Published in final edited form as:

IEEE Trans Biomed Eng. 2015 August ; 62(8): 2070–2078. doi:10.1109/TBME.2015.2412533.

A Stochastic Algorithm for Generating Realistic Virtual Interstitial Cell of Cajal Networks

Jerry Gao,

Auckland Bioengineering Institute, University of Auckland, New Zealand

Shameer Sathar,

Auckland Bioengineering Institute, University of Auckland, New Zealand

Gregory O'Grady,

Auckland Bioengineering Institute, University of Auckland, New Zealand

Rosalind Archer, and

Department of Engineering Science, University of Auckland, New Zealand

Leo K. Cheng

Auckland Bioengineering Institute, University of Auckland, Auckland 1020, New Zealand

Department of Surgery, Vanderbilt University, Nashville, TN 37240 USA

Leo K. Cheng: l.cheng@auckland.ac.nz

Abstract

Interstitial cells of Cajal (ICC) play a central role in coordinating normal gastrointestinal (GI) motility. Depletion of ICC numbers and network integrity contributes to major functional GI motility disorders. However, the mechanisms relating ICC structure to GI function and dysfunction remains unclear, partly because there is a lack of large-scale ICC network imaging data across a spectrum of depletion levels to guide models. Experimental imaging of these large-scale networks remains challenging because of technical constraints, and hence, we propose the generation of realistic virtual ICC networks *in silico* using the single normal equation simulation (SNESIM) algorithm. ICC network imaging data obtained from wild-type (normal) and 5-HT_{2B} serotonin receptor knockout (depleted ICC) mice were used to inform the algorithm, and the virtual networks generated were assessed using ICC network structural metrics and biophysically based computational modeling. When the virtual networks were compared to the original networks, there was less than 10% error for four out of five structural metrics and all four functional measures. The SNESIM algorithm was then modified to enable the generation of ICC networks across a spectrum of depletion levels, and as a proof-of-concept, virtual networks were successfully generated with a range of structural and functional properties. The SNESIM and modified SNESIM algorithms, therefore, offer an alternative strategy for obtaining the large-scale ICC network imaging data across a spectrum of depletion levels. These models can be applied to accurately inform the physiological consequences of ICC depletion.

Index Terms

Computational modeling; confocal microscopy; gastrointestinal (GI); gastroparesis; SNESIM

I. Introduction

Gastrointestinal (GI) motility encompasses the muscular contractions in the GI tract required for digestion and transportation of contents. Throughout much of the tract, this process is facilitated by specialized cells called interstitial cells of Cajal (ICC) [1]. One of the most prominent roles of ICC is the pacemaking of GI motor patterns, achieved by actively initiating and propagating electrical oscillations that excite and cause contractions in the GI musculature [2], [3]. ICC loss and injury is now a major research focus as it is recognized as a hallmark of several GI functional motility disorders [4], notably gastroparesis [5], [6], intestinal pseudo-obstruction [7], [8], and slow-transit constipation [9], [10].

Although the importance of ICC in GI health and functional disorders is now well established, the mechanism relating ICC structure to GI function and dysfunction remain poorly defined. A major constraint in resolving this problem is the limitations of current ICC imaging data. The imaging of *ex vivo* ICC networks is technically challenging, and the obtained data are still generally limited to small fields-of-view in the order of a few hundred micrometers to millimeters [11]. Also, most studies on ICC loss compare normal networks against depleted networks at some set depletion severity, such as that induced by a gene knockout (KO) [3], [12] or disease [6], [7], and there is no systematic method to experimentally control the depletion severity so that networks at an intermediate depletion level can be imaged and investigated. A comprehensive imaging dataset encompassing large-scale ICC networks across a spectrum of depletion levels would be of substantial benefit in investigating the pathophysiology of ICC loss.

In a preliminary report, a computational strategy for obtaining a comprehensive ICC imaging dataset was proposed for generating realistic virtual ICC networks *in silico* using the stochastic single normal equation simulation (SNESIM) algorithm [13]. The SNESIM algorithm was originally developed in the petroleum industry for building realistic statistical models of the geological formations, which host oil reservoirs [14], [15]. This study now aims to advance, validate, and demonstrate proof-of-principle of the SNESIM algorithm for generating realistic virtual ICC networks. The structural and functional similarity between experimentally imaged and virtually generated networks was assessed using ICC network structural metrics and biophysically-based computational modeling. Following validation, the SNESIM algorithm was also adapted to enable the generation of ICC networks across a spectrum of depletion levels.

II. Materials and Methods

A. SNESIM Algorithm

In summary, the SNESIM algorithm generates images of any size with similar structural properties to a user-supplied training image that contains the desired image characteristics.

Full details on the algorithm and its user-supplied input parameters can be found in previous reports [14]–[16].

