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Chapter 6: Multiscale Microscopic Analysis of the Paper and Fibres of the *Tsakali*

Abstract: In this chapter, both digital and optical microscopy were employed to study the raw material composition of *tsakali* paper. The fibre analysis reveals what the *tsakali* paper is made of, since the primary component of paper is the type of raw material used in its production. This ability to distinguish separate plant species in paper fibre is why fibre analysis, if and when applicable, is helpful in determining regional origin and sometimes in dating, especially when using a comparative study that entails overlapping typologies. Other aims were to learn about the relationship between the raw materials used and the properties of the resultant paper; and to deduce the origins of the paper by comparing the fibre identification results with local occurrences of the same plant. At the methodological level, this study also discusses the possibilities and limitations of different types of microscopes in the characterisation of both fibres and other components of paper, and how this may potentially contribute to the provenance study.

1 Microscopy and fibre analysis

Whenever digital or standard optical microscopes are used for fibre analysis, the aim of the procedure is exactly the same: to identify the fibres used in a manuscript's paper support on the basis of the morphological features of these fibres.[1] The crucial difference between these tools, however, in the context of heritage object analysis, is that digital microscopy allows for non-destructive testing, and optical microscopy requires that micro-samples be taken from the object to perform an analysis. Despite this fact, it is not so obvious when one method or tool should be selected over the other. In specific cases, optical microscopy may yet be more efficient in allowing for higher-precision identification. The samples to be observed under an optical microscope require preparation (boiling in water and

1 Digital microscopy is not a microscope with a camera attached to it: it's an optical inspection system that is designed with the screen as the only output, so that the complete concept and construction of the microscope is different than a binocular-based microscope. Digital microscopes primarily offer speed and convenience, as with digital cameras. They can produce very high-quality images, limited only by the optical system of the particular tool.

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then separating on the microscopic slide). This process aims to remove other components (than fibres) from the paper. Thus, on the one hand, after such preparation, it is possible to observe the fibres as whole cells, but on the other hand, it is no longer possible to document the paper's other components, as they are usually removed during sample preparation. It should be noted that most of the non-destructive spectroscopic techniques can provide information on paper components, not just on fibres (see Chapters 8 and 9 in this volume).[2] Thus, despite the development of many new methods in recent decades, optical microscopy is still the most useful for the botanical identification of plant tissue. In many cases, the optics and resolution of standard microscopes are better than digital ones, but this also depends on the specific type of microscope used.

The fibres belong to one of two general categories, natural and synthetic; then, depending on their origin, they can be classified as vegetable, animal or mineral.[3] However, most of the fibres that were traditionally used for papermaking in Asia were of natural, vegetal (papermaking plants) or animal (silk, wool) origin.

The scientific protocol for analysing handmade paper through the identification of fibre materials by observing similarities in morphological features was first established by Julius Wiesner in the late nineteenth century.[4] In the early stage, most microscopic analyses of paper fibres relied on a staining method to colour and distinguish the morphological features for identification. Staining is an auxiliary method, useful especially in cases of mixed fibre component paper. However, it is usually not sufficient for the identification of fibres on its own, due to the fact that the same colour can indicate rather large groups of possible fibre components. Furthermore, it requires a sample that can only be observed for about an hour after staining, and that becomes unsuitable for subsequent analysis. This is why staining is rarely used for the analysis of paper in heritage objects.

Digital microscopy allows for the study of paper in its original structure of interlaced fibres and all other possible components together. Furthermore, 3D digital microscopy offers fast 2D and 3D measurement, a larger depth of field and working distance than an optical microscope, as well as a very large range of magnification which can go from macro to micro, and many illumination techniques (including polarisation, differential interference contrast (DIC), transmission lighting and fluorescence).

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2 See Almogi et al. 2013–2014 [2015].

3 For a general overview of fibre categories and classification, see Herzog 1955; Ilvessalo-Pfäffli 1995; and Robertson, Roux and Wiggins 2018.

4 Wiesner 1892; Wiesner 1902; Wiesner 1903; Wiesner 1911.

The advantage of a digital microscope is that there is no need to interfere with the original object: the method is almost contactless. However, for this reason, it only allows us to see fragments of a fibre, and never the whole fibre as a cell. It is also challenging when the paper is coated with other substances, such as glue, primers or any other coating substances that limit the possibility of seeing anatomical features of the original plant cells. This makes identification much more difficult, and often impossible to conclude. Furthermore, there is no fixed protocol of observation, and it is challenging to trace the exact coordinates of the observed spot or area (depending on magnification). Thus, the measurements and observation of singular fibres may not always be possible to repeat in this case.

