Handling Creutzfeldt-Jakob Disease Tissues in the Histology Laboratory

Michael Titford and Frank O. Bastian
University of South Alabama

Creutzfeldt-Jakob disease (CJD) resembles two other diseases, kuru (found in New Guinea tribesmen) and scrapie (found in sheep). CJD is a slow progressive dementia of the central nervous system. The transmissible agent of CJD is unusual for several reasons: It has a long incubation period, up to eight years in some cases; it does not provoke an inflammatory reaction within infected tissues; it has never been isolated; it is resistant to routine paraffin processing. Histotechnologists need to be informed of the potential hazards and of the correct handling of tissues infected with this unconventional agent, even though it has low transmissibility. Treatment of CJD tissues with long sterilization at high temperatures or prolonged exposure to bleach has proven effective in deactivating the agent. This article outlines the precautionary procedures used in our laboratory when processing CJD tissues. (*J Histolechnol* 12:214, 1989).

Introduction

The histology laboratory must give careful attention to the handling of infectious tissues because of the rising incidence of AIDS and new awareness of other infectious diseases. The College of American Pathologists and other medical laboratory inspection agencies also expect increased precautions as a requirement for accreditation.

Creutzfeldt-Jakob disease (CJD) is one infectious disease requiring histological examination of tissues for diagnosis. This progressively fatal disease of the central nervous system affects 1-2 people per million each year worldwide; therefore, 1 to 2 cases may occur in a large metropolitan area per year. Of interest to the histotechnologist, the infectious agent is resistant to formalin fixation, alcohol dehydration, paraffin embedment and moderate temperatures[1]. As a result, the paraffin block and tissue section are potentially infectious.

This article describes the disease and outlines the precautions to be followed when handling CJD tissues in the histology laboratory.

The Transmissible Spongiform Encephalopathies

These chronic brain infections have been categorized together because of the unusual vacuolar encephalopathy they exhibit. These diseases include CJD and kuru in humans, scrapie in sheep and goats, and transmissible mink encephalopathy (TME).

Creutzfeldt-Jakob Disease

The patient with CJD presents with a progressive dementia that may include personality changes, abnormal EEG, and jerking movements. The neurological findings in CJD may mimic other brain diseases like Alzheimer's disease. Fifteen percent of cases of CJD occur in families with a history of dementia[2]. Laboratory tests in CJD patients are generally normal and a brain biopsy is essential for diagnosis. Whereas most patients with CJD are in their sixties, patients as young as 17 years have been reported[3]. Death usually occurs within one year of diagnosis.

The classical pathological findings on brain biopsy are characterized by a fine meshwork of vacuoles in the grey matter accompanied by astrocyte proliferation, fibrillary gliosis, and loss of neurons (Figure 1). Transmission electron microscopy (TEM) shows typical membrane changes. The meshwork of holes in the brain as shown by light and TEM has rendered the disease the more descriptive title of spongiform encephalopathy.

There are several unusual features about the disease. The incubation period is long, up to 8 years in some cases. The agent responsible has never been satisfactorily isolated, and no inflammatory process is seen in diseased brain tissue.

Historically, kuru was first described in 1957 and is confined to the Fore people of the Eastern Highlands of New Guinea. The disease was passed down, generation to generation, by ritualistic cannibalism, during which the women and children ate the brains of the deceased[4]. During the 1950s, 90% of deaths among the women of the tribe were attributed to this disease[5]. With the cessation of cannibalism, the disease began to die out; it now affects only adults born before 1959. A study has shown that the incubation period for kuru ranges from 4-30 years[4]. Symptoms and pathology of kuru resemble CJD.

Scrapie

Scrapie is a spongiform encephalopathy of sheep and has been known to be transmissible for over 100 years. The name derives from the habit of sheep to rub fleece from their bodies against fence posts, wire, or trees during the clinically visible period of the disease. Other symptoms include progressive ataxia, lack of coordination, tremors, wasting, and itching. The pathology of scrapie closely resembles CJD[6].

