Two-Photon microscopy with High Depth of Field using an Axicon

P. Dufour[1], Y. De Koninck[2] and N. McCarthy[1]

[1]COPL, Université Laval
[2]Centre de recherche Université Laval Robert-Giffard
Québec, Canada

COPL
Centre d'optique, photonique et laser

June 2005

Le Centre de recherche
Université Laval Robert-Giffard

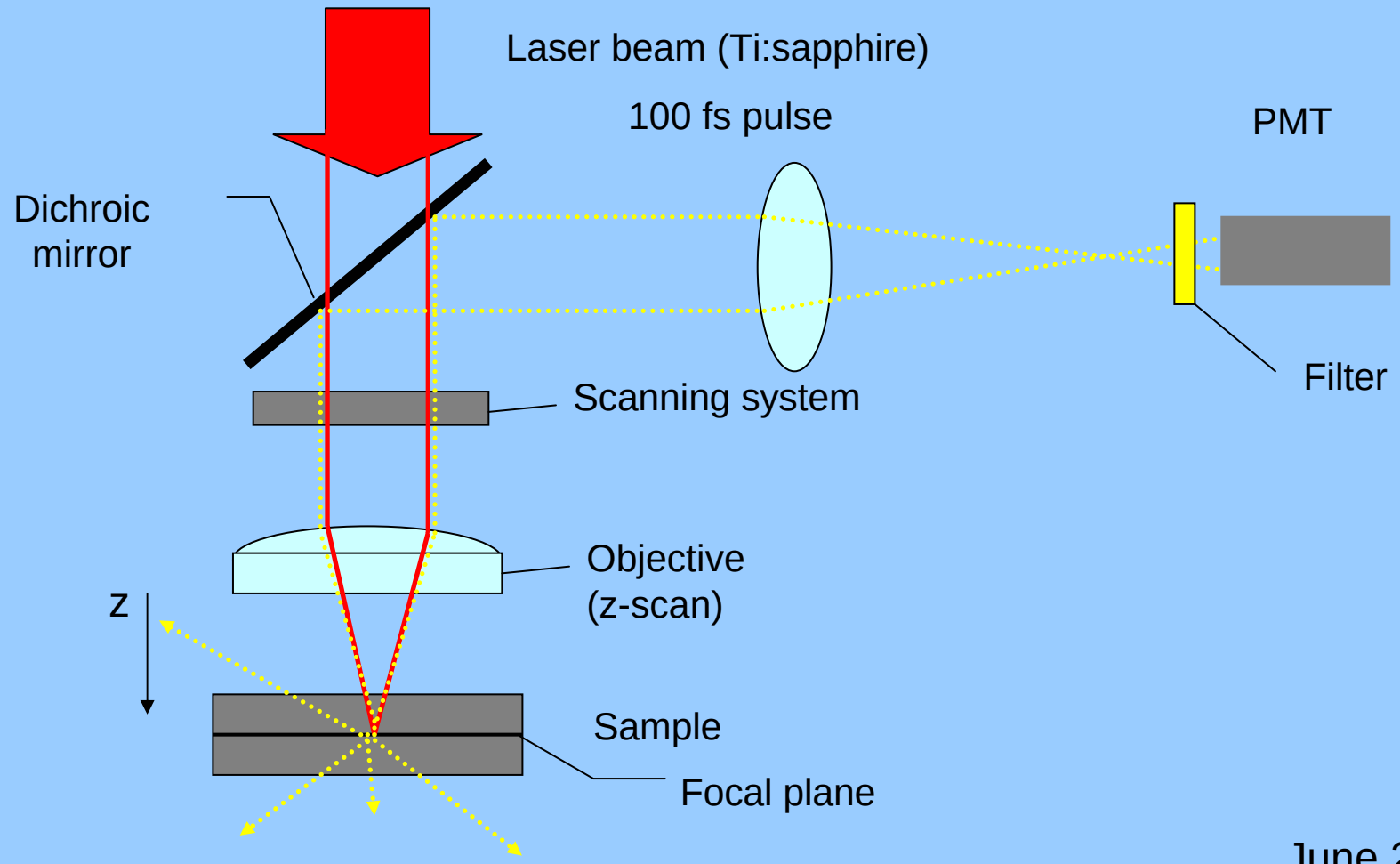
June 2005

Contents

- Conventional two-photon microscopy; advantages and limitations
- What is an axicon?
- Two-Photon microscopy with an axicon
- Comparison of an axicon and a microscope objective
- Examples of thick sample images
- Conclusion

