Tissue Clearing of Embryo and Hard Tissue with the PEGASOS Method

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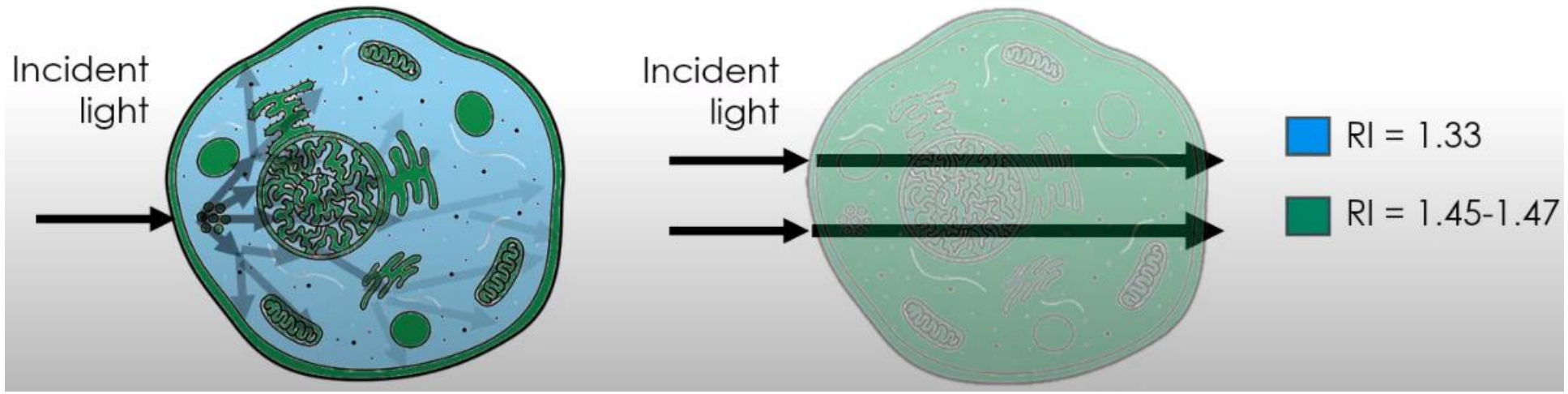
中研院NPAS神經科學研討報告

Concepts of Tissue Clearing



Eliminating RI mismatch &

decolorizing pigment element.

Restrictions of Current Tissue Clearing

1. **Limited type of tissues to be cleared, including hard tissue.**
2. **Loss of endogenous fluorescence.**

Polyethylene Glycol (PEG)-associated Solvent System (PEGASOS) could overcome these problems.

Jing, D., Zhang, S., Luo, W. *et al.* Tissue clearing of both hard and soft tissue organs with the PEGASOS method. *Cell Res* **28,** 803–818 (2018).

PEGASOS Method Procedure

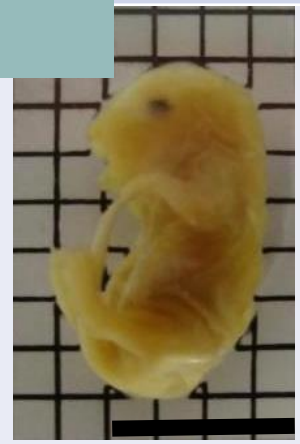
Brief description of procedure

Embryo

Bone

Step	Chemical Agents (v/v)	Purpose
Fixation	4% PFA	Preservation of sample
Decalcification(for hard tissue)	20% EDTA	Chelating agents
Decolorization	25% Quadrol + 5% Ammonium	Decolorizing, Dissolving Heme, Whitening bone samples.
Delipidation	30%, 50%, 70% tB gradient	Better protection of fluorescence.
Dehydration	70% tB-PEG	Dehydrate
Clearing	75% BB-PEG	Clearance of impurities，RI matching.

