Imaging the Developing Embryonic Heart: Combining Fast Confocal Microscopes with 4D Reconstruction Techniques

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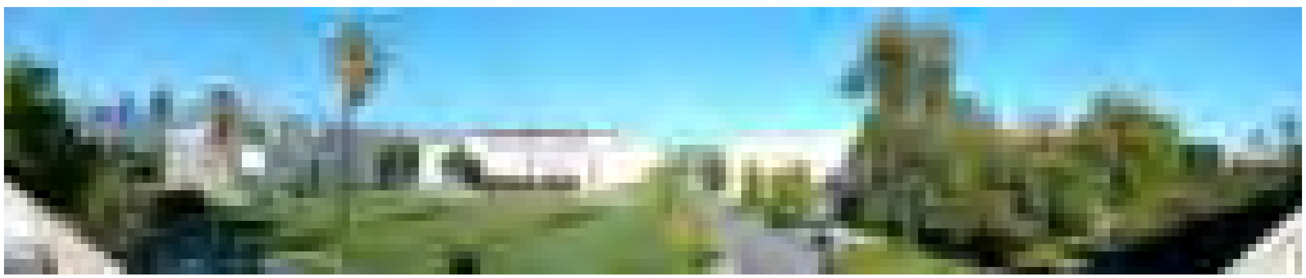
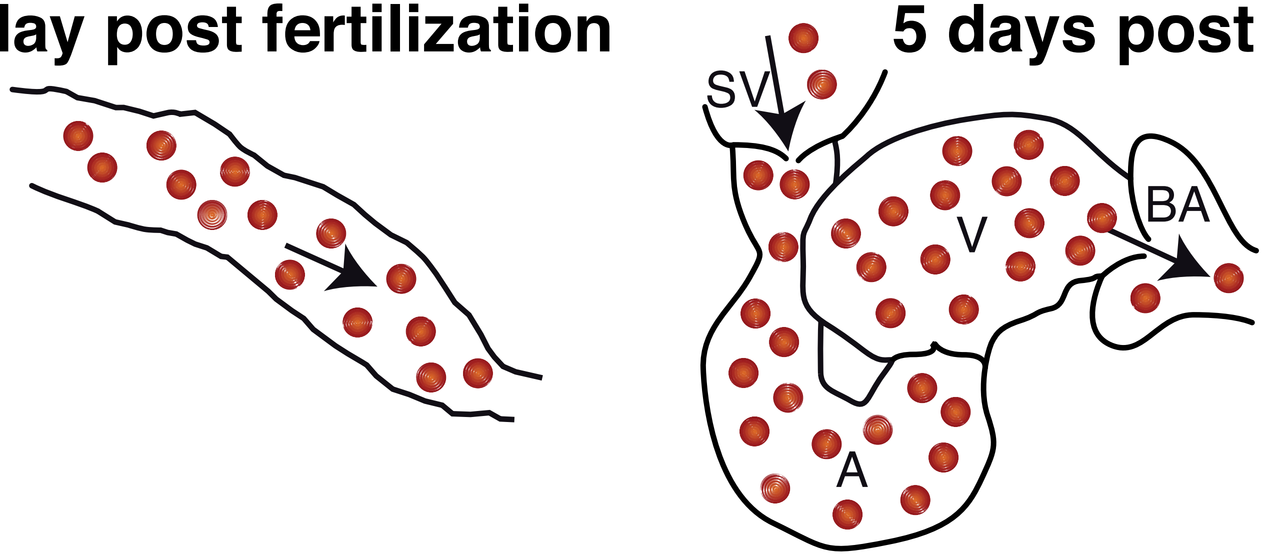
`http://www.its.caltech.edu/~liebling/`

FNSNF
Fonds national suisse
Schweizerischer Nationalfonds
Fondo nazionale svizzero
Swiss National Science Foundation



Studying the Developing Zebrafish Heart

1 day post fertilization **5 days post fertilization**



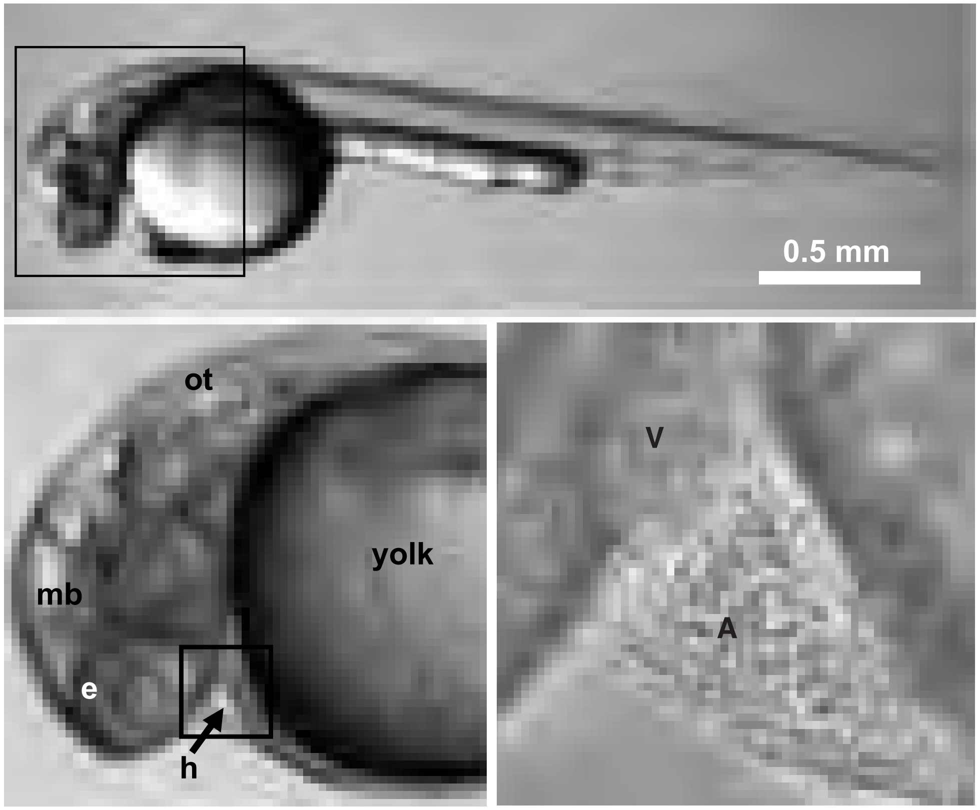
Goals

- Determine genetic & epigenetic factors contributing to heart development
- Dynamic 3D imaging in vivo, over various developmental stages
- Measure volume changes, blood flow, shear stress, etc.

Challenge

Even state-of-the-art laser scanning confocal microscopes are not fast enough for high-speed, real-time 3D image acquisition

Imaging in the Zebrafish Embryo

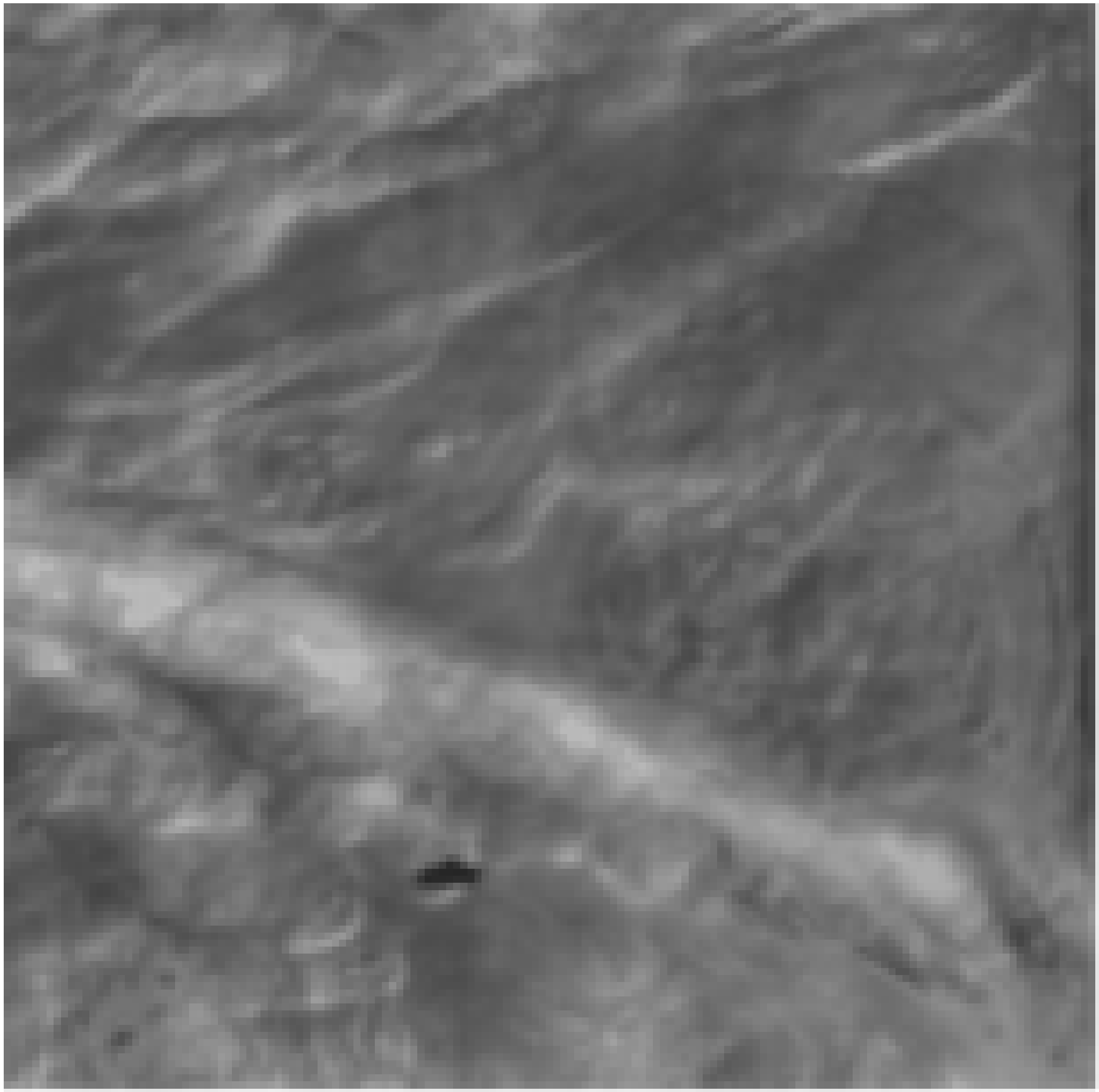


48 hpf (hours post fertilization)
mb: midbrain
ot: otocyst
e: eye
h: heart
yolk: yolk mass

Advantages:

- zebrafish are vertebrates
- reproduce externally and rapidly
- relatively transparent
- may be genetically engineered to express fluorescent markers in specific tissues [e.g. Tg(*gata1*::GFP)]

Fluid forces contribute to heart formation



Acquisition:

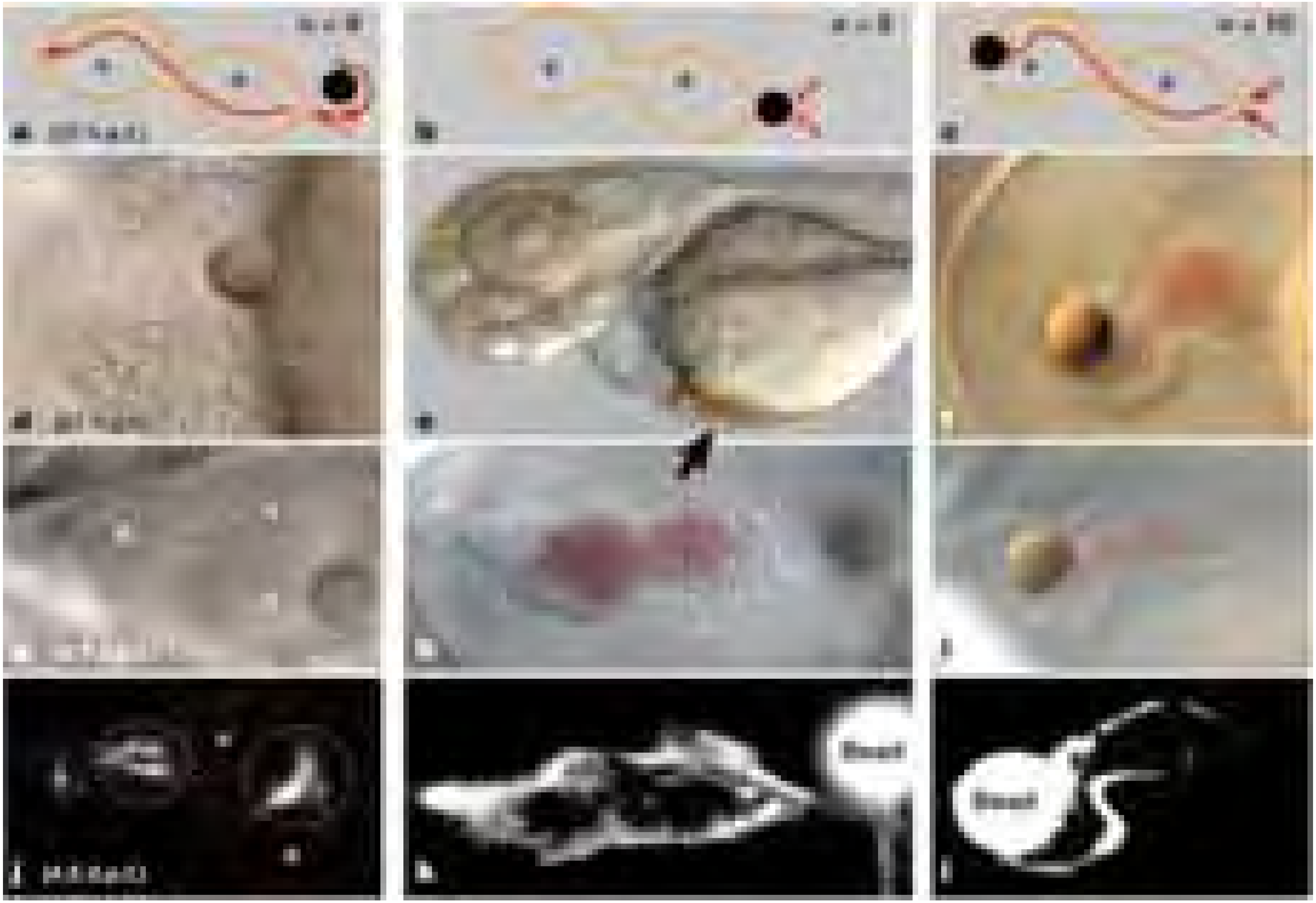
Widefield, 440 frames/s

Analysis:

Digital Particle Image Velocimetry

J.R. Hove, R.W. Köster, A.S. Forouhar, G. Acevedo-Bolton, S.E. Fraser, and M. Gharib,
"Intracardiac fluid forces are an essential epigenetic factor for embryonic cardiogenesis,"
Nature, 421, 172–177 (2003).

Fluid forces contribute to heart formation



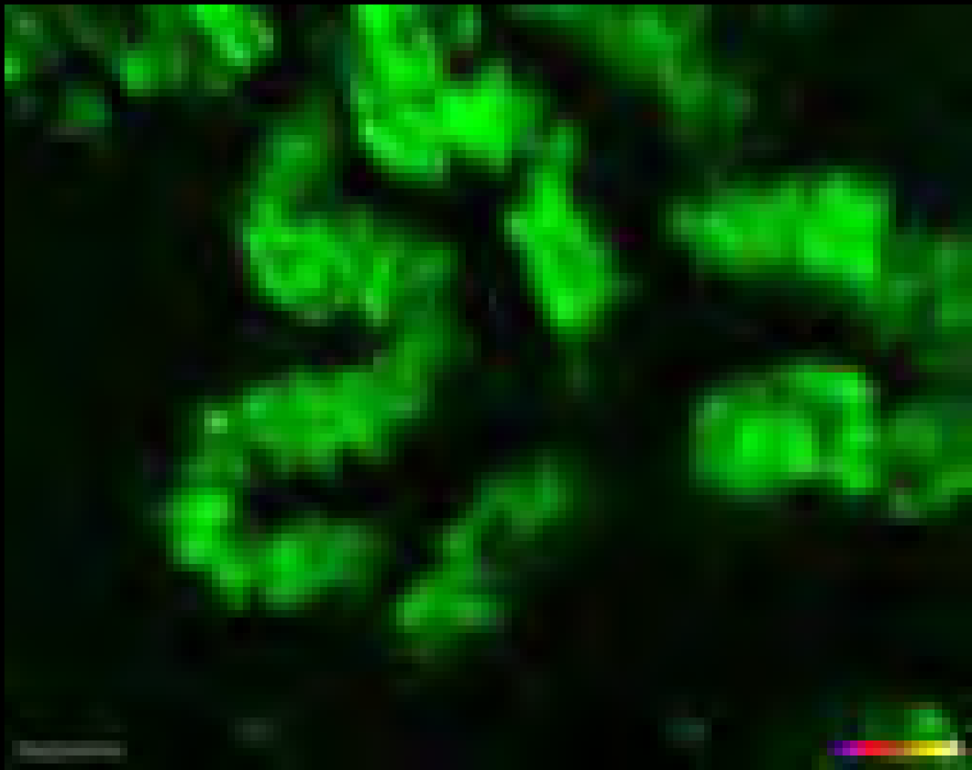
J.R. Hove, R.W. Köster, A.S. Forouhar, G. Acevedo-Bolton, S.E. Fraser, and M. Gharib, “Intracardiac fluid forces are an essential epigenetic factor for embryonic cardiogenesis,” Nature, 421, 172–177 (2003).

Speed of some Biological Processes

Process	Speed / Frequency
Microtubule growth	300-500nm/s
Cell migration (EC over fibroblasts)	20-50 μm/h
Cellular trafficking (protein transp. by vesicle, . . .)	20-80 μm/min
Chromosome dynamics	$>$ 0.5 μm/10 sec
Calcium signaling (sparks)	1 nm/s to 50 μm/sec
Blood flow	mm/s
Heart beat	1-10 Hz
Beating ciliated cells	3-40 Hz

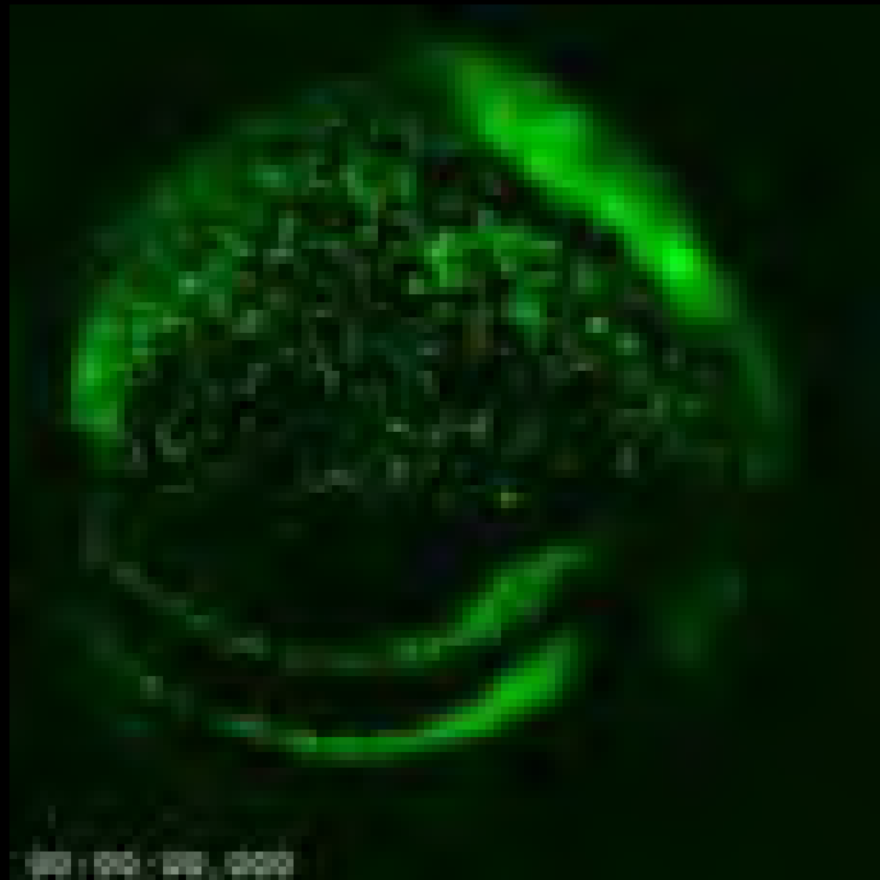
Moving at the Speed of Cells

Migrating germ cells @ 6 frames/h



C. Readhead, M. Liebling
18.5 dpc Tg(*oct4*::GFP) mouse.
Grid: 50μm, 6 frames/h, over 6 hours

Blood circulation @ 12 frames/h



Liz Jones, Mary Dickinson
8.5dpc Tg(ϵ-globin::GFP) mouse, 1 frame/5 minutes
[Jones *et al.* (2002) Genesis 34, 228–235]

	Cell migration/division	**Heart motion, blood flow**
Speed	10 μm/s	mm/s
Required frame rate	1–10 frames/s	100–200 frames/s

