

Distinguishing Mesoscale Polar Order (Unidirectional vs. Bidirectional) of Cellulose Microfibrils in Plant Cell Walls using Sum Frequency Generation Spectroscopy

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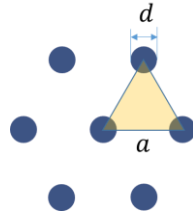
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Calculation of distances between crystals

The aqueous suspension of rod-shape cellulose nanocrystals in the capillary tube forms a hexagonal array with the rod axis aligned along the long axis of the capillary tube. Then, the distance between crystals can be calculated by:



$$\Phi = \frac{\pi \left(\frac{d}{2}\right)^2 \frac{3}{6}}{\frac{\sqrt{3}}{4} a^2} \rightarrow a = \sqrt{\frac{\pi d^2}{2\sqrt{3}\Phi}}$$

Where Φ is the volume fraction of cellulose crystal in the suspension, d is the diameter of crystals, and a is the distance between crystals. By considering the cross sectional diameter of the cellulose nanocrystals isolated from tunicates (≈ 10 nm) and the density of cellulose (≈ 1.5 g/cm³), the distance between crystals is estimated to be around 58 nm for the cellulose content of 4 wt%.

This model can be used for rough estimation of the average inter-CMF distance in plant cell wall. Let's assume that the elementary microfibril diameter is ~ 4 nm and they are fully isolated and dispersed as in the case of liquid suspension. The typical cellulose content in the secondary cell wall is about 40% in dry mass base; then, Δl is estimated to be about 6 nm. In the primary cell wall, typical cellulose content is about 15-20% in dry mass base and typical water content is 70-90%; then, Δl is estimated to be around 20-30 nm.

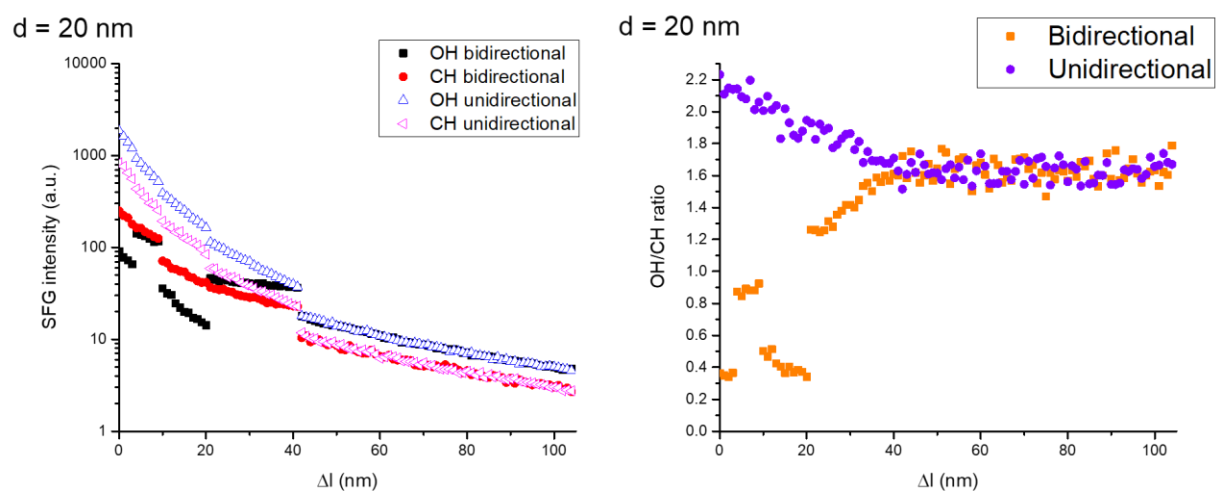
SFG calculations for 20 nm size crystals

Figure S1. (a) Theoretical calculations of the CH and OH stretch peaks for 20 nm diameter crystals with unidirectional and bidirectional packing as a function of Δl . (b) OH/CH intensity ratio for 20 nm crystals as a function of Δl for parallel and antiparallel packing.

