Synchrotron Radiation-Based FTIR Microspectroscopic Imaging of Traumatically Injured Mouse Brain Tissue Slices

Yuansen Guo, Tunan Chen, Shi Wang, Xiaojie Zhou, Hua Zhang, Dandan Li, Ning Mu, Mingjie Tang, Meidie Hu, Dongyun Tang, Zhongbo Yang, Jiajia Zhong, Yuzhao Tang, Hua Feng, Xuehua Zhang, and Huabin Wang*

ABSTRACT: Traumatic brain injury (TBI) is a health problem of global concern because of its serious adverse effects on public health and social economy. A technique that can be used to precisely detect TBI is highly demanded. Here, we report on a synchrotron radiation-based Fourier transform infrared (SR-FTIR) microspectroscopic imaging technique that can be exploited to identify TBI-induced injury by examining model mouse brain tissue slices. The samples were first examined by conventional histopathological techniques including hematoxylin and eosin (H&E) staining and 2,3,5-triphenyltetrazolium chloride staining and then spectroscopically imaged by SR-FTIR. SR-FTIR results show that the contents of protein and nucleic acid in the injured region are lower than their counterparts in the normal region. The injured and normal regions can be unambiguously distinguished from each other by the principle component analysis of the SR-FTIR spectral data corresponding to protein or nucleic acid. The images built from the spectral data of protein or nucleic acid clearly present the injured region of the brain tissue, which is in good agreement with the H&E staining image and optical image of the sample. Given the label-free and fingerprint features, the demonstrated method suggests potential application of SR-FTIR spectroscopic mapping for the digital and intelligent diagnosis of TBI by providing spatial and chemical information of the sample simultaneously.

1. INTRODUCTION

Traumatic brain injury (TBI) is caused by an external force exceeding the brain protective capacity, such as shock waves, striking an object, being struck by an object, or being penetrated by a foreign body and can lead to the alteration of brain function and pathology.1 It is estimated that ~10 million people worldwide are newly affected by TBI each year, and TBI is now becoming a major cause of long-term disability in young adults and has serious adverse effects on the patients in terms of health and economic burden.2 Therefore, unambiguous diagnosis of TBI is not only a prerequisite for its clinical treatment but also fundamental for its research, which ultimately strongly affects the lives of patients and their families as well as the society.3

Previous studies indicate that the pathophysiology of TBI is very complex and that a cascade of molecular and biochemical changes can occur after TBI.3 Although histopathological methods such as hematoxylin and eosin (H&E) staining and 2,3,5-triphenyltetrazolium chloride (TTC) staining can be employed to diagnose TBI qualitatively, they are difficult to be used to detect the composition, content, and distribution of biomolecules, intrinsically limiting their applications in the precise detection of TBI.4 Some advanced imaging techniques such as computed tomography (CT),5 magnetic resonance imaging (MRI),6 positron emission tomography (PET),7 and fluorescence molecular imaging (FMI)8 have been utilized to diagnose TBI or explore the disease mechanisms of TBI. Despite their advantages, the intrinsic limitations of these approaches hinder us to a certain degree to precisely detect pathological changes and comprehensively capture disease pathogenesis of TBI. For example, mild TBI appears normal on conventional CT and MRI,6,9 PET is challenged by the exposure to radioactive elements7 and its low resolution, and FMI is restricted by the requirement to design a suitable fluorophore with high biocompatibility, photostability, and quantum yield.8 Hence, new techniques are urgently needed for in-depth TBI diagnosis and research, which should be, ideally, independent of fluorescence or radioactive labeling and...
have high sensitivity and high resolution in the detection of biochemical changes of the tissue after TBI.

Relying on purely constituent molecular species of a sample rather than exogenous contrast agents, radioactive elements, or coloring chemicals, Fourier transform infrared (FTIR) spectroscopic imaging techniques enable label-free detection of biochemical information on biological samples and have the potential to revolutionize histopathology for improved disease diagnosis. Various biological tissue samples such as prostate tissue sections from patients, lung cross sections from a mouse inoculated with tumor cells, and brain tumor or diseased brain tissue sections from patients or animal models have been successfully investigated, and the capability of biochemistry-based recognition of the diseased state of biological tissues by FTIR has been confirmed. Particularly, the emergence of synchrotron radiation (SR)-based FTIR spectroscopic imaging techniques further improves the performance of FTIR in revealing detailed spectral information necessary for the identification of a sample’s constituent species due to its much higher signal-to-noise ratio and less damage to biological samples than conventional FTIR techniques. Using SR-FTIR spectroscopic mapping techniques, researchers in different fields have made numerous significant findings such as elevated lipid-enveloping dense-core amyloid plaques in model mouse and human Alzheimer’s disease brain sections, constituent changes of proteins, lipids, and nucleic acids within single human mesenchymal stem cells, and nonpolar phospholipid-rich multilayered myelin sheath possessing higher refraction indexes within certain wavelengths than axon in a myelinated neural axon. These studies strongly support that SR-FTIR spectroscopic mapping may offer great promise for the accurate diagnosis of diseased biological samples.

