DELIVERABLES REPORT

MultiHemp

Multipurpose hemp for industrial bioproducts and biomass

(Ref n. 311849)

Task: 6.5 Report on the characterisation of hemp cultivar strength and dislocations

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Part 1: Dislocation analysis

1. Introduction

Hemp and other bast fibres are prone to damage caused through compressive failure of the cell wall (Fig. 1). This damage commonly referred to as nodes, kink bands, micro-compressions or, more commonly, simply dislocations, can occur in the plant during growth, but when hemp fibres are processed industrially, significantly more dislocations are produced in the fibres (Hänninen *et al.,* 2012). The causes and implicates of dislocations have been comprehensively reviewed by Hughes (2012). These dislocations can be visualized using cross-polarized light microscopy. So far, methods based on microscopy have been developed (Thygesen and Hoffmeyer, 2005) in order to quantify the number of defects, but they are time consuming and not easy to implement industrially. Alternative methods, relying upon the increased sensitivity of the fibre to acid hydrolysis at the defects to break them into shorter segments have also been developed (Thygesen, 2008) and this approach has been adopted in this work. The aim of this procedure is to offer an alternative to conventional, microscopy-based methods, to quantify the amount of dislocations present in the fibres. Fig. 1 shows hemp fibres after three stages of production, where the dislocations are very clear (the black bands across the fibres) in the decorticated and carded fibres.

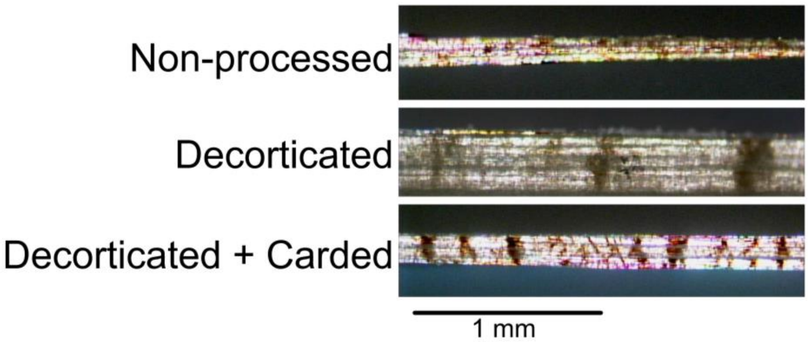

Fig. 1: Microscopic images of hemp fibres at different stages of production

The procedure uses a combination of acid hydrolysis of the fibres followed by fractionation using a Bauer McNett fibre classifier. The acid hydrolysis breaks the fibres at the dislocations into smaller fibre segments (Thygesen, 2008; Hänninen *et al.,* 2012) and after that, the fibre classifier separates the fibre fragments into fractions according to the fibre segment's length. Both the equipment and procedures are detailed in the protocol developed as part of this work package. At the end, the expected results are that fibre samples that have fewer dislocations should exhibit a larger proportion of longer fibre length fractions and conversely, samples with more dislocations will show a larger proportion of fibres of shorter fibre length fractions.

2. Materials and methods

2.1 Origin of Raw Material and Tests Performed

Raw material, in the form of scotched and carded fibre, was supplied by Hochschule Bremen (partner 4) to Aalto University (partner 7), and consisted of 4 varieties of stems grown in 3 different locations (with 3 plots per location) and processed in two different ways, as summarized below:

- 4 Varieties: VDS-303, AGM-703, AGM-705 and IWNRZ-903
- 4 Locations: FNPC, France (partner 9), UCSC, Italy (partner 1) and VDS, Netherlands (partner 10)
- Processing: water retted and green
- Plot: B1, B2 and B3

2.2 Defect characterisation

Characterisation of the defects was carried out in accordance wit the protocol developed in this work package and reported previously (D_6-2_Protocol_2015_Aalto). Please refer to the apended protocol for details of the characterisation methodology. The protocol was revised and updated to incorporate minor modifications. The modifications were agreed beforehand with the other partners in WP6, and all tests were performed according to the revised protocol.

The key revisions to the protocol consisted of:

- Reduction of the sample from 10 grams to 4 grams, because limited amount of sample was available.
- Taken out the process of measuring the moisture content, and changed that the sample mass measured is considered with the fibre moisture content, for the same reason mentioned above, of having limited amount of fibre.

3. Results

3.1 Interpretation of results

The process of fractionation in Bauer McNett separates the fibre segments of a sample of acid hydrolysed hemp fibre into fractions depending by the length of the fibre segment. The device is equipped with sieves of a different aperture, from the biggest to the smallest in a decreasing progression, so if a fibre segment if small enough it will be able to pass through the sieves and it will be retained by the sieve that it's not able to pass through.

Based on this, every sieve retains the fibre segments that are able to pass the sieve before, and that are not able to pass the current sieve. And all the fibre segments retained by each sieve are captured by a filter paper when draining the deposits of the Bauer McNett device. This allows transforming the fibre segment weight into the graphs that will be shown later on this report.

So, the bigger the size of a fraction in the graphs presented below, the greater the number of fibre segments it contains (though it must be noted that only the <u>same fractions</u> can be directly compared since the measurements are based on mass, thus different fractions containing the <u>same mass</u> of fibre will have <u>different numbers</u> of segments in each). Comparison shows the amount of dislocations, since if a fibre is more damaged it will results in bigger amount of smaller segments (and bigger fractions 5, 6, 7 and 8) and if the fibre contains a lower amount of dislocations it will show greater values in fractions 1, 2, 3, and 4.

3.2 Results From Testing the Plot B1 samples for all Varieties, Processings and Locations

Key remarks from Figure 2

Figure 2 shows the fractions for all varieties, locations and processing methods. As may be seen, the green fibres (i.e. not retted), generally show a greater proportion of larger fractions (i.e. fractions 1, 2, etc.). The fibres grown in the Netherlands and Italy (UCSC and VDS) show similar trends in fractions, however, the fibres from stems grown in France (FNPC) show a rather lower proportion of fibres form the lower fractions, indicating a greater proportion of damage in these fibres. There is some evidence to suggest that INWRZ-903 has slightly lower damage than the other varieties.

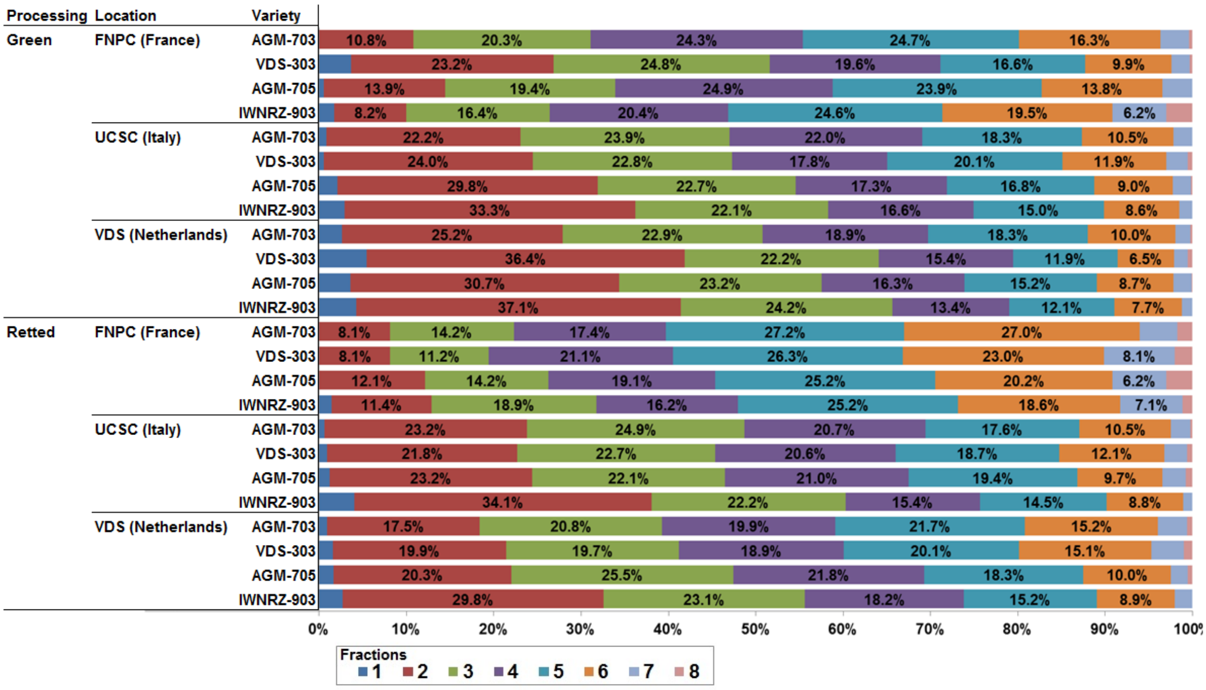

Fig. 2: Fractions for all varieties, locations and processing methods

Key remarks from Figure 3

Figure 3 shows the same results than Figure 2, but in this graph the samples have been grouped all the varieties in the same fraction distribution, to analyse the differences among locations. This graph in figure 3 confirms the trend mentioned before, that he fibres grown in France (FNPC) show a lower proportion on the lower fractions compared with the fibres grown in the Netherlands and Italy (UCSC and VDS) that show higher proportion in the lower fractions, as well as similar trends in the fraction distribution.

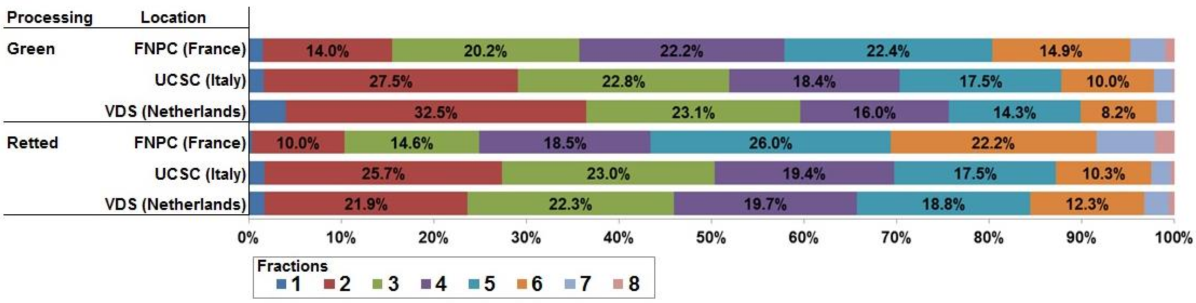

Fig. 3: Data from Figure 2, sorted by Processing and Location (Varieties grouped)

Key remarks from Figure 4

Figure 4 shows the same results than Figure 2, grouping all the locations on the same fraction distribution, to analyse the differences among varieties and processing. The fraction distribution supports what was mentioned previously, that variety IWNRZ-903 has lower damage than other varieties, even though VDS-303 and AGM-705 have similar fraction distribution in the case of green fibres (i. e. non-retted)

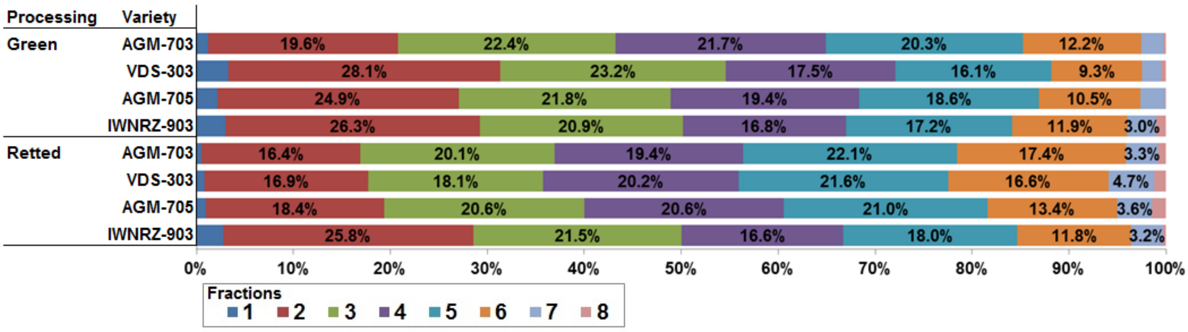

Fig. 4: Data from Figure 2, sorted by Processing and Variety (Locations grouped)

