

**FRACTAL ANALYSIS OF
CANCELLOUS BONE IN DISEASE**

IAN HENRY PARKINSON

**Thesis submitted for the degree of
Doctor of Philosophy
in
The Department of Pathology
The University of Adelaide**

JUNE 2002

"Over the mountains
Of the Moon,
Down the Valley of the Shadow,
Ride, boldly ride,"
The shade replied -
"If you seek for Eldorado!"

from Eldorado

Edgar Allan Poe

Errata Sheet

Page	Para	Line	
Viii	3	4	"effect" should be "affect".
X	2	2	"remodeling" should be "remodelling".
10	5	5	"Figure 1.2d" should be "Figure 1.2c".
11	legend		"F _D =Maximum Feret's diameter, δ = Fractal dimension" inserted.
12	1	5-6	Should be "(Figure 1.3a), where the perimeter is the ...".
17	2	14	"modeling" should be "modelling".
24	1	3	"where" should be "were".
36	1	1	"figure x" should be "Figure 2.2d".
51	2	4	"difficult" should be deleted.
66	1	7	"remodeling" should be "remodelling".
66	2	2	second instance of "was constructed" should be deleted.
67	1	2	"bone multicellular unit" should be "basic multicellular unit".
68	legend		"remodeling" should be "remodelling".
69	1	2	"remodeling" should be "remodelling".
69	1	5	"remodeling" should be "remodelling".
114	1	5	"Aaron <i>et al</i> (1985)" should be "Aaron <i>et al</i> (1987)".
114	1	8	"Thomson" should be "Thomsen".
114	1	8	"Chavassieux and Meunier 2001" should be deleted.
116	2	9	"modeling" should be "modelling".
116	2	10	"remodeling" should be "remodelling".
125	legend 1		"between" should inserted after "differences".
125	legend 2		"between" should inserted after "differences".
131	1	1	"Hildebrand and Ruegsegger 1997" should be deleted.
135	legend		"Parfitt <i>et al</i> 1987" inserted at end.
135	2	7	"P1" should be "P2".
136	1	2	"Hahn <i>et al</i> 1992" inserted at end.
154	1	11	"Figure 4.4x and 4.4y" should be deleted.
154	2	7	"Figures 4.4x and 4.4y" should be deleted.
154	2	8	"independent" should be "different".
155	1	2	"Figures 4.4x and 4.4y" should be deleted.
169	1	1	"with the normal" should be "and normals".
173	1	3	"stained" should be deleted.
197	2	4	"is" should be deleted.
241	2	6	"remodeling" should be "remodelling".
244	3	9	"The" should be "the".
245	2	6	"modeling" should be "modelling".
245	3	5	"remodeling" should be "remodelling".

TABLE OF CONTENTS

ABSTRACT	viii
DECLARATION	xiii
ACKNOWLEDGEMENTS	xiv
PUBLICATION ARISING	xv
AWARDS	xvi
<u>CHAPTER ONE</u>	
INTRODUCTION AND LITERATURE REVIEW	1
1.1 INTRODUCTION	2
1.2 THEORY	6
1.3 MEASURING THE FRACTAL DIMENSION	12
1.4 APPLICATIONS OF FRACTAL ANALYSIS	16
1.4.1 Introduction	16
1.4.2 Fractal Analysis in Medicine	16
1.4.3 Fractal Analysis of Bone	18
1.5 SUMMARY	26
<u>CHAPTER TWO</u>	
METHODOLOGY FOR FRACTAL ANALYSIS	28
2.1 Introduction	30
2.2 Analytical Method	33
2.3 Objects and Specimens	39
2.4 Efficacy of Fractal Analysis	42
2.4.1 Introduction	42
2.4.2 Methods	42
2.4.3 Results	44

