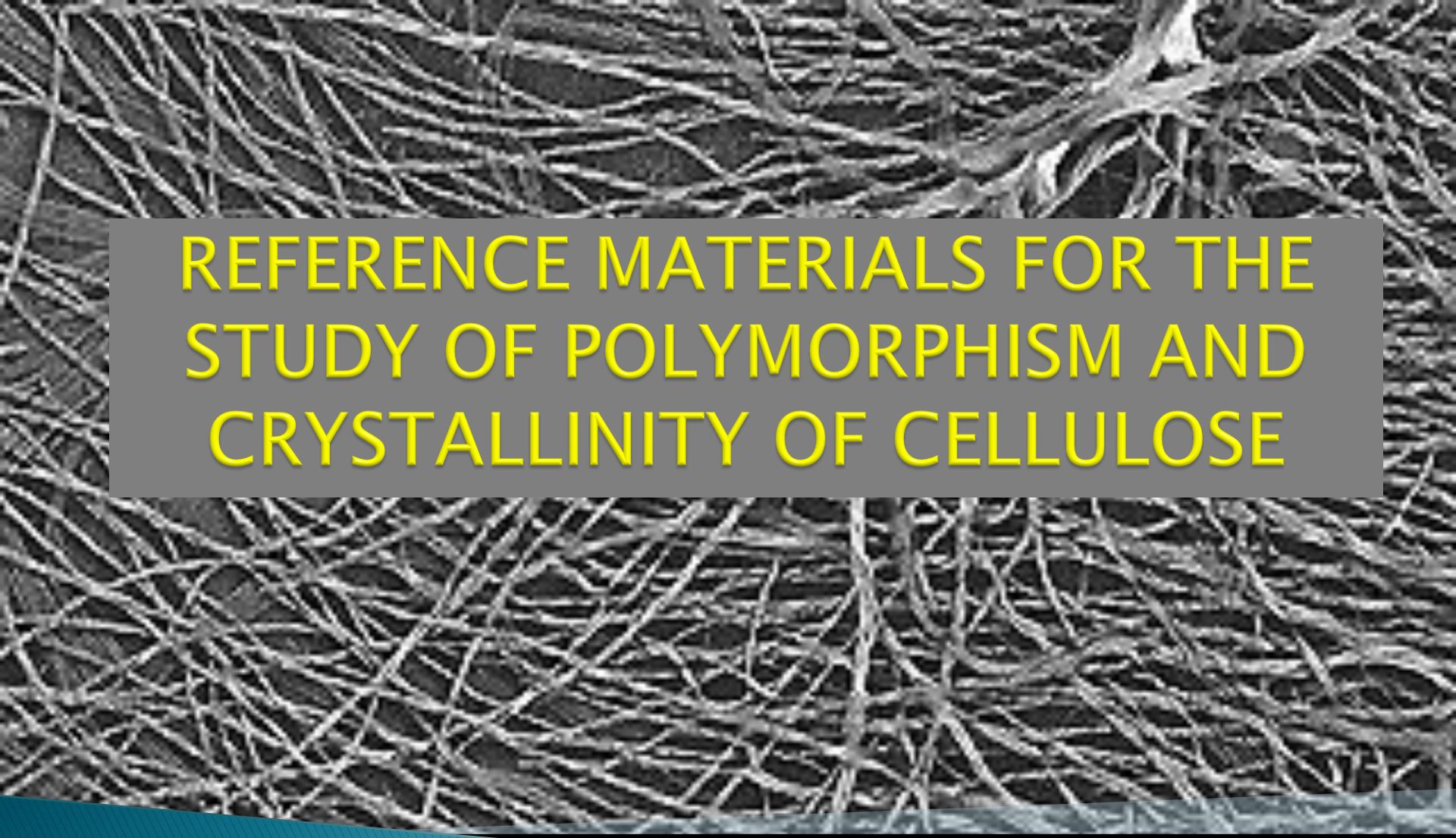

REFERENCE MATERIALS FOR THE STUDY OF POLYMORPHISM AND CRYSTALLINITY OF CELLULOSE

T. G. Fawcett, C. E. Crowder and S. Kabbekodu, International Centre for Diffraction Data

J. A. Kaduk, Poly Crystallography Inc.

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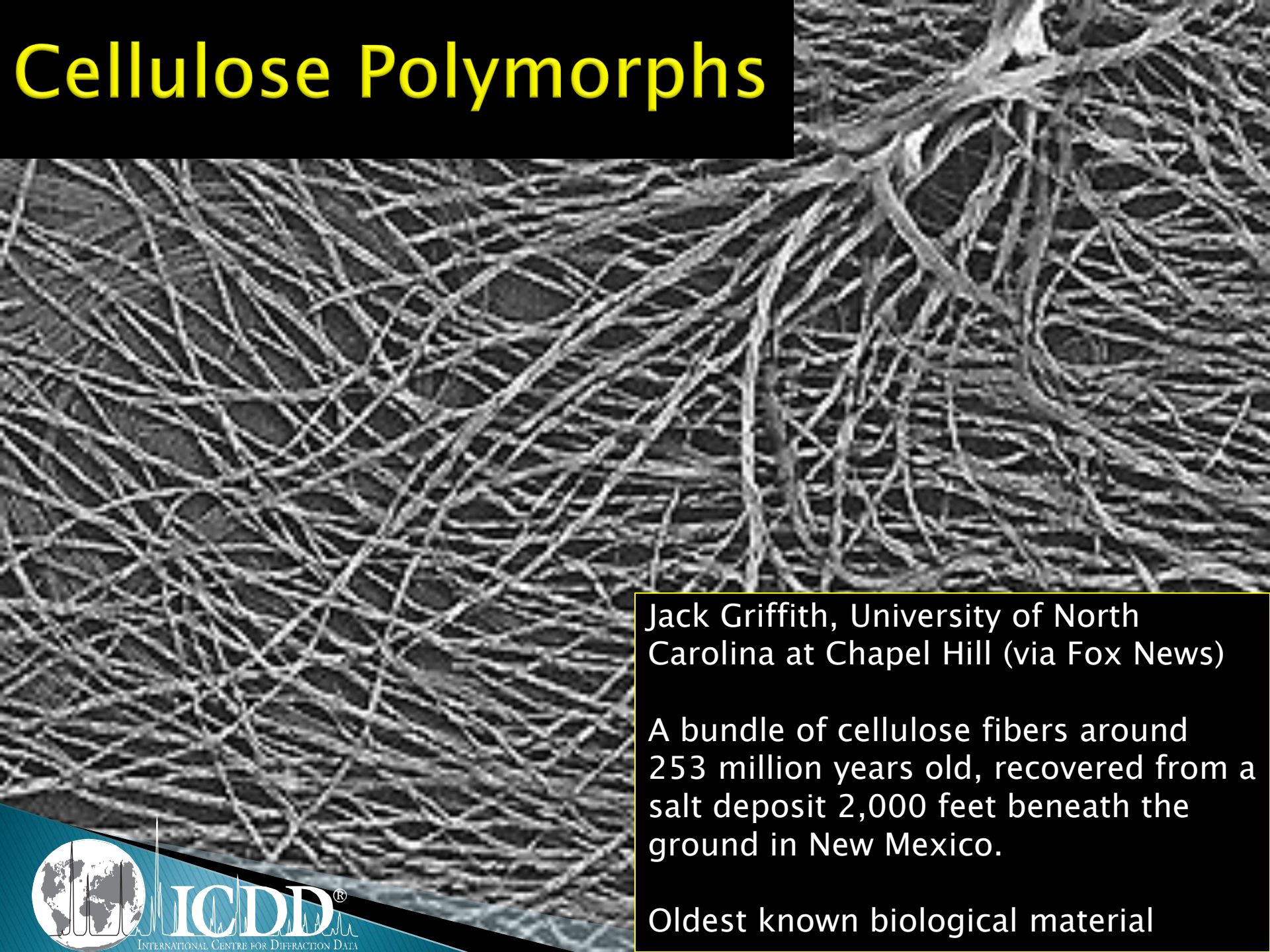
This document was presented at PPXRD - Pharmaceutical Powder X-ray Diffraction Symposium

Sponsored by The International Centre for Diffraction Data

PPXRD Website – www.icdd.com/ppxrd

ICDD Website - www.icdd.com

Cellulose Polymorphs



Jack Griffith, University of North Carolina at Chapel Hill (via Fox News)

A bundle of cellulose fibers around 253 million years old, recovered from a salt deposit 2,000 feet beneath the ground in New Mexico.

Oldest known biological material

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Collaborators and Contributors

- Jim Kaduk, Poly Crystallography Inc
- Tom Blanton, Eastman Kodak Co.
- Ewa Bucher, International Paper Company
- Fangling Needham, ICDD
- Cam Hubbard, Oak Ridge National Laboratory
- Valeri Petkov, Central Michigan University
- Roman Shpenchanko, Moscow State University

- Bruker-AXS & Glascow University (PolySnap), PANalytical (HighScore Plus), CrystalMaker Software LTD (CrystalMaker)
- Rigaku, Bruker-AXS, PANalytical, Argonne NL Light Source- instrument time and expertise

Data Collection and analysis

2002-2007

- 12 Pharmacuetical Tablets – Fangling Needham, ICDD clinics, Cam Hubbard, Oak Ridge National Lab, Jim Kaduk, Argonne Light Source
- 3 Natural Products
- 18 Wood Pulps, Cotton Linters – Eva Bucher, International Paper

2010-2011

- 21 Wood chips – Jim Kaduk, Poly Crystallography Inc
- 6 USP references – ICDD editors, Joel Reid and Suri Kabekkodu, ICDD grantees, Victor Petkov, Roman Shpanchenko
- 6 Substituted celluloses – Tom Blanton, Eastman Kodak, Suri Kabekkodu, ICDD

Cellulose Studies – PPXRD

CRYSTAL STRUCTURES AND BONDING IN CELLULOSE POLYMORPHS

James A. Kaduk, BP Chemicals, Naperville IL 60566 and
Paul Langan, Los Alamos National Laboratory, Los Alamos NM 87545

PPXRD-2, 2002 Denver X-ray Conference, 2002, 2007

Elucidation of the **structures of cellulose 1 alpha, cellulose 1 beta and cellulose II.**

Ab-initio refinements constrained by XRD, ED, nmr and SEM data

REEXAMINING STRUCTURE AND CRYSTALLINITY IN CELLULOSE

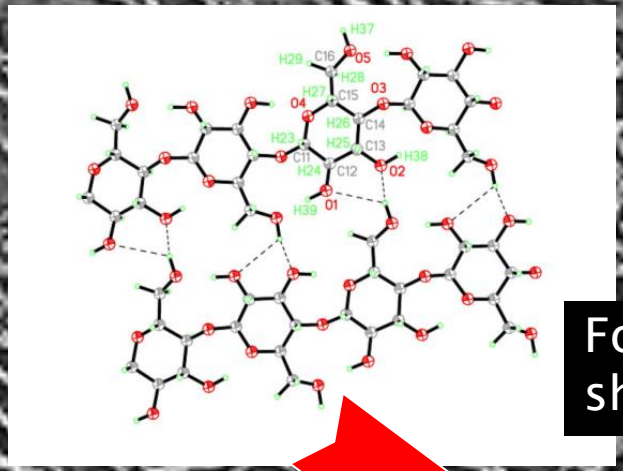
T. G. Fawcett, International Centre for Diffraction Data
J. A. Kaduk, INEOS Technologies
E. Bucher, International Paper Company

PPXRD-6, 2007

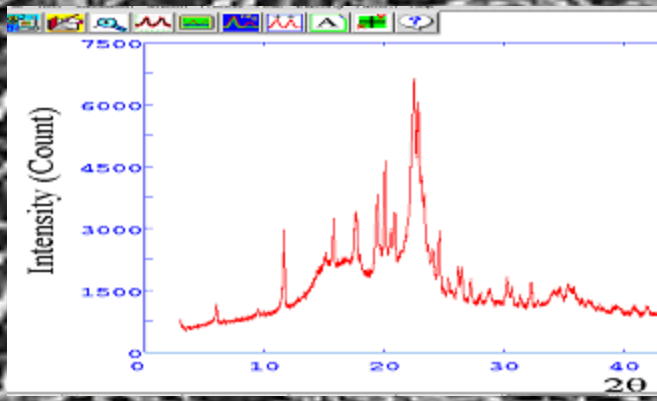
Structures applied to powder patterns and used to identify polymorphism in wood pulps and pharmaceuticals.

Reported the **pattern of amorphous cellulose**

Polymorphs of Cellulose

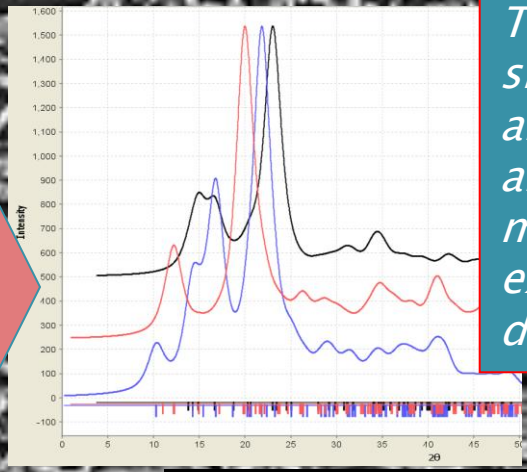


Form 1 beta shown



Pepcid AC

PDF-4+
Faber, Scardi Leone

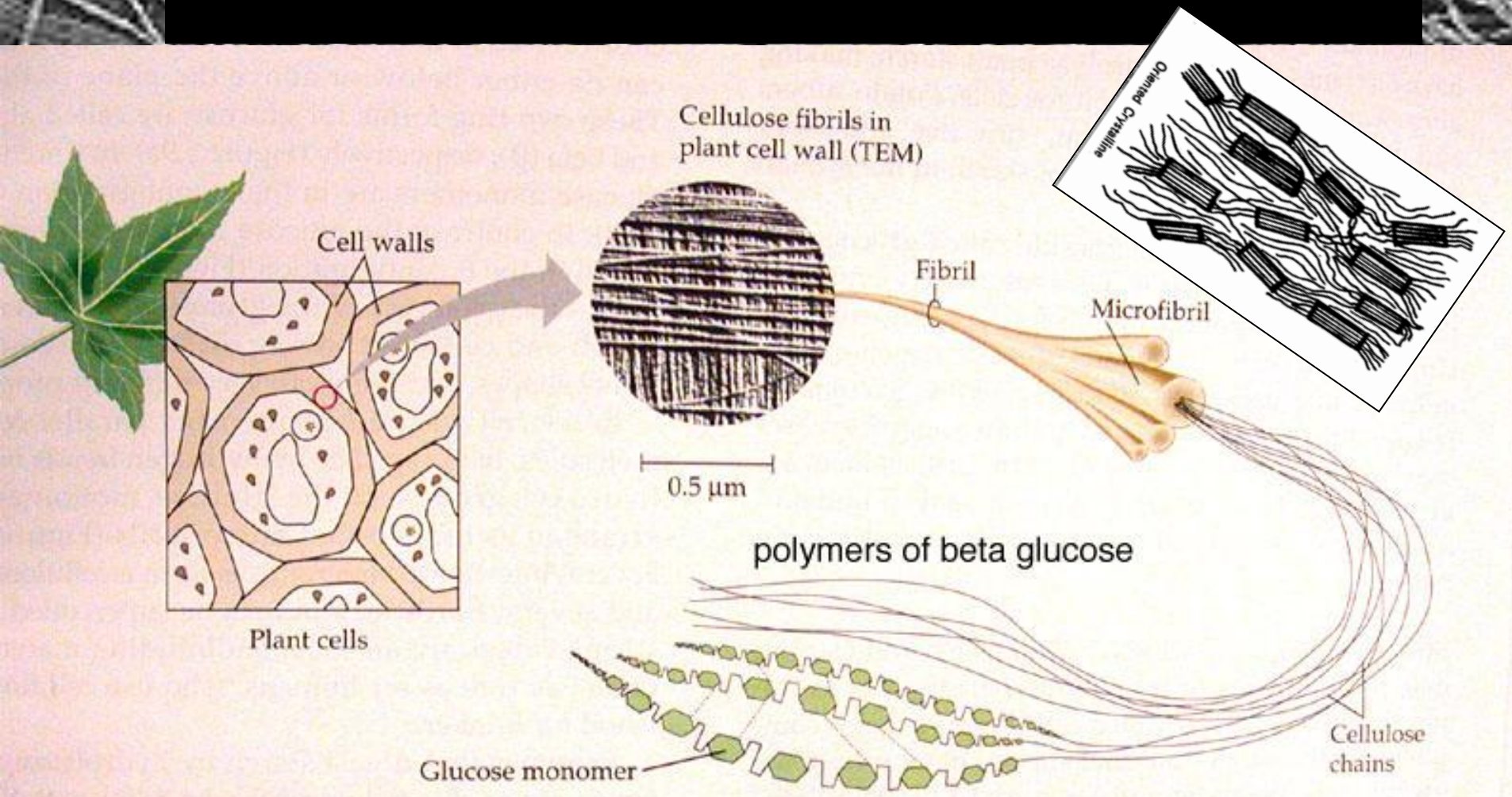


These simulations are exported and used to model experimental data

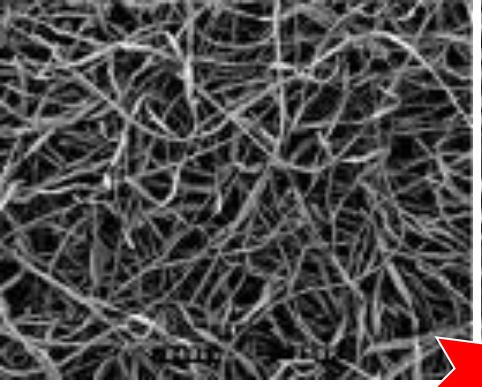
References for Form I alpha, Form I beta and Form II

Simulation of microcrystalline states of cellulose

Macro to micro to nano



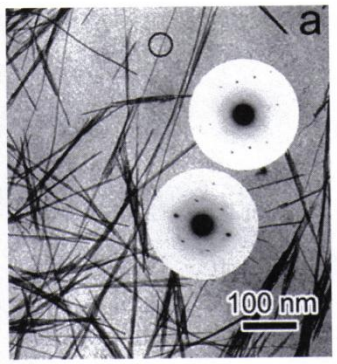
Microscopy
Macro to Micro



Surface of a tissue

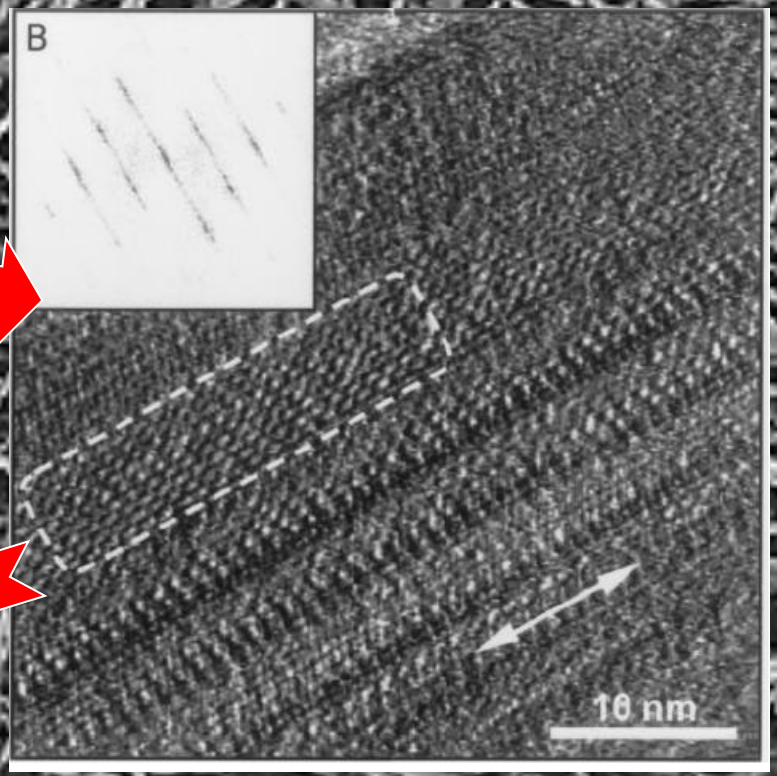
Bundled fibers

Ref. 7



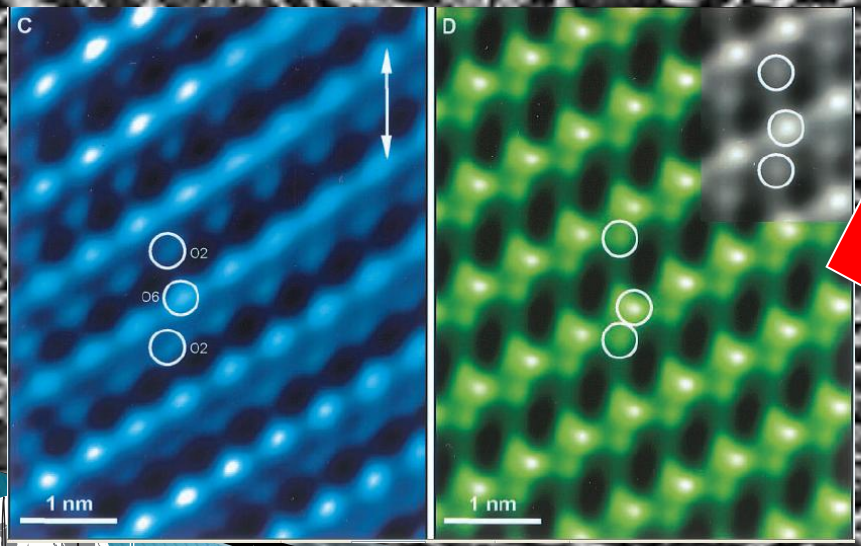
Fibril

AFM of Cellulose I alpha



Baker, Helbert, Sugiyama and Miles

Biophysical Journal Volume 79 August 2000 1139–1145

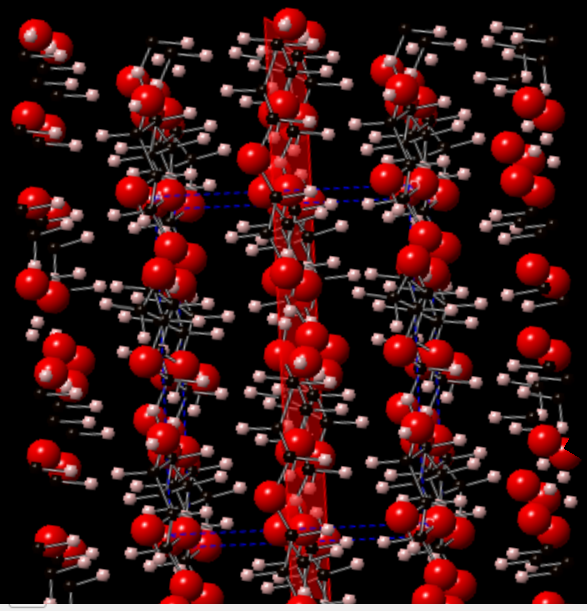


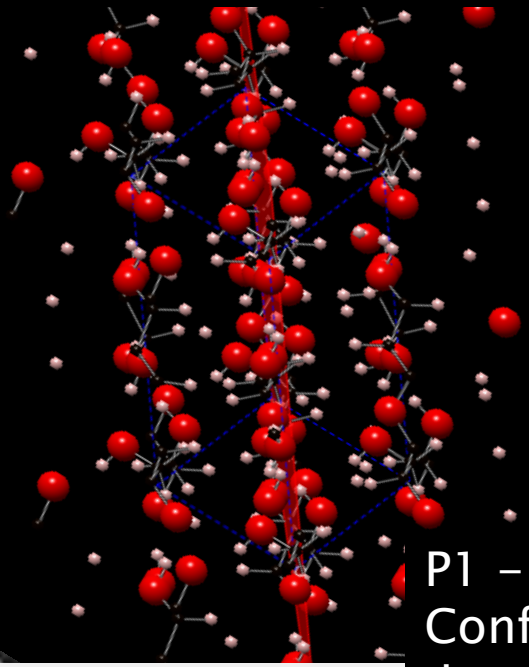
Simulation from AFM® images, oxygens circled

Microfibrils

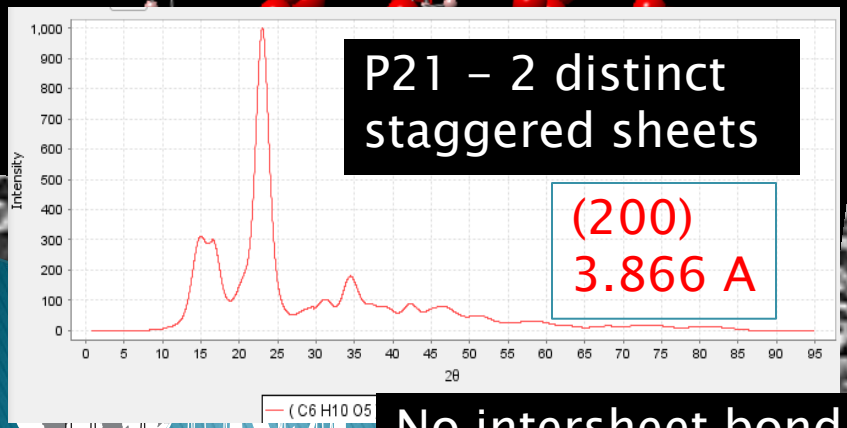
Cellulose 1 beta and alpha

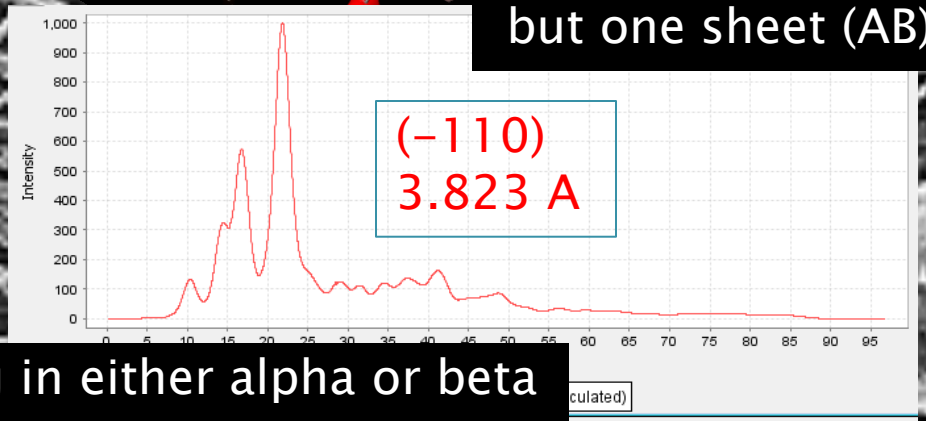
- (200) Cellulose I beta
- (-110) Cellulose 1 alpha





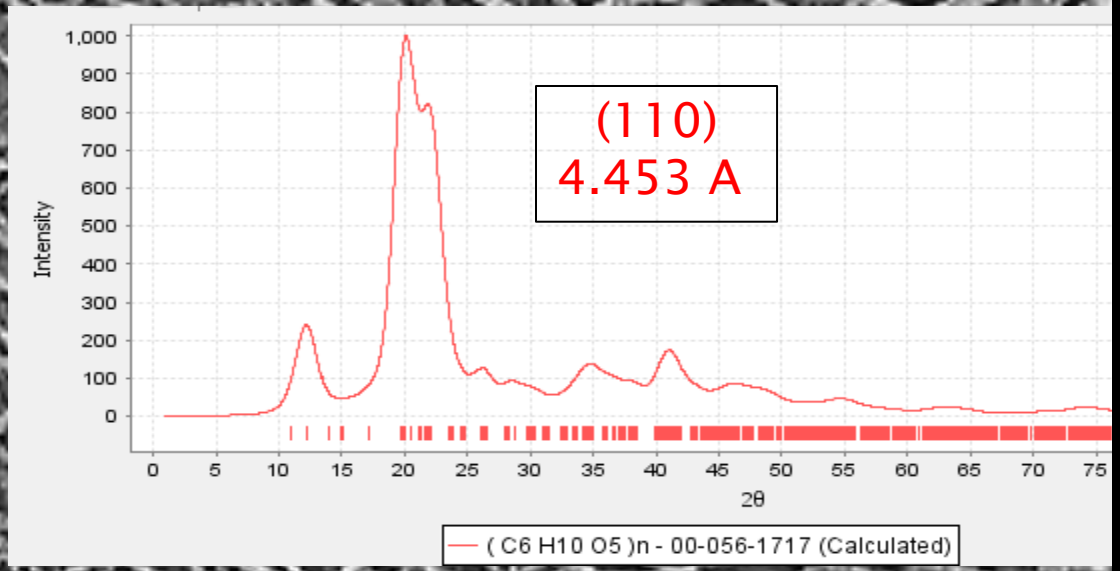
P1 – Alternating Conformers in dimer but one sheet (AB)

