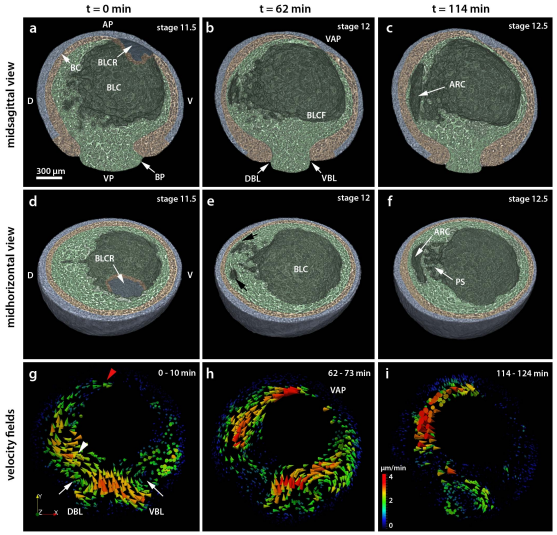
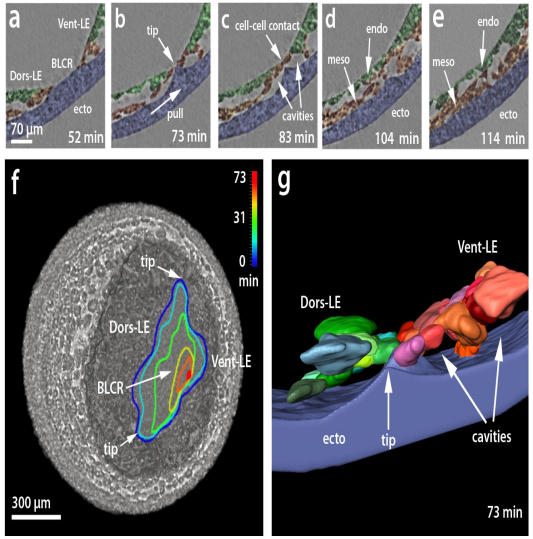
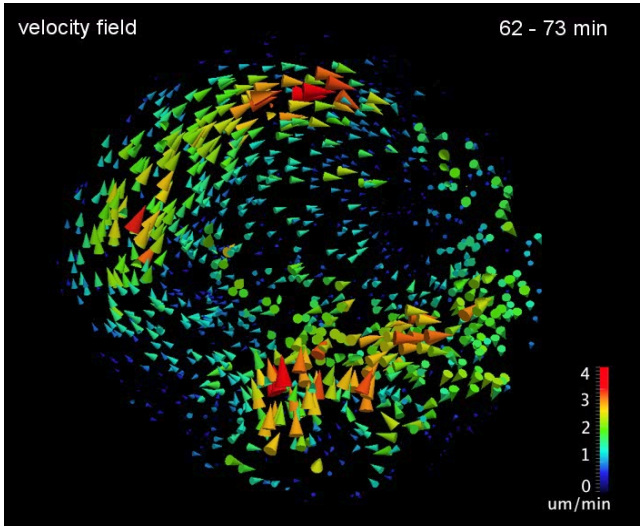
Phase-contrast X-ray *in vivo* microtomography and developmental biology

Ralf Hofmann, KIT





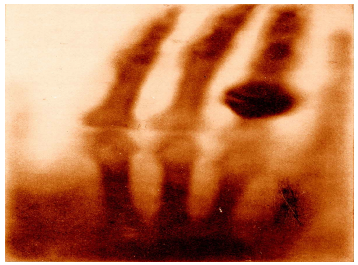


Outline

- Introduction and motivation
- Phase-contrast X-ray microtomography
- Gastrulation in *Xenopus laevis*: time lapse microtomography *in vivo*
- Dose issues
- Improving contrast and resolution: APS lattice upgrade plus virtual source

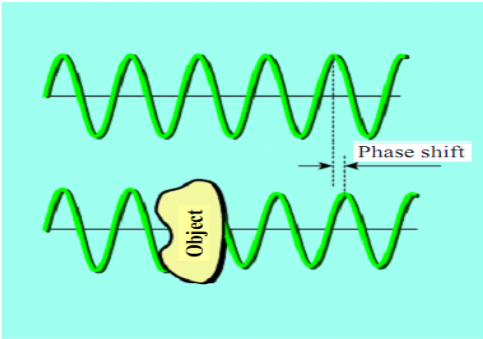
Introduction and motivation (soft-tissue imaging)

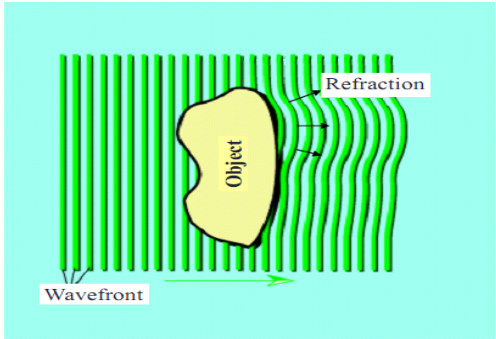
- Hard X-rays strongly penetrating electromagnetic waves



(courtesy: Mrs Röntgen 1895)

- Interaction with matter described by refractive index: $n = 1 - \delta + i\beta$





phase shift

absorption:
destroys organisms

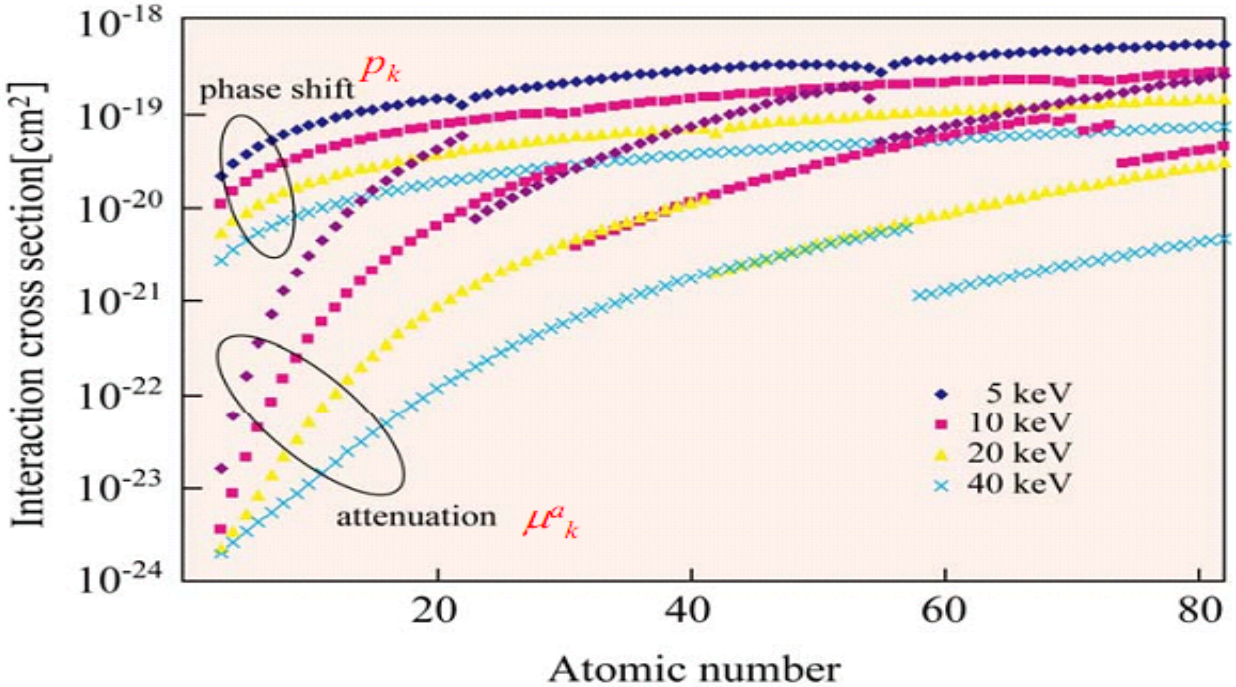
phase shift:
- characterizes organisms without destroying them
- to be viable: good spatiotemporal **coherence** and **high flux density**

$\Rightarrow$ **Use monochromatic synchrotron radiation and X-ray energies such that**

$$\frac{\delta}{\beta} \gg 1$$

Phase shift vs. attenuation

since:



⟹ **Cartillage-, bone-, and blood vessel-free biological tissues** essentially act as **pure-phase objects** for X-ray energies **E > 20 keV**. (early embryonic stages in Developmental Biology)

However: Phase-contrast imaging requires (partial) coherence.

Pure-phase objects: early stages in vertebrate models

Vertebrate models with high degree of conservation w.r.t. to humans:

Xenopus laevis (African clawed frog)

Lamprey

$\Rightarrow$

Optically opaque embryos (X-rays for 3D imaging!)

Zebrafish

Medaka

Partially optically transparent embryos (light-sheet, confocal microsc.)

Motivation cntd. (digital embryo)

- **Fluorescent nuclei by** marker protein expression
- **Tracking of nuclei only:** no dense structural info
- **Limited depth information:** a few cell layers only

Reconstructed nuclei motion from
Digital scanned laser light sheet fluorescence
microscopy in entire wild-type Zebrafish embryos over the first 24 hours of development
(Keller, Schmidt, Wittbrodt, Stelzer, Science 2008)

Gastrulation in *Xenopus laevis*

Stitching-in-time of sagittal slices from different, fixed embryos.

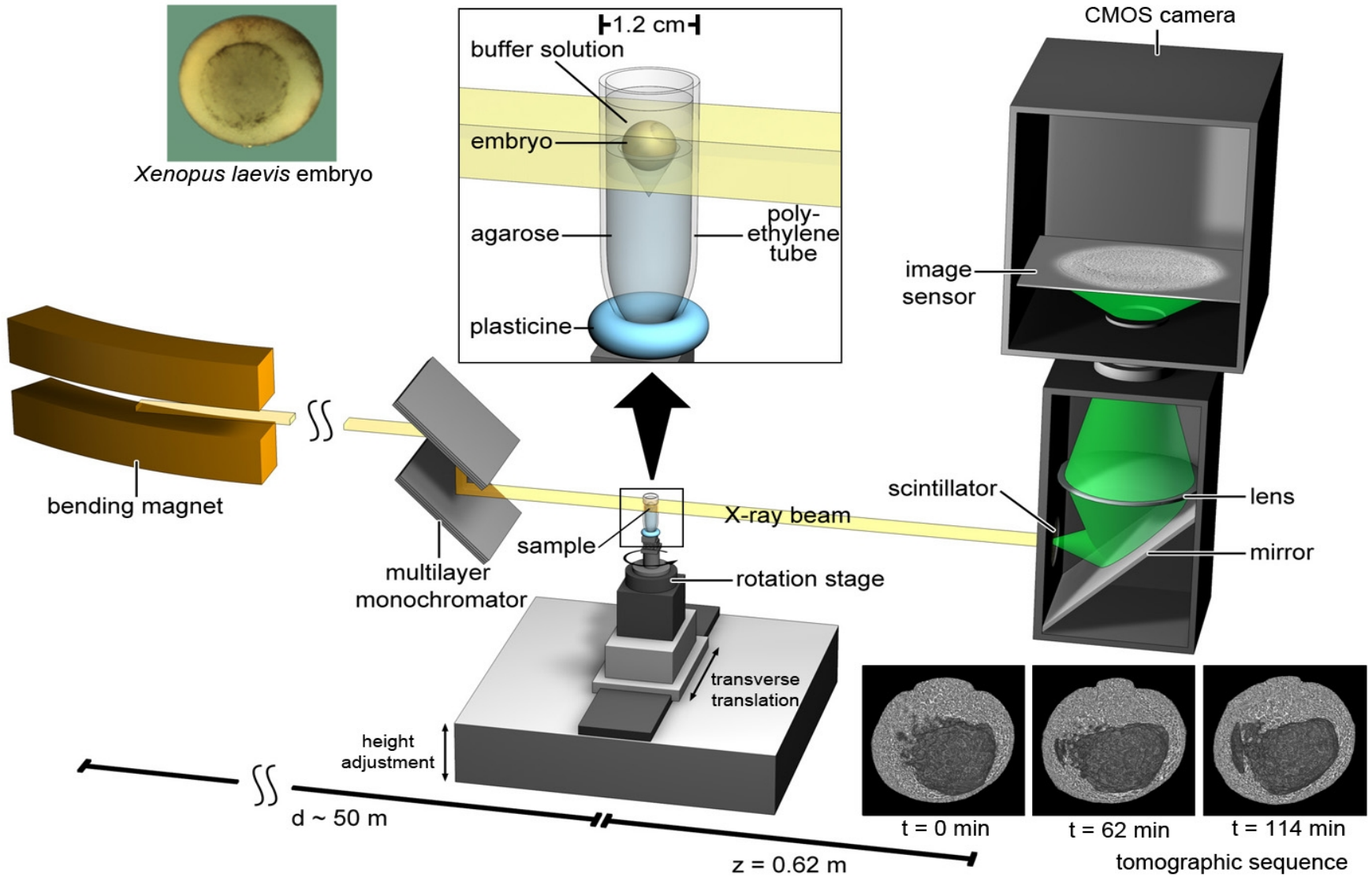
Gastrulation in *Xenopus laevis*: 4D *in vivo* imaging using PCXMT I

sagittal horizontal

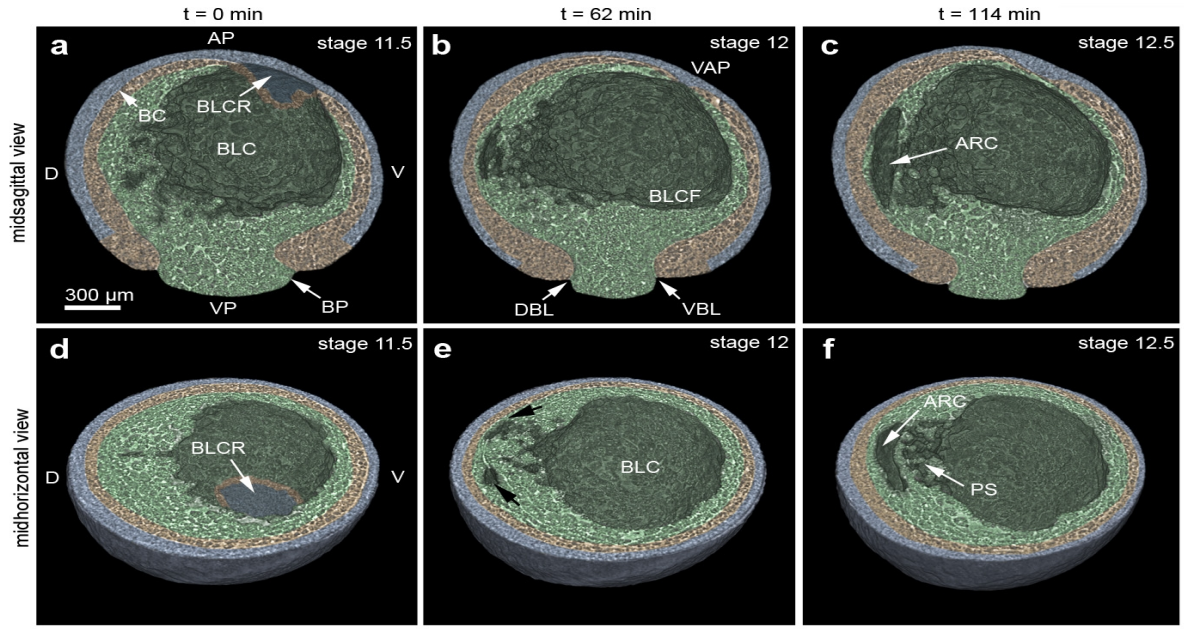
data acquired at APS beamline 2BM (and 32ID):
E=30 keV, z=62 cm, $\Delta x = 2\mu m$, dE/E=10^{-4}, 1200 projts, 15ms exposure per projt

Moosmann et al., Nature **497**, 374 (2013);
Moosmann et al. Nature Protocols, accepted;
Nawy, Nature Methods **10**, 603 (2013)

