



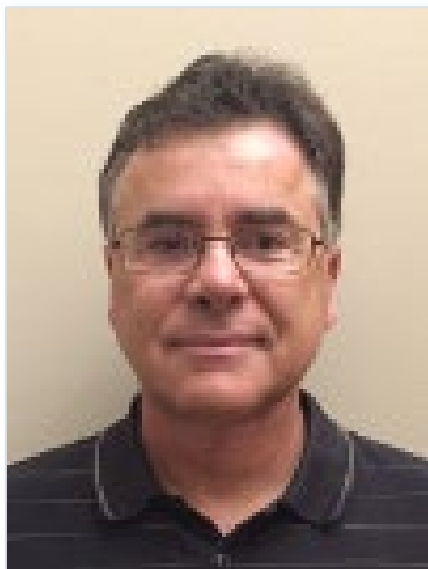
# Macromolecular Crystallography

## Redefining the Role of the Home-Lab X-ray Facility

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# Welcome



Dr. Matt Benning  
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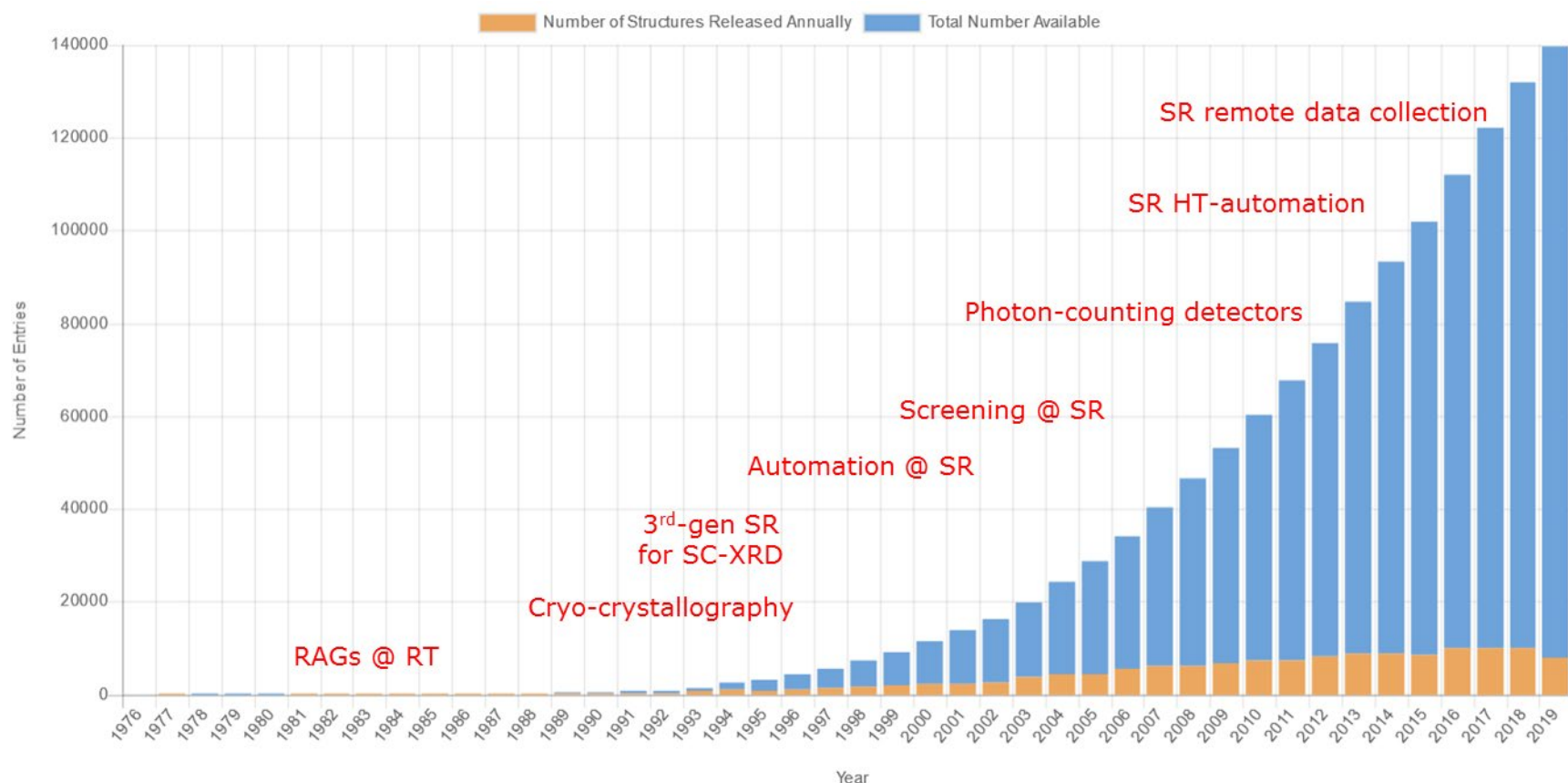
Dr. Vernon Smith  
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# Redefining the Role of the Home-lab X-ray Facility



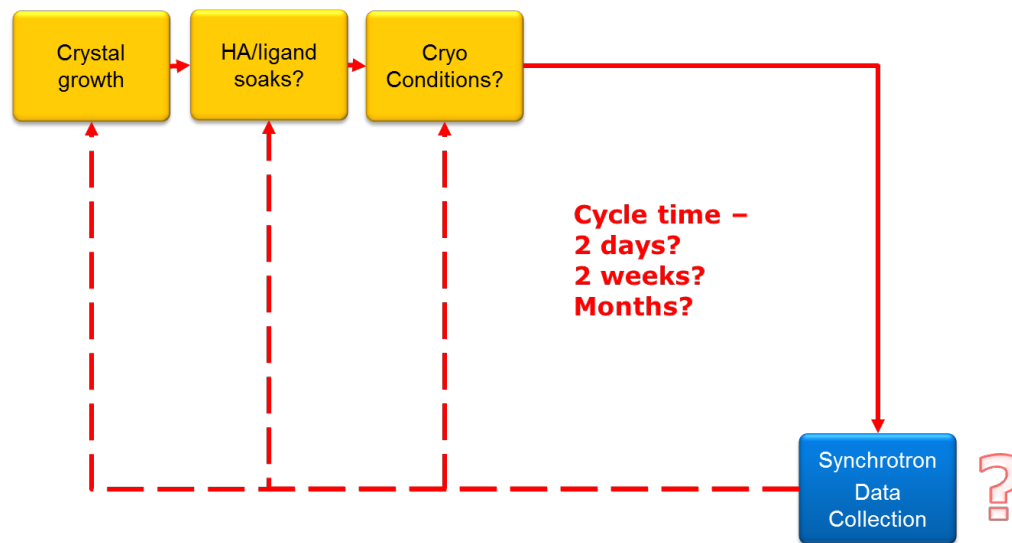
- A look at the typical crystal-to-structure workflow
- Potential to reduce time from crystal to structure
- Overview of D8 VENTURE with I $\mu$ S DIAMOND X-ray source and PHOTON III CPAD
- D8 VENTURE case studies

# Technological Developments in MX



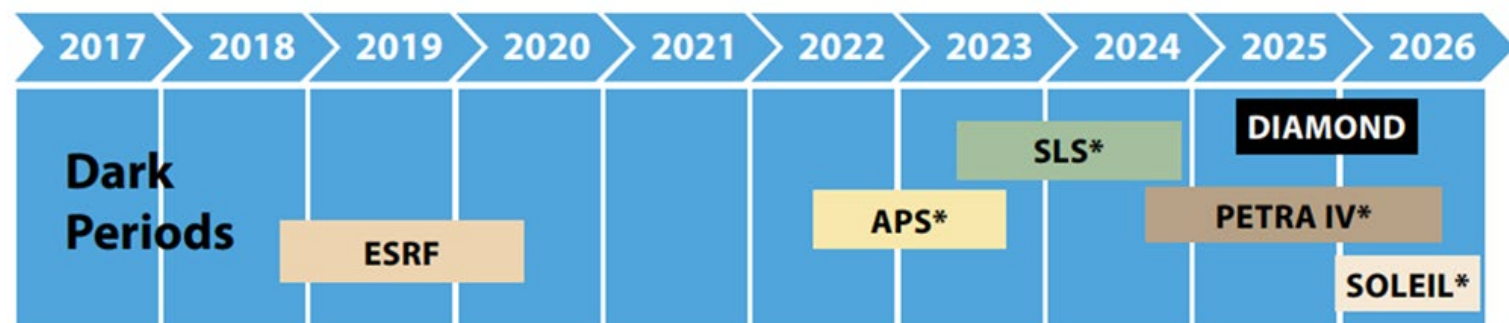
# Typical Crystal-to-Structure Workflow

- Heavily reliant on synchrotrons
  - Perceived excess of beamtime
  - Remote data collection
- Diffraction screening and data collection remote from crystallization facility – long turnaround times prolong optimization cycle
- Beamtime not efficiently used testing crystals
- The generation rate of useful datasets collected is not optimal
- Collect large amounts of non-optimal data



