Virtual Histology: Basics, Practice and Pitfalls

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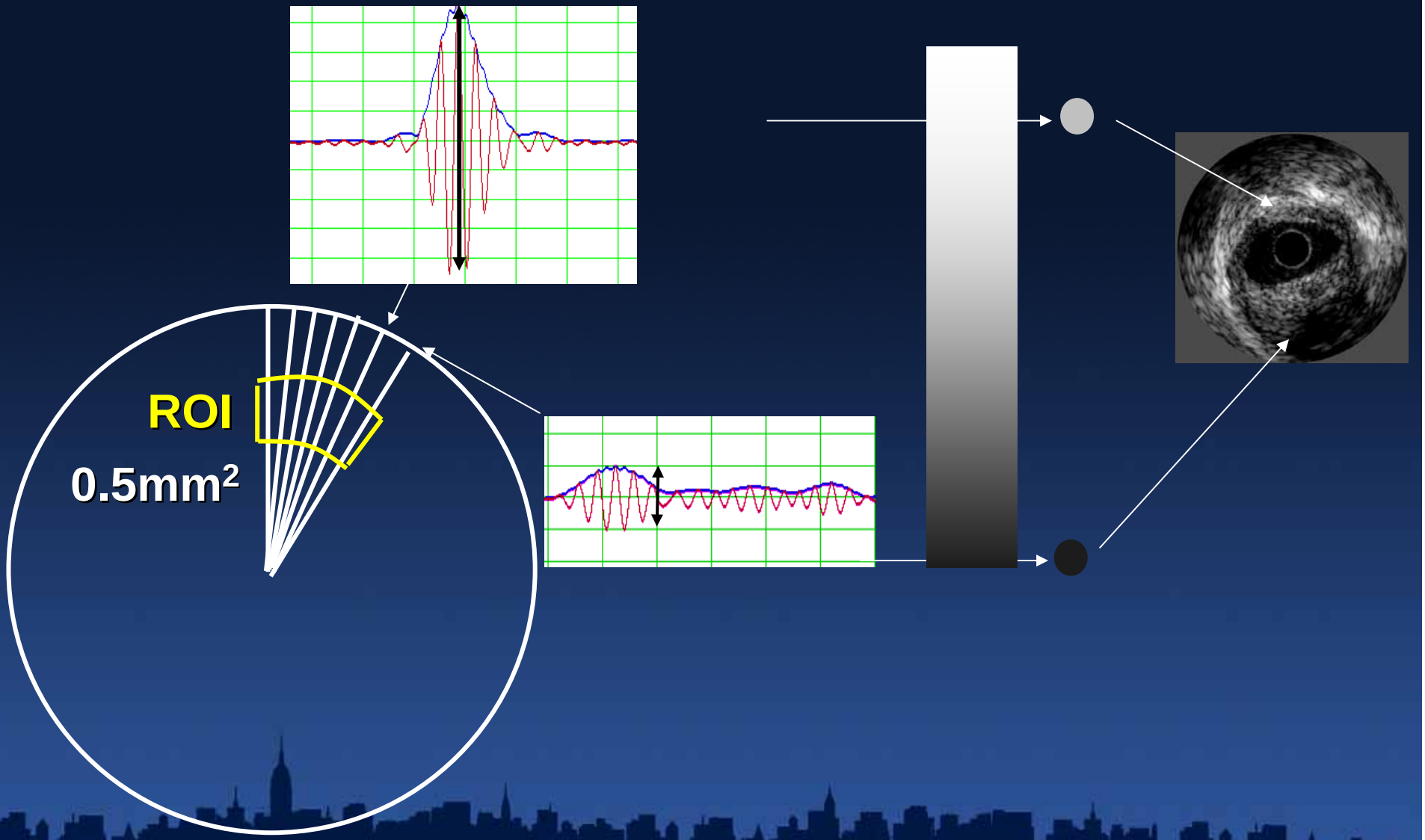
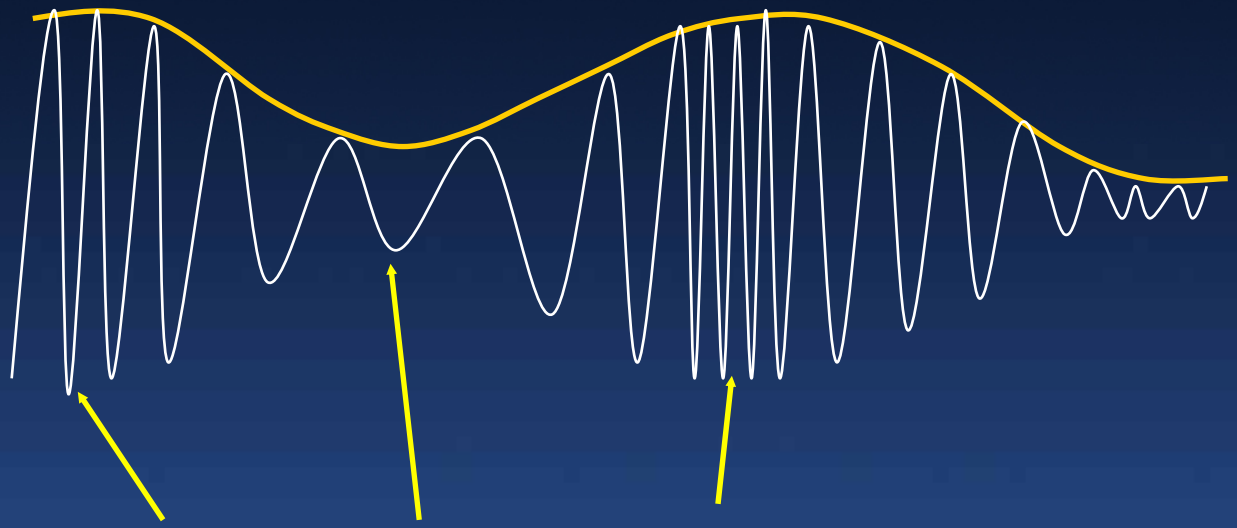
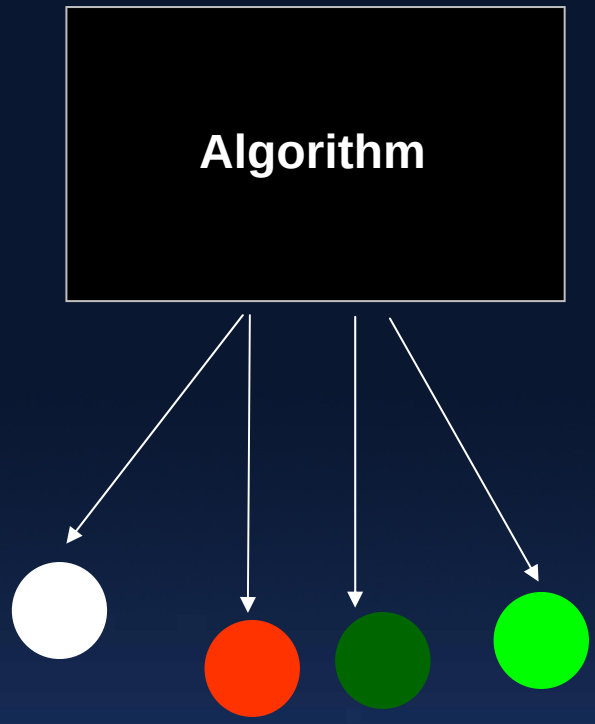
Grayscale Image uses only amplitude

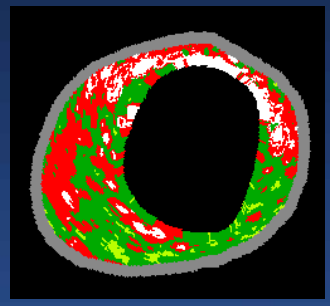


RF/VH analysis uses 8 amplitude and frequency parameters



Frequency of echo signal varies, depending on the tissue





30MHz Mechanical Transducer

VH IVUS vs histopathology from fresh 51 fresh, post mortem LADs (88 plaques and 208 regions of interest)

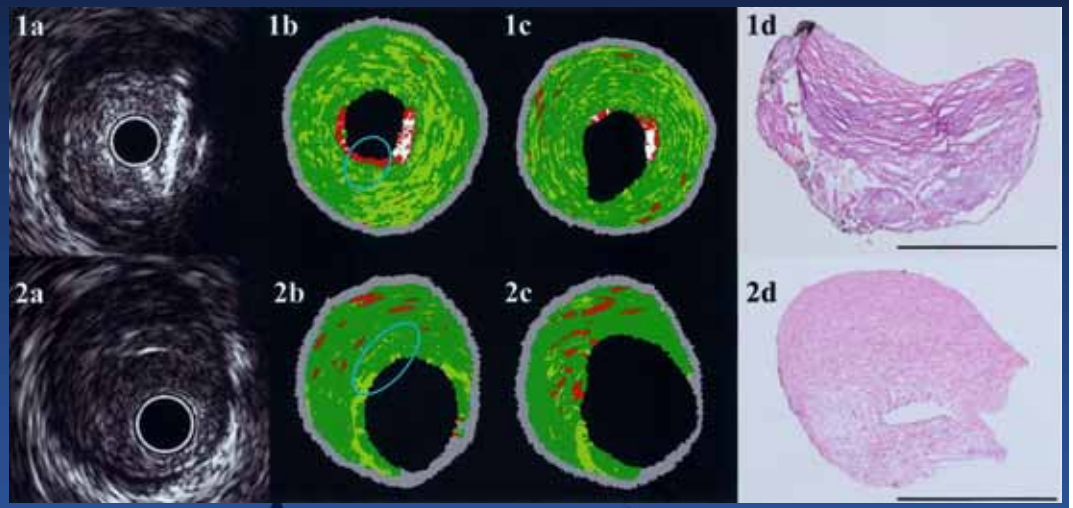
	Training set predictive accuracy	Validation set predictive accuracy
Fibrous tissue (n=101)	90.4%	79.7%
Fibrofatty (n=56)	92.8%	81.2%
Necrotic core (n=70)	89.5%	85.5%
Dense calcium (n=50)	90.9%	92.8%

Nair et al. Circulation 2002;106;2200-6

Validation of of VH-IVUS in 30MHz Mechanical Transducers (with Blinded Deconvolution) Against DCA Specimens (307 Sections in 30 Pts)

	Sens	Spec	Predictive Accuracy
Fibrous tissue	86.0%	90.5%	87.%
Fibrofatty	79.3%	100%	87.1%
Necrotic core	67.3%	92.9%	88.3%
Dense calcium	50.0%	98.9%	96.5%

- 15 stable angina and 15 ACS pts.
- NC was more common in ACS vs SAP
  - Histopathology: 22.6% vs. 12.6%, p=0.02
  - In vivo VH-IVUS: 24.5% vs. 10.4%, p=0.002



Nasu et al. J Am Coll Cardiol 2006;47:2405-12

Eagle Eye (20MHz Electronic Array Transducer)

VH IVUS vs histopathology from fresh 51 fresh, post mortem LADs (115 sections and 407 regions of interest)

	Sensitivity	Specificity	Predictive Accuracy
Fibrous tissue (n=162)	84.0%	98.8%	92.8%
Fibrofatty (n=84)	86.9%	95.1%	93.4%
Necrotic core (n=69)	97.1%	93.8%	94.4%
Dense calcium (n=92)	97.8%	99.7%	99.3%

Nair et al. Eurointervention 2007;3:113-20

Reproducibility

- **Hartmann, et al. Reproducibility of volumetric intravascular ultrasound radiofrequency-based analysis of coronary plaque composition in vivo. Int J Cardiovasc Imaging. 200;25:13-23.**
  - **Intraobserver comparison, same pullback: relative differences = 0.45±2.1% (FT); -1.12±4.9% (FF); -0.84±2.1% (DC); -0.22±1.8% (NC)**
  - **Intraobserver comparison, repeated pullback: relative differences = 1.40±4.1% (FT); 1.26±6.7% (FF); 2.66±7.4% (DC); 0.85±4.4% (NC)**
  - **ilrobserver comparison, same pullback: relative differences = -1.60±4.9% (FT); 3.85±8.2% (FF); 1.66±7.5% (DC), and -1.58±4.7% (NC)**
  - **NC volume showed on average the lowest measurement variability.**
- **Huisman et al. Between-centre reproducibility of volumetric intravascular ultrasound radiofrequency-based analyses in mild-to-moderate coronary atherosclerosis: an international multicentre study. EuroIntervention. 2010;5:925-31**
  - **Intraclass correlations were 0.95 (FT), 0.93 (FF), 0.99 (NC), and 1.00 (DC)**
  - **Of the plaque components NC and DC volume showed on average the highest reproducibility.**

Reproducibility of Repeated Imaging

Following IVUS-VH acquisition and after the disengagement and re-engagement of the guiding catheter, an additional acquisition was performed using a new IVUS catheter.

