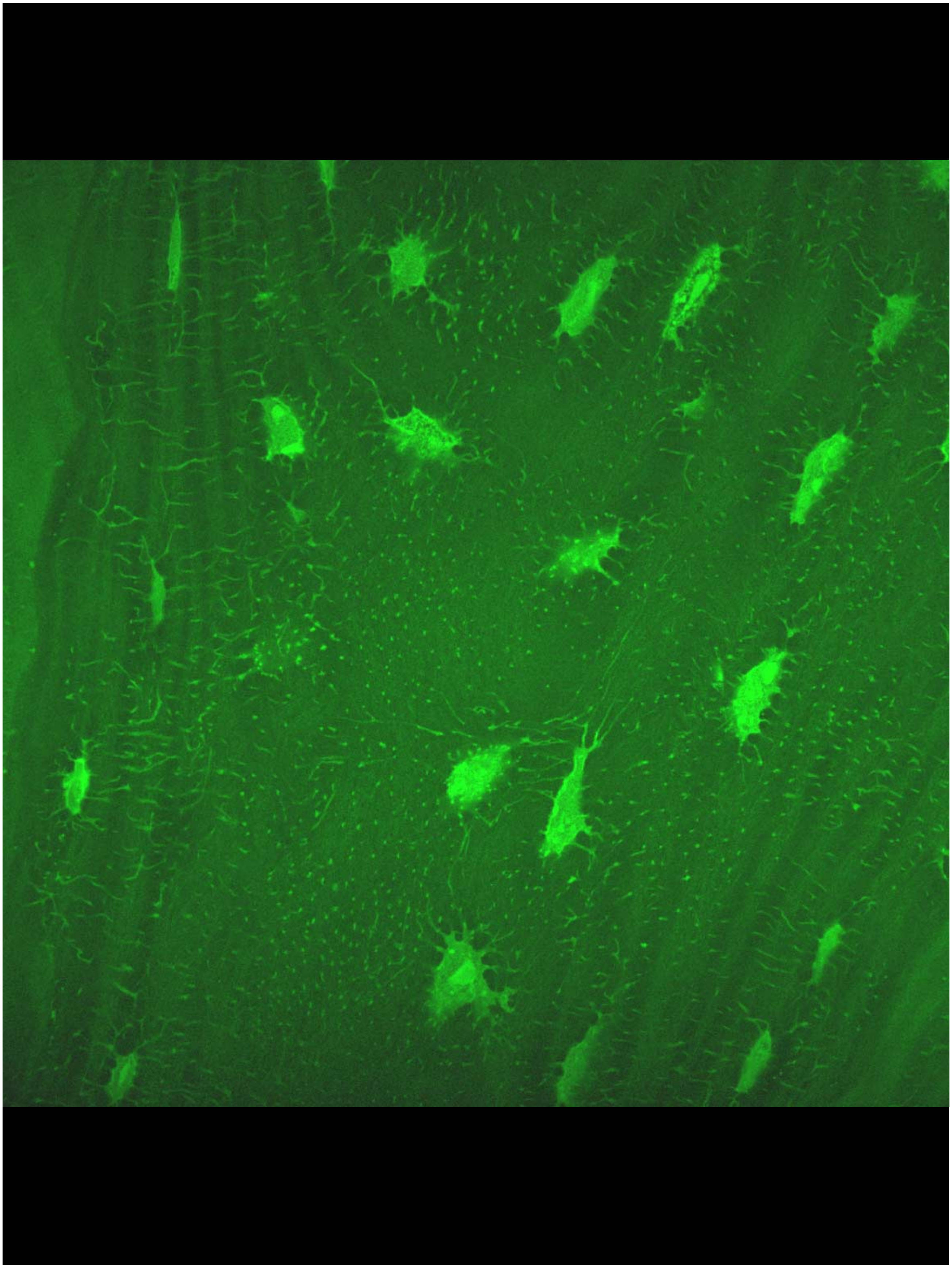
Section from femoral head stained with basic fuschin and embedded in PMMA