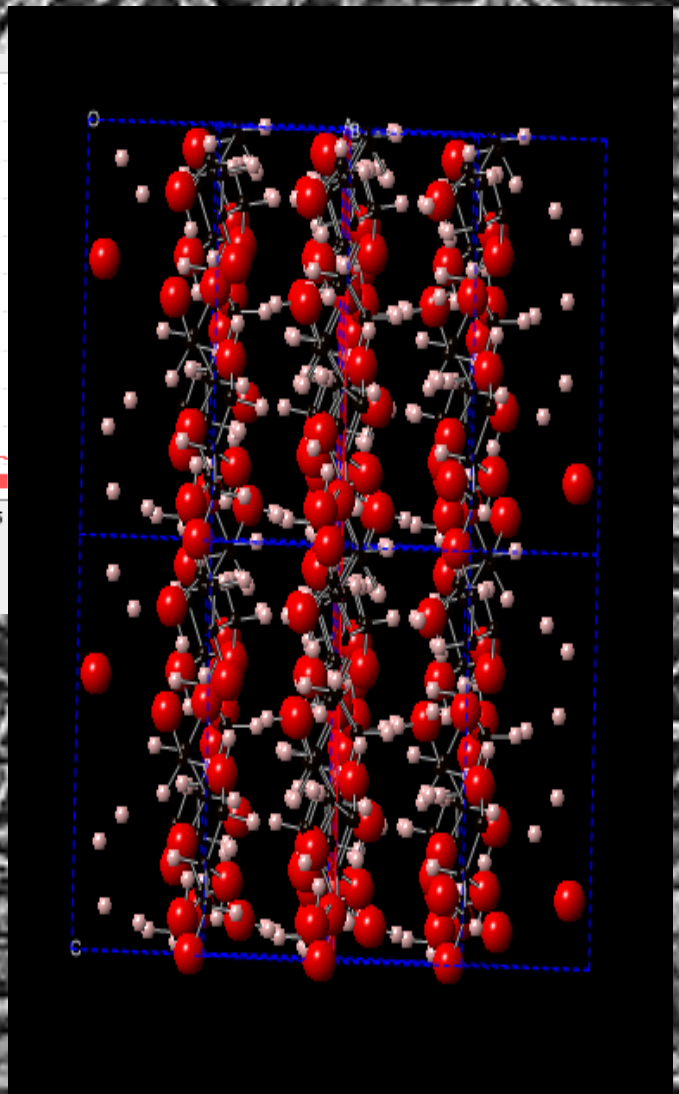




No intersheet bonding in either alpha or beta

Cellulose II





Stable form
Intersheet hydrogen bonding
2 chains (AA or BB) antiparallel
Large –OH disorders (10–30%)

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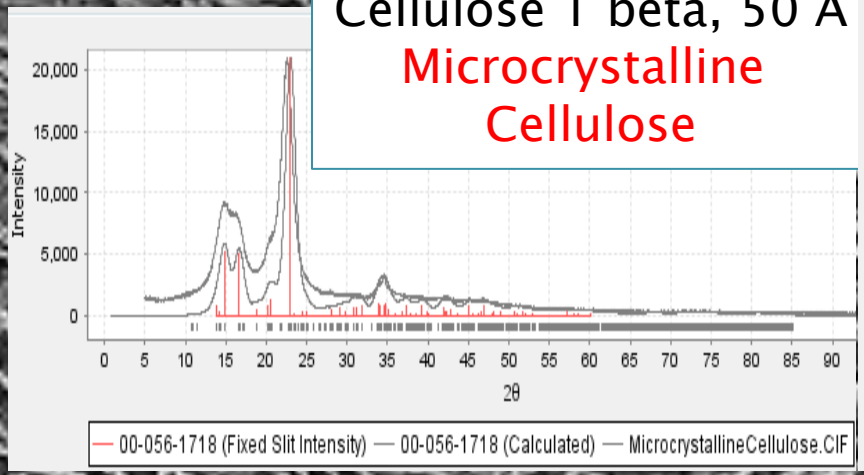
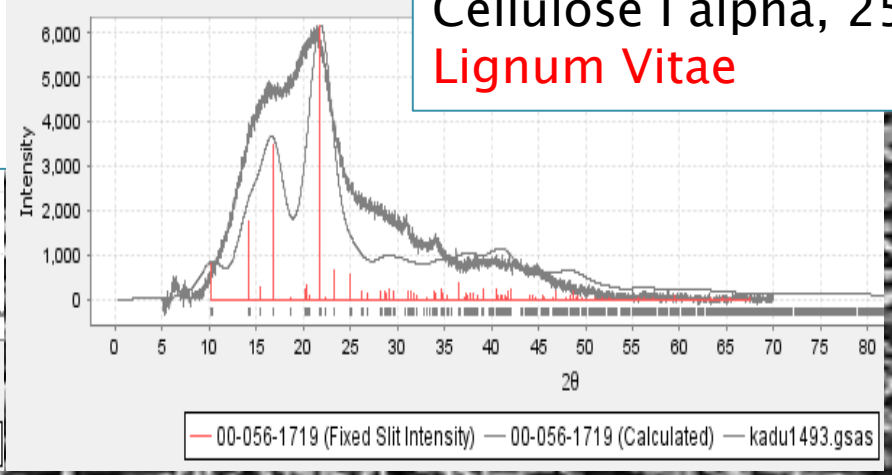
Confidence In Reference Standards

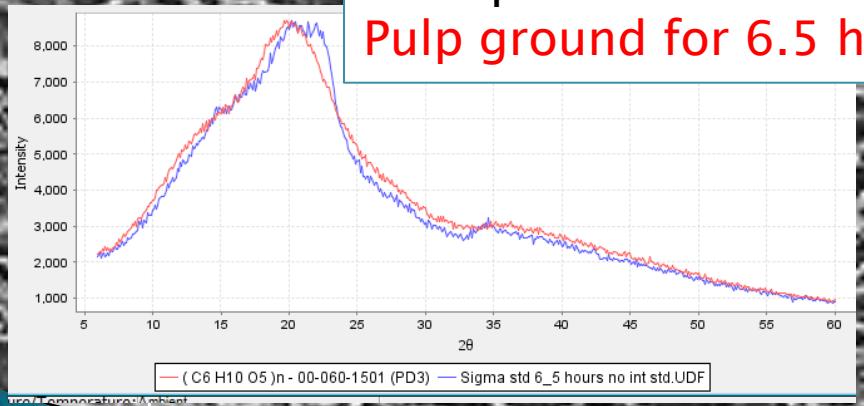
Cellulose 1 beta, 50 A
Microcrystalline Cellulose



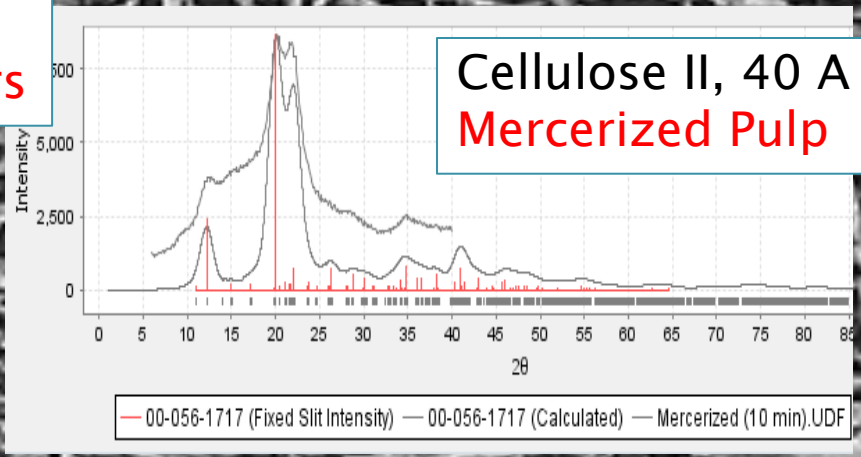
Cellulose I alpha, 25 A
Lignum Vitae



Amorphous Ref
Pulp ground for 6.5 hrs



Cellulose II, 40 A
Mercerized Pulp



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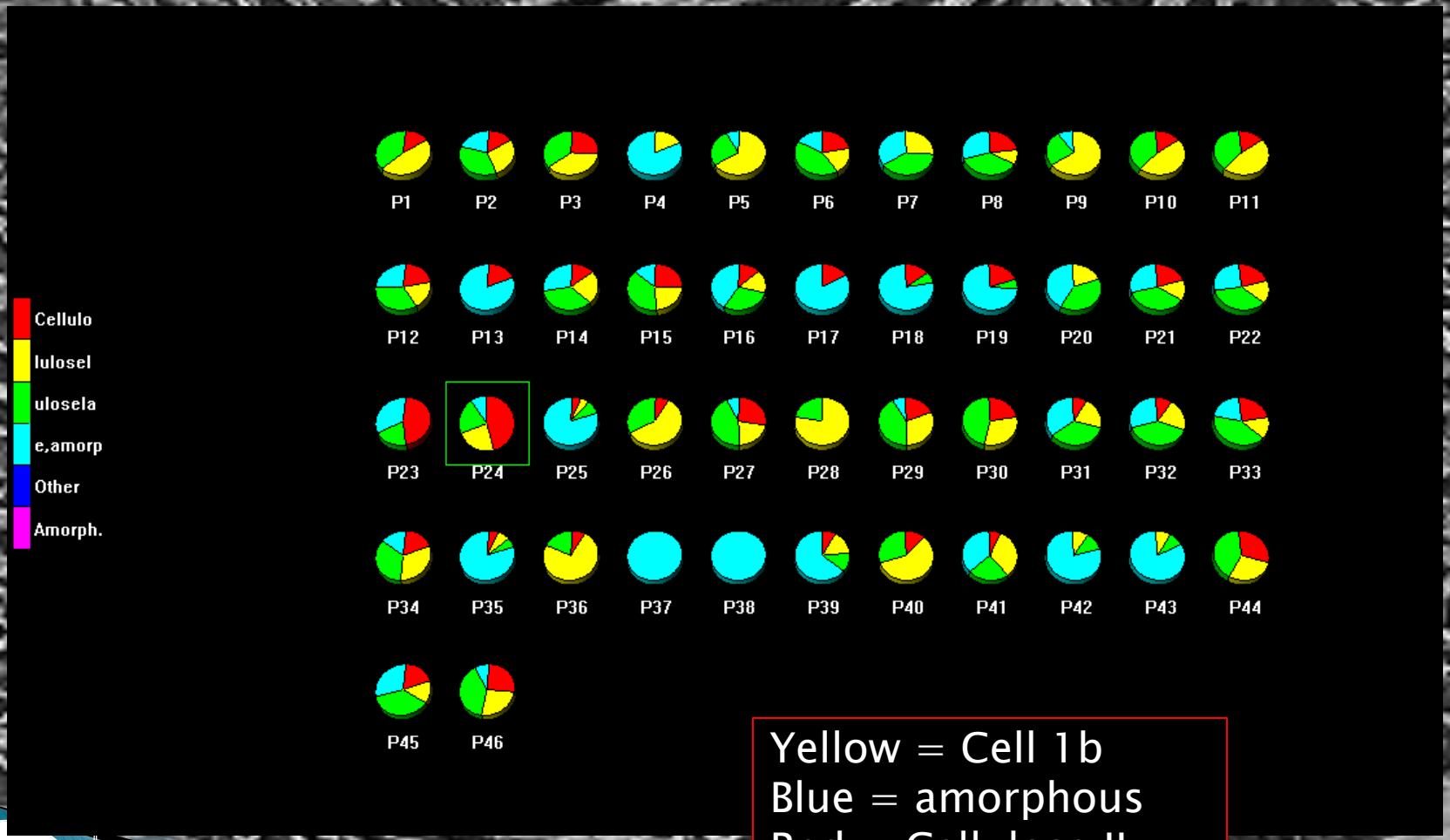
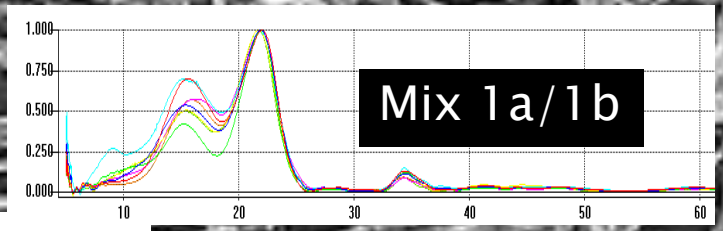
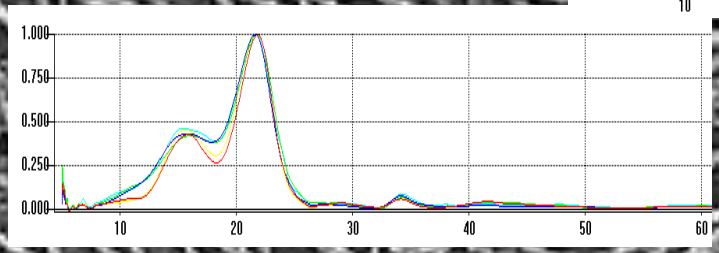
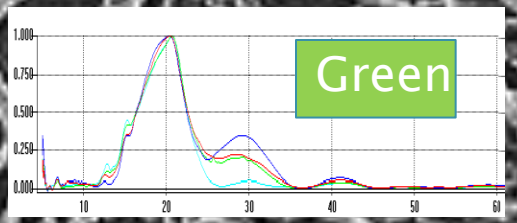
46 Experimental cellulose specimens compared to 4 cellulose standards (50A)

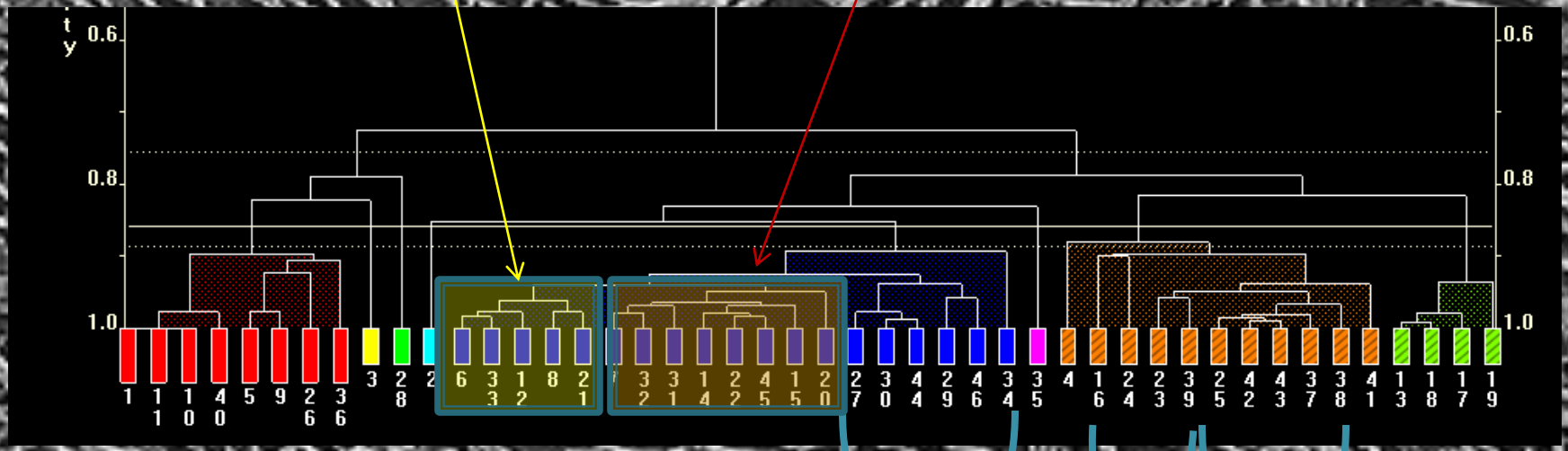


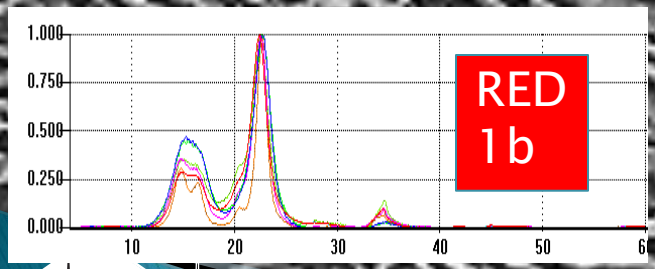
Yellow = Cell 1b
Blue = amorphous
Red = Cellulose II
Green = Cell 1a

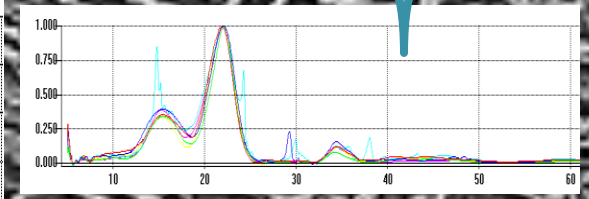


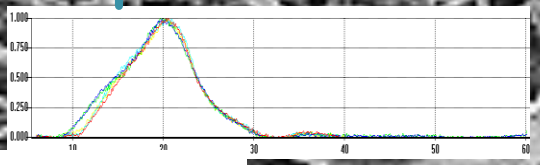


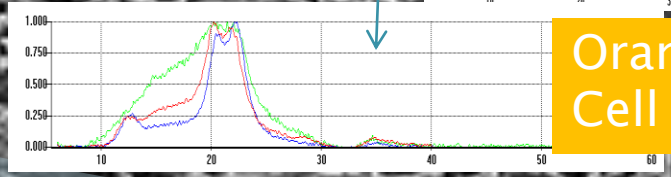












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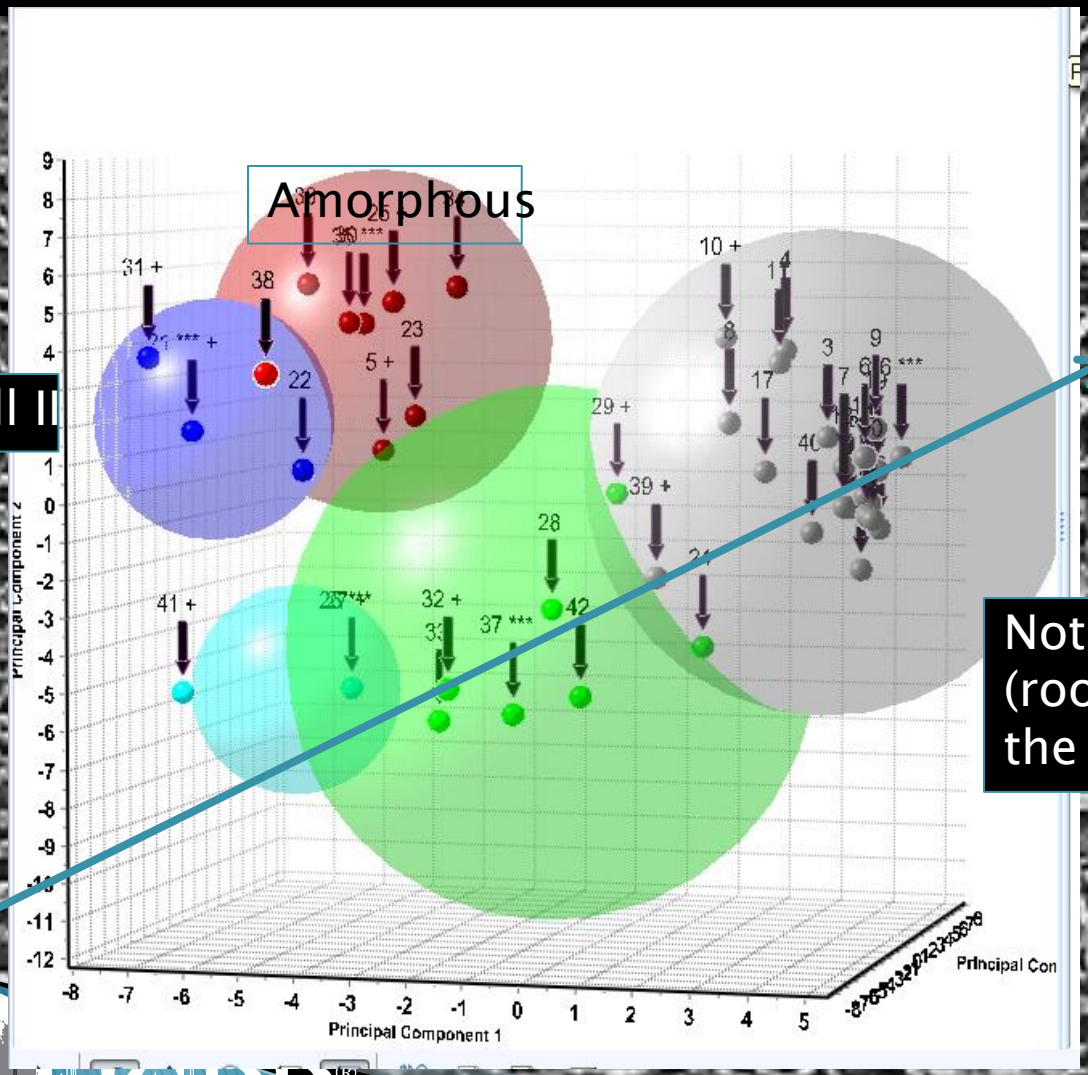
Crystallinity in processed and ground celluloses

			Major Phase			% Crystallinity
						1b
Sigma	0 hr		1b	0.892		100
Filter Paper			1b	0.818		100
USP Microcrystalline			1b			88.2
Paper Pulp A handsheet			1b	0.921		87
Paper Pulp C Handsheet			1b	0.92		86.2
Micro crystall Aldrich			1b	0.818		80.2
Sigmacell			1b	0.892		80.1
Sigmacell	1 hr	Ground	Amorph	0.911		46.3
Mercerized	sheet	Pretreated	Amorph	0.871		43.9
Sigma	6.5 hr	Ground	Amorph	0.925		21.1
Mercerized	10 min	Ground	Amorph	0.923		13.6
Sigmacell	2 hr	Ground	Amorph	0.974		13.5
Sigmacell	3 hr	Ground	Amorph	0.982		11.7
Mercerized	1 hr	Ground	Amorph	0.97		8.3
Sigma	13 hr	Ground	Amorph	0.959		0
Sigma	10 hr	Ground	Amorph	0.941		0

Macro clustering reflects polymorphism and crystallinity



Note the natural products (roots) are mixed with the wood pulps

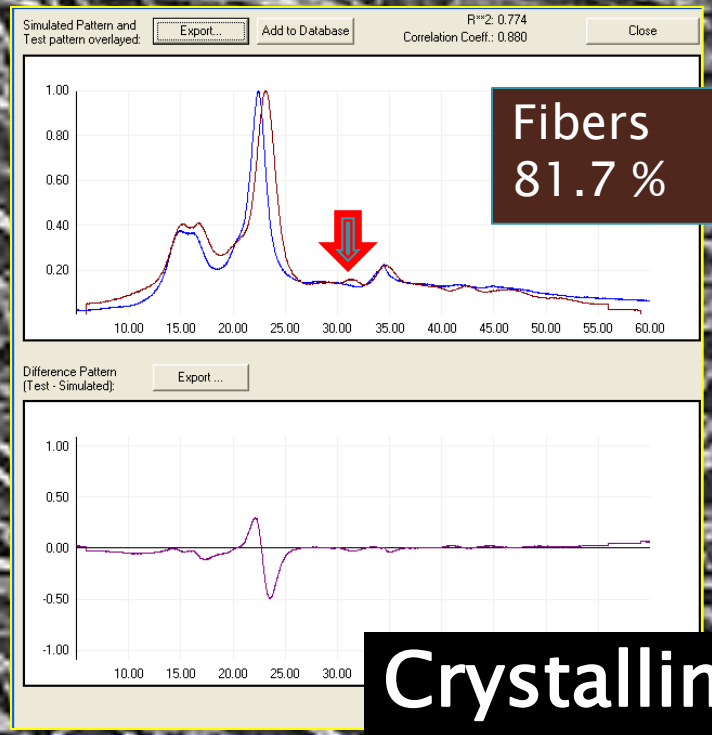
Some conclusions

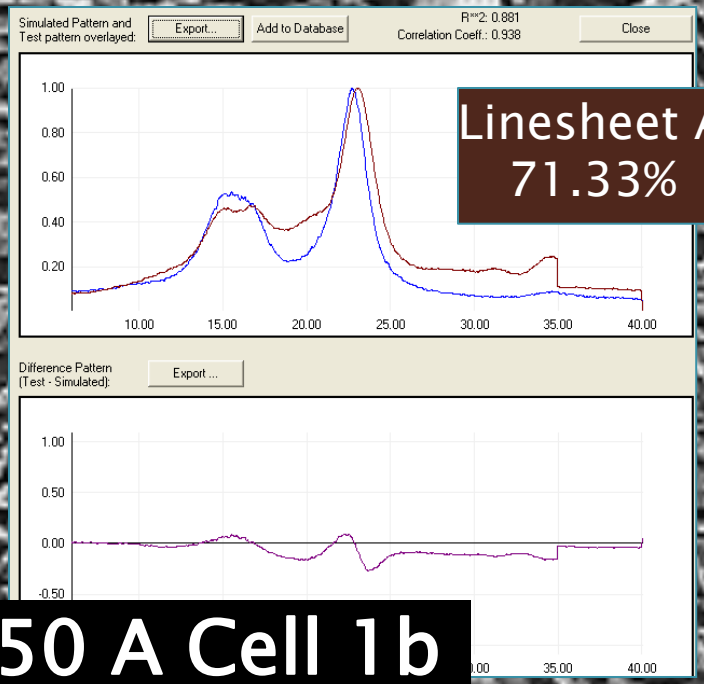
- Structures of Cellulose 1a, 1b and II along with experimentally derived amorphous cellulose can be used as references for polymorph identification and crystallinity measurements
- Similarity indices used in PolySNAP 2.0 and HighScorePlus 3.0 cluster analyses do a good job in separating out cellulose materials based on polymorphism and crystallinity

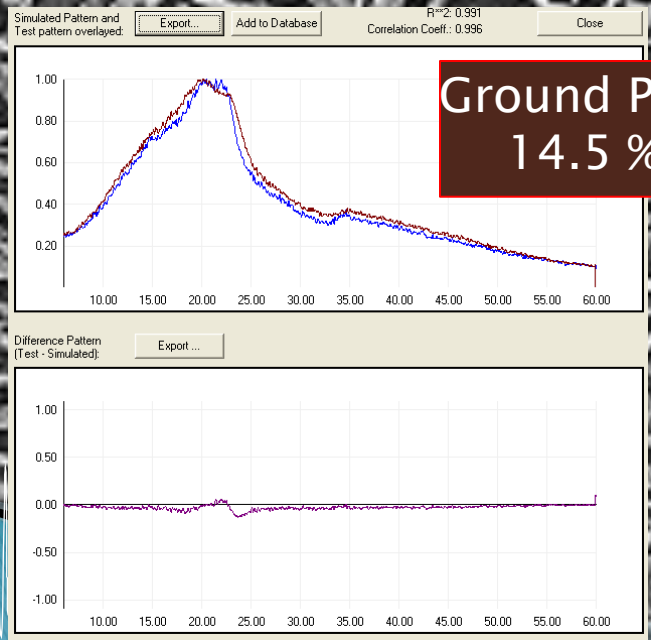
PolySNAP – Calculated Fits

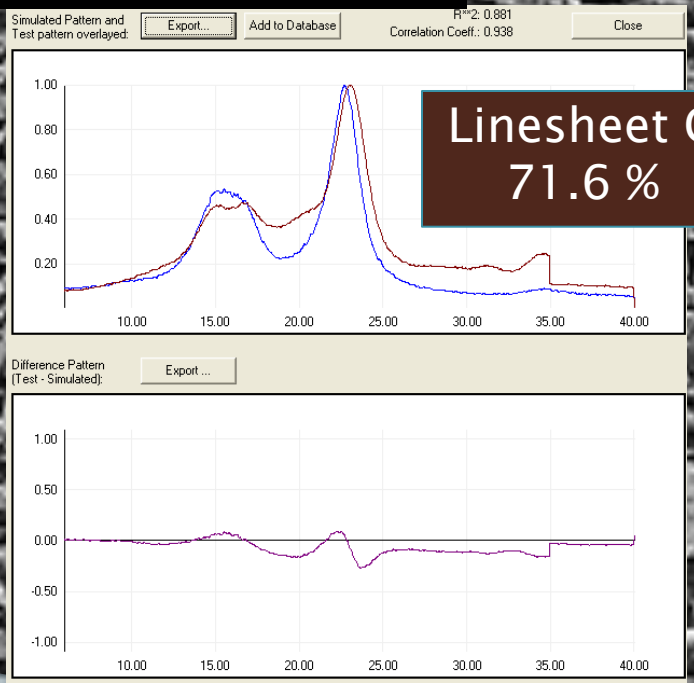
- Zero Shift correction
- Autoscaling
- **Automated background subtraction**
- **Forces fit to set number of references – but fundamentally unlimited in number, algorithms choose which ones to use**