Two-photon microscopy

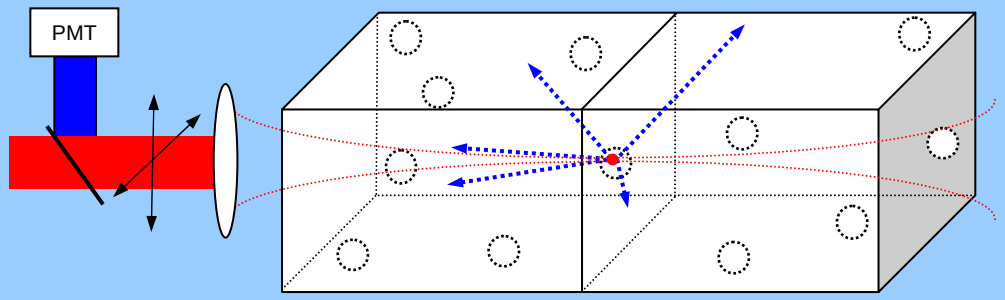
- Scheme



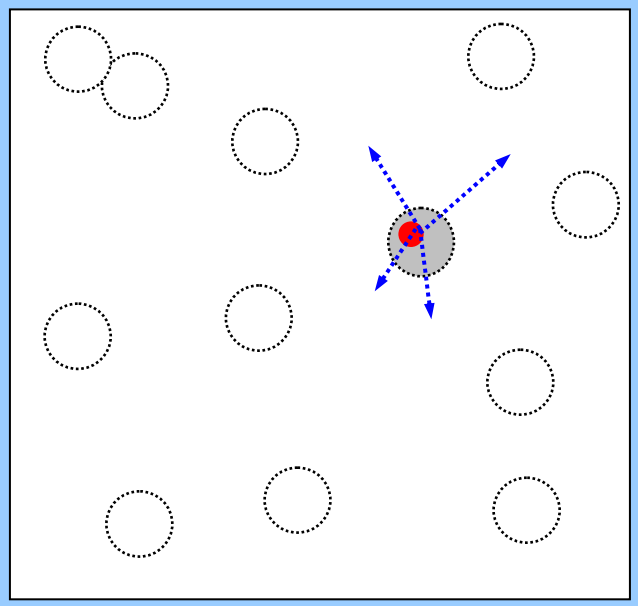
June 2005

Two-photon microscopy

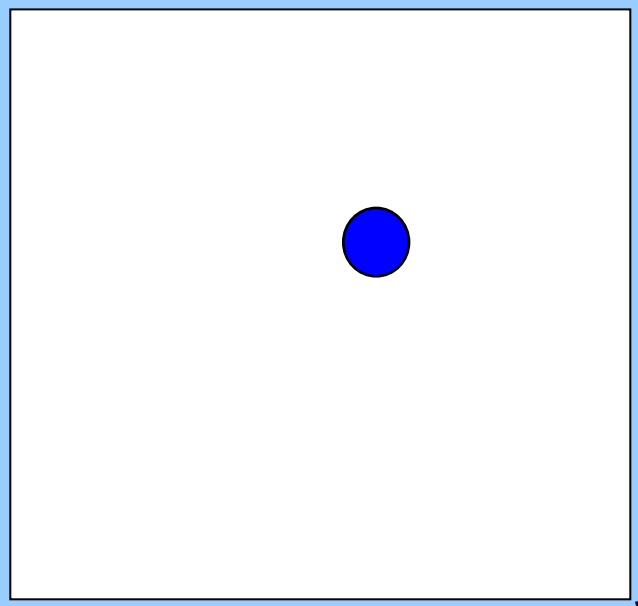
Two-photon microscopy (low depth of field)