2.4.4 Discussion	45
2.5 Linear Limits of Fractal Analysis	46
2.5.1 Smallest Box Size Study	46
2.5.1.1 Introduction	46
2.5.1.2 Methods	46
2.5.1.3 Results	47
2.5.1.4 Discussion	48
2.5.2 Largest Box Size Study	49
2.5.2.1 Introduction	49
2.5.2.2 Methods	49
2.5.2.3 Results	50
2.5.2.4 Discussion	51
2.6 Image Analyser Settings	52
2.6.1 Magnification Study	52
2.6.1.1 Introduction	52
2.6.1.2 Methods	52
2.6.1.3 Results	53
2.6.1.4 Discussion	53
2.6.2 Angle of Presentation Study	54
2.6.2.1 Introduction	54
2.6.2.2 Methods	54
2.6.2.3 Results	54
2.6.2.4. Discussion	55
2.6.3 Detection Study	56
2.6.3.1 Introduction	56
2.6.3.2 Methods	56
2.6.3.3 Results	57
2.6.3.4 Discussion	57
2.7 Analysis of Richardson Plot	59
2.7.1 Plateau Removal study	59
2.7.1.1 Introduction	59

2.7.1.2 Methods	59
2.7.1.3 Results	60
2.7.1.4 Discussion	60
2.7.2 Sectional Fractal Dimensions Study	61
2.7.2.1 Introduction	61
2.7.2.2 Methods	62
2.7.2.3 Results	64
2.7.2.4 Discussion	65
2.7.3 Schematic Study	66
2.7.3.1 Introduction	66
2.7.3.2 Methods	66
2.7.3.3 Results	68
2.7.3.4 Discussion	69
2.7.4 Goodness of Fit Study	71
2.7.4.1 Introduction	71
2.7.4.2 Methods	71
2.7.4.3 Results	72
2.7.4.4 Discussion	73
2.8 Discussion	75
2.9 Appendix	80

CHAPTER THREE

FRACTAL DIMENSIONS AND THE NORMAL SKELETON 91

3.1 Introduction	92
3.2 Methods	96
3.2.1 Specimen Preparation	96
3.2.2 Data Acquisition	102

3.2.3 Statistics	102
3.3 Results	106
3.3.1 Descriptive Statistics and Sex Differences	106
3.3.2 Relationships with Age	107
3.3.3 Differences Between Skeletal Groups	111
3.4 Discussion	114
3.5 Appendix	120

CHAPTER FOUR

FRACTAL DIMENSIONS AND HISTOMORPHOMETRY IN NORMALS 129

4.1 Introduction	130
4.2 Methods	134
4.2.1 Specimen Preparation	134
4.2.2 Data Acquisition	134
4.2.3 Statistics	136
4.3 Results	140
4.3.1 Descriptive Statistics and Sex Differences	140
4.3.2 Relationship of Age with Structural Parameters of Cancellous Bone	142
4.3.3 Differences Between Skeletal Groups	144
4.3.4 Relationships of Fractal Dimensions with Structural Parameters of Cancellous Bone	145
4.4 Discussion	152
4.5 Appendix	157

CHAPTER FIVE

FRACTAL DIMENSIONS AND PATHOLOGY 166

5.1 Introduction	167
-------------------------	------------

5.2 Methods	171
5.2.1 Specimen Preparation	171
5.2.2 Data Acquisition	174
5.2.3 Statistics	175
5.3 Results	179
5.3.1 Descriptive Statistics and Sex Differences	179
5.3.2 Age Relationships	185
5.3.3 Relationships of Fractal Dimensions with Structural Parameters of Cancellous Bone.	198
5.3.4 Differences Between Normal and Disease Groups	202
5.4 Discussion	214
5.5 Appendix	221

CHAPTER SIX

SUMMARY AND CONCLUDING REMARKS 239

6.1 Unifying Discussion	240
6.2 Limitations	244
6.3 Future Directions	245

BIBLIOGRAPHY 247

ABSTRACT

The principal aim of this thesis was to develop and implement a standardised protocol for the fractal analysis of cancellous bone architecture. Cancellous bone structure from different skeletal sites in groups of osteoporotic, osteoarthritic and normal individuals was analysed. The results of fractal analysis were explained in the context of conventional bone histomorphometry and *a priori* knowledge to advance the understanding of cancellous bone architecture.