Crystallinity, 50 A Cell 1b

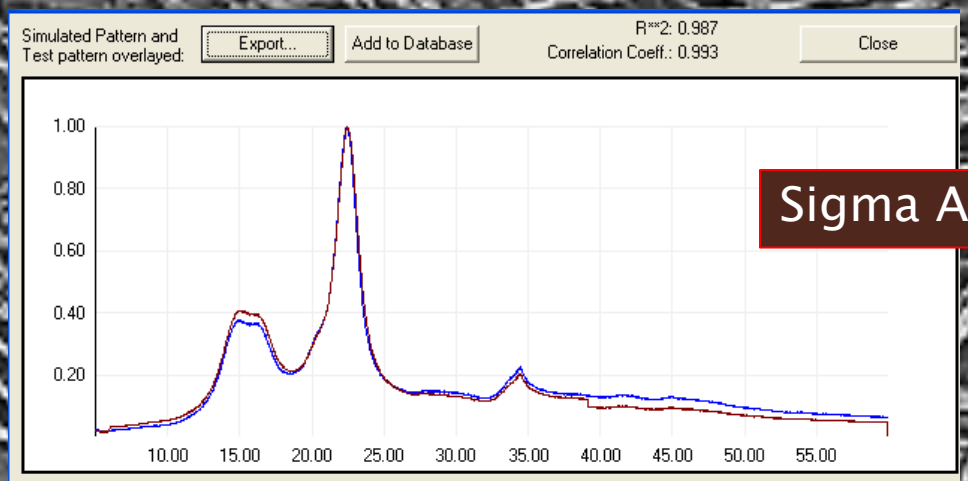






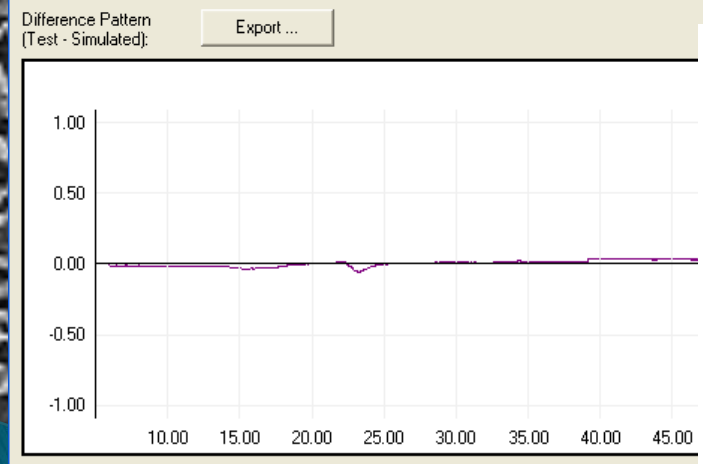


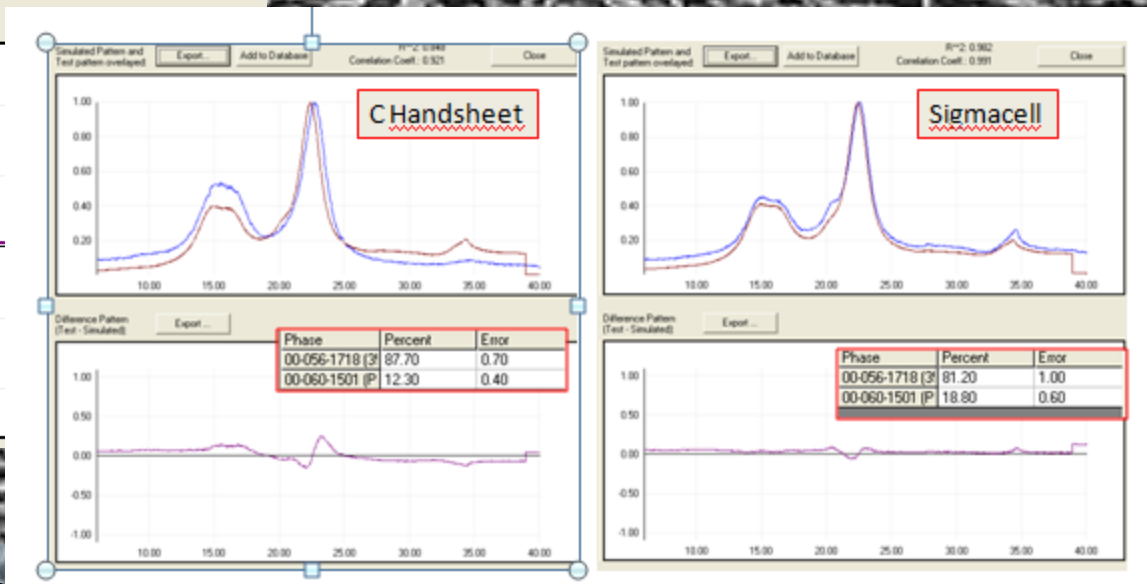
PolySNAP – Pattern summations Great fit with 98.7 R2, 35 A Cell Ib



Sigma Aldrich 00-060-1502

Phase	Percent	Error
00-056-1718 (3!	82.60	1.10
00-060-1501 (P	17.40	0.60





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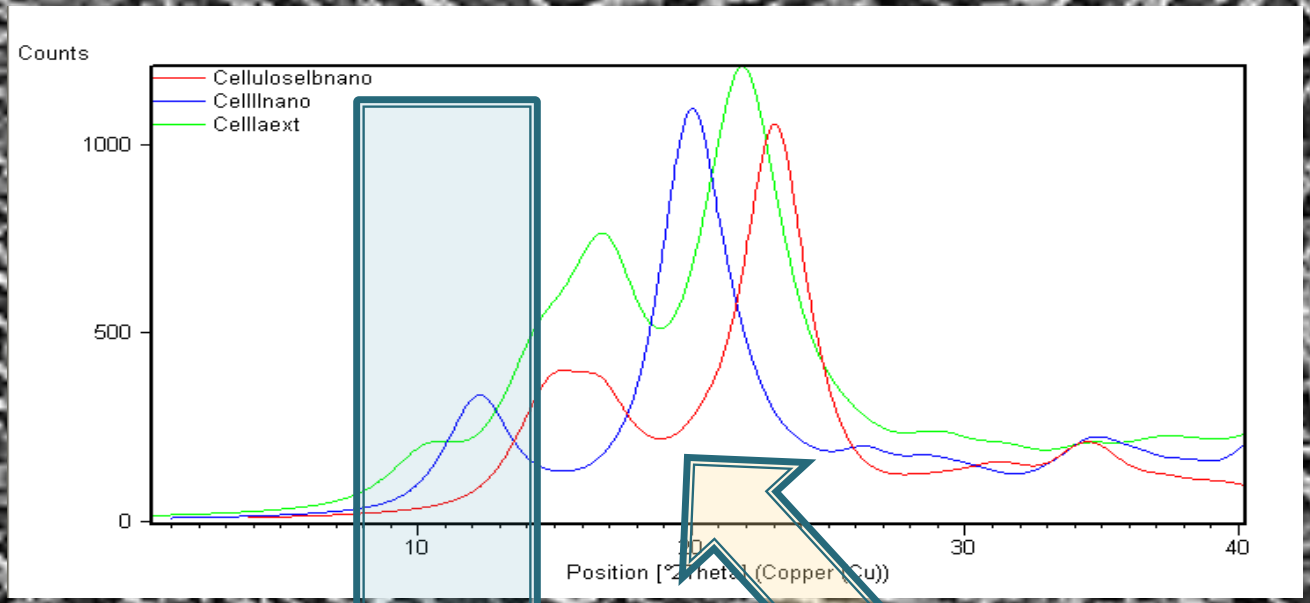
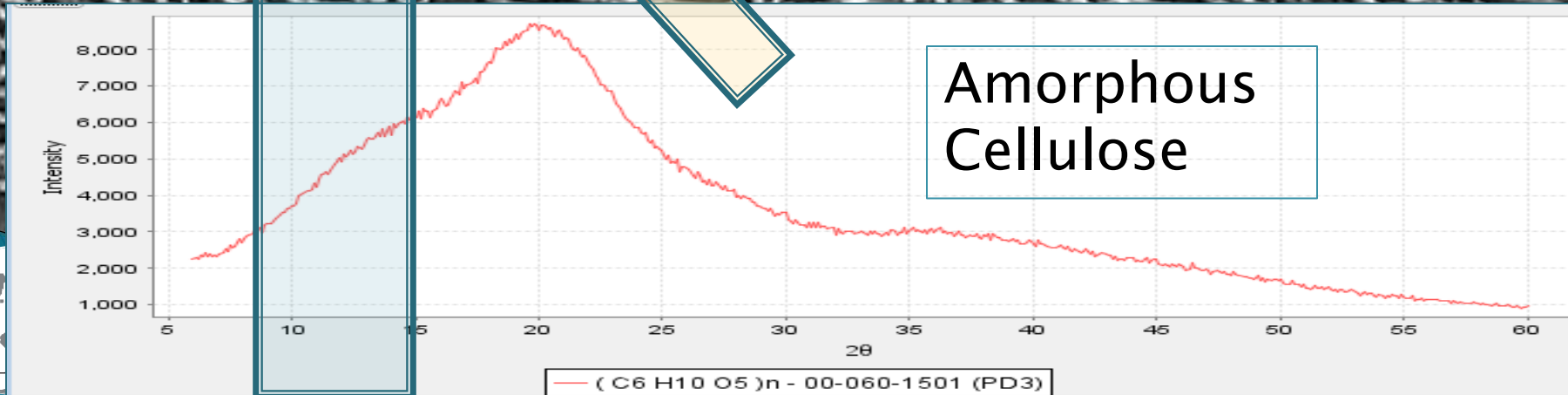
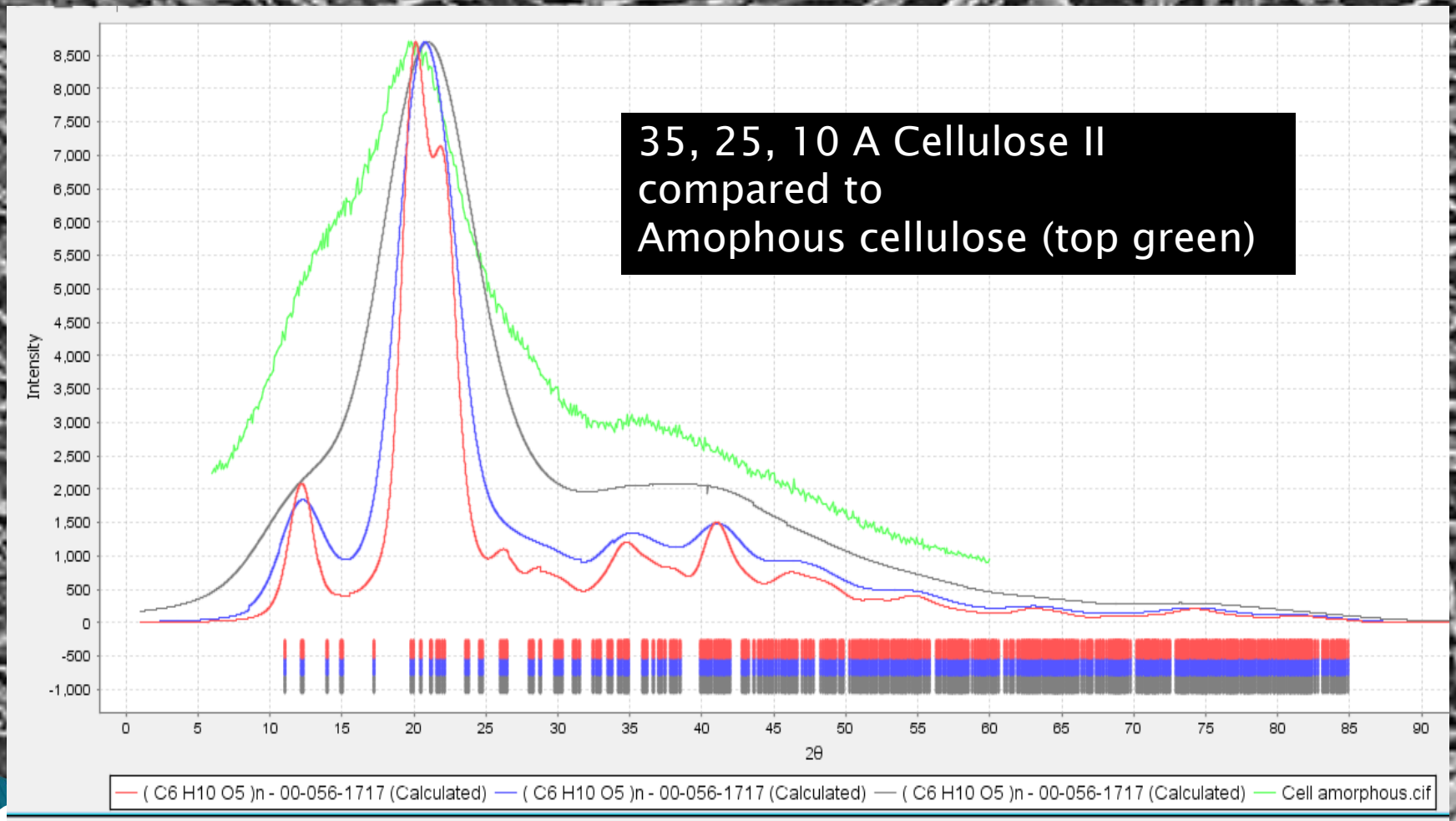
Nanocrystalline cellulose (30 A)





cellulose II compared to amorphous cellulose

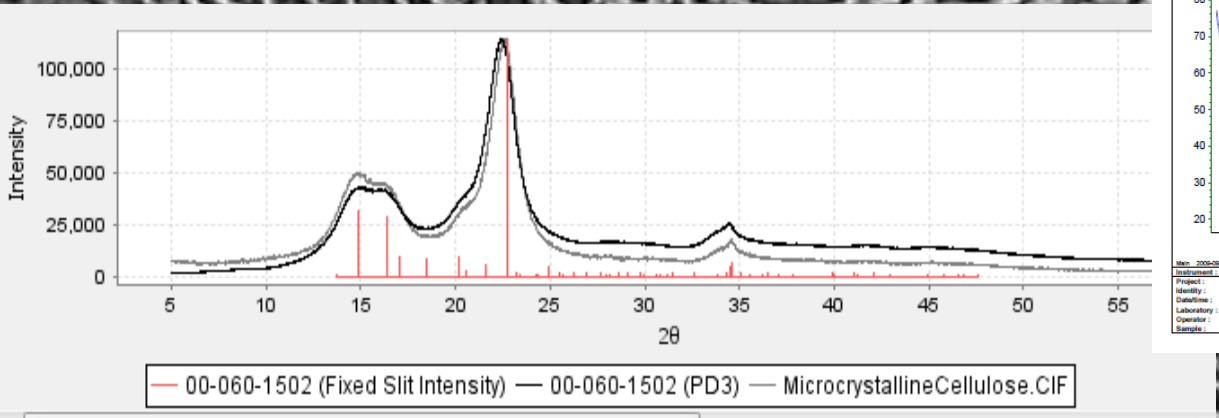


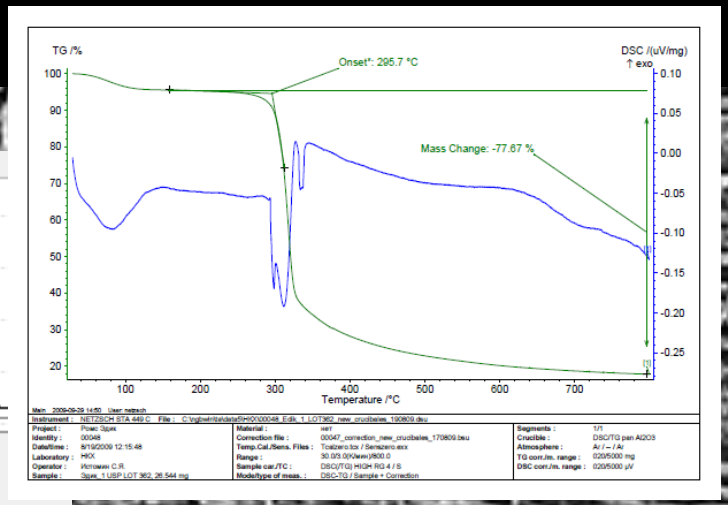
More Conclusions

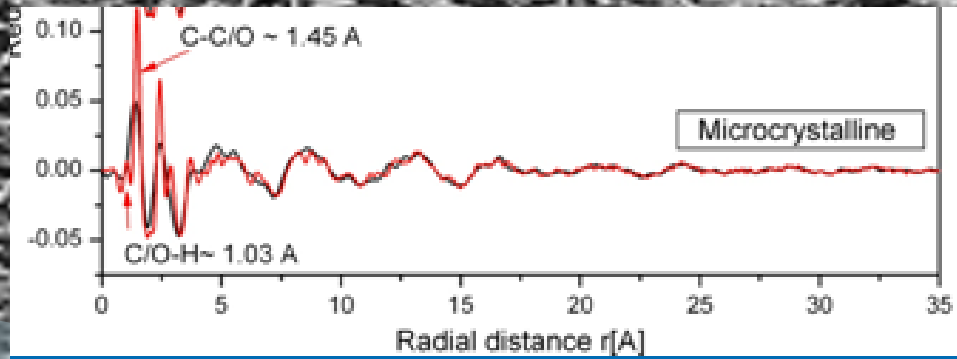
- Under 30 A distinctions between the diffraction patterns blur
- Cellulose 1 alpha and 1 beta are highly correlated (dmax 3.82 and 3.87)
- Cellulose II and amorphous cellulose are highly correlated (dmax 4.45 and 4.48)

- Grind cellulose Ia, 1b see the amorphous "jump" but not with cellulose II

Microcrystalline Cellulose Sigma Alrich (00-060-1502) and US Pharmacopea







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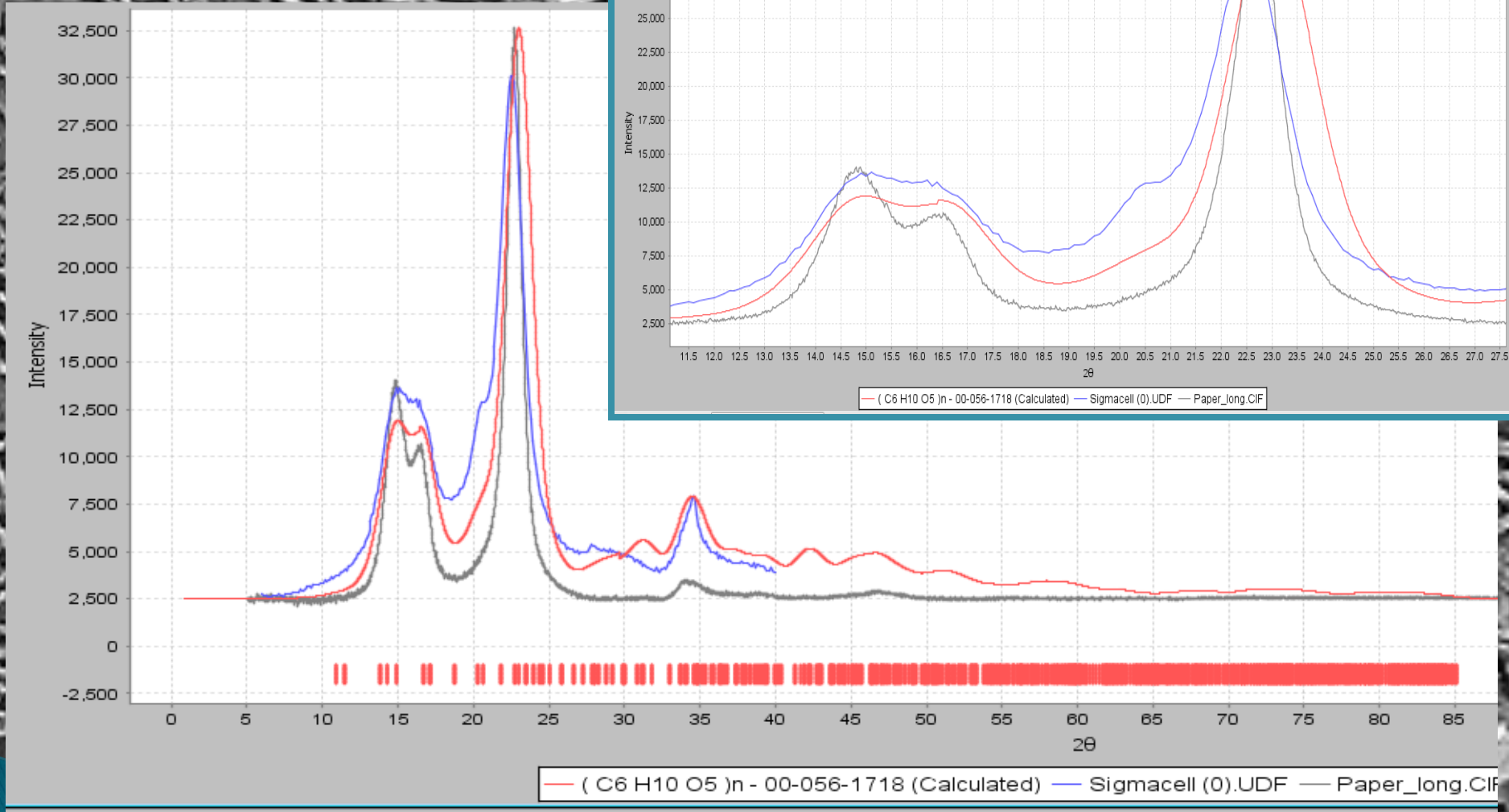
Microcrystalline Cellulose

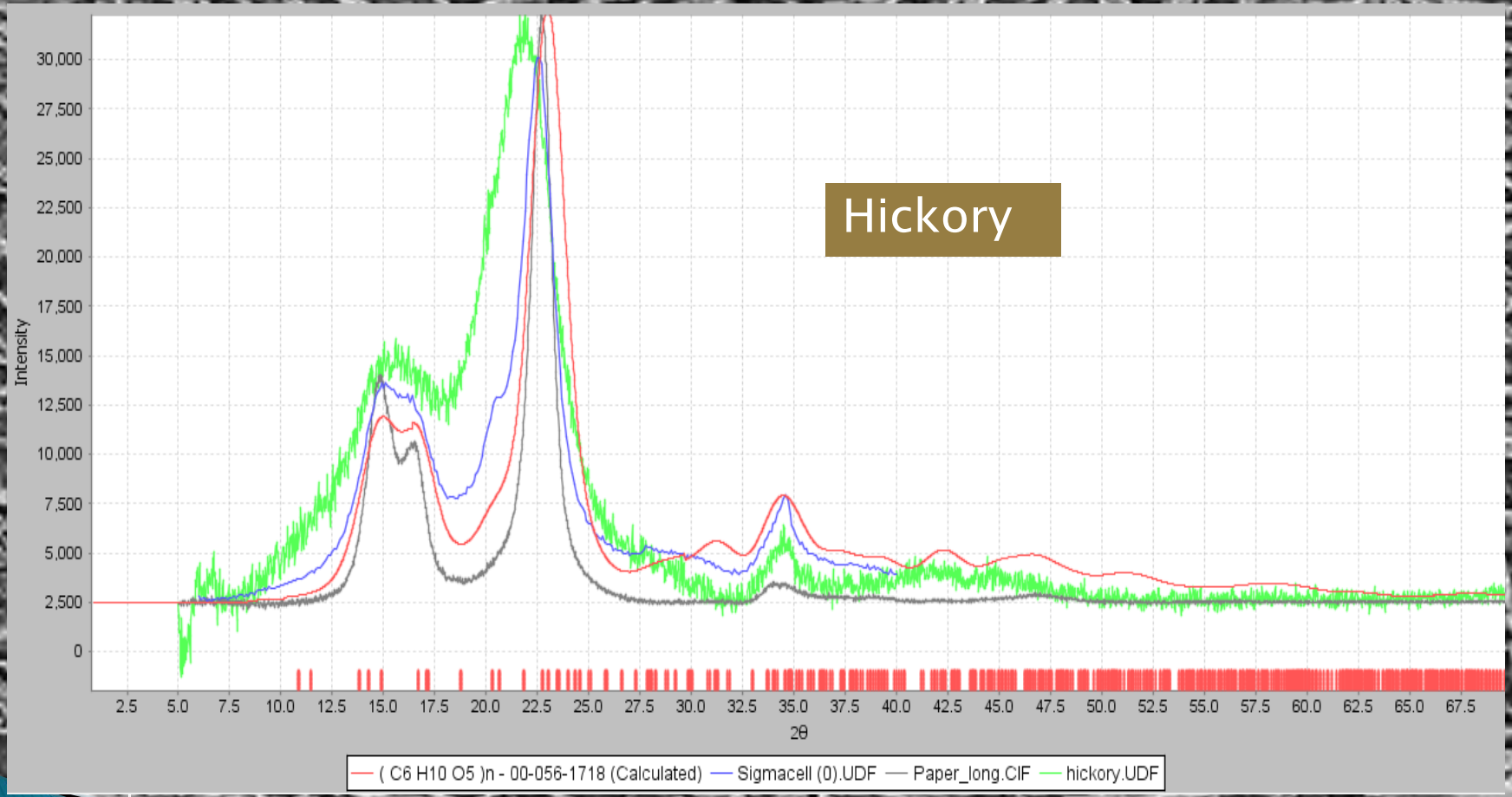
- Predominately Cellulose I beta (XRD)
- ~ 40 A Crystallites (XRD)
- 20 um particles (Sigma-Alrich specification)
- ~ 3 % absorbed water at RT (DTA)
- 1-3 % amorphous cellulose (XRD-FULLPat)
  (other programs estimate 10-20%)
- Microcrystallinity confirmed by PDF analysis showing long coherence lengths and bond distances typical of Cellulose I's

<u>Suggested by published studies</u>

- The particle size and crystallite size are in the known magnitude of the fibril and microfibril widths, respectively
- Derived from native cellulose the microcrystalline cellulose may have polymorph 1 alpha on fibril surfaces, XRD may not detect a few %

Cellulose


Intensity
2θ
(C6 H10 O5)n - 00-056-1718 (Calculated)
Sigmacell (0).UDF
Paper_long.CIF