# Planned Synchrotron Shutdowns

- Globally coordinated program of synchrotron upgrades over coming years
- Will result in additional pressure on beamtime worldwide

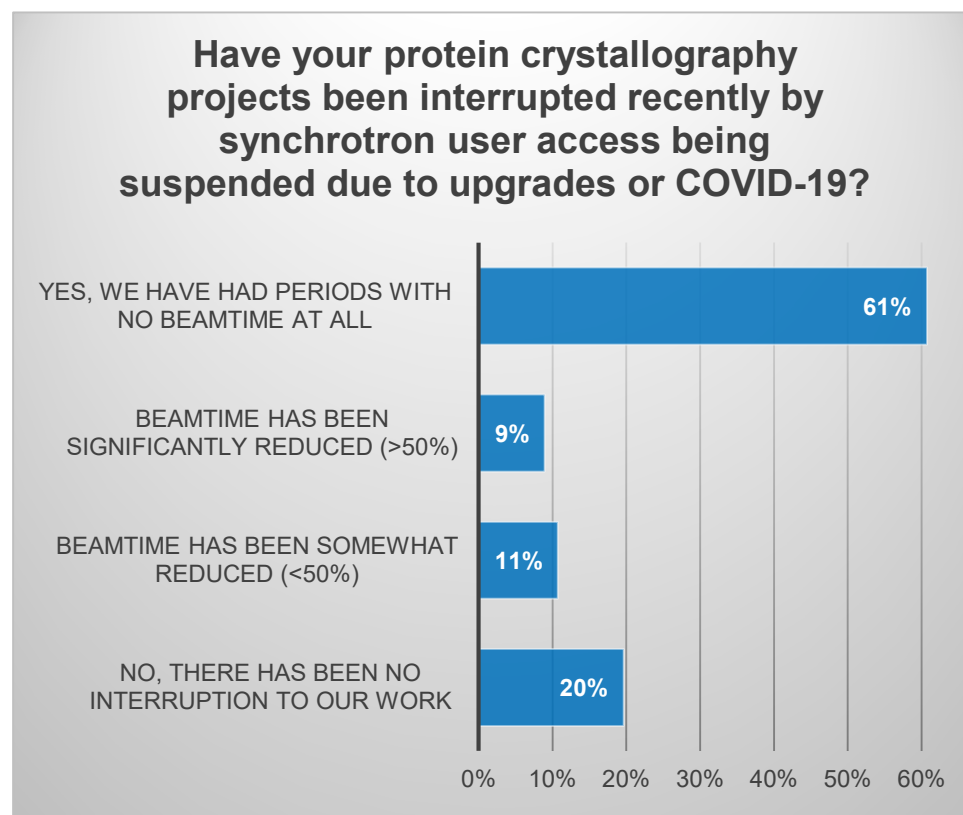


\*to be confirmed. Discussions ongoing with European facilities

<https://www.diamond.ac.uk/Home/About/Vision/Diamond-II.html#>

# Webinar Registration Question - Results

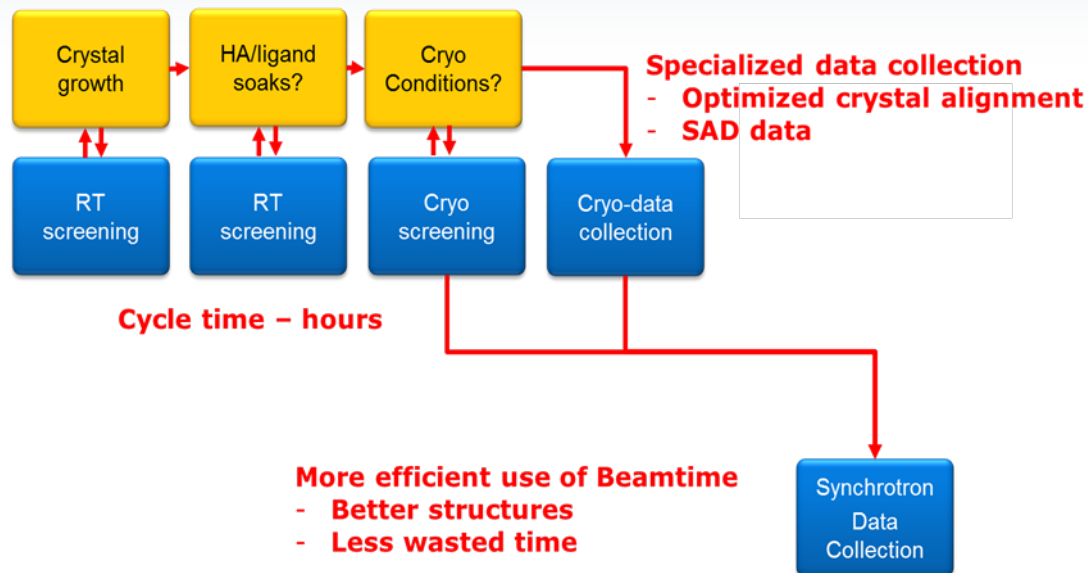
- Thank you for your responses!
- Over 200 respondents
- Only 20% of crystallographers reported no recent disruption to your research



# Optimised Crystal-to-Structure Workflow

- Crystal screening adjacent to crystallization facility
- Immediate feedback into crystal optimization
- Investment in home-lab diffractometers can be difficult to justify
  - Performance does not match synchrotrons
  - Funding pressure
  - Operational costs
  - Maintenance requirements

**Maximize the performance-to-cost ratio**



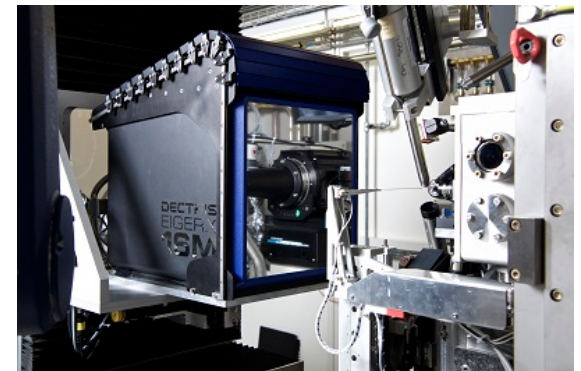


# Beamline performance

## What is *most important*?



- X-ray source
  - High intensity
    - *Brilliance*
  - Small beam
  - High spectral purity
  - Low divergence
  - High stability, no downtime
- Detector
  - High sensitivity
    - Photon counting
    - *Detective quantum efficiency (DQE)*
  - Large active area
    - *Detective collection efficiency (DCE)*
    - PILATUS 6M 423.6 x 434.6 mm<sup>2</sup>
    - EIGER 16M 311 x 328 mm<sup>2</sup>
  - Dynamic range, high linear count rate



# Requirements of modern home-lab X-ray facilities



- Located in crystallization facility
- Beam must always be available
- Must be accessible to wide variety of users
- Diffraction relatable to modern synchrotrons
- Must be fast
- Low downtime
- Low cost of ownership
- **Accelerate protein-to-structure**



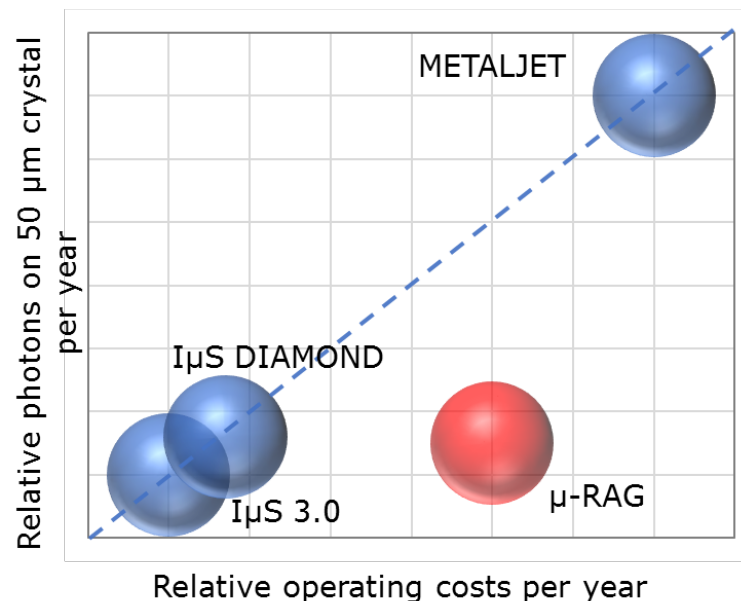
**D8 VENTURE**

# Development of modern X-ray sources

## Maximizing Productivity / Cost



- Modern X-ray sources are designed to provide small, bright beams suited to current crystallographic challenges
- Also designed to have maximal uptime and minimal operating costs
- **Source development drives towards maximizing output and minimizing costs**

