

Micropatterned Human iPSC-Derived Cardiomyocytes

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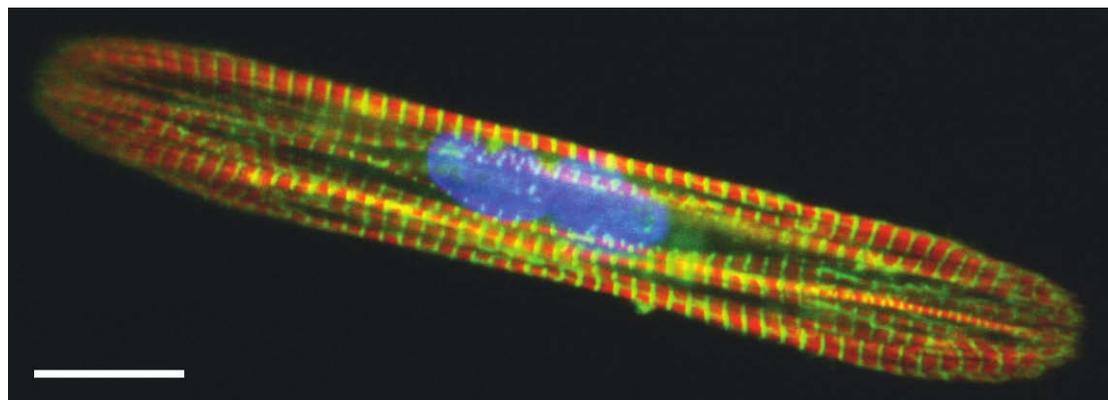
Division of Applied Regulatory Science

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- Disclaimer: The opinions expressed in this presentation are mine and do not necessarily reflect the official views of the U.S. Food and Drug Administration (FDA)
- No conflicts of interest

Additional disclaimer

- Most of the work presented here was not developed at the FDA:
 - Stanford University
 - Stanford Cardiovascular Institute
 - Gladstone Institute of Cardiovascular Disease
- At FDA, we are now evaluating the regulatory potential of micropatterned iPSC-derived cardiomyocytes

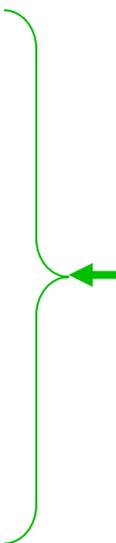
FDA Division of Applied Regulatory Science (DARS) in CDER



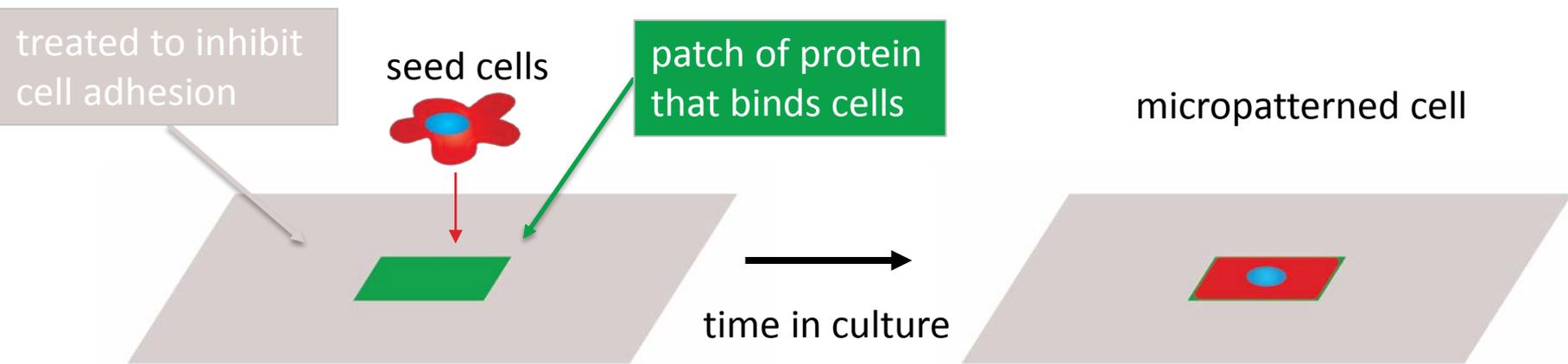
Vision

- To move new science into the CDER review process and close the gap between scientific innovation and product review

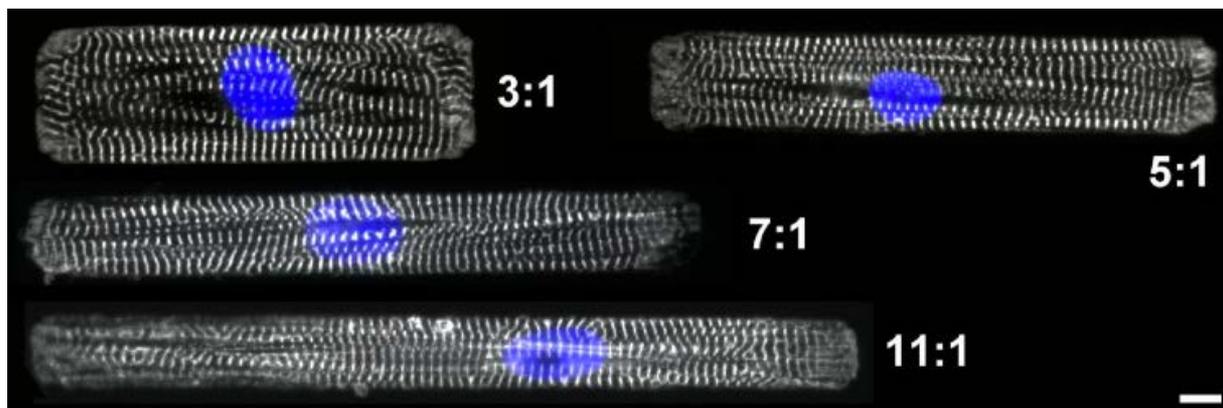
What does DARS do?

- Performs mission-critical applied research to develop and evaluate tools, standards and approaches to assess the safety, efficacy, quality and performance of drugs  Evaluate the regulatory potential of cellular systems
- Performs expert regulatory review consultations for mechanistic safety evaluation for immediate regulatory needs combining
 - Critical review of existing knowledge
 - Computational analyses with informatics tools and disease-pharmacology models
 - In vitro and in vivo laboratory studies
 - Translational analysis of preclinical studies, clinical trials and postmarket data support internal reviews

Micropatterning: engineer cell shape and size in 2D, 2.5D and 3D



Murine neonatal CMs patterned with different aspect ratios: **3:1, 5:1, 7:1, 11:1**



The regulatory use of *in vitro* cellular systems stands on two principles

- Cell function is physiologically relevant:
 - Mature, mature-like or mature enough for context of use/ fit for purpose
 - Predict clinical effects in specific contexts of use/ fit for purpose
- Functional endpoints should be comprehensively assessed:
 - Metrics of contractile function (i.e. force, tension, sarcomere length, velocity, rate dependent effects, etc.)
 - Electrophysiology parameters (i.e. upstroke velocity, resting membrane potential, action potential amplitude, duration, etc.)

Key takeaways today

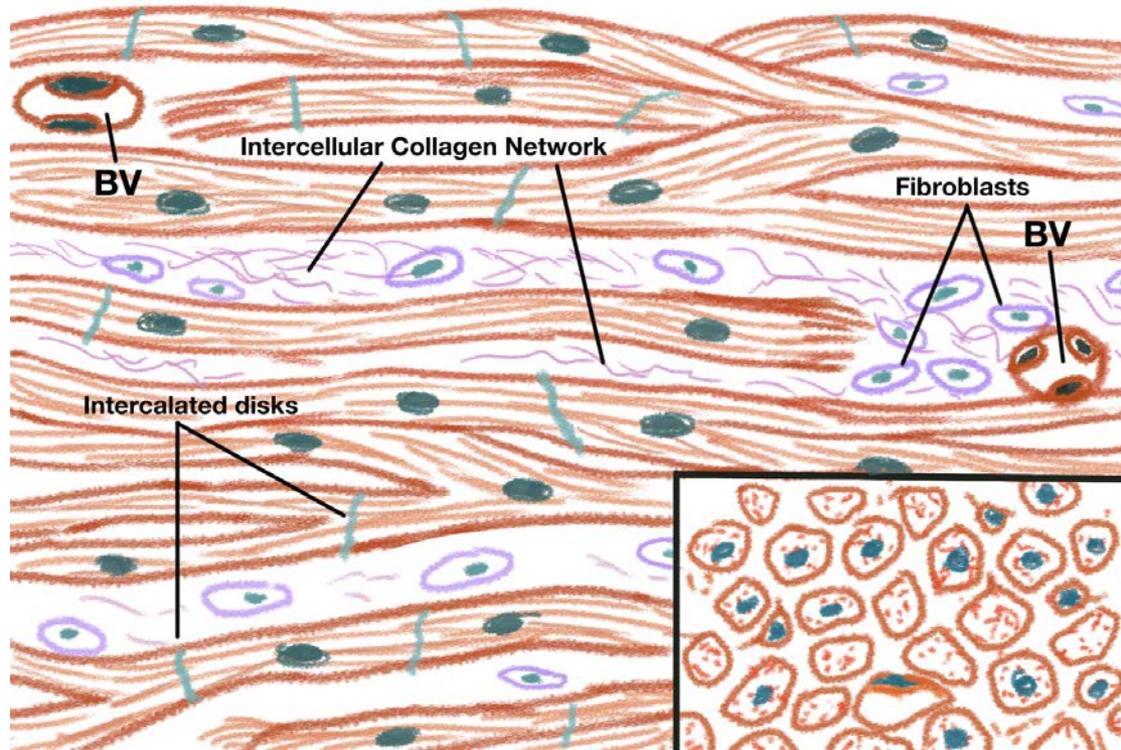
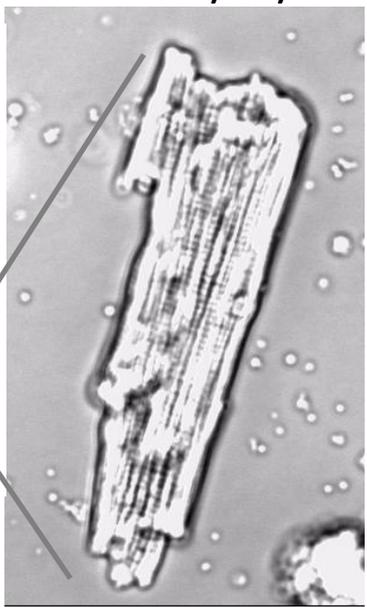
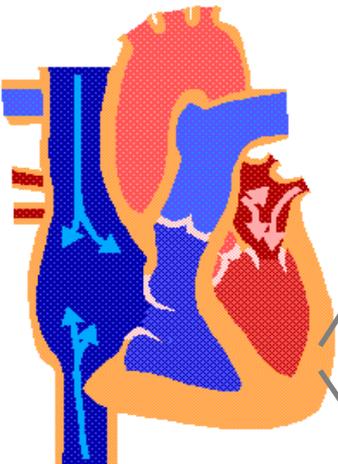
1. Micropatterning iPSC-cardiomyocytes into a physiological shape on soft materials enhances their physiological relevance
2. Contractility can be comprehensively assessed with micropatterned cells
3. The regulatory potential of this technology still needs to be evaluated

