BioCAT

The Biophysics Collaborative Access Team

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CSRRI

Cardiac muscle sample preparation for X-ray diffraction

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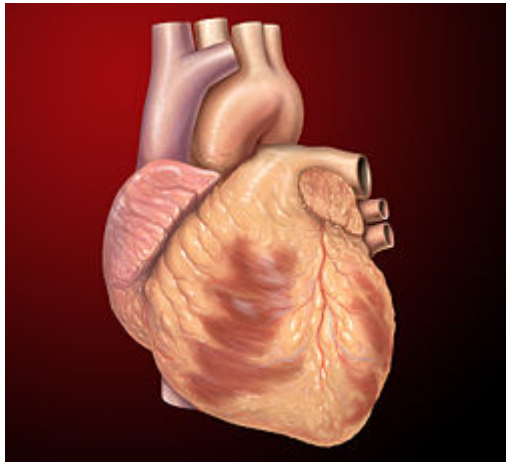
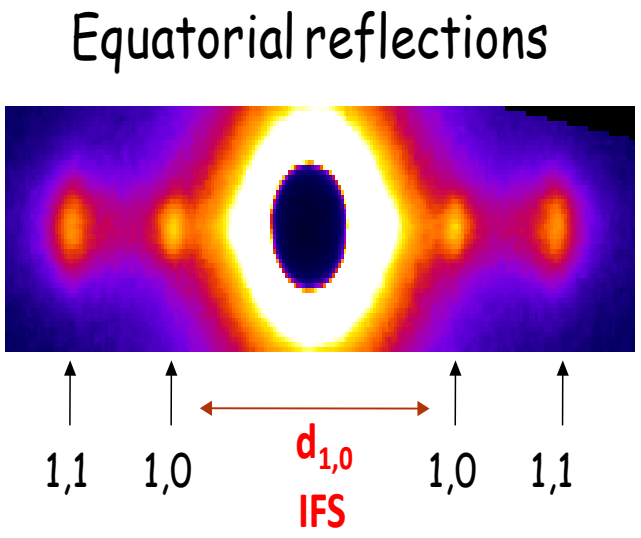
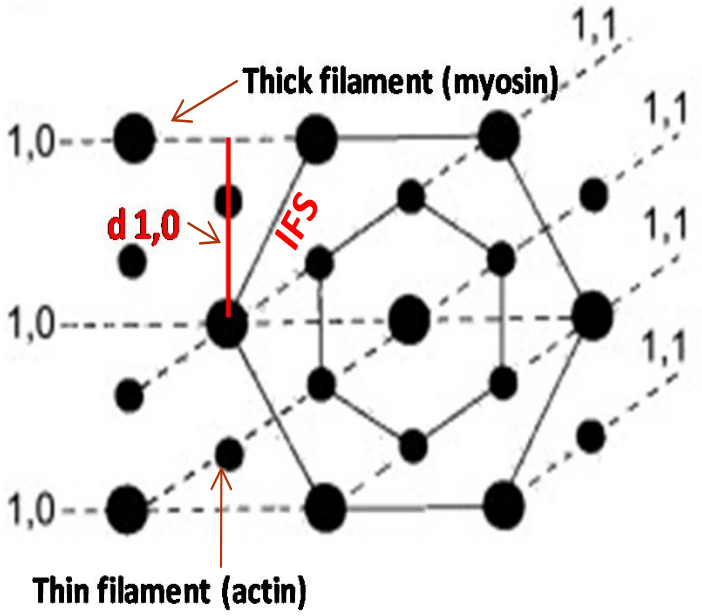
05/23/2018 Madison



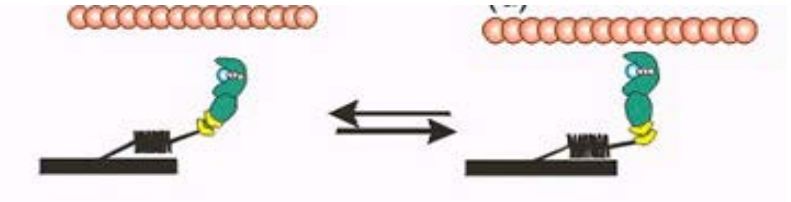
Small Angle X-ray Diffraction Study:

Equatorial reflections: 1,0–myosin filaments; 1,1 –actin & myosin filaments.
The $I_{1,1}/I_{1,0}$ ratio represents the distribution of cross-bridge mass.





