Actin droplet machine

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Abstract

The actin droplet machine is a computer model of a three-dimensional network of actin bundles developed in a droplet of a physiological solution, which implements mappings of sets of binary strings. The actin bundle network is conductive to travelling excitations, i.e. impulses. The machine is interfaced with an arbitrary selected set of \(k\) electrodes through which stimuli, binary strings of length \(k\) represented by impulses generated on the electrodes, are applied and responses are recorded. The responses are recorded in a form of impulses and then converted to binary strings. The machine’s state is a binary string of length \(k\): if there is an impulse recorded on the \(i\)th electrode, there is a ‘1’ in the \(i\)th position of the string, and ‘0’ otherwise. We present a design of the machine and analyse its state transition graphs. We envisage that actin droplet machines could form an elementary processor of future massive parallel computers made from biopolymers.

Keywords: actin network, computing, waves, logical gates, finite state machine, automata

1 Introduction

Actin is a protein presented in forms of monomeric, globular actin (G-actin) and filamentous actin (F-actin) \([34, 18, 38]\). G-actin polymerises into filamentous actin forming a double helical structure \([35, 19, 13]\). The filaments can be further arranged into bundles by various different mechanisms such as crowding effects, cross-linking or counterion condensation \([16, 45, 7, 29, 30, 37, 36, 14, 15]\). The bundles are conductive to travelling localisations — defects, ionic waves, solitons \([11, 42, 13, 10, 22, 14, 25, 27, 26, 17]\). By interpreting presence or absence of a travelling localisation at a given site of the network at a given time step, we can implement a logical function. This approach was comprehensively developed and successfully tested on chemical systems in the framework of collision-based computing \([41, 16, 8, 39, 49, 2]\).
Our approach — computing with excitation waves propagating on overall ‘density’ of the conductive material — has previously been presented by us in [5]. As conductive material we looked at networks of actin bundles which were arranged by crowding effects without the need of additional accessory proteins [29, 30]. We demonstrated how to discover logical gates on a two-dimensional slice of the actin bundle network by representing Boolean inputs and outputs as spikes of the network activity.

In the present paper we develop a novel concept and computer modelling implementation of the actin network machine, which implements a mapping \( F : \{0, 1\}^k \rightarrow \{0, 1\}^k \), where \( k \) is a number of electrodes, and ‘1’ signifies a presence of an impulse on the electrode and ‘0’ the absence. At a higher level, the machine acts as a finite state machine, at the lower level a structure of the mapping \( F \) is determined by interactions of impulses propagating on the three-dimensional network of actin bundles.

We also offer an alternative to a numerical integration used in [5]: an automaton model of a three-dimensional actin network. There is a substantial body of evidence confirming that automaton models are sufficient and appropriate discrete tools for modelling dynamics of spatially extended non-linear excitable media [21, 10, 47], propagation [20], action potential [48, 6], electrical pulses in the heart [28, 9, 33]. A major advantage of automata is that they require less computational resources than typical numerical integration approaches.

The paper is structured as follows. Our modelling approach is described in detail in Sect. 2. This includes a representation of a three-dimensional actin bundle network (Subsect. 2.1), a structure of an automaton model to simulate propagation of impulses on the actin bundle network (Subsect. 2.2), and an interface with the actin network (Subsect. 2.3). In Sect. 3 we analyse dependencies of a number of Boolean gates implemented in the network on an excitation threshold and refractory period. Thus, we justify the selection of these parameters for the construction of the actin machine. The actin droplet machine is designed and analysed in Sect. 4. Section 5 discusses the results in a context of cytoskeleton computing and outlines directions for future research.

2 Methods

The overall approach is the following: we simulate the actin bundle network using three-dimensional arrays of finite-state machines, cellular automata. We select several domains of the network and assign them as inputs and outputs. We represent Boolean logic values with spikes of electrical activity, which are schematically represented as a virtual experiment in Fig. 1. We stimulate the network with all possible configurations of input strings and record spikes on the outputs. Based on the mapping of configurations of input spikes to output spikes, we reconstruct logical functions implemented by the network. In our design of the actin droplet machine we consider outputs recorded on all electrodes at a given time step as a binary string and then represent the actin droplet machine as a finite-state machine whose states are binary strings of a given length.