Gastrulation in *Xenopus laevis*: 4D *in vivo* imaging using PCXMT II



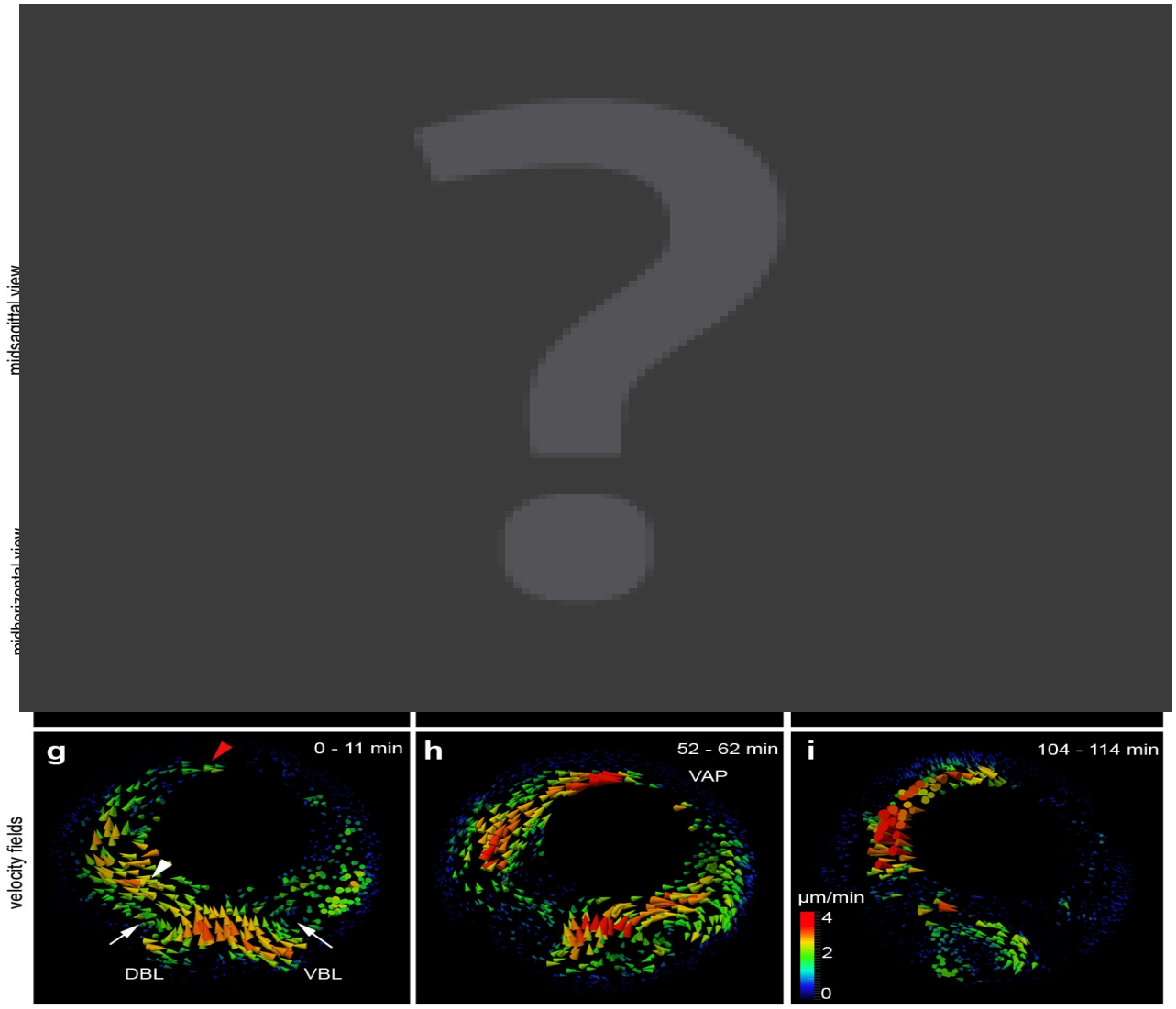
gastrulation in *Xenopus laevis:* structure in 3D, I



gastrulation in *Xenopus laevis:* structure in 3D, II

gastrulation in *Xenopus laevis:* archenteron inflation

gastrulation in *Xenopus laevis:* snapshots of 3D flow field

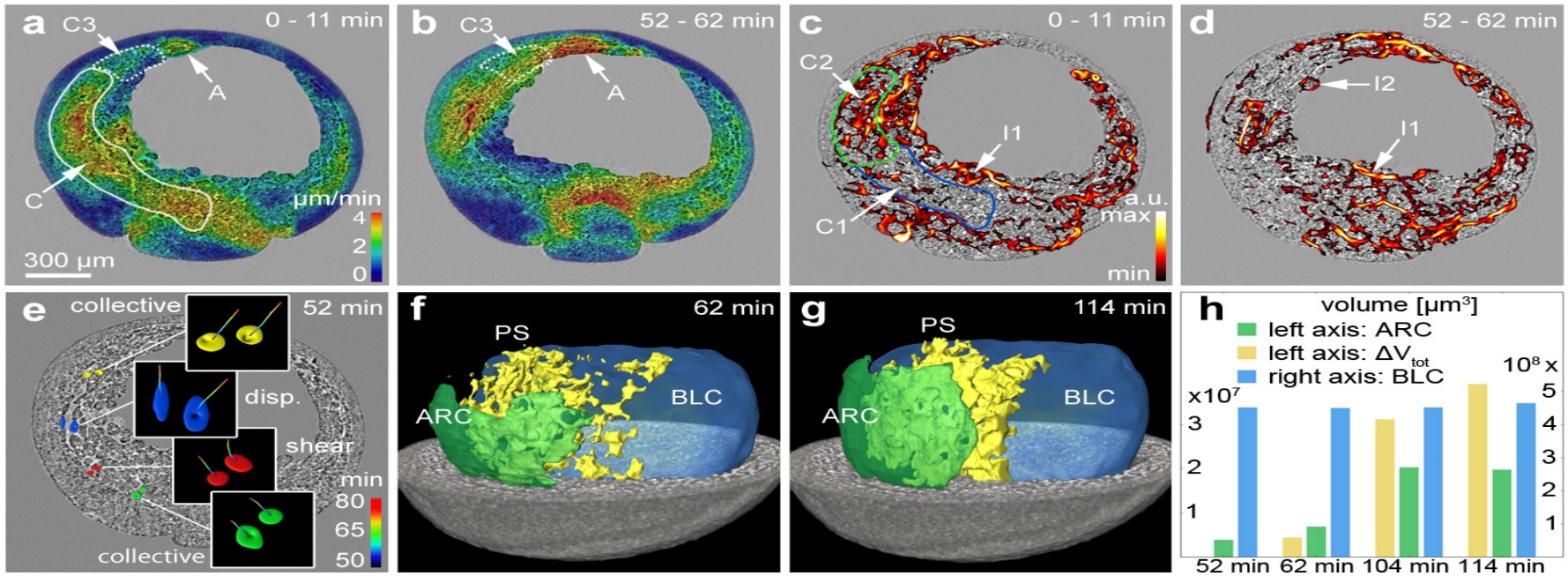


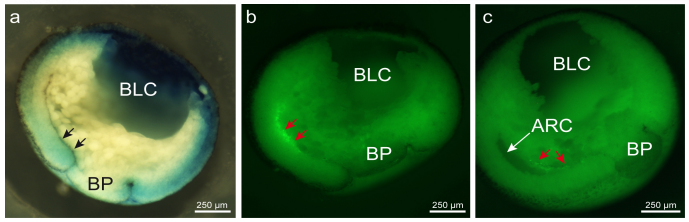
gastrulation in *Xenopus laevis*: differential flow, tissue propulsion, fluid exchange, etc.

Karlsruher Institut für Technologie

(i) spatially differential flow field ⟷ tissue/cell propulsion (converg. extens., crawl)
(ii) time dependence of cavity volumes ⟷ mechanism of water uptake

[Ewald et al, Development 131, 6195 (2004) clashes with Tuft, J. Exp. Biol. 39, 1 (1962)]

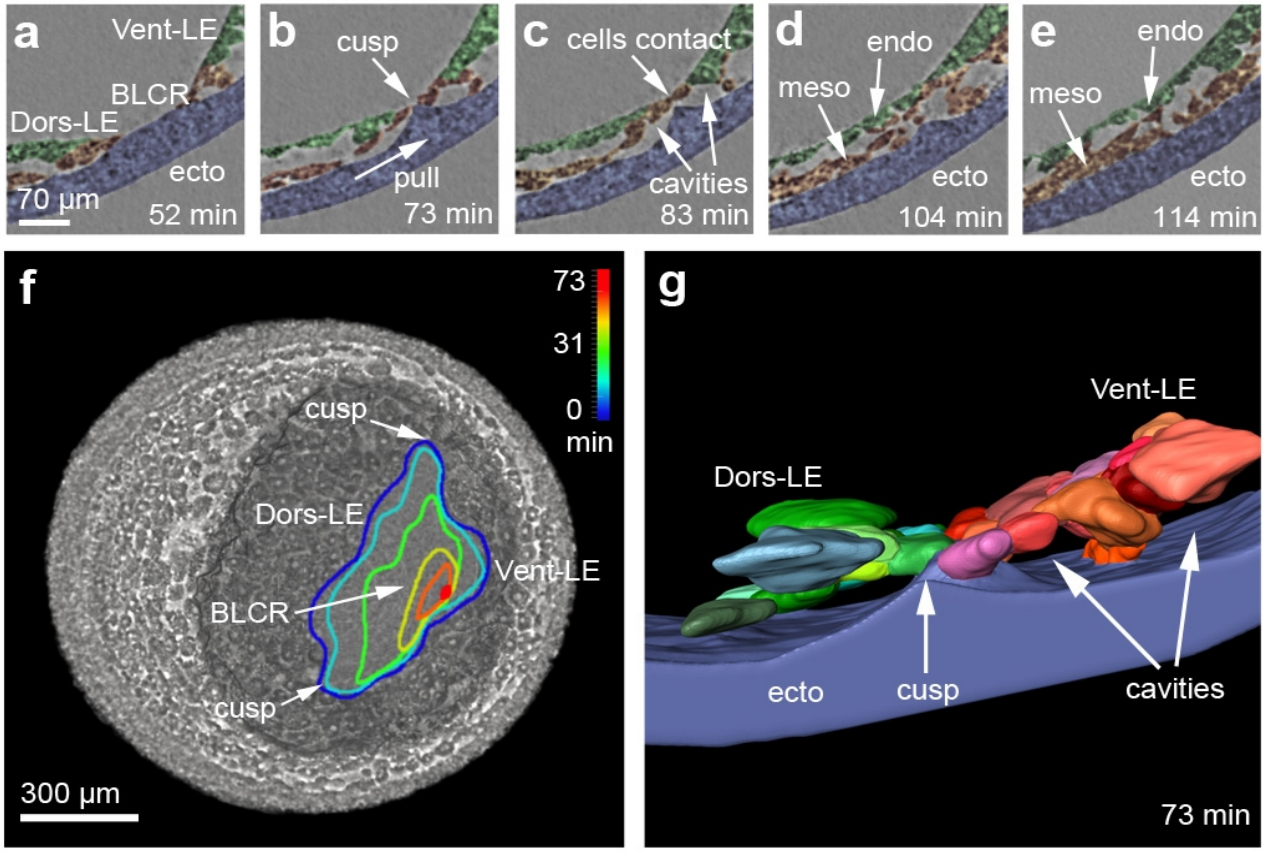




dye experiments

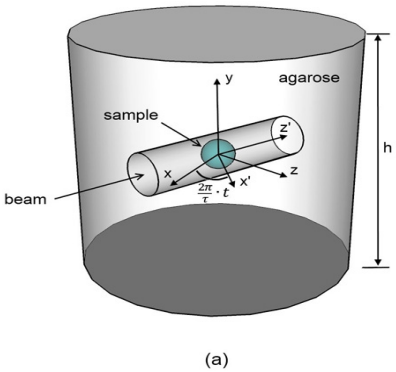
Archenteron inflation in the affirmative by uptake of external water.

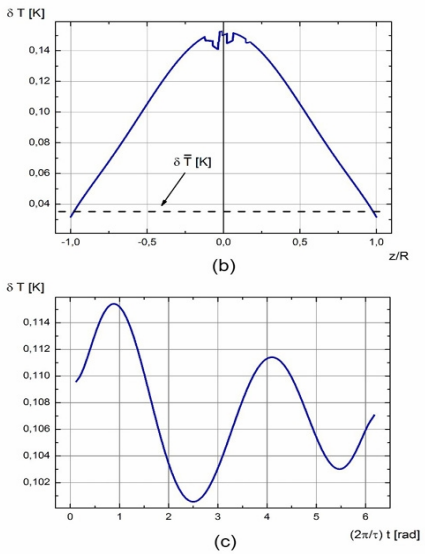
gastrulation in *Xenopus laevis*:
transient ectodermal ridge in collision zone of head- and ventral mesendoderm

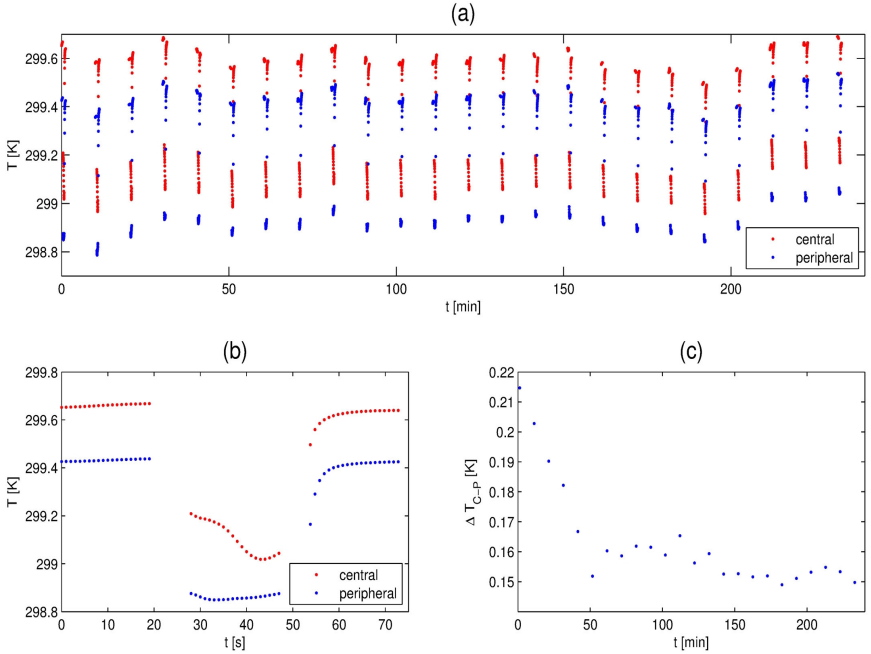


Definition of neural plate ?