3.3 Results From Testing the <u>Variety IWNRZ-903</u> for all Plots, Processings and Locations

<u>Note:</u> Data from plots B1 is the same than in Figure 2. Tests of fibres from plots B2 and B3 is added in the following graph of Figure 5 to test the repeatability

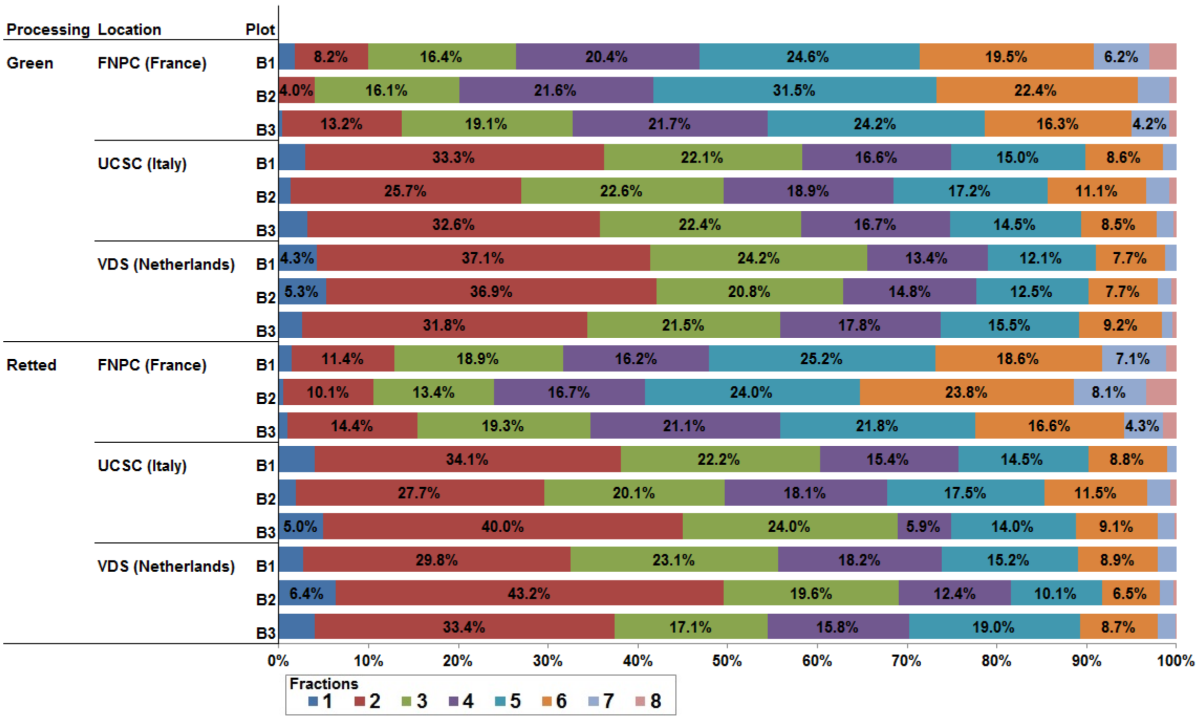

Fig. 5: General graph for all tests performed with the variety IWNRZ-903

Key remarks from Figure 5

Figure 5 shows the results for all the samples from plots B1, B2 and B3, for all locations and processings for the variety IWNRZ-903. All samples from plots grown in France (FNPC) locations follow a similar trend, clearly different than the fibres grown in the Netherlands and Italy (UCSC and VDS).

<u>Key remarks from Figure 6</u>

Graph in Figure 6 shows from the same data than Figure 5, grouping all the plots together, and showing the results classified by the location and the processing, to analyse the differences among locations and processing (excluding the plots). It is clearly seen the same trend observed before, that fibres grown in France (French) show a very different pattern in the fraction's distribution, as well as that green fibres show a little less presence of damage than retted fibres, with the exception of the fibres processed in the Netherlands (VDS) that both fibres, green and retted, show a very similar pattern.

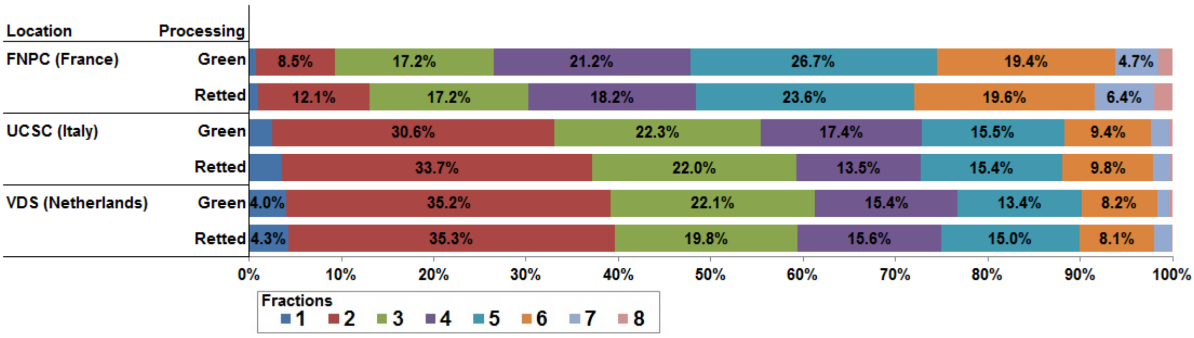

Fig. 6: Data from Figure 5 sorted by Processing and Location (Plots grouped)

4. Dissemination of Knowledge

There is one manuscript almost ready, in process to be sent to other partners and to be submitted to a peer reviewed journal during spring 2015, and there is a second manuscript in preparation with submission planned during 2015.

- Tentative Title for the First Manuscript: The influence of processing on the physical structure of hemp fibres
- Tentative Title for the Second manuscript: Characterization of defects in hemp fibres and correlation with production processes

5. References

Hughes, M. (2012). Defects in natural fibres – their origin, characteristics and implications for natural fibre reinforced composites: a review J Mater Sci, 47(2), 599-609

Hänninen, T., Thygesen, A., Mehmood, S., Madsen, B. and Hughes, M. (2012). Mechanical processing of bast fibres: The occurrence of damage and its effect on fibre structure. Industrial Crops and Products, 39:7–11

Thygesen, L. G. (2008). Quantification of dislocations in hemp fibers using acid hydrolysis and fiber segment length distributions. Journal of Materials Sciences, 43:1311–1317

Thygesen, L. G. and Hoffmeyer, P. (2005). Image analysis for the quantification of dislocations in hemp fibres. Industrial Crops and Products, 21:173–184

Part 2: Strength analysis

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WP6 (task 6.1) Fibre mechanics

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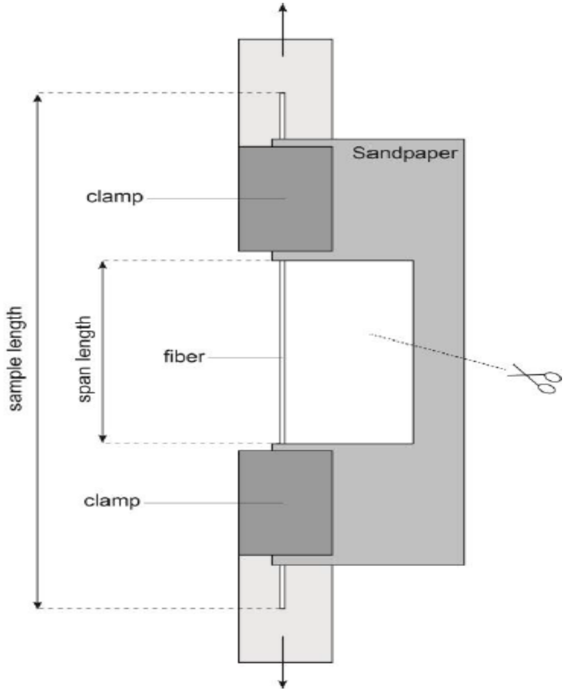
AW Van Vuure

MultiHemp

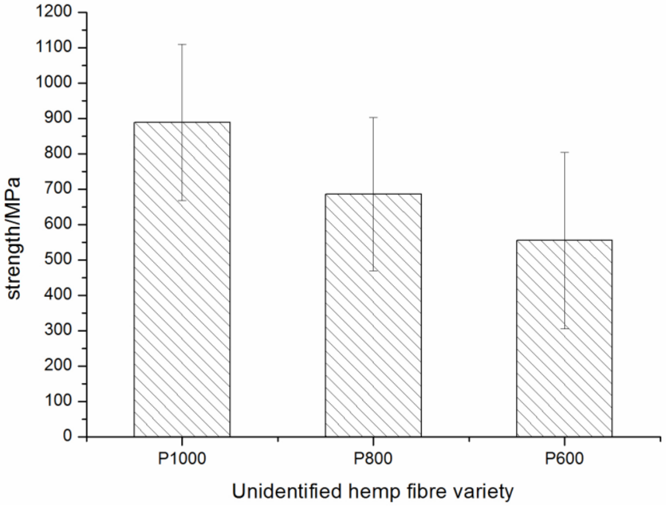
Fibre quality: Single fibre tensile tests

Span length	20 mm
Tensile speed	0.5 mm/min
Clamp pressure	7 bar
Roughness of the sandpaper	P1000

