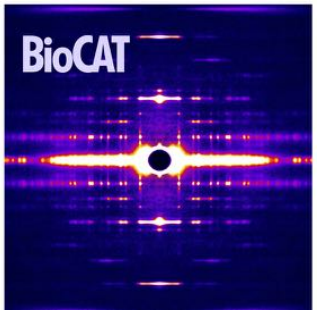
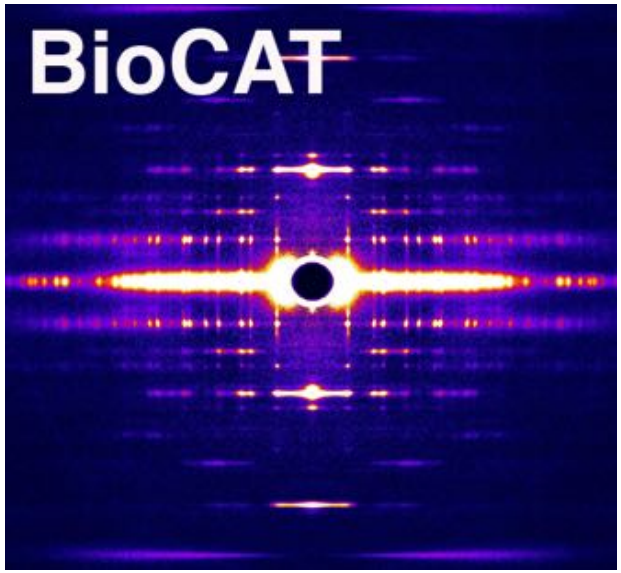


MuscleX Workshop



May 23rd 2018 Madison Wisconsin

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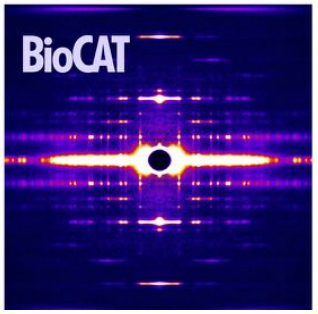

Welcome!

- This is the first of a series of MuscleX workshops where we come together to discuss how X-ray diffraction at the BioCAT Beamline 18ID at the Advanced Photon Source can be used to increase the value of muscle biophysics experiments.
- Outline of this talk:
  - What is BioCAT
  - Some history
  - Recent developments
  - Intro to the rest of today's program

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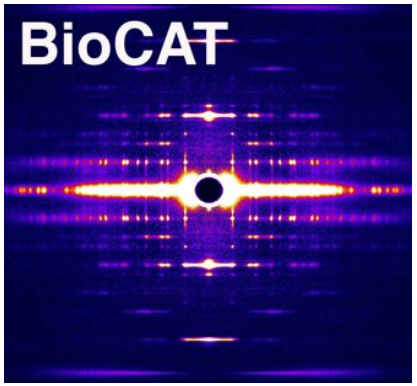

What is BioCAT?

- BioCAT operates undulator beamline 18-ID providing scientific staff support, wet laboratory and computational facilities
- Operated by the Center for Synchrotron Radiation Research and Instrumentation (CSRRI) of the Illinois Institute of Technology under a P41 Biotechnology Research Resource grant (P41) from NIGMS
- In operation since 1998
- Available to all scientists through APS General User Proposals or collaboration with BioCAT staff

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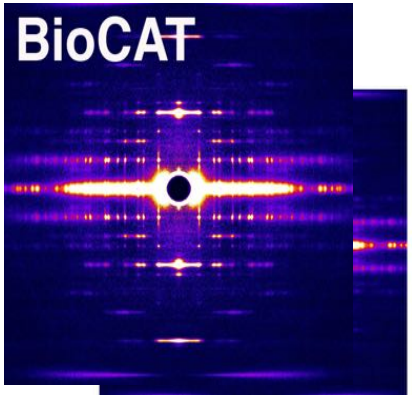

The Advanced Photon Source

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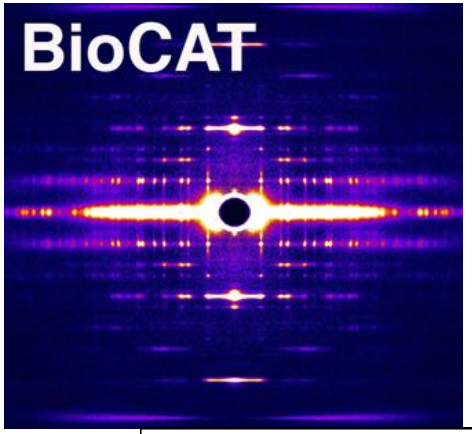

Scientific Mission of BioCAT

Modality		Sample Applications
Fiber diffraction	Muscle Diffraction	Muscle regulation, heart disease, muscular dystrophy, other skeletal muscle diseases
	Fiber Crystallography	Neurodegenerative disease, arthritis, connective tissue
	Fiber Diffraction Imaging	Neurodegenerative disease, arthritis, cancer metastasis, traumatic brain injury, connective tissue diseases
SAXS	Equilibrium SAXS	Structure of Macromolecules: complexes, protein-ligand interactions, flexible and intrinsically disordered proteins
	Time Resolved SAXS	Kinetics, protein and RNA folding, enzymatic reactions

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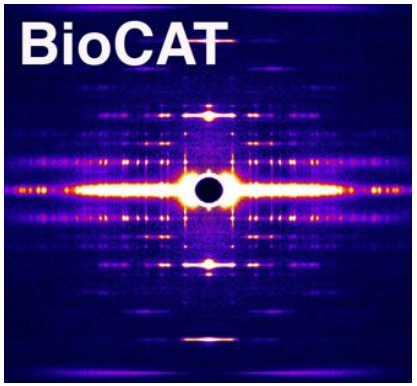

Synchrotrons and Muscle Diffraction

- Early work all done with conventional sources - why need synchrotrons?
- Patterns weak, have high backgrounds, frequently have multiple closely spaced lattices
- Studies benefit from greatly increased beam quality
- Greatly increased flux permits time-resolved experiments

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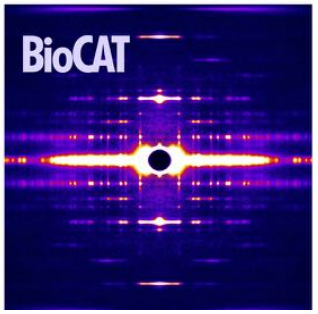

First Diffraction Pattern Using Synchrotron Radiation

Equatorial pattern from insect flight muscle

August, 1970, DESY, Hamburg

Rosenbaum, Holmes,. & Witz (1971).

Nature **230**, 434-437.