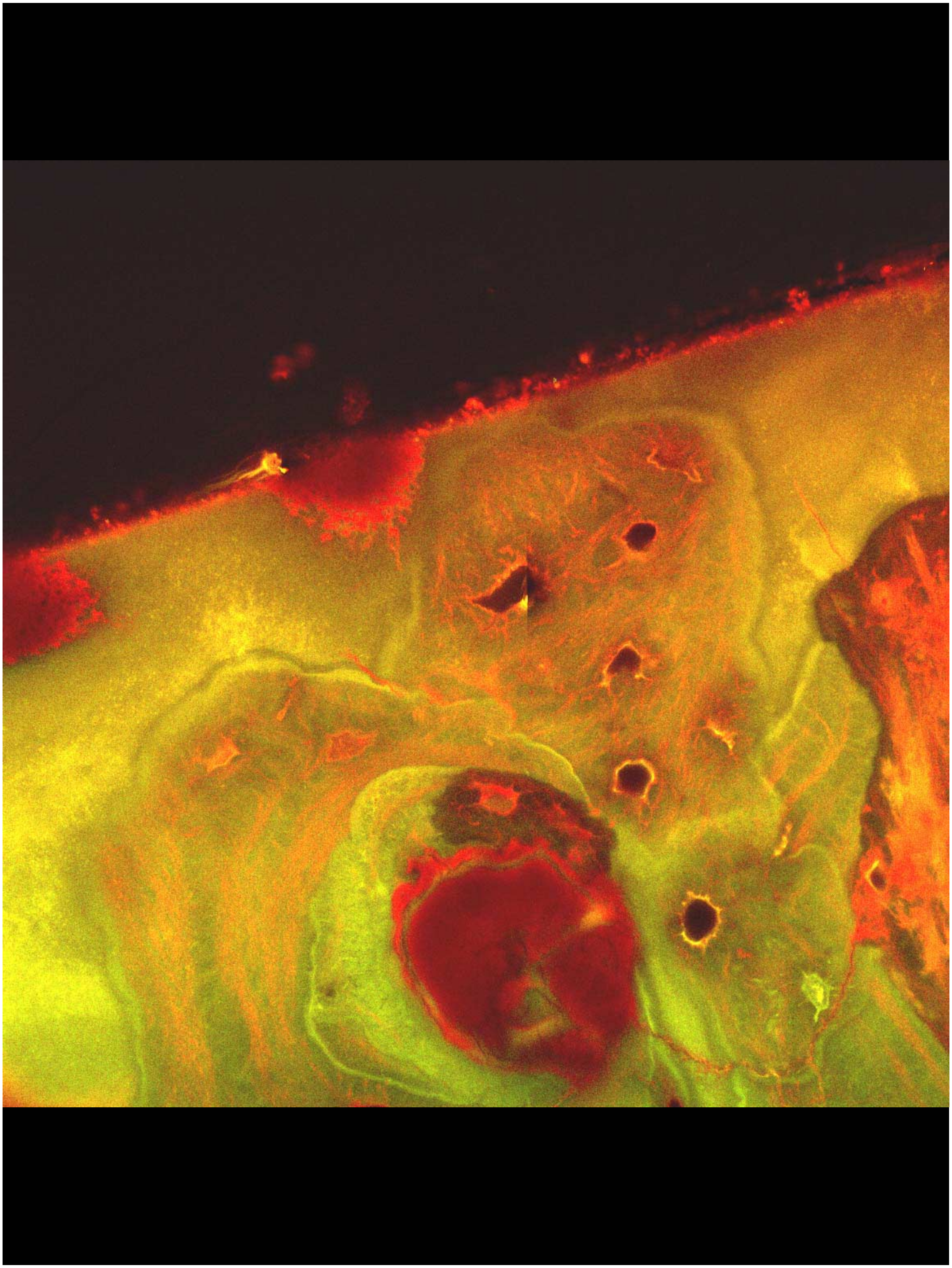

Bone section,
12 µm depth,
$\lambda_{ex.}$: 810 nm
435-485 nm
Autofluorescence
Mineralized matrix ?
640-700 nm
Autofluorescence
Collagen matrix
590-640 nm
Basic fuschin stain