In general, the SNESIM algorithm replicates the underlying multiple-point statistics of the training image, which express the conditional probabilities of the values that can be taken by a pixel of interest based on the values of multiple neighboring pixels. The relative locations of these neighboring pixels to the pixel of interest are defined in the user-supplied data template, and the set of neighboring pixels is termed the “data search neighborhood” (DSN).

To capture large-scale structural properties, the DSN needs to contain far-away neighboring pixels, whereas for small-scale structural properties close-by neighboring pixels are required. However, as a larger number of neighboring pixels within the DSN incurs greater computational costs, employing a DSN encompassing both small- and large-scale structural properties is often infeasible. The multigrid approach overcomes this difficulty by sequentially generating the image over coarse to fine grids, while scaling the DSN accordingly. Specifically, the algorithm begins with the coarsest grid, where adjacent pixels are further apart, and hence, the DSN efficiently covers a larger area. The grid is then recursively refined by including all pixels halfway between any two currently adjacent pixels, thereby shrinking the DSN by a factor of 2 with each refinement, until the finest grid is reached. With this approach, only close-by neighboring pixels are required in the DSN, as the scaling applied with the coarser grids allow large-scale structural properties to be captured. The coarseness of the initial grid is determined by the user-supplied number of multigrids. Each time the grid is refined, the multigrid level decreases by one, and a multigrid level of one corresponds to the finest grid. The DSN and number of multigrids are, therefore, important input parameters to accurately replicate structural properties across a range of spatial scales.

The specific steps taken by the SNESIM algorithm are summarized below (see Fig. 1), and the terminology used associated with probability density functions (PDFs) is described in Table I. See [17, Fig. 4.3] for an illustrative figure depicting the critical steps of the algorithm.

1. Starting from the highest multigrid level, each pixel in the training image is scanned using the data template to generate the search tree, which records the multiple-point statistics of the training image for the current multigrid level.
2. A path that visits all pixels in the current multigrid once only is defined. Pixels with more inferable neighboring pixels are visited first, and the ordering of pixels with the same number of inferable neighboring pixels is determined randomly.
3. Each undetermined pixel along the random path is scanned by the data template, and the values of the neighboring pixels within the DSN are used to retrieve the conditional PDF for the current pixel of interest from the search tree generated in step one. If the values presented by the neighboring pixels do not exist in the search tree (i.e., the pattern presented by the pixels in the DSN do not appear in the training image), the pixel in the DSN with furthest distance from the pixel of interest is dropped, and the conditional PDF for the current pixel of interest is re-retrieved. It is known that the DSN have the same furthest distance from the pixel

of interest, in the training image to one pixel is selected arbitrarily and dropped. If all neighboring pixels within the DSN are dropped, the value of the pixel of interest is generated from the user-defined global marginal PDF.

4. The PDF computed in step 3 is adjusted to account for the local marginal PDF, and a value for the current pixel of interest is simulated from this adjusted PDF. This pixel is added into the image to inform subsequently simulated pixels.
5. Steps 3 and 4 are repeated until all pixels in the random path are simulated.
6. The multigrid level is decreased by 1 and steps 1–5 are repeated. All previously simulated pixels in the higher multigrid levels are retained to inform the subsequently simulated pixels. The algorithm is terminated when multigrid level one is completed.

In the context of generating virtual ICC networks, the SNESIM algorithm can only generate virtual networks at the depletion level of the training network as it is simply replicating the multiple-point statistics. Therefore, the SNESIM algorithm was modified to enable the generation of ICC networks across a spectrum of depletion levels. The specific modifications are as follows:

1. Two training networks are used instead of one. These two training networks represent two points on the spectrum of depletion levels and, hence, by merging the structural properties of these two training networks, ICC networks across a spectrum of depletion levels can be generated. A search tree is constructed from each of the training networks.
2. A merge factor (ϕ) is computed as:

$$\phi = \frac{\rho - \rho_1}{\rho_2 - \rho_1} \quad (1)$$

where ρ is the user-supplied global marginal PDF, and ρ_1 and ρ_2 are the global marginal PDFs of training networks one and two respectively. The user defines the desired depletion level of the network to be generated through ρ , with a lower ICC probability corresponding to a more severe depletion level. ϕ determines the severity of the depletion level relative to those of the training networks, with $\phi=0$ indicating the same depletion level as training network one, and $\phi=1$ indicating the same depletion level as training network two. However, note that ϕ may lie outside the range $[0 - 1]$, when ρ lies outside the range $[\rho_1 - \rho_2]$ (i.e., when the desired depletion level of the virtual network is outside the spectrum presented by the two training networks).

3. When simulating a pixel, a PDF is retrieved from each of the two search trees separately. The PDFs retrieved from the search trees corresponding to training networks one and two are weighted by $(1-\phi)$ and ϕ , respectively, and the sum of these weighted PDFs is used to simulate the pixel. Any negative probabilities are limited to zero, and probabilities over one are limited to one.