Insufficient frame-rates introduce artifacts

Blood flow visualization

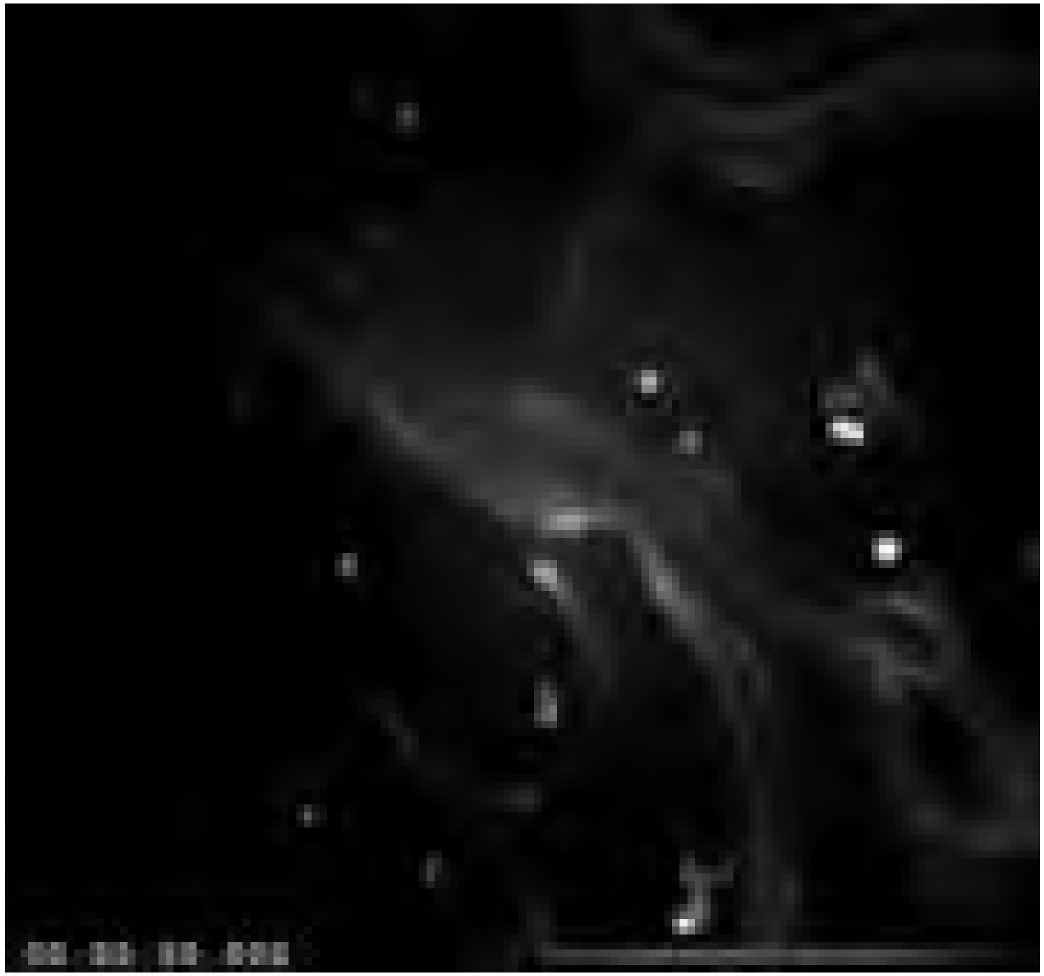
@ 2 frames/s (confocal)

Liz Jones, Mary Dickinson

Tg(ϵ-globin:GFP) mouse, Zeiss LSM 5 PASCAL

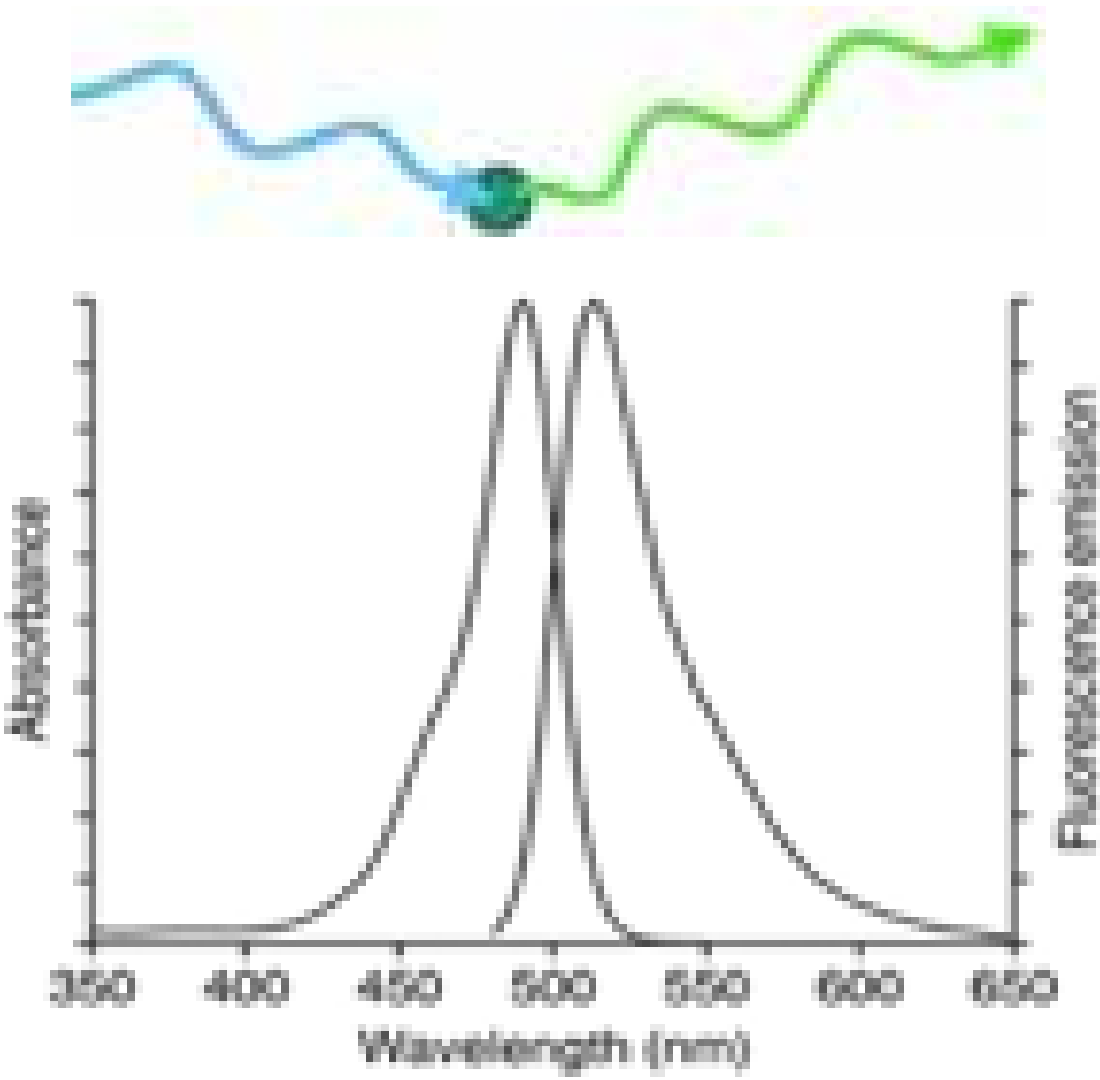
Transmitted light / 488nm ex/ LP 505 em, 2 frames/s

@ 30 frames/s (video)

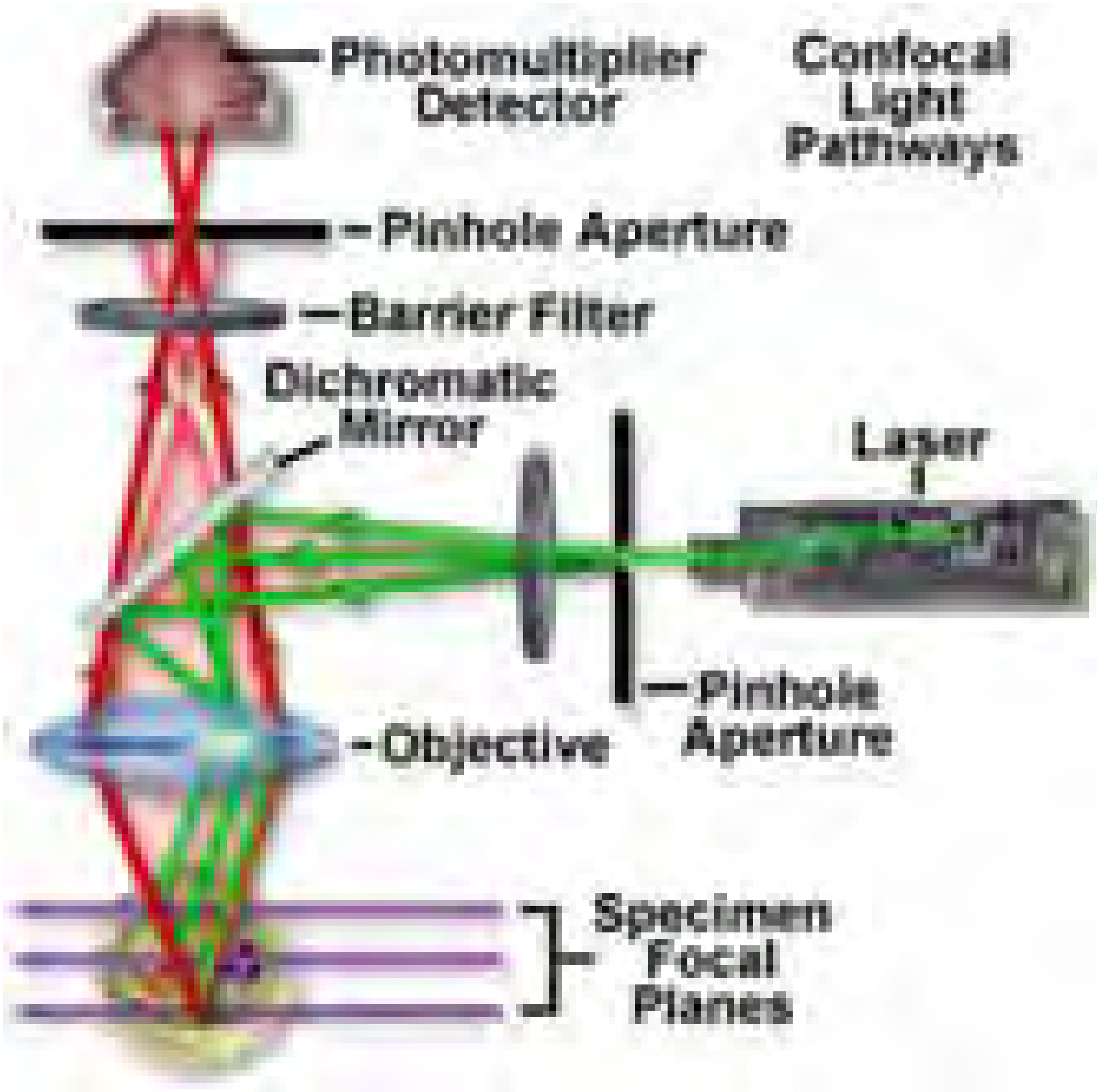


Liz Jones, Mary Dickinson

Fluorescence: Reminder

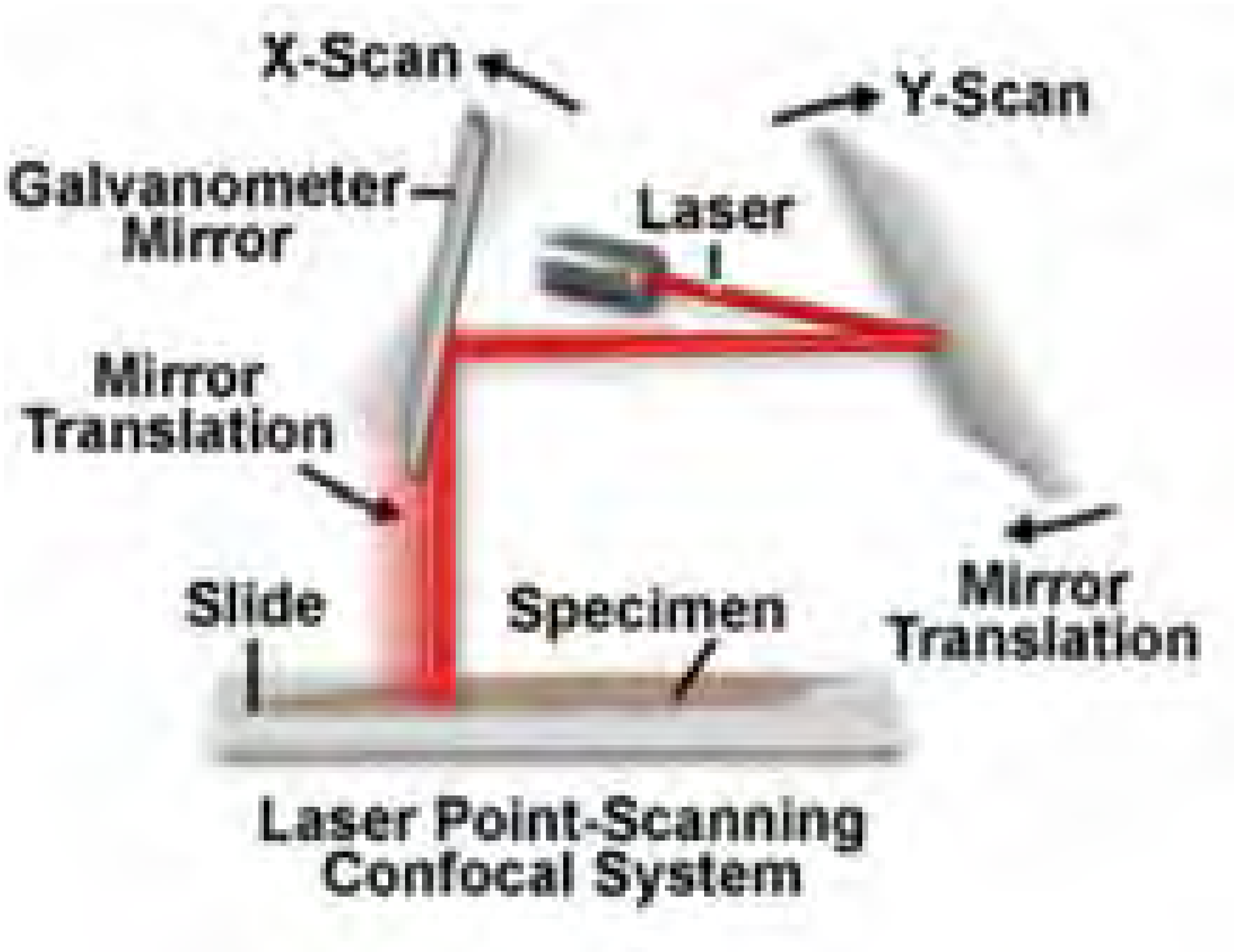


Confocal Microscopy Principle: Reminder



From: `http://www.olympusfluoview.com/theory/index.html`

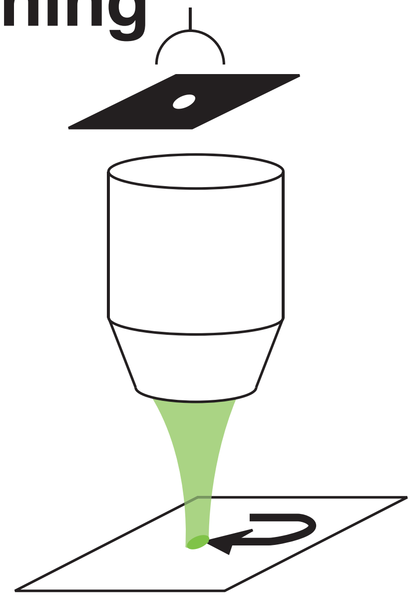
Point Scanning



Adapted from: `http://www.olympusfluoview.com/theory/confocalscanningsystems.html`

Making a Fast Scanning Confocal Microscope

Point Scanning

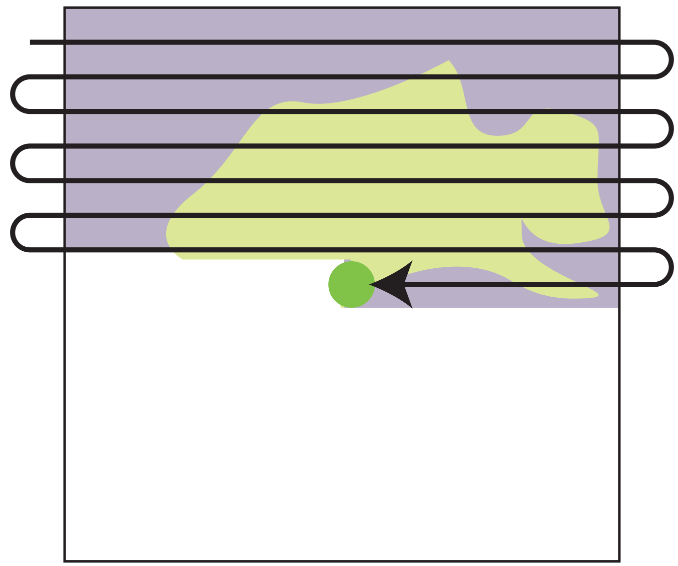


Point detector

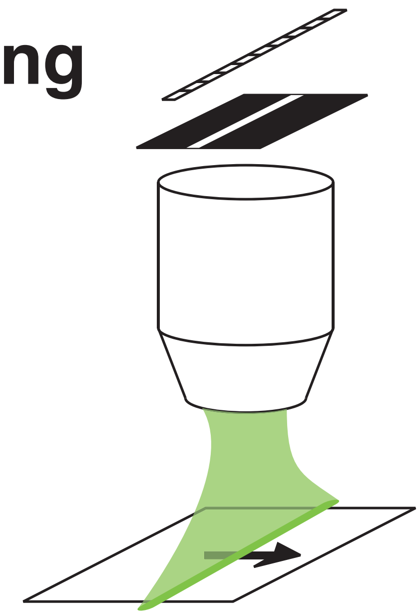
Confocal
Pinhole

Microscope
Objective

Focused
Point



Slit Scanning

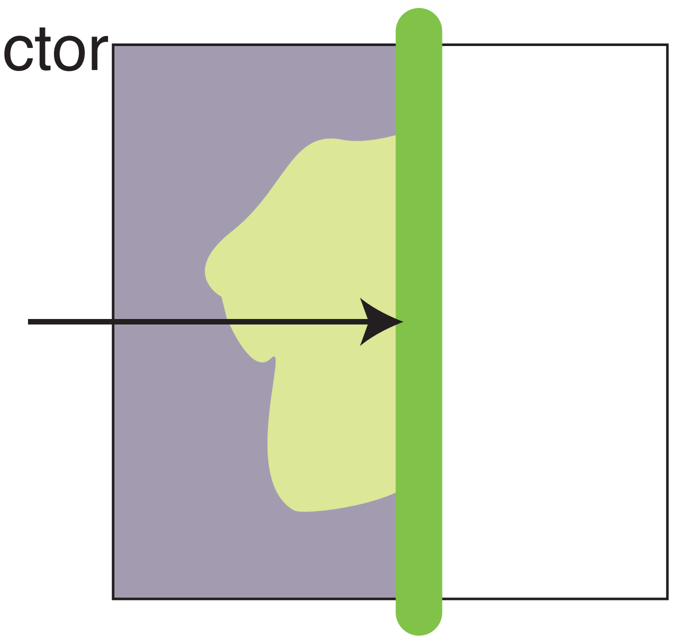


Line array detector

Confocal Slit

Microscope
Objective

Focused
Line



Zeiss LSM 5 LIVE

Parallel Acquisition for Increased Speed

Zeiss LSM 5 *LIVE*

Fast confocal microscopy

Parallel illumination/detection

120 frames per s at 512×512

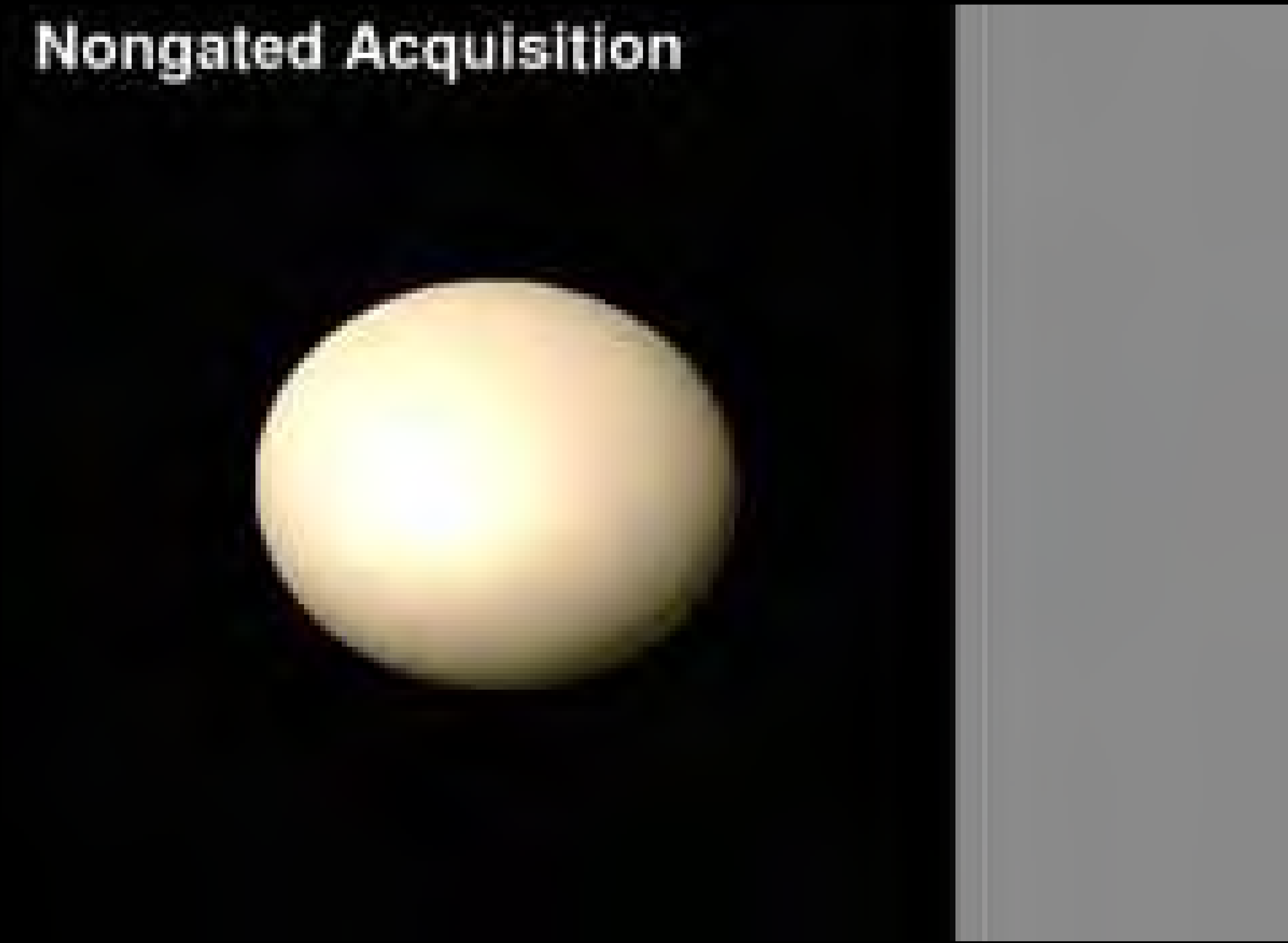
2D slice of zebrafish heart (60 fps)

