The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific and
Cultural Organization

IAEA
International Atomic Energy Agency

2332-8

School on Synchrotron and FEL Based Methods and their Multi-Disciplinary Applications

19 - 30 March 2012

Thin film analysis by GISAXS

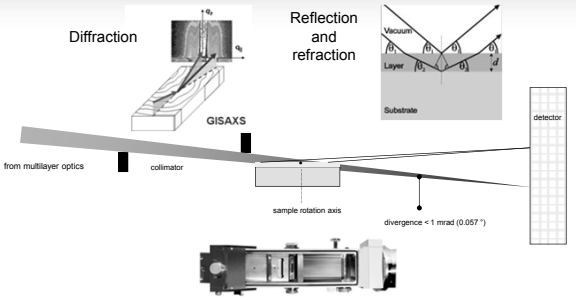
Peter Laggner
University of Graz (Austria)

Thin film analysis by GISAXS

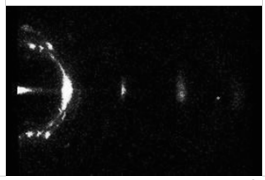
XRR

Diffraction

Reflection and refraction

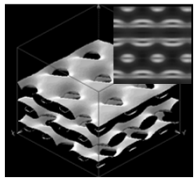


Lab-GISAXS of phospholipid mesophase



DOPC on Si chip

Hecus S3-MICROpix , 1000 s



This structure has highly promising features for thin-film biotechnology:

- controlled pore size,
- large inner surface
- easy to produce

Can we functionalize it (e.g. cytotoxic) ?

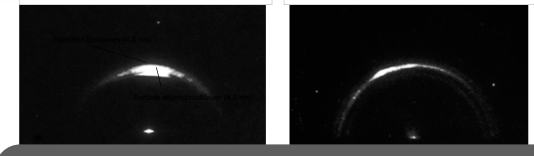
… tests with melittin (bee venom peptide)

Functionalized Peptide – Lipid Film

DOPC/Melittin R = 10^{-4} in vacuo on Si

S3-MICROpix 10 min

20 hours later, air, RT



- The original cubic film structure of DOPC is transformed into lamellar, isotropic multilayers already at molar ratios of 1:10.000 of melittin.
- Two (or more) domains with different lamellar repeats appear

S3-MICROcaliX

Combining SWAX & Calorimetry*

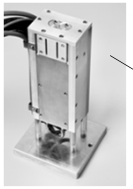
- Calorimetry provides only the enthalpy e.g. of phase changes, no structure
- SAXS is essential to identify long-period structures, e.g. in fats
- detection of pre-transitional changes in nanostructure – domains, interfaces
- simultaneous measurement is crucial with unstable materials

*Cooperation started with Michel Ollivon, Greard Keller, Claudie Bourgeaux et al in 2000,ontinued with Setaram, Caluire, France

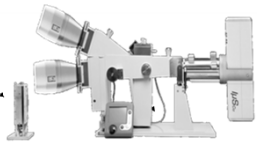
84

S3-MICROcaliX

Microcalorimeter



Microbeam X-Ray Camera



Integrated Laboratory Benchtop System

Calorimeter unit by Setaram , Caluire France

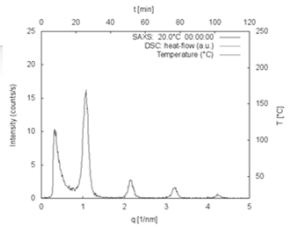
85

S3-MICROcaliX

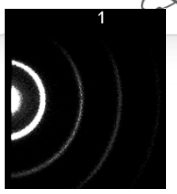
Calorimeter Specs

- Sample volume: 20µl (open capillary)
- Temperature range: -25 °C➔ 200°C (-30°C ➔ 200°C reached when RT=20°C)
- **Average sensitivity: 410µV/mW**
- **Isothermal detection limit:1.5µW**
- **Scanning detection limit: 2.5µW**
- Static drift (25 min isotherm at 26°C): 3µW
- Repeatability< 1%
- Maximum heating rate : 5K/min
- Maximum cooling rate
  - 5K/min(200°C ➔ 50°C)
  - 1K/min (50°C ➔ 0°C)
  - 0.5K/min (0°C ➔ -30°C)

