

## Antileishmanial activity of a new 8-hydroxyquinoline derivative designed 7-[5'-(3'-phenylisoxazolino)methyl]-8-hydroxyquinoline: preliminary study

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### Abstract

7-[5'-(3'-phenylisoxazolino)methyl]-8-hydroxyquinoline a new synthetic 8-hydroxyquinoline derivative, was found, for the first time, to inhibit the multiplication in vitro of *Leishmania tropica*, *Leishmania major* and *Leishmania infantum* at micromolar concentrations. For each test we calculated a 50% inhibitory concentration ( $IC_{50}$ ) and the  $IC_{50}$  values found after 48 h are: 0.4  $\mu$ g/ml for *L. tropica*, 0.88  $\mu$ g/ml for *L. major* and 0.62  $\mu$ g/ml for *L. infantum*. As positive control, amphotericin B, a standard antileishmanial drug was used.

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### 1. Introduction

Leishmaniasis is a major health problem that affects the population of 88 countries at a rate of 2 million cases per year with a prevalence of 12 million and 350 million at risk around the world. It is a significant cause of morbidity and mortality [1]. A causative agent of this disease are parasites of the genus *Leishmania*, which infect and replicate in macrophage of the vertebrate host. Depending on the parasite species, different forms of leishmaniasis may develop in the mammalian host, ranging from chronic skin ulcers to fatal visceral disease if untreated.

Three different forms of cutaneous leishmaniasis (CL) occur in distinct geographical areas of Morocco. First, zoonotic cutaneous leishmaniasis (ZCL) is epidemic in the South of the country, it is known to be caused by *Leishmania major* (Zymodeme MON25) [2]. Second, a chronic form of CL is encountered in the Center and North of Morocco, it is due to *Leishmania tropica* [3,4]. Third, a sporadic form

occurs in the North Morocco, it is caused by *Leishmania infantum*, which is responsible of human and canine visceral leishmaniasis in the same area [5,6].

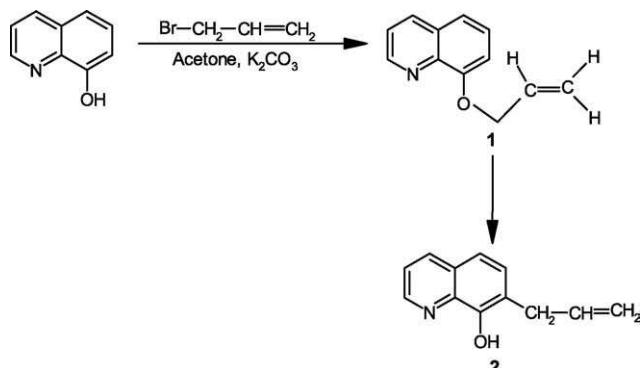
Chemotherapy is limited to the use of pentavalent antimoniais such as sodium stibogluconate (Pentostam), N-methylglucamine (Glucantime) or amphotericin B. These drugs are toxic and difficult to administrate because of their long duration of therapy and high cost [7]. Treatment failure, especially in patients with kala-azar, mucosal leishmaniasis, and diffuse CT, is becoming a common problem in many areas of endemicity [8]. Thus, other drugs that are more effective, less toxic, and easier to use are urgently needed.

A large number of 8-hydroxyquinoline derivatives have already been synthesized and shown to be active agents. Indeed, the 8-hydroxyquinoline derivatives have been reported to possess antitumor [9–11], antimicrobial activities [9,12,13]. However, in spite of the existing interest on this class of compounds, studies on other effects of 8-hydroxyquinoline are lacking and virtually nothing is known about its activity on *Leishmania* species.

Thus, in the course of the research completed in our respective laboratories for the development of heterocyclic

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Scheme 1

compounds of both synthetic and biological interest and the increased interest on new antileishmanial agents due to the lack of effective drug, we reported here the synthesis and the antileishmaniasis activity of a new 8-hydroxyquinoline derivative designed 7-[5'-(3'-phenylisoxazolino)methyl]-8-hydroxyquinoline.

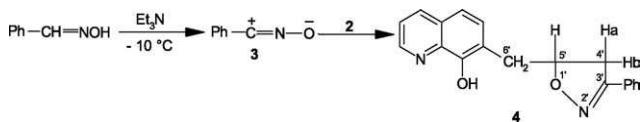
## 2. Experimental procedures

### 2.1. Chemistry

The melting points were given with a Buchi 510 apparatus. Spectra NMR were carried out in  $\text{CDCl}_3/\text{TMS}$  using Bruker spectropocin AC200 (200 MHz for  $^1\text{H}$  62 MHz for  $^{13}\text{C}$ ). The spectra IR were recorded with Perkin Elmer 577, the solid products were pelletized in KBr. The ultimate analysis was carried out by the central service of microanalysis of CNRS Vernaison, France.

### 2.2. 7-[5'-(3'-phenylisoxazolino)methyl]-8-hydroxyquinoline 4

7-Allyl-8-hydroxyquinoline **2** was obtained by a Williamson synthesis from 8-hydroxyquinoline and allyl bromide via allyloxyquinoline **1** which underwent a Claisen rearrange-



Scheme 2

Table 1

Percentage growth inhibition of *Leishmania* promastigotes by the 7-[5'-(3'-phenylisoxazolino)methyl]-8-hydroxyquinoline

Strains	Time (h)	Compound*				Amphotericin B*			
		1.25	2.50	5	10	1.25	2.50	5	10
<i>L. tropica</i>	24	50	62.5	87	95	76.5	94.5	100	100
	48	80.3	92	97	98	87.2	96	100	100
<i>L. major</i>	24	51	81	94	97	69	92.1	100	100
	48	66	98	97	98.2	80.4	95.3	100	100
<i>L. infantum</i>	24	45	78	94.5	100	73	93	100	100
	48	75.2	93	99.5	100	83.4	94.1	100	100

\* Concentration ( $\mu\text{g}/\text{ml}$ ).

ment to yield **2** [13] (Scheme 1). 7-allyl-8-hydroxyquinoline **2** (13.5 mmol) was dissolved in 50 ml of tetrahydrafuran (THF) and added to an etheric solution enclosing the precursor of benzonitriloxide **3** (Scheme 2) prepared as described [14–17]. The mixture was kept at  $-10^\circ\text{C}$  and triethylamine was slowly added. The crude product was then filtered, washed with a mixture of water–chloroform, dried on  $\text{Na}_2\text{SO}_4$  and recrystallized from ethanol [18].

Yield of the product was 85%; m.p. =  $115^\circ\text{C}$ ;  $^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ ): 3.31 (dd, 4'H); 3.19 (dd, 4'H); 5.25 (m, 5'H); 3.38 (d, 6'H);  $^{13}\text{C}$  NMR (62 MHz,  $\text{CDCl}_3$ ): 39.00 (4'C); 81.00 (5'C); 35.00 (6'C); IR/ $\gamma_{\text{C}=\text{N}}$ : 1580  $\text{cm}^{-1}$ . Anal. Calcd. for  $\text{C}_{19}\text{H}_{16}\text{N}_2\text{O}_2$ : C, 75.00; H, 5.26. Found: C, 75.24; H, 5.13.

### 2.3. Microorganisms and growth conditions

The synthetic compound was tested for its antimicrobial activity against the following *Leishmania* species: *L. tropica* (MHOM/SY/86/LIPA154), *L. major* (MHOM/MA/95/9Y) and *L. infantum* (MHOM/FR/78/LEM75) were used in this study. Promastigotes of each species were grown at  $25^\circ\text{C}$  in RPMI-1640 medium (Aqual) supplemented with 15% heat-inactivated fetal calf serum (Aqual) and antibiotics.

### 2.4. Promastigotes drug susceptibility assay

Promastigotes drug susceptibility determinations were made using a previously described direct counting assay based on growth inhibition [19]. Promastigotes were seeded at an initial concentration equivalent to  $1.5 \times 10^6$  promastigotes/ml and allowed to multiply for 8 d in medium alone or in the presence of serial dilutions of drug ranging from 1.25 to 10  $\mu\text{g}/\text{ml}$ . Amphotericin B was used in the same concentrations as a positive control. The protozoal counts were taken using Thomas haemocytometer.

### 2.5. Determination of percentage of growth inhibition

Growth rate (GR) is the relation between the number of viable *Leishmania* at 48 h and the number counted at 0 h. the percentage of growth inhibition (%GI) was calculated with respect to the growth control as follows: %GI =  $1 - (\text{GR}_{\text{extract}}/\text{GR}_{\text{control}}) \times 100$ .

### 2.6. Statistical analysis

Statistical analysis was done using Statview software. The log dose-response curves allowed determination of the con-

Table 2

$IC_{50}$  values after 48 h of promastigotes incubation with 7-[5'-(3'-phenylisoxazolino)methyl]-8-hydroxyquinoline and amphotericin B

Species	$IC_{50}$ ( $\mu\text{g/ml}$ )	Amphotericin B
	Compound	
<i>L. tropica</i>	0.4	0.25
<i>L. major</i>	0.88	0.50
<i>L. infantum</i>	0.62	0.34

centration causing a 50% reduction in the promastigotes number ( $IC_{50}$ ). The standard deviation for the range of  $IC_{50}$  values for the compound for assays on promastigotes was determined by least-square regression analysis of the relative growth rate (% control) against the logarithm of the 8-hydroxyquinoline concentration at  $P = 0.0281$  for *L. tropica*,  $P = 0.2569$  for *L. major* and  $P = 0.0780$  for *L. infantum*. The  $IC_{50}$  for the amphotericin B were at  $P = 0.0211$  for *L. tropica*,  $P = 0.0682$  for *L. major* and  $P = 0.0322$  for *L. infantum*.

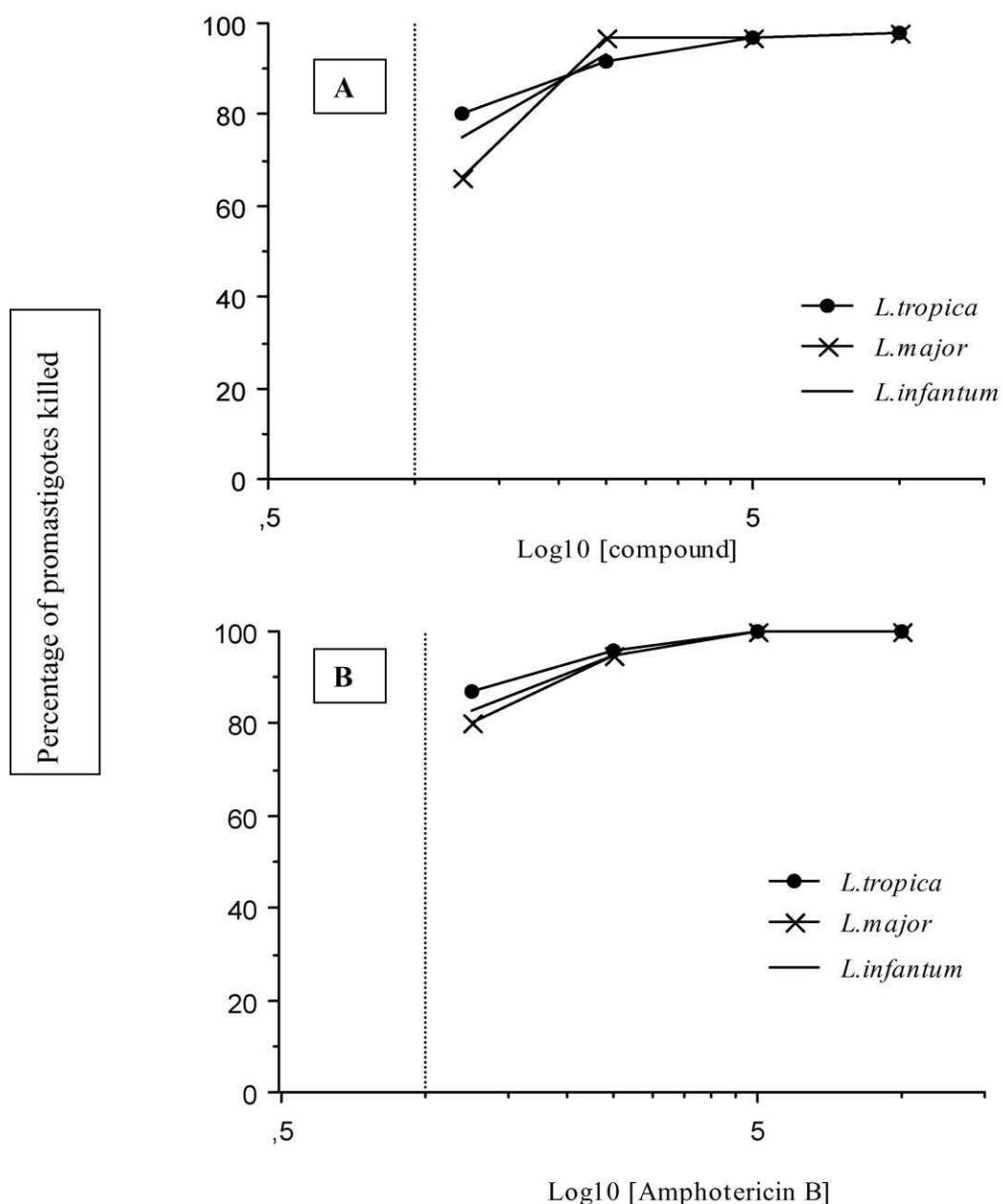


Fig. 1. Effect of different concentrations of the compound and amphotericin B against *L. tropica*, *L. major* and *L. infantum*. (A): Curve A allowed the determination of  $IC_{50}$  of the compound; *L. tropica*:  $Y = 75.344 + 51.81X - 29.243X^2$ ,  $R^2 = 0.999$ ; *L. major*:  $Y = 56.514 + 121.867X + 81.936X^2$ ,  $R^2 = 0.934$ ; *L. infantum*:  $Y = 68.197 + 78.352X - 46.9X^2$ ,  $R^2 = 0.994$ . (B): Curve B allowed the determination of  $IC_{50}$  of the amphotericin B; *L. tropica*:  $Y = 83.26 + 41.52X - 24.829X^2$ ,  $R^2 = 1$ ; *L. major*:  $Y = 74.147 + 66.985X - 41.382X^2$ ,  $R^2 = 0.995$ ; *L. infantum*:  $Y = 78.089 + 54.917X - 33.106X^2$ ,  $R^2 = 0.999$ .

