CHAPTER 4

Live fluorescent imaging of F-actin organization in chick whole embryo cultures using SiR-actin

Manuel Schmitz
Grazvydas Lukinavicius
Theodoor H. Smit

(In preparation for Methods and Applications in Fluorescence)
ABSTRACT

Key to understanding morphogenetic processes is to study them in vivo. Changes in tissue shape are initiated on cellular level as the architecture and contractility of intracellular F-actin networks determine shape and motility of cells, influence differentiation and cytokinesis and mediate mechanical signaling. Here, we present the first protocol to use the new fluorogenic probe SiR-actin to stain F-actin in vitro in whole embryo cultures of chicken embryos for live fluorescence imaging. We determined a concentration of 50 nM SiR-actin in the culture medium as optimal for live fluorescent imaging of early chicken embryos, as it provides high labelling density without occurrence of morphological abnormalities during development. Additionally, we used SiR-actin in combination with our custom-made embryos stretching setup (CHAPTER 3) and show some first results indicating that daughter somite formation is initiated on the level of somitic actin organization prior to the complete organization of the mother somite.

INTRODUCTION

Embryogenesis is a complex process of tissue formation, driven by the tightly coordinated behavior of numerous cells. Next to proliferation and programmed cell death, this involves shape changes and relative movements of cells [1]. Shape and motility of a cell, but also mechanotransduction, differentiation and cytokinesis are mainly determined by the architecture and contractility of intracellular filamentous actin (F-actin) networks [2]–[4]. In fixated samples, F-actin is often visualized by fluorescently labelled phalloidin, a filament binding toxin of low molecular weight, assumed “to provide the most complete and accurate picture of the actin cytoskeleton” [5]–[7]. Phalloidin circumvents possible disadvantages of antibody-based labeling techniques, such as non-specific binding, actin scavenging, high background or epitope variations between actin species [8], [9]. However, key to a deeper understanding of morphogenetic processes on cell and tissue level is to study them dynamically in vivo. Until recently, actin dynamics could be visualized in vivo after loading cells with fluorescently labelled actin and phalloidin via microinjection, by using the “bead loading” method [10]–[12] or after transfection with genetically encoded actin reporters.
like Lifeact and GFP-actin [13]. However, each of these approaches comes with disadvantages: microinjection of fluorescently labelled actin is technically demanding and phalloidin binding disturbs actin dynamics by filament stabilization. Additionally, microinjection is a low throughput method and involves temporal disruption of the cell plasma membrane. The bead loading method relies on temporal mechanical disruption of the plasma membrane and is only applicable to cell monolayers grown on glass slides [12]. Genetically encoded actin reporters turned out to be biased with respect to their inability to stain different F-actin species equally well [7].

Recently, a new powerful probe has been introduced to overcome many of the mentioned hurdles in fluorescent imaging of actin dynamics in living cells: SiR-actin [14] is a combination of the fluorophore silicon-rhodamine (SiR) [15] and a synthetic and less cytotoxic derivative of jasplakinolide [16], a naturally occurring cyclic peptide known to bind F-actin competitively with phalloidin [17]. Unlike fluorescent phalloidin derivatives, SiR-actin is cell permeable. It is biocompatible through its excitation and emission in the near-infrared and disturbs the cell’s actin dynamics only minimally. It functions as bright and photo stable fluorogenic probe, increasing its fluorescence intensity 100-fold upon binding to F-actin in vitro [14]. Thereby, SiR-actin provides a high signal-to-noise ratio, without the need for additional washing steps before in vivo imaging. So far, SiR-actin has been successfully used for high resolution (and super-resolution) fluorescence microscopic imaging and has, for example, helped to reveal the ubiquitous subcortical cytoskeleton periodicity in the nervous system [18], [19] and the dynamic nature of the actin cytoskeleton in mammalian red blood cells [20]. The list of possible applications is constantly being extended, but as of yet, SiR-actin has not been successfully used for live fluorescent imaging of F-actin in whole embryo cultures.