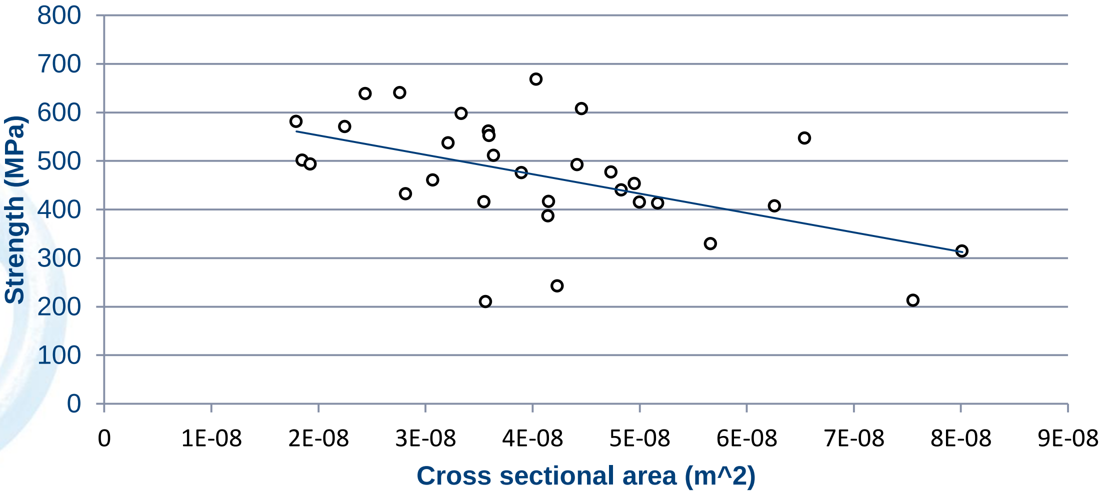


Tensile test set-up

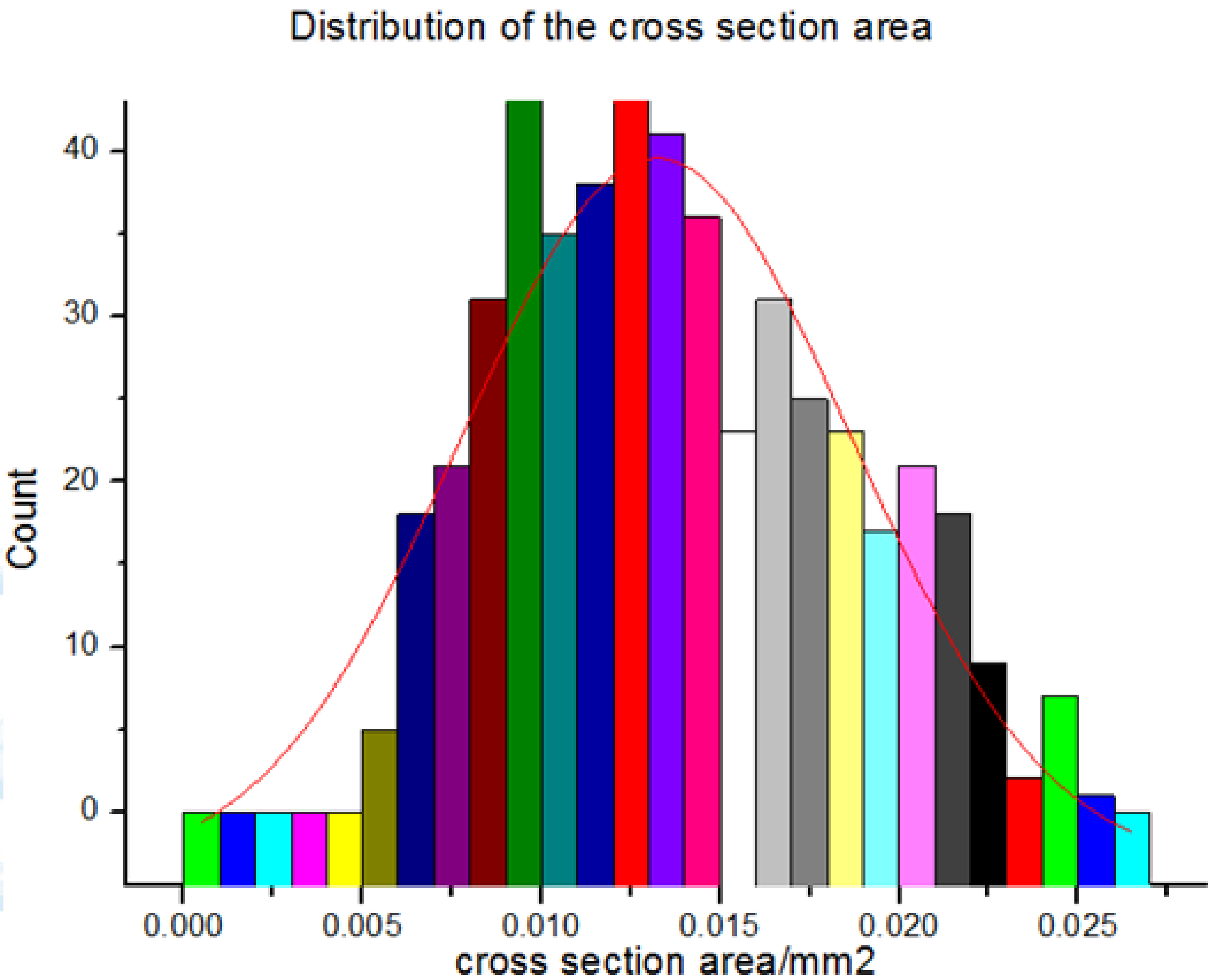
Tensile strength using different sandpaper



Size of the samples



Cross section area statistical analysis



Data processing (Strength)

a) Weibull Analysis of the tensile strength

- Firstly, the results are analyzed on normality.
- A confidence level of 0.95 is used to determine the confidence interval.
- Only results inside the confidence interval are included for the following Weibull analysis.

Normality test

Long Name	FI903
Units	Mpa
Comments	
2	348.02025
3	414.04055
4	418.29587
5	451.40184
6	522.66134
7	531.65389
8	569.28564
9	580.7109
10	586.97285
11	620.12412
12	652.9292
13	664.33051
14	666.22614
15	670.5138
16	732.6023
17	772.7643
18	821.56916
19	851.17164
20	908.74753
21	944.52348

Normality Test (2014/12/13 11:35:32)

Notes

Input Data

Descriptive Statistics

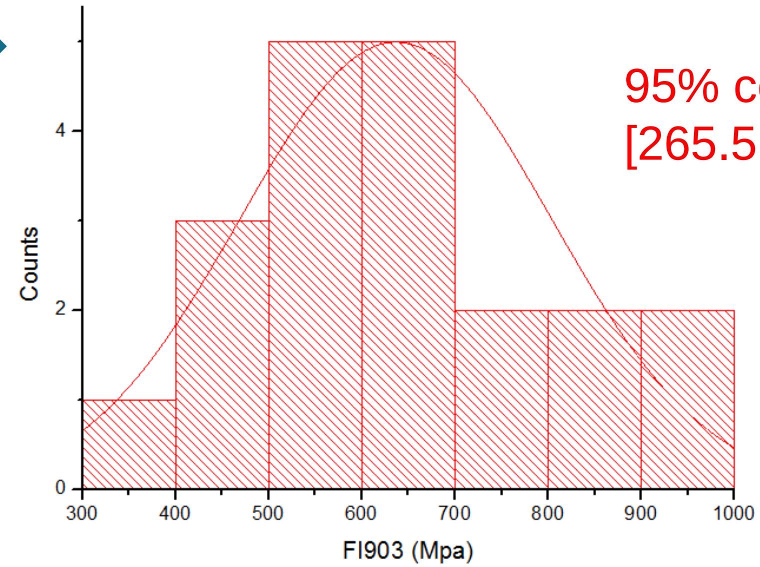
	Sample Size	Mean	Standard Deviation	SE of Mean
FI903	21	620.83108	177.66089	38.76878

NormalityTest

Shapiro-Wilk

	DF	Statistic	Prob<W
FI903	21	0.97822	0.89784

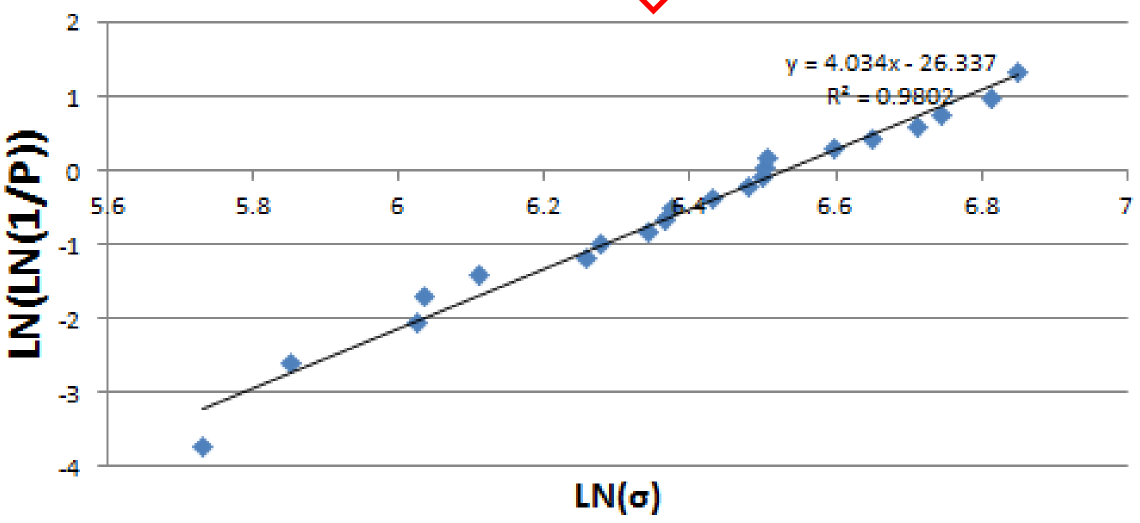
FI903: At the 0.05 level, the data was significantly drawn from a normally distributed population.



95% confidence interval:
[265.51, 976.15]

Weibull analysis of the strength for FNPC-IWNRZ-903-B3

		x	i	F(x) = (i-0,5)/n		Estimator 1 Y	X
	Fibre Nr.	Data	Rank	Median Rank	1/(1-Median Rank)	ln(ln(1/(1-Median Rank)))	ln(Data)
1		308.90742	1	0.023809524	1.024390244	-3.725645038	5.733041605
2		348.02025	2	0.071428571	1.076923077	-2.602232166	5.852260673
3		414.04055	3	0.119047619	1.135135135	-2.06552518	6.025963916
4		418.29587	4	0.166666667	1.2	-1.701983355	6.036188996
5		451.40184	5	0.214285714	1.272727273	-1.422286137	6.112357937
6		522.66134	6	0.261904762	1.35483871	-1.191772815	6.258933719
7		531.65389	7	0.30952381	1.448275862	-0.993242545	6.275992699
8		569.28564	8	0.357142857	1.555555556	-0.816823857	6.344382312
9		580.7109	9	0.404761905	1.68	-0.65624879	6.364253043
10		586.97285	10	0.452380952	1.826086957	-0.50720651	6.374978563
11		620.12412	11	0.5	2	-0.366512921	6.429919652
12		652.9292	12	0.547619048	2.210526316	-0.231641256	6.481468701
13		664.33051	13	0.595238095	2.470588235	-0.100421318	6.498779782
14		666.22614	14	0.642857143	2.8	0.029189236	6.501629159
15		670.5138	15	0.69047619	3.230769231	0.159326059	6.508044284
16		732.6023	16	0.738095238	3.818181818	0.292501201	6.596602987
17		772.7643	17	0.785714286	4.666666667	0.432071362	6.649974086
18		821.56916	18	0.833333333	6	0.583198081	6.711216127
19		851.17164	19	0.880952381	8.4	0.75529145	6.7466138
20		908.74753	20	0.928571429	14	0.970421781	6.812067313
21		944.52348	21	0.976190476	42	1.318462321	6.85068055



Shape parameter m	**4.0**
Mean strength	620.7Mpa
Standard deviation	172.8Mpa

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Results of Weibull analysis of tensile strength for 70 species

Sample code	Abbreviation	Strength/MPa	STDEV/MPa
VDS-FNPC-255-B3	Min. VF255	338.62	83.74
VDS-FNPC-254-B3	VF254	416.05	206.42
VDS-FNPC-252-B3	VF252	417.70	149.92
FNPC-AGM-705-B3	FA705	419.03	107.62
VDS-AGM-704-B3	VA704	423.19	280.68
VDS-FNPC-254-B3	VF254	427.44	122.86
FNPC-AGM-703-B3	FA703	444.32	158.12
VDS-CRA-411bis-B3	VC411	471.86	214.26
FNPC-AGM-704-B3	FA704	482.27	130.61
VDS-IWNRZ-902-B3	VI902	488.3	152.68
VDS-FNPC-253-B3	VF253	500.75	176.48
FNPC-IWNRZ-901-B3	FI901	500.84	186.84
FNPC-FNPC-252-B3	FF252	502.11	84.06
FNPC-FNPC-251-B3	FF251	503.97	159.51
VDS-IWNRZ-901-B3	VI901	508.75	141.31
VDS-CRA-411bis-B3	VC411	513.45	185.77
FNPC-FNPC-254-B3(plateau)	FF254	514.41	74.16
UCSC(WR)-IWNRZ-902-B3	UWI902	520.14	118.21
VDS-FNPC-251-B3	VF251	524.44	251.37
VDS-AGM-705-B3	VA705	537.33	218.40
VDS-FNPC-251-B3	VF251	540.87	223.86
VDS-FNPC-252-B3	VF252	545.28	105.31
FNPC-VDS-303-B3	FV303	545.91	169.92
IWNRZ 901	I901	546.70	223.81