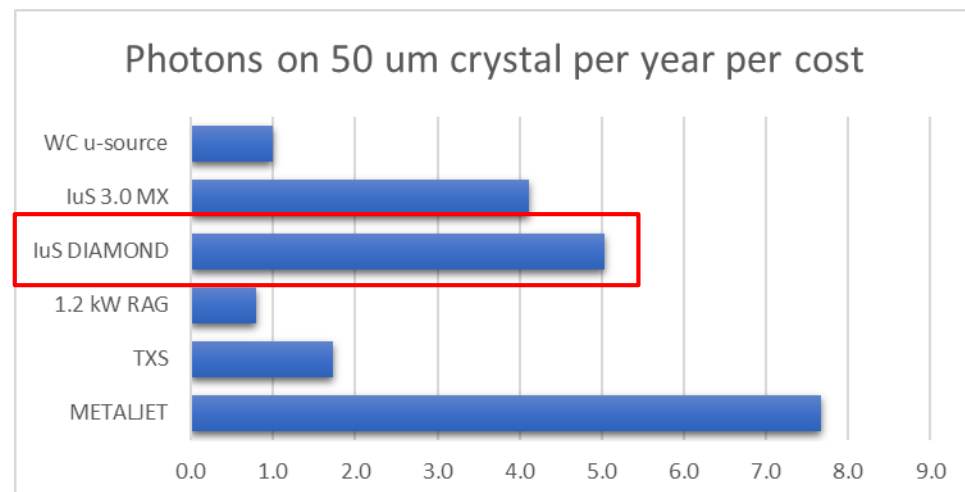
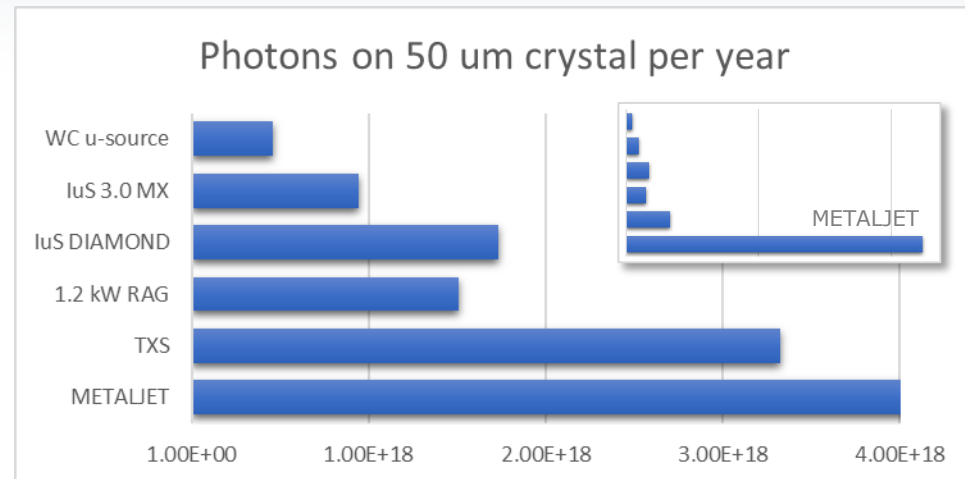


# Development of modern X-ray sources

## Maximizing value of investment



- Typical crystal size 50  $\mu\text{m}$
- How many photons are available for such a crystal per year?
  - X-ray flux in 50  $\mu\text{m}$  beam diameter
  - Deterioration of beam flux over time
  - System uptime
- Costs:
  - Downtime
  - Staff operation time
  - Utilities costs (electricity, water)
  - Maintenance costs, service contracts
- **Today focusing on the IpS DIAMOND**
  - Highest productivity per unit cost
  - Can reduce time to structure



# D8 VENTURE

## Source

- METALJET Liquid Gallium
- I $\mu$ S DIAMOND microfocus tube

## Helios MX optics

- 2x reflectivity over previous generation
- Higher intensity, lower background

## PHOTON CPAD detectors

- First in-house CPAD (charge-integrating pixel array detector)
- Large active area
- Photon-counting capabilities
- Mixed-mode data collection



# I $\mu$ S DIAMOND Microfocus Source

## Advanced anode and cathode technology

- 60 watt, air-cooled source
- Hybrid diamond anode dissipates the heat load much more efficiently than standard copper anode
- Lifetime average intensity higher than microfocus rotating anodes
  - Doesn't suffer the high anode degradation like traditional RAGs (30% per year)

## Ultra-low maintenance

- No external cooling
- No 3-phase power
- No filament changes

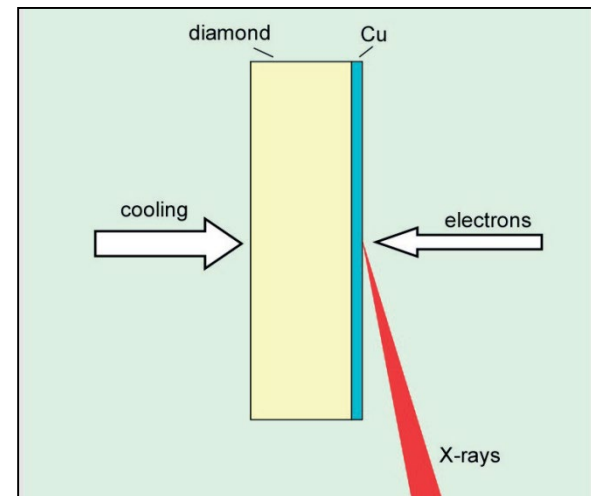
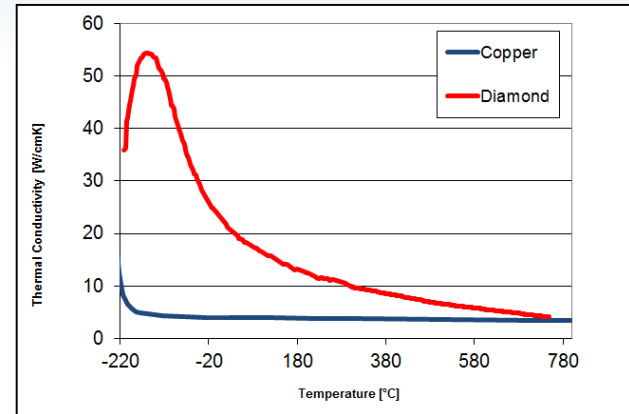


# Microfocus Tube Sources

## Hybrid diamond anode



- If you could remove the heat more efficiently on a stationary target, you could apply a higher power load and increase the intensity output
- Copper has a high thermal conductivity, but diamond is much better, 5x
- Hybrid diamond anode
  - Bruker I $\mu$ S DIAMOND
  - High-quality synthetic diamond substrate
  - Thin layer of copper on surface
  - Allows a higher power load on the anode without degradation
  - Intensity output is >2x compared with a solid copper anode



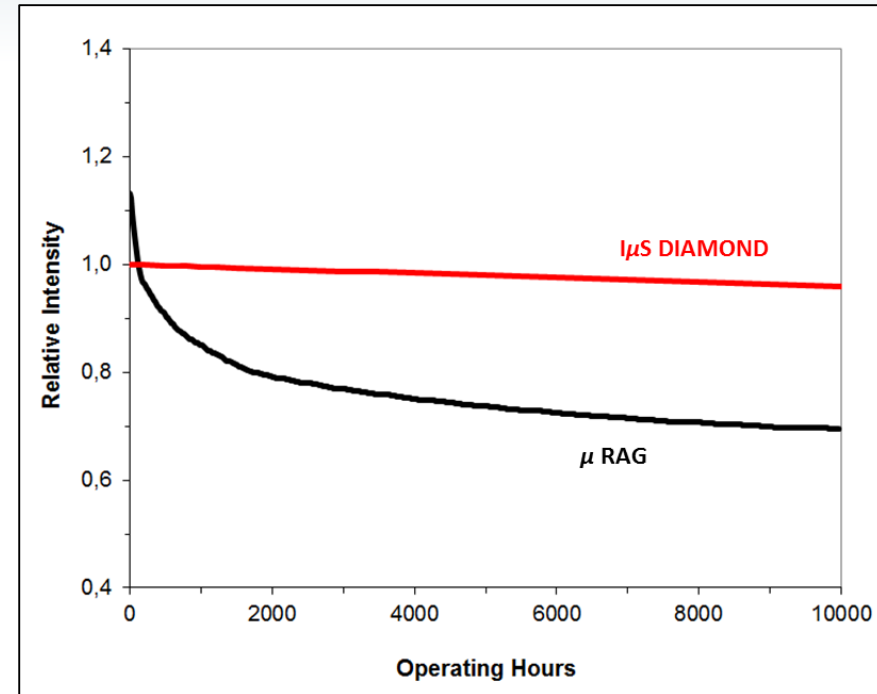
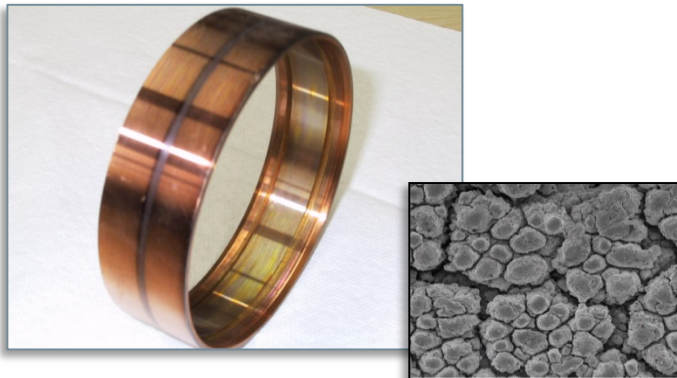