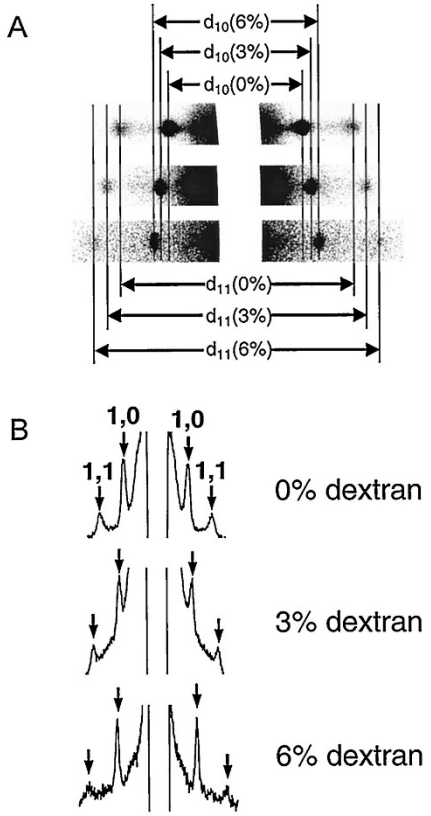
Intensities ratio:
$I_{1,1}/I_{1,0}$



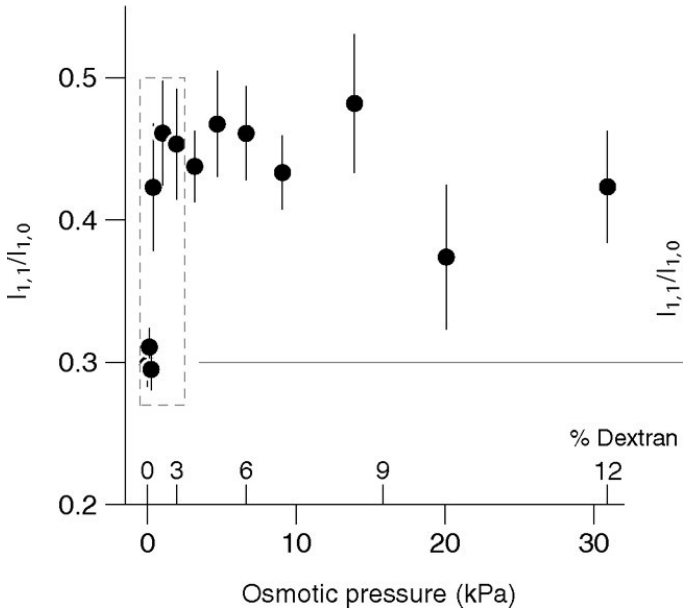
Lower Higher

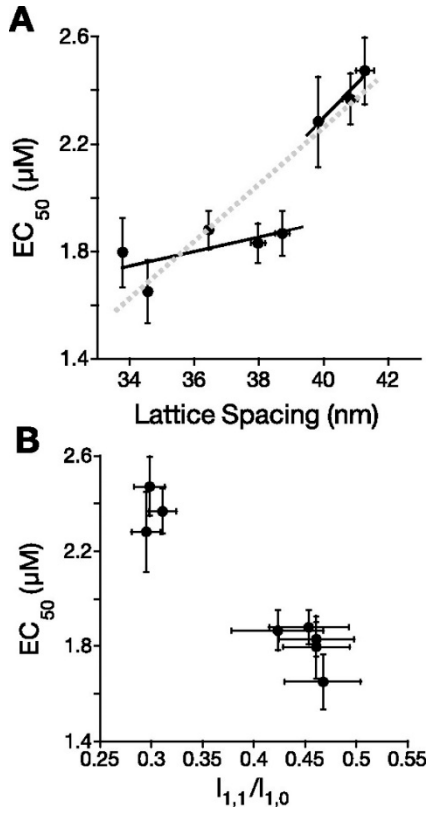
What can you do with skinned cardiac muscle?

- 1. Konhilas JP, Irving TC and de Tombe PP**. Myofilament calcium sensitivity in skinned rat cardiac trabeculae: role of interfilament spacing**. *Circulation research*. 2002;90:59-65.
- 2. Farman GP, Walker JS, de Tombe PP and Irving TC**. Impact of osmotic compression on sarcomere structure and myofilament calcium sensitivity of isolated rat myocardium. *American journal of physiology Heart and circulatory physiology*.** 2006;291:H1847-55.



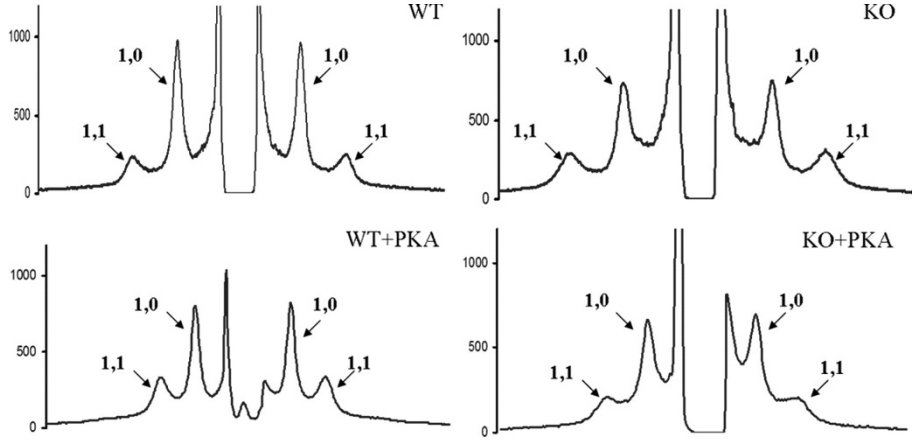
John P. Konhilas et al. Circ Res. 2002;90:59-65

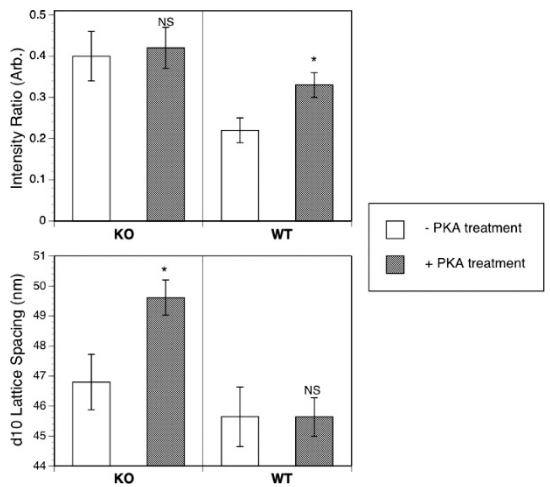


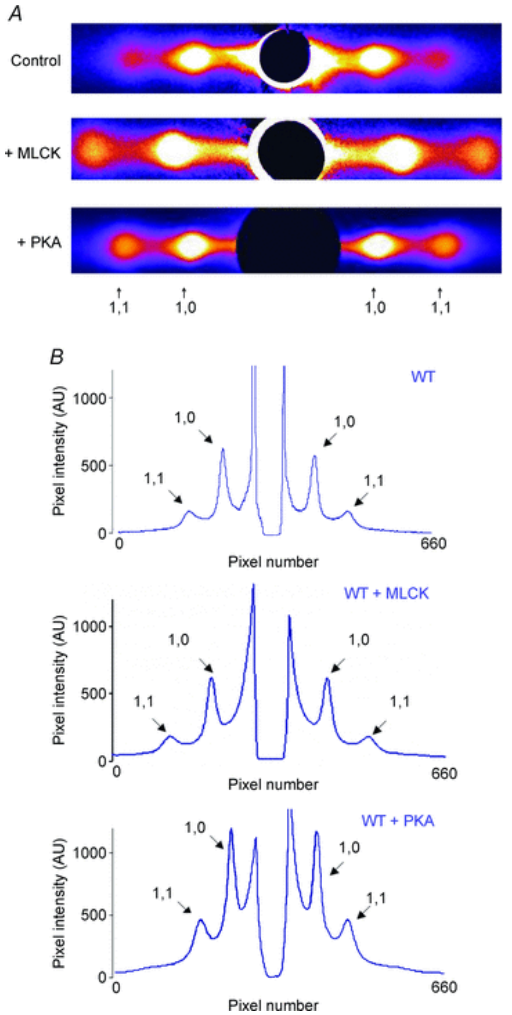


Gerrie P. Farman; et al. *American Journal of Physiology-Heart and Circulatory Physiology* **2006,** 291, H1847-H1855.

