EIGHTEEN YEARS OF MULTI-PHOTON MICROSCOPY (MPM) AT PROGRAM IN CELLULAR & MOLECULAR MEDICINE (PCMM)

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Introduction: *During the 90s, Cornell Physicist Watt Webb and his colleague introduced 2 and 3 photon excitation that revolutionized laser scanning microscopy. The term "multi-photon" was used to describe excitation of 2 or more photons in general. At that time, the technology was hailed as something that would someday replace confocal microscopy. Now, twenty seven years after the initial paper was published (1), MPM is a mature technology in its own right, an indispensable tool in high resolution in vivo imaging. This past October was the 18th anniversary of the initial MPM installation here at PCMM. We have lived through the challenges and struggles of MPM development from the early years through to its present glory. What works, what doesn't, the pitfalls and the hypes are summarized below.*

MPM has unique advantages when imaging into highly light scattering materials

- Intrinsic optical sectioning capability
- Minimal photo-damage (confined to focus volume). (Fig 1)
- Non de-scanned detection collects both scattered and un-scattered signals. (Fig 2)
- Imaging of collagen with Second Harmonic Generation (SHG) (Fig 3).
- The only in vivo technology, offering high spatial and temporal resolution, beyond 300 mm deep into opaque tissues.
- Practical isssues - MPM excitation spectra are blue shifted and not the exact half of its single photo counterpart. (SHG is exactly half)

However, there is no indication of MPM to replace Confocal, rather, both complementing, each has its unique role in a modern biomedical research facility

- There is no benefit in imaging thin specimens with multi-photon, except for UV excitation.
- Needs improvement - video rate acquisition remains challenging despite advances in laser and detector technologies.
- Other oncerns - photo damage at the focal region alone could be severe; prolonged continuous image acquisition, in some case, may cause overheating (cooking) of sample; samples containing dark pigments could risk complete destruction.

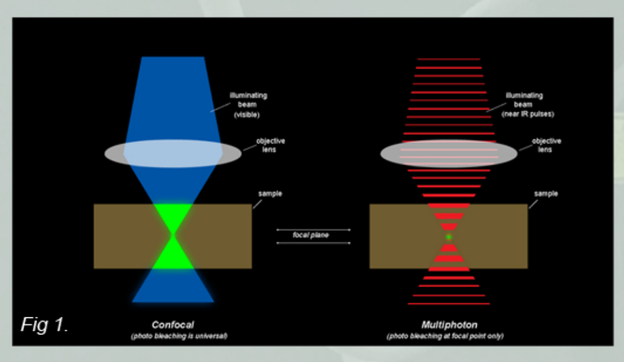


Fig 1.

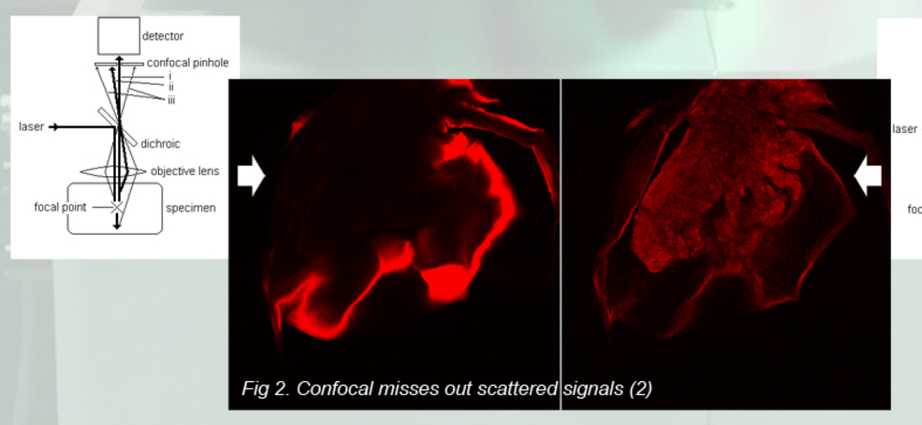


Fig 2. Confocal misses out scattered signals (2)