There has been much effort devoted to the pursuit of descriptors of cancellous bone complexity. The aim of these endeavours has been to develop morphological descriptors of bone quality that explain the functional properties of the cancellous bone structure for age-related changes, the effect of disease processes or the effect of therapeutic agents on the diseased skeleton. The fractal analysis of the complexity of cancellous bone architecture promises to be an exciting addition to existing analytical techniques.

The establishment of a standardised methodology for the fractal analysis of cancellous bone encompassed many components. Knowledge of the stereological and histomorphometric principles that are employed in currently available techniques enabled a comprehensive examination of the factors that effect the measurement of the fractal dimensions. The methodology presented in this thesis has been optimised specifically for measuring sectional fractal dimensions in histological sections of cancellous bone.

The sectional fractal dimensions show that, over three ranges of scale, cancellous bone is effectively fractal at multiple sites in the normal skeleton. The three sectional fractal dimensions describe different morphological

compartments of the cancellous bone structure. Fractal 1 describes the surface texture of the trabeculae, fractal 2 describes the shape or form of individual trabeculae and fractal 3 describes the spatial arrangement or overall architecture of the cancellous bone.

This thesis reports that in the normal skeleton there are differences between skeletal sites for the fractal dimensions, which are dependent on the functional properties of the skeletal sites. Fractal 2 and fractal 3 for subchondral cancellous bone is greater than vertebral body and iliac crest cancellous bone, which indicates greater morphological complexity. Also, fractal 2 and fractal 3 in subchondral cancellous bone show an age-related decrease, which suggests that the cancellous bone structure becomes less complex with age. This interostotic variability in response to ageing is indicative of the heterogeneity in functional properties of cancellous bone in the skeleton.

In this thesis, fractal analysis has been shown to detect morphological differences in the cancellous bone between normals, osteoporotics and osteoarthritics in the compressive and tensile trabeculae of the femoral head and the iliac crest. These data have provided new insights into the mechanisms of change to cancellous bone structure in ageing and in disease.

Age-related changes in the structural parameters of cancellous bone are seen at all the skeletal sites in the normals but are only present in the compressive trabeculae of the femoral head in the osteoporotics and not at all in the osteoarthritics. These observations indicate that these disease processes are associated with an uncoupling of the control mechanisms that affect cancellous bone structural complexity. In the normals, the fractal dimensions only show age-related change in the tensile trabeculae of the femoral head, suggesting that fractal analysis is not suitable for detecting the age-related changes that are quantified by the structural parameters of cancellous bone in these study groups

but the fractal dimensions detect underlying cancellous bone complexity independent of age.

For the osteoporotics, fractal 1 is the same at all skeletal sites. This suggests that the relative levels of remodeling activity are the same for both normals and osteoporotics. Fractal 2 for both the compressive and tensile trabeculae in the femur is significantly lower for the osteoporotics than the normals but in the iliac crest, fractal 2 is the same. This implies that in the femoral head the osteoporotics have trabeculae that are significantly less complex in shape than the normals. This phenomenon is not seen in the iliac crest, which is usually the site of diagnostic biopsy. Therefore, biopsies for diagnosis of osteoporosis may not show changes in cancellous bone structural complexity that are evident in disease affected sites. The structural parameters of cancellous bone show that osteoporotics lose whole trabeculae due to perforation of trabeculae, through decreased Tb.N and increased Tb.Sp. This leads to less interconnection between trabeculae, loss of branching and more rounded trabeculae, hence the trabeculae are less complex in shape. For fractal 3, in compressive and tensile regions of the femur the osteoporotics are significantly lower than the normals and in the iliac crest the osteoporotics are the same as the normals. This indicates that in the femoral head the spatial arrangement of the trabeculae within the cancellous structure of the osteoporotics is less complex. The structural parameters of cancellous bone show that there is loss of whole trabeculae, which is associated with increased spatial separation between the trabeculae as bone is lost.