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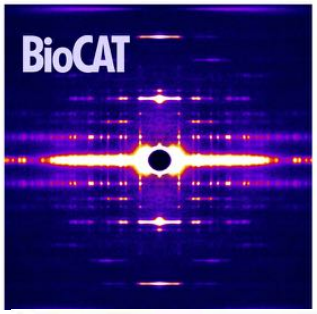
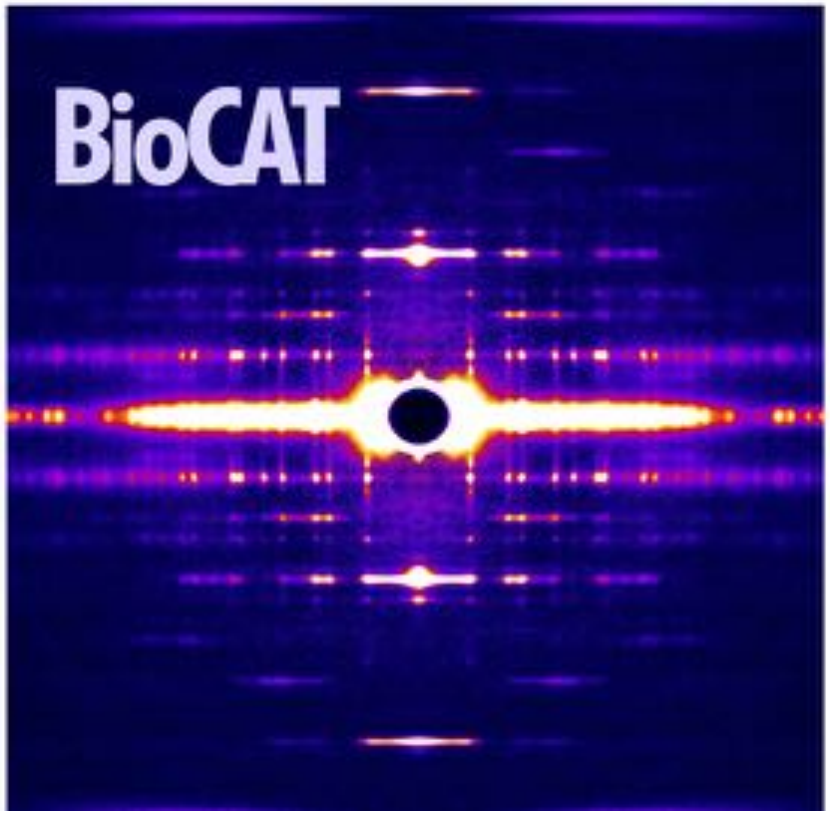


Fast Forward 30 Years:



- Gerd Rosenbaum also designed the BioCAT Beamline 18ID

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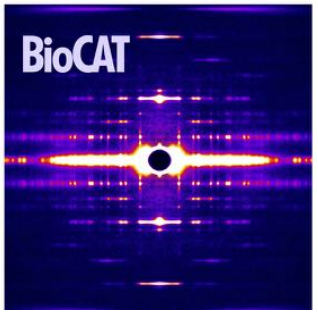

Muscle Diffraction Program

- Muscle fiber diffraction is the method of choice to obtain nm scale structural information under physiological conditions in real physiological time
- Was envisaged as a key component of the BioCAT scientific program from the start
- An important consideration in the design of the beamline
- Emphasis on small focal spots with high flux
- Matched to fast and/or high resolution area detectors

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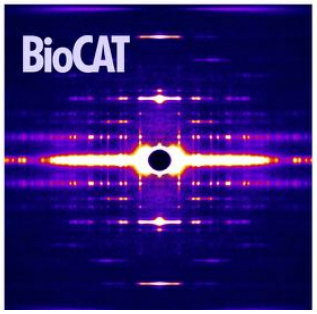

Some History

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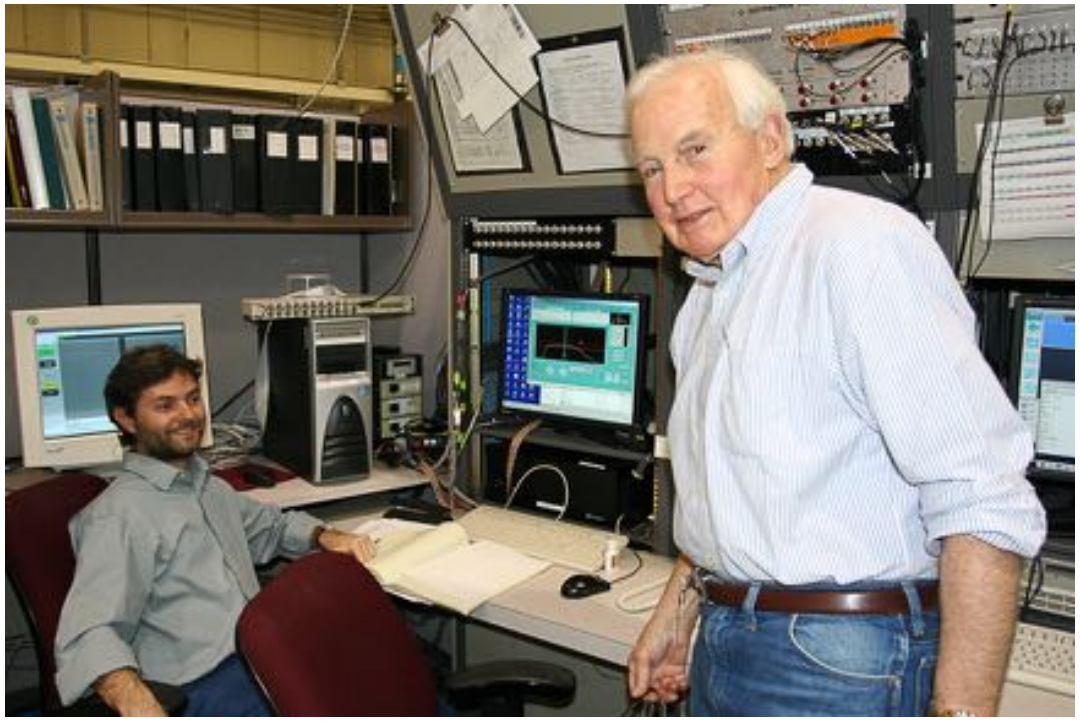
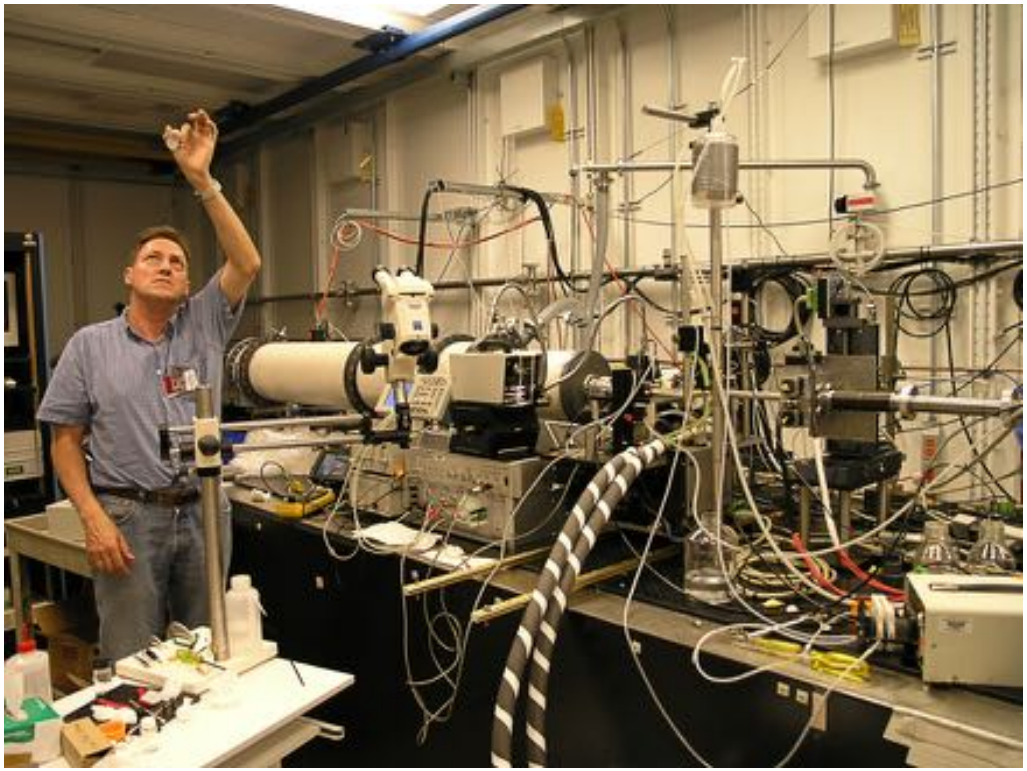
Huxley Collaboration