- Cell function is physiologically relevant

- Functional endpoints should be comprehensively assessed

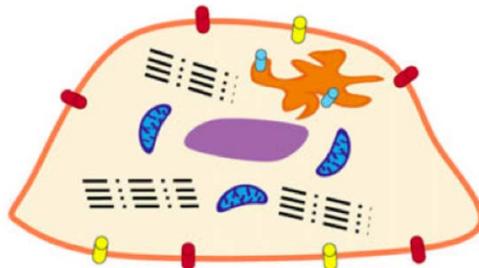
Cellular morphology and alignment of myofibrils are markers of cardiomyocyte maturity

mouse single cardiomyocyte



iPSC-cardiomyocytes have fetal-like morphology and myofibril organization

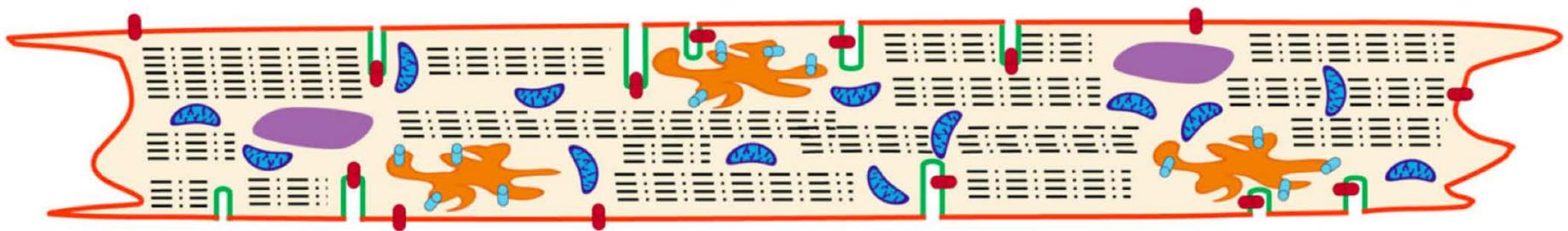
fetal-like cell



iPSC-cardiomyocytes

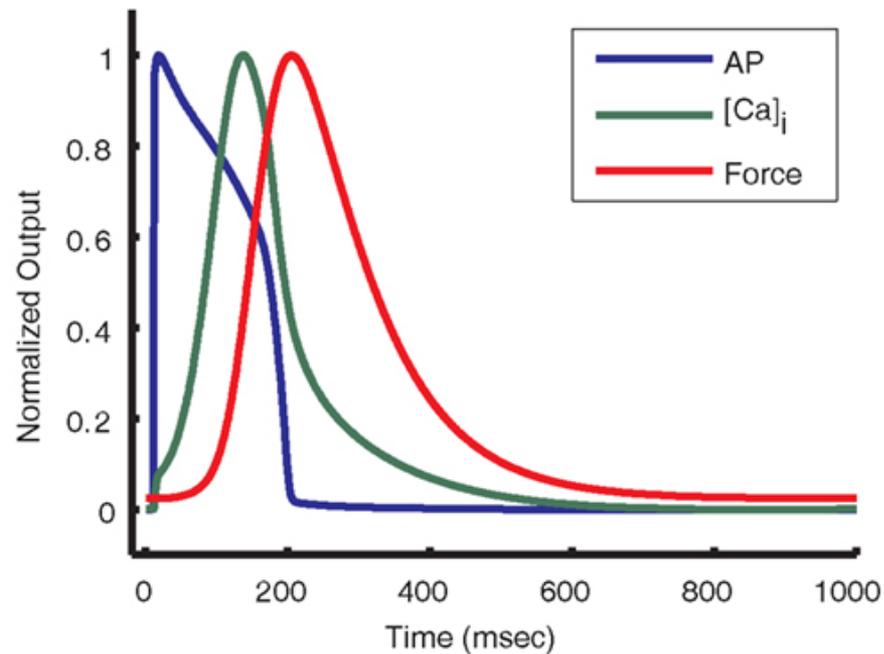
- Nuclei
- Sarcomere
- Mitochondria
- Sarcoplasmic Reticulum
- L-Type Ca^{2+} Channel
- T-Type Ca^{2+} Channel
- SR Ca^{2+} -ATPase
- Sarcolemma
- T-Tubule

mature cell

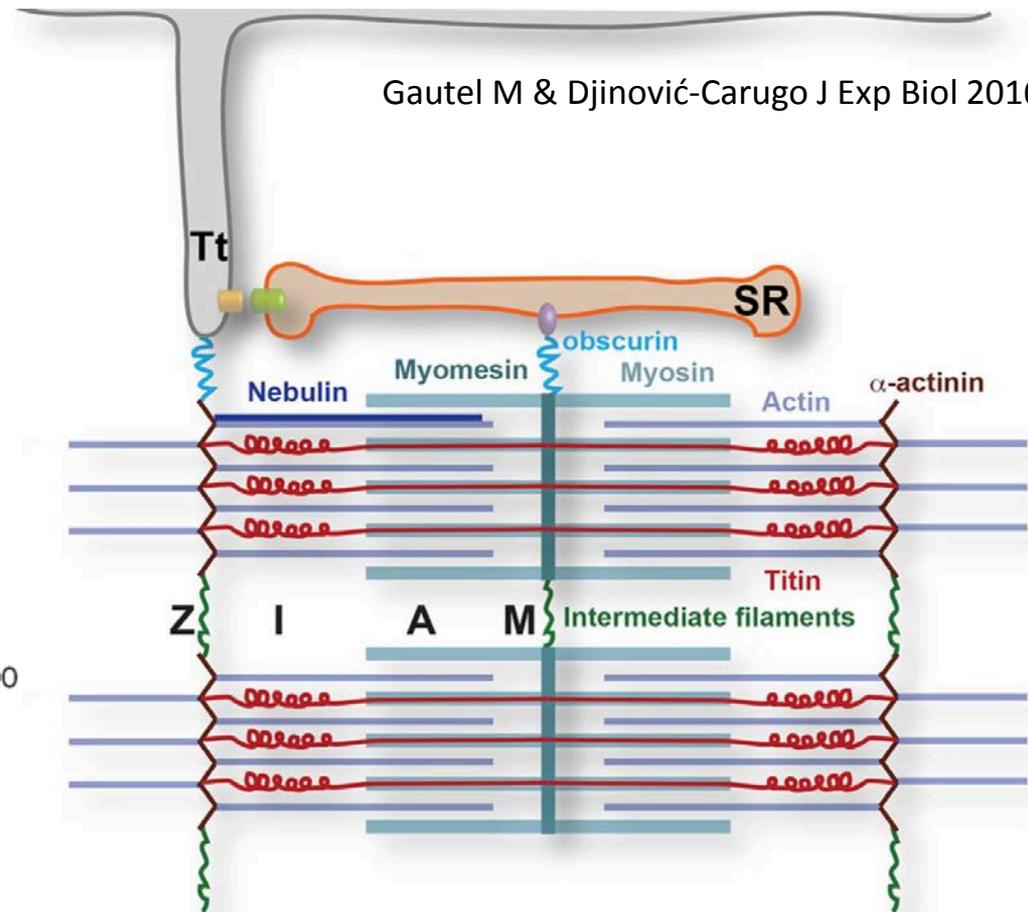


primary adult cardiomyocytes

Contractility is a cellular function downstream of action potential and calcium release

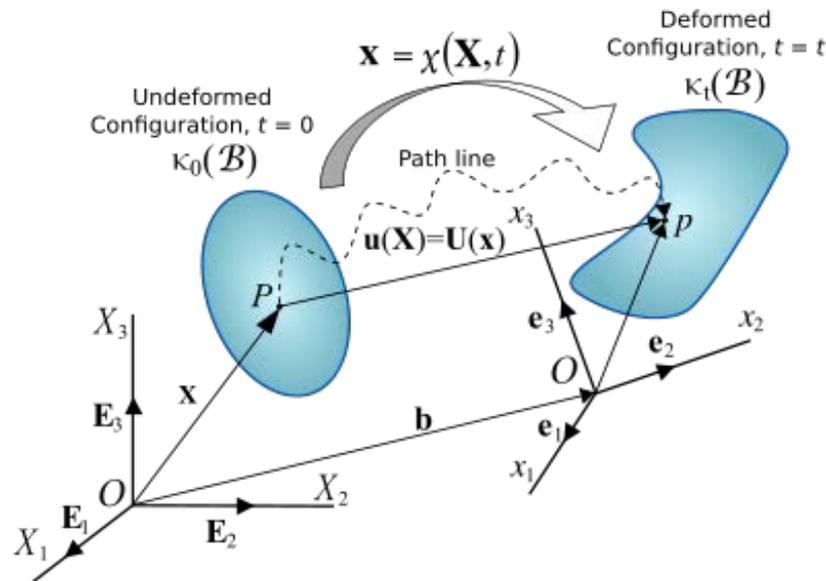


Gautel M & Djinović-Carugo J Exp Biol 2016



Gauthier LD et al. Circ Res 2012

Contractility is a biomechanical endpoint



Mechanics studies how **Force Fields** in a **Body** relate to variations in its **Morphology**

mechanical endpoints of cardiomyocytes

contractility

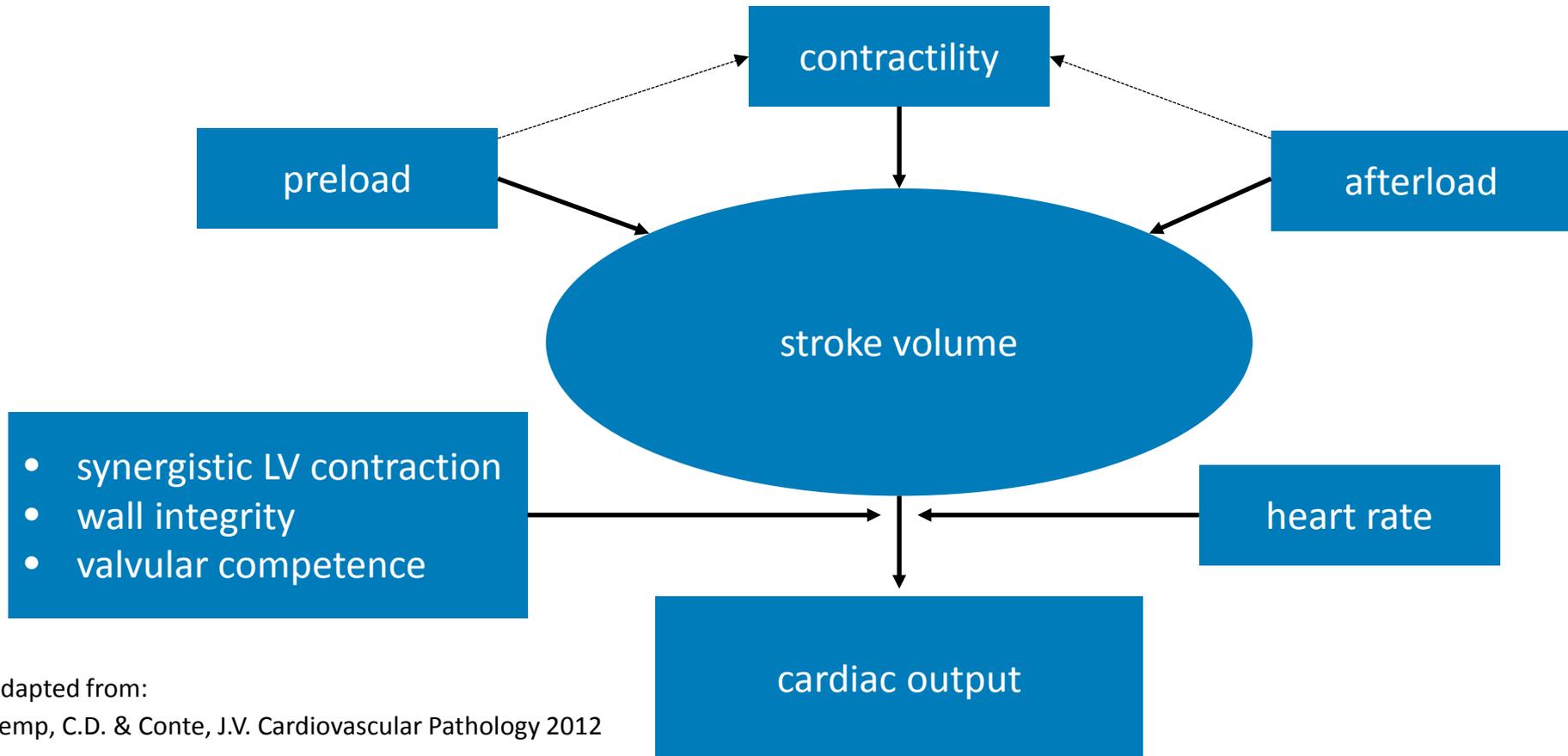
- force (N)
- stress (Pa)
- tension (N)
- extracellular load (N)
- cell/tissue rigidity (N/m)

kinetics

- beat rate (bps)
- contraction velocity (m/s)
- relaxation velocity (m/s)
- duration of contractions (s)

