# New methods in digital wood anatomy: automatization of pixel-contrast densitometry and its application to angiosperms

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## Introduction

Digital wood anatomy as a new direction in the study of xylogenesis and the influence of environmental factors on seasonal growth and structure of annual rings is intensively using new technologies and developing software tools for automated analysis of annual rings. In a recently published article, a PiC densitometry technique developed to obtain an estimate of the annual ring density profile was proposed.

Silkin P.P. et al. proposed to use software that analyzes the image as a substitute for the X-ray densitometry. A photograph of wood cross-section consists of pixels - points with equal area and belonging either to a cell wall or to a lumen. This allows to find both the total areas of cell walls and lumen and the ratio of cell wall area to the total area of the scan line. This ratio, when multiplied by the cell wall density value, gives the wood density value of the scan line.

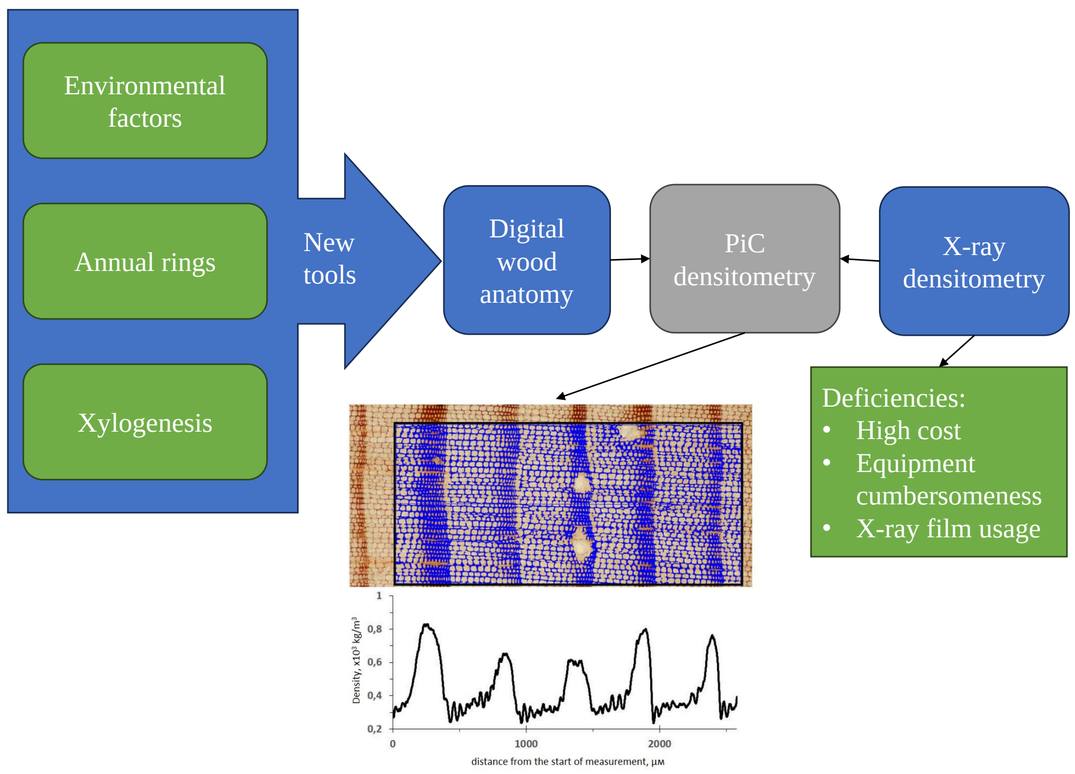


Figure 1. Advances in quantitative wood anatomy methodology

## Study Area

The work was carried out in the Western Sayan Mountains (South Siberia), in the Sayano-Shushensky Nature Reserve, coastal zone of the Yenisei River.

Samples of eight species (shrubs and small trees) were collected in 2022 :

(1) *Caragana altaica*, *Spiraea hypericifolia*—52◦04.566 N 92◦13.221 E

(2) *Caragana arborescens*, *Rhododendron ledebourii*—52◦04.543 N 92◦13.073 E

(3) *Prunus padus*, *Cornus alba*, *Alnus alnobetula*, *Ribes nigrum*—52◦04.529 N 92◦13.174 E


Figure 2. Geographical location of the study area.

## Materials

Samples of eight species of shrubs and small trees were taken in the form of a transverse cut of a trunk, one cut per specimen; height of cuts was 30-40 cm from the ground for *Prunus padus*, 10-15 cm for other species. These species are all angiosperms and have various wood structure from ring-porous to diffuse-porous.

| Species | Wood structure | Number of samples | Age, years |
|---|---|---|---|
| *Prunus padus L.* | diffuse-porous | 10 | 17-32 |
| *Caragana arborescens Lam.* | ring-porous | 13 | 28-42 |
| *Alnus alnobetula Ehrh.* | diffuse-porous | 12 | 13-25 |
| *Caragana altaica (Kom.) Pojark. = Caragana pygmaea (L.) DC.* | ring-porous | 1 | 19 |
| *Cornus alba L.* | diffuse-porous | 1 | 5 |
| *Rhododendron ledebourii Pojark. = Rhododendron dauricum L.* | diffuse-porous | 1 | 37 |
| *Ribes nigrum L.* | diffuse-porous | 1 | 7 |
| *Spiraea hypericifolia L.* | semi-ring-porous | 1 | 18 |

Figure 5. characterization of collected shrub specimens

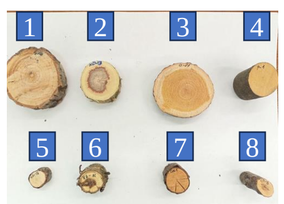


Figure 4. Examples of obtained transverse sections :
*Prunus padus (1), Caragana arborescens (2), Alnus alnobetula (3), Rhododendron ledebourii (4), Cornus alba (5), Caragana altaica (6), Ribes nigrum (7), Spiraea hypericifolia (8)*

Cuts were sectioned on the microtome and fixed in glycerol. One of the peculiarities of this method is that the cell walls of slices in glycerol fixation are in the conditions of the highest water absorption/ It decreases density of cell walls. Thus, instead of density, we use an alternative value – wood porosity, which is ratio of lumen area to total area.