### 3. Results

As shown in Table 1, 7-[5'-(3'-phenylisoxazolino)methyl]-8-hydroxyquinoline inhibited leishmanial growth, a concentration of 10 µg/ml exerted a strong inhibitory effect on promastigotes multiplication of the three species tested (i.e. *L. tropica*, *L. major* and *L. infantum*).

The IC<sub>50</sub> values of the product and the amphotericin B obtained after 48 h for the promastigotes growth of *L. tropica*, *L. major* and *L. infantum* were shown in Table 2 and Fig. 1.

### 4. Discussion

Although members of the class of 8-hydroxyquinoline derivatives have been studied in several areas, they have been reported to have diverse activities. To our knowledge, this is the first report showing an antileishmanial activity of the 8-hydroxyquinoline derivatives. Our results clearly show that 7-[5'-(3'-phenylisoxazolino)methyl]-8-hydroxyquinoline inhibited leishmanial growth at micromolar concentrations. In fact, the assay was based on direct counting of *Leishmania* promastigotes, the result obtained with the compound were homogeneous, and all tested *Leishmania* showed a significant reduction of the promastigotes concentration to 50% after 24 h of incubation in presence of the minimal concentration of the compound (1.25 µg/ml). The percentage of growth inhibition reported in Table 1 showed that a concentration of 10 µg/ml inhibited completely the promastigotes growth of *L. infantum* after 24 h, whereas, only 95% and 97% of promastigotes of *L. tropica* and *L. major* respectively was inhibited. It is of interest to note, that in Mediterranean area, *L. infantum* causes CT [20–24], lethal visceral leishmaniasis in dog and human. Furthermore, this last form is reported more and more frequently in association with the human immunodeficiency virus (HIV) [25].

To further validate the assay, we determined the IC<sub>50</sub> values for the compound and standard antileishmanial drug (amphotericin B). The IC<sub>50</sub> values reported in Table 2 indicate that *L. tropica* was the most sensitive species to the compound as well as to the amphotericin B. *L. major* which causes the ZCL was slightly less susceptible.

In the present study, amphotericin B was used as the standard antileishmanial drug and all the *Leishmania* tested were highly susceptible to this compound. The IC<sub>50</sub> values of amphotericin B are slightly better than those of the 7-[5'-(3'-phenylisoxazolino)methyl]-8-hydroxyquinoline. Amphotericin B is one of the most active antileishmanial agents [26]. In cases of treatment not responding to Pentostam, it was used as a satisfactory substitute [27]. However, drawbacks to amphotericin B include the requirement for infusions, length of therapy, adverse reactions, close laboratory monitoring for potential toxicity and, to some extent cost. In contrast, adequate data on acute toxicity of 8-hydroxyquinoline were rare [28]. Considering the effect of 7-[5'-(3'-phenyl-

soxazolino)methyl]-8-hydroxyquinoline on *Leishmania* strains which is an interesting finding, we can raise the question of whether this compound may be a substitute to antileishmanial agents used classically. But, to be able to do so more studies are needed.

In conclusion, we have described for the first time the antileishmanial activity of the 7-[5'-(3'-phenylisoxazolino)methyl]-8-hydroxyquinoline against *L. tropica*, *L. major* and *L. infantum* promastigotes. We also found that the sensitivity of promastigotes to amphotericin B closely parallels the sensitivity to the compound. Moreover, the compound could have more favorable biological properties, requiring further studies. Thus, more investigations should be done, first to test antileishmanial effect using *Leishmania* amastigote (parasite vertebrate stage) and further investigate in vivo activity in experimentally infected animals, second to better define the spectrum of the antimicrobial activity of this compound and to improve the biological activity by a suitable structural modifications.

### Acknowledgements

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The figure consists of three side-by-side spectrograms. Each spectrogram displays frequency on the vertical axis and time on the horizontal axis. The left panel shows a single speaker's voice, with a prominent, relatively stable vowel-like formant. The middle panel shows two speakers' voices simultaneously. The right panel shows three speakers' voices simultaneously. In all panels, the speakers' voices are represented by distinct vertical bands of energy, often appearing as darker, more solid regions against a lighter background. The overall pattern of energy distribution changes over time, reflecting the movement of the vocal folds and the opening and closing of the oral cavity.

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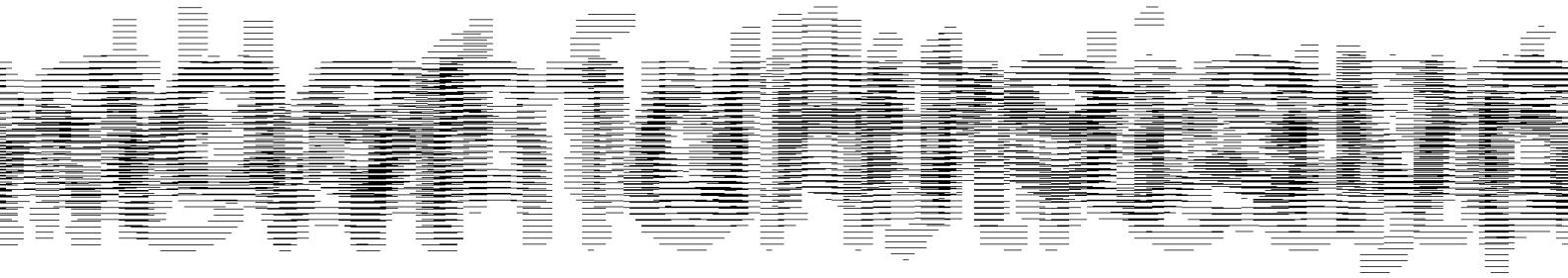
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A spectrogram visualization showing frequency over time. The horizontal axis represents time, and the vertical axis represents frequency. Numerous vertical lines of varying intensities are plotted, representing the presence of specific frequencies at different times. The pattern shows several distinct clusters of energy, likely corresponding to different notes or chords being played.

