Artificial Intelligence and Digital Microscopy

25-26 June 2018,
Reed Hall Conference Centre
University of Exeter, Exeter, UK

Abstracts

A multidisciplinary meeting on applications of Artificial Intelligence methods to Digital Microscopy.

Meeting hosted as part of the project *Novel context-based segmentation algorithms for intelligent microscopy*, funded by the Engineering and Physical Sciences Research Council (EPSRC), Universities of Birmingham and Exeter. (Grant number EP/M023869/1)

Organising Committee: David A Randell, Gabriel Landini and Antony Galton.

Programme

Monday 25 June

12:00 - 12:30 Registration

12:30 - 13:30 Buffet Lunch

13:30 - 13:40 Introduction to the event and orientation

13:40 - 14:00 ***AI – a (very) quick overview.*** Antony Galton, University of Exeter, UK

14:00 - 14:40 Keynote talk: ***Machine learning for 3D image segmentation.*** Prof. Richard Everson, University of Exeter, UK

14:40 - 15:00 ***Ontological levels in histological imaging.*** Antony Galton, University of Exeter, UK

15:00 - 15:20 Coffee/tea

15:20 - 15:40 ***Discrete Mereotopology: an introduction.*** David Randell, University of Birmingham, UK

15:40 - 16:00 ***Discrete Mereotopology in histological Imaging.*** Gabriel Landini, University of Birmingham, UK

16:00 - 16:40 Keynote talk: ***The Promise of Computational Pathology.*** Prof. Nasir Rajpoot, University of Warwick, UK

16:40 - 17:00 Discussion + announcements

18:30 Dinner

Tuesday 26 June

08:45 - 09:00 Coffee/tea

09:00 - 09:20 ***Resegmenting digitised histological images using discrete mereotopology.*** David Randell, University of Birmingham, UK

09:20 - 09:40 ***Unsupervised and semi-supervised learning frameworks for the epithelium and stroma segmentation in histopathological images.*** Shereen Fouad, University of Birmingham & Birmingham City University, UK

09:40 - 10:00 ***Using transfer learning for deep classification of tumours in whole slide image patches.*** Taran Rai, University of Surrey, UK

10:00 - 10:20 ***Tissue segmentation for HPV status assessment*****.** Tzu-Hsi (Mike) Song, University of Birmingham, UK

10:20 - 10:40 Coffee/tea

10:40 - 11:00 ***Persistent homology as a tool to probe structure in single molecule localization microscopy datasets.*** Jeremy Pike, University of Birmingham, UK

11:00 - 11:20 ***Deep learning for the analysis of label-free ptychographic imaging data*****.** Tristan Henser-Brownhill, University of Manchester, UK

11:20 - 11:40 ***Automating FLImP - macromolecular structure fingerprints for personalised cancer therapy.*** Dan Rolfe, Rutherford Appleton Laboratory, Oxford, UK

11:40 - 12:00 ***A logic for spatial reasoning at different levels of details.*** Giulia Sindoni, University of Leeds, UK

12:00 - 12:20 ***Detection of leukemic blasts in white blood cell mixture using deep learning and high-content analysis of imaging flow cytometry data.*** Claire Barnes, University of Swansea, UK

12:20 - 13:20 Buffet Lunch

13:20 - 13:40 ***Automated segmentation of HeLa nuclear envelope from electron microscopy images.*** Cefa Karabag, City, University of London, UK

13:40 - 14:00 ***Image analysis and tracking of migrating macrophages.*** José Alonso Solís Lemus, City, University of London, UK

14:00 - 14:20 ***Assessing tissue and cellular morphometry in the foetal lung in a murine oligohydramnios model.*** Rasha Abu Eid, University of Aberdeen, UK

14:20 - 14:40 ***3d collective cell motility and extra cellular matrix invasion.*** Emanuele Martini, IFOM/FIRC Inst. Molec. Oncology, Milano, Italy

14:40 - 15:00 ***Advanced time-resolved microscopy reveals the nanostructure and function of human dental tissue.*** Tan Sui, University of Surrey, UK

15:00 - 15:30 Future directions and closing remarks, followed by coffee/tea

Introduction to AI

Antony Galton

Department of Computer Science, University of Exeter, Exeter EX4, UK.

In this short talk I will present a very broad-brush overview of different approaches to Artificial Intelligence (AI), and will present a tentative view of how these different approaches are represented in the work to be presented at this workshop.

Ontological Levels in Histological Imaging

Antony Galton[1], Gabriel Landini [2], David Randell [2] and Shereen Fouad [2,3]

[1] Department of Computer Science, University of Exeter, Exeter EX4, UK.
[2] School of Dentistry, University of Birmingham, Birmingham Birmingham, Birmingham, B5 7EG, UK.
[3] Current address: School of Computing and Digital Technology, Birmingham City University, Birmingham B4 7XG, UK.

In this talk we present an ontological perspective on our work in histological and histopathological imaging involving the quantitative and algorithmic analysis of digitised images of cells and tissues. We present the derivation of consistent histological models from initially captured images of prepared tissue samples as a progression through a number of ontological levels, each populated by its distinctive classes of entities related in systematic ways to entities at other levels. We believe that adopting such a perspective can provide not only a clearer understanding of what we are doing at different stages of analysis, but also a useful framework within which to characterise the various different kinds of errors and artefacts arising throughout the process.

Discrete Mereotopology: An Introduction

David A Randell [1], Antony Galton [2], Gabriel Landini [1] and Shereen Fouad [1,3]

[1] School of Dentistry, College of Medical and Dental Sciences, University of Birmingham, Birmingham B152TT, UK.
[2] Department of Computer Science, University of Exeter, Exeter EX4, UK.
[3] School of Computing and Digital Technology, Birmingham City University, Birmingham B4 7XG, UK.