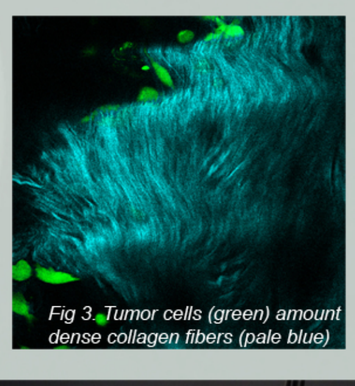
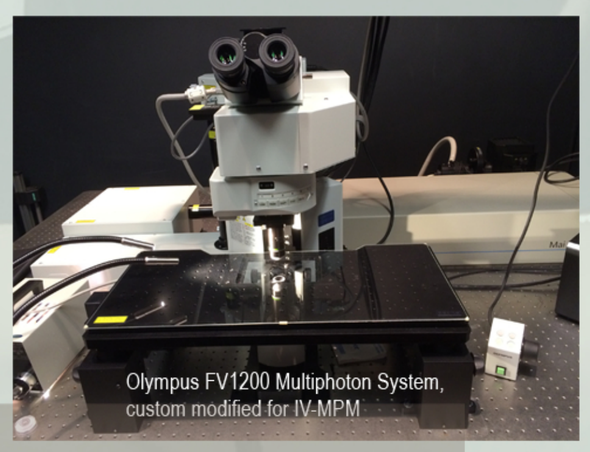


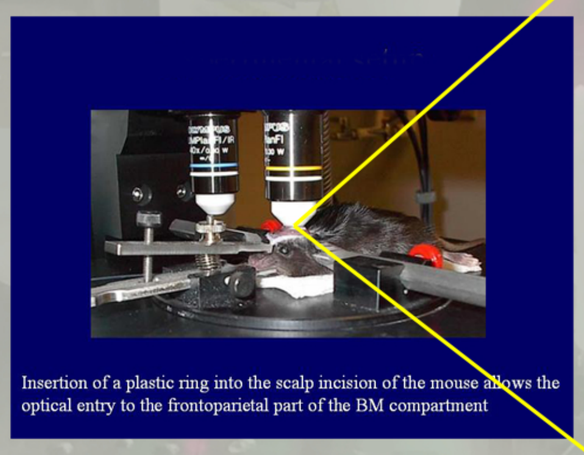
Fig 3. Tumor cells (green) amount dense collagen fibers (pale blue)

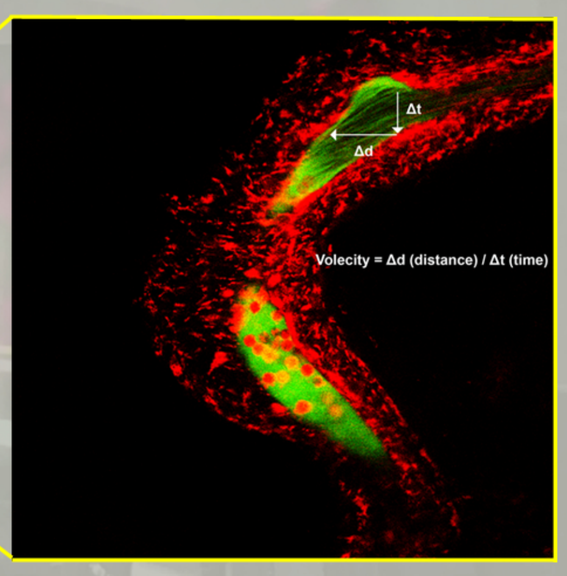
MPM developmen at PCMM: In the year 2000, PCMM (then Center for Blood Research) established an Optical Microcopy Core, equipped with Confocal and MPM systems, serving the academic and research Community in Boston. It was the very first shared microscopy resource in the Longwood Medical Area. While confocal was a well-established technology at that time, MPM was still emerging. However, within a short two years period, we succeeded in mastering the technology and use it exclusive for intravital imaging (IV). The technique was then evolved into IV-MPM. Although the original instrument used for such development has already been retired, the Core continues to maintain a state-of-the-art IV-MPM system, with expert training and technical support. Our current IV-MPM system is housed in a custom-modified room, complete with water bath, perfusion and anesthesia setup, designed specifically for small animal imaging. It is located in Warren Alpert Building (WAB), at the "Quad", and remains the only facility of its kind in the Longwood Community.