Dose issues with *in vivo* PCXMT:
estimation vs. measurement: heat load







Simulation (heat equation):
Eppendorf with R=0.6cm, E=30 keV,
beam profile $2 \times 1\,\mathrm{mm}^2$, $I_0 = 10^{12}\,\frac{\mathrm{photons}}{\mathrm{mm}^2\mathrm{s}}$

exposure per tomogram: 18 seconds

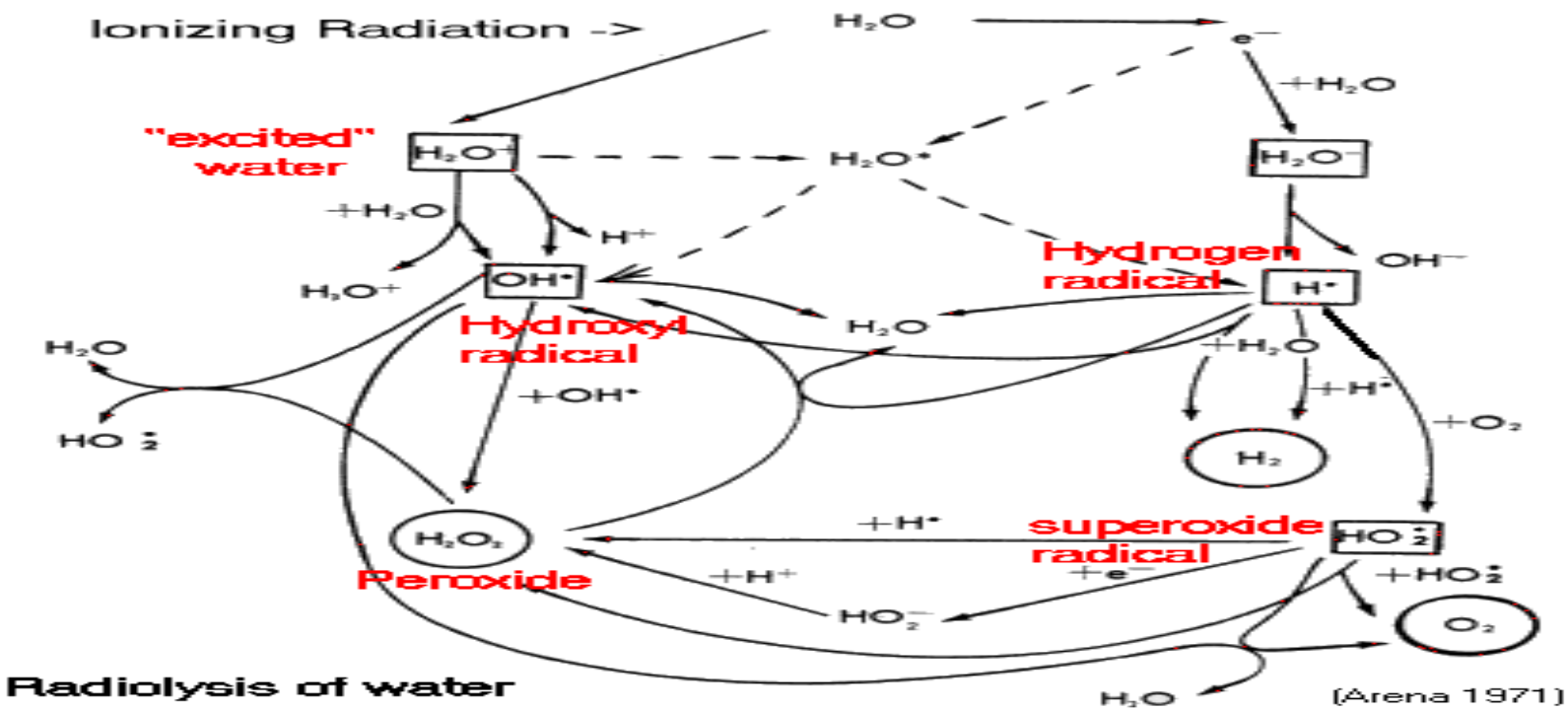
Measurement:
2 thermistors,
central & peripheral
[Xianghui Xiao]

Heat load: no concern in *in vivo* imaging of Xenopus embryos!

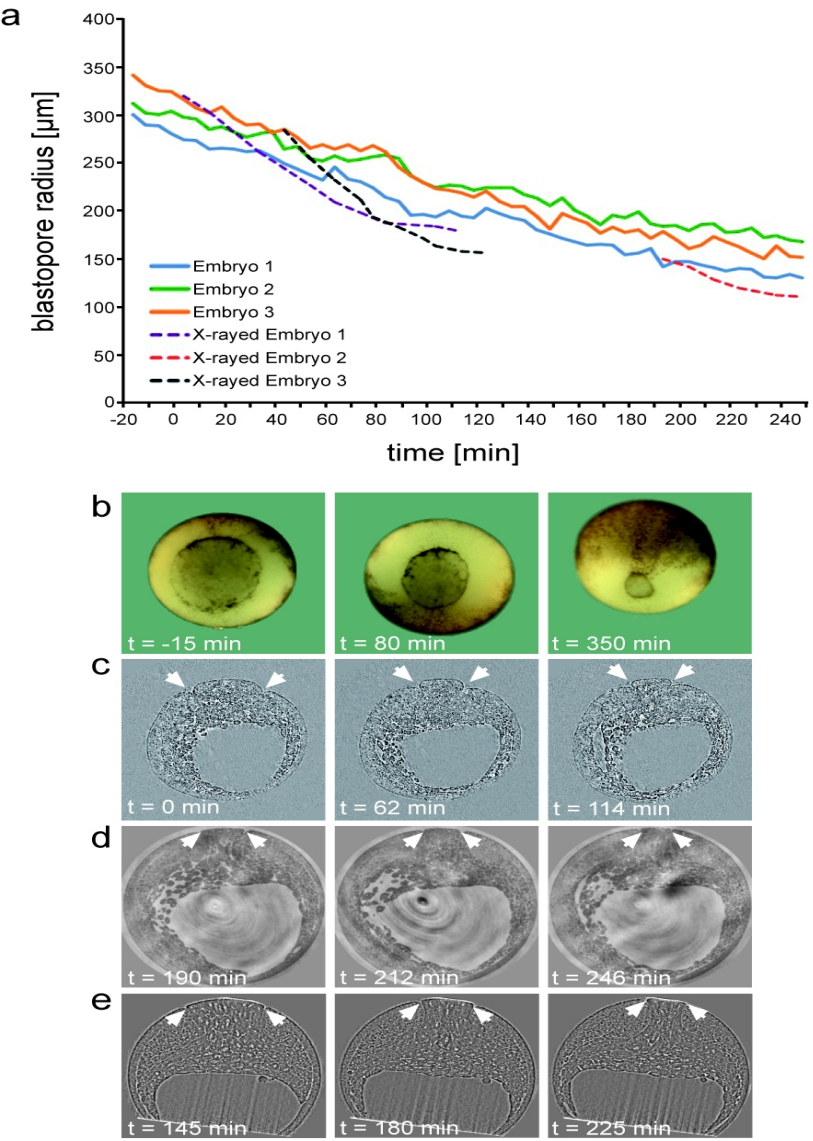
Dose issues with *in vivo* PCXMT:
effect estimation: radiolysis I

- estimate of **direct impact** of ionizing radiation on breaking of hydrogen bonds (e.g. DNA strand breaking): **no problem**
  [Howells, Hitchcock, Jacobsen, J. Electr. Spectr. and Rel. Phen. 2009]

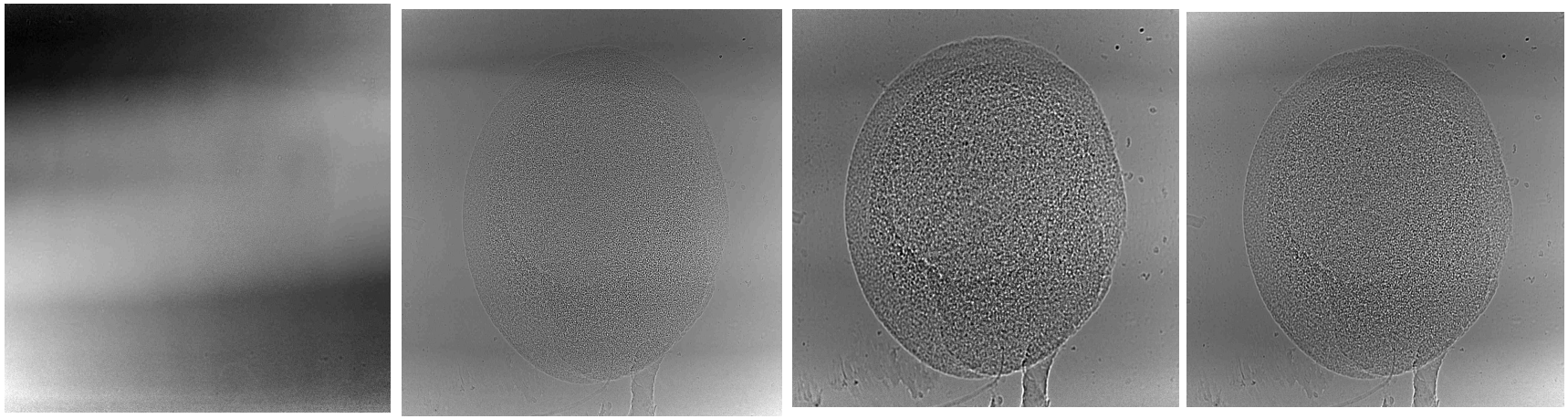
- **hard-to-control** effects of **Radiolysis of Water**
  (protein erosion, DNA/RNA destruction by **free radicals**):



Dose issues with *in vivo* PCXMT:
phenomenological effect estimation: radiolysis II



Increasing contrast and spatial resolution of *in vivo* PCXMT, I



z=0.5cm z=18cm z=58cm z=108cm

increasing signal-to-noise ratio with **increasing object-detector distance**

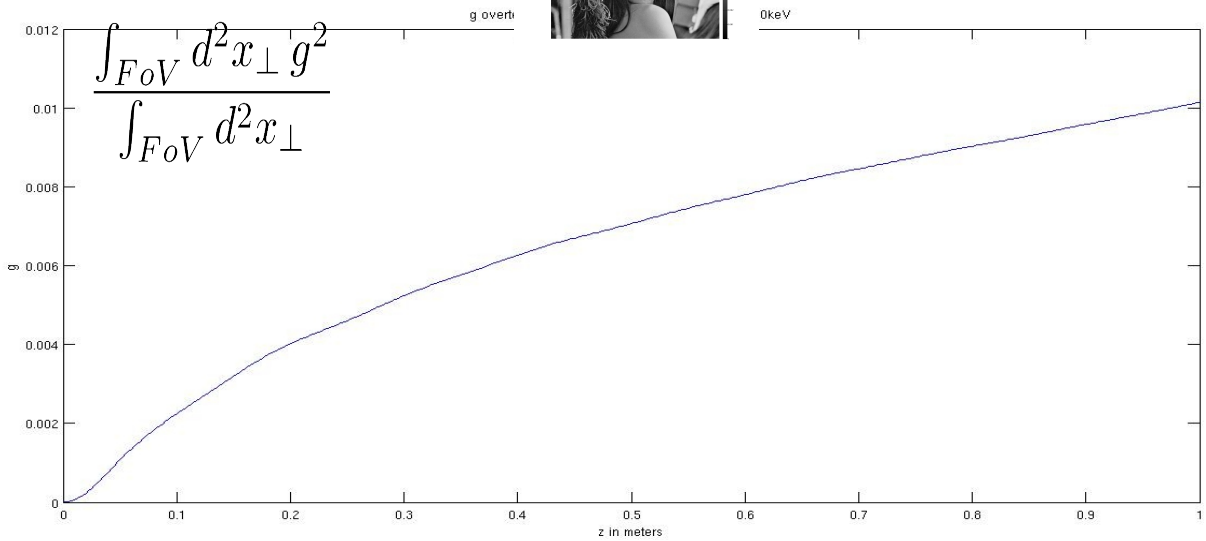
[fixed Xenopus embryo, stage 10.5] TOPO-TOMO@ANKA, E=12 keV, exp. = 180 s]

Increasing contrast and spatial resolution of *in vivo* PCXMT, II:

simulated forward propagation of at E=20 keV:

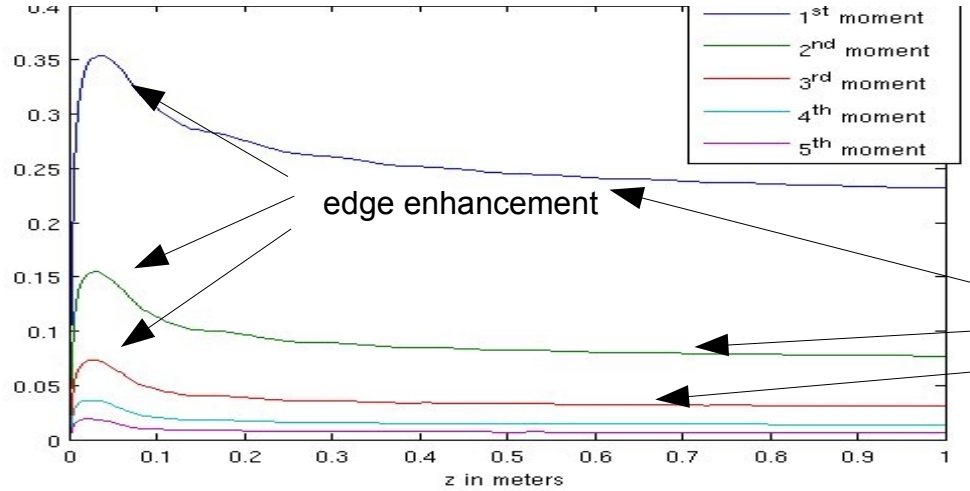
mean g^2

$$\frac{\int_{FoV} d^2x_\perp\, g^2}{\int_{FoV} d^2x_\perp}$$



z

moments of $|\mathcal{F}g|(\vec{\xi})$



edge enhancement

$[g = 2\sin(\lambda z \Delta_\perp)\phi]$

population of lower frequencies (fringes)

z

Increasing spatial resolution of *in vivo* PCXMT, III:

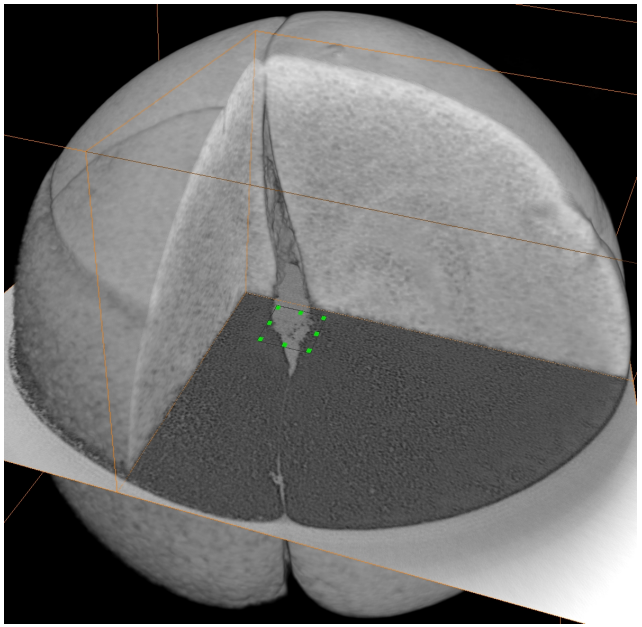
- measure intensity contrast at maximum distance z allowed by tranverse coherence length l_t
  (limited through effective frequency cutoff $\xi_b = \frac{l_t}{z\lambda}$ becoming **smaller** than inverse effective pixel size Δx^{-1}: **source blurring**)

- presently, with l_t available at 2 BM or 32 ID one has $z < 1$ m

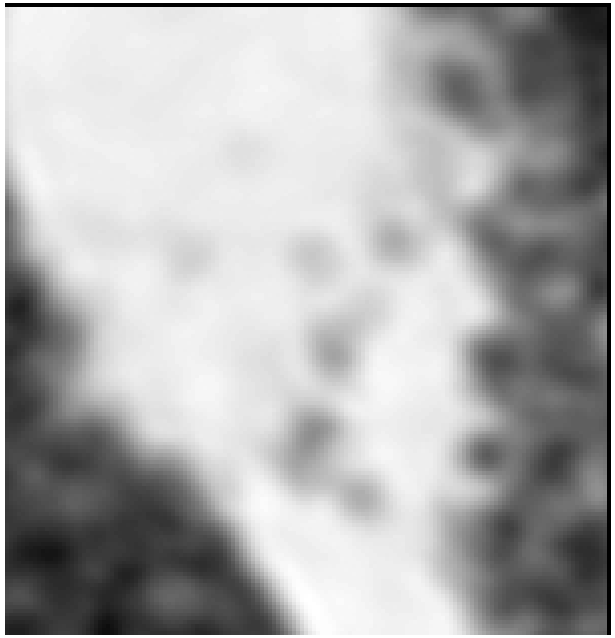
- APS lattice upgrade: increase of l_t by two orders of magnitude
  $\Longrightarrow$ substantial increase of z at decreasing Δx (virtual source!)
  $\Longrightarrow$ increase of signal-to-noise in intensity contrast g
  at similar (limited!) integrated flux
  $\Longrightarrow$ substantial increase of attainable resolution

- however: efficient, single-distance, local but
  nonlinear phase retrieval algorithm required (large exit phase variations):
  QUASI-PARTICLE
  [Moosmann et al, Optics Express 19, 25881 (2011),
  Hofmann et al. Optics Express 19, 12066 (2011), Schober et al.,work in progress]