This talk covers the theoretical foundations and model-based methods used that underpins our use of Discrete mereotopology (DM) in histological image processing and analysis. DM is a variant of the well-known spatial logic RCC that integrates *mereology* (that part of the theory deals with parts, wholes and the notion of overlap) and *topology* and to model discrete space. It is one of several mereotopological logics that have been developed within AI. Unlike RCC which is defined on a continuous space, DM is defined on a discrete space and it is this property which makes it suitable for representing and reasoning about regions that are ultimately decomposable into atoms or pixels where digitised images are its models. We show how a subset of DM's discrete topological operators (i.e. interior and closure) map directly to the erosion and dilation operators in Mathematical Morphology. Using these operators discrete analogues of the well-known eight and five-element relation sets (respectively RCC8D and RCC5D), can be defined. This mapping enables one to directly implement these operators and relation-sets using morphological libraries that are common to many popular image processing programs. The RCC5D and RCC8D relation sets are embedded in relational lattices where the five and eight element sets of relations form base relations. The lattices provide the means to define weaker relations that are used to describe more general relations between e.g. cells and their parts or abutting and overlap relationships between segmented tissue compartments. The discrete topological operators are used to define notions of minimal change to a region of a region-pair. This is used to define a set of directed graphs, where paths through the network can be interpreted as sequences of operations on regions taking us from one specified relation to another. This property enables one to guide segmentation algorithms so that the end result is in conformity with an assumed histological model.

References

[1] David A. Randell, Zhan Cui, and Anthony G. Cohn (1992): "A Spatial Logic Based on Regions and Connection," *Proc. Third Int'l Conf. Knowledge Representation and Reasoning*, pp. 165-176, 1992.

[2] David A. Randell, Gabriel Landini and Antony Galton (2013): Discrete Mereotopology for Spatial Reasoning in Automated Histological Image Analysis, *IEEE Transactions on Pattern Analysis and Machine Intelligence*, Vol 35, Issue 3, pp 568-581

[3] Antony Galton. (2014): "Discrete Mereotopology". In: Calosi C., Graziani P. (eds) *Mereology and the Sciences. Synthese Library (Studies in Epistemology, Logic, Methodology, and Philosophy of Science)*, vol 371. Springer, Cham

Discrete Mereotopology in Histological Imaging

Gabriel Landini [1*], David A. Randell [1], Antony Galton [2], Shereen Fouad [1,3].

[1] School of Dentistry, University of Birmingham 5 Mill Pool Way, Edgbaston, Birmingham, B5 7EG, UK.
[2] Department of Computer Science, University of Exeter, Exeter, UK.
[3] Current address: School of Computing and Digital Technology, Birmingham City University, Birmingham B4 7XG, UK..
*Email: G.Landini@bham.ac.uk

We present an overview of a range of applications where Region Connection Calculus (RCC) can provide algorithmic solutions to some histological imaging problems. Our implementation of RCC5D and RCC8D relation sets uses mathematical morphology to enable algorithmic spatial reasoning in terms of imaging procedures. This allows computing relationships held between regions that carry histologically-relevant meaning. This, in turn, enables the construction of region-based cellular and tissue models that go beyond the traditional pixel-based and 'region of interest' (ROI)-based approaches commonly used in the analysis of cyto- and histological data.

For this purpose we developed open source software (ImageJ plugins) to perform RCC analysis between region pairs as well as across multiple regions in pairs of images. The presentation will showcase examples of explicitly defined 'model cells', detection of segmentation errors and construction of layered multicellular structures.

In terms of generic image processing, the implementation of Discrete Mereotopology also opens the possibility of designing new morphological operators where 'minimal changes' fulfil given spatial constraints. Within this remit we have identified convenient representations of the relations held between image regions to compute, for example spatial hierarchy (i.e. parent/child region relations) and a mereotopological interpretation of hysteresis thresholding.

References

[1] Randell DA, Landini, G, Galton A. Discrete Mereotopology for spatial reasoning in automated histological image analysis. I*EEE Trans Patt Rec Mach Intell* 35(3): 568-581, 2013. DOI: 10.1109/TPAMI.2012.128

[2] Landini G, Randell DA, Galton. Discrete Mereotopology in Histological Imaging. Medical Image Understanding and Analysis, In: E Claridge, AD Palmer and WTE Pitkeathly (editors), *Proceedings of the 17th Conference on Medical Image Understanding and Analysis*, 17th-19th July 2013, Birmingham, UK, pages 101-106.

[3] Galton A, Landini G, Randell D, Fouad S. Ontological levels in histological imaging. *9th International Conference on Formal Ontology in Information Systems*, *FOIS 2016*, Annecy, Jul 4-6, 2016.

[4] Randell DA, Galton A, Fouad S, Mehanna H, Landini G. Model-based correction of segmentation errors in digitised histological images. *Medical Image Understanding and Analysis, 21st Annual Conference, MIUA 2017*, Edinburgh, UK, July 11–13, 2017, *Proceedings, Communications in Computer and Information Science* book series (CCIS, volume 723), p. 718-730.

[5] Galton A, Fouad S, Landini G, Randell D. Errors and Artefacts in Histopathological Imaging. *8th International Conference on Biomedical Ontology (ICBO)*, Newcastle upon Tyne, Sep 13-15, 2017.

[6] Randell DA, Galton A, Fouad S, Mehanna H, Landini G. Mereotopological correction of segmentation errors in histological imaging. *Journal of Imaging*, Selected Papers from "MIUA 2017", 2017, 3(4), 63.