Unfortunately, although FTIR and/or SR-FTIR imaging techniques provide an ideal platform to directly probe the biochemical and structural composition of tissues subjected to TBI, hitherto only relatively little attention has been received by the scientific community, and only very limited information is available on this regard. As far as we know, the best example is that Zhang et al. identified axonal injury following TBI by analyzing infrared absorption intensity of amide I band using a conventional FTIR technique. So far, SR-FTIR has not even been exploited to investigate TBI in spite of its great potential in obtaining rich biochemical information on the measured samples for its prominent capability of achieving high-quality spectra and high-resolution spectroscopic images of the samples. In order to evaluate the performance of SR-FTIR in TBI detection and understand more about the biochemistry involved in the tissue samples following TBI, in the present work we employed a SR-FTIR microspectroscopic imaging technique to investigate TBI slices of model mice. We found that the contents of protein (either amide I and/or amide II) and nucleic acid in the TBI region were lower than those in the normal region. The content of protein and/or nucleic acid could be used as a good biomarker to unambiguously distinguish the two regions, which was confirmed by both H&E staining and optical observation of the sample. Thus, we provide evidence that SR-FTIR spectroscopic imaging is an effective technique for the detection of TBI. The demonstrated technique may be further developed into a reliable, label-free, digital, and intelligent method for the diagnosis of biological samples.

2. RESULTS AND DISCUSSION

The animal model of TBI was prepared essentially by following a previously established protocol, which is illustrated in Figure 1 and detailed in the Experiment Section. This approach is reliable for the establishment of a cortical contusion model, and the lesion was restricted to the cortex and white matter near the impact and no puncture or tearing of the cortex or dura was observed during our experiments by checking the brain tissues and sectioned brain slices after TBI.

Representative brain tissue is shown in Figure 2a, from which the local lesion is clearly observed on the right side. Since TTC can be used to identify metabolically active normal brain tissue from metabolically inactive dead/damaged tissue, we stained the brain tissue slices with TTC after TBI. It can be seen from Figure 2b,c that the normal region of brain

Figure 1. TBI model establishment. (a) Schematic illustration of the front view of the contusion device and a mouse with a bone window (red dot) drilled to expose the dura. A footplate with a diameter of 2.5 mm was located on the dura and positioned 0.5 mm posterior and 0.5 mm lateral to the bregma. The TBI is induced by free fall of the steel rod to hit the footplate to impact the brain to a depth of 2.5 mm. (b) Schematic illustration of the overlooking view of the mouse head fixed in a stereotaxic apparatus. The scalp of the mouse was opened and a bone window (red dot) was created to expose the dura. (c) Photograph showing experimental setup as well as the fixed mouse. (d) Close-up of the trauma after TBI, as indicated by an arrowhead.

Figure 2. TTC stain of a slice from the mouse brain tissue after TBI. (a) Optical visible image of a mouse brain tissue with TBI. (b,c) Two TTC-stained coronal mouse brain tissue slices sectioned approximately along the white dotted lines marked by “1” and “2” in (a), respectively. The injured region is pale and marked by a black dotted line.
tissue can be easily distinguished from the injured region for that the former is stained to red due to TTC reacting with dehydrogenase in normal tissue, while the latter appears pale due to the lack of such a reaction.32

The morphology and architecture of the brain tissue slices after TBI were further examined by H&E staining, by which protein-rich areas such as cytoplasm, blood cells, and collagen were stained to pink while the nucleic acid-rich areas stained to blue.12 From Figure 3a, it can be observed that the normal region (marked by “1”) has a deeper color than the injured region (marked by “2”). A close examination of the two regions is shown in Figure 3b,c, respectively. From Figure 3b, neuronal cell structure is clear and the cells are relatively uniformly and densely arranged (37 ± 4 cells in 80 × 160 μm², n = 30). In contrast, Figure 3c shows severe pathological alterations in the injured region such as significantly decreased cellular density (21 ± 4 cells in 80 × 160 μm², n = 30), uneven distributed cells, and cytoplasmic vacuolization. These observations are highly consistent with the previous H&E staining results of TBI tissues.5,33

To perform SR-FTIR measurement, the TBI slice was first observed optically by an optical microscope coupled with the SR-FTIR system, and the injured region can be found in the upper-right part of the sample (Figure 4a). As shown in the corresponding SR-FTIR mapping images based on either the integrated area of the spectral region (1600–1715 cm⁻¹) for amide I group of protein peaked at 1655 cm⁻¹ (Figure 4b) or the integrated area of the spectral region (1184–1274 cm⁻¹) for phosphodiester group of nucleic acid peaked at 1235 cm⁻¹ (Figure 4c)20,34 the injured region and normal region can be clearly identified, which is consistent with the visible optical image in terms of the morphological structure of the sample. Figure 4d,e shows high-resolution SR-FTIR images constructed from the integrated area of amide I (1600 to 1715 cm⁻¹) of the spectra collected from the normal and injured regions at each pixel, respectively. Similarly, high-resolution SR-FTIR images constructed from nucleic acid fingerprint (1184 to 1274 cm⁻¹) for the normal and injured regions are shown in Figure 4f,g, respectively. In either case, the normal and injured regions have evident different contrast, which means that both protein content and nucleic acid content are different from the counterparts in the two different regions. The images constructed from the integrated areas of the spectral region (1477–1600 cm⁻¹) for amide II group of protein peaked at 1541 cm⁻¹ can also be used to well represent the normal and injured regions. For simplicity, we take amide I to represent protein and will not discuss amide II in the following context. These observations indicate the feasibility of acquisition of biochemical composition distributions of TBI samples with the SR-FTIR microspectroscopy imaging technique.

