Quantitative Analysis of Large Scale Image Datasets

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Outline of the Presentation

• Who We Are
• What We Do - Scientific Capability & Industrial Applications
• Quantitative Analysis of Large Scale Image Datasets
• Summary
Who We Are

• **CSIRO**
  - Australia’s national science agency and one of the largest and most diverse research organisations in the world
  - Undertakes research in:
    - agriculture, environment, manufacturing, minerals and energy, ICT, infrastructure and services
  - Non-government revenue from industrial work $634M (2008/2009)
  - Over 6500 staff at 56 sites throughout Australia and overseas

• **CSIRO Quantitative Imaging Group**
  - 12 scientists & engineers specialising in fast, automated & quantitative analysis of complex images & spectra
  - Our strategic image analysis research is motivated by industrial applications
Engagements

- Transformational
- Integrated

Regenerative
- Neurosphere cell segmentation and dynamics.
- Stem cell profiling under spectral imaging

Oncology
- Cell migration
- Breast cancer

Neuro
- Neurite tracing, assay development
- Alzheimer’s (neurite dynamics, amyloid plaque counting)

Infectious
- Bacterial dynamics in biofilms.
- Imaging virus entry into cells

Diabetes
- Vesicle fusion quantification (TIRF Explorer)
- Retinal imaging
More Engagements

- Commercial or political impact
- Naturally challenging
- Scientifically exciting

Industrial

- Objective assessment of opals and other gems (OPAL).
- Quality control of solar panels (BT Imaging)
- Stem cell image analysis

Biodiversity

- Automated insect identification
- Automated pollen classification

Agri

- Biomass estimation
- Plant phenotyping

Technology

- GPU image analysis libraries (CSS)
- 3D segmentation of rocks (WFO, CSS)

Capability dev.

- Compressive sampling
- Pattern spectra
- Shortest paths
- Stereo & 3D reconstruction
- Libraries
Scientific Capability - Object segmentation

- Sophisticated object segmentation increases the robustness and accuracy of automated identification of objects in images.

- Segmentation techniques include:
  - mathematical morphology, level sets, attribute morphology, graph morphology, scale space and shortest path methods.

- Example - to segment nuclei despite:
  - nucleus touching
  - variation in nuclei brightness
  - variation in background brightness

Fluorescence cell image

Nucleus Segmentation
Scientific Capability - Feature extraction & Statistical analysis

- Customised feature extraction of complex objects provides detailed characterisation of those objects
- When analysed with sophisticated statistical classification techniques, these features allow reliable recognition of objects
- Example - to classify phenotypes of mitotic arrest (Roche):
  - custom designed features measure size, shape, symmetry, orientation wrt neighbours, and intensity distribution for each figure
  - these features are more robust to changes in imaging platform, cell type, objective and/or staining than generic features
Scientific Capability - Stereo Vision

• Automated extraction of matching image features in stereo pairs of images
• Extraction of depth map from disparity in position of matching features
• Example – looking for retinal surface deformations resulting from macula degeneration (Lions Eye Institute):

Stereo images of Retina

Depth map - Raw (L) and Smoothed (R)
Scientific Capability - Image Stitching, Real-time Fisheye Mapping

LadyBug™ 360° spherical digital camera

6 video cameras

Convert to spherical camera views

Convert to single fisheye projection

3 metre dome projection
• Automated segmentation of objects in each frame
• Tracking of objects between frames in the presence of ambiguity in matching
• Example - tracking & analysing platelets (Monash University):
  • platelets move out of focal plane so impossible to match all objects
  • clumping behaviour evident in velocity statistics for whole population
Hyperspectral images enable us to map spatial variations in chemistry

- The chemistry is revealed by a material’s spectral signature - the amount of light that an object reflects, absorbs, transmits, scatters or fluoresces at each wavelength.
- In many applications, most pixels contain a mixture of materials. So their spectra are a mixture of the spectra of their constituent materials.