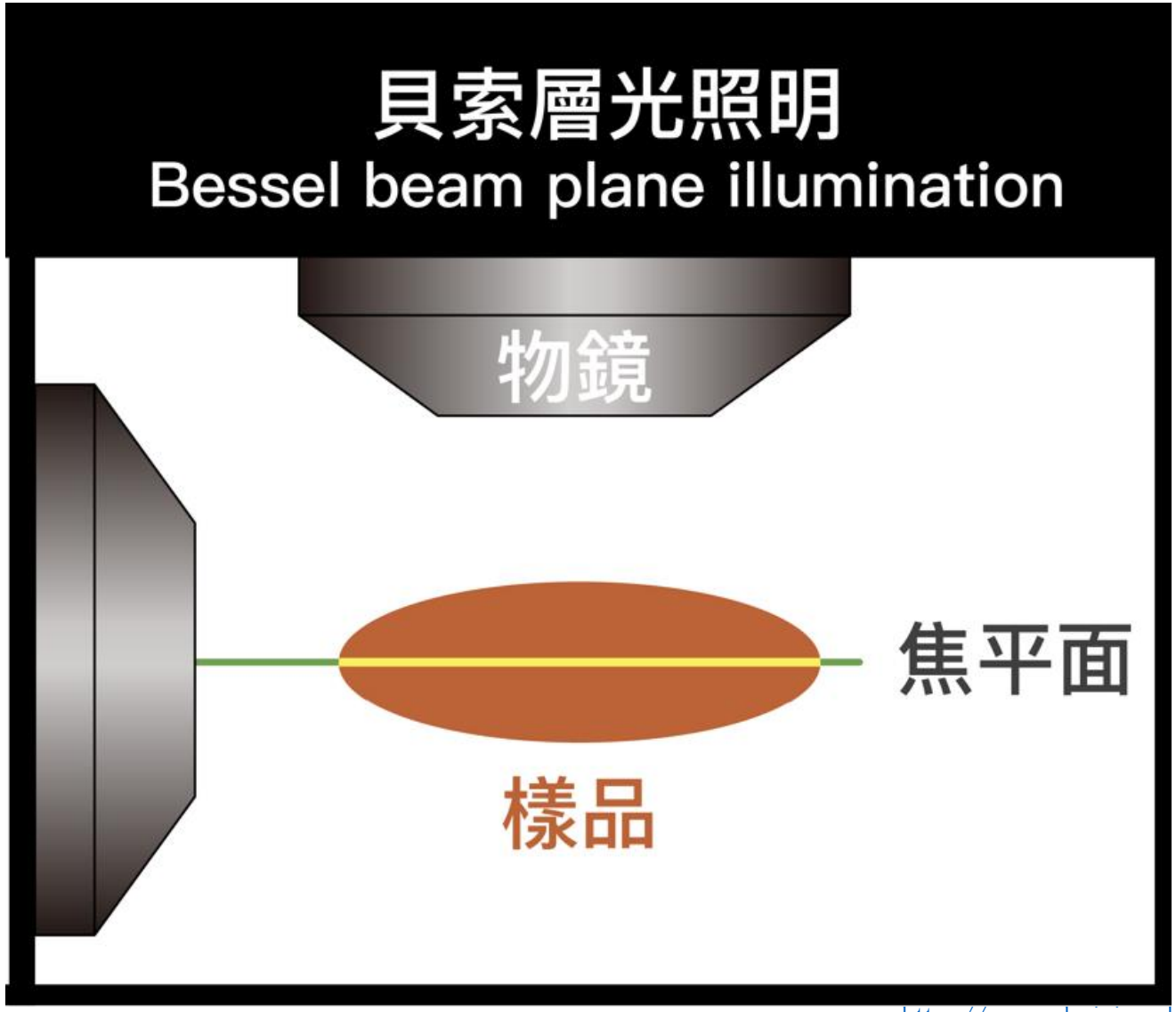
Experiment 1 : Embryo

Sample	Fixation	Decolorization	Delipidation	Dehydration	Clearing
E_1					
E_2					

We chose E_2 for further image processing.

