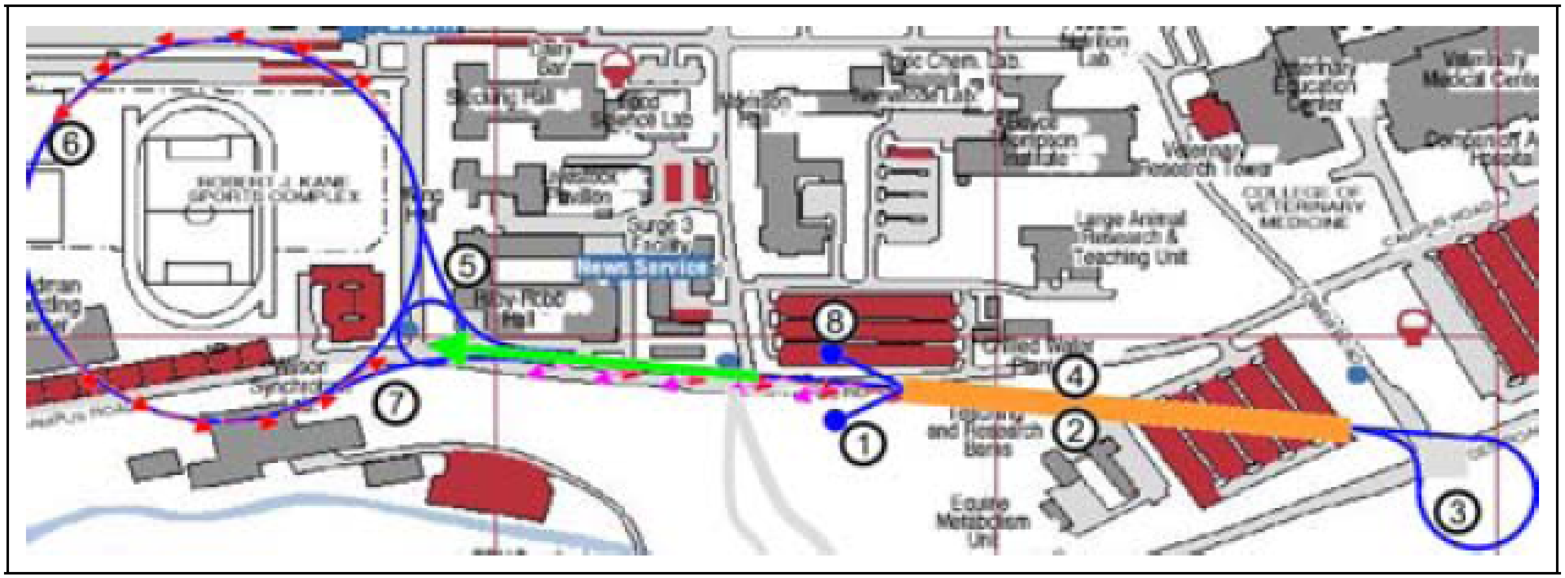
Femtosecond Time-resolved Laue crystallography: Using an ERL to Watch Proteins Function on the Chemical Time Scale

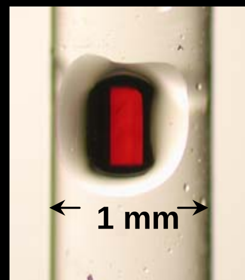
<u>Philip Anfinrud</u>

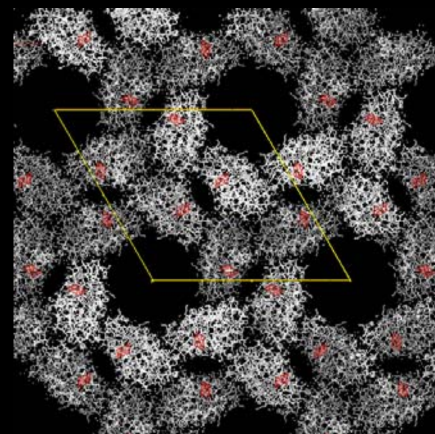
Laboratory of Chemical Physics, NIDDK

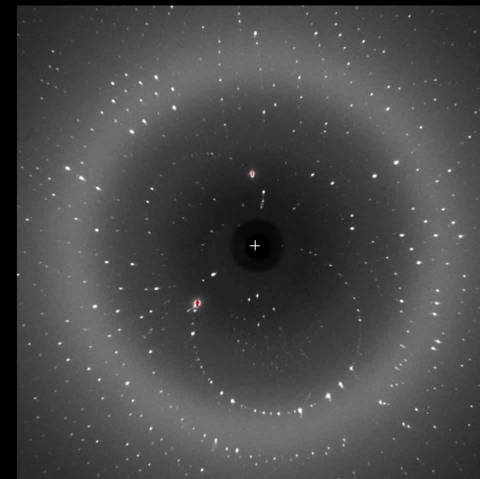
National Institutes of Health, Bethesda, MD USA

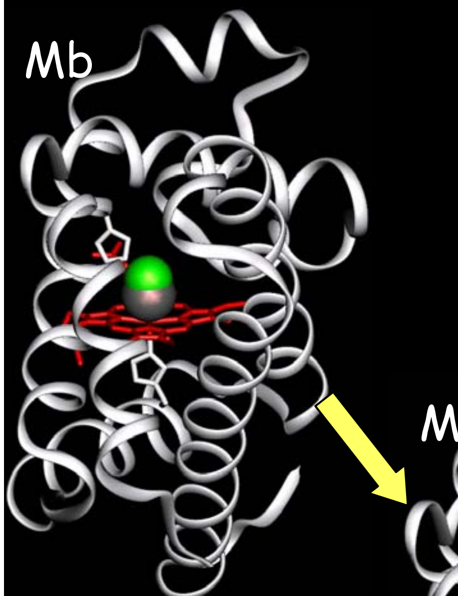


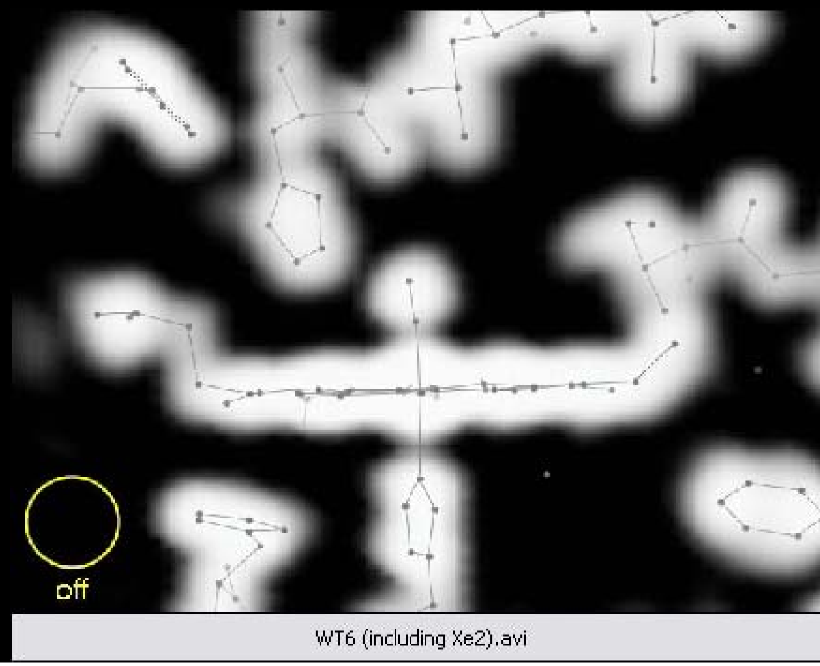
Time-resolved Laue Crystallography: Probing ligand migration and correlated protein motion in photolyzed carbon monoxy myoglobin