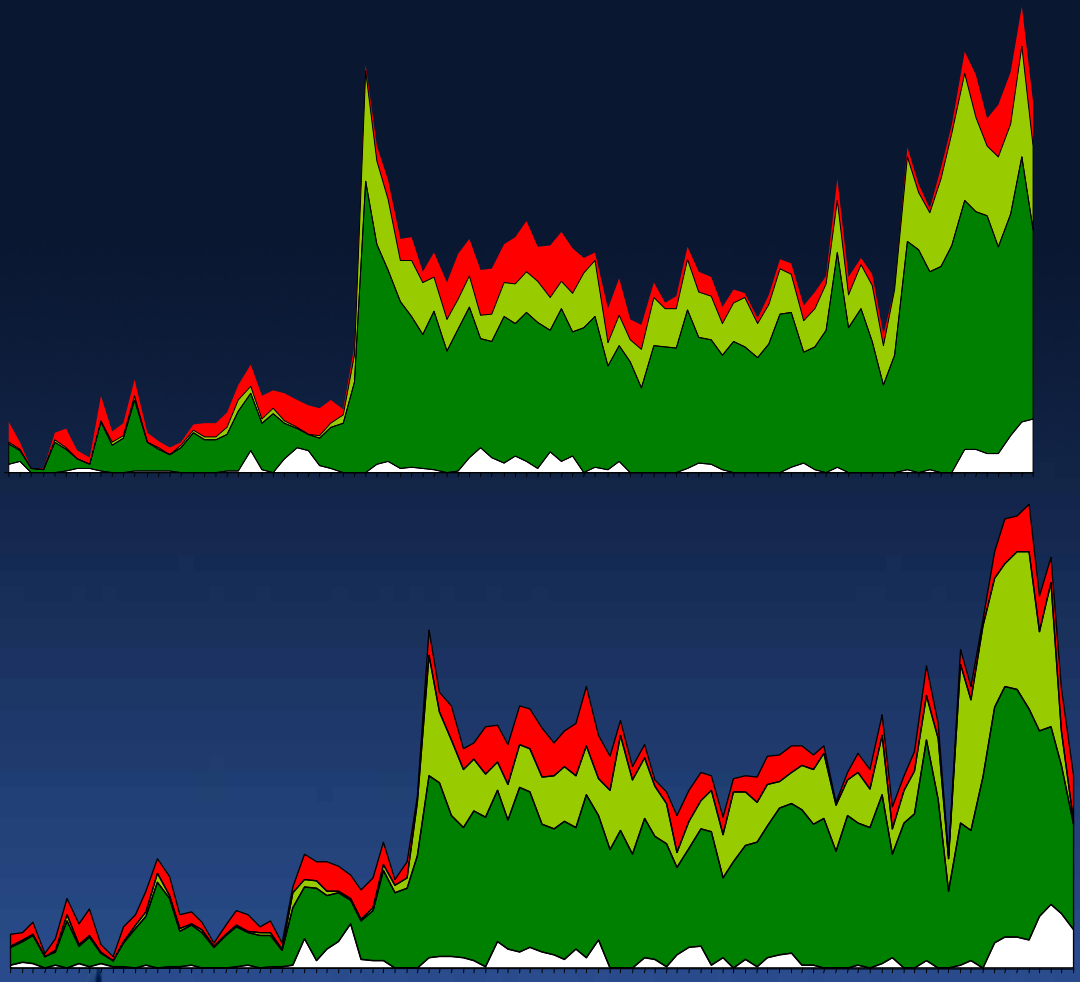
Table 3. Mean CSA compositional measurements of matched ROI with two subsequent 20 MHz IVUS imaging catheters (*n*:16).

	Catheter 1	Catheter 2	Absolute Δ	Relative Δ (%)
Observer 1				
Calcium CSA (mm^2)	0.17 ± 0.3	0.16 ± 0.2	0.01 ± 0.1	8.0
Calcium (%)	4.27 ± 5.2	3.82 ± 3.8	0.45 ± 2.6	11.1
Fibrous CSA (mm^2)	1.96 ± 1.0	2.11 ± 1.1	0.16 ± 0.4	7.7
Fibrous (%)	60.37 ± 9.2	62.15 ± 8.7	1.78 ± 10.0	2.9
Fibrolipidic CSA (mm^2)	0.66 ± 0.4	0.63 ± 0.3	0.02 ± 0.3	3.5
Fibrolipidic (%)	21.10 ± 9.8	19.58 ± 7.0	1.53 ± 8.3	7.5
Necrotic core CSA (mm^2)	0.40 ± 0.4	0.43 ± 0.4	0.02 ± 0.2	5.7
Necrotic core (%)	11.27 ± 6.8	10.87 ± 6.6	0.40 ± 5.4	3.6
Observer 2				
Calcium CSA (mm^2)	0.17 ± 0.3	0.16 ± 0.2	0.01 ± 0.1	8.7
Calcium (%)	4.08 ± 5.0	3.74 ± 3.4	0.34 ± 2.4	8.7
Fibrous CSA (mm^2)	2.21 ± 1.1	2.28 ± 1.2	0.07 ± 0.4	3.1
Fibrous (%)	58.12 ± 10.5	60.63 ± 7.9	2.51 ± 10.1	4.2
Fibrolipidic CSA (mm^2)	0.88 ± 0.6	0.77 ± 0.4	0.11 ± 0.4	13.1
Fibrolipidic (%)	22.97 ± 11.7	20.95 ± 10.8	2.01 ± 9.6	9.2
Necrotic core CSA (mm^2)	0.42 ± 0.4	0.45 ± 0.5	0.03 ± 0.2	6.1
Necrotic core (%)	10.75 ± 6.9	11.02 ± 6.5	0.26 ± 5.5	2.4

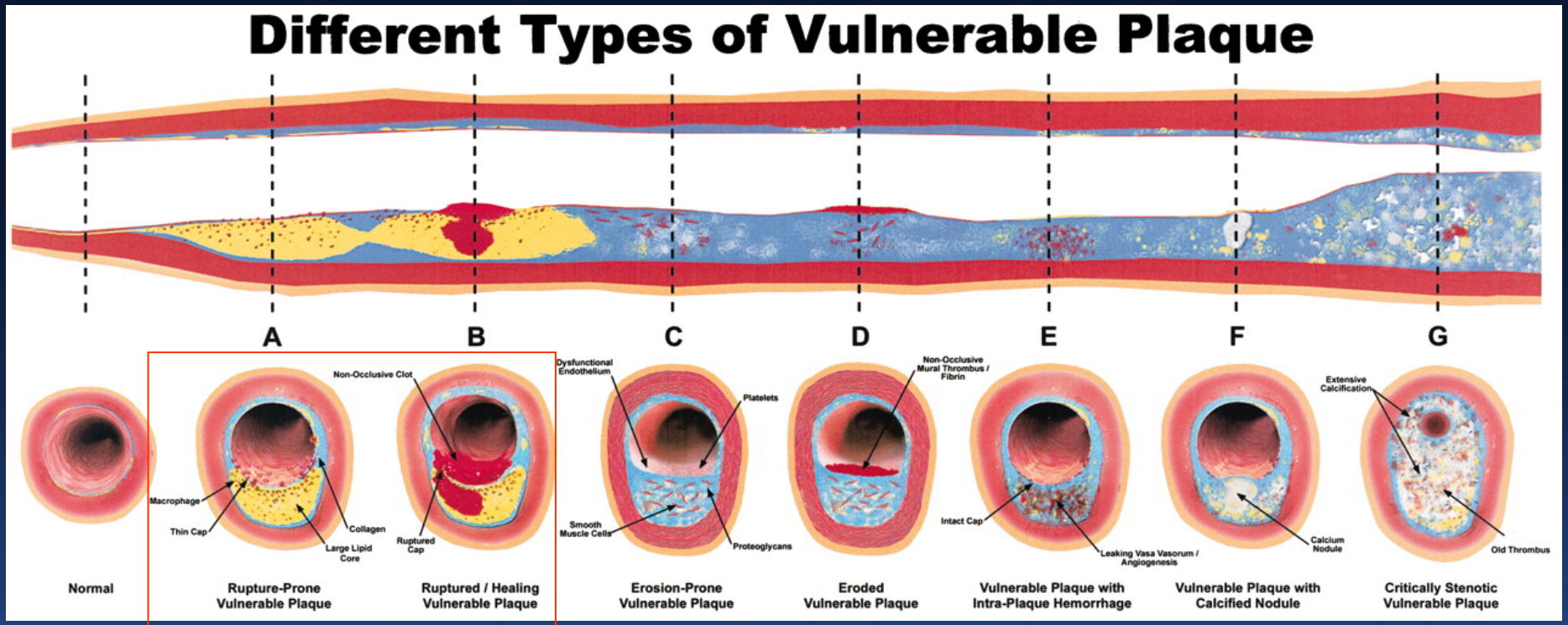
Rodriguez-Granillo et al Int J Cardiovasc Imaging. 2006;22:621-31

In vivo Reproducibility

Two 20MHz catheters and two pullbacks



"Vulnerable Plaque" = thrombosis-prone plaque and plaque with a high probability of undergoing rapid progression

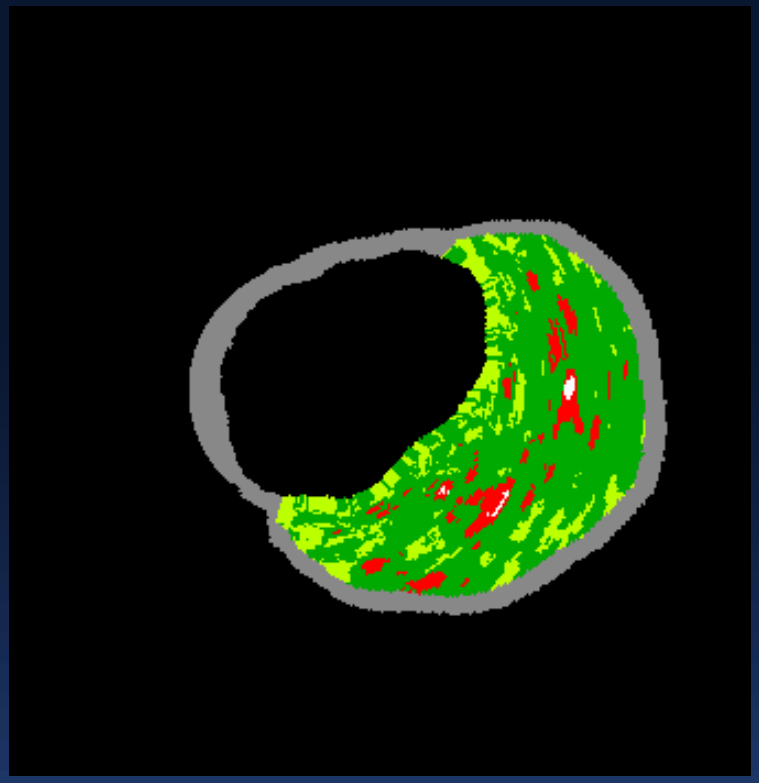


70% of ACS culprit lesions

30% of ACS culprit lesions

Naghavi et al. Circulation 2003;108:1664-72

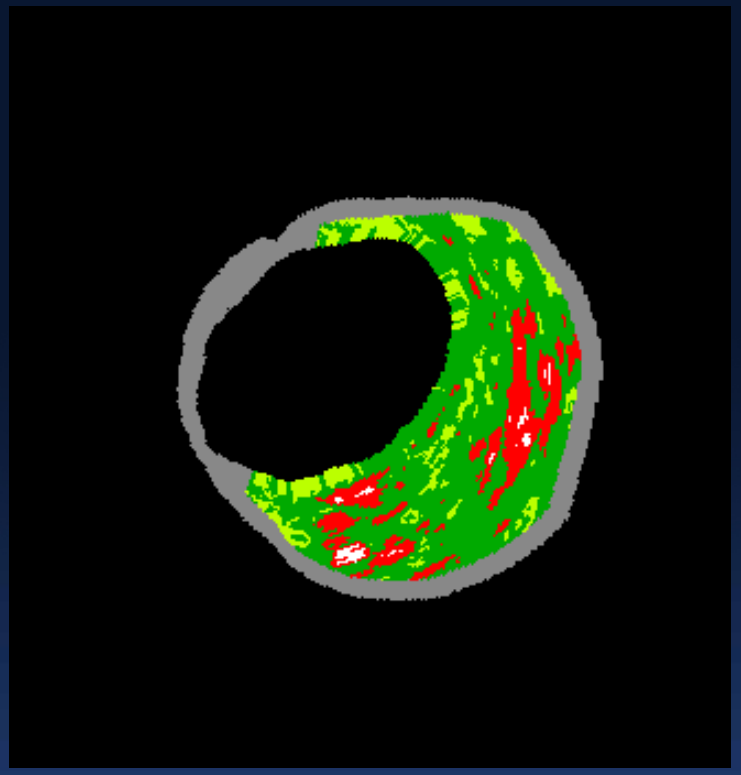
Search for TCFA-II: PIT vs FA



Non-Confluent NC

↓

Pathological Intimal Thickening



Confluent NC

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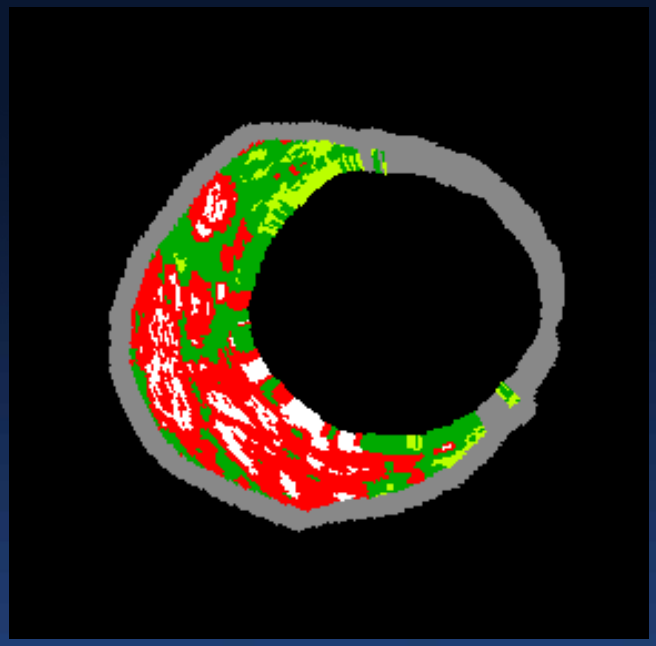
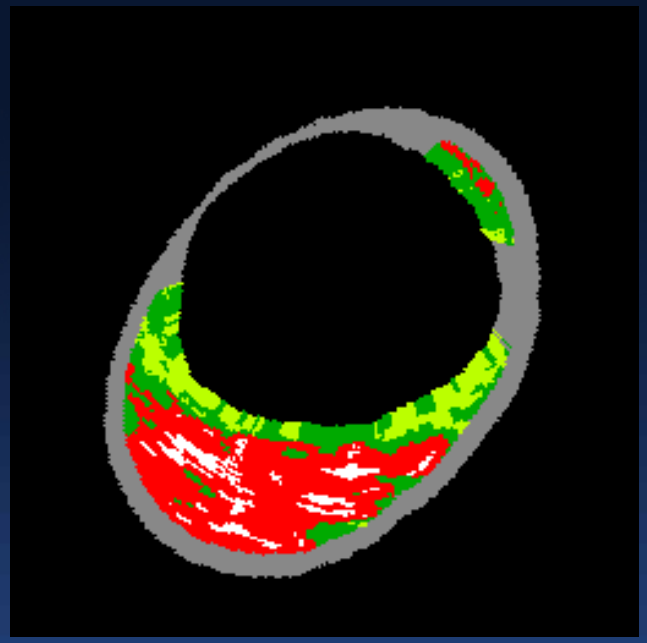
Fibroatheroma