We could successfully fill this gap by adding SiR-actin to the culture medium of early chick embryos grown in the ‘submerged filter paper sandwich’ [21], a variant of the well-established filter paper carrier technique [22]. As described in CHAPTER 2, this new technique allows for the culturing of the embryos fully submerged in the culture medium. As the medium surrounds the embryo at all times, our idea was that it could function as reservoir for SiR-actin throughout the embryo’s development and allow the dye to constantly bind to F-actin without further manipulations or washing. The use of
SiR-actin was of special interest to our work as a follow-up to our stretching experiments on live chicken embryos. Until then, we could visualize the F-actin organization of stretched somites undergoing daughter somite formation only in fixated samples after immunohistochemical staining (compare Figure 2 in CHAPTER 3). As these fixated samples contained somites during different stages of daughter somite formation, we were able to generate a potential temporal order of the reorganization process. However, two essential questions could not be answered from these still images: 1. How and where does the apical actin cortex of stretched somites rupture and how exactly do the mesenchymal cells of the somitocoel react to this new epithelial-mesenchymal interphase? 2. Do daughter somites only form from somites that already have a complete apical actin cortex or does the organization into smaller daughter somites already happen earlier, during the onset of epithelialization and cell polarization in the anterior tip of the PSM?

Using the static version of the submerged filter paper sandwich (CHAPTER 2), we determined a SiR-actin concentration of 50nM as optimal for live fluorescent imaging of early chicken embryos, as it provides high labelling density without occurrence of morphological abnormalities, such as head malformations or incomplete somite separations which can be caused by interference of the fluorescent probe with actin dynamics. Subsequently, we executed stretch experiments (as described in CHAPTER 3) in presence of SiR-actin and present some first results.

MATERIALS AND METHODS

Culture medium
We prepared the culture medium as a mixture of Pannett-Compton (PC) -saline [23], [24] and freshly harvested thin albumen, as described before [21], but reduced the thin albumen percentage from 40% to 2%. Preliminary experiments had shown that SiR-actin uptake into the embryonic tissue was restrained in presence of high percentages of thin albumen. Presumably, most SiR-actin molecules were retained the culture medium by unspecific interaction with proteins, which make up about 10% of the thin albumen volume in the egg [25] and made up about 4% of the volume in our original
culture medium. Here, Ovalbumin (54%), Ovotransferrin (12%) and Ovomucoid (11%) are the most prevalent proteins. The reduction of thin albumen percentage to 2% did not impair embryonic development, as shown below. Penicillin/Streptomycin (10000 U/ml) in 100x dilution were added to prevent bacterial infections. SiR-actin was purchased from tebu-bio BV, Heerhugoward (NL), stored as 1 mM stock solution with anhydrous DMSO at -20°C and, after thawing, added to the culture medium in varying concentrations.

**Submerged filter paper sandwich**
Fertilized chicken eggs (*Gallus gallus*, Drost BV, Loosdrecht, NL) were incubated at 37.5°C in a humidified atmosphere for 32-36 hours and subsequently transferred into submerged filter paper sandwiches [21]. In short, embryos were clamped between two small sheets of thick filter paper with a central aperture. Thereby, the filter paper surrounded the embryo like a picture frame from both sides. In the static version, explants were cultured in 6 cm petri dishes, stabilized by stainless steel rings and kept fully submerged in the culture medium at all times [21] (see also Figure 1 in **CHAPTER 2**). For stretching experiments, filter paper sandwiches were clamped into our custom-made embryo stretching setup and incubated in the presence of SiR-actin in the culture medium (**CHAPTER 3**). Where possible, the culture medium was covered with a layer of light mineral oil to prevent evaporation.

