MICROSCOPIA OTTICA IN BIOLOGIA CELLULARE [675SM]

MICROSCOPY IN CELL BIOLOGY –

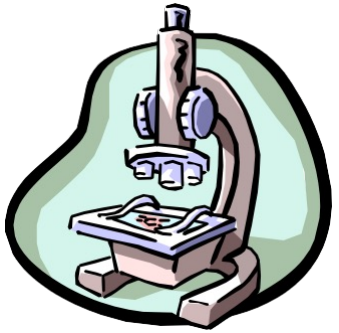
aa 2022/2023, 2nd semester

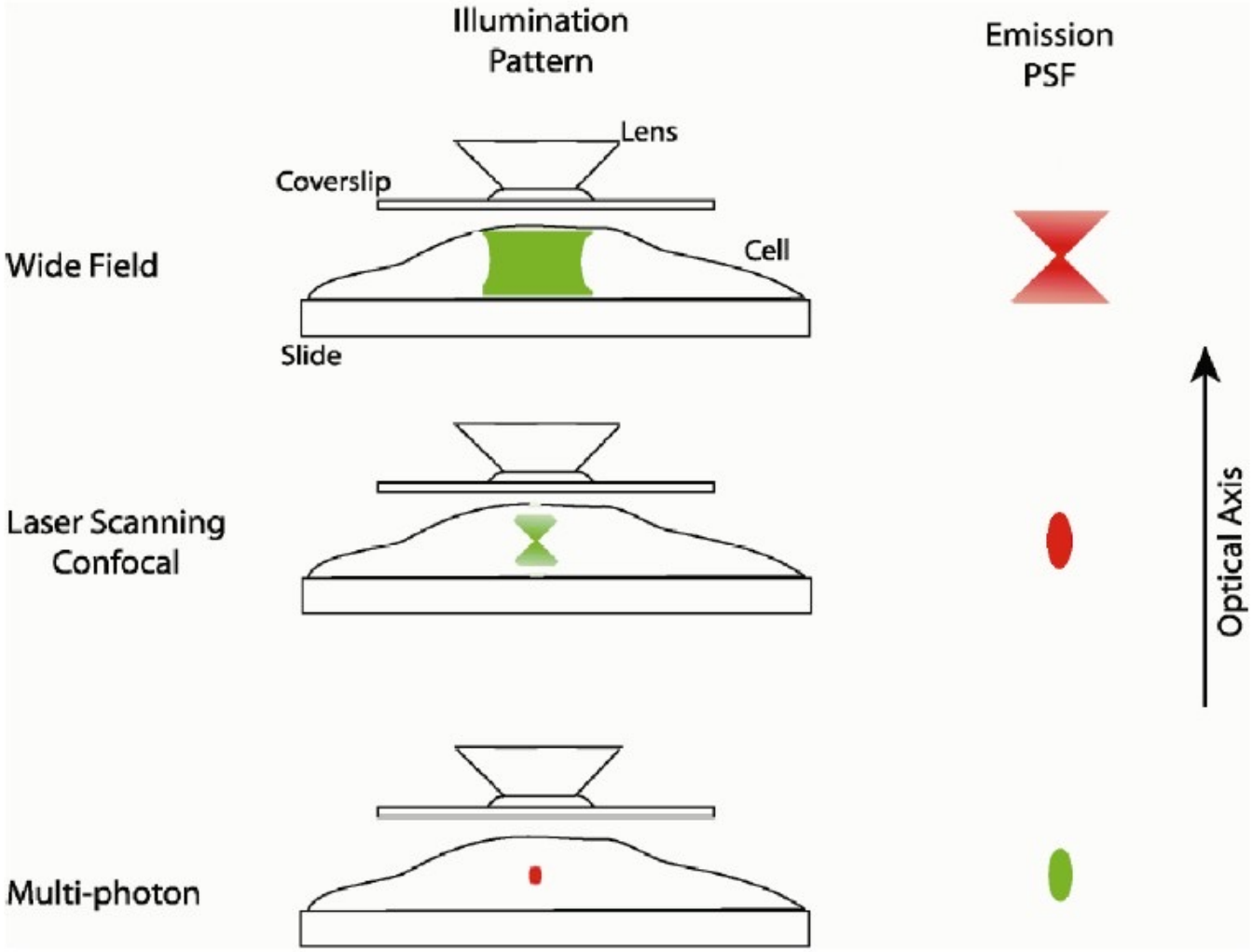
Aula 5A, edificio H2bis 15-18

Agnes Thalhammer
agnes.thalhammer@units.it

cima Centro Interdipartimentale di Microscopia Avanzata
UNIVERSITÀ DEGLI STUDI DI TRIESTE
Dipartimento di Scienze della Vita