Need to “unmix” the spectra into their constituent materials

- with a spectral library: The Spectral Assistant (TSA) – trademarked
- without a spectral library: ICE (Iterated Constrained Endmembers) – patented.

Examples:
- monitoring inflammatory response to drugs (Astra Zeneca)
- flow cytometry (Purdue University)
Scientific Capability - Quantitative Analysis of 3D Tree Structures

- 3D Neurite Outgrowth
- Micro-Vascular Tree of a mouse brain

Data courtesy of Shanghai Synchrotron Radiation Facility (SSRF)
Scientific Capability - Hardware speed-up for Image Analysis

• General-Purpose Graphical Processor Units (GPGPUs) can be used to speed up image analysis application – particularly important for HCA
  • new graphics cards designed for gamers now available with general processing libraries
  • ~512 CUDA Cores, <6GB RAM
  • $500-3K
• Example: Deconvolution
  • correction of images using 3D point spread function of the microscope
  • multiple GPUs - perform 3D spatially-variant deconvolution up to 100X faster than a single CPU
  • single desktop or small cluster - high quality deconvolution of medium datasets < 2-3 seconds
  • deconvolution of 3D movies – e.g. 100 frame 3D video: 1xGPU=1hr, 4xGPU=5min, 64xGPU=40sec

David Biggs, Biophotonics Int.
Standard HCA Assays – Nucleus-cytoplasm and Membrane-cytoplasm translocation

- **Cytoplasm segmentation for translocation assays in confocal images**
  - Because the outer margin of the cytoplasm is more clearly defined in confocal images, the standard solution is to segment the full cytoplasm extent.
  - Our solution works robustly on cells with variable stain levels.

- **Membrane segmentation for translocation assays**
  - Our membrane segmentation is derived from the outermost rim of the cytoplasm mask.
  - Our solution works robustly on cells with variable stain levels.
Standard HCA Assays – Cytoplasm-vesicle translocation

- Dot segmentation for Vesicle or Membrane Pit translocation assays
  - Standard solutions just count the vesicles in the full image
  - Our solution does per cell counts – so atypical cells can be detected

**Negative result**

**Positive result**

Raw image  | Standard dot detection  | Our per cell allocation
---|---|---

Standard HCA Assays – 2D Neurite outgrowth

• **Standard measures**
  - average length of neurites
  - sometimes a simple measure of neurite outgrowth complexity – the number of branch points

• **CSIRO measures**
  - multiple features to fully characterise the complexity of neurite branching
    - Cell body area & circumference
    - Total neurite length
    - Longest neurite length
    - No. of primary (or root) neurites
    - No. of branching layers
    - No. of end segments
    - No. of branch points
    - No. of segments
  - allows you to selectively screen for compounds which generate different types of neurite outgrowth behaviour
Morphology Analysis

- Need to detect and quantify punctate and linear structures on a per cell basis
- Punctate features – number, mean brightness, area, density
- Linear features - number, mean brightness, length, width, density, orientation, orientation variance

Astrocyte image
R – membrane protein
G – actin
B – nuclei

Nuclei
Cell ‘extent’
Punctate protein
Actin filaments
Research Collaborations – Neuron-astrocyte co-culture analysis

• Broad Institute, MIT & Harvard - algorithms for neuronal co-culture analysis
  • for 2D images of co-cultures of neurons on a support layer of astrocytes
  • closer to in vivo behaviour of neurons
  • customised features to describe the morphology at the whole image level
    i.e. population features rather than single cell features
    • No. of (neurons, astrocytes, other glial cells)
    • Clumping tendency (neurons, astrocytes, other glial cells)
    • Co-localization of neurons and astrocytes
    • Area of neurons and astrocytes
    • Neuron webbiness
    • Astrocyte starriness & roundness

2D co-culture morphologies very different from monolayer behaviour
Research Collaborations – Micronucleus Cytome assay