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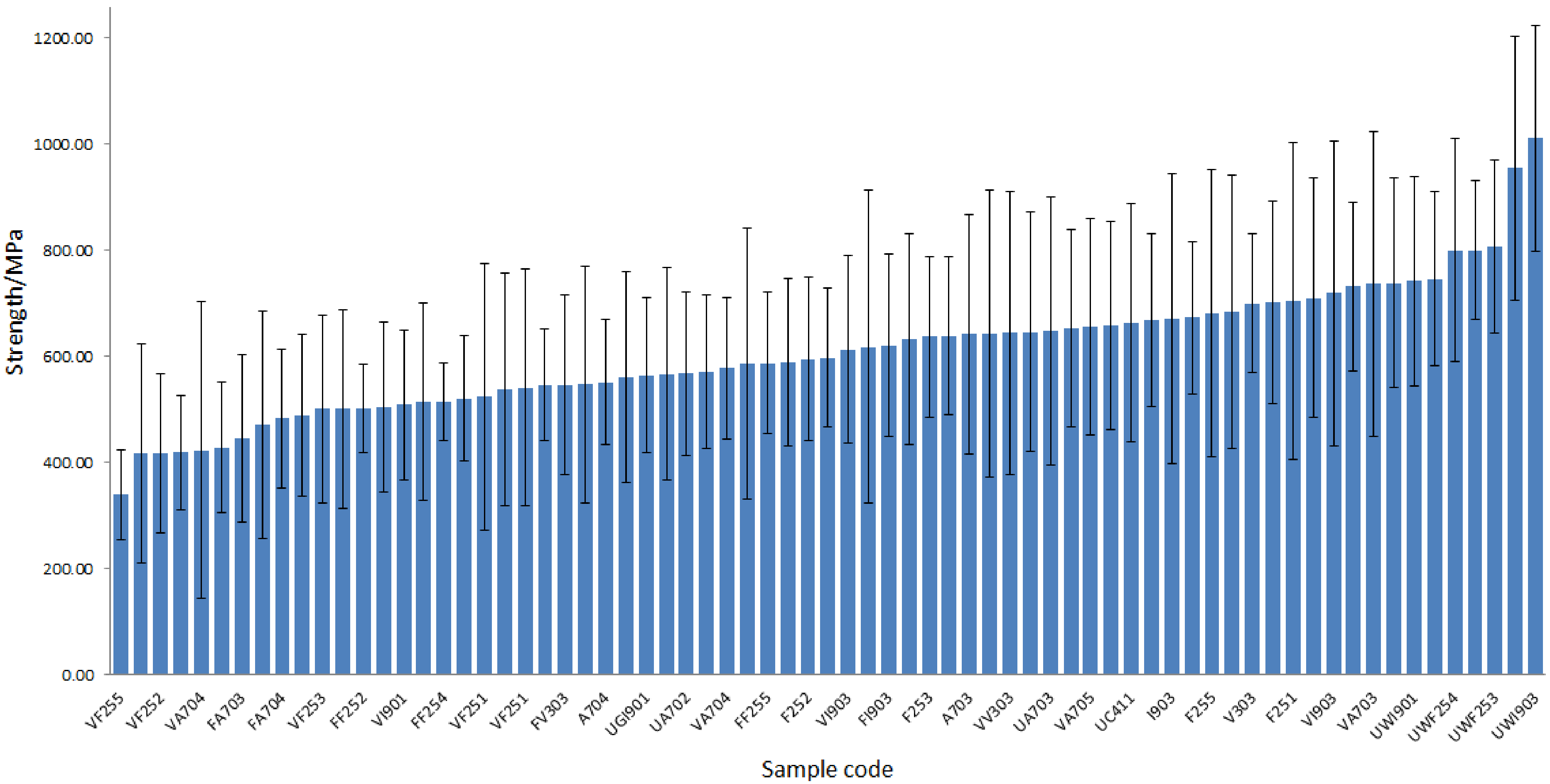
Sample code	Abbreviation	Strength/MPa	STDEV/MPa
AGM 704	A704	551.52	117.27
AGM 705	A705	560.22	198.08
UCSC(green)-IWNRZ-901-B3	UGI901	564.32	145.37
VDS-VDS-303-B3	VV303	566.62	199.27
UCSC-AGM-702-B3	UA702	567.74	154.01
FNPC-IWNRZ-903-B3	FI903	570.11	144.70
VDS-AGM-704-B3	VA704	577.39	134.43
VDS-IWNRZ-901-B3	VI901	586.31	255.63
FNPC-FNPC-255-B3	FF255	587.21	133.61
UCSC-FNPC-252-B3（2mm/min）	UF252	589.39	157.38
FNPC 252	F252	594.48	153.65
UCSC(WR)-AGM-702-B3	UWA702	597.37	130.69
VDS-IWNRZ-903-B3	VI903	613.02	175.91
FNPC-IWNRZ-901-B3	FI901	617.72	294.87
FNPC-IWNRZ-903-B3	FI903	620.68	172.8
VDS-AGM-702-B3	VA702	632.39	198.95
FNPC 253	F253	636.34	151.00
UCSC-AGM-704-B3（2mm/min）	UA704	638.15	149.32
AGM 703	A703	641.41	225.72
VDS-IWNRZ-902-B3	VI902	643.22	270.70
VDS-VDS-303-B3	VV303	644.50	267.14
VDS-AGM-703-B3	VA703	646.24	224.81
UCSC-AGM-703-B3（2mm/min）	UA703	647.77	251.96

Sample code	Abbreviation	Strength/MPa	STDEV/MPa
UCSC-FNPC-255-B3	UF255	653.06	186.61
VDS-AGM-705-B3	VA705	655.72	204.26
UCSC(WR)-AGM-703-B3	UWA703	657.84	195.72
UCSC-CRA-411bis-B3	UC411	663.24	224.7
UCSC(WR)-VDS-303-B3	UWV303	668.8	163.22
IWNRZ 903	I903	671.83	273.15
FNPC-CRA-411bis-B3	FC411	673.03	143.60
FNPC255	F255	681.32	270.38
UCSC-FNPC-253-B3（2mm/min）	UF253	684.26	257.85
VDS 303	V303	700.12	131.57
AGM 702	A702	701.71	191.35
FNPC 251	F251	704.56	299.18
UCSC(green)-CRA-411bis-B3	UGC411	710.59	225.83
VDS-IWNRZ-903-B3	VI903	718.47	286.36
UCSC(WR)-AGM-704-B3	UWA704	731.57	159.86
VDS-AGM-703-B3	VA703	737.20	287.22
UCSC-IWNRZ-902-B3(plateau)	UI902	738.75	196.53
UCSC(WR)-IWNRZ-901-B3	UWI901	741.8	197.36
FNPC-FNPC-253-B3(plateau)	FF253	745.86	164.55
UCSC(WR)-FNPC-254-B3	UWF254	800.04	210.73
UCSC(WR)-FNPC-251-B3	UWF251	800.09	130.43
UCSC(WR)-FNPC-253-B3	UWF253	806.74	162.87
UCSC(WR)-FNPC-252-B3	UWF252	954.5	248.95
UCSC(WR)-IWNRZ-903-B3	Max. UWI903	1011.13	213.48

Histogram with error bars of tensile strength for 70 species



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Data processing

b) One-way ANOVA

- To compare the strength of every species and to select the best species

Data processing

b) One-way ANOVA

- To compare the strength of every species and to select the best species

Results

One-way ANOVA of top 10 species with high strength

Overall ANOVA

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	9	1.0112E6	112355.77712	3.09766	0.00244
Error	105	3.80848E6	36271.22381		
Total	114	4.81968E6			

Null Hypothesis: The means of all levels are equal.

Alternative Hypothesis: The means of one or more levels are different.

At the 0.05 level, the population means are significantly different.

⇩

The strength difference for the 10 species is significant at 95% confidence level.

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Results

Fisher test for top 10 species with high strength

Fisher Test

	MeanDiff	SEM	t Value	Prob	Alpha	Sig	LCL	UCL
Level10 Level1	280.66966	79.49834	3.53051	6.17314E	0.05	1	123.03914	438.30018
Level10 Level2	292.49515	90.57707	3.22924	0.00166	0.05	1	112.89755	472.09275
Level10 Level3	263.54783	74.92265	3.5176	6.4478E-	0.05	1	114.99005	412.10561
Level10 Level4	270.08567	76.24104	3.54252	5.92751E	0.05	1	118.91377	421.25757
Level10 Level5	265.53633	83.98058	3.16188	0.00205	0.05	1	99.01835	432.05431
Level10 Level6	212.52981	76.24104	2.7876	0.0063	0.05	1	61.35791	363.70171
Level10 Level7	211.56515	76.24104	2.77495	0.00654	0.05	1	60.39326	362.73705
Level10 Level8	204.78081	79.49834	2.57591	0.01139	0.05	1	47.15029	362.41134
Level10 Level9	59.46031	77.75091	0.76475	0.44613	0.05	0	-94.70538	213.62601

Sig equals 1 indicates that the means difference is significant at the 0.05 level.
Sig equals 0 indicates that the means difference is not significant at the 0.05 level.

⇩

The means difference between UWI903(level 10) and others except UWF252(level 9) is significant at 95% confidence level.

⇩

UWI903 and UWF252 are the best two fibre species with high strength.

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Results

One-way ANOVA of bottom 10 species with low strength

Overall ANOVA

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	9	214217.24328	23801.91592	0.87754	0.54754
Error	114	3.09208E6	27123.51136		
Total	123	3.3063E6			

Null Hypothesis: The means of all levels are equal.

Alternative Hypothesis: The means of one or more levels are different.

At the 0.05 level, the population means are not significantly different.

⇩

The strength difference for the 10 species is not significant at 95% confidence level.

⇩

VF255 and VF254 may be the worst two fibre species with low strength.