**SiR-actin live fluorescence microscopy**
Long-term darkfield microscopic and widefield fluorescent time-lapse imaging was realized using a long working distance, upright zoom microscope (Zeiss Axio Zoom V.16), equipped with a PlanNeoFluar Z 1.0x objective, a Cy-5 filter cube (excitation wavelength filter 625-655 nm, emission wavelength filter 665-715nm) and a Hamamatsu ORCA Flash 4.0 camera. Time lapse images were acquired at 5 min intervals. 16-bit grayscale images of 2048 x 2048 pixels were acquired at different magnifications of the zoom objective. The tiles module of the Zeiss Zen software was used to acquire panorama images with a 10% overlap of single tiles. Single tiles were stitched afterwards using the Zen software.
240nM low magnification (Figure 1 A, B):
Zoom 2.5, single tile acquired, image size 2048 x 2048 pixels, pixel size 2.6 µm x 2.6 µm, exposure time Cy5 channel 450 ms, darkfield channel 3.5ms.

240nM high magnification (Figure 1 I):
Zoom 11.2, two tiles (10% overlap) acquired, resulting image size 3880 x 2052 pixels, pixel size 0.580 µm x 0.580 µm, exposure time Cy5 channel 250 ms, darkfield channel 25 ms.

50nM low magnification (Figure 1 D, E):
Zoom 3.1, three tiles images (10% overlap) acquired, resulting image size 5735 x 2048 pixels, pixel size 2.08 µm x 2.08 µm, exposure time Cy5 channel 600ms, darkfield channel 5ms.

50nM high magnification (Figure 1 K):
Zoom 7.8, single tile acquired, image 2048x2048 pixels, pixel size 0.832 µm x 0.832 µm, exposure time Cy5 channel 2.61s, darkfield channel 19.4ms.

Stretch timelapse (Figure 3):
Zoom 7.8, three tiles acquired (10% overlap), image size 5735 x 2048 pixels, pixel size 0.832 µm x 0.832 µm, exposure time Cy5 channel 1 s, darkfield channel 12 ms.

Confocal imaging in vivo (Figure 2 B, C, D):
Images were acquired using an upright confocal microscope (Leica SP5), either using a 10x air objectives (NA=0.40) or a 63x water immersion objective (NA=0.90). Due to the short working distance of the objective, images were acquired without a layer mineral oil on top of the medium. Exact settings of the confocal microscope can be found in the supplementary material (Table S1 and S2).

SiR-actin and Phalloidin costaining in fixated samples (Figure 2 A):
Embryos were transferred into filter paper sandwiches [21] and carefully washed in PBS. Then, embryos (still in filter paper sandwiches) were fixated overnight in 4% PFA in PBS at 4 °C. After washing in PBS (3x 5 min), embryos were permeabilized in 1% Triton-X-100 in PBS for minimum 1.5 hrs. After washing (3x 5min), embryos were cut out of the filter paper carriers, transferred to 1.5 ml Eppendorf tubes and incubated for 1.5 hrs with
250 nM SiR-actin and 250nM Phalloidin (Alexa Fluor™ 555 Phalloidin, Thermo Fisher Scientific) and 0.1 % Triton-X-100 in PBS. After washing (3 x 5 min), embryos were mounted on microscope slides with a drop of mounting solution (ProLong™ Gold Antifade Mountant, Thermo Fisher Scientific) and sealed with a cover slip. A layer of parafilm with a rectangular aperture was used as spacer between microscope slide and coverslip. Samples were imaged on an inverted spinning disc confocal microscope (Nikon Ti2-E Eclipse + Yokogawa CSU WI confocal scanner unit), equipped with a Nikon CFI Plan Apochromat Lambda 20x air objective (NA = 0.70) and an Andor iXon-Ultra-888 Back illuminated EMCCD camera. Images were acquired with a depth of 16 bit and had a size of 1024 x 1024 pixels (pixel size 0.65 µm x 0.65 µm). Laser power was set to 10 % (561 Cobolt Jive 200 mW and 639 Toptica iBeam Smart 500 mW), a gain of 300 and an exposure time of 50 ms was used for both channels.