Note that the PDF adjustment to account for the local marginal PDF in the SNESIM algorithm (step 4) involves the global marginal PDF of the training network, and as the modified SNESIM algorithm utilizes two training networks, this PDF adjustment was not implemented.

B. ICC Network Imaging Data

Confocal ICC network imaging data were used as training networks to inform the SNESIM and modified SNESIM algorithms [12]. Briefly, two-dimensional bitmap images of the *Kit*-positive ICC structures at the plane of the myenteric plexus (ICC-MP) were obtained from the jejunum of four-week-old wild-type (WT; normal) and 5-HT_{2B} serotonin receptor knockout (KO; depleted ICC) mice. Normally, serotonin acts on 5-HT_{2B} receptors to increase ICC proliferation and numbers [18], and a lack of 5-HT_{2B} receptors has been demonstrated to decrease ICC proliferation, numbers, and network volume [12]. The orientation of the networks relative to the circumferential direction was extracted from the *Kit*-positive ICC structures at the plane of the deep muscular plexus [19], and the ICC-MP structures were reoriented such that the circumferential direction was horizontal. These networks were also pre-processed to remove artifacts as previously described [19]. Briefly, small gaps with radius of one pixel ($\approx 0.6 \mu\text{m}$) or less in the network were joined, and small objects of less than four pixels ($\approx 1.54 \mu\text{m}^2$) were removed. One ICC-MP network was obtained from each of six mice (three WT, three KO) as described previously [12]. Each network was 362×362 pixels, and corresponded to physical dimensions of $0.225 \times 0.225 \text{ mm}$.

Three virtual ICC networks were generated for each WT and KO training network using the SNESIM algorithm (i.e., 9 WT and 9 KO virtual networks). The global marginal PDF ICC probabilities of the WT and KO training networks were calculated to be approximately 0.55 (0.51–0.59) and 0.40 (0.40–0.41), respectively, and the virtual networks were generated with global marginal PDF ICC probabilities matching their training counterparts.

To generate virtual ICC networks across a spectrum of depletion levels, the WT and KO training networks were arbitrarily paired and used to inform the modified SNESIM algorithm. For each of the three training network pairs, three virtual ICC networks were generated with global marginal PDF ICC probabilities of 0.30, 0.35, 0.40, 0.45, 0.50, and 0.55 (i.e., nine virtual networks for each global marginal PDF; a total of 54 virtual networks). The highest ICC probability (0.55) was selected to match the WT training networks, but ICC probabilities lower than that of the KO training networks (0.30–0.35) were selected. This was because although the KO mice showed depleted ICC networks, their intestinal transit times were not affected [12], and hence, we attempted to generate virtual networks at depletion levels of yet more marked severity. All virtual networks were generated using a multigrid level of five and a circular DSN with radius of four pixels and one pixel spacing. All generated virtual networks were the same size and at the same resolution as the training networks.

C. Structural Validation

Five numerical metrics were used to quantify the structural properties of the training and virtual ICC networks (see [19] for details on the computation of these metrics):

1. *Density*: measures the ICC network volume, representative of the amount of bioelectrical current generated and propagated;
2. *Thickness*: measures the width of cellular structures within the ICC network plane, which may impact electrical activity propagation through the network;
3. *Hole size*: measures the radius of non-ICC regions within the ICC network plane. Reflects the distribution of ICC throughout the MP (i.e., the “tightness” of the network), and may relate to the uniformity of smooth muscle cell activation;
4. *Anisotropy*: indicates the degree of preferential alignment of ICC structures, and may reflect the dominant slow-wave propagation direction, potentially affecting predilection and resilience to dysrhythmia [20];
5. *Connectivity*: measures the connectivity of the ICC network. Reflects the structural integrity of the ICC network, indicating the cohesion of entrainment pathways [21].

Prior to the application of structural metrics, the virtual networks were preprocessed to remove artifacts in the same manner as conducted on the training networks (see Section II-B.). Structural validation of the SNESIM algorithm was performed by comparing the structural metric values of the training and virtual WT and KO networks. For the non-normalized metrics of thickness, hole size, and connectivity (i.e., these metric have an infinite range), the relative errors of the metric values were computed, whereas for the normalized metrics of density and anisotropy, the errors were computed relative to the range of the metric [19]. Due to the logarithmic nature of the connectivity metric, the logarithm (base 10) of the metric value was used in the error computation.

D. Functional Validation

ICC pacemaker activity was simulated over the training and virtual ICC networks using a biophysically-based computational model [22]. The ICC networks were discretized into a finite element triangular mesh, with each node point corresponding to a pixel in the ICC network imaging data. The cellular activity of the ICC node points was represented using a biophysically based ICC model, based on the Corrias and Buist model, which was modified to incorporate a finite-state machine approach [23], [24]. The activity of the non-ICC grid points was represented using a passive cell model with a zero active ionic current. The continuum-based bidomain equations were employed to model the tissue level activity, and the model was solved using the CHASTE computational framework [24], [25].