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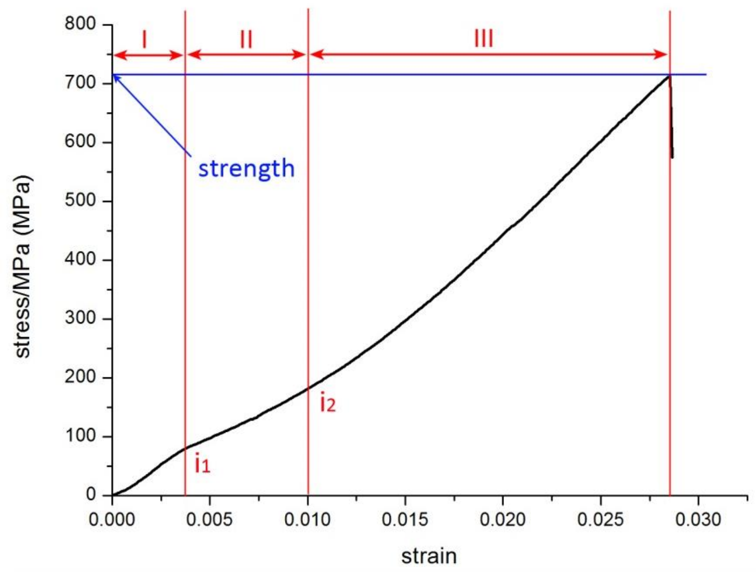
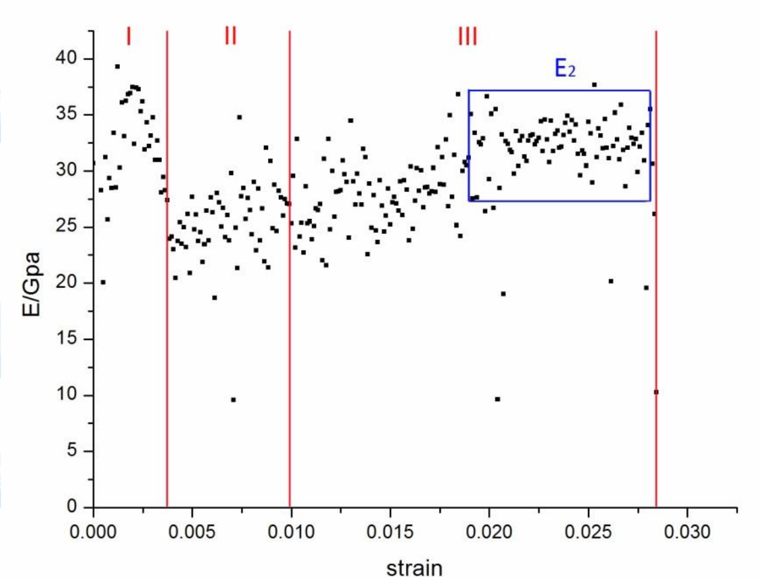
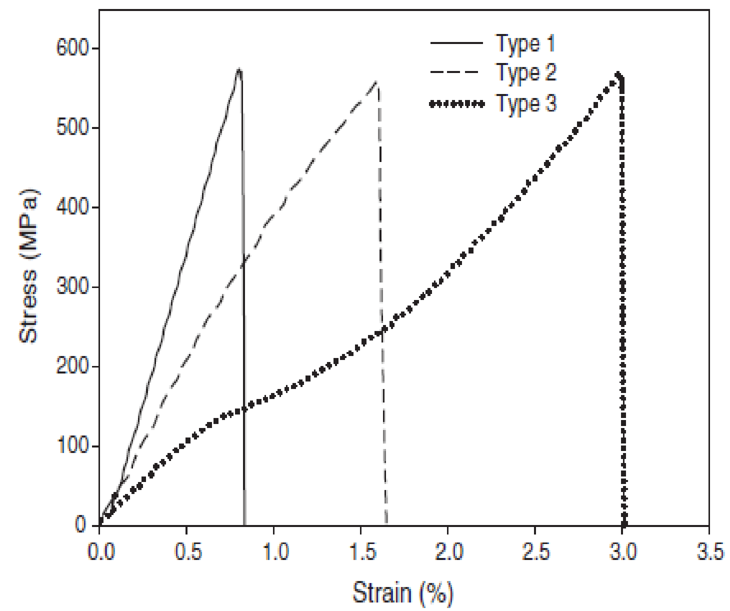
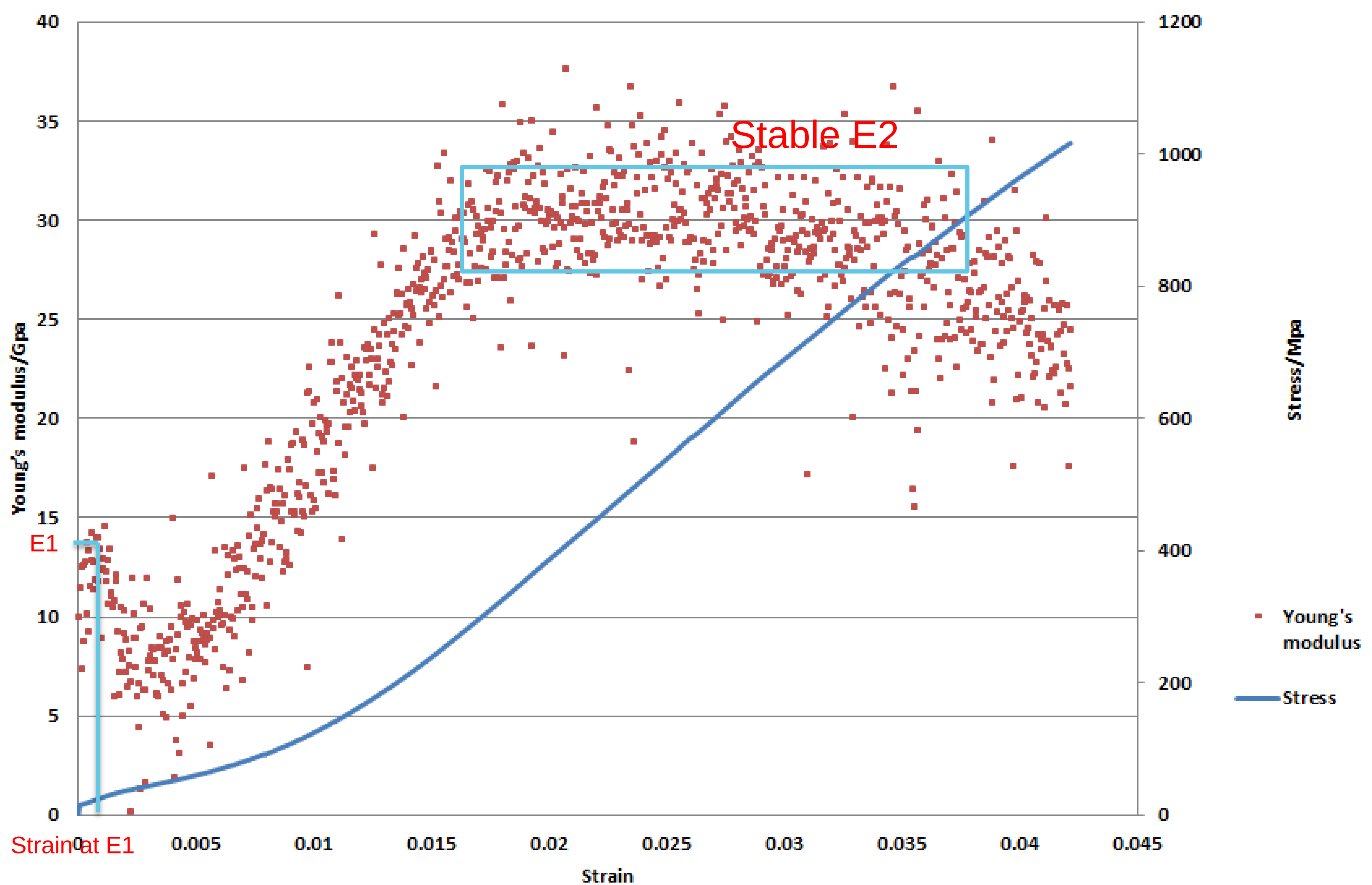
Data processing (Young's modulus)



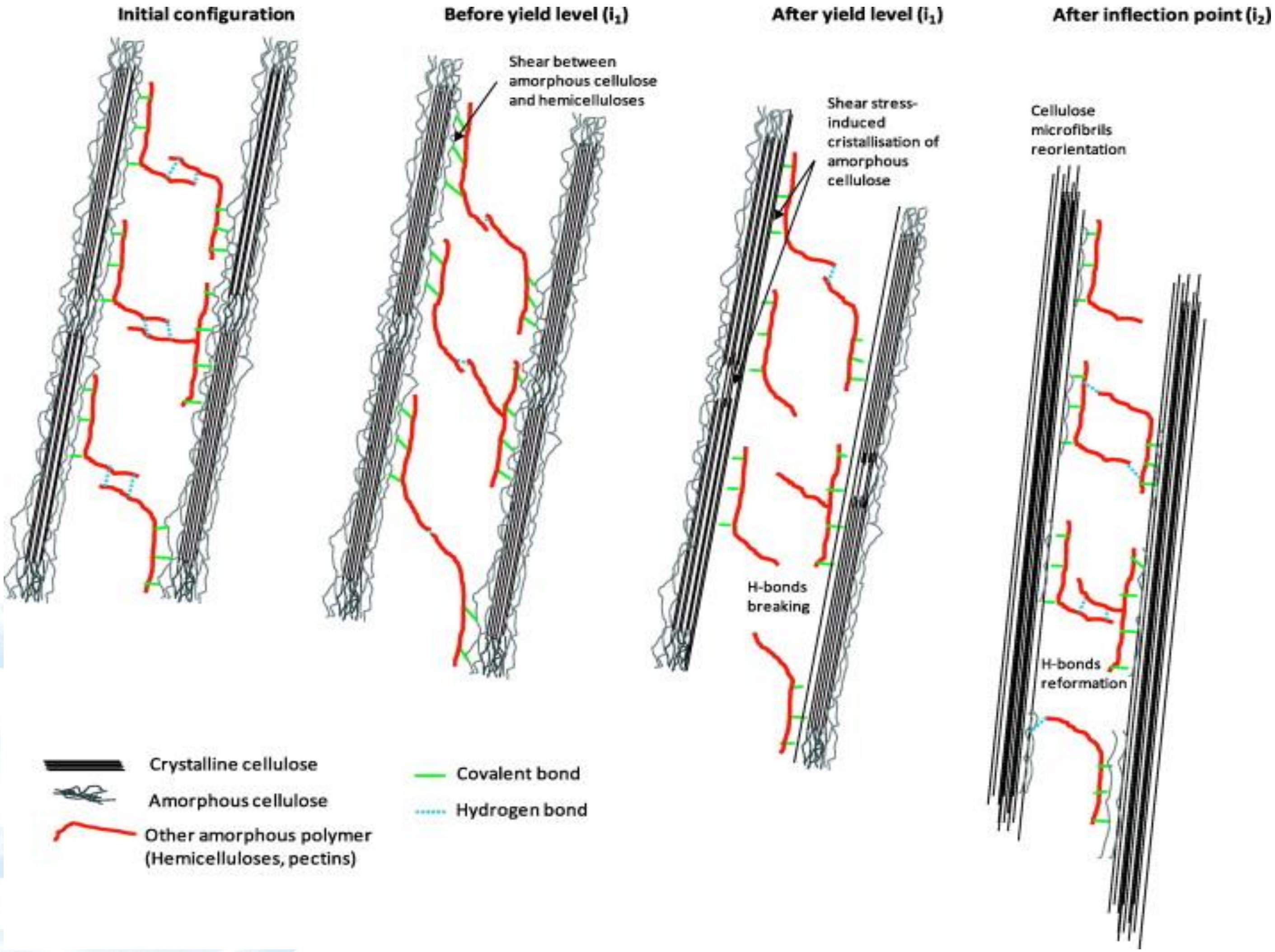




Stress and Young's modulus as a function of the strain



Tensile behaviour



Schematic representation of the scenario proposed to explain the complex tensile behaviour of hemp fibre[10]

[10]Placet, V., O. Cissé, and M. Lamine Boubakar, *Nonlinear tensile behaviour of elementary hemp fibres. Part I: Investigation of the possible origins using repeated progressive loading with in situ microscopic observations.* Composites Part A: Applied Science and Manufacturing, 2014. **56**: p. 319-327.