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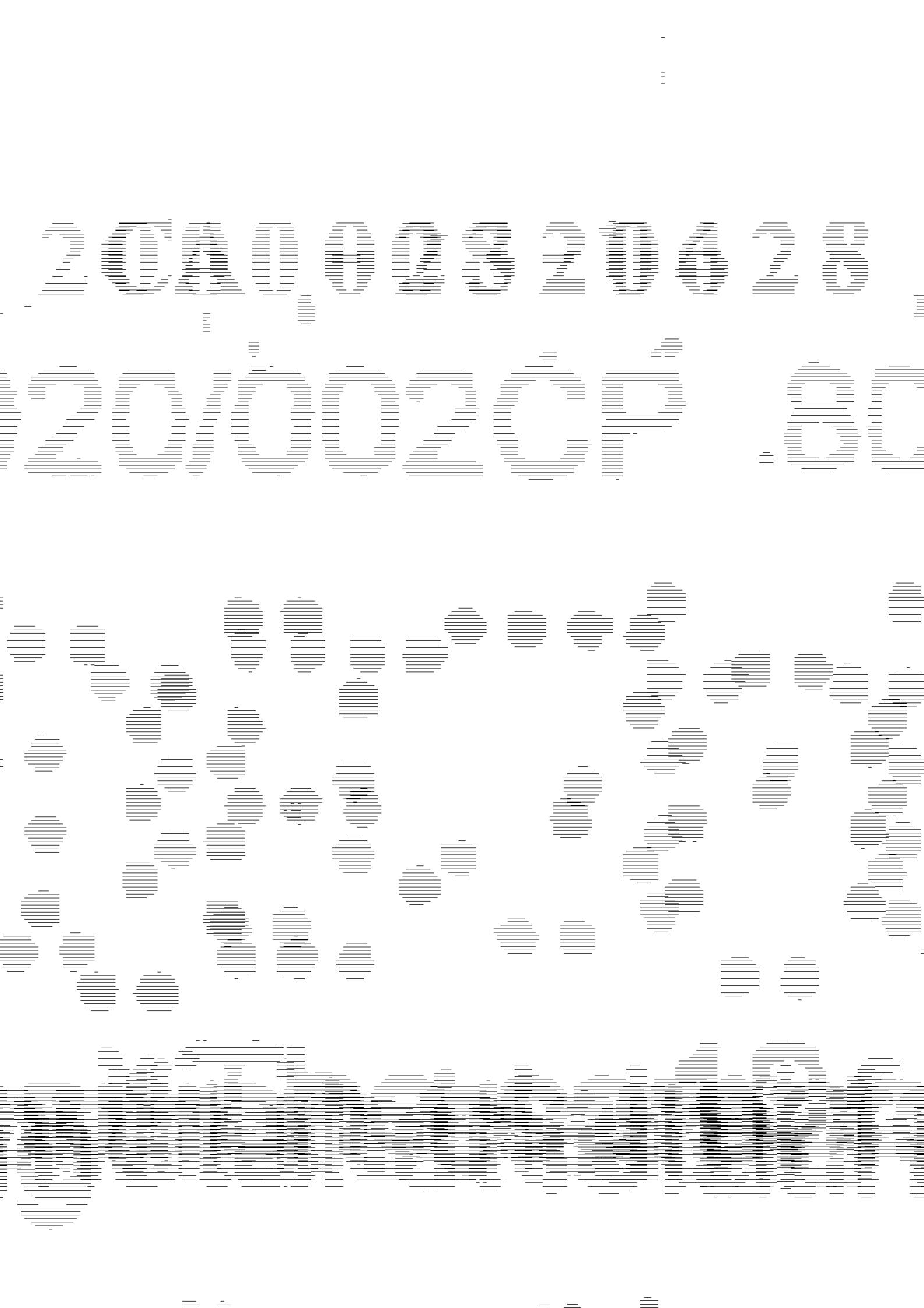
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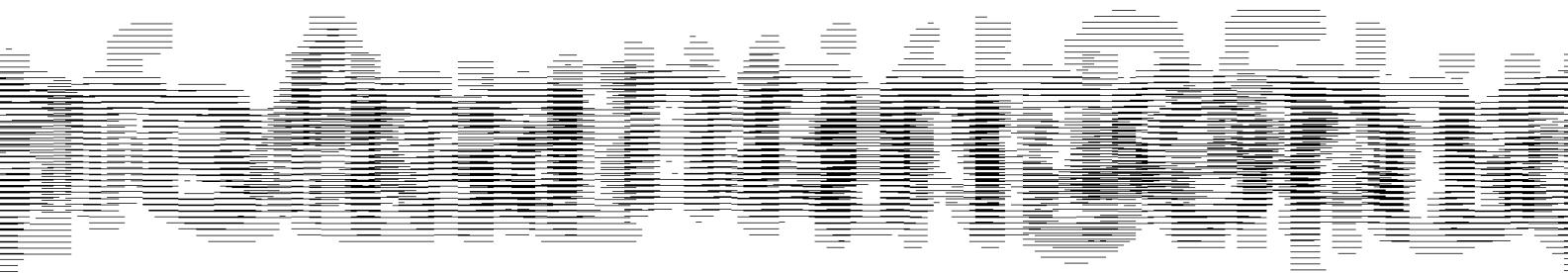
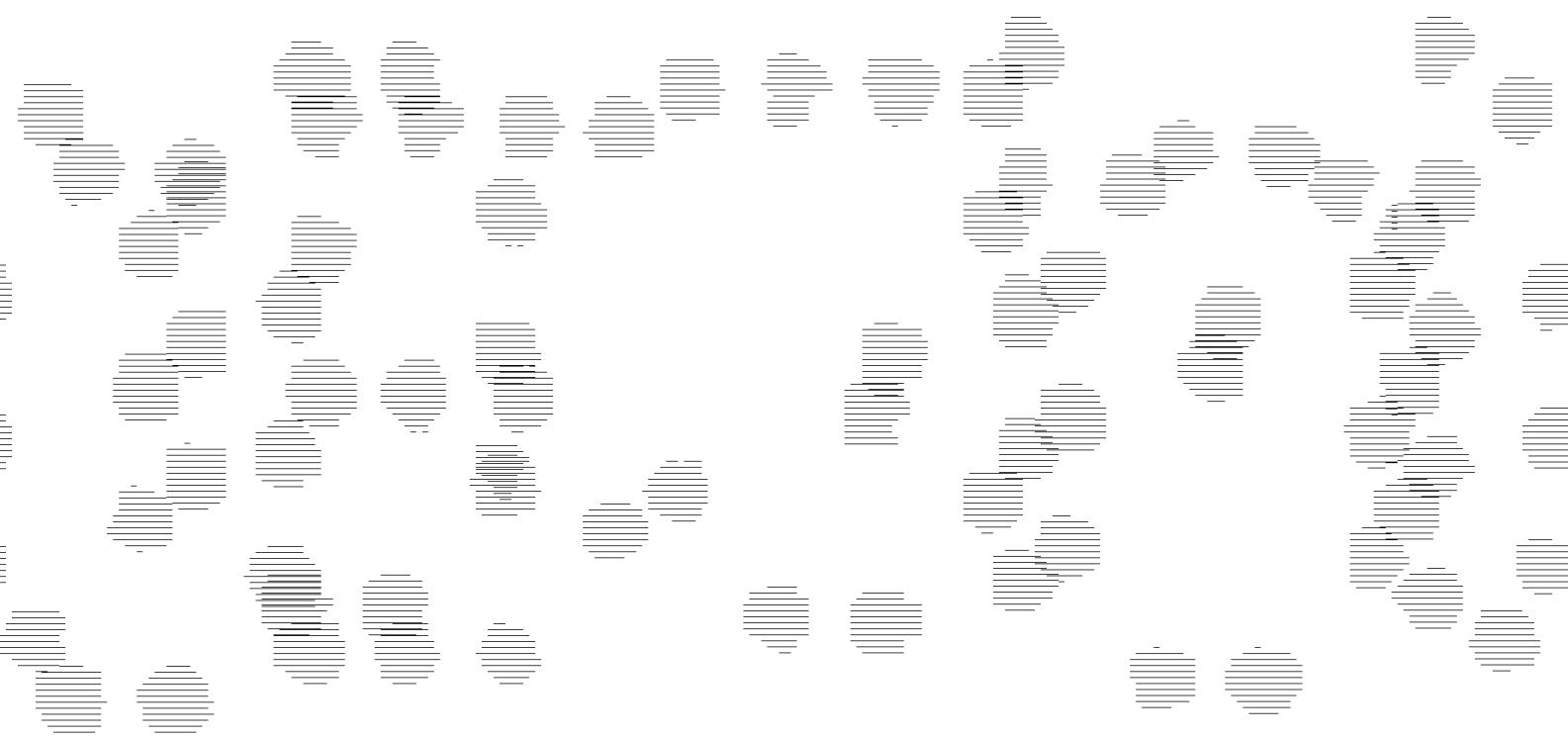
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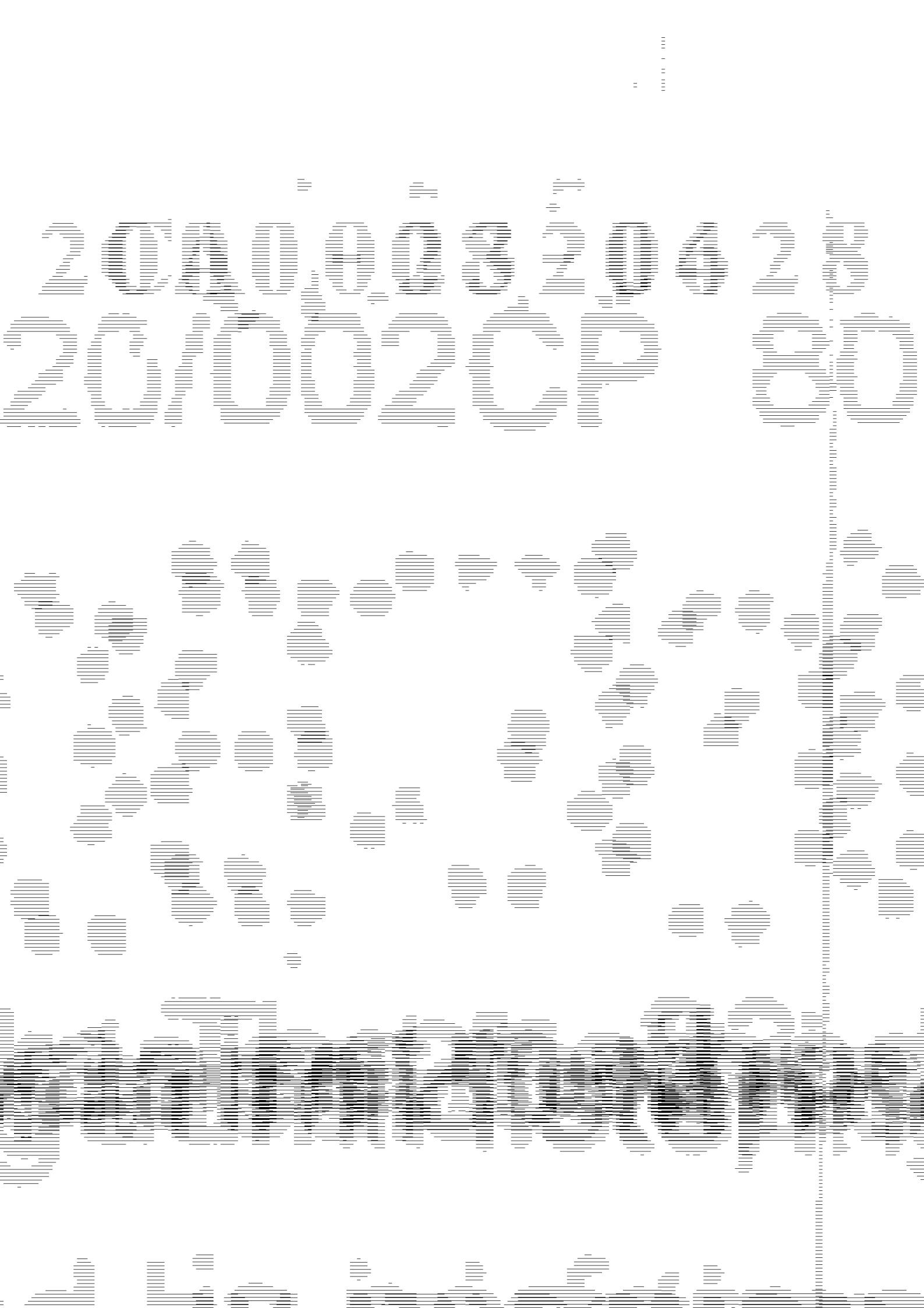
The image consists of a dense collection of black horizontal lines of varying lengths and orientations. These lines are arranged in a way that creates a sense of depth and movement, resembling a stylized map or a microscopic view of a cellular structure. The overall effect is organic and abstract, with no clear text or other discernible features.



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## Expert Review

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# A Review of Poloxamer 407 Pharmaceutical and Pharmacological Characteristics

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**Abstract.** Poloxamer 407 copolymer (ethylene oxide and propylene oxide blocks) shows thermoreversible properties, which is of the utmost interest in optimising drug formulation (fluid state at room temperature facilitating administration and gel state above sol-gel transition temperature at body temperature promoting prolonged release of pharmacological agents). Pharmaceutical evaluation consists in determining the rheological behaviour (flow curve or oscillatory studies), sol-gel transition temperature, *in vitro* drug release using either synthetic or physiological membrane and (bio)adhesion characteristics. Poloxamer 407 formulations led to enhanced solubilisation of poorly water-soluble drugs and prolonged release profile for many galenic applications (e.g., oral, rectal, topical, ophthalmic, nasal and injectable preparations) but did not clearly show any relevant advantages when used alone. Combination with other excipients like Poloxamer 188 or mucoadhesive polymers promotes Poloxamer 407 action by optimising sol-gel transition temperature or increasing bioadhesive properties. Inclusion of liposomes or micro(nano)particles in Poloxamer 407 formulations offers interesting prospects, as well. Besides these promising data, Poloxamer 407 has been held responsible for lipidic profile alteration and possible renal toxicity, which compromises its development for parenteral applications. In addition, new findings have demonstrated immuno-modulation and cytotoxicity-promoting properties of Poloxamer 407 revealing significant pharmacological interest and, hence, human trials are in progress to specify these potential applications.

**KEY WORDS:** adhesion; copolymer; gelation; prolonged drug delivery; rheology.

## INTRODUCTION

*In situ*-forming systems are based on different approaches including solvent exchange, UV-irradiation, pH change and temperature modulation. Among thermosensitive systems, polysaccharides (e.g., cellulose derivatives, xyloglycan, chitosan), N-isopropylacrylamide and poloxamers have commonly been quoted in the literature (1). Poloxamer block copolymers have been introduced in the late 1950s and since then they have been proposed for diverse pharmaceutical applications (2–4). They are listed in the US and European Pharmacopoeia (5). This group of copolymers consists of ethylene oxide (EO) and propylene oxide (PO) blocks arranged in a triblock structure  $\text{EO}_x\text{-PO}_y\text{-EO}_x$ . Their chemical formula is  $\text{HO}[\text{CH}_2\text{-CH}_2\text{O}]_x[\text{CH}(\text{CH}_3)\text{-CH}_2\text{O}]_y[\text{CH}_2\text{-CH}_2\text{O}]_x\text{OH}$ ,  $y$  is higher than 14. Registered trademarks of these copolymers (e.g., Pluronic®, Synperonic® or Tetronic®...) cover a large range of liquids, pastes and solids. They are

synthesised by sequential polymerisation of PO and EO monomers in the presence of sodium hydroxide or potassium hydroxide (6). Chromatographic fractionation can be used to purify the block copolymers. These copolymers have amphiphilic properties characterised by their HLB values (hydrophilic-lipophilic balance), which highly depend on  $x$  and  $y$  values. By varying the values of these parameters, size, lipophilicity and hydrophilicity can be easily modified.

Poloxamer 407 (European Pharmacopoeia 5th Edition), principally available in the registered trademark of Pluronic F127® (BASF laboratories, Wyandotte, USA) and Synperonic F127® (ICI laboratories, Wilton, UK), has a molecular weight of about 12,600 (9,840–14,600) (7)  $x$  and  $y$  are equal to 95–105 and 54–60, respectively. Its HLB is 22 at 22°C (6,8). FDA guide has presented Poloxamer 407 as an “inactive” ingredient for different types of preparations (e.g., IV, inhalation, oral solution, suspension, ophthalmic or topical formulations) (5).

Poloxamer 407 aqueous solutions show thermoreversible properties, which present great interest in optimising drug formulation. A considerable number of patents associating Poloxamer 407 have been registered in the USA www.uspto.gov. The phenomenon of thermogelling is perfectly reversible and is characterised by a sol-gel transition temperature ( $T_{\text{sol}} \rightarrow \text{gel}$ ). Below this temperature, the sample remains fluid though above the solution becomes semi-solid. The thermo-gelation results from interactions between different segments

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of the copolymer (9). As temperature increases, Poloxamer 407 copolymer molecules aggregate into micelles. This micellization is due to the dehydration of hydrophobic PO blocks, which represents the very first step in the gelling process (Fig. 1). These micelles are spherical with a dehydrated polyPO core with an outer shell of hydrated swollen polyEO chains (10). This micellization is followed by gelation for sufficiently concentrated samples. This gelation was attributed to the ordered packing of micelles. The different structures that can be observed with Poloxamer 407 in water or in mixed solvents were mainly elucidated by small angle X-ray scattering (SAXS) (11). According to Liu and Chu (11), a face centred cubic structure is obtained for Poloxamer 407 concentrations in water ranging between 20 and 40%. At higher concentrations (50%), a body centred cubic packing of micelles is observed. These micellar cubic structures and possible micellar entanglements produce high viscosity, partial rigidity and slow dissolution of the gels. Such properties facilitate incorporation of both hydrophilic and hydrophobic drugs.

This article addresses the physical and pharmaceutical properties of Poloxamer 407, specifies possible applications of this copolymer, emphasises development pathways to promote drug delivery (e.g., Poloxamer 407 combination with other polymers, inclusion of liposomes or nanoparticles in a Poloxamer 407 thermoreversible gel...). Moreover, pharmacological and toxicological data on Poloxamer 407 recently highlighted are presented at the end of this review, as well.

## PREPARATION AND PHARMACEUTICAL EVALUATION OF POLOXAMER 407 FORMULATIONS

If the preparation of Poloxamer 407 gel presents no particular difficulty and is approximately the same, *in vitro* evaluation highly differs according to the authors. Table I shows the key-steps, which should be used to develop a formulation.