On the other hand, an obvious advantage of digital microscopy is that one can use digital microscopes in conditions that do not support the use of an optical microscope, for example in libraries or museums, allowing for work *in situ*. It is also more useful when many different recordings of a larger area are needed. In fact, the whole surface of the paper can be documented if needed, unlike with optical microscopy, which is performed on micro-samples taken from only one specific place. Thus, the unique flexibility of digital microscopy is an advantage, as the optical system can be attached to any support, enabling contact inspection of the paper surface (interaction of paper and ink) or non-contact inspection of an entire written artefact of any shape at a high resolution. This tool, therefore, is perfect for macroscopic analysis of the whole paper object, allowing for documenting and registering features observed at lower magnifications. This includes a wide range of features, such as surface texture, hue, interaction of paper and ink (or paints), presence of fillers, fibre distribution (if its visibility is not prevented by coatings), quality and impression of the screen (papermaking sieve print), the imprint of the material texture on which a paper has dried, the finishing process and remains of ancient repairs or conservation treatments.

This is why, though digital microscopy is preferable in general, it compromises the chances for precise identification. Thus, each method has its possibilities and limitations. One needs to know which methods to choose from to get the right type of information they may need. Considering all the facts discussed above, both methods complement each other, and are best used in combination. Furthermore, in both cases, the current state of microscopy offers much greater possibilities to observe specific anatomical features and indeed much better chances for successful identification.

2 Microscopic analysis of *tsakali* paper

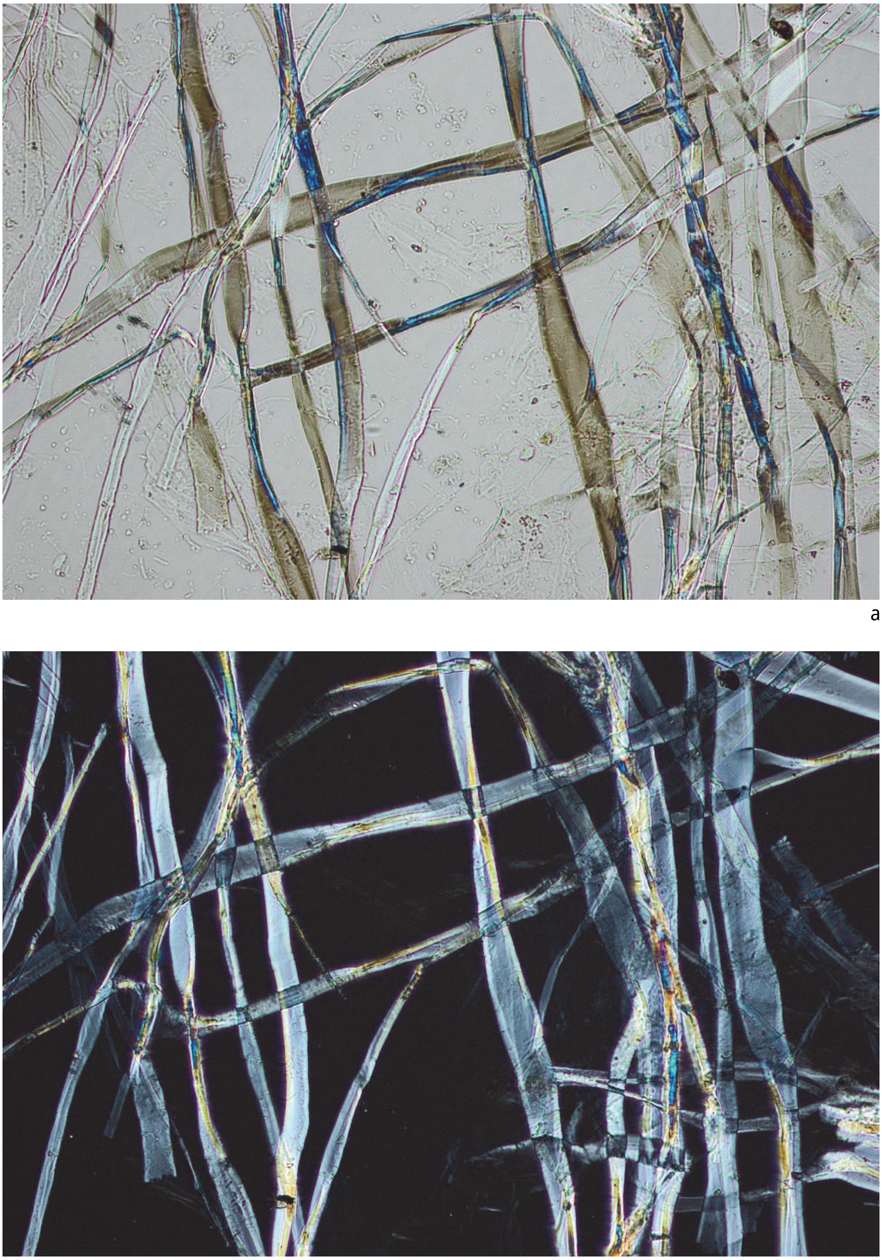
Detailed analysis of these samples was performed at the Centre for the Study of Manuscript Cultures at Universität Hamburg. The paper samples were first immersed in distilled water in a small beaker and boiled for ten to fifteen minutes. The water was decanted and the samples drained. About 0.2 g of paper pulp was placed on a microscopic slide and separated into a fine suspension of individual fibres. After water was dried, a drop of mounting solution was added and the microscope slide was closed with a coverslip. Then the slide, pressed between a metal plate and a magnet, was left until the mounting resin had dried. The fibres were then observed using an Olympus BX51 microscope with polarised light, and an Olympus UC30 digital camera that was connected to a computer and controlled by STREAM 2.4 PRO image analysis software, used for photographic acquisition. A range of magnification from 50× to 400× with both non-polarised and polarised light was used.