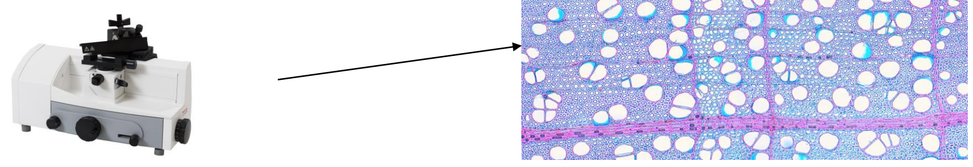

Figure 5. Image of cell structure of *Prunus padus* wood

## Methods

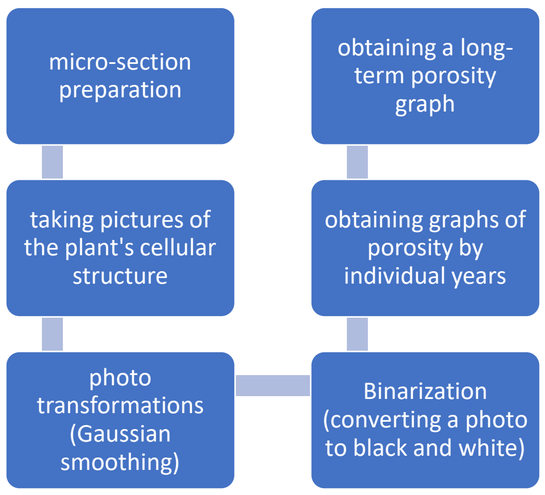


Figure 6. Methodology diagram

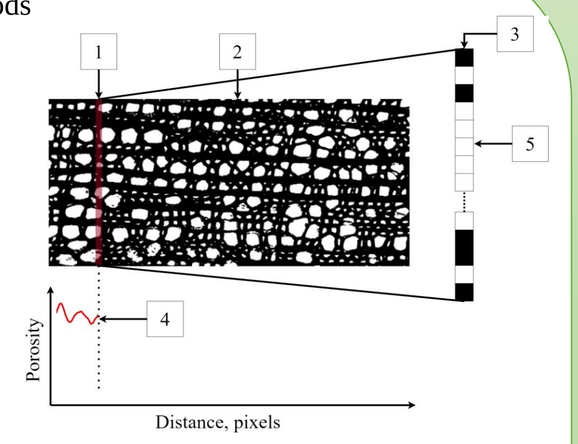


Figure 7. An example of the operation of a virtual sensor:
1—a 1-pixel-thick virtual sensor,
2—the scanning area,
3—the scanning line on an enlarged scale
4—the porosity profile (calculated as fraction of a unit).

The sensor counts the number of white pixels (5) associated with the voids and calculates the porosity as its ratio to the total number of pixels on the scan line.

## Results

In all three species, a strong correlation between the series of maximum, minimum, and average porosity values was observed, which suggests a functionally determined limitations or strict interrelations of these values. At the same time, a significant relationship between annual ring width and porosity profile values was observed only in *Caragana arborescens*.

| | *Caragana arborescens* | | | *Prunus padus* | | | *Alnus alnobetula* | | |
|---|---|---|---|---|---|---|---|---|---|
| | RW | MEANPor | MAXPor | RW | MEANPor | MAXPor | RW | MEANPor | MAXPor |
| MEANPor | **-0.30** | | | 0.04 | | | -0.01 | | |
| MAXPor | **-0.21** | **0.38** | | -0.01 | **0.63** | | 0.07 | **0.71** | |
| MINPor | 0.04 | **0.67** | 0.12 | -0.03 | **0.80** | **0.35** | -0.02 | **0.81** | **0.46** |

Figure 8. Correlations between porosity profile characteristics and ring width (RW). Bold values are significant at $p < 0.05$; All values are marked with color gradient: from red (−1) through white (0) to green (+1).

The average inter-serial correlation coefficients for maximum, minimum, and average porosity in the ring are comparable or even higher than for annual ring width, which suggests that these indices of the wood structure have a common signal for external factors influencing its formation. Of course, it is difficult to speak about the significance level for such short series, but even these preliminary results are already indicative, since they are comparable to the inter-serial correlation coefficients observed for other anatomical parameters of wood structure of both coniferous and deciduous trees.

| Characteristics | Species | | |
|---|---|---|---|
| | *Caragana arborescens* | *Prunus padus* | *Alnus alnobetula* |
| RW | 0.41 | 0.15 | 0.28 |
| MEANPor | 0,09 | 0.50 | 0.17 |
| MAXPor | 0.19 | 0.30 | 0.14 |
| MINPor | 0.03 | 0.46 | 0.29 |

Figure 9. Mean inter-series correlation coefficients of the tree-ring parameters

## Conclusions

- PiC densitometry proved to be applicable to angiosperm woody plants with various wood structures
- We developed algorithm of image processing and measurement, and implemented it as program to perform these steps automatically.
- Porosity profiles register both intra- and inter-annual variability of the wood structure.
- Testing of the more long-lived species revealed that the maximum porosity series contain external signals common among specimens of the same species; for diffuse-porous species, this is also true for mean and minimum porosity.