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AN EXPERIMENTAL METHODOLOGY FOR STUDYING THE PENETRATION OF ADHESIVES INTO PAINTED TEXTILES WITH FLUORESCENT LABELLING AND CROSS- SECTIONS: A CASE STUDY IN THANGKA PAINTING SAMPLES

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ABSTRACT

The current dissertation examines labelled Isinglass and funori with 5-DTAF [5-(4,6-Dichlorotriazinyl)aminofluorescein] after application on Tibetan thangka painting samples, with fluorescent light microscopy.

Different types of preparation were tested included samples with added iron red in the pigmentation and/or starch paste for sizing the ground fabric. This aimed in finding the most appropriate methodology for creating samples with low autofluorescence of the samples. Since, samples observed embedded in cross-section, materials and techniques of coss-sectioning was reviewed and evaluated for examination with fluorescent microscopy

This study was conducted in order to provide an experimental methodology and guidelines for preparing labelled adhesives and painted textiles samples, for the study of their penetration.

The conclusions of this dissertation can be used by any researcher in order to advance the research about adhesives' penetration, and potentially help conservators decide about the proper consolidation practices for painted textiles.

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INTRODUCTION

The term painted textiles is mainly used for defining objects that are not supposed to remain on wooden stretchers like canvas paintings. The main reason for creating painted textiles, that were not works of art/painting, was to deliver a message. Hence, the main types of painted textiles we can find in museum collections are religious banners, trade union banners, and military/naval flags.

Due to their nature painted textiles are usually hanging either free or from scrolls that are attached on them and made so they can rolled and unrolled. Due to those facts, about their construction and use, impact their preservation condition as well. More specifically, the mechanical stress that painted textiles undergo, usually results in wear, tear abrasion of the fabric and also cracking and flaking (delamination) of the paint layer from the fabric.[1] Flaking, that can visually identified as “islands of paint of varying sizes, often with cupped edges”, is usually caused by folding or excessive flexing of the textile.[2] In order to avoid irrevocable losses of the delaminated paint layer, it needs to be stabilised by applying adhesive solutions. This process is known in the conservation field as consolidation. Respectively, adhesives used for the consolidation of paint are often referred as consolidants.[3]

Consolidation between delaminated layers is a very common and important conservation technique, particularly in the painting conservation field. There is a great number of consolidants, described in a very informative article

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[1] C. B. Gupta, "Conservation of Thangka Paintings: A Cultural Heritage from the Himalayan Region" (paper presented at the Special Session of the ICOM-CC 15th Triennial Conference, New Delhi, India, New Delhi, India, 2009 26 September 2008): 62-72.

[2] Nancy Pollack and Jan Vuori, "Moving Pictures: Adapting Painting Conservation Techniques to the Treatment of Painted Textiles," *Tales in the textile. The conservation of flags and other symbolic textiles. Preprints, NATCC, Albany NY* (2003): 127-134.

[3] Sylvia M. Rodgers, "Consolidation, Fix-Ing, Facing," *Paper conservation catalog. Washington, DC: American Institute for Conservation* 1, no. 20 (1994): 07417-01884.

published by the Paper Conservation Catalog.[4] For selecting the most appropriate consolidant for stabilising delaminate paint, factors such as the presence of varnish and the type and quantity of binding medium (in the paint layer which needs consolidation) should be considered. Specifically, there are recommendations for using certain adhesives for paint layers with oil binding media, which have a glossy appearance, and different ones are ~~suggested~~ for paint layers with water and protein based binding media- resulting in a matte appearance.[5]

In particular for consolidating painted textiles as Pollack highlights, most materials and techniques are adapted form those for matte paint, with special consideration in maintaining the flexibility of the object after the treatment.[6] The type and quantity of adhesive applied must be absorbed from the top of the paint layer in order to avoid the formation of glossy areas and penetrate enough to ensure stabilisation of the delaminated paint. Ideally, it should be placed exactly between the delaminated paint layer and the fabric in order to re-join them (Figure 1).

As Hummert et all. mention, “studying the penetration behaviour of adhesive allows observation of whether the adhesive is distributed to the desired position and permits adaption and improvements of the application methods”.[7] However, it is extremely difficult to trace the allocation and penetration behaviour of an applied adhesive visually, since adhesives are colourless.

One option for making adhesives visible, in order to study them, is simply by mixing them with a colourant. Nevertheless, because chemical bonds do not

[4] Ibid.
[5] Nancy Pollack and Jan Vuori, "Moving Pictures: Adapting Painting Conservation Techniques to the Treatment of Painted Textiles."
[6] Ibid.
[7] Eva Hummert, Ute Henniges, and Antje Potthast, "Fluorescence Labeling of Gelatin and Methylcellulose: Monitoring Their Penetration Behavior into Paper," *Cellulose* 20, no. 2 (2013): 919-931.

exists between the adhesive and the colourant, they can be easily separated making this method unreliable.[8, 9]

Another early attempt for studying the penetration of binding media, also in the painting conservation field, was by applying histochemical staining techniques.[10, 11] Nonetheless, histochemical staining is also unreliable, since similar chemical groups of the sample itself could be stained as well.[12, 13]

Fourier transform infrared spectroscopy (FTIR) has been also used as an indirect method for studying the penetration of adhesives, though the detection of molecule bands that belong to them; it is worth mentioning Barret (1989) and Rouchon et al. (2010) studies, who determined gelatin distribution in paper.[14, 15] Nevertheless, this method does not allow the visualisation of the penetration.[16] Using this method for studying paintings and painted textiles, as with staining, similar chemical groups that occur in the original material, such as the binding media of the paint layer, would also be detected, making the interpretation of the results very difficult.

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[8] Iris Winkelmeyer and Staatliche Akademie der Bildenden Künste Stuttgart, *Zeitgenössische Kunst Aus Polyurethanweichschaum: Entstehung-Alterung-Restaurierung-Lagerung*(Institut für Museumskunde, 2000).
[9] Karolina Soppa, Tilly Laaser, and Christoph Krekel, "Visualizing the Penetration of Adhesives Using Fluorescent Labelling" (paper presented at the Proceedings of the symposium in adhesives and consolidants for conservation, Ottawa, 2011): 1-18.
[10] Joyce Plesters, "Cross-Sections and Chemical Analysis of Paint Samples," *Studies in Conservation* 2, no. 3 (1956): 110-157.
[11] Richard Wolbers and Gregory Landrey, "The Use of Direct Reactive Fluorescent Dyes for the Characterization of Binding Media in Cross Sectional Examinations" (paper presented at the Preprints of Papers Presented at the Fifteenth Annual Meeting, American Institute for Conservation of Historic and Artistic Works, Vancouver, British Columbia, May 20-24, 1987): 168-202.
[12] Karolina Soppa, Tilly Laaser, and Christoph Krekel, "Visualizing the Penetration of Adhesives Using Fluorescent Labelling" (2011): 1-18.
[13] Eva Hummert, Ute Henniges, and Antje Potthast, "Fluorescence Labeling of Gelatin and Methylcellulose: Monitoring Their Penetration Behavior into Paper."
[14] Timothy D. Barret, "Early European Papers/Contemporary Conservation Papers: A Report on Research Undertaken from Fall 1984 through Fall 1987," *The Paper Conservator* 13(1989): 39-41.
[15] Véronique Rouchon, Eleonora Pellizzi, and Koen Janssens, "Ftir Techniques Applied to the Detection of Gelatine in Paper Artifacts: From Macroscopic to Microscopic Approach," *Applied Physics A* 100, no. 3 (2010): 663-669.
[16] Eva Hummert, Ute Henniges, and Antje Potthast, "Fluorescence Labeling of Gelatin and Methylcellulose: Monitoring Their Penetration Behavior into Paper."

All difficulties mentioned previously seem to be overcomed with fluorescent labelling of adhesives. Labelling is carried out before the adhesive's application, allowing its detection after application by examining samples embedded in resin with fluorescence light microscopy (FLM),

More specifically, labelling adhesives with fluorescent dyes ensures the formation of stable chemical bonds between the dye and the adhesive that prevents, if performed correctly, their separation during application. Also penetration can be easily visualised and documented by using a Fluorescent light microscope with a camera attached to it.

Fluorescent labelling as technique was initially introduced and developed for biological and medical research.[17] In the conservation field it first appeared in 1995 in a study conducted by Ream for the consolidation of gouache paint on paper. The aim of this study was to address the penetration of two consolidants (gelatin and methyl cellulose) by comparing different combinations of diluents and application techniques. She used a fluorescent dye named Rhodamine B for labelling both consolidants and examined thin section of embedded samples in polyester resin.[18]

It took quite few years for the next research in the conservation field to appear. This time the publication came from the paintings field and was published by Soppa et al 2011.[19] This research employed the use of three different fluorescent dyes (mentioned more accurately as fluorochromes), TRITC, 5-DTAF and FITC studying and visualise the penetration of gelatin, methyl cellulose and poly(isobutyl methacrylate) (common brand names Degalan PQ611, and Acryloid B-67 respectively). Adhesives were also examined in thin section, embedded in acrylic resin (Technovit™7100). This study provided a more detail information for the chemistry behind fluorescent labelling and fluorescence microscopy and provided interested and worthwhile

[17] Julie Dennin Ream, "Observations on the Penetration of Two Consolidants Applied to Insecure Gouache on Paper," *Book Paper Group Annua* 14(1995): 27-104.
[18] Ibid.
[19] Karolina Soppa, Tilly Laaser, and Christoph Krekel, "Visualizing the Penetration of Adhesives Using Fluorescent Labelling" (2011): 1-18.

guidelines for the sample preparation. Nevertheless, in order to prepare samples that would not interfere with the fluorescence emitted form the labelled adhesives the authors excessively manipulated the material for the sample preparation. Namely, as binding medium they used a type of epoxy resin instead of anima glue.

The most resent study in the conservation field was published by Hummert et al. in 2013.[20] This research employed the use of fluorescent labelling of adhesives (also gelatin and methyl cellulose) in order to study their penetration behaviour into paper. This study further contributed to the systematical study of adhesives' penetration behaviour through fluorescent labelling. It examined the potential of using this method for detecting very low concentrations of adhesives (0.5-1%), which were applied by immersion or with aerosol-misted application. Moreover, it provides detailed information for the chemistry related to fluorescent labelling and more advanced technical guidelines for the purification process of the labelled adhesives, which is necessary after the fluorescent labelling. Purification methods mainly aim in removing any unbonded fluorescent dye molecules from the adhesive after labelling.[21] Hummert et al. also examined thin sections of samples embedded in acrylic resin (Technovit™7100) cut with a rotary microtome.

According to the aforementioned, the main aim of this project is to determine an accurate experimental methodology and guidelines for preparing adhesives and painted textiles samples in order to study their penetration with fluorescent labelling.

Special attention will be paid in explaining the rational behind selecting the materials and methods, and highlighting the aspects that need to be considered when choosing them. Some of these aspects include the avoidance of over complex processes, when simpler are sufficient, and the use of resources that are readily available in an academic conservation

[20] Eva Hummert, Ute Henniges, and Antje Potthast, "Fluorescence Labeling of Gelatin and Methylcellulose: Monitoring Their Penetration Behavior into Paper."
[21] Ibid.

laboratory (Centre of Textile Conservation and Technical Art History, University of Glasgow in our case). Hence, techniques that would make such a research task difficult to be replicated were avoided, in order to encourage future research and improvements on it.

This study will be based on previous recommendations on sample preparation made by Soppa et al., in order to examine and evaluate three different reconstruction methodologies for sample preparation. All, samples in this project will be examined embedded in cross-section, as it was a readily available technique included and studied in previews Textile Conservation dissertation projects.

For the purpose of this study all samples were prepared according to the traditional preparation of the painting surface of Tibetan thangkas, by making a reconstruction. Thangkas were particularly chosen because there is sufficient literature about the materials and methodology for preparing them. Moreover, due to the fact that thangkas are basically composite objects containing other textile elements, apart of the painted surface, their treatment often concerns textile conservators.

This dissertation is divided as following: Chapter 1 explains the basics about thangka paintings; Chapter 2 presents the method of consolidation. Chapter 3 and 4 give a brief explanation of the scientific background (“Fluorescent Labelling And Fluorescence Microscopy” and “Cross-section” respectively). Chapter 5 demonstrates the experimental rational behind this project; Chapter 6 describes the materials and methods for the experimental part; Chapter 7 presents the results. Finally two last chapters, the “Discussion And Future Work” and the “Conclusions”, critically discuss the results and propose future work and concludes respectively.

CHAPTER 1: Thangka Paintings

Tibetan Thangkas scroll-banners as shown in Figure# are composite objects made of a painted part and different textile elements. As this study is focused on the painted part, this chapter aims to provide essential details about thangka paintings; their materials and construction technique. The information provided aims to illustrate the reconstruction process.

Subsequently the conservation literature will be reviewed.

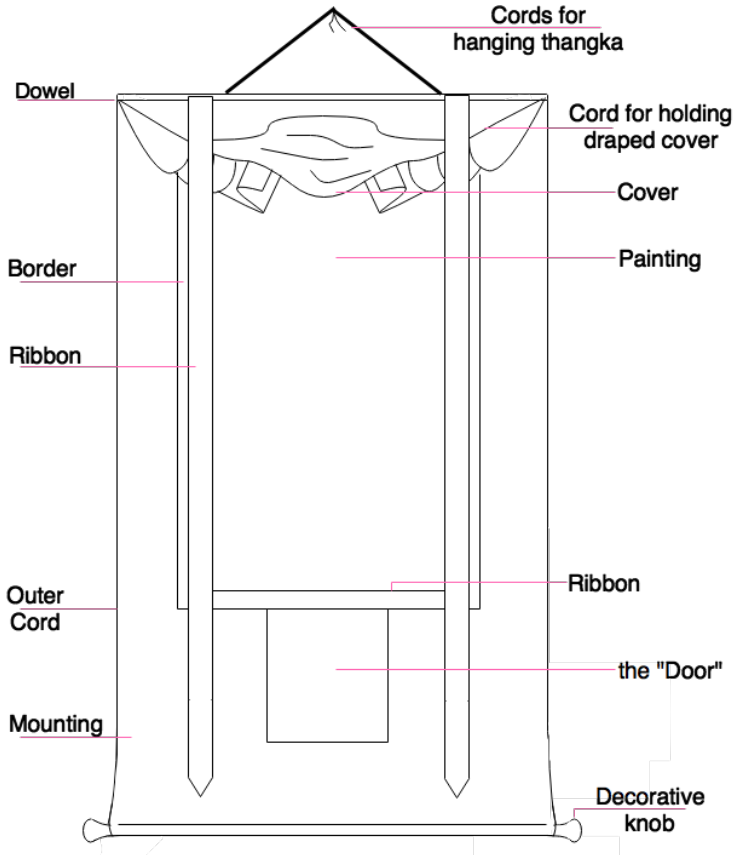

Figure 1: Diagram showing the different elements of a thangka scroll.

1.1. Tibetan Thangka painting: Materials and Techniques

Thangka paintings are religious banners from Tibet, Nepal and Bhutan. These are generally made on coarse cotton cloth (support fabric) with only few exemptions made from other materials.[22]

David and Janice Jackson in the 1970s and 1980s conducted a valuable field study of the methods and materials of thangka paintings by collecting data from living thangka painters in Tibet. This study offers the most comprehensive and detailed account so far for the traditional methods used by the artists, the iconometry, the sketching techniques, the materials and ground techniques, the techniques of painting and shading, the pigments and even for the brushes.[23]

A brief summary of the basic construction steps for the preparation of the painting surface is presented in the following sections. Information about artistic aspects (iconography and iconometry) for of the painting will not be included, as the focus of this project is material oriented. Hence only few information will be presented for the paint preparation at the end of this subchapter.

1.1.1. The support fabric

The support is generally a plain weave cotton of various weave counts, from fine and tight weaves to loosely woven.[24] There are rare cases were linen, silk, leather or skin have been used for support fabric.[25, 26, 27, 28] More

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[22] C. B. Gupta, "Conservation of Thangka Paintings: A Cultural Heritage from the Himalayan Region" (paper presented at the Special Session of the ICOM-CC 15th Triennial Conference, New Delhi, India, September 26, 2008, 2009).
[23] David Jackson and Janice Jackson, *Tibetan Thangka Painting: Methods and Materials*(Shambala Publications. Inc., 1984).
[24] Sabine Cotte, "An Evaluation of the Role of Semi-Transparent Relining in the Conservation of Thangka Paintings," *Studies in conservation* 52, no. 1 (2007): 2-12
[25] John C. Huntington, "The Technique of Tibetan Paintings," ibid.15, no. 2 (1970): 122-123
[26] David Jackson and Janice Jackson, *Tibetan Thangka Painting: Methods and Materials*, p. 16.
[27] Robert Bruce-Gardner, "Himalayan Scroll Paintings: Conservation Parameters," *The Conservator* 12, no. 1 (2010): 3-14.
[28] Jacki Elgar, "Tibetan Thang Kas: An Overview," *The Paper Conservator* 30, no. 1 (2006): 99-114

specifically, according to the tradition, the most common cotton cloth used was Indian muslin, or similar cotton cloths from China (for the eastern parts of Tibet). Later on, Thangka modern artists in India and Nepal have started using light-weight, fine, slightly open weave, Indian cotton.[29] Furthermore, it was a common practice in Tibet, stitching two pieces on cotton cloth together to form a big enough support for large paintings.[30]

1.1.2. Stretching the fabric

The first practical step, after choosing the type of fabric, was to stretch it in a wooden stretcher frame and then coating it with a layer of white paint for the ground. For stretching the cloth two stretching frames were used, a small inner frame that was attached to the edges of the cloth, and a bigger heavier one. The two frames were connected together by looping a twine. The inner was made of bamboo sticks that was attached to the edges usually by stitching and helped to distribute the tension evenly and the outer was basically the stretcher.[31]

Hence, the cloth was much smaller than the stretcher in order to fit inside it. The cloth and stretcher, after the fastened, looked like a miniature trampoline.[32]

1.1.3. Sizing of the support fabric

The next step after stretching the support was to apply a layer of glue which aimed in the coating and stiffening of the support fabric. The adhesive used for sizing was a solution of warm hide glue, applied on both sides of the support fabric by using a large brush or wadded-up rag. After the twine that connected the cloth to the stretcher was tightened again, it was set to dry. It should be mentioned at this point that, many artists did not apply size to the

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[29] David Jackson and Janice Jackson, *Tibetan Thangka Painting: Methods and Materials*, 16.
[30] Ibid.
[31] Ibid., 16-18.
[32] Ibid.

cloth in a separate operation but were directly applying a coat of gesso. As for the adhesive used for sizing, the painters of Tibet preferred the purest size available, which was usually a gelatin, made only from skins without fat, hairs and other impurities.[33]

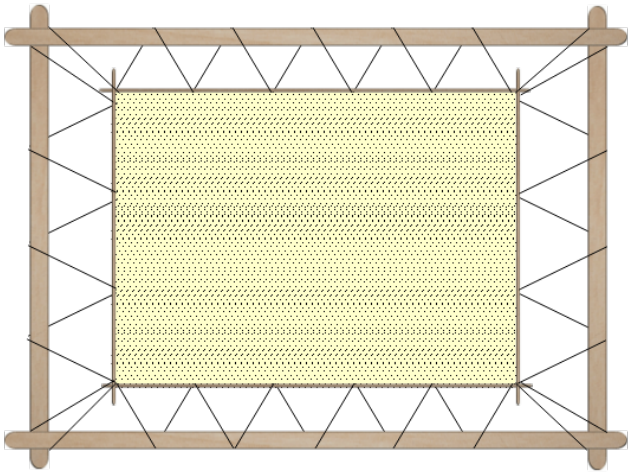

Figure 2: Fabric stretched with the wooden stretchers (inner and outer).

