Formation of Stress Fibres in Adult Stem Cells

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Contributors

- Max Sommerfeld (SAMSI 2013/14)
- Kwang-Rae Kim (now at the Univ. of Nottingham)
- Florian Rehfeldt and Carina Wollnik (Physics III/Biophysics, Göttingen)
- Carsten Gottschlich, Benjamin Eltzner and Axel Munk (Univ. Göttingen)
- DFG CRC 755 “Nanoscale Photonic Imaging”
- SAMSI LDHD

SFB 755

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One Motivation: Stem Cell Therapy

- Medical condition e.g. post heart attack,
- medical goal e.g. grow new heart muscle tissue,
- intervention strategy: inject stem cells.

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- pluripotent = differentiate e.g. into
- myoblasts = muscle precusor cells,
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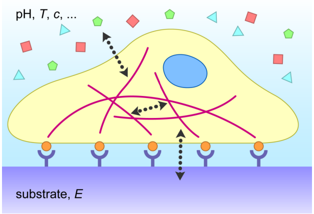
- Previous research by Engler et al. (2006) indicates that surrounding tissue elasticity influences the blast – type.

Problem at Hand: Study Early Stem Cell Differentiation



- put cells on gel of varying elasticity (kPa),
- flourescence labeling of actin-myosin filaments,
- photograph after 24 hrs. (before duplication).

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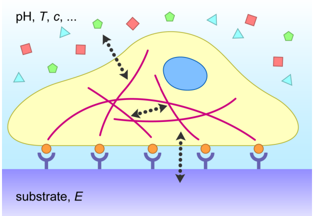

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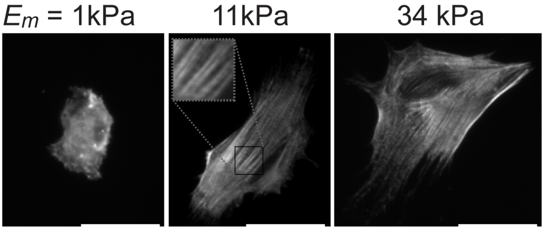
From Zemel et al. (2010)



Biomechanical Hypotheses

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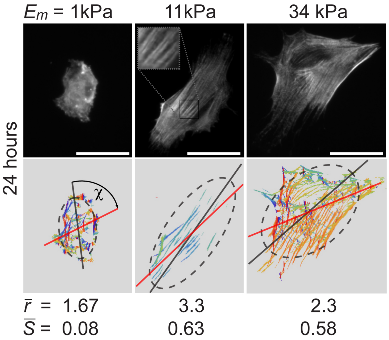

Orientation detection: elongated Laplacians of a Gaussian

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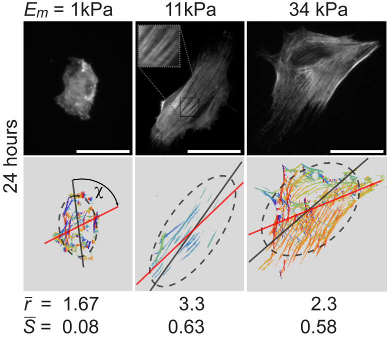

Orientation detection: elongated Laplacians of a Gaussian

- Low rigidity (1kPa) $\Rightarrow$ few short non-oriented filaments.
- Resonance rigidity (11 kPa) $\Rightarrow$ many long aligned filaments.
- High rigidity (34 kPa) $\Rightarrow$ many long filaments with varying directions.

Challenges

1. Good data: reliably digitize filament structure $\to$ filament process

$$(\lambda, \phi)_{z_i},\ i = 1, \ldots, N,\ \lambda \in \mathbb{R}^+,\ \phi \in [0, \pi)\,.$$

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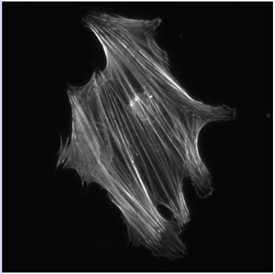

Good quality image

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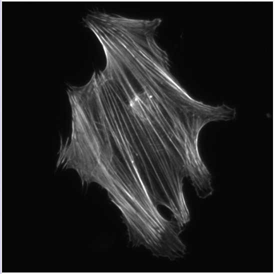

