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Telepathology and Imaging Spectroscopy as a New Modality in Histopathology

Sandor G. VARI, MD
VariMed, Szalanci u 5, H-1124 Budapest XII, Hungary
Gerhard MÜLLER Dr. h.c. Dr.-Ing
Laser-und Medizin Technologie, gGmbH, Berlin, Krahmerstr 6-10, 12207Berlin Germany
Jeremy M. LERNER
LightForm Inc. 601 Route 206, Suite 26-479, Belle Meade New Jersey 08502, USA
Rolf-Dieter NABER
Tritech GmbH, Hubert-Netzer-Weg 2/1, 88316 Isny im Allgäu, Germany

Abstract. Telemedicine started in the late 1950's by transmitting data on patients' pulse and heart rates. In the 1980's it expanded to radiology and orthopedics. The technology is now expanding to other specialties that can digitally gather patient data. Telepathology comprises the transmission of microscopic images via telecommunication network. Image compression and multiplexing technologies enabled high-resolution telepathology as well as real-time video consultations over international telephone lines. Organ transplantation has become a viable treatment and offers new life to an increasing number of patients suffering from chronic end stage diseases and from irreversible organ failure. Rejection is still a major problem in kidney, liver, and heart transplantation. To gain further insight into the complex interactions within the components of the immune system, it has become increasingly necessary to develop rapid and simple methods to monitor the status of the immune system in patients. Clinical signs suggest organ rejection and abnormal laboratory test results, but only histological signs on biopsy specimens are adequately specific. The financial cost of organ transplant makes it imperative to develop tools for the early identification and treatment of organ rejection. An increasingly sensitive and accurate way of localizing key structures and abnormalities is through spectroscopy of either H&E stained samples or with a fluorescent tag (fluorophore) or by relying on natural fluorescence. The system is based on a unique Prism and Mirror Imaging Spectroscopy System ("PARISS ™"), spectrometer originally designed and implemented for remote Earth monitoring from space and aircraft and astronomical imaging spectroscopy. Compact and lightweight both the mirror and prism are presently constructed in inexpensive glass but can also be injection molded in plastic. Any number of vendors anywhere in the world can produce all parts of the assembly. This greatly enhances the chances of future commercial viability. The Interactive Histopathology Consultation Network INTERPATH (PL961121) project integrated of remote control imaging microscopy system, imaging spectroscopy, and communication networks called SPECTROMIC. This telepethology unit will be a useful tool in the Regional and International Integrated Telemedicine Network for Medical Assistance in End Stage Diseases and Organ Transplant, RETRANSPLANT HC 4028 (HC) & IN 4028 (HC).

1. Introduction

Quality improvement and standardization of diagnosis in histopathology and cytology are important for the future of the discipline. Nominal scale diagnoses dominate the practice

and their standardization depends on relevant and reproducibly identifiable criteria as well as on communication of these among pathologists. Telepathology, i.e. the transmission of adequately detailed color images of microscopic fields over the telephone network is now a realistic possibility [1,2]. Telecommunication links available inexpensive worldwide in the form of the public telephone net, with the restriction of a limited channel capacity. The restrictions imposed by the application of the telephone net (preferentially the ISDN service) are investigated by Schwarzmann et al and strategies outlined to overcome these restrictions [3,4]. Working at the microscope in the framework of the pathology laboratory daily routine was thus improved for quality of decision-making, management of medical information [1].

Multi-spectral imaging (MSI), developed by NASA for planetary probes and remote sensing, has recently come into wide use for earth studies. It is used for oceanography, geology, climatology and environmental studies. It is also used commercially, with applications in agriculture, process control and graphics. All of these applications rely upon the unique combination of imaging and spectral analysis that MSI supplies. Although most of the digital imaging techniques developed for monochrome microscopy work well with color images, there are a few considerations specific to the analysis of multispectral imagery. The method consists of the determination of spectral characteristics of different elements of interest. These include obtaining a properly balanced multicolor digital image, specifying the color components of the image, and multispectral imaging yields functional maps, showing *what* and *where* biological molecules are located within a structure. Histopathologists recognize the need for more accurate, automated data analysis to enable better decision making. The use of the Prism and Mirror Imaging Spectroscopy System ("PARISS ™"), as an imaging spectrograph will go a long way to adding the extra degrees of freedom necessary for complete spectral object identification. The workstation of the pathologist in the year 2000 could be the combination of a microscope, a HD-TV-camera, imaging spectroscopy and a personal computer.