1.1.4. The preparatory layer (preparation)

The sizing of the cloth followed the preparation and application of the preparatory layer, a technique also known as preparation or “gesso”. The function of this layer was to make the support fabric suitable for painting. The type of gesso used by Tibetan painters was a mixture of the most available white earth pigment, either a chalk or a white clay (kaolin) – with some size solution. As David and Janice Jackson state, thangka painters, did not use precise measurements for preparing the gesso mixture, because “they preferred to mix their binders by "feel" and not by measure”.[34] Nevertheless, David and Janice Jackson reported in their book that the artists, they interviewed, used approximately two parts of white pigments to one part of size solution for preparing it.[35] In terms of the strength of the size solution used for the gesso preparation, there are no specifications or formal

[33] Ibid., 18-20.
[34] Ibid., 20.
[35] Ibid.

guidelines. Some artists use a size solution twice the strength of the ordinary solution applied for sizing the support fabric while others used a size solution with almost the same strength as they ordinary sizing solutions. For applying the gesso to the support fabric, a wadded-up rag or a gesso knife were usually used and occasionally oversized brushes. Whichever applicator was used, the gesso had to be applied evenly to both sides of the stretched cloth in thin coats of the same thickness.[36] It is worth mentioning that the difficulty in preparing the support fabric was linked to the properties of the fabric. Fabrics of fine weave required less time for applying the gesso since it was easier to do it, whereas "coarser" fabrics required more layers of gesso.[37]

1.1.5. Polishing

After the application of each coat of gesso, polishing was taking place in order to create a smooth and even surface for painting. Basically, two types of polishing were used: "damp polishing" over a slightly wet gesso or "dry polishing" where the "gesso" was dry. In the case of the former, both sides of the working area had to be dampened usually with a cotton rug. For both polishing methods, tools with hard smooth surfaces and rounded edges were used (such as stones and conch-shells). Even when dry polishing was used, the final coat of the front side was always damp polished. This aimed in creating a less slippery surface with more texture for the paint layer, instead of the more shiny surface that dry polishing creates.

1.1.6. The paint layer

Cotte, in her review published in 2011, mentions that "the painting technique is distemper, animal glue being the main binding medium for the ground and the pigments".[38] Sankrityayana and Tucci, who are recognized for their contribution on Tibetan painting research and formed the main literature for

[36] Ibid.
[37] Ibid., 16.
[38] Sabine Cotte, "Conservation of Thangkas-a Review of the Literature since the 1970s," *Studies in Conservation* 56, no. 2 (2011): 81-93

referencing materials and technique, list predominantly inorganic pigments and few organic pigments occasionally used.[39, 40]

Nevertheless, since this dissertation is focused on modifications of the preparatory layer, the composition of the paint layer will not be further analysed.

1.2. Thangkas in the conservation literature

As mentioned in previously, thangkas combine many different materials such as painting, silk textiles, ribbons and wood/bamboo and sometimes leather and metallic elements. Thus, thangkas are considered by conservators as composite objects and as Shaftel highlights, “require a balanced approach in conservation treatment”.[41, 42] Moreover, conservation treatments for preserving thangkas should not solely be focused on materials, but also encompass ethical and cross-cultural aspects, as they constitute sacred objects and require analogous treatment approaches.[43]

The majority of the publications for thangkas before 1970s are within a technical and art historical context. Characteristic examples of early publications on thangkas are the 'Technique in Tibetan paintings' by Rahula Sankrityayana published in 1937 and the thesis 'Tibetan painted scrolls', 1949

[39] Rahula Sankrityayana, "Technique in Tibetan Paintings," *Asia magazine* 16, no. 4 (1963): 30-33.
[40] Giuseppe Tucci, *Tibetan Painted Scrolls*(SDI Publications, 2006).
[41] Sabine Cotte, "Conservation of Thangkas-a Review of the Literature since the 1970s," p.81.
[42] Ann Shaftel, "Conservation Treatment of Tibetan Thangkas," *Journal of the American Institute for Conservation* 30, no. 1 (1991): pp. 3-11
[43] Sabine Cotte, "Conservation of Thangkas-a Review of the Literature since the 1970s."

by Giuseppe Tucci.[44, 45] The latter was published in limited copies and reviewed in 2000 by Erberto Lo Bue and republished in 2006.46, [47]

According to Cotte, who carried out a recent literature review in 2011, there is a considerable number of publications on thangkas in the conservation field. These are either studies for their materials and techniques, or articles that discuss conservation issues and ethics related to their sacred nature in western museums and collections. The latter indicates the increased consideration of the western conservation professionals for ethnographic objects.[48]

The very first papers for the conservation of Tibetan thangka paintings were a series of articles published in *Studies in Conservation* by Huntington (1969-1970). [49, 50, 51] He described the materials and technique of these paintings, published the first reports of conservation practices related to Thangkas, and conducted a valuable reference work that helped conservators in understanding the structure of the textile part of Thangkas.

The next major contribution to the conservation field made by ICCROM (International Centre for Conservation, Rome), in collaboration with New Delhi's National Museum who hosted a Seminar on Conservation of Cultural Property called 'Conservation in the Tropics' in 1972.[52] From this seminar and its proceedings, valuable information came into surface from Indian and

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[44] Rahula Sankrityayana, "Technique in Tibetan Paintings."
[45] Giuseppe Tucci, "Tibetan Painted Scrolls (Rome, 1949)," *Janos Szerb,"Glosses on the Oeuvre of Bla-Ma'Phags-Pa: III. The 'Patron-Patronized'Relationship" in Aziz and Kapstein, eds. Soundings in Tibetan Civilization* (1985): 165-173.
[46] Erberto Lo Bue, "Reviewed Work: Tibetan Painted Scrolls by Giuseppe Tucci," *East and West* 50, no. 1/4 (2000): 597-603.
[47] Giuseppe Tucci, *Tibetan Painted Scrolls*.
[48] Sabine Cotte, "Conservation of Thangkas-a Review of the Literature since the 1970s," p.82.
[49] John C. Huntington, "The Technique of Tibetan Paintings."
[50] John C. Huntington, "On the Conservation of Tibetan Thang-Kas," *Studies in Conservation* 14, no. 4 (1969): 152-154.
[51] John C. Huntington, "The Iconography and Structure of the Mountings of Tibetan Paintings," *Studies in Conservation* 15, no. 3 (1970): 190-205.
[52] Sabine Cotte, "Conservation of Thangkas-a Review of the Literature since the 1970s," ibid.56, no. 2 (2011): 81-93

Eastern conservators in term of material and techniques used in the local conservation practice of the East, as Sabine Cotte highlights.[53] At this seminar Mehra and Agrawal were the first Indian conservators who shared with conservators from the western schools their technical skills and conservation approaches for the care mobile Buddhist paintings.[54, 55, 56]

Agrawal in 1984 goes a step further by publishing a book for the Conservation of Manuscripts and paintings of South East Asia. More specifically in the chapter 6 of this book, specifically discusses the treatment of Thangkas. In this chapter also explains the symbolism behind of all the construction elements of a Thangka and separates them from 'ordinary' paintings, because of their deep significant religious character. Apart from the theoretical approach, he also identified and analysed the practical challenges surrounding the conservation of Thangkas, worth mentioning their need to remain flexible after treatment, and also to maintain the damaged borders together with the painting.[57] He also underlines that is a composite object that should be separated only in order to treat each material with appropriate techniques for the woven textiles and the painting surface.[58]

Boon Nee Loh in 2002 investigated treatments carried out in various Western museums and the decision-making processes related to thangkas, their significance, the time available for conservation treatment and the causes of their damage. He also encouraged the use of 'decision trees' for decision-

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[53] Ibid.
[54] V. R. Mehra, "Note on the Technique and Conservation of Some Thang-Ka Paintings," ibid.15, no. 3 (1970): 206-214.
[55] O.P. Agrawal, "Conservation Des Biens Culturels Dans I'asie Du Sud Est. La Conservation: Ie Point De Vue De L'asie," *Museum XXVII* (1975): 157-160.
[56] O.P. Agrawal, "Conservation: Les Conditions Climatiques Et Naturelles Regionales," *Museum XXVII* (1975): 161-165.
[57] O.P. Agrawal, *Than-Kas*, ed. O.P. Agrawal, Conservation of Manuscripts and Paintings in South East Asia, (London: Butterworths, 1984), 256.
[58] Ibid., 257.

making based on previews published models for preventive conservation strategies.[59, 35]

1.3. Deterioration and causes of Thangkas painting surface

The fact that Thangkas were made to be rolled and unrolled is one of the major reasons for their deterioration, as Gupta underlines, since these actions result in cracking and flaking of the paint. The cause of these damages is the unequal mechanical stress applied on painting areas with different thickness.[60] Also another possible cause of the damage at the paint surface, due to rolling, could be the fact that thangkas are rolled with the painted side in. this action creates more mechanical stress to the preparation and paint at the main surface and less to the preparatory layer at the back of the support fabric.

Cotte in 2007 suggests another potential reason for cracking and flaking. She links cracking and flaking with the construction technique and more specifically with the application of the gesso that was probably not very "carefully worked on the cloth".[61]

The fact that cracking and flaking is strongly connecting with the construction of the preparatory layer is also supported by the conservation literature in the paintings conservation field. In 1992 Pocobene and Hodkinson mention that if the size was applied cold on the cloth the paint and preparation layer is more likely to delaminate due to the lack of bonding between the size and the fabric.[62] Moreover D and J Jackson in their book underline that the composition of the gesso and the percentage of the sizing in it is of paramount importance. More specifically, too much sizing could result in very brittle pain

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[59] Jacinta Boon Nee Loh, "Decision from Indecision: Conservation of Thangka Significance, Perspectives and Approaches," *Journal of Conservation and Museum Studies* 8(2011): 1-5.
[60] C. B. Gupta, "Conservation of Thangka Paintings: A Cultural Heritage from the Himalayan Region" (2009 26 September 2008): 62-72.
[61] Sabine Cotte, "An Evaluation of the Role of Semi-Transparent Relining in the Conservation of Thangka Paintings."
[62] Gianfranco Pocobene and Ian Hodkinson, "Use of a Pressure-Sensitive Adhesive to Facilitate the Transfer of a Severely Tented Painting," *Journal of the American Institute for Conservation* 31, no. 2 (1992): 161-173.

surface particularly prone in cracking and flaking on the paint layer applied over it while not enough sizing could also cause flaking and powdering after rolling and unrolling the Thangka many times.[63]

[63] David Jackson and Janice Jackson, *Tibetan Thangka Painting: Methods and Materials*, 20-21.

CHAPTER 2: Consolidation

As mentioned previously, one of the main conservation issues of thangkas in terms of their paint surface is cracking and flaking. It is caused by extensive use, rolled storage and by factors related with their materials and construction techniques. In order to overcome these issues, consolidation of the paint surface is necessary. As discussed in the Introduction and #### the fact that the binding medium of thangkas paintings is water/protein-based and the absence of varnishes, provides them a matte rather than glossy appearance.

The recent literature available for painted textiles' consolidation is quite limited. Most of it is based on case studies for painted flags, banners and hangings.

Nevertheless, particularly, for thangkas in the literature we can find accumulated information for their conservation from the Proceedings of the Forum on the Conservation of Thangkas published by ICOM-CC 15th (triennial conference that took place in India in 2008).

In the following subchapters issues related with the consolidation process, such as consolidants and penetration behaviour will be reviewed. Moreover, few case studies for the consolidation of matte paintings will be presented.

2.1. Consolidants for painted textiles

According to Pollak, painted textiles are usually treated using consolidants and consolidation techniques for matte paintings.[64]

Matte paintings are generally characterised by a higher pigment volume concentration (PVC) and/or air gaps around the pigment particles. This means that they have a low percentage of bind media within the pigment. This leads to higher porosity, poor cohesion with the paint and also poor adhesion with the ground layer. All these reasons make matte paint particularly prone in

[64] Nancy Pollack and Jan Vuori, "Moving Pictures: Adapting Painting Conservation Techniques to the Treatment of Painted Textiles."

becoming flaky and powdery.[65, 66] Research into consolidants for matte paints has a long history and it is expanding, due to the use of matte paints and powdered pigment by contemporary artists.[67]

Specifically for the consolidation of matte paint, acrylic resins such as Paraloid® B72 and B67 in appropriate solvents (that do not dissolve the paint) but also the used of water based consolidants such as Klucel G, carboxyl methyl cellulose, and acrylic dispersions (Primal B60A) have been reported by the British Museum.[68] Aquazol, "Lascaux® Medium for Consolidation" and gelatine are mentioned as good adhesives for a cohesive flaking paint layer but by are not good for consolidation of under-bound (with not enough binding medium) or powdering paint.[69] Also, in early treatments there are case studies of using PVA adhesives such as Vinamul 3252, but nowadays they are not generally used.[70] Moreover, a "trend" during 1960s and 1970 appeared to be consolidation with soluble nylon (reaction of nylon with formaldehyde) for consolidating matte painting but as it had very bad ageing properties it was discarded from consolidation practices.[71]

As the ideal adhesive could not be found, the research continued during 1990s, with the return to a traditional consolidant, isinglass. Characteristically, according to a research project that compared many synthetic consolidants a solution of 2.5% isinglass in deionised water, had the best consolidation

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[65] Thomas Geiger and Françoise Michel, "Studies on the Polysaccharide Junfunori Used to Consolidate Matt Paint," *Studies in Conservation* 50, no. 3 (2005): 193-204.
[66] Eric F. Hansen and Rosa Lowinger, "Investigations into Techniques for the Consolidation of High Pigment Volume Concentration Paint at the Getty Conservation Institute," *WAAC Newsletter 12,* no. 3(Accessed, http://cool.conservation-us.org/waac/wn/wn12/wn12-3/wn12-307.html.
[67] Chantal-Helen Thuer, *Scottish Renaissance Interiors: Facings and Adhesives for Size-Tempera Painted Wood*, vol. 11, Historic Scotland Technical Paper (2011), 16.
[68] Ibid., 24.
[69] Ibid.
[70] Ibid.
[71] Elizabeth C. Welsh, "A Consolidation Treatment for Powdery Matte Paint" (paper presented at the Preprints of papers presented at the eighth annual meeting of the American Institute for Conservation of Historic and Artistic Works, San Francisco, California, 22-25 May 1980, 1980): 141-150.

properties compared to 5% Paraloid B72 in p-xylene, 5% Plextol 500 in isopropanol, 4% Klucel EF in ethanol and 4% Klucel L in ethanol.[72]

Funori is a traditional Japanese adhesive, which is used for centuries in Japan, but has only recently found its way into conservation outside, especially for matte paint. It is occasionally used by Swedish conservators for size-tempera at the University of Northumbria.[73] The purified version of funori, junfunori® by Lascaux, developed a few years ago, is currently used by Swiss and German conservators predominantly.[74] Finally, worth mentioning, the publications on funori by Swider and Smith and Geiger, both in 2005.[75, 76]

Another, not widely known consolidant that has been in use for a while for consolidating matte paint is Aquazol, available with three different chain lengths. Arslanoglu and Bosetti have provided concise information on its properties.[77,78] Aquazol, as reported by Thuer (2011), is used as consolidant in Estonia and at Berne University of the Arts.[79] Moreover, worth mentioning, a case study on Aquazol for the treatment of Vietnamese matte paintings.[80] Aquazol was also included in a Texile Conservation dissertation project conducted by Blair in 2013 together with isinglass and funori.[81]

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[72] Chantal-Helen Thuer, *Scottish Renaissance Interiors: Facings and Adhesives for Size-Tempera Painted Wood*, 11, 24.
[73] Ibid., 25.
[74] Ibid., 25-26.
[75] Joseph R. Swider and Martha Smith, "Funori: Overview of a 300-Year-Old Consolidant," *Journal of the American Institute for Conservation* 44, no. 2 (2005): 117-126.
[76] Thomas Geiger and Françoise Michel, "Studies on the Polysaccharide Junfunori Used to Consolidate Matt Paint."
[77] Julie Arslanoglu, "Aquazol as Used in Conservation Practice," *Western Association for Art Conservation (WAAC)* 26, no. 1 (2004).
[78] Elisabetta Bosetti, "A Comparative Study of the Use of Aquazol in Paintings Conservation," *e-Conservation magazine*2012, 72-87.
[79] Chantal-Helen Thuer, *Scottish Renaissance Interiors: Facings and Adhesives for Size-Tempera Painted Wood*, 11, 26.
[80] Bettina Ebert, Brian Singer, and Nicky Grimaldi, "Aquazol as a Consolidant for Matte Paint on Vietnamese Paintings," *Journal of the Institute of Conservation* 35, no. 1 (2012): 62-76.
[81] Kate Blair, "The Consolidation of Mud-Silk and Painted Three-Dimensional Textiles" (MPhil Thesis, Centre for Textile Conservation and Technical Art Histoty, University of Glasgow, 2013).

2.2. Consolidant penetration

The consolidant penetration, as reported by Berger et al., can affect both the appearance and flexibility of objects. When the adhesive penetrates a fabric, it fills air gaps within the fibres and yarns and decreases its bending properties.[82] This becomes even more crucial in the case of painted textiles that unlike canvas paintings, are required to flex. Moreover, according to Soppa et al. the adhesives used as consolidants should also be placed exactly at the desired position. More specifically, for flaking paint this position is the space between two neighbouring separated layers. Those layers are usually the ground fabric and the preparatory layer, but separation may also occur between the preparatory and the paint layer. Hence, the consolidant should penetrate the paint and settle between the loose surface.[83]

According to the aforementioned, it becomes clear that the penetration of the consolidant is quite an important factor. Based on Hansen and Lowinger, effective consolidation of matte paint (high pigment volume concentration paints), that causes minimal change of appearance, is found to be linked with application procedures (e.g. wetting) and properties of the consolidation solutions such as the viscosity.[84] Wetting in particular, is important in controlling the flow of the consolidant. When a paint surface is formed with waxes or oils, it will not be penetrated effectively by water-based consolidants.

Nevertheless, the ability of a consolidant to penetrate a surface may be enhanced by pre-wetting the paint surface or by preparing the consolidants with surfactants added in them.[85] By decreasing the viscosity, a consolidant will flow and penetrate more through a paint layer with higher porosity.