Projection image

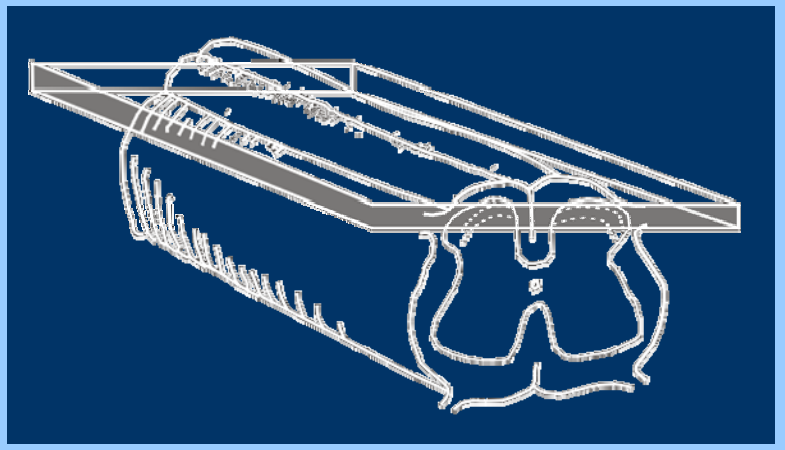


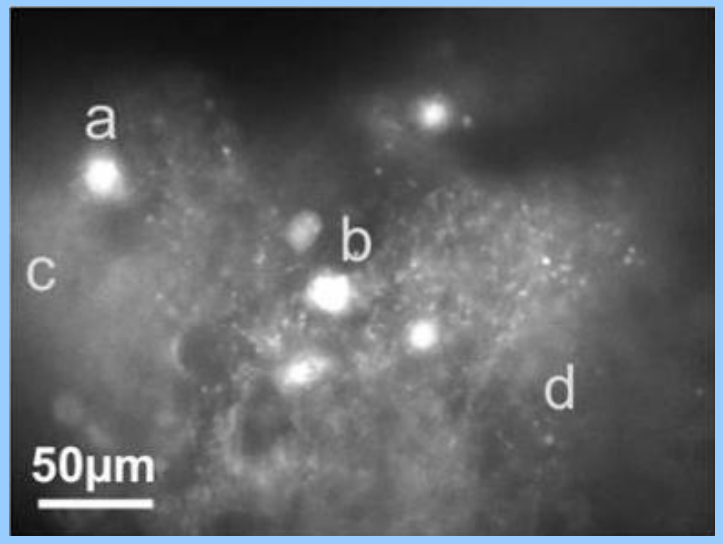
Fluorescence image

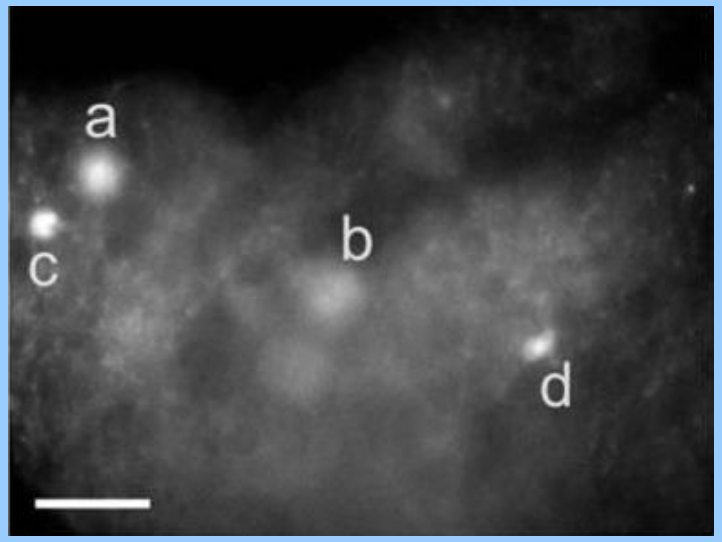


Two-photon microscopy (limitations)

- Challenge for rapid imaging from several cells at different focal point.