### Preparation of Thermoreversible Formulation

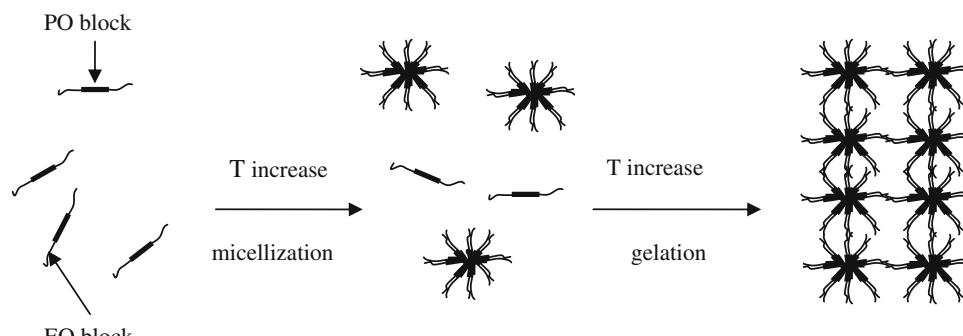
Compared to methods using high temperature, the cold method shows more advantages. This method facilitates poloxamer dissolution and limits possible alterations. Poloxamer 407 is mixed at 4–5°C with other components (e.g., drug) and water previously cooled until a homogeneous

solution is obtained. It is possible to easily prepare 20–30% (w/w) Poloxamer 407 while 35% (w/w) concentration requires placing the Poloxamer 407 solution in a freezer for a few minutes to liquefy the preparation (6,9,12). pH and osmolarity are adjusted to favour formulation stability and satisfactory tolerance (13). Sterilisation by autoclaving (120°C, 15 min, 1 bar) appears compatible and does not significantly alter viscosity characteristics of Poloxamer 407 solution, which is interesting to prepare sterile formulations (i.e., ophthalmic or injectable) (14,15). Nevertheless, no studies have been conducted to specify possible degradation of this copolymer during autoclaving.

### Solubilisation Promotion

The poloxamer copolymers exist in solution as unimers but self-assemble into micelles. CMC and CMT define critical micelle concentration (at a constant temperature) and critical micelle temperature (at a constant concentration), respectively (16,17). At concentrations above the CMC, the copolymer self-aggregates forming micelles of various aspects (18). The hydrophobic PO core can incorporate water insoluble molecules and then protects fragile agents from exterior components (19–23).

Poloxamer 407 facilitates solubilisation of poorly water-soluble molecules like indomethacin (14) or insulin (24–26). Solubility of piroxicam in water was increased by 11-fold by adding 22.5% w/w Poloxamer 407 (27). It appears more effective than polyol or polysorbate. Incorporation of Poloxamer 407 in solid dispersion of poorly water-soluble molecules, like nifedipine or piroxicam, led to marked improvement and thus promoted faster and more complete dissolution (27,28). The wettability determined by the contact angle directly measured by photograph and the dissolution using US Pharmacopoeia dissolution test apparatus were dramatically improved in comparison with cyclodextrin formulation. Solubility of nifedipine was increased 27-fold from that in purified water (28) in the presence of 4% poloxamer (w/v). Intermolecular hydrogen binding between nifedipine and Poloxamer 407 was shown by infrared spectroscopy. It has also been suggested that nifedipine was converted to an amorphous state in crystalline Poloxamer 407 enhancing dissolution. In addition, when a Poloxamer 407 solid dispersion is prepared by the melting method and physical mixing, a smoother nifedipine crystal surface is observed. A higher dissolution rate of phenylbutazone was yielded with solid dispersions containing Poloxamer 407, as well (29).



**Fig. 1.** Schematic representation of the association mechanism of Poloxamer 407 in water.

**Table I.** Key-Steps to Characterise Poloxamer 407 Preparations (Precise Procedures and Bibliography are Detailed in the Text)

	Objectives	Methods
Preparation	<ul style="list-style-type: none"> <li>-Promotion of satisfactory tolerance</li> <li>-Promotion of solubilization for poorly water soluble drugs</li> <li>-Determination of stability (e.g., during storage or after autoclaving) (drug and Poloxamer 407)</li> </ul>	<ul style="list-style-type: none"> <li>-Physical determinations (pH, osmolarity...)</li> <li>-Analytical determinations (UV-Vis spectrophotometry, Chromatography...)</li> </ul>
Sol-gel transition temperature	<ul style="list-style-type: none"> <li>-Optimisation of transition temperature according to the site of administration</li> <li>-Determination of possible alterations relative to the addition of drug(s) or other agents</li> </ul>	<ul style="list-style-type: none"> <li>-Glass tube method</li> <li>-Apparatus using a stirring bar</li> </ul>
Rheological behaviour	<ul style="list-style-type: none"> <li>-Study of the rheological behaviour and possible alterations due to the formulation or to the possible dilution</li> </ul>	<ul style="list-style-type: none"> <li>-Rheological measurements</li> <li>-Flow curve studies (rheogram)</li> </ul>
Gel strength <i>In vitro</i> adhesion	<ul style="list-style-type: none"> <li>-Optimisation of gel consistency</li> <li>-Optimisation of adhesive properties (e.g., addition of adhesive polymers)</li> <li>-Determination of possible alterations due to the addition of drug(s) or other agents</li> </ul>	<ul style="list-style-type: none"> <li>-Oscillatory studies</li> <li>-Apparatus submitting the sample to a mass force</li> <li>-Apparatus determining a detachment force on an inert synthetic support</li> </ul>
Bioadhesion <i>In vitro</i> release	<ul style="list-style-type: none"> <li>-Prolongation of drug residence time at the administration site</li> <li>-Optimisation and control of drug release</li> </ul>	<ul style="list-style-type: none"> <li>-Device determining detachment force on a physiological tissue</li> <li>-Dissolution apparatus (models with or without a membrane) under stirred conditions (static or dynamic device)</li> <li>-Model using freshly excised physiological tissues (Guyot or Franz diffusion cells)</li> </ul>
<i>In vitro</i> diffusion cell using a physiological tissue	<ul style="list-style-type: none"> <li>-Optimisation and control of drug release with a physiological model</li> </ul>	<ul style="list-style-type: none"> <li>-Preliminary administration in animals to research possible intolerance, dilution, leakage, etc...</li> </ul>
<i>In vivo</i> behaviour	<ul style="list-style-type: none"> <li>-Verification of satisfactory behaviour and tolerance with animals</li> </ul>	<ul style="list-style-type: none"> <li>-Determination in the physiological fluid</li> </ul>
Prolongation of the drug release	<ul style="list-style-type: none"> <li>-Assessment of the prolongation in comparison with conventional drug solutions in animals</li> </ul>	<ul style="list-style-type: none"> <li>-Pharmacological effect profile</li> <li>-Irritation score</li> </ul>
Tolerance	<ul style="list-style-type: none"> <li>-Study of local tolerance and general side effects</li> </ul>	<ul style="list-style-type: none"> <li>-Animal experiment (e.g., LD50...)</li> </ul>
Clinical trials	<ul style="list-style-type: none"> <li>-Definition of the benefit to risk ratio</li> </ul>	<ul style="list-style-type: none"> <li>-Pre-clinical and clinical trials in humans</li> </ul>

This Poloxamer 407 property has been combined with other engineering technologies used to enhance dissolution of poorly water-soluble drugs (e.g., spray freezing into liquid) (30–32). Danazol, a water insoluble drug, has been incorporated in powders loaded with various polymers including Poloxamer 407. The spray-micronized porous powder contained amorphous danazol embedded within a hydrophilic excipient. This formulation was characterised by high surface areas and consequently produced a rapid and complete dissolution in water. Scanning electron microscopy showed relevant molecular interactions between danazol and Poloxamer 407. Taking advantage of surfactant properties, Poloxamer 407 has been proposed to inhibit microbial but the pharmaceutical applications of this property do not clearly appear (33,34).

### **Stabilisation Properties**

Poloxamer 407 promotes stabilisation of included drugs in particular proteins (35–40). Poloxamer 407 also reduces the propensity for peptide unfolding in relation with low CMC and lack of electrostatic binding. The three dimensional structure of proteins is better preserved in the presence of Poloxamer 407 (10,35–38). Micro- or nanoparticles were optimised by addition of Poloxamer 407. Incorporating Poloxamer 407 in protein-loaded poly(epsilon-caprolactone) microparticles increased hydrophilicity and prevented micro-particles from aggregating (41). In a similar way, urease incorporated into poly (lactide-co-glycolide) microspheres was stabilised and the retention of its bioactivity was favoured (42). Interesting findings on interactions between Poloxamer 407 and liposomes have been documented, as well. Poloxamer 407 is either co-solubilised with lipid during preparation of liposomes or added afterwards to the already formed liposomes. Including Poloxamer 407 in liposomal delivery system sterically stabilised liposomes and, hence, prolonged their half-life (43). Poloxamer 407 prevented the steric aggregation and the fusion of thawed egg phosphatidylcholine multilamellar vesicles (44). Poloxamer 407 causes significant size-reduction of the multilamellar vesicles using quasi-elastic light scattering. Differential scanning calorimetry and photon correlation spectroscopy have been used to specify Poloxamer 407-liposome interactions (45,46). It has been suggested that Poloxamer 407 might penetrate into the liquid crystalline bilayers. Stabilisation of liposome is directly related with incorporation or adsorption of Poloxamer 407, a phenomenon occurring above the CMT (47). On the contrary, below CMT, Poloxamer 407 molecules remain individual (non-associated or unimers). Poloxamer 407 has also been used with W/O/W multiple emulsion to promote the stability of the preparation (48).

### **Gel Strength**

*In vitro* determination of gel strength gives precious information to formulate a preparation with adequate consistency and strength (49). To determine *in vitro* gel strength, authors placed the gel in a cylinder and submitted it to a mass force using a piston. The time the apparatus takes to sink down a predetermined distance through the formulation is representative of the gel strength (50,51). Gel

strength increases with temperature (i.e., thermally reversible properties) and Poloxamer 407 concentration and may be altered in the presence of drugs or additives. On the one hand, diclofenac, ethanol and propylene glycol weaken Poloxamer 407 gel; on the other hand sodium chloride, sodium monohydrogen phosphate and glycerine do the opposite (50,52). The determination of this parameter necessitates to be completed with adhesive studies to interpret the results.

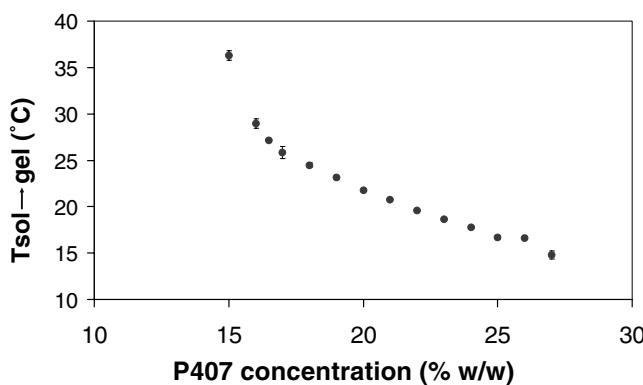
### **Adhesive Properties**

Defining the (bio)adhesive characteristics is of great importance when prolonged residence-time is required, in particular with topical formulations (e.g., rectal, cutaneous or ophthalmic preparations). (Bio)adhesive force generally increases with gel strength and its value is modified by the same parameters (i.e., temperature and Poloxamer 407 concentration). The presence of various solvents or ionic agents may alter the adhesion characteristics of poloxamer formulations as it has been previously noted. Because of its adhesion-promoting action, NaCl has been included in some Poloxamer 407 gels to prolong residence-time in the site of administration (52). Experimental models determine the detachment force using different inert or physiological media and are completed with *in vivo* preliminary behaviour studies conducted with animals.

Determination of bioadhesive characteristics is of great interest because leakage is frequent with rectal forms. Retaining the drug in the rectum is a very important factor in avoiding first-pass hepatic elimination. Le Ray *et al.* (53) have performed (bio)adhesion tests using galls tube made of flexible inert material (polyethylene) or freshly excised rabbit small intestine membrane. A dynamic continuous-flow adhesion cell was developed by the authors. A coloured marker (e.g., Ponceau S) has been incorporated in the Poloxamer 407 gel to facilitate the visualisation of adhesive properties (53). A similar system incorporating tissues cut from ampulla of rabbit rectum has been described with Poloxamer 407 suppositories including diclofenac (52). To verify bioadhesive behaviour *in vivo*, suppositories have been administered in the rectum of rats. *In vivo* migration distance in rectum has been determined by means of a colorant (e.g., Blue n°1 lake 0.1% w/w) and appeared to be an interesting predictive indicator of bioadhesive properties (51). Generally, as mucoadhesive force increased, migration distance decreased and thus the area under the curve in plasma increased. For liquid suppositories formulated with Poloxamer 407/Poloxamer 188 as thermoreversible copolymers and propanolol as the pharmacological agent (51), rectal bioavailability was correlated with mucoadhesive force ( $r = 0.984$ ) and migration distance ( $r = -0.951$ ). Other physiological tissues have been used like rabbit cornea in the case of ophthalmic applications but the number of these studies is very limited (54).