ICC pacemaker activity was simulated over each of the training and virtual networks for 1000 ms with an ODE time step of 0.1 ms and a PDE time step of 1 ms. These ODE and PDE time steps were selected based on the convergence analysis to obtain a stable solution to the model. The PDE component of the model represents the diffusion of the ICC network pacemaker activity, which is slow in comparison to the cellular activity represented by the ODE component. Therefore, the PDE time step does not need to be as refined as the ODE time step. ICC node points within a square corresponding to 1% of the total network area at the top-right corner of the grid were set to activate at time $t=0$ ms as the initial stimulus to the simulations, whereas the remaining ICC grid points were activated via the voltage-dependent entrainment mechanism of the ICC model [24]. The conductivity parameters of

the model were selected such that the pacemaker activity propagated through the WT training networks at approximately 5 mm/s, as experimentally recorded in the healthy rat jejunum [26]. The model parameters used are the same as in [23] and [24]. Key parameters of the ICC model are reproduced in Table II of the appendix.

Four measures were then used to quantitatively assess the simulated ICC pacemaker activity [22]:

1. *Activation rate*: measures the rate at which the network achieves an average V_m of -30 mV;
2. *Peak $[Ca^{2+}]_i$* ;
3. *Time to peak $[Ca^{2+}]_i$* ;
4. *Half peak $[Ca^{2+}]_i$ time ratio*: gauges the dynamics of the $[Ca^{2+}]_i$ upstroke.

Functional validation of the SNESIM algorithm was performed by comparing the functional measure values of the training and virtual WT and KO networks. For the non-normalized measures of activation rate, peak $[Ca^{2+}]_i$, and time to peak $[Ca^{2+}]_i$, the relative errors of the measure values were computed, whereas for the normalized measure half peak $[Ca^{2+}]_i$ time ratio, the errors were computed relative to the range of the measure [22].

III. Results

The three WT and three KO training networks along with an example virtual network generated from each training network are shown in Fig. 2.

The errors in the structural metric values of the virtual networks compared to their training counterparts are shown in Fig. 3. The virtual networks accurately replicated the density metric (average absolute errors: WT 1%; KO 2%), and in the majority of networks the thickness metric as well (WT 5%; KO 2%). Replication of the anisotropy (WT 4%; KO 5%) and connectivity metrics (WT 8%; KO 6%) in the majority of networks was reasonable. The hole-size metric was relatively underestimated in the virtual networks (WT 16%; KO 17%).

The errors in the functional measure values of the virtual networks compared to their training counterparts are shown in Fig. 4. The virtual networks accurately replicated the peak $[Ca^{2+}]_i$ (WT 2%; KO 2%) and half peak $[Ca^{2+}]_i$ time ratio measures (WT 2%; KO 3%). The activation rate (WT 8%; KO 14%) and time to peak $[Ca^{2+}]_i$ measures (WT 5%; KO 8%) were also reasonably replicated for the majority of networks.

Example virtual networks generated across a spectrum of depletion levels using the modified SNESIM algorithm are shown in Fig. 5. The structural metrics and functional measures varied with respect to the global marginal PDF (see Figs. 6 and 7), demonstrating that by altering the global marginal PDF, virtual networks with a range of structural and functional properties could be generated from two training networks of different depletion levels. In general, the structural metrics and functional measures of the virtual networks displayed a monotonically increasing or decreasing trend with respect to the global marginal PDF ICC probability the virtual networks were generated with. However, the trends differed

between the interpolated virtual networks [i.e., generated with global marginal PDF ICC probabilities of 0.40 (KO) to 0.55 (WT)] and the extrapolated ones [i.e., generated with global marginal PDF ICC probabilities of 0.30 or 0.35, below 0.40 (KO)]. This is further discussed below.

Less than 1 min was needed to generate a virtual network using the SNESIM algorithm on a quad-core Intel Xeon W3530 CPU, whereas it took less than 2 min to generate a virtual network using the modified SNESIM algorithm on the same machine.

IV. Discussion

ICC depletion is thought to play a key role in GI dysmotility; however, a major constraint restricting the investigation of ICC network structure–function relationships is the paucity of imaging data. In particular, there is a lack of ICC network imaging data covering large fields-of-view and at a range of depletion levels spanning health and disease states. This study addresses these challenges by generating realistic virtual ICC networks *in silico* using the stochastic SNESIM algorithm. The fidelity of the virtual networks was validated both structurally and functionally, by applying ICC network structural metrics and biophysically based computational modeling, respectively. Modifications to SNESIM were also proposed to facilitate the generation of ICC networks across a spectrum of depletion levels, and as proof-of- concept, virtual networks were successfully generated with a range of structural and functional properties.