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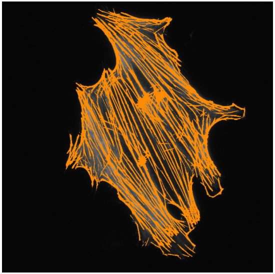

Elongated Laplacian of a Gaussian following Zemel et al. (2010)

filament pixel $\mapsto$ orientation

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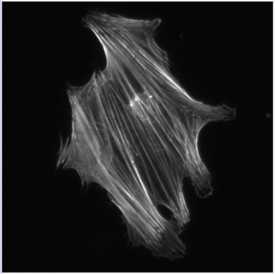

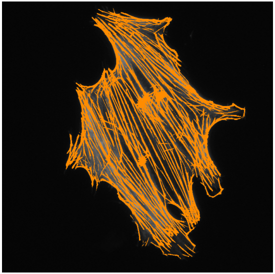

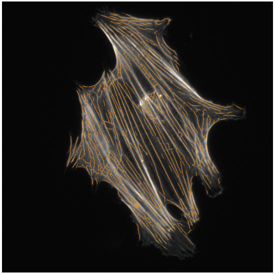

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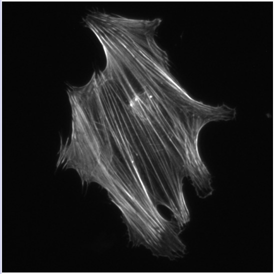

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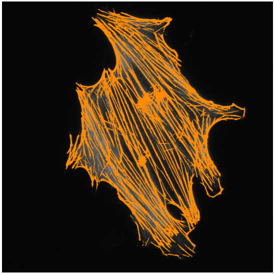

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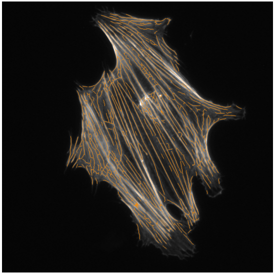

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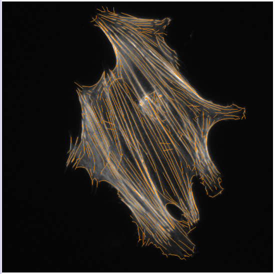
Ground truth?

Methods Against Ground Truth



Manually expert marked ground truth database

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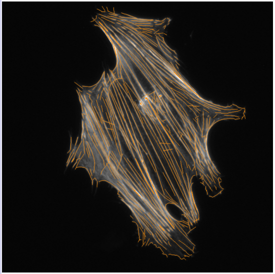

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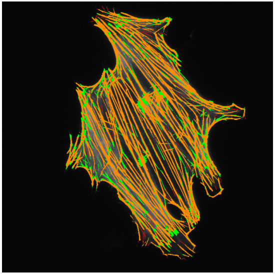
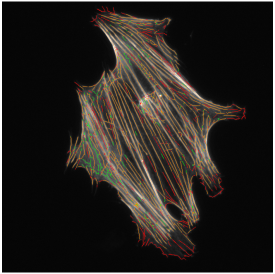


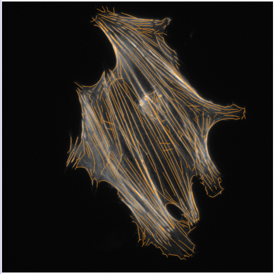
eLoGs



CRD

- Yellow: correctly traced
- Green: false detects
- Red: not detected

Tracing: The Filament Sensor



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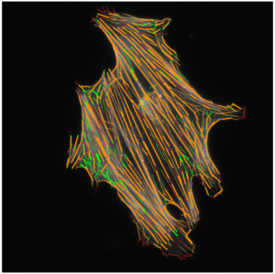

Filament sensor

- individual filaments: offset, length, angle, width
- incorporate expert knowledge

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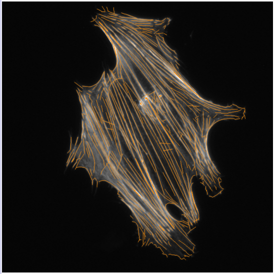