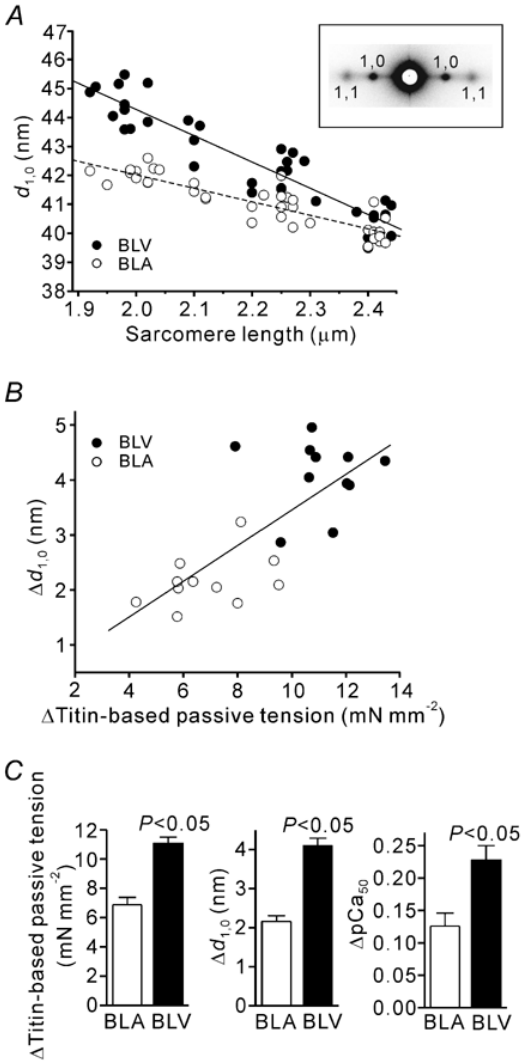
- 3. Colson BA, Bekyarova T, Locher MR, Fitzsimons DP, Irving TC and Moss RL. **Protein kinase A-mediated phosphorylation of cMyBP-C increases proximity of myosin heads to actin in resting myocardium.** ***Circulation research*****.** 2008;103:244-51.
- 4. Colson BA, Locher MR, Bekyarova T, Patel JR, Fitzsimons DP, Irving TC and Moss RL. **Differential roles of regulatory light chain and myosin binding protein-C phosphorylations in the modulation of cardiac force development.** *The Journal of physiology*. 2010;588:981-93

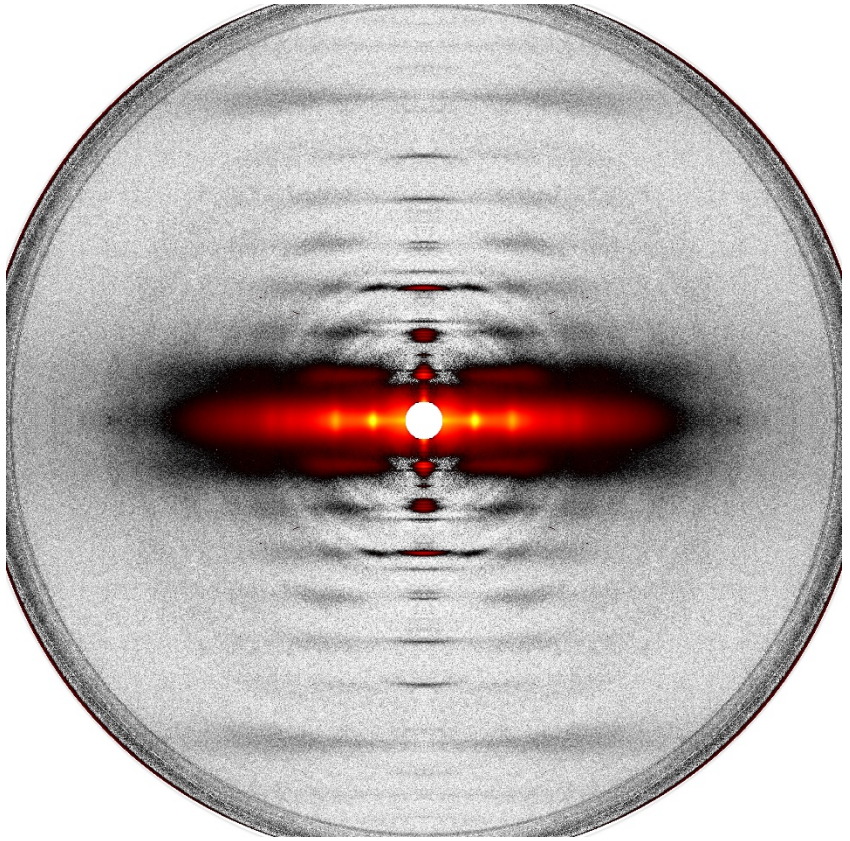






- 5. Fukuda N, Wu Y, Farman G, Irving TC and Granzier H. **Titin isoform variance and length dependence of activation in skinned bovine cardiac muscle.** *The Journal of physiology*. 2003;553:147-54.
- 6. Anderson RL, Trivedi DV, Sarkar SS, Henze M, Ma W, Gong H, Rogers CS, Wong FL, Morck M, Seidman JG, Ruppel KM, Irving TC, Cooke R, Green EM and Spudich JA. **Mavacamten stabilizes a folded-back sequestered super-relaxed state of β-cardiac myosin**. *bioRxiv*. 2018.