In general, the virtual networks generated by the SNESIM algorithm possessed reasonably similar structural and functional properties to their training counterparts. For the virtual WT networks, four out of five structural metrics and four out of four functional measures had average absolute errors under 10%, whereas for the virtual KO networks, four out of five structural metrics and three out of four functional measures had average absolute errors under 10%. The structural metric associated with the greatest errors was the hole-size metric, which focused on examining the non-ICC regions within the ICC network. For a non-ICC region to remain intact, it must be void of ICC pixels (otherwise, the region essentially divides into multiple smaller regions). The multigrid approach of SNESIM only simulates sparse pixels initially, leaving the intermediate pixels to be simulated subsequently, but when these intermediate pixels are being simulated, the multigrid level has decreased, and hence, the scale of the DSN has also decreased. The algorithm, therefore, has a more restricted recognition of large-scale structures and, hence, probabilistically, ICC pixels may be simulated, consequently reducing the hole-size metric. We tested whether increasing the DSN would improve the fidelity of the generated virtual networks. It is known that DSN should be larger than the size of the largest scale structures in the training to accurately generate images with high similarity, so the DSN size was increased to 13 pixels or inclusion of pixels up to 50 pixels away while maintaining a multigrid level of five (at the coarsest level the 13 pixel DSN would span 416 pixels). This resulted in virtual networks with comparable average absolute errors in the structural metrics (of the metrics which improved errors were reduced by less than 5%; data not shown). However, the computational cost of using these increased DSNs was up to 11 times more expensive than

when using the original DSN with a radius of four pixels, and hence, the original DSN was retained.

The errors in the time-related functional measures (activation rate, time to peak $[Ca^{2+}]_i$, and half peak $[Ca^{2+}]_i$ time ratio) were slightly higher for the virtual KO networks in comparison to the virtual WT networks. This was because training network KO1 was particularly sparse in the top-right corner where the initial stimulus for the ICC network pacemaker activity simulation was applied. Therefore, the onset of the simulated activity was delayed considerably, and the functional measures for the virtual networks generated from training network KO1 consistently showed faster activation rates, shorter times to peak $[Ca^{2+}]_i$ and smaller half peak $[Ca^{2+}]_i$ time ratios. The non-time-related measure of peak $[Ca^{2+}]_i$, however, was not affected. Therefore, taking into account these virtual KO networks with particularly high functional measure errors, the functional fidelity of virtual WT and KO networks are comparable.

The pre-processing of the training ICC networks before informing the SNESIM and modified SNESIM algorithms is an important step to generate realistic virtual ICC networks. As these algorithms generate the pixels of the virtual network sequentially, pixels simulated earlier on influence subsequently simulated pixels. Any artifacts present in the training network may be stochastically reproduced in the virtual network, and once reproduced, subsequently simulated pixels may therefore also be affected by this artifact, leading to a cascade of error.

The modified SNESIM algorithm was capable of generating virtual ICC networks with a range of structural and functional properties by retrieving a PDF from each of the two search trees corresponding to the two training networks, and then simulating pixels from a weighted sum of these PDFs. However, during extrapolation (i.e., when a virtual network is generated with depletion severity outside the spectrum presented by the two training networks), the merge factor takes on values less than zero or greater than one, so a negative PDF weight will be present. The merging of the two PDFs, hence, becomes the subtraction of one from the other, albeit weighted, as opposed to simply taking an intermediate value in the case of interpolation. Therefore, interpolated virtual networks are likely to be more reliable in terms of fidelity than extrapolated virtual networks. The PDF adjustment to account for the local marginal PDF in the SNESIM algorithm (step 4) allows the generated virtual network to match the user-defined global marginal PDF closer by compensating other structural properties [16]. The density structural metric is analogous to the global marginal PDF ICC probability and, hence, can be used to assess the degree to which the user-defined global marginal PDF is met. Although the PDF adjustment was not implemented in the modified SNESIM algorithm, the density metric values of the virtual networks still matched the user-defined global marginal PDF closely. This could be because the merge factor is computed based on the user-defined global marginal PDF and is then used to form the merged PDF for generating the virtual network. Therefore, the user-defined global marginal PDF is inherently present in the virtual network generation process.

The computational cost of using the modified SNESIM algorithm to generate virtual networks is approximately double that of using the SNESIM algorithm as there are two

training networks, and for each pixel simulated PDFs need to be retrieved from two search trees. However, with the current input parameters, virtual networks can still be generated quickly using both SNESIM and modified SNESIM. As these algorithms are capable of generating virtual networks of any size, experimentally obtained small-scale ICC network imaging data can be used to inform these algorithms to generate large-scale virtual ICC network imaging data at fields-of-view extending beyond the limitations presented by experimental imaging.

In this study, virtual ICC networks were generated across a spectrum of depletion severities by interpolating the PDFs drawn from the search trees of the training networks. This was an improvement from our previous strategy [13], which directly interpolated the search trees because in the previous strategy, if the pattern of pixels presented by the DSN during virtual network generation only appeared in one training network but not the other, the PDF used to simulate the pixel of interest was only drawn from the search tree corresponding to that training network regardless of the interpolation factor. It is, therefore, possible that pixel values are being simulated solely based on a training network, which is not similar to the desired network.