2. Materials and Methods

The telepathology imaging spectroscopy (TISP) system will add a new feature to the integrated microscopy workstation. The system consists of at least two main parts:

The PARISS spectrograph is based on an imaging spectrometer originally designed for Remote Earth Monitoring and Astronomy and is currently in use for both applications in military and civilian environments. The original spectrograph was patented

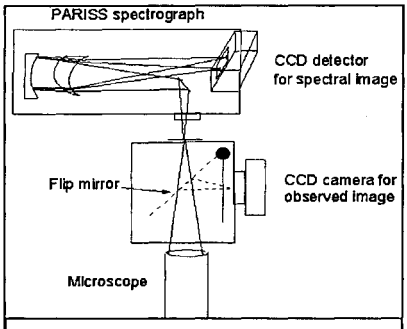

Figure 1. Schematic of PARISS Imaging Spectrometer

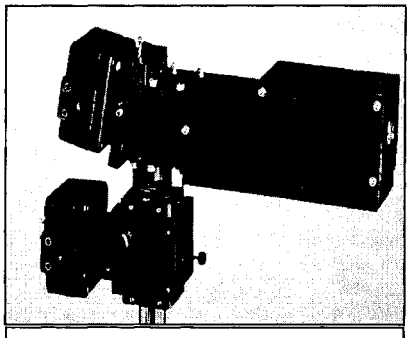

Figure 2. PARISS mounted on an epi-fluorescence microscope

by The Aerospace Corporation (TAC) for use in the infrared from 3 to 15μm. LightForm acquired the rights to the patent and redesigned the optics for use from 360 to 800nm. The acronym "PARISS" is derived from "Prism and Mirror Imaging Spectroscopy System". It was the goal of the investigators to demonstrate that the device, with an origin in a military environment, could be of value in the life sciences. The system works by accepting an image projected by a microscope that presents a particular field containing "objects" onto the entrance slit of the PARISS spectrometer. A slice of image passes through the slit and strikes the first curved surface of the prism, is refracted, strikes the second surface, and exits to strike the spherical mirror. The light then returns through the prism to be focused onto the CCD matrix array, wavelength dispersed (see ***Figure 1***). Over 80% to 90% of all light, over the entire wavelength range, is transmitted through the system. The system uses a prism made of inexpensive flint glass, as illustrated in ***Figure 1 & 2***, but the support and body of the unit will be in either cast aluminum, or ribbed metal plate for enhanced rigidity. The optical system was fully ray-traced and performance was found to meet or exceed expectations. ***Figure 2*** shows the finished PARISS assembly on a microscope. The system generates spectrally and spatially resolved topographical maps of histopathology samples and will recognize natural fluorescence of a formalin fixed H&E stained tissue sample, a frozen sample, immunofluorescence of immunoglobulin deposits that have been fluorescence tagged of allografts with manifesting organ rejection. The PARISS spectrograph receives light projected by the microscope objective onto its entrance slit. The spectrum is wavelength dispersed onto a CCD detector. The data is sent to a trained Hybrid Neural Network (HNN) that automatically classifies each object in terms of characteristic spectral features (CSF) and paints the results in false colours to a computer screen superimposed onto a white light image. The HNN provides the intelligence to automate the processing of these multi-spectral images (MIS) and performs non-linear transformations providing the sophisticated deconvolution and correlation routines needed to handle real-life-convoluted samples.

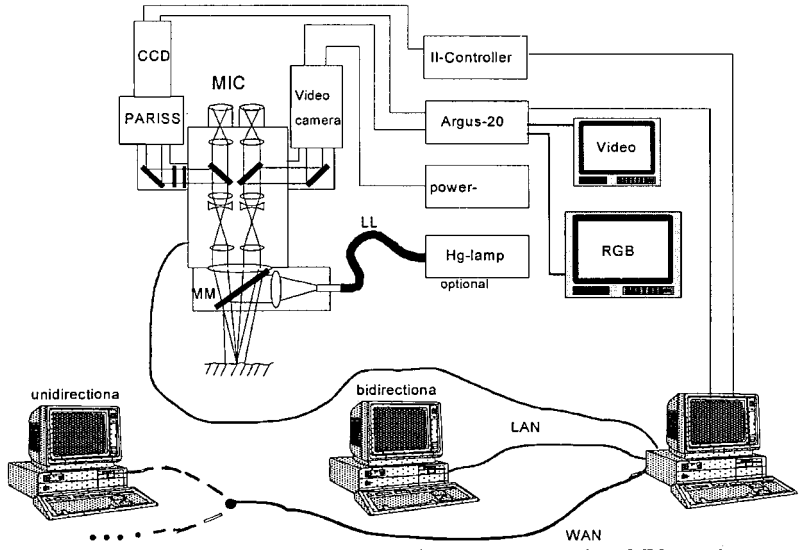

Figure 3: Technical setup of the TELEPATH microscope workstation. MIC = microscope, LAN = local area network based on optical fiber system, WAN = wide area network based on ISDN telecommunication network, LL = liquid light guide (if necessary), MM = movable diachronic mirror, MCP = multi channel plate