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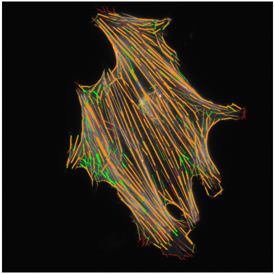

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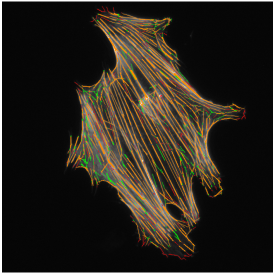
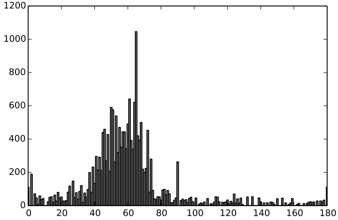


Filament sensor with expert knowledge

- individual filaments: offset, length, angle, width
- incorporate expert knowledge
- 20 secs per image (eLoG: 20 mins, CRD: 20 hrs)

Angular Histograms



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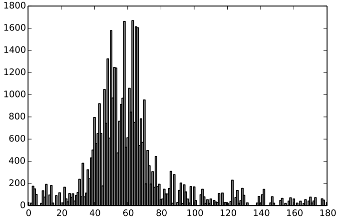
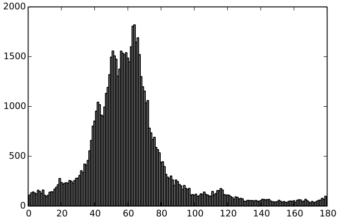
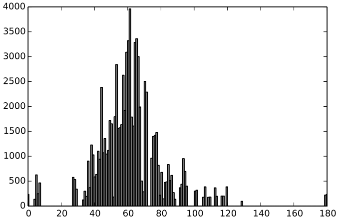


line sensor

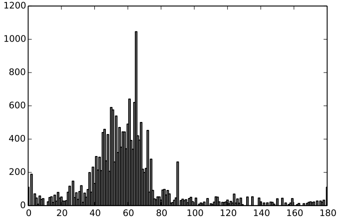


eLoGs



Hough transform

Angular Histograms



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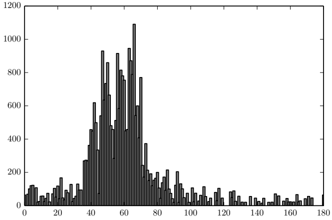
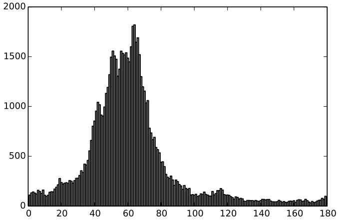


expert knowledge line sensor



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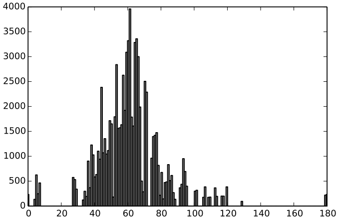
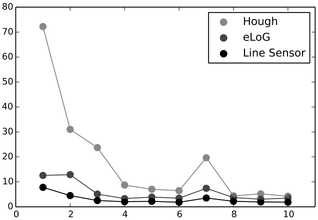
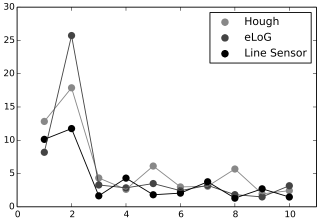


Hough transform

Benchmarking

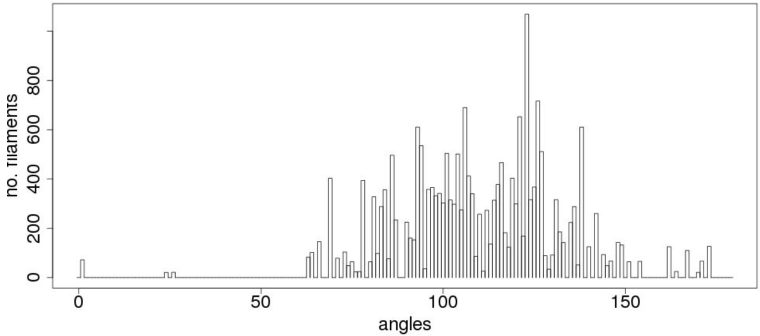




Histogram mass ratios   Normalized histogram distances

Movie

How Many Modes?



