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María S Guillem PhD[§], Andreu M Climent PhD[&], Miguel Rodrigo MS[§], Felipe Atienza MD[&], Omer Berenfeld PhD FHRS[#]

§ ITACA, Universitat Politécnica de Valencia, Valencia, Spain

& Hospital General Universitario Gregorio Marañón, Madrid, Spain

# Center for Arrhythmia Research, University of Michigan, Ann Arbor, Michigan

**Title:**

# Presence and stability of rotors in atrial fibrillation: Evidence and therapeutic implications

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**Corresponding Author:**

Omer Berenfeld, PhD, FHRS

Center for Arrhythmia Research

2800 Plymouth Rd.

Ann Arbor, MI 48109

Phone: (734) 998-7560

Email: oberen@umich.edu

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**Abstract**

Rotor-guided ablation has opened new perspectives into the therapeutical treatment for atrial fibrillation (AF). However, the driving role of rotors in human AF is still controversial. In this review the current knowledge gained through research models and patient data that supports that rotors are key players for AF maintenance is summarized. We address the reported divergences regarding rotor prevalence and stability, which can be attributed to methodological differences among mapping technologies. Improvement of current clinical mapping technologies will be crucial for developing mecanistic based ablation strategies that may help in selecting the best therapeutical strategy in a patient basis.

## 1. Introduction

Atrial fibrillation (AF) is the most common arrhythmia seen in clinical practice and is associated with increased risk of stroke, heart failure and death [Wann 2011]. In spite of the high prevalence of AF, the success of current therapies for restoring sinus rhythm in AF patients by either administration of antiarrhythmic drugs, electrical cardioversion or catheter ablation are suboptimal.

Catheter ablation has been reported to be more effective in maintaining sinus rhythm than antiarrhythmic drugs [Dobrev 2010, Wilber 2010, Parkash 2011], with reported success rates of around 70% [Cappato 2010]. After the identification of the critical role of the pulmonary veins in the initiation of AF [Haïssaguerre 1998], pulmonary vein isolation (PVI) has been established as the recommended catheter ablation approach [Calkins 2012, Parkash 2011, Dewire 2010] with overall success rates of up to 87% in paroxysmal AF patients [Tzou 2010, Pappone 2011, Medi 2011]. However, up to 43% paroxysmal AF patients may develop AF recurrence after a single procedure if antiarrhythmic drugs are discharged [Medi 2011] and long-term evaluation of catheter ablation outcome reveals a decline in arrhythmia-free survival even after repeated ablations [Weesasooriya 2011]. Moreover, success rates of catheter ablation in non-paroxysmal AF patients are disappointing, with AF-free rates for single a procedure as low as 28% or 51% after multiple repeat procedures [Chao 2012].

As opposed to the anatomical-only PVI isolation strategy, ablation approaches that rely in the detection of atrial sources have also been proposed. Isolation of atrial sources identified as those that could initiate AF after stimulation [Dixit 2008] or as those with highest activation rates [Atienza 2014] has been reported to be as efficient as isolation of all PVs. But recent reports on the success ratio of rotor-based ablation strategies, which outperform PVI isolation [Narayan 2012, Narayan 2013, Haïssaguerre 2014] now put in the spotlight the development of mechanistic-based strategies for selecting the best therapeutic option in an individual patient basis.

## 2. Mechanisms of atrial fibrillation

There is still an open debate regarding the mechanisms that initiate and maintain atrial fibrillation [Jalife 2011, Waks 2014]. While some authors advocate for the presence of multiple wavelets with a random propagation as the main mechanism sustaining AF [Moe 1962, Allesie 1985], some other authors argue that there are spatially localized drivers that maintain the arrhythmia [Vaquero 2008, Jalife 2004, Lee 2013].

Supporters of the multiple wavelet hypothesis have in their favor a strong evidence of a highly disorganized electrical activity during fibrillation and the unquestionable presence of multiple simultaneous propagation wavelets that can be observed during AF no matter which mapping technology is used [Konings 1994, Eckstein 2013, Jalife 2002, Narayan 2012] and whose complexity is increased in persistent AF patients [de Groot 2010].

However, there is strong evidence of the presence of some spatiotemporal organization of AF both in animal models and in humans [Gerstenfeld 1992, Mansour 2001, Sarmast 2003, Kalifa 2003, Sanders 2005, Atienza 2009] that contradicts the multiple wavelet hypothesis and advocates for the maintenance by localized atrial sources. Maybe the strongest evidence against the multiple wavelet hypothesis is the consistent finding of localized drivers in the atria [Calkins 2012], predominantly located in the pulmonary veins (PV) [Haissaguerre 1998], but not confined to the PV area [Schmitt 2002, Weber 2007, Lau 2014, Hsu 2004, Lin 2003], whose driving role can be confirmed by cessation of the arrhythmia after their isolation from the remaining atrial tissue [Haissaguerre 1998, Herweg 2003, Atienza 2009, Calkins 2012].

Atrial fibrillatory sources have been hypothesized to be either ectopic foci, or driven by a group of cells consistently firing ectopic beats [Jais 1997, Schmitt 2002, Voigt 2012 ], or rotors, defined as functional reentries around an unexcited core [Gray 1995, Jalife 2002, Vaquero 2008]. Nobody questions today that trigger activity is involved in AF initiation by either early or delayed afterdepolarizations, most likely due to sarcoplasmic reticulum Ca2+ leaks [Hove-Madsen 2004,

Oral 2008, Voigt 2014]. On the other side, rotors by themselves have been shown to sustain fibrillatory processes in cardiac tissue slices [Cabo 1996], cardiac monolayers [Zlochiver 2008, Herron 2012, Climent 2015] and computer model simulations [Kneller 2002, Pandit 2013] but their role in whole-heart AF as drivers or bystanders is still a matter of debate [Waks 2014, Narayan 2014]. However, these two hypotheses are nonexclusive since propagation of a wavefront originated in an ectopic trigger can reach a line of unidirectional block which its increases curvature and then this curved wavefront self-sustains in the form of a rotor [Jalife 2002], see Figure 1.A-B. Both ectopic triggers and rotors can be seen as either endocardial or epicardial breackthroughs when the core of the rotor lays underneath the mapped cardiac face [Hansen 2015], see Figure 1.C-D.