Detailed spectral information of the normal and damaged regions is shown in Figure 4b, in which each of the spectra represents averaged spectra randomly sampled from the normal region or injured region (n = 100 for each region). In this study, the analysis was focused on the region of 1000–1800 cm⁻¹, mainly including protein and nucleic acid. The region above 1800 cm⁻¹ was not considered in order to exclude the uncertainty likely caused by the deparaffinization process.33 It can be observed that the intensities of the peaks positioned at 1655 cm⁻¹ (amide I), 1541 cm⁻¹ (amide II), and 1235 cm⁻¹ (asymmetric P=O stretch of phosphodiester groups in nucleic acids) for the injured tissue are obviously lower than those for the normal tissue,13,35–37 indicating that the content of protein and nucleic acid in the injured region are lower than those in the normal region.

Principal component analysis (PCA) has been used to evaluate the spectral differences for the normal and injured regions, based on the spectral regions between 1600 and 1715 cm⁻¹ for amide I and between 1184 and 1274 cm⁻¹ for nucleic acid. As shown in Figure 4i,j, an evident separation of clusters of the normal region and injured region can be observed in the PCA score plots with 68% confidence ellipsoids included. The 1st and 2nd principal components (PC), that is, PC1 and PC2, respectively, incorporate 97.9 and 1.58% of the variance in Figure 4i and 99.16 and 0.54% in Figure 4j. The results imply that it is feasible to detect TBI on the basis of the biochemical components of the mouse brain tissue.

Considering k-means clustering analysis is an easy unsupervised learning algorithm often used for spectral image analysis based on spectral similarity,38 we also performed k-means clustering classification to generate three clusters on the regions between 1600 and 1715 cm⁻¹ for proteins (Figure 5a) and between 1184 and 1274 cm⁻¹ for nucleic acids (Figure 5b) of the spectra collected on the whole sample. For both images, the injured region and normal region can be discriminated, which are in line with the corresponding

![Figure 3. H&E stain of a slice from the mouse brain tissue after TBI. (a) H&E stained image of the slice. (b,c) Enlarged images to show the areas marked by “1” and “2” in (a). The insets in (b,c) are enlarged images corresponding to the regions marked by the boxes in (b,c), respectively. The areas marked by “1” and “2” are positioned symmetrically with respect to each other and located in the intact region and damaged region, respectively. A little blood covered on the tissue due to slight hemorrhage caused by the TBI can be observed in (ac), as marked by “3”.](https://dx.doi.org/10.1021/acsomega.0c03285)
and SR-FTIR images constructed from the integrated spectral peak areas between 1600 and 1715 cm\(^{-1}\) for proteins (Figure 4b) and 1184 and 1274 cm\(^{-1}\) for nucleic acids (Figure 4c), respectively. The results indicate that \(k\)-means can be used to distinguish different regions in the sample. It needs to mention that although the injured region can be identified fairly well from both Figure 5a,b, some subtle differences exist between the two images, which may be caused by a number of factors, including different spatial distributions of nucleic acids and proteins, limited imaging resolution, and limited categorizing numbers. It is expected that the combination of the two images should provide a more accurate diagnosis of the injured region. In addition, improving the spatial resolution and increasing the categorizing number may also be helpful for identifying the injured region more precisely.

Previously, it has been reported that TBI can cause the loss of neuronal cells (e.g., dopaminergic neurons) and promote cell apoptosis,\(^{39,40}\) which has also been found in our experiment, as manifested by the H&E staining results. Since the cytoskeletal components including microtubules, microfilaments, and intermediate filaments are rich in protein and the cellular nuclei contains a lot of nucleic acids, the loss of

![Figure 4](https://example.com/figure4)

**Figure 4.** SR-FTIR spectroscopic imaging of a slice from the mouse brain tissue after TBI. (a) Optical image of the slice, in which the injured region is indicated by an arrowhead. (b,c) SR-FTIR images of the slice that were respectively constructed from the integrated areas of the spectra between 1600 and 1715 cm\(^{-1}\) for protein (amide I band) and between 1184 and 1274 cm\(^{-1}\) for nucleic acid at each pixel. (d,e) SR-FTIR images corresponding to the areas marked by “1” and “2” in (b) constructed from the integral area of the spectra between 1600 and 1715 cm\(^{-1}\). The two areas are positioned symmetrically with respect to each other and are located in the intact and damaged regions, respectively. (f,g) SR-FTIR images corresponding to the areas marked by “1” and “2”, constructed from the integral area of the spectra between 1184 and 1274 cm\(^{-1}\). (h) SR-FTIR spectra obtained from the normal region and the injured region. Each of the spectra is an average of the spectra measured at more than 100 different points. PCA plots of the SR-FTIR data (i) between 1600 and 1715 cm\(^{-1}\) and (j) between 1184 and 1274 cm\(^{-1}\) for the spectra collected at more than 100 different points in either the normal region or the injured region. The data were projected onto PC1 vs PC2 coordinate systems, included with 68% confidence ellipsoids. The color bars in (b–g) indicate the relative value of the integrated area of the specified spectral region for a certain point/pixel in the images.

