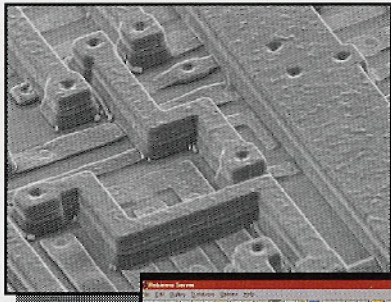


## Retriever32

Makes capturing, storing, finding and printing Digital Images a Breeze



Slow Scan Image captured using the ADS slow scan SEM card. The ADS slow scan card works with most

Retriever32 image database screen view with various



### Features of Retriever32:

- \* Windows '95/'98, NT 4.0/5.0 compliant
- \* User definable Access or Paradox database engine
- \* Keyword or text search for information
- \* Capture images directly into the database
- \* Import raster, vector, and MultiMedia files
- \* Support of PDF and Oxford spectral files
- \* Twain compliant image capture (scanners)
- \* Drag and drop images into other applications
- \* Print any combination of images/page
- \* User definable galleries of images
- \* Slid show, image editor, image conversion tools
- \* Network sharing of images

### Hardware ADS can supply:

- \* High resolution SEM slow scan capture card
- \* Video capture cards and cables
- \* Computers and peripherals
- \* Digital and video cameras

phone, write, e-mail or fax us for a

**Free Trial Demo Disk of**

## Retriever32

ADVANCED DATABASE SYSTEMS, Inc.

2750 S. Shoshone St. #325

Englewood CO 80110

<http://www.adsdb.com> 303-761-5635 fax 303-761-3780

## Detection of *Ophiostoma Piceae* in Radiata Pine using Immunofluorescence Labeling and Confocal Laser Scanning Microscopy

Ying Xiao<sup>1</sup>, Bernhard Kreber<sup>1</sup> and Colette Breuil<sup>2</sup>

1. New Zealand Forest Research Institute Limited, Rotorua, New Zealand

2. Faculty of Forestry, University of British Columbia, Vancouver, Canada

The use of fluorescence microscopy to investigate fungal growth in wood often causes interference due to the strong autofluorescence of wood lignin, unless fluorescent probes which specifically react to fungal hyphae, are used. Techniques to enable differentiation of hyphae from wood have been recently reported (Singh *et al.*, 1997; Xiao *et al.*, 1997). The authors demonstrated that while glutaraldehyde can be used to detect fungal native proteins, wheat germ agglutinin (WGA) which reacts with cell wall chitin of hyphae, considerably improved detection of fungi growing in wood.

Confocal laser scanning microscopy (CLSM), a new technique in wood biodeterioration research, has recently been employed because it produces blur free images and allows optical sectioning across the thickness of the specimen (Xiao *et al.*, 1998). CLSM also enables multi-channel, fluorescent imaging which can be monitored on a big screen and controlled by computer.

In the current study, an immunofluorescence technique was developed to detect *Ophiostoma piceae*, a common sapstain fungus in New Zealand, in radiata pine wood using a monoclonal antibody, 1F3(1), and CLSM. Production and characterisation of the monoclonal antibody used in this investigation, has been described previously (Banerjee *et al.*, 1994).

Wood wafers which were infected with *O. piceae*, were cut into 20  $\mu$ m thick sections using a microtome prior to incubation in 1% (w/v) casein in phosphate buffered saline (PBS, pH 7.4) for 20 minutes to block non-specific antibody binding. Wood sections were then incubated for 1 hour in monoclonal antibody diluted 1:500 in PBS containing 0.01% Triton X-100 (Breuil *et al.*, 1992). Sections were repeatedly washed in water and then incubated in 0.5 mg/mL Oregon green 514 Goat anti-mouse IgG (H+L) fluorescent dye (excitation 511 nm, emission 530 nm; Molecular Probes, Inc., Eugene, OR) in the dark for 1.5-2 hours. After four washes in water, sections were individually mounted in glycerol on glass slides with a cover slip and the edges were sealed with nail varnish. Microscopic examination was performed using a Leica TCS NT CLSM with an Argon-Krypton laser excitation source (488/568/647 nm). Images were captured in green channel through a band pass filter BP 530/30 nm and in red channel through a long pass filter LP 590 nm using a 63x oil lens with numerical aperture setting of 1.4. Digital image stacks obtained on

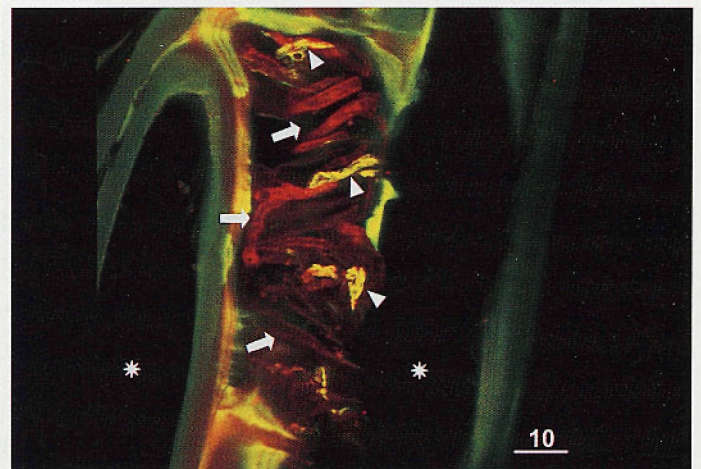


Figure 1: Dual channel projection in maximum intensity mode of an image stack with 2.4  $\mu$ m thickness. Because the colour of the green and red channel were inverted fungal hyphae appear in red (arrows), and wood cell walls in yellow-green. Arrowheads indicate wood extractives and asterisks wood lumina. Bar = 10  $\mu$ m.

