Histochemical studies in wood

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ACADEMY LECTURE (IAWS 2007 Meeting)

What is the microspectrophotometry?

- **Microscopic system with a spectrophotometric system**
- **Allowing qualitative and quantitative chemical analyses of microscopic specimen**

Early development of microspectrophotometry

1904 Koehler; prototype of UV microscope (Zeiss)

1936 Caspersson; might be called “the father of microdensitometry and microspectrophotometry”

1947 Pollister and Ris; DNA content of nuclei

1954 Olympus introduced model MSP

1959 Carl Zeiss followed with model UMSP Ⅰ

1961 Leitz UV microscope

History of Zeiss MPM for UV-spectra range

1904 Prototype of UV- microscope (Koehler)

1959 UMSP Ⅰ (XBO450W, Prism-M4QⅢ, Build-in Microscope)

1962 UV-Microscope (XBO450W, Prism-M4QⅢ, UNIVERSAL)

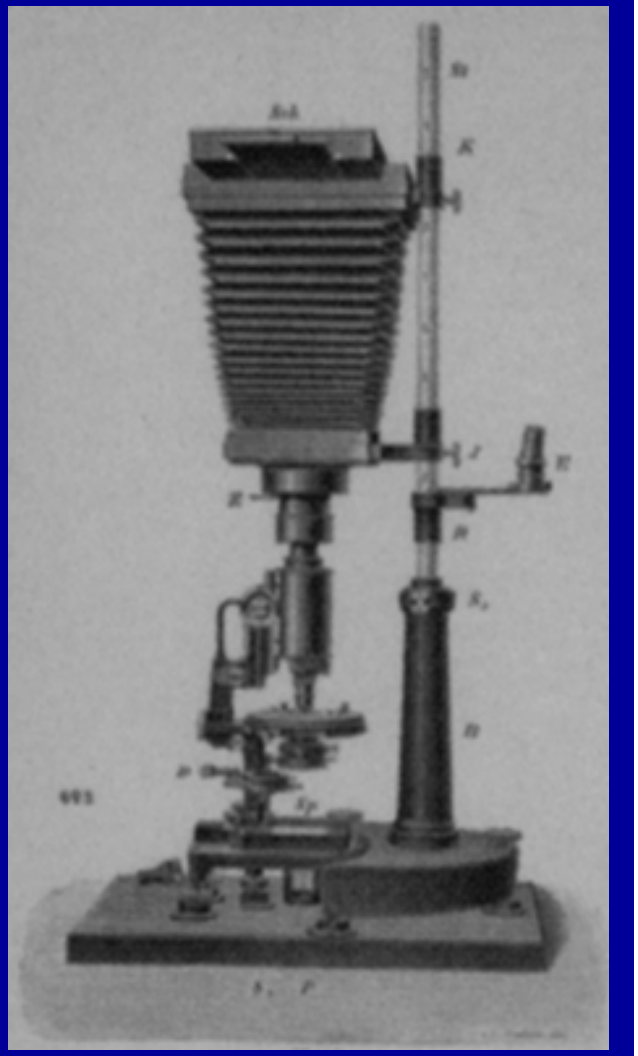
1970 MPM 01 (XBO150W, Prism-M4QⅢ, UNIVERSAL)

1972 SPM 05 (MPM 05, XBO150W, Prism-M4QⅢ, UNIVERSAL)

1977 MPM 03 (XBO150W, Prism-M4QⅢ, UNIVERSAL)

1985 UMSP 80 (XBO75W, Grating-Monochro., AXIOPHOTO)

1992 MPM 800 (XBO75W, Grating-Monochro., AXIOTRONE)



(UVM 1904)

The laboratory instruments in use
(at Gifu Univ. and Hokkaido Univ.)

(1) Olympus MPM (MSP-ATV), 1962-1967

(2) Carl Zeiss MPM 01, 1971-1986

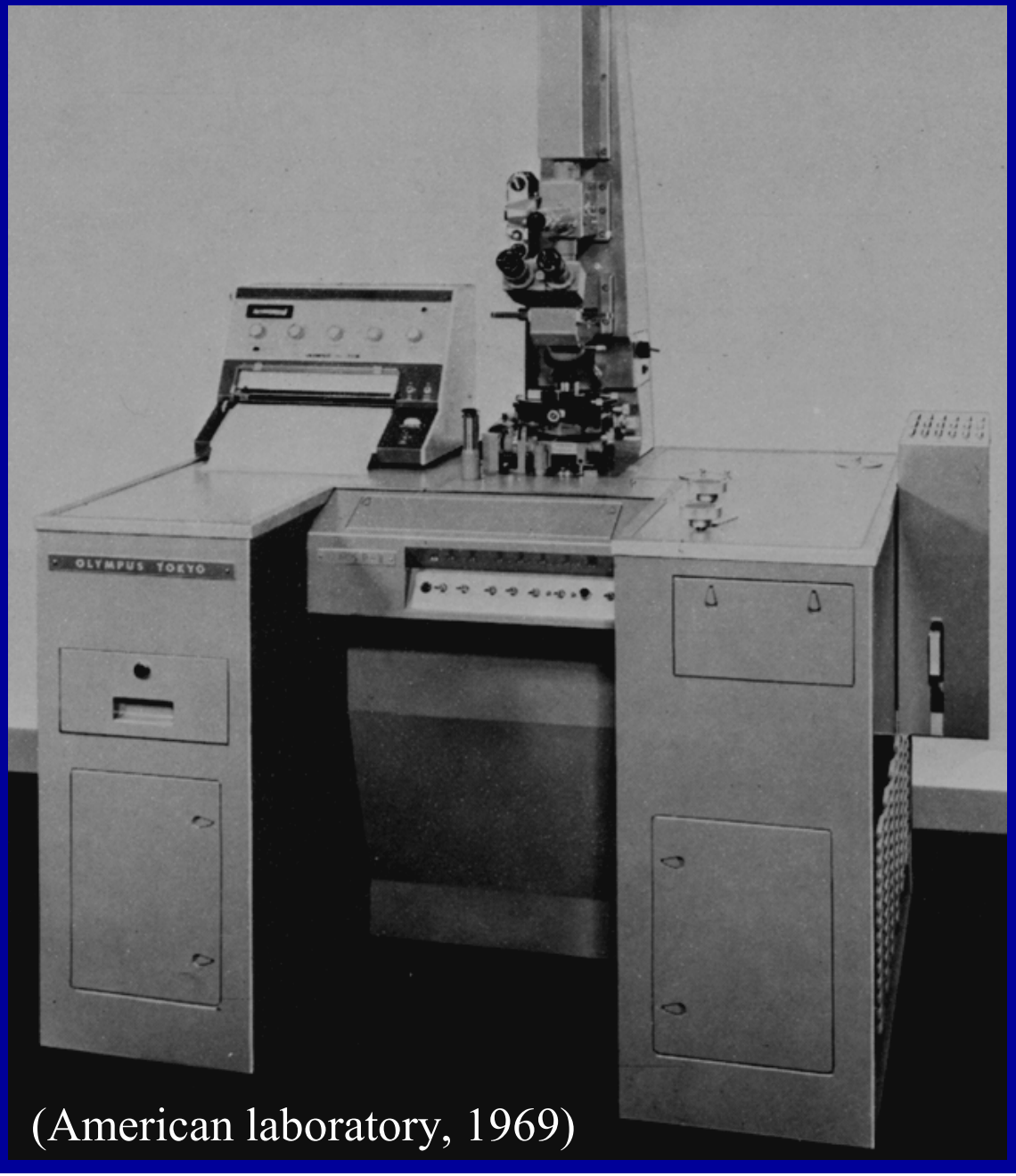
(3) Carl Zeiss UMSP 80, 1986-1995

(4) Carl Zeiss MPM 800, 1993-

(5) Carl Zeiss LSM-310 (confocal laser scanning microscope), 1993-

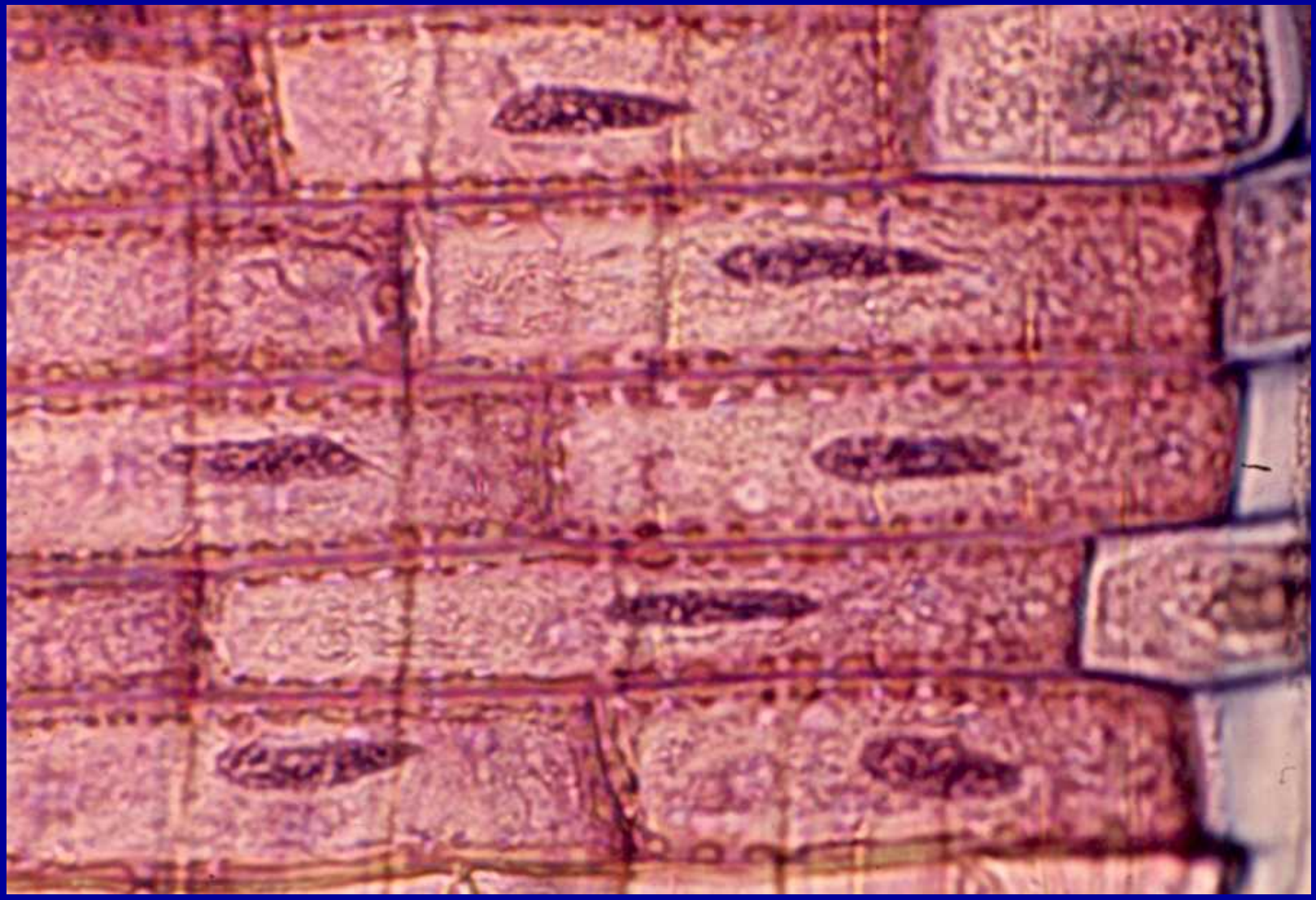
* also used Leitz UV microscope at PAPRICAN, Montreal, temporarily in 1979-1980

(1) Olympus MSP (MSP-ATV), 1962~1967



(American laboratory, 1969)

Some observations on nucleus and DNA & RNA contents in ray parenchyma



Higuchi T., Fukazawa K. et al.: *J. Japan Wood Res. Soc.* & *others* (1964-1967)

Early papers for wood anatomy using UVM (1)

- Lange (1954) lignin content in the middle lamella and secondary wall from UV photos
- Wardrop (1957) lignification in differentiating xylem from UV photos
- Frey-Wysslings & Bossard (1959)
- Wergin (1965) unusual lignin distribution in compression wood from UV photos (Zeiss UMSP1)
- Jayme et al. (1967) topochemistry of delignification in pulping from Leitz UVM

Reflecting objectives:

- Transmission is over wide range
- Glare is reduced through the use of aspherical mirror
- Aperture is small, therefore, desirable for microphotometry
- Refocusing is not required from the focus in visible light

Refracting achromats:

- Difficult to design for transmission between 240-300 nm
- Low transmission
- Glare
- Slight refocusing is required in the UV region

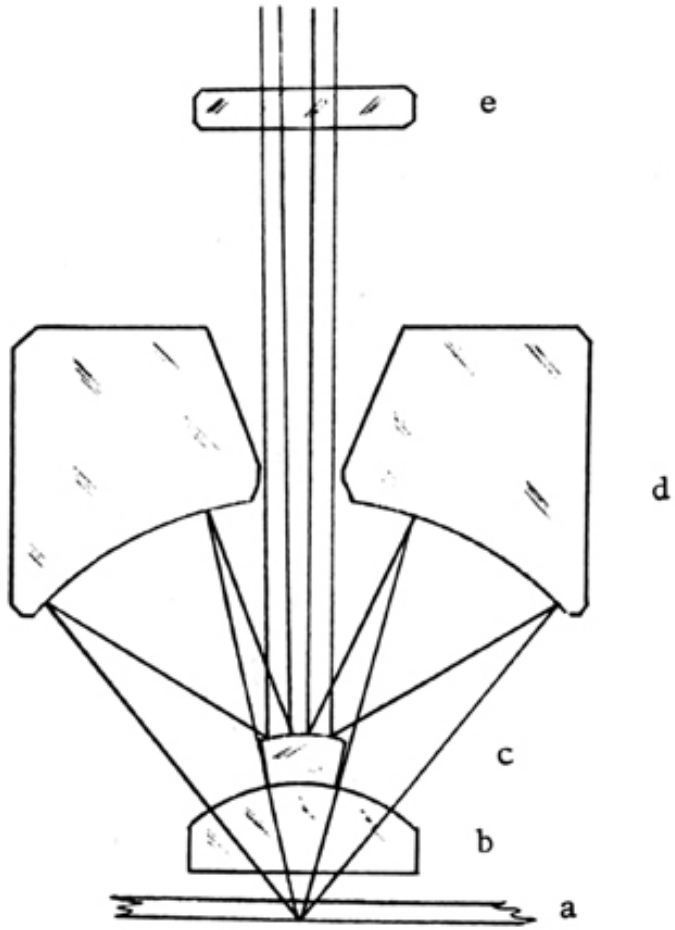


Figure 3 *Schematic diagram of the Olympus MO reflecting objectives.*

a) Quartz O.7mm thickness cover glass.
b) Front lens (quartz).
c) Small spherical mirror.
d) Large aspherical mirror.
e) Top dust cover glass (quartz).