The microcalorimeter of MICROcaliX matches the best stand-alone instruments

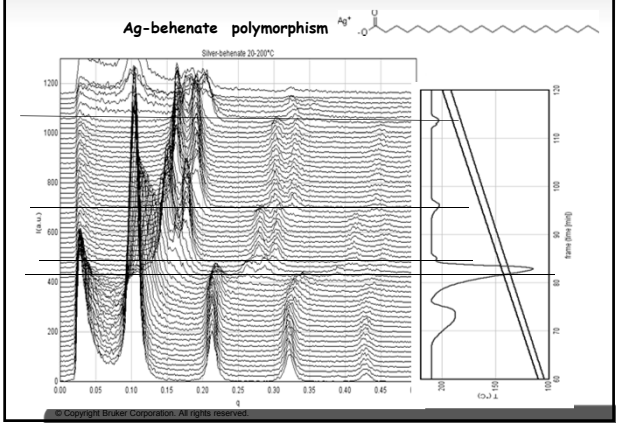


Ag-behenate powder:
20°-200°C (1.5°/min)
1D-SAXS: 1 frame/min

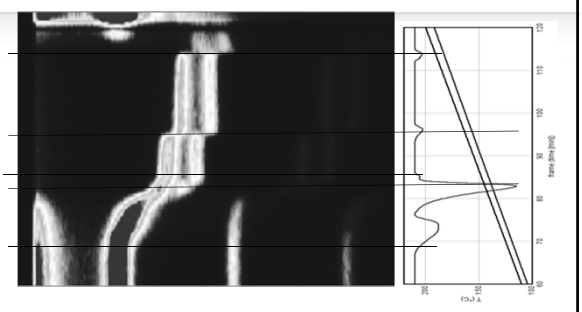


2 D-SAXS pattern of Ag-behenate heating scan: 110°C-200°C (1°C/min) 60 frames (1min each, 1°C/frame). Animation is not time-synchronous with 1D (left)

Ag-behenate polymorphism



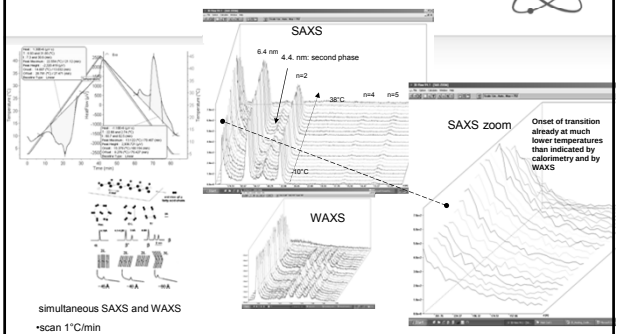
Silver Behenate SAXS-DSC
with Bruker MicroCaliX



COCOA BUTTER polymorphism - SWAXS/DSC

with Bruker MICROcaliX

Cocoa Butter: SAXS shows pre-transitional nanodomain effects



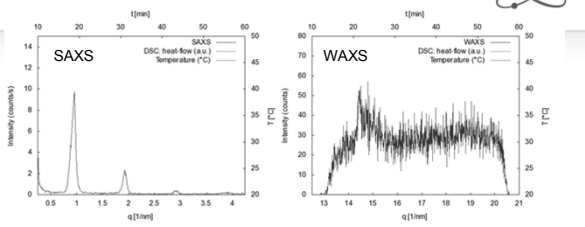
simultaneous SAXS and WAXS

- scan 1°C/min
- signal strength sufficient to measure SWAXS with time resolution of ~ 20 s, i.e. faster scanning is technically possible.

Liposome Thermal Polymorphism

with Bruker MICROcaliX

DPPC/H$_2$O, Thermotropic phase transitions $L_{\beta'}$ - $P_{\beta'}$ - L_α



DPPC
23°- 48°C
DSC: 0.5°/min
SAXS: 1 frame/min

2 D-SAXS pattern of DPPC-heating scan:
70 frames (1min exposures).
Animation is not time-synchronous with 1D from above.

MICROcaliX - Fields of Application

	Component	Application
Food	protein	denaturation, aggregation
	Protein powder	crystallization
	starch	gelatinization, retrogradation, glass transition
	milk	Melting, crystallization, denaturation, aggregation
	fat, chocolate	Melting, crystallization, lipidic transition, polymorphism
	fat	crystallization
	hydrocolloids	melting, gelation
	sugar	Melting, crystallization, glass transition (amorphizer)
Pharmaceutical	**drug, excipient**	**Melting, polymorphism**
Bio	protein	denaturation, aggregation, polymorphism
	Lipid, membrane, vesicle	Lipidic transition
Others	Liquid crystal	transitions
	Gas hydrate	Formation, dissociation