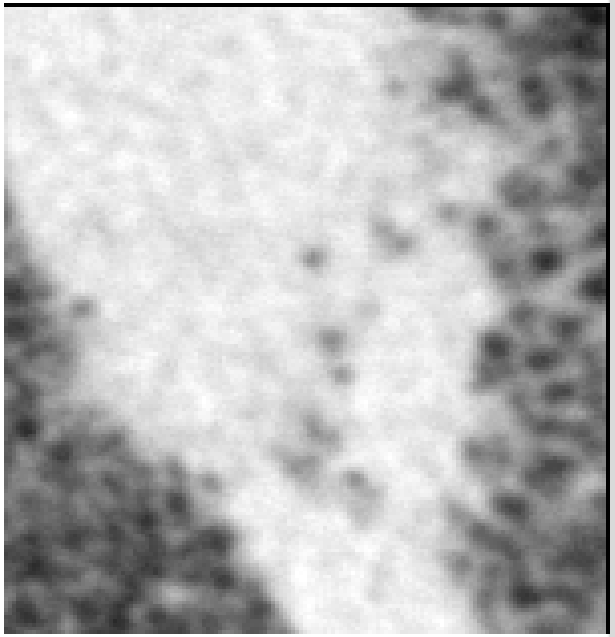
Xenopus laevis: 4-cell stage (fixed), ID19_ESRF



2mm



Paganin



quasi-particle

E = 20 keV, *z* = 95 cm, $\frac{\Delta E}{E} = 10^{-4}$, $\Delta x = 0.75\,\mu\mathrm{m}$

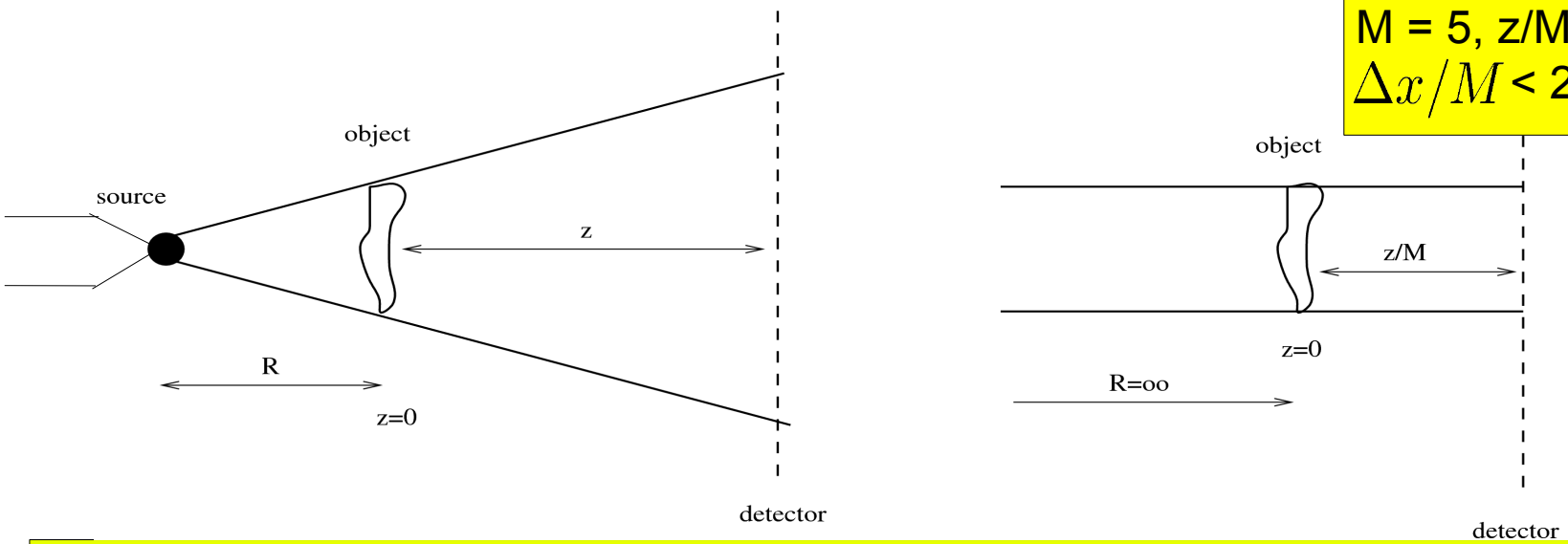
1600 projections, 2 s exposure per frame

Increasing spatial resolution of *in vivo* PCXMT, III:

decrease of effective pixel size Δx at higher propagation induced contrast :
virtual source and Fresnel scaling

M=1+z/R>1 ⟷ M=1

e.g.:
R = 2.5 m, z =10 m,
M = 5, z/M = 2 m,
$\Delta x / M$ < 200 nm



$$\Rightarrow \Delta x \rightarrow \frac{\Delta x}{M}\,, \quad z \rightarrow z/M\,, \quad M^2$$ times present flux density

Summary and Outlook:

- principles of phase-contrast tomography

- early stage vertebrate model systems:
  essentially pure phase for E>20 keV

- results on *in vivo* imaging of gastrulation in *Xenopus l.*

- dose issues: only some phenomenological understanding so far

- improving resolution at limited dose:
  - increase of (rescaled) propagation distance allowed by substantial increase of coherence length through APS machine upgrade
  - more info at high spatial frequencies non-linearly retrieved
  - decrease of effective pixel size by virtual source

- fewer projections with improved Poisson noise statistics using ART instead of FBP

- application to other dev. stages (neurulation, migration of neural crest cells) and model systems

Acknowledgement

Xianghui Xiao

Julian Moosmann

Alexey Ershov

Venera Weinhardt

Tilo Baumbach

Carole LaBonne

Maneeshi Prasad

Jubin Kashef

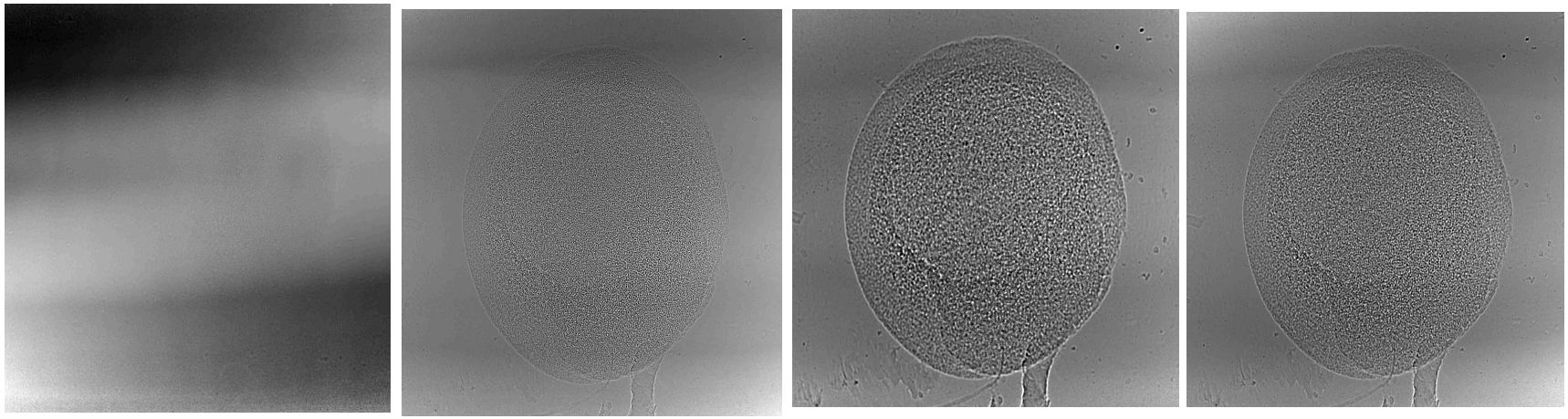
Ray Keller
Rudi Winklbauer
Doris Wedlich
Jochen Wittbrodt
Herbert Steinbeisser
Thomas van der Kamp
Martin Köhl
Francesco deCarlo
Kamel Feeza
AG Kashef

Karlsruher Institut für Technologie

Thank you!

ANKA/IPS imaging/image analysis

Propagation based phase contrast:



z=0.5cm z=18cm z=58cm z=108cm

increasing signal-to-noise ratio with **increasing object-detector distance**

[fixed Xenopus embryo, stage 10.5] TOPO-TOMO@ANKA, E=12 keV, exp. = 180 s]

High-resolution, single-distance, nonlinear and noniterative phase-retrieval algorithms (Bio-Imaging)

- **Acceptable signal-to-noise** at **limited dose** deposition:
  - **large object-detector distances**
  - **single-distance** imaging
  - reduction of required **number of projections** (ART versus FBP)

- **Large throughput with online feedback option (Atlases):**
  - **single-distance** imaging
  - fast reconstruction

  $\Longrightarrow$ **noniterative phase retrieval**

- **High-resolution of phase retrieval:**
  - **simple** linear **algorithms too coarse**
  - highly-resolving **linear algorithms inapplicable**
    (imaging of **entire** embryos, **strong phase objects,** $\Delta\phi \sim 1$)

  $\Longrightarrow$ **nonlinear phase retrieval**

Fresnel theory (perfect coherence, parallel beam, pure phase)

- Re-writing Fresnel diffraction integral [Guigay 1977]:

$$(\mathcal{F}\, I_z)(\vec{\xi}) = \int d^2r\, \exp(-2\pi i \vec{r}\cdot\vec{\xi}) \times \psi_0\left(\vec{r} - \frac{\pi z}{k}\vec{\xi}\right)\psi_0^*\left(\vec{r} + \frac{\pi z}{k}\vec{\xi}\right)$$

- Expansion **of exponential in exit wave-field:**

$$\psi_0 = \sqrt{I_0}\exp(i\phi_0) = \sqrt{I_0}(1 + i\phi_0 - \frac{1}{2}\phi_0^2 + O(\phi_0^3))$$

- Guigay up to quadratic order:

[Moosmann, RH, Baumbach, Opt. Exp., 2011;
RH, Moosmann, Baumbach, Opt. Exp. 2011]

$$(\mathcal{F}\, g_z)(\vec{\xi}) = 2\sin(s)\ (\mathcal{F}\,\phi_0)(\vec{\xi}) - \cos(s)\int d^2\xi'\,(\mathcal{F}\,\phi_0)(\vec{\xi'})(\mathcal{F}\,\phi_0)(\vec{\xi} - \vec{\xi'})$$

linear order

$$+\exp(is)\int d^2\xi'\,\exp(-\frac{4\pi^2 i z\vec{\xi}\cdot\vec{\xi'}}{k})(\mathcal{F}\,\phi_0)(\vec{\xi'})(\mathcal{F}\,\phi_0)(\vec{\xi} - \vec{\xi'}) + O((\mathcal{F}\,\phi_0)^3)$$

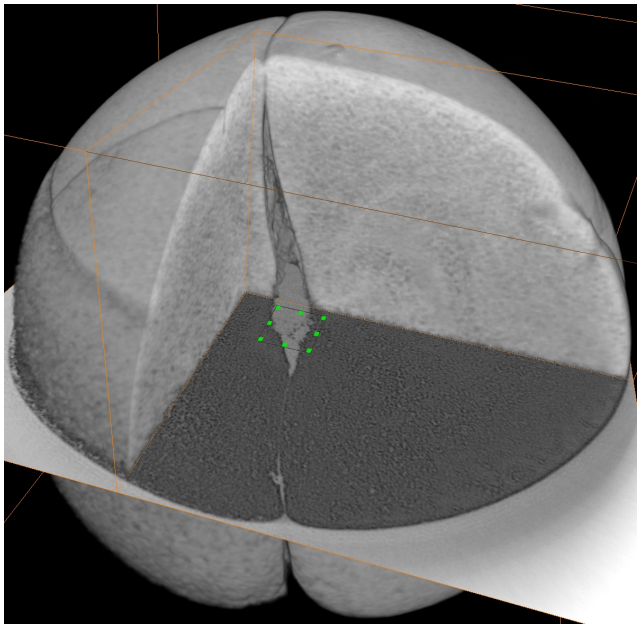
$$(s \equiv \pi\vec{\xi}^2 z\lambda)$$

Theory: Quasiparticle approach

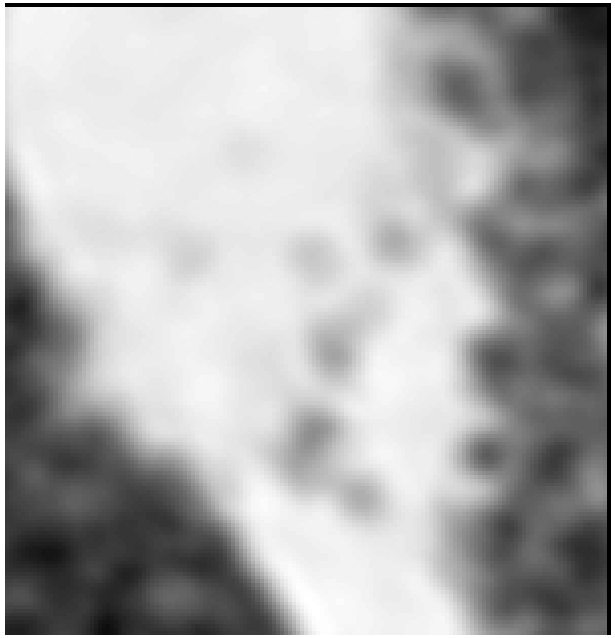
$|\mathcal{F} g_z|$

Fourier-transformed intensity
under exit-phase upscaling: $\phi_0 \rightarrow S\,\phi_0 \quad (S > 1)$

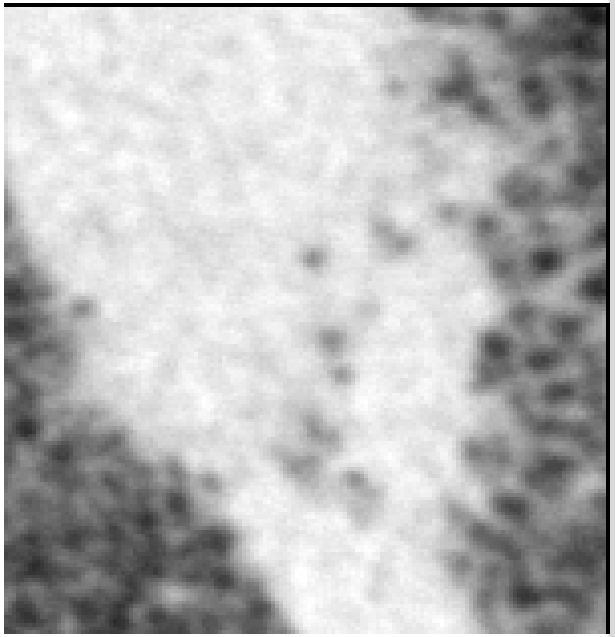
Xenopus laevis: 4-cell stage (fixed), ID19_ESRF



2mm



linearized TIE

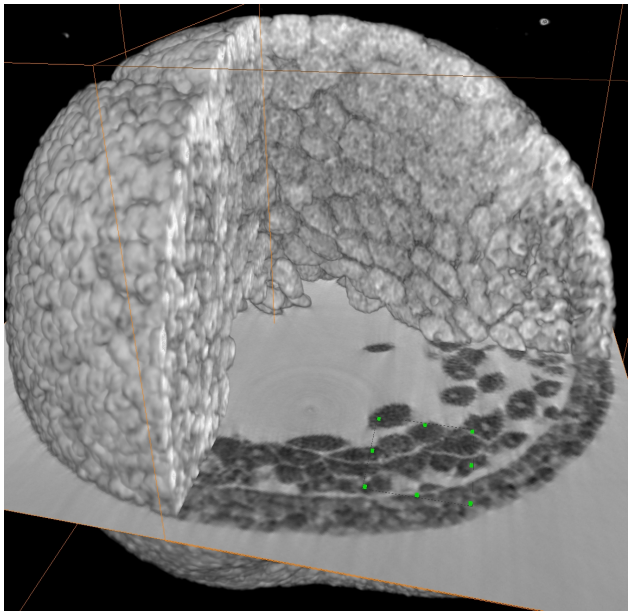


quasiparticle

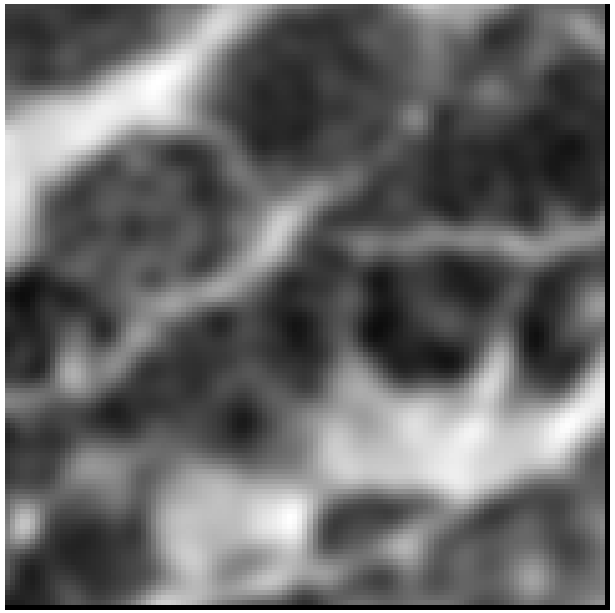
E = 20 keV, *z* = 95 cm, $\frac{\Delta E}{E} = 10^{-4}$, $\Delta x = 0.75\,\mu\mathrm{m}$