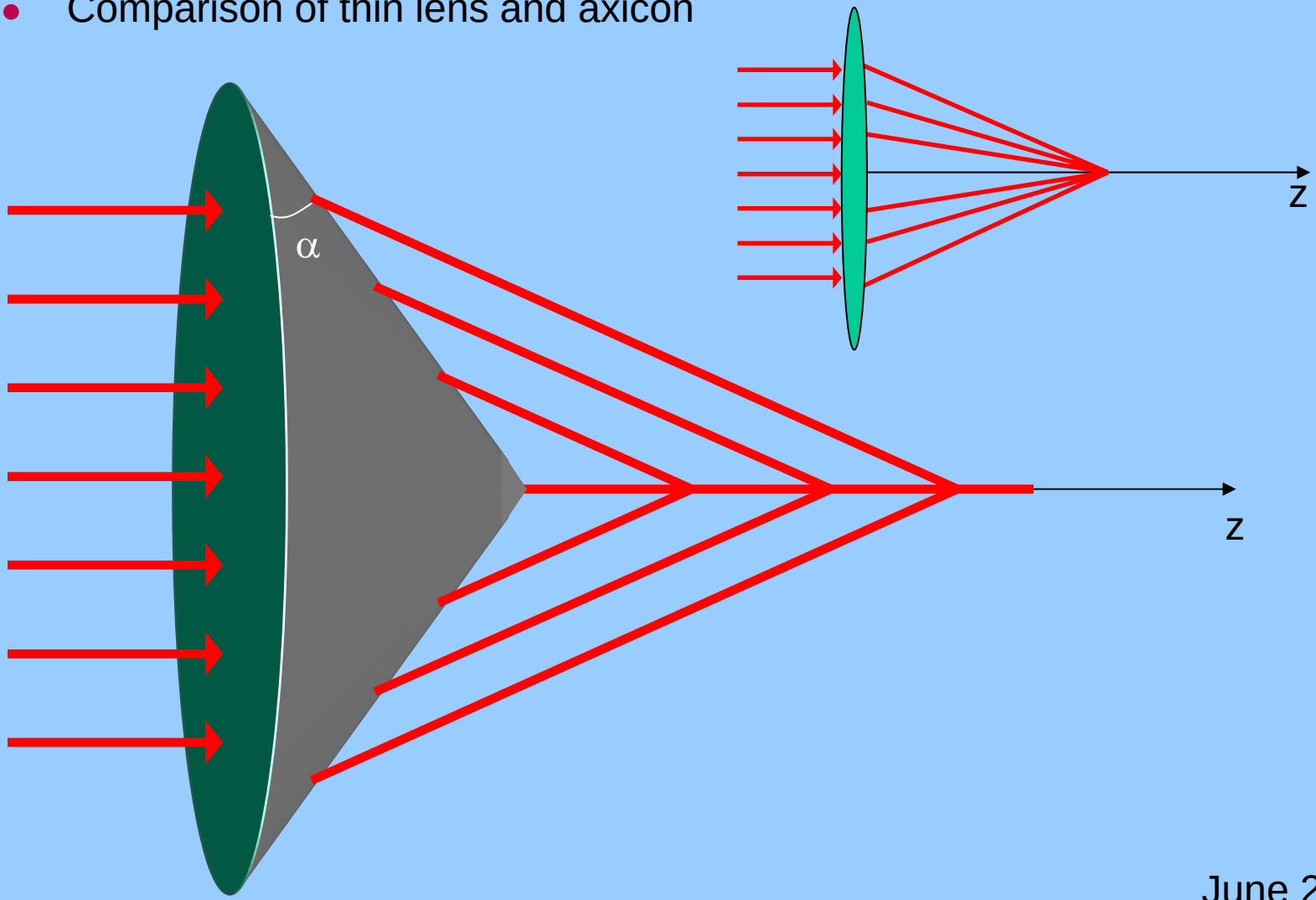






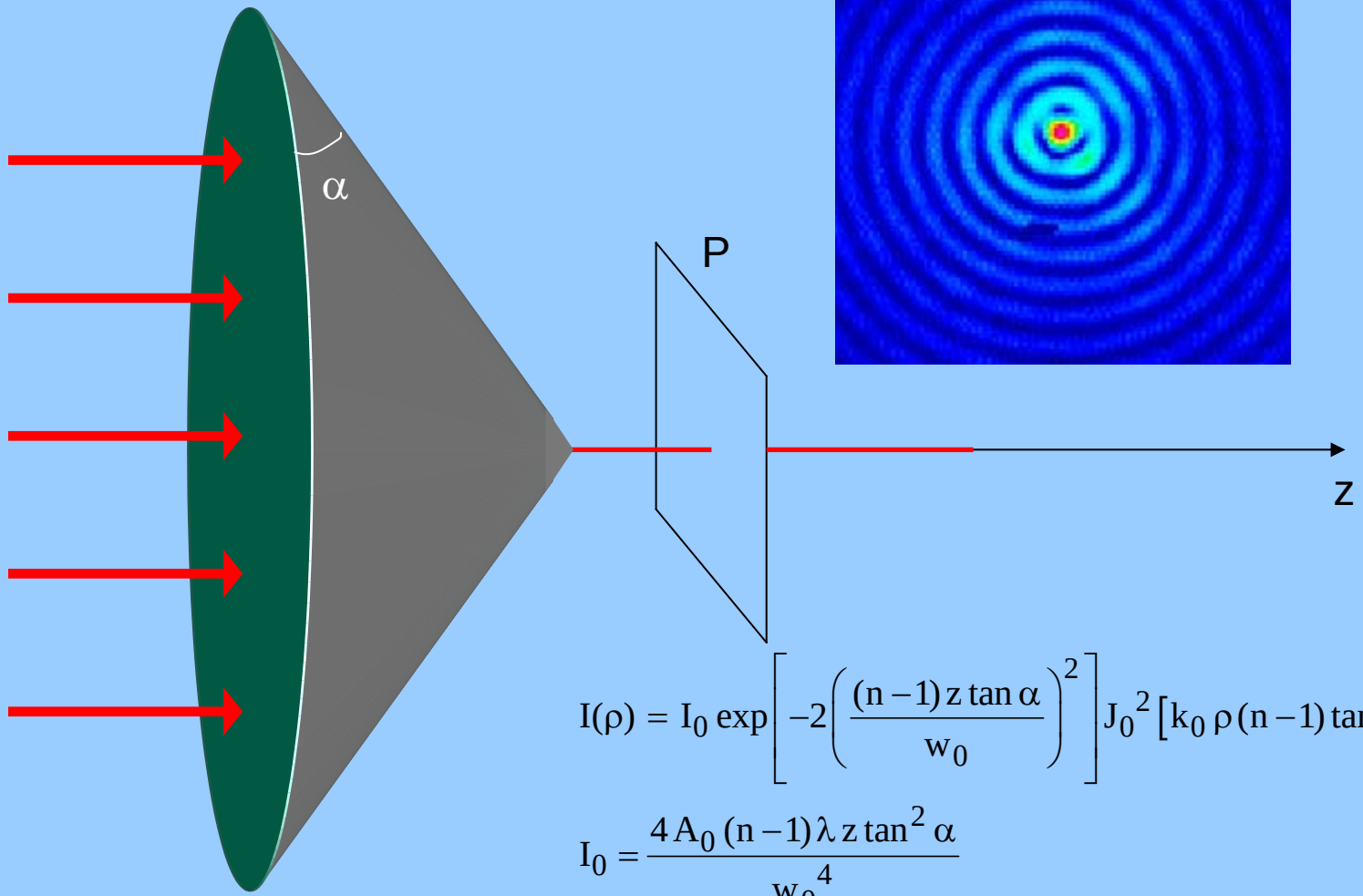
What is an axicon?

- Comparison of thin lens and axicon



What is an axicon? (more details)