![Figure 5](https://example.com/figure5)

**Figure 5.** \(k\)-means clustering maps of a TBI tissue slice. The maps were constructed from the integrated area (a) between 1600 and 1715 cm\(^{-1}\) (amide I band) and (b) between 1184 and 1274 cm\(^{-1}\) (nucleic acid) of the SR-FTIR spectra collected at each pixel. Three clusters were applied to the data; blue, green, and red represent background, normal region, and injured region, respectively.

optical image (Figure 4a) and SR-FTIR images constructed from the integrated spectral peak areas between 1600 and 1715 cm\(^{-1}\) for proteins (Figure 4b) and 1184 and 1274 cm\(^{-1}\) for nucleic acids (Figure 4c), respectively. The results indicate that \(k\)-means can be used to distinguish different regions in the sample. It needs to mention that although the injured region can be identified fairly well from both Figure 5a,b, some subtle differences exist between the two images, which may be caused by a number of factors, including different spatial distributions of nucleic acids and proteins, limited imaging resolution, and limited categorizing numbers. It is expected that the combination of the two images should provide a more accurate diagnosis of the injured region. In addition, improving the spatial resolution and increasing the categorizing number may also be helpful for identifying the injured region more precisely.

Previously, it has been reported that TBI can cause the loss of neuronal cells (e.g., dopaminergic neurons) and promote cell apoptosis,\(^{39,40}\) which has also been found in our experiment, as manifested by the H&E staining results. Since the cytoskeletal components including microtubules, microfilaments, and intermediate filaments are rich in protein and the cellular nuclei contains a lot of nucleic acids, the loss of
neuronal cells will lead to decreased content of protein and nucleic acid. This can interpret what was revealed by the SR-FTIR spectroscopic mapping in the present work that the contents of both protein and nucleic acid in the injured region are lower than in the normal region. The protein content decrease has also been observed on homogenized rat brain samples subjected to hypoperfusion by FTIR spectroscopy,41 which can further support our current results. Nevertheless, it needs to be mentioned that besides the cellular change mentioned above, other biochemical changes such as high levels of oxidative stress, abnormal levels of oxygenation, altered metabolism, disrupted energy levels and systemic hormonal secretion, and up-regulation of inflammatory activities can also occur in TBI tissue,42 which may contribute to our observations and need further investigations in the future.

3. CONCLUSIONS

In conclusion, the results of our work suggest that TBI causes significant effects on the morphological and biochemistry changes in mouse brain tissue. A decrease in the protein and nucleic acid content was observed by SR-FTIR spectroscopic mapping, which was confirmed by the comparison of the spectra collected on different regions, PCA classification of the spectral bands for either protein or nucleic acid, constructed images from the integrated spectral peak area of protein or nucleic acid, and images obtained by k-means clustering of the spectral bands of either protein or nucleic acid. The above-mentioned composition changes can be supported by our H&E staining results as well as the previous literature. Our findings clearly show that SR-FTIR spectroscopic imaging can be used to extract valuable chemical information at the molecular level so as to have a better understanding of histopathological changes in TBI tissue. Although a severe TBI model was investigated in our present work,4,30 it is possible to detect moderate and mild TBI using the demonstrated technique by considering the excellent performance of SR-FTIR. The demonstrated technique has the potential to be developed to a label-free, digital, and intelligent approach for the diagnosis of diseased states of biological samples with high accuracy.

4. EXPERIMENT SECTION

4.1. TBI Model Induction. Approval of the animal experiments was granted by the Third Military Medical University (Army Medical University) Committee on Ethics in the Care and Use of Laboratory Animals. All experiments were carried out by complying with the Chinese Animal Welfare Legislation for Protection of animals used for scientific purposes. Three month-old C57 mice (Animal Center of the Third Military Medical University, Chongqing, China) with a weight of 20 ± 2 g for each were used in the experiments. The mice (n = 15) were subjected to TBI using a contusing device (68093, RWD Life Science Co., Ltd, Shenzhen, China) essentially following the method proposed by Feeney et al.28 The mice were first anesthetized by intraperitoneal injection of chloral hydrate (40 mg/kg) to achieve deep anesthesia that was confirmed by checking for pain reflexes. Next, individual mice were fixed in a stereotactic apparatus, and the head was disinfected and shaved. Then, the scalp was opened by a midline incision, and the peristium was gently removed to expose the skull. Afterward, left parietal craniotomy was carefully conducted by a dental drill to expose the dura with a diameter of ~3 mm but without damaging the meninges. Shortly thereafter, a 40 g stainless steel rod (~1.2 cm in diameter, 4.5 cm in height) was allowed to fall from a height of 20 cm to hit the footplate located on the exposed dura to cause brain injury. Finally, the hole was sealed by medical bone wax, the skin sutured after careful local disinfection, and the mouse was allowed to recover in the laboratory animal room with normal feeding and close monitoring.

4.2. TTC Staining. One day after the TBI, the mice (n = 6) were anesthetized by intraperitoneal injection of chloral hydrate (40 mg/kg) and then perfused intracardially with 0.9% saline. The mouse brains were then rapidly removed and frozen at −20 °C for 5 min.43 Individual brains were then sectioned into 1.0 mm thick slices by the blade of a freezing microtome (CM3050S, Leica Biosystems Nussloch GmbH, Nussloch, Germany). Afterward, the slices were stained by using a commercial TTC stain kit (D025-1-1, Nanjing Jiancheng, Bioengineering Institute, Nanjing, China) as per the manufacturer’s instructions. After being fixed with 4% paraformaldehyde (PFA, Beijing Solarbio Science & Technology Co., Ltd, Beijing, China) at 4 °C for a minimum of 24 h, the stained slices were photographed by a camera. Brain damage was revealed by a lack of TTC staining, which indicates that tissue is dehydrogenase deficient.