Resegmenting Digitised Histological Images using Discrete Mereotopology

David A Randell [1], Antony Galton [2], Gabriel Landini [1], Shereen Fouad [1] and Hisham Mehanna [3]

[1] School of Dentistry, College of Medical and Dental Sciences, University of Birmingham, Birmingham B152TT, UK; {d.a.randell, g.landini, s.a.fouad}@bham.ac.uk
[2] Department of Computer Science, University of Exeter, Exeter EX4, UK; apgalton@ex.ac.uk
[3] Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham B15 2TT, UK; h.mehanna@bham.ac.uk

This talk covers the use of Discrete Mereotopology (DM) in the segmentation and resegmentation of digitised histological images. DM is a variant of the well-known spatial logic RCC that integrates *mereology* (that part of the theory deals with parts, wholes and the notion of overlap) and *topology* and to model discrete space. It is one of several mereotopological logics that have been developed within AI. A set of set-theoretic operators are added to a set of discrete topological operators and used to generalise a notion of minimal change to a region of a region-pair. Using these operators discrete analogues of the well-known eight and five-element relation sets used in the spatial logic RCC (respectively RCC8D and RCC5D) can be defined. The relations and operators are used to define a set of 20 directed graphs, where paths through the graph network (edges linking together the graph vertices) can be interpreted as sequences of operations on regions, taking us from one specified relation to another. Examples are given of the range of resegmentations made possible by combining these operations on regions. This property enables one to guide segmentation algorithms so that the end result is in conformity with an assumed histological model. Connections are drawn with other methods used to solve symbolic-based constraint satisfaction problems (CSPs) and qualitative simulation programs that have been developed in AI.

References

[1] David A. Randell, Zhan Cui, and Anthony G. Cohn (1992): “A Spatial Logic Based on Regions and Connection,” *Proc. Third Int’l Conf. Knowledge Representation and Reasoning*, pp. 165-176, 1992.

[2] David A. Randell, Gabriel Landini and Antony Galton (2013): “Discrete Mereotopology for Spatial Reasoning in Automated Histological Image Analysis”, *IEEE Transactions on Pattern Analysis and Machine Intelligence*, Vol 35, Issue 3, pp 568-581, 2013.

[3] David A Randell, Antony Galton, Shereen Fouad, Hisham Mehanna and Gabriel Landini (2017): “Mereotopological Correction of Segmentation Errors in Histological Imaging”, *Journal of Imaging*, Vol 3 (4), 2017.

Unsupervised and semi-supervised learning frameworks for the epithelium and stroma segmentation in histopathological images

Shereen Fouad [1,2], Gabriel Landini [1], David Randell [2], Antony Galton [3], Hisham Mehanna [4]

[1] School of Dentistry, University of Birmingham, Birmingham Birmingham, Birmingham, B5 7EG, UK
[2] School of Computing and Digital Technology, Birmingham City University, Birmingham B4 7XG, UK.
[3] Department of Computer Science, University of Exeter, Exeter EX4, UK.
[4] Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham B15 2TT, UK

Epithelium and stroma are two types of tissues found in many types of pathological specimens. In the case of oropharyngeal cancer samples, automatic identification of these two tissues is essential for the spatial localisation and the analysis of tumour micro-environment. Current supervised methods for epithelium and stroma segmentation have reported promising results however, they require large volumes of manually segmented training sets that are difficult and time-consuming to generate. Here, we present unsupervised and semi-supervised learning frameworks for the epithelium-stroma regions recognition in H&E stained images of oropharyngeal tissue microarrays. Our framework initially applies a superpixel segmentation algorithm which splits-up the image into binary regions. Stain colour features are then extracted and fed into several base clustering algorithms with various parameter initializations. Consensus Clustering (CC) formulations are then used to combine the base clustering outcomes into a more robust detection of tissue compartments than the base clustering methods on their own. The obtained CC result is then used to build a Self-Training Semi-Supervised Classification (ST-SSC) model. Unlike supervised segmentations, which rely on large number of labelled training images, the SSC approach performs a quality segmentation while relying on few labelled samples. Experiments conducted on forty-five hand-annotated images of oropharyngeal cancer tissue microarrays show that the CC algorithm generates more accurate and stable results than individual clustering algorithms. It also show that the proposed ST-SSC algorithm outperforms supervised methods, trained with only a few labelled instances.

Using Transfer Learning for Deep Classification Of tumours in Whole Slide Image patches

Rai T[1], Morisi A[2], Wells K[1], Bober M[1], La Ragione R[2], Bacci B[2]

[1] CVSSP, University of Surrey, Guildford, GU2 7AL, United Kingdom.
[2] School of Veterinary Medicine, University of Surrey, Guildford, GU2 7AL, United Kingdom.

BACKGROUND: Breast cancer is the most common malignant disease in women worldwide (Ghoncheh, Pournamdar and Salehiniya, 2016). Pathological assessment is the gold standard for surgical and oncological treatment decision making, as tumour morphology remains the strongest predictor of clinical outcome (Robertson *et al.*, 2018). As only a relatively small portion of the information present in histological sections is perceived by microscopy viewing with the human visual system, there is opportunity to mine such data for characteristic recognition and quantification of complex patterns and relationships that may not be apparent from human visual inspection. This relies on digital image analysis and the use of 'Deep Learning' methods. However, one primary concern is the availability of large datasets for training on Deep Learning algorithms. Transfer Learning has been suggested as a technique that might address the paucity of large datasets needed for such analysis.

OBJECTIVE: The aim of this presentation is to show the effectiveness of transfer learning on a small histopathological dataset.

METHODS & RESULTS: An original dataset was used consisting of 500 whole-slide images (WSI) of haematoxylin and eosin (H&E) stained lymph node sections released by a researcher challenge competition (CAMELYON17) in March 2017 (Bejnordi *et al.,* 2017). Nine WSIs were randomly selected and patches of both normal and metastatic tissue were created from previously annotated regions. The size of these patches was constrained to 256 x 256 pixels for faster computation. The dataset was created randomly and completely balanced, where training, validation and testing set consisted of 1200, 200, 200 images respectively. Pretrained models were originally trained on the ImageNet dataset. Bottleneck features were extracted from the input dataset and used to train a fully connected model. A number of different Deep Learning models were trained, including VGG, Xception and ResNet50. Results are presented below in Table 1.

