

# Multi-photon Microscopy for Characterization of Renal Cell Carcinoma Pseudocapsule: Implications for Tumour Enucleation

Tan Yi Quan<sup>1</sup>, Tay Wy Keat<sup>1</sup>, Ooi Li Yin<sup>2</sup>, Teo Zui Chih Rachel<sup>3</sup>, Tiong Ho Yee<sup>1</sup>

<sup>1</sup>Department of Urology, National University Health System, Singapore; <sup>2</sup>Department of Pathology, National University Health System, Singapore; <sup>3</sup>Department of Medicine, National University Health System, Singapore

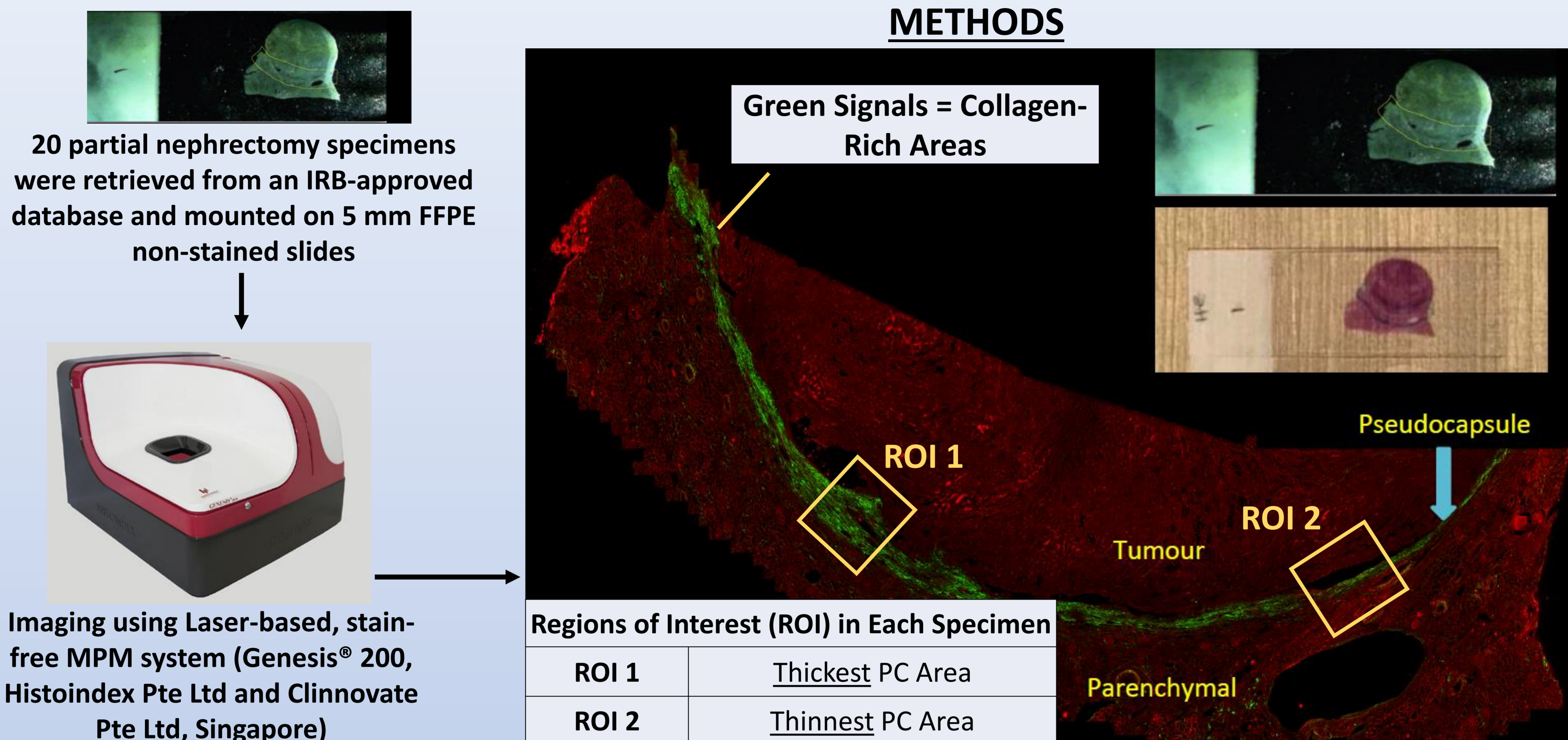
## INTRODUCTION

RCC Tumour Enucleation depends on dissection along its fibrous Pseudocapsule (PC), an Extracellular Matrix (ECM) structure. Collagen, being an abundant ECM component, is the strongest and hardest to penetrate by tumour cells. During tumour expansion, collagen remodeling and reorientation occurs, with increased collagen deposition and density for mechanical strength to resist tumour growth. Degradation of collagen has been shown necessary for tumour invasion.<sup>1</sup> The presence and integrity of PCs have been associated with improved overall survival in RCC.<sup>2,3</sup> Multi-Photon Microscopy (MPM) has been successfully employed as a stain-free imaging technique for collagen quantification in other biological tissues.<sup>4</sup>

## OBJECTIVES

To use Multi-Photon Microscopy to quantify the collagen content and structure of the PC. The study hypothesised that the PC is not a homogenous layer and has quantifiable intra-tumoral variation.

## METHODS



20 partial nephrectomy specimens were retrieved from an IRB-approved database and mounted on 5 mm FFPE non-stained slides

Imaging using Laser-based, stain-free MPM system (Genesis<sup>®</sup> 200, Histoindex Pte Ltd and Clinovate Pte Ltd, Singapore)

Green Signals = Collagen-Rich Areas

Regions of Interest (ROI) in Each Specimen

ROI 1	Thickest PC Area
ROI 2	Thinnest PC Area

At each ROI, clinically validated algorithms quantify collagen content and structure. These algorithms are "machine learned" with pathologists inputs and validated against conventional stained H&E images.

Collagen Parameters Quantified		Definition	Unit
Collagen Content	Collagen Area Ratio (CAR)	Percentage of collagen within given area	%
	Collagen Fibre Density (CFD)	Collagen density within collagen fibre	%
Collagen Structure	Collagen Reticulation Index (CRI)	Number of collagen crosslinks within a given area	Unitless