For the local area network preferable to use an optical fiber network with Novell multi-user software connected to a bare fiber-AUI converter and an Ethernet AUI Interface. So on this way a high-speed data transfer achievable, and in the other direction, a remote control of the microscope workstation could be performed. In ***Figure 3*** a wide area network demonstrated. A high speed active ISDN PC-card embedded in a special mailbox software delivers a fast image transfer to a workstation in a foreign country.

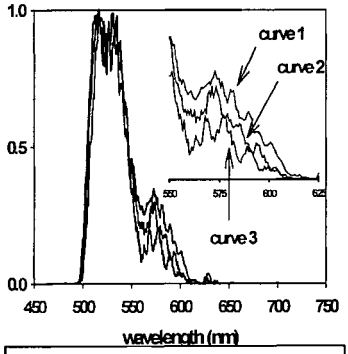

Figure 4: Comparison of spectral features, immunofluorescence stained for IgA, IgG and IgM.

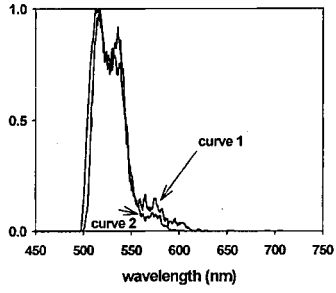

Figure 5: Comparison of spectra from transplant biopsies negative for humoral

3. Results of Kidney and Heart Biopsies

System operating parameters

The microscope objective: 40X. Entrance slit to the spectrometer: 50 μm. Average acquisition time of spectra: 9 Sec. Average acquisition time of Observed image:1 Sec. Number of objects per acquisition: 240. Size of each object: 0.5 μm by 2.5 μm. Spectral range per acquisition: 400 to 800nm. Number of Renal disease patients: 7. Number of repeat tests per slide: 6. The excitation wavelength was 405nm and spectra were acquired through a long pass filter that allowed all light of greater than 470nm to pass, and blocked the excitation wavelength and all wavelengths shorter than 470nm.

Figure 4 shows clear differentiation between the spectra of immunofluorescence staining for IgA, IgM and IgG in the spectral region from 550nm to 650nm. Curve 1: Patient with chronic diabetic nephropathy and glomerulosclerosis. Stained for IgA.

Curve 2: Patient with chronic diabetic nephropathy and glomerulosclerosis. Stained for IgG.

Curve 3: Patient with chronic diabetic nephropathy and glomerulosclerosis. Stained for IgM.

In the heart transplant Non-rejection pathology is frequently seen post transplant to include ischemia or catacholamine effects, interstitial fibrosis, myocardial calcification, and cyclosporine-associated endocardial infiltrates called the Quilty effect. It is therefore very important to be able to automatically diagnose evidence of immunosuppressive disorders even if there is no evidence of cellular rejection. The International Society of Heart Transplantation (ISHT) provides criteria to enable grading of heart rejection ranging from Grade 0 to +4. ***Figure 5*** shows two spectra from two patients, both negative for humoral rejection with an ISHT Grade 0 and also negative for Quilty effect. Both were of right ventricular septal endocardial biopsies of heart transplant patients. The slides were stained with FITC conjugated antisera to immunoglobulins, the third component of complement and fibrinogen. Both spectra are fundamentally the same. Curve 1: Negative Quilty effect, negative rejection, ISHT Grade 0. Curve 2: Negative Quilty effect, negative rejection, ISHT Grade 0. This example provides a

good indication that spectra are consistent between patients with similar findings. Identification and differentiation between patients with and without the Quilty effect.

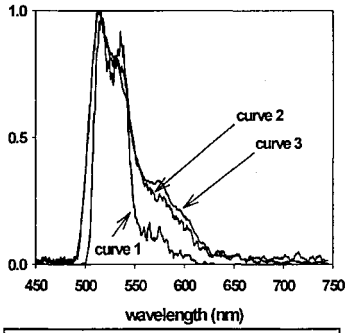

Figure 6: Comparison of transplant biopsies with and without Quilty effects.