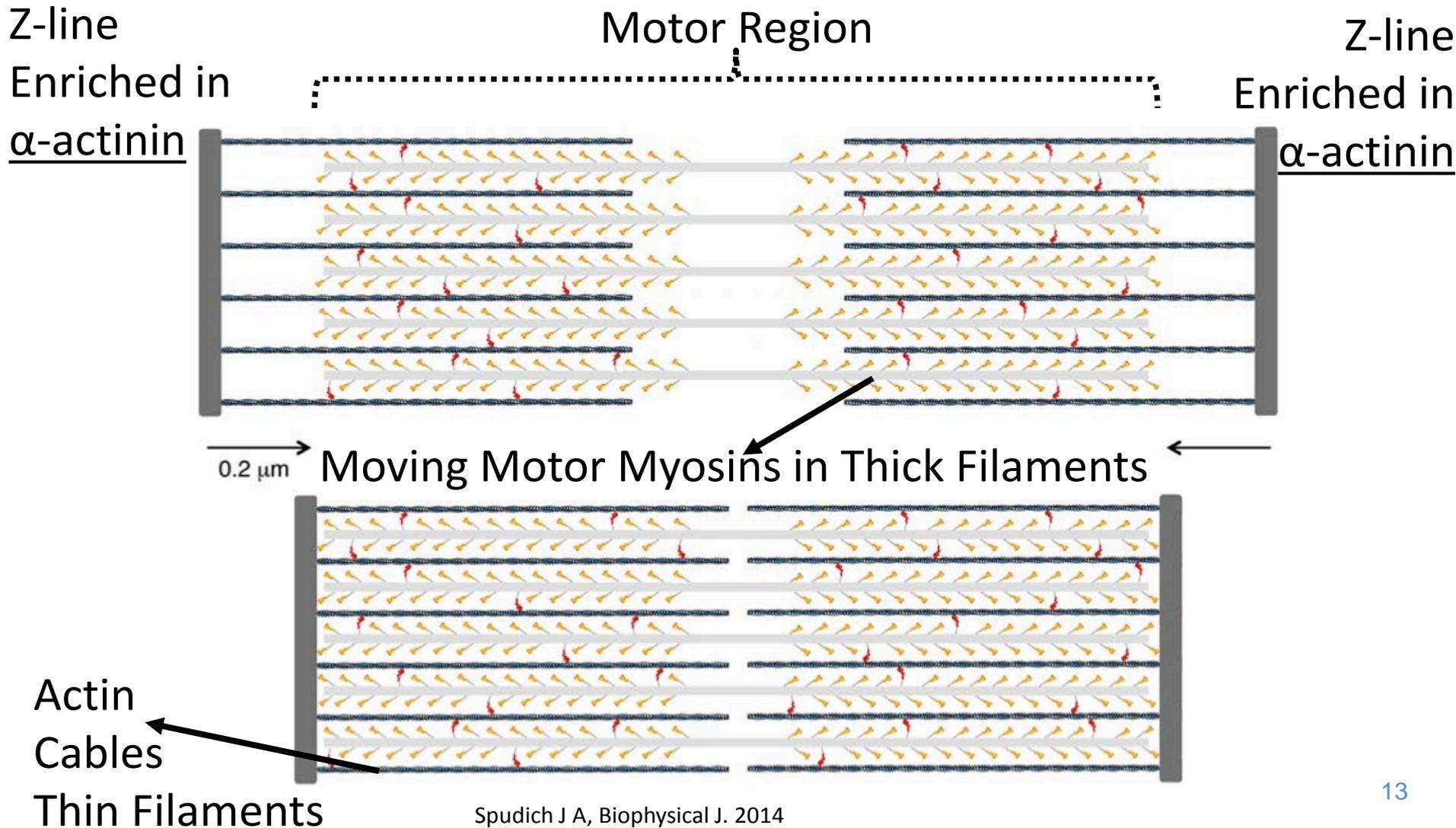
power = force x velocity

Intracellular contractile and biophysical mechanisms affect cardiac output

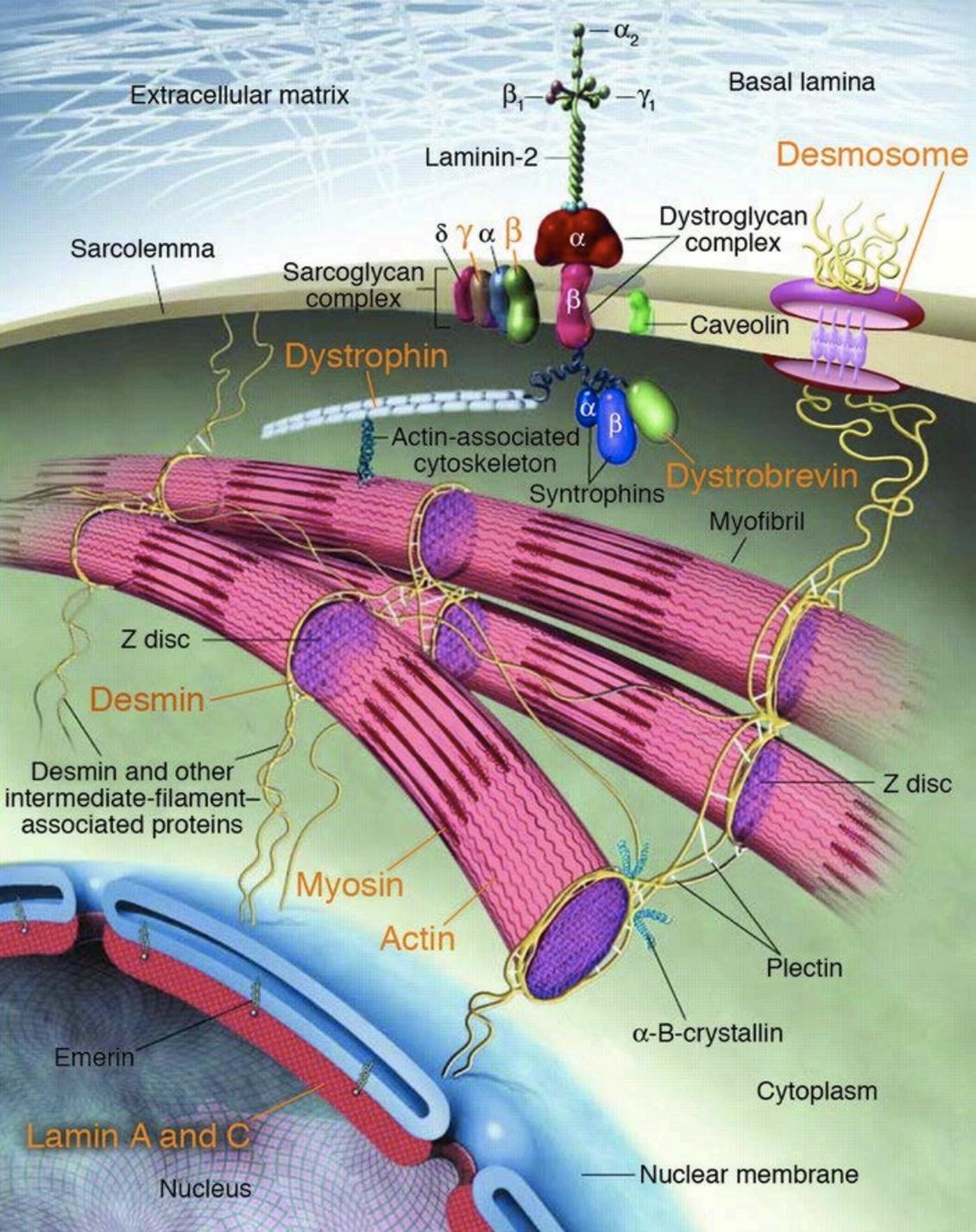


Adapted from:
Kemp, C.D. & Conte, J.V. Cardiovascular Pathology 2012

Sarcomeres are the contractile functional units



Z-lines physically connect each other and connect other mechanical structures

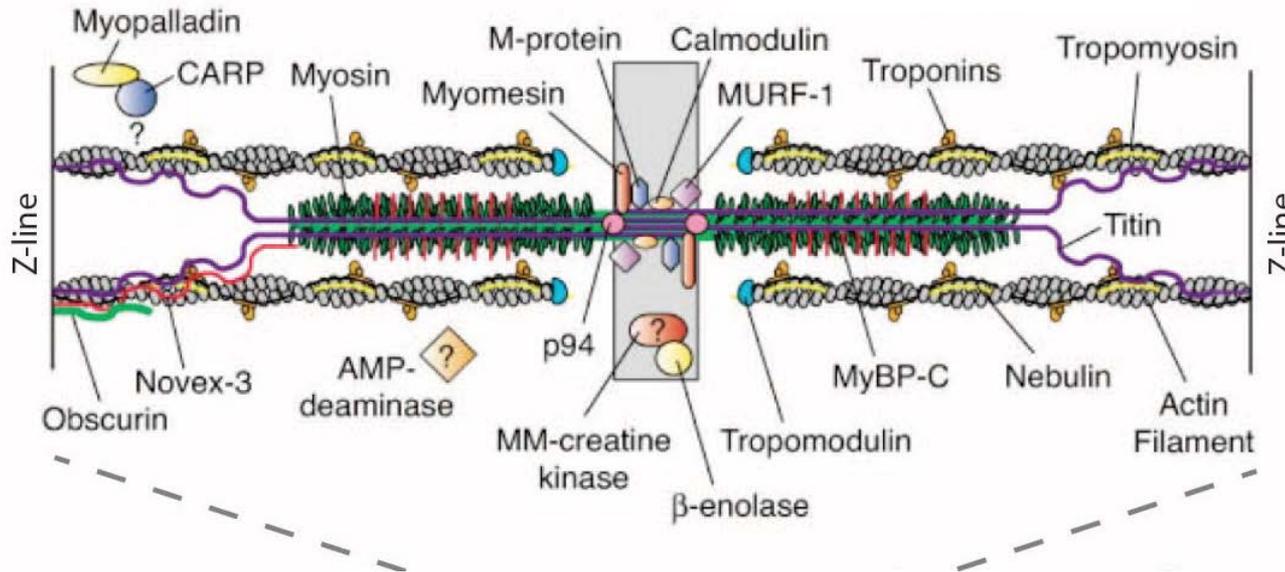


Morita J et al. J Clin Res 2005

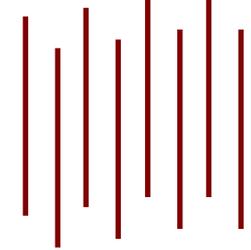
Primary cardiomyocytes have aligned contractile fibers and well registered Z-lines