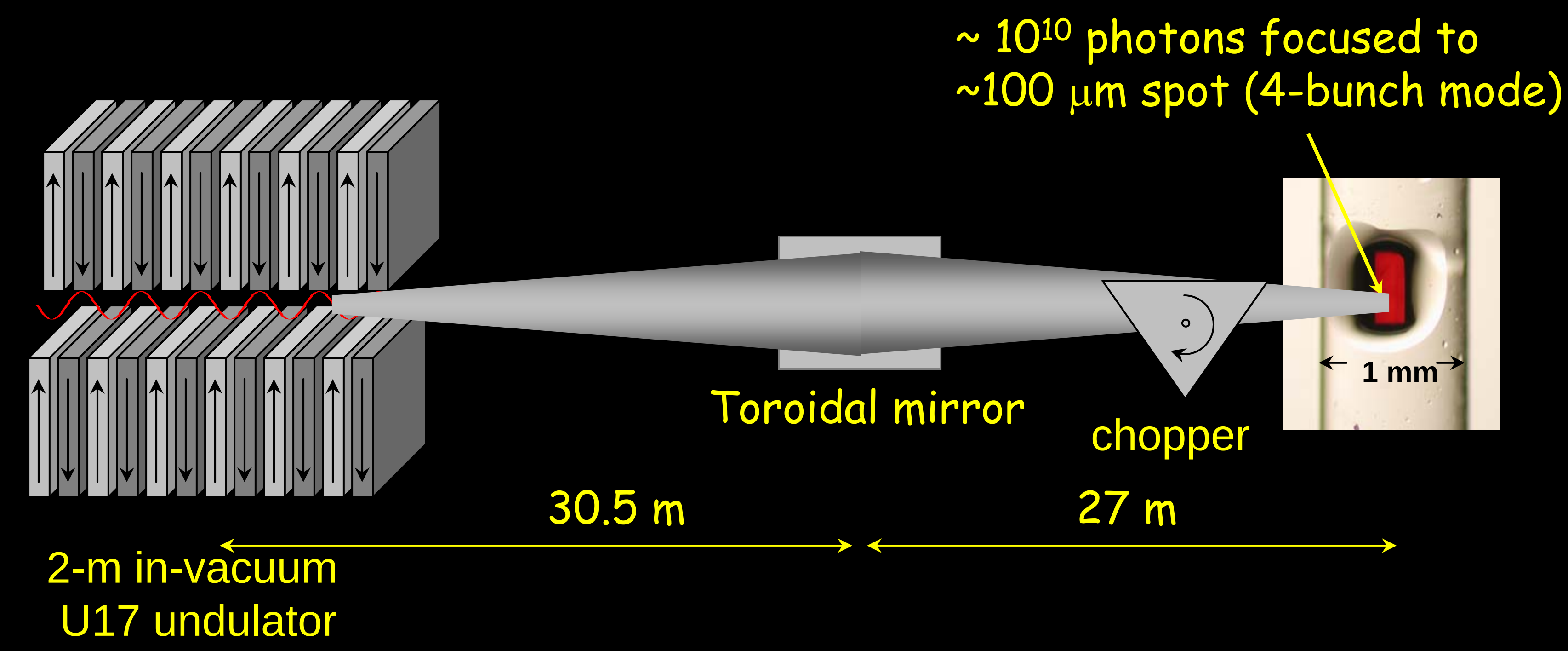


European Synchrotron and Radiation Facility, Grenoble, FRANCE

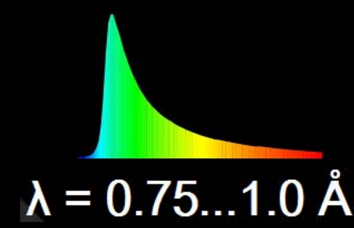
ESRF

- Time-Resolved X-ray (TReX) Studies

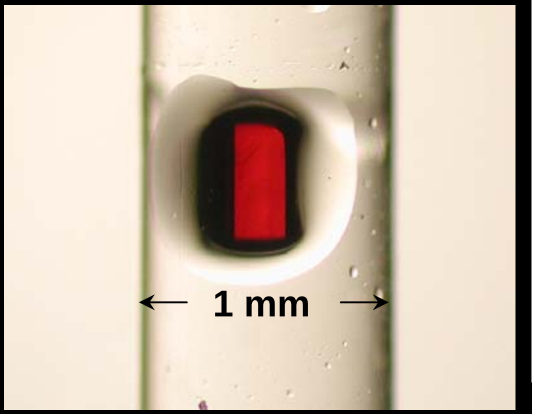
X-ray Generation at ID09B (ESRF)

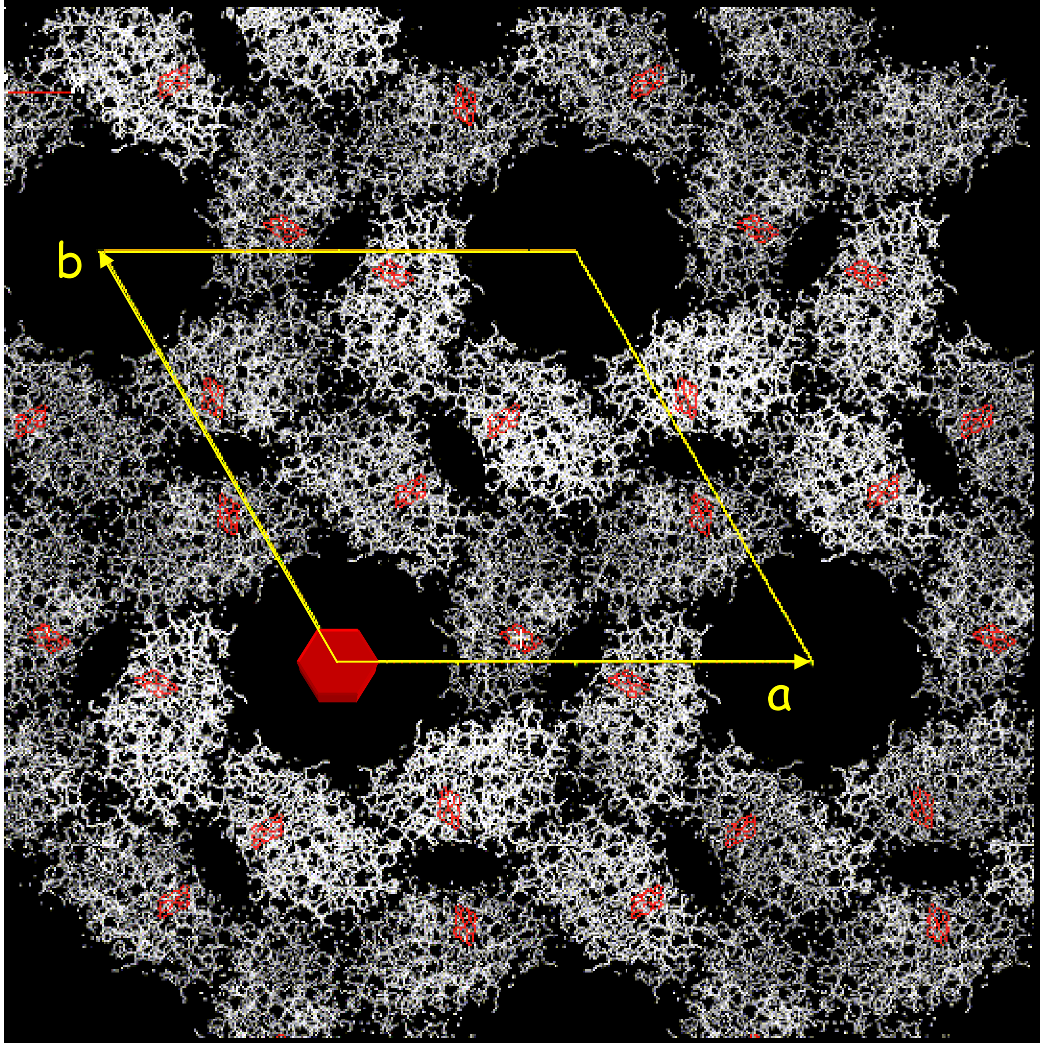


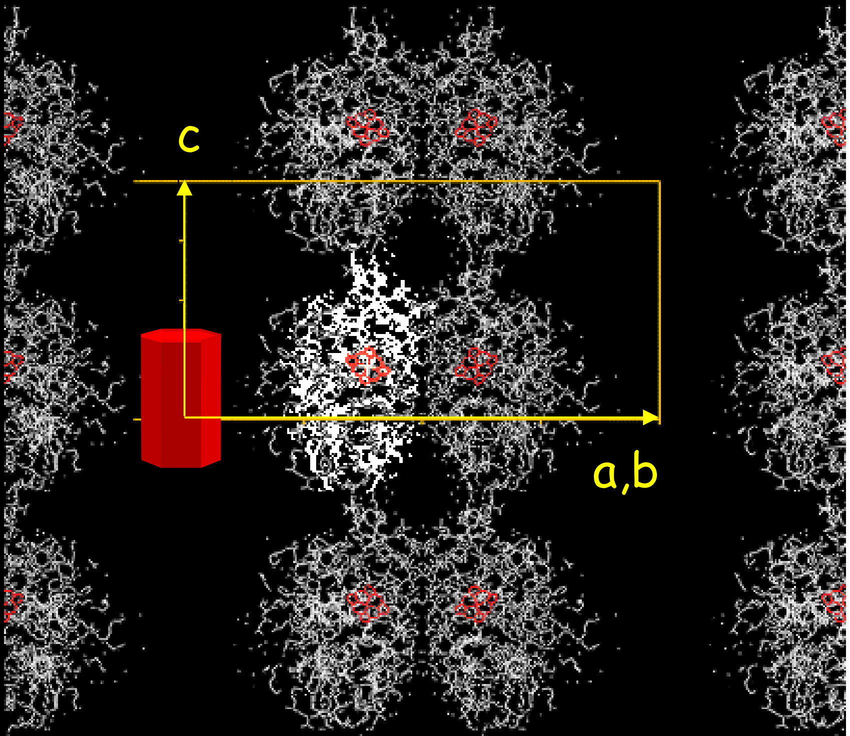
- 4-bunch mode (~ 30-nC/pulse; 704-ns spacing)
- Low-beta straight section (H source size: 130-μm FWHM)
- In-vacuum undulator (6-mm gap; 15-keV fundamental)
- Toroidal mirror (maximizes flux via single reflection)
- High-speed chopper (164-ns opening time with 100-μm vertical aperture)



Packing of P6 Myoglobin Crystal
(D122N)