1600 projections, 2 s exposure per frame

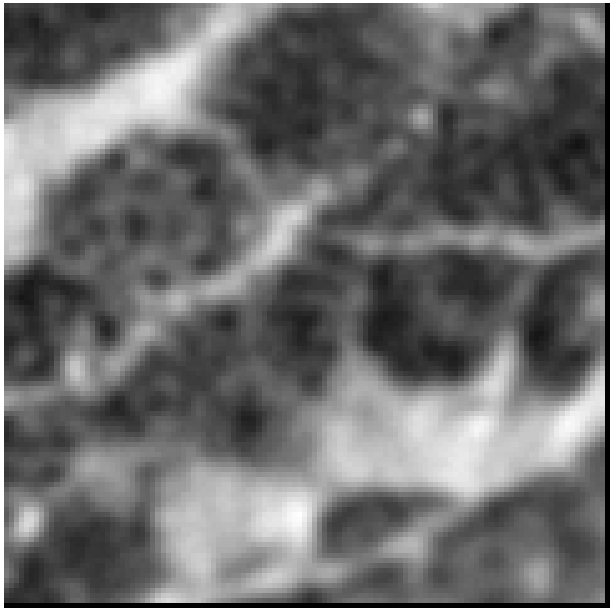
Xenopus laevis: stage 10.5 (fixed), ID19_ESRF



2mm



linearized TIE



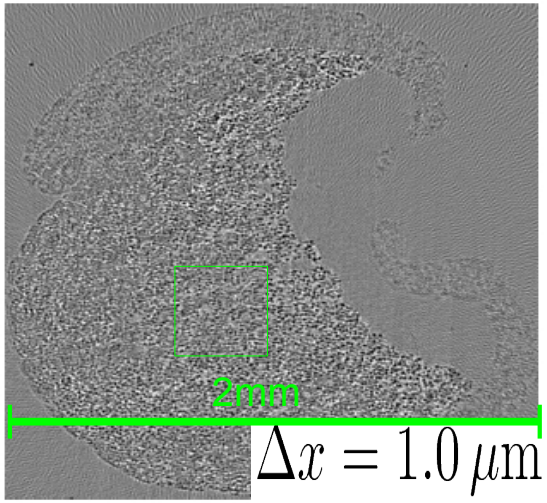
quasiparticle

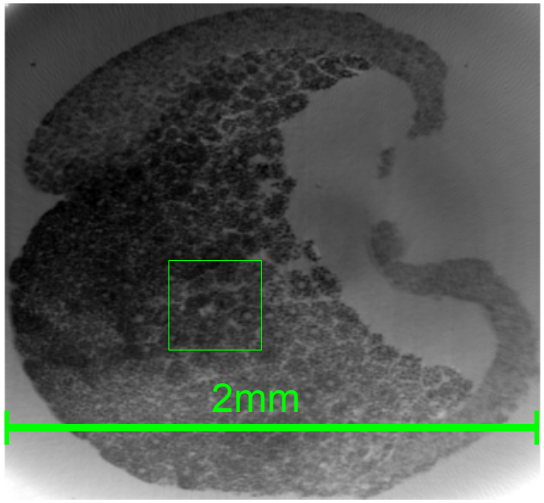
E = 20 keV, *z* = 95 cm, $\frac{\Delta E}{E} = 10^{-4}$, $\Delta x = 1.5\,\mu\mathrm{m}$

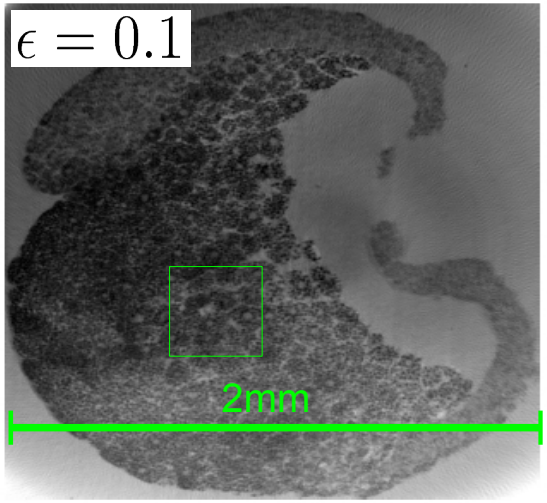
1600 projections, 1 s exposure per frame

Xenopus: stage 10, TOPO/TOMO_ANKA

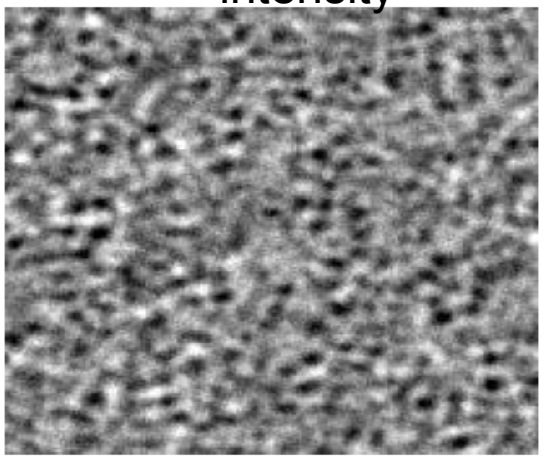
E = 12 keV, *z* = 37.5 cm, $\frac{\Delta E}{E} = 10^{-2}$, slice through 3D reconstructions



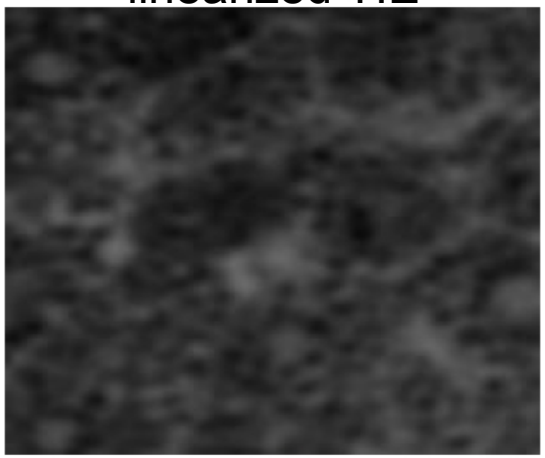




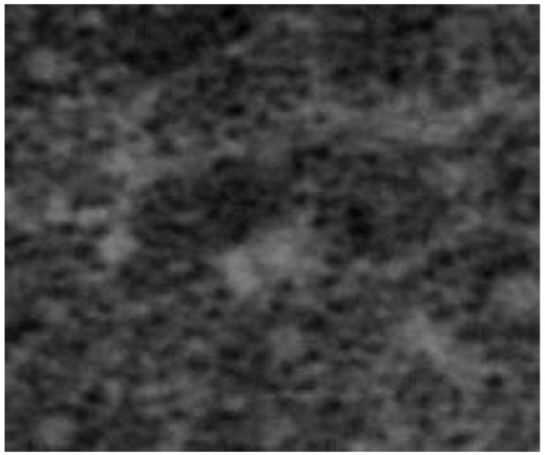
intensity



linearized TIE



quasiparticle



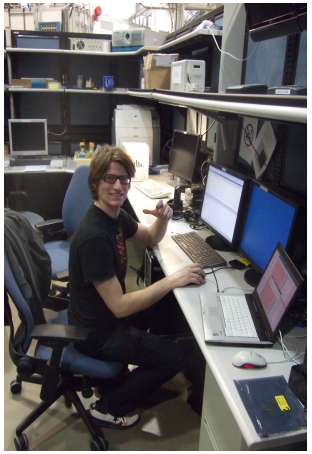
650 projections, 50 s exposure per frame

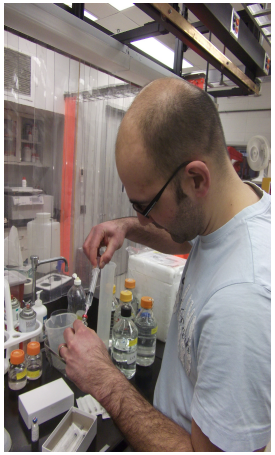
3D in-vivo time lapse imaging, Xenopus Laevis

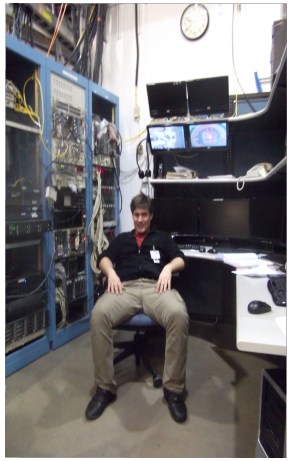
- Experiment at 2BM_APS (Feb 2012, frogs from C. La Bonne, North Western U)

- Parameters varied:
  - E = 22 … 30 keV
  - radical scavengers of multiple concentrations (radiolysis of water!)
  - exposure per projection 15 ms … 100 ms
  - developmental stages: 11 (gastrulation) … 16 (neurulation)

- On-the-fly tomography: 1200 projections (periph. rot. blurring, cell motion)

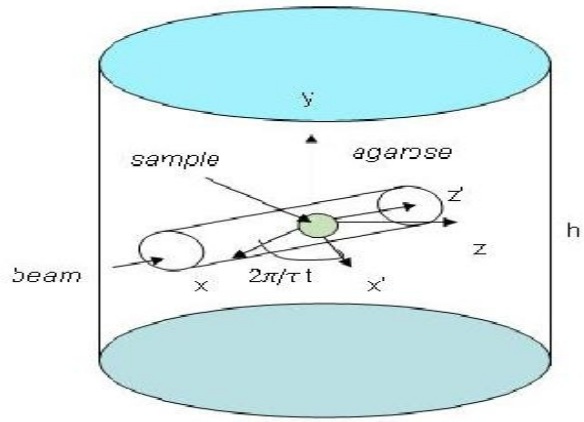




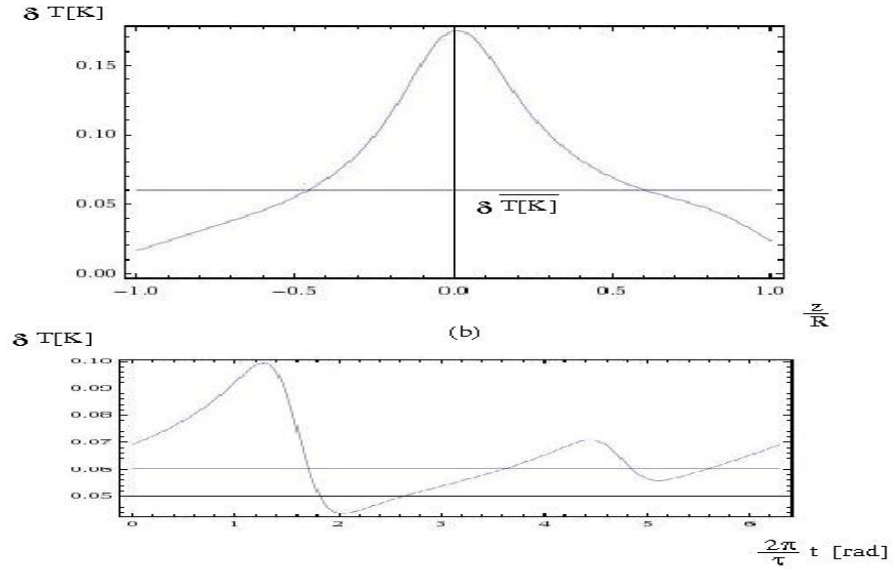


3D in-vivo time lapse imaging, Xenopus: Heat load

$$I_0 = 10^{12}\,\frac{\text{photons}}{\text{mm}^2\text{s}}$$



(a)



(c)

Simulation (heat equation):

Eppendorf with R=0.6cm, E=30 keV, beam profile ,

2-BM photon flux density $10^{12}\text{ph s}^{-1}\text{mm}^{-2}$, $1 \times 1\,\text{mm}^2$

exposure per tomogram = 18 seconds

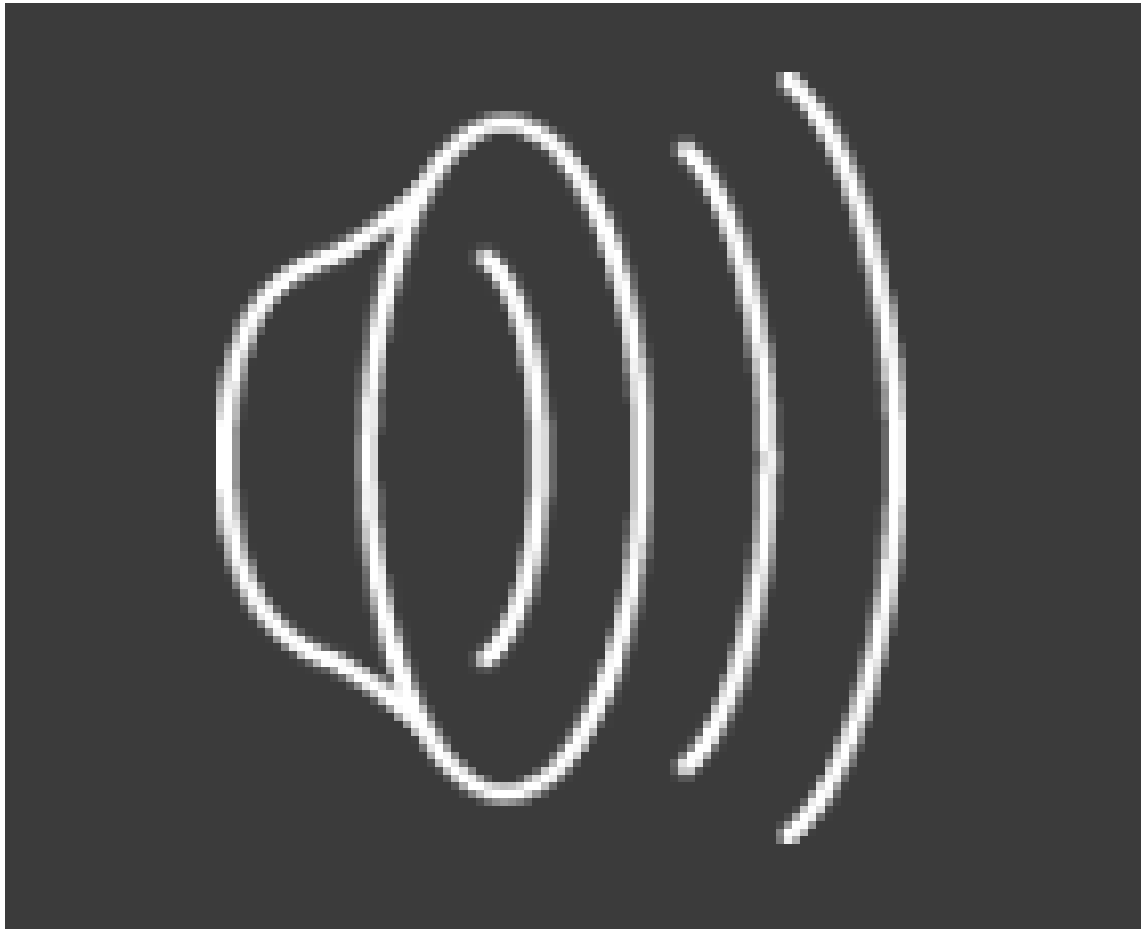
Heat load: No concern in in-vivo imaging of Xenopus embryos!