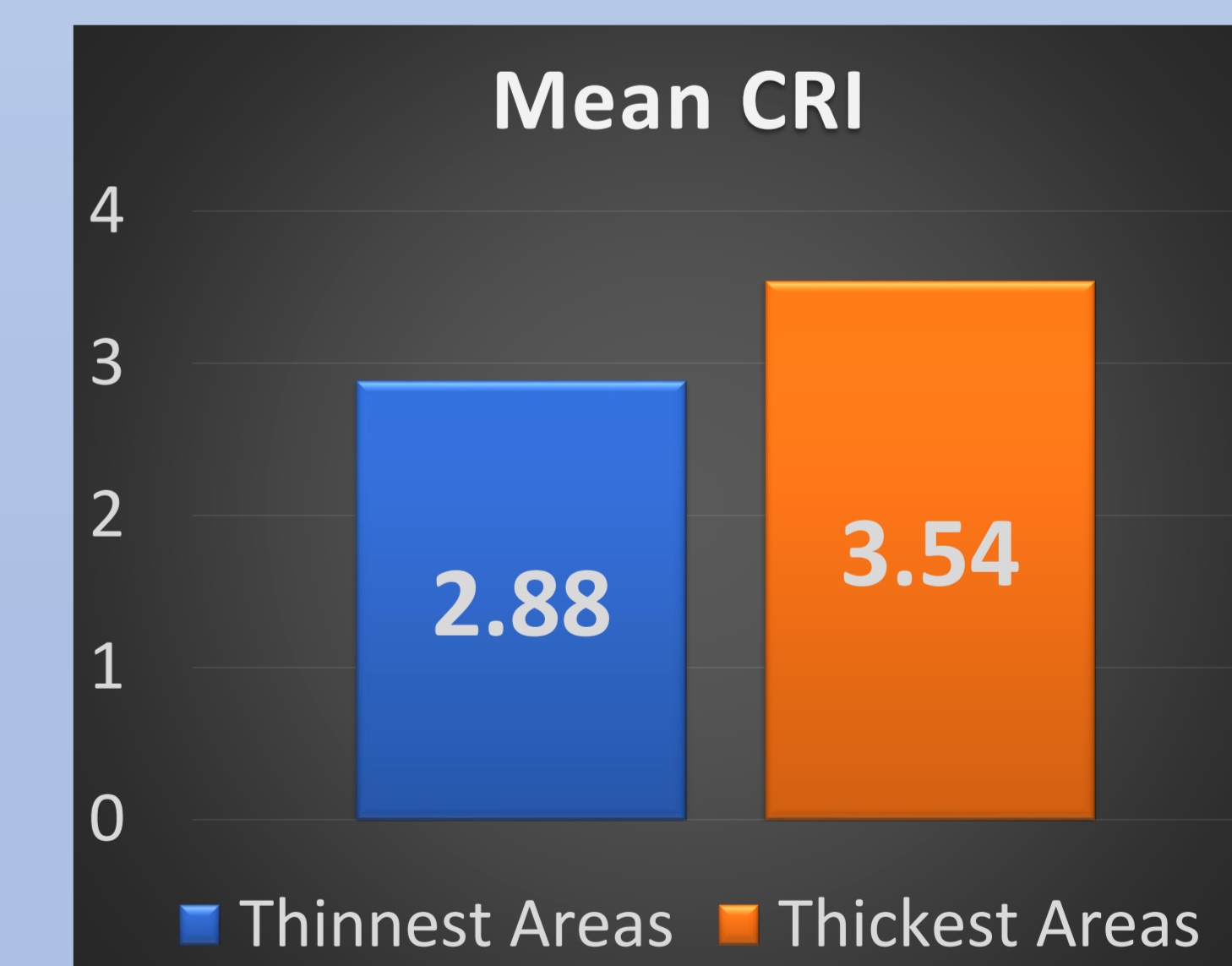
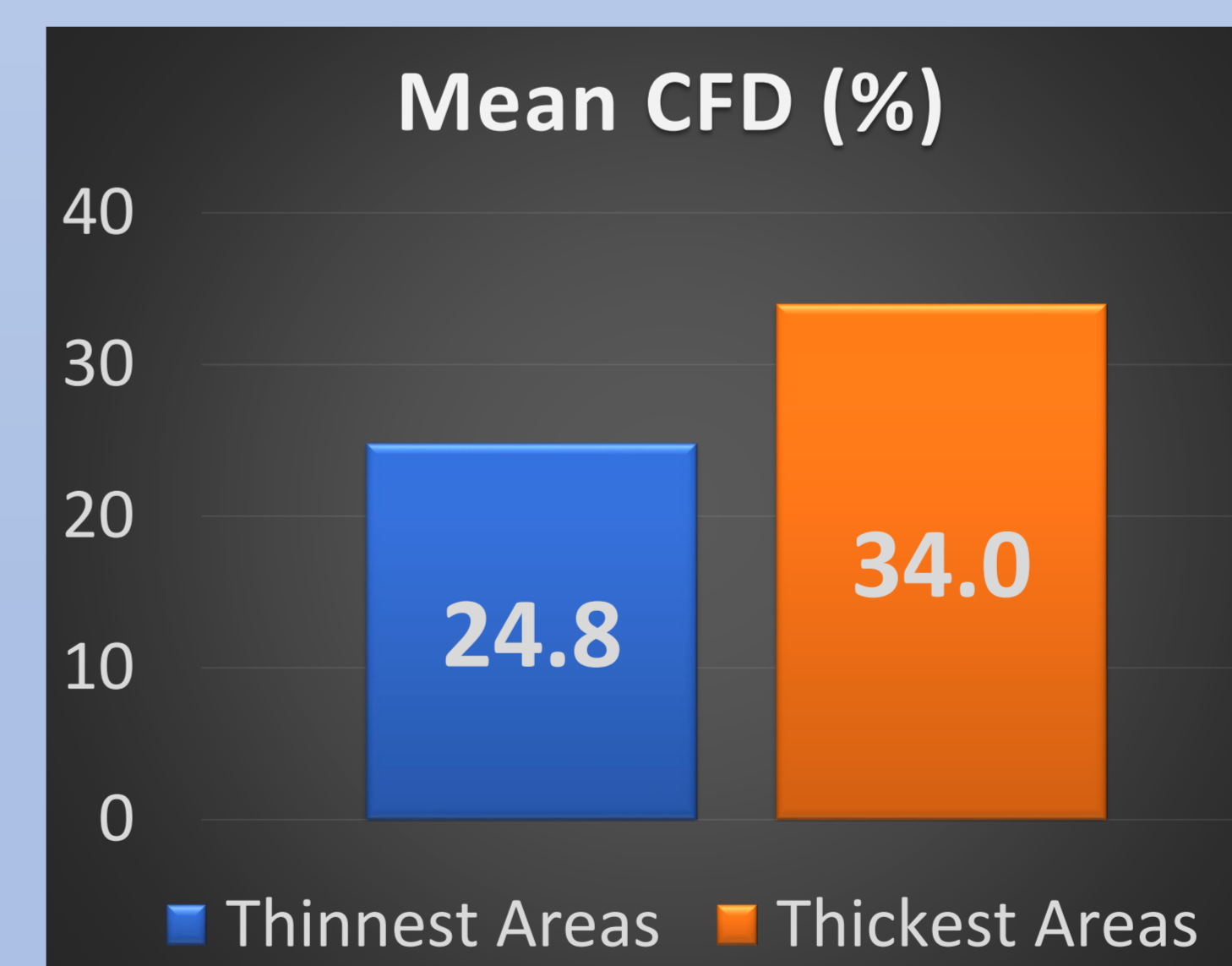