- **CSIRO Human Nutrition - algorithms for Cytokinesis-Block MicroNucleus (CBMN) Cytome assay**
  - characterises a much wider range of genome damage effects than standard CBMN assay
  - indicator of genome damage due to:
    - Exposure to genotoxic chemicals; Oxidative Stress or Radiation exposure; Ageing; Dietary or micronutrient deficiency or excess
  - image analysis to detect:
    - in Mono-nucleated cells - MicroNuclei (MN), Nuclear Buds (NBUDs)
    - in Binucleated cells - MN, NBUDs and nucleoplasmic bridges (NPBs)
Micronucleus *Cytome* assay – Nucleoplasmic bridge detection

- Uses shortest paths techniques on linear feature enhanced images
  - for single bridges

- And also for multiple bridges
Research Collaborations – TIRF microscopy

- Garvan Institute - algorithms for detecting vesicle-membrane fusion events in live cells
  - tracking of labelled proteins in vesicles to distinguish between those vesicles which move out of plane and those which fuse with the membrane to release their contents

![TIRF video sequence](image)

3D plot of tracks: x,y = position, z = time
TIRF analysis - spatial distribution

- To understand the spatial distribution over time of insulin-stimulated fusion events of GLUT4 storage vesicles
  - Need to analyse spatial statistics in presence of confounding factors such as non-attachment of cell to cover slip
  - Need to compare spatial patterns with randomly occurring patterns to assess significance of observed patterns

Beta cell – real events

Simulated random distribution
Automated SEM Image Analysis

Bioengineering and Surface Engineering Materials Group, CSIRO Material Science and Engineering

• Coating Process for Implant materials
  • Discriminate between all samples in terms of average hole size and average separation as a function of coating conditions.
  • Refine the coating process to achieve greater uniformity of hole size and separation
Public Health Application – Automated Breast Density Estimation

• **An Australian platform for epidemiological validation of automated breast density measurement methods**
  - Collaboration with Melbourne University and Australian E-Health Research Centre
  - Designed to identify optimal methods for fully-automated measurement of dense tissue in the breast

• **Why is it needed?**
  - Breast cancer is the most common cancer in Australian women
  - Increased mammographic breast density strongly associated with
    - an elevated risk of breast cancer
    - poorer screening test sensitivity
  - Easier identification of women at high and low risk of breast cancer at screening

- Breast segmented from background
- Breast density segmented automatically by N methods
- Results compared with radiologists’ performance
- Best method to use in a pilot study with BreastScreen VIC
What We Do - Image analysis products

• HCA-Vision (http://www.hca-vision.com)
• SPOT
High throughput HCA systems incorporating our software modules

- **Molecular Devices’ ImageXpress®**
  - full suite of rapid assay analyses of fluorescence images

- **BD Biosciences’ Pathway HT™**
  - neurite outgrowth assay analysis

- **PerkinElmer’s Opera™**
  - neurite outgrowth assay analysis
Developed for Polartechnics Limited

The automated melanoma detection system was based on dermoscopy (colour surface microscopy) images of skin lesions.
• **Developed for Enterix P/L**

  • Presence of globin in stool water samples is an indicator of bleeding in the bowel - a symptom of bowel cancer.

  • Globin is detected using immunochromatography (colour change in antibodies which bind to a specific antigen, in this case globin).

  • Machine reading has several advantages over human reading:

    • reproducible results
    • threshold can be adjusted to give known sensitivity

The validity of the chemistry of the test line (top) is checked using a control line (bottom).
Industry Application – Opal Grading: GDA (Gemmological Digital Analyser)

- **Developed for Opal Producers of Australia Ltd (OPAL) to measure:**
  - *Flash* - colour caused by diffraction from lattice of silica spheres; Variable sphere sizes give different colours
  - *Play of Colour* - change of colour with viewing angle
  - *Bodytone* - colour of the body of the opal between regions of flash

- **The Challenge:**
  - Design the hardware (implemented by Applied Robotics)
  - Develop fast image analysis algorithms to process 720 images for each opal
  - Deliver commercial grade software

At each rotation angle:
- Find darkest regions
- Exclude glint and background to derive total *Flash* histogram