Attention was paid to the morphology of the fibres and other elements of the pulp, such as

– the general shape of fibres and its flexibility (for this purpose, it was important to observe the entire fibre, not just parts of it);
– the regularity of the fibre structure (or lack thereof);
– the ratio of the lumen width to the cell wall of the fibres;
– the presence and characteristics of cross-markings and dislocations, as well as longitudinal striation;
– the shape of the natural fibre endings; and
– the colour that the fibres developed in the reagents.

The aim was to learn what plants were used to make the paper for the sixty-five cards, as well as how homogenous or mixed this paper was across the full set. A digital microscope (Dino-Lite AD413T-I2V) was first used to study the surface morphology and typology of the paper of all sixty-five *tsakali*. Then a Keyence VHX-5000 digital microscope, equipped with a VH-Z100UR objective, was used to observe the paper-surface characteristics and the interaction between paper and ink in these cards. Observation under both digital microscopes suggested that all sixty-five *tsakali* were made on the same type of paper, quite homogenous in its structure; however, the fibre identification has proved impossible. The cards were in fact made of a very similar type of paper, but, so far, only its smooth texture and fragments of fibres have been possible to characterise. Thus, aiming at the identification of the fibre material, thirteen *tsakali* were sampled for fibre analysis (see Table 1).[5]

5 The results are also in the appendix.



a

b

Figs 1a–b: *Stellera chamaejasme* fibres identified in Tsakali 8, observed under polarised light (parallel (a) and crossed (b) Nicol prisms) at a magnification of 200×.

As microscopic analysis showed, the paper of the *tsakali* under study, independently of its technological features (both woven and laid), is mostly homogenous and made of *Stellera chamaejasme.* This plant belongs to the Thymelaeaceae family. Most of the fibres observed were flabby, ribbon-like in shape and with an impression of transparency: similar to cotton fibres, yet without the twisting typical of cotton, and shorter (see Figs 1a–b and 2). There were broad sections within the length of fibres that were typical of papermaking plants belonging to Thymeleaceae family, such as *Daphne* sp. The wavy fibre walls and irregular lumens, however, clearly suggest that these are *Stellera* fibres, rather than *Daphne*. The fibre walls and lumen are much more irregular compared to *Daphne.* The lumen is often delineated by wavy fibre walls, which creates a very irregular space inside the fibre cell (see the arrows on Figs 1a–b). The colour that developed after treating one sample with Herzberg stain was olive-grey, the same as others from this family.[6] The spiral vessels and minor woody fibres were present in the pulp of samples taken from Tsakali 1, 3 and 7. A singular fibre of silk was detected in Tsakali 2.