M. Liebling, J. Vermot

[38 hpf wildtype zebrafish, BODIPY FL C_5-ceramide]

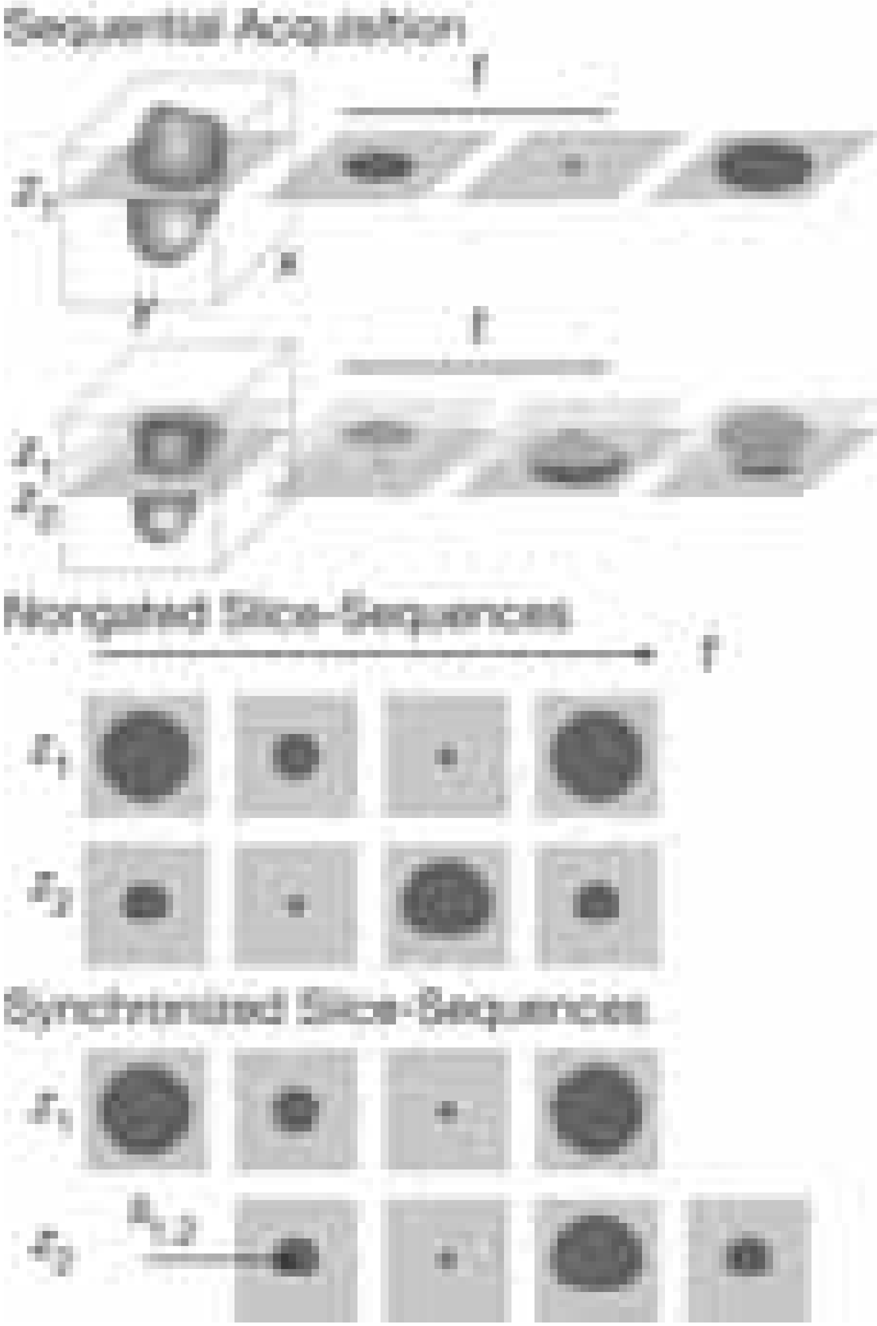
4D Imaging via Gated Image Acquisition

Nongated Imaging Requires Synchronization



4D Imaging via Post-Acquisition Synchronization

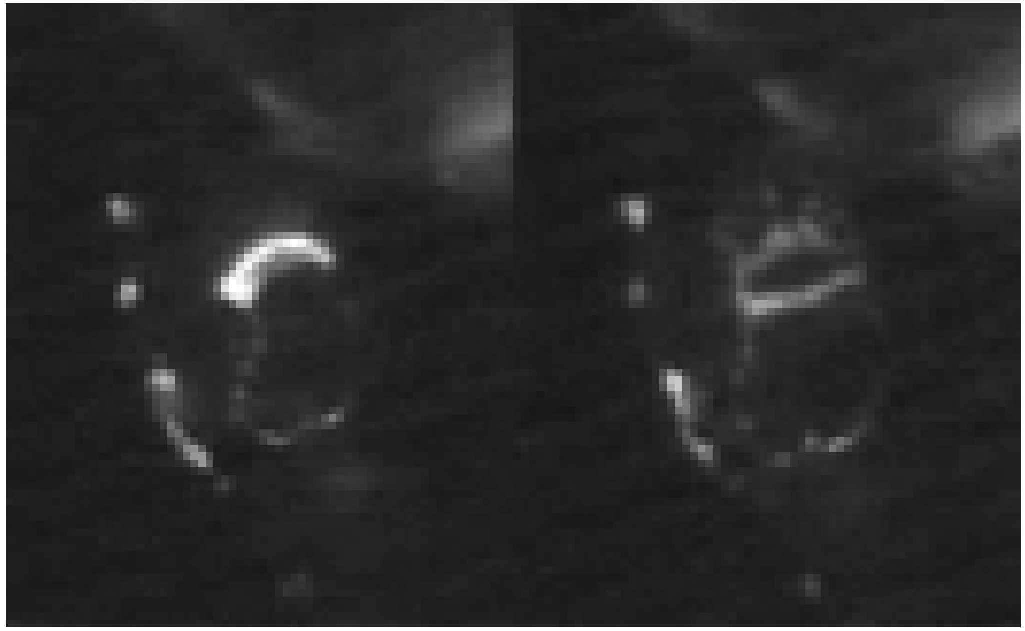
Method



Adjacent 2D slices of zebrafish heart

Before synchronization (raw data)

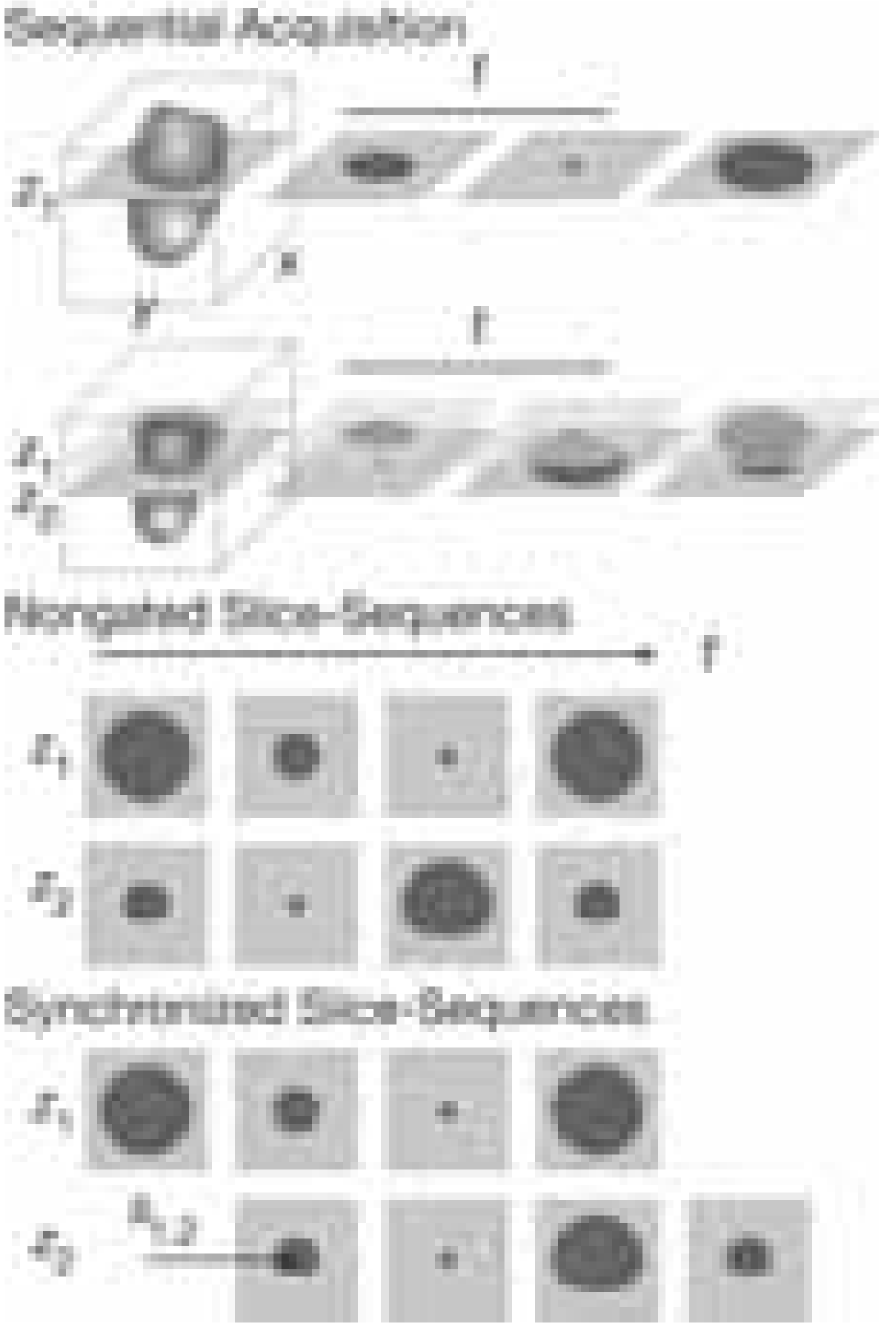
z_1 z_2



Mary Dickinson, Arian Forouhar

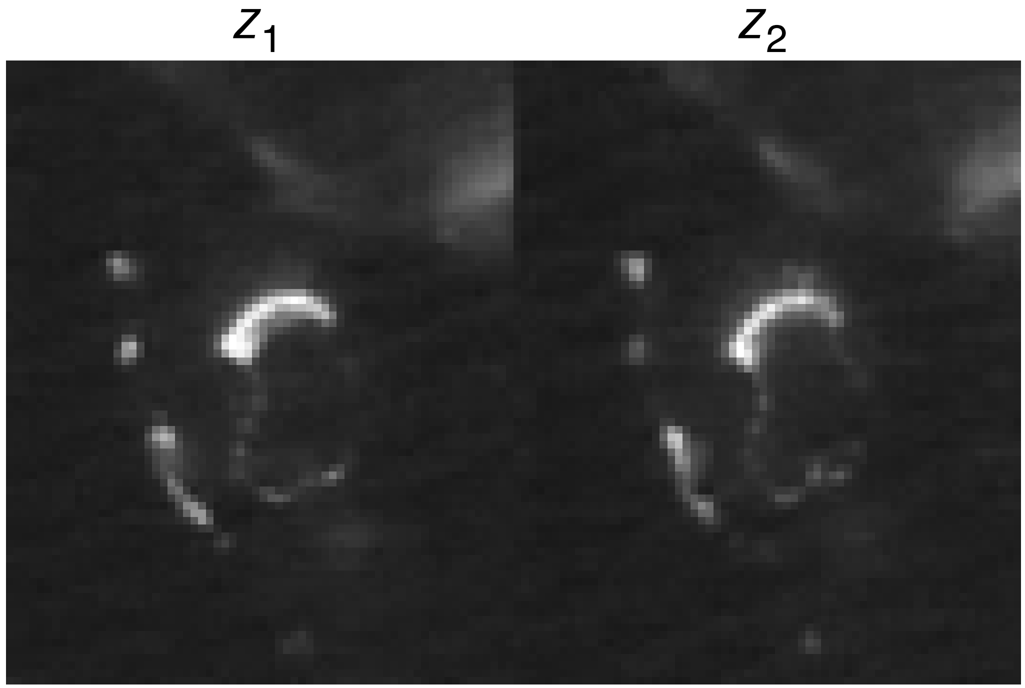
4D Imaging via Post-Acquisition Synchronization

Method



Adjacent 2D slices of zebrafish heart

After synchronization



Mary Dickinson, Arian Forouhar

Manual 4D Reconstruction

Automatic Registration Challenges

Limitations of manual registration

Tedious, non-reproducible, error-prone.

Challenges

Large data size, limited time, fluorescence imaging artifacts (bleaching, photon/detector noise, etc.).

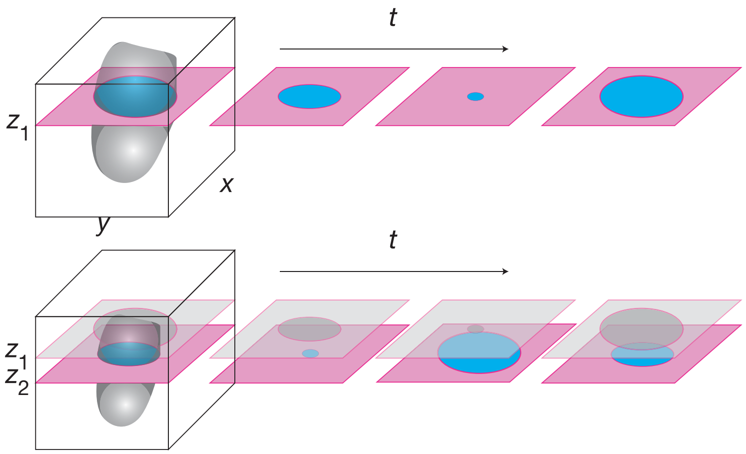
Requirements

No a priori information (electrocardiogram, etc.), robust (accurate and reproducible)

X	Y	Z	Time	Channels	Depth	Memory
512	512	128	512	4	16 bits	128GB
512	512	128	512	2	8 bits	32GB
256	256	64	256	1	8 bits	1 GB
256	256	64	128	1	8 bits	512 MB

4D Confocal Imaging of the Beating Heart

Sequential z-acquisition:



Measurement model:

$$I_m(\mathbf{x}, z_k, t) = \iiint I(\mathbf{x}', z, t - s_k)$$
$$\mathrm{PSF}(\mathbf{x} - \mathbf{x}', z - z_k)\, \mathrm{d}\mathbf{x}'\mathrm{d}z$$

for $k = 1, \dots, N_z$. Unknowns shifts s_k.

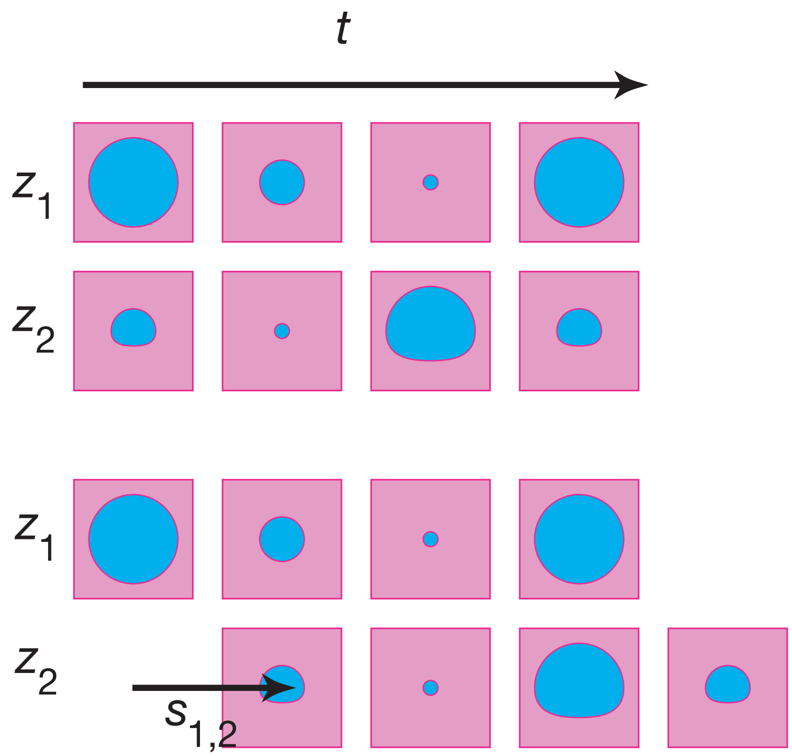
Assumptions:

- Periodicity: $|I(\mathbf{x}, z, t) - I(\mathbf{x}, z, t + T)| \ll I_{\max}$
- Same period T at all depths
- Acquisitions overlap: $\mathrm{supp}_z(\mathrm{PSF}) > \Delta z$

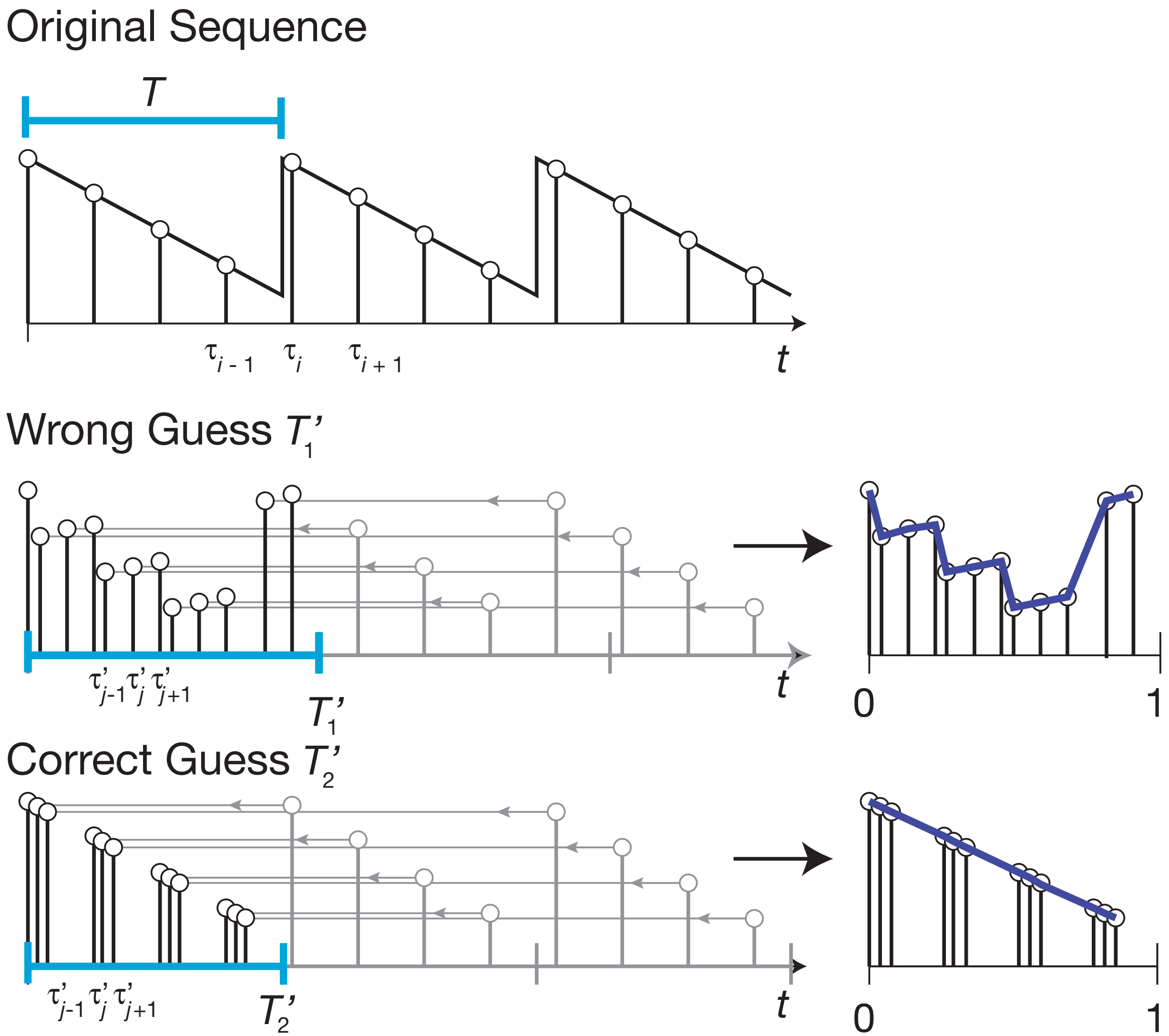
Synchronization (time-registration):

Error metric

$$|I_m(\mathbf{x}, z_1, t) - I_m(\mathbf{x}, z_2, t - s_{1,2})|^2$$



. . . with some help from astronomy



R. F. Stellingwerf, "*Period determination using phase dispersion minimization*," Astrophysical Journal, 224(3), 953–960 (1978)

M. M. Dworetsky, "*A period-finding method for sparse randomly spaced observations or how long is a piece of string*," Monthly Notices of the Royal Astronomical Society, 203(3), 917–924 (1983).