## 3. Evidence of rotor presence in atrial fibrillation

### 3.1 Rotors in research models

Most of the knowledge gained about rotors and their role in AF is based in mapping experiments in research models, such as Langendorff-perfused isolated hearts or cardiac monolayers.

The isolated heart model imposes conditions to the preparation that are certainly far from being physiological, it has the advantage of allowing wide-field and high spatial resolution mapping. By performing epicardial electrical mapping in 11 Langendorff-perfused canine hearts, Schuessler et al. investigated the driving role of rotors in sustaining AF [Schuessler 1992]. They quantified the presence of rotors and simultaneous wavefronts by drawing isochronal maps during induced atrial fibrillation with and without infusion of acetylcholine (ACh). They hypothesized that an increased number of simultaneous wavelets due to ACh, which reduces the atrial refractory period and thus "enlarges" the atria, would confirm the multiple wavelet theory. However, they found that below a critical refractory period a single rotor stabilized and maintained the electrical activity and that the number of simultaneous wavefronts was reduced.

But the driving role of rotors has been mainly pushed forward by the use of the optical mapping technology, which allows for an increased spatial resolution and field of view and a more reliable detection of activation times than electrical recordings. In an isolated sheep heart model, Skanes et al. showed that the activation at rotor sites was faster and more regular than that of other sites during induced fibrillation [Skanes 1998]. This observation was attributed to a fast reentrant activity at rotor domains that cannot be followed at other atrial sites thus conduction blocks were observed at the transition between these domains. In a latter study, Berenfeld et al. showed that spectral analysis allowed identification of atrial sources by displaying dominant frequency (DF) maps [Berenfeld 2000] and highlighting a hierarchical activation pattern.

Further evidence of the driving role of rotors was provided by Mandapati et al. [Mandapati 2000], who found a highly significant correlation between the rotation period of rotors and the DF at the same sites. In the same study, they found that these reentrant sources were more frequently located in the posterior free wall of the left atrium and thus there was a left-to-right gradient in activation frequencies. This driving role of left atrial rotors was confirmed by Mansour et al. [Mansour 2001], who showed that ablation of interatrial conduction paths decreased the DF of the right atrium while did not modify the DF of the left atrium, which was typically higher.

According to all these observations, there is a rotor-driven hierarchical activation pattern during atrial fibrillation that is summarized in Figure 1.

In addition to isolated heart experiments, optical mapping studies of cardiac cell cultures, which allow the recording of the entire activated tissue without any hidden areas, were used to demonstrated that in-vitro fibrillation was sustained by stable reentries [2000 Entcheva, 2003 Iravanian]. Cell cultures also allow controlling the cell microenvironment and the monolayer composition in co-cultures and thus mechanistic hypothesis can be tested. The co-culture of cardiomyocytes and myofibroblasts allowed determining that an increased myofibroblast content, reduces the conduction velocity and thus increases the complexity of reentrant patterns [Zlochiver

2008], which may explain the increased complexity in persistent AF patients with remodelled atria. Atrial cell cultures with AF-induced electrical remodelling, with a decreased expression of connexins and an altered expression of some ion channel proteins have been recently used to demonstrate that rotors, and their dynamics, govern the electrical activity even in a substantially remodelled atria [Climent 2015].

### 3.2 Rotors in human AF

But evidence of the important role of rotors in AF is not restricted to research models and has been found in human AF. Since it is not possible to perform optical mapping in human patients because of current technological limitations, most of the efforts in locating rotors in human AF have been directed towards the identification of highest DF sites. Sanders et al. [Sanders 2005] developed a real time analysis tool to find the highest DF sites in patients during an electrophysiological study. By performing real time DF mapping they demonstrated a hierarchical pattern of activation that was consistent with previous observations in isolated sheep hearts, with highest DF sites typically located in the PV area ,although the highest DF sites were more widespread in persistent AF patients that paroxysmal AF patients. This left-to-right DF gradient in paroxysmal AF was independently found in other laboratories [Lazar 2004, Lin 2006, Atienza 2006] and can be attributed to a left-to-right gradient in inward-rectifier potasium currents [Atienza 2006, Voigt 2010]. Persistent AF patients do not consistently show a left-to-right DF gradient, which evidences that atrial tissue remodeling modifies the distribution of AF drivers and thus the RA is involved in the maintenance of AF in persistent AF patients [Hocini 2010, Atienza 2006, Atienza 2009].

Atienza et al. [Atienza 2006] demosntrated the driving role of the highest DF sites by infusion of adenosine. Adenosine increases the conductivity of inward potasium rectifier channels and thus shortens the action potential and reduces excitability and automaticity, similar to the effect of ACh infusion already reported in animal studies [Schuessler 1992, Sarmast 2003]. In this study they

found that adenosine accelerated the highest DF sites, especially at the PVs of paroxysmal AF patients, which suggested that the highest DF sites were rotor-driven.