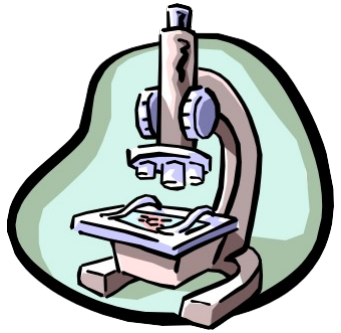
WIDE-FIELD VS. CONFOCAL VS. 2-PHOTON



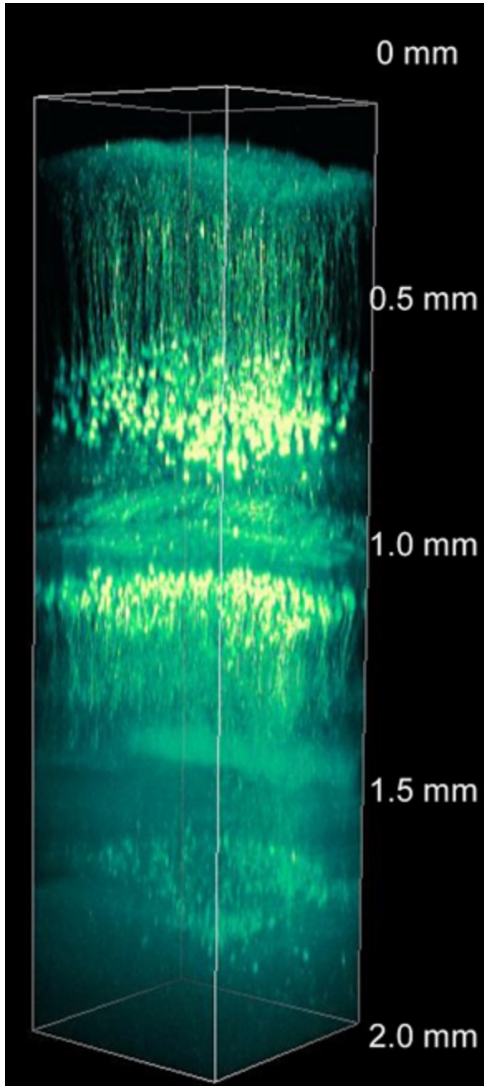


Drawing by P. D. Andrews, I. S. Harper and J. R. Swedlow

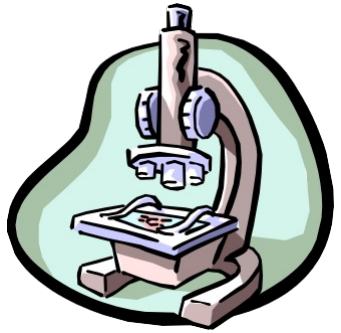
FAR FIELD: TWO-PHOTON



- Non-linear 2-photon excitation and pinhole detection decrease SPF beyond classical limits
- $2^{1/2}$ improvement in resolution
- High penetration depth (IR wavelengths for stimulation)