From *literature of Olympus Co.* (Schlueter 1969)

Reflecting objectives (mirror type)

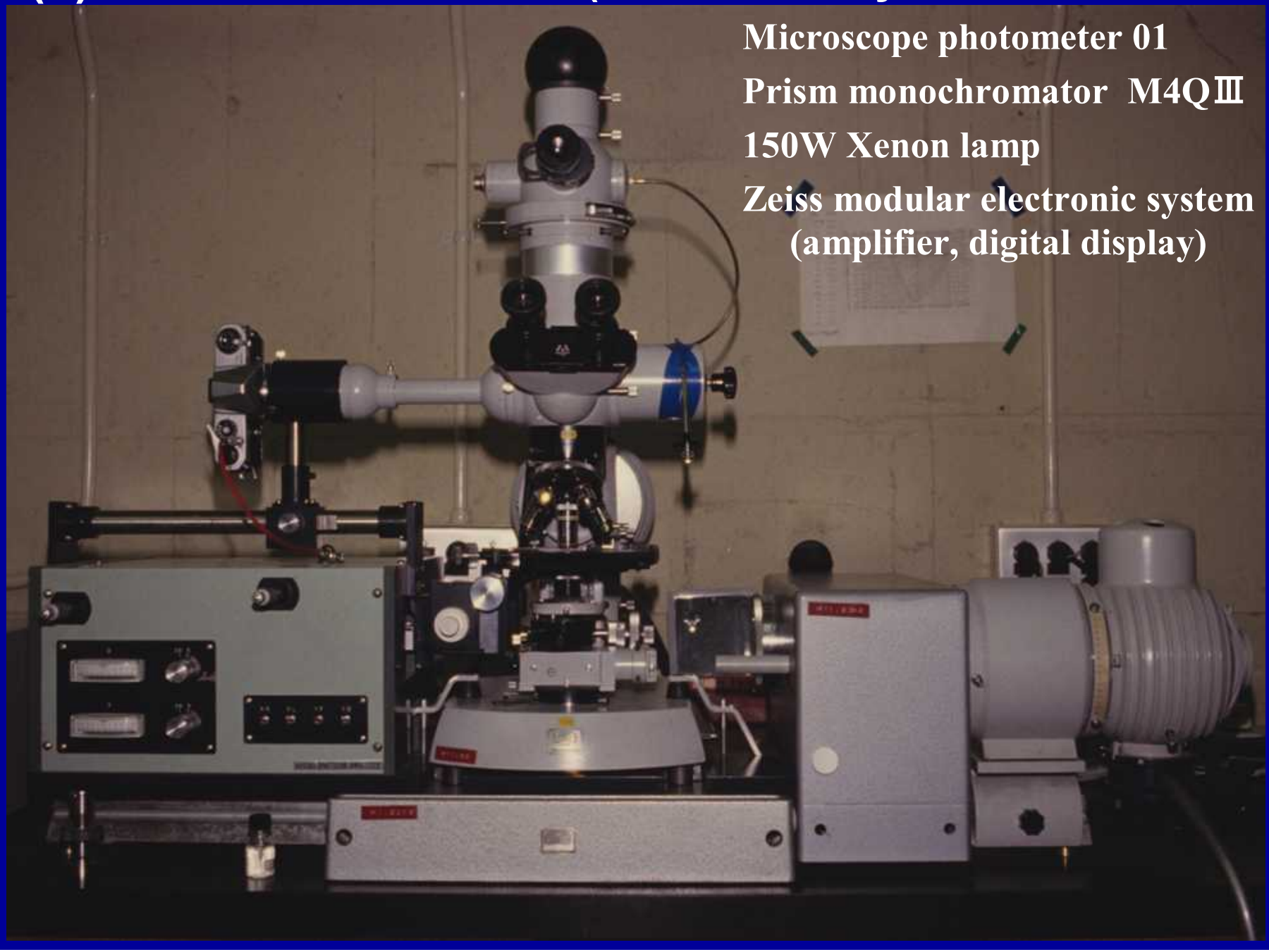
- Low quality of central image formation; due to the arrangement of spherical and aspheric mirror
- Low image quality; due to the spherical aberration
- Low N.A.; not exceeding 0.7-0.8
- Shock susceptibility

Ultrafluar (Zeiss refracting objectives)

- Excellent image quality
- High resolving power (2 times of reflecting objectives on N.A. 1.25)
- Transmission; λ 250nm; ca.50% (32/0.40), ca.40% (100/1.25)
  λ 280nm; ca.60% (32/0.40), ca.50% (100/1.25)
- Focal length; λ 285nm; 6.0 mm (32/0.40), 1.77 mm (100/1.25)
  λ 546nm; 6.4 mm (32/0.40), 2.00 mm (100/1.25)

(From *Zeiss information*)

(2) Carl Zeiss MPM 01 (the laboratory use in 1971-1986)



Microscope photometer 01

Prism monochromator M4QⅢ

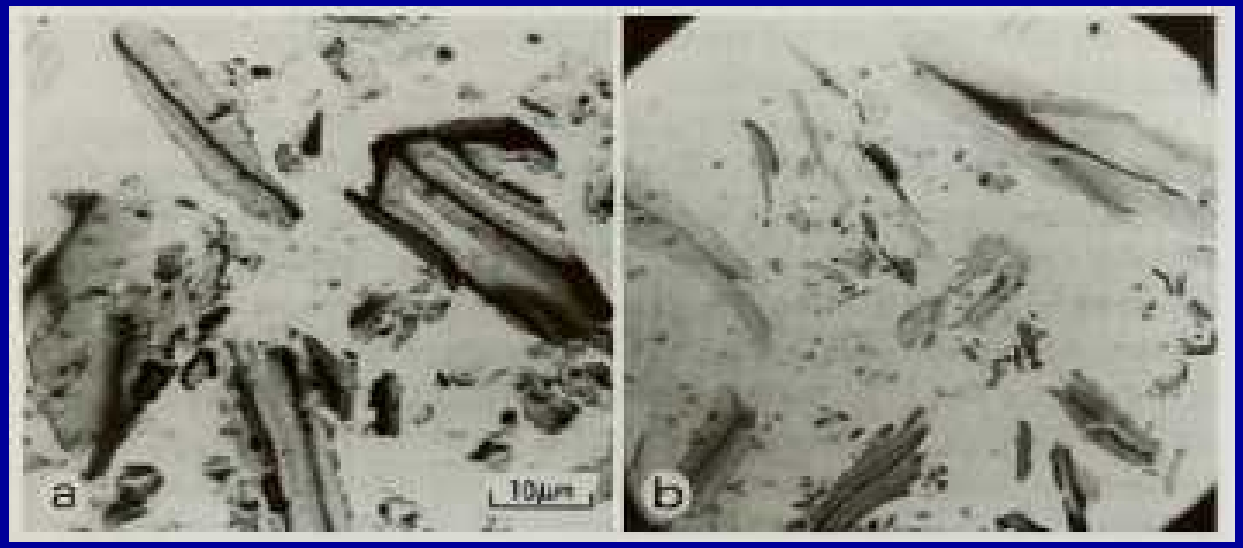
150W Xenon lamp

Zeiss modular electronic system
(amplifier, digital display)

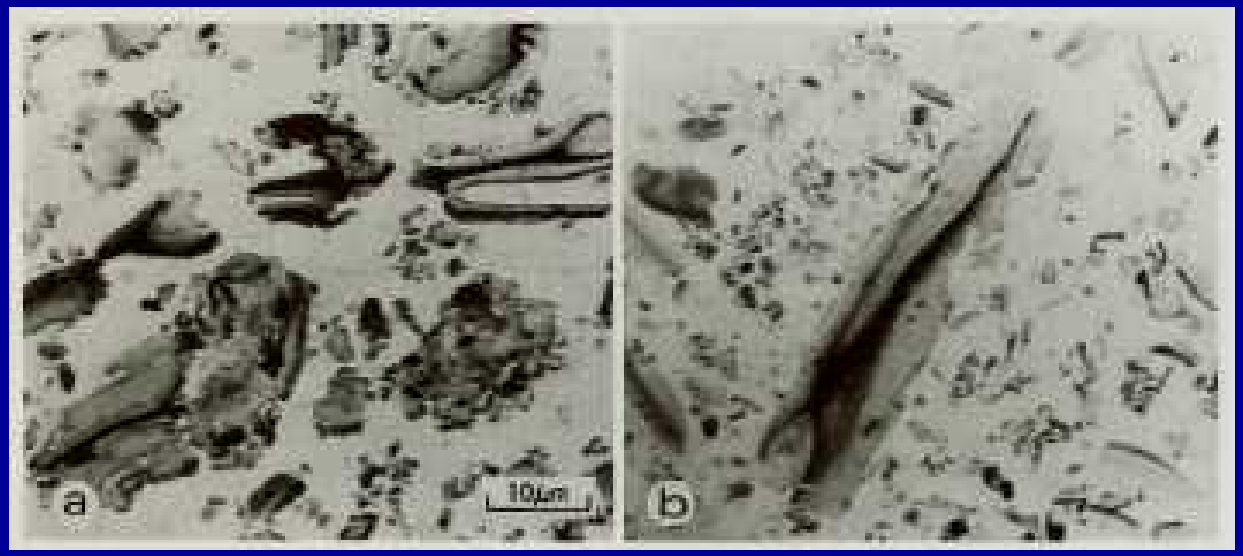
Leitz UV microscope

150W Xenon lamp (Osram)
Linear mirror monochromator
Illuminating system (a quartz collimator and two quartz lenses
for adjustment in order to preserve the image conditions
at each wavelength)
Reflecting objectives (Q 170/0.50, Q Gly. 300/0.85)
Reflecting condensers (Q Gly.0.60, Q Gly 0.85)
Photography (Leica camera body)
Image converter (for visual observation of the specimen in UV light)
Microscope (Leitz ORTHOLUX)

UV photomicrograph of sample after ball milling, before and after enzyme treatment (1)



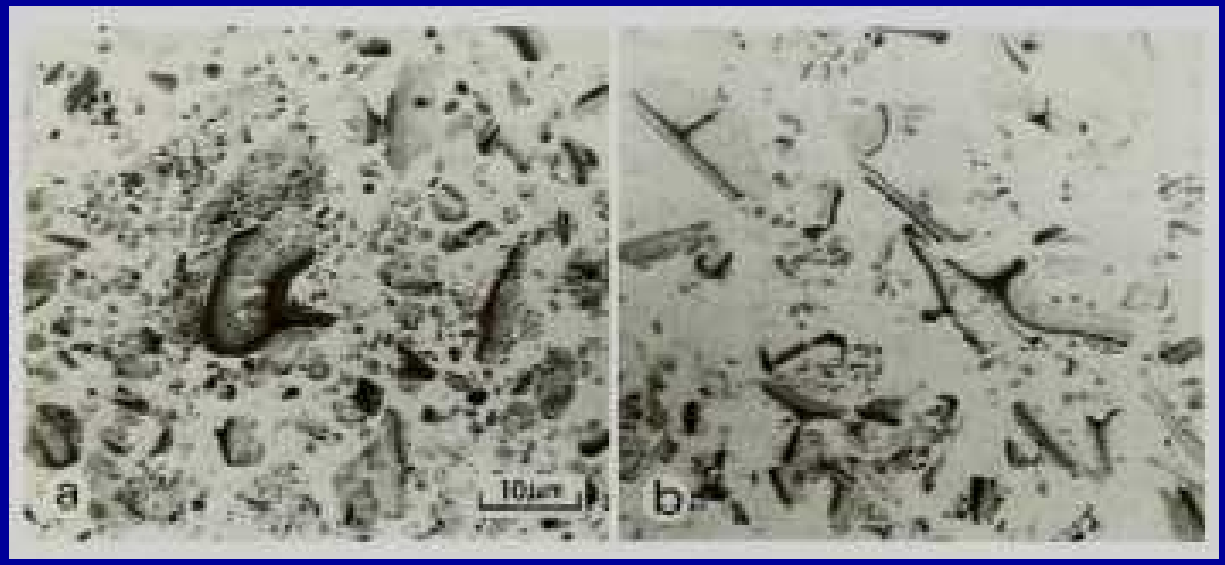
one day



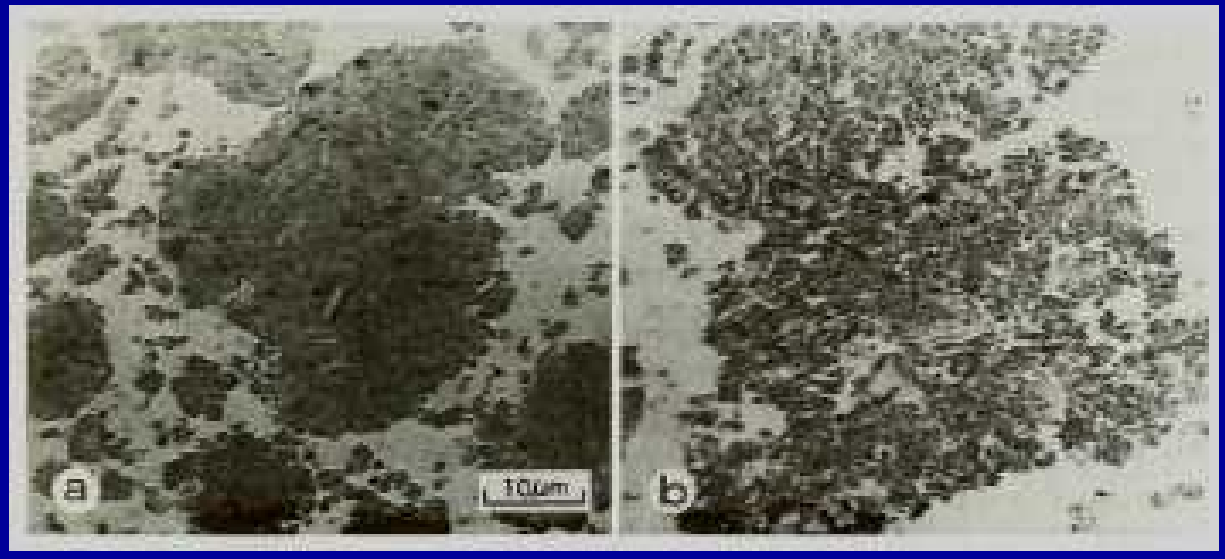
two days

Fukazawa K., Goring D. A. I. et al.: *Wood Sci. Technol.* (1982)

UV photomicrograph of sample after ball milling, before and after enzyme treatment (2)



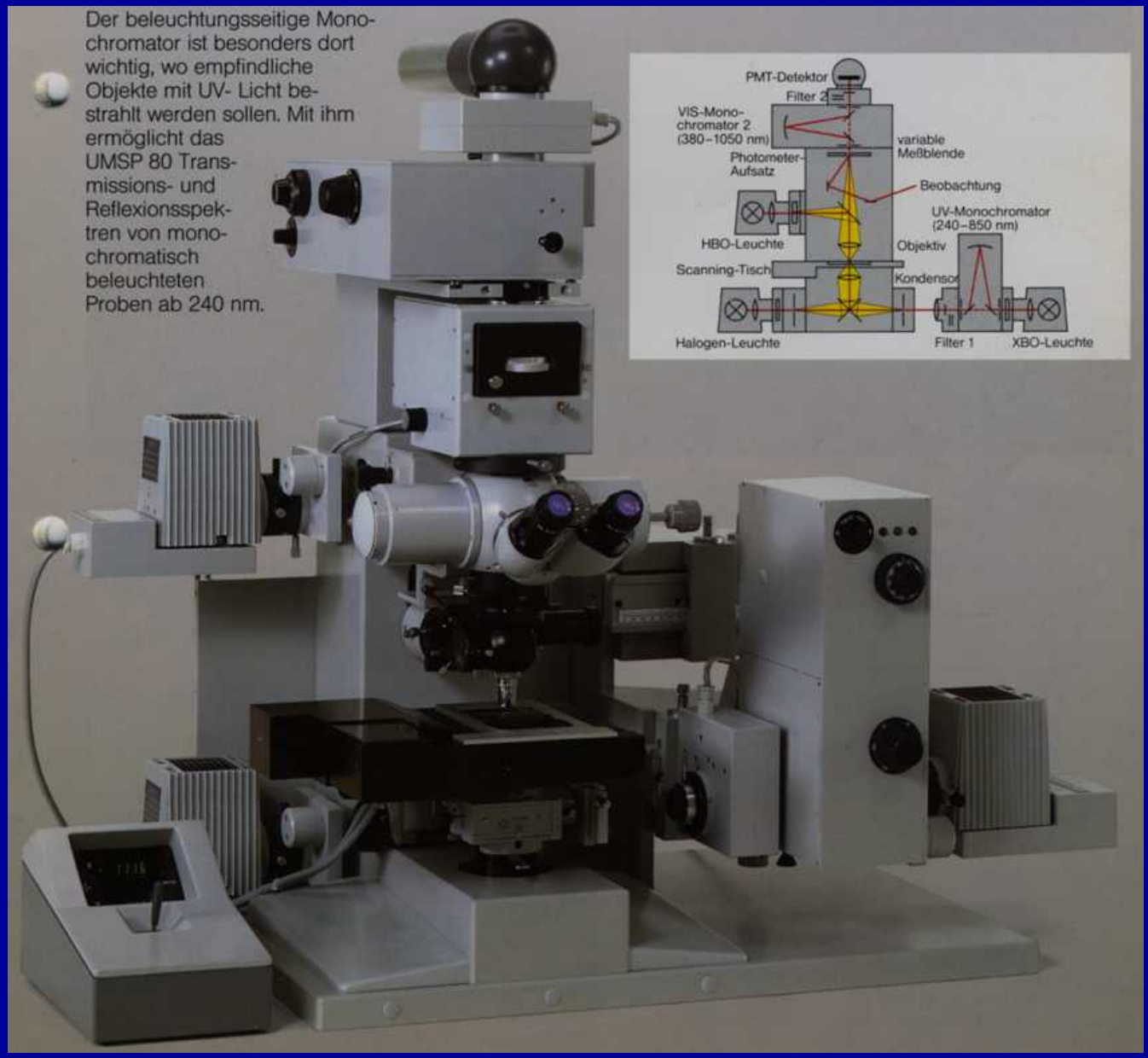
three days



ten days

Fukazawa K., Goring D. A. I. et al.: *Wood Sci. Technol.* (1982)