Direct visualization of rotors by either activation maps or phase analysis using conventional mapping tools is technologically demanding because requires the use of multipolar catheters and intensive signal processing tools. Lin et al. [Lin 2013] reported their approach to localize rotors by sequential point-by-point mapping of the atria in 53 patients. They identified possible rotor sites in the LA as those with some degree of fractionation, high DF and some regularity and then obtained activation maps by sequentially mapping a mean of 9 sites around the putative rotor. By using this approach, they found that 15% of patients presented activations consistent with rotors. Although this may seem a very low incidence, the conditions they imposed to rotors were very strict (i.e. the rotor had to be stable for the few minutes). Only very stable rotors anchored at very fixed locations restricted to the LA could fulfilled their imposed criteria and under these circumstances, a rotor prevalence of 15% does not appear to be only anecdotical and, instead, highlights the importance of rotors in human AF.

Multipolar catheters are, in principle, more suitable for indentifying rotors than bipolar catheters. Rotational activity consistent with rotors could be observed by using a spiral catheter [Atienza 2011]. By using this spiral catheter, organized maps showing incoming directions that were frequently consistent with activations from the PVs accounted for 31% of activations. Rotors in the form of transient rotational patterns were also observed by [Ghoraani 2013] in the LA of 66% of 32 patients. However, these methods underestimate the number of possible rotors to be observed because due to the limited atrial area covered by the catheter.

The development of multipolar basket catheters that allow mapping a wider atrial region together with very specific signal processing algorithms [Narayan 2012] allowed Narayan et. al to construct spatiotemporal maps showing either rotational patterns or focal sources. This technique named Focal Impulse Rotor Modulation (FIRM) mapping is based in the use of a 64-pole catheter,

activation detection and a specific “physiological filtering”. By using this approach they reported that as much as 97 % of 101 patients presented either focal sources or sustained rotors that lasted for tens of minutes, predominantly found in the LA (76%) [Narayan 2013]. The large prevelance of detected sources reported by Narayan was striking to the clinical and research community since they contradicted most observations in human AF for years [Konings 1991, de Groot 2010, Lee 2014] who failed to find rotors in human AF. While many authors question the validity of Narayan’s approach, Benharash et al. has been the first author to do so based on his own experience with FIRM mapping [Benharash 2015]. Benharash et al. reported no differences in DFs at rotor and non-rotor sites, which indeed contradicted most rotor-based studies. Unfortunately they defined the DFs in a two-wide range (1-20 Hz, instead of a more physiological 4-12 Hz range) and thus they may be defining DFs that do not correspond to actual activation frequencies of the atrial tissue. Further studies are required in order clarify the driving role of driver sites identified by FIRM mapping.

Additional support for the driving role of rotors in human AF was recently provided by Hansen et al. [Hansen 2015]. In their study, Hansen et al. performed simultaneous sub-epicardial and sub-endocardial optical mapping in atrial preparations from 8 excised human hearts together with 3D gadolinium-enhanced magnetic resonance imaging to quantify fiber orientations. They found stable reentries anchored at anatomical tracks with increased transmural fiber angle differences and interstitial fibrosis. Ablation at these sites confirmed the primary role of these rotors as AF drivers.

## 4. Noninvasive mapping of rotors in human AF

The ability of body surface potentials to detect rotors and stable propagation patterns during AF was described several years ago by our group [Guillem 2009]. A total of 64 electrodes were placed in both the anterior and posterior torso and only TQ segments free from ventricular content were analyzed, but the observation of rotors in our studied patients was sporadic. Phase maps

computed from surface potentials also showed complex patterns in which reentries could be identified, but they were unstable and lasted for very short time.

Very similar observations were reported in an early inverse problem report during AF [Cuculich 2010]. Patients wore a vest with 256 electrodes and torso and heart volumes, required for solving the inverse problemand thus estimating epicardial potentials, were segmented from CT images. Inverse problem was validated by comparing inverse-reconstructed maps with CARTO activation maps during atrial pacing. After quantification of activation patterns, most activation maps presented multiple wavelets and only 15% of patients presented activation maps that could be attributted to rotors. Unfortunately, the lack of simultaneous endocardial mapping technology did not allowed the validation of those AF inverse problem solutions propagation patterns. However, indirect location of rotors in the form of highest DF sites and DF gradients was found to be possible from surface recordings in a latter study [Guillem 2011]. We performed real-time endocardial DF mapping in order to find the highest DF sites and obtained simultaneous endorcardial and body surface potentials. We found that it was possible to determine the presence of a DF gradient and to identify which atrium was faster in basis of the surface DF pattern.

Since highest atrial activation frequencies can be detected from the body surface, we decided to use this information to detect rotor presence, which should take place at the highest DFs in the atria. Indeed, by band-pass filtering the potential signals around the highest DF we could observe stable reentries during 73.1±16.8% of the time vs. 8.3±5.7% for unfiltered potentials [Rodrigo 2014]. Our BSPM phase maps obtained after filtering surface potentials displayed very simple propagation patterns that resembled those reported by Haissaguerre et al. after solving the inverse problem of the electrocardiography after adding filtering and phase map analysis to their reconstructed potentials [Haissaguerre 2013]. Latter studies by Haissaguerre et al. in a cohort of 103 persisent AF patients [Haissaguerre 2014] reported up to 80.5% activations caused by reentries.

However, there is indeed room for skepticism around inverse-problem AF maps because the activation patterns they report are simpler than epicardial maps recorded both by electrical or optical mapping during AF and have not been validated with simultaneous intracardiac data.

In order to clarify the relation between noninvasive mapping recordings and intracardiac AF activity, we performed mathematical model simulations to help us interpret some of these observations that arise from noninvasive studies in AFBy using a simplified model of atria and torso we could track phase singularities of potentials at both the inner and outer spheres but also at the intermediate layers and describe the evolution of these phase singularities inside the torso volume and we termed filaments the connection of phase singularities across layers in our model. Filaments arising from the driving rotors did reach the outer surface whereas filaments arising from fibrillatory conduction decreased in number with increasing distances from the atria. This decrease in the number of phase singularities with the distance was a consequence of mutual cancellation between nearby filaments with opposed chiralities (see Figure 3). This explains why our body surface phase maps are quite simpler as compared to the expected complexity of epicardial potentials.