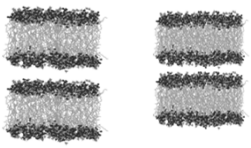
BRUKER

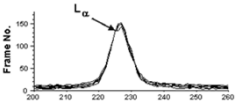
Michel Ollivon - The Pioneer

He initiated the development of SAXS/DSC at synchrotrons. A laboratory bench differential microcalorimeter coupled with a SWAXS (Small- and Wide-Angle X-ray Scattering) spectrometer allowing both types of analysis simultaneously has been developed in collaboration between SETARAM Instrumentation and former Hecus, now Bruker ASX, following a research work performed by Prof. Michel Ollivon at the University of Chatenay Malabry (Paris, France).

DYNAMICS
Nano-Cinematography

Membrane transitions





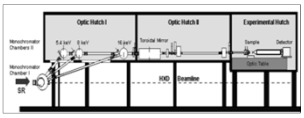
- *Detect short-lived intermediates*
- Combination of:
  - brilliant source
  - fast detectors

- high X-ray brillance
- efficient optics

Most of todays third-generation synchrotron sources offer suitable beam-lines for fast time-resolved diffraction/scattering

e.g. the Austrian-SAXS beamline at ELETTRA / Trieste

Flux ~ 10^{12} / s.mm^2



Requirements:

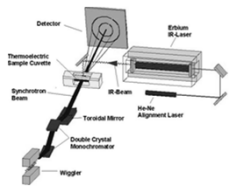
- high X-ray brillance
- efficient optics
- fast position-sensitive detectors
- synchronization/trigger methods

- synchronization/trigger methods

Jump-Relaxation Techniques: e.g. pressure-,temperature-, stopped-flow (concentration-), magnetic field jumps

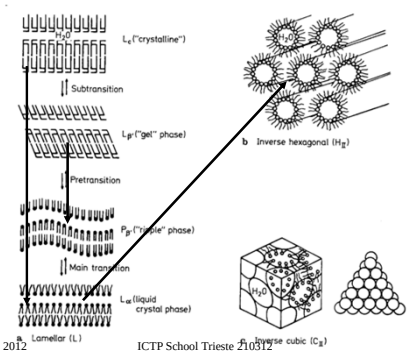
Steady-State Techniques: time-upon mixing / continuous-flow techniques

Non-Equilibrium Dynamics
MILLISECOND TEMPERATURE-JUMP EXPERIMENT

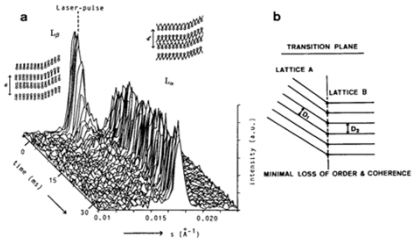


IR-Laser:
λ = 1.54 μm
E_{max} = 4 J
τ = 2 ms
ΔT_{max} = 20°C

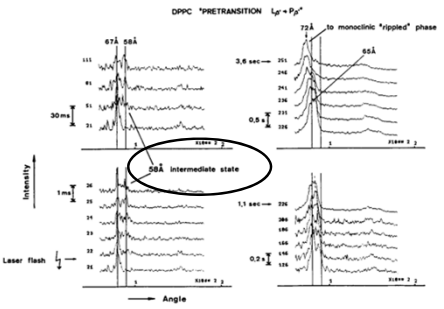
Polymorphism of Lipids –
Mechanisms of Phase Transitions ?



Martensitic transisiton between two lamellar, *affine* lattices

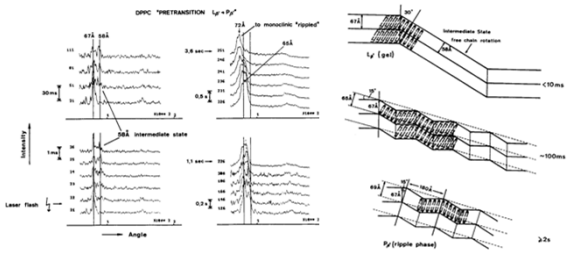


Short-lived intermediates in transitions between *nonaffine* latices
e.g. $L_{\beta'}$ - $P_{\beta'}$