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[82] Gustav A. Berger and Harold I. Zeliger, "Effects of Consolidation Measures on Fibrous Materials," *Journal of the American Institute for Conservation* 14, no. 1 (1973): 43-65.
[83] Karolina Soppa, Tilly Laaser, and Christoph Krekel, "Visualizing the Penetration of Adhesives Using Fluorescent Labelling" (2011): 1-18.
[84] Eric F. Hansen and Rosa Lowinger, "Investigations into Techniques for the Consolidation of High Pigment Volume Concentration Paint at the Getty Conservation Institute".(Accessed:
[85] Ibid.

Consolidants that are more viscous can be more efficient in “following” the morphology of a rough surface that results in a matte appearance of the paint.[86, 87]

One way of manipulating the viscosity of a solution is through solvent’s volatility. Choosing to dissolve the adhesives in a low volatility solvent helps in having consolidants of low viscosity for a longer period, as they dry out slower.[88] Another way of controlling viscosity is by working in a saturated atmosphere of a solvent that can dissolve the consolidant.[89]

Alternatively, based on recent publications and recommendations for the use of consolidants, which will be discussed below, viscosity can be manipulated by combining two consolidants, a viscous and a less viscous one. Paper conservators are more familiar with the mixing concept as they often combine adhesives together to get a blend that tweaks desired working properties. For instance, methyl cellulose and wheat starch paste are mixed for an adhesive with “more slip.” Also in book conservation, wheat starch paste or methyl cellulose adhesives are mixed alone, or in combination, with Jade 403 PVA to acquire longer working times, while maintaining the flexible character of the PVA film.[90]

Lascaux Colours & Restauro that worked on funori and managed to purify it (junfunori) provided recommendations for the modification of funori solution to make it less viscous. Specifically, they indicated that even though the adhesive strength of JunFunori is sufficient to consolidate powdering paint layers, sturgeon glue (isinglass) could be added to improve the penetration of

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[86] Ibid.
[87] Robert L. Feller, Nathan Stolow, and Elizabeth H. Jones, *On Picture Varnishes and Their Solvents*(The Press of Case Western Reserve University, 1971).
[88] Kate Blair, "The Consolidation of Mud-Silk and Painted Three-Dimensional Textiles," 26.
[89] Eric F. Hansen, Rosa Lowinger, and Eileen Sadoff, "Consolidation of Porous Paint in a Vapor-Saturated Atmosphere a Technique for Minimizing Changes in the Appearance of Powdering, Matte Paint," *Journal of the American Institute for Conservation* 32, no. 1 (1993): 1-14.
[90] Crystal Maitland, "Microscopy for Paper Conservation: Comparing Various Adhesives and Examining Wheat Starch Paste Preparation Methods" (paper presented at the Poster presented at AIC’s 38th Annual Meeting, 2010): 129-138.

funori while the latter acts as a thickener, preventing the sturgeon glue from being absorbed into the substrates. The isinglass added can help by improving the adhesive strength for the consolidation and reattachment flaking paint.[91]

All these manipulations that both adhesives and application methods might undergo, aim to force the adhesive to be placed between the delaminated surfaces that need stabilisation. Nevertheless, the evaluation and conclusion on weather an adhesive combination or a specific application method is successful would be far more precise if we could visualise adhesives and their penetration. As mentioned in the Introduction, considerable effort is paid form scientist and conservators towards this direction. The most promising of evaluation method seems to be fluorescent labelling of adhesives and their detection though fluorescent light microscopy.

The basic principles and consideration regarding these techniques, in order to employ them for studying adhesives in the conservation field, will be reviewed in the following chapters.

[91] Lascaux Colours & Restauro, "Lascaux Junfunori," Lascaux Colours & Restauro, http://talasonline.com/photos/msds/junfunori.pdf.

CHAPTER 3: Fluorescent Labelling And Fluorescence Microscopy

Certain materials have the ability to emit energy, which can be detected as visible light when irradiated with light of specific wavelengths.[92] More specifically, when a compound absorbs energy (in this case in the form of light), electrons are raised from the ground state (of a lower energy) to an excited state (higher energy). When electrons return to their initial state, they emit light as result of this transition. This phenomenon of re-radiation of light by organic and inorganic specimens is termed fluorescence or phosphorescence and is depicted in Figure #. [93]

Figure 3: Fluorescence

Fluorescence was first described in 1852 by British scientist Sir George G. Stokes when he examined mineral fluorspar under the ultraviolet light. Further investigations on the fluorescence phenomenon were made during the 19th century, when scientist with the use of microscopes with ultraviolet light

[92] Nobel Media, "The Fluorescence Microscope," http://www.nobelprize.org/educational/physics/microscopes/fluorescence/.
[93] Michael G. Ormerod and David Novo, "Fluorescence & Fluorochromes," in *Flow Cytometry: A Basic Introduction*(MG Ormerod, 2008).

reported relevant fluorescence of other materials such as minerals, crystals, resins, crude drugs, butter, chlorophyll, vitamins, and inorganic compounds.[94] Nevertheless, the invention of fluorescing chemical compounds in 1930's, which act as fluorescing dyes named fluorochromes (also found as fluorophores or fluorescent probes), was the stepping stone for the development of a fluorescent light microscopy.[95]

3.1. Fluorescent labelling

3.1.1. Introduction to Technique

Fluorescent labelling is a practical application of the fluorescence phenomenon in order to prepare samples for fluorescent microscopy. It is one of the most common methodologies used for bioanalytical purposes.[96] Labelling can be carried out with radioactive compounds but also with non-radioactive that absorbs light and emits fluorescence in wavelengths of electromagnetic spectrum from the ultraviolet to the near infrared.[97] In the conservation field, fluorochromes were initially used as stains for material identification purposes. Staining technique was detailed reviewed in an early publication by Wolbers and Landrey in 1987 for the characterization of binding media in cross sections.[98] Even though the staining technique used in this study is not recommended nowadays, this paper provides useful information for fluorochromes.

Fluorescent labelling, in bioanalysis, as Ream quotes "typically involves the injection of the chemically attaching a fluorescent dye to a protein, and subsequently observation of tissues from the animal by fluorescence

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[94] Kenneth R. Spring and Michael W. Davidson, "Introduction to Fluorescence Microscopy," https://www.microscopyu.com/articles/fluorescence/fluorescenceintro.html.
[95] Ibid.
[96] M. Sameiro T. Gonçalves, "Fluorescent Labeling of Biomolecules with Organic Probes," *Chemical reviews* 109, no. 1 (2008): 190-212.
[97] Ibid.
[98] Richard Wolbers and Gregory Landrey, "The Use of Direct Reactive Fluorescent Dyes for the Characterization of Binding Media in Cross Sectional Examinations" (1987): 168-202.

microscopy." The process of reacting a protein with the dye is described as conjugation reaction, and the combined molecule after the labelling is called conjugate.[99] Generally, organic fluorochromes may form covalent or noncovalent linkages with the sample to be analysed. The conjugates formed, can emit fluorescence from short to very long wavelengths, depending on the fluorescent dye used. [100] The colours of the exciting and emitting light of each fluorochrome are different. They can be separated from one another by the special developed optical filters that fluorescent microscopes have.[101] In the case of applications of fluorescent labelling for labelling adhesives, the conjugation reaction should be precede and the labelled adhesive should be then applied on the surrogate to be studied.

The aforementioned process is not appearing to cause any deterioration of the labelled compound, as an early research indicates. Specifically, apart from the change in absorption and fluorescence emission, all the physical properties of the conjugate (molecular size and viscosity) did not differ appreciably from the starting one.[102]

3.2. Fluorescent Dyes (Fluorochromes)

Nowadays, it is very common to use for fluorescent labelling a wide array of added synthetic and naturally occurring fluorochromes which are excited by specific wavelengths of irradiating light and emit light of defined and useful intensity.[103]

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[99] Julie Dennin Ream, "Observations on the Penetration of Two Consolidants Applied to Insecure Gouache on Paper."
[100] M. Sameiro T. Gonçalves, "Fluorescent Labeling of Biomolecules with Organic Probes."
[101] Michael G. Ormerod and David Novo, "Fluorescence & Fluorochromes."
[102] Alfred A. Schiller, Richard W. Schayer, and E. L. Hess, "Fluorescein-Conjugated Bovine Albumin Physical and Biological Properties," *The Journal of general physiology* 36, no. 4 (1953): 489-506.
[103] Kenneth R. Spring and Michael W. Davidson, "Introduction to Fluorescence Microscopy".

Fluorochromes used for fluorescent labelling are basically stains that have the ability to be attached to “visible or sub-visible structures”. Most of them are highly selective in terms of what they are targeting for reaction, and have a significant quantum yield (ratio of photon absorption to emission).[104] These specifically developed fluorochromes have documented intensity profiles of excitation and emission and are compatible with a range of different filter sets for fluorescent microscopes.

The properties of fluorochromes are strongly linked and depend on its environment. For example, propidium iodide (PI), used for labelling DNA, has a weakly fluorescence in aqueous solutions but when intercalating with DNA in hydrophobic environment, its fluorescence becomes highly increased. Moreover, fluorochromes, of the fluorescein group, are pH-sensitive.[105]

The most common fluorochrome used to label proteins is fluorescein isothiocyanate. During the labelling process, the isothiocyanate group reacts with the amino groups on the lysine residues in the protein.[106]

Dichlorotriazinyl aminofluorescein (DTAF) is another group of fluorescein-bearing ligand that binds covalently to amino groups of protein under regular conditions. DTAF has been widely used for labelling tubulin and localise the site of tubulin subunit dissociation, labelling blood cells, labelling stromal collagen and in immunologic studies.[107] DTAF is prone to hydrolytic degradation and the rate of its reaction is increasing with higher pH. According to Wee et al. It is dissolved in sodium bicarbonate buffer for topical use in order to stain stromal collagen.[108]

As for labelling (poly)saccharides the most commonly used fluorochromes are namely 5-aminofluorescein, O-2-[aminoethyl]fluorescein, rhodamine 110, 8-aminonaphthalene-1,3,6-trisulfonate, 3-amino-9-ethylcarbazole. Even though

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[104] Ibid.
[105] Michael G. Ormerod and David Novo, "Fluorescence & Fluorochromes."
[106] Ibid.
[107] Won Ryang Wee et al., "The Effect of Dichlorotriazinyl Aminofluorescein on Human Keratocytes in Vitro," *Korean J. Ophthalmol* 10(1996): 63-67.
[108] Ibid.

these fluorochromes are widely used for high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE) analysis, they have quite low quantum yields, a fact that makes them unsuitable for fluorescence microscopy.[109] Due to this reason there is a great interest in developing fluorescent labelling protocols for (poly)saccharides with fluorochromes used for labelling proteins such as fluorescein isothiocyanate (FITC), fluoresceinamine and lissamine rhodamine B sulfonyl chloride (LRSC).[110] Apart from the aforementioned, based on recommendations provided by manufactures of fluorochromes 5-DTAF [5-(4,6-Dichlorotriazinyl)aminofluorescein], that was developed as a fluorochrome for labelling proteins, directly reacts with hydroxy groups such as polysaccharides and other alcohols in aqueous solution at pH above 9.[111]

3.3. Fluorescent Microscopy

3.3.1. Introduction to Technique

Fluorescence microscopy is an advanced microscopic method, because it has properties that are not instantly available (without computer processing) in regular contrast modes of optical microscopy (Brightfield / Darkfield microscopy, Phase Contrast, Polarization Contrast, Differential Interference Contrast).[112] For this reason, it became a very important tool not only for the biological and biomedical fields but also for material science.[113]

Samples, in order to be observed with fluorescence microscopy, can either be labelled (terms stained or tagged also used) with fluorochromes, or be observed through their natural fluorescing, such as chlorophyll or other minerals.[114] From the previous two methods, labelling is the main

[109] Ying Zhang et al., "One-Pot Fluorescent Labeling of Saccharides with Fluorescein-5-Thiosemicarbazide for Imaging Polysaccharides Transported in Living Cells," *Carbohydrate research* 346, no. 14 (2011): 2156-2164.
[110] Ibid.
[111] Inc AnaSpec, "5 - Dtaf [5 - (4,6 - Dichlorotriazinyl)Aminofluorescein]," (Accessed: 10 July 2015), http://www.anaspec.com/products/product.asp?id=28815.
[112] Kenneth R. Spring and Michael W. Davidson, "Introduction to Fluorescence Microscopy".
[113] Ibid.
[114] Nobel Media, "The Fluorescence Microscope".

methodology used for the examination of samples under the fluorescence microscope.[115]

3.3.2. The Fluorescent Microscope

The basic function of a fluorescence microscope is to collect only the desired fluorescence emission from the sample, while minimizing the background illumination (unwanted excitation light and autofluorescence). The intensity of fluorescence from the sample can be much weaker than the intensity of the exciting light, a fact that makes the aforementioned process very complicated.[116] In order to overcome this issue, an advanced technology in terms of the microscope's optical system is used.

There are two basic types of illumination for fluorescence microscopy, transmitted light fluorescence microscopy and incident light fluorescence microscopy also described as reflected light fluorescence microscopy or epi-fluorescence.[117, 118]

The optical system of reflected light fluorescence microscopy, which became quite popular after its invention in 1967 by Ploem, will be further described since it was the one used for the purpose of this research. [119,120]

The advantage of reflected light fluorescence microscopy is that "the same lens system acts as objective and condenser." This is possible due to the use of a special type of mirror known as chromatic beam-splitter (CBS) or dichroic (dichromatic) mirror which is mounted in filter cube-like units together with an

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[115] Kenneth R. Spring and Michael W. Davidson, "Introduction to Fluorescence Microscopy".
[116] Johan S. Ploem, "Fluorescence Microscopy," in *Fluorescent and Luminescent Probes for Biological Activity: A Practical Guide to Technology for Quantitative Real-Time Analysis*, ed. William T. Mason(Academic Press, 1999), 4-13.
[117] M. R. Young, "Principles and Technique of Fluorescence Microscopy," *Quarterly Journal of Microscopical Science* 3, no. 60 (1961): 419-449.
[118] Kenneth R. Spring and Michael W. Davidson, "Introduction to Fluorescence Microscopy".
[119] P. T. Tran and Fred Chang, "Transmitted Light Fluorescence Microscopy Revisited," *The Biological Bulletin* 201, no. 2 (2001): 235-236.
[120] Kenneth R. Spring and Michael W. Davidson, "Introduction to Fluorescence Microscopy".

excitation and emission (barrier) filter sets. The former lets only the wavelength that can excite the specific fluorochrome of the sample while the later (emission) lets only the main emission wavelength of the fluorescence to be detected from the eyepieces. The dichroic mirror, which has a special coating on each side and is positioned diagonally between the aforementioned filters, has a double function. It directs only the shorter wavelengths of the exciting light onto the specimen and transmits only the longer wavelengths of the emitted fluorescence towards emitted filter and the eyepieces.[121, 122]

There are individually designed units with the structure described above for UV, violet, blue, green and red excitation and are provided by several microscope manufacturers. Since for this research a filter unit that provides green excitation have been used, a more detail customised diagram for the light path of green fluorescence is shown in Figure 4.

[121] Johan S. Ploem, "Fluorescence Microscopy," 4-13.
[122] Karolina Soppa, Tilly Laaser, and Christoph Krekel, "Visualizing the Penetration of Adhesives Using Fluorescent Labelling" (2011): 1-18.

Figure 4: Fluorescence light microscope's setup when green fluorescence is observed

Exciting light which does not correspond well with the excitation peak of the fluorochrome will often cause unnecessary autofluorescence of the sample and provide excess of unwanted excitation light from the microscope's optical system. This will result in an image with a much decreased contrast. Nevertheless, in order to observe the sample easier, compromise between the "intensity of the fluorescence and the level of background illumination" is usually necessary as Johan S. Ploem highlights.[123]

The choice of light source is determined by the excitation spectrum of the fluorochrome, its quantum efficiency, the number of fluorochromes in the sample and the sensitivity of the detector used (human eye, film, TV /CCD camera). Halogen, mercury and xenon high-pressure arc lamps, and various laser light sources are available. Mercury lamps are available at 50, 100 and 200 W.

[123] Johan S. Ploem, "Fluorescence Microscopy," 4-13.

CHAPTER 4: Cross Sections

Cross-sections constitute an established method for paint study and research. Key early publications for cross-sections were published in 1936 by Gettens and in 1956 by Plesters.[124, 125] The latter article provides valuable information on the methodology and their application. Even though the materials now available are improved, the basic principles of this technique remain the same. Cross-sections have found a wide range of applications in the documentation and examination of a number of materials, including paintings, stone, ceramic, wood, plaster and textiles.[126]

4.1. Methods and Materials

The methodology for preparing cross sections involves embedding a sample in a block of clear, transparent resin set in moulds usually made of rubber. The block is then cut up to the edge of the sample to be examined and then highly polished for viewing under the microscope. Detailed guidelines for preparing cross-sections can be found in technical manuals and relevant publications.[127, 128] Moreover, past dissertations by the Textile Conservation Centre provide accumulated information and guidelines for preparing cross-section, worth mentioning Rogerson's dissertation project, published in 1999.[129, 130]

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[124] Rutherford J. Gettens, "The Cross-Sectioning of Paint Films," *Technical studies in the field of the fine arts* 5(1936): 18-22.
[125] Joyce Plesters, "Cross-Sections and Chemical Analysis of Paint Samples."
[126] Cordelia Rogerson, "Evaluating the Application of Cross-Sectional Analysis to the Documentation and Examination of Textiles. " (Textile Conservation Centre, Courtauld Institute of Art 1997), 27-29.
[127] Walter C. McCrone, "The Microscopical Identification of Artists' Pigments," *Journal of the International Institute for Conservation-Canadian Group* 7, no. 1-2 (1982): 11-34.
[128] Jia-Sun Tsang and Roland H. Cunningham, "Some Improvements in the Study of Cross Sections," *Journal of the American Institute for conservation* 30, no. 2 (1991): 163-177.
[129] Cordelia Rogerson, "Evaluating the Application of Cross-Sectional Analysis to the Documentation and Examination of Textiles. ."
[130] Cordelia Rogerson and Dinah Eastop, "The Application of Cross-Sections in the Analysis of Historic Textiles," *The Conservator* 23, no. 1 (1999): 49-56.