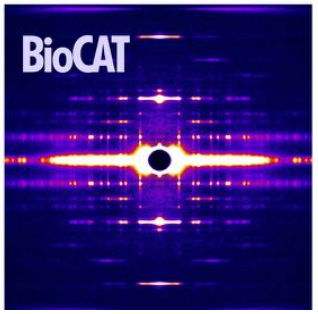


- Huxley et al., 2006 "X-ray Interference Studies of Crossbridge Action in Muscle Contraction: Evidence from Quick Releases," J. Mol. Biol. **363** (4), 743-761

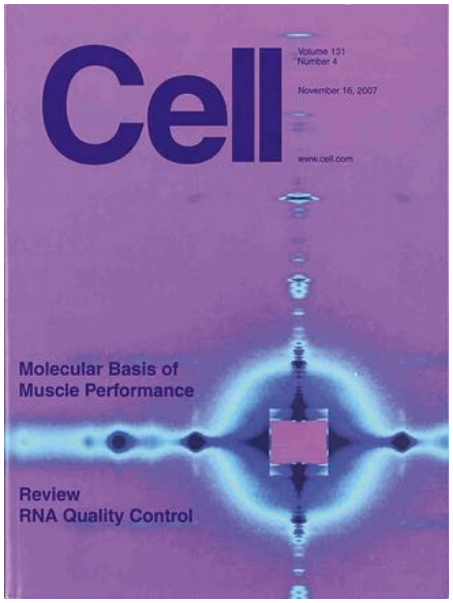
de Tombe Collaboration



- Irving et al., 2000 "Myofilament lattice spacing as a function of sarcomere length in isolated rat myocardium," Amer. J. Physiol. Heart. Circ. Physiol. **279**, H2568-H2573.



Lombardi/M. Irving Collaboration



- Piazzesi et al., 2007, "Skeletal Muscle Performance Determined by Modulation of Number of Myosin Motors Rather Than Motor Force or Stroke Size," Cell **131** (4), 784-795 (2007)

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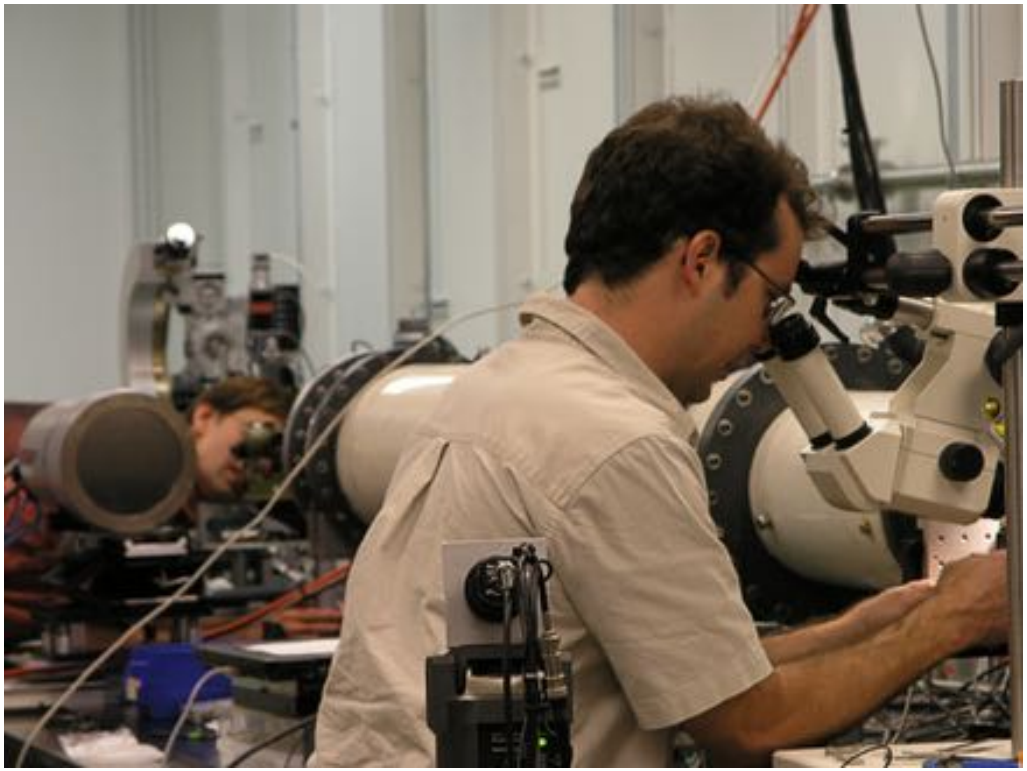
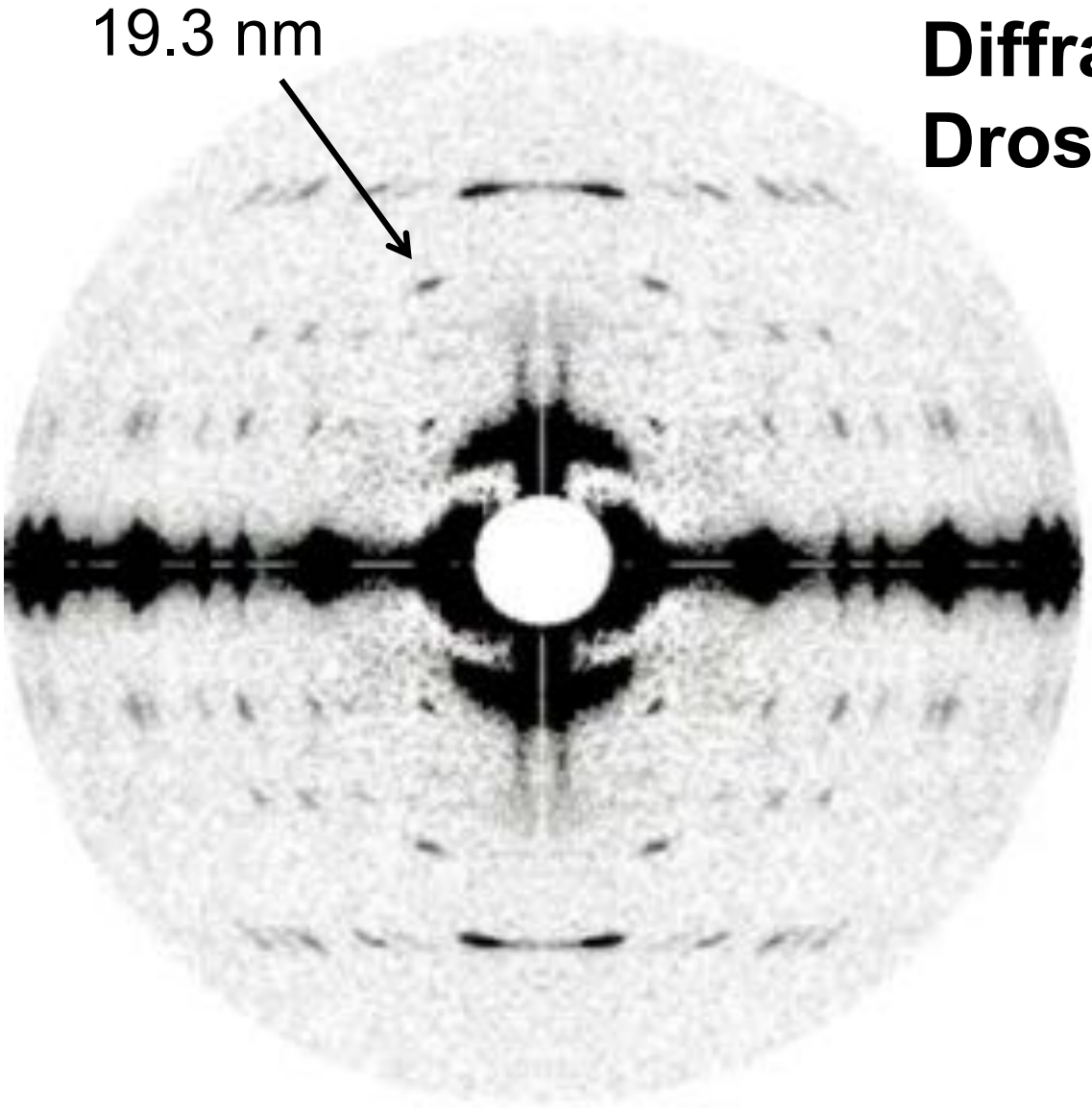
Maughan/Dickinson Collaboration



