Presented at the BIG4 Workshop "3D Imaging and Innovative Approaches in Biosystematics" 8-13 May 2017, Vienna

> Dr. Brian Metscher Senior Scientist Department of Theoretical Biology



brian.metscher@univie.ac.at

All content © 2017 Brian Metscher, made available under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made. <u>http://creativecommons.org/licenses/by-nc-nd/3.0/</u>









Contrast-enhanced MicroCT: 3D images in various life sciences applications

> Brian D. Metscher Department of Theoretical Biology



brian.metscher@univie.ac.at

MicroCT imaging has revealed that *Drosophila melanogaster* is actually a real insect.

oatsue C1217/0115

500µm

Termite, three-dimensional:



Image made by S. Handschuh for Industrial Motion Art's film "Termiten" (www.industrial-motion-art.com)

Phthirapteran imagery through the centuries



1665 Louse, by Robert Hooke

1786

"Ye ugly, creepin, blastit wonner..."

To A Louse, by Robert Burns

2011



Louse, by Stephan Handschuh



MicroCT is a powerful complement to SEM and semithin sections



Vibration sense organs in mantophasmatodean insects



Eberhard et al. (2010) Arthropod Struct. Devel. 39:230-241

Size-calibrated digital volume images can provide quantitative data on function



Project practicum work by Bela Hausmann & Bernhard Rosensteiner



Contrast is everything

Freshwater bryozoan *Cristatella* 4.2µm voxels

PTA imparts high contrast to almost anything...



Idiosepius ca. 2mm long 4.0µm voxels



...but penetration is a problem



Mantophasmid tibia, scolopidial organs ca. 300μ m diameter, 0.9 μ m voxels

Calliphora vicinia (Diptera) late pupa SkyScan 1174, 7.7 μm voxels

From Metscher (2009) BMC Physiol 9:11

lodine works for larger samples, and those with more serious integuments



Chrysopa (Neuroptera) virtual sections



Introduction to digital images:

What do we use them for?

1. Illustration



2. Analysis

Th21 mouse embryo, PTA stained 2.7µm voxels

Why 3D images?

What can't we see in 2D?



14-day rat embryo, iodine stained, in utero

Muscles and glands in Vespula germanica



Elements of a digital picture: a **pixel** is a location + value(s)



mouse: 1078 x 1078 pixels

nose: 10 x 10 pixels

Elements of a digital picture: a **pixel** is a location + value(s)



mouse: 1078 x 1078 pixels

nose: 10 x 10 pixels

94	100	104	119	125	136	143	153	157	158
103	104	106	98	103	119	141	155	159	160
109	136	136	123	95	78	117	149	155	160
110	130	144	149	129	78	97	1 5 1	1 6 1	158
109	137	178	167	119	78	101	185	188	161
100	143	167	134	87	85	134	216	209	172
104	123	166	161	155	160	205	229	218	181
125	131	172	179	180	208	238	23 7	228	200
131	148	172	175	188	228	239	238	228	206
161	169	162	163	193	228	230	237	220	199

Pixels, bit depth, and variable types

Pixel: location + grey or brightness value

8-bit (256 values) "UCHAR"
16-bit (65536 values) "SHORT"
32-bit (65536² values) "FLOAT"



Image histogram

StdDev: 42.263



Max: 255 Mode: 11 (557758)

L = 2048 W = 4096

Digital image file formats

- JPG or JPEG: common; compressed
- TIFF: near-universal; LZW compression
- PNG: open-source; becoming more common
- GIF: 8-bit, animation; for web
- BMP: Windows thing. Don't bother.
- RAW: complete, diverse

What about colour?

RGB: 3 x 8-bit; 4.3 billion colours (sort of)

CMYK: for printing

RGBA: RGB + alpha (transparency)

L*a*b*: lightness + green-red + yellow-blue

"Resolution" refers to some measure of the ability to separate (resolve) objects



Pixel size is NOT equal to image resolution! --But it DOES place a limit on image resolution

Finer details (higher spatial frequencies) are imaged with less contrast



From 2D to 3D: How to visualise volumes?

Sculpture: e.g. Blaschka glass models

Multi-angle views: rotating SEM, photogrammetry

Selective focal planes: CSLM

Slice & shoot: serial reconstruction

Tomographic methods

1880s: Wax embryo models by His and Ziegler, based on dissection and sections



Anatomisches Museum Basel

3D models of mouse tooth germs, manually reconstructed from serial sections



Peterková et al. 2002. *Connective Tissue Research, 43: 120–128.*