E15_mito-dendra2

Bessel Beam Light Sheet Microscope

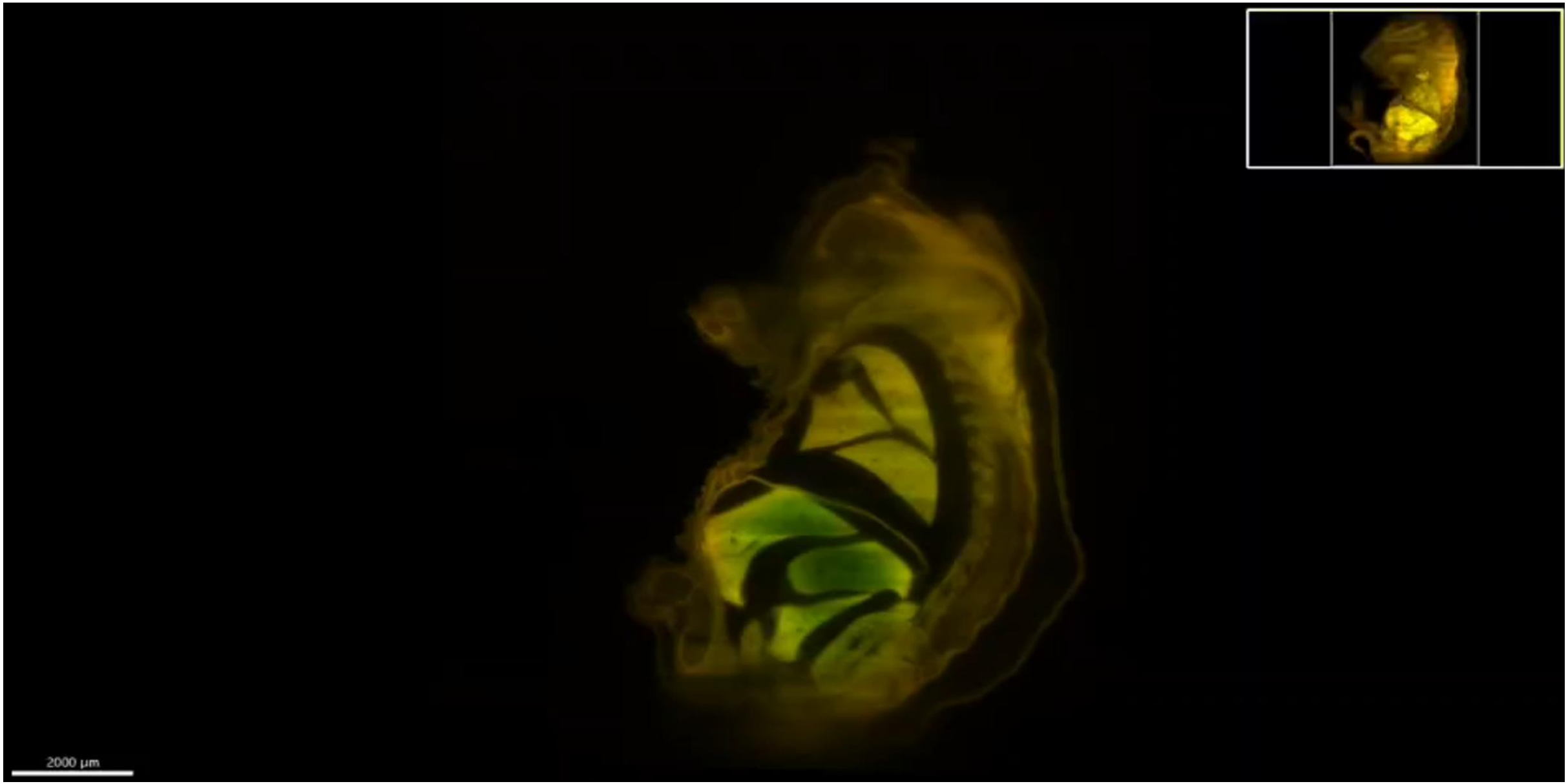


https://research.sinica.edu.tw/chen-bi-chang-lattice-light-sheet-microscopy/

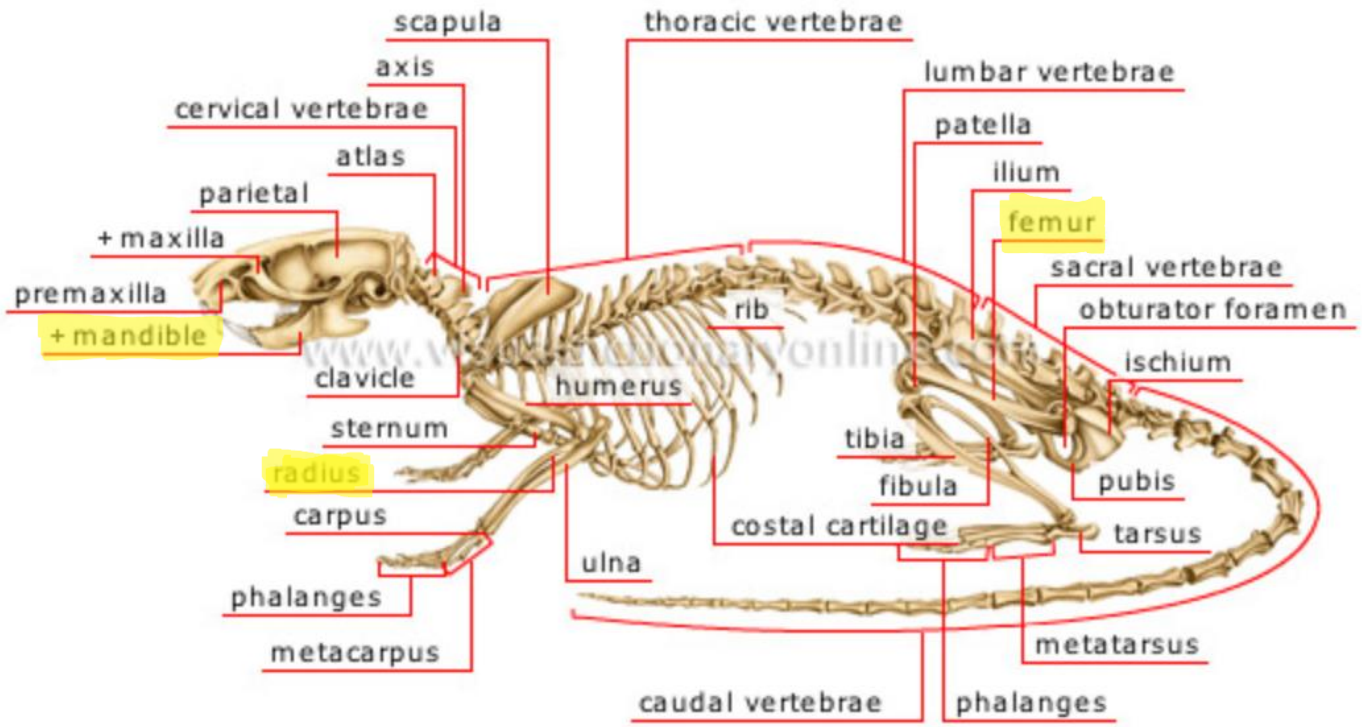
Bessel Beam Light Sheet Microscope cont.

Embryo IMARIS Processing

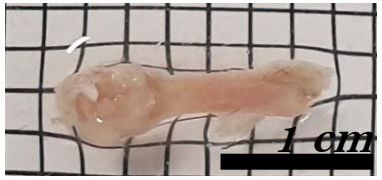
IMARIS



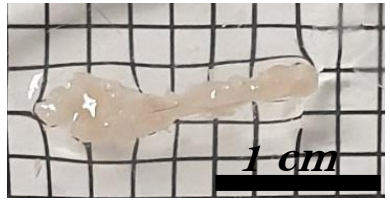
Experiment 2 : Hard Tissue



Mandible



Femur



Radius

Adult *C57BL/6* WT mouse

http://www.visualdictionaryonline.com/animal-kingdom/rodents-lagomorphs/rodent/skeleton-rat.php