2.1 Three-dimensional actin network

As a template for our actin droplet machine we used an actual three-dimensional actin bundle network produced in laboratory experiments with purified proteins (Fig. 2). The underlying experimental method was shown to reliably produce regularly spaced bundle networks from homogeneous filament solutions inside small isolated droplets in the absence
Figure 1: A scheme of a virtual experiment. The actin bundle network is shown as a three-dimensional Delaunay triangulation. Electrodes are shown by thick lines and labelled $E_1$ to $E_5$. Exemplary trains of spikes are shown near the electrodes.

of molecular motor-driven processes or other accessory proteins [15]. These structures effectively form very stable and long-living three-dimensional networks, which can be readily imaged with confocal microscopy resulting in stacks of optical two-dimensional slices (Fig. 2). Dimensions of the network are the following: size along $x$ coordinate is 225 $\mu$m (width), along $y$ coordinate is 222 $\mu$m (height), along $x$ coordinate is 112 $\mu$m (depth), voxel width is 0.22 $\mu$m, height 0.22 $\mu$m and depth 4 $\mu$m.

Original image: $A_z = (a_{ijz})_{1 \leq i,j \leq n, 1 \leq z \leq m}$, where $n = 1024$, $m = 30$, $r_{ijz}, g_{ijz}, b_{ijz}$ are RGB values of the element at $ijz$, 1 $\leq r_{ijz}, g_{ijz}, b_{ijz}$ $\leq 255$ was converted to a conductive matrix $C = (c_{ijz})_{1 \leq i,j \leq n, 1 \leq z \leq m}$ as follows: $c_{ijz} = 1$ if $r_{ijz} > 40$, $g_{ijz} > 19$ and $b_{ijz} > 19$. The conductive matrices are shown in Fig. 3. The 3D conductive matrix is compressed along $z$-axis to reduce consumption of computational resources, scenario of the non-compressed matrix will be considered in future papers.

2.2 Automaton model

To model activity of an actin bundle network we represent it as an automaton $A = (C, Q, r, h, \theta, \delta)$. $C \subset \mathbb{Z}^3$ is a set of voxels, or a conductive matrix $C$ defined in Sect. 2.1. Each voxel $p$ in $C$ takes states from the set $Q = \{\star, \bullet, \circ\}$, excited ($\star$), refractory ($\bullet$), resting ($\circ$) and is complemented by a counter $h_p$ to handle the temporal decay of the refractory state. Following discrete time steps, each voxel $p$ updates its state depending on its current state and the states of its neighbourhood $u(p) = \{q \in C : d(p,q) \leq r\}$, where $d(p,q)$ is an Euclidean distance between voxels $p$ and $q$; $r \in \mathbb{N}$ is a neighbourhood radius. $\theta \in \mathbb{N}$ is an excitation threshold and $\delta \in \mathbb{N}$ is refractory delay. All voxels update
Figure 2: Exemplary $z$-slices of a three-dimensional actin bundle network reconstructed as described in [15].
Figure 3: Exemplary z-slices of ‘conductive’ geometries $C$ selected from the three-dimensional actin bundle network shown in Fig. 2, which were reconstructed as described in [15].
Table 1: Coordinates of electrodes in experiments family $E_1$.

| $e$ | $i$   | $j$   | $z$ |
|-----|-------|-------|-----|
| 1   | 369   | 567   | 6   |
| 2   | 509   | 580   | 10  |
| 3   | 631   | 590   | 10  |
| 4   | 382   | 322   | 12  |
| 5   | 533   | 331   | 23  |
| 6   | 626   | 463   | 7   |
| 7   | 358   | 676   | 22  |
| 8   | 369   | 424   | 7   |
| 9   | 572   | 691   | 17  |
| 10  | 705   | 394   | 17  |