(3) Carl Zeiss UMSP 80 (the laboratory use in 1986-1995)



(Carl Zeiss catalogue)

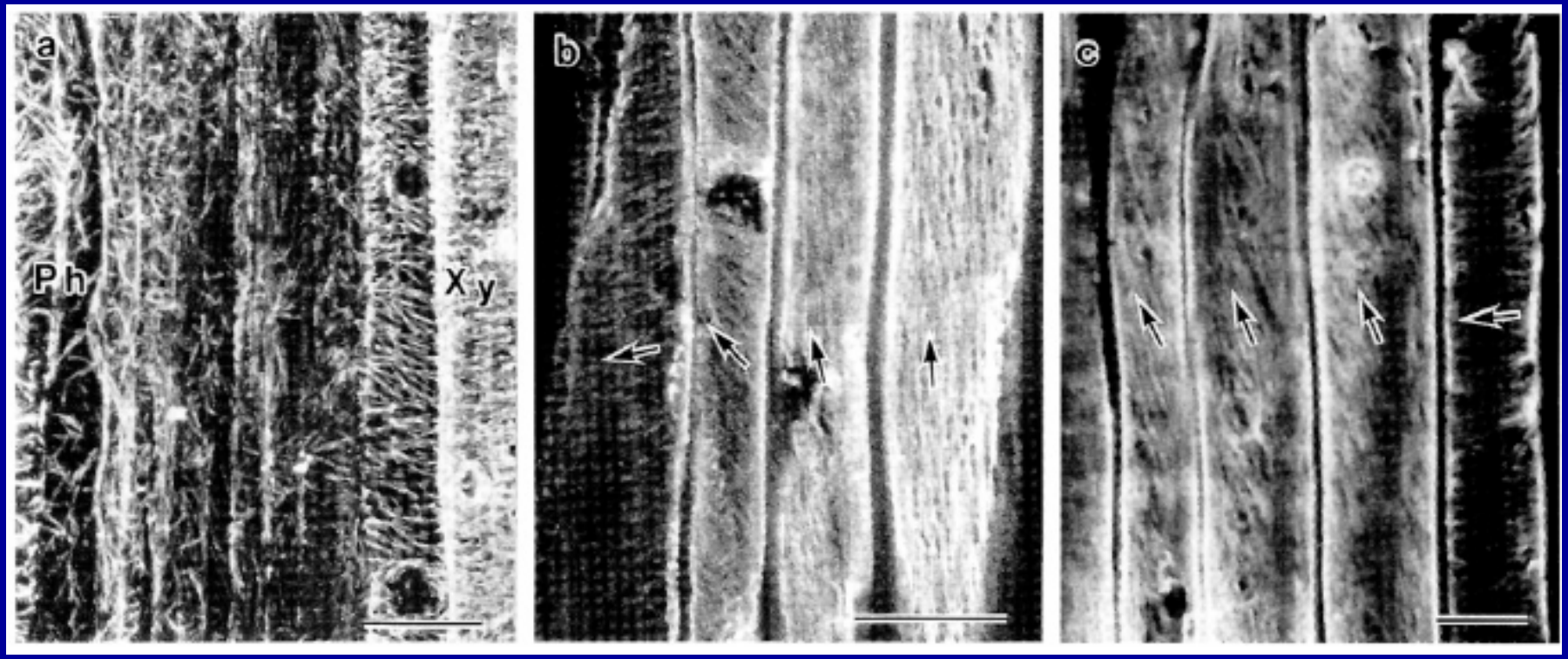
(4) Carl Zeiss MSM 800 (the laboratory use in 1993-)

(Carl Zeiss catalogue)

(5) Carl Zeiss LSM-310 (confocal laser scanning microscope, the laboratory use in 1993-) (1)

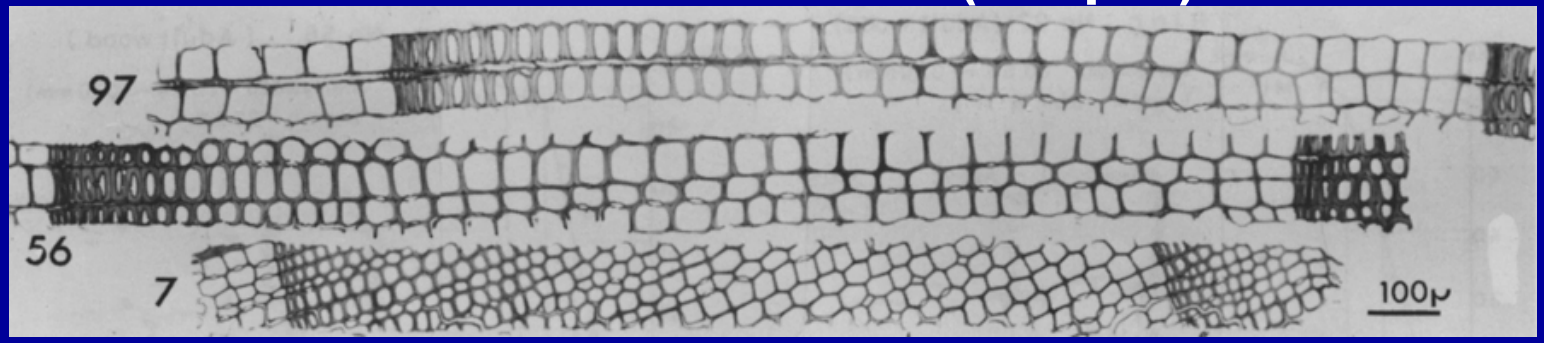
(Carl Zeiss catalogue)

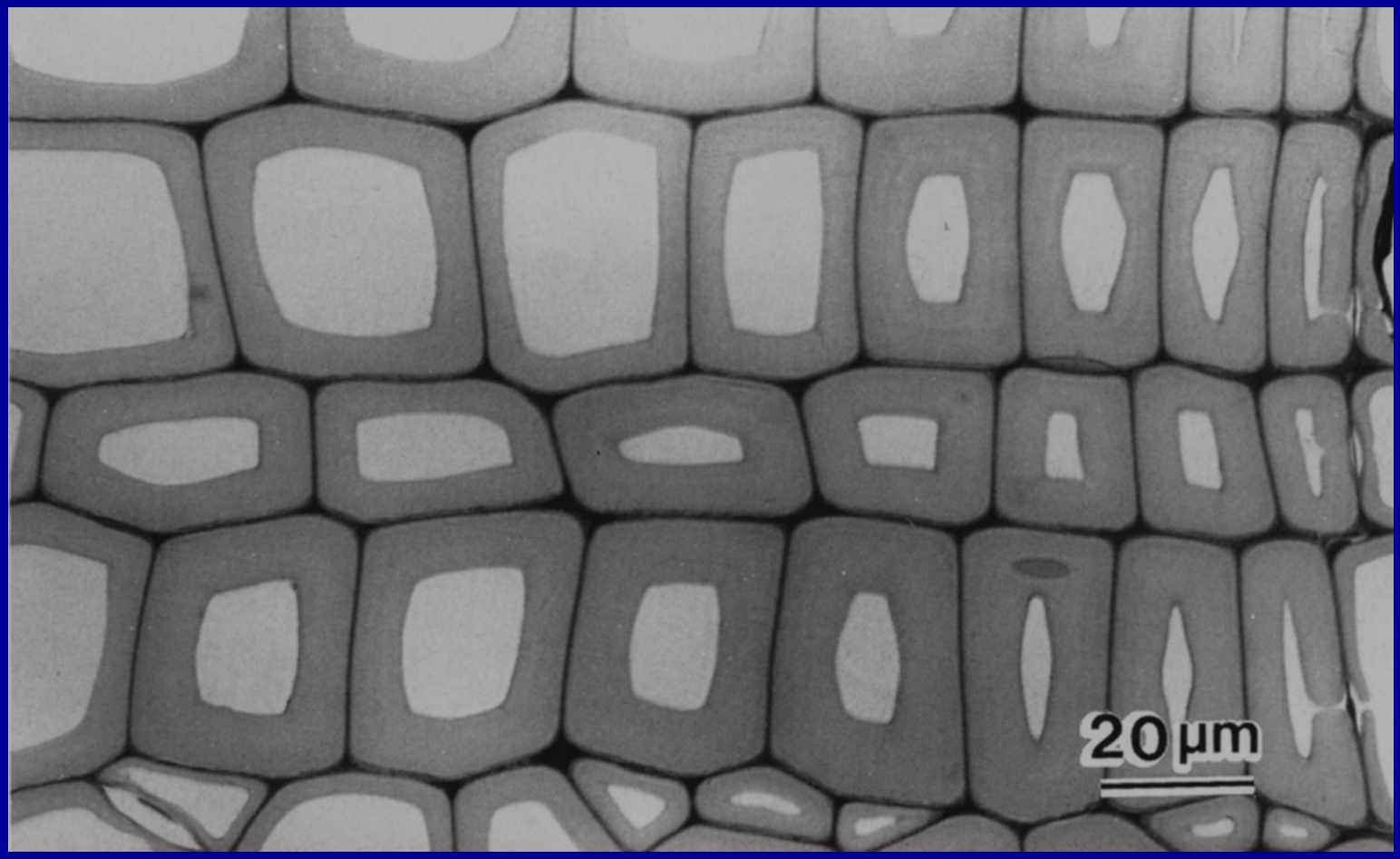
Dynamic changes of the arrangement of cortical microtubules



Abe H., Funada R. et al.: *Planta* & *other publications* (1995-)

Section thickness (0.5 µm)



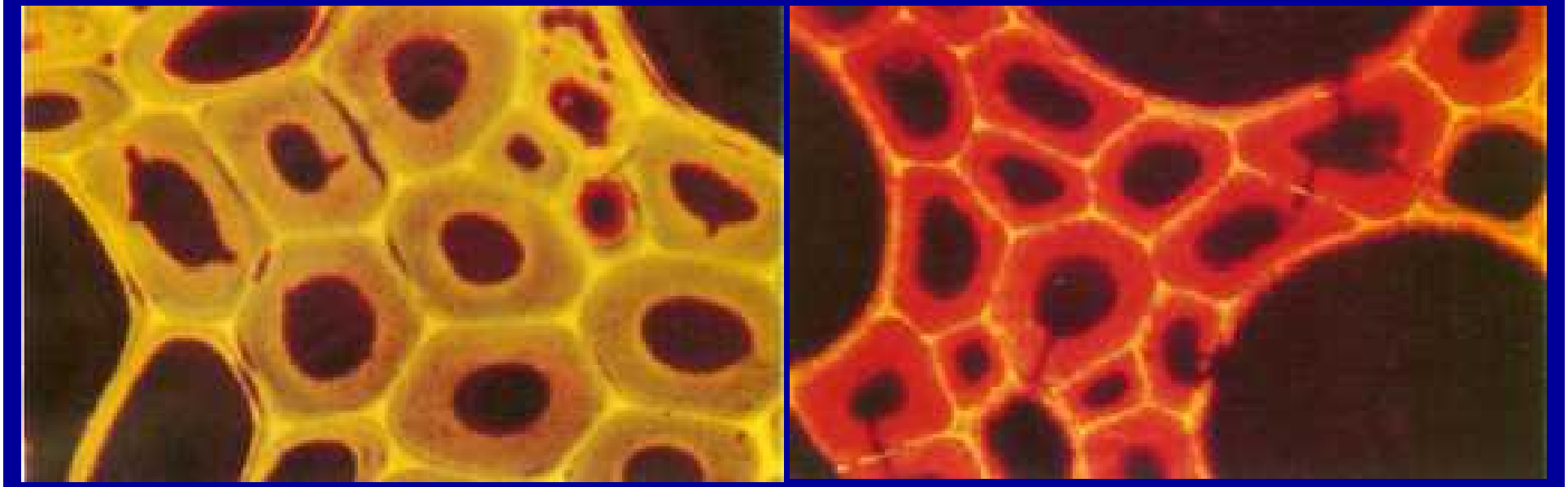


Takano T. et al.: *Res. Bull. Exp.For. Hokkaido Univ.* (1983)

Specific stain for wood sections

- **Basic dyes**
  safranin, basic fuchsin, toluidine blue O, methylen blue, nile blue, etc.
- **Acidic dyes**
  fast green, acid fuchsin, etc.
- **Metachromatic dyes**
  toluidin blue, etc.
- **Fluorochrome and metachromatic dyes**
  acridine orange etc.
- **Wiesner color reaction**
- **Mäule color reaction**

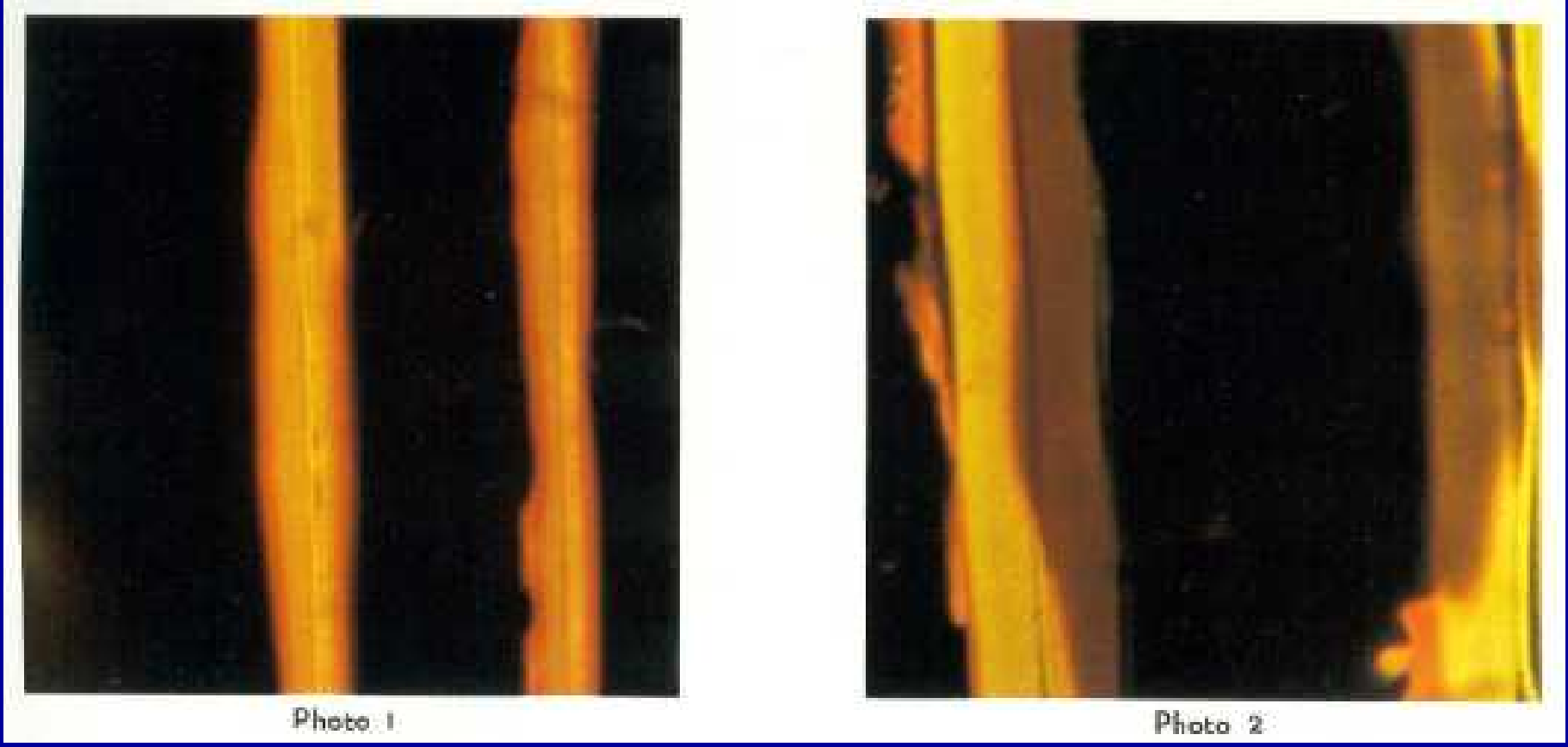
Fluorochrome & metachromatic dyes: acridine orange (2)