Hard Tissue Procedure

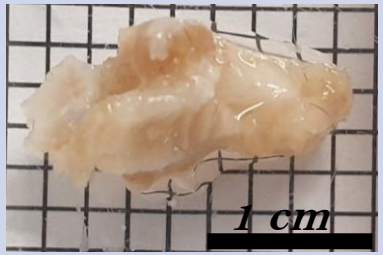
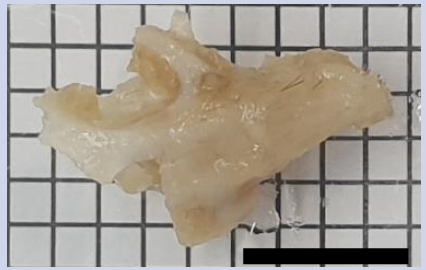
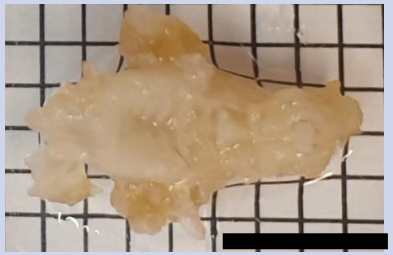
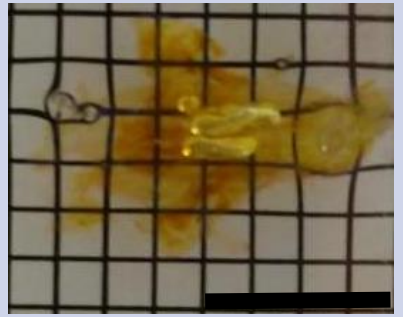
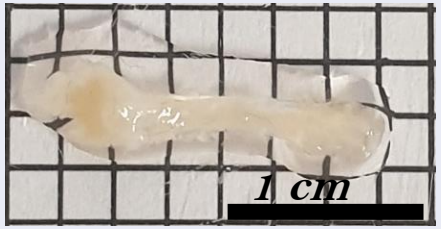
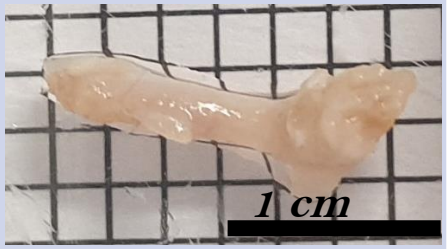
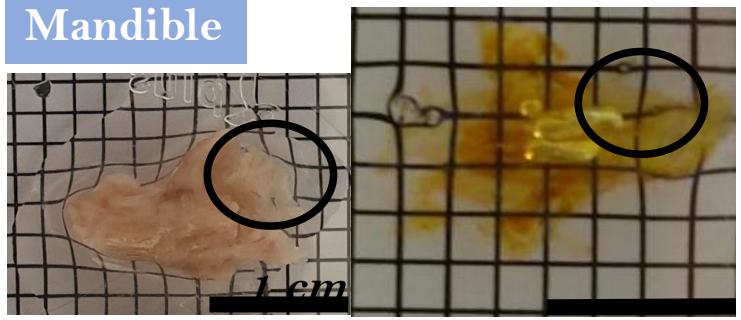
Sample	Decalcification	Decolorization	Delipidation	Dehydration	Clearing
Mandible					
Radius					
Femur					

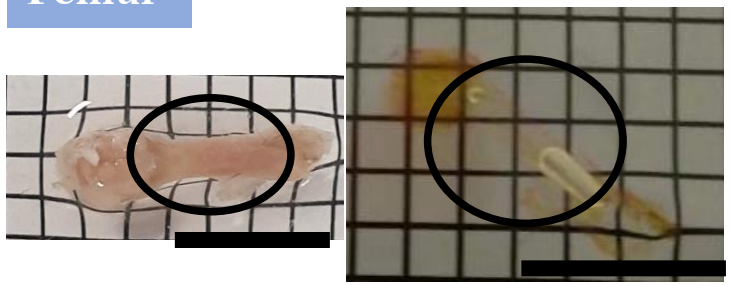
Image of Hard Tissues

Mandible



Radius

Femur



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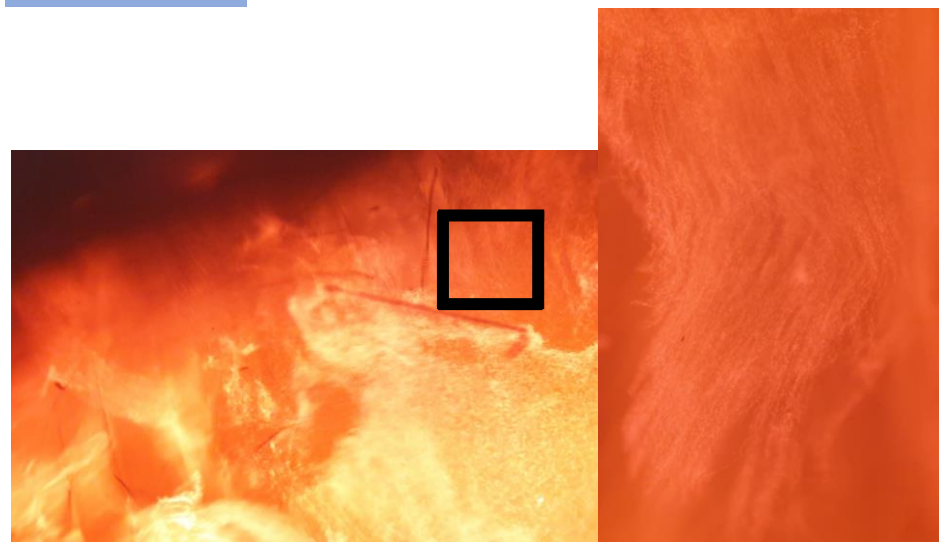
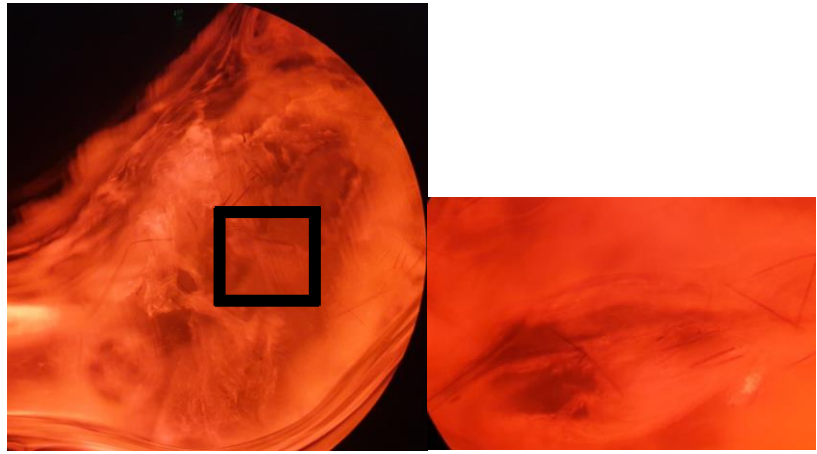


