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# Assessing fibrinogen extravasation into Alzheimer's disease brain using high-content screening of brain tissue microarrays

Pritika J. Narayan[a,b,c], Sue-Ling Kim[b], Claire Lill[b], Sheryl Feng[b], Richard L.M. Faull[b], Maurice A. Curtis[b], Michael Dragunow[a,b,*]

[a] Gravida, National Centre for Growth and Development, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand
[b] Centre for Brain Research, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand
[c] Biomedical Imaging Research Unit, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand

## HIGHLIGHTS

- Fibrinogen extravasation is significantly increased in Alzheimer's disease brain.
- High content analysis of brain tissue microarrays is demonstrated.
- Novel coupling of VSlide imaging and MetaXpress image analysis is used.
- HCS findings corroborated with Western blot results and manual *H*-scores.

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## ABSTRACT

*Background:* Tissue microarrays are commonly used to evaluate disease pathology however methods to automate and quantify pathological changes are limited.
*New method:* This article demonstrates the utility of the VSlide scanner (MetaSystems) for automated image acquisition from immunolabelled tissue microarray slides, and subsequent automated image analysis with MetaXpress (Molecular Devices) software to obtain objective, efficient and reproducible data from immunolabelled tissue microarray sections.
*Results:* Significant increases in fibrinogen immunolabelling were observed in 29 Alzheimer's disease cases compared to 28 control cases analysed from a single tissue microarray slide. Western blot analysis also demonstrated significant increases in fibrinogen immunolabelling in 6 Alzheimer's cases compared to 6 control cases. The observed changes were also validated with gold standard blinded manual *H*-scoring.
*Comparison with existing method:* VSlide Metafer software offers a 'tissue microarray acquisition' plugin for easy mapping of tissue cores with their original position on the tissue microarray map. High resolution VSlide images are compatible with MetaXpress image analysis software. This article details the coupling of these two technologies to accurately and reproducibly analyse immunolabelled tissue microarrays within minutes, compared to the gold standard method of manual counting using *H*-scores which is significantly slower and prone to inter-observer variation.
*Conclusions:* Here, we couple brain tissue microarray technology with high-content screening and automated image analysis as a powerful way to address bottle necks in data generation and improve throughput, as well as sensitivity to study biological/pathological changes in brain disease.


## 1. Introduction

The use of multiple-tissue blocks in histology was first described over two decades ago (Battifora, 1986), and has since evolved into what is known today as a tissue microarray (TMA) (Kononen et al., 1998). A TMA consists of an array of cylindrical tissue cores extracted from different paraffin-embedded tissue 'donor' blocks,

*Abbreviations:* TMA, tissue microarray; PBS, Phosphate buffered solution.

* Corresponding author at: Department of Pharmacology and Clinical Pharmacology, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland, New Zealand. Tel.: +64 9 9236403; fax: +64 9 3737556.
*E-mail address:* m.dragunow@auckland.ac.nz (M. Dragunow).

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which are subsequently punched into a 'recipient' paraffin block. The utility of this technology far surpasses that of previous histological methods, particularly as it enables tens, if not hundreds, of anatomical regions or cases to be arranged as small adjacent cores for assessment on a single glass slide (Decaestecker et al., 2009). This technology improves the processing time, reduces the amount of tissue resources and consumables required, and also minimises technical error (Kumar et al., 2004). Additionally, the sources of variation arising from immunolabelling protocols are consistent across all tissue cores on any given TMA section, allowing for standardisation of any technical error across all cases and/or anatomical regions (Kumar et al., 2004).

In diagnostic applications, there has been concern as to whether 0.6 mm or 1 mm cores are accurately representative of a tissue cross-section containing heterogeneous tumour cell populations. These concerns have been addressed by studies showing a strong correlation between the results obtained from immunolabelled TMA sections and traditional larger tissue sections (Gillett et al., 2000; Kyndi et al., 2008; Sauter and Mirlacher, 2002). As a result, TMA technology has been particularly useful in cancer diagnostics (Hoos and Cordon-Cardo, 2001; Horvath and Henshall, 2001; Kallioniemi et al., 2001; Moch et al., 1999; Mohamed et al., 2007) and, in more recent years, it has been applied to the study of non-neoplastic intracranial disorders (Goldstine et al., 2002; Kauppinen et al., 2006; Martikainen et al., 2006; Qu et al., 2009; Sjobeck et al., 2003; Wang et al., 2002).