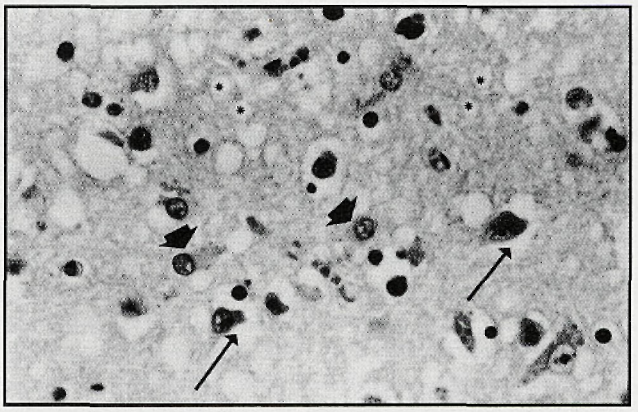
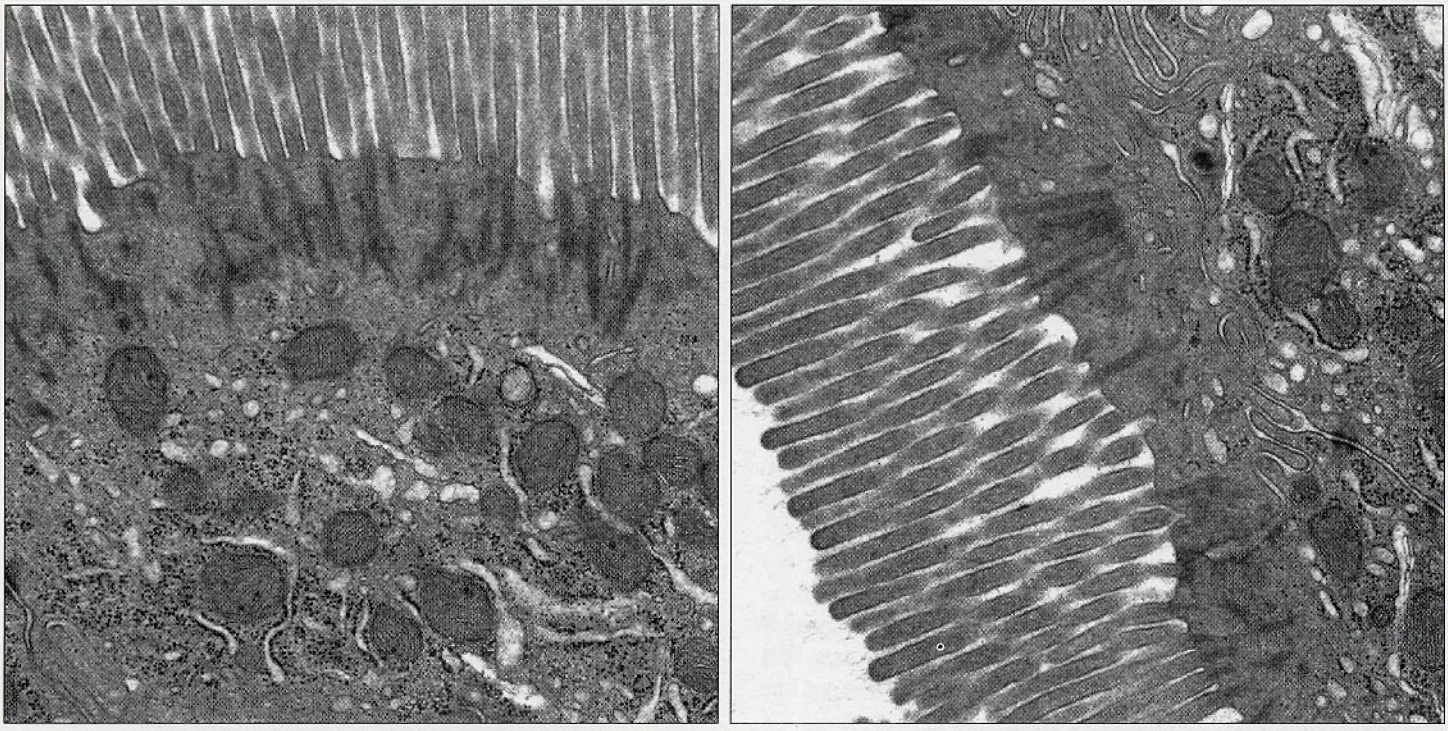


Figure 1: Typical histological findings in CJD include perineuronal vacuolization (long arrow), hypertrophic astrocytes (short arrows), and spongiosis of neurophil (stars). Lipofuchsin accumulation within neurons often accompanies these changes.

