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Choices for tissue visualization with IR microspectroscopy

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Abstract

In this paper, we address some of the issues associated with infrared (IR) imaging, with reference to our work on brain tissue from the TgCRND8 mouse, a transgenic model of Alzheimer's disease (AD). AD is the most common cause of dementia in the aging population. One of the characteristic hallmarks of this chronic neurodegenerative disorder is the accumulation of plaques in the brain, usually visualized with histochemistry and immunostaining. Although these methods are extremely useful, they illustrate only certain aspects of the sample, require a great amount of tissue processing, and are highly dependent on experimental protocols and reagent quality. IR imaging provides information on multiple components, with a minimal amount of sample processing. However, in order to interpret the data successfully, the issues of spectral acquisition parameters, pre-processing, and spectral artifacts need to be considered. The methods commonly used to process the data, such as uni- and bi-variate spectral analysis, and multivariate methods, such as hierarchical cluster analysis, and some issues concerning the use of second derivatives of IR spectra are discussed.

Keywords: Infrared microspectroscopy; Alzheimer's disease; Spectral data processing; Second derivative

1. Introduction

The long-term goals for the use of infrared (IR) imaging range from application as a mainstream tool for rapid-throughput diagnostics to an important complementary tool in detailed pathology studies. There are several definable advantages for choosing IR over the many alternatives: (a) the spectra contain information about multiple components in situ, (b) the preparation of tissue sections, usually cryotomed from frozen, unfixed tissue blocks, is simple, (c) apart from desiccation, the tissues are typically not altered in any way, i.e. nothing has been added to the tissue and, except for volatile components (water), nothing has been removed, (d) the IR light is not damaging to the sample, thus subsequent analyses are readily feasible, (e) with a bright synchrotron source or with focal plane array detectors, good spectra can be recorded at the diffraction limit. Recent papers on applications of IR to tissue visualization illustrate and emphasize these points [1–4]. Nevertheless, if the long-term goals are to be achieved, there must be further dialogue on how best to interpret and illustrate the spectral differences recorded in IR spectra.

IR spectral data maps present some interesting challenges for analysis, both from the viewpoint of the quantity of raw data and the choices for processing that data to obtain useful, meaningful information. Here, our focus is on the illustration of some of these options and a discussion of advantages and disadvantages, with specific reference to our work on Alzheimer's disease tissue. In addition, we consider some of the common practices in data acquisition that can increase the difficulty in extracting meaningful information.

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Alzheimer's disease (AD) is the most common cause of dementia in the aging population. One of the characteristic hallmarks of this chronic neurodegenerative disorder is the accumulation of plaques in the brain [5]. The main component of plaques is the amyloid peptide (Aβ), which consists of 40 or 42 amino acids and adopts a highly aggregated β-pleated sheet fibril structure, known as an amyloid [6], in the plaque cores. We are studying brain tissue from TgCRND8 mice, a transgenic model of AD [7]. The animals express a double mutant form of human amyloid precursor protein 695 (KM670/671NL + V717F), under the control of the brain-specific PrP gene promoter. The characteristics include robust plaque pathology and cognitive impairment from an early age; small dense-cored plaques are detected by 3 months of age, concentrated in the cortex and hippocampus. As the animal ages, the plaques become larger and acquire a halo of diffuse amyloid. Purely diffuse plaques become more common in older animals and are most concentrated in the caudate and the molecular layer of the dentate gyrus. The dense-cored plaques are accompanied by dystrophic pathology and inflammation.

Classic histochemistry and immunohistochemistry are regularly used to illustrate different aspects of AD pathology [8–10]. However, these techniques are very specific, illustrating only one or two different targets, and require extensive tissue manipulation that can affect other features. Infrared (IR) spectroscopy has been used to study protein aggregation, since the position of the amide I band is dependent on the secondary structure of the protein [11–13]. Infrared microspectroscopy, which combines high spatial resolution with molecular-level information, provides a unique tool for studying plaques and associated changes in situ.

In this paper, we present a comparison of classic histology and immunohistochemistry with microspectroscopic visualization achieved through the analysis of a representative IR map that contains many features typical of TgCRND8 mouse brain. The data are processed in several ways, to reveal both the power and the potential pitfalls of this technique. Specific consideration is given to spectral acquisition parameters, pre-processing, some spectral artifacts, uni- and bi-variate spectral analysis contrasted to cluster methods with software such as CytoSpec, and some issues concerning the proper use of second derivatives of spectra.

2. Experimental

2.1. Animals

The animals in this study were treated in accordance with the guidelines of the Canadian Council on Animal Care and the protocols were approved by the University of Toronto and University of Manitoba. TgCRND8 mice and non-transgenic littermates were sacrificed by cervical dislocation. The brains were removed immediately and divided into left and right hemispheres. One half was fixed in 3% buffered paraformaldehyde, dehydrated and embedded in paraffin; the other half was covered with OCT medium and snap frozen on dry ice. For the purposes of this paper, only one map, from an 11 month TgCRND8 mouse, will be considered in detail.

2.2. Sample preparation and tissue staining

For an initial examination of plaque morphology, the paraffin-embedded brains were cut into 6 μm sections in the sagittal plane and placed on glass slides. Serial sections were stained with hematoxylin and eosin, Congo red, modified Bielschowsky silver stain and immunohistochemistry against ubiquitin (rabbit polyclonal, Dako, Carpinteria, CA). The frozen brains were cryotomed sagittally at 8 μm thickness. For IR spectroscopic analysis, sections were thaw-mounted on reflective MirrIR™ slides (Kevley Technologies, Chesterland, OH). After IR analysis, they were stained with Congo red. Additional sections were placed on glass slides for immunofluorescence staining with anti-Aβ peptide antibody 4G8 (Signet Laboratories, Dedham, MA), visualized with Alexa Fluor® 488 goat anti-mouse IgG secondary antibody (Molecular Probes, Eugene, OR), and counterstained with 4′,6-diamidino-2-phenylindole. Immunofluorescence slides were imaged on a Zeiss Axiophot microscope equipped with a SenSys digital camera, and MacProbe v4.4 software (Applied Imaging, San Jose, CA).

2.3. Synchrotron IR mapping and data analysis

The map analyzed here was recorded with a Nicolet Magna 860 FTIR with a Spectra Tech Continuum IR microscope (U10B beamline, NSLS, BNL, Upton, NY). The spectra were recorded at 4 cm^{-1} resolution, 12 μm aperture, 10 μm step size. Neither zero filling nor spectral smoothing was employed. A suitable region in the hippocampus was selected for spectral mapping by inspection, as the dense plaques are readily visible under bright light in the unfixed thaw-mounted sections. Uni- and bi-variate data analyses were performed with OMNIC/Atlμs software (Thermo-Nicolet), while multivariate analyses were done with CytoSpec software (http://www.cytospec.com).

2.4. Second derivative analysis with simulated spectra

Model spectra with pure Lorentzian bands were created in Mathcad (MathSoft Engineering and Education, Inc., Cambridge, MA) to investigate some practical limits on the use of second derivatives in data analysis. We simulated each multicomponent spectrum as a sum of a preset number of Lorentzians with random numbers for the amplitude, wavenumber and bandwidth, to represent bands assigned as functional groups. Units are not relevant, so the ''spectral'' range was from 3 to 5. The first member of the set was centered at wavenumber 4 and had a constant bandwidth. The amplitudes were varied randomly each time