In addition, commercially available systems such as the VSlide scanner (MetaSystems) coupled with MetaMorph software (Molecular Devices) can streamline image acquisition and analysis of cores. This coupling of scanner and software enables high-throughput processing of immunolabelled TMA sections, and thus avoids the effects of inter- and intra-observer subjectivity that is intrinsic to manual microscope analysis (Cregger et al., 2006). This coupling also improves the objectivity and accuracy of the data obtained and accelerates data processing capability. Image analysis-based segmentation of staining/labelling and the biological parameters chosen for extraction of quantitative features are pivotal in this process and also for ensuring the reproducibility of the data generated (Decaestecker et al., 2009).

This article demonstrates the utility and importance of high-content screening of immunolabelled, paraffin-embedded TMA sections to quantify fibrinogen immunolabelling in middle temporal gyrus from post-mortem control and Alzheimer's disease cases. This specific example aids in the assessment of fibrinogen extravasation across a potentially leaky blood brain barrier in Alzheimer's disease cases.

## 2. Methods

### 2.1. TMA design, production and case information

There are several important factors to consider in the design and production of TMAs. An excellent resource which provides important guidelines and considerations for the use of TMAs is provided in this review (Ilyas et al., 2013). The typical workflow used in our lab to prepare a TMA comprises of case selection, TMA template preparation, careful transfer of donor cores in to a recipient block and cutting sections using a rotary microtome (Leica, Model RM2235; see Supplementary Fig. 1 for further details on the workflow involved).

Case selection was a critical step in TMA preparation. Matching control and disease group cases for post-mortem delay (PMD) and age at death was important in order to minimise variation caused by these parameters. TMA#V, was used for this study. This TMA consists of control and Alzheimer's disease cases that were matched for age at death (control, mean 75 years; AD mean 76 years), PMD (control, mean 14 h; AD, mean 14 h), days in storage (control, mean 2735 days; AD, mean 2284 days) and where possible, sex (control 28% female; AD 55% female). This TMA has previously been used in two publications (Coppieters et al., 2014; Narayan et al., 2014) which provide detailed clinopathological information for each case including details of the date of perfusion, corresponding to the storage time of cases in the Brain Bank.

All brain tissue collected at autopsy from Auckland City Mortuary was delivered to the Neurological Foundation of New Zealand Human Brain Bank, in compliance with the University of Auckland Human Participants Ethics Committee. Alternatively tissue from outside Auckland was airfreighted to Auckland on ice for further processing. All brain tissue was processed using uniform methods as previously described (Waldvogel et al., 2008). Brain donations were accepted from individuals who had gone through a process of informed consent during life and then informed consent was obtained from the next of kin at the time of death. All procedures are governed by the Human Tissue Act of 2008 and all procedures were approved by the University of Auckland Human Participants Ethics committee. Brain tissue blocks used for the preparation of donor blocks were fixed, dehydrated and processed through standard histological protocols for wax embedding, as described previously (Waldvogel et al., 2006). Before preparation of the TMA, 7-μm thick sections were taken from each of the donor blocks and labelled using standard paraffin immunolabelling protocols for microtubule-associated protein 2 (MAP2). This ensured that all cases selected for the final TMA were exempt of fixation irregularities and labelled accurately for an antibody against a ubiquitous protein for this anatomical region. Once cases were confirmed, an additional 7 μm section was taken from each case and labelled with Nissl. Nissl staining was particularly important for identifying specific cell layers of interest (for example, layer I–V of the cortical ribbon), to assist with choosing specific regions to core from the donor block (see also Section 2.3) (Gittins and Harrison, 2004). A TMA template was also prepared to assign the location of each case and of the blank reference points that served as orientation markers for mounting onto glass slides. An example of the template used to prepare TMA#V is provided in Supplementary Table 2.

The Advanced Tissue Arrayer, model VTA-100 (Veridiam) was used for the extraction of 2 mm tissue cores from different donor blocks and their insertion into the blank recipient paraffin block (see Fig. 1). Core cylinder lengths varied from 4 to 6 mm depending on the size of the donor tissue block. Careful, slow and consistent coring into donor blocks was imperative to avoid causing any cracks or breakages in the cores taken and the donor block. Once all donor cores had been transferred, the recipient block was incubated overnight at 37 °C, followed by 7–8 min incubation at 60 °C. This enabled the paraffin between each core and the recipient paraffin block to amalgamate upon heating.

Once cooled, the recipient block was cut into 7 μm thick sections using a rotary microtome. The block was initially cut until all cases were represented as a single section. Approximately 200 sections were obtained from any given TMA. It was important to take care when mounting serial sections onto corresponding numbered charged glass slides. Blanks were used to ensure consistency in the orientation of the section when mounted onto charged slides in the water bath. Once mounted and dried overnight at room temperature, the TMA sections were stored at room temperature until further use.

### 2.2. Immunohistochemistry of paraffin-embedded sections

Standard immunohistochemistry was applied, as described previously (Waldvogel et al., 2008). Briefly, mounted slides were