RESULTS

**Proof of principle - increasing fluorescence signal while development progresses**

Figure 1 shows selected time-lapse frames of two chicken embryos, cultured ex ovo as submerged filter paper sandwiches [21] in the presence of SiR-actin at different two different concentrations: 50 and 240 nM. A young embryo, cultured in the presence of 240 nM SiR-actin, starting from the 1-3-somite-stage (HH7 to 8- [26], Fig. 1 A and B), progressed its development towards the 10-somite stage (HH10) over 12 hours of incubation ([Movie SI Young embryo 240nM SiR-actin](#)). Simultaneously, the SiR-actin signal continuously increased in strength. The staining started with a spotted appearance of medial parts of the early embryonic heart, probably due to its high morphogenetic activity and its accessibility for the dye. Then, the embryonic midline with the highly contractile cells of the closing neural tube and the remains of the primitive streak became visible. And finally, the somites, segmenting from the presomitic mesoderm on both sides of the embryonic midline, were stained specifically for F-actin, indicating that it takes about 5-7 hours for sufficient amounts of SiR-actin to penetrate approximately 100 µm into the tissue (Figure 1 B). Pre-incubation overnight, in the presence of 240 nM SiR-actin, allowed to acquire time-lapse images of somite formation at higher resolution ([Movie S2 240 nM SiR-actin high magnification](#)). Here
we could observe the gradual formation of the apical actin cortex in the newly forming somites, starting from the posterior and progressing anteriorly.

However, we found that young chick embryos, cultured in the presence of 240 nM SiR-actin for more than 10 hours, revealed morphological abnormalities in the form of incompletely separated somites (white asterisk in Figure 1 I), indicating that SiR-actin can disturb the actin machinery. Therefore, we decided to reduce the SiR-actin concentration in the culture medium considerably by about 80 %. We incubated older embryos (8-somite stage, HH9 to 9+) with 50nM SiR-actin for more than 28 hours and did not observe any malformations (Figure 1 D and E, Movie S3_older embryo 50nM SiR-actin). In fact, comparison of average somite formation time between experimental embryos and controls did not indicate any impairment of developmental speed (79 ± 7 min in presence of 50 nM SiR-actin and 86 ± 5 min in controls, n=3 for each, average ± SD, Table S3).

The SiR-actin signal at 50nM was hardly visible at lower magnification (Figure 1 E), but image acquisition at higher magnification, and therefore higher numerical aperture of the zoom objective used (change in NA from 0.12 to 0.24, see Figure S1), revealed an intense and highly specific staining of somitic F-actin (Figure 1 K).

To compare the temporal development of the SiR-actin fluorescence signal at the two different concentrations, we acquired line intensity profile plots (along white dashed lines in Figure 1 B and E) and determined the integrated fluorescence intensity originating from the embryonic neural tube at different time points. Figure 1 G presents the results as normalized intensities. The fluorescence signal behaves very similarly for both SiR-actin concentrations: An initial linear increase of fluorescence leads to a peak period followed by a gradual decrease. Linear fitting of the initial incubation phase shows that the fluorescence increases much quicker for 240 nM SiR-actin (ca. 21 %/h) compared to 50 nM SiR-actin (ca. 7 %/h). During the decrease phase, the fluorescence intensity drops to 60-70% of the maximum value. This could partly be due to the overall aging and thickening of the embryonic tissue at the selected region, causing additional scattering of the fluorescent signal. We also determined the relation between fluorescent signal and background by calculating the ratio between integrated fluorescent intensities of vertebral tube (Figure 1 C and F) and averaged background value (dashed
cyan line in Figure 1 B and E) for both SiR-actin concentrations. Figure 1 H shows that the signal-to-background ratio (SBR) increases steadily for both concentrations over the first hours of incubation. However, while the SBR reaches a maximum for 240 nM SiR-actin after approx. 6 hours of incubation and decreases thereafter, it keeps increasing for 28 hours of incubation with 50nM SiR-actin. Higher magnification imaging results into similar signal and background intensities for both SiR-actin concentrations (Figure 1 J and L), where preference should be given to lower concentrations to avoid malformations (Figure 1 I).