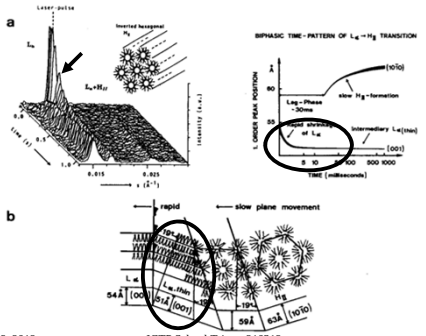


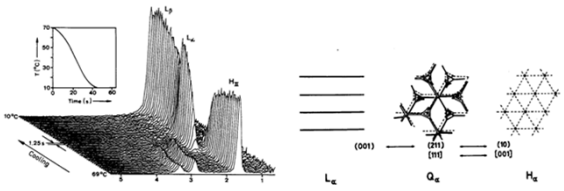
Short-lived intermediates in transitions between *nonaffine* latices

e.g. $L_{\beta'}$ - $P_{\beta'}$



Intermediate also in lam/hex transition





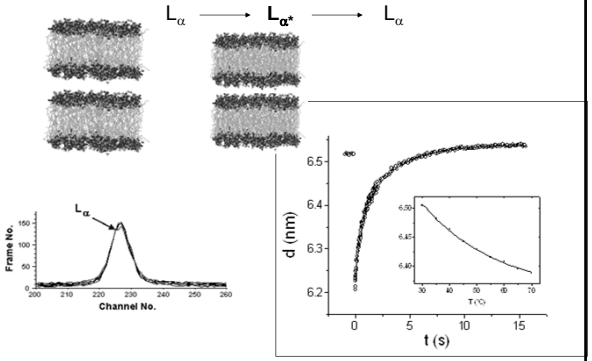
Intermediates not detectable in slow-scan mode

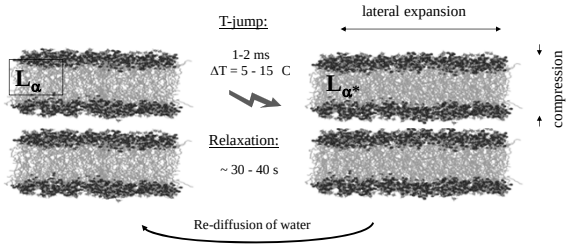
Conclusion:

Mesomorphic phase transitions follow different mechanisms depending on how far from equilibrium they are.

T-jump within one-phase L_α region

$L_\alpha \longrightarrow L_{\alpha^*} \longrightarrow L_\alpha$

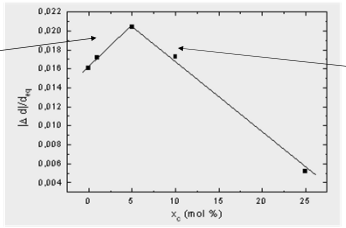




T-jump probes the elastic properties of liposomes

Effects of Cholesterol on Bilayer Response in T-Jumps

Softening



Hardening

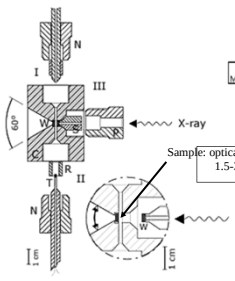
High-pressure SAXS

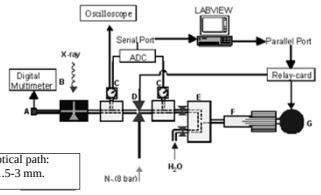
Monica Vidal (IBR)
Cilaine Teixera (IBR)
Manfred Kriechbaum (IBR)

Heinz Amenitsch (IBR)
Sigrid Bernstorff (Sincrotrone Trieste)

Milos Steinhart
(IMC Prague)

The HP-X-ray Cell





Cell body: Stainless steel (5 x 3.5 x 2 cm)

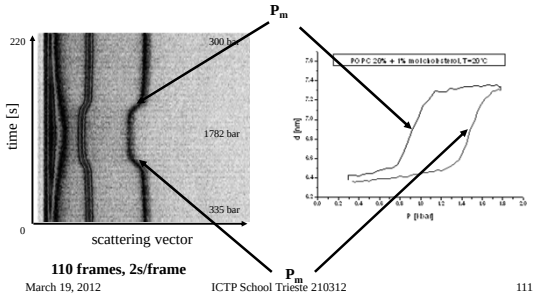
Max. **pressure**: 2-3 kbar (5-90 C)

Windows: Be or diamond C (2r = 3 mm, d=1.5 and 0.75 mm, respectively)

Aperture: entrance: 1.5 mm, exit: 2 mm (2r); 60 angle cone (covers SAXS and WAXS).