# Microfocus Tube Sources

## Hybrid diamond anode



- Stationary microfocus tubes offer reduced loss of intensity over time
  - The loss is typically <5% over 10,000 hours of full power operation
  - There are many fewer thermal cycles in a stationary anode (cycles occur only when the tube is turned off and on) and thus micro-cracking due to thermal fatigue is much reduced
  - No moving parts that get worn out and then cause further loss in intensity





# Source Comparison

## Operational costs and maintenance



- Rotating anodes require an external cooling water source (Haskris chiller). The I $\mu$ S DIAMOND source is air cooled.
- Rotating anodes require 220 V, 3-phase power. I $\mu$ S DIAMOND has no special power requirements using 110 V, single-phase power. With its self-contained enclosure, the I $\mu$ S DIAMOND can be installed in almost any lab space.
- Rotating anodes required 2-3 filament changes a year, plus a yearly anode rebuild. The cost just for the parts is ~\$25,000. Anodes cannot be rebuilt by the user. The I $\mu$ S DIAMOND has no annual maintenance costs.

	RAG	I $\mu$ S DIAMOND
Power setting	1200 W	60 W
Power requirement	3-phase	single-phase
Anode cooling	Water cooled	Air
Yearly maintenance costs, parts	~\$25k	\$0
Scheduled yearly downtime	3-4 weeks	0

# Source Comparison

## Brilliance



- The performance of the I $\mu$ S DIAMOND is very similar to the current microfocus rotating anode generators

Source	Power (W)	Brilliance (photons s <sup>-1</sup> mm <sup>-2</sup> mrad <sup>-2</sup> )
Standard sealed tube*	2000	0.6 x 10 <sup>9</sup>
Microfocus sealed tube*	50	2.0 X 10 <sup>9</sup>
Microfocus RAG*	1200	6.0 X 10 <sup>9</sup>
I $\mu$ S DIAMOND	60	5.0 X 10 <sup>9</sup>

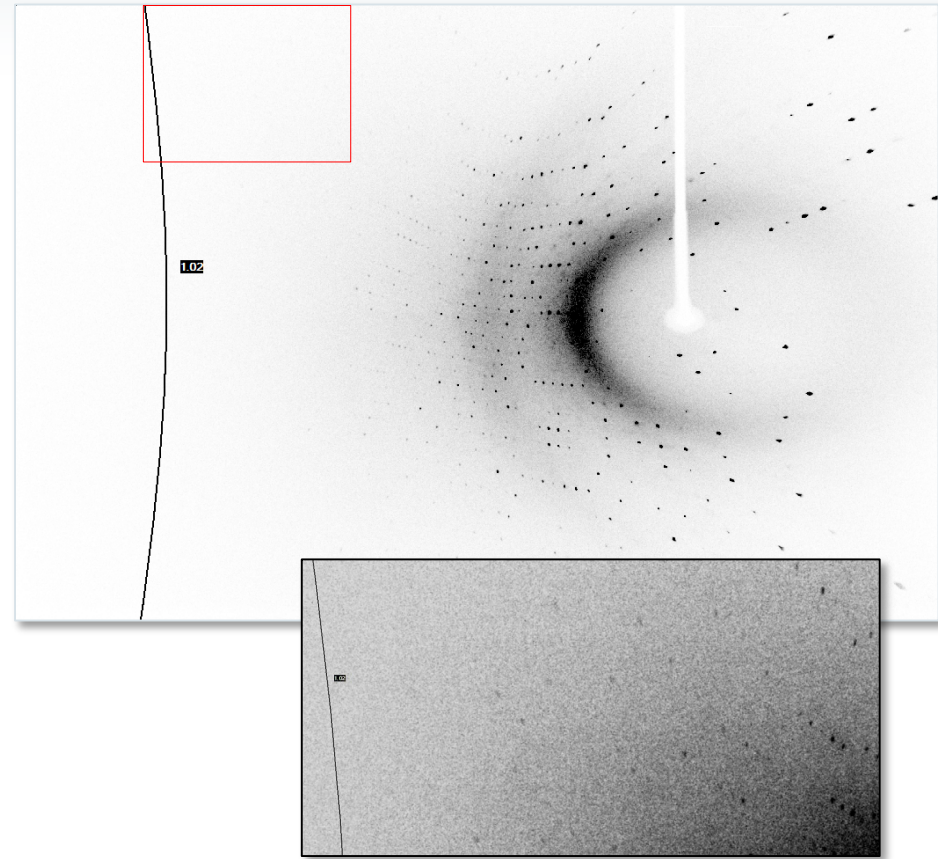
\*"Collecting data in the home laboratory: evolution of X-ray sources, detector and working practices". Acta Cryst., **D69** 2013.

# D8 VENTURE

## Monoclinic HEW Lysozyme



- Space group  $P2_1$
- Cell constants  
 $a=27.5$ ,  $b=62.4$ ,  $c=59.6$  Å,  $\beta=90.5^\circ$
- DX 40 mm
- Max resolution 1.02 Å
- Detector PHOTON III (M28)
- Source I $\mu$ S DIAMOND
- Crystal dimensions  
0.125 x 0.185 x 0.300 mm
- The unique attributes of the I $\mu$ S DIAMOND source and PHOTON III detector provide the D8 VENTURE with an overall system performance similar to the current generation rotating anode generators and HPAD detectors without the high operational costs.



# D8 VENTURE

## Monoclinic HEW Lysozyme



- To illustrate this, a data set on a monoclinic form of HEW lysozyme was collected on the D8 VENTURE. This data was compared to an XtaLAB data set submitted to the PDB (code 3WL2) using a MICROMAX-007 HF source and DECTRIS Pilatus 200K detector.
- The D8 VENTURE produced very good merging statistics across reciprocal space. The only data statistic available from the PDB submission was Rmerge. Both the diffraction limit and Rmerge values were similar for the two data sets.

Intensity statistics D8 VENTURE			
	Overall	Inner shell	Outer shell
Low res limit	29.81	29.81	1.04
High res limit	1.02	5.59	1.02
Rmerge	0.046	0.04	0.999
Rmeas	0.048	0.041	1.195
Rpim	0.013	0.009	0.645
I/ $\sigma$ (I)	16.5	89.6	1.5
CC(1/2)	1	0.999	0.505
Completeness	100	98.5	99.9
Multiplicity	6.3	19.2	3.2

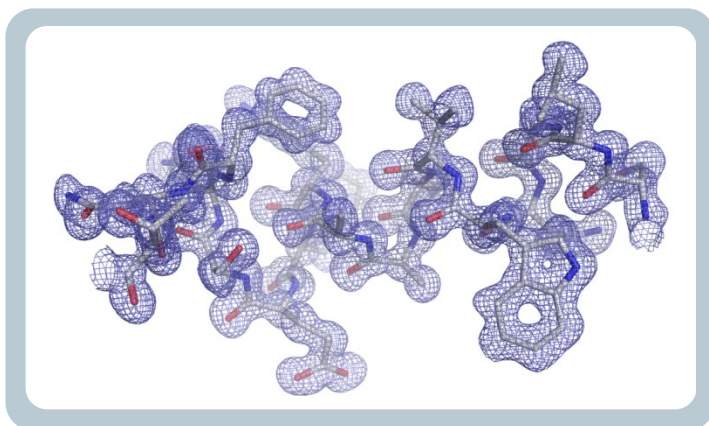
\* Statistics generated by AIMLESS

# D8 VENTURE

## Monoclinic HEW Lysozyme



- The lysozyme model was refined against the D8 VENTURE data in the same manner listed in the PDB submission. With minimal manual fitting, there was good agreement between the observed and calculated data.