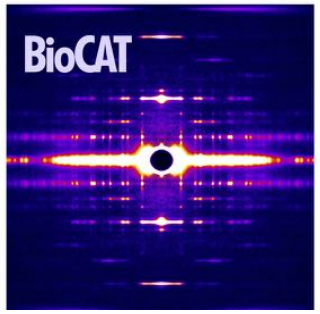
- Dickinson et al. 2005 "Molecular dynamics of cyclically contracting insect flight muscle in vivo," Nature **433** (7023), 330-333.

Time-Resolved X-ray Diffraction from Living Drosophila Flight Muscle

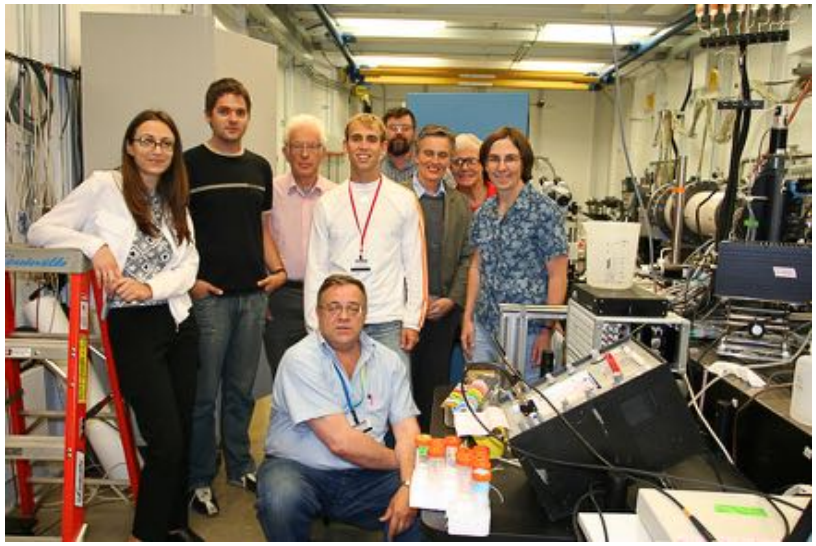


0.06 phase

Dickinson et al., 2005
Nature 433:330



Reedy Collaboration



- Perz-Edwards et al. (2011) "X-ray diffraction evidence for myosin-troponin connections and tropomyosin movement during stretch activation of insect flight muscle," Proc. Natl. Acad. Sci. USA **108** (1), 120-125.

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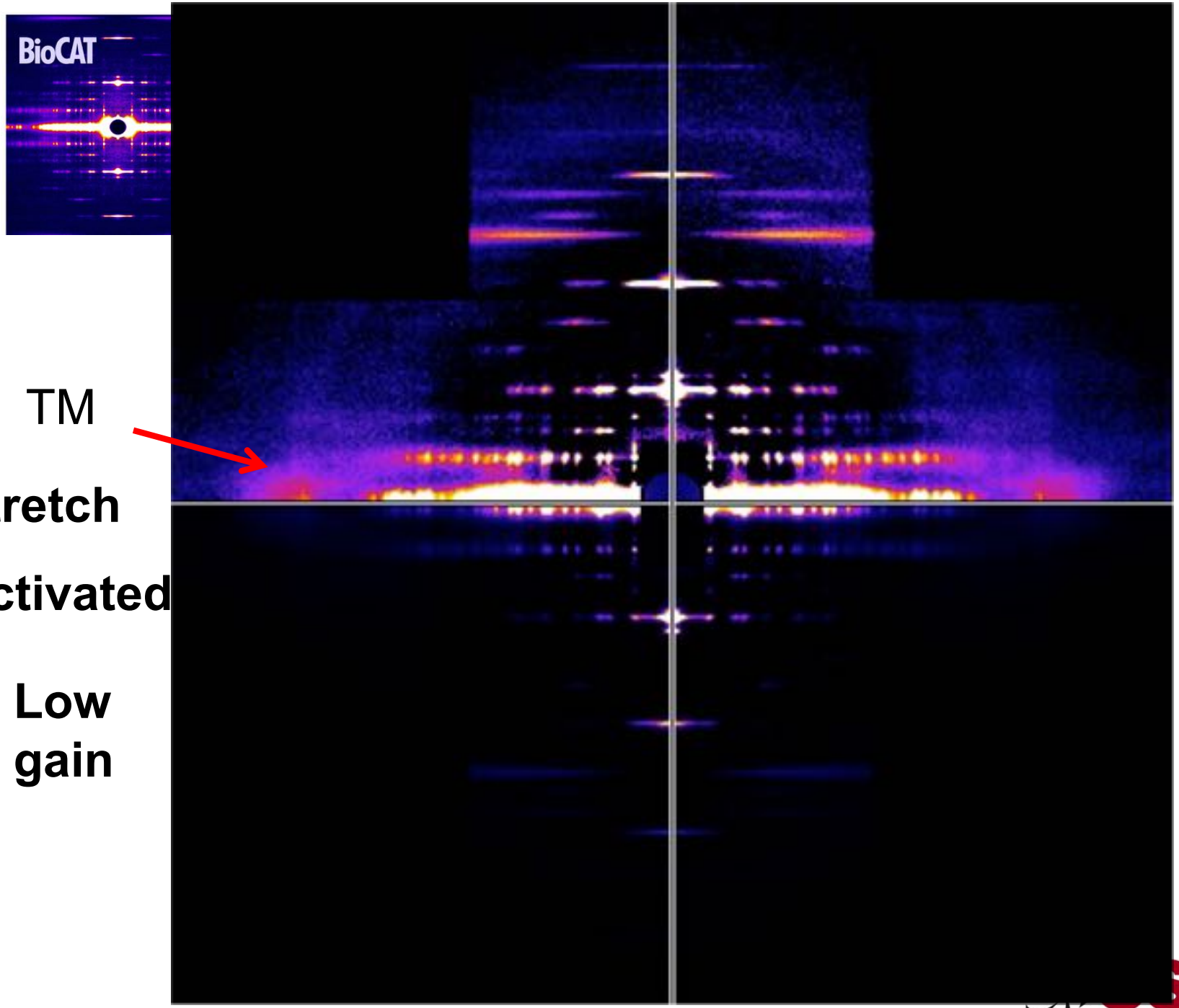

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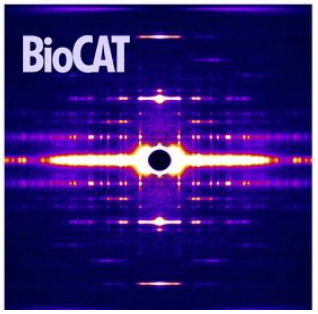

Time's Are Changing

- We still like to do heroic time resolved experiments with hard-core experts but most of our users these days are main stream physiologists wanting to test structural hypotheses with transgenic mouse models.
- We have built up apparatus to cater to these users
- Streamline ability to ship mice and prepare samples on site
- New efficiencies in operations allow us to accommodate more users
- Greatly improved analysis tools

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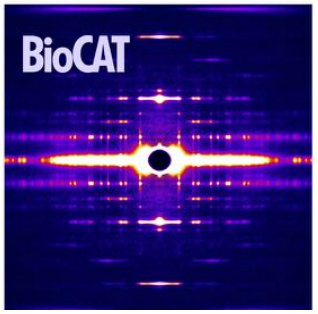

Facilities for Muscle Diffraction

- Flexible small-angle diffraction instrument that can deliver > 10^{13} 12 keV photons/s into focal spots of < 140 x 30 μm
- Diverse set of high performance X-ray detectors
- Well-equipped biochemistry wet lab
- Comprehensive set of muscle chambers and physiological apparatus
- Talented and dedicated beamline scientist – Dr. Weikang Ma.