Our simulations also helped us to understand the instability of rotors on the body surface We found that deflection of the filament on the outer layer had the same periodicity than the propagation pattern on the passive hemisphere and thus the electrical activity of the remaining tissue would most likely be the cause of the filament deflection (see Figure 3). Therefore, an irregular propagation at distal regions to the driving rotor results in a magnified instability of the rotor on the torso surface. Subsequent filtering of potentials on the surface at the frequency of the rotor in presence of a DF gradient reduced the deflection of the filament and thus stabilized the phase singularity on the outer surface.

According to our results, it becomes evident that the signal processing applied is crucial for detecting stable phase singularities in non-contact mapping because, even if rotors are present,

the electrical activity that does not follow a rotational pattern deflects the projection of this rotation which may lead to misinterpretation of the propagation pattern. Overall, it seems that the smoothing effect of the torso may be responsible for blurring the most disorganized electrical activity (i.e. fibrillatory conduction) while emphasizing the more organized activity. In addition, time course filtering of potentials at the frequency of the rotor may cancel out the activity at other frequencies than that of the rotor and thus may help in identifying the propagation patterns at the frequencies of interest.

An additional observation from our simulations was that by recording non-contact potentials of rotational patterns a mirroring can be found in panoramical mapping. This is the consequence of the projection of rotational patterns on two contralateral views: the filament can be seen as the center of rotation that projects in a normal direction to the rotation plane and intersects the torso surface at two sites, producing two phase singularities. This “mirror effect” may also explain the consistent finding of at least two simultaneous rotors in non-contact mapping studies [Haissaguerre 2014].

## 4. Stability of rotors in atrial fibrillation

Rotors, defined as functional reentries, are not stationary and may meander or drift, as opposed to anatomical reentries in which there is a non-excitable anatomical obstacle. Rotor meandering occurs as a consequence of beat-to-beat variations in core excitability and ionic dynamics while drifting may occur because of tissue heterogeneities. Computer model simulations allowed determining that rotor drift aligns with fiber orientation [Berenfeld 1999] and is governed by inhomogeneities in ion channel expressions [Calvo 2014]. In particular, the different gradients of main ion channels proteins and particularly the inward rectifier potassium current (IK1) may explain the attraction of and perpetuation of rotors to in pulmonary veins. [Calvo 2014]. Both in animals models [Yamazaki 2012] and humans [Hansen2015], rotors seem to anchor at sites that represent boundaries of areas with different wall thickness [Yamazaki 2012] or sites with

transmural differences in fiber orientation and increased interstitial fibrosis [Hansen 2015], which again is coherent with the reported higher incidence of rotors and high DF sites near the PVs [Sanders 20015, Haïsaguerre 1998].

The fundamental role of ion channels expression (mainly sodium, L-type calcium and inward rectifier currents) in rotors behaviors has been also demonstrated both in animals and in-vitro studies [Martins 2014, Climent 2015]. In a sheep model of long-term AF, an increase of DF during the transition from paroximal to persistent AF was associated with changes in aciton potential duration and densities of soldium, L-type calcium and inward rectifier currentss which suggested that rotors are more stable with the progression of AF [Martins 2014]. In the same direction, Climent et al. demonstrated that AF induced electrical remodeling harbours rotors with an increased stability in spite of their increased number of rotors becuase their spatial stability is also increased [Climent 2015].

In addition to tissue heterogeneities and remodeling, infusion of drugs has also been shown to affect rotor stability. Sarmast *et al.* [Sarmast 2003], demonstrated that the number of phase singularities and their DF in both atria monotonically increased with ACh concentration although rotors life span decreased. In humans, infusion of adenosine increases the mean DF at the posterior left atrial wall, leading to an increase in electrogram duration and number of spikes in surrounding electrodes [Atienza 2011]. We also compared the DFs at peak adenosine effect during consecutive infusions and found no significant differences between consecutive adenosine infusions with time (Atienza 2006). Thus, although the temporal stability and reproducibility of the DFs gradients distribution at peak adenosine effect is preserved, adenosine mediated acceleration of AF drivers gives rise to electrogram fragmentation of the tissue surrounding the DFmax domain. On the other side verapamil, a calcium channel blocker, lowers the atrial rate in AF patients [Bollmann 2002], most likely due to a reduction in rotor stability and rotation frequency that contributes to fibrillation termination [Climent 2015]. Chloroquine, a blocker of inward-rectifier

$K^+$ channels showed a similar antiarrhythmic effect with increased rotor meandering and decreased DFs in a stretch AF model in sheep [Filguerias 2012]. In fact, stretch is another variable that may affect the stability of reported AF drivers. Already in 2003, Kalifa *et al* [Kalifa 2003] showed that an increase in intra-atrial pressure increases the rate and organization of waves emanating from the superior pulmonary veins underlying stretch-related perpetuation of AF.

Rotor stability also affects EGM characteristics and their stability. Rotor drift may cause electrogram fractionation as a consequence of: 1) beat-to-beat changes in local directionality of successive activations wavefronts from the rotor core to the point of recording due to instant variations on frequency activation [Zlochiver 2008] and 2) Doppler effect due to wave front acceleration ahead of drifting rotors giving rise to intermittent local fractionation [Atienza 2011]. When a rotor drifts towards the recording electrode, there is a shortening in the atria-to-atria activation times that results in EGM fractionation, whereas when the rotor is stable the EGMs are periodic and monomorphic. Therefore, from a theoretical point of view, rotors themselves present some instability that is amplified at their periphery, which complicates the detection of stable rotors by any mapping technology.