Table 2: Coordinates of electrodes in experiments family $E_2$.

| $e$ | $i$   | $j$   | $z$ |
|-----|-------|-------|-----|
| 1   | 369   | 567   | 6   |
| 2   | 509   | 580   | 10  |
| 3   | 631   | 590   | 10  |
| 4   | 382   | 322   | 12  |
| 5   | 533   | 331   | 23  |
| 6   | 626   | 463   | 7   |
| 7   | 358   | 676   | 22  |
| 8   | 369   | 424   | 7   |
| 9   | 572   | 691   | 17  |
| 10  | 705   | 394   | 17  |

table 1: Coordinates of electrodes in experiments family $E_1$.

table 2: Coordinates of electrodes in experiments family $E_2$.

their states in parallel and by the same rule:

$$p^{t+1} = \begin{cases} 
\ast, & \text{if } (p^t = \circ) \text{ and } (\sigma(p)^t > \theta) \\
\bullet, & \text{if } (p^t = \circ) \text{ or } ((p^t = \bullet) \text{ and } (h^t_p > 0)) \\
\circ, & \text{otherwise}
\end{cases}$$

$$h^{t+1}_p = \begin{cases} 
\delta, & \text{if } (p^{t+1} = \bullet) \text{ and } (p^t = \ast) \\
h^t_p - 1, & \text{if } (p^{t+1} = \bullet) \text{ and } (h^t_p > 0) \\
0, & \text{otherwise}
\end{cases}$$

Every resting ($\circ$) voxel of $C$ excites ($\ast$) at the moment $t + 1$ if a number of its excited neighbours at the moment $t$, $\sigma(p)^t = |\{q \in u(p) : q^t = \ast\}|$, exceeds a threshold $\theta$. An excited voxel $p^t = \ast$ takes the refractory state $\bullet$ at the next time step $t + 1$ and at the same moment a counter of refractory state $h_p$ is set to the refractory delay $\delta$. The counter is decremented, $h^{t+1}_p = h^t_p - 1$ at each iteration until it becomes 0. When the counter $h_p$ becomes zero the voxel $p$ returns to the resting state $\circ$. For all results shown in this manuscript, the neighbourhood radius was set to $r = 3$. Choices of $\theta$ and $\delta$ are considered in Sect. 3.

### 2.3 Interfacing with the network

To stimulate the network and to record activity of the network we assigned several domains of $C$ as electrodes. We calculated a potential $p^t_x$ at an electrode location $c \in C$ as $p^t_x = |z : d(c,z) < r_e$ and $z^t = +|$, where $d(c,z)$ is an Euclidean distance between sites $x$ and $z$ in 3D space. We have chosen an electrode radius of $r_e = 4$ voxels and conducted two families of experiments with two configurations of electrodes.

In the first family of experiments $E_1$ we studied frequencies of two-input-one-output Boolean functions implementable in the network. We used ten electrodes, their coordinates are listed in Tab. 2.3 and a configuration is shown in Fig. 4(a). Electrodes $E_0$ representing input $x$ and $E_9$ representing input $y$ are the input electrodes, all others are output electrodes representing outputs $z_1, \ldots, z_8$. Results are presented in Sect. 3. In the second family of experiments $E_2$ we used six electrodes (Tab. 2.3 and Fig. 4(b)). All electrodes were considered as inputs during stimulation and outputs during recording of the network activity.
Figure 4: Configurations of electrodes in the three-dimensional network of actin bundles used in (a) $E_1$ and (b) $E_2$. Depth of the network is shown by level of grey. Sizes of the electrodes are shown in perspective.

Exemplary snapshots of excitation dynamics on the network are shown in Fig. 5. Domains corresponding to the two electrodes $e_0$ and $e_9$ (Tab. 2.3 and Fig. 4(a)) have been excited (Fig. 5(a)). The excitation wave fronts propagate away from $e_0$ and $e_9$ (Fig. 5(b)). The fronts traverse the whole breadth of the network (Fig. 5(c)). Due to the presence of circular conductive paths in the network, the repetitive patterns of activity emerge (Fig. 5(d)). Videos of the experiments can be found in http://doi.org/10.5281/zenodo.2649293.