Intensity-based Registration—Periodic Case

Mean square intensity difference

$$Q_{k,k'}(s) = \iint_{\mathbf{R}^2} \int_0^L |I_m(\mathbf{x}, z_k, t) - I_m(\mathbf{x}, z_{k'}, t-s)|^2 \, \mathrm{d}t \, \mathrm{d}\mathbf{x} \text{ where } s \in \mathbf{R}$$

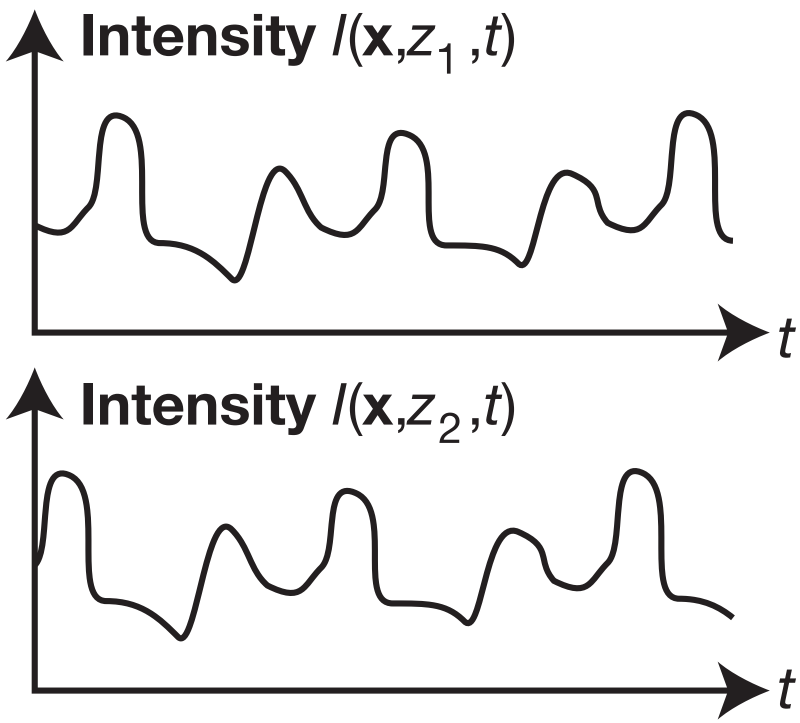
Periodicity implies:

$$\begin{aligned} Q_{k,k'}(s) &= \iint_{\mathbf{R}^2} \int_0^L |I_m(\mathbf{x}, z_k, t)|^2 + |I_m(\mathbf{x}, z_{k'}, t-s)|^2 \, \mathrm{d}t \, \mathrm{d}\mathbf{x} \\ &\quad - 2 \iint_{\mathbf{R}^2} \int_0^L I_m(\mathbf{x}, z_k, t) I_m(\mathbf{x}, z_{k'}, t-s) \, \mathrm{d}t \, \mathrm{d}\mathbf{x} \\ &= C - 2 \iint_{\mathbf{R}^2} \underbrace{\int_0^L I_m(\mathbf{x}, z_k, t) I_m(\mathbf{x}, z_{k'}, t-s) \, \mathrm{d}t}_{\text{Correlation}} \, \mathrm{d}\mathbf{x}. \end{aligned}$$

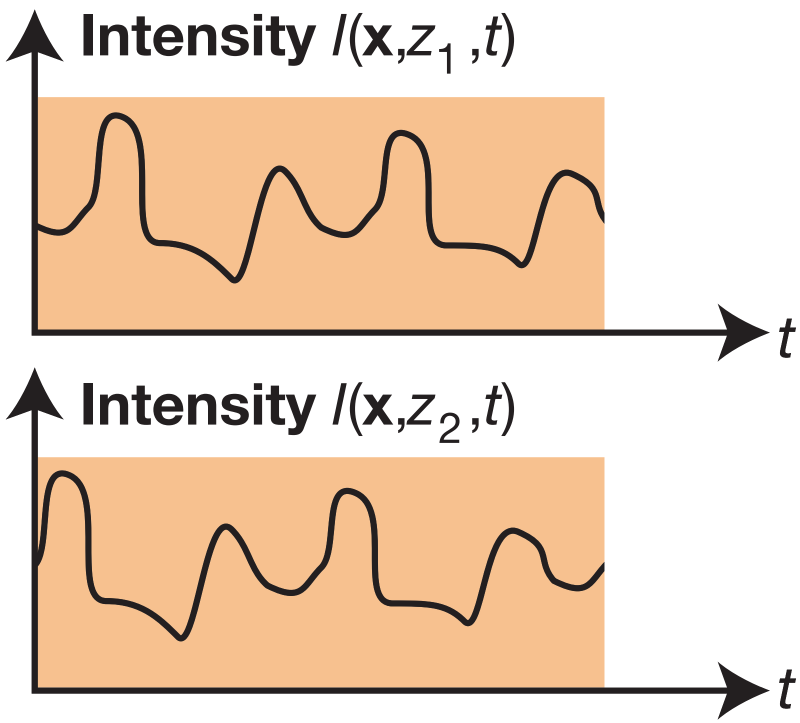
Least-squares error minimization (≡ correlation maximization)