Model	Stop training @ epoch #	Accuracy (%)	Normal/ Tumour	Precision	Recall	f1-score	No. of Test Samples
VGG16	9	89.5	0	0.85	0.96	0.90	100
			1	0.95	0.83	0.89	100
			Avg/Tot	0.90	0.90	0.89	200
VGG19	**4**	**90.5**	**0**	**0.91**	**0.90**	**0.90**	**100**
			1	**0.90**	**0.91**	**0.91**	**100**
			Avg/Tot	**0.91**	**0.91**	**0.90**	**200**
InceptionV3	3	50	0	0.50	1.00	0.67	100
			1	0.00	0.00	0.00	100
			Avg/Tot	0.25	0.50	0.33	200
ResNet50	473	85	0	0.83	0.88	0.85	100
			1	0.87	0.82	0.85	100
			Avg/Tot	0.85	0.85	0.85	200
InceptionResNetV2	5	86	0	0.83	0.91	0.87	100
			1	0.90	0.81	0.85	100
			Avg/Tot	0.86	0.86	0.86	200

Table 1: Transfer Learning model results. VGG19 produced the best accuracy of 90.5% and the highest average f1-score of 0.90.

CONCLUSIONS: Transfer Learning has much potential for Medical Imaging as large datasets can be scarce for training in Deep Learning. Computationally deeper models such as VGG19 appear to be most effective for this type of learning. This supports the idea of transfer learning in alternative tasks where they find most success with VGG nets. Future work will include investigation of different CNN architectures, with different layers from which bottleneck features are extracted, depending on model type.

REFERENCES:

Bejnordi, B.E. et al, 2017. Jama, 318(22), pp.2199-2210.

Ghoncheh, M et al APJCP, 17(S3), pp. 43–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/27165206 (Accessed: 15 May 2018).

Robertson, S. et al. (2018) Translational Research, 194, pp. 19–35. doi: 10.1016/j.trsl.2017.10.010.

Tissue segmentation for HPV status assessment

Tzu-Hsi (Mike) Song [1], Gabriel Landini [1], David A. Randell [1], Shereen Fouad [1,2] and Hisham Mehanna[3]

[1] School of Dentistry, University of Birmingham, Birmingham Birmingham, Birmingham, B5 7EG, UK
[2] School of Computing and Digital Technology, Birmingham City University, Birmingham B4 7XG, UK.
[3] Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham B15 2TT, UK

We report our ongoing work on detection of Human Papilloma virus (HPV) infection in oropharyngeal carcinoma tissue micro-array sections by *in situ* hybridisation (ISH). We investigated using colour deconvolution for the detection of HPV+ samples processed for detection of 'high-risk' HPV strains using the INFORM (Ventana, Roche) system. The ISH technique uses NBT/BCIP (blue) as a chromogen to visualise the result of the hybridisation, while Red Counterstain II dye (pink) serves as contrast stain to reveal the morphology of tissues. However the contrast stain is not able to clearly differentiate between tissue types and makes HPV assessment a complicated task. Current practice relies on expert observers identifying where the hybridisation products are localised: to consider a carcinoma to be HPV+ it is expected that the blue ISH product is localised in the epithelial tissue cells. This presentation does not concentrate on the hybridisation result, but instead on the identification of tissue types in ISH preparations. We investigated an *unsupervised* machine learning approach for grouping image pixels into clusters corresponding to background, stroma, and epithelium based solely on data from the deconvolved counterstain ('pink') channel. It was found that this channel provides similar results (accuracy: 78.7%, F1: 48.6%) to those obtained with the greyscale version of the image for segmentation in the three classes of interest (accuracy: 78.7%, F1: 47.6%). We also examined *supervised* approaches to classify tissue patches (64x64 pixels) into the three classes. The patch-based accuracy of a Support Vector Machine algorithm (using colour and texture features as input) was 80.0% (F1 score: 52.5%) when using the 'pink' channel while for greyscale the accuracy was 79.7% (but achieving a lower F1 score of 36.2%).
Finally an approach based on *deep learning* was also considered in the search for suitable and efficient models that might identify epithelium regions efficiently. First, we investigated a convolutional neural network (patch-based accuracy 77.8%, F1 68.8%) as well as and a state-of-the-art deep learning model, Unet, commonly used for object segmentation to output a predicted mask of epithelium. The preliminary results indicate that single-dye segmentation is possible and might allow automation of ISH analysis.

Persistent homology as a tool to probe structure in single molecule localization microscopy datasets

Jeremy A. Pike[1] , Abdullah Khan[2], Iain B. Styles[1,3]

[1] Centre for Membrane Proteins and Receptors, The Universities of Birmingham and Nottingham, UK.
[2] Birmingham Platelet Group, College of Medical and Dental Sciences, University of Birmingham, UK
[3] School of Computer Science, University of Birmingham, UK
Email: j.a.pike@bham.ac.uk

After performing localization, the data from a single molecule localization microscopy (SMLM) experiment is represented by a set of spatial coordinates, each corresponding to a single detection, that form a point cloud. This can be analyzed either by rendering an image from these coordinates and using image-based analysis methods, or by analyzing the point cloud directly. Several analysis methods of this type have focused on clustering the point cloud into distinct groups [1]-[3]. Per-cluster statistics such as area and detection density can then be calculated.
Persistent homology provides a robust mathematical framework for probing the topology, or shape of a point cloud [4]. By creating a sequence of simplicial complexes of increasing search radius, persistence barcodes and diagrams can be plotted that reveal the emergence and disappearance of features as a function of length scale. This provides new information about the organization and topology of the point cloud including the existence of both 2D holes and 3D voids.
We apply persistent homology analysis to SMLM datasets and show, for the first time, how this technique can quantify structure across a range of scales, revealing new information about single molecule organization and distribution.
Finally we use KNIME as a platform for analysing and batch processing SMLM datasets. We will present workflows for topological data analysis and also for standard techniques such as Ripley's K function and DBSCAN.

[1] P. Rubin-Delanchy, G. L. Burn, J. Griffié, D. J. Williamson, N. A. Heard, A. P. Cope, and D. M. Owen, "Bayesian cluster identification in single-molecule localization microscopy data," Nature methods, vol. 12, no. 11, p. 1072, 2015.