Search for TFCA-II: TCFA vs ThFCA

Thin Cap

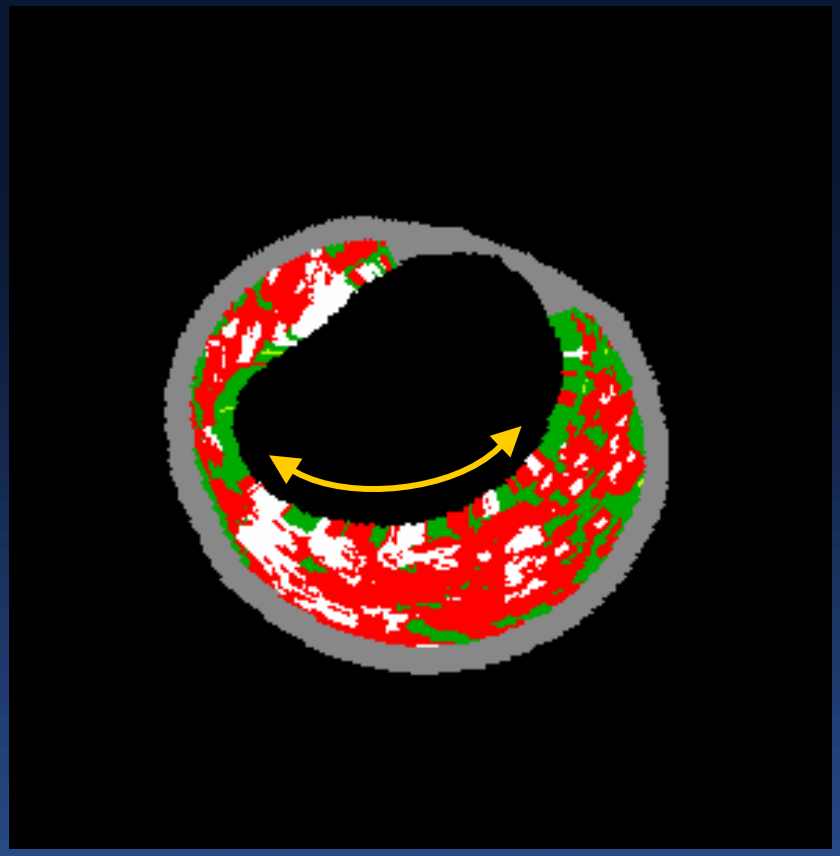


Thick Cap



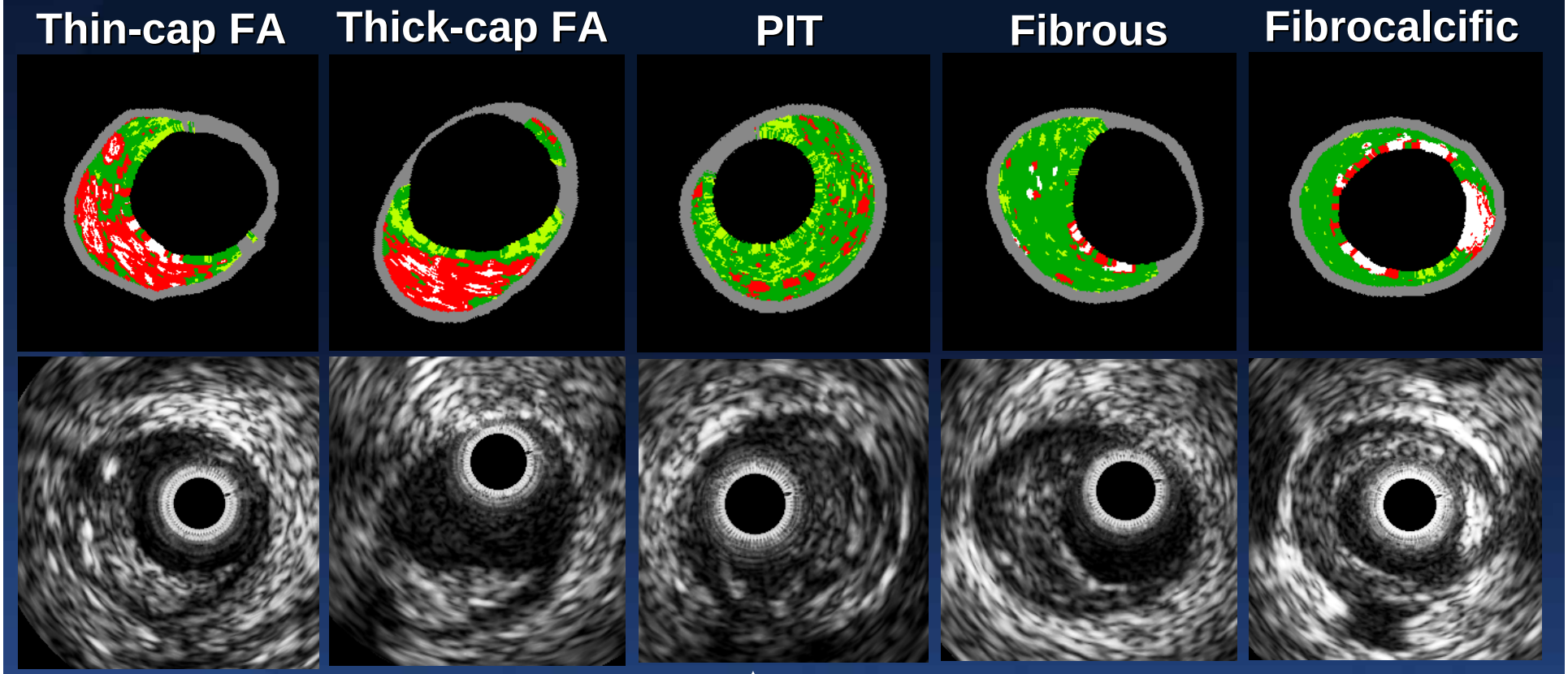
- Fibrous cap of typical TCFA is <65µm
- However, all fibrous caps <150µm will abut the lumen because the resolution of VH-IVUS is 150µ

Thin cap fibroatheroma (TCFA)



1. Confluent NC>10%
2. 30 degree abutting
3. 3 consecutive frames

VH-IVUS Classification



> 10%
Confluent
Necrotic Core

> 15%
Fibrofatty

< 10% Confluent
Necrotic Core

> 10%
confluent
calcium

Unreliable Assessment of Necrotic Core by Virtual Histology Intravascular Ultrasound in Porcine Coronary Artery Disease

Troels Thim, MD; Mette Kallestrup Hagensen, MSc; David Wallace-Bradley, MSc; Juan F. Granada, MD; Greg L. Kaluza, MD, PhD; Ludovic Drouet, MD, PhD; William P. Paaske, MD, DMSc; Hans Erik Bøtker, MD, PhD, DMSc; Erling Falk, MD, DMSc

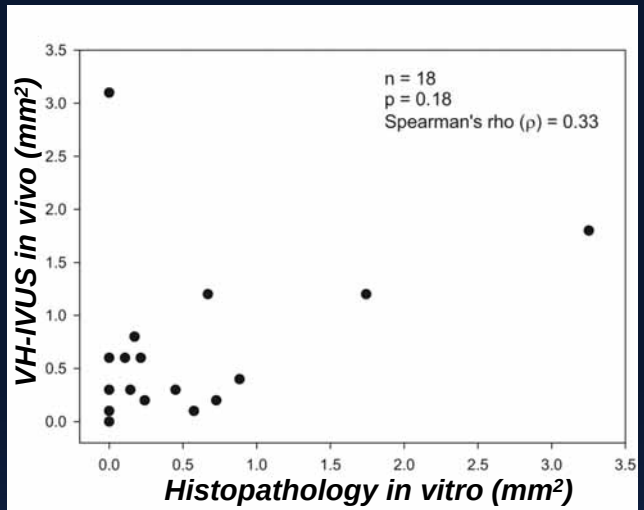
Background—Intravascular ultrasound–derived virtual histology (VH IVUS) is used increasingly in clinical research to assess composition and vulnerability of coronary atherosclerotic lesions. However, the ability of VH IVUS to quantify individual plaque components, in particular the size of the destabilizing necrotic core, has never been validated. We tested for correlation between VH IVUS necrotic core size and necrotic core size by histology in porcine coronary arteries with human-like coronary disease.

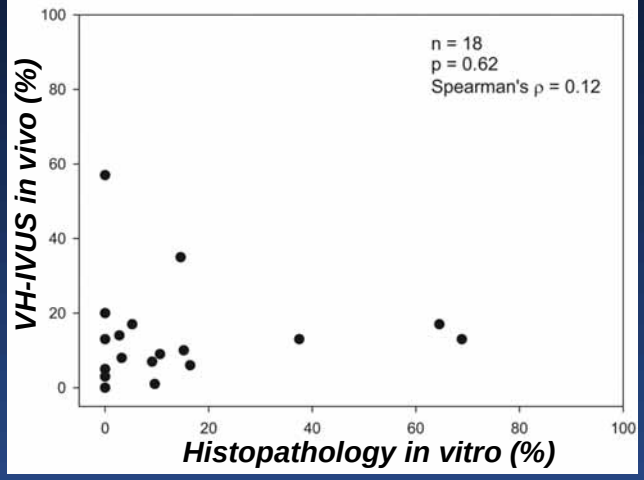
Methods and Results—In adult atherosclerosis-prone minipigs, 18 advanced coronary lesions were assessed by VH IVUS in vivo followed by postmortem microscopic examination (histology). We found no correlation between the size of the necrotic core determined by VH IVUS and histology. VH IVUS displayed necrotic cores in lesions lacking cores by histology.

Conclusions—We found no correlation between necrotic core size determined by VH IVUS and real histology, questioning the ability of VH IVUS to detect rupture-prone plaques, so-called thin-cap fibroatheromas. **(*Circ Cardiovasc Imaging*. 2010;3:384-391.)**

Key Words: intravascular ultrasound ■ virtual histology ■ vulnerable plaque ■ coronary disease ■ animal model

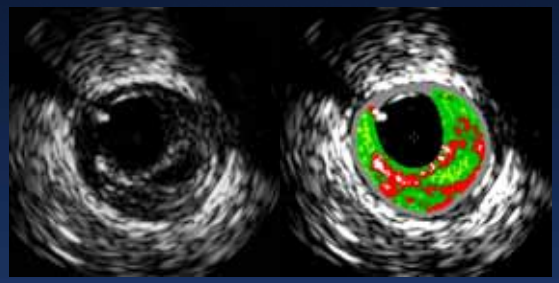
Thim et al. Circ Cardiovasc Imaging 2010;3:384-391



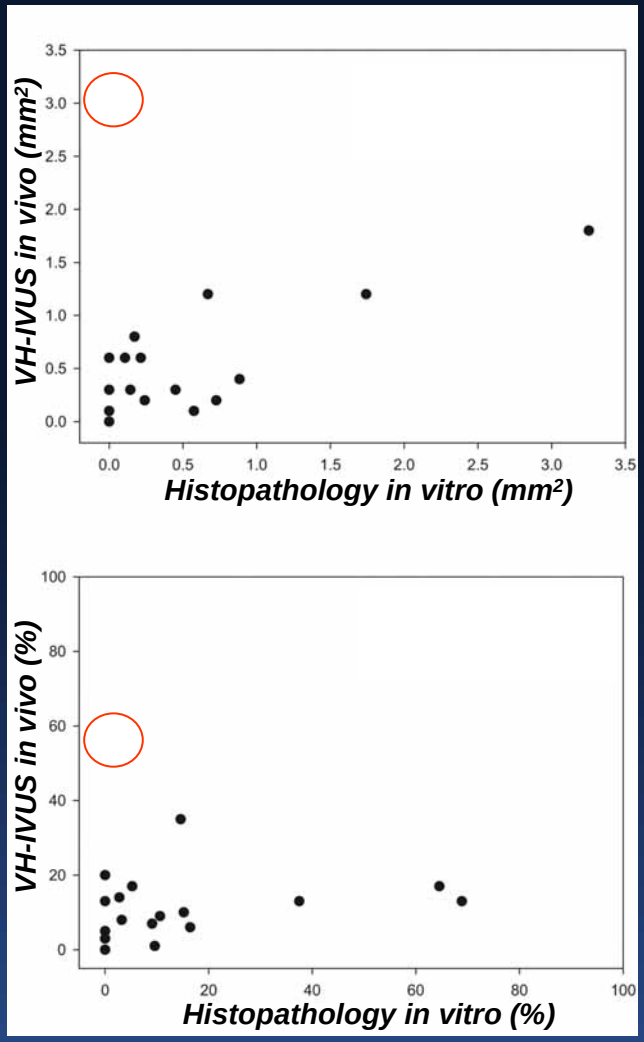


Thim et al. Circ Cardiovasc Imaging 2010;3:384-391

- **Co-registration of VH IVUS and histologic sections may be problematic. VH IVUS is sampled every 0.5 mm (on average) vs every 4 mm for pathologic sections. This cannot be overcome by use of fiduciary branch points or angioplasty footprints.**
- **The VH IVUS algorithm was "trained" on human histology. Swine atherosclerosis balloon injury model is different from human necrotic cores. Ultrasonic reflection from a lipid pool (with very few interfaces) is markedly different from an area with "cellular debris" with several interfaces on which to reflect ultrasound.**