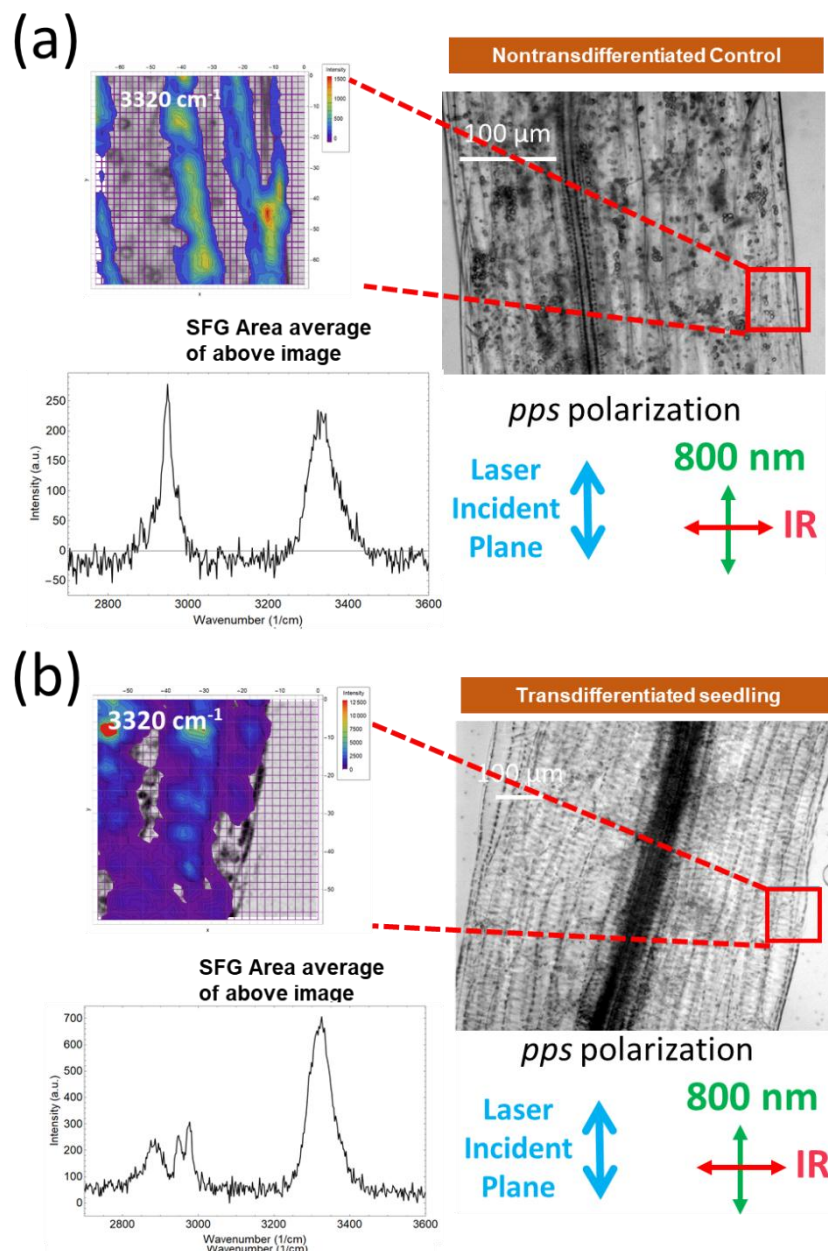


Figure S2. Control experiments of (a) nontransdifferentiated and (b) transdifferentiated epidermis of *Arabidopsis* hypocotyl never-dried and hydrated in D_2O . An area of $70 \mu\text{m} \times 70 \mu\text{m}$ (marked with a box) was analyzed with a $2 \mu\text{m}$ step interval. The hyperspectral SFG imaging was done with the *pps* polarization. The nontransdifferentiated epidermis is a primary cell with low cellulose content and high water content; thus, the average inter-CMF distance is large and the OH/CH intensity ratio is $0.7\sim 0.8$. The xylem induced in the epidermis is a secondary cell wall with high cellulose content and low water content; thus, the average inter-CMF distance is small. Then, the OH/CH intensity ratio of ~ 3 must be due to the biased unidirectionality of CMFs within the probe volume.