3 Frequencies of gates

To map dynamics of the network onto sets of gates, we undertook the following trials of stimulation

1. fixed refractory delay $\delta = 20$ and excitation threshold $\theta = 4, 5, \ldots, 12$,
2. fixed excitation threshold $\theta = 7$, and refractory delay $\delta = 10, 15, 17, \ldots, 24, 30$.

For each combination $(\rho, \theta)$ we counted numbers of gates OR, AND, XOR, NOT-AND, AND-NOT and SELECT. We found that in overall a total number of gates $\nu(\theta)$ realised by the network decreases with increase of $\theta$ (Fig. 6(a)). The function $\nu(\theta)$ is non-linear and could be adequately described by a five degree polynomial. The function reaches its maximal value at $\theta = 7$ (Fig. 6(a)). OR gates are most commonly realised at $\theta = 11$, AND gates at $\theta = 6$ and xor gates at $\theta = 5$ as well as $\theta = 7$ (Fig. 6(b)). A number of AND-NOT gates implemented by the network reaches its highest value at $\theta = 6$ then drops sharply after $\theta_8$ (Fig. 6(c)). NOT-AND gates are more common at $\theta = 5, 7, 9, 11$, while SELECT(x) has its peak at $\theta = 7$ and SELECT(y) at $\theta = 8, 9$ (Fig. 6(c)). A total number of gates realised in the network with the excitability threshold fixed to $\theta = 7$ decreases with the
Figure 5: Snapshots of excitation dynamics on the network. The excitation wave front is red and the refractory tail is magenta. The excitation threshold is $\theta = 7$ and the refractory delay is $\delta = 20$. 
Figure 6: An average number $\nu$ of gates realisable on each of the electrodes $e_1, \ldots, e_8$ depends on threshold $\theta$ of excitation when the refractory delay $\delta$ is fixed to 20 (abc) and on refractory delays $\delta$ when the threshold $\theta$ is fixed to 7 (def). (a) Number of gates $\nu$ versus threshold $\theta$, $\delta = 20$. (b) Number of OR (black circle), AND (orange solid triangle) and XOR (red blank triangle) gates, $\delta = 20$. (c) Number of NOT-AND (yellow blank triangle), AND-NOT (magenta solid triangle), SELECT($x$) (cyan blank rhombus), SELECT($y$) (light blue disc), $\delta = 20$. (d) Number of gates $\nu$ versus delay $\delta$, $\theta = 7$. (e) Number of OR (black circle), AND (orange solid triangle) and XOR (red blank triangle) gates, $\theta = 7$. 
Figure 7: All spikes recorded at each electrode for input binary strings from 1 to 63. The representation is implemented as follows. We stimulate the $\mathcal{M}$ with strings from $\{0, 1\}^6$ and represent a spike detected at time $t$ by a black pixel at position $t$ along horizontal axis. A plot of each electrode $e_i$ represents a binary matrix $S = (s_{zt})$, where $1 \leq z \leq 63$ and $1 \leq t \leq 1000$: $s_{zt} = 1$ if the input configuration was $z$ and a spike was detected at moment $t$, and $s_{zt} = 1$ otherwise.

increase of $\delta$. Oscillations of $\nu(\delta)$ are visible at $15 \leq \delta \leq 25$ (Fig. 6(d)). The three highest values of $\nu(\delta)$ are achieved at $\delta = 10, 17$ and $20$. Let us look now at the dependence of the numbers of OR, AND and XOR gates of the refractory delay $\delta$ in Fig. 6(e). The number of OR gates increases with $\delta$ increasing from 10 to 15, but then drops substantially at $\delta = 18$ to reach its maximum at $\delta = 19$. Numbers of gates AND and XOR behave similarly to each other. They both have a pronounced peak at $\delta = 20$ (Fig. 6(e)). Thus, to maximise a number of logical gates produced and their diversity we selected $\theta = 7$ and $\delta = 20$ for our construction of the actin droplet machine.