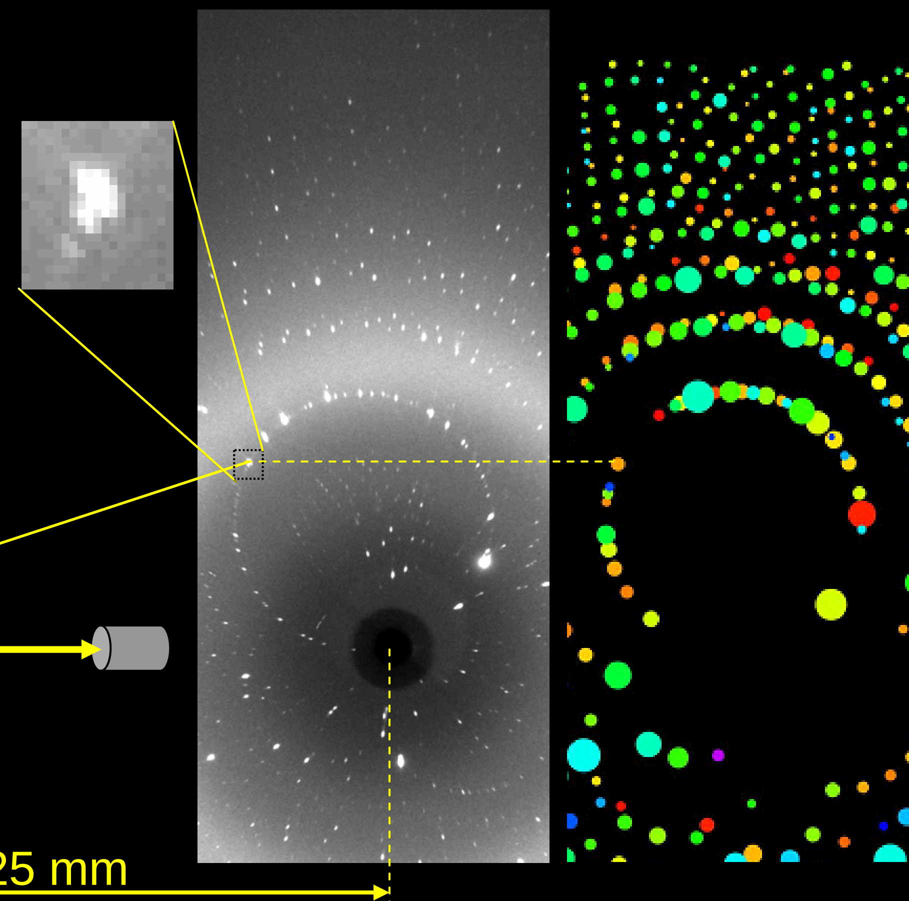


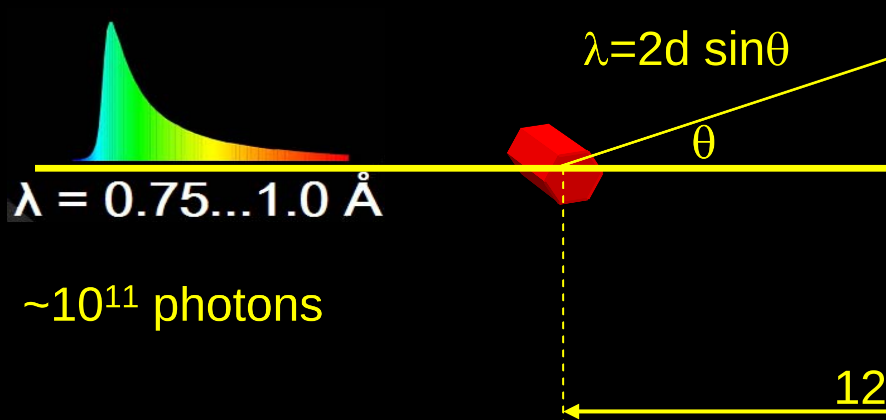
a = b = 91.20 Å, c = 45.87 Å, $\alpha = \beta = 90^{o}$, $\gamma = 120^{o}$; heme plane to a,b plane ∠ = 55°

X-ray characteristics (ESRF)

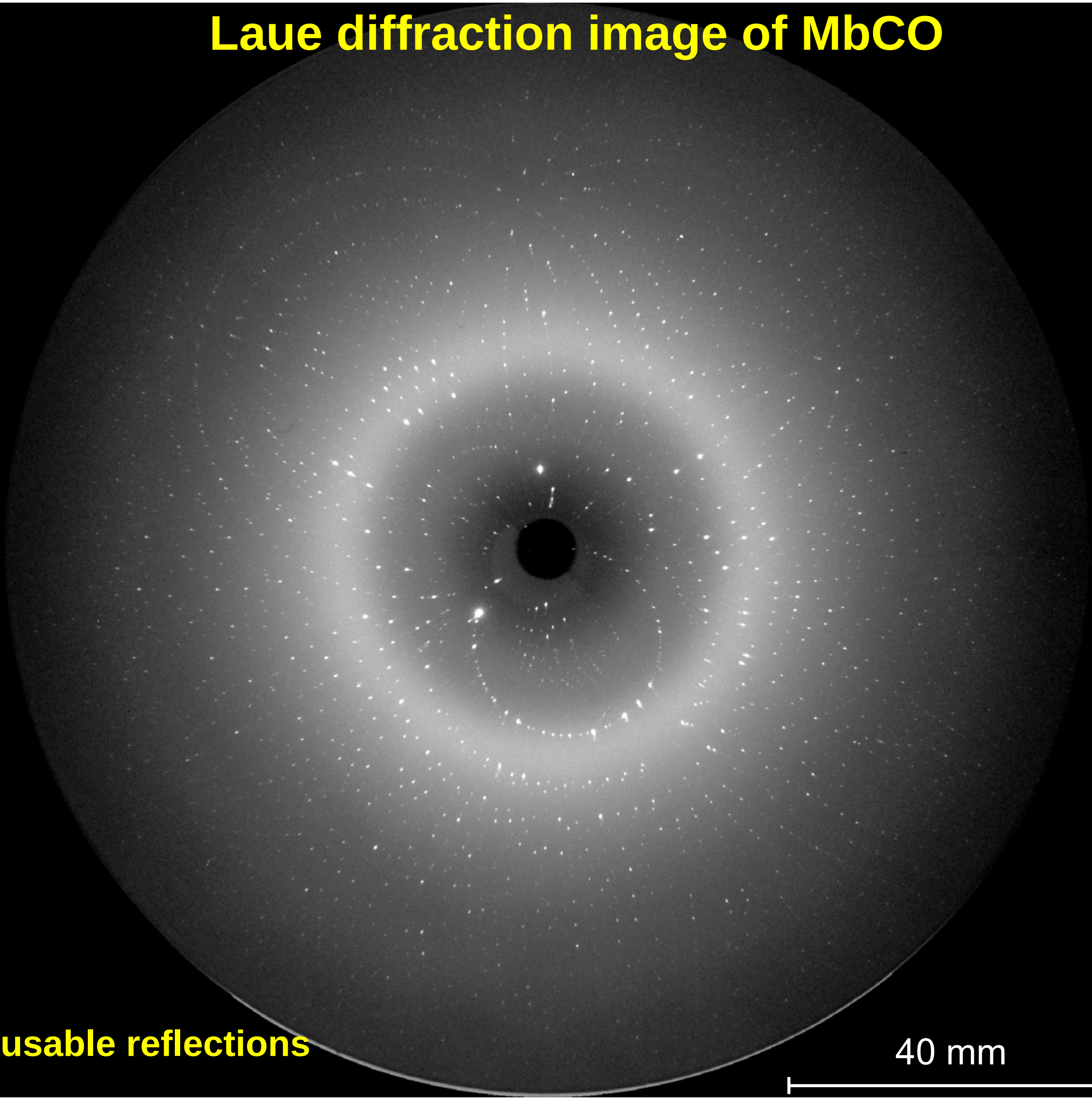
- **“Pink” beam peaked around ~ 15 keV (0.8 Å)**
- **~10^{10} photons per shot; ~ 8 shots per image**