Electron density contoured at  $1\sigma$

REFMAC refinement statistics		
	D8 VENTURE	XtaLAB P200
Resolution range (Å)	30 - 1.02	30 - 0.96
Rmerge	0.05	0.054
Completeness (%)	99.9	91.0
Rwork	0.168	0.173
Rfree	0.196	0.197
Bonds RMS	0.014	0.015
Angles RMS	1.679	1.815
CC Fo-Fc	0.949	0.950
CC Fo-Fc Free	0.937	0.939

# PHOTON III Series

## First photon-counting CPAD for the home lab



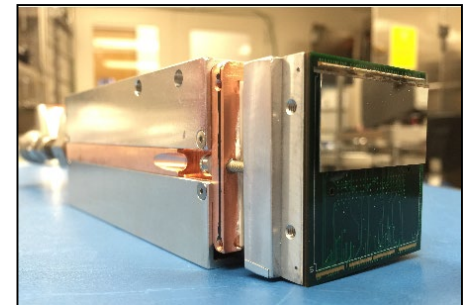
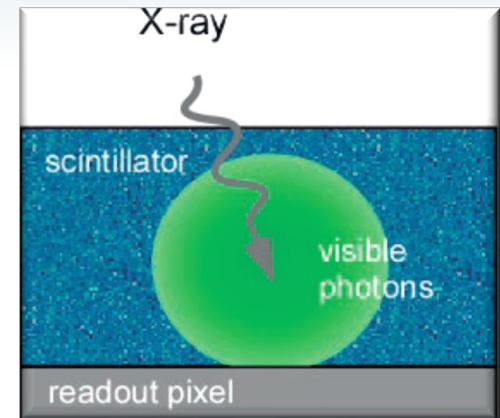
- Largest active areas for a home laboratory detector
  - 200 x 140 mm<sup>2</sup>
  - 140 x 100 mm<sup>2</sup>
- 100% of active area is sensitive
  - No gaps
  - No dead areas in pixels
  - Frame rate 70 fps
- *Advanced Mixed-Mode photon counting*
  - Photon-counting and integration at the same time
  - You don't compromise the strong low angle data while improving the accuracy of the weak reflections



	PHOTON III 28	PHOTON III 14
Active area (mm)	208 × 139	104 × 139
Sensor format (pixels)	1,536 × 1,024	768 × 1,024
Number of modules	2	1
Gaps / dead area	0%	

# What is a Charge-Integrating Pixel Array?

- A CPAD *is a pixel array* but has additional features
  - Full charge is measured, not a simple threshold
  - Effective gain is variable to achieve high dynamic range
  - Massively parallel readout to achieve high speed
  - Photon-counting capabilities
- CPADs eliminate the two largest drawbacks of photon-counting HPADs
  - No count rate saturation
  - No charge sharing noise
- CPAD detectors were recently developed for applications at 4<sup>th</sup> Gen beamlines\*
  - Jungfrau, Mönch (SwissFEL), AGIPD (European XFEL), CSPAD, ePIX (LCLS)



ePIX (LCLS)

\*J Synch. Radiat. 2014 Sep 1; 21(Pt 5): 1006–1010, Pixel detectors for diffraction-limited storage rings, P. Denes and B. Schmitt

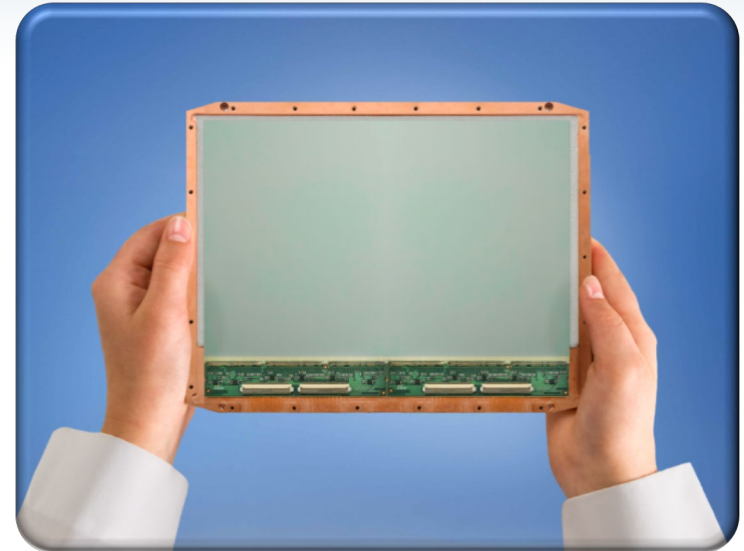


# PHOTON III

## Mixed-mode data collection



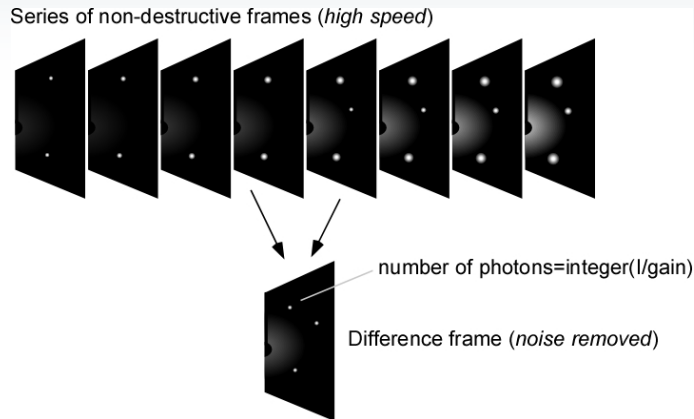
- The strong reflections are integrated and weak reflections are measured using photon-counting
  - Possible to achieve Poisson-limited DQE across the entire dynamic range
  - E. Kraft et al., *Counting and Integrating Readout for Direct Conversion X-ray Imaging: Concept, Realization and First Prototype Measurements*.  
[doi:10.1109/TNS.2007.891571](https://doi.org/10.1109/TNS.2007.891571) IEEE Trans.Nucl.Sci.,54,383.
- Mixed-mode is just an integrating detector that is run at a very high frame rate



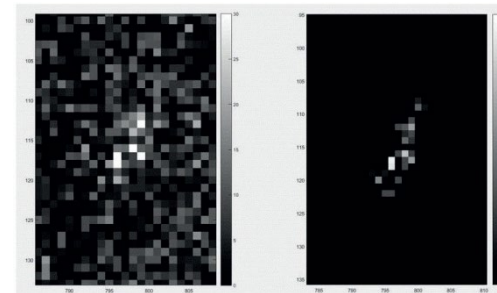
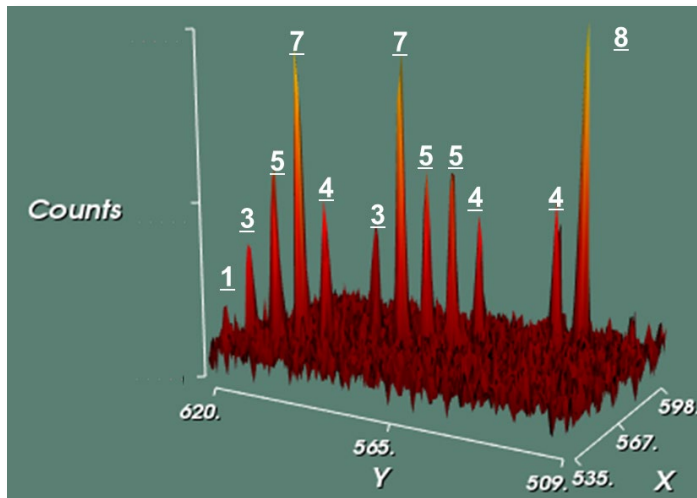


# PHOTON III

## Mixed-mode data collection



- Sub-frames quickly integrated
- A threshold is applied in software rather than while the frame is being acquired
- Photons counted in the space domain
- Sub-frames are compiled to produce full frame



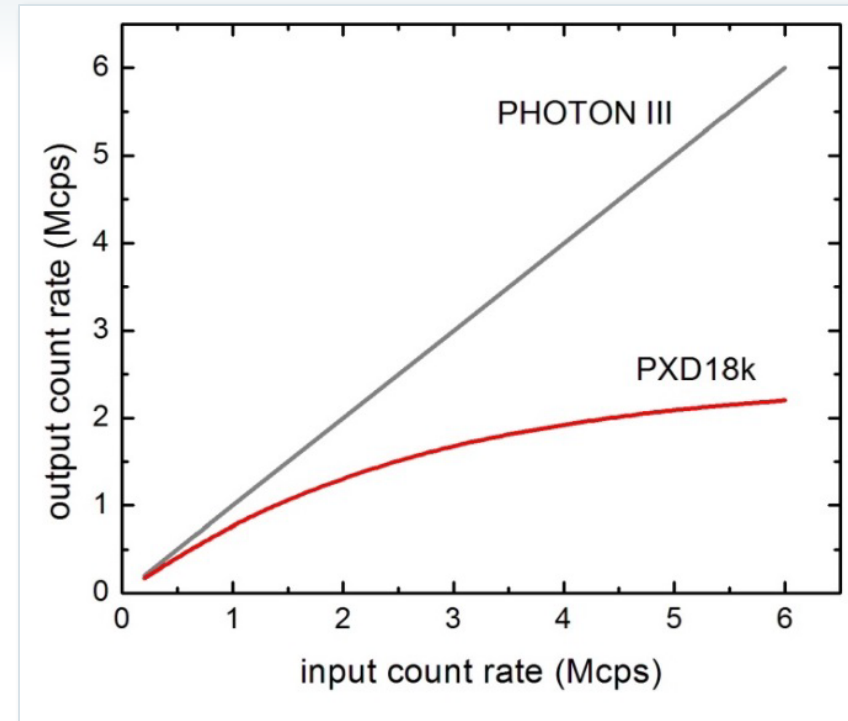
Single Bragg reflection, 5 min exposure

# Mixed-Mode Detection

## No lost counts at high X-ray frequency



- Conventional photon counting detectors suffer saturation at high count rates
  - Typically more than half of incident X-rays lost at counts rates above 2 Mcps