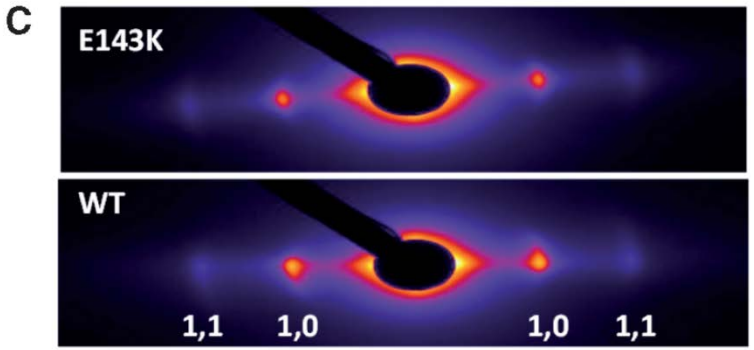




Porcine cardiac muscle

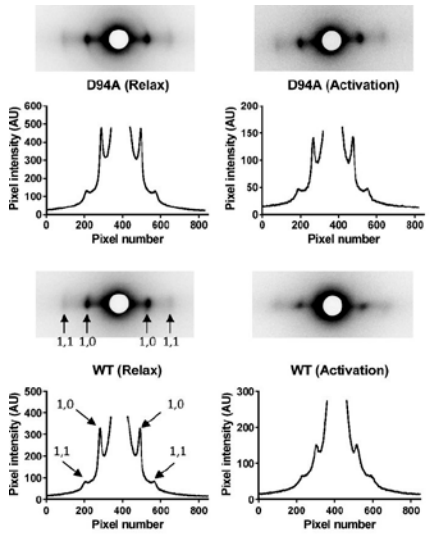
- 7. Yuan CC, Muthu P, Kazmierczak K, Liang J, Huang W, Irving TC, Kanashiro-Takeuchi RM, Hare JM and Szczesna-Cordary D. **Constitutive phosphorylation of cardiac myosin regulatory light chain prevents development of hypertrophic cardiomyopathy in mice**. *Proceedings of the National Academy of Sciences of the United States of America*. 2015;112:E4138-46.
- 8. Yuan CC, Kazmierczak K, Liang J, Kanashiro-Takeuchi R, Irving TC, Gomes AV, Wang Y, Burghardt TP and Szczesna-Cordary D. **Hypercontractile mutant of ventricular myosin essential light chain leads to disruption of sarcomeric structure and function and results in restrictive cardiomyopathy in mice**. *Cardiovascular research*. 2017;113:1124-1136.
- 9. Yuan C-C, Kazmierczak K, Liang J, Zhou Z, Yadav S, Gomes AV, Irving TC and Szczesna-Cordary D. **Sarcomeric perturbations of myosin motors lead to dilated cardiomyopathy in genetically modified mice.** *Proceedings of the National Academy of Sciences*. 2018;115:E2338.

RCM



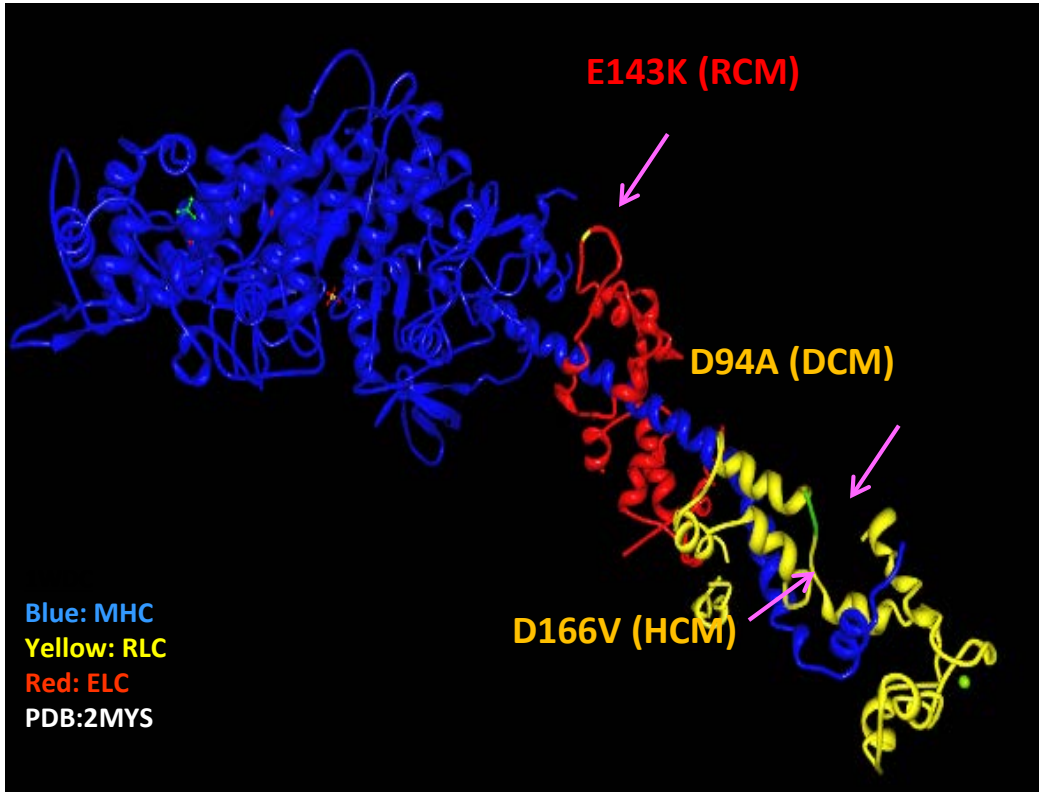
Parameter	E143K	WT
No. fibers (no. of shots)	11 (38)	16 (47)
$I_{1,1}/I_{1,0}$	0.33±0.03	0.34±0.03
No. fibers (no. of shots)	9 (36)	7 (45)
$d_{1,0}$ (nm)	39.51±0.35*	41.26±0.35