[2] F. Levet, E. Hosy, A. Kechkar, C. Butler, A. Beghin, D. Choquet, and J.-B. Sibarita, "SRTesseler: a method to segment and quantify localization-based super-resolution microscopy data," Nature methods, vol. 12, no. 11, p. 1065, 2015.

[3] M. Ester, H.-P. Kriegel, J. Sander, X. Xu, et al. "A density-based algorithm for discovering clusters in large spatial databases with noise.," in Kdd, 1996, vol. 96, no. 34, pp. 226–231.

[4] R. Ghrist, "Barcodes: the persistent topology of data," Bulletin of the American Mathematical Society, vol. 45, no. 1, pp. 61–75, 2008.

Deep Learning for the Analysis of Label-Free Ptychographic Imaging Data

Tristan Henser-Brownhill[1,2], Christoph Ballestrem[2], Patrick Caswell[2] and Timothy F. Cootes[1]

[1]Division of Informatics, Imaging & Data Sciences, School of Health Sciences, University of Manchester

[2]Wellcome Centre for Cell Matrix Research, School of Biological Sciences, University of Manchester

Cells are mostly transparent, with details difficult to visualise using conventional microscopy techniques. Contrast enhancing methods normally involve staining with chemical dyes, or labelling with fluorescent markers. Labelling can perturb living cells, and excitation of fluorophores with high-power lamps or lasers can trigger phototoxic stress responses, especially at short wave-lengths. Ptychography is a quantitative phase imaging (QPI) technique used to generate information-rich, high-contrast, label-free images of living cells. QPI accurately computes the phase-delay produced by objects as light passes through them towards a charge-coupled device (CCD) detector. This information can be used to calculate dry mass, granularity, cell volume, and a wide range of morphological features.

We hypothesise that raw, information-rich, ptychographic images can be used as direct input to deep-learning models for the improvement of both semantic segmentation and time-lapse tracking of individual live cells from QPI experiments. Using machine learning models, we further predict that different phenotypes, behaviours, and disease states can be accurately classified from both hand-crafted and learned image features, eliminating the need for fluorescent labels. To address the former, we created a dataset of hand-labelled ground-truth QPI images comprising different types of cells with distinct behaviours and morphologies, and used these to train an ensemble of convolutional neural networks (CNNs) capable of both semantic segmentation and seed-point prediction. To predict biologically relevant information from segmented cells, we developed machine learning models to classify both normal and malignant breast cells grown on a variety of different substrates.

As the project develops, we hope to address more complex problems such as classifying unmodified cells from cells with induced mutations, assessing the effects of drugs on cell appearance, and predicting the lineage choice of stem/progenitor cells. Our long-term aims are to produce a toolkit that will improve analysis in QPI experiments, and to highlight the power of label-free ptychographic imaging for addressing key biomedical questions.

Automating FLImP – macromolecular structure fingerprints for personalised cancer therapy

Daniel Rolfe

OCTOPUS Imaging Facility,
Central Laser Facility, Research Complex at Harwell, UKRI/STFC Rutherford Appleton Laboratory

FLImP (Fluorescence Localisation Imaging with Photobleaching) [1,2] is a single-molecule fluorescence microscopy-based method which reveals fingerprints of macromolecular structures in cell membrane receptors with ~5nm resolution. It was developed in STFC to investigate interactions of the Epidermal Growth Factor Receptor (EGFR) family, whose interactions are critical to the development and treatment of many cancers. The FLImP fingerprints of these receptors, essentially high-resolution distributions of receptor-receptor separations in cells, reveal structures and interactions in unprecedented detail in-situ in cells where their effects are evident and have life-changing implications.

Exploited in tandem with single-molecule tracking, molecular simulations and prior structural knowledge FLImP provides a powerful tool for biomedical research into cancer. Translating this partially-automated but still labour-intensive method to a fast, automated assay accessible in the clinic will provide a tool with unprecedented precision for personalised diagnosis and treatment of cancer. We are now planning this translation to the clinic, using machine learning methods to achieve full automation of our pipeline from data acquisition to the final FLImP fingerprint and its clinical exploitation.

I will briefly outline the FLImP method and our plans for translating it to the clinic.

References

[1] Needham, S. R. et al. Measuring EGFR separations on cells with similar to 10 nm resolution via fluorophore localization imaging with photobleaching. PLoS ONE 8, e62331 (2013).

[2] Needham, S. R. et al. EGFR oligomerization organizes kinase-active dimers into competent signalling platforms. 7, 13307, doi:10.1038/ncomms13307 (2016).

A Logic for Spatial Reasoning at Different Levels of Details

Giulia Sindoni, John G. Stell

Qualitative spatial relations are used in artificial intelligence to model commonsense notions such as regions ofspace overlapping, touching only at their boundaries, or being separate. Various spatial calculi have been developed, as the Region-Connection Calculus (RCC). The RCC models topological relations in dense spaces, and defines relations using first-order logic. We present a work on qualitative relations in discrete space using a bi-intuitionistic modal logic with universal modalities, called UBiSKt. This logic has a semantics in which formulae are interpreted as subgraphs of a multi-graph space. We show how a variety of qualitative spatial relations can be defined in UBiSKt. We use an axiomatisation of the logic and a tableau based theorem prover to establish properties of these spatial relations.

Our logic can deal with spatial relations at different levels of detail. The idea of zooming out, or viewing a situation in a less detailed way, is commonplace. For instance zooming out on an image (a set of pixels) we expect narrow cracks to fuse and narrow spikes to become invisible. This idea is formalized in mathematical morphology: instead of being able to see individual pixels, only groups of pixels of a particular pattern (copies of a structuring element) can be seen. This idea is known to extend to graphs (not just sets of pixels). We are using the logic to define qualitative relations between regions (subgraphs) in a “zoomed out” fashion as well as at the familiar detailed level. The interplay between two levels is a novel feature of this work.

References

[SS17] G. Sindoni and J. G. Stell. The logic of discrete qualitative relations. In *LIPIcs-Leibniz International Proceedings in Informatics*, volume 86. Schloss Dagstuhl-Leibniz-Zentrum fuer Informatik, 2017.