Spot sizes:
~125-μm FWHM

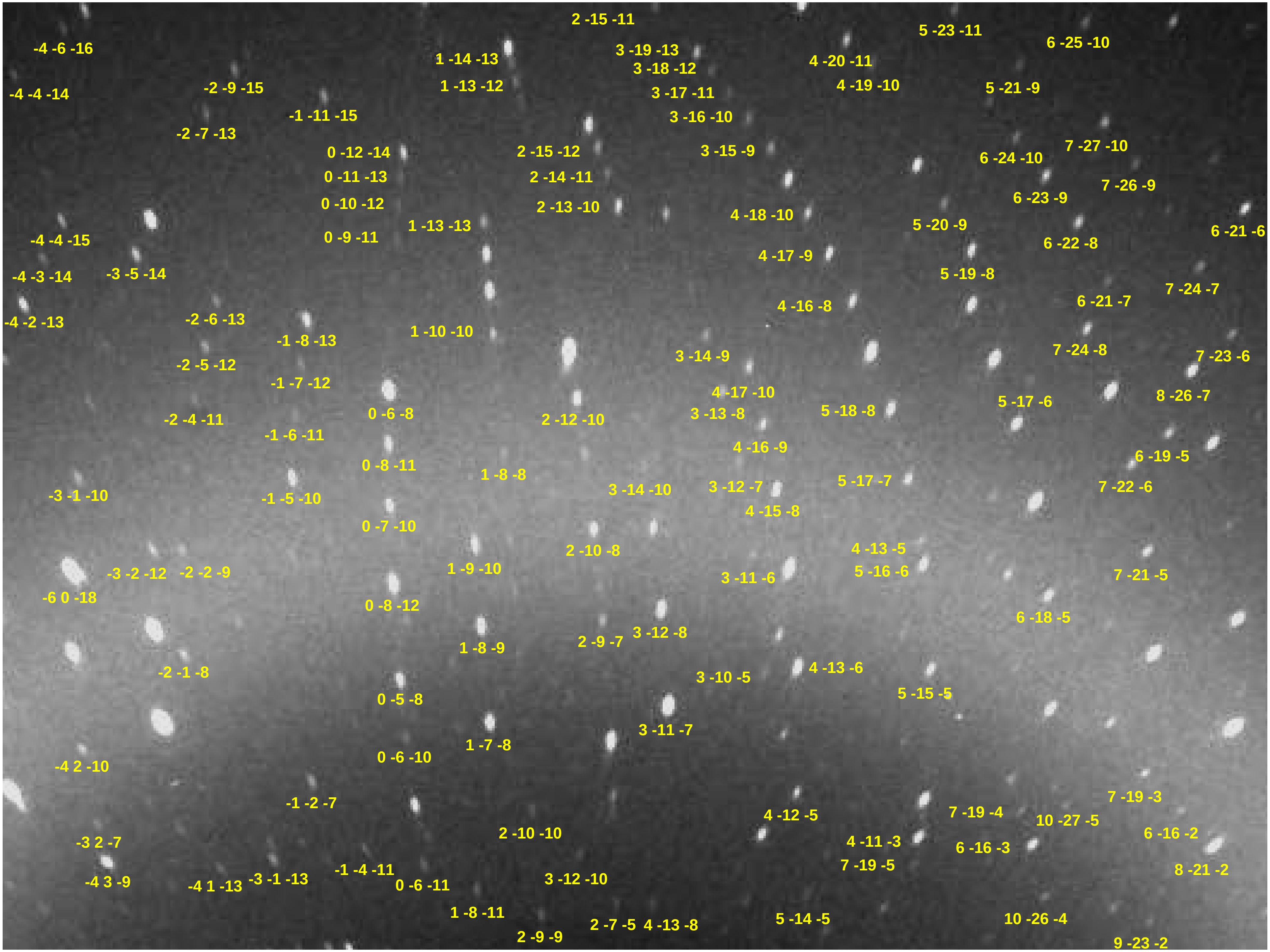




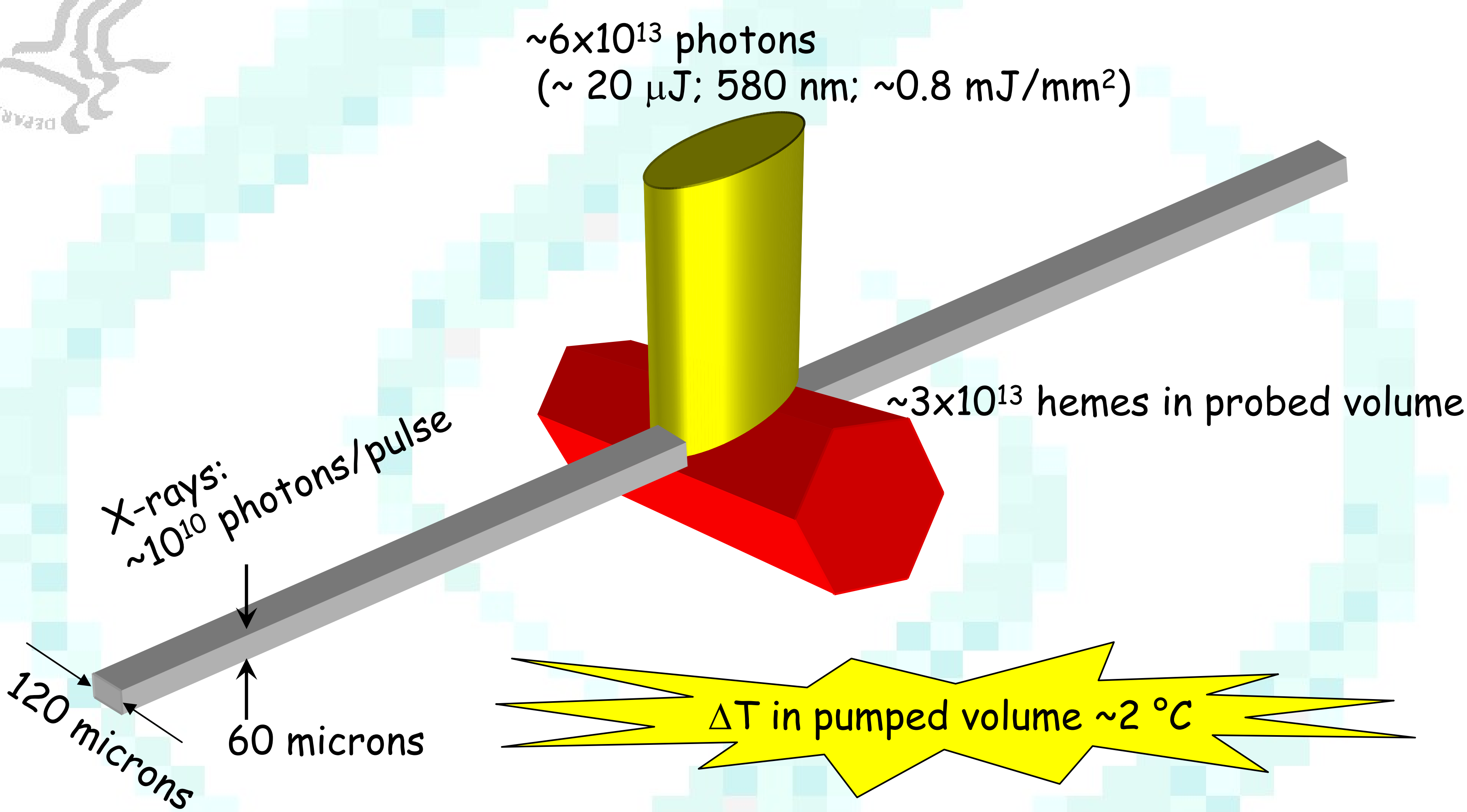
Laue diffraction image of MbCO

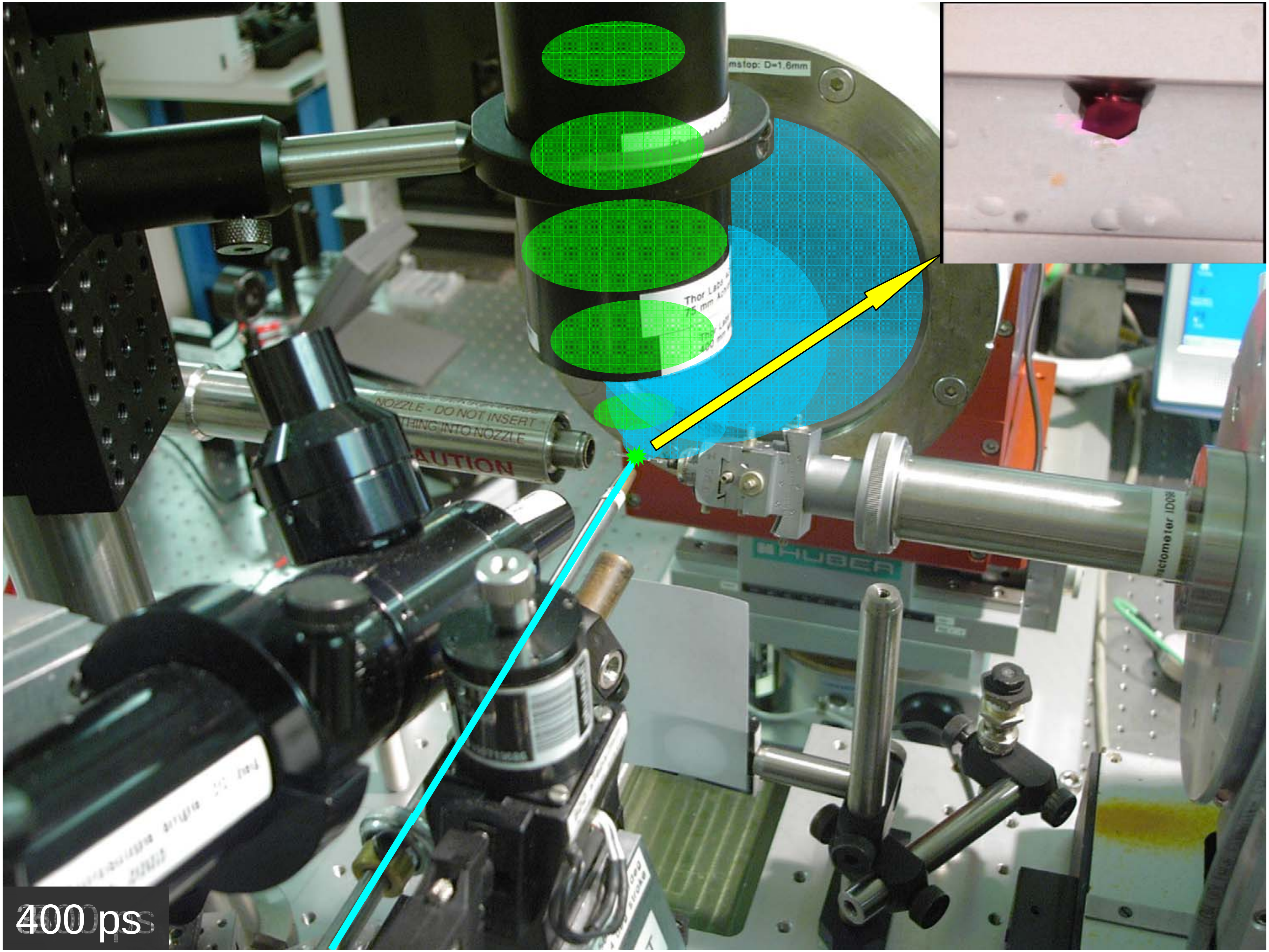


ca. 4000 usable reflections

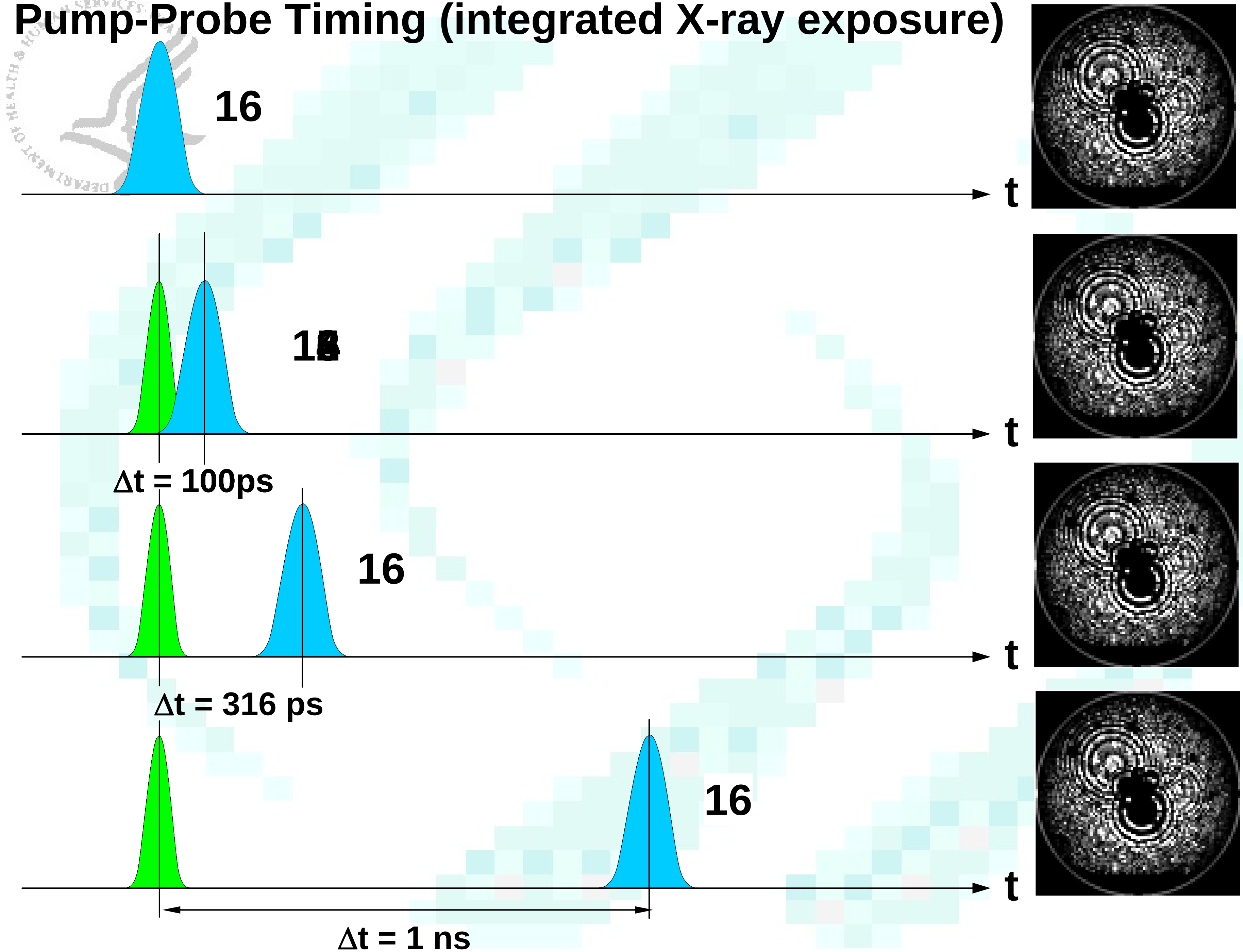

2 -15 -11
5 -23 -11
-4 -6 -16
6 -25 -10
1 -14 -13
3 -19 -13
4 -20 -11
3 -18 -12
-4 -4 -14
-2 -9 -15
1 -13 -12
4 -19 -10
5 -21 -9
3 -17 -11
-1 -11 -15
3 -16 -10
-2 -7 -13
7 -27 -10
0 -12 -14
2 -15 -12
3 -15 -9
6 -24 -10
0 -11 -13
2 -14 -11
7 -26 -9
6 -23 -9
0 -10 -12
2 -13 -10
4 -18 -10
1 -13 -13
5 -20 -9
6 -21 -6
-4 -4 -15
0 -9 -11
6 -22 -8
4 -17 -9
-4 -3 -14
-3 -5 -14
5 -19 -8
7 -24 -7
6 -21 -7
4 -16 -8
-4 -2 -13
-2 -6 -13
1 -10 -10
-1 -8 -13
3 -14 -9
7 -24 -8
7 -23 -6
-2 -5 -12
-1 -7 -12
4 -17 -10