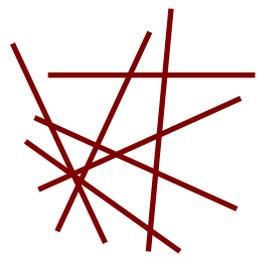
Between Z-lines



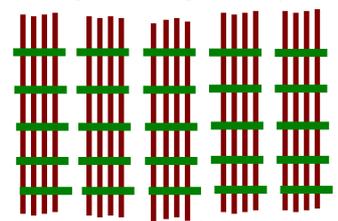
aligned



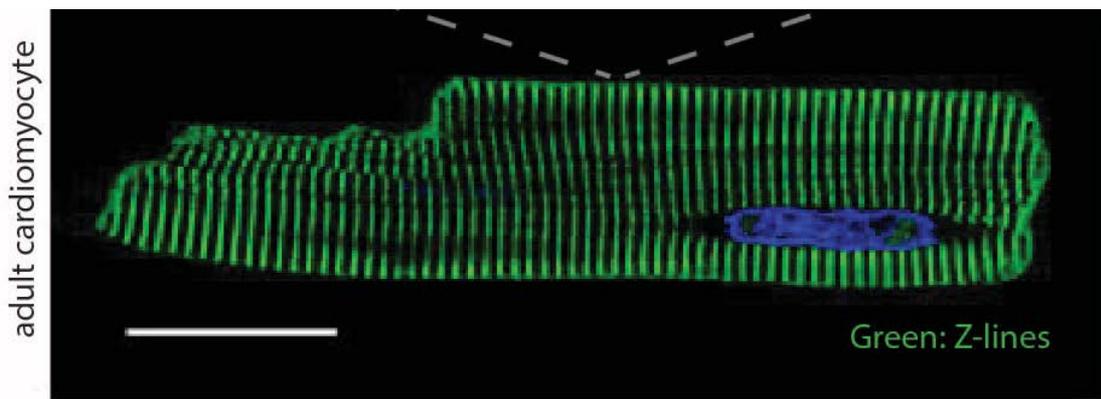
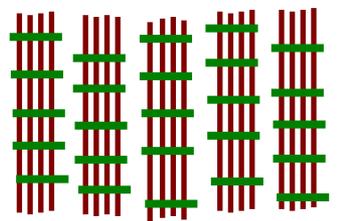
non-aligned



high registry

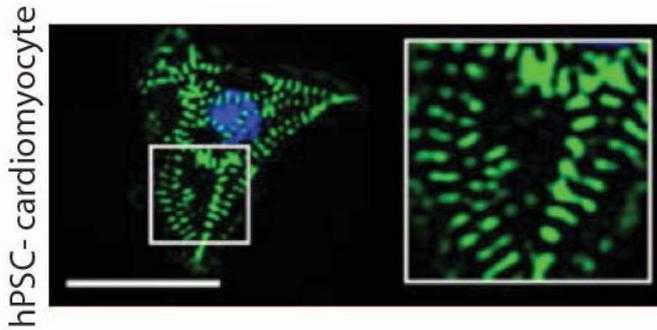


low registry

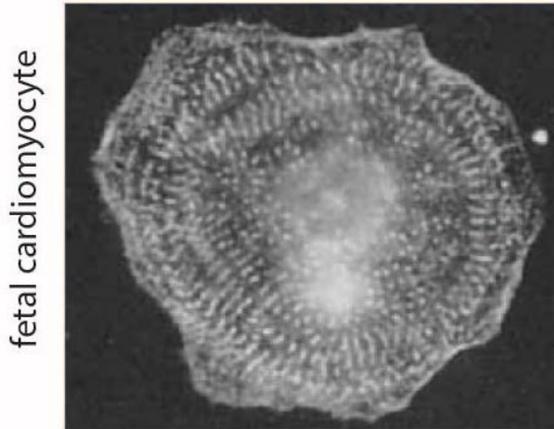


Clark, KA et al. Annu Rev Cell Dev Biol 2002

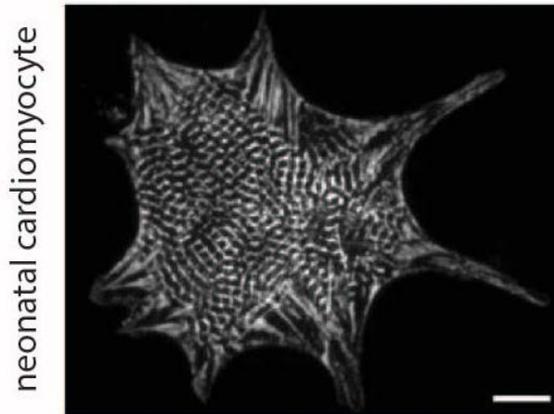
Yang, X et al. Circ Res 2014



Yang, X et al. Circ Res 2014



Rhee, D et al. Cell Motil Cytoskeleton 1994

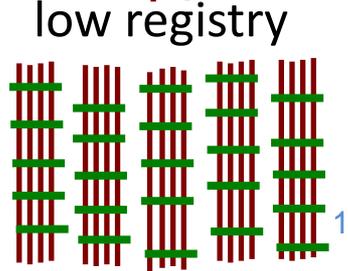
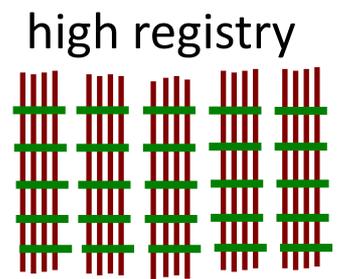
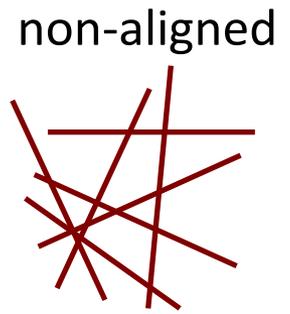
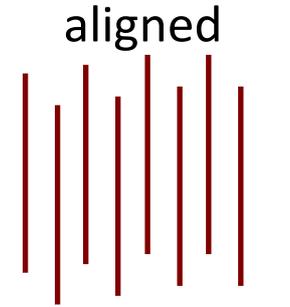


Bray, MA et al. Cell Motil Cytoskeleton 2008

Stem cell derived cardiomyocytes have:

- Non-aligned fibers
- Low registry of Z-lines

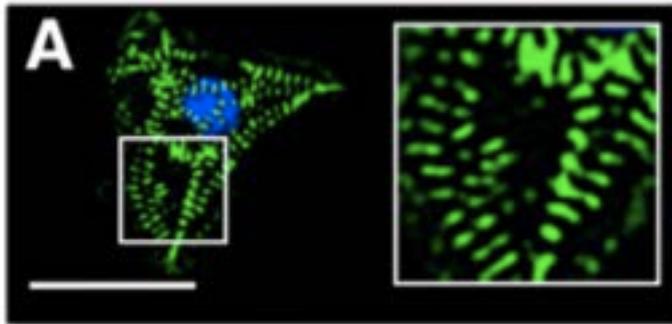
immature



The notion of cell immaturity is a moving target

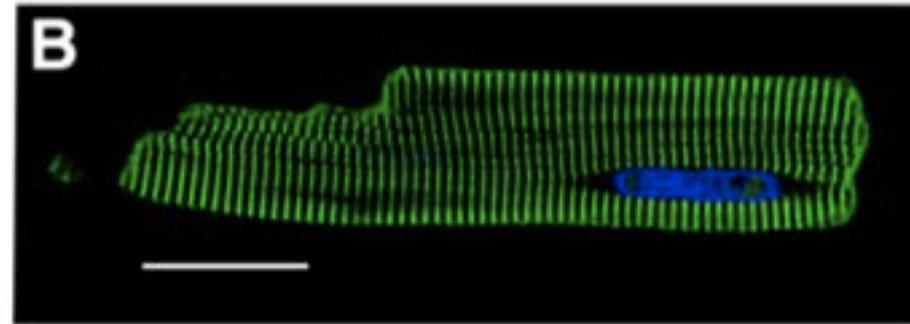


Parameters		Immature CMs	Mature CMs	Methods / Metrics
Sarcomere	Structure / Length / Stability	Disarrayed / 1.6 μm / fluid	Organized / 2.2 μm / fixed	FFT / measure length / dynamic of myofibrils
Mitochondria	Distribution	Occupies a small fraction	\approx 40% of cell	Mitochondria Dies
Binucleation	Number	Mononucleated	\approx 25% binucleated	Counting
Electrophysiology	Upstroke velocity / Resting potential	\approx 50 V/s / -60mV	\approx 250 V/s / -90 mV	Patch clamp
Excitation-Contraction Coupling	Pacing of Cells	Partially Developed - relate to calcium	Mature	Calcium imaging
Contractile Force	Biomechanical Phenotypes	\approx nN range/cell	\approx μN range/cell	Traction Force Microscopy



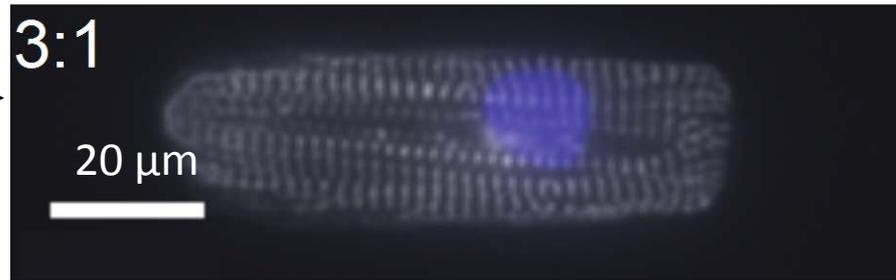
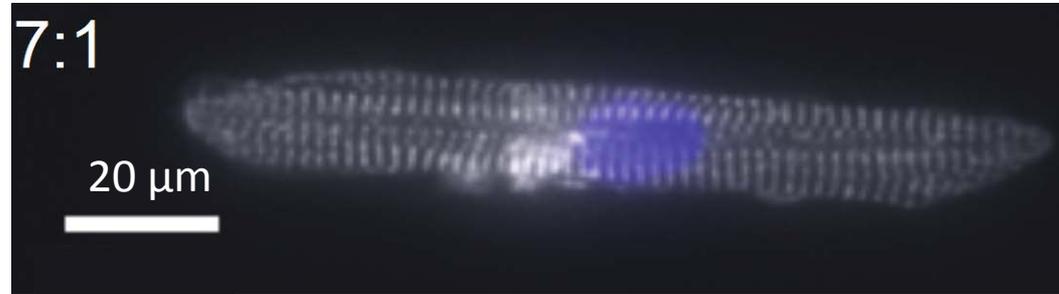
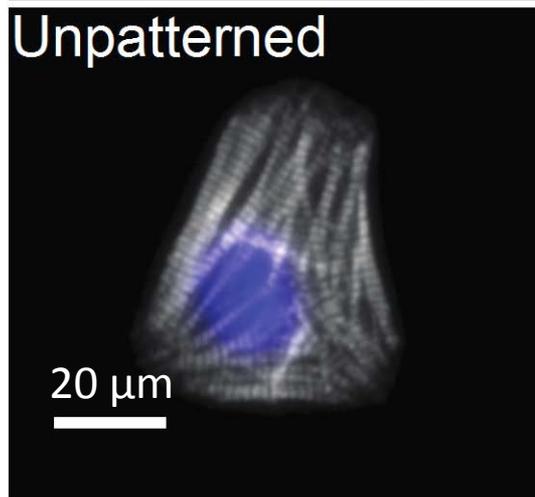
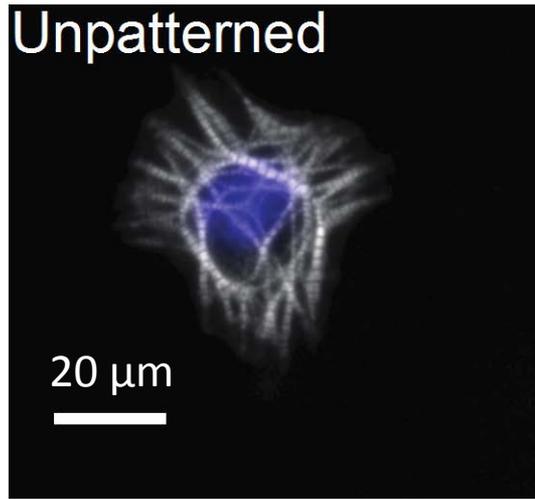
Stem Cell – CM
Immature

Yang, X et al. Circ Res 2014



Adult CM
Mature

We use micropatterning with iPSC-cardiomyocytes



- micropatterning of extracellular matrix
- soft material to measure contractility

2015: four manuscripts reporting an enhancement of physiology when iPSC-cardiomyocytes were cultured on soft materials and rectangular shape



Biomaterials. 2015 May;51:138-150.