4x/0.13 objective

10x/0.25 obj.

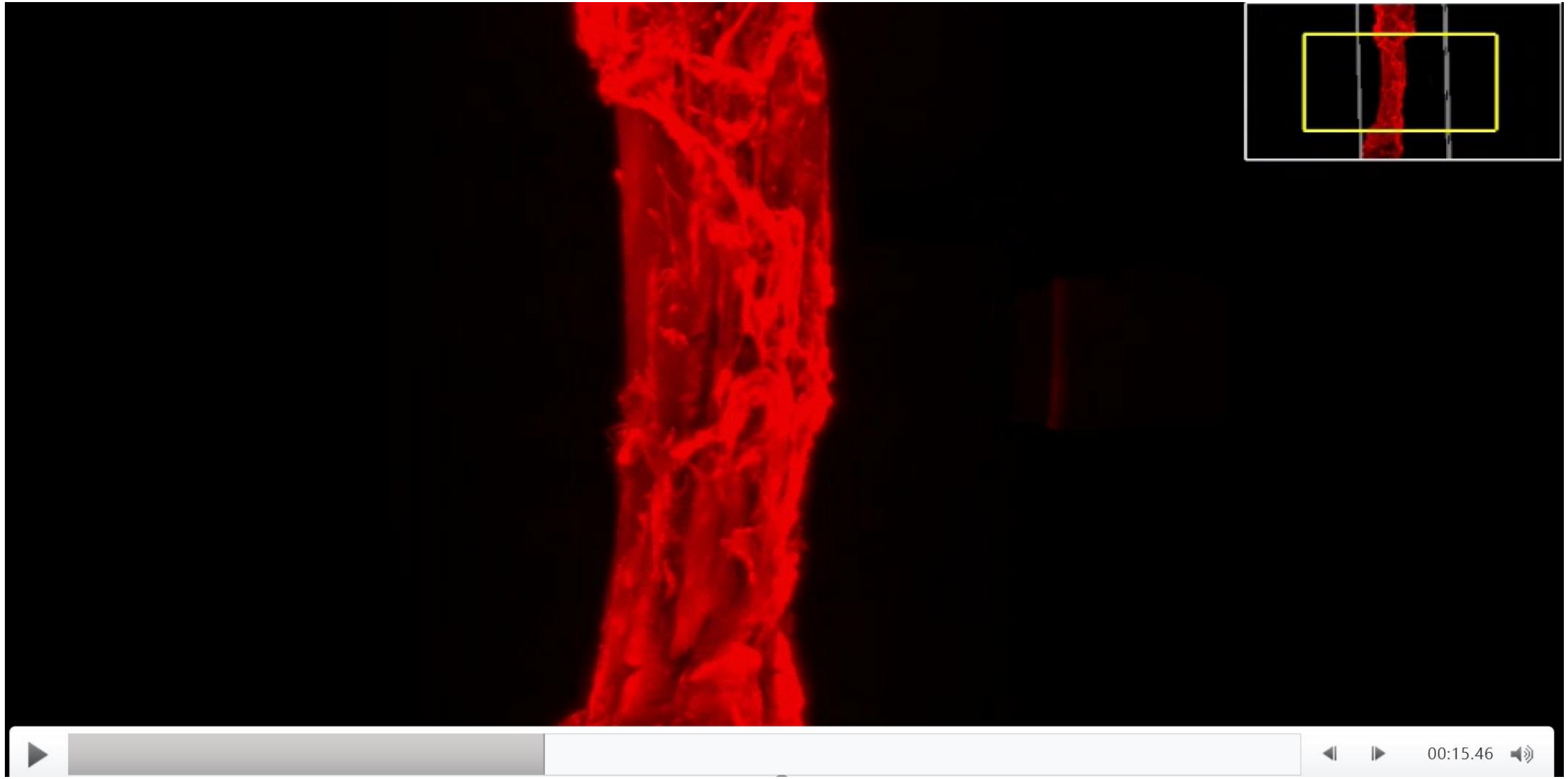
Radius

Femur



Radius IMARIS Processing

IMARIS



Summary

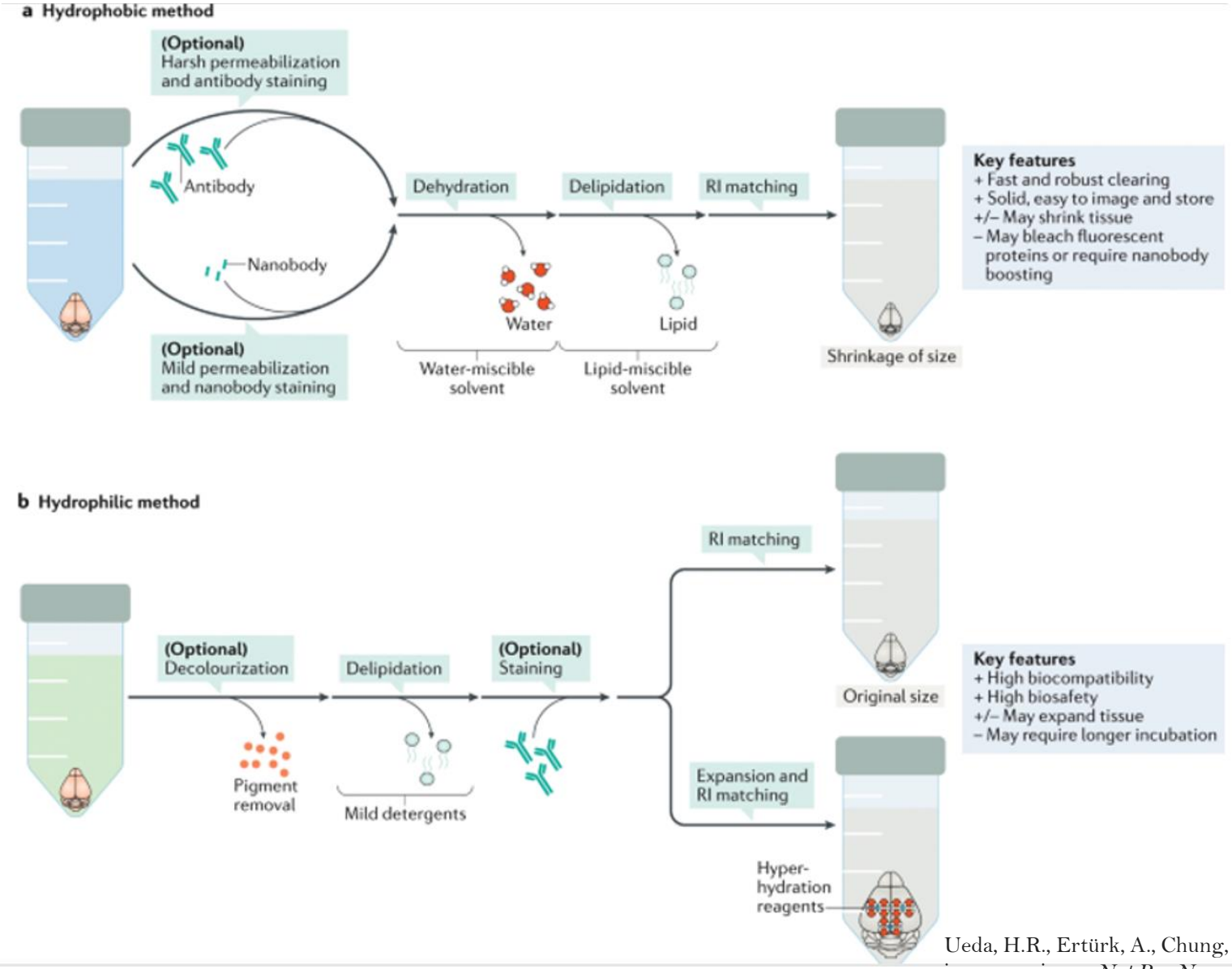
PEGASOS achieved transparency for soft and hard tissues.

PEGASOS combined with IMARIS could achieve single cell resolution without dissection.

Soft tissue shrinkage remains to be the drawback of the PEGASOS method.

Thank you for your listening

Different Clearing Methods



Ueda, H.R., Ertürk, A., Chung, K. *et al.* Tissue clearing and its applications in neuroscience. *Nat Rev Neurosci* **21,** 61–79 (2020).

PEGASOS Passive Immersion Method

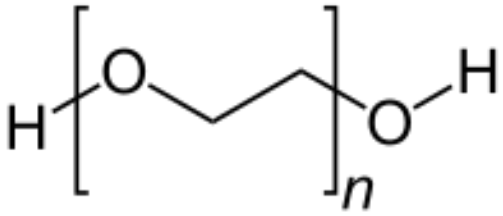
Soft tissue

Hard tissue

Jing, D., Zhang, S., Luo, W. *et al.* Tissue clearing of both hard and soft tissue organs with the PEGASOS method. *Cell Res* **28,** 803–818 (2018).