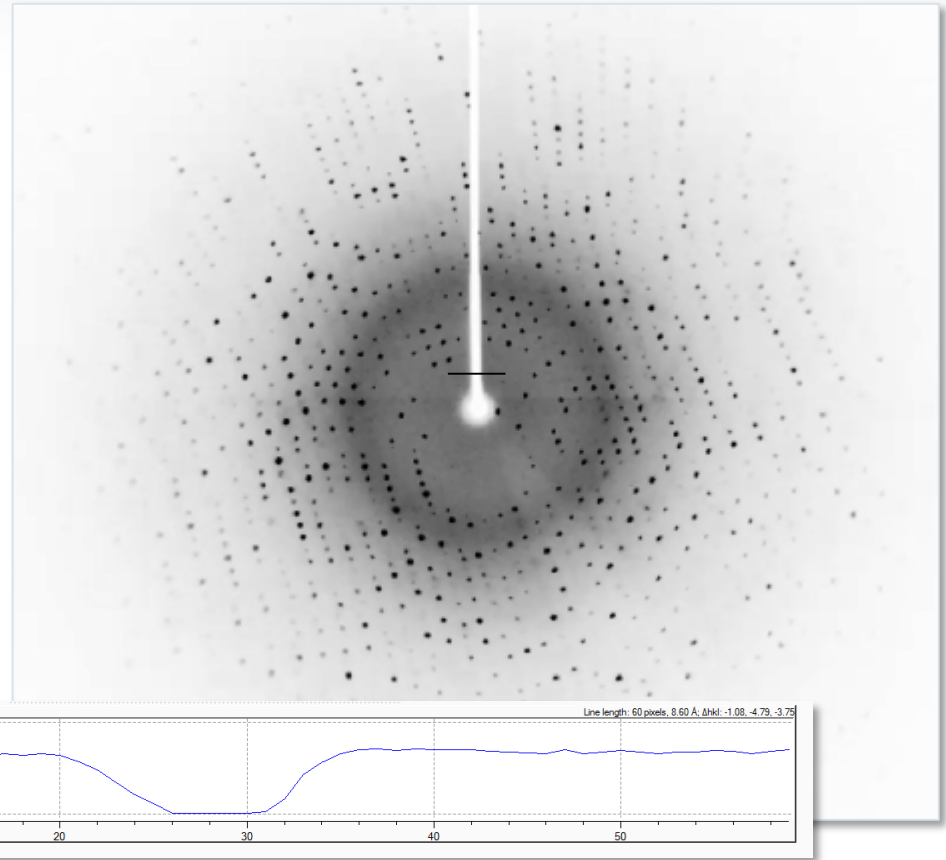
\*M.W. Tate, et al., *A Medium-Format, Mixed-Mode Pixel Array Detector for Kilohertz X-ray Imaging*, [Journal of Physics: Conference Series](#), [Volume 425](#), [Part 6](#), 2013.

# PHOTON III

## First photon-counting CPAD for the home lab



- Lysozyme image
  - I $\mu$ S Diamond source
  - PHOTON III (M28)
  - DX 50 mm
  - Exp time 7 sec
  - Rotation width 0.5°
- No background counts behind the beamstop shadow

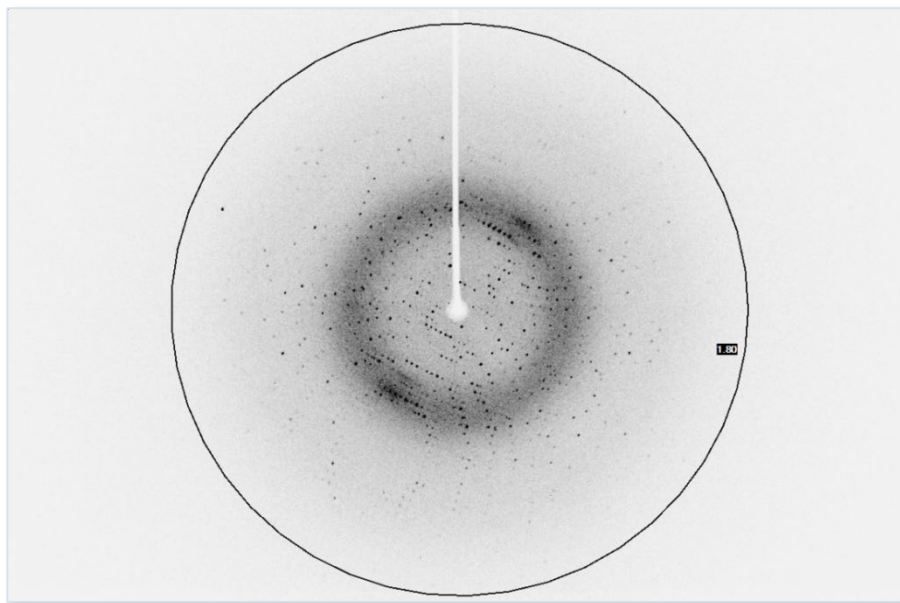


# D8 VENTURE

## Neurotoxin serotype A light chain



- Source *Clostridium botulinum*
- Space group  $P2_1$
- Cell constants  $a=49.1$ ,  $b=65.8$ ,  $c=63.8$  Å  
 $\beta=98.8^\circ$
- Crystal dimensions  $0.040 \times 0.095 \times 0.143$  mm
- Allen lab, Boston University



### Data Collection Parameters

Data collected ( $^\circ$ )	225
Exposure time (s)	10
Rotation width ( $^\circ$ )	0.5
Divergence (mrad)	7.5
DX (mm)	55
Wall time (hrs)	1.25
Diffraction limit (Å)	1.70

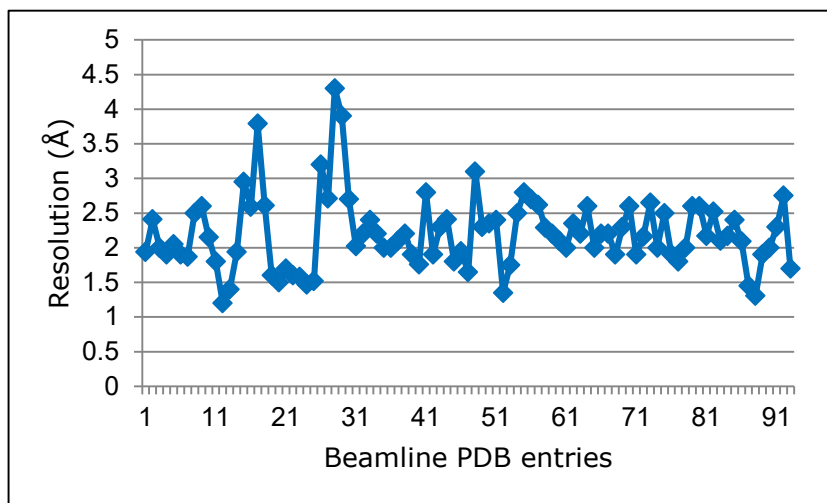
- Data collected using a I $\mu$ S DIAMOND and PHOTON III M28

# D8 VENTURE

## Neurotoxin serotype A light chain



- Data statistics look very good out to the diffraction limit of 1.70 Å
- There are many protein-inhibitor complexes submitted to the PDB, the diffraction limits generally range between 1.5 – 2.5 Å
- In around 1.5 hours, you can get similar results with the D8 VENTURE



### Data Statistics

	Overall	Inner shell	Outer shell
Low res limit	24.7	24.7	1.73
High res limit	1.7	9	1.7
Rmerge	0.102	0.031	1.076
Rmeas	0.114	0.034	1.241
Rpim	0.051	0.015	0.603
I/σ(I)	9.3	30.2	1.1
CC(1/2)	0.997	0.998	0.582
Completeness	99.8	90.5	100
Multiplicity	4.8	4.8	4.1