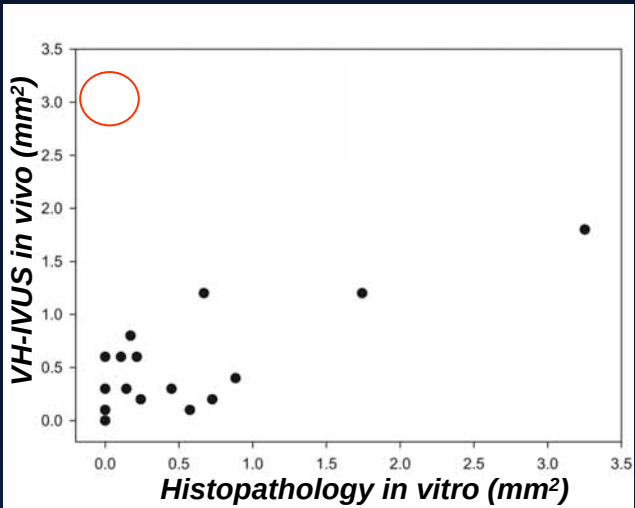


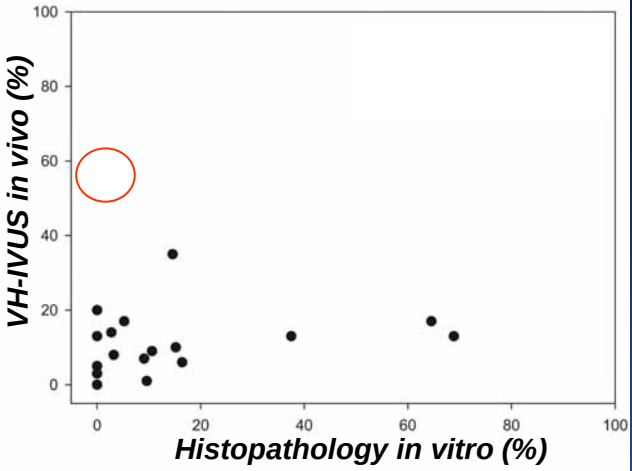
- **Fixation artifacts distort absolute necrotic core measures. To overcome this limitation, geometric transformation was used in the validation studies.**
- **Although not stated in the Methods, animal studies typically re-use IVUS catheters. VH is only as good as the signal available from the IVUS transducer and the signal processing available. (Int J Card Imaging. 2000;16:23-7)**



Thim et al. Circ Cardiovasc Imaging 2010;3:384-391

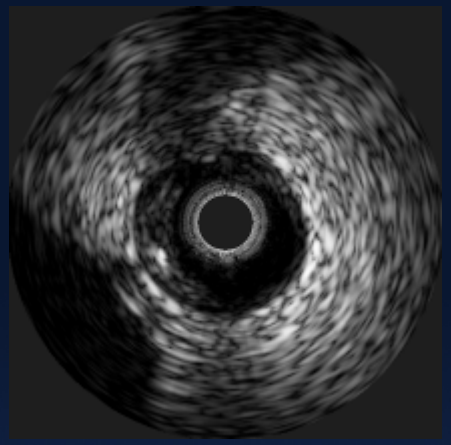
- **The lack of correlation between VH IVUS and histology necrotic core area in the present report was driven primarily by one point in the upper left quadrant. Using the data in this graph, if this outlier is removed, one can calculate that the Pearson improves to r = 0.79 (P=0.0003), indicating very good correlation.**

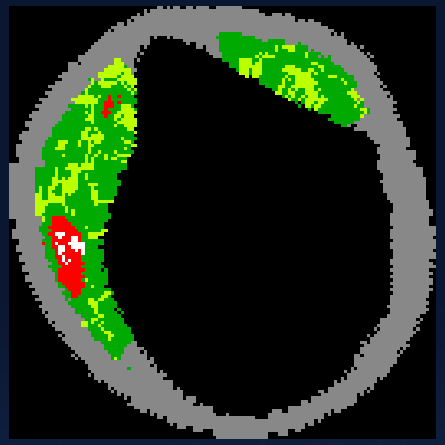


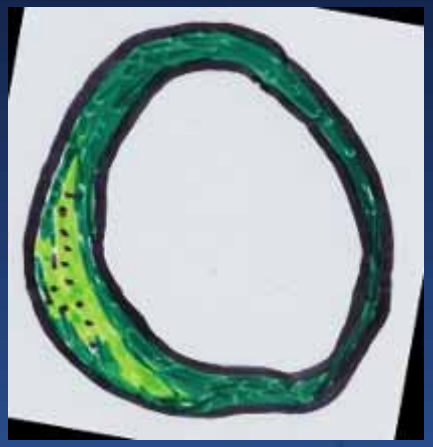


Thim et al. Circ Cardiovasc Imaging 2010;3:384-391

- **There is often major disagreement among pathologists when assessing histology. From a study in which 4 highly experienced cardiac pathologists assessed 30 Movat pentachrome coronary histology slides, in only 7 cases (23%) was there agreement as to histologic classification with the greatest source of variability being in the location and quantity of necrotic core**

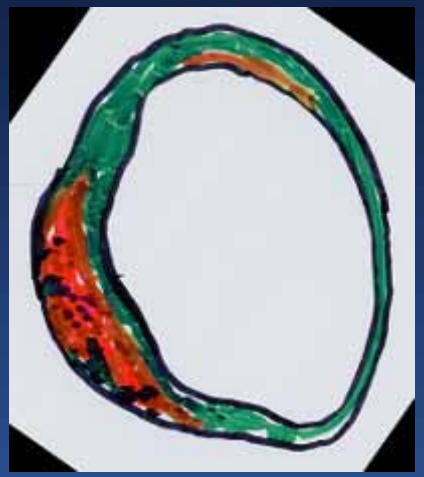






PIT

Ca FA



FA

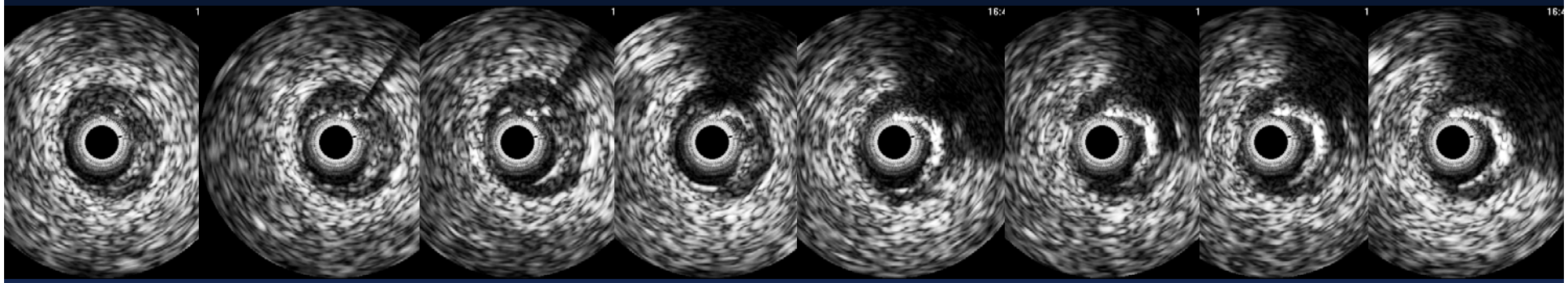
PIT

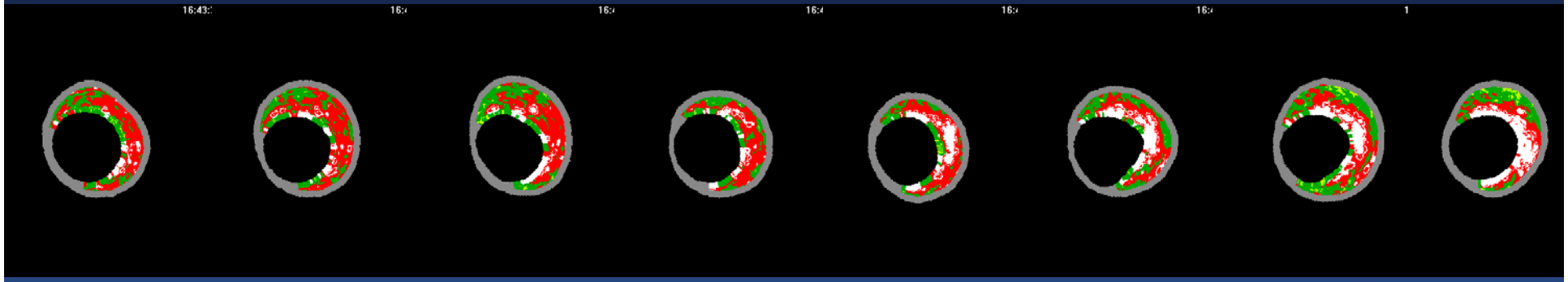
VH-IVUS and Plaque Behind Calcium

- **Using the 20MHz transducer, 80% of ROI behind calcium contained both reflected ultrasound information and noise although the signal-to-noise ratio was low. 20% of ROIs behind calcium had only noise** *(Tanaka et al. J Am Coll Cardiol 2007;49:29B)*
- **When inaccurate, tissue is classified as NC 65% of the time, FT 18% of the time, and FF 14% of the time** *(Vince. Volcano Corp)*

		Correct	Incorrect	ROIs	Accuracy
Mild microcalcium	IVG	2	0	2	100%
	S5	1	1	2	50%
Heavy microcalcium	IVG	3	6	9	33.3%
	S5	18	9	27	66.7%
Dense calcium	IVG	27	10	37	73%
	S5	27	16	43	62.8%
Overall	IVG	32	16	48	66.7%
	S5	46	26	72	63.9%