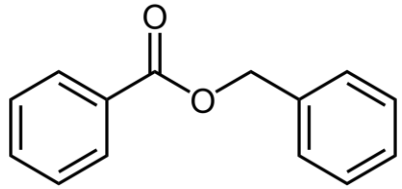
BB-PEG preserves endogenous fluorescence

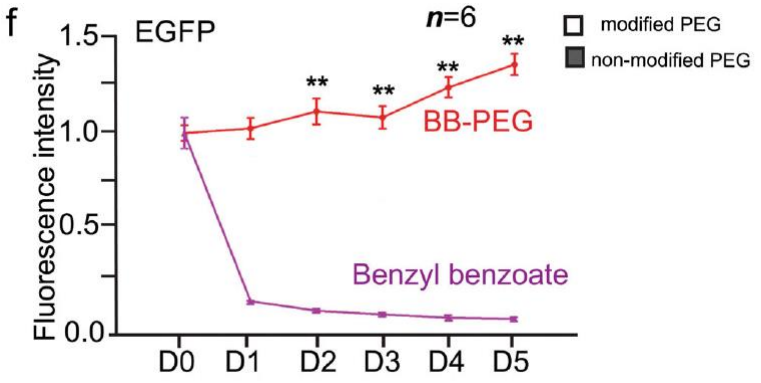
PEG (PEGASOS)

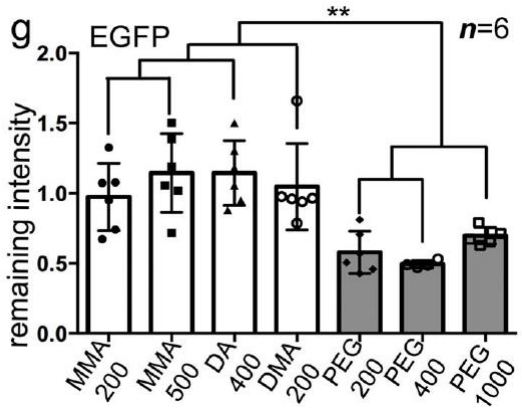


Dibenzyl ether (uDISCO)