4.3. H&E Staining. One day after the TBI, the mice (n = 9) were anesthetized by intraperitoneal injection of chloral hydrate (40 mg/kg) and perfused individually with 25 mL of 0.9% saline followed by 25 mL of 4% PFA (buffered by 0.1% PBS). Then, the brain was removed and immediately fixed in 4% PFA at 4 °C for 48 h to prevent possible autolysis.44 PFA-fixed brain tissues were embedded in paraffin and sectioned into slices of 5 μm by a rotary microtome (CUT4062, SLEE medical GmbH, Mainz, Germany) to ensure even thickness of each slice. The slices were then de-paraffinized in xylene twice (5 min each time) and rinsed by each of 100, 95, 80, and 75% ethanol solutions for 1 min and then rinsed by each slice. The slices were mounted on BaF2 slides (3 mm × 3 mm × 1 mm, Shanghai Duopu Optical Material Co., Ltd, Shanghai, China) and kept in a drying box for later SR-FTIR experiments.

4.4. SR-FTIR Microspectroscopy. TBI samples were measured by a Nicolet 6700 FTIR Spectrometer with Nicolet Continuum IR Microscope in the transmission mode at the beamline BL01B of Shanghai Synchrotron Radiation Facility (SSRF). Each of the samples was first completely mapped spectroscopically in the mid-infrared range of 650–4000 cm⁻¹ at a resolution of 8 cm⁻¹ with 16 coadded scans and an aperture size of 80 μm × 80 μm. Then, small areas in the injured region and the corresponding normal region were mapped in the mid-infrared range of 650–4000 cm⁻¹ at a resolution of 4 cm⁻¹ with 32 coadded scans and an aperture size of 20 μm × 20 μm. All spectral data were collected by OMNIC9.2 software (Thermo Fisher Scientific, Waltham, MA, USA). Data mapping was performed with a step size the same as the aperture size. Raw spectral data were baseline corrected, smoothed by a 9-point Savitzky–Golay algorithm.45
4.5. Data Analysis. All raw spectra in the 1000−3000 cm−1 were corrected for the Mie scattering using MATLAB R2018a (MathWorks Inc., Natick, MA, USA) according to the RMie-EMSC algorithm described by Paul Bassan et al.36 PCA,44−49 and k-means clustering method were used to classify the data using MATLAB.30,31 Score plots of PCA were prepared using Origin 8.5 software (OriginLab Co., Northampton, MA, USA). PCA is a broadly used method for the reduction of the dimension or number of variables in a multidimensional dataset. The order of the PCs obtained through PCA indicates their importance to the original dataset. PC1 accounts for the highest amount of variance in the original dataset, PC2 the second highest, and so on. Normally, the first two or three PCs can be used to represent the original dataset if their total variance is no less than 90% of the total variance of the original dataset.52 k-means clustering analysis is a widely used efficient unsupervised data classification algorithm, by which the data can be grouped into k clusters through minimizing the average squared Euclidean distance of data points from their cluster centers. With the implementation of k-means clustering analysis, the spectral data can be grouped into k groups according to their similarity, and each group represents a region with identical/similar chemical/molecular properties.53

The cell densities have been examined by counting the cell numbers in the normal region and the injured region. The statistical data were based on six different mouse brain tissues subjected to TBI. For each stained slice, the cells were counted from randomly picked five different areas (80 μm × 160 μm) in the normal region and the injured region symmetrical to the normal region. Thus, the cells in 30 different areas for each kind of region have been counted. The cell densities have been compared by pair sample t-test analysis of hypothesis testing (Origin 8.5 software) and p < 0.01 was considered statistically significant in the comparison of data. The data are presented in mean ± standard deviation. The significance of the data was analyzed.

AUTHOR INFORMATION

Corresponding Authors

Yuzhao Tang – National Facility for Protein Science in Shanghai, Zhangjiang Lab, Shanghai Advanced Research Institute, Chinese Academy of Sciences, Shanghai 201210, China; Email: tangyuzhao@zjlabs.org.cn

Huabin Wang – Center of Applied Physics & Chongqing Engineering Research Center of High-Resolution and Three-Dimensional Dynamic Imaging Technology, Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, Chongqing 400714, China; orcid.org/0000-0001-5342-0672; Email: wanghuabin@citig.ac.cn

Authors

Yuansen Guo – Center of Applied Physics & Chongqing Engineering Research Center of High-Resolution and Three-Dimensional Dynamic Imaging Technology, Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, Chongqing 400714, China; University of Chinese Academy of Sciences, Beijing 100049, China

Tunan Chen – Department of Neurosurgery and Key Laboratory of Neurotrauma, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing 400038, China

Shi Wang – Department of Neurosurgery and Key Laboratory of Neurotrauma, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing 400038, China

Xiaojie Zhou – National Facility for Protein Science in Shanghai, Zhangjiang Lab, Shanghai Advanced Research Institute, Chinese Academy of Sciences, Shanghai 201210, China

Hua Zhang – Center of Applied Physics & Chongqing Engineering Research Center of High-Resolution and Three-Dimensional Dynamic Imaging Technology, Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, Chongqing 400714, China

Dandan Li – Center of Applied Physics & Chongqing Engineering Research Center of High-Resolution and Three-Dimensional Dynamic Imaging Technology, Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, Chongqing 400714, China