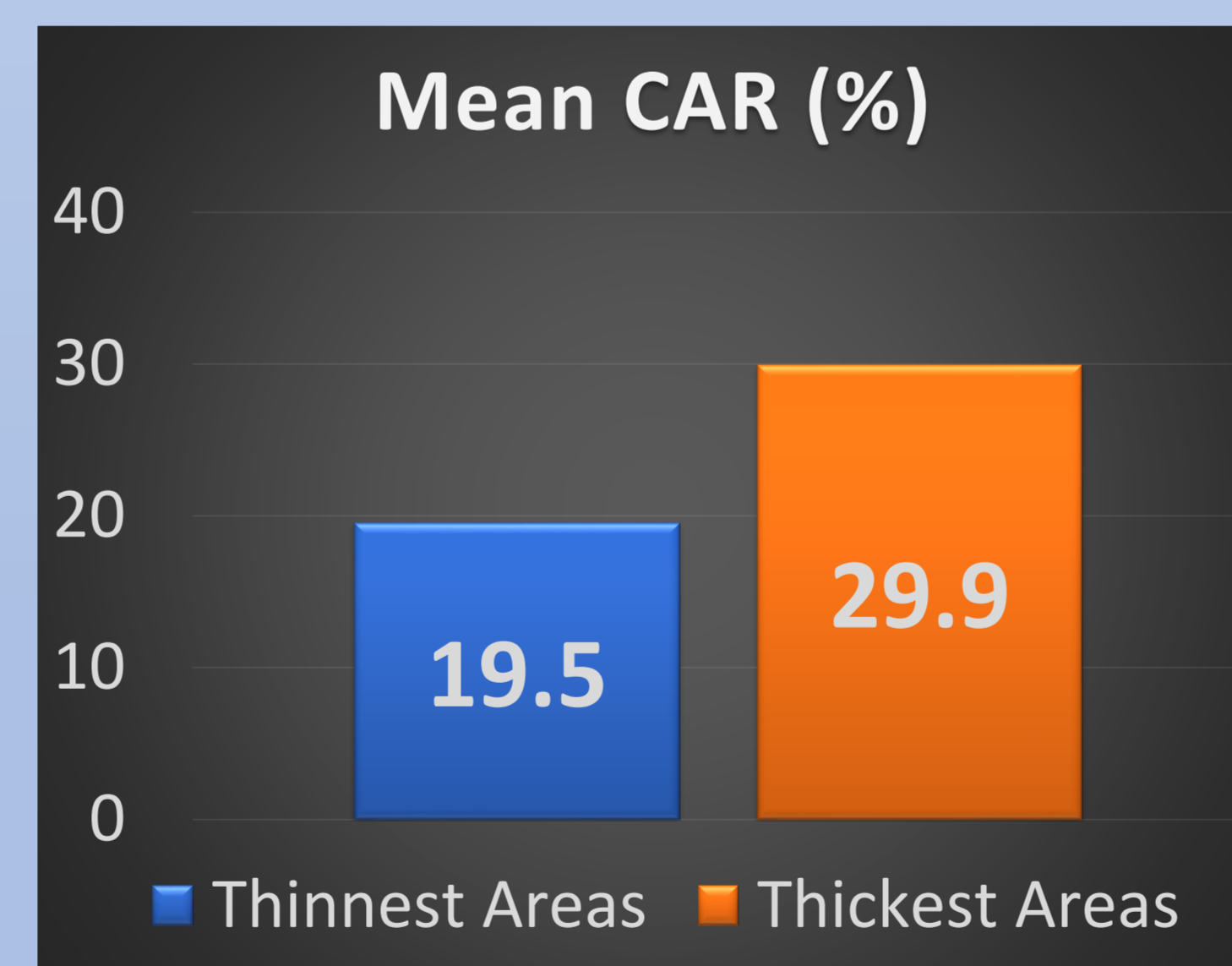
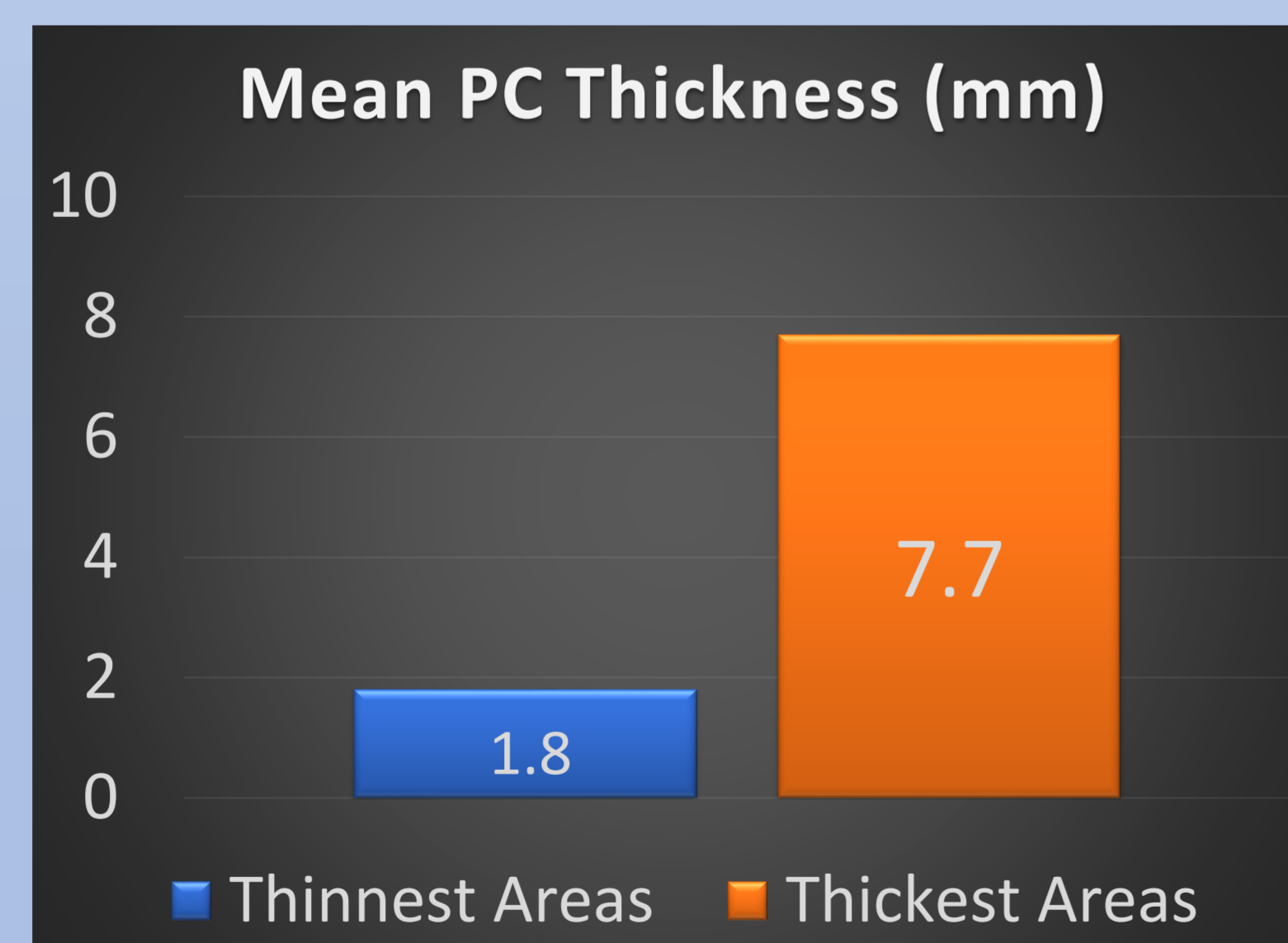
Statistical analysis was performed using Paired T-Test to compare if CAR, CFD and CRI between the thickest PC areas (ROI 1) and thickest PC areas (ROI 2)