Figure 1: Embryonic development and gradual increase of SiR-actin fluorescence in chick embryos cultured ex ovo, representative images. Time-points indicate incubation time with
SiR-actin. Anterior is always to the left. Scale bars, 1 mm (A, B, D, E), 250 µm (I, K). A Darkfield (DF) microscopic time-lapse frames of 3-somite stage embryo (HH7), cultured in presence of 240 nM SiR-actin. Development progressed up to 10-somite stage (HH10) during 12 hours of incubation. B Corresponding widefield fluorescence (WF) time-lapse frames of embryo shown in A. Dashed lines indicate line ROI used to measure fluorescence intensities of embryonic midline (white) and surrounding structures (background, cyan). C Line intensity profile plots across embryonic midline as indicated in B, selected time points. Central peak originates from neural tube fluorescence, lateral peaks from somites. Plots are aligned for the central peak. D DF images of 8-somite stage embryo (HH9 to 9+), incubated with 50 nM SiR-actin. Development progressed up to the 21-somite stage (HH13-14) over 28 hours of incubation. E Corresponding WF frames of the embryos shown in D. Fluorescence signal builds up slower than and not as strong as in B. F Line intensity profile plots across the embryonic midline as indicated in E. Plots are aligned to the single peak originating from the fluorescence of neural tube. G Temporal development of the normalized integrated intensity of fluorescence originating from neural tube at 240 nM and 50 nM SiR-actin concentration. Linear fitting of initial phase shows: fluorescence increases by 21 %/h (240 nM) and 7 %/h (50nM). H Temporal development of signal-to-background ratio (SBR) for different SiR-actin concentrations. SBR was calculated as ratio between integrated intensity of midline fluorescence and averaged intensity of background fluorescence (white dotted line parallel to embryonic midline in B and E). I DF and WF-zoom of the posterior somitic mesoderm of embryo in A and B. Incomplete separation of somites indicated (*). J Line intensity profile plot along dashed lines in I. K DF and WF-zoom of the posterior somitic mesoderm of embryo in D and E. Regularly shaped somites and specific F-actin signal visible, showing that 50 nM SiR-actin is sufficient for imaging at higher magnifications. L Line intensity profile plot along the dashed lines indicated in K.

Confocal imaging of whole embryos in fixated samples and in vivo

To compare the staining characteristics of SiR-actin and Phalloidin, we performed a costaining of fixated chicken embryos with the same concentration of both probes (250 nM). We observed an almost perfect overlap between the two staining patterns (Figure 2 A), thereby confirming the usefulness of SiR-actin to visualize actin dynamics in vivo. To further explore the potential of our culture technique, we incubated live embryos with 50 nM SiR-actin overnight and imaged them, subsequently, on an upright confocal microscope. Figure 2 B and C shows representative micrographs, illustrating the performance of SiR-actin in labelling F-actin in live chick whole embryo cultures. We could image the F-actin localization during somite formation in overview images and at cellular resolution (Figure 2B and D), as well as the F-actin cortices of endodermal cells (Figure 2 C). Especially, the difference in F-actin polarization between the anterior and
the posterior part of somite S1, the somite that has just separated from the anterior tip of the unsegmented mesoderm, could be visualized (Fig 2 D).

Figure 2: Confocal SiR-actin imaging in fixated samples and in vivo. Representative micrographs, acquired using an inverted spinning disc confocal microscope (A) or an upright confocal microscope (B - D). Anterior is always to the left, ventral view. A Costaining of fixated chicken embryos with 250 nM SiR-actin and Alexa-555 conjugated Phalloidin showing an almost perfect overlap in staining. B Overview of segmenting paraxial mesoderm on both sides of the neural tube, in vivo staining with 50 nM SiR-actin. Somite numbers of newly forming somites
are indicated according to [27]. C F-Actin cortices of endodermal cells, in vivo staining. D High resolution micrograph of live embryo. Formation of the apical actin cortex in somite S1 is more progressed in the posterior half of the somite (white arrow).