4 Actin droplet machine

An actin droplet machine is defined as a tuple $\mathcal{M} = (\mathcal{A}, k, \mathcal{E}, S, F)$, where $\mathcal{A}$ is an actin network automaton, defined in Sect. 2.2, $k$ is a number of electrodes, $\mathcal{E}$ is a configuration of electrodes, $S = \{0, 1\}^k$, $F$ is a state-transition function $F : S \rightarrow S$ that implements a mapping between sets of all possible configurations of binary strings of length $k$. In the experiments reported here $k = 6$.

In our experiments we have chosen six electrodes, their locations are shown in Fig. 4(b) and exact coordinates in Tab. 2.3. Thus, $F : \{0, 1\}^6 \rightarrow \{0, 1\}^6$ and the machine $\mathcal{M}$ has 64 states. We represent the inputs and the machine states in decimal encoding. Spikes detected in response to every input from $\{0, 1\}^6$ are shown in Fig. 7.

Global transition graphs of $\mathcal{M}$ for selected inputs are shown in Fig. 8. Nodes of the graphs are states of $\mathcal{M}$, edges show transitions between the states. These directed graphs are defined as follows. There is an edge from node $a$ to node $b$ if there is such $1 \leq t \leq 1000$ that $\mathcal{M}^t = a$ and $\mathcal{M}^{t+1} = b$.

Let us now define a weighted global transition graph $\mathcal{G} = (Q, E, w)$, where $Q$ is a set of nodes (isomorphic to the $\{0, 1\}^k$), and $E$ is a set of edges, and weighting function $w : E \rightarrow [0, 1]$ assigning a number of a unit interval to each edge. Let $a, b \in Q$ and $e(a, b) \in E$ then a normalised weight is calculated as $w(e(a, b)) = \frac{\sum_{s,t \in T} \chi(s^t = a \text{ and } s^{t+1} = b)}{\sum_{e \in Q, t \in T} \sum_{s \in T} \chi(s^t = a \text{ and } s^{t+1} = d)}$, with $\chi$
Figure 8: State transitions of machine $\mathcal{M}$ for selected inputs $I$. A node is a decimal encoding of the $\mathcal{M}$ state $(e_0^I \ldots e_5^I)$. 
Figure 9: (a) Global graph of $\mathcal{M}$ state transitions. Edge weights are visualised by colours: from lowest weight in orange to highest weight in blue. (b) Pruned global graph of $\mathcal{M}$: only transitions with maximum weight for any given predecessors are shown, each node/state has at most one outgoing edge.
Figure 10: Graph of $g$ at $t = 41$.

The function $g$ takes value ‘1’ when the conditions are true and ‘0’ otherwise. In words, $w(e(a, b))$ is a number of transitions from $a$ to $b$ observed in the evolution of $\mathcal{M}$ for all possible inputs from $\mathcal{Q}$ during time interval $\mathbf{T}$ normalised by a total number of transition from $a$ to all other nodes. The graph $\mathcal{G}$ is visualised in Fig. 9(a). Nodes which have predecessors are 1–6, 8–10, 12, 16–21, 24, 25, 28, 32–34, 36–38, 40, 41, 44, 48–50, 52, 53, 56. Nodes without predecessors are 7, 11, 13–15, 22, 23, 26, 27, 29–31, 35, 39, 42, 43, 45–47, 51, 54, 55, 57–63.

Let us convert $\mathcal{G}$ to an acyclic non-weighted graph of more likely transitions $\mathcal{G}^*(\mathcal{Q}, \mathcal{E}^*)$, where $e(a, b) \in \mathcal{E}^*$ if $w(e(a, b)) = \max\{w(e(a, c))|e(a, c) \in \mathcal{E}\}$. That is for each node we select an outgoing edge with maximum weight. The graph is a tree, see Fig. 9(b).