Beech wood fibers in an early stage of decay by white rot and brown rot

Fukazawa K. et al.: ***Res. Bull. Exp.For. Hokkaido Univ.*** **(1976)**

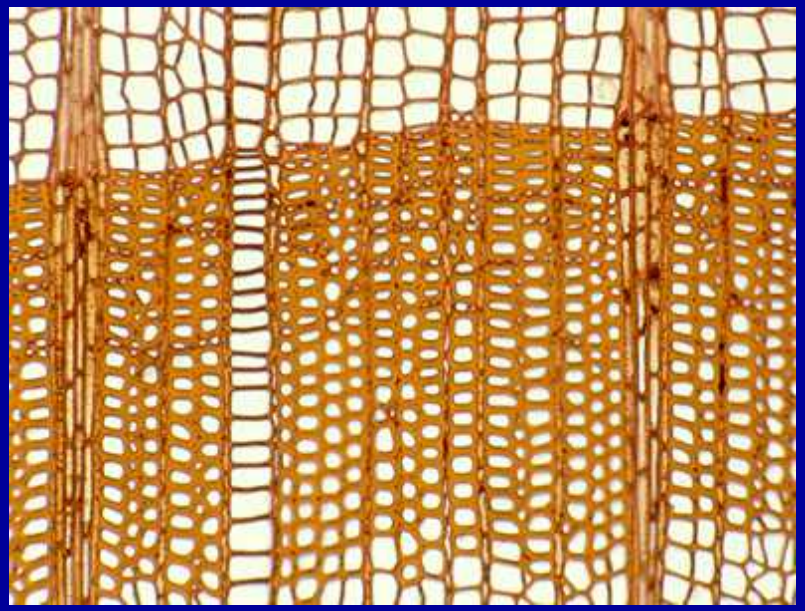
Fluorochrome & metachromatic dyes: acridine orange (3)

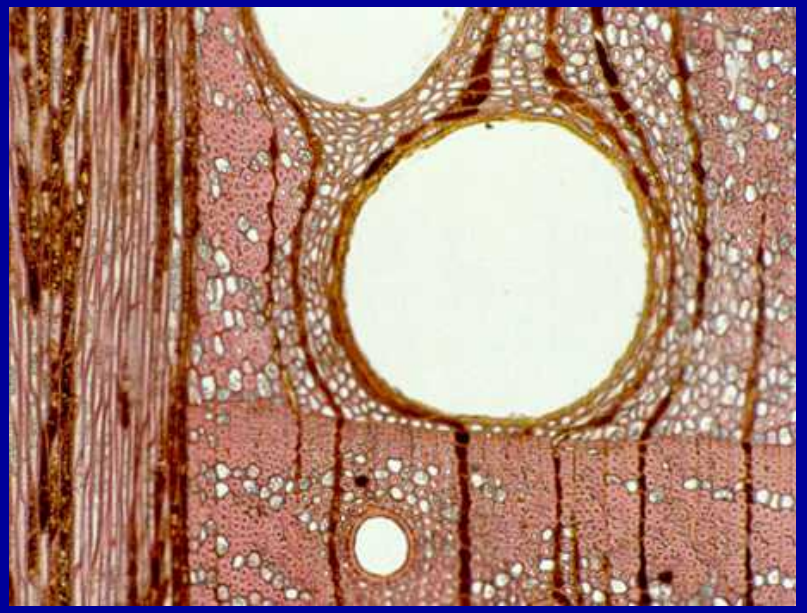


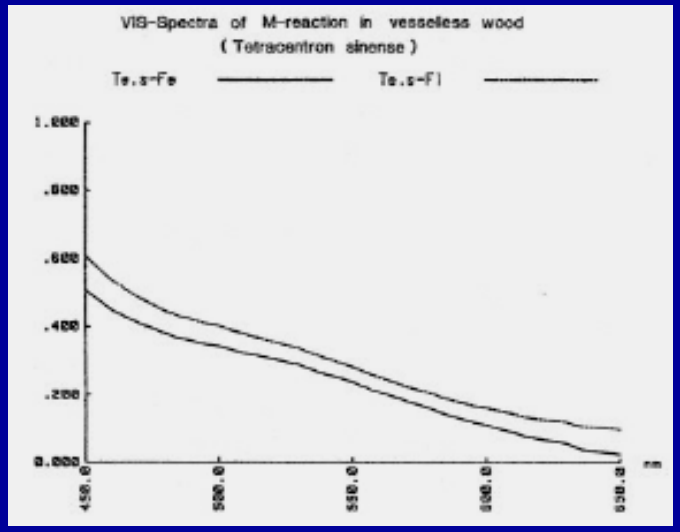
Tracheid walls decayed by white rot fungi

Yamashita Y. et al.: *Res. Bull. Exp.For. Hokkaido Univ.* (1977-79)

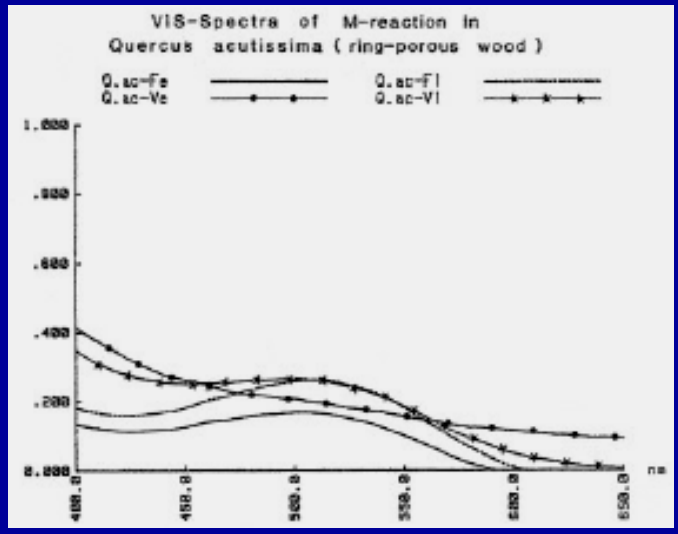
Mäule color reaction







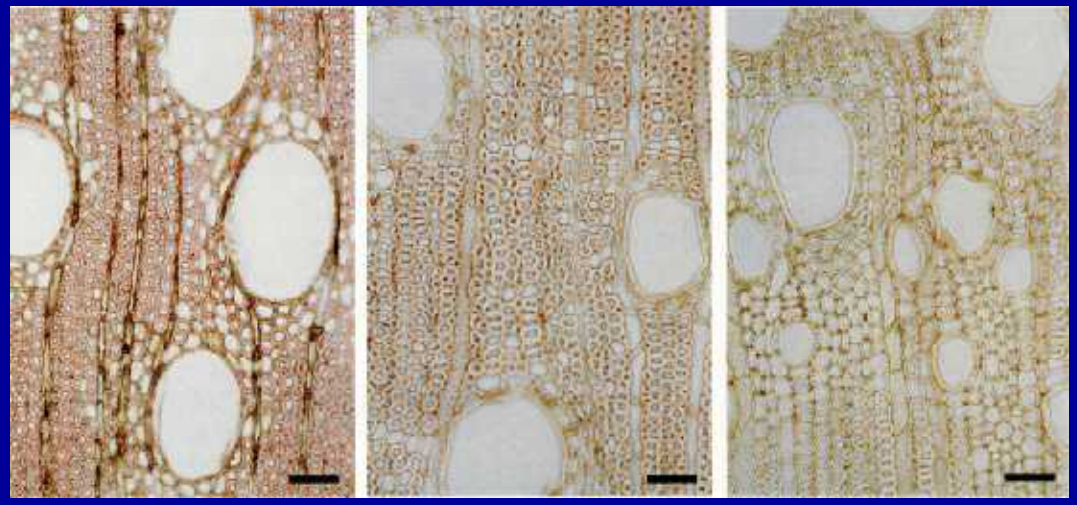
Tetracentron sinense

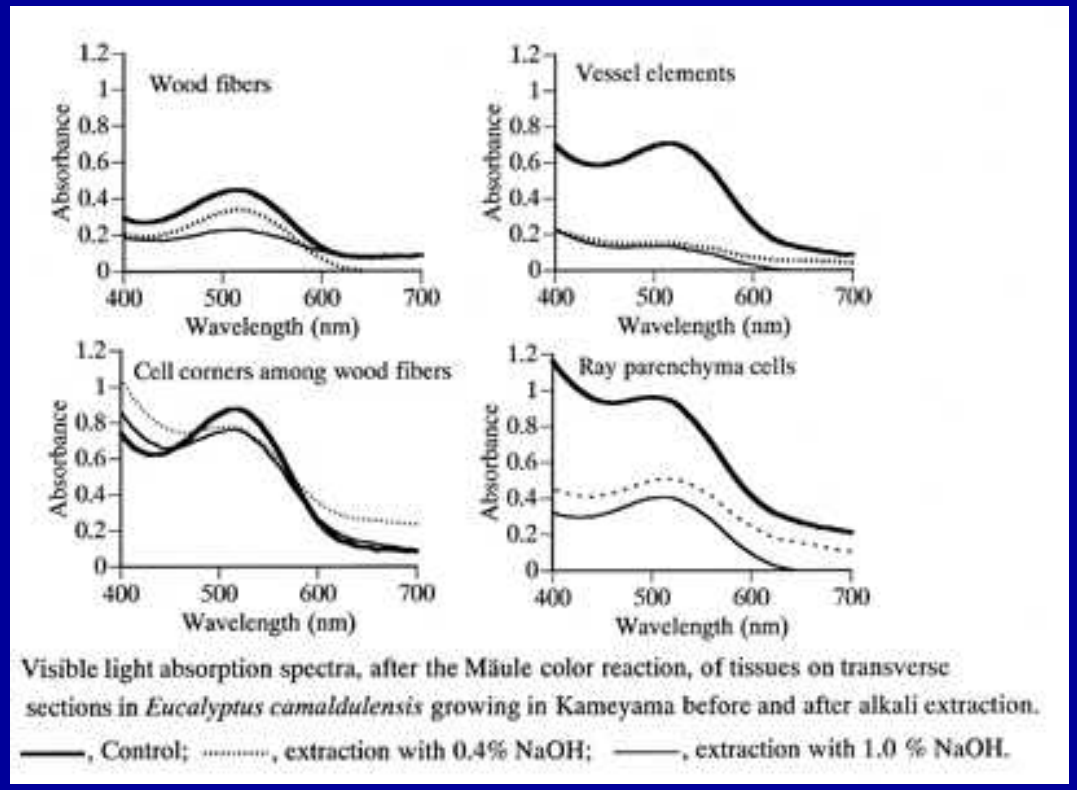


Quercus acutissima

Wu J. et al.: *Holzforschung* (1992)

Influence of wood extractives on Mäule color reactions





Visible light absorption spectra, after the Mäule color reaction, of tissues on transverse sections in *Eucalyptus camaldulensis* growing in Kameyama before and after alkali extraction.
——, Control; ·····, extraction with 0.4% NaOH; ——, extraction with 1.0 % NaOH.

***E. camaldulensis*: control, 0.4 % NaOH and 1.0 % NaOH**

Watanabe Y. et al.:
***Mokuzai Gakkaishi* (1997)**

Influence of wood extractives on UV mcroscopy for lignin determination

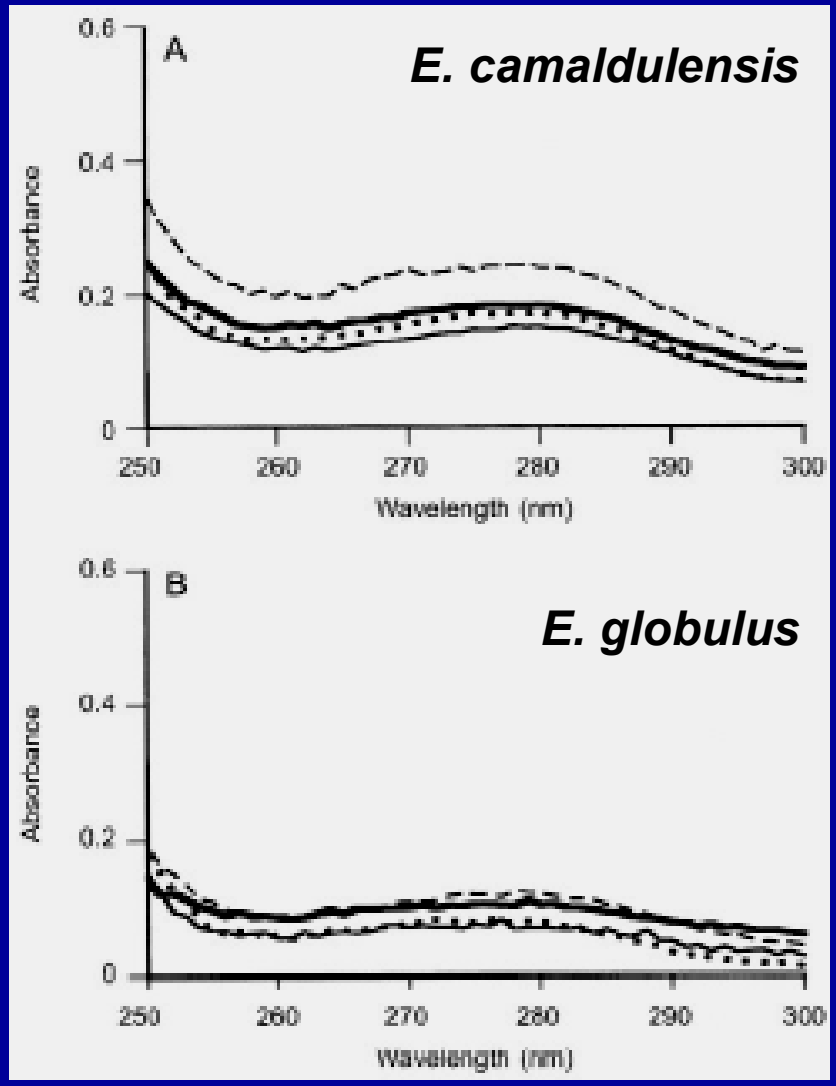
Bland & Hillis (1962, 1969)
Eucalyptus species

Imagawa & Fukazawa (1978)
Larix species

Wu et al. (1959)
Tropical hardwoods

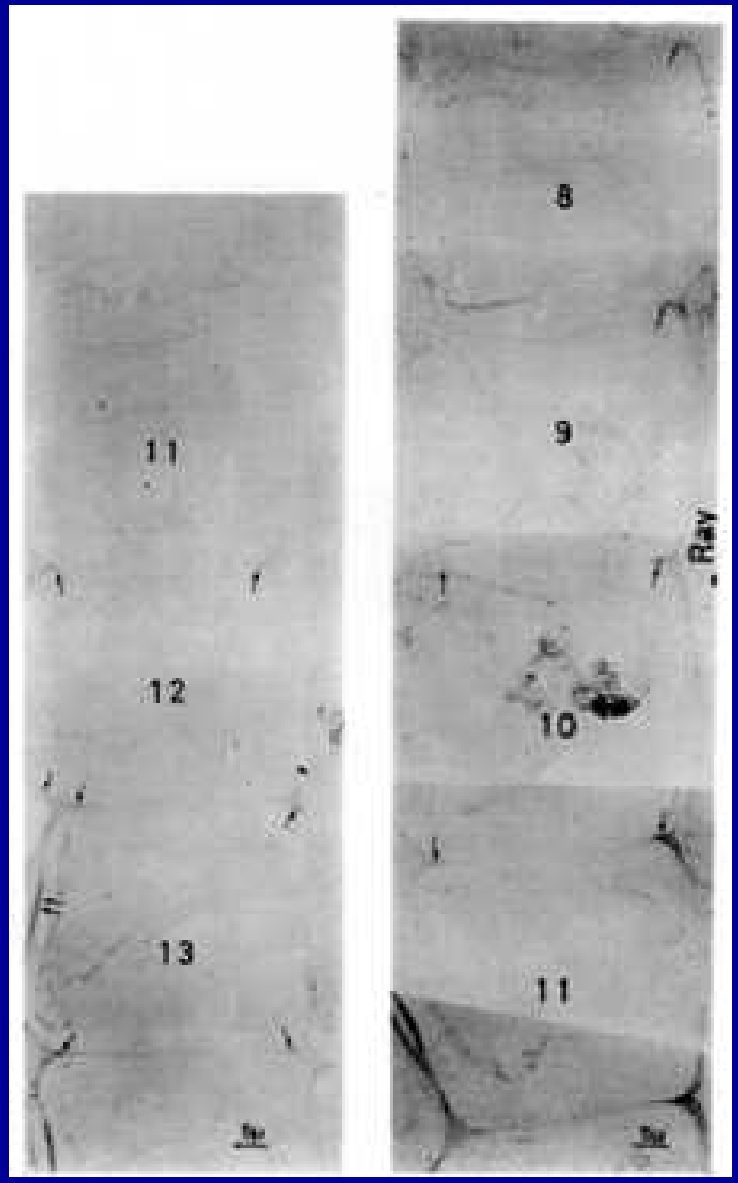
Kleist & Bauch (2001)
Tropical hardwoods

