

IMAGE ANALYSIS ON BIOPOLYMER NETWORKS

CHARACTERIZATION USING GRAPHS

Pablo Hernandez-Cerdan

Main Advisor: Prof. M.A.K Williams

Leipzig University – November 16, 2015

PhD. Student

Institute of Fundamental Sciences, Massey University

MacDiarmid Institute for Advanced Materials and Nanotechnology

Riddet Institute

New Zealand

TABLE OF CONTENTS

1. Motivation: Role of network architecture
2. Gathering structure data:
3. Image Analysis
4. Graph Characterization

MOTIVATION: ROLE OF NETWORK AR- CHITECTURE

SOURCES OF STRAIN-STIFFENING

- [Storm et al., 2005]: strain stiffening arises from non linearity of single chains. Under the assumption that the network is isotropic and homogeneous. What does happens under other architectures?
- [Onck et al., 2005], at the same time an alternative explanation of strain stiffening arised exclusively from network connectivity and relative orientation of the fibers.

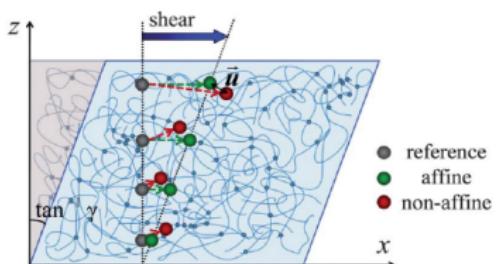
STRAIN-STIFFENING: VALIDITY OF AFFINE DEFORMATION APPROXIMATION IN SEMI-FLEXIBLE POLYMERS

Affine transformations valid when:

In Homogeneous and Isotropic Network

- Stiff Chains
- Dense Networks

[Wilhelm and Frey, 2003]



[Basu et al., 2011; Wen et al., 2012]

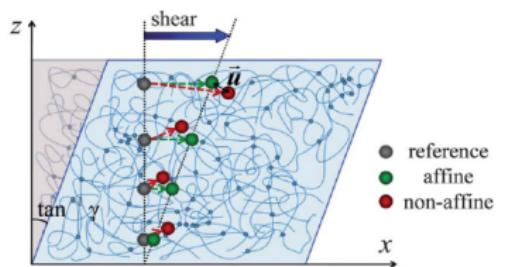
STRAIN-STIFFENING: VALIDITY OF AFFINE DEFORMATION APPROXIMATION IN SEMI-FLEXIBLE POLYMERS

Non-Affine range when:

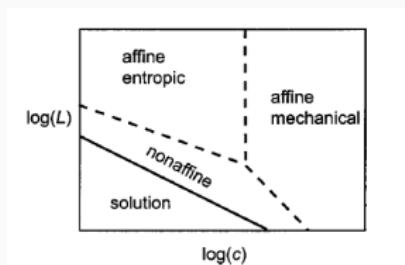
In Homogeneous and Isotropic Network

- Compliant Chains
- Coarse Networks

[Wilhelm and Frey, 2003]



[Basu et al., 2011; Wen et al., 2012]



L: Molecular Weight, c: Concentration
[Head et al., 2005]

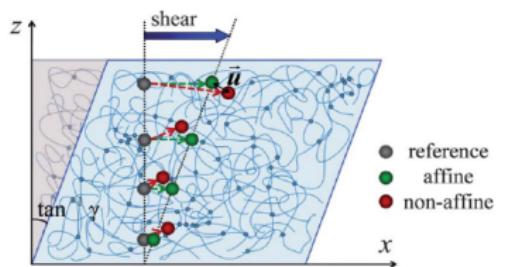
STRAIN-STIFFENING: VALIDITY OF AFFINE DEFORMATION APPROXIMATION IN SEMI-FLEXIBLE POLYMERS

Affine transformations valid when:

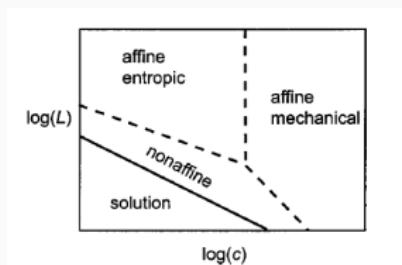
In Homogeneous and Isotropic Network

- Stiff Chains
- Dense Networks

[Wilhelm and Frey, 2003]



[Basu et al., 2011; Wen et al., 2012]



L: Molecular Weight, c: Concentration
[Head et al., 2005]

Pre-stress and architecture role:

Affine approx. is valid at constrained networks

- Initial pre-stress constrains networks. [Cioroianu et al., 2015]

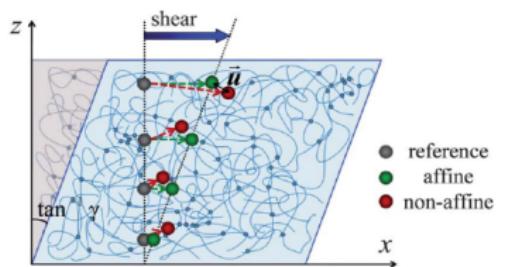
STRAIN-STIFFENING: VALIDITY OF AFFINE DEFORMATION APPROXIMATION IN SEMI-FLEXIBLE POLYMERS

Affine transformations valid when:

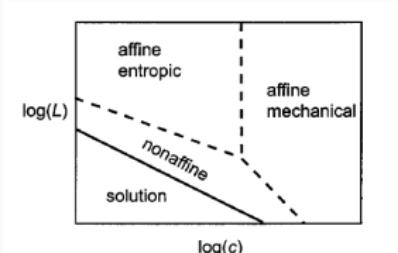
In Homogeneous and Isotropic Network

- Stiff Chains
- Dense Networks

[Wilhelm and Frey, 2003]



[Basu et al., 2011; Wen et al., 2012]



L: Molecular Weight, c: Concentration
[Head et al., 2005]

Pre-stress and architecture role:
Affine approx. is valid at constrained networks

- Initial pre-stress constrains networks. [Cioroianu et al., 2015]
- Role of Anisotropy or Architecture?

GOALS AND RESEARCH QUESTIONS

- Going beyond the homogeneous and isotropic model of biopolymer networks: **Characterize the architecture** of connected filaments using images from different microscopy techniques depending on the size of the biopolymer.

GOALS AND RESEARCH QUESTIONS

- Going beyond the homogeneous and isotropic model of biopolymer networks: **Characterize the architecture** of connected filaments using images from different microscopy techniques depending on the size of the biopolymer.
- How different architectures do influent the **mechanical properties**? Are completely different biopolymers sharing similar geometries?

GOALS AND RESEARCH QUESTIONS

- Going beyond the homogeneous and isotropic model of biopolymer networks: **Characterize the architecture** of connected filaments using images from different microscopy techniques depending on the size of the biopolymer.
- How different architectures do influent the **mechanical properties**? Are completely different biopolymers sharing similar geometries?
- Develop an open-source, free, user friendly, tested, and well documented software for others to use with microscopy images.

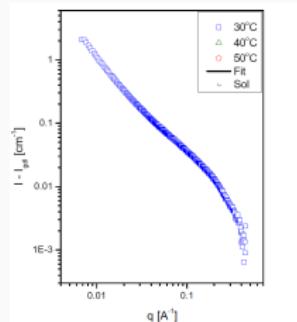
STEPS TO CHARACTERIZE THE NETWORK

- Gathering the images. Microscopy.
- Image analysis. Skeletonization.
- Characterize the image with a graph representation.

GATHERING STRUCTURE DATA:

SCATTERING: SAXS AND SANS

- Study of peaks and fractality at different q values provides an averaged structural information (mesh size, width of single-chains)
- It does not require special sample preparation.
- Good statistics, fast, allow study of dynamics.
- **But**, it does not provide explicit 3D structure of exact connectivity.