Continued on page 24

TV-rate Speed and Digital Image Capture in One Camera



Canine brush border acquired with Gatan's new DualView CCD Camera at 120 kV.

DualView

Digital Acquisition and TV-rate viewing in one low cost package

The Gatan DualView CCD camera is the only high resolution, high contrast camera designed to view both video-rate and acquire high quality digital images.

Superior design. The lens-coupled, cooled CCD with 1039 x 1300 pixels, 12-bit resolution, and a larger field-of-view than film.

TV-rate imaging. Quickly scan the sample in TV mode to find the area of interest at up to 15 frames per second.

Rotatable Flange. The only retractable 35mm port camera that can be rotated 360° to orient sample features in the image window for best presentation.

Choose your imaging solution. The DualView runs on both the Power Macintosh and Windows platforms.

Increase productivity, lower costs. Real-time image viewing allows easy optimization of imaging conditions and reduces darkroom costs. Save only the images you want and archive them for online retrieval.

NEW! DigitalMicrograph 3.x software. Use the industry leading image processing tool, with new image analysis tools and enhanced page layout capabilities, to acquire and process images at the microscope.

Online teaching. The large monitor display and fast read-out is ideal for group demonstrations and presentations.

For more information on the new DualView camera, contact Gatan or a Gatan representative for more information about the new CCD Camera for TEM.

gatan

Gatan Inc.
Tel 510 463 0200
Fax 510 463 0204

Gatan Inc.
Tel 412 776 5260
Fax 412 776 3360

Gatan GmbH
Tel (089) 352 374
Fax (089) 359 1642

Gatan Ltd.
Tel 01536 743150
Fax 01536 743154

Gatan Inc. (Hong Kong)
Tel: 852 2658 6003
Fax: 852 2658 6002

Nippon Gatan (Japan)
Tel: 81-03-5387-3585
Fax: 81-03-5387-3585

Gatan Online
http://www.gatan.com
info@gatan.com
help@gatan.com

Circle Reader Inquiry #10

Handling Creutzfeldt-Jakob Disease Tissues in the Histology Laboratory - Continued from page 22

Spongiform encephalopathies with similar symptoms have been reported in other animals. Transmissible mink encephalopathy was first reported in 1950 in Wisconsin and presumably results from the minks' feeding on scrapie infected sheep. Captive mule deer and white tigers have been documented to have contacted a similar disease process[6].

The Nature of the Transmissible Agents(s)

The infectious agents of transmissible spongiform encephalopathies have not been isolated. The usual method researchers have used to study these diseases is to inoculate laboratory animals with infectious tissues. CJD has been transmitted to chimpanzees, guinea pigs, hamsters and mice by these means. Similarly, kuru has been transmitted to chimpanzees, and scrapie introduced into mice, goats and rats[6].

Unconventional characteristics of the Agent

In these transmission experiments, there is an unusually long incubation after the infectious tissue is introduced into a new species of animal. The incubation time may be reduced by the mode of infection, which will determine how long the incubation will last and which symptoms will occur. Intramuscular, abdominal, or subcutaneous inoculation will result in longer incubation periods and different symptoms than by intracranial inoculation[6]. Overall, the same type of infectious agent is responsible for CJD, kuru, and scrapie. Variants of the same organism and a genetic predisposition to infection may result in different clinical presentations. The presence of infectious agent or its concentration is detected in the animal model by a method known as titration. This inexact assay method is expensive and time consuming.