**Live fluorescence time lapse imaging in combination with embryo stretching**

To address the open questions regarding the reorganization of the apical actin cortices in stretched somites undergoing daughter somite formation, we performed a stretching experiment (as described in Chapter 3) in the presence of 50 nM SiR-actin in the culture medium. Figure 3 shows the segmenting paraxial mesoderm of a stretched embryo at selected time points after the application of the second pull. In stretched somites undergoing daughter somite formation, we observed that the appearance of the somitic furrow that indicates the separation of the mother somite from the anterior tip of the PSM coincides with the concentration of F-actin into two centers. These centers initially are located very close to each other and separate thereafter (Movie S4_SiR-actin stretched embryo).

![Figure 3: F-actin organization during daughter somite formation.](image)

**Figure 3: F-actin organization during daughter somite formation.** DF and WF microscopic time lapse images of a stretched embryo incubated with 50 nM SiR-actin at selected time points after the end of pull protocol. A DF images showing the separation of the mother somite from the anterior tip of the PSM and its organization into two daughter somites (white asterisk). B WF images of the F-actin organization during daughter somite formation. Note that with the appearance of the somitic furrow visible in the DF image (A) at 6:10 hrs, already two actin centers are visible (white arrows). These are close next to each other and later separate from each other.
DISCUSSION

In summary, we established a simple protocol for staining F-actin in whole embryo cultures of early chick embryos using SiR-actin in combination with the ‘submerged filter paper sandwich’ [21]. The culture medium, surrounding the embryos at all times, functions as a reservoir for SiR-actin dye. Direct manipulations on embryos, like microinjection or electroporation, are dispensable for this staining protocol. By reducing the percentage of thin albumen in the culture medium, we successfully minimized its blocking effect on the SiR-actin uptake into the embryonic tissue. Thereby, we were able to use only small volumes of SiR-actin stock solution, making the protocol very cost effective. Using confocal microscopy, we could confirm that a high signal intensity and a specific staining pattern, very similar to the results obtained for fluorescently labelled phalloidin, was achieved. We showed that the SiR-actin concentration could be lowered sufficiently to avoid morphological abnormalities, like incompletely separated somites, while still delivering a good signal-to-noise ratio.

In a preliminary experiment, we used SiR-actin in combination with our custom-made embryo stretching setup. While the imaging modalities still have to be optimized, we already learned from this experiment that the reorganization of stretched somites into daughter somites is initiated at the level of somitic actin organization prior to the complete epithelialization. We observe that during daughter somite apical F-actin localizes in two discrete centers that first appear interconnected, but then gradually release from each other. This organization into two centers coincides with the appearance of the somitic furrow that indicates where the mother somite buds off the anterior tip of the PSM.

This supports our conclusion drawn in CHAPTER 3 that the organization of epithelializing cells into somites at the anterior tip of the PSM is a process of self-organization that is determined by the geometrical or mechanical boundary conditions of the segmenting tissue. While the position of the somitic furrow is most probably determined by the outcome of the genetic patterning of the clock and wavefront, the subsequent organization of the somitic rosettes can be guided by the mechanical microenvironment.
Technical and time limitations prevented the acquisition of time lapse images at confocal resolution so far. The upright confocal microscope we used was equipped only with a 10x air objective and a 63x water immersion objective. The working distance of the 10x objective was too small to image the embryo through a layer of mineral oil on top of the medium and imaging without this protective layer led to a quick loss of focus due to evaporation. In the small field of view of the 63x objective, on the other hand, the imaging was massively disturbed by the heartbeat of the embryo. But we are confident that water immersion objectives (ideally 10x or 20x), potentially in combination with light-sheet microscopy, will allow the acquisition of high resolution time lapse series.

We exemplarily showed that SiR-actin penetrates deep enough into the embryonic tissue to stain even the apical actin cortices of the somites. Other embryonic structures, like the early embryonic heart or the epithelial cells of the endoderm (Figure 2 C), are even more accessible to the dye and their imaging would suffer less from scattering of the fluorescent signal. Therefore, we think that our protocol holds large potential for the high resolution live imaging of many morphogenetic events during embryogenesis.
REFERENCES


SUPPLEMENTARY MATERIAL

Movie S1_Young embryo 240nM SiR-actin
Darkfield and widefield fluorescent time lapse movie of chicken embryo cultured in vitro in a submerged filter paper sandwich in the presence of 240nM SiR-actin in the culture medium. Developed progressed from the 3-somite stage embryo (HH7 up to 10-somite stage (HH10) during 12 hours of incubation.