$$s_{k,k'} = \min_{s=kT, k=1,\ldots,N_t} Q_{k,k'}(s).$$

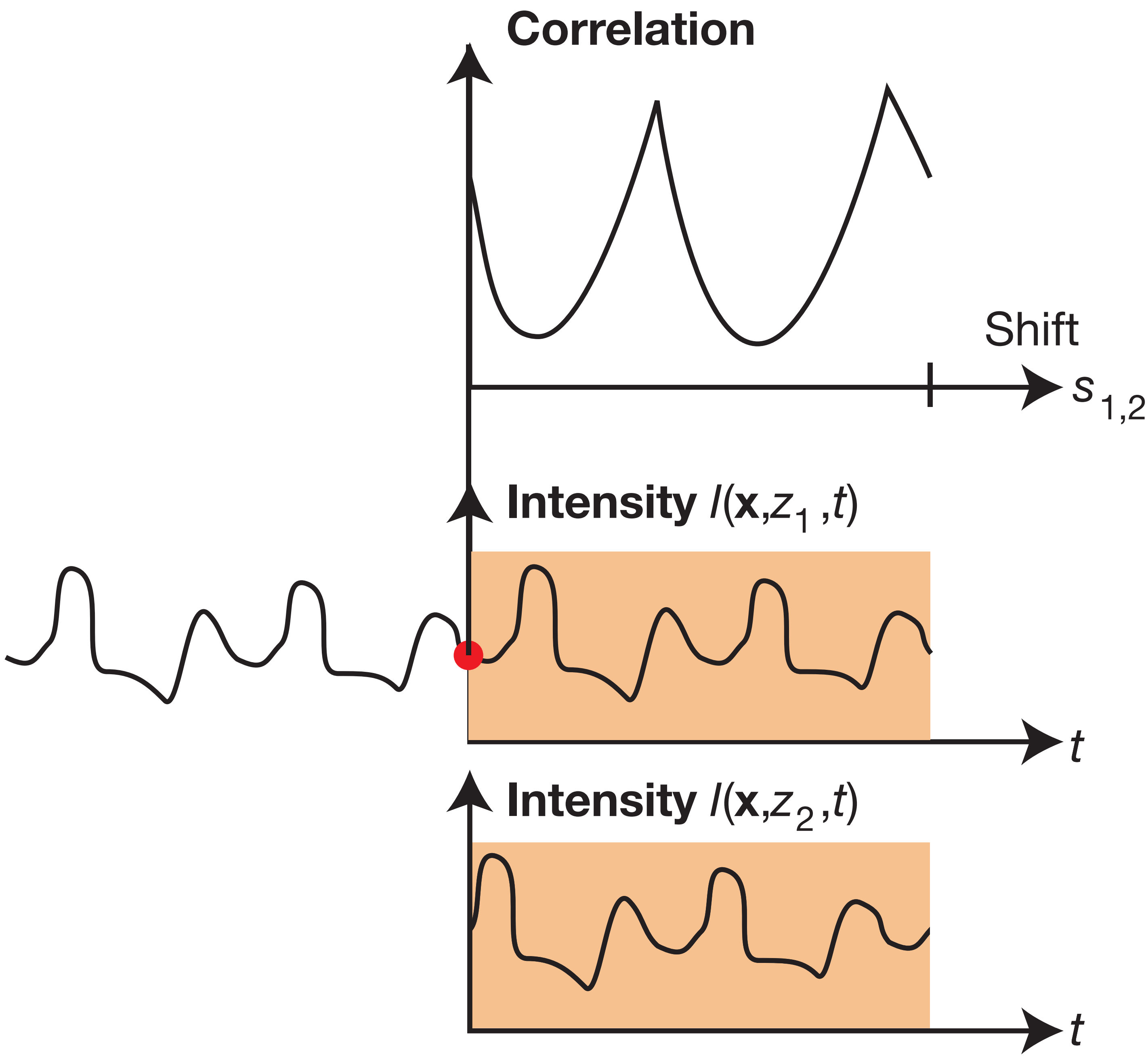
Correct Shift Maximizes Correlation



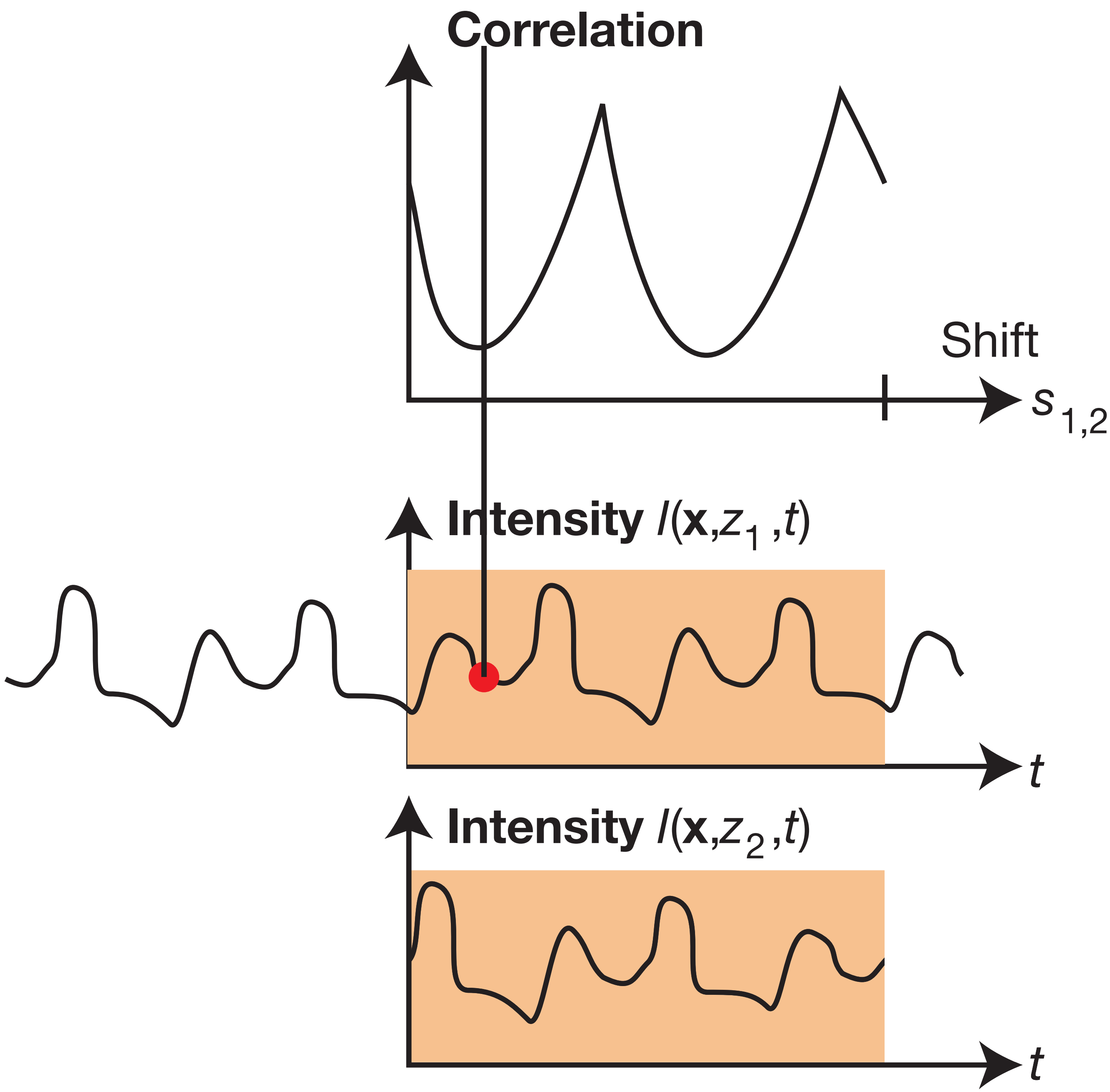
Correct Shift Maximizes Correlation



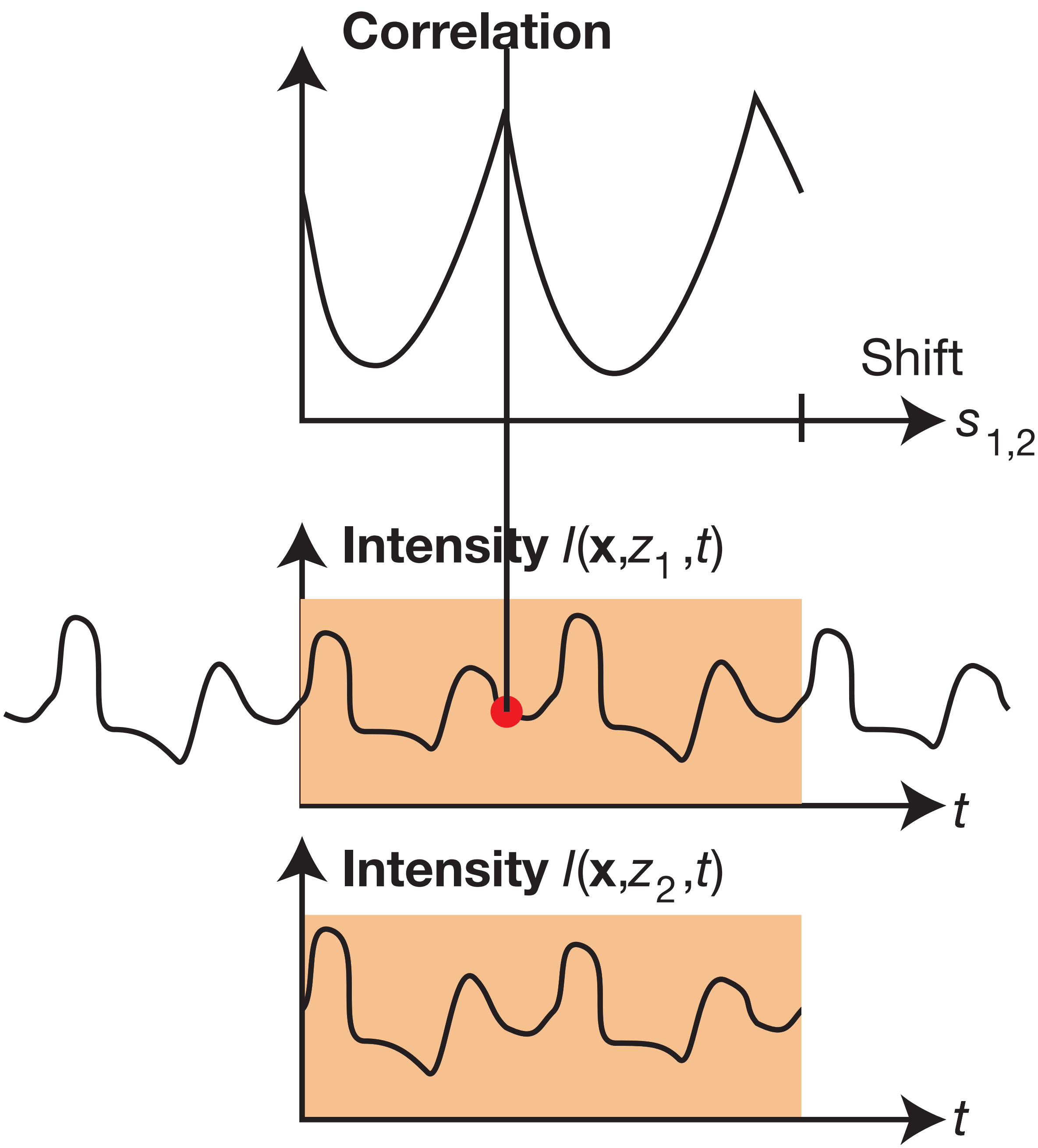
Correct Shift Maximizes Correlation



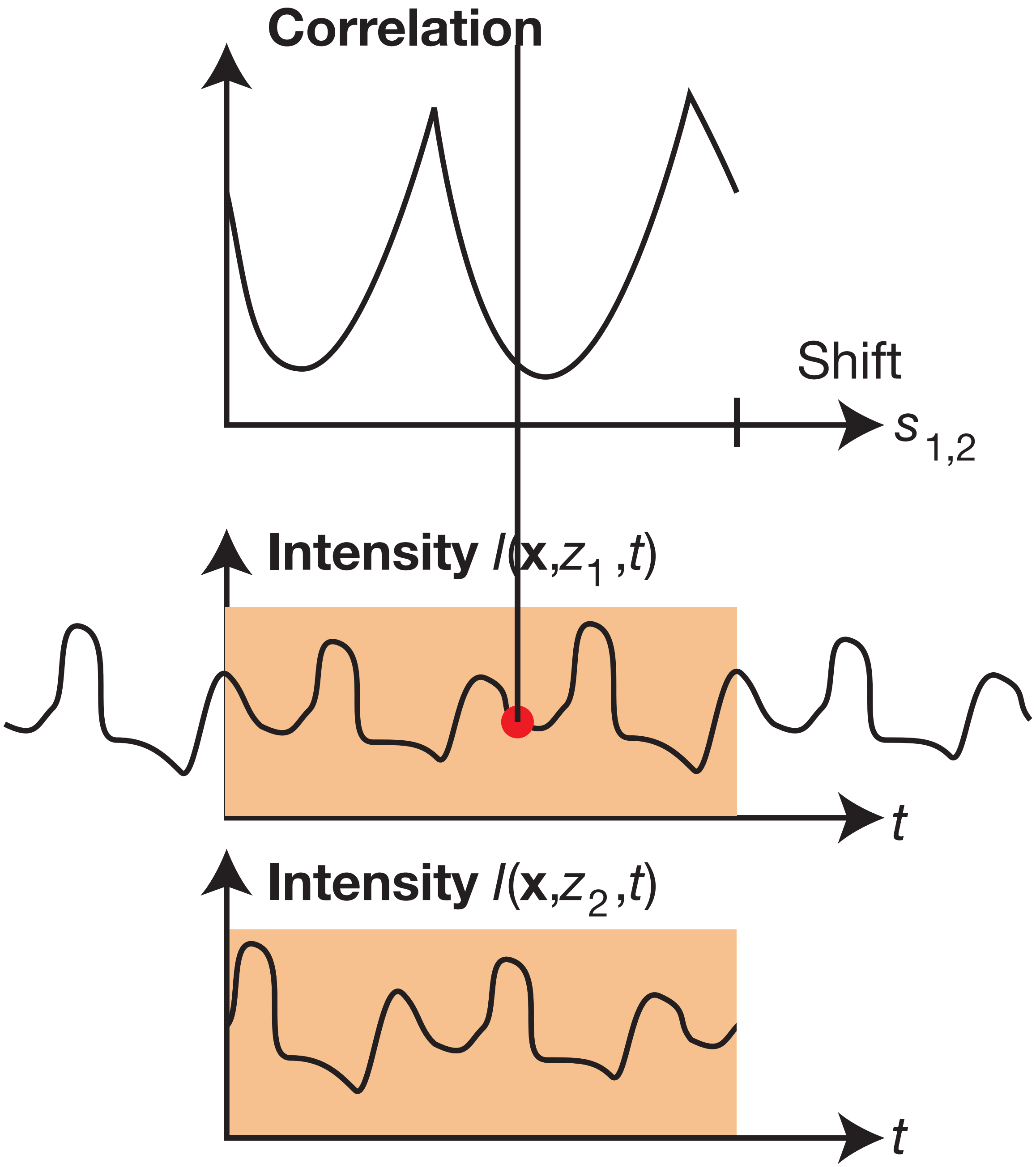
Correct Shift Maximizes Correlation



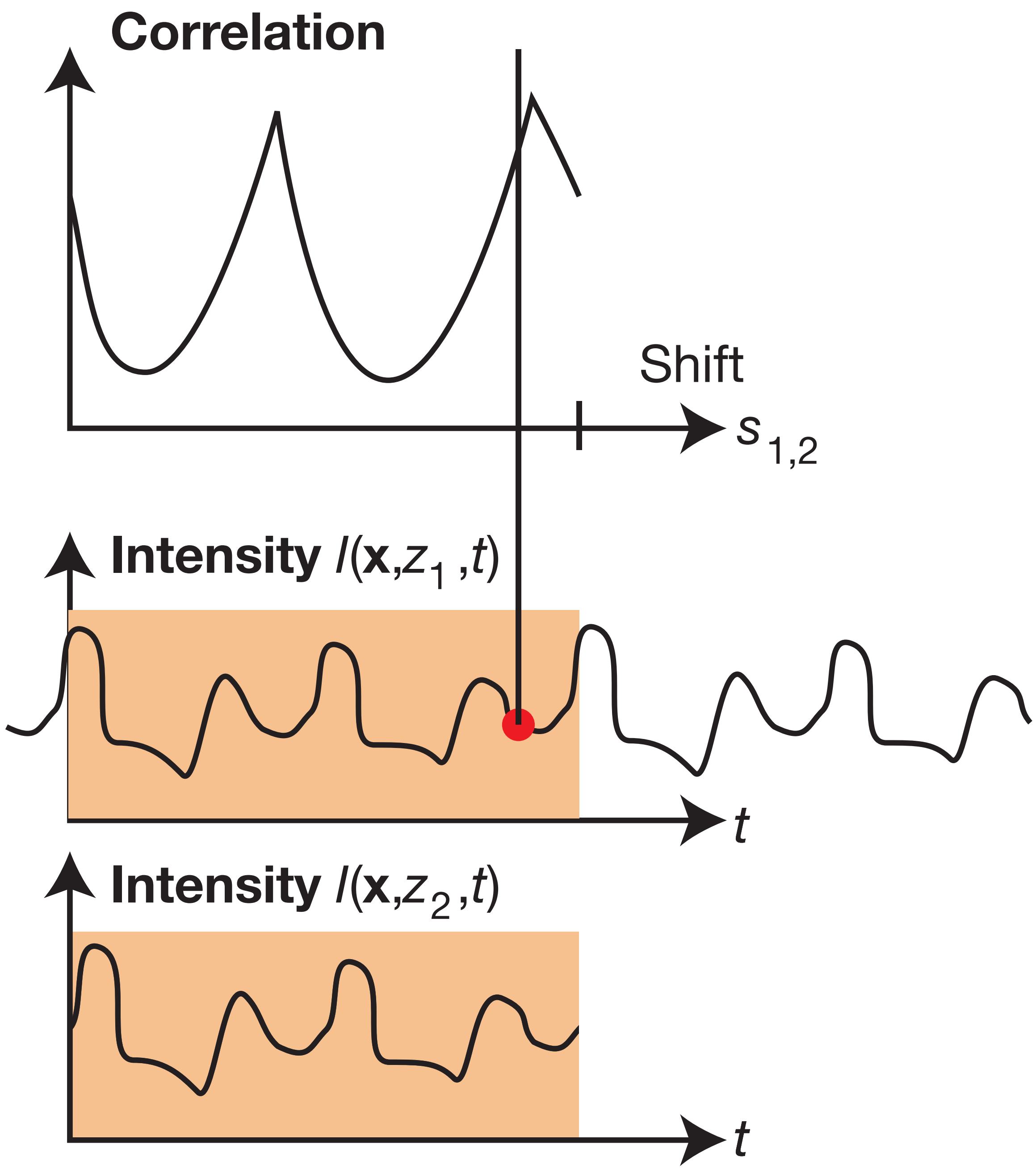
Correct Shift Maximizes Correlation



Correct Shift Maximizes Correlation



Correct Shift Maximizes Correlation



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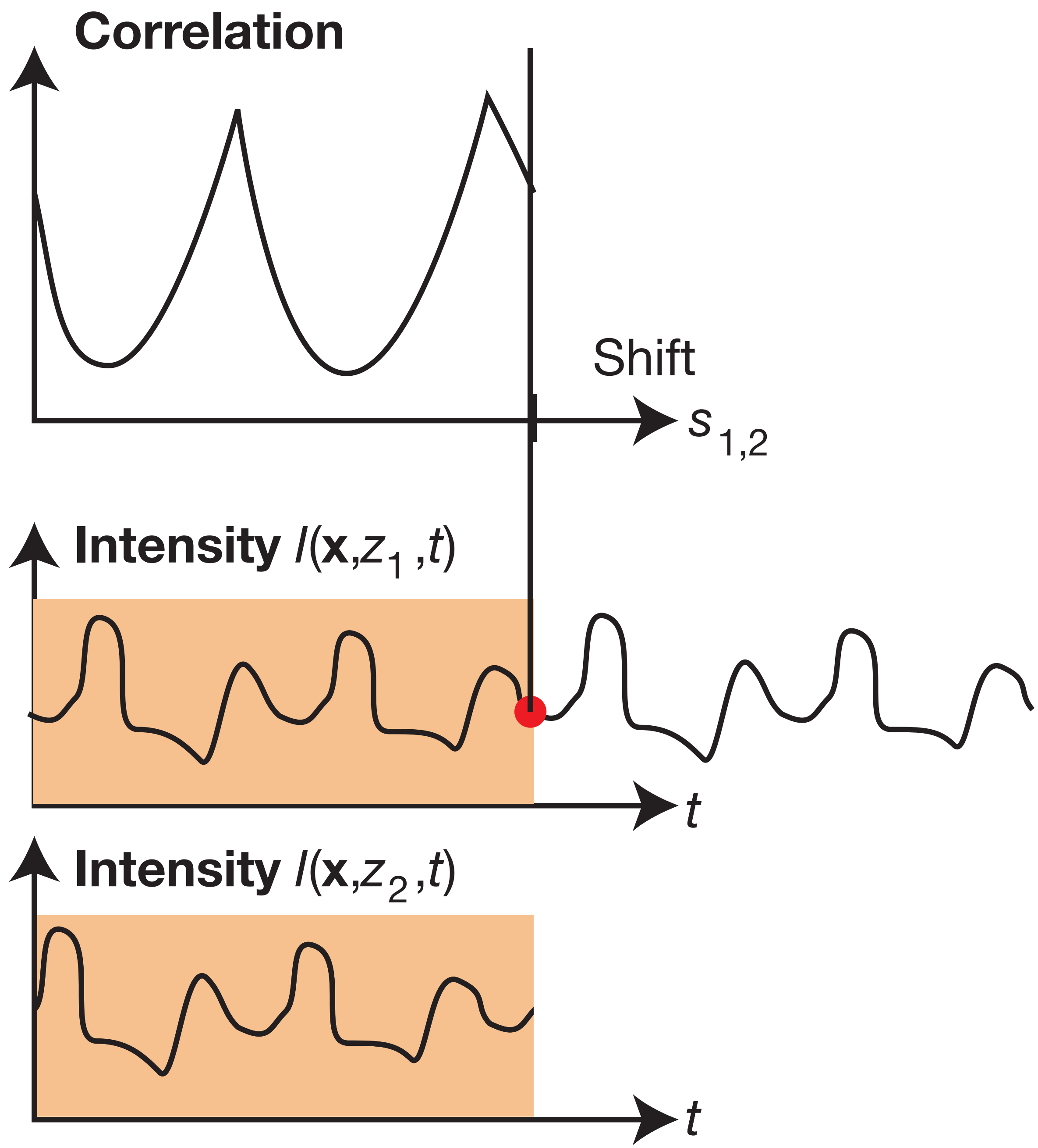
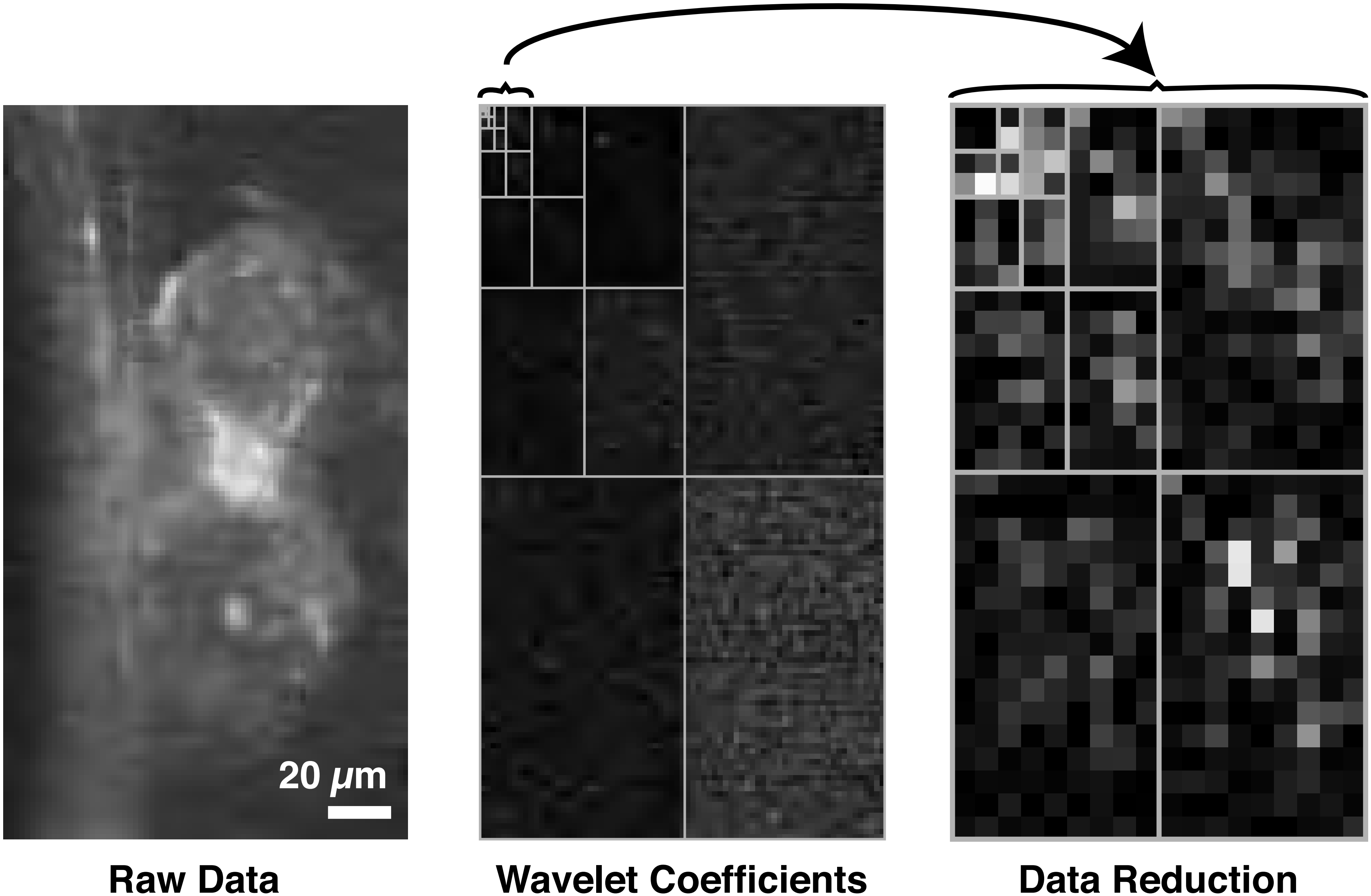
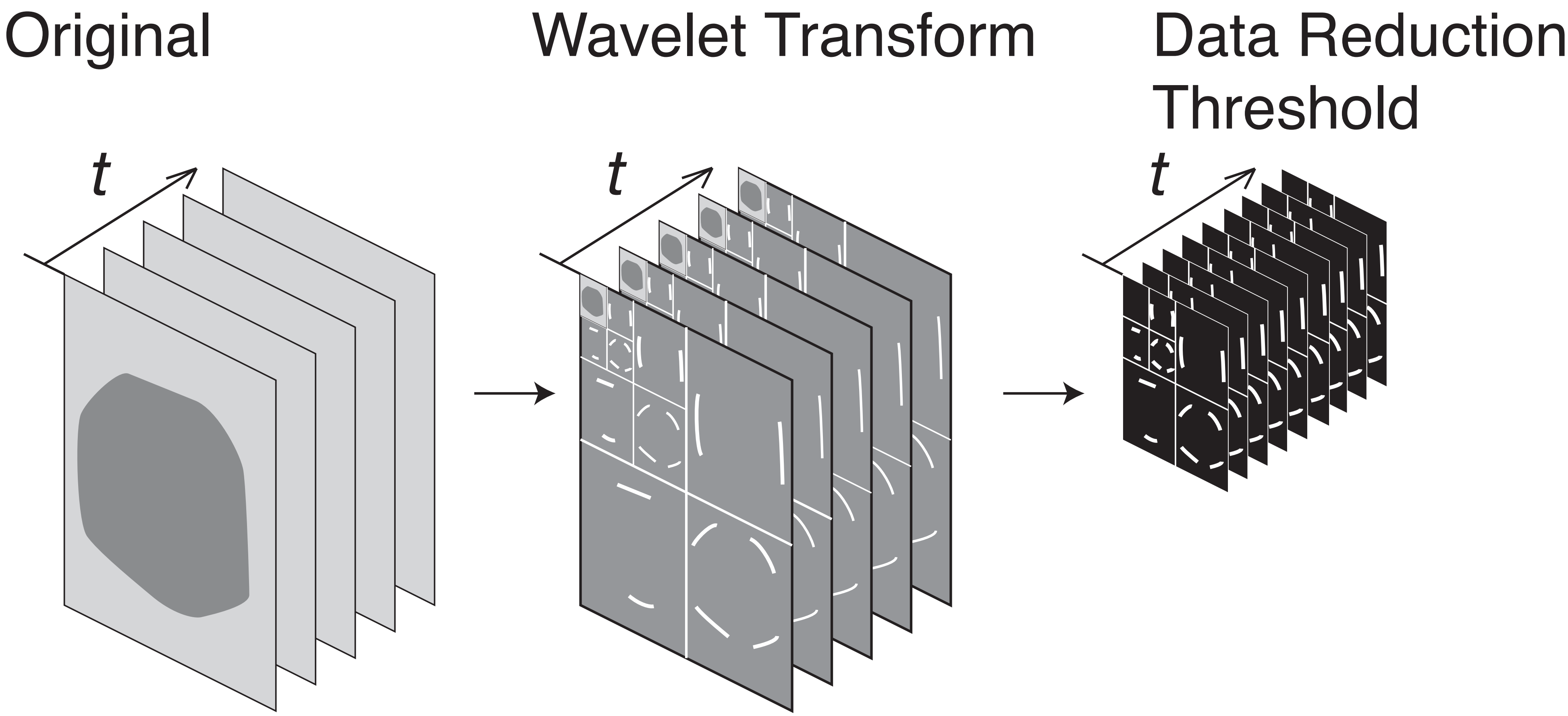


Overcoming Fluorescence Imaging Artifacts



Thresholded Wavelet Coefficient Synchronization



Features:

Cropping → increase speed, save memory

Remove high pass bands, threshold → robustness to photon / acquisition noise

Remove low pass band → robustness to bleaching artifacts

Time interpolation → increase accuracy

M. Liebling, A.S. Forouhar, M. Gharib, S.E. Fraser, M.E. Dickinson, *J. Biomedical Optics*, 10 (5), 2005.

Absolute Shift Determination

Relation between **absolute shifts** s_k and **relative shifts** $s_{k,k'}$:

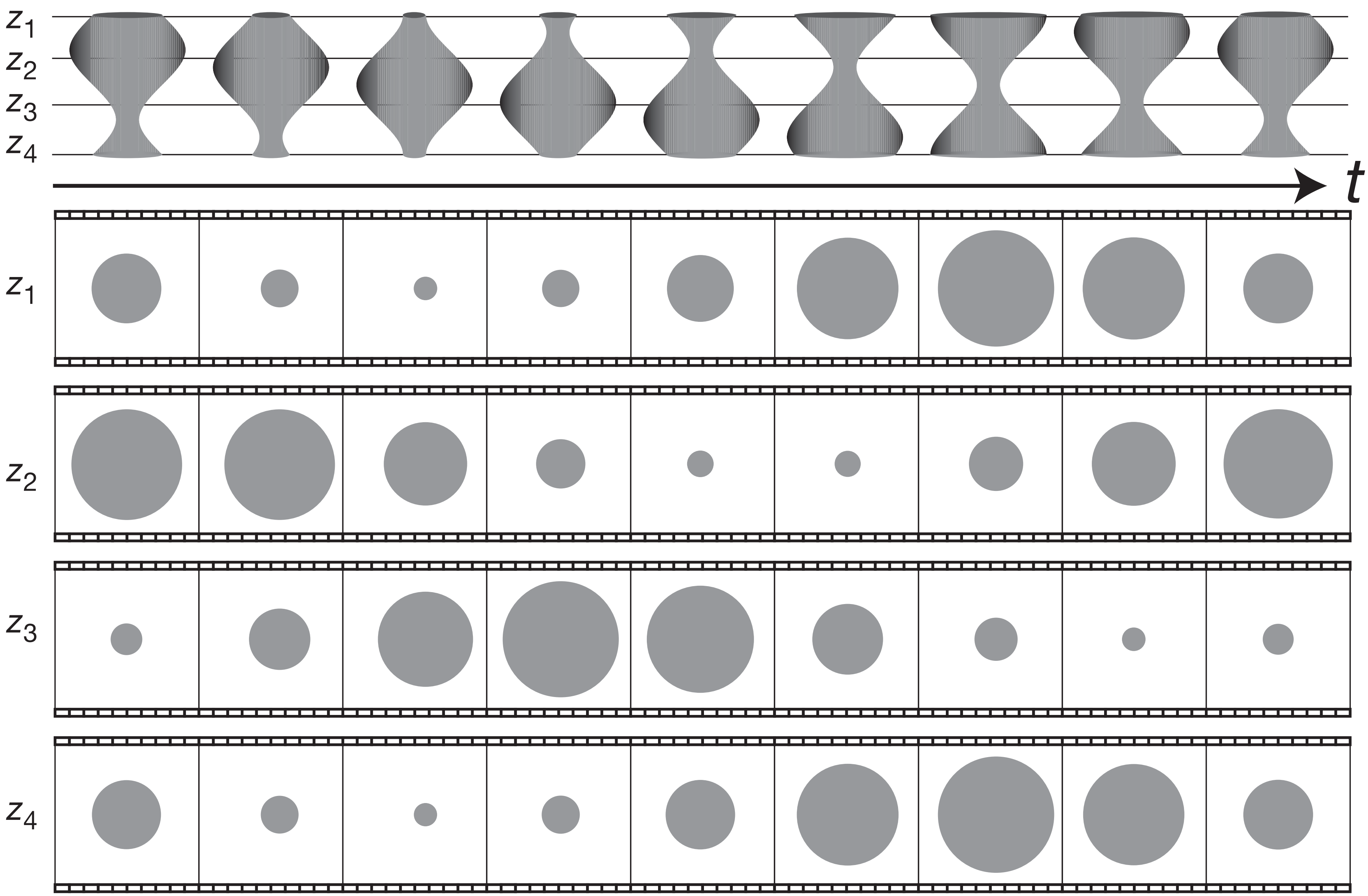
$$\underbrace{\begin{bmatrix} 1 & 0 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 1 & -1 \\ 1 & 0 & -1 & 0 & 0 \\ 0 & 1 & 0 & -1 & 0 \\ 0 & 0 & 1 & 0 & -1 \end{bmatrix}}_{\mathbf{A}} \underbrace{\begin{pmatrix} s_1 \\ s_2 \\ s_3 \\ s_4 \\ s_5 \end{pmatrix}}_{\mathbf{t}} = \underbrace{\begin{bmatrix} 0 \\ s_{1,2} \\ s_{2,3} \\ s_{3,4} \\ s_{4,5} \\ s_{1,3} \\ s_{2,4} \\ s_{3,5} \end{bmatrix}}_{\mathbf{s}}$$

Weighted least-squares solution (via normal equations):

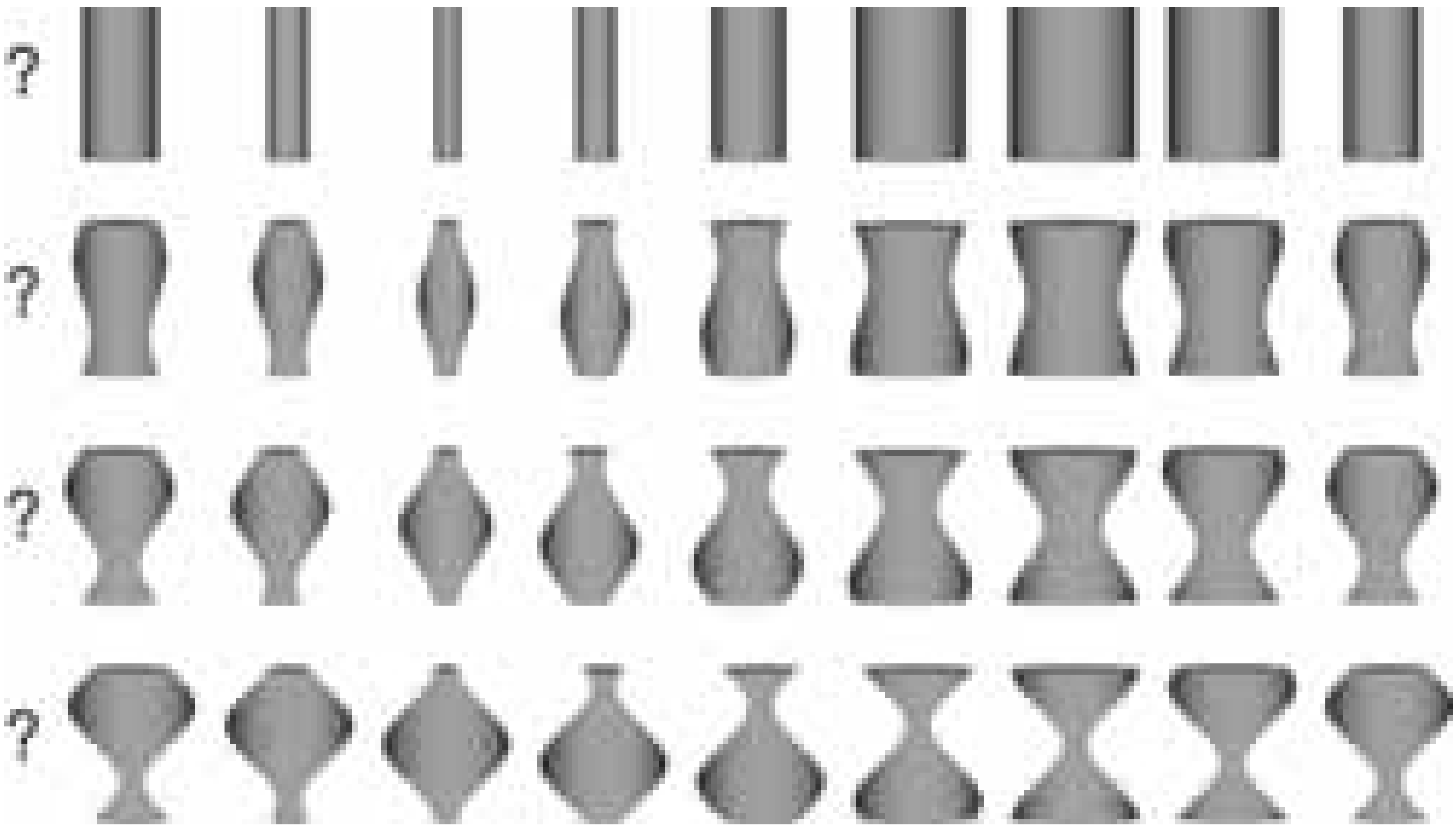
$$\mathbf{A}^\dagger \mathbf{W}^\dagger \mathbf{W} \mathbf{A} \mathbf{t} = \mathbf{A}^\dagger \mathbf{W}^\dagger \mathbf{W} \mathbf{s}$$

with **weights** $\mathbf{W} = \mathrm{diag}(1, w_1, w_1, w_1, w_1, w_1, w_2, w_2, w_2)$.