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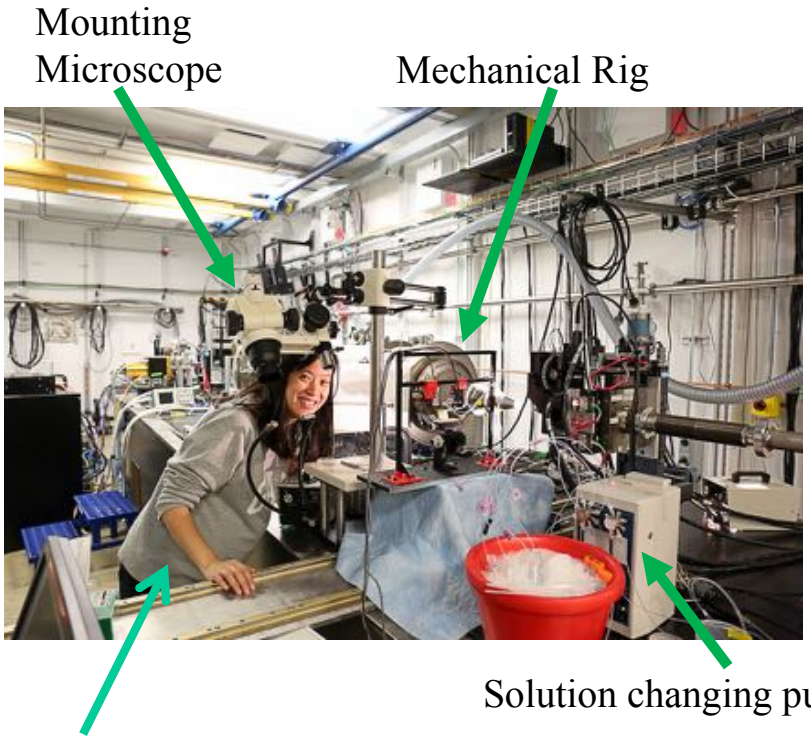
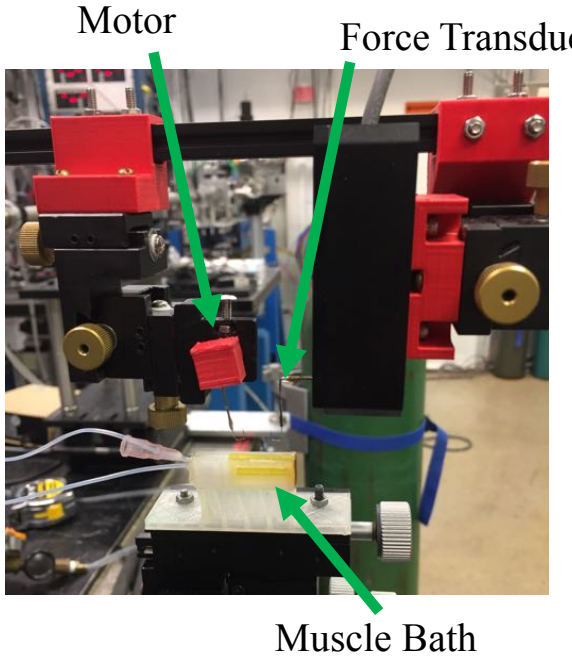
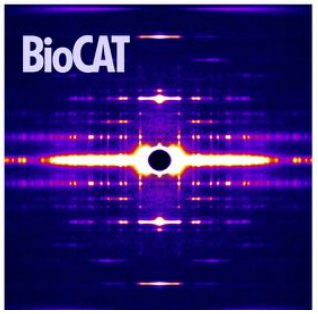
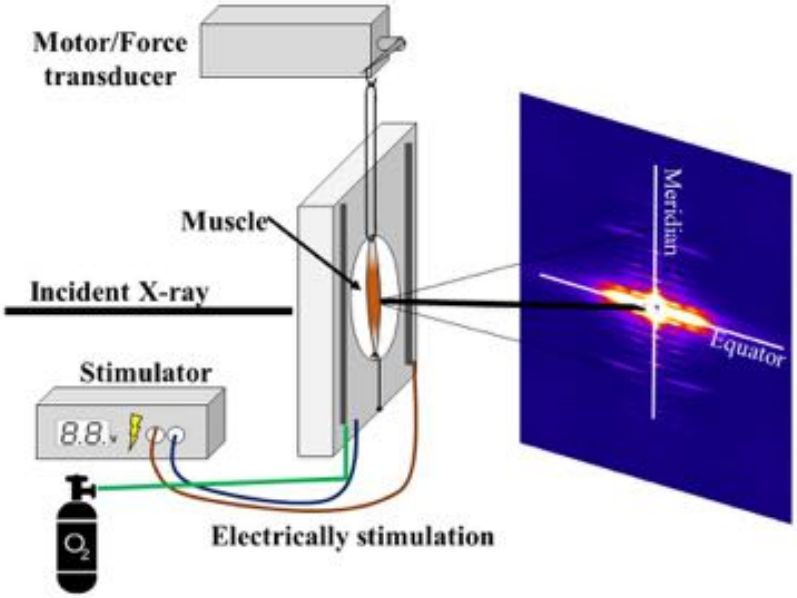
Mechanical Setups-Skinned Muscle

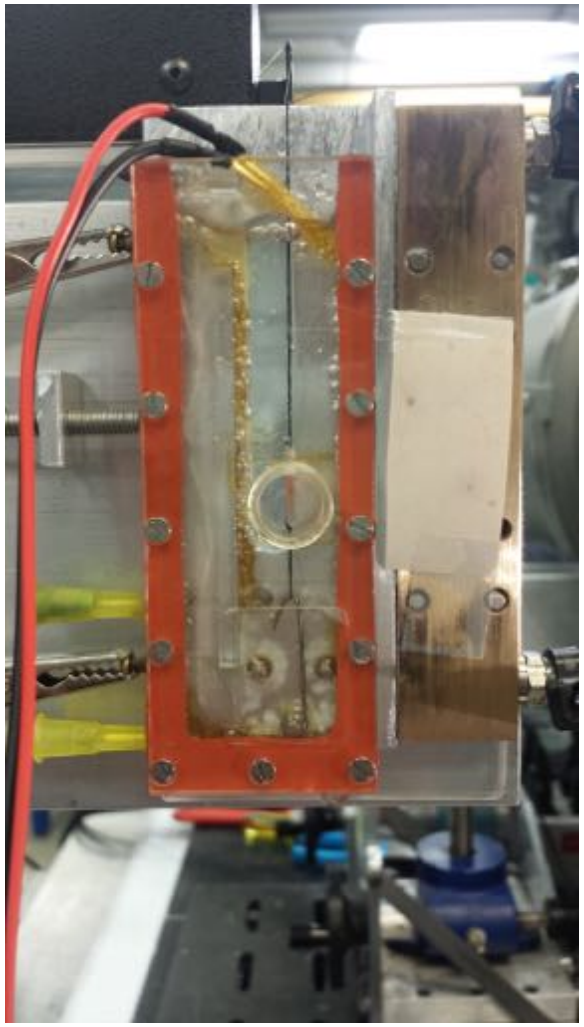






Mechanical Setups-Intact Muscle





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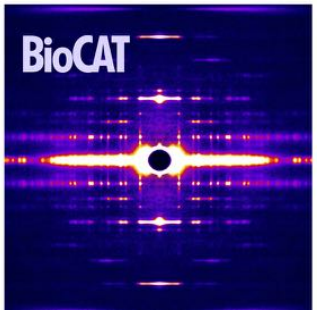