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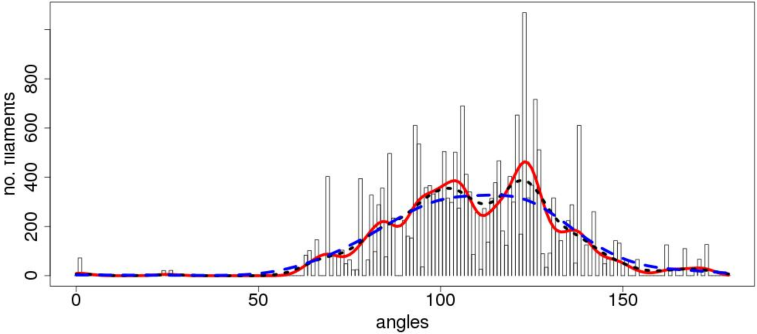

After kernel smoothing:

- six modes ($h = 2$)?
- Two modes ($h = 5$)?
- One mode ($h = 10$)?
- What is the right scale (bandwidth h)?
- How persistent are modes over bandwidths?

The Linear Scale Space / SiZer of Chaudhuri and Marron (1999, 2000)

- Unknown density $f : \mathbb{R} \to \mathbb{R}^+$,
- f_n its empirical histogram,
- $\hat{f}_n^{(h)} := g^{(h)} * f_n$ its kernel smoothed version,
- $\hat{f}^{(h)} := g^{(h)} * f$ the true kernel smoothed version,
- all with bandwidth $h \in \mathbb{R}^+$.

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- all with bandwidth $h \in \mathbb{R}^+$.
- We have confidence that $\hat{f}_n^{(h)}$ has a mode "around" $t \in \mathbb{R}$ if $\exists \epsilon_1, \epsilon_2 > 0$ such that

$$\partial_t \hat{f}_n^{(h)}(t + \epsilon_2) < 0 < \partial_t \hat{f}_n^{(h)}(t - \epsilon_1)$$

with significance.

The Linear Scale Space / SiZer

(a) If $\left(\partial_t \hat{f}_n^{(h)}(t)\right)_{h,t} \to \partial_t f^{(h)}$ weakly

- obtain asymptotic confidence levels for the number modes of $f^{(h)}(t)$.

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$$\sharp \text{ modes of } f^{(h)} \leq \sharp \text{ modes of } f^{(h')} \; \forall h \geq h' > 0$$

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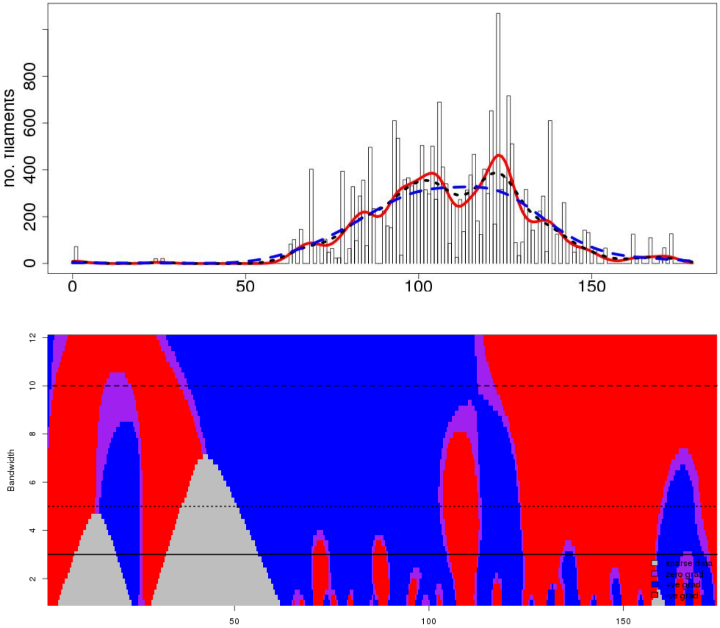
Theorem (Chaudhuri and Marron (1999, 2000))

If f is sufficiently regular and $g^{(h)}$ the Gaussian heat kernel then causality holds and

$$\sqrt{n}\left(\partial_t \hat{f}_n^{(h)}(t) - \partial_t \hat{f}^{(h)}(t)\right) \to (G_h)_t \textit{ weakly}$$

with a Gaussian process $(G_h)_t$.