- ***For plaques with arc of calcium >90°, NC does not correlate with LCBI.***



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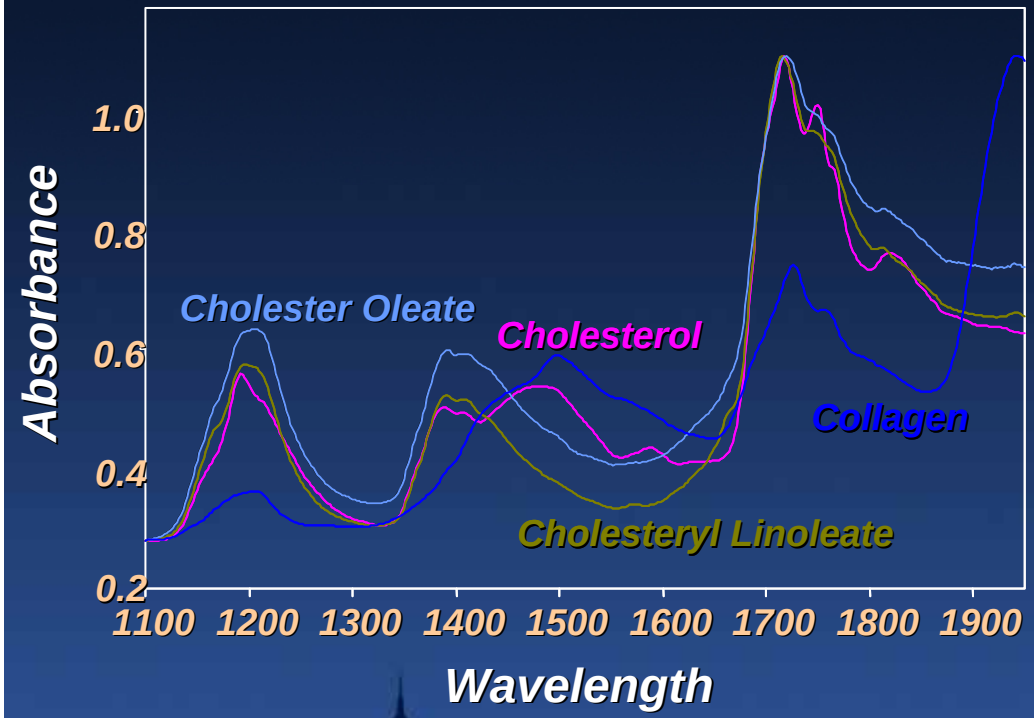
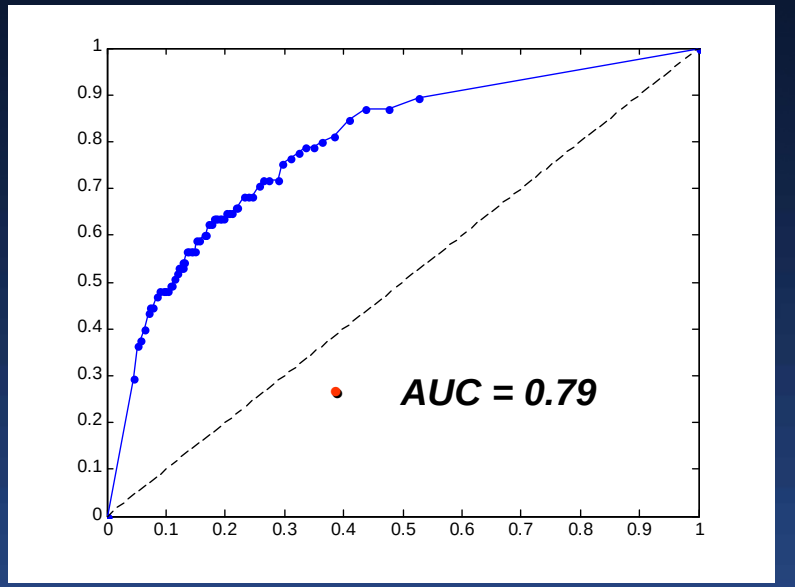
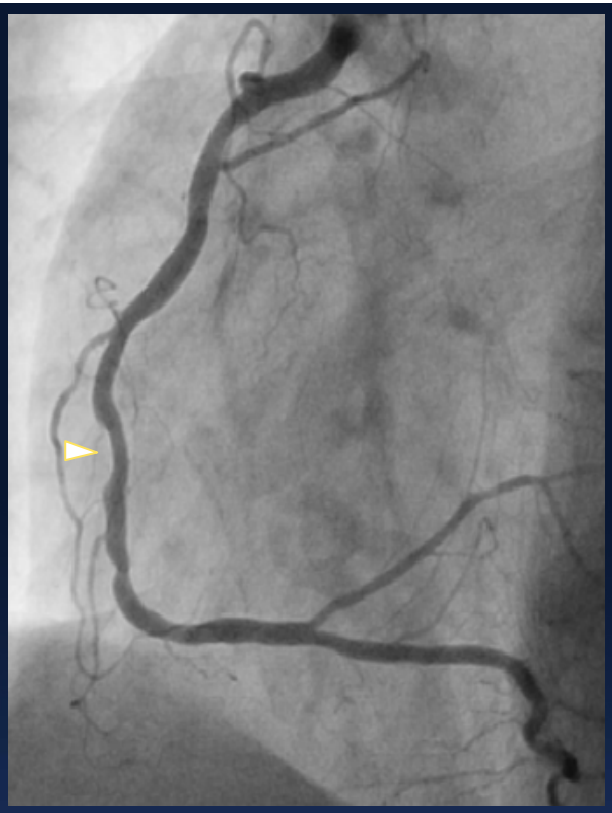
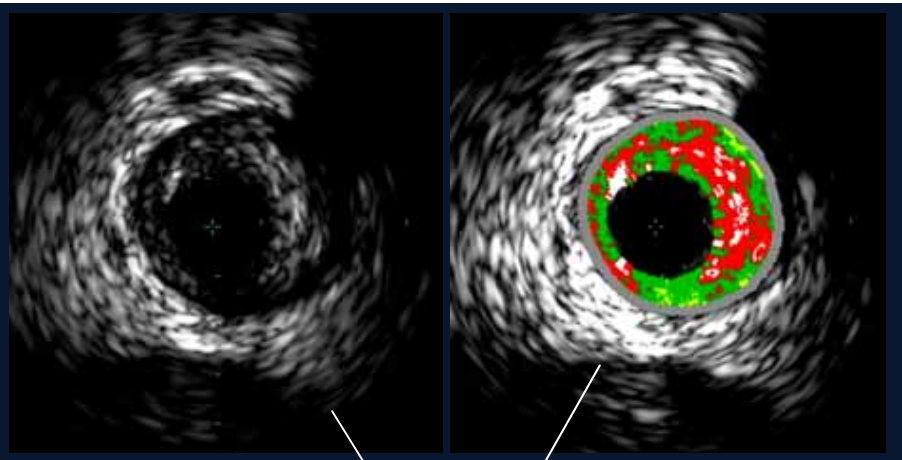
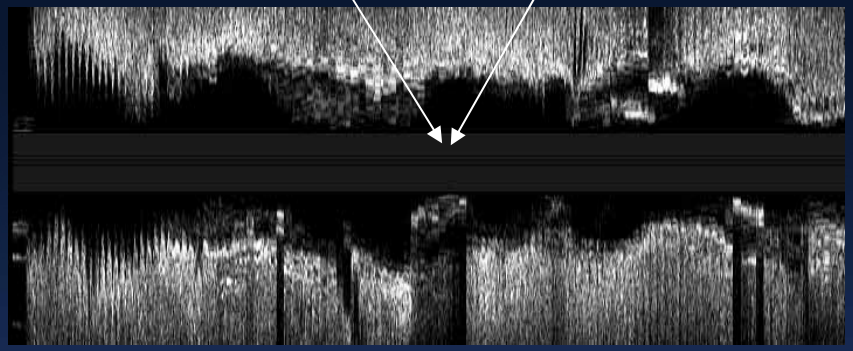
NIR Spectroscopy can identify the chemical composition of unknown substances and distinguish cholesterol from collagen. ROC validation of NIR Spectroscopy in 51 autopsy hearts (algorithm for detection of confluent [>0.2mm thick and >60°] and relatively superficial necrotic core [overlying mean fibrous cap thickness <0.45microns])



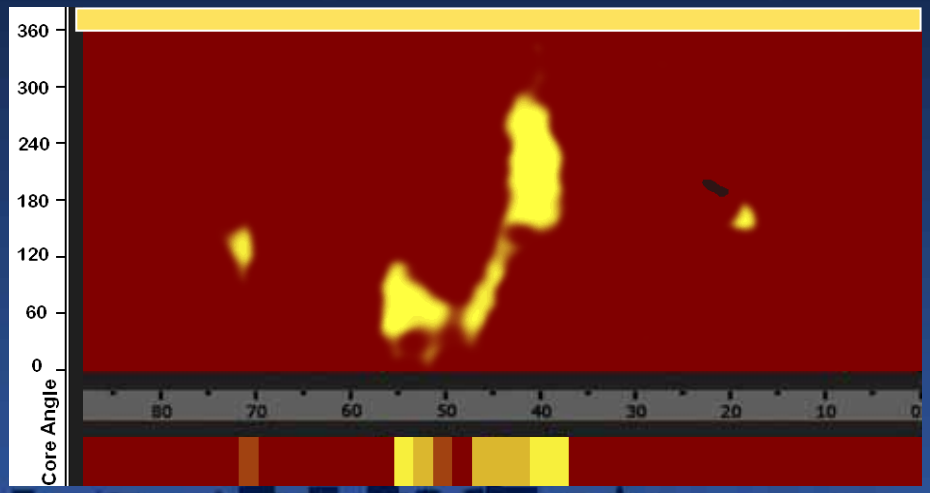


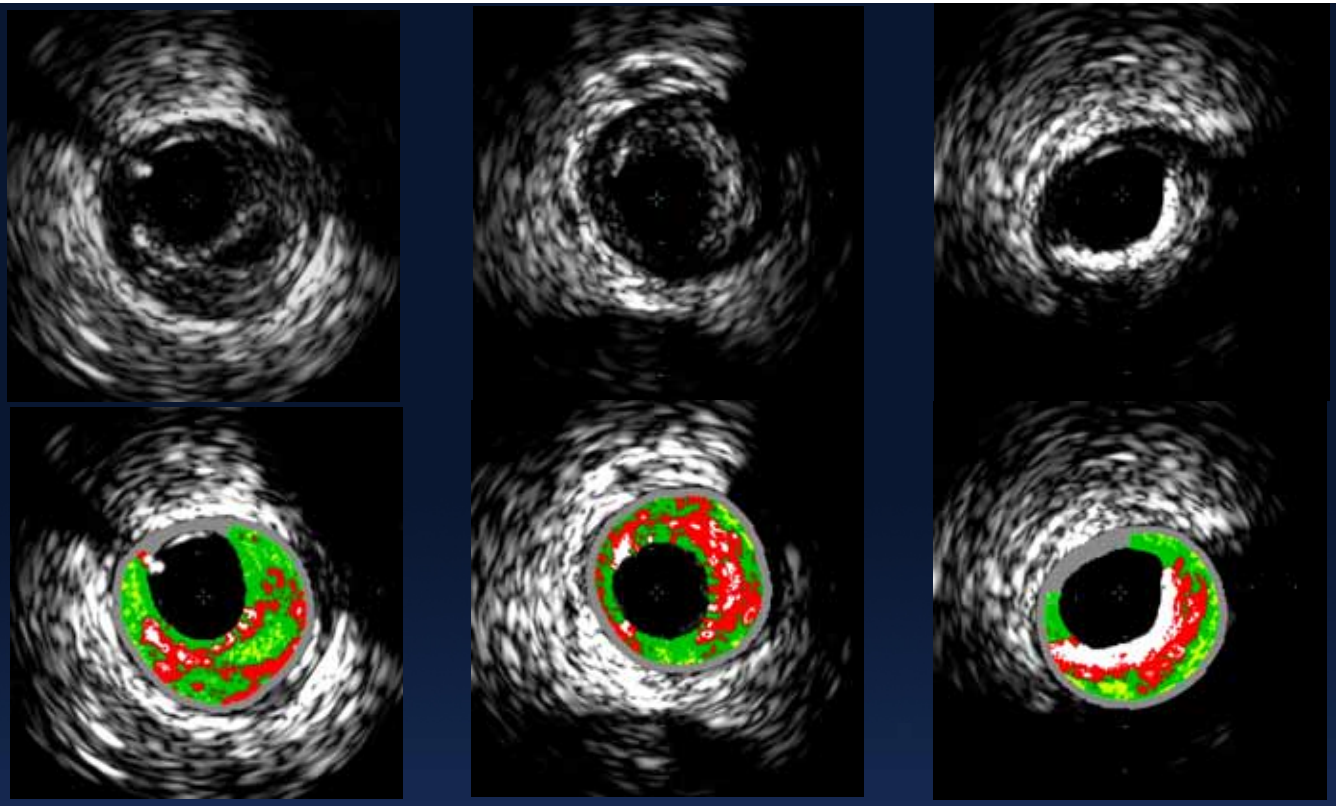


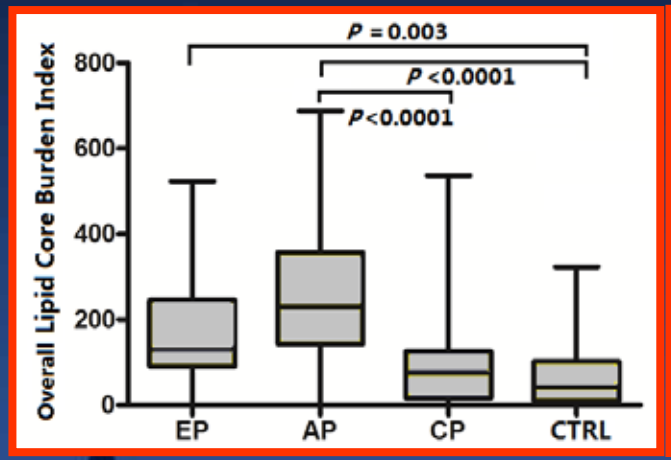


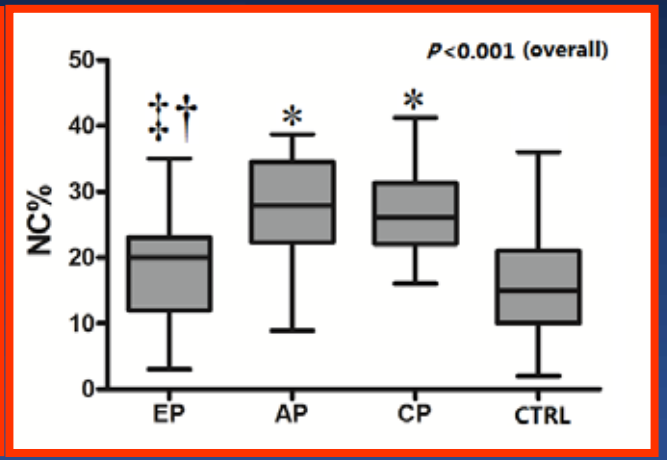


When calcified plaques were excluded, there was a significant correlation between VH-IVUS %NC vs. NIR-derived LCBI (Rho=0.496, P=0.001).