36 images every 10° of stage rotation x 10 tilt angles in 10° steps

At each rotation angle:
- Find darkest regions

CSIRO Quantitative Imaging
Quantitative Analysis of Large Image Datasets

• Large Quantities of Images and Many Measurements
  • Thousands of images generated from HCS for a single experiment (384 Wells, 16R x 24C x 12)
  • Image Size and Complexity (1280 x 1280; number of neurons from 100 – 1100; dense wells)
  • Overall, millions of parameters to be measured

• Large 3D Image Datasets
  • Vascular Networks
  • Neurite Outgrowth

Image courtesy of Marjo Götte, Novartis Institutes for BioMedical Research
HCA-Vision for Neurite Analysis

- Permits detailed characterisation of cellular phenotype, including measurement of:
  - Neurites
  - Nuclei
  - Cell membrane
  - Cytoplasm
  - ...

 Quantitative Analysis of 3D Tree Structures

• **3D Micro-Vascular Tree Visualisation and Analysis**
  • The samples were acquired using SSRF from a mouse brain (cerebellum, olfactory bulb, and other part).
  • The purpose is to compare cerebral vasculature of an adhesion molecule conditional knockout (KO) mouse and that of wild-type littermates.

Raw Image Data (Tiff Stacks) and Rendered 3D Volume
Quantitative Analysis of 3D Tree Structures

- Visualised 3D Volume of Micro-Vascular Tree - Cerebellum, olfactory bulb, and other part (resolution 3.7 um, SSRF)
Quantitative Analysis of 3D Tree Structures

• 3D Neurite Outgrowth
Quantitative Analysis of 3D Tree Structures

Branching layers

Individual neurite trees
Detect Neurons

- **Gaussian smoothing**
  
  \[
  f(x, y, z) = Ae^{-\frac{(x-x_0)^2+(y-y_0)^2+(z-z_0)^2}{2\sigma^2}}
  \]

- **Background correction (Morphological Top-Hat Filtering, the difference between the original image and its opening)**
  
  \[
  T_w(f) = f - \gamma(f)
  \]

- **Remove neurites (Morphological 3D opening)**

- **Automated Thresholding Using Bivariate Histogram**
  
  - The threshold is determined based on gradient strength information
  - The gradient strength shows how the image changes at each voxel and therefore how likely this voxel is part of the surface of the objects being detected. Here, \(\delta_B(f)\) is dilated image, \(f_e\) is the edge image.
  
  \[
  f_e = \delta_B(f) - f
  \]

  - Calculate the gradient histogram \(G(i,j,k)\)
  - Calculate the average gradient strengths for each grey-level, which are treated as a histogram.
  - An input parameter, threshold sensitivity, is used to determine the quantile of the gradient distribution to be used as the threshold.
Detected Neurons
Detect Neurite Trees

- Biotech Imaging Group’s patented algorithms: Multiple Directional Non-Maximum Suppression (MDNMS)


3D linear windows at different directions
Detected Neurite Trees
Chamfer Distance Transform Based Skeletonization

- **Chamfer Distance** – An integer approximation to the Euclidean Distance
  - Euclidean Distance
  - Integer approximation
    \[
    d(p, q) = \sqrt{\sum_{i=1}^{n} (q_i - p_i)^2}
    \]
  - \(d(p, q) = \begin{cases} 
    n_1, \|p - q\|^2 = 1 & \text{(only one coordinate changes from } p \text{ to } q) \\
    n_2, \|p - q\|^2 = 2 & \text{(two coordinates change from } p \text{ to } q) \\
    n_3, \|p - q\|^2 = 3 & \text{(all three coordinates change from } p \text{ to } q) 
  \end{cases} \]
  - \(n_1 = 3, \quad n_2 = 4, \quad n_3 = 5\)
  - \(d(p) = \min_{q \in N_{26}(p)} \{d(q) + d(p, q)\}, p \in F(\text{foreground})\)
  - \(0, p \notin F\)