With the updated strategy employed in this study, the PDFs are drawn separately and then interpolated and, hence, both training networks are always considered appropriately according to the interpolation factor. We, therefore, also expect the updated algorithm to generate more realistic virtual networks than previously described [13].

Various studies have attempted to explain and predict GI electrical activity in health and disease states by utilizing computer models. Since the 1960s, when the pacemaker role of ICC was still unclear, GI electrical activity has been modeled as a series of coupled Van der Pol relaxation oscillators [27]. This concept was further expanded to demonstrate entrainment in a network of bi-directionally coupled series of relaxation oscillators [28], and was used to simulate the effects of partial cuts in GI organs on the electrical activity [29]. More recently, a cellular automaton model was developed to investigate the effects of tissue degradation on the overall GI electrical activity propagation, with the degradation being modeled as randomly distributed nodes throughout the simulation grid [26]. Although these studies were insightful in simulating GI electrical activity under a variety of conditions, they lacked the underlying electrophysiological basis of ICC. The generation of virtual ICC networks proposed here can establish a more sophisticated model of GI tissue and, therefore, offers potential for the previous studies to be repeated and extended in a more physiologically realistic manner.

Simulation studies using experimentally imaged ICC networks have successfully related ICC network structure to function [30]. This framework can be further enhanced by incorporating the virtual network generation algorithms, such that the simulations are not limited to the small spatial scale of the experimental data. The augmented framework can then be used as a virtual platform to investigate ICC structure–function relationships in, for example, gastroparesis and slow-transit constipation where multiple cellular pathologies and competing theories coexist [5], [10]. Another potential application of the virtual network generation algorithms is to generate large-scale networks to inform multiscale models [31],

[32], which, for instance, can be used to investigate the mechanisms of conduction slowing and dysrhythmias, recently observed in a high-resolution electrical mapping study of gastroparetic patients with ICC depletion [6].

As future work, the modified SNESIM algorithm can be validated against experimental imaging data of ICC networks in obstruction. During obstruction, ICC networks exhibit a gradient of depletion in the oral direction from the site of obstruction [33], and hence, ICC network imaging data can be obtained at various depletion levels throughout this gradient. The networks at the extremes of the spectrum (i.e., normal and severely depleted) can be used as the training networks to the modified SNESIM to test if virtual networks generated at intermediate depletion severities have similar structural and functional properties to those obtained experimentally. Following validation, the algorithm may also be further adapted to generate large-scale virtual networks, which in fact capture the transition in network properties through space. This additional functionality can reveal a holistic view of, for example, the natural spatial gradients present along the curvature of the stomach [34].

In summary, this paper validated the efficacy of using the SNESIM algorithm to generate realistic virtual ICC networks, and presented modifications to the algorithm to enable virtual networks with a range of structural and functional properties to be generated. These algorithms offer an alternative strategy for obtaining comprehensive imaging datasets encompassing large-scale ICC networks across a spectrum of depletion levels. The algorithms can now be applied in modeling studies to elucidate the structure–function relationships of ICC depletion in GI motility disorders.

ACKNOWLEDGMENT

The authors would like to thank Dr. S. Strebelle and Prof. A. Journel for making the SNESIM algorithm source code available, and Dr. G. Farrugia and Dr. S. Gibbons for providing the training ICC network imaging data. The authors would also like to thank the contribution of the NeSI high-performance computing facilities at the University of Auckland and the staff at NeSI and Center for eResearch.

This work was supported in part by the Riddet Institute, New Zealand Health Research Council, and the National Institutes of Health under Grant R01 DK64775. The work of J. Gao was supported by the University of Auckland Health Research Doctoral Scholarship, the Freemasons Postgraduate Scholarship, and the R. H. T. Bates Postgraduate Scholarship. The work of L. K. Cheng was supported by the Fraunhofer-Bessel Research Award from the Alexander von Humboldt Foundation and the Fraunhofer-IPA.

APPENDIX

TABLE II

Key Parameters of the ICC Model (for Reinitializing the State Variables, see [24] for Details)

State variables	Value	Units
Ca_i	1.00314e−5	millimolar
d_{Ltype}	8.21294e−6	dimensionless
f_{Ltype}	9.40876e−1	dimensionless
f_{ca_Ltype}	1.00000e+0	dimensionless
d_{VDDR}	9.80477e−4	dimensionless