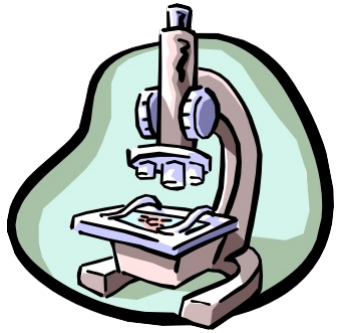


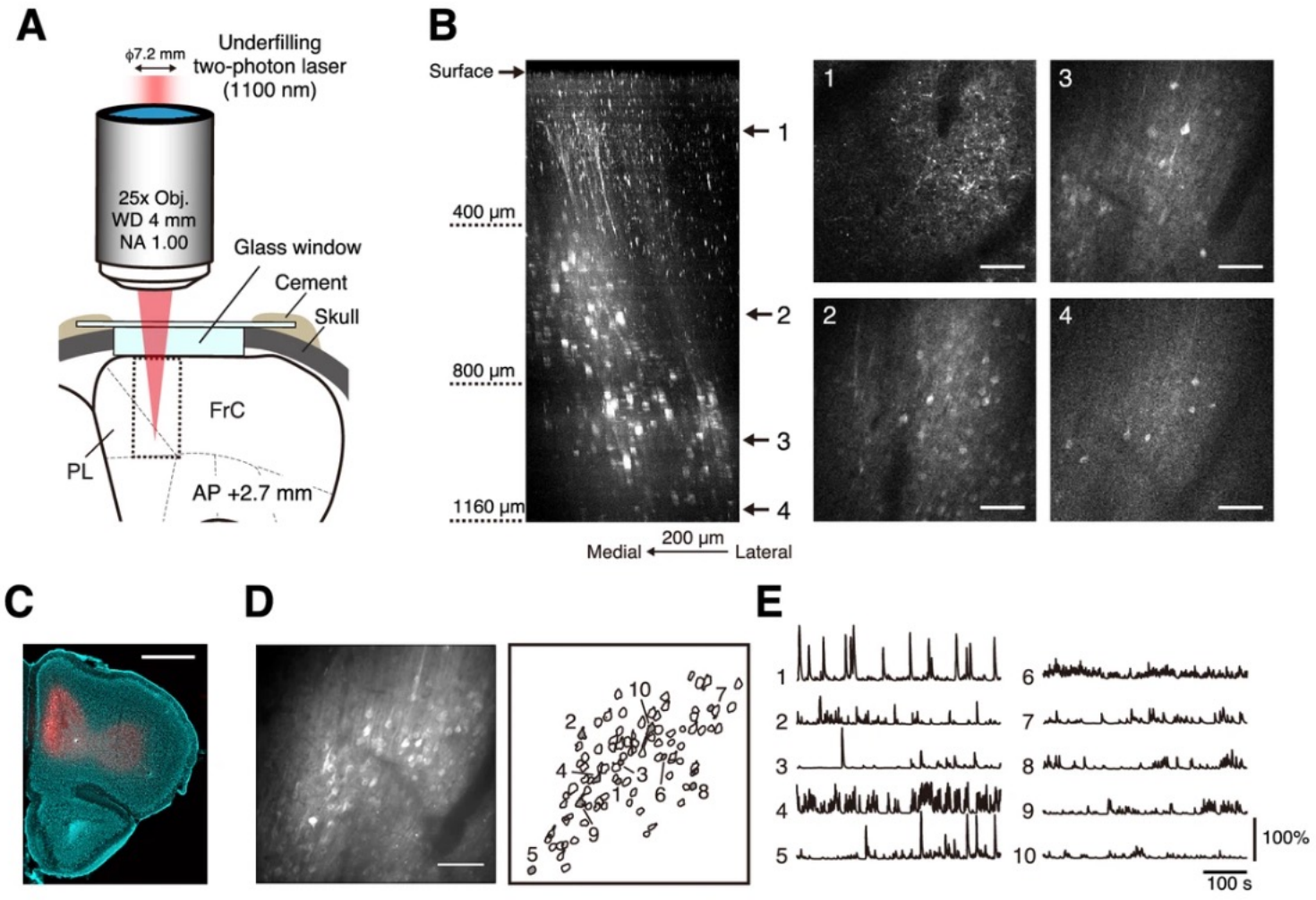
TWO-PHOTON MICROSCOPY – DYE examples



	Alexa Fluor® 350	Alexa Fluor® 488	Alexa Fluor® 546	Alexa Fluor® 555	Alexa Fluor® 568	Alexa Fluor® 594	Alexa Fluor® 647
Target	label/conjugate	label/conjugate	label/conjugate	label/conjugate	label/conjugate	label/conjugate	label/conjugate
Bibliography	Citations	Citations	Citations	Citations	Citations	Citations	Citations
TPE excitation (nm)	720	720, 830	810	810	770	810	800
Laser line (nm)	350/405	488	488	488	561	594	594/633
Standard filter set	DAPI	FITC	TRITC	TRITC	RFP	Texas Red®	Cy®5
Ex/Em (nm)	346/442	490/525	556/573	555/580	578/603	590/617	650/665