## RESULTS

Table 1 shows the baseline patient characteristics. The mean variation difference in PC thickness between the thickest and thinnest PC areas (ROI 2) was 5.9 +/- 1.21mm. In the thickest areas of the PC, collagen content was higher compared to the thinnest areas. Mean Collagen Area Ratio was higher in the thickest (29.9%, 95% CI: 19.9-39.9%) compared to the thinnest areas (19.5%, 95% CI: 17.2-21.8%) of the PC (p=0.12). Mean Collagen Fibre Density was also higher in the thickest (34.0%, 95% CI: 25.2-42.8%) compared to the thinnest areas (24.8%, 95% CI: 21.1-28.6%) of the PC (p=0.09). Compared to the thinnest PC areas, the thickest PC areas showed greater degrees of collagen cross-linking and collagen network complexity. Mean Collagen Reticulation Index was higher in the thickest PC areas (3.54, 95% CI: 2.79-4.29) compared to the thinnest (2.88, 95% CI: 2.15-3.60) PC areas (p=0.13). However, these differences did not reach statistical significance.

Table 1: Baseline Patient Characteristics

Age (mean+/-SD)	60.6 +/- 10.6 years
Gender	15 male (75%)
	5 female (25%)
Tumour Size (mean+/-SD)	3.32 +/- 1.47cm
Nephrometry Score	5 Low (4-6)
	13 Medium (7-9)
	2 High (10-12)
Tumour Subtype	11 Clear Cell
	6 Papillary
	3 Chromophobe
Fuhrman Grade (for CCRCC)	3 (1-2)
	8 (3-4)



## CONCLUSIONS

For oncological safety, it is important for surgeons to be aware that Pseudocapsules at the tumour-parenchyma interface are not homogenous during Tumour Enucleation. With MPM, we can quantify PC collagen content and structure objectively, as an indicator of mechanical strength. As a proof-of-concept study, these findings will lead to larger studies using MPM to compare PCs between tumours of different subtypes and nuclear grade, with the long-term goal of correlating with survival outcomes.

## REFERENCES

1. F Min, JP Yuan, CW Peng, L Yan. Collagen as a Double-edged Sword in Tumour Progression. *Tumor Biol* 2014 Dec; 35:2871-2882.
2. X Wei *et al.* Evaluation of Tumor Pseudocapsule Status and its Prognostic Significance in Renal Cell Carcinoma. *J Urol* 2018 Apr; 199:915-920.
3. M Kim *et al.* Differences in Peritumoral Pseudocapsule Characteristics According to Clinicopathological Factors in Clinical T1a Renal Tumors. *Int J Clin Exp Pathol* 2015;8(9):11323-11331.
4. XF Wu *et al.* Visualization of Basement Membranes in Normal Breast and Breast Cancer Tissues Using Multiphoton Microscopy. *Oncology Letters* 2016; 11:3785-3789.