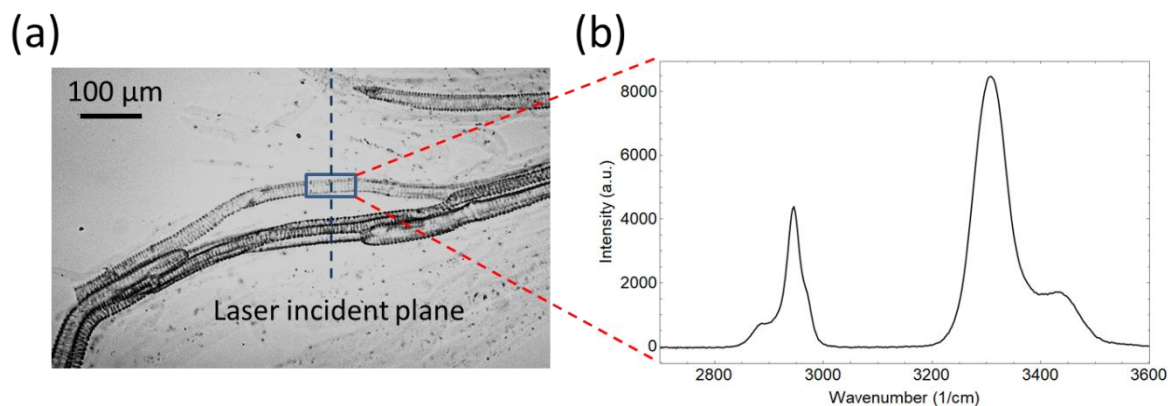


Figure S3. (a) The 90° rotation of celery coils with respect to laser incident plane. (b) The average *pps*-SFG spectra of the marked area. The OH/CH ratio follows similar trend observed in Figure 7. The difference in the CH and OH peak shape is due to the azimuth orientation dependence.

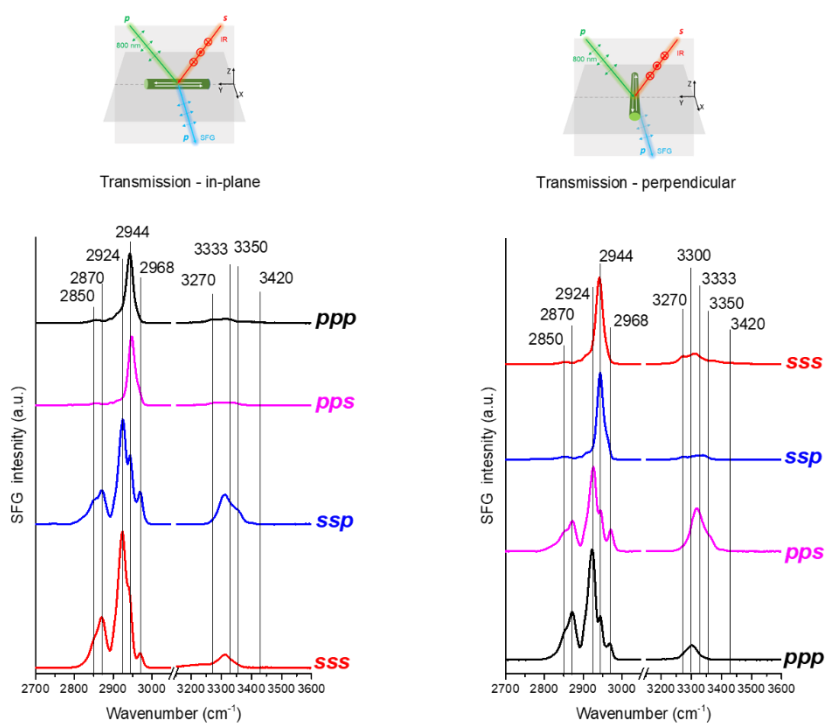


Figure S4. SFG spectra of the uniaxially-packed cellulose I β with unbiased bidirectionality. This is the case where $\Delta l \approx 0$ nm (volume fraction ≈ 100 %, except some voids between crystals).