Benzyl benzoate



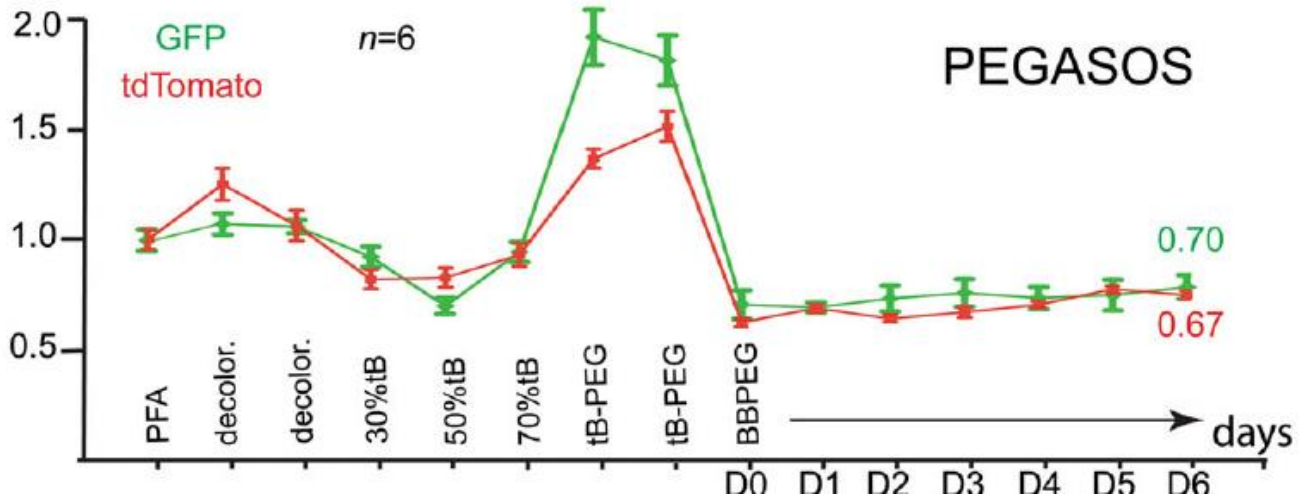


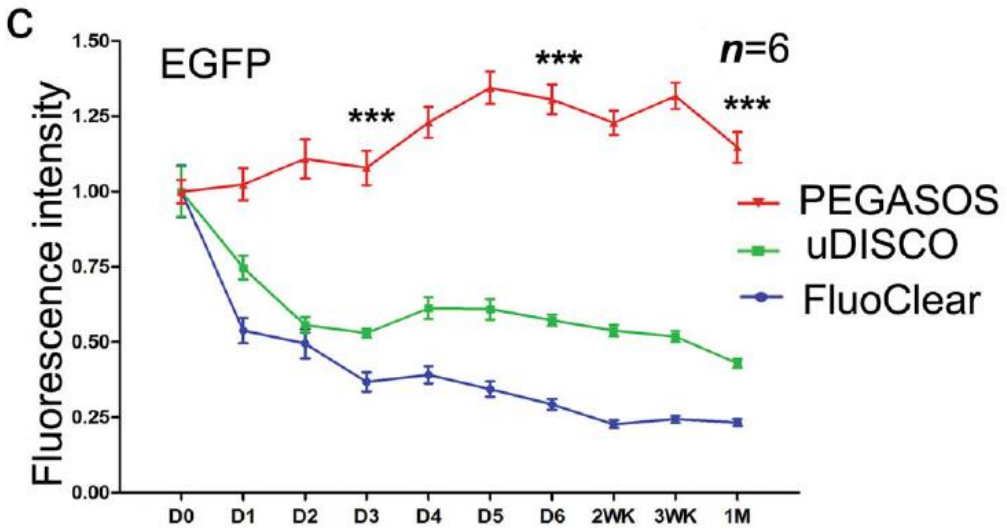


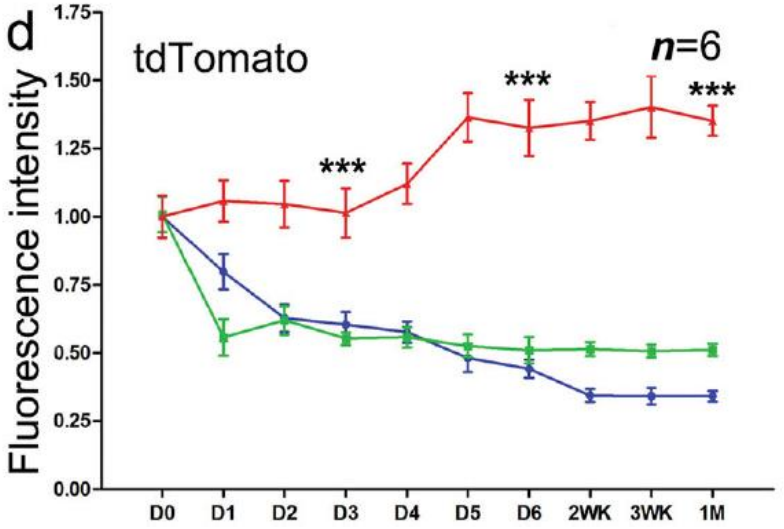
PEG MMA & DA are best forms of PEGs.

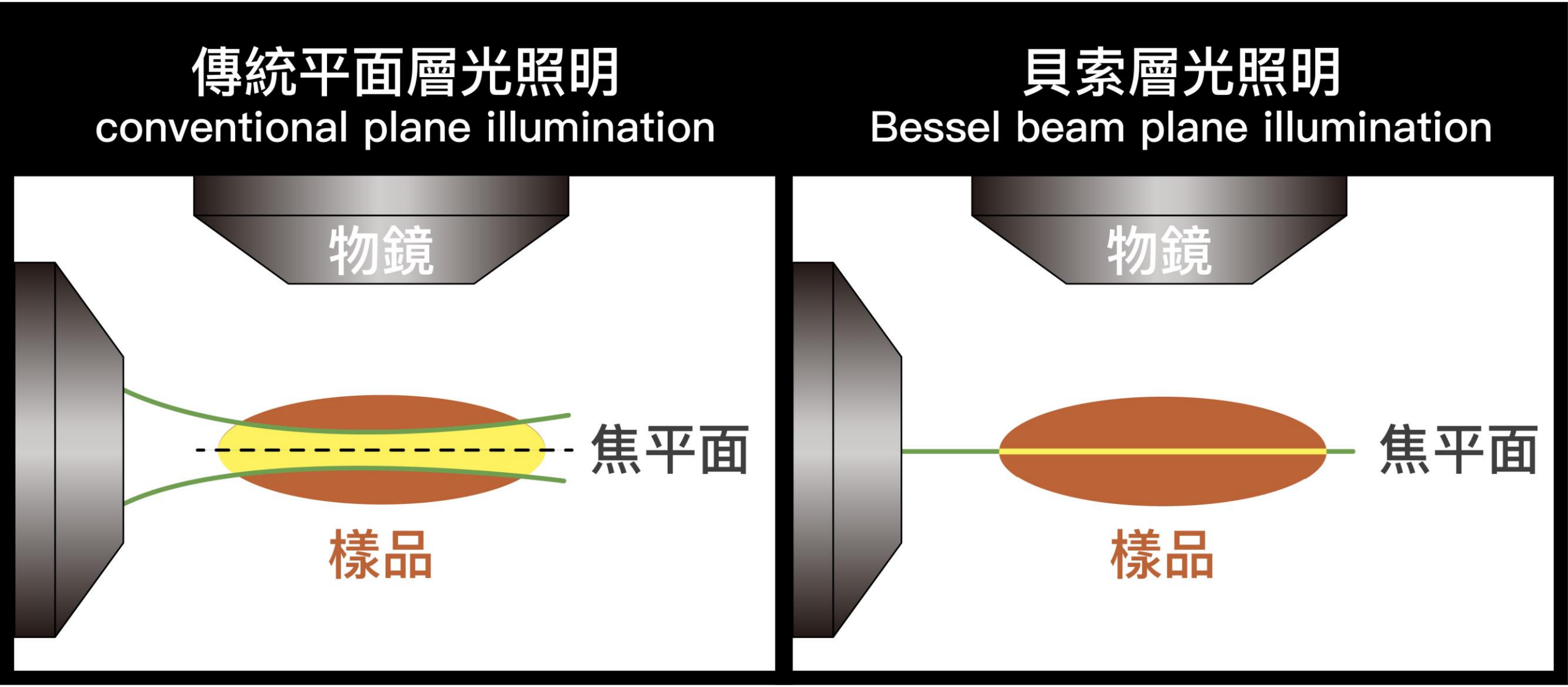
https://en.wikipedia.org/wiki/Benzyl_benzoate
https://en.wikipedia.org/wiki/Polyethylene_glycol

BB-PEG preserves endogenous fluorescence








傳統平面層光照明
conventional plane illumination
物鏡
焦平面
樣品
貝索層光照明
Bessel beam plane illumination
物鏡
焦平面
樣品