8 -26 -7
5 -17 -6
0 -6 -8
3 -13 -8
5 -18 -8
-2 -4 -11
2 -12 -10
-1 -6 -11
4 -16 -9
6 -19 -5
0 -8 -11
1 -8 -8
5 -17 -7
3 -14 -10
3 -12 -7
7 -22 -6
-3 -1 -10
-1 -5 -10
4 -15 -8
0 -7 -10
2 -10 -8
4 -13 -5
1 -9 -10
5 -16 -6
-3 -2 -12
-2 -2 -9
3 -11 -6
7 -21 -5
-6 0 -18
0 -8 -12
6 -18 -5
3 -12 -8
1 -8 -9
2 -9 -7
-2 -1 -8
4 -13 -6
3 -10 -5
0 -5 -8
5 -15 -5
3 -11 -7
1 -7 -8
0 -6 -10
-4 2 -10
-1 -2 -7
7 -19 -3
7 -19 -4
4 -12 -5
10 -27 -5
2 -10 -10
-3 2 -7
4 -11 -3
6 -16 -3
6 -16 -2
7 -19 -5
-1 -4 -11
8 -21 -2
-4 3 -9
-4 1 -13
-3 -1 -13
0 -6 -11
3 -12 -10
1 -8 -11
5 -14 -5
10 -26 -4
2 -7 -5
4 -13 -8
2 -9 -9
9 -23 -2

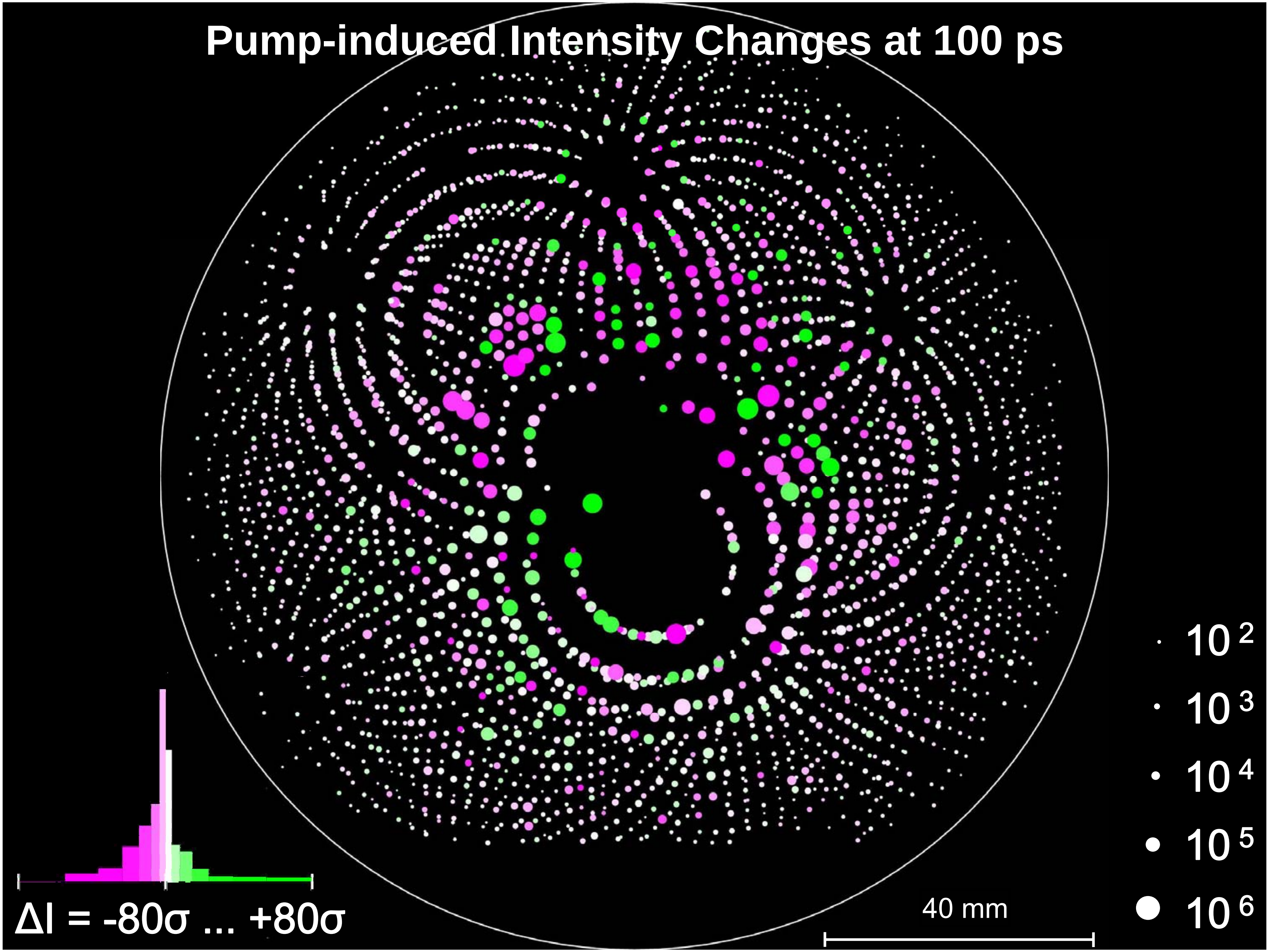
Pump-Probe Geometry




NOZZLE - DO NOT INSERT
400 ps

Pump-Probe Timing (integrated X-ray exposure)