Gastrulation, Xenopus:

Opening of archenteron I, blastoporus closing, mesodermic involution, mid-sagittal plane

blastoporus

dorsal

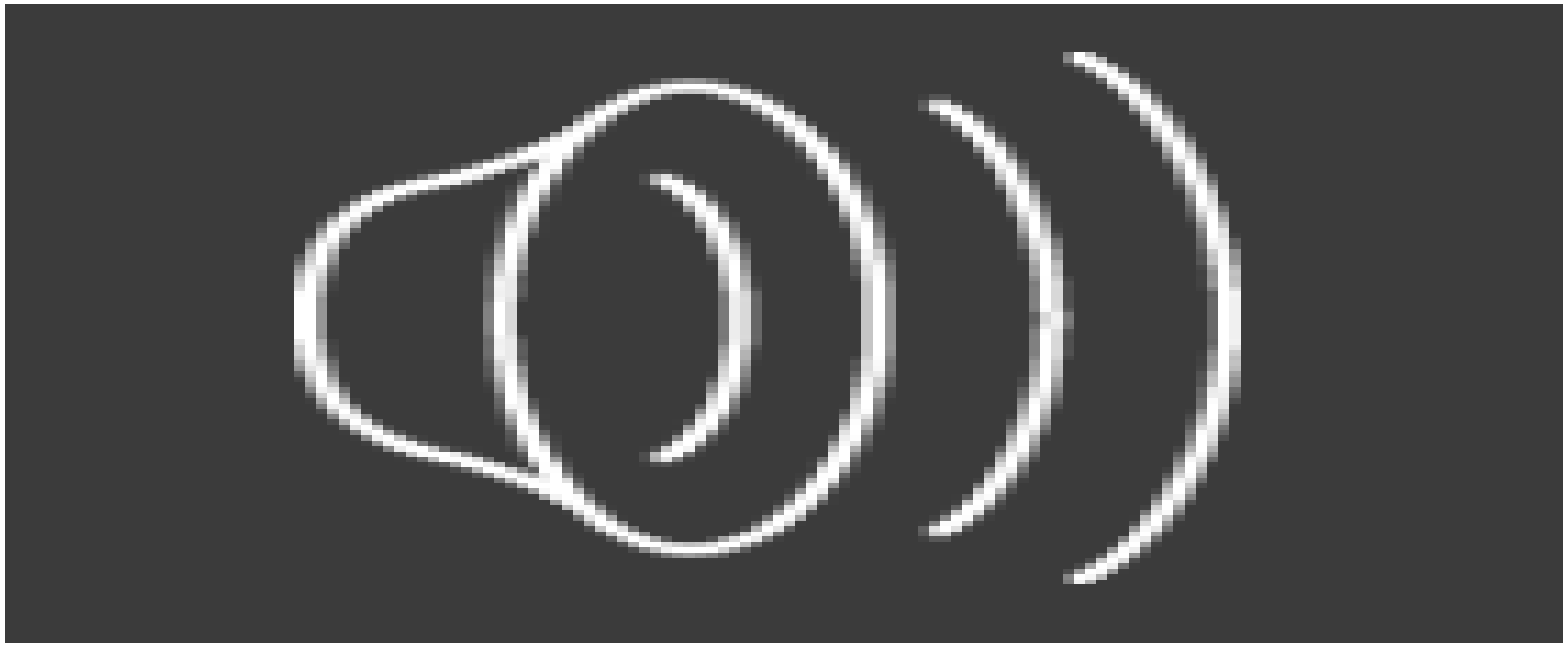


ventral

midsagittal plane

confidential

Gastrulation, Xenopus:
Dense flow field



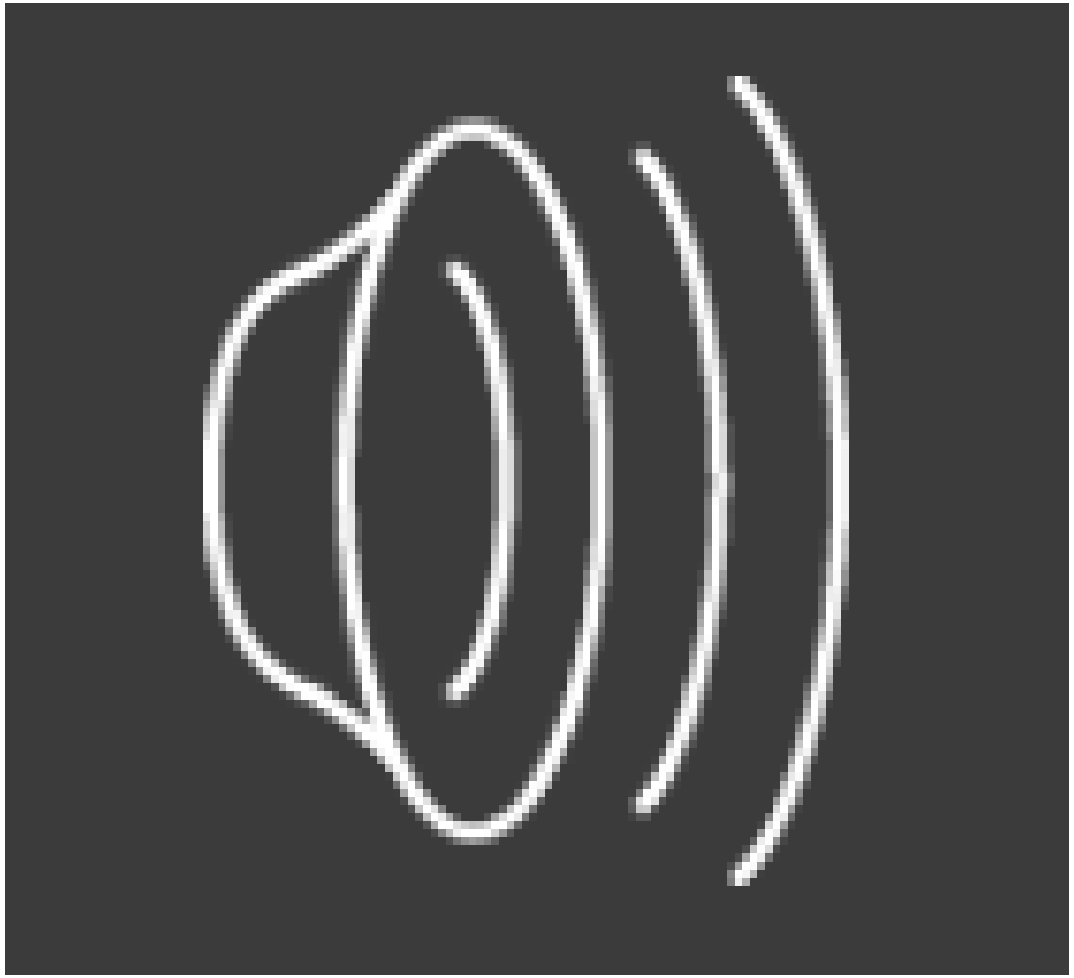
confidential

Gastrulation, Xenopus:
Cell trajectories, archenteron walls

confidential

Gastrulation, Xenopus:

Cell trajectories: bottle, blastopore, invol. mesoderm, endoderm



confidential

Outlook: Digital Embryo with cellular structure

Karlsruher Institut für Technologie

- Test hypotheses on cellular-level genotype-phenotype correlation in morphogenesis of mutants
- Test hypotheses on functional transmutation of cell clusters (e.g. bottle cells)
- Rate of continuous/discrete space-symmetry breaking
- Rates of tissue volume growth
- Forces exerted on single cells (**flow lines vs. trajectories**)
- Cell-shape changes (3D rendering)
- Mechanisms of collective cell motion
- Etc., etc.

Acknowledgement

Xianghui Xiao

Julian Moosmann

Alexey Ershov

Venera Weinhardt

Tilo Baumbach

Carole LaBonne

Maneeshi Prasad

Jubin Kashef

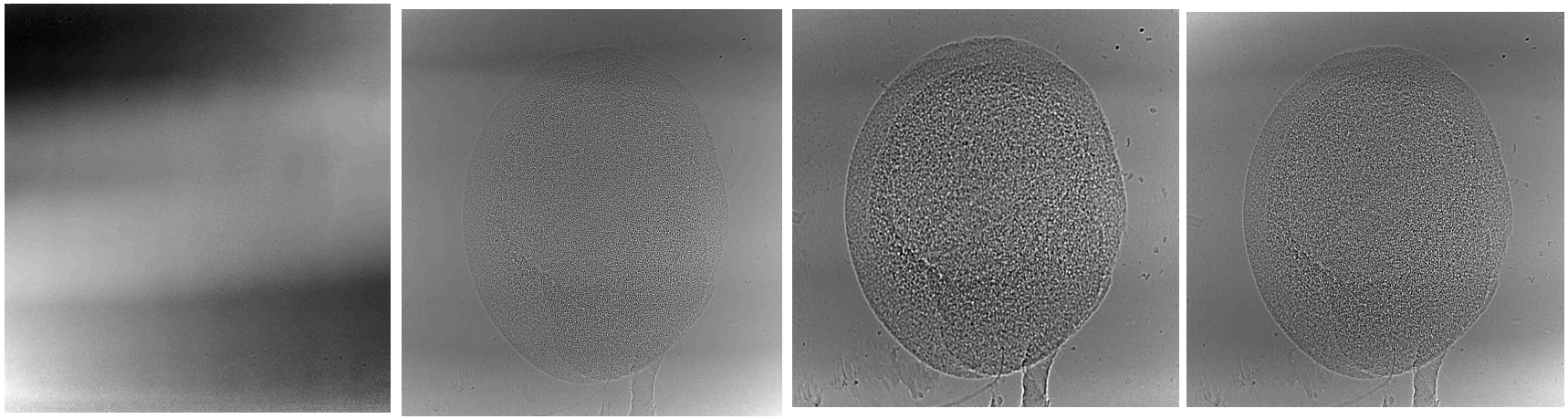
Ray Keller
Rudi Winklbauer
Doris Wedlich
Jochen Wittbrodt
Herbert Steinbeisser
Thomas van der Kamp
Martin Köhl
Francesco de Carlo
Kamel Feeza
AG Kashef

Karlsruher Institut für Technologie

Thank you!

ANKA/IPS imaging/image analysis

Propagation based phase contrast:



z=0.5cm z=18cm z=58cm z=108cm

increasing signal-to-noise ratio with **increasing object-detector distance**

[fixed Xenopus embryo, stage 10.5] TOPO-TOMO@ANKA, E=12 keV, exp. = 180 s]

Motivation cntd.: Digital embryo

- **Fluorescent nuclei by** marker protein expression
- **Tracking of nuclei only**
- **X-ray phase contrast: structural info** (electron density)

Reconstructed nuclei motion from
Digital scanned laser light sheet fluorescence
microscopy in entire wild-type Zebrafish embryos over the first 24 hours of development
(Keller, Schmidt, Wittbrodt, Stelzer, Science 2008)

High-resolution, single-distance, nonlinear and noniterative phase-retrieval algorithms (Bio-Imaging)

- **Acceptable signal-to-noise** at **limited dose** deposition:
  - **large object-detector distances**
  - **single-distance** imaging
  - reduction of required **number of projections** (ART versus FBP)

- **Large throughput with online feedback option (Atlases):**
  - **single-distance** imaging
  - fast reconstruction

  $\Longrightarrow$ **noniterative phase retrieval**

- **High-resolution of phase retrieval:**
  - **simple** linear **algorithms too coarse**
  - highly-resolving **linear algorithms inapplicable**
    (imaging of **entire** embryos, **strong phase objects,** $\Delta\phi \sim 1$)

  $\Longrightarrow$ **nonlinear phase retrieval**

Fresnel theory (perfect coherence, parallel beam, pure phase)

- Re-writing Fresnel diffraction integral [Guigay 1977]:

$$(\mathcal{F}\, I_z)(\vec{\xi}) = \int d^2r\, \exp(-2\pi i \vec{r}\cdot\vec{\xi}) \times \psi_0\left(\vec{r} - \frac{\pi z}{k}\vec{\xi}\right)\psi_0^*\left(\vec{r} + \frac{\pi z}{k}\vec{\xi}\right)$$

- Expansion **of exponential in exit wave-field:**

$$\psi_0 = \sqrt{I_0}\exp(i\phi_0) = \sqrt{I_0}(1 + i\phi_0 - \frac{1}{2}\phi_0^2 + O(\phi_0^3))$$

- Guigay up to quadratic order:

[Moosmann, RH, Baumbach, Opt. Exp., 2011;
RH, Moosmann, Baumbach, Opt. Exp. 2011]

$$(\mathcal{F}\, g_z)(\vec{\xi}) = 2\sin(s)\ (\mathcal{F}\,\phi_0)(\vec{\xi}) - \cos(s)\int d^2\xi'\,(\mathcal{F}\,\phi_0)(\vec{\xi'})(\mathcal{F}\,\phi_0)(\vec{\xi} - \vec{\xi'})$$

linear order

$$+\exp(is)\int d^2\xi'\,\exp(-\frac{4\pi^2 i z\vec{\xi}\cdot\vec{\xi'}}{k})(\mathcal{F}\,\phi_0)(\vec{\xi'})(\mathcal{F}\,\phi_0)(\vec{\xi} - \vec{\xi'}) + O((\mathcal{F}\,\phi_0)^3)$$

$$(s \equiv \pi\vec{\xi}^2 z\lambda)$$

Theory: Quasiparticle approach

$|\mathcal{F} g_z|$

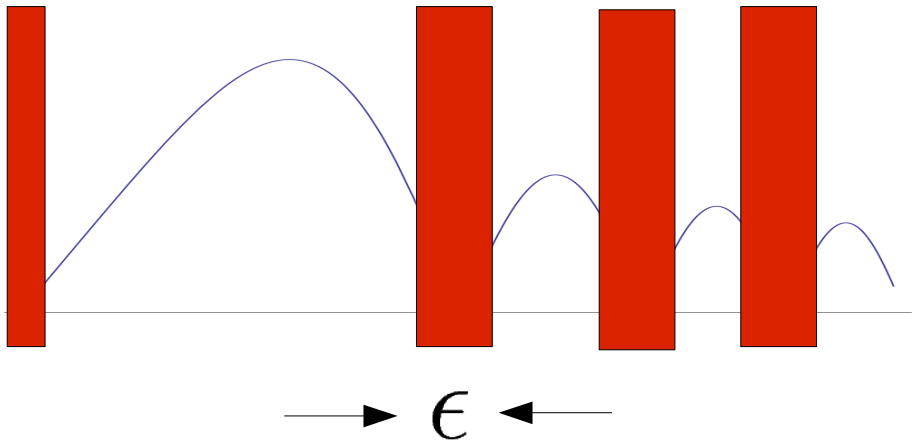
Fourier-transformed intensity
under exit-phase upscaling: $\phi_0 \rightarrow S\,\phi_0 \quad (S > 1)$

Theory: Quasiparticle approach

$$\Theta\left(|\sin(s)| - \epsilon\right) \times (\mathcal{F}\, g_z)(\vec{\xi}) = 2\sin(s)\ (\mathcal{F}\, \phi_0)(\vec{\xi})$$

Quasiparticle dispersion!