[SSR16] J.G. Stell, R.A. Schmidt, and D. Rydeheard. A bi-intuitionistic modal logic: Foundations and automation. *J. Logical and Algebraic Methods in Programming*, 85(4):500–519, 2016.

Detection of leukemic blasts in white blood cell mixture using deep learning and high-content analysis of imaging flow cytometry data.

Claire M. Barnes[4], Minh Doan[1], Holger Hennig[1,2] , Claire McQuin[1], Allen Goodman[1], Juan Caicedo[1], Marian Case[3], Andrew Filby[3], Olaf Wolkenhauer[2], Huw D. Summers[4], Julie Irving[3] and Anne E. Carpenter[1,*] Paul Rees[1,4,*]

Affiliations:
[1]Imaging Platform, Broad Institute of MIT and Harvard, Cambridge, Massachusetts 02142, USA
[2]Dept. of Systems Biology & Bioinformatics, University of Rostock, Rostock, 18051 Rostock, Germany
[3]Medical School, Newcastle University, United Kingdom
[4]College of Engineering, Swansea University, Singleton Park, Swansea, Swansea SA2 8PP, United Kingdom

Abstract: Leukemia refers to a group of cancers of the blood-forming tissues in the body which result in high numbers of abnormal white blood cells. There are four main types of Leukemia - acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL) and chronic myeloid leukemia (CML) each with its own etiology. Acute forms of the disease result in a rapid onset of symptoms and can be fatal if left untreated thus making early diagnosis a key motivation for research. Imaging flow cytometry has potential in the area of diagnostics applying the power and speed of a flow cytometry coupled with the high resolution of a microscope to generate hundreds of thousands of high-content cell images within minutes. Features of the images provided can subsequently be extracted to generate multivariable parameters of interest, including pixel intensities, size, shapes and textures along with subcellular components. By applying some of the latest analytical tools, such features can be used to distinguish between various subtypes of blood cells and thus aid early diagnosis of the disease.

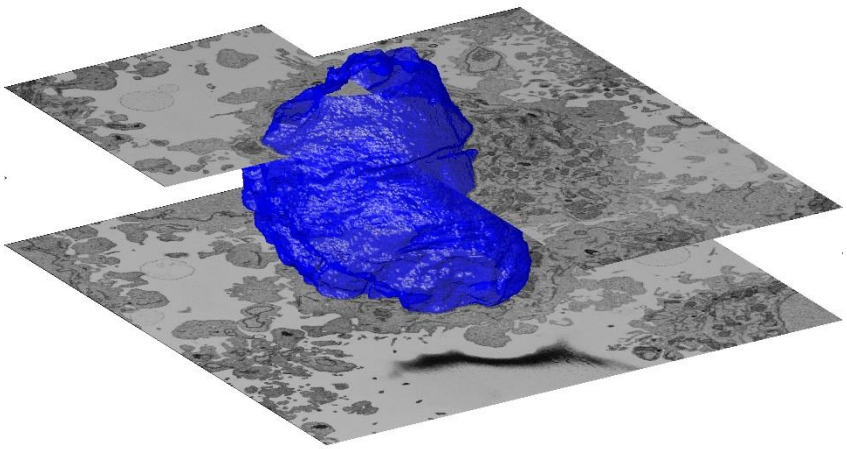
This work focuses on the use of deep learning techniques along with high-content IFC images to detect leukemic blasts from a mixture of white blood cells in blood samples. A remarkable finding of this work indicated that using just the bright field (forward scatter) and dark field (Side scatter) channels plus a nucleus stain (DAPI) achieved 75-81% prediction accuracy to automatically differentiate leukemic blasts from a mixture of white blood cells. A ResNet50 convolutional neural network (CNN) and a linear support vector machine (SVM) yielded similar results. This work also investigates the effect of different biomarkers on the ability of these techniques to detect leukemic cells. The possibility of using fewer biomarkers whilst maintaining sufficient predictive power was demonstrated, an important finding for clinicians due the complex and time-consuming nature of biomarker labelling. These techniques were also used to study heterogeneity within a cohort of Leukaemia patients taking part in a clinical trial. Classifications using these deep learning techniques were compared with classical machine learning and unsupervised clustering. It was found that the utilization of deep learning helped to extract unique cellular features that could be otherwise overlooked. In summary, this work demonstrates the potential application of combining IFC and deep learning as a diagnostic tool in a clinical setting. Moreover, patient biodiversity shown by these techniques, provide valuable insight into the condition and will undoubtably form the basis of work on personalised medicine in the future.

Automated Segmentation of HeLa Nuclear Envelope from Electron Microscopy Images

Cefa Karabag (1), Martin L. Jones (2), Christopher J. Peddie (2), Anne E. Weston (2), Lucy M. Collinson (2), and Constantino Carlos Reyes-Aldasoro (1)
(1) - Research Centre for Biomedical Engineering, School of Mathematics, Computer Science and Engineering, City, University of London, Northampton Square EC1V 0HB, UK
(2) - Electron Microscopy Science Technology Platform
The Francis Crick Institute, 1 Midland Road, London NW1 1AT, UK

Abstract Modern Electron Microscopes (EM) can acquire very high resolution images and a core facility can produce tens of thousands of data sets that can easily exceed gigabytes of data every month. The identification of individual cells, and the shape of the cell and its parts like the nuclear envelope is of great interest to scientists, as the structure of the cell may reveal some conditions of health or disease. Despite the significant disadvantages in time and inter- and intra-user variability, manual or semi-automatic delineation of nuclear envelope is widely used. The aim of this work was to find an image-processing algorithm for the automatic segmentation of the nuclear envelope of *HeLa* cervical cancer cells acquired via EM.
The data sets consisted of EM images of cervical cancer "HeLa" cells. Wild type HeLa cells were prepared and embedded in Durcupan resin following the method of the National Centre for Microscopy and Imaging Research (NCMIR). Serial blockface scanning electron microscopy (SBF SEM) data was collected using a 3View2XP (Gatan, Pleasanton, CA) attached to a Sigma VP SEM (Zeiss, Cambridge). Images were acquired at 8192 x 8192 pixels over a total of 518 slices, with 10 x 10 x 50 nm voxel size and the intensity of each voxel was between 0 and 255. Individual cells were manually cropped as volumes of interest. The single cell used in this study was a substack of 2000 x 2000 x 300 voxels.
The algorithm consists of several image processing steps: low-pass filtering, edge detection, determination of superpixels, distance transforms, morophological operators and the final delineation of the nuclear envelope. The algorithm assumes that there is a single HeLa cell of interest in the field of view, the centre of the cell is located at centre of a 3D stack of images, the nuclear envelope is darker than the nuclei or its surroundings, and the background is brighter than any cellular structure.