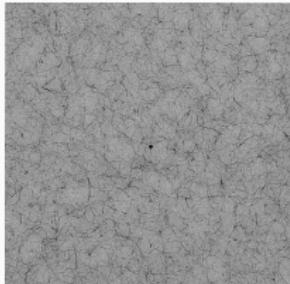
I vs q Pectin from SAXS

MICROSCOPY: TEM AND CONFOCAL

To get better architecture we need 3D microscopy.

TEM tomography

- Necessary to reach polysaccharides scale.
- Sample preparation is hard. Artifacts?
- **Working on validation method** comparing it with scattering.



Confocal

- Suitable for most protein biopolymer networks.
- Sample preparation is more reliable.

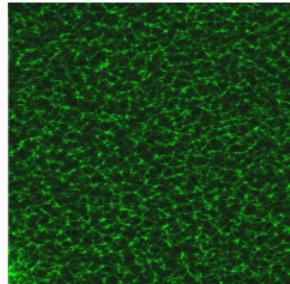


IMAGE ANALYSIS

SKELETONIZATION I: RIDGES OF DISTANCE MAP

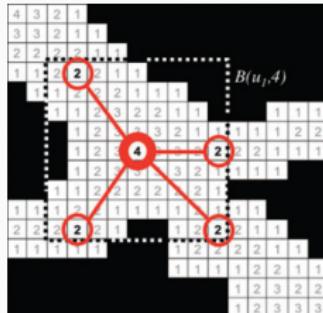
Goal:

Process raw image data to get a spatial graph representation of the network.

Anisotropic denoise: Denoise conserving edges.

Binarization: Choose threshold that best conserves architecture.

Distance Map: Distance to the background for each pixel.



SKELETONIZATION I: RIDGES OF DISTANCE MAP

Goal:

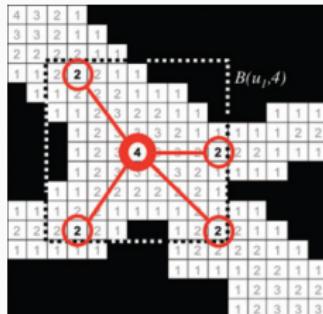
Process raw image data to get a spatial graph representation of the network.

Anisotropic denoise: Denoise conserving edges.

Binarization: Choose threshold that best conserves architecture.

Distance Map: Distance to the background for each pixel.

Ridge Detection: Follow the highest values of the distance map.



SKELETONIZATION I: RIDGES OF DISTANCE MAP

Goal:

Process raw image data to get a spatial graph representation of the network.

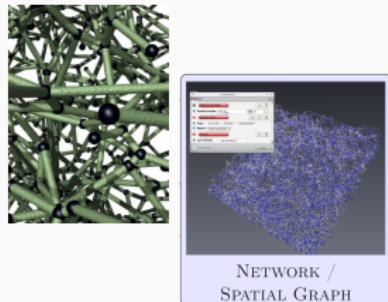
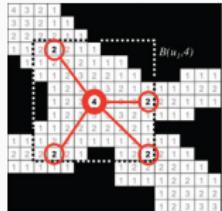
Anisotropic denoise: Denoise conserving edges.

Binarization: Choose threshold that best conserves architecture.

Distance Map: Distance to the background for each pixel.

Ridge Detection: Follow the highest values of the distance map.

Branch Merging and Pruning: Fill gaps and remove short branches.



Pre-processing techniques

1. Hessian Matrix:

Eigenvalues to detect vesselness.

2. Anisotropic Diffusion:

Denoise, keeping edges information.

3. Multiscale

characterization with gaussian operator.

Other Skeleton Methods

1. Open Contours: Snakes

2. Template Matching

3. Morphology Operators

GRAPH CHARACTERIZATION

GRAPHS: A LINK TO COMPLEX SYSTEMS

We can now link our spatial graph to standard complex network libraries.