### **Sol→Gel Transition Temperature**

Sol→gel transition temperature increases when Poloxamer 407 concentration decreases (Fig. 2) (3). Simple and easy experimental procedures were extensively carried out to evaluate sol→gel transition temperature ( $T_{sol\rightarrow gel}$ ), which



**Fig. 2.** Transition temperature ( $T_{\text{sol} \rightarrow \text{gel}}$ ) versus P407 concentration (with standard error bar) (3).

represents a key-parameter to define the preparation. These methods use either a glass microcapillary tube or a stirring magnetic bar. In the first one, temperature was generally decreased until the sample became fluid and fell in the lower part of a glass tube ( $T_{\text{gel} \rightarrow \text{sol}}$  considered to be equivalent to  $T_{\text{sol} \rightarrow \text{gel}}$  due to the perfectly reversible properties of thermogelation) (55,56). Although not very sensitive, this “easy to carry out” procedure may point out significant modification of this parameter. Thus, using this method, the authors have detected increment of  $T_{\text{sol} \rightarrow \text{gel}}$  by adding delta-5-amino levulinic acid in a 19% Poloxamer 407 gel (55). With the second method, Poloxamer 407 solution was heated progressively and submitted to a constant stirring. When the magnetic bar stopped moving due to gelation, the temperature displayed was considered to be  $T_{\text{sol} \rightarrow \text{gel}}$  (51,54).

Including drugs or various additives may greatly modify bioadhesive forces, generally in the opposite way (57) (i.e., when  $T_{\text{sol} \rightarrow \text{gel}}$  increases, (bio)adhesive forces decreases and *vice versa*). These agents interfere in the Poloxamer 407 micellization and alter the dehydration of hydrophobic PO blocks (9,58,59). Molecules like diclofenac, ethanol, propylene glycol, and HCl reduce the gel strength and bioadhesive force and increase  $T_{\text{sol} \rightarrow \text{gel}}$  while others do the opposite (e.g., NaCl, Na<sub>2</sub>HPO<sub>4</sub>, NaH<sub>2</sub>PO<sub>4</sub>) (50,52). As ethanol is often added in gel formulation to enhance solubility (60,61), preliminary studies must be carried out to specify influence of ethanol on Poloxamer 407 gel characteristics. Adding sodium alginate, polycarbophil or carbopol has been reported to lower gelation temperature (51), as well. Including complex formulations like cyclodextrins in Poloxamer 407 gel produced an elevation of  $T_{\text{sol} \rightarrow \text{gel}}$  by disturbing the micellar packing and entanglements of Poloxamer 407 (54). The precise influence of conservative agents like disodium edetate remains undocumented despite their extensive use in pharmaceuticals but the use of benzalkonium chloride decreased  $T_{\text{sol} \rightarrow \text{gel}}$  of Poloxamer 407 gel (56).

Conjugation 3, 4 dihydroxyphenyl-L-alanine (DOPA) moieties to Poloxamer 407 block copolymer has been proposed to modify the temperature range of gelation and to offer better bioadhesive properties (17).  $T_{\text{sol} \rightarrow \text{gel}}$  of DOPA-Poloxamer 407 ranged from ~22 to 31°C according to the concentration. In comparison,  $T_{\text{sol} \rightarrow \text{gel}}$  of non-modified-Poloxamer 407 pharmaceutical preparations generally range from 15 to 35°C.

Nevertheless, these methods show significant limitations because the sol→gel transition is operating within a large temperature interval, thus the experimental set-up greatly influences  $T_{\text{sol} \rightarrow \text{gel}}$  value. Other methods like rheological one present more interests.

### Rheological Properties

Rheological behaviour represents a key-part in the formulation of Poloxamer 407 preparations and must be fully studied. This study determines the temperature range and consequently detects, with great sensitivity, interactions between additives and Poloxamer 407. A new interesting angle has been proposed to optimise *in vivo* behaviour thanks to rheological tests. In particular, this method offers key-information on thermogelation kinetic (62,63). Most of the authors determined either rheograms (i.e., shear stress *versus* shear rate or flow curve studies) or oscillatory studies. Rheograms have applications in the development and the control phases whereas oscillatory tests give more information on the structure and the behaviour of Poloxamer 407 in the research studies.

#### 1. Flow curves studies (shear stress *versus* shear rate)

Couettetype or cone/plate geometry rheometers are classically used (9,64,65). The rheological behaviour is either Newtonian or non-Newtonian depending on the temperature. Below  $T_{\text{sol} \rightarrow \text{gel}}$ , Poloxamer 407 solutions exhibit a Newtonian behaviour. Viscosity slightly increases with temperature. Above  $T_{\text{sol} \rightarrow \text{gel}}$ , the rheological type becomes non-Newtonian and is characterised by a significant measured yield value (49,58,59).  $T_{\text{sol} \rightarrow \text{gel}}$  has been determined as the inflection point on the curve of the apparent viscosity as a function of the temperature (49). Nevertheless, the calculated value highly depends on the shear stress used during the experiment (9). The rheograms were better fitted by the Herschel–Bulkley equation [Eq. (1)] than by the Ostwald equation [Eq. (2)], underlining a plastic behaviour.

$$\tau - \tau_0 = K \dot{\gamma} \quad (1)$$

$$\tau = K \dot{\gamma} \quad (2)$$

$K$  is a constant of the model.  $\dot{\gamma}$ ,  $\tau$  and  $\tau_0$  represent the shear rate, the shear stress and the yield value, respectively.

Using a 20% (w/v) Poloxamer 407 solution, the calculated yield value and the  $K$  value tended to increase with temperature, whilst the  $n$  value tended to decrease; then these three values tended to plateau out above 25°C.

#### 2. Oscillatory studies

Oscillatory studies determine the visco-elastic properties by subjecting the sample to a sinusoidal shear stress  $\tau$  (2,9,64,65). Oscillatory measurements are conducted in the linear viscoelastic range (non destructive dynamic conditions) (66). From these different values, two moduli can be calculated, the storage modulus  $G'$  and the loss modulus  $G''$ , which are characteristic of the stored elastic energy and the viscous dissipated energy, respectively.  $T_{\text{sol} \rightarrow \text{gel}}$  was chosen as the temperature at which both moduli were equal, reflecting similar elastic and viscous properties (“ $G'G''$  crossover”

criterion) (9). However, the crossover of  $G'$  and  $G''$  might be frequency dependant. To avoid this problem, Cabana *et al.* (67) extrapolated for Poloxamer 407 the approach of Winter, which was originally developed for chemically crosslinked gels. A linear relationship was observed between Poloxamer 407 concentration and  $T_{sol \rightarrow gel}$  in the 18–25% Poloxamer 407 concentration range (64).  $T_{sol \rightarrow gel}$  temperature decreased with Poloxamer 407 concentration (32 and 19°C for 18 and 25% Poloxamer 407 concentrations in phosphate buffer, respectively) (64). It was observed that both the maximal force of detachment and the work of adhesion ( $W$ ), using tests on the rabbit rectal mucous membrane, increased with  $G'$ . A nonlinear regression between  $W$  and  $G'$  led to a logarithmic equation ( $r = 0.889$ ) which might be a useful tool to predict the gel behaviour *in vivo* (2). In addition, the yield values resulting from oscillatory studies were very similar to those obtained with other methods, confirming the plastic behaviour of poloxamer solutions (9). Using rheological procedures, it has been demonstrated that additives like  $Na_2CO_3$ , PEG400, short-chain fatty acids and propanediol 1,2 dramatically modified  $T_{sol \rightarrow gel}$ , whereas lidocaine or morphine did not significantly influence this parameter (2,9,49,66) (Table II).

However, like other *in vitro* evaluations, the interest in rheological studies is restricted by the dilution, which imme-

dately follows the administration. To better simulate *in vivo* dilution that may hinder appropriate gelation, Chang *et al.* (58) have mixed 3 ml of liquid clomitiazole/ Poloxamer 407 vaginal formulation with 0.9 ml of simulated vaginal fluid. This dilution has drastically decreased the elastic modulus. To mimic the properties in the eye, a 25- $\mu$ l volume of Poloxamer 407 solution has been mixed with 7  $\mu$ l of simulated tear fluid to study the influence of possible dilution that may alter gelation after ocular application in fluid state (i.e., before *in situ* gelation). Thermogelation was satisfactory only with the 25% Poloxamer 407 solution whereas the lesser concentrated formulation lost this fundamental property (64).

In addition, rheological studies give interesting information on the kinetics of gelation and in particular on gelation time. Gelation time describes time-dependent changes occurring during the gelation. It is defined as the time after which the elasticity modulus becomes higher than the viscosity modulus at a constant temperature above the transition. At 37.2°C, time-dependent changes of the viscoelastic properties showed great increase of both the elastic and viscosity moduli and reached a plateau after 50–150 s according to the formulation (58). The shorter the gelation time is, the lesser the risk of dilution with physiological fluid and the possibility of drastic drainage are. Such information is of great interest to

**Table II.** Influence of Drugs or Various Agents on the Transition Temperature of Poloxamer Formulation

Drug or Agent	Influence on the Transition Temperature (Method Used)	
	±Influence on Gel Strength and Mucoadhesive Force	
Benzalkonium chloride	Transition temperature ↓ (glass tube)	(56)
Cysteine	Transition temperature ↓ (magnetic bar method)	(3)
Carbopol	Gel of strength ↑ Transition temperature ↓ (magnetic bar method) Gel strength and mucoadhesive force ↑	(51)
Delta 5-aminolevulinic acid	Transition temperature ↑ (glass tube)	(55)
Diclofenac	Transition temperature ↑ (magnetic bar method) Gel strength and the bioadhesive force ↓	(50)
Ethanol	Transition temperature ↑ (magnetic bar method) Gel strength and the bioadhesive force ↓	(52)
Hydrochloric acid	Transition temperature ↑ (magnetic bar method) Gel strength and the bioadhesive force ↓	(52)
Hydroxypropylcellulose	No significant modification of the Transition temperature (magnetic bar method) and gel strength	(51)
Lidocaine	No significant modification (rheological method)	(66)
Morphine	No significant modification (rheological method)	(9)
Poloxamer 188	Transition temperature ↑ (magnetic bar method)	(3)
PEG 15000	Transition temperature ↑ (glass tube)	(56)
Polycarbophil	Transition temperature ↓ (magnetic bar method) Gel strength and mucoadhesive force ↑	(51)
Polyvinylpyrrolidone	No significant modification of the Transition temperature (magnetic bar method) and gel strength	(51)
Propylene glycol	Transition temperature ↑ (magnetic bar method) Gel strength and the bioadhesive force ↓	(52)
Short-chain fatty acid	Transition temperature ↓ (rheological method)	(49)
Sodium alginate	Transition temperature ↓ (magnetic bar method) Gel strength and mucoadhesive force ↑	(51)
Sodium chloride	Transition temperature ↓ (magnetic bar method) Gel strength and mucoadhesive force ↑	(52)
Sodium dihydrogen phosphate	Transition temperature ↓ (magnetic bar method) Gel strength and mucoadhesive force ↑	(52)
Sodium monohydrogen phosphate	Decrease of the Transition temperature ↓ (magnetic bar method) Gel strength and mucoadhesive force ↑	(52)
Vitamin B12	Transition temperature ↓ (glass tube)	(56)

understand phenomena like dilution, which may occur in the time interval before complete gelation, leading to a local drainage of the formulation (i.e., a rapidly gelled formulation may face the less elimination from the site of application) (58).

### In Vitro Drug Release Study

*In vitro* kinetics of drug release from Poloxamer 407 formulations in a receptor medium appear to be essential for the study of Poloxamer 407 preparations (Table III). These tests serve as a comparative tool during the development of topical formulations. Drug release into the receptor medium is consecutive to either drug diffusion in the Poloxamer 407 gel matrix or Poloxamer 407 dissolution. Drug diffusion is predominant with membrane models. Conversely, dissolution is the major mechanism observed with membraneless models. In addition, it has been supported that they may be valuable in predicting *in vivo* behaviour but the results highly depend on the experimental set-up (75). Drug release and Poloxamer 407 dissolution are studied *in vitro* by means of various experimental devices, generally close to the US Pharmacopoeia dissolution apparatus under stirring systems like paddles or rotating discs. Experimental conditions vary according to formulation and pharmaceutical use and can be presented as follows (Table IV):

1. Agitation system usually 20–100 rpm (12,89).
2. Analytical detection of the released drug (or Poloxamer 407).

Various methods for determining the drug released have been described such as UV-Vis spectrophotometry or liquid chromatography by periodically withdrawing aliquots from the receptor medium. UV-Vis detection presents the possibility of a direct determination using a peristaltic pump and flow-through cell system (90) but may present lack of specificity. The use of radiolabelled molecules has been developed for complex formulations (e.g., liposomes of oligonucleotide included in a Poloxamer 407 gel) (80). The Poloxamer 407 dissolution is indirectly measured by weight. The amount of dissolved gel is gravimetrically determined by removing the gel container, blotting it dry and quickly weighing it (12). An alternative procedure consists in evaluating poloxamer dissolved using a colorimetric assay (80). The colorimetric method is based on a water-insoluble complex with cobalt(II) thiocyanate (91). The poloxamer complex concentration is determined by UV absorbance (624 nm) after extraction by acetone. Size exclusion chromatography has recently been developed for determining Poloxamer 407 concentration (91).