Functional maturation of human pluripotent stem cell derived cardiomyocytes in vitro-correlation between contraction force and electrophysiology.

Ribeiro MC, Tertoolen LG, Guadix JA, Bellin M, Kosmidis G, D'Aniello C, Monshouwer-Kloots J, Goumans MJ, Wang YL, Feinberg AW, Mummery CL, Passier R.

Proc Natl Acad Sci U S A. 2015 Oct 13;112(41):12705-10.

Contractility of single cardiomyocytes differentiated from pluripotent stem cells depends on physiological shape and substrate stiffness.

Ribeiro AJ, Ang YS, Fu JD, Rivas RN, Mohamed TM, Higgs GC, Srivastava D, Pruitt BL.

Circ Res. 2015 Dec 4;117(12):995-1000.

Matrigel Mattress: A Method for the Generation of Single Contracting Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes.

Feaster TK, Cadar AG, Wang L, Williams CH, Chun YW, Hempel JE, Bloodworth N, Merryman WD, Lim CC, Wu JC, Knollmann BC, Hong CC.

Stem Cell Reports. 2015 Dec 8;5(6):1226-1238.

Integrated Analysis of Contractile Kinetics, Force Generation, and Electrical Activity in Single Human Stem Cell-Derived Cardiomyocytes.

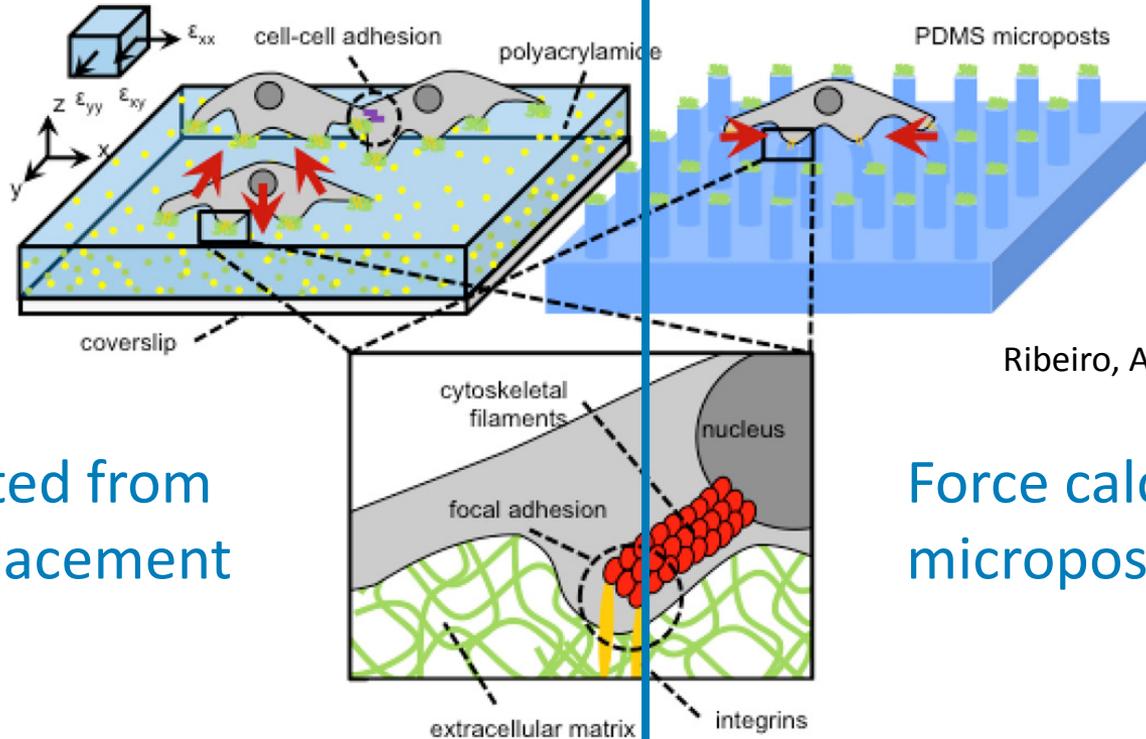
Kijlstra JD, Hu D, Mittal N, Kausel E, van der Meer P, Garakani A, Domian IJ.

Observed phenotypes of maturity (**in bold**)



- Function
 - **Electrophysiology**
 - Positive force-frequency relation
 - **Calcium flow**
 - Excitation-contraction coupling
 - **Contractile force**
 - Response to **inotropic**, chronotropic and dromotropic **drugs**
- Structure
 - **Size and shape**
 - **Aligned myofibrils**
 - **Sarcomeres laterally registered**
 - Composition of sarcomeres
 - **T-tubules**
 - **Binucleation**

Attaching cardiomyocytes to force sensors enables contractility measurements



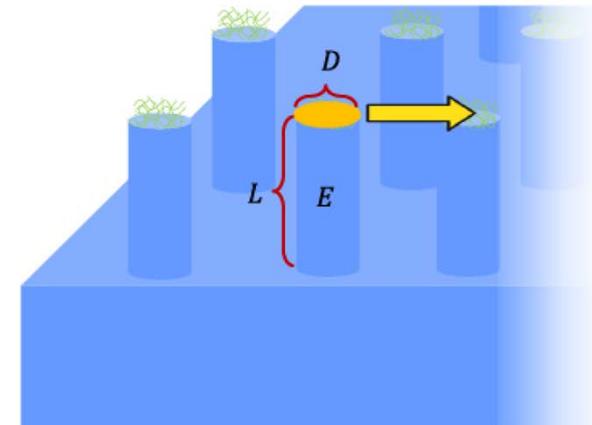
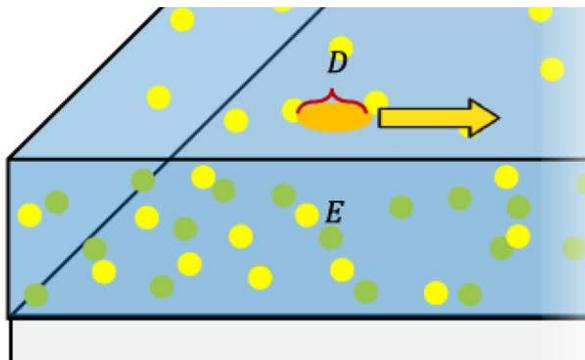
Ribeiro, AJ et al. Methods 2016

Force calculated from material displacement

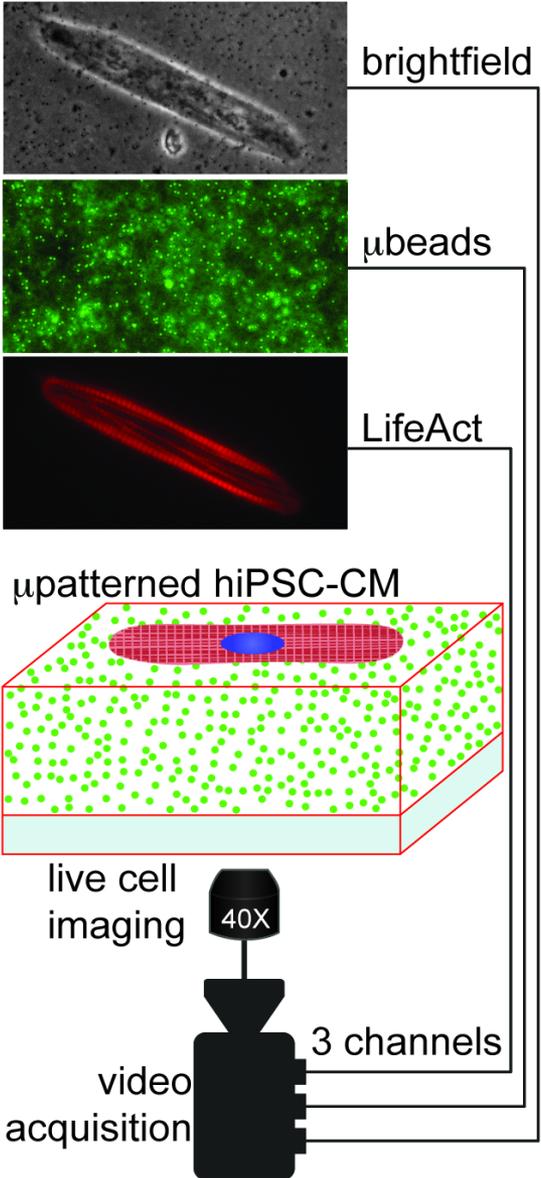
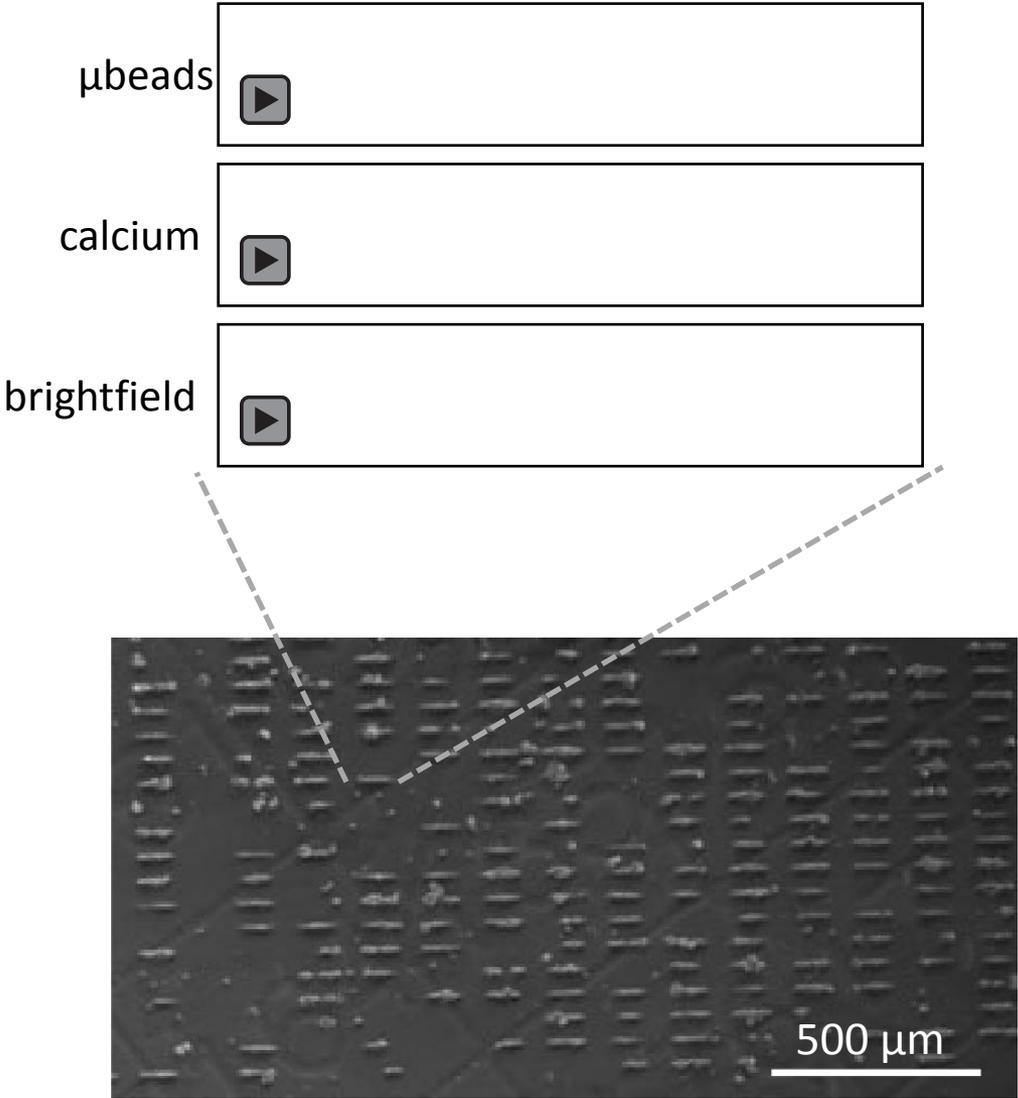
Force calculated from micropost bending