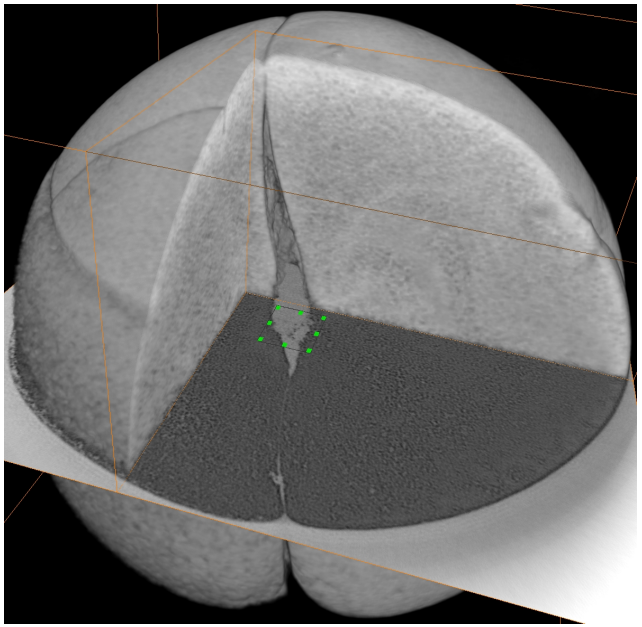
(Fermi liquid: [Landau 1953])



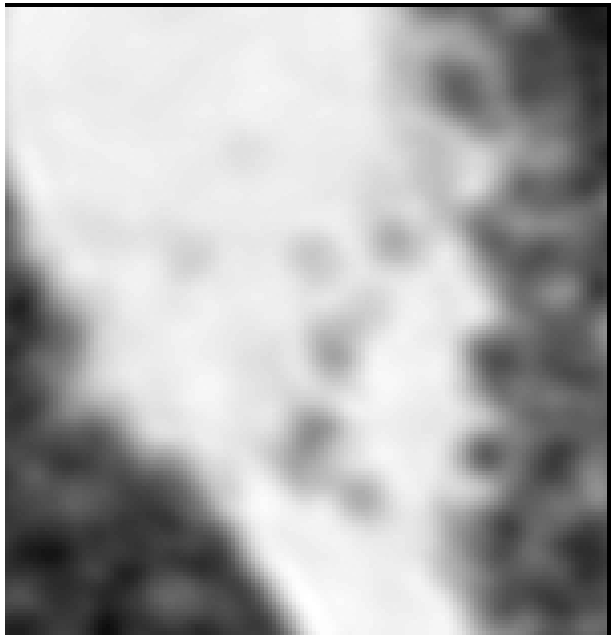
- Results do not **depend on** $\epsilon = 0.1 \cdots 0.001$
- **Generalization to absorption** under **global attenuation-phase duality** [Wu, Liu, and Yan, Opt. Lett. 2005]

[RH, Moosmann, Baumbach 2012]

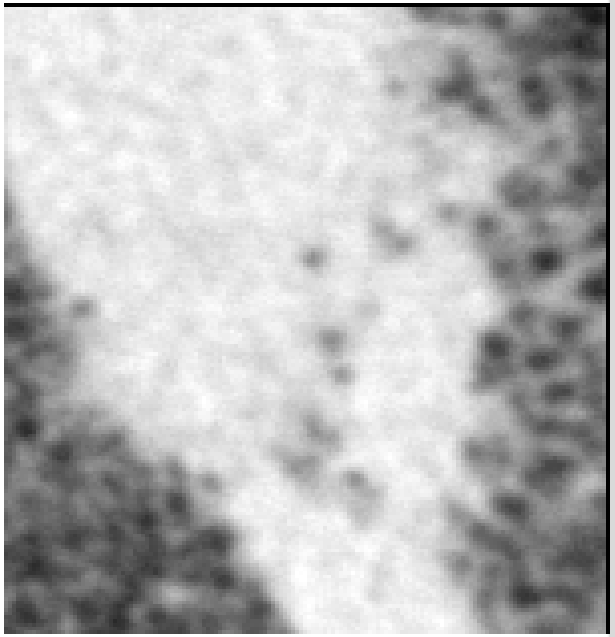
Xenopus laevis: 4-cell stage (fixed), ID19_ESRF



2mm



linearized TIE

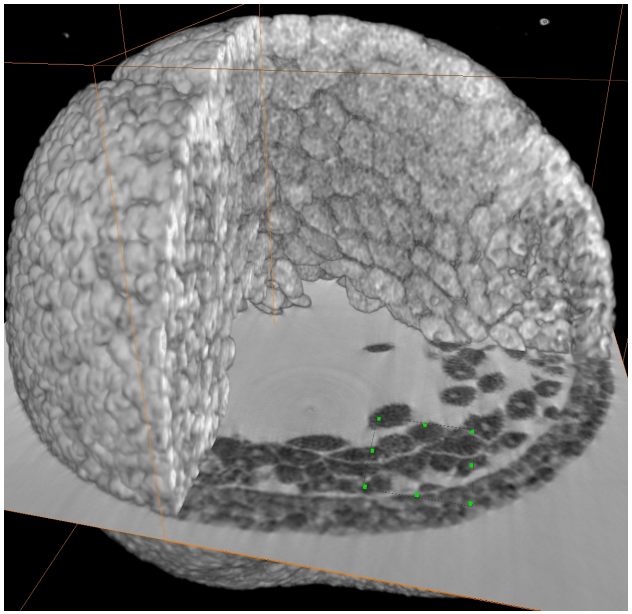


quasiparticle

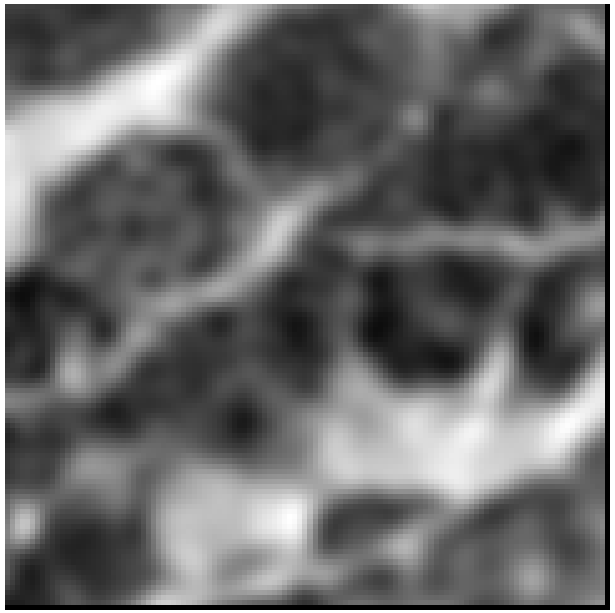
E = 20 keV, *z* = 95 cm, $\frac{\Delta E}{E} = 10^{-4}$, $\Delta x = 0.75\,\mu\mathrm{m}$

1600 projections, 2 s exposure per frame

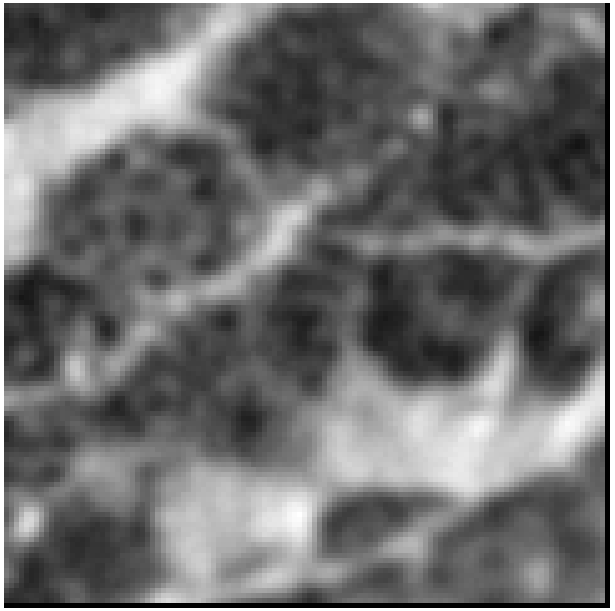
Xenopus laevis: stage 10.5 (fixed), ID19_ESRF



2mm



linearized TIE



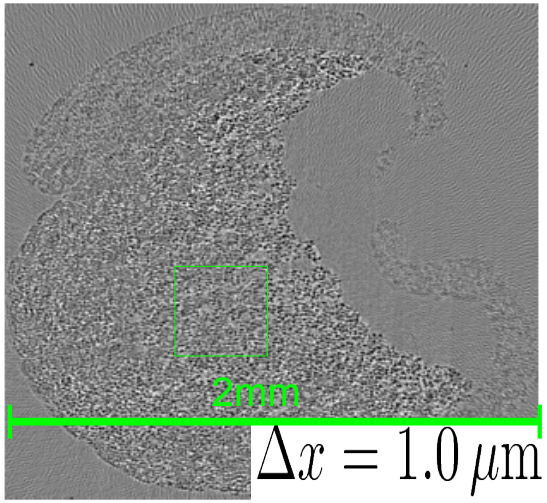
quasiparticle

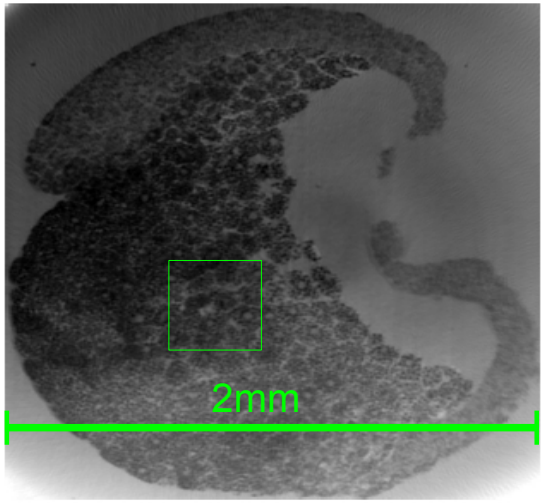
E = 20 keV, *z* = 95 cm, $\frac{\Delta E}{E} = 10^{-4}$, $\Delta x = 1.5\,\mu\mathrm{m}$

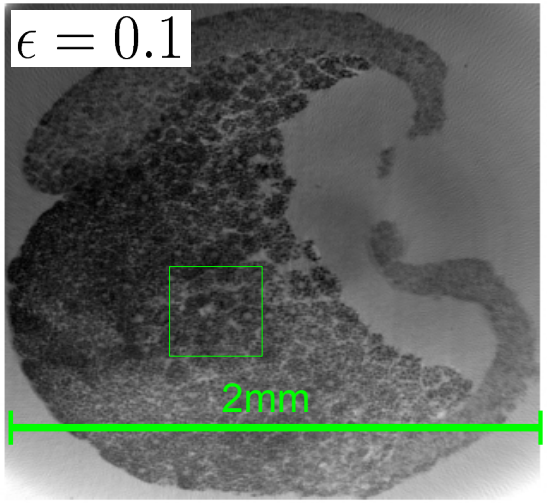
1600 projections, 1 s exposure per frame

Xenopus: stage 10, TOPO/TOMO_ANKA

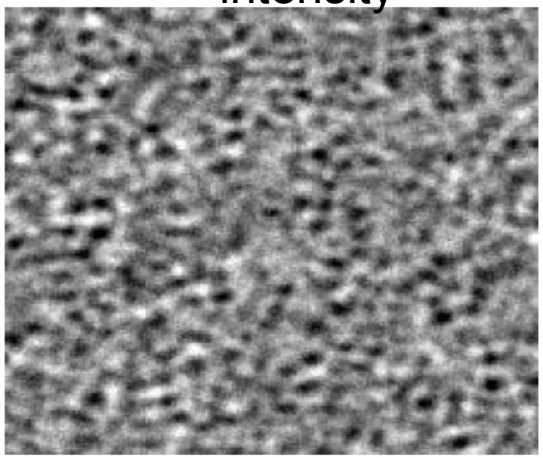
E = 12 keV, *z* = 37.5 cm, $\frac{\Delta E}{E} = 10^{-2}$, slice through 3D reconstructions



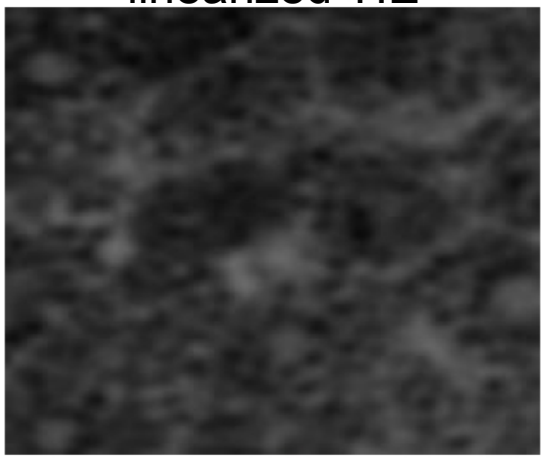




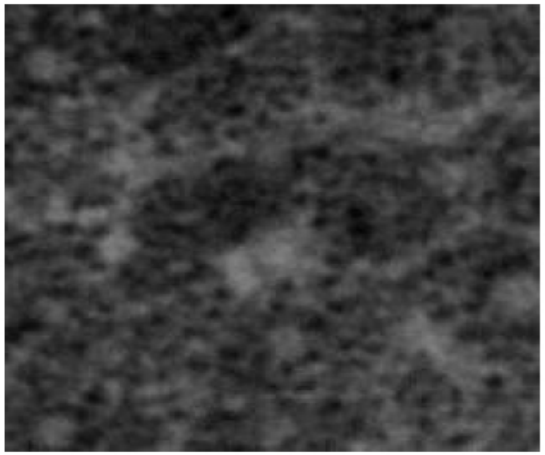
intensity



linearized TIE



quasiparticle



650 projections, 50 s exposure per frame

In-vivo imaging of entire embryos

Karlsruher Institut für Technologie

- **Fresnel theory and reconstruction:**
  - high resolution, single-distance, nonlinear phase retrieval
  - low number of projections (ART vs. FBP)

- **Experimental conditions:**
  - Heat and radical diffusion (dose)
  - Partial coherence/signal-to-noise

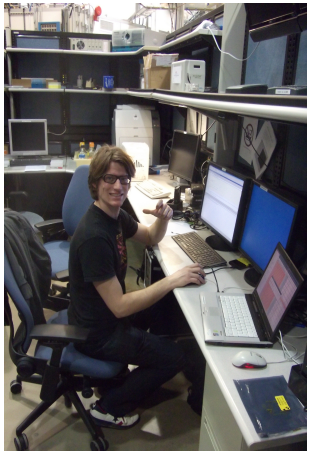
- **Image analysis:**
  - Dense flow-fields
  - Morphogenetic metrics
    (fluid dynamics: flow lines vs. trajectories,
    forces, angles, shapes, symmetry breaking, etc.)

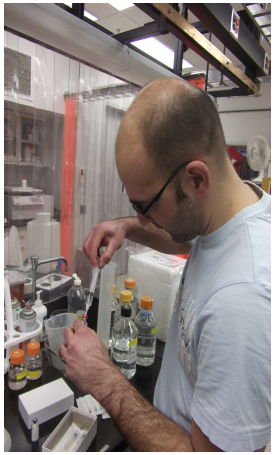
3D in-vivo time lapse imaging, Xenopus Laevis

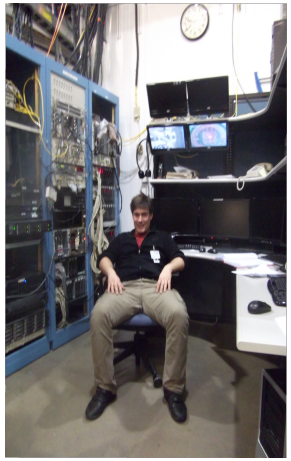
- Experiment at 2BM_APS (Feb 2012, frogs from C. La Bonne, North Western U)

- Parameters varied:
  - E = 22 … 30 keV
  - radical scavengers of multiple concentrations (radiolysis of water!)
  - exposure per projection 15 ms … 100 ms
  - developmental stages: 11 (gastrulation) … 16 (neurulation)

- On-the-fly tomography: 1200 projections (periph. rot. blurring, cell motion)

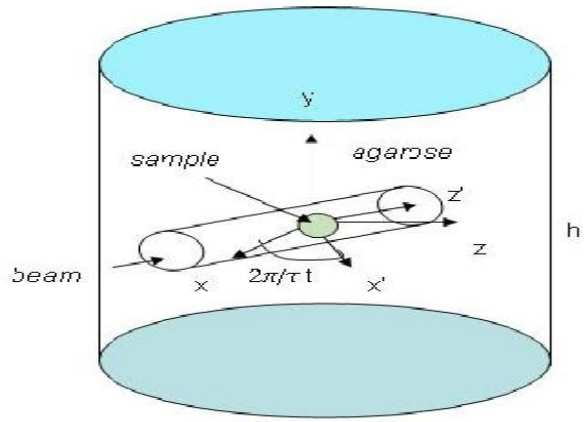




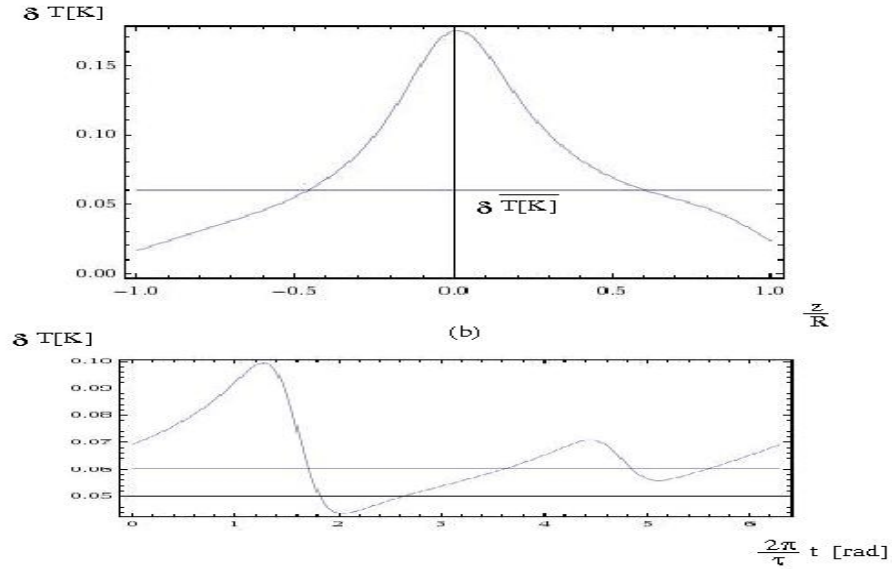


3D in-vivo time lapse imaging, Xenopus: Heat load

$$I_0 = 10^{12}\,\frac{\text{photons}}{\text{mm}^2\text{s}}$$



(a)