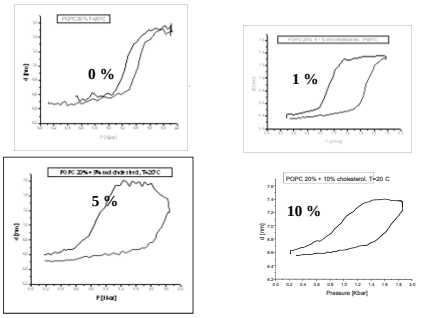
Reference: M.Steinhart, M.Kriechbaum, K.Pressl, H.Amenitsch, P.Laggner and S.Bernstorff, Rev.Sci.Instrum. 70, 1540-1545 (1999).

P-scans of POPC/cholesterol (20% in water)



110 frames, 2s/frame

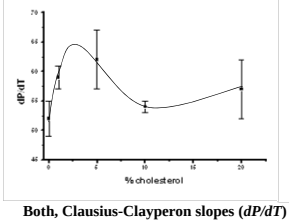
Pressure-Hysteresis



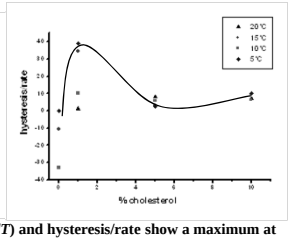
Hysteresis for POPC 20% and different molar concentrations of cholesterol at 20 C
d-spacing as a function of pressure during a p-scan (red: compression and blue: decompression)

Pressure scanning of PC / Cholesterol mixtures

dP/dT



Rate-dependence of hysteresis

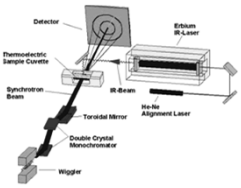


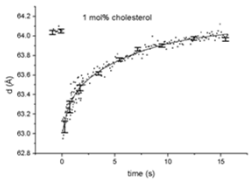
Both, Clausius-Clayperon slopes (*dP/dT*) and hysteresis/rate show a maximum at low concentrations of cholesterol (1-5 mol%). This agrees with the notion that low amounts of cholesterol induce a softening/plastification and higher concentration cause hardening.

What drives lateral organisation in fluid membranes ?

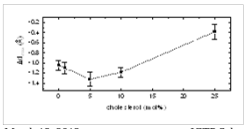
Is it the tendency to minimize lateral compressibility that drives the spontaneous redistribution of components?

Membrane Softness: the Effect of Cholesterol





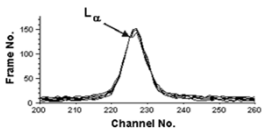
T-jumps of 3 *C* (T_{Start} = 10 *C*)



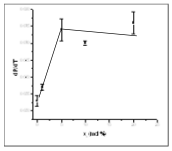
Result: Low amounts of cholesterol soften the bilayer whereas above 5 mol% the membrane gets more and more rigid.

Phospholipid Bilayers: Ordered Intermediate

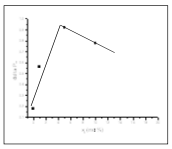
$L_\alpha \longrightarrow \mathbf{L_{\alpha^*}} \longrightarrow L_\alpha$



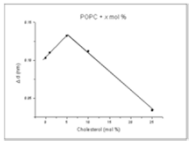
p-SCAN AND T-JUMP RESULTS



Transition pressure



Pressure hysteresis



T-jump: change of d-spacing

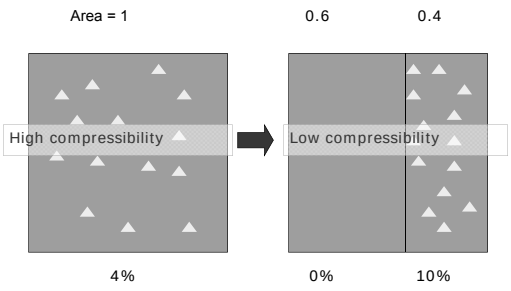
Pabst G., Rappolt M., Amenitsch H., Bernstorff S., and Laggner P. (2000). *Langmuir* **16**, 8994-9001