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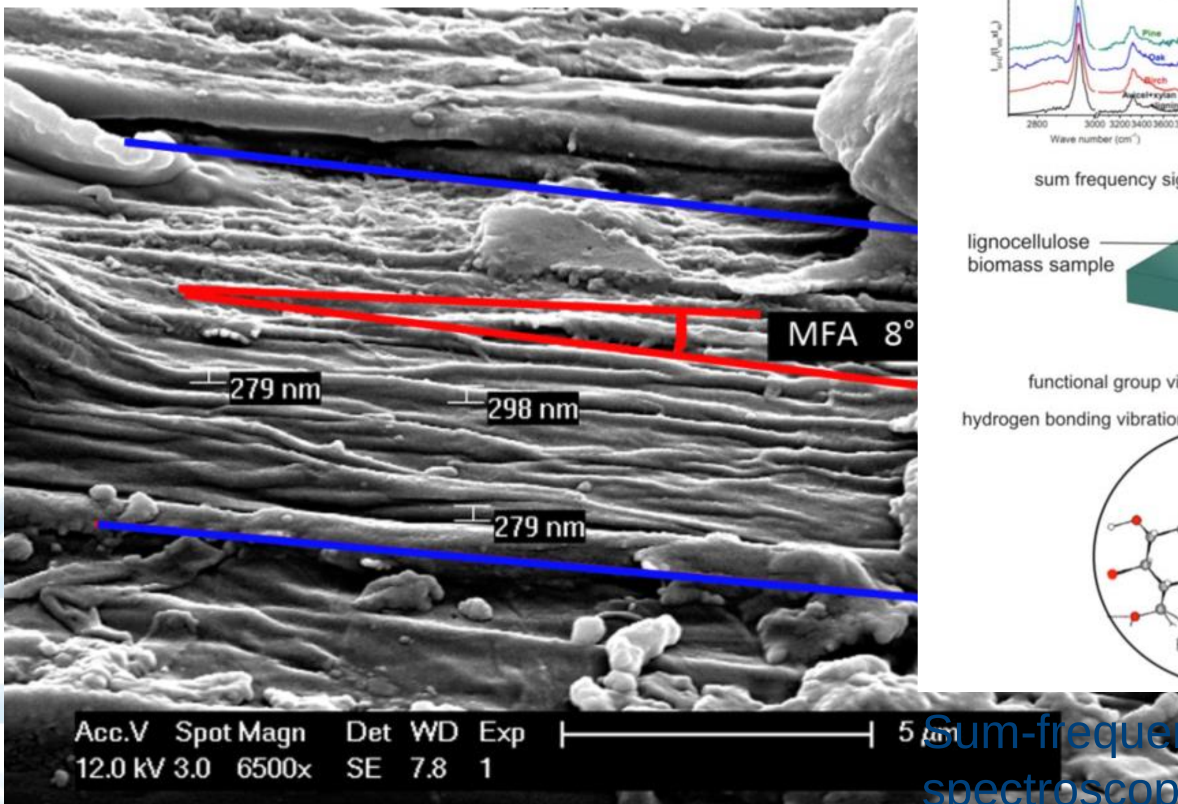
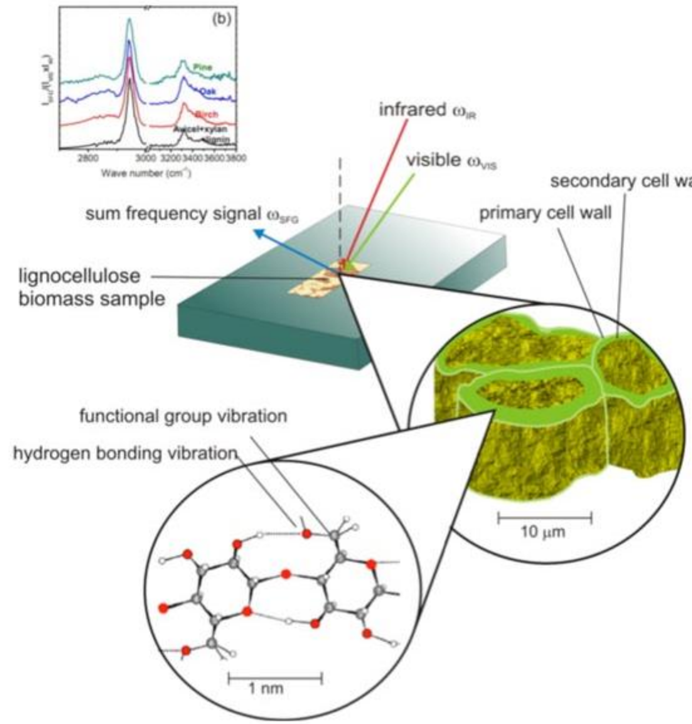
Evaluation of the reorientation of the nanofibril angle

Microtome + nanoSEM or SEM



Study of the variation of nanofibrillar orientation (before and after tensile test)



Sum-frequency-generation vibrational spectroscopy (PennState University)
-Prof Seong Kim (David's Contact)

Strain mapping

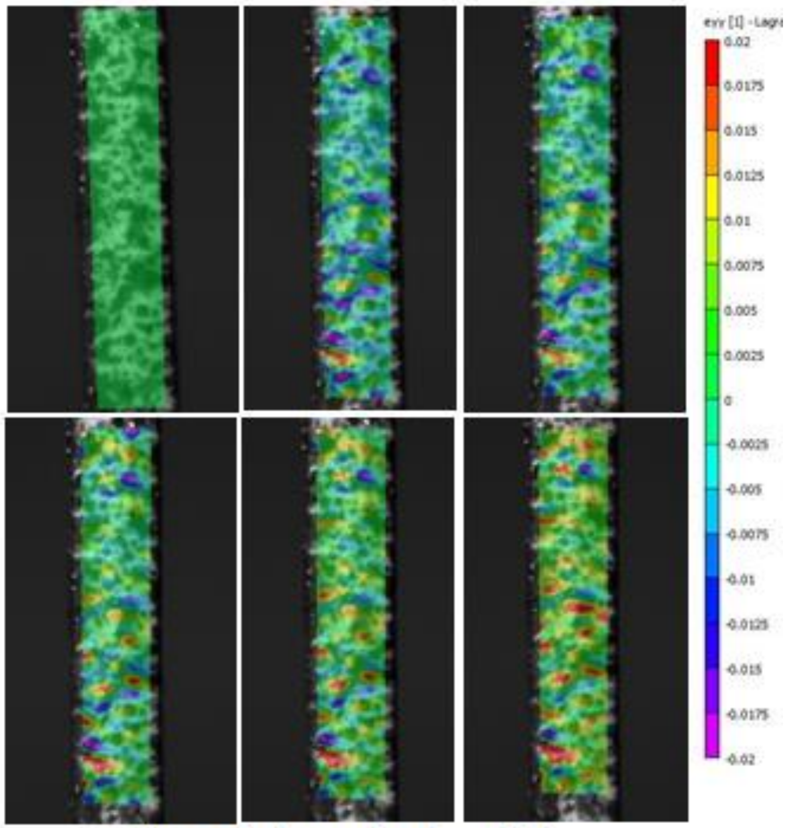
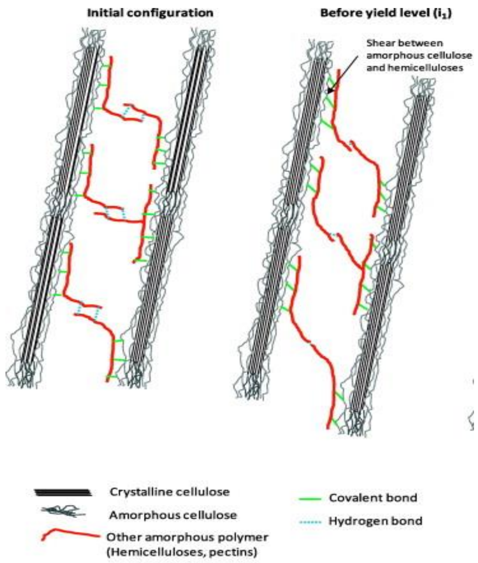
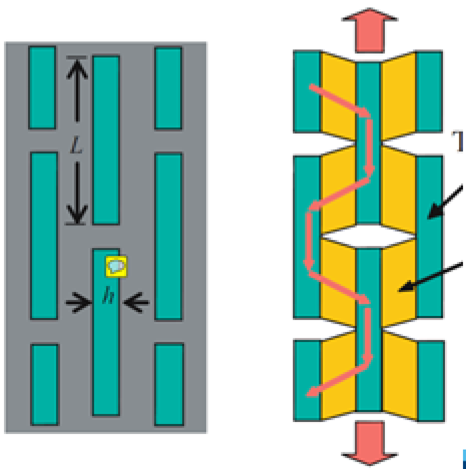