There are three basic categories of embedding resins used, polyester, epoxy and acrylic resins. The former were widely used in the past for embedding paint cross-sections while epoxies and acrylics were more common in use from forensic scientists.[131] More information and discussion on the use of polyester resins can be found in an article published by Derrick *et al.* in 1994.[132] The literature available indicates that polyester resins were mostly used in earlier publications. In more recent ones, the use of acrylic resins is more common. Nowadays, Technovit™ acrylic resins appear to be widely used in scientific publications from the conservation and also biomedical and metallographic field, worth mentioning Technovit™7100, 7200 LVC and Technovit™ 2200 LC.[133, 134,135] Technovit™ acrylic resins have quite different properties that makes the suitable for different materials and applications, such as light or temperature sensitive materials and microtoming. Information for each type of resin can be found online by manuals published by their manufacturer and suppliers.[136]

[131] Michele Derrick et al., "Embedding Paint Cross-Section Samples in Polyester Resins: Problems and Solutions," *Journal of the American Institute for Conservation* 33, no. 3 (1994): 227-245.
[132] Ibid.
[133] W. L. H. Van Veenendaal and R. W. Den Outer, "Distribution and Development of the Non-Articulated Branched Laticifers of Morus Nigra L.(Moraceae)," *Acta botanica neerlandica* 39, no. 3 (1990): 285-296.
[134] Annie Major et al., "Implant Degradation and Poor Healing after Endovascular Repair of Abdominal Aortic Aneurysms: An Analysis of Explanted Stent-Grafts," *Journal of Endovascular Therapy* 13, no. 4 (2006): 457-467.
[135] Marta Monjo et al., "In Vivo Performance of Absorbable Collagen Sponges with Rosuvastatin in Critical-Size Cortical Bone Defects," *Acta biomaterialia* 6, no. 4 (2010): 1405-1412.
[136] Heraeus Kulzer, "Technovit ®," no. (Accessed: 08 August 2015), http://www.equilab.es/pdf/Prospekt_Technovit_englisch.pdf.

CHAPTER 5: Experimental Rationale

In order to design the experiment and decide on the methods of evaluation it was of key importance to define the experimental rationale for each task of this project. More practical questions such as methods of sample preparation and materials chosen for the fluorescent labelling as well as methods for testing the stability of the labelled adhesives, are presented, answered and explained in the following subchapters.

5.1. Preparation of Thangka Samples

For the preparation of the Thangka paintings samples it was decided to choose three different processes based on recommendations from relevant publications. The issue that the modified samples aim to overcome is the autofluorescence that the animal glue (used for sizing the fabric and as a binding for the preparatory and paint layer) according to Soppa et al. the sample will have.[137] The question to be answered from the examination of all three types of samples would be: “which sample provides the best result based on its autofluorescence for studying the penetration of adhesives under the fluorescent microscope?”

The three different samples will be reconstructed and be examined

1. A Thangka sample without any alteration based on the traditional method described in Chapter 1,
2. A Thangka sample slightly modified by adding in the white pigments mixture for the preparatory layer iron red.
3. A highly modified sample where apart from adding iron red, starch paint have been used for sizing the fabric instead of animal glue (rabbit skin glue).

[137] Karolina Soppa, Tilly Laaser, and Christoph Krekel, "Visualizing the Penetration of Adhesives Using Fluorescent Labelling" (2011): 1-18.

Both the addition of iron red and the replacement of starch paste was recommended (together with the replacement of the animal glue as a binding media with an epoxy resin) in order to create painting samples with minimum autofluorescence. As this research aims in creating Thangka samples, that are closer to the original materials and its properties, the recommendation for using epoxy as a binding media has not been taken into account. The quantity of the iron red in this experiment is higher than the recommended by Soppa, since 1:60 of iron red in the pigmentation, as mentioned, is very low to cause any significant change on the pigment colour. Hence, this could be a typographical error (1 instead of 10).

As a ground fabric, unbleached cotton with weave-count 11x12 before stretching and 12x13/cm^2 after stretching was chosen. The use of bleached cotton muslin that was treated with optical brighteners and provided strong autofluorescence under a UV lamp has been avoided.

For sizing the fabric and for binding the pigments, rabbit skin animal glue has been used with concentration 5% and starch paste 13%.[138] For the preparatory layer a mix of chalk and kaolin china clay and occasionally iron red has been chosen. For the paint layer, the same pigments mix with more iron red and binding medium was chosen for minimizing the complexity of the preparation.

5.2. Adhesives

The primary consideration when deciding on the adhesives to be used for labelling during this project was to use one form animal origin (protein based) and one of plant origin (polysaccharide based). Both these two groups of natural origin adhesives are widely used for consolidating matte paintings and painted textiles. The secondary consideration was to choose adhesives that were included in past dissertation projects, in order to build on previous research and use resources that already existed.

[138] Ibid.

Hence, isinglass as a protein based and funori as a polysaccharide-based adhesive were chosen. Both were included in a dissertation project conducted in 2013 by Kate Blair, and for isinglass there was the additional contribution by Sarah's Foskett dissertation project conducted in 1994.[139, 140]

In terms of the concentrations, based on a publication for the consolidation of matte paintings, it was decided to prepare funori in 3% concentration. This quantity is the maximum recommended for funori according to Thuer (2011), as he indicates that higher percentage than 3% is not producing a stronger adhesive, "because of a limited amount of adhesive agent that can be dissolved from the algae substrate at any time."[141] In order to minimise the complexity of the experimental methodology, the same concentration (3%) was decided to be used for isinglass as well. This was further attested by the literature as a sufficient concentration for the consolidation of paint.[142]

5.3. Fluorochromes and Conjugation Reactions

The primary action before choosing a fluorochrome, is to see which type of filter sets the fluorescence microscope, to be used, has. It the case of this research at the Centre for Textile Conservation at the University of Glasgow, the microscope used was a Zeiss Axioscope 2 with filter sets 00, 02 and 09 (Zeiss no). Information on the emission and excitation wavelengths of each filter and lists with the fluorochromes that can be used with each filter can be found in Appendix 1.From all the dyes available it was decided to use 5-(4,6-dichlorotriazinyl)aminofluorescein (5-DTAF) for the following reasons:

- It is a fluorochrome that can be used for labelling both proteins and polusaccharides.

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[139] Kate Blair, "The Consolidation of Mud-Silk and Painted Three-Dimensional Textiles."
[140] Sarah Foskett, "An Investigation into the Properties of Isinglass in Conservation" (Textile Conservation Centre, Courtauld Institute of Art 1994).
[141] Chantal-Helen Thuer, *Scottish Renaissance Interiors: Facings and Adhesives for Size-Tempera Painted Wood*, 11.
[142] Ibid.

- It was used in the conservation field for labelling gelatin and Methyl Cellulose.
- It is not expensive, considering that the minimum quantity that can be bought (100mg) is sufficient for labelling more adhesives than needed for this project.

As for the labelling methodology the guidelines by Soppa et al. were used, slightly modified to fit with the preparation process of isinglass and funori.[143] The conjugation reactions of both isinglass and funori with 5-DTAF are depicted in Table 1.

5.4. Buffers for the labelling process

From academic publications, guidebooks with protocols for labelling and technical information sheets by suppliers of fluorochromes, it became clear that for raising the pH above 9 for the labelling process of isinglass and funori it was necessary to use sodium bicarbonate buffer.[144, 145, 146]

The pH of sodium bicarbonate buffer of 0.1M was 8.3 and thus a buffer solution of 0.1M sodium carbonate with a pH 11.3 was used to raise its pH. Sodium carbonate was used, it is also recommended for labelling.[147]

5.5. Purification after labelling

In all the reviewed publications on fluorescent labelling of adhesives after the conjugation reaction it was highlighted that the adhesives should undergo purification. This process aimed in removing any free fluorochromes, the buffers and the solvent used for dissolving the fluorochrome that comes in a powdery form.

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[143] Karolina Soppa, Tilly Laaser, and Christoph Krekel, "Visualizing the Penetration of Adhesives Using Fluorescent Labelling" (2011): 1-18.
[144] Ibid.
[145] Andrew J. Garman, *Non-Radioactive Labelling: A Practical Introduction*(Academic Press, 1997), 57-58.,
[146] Inc AnaSpec, "Reactive Fluorescent Dyes," (Accessed: 12 July 2015), http://www.anaspec.com/content/pdfs/c_literature167.pdf.
[147] Ibid.

Table 1: Conjugation reactions of isinglass and funori with 5-DTAF

Isinglass			
	Reactive group of isinglass	**5-DTAF**	**Conjugate**
Funori			
	Reactive group of funori	**5-DTAF**	**Conjugate**

Soppa et al. and Ream used for the purification of the labelled adhesives cellulose dialysis tubing (also termed visking tubing).[148,149] Cellulose dialysis tubing is a semipermeable membrane, made of regenerated cellulose that

[148] Karolina Soppa, Tilly Laaser, and Christoph Krekel, "Visualizing the Penetration of Adhesives Using Fluorescent Labelling" (2011): 1-18.
[149] Julie Dennin Ream, "Observations on the Penetration of Two Consolidants Applied to Insecure Gouache on Paper."

enables the removal and transition of molecules between the solutions in the membrane and outside of it due to osmosis and differential diffusion.[150]

Hummert et al. in 2013 on the other hand, used two different methods for purification; ultrafiltration with a polyethersulfone membrane disc filter for removing the buffers from the labelled adhesives (desalting) and they were purified by unbounded fluorochromes by performing aqueous size-exclusion chromatography (SEC).[151]

During this research it was decided to use cellulose dialysis tubing as a purification method because it needs far less sophisticated equipment and can be easily performed with the facilities and equipment available.

Dialysis tubing comes in several sizes in terms of the diameter of the tube, the thickness of the membrane and the most important, the size of the pores that the membrane has and through which the molecules pass. The latter is described by the by the acronym MWCO (molecular weight cut-off) which indicates the minimum molecular weight of the molecules (in Daltons) that can the membrane can retain.[152] The regular MWCO is about 15,000 Da (or 15 kDa) for retaining proteins and can be used with confidence for retaining molecules with molecular weight larger than 20,000 Da. For smaller molecules there are also membranes available with smaller MCWO.[153]

Both isinglass and funori, that had to be retained in the tubing, have molecular weight larger than 20,000 Da. More specifically isinglass which is a type II collagen structure is a high molecular weight protein that weight over 100,000 Da (100 kDa).[154, 155] On the other hand funori which is a type of agar extracted

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[150] Lyman C. Craig, "Differential Dialysis," *Advances in analytical chemistry and instrumentation* 4(1965): 35-74.
[151] Eva Hummert, Ute Henniges, and Antje Potthast, "Fluorescence Labeling of Gelatin and Methylcellulose: Monitoring Their Penetration Behavior into Paper."
[152] Zbigniew Darzynkiewicz, J. Paul Robinson, and Mario Roederer, *Essential Cytometry Methods*(Academic Press, 2009), 89.
[153] Ibid.
[154] Sarah Foskett, "An Investigation into the Properties of Isinglass in Conservation."
[155] D. Hickman et al., "Isinglass/Collagen: Denaturation and Functionality," *Journal of biotechnology* 79, no. 3 (2000): 245-257.

by seaweed has an average molecular of 30,000 to 150,000 Da.[156] Generally, every smaller size of membrane could work as happed in the study of Soppa et al. where a membrane with MWCO 3,500 Da was used.[157]

For this project, cellulose dialysis tubing with MWCO 12,000-14,000 Da was used, since it had the lowest price.

5.6. Determining the success of the purification method.

In order to determine the success of the dialysis process in removing the free fluorochromes for the adhesives, methods such as thin layer chromatography and UV spectrometry have been used. The former on the labelled adhesives and the latter on the water samples taken form the bakers at successive rinse changes of the water outside the tubing.[158, 159] Hummert et al. confirmed the absence of unreacted fluorochromes by performing organic size-exclusion chromatography (SEC) for methyl cellulose and aqueous SEC for gelatin. Samples of the conjugates were prepared by diluting the conjugate in the mobile phase of chromatography.[160]

For this project it was decided to perform thin layer chromatography (TLC) to determine the success of the purification method. This was based on the fact that it was used successfully by Soppa et al., for both gelatin and Methyl Cellulose.[161]

Thin Layer Chromatography is a separation technique. Chromatographic separations take advantage of the substances' ability to differentiate between

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[156] Katsunori Suzuki and Toshiharu Fukushima, "Comprising an Agar Selected to Have an Average Molecular Weight of 30,000 to 150,000 and Water, Wherein Said Binder Leaves Less Than About 0.4% Carbon Residue in a Sintered Molded Body,"(Google Patents, 2001).
[157] Karolina Soppa, Tilly Laaser, and Christoph Krekel, "Visualizing the Penetration of Adhesives Using Fluorescent Labelling" (2011): 1-18.
[158] Ibid.
[159] Julie Dennin Ream, "Observations on the Penetration of Two Consolidants Applied to Insecure Gouache on Paper."
[160] Eva Hummert, Ute Henniges, and Antje Potthast, "Fluorescence Labeling of Gelatin and Methylcellulose: Monitoring Their Penetration Behavior into Paper."
[161] Karolina Soppa, Tilly Laaser, and Christoph Krekel, "Visualizing the Penetration of Adhesives Using Fluorescent Labelling" (2011): 1-18.

two phases, a mobile and a stationary phase.[162] In thin layer chromatography (TLC), the mobile phase is a liquid and the stationary phase is a solid absorbent.

In TLC, the mixture starts as a small spot (near the bottom of the plate) and the solvent (mobile phase) carries, through capillary action, the compounds higher at the plate. The measure of the distance that a compound moved is called Rf value. [163] It can be determined by measuring the distance that the compound moved from the baseline, divided by the distance the solvent moved from the baseline.

In most cases, the stationary phase (adsorbent) is very polar and the mobile phase (eluent) is fairly non-polar. Molecules that are more polar stick to the polar stationary phase more than fairly non-polar molecules, which are carried along in the mobile phase.[164]

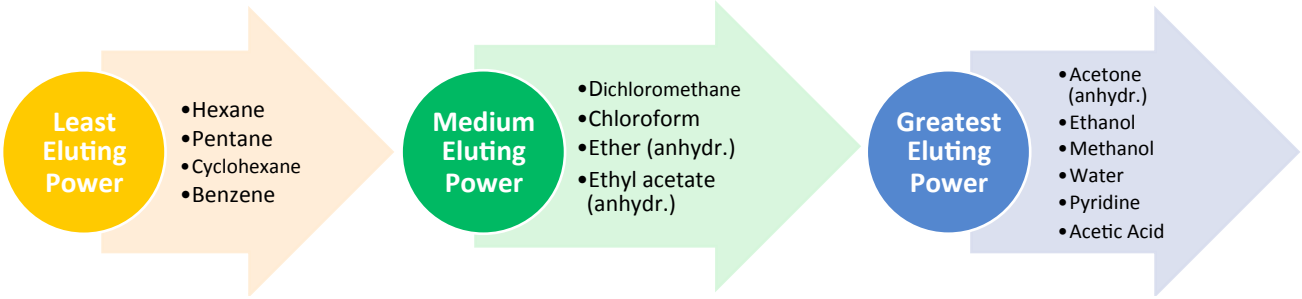


Figure 5: Most common solvents used as mobile phases for TLC.

The most common factor that is adjusted to achieve good separation is the solvents used in the mobile phase. There are many choices for solvents with less or greater eluting power that can be used and solvent mixtures with less or greater eluting power that can be used separately or as mixtures.[165] The

[162] Peter E. Wall and Roger M. Smith, *Thin-Layer Chromatography: A Modern Practical Approach*, vol. 12(Royal Society of Chemistry Cambridge, 2005), 1-2.
[163] Egon Stahl, *Thin-Layer Chromatography. A Laboratory Handbook*, Thin-Layer Chromatography. A Laboratory Handbook. (1967), 42.
[164] Ibid., 29-31.
[165] Peter E. Wall and Roger M. Smith, *Thin-Layer Chromatography: A Modern Practical Approach*, 12, 92-97.

most common solvents used as mobile phases are included in the diagram of Figure 1.

For choosing the solvent system for this project, a series of TLC tests were performed. In order to save time and recourses, the tests were performed in bakers of 250 ml covered with glass petri dishes instead of using the chromatographic chamber.

Since ethanol that was used as a mobile phase by Soppa et al.[166] is not available in the UK, IDA (Industrial Denatured Alcohol) that contains ethanol with 10% of methanol was used. Nevertheless, it was combined with less polar solvents to bring down the polarity, due to the presence of methanol.

From Thin Layer Chromatography it is expected to identify if the free dye molecules that are still present in the labelled adhesive. This can be achieved by comparing the spots form the fluorochrome itself with the adhesives before the dialysis tubing and after.

The basic principles and methodology for performing thin layer chromatography will be included in a background information subchapter, before the relevant experiment.

5.7. Choosing material and method for preparing cross-sections

The basic consideration for choosing the embedding resin was to avoid light curing resin, as the UV light would “destroy” the fluorescence form the labelled adhesives. Since epoxy resin was readily available and does not require light curing, it was decided to use this as a more accessible material.

[166] Ibid.

CHAPTER 6: Experimental Part – Materials and Methods

6.1. Preparation of Thangka Samples

- Sample 1 (Traditional Thangka preparation)

The ground fabric used was a natural (unbleached) cotton fabric. The four edges of the fabric were sewed to form “sleeves” of approximately 1cm on each edge. The sleeves were used for passing through them bamboo sticks, which helped with stretched the fabric. The fabric was then stretched inside a wooden frame/stretcher with the use of cotton strings.

For sizing the fabric (Figure 6), rabbit skin glue was prepared with 5% w/v concentration in deionised water. In order to prepare the glue, the baker was placed in a “bain-marie”. A heating source with magnetic stirrer was used (HB502, BIBBY). The rabbit skin glue was ready after 30 minutes of heating, with continuous stirring, at 65°C. The glue was then applied with a Japanese soft brush on the stretched fabric from both sides and let to dry.

For the preparatory layer, one part of rabbit skin glue and two parts of white pigments were used. As white pigment, a mix of chalk and kaolin clay, which consisted of equal parts of each measured by weight, was used. Then 20 g of the gesso were applied on both sides, on a squared surface of 24 cm of the sized fabric, by using a flat spatula with curved edges. Before each application the sample was set aside to dry. The preparatory layer was polished with “damp-polishing” by using a piece of cotton, slightly damped and a small plastic container with curved edges.

For the paint layer (Figure 7), the same mixture of the white pigments as the preparatory layer was used with the addition of iron red. It was prepared by mixing 3 parts of rabbit skin glue with one part of pigment mixture of chalk, kaolin and iron red (4:4:2 parts by weight). The paint was mixed in a glass mortar (Figure 8). 10 g of the paint were applied to cover the surface of 24cm.

Figure 6: Sizing the Thangka sample

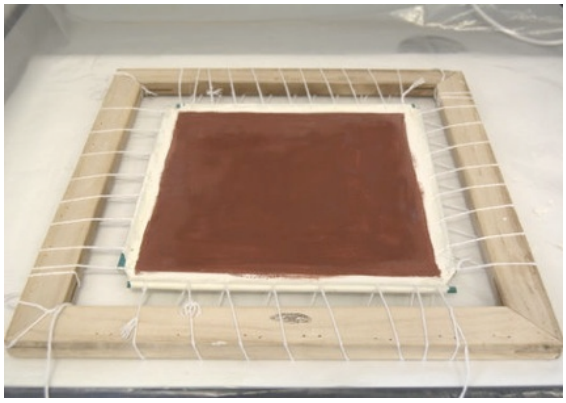


Figure 7: The Thangka sample with a layer of red pigment

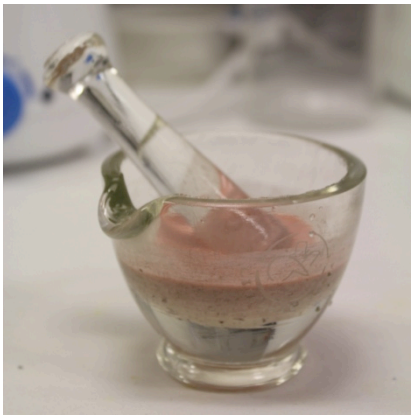


Figure 8: Preparing the pigment for the paint layer by using a glass mortar

- Sample 2 (Traditional preparation with iron red added in the gesso)

The preparation of Sample 2 was the same methodology as Sample 1, apart from the composition of the pigment mixture for the preparatory layer (gesso) that contained iron red (Figure 9 & 10). More specifically, a mixture of chalk, kaolin and iron red was used, 20:20:5 (parts by weight).[167] The overall composition was the same with Sample 1, 1:2 rabbit skin glue (5%)-pigment mixture.