*Mathematica code***(*Coherence Length Calculations*)**

```

c=299792458 ;(*speed of light m/s*)
lambda1=3500*10^-9;(*wavelength*)
lambda2=800*10^-9; (*wavelength*)
lambda3=(lambda1*lambda2)/(lambda1+lambda2) //N;
6.511627906976744`*^-7;
teta1air=45;(*degrees*)
teta2air=45;(*degrees*)

(*snells law:Subscript[n, air]sin(Subscript[teta,
air])=Subscript[n, cellulose]sin(Subscript[teta,
cellulose]*)

nair=1;
n1cellulose=1.4577;
n2cellulose=1.464;
n3cellulose=1.467;
teta1cellulose=ArcSin[nair*Sin[teta1air Degree]/n1cellulose
]*180/Pi;
13.569813240933804;
teta2cellulose=ArcSin[nair*Sin[teta2air Degree]/n2cellulose
]*180/Pi;
13.510309104885206;
(*phase matching condition Subscript[n3,
cellulose]*(c/lambda3)*Sin[teta3 Degree]=Subscript[n2,
cellulose]*(c/lambda2)*Sin[teta2 Degree]-Subscript[n1,
cellulose]*(c/lambda1)*Sin[teta1 Degree]*)

teta3cellulose=ArcSin[(n2cellulose*(c/lambda2)*Sin[teta2cellulose
Degree]+n1cellulose*(c/lambda1)*Sin[teta1cellulose
Degree)]/(n3cellulose*(c/lambda3))]*180/Pi;
8.417886067354257;
(*deltak=k1+k2+k3 for reflection and deltak=k1+k2-k3
Lc=2/deltak*)
k1=(2Pi*n1cellulose/lambda1)*Cos[teta1cellulose Degree];
k2=(2Pi*n2cellulose/lambda2)*Cos[teta2cellulose Degree];
k3=(2Pi*n3cellulose/lambda3)*Cos[teta3cellulose Degree];
Δk=k1+k2+k3
Lc=(Pi/Δk) (*reflection*)

```

(*OH - Antiarallel*)

```

F=Table[0,{w,1,100}];
c=Table[{0,0,0},{w,38*123}];
p=1;
q=1;

For[u=3, u<41,u++ ,

  For[Δz=0, Δz<123,Δz++,

    c[[ (p-1) 123+q, 2]] = u;
    c[[ (p-1) 123+q, 1]] = Δz;

    m = (Lc*109) / (u+Δz);

    If [ (Δz+u) < (Lc*109+1) ,

      For[w=1,w<101,w++,

        temp = ((Exp[-I Δk u*10-9]-1)/Δk) (Sum[m u2 (-
1) i * Cos[RandomVariate[NormalDistribution[Pi/6,0.15]]] Exp[-
I Δk i (u*10-9+Δz*10-9)] Exp[-i (u*10-9+Δz*10-9)], {i,0,m-
1}]+Sum[m u2
RandomReal[{0,1}] Sin[RandomVariate[NormalDistribution[Pi/6,
0.15]]] Exp[- I Δk i (u*10-9+Δz*10-9)] Exp[-i (u*10-9+Δz*10-
9)], {i,0,m-1}]);
        F[[w]] = (Conjugate[temp]*temp);
        ];
        c[[ (p-1) 123+q, 3]] = Mean[F];

      ];
      q++;
    ];

  p++;
  q=1;
];

```

(*OH - Parallel*)

```

G=Table[0,{w,1,100}];
dd=Table[{0,0,0},{w,38*123}];
p=1;
q=1;
For[u=3, u<41,u++ ,

  For[Δz=0, Δz<123,Δz++ ,

    dd[[ (p-1) 123+q,2]]=u;
    dd[[ (p-1) 123+q,1]]=Δz;

    m=Lc/(u*10-9+Δz*10-9);

    If[(Δz+u)<(Lc*109+1),

      For[w=1,w<101,w++,
        temp=((Exp[-I Δk u*10-9]-1)/Δk) (Sum[ m
u2*Cos[RandomVariate[NormalDistribution[Pi/6,0.15]]]Exp[- I
Δk i (u*10-9+Δz*10-9)]Exp[-i (u*10-9+Δz*10-9)] ,{i,0,m-
1}]+Sum[m u2
RandomReal[{0,1}]Sin[RandomVariate[NormalDistribution[Pi/6,
0.15]]]Exp[- I Δk i (u*10-9+Δz*10-9)]Exp[-i (u*10-9+Δz*10-
9)] ,{i,0,m-1}]);
        G[[w]]=(Conjugate[temp]*temp);
        ];
        dd[[ (p-1) 123+q,3]]=Mean[G];

      ];
      q++;
    ];

  p++;
  q=1;
];