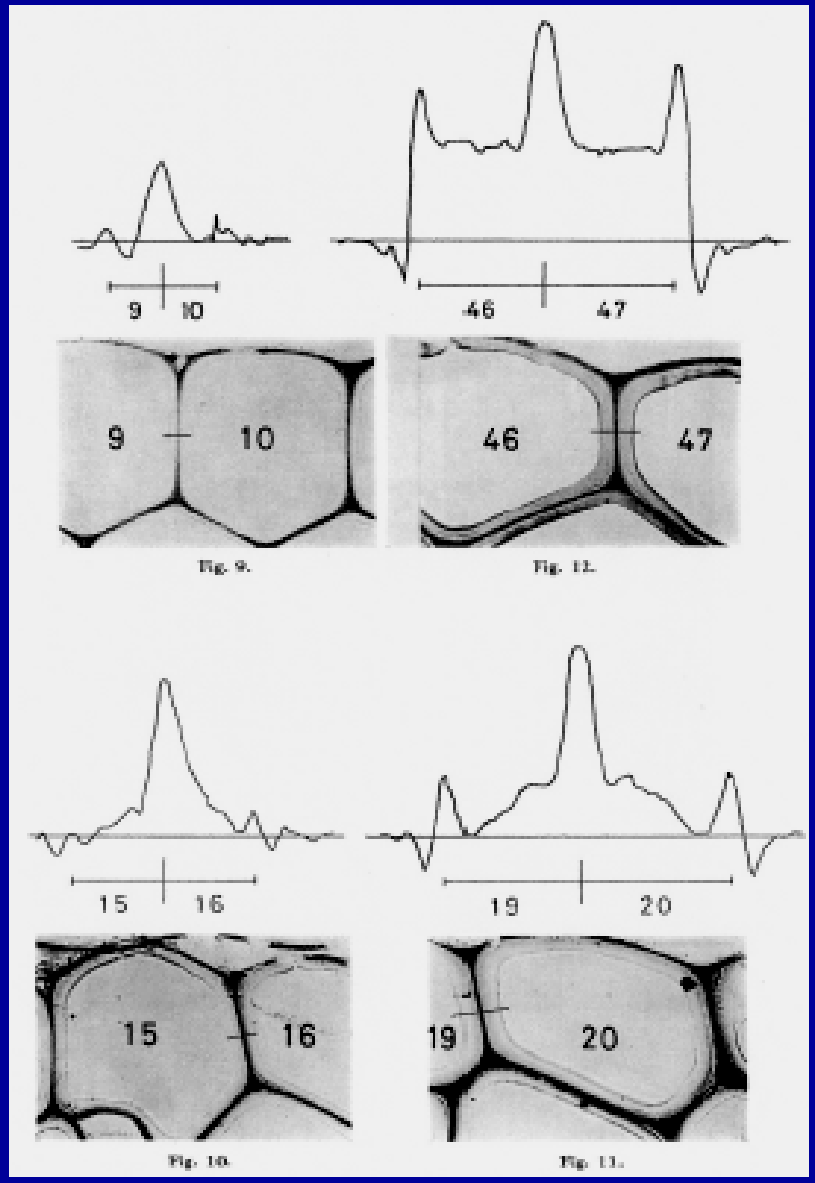
Watanabe et al. (1997, 2004)
Eucatyptus species



Watanabe Y. et al.: *IAWA J.* (2004)

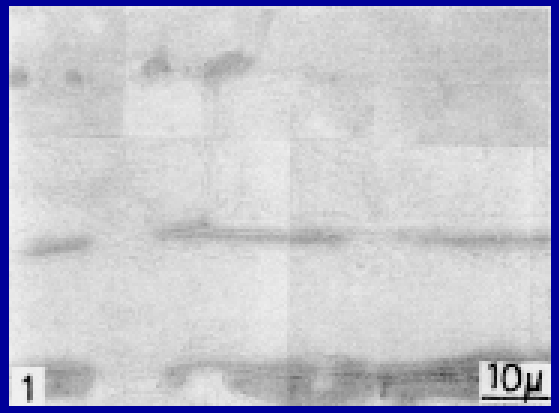
Lignification at cambial zone (1)

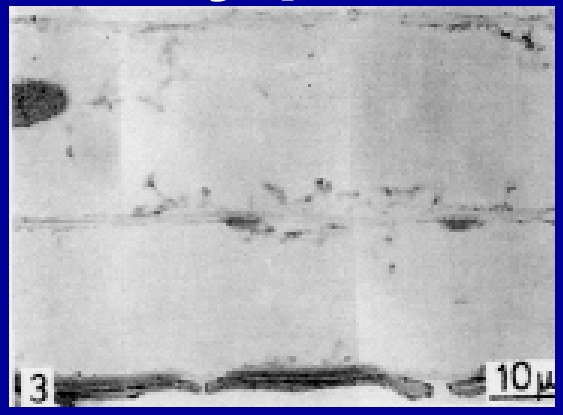




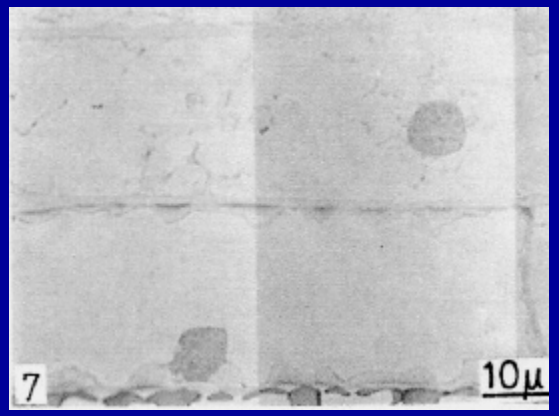
Imagawa H. et al.: *Res. Bull. Exp. For. Hokkaido Univ.* (1976)

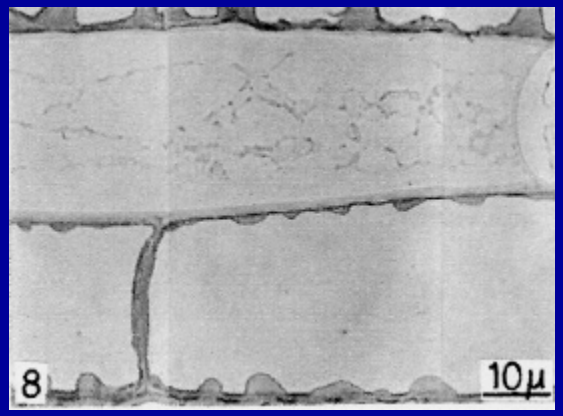
Lignification of ray parenchyma in *Pinus*



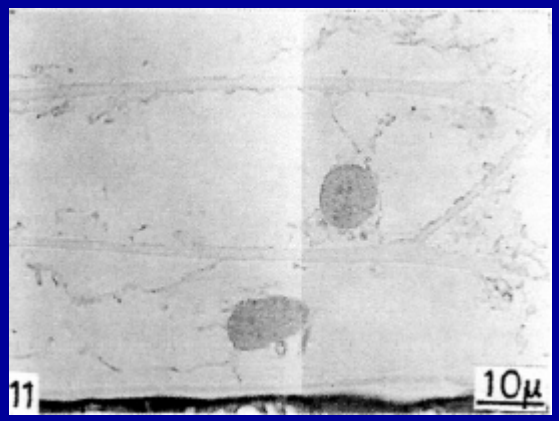


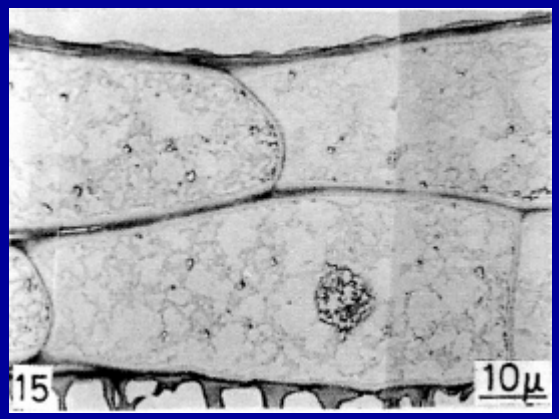
Type A
outermost sapwood





Type B
intermediate wood

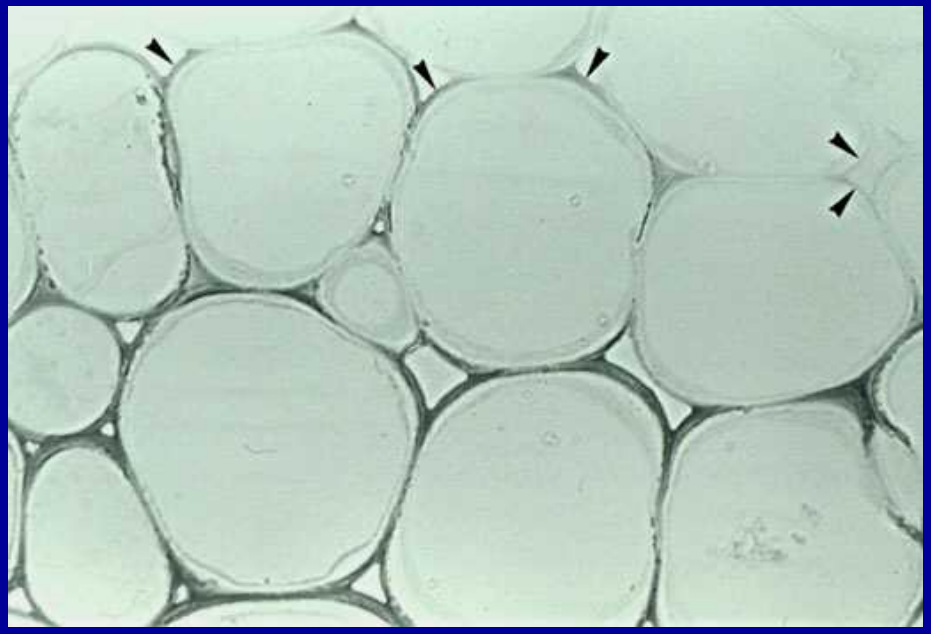


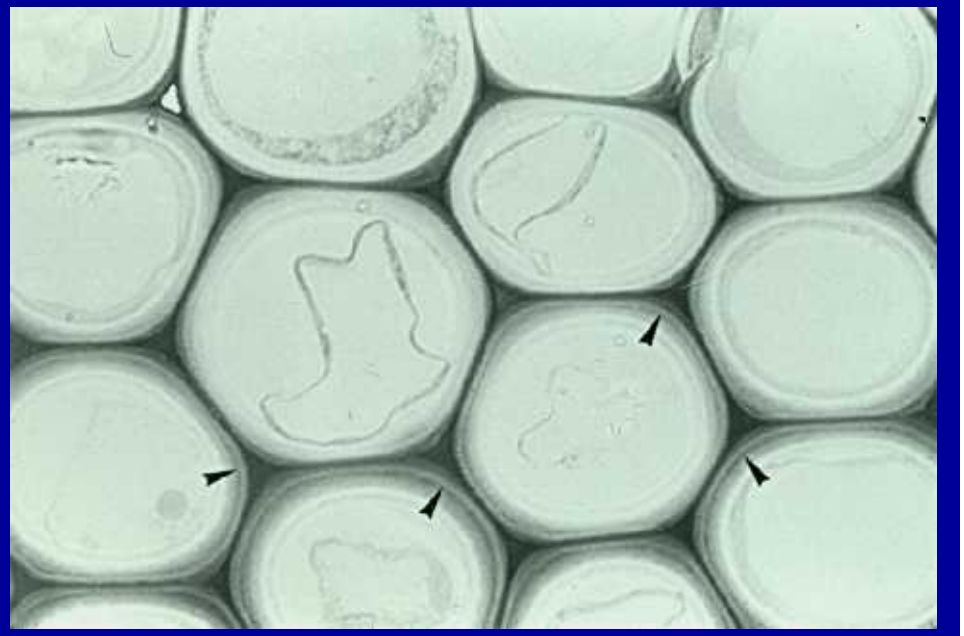


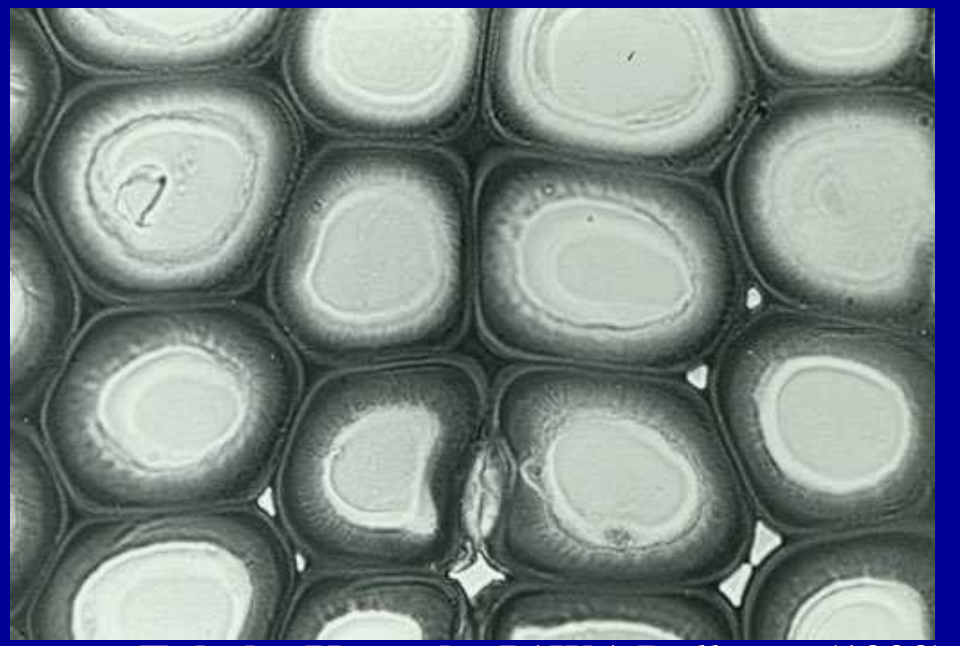
Type C
intermediate wood

Yamamoto K. et al.: *Res. Bull. Exp. For. Hokkaido Univ.* (1977-82)

Lignification in compression wood (1)