- Axicon of diameter D

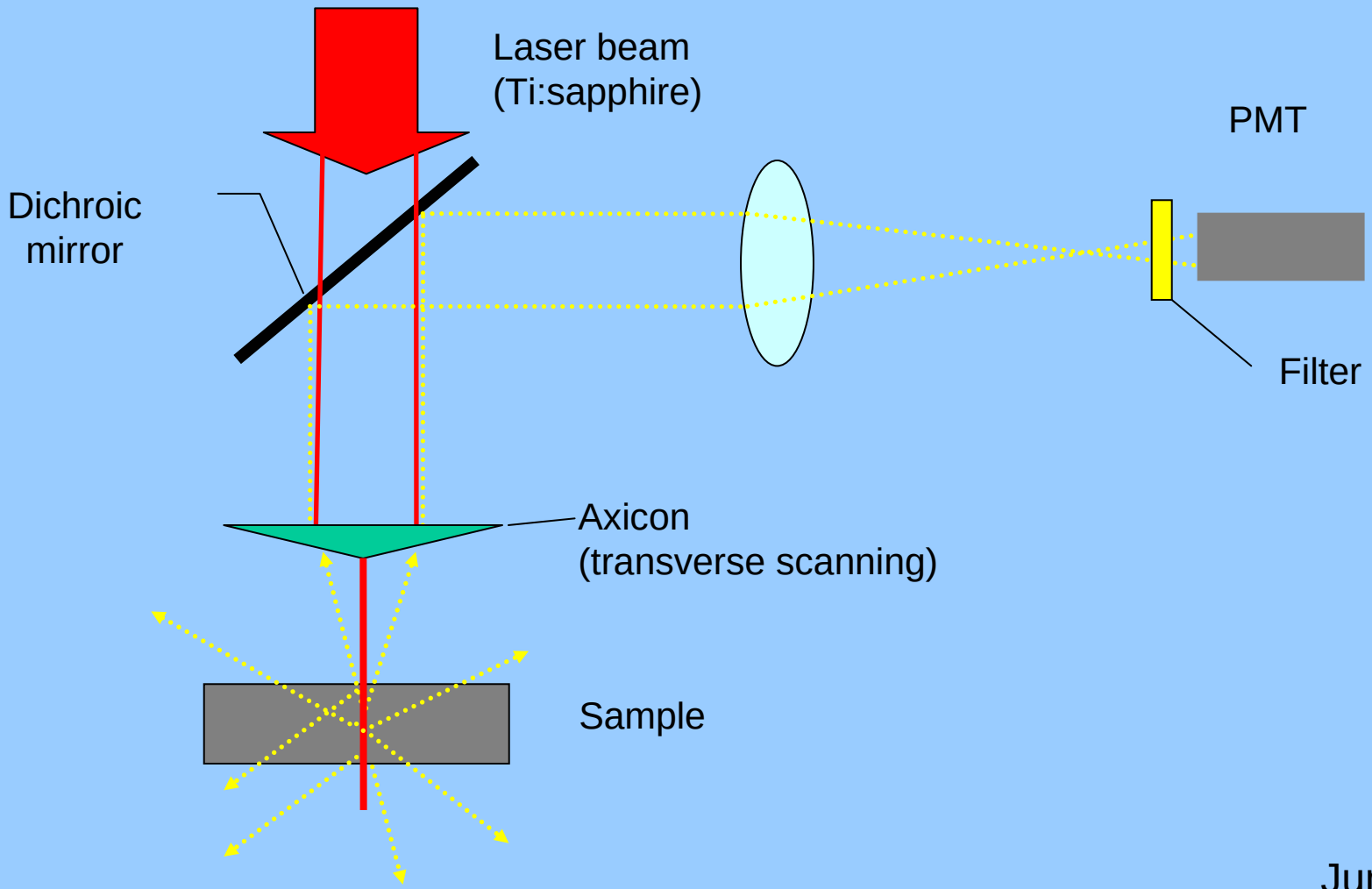


$$I(\rho) = I_0 \exp\left[-2\left(\frac{(n-1)\,z\tan\alpha}{w_0}\right)^2\right] J_0{}^2\left[k_0\,\rho\,(n-1)\tan\alpha\right]$$