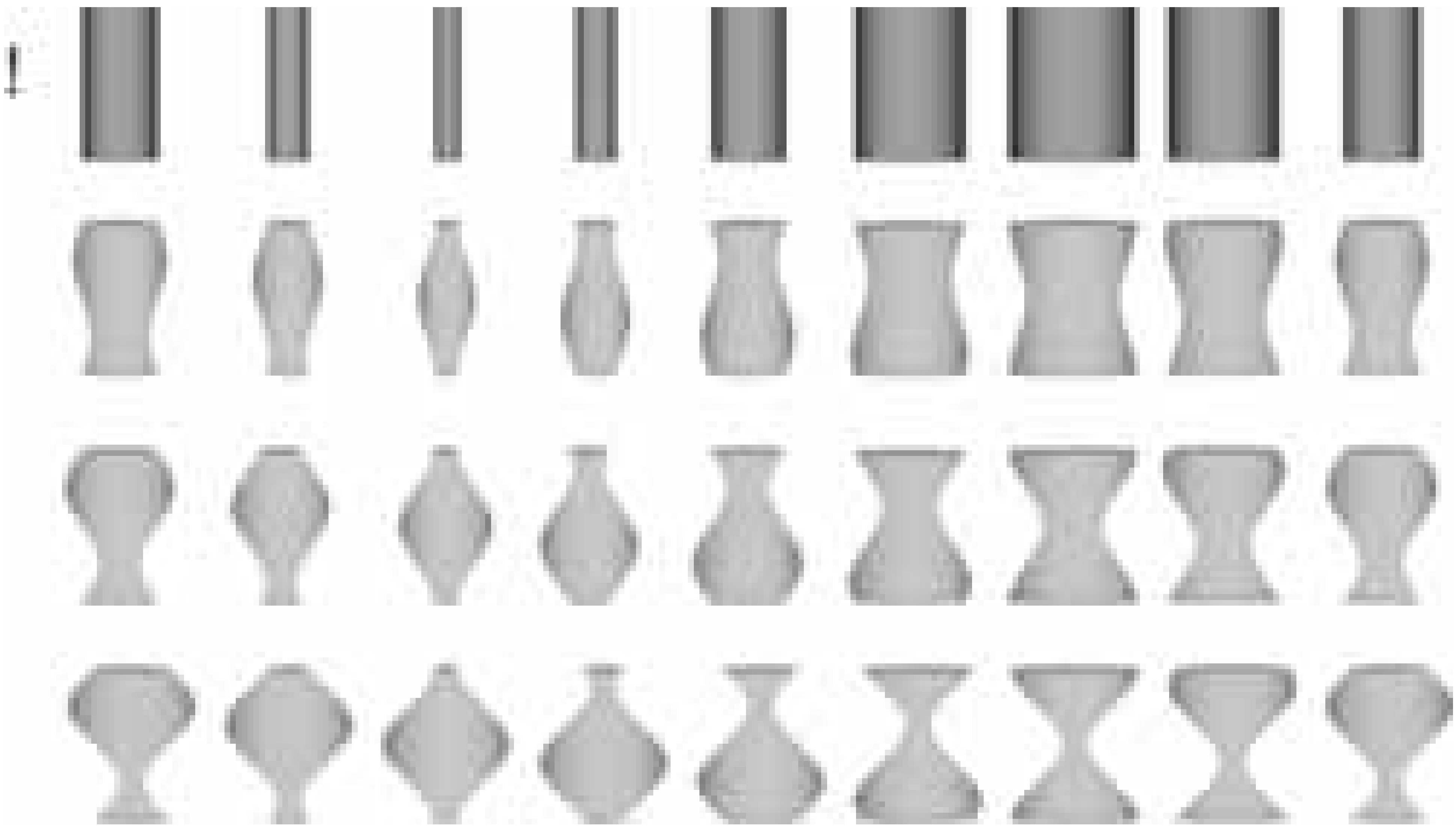
A Special Case. . .



4 Possible Reconstructions



. . . an ill-Posed Problem



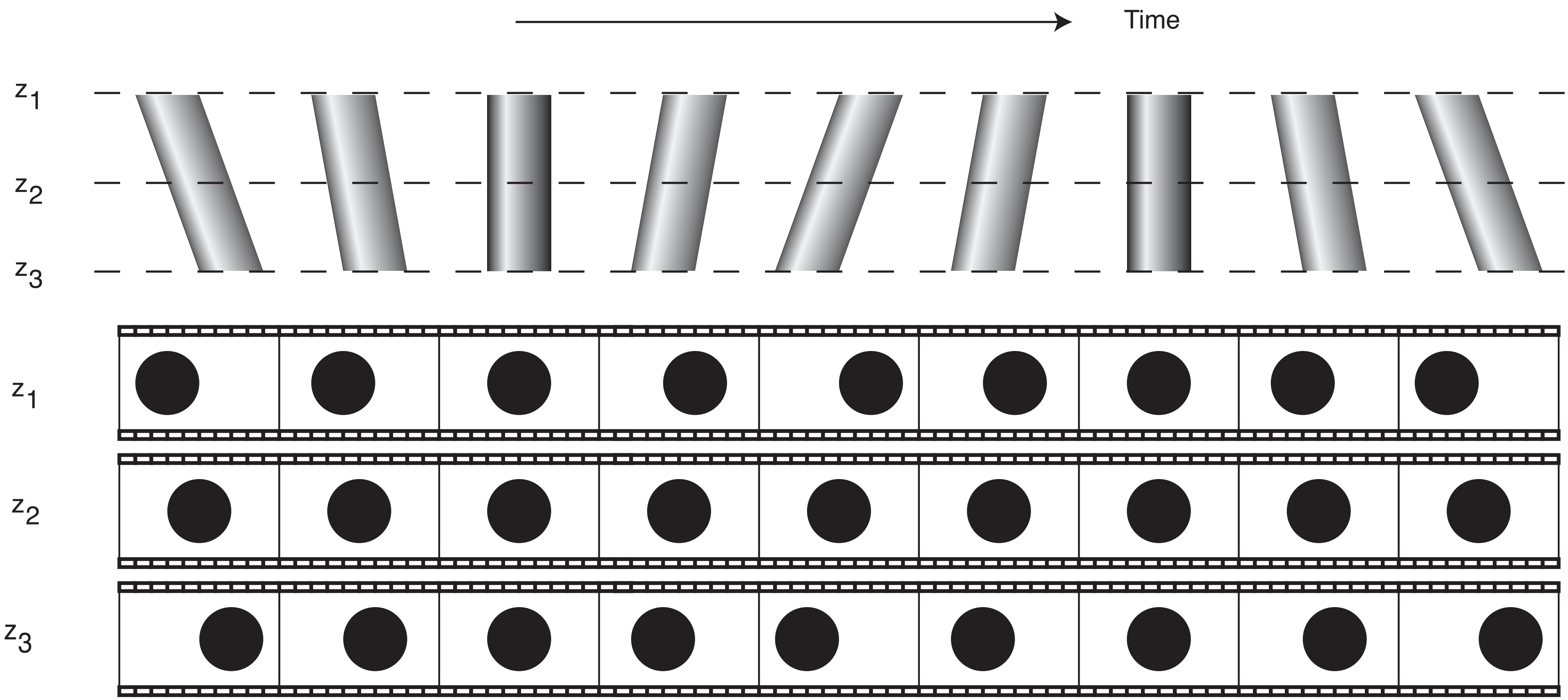
When is reconstruction possible?

Conversion from 2D+time to 3D+time is possible when:

a) Motion is homogeneous along *z* (→ hypothesis: PSF overlap).

b) Availability of supplementary knowledge:
ECG, triggered/gated measurements, etc.

Shear is allowed!



What is the accuracy?

Validation method:

1. Random shifts definition
2. Simulated data acquisition (random periodic deformation)
3. Reconstruction
4. Comparison

Average error of *absolute* shifts

$\bar{\epsilon} = 0.31 \pm 0.08$ frames

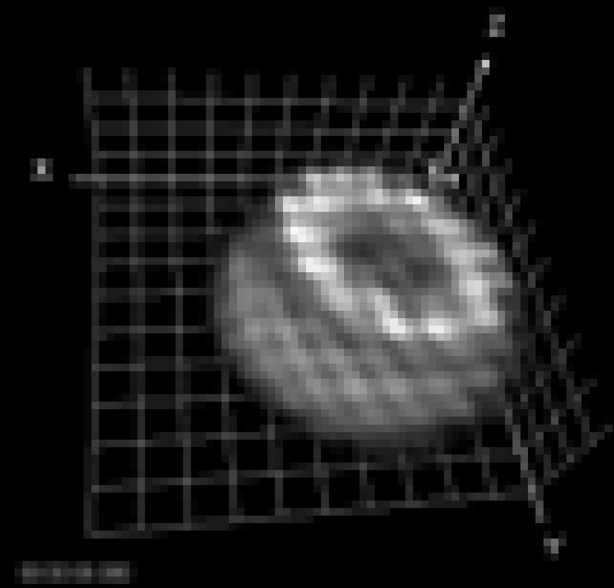
(Calculated over 100 random periodic homogeneous deformations

40 frames, 2× time-oversampling)

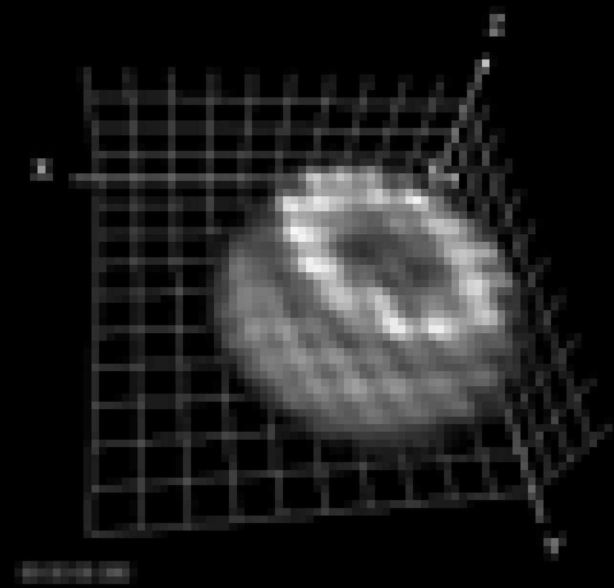
⇒ **Sub-frame accuracy**

M. Liebling *et al.*,
J. Biomed. Opt., 10(5), 2005.

Simulated original



Reconstruction



Specific Imaging by Use of Transgenic Fish

Nonuniform temporal registration

Acquisition Model

$$I_m(\mathbf{x}, z_k, t) = \iiint I[\mathbf{x}', z, \tau_k(t)]\, h(\mathbf{x} - \mathbf{x}', z_k - z)\, \mathrm{d}\mathbf{x}'\mathrm{d}z\,,$$

$\tau_k(t)$: unknown warping functions. $h(\mathbf{x}, z)$: Microscope point spread function (PSF) **Hypothesis**

$$|I[\mathbf{x}, z, \tau] - I[\mathbf{x}, z, \tau + T]| \ll I_{\max}.$$

Objective criterion (absolute difference):

$$Q_{k,k',w_k}\{w'_k\} = \int_0^L \iint_{\mathbf{R}^2} |I_m[\mathbf{x}, z_k, w_k(\tau)] - I_m[\mathbf{x}, z_{k'}, w_{k'}(\tau)]|\, \mathrm{d}\mathbf{x}\, \mathrm{d}\tau.$$

Goal:

Recover warping functions $w_{k'}(\tau)$ for $k' = \{0, \ldots, N_z - 1\}\backslash\{\bar{k}\}$.

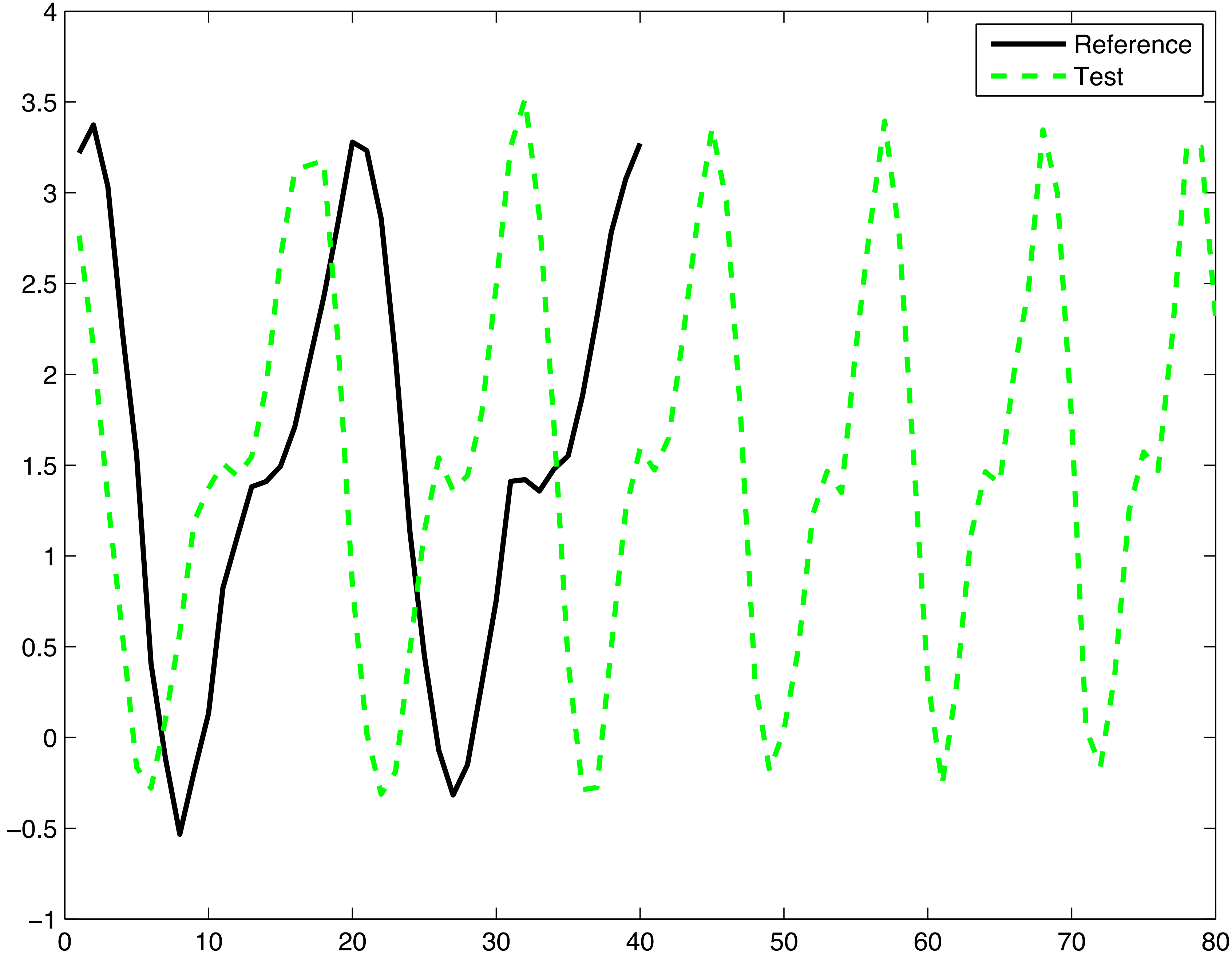
Approach:

(a) Interpolation and oversampling of reference sequence $\Rightarrow$ discrete $w_k(\tau)$

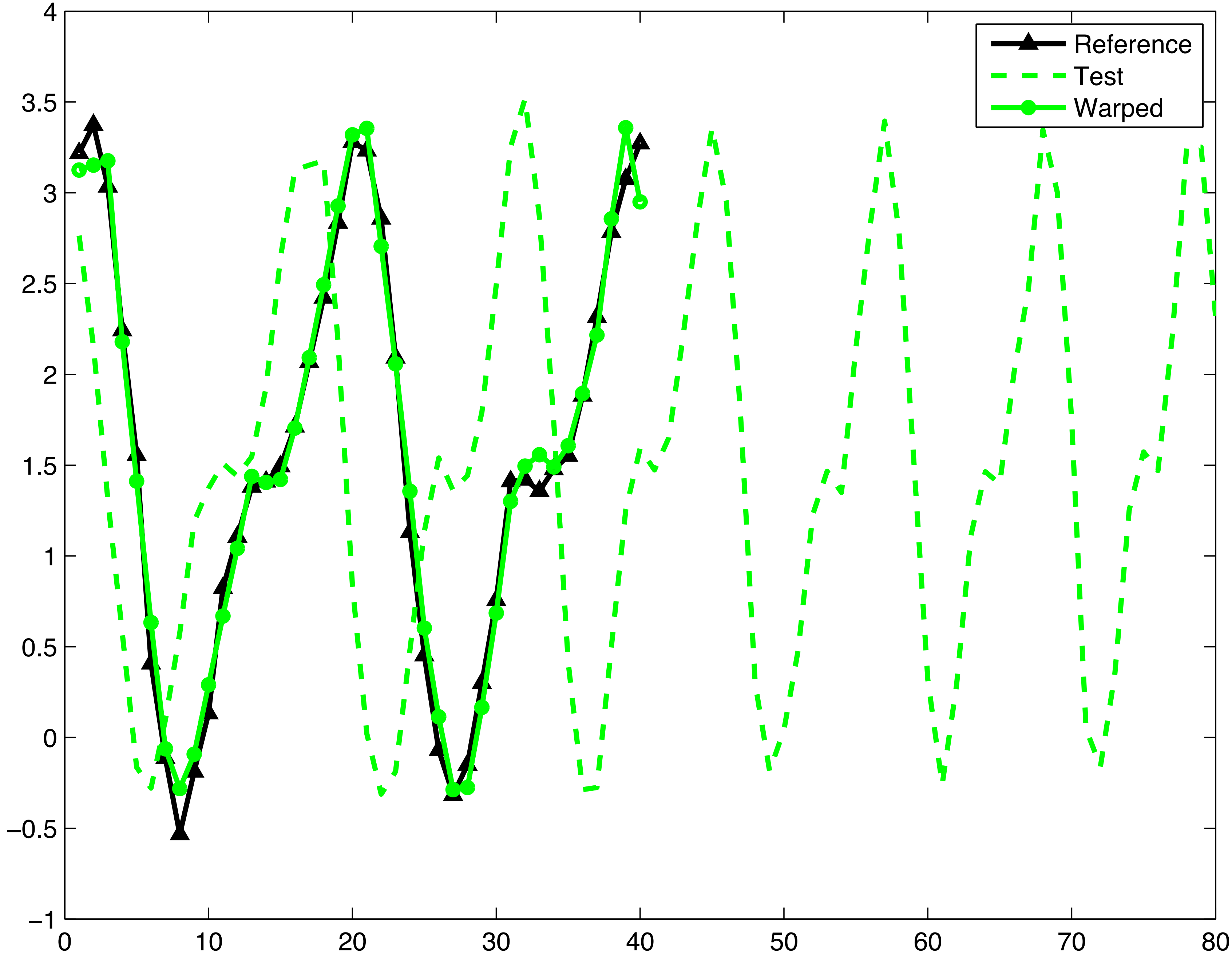
(b) Frame matching via dynamic programming

$\Rightarrow$ Reduce complexity from $\mathcal{O}\left((N_r)^{N_t}\right)$ to $\mathcal{O}(N_r N_t)$.