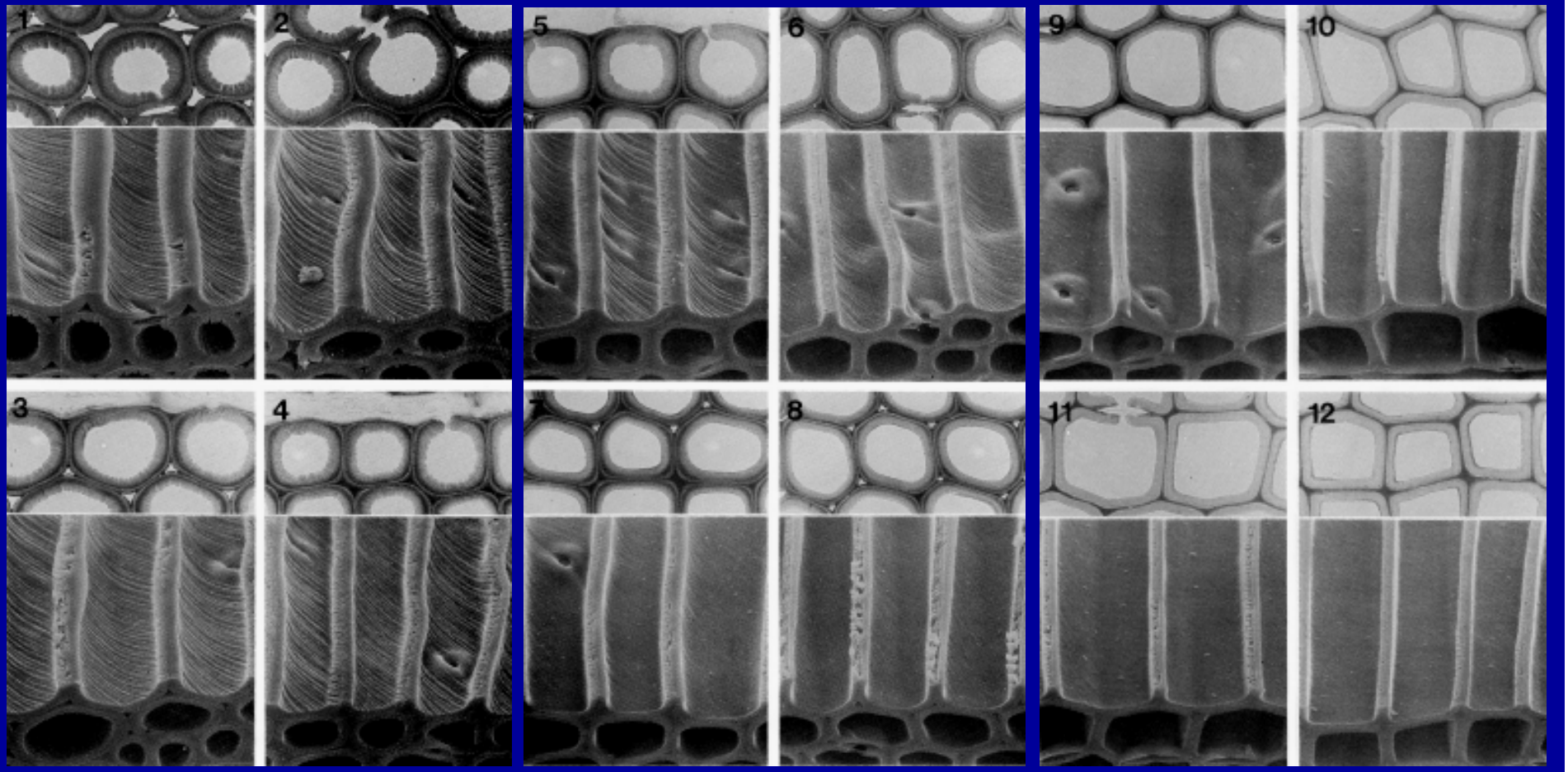






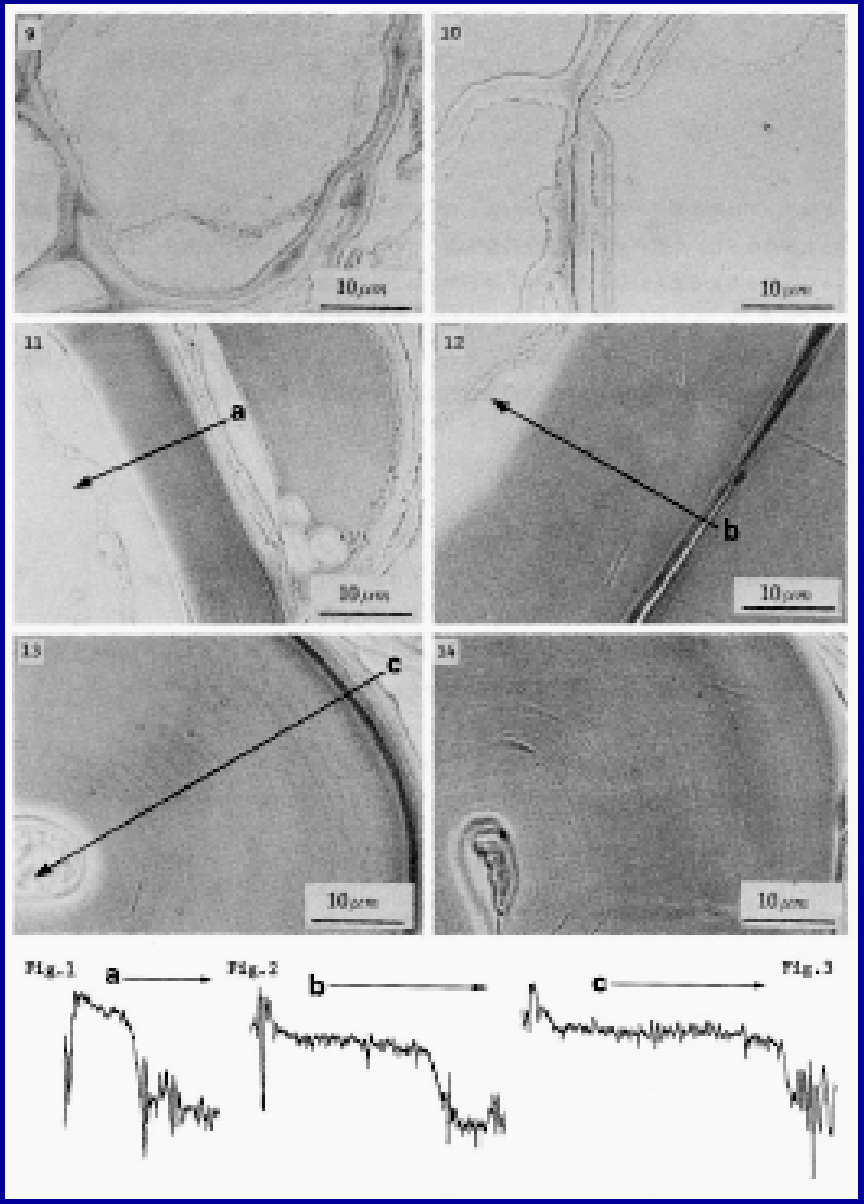
Takabe K. et al.: *IAWA Bull. n.s.* (1992)

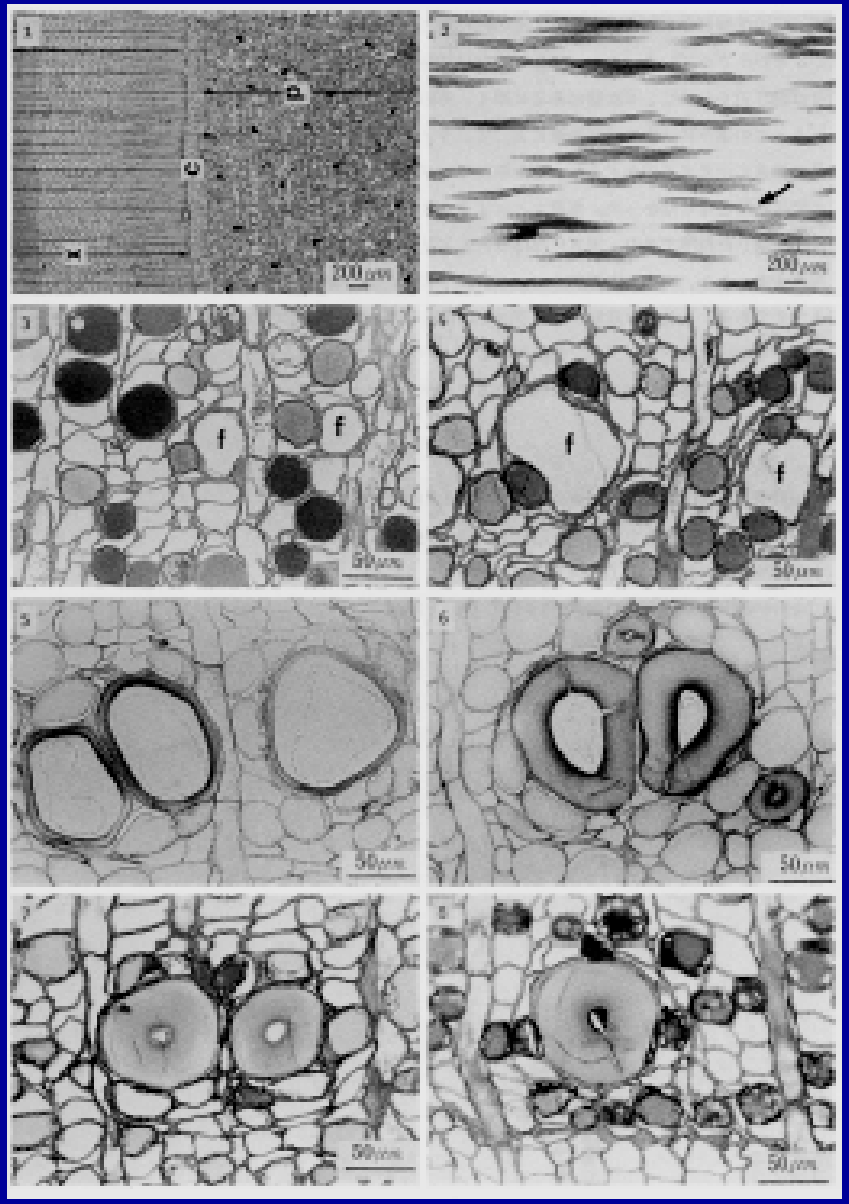
Lignification in compression wood (2)



Yumoto M. et al.: *Res. Bull. Exp. For. Hokkaido Univ.* (1982-83)

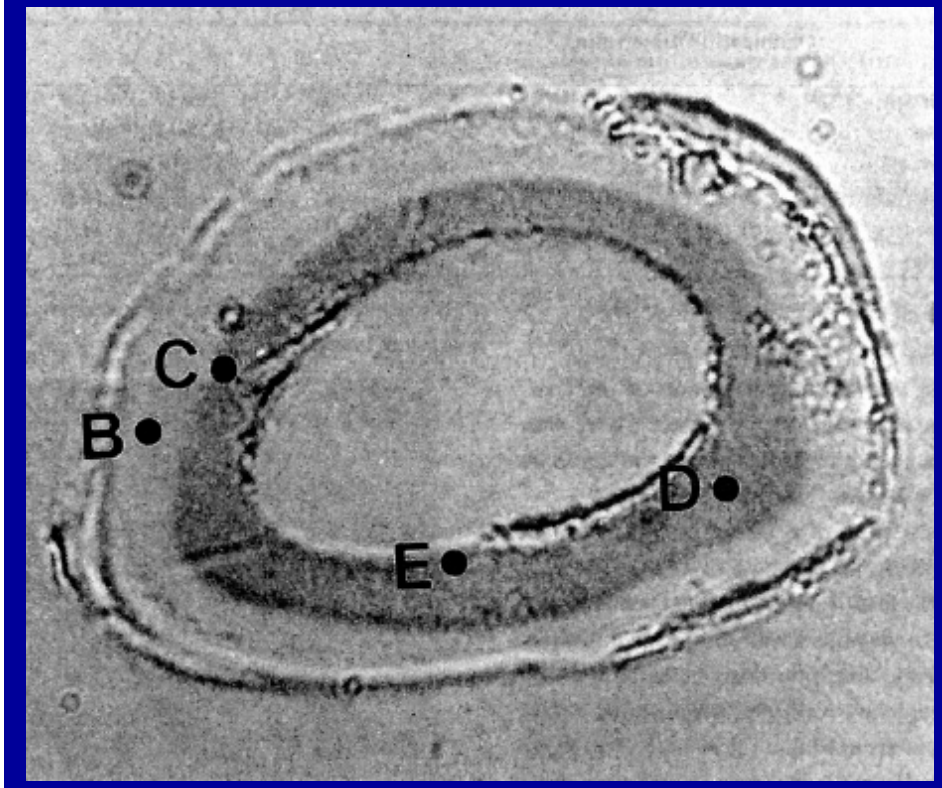
Maturation of phloem fiber sclereid in *Larix*



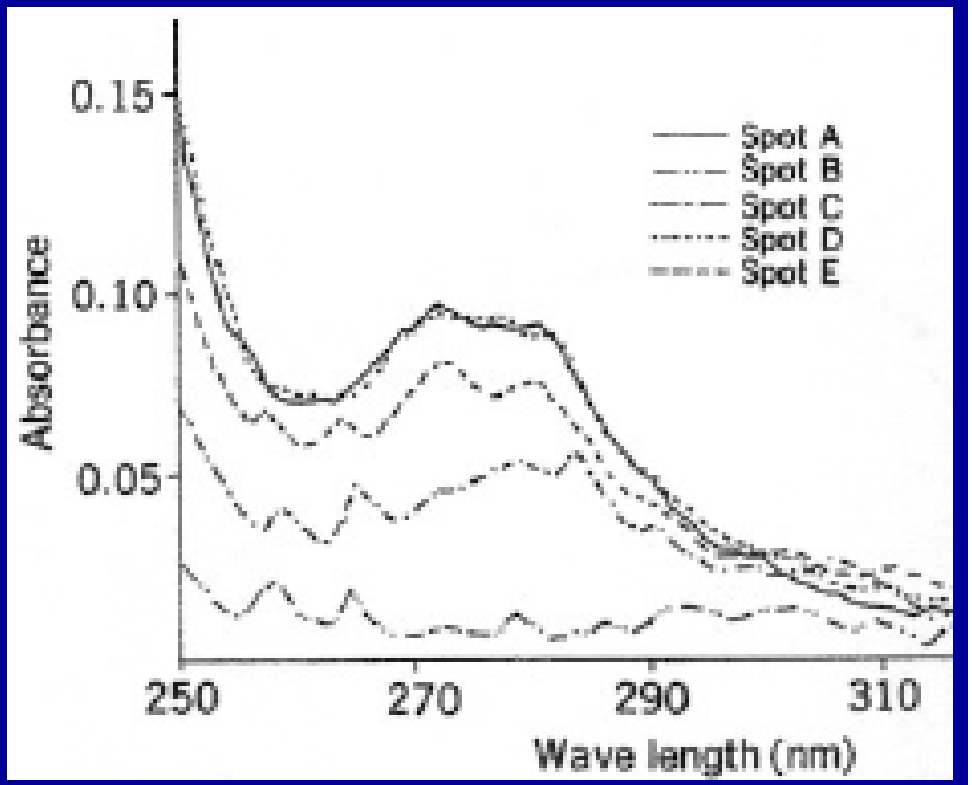


Imagawa H. et al.: *Report from the Grand in Aid for Sci. Res.* (1984)