Olympus FV1200 Multiphoton System, custom modified for IV-MPM

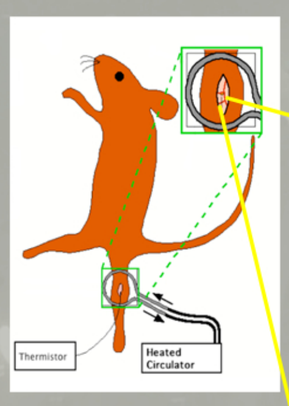
Project examples:

Immune cells trafficking in the skull bone marrow (BM)

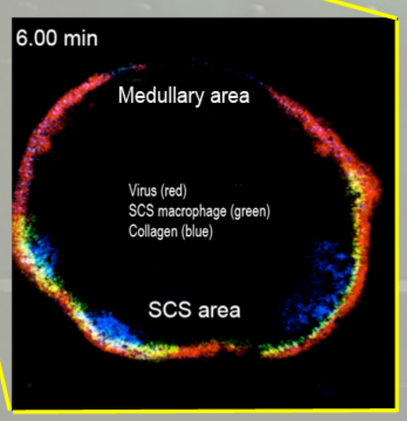

Insertion of a plastic ring into the scalp incision of the mouse allows the optical entry to the frontoparietal part of the BM compartment



Bone marrow cavity in the skull of a live mouse: The blood plasma is labeled with FITC-dextran (green). Note the rapid blood flow in the vessel center in the upper portion of the vessel. Rhodamine 6G (red) stains nuclei and mitochondria in the hematopoietic cells that adhere within the vessel (the round spheres inside the green dextran-filled vessel) and the sur-rounding tissue. The cavity is enclosed in solid bone, which is not penetrated by rhodamine 6G and, hence, appears black.

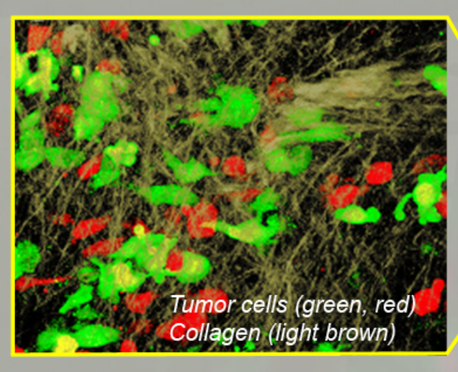


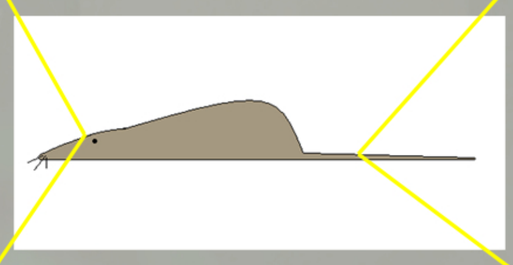
Uptake of influenza virus by innate cells in popliteal lymph node

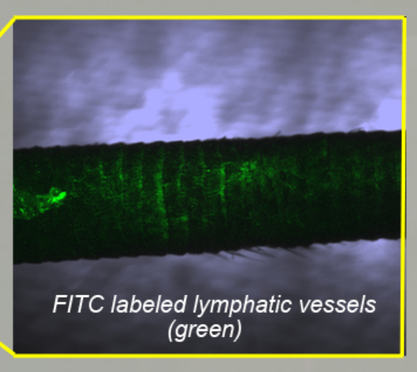


Tumor angiogenesis

Tumor cell migration in mouse dermis (3)

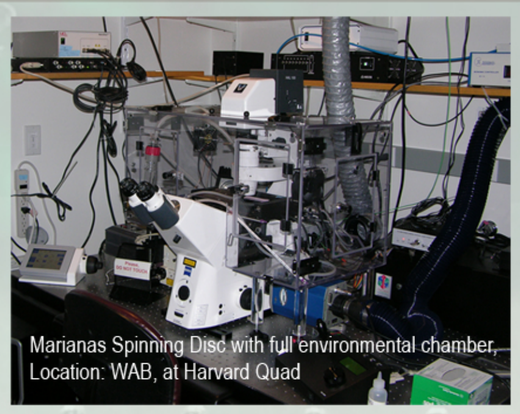






Lymphatic-drainage along mouse tail

Besides IV-MPM microcopy, PCMM's Microscopy Core also provides researchers access to high-end Confocal, Spinning Disk and Transmision Electron Microscopy with expert training and assistance to all imaging procedures as needed by the Investigators.


Marianas Spinning Disc with full environmental chamber, Location: WAB, at Harvard Quad


Olympus FV1000 Confocal System, Location: CLS 3rd/FI


JEOL JEM1400 Tranmission Electron Microscope, Location: CLS 3rd/FI

References

1. Denk, W., Strickler, J.H., & Webb, W.W., Science 248:73-76. 1990.
2. Leung, H., Bulletin Micros. Society Canada 29(1):13-15. 2001
3. Mazo, I.B. *et al*, Immunity, 22(2): 259-70, 2005.

Web page:
<childrenshospital.corefacilities.org/service_center/show_external/3145>