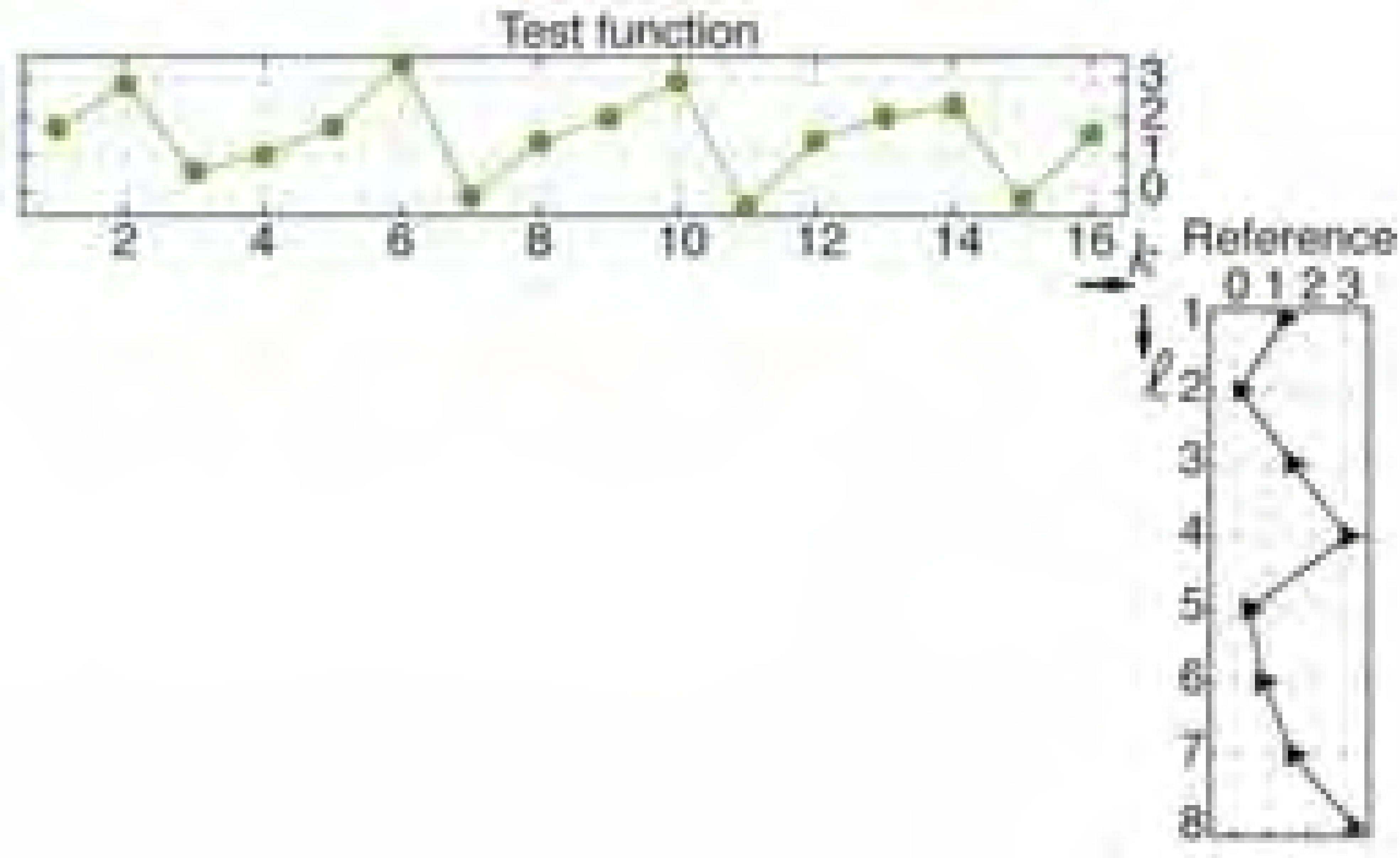
Nonuniform Registration



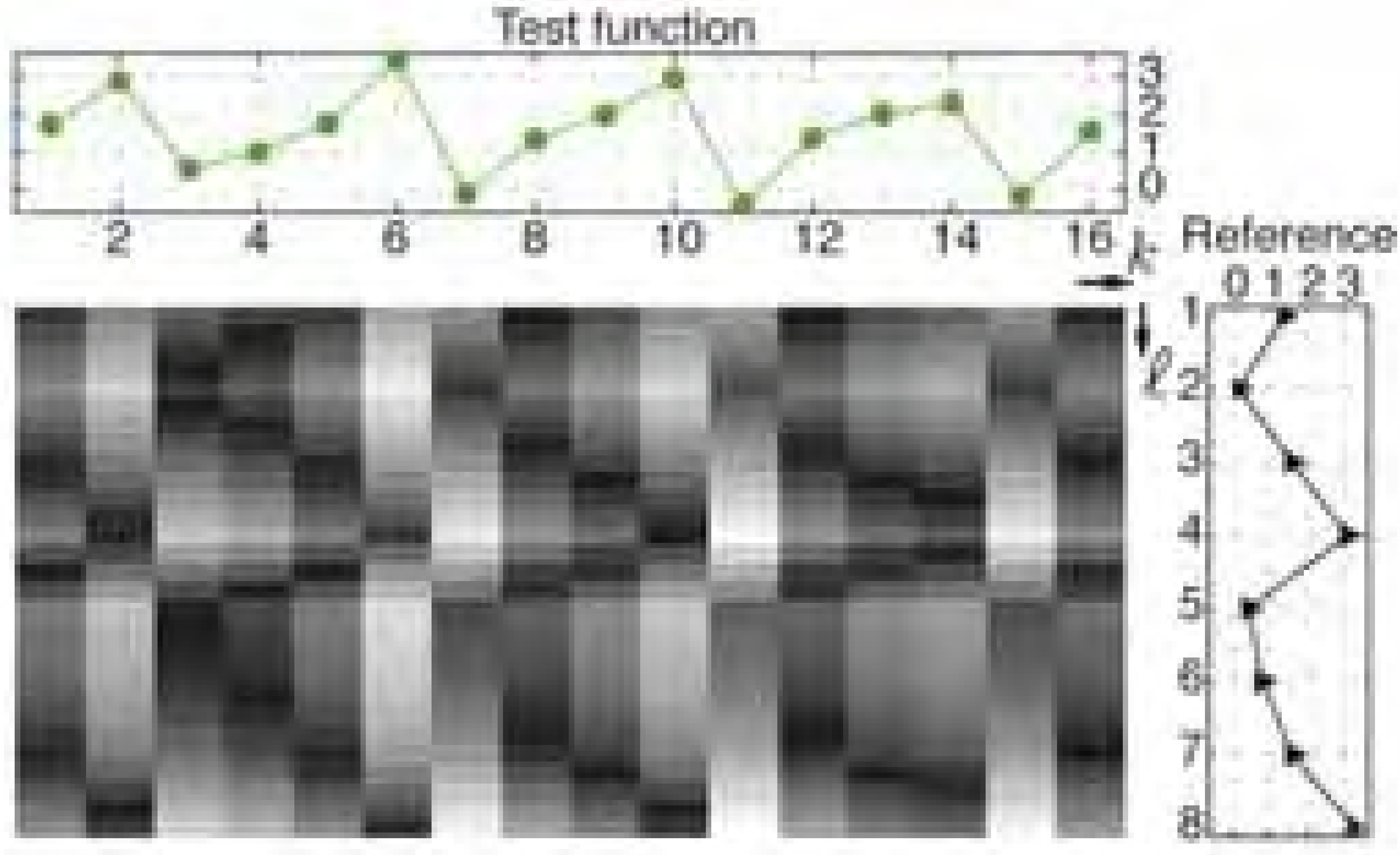
Nonuniform Registration



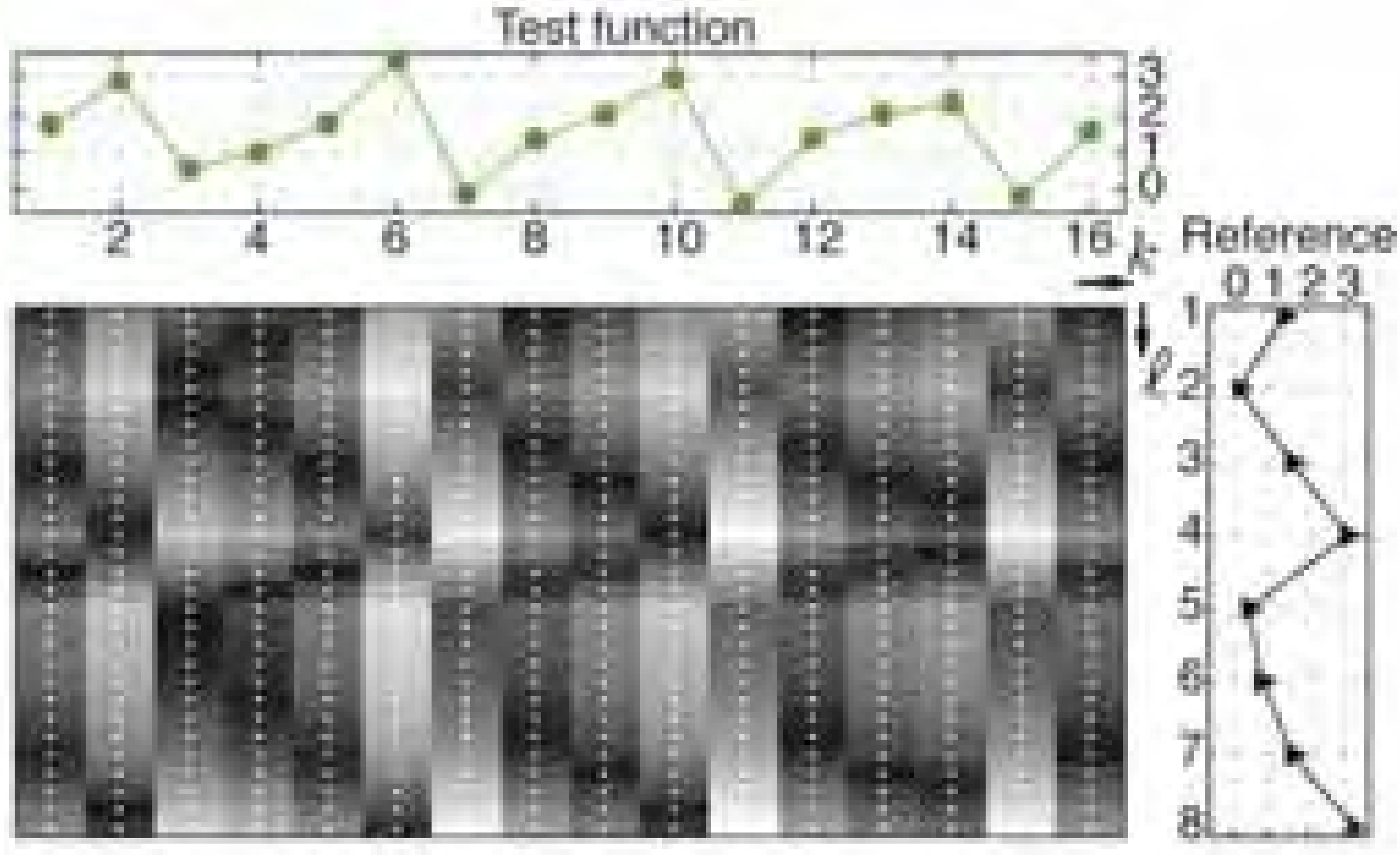
Nonuniform Dynamic Programming Registration



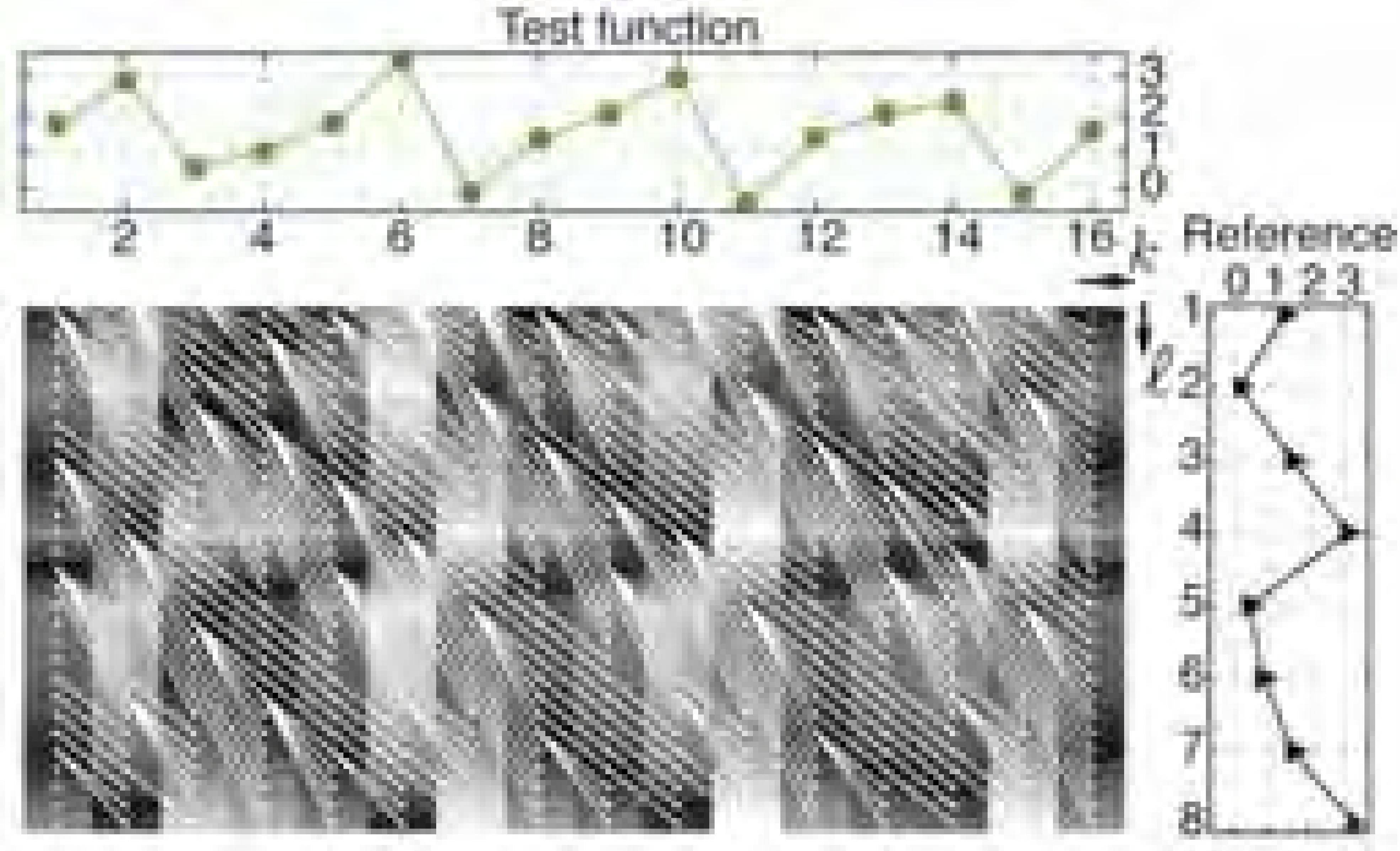
Nonuniform Dynamic Programming Registration



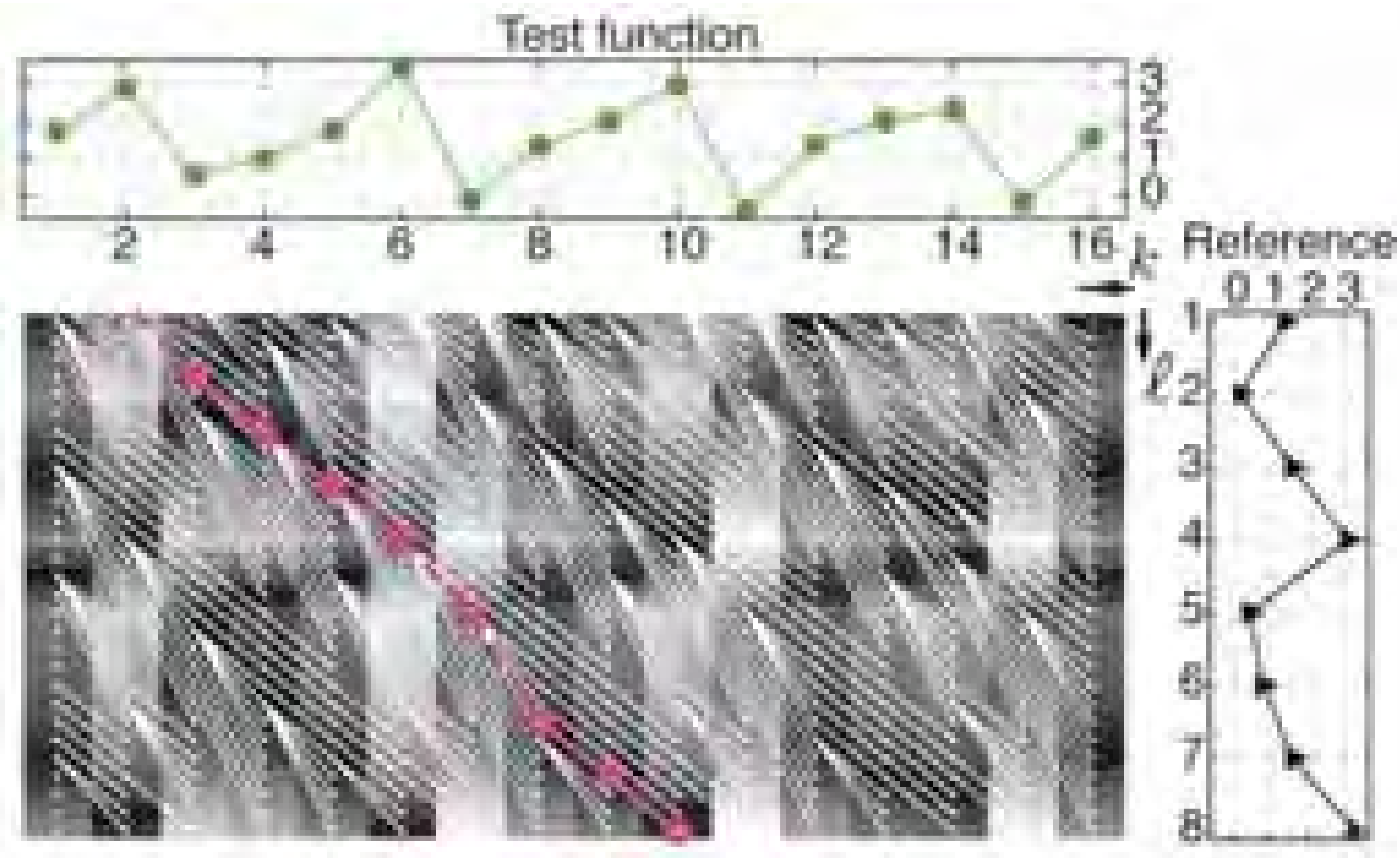
Nonuniform Dynamic Programming Registration



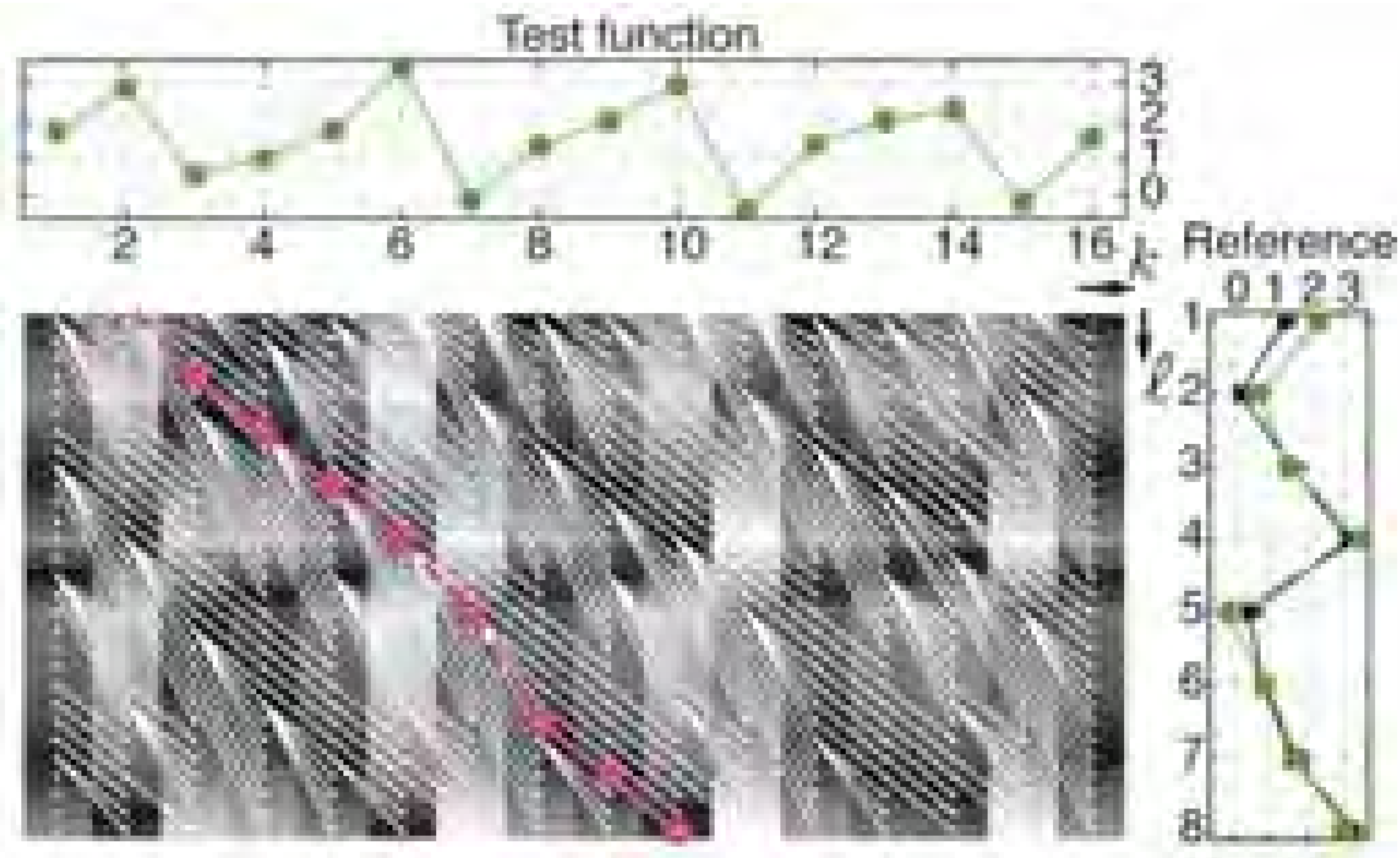
Nonuniform Dynamic Programming Registration