For his studies in this field, Gajdusek was awarded the Nobel Prize for Medicine in 1976. In spite of a great amount of research, the mode of transmission outside the laboratory has not been clearly established. In animals infected with scrapie, it is possible that disease is transmitted via contamination of bites, scratches, or by the eating of placenta. The route of transmission of CJD in humans is unknown. Spouses of CJD patients rarely contract the disease in spite of close contact [3].

Theories About the Identity of the Agent

Up to this time, the agent causing CJD, kuru and scrapie has not been identified. Reports of finding virus and spiroplasma (a mycoplasma-like organism) in CJD diseased brains are awaiting confirmation[7,8]. Today, fibrillar proteins found in CJD and scrapie brains and not in controls represent a morphological marker of the transmissible agent[9]. It is of interest that these infection-related proteins resemble fibrils found in spiro-plasma[10]. CJD fibrils and spiroplasma react consistently immunologically with scrapie antibodies[11].

Because of the long incubation period of CJD, the term "slow virus" has been applied, meaning the infectious agent lays dormant for a long period and is filterable through a 220 nm filter that removes bacteria. In reality, because no virus has been identified and other microorganisms like mycoplasmas are also filterable, "slow infectious agent " would be more applicable[6].

Using brain material from CJD, kuru, and scrapie cases, researchers have determined that the size of the agent is between 27nm and 47nm[12]. Having never found an infectious agent for these diseases, researchers can only speculate as to its structure. Besides virus and spiroplasma, another theory promotes the idea of a new infectious particle, the prion, composed only of protein, without nucleic acid[13]. However, recent studies of the agent suggest that it could be a conventional microorganism without need of heretical concepts[14].

Dangers of Handling CJD Tissues in the Histology Laboratory

For the histotechnologist, the most worrisome aspect of this group of diseases is the resistance of the organism to inactivation. CJD is resistant to formalin and other aldehyde fixatives, alcohol, moderate heat (up to 100°C), ultraviolet radiation, and ionizing radiation[3,6]. Early studies created a great deal of fear in the medical community, in the setting of which theories like "the prion" evolved. Subsequent evaluation of these early studies showed the transmissible agent to be resistant to treatments similar to unknown viruses and bacteria. Even so, studies that show transmission by paraffin processed materials indicate that precautions should be taken until more is known about the agent. A controlled handling of the tissues is acceptable rather than a hysterical approach.

Transmissibllity in Humans

Cases of infection have occurred following medical procedures. In one case, electrodes implanted in the brain of a CJD patient were removed, gas sterilized, and used again in a second patient who later contracted CJD[15]. Another case occurred following corneal transplant, where the donor was shown to have CJD at necropsy[16]. Four cases were reported to develop CJD following administration of growth hormone that had been processed from pituitaries[3]. Recently, two cases of CJD have occurred in histopathology technicians. In one case, a 62 year old woman contracted the disease following a 22 year career in a neuropathology laboratory[17]. Her duties involved histological technique but not the gross brain cutting. In another case, a 75 year old man who had been exposed to infected animal and human brains during his laboratory career contracted the disease[18]. Although the disease could be contracted during their work, the incidence of CJD among the medical community is reported to be no higher than in general population[4].

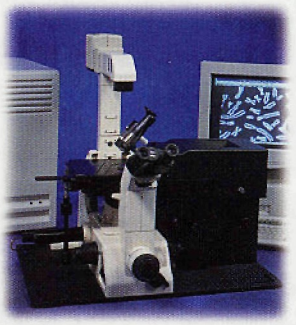
Precautions for the Histotechnologist

In this laboratory, a local referral center for CJD, brain specimens are received as part of a research protocol in two parts, fresh frozen and formalin fixed, accompanied by serum and cerebrospinal fluid (CSF). The fresh frozen

Continued on page 26

TopoMetrix AFMs Get Rid of the Limits

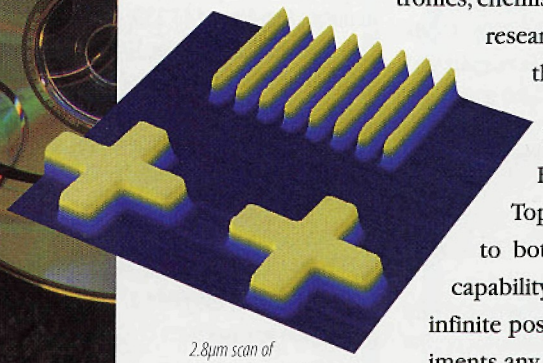
To Nano-World Research.