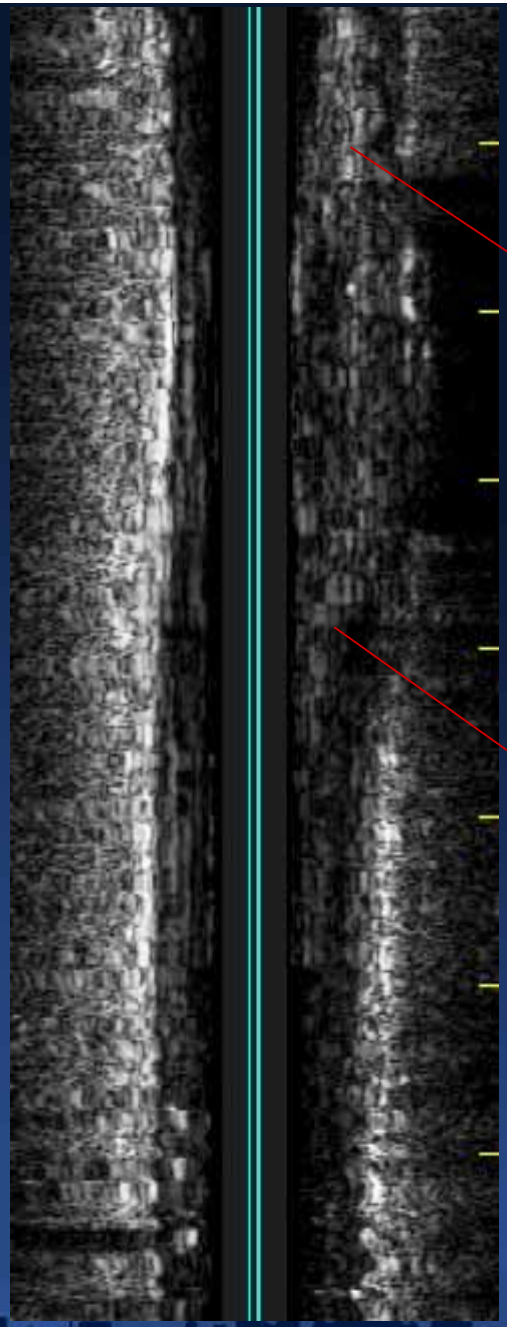

P = 0.003
P <0.0001
P<0.0001
Overall Lipid Core Burden Index
800
600
400
200
0
EP
AP
CP
CTRL

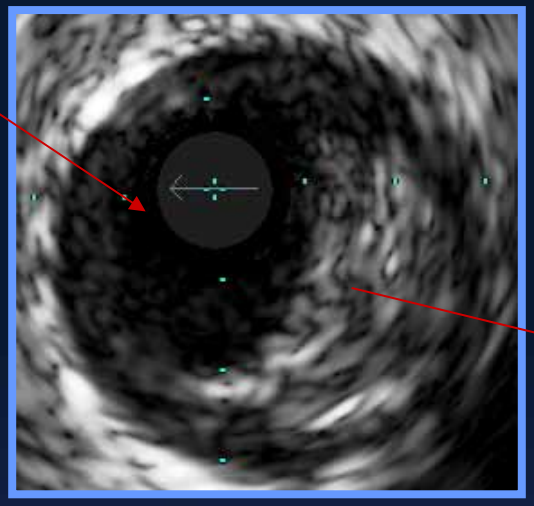

P<0.001 (overall)
NC%
50
40
30
20
10
0
EP
AP
CP
CTRL

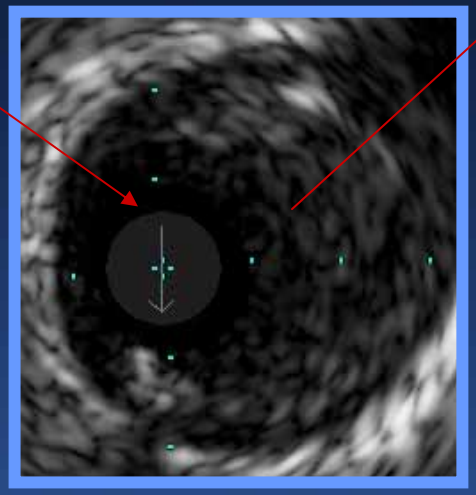
Thrombus

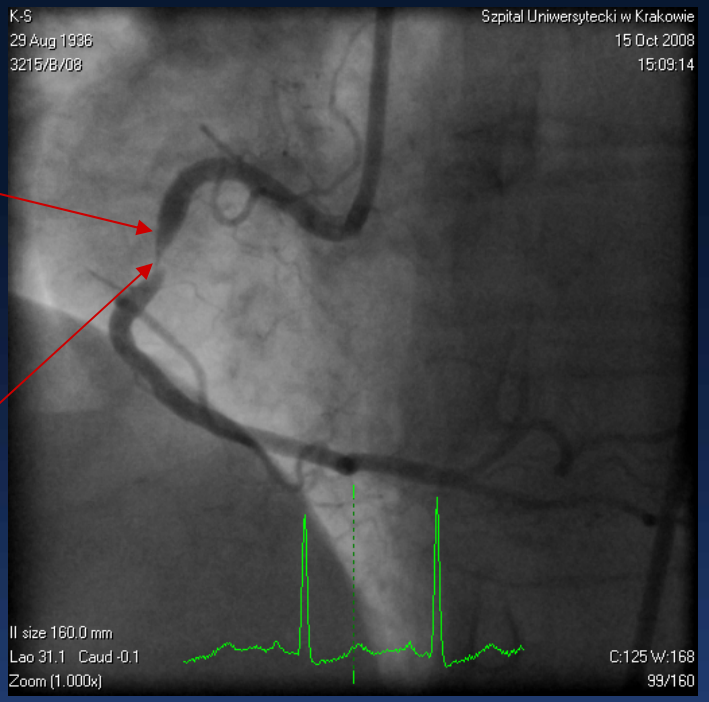
- Thrombus was detected in 81 of 259 histology slices. Thrombus was colored as fibrous or fibro-fatty by VH-IVUS.
- As a result. . .
  - Superficial thrombus will cause a TCFA to be classified as a ThFCA
  - A thrombus-containing lesion may be classified as PIT or fibrotic (stable) rather than unstable
- In all probability RF-IVUS detection of thrombus will not be possible by any technique since the IVUS signal changes with the “age” of the thrombus

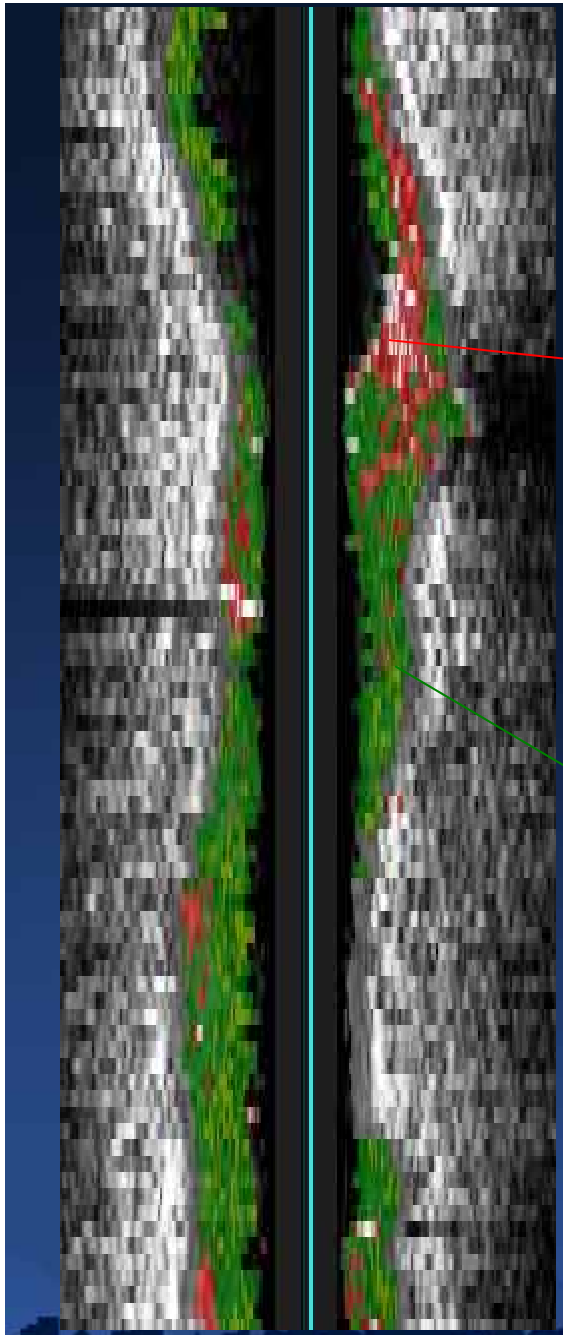
Nasu et al Am J Cardiol 2008;101:1079-83

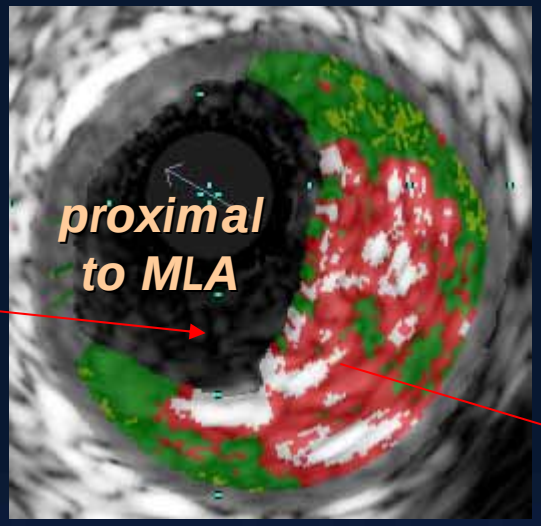


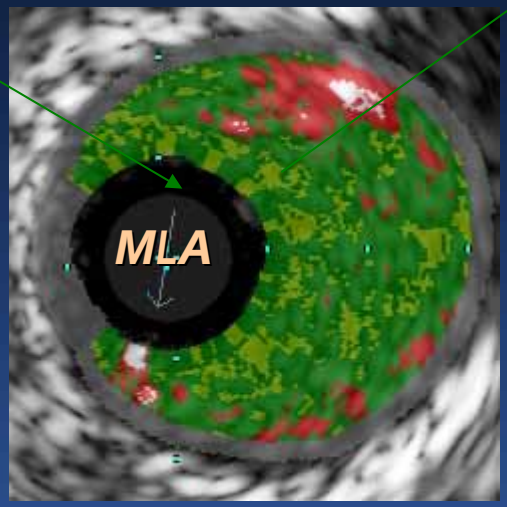


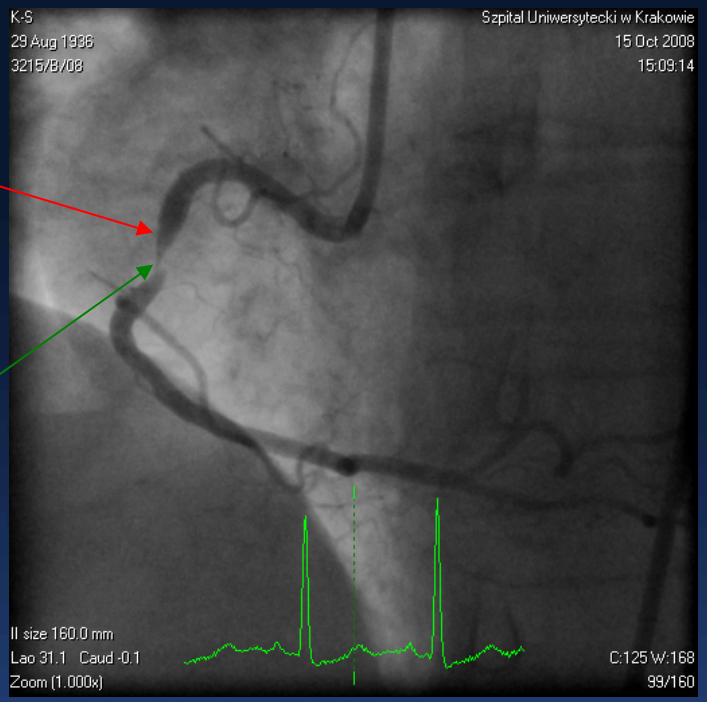



K-S
29 Aug 1936
3215/B/08
Szpital Uniwersytecki w Krakowie
15 Oct 2008
15:09:14
II size 160.0 mm
Lao 31.1 Caud -0.1
Zoom (1.000x)
C:125 W:168
99/160