- Recursively delete points if doing so preserves topology, and until no more points can be deleted. The deletion order is based on chamfer distance order (ascending).
Neurite Tree Skeleton
Quantitative Analysis of 3D Tree Structures

- **Split neurite trees into segments**
  - Creating junction mask by detecting voxels with more than 2 neighbours
- **Dilate and label all junction points**
- **Count and label all segments**
- **Measure individual segment intensities and lengths**
- **Grow 3D trees**
  - Identify roots/trunks
  - Find all siblings of individual roots/trunks
- **Compute per segment statistics, per branching layer features, per tree measurements and image wide morphometric features**
Result Images

Branching layers

Individual neurite trees
Image Wide Statistics

- Total outgrowth: 1014
- Total number of segments: 106
- Longest neurite: 143
- Total number of extreme branches: 36
- Total number of branch points: 26
Quantification Results at Tree Level

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<th>Tree Label</th>
<th>Total Length</th>
<th>Longest Neurite</th>
<th>Max Branch Layer</th>
<th>Mean Branch Layer</th>
<th>Number of Branch Points</th>
<th>Number of Neurite Segments</th>
<th>Number of Extreme Neurites</th>
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Challenges in Quantitative Analysis of Large Image Datasets

• **Computational Bottleneck due to**
  • Large Quantities of Images and Many Measurements
  • Large 3D Image Datasets
  • Disk IO Bottleneck

• **Throughput Problems**
  • Take several hours or even days to process all images produced from a single experiment
  • Throughput time unacceptable for the workflows in laboratories
How to Tackle the Challenges

• **High Performance Computing**
  • Multi-Core Based High Throughput Image Analysis
  • GPU Enabled Image Analysis
  • Optimisation of Image Analysis Routines
  • Parallel Disk I/Os

• **Goals**
  • Parallel Image analysis algorithms for fast execution to take advantages of the processing power in mid to high end computers (Multi-cores CPUs, Multi-cores CPUs + GPUs, GPU Clusters)
  • Image Processing Strategies to handle a large number of images or very large individual images – carry out in parallel the low level processing on individual images or image tiles for a large image, then extract image wide features on the stitched result images.
  • GPU Enabled Image Analysis Library including routines of Mathematical Morphology, Watershed, Seed Region Growing, Labelling etc.
Parallel Disk I/Os

• **Parallel File System**
  • HDF5, [http://www.hdfgroup.org/HDF5/](http://www.hdfgroup.org/HDF5/)

• **High Performance Data Storage**
  • IBM Scale Out Network Attached Storage
  • Isilon Clustered Storage, Isilon Systems
  • High-performing network-attached storage (NAS) such as EMC VNX Series Gateways
Neurite Analysis Results – Need a Solution to Generate These Faster

• Mixed cell types and multiple features to fully characterise the complexity of neurite branching (34 measures/Cell)
  • average length of neurites
  • sometimes a simple measure of neurite outgrowth complexity – the number of branch points
  • Cell body area & circumference
  • Total neurite length
  • Longest neurite length
  • No. of primary neurites
  • No. of branching layers
  • No. of end segments
  • No. of branch points
  • No. of segments
  • …

Cytoplasm and membrane segmentation for translocation assays
Experiments

• Testing Batch Processing for
  • Neuron Body Detection
  • Neurite Analysis
• Hardware
  • 4-Core Intel® Xeon™ 3.2Ghz
  • 8 GB of Ram
Experimental Results - Performance

• Neuron Body Detection
  • 96 Well x 6 images/well
  • Reduced to 38% of the original processing time

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<tr>
<td>3</td>
<td>69 Min 18 Sec</td>
<td>25 Min 9 Sec</td>
</tr>
</tbody>
</table>

Image courtesy of Marjo Götte, Novartis Institutes for BioMedical Research
Experimental Results - Performance

• **Neurite Analysis**
  - 96 Well x 6 images/well
  - Reduced to 46% of the original processing time