Movie S1_Young embryo 240nM SiR-actin
Video URL:  https://youtu.be/DtfjRQWlqvg

Movie S2_240 nM SiR-actin high magnification
Formation of somites in chicken embryo, pre-incubated with 240nM SiR-actin overnight prior to imaging.

Movie S2_240 nM SiR-actin high magnification
Video URL:  https://youtu.be/6le4w6mynZI

Movie S3_older embryo 50nM SiR-actin
Darkfield and widefield fluorescent time lapse movie of chicken embryo cultured in vitro in a submerged filter paper sandwich in the presence of 50nM SiR-actin in the culture medium. Developed progressed from the 8-somite stage embryo (HH9 to 9+) up to 21-somite stage (HH13-14) during 28 hours of incubation.

Movie S3_older embryo 50nM SiR-actin
Video URL:  https://youtu.be/VBQlaEGPV20

Movie S4_SiR-actin stretched embryo
DF and WF microscopic time lapse images of the segmenting paraxial mesoderm in a stretched chicken embryo cultured in vitro in a submerged filter paper sandwich in presence of 50 nM SiR-actin. The movie starts after the end of stretching protocol.

Movie S4 SiR-actin stretched embryo
Video URL:  https://youtu.be/2b-gCWH-Gf0
Table S1: Microscope settings Leica SP5. Overview of settings used to acquire micrograph presented in Figure 2 B (left table) and Figure 2C (right table).

### Scanner Settings

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective</td>
<td>63x</td>
</tr>
<tr>
<td>Magnification</td>
<td>63x</td>
</tr>
<tr>
<td>Objective N.A.</td>
<td>1.4</td>
</tr>
<tr>
<td>Andor resurrection</td>
<td>95 x</td>
</tr>
<tr>
<td>Andor Magnification</td>
<td>3 nits</td>
</tr>
<tr>
<td>Andor Cauter</td>
<td>1</td>
</tr>
<tr>
<td>Andor Bandwidth</td>
<td>20 nits</td>
</tr>
<tr>
<td>Andor AC coupled</td>
<td>1</td>
</tr>
<tr>
<td>Andor Freeze Frame</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera X, Y, Z</td>
<td>5</td>
</tr>
<tr>
<td>Andor Camera A, B, C</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera D, E, F</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera G, H, I</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera J, K, L</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera M, N, O</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera P, Q, R</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera S, T, U</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera V, W, X</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera Y, Z, A</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera B, C, D</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera E, F, G</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera H, I, J</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera K, L, M</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera N, O, P</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera Q, R, S</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera T, U, V</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera W, X, Y</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera Z, A, B</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera C, D, E</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera F, G, H</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera I, J, K</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera L, M, N</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera O, P, Q</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera R, S, T</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera U, V, W</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera X, Y, Z</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera A, B, C</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera D, E, F</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera G, H, I</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera J, K, L</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera M, N, O</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera P, Q, R</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera S, T, U</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera V, W, X</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera Y, Z, A</td>
<td>1</td>
</tr>
</tbody>
</table>

### Beamline Settings

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective</td>
<td>63x</td>
</tr>
<tr>
<td>Magnification</td>
<td>63x</td>
</tr>
<tr>
<td>Objective N.A.</td>
<td>1.4</td>
</tr>
<tr>
<td>Andor resurrection</td>
<td>95 x</td>
</tr>
<tr>
<td>Andor Magnification</td>
<td>3 nits</td>
</tr>
<tr>
<td>Andor Cauter</td>
<td>1</td>
</tr>
<tr>
<td>Andor Bandwidth</td>
<td>20 nits</td>
</tr>
<tr>
<td>Andor AC coupled</td>
<td>1</td>
</tr>
<tr>
<td>Andor Freeze Frame</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera X, Y, Z</td>
<td>5</td>
</tr>
<tr>
<td>Andor Camera A, B, C</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera D, E, F</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera G, H, I</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera J, K, L</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera M, N, O</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera P, Q, R</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera S, T, U</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera V, W, X</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera Y, Z, A</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera C, D, E</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera F, G, H</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera I, J, K</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera L, M, N</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera O, P, Q</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera R, S, T</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera U, V, W</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera X, Y, Z</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera A, B, C</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera D, E, F</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera G, H, I</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera J, K, L</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera M, N, O</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera P, Q, R</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera S, T, U</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera V, W, X</td>
<td>1</td>
</tr>
<tr>
<td>Andor Camera Y, Z, A</td>
<td>1</td>
</tr>
</tbody>
</table>

