Comparison of Laser Scanning Confocal Microscopy & Light Microscopy in Forensic Histo-Osteology

Watamaniuk, L[1], Smith, A.C.[2], Dion, N[3], Ste-Marie, L.G.[3]

[1]McMaster University, [2]University of Toronto – Mississauga, [3]Centre Hôspitalier de l'Université de Montréal

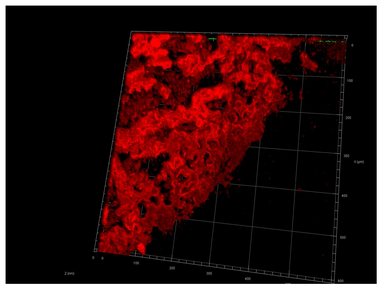

Fig 1

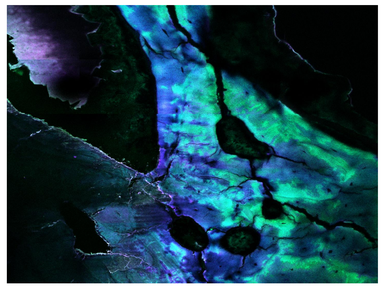

Fig 2

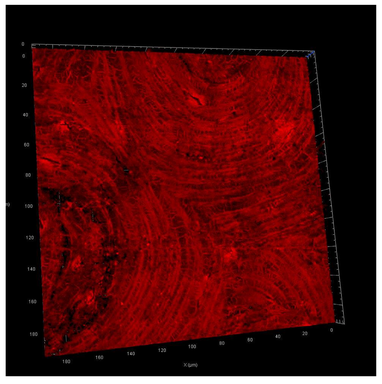

Fig 3

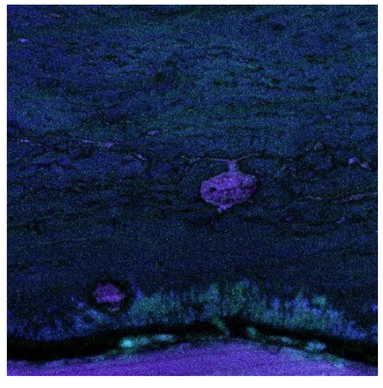

Fig 4

Introduction

Forensic anthropologists have applied histological methods to undecalcified bone specimens throughout the development of the discipline to answer questions ranging from whether fragmentary or burned remains are human, to estimating age at death. This pursuit has involved the use of ground sections of bone viewed under plane and polarized light microscopy, and has increasingly included the use of special stains to increase the visibility (and quantifiability) of microstructures. The use of microscopy in forensic anthropology, however, is limited by the condition of the remains under investigation. In particular, diagenetic alteration (chemical and biological changes to the content and structure of bone from the time of deposition to discovery) can complicate analysis. The purpose of this pilot study was to determine whether the application of an alternate modality, laser scanning confocal microscopy (LSCM), to stained and unstained specimens, improves the ability of forensic anthropologists to perform histological analysis on recovered human remains.

Methodology

Samples/Stains Used

- Clinical trans-iliac biopsies (*n*=16)
- Rib sections (3-5th Century BCE) (*n*=20)
- Human anatomical femoral sections (*n*=5)
- Porcine iliac fragments (*n*=20)
- Each sample was stained with Basic Fuchsin and Toluidine Blue

Each sample was viewed under low (4-20x) to high (40-150x) magnification using the following microscopes:

- Keyence VHX1000 Digital Light Microscope
- Nikon Eclipse E200 with Nikon DSLR7000 Camera
- Carl Zeiss LSM800 Laser Scanning Confocal Microscope
- Carl Zeiss LSM880 Laser Scanning Confocal Microscope
- Leica Spinning Disc Confocal Microscope

Each sample was assessed under each modality for its resolution and speed of processing by the authors.

Results

Analysis	LSCM	TLM
Characterizing Diagenesis	☑☑☑	☑☑
Edge Geometry of Bone Elements	☑☑☑	☑
Pathological Detail	☑☑☑	☑☑
Microstructure of Bone	☑☑☑	☑
Age Estimation	x	☑☑☑
Animal vs Human	x	☑☑☑
3 Dimensional (Z-stack) Resolution	☑☑☑	☑

Each ☑ represents the authors collective decision on resolution and speed of processing of the samples under microscopy for a given task.

Discussion & Conclusion

This pilot study has demonstrated that the use of LCSM in forensic anthropology has some advantages over traditional light or digital light microscopy in higher order investigations: z-stack resolution at high magnification, assessment of fracture edge geometry, assessment of bone diagenesis, and high magnification bone microstructure studies (Fig 1-4). When combined with stains and dyes (e.g. Basic Fuchsin or Toluidine Blue) it can further elucidate protein location and protein degradation for estimation of the post mortem interval. However, for low to middle order investigations: animal versus human, age estimation, and assessment of histopathology, light and digital light microscopy, along with the use of special stains such as Toluidine Blue and/or a light polarizing filter (Fig. 5-8) is still a more efficient and effective method of investigation.

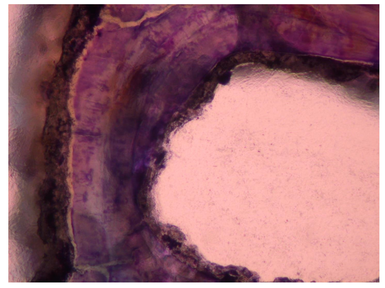

Fig 5

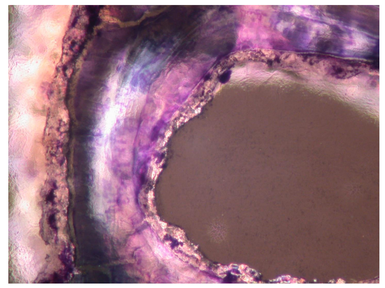

Fig 6

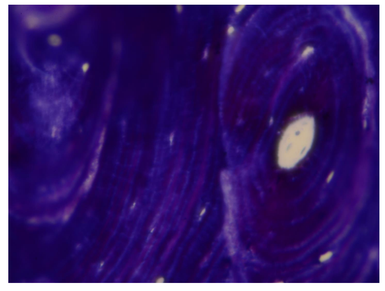

Fig 7

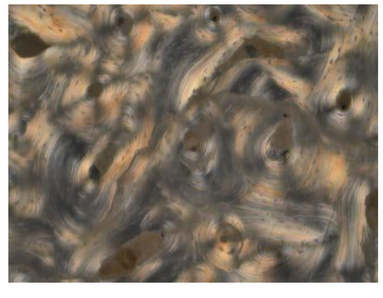

Fig 8

With special thanks to : Dr. Anne Keenleyside, Trent University, Dr. Tracy Rogers, University of Toronto, Dr. Tracy Prowse, McMaster University, UTM Department of Biology, Vanessa Rossi, MSc., University of Toronto