#### 3. Nature of the receptor medium

The temperature is chosen according to the pharmaceutical use of the formulation (i.e., close to physiological fluids: 33–34°C and 37°C for ophthalmic and injectable formulations, respectively) (13). The formulation is generally gelled above  $T_{sol} \rightarrow$ gel before the experimental set-up (68). Otherwise, the formulation might rapidly be dissolved in the aqueous receptor medium, in particular with a membraneless set-up. Nevertheless, this experimental preheating does not

mimic *in vivo* conditions. According to possible dilution resulting from incomplete gelation, *in vitro* drug release must be completed by rheological studies to guarantee that gelation is operating during a restricted time interval, as it has previously been advised.

An isotonic aqueous buffer is generally used as the receptor medium (e.g., phosphate or HEPES buffer). pH is chosen according to the site of administration. pH modification may alter drug solubility and thus lead to change drug release kinetics. Being closer to physiological fluids, artificial receptor media like artificial tears are classically retrieved (13,86). Veyries *et al.* (15) used a gel–gel diffusion system in which the donor compartment was filled with a vancomycin Poloxamer 407 gel and the receptor compartment was filled with the same but drug-free formulation. This model was close to *in vivo* transfer of the drug from the gel matrix to a prosthetic or a bone tissue. Hydrophobic media like isopropyl myristate have been quoted for topical formulations. Such a medium presents the advantage of not dissolving Poloxamer 407 formulation. Thus, it does not necessitate any separating membrane or preheating of the sample (60,61,70). In addition, the use of a hydrophobic receptor medium has been presented as one, which better considers factors influencing drug partitioning (70).

The volume of the receptor medium, generally being large, ensures sink conditions, which raise critical issues because of its remoteness from physiological conditions. Limiting the volume of receptor medium better mimics *in vivo* conditions especially for injectable formulations. In this case, a receptor medium whose volume is limited (e.g., 1 ml) is carefully layered over the surface of the gel (68,74). Then, the tube is submitted to a rotating or shaking system.

Other characteristics, like surface area or quantity of formulation placed in the apparatus, are predominant factors in the exchange phenomena between both compartments but these factors are rarely detailed in the studies. Modified Keshary–Chien diffusion cells have been used for a sublingual formulation of triamcinolone and a surface area of 0.79 cm<sup>2</sup> has been selected to simulate *in vivo* conditions (87).

#### 4. Membrane

Drug release from Poloxamer 407 gel differs according to whether or not a membrane is used. Thereby, this choice depends on the objectives and the indications of Poloxamer 407 formulations.

##### a. Membrane-free set-up

When both the drug and Poloxamer 407 are expected to dissolve and be released, the membrane free models are generally used, in particular for ophthalmic or injectable formulations (12,13,25,68,81). In membrane free models, two phenomena are involved; on the one hand the Fickian diffusion of the drug and on the other hand the dissolution of poloxamer. Water uptake and subsequent dilution of Poloxamer 407 micelles generally govern drug release into the receptor solution. Most of the times, drug release and Poloxamer 407 dissolution follow a zero-order kinetics due to the rapid dissolution of Poloxamer 407 in the receptor fluid (12,88). A linear relation between time *versus* the weight of dissolved gel and the released pilocarpine was retrieved *in vitro* with a Poloxamer 407 [25%]/Pilocar-

**Table III.** *In Vitro* Release Studies Conducted with Poloxamer 407 (P407)

Drug	Route	<i>In Vitro</i> Release Model Receptor Medium	Other Evaluations	Authors
Melanotan-I, P407: 25%	Peritoneal	Membraneless Isotonic phosphate buffer	*Guinea pigs Plasma concentration	(68) Bhardwaj 1996
Diclofenac, P407: 15%	Rectal route	Semi-permeable membrane tube model Phosphate buffer	*Sprague-Dawley rats Plasma concentration	(69) Park 2003
Flurbiprofen P407: 20–30%	Topical	Membraneless Isopropyl myristate Membrane model (Cellulose acetate) Phosphate buffer		(70) El Gendy 2002
Human epithelial growth factor included in cyclodextrin complex, P407: 16%	Ophthalmic	Membrane (inert cellulose)	*New Zealand rabbits	(54) Kim 2002
Ibuprofen included in a liposomal formulation, P407: 25%	Epidural	Phosphate buffer	Lachrymal concentration *Permeation through the dural membrane	(71) Paavola 2000
Ibuprofen (or ketoprofen), P407: 25%	Topical	Membrane inert (cellulose) Buffer	*Permeation freshly-excised rat skin *Plasma concentrations (rats)	(72) Takahashi 2002
Interleukin-2, P407: 30/35%	Intraperitoneal	Membraneless	*Mice (Cytotoxicity using a murine malignant cell line)	(73) Johnston 1992
Insulin included in nanoparticles, P407: 20–30%	Subcutaneous	Phosphate buffer Membraneless	*Wistar rats	(25) Barichello 1999
Insuline + enhancers, P407: 20%	Sublingual	Water bath Membraneless Phosphate buffer solution	Serum glucose levels *Wistar rats Serum glucose levels	(74) Morishita 2001
Ketoprofen	Topical	Isopropyl myristate soaked membrane		(75) Proniuk 2001
Ketoprofen, P407: 20/25/30%	Topical	Membraneless		(61) Chi and Jun 1991
Lidocaine, P407: 25%	Epidural	Isopropyl myristate Membrane (inert cellulose) Phosphate buffer	*Wistar rat (paw-pressure test)	(76) Paavola 1995
Lidocaine microparticle, P407: 25%	Intra-sciatic nerve	Semipermeable membrane bags Phosphate buffer	*Sprague-Dawley rats Nociceptive blockade test	(77) Chen 2004
Lidocaine P407 : 20/25/30%	Injectable	Membrane model (cellulose dialysis tube) Phosphate buffer	<i>In vivo</i> biocompatibility (rats)	(78) Ricci 2005

Mitomycin C, P407: 20/25/30%	Intraperitoneal		*Rabbit and mouse Toxicological studies	(79) Abe 1990
Oligonucleotide pdT 16 within liposomes, P407: 20/27%	Ophthalmic	Membraneless		(80) Bochot 1998
Paclitaxel, P407: 20%	Intratumoral	HEPES buffer Membraneless Phosphate buffer	*Adult mice (B16F1 murine melanoma model)	(81) Amiji 2002
Pilocarpine, P407: 25%	Ophthalmic	Membraneless Artificial isotonic tear	*New Zealand rabbits Pupil diameter	(13, 82) Desai 1998
Pilocarpine, P407: 25%	Ophthalmic	Membrane model (dialysis cells) Simulated tear fluid	*Rabbits	(83) Miyazaki 2001
Piroxicam + enhancers, P407: 10–25%	Topical	Membrane model (cellulose) Sörensen's phosphate buffer	Pupil diameter *Permeation through rat skin	(84) Shin 1999
Propanolol , P407: 15%	Rectal	Membrane model Phosphate buffer	*Sprague–Dawley rats Bioavailability study	(51) Ryu 1999
Propranolol HCl, metronidazole and cephalexin P407: 20/25/30/35%	No specified	Membraneless		(12) Moore 2000
Quinine, P407: 18%	Rectal	Deionized water Membrane model (dialysis tubing) US Pharmacopoeia dissolution apparatus	*New Zealand rabbits Plasma concentrations	(85) Fawaz 2004
Short-chain fatty acids, P407: 17–20%	Rectal	Deionized water Membrane model (cellulose) Distilled water		(49) Charrueau 2001
Timolol, P407: 15/20/25%	Ophthalmic	Membraneless Artificial isotonic tear	*New Zealand rabbits Conc. in aqueous humor	(86) El-Kamel 2002
Triamcinolone acetonide P407: 20%	Sublingual	Cellulose membrane (Keshary-Chien Diffusion cell) Propylene glycol:phosphate buffered solution	*Permeation through freshly excised buccal tissue (rat)	(87) Shin 2000
Urease P407: 20/30/35%	Not specified	Membraneless Phosphate buffer		(88) Fults 1990
Vancomycin P407: 25%	Injectable	Membrane model (cellulose) P407 receptor medium	*Wistar rat <i>In vivo</i> kinetic studies	(15) Veyries 1999

**Table IV.** *In Vitro* Drug Release Study—Key Parameters

Study	Key Parameters	References
Agitation System	20–100 rpm	(12, 89)
Analytical determination of the released drug	UV-Visible spectrophotometry (non specific) Liquid chromatography Radiolabelled molecules Gravimetical determination	(80, 90) (12, 80, 91)
Analytical determination of the Poloxamer dissolution	Colorimetric method (cobalt(II) thiocyanate) Size exclusion chromatography	
Nature of the receptor medium	Aqueous *Non buffered (Rectal preparation) *Buffered (pH close to physiology) *Composition close to physiological parameter (e.g., artificial tears) Isopropyl myristate (topical) Static (“dissolution apparatus”) or dynamic (ophthalmic preparation) system Temperature: 33–34°C (ophthalmic preparation) or 37°C	(13, 68, 86)
Membrane free models	Volume of sample and receptor medium (surface area) Applications: Ophthalmic formulations (+ +) Profile of drug release: zero-order kinetics (dissolution in aqueous medium) or Higuchi kinetics (non aqueous medium) Limits: influenced by the agitation system	(12, 13, 25, 61, 68, 70, 81, 88, 89)
Models with synthetic membrane	Applications: Topical formulations (+ +) Semi-permeable membrane (e.g., inert cellulose, cut off ~6,000–8,000) Profile of kinetics: Higuchi equation	(15, 51, 69, 71, 72, 75, 76, 83, 84, 85, 87, 92, 93)
Models with excised physiological membrane	Permeation study (rectal, topical formulations) Evaluation of enhancers	(72, 87, 92, 93)

pine[1%] gel (13). This experimental set-up was performed to select a combination of Poloxamer 407/[Methylcellulose or Hydroxypropylcellulose] gel among various mucoadhesive polymers–Poloxamer 407 combinations (13). Similar findings were obtained with various drugs like propranolol HCl, metronidazole and cephalexin (12): a zero-order process was observed up to a 90% Poloxamer 407 release.

This procedure has the disadvantage to greatly be influenced by stirring speed and experimental set up (e.g., receptor medium temperature). Anderson *et al.* (89) have linearly related gel dissolution to agitation rates ( $r = 0.9955$ ) and for a 30% Poloxamer 407 gel concentration, approximately 5% of the marker (i.e., nile blue chloride and toluidine blue dyes) release was due to diffusion at the interface while the remainder was released during gel dissolution.

In the case of a topical formulation, the authors used a membraneless model with a lipophilic receptor medium like isopropyl myristate. Isopropyl myristate presents high solvent action for poorly hydrosoluble drug and immiscibility with Poloxamer 407. Release of drugs like flurbiprofen or ketopro-

fen was an inverse function of Poloxamer 407 concentration, higher concentrations of Poloxamer 407 showing a slower diffusivity of the drug (70). The drug release profile obeys to the Higuchi equation ( $r > 0.997$ ) due to insolubility of Poloxamer 407 in the receptor medium (61,70). The amount released per unit area of exposure is function of square root of time. A linear relationship was found between the logarithm of the apparent diffusion coefficient and Poloxamer 407 concentration (%w/w) with ketoprofen Poloxamer 407 gel (61).

#### b. Models with a membrane

Such models have been fully published and involve injectable or topical formulations in which the gel is not expected to be quickly dissolved in physiological fluids (15,71,72,75,76,84,87) but have also been proposed for liquid suppositories (semi-permeable tube containing the liquid suppository secured with a thread and placed in the dissolution medium) (51,69,85). In this model, a membrane, which is permeable to the drug isolates both media (i.e., formulation and receptor medium, respectively) and thus

presents the advantage of avoiding direct dissolution of Poloxamer 407 in the receptor medium. Also described are plastic dialysis placed in a donor compartment (simulated tear fluid) developed for ophthalmic preparations (83). Nevertheless, in this case, the use of a plastic membrane is not relevant because it does not mimic the physiology of the lachrymal system and should not be recommended (54).

Semi-permeable inert cellulose (or cellulose acetate) membrane allows drug release in the receptor medium without any diffusion of poloxamer by means of an appropriate molecular weight cut-off ( $\sim 6,000\text{--}8,000$ ) (51). The cumulative amount of drug released per unit area generally approaches the Higuchi square root equation, especially when the quantity released does not exceed 50–60% (72,83). Nevertheless, this model efficiency highly depends on whether or not additives are present, because these one have been held responsible for significant alteration. For example, kinetics close to zero-order have been retrieved with quinine rectal Poloxamer 407 gel containing 20% propylene glycol (85) and with a diclofenac sodium rectal Poloxamer 407/Poloxamer 188 gel (69).

In the case of a transdermal formulation, a membrane model has been developed using excised physiological membranes, which are mounted on cells (e.g., Valia-Chien diffusion cell) (92,93). Predictive interest of *in vitro* release has been retrieved with ibuprofen and ketoprofen (72). These authors compared a 25% Poloxamer 407 gel containing non-steroidal anti-inflammatory drugs with a xyloglycan thermoreversible gel. Both *in vitro* (cellulose membrane) and *ex vivo* (excised skin) releases were correlated with plasma concentration and suggested that xyloglycan allowed better prolongation of drug release and pharmacokinetic profile. A similar release profile was retrieved between a cellulose membrane and freshly excised buccal tissues for a 0.1% triamcinolone acetonide formulation prepared with 1% carbopol and 20% Poloxamer 407 (87). Freshly excised membranes offer interests to better predict *in vivo* performance and evaluate the permeation promotion due to the inclusion of enhancers (87,92).