IGraph C, R, Python (GPL licence)

Boost: Graph and GraphParallel C++ (MIT licence)

NetworkX Python (MIT licence)

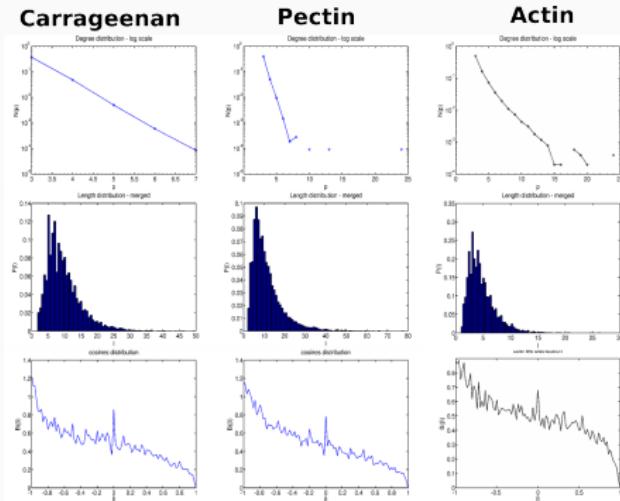


GENERATING SOFTWARE TOOLS

RESULTS: PROOF OF CONCEPT

Three Networks analyzed:

- Polysaccharides using TEM: Pectin, Carrageenan
- Proteins using Confocal: Actin (Collagen from literature [Lindström et al., 2013]).



Degree: Geometrical distribution

$$N(p) = q(1-q)^{p-3}$$

where $q = 1/(Z - 2)$, and Z is the average node degree.

Node-to-node dist.: Log-normal

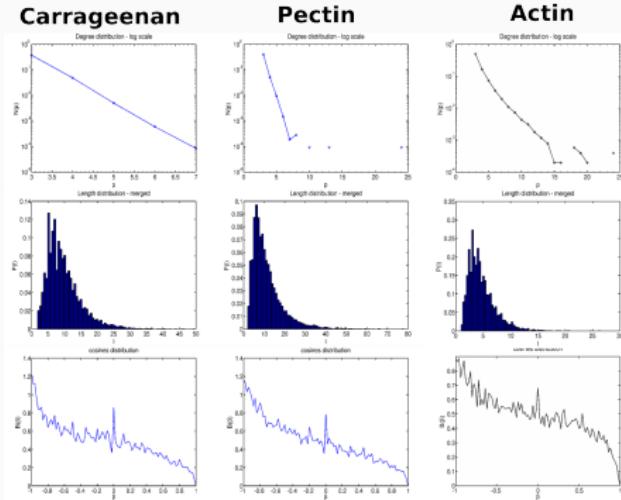
$$P(\ell) = \frac{1}{\ell s \sqrt{2\pi}} \exp\left(-\frac{(\mu - \ln \ell)^2}{2s^2}\right)$$

Edge relative orientation: Power-Series

$$B(\beta) = \sum_{k=1}^m b_k (1-\beta)^{2k-1}$$

RESULTS: PROOF OF CONCEPT

Statistical distributions of local graph properties: **Showing same functional forms**



Degree: Geometrical distribution

$$N(p) = q(1-q)^{p-3}$$

where $q = 1/(Z - 2)$, and Z is the average node degree.

Node-to-node dist.: Log-normal

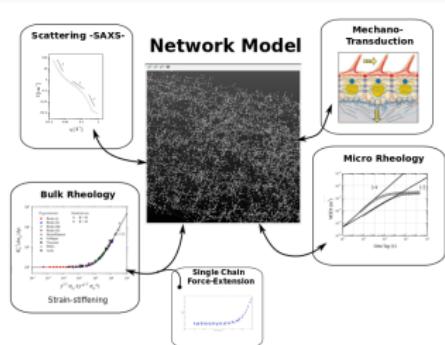
$$P(\ell) = \frac{1}{\ell s \sqrt{2\pi}} \exp\left(-\frac{(\mu - \ln \ell)^2}{2s^2}\right)$$

Edge relative orientation: Power-Series

$$B(\beta) = \sum_{k=1}^m b_k (1-\beta)^{2k-1}$$

Digging up the network geometry

- When does architecture matter?
What is its role at lower scales.
- Gather best image analysis techniques for biopolymers.
- Link to complex network libraries for graph analysis. **Reconstruct in-silico networks from it**
- **The dream** would be to input force-extension curve of single chains + network architecture to get bulk rheology. But initial configuration setup and pre-stress play a role in simulations.