The algorithm was applied to a 3D stack of 300 images with satisfactory results (Figure attached). Comparison with manual segmented ground truth (GT) from a citizen-science called *Etch a Cell* reported Jaccard Similarity Index (JI) between 94-98 % on the central slices but it decreases towards the top and bottom of the cell as the structure was considerably more complex. In order to compare with an alternative approach the active contour segmentation was also applied on the same images and results from both methods were compared. The difference in JI suggests that segmentation of the algorithm gives better results than that of active contour. In order to determine the optimal parameters sensitivity analyses were also performed. The code is freely available in https://github.com/reyesaldasoro/HeLa-Cell-Segmentation.



This work was supported by the Francis Crick Institute.

Image Analysis and Tracking of Migrating Macrophages

José Alonso Solís Lemus*, Brian Stramer†, Greg Slabaugh‡
Constantino Carlos Reyes-Aldasoro*

AI and Digital Microscopy Meeting. 25-26 June 2018, University of Exeter, Exeter.

Abstract

Cell migration is crucial in many processes of development and maintenance of multicellular organisms. Understanding the migrating patterns of cells in the immune system is of great importance, for example in macrophages, excessive migration could lead to autoimmune diseases and cancer. A precise analysis of the cell shapes in biological studies could lead to insights about migration. However, in some cases, the interaction and overlap of cells can complicate the detection and interpretation of their shapes.

In this work, two aspects of this problem will be discussed: (i) correcting the segmentation of overlapping cells and (ii) measuring the impact of cell interactions in the change of the direction of movement. The work is based on fluorescently labelled macrophages observed on embryos of *Drosophila melanogaster*. When dealing with overlapping cells, a description of the algorithms to analyse the shape and movement of cells will be presented. After segmenting image sequences of macrophages, an analysis of the shape of the segmented macrophages is done through a 2D matrix with multiscale angle variation based on the angles between points of the boundary of an object. The matrix, called the *anglegram*, produces a signature of the shape from which morphological information can be extracted, such as relevant junctions like corners and bends. Then, the junctions found can be used to correct segmentation of some cases of overlapping cells.

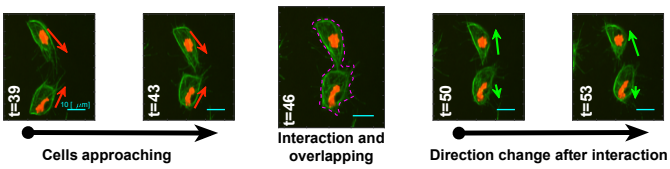


Figure 1: Two problems arise when analysing moving macrophages: (1) the ambiguity when segmenting overlapping cells (centre frame) and (2) measuring the observed change of direction after interactions.

Finally, preliminary results will be presented from an algorithm which integrates the previous segmentation technique with a tracking framework into a tool for the analysis of the trajectories of cells before and after they overlap. The main objective is to provide insights into the notion that interactions between cell structures appear to alter the trajectories of the cells. The results show promise into the analysis of movement and the impact cell interactions have on the moving patterns of the cells.

*School of Mathematics, Computer Science and Engineering, City, University of London, UK
†Randall Division of Cell & Molecular Biophysics, King's College London, UK
‡Huawei Technologies Research & Development, London, UK

Assessing Tissue and Cellular Morphometry in the Foetal Lung in a Murine Oligohydramnios Model

Tanbir Najrana [1], Juan Sanchez-Esteban [1] and Rasha Abu Eid [2]

[1] Department of Pediatrics, Alpert Medical School of Brown University, Women & Infants Hospital of Rhode Island, 101 Dudley Street, Providence 02905, Rhode Island

[2] Institute of Dentistry, School of Medicine, Medical Sciences & Nutrition, University of Aberdeen, Foresterhill Campus, Aberdeen AB25 2ZR, Scotland

Background:

Foetal lung development is greatly driven by mechanical forces. Therefore, Oligohydramnios (OH) due to the decrease of fluid pressure results in severe pulmonary hypoplasia.

Methods:

In this murine model, amniotic sacs were punctured in pregnant mice to mimic OH, with untouched fetuses serving as controls. Fetuses were then terminated, and lung tissues collected. Proliferation, differentiation, apoptosis, and angiogenesis were assessed in OH and control foetuses. Histological sections of the lungs were imaged and analyzed at the tissue level and the cellular level. Various morphometric descriptors of size and shape were used to compare tissues from OH and untouched controls.

Results:

We found that OH leads to a significant reduction in lung weight, lung to total body weight ratio, and lung water content. Furthermore, OH was associated with smaller, less developed air spaces when compared to controls. Cells from OH fetuses were smaller and less regular in shape compared to controls.