Figure 6 shows that the spectra for patients with the Quilty effect, curves 2 and 3, are very similar, even though one is negative and the other positive for rejection. Both are different from the spectrum of the patient negative for both rejection and Quilty effect. This is a good example of how the fluorescent spectral envelope can change as a function of cellular condition and would not be detected with single filter observation. Curve 1: Negative Quilty effect, negative rejection, ISHT Grade 0. Curve 2: Positive Quilty effect, negative rejection, ISHT Grade 0. Curve 3: Positive Quilty effect, positive for cellular rejection, ISHT Grade 1A.

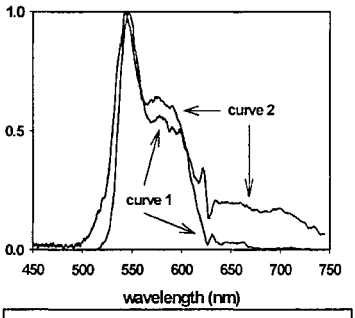

Figure 7: Comparison of spectra from normal aorta wall and aorta showing degeneration.

Figure 7 shows Marfan's Syndrome: aorta stained with Hematoxylin and Eosin (H&E). The samples were examined under the epi-fluorescent microscope excited at 436nm. There was a strong blue-green fluorescence. The spectral features shown in Figure 7 clearly differentiate between a normal aorta wall and an aorta showing degeneration. Curve 1: Normal aorta wall. Curve 2: Degenerating aorta wall.

4. Neural Network development

During the initial part of the project there was insufficient data to train a Supervised Neural Network. It was therefore decided to implement an Unsupervised Neural Network that was designed to self-train on subtle differences between spectral signatures. This approach proved highly successful. The Windows 95 platform caused many memory management problems that reduced the number of spectral objects that could be processed simultaneously from 240 to about 100. These issues will be resolved with the possibility either of changing the existing code to Windows NT or converting to LabVIEW code written for the Windows NT environment. Pathologists are used to viewing slides through a microscope with excellent optics. They observe high resolution images with a large field of view. The Phase I camera was a low cost 8-bit black and white model with 177K pixels made by Electrim Corporation of Princeton, NJ. Although this camera was very effective, it is also low resolution. Future PARISS systems will be equipped with a higher resolution (higher pixel density) color CCD manufactured by Eastman Kodak Corporation. We believe that many pathologists and researchers will consider the improved resolution and performance to be worth the additional cost.

5. Discussion

The use of image transmission in pathology - telepathology - is applied mainly three purposes:

1. To enable rapid diagnosis of frozen sections prepared by technician in a operating theater lacking present of expert pathologist, using spectroscopic imaging method integrated into a remote imaging microscope.
2. To establish on-line consultation with experts throughout Europe, on difficult and rare cases of histopathology / cytopathology.
3. To join the European telecommunication systems via Euro-ISDN connection in order to ensure more tasks, as diagnostic standardization, quality assurance and education programs.

In general the results were most encouraging even with low signal strength. It would appear that the technique is robust. There were strong indications that it is possible to differentiate between acute and chronic renal transplant rejection and differentiate cyclosporine toxicity. It appears that the wavelength range from 550 to 650nm shows the greatest change as a function of abnormality. This is evident in Figures 4 through 7. A comparison of Figures 4 and 5 clearly shows that there are differences in the shape of the spectral envelope depending on the target feature. Even though fluroresceinated antisera specific to IgA, IgM or IgG all are usually observed by eye with the same filter set, the PARISS system reveals that the total envelopes of each are different. It was also shown that the presence of the Quilty effect and cyclosporine toxicity also have a tangible effect on the spectral envelope emitted by the stained organ.

6. Conclusion

The workstation of the pathologist in the year 2000 could be the combination of a microscope, a HD-TV-camera, elector-optics for imaging spectroscopy and a personal computer with many software's allowing treatment and transmission of numerous pictures. Archival immunofluorescence-stained renal and heart transplant samples were investigated and weak fluorescence acquired. The slide specimens under study had already been subject to fluorescence investigation and many samples were already substantially photo-bleached. In spite of this, spectral acquisitions were surprisingly sensitive given the weak signals. In the Countries of Central and Eastern Europe (CEEC), an integrated network would significantly reduce the cost of histopathology and record management. For small hospitals with limited availability of local pathology services and for hospitals with a deficiency of specialists, telepathology may be a worthwhile substitute. The system can be expanded to include interactive audiovisual technology in continuing medical education and training, to video conferencing.

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