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<td>3</td>
<td>110 Min 33 Sec</td>
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Image courtesy of Marjo Götte, Novartis Institutes for BioMedical Research

CSIRO Quantitative Imaging
**GPU Hardware & Software**

**Hardware**

- NVIDIA Tesla GPU
  - Brings supercomputing power to any workstation or server.
  - Massively multi-threaded architecture with a 128-processor computing core
- PCI Express Bus (PCIe) – Support GPU card with a dedicated bandwidth of 8GB/s
- Single GPU/CPU Image Processing - General-purpose computing on graphics processing units (GPU)
- GPU Cluster

**Software**

- CUDA (Compute Unified Device Architecture) – C for GPU
- Algorithm mapping (mapping image processing/analysis algorithms to run on a GPU/GPU cluster)
NVIDIA’s GPUs: Ever Increasing Performance

GFlops

T10 = Tesla 10-series
G9x = GeForce 9800 GTX
G80 = GeForce 8800 GTX
G71 = GeForce 7900 GTX
G70 = GeForce 7800 GTX
NV40 = GeForce 6800 Ultra
NV35 = GeForce FX 5950 Ultra
NV30 = GeForce FX 5800

Double Precision debut

Intel Xeon Quad-core 3 GHz


Courtesy of Mark Harris, NVIDIA Corporation 2009
Host and Device - The Big Picture

- **GPU (device)**
  - Massively multi-core processor
  - Executes thousands of threads concurrently
  - Good at running isolated data parallel operations
  - Handles thread management and scheduling
  - Minimal-No access to Host resources

- **PC/Workstation (host)**
  - Higher-level management/organisation
  - Responsible for controlling GPU
    - Device Memory Management
    - Copying data to and from device (PCI-Express)
    - Launches parallel programs on GPU
Neurite Detection - GPU Speed Up

- 20Xsynaptophysin.tif
- lifcpic5control.tif
- neuro_5_overlay.tif
- neuro_3_overlay.tif
- neuro_2_overlay.tif
- sample.tif

Graph showing speedup for different files with categories Overall, Gap Fill, and DNMS.
Case study 1 – Neurite Detection

- **Simple Image**
  - Res: 694x520 (1MB)
  - Speed-up over CPU: NONE

- **Moderate Complexity**
  - Res: 1300x1030 (3.8MB)
  - Speed-up over CPU: 8x

- **Very Complex**
  - Res: 1280x1280 (4.7MB)
  - Speed-up over CPU: 12.7x

Images courtesy of: (1) Prof. Pat Doherty, Kings College, London, UK. (2) Dr. Xiaokui Zhang, Helicon Therapeutics, Inc., USA. (3) Marjo Götte, Novartis Institutes for BioMedical Research

CSIRO Quantitative Imaging
## Speedup of Some GPU Enabled Image Analysis Functions

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<th>Function</th>
<th>Description</th>
<th>Speedup</th>
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<td>DilateRect</td>
<td>dilation with rectangular SE</td>
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<tr>
<td>ErodeRect</td>
<td>erosion with rectangular SE</td>
<td>10.55</td>
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<tr>
<td>OpenRect</td>
<td>opening with rectangular SE</td>
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<tr>
<td>CloseRect</td>
<td>closing with rectangular SE</td>
<td>6.86</td>
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<td>Asf</td>
<td>Alternating sequential filter</td>
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<td>Binfill8</td>
<td>Binary hole filling, 8 connected</td>
<td>7.66</td>
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<td>Label</td>
<td>Labelling</td>
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<td>erosion with circular SE</td>
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Summary

- Challenges in Dealing with large image datasets
- Significant Speedup can be achieved using multi-Core based batch processing
- GPU enabled image analysis has great potential
- The study shows that the High Performance Image Analysis can significantly speedup the quantitative analysis of large image datasets.
Collaborations

• Joint Product development
• Image Analysis Services
• Joint Grant Application
• Joint publications
• Co-supervising PhD Students
• Image Analysis Product Distribution
• ...

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