Physiological Equipment

- Several Aurora 400 series transducers
  - 2 500 mN, 1 100 mN, 1 mN
- Several Aurora Motors
  - 2 308B's, 3 315's
- 2- 300 series muscle levers
  - 1LR and 1 custom modified for speed
- 2 Aurora DAQ systems
  - – 1 Linux, 1 Windows
- Laser diffraction
- 3D Printer allows fast modifications to set ups

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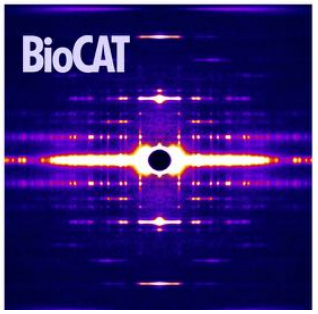
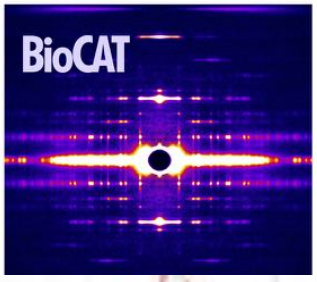


CCD - Detectors

- Mar 165
  - 165 mm circular active area. 40 or 80 μm pixels 65 μm psf phosphor
- Pros-
  - easy to use and reliable
  - Good spatial resolution
- Cons
  - sensitivity, triggering not suitable for time resolved experiments



Pilatus 3 1M Detector

- Delivered October, 2014
- 1k x 1k, 172 × 172 μm^2 pixels
- 170 x 180 mm^2 active area,
- Pros:
  - Photon-counting detector able to read at 500 fps
  - No read noise
  - Very easy to incorporate into time resolved protocols
- Cons:
  - Gaps between individual chips obnoxious.
  - Large pixels can be a problem.
- Very useful for many classes of experiments but not all

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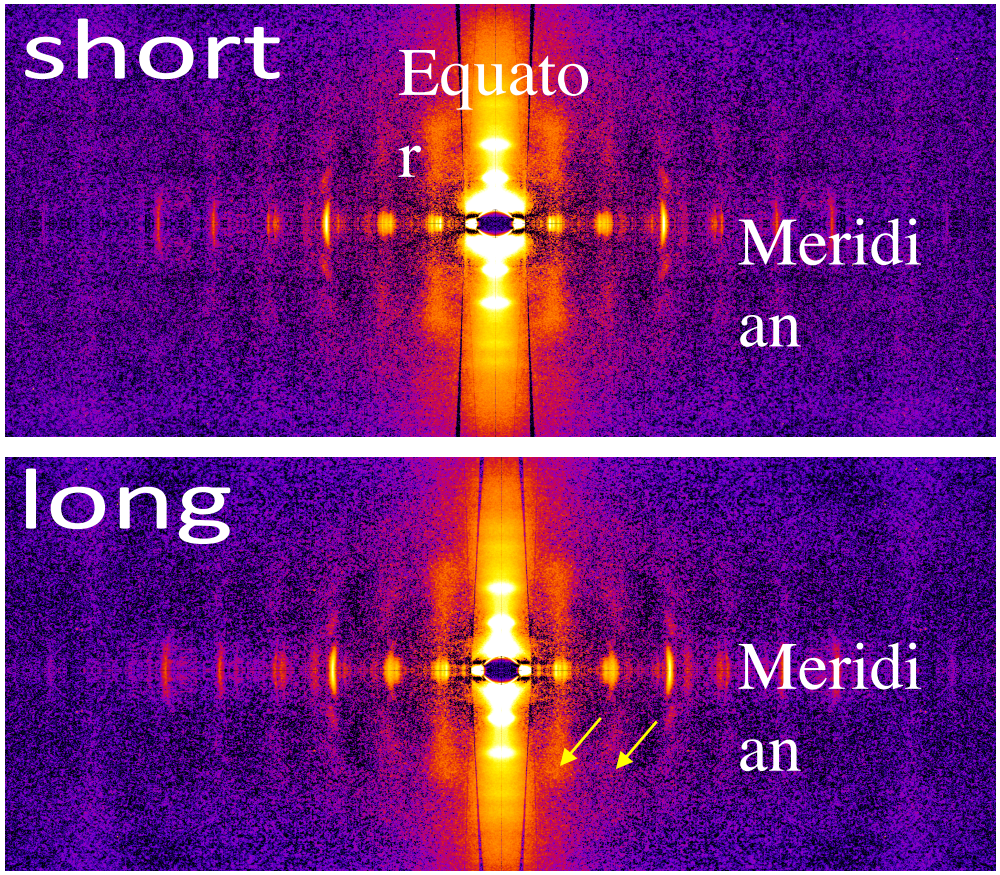
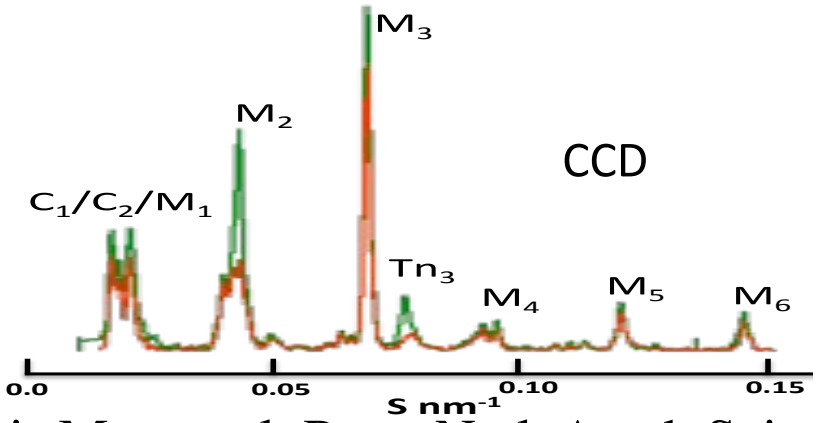
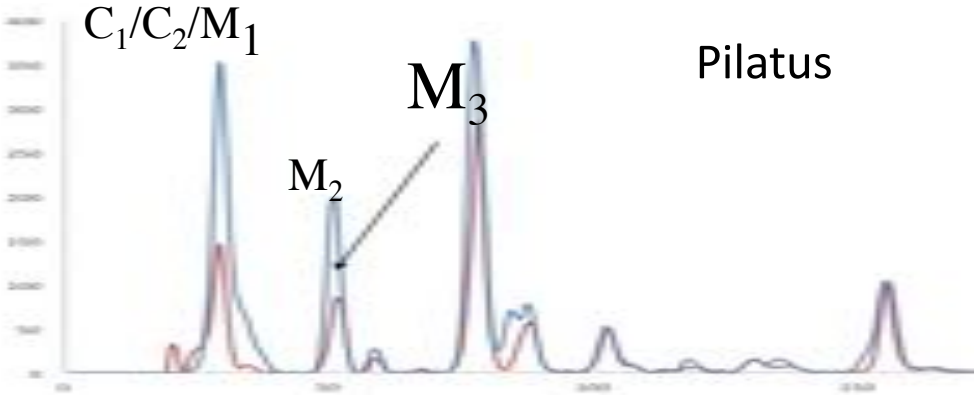


Pilatus Pixel Size Not Optimal for Muscle

- 172 µm Pilatus vs ~ 40 µm (~65 µm psf) pixels CCD detectors
- Fine diffraction features on meridian hard to resolve



Ait-Mou et al. Proc. Natl. Acad. Sci. **113**, 2306-2311 (2016).