OH was also associated with a defect in the differentiation of Type I alveolar epithelial cells and compromised apoptosis and angiogenesis

Conclusions:

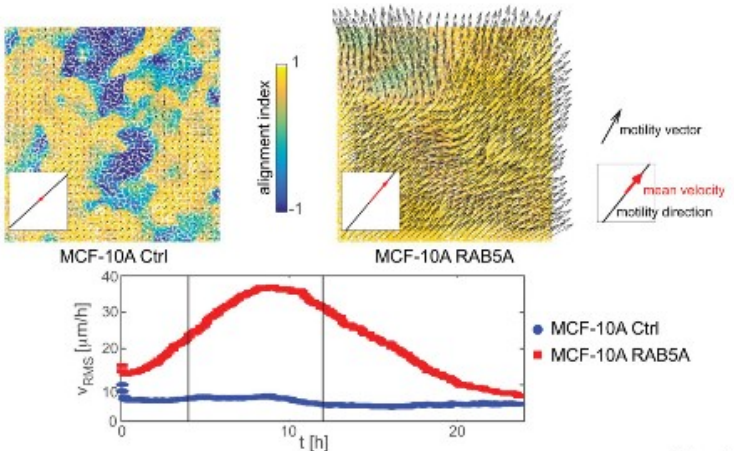
Although, the hypoplasia in the lung could be explained by a decreased of lung fluids, our data suggest that increased of external compression secondary to severe OH can compromise cell size and interfere with epithelial and endothelial development. Type I epithelial cells could have an unrecognized key role in the differentiation of the distal lung mediated by mechanical signals

3D Collective Cell Motility and Extra Cellular Matrix invasion

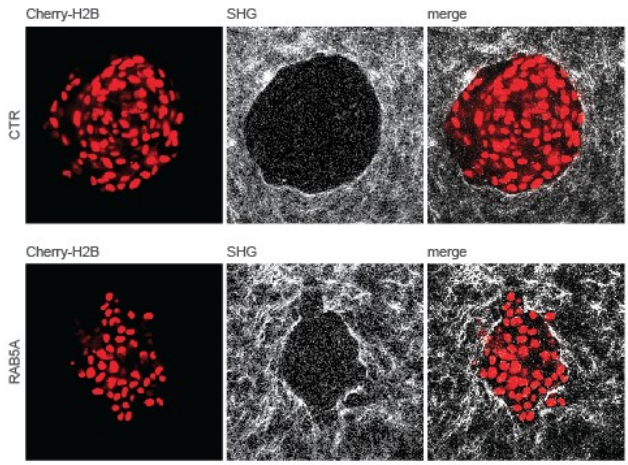
Emanuele Martini

IFOM/FIRC Inst.Molec. Oncology, Milano, Italy

Collective cell migration, a widely recognized mode of migration during embryogenesis, wound repair and cancer, refers to the process of many cells migrating as a cohesive group with each individual cell correlating its own movement with that of its neighbors. A cellular process that influences different aspects of cellular and multicellular plasticity and collective motility strategies is membrane trafficking. This process is controlled by a myriad of different proteins. However, the small GTPases of the RAB family have emerged as critical trafficking nodes between the different stations of the endocytic pathway. We found that endocytic circuitries controlled by RAB5A, a key regulator of early endosomes necessary to promote a proteolytic, mesenchymal program of individual cancer cell invasion, have a dramatic impact on the mechanics and dynamics of multicellular, normal and tumorigenic, cell assemblies. Perturbations of RAB5A levels are sufficient to re-awaken the motility of otherwise solid-like and kinetically arrested, jammed monolayers.



We observed the same transition, from static to dynamic state that promote coherent angular motion in 3D MCF10.DCIS-tumor spheroids embedded in stiff collagen matrix. We additional found that the reawakening of circular angular motion in 3D spheroids correlates with the ability of individual cells to escape the cage imposed by 3D confinement at short time scale, while it promotes matrix remodelling and collective invasion at longer time scale. Thus, the acquisition of fluid-like features linked to unjamming may promote an alternative or complementary strategy with respect to canonical epithelial-to-mesenchymal transition for cell dissemination and distal metastasis formation.



Advanced time-resolved microscopy reveals the nanostructure and function of human dental tissue

Tan Sui[1]*, Gabriel Landini[2]

[1] Department of Mechanical Engineering Sciences, University of Surrey, Guildford, Surrey, GU2 7XH, U.K.
* t.sui@surrey.ac.uk
[2] School of Dentistry, College of Medical and Dental Sciences, University of Birmingham, 5 Mill Pool Way, Edgbaston, Birmingham B5 7EG, U.K.

Abstract: The continued demand for improved dental treatment and prosthetics has driven the pursuit of understanding of the structure and thermo-chemical-mechanical properties of human teeth. It constitutes a superb lesson from nature on how to achieve strong with layered structures of dentine and enamel, and durable bonding of dentine-enamel junction (DEJ) between those significantly dissimilar materials. It is the desire to learn from nature's architecture of mineral-organic composite, hierarchically structured, property gradient materials that provided the motivation for this study. The advanced time-resolved synchrotron X-ray (e.g. Small- and Wide- angle X-ray Scattering (SAXS-WAXS), radiography and tomography) and multi-modal microscopy techniques (e.g. Focused Ion Beam (FIB), Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDS) and Atomic Force Microscopy (AFM)) [1-5] allowed us to create functional and structural probes across the length scales, from lattice-scale to nano-scale to macroscopic scale, and obtain critical insights into the microstructure characteristics, micro-scale residual stress evaluation and structural integrity under mechanical load, thermal shock and chemical reaction of human teeth, which is beneficial to biology and therapy. Potential benefits from artificial intelligence and machine learning in time-resolved advanced microscopy will be discussed.

Acknowledgment: Tan Sui acknowledges the invaluable support from colleagues in the MBLEM laboratory led by Prof Alexander M. Korsunsky at Oxford and all the project partners.

[1] T. Sui *et al.*, *Acta Biomater.*, 2013, 9: 7937-7947.
[2] T. Sui *et al.*, *Acta Biomater.*, 2014, 10: 343-354.
[3] T. Sui *et al.*, *J R Soc Interface*, 2014, 11.
[4] T. Sui *et al.*, *J Mech Behav Biomed*, 2014, 32: 113-124.
[5] T. Sui *et al.*, *Acta Biomater.*, 2016, 32: 256-263.