[167] Karolina Soppa, Tilly Laaser, and Christoph Krekel, "Visualizing the Penetration of Adhesives Using Fluorescent Labelling" (2011): 1-18.

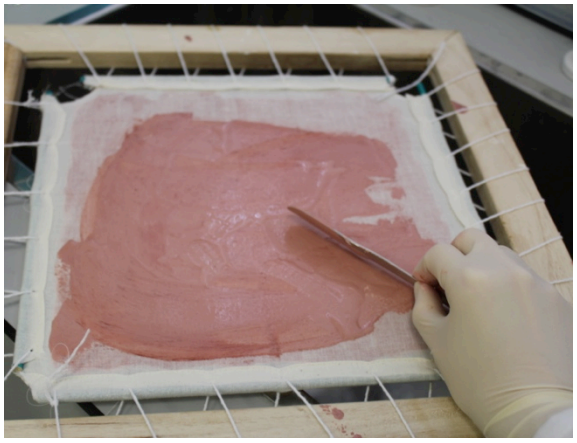


Figure 9: Applying gesso that contains Iron Red in pigmentation.

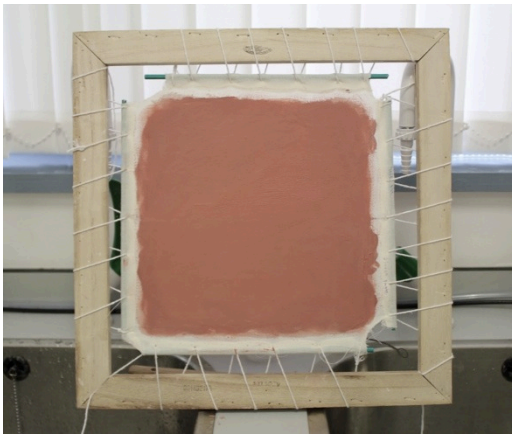


Figure 10: Letting the sample dry after the application of gesso.

- Sample 3 (Ground fabric sized with starch paste and iron red added in the gesso)

In Sample 3, the same modification followed in terms of the percentage of iron red added in the preparatory layer. The difference of this sample from the former is that instead of rabbit skin glue the fabric was sized with 13% starch paste.

Starch paste prepared by mixing 1 part of starch powder with 4 parts of water, measured by volume. It was heated in a pot, and stirred continuously until it turned into a thick translucent gel. It was then sieved though a very fine metal mesh. Starch paste was then mixed with water to form a very thin, almost watery solution.

6.2. Fluorescent Labelling of Adhesives

The preparation for the labelling process started with the preparation of a stock solution of sodium bicarbonate-carbonate buffer system and 5-DTAF.

For the buffer system, 0.1M solution of sodium bicarbonate and 0.1M solution of sodium carbonate were prepared separately. Then the sodium carbonate was added gradually into the sodium bicarbonate in order to raise its pH at

9.3, the desired level for labelling both, isinglass and funori. For measuring the pH during this process a Hanna Instruments portable pH meter was used.

Based on recommendation by the literature and technical information sheets for fluorochromes from suppliers, the stock solution was prepared by dissolving 5-DTAF in DMSO (5 mg ml^{-1}).[168, 169]

- Fluorescent labelling of isinglass with 5-DTAF

Isinglass was first chopped in small pieces and then was additionally finely cut with the use of an electric coffee grinder. Afterwards, the finely chopped isinglass was soaked for 6 hours in 0.1M sodium bicarbonate buffer with pH 9.3 to form an isinglass solution with concentration 3%. In total 1.5g of isinglass were used in 50 ml of buffer solution.

The isinglass reaction carried out for 30 minutes in a bain-marie at a temperature of 60°C. The fluorochrome 5-DTAF, dissolved in DMSO, was then added drop wise during 15 minutes with continuous stirring (Figure 11). A heating source with a magnetic stirrer was used for this reason (HB502, BIBBY). As 5 mg of 5-DTAF required per 1g of adhesive, for labelling 1.5g of isinglass, in total, 1.5ml form the stock solution of 5-DTAF in DMSO was used.

[168] Ibid.
[169] Inc AnaSpec, "Reactive Fluorescent Dyes".(Accessed: 12 July 2015)

Figure 11: Stirring of isinglass during the conjugation reaction

- Fluorescent labelling of funori with 5-DTAF

Finely grinded funori was soaked for 6 hours in 0.1M sodium bicarbonate-carbonate buffer system, with pH 9.3 in order to form a solution of 3%. 1.5g of funori powder were soaked in 50ml of buffer. Based on the preparation method suggested by Swider and Smith the funori reaction carried out at a temperature of 60°C for 2 hours.[170] The fluorochrome 5-DTAF dissolved in DMSO, was then added drop wise during 30 minutes with stirring. Since the funori was too viscous for using the magnetic stirrer, a vibrating stirrer was used instead. For maintaining the temperature at 60°C a bottle warmer with a temperature sensor was used. In total 1.5ml of 5-DTAF in DMSO from the stock solution was added for the conjugation reaction (5mg per 1g of adhesive). The funori was then sieved by using a very fine metal mesh (Figure 12).

[170] Joseph R. Swider and Martha Smith, "Funori: Overview of a 300-Year-Old Consolidant."

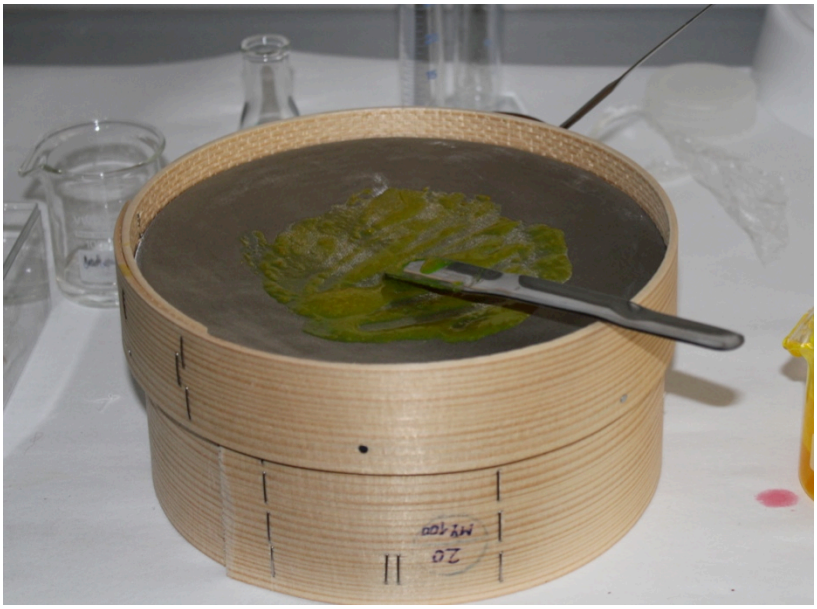


Figure 12: Funori was sieved by using a very fine metal mesh

6.3. Purification with Cellulose Dialysis Tubing

Aqueous dialysis was performed for approximately 30 hours, by using cellulose dialysis tubing, in order to separate the conjugate from free (unreacted) fluorochromes, DMSO and buffer agent.

During this process a cellulose dialysis tubing with MWCO 12,000-14,000 Da, with 14mm diameter used. Since the tubing comes dehydrated in a flat form, it was first soaked in deionized water for 3 minutes in order to become flexible. One of its edges was then twisted and tied with a cotton thread multiple times to avoid any leak form/to inside.

Approximately, 10cm of cellulose dialysis tubing were filled with adhesive. The upper edge was then twisted leaving as less air as possible in the tubing and was tied once with a cotton thread. After securing the bottom edge, the tubing was placed half in a baker with deionised water to avoid being dehydrated. An additional ordinary plastic bag clip was placed at the top edge. The clip was floating and thus prevented the tube form sanking into the water, and

additionally it was securing the edge from leaking. The experimental setup for cellulose dialysis tubing is described in Figure 13.

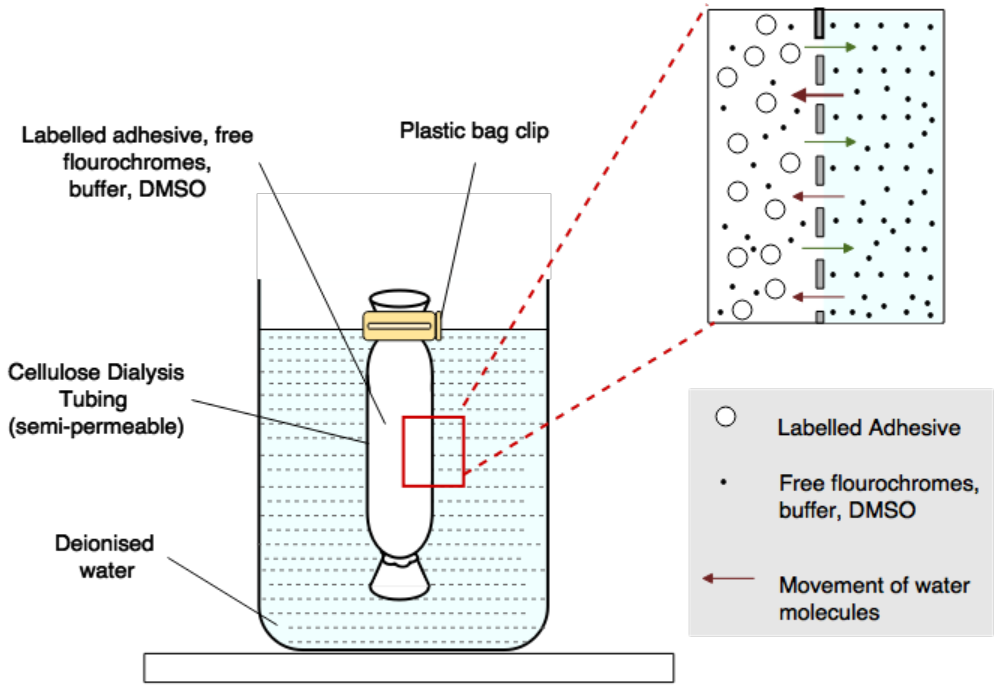


Figure 13: The experimental setup for cellulose dialysis tubing.

The process repeated for preparing two tubes, one for isinglass and one for funori.

Since the isinglass and funori become too viscous in low temperature, a fact that would discourage the mobility of the molecules inside the labelled solution, dialysis took place in controlled temperature around 35°C (temperature of the water outside the tubing).

Based on the literature, after 3-4 hours with stirring -or after 6 if not stirred- the rinse solution reaches equilibrium with the solution inside the tubing.[171] Therefore, when the pH of the rinse solution remained stable, it was changed with fresh deionised water, slightly warmed (around 35°C). Three changes of the rinse solution performed until the pH of the water was slightly below 7 (regular price for deionised water) and appeared crystal clear, without the yellowish shade form the fluorochrome.

[171] Zbigniew Darzynkiewicz, J. Paul Robinson, and Mario Roederer, *Essential Cytometry Methods*, 89.

Figure 14: First rinse water from cellulose dialysis tubing. The more yellow (left) is funori.

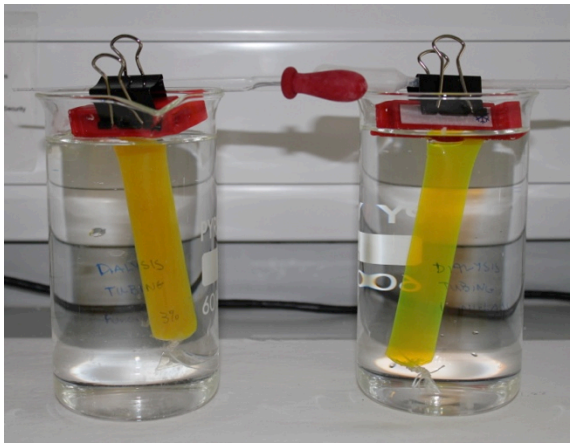


Figure 15: last rinse water from cellulose dialysis tubing. Both appear clear.

6.4. Thin Layer Chromatography (TLC) of Adhesives Before and After Dialysis

6.4.1. TLC of isinglass

In order to perform TLC of isinglass, a plate with silica gel coating was used as stationary phase with dimensions approx. 15x7cm.The basic solvent, used as mobile phase, was IDA (Industrial Denatured Alcohol) which was more polar than ethanol, recommended by Soppa et al.[172] For this reason drops of a solvent system with 60:40 Hexane in Ethyl acetate were added to minimise the speed that the chromatography run.

In order to start the chromatography, 30 ml of IDA were placed in the TLC chamber with dimensions 22x8 cm (approx.). Additionally, four strips of chromatographic paper soaked in IDA, were placed in the chamber (attached on the walls with double-sided self-adhesive tape), for helping to saturate the atmosphere inside the TLC chamber with solvent vapour.[173] This made the

[172] Karolina Soppa, Tilly Laaser, and Christoph Krekel, "Visualizing the Penetration of Adhesives Using Fluorescent Labelling" (2011): 1-18.
[173] Egon Stahl, *Thin-Layer Chromatography. A Laboratory Handbook*, 18.

TLC plate run faster by slowing the evaporation of solvent from the plate(Figure 16).

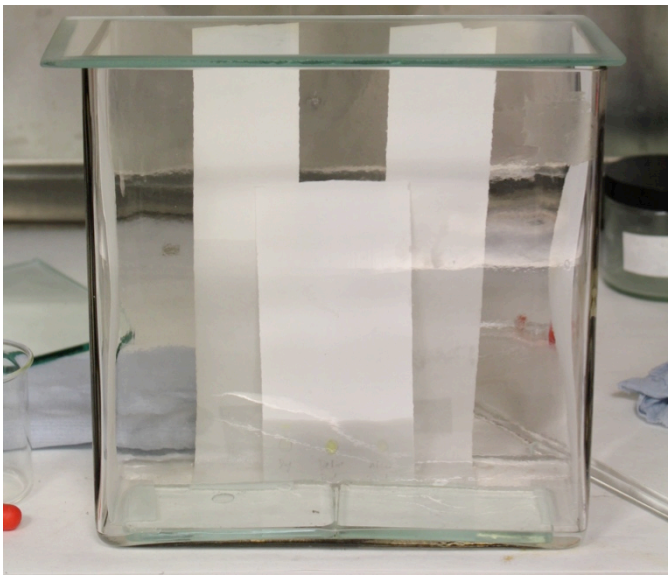


Figure 16: TLC plate inside the chamber.

Samples of isinglass before dialysis, after dialysis and from 5-DTAF was first diluted in 60:40 Hexane:Ethyl acetate (approx. 1:4 solvent to isinglass), based on recommendations by Hummert et al. and Wall and testing.[174, 175] Afterwards, by using micro-pipettes,, the three spots of 0,02 ml each, were placed on the baseline of the TLC plate marked with a pencil. The TLC plate was placed gently into the TLC chamber, without letting the solvent level to get above the baseline and covered with its cap. In few minutes the solvent front started traveling up the plate. Few drops of ethyl acetate-Hexane were added in the chamber to slow down the speed that the solvent travelled on the plate. When the solvent front has gotten close to the top of the TLC plate, it was removed from the chamber. The solvent front was then marked before it evaporates with a pencil line in order to calculate the Rf values of the spots.

[174] Zbigniew Darzynkiewicz, J. Paul Robinson, and Mario Roederer, *Essential Cytometry Methods*, 89.
[175] Eva Hummert, Ute Henniges, and Antje Potthast, "Fluorescence Labeling of Gelatin and Methylcellulose: Monitoring Their Penetration Behavior into Paper."

6.4.2. TLC of funori

In order to perform TLC of funori, the same type of plate and the same saturated chamber system used. The basic solvent used as mobile phase was ethyl acetate as recommended by Soppa et al.[176] The polarity of the solvent was slightly modified by adding drops of a solvent system with 60:40 hexane:ethyl acetate to modify the speed that the chromatography run for better results

The chromatography started by placing 40ml of Ethyl acetate in the TLC chamber. Samples of funori before dialysis, after dialysis and from 5-DTAF, were also diluted in Hexane:ethyl acetate (1:4 solvent to funori). Afterwards, by using micro-pipettes, one spot of 0.02ml form each sample was placed on the baseline of the TLC plate. The plate was placed in the chamber, same as with isinglass. The solvent front was also marked for calculating the Rf values of the spots.

6.4.3. TLC results and conclusion

Both TLC plates (funori and isinglass) were examined under a UV lamp, as the spots were fluorescent from the presence of the fluorescent dye (5-DTAF).

In both TLC plates it was possible to identify the same Rf value, 0.75 for the distant the unreacted dye travelled as a reference spot for the presence of free fluorochromes.

[176] Karolina Soppa, Tilly Laaser, and Christoph Krekel, "Visualizing the Penetration of Adhesives Using Fluorescent Labelling" (2011): 1-18.

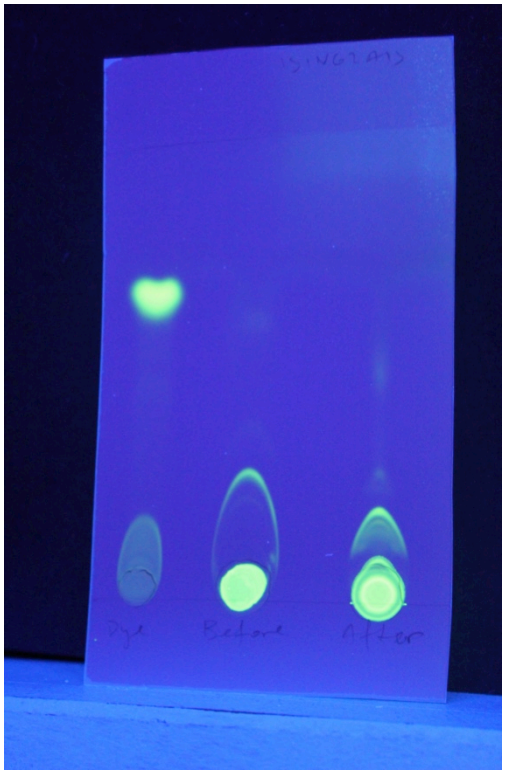


Figure 17: TLC plate of isinglass. No separation occurred at spot 3 (after dialysis) with the same travel distance with spot 1.