Most states apart of 1, 2, 4, 8, 16, 20, 32 are Garden-of-Eden configurations, which have no predecessors. Indegrees $\nu()$ of not-Garden-of-Eden nodes are $\nu(20) = 1, \nu(32) = 2, \nu(2) = 3, \nu(4) = 4, \nu(1) = 5, \nu(16) = 6, \nu(8) = 12$. There is one fixed point, the state 1, corresponding to the situation when a spike is recorded only on electrode $e_5$; it has no successors.

By analysing $\mathcal{G}$ we can characterise a richness of $\mathcal{M}$’s responses to input stimuli. We define a richness as a number of different states over all inputs, as shown in Tab. 3 and distribution in Fig. 11(a). A number of states produced increases from under five for beginning of $\mathcal{M}$ evolution and then reaches circa seven states in average. Oscillations around this value are seen in (Fig. 11(a)). Figure 11(b) shows a number of different nodes, generated in evolution of $\mathcal{M}$, stimulated by a given input. There is below fifteen different states found in the evolution in responses to inputs 1 to 21 (21 corresponds to binary input string 010101); then a number of different nodes stay around 25. The diagram Fig. 11(c) shows how many inputs might lead to a given state/node of $\mathcal{M}$. Some of the states/nodes are seen to be Garden-of-Eden configurations $\mathcal{E}$ (nodes without predecessors) and thus could not be generated by stimulating $\mathcal{M}$ by sequences from $\mathcal{Q} - \mathcal{E}$.

Assume $\mathbf{T}$ is a set of temporal moments when the machine responded at least to one input string with a non-zero state. Configurations at each transition $t$ can be considered as outputs representing the function $g : 0, 1^6 \to 0, 1^6$. As we can see in Tab. 3 transitions at $t = 41$ and $t = 53$ correspond to the highest number of different binary strings ($e_1, \ldots, e_6$).
| $t$ | $\mu(t)$ | $P(t)$ |
|-----|-----------|--------|
| 1   | 3         | 8, 9, 1 |
| 2   | 3         | 16, 8, 8 |
| 3   | 3         | 1, 16, 32 |
| 4   | 3         | 8, 1, 16 |
| 5   | 3         | 1, 8, 16 |
| 6   | 3         | 16, 8, 1 |
| 7   | 4         | 8, 1, 16, 4 |