$$k = \frac{\pi ED}{2(2 - \nu)(1 + \nu)}$$

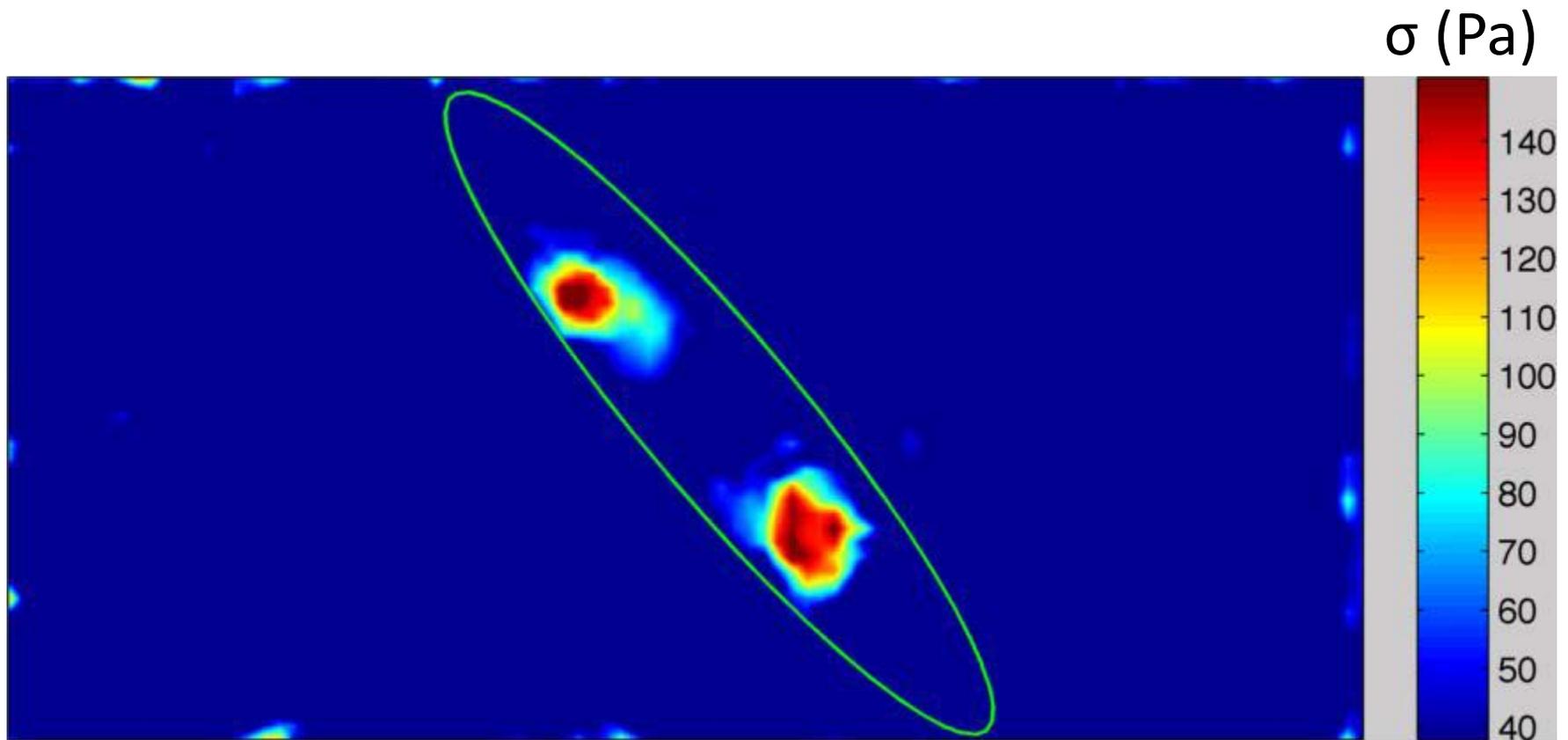
$$k = \frac{3\pi ED^4}{64L^3}$$



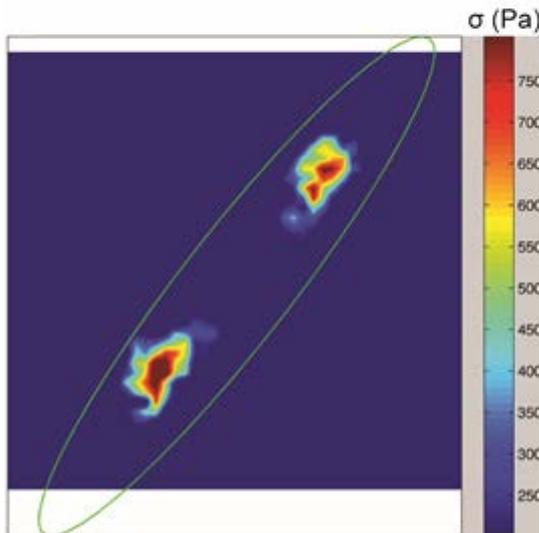
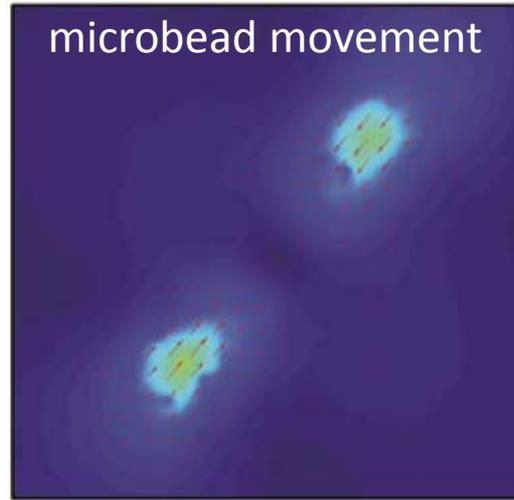
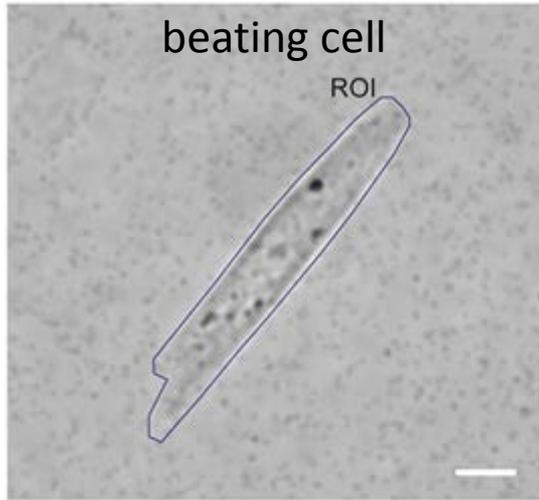
Traction force microscopy estimates cell-generated forces from the displacement of the soft material



We calculate force from the movement of the soft material under each cell

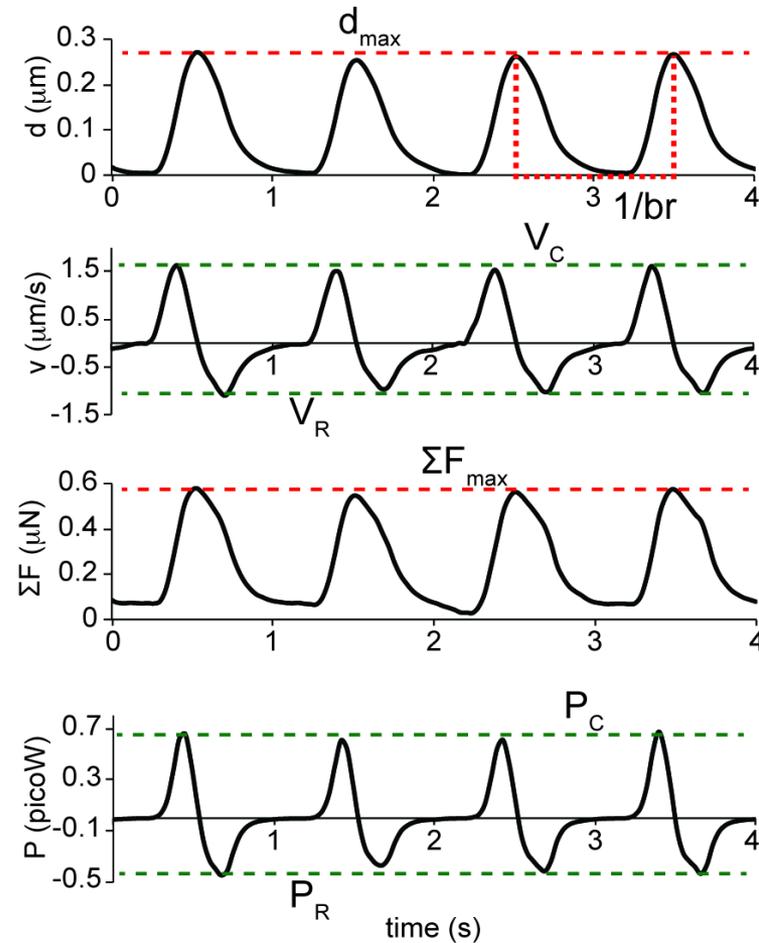


We obtain parameters that quantify the contractile performance of each cell

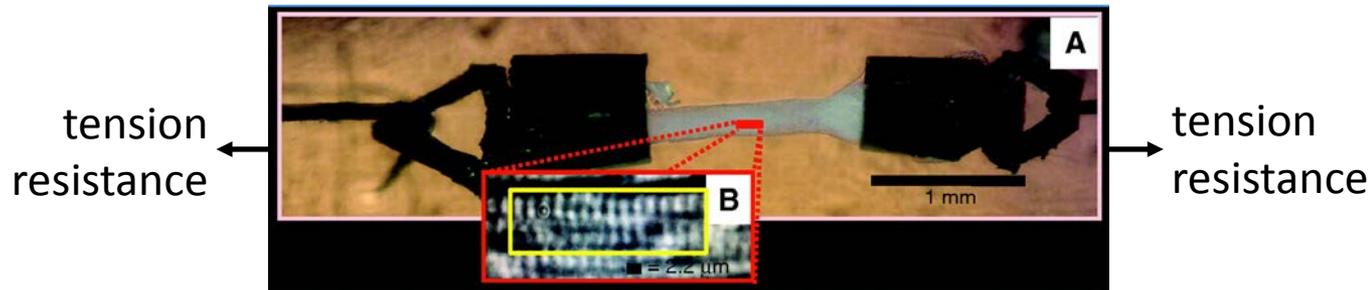
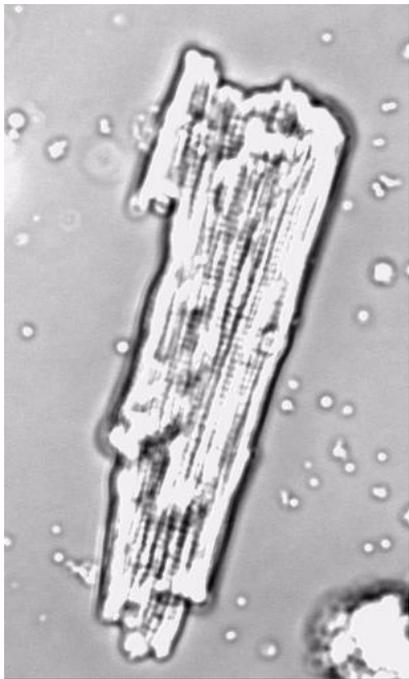


$$\text{Map of traction stresses} = \frac{\text{Force}}{\text{Pixel Area}}$$

contractile parameters



Isolated cardiomyocytes or cardiac trabeculae are traditional systems for assaying contractility



- endpoints:
- cell shortening
 - sarcomere shortening
 - frequency dependence

- endpoints:
- force
 - isometric tension
 - sarcomere shortening
 - load-dependent relations