$$I_0 = \frac{4A_0\,(n-1)\,\lambda\,z\tan^2\alpha}{w_0{}^4}$$

Two-photon microscopy using an axicon

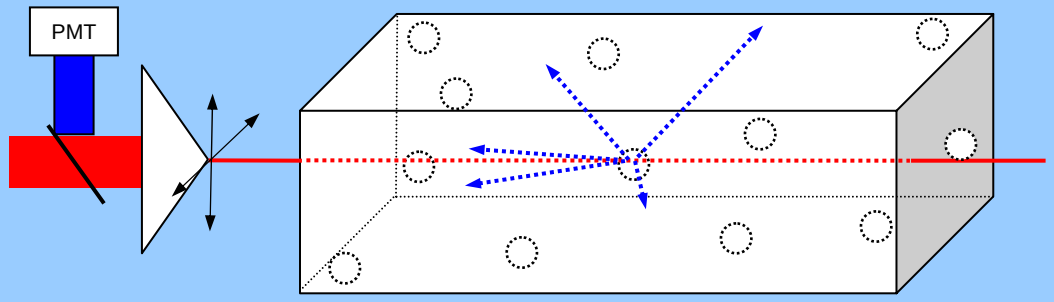
- Scheme



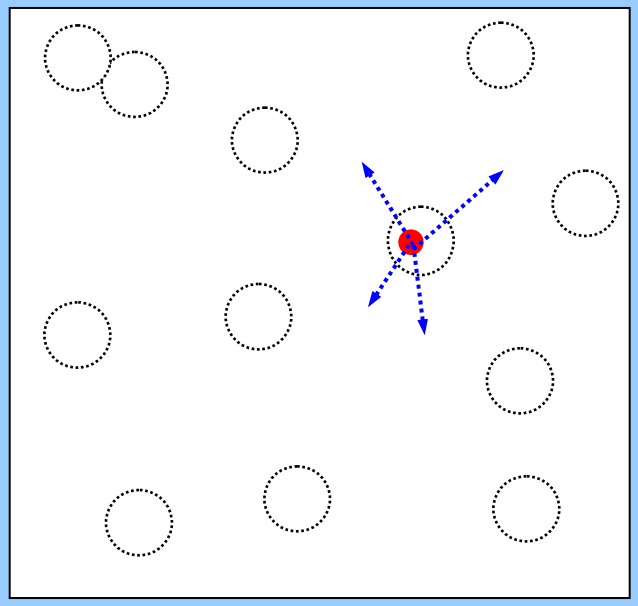
June 2005

Two-photon microscopy using an axicon

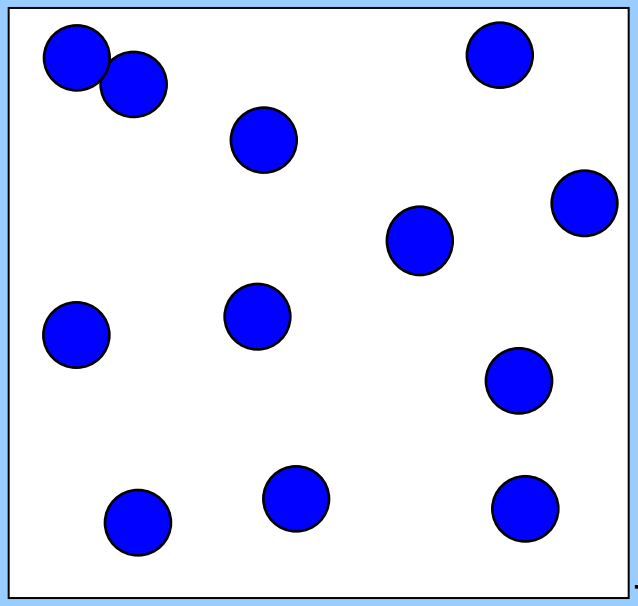
Two-photon microscopy (high depth of field)



Projection image



Fluorescence image

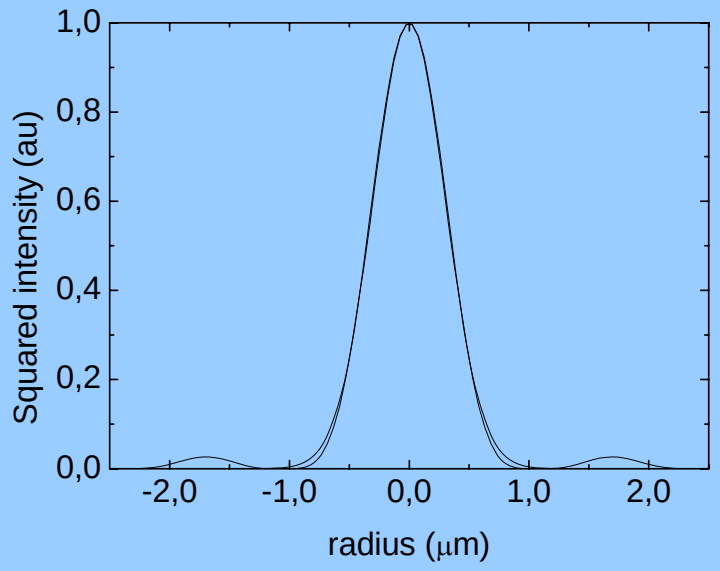


June 2005

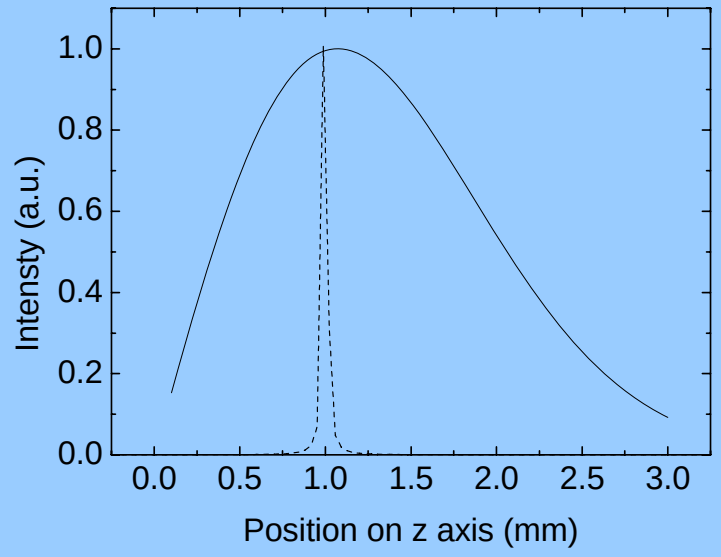
Comparison of an axicon and an objective

Axicon : 30 ° (continuous line)
Objective : NA = 0.5 (dashed line)

Radial resolution



Axial resolution



Axicon : ρ_0 = 1 μm
Objective : ρ_0 = 1 μm
I_{max}/I_{max2} = 2 % (16 % confocal)