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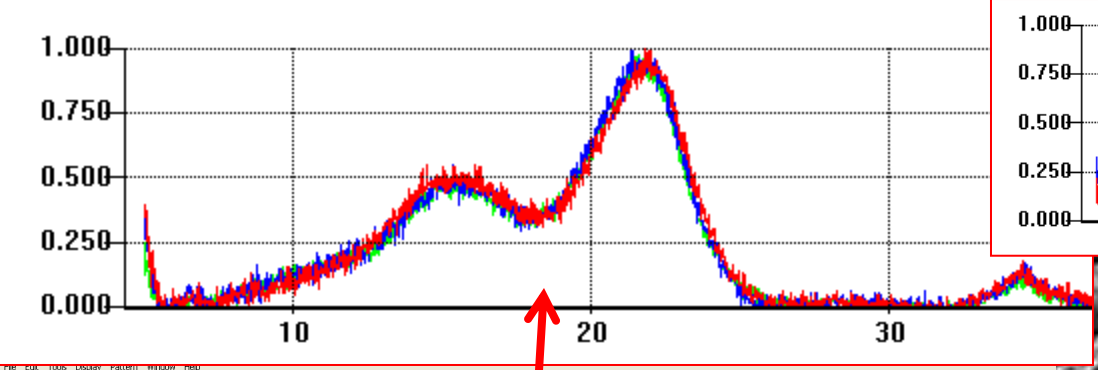
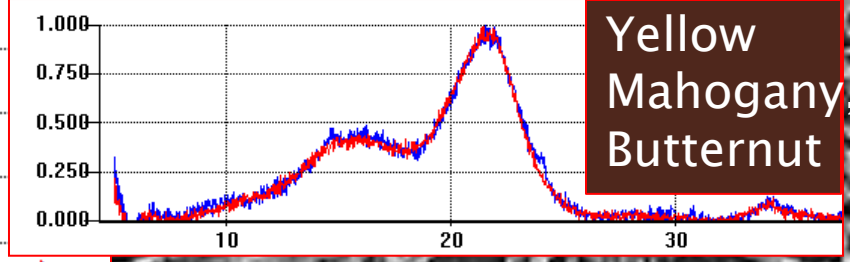
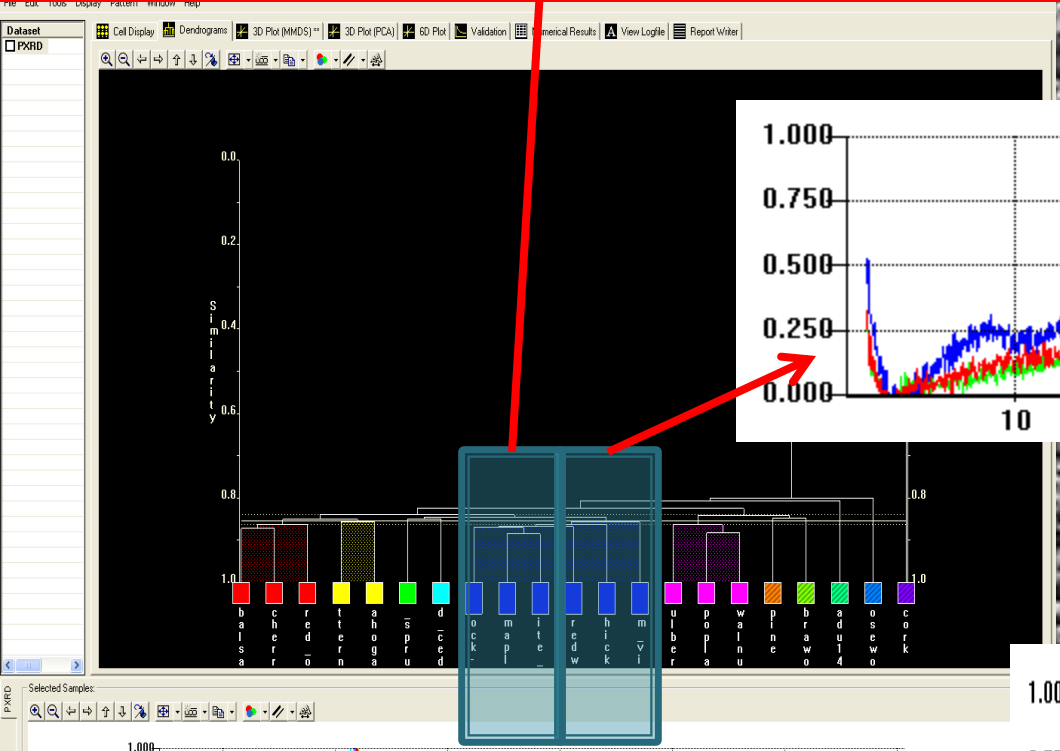
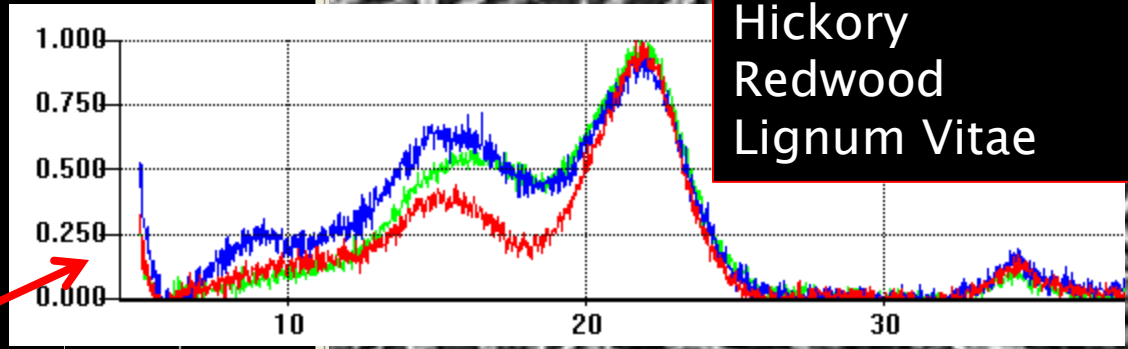


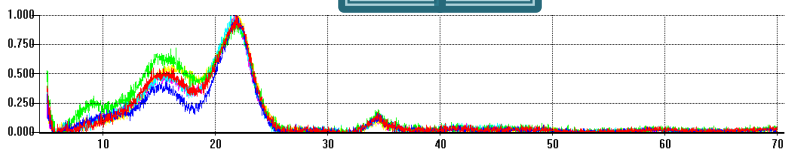


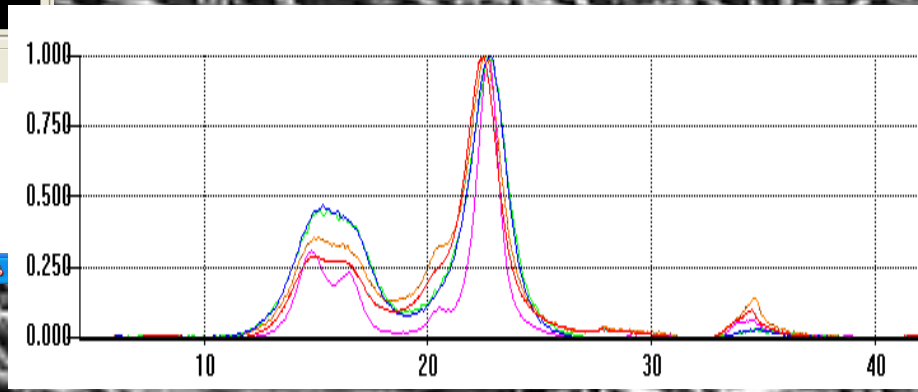
Yellow
Mahogany,
Butternut





Hickory
Redwood
Lignum Vitae





8 New Reference Materials 2006-2011

PDF #	QM	Chemical Formula	Compound Name	SYS	Author	Journal	SPGR	Coords
00-056-1717	S	(C6 H10 O5)n	Cellulose II	M	Kaduk, J., BP Chemical, Naperville, IL, USA.	Private Communication	P21	√
00-056-1718	S	(C6 H10 O5)n	Cellulose Iβ	M	Kaduk, J., BP Chemical, Naperville, IL, USA.	Private Communication	P21	√
00-056-1719	S	(C6 H10 O5)n	Cellulose Iα	A	Kaduk, J., BP Chemical, Naperville, IL, USA.	Private Communication	P1	√
00-060-1501	M	(C6 H10 O5)n	Cellulose, amorphous	X	Bucher, E., International Paper.	Private Communication		
00-060-1502	R	(C6 H10 O5)n	Cellulose-Iβ	M	Needham, F., Reid, J., International Centre for Diffraction Data, Newtown Square, P...	Private Communication	P21	√
00-061-1407	I	(C6 H7 O2 (C2 H3 O2)3)n	Cellulose triacetate	O	Blanton, T., Eastman Kodak Company, Research Laboratories, Rochester, NY, USA.	Private Communication	P212121	
00-061-1408	M	(C6 H7 O2 (C2 H3 O2)3)n	Cellulose triacetate	X	Blanton, T., Eastman Kodak Company, Research Laboratories, Rochester, NY, USA.	Private Communication		
00-061-1409	M	(C6 H7 O2 (C2 H3 O2)3)n	Cellulose triacetate	X	Blanton, T., Eastman Kodak Company, Research Laboratories, Rochester, NY, USA.	Private Communication		

8 New Reference Materials –

4 have crystal structures, 4 have full experimental patterns

3 are amorphous references (SYS = X), 5 are crystalline

In progress **(ICDD grant data ,collected ,being processed for publication)**

Cellulose triacetate (USP), microcrystalline cellulose (USP), Cellulose acetate pthalate, cellulose acetate butyrate – both amorphous (Support elemental analyses, DSC, DTA)

Povidone, crospovidone

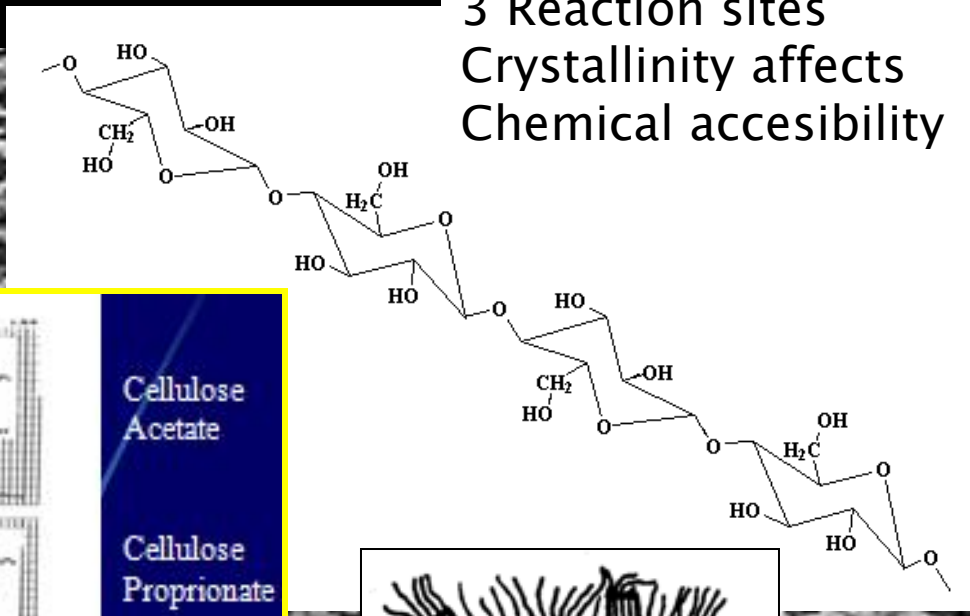
Roman Shpanchenko, Moscow State University

Pair distribution function analysis of all in-progress materials

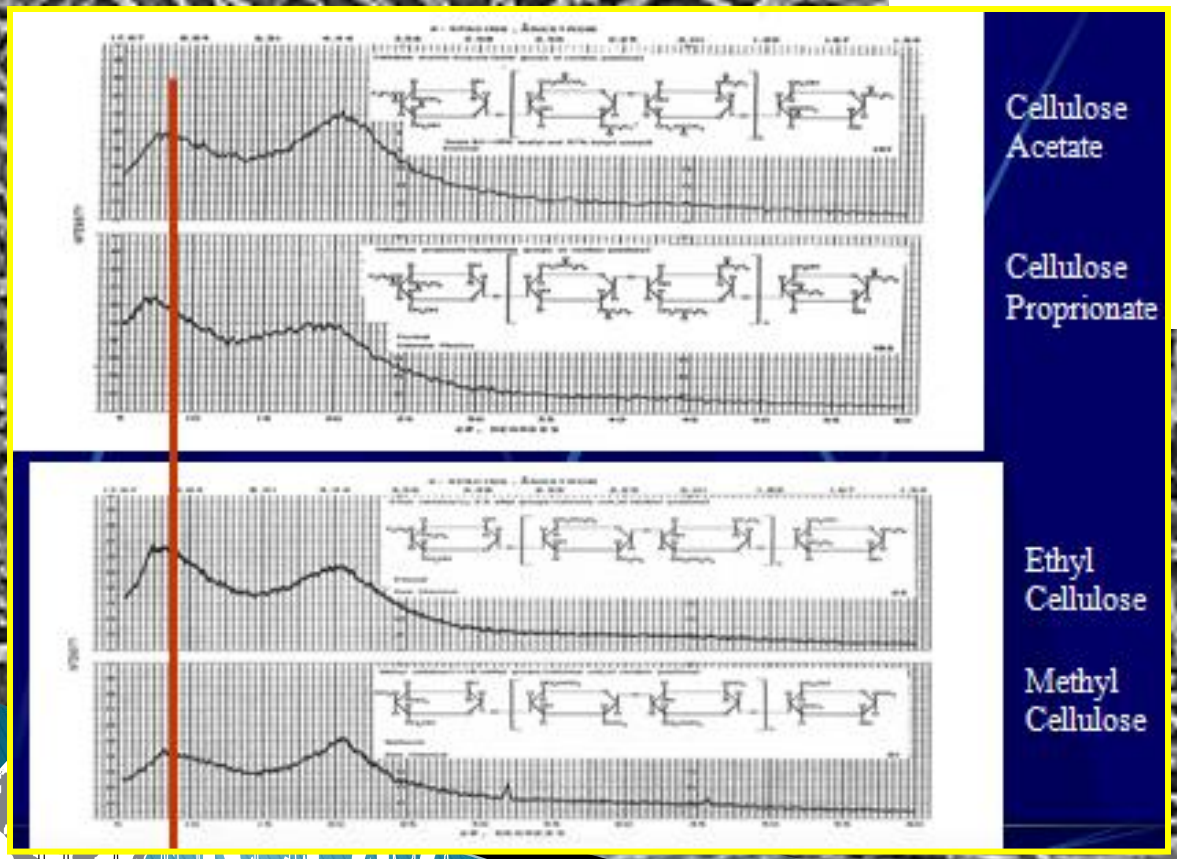
Valeri Petkov, Central Michigan University

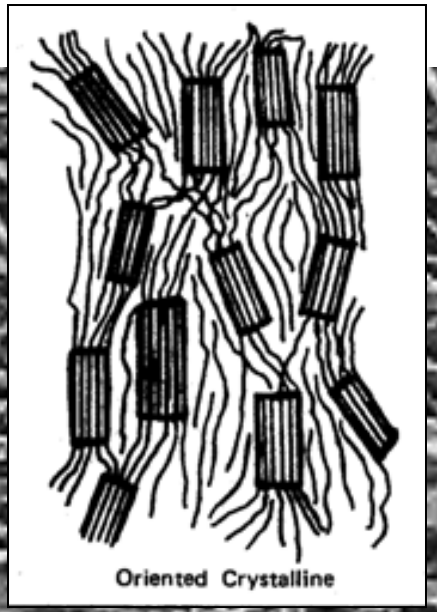
Substituted Celluloses

June Turley, Dow Chemical, 1965



3 Reaction sites
Crystallinity affects
Chemical accesibility

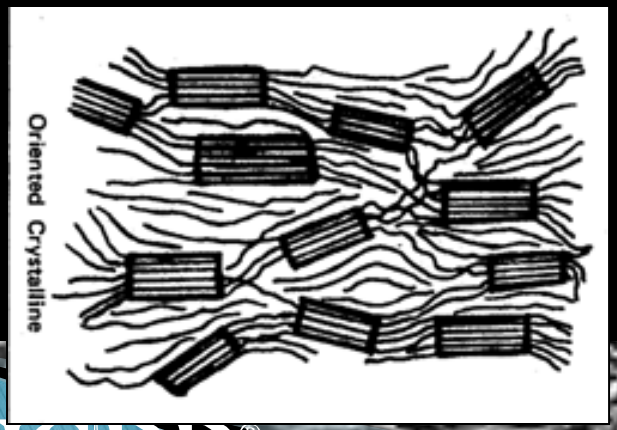


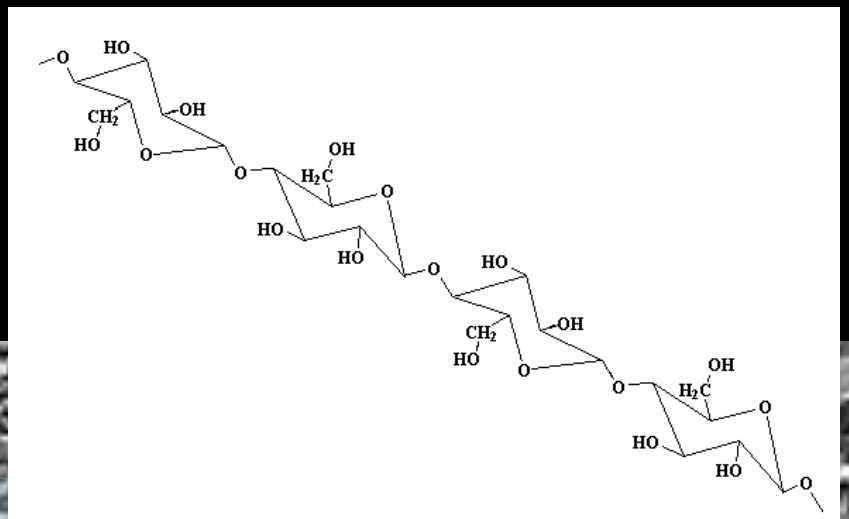


Substitution in Cellulose

- 3 – OH sites per glucose monomer

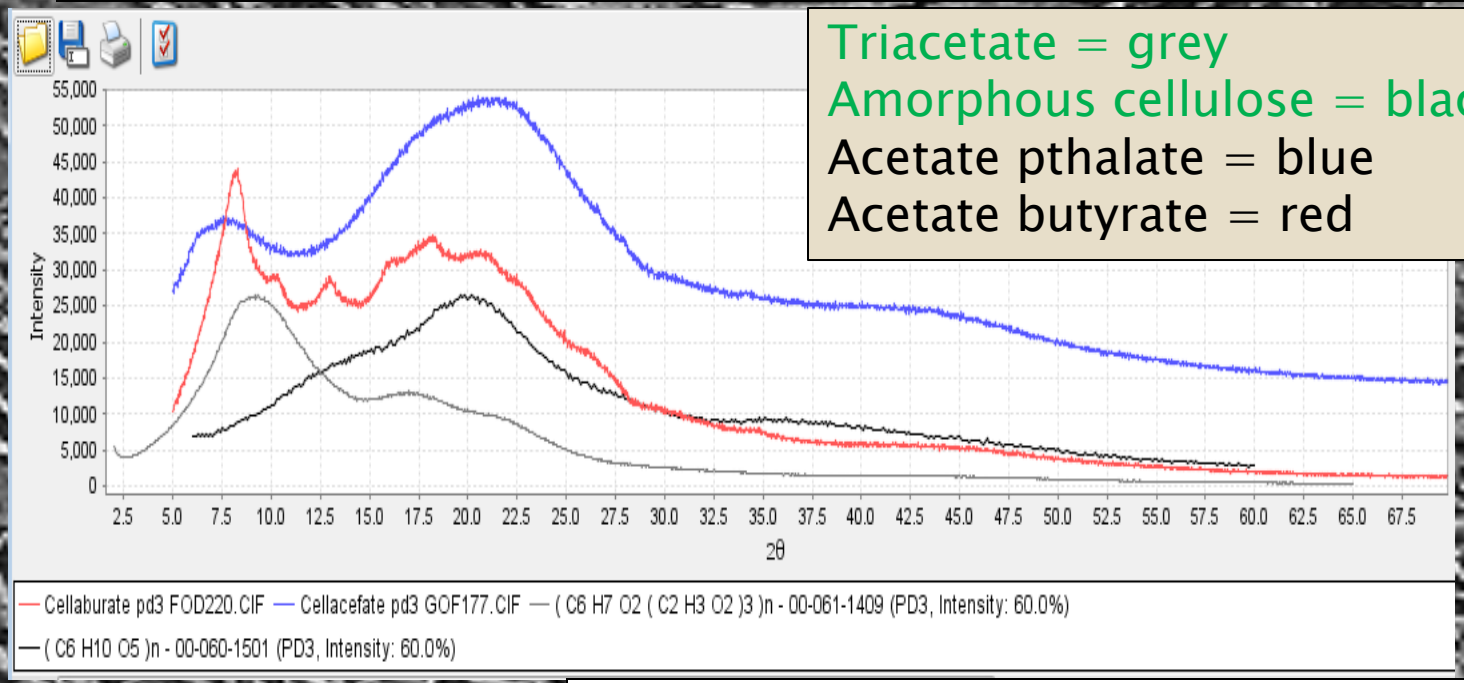
- Unsubstituted (0) and fully substituted (3)
- 3 mono substitution choices (site 1, 2, 3)
- 3 disubstituted choices (1,2…1,3 …2,3)

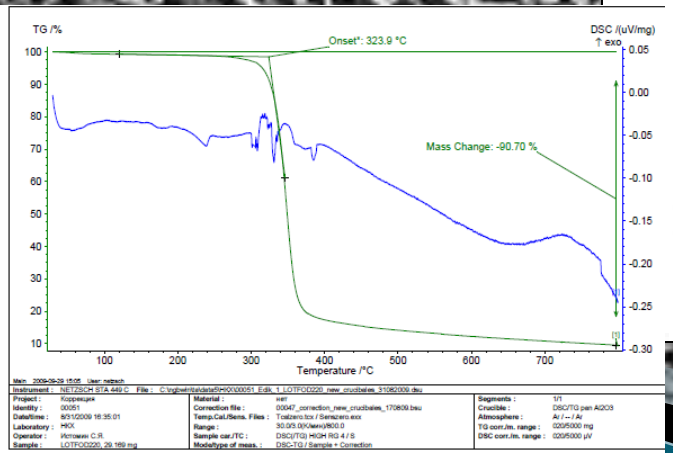


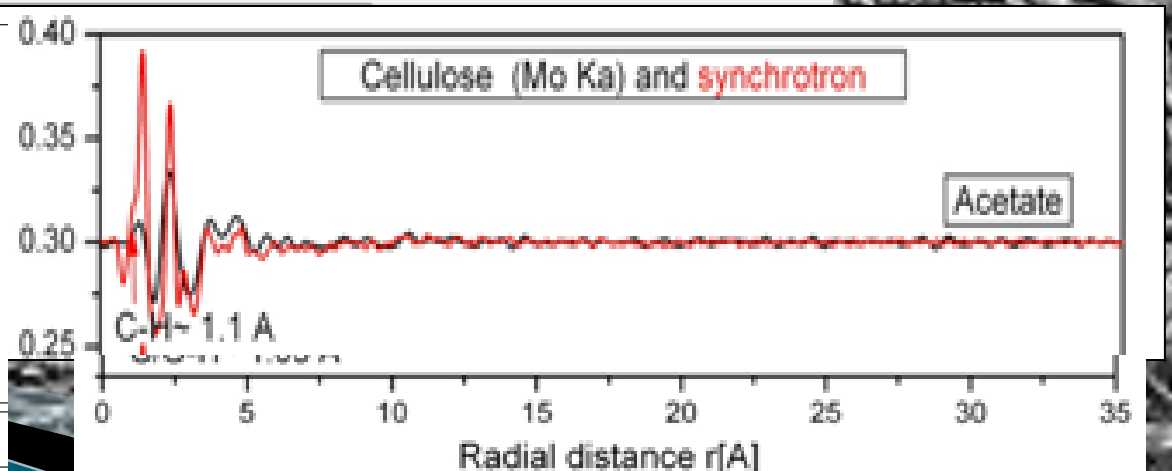


New substituted Celluloses

Triacetate = grey
Amorphous cellulose = black
Acetate pthalate = blue
Acetate butyrate = red



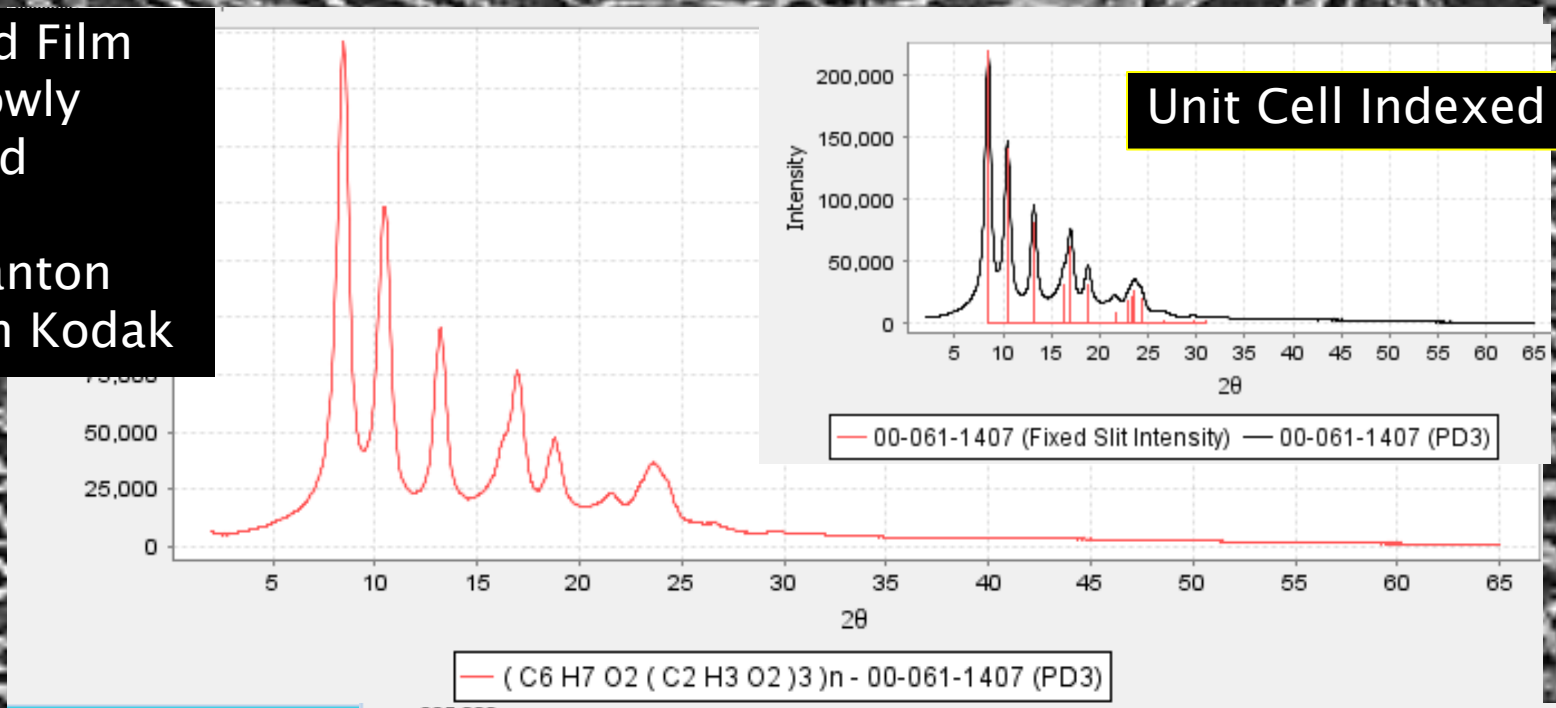


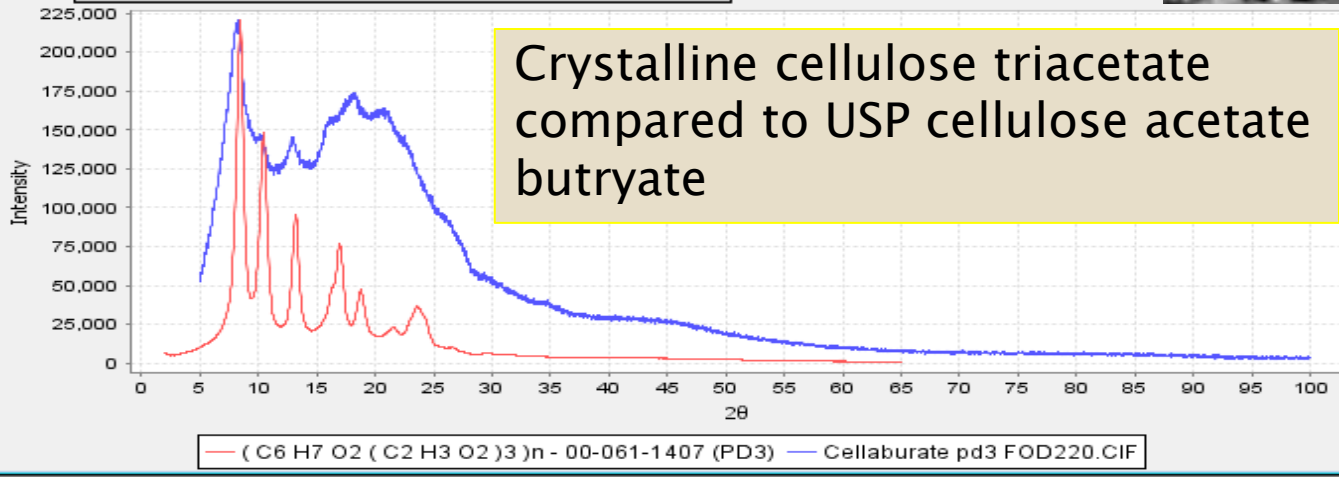


Substituted Cellulose (Not always amorphous)

