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SUPPLEMENTARY METHODS

Calibration and Alignment of Optical and Acoustical Imaging

Fluorescence imaging (optical mapping) was performed simultaneously with (either 2D or 3D/4D) ultrasound imaging. Fluorescence imaging was performed using either a panoramic imaging setup consisting of 4 cameras situated at a 90° degree angle around the heart (pig + 4D ultrasound) or using a monocular single-camera setup (rabbit + 2D ultrasound). With both the multi-camera and the single-camera setup, the cameras were aligned filming horizontally through the glass walls of an 8-sided aquarium, see Fig. 1c and Extended Data Fig. 2 and 3, such that the heart surface was filmed approximately co-planar to the CCD camera plane, see Fig. 1c and Extended Data Fig. 3. The 2D ultrasound probe beam was directed at the heart walls from the top scanning vertically into the bath and into the heart walls, the 2D imaging cross-section being aligned co-planar to the CCD camera plane. The 3D ultrasound probe beam was directed from underneath the bath at the heart, scanning vertically into the bath, scanning a pyramid-shaped volume. The main axis of the pyramid was aligned perpendicularly to the optical axis of the CCD cameras, see Fig. 1c and Extended Data Fig. 3 c.

The alignment of the two imaging modalities with respect to each other was determined either manually using photographs or by using an opto-acoustical calibration procedure

(Extended Data Fig. 2). The positions, optical axes and fields of views of the cameras could be determined using an optical 2D calibration target pattern, see Extended Data Fig. 2e, inserted into the bath before or after the experiment and a numerical calibration routine. The position, lateral axis and field of view of the 3D ultrasound probe was determined using a three-dimensional calibration grid, see Extended Data Fig. 2f. Both calibrations can be obtained with respect to a common coordinate system.

Computation of Measures of Deformation and Spatial-Temporal Strain-rate Patterns from Optical and Acoustical Motion Tracking Data

Deformation was computed from the tracked motion and computed displacement data for both optical fluorescence video data and 2D and 3D ultrasound data as follows: deformation is a relative change of tissue points or coordinates with respect to each other. Motion tracking yields the trajectories of such tissue coordinates through space. The trajectories can be reconstructed from displacement vector fields computed in between (2D or 3D) images. A change of the relative locations of the tracked tissue coordinates with respect to each other is accordingly a deformation or a change of the mechanical configuration of the tissue or non-rigid body motion. Neighboring tissue segments and their length changes with respect to each other were measured in every voxel or pixel of the imaging data or a subset of voxels or pixels of the imaging data (i.e. a pre-defined masked region showing the tissue only). Either instantaneous displacement vectors from one instance in time or image to the next were measured, or displacement vectors indicating the shifts of the tissue with respect to one particular reference image were measured. In case that a particular reference frame was chosen, differencing of the displacement vectors between consecutive frames yielded instanta-

neous displacement vectors from one frame to the next. For ultrasound data (2D and 3D) instantaneous displacement vectors were computed, whereas for optical data a reference frame was chosen.

In every voxel or pixel, we could then determine deformation tensors combining the displacement data and relative changes of the displacement data from neighbouring voxels or pixels. As the measured deformation data is a tensorial quantity (it contains the relative length changes of the components in all spatial directions in a second order tensor), it was required to simplify the tensorial data to scalar-valued data to be able to compare maps of deformation to maps of action potential wave spread. We therefore computed scalar-valued measures of deformation (red-blue colorcode with red indicating contractile and blue indicating tensile strain-rates) and compared those to maps of the electrical activity. More specifically, the gradient deformation tensors and the Green Lagrangian strain tensors were computed from the smoothed displacement vector field described above. The tensorial data (Green Lagrangian) was reduced to scalar-valued data calculating tensor invariants, i.e. the tensor trace, principal stretches or principal eigenvalues. The strain invariant (tensor trace) was normalized using a sliding-window normalization similarly as described for the optical data with a window width w of at least 1x the period of the dominant frequency and typically not more than 1.5-2.5 periods of the dominant frequency. The resulting time-varying two- or three-dimensional strain-rate map was smoothed in space and over time using spatial-temporal filter kernels with Gaussian profile (diameter d = 7 - 11 voxels / pixels, 3-5 frames linear averaging). All computational routines for extraction, processing and visualization of the ultrasound imaging data were custom-made routines written in C/C++, VTK and OpenGL.