| 8   | 4         | 1, 16, 8, 5 |
| 9   | 5         | 16, 1, 8, 4, 5 |
| 10  | 4         | 16, 1, 8, 4 |
| 11  | 5         | 8, 1, 16, 20, 4 |
| 12  | 4         | 1, 16, 8, 20 |
| 13  | 6         | 16, 8, 1, 17, 4, 20 |
| 14  | 8         | 8, 16, 17, 4, 20, 1, 32, 2 |
| 15  | 8         | 1, 16, 8, 4, 2, 10, 20, 32 |
| 16  | 6         | 16, 4, 8, 1, 10, 32 |
| 17  | 5         | 16, 1, 4, 8, 9 |
| 18  | 7         | 8, 16, 4, 1, 17, 10, 9 |
| 19  | 6         | 1, 8, 16, 17, 4, 10, 20 |
| 20  | 8         | 16, 1, 8, 17, 4, 24, 10, 2 |
| 21  | 9         | 8, 16, 1, 17, 32, 24, 9, 4, 10 |
| 22  | 6         | 16, 1, 8, 32, 9, 4 |
| 23  | 7         | 8, 1, 16, 4, 32, 9, 17 |
| 24  | 6         | 1, 16, 17, 4, 32, 8 |
| 25  | 7         | 16, 1, 8, 4, 17, 32, 9 |
| 26  | 6         | 8, 16, 4, 12, 1, 17 |
| 27  | 6         | 1, 8, 16, 4, 17, 32 |
| 28  | 6         | 16, 8, 4, 1, 24, 32 |
| 29  | 7         | 8, 1, 4, 16, 12, 24, 32 |
| 30  | 7         | 16, 1, 8, 4, 17, 2, 32 |
| 31  | 9         | 8, 1, 24, 16, 12, 4, 2, 17, 32 |
| 32  | 7         | 1, 16, 8, 24, 17, 2, 40 |
| 33  | 9         | 16, 8, 1, 4, 40, 17, 24, 32, 2, 34 |
| 34  | 7         | 8, 1, 16, 24, 40, 4, 32 |
| 35  | 6         | 1, 16, 8, 4, 24, 2 |
| 36  | 6         | 16, 8, 1, 17, 4, 32 |
| 37  | 7         | 8, 16, 17, 4, 1, 40, 2 |
| 38  | 7         | 1, 8, 16, 17, 4, 24, 2 |
| 39  | 7         | 16, 1, 8, 17, 9, 4, 2 |
| 40  | 7         | 8, 16, 4, 1, 24, 40, 2 |
| 41  | 10        | 1, 8, 16, 9, 17, 4, 18, 24, 40, 2 |
| 42  | 8         | 16, 1, 8, 4, 18, 33, 40, 24 |
| 43  | 9         | 8, 1, 16, 4, 24, 33, 18, 32, 34 |
| 44  | 9         | 1, 16, 8, 4, 17, 33, 24, 32, 40 |
| 45  | 7         | 16, 8, 4, 1, 12, 24, 34 |
| 46  | 7         | 8, 1, 16, 4, 24, 18, 34 |
| 47  | 5         | 1, 16, 8, 4, 33 |
| 48  | 5         | 16, 8, 1, 4, 17 |
| 49  | 8         | 8, 1, 16, 8, 4, 20, 32, 24, 19 |
| 50  | 6         | 1, 16, 8, 4, 17, 32 |
| 51  | 8         | 16, 8, 1, 4, 17, 32, 41, 19 |
| 52  | 9         | 8, 16, 4, 1, 32, 33, 41, 2, 19 |
| 53  | 10        | 1, 8, 16, 4, 20, 10, 2, 41, 32, 19 |
| 54  | 9         | 16, 1, 8, 5, 17, 4, 2, 32, 19 |