ACKNOWLEDGMENTS



**The MacDiarmid
Institute**
*for Advanced Materials
and Nanotechnology*



Main Advisor: Prof. M.A.K Williams

- Andrew Leis. CSIRO Animal Health. Australia.
- Leif Lundin. SP Food and Bioscience. Sweden.



Brad Mansel, New Zealand

Visit us in NZ or online at:
www.biophysics.ac.nz

THANKS FOR YOUR ATTENTION!

FOLLOW DEVELOPMENT LIVE IN GITHUB

Keep up to date with further development at my github page:

github.com/phcerdan

Get this beamer theme from:

github.com/matze/mtheme



REFERENCES

Anindita Basu, Qi Wen, Xiaoming Mao, T. C. Lubensky, Paul A. Janmey, and A. G. Yodh. Nonaffine Displacements in Flexible Polymer Networks. *Macromolecules*, 44(6):1671–1679, March 2011. ISSN 0024-9297, 1520-5835. doi: 10.1021/ma1026803. URL <http://pubs.acs.org/doi/abs/10.1021/ma1026803.00025>.

Adrian Cioroianu, Ewa Spiesz, and Cornelis Storm. Disorder, pre-stress and non-affinity in polymer 8-chain models. arXiv preprint arXiv:1507.04156, 2015. URL <http://arxiv.org/abs/1507.04156.00000>.

REFERENCES II

- D. Head, A. Levine, and F. MacKintosh. Mechanical response of semiflexible networks to localized perturbations. *Physical Review E*, 72(6), December 2005. ISSN 1539-3755, 1550-2376. doi: 10.1103/PhysRevE.72.061914. URL <http://link.aps.org/doi/10.1103/PhysRevE.72.061914>.
- Stefan B. Lindström, Artem Kulachenko, Louise M. Jawerth, and David A. Vader. Finite-strain, finite-size mechanics of rigidly cross-linked biopolymer networks. *Soft Matter*, 9(30):7302, 2013. ISSN 1744-683X, 1744-6848. doi: 10.1039/c3sm50451d. URL <http://xlink.rsc.org/?DOI=c3sm50451d>.
- P R Onck, T Koeman, T van Dillen, and E van der Giessen. Alternative explanation of stiffening in cross-linked semiflexible networks. *Physical Review Letters*, 95(17):178102, October 2005. ISSN 0031-9007.

REFERENCES III

- Cornelis Storm, Jennifer J. Pastore, F. C. MacKintosh, T. C. Lubensky, and Paul A. Janmey. Nonlinear elasticity in biological gels. *Nature*, 435(7039):191–194, May 2005. ISSN 0028-0836. doi: 10.1038/nature03521. URL <http://www.nature.com/nature/journal/v435/n7039/full/nature03521.html>. 00641.
- Qi Wen, Anindita Basu, Paul A. Janmey, and Arjun G. Yodh. Non-affine deformations in polymer hydrogels. *Soft Matter*, 8(31):8039–8049, July 2012. ISSN 1744-6848. doi: 10.1039/C2SM25364J. URL <http://pubs.rsc.org/en/content/articlelanding/2012/sm/c2sm25364j>. 00025.
- Jan Wilhelm and Erwin Frey. Elasticity of Stiff Polymer Networks. *Physical Review Letters*, 91(10):108103, September 2003. doi: 10.1103/PhysRevLett.91.108103. URL <http://link.aps.org/doi/10.1103/PhysRevLett.91.108103>. 00240.