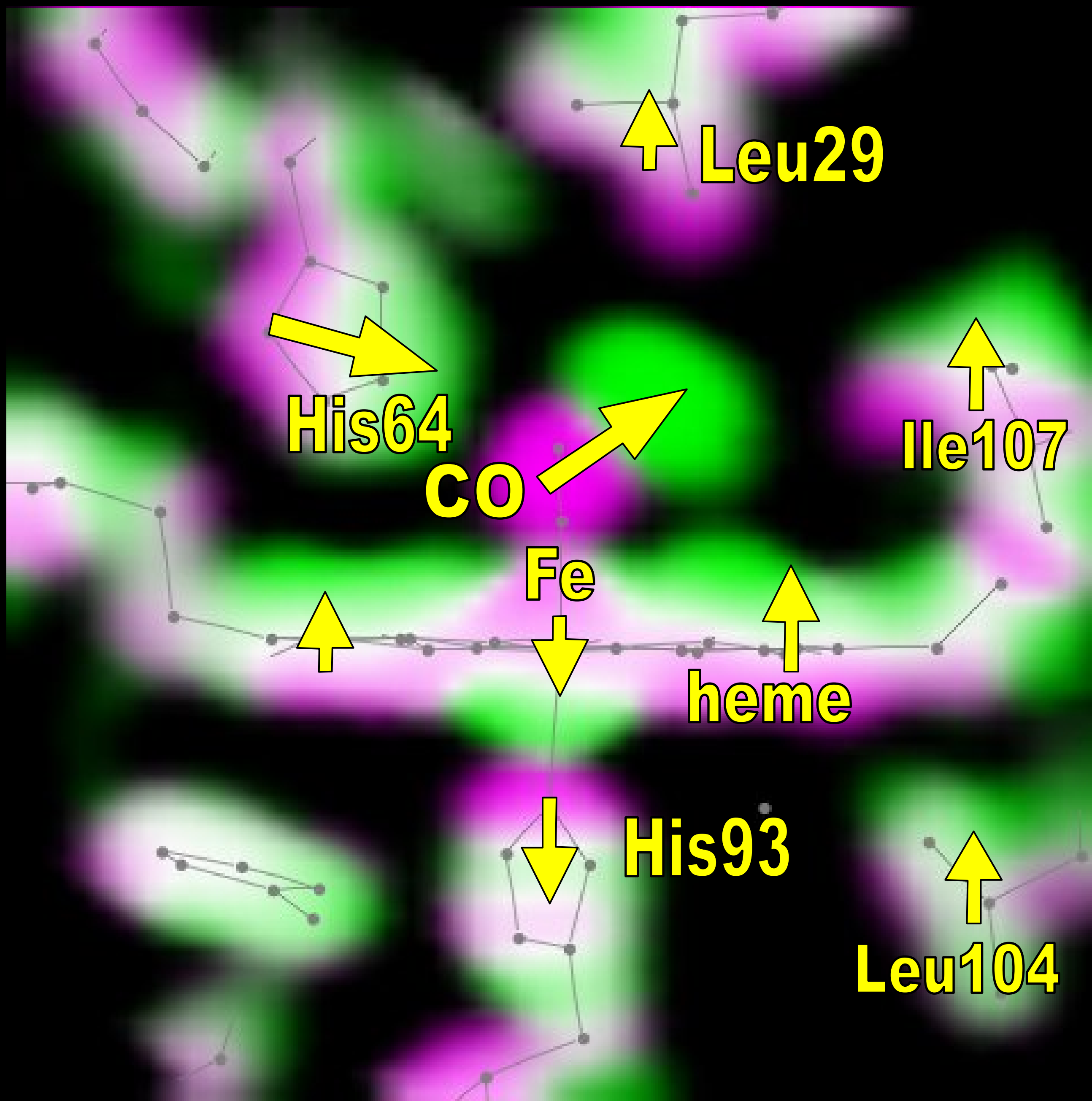



Pump-induced Intensity Changes at 100 ps
10 2
10 3
10 4
10 5
10 6
ΔI = -80σ ... +80σ
40 mm

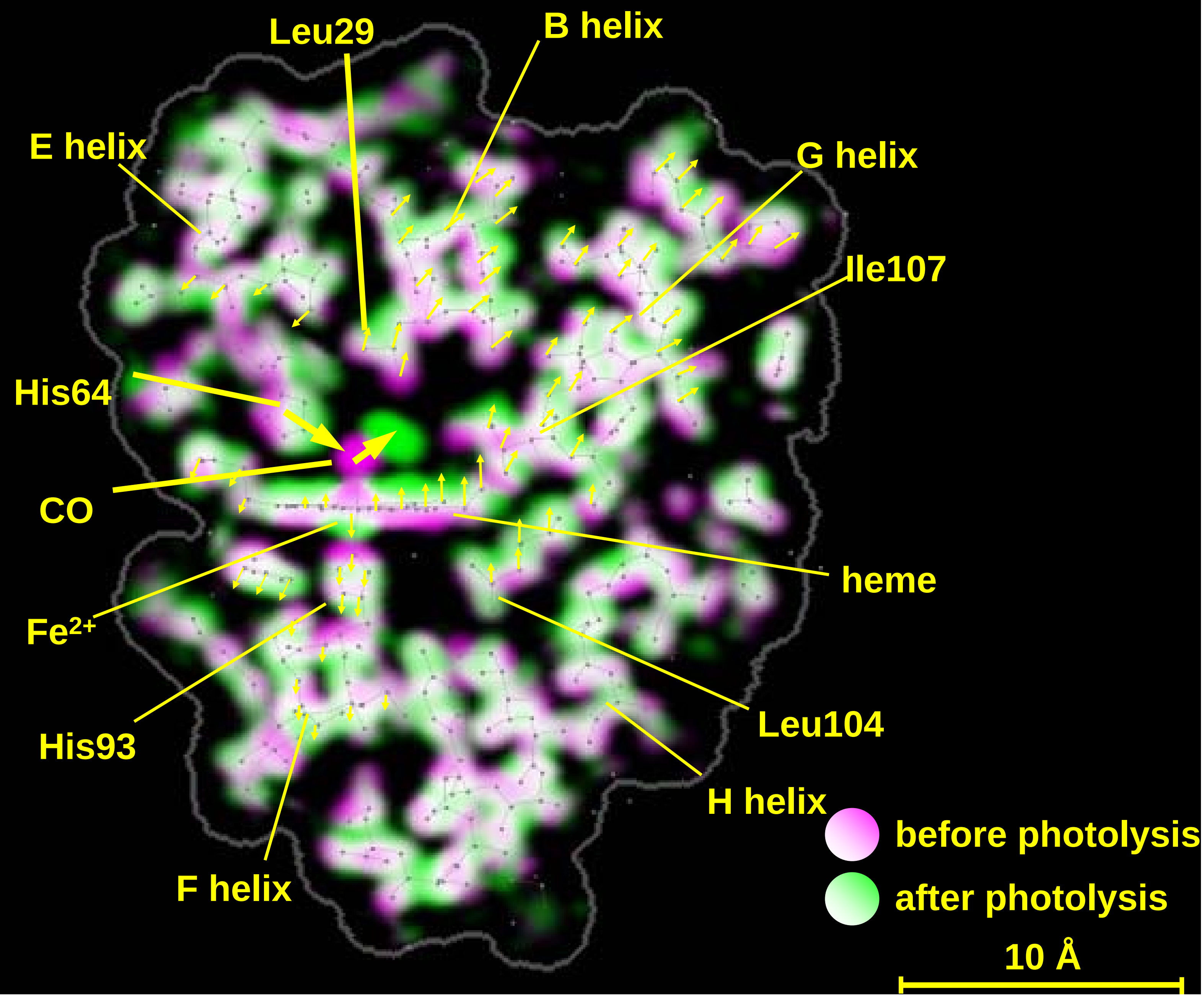
Color-coded maps superimposed: MbCO at 100 ps

Unphotolyzed

Photolyzed



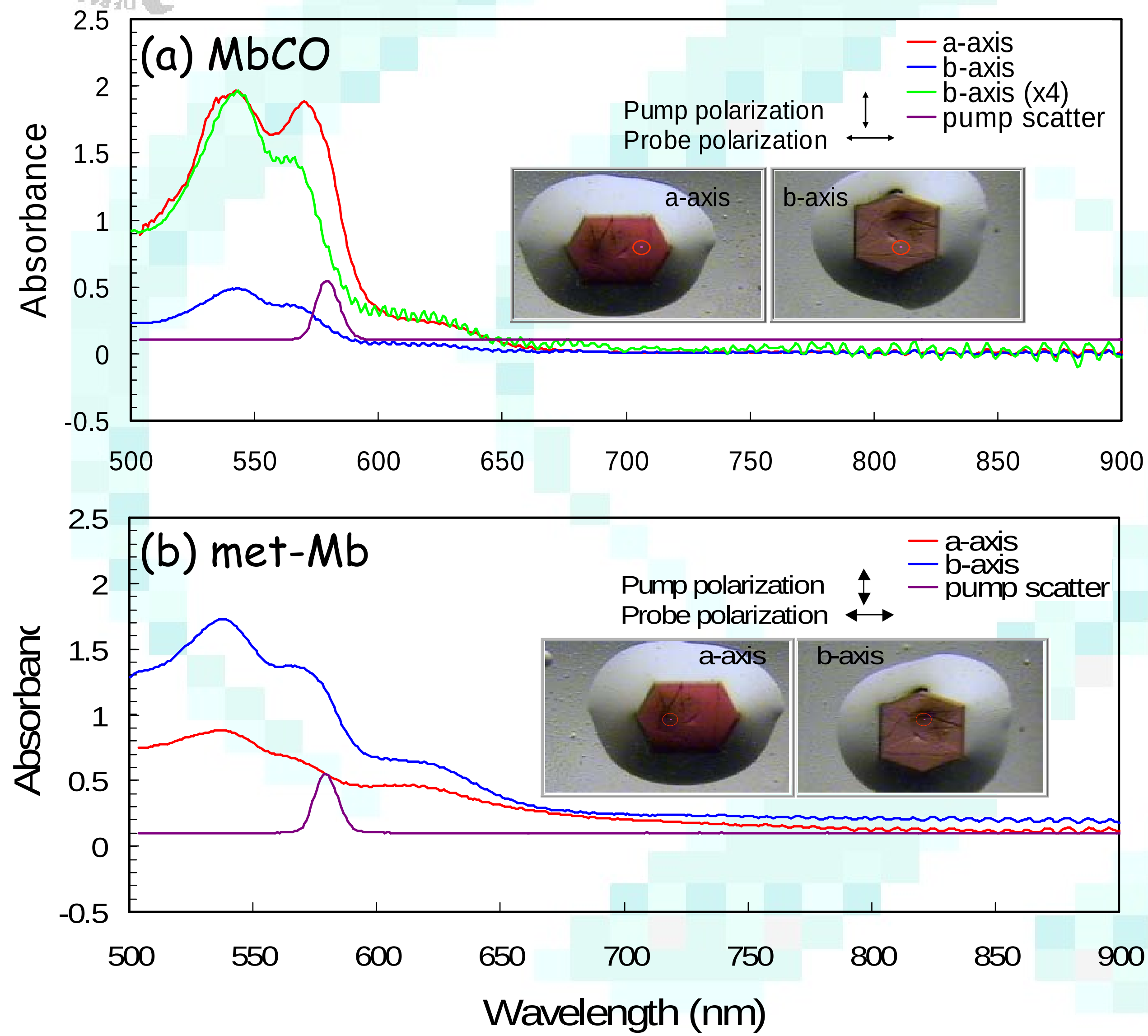
Global Tertiary structure changes of photolyzed MbCO are unresolvably fast (t = 100 ps)



Extending time-resolved Laue crystallography to the femtosecond time domain: What are the issues?