Oriented Film then slowly annealed

Tom Blanton
Eastman Kodak





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CONCLUSIONS – References

1. Ab-initio structures used to calculate cellulose polymorphs powder pattern references have been validated in the study of pulps and papers to aid in the determination of polymorphic composition
2. Amorphous cellulose references have similarly been validated and can be used in the determination of crystallinity.
3. Using the references, a wide variety of cellulose containing material have been studied, polymorphs analyzed, and crystallinities measured

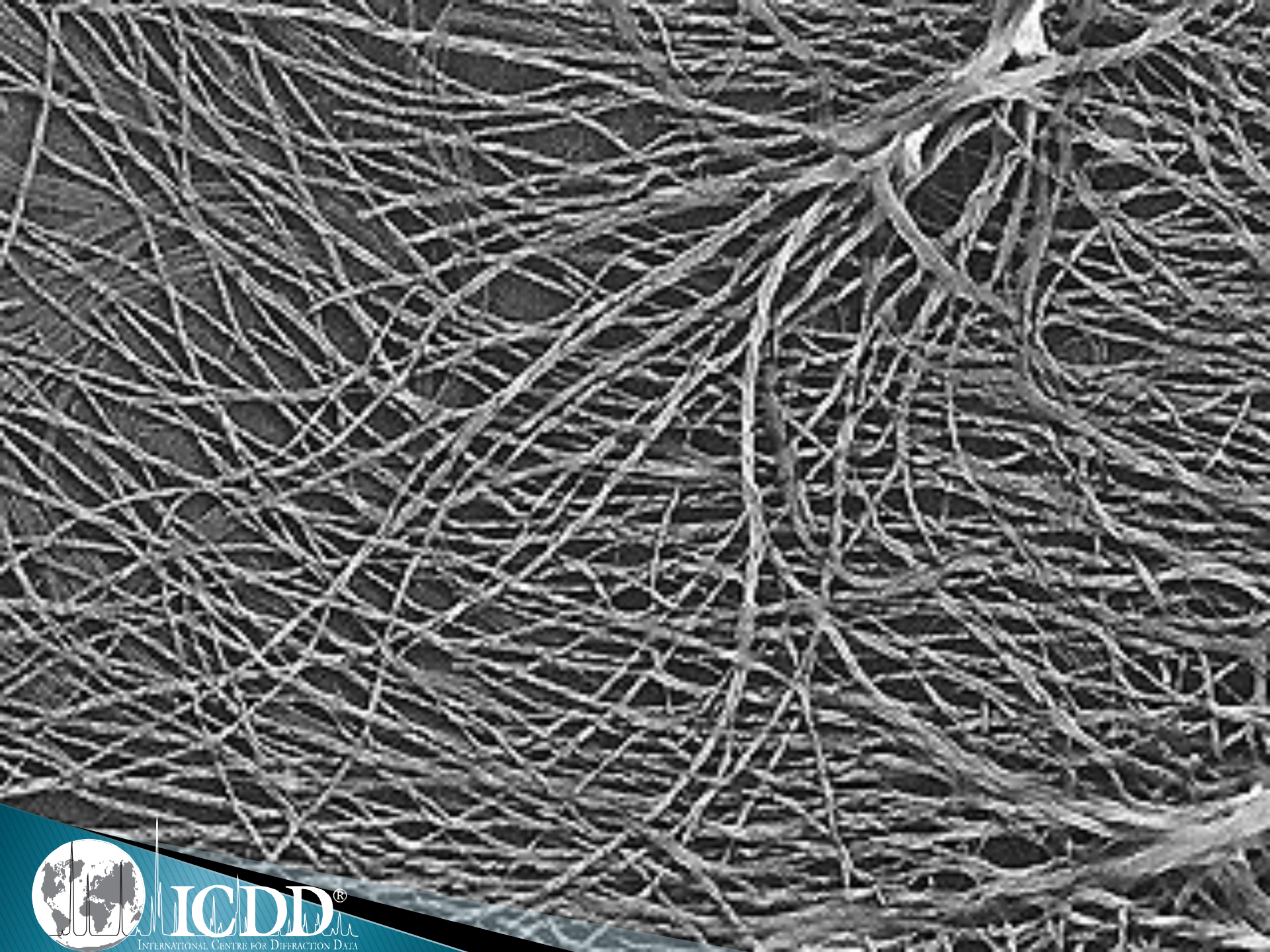
CONCLUSIONS – Methods

- Cluster Analyses – Have been shown to be very valuable in separating out clusters of cellulose containing materials based on polymorphism and crystallite size
- Integral Index – A nice tool for non-crystalline and small crystallite materials to identify phase and polymorphism. Has an advantage when applied to subfiles
- Rietveld – May be too powerful for these relatively simple patterns too many refined variables with too little data. Often refines to an averaged structure with a small crystallite size when other data may indicate a polymorphic mix. Best used with the highest quality data (i.e synchrotron) and/or with constrained refinement.
- Pattern Fitting Methods –Three different programs used, often worked well for crystallinity measurements and polymorphic identification. These methods are very dependent on using the correct crystallite size for the references. This require reiteration – pattern fit, adjust crystallite size, pattern fit again

- *All methods* were *highly dependent* on accurately removing background and cleanly separating background from amorphous or microcrystalline contributions. This also means that specimen preparation and data collection methods must be reproducible and aimed at reducing background effects as much as possible.

Conclusions Materials

- Cellulose is wonderfully versatile and chemically complex – it will provide work for scientists for generations to come
- Most wood pulps, pharmaceutical cellulose and paper pulps can be described as a mixture of cellulose 1a, 1b and amorphous cellulose.
- The most common combination for commercial materials, made from cotton and wood, is a high cellulose 1b content (>60%) with smaller amounts of cellulose 1a and amorphous cellulose
- We can measure polymorph and crystallinity changes in grinding studies and mercerization processes
- Lignum vitae, an extremely hard wood, also appears to be unusual in that it is predominately cellulose 1a polymorph, several other types of woods also appear to have significant 1a contributions



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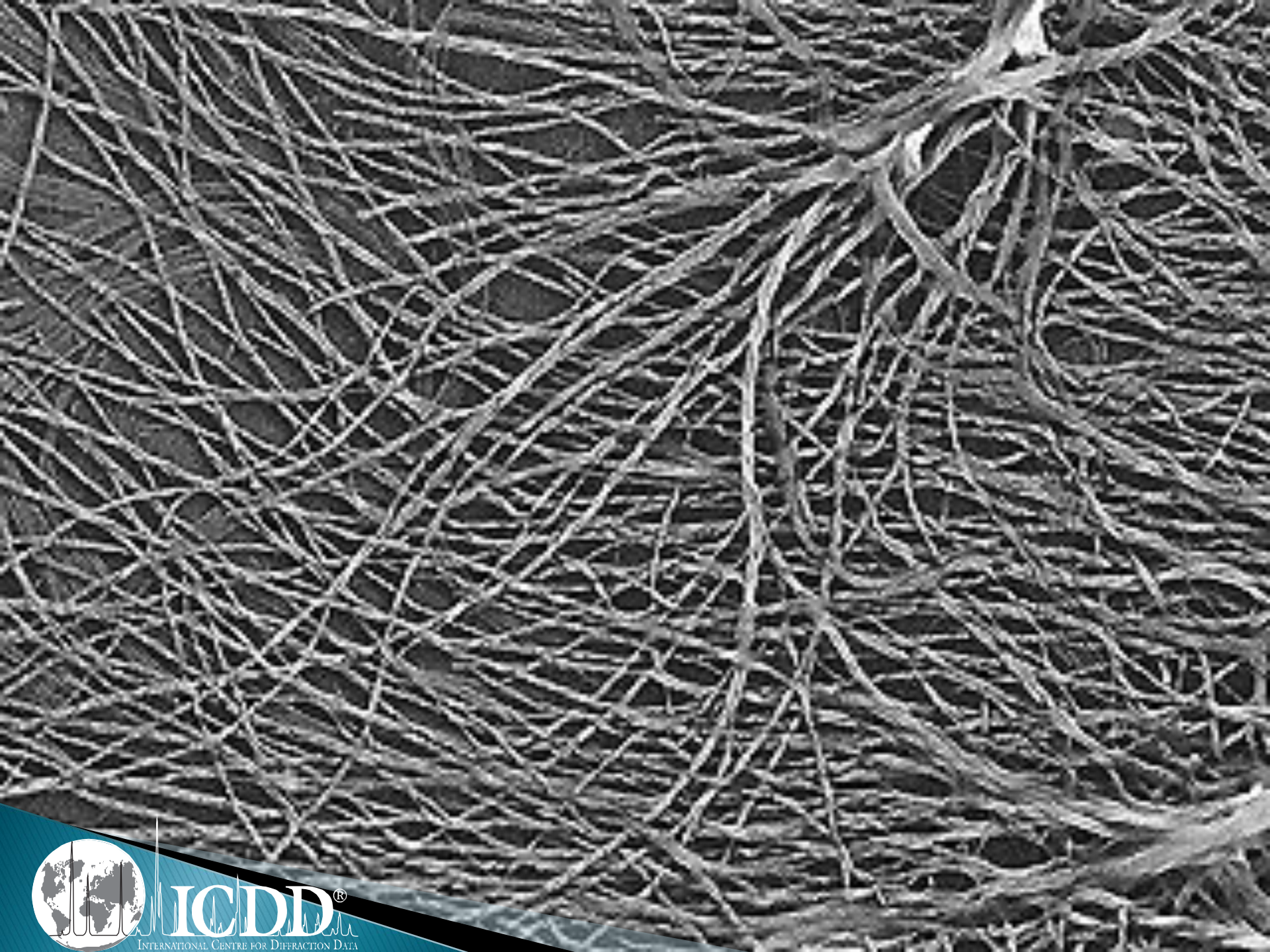

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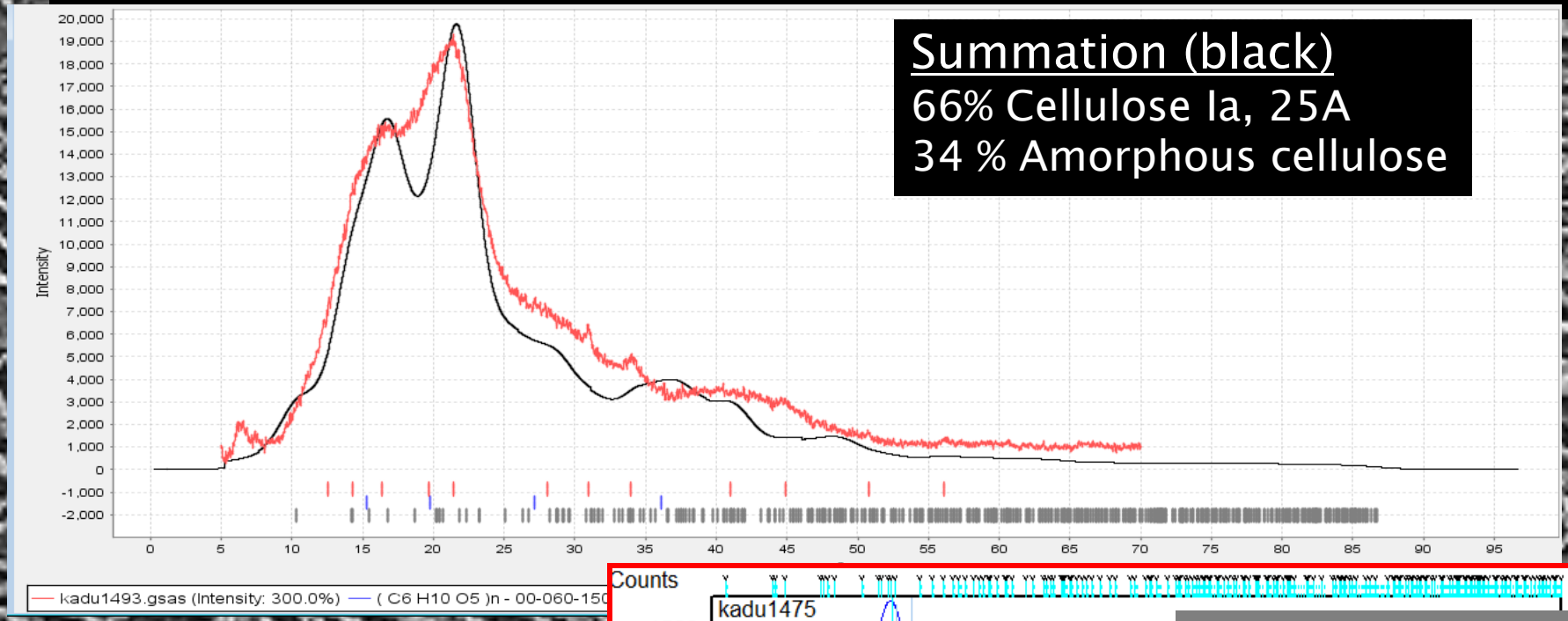
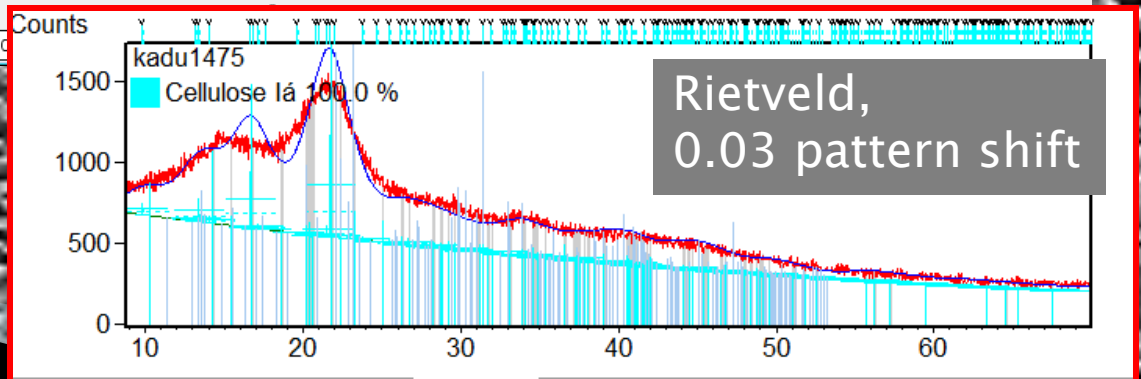
ICDD Pattern Summation – Lignum Vitae Experimental data (red)



Summation (black)
66% Cellulose Ia, 25A
34 % Amorphous cellulose



Rietveld,
0.03 pattern shift

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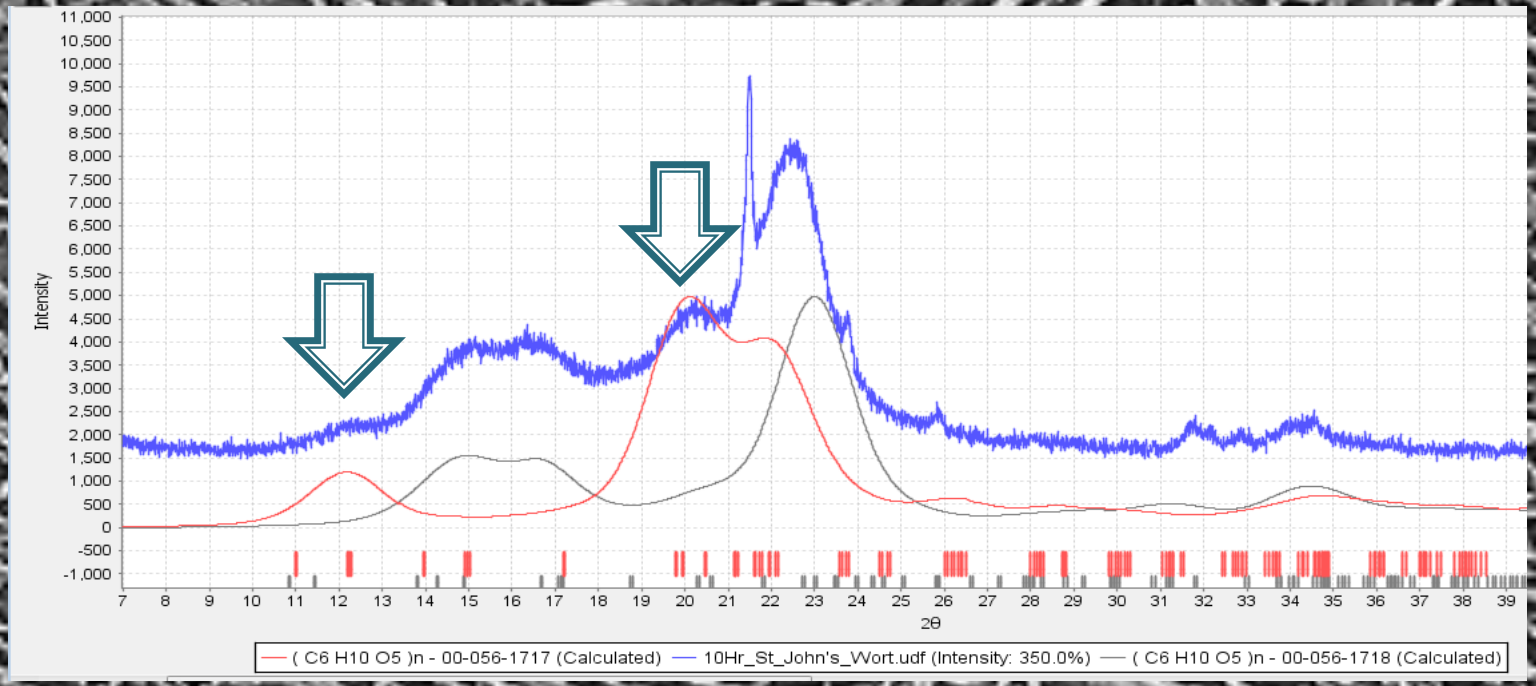
St-John's Wort
Cellulose 1 b and Cellulose II (red)



A sample of St John's Wort showing distinct features of Cellulose II (35 A)

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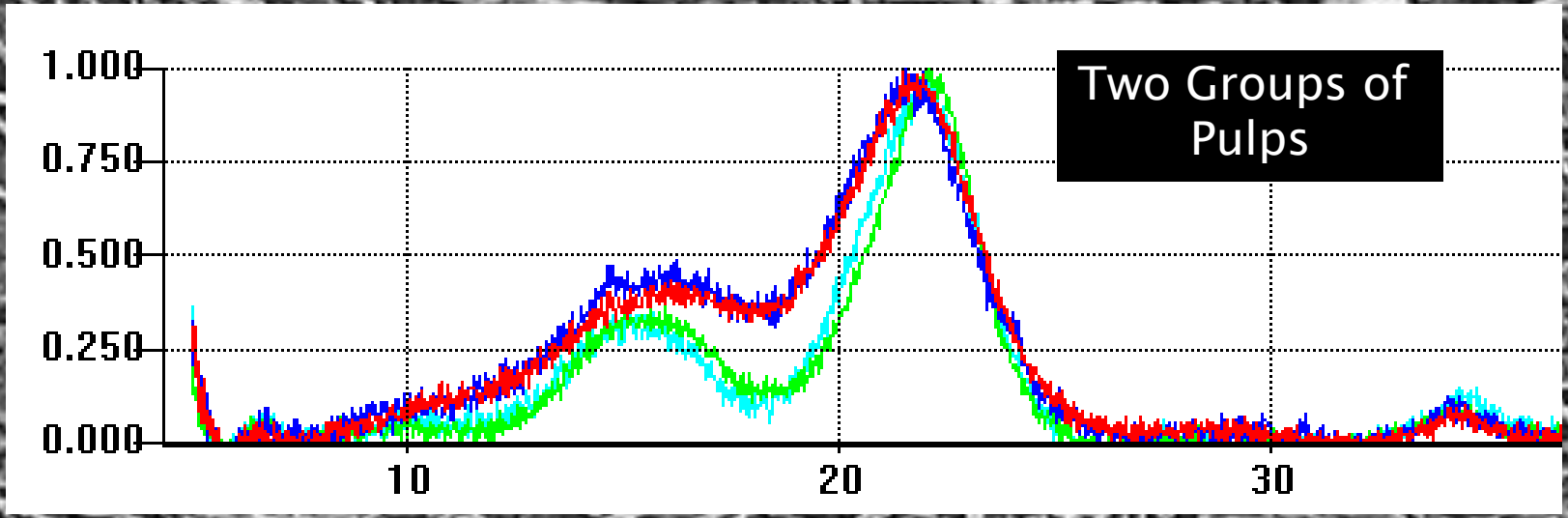
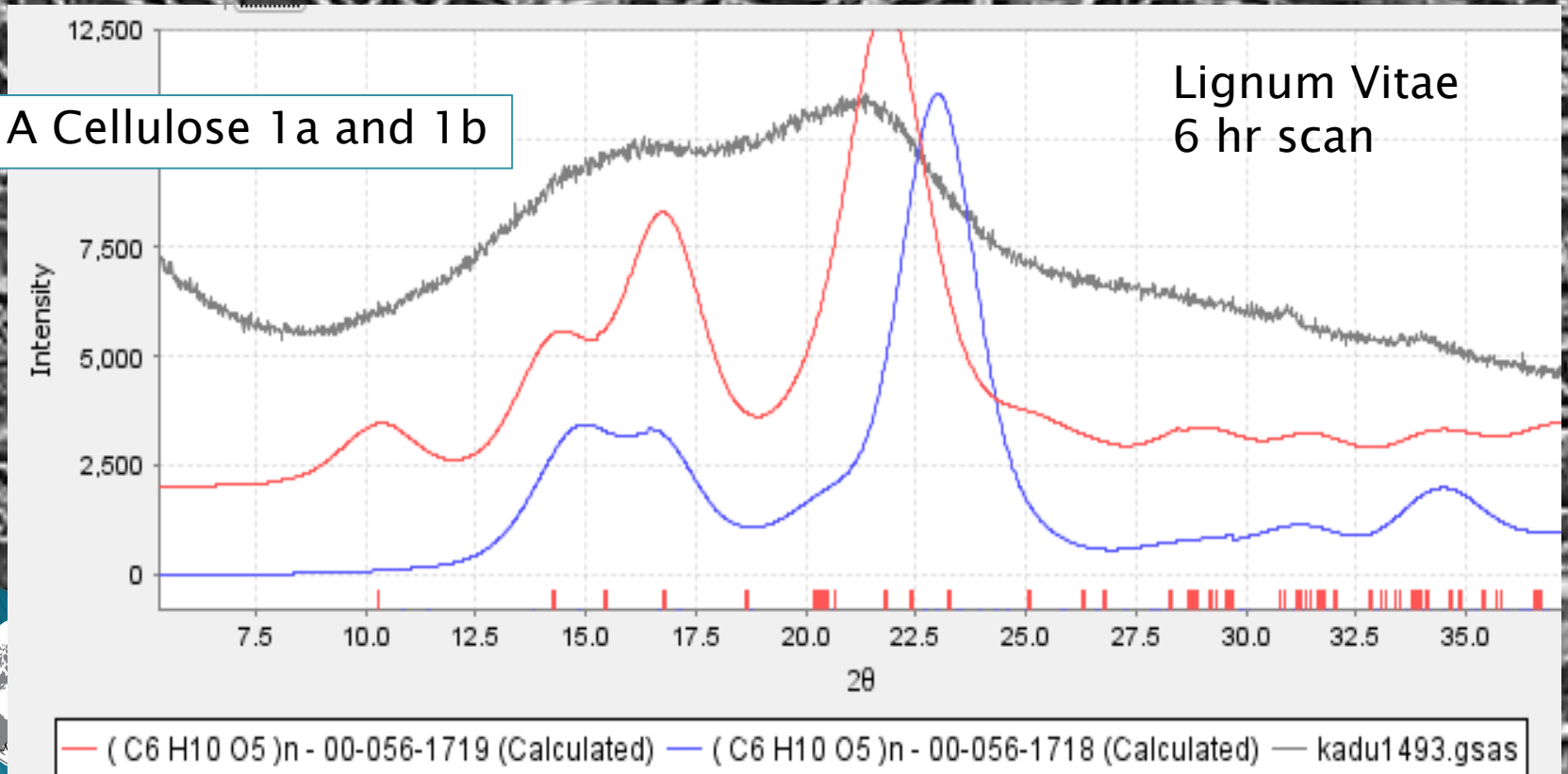


35 A Cellulose 1a and 1b



Samples – Data Collection and analysis

2002-2007

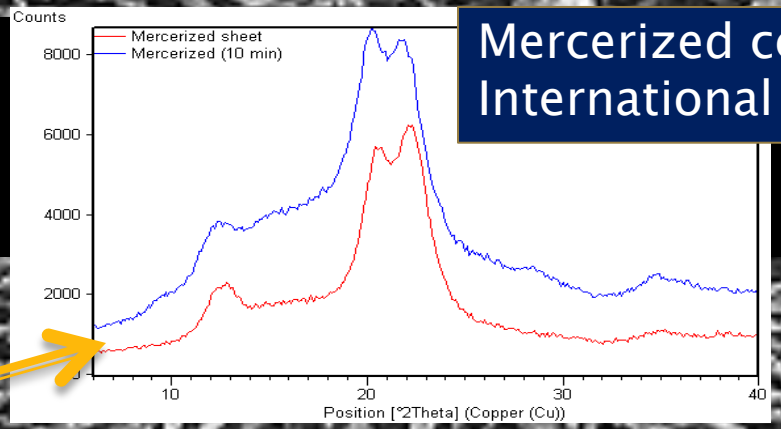
- 12 Pharmacuetical Tablets – Fangling Needham, ICDD clinics, Cam Hubbard, Oak Ridge National Lab, Jim Kaduk, Argonne Light Source
- 18 Wood Pulps, Cotton Linters – Eva Bucher, International Paper

2010-2011

- 21 Wood chips – Jim Kaduk, Poly Crystallography Inc
- 6 USP references – ICDD editors, Joel Reid and Suri Kabekkodu, ICDD grantees, Victor Petkov, Roman Shpanchenko

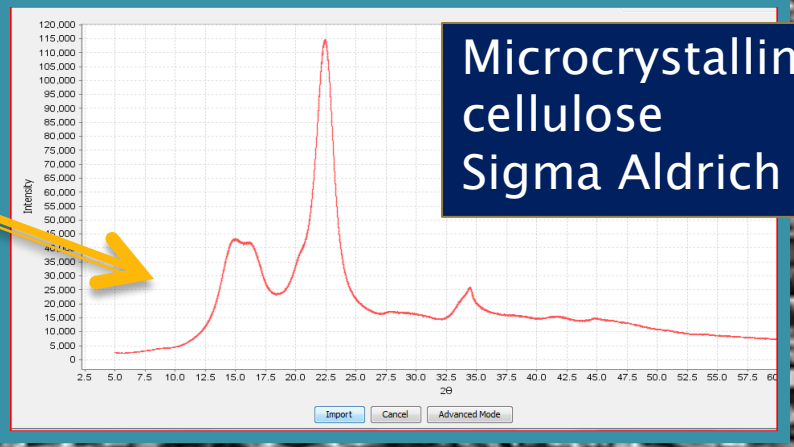
Challenges

Where is the baseline ?