DF mapping has been clinically used to identify rotor location, considering that sites activating at highest DF would be those driving AF. Consequently, the stability of DF regions may indicate that AF drivers are stable and could be isolated. To date, most of these studies analyzing AF spectral features acquired signals using sequential mapping with varying recording durations, casting doubts with regards to the stability and reproducibility of the DF determinations, since the spatial distribution of DF on maps depends upon the time at which each site is sampled. Indeed, in the study of Sanders et al. fluctuations in DF values as measured in the coronary sinus over a period of 50 min were found during sustained AF, but without a significant slowing or acceleration trend. Spatio-temporal stability analyses were also reported by Atienza et al. [Atienza 2006], and found that in 33 patients in whom consecutive DF measurements at 3 stable biatrial positions every 2

minutes fluctuated with an average standard deviation of 0.25 and 0.21 Hz in paroxysmal and persistent AF patients, respectively, without significant temporal trend. Similarly, Lazar et al [Lazar 2004], found an excellent agreement among recorded RA frequencies (r=0.99) and PV frequencies (r=0.93) during longer-term recordings. Moreover, a similar DFmax sites location and DF values was observed in five patients undergoing a redo procedure following a first DF guided ablation with clinical recurrence [Atienza 2009]. Thus, long-term measured DFs in different parts of the atria using several approaches and varying order consistently demonstrated the presence of spatio-temporal stability of DF distribution over periods of time spanning several minutes in the atria of patients with both paroxysmal and persistent AF.

There are, indeed, large discrepancies regarding rotor stability across studies are summarized in Table 1. Whereas rotors appear as unstable in some studies and account for very few consecutive rotations [Yamazaki 2009, Ghoraani 2013, Haïsaguerre 2014, Rodrigo 2014], they have been reported to last for up to several minutes [Schluesser 1992, Narayan 2012]. These discrepancies may be largely attributed to methodological differences.

First important difference among studies is the size of the mapped area. Obviously, the larger the mapped area, the greater the chances of finding rotors. This may explain the differences between wide-area optical mapping [Skanes 1998] and high-density epicardial or endocardial electrical maps [Konings 1994, Lee 2014] but also between point-by-point [Lin 2013] or multipolar catheters sequential mapping [Atienza 2011, Ghoraani 2013] and endocardial baskets that provide simultaneous wide endocardial recordings [Narayan 2013].

There are also relevant differences in rotor stability between invasive [Narayan] and noninvasive [Rodrigo 2014, Haïsaguerre 2014] approaches that can be attributed to the distance from the electrical sources and the recording point. According to our mathematical simulations, electrical activity at sites different than the rotor distorts the pattern caused by the rotor itself [Rodrigo 2014]. Although rotors can be stabilized by signal processing, filtering does not completely remove the

effect on the ECG at other frequencies and it does not remove at all the effect of planar waves and wavebreaks taking place at the rotor frequency.

In addition to the different mapping technologies, the significant reported differences between clinical epicardial [Konings 1994, de Groot 2010] and endocardial [Narayan 2013] electrical mapping would be explained attending to recent optical mapping experiments from isolated human atria which reported that there is a higher incidence of rotors in the endocardium than in the epicardium [Hansen 2015] and that reentries taking place at a distant plane or at a plane different than that mapped may appear as unstable breakthroughs and multiple-wavelets.

Finally, the disparity in the reported presence of rotors among studies can be attributed to the the applied signal processing and analysis. Narayan [Narayan 2013], Haïsaguerre [Haïssaguerre 2014] and ourselves [Rodrigo 2014] applied a quite restrictive band-pass filtering in order to stabilize rotors. Although this can be a matter of debate, we have shown that this filtering mode attenuates the effect of atrial regions activating at frequencies different than those driving the rotor [Rodrigo 2014].

## 5. Clinical implications of rotors and rotor-guided ablation

Rotor-guided ablation has emerged in the recent years and has opened new perspectives into the therapeutical approaches for AF. Sanders et al. [Sanders 2005] were able to identify localized sites of high-frequency activity during AF in humans and showed the different DF distributions in paroxysmal and permanent AF. A latter study by Atienza et al. showed that it was feasible and effective to ablate the highest DF sites by performing real-time DF mapping in humans [Atienza 2009]. The multicenter RADAR-AF study showed that in paroxysmal AF patients, highest DF ablation is noninferior to the empirical isolation of PVs and was associated with a lower incidence of adverse events [Atienza 2014]. However, in persistent AF patients, the combination of CPVI with DF sites ablation offered no incremental benefit and tended to increase complications rate.

In contrast, rotor-guided ablation using either endocardial or inverse computed epicardial recordings have reported higher AF freedom rates that the standard CPVI approach in persistent AF patients. Narayan et al. reported a significantly improved outcome in persistent AF patients when the sources found by FIRM mapping were ablated together with a conventional anatomical ablation [Narayan 2013]. Their reported success for persistent AF patients is striking: 82% vs. 45% for empirical PVI. Similar results were reported for rotor-guided ablation based on the inverse problem resolution of body surface recordings, with an 85% freedom of AF at 1 year.

Acute endpoint termination is not achieved in most patients after ablation of FIRM-identified sources, or DF-targeted patients, and this constitutes another source of criticism for these rotor-guided ablation strategies. However, their supporters claim that even after the critical sites for reentry are ablated, reentry can take place at other sites, but their elimination hampers the appearance of new sustained episodes.

## 6. Future perspectives

Rotor-guided ablation has opened new perspectives into the therapeutical treatment for AF. However, there are still open questions that will need to be addressed in the near future. A wider use of both FIRM and DF mapping based ablation will help to confirm the reproducibility of such approaches by independent laboratories. In addition, the noninvasive detection of AF drivers will potentially help in selecting patients for AF ablation and planning their ablation procedures. Current studies are underway that aim at validating the noninvasively computed propagation patterns with the actual electrical patterns in AF patients. These studies may clarify the accuracy of noninvasive approaches and its potential application to patient identification. In the long run, development of new technological solutions together with more realistic research models may be the key for understanding AF mechanisms and develop effective therapeutical approaches.