Ning Mu – Department of Neurosurgery and Key Laboratory of Neurotrauma, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing 40038, China

Mingjie Tang – Center of Applied Physics & Chongqing Engineering Research Center of High-Resolution and Three-Dimensional Dynamic Imaging Technology, Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, Chongqing 400714, China

Meidie Hu – Center of Applied Physics & Chongqing Engineering Research Center of High-Resolution and Three-Dimensional Dynamic Imaging Technology, Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, Chongqing 400714, China

Dongyun Tang – Center of Applied Physics & Chongqing Engineering Research Center of High-Resolution and Three-Dimensional Dynamic Imaging Technology, Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, Chongqing 400714, China

Zhongbo Yang – Center of Applied Physics & Chongqing Engineering Research Center of High-Resolution and Three-Dimensional Dynamic Imaging Technology, Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, Chongqing 400714, China

Jiajia Zhong – National Facility for Protein Science in Shanghai, Zhangjiang Lab, Shanghai Advanced Research Institute, Chinese Academy of Sciences, Shanghai 201210, China

Hua Feng – Department of Neurosurgery and Key Laboratory of Neurotrauma, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing 400038, China

Xuehua Zhang – Department of Chemical & Materials Engineering, University of Alberta, Alberta T6G1H9, Canada; orcid.org/0000-0001-6093-5324

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c03285

Author Contributions

Y.G. and T.C. contributed equally. The manuscript was written through contributions of all authors. All authors have given their approval to the final version of the manuscript.
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

The authors would like to thank the staff from BL01B beamline of National Facility for Protein Science in Shanghai Synchrotron Radiation Facility for assistance during data collection. This work was supported by National Natural Science Foundation of China (U1932132, U1632273 and U1732130), Central Government Supported Key Instrument Program of China (YXYGQ201700136), Natural Science Foundation of Chongqing (cstc2018jcyjAX045 and cstc2019jcy-msxmX0654), and the Chongqing Collaborative Innovation Center for Brain Science.

**REFERENCES**

1. Menon, D. K.; Schwab, K.; Wright, D. W.; Maas, A. I. Position statement: definition of traumatic brain injury. *Arch. Phys. Med. Rehabil.* 2010, 91, 1637–1640.

2. Feigin, V. L.; Theadom, A.; Barker-Collo, S.; Starkey, N. J.; McPherson, K.; Kahan, M.; Dowell, A.; Brown, P.; Parag, V.; Kydd, R.; Jones, K.; Jones, A.; Ameratunga, S. Incidence of traumatic brain injury in New Zealand: a population-based study. *Lancet Neurol.* 2013, 12, 53–64.

3. Deng, Y.; Thompson, B.; Gao, X.; Hall, E. Temporal relationship of peroxynitrite-induced oxidative damage, calpain-mediated cytoskeletal degradation and neurodegeneration after traumatic brain injury. *Exp. Neurol.* 2007, 205, 154–165.

4. Zhao, H.; Wang, Y.; Chen, L.; Shi, J.; Ma, K.; Tang, L.; Xu, D.; Yao, J.; Feng, H.; Chen, T. High-sensitivity terahertz imaging of traumatic brain injury in a rat model. *J. Biomed. Opt.* 2018, 23, 036015.

5. Maas, A.I.; Menon, D.K.; Adelson, P.D.; Andelic, N.; Bell, M.J.; Belli, A.; Bragge, P.; Brazinova, A.; Buki, A.; et al. Traumatic brain injury: integrated approaches to improve prevention, clinical care, and research. *Lancet Neurol.* 2017, 16, 987–1048.

6. Shenton, M. E.; Hamoda, H. M.; Schneiderman, J. S.; Bouix, S.; Pastorak, O.; Rath, Y.; Vu, M.-A.; Purohit, M. P.; Helmer, K.; Koerte, I.; Lin, A. P.; Westin, C.-F.; Kikinis, R.; Kubicki, M.; Stern, R. A.; Zafonte, R. A review of magnetic resonance imaging and diffusion tensor imaging findings in mild traumatic brain injury. *Brain Imag. Behav.* 2012, 6, 137–192.

7. Spadoni, A. D.; Huang, M.; Simmons, A. N. Emerging approaches to neurocircuits in PTSD and TBI: imaging the interplay of neural and emotional trauma. *Curr. Top. Behav. Neurosci.* 2018, 38, 163–192.

8. Zhang, X.-D.; Wang, H.; Antaris, A. L.; Li, L.; Diao, S.; Ma, R.; Nguyen, A.; Hong, G.; Ma, Z.; Wang, J.; Zhu, S.; Castellano, J. M.; Wys-Coray, T.; Liang, Y.; Luo, J.; Dai, H. Traumatic brain injury imaging in the second near-infrared window with a molecular fluorophore. *Adv. Mater.* 2016, 28, 6872–6879.

9. Katti, G.; Ara, S. A.; Shireen, A. Magnetic resonance imaging (MRI) – a review. *Int. J. Dent. Clin.* 2011, 3, 65–70.

10. Fernandez, D. C.; Bhargava, R.; Hewitt, S. M.; Levin, I. W. Infrared spectroscopic imaging for histopathologic recognition. *Nat. Biotechnol.* 2005, 23, 469–474.

11. Miller, L. M.; Dumas, P. From structure to cellular mechanism with infrared microscopy. *Curr. Opin. Struct. Biol.* 2010, 20, 649–656.