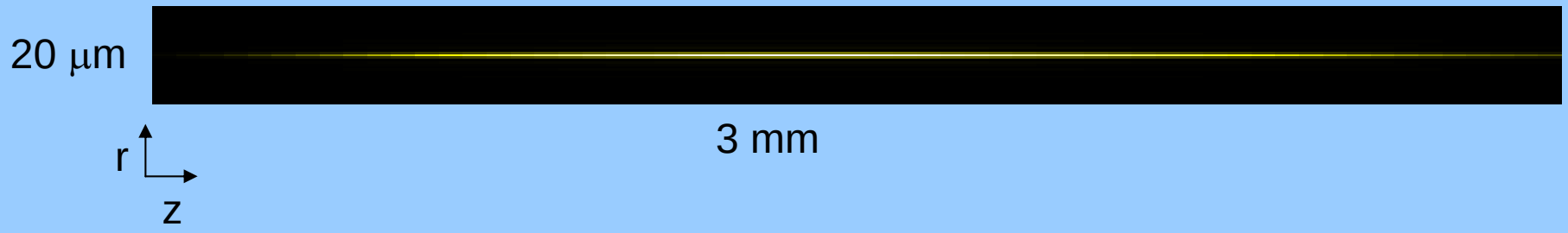
Axicon : L = 3 mm
Objective : L = 7 μm

June 2005

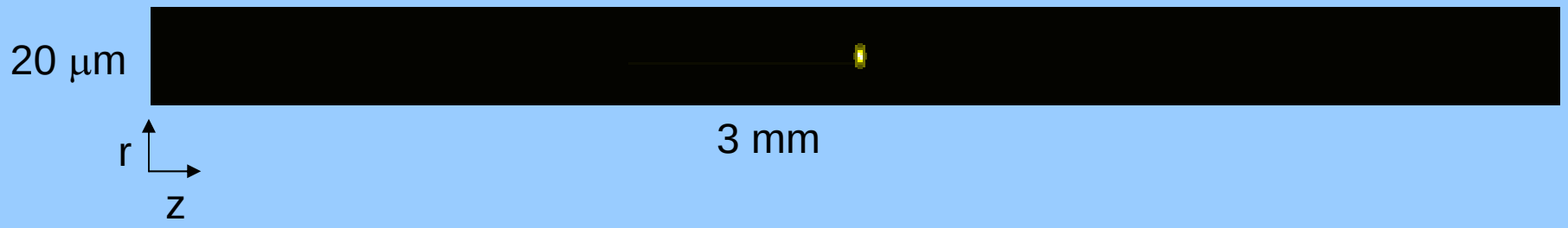
Comparison of an axicon and an objective