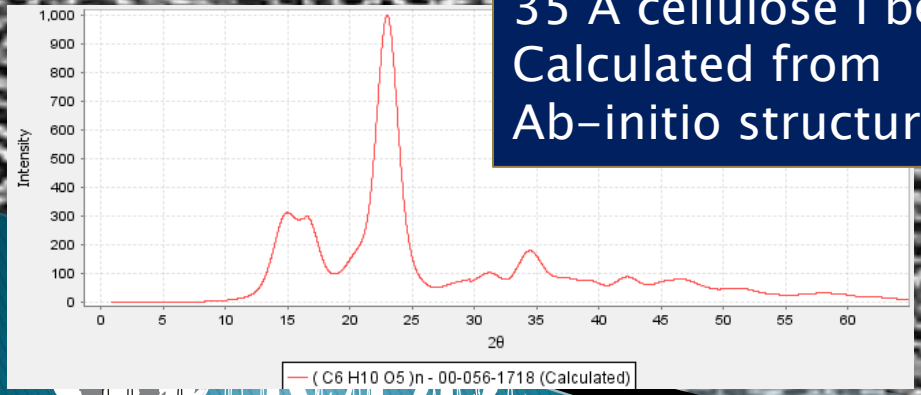
Mercerized cellulose
International Paper

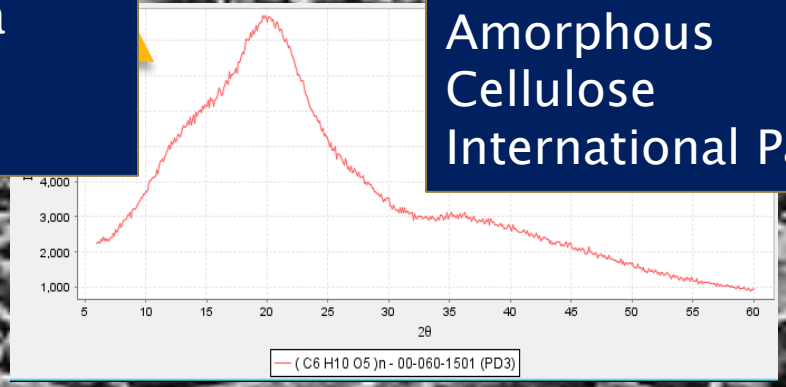
How do you separate Microcrystalline line broadening from the amorphous content or air scatter or Brehmstralung radiation



Microcrystalline cellulose
Sigma Aldrich

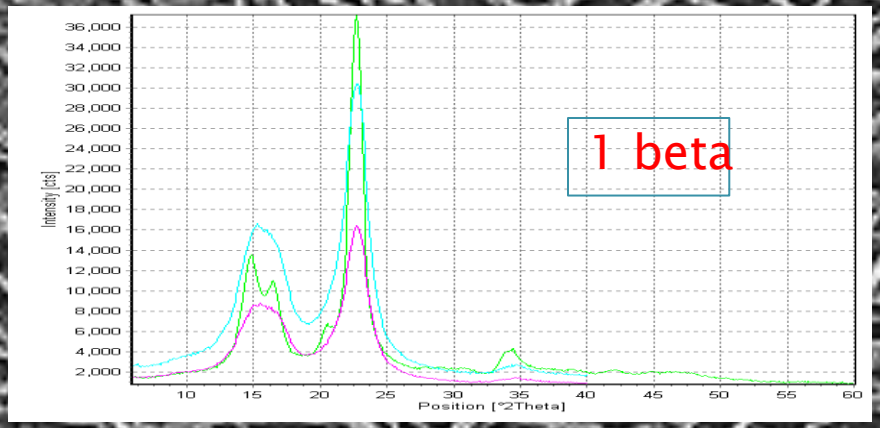
35 A cellulose I beta
Calculated from
Ab-initio structure

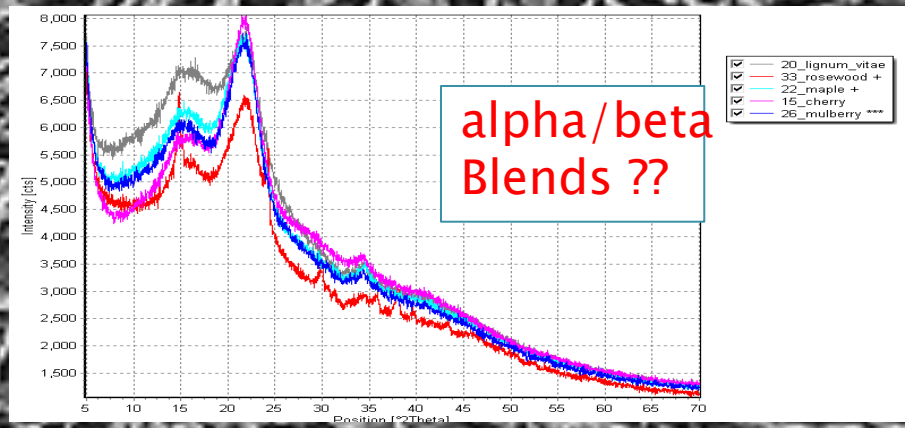


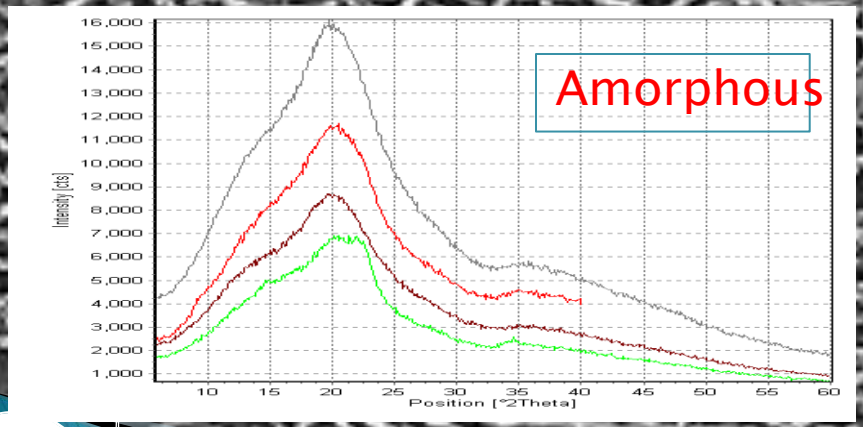


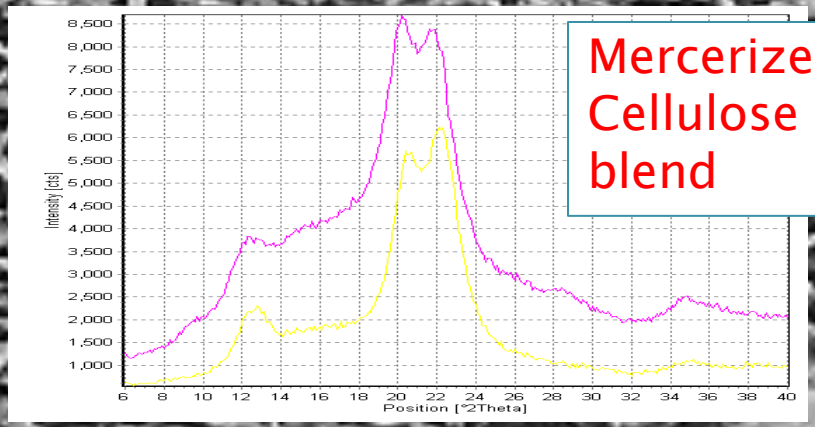
Amorphous
Cellulose
International Paper

Polymorph based clusters
Cluster groups from a cluster analysis









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Software Toolkit

Deconvolution Software

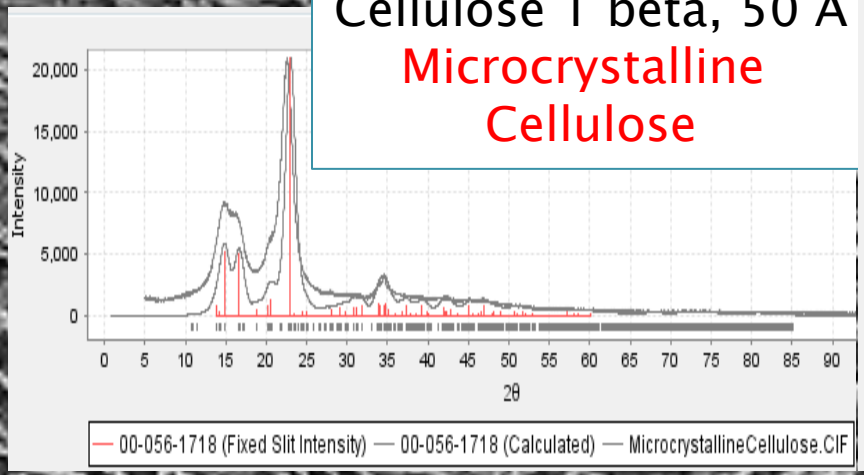
Similarity Indices
Cluster Analyses
(PANaltycial HighScore Plus 3.0.2)
Similarity Index
(ICDD PDF-4 Release 2011)
PolySnap
(Bruker-AXS Version 2.0)

Refinements
Rietveld Refinement
LeBail Refinement
Pattern Fitting (FULLPat)
(PANalytical HighScore Plus 3.0.2
Pattern Summation - ICDD Release 2011

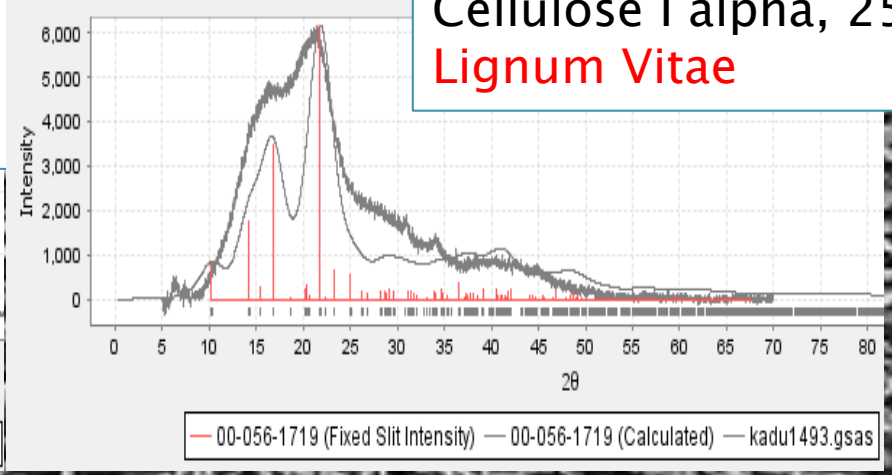
Pair Distribution Functions
RAD – Valeri Petkov

Confidence In Reference Standards

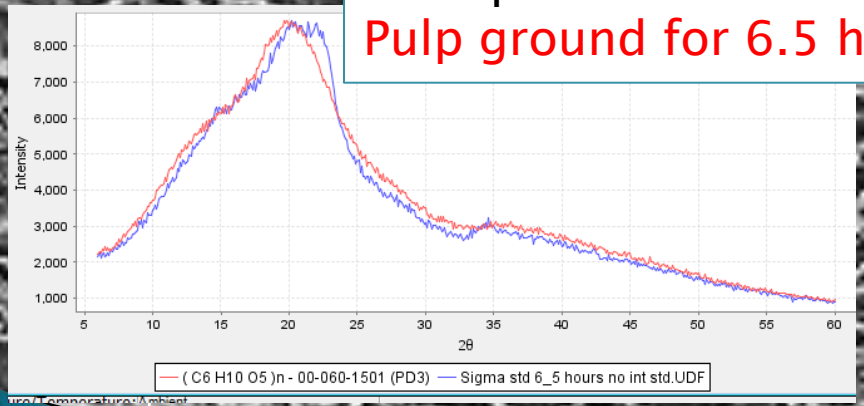
Cellulose 1 beta, 50 A
Microcrystalline Cellulose



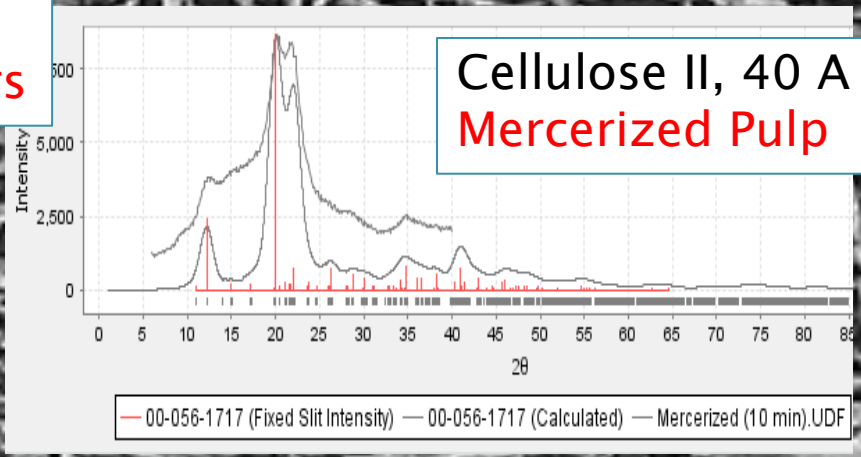
Cellulose I alpha, 25 A
Lignum Vitae



Amorphous Ref
Pulp ground for 6.5 hrs



Cellulose II, 40 A
Mercerized Pulp



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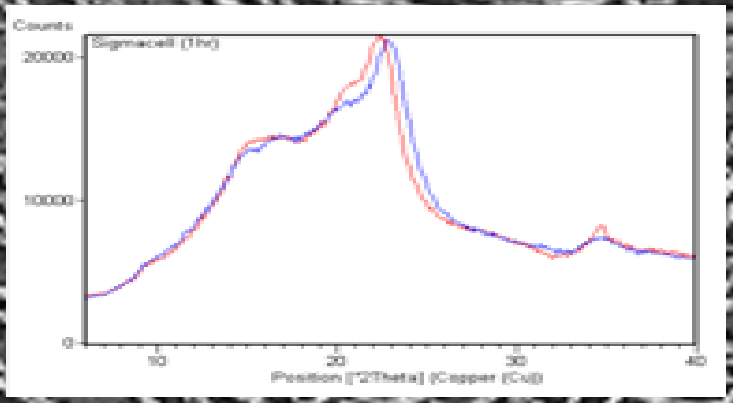
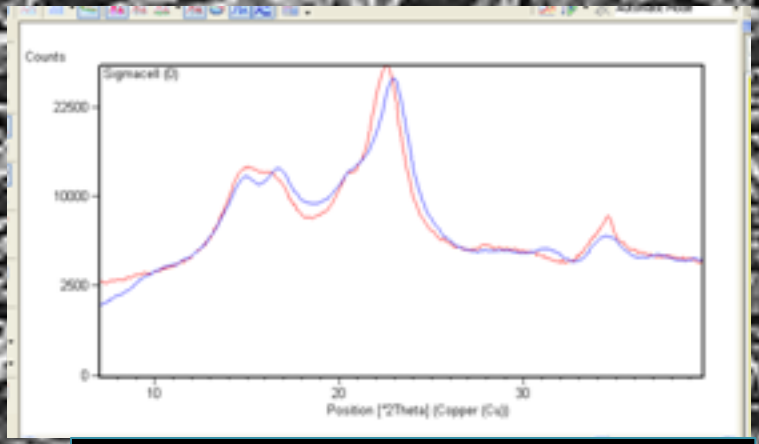
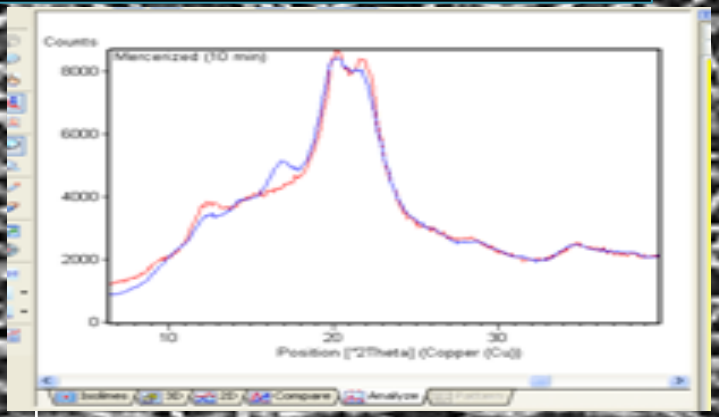
Confidence in Standards Experimental Data compared to pattern fit from standards



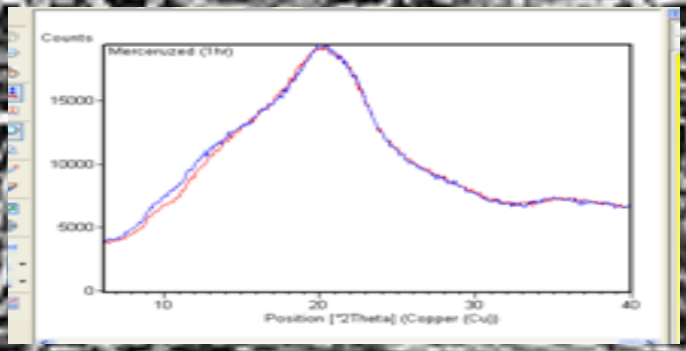
Ground pulp with amorphous/ 1 beta blend



Sigmacell with amorphous/ 1a/1b

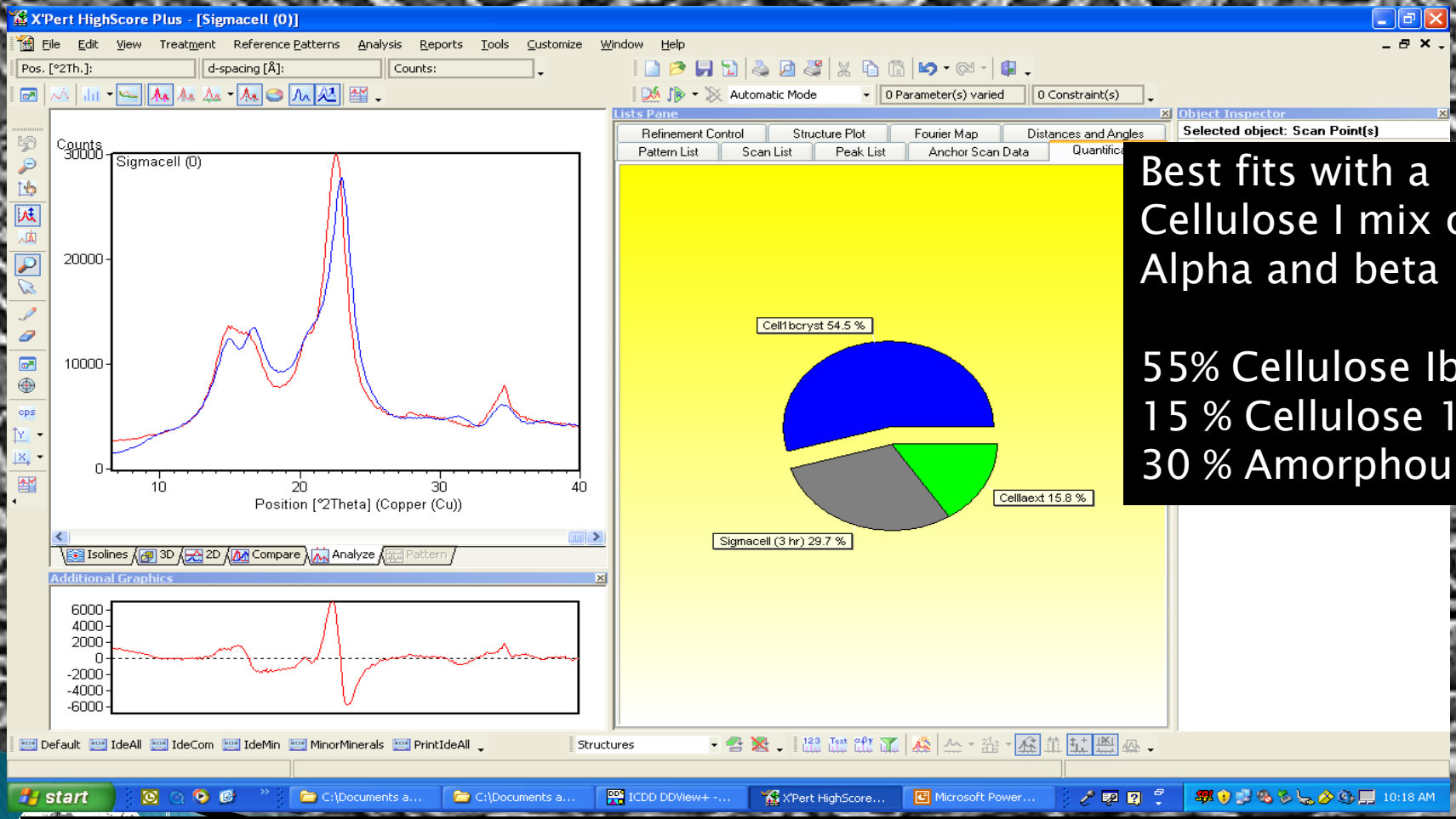


Mercerized pulp – II/1a/amorphous



Ground pulp – 1b and amorphous

Pattern Fitting – Sigmacell FULLPat in Highscore Plus

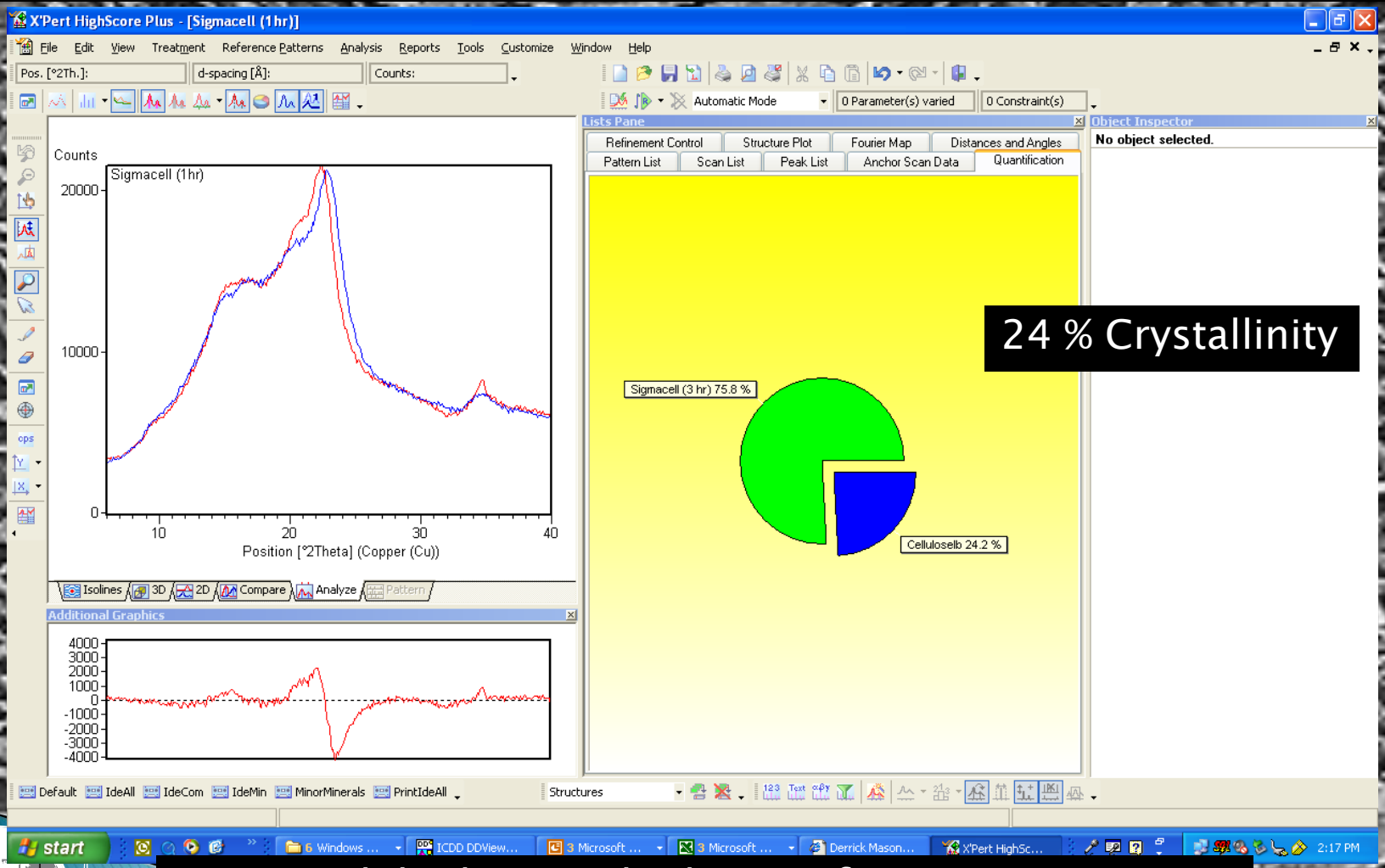


Best fits with a Cellulose I mix of Alpha and beta

55% Cellulose Ib
15 % Cellulose 1a
30 % Amorphous

Step 3a. Added in a very small size Cell Ia (width = 1.86), increases

Pattern Fitting – Ground Pulp FULLPat in Highscore Plus

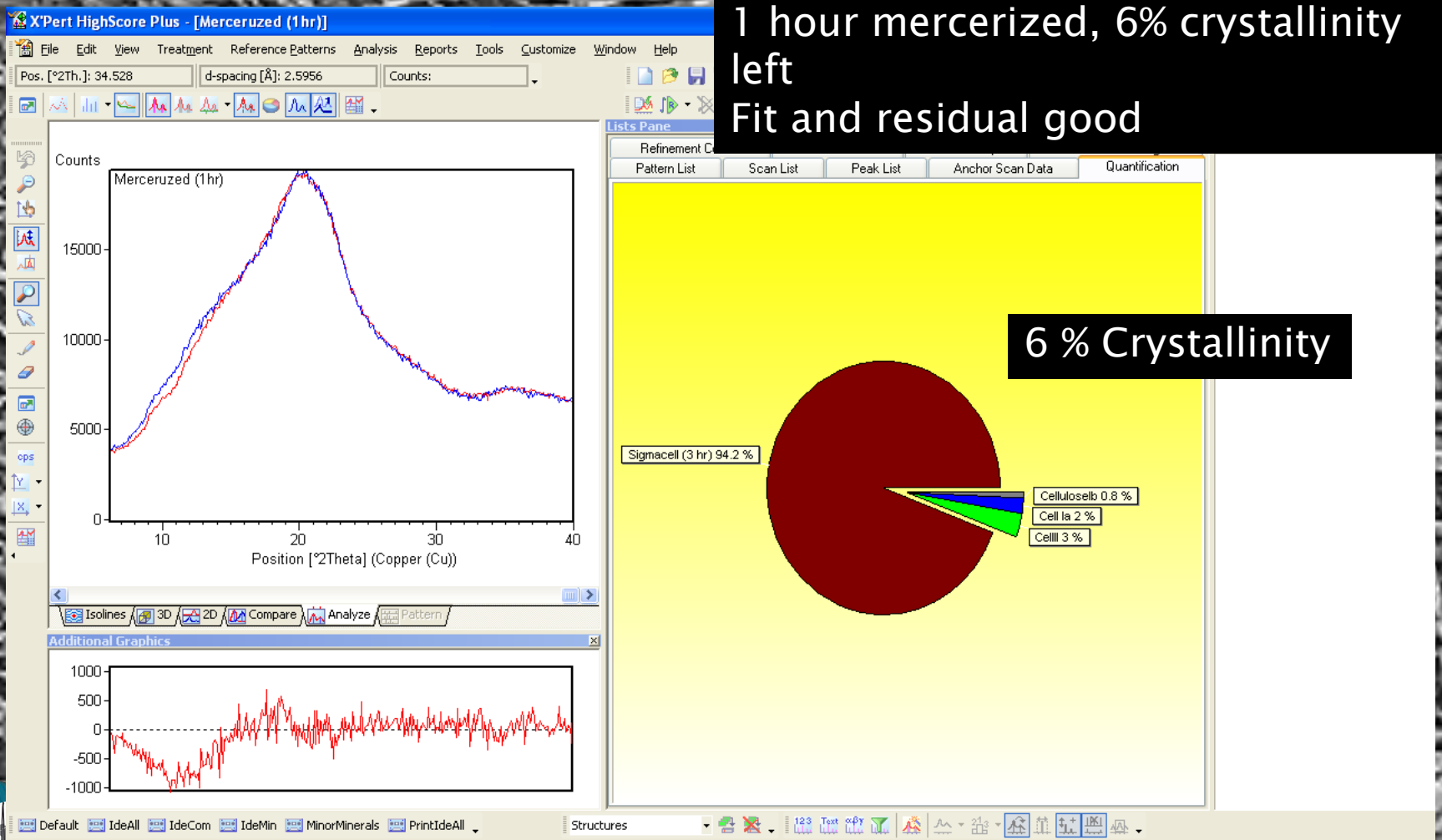


24 % Crystallinity

Using Kaduk 1beta and 3 hr as reference pts
Crystallinity calculated as 24.2 % for the 1 hour grind