SUPPLEMENTARY DISCUSSION

Potential Limitations of the Study

While there exist differences between *in vivo* and *ex vivo* (Langendorff), we have observed[15] the electrical behavior of cardiac fibrillation to be very similar *in vivo* and later *ex vivo* in the same heart. During fibrillation, we expect also the mechanics to be represented sufficiently well as the overall pumping forces are decreased, pressure and resistance changes are small and resistance-related effects on the electro-mechanical dynamics are likely to stay small. In the following we address the in our opinion most critical potential limitations of our study.

Degeneration of Electromechanical Coupling

The most critical limitations of our study are conditions or instances that can lead to a compromised excitation-contraction coupling mechanism or a decoupling between the electrical and mechanical dynamics. The degeneration of the cellular excitation-contraction coupling mechanism could lead to seemingly unrelated electrical and mechanical activity in the heart. In this case, mechanical activity as a surrogate for electrical activity is not valid. At least, a direct correlation between electrical and mechanical phase singularities, for instance, cannot be expected to be immediately existent. Instead it could be degenerated to a degree, that our imaging approach could not be used to measure, for instance, scroll or spiral waves. However, from a clinical standpoint, mechanical activity per se is a vital prerequisite that can be expected to be present in most patients. Moreover, a degenerated or unphysiologically altered coupling

between electrics and mechanics does not necessarily imply that it is in general impossible to gain insightful information on the electrophysiological state of the heart by using an imaging approach that analyzes the heart's mechanical activity. In the following, we would like to briefly discuss some of the potential complications that could impede or challenge the aim to directly use mechanical activity as a surrogate for electrical activity. For instance, it has been found that the bidirectional coupling between voltage and calcium dynamics can degenerate during ventricular fibrillation, such that depolarizations of the cardiac myocyte may not necessarily trigger intracellular calcium release[41,42]. Moreover, ryanodine receptors have been found to remain continuously refractory during ventricular fibrillation[44]. The refractoriness of ryanodine receptors prevents intracellular calcium release from the sarcoplasmic reticulum. With calcium mediating the contractions of cardiac myocytes, the refractoriness could potentially disrupt excitation-contraction coupling. Nevertheless, despite the absence of calcium release from the sarcoplasmic reticulum[44], the intracellular calcium concentration has also been found to exhibit a finite residual modulation[44], which is immediately coupled to the course of the action potential and exhibits an amplitude of about 30 % of the amplitude with intact calcium release from the sarcoplasmic reticulum. In all our measurements, we found that the fibrillating hearts were contracting substantially during ventricular fibrillation, which suggests that mechanical activity occurs even with refractory ryanodine receptors and that the residual modulation of intracellular calcium is responsible for the contractions during ventricular fibrillation. Therefore, refractory ryanodine receptors are unlikely to lead to a full decoupling between electrical and mechanical patterns and should not impede our imaging approach. On the other hand, calcium-related feedback mechanisms have been identified to trigger depolarizations of cardiac

mycoytes during early afterdepolarizations[43]. Such early afterdepolarizations could lead to substantially altered electromechanical dynamics and to seemingly uncorrelated electrical and mechanical wave patterns. Lastly, it is hypothesized that stretch-activated ion channels produce the phenomenon of electromechanical feedback, which in itself could alter the electromechanical coupling dynamics, such that macroscopic wave dynamics could become strongly altered as well[36]. While the circumstances and potential limitations described above do not principally invalidate our imaging approach, it is clear that they could restrict its applicability in the clinical setting.

Deviations from the *in vivo* Configuration

We aimed to mimic the *in vivo* imaging configuration in our *ex vivo* experimental Langendorff setup. During ultrasound examinations with patients, the human heart is either filmed from outside the body through the chest in a *transthoracic* echocardiography examination (TTE), or from within the body in a *transesophageal* echocardiography examination (TEE) using a transesophageal ultrasound probe. The first measurement provides a clearer picture of the ventricles, whereas the latter measurement provides a clearer picture of the atria. We aimed to mimic the TTE measurement filming the ventricles using a probe that is routinely used in TTE examinations. In a TTE measurement, the transducer is positioned on the chest below the heart with the field of view pointing upwards through the ribs or from underneath the ribs, see Extended Data Fig. 3 e. In the apical view, the apex of the heart is facing the transducer, being located closest to the transducer array. The atria are located furthest away from the transducer array. The imaging configuration used in our experiment is very similar to this situation. In our setup, the transducer is filming through an acoustical window from

underneath the heart, and the heart is aligned very similarly within the pyramid-shaped field of view as in the TTE examination (apical view). The size of the human heart is comparable to the size of the pig hearts used in the *ex vivo* experiments. A description of the imaging configurations can be found in Extended Data Fig. 3.