Yesterday, there were finite limits to angstrom- and nanometer-level research. But today, those limits are gone.

Whether you're examining the structure of complex composites or the interaction of biomolecules in solution, TopoMetrix' complete line of scanning probe microscopes (SPM) knows no boundaries. Think about topography, thermal conductivity, chemistry, electro-chemistry, and magnetic fields. Or near-field optical and optical contrast mechanisms, like polarization, fluoroscopy and spectroscopy. These are just some of the impressive capabilities of TopoMetrix SPMs. And they certainly help get rid of limits, don't you think?

But can a TopoMetrix SPM really help you in your research? There's a good chance one can, if you're involved in biotechnology, materials research, microelectronics, chemistry, mass data storage, or any of the basic or applied research disciplines where observing microstructures is the key to understanding.



2.8μm scan of NIST Standard SRM-209A with 200nm pitch.

Unlimited is a good description of TopoMetrix SPMs. But infinite might be even better. For example, TopoMetrix' unique open architecture concept applies to both hardware and software. This industry-exclusive capability provides scientific investigators just like you with infinite possibilities to configure their systems and their experiments any way they desire.

Hundreds of organizations throughout the world use TopoMetrix SPMs, every day. Top industrial companies. Leading universities. Major government laboratories. And respected independent researchers. Organizations and scientists worldwide who have learned to work without limits.

Sound promising? Just call, e-mail, fax or write today. We'll show you why so many scientists in so many industries engaged in so many different branches of science select TopoMetrix SPMs. You'll see that their reasons are...well, unlimited.

TOPOMETRIX
EXPLORING THE NANO WORLD

5403 Betsy Ross Drive
Santa Clara, CA 95054-1162

Tel: 408.982.9700
Fax: 408.982.9751

E-mail: inquiry@topometrix.com
Web: www.topometrix.com

Circle Reader Inquiry #8

Handling Creutzfeldt-Jakob Disease Tissues in the Histology Laboratory - Continued from page 24

brain, serum, and CSF are sent to the research laboratory. The formalin fixed brain is processed in the histology laboratory using special procedures. Brain biopsies from suspected CJD patients are also received and processed in the same manner.

Gloves are worn at all stages of handling CJD tissues and during sectioning and staining. Autopsy brains and brain biopsies are grossly examined and dissected using the Baxter infectious tissue kit (Baxter #D3500-1, Scientific Products Division, Baxter Healthcare, McGraw Park, IL). Specimens selected for histology are placed in green prelabled cassettes. In this laboratory, green cassettes are reserved for infectious specimens. Remaining tissue is replaced in formalin, double bagged, and appropriately labeled with the patient's name, case number, and with an "infectious" label.

Histology specimens are processed separately using a single layer of a Technicon Duo (Technicon, Tarrytown, New York) programmed with a 12-hour schedule. Tissues are embedded normally and sectioned with a microtome reserved for CJD specimens. Additional sections are cut at the time of sectioning for possible Bodian and Congo red stains.

Clean Up

Following each step of the procedure, the area is cleaned and sterilized using 10% commercial bleach. This is prepared by mixing 1 liter of commercial bleach *(5.25%* sodium hypochlorite) with 9 liters of water. Bleach has been shown to be an effective agent to chemically destroy the CJD organism[19]. The infectious tissue kit used for gross examination, trimmed wax from microtomy, waste sections, gauzes, gloves, etc. are incinerated.

Processing fluids from the tissue processor and stain solutions are placed in one gallon plastic bottles, appropriately labeled, and sent to the institution's environmental safety officer for disposal. Alternatively, fluids and instruments can be steam autoclaved for 1 hour at 132°C (15 psi)(20). Fluids can then be discarded normally. They should not be reused[4]. Containers and baskets from the tissue processors, forceps, moulds, etc. are each soaked for 1 hour in 10% in bleach, washed well in running water, and dried. This will cause some minor corrosion of the metal wax pots, but the sacrifice is worthwhile.