The SiZer Map



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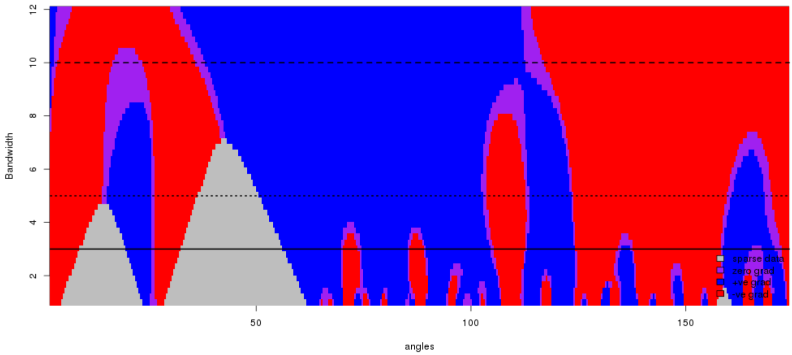

- Many (noisy) modes for $h \leq 4$
- Four modes persist from $h = 4$ to $h = 7$
- Two modes from $h = 7$ to $h = 15$
- One mode from $h = 15$ to $h = \infty$

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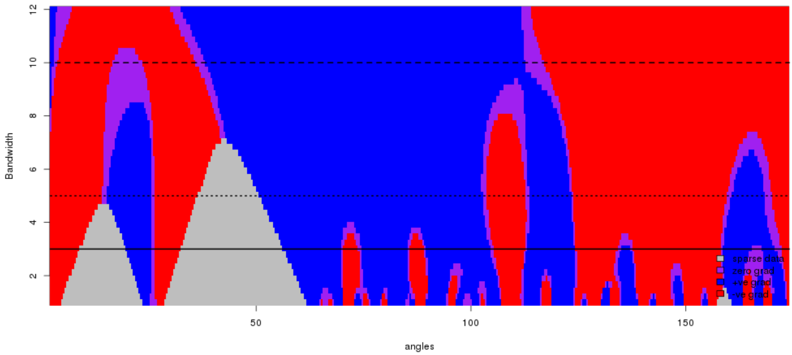

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- One mode from $h = 15$ to $h = \infty$
- The data is cyclic!

The Circular SiZer

Which smoothing kernel on the circle $[-\pi, \pi)$ gives

1. empirical scale space tube $\to$ Gaussian process?
2. causality of the scale space tube?

$\Rightarrow$ confidence bounds from below for number of true modes.

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1. Kernels with second moments, e.g. the von Mises density, making the CircSiZer by Oliveira et al. (2013):

$$m_\kappa(x) := \frac{1}{2\pi\, I_0(\kappa)}\, e^{\kappa \cos(x)}\,.$$

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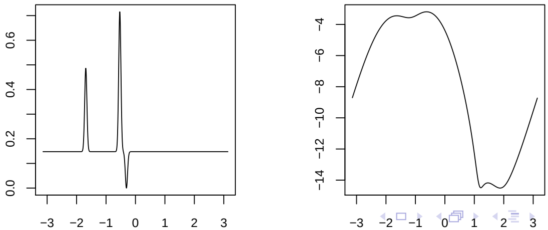
2. Not the CircSiZer (cf. also Munk (1999)):



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2. **Theorem (The WiZer)**

The solution of the circular heat equation: the wrapped Gaussian

$$g_h^{(w)}(x) := \sum_{m=-\infty}^{\infty} \frac{1}{\sqrt{2\pi}\, h}\, e^{-\frac{(x+2\pi m)^2}{2h^2}}\,.$$

guarantees causality of the scale space tube.

Circular Scale Space Axiomatics

A family $\{L_h : h > 0\}$ of convolution kernels ($\int L_h = 1$) is

- a semi-group if $L_{h+h'} = L_h * L_{h'}$ for all $h, h' > 0$
- causal if $S(L_h * f) \leq S(f)$ for all f
- strongly Lipschitz if $\exists r > 0$

$$\forall \epsilon > 0 \; \exists h_0 = h_0(\epsilon) > 0 \text{ such that } |(\mathcal{F}L_h)_k - 1| < \epsilon h |k|^r$$

for all $k \in \mathbb{Z}$ and all $0 < h \leq h_0$.