Another deviation from the *in vivo* measurement is that the hearts were done in a non-working heart Langendorff setup. In a non-working heart Langendorff setup, the heart pumps against a vanishing resistance compared to the working heart setup. This could alter the contractile dynamics of the heart significantly. However, if overall pulsatile pumping forces are strongly decreased, as it is the case during ventricular fibrillation, then pressure and resistance changes are small and resistance-related abnormal effects onto the electro-mechanical dynamics are likely to stay small as well. Therefore, we anticipate that our observations can similarly be found in *in vivo* conditions. We acknowledge existing concerns as to whether and how cardiac fibrillation is altered in non-working compared to working heart conditions and in an *ex vivo* compared to an *in vivo* situation. Nevertheless, while scroll waves are very likely to be the driving mechanism of cardiac fibrillation in any case, rotational electrical patterns should lead very generally to rotational mechanical patterns as long as the coupling between the two physical systems is intact. We anticipate that the electromechanical rotor phenomena, which we describe in this manuscript and which we observed in the isolated non-working heart, may have slightly different characteristics under varying conditions, but should principally represent a universal phenomenon. We anticipate that it will be possible to observe very similar phenomena in working hearts *ex vivo* as well as *in vivo*.

Another deviation from the *in vivo* imaging situation is that the heart is isolated and situated alone in the tank of the Langendorff setup in the absence of surrounding tis-

sue. The surrounding water facilitates post-processing of the imaging data, as the ultrasound beam does not get attenuated. This leads to an excellent image quality and maximal contrast of the tissue, which in turn facilitates the identification and separation of tissue from background and promotes segmenting the heart shape. However, we found that the tank introduces other acoustic artifacts, which are unlikely to be present in *in vivo* imaging data. The artifacts appear as stripes or other bright flare-like structures in the data and are presumably caused by acoustic reflections off of the walls of the tank. They can be minimized or entirely avoided by, firstly, aligning the heart properly inside the field of view of the transducer and, secondly, by using acoustic absorber materials on the walls of the tank. Remaining residual artifacts can be removed numerically post-acquisition. The artifacts are critical as they can impede motion tracking if present. Similar artifacts are absent in the *in vivo* imaging situation. In *in vivo* imaging data, surrounding tissue could lead to higher attenuation of the ultrasound beam and accordingly to lower image quality, which could impede motion tracking. Low image quality could also impede segmenting the heart shape properly. However, existing motion tracking and segmentation technology that is used routinely in commercial echocardiography systems shows that these factors are not fundamental limitations and can be addressed accordingly.

Another deviation from the *in vivo* imaging situation is the lacking support by surrounding body tissue in the tank. The heart is freely floating in water, being attached to the setup only at the aorta and can easily start to exhibit swinging and other bulk motion. Excessive bulk motion can complicate data analysis and could pose a generally unphysiological condition that could alter the overall electromechanical behavior of the heart, similarly as the non-working heart condition.

Spatial Resolution and Resolving Structural Features of Atria

The spatial resolution of the 3D ultrasound scanner that was used in our study is optimized for imaging the ventricles of human hearts. Resolving finer structures such as the atria or smaller hearts from children or small animals requires different ultrasound transducer hardware operating at higher carrier frequencies (>5 MHz). In principle, such transducers can be fabricated, but were not available within the scope of this study. Higher spatial resolutions would be necessary to resolve the thin walls and trabeculae of the atria. In principle, with higher spatial resolutions also arrhythmic activity in the atria could be imaged.

Characteristics of Mechanical Phase Singularity and Electromechanical Vortex Filaments