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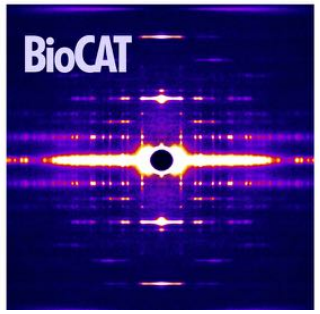
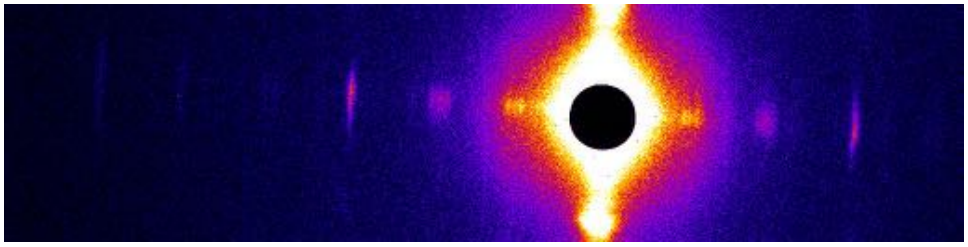


Merlin Pixel Array Detectors

- Able to collect data from muscle continuously at 200 Hz and for 1 s at 1000 Hz.
- Direct photon detection (high spatial resolution)
- Photon counting (noiseless)
- 1024 x 256 55 µm pixels
- Have an additional 512 x 512 pixel unit
- Very small active areas but small form factor allows creative experimental conformations



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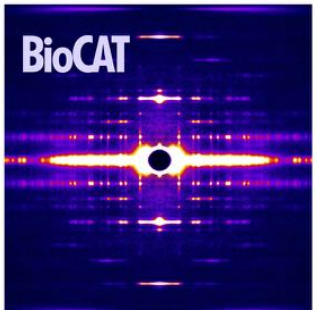
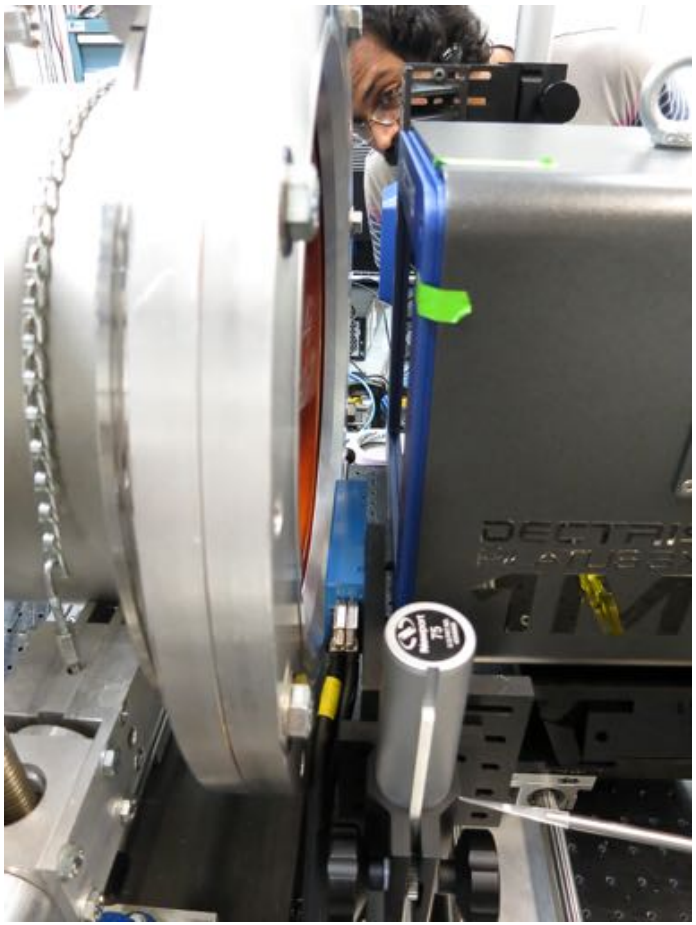


Two Detector Configuration



- Merlin fits in front of the Pilatus
- Can collect higher order meridionals (eg. 27.2 actin and 28.3 nm myosin reflections)
- Or high resolution pattern from the meridian on one side of the pattern
- Particularly useful to collect 2nd order actin meridional

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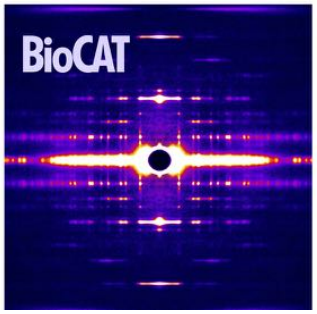
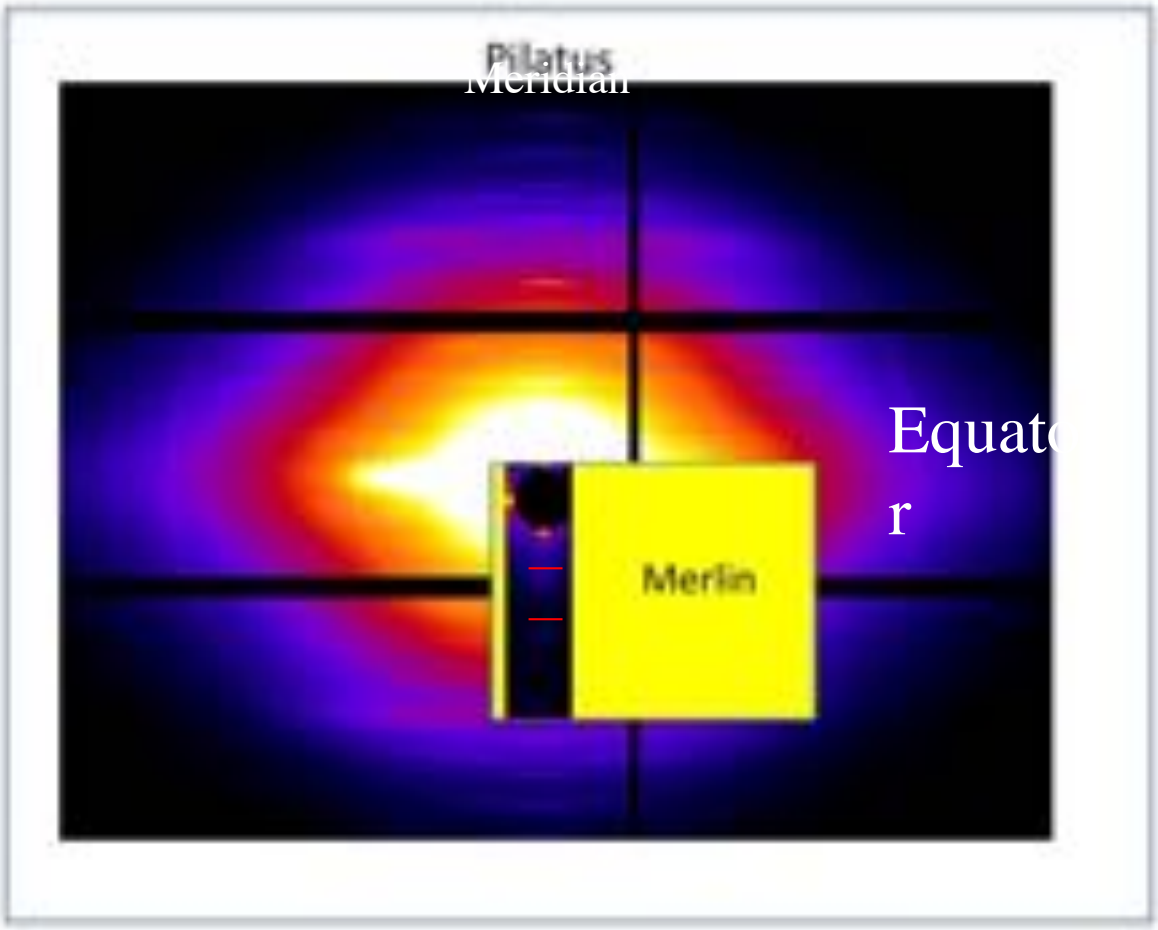