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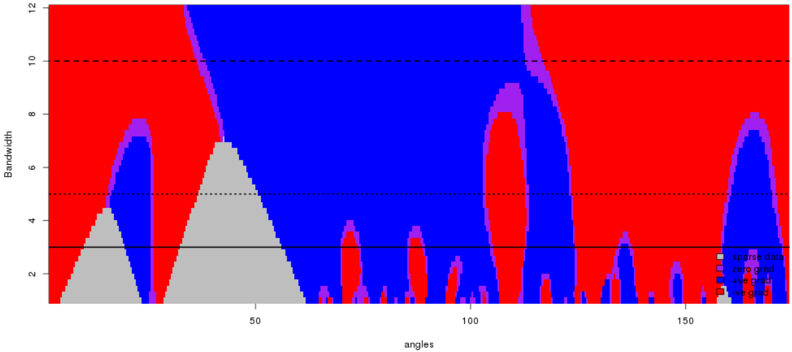
Theorem

The only casual and strongly Lipschitz semi-group on the circle is given by the wrapped Gaussians.

For Euclidean analogs, e.g. Weickert et al. (1999); Lindeberg (2011).

The WiZer Map



- Many (noisy) modes for $h \leq 4$
- Four modes persist from $h = 4$ to $h = 7$

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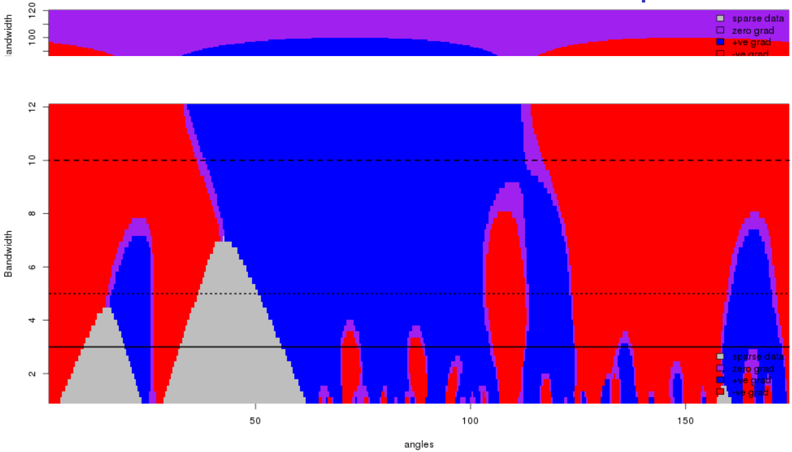
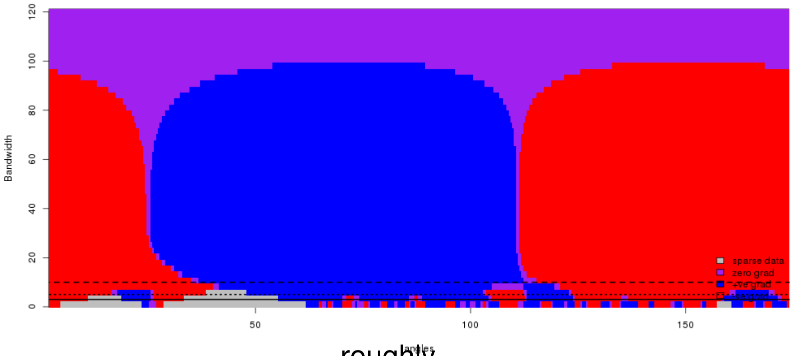


- Many (noisy) modes for $h \leq 4$
- Four modes persist from $h = 4$ to $h = 7$
- One mode from $h = 8$ to $h = 100$
- No mode from $h = 100$ to $h = \infty$