The filaments observed in numerical simulations (Fig. 2c,d) show paired electrical and mechanical filaments (co-aligned green and red lines) as well as additional mechanical filaments (single red lines), which are apparently not immediately paired with a particular electrical filament. Electromechanical vortex filaments appear to be composed of paired and unpaired electrical and mechanical filaments, the electrical and mechanical filaments not necessarily having to retain a one-to-one correspondence. We observed the emergence of additional unpaired mechanical filaments in our data. The filaments shown in Fig. 2c,d were obtained in computer simulations, in which mechano-electrical coupling was maintained at all times. Unpaired mechanical filaments or phase singularities emerge in 2D and 3D, even when mechano-electric coupling is maintained, due to

elastic inhomogeneities. Such mechanical inhomogeneities result from a combination of (1) muscle fiber anisotropy, (2) the passive elastic relaxational response of the tissue and (3) boundary effects. Extended Data Fig. 8 illustrates the emergence of additional unpaired mechanical phase singularities in a 2D electro-mechanic computer model. Extended Data Fig. 8 a-d shows the effect of muscle fiber anisotropy onto the morphology of the mechanical deformation pattern induced by a spiral wave. We found that, generally, the deformation patterns emerging during spiral wave activity (with isotropic conduction) are highly anisotropic due to muscle fiber anisotropy. They reflect the underlying isotropic spiral wave, but are deformed and align accordingly with the muscle fiber orientation (here shown for uniform horizontal vs. vertical linear transverse alignment). This effect can also be seen in Fig. 2d (see also Supplementary Video 3). Here, the muscle fibers, which are located close to the front surface of the bulk are aligned in parallel to the surface at an angle of approx. 45 degree to the horizontal / vertical plane (from top left to bottom right corner). The amplitude of the rate of deformation is smaller along the perpendicular direction to the fiber orientation as the wave propagates through the medium. Extended Data Fig. 8 e-h shows that elastic perturbations can lead to elastic inhomogeneities under these circumstances. In the presence of elastic perturbations, the mechanical deformation pattern that was directly caused by electrical activity may be altered in locations where the electromechanical wave propagates in perpendicular direction to the muscle fiber orientation and produces accordingly a weaker strain-rate amplitude. The weaker strain-rate signal is more likely to be affected and superimposed by other elastic activity. These perturbations can emerge due to the non-local nature of elastic deformation waves inside the active elasto-mechanical medium. Contractile activity can cause elsewhere deformations mediated

over long distances through the elastic medium. Extended Data Fig. 8 g shows accordingly that, when computing phase maps, additional unpaired mechanical phase singularities emerge further away from and outside of the spiral wave core in regions in which wave propagation occurs in perpendicular direction to the muscle fiber orientation. However, a co-localized pair of electrical and mechanical phase singularities emerges at the spiral wave core. This idealized situation translates to the 3D case and similar effects create the unpaired mechanical filaments shown in Fig. 2 d.

As a result in 3D, the degree of pairing and entanglement of electrical and mechanical filaments may fluctuate over time and may depend on factors such as (1) the alignment of the electrical filament with respect to the local muscle fiber orientation, the complexity of the electrical wave activity, the overall passive elastic response of the tissue. Mechanical filaments generally possess the same topological constraints as electrical filaments in that they can appear only as filaments touching with both their ends the medium boundary or as closed loops (Fig. 2 b).

The emergence of unpaired mechanical phase singularities in the simulations agrees with the observations that we made during VF on the heart surface in the experiments. We found consistently a greater number of mechanical phase singularities than electrical phase singularities (Extended Data Fig. 6 a-c). The emergence of unpaired mechanical phase singularities could also be a reason for a partially low degree of co-localization, which is indicated by the tail in the distributions displayed in Fig. 3g and 4d. The distributions show the average co-localization statistics of electrical and mechanical phase singularities measured on the heart surface. The tails indicate that yet

in many cases there is only little or no co-localization between electrical and mechanical phase singularities. Nevertheless, we anticipate that, in principle, unpaired mechanical filaments can be discriminated from paired mechanical filaments by distinct properties in their dynamics (higher motility, different topological behavior, such as lacking vorticity and reversal or change in topological charge, and different lifetimes), see Extended Data Fig. 8 e-g.

Entanglement of Electrical and Mechanical Vortex Filaments

Fig. 1 and 2a,b display elasto-mechanical vortex filaments, which were measured during ventricular tachycardia and fibrillation in pig hearts. Figure 2c,d display electromechanical vortex filaments, which were generated using computer simulations. We refer to the *entanglement* of these electromechanical vortex filaments, as the computer simulations suggest that electromechanical vortex filaments are composed of pairs of co-existing, partially co-aligned and co-localized electrical and mechanical vortex filaments, which each describe an individual electromechanical scroll wave. However, the entirety of filaments one finds in a fibrillating bulk of tissue is not only composed of pairs of electrical and mechanical filaments.