State variables	Value	Units
f_{VDDR}	5.67153e-1	dimensionless
d_{CaCl}	3.67480-4	dimensionless
d_{ERG}	2.00000e-1	dimensionless
d_{kv11}	3.98797e-3	dimensionless
f_{kv11}	9.97147e-1	dimensionless
d_{Na}	1.37236e-2	dimensionless
f_{Na}	1.81419e-1	dimensionless
d_{NSCC}	5.04356e-3	dimensionless
PU_unit-Ca _{PU}	7.54078e-5	millimolar
PU_unit-Ca _m	2.30866e-4	millimolar
PU_unit-Ca _{ER}	3.22894e-3	millimolar
PU_unit-ADP _m	2.60102e+0	millimolar
PU_unit-ADP _i	7.72883e-3	millimolar
PU_unit-NADH _m	1.01697e-1	millimolar
PU_unit-h	9.39673e-1	dimensionless
PU_unit-deltaPsi	1.63999e+2	millivolt

References

1. Farrugia G. Interstitial cells of Cajal in health and disease. *Neurogastroenterol Motil.* 2008; 20:54–63. [PubMed: 18402642]
2. Ward SM, et al. Mutation of the proto-oncogene c-kit blocks development of interstitial cells and electrical rhythmicity in murine intestine. *J Physiol.* 1994; 480:91–97. [PubMed: 7853230]
3. Huizinga JD, et al. W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. *Nature.* 1995; 373:347–349. [PubMed: 7530333]
4. Huizinga JD, et al. Physiology, injury and recovery of interstitial cells of Cajal: Basic and clinical science. *Gastroenterology.* 2009; 137:1548–1556. [PubMed: 19778538]
5. Grover M, et al. Cellular changes in diabetic and idiopathic gastroparesis. *Gastroenterology.* 2011; 140:1575–1585. [PubMed: 21300066]
6. O'Grady G, et al. Abnormal initiation and conduction of slow-wave activity in gastroparesis, defined by high-resolution electrical mapping. *Gastroenterology.* 2012; 143:589–598. [PubMed: 22643349]
7. Isozaki K, et al. Deficiency of c-kit+ cells in patients with a myopathic form of chronic idiopathic intestinal pseudo-obstruction. *Am. J. Gastroenterol.* 1997; 92:332–334. [PubMed: 9040218]
8. Feldstein AE, et al. Chronic intestinal pseudoobstruction associated with altered interstitial cells of Cajal networks. *J Pediatr Gastroenterol Nutr.* 2003; 36:492–497. [PubMed: 12658043]
9. Lyford GL, et al. Pan-colonic decrease in interstitial cells of Cajal in patients with slow transit constipation. *Gut.* 2002; 51:496–501. [PubMed: 12235070]
10. Wedel T, et al. Enteric nerves and interstitial cells of Cajal are altered in patients with slow-transit constipation and megacolon. *Gastroenterology.* 2002; 123:1459–1467. [PubMed: 12404220]
11. Gibbons, SJ., et al. *New Advances in Gastrointestinal Motility Research.* New York, NY, USA: Springer-Verlag; 2013. ICC network density: Regulation and consequences; p. 29-49.
12. Tharayil VS, et al. Lack of serotonin 5-HT_{2B} receptor alters proliferation and network volume of interstitial cells of Cajal in vivo. *Neurogastroenterol Motil.* 2010; 22:462–469. [PubMed: 19941613]
13. Gao J, et al. A stochastic multi-scale model of electrical function in normal and depleted ICC networks. *IEEE Trans. Biomed. Eng.* 2011 Aug; 58(12):3451–3455. [PubMed: 21843981]