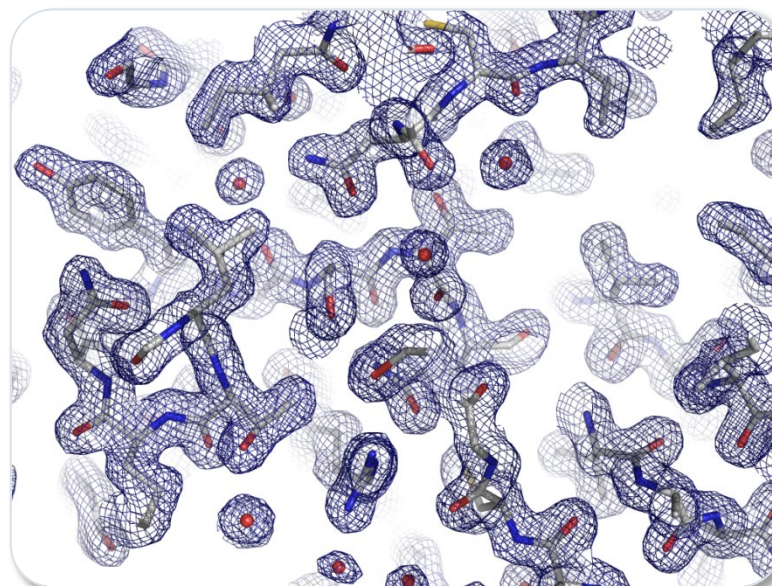
# D8 VENTURE

## Neurotoxin serotype A light chain



- Using Phenix, the data set refined well against the 3BOK PDB model
  - Model placed in the cell using Phaser
  - Simple refinement with solvent and hydrogens added

Resolution (Å)	25 – 1.70
R-work	0.182
R-free	0.220
RMS(bonds)	0.012
RMS(angles)	1.127
MolProbity score	1.05



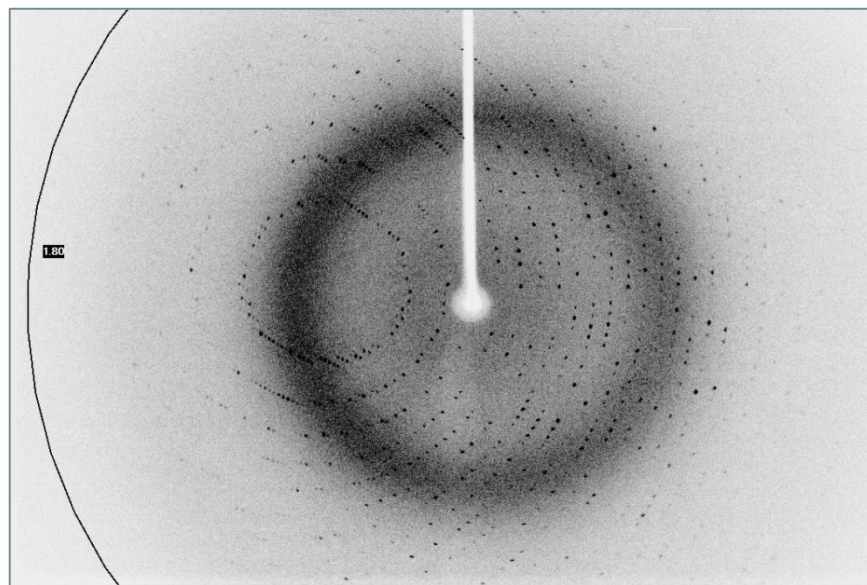
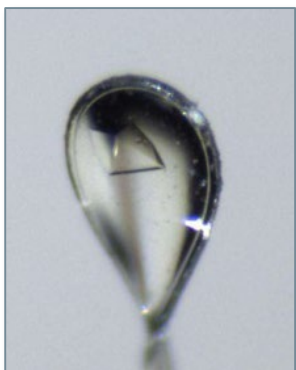
- Electron density from the fast data refinement contoured at  $1\sigma$

# D8 VENTURE

## RIVAX, Human Ricine Vaccine



- Space group  $P2_1$
- Cell constants  $a=b=67.7, c=139.6 \text{ \AA}$
- Exposure time 60 sec
- DX 80 mm
- Rotation angle  $0.3^\circ$
- Wall time 11 hrs
- Max resolution  $1.80 \text{ \AA}$
- Detector PHOTON III (M28)
- Source  $I_{\mu}S \text{ DIAMOND}$
- Crystal dimensions  
 $0.050 \times 0.090 \times 0.105 \text{ mm}$



- Another example of D8 VENTURE data which is comparable to or better than rotating anode data. The original structure was published from data collected on a Bruker MICROSTAR rotating anode with a SMART 6000 CCD.



# D8 VENTURE

## RIVAX, Human Ricine Vaccine



- The merging statistics indicated that the data precision was very good out to the diffraction limit
- The combination of high intensity DIAMOND I $\mu$ S and photon-counting PHOTON III greatly expands the type of samples that can be collected with a maintenance-free system.

### Data Statistics\*

	Overall	Inner shell	Outer shell
Low res limit	24.12	24.12	1.84
High res limit	1.8	9	1.8
Rmerge	0.083	0.016	0.845
Rmeas	0.087	0.017	0.952
Rpim	0.025	0.005	0.428
I/ $\sigma$ (I)	23	96	1.8
CC(1/2)	0.999	1	0.602
Completeness	99.7	95.4	98.5
Multiplicity	11.1	11.1	4.5

\* Statistics generated by AIMLESS

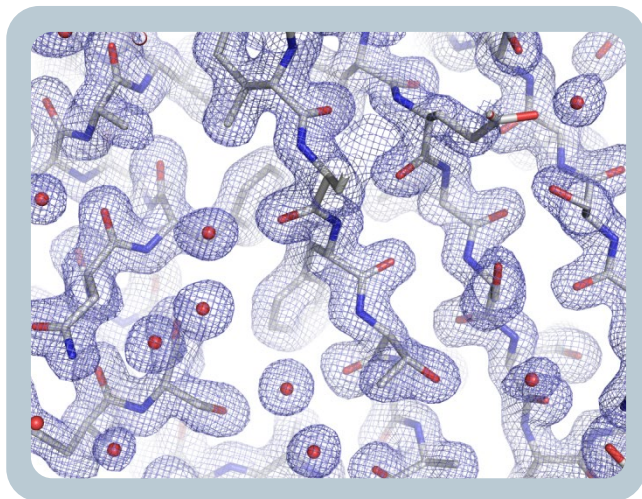


# D8 VENTURE

## RIVAX, Human Ricine Vaccine



- The PDB:3SRV model refined well against the D8 VENTURE data using PHENIX
- The diffraction limit for the D8 VENTURE was higher than that published for the MICROSTAR



D8 VENTURE electron density contoured at  $1\sigma$

### Refinement statistics

	D8 VENTURE	MICROSTAR*
Resolution range (Å)	24 - 1.80	24 - 2.14
Rmerge	0.083	0.098
Completeness (%)	99.7	98.4
Rwork	0.195	0.214
Rfree	0.225	0.254
Bonds RMS	0.011	0.008
Angles RMS	1.170	0.980

(\*Legler et al., 2011)