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699 **Table 1. Selected references for rotor/reentry presence in the atria and atrial fibrillation**

| Reference | Mapping technique | Patients/animals | Prevalence | Duration/number of rotations |
|---|---|---|---|---|
| Allessie 1976 | Multiple synchronous microelectrode recordings | Isolated segments (15 X 15 mm) of rabbit left atrium | Not reported | Not reported |
| Allessie 1984 | Two endocavitary mapping electrodes containing 960 leads and recording from 192 different sites simultaneously. | 6 isolated blood-perfused canine hearts, perfused with ACh | | Atrial fibrillation or flutter lasting from several seconds to more than half an hour |
| Schuessler 1992 | Electrical mapping, 256 electrodes | 11 Dogs (Langendorff perfused) with ACh | 57% to 100% in a dose dependent manner with ACh concentrations | Up to 2 minutes (2100 cycles) |
| Skanes Circulation. 1998 | Optical mapping | 6 sheep (Langendorff perfused with ACh) | 12/20 recordings showed spatiotemporal periodicity | Up to 3-4 sec, 4-14 consecutive |
| Mandapati Circulation. 2000 | Optical mapping | 7 sheep (Langendorff perfused with Ach) | Not reported | Not reported |
| Chen Cardiovasc Res. 2000 | Optical mapping | 6 sheep (Langendorff perfused with ACh) | Not reported | Not reported |
| Sarmast Cardiovasc Res. 2003 | Optical mapping | 7 sheep (Langendorff perfused with Ach) | Not reported | 1 to 2.1 rotations depending on ACh dose and chamber |
| Yamazaki 2009 | Optical mapping | 24 sheep (Langendorff perfused) | 16.6% to 93.2% depending on stretch, Ach, RYA, CAFF | 1.1 to 6.2 consecutive rotations |
| Atienza 2011 | Spiral catheter in LA | 5 paroxysmal AF | 62% | Not reported |

| | | | | |
|---|---|---|---|---|
| Filgueiras-Rama | Endocardial - epicadial optical mapping | 30 intact isolated sheep hearts | Not reported | Up to 3.7 rotations depending on chloroquine dose |
| Cuculich Circulation. 2010 | ECGi | 26 patients (11 paroxysmal) | 15% of patients (only in non-paroxysmal patients) | Rarely > 1 rotation |
| Narayan 2012 | FIRM mapping | 92 patients (72% persistent) | 97% of patients with sources, 70% of time | Tens of minutes |
| Ghoraani 2013 | Circular catheter in LA | 32 patients, 88% persistent. | 66% of patients | Few (9%) lasted 2.5 seconds, most non-sustained (610 ms) |
| Lin 2013 | Sequential electroanatomical mapping in LA | 53 patients (31 persistent, 22 long-standing) | 15% patients | Not reported |
| Haïssaguerre 2014 | ECGi | 103 persistent AF patients | 80.5% of time | Average 2.6 rotations. Max 7 rotations |
| Rodrigo 2014 | BSPM | 14 AF patients | 73.1% of time | 2.8 rotations (I THINK OUR DATA SHOWS A NAVERAGE OF ABOUT 7 ROTATIONS - ~340 msec) |
| Hansen 2015 | Endocardial - epicadial optical mapping | 6 explanted human heart preparations | 75% preparations | Not reported |

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## Figure legends

**Figure 1.** Schematic representation of trigger initiation of AF and rotor maintenance. An ectopic beat causes a concentrical propagation (A) that can find a discontinuous propagation or line of block that can curve the propagation wavefront and initiate a rotor(B). A rotational pattern can then be observed at the plane of rotation of the rotor, such as the epicardium in (C) whereas at the epicardium the propagation pattern is consistent with a breakthrough. When the rotor seats transmurally, then a a rotational pattern may not be seen neither in the epicardium nor the endocardium and breackthroughs may be seen in both layers (D)

**Figure 2.** Schematic representation of the hierarchical activation during AF. Rotors,or functional reentries around an unexcited core, present some degree of spatiotemporal periodicity and thus EGMs are quite regular. Spectral analysis at these sites allows identifying a dominant peak which matches the activation frequency of the rotor, which is the fastest in the tissue. At nearby sites, the wavefront cannot rotate at the same frequency than the rotor, beause this wold require and exceedingly high propagation velocity and thus the propagation wavefront fractionates and some activations are blocked. Since there are beat-to-beat variations in activation times and directions at the boundaries of the rotor, EGMs at these sites have variable morphology and are fractionated. In the frequency domain, these beat-to-beat variations in activation times result in multiple peaks in the spectrum. At more distal sites, the wavefront is less curved and thus there are less wavebreaks and a more regular activity. Since some activations are missed at the boundaries of the rotor, the activation frequency that can be observed in the frequency domain is lower than at the rotor site.

**Figure 3.** Propagation of rotational patterns from the epicardium to the torso surface. Rotational patterns are projected from the epicardium towards the torso surface.The center of rotation of rotors across intermediate layers (filament) is deflected by the main propagation direction in the remaining atria. Complexity is decreased across layers because filaments arising from counter-rotating sources cancel out with each other. Instability in the main propagation pattern in the tissue causes an unstable pattern on the body surface, that can be stabilized by band-pass filtering of surface potentials at the rotor frequency.