Dissolution of lignin from the cell wall of pulp fibers



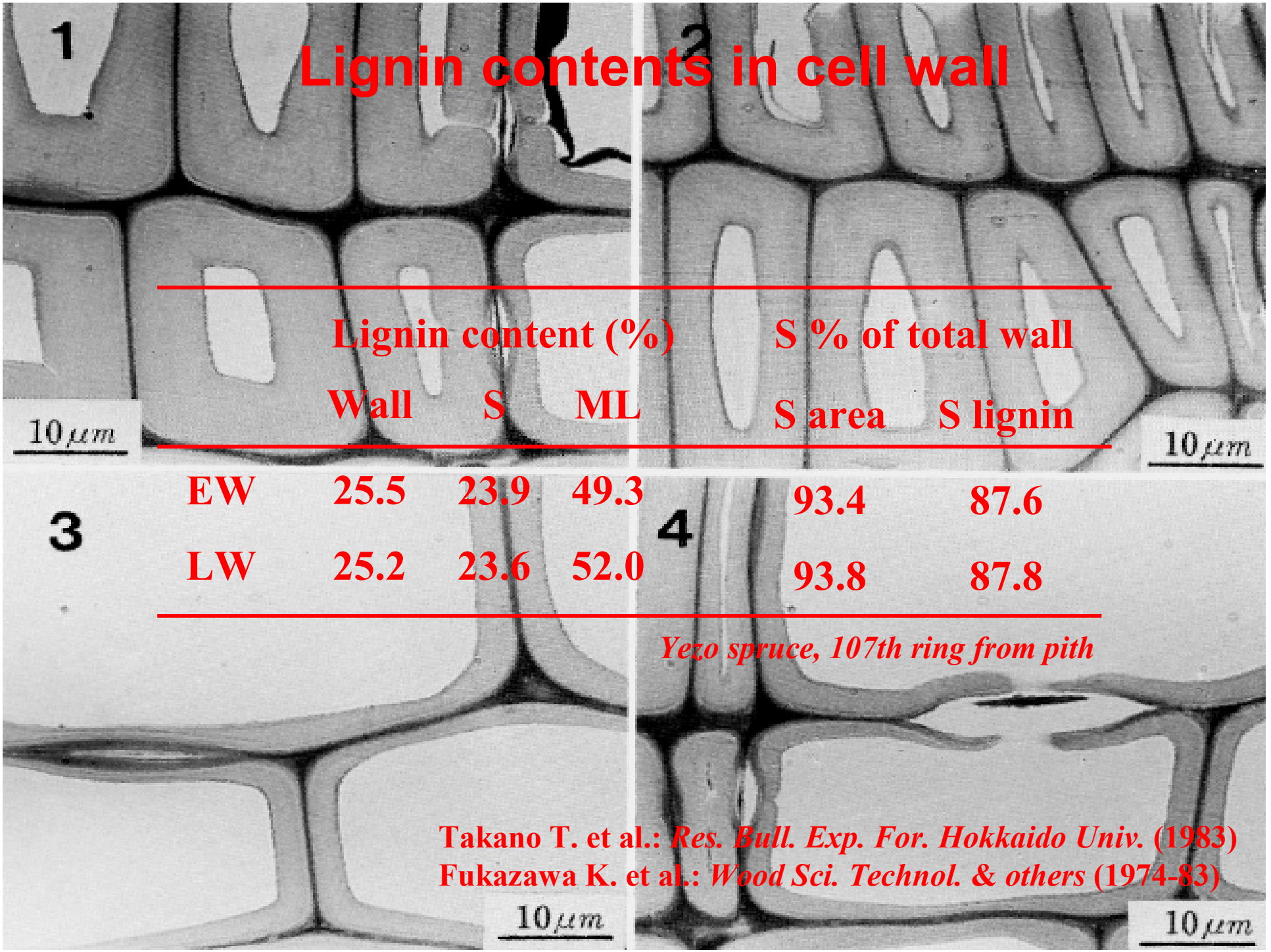
UV micrograph



UV absorption spectra

Kojima Y. et al.: *Mokuzai Gakkaishi & others* (1988)

Lignin contents in cell wall

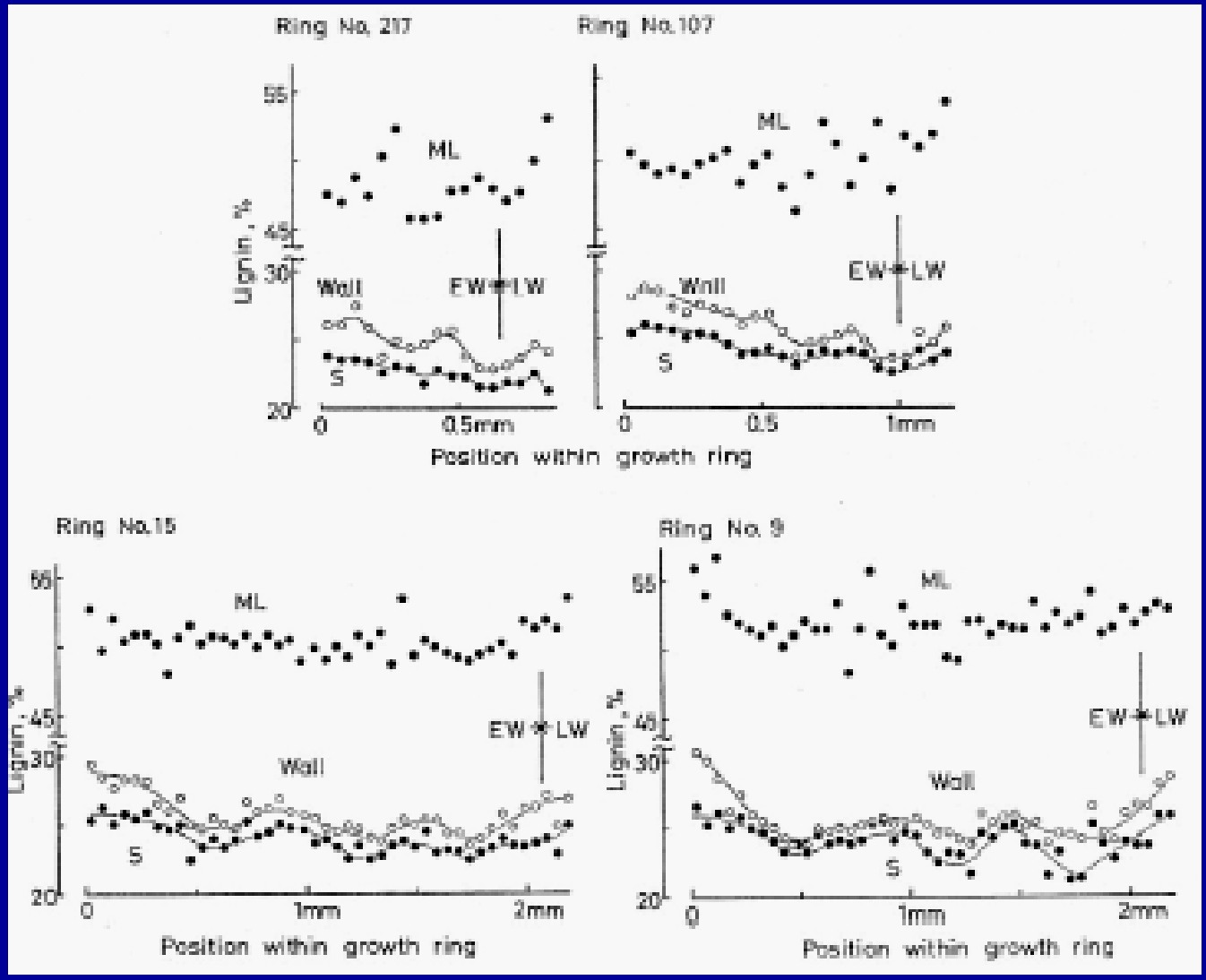


	Lignin content (%)			S % of total wall	
	Wall	S	ML	S area	S lignin
EW	25.5	23.9	49.3	93.4	87.6
LW	25.2	23.6	52.0	93.8	87.8

Yezo spruce, 107th ring from pith

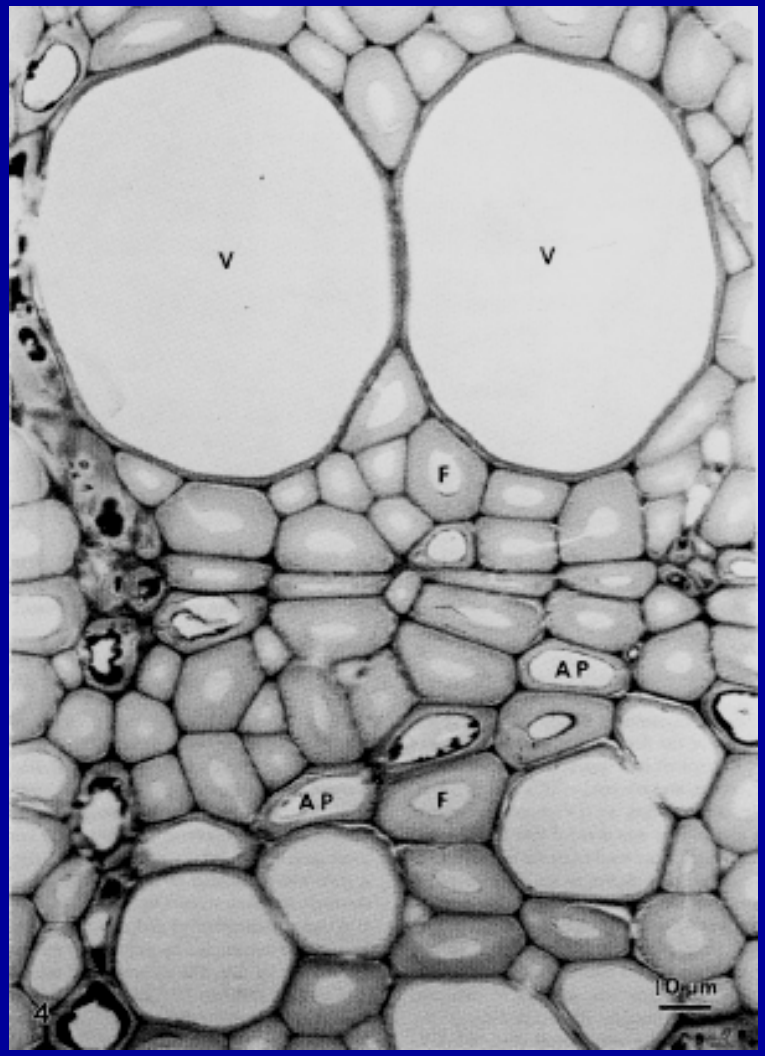
Takano T. et al.: *Res. Bull. Exp. For. Hokkaido Univ.* (1983)
Fukazawa K. et al.: *Wood Sci. Technol. & others* (1974-83)

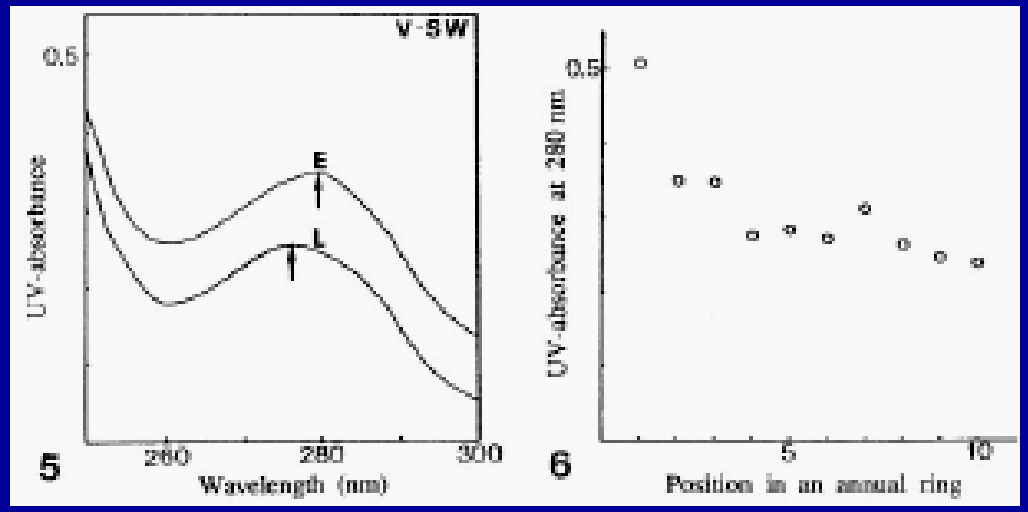
Variation of lignin contents within one growth increment (1)

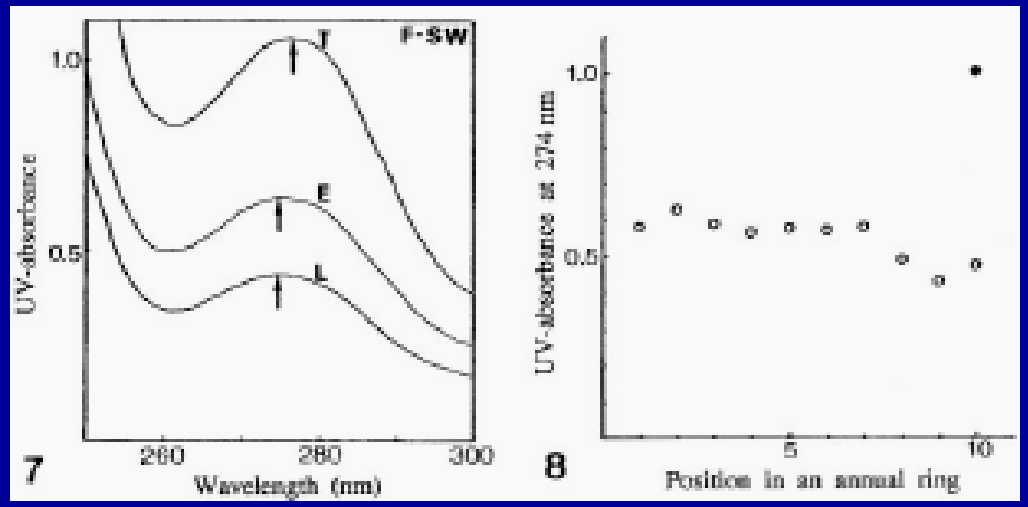


Takano T. et al.: *Res. Bull. Exp. For. Hokkaido Univ.* (1983)
Fukazawa K. et al.: *Wood Sci. Technol. & others* (1974-83)

Variation of lignin contents within one growth increment (2)

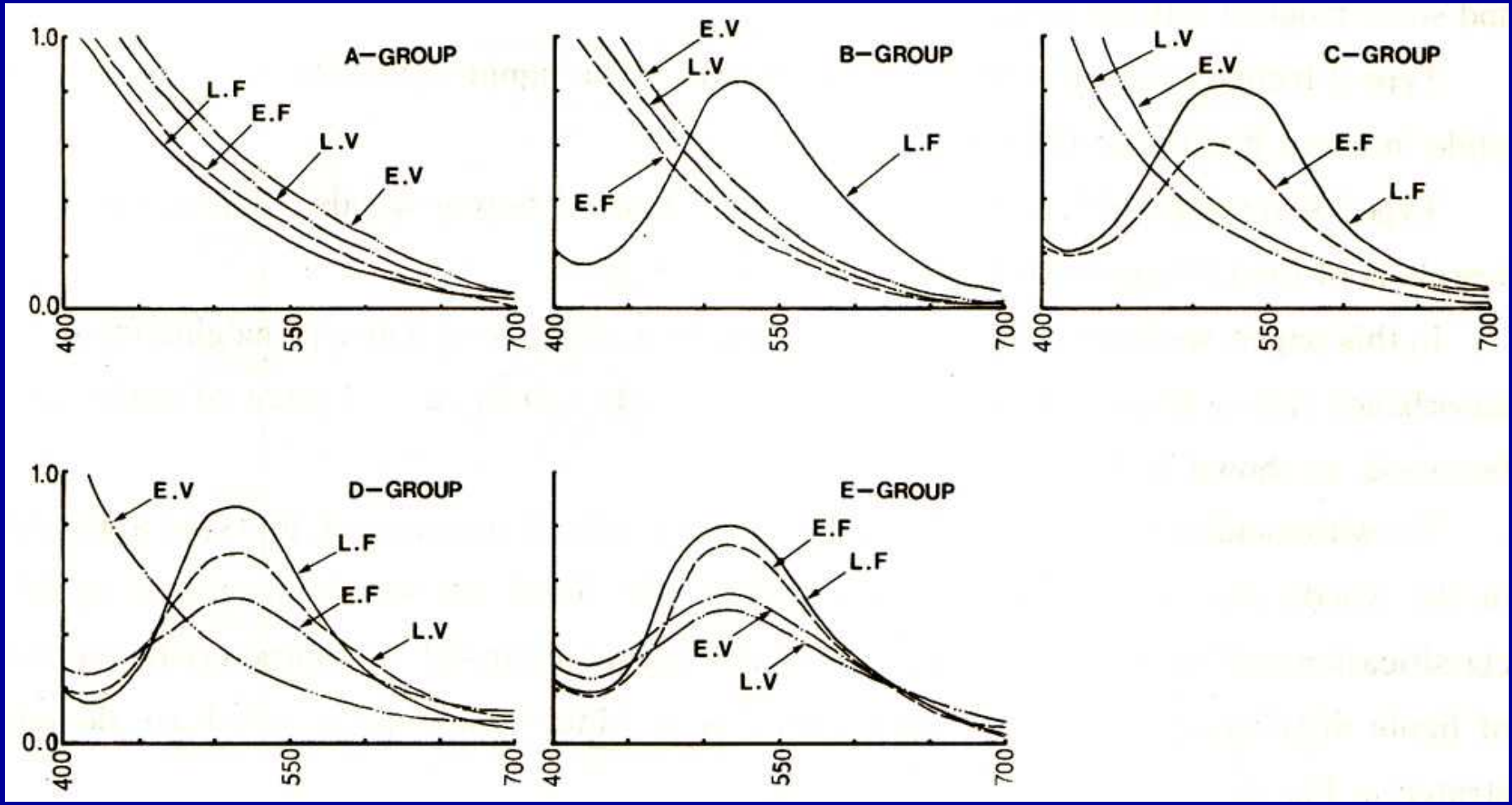






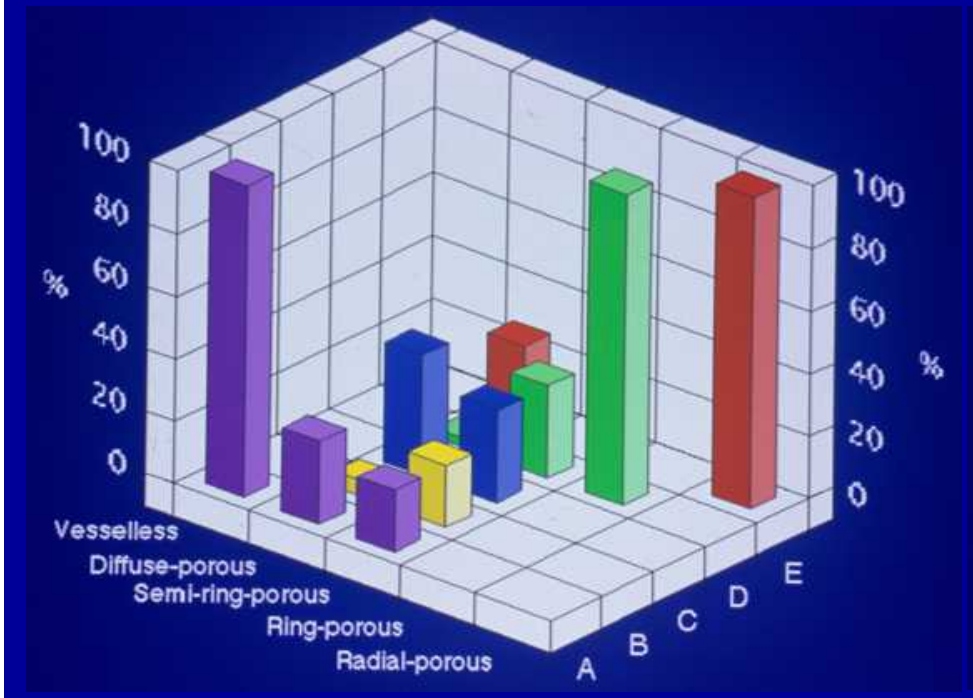
Takabe K. et al.: *IAWA Bull. n.s.* (1992)