Axial resolution

Axicon : 30 °



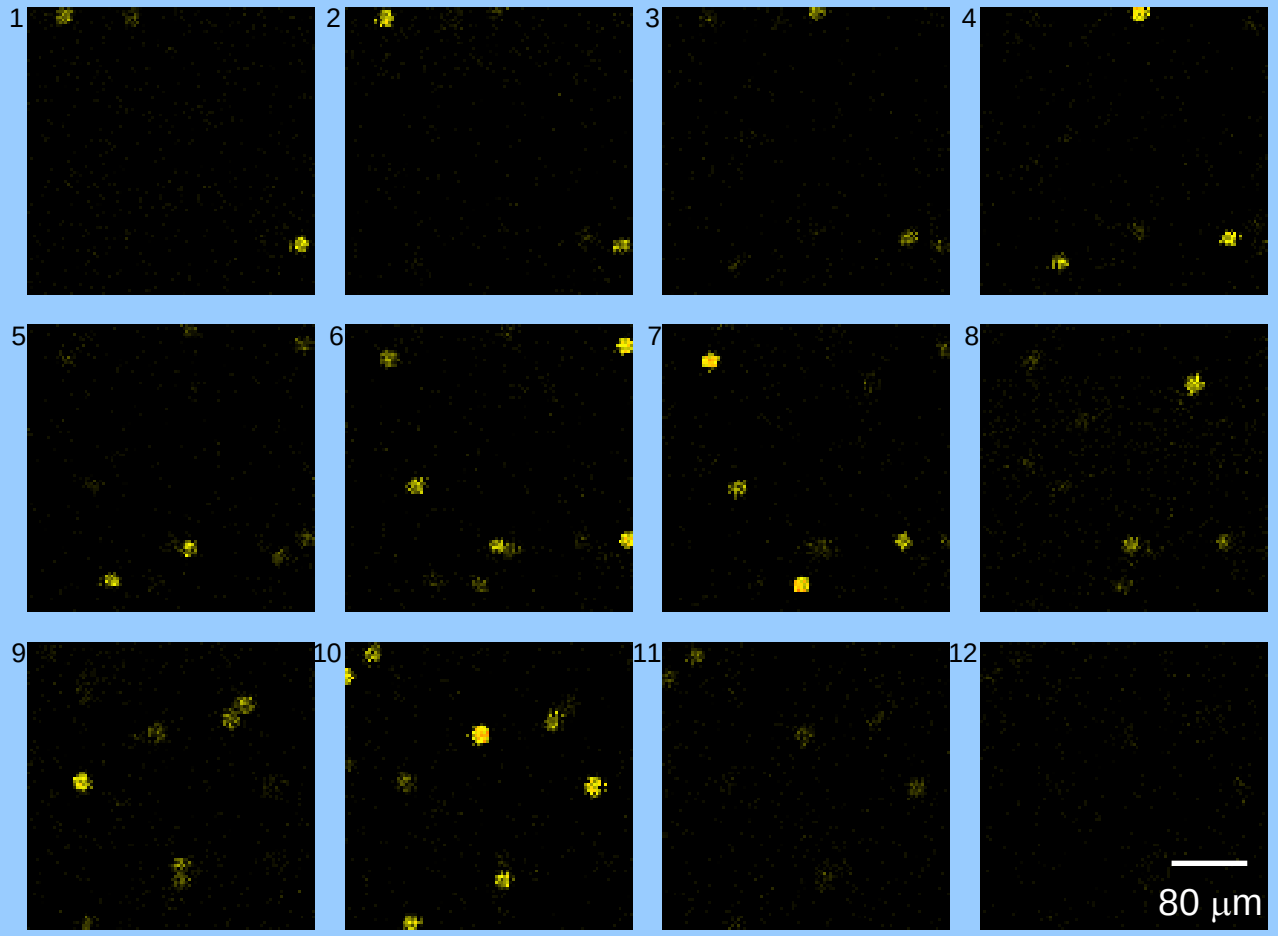
Objective : NA = 0.5



June 2005

Results with a conventional two-photon

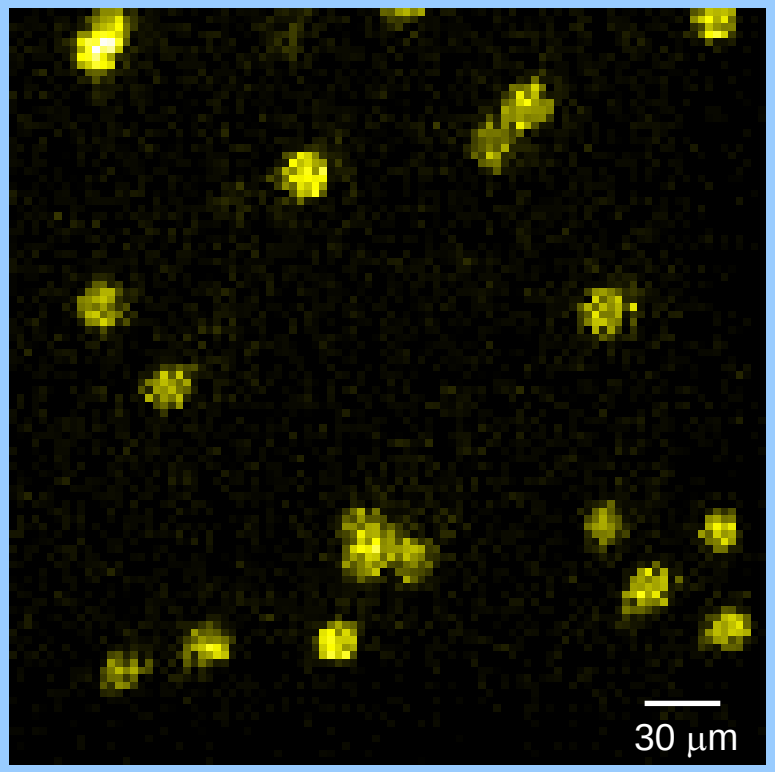
- Twelve scans (300 μm x300 μm) taken with the conventional two-photon microscope (distance between consecutive plane is 50μm)



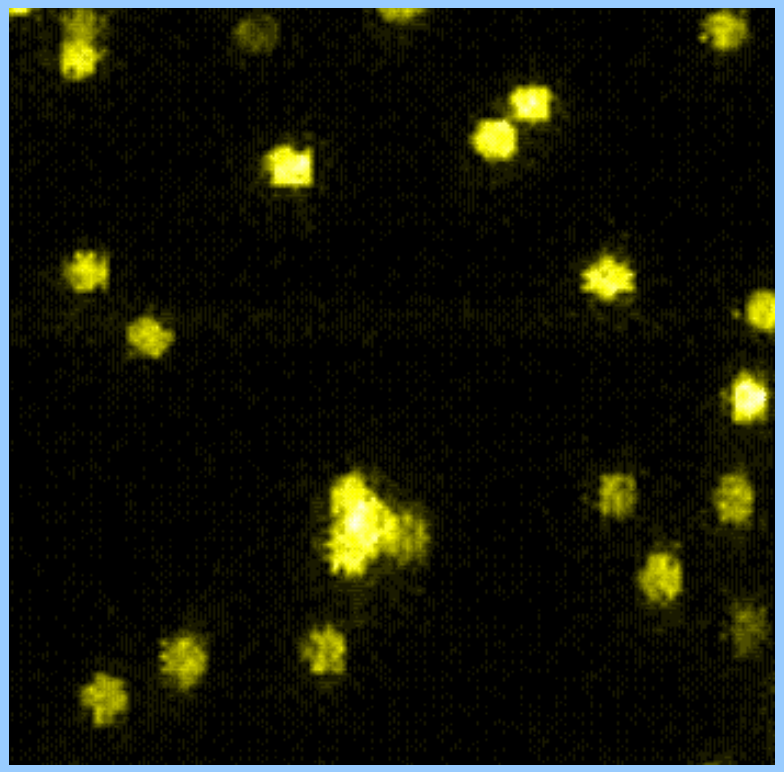
June 2005

Comparison of both systems

With an objective
(sum of 12 scans)



With an axicon
(1 scan)



- Sphere diameter = 15 μm; Sample thickness > 1 mm.

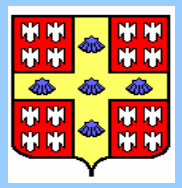
June 2005

Conclusion

- We have demonstrated that a two-photon microscope using an axicon can be used to get fluorescence image of a sample.
- We can increase the depth of field to get a complete image of a thick sample with a single scan.
- The resolution of a 30 ° axicon is as good as a NA = 0.5 objective.
- It is possible to modify the transverse profile of the incident beam to obtain a constant intensity along the z axis in the sample.
- This system can be use to observe dynamic phenomena over short time scales in thick samples.
- Axicon-based microscopy maintains the advantage of two-photon excitation, namely small excitation volume and low dispersion in biological tissue (allowing deep tissue imaging).

Acknowledgments

CIHR NeuroPhysics Training Program



Le Centre de recherche
Université Laval Robert-Giffard

June 2005