DCM

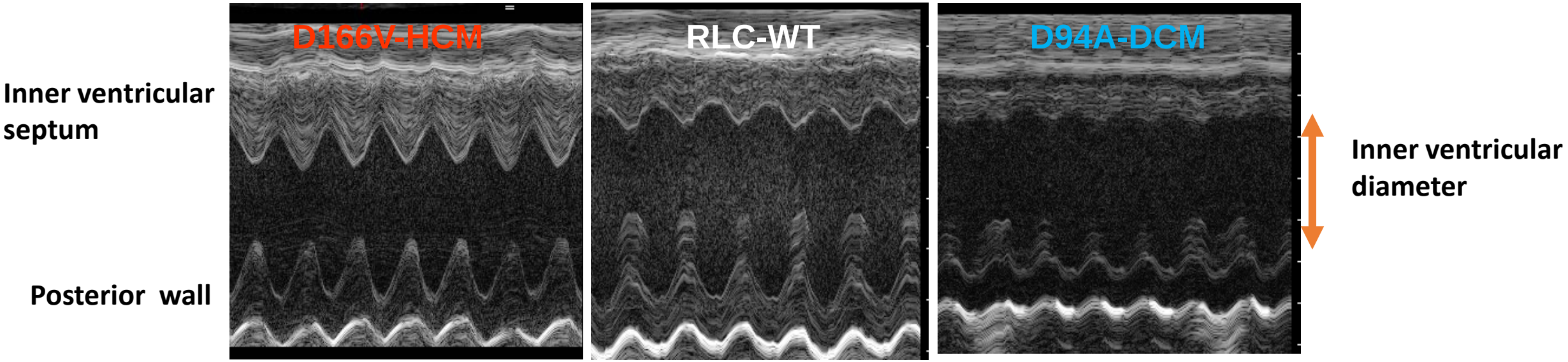


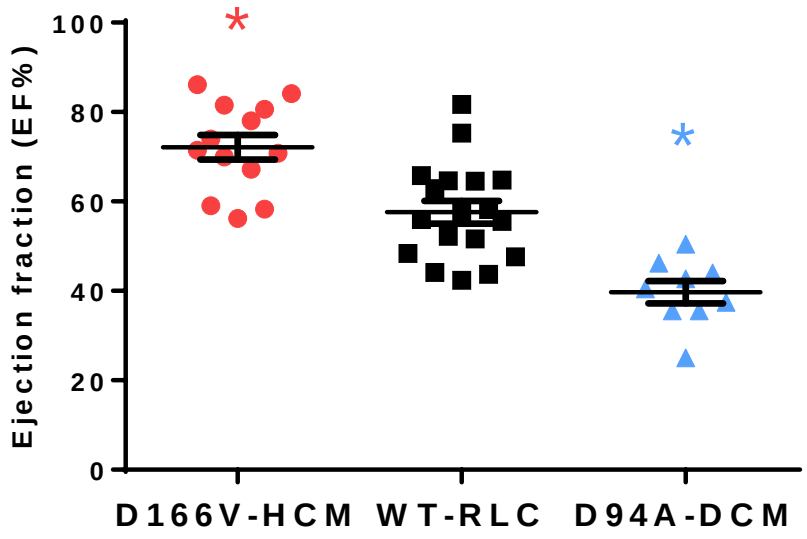
	WT	Nº fibers	D94A-L1	Nº fibers	P-value
Intensities ratio ($I_{1,1}/I_{1,0}$)					
pCa 8 (Relaxation)	0.22±0.06	15 (7F, 8M)	0.26±0.08	15 (8F, 7M)	0.206
pCa 5.2 (Activation)	0.43±0.02	14 (7F, 7M)	0.35±0.03*	12 (4F, 8M)	0.043
Lattice spacing ($d_{1,0}$ in nm)					
pCa 8 (Relaxation)	41.54±0.3	16 (8F, 8M)	41.38±0.24	15 (8F, 7M)	0.722
pCa 5.2 (Activation)	41.45±0.31	15 (7F, 8M)	41.64±0.29	14 (7F, 7M)	0.701

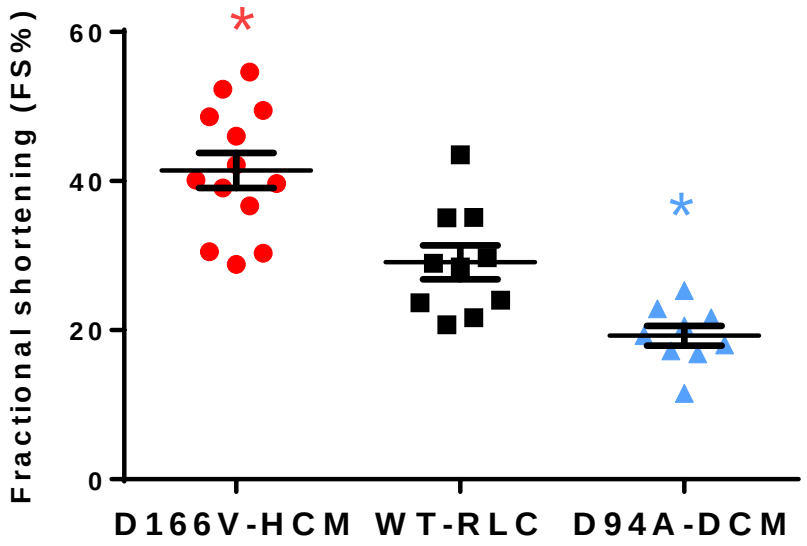
Lattice structure alterations in DCM HCM and RCM mouse models associated with mutations in myosin regulatory light chain