Figure 2d depicts a highly idealized situation, in which a single scroll wave rotates in an accordingly deforming bulk of tissue with rotational muscle fiber anisotropy. The scroll wave retains an electrical vortex filament that is accompanied by a mechanical filament with an almost identical alignment. In this case, the filament pair exhibits a high entanglement. Fig. 2c depicts a double scroll wave rotor with two scroll waves rotating on the opposite side of a rabbit heart. Each scroll wave can be associated with one electrical

and one mechanical filament, both indicating the scroll wave's rotational center and both emerging in the same region of the heart. Both filaments share the same alignment and topological charge. Nevertheless, there is a spatial offset between electrical and mechanical filament and the filaments do not co-localize perfectly. However, in both computer simulations mechano-electrical coupling was maintained. In the numerical models, the electrical activity was immediately converted into the development of active stress, homogeneously throughout and equally in each part of the tissue modulated by the local active stress. Due to the nature of cardiac mechano-electric coupling, electrical activation patterns do not necessarily translate one to one into corresponding elasto-mechanical patterns. Therefore, also electrical and mechanical filaments do not necessarily always have to be paired couples that show a high degree of entanglement, even when the excitation-contraction coupling is fully intact. We anticipate that the entanglement can fluctuate and is largely determined by long-range passive elastic effects. Eventually, long-range passive elastic responses of the tissue may pull mechanical filaments away from the electrical filament. The situation shown in Fig. 2d is an idealized situation, in which a volume of tissue is occupied by one electromechancical scroll wave. Accordingly, the deformations of the bulk are determined by this *one* scroll wave. As there are no other perturbations, the single scroll wave is able to generate a mechanical deformation pattern that retains a highly similar morphology. We anticipate that multiple scroll waves will influence each other and will perturb the other scroll wave's domain and its locally induced own scroll deformation pattern. We anticipate that this effect will become most pronounced during fibrillatory activity with large meandering rotors, which each activate a large portion of the heart and cause large deformations of the entire heart muscle.

Periodic Pacing of the Heart

Baseline measurements have been obtained during periodic pacing of a pig heart using a biopolar electrode. The location of the pacing electrode on the left-ventricular wall is shown in Extended Data Fig. 2a,b,g. Extended Data Fig. 2h shows the propagation of an excitation wave outwards at a pacing frequency of 3.6 Hz. Simultaneously to the measurement of membrane potential using optical mapping, 4D ultrasound imaging is used (Extended Data Fig. 2c,i). The electrode is flexible and touches continuously the heart surface as it deforms. Correspondingly, the videos show concentric electrical action potential and mechanical strain rate waves emanating from the stimulation site at a frequency of 3.6 Hz. Extended Data Fig. 3h,j shows a series of action potential maps together with phase maps, which were computed from the action potential and strain rate waves respectively. Both phase maps indicate a focus from where the activity starts. Both foci coincide with the location of the electrode touching the heart wall. The data demonstrates that electromechanical wave phenomena can similarly be observed during a basal non-arrhythmic state. In the case, in which rotors or vortex activity is not present in the myocardial muscle tissue, we can identify concentric wave patterns, which include focal points, which indicate the locations from which the electromechanical activation starts.

Mode	Manufacturer	Model	Transd.	Acq. Rate	Field of View	Species
3D	Siemens	Acuson sc2000	4Z1c	51 vps	90° x 90°, 12cm lat.	Pig
3D	Siemens	Acuson sc2000	4Z1c	85 vps	90° x 90°, 6cm lat.	Pig
3D	Siemens	Acuson sc2000	4Z1c	134 vps	52° x 90°, 6cm lat.	Pig
3D	Siemens	Acuson sc2000	4Z1c	154 vps	86° x 44°, 6cm lat.	Pig
3D	Siemens	Acuson sc2000	4Z1c	188 vps	62° x 44°, 6cm lat.	Pig
2D	FUJIFILM Visualsonics	Vevo 2100	MS-550D	202 fps	14 mm width 10 mm depth	Rabbit
2D	FUJIFILM Visualsonics	Vevo 2100	MS-550D	279 fps	10 mm width 10 mm depth	Rabbit
2D	FUJIFILM Visualsonics	Vevo 2100	MS-550D	309 fps	9 mm width 10mm depth	Rabbit

Supplementary Table 1: Ultrasound imaging systems used with either pig (3D) or rabbit hearts (2D) and exemplary imaging parameters. Field of views given for pyramid-shaped 3D volume images (x cm lat. = lateral depth or pyramid height in cm) and rectangular 2D images respectively (width x depth).

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