734 **Figure 1**

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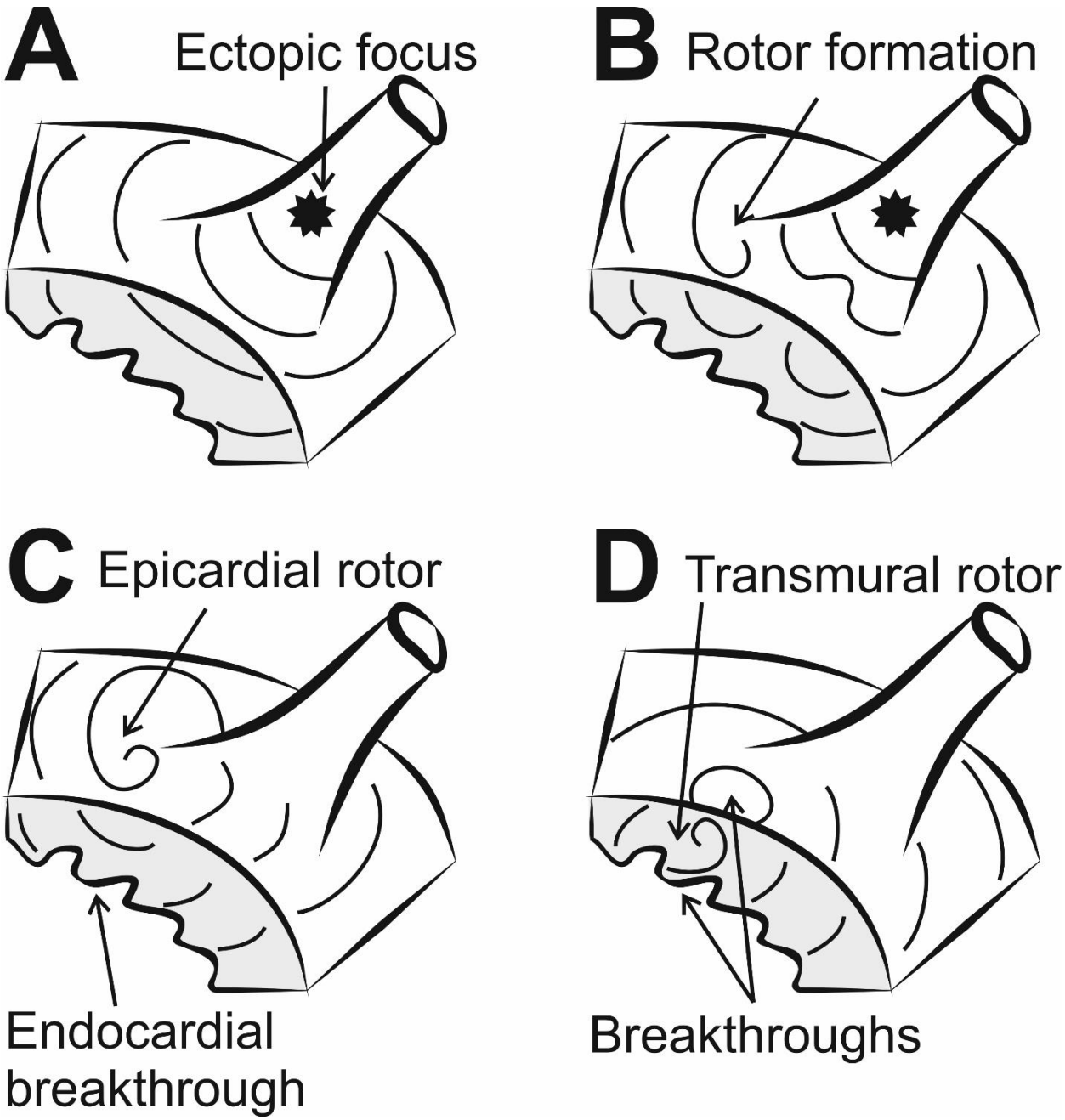