## PHARMACEUTICAL FORMULATIONS EFFICIENCY

*In vitro* evaluation serves as a research tool in the course of developing formulations and thus, considerable works have been conducted in order to specify the characteristics of Poloxamer 407 preparations. *In vitro*, zero-order release is researched although *in vivo* an optimisation of the pharmaceutical or pharmacokinetic profiles is expected. Results and potential interests highly depend on the route of administration. The evaluation is based on pharmacokinetic profile, tolerance study and experimental model (miming a pathology using animals), generally conducted in comparison with other formulations without Poloxamer 407. For each route of administration, we present the key-studies carried out with simple Poloxamer 407 preparations, which generally show little improvement, then we emphasise the advantage of more complex formulations like either combination with other agents (e.g., mucoadhesive agents, enhancers or other Poloxamers) or inclusion in a targeted vehicle (e.g., liposome, nanoparticle...).

## Oral Formulation

Poloxamer 407 has been used to formulate sublingual or oral preparations. Sublingual triamcinolone acetonide formulation containing Poloxamer 407 (20%) and carbopol (1%) presents promising results according to higher viscosity and bioadhesiveness, which may increase residence-time in the sublingual sites (94). Freshly excised porcine buccal tissue was mounted on a diffusion cell to study the influence of enhancer inclusion. The enhancement factor was calculated showing that propylene glycol when included in Poloxamer 407 (20%)/Carbopol(1%) highly promoted triamcinolone permeation (87). A buccal delivery system combining Poloxamer 407 (20%) and sodium deoxycholate showed significant improvement of bioavailability (Enhancement ratio of 1.74). Unfortunately, most of the time, sublingual administration shows limitations due to unsuitable adhesion on account of salivation, tongue movement and swallowing (87) and consequently did not present clear advantages.

Besides sublingual application, some authors made use of Poloxamer 407 adhesive properties to lengthen residence-time of agents in the gastro-intestinal tract. A Poloxamer 407 (19%)/delta-5-aminolevulinic acid thermosetting gel characterised by a Tsol $\rightarrow$ gel of 26°C was assessed for photodynamic therapy of gastrointestinal tract lesions (55). Good adhesion in the oesophagus with efficient diffusion of the drug into the mucosa was observed in the mouse using an optical fibre spectrofluorimetric method.

## Rectal Formulation

Retaining the drug at the rectal site after administration is a very important factor in avoiding first-pass hepatic elimination and thus, enhances bioavailability. A thermosensitive liquid-type suppository could alleviate the discomfort felt by patients, which may promote compliance. A (17–20%) Poloxamer 407 rectal liquid formulation of short chain fatty acids was developed to treat distal ulcerative colitis or short bowel syndrome. Despite interesting properties observed during the determination of adhesive properties and prolongation of the *in vitro* release test carried out with Guyot-Herman's device, no information has been given on the *in vivo* efficiency of this formulation (49).

With liquid suppositories, the new trend corresponds to add extra-adjuvants (e.g., combination of poloxamers, bio-adhesion enhancers) to significantly increase drug absorption. Synergetic associations may use other poloxamers to optimise Tsol $\rightarrow$ gel, bioadhesive agents or various promoters. Nevertheless, Poloxamer 407 may interact with other polymers. Incompatibility has been observed between hydroxypropylmethylcellulose and Poloxamer 407 which has been resolved by the addition of propanediol 1, 2. Using combination of poloxamers (e.g., 15% Poloxamer 407/15% Poloxamer 188) offers an interesting perspective to optimise formulation and presents an adequate 30–36°C Tsol $\rightarrow$ gel (i.e.,  $35.7^{\circ}\text{C} \pm 0.3$ ) (50). Absence of leakage and easy insertion were obtained using coloured markers like blue lake. The position of the coloured poloxamer gel in the rectum did not change with time and was related with the formulation bioadhesive force, itself reinforced by the presence of sodium chloride (50). Poloxamer 407 bioadhesive

properties can be significantly reinforced by adding mucoadhesive polymers like, hydroxypropylcellulose (HPC), polyvinylpyrrolidone (PVP), carbopol, polycarbophil or sodium alginate (51). Rectal mucous lining consists of oligosaccharide chains with sialic acid. The hydrophilic groups such as the OH group can strongly bind to oligosaccharide chains resulting in a considerable bioadhesive force. In this study, bioavailability of propanolol increased from 62% (in a Poloxamer 407/Poloxamer 188 solution) to 84.7% when alginate was added to the preparation. However, no relationship was observed between the value of  $T_{sol \rightarrow gel}$  and the corresponding AUC. Nevertheless, the interest to increase bioadhesion has been highly debated and remains questionable. Excessive adhesion can damage rectal mucus and adding a bioadhesive agent to poloxamer liquid suppositories may induce irritations. Irritation scores were rated using light microscopy after cervical dislocation of rectum from sacrificed rats. Alginate seems to induce only minimal irritation compared to other bioadhesive polymers (51). Satisfactory rectal tolerance of Poloxamer 407 was confirmed by Park *et al.* (69), as well.

Synergistic combinations with Poloxamer 407 have been proposed and included promoters like unsaturated fatty acids (e.g., oleic acid, eicosapentaenoic acid, docosahexaenoic acid) known to markedly enhance bioavailability of proteins like insulin. Adding these promoters in a thermoreversible Poloxamer 407 [20%] rectal solution produced significant increase in plasmatic absorption compared to a simple Poloxamer 407 formulation (Bioavailability: 36.3–38.7 *versus* 5–6%) (24).

### Ophthalmic Formulation

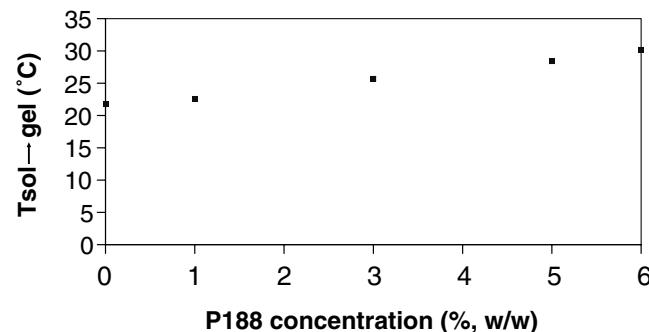
Classically, the *in vivo* ophthalmic evaluation consists in comparing the thermoreversible Poloxamer 407 formulation *versus* a non-thermoreversible solution in the New-Zealand rabbits. Many procedures have been proposed to study the efficiency of ophthalmic formulations such as determination of a pharmacological response (83), recovery in the case of an experimental model, specific dosage in tears (lachrymal kinetics) or in aqueous humor (intraocular bioavailability) (54,95).

Poloxamer 407 presents satisfactory inertia for ophthalmic mucosa. Recently, an injectable intraocular lens formulation containing 25% Poloxamer 407 produced no inflammatory response or toxicity in the conjunctiva, iris, vitreous or retina using New-Zealand white rabbits (96). To reduce gradual elimination of Poloxamer 407 after intraocular injection, a photoinitiator was added to induce irreversible gelation and resolved the problem of the poloxamer slowly dissolving in the lens capsule (96). *In vivo* studies of contact-time were performed with two human subjects using Poloxamer 407 gel containing fluorescent microparticles (Fluorescein-5-isothiocyanate-labeled Sephadex G25 superfine<sup>®</sup>) to facilitate visualisation. Contact-time increased as Poloxamer 407 concentration increased (16–24 and 30–55 min for 18 and 25% Poloxamer 407, respectively) (64). By promoting the solubility and stability of drugs like indomethacin, Poloxamer 407 increased the aqueous humour concentrations of indomethacin and led to a faster resolution in an immunogenic uveitis model (95). A micellar solution of low concentration of

Poloxamer 407 (2%) with pilocarpine exhibited prolongation of miotic activity and increase in AUC compared to Poloxamer 407-free solutions (97). The reduction of the volume (e.g., 20  $\mu$ l instead of 60–100  $\mu$ l) administrated in the eyes generally improved the AUC. Indeed, limitation of reflex tearing reduces lachrymal drainage and thus favours drug residence in the pre-ocular structure (82).

Nevertheless, the interest of simple Poloxamer 407 preparations for ophthalmic applications is highly questionable. One of the critical points corresponds to  $T_{sol \rightarrow gel}$ , considering average room temperature. Because the 20–25°C range of sol $\rightarrow$ gel temperature generally described with Poloxamer 407 formulations does not appear suitable, the optimal  $T_{sol \rightarrow gel}$  should range in the 26–29°C interval to facilitate fluid administration. In addition, the concentrations that are necessary to obtain thermoreversible properties are high (i.e., 20–30%) and present the drawback to increase osmolarity due to the other components of the formulation (i.e., drug, additives...). Edsman *et al.* observed in two human subjects fast elimination of fluorescent particles after ocular application of a (14–25%) Poloxamer 407 gel due to the dilution that occurred in the eye (64). Similar results have been retrieved with morphine ophthalmic Poloxamer 407 gel (4). According to Miyazaki *et al.* (83), alternative thermoreversible polymers may offer better perspectives.

Nevertheless, Poloxamer 407 remains a promising polymer for ophthalmic use when combined with other polymers (e.g., other poloxamers or thickening agents). Addition of Poloxamer 188 led to optimise the  $T_{sol \rightarrow gel}$  in order to target a specific range of temperature (Fig. 3) (3). An application of such combination is illustrated in a work published by Wei *et al.* (65). In this work, this combination promoted local availability of a radiotracer (technetium-99 m-diethylentriamine pentacetate acid (Tc99 m-DTPA)) in relation with the optimisation of  $T_{sol \rightarrow gel}$  from 17.5°C [Poloxamer 407 21%] to 26.5°C [Poloxamer 407/Poloxamer 188 (21%/10%)]. This ophthalmic formulation produced a three-fold increase in corneal residence-time compared to simple Tc99 m-DTPA solution using single photon emission computed tomography in rabbits (65). Including bioadhesive polymers like methylcellulose increased local bioactivity about 2.6-fold compared to isotonic solutions (82). The addition of a thickening agent enables decreases in Poloxamer 407 concentration with similar results. Thereby, a Poloxamer 407/methylcellulose (15%/3%), like Poloxamer



**Fig. 3.** Temperature of transition ( $T_{sol \rightarrow gel}$ ) *versus* Poloxamer 188 concentration 1(w/w) in a combination with 20% (w/w) P407 formulations (with standard error bar) (3).

407 (25%) solution enhanced ocular bioavailability of timolol in albino rabbits 2.4- to 2.5-fold compared to an aqueous solution (86).

Adding Poloxamer 407 in pharmaceutical encapsulation (e.g., nanocapsule, liposomes) or inclusion (cyclodextrin) leads to significantly improve the drug release profile. Biodegradable polyisobutylcyanoacrylate nanocapsules of pilocarpine that have been incorporated into a Poloxamer 407 gel enabled a longer miotic response than a simple dispersion without Poloxamer 407, in the albino rabbit eye (98). Liposomes included in a Poloxamer 407 thermogelling gel have been developed for ophthalmic applications with an oligonucleotide: pdT16 (80). Results indicated that both the free oligonucleotide and the liposomally encapsulated oligonucleotide were released from Poloxamer 407 gel in the receptor medium following first order kinetics. The 27% Poloxamer 407 gel showed significantly slower dissolution of Poloxamer 407 compared to the 20% Poloxamer 407 formulation (T<sub>1/2</sub>: 5.1 *versus* 1.45 h). Similarly, the release of free oligonucleotide was slowed down with the 27% Poloxamer 407 gel (T<sub>1/2</sub>: 2.45 h) *versus* the 20% Poloxamer 407 gel (T<sub>1/2</sub>: 1.28 h) and the total drug release was prolonged (T<sub>1/2</sub>: 4.81 h for the liposomes dispersed in the 27% Poloxamer 407 gel) (80). In contrast, the dispersion of liposomes within a dilute 2% Poloxamer 407 gel resulted in a considerable leakage of the oligonucleotide whereas this polymer exerted an interesting inhibition on liposome aggregation at a higher concentration (27%). This observation may explain the lack of pharmacological improvement after ophthalmic administration due to *in vivo* dilution of Poloxamer 407 (80).

Suitable combinations of copolymer with Poloxamer 407 may be determined with the objective of optimising Tsol→gel. A formulation containing Poloxamer 407/Poloxamer 188 (16%/14% ratio) and rhEGF (human epithelial growth factor)/HP-beta-CD (hydroxy-beta-cyclodextrin) complex was selected to obtain a Tsol→gel of 35.5°C. The concentrations of rhEGF in tears followed a first-order elimination and this formulation produced a drastic increase in lachrymal AUC compared to a rhEGF simple solution (i.e., 1.6–3.8 times greater) in New Zealand rabbits (54).

### Topical Formulation

A reversible state-transition (sol↔gel) property enables a cool solution to flow onto skin. It contacts intimately to generate a non-occlusive gel at body temperature. Poloxamer 407 gel absorbs sweat gland secretion (70). Local tolerance was described as very satisfactory (15) even in the treatment of thermal burns (99). In contrast, little added values were found to reduce the bacterial colonisation of human skin despite a limitation of biofilm formation (100).