```


(*CH - Antiparallel*)

```

H=Table[0,{w,1,100}];
e=Table[{0,0,0},{w,38*123}];
p=1;
q=1;

For[u=3, u<41,u++ ,

  For[Δz=0, Δz<123,Δz++ ,

    e[[ (p-1)123+q,2]]=u;
    e[[ (p-1)123+q,1]]=Δz;

    m=(Lc*109)/(u+Δz);

    If[(Δz+u)<(Lc*109+1),

      For[w=1,w<101,w++,
        temp=((Exp[-I Δk u*10-9]-1)/Δk)(Sum[m u2 (-1)i
Cos[RandomVariate[NormalDistribution[Pi/3,0.15]]]Exp[- I Δk
i(u*10-9+Δz*10-9)]Exp[-i(u*10-9+Δz*10-9)0.5],{i,0,m-
1}]+Sum[m u2
RandomReal[{0,1}]Sin[RandomVariate[NormalDistribution[Pi/3,
0.15]]]Exp[- I Δk i(u*10-9+(Δz*10-9)/1)]Exp[-i(u*10-
9+(Δz*10-9)/1)0.5],{i,0,m-1}]);
        H[[w]]=(Conjugate[temp]*temp);
      ];
      e[[ (p-1)123+q,3]]=Mean[H];

    ];
    q++;
  ];

p++;
q=1;
];

```

(*CH - Parallel*)

```

J=Table[0,{w,1,100}];
ff=Table[{0,0,0},{w,38*123}];
p=1;
q=1;

For[u=3, u<41,u++ ,

  For[Δz=0, Δz<123,Δz++ ,

    ff[[ (p-1) 123+q,2]] =u;
    ff[[ (p-1) 123+q,1]] =Δz;

    m=Lc/(u*10-9+Δz*10-9) ;

    If[(Δz/1+u)<(Lc*109+1) ,

      For[w=1,w<101,w++,
        temp=((Exp[-I Δk u*10-9]-1)/Δk) (Sum[m u2
Cos[RandomVariate[NormalDistribution[Pi/3,0.15]]]Exp[- I Δk
i (u*10-9+Δz*10-9)]Exp[-i (u*10-9+Δz*10-9)] ,{i,0,m-1}]+Sum[m
u2
RandomReal[{0,1}]Sin[RandomVariate[NormalDistribution[Pi/3,
0.15]]]Exp[- I Δk i (u*10-9+Δz*10-9)]Exp[-i (u*10-9+Δz*10-9)] ,{i,0,m-1}]);
        J[[w]]=(Conjugate[temp]*temp) ;
      ];
      ff[[ (p-1) 123+q,3]] =Mean[J] ;

    ];
    q++;
  ];

  p++;
  q=1;
];

```

```

U=5;
V=10;
W=20;
Y=30;

dU=Table[{Re[c[[s+(V-1)123,1]]],Re[c[[s+(U-
1)123,3]]]/Re[e[[s+(U-1)123,3]]]}, {s,1,123}];

dV=Table[{Re[dd[[s+(V-1)123,1]]],Re[dd[[s+(U-
1)123,3]]]/Re[ff[[s+(U-1)123,3]]]}, {s,1,123}];

dX=Table[{Re[c[[s+(V-1)123,1]]],Re[c[[s+(U-
1)123,3]]]}, {s,1,123}];

dW=Table[{Re[c[[s+(W-1)123,1]]],Re[e[[s+(U-
1)123,3]]]}, {s,1,123}];

dT=Table[{Re[c[[s+(W-1)123,1]]],Re[dd[[s+(U-
1)123,3]]]}, {s,1,123}];

dH=Table[{Re[c[[s+(W-1)123,1]]],Re[ff[[s+(U-
1)123,3]]]}, {s,1,123}];

ListPlot[{MovingAverage[dV,1],MovingAverage[dU,1]},PlotRang
e->All]
ListPlot[{MovingAverage[dX,1],MovingAverage[dW,1],MovingAve
rage[dT,1],MovingAverage[dH,1]},PlotStyle-
>{{Black},{Red},{Blue},{Green},{Pink}},PlotRange->{All,
{0,10^-10}}]

```