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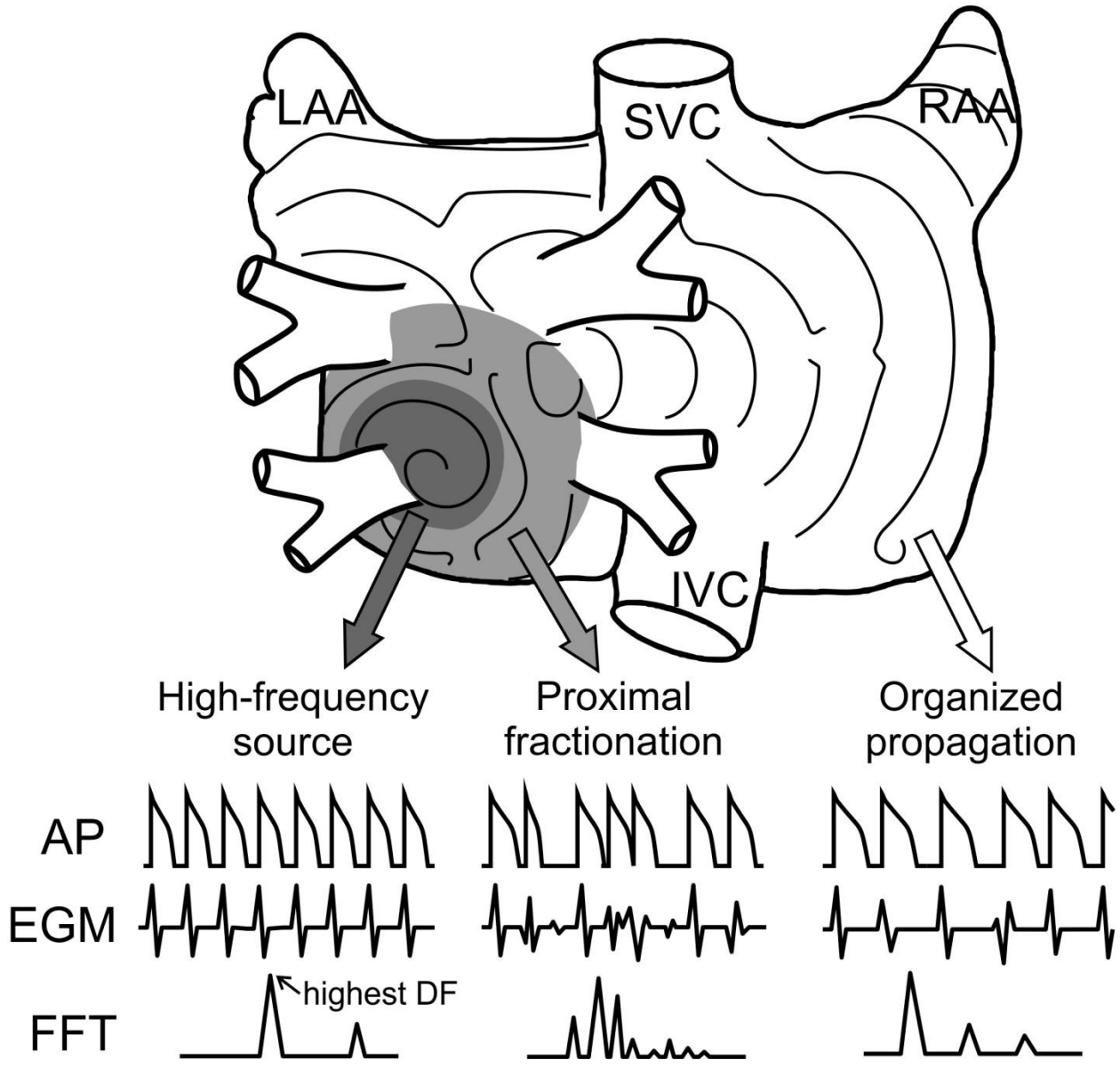
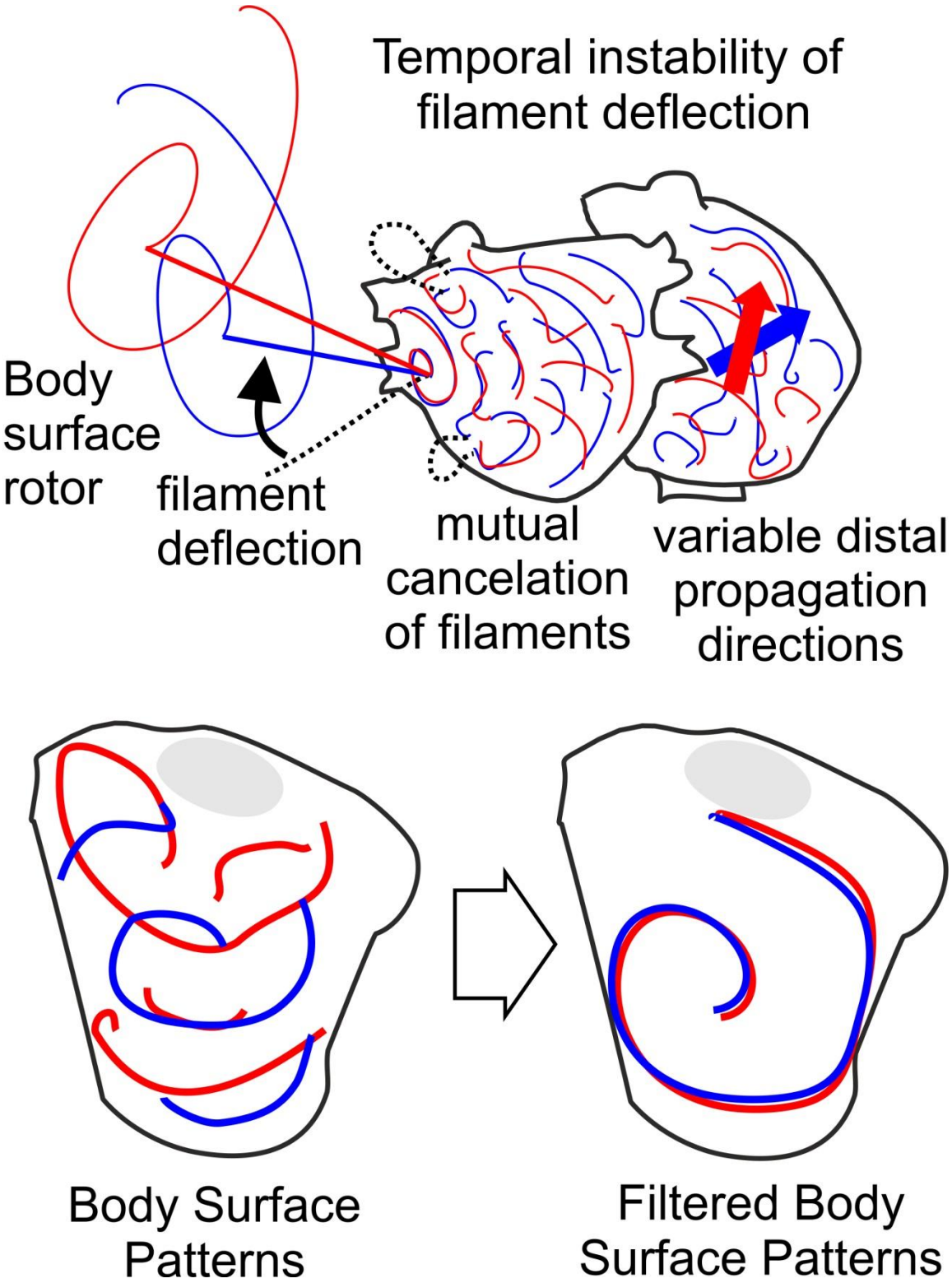
**Figure 2**


LAA
SVC
RAA
IVC
High-frequency source
Proximal fractionation
Organized propagation
AP
EGM
FFT
highest DF

744 **Figure 3**

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