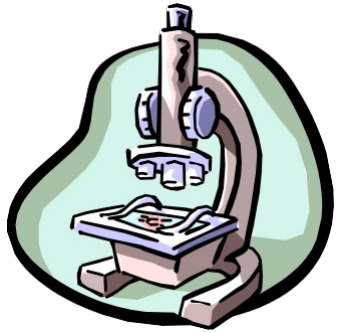
TWO-PHOTON MICROSCOPY – DYE examples





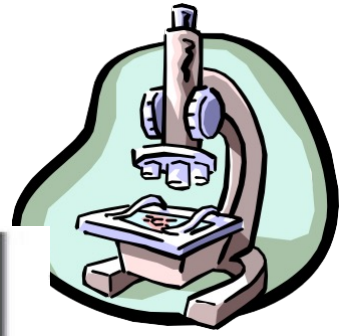
Kondo et al, 2017

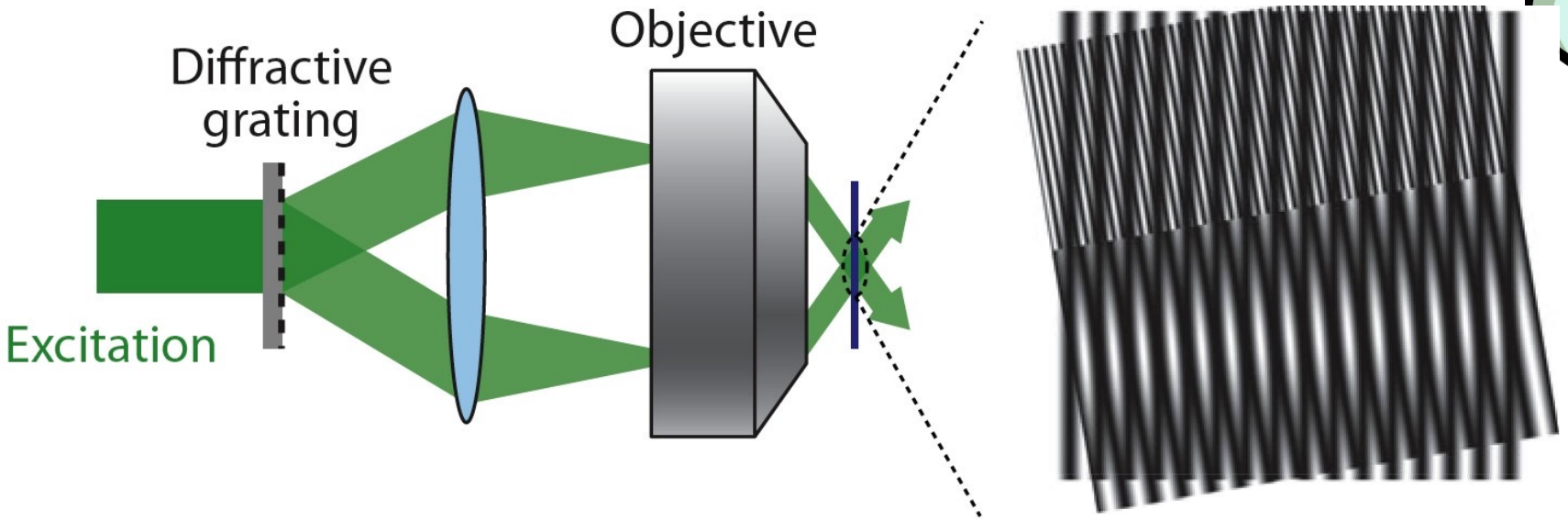
TWO-PHOTON MICROSCOPY – DYE examples



Kondo et al, 2017

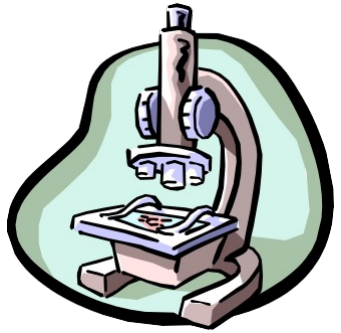
STRUCTURED-ILLUMINATION MICROPSCOPY (SIM)





100 nm resolution
possible

STRUCTURED ILLUMINATION – HISTORY



Optischen Abbildung unter Überschreitung der beugungsbedingten Auflösungsgrenze

von W. LUKOSZ und M. MARCHAND
Physikalisches Institut, Technische Hochschule, Braunschweig, Germany

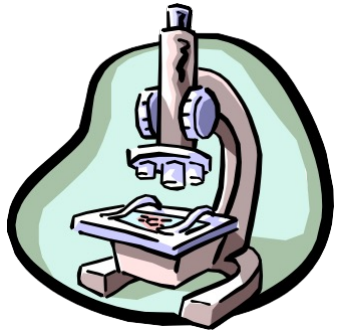
(*Received* 5 *February* 1963, *and in revised form* 1 *July* 1963)

Bekanntlich setzt die Beugung dem mit einem optischen System erreichbaren Auflösungsvermögen (präziser formuliert : der Bandbreite des vom System durchgelassenen Orts-Frequenzbandes) eine prinzipielle Grenze.