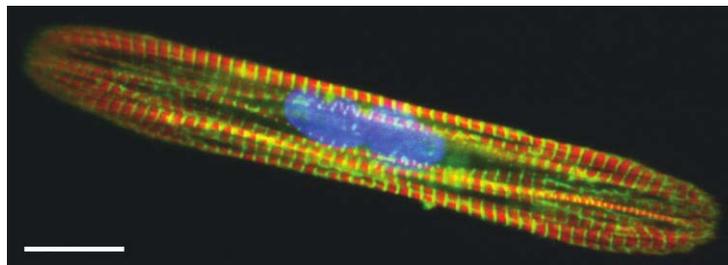
main gaps:

- human material is rare
- genetic manipulation limited
- not suitable for chronic measurements of drug effects

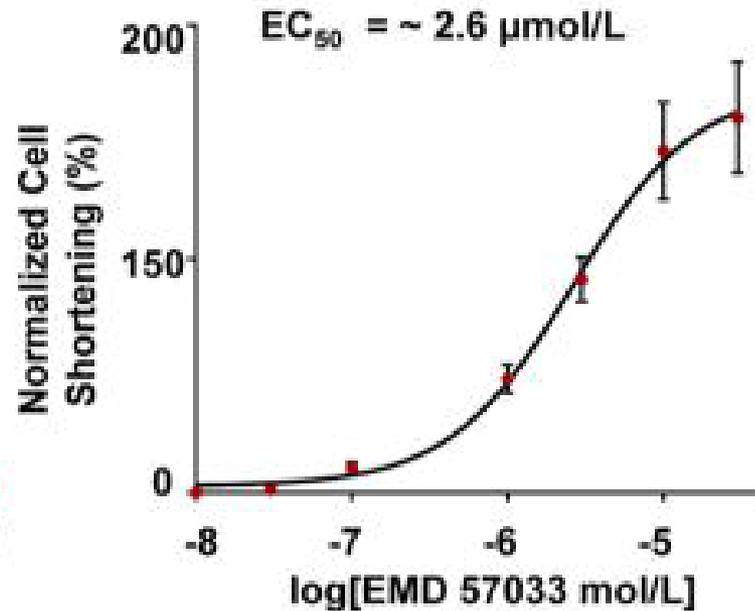
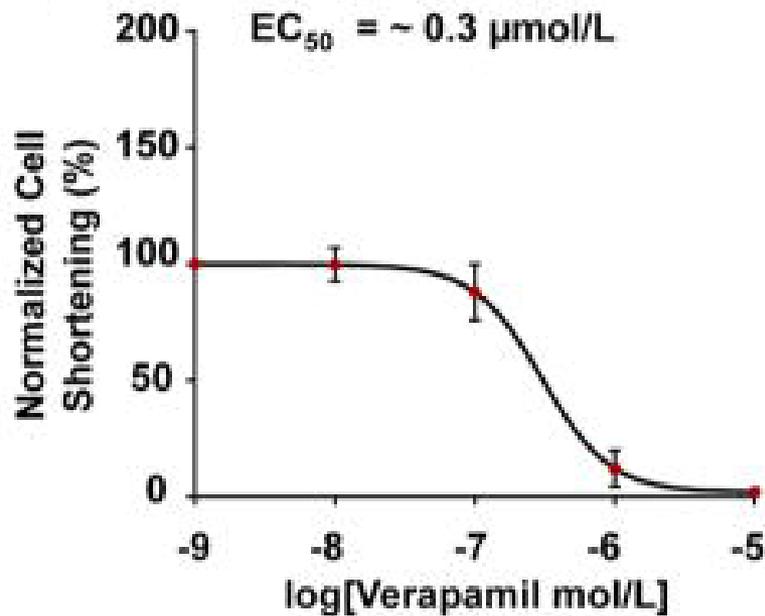
Difficult to attach primary cardiomyocytes to force sensors

Contractility of single matured iPSC-cardiomyocytes on soft materials for drug testing

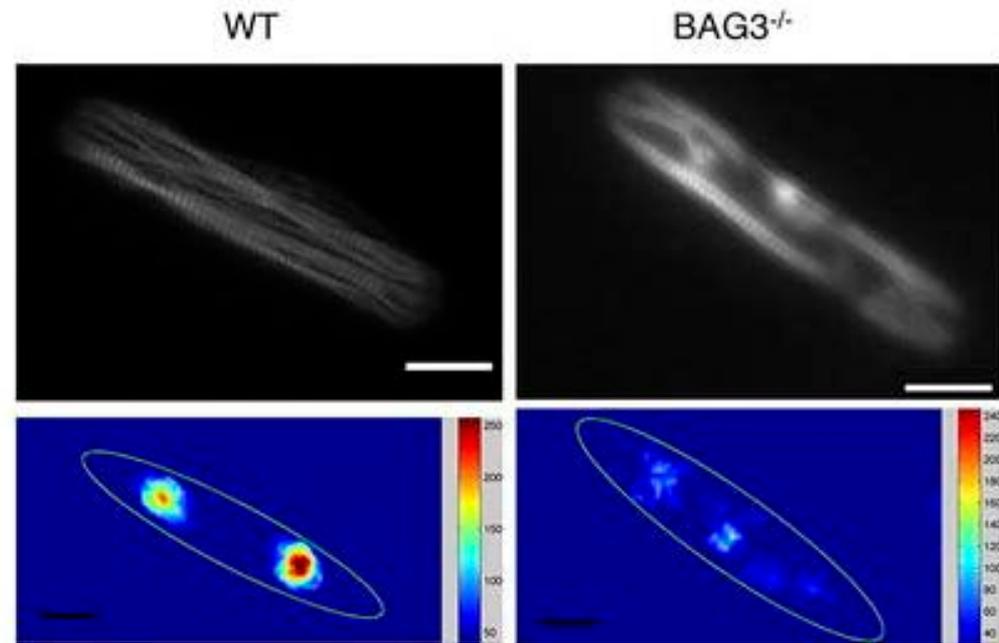
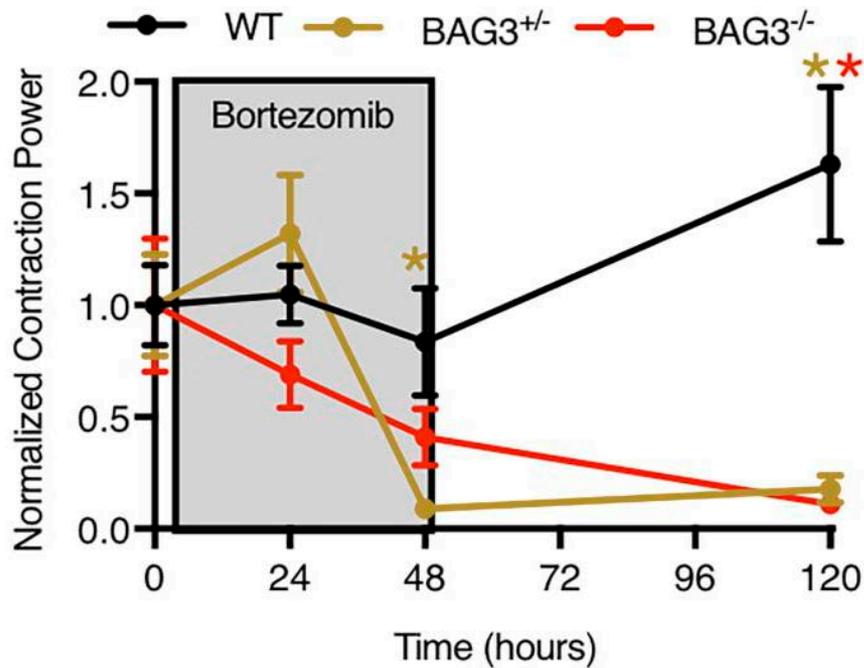
- EC_{50} curves
- Sub-chronic drug effects (minimally invasive and non-destructive)
- Disease modeling



Contractile response to inotropes: EC50



Functional recovery from bortezomib-induced toxicity



Structural defects related to differences in toxicity

Selected studies of cardiac disease with single micropatterned iPSC-cardiomyocytes



Published:

[Cell Rep.](#) 2015 Oct 27;13(4):733-745

Contractile Defect Caused by Mutation in MYBPC3 Revealed under Conditions Optimized for Human PSC-Cardiomyocyte Function.

[Birket MJ](#), [Ribeiro MC](#), [Kosmidis G](#), [Ward D](#), [Leitoguinho AR](#), [van de Pol V](#), [Dambrot C](#), [Devalla HD](#), [Davis RP](#), [Mastroberardino PG](#), [Atsma DE](#), [Passier R](#), [Mummery CL](#)

[Cell.](#) 2016 Dec 15;167(7):1734-1749.e22

Disease Model of GATA4 Mutation Reveals Transcription Factor Cooperativity in Human Cardiogenesis.

[Ang YS](#), [Rivas RN](#), [Ribeiro AJS](#), [Srivas R](#), [Rivera J](#), [Stone NR](#), [Pratt K](#), [Mohamed TMA](#), [Fu JD](#), [Spencer CI](#), [Tippens ND](#), [Li M](#), [Narasimha A](#), [Radzinsky E](#), [Moon-Grady AJ](#), [Yu H](#), [Pruitt BL](#), [Snyder MP](#), [Srivastava D](#)

Submitted:

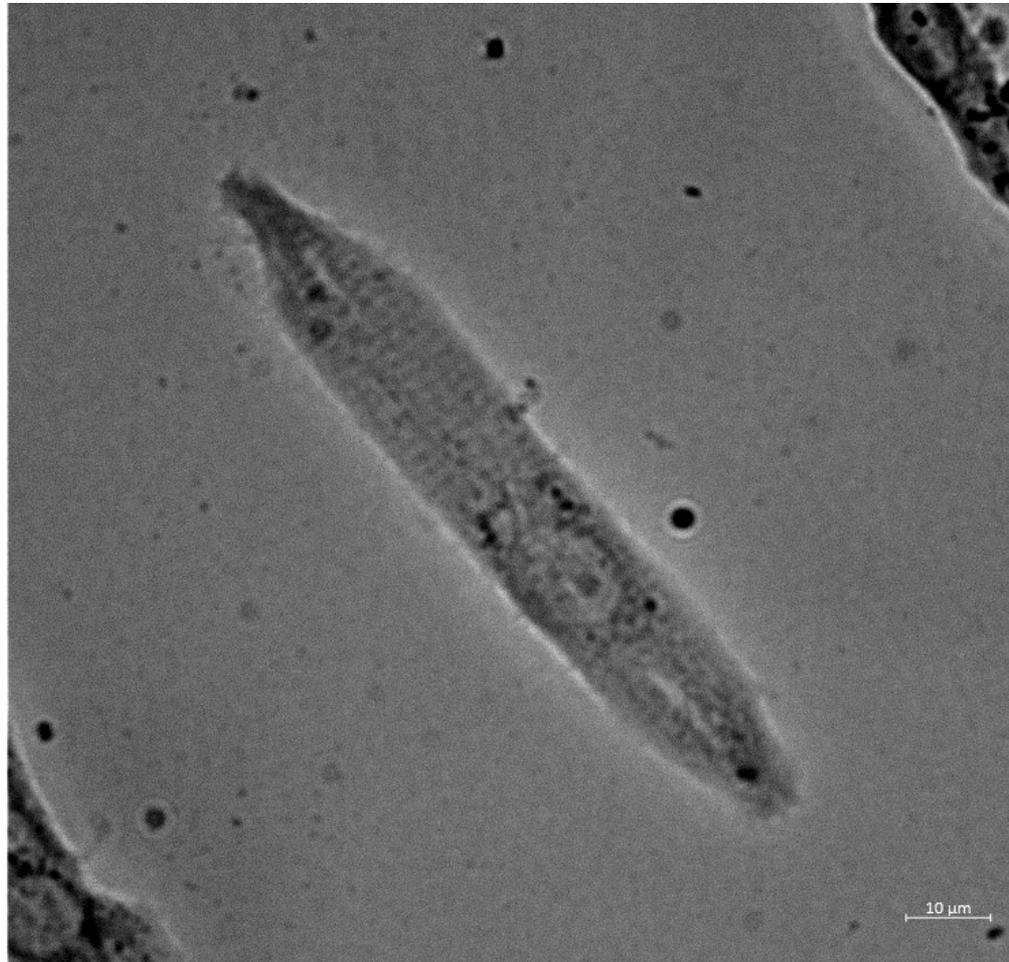
2019

Duchenne cardiomyopathy

2019

Hypertrophic cardiomyopathy

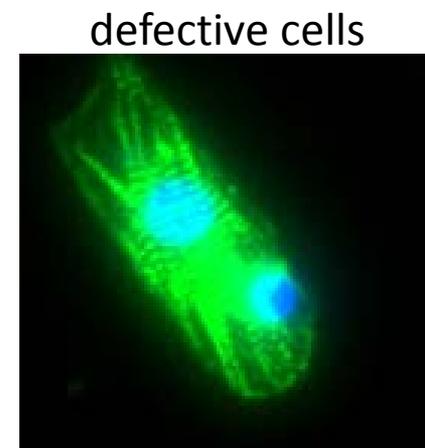
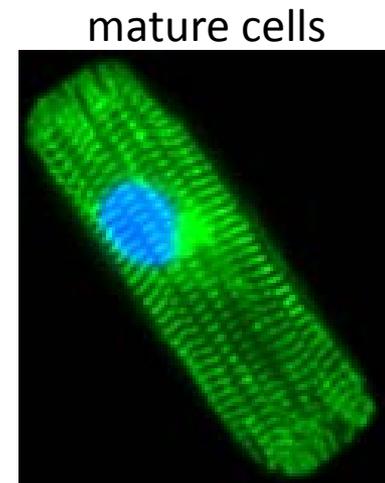
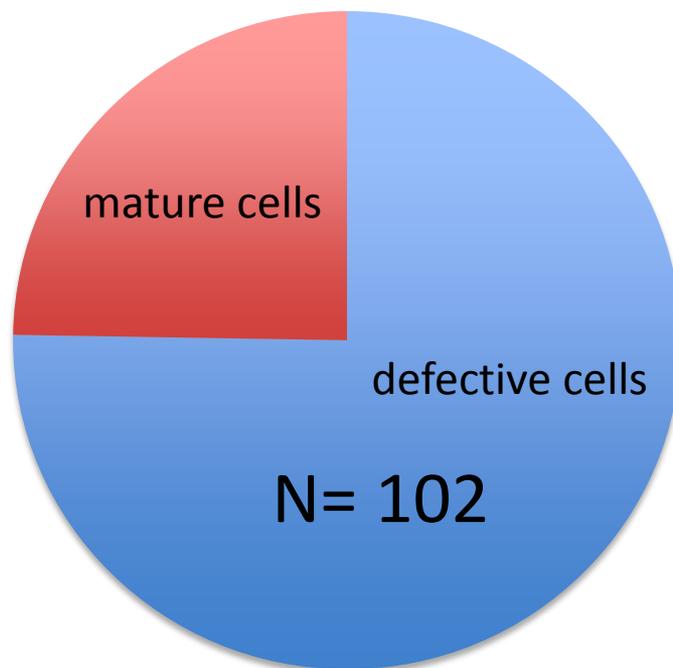
We are now evaluating this technology in CDER labs



The potential of micropatterning: high content screening, not yet high throughput

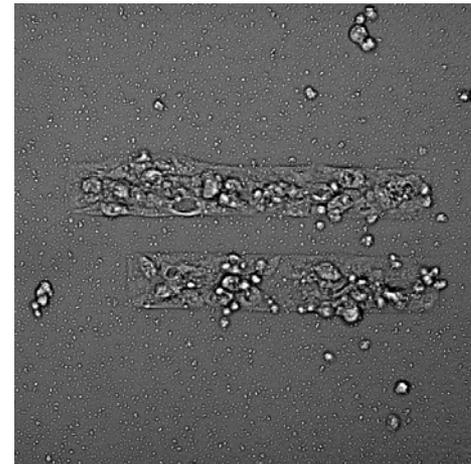
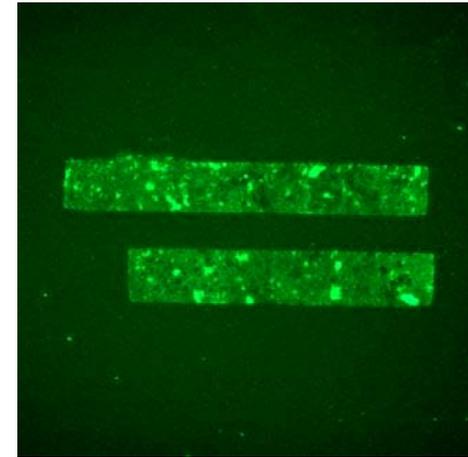
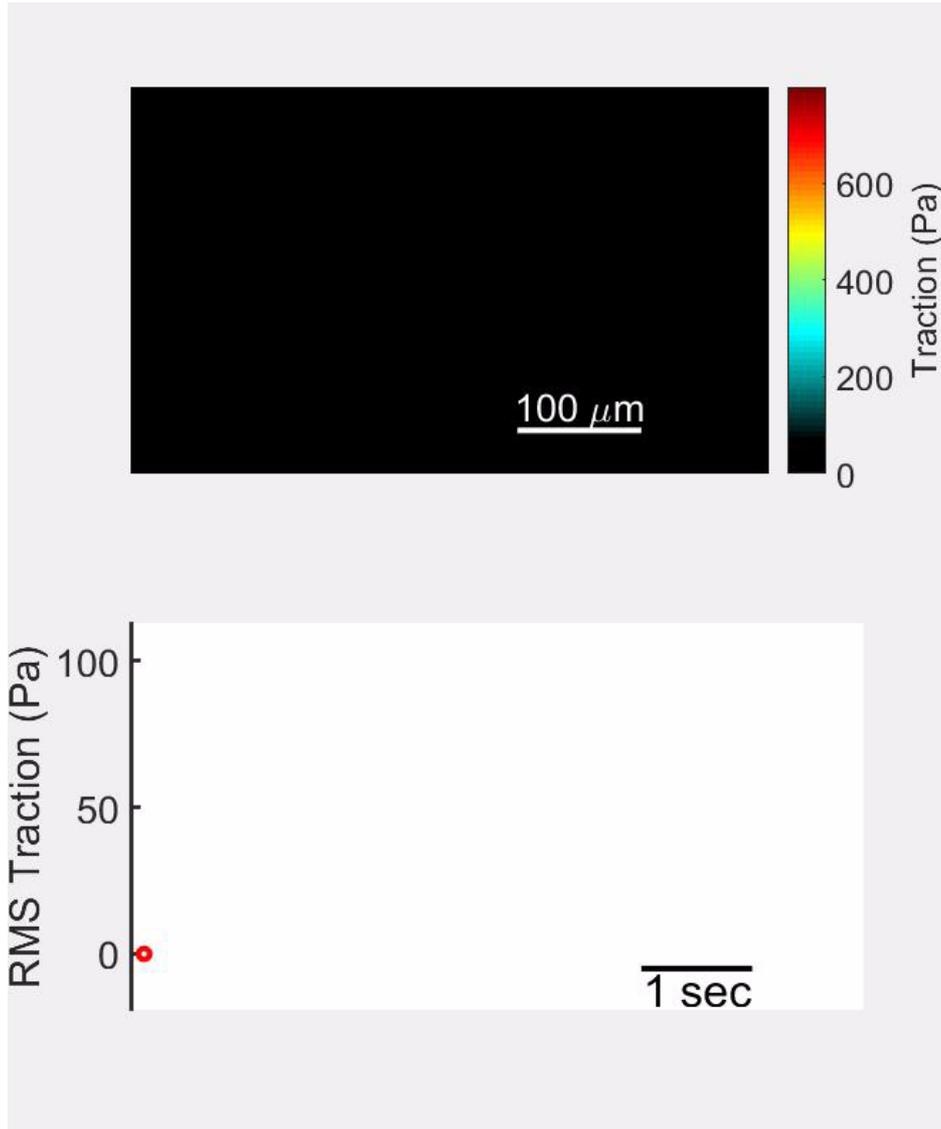
For each single cell:

- Contractility
- Calcium
- Sarcomere organization
- Voltage sensitive dyes
- Electrophysiology
- Gene expression?
- Protein expression?
- Metabolism?
- Epigenetics?



Cell to cell variability and lack of automated tools limit the potential for high throughput

Future work: aligned 2D tissues for high throughput



Data by Niccole Schaible
Ramaswamy Krishnan Laboratory
Harvard School of Medicine

Key takeaways today

1. Micropatterning iPSC-cardiomyocytes into a physiological shape on soft materials enhances their physiological relevance
2. Contractility can be comprehensively assessed with micropatterned cells
3. The regulatory potential of this technology still needs to be evaluated

- Cell function is physiologically relevant

- Functional endpoints should be comprehensively assessed

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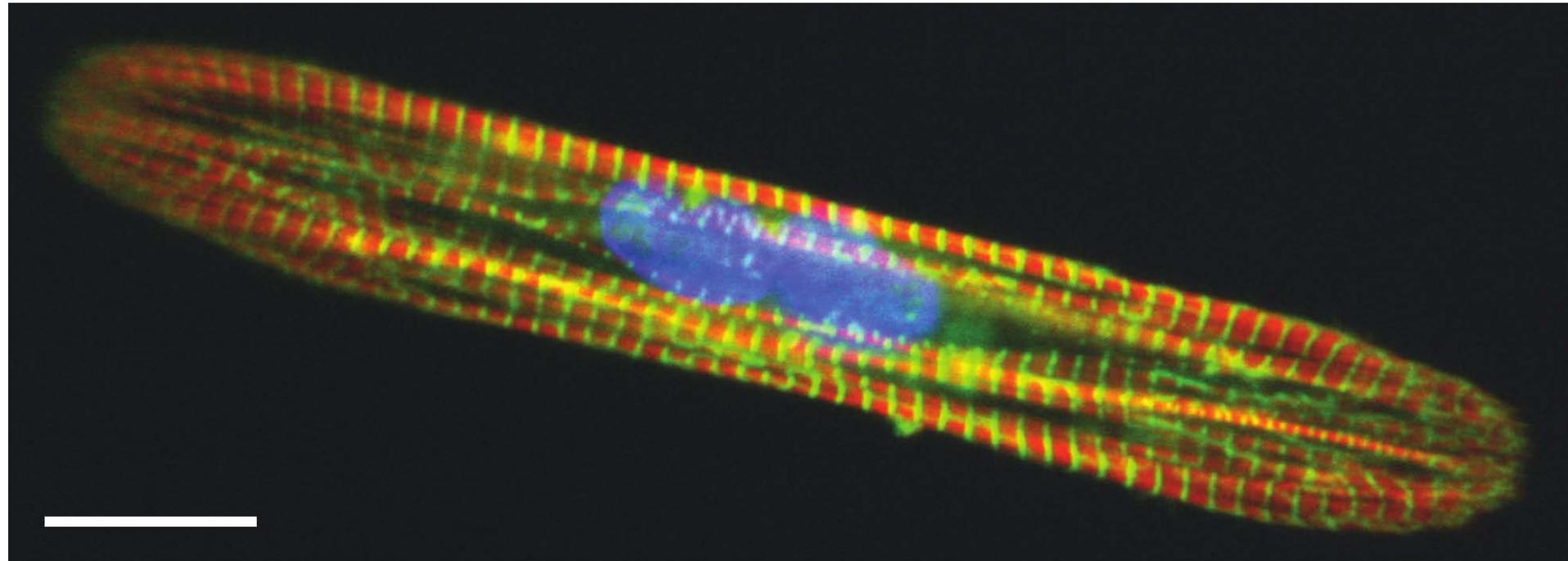
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Questions



Green: α -actinin

Red- Actin (LifeAct)

Blue: nucleus