3 What can the results of the fibre analysis tell about the *tsakali* set?

Plants of the *Stellera chamaejasme* species thrive at high altitudes, and in some regions of Tibet, they have been used as the main raw material in papermaking (Fig. 2).[7] *Stellera* is a small genus of less than ten species, belonging to the Thymelaeaceae family.[8] It is also found growing in comparatively dry conditions in parts

6 The sample with Herzberg reagent was always prepared immediately before the analysis. First, a previously boiled sample was placed on a microscope slide, and a drop of Herzberg reagent was added. The sample was carefully dissected, and a coverslip gently placed on top so that no air bubbles formed.

7 Boesi and Helman-Ważny 2020.

8 Thymelaeaceae is a family of dicotyledonous flowering plants with 898 species in 50 genera. The phloem contains very strong fibres with a large quantity of hemicelluloses, which makes the bark of many Thymelaeaceae species very suitable for the manufacture of high-quality paper, such as that used for bank notes and writing supports. These fibres are long and narrow, and supportive cells provide tensile strength without limiting flexibility – further characteristics that render the bark a valuable material for papermaking. Yet another quality that makes Thymelaeaceae appropriate for papermaking is that most species are poisonous (while some are important medicinally); such paper can resist insect infestation longer than papers made of other plants and is therefore more durable and long-lasting.

of Central Asia, Bhutan, Nepal, Mongolia, Russia and China. It is widely distributed throughout the Himalayan range, where it is found on sunny, dry slopes and sandy places at altitudes of 2600 to 4500 m above sea level.

The plant is named *re lcag pa* in Tibetan, and as such is a well-known paper plant in many Tibetan cultural regions, such as A mdo, Khams and dBus gtsang, as well as in Nepalese high valleys like Dolpo and lower Mustang. Yet each region uses a local vernacular name for *Stellera*, such as *mgo re lcag re* in Lithang; *mgo nara* in Nepalese Dolpo; *mgo bo rol gdangs* in the lower Mustang District (Nepal); and *ra ma rwa co* in the Xining area (Qinghai Province, PRC).[9] To my knowledge, *Stellera chamaejasme* is also known locally in Mustang as *sibri mentok* (*sibri* means 'smelling of sweat'; *mentok* means 'flower').[10]

This plant was first described at the end of the nineteenth century.[11] It is a pest plant, which successfully competes with other species and quickly colonises degraded pasturelands. From the 1960s onwards, pasture over-exploitation has been a widespread phenomenon all over the Tibetan plateau and *Stellera* has been increasingly significant.[12] This implies that, when considering the amount of source material for papermaking that was available in the past, we cannot rely on the present abundance and distribution of this species. On the other hand, it is the only papermaking species that grows at over 3500 m above sea level. Thus, *tsakali* paper from *Stellera* roots must have been produced in the high Himalayan area, where practically nothing else grows, and by Tibetan craftsmen.

The altitude range of *Daphne* sp. reaches 3600 m above sea level, and *Stellera* sp. grows up to 4500 metres (*Daphne* plants need much more moisture than *Stellera*) (Figs 3a–b). Thus, these two species very rarely grow in the same habitat. Besides the different ecosystems in which these plants occur, their use is also conditioned by different technological requirements in the process of papermaking, such as (1) the hardship of collecting these roots growing deep in stones in the mountains, as well as (2) the extra steps of the papermaking process required to neutralise its poisonous qualities.

Identifying *Stellera* fibres in the paper of thirteen *tsakali* obviously confirms that they were created on Tibetan paper; we must additionally consider the dis-

9 Boesi 2016, 504.

10 Helman-Ważny and Ramble 2021.

11 Kingdon-Ward 1934, 64; see also the sample of *Stellera chamaejasme* collected on 10 June 1922 in the *Herbarium of the L. H. Bailey Hortorium* at Cornell University, Department of Botany, College of Agriculture, Ithaca, NY.

12 Boesi 2016, 507.

tribution of this plant suggesting that the *tsakali* were produced at the place located above 3600 m above sea level.

What are the implications for dating the *tsakali* production? From the perspective of manuscript studies, most of the early manuscripts from central and western Tibet – other than the Dunhuang manuscripts, which were written on rags or *Daphne* paper – were written on *Stellera* paper, or on paper in which *Stellera* is a predominant component.