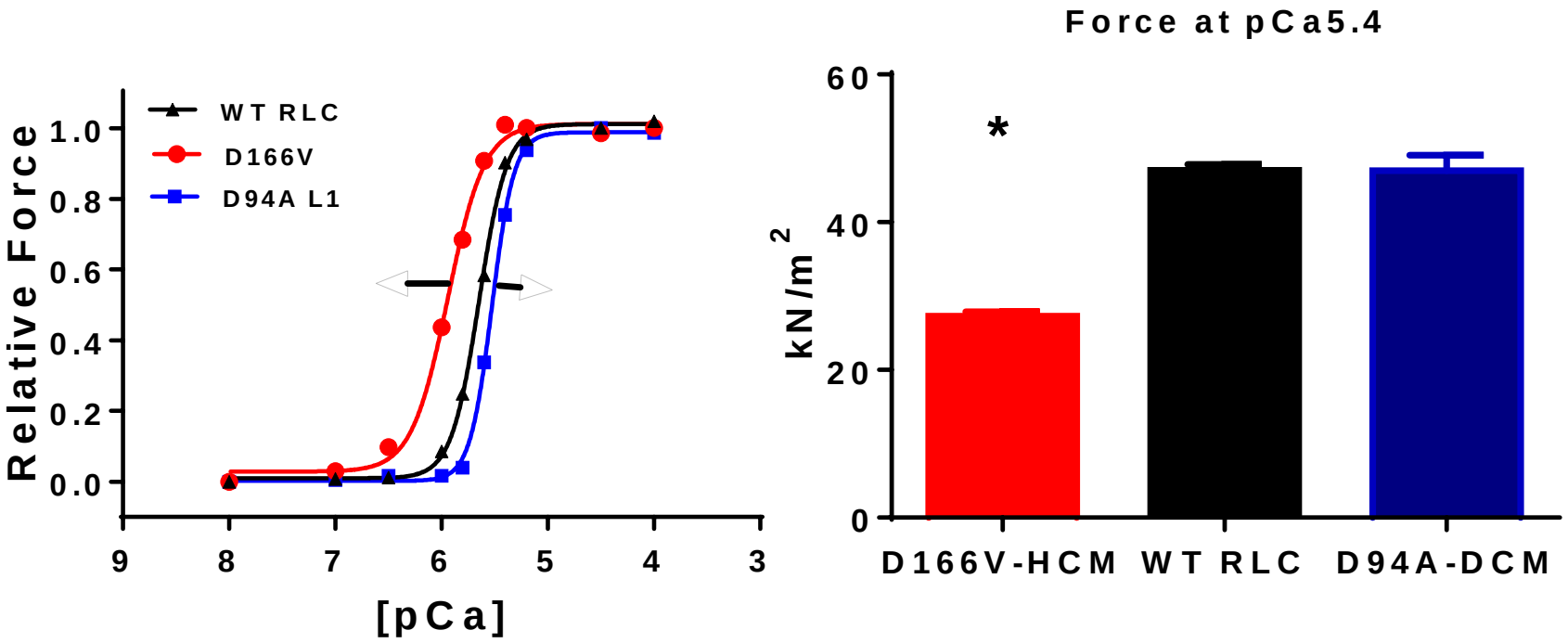
Hypercontractility in D166V-HCM, and hypocontractility in D94A-DCM hearts







Force-pCa in skinned papillary muscle strips



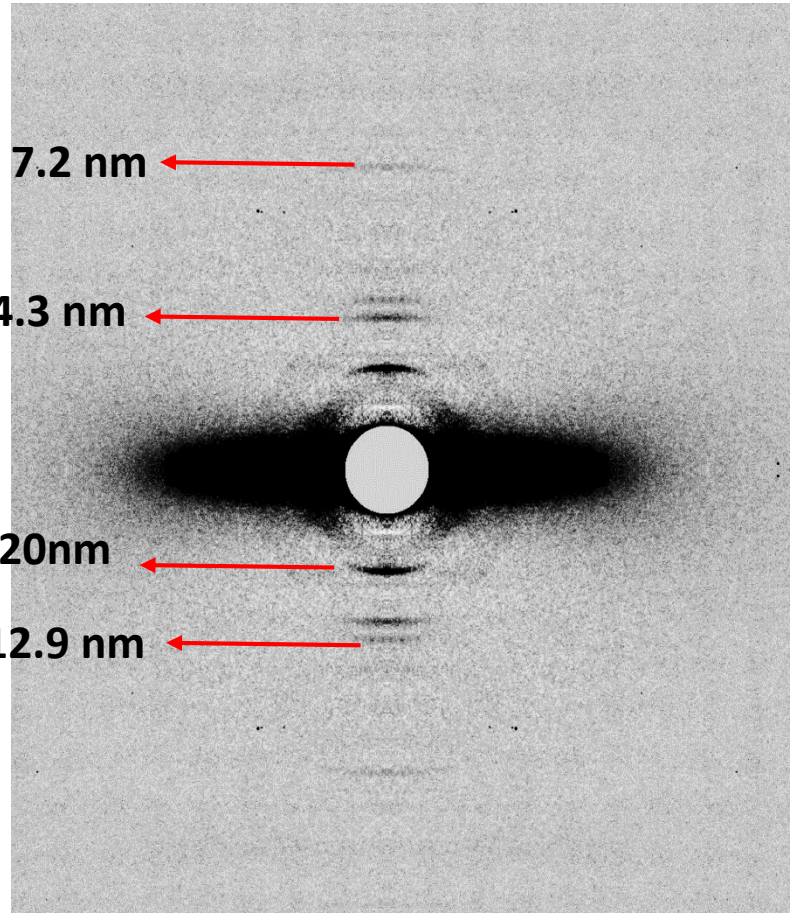
Decreased maximal tension and increased Ca^{2+} sensitivity in **D166V-HCM**

No change of maximal tension but decreased Ca^{2+} sensitivity in **D94A-DCM**

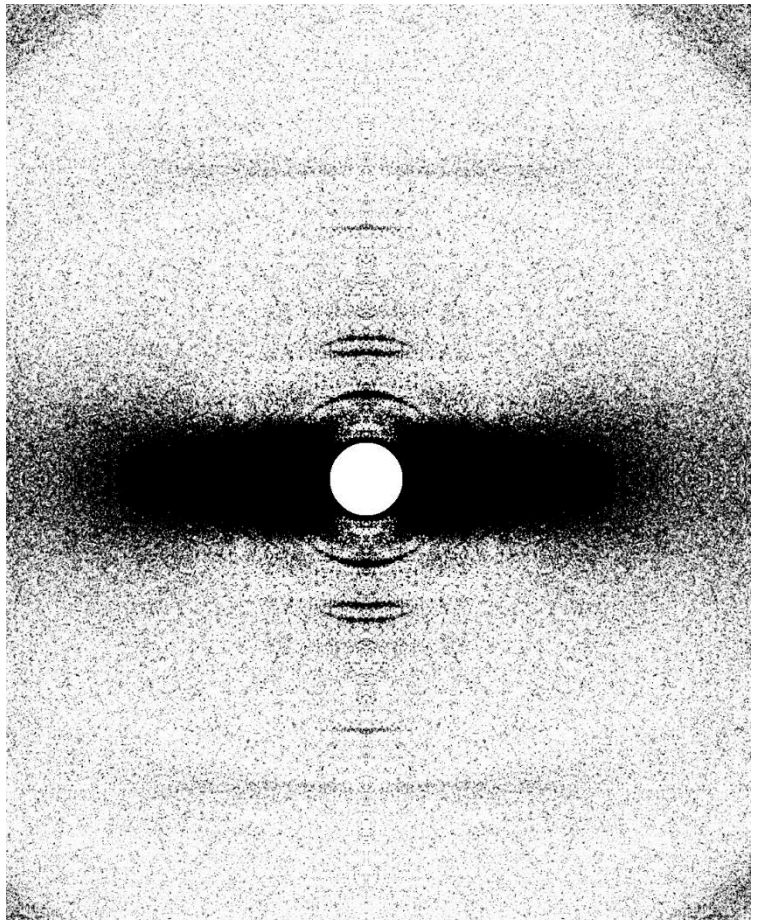
Small angle X-ray data acquired at serial pCa solutions