It should be noted that modern enclosed tissue processors use internal tubing and parts that are not readily sterilized. The older model Technicons lend themselves to sterlization.

Following sectioning, the exterior of the microtome and flotation bath are wiped with bleach, then with water, and dried. Moving surfaces on the microtome are lubricated. Disposable microtome knives are incinerated. Due to the complex mechanical nature of the microtome, a thorough cleaning of the microtome is not possible and it is always considered infectious[3].

Conclusion

CJD is a rare central nervous system disease caused by an organism that is infectious but has low transmission[21]. Histotechnologists need to be aware of the disease and the precautions to be used when handling the tissues. With aseptic technique, sterilization of areas with bleach, and disposal of processing and staining fluids, risk of infection is low.

The greatest danger to the histotechnologist handling CJD tissue is when a diagnosis has not been established and is unknown to physician and technologist alike.

Some reports have recommended that tissues be autoclaved for one hour at 132°C following formalin fixation and prior to processing[21]. The combination of fixation and sterilization is believed to inactivate the organism. Staining techniques appear to work well with this protocol, but the high heat precludes the use of immunoperoxidase technique afterwards. Another method recommends fixation for 48 hours in phenol-formalin, with three weeks fixation for autopsy specimens[22]. Another method recommends mixing bleach in intital formalin fixative[23]. These modifications are unnecessary when the histotechnologist handles the tissues as outlined above. As always, the histotechnologist must handle all tissues as if they were potentially infectious. ■

Acknowledgement: The authors wish to thank Janice Brown for secretarial assistance.

References

1. Brown P, Gibbs CJ, Gajdusek DC, *et al:* Transmission of Creutzfeldt-Jakob disease from formalin-fixed, paraffin-embedded human brain tissue. *N Eng J Med* 315:1614, 1986.

2. Masters CL, Gajdusek DC, Gibbs CJ: The familial occurrence of Creutzfeldt-Jakob disease and Alzheimer's disease. *Brain* 104:535, 1981.

3. Asher DM, Gibbs CJ, Gajdusek DC: Slow viral infections: Safe handling of the agents of subacute spongiform encephalopathies; Chapter 10. In *Laboratory Safety: principles and Practice*, BM Miller (ed). American Society of Microbiology, Washington, DC, 1986, p 59.

4. Gajdusek DC, Gibbs CJ, Asher DM, *et al:* Precautions in medical care of, and in handling materials from patients, with transmissible virus dementia (Creutzfeldt-Jakob disease). *N Eng J Med* 297:1253, 1977.

5. Robbins SL, Cotran RS, Kumar V: *Pathogenic Basis of Disease*, 3rd edition, WB Saunders, Philadelphia, 1984.

6 Dinn JJ: Slow infections - a challenge to traditional concepts. *Irish Med J78:293*, 1985.

7 Narang HK: Virus-like particles in Creutzfeldt-Jakob biopsy material. *Acta Neuropathol* (Berl) 32:163, *1975.*

8. Bastian FO: Spiroplasma-like inclusions in Creutzfeldt-Jakob disease. *Arch Path Lab Med* 103:665, 1979.

9. Merz PA, Rohwer RG, Kascsak R, *et al:* Infection-specific particle from the unconventional slow virus diseases. *Science* 225:437, 1984.

10. Bastian F, Jennings R, Quindlen E: Spiroplasma-associated fibrils. *JNeuropathol Exp Neurol* 43:333, 1984.

11. Bastian FO, Jennings RA, Gardner WA: Antiserum to scrapie-associated fibril protein cross-reacts with spiroplasma mirum fibril proteins. *J Clin Micr* 25:2430, 1987.

12. Lampert PW, Gajdusek DC, Gibbs CJ: Subacute spongiform virus encephalopathies. *Am J Path* 68:626, 1972.

13. Prusiner SB: Prions. *Sci Amer* 251:50, 1984.

14. Manuelidis L, Manuelidis EE: Recent developments in scrapie and Creutzfeldt-Jakob disease. *Prog Med Virol* 33:78, 1986.