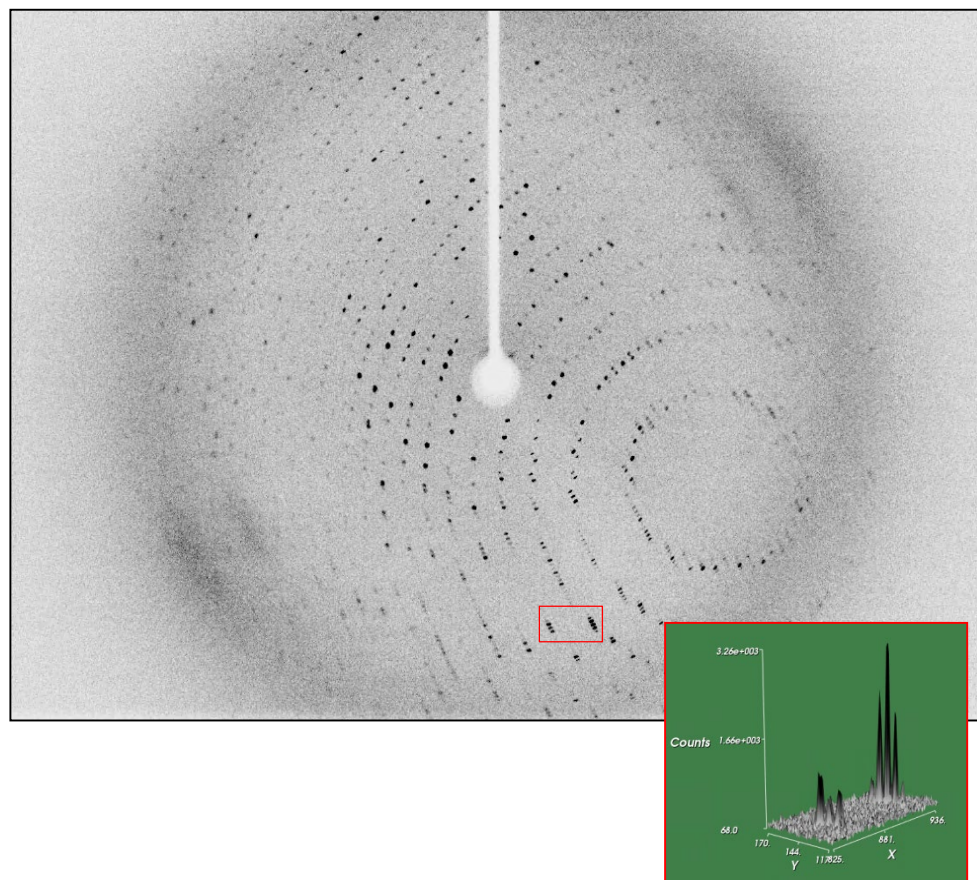
# D8 VENTURE

## Yeast 20S Proteasome



- Space group  $P2_1$
- Cell constants  $a = 136.5 \text{ \AA}$   $b = 300.5 \text{ \AA}$   $c = 145.6 \text{ \AA}$   $\beta = 113.1^\circ$
- Exposure time 70 s
- Divergence 5 mrad
- DX 160 mm
- Rotation angle  $0.3^\circ$
- Wall time 19 hrs
- Max resolution  $3.0 \text{ \AA}$
- Detector PHOTON III (M28)
- Source  $I\mu\text{S DIAMOND}$

- The 20S Proteasome is a large biological assembly and of pharmaceutical relevance
- Despite low symmetry and weak diffraction properties of the proteasome crystals, a complete data set was collected fast. This highlights the benefits of the large active detector area of the M28.

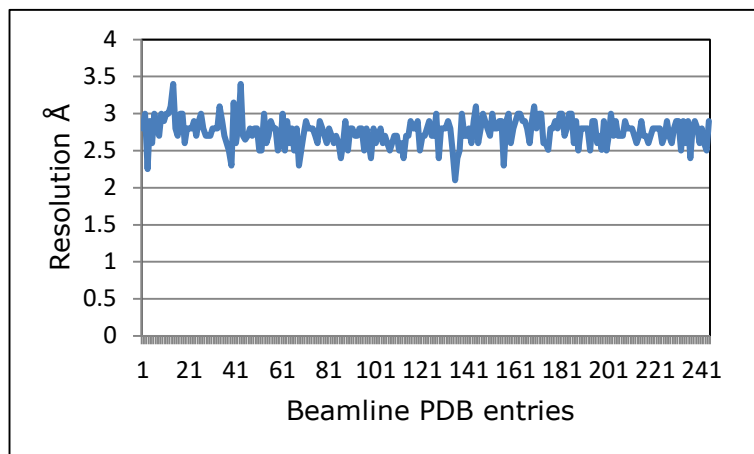


# D8 VENTURE

## Yeast 20S Proteasome



- The data statistics don't match those found for most of the beamline data sets but you still get a data set in-house that gives you useful information



### Data Statistics\*

	Overall	Inner shell	Outer shell
Low res limit	25	25	3.05
High res limit	3	16.43	3
Rmerge	0.229	0.037	0.967
Rmeas	0.258	0.041	1.157
Rpim	0.117	0.017	0.623
I/ $\sigma$ (I)	7.5	34.4	1.3
CC(1/2)	0.97	0.999	0.679
Completeness	98.9	99.0	92.1
Multiplicity	4.7	5.6	3.1

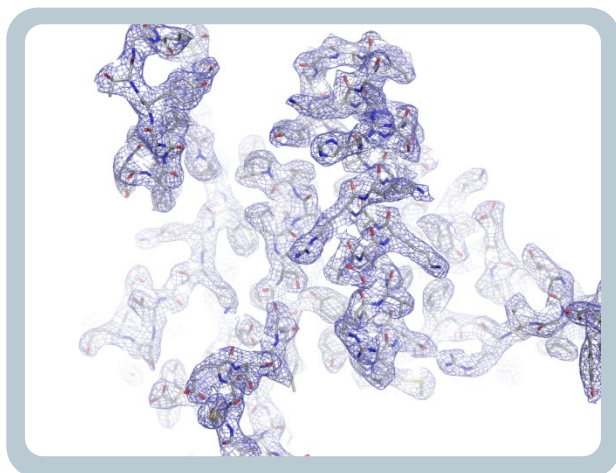
\* Statistics generated by AIMLESS

# D8 VENTURE

## Yeast 20S Proteasome



- The proteasome complex was solved by molecular replacement using the program MolRep (CCP4) and further refined using REFMAC (CCP4)
- Good model statistics and electron density maps can be obtained in house even for challenging large biological assemblies



### Refinement statistics

Resolution range (Å)	25 – 3.0
Completeness (%)	98.6
Rwork	0.212
Rfree	0.272
Bonds RMS	0.008
Angles RMS	1.246
CC Fo-Fc	0.891
CC Fo-Fc Free	0.867

# Summary

- The I $\mu$ S DIAMOND source is a great alternative to high-performance sources that require routine maintenance.
- The I $\mu$ S DIAMOND significantly lowers operational costs while providing the performance needed for most research projects.
- The PHOTON series CPAD detectors combine photon-counting performance with a large active area for the home-lab. Its novel mixed-mode approach to data acquisition provides the best way to measure both strong and weak Bragg reflections at the same time.
- The D8 VENTURE allows not only sample characterization but also data collection for a wide range of projects.
- The D8 VENTURE with I $\mu$ S DIAMOND with PHOTON III CPAD provides the highest performance/price ratio of any home-lab system.



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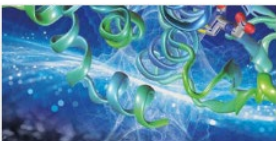
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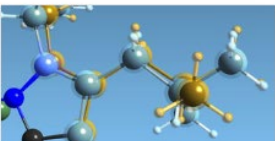


4月 24日, 2020 ☒ ON-DEMAND

**SHELXリファインに慣れる – 最も簡単で確実な方法 –**

最近の単結晶X線構造解析向けソフトウェアは総じて使いやすくなっている。その原由は、ファイルの取扱いなどについてほとんど苦慮しなくても解析できてしまう状況にあります。一方で、その簡便さゆえ、簡単な問題でつまづくこともしばしば。そうならないためにSHELXリファインに慣れ、基礎を習得することも目...

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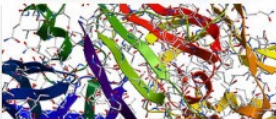



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**Disorder bogging you down**

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# Questions and Answers

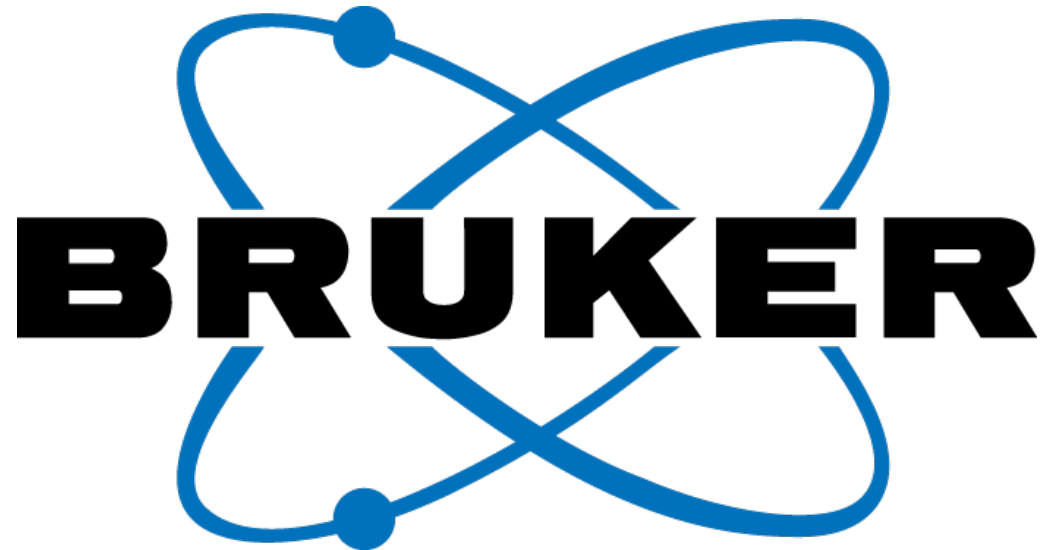
## Any questions?

Please type any questions you may have for our speakers in the [Q&A panel](#) and click Send.

Thank you!







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