Rat cardiac muscle fiber diffraction: Meridian

ATP pCa9 170mM SL:2.3µm



dATP pCa9 170mM SL:2.3µm



Sample Types-skinned cardiac muscle bundle

- Mouse:

Left ventricular papillary muscle

Cross section diameter : ~200-300 μm

3-4 samples from one heart

- Rat:

Right ventricular trabeculae and papillary muscle

Cross section diameter: ~200-400μm

3-4 samples from one heart

Heart dissection-solutions

- Mice

1. Wash heart with 0.9% NaCl

2. Dissect the bundle in Solution A (pCa8 solution+ 30mM BDM+15 units per mL of creatine phosphokinase (CPK) + Protease inhibitors (sigma p8340))+ 15% glycerol

3. Muscle bundle samples will transfer to solution A without 15% glycerol for 15 min on ice to remove glycerol.

- Rat

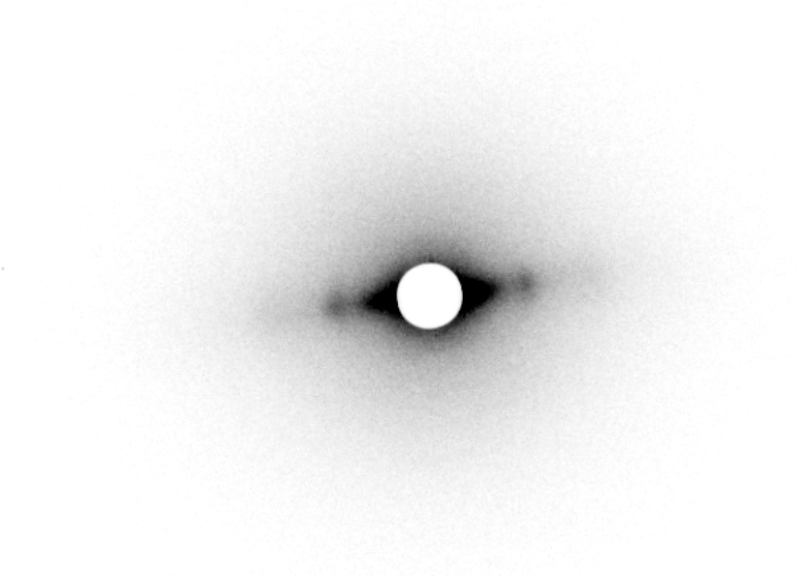
Krebs–Henseleit (KH) : 118.5 mM NaCl, 5 mM KCl, 2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 10 mM glucose, 25 mM $NaHCO_3$, and 0.1 mM $CaCl_2$, as well as 20 mM (BDM)

BDM, Protease inhibitors and CPK freshly add everyday

Skinned protocol

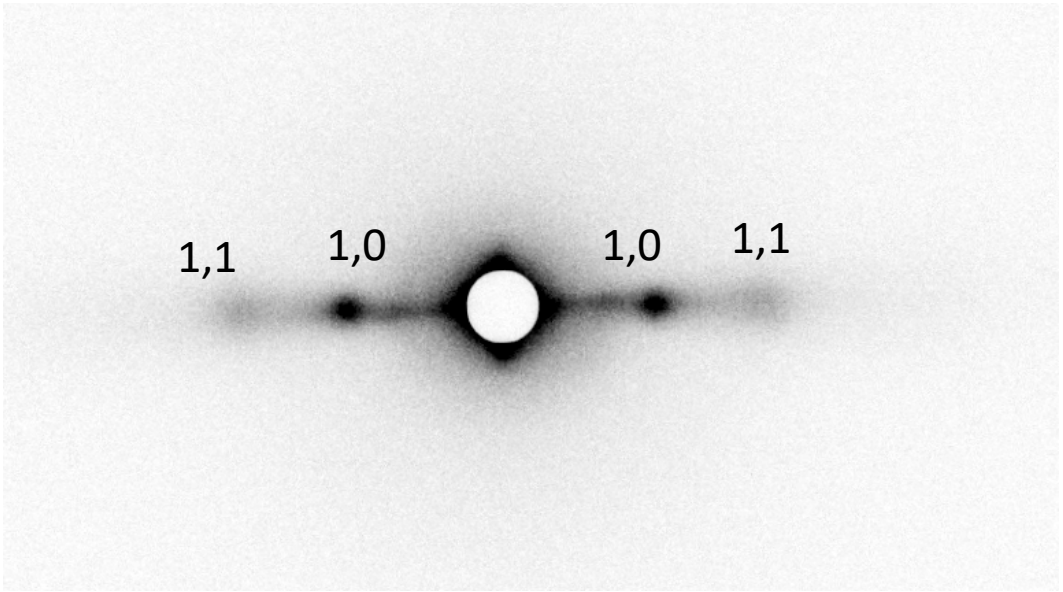
- Skinned overnight with 1% Triton-X 100 in 4°C and stored in 50% of glycerol
- Freshly skinned in room temperature for 1.5 to 2 hours

Skinned overnight



pCa9 Rat trabecula

Freshly skinned in room temperature



pCa9 Rat trabecula

Sample preparation

- Isolated muscle fibers out from the heart
- Using insect pin to fix muscle length
- Stored in 1.5ml of tubes with skinning solution
- Put on the shaker and skinning for 1.5 to 2 hours
- Change freshly skinned solution ones during the process
- Washing 3X 5min with solution without tritonX-100 on ice
- Stored skinned muscle fibers in 4 °C and use same day for x-ray experiment

Skinned solution:

Miami for mice

Solution A + 1% of tritonX-100. pH adjust by K-propionate to 7 (IS:150mM)