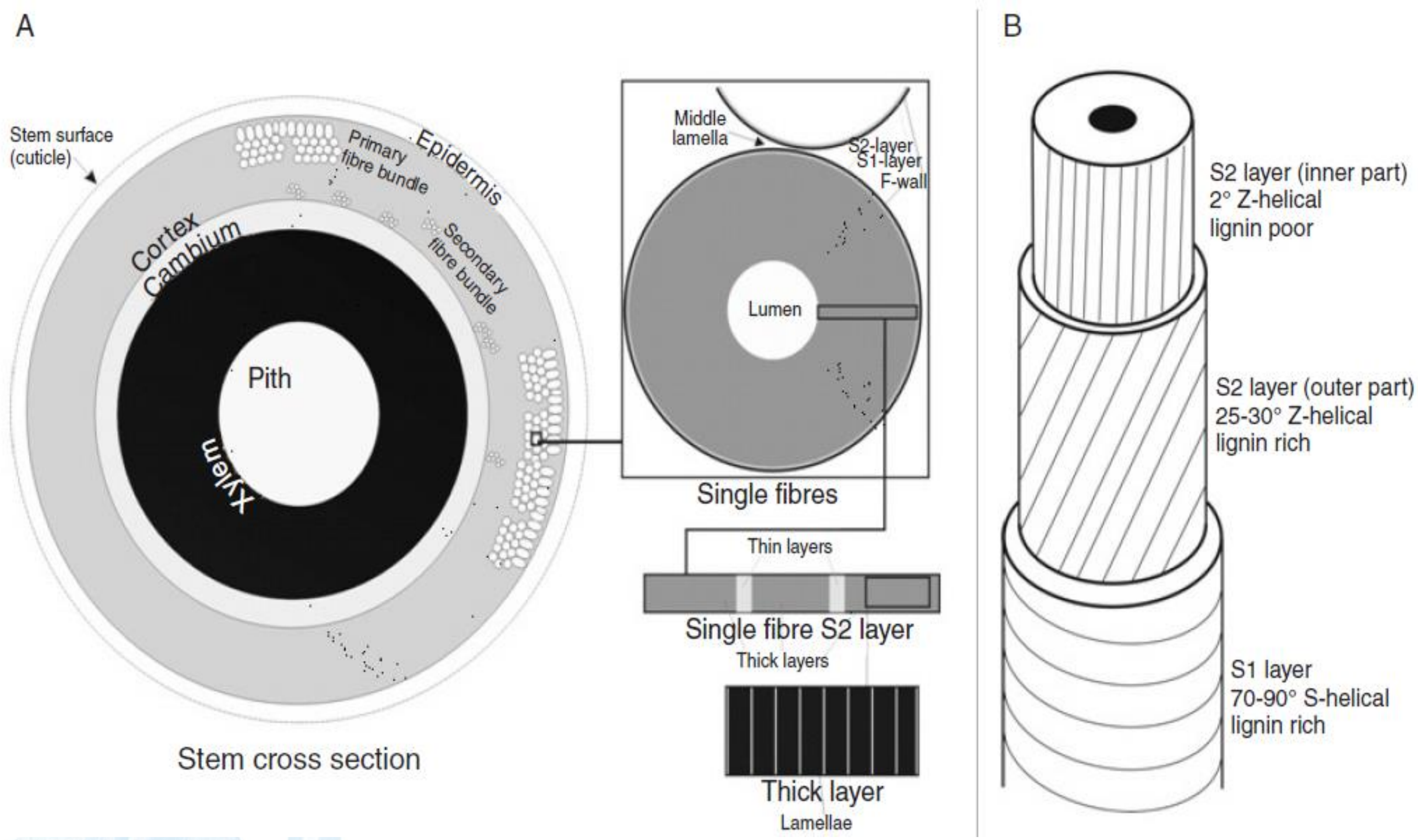
Figure a: Strain mapping of sample 01

Ichu fibre





Microstructure

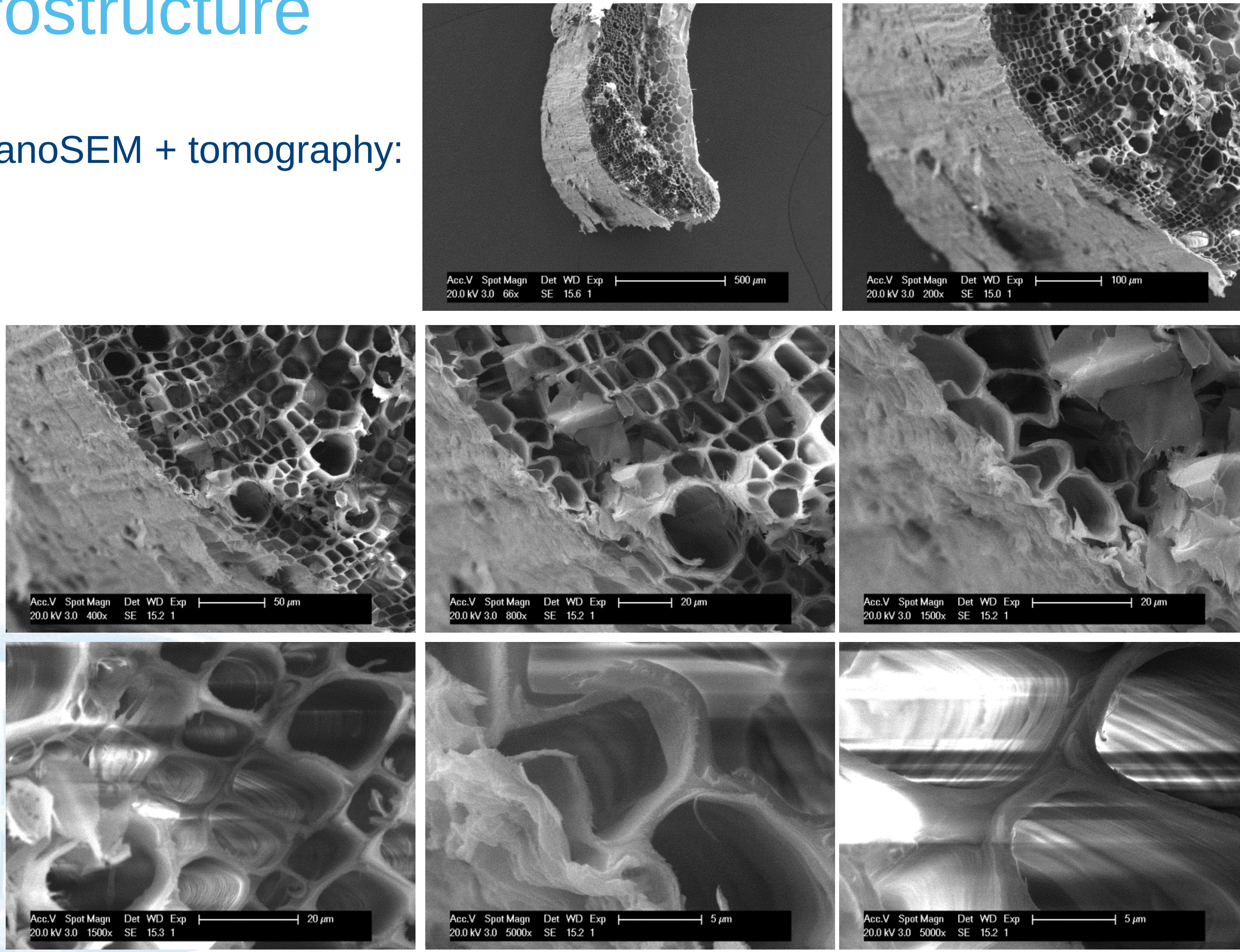


A: Model of transverse stem section zooming to single fibres, secondary cell wall and finally the cell wall lamella structure.
B: Model of the microfibril orientation throughout the secondary cell wall.[5]

[5] Thygesen, A., et al., *Hemp Fiber Microstructure and Use of Fungal Defibration to Obtain Fibers for Composite Materials.* Journal of Natural Fibers, 2006. **2**(4): p. 19-37.

Microstructure

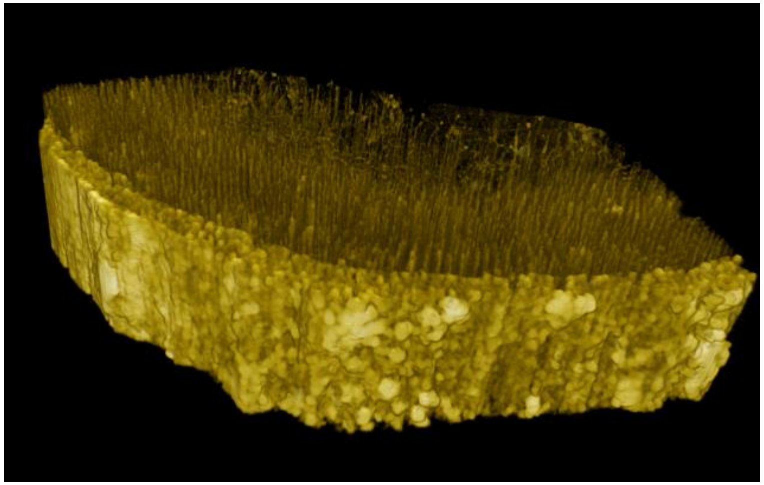
SEM + nanoSEM + tomography:

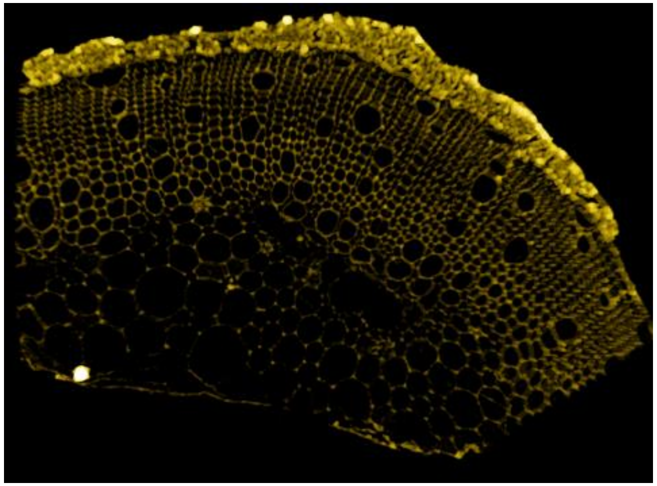


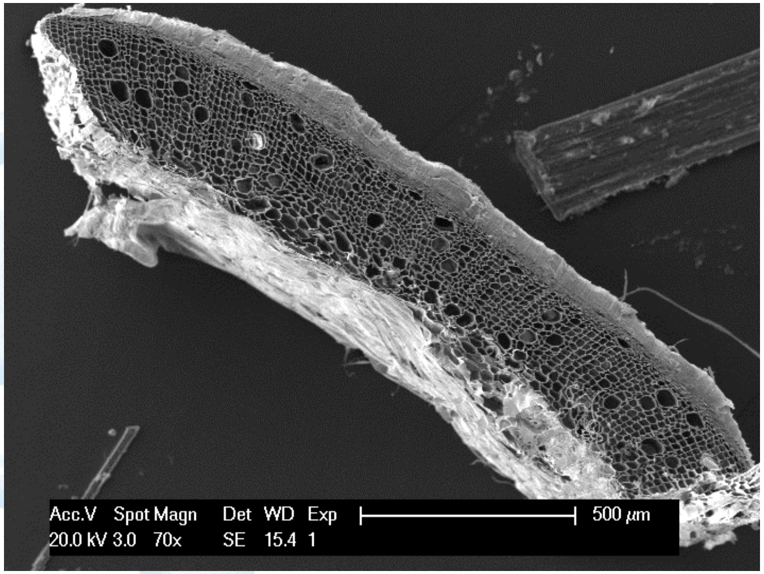
SEM images of a hemp stem

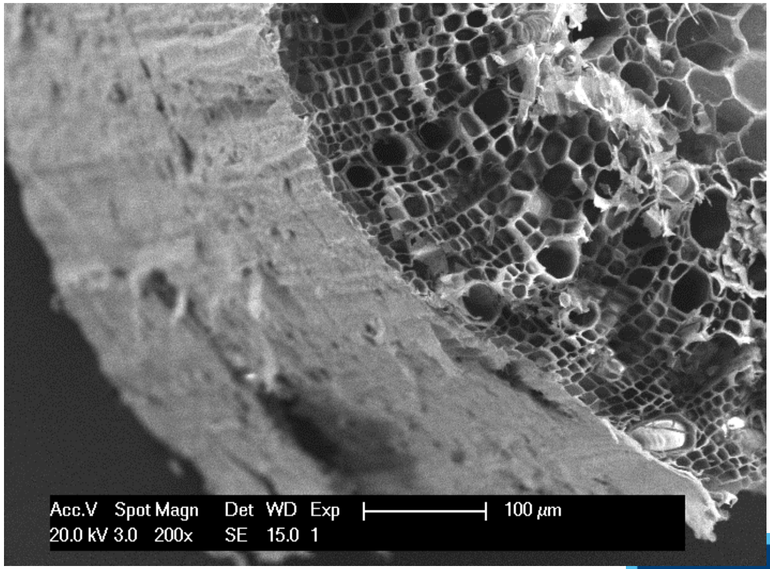
[7]Dupeyre, D. and M. Vignon, *Fibres from semi-retted hemp bundles by steam explosion treatment.* Biomass and Bioenergy, 1998. **14**(3): p. 251-260.