Pattern Fitting – Mercerized pulp FULLPat in Highscore Plus

1 hour mercerized, 6% crystallinity left
Fit and residual good

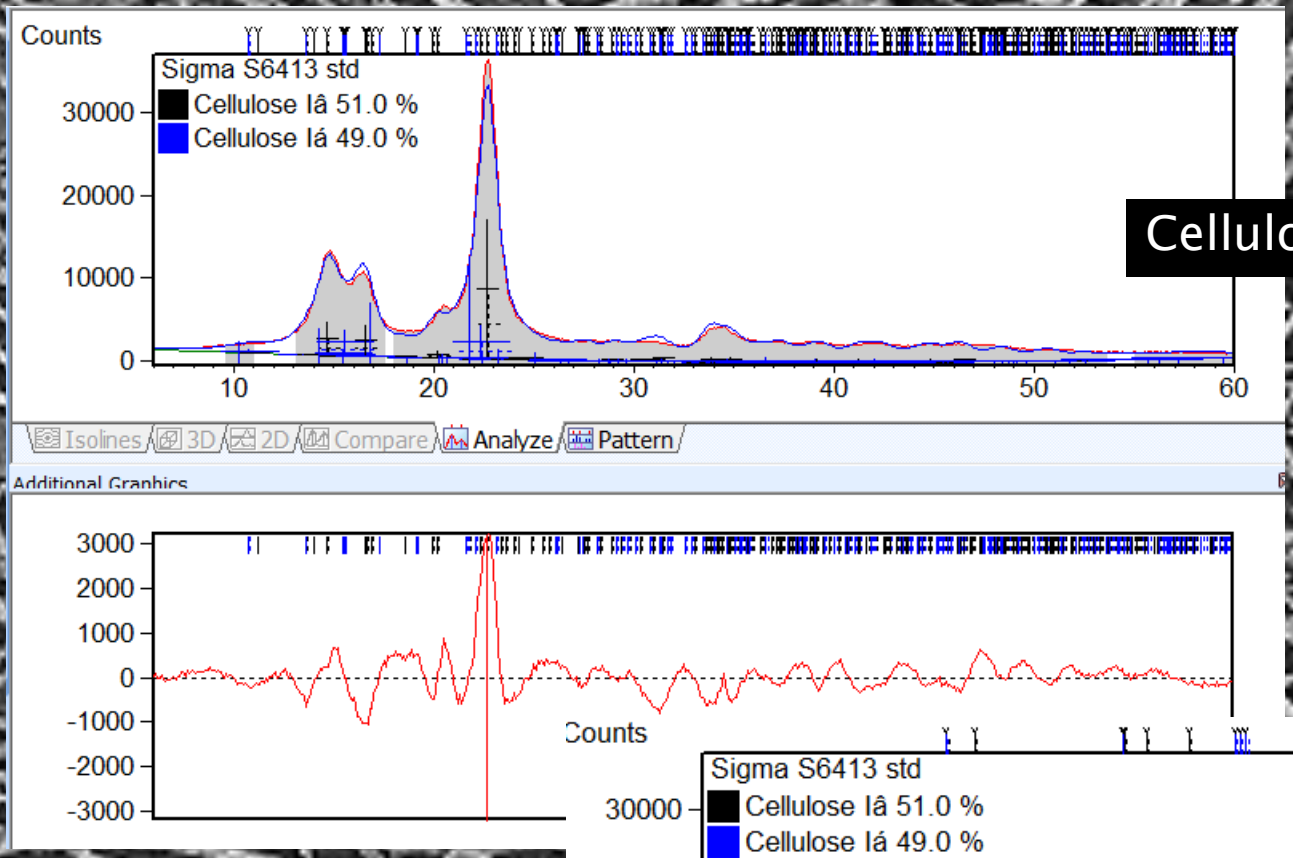


Best Results

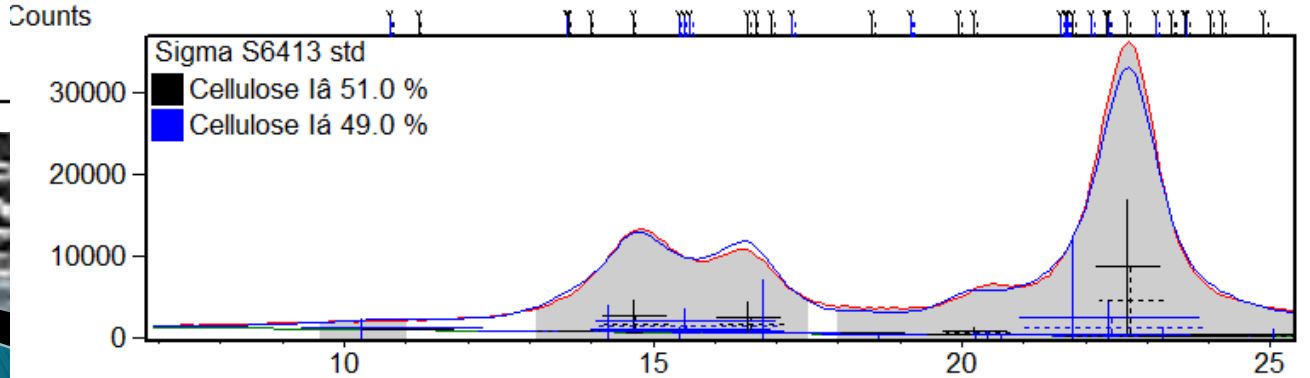
Sample	Treatment	% Cry	Polymorph
Sigmacell		70 %	54 Ib 16 Ia
Sigmacell	1hr	24.2 %	24.2 Ib
Sigmacell	2hr	3 %	
Sigmacell	3hr	0 %	
Mercerized*		70 %	30 Ib 40 II
Mercerized	10 min	32 %	8 Ib 24 II
Mercerized	1hr	6 %	1 1b 2 1a 3 II

* Statistically poor fit

Pattern Fitting – Cotton Linters Rietveld



Cellulose I beta



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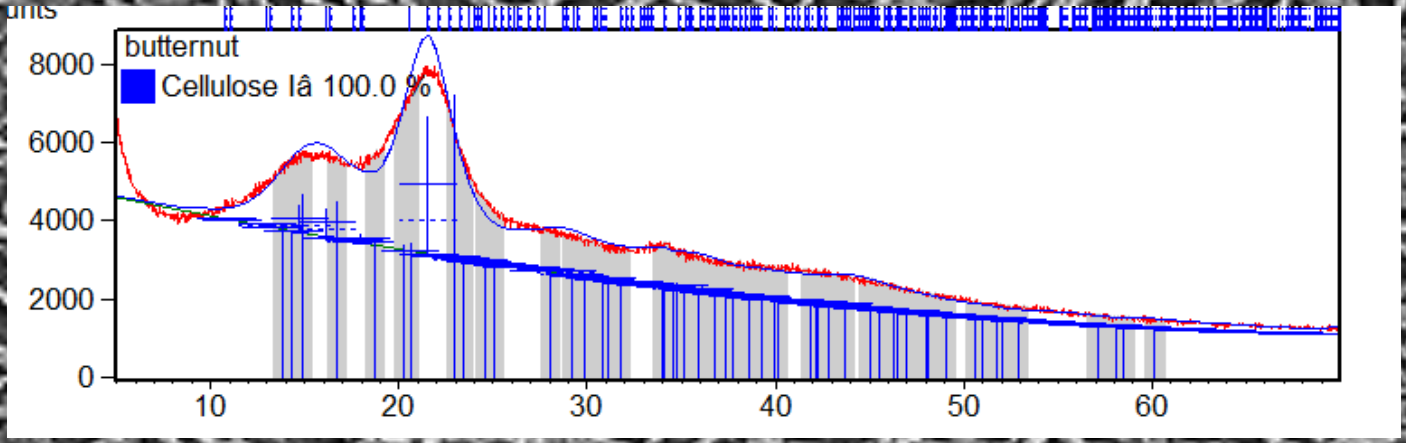
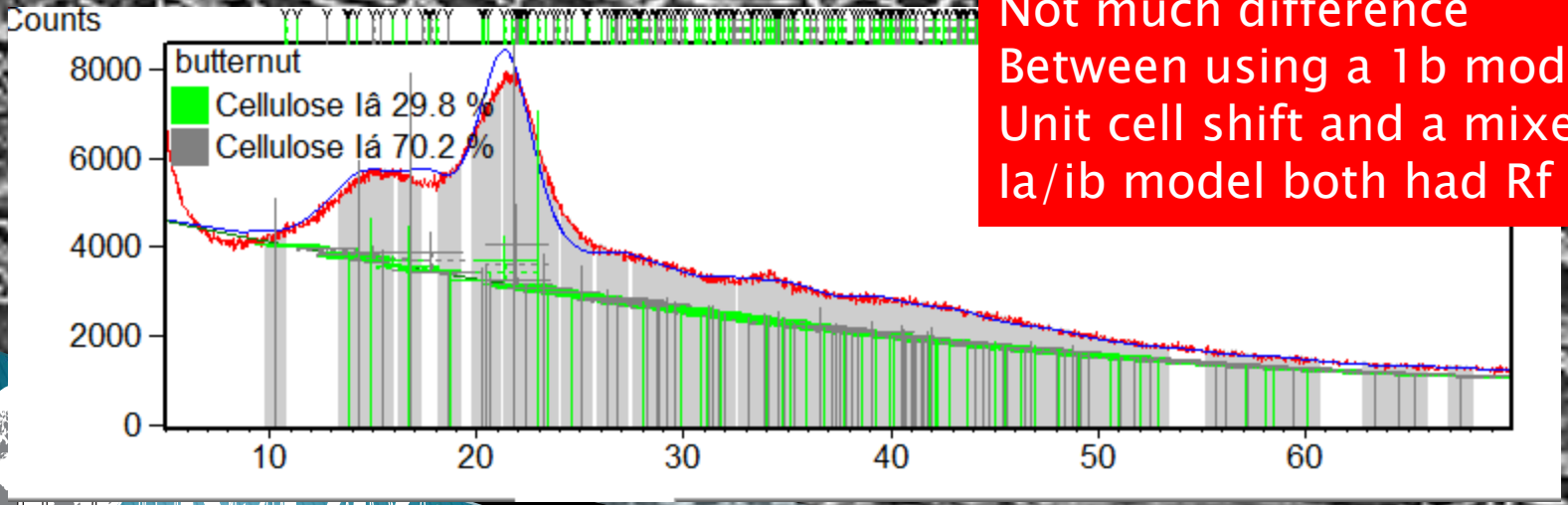
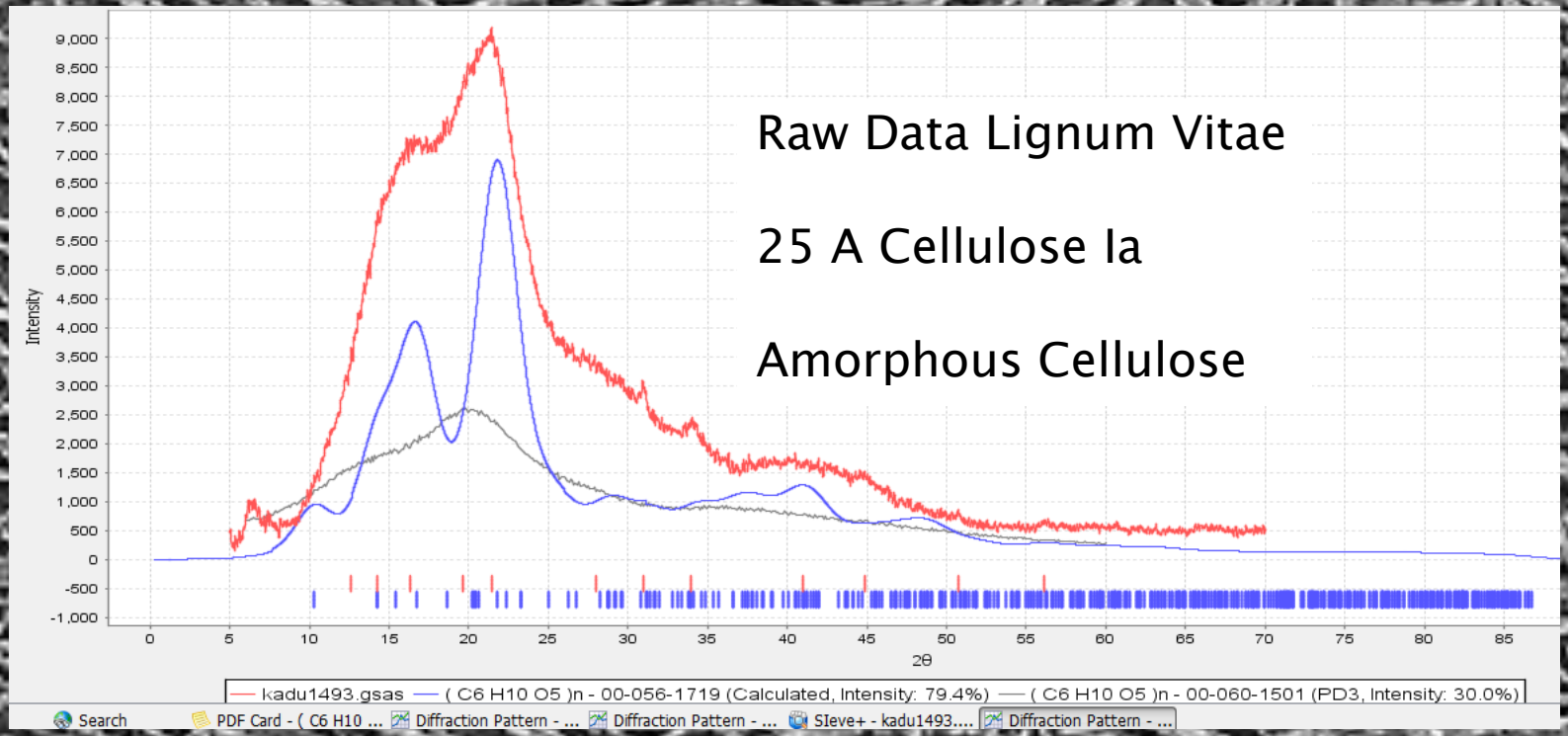
Butternut: Small crystallite size shifted unit cell a lot





Not much difference
Between using a 1b model with
Unit cell shift and a mixed
Ia/ib model both had Rf below 5

ICDD Pattern Summation 80/30



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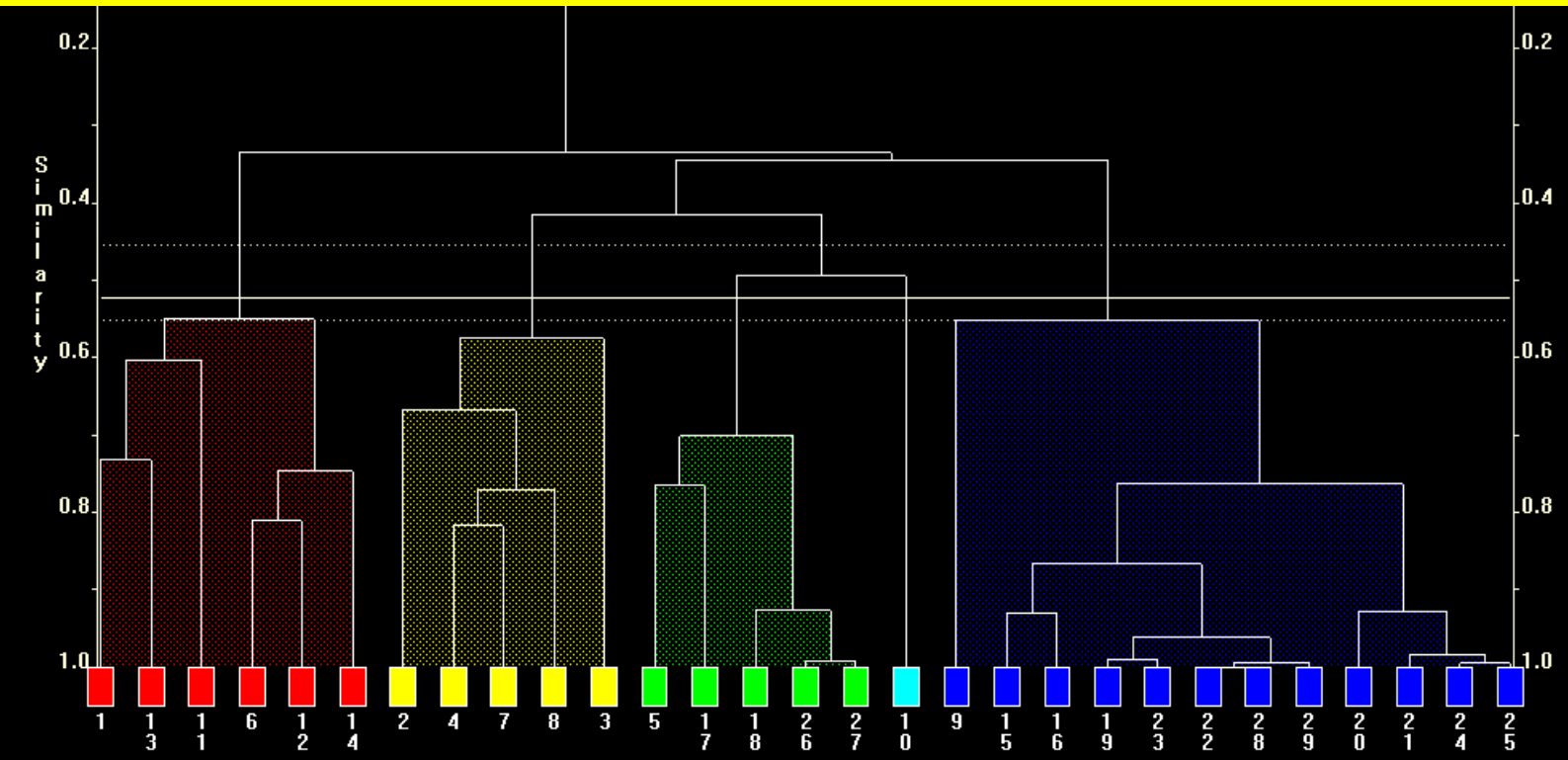
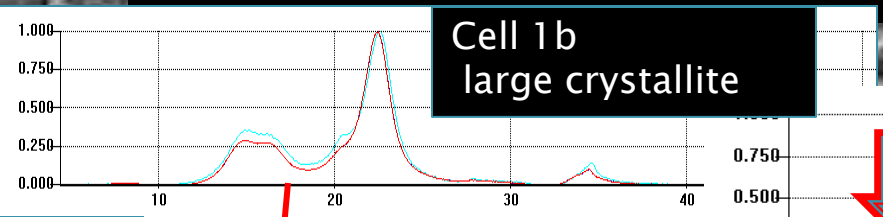
All ICDD References – historic

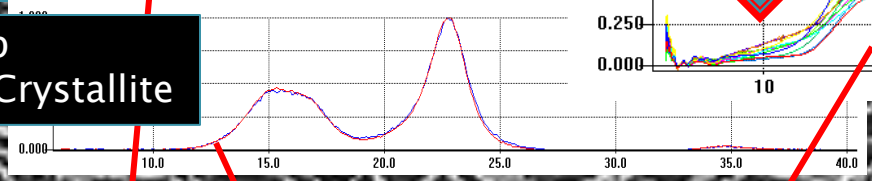


Blue is cellulose Ia and cellulose Ib characterisitics
Green is cellulose II characteristic
Yellow is cellulose III characteristic
Red are substituted celluloses – generally peaks at lower angle (triacetyl, tripropionate, nitrate, perchlorate, glycerine and trimethyl)

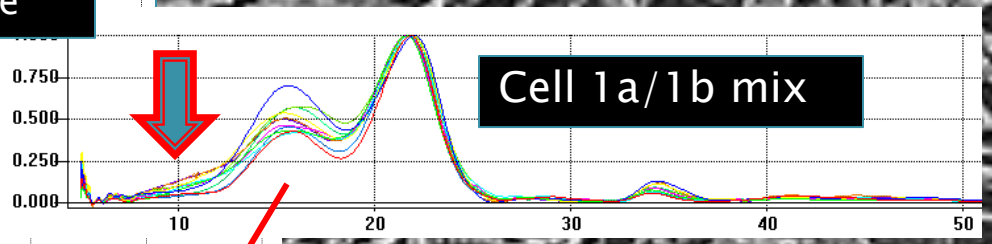
Cluster Analysis Dendrogram – Wood chips, pulps, and papers



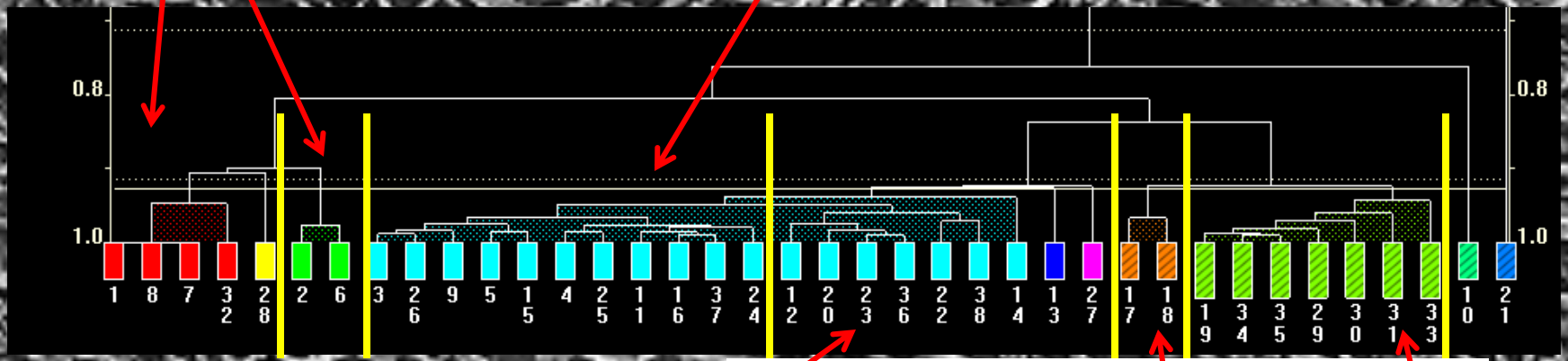
Cell 1b
large crystallite

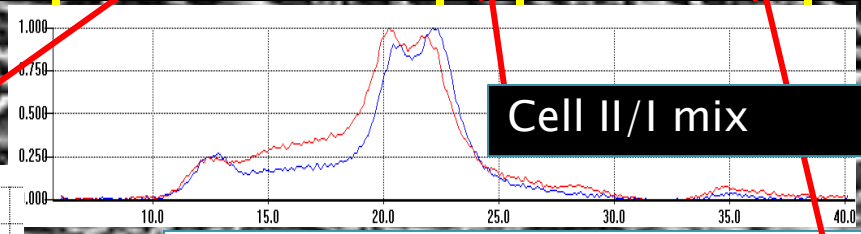


Cell 1b
Small Crystallite

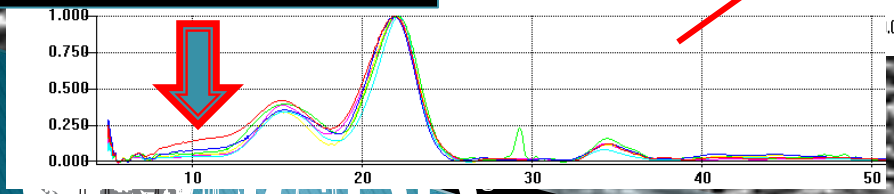


Cell 1a/1b mix

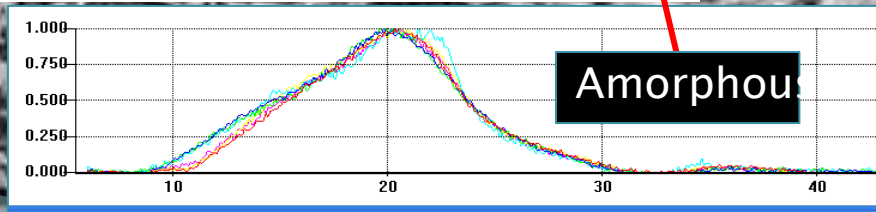




Cell II/I mix

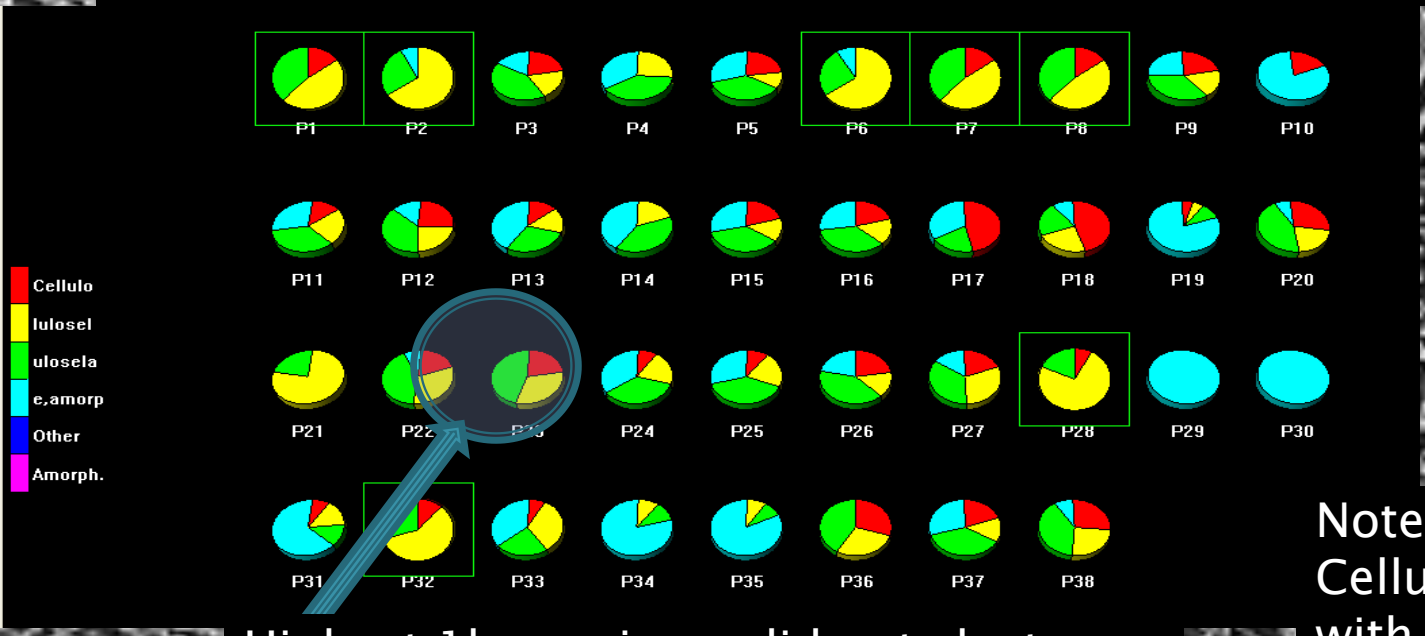


Cell 1a/1b mix

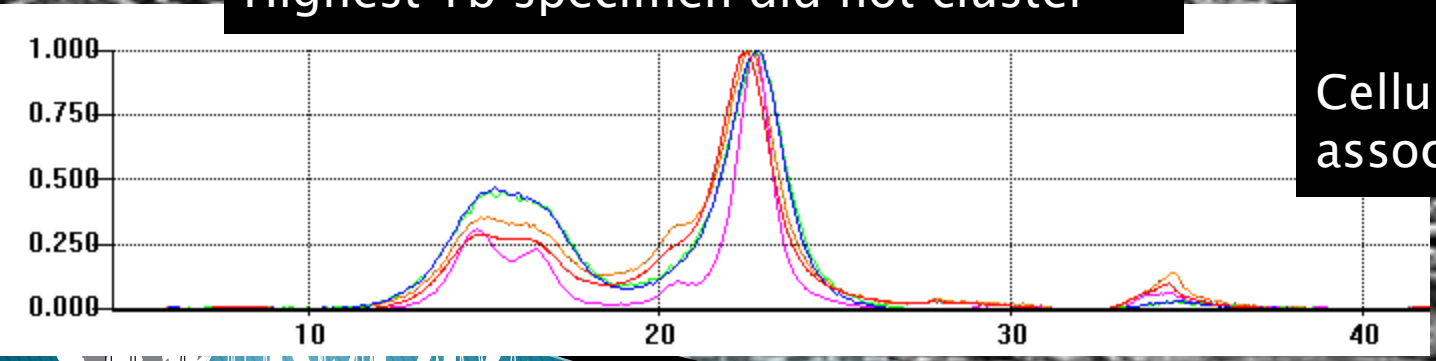


Amorphous

PolySNAP – 4 Reference Set Used Cell II (red), cell 1b (yellow), cell Ia (green) and amophous (blue)