The five VIS-spectra models classified by the difference of GS-lignin distribution

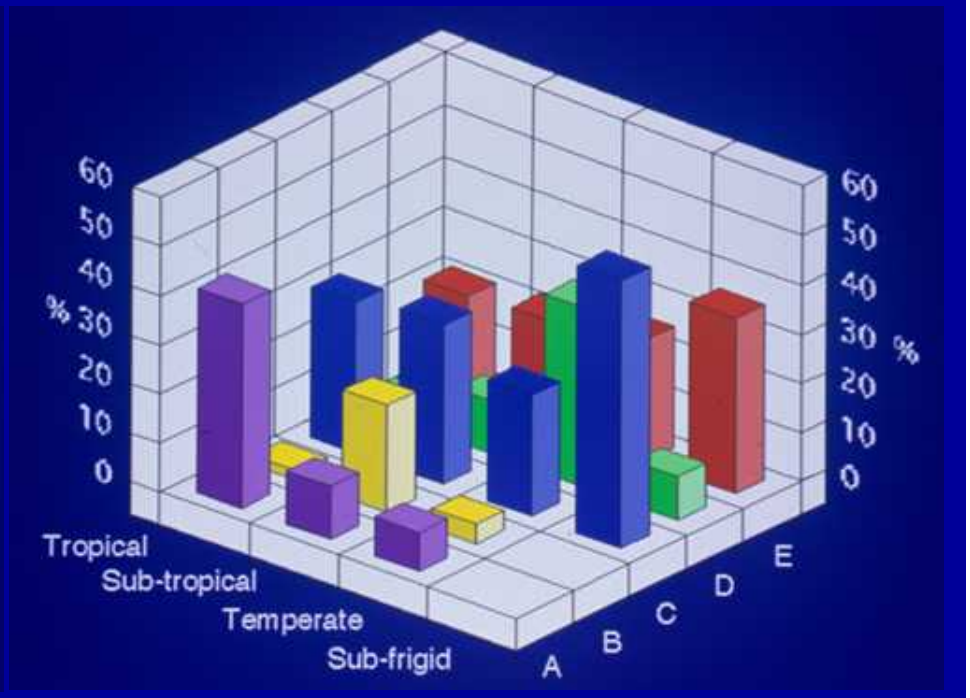


Wu J. et al.: *Holzforschung & others* (1990-93)

Variation pattern of the difference of GS-lignin distribution



porosity

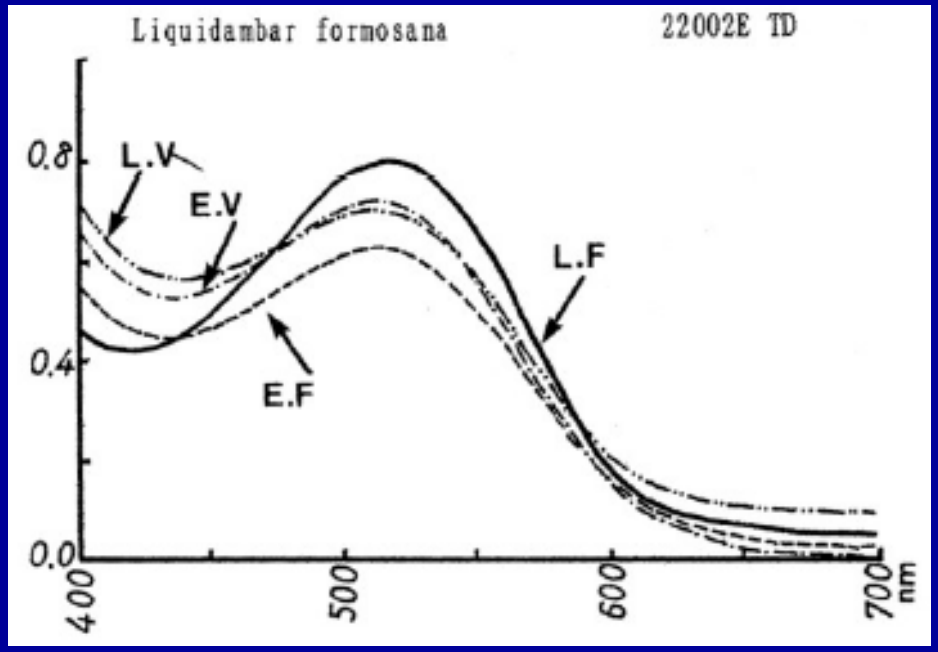


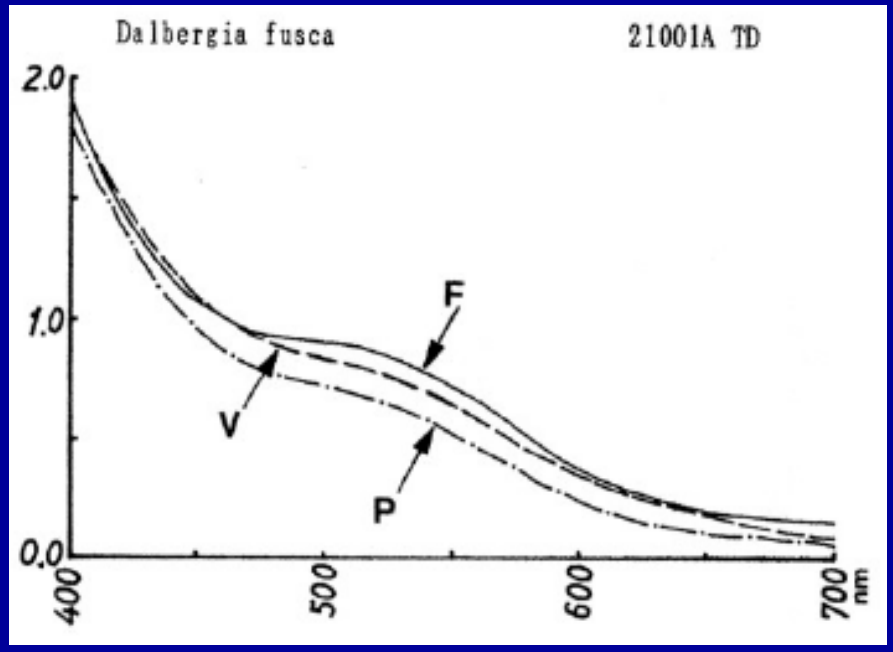
habitat

101 species, Yunnan Province, China

Wu J. et al.: *Holzforschung* & *others* (1990-93)

Variation pattern of GS-lignin related to size of elements and wall thickness



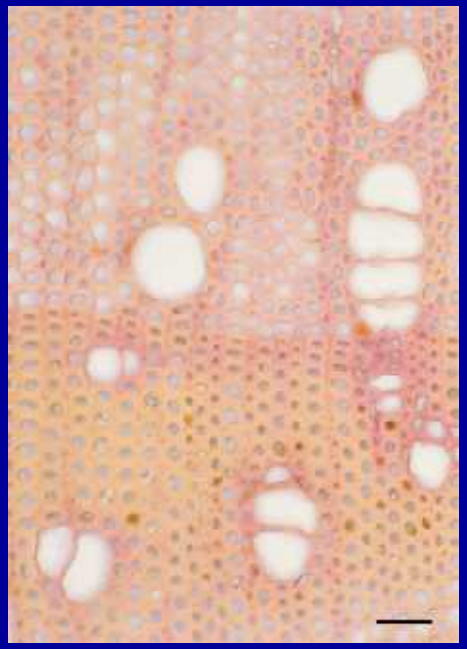


- The more size of elements becomes small, the more S-lignin is rich.
- The more cell wall becomes thick, the more S-lignin rich.

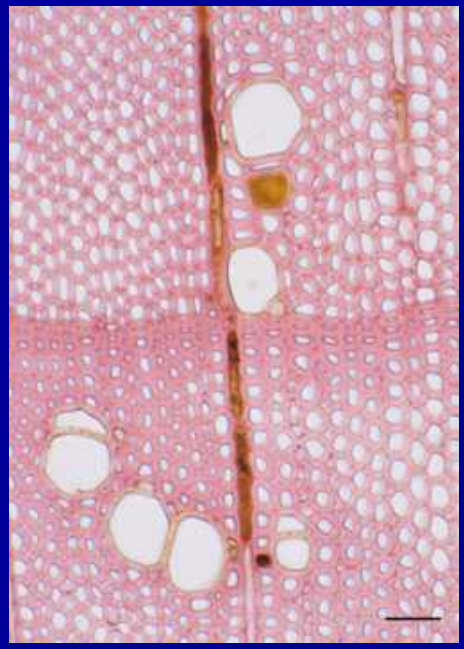
Wu J. : *Memoirs of the Fac. Agr. Hokkaido Univ.* (1993)

Lignin heterogeneity of the cell walls on the genus *Acer*

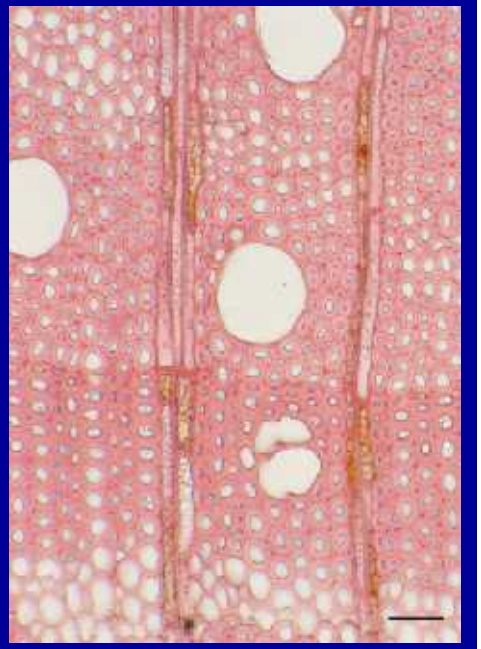
A. carpinifolium
チドリノキ



A. cissifolium
ミツデカエデ



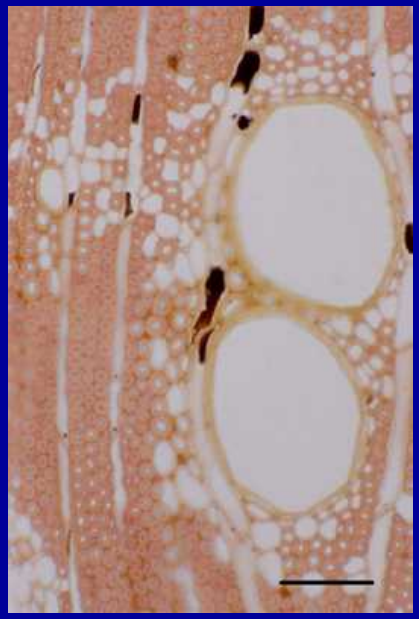
A. micranthum
コミネカエデ



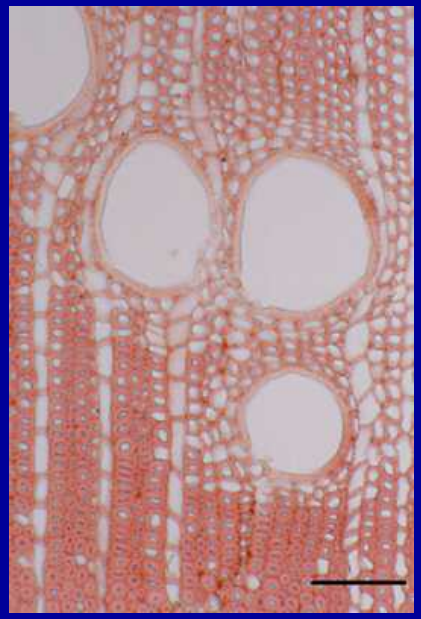
A. japonicum
ハウチワカエデ

Watanabe Y. et al.: *Res. Bull. Exp. For. Hokkaido Univ.* (1993)

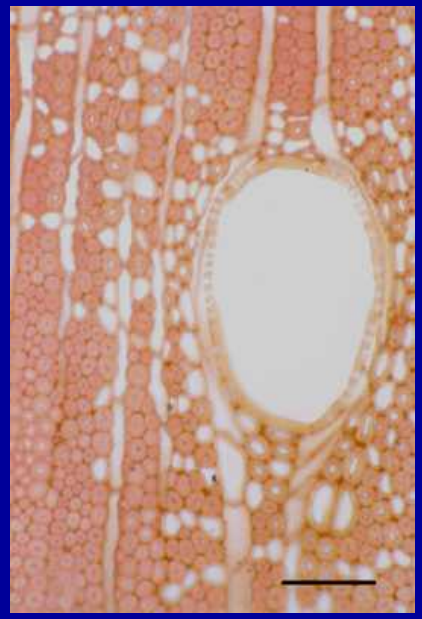
Lignin heterogeneity of the cell walls on the genus *Eucalyptus*



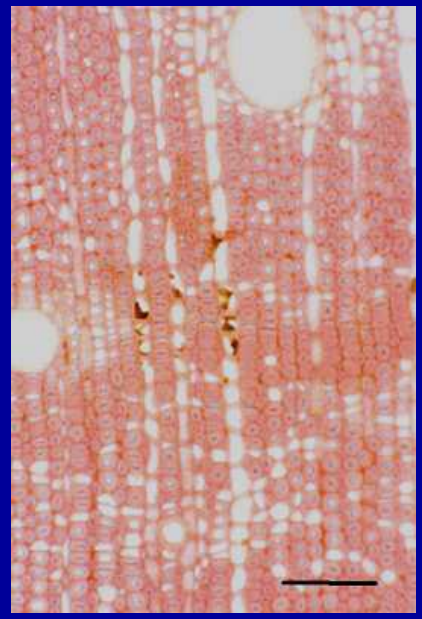
E. camaldulensis



E. nitens



E. macarthurii



E. viminalis

	type 1	type 2	type 3	type 4
F	S	S	S	S
V	G	G	G	S
CC	G	G	S	S
R	G	S	S	S

Watanabe Y. et al.: *IAWA J.* & others (1993-2004)


I thank you very much for your attention.
I also thank Dr. Sano and Dr. Watanabe, Hokkaido University, fellows Funada and Takabe for valuable advice and preparation of lecture materials.