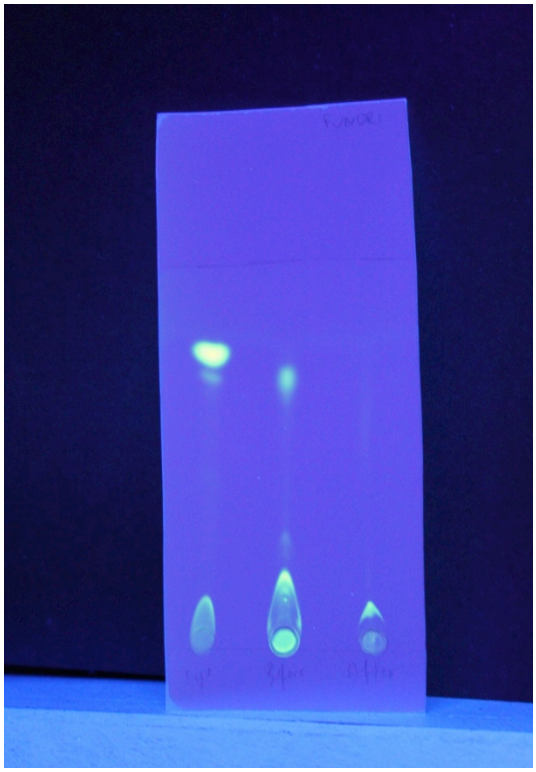


Figure 18: TLC plate of isinglass. Same as with Figure 15.

No such a spot with the same or close Rf value appeared neither from isinglass nor form funori after dialysis with cellulose tubing. Hence, cellulose dialysis tubing appeared to be successful in removing free fluorochromes form the adhesives after fluorescent labelling.

6.5. Application of labelled adhesives on thangka samples

Two squared pieces (1.5x1.5 cm) were cut from each of the three thangka samples with scissor. For creating flaking paint effect, the paint and preparatory layer were slightly cut with scalpel in both directions (approx. 3 mm distance between each cut line) and with additional mechanical stress the preparatory layer slightly delaminated from the ground fabric.

By using a micro-pipette, 0.08 ml from each adhesive were applied on each square sample. Both adhesives were warm when applied (temperature of the water from the bain-marie 50°C. Since funori was too viscous, a flat brush was additionally used. The samples were covered with silicon release paper, weighted on top with glass weights and set aside to dry. In total 6 samples prepares, a set of the three different preparation methods with each of the two adhesives.

6.6. Preparation of cross-sections

For the cross-sections half of the epoxy resin required (Pebeo glazing Resin) was placed in the rubber mould and set aside to dry for 1 hours in order to become more viscous. Then thin rectangular shaped samples were placed in the resin and let to dry for an hour. When the resin became more viscous (not to much to form a “skin” layer, the rest of the resin was places in the mould. For saving time and resin, two samples were placed in each mould where possible. The samples with this type of epoxy resin were ready for to be cut and polished after 24 hours in room temperature.

Samples were cut after drying with a saw and then grinded and polished with using a single wheel grinding machine (Buehler Metaserv) with a set of different grinding and polishing textiles. The resin was grinded until only a tiny layer of resin covered the sample and then polished. For polishing, six different grades from 1500 to 12000 of a micro-mesh® polishing kit were used. Since they come in a rectangular shape, they were attached on the rotary surface of the grinder with double-sided self-adhesive tape. Few drops of white spirit were applied on the surface of the sample that acted as a lubricant during polishing.

CHAPTER 7: Results

7.1. Part 1: Examination of reference samples

For the examination of the samples, a Zeiss Axioscope2 fluorescence light microscope with transmitted light coming from an HBO mercury lamp 50Hz was used. The main filter set used was the 09 (Zeiss filters) which is recommended for detecting 5-DTAF. The Zeiss filter set 02 was also used as it provided more stratigraphic information for examining the samples.

Images form the fluorescence light microscopy (FLM) captured by using the following equipment, Zeiss camera (AxioCam) and a ZEN 2 (blue edition) microscope software.

- Examination of samples taken from each one of the three different Thangka preparation methods

Samples from each of the three different preparation methods examined in order to assess the level of the autofluorescence for each preparation method.

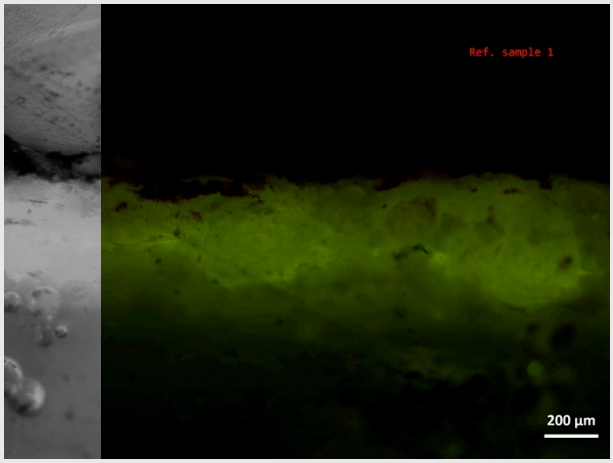
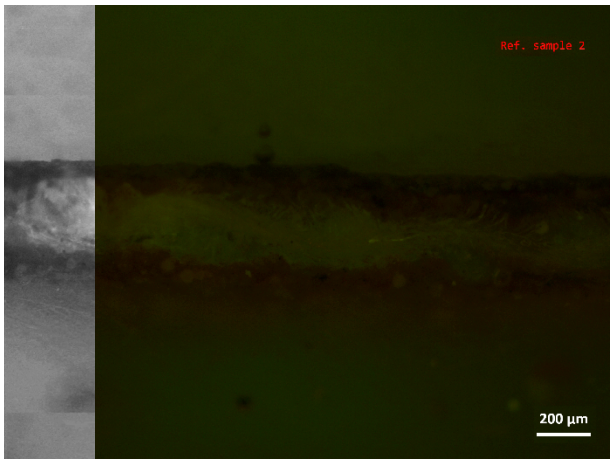
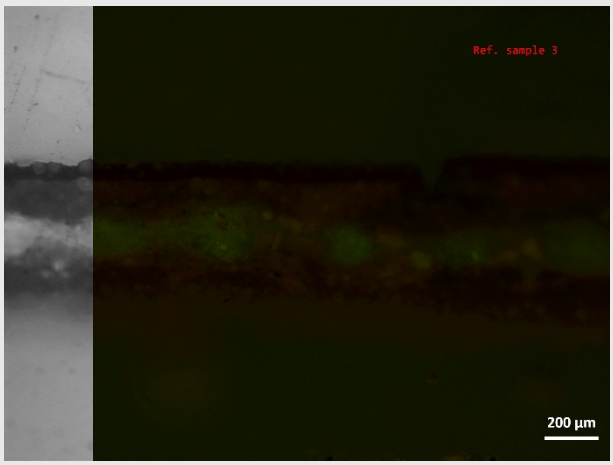
Sample one (1) that was constructed based on traditional material and methods had very strong levels of autofluorescence as the sample appeared bright green.

Sample two (2) that contained iron red in the preparatory layer emitted much less autofluorescence; as the sample appeared very dark compared with sample one. Most of its very light-green autofluorescence that was coming from the fabric layer, which was sized with rabbit, skin glue.

Sample three (3) that contained iron red in the preparatory layer and its fabric was sized with starch paste, appeared slightly darker than the sample two. It had a very light-green autofluorescence that was coming from the layer of the fabric that was sized in this case with starch paste.

Images taken during the fluorescence light microscopy (FLM) are presented in Table 2. All images, processed by the microscope software, are presented with the same display settings.

Table 2. Images taken during the cross-section examination of samples from the three different thangka preparation methods without the any adhesive applied on them. The gray section at the left depicts the actual size of the sample. All Scales: 200 µm. All samples are embedded in epoxy resin.

Reference Sample	Comments	Image from FLM (Filter set 09)
No. 1 Fabric: unbleached cotton sized with rabbit skin glue. Preparation: white pigments with rabbit skin glue. Paint layer: iron red, white pigments and animal skin glue	Strong level of autofluorescence. The sample appeared bright green.	
No. 2 Fabric: unbleached cotton with rabbit skin glue. Preparation: white pigments and iron red with rabbit skin glue. Paint layer: iron red, white pigments and rabbit skin glue	Light level of green autofluorescence. It is mostly emitted from the fabric layer, which is sized with rabbit skin glue.	
No. 3 Fabric: unbleached cotton with starch paste. Preparation: white pigments and iron red with rabbit skin glue. Paint layer: iron red, white pigments and rabbit skin glue	Very light level of green autofluorescence. It is emitted from the fabric layer, which is sized with starch paste.	

- Examination of cotton samples impregnated with the labelled adhesives

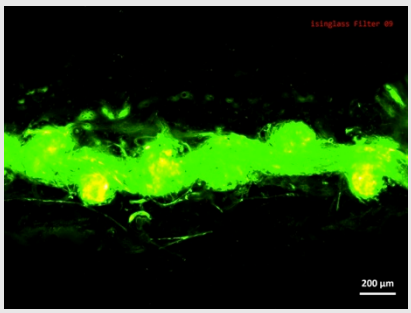
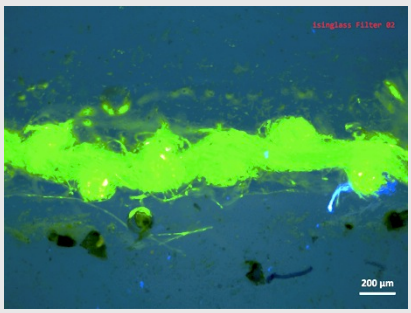
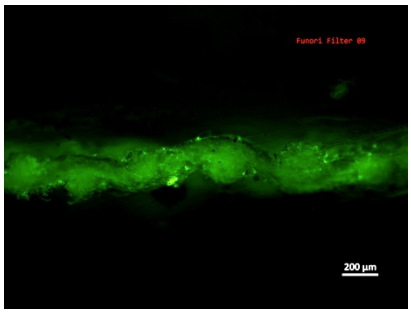
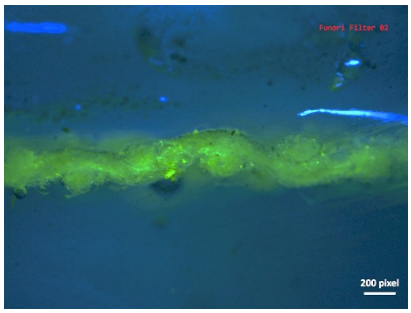
Samples of unbleached cotton substrate impregnated with both adhesives in order to be examined with FLM.

Isinglass had a very bright green fluorescence when examined with filter 09. This bright fluorescence was also detectable with the filter 02.

Even though funori had much paler colour under regular and UV light coming from a lamp, it emitted quite strong fluorescence when examined with filter 09 under the fluorescence microscope. A paler but still detectable green fluorescence was also detectable with the filter 02.

Images taken during the fluorescence light microscopy (FLM) are presented in Table 3 below. Images captured using the same filter sets are presented with the same display settings

Table 3: Images taken during the cross-section examination of the labeled adhesives on a cotton substrate observed with filter set 09 and 02. All Scales: 200 μm.

Sample	Comments	Image from FLM (Filter set 09)	Image from FLM (Filter set 02)
Isinglass on cotton fabric	-Very strong fluorescence with filter set 09. -Less, but still very strong, with filter set 02 .		
Funori on cotton fabric	-Strong fluorescence with filter set 09. -Less, but still detectable, with filter set 02.		

7.2. Part 2: Examination of thangka samples with the labeled adhesives

- Examination of thangka samples with isinglass labeled with 5-DTAF

Samples impregnated with labelled isinglass named I1, I2 and I3, where the number indicates the sample preparation method. Three samples of each different preparation method prepared and observed with FLM.

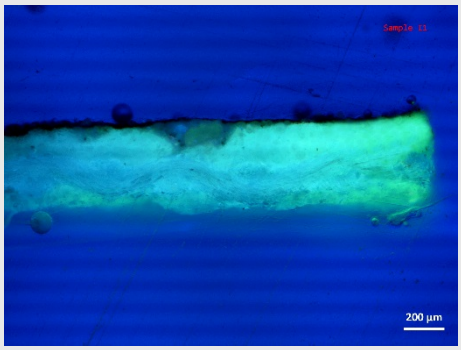
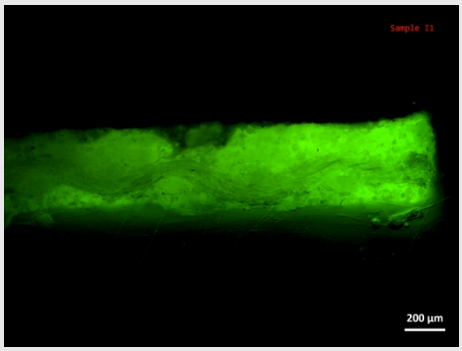
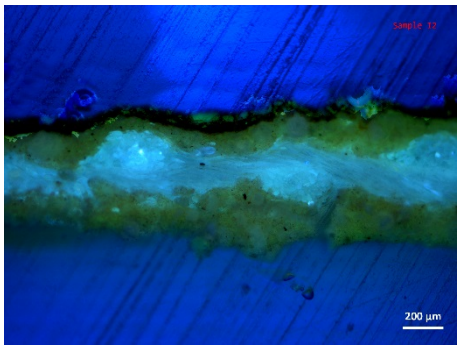
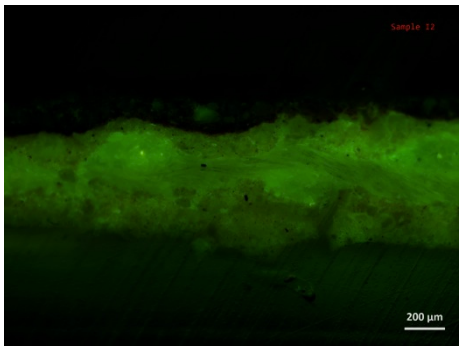
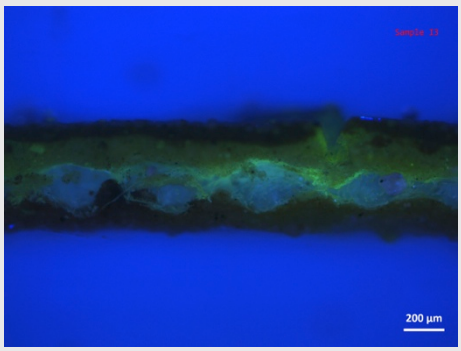
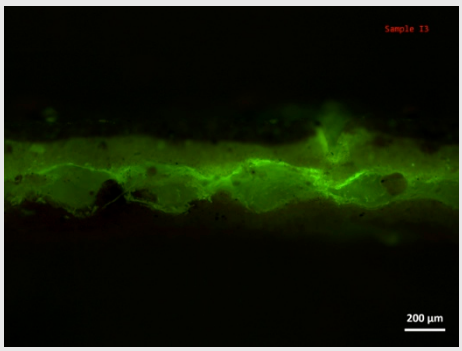
The sample I1 revealed, very strong green fluorescence coming form all layers (fabric, preparation of both its sides and paint layer at the top). It was not possible form this type of sample preparation, to distinguish autofluorescence form the green fluorescence coming form the adhesive.

Sample I2, which had very low levels of autofluorescence, green fluorescence coming form the adhesive observed though all the layers of the sample

Sample I3, which had also low level of autofluorescence, green fluorescence form the adhesive detected only at half the sample. More specifically, below the layer of the fabric (preparatory layer applied at the back of the Thangka sample no adhesive penetrated. Most adhesive was located on top of the fabric that was sized with starch paste. The fluorescence due to the amount of the adhesive at this point was that strong that could even be detected with the filter set 02.

One characteristic image of each is presented in the Table 4. Same images observed with the filter set 02 are also presented as they provide supplementary information for the size of the sample and its stratigraphy. Moreover, if the fluorescence is very strong (as with samples I3) is also detectable with the filter set 02 as well.

Table 4: Images taken during the cross-section examination of samples from the three different thangka preparation methods with labelled isinglass applied on them. All Scales: 200 µm.

No.	Comments	Image from FLM (Filter set 02)	Image from FLM (Filter set 09)
I1	Very strong green fluorescence coming from all layers		
I2	Green fluorescence coming from the adhesive observed though all the layers of the sample		
I3	Green fluorescence form the adhesive at the top half of sample. Most adhesive deposition on top of the fabric.		

- Examination of thangka samples with the labelled funori.

Samples impregnated with labelled funori named F1, F2 and F3, where the number indicates the sample preparation method. Three samples of each different preparation method prepared and observed with FLM.

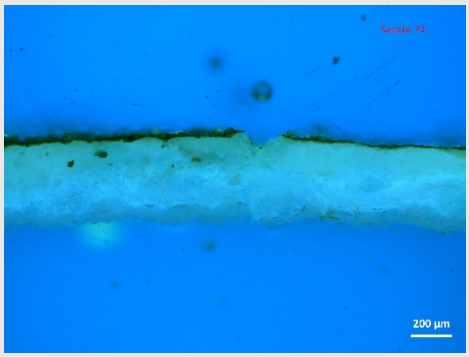
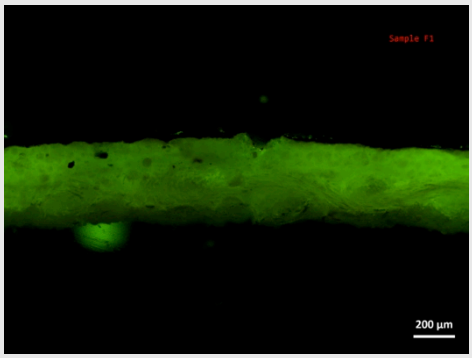
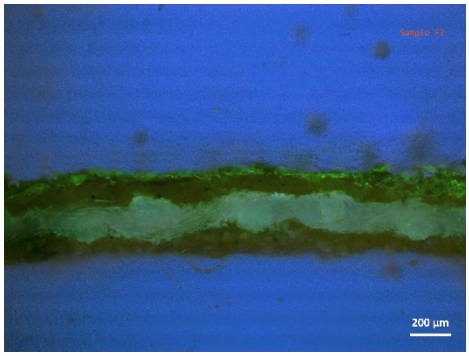
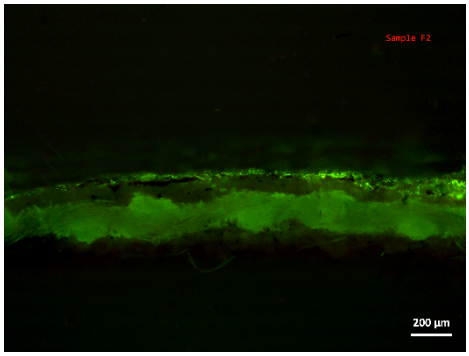
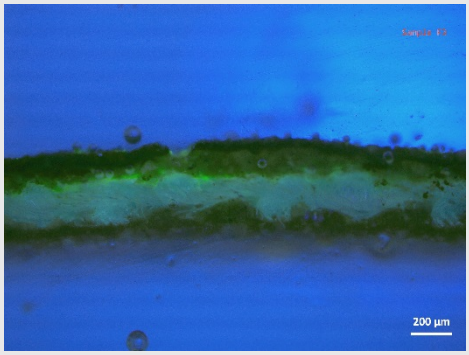
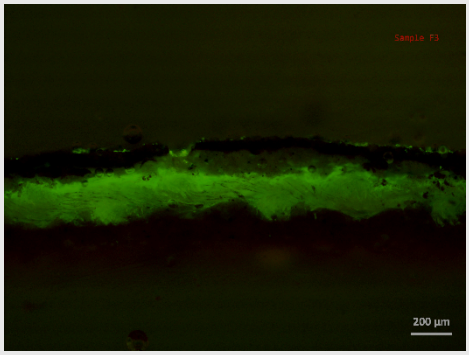
The sample F1 illustrated, very strong green fluorescence coming form all layers (fabric, preparation of both its sides and paint layer at the top). It was not possible form this type of sample preparation, to distinguish autofluorescence form the green fluorescence coming form the adhesive.