14. Strebel, S.; Journel, A. Reservoir modeling using multiple-point statistics. presented at the SPE Annu. Tech. Conf. Exhib.; New Orleans, LA, USA. 2001.
15. Strebel S. Conditional simulation of complex geological structures using multiple-point statistics. *Math. Geol.* 2002; 34:1–21.
16. Liu Y. Using the Snesim program for multiple-point statistical simulation. *Comput. Geosci.* 2006; 32:1544–1563.
17. Arpat, GB. Ph.D. dissertation. Stanford, CA, USA: Dept. of Petroleum Eng., Stanford University; 2005. Sequential simulation with patterns. [Online]. Available: <https://pangea.stanford.edu/ERE/pdf/pereports/PhD/Arpat05.pdf>
18. Wouters MM, et al. Exogenous serotonin regulates proliferation of interstitial cells of Cajal in mouse jejunum through 5-HT_{2B} receptors. *Gastroenterology*. 2007; 133:897–906. [PubMed: 17854596]
19. Gao J, et al. Numerical metrics for automated quantification of interstitial cell of Cajal network structural properties. *J R Soc Interface*. 2013; 10:20130421. [PubMed: 23804441]
20. O'Grady G, et al. Rapid high-amplitude circumferential slow wave propagation during normal gastric pacemaking and dysrhythmias. *Neurogastroenterol Motil.* 2012; 24:e299–e312. [PubMed: 22709238]
21. van Helden DF, et al. Generation and propagation of gastric slow waves. *Clin. Exp. Pharmacol. Physiol.* 2010; 37:516–524. [PubMed: 19930430]
22. Gao J, et al. Developmental changes in postnatal murine intestinal interstitial cell of Cajal network structure and function. *Ann. Biomed. Eng.* 2014; 42:1729–1739. [PubMed: 24866568]
23. Corrias A, Buist ML. Quantitative cellular description of gastric slow wave activity. *Am. J. Physiol. Gastrointest Liver Physiol.* 2008; 294:G989–G995. [PubMed: 18276830]
24. Sathar S, et al. A biophysically based finite-state machine model for analyzing gastric experimental entrainment and pacing recordings. *Ann. Biomed. Eng.* 2013; 42:858–870. [PubMed: 24276722]
25. Mirams GR, et al. Chaste: An open source C++ library for computational physiology and biology. *PLoS Comput. Biol.* 2013; 9:e1002970. [PubMed: 23516352]
26. Lammers WJ, et al. Slow wave propagation and plasticity of interstitial cells of Cajal in the small intestine of diabetic rats. *Exp. Physiol.* 2011; 96:1039–1048. [PubMed: 21742753]
27. Nelsen TS, Becker JC. Simulation of the electrical and mechanical gradient of the small intestine. *Am. J. Physiol.* 1968; 214:749–757. [PubMed: 5642936]
28. Sarna SK, et al. Simulation of slow-wave electrical activity of small intestine. *Am. J. Physiol.* 1971; 221:166–175. [PubMed: 5555782]
29. Sarna SK, et al. Effects of partial cuts on gastric electrical control activity and its computer model. *Am. J. Physiol.* 1972; 223:332–340. [PubMed: 5046751]
30. Du P, et al. Tissue-specific mathematical models of slow wave entrainment in wild-type and 5-HT(2B) knockout mice with altered interstitial cells of Cajal networks. *Biophys. J.* 2010; 98:1772–1781. [PubMed: 20441740]
31. Buist ML, et al. A model of slow wave propagation and entrainment along the stomach. *Ann. Biomed. Eng.* 2010; 38:3022–3030. [PubMed: 20437204]
32. Du P, et al. A multiscale model of the electrophysiological basis of the human electrogastragram. *Biophys. J.* 2010; 99:2784–2792. [PubMed: 21044575]
33. Chang IY, et al. Loss of interstitial cells of Cajal and development of electrical dysfunction in murine small bowel obstruction. *J Physiol.* 2001; 536:555–568. [PubMed: 11600689]
34. Burns AJ, et al. Interstitial cells of Cajal mediate inhibitory neurotransmission in the stomach. *Proc. Nat. Acad. Sci. USA.* 1996; 93:12008–12013. [PubMed: 8876253]

Fig. 1.
Flowchart of the specific steps taken by the SNESIM algorithm.

Fig. 2.

The three WT and three KO training networks, and corresponding virtual networks generated from each training network using the SNESIM algorithm. The white regions represent the ICC network. Each network is 362×362 pixels, and corresponds to physical dimensions of 0.225×0.225 mm. Scale bar (bottom right) = 0.1 mm.

Fig. 3.
The percentage errors in the structural metric values of the WT and KO virtual networks compared to their training counterparts.

Fig. 4.

The percentage errors in the functional measure values of the WT and KO virtual networks compared to their training counterparts.

Fig. 5.

Example virtual networks generated across a spectrum of depletion levels using the modified SNESIM algorithm. The white regions represent the ICC network. The headings along the left denote the training networks used to generate the virtual networks (i.e., WT1×KO1 denotes that the WT1 and KO1 training networks were used to generate the virtual networks in that row), whereas the headings along the top indicate the global marginal PDF ICC probabilities used to generate the virtual networks in that column. Each network is 362×362 pixels, and corresponds to physical dimensions of 0.225×0.225 mm. The WT and KO training networks correspond approximately to global marginal PDF ICC probabilities of 0.55 and 0.40 respectively, and hence virtual networks generated at global marginal PDF ICC probabilities within this range were interpolated, whereas those generated outside this range were extrapolated. Scale bar = 0.1 mm.

Fig. 6.

The structural metric values of the virtual networks generated using the modified SNESIM algorithm against the global marginal PDF ICC probabilities they were generated at. Crosses represent the average metric value of the nine virtual networks generated at each of the global marginal PDF ICC probabilities.

Fig. 7.

The functional measure values of the virtual networks generated using the modified SNESIM algorithm against the global marginal PDF ICC probabilities they were generated at. Crosses represent the average measure values of the nine virtual networks generated at each of the global marginal PDF ICC probabilities.

TABLE I

Terminology Associated With PDFS

Terminology		Description
Local		Associated with only the pixels which have already been simulated.
Global		Associated with all pixels in the image. Opposite of Local.
Conditional		Dependent upon the values of other pixels.
Marginal	Relates to only which category (ICC or non-ICC) a pixel should belong independent of the values of other pixels. Opposite of Conditional.	