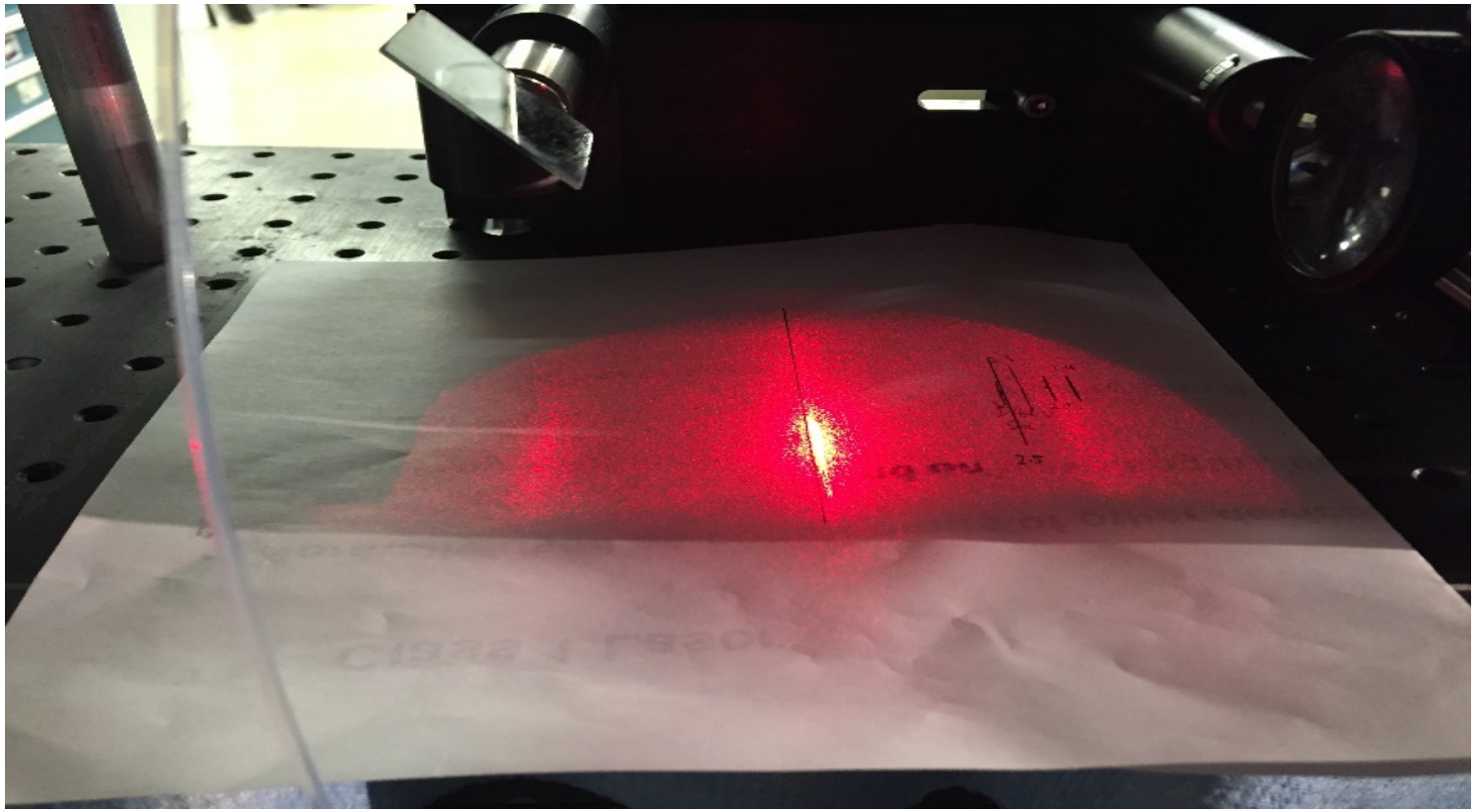
PS. Washing with pCa8 +CPK + protease inhibitor solution without BDM.

Washington for Rat

Relaxing solution: 0.1 M KCl, 5mM K_2EGTA, 9mM $MgCl_2$, 4mM Na_2ATP
with 1% of TrintonX-100 and Protease inhibitor (sigma P8340) , pH adjusted by KOH to 7

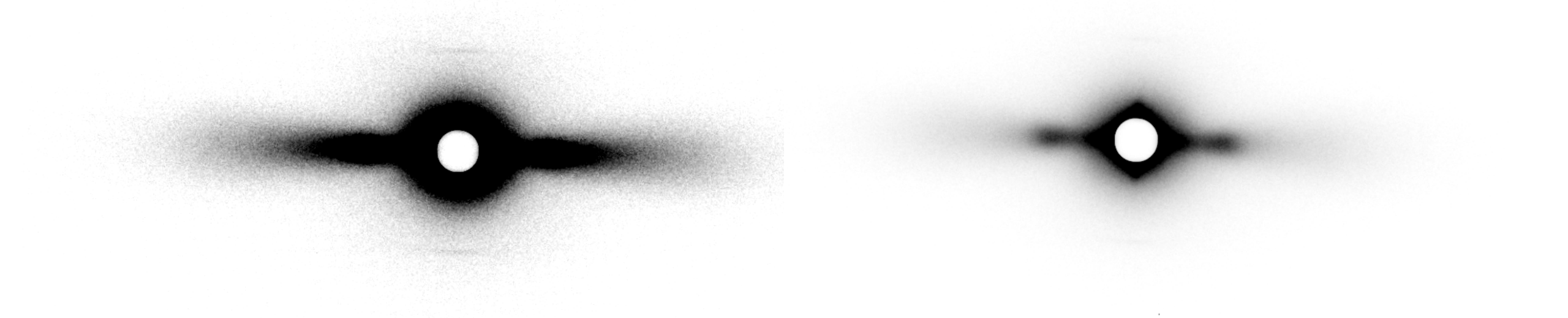
Note: many people prefer K-propionate to KCl , but sample looks similar in my hand

How to check the quality of preparation-Laser



D94A DCM mouse cardiac muscle SL=2.1µm

Freshly skinned in room temperature without BDM treatment and 2% of TritonX-100 for 2 hours



Rat EDL skeletal muscle

Summary

- Freshly prepare fiber sample
- Freshly add BDM, protease inhibitor into the skinning solution
- Check the quality skinning sample by laser before coming to Argonne
- Well trained and energetic student, postdoc…… better data more data . Optimal your protocol will bring more success.

Question? Discussion

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