Discrete Mereotopology in Histological Imaging

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Abstract

In this paper we describe methods suited for developing intelligent histological imaging procedures based on mathematical morphology and a discrete version of the Region Connection Calculus (*RCC*) known as Discrete Mereotopology. The implementation of the discrete versions of *RCC5* and *RCC8* relation sets enables computation of the spatial relationships between image regions and reasoning about those relations in segmented digitised images. It also opens the possibility of defining histologically relevant models of biological structures (cells and tissues) so the relations of their components can be assessed algorithmically. A Java plugin implementing the *RCC5D* and *RCC8D* relations sets for the popular imaging tool ImageJ was developed. We illustrate an application for automated cell sorting on cultured fibroblasts.

1 Introduction

The term "intelligent imaging" covers applications designed to perform a certain level of mechanical reasoning about image contents. The usefulness of such procedures for histological imagery relies on: 1) the possibility of relaxing the need for expert (human) supervision (e.g. in high throughput applications) where the size of the data or time requirements make it impractical to rely on observer-based confirmation of results and 2) enabling algorithmic quantification and categorisations of imaging results. For example, segmentation correctness could be mechanically tested against an expected model of image contents and used to qualify the performance of segmentation procedures. The methods presented here graft sets of relations defined in a spatial logic called *Discrete Mereotopology* (DM) [1, 2] onto Mathematical Morphology (MM). DM is a discrete version of the well-known spatial logic *RCC* [3]. These relations can be used to describe the topology and organisation of organelles, cells and tissue components in images. These relations comprise a set of contact, overlap and part-whole relationships in discrete 2D space that can hold between pairs of binary regions in a single image or between regions across different images. Two jointly exhaustive and pairwise disjoint (JEPD) relation sets (*RCC5D* and *RCC8D*) are factored out which are *discrete* versions of the *RCC5* and *RCC8* relation sets well known in Qualitative Spatial Reasoning [1-4]. The *RCC5D* and *RCC8D*

relation sets were implemented as a plugin for ImageJ [5] written in Java [6]. These relations model "external contact", "partial overlap" and "tangential" and "non-tangential" connections, among others. It has been shown [1, 6] that the discrete quasi-topological interior and closure functions defined within DM map directly to the erosion and dilation operators of MM respectively. Therefore, the DM relations can be implemented using standard morphological routines available in most image processing environments. In addition the nature of the logic enables imaging packages to be used as a front-end for Artificial Intelligence methods for querying and analysing images as well as exploiting automated, mechanical reasoning programmes.

2 Implementing Discrete Mereotopology

The domain is modelled using a two-sorted mereotopological logic [2] as a specification language. Here, pixels are denoted by lower-case letters (x, y, z,...) and regions by upper-case letters (X, Y, Z,...). Predicates are strings of upper-case or lower-case letters prefixed with upper-case letters, and functions are strings of lower-case letters. Standard readings are assumed: the symbols: $\forall$, $\exists$, &, $\lor$, $\rightarrow$, $\neg$, $\equiv$ are respectively read as *for all*, *there exists*, *and*, *or*, *materially implies*, *not*, and *if and only if*.

In DM, regions mapping to digital images as a model are defined as (possibly empty) sets of pixels. The spatial relations are defined as follows. *Inclusion* is: $X \subseteq Y \equiv \forall x \ (x \in X \rightarrow x \in Y)$, and the mereological (non-null) *part/whole* relation is: $P(X,Y) \equiv X \subseteq Y \ \& \ X \neq \varnothing$. The mereological *proper-part* relation is defined as: $PP(X,Y) \equiv P(X,Y) \ \& \ X \neq Y$ and *overlap* is: $O(X,Y) \equiv X \cap Y \neq \varnothing$. The *connection* (or *contact*) relation between regions is: $C(X,Y) \equiv \exists x \exists y (x \in X \ \& \ y \in Y \ \& \ A(x,y))$. The dyadic adjacency relation A is axiomatised to be reflexive and symmetric. We additionally assume an 8-connected square-based pixel array so that two pixels x and y are adjacent if they are nearest neighbours or equal, meaning $A(x,y)$ is satisfied if $d(x,y) \leq \sqrt{2}$ where d: $\mathbb{Z}^2$ x $\mathbb{Z}^2 \rightarrow \Re$. The introduction of adjacency extends the set of overlap relations (used to define the *RCC5D* relations set) enabling the remaining discrete counterparts of *RCC8* [6] relations to be defined. The "*D*" suffix is added to *RCC5/8* to emphasise that the relations defined have models in *discrete* space. Models of the five relations of *RCC5D* and the eight relations of *RCC8D* are shown in Figure 1.