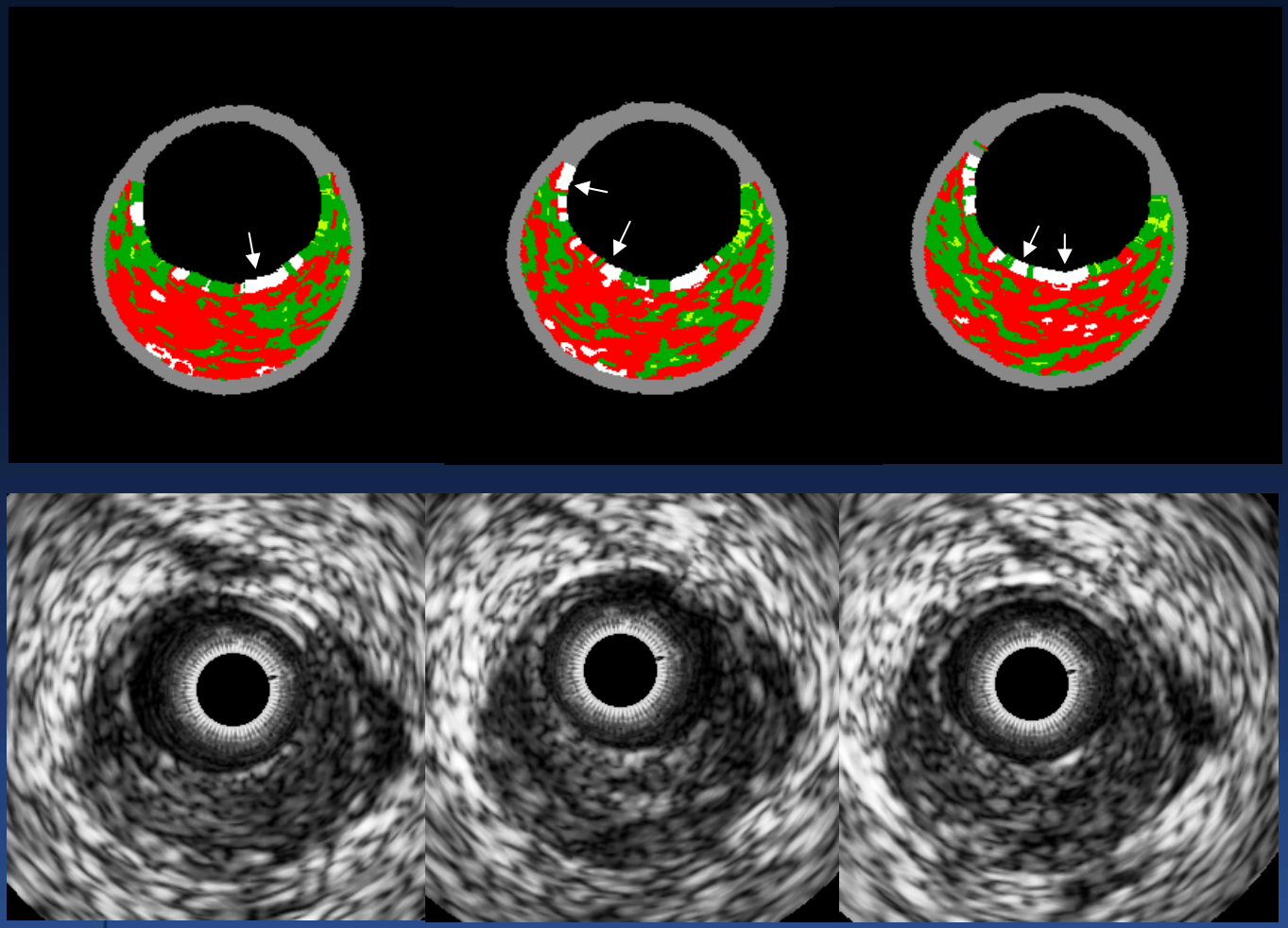



proximal
to MLA

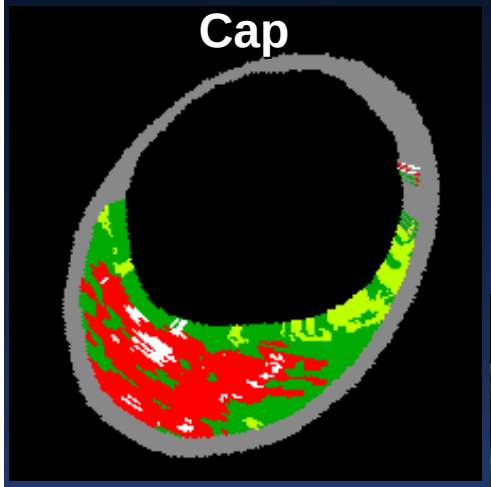

MLA

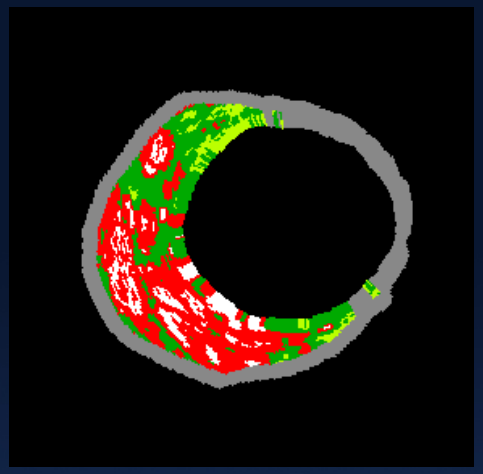

K-S
29 Aug 1936
3215/B/08
Szpital Uniwersytecki w Krakowie
15 Oct 2008
15:09:14
II size 160.0 mm
Lao 31.1 Caud -0.1
Zoom (1.000x)
C:125 W:168
99/160

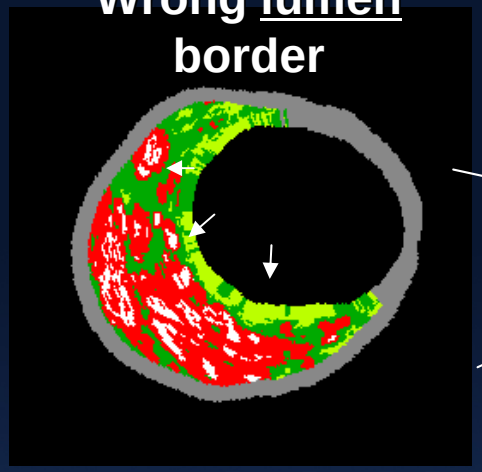
One Pixel White Border on the Surface

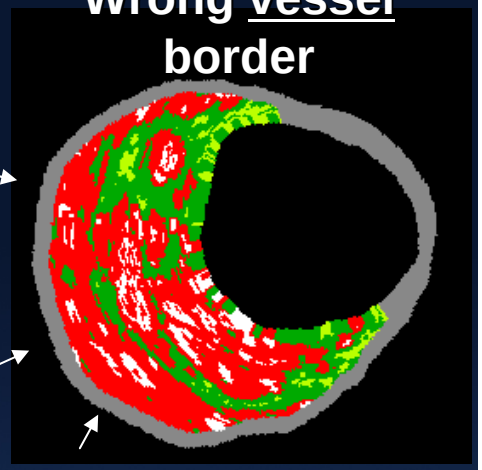


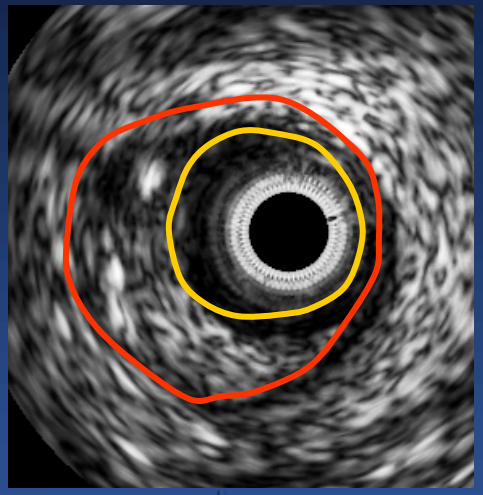
Wrong Lumen/Vessel Border

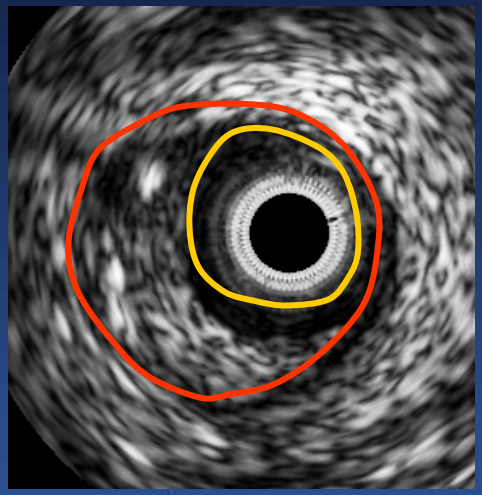


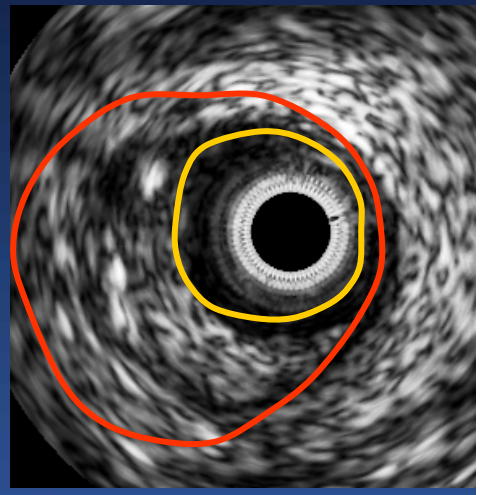








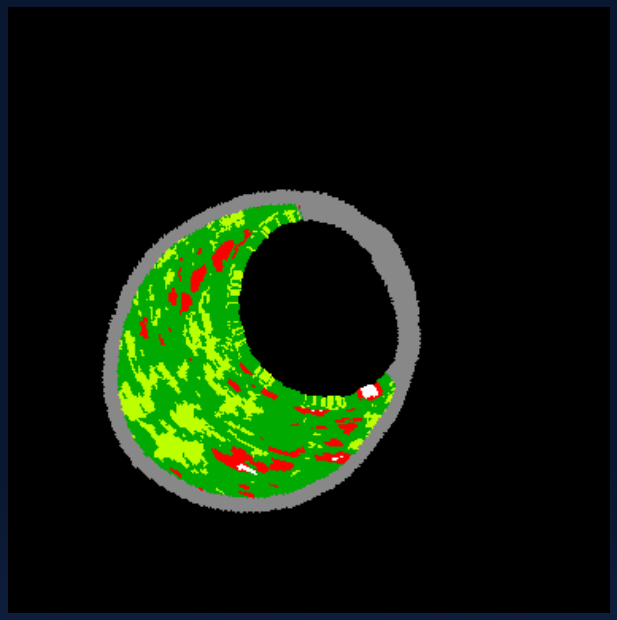


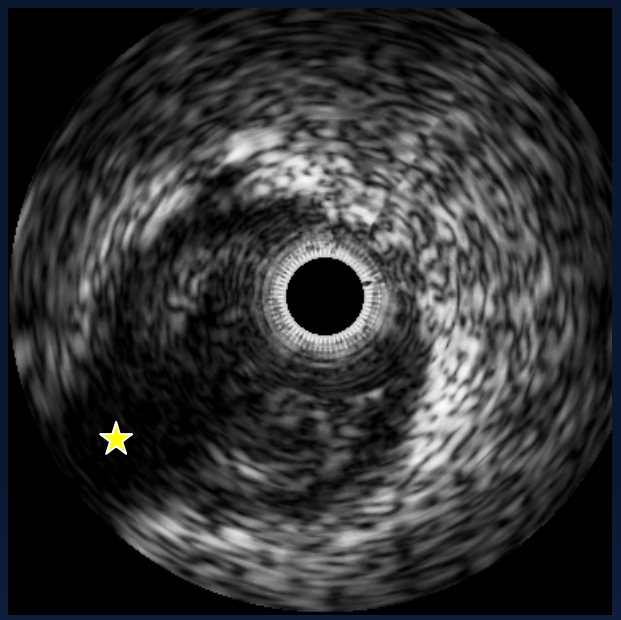


Stents

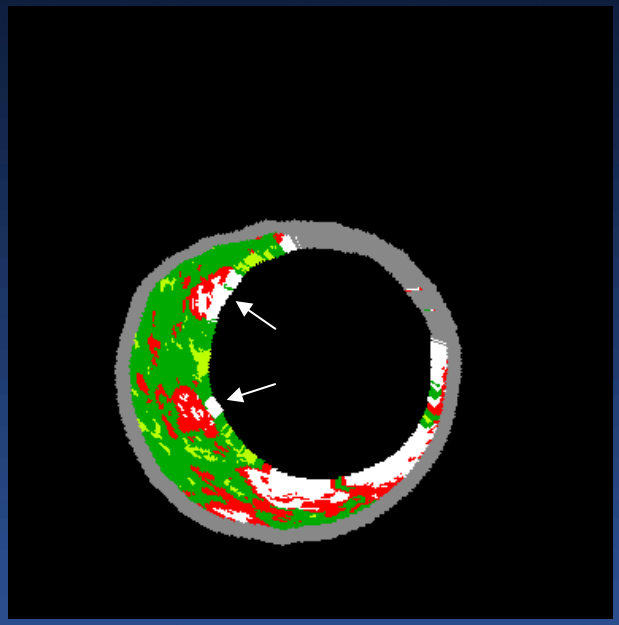
- Stent metal appears as calcium surrounded by necrotic core even when implanted acutely and should ont be interpreted as inflammation or necrotic core unless there is a baseline study for comparison
  - Kim et al. Am J Cardiol 2008;102:1182-6
- No validation for intimal hyperplasia