CLSM were transferred to a SGI Indy workstation and processed using Image Space™ software (Molecular Dynamics Inc.). Dual channel projection was obtained by combining the images collected of two channels and image slices were composed together using Volume Workbench.

This investigation showed that immunofluorescent labeling yielded strong fluorescence of *O. piceae* in the green channel whereas wood strongly autofluoresced in both, green and red channels. Therefore it was possible to differentiate hyphae of *O. piceae* from wood using a dual channel projection (Figure 1). Furthermore, the immunofluorescence labeling technique described enabled us to distinguish readily between *O. piceae* hyphae and any autofluorescing of wood extractives which were associated with ray parenchyma cells. This latter differentiation is of particular importance in investigations on the early stages of colonisation when *O. piceae* preferentially invades ray parenchyma cells where readily accessible nutrients are present.

Immunofluorescence labeling of additional sections which were infected respectively with other sapstain fungi, such as *Diolodia* sp, *O. piliferum* *O. floccosum*, was performed using the procedure described above. It failed to detect fungal hyphae in these sections. However WGA staining, which was subsequently performed on the same sections, confirmed their presence.

In conclusion, the immunofluorescence labeling technique described in this paper does not involve any fixation and dehydration of wood sections; therefore damage which often occurs during sample preparation is minimized and the inconvenience of reducing possible autofluorescence induced by glutaraldehyde, avoided. Furthermore, the technique is easy to carry out and provides a useful tool for specific detection of *O. piceae* in radiata pine. The method described herein is currently being used to investigate fungal interactions between *O. piceae* and other sapstain fungi as well as the effect of fungicides on development of *O. piceae*. ■

Breuil C., B.T. Luck, L. Rossignol, J. Little, C.J. Echeverri, S. Banerjee and D.L. Brown. 1992. Monoclonal antibodies to *Gliocladium roseum*, a potential biological control fungus of sapstaining fungi in wood. *J. Gen. Microbiol.* 138:2311-2319.

Banerjee, S., J. Little, M. Chan, B.T. Luck, C. Breuil and D. L. Brown. 1994. Production and characterization of monoclonal antibodies to the sapstaining fungus *Ophiostoma piceae*. *Can. J. Microbiol.* 40:35-44.

Singh, A. P., Y. Xiao and R.N. Wakeling. 1997. Glutaraldehyde autofluorescence useful in confocal studies of fungi. *Microscopy in Focus, Newsletter for Microscopy New Zealand Inc.* No.2.

Xiao S.Y., A.P. Singh and R.N. Wakeling. 1997a: Detection of fungal hyphae in wood using a chitin selective fluorescent probe. *Microscopy in Focus, Newsletter for Microscopy New Zealand Inc.* No.3.

Xiao S.Y., A.P. Singh and R.N. Wakeling. 1998. Using Confocal laser scanning microscope and post image processing technologies to describe fungal attacking mode in wood. In proceedings of the 15<sup>th</sup> Australia conference for Electron Microscopy, Hobart, Australia, Abstract, p.61.

## VACUUM FORELINE TRAPS

.....For a cleaner SEM/TEM

- Easy to install
- Available in Aluminum and Stainless Steel
- Replaceable elements

M.E. TAYLOR ENGR., INC.  
21604 Gentry Lane  
Brookeville, MD 20833  
Phone: 1-301-774-6246  
FAX: 1-301-774-6711  
E-Mail: Metengr@aol.com

# First, a High Quality, Desktop Cold Sputter/Etch System.



# Now, Guaranteed Next Day Shipment.

Order Denton's standard Desk II System by noon today and get guaranteed shipment tomorrow or Denton pays the freight.

The Desk II produces uniform, conductive, fine grain Au/AuPd coatings in under three minutes and is the highest quality desktop system available.

### The Desk II features:

- Automatic or manual operation
- Easy to read digital vacuum and current gauges
- Optional carbon evaporation accessory

For more information, look no further than Denton... where both Quality and Delivery invite comparison.

**DENTON  
VACUUM  
INC.**

1259 North Church St.  
Moorestown, NJ 08057 USA  
Tel: (609) 439-9100  
FAX: (609) 439-9111

★ **MADE IN AMERICA**

Next day shipment must be requested at time of order.

**Decades of Experience In EM Specimen Prep Equipment**