Table 3: Fifty four state transitions of $\mathcal{M}$ over all possible inputs: $t$ is a transition step, $\mu(t)$ is a number of different states appeared over all possible inputs, $P(t)$ is a set of nodes appeared at $t$. 
The graph corresponding to \( g(41) \) at \( t = 41 \) is shown in Fig. 10 and is not connected. The small component consists of fixed point 40 (string ‘101000’) with two leafs 39 (‘100111’) and 38 (‘100110’). The largest component has a tree structure at large, with cycle 2 (‘000010’) – 1 (‘000001’) as a root. Other nodes with most predecessors are 8 (‘001000’), 16 (‘010000’), and 18 (‘010010’).

From the transitions \( g(41) \) we can reconstruct Boolean functions realised at each of six electrodes (the functions are minimised and represented in a disjunctive normal form):

\[\begin{align*}
e_0 : f_0(x_0, \ldots, x_5) &= x_0 \cdot \overline{x_1} \cdot x_2 \cdot x_3 + x_0 \cdot x_1 \cdot \overline{x_2} \cdot x_3 \cdot \overline{x_4} + x_0 \cdot \overline{x_1} \cdot x_2 \cdot \overline{x_3} \cdot \overline{x_4} + x_0 \cdot \overline{x_1} \cdot \overline{x_2} \cdot x_3 \cdot \overline{x_4} \\
e_1 : f_1(x_0, \ldots, x_5) &= x_0 \cdot \overline{x_1} \cdot x_2 \cdot x_3 + x_0 \cdot \overline{x_1} \cdot x_2 \cdot \overline{x_3} \cdot \overline{x_4} + x_0 \cdot \overline{x_1} \cdot x_2 \cdot \overline{x_3} \cdot \overline{x_4} + x_0 \cdot \overline{x_1} \cdot \overline{x_2} \cdot x_3 \cdot \overline{x_4} \\
e_2 : f_2(x_0, \ldots, x_5) &= x_0 \cdot \overline{x_1} \cdot x_2 \cdot x_3 + x_0 \cdot \overline{x_1} \cdot x_2 \cdot \overline{x_3} \cdot \overline{x_4} + x_0 \cdot \overline{x_1} \cdot x_2 \cdot \overline{x_3} \cdot \overline{x_4} + x_0 \cdot \overline{x_1} \cdot \overline{x_2} \cdot x_3 \cdot \overline{x_4} \\
e_3 : f_3(x_0, \ldots, x_5) &= x_0 \cdot \overline{x_1} \cdot x_2 \cdot x_3 + x_0 \cdot \overline{x_1} \cdot x_2 \cdot \overline{x_3} \cdot \overline{x_4} + x_0 \cdot \overline{x_1} \cdot x_2 \cdot \overline{x_3} \cdot \overline{x_4} + x_0 \cdot \overline{x_1} \cdot \overline{x_2} \cdot x_3 \cdot \overline{x_4} \\
e_4 : f_4(x_0, \ldots, x_5) &= x_0 \cdot \overline{x_1} \cdot x_2 \cdot x_3 + x_0 \cdot \overline{x_1} \cdot x_2 \cdot \overline{x_3} \cdot \overline{x_4} + x_0 \cdot \overline{x_1} \cdot x_2 \cdot \overline{x_3} \cdot \overline{x_4} + x_0 \cdot \overline{x_1} \cdot \overline{x_2} \cdot x_3 \cdot \overline{x_4} \\
e_5 : f_5(x_0, \ldots, x_5) &= x_0 \cdot \overline{x_1} \cdot x_2 \cdot x_3 + x_0 \cdot \overline{x_1} \cdot x_2 \cdot \overline{x_3} \cdot \overline{x_4} + x_0 \cdot \overline{x_1} \cdot x_2 \cdot \overline{x_3} \cdot \overline{x_4} + x_0 \cdot \overline{x_1} \cdot \overline{x_2} \cdot x_3 \cdot \overline{x_4} 
\end{align*}\]
5 Discussion

Early concepts of sub-cellular computing on cytoskeleton networks as microtubule automata [12, 24, 11] and information processing in actin-tubulin networks [23] did not specify what type of ‘computation’ or ‘information processing’ the cytoskeleton networks could execute and how exactly they do this. We implemented several concrete implementations of logical gates and functions on a single actin filament [32] and on an intersection of several actin filaments [31] via collisions between solitons. We also used a reservoir-computing-like approach to discover functions on a single actin unit [3] and filament [4]. Later, we realised that it might be unrealistic to expect someone to initiate and record a travelling localisations (solitons, impulses) on a single actin filament. Therefore, we developed a numerical model of spikes propagating on a network of actin filament bundles and demonstrated that such a network can implement Boolean gates [5].

In present paper, we reconsidered the whole idea of the information processing on actin networks and designed an actin droplet machine. The machine is a model of a three-dimensional network, based on an experimental network developed in a droplet, which executes mapping $F$ of a space of binary strings of length $k$ on itself. The machine acts as a finite state machine, which behaviour at a low level is governed by localisations travelling along the networks and interacting with each other. By focusing on a single element of a string, i.e. a single location of an electrode, we can reconstruct $k$ functions with $k$ arguments, as we have exemplified at the end of the Sect. 4. Exact structure of each $k$-ary function is determined by $F$, which, in turn, is determined by the exact architecture of a three-dimensional actin network and a configuration of electrodes.

Thus, potential future directions could be in detailed analysis of possible architectures of actin networks developed in laboratory experiments and evaluation on how far an exact configuration of electrodes affects a structure of mapping $F$ and corresponding distribution of functions implementable by the actin droplet machine. The ultimate goal would be to implement actin droplet machines in laboratory experiments and to cascade several machines into a multi-processors computing architecture.

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**Author contributions statements**

A.A., F.H., J.S. undertook the research and wrote the manuscript.

**Competing interests**

The authors declare that they have no competing interests.