Nonuniform Dynamic Programming Registration



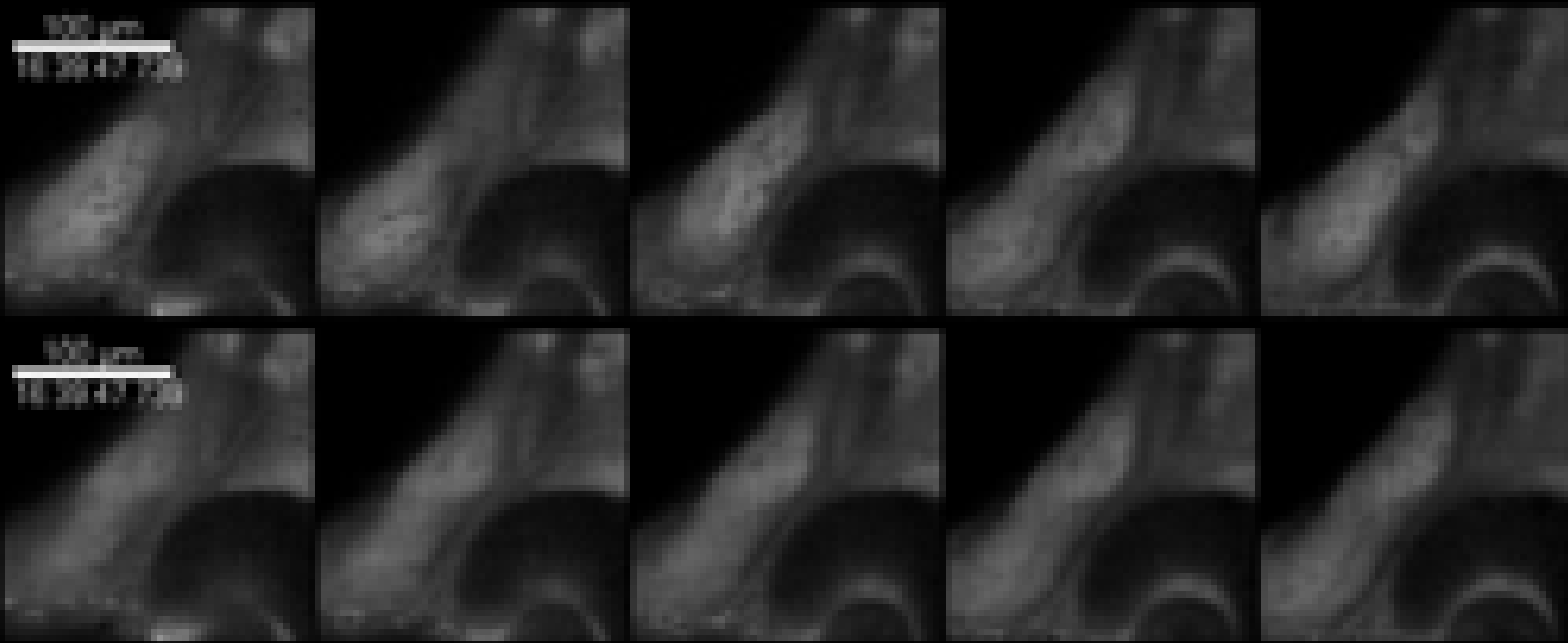
Nonuniform Dynamic Programming Registration



Robust and Reproducible Synchronization

Slices before synchronization

z = 0 μm | z = 10 μm | z = 20 μm | z = 30 μm | z = 40 μm

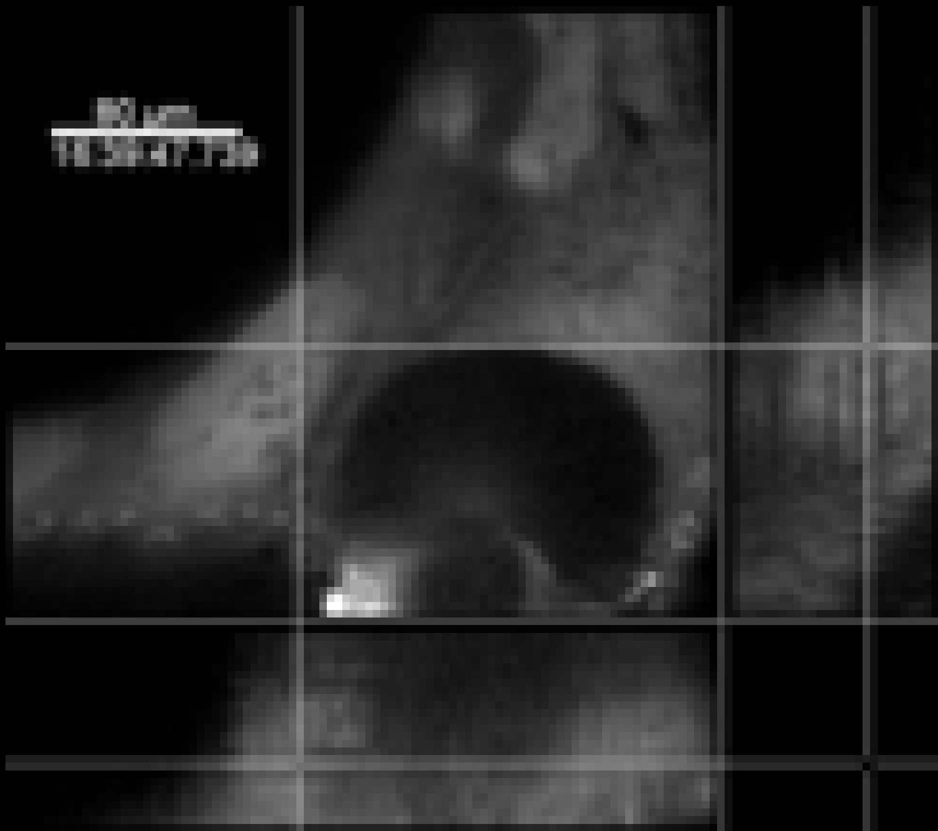


Slices after synchronization

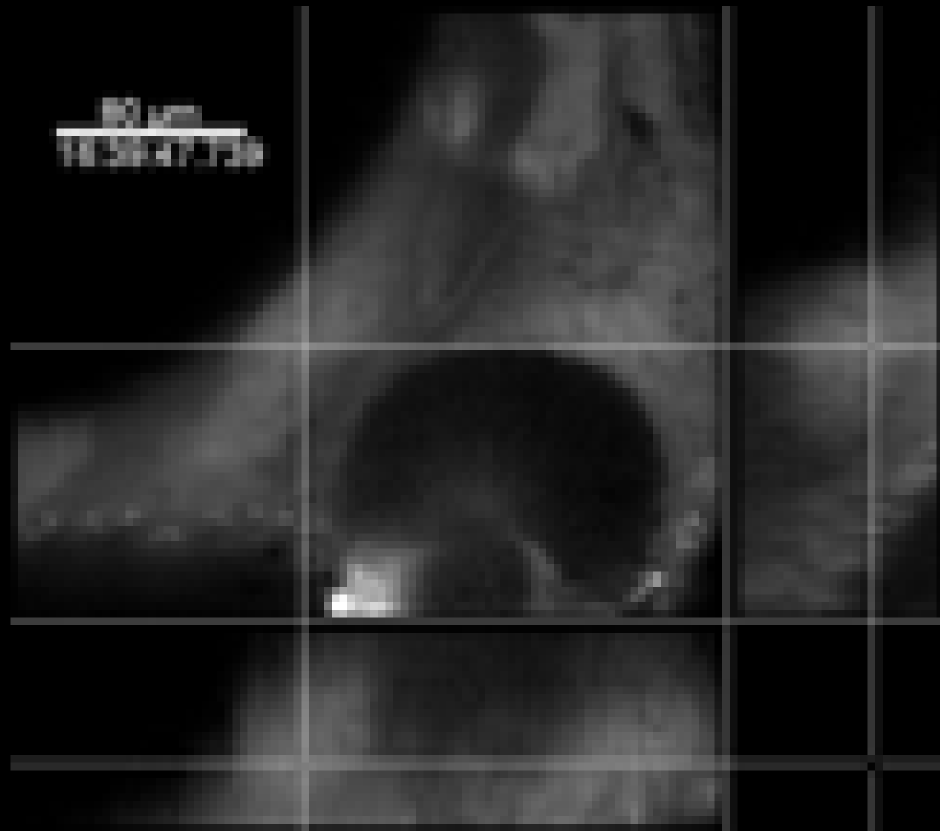
M. Liebling, J. Vermot [38 hpf wildtype zebrafish, BODIPY FL C_5-ceramide]
M. Liebling, J. Vermot, A.S. Forouhar, M. Gharib, M.E. Dickinson, S.E. Fraser, Proc. ISBI'06, in press.

Automatic synchronization allows 4D reconstruction

Before synchronization



After synchronization



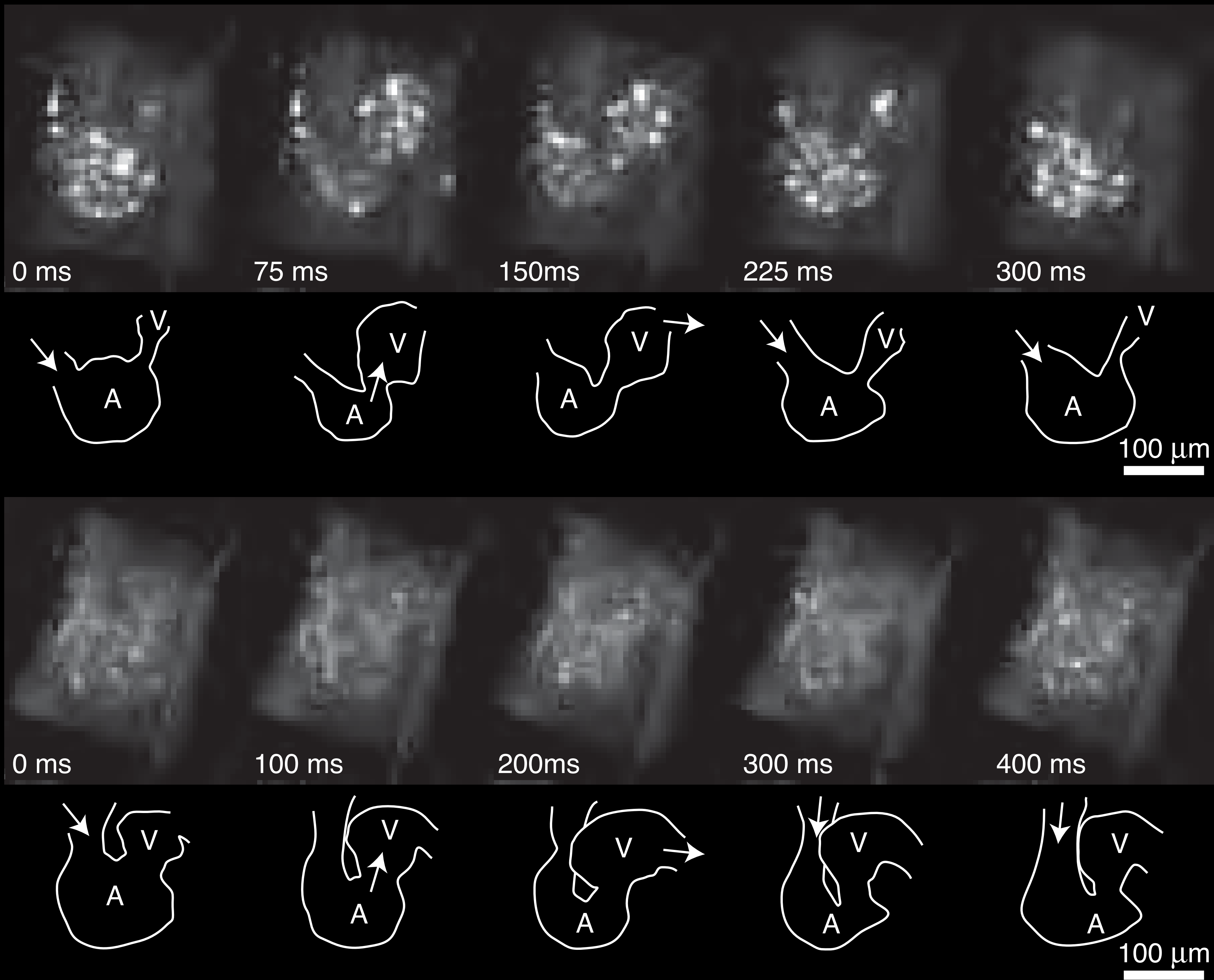
M. Liebling, J. Vermot [38 hpf wildtype zebrafish, BODIPY FL C_5-ceramide]

M. Liebling, J. Vermot, A.S. Forouhar, M. Gharib, M.E. Dickinson, S.E. Fraser, Proc. ISBI'06, in press.

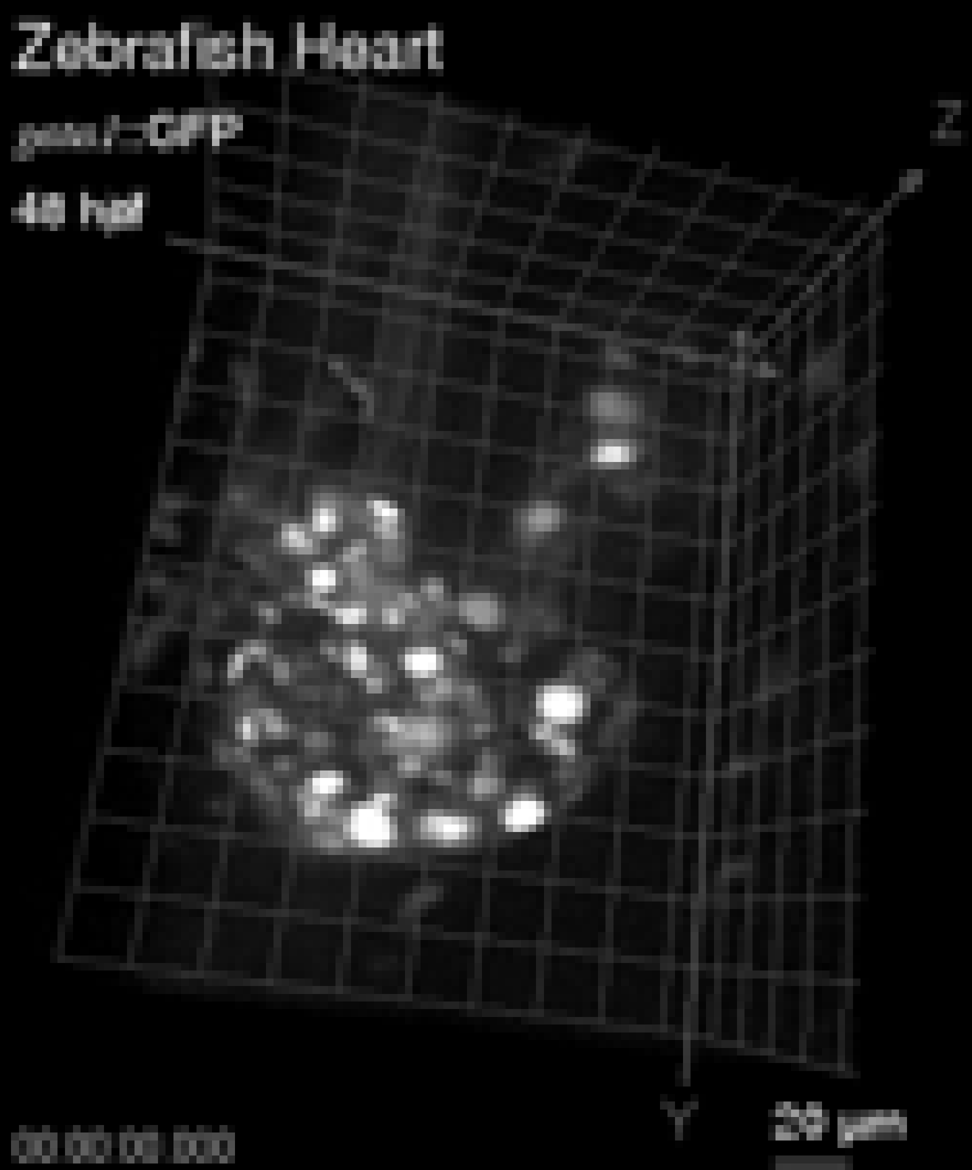
Synchronized dataset is suited for 3D rendering

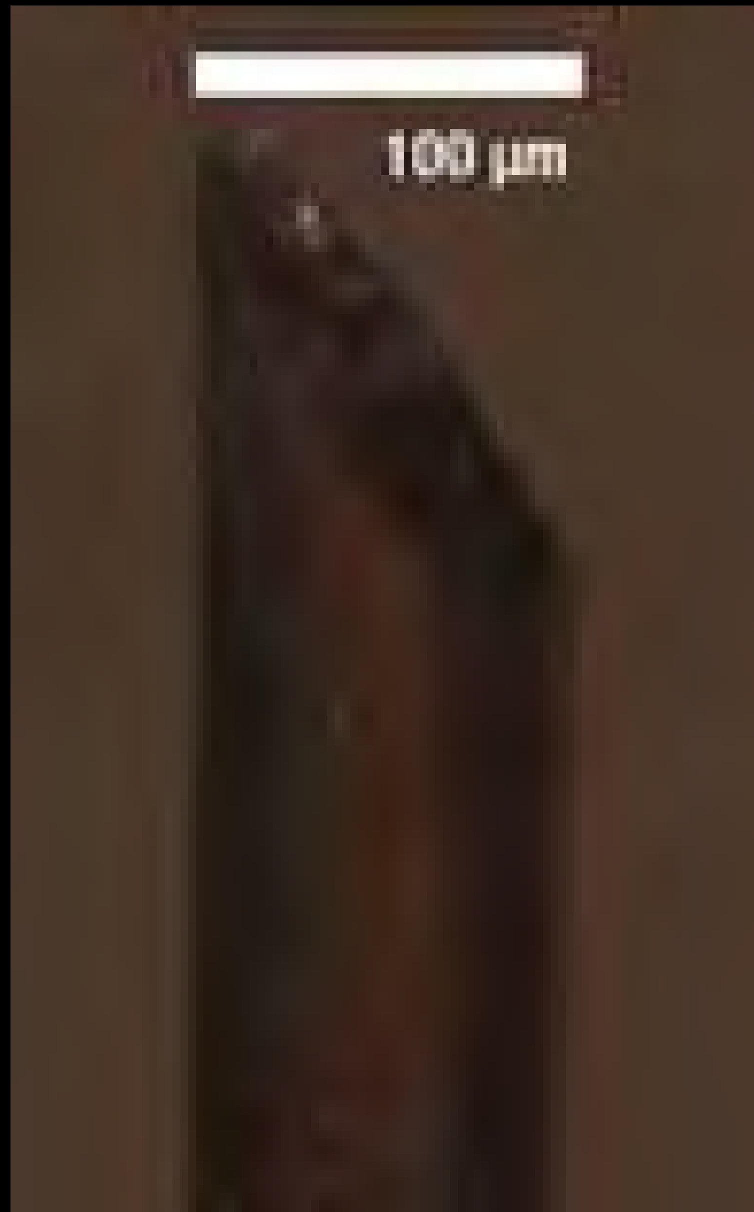
M. Liebling, J. Vermot [38 hpf wildtype zebrafish, BODIPY FL C_5-ceramide]

Heart Cycle @ 48hpf, *gata1*::GFP Zebrafish



Microscopic Heart





Summary

Fast 3D+time imaging is possible
in the living embryonic heart

Automatic reconstruction procedure
allows quantitative measurements
in a large number of fish

Automated Reconstruction:

- Robust to confocal microscopy artifacts
- Adapted to large data size
- Accuracy validated in silico and in vivo
- Allows for systematic and quantitative measurements

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Carol Readhead
Julien Vermot
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Shuo Lin, UCLA [Tg(*gata1*::GFP)]
Huai-Jen Tsai, Nat'l Taiwan U. [Tg(*cmlc2*::GFP)]

Colophon

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Postprocessing:
ImageMagick, GraphicConverter, pdfLaTeX

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Index

Studying the Developing Heart
Zebrafish Embryos
Genetic and Epigenetic Factors
Speed Biological Processes Moving at the Speed of Cells
Insufficient frame-rate artifacts
Fluorescence: Reminder
Confocal Microscopy Reminder
Point Scanning
Points vs line scan
Zeiss LSM 5 LIVE
Parallel Acquisition
Gated 4D Imaging
Nongated 4D Imaging
4D Imaging
Manual 4D Reconstruction
Automatic Registration Challenges
Problem definition
Finding the Period...
Intensity-based Registration
Correct Shift Maximizes Correlation
Wavelets and Fluorescence
Wavelet Synchronization
Absolute Shift Determination
A Special Case...
When is the reconstruction possible?
What is the accuracy?
cmlc2 3D
Dev. of Zfish Heart (cmlc2)
Nonuniform temporal registration
DP example
DP Registration
Bodipy Galery
Bodipy Slice
Bodipy 3D
Heart Cycle @ 48hpf
Dev. of Zfish Heart (gata1)
Volume Measurements
Peristaltic vs Impedance Pumping
Impedance Pump: Mechanical Model
Early embryonic heart tube
Is pump peristaltic?
Summary