Dual Detector Configuration for Muscle Diffraction



- Small profile of Merlin detector allows dual detector configuration with Pilatus 1M
- large active area and high spatial resolution only where it is needed

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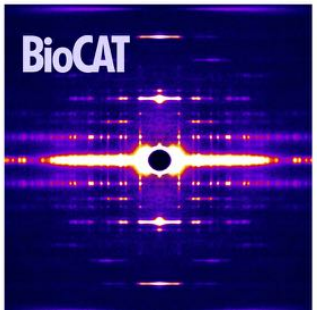

EIGER 500k Detector

Sensitive area (width x height) [mm^2] 77.2 x 38.6
(Brandeis detector was 86 x 49 mm^2)
Pixel size [μm^2] 75 x 75
Number of pixels (horiz. x vert.) 1030 x 514 = 529,420
Maximum frame rate [Hz](30 s burst) 9000
Maximum frame rate [Hz] (continuous) 3000
Readout time [µs] continuous readout, 3 µs dead time
Point-spread function 1 pixel
Maximum count rate [phts/s/mm^2] $5 * 10^8$
Dimensions (mm) 114 x 92 x 242

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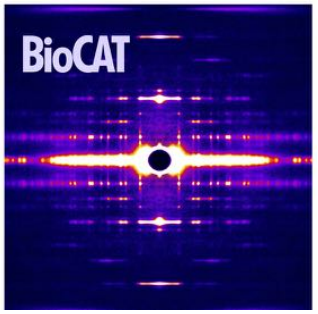
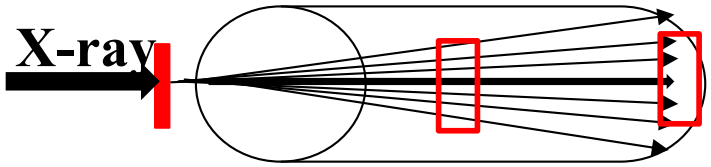


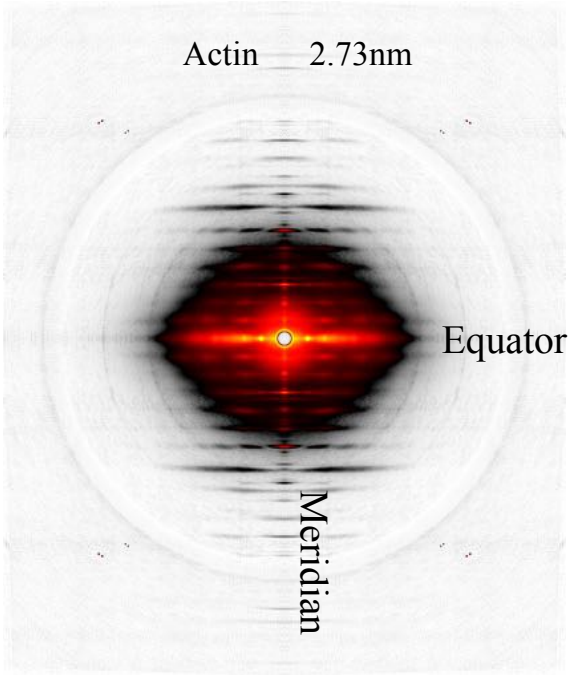
Camera Lengths

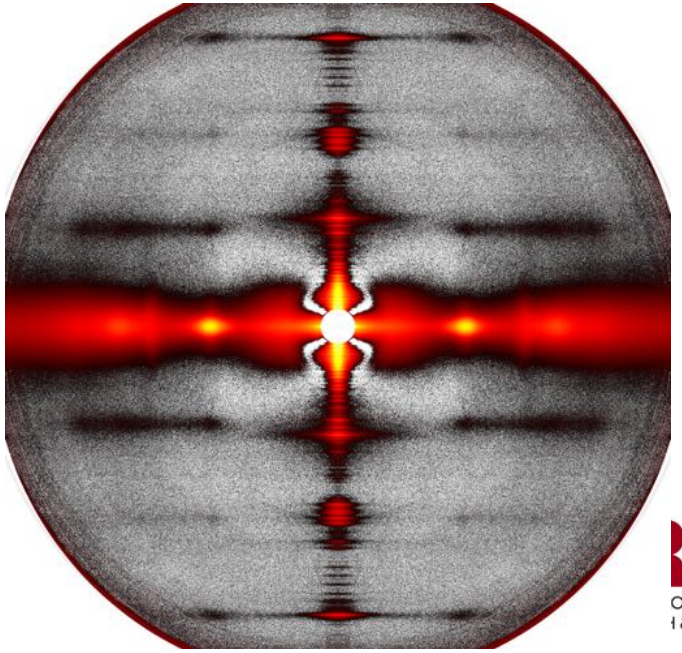
- Typical setup 2-3.5 meter
- Possible range: 1.5 - 9.5 meters



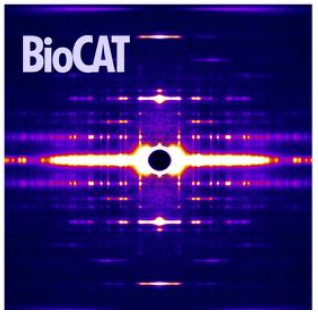
9.5M

2M





ON RADIATION
& INSTRUMENTATION



MuscleX

- User friendly program package that greatly speeds up process of analyzing data
- Open source and runs natively on Linux and Mac OS
- Windows installer available
- Weikang Ma will explain its capabilities later in this session
- https://github.com/biocatiit/musclex

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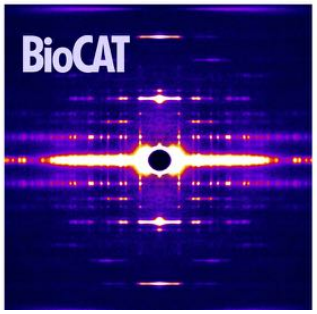

Useful Links

- BioCAT website
- http://www.bio.aps.anl.gov
- MuscleX website
- https://musclex.readthedocs.io/en/latest/
- Can always email me at:
- irving@iit.edu

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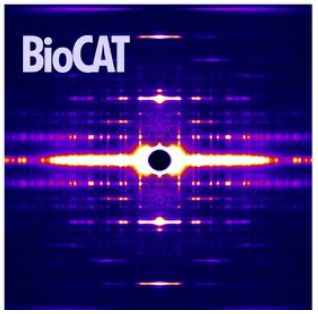

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