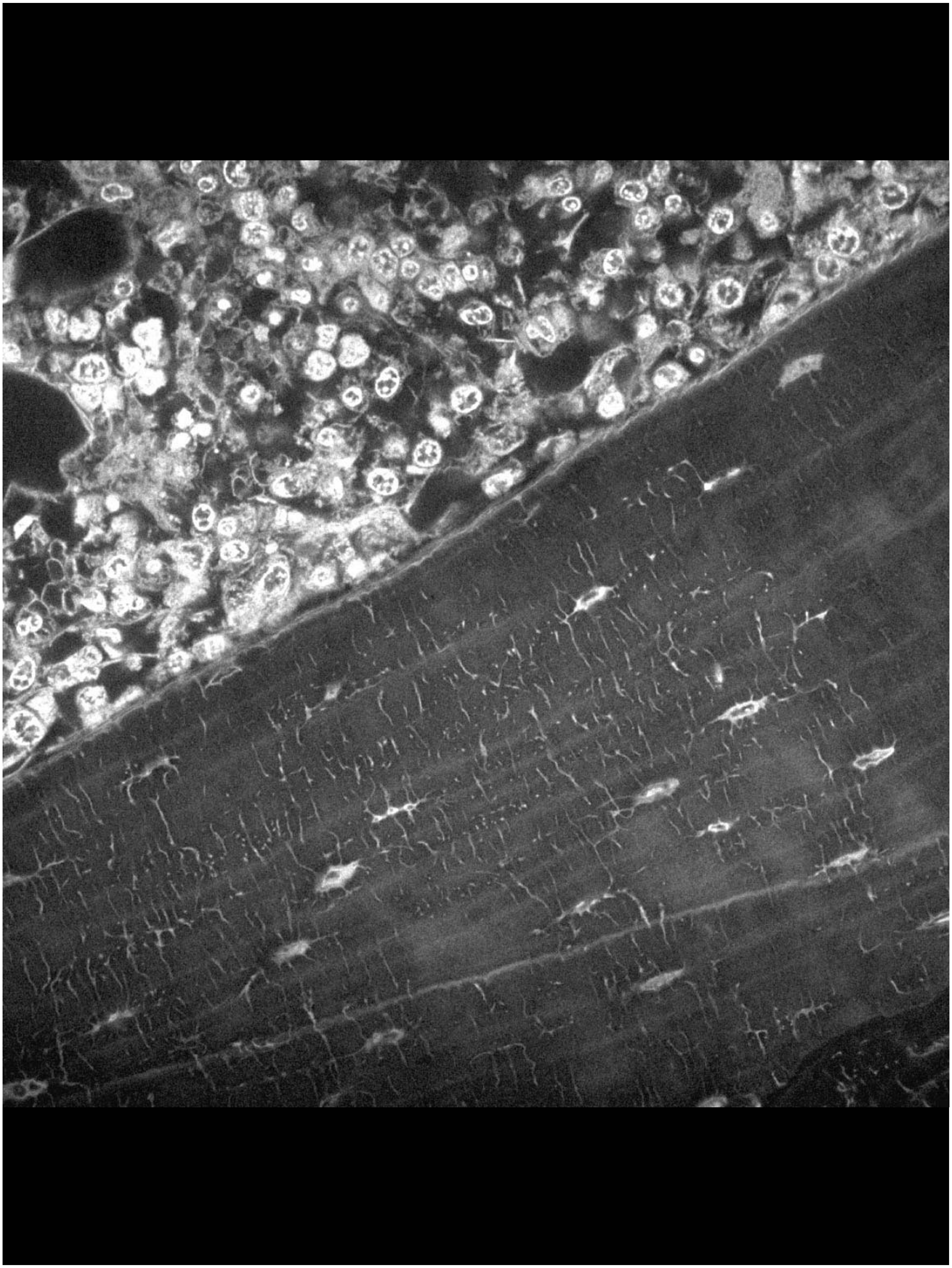




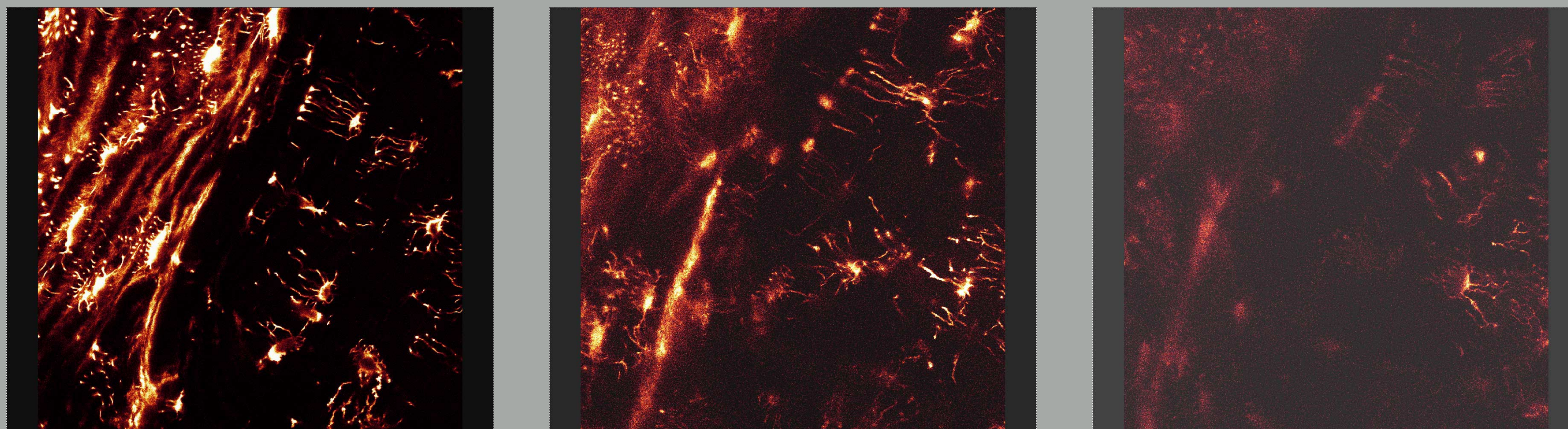




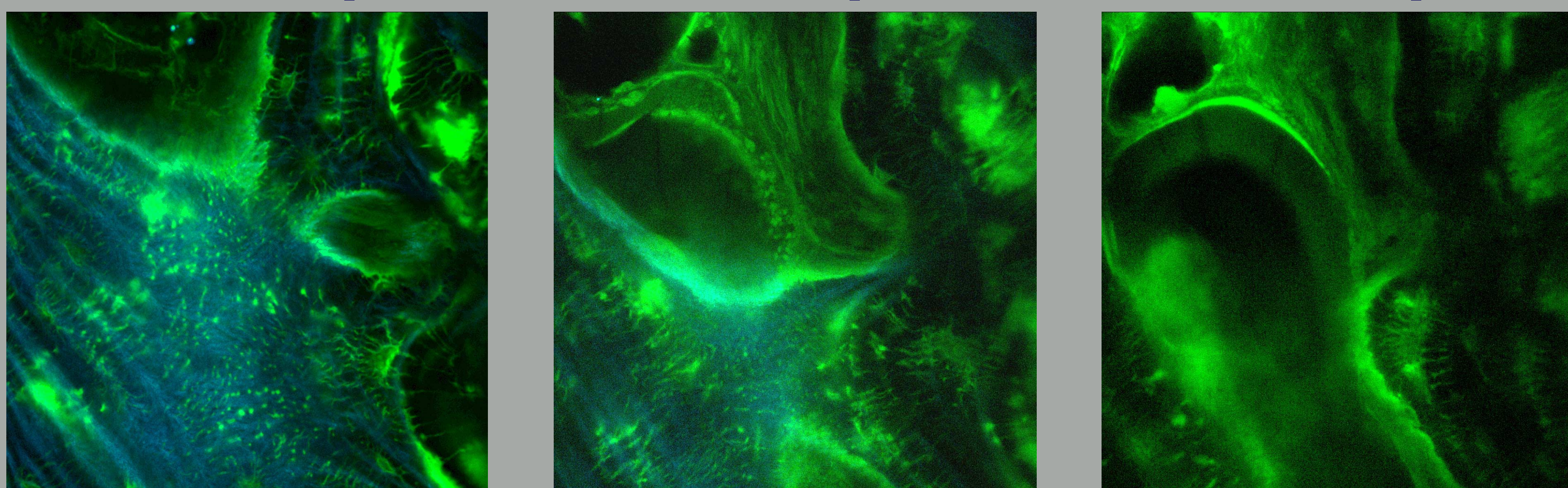




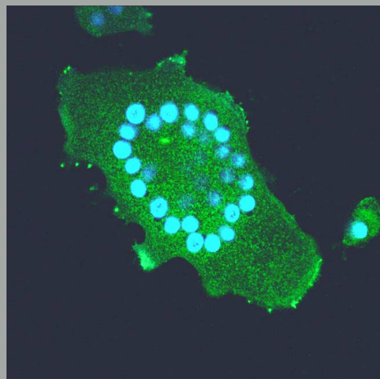
Confocal (top, 568 nm) versus Two-photon (bottom, 910 nm)



20 micron depth | 50 micron depth | 70 micron depth



Cortical bone (femur)



Producing osteoclasts in-vitro:

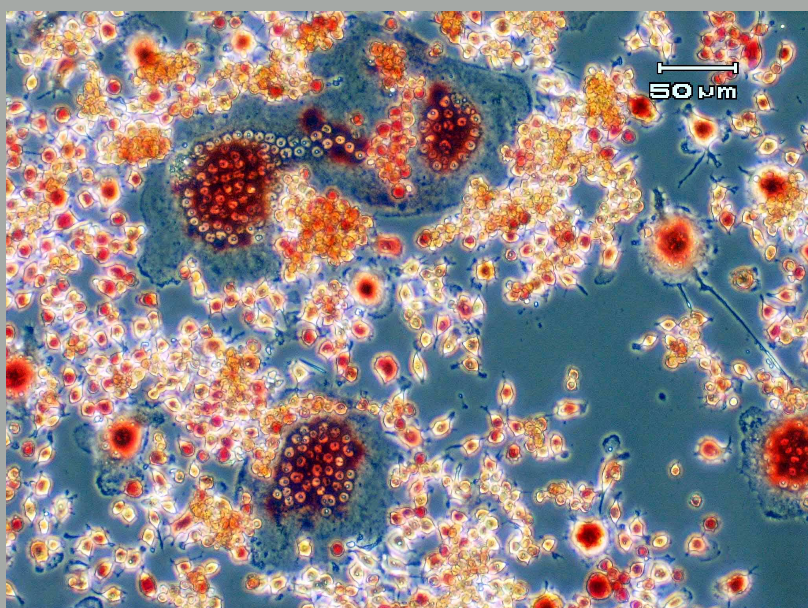
macrophage

+ RANKL

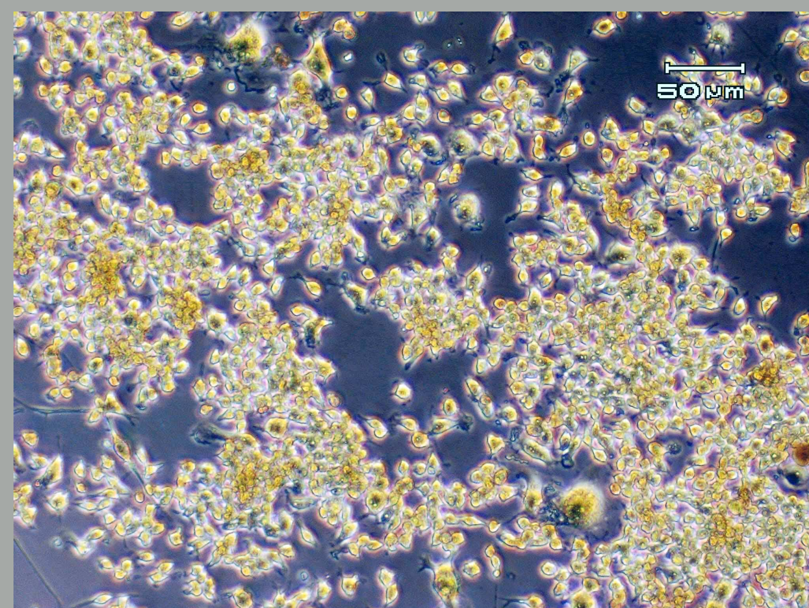
osteoclast precursor — macrophage

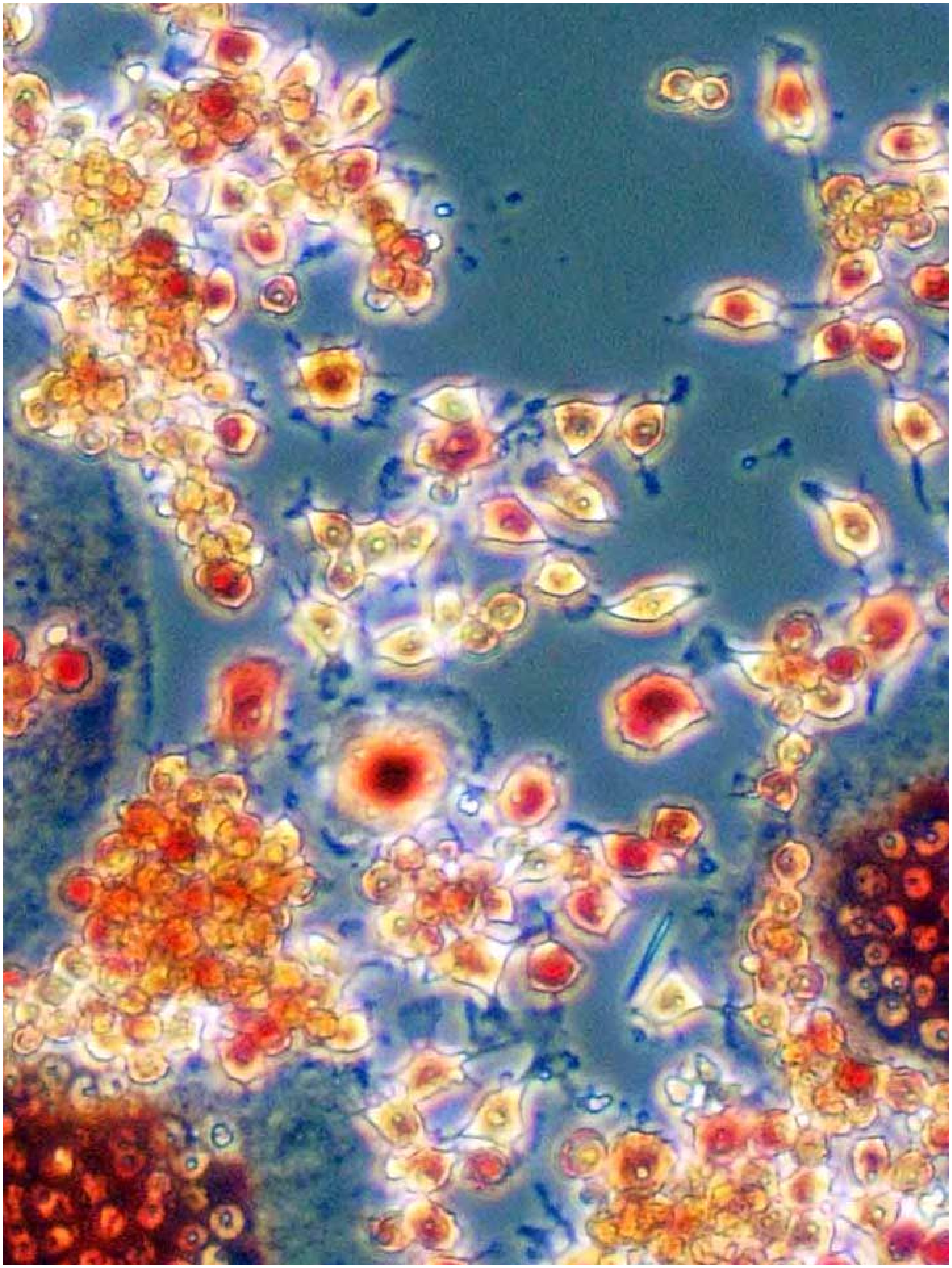
multinucleated osteoclast

TRAP+



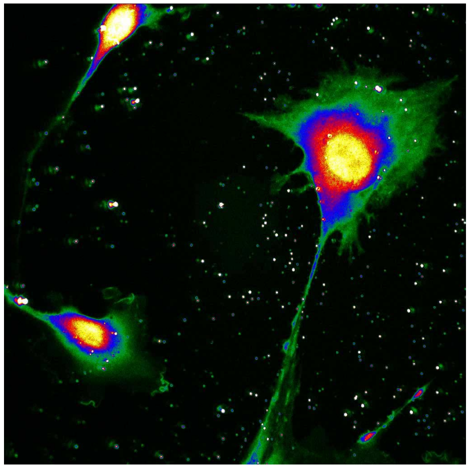
TRAP- **(no RANKL treatment)**





Fluorescence Lifetime Imaging Microscopy (FLIM)

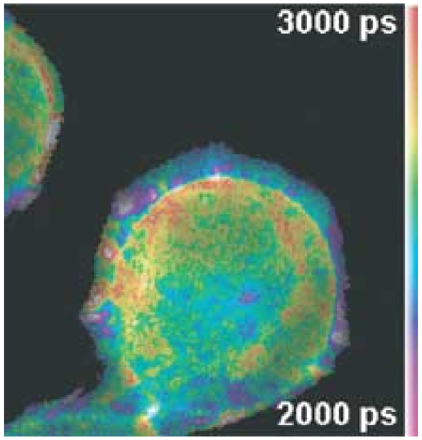
- Presently adding FLIM capability to the microscope



Fura-2 dye (Calcium indicator), MC3T3 cells

Intensity is a function of many variables (dye and Ca conc., excitation/collection efficiency)

- **OK for qualitative imaging**
- **Quantitative data is possible but difficult**



Example FLIM image: Elson et al., Optics and Photonics News, Nov '02

Fluorescence lifetime $\tau = \dfrac{1}{\Gamma + k}$ Γ, radiative decay rate; k, non-radiative decay rate

k=k(pH, Ca^{++}, viscosity, membrane potential)

- **Provides quantitative data regarding cell physiology**

Summary

- We are applying two-photon fluorescence microscopy techniques to the study of bone tissue and bone cell biology
- Ultimate goal is to understand bone loss in microgravity
- FLIM/FCS/protein expression will be used to study effects of fluid flow, acoustic vibrations, electro-mechanical forces on bone cells

Acknowledgements:

Special thanks to the …

NASA John Glenn Biomedical Engineering Consortium

… for financial support!