Microstructure characterization – Stem level
tomography



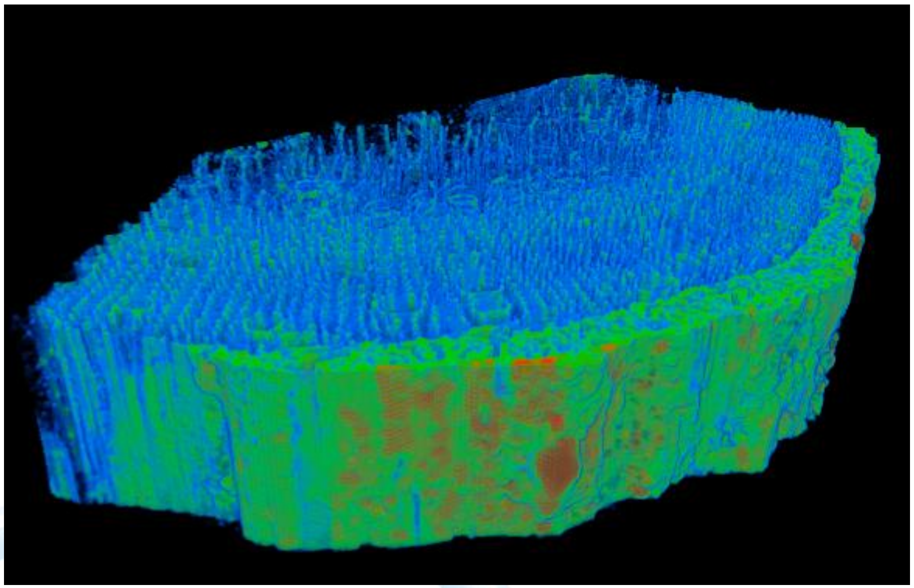


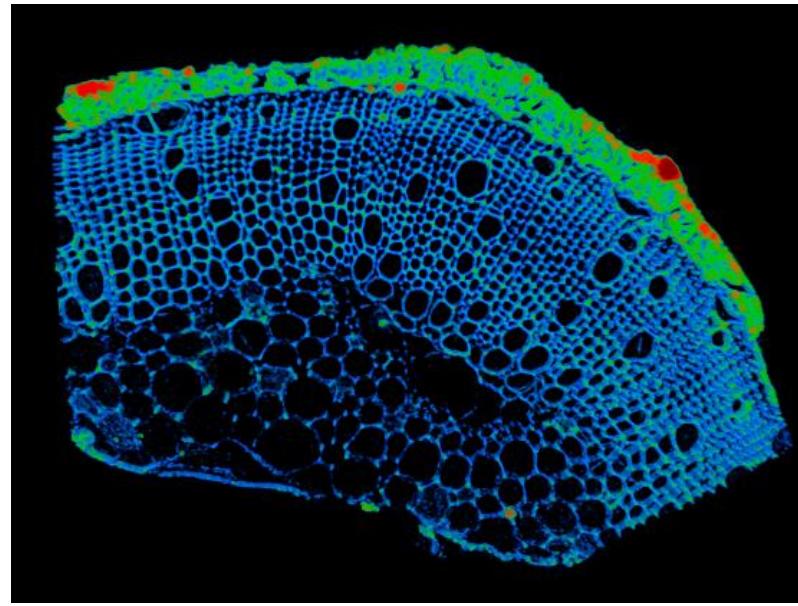


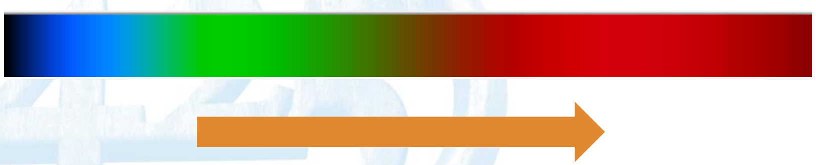


Mapping of the density – Stem Level

tomography



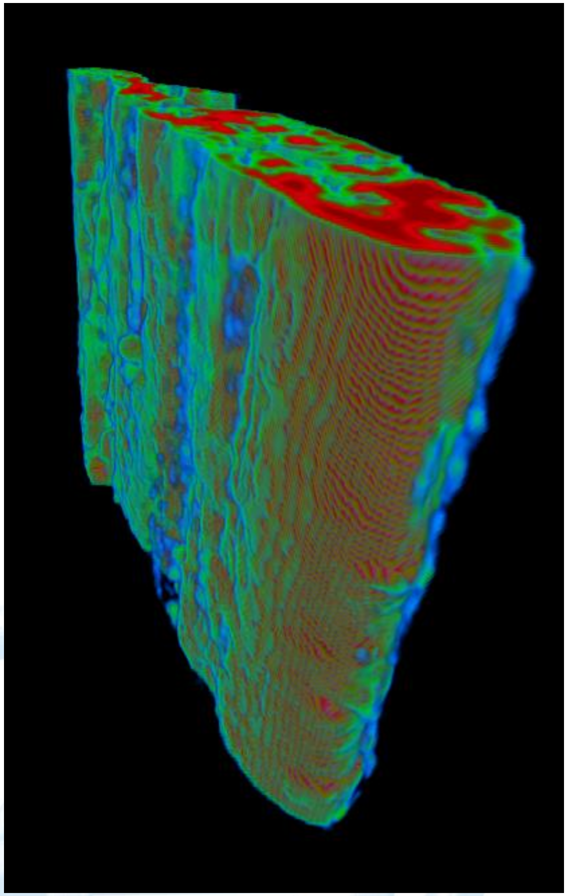


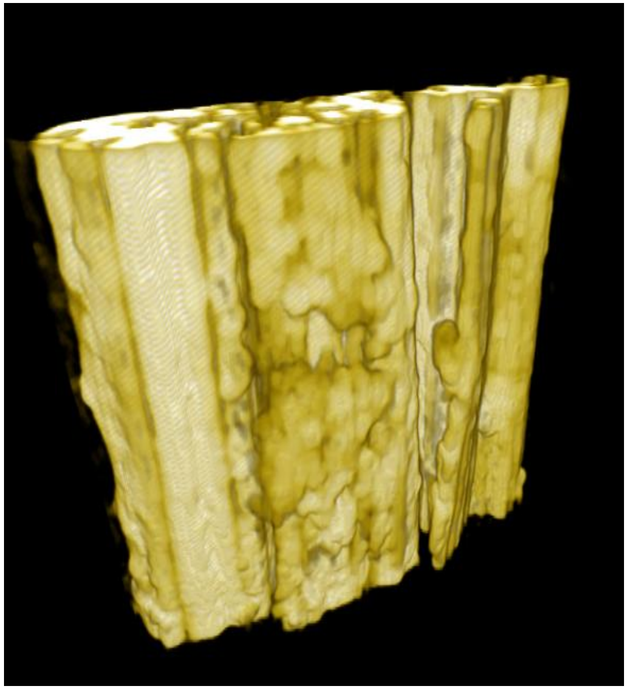


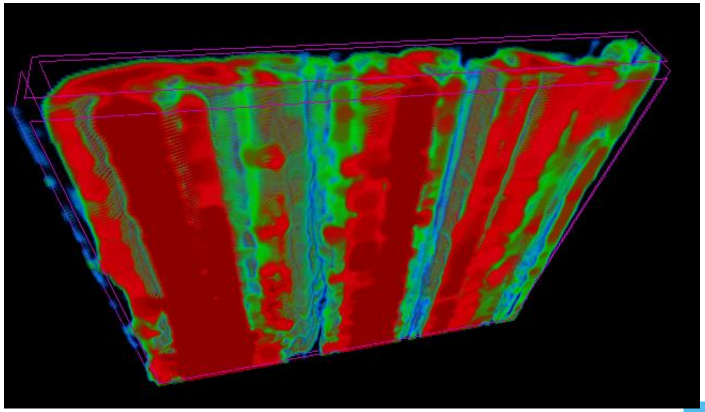
Higher density

Microstructure characterization – Technical fibre level

tomography

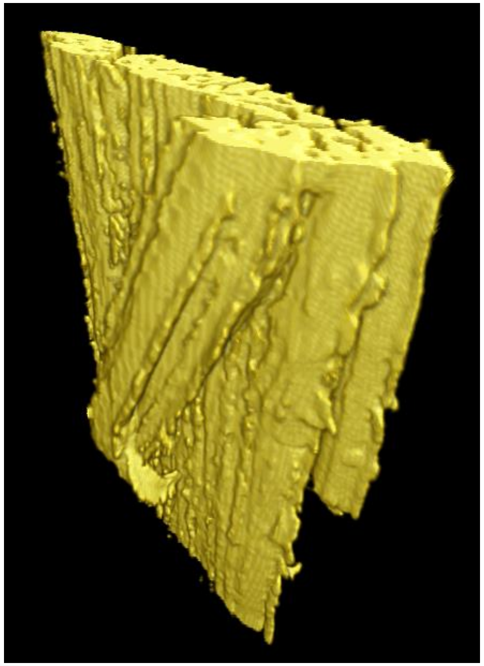


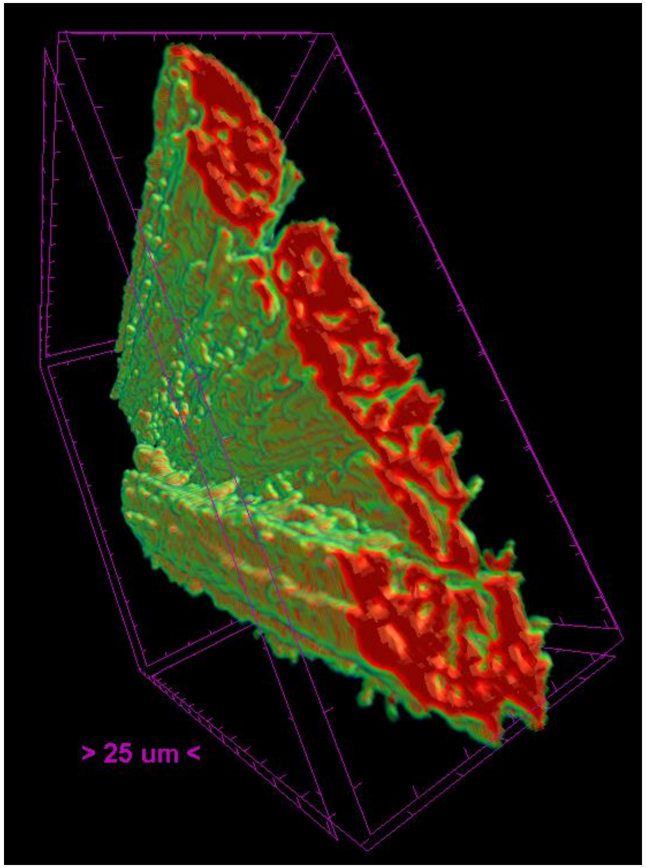


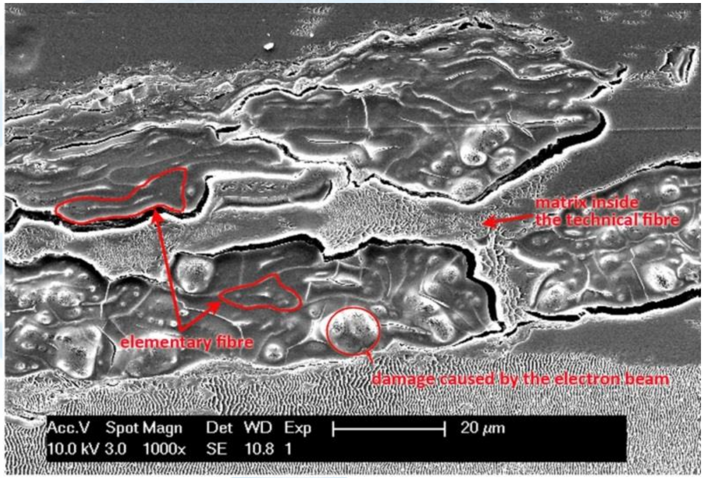


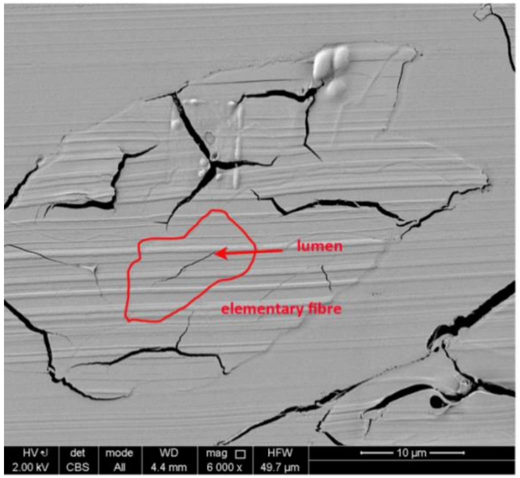
Higher density

Microstructure characterization – Technical fibre level









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Study of the chemical composition

By using XPS, and TOFsims (surface)

Chemical extraction of cellulose, lignin, hemicellulose (bulk)

Thank you for your attention!