(c)

Simulation (heat equation):

Eppendorf with R=0.6cm, E=30 keV, beam profile ,

2-BM photon flux density $10^{12}\text{ph s}^{-1}\text{mm}^{-2}$, $1 \times 1\,\text{mm}^2$

exposure per tomogram = 18 seconds

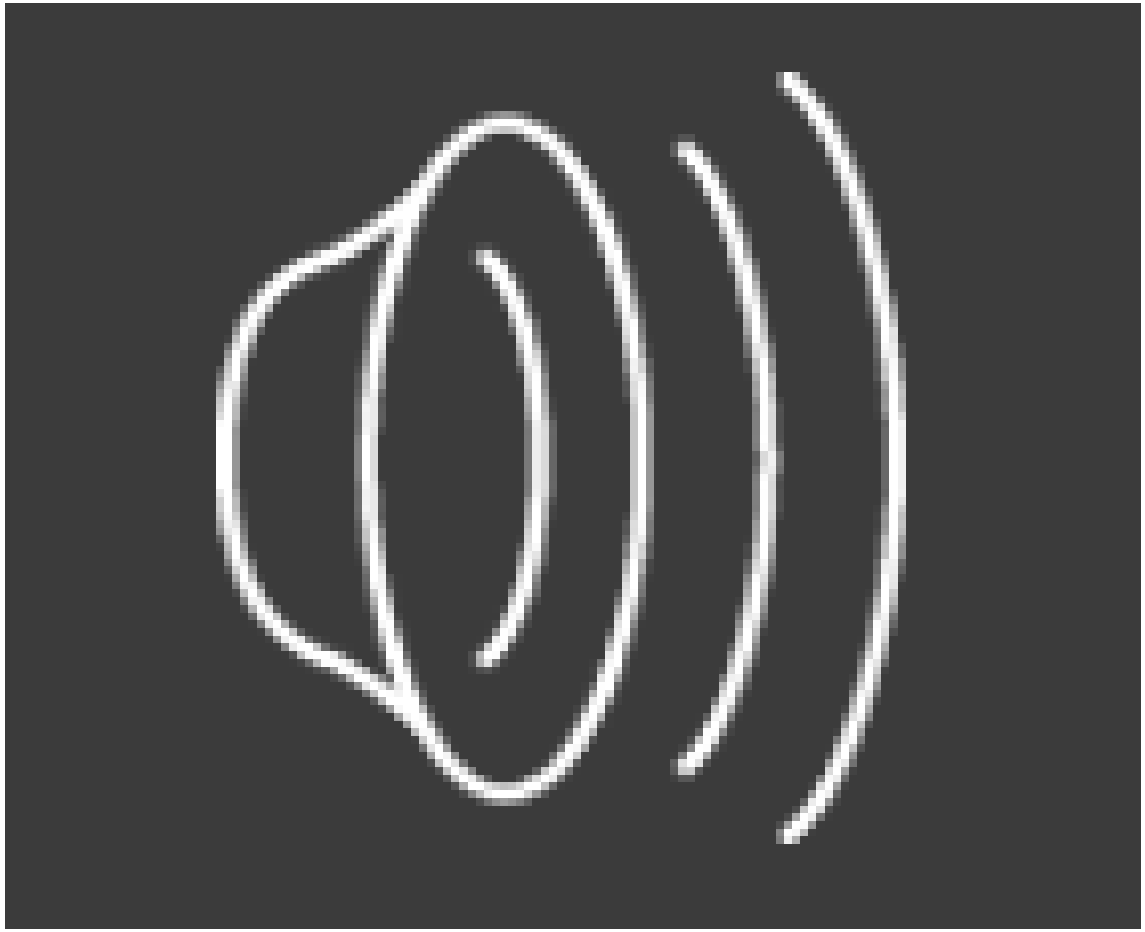
Heat load: No concern in in-vivo imaging of Xenopus embryos!

Gastrulation, Xenopus:

Opening of archenteron I, blastoporus closing, mesodermic involution, mid-sagittal plane

blastoporus

dorsal

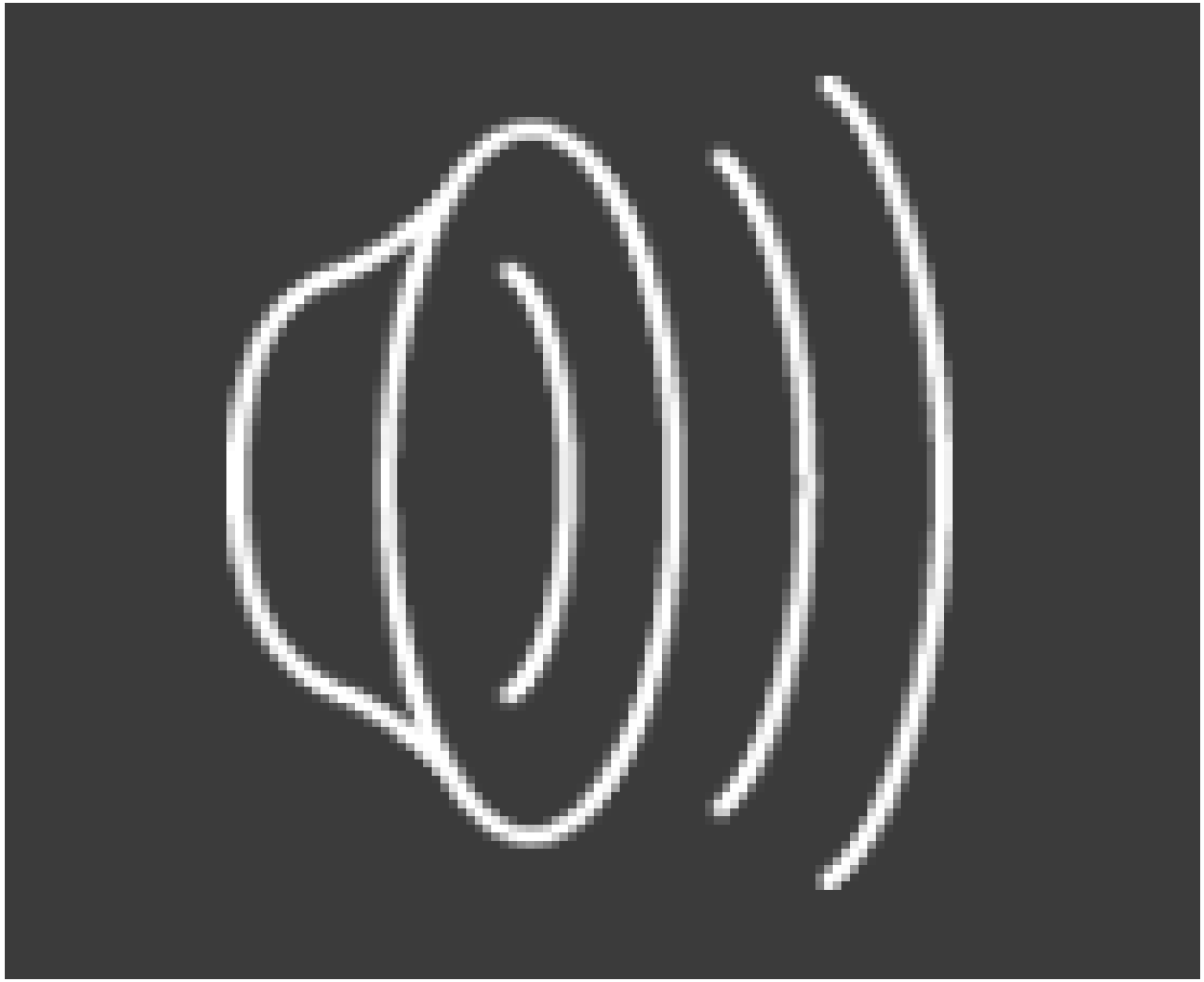


ventral

midsagittal plane

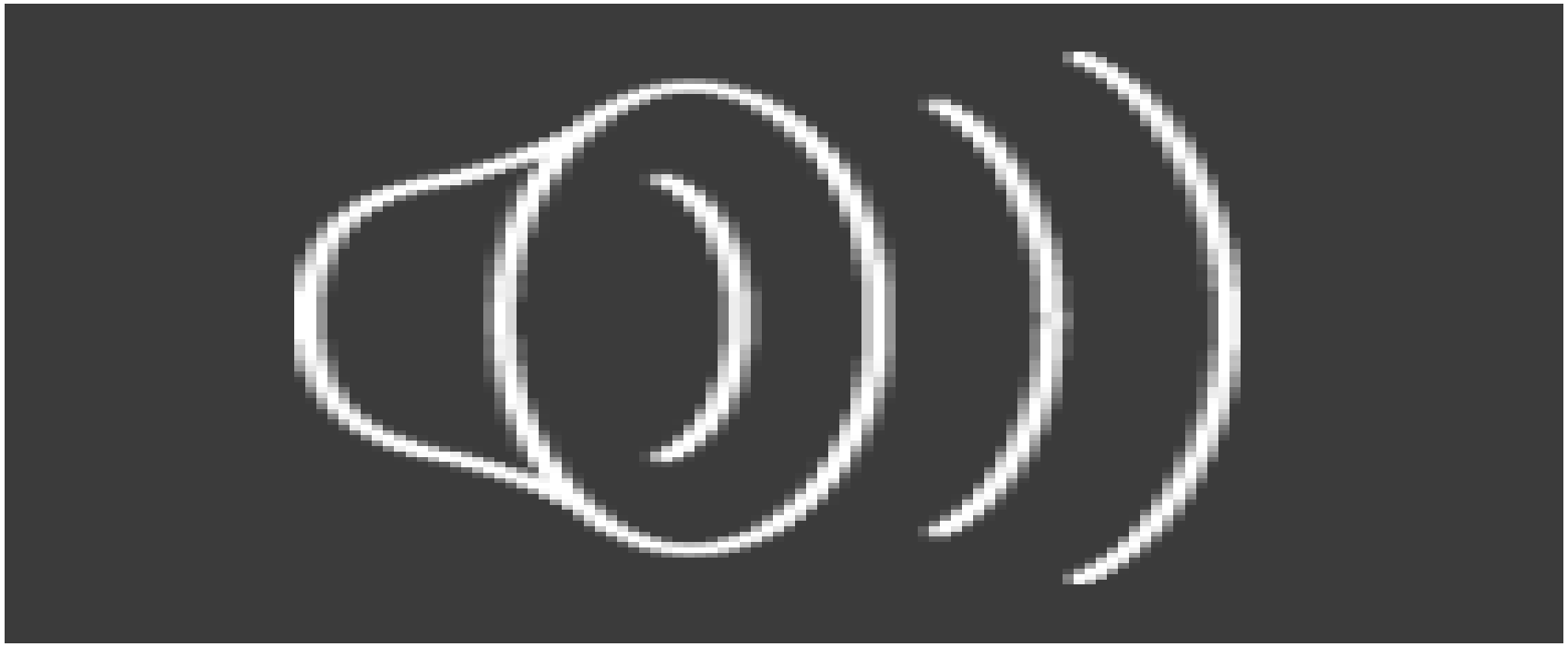
confidential

Gastrulation, Xenopus:
Archenteron inflation



confidential

Gastrulation, Xenopus:
Dense flow field



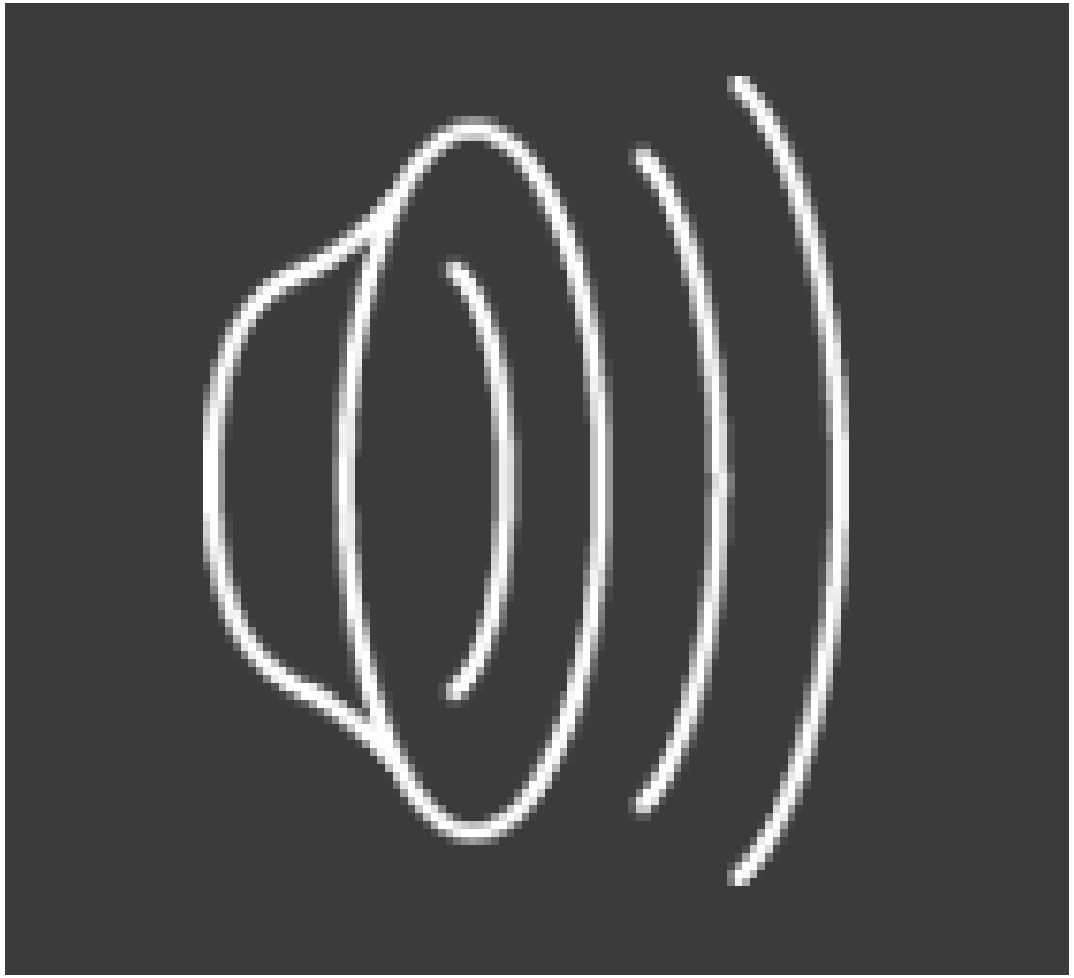
confidential

Gastrulation, Xenopus:
Cell trajectories, archenteron walls

confidential

Gastrulation, Xenopus:

Cell trajectories: bottle, blastopore, invol. mesoderm, endoderm



confidential

Summary:

Outlook: Digital Embryo with cellular structure

Karlsruher Institut für Technologie

- Test hypotheses on cellular-level genotype-phenotype correlation in morphogenesis of mutants
- Test hypotheses on functional transmutation of cell clusters (e.g. bottle cells)
- Rate of continuous/discrete space-symmetry breaking
- Rates of tissue volume growth
- Forces exerted on single cells (**flow lines vs. trajectories**)
- Cell-shape changes (3D rendering)
- Mechanisms of collective cell motion
- Etc., etc.

Karlsruher Institut für Technologie

Thank you!