The eight relations are defined below (Table 1) with their *MM* counterparts. For the latter, a structuring element *B* is assumed and defined as a filled 3x3 neighbourhood pixel array. The main relations are read as follows: DC ("is disconnected from"), EC ("is externally connected with"), PO ("partially overlaps"), TPP ("is a tangential proper part of"), NTPP ("is a non-tangential proper-part of"), EQ ("is identical with"), while TPPi and NTPPi are inverses of TPP and NTPP respectively. Using this approach, weaker JEPD relation sets become easily definable, for example the spatial constraint language *RCC5* with five base relations {DR,PO,PP,PPi,EQ}, with DR ("is discrete from") as the least upper bound (lub) of DC and EC, PP ("is a proper part of") mapping to TPP lub NTPP, and similarly for PPi as TPPi lub NTPPi. It is worth noting that the set of eight JEPD relations defined in *RCC8D* can be extended further, however here we restrict the relationships to *RCC5D* and *RCC8D*.
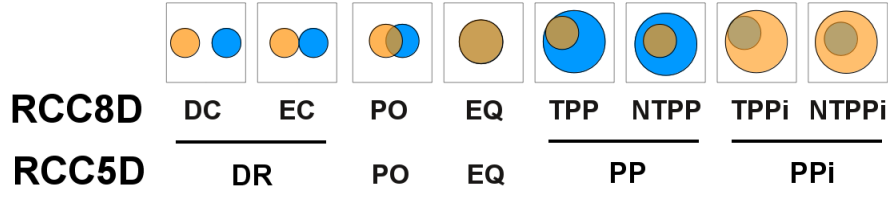
Figure 1. The *RCC5D* and *RCC8D* relations. Regions are shown as 2D discs (the bright disc is X and the dark is Y). The models for *RCC5D* cover the cases shown in *RCC8D*. Where one or both regions are null, the *RCC5/8D* relation DR holds. DR: *discrete from*, DC: *disconnected from*, EC: *externally connected*, PO: *partially overlaps*, PP: *proper part*, TPP: *tangential proper part*, NTPP: *non-tangential proper-part*, EQ: *identical with*, PPi, TPPi and NTPPi are inverses of PP, TPP and NTPP respectively.

Table 1. The *RCC8D* relations implemented using mathematical morphology.

Discrete Mereotopology	Mathematical Morphology	
$DC(X,Y) \equiv \neg C(X,Y)$	$DC(X,Y) := (X \oplus B) \cap Y = \varnothing$	(1)
$EC(X,Y) \equiv C(X,Y) \,\&\, \neg O(X,Y)$	$EC(X,Y) := (X \cap Y = \varnothing) \,\&\, ((X \oplus B) \cap Y \neq \varnothing)$	(2)
$PO(X,Y) \equiv O(X,Y) \,\&\, \neg P(X,Y) \,\&\, \neg P(Y,X)$	$PO(X,Y) := (X \cap Y \neq \varnothing) \,\&\, (X\text{-}Y \neq \varnothing) \,\&\, (Y\text{-}X \neq \varnothing)$	(3)
$TPP(X,Y) \equiv PP(X,Y) \,\&\, \exists Z \,(EC(Z,X) \,\&\, EC(Z,Y))$	$TPP(X,Y) := (X\text{-}Y = \varnothing) \,\&\, (Y\text{-}X \neq \varnothing) \,\&\, (((X \oplus B)\text{-}Y) \neq \varnothing)$	(4)
$NTPP(X,Y) \equiv PP(X,Y) \,\&\, \neg \exists Z \,(EC(Z,X) \,\&\, EC(Z,Y))$	$NTPP(X,Y) := (X\text{-}Y = \varnothing) \,\&\, (Y\text{-}X \neq \varnothing) \,\&\, ((X \oplus B)\text{-}Y) = \varnothing$	(5)
$TPPi(X,Y) \equiv TPP(Y,X)$	$TPPi(X,Y) := TPP(Y,X)$	(6)
$NTPPi(X,Y) \equiv NTPP(Y,X)$	$NTPPi(X,Y) := NTPP(Y,X$	(7)
$EQ(X,Y) \equiv P(X,Y) \,\&\, P(Y,X)$	$EQ(X,Y) := (X\text{-}Y = \varnothing) \,\&\, (Y\text{-}X = \varnothing)$	(8)

$\oplus$: morphological dilation, *B*: structuring element, "-" is the *diff* or *logical subtraction o*peration.

2.1 Implementing *RCC5/8D*

A plugin for ImageJ [5] was written in Java [6] to compute the set of *RCC5D* and *RCC8D* relations. The input is two binary images, X and Y, with the regions to be tested. These can represent, e.g. labelled cells, nuclei or tissue compartments segmented with separate procedures or imaging modalities. The image frame sizes are expected to correspond to the same scene position exactly. The relation between the objects in X and Y can be computed from a series of nested tests on the overlaps (logical AND) and set-theoretical differences (diff operation, Table 1) between various combinations of original and dilated versions of the images.