12. Pilling, M.; Gardner, P. Fundamental developments in infrared spectroscopic imaging for biomedical applications. *Chem. Soc. Rev.* 2016, 45, 1935–1957.

13. Tang, M.; McEwen, G. D.; Wu, Y.; Miller, C. D.; Zhou, A. Characterization and analysis of mycobacteria and Gram-negative bacteria and co-culture mixtures by Raman microscopy, FTIR, and atomic force microscopy. *Anal. Bioanal. Chem.* 2013, 405, 1577–1591.

14. Pilling, M. J.; Bassan, P.; Gardner, P. Comparison of transmission and translucence mode FTIR imaging of biological tissue. *Analyst* 2015, 140, 2383–2392.

15. Augustyniak, K.; Chrabaszcz, K.; Jasztal, A.; Smeda, M.; Quintas, G.; Kuligowski, J.; Marzec, K. M.; Malek, K. High and ultra-high definition of infrared spectral histopathology gives an insight into chemical environment of lung metastases in breast cancer. *J. Biophot.* 2019, 12, No. e20180345.

16. Krafft, C.; Thümmler, K.; Sobottka, S. B.; Schackett, G.; Salzer, R. Classification of malignant gliomas by infrared spectroscopy and linear discriminant analysis. *Biopolymers* 2006, 82, 301–305.

17. Bambery, K. R.; Schüttke, E.; Wood, B. R.; MacDonald, S. T. R.; Ataelammann, K.; Griebel, R. W.; Juirlink, B. H. J.; McNaughton, D. A. Fourier transform infrared microspectroscopic imaging investigation into an animal model exhibiting glioblastoma multiforme. *Biochim. Biophys. Acta Biomembr.* 2006, 1758, 900–907.

18. Surowka, A. D.; Pilling, M.; Henderson, A.; Boutin, H.; Christie, L.; Szczerszowska-Boruchowska, M.; Gardner, P. FTIR imaging of the molecular burden around Abeta deposits in an early-stage 3-Tg APP-PSI-TAU mouse model of Alzheimer’s disease. *Analyst* 2016, 142, 156–168.

19. Caine, S.; Herea, P.; Tobin, M. J.; McNaughton, D.; Bernard, C. C. A. The application of Fourier transform infrared microspectroscopy for the study of diseased central nervous system tissue. *Neuralmage* 2012, 59, 3624–3640.

20. Miller, L. M.; Dumas, P. Chemical imaging of biological tissue with synchrotron infrared light. *Biochim. Biophys. Acta Biomembr.* 2006, 1758, 846–857.

21. Nasse, M. J.; Walsh, M. J.; Mattson, E. C.; Reinner, R.; Kajdacsy-Balla, A.; Macias, V.; Bhargava, R.; Hirschmugl, C. J. High-resolution Fourier-transform infrared chemical imaging with multiple synchrotron beams. *Nat. Methods* 2011, 8, 413–416.

22. Stem, M. R. Understanding why researchers should use synchrotron-enhanced FTIR instead of traditional FTIR. *J. Chem. Educ.* 2008, 85, 983–989.

23. Liao, C. R.; Rak, M.; Lund, J.; Unger, M.; Platt, E.; Albensi, B. C.; Hirschmugl, C. J.; Gough, K. M. Synchrotron FTIR reveals lipid around and within amyloid plaques in transgenic mice and Alzheimer’s disease brain. *Analyst* 2013, 138, 3991–3997.

24. Surowka, A. D.; Adamek, D.; Szczerszowska-Boruchowska, M. The combination of artificial neural networks and synchrotron radiation-based infrared micro-spectroscopy for a study on the protein composition of human glial tumors. *Analyst* 2015, 140, 2428–2438.

25. Liu, Z.; Tang, Y.; Chen, F.; Liu, X.; Liu, Z.; Zhong, J.; Hu, J.; Liu, J. Synchrotron FTIR microspectroscopy reveals early adiogenic differentiation of human mesenchymal stem cells at single-cell level. *Biochem. Biophys. Res. Commun.* 2016, 478, 1286–1291.

26. Liu, G.; Chang, C.; Qiao, Z.; Wu, K.; Zhu, Z.; Cui, G.; Peng, W.; Tang, Y.; Li, J.; Fan, C. Myelin sheath as a dielectric waveguide for signal propagation in the mid-infrared to terahertz spectral range. *Adv. Funct. Mater.* 2019, 29, 1807862.

27. Zhang, J.; Niu, F.; Dong, H.; Liu, L.; Li, J.; Li, S. Characterization of protein alterations in damaged axons in the brainstem following traumatic brain injury using Fourier transform infrared microspectroscopy: a preliminary study. *J. Forensic Sci.* 2015, 60, 759–763.

28. Feeney, D. M.; Boyesen, M. G.; Linn, R. T.; Murray, H. M.; Dad, W. G. Responses to cortical injury: I. Methodology and local effects of contusions in the rat. *Brain Res.* 1981, 211, 67–77.

29. Shi, J.; Wang, Y.; Chen, T.; Xu, D.; Zhao, H.; Chen, L.; Yan, C.; Tang, L.; He, Y.; Feng, H.; Yao, J. Automatic evaluation of traumatic brain injury based on terahertz imaging with machine learning. *Opt. Express* 2018, 26, 6371–6381.