- Sample reversibility
  - nonlinear absorption damages chromophore and compromises sample reversibility
  - Can we record "single-shot" Laue diffraction images?
- Flux requirements
  - High-dynamic range diffraction image requires ~16 shots at ESRF
  - Can the Cornell ERL generate suitable X-ray pulse energy for "single-shot" Laue diffraction?
- Repetition frequency limits (for non-exchangeable, crystalline samples)
  - Limited by laser pulse energy deposited in the crystal
    - 3.3 Hz at ESRF with 100 micron spot size
  - To what extent can tighter focusing boost the pump-probe repetition frequency?
- Group velocity mismatch between laser and X-ray pulses
  - Which sample excitation geometries preserves maximum time resolution?

Intense femtosecond excitation converts MbCO (a) to met-Mb (b); (see darkening at the site of exposure).



- Photo-oxidation is triggered by multi-photon absorption via a strongly-absorbing shot-lived (<100 fs) excited state
- Stretching the optical pulse shuts down this channel, but broadens the time resolution
- Can we record "single-shot" Laue diffraction images?

Extending time-resolved Laue crystallography to the femtosecond time domain: What are the issues?

- Sample reversibility
  - nonlinear absorption damages chromophore and compromises sample reversibility
  - Can we record "single-shot" Laue diffraction images?
- Flux requirements
  - High-dynamic range diffraction image requires ~16 shots at ESRF
  - Can the Cornell ERL generate suitable X-ray pulse energy for "single-shot" Laue diffraction?
- Repetition frequency limits (for non-exchangeable, crystalline samples)
  - Limited by laser pulse energy deposited in the crystal
    - 3.3 Hz at ESRF with 100 micron spot size
  - To what extent can tighter focusing boost the pump-probe repetition frequency?
- Group velocity mismatch between laser and X-ray pulses
  - Which sample excitation geometries preserves maximum time resolution?

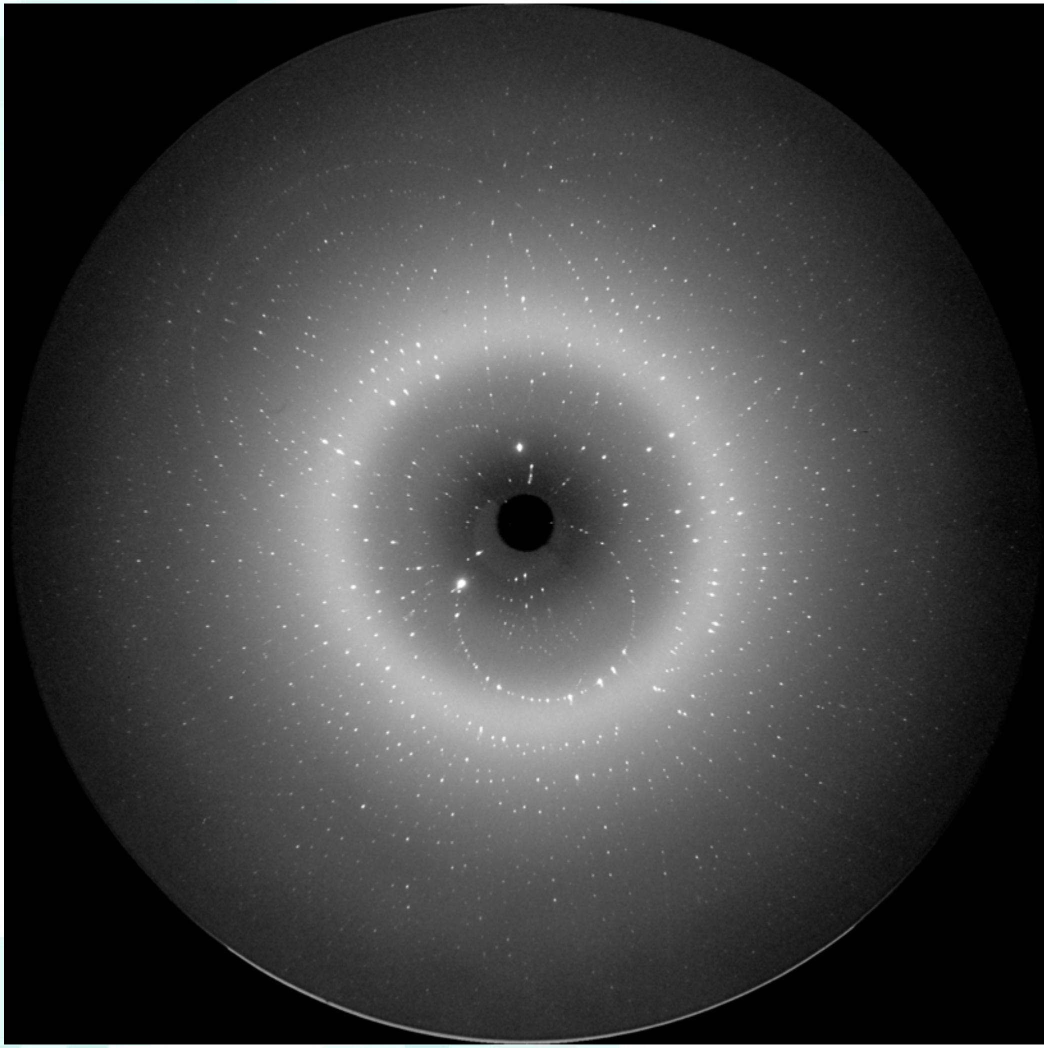
X-ray flux needed for "single-shot" Laue diffraction

 # X-ray Photons ∝ (bunch charge) x (undulator length)

- **ESRF Flux (~10^{10} photons/shot)**
  - 30 nC at 6 GeV
  - 2 m U17 undulator
  - 16 shots
  - ~10^{11} incident photons

- **ERL**
  - 10 nC at 5 GeV
  - 100 m U17 undulator
  - 1 shot

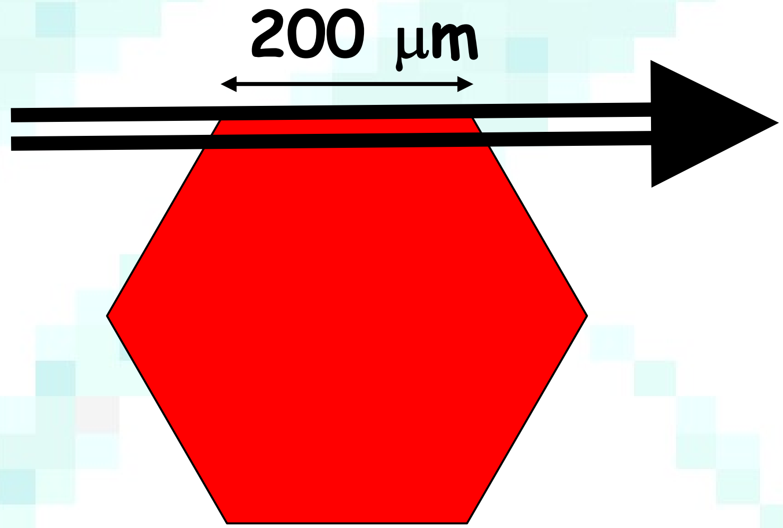
"FAT" bunch



Extending time-resolved Laue crystallography to the femtosecond time domain: What are the issues?

- **Sample reversibility**
  - nonlinear absorption damages chromophore and compromises sample reversibility
  - Can we record "single-shot" Laue diffraction images?
- **Flux requirements**
  - High-dynamic range diffraction image requires ~16 shots at ESRF
  - Can the Cornell ERL generate suitable X-ray pulse energy for "single-shot" Laue diffraction?
- **Repetition frequency limits (for non-exchangeable, crystalline samples)**
  - Limited by laser pulse energy deposited in the crystal
    - **3.3 Hz at ESRF with 100 micron spot size**
  - To what extent can tighter focusing boost the pump-probe repetition frequency?
- **Group velocity mismatch between laser and X-ray pulses**
  - Which sample excitation geometries preserves maximum time resolution?

Optimum X-ray Focus (~20 micron spot?)



- **Volume intercepted by 20 micron X-ray beam:**
  - Contains ~ 1.4×10^{12} hemes (37 mM for P6 MbCO)
- **X-ray induced T-jump when focusing ~1×10^{11} photons at 8 keV down to 20 microns:**
  - ~ 50 K
- **Laser pulse energy required to photoexcite twice this volume (~2 photons/chromophore):**
  - ~2 μJ @ 525 nm
  - T-jump of ~ 4 K
  - ~30 Hz acquisition should be possible (requires fast readout detector)

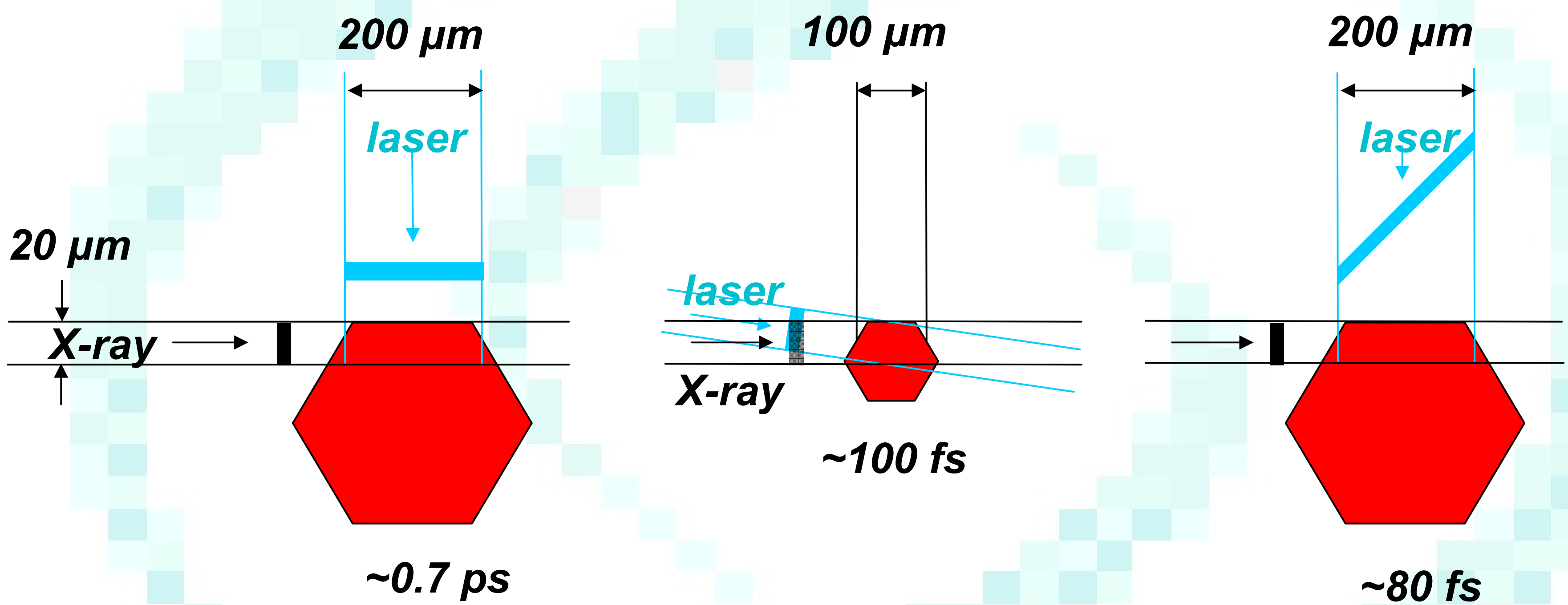
Extending time-resolved Laue crystallography to the femtosecond time domain: What are the issues?

- **Sample reversibility**
  - nonlinear absorption damages chromophore and compromises sample reversibility
  - Can we record "single-shot" Laue diffraction images?
- **Flux requirements**
  - High-dynamic range diffraction image requires ~16 shots at ESRF
  - Can the Cornell ERL generate suitable X-ray pulse energy for "single-shot" Laue diffraction?
- **Repetition frequency limits (for non-exchangeable, crystalline samples)**
  - Limited by laser pulse energy deposited in the crystal
    - 3.3 Hz at ESRF with 100 micron spot size
  - To what extent can tighter focusing boost the pump-probe repetition frequency?
- **Group velocity mismatch between laser and X-ray pulses**
  - Which sample excitation geometries preserves maximum time resolution?

Group Velocity Mismatch limits time resolution

$n_{vis} = 1.33$

$n_{x\text{-}ray} = 1.00$

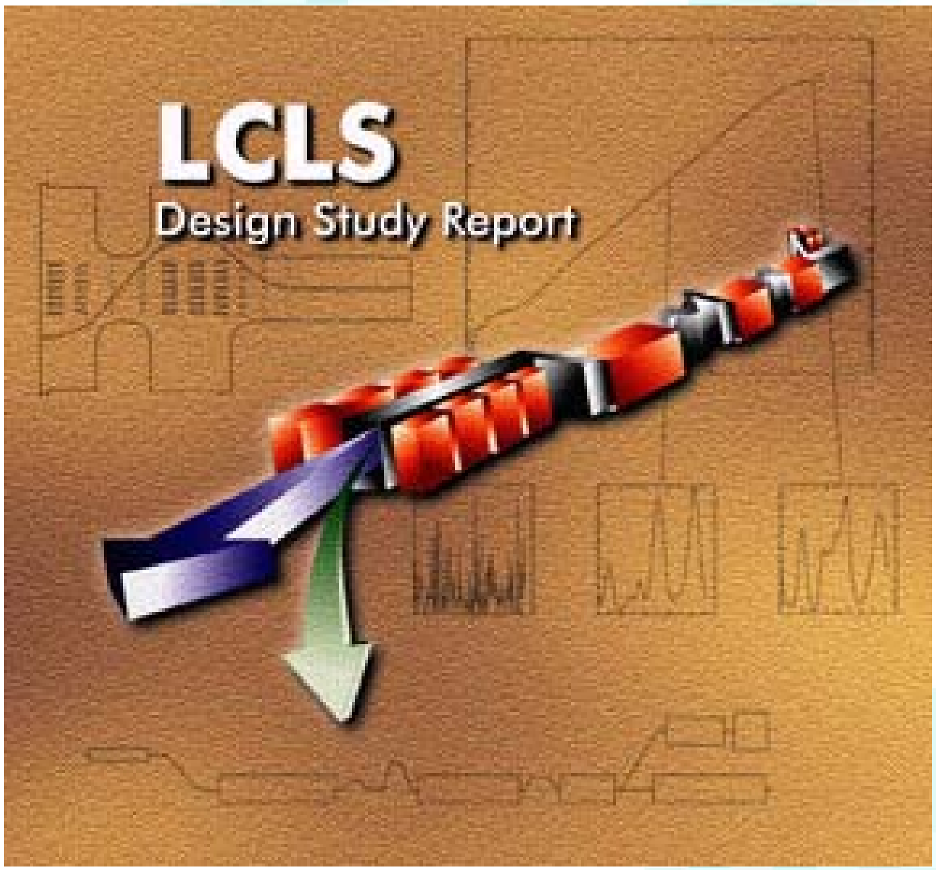


4^{th} Generation X-ray source: Free Electron Laser

~10^{12} photons/shot

~100 fs pulse duration

LCLS at Stanford in 2009?



XFEL in Germany in 2012?

X-ray Characteristics

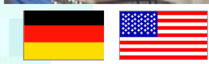
	ESRF	XFEL	LCLS	ERL
Electron energy:	6 GeV	10 GeV	14.35 GeV	5.3 GeV
X-ray pulse duration:	~150 ps ☹	~100 fs ☺	~100 fs ☺	~200 + fs
single bunch charge:	~28 nC ☺	~1 nC	~1 nC	1 or 10 nC
undulator length	2 m	50 m	100 m	100 m
Spontaneous:				
X-ray energy (fundamental):	15 keV (U17)	15 keV (U20.9)	8.2 keV (U30) 😐	8.27 keV (U17) 😐
X-ray bandwidth (fund.):	~3%	~5%	~5%	
X-ray photons/pulse	~1.4x10^{10}	~0.9x10^{10}	~2x10^{10}	
SASE1:				
X-ray energy:	-	12.4 keV	8.2 keV	-
X-ray bandwidth:	-	0.09%	0.1%	-
X-ray photons/pulse	-	~1.2x10^{12} ☺	~1.1x10^{12} ☺	-
Beam size at crystal/detector (VxH):	~60x100 μm	110 μm	82 μm	20 μm
Repetition frequency	1 kHz	10 Hz	120 Hz	1 MHz

Outlook:

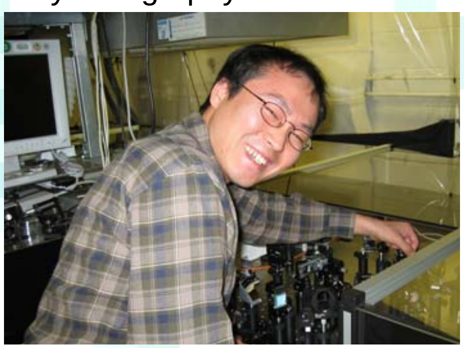
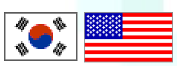
- Dual-mode operation of the Cornell ERL would allow no-compromise optimization of time-resolved capabilities (bunch charge, pulse compression, etc.)
- “Fat” bunch operation with a long undulator would enable single-shot Laue diffraction with spontaneous radiation
- Structural studies of proteins on the chemical time scale with near-atomic resolution would unveil mechanisms of protein function at an unprecedented level of detail. Such information is desperately needed to establish a solid foundation for rational drug design.

Acknowledgements

Post-Doctoral fellows

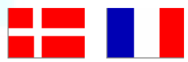
Dr. Friedrich Schotte
NIH
Time-resolved x-ray crystallography

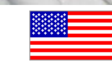
Dr. HyunSun Cho

Dr. Nara Dashdorj

Collaborators

Dr. Michael Wulff
ESRF
X-ray Instrumentation

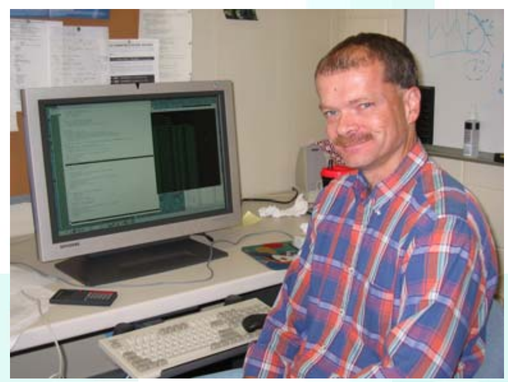
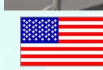



Dr. Gerhard Hummer
LCP, NIH
MD simulations

Harry Ihee
KAIST
PYP, bR

Marco Cammarata
ESRF
Time-resolved SAXS


Prof. John Olson
Rice University
Myoglobin mutants
(Dr. Jayashree Soman)

Dr. Eric Henry
LCP, NIH
Laue Data Analysis


Prof. George Phillips
Univ. Wisconsin
Refinement
(Roman Aranda and Elena Levin)