Sample F2, which very low levels of autofluorescence, green fluorescence form the adhesive observed on the paint layer and on the fabric.

Sample F3 that had also low level of autofluorescence emitted also green fluorescence form the paint layer but the fluorescence coming form the fabric was much stronger.

One characteristic image of each is presented in the Table 5. Same images observed with the filter set 02 are also presented as they provide supplementary information for the size of the sample and its stratigraphy. Moreover, if the fluorescence is very strong (as with samples F2 and F3) is also detectable with the filter set 02 as well.

Table 5: Images taken during the cross-section examination of samples from the three different thangka preparation methods with labelled funori applied on them. All Scales: 200 µm.

No.	Comments	Image from FLM (Filter set 02)	Image from FLM (Filter set 09)
F1	Very strong green fluorescence coming form all sample layers.		
F2	Fluorescence from the adhesive on the paint layer and the fabric. Stronger emission from the paint layer		
F3	Fluorescence from the adhesive on the paint layer and the fabric. Stronger emission from the fabric.		

DISCUSSION AND FUTURE WORK

During dialysis of both adhesives, funori released more unbonded fluorescent dye molecules of 5-DTAF than funori. This was further attested by examining the labelled adhesives macroscopically, since funori had a paler yellow colour in comparison with the very bright saffron yellow colour of isinglass. Even though fluorescent labelled funori's colour was paler, its fluorescence emission was sufficient in order to be examined with fluorescence light microscopy.

Another practical observation was that the labelled isinglass after dialysis lasted approximately one week in the fridge (after this time it became watery and smelled bad), much less comparing to isinglass that did not undergo dialysis. This can be easily explained since the adhesive remained in the dialysis tubing in a higher temperature, a fact that encouraged its deterioration.

Funori on the other hand, appeared more resistant after dialysis in terms of its deterioration, but it became slightly more viscous. This could have happened because some water passed in the tubing during dialysis. In order to overcome this issue, dialysis was repeated before shivering funori to keep the undissolved residues.

On the next phase, thin layer chromatography was effective for determining the success of the purification method for funori. More specifically, labelled funori's spot before dialysis had its Rf value close to the Rf value of the unbonded 5-DTAF. Such a relevant spot was not detected at the sample after dialysis, meaning that unbonded dye was not present after dialysis.

Accordingly, the TLC plate from isinglass this spot was not detected neither before nor after dialysis. This could be explained by the higher reactivity that 5-DTAF had with isinglass compared to funori. Nevertheless, before recommending the labelling of isinglass with more 5-DTAF than 5mg per 1g of adhesive, further practice with TLC of isinglass with slightly modified polarity of the solvent system is necessary. Moreover, apart from analising the

labelled adhesive the rinse water after dialysis could be analysed as well, in order to detect the presence of unbounded dye molecules.

Figure 19: Funori's tail marks on the TLC plate

All spots when travelled on the TLC plate were leaving some tail marks as shown in Figure 19. These marks according to Wall could either be caused form sample overloading or due to a damaged layer (Figure #).[177] The second reason is more likely, since the TLC plates used were very old, nevertheless repetition with less sample deposition would worth performed.

In terms of the sample preparation, the use of iron red oxide is necessary during the pigmentation of the gesso. It helps to avoid the transmition and reflection of the labelled adhesives and also to decrease the autofluorescence of the samples.

The pigment mixture used in sample 2 and 3 of this project consisted of 20:20:5 parts by weight of chalk, kaolin and iron red respectively. This specific pigment mixture showed a significant decrement of the samples' autofluorescence.

The use of rabbit skin glue (5% in water) for sizing the ground fabric caused low autofluorescence when iron red was included in the pigment mixture of the preparatory layer. More specifically, the autofluorescence of the reference sample 2, where only slightly higher in comparison to sample 3 (sized with

[177] Peter E. Wall and Roger M. Smith, *Thin-Layer Chromatography: A Modern Practical Approach*, 12.

13% starch paste). Hence, the use of starch paste provides no benefit and also appears to cause some unexpected implications on the penetration behaviour.

As for the penetration of the two consolidants, generally, isinglass was more penetrating in all three samples, while funori was leaving a thin layer of adhesive on the paint surface.

Isinglass (collagen) appears to penetrate less within the fibres that were impregnated with starch, which is a polysaccharide (sample I3). In addition it more easily can penetrate the fabric when sized with rabbit skin glue, which is also a collagen (sample I2). Funori, which is a polysaccharide, was penetrating and placed within the fabric layer when it was sized with starch paste (sample F3), compared to sample F2 with rabbit skin glue where it did not penetrate the fabric that much. Hence, it seems that adhesives used for sizing appear to attract and incorporate easier those consolidants that are closed to their chemical composition.

Nevertheless, further modifications with more iron red and experiments to dye the cotton fabric d in a colour that provides even less autofluoresences when examined under the filter set 09 (zeiss no.) or other with relevant filters are strongly encouraged.

Cross sections

Cross sectioning of sample appear to be a suitable method for sample preparation. Nonetheless, the sample needs to be as close as possible to the surface and be very smooth as any irregularities can scatter and reflect the emitted light. Thin sectioning would overcome this issue since all the resin form the surface is cut. On the other hand thin sectioning has also disadvantages, as it can cause delamination between the paint layer and the textile, as reported by Soppa et al.[178] Therefore, a combination of the two methods, examining cross sections with their surface cut by a microtome, could result in having the benefits of both methods.

[178] Ibid., 65-66.

The use of epoxy resin for jewellery making and handcraft, seems suitable for fluorescent light microscopy. The only issue regarding its use is that, as a two part resin, it requires mixing that creates bubbles. Mixing the two parts by pouring the resins between two containers created less bubbles than direct stirring with a stick/spatula. It should be done though many times and between changing containers, residues from the walls should be gathered too.

Zeiss camera software

Zeiss camera software was very easy to use, and moreover had rich functionality for manipulating the visual aspects of captured images. It is worth mentioning, the option of combining two photos taken from different filters in one image. This function, allows the examination and visualisation of mixtures of labelled consolidants with different fluorescent dyes. Hence, the use of the functionality could be a more affordable than using a Dual filter set.

On the other hand excessive use and manipulation through this software, can lead in loss of information, which then might be used to mislead. Consequently, the option of coping/pasting the exact same display settings (Figures 20 & 21) between different images, was used for comparing them in this project, and is strongly suggested for any relevant future study.

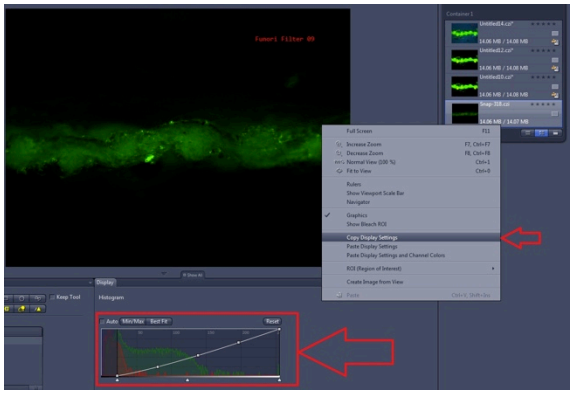


Figure 20: Coping the display settings of an image

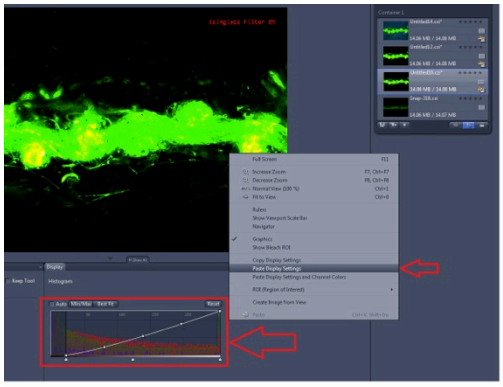


Figure 21: Pasting the display settings to another image

CONCLUSION

From the experiments conducted during this project it is possible to make the following recommendations for any relevant future work.

Labelling with 5-DTAF is possible for a wide range of adhesives/consolidants of animal origin, which are proteins, but also of plant/cellulose origin (polysaccharides). In any case the pH during the conjugation reaction should be above 9. It is recommended to raise the pH at 9.3 by using a 0.1M sodium bicarbonate-sodium carbonate buffer system. Finally, the adhesive should be dissolved in the buffer system.

In terms of dialysis after labelling, particularly for funori, it is suggested to conduct the dialysis while the residues are still present. Afterwards residues can be removed by shivering.

Continuing with the sample preparation, rabbit skin glue can be used for sizing the samples, and also as binding medium for pigments, only if combined with iron red oxide. Iron red also, decreases the transmission and reflectance of fluorescence, from both the labelled adhesive and the rabbit skin glue, and thus provides a very dark background for better observation. The use of approximately 10% iron red in the white pigment mixture was sufficient for observation.

The next experimental part of cross sectioning showed that, embedding samples in epoxy resin was a successful preparation method. Particularly for epoxy raisins, special care should be given when stirring the two parts of the resin in order to avoid bubbles, and for creating a very smooth surface with grinding and polishing. Alternatively, the use of Technovit™7100 and microtiming the surface of the samples is proposed but first should be tested for effectiveness. The use of light curing resins (such as Technovit™2200) should be avoided as the UV light used for curing “destroys” the fluorchromes.

In terms of using the fluorescence microscope, special consideration should be paid in following the recommendations for the use of mercury lamp. Briefly,

the lamp when turned should remain on for at least half an hour and, when turned off should remain closed for at least an hour. Moreover, the use of an 100W HBO mercury lamp is recommended, because when an image is captured using 50W and the exposure time is set low, there are interference lines in the image.

When fluorescence between different samples is about to be compared it is suggested to copy/paste the same display setting between images for more reliable results.

As a final point, images captured in very dark background, as when using filter 09 (Zeiss no.), cannot readily provide sufficient information for conclusions. The information about the samples' size and stratigraphy (topography of layers) are not visible. In this case an image should be presented next to the exact same image taken with visible light or other filter that provides the missing information (in this project with Zeiss filter 02). Alternatively, marks or diagrams could be added over the dark image in order to provide such information.

All the above conclusions constitute an experimental methodology and guidelines for preparing adhesives and painted textiles samples, in order to study their penetration with fluorescent labelling. These conclusions can be used by any researcher in order to advance the research about adhesives' penetration, and potentially help conservators decide about the proper consolidation practices for painted textiles.

APPENDIX 1

Filter Assistant

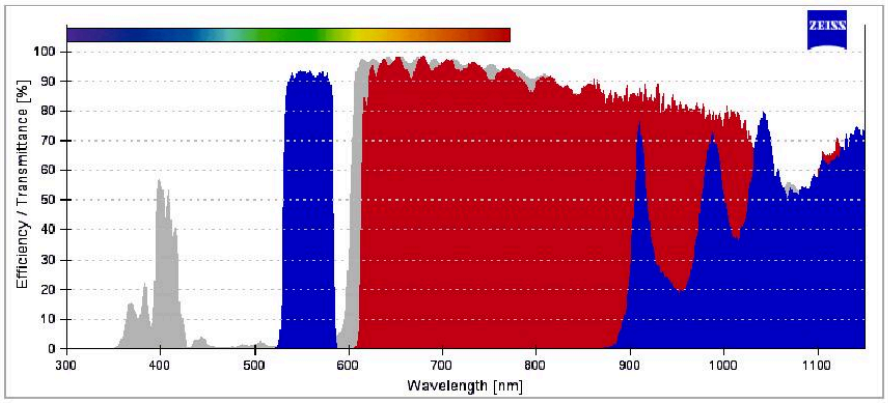
→ Deutsch

→ Fluorescence Dye and Filter Da... → Overview Filter Sets

Print

Filter Set

Filter Set 00 (488000-0000-000) → Price Basket



Show total efficiency

Note:
Additional components can influence efficiency.

Filter Set	00
Excitation	BP 530-585
Beam Splitter	FT 600
Emission	LP 615

37 possible dyes found:

Refresh view

Please note that the given values are typical only and not guaranteed.

37 possible dyes found:

Sample Fluorescent Dyes	Excitation	Emission
5-ROX (5-Carboxy-X-rhodamine, triethylammonium salt)	578 nm	604 nm
5-ROX pH 7.0	578 nm	604 nm
Alexa 568	576 nm	603 nm
Alexa 594	590 nm	619 nm
Alexa Fluor 568 antibody conjugate pH 7.2	579 nm	603 nm
Alexa Fluor 610 R-phycoerythrin streptavidin pH 7.2	567 nm	627 nm
Alexa Fluor 647 R-phycoerythrin streptavidin pH 7.2	569 nm	666 nm
BO-PRO-3-DNA	574 nm	604 nm
BOBO-3-DNA	570 nm	605 nm
BODIPY TR-X phallacidin pH 7.0	590 nm	621 nm
BODIPY TR-X, MeOH	588 nm	621 nm
BODIPY TR-X, SE	588 nm	621 nm
BOPRO-3	574 nm	604 nm
Calcium Crimson	589 nm	608 nm

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User ID:
Password:
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› Forgot Password?

Applications
› Biomedical Applications
› Materials Applications
Microscopes
› Upright microscopes
› Inverted microscopes
› Stereomicroscopes
› Zoom Microscopes
› Digital Microscopes
Computers
› Computers
Software
› AxioVision Software
› ZEN Software
Accessories
› Accessories
Tools
› Camera Assistant
› Filter Assistant
› Objective Assistant

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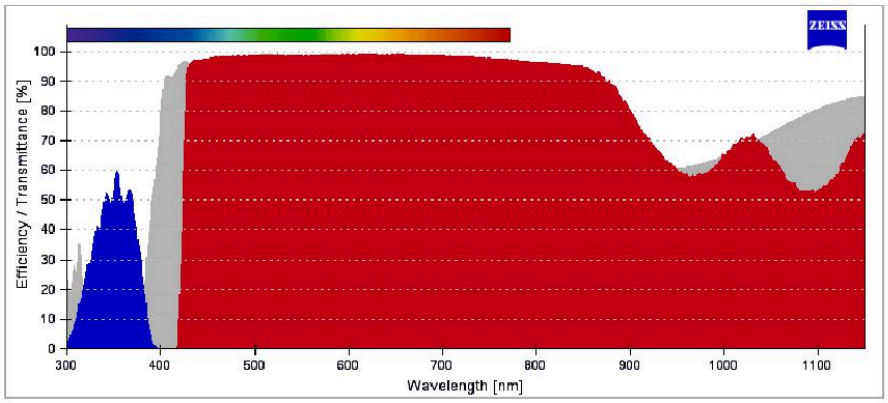
Calcium Crimson Ca2+	590 nm	608 nm
Carboxynaphthofluorescein pH 10.0	600 nm	674 nm
DDAO pH 9.0	648 nm	657 nm
dTomato	554 nm	581 nm
DyLight 594	592 nm	616 nm
Ethidium Bromide	524 nm	605 nm
Ethidium homodimer	528 nm	617 nm
Ethidium homodimer-1-DNA	528 nm	617 nm
FM 4-64	508 nm	751 nm
FM 4-64, 2% CHAPS	506 nm	751 nm
HcRed	590 nm	614 nm
mCherry	587 nm	610 nm
MitoTracker Red	578 nm	599 nm
MitoTracker Red, MeOH	578 nm	599 nm
mPlum	587 nm	649 nm
mRFP	585 nm	608 nm
mStrawberry	575 nm	596 nm
Nile Red	559 nm	637 nm
Nile Red-lipid	553 nm	636 nm
Propidium Iodide	538 nm	617 nm
Propidium Iodide-DNA	538 nm	619 nm
ReAsH	597 nm	608 nm
X-Rhod-1 Ca2+	580 nm	602 nm

Filter Assistant

→ Deutsch

Filter Set

Filter Set 02 (488002-9901-000) → Price Basket



Show total efficiency

Note:
Additional components can influence efficiency.

Filter Set	02
Excitation	G 365
Beam Splitter	FT 395
Emission	LP 420

35 possible dyes found:

Refresh view

Please note that the given values are typical only and not guaranteed.

35 possible dyes found:

Sample Fluorescent Dyes	Excitation	Emission
1,8-ANS (1-Anilinonaphthalene-8-sulfonic acid)	375 nm	480 nm
1-Anilinonaphthalene-8-sulfonic acid (1,8-ANS)	375 nm	479 nm
6,8-Difluoro-7-hydroxy-4-methylcoumarin pH 9.0	358 nm	450 nm
7-Amino-4-methylcoumarin pH 7.0	346 nm	442 nm
7-Hydroxy-4-methylcoumarin	360 nm	447 nm
7-Hydroxy-4-methylcoumarin pH 9.0	361 nm	448 nm
Alexa 350	343 nm	441 nm
AMCA conjugate	347 nm	444 nm
Amino Coumarin	345 nm	442 nm
BFP (Blue Fluorescent Protein)	380 nm	439 nm
Cascade Blue	398 nm	420 nm
Coumarin	360 nm	447 nm
Dansyl Cadaverine	335 nm	524 nm
Dansyl Cadaverine, MeOH	335 nm	526 nm

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DAPI	358 nm	463 nm
DAPI-DNA	359 nm	461 nm
Dapoxyl (2-aminoethyl) sulfonamide	372 nm	582 nm
DyLight 350	332 nm	435 nm
Ethidium homodimer	528 nm	617 nm
Fura-2 Ca2+	336 nm	505 nm
Fura-2, high Ca	336 nm	504 nm
Fura-2, no Ca	367 nm	515 nm
Hoechst 33258	352 nm	455 nm
Hoechst 33258-DNA	352 nm	455 nm
Hoechst 33342	352 nm	455 nm
Indo-1 Ca2+	346 nm	404 nm
Indo-1, Ca free	346 nm	479 nm
Indo-1, Ca saturated	331 nm	404 nm
LysoSensor Blue	374 nm	424 nm
LysoSensor Blue pH 5.0	374 nm	424 nm
LysoSensor Yellow pH 3.0	389 nm	542 nm
LysoSensor Yellow pH 9.0	335 nm	530 nm
LysoTracker Blue	373 nm	421 nm
Marina Blue	362 nm	464 nm
SBFI-Na+	336 nm	527 nm

Filter Assistant

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→ Fluorescence Dye and Filter Da... → Overview Filter Sets

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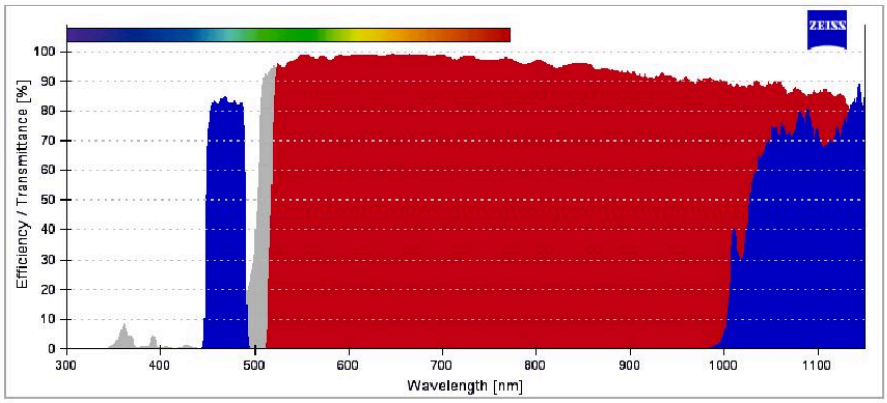
Filter Set

Filter Set 09 (488009-9901-000) → Price Basket



Show total efficiency

Note:
Additional components can influence efficiency.