15. Bernoulli C, Siegfried J, Baumgartner G, *et al:* Danger of accidental person-to--person transmission of Creutzfeldt-Jakob disease by surgery. *Lancet* 1:478, 1977.

16. Duffy P, Wolf J, Collins G, *et al:* Possible person-to-person transmission of Creutzfeldt-Jakob disease. *N Eng J Med 290:692,* 1974.

17. Miller DC: Creutzfeldt-Jakob disease in histopathology technicians. *N Eng J Med* 3l8:853, 1988.

18. Sitwell L, Lach B, Atack E, *et al:* Creutzfeldt-Jakob disease in histopathology technicians. *N Eng J Med* 318:854, 1988.

19. Brown P, Gibbs CJ, Amyx HL, *et al:* Chemical disinfection of Creutzfeldt-Jakob disease virus. *N Eng J Med* 306:1279, 1982.

20. Masters CL, Jacobsen PF, Kakulas BA: Decontamination of formaldehyde-fixed tissues of Creutzfeldt-Jakob disease. *J Neuropathol Exp Neurol 45:760,* 1986.

21. Masters CL, Jacobsen P, Kakulas BA: Letter to the editor. J *Neuropathol Exp Neurol* 44:304, 1985.

22. Case Records of the Massachusetts General Hospital. (Case 45-1980) *N Eng J Med* 303:1161, 1980.

23. Armbrustmacher VW: Armed Forces Institute of Pathology, Washington, DC, Personal Communication.

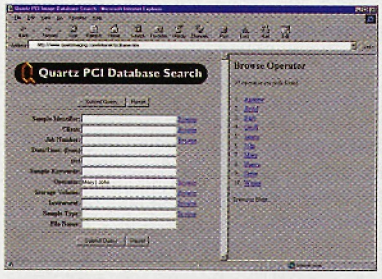
Reprinted from the Journal of Histotechnology, Sept. 1989, Volume 12, No.3

Keeping a good image around the lab just got a lot easier. Quartz PCI.

With the Windows®-based Quartz PCI™ system, you can capture and manage high-resolution digital images from just about any image-producing device. And do so with a single—and familiar—family of software that lets you smoothly work with SEMs, STEMs, TEMs, light microscopes, video sources, TWAIN devices and CD SEMs. You end continuous learning of new software. Easily network diverse instruments. And conveniently enter all types of images into one database.

Further, after acquiring an image, PCI's processing and display capabilities let you do just about anything you want with it—from annotating, zooming, smoothing and sharpening it to making gamma adjustments and constructing anaglyph stereo images. And you're free to add your own custom functions as well.

Now, think about your database. PCI's scalable family of database solutions ensures your images—plus related documents, custom fields and tables—become part of a powerful database that provides data when, where, and in the form needed. OBDC compliance ensures compatibility with third-party tools like Microsoft® Access™ And PCI's Workgroup Database, for facilities with up to 25 users, is upgradable to our Enterprise Database which—based on either Oracle® or Microsoft® SQL Server™—supports thousands. With our Intranet Image Server, you can perform queries and retrieve images via standard Web browsers. And, for off-line storage, there's built-in support for removable media such as CD-ROMs or magneto-optical disks.



The PCI Intranet Image Server lets you use your Web browser to search for images.

So. If easily acquiring, analyzing, manipulating, annotating, transmitting, archiving and retrieving high-resolution images from various sources is in your future, you'll want Quartz PCI details in your hands. You can arrange that easily, too. Visit the Nissei Sangyo Web site for a demonstration, or let us hear from you by phone, FAX or E-mail.

NSA

Nissei Sangyo America, Ltd.

755 Ravendale Drive
Mountain View, CA 94043
(800) 227-8877
E-mail: sidsales@nissei.com
www.nissei.com

25 West Watkins Mill Road
Gaithersburg, MD 20878
(800) 638-4087

Circle Reader Inquiry #9