An alternative, more efficient, practical approach consists of encoding the binary images with values 0, 1 for X and 0, 2 for Y, and inspect the histogram of the sum of the two images (which will have for values from 0 to 3). This gives an indication of which of the 5 *RCC5D* relations holds. From those, a further test provides the eight *RCC8D* relations. A diagram of this algorithm (implemented in our plugin *RCC8D*) is shown in Figure 2. An additional plugin was written to deal with multiple regions in each image and an application is described in section 3.
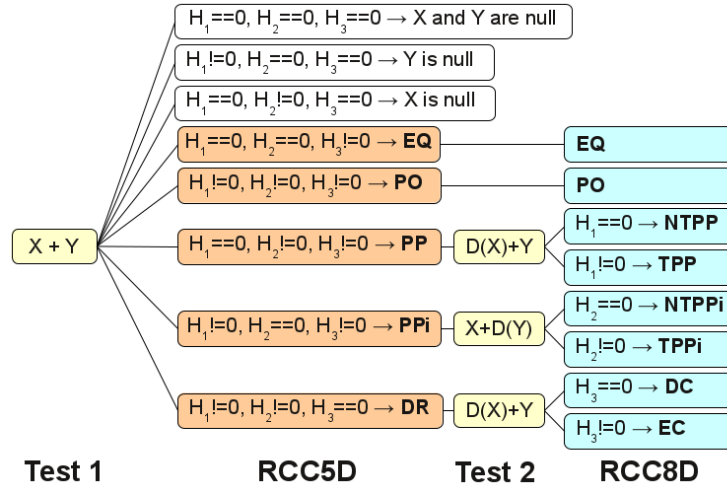
Figure 2. The alternative *RCC* algorithm. Images X and Y are encoded with pixel values 0, 1 and 0, 2 respectively (0 is 'background', non-zero values are region pixels). The histogram H of the arithmetical sum of the images (X+Y, Test 1) can result in various combinations of zero and non-zero counts in bins H_0 (background), H_1 (pixels occupied only by region X), H_2 (pixels occupied only by region Y), or H_3 (overlapping regions). This allows finding the *RCC5D* relationship held. E.g. if the number of counts in histogram bins H_1 and H_2 is both 0 and for the sum (H_3) is >0 (overlap), EQ holds. This is shown in the figure as (H_1==0 H_2==0 H_3!=0). In the case of *RCC8D*, the relations PP, PPi and DR are subjected to an additional operation (Test 2), depending on the Test 1 result. D(X) and D(Y) symbolise the morphological dilation of images X and Y respectively.

3 Intelligent imaging in the histological domain

When performing tests between two images holding one region each, the *RCC8D* plugin results can be output to a text window or retrieved programmatically by querying the image *via* ImageJ's *image attributes* (variables stored in the images). However, storing multiple object relations (between all pairs of regions in both images) requires a table. We developed an additional plugin (*RCC8D_Multi*) that generates a table of relationships between all the objects in the two images restricted to connected components. Those are stored as an 8 bit image (named "*RCC*") where the pixel coordinates *x* and *y* encode the index of the regions in images *X* and *Y* and the pixel value is a numerical relation code. For example, row 0 in the *RCC* image table (Figure 3C) encodes the relationships between the first region (index 0) in image *X* and all the other regions in image Y; likewise, the relation between region index 4 of image *X* and region index 15 of image *Y* is given by the value of pixel with coordinates (4, 15). Again, attributes are set to the *RCC* table via the following keys to facilitate querying the table programmatically: *mode* (values indicating which logic was used *RCC5D* or *RCC8D*), *imageX* and *imageY* (the images' names).