Filter Set	
Excitation	BP 450-490
Beam Splitter	FT 510
Emission	LP 515

54 possible dyes found:

Refresh view

Please note that the given values are typical only and not guaranteed.

54 possible dyes found:

Sample Fluorescent Dyes	Excitation	Emission
5-FAM pH 9.0	492 nm	518 nm
Alexa 430	431 nm	540 nm
Alexa 488	493 nm	520 nm
Alexa Fluor 430 antibody conjugate pH 7.2	431 nm	540 nm
Alexa Fluor 488 hydrazide-water	493 nm	518 nm
BCECF pH 5.5	485 nm	521 nm
Calcein	493 nm	514 nm
Calcein pH 9.0	494 nm	514 nm
CFDA	495 nm	517 nm
CI-NERF pH 2.5	504 nm	541 nm
Di-8 ANEPPS	469 nm	630 nm
Di-8-ANEPPS-lipid	469 nm	631 nm
DM-NERF pH 4.0	493 nm	530 nm
DTAF	495 nm	517 nm

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DyLight 488	493 nm	518 nm
eGFP (Enhanced Green Fluorescent Protein)	488 nm	509 nm
evoglow-Bs1	448 nm	496 nm
evoglow-Bs2	448 nm	496 nm
evoglow-Pp1	448 nm	495 nm
FDA	495 nm	517 nm
FITC	495 nm	517 nm
FITC antibody conjugate pH 8.0	495 nm	519 nm
Fluo-4	494 nm	516 nm
Fluorescein	495 nm	517 nm
Fluorescein 0.1 M NaOH	493 nm	513 nm
Fluorescein antibody conjugate pH 8.0	493 nm	517 nm
Fluorescein pH 9.0	490 nm	514 nm
Fluoro-Emerald	495 nm	524 nm
FM 1-43	472 nm	578 nm
FM 1-43 lipid	473 nm	579 nm
FM 4-64	508 nm	751 nm
FM 4-64, 2% CHAPS	506 nm	751 nm
Fura Red Ca2+	435 nm	670 nm
Fura Red, high Ca	434 nm	659 nm
Fura Red, low Ca	472 nm	673 nm
LysoSensor Green	447 nm	504 nm
LysoSensor Green pH 5.0	447 nm	502 nm
mHoneydew	478 nm	562 nm
MitoTracker Green	490 nm	512 nm
MitoTracker Green FM, MeOH	490 nm	512 nm
NBD-X	466 nm	534 nm
NBD-X, MeOH	467 nm	538 nm
NeuroTrace 500/525, green fluorescent Nissl stain-RNA	497 nm	524 nm
Nissl	497 nm	524 nm
Rhodamine 110	497 nm	520 nm
Rhodamine 110 pH 7.0	497 nm	520 nm
Rhodamine Green	497 nm	524 nm
Rhodaminen Green pH 7.0	497 nm	523 nm
Rhodol Green antibody conjugate pH 8.0	499 nm	524 nm
SYBR Green I	498 nm	522 nm
SYPRO Ruby	467 nm	618 nm
SYTO 13-DNA	488 nm	506 nm
YO-PRO-1-DNA	491 nm	507 nm
YOYO-1-DNA	491 nm	509 nm

APPENDIX 2

University of Glasgow

RISK ASSESSMENT FORM

School: Culture and Creative Arts	Section: Centre For Textile Conservation and Technical Art History	Location: Room number(s) 309A / 310	Reference No: R68/2014-15	Related COSHH Form (if applicable): C 102-107/2014-15

Description of activity:
Preparation of painted Samples with animal glue and mineral pigments (Chalk, kaolin, Iron red oxide). The animal glue will be prepared from rabbit skin pellets and heated using a bain-marie system.

Preparations of cross-sections with epoxy resin Pebeo-gedeO. The block will be polished using an electric grinder/polisher and by hand.

Preparation of buffer solutions, sodium carbonate and sodium bicarbonate and pH adjustment with NaOH and HCl if needed.

Thin layer chromatography (TLC). Use of various solvents as a mobile phase.

Persons at risk: Student and tutors

Is operator training/supervision required? If yes, please specify: Yes, demonstration in the use of the grinder/polisher

Hazards/ Risks	Current controls	Are these adequate?	What action is required if not adequately controlled?
Mineral pigments	Avoid exposure to dust and skin contact. Use of mask, gloves, goggles, lab coat (PPE), read material safety data sheet	**Yes**	
Epoxy resin	PPE avoid skin contact	**No**	**COSHH form for epoxy resin**

Use of chemicals for buffer solutions and TLC	PPE, work under fume extraction	**No**	**Fill COSHH form fro HCl and NaOH, IDA +++**
Use of boiling water	Wear heat resistant gloves, mop up spillages	**Yes**	
Use of electrical equipment	Unplug all devices and equipment after use. Trip hazard – cover trailing leads	**Yes**	
Glassware	Dispose broken or damaged glasses in the appropriate boxes at the web/chemical lab	**Yes**	
Using sharp tool to cut materials for storage mounts	Careful handing of the object. Be aware of the location of the pharmacy box (wet lab/tutor's office). Dispose of sharps in the appropriate box in the chemical lab.	**Yes**	
Spillages for chemicals	Mop up spillages following COSHH guidelines if necessary, leave to evaporate in fume cupboard	**Yes**	
Electric polisher/grinder	Wear goggles, gloves and lab coat. User needs to dc shown how to operate the equipment before it is used the first time. Make sure there is someone responsible within earshot, and that they know that you plan to carry out this work. Avoid touching surface when turned on.	**Yes**	

Completed by (print name and position, and sign): Maria Kinti	**Date:** 12/06/2015
Approved by (print name and position, and sign): KAREN THOMPSON (TUTOR) Karen Thompson	**Date:** 15.6.15

University of Glasgow

RISK ASSESSMENT FORM

School: Culture and Creative Arts	**Section:** Centre For Textile Conservation and Technical Art History	**Location:** Room number(s) 313	**Reference No:** R 66 /2014-15	**Related COSHH Form (if applicable):** C_____

Description of activity:

Use of Axioskop with incident light fluorescence with an HBO 50 mercury short arc lamp.

Persons at risk:

Is operator training/supervision required? If yes, please specify:

Hazards/ Risks	Current controls	Are these adequate?	What action is required if not adequately controlled?
Failure of mercury lamp * Explosion of lamp	After turning off lamp, do not turn on again for 1 hour. Leave room & close door if lamp blows - DO NOT RE-ENTER for 1 hour.	YES	

PTO

	Bulb needs replacing after 100 hours		

Completed by (print name and position, and sign): KAREN THOMPSON, TUTOR Karen Thomp	**Date:** 2.7.15
Approved by (print name and position, and sign):	**Date:**

University of Glasgow

COSHH Assessment Form

School: Culture and Creative Arts
Section: Centre for Textile Conservation and Technical Art History
Project Title:

File ref: C103/2014-15
Related Assessment Form: R 68/2014-15
Date: 10/07/2015

Room Number(s): 310 Chemistry Lab

Building: Robertson Building, Level 3

Persons involved: Student and Supervising tutors

Description of procedure:

Using DMSO (dimethyl sulfoxide) for dissolving fluorescent dyes

Substance used	Quantities used	Frequency of use	Hazards identified	Exposure route
Dimethyl sulphoxide (DMSO)	3 ml	I day	R: 36/37/38 Do not breathe vapour. Avoid contact with eyes and skin. May promote skin absorption of other chemicals. Stench. Irritant.	Eyes Skin Ingestion Inhalation

Could a less hazardous substance (or form of the substance) be used instead? ~~yes~~ / no

Justify not using it:

What measures have you taken to control risk?

Engineering controls: Use in fume cupboard with vent extraction

Personal Protective Equipment: Gloves, goggles, lab coat, respirator mask if not working in fume cupboard.

Management measures: Keep away from heat and flame, keep containers tightly closed in a dry cool place

Checks on control measures:

Is health surveillance required? yes/no

Training requirements: none

Emergency procedures:
Consult a physician. Show this safety data sheet to the doctor in attendance.
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.
In case of skin contact -Wash off with soap and plenty of water. Consult a physician.
In case of eye contact -Flush eyes with water as a precaution.
If swallowed - Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

Waste disposal: Departmental procedures for disposal. For splits, soak up with tissues and place in plastic bag for disposal. Contact safety officer. Disposal via combustion. DMSO (5-10%) is in the sink (diluted small volumes)

Name and position of assessor: Maria Kinti, Student
Signature:

Name of supervisor (student work only): KAREN THOMPSON
Signature:

Name of Head of School or nominee:
Signature: Karen Thomp

University of Glasgow

COSHH Assessment Form

C.104/2014-15 R68/2014-15

School: Culture and Creative Arts
Section: Centre for Textile Conservation and Technical Art History
Project Title: Dissertation

File ref: ~~C80/2014-15~~
Related Assessment Form: ~~R47/2014-15~~
Date: ~~23/01/2015~~ 20/07/2015

Room Number(s):

Building: Robertson Building, Level 3

Persons involved: Students and tutor

Description of procedure:
Cross section polishing, coating cross sections before examination uders the microscope

Substance used	Quantities used	Frequency of use	Hazards identified	Exposure route
White spirit/Stoddard solvent	up to ~~150 ml~~ 5ml	~~3~~ days	R10, R65, R66, R48/22/24/25	Exposure to fumes/swallowing/inhalation/repeated exposure/waste disposal

Could a less hazardous substance (or form of the substance) be used instead? ~~Yes~~/no

Justify not using it:

What measures have you taken to control risk?

Engineering controls: fume cupboard, extractor hood

Personal Protective Equipment: mask, goggles, gloves, lab coat

Management measures: measure under fume cupboard, storage with flammable liquids

Checks on control measures:

Is health surveillance required? ~~Yes~~/no

Training requirements:

Emergency procedures: eye contact: wash with water, skin: wash with water, ingestion: seek medical advice

Waste disposal: dispose with non chlorinated solvents, small amounts on blotter to be left to evaporate in fume cupboard

Name and position of assessor: Maria Kinti **Signature:**

Name of supervisor (student work only): Karen Thompson **Signature:** Karen Thomp~

Name of Head of School or nominee: **Signature:**

University of Glasgow

COSHH Assessment Form

School: Culture and Creative Arts
Section: Centre for Textile Conservation and Technical Art History
Project Title:

File ref: C 105 / 2014-15
Related Assessment Form: R 68 / 2014-15
Date: 14/07/2015

Room Number(s): 310 Chemistry Lab

Building: Robertson Building, Level 3

Persons involved: Student and Supervising tutors

Description of procedure:

Using Ethyl Acetate mixed with Hexane as a solvent mixture for Thin Layer Chromatography

Substance used	Quantities used	Frequency of use	Hazards identified	Exposure route
Hexane (altern. n-Hexane)	Up to 50 ml	I day	R:11-38-48/20-51/53-62-65-67 Highly flammable (flash point -22₀ C). Harmful by inhalation Harmful: danger of serious damage to health by prolonged exposure. Possible risk of impaired fertility	Eyes Skin Ingestion Inhalation

Could a less hazardous substance (or form of the substance) be used instead? ~~yes~~ / no

Justify not using it:

What measures have you taken to control risk?

Engineering controls: Use in fume cupboard with vent extraction

Personal Protective Equipment: Gloves, goggles, lab coat, respirator mask if not using in fume cupboard.

Management measures: Keep away from heat and flame, keep containers tightly closed in a dry cool place

Checks on control measures:

Is health surveillance required? ~~yes~~/no

Training requirements: none

Emergency procedures: In case of accident, or if you feel unwell, seek medical advice (show label).
If inhaled: Remove victim to fresh air and keep at rest in a position comfortable for breathing
If skin irritation occurs: Get medical advice/attention. Take off immediately all contaminated clothing. Rinse skin with water/shower
Eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
If eye irritation persists: Get medical advice/attention.
If swallowed: Immediately call a **POISON CENTRE** or doctor/physician. Do NOT induce vomiting

Waste disposal: Non-chlorinated waste solvent bottle. For spills, soak up with tissues and leave in fume cupboard to evaporate.

Name and position of assessor: Maria Kinti, Student
Signature:

Name of supervisor (student work only): KAREN THOMPSON
Signature: Karen Thomp

Name of Head of School or nominee:
Signature:

University of Glasgow

COSHH Assessment Form

School: Culture and Creative Arts
Section: Centre for Textile Conservation and Technical Art History
Project Title: Dissertation

File ref: C 107/2014-15
Related Assessment Form: R 68/2014-15
Date: 14/07/2015

Room Number(s): 310 Chemistry Lab

Building: Robertson Building, Level 3

Persons involved:
Student and Supervising tutors

Description of procedure:

Using IDA mixed with Hexane/ethyl acetate as a solvent mixture for Thin Layer Chromatography

Substance used	Quantities used	Frequency of use	Hazards identified	Exposure route
IDA [redacted]	Up to 50 ml	1 day	R11, R20/21/22, R68/20/21/22	Skin/Inhaled/swallowed/damage to organs/ flammable

Could a less hazardous substance (or form of the substance) be used instead? ~~yes~~ / **no**

Justify not using it:

What measures have you taken to control risk?

Engineering controls: Use in fume cupboard with vent extraction

Personal Protective Equipment: Gloves, goggles, lab coat, respirator mask if irritation occurs.

Management measures: Keep away from heat and flame, keep containers tightly closed in a dry cool place

Checks on control measures:

Is health surveillance required? ~~yes~~/no

Training requirements: none

Emergency procedures: Keep away from sources of ignition. Do not breath vapour In case of contact with eyes, rinse with plenty of water and seek medical advice. Take precautions against static discharge.

Waste disposal: Non-chlorinated waste solvent bottle. For splits, Soak up with tissues and leave in fume cupboard to evaporate.

Name and position of assessor: Maria Kinti, Student — **Signature:**

Name of supervisor (student work only): Karen Thompson — **Signature:** Karen Thompson

Name of Head of School or nominee: — **Signature:**

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COSHH Assessment Form

C.102/2014-15

School: Culture and Creative Arts
Section: Centre for Textile Conservation and Technical Art History
Project Title: Treatment CTC.244

File ref: ~~C79/2014-15~~ R68/2014-15
Related Assessment Form: R472014-15
Date: ~~23/01/2015~~ 20/07/2014

Room Number(s): 309A/310

Building: Robertson Building, Level 3

Persons involved: Students and tutor

Description of procedure:

Preparation of cross sections with epoxy resin

Substance used	Quantities used	Frequency of use	Hazards identified	Exposure route
Epoxy resin (pebeo ~~crystal~~ glazing resin)	40 ml	6 days	Harmful-R22,R21, R52/53, Corrosive-R34	Skin/eye contact, /swallowed/Hazardous to the aquatic environment - Chronic hazard/flamable

Could a less hazardous substance (or form of the substance) be used instead? ~~Yes~~/no

Justify not using it:

What measures have you taken to control risk?

Engineering controls: Ensure that there is adequate ventilation

Personal Protective Equipment: PPE: gloves, mask, goggles, lab coat,

Management measures: Use personal protective equipment that is clean and has been properly maintained.
Store personal protective equipment in a clean place, away from the work area.
Never eat, drink or smoke during use. Remove and wash contaminated clothing before re-using.
Individuals wearing contact lenses should wear prescription glasses during work where they may be exposed to irritant vapours.

Checks on control measures: Tutor's supervision

Is health surveillance required? ~~Yes~~/no

Training requirements:

Emergency procedures: IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
ON SKJN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310: Immediately call a POISON CENTER or doctor/physician

Waste disposal: Do not pour into drains or waterways
Do not contaminate the ground or water with waste. Do not dispose of waste into the environment.

Name and position of assessor: Maria Kinti **Signature:**

Name of supervisor (student work only): Karen Thompson **Signature:** Karen Thompson

Name of Head of School or nominee: **Signature:**

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COSHH Assessment Form

School: Culture and Creative Arts
Section: Centre for Textile Conservation and Technical Art History
Project Title: Dissertation

File ref: C106/2014-15
Related Assessment Form: R 68/2014-15
Date: 14/07/2015

Room Number(s): 310 Chemistry Lab

Building: Robertson Building, Level 3

Persons involved:
Student and Supervising tutors

Description of procedure:

Using IDA mixed with Hexane/ethyl acetate as a solvent mixture for Thin Layer Chromatography

Substance used	Quantities used	Frequency of use	Hazards identified	Exposure route
Ethyl acetate (EtOAc)	Up to 50 ml	1 day	R11, R20/21/22, R68/20/21/22	Skin/Inhaled/swallowed/damage to organs/ flammable

Could a less hazardous substance (or form of the substance) be used instead? ~~yes~~ / no

Justify not using it:

What measures have you taken to control risk?

Engineering controls: Use in fume cupboard with vent extraction

Personal Protective Equipment: Gloves, goggles, lab coat, respirator mask if irritation occurs.

Management measures: Keep away from heat and flame, keep containers tightly closed in a dry cool place

Checks on control measures:

Is health surveillance required? ~~yes/no~~

Training requirements: none

Emergency procedures: Keep away from sources of ignition. Do not breath vapour In case of contact with eyes, rinse with plenty of water and seek medical advice. Take precautions against static discharge.

Waste disposal: Non-chlorinated waste solvent bottle. For splits, Soak up with tissues and leave in fume cupboard to evaporate.

Name and position of assessor: Maria Kinti, Student — **Signature**:

Name of supervisor (student work only): Karen Thompson — **Signature**:

Name of Head of School or nominee: — **Signature**:

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