For the osteoarthritics, fractal 1 is the same as the normals at all skeletal sites. Fractal 2 for the compressive trabeculae in the femoral head is significantly higher for the osteoarthritics than the normals but in the tensile trabeculae of the femoral head and the iliac crest fractal 2 for the osteoarthritics is the same as the normals. This implies that in the compressive trabeculae of the femoral head the osteoarthritics have trabeculae that are significantly more complex in

shape than the normals. The structural parameters of cancellous bone show that the compressive trabeculae of the femoral head are thicker, more numerous and less widely separated with greater BV/TV than the normals. This leads to greater interconnectivity between trabeculae and more complex branching, hence the trabeculae are more complex in shape. For fractal 3, in the compressive and tensile regions of the femoral head the osteoarthritics and the normals are the same but in the iliac crest the osteoarthritics are lower than the normals. This indicates that the spatial arrangement of the trabeculae within the cancellous structure of the osteoarthritics does not change in response to the disease process in subchondral cancellous bone adjacent to the articular lesion but in the iliac crest the spatial arrangement of the trabeculae in osteoarthritics is less complex in shape. The structural parameters of cancellous bone show that BV/TV is increased in the compressive and tensile trabeculae of the femoral head but not in the iliac crest of the osteoarthritics. This indicates that the spatial complexity of the trabecular arrangement within the cancellous structure of osteoarthritics changes independently of changes in cancellous bone structure detected by the structural parameters of cancellous bone.

The sectional fractal dimensions have detected differences in morphological complexity between the normals and disease groups and between the skeletal sites. These novel data have been obtained using an innovative technique that is not dependent on assumptions based on conceptual models of cancellous bone structure. *A priori* knowledge of bone biology is utilised to enable the fractal analysis to measure specific morphological entities within the cancellous bone structure. The fractal dimensions have identified changes in the morphological complexity of specific components of the cancellous structure, which are not identified by existing model-based morphometric techniques. This has enabled new understanding of how change to cancellous bone structure occurs as a result of a disease process. Fractal analysis is a novel tool that will prove useful for the study of changes in cancellous bone structure due to disease and to

study the use of therapies to alter or maintain the quality of cancellous bone architecture.

DECLARATION

This work contains no material that has been accepted for the award of any degree or diploma in any university or other tertiary institution and to the best of my knowledge contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis when deposited in the University library, being available for loan and photocopying.

Ian H Parkinson

27/6/2002

ACKNOWLEDGEMENTS

Associate Professor Nick Fazzalari, my supervisor. Nick has provided continual support and encouragement for the duration of my candidature. His insightful input into all aspects of this work has been invaluable in enabling me to complete the analytical components and in particular the writing of this thesis. For all of his assistance I will be forever grateful.

Phil Leppard, statistical consultant (formerly) at the University of Adelaide. Phil's contribution at an early stage of this work in helping to formulate the critical methodological algorithm overcame a significant hurdle that enabled the subsequent analyses to be performed. I thank Phil for this invaluable contribution.

Len Wylde, engineer (formerly) Australian Construction Services. Len with his background in material sciences brought to our research group novel ideas for the description of complex shapes. His development of a methodology for the fractal analysis of road materials provided an impetus to apply this novel technique to the study of cancellous bone. Thank you Len.

PUBLICATIONS ARISING

Parkinson IH, Fazzalari NL.

Methodological principles for fractal analysis of trabecular bone.

Journal of Microscopy 198:134-142 (2000).

Parkinson IH, Fazzalari NL.

Fractal analysis of trabecular bone: A standardised methodology.

Image Analysis and Stereology 19:45-49 (2000).

Parkinson IH, Fazzalari NL.

Goodness of fit on a modified Richardson plot by residual analysis.

Fractals 8:261-265 (2000).

Parkinson IH, Fazzalari NL.

Structural Complexity of Cancellous Bone: A Fractal Analysis.

Bone 28:S200 (2001).

AWARDS

Best Paper: International Quantimet and Stereoscan User Conference,
Cambridge, UK, October 1995.

Postgraduate Travelling Fellowship 2001, Faculty of Health Sciences,
University of Adelaide.