3.1 Example – model-based cell sorting

Figure 3 shows an example analysis based segmented images NIH/3T3 fibroblasts cultured on glass coverslips. Figure 3B represents the binarised nuclei obtained with a greyscale threshold, and 3C shows the cell profiles. We aim to extract cells which have been segmented successfully based on the relationship between their nuclei and cytoplasms.
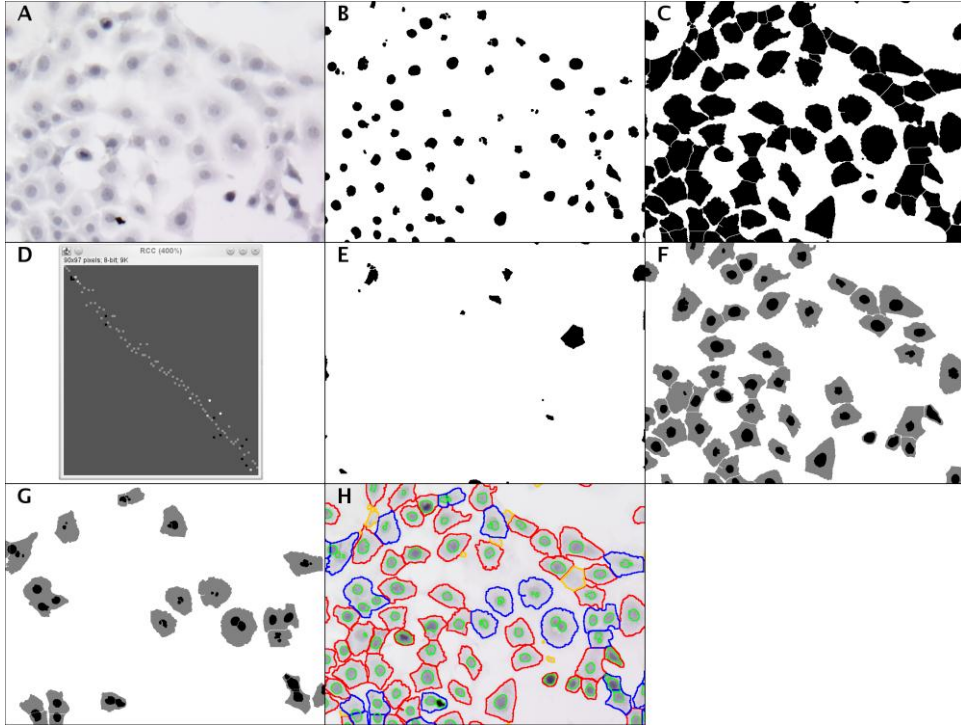

Figure 3. An image of cells in culture (A), the set of 90 segmented nuclei (B) and 97 cell/cytoplasm profiles (C). To identify "model cells" (i.e. with a single associated nucleus), image D is computed by the *RCC8D_Multi* plugin to encode in a grey value the *RCC8D* relations between the nuclei (indexed in the x axis) and cytoplasms (y axis). This enables identifying cells without a nucleus (E), model cells satisfying the mereotopological relations EQ, TPP and NTPP (54 instances, composite in F) and cells not fulfilling the model (G). The latter contains several mismatching types (cells with >1 nucleus and cells sharing partially overlapping nuclei). Panel H shows the classified relations colour coded on the boundary of segmented objects: nuclei (green), cytoplasm of model cells with 1 nucleus (in red), cytoplasm without a nucleus (yellow) and incorrectly segmented cells with multiple or overlapping nuclei (blue).

A "model cell" is defined in this example as a region containing a single nucleus, even though, biologically speaking, multinucleated (e.g. osteoclasts, Langhans-type giant cells, etc.) and non-nucleated cell types (human erythrocytes, bacteria) also exist. In *RCC8D* the three "part" (P) relations (i.e. TPP, NTPP and EQ) are used to define a "model cell" such that CellBody(X) & ∃Y (Nuc(Y) & P(Y,X)), where a CellBody is a candidate cell profile

(regions in 3C) regardless of the relationship with any nuclei in 3B. By examining the relationships table generated by the *RCC8D_Multi* plugin (Figure 3D), we can query the relations of any nucleus with any cell to find those cases where P(nuc$_i$, cellbody$_j$) occurs only once combined with a single instance of nuclei relations with a particular cellbody (Figure 3F). Cell bodies with no associated nuclei can be identified as the rows in the table labelled only with DC relations (21 instances, shown in Figure 3E) All the other possible relations (Figure 3G) not fulfilling the "nucleated cell" model (i.e, cells with multiple nuclei or cell bodies sharing a nucleus, thus partially overlapping cells) can also be specified in DM terms. Note that while model cells could also be extracted using *RCC5D*, nuclei forming TPP or NTPP relationships with the cytoplasm would not be distinguished.

One further advantage of these approaches is that knowledge of the relations between image regions can be enhanced by means of *conceptual neighbourhood diagrams* [2-4, 6]. These are pre-computed graphs encoding the possible changes in a relation between regions when one of the regions undergoes a "minimal change" (e.g. after a morphological dilation). Knowledge of these relation changes helps understanding which additional morphological operations in the segmentation process can be applied so the expected image content model is better fulfilled.

4 Conclusions

We presented an implementation of DM for two related sets defined by means of MM computations. Those relations can be used to model topological cell and tissue organisation in histological sections (further examples on tissue architectural features have been suggested in [6]). While histological imagery is commonly modelled as 2D discrete space, the models for DM are by no means restricted to 2D space and regions can in principle be modelled as volumes or include an additional temporal dimension.

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