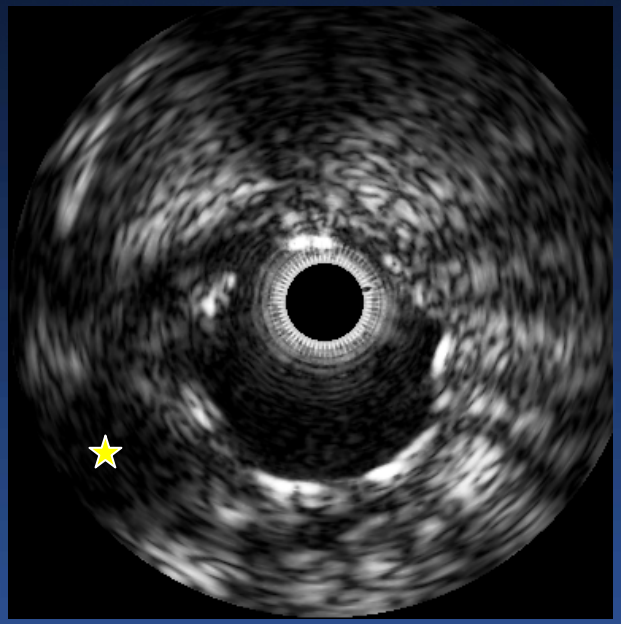
Before-Stent





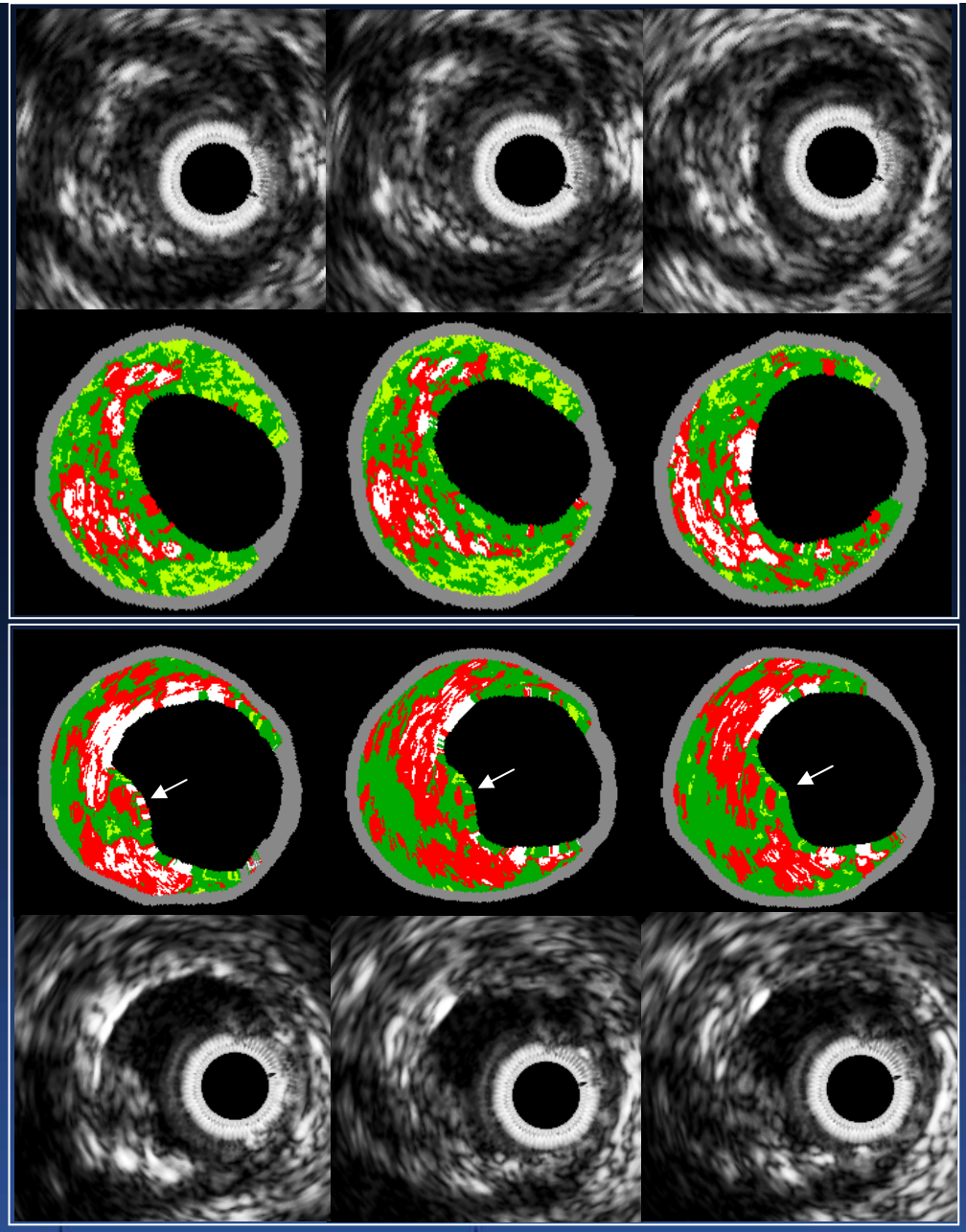
Post-Stent



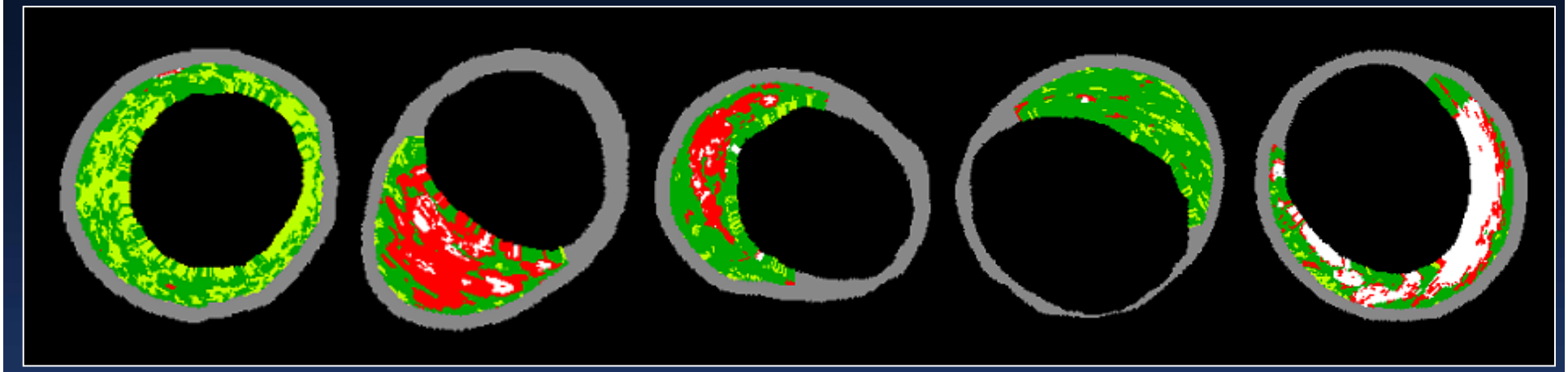


Pre-PCI

Post-Stent



Change in non-culprit lesion phenotype in 106 patients (201 lesions) with plaque burden >40%) from the Global VH Registry with baseline and 8-month follow-up VH analysis



Pathological intimal thickening (PIT)

Thin-cap fibroatheroma (TCFA)

Thick-cap fibroatheroma (ThFA)

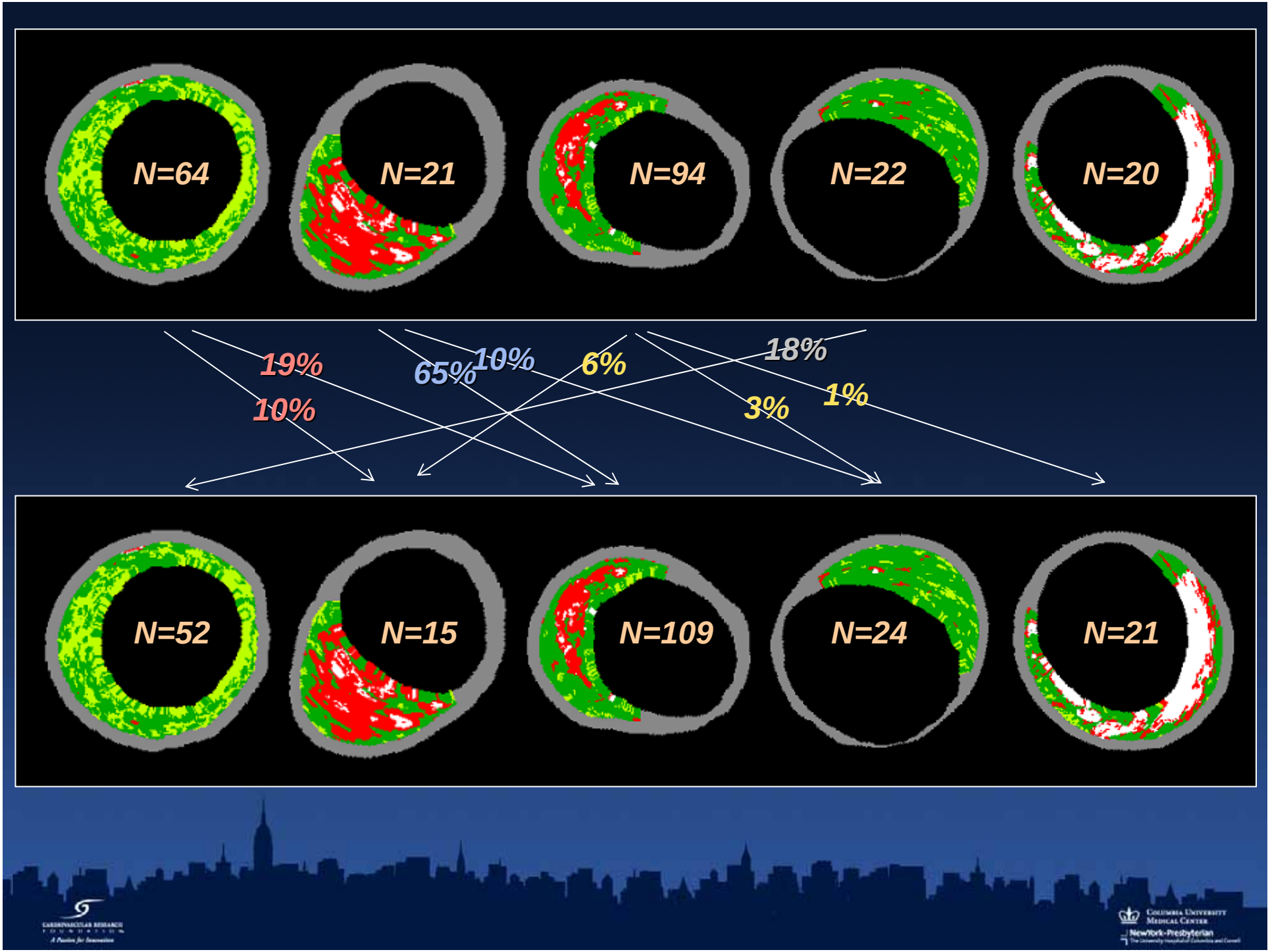
Fibrotic

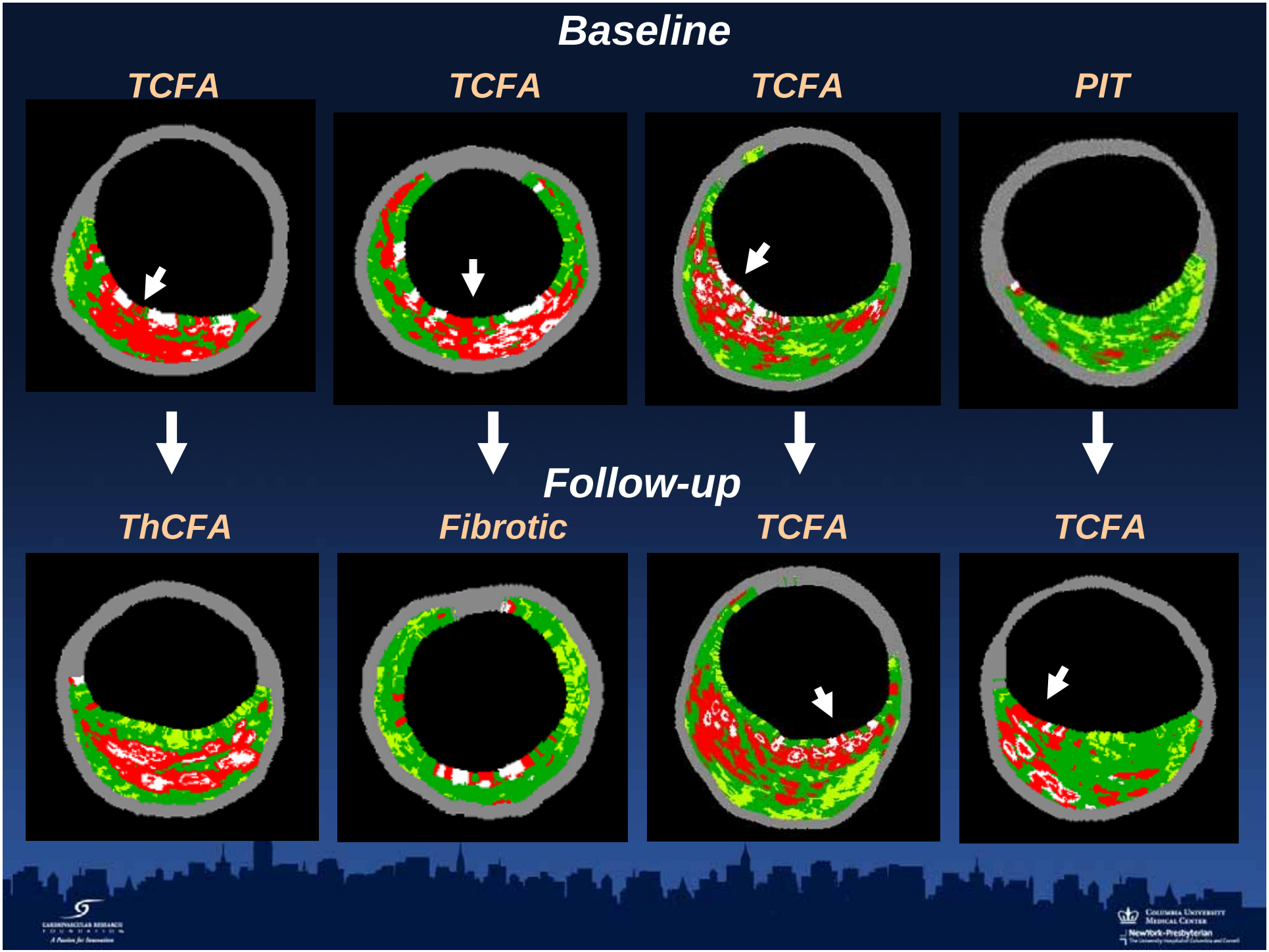
Fibrocalcif

Kubo et al. J Am Coll Cardiol 2010;55:1590-7

- During follow-up. . .
  - 75% of TCFAs healed and 25% remained unchanged although the location of the necrotic core in contact with the lumen shifted axially.
- Compared to TCFAs that healed, TCFAs that did not change were more proximal in location and had larger lumen area, vessel area, plaque area, calcium area, and necrotic core area.
- 12 new TCFAs were noted
  - 6 late-developing TCFAs were PIT and 6 were ThFA at baseline.
- No fibrotic or fibrocalcific plaques evolved into a TCFA.

Kubo et al. J Am Coll Cardiol 2010;55:1590-7


N=64
N=21
N=94
N=22
N=20
19%
10%
65%
10%
6%
18%
3%
1%
N=52
N=15
N=109
N=24
N=21


Baseline
TCFA
TCFA
TCFA
PIT
Follow-up
ThCFA
Fibrotic
TCFA
TCFA

HORIZONS-AMI non-culprit