Was *Stellera* used specifically for its properties? From the technological point of view, since manuscript paper must be processed before writing, it is possible to use a variety of fibres in its production, regardless of their individual properties and strengths. The only factor influencing the selection of the paper would have been the size of the manuscript. *Stellera* fibre alone is not stable enough for large formats (*Stellera* paper can easily bend or break). Smaller formats, like *tsakali*, were more easily produced on pure *Stellera* paper.

Besides paper made of *Daphne* fibre, Tibetans usually claim that paper made from *Stellera* sp. is the 'original' Tibetan paper. The reason is probably that Tibetans associate this type of paper exclusively with the 'heart of their culture', since the plant grows over 4000 m above sea level and is not mentioned anywhere else in the context of papermaking. There is no evidence so far that it was used in places other than the Tibetan plateau. The oldest samples of *Stellera* sp. in Tibetan manuscripts have been identified in a manuscript from central Tibet that dates to the tenth century and one from western Tibet that dates to the eleventh century.[13] It should be noted, however, that there is no evidence of these plants in the Tibetan manuscripts found in Dunhuang. After the fifteenth century, this plant was more often used in addition to *Daphne* and *Edgeworthia*, possibly to add some softness to the *Daphne* paper and make it more suitable for printing.[14]

These *Stellera* root fibres alone create a very specific, soft type of paper that Tibetan papermakers later considered to be of lower quality than the bark paper made of *Daphne*, *Wikstroemia* and *Edgeworthia*. *Stellera* fibres are not considered as strong. The roots are especially difficult to harvest, which places a serious limitation on the quantity of paper that can be produced. They additionally require a longer time for processing. Thus, in general, they are only used in papermaking when no other sources are available. On the other hand, the poisonous properties of these plants make the paper resistant to damage caused by insects, meaning that it may ultimately be more durable than other types of paper.

13 Helman-Ważny 2016a.

14 Helman-Ważny 2016b.

Fig. 2: *Stellera* plant collected by paper makers in Dobe Shang; photo by Agnieszka Helman-Ważny during fieldwork in Tibet, 2013.

a

b

Figs 3a–b: (a) Digging out a *Stellera* plant in the sKyid grong area of Tibet; (b) a *Stellera* plant growing in its natural habitat; photo by Agnieszka Helman-Ważny during fieldwork in Tibet, 2013.

Table 1: *Tsakali* paper.

Ordinal no.	Original *tsakali* no. and name	Type of paper (papermaking sieve print)	Fibre composition
1	**0** Bum pa (vase)	**Woven** paper without fibre bundles (cloudy fibre distribution)	***Stellera chamaejasme***
2	**1** Ye nyid kyis ston pa	**Woven** paper without fibre bundles (cloudy fibre distribution)	***Stellera chamaejasme*** (spiral vessels and minor woody fibres present in the pulp)
3	**2** Thug[s] [b]rtse'i ston pa	**Woven** paper without fibre bundles (cloudy fibre distribution)	***Stellera chamaejasme*** (singular fibre of silk)
4	**3** Cir yang sprul pa'i ston pa	**Woven** paper without fibre bundles (cloudy fibre distribution)	***Stellera chamaejasme*** (spiral vessels and minor woody fibres present in the pulp)
5	**4** Tshad med 'od ldan	**Woven** paper without fibre bundles (cloudy fibre distribution)	***Stellera chamaejasme*** (short fibres, more damaged)
6	**5** Yab 'Phrul gshen snang ldan	**Woven** paper without fibre bundles (cloudy fibre distribution)	***Stellera chamaejasme***
7	**6** rGyud khungs kyi ston pa	**Woven** paper with many fibre bundles	***Stellera chamaejasme***
8	**7** Yum Zang za rig btsun	**Woven** paper without fibre bundles (cloudy fibre distribution)	***Stellera chamaejasme*** (spiral vessels and minor woody fibres present in the pulp)
9	**8** Sras 'Chi med gtsug phud	**Woven** paper with many fibre bundles	***Stellera chamaejasme***
10	**9** gShen gSang ba 'dus pa	**Woven** paper without fibre bundles (cloudy fibre distribution)	***Stellera chamaejasme***
12	**25** De sras dGe 'phan	**Laid paper**	***Stellera chamaejasme***
13	**A** Zhing lnga gtso' bo	**Woven** paper	***Stellera chamaejasme***