Gels and ointments containing non-steroidal anti-inflammatory drug (e.g., flurbiprofen, indomethacin, ketoprofen, piroxicam) have been formulated with Poloxamer 407. Most of the time, these preparations included a thickening agent like carbopol or cellulose derivatives (70,72,93,101,102). Despite solubilisation enhancement of Poloxamer 407 by means of micelle formation, a non-aqueous solvent like ethanol may be added to formulate a topical preparation with insoluble drugs like ketoprofen

(insolubility at a concentration greater than 3%) (61). Ethanol (0–20%) increased linearly ketoprofen coefficient of diffusion and this observation was attributed to the decreased viscosity of the ketoprofen gel due to ethanol. A naproxen/ Poloxamer 407 formulation was compared to IV bolus administration. A bioavailability of 2% and a significant concentration in the epidermis, dermis and muscle tissue beneath gel application site were retrieved in dogs (103). These concentrations were much more important than after oral administration. In particular, the muscle *versus* blood ratio after administration of the topical dose was found to be significantly higher than that of the oral dose (interesting targeting effect). The serum half-life of naproxen was increased when topical administration was performed compared to IV bolus, which may be ascribed to the large accumulation of drug in the skin. Accumulation of methotrexate into muscle beneath the application site was observed with a Poloxamer 407 preparation (20% w/w) combined with 16% ethanol, as well (104).

In order to yield an increase in the transdermal permeation, ketoprofen and piroxicam preparations have been formulated with Poloxamer 407 and enhancers, which produced an increase in the anti-inflammatory activity with animal models (i.e., carragenin-induced rat foot swelling) (102). Including absorption promoters in Poloxamer 407 gel led to increase the permeation of drugs like ketoprofen. Four terpenes significantly increased ketoprofen *in vitro* diffusion through hairless mouse skins (105). Combining terpene derivatives with ethanol led to a synergistic effect on enhancing drug activity. Similar *in vitro* observations were retrieved with piroxicam or indomethacin Poloxamer 407 gels combined with enhancers like limonene or linoleic acid using excised rat skin (92,101). Shin *et al.* have studied piroxicam (1%) included in a Poloxamer 407 topical gel. An enhancing factor of 2.84 was obtained with polyoxyethylene-2-oleyl ether as the absorption promoter of piroxicam using *in vitro* Franz diffusion cells fitted with excised rat skin. These observations were correlated with *in vivo* results determining the extent of inhibition of swelling in the carragenin-induced rat foot-swelling model (93,102). The relative bioavailability was increased approximately 1.8-fold compared to that in the enhancer-free gel. Nevertheless, the use of enhancers is limited for tolerance issues, use which remains highly debated despite significant increment of bioavailability.

### Nasal Formulation

At the present time, we could not recommend Poloxamer as a suitable vehicle for nasal formulation. Slow release kinetics at the nasal absorption site may present a disadvantage due to a longer exposure to peptidase as it was observed with ACTH in Poloxamer 407 solution. Addition of Poloxamer 407 [20%] to formulations containing enhancers like sodium glycocholate and bacitracin did not induce any increase in the bioavailability (106).

### Injectable Formulation

At low temperature, the Poloxamer 407 solution containing the drug to be released is a fluid solution that can

easily be injected intramuscularly into the body via a syringe. At a higher temperature (above the transition temperature at body temperature), the formulation becomes a gel and drug release can be significantly prolonged (89).

Local injectable Poloxamer 407 formulations were assessed to promote slow drug release directly at the site of interest. Poloxamer 407 thermosetting gel reduces drug degradation in muscle tissue and contributes to slow down release into plasma. Thus, the kinetic profile shows, after IM administration, a broader plasmatic peak, which is lower and delayed, compared to classical formulations. Poloxamer 407 thermosetting gel improved stability of the included drug in particular for peptides and proteins like insulin or deslorelin (25,107). Paclitaxel included in a 20% Poloxamer 407 formulation was injected intratumorally in melanoma-bearing mice. The initial tumour growth was delayed by 67% and the tumour volume doubling time was increased by 72% relative to saline control (81). A lidocaine (2%) formulation injected into the vicinity of the sciatic nerve has been developed with Poloxamer 407 (25%) as the thermally reversible polymer or cellulose derivatives as the thickening agent. The Poloxamer 407 formulation produced best the prolongation in concordance with the *in vitro* release tests. A correlation between *in vitro* cumulative lidocaine released and *in vivo* antinociceptive response (paw-pressure test) has been found ( $r = 0.987\text{--}0.997$ ,  $p < 0.01$ ). Tolerance analysis was satisfactory; only mild-or absence-of irritation of skeletal muscle tissue was detected after administration. No sign of marked inflammatory change was found in the muscle tissue surrounding the sciatic nerve (81). After intraperitoneal injection in rats, the bioavailability of urease activity was increased twofold by the use of 33% Poloxamer 407 compared to a simple aqueous solution (36). Similar findings were retrieved with a Poloxamer 407 (33%)/Interleukin 2 formulation after intra-peritoneal injection (37,73). Poloxamer 407 has been used as drug carrier for cells like autologous porcine auricular chondrocytes. This suspension turns out to be biodegradable and biocompatible in relation with satisfactory tolerance of Poloxamer 407 (108). More recently, Poloxamer 407 (21%) was used as vehicle to enhance transgene expression and to reduce virus dissemination after viral infusion into tumours in mice showing a potential to be used in viral gene therapy (109).

Thickening agents improve drug retention in Poloxamer 407 gel. Methylcellulose and hydroxypropylcellulose (HPC) significantly slowed down *in vitro* dissolution of gel and release of a analogue of  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH) whereas polyvinylpyrrolidone did the opposite. These results were correlated with *in vivo* observations conducted with guinea pigs following intraperitoneal administration. *In vivo* studies underlined a 71% greater AUC for the gel compared to a simple solution (68). Likewise, using mice, the combination of 17% Poloxamer 407 with HPC increased the bioavailability and elimination half-life of granulocyte colony-stimulating factor (G-CSF) leading to mobilise more rapidly to the spleen hematopoietic stem cells compared to a saline formulated G-CSF (110).

Nevertheless, as previously described with other routes of administration, new axis of research corresponds to evaluate this copolymer in new formulations like liposomes or microspheres included in Poloxamer 407 gels. Liposomes

containing ibuprofen were included in Poloxamer 407 gel. Based on *in vitro* release, liposomal Poloxamer 407 gel significantly slowed down ibuprofen release compared to a simple solution, Poloxamer 407 gel or liposomes (71). Permeation experiments were carried out using porcine lumbar membrane mounted on donor tubes. In addition, cumulative amounts of ibuprofen permeated in 24 h were in agreement with *in vitro* release. Including drug-loaded microspheres in Poloxamer 407 gel limits possible fusion of these microspheres and, hence, increases their mechanical stability and possible clogging of the needle. Prolongation of drug action is generally observed for localised or generalised effects. Lidocaine-loaded poly(D,L-lactic acid) microspheres included in a 25% Poloxamer 407 gel yielded longer duration of sensory and motor block than a simple solution of lidocaine-loaded microspheres or lidocaine in Poloxamer 407 gel (77). Polylactic-co-glycolic acid nanoparticles dispersed in Poloxamer 407 gel (20–30%) produced the longest lasting hypoglycaemic effect compared to other presentations (25). The insulin AUC in plasma was increased up to 3.1-fold compared with a simple solution. Buffering the formulations allows modulating the release rate of drugs whose solubility is pH-dependent, such as insulin. Insulin release from various Poloxamer 407 gels, whether buffered or not, showed linear release *versus* time but was accelerated with the use of a pH 7 buffered gel (in comparison with results of pH 5.5 or pH 4-buffered gels) (25). In this study, nanoparticles loaded with insulin dispersed in Poloxamer 407 gels optimised the prolongation of insulin release in the receptor medium. A zero-order profile suggested erosion as the main mechanism overriding the release of insulin from the nanoparticles.

## PHARMACOLOGICAL PROPERTIES

Besides its galenic use as a thermoreversible drug carrier, Poloxamer 407 possesses specific pharmacological actions quoted in the literature. Temporary vascular occlusion obtained with poloxamer gel may have therapeutic application (111). In a similar way, data suggested that oil-in-water Poloxamer 407-based microemulsion might be an effective agent to extract lipophilic drugs (i.e., bupivacaine) from plasma in case of surdosage (112). Moreover, Poloxamer 407 has been the subject of considerable pharmacological research on various fields like immunity, cell multiplication, cancers and lipid metabolism.

## Promotion of Cell Multiplication

Poloxamer has been proposed as a carrier for osseous graft material either topically or systemically (113,114). It facilitates early collagen synthesis and microcirculation. A Poloxamer 407 hydrogel enveloping a non-biodegradable endoskeletal scaffold shows interesting prospects to generate tissue of intricate shape (e.g., human ear) (113,115). Very low concentrations of Poloxamer 188 and Poloxamer 407 increase attachment and growth of human gingival fibroblasts and may have applications in early post surgical wound healing (116). Nevertheless, further studies are necessary to better understand this action and its possible application.

### Immuno-Modulation Properties

Poloxamer 407 does exert significant modulation of immune function and promotes the action of various agents. Poloxamer 407 with chitosan exerts an additive or synergistic effect on the systemic and mucosal immune response obtained with tetanus toxoid after intranasal administration (117). The mechanism remains unspecified and the authors hypothesised that the poloxamer gel increased antigen delivery through reverse gelation properties and promoted antigen stability. Nevertheless, a specific action of Poloxamer 407 clearly appears in different studies. Significant enhancement of both cell-mediated and humoral immune response like rate of wound or burn healing has been retrieved in many research papers. Various poloxamers such as Poloxamer 407 increase transgene expression and enhance expression of plasmid DNA in mouse skeletal muscle (6). This faculty to promote gene transfer has been observed using various experimental models (118). Poloxamer 407 improved gene transfer to myogenic cells using virus like particles conjugate. The presence of Poloxamer 407 increased the expression level of beta-galactosidase in human cervical cancer cells lines (C-33A) by four times (119).

In addition, poloxamers in general strongly adsorb onto the surface of hydrophobic nanospheres like poly-styrene, poly(lactide-co-glycolide) acid, poly(phosphazene), poly(methylmethacrylate) and poly(butyl 2-cyano-acrylate) (8). Pre-treatment of microspheres seemed to favour drug distribution to specific target-areas. Small colloidal particulate (polystyrene microsphere) coated with Poloxamer 407 led specifically to redirect drugs to the sinusoidal endothelial cells of bone marrow following IV administration in the rabbit. This effect is believed to be due to the stearic repulsive effect generated by the hydrophilic OE segment that alters protein interaction and particle-microsphere adhesion.

Poloxamer 407 coated nanoparticles offer an interesting alternative to reduce significant hepatic uptake. The precise mechanism of retargeting is still unclear but the copolymer work as "camouflage" to avoid macrophage recognition (120,121). A low degree of distribution in various organs—especially the liver—was found. Therefore, the critical stage influencing biodistribution known as opsonisation is optimised.

Coverage consists in incubating Poloxamer 407 solution overnight at room temperature with a suspension of nanoparticles, the suspension then is centrifuged at high rpm. Different percentages of surface coverage (0–100%) may be obtained by incubation with increasing volumes of 1% Poloxamer 407 (122). The adsorption of copolymer onto the surface is determined by measuring the surface layer thickness and surface potential by means of photocorrelation spectroscopy. Adsorption isotherm studies depend on particle diameter (123). The hydrodynamic layer thickness (Delta) is calculated as follows (122):

$$\text{Delta} = (da - di)/2$$

da: nanoparticle diameter after poloxamer adsorption  
di = initial diameter

14 C-poly(methylmethacrylate) nanoparticles coated with various poloxamers (Poloxamer 407, Poloxamer 908) led to long-acting concentration in the melanoma and breast cancer models in mice in relation with reduction of Kupffer cell uptake (124). Coating another type of nanoparticle (i.e., poly(lactide-co-glycolide) with the same poloxamers produced extended half-life of the Rose Bengal (plasma marker) (125). Polystyrene nanoparticles of 40 nm in diameter with a Poloxamer 407 surface coverage approximately above 25% showed improved circulation profiles. This finding has been explained by a reduction in the amount of serum protein absorbed, particularly for high molecular weight proteins (122).

These immuno-modulation properties have been proposed to solve biocompatibility problems that raise critical issues in pharmaceutical development. Poloxamer 407 reduced the neutrophil activation observed with microspheres of various compositions [e.g., (poly(DL-lactic acid), poly(epsilon caprolactone), poly(methylmethacrylate)]. Pre-treatment of microspheres with Poloxamer 407 was achieved by incubation with a 2% Poloxamer 407 solution in water for 1 h followed by four washes in water (126). Two amphiphilic block copolymers, Poloxamer 181 (Pluronics® L61) and Poloxamer 407 (Pluronics® F127) have been combined under the name of SP1017® for gene transfer to achieve local or systemic production of therapeutic proteins, as well (127). This combination has been evaluated