30. Yu, S.; Kaneko, Y.; Bae, E.; Stahl, C. E.; Wang, Y.; van Loveren, H.; Sanberg, P. R.; Borlongan, C. V. Severity of controlled cortical...
impact traumatic brain injury in rats and mice dictates degree of behavioral deficits. *Brain Res.* 2009, 1287, 157−163.

(31) Kramer, M.; Dang, J.; Baertling, F.; Denecke, B.; Clarner, T.; Kirsch, C.; Beyer, C.; Kipp, M. TTC staining of damaged brain areas after MCA occlusion in the rat does not constrict quantitative gene and protein analyses. *J. Neurosci. Methods* 2010, 187, 84−89.

(32) Yu, G.; Xu, L.; Hadman, M.; Hess, D. C.; Borlongan, C. V. Intracerebral transplantation of carotid body in rats with transient middle cerebral artery occlusion. *Brain Res.* 2004, 1015, 50−56.

(33) Li, Z.; Dong, X.; Zhang, J.; Zeng, G.; Zhao, H.; Liu, Y.; Qiu, R.; Mo, L.; Ye, Y. Formononetin protects TBI rats against neurological lesions and the underlying mechanism. *J. Neurol. Sci.* 2014, 338, 112−117.

(34) Diem, M.; Romeo, M.; Matthäus, C.; Miljkovic, M.; Miller, L.; Lasch, P. Comparison of Fourier transform infrared (FTIR) spectra of individual cells acquired using synchrotron and conventional sources. *Infrared Phys. Technol.* 2004, 45, 331−338.

(35) Jamin, N. g.; Miller, L.; Moncuit, J.; Fridman, W.-H.; Dumas, P.; Teillaud, J.-L. Chemical heterogeneity in cell death: combined synchrotron IR and fluorescence microscopy studies of single apoptotic and necrotic cells. *Biopolymers* 2003, 72, 366−373.

(36) Maziak, D. E.; Do, M. T.; Shamji, F. M.; Sundaresan, S. R.; Perkins, D. G.; Wong, P. T. T. Fourier-transform infrared spectroscopic study of characteristic molecular structure in cancer cells of esophagus: an exploratory study. *Canc. Detect. Prev.* 2007, 31, 244−253.

(37) Holman, H.-Y. N.; Miles, R.; Hao, Z.; Wozei, E.; Anderson, L. M.; Yang, H. Real-time chemical imaging of bacterial activity in biofilms using open-channel microfluidics and synchrotron FTIR spectromicroscopy. *Anal. Chem.* 2009, 81, 8564−8570.

(38) Ling, S.; Qi, Z.; Watts, B.; Shao, Z.; Chen, X. Structural determination of protein-based polymer blends with a promising tool: combination of FTIR and STXM spectroscopic imaging. *Phys. Chem. Chem. Phys.* 2014, 16, 7741−7748.

(39) Rosas-Hernandez, H.; Burks, S. M.; Cuevas, E.; Ali, S. F. Stretch-induced deformation as a model to study dopaminergic dysfunction in traumatic brain injury. *Neurochem. Res.* 2019, 44, 2546−2555.

(40) Hutson, C. B.; Lazo, C. R.; Mortazavi, F.; Giza, C. C.; Hovda, D.; Chesselet, M.-F. Traumatic brain injury in adult rats causes progressive nigrostriatal dopaminergic cell loss and enhanced vulnerability to the pesticide paraquat. *J. Neurotrauma* 2011, 28, 1783−1801.

(41) Toyran, N.; Zorlu, F.; Dönmez, G.; Öge, K.; Severcan, F. Chronic hypoperfusion alters the content and structure of proteins and lipids of rat brain homogenates: a Fourier transform infrared spectroscopy study. *Eur. Biophys. J.* 2004, 33, 549−554.

(42) Cornelius, C.; Crupi, R.; Calabrese, V.; Graziano, A.; Milone, P.; Pennisi, G.; Radak, Z.; Calabrese, E. J.; Cuzzocrea, S. Traumatic brain injury: oxidative stress and neuroprotection. *Antioxid. Redox Signaling* 2013, 19, 836−853.

(43) Xu, S.-y.; Jiang, X.-l.; Liu, Q.; Xu, J.; Huang, J.; Gan, S.-w.; Lu, W.-t.; Zhuo, F.; Yang, M.; Sun, S.-q. Role of rno-miR-124-3p in regulating MCT1 expression in rat brain after permanent focal cerebral ischemia. *Gene Dis.* 2019, 6, 398−406.

(44) Dernby, K. G. A study on autolysis of animal tissues. *J. Biol. Chem.* 1918, 35, 179−219.

(45) Savitzky, A.; Golay, M. J. E. Smoothing and differentiation of data by simplified least squares procedures. *Anal. Chem.* 1964, 36, 1627−1639.

(46) Bassan, P.; Kohler, A.; Martens, H.; Lee, J.; Byrne, H. J.; Dumas, P.; Gazi, E.; Brown, M.; Clarke, N.; Gardner, P. Resonant Mie Scattering (RMieS) correction of infrared spectra from highly scattering biological samples. *Analyst* 2010, 135, 268−277.

(47) Geng, G.; Dai, G.; Li, D.; Zhou, S.; Li, Z.; Yang, Z.; Xu, Y.; Han, J.; Chang, T.; Cui, H. L.; Wang, H. Imaging brain tissue slices with terahertz near-field microscopy. *Biotechnol. Prog.* 2019, 35, No. e2741.