- 110 -
Table S2: Microscope settings Leica SP5. Overview of settings used to acquire micrograph presented in Figure 2 D.

<table>
<thead>
<tr>
<th>Objective</th>
<th>Logical Size</th>
<th>Physical Length</th>
<th>Physical Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>21.2</td>
<td>205.51 pm</td>
<td>3 pm</td>
</tr>
<tr>
<td>Y</td>
<td>4.2</td>
<td>36.25 pm</td>
<td>8 pm</td>
</tr>
</tbody>
</table>

**Scanner Settings**

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BitShifter</td>
<td>0</td>
</tr>
<tr>
<td>ValveShutter</td>
<td>1</td>
</tr>
<tr>
<td>Excitation</td>
<td>Ar</td>
</tr>
<tr>
<td>Pinhole (um)</td>
<td>250</td>
</tr>
<tr>
<td>Zoom (um)</td>
<td>2.25</td>
</tr>
<tr>
<td>Slit (um)</td>
<td>234-32 pm</td>
</tr>
<tr>
<td>Slit (um)</td>
<td>215-52 pm</td>
</tr>
<tr>
<td>ScanDepth</td>
<td>0</td>
</tr>
<tr>
<td>Magnification</td>
<td>0.380 pm</td>
</tr>
<tr>
<td>Visual Width</td>
<td>0.097 m</td>
</tr>
<tr>
<td>Visual Height</td>
<td>0.095 m</td>
</tr>
<tr>
<td>Zoom</td>
<td>1</td>
</tr>
<tr>
<td>Iris Diaphragm</td>
<td>0.5</td>
</tr>
<tr>
<td>Aperture</td>
<td>0</td>
</tr>
<tr>
<td>Image-Average</td>
<td>1</td>
</tr>
<tr>
<td>Line-Average</td>
<td>1</td>
</tr>
<tr>
<td>Resolution</td>
<td>0.59 pm</td>
</tr>
<tr>
<td>Contrast</td>
<td>0.25</td>
</tr>
<tr>
<td>FrameHeight</td>
<td>256 pixels</td>
</tr>
<tr>
<td>FrameWidth</td>
<td>320 pixels</td>
</tr>
<tr>
<td>Line-Accumulation</td>
<td>1</td>
</tr>
<tr>
<td>Sections</td>
<td>1</td>
</tr>
</tbody>
</table>

- - III -
Table S3: Average somite formation time. Number of somites that were completely separated from the anterior tip of the PSM were counted at selected time-points during imaging. Darkfield microscopic time lapse images were used.

<table>
<thead>
<tr>
<th>Sample</th>
<th>SiR-actin concentration [nM]</th>
<th>No. somites formed</th>
<th>Elapsed time [min]</th>
<th>Average time/somite [min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>11</td>
<td>770</td>
<td>77</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>16</td>
<td>1300</td>
<td>86.7</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>19</td>
<td>1329</td>
<td>73.8</td>
</tr>
<tr>
<td>SiR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>14</td>
<td>1166</td>
<td>89.7</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>15</td>
<td>1130</td>
<td>80.7</td>
</tr>
<tr>
<td>3</td>
<td>240</td>
<td>9</td>
<td>711</td>
<td>88.9</td>
</tr>
</tbody>
</table>
Figure S1: Numerical aperture Zeiss PlanNeofluar 1.0x objective. NA of objective in dependence of zoom value as received as information from Zeiss (black line).