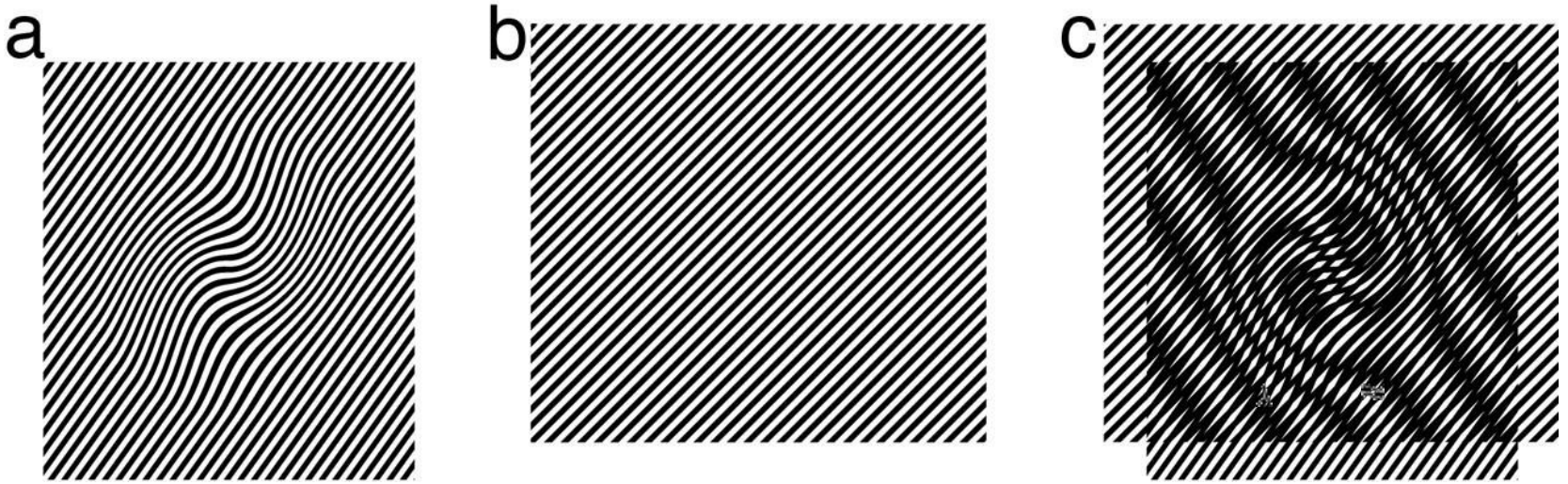
In der vorliegenden Arbeit wird ein neues Verfahren zur optischen Abbildung mit einem über die beugungsbedingten Grenzen hinausgehenden Auflüsungsvermögen erläutert : Das optische System selbst wird unverändert benutzt. In (bzw. in der Nähe) der Objektebene wird aber eine Maske mit örtlich variabler Transmission (z.B. ein Gitter) angebracht oder

- Lukosz and Marchand suggested in 1963 that lateral light patterns could be used to enhance resolution
- Practical implementation was reported by T. Wilson et al. in 1997. (Neil, M. A. A., Wilson, T. & Juskaitis, R. (1997) *Opt. Lett.* **22**, 1905–1907.)

RESOLUTION EXTENSION THROUGH THE MOIRÉ EFFECT



The word moiré is French (from the past participle of the verb moirer, meaning to water).



If an unknown sample structure (*a*) is multiplied by a known regular illumination pattern (*b*), moiré fringes will appear (*c*). The Moiré fringes occur at the spatial difference frequencies between the pattern frequency and each spatial frequency component of the sample structure and can be coarse enough to observe through the microscope even if the original unknown pattern is unresolvable. Otherwise-unobservable sample information can be deduced from the fringes and computationally restored.

Gustafsson, M.G.L. (2005) Proc. Natl. Acad. Sci. USA 102, 13081-13086

Advanced microscopy techniques

SIM-Structural illumination microscopy