Persistence of Modes



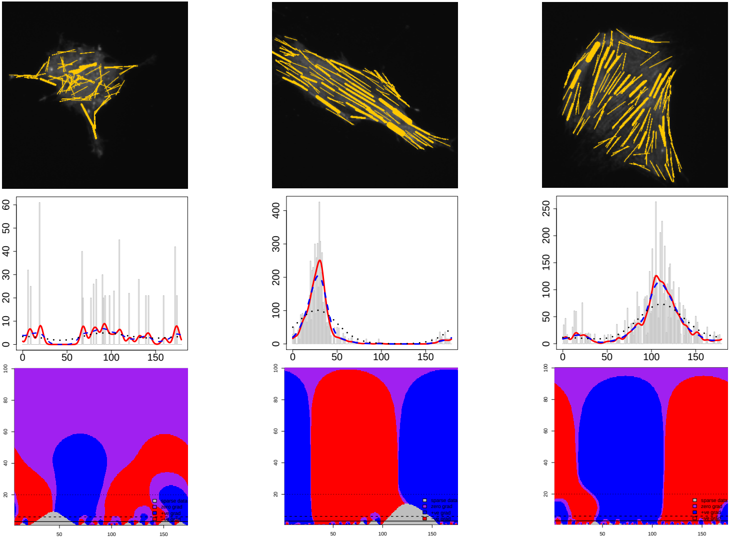
roughly

$h \in$	$(h_0, 4)$	$(4, 7)$	$(8, 100)$	$(100, \infty)$
	many modes	4 modes	1 mode	0 modes

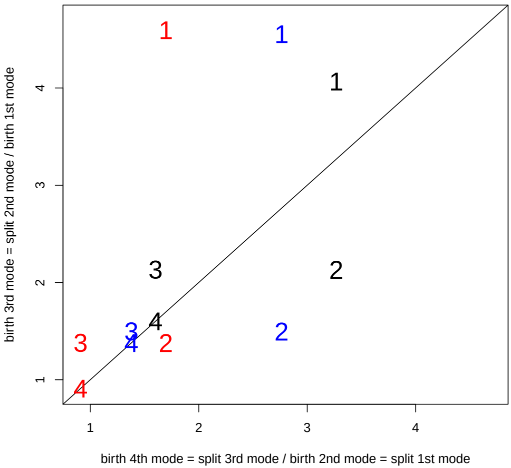
How to measure persistence?

- Not within a single WiZer map
- but across several WiZer maps.

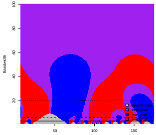
Three Elasticities



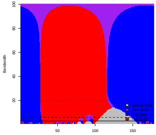
Persistence Diagram of Modes



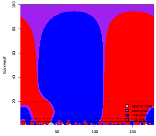
black:



red:

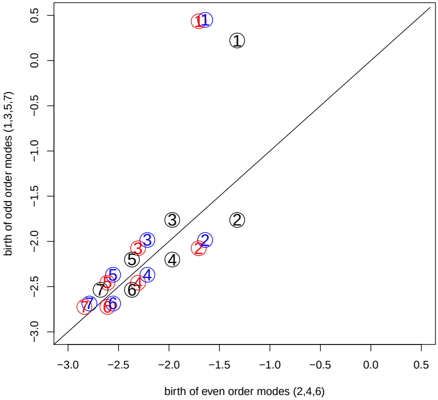


blue:



Appliction: Log Persistence Diagram

Data: $\approx$ 60 cells each of 1 kPa (black), 11 kPa (red) and 34 kPa (blue) after 24 hrs. with respective means.



- 1 kPa: least persistent first mode, most persistent higher modes,
- 11 kPa: least persistent modes,
- 34 kPa: almost like 11 kPa but intermediate persistent modes

Summary and Outlook

- Good data: entire cell filament process
- New circular scale space theory
- Bound the number of shape features from below with confidence:
  - above a given bandwidth,
  - truly statistical,
  - bound number of shape features simultaneously over all bandwidth (Max's master thesis)
- Corroborating early biomechanically induced stem cell differentiation.

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- Good data: entire cell filament process
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- Corroborating early biomechanically induced stem cell differentiation.
- Outlook:
  - Include locality, statistics of more than just $\sharp$ modes,
  - statistics of bounded inhomogeneous filament processes
  - temporal evolution of filaments:
    from mode hunting $\to$ change point hunting

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Mode Persistence Boxplots