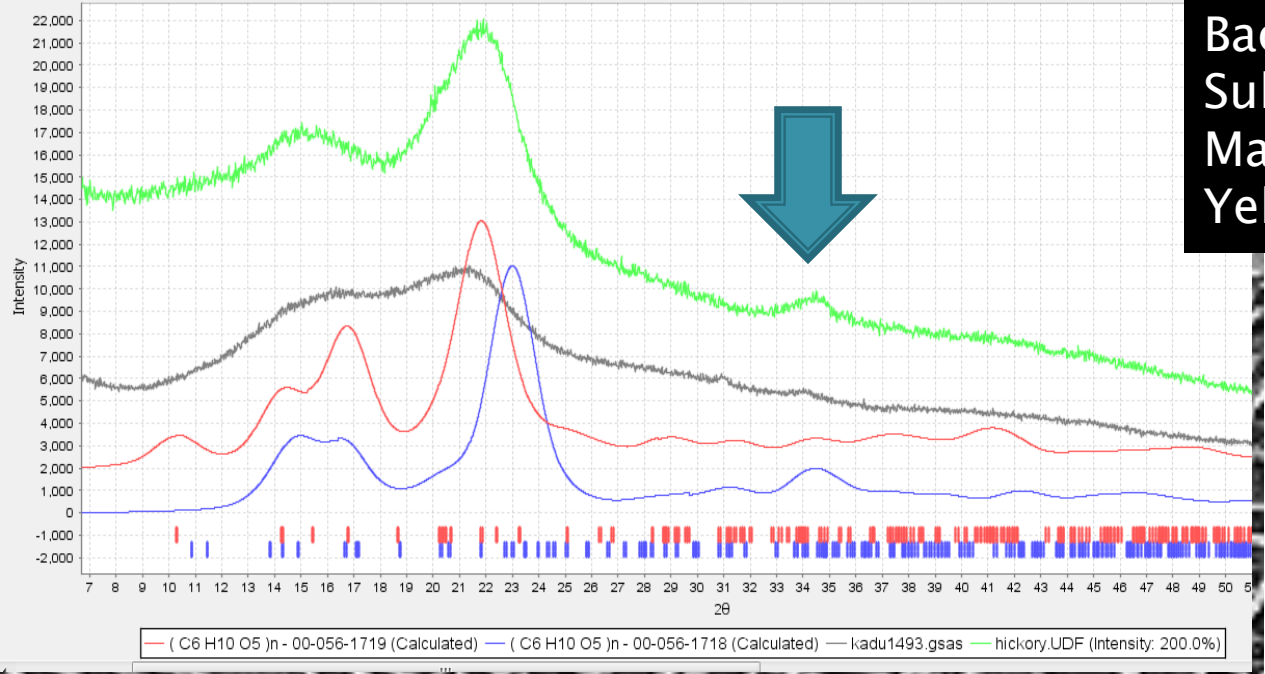


Highest 1b specimen did not cluster

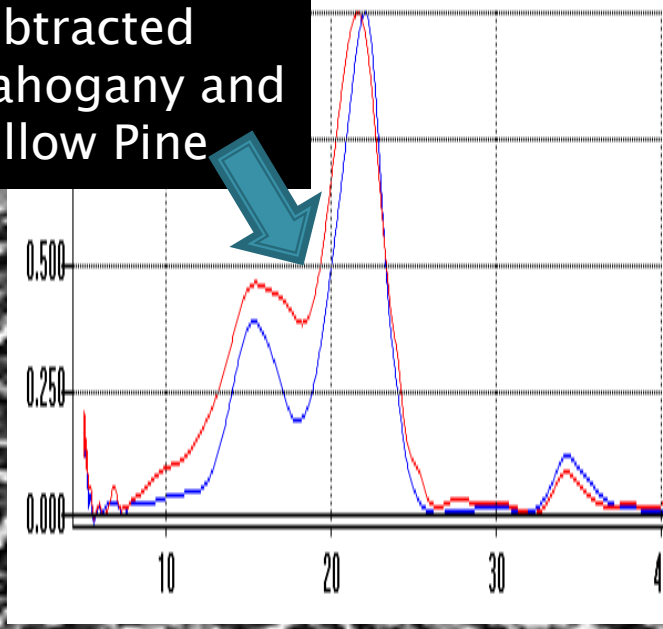


Note
Cellulose II associates with amorphous

Cellulose 1a and 1b associate



Backgroud Subtracted Mahogany and Yellow Pine



Hickory, Mahogany and Lignum Vitae

All show intensity around 10 degrees two theta

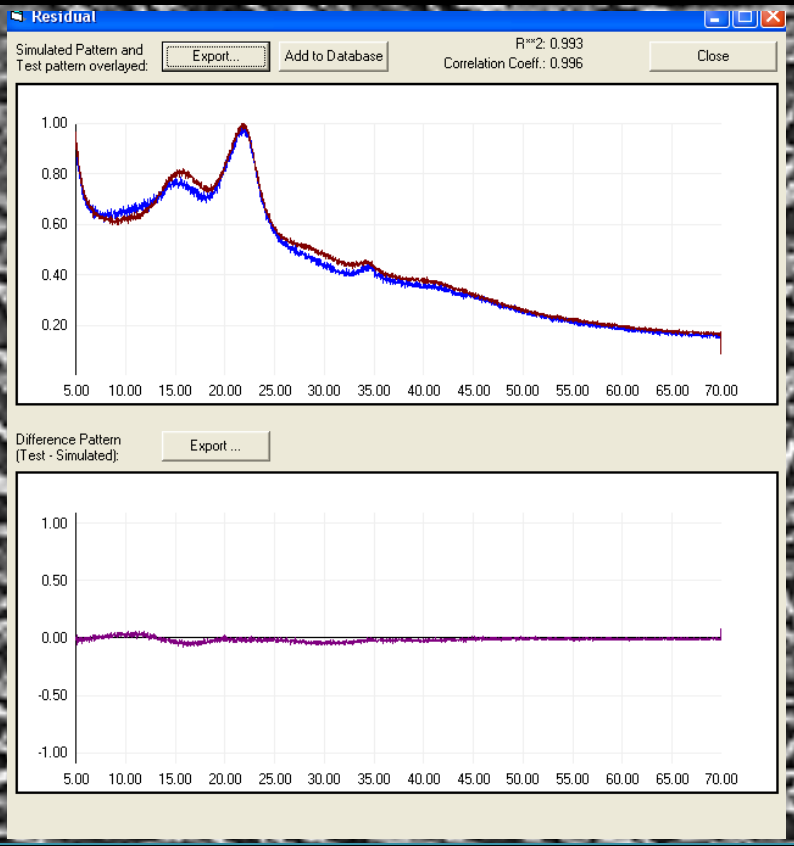
Hickory and Magoney both exhibits a peak at 34-35 degrees that is usually associated with cellulose Ib, the pattern looks to be predominately small crystallite size cellulose Ib, but it may be a 1b/1a mix

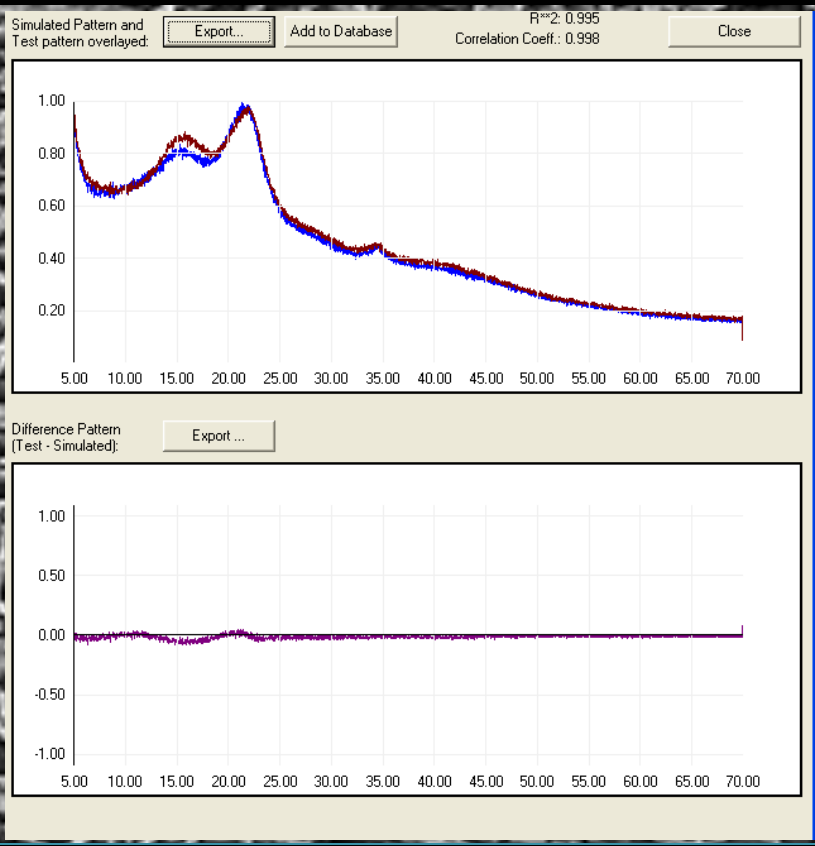
Lignum Vitae

Exhibits the character of a small crystallite size 1a with an amorphous component

In both specimens it is very difficult to say whether the intensity at 10-12 degrees is from cell Ia or an amorphous contribution. In mahogany and lignum Vitae there does appear to be slight but distinct slope changes

Small crystallites – different polymorphs





Hickory – 58 % Crystalline, 20 A Cell Ib

Maple – 85 % Crystalline 20 A Cell I a

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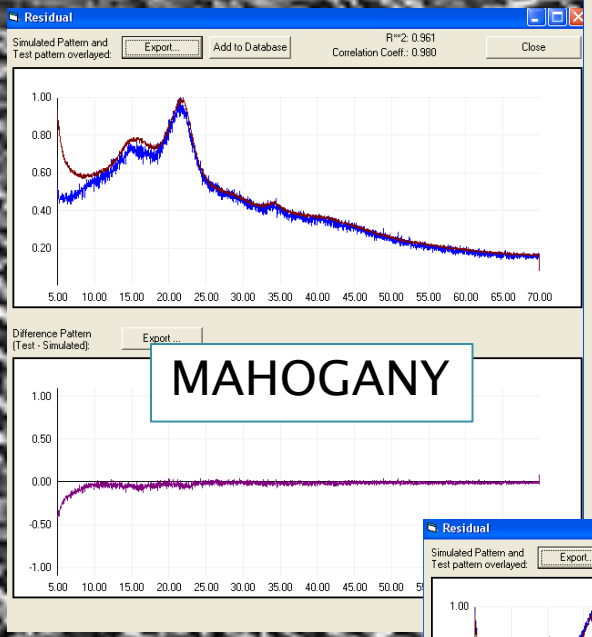
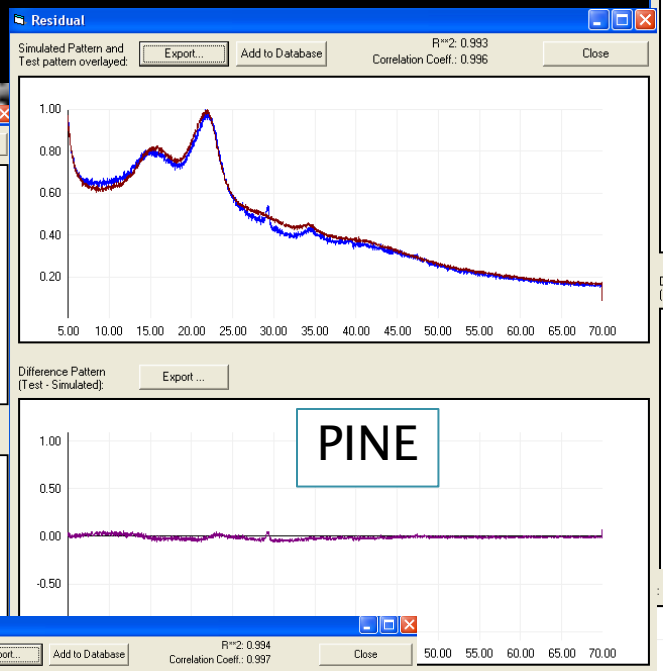
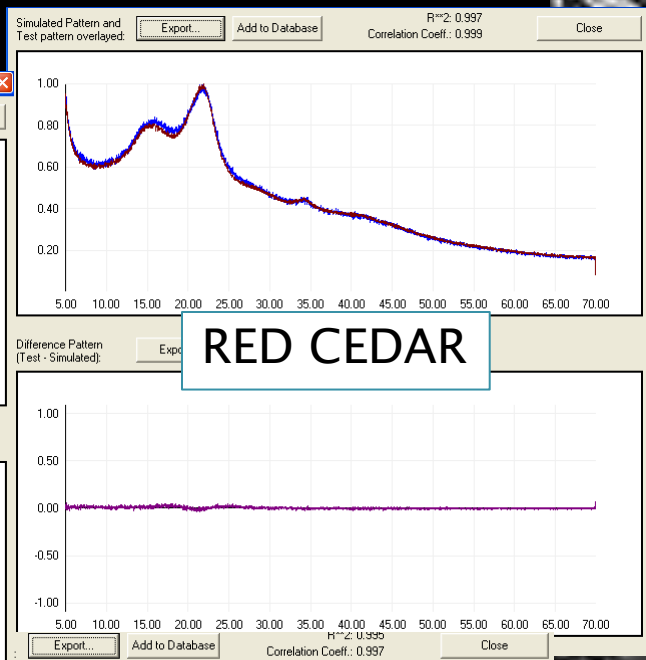
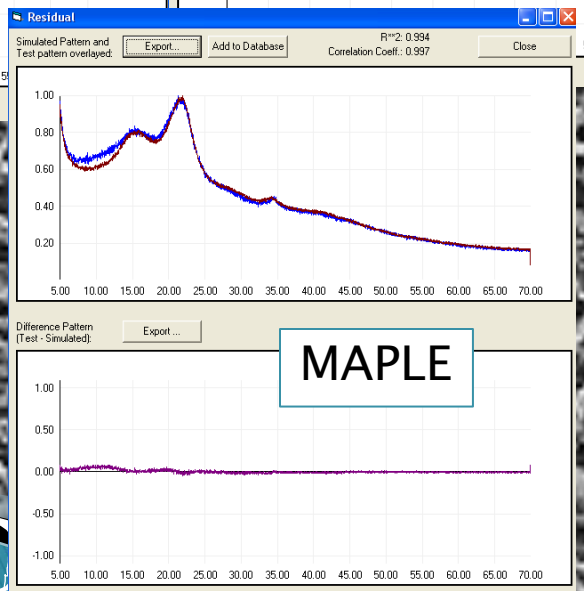
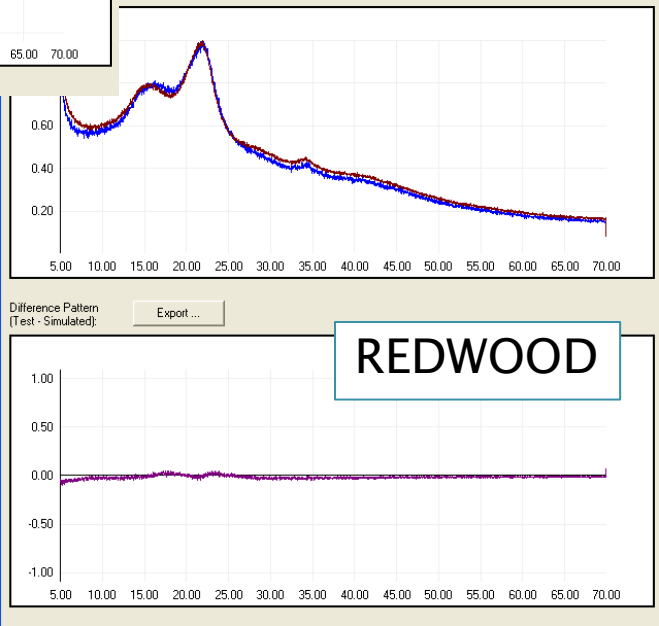
3 phase Fits



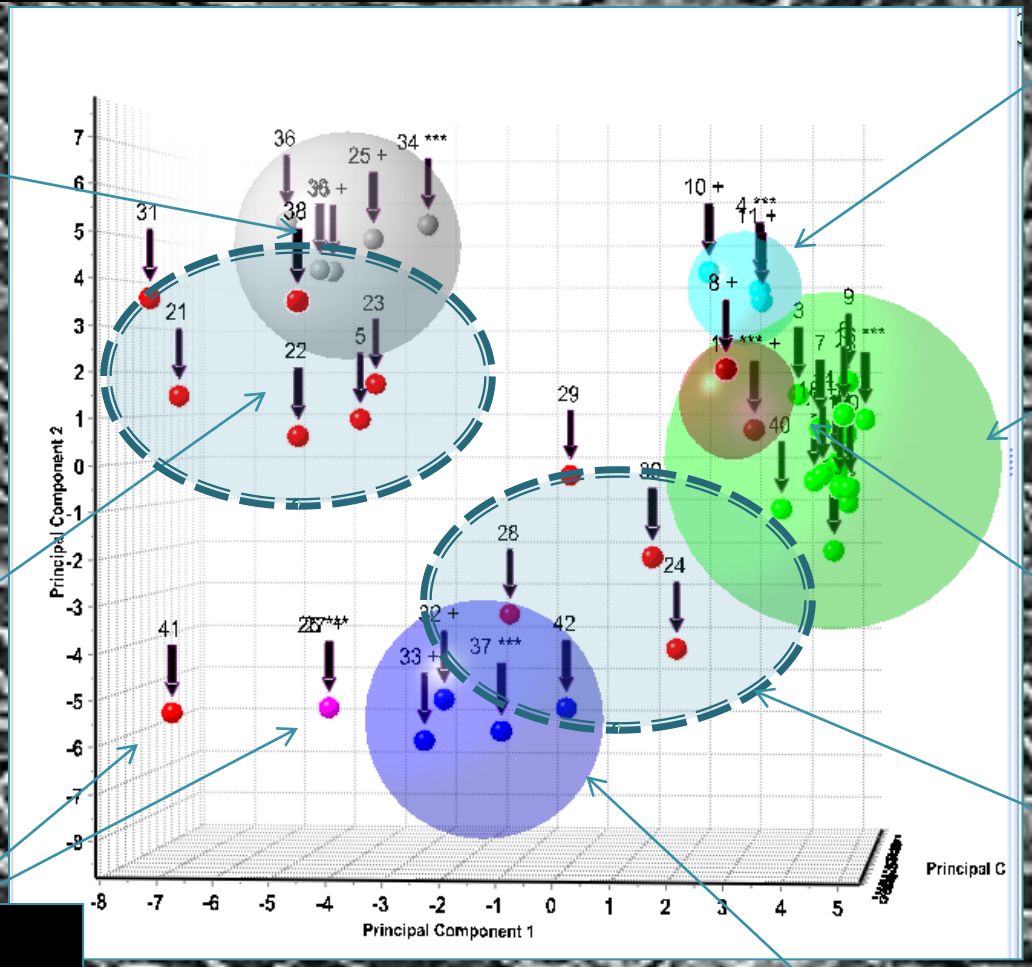








New PCA



Amorphous

Maple, Cherry, Mulberry

Wood pulps

Not clustered But mostly Cellulose II

Lignin Rosewood

Not clustered but mostly Ib

Very High Crystallinity Ib's

Highly crystalline cellulose Ib's Standards and filter paper

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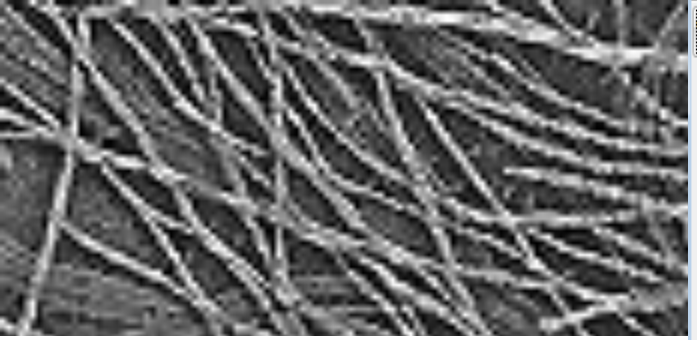
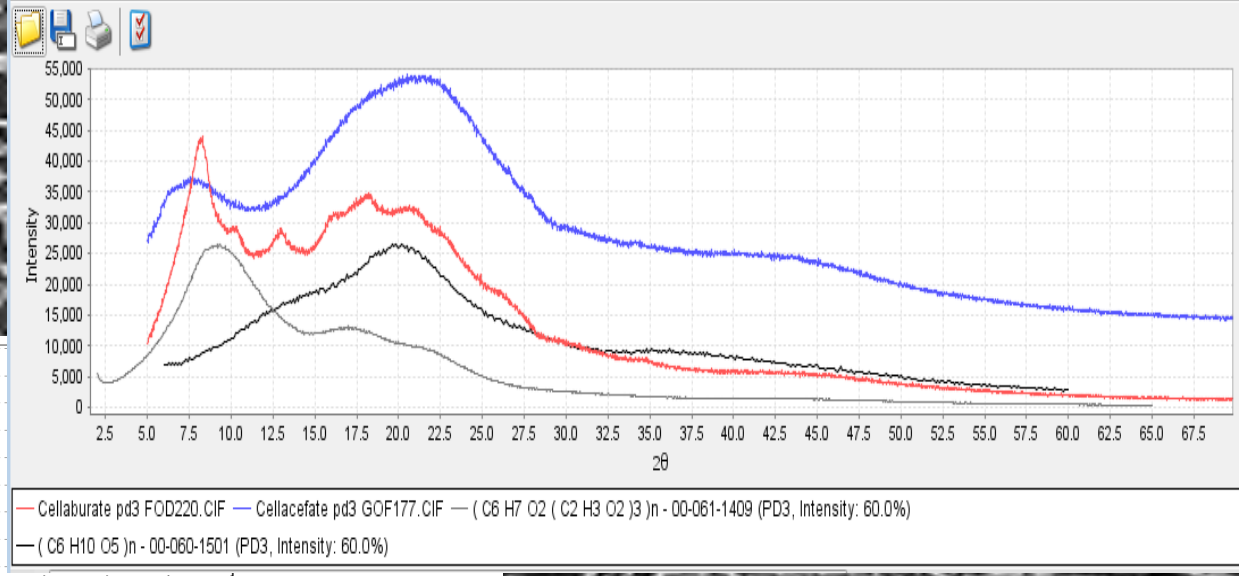
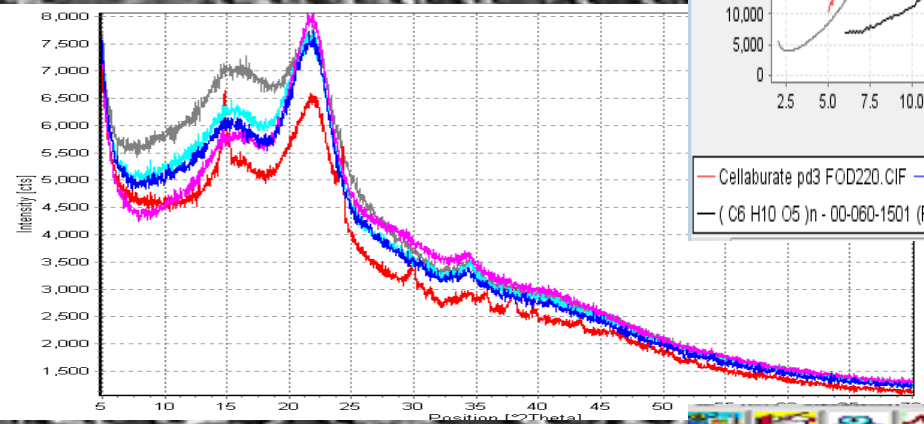
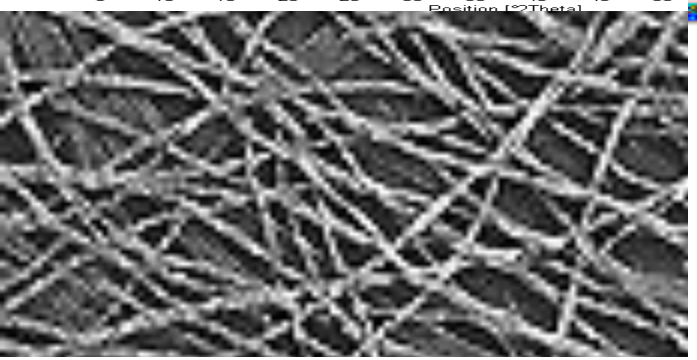
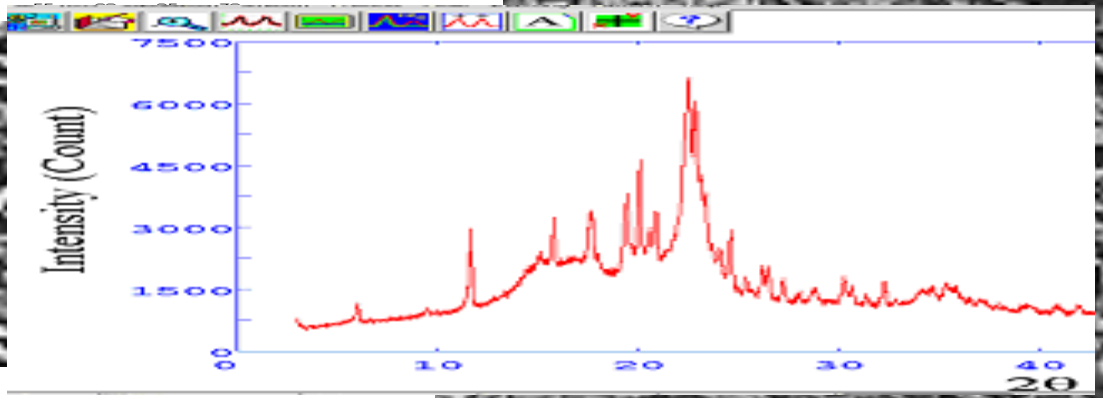



Intensity
2θ
Cellaburate pd3 FOD220.CIF
Cellacefate pd3 GOF177.CIF
(C6 H7 O2 (C2 H3 O2)3)n - 00-061-1409 (PD3, Intensity: 60.0%)
(C6 H10 O5)n - 00-060-1501 (PD3, Intensity: 60.0%)


Intensity [cts]
Position [°2Theta]




Intensity (Count)
2θ