HYPOTHESIS:

Compressibility is the main determinant

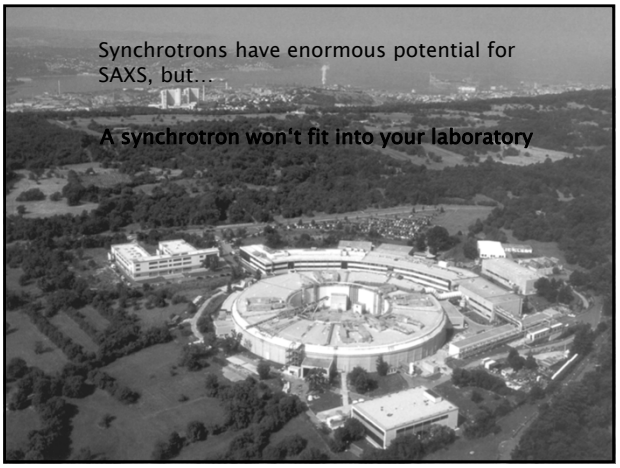
Components are pressed into the arrangement of lowest overall compressibility

Example : 4 Mol-% cholesterol



Membranes with high flexibility and shape differentiation have low cholesterol contents, e.g. endoplasmic reticulum, mitochondrial inner membranes, sarcoplasmic reticulum.

Membrane cholesterol increases with age

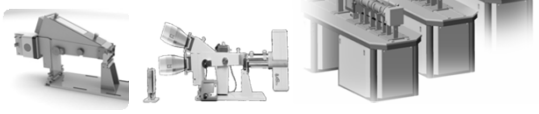


Advanced Laboratory Instrumentation
The Bruker-AXS SAXS Platform

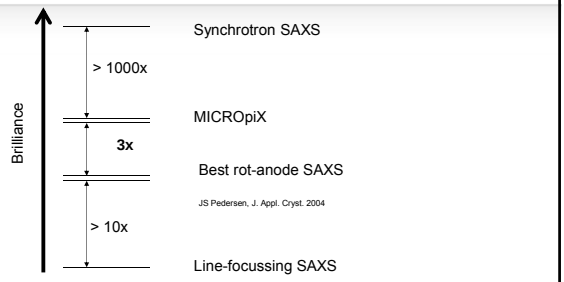
BRUKER

The Bruker SAXS Platform covers the two key typologies:

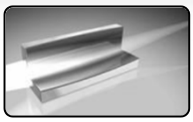
I. The versatile **NANOSTAR™ series**
for higest power in physics and material science applications
e.g. nanography, GISAXS, erc



II. The compact **MICROPix ™ and MICROCaliX ™** series
for high-flux / high resolution applications in chemistry and biology with micro-point-focussing optics .



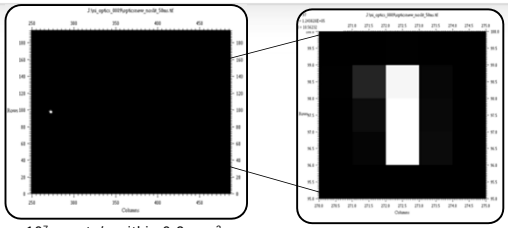
Bruker Micro SAXS Platform – X-ray Source



- 50 W, air cooled, IμS (Incoatec micro-focus X-ray source)
- 3 years of warranty

- Choice of focusing, parallel beam 2D or 1D Montel or Göbel Mirrors for various wavelengths

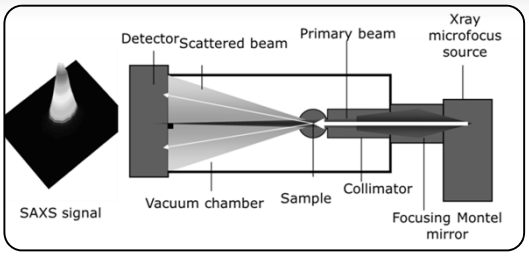
The MICRO – Primary Beam



- 10^7 counts/s within 0.2 mm^2
- flux density: > 10^8 / mm^2
- FWHM : ~ 0.2 x 0.43 mm^2 (z/y)
- Imax= ~ 3 x 10^6 counts/s

- (recorded with Pilatus 100k, 172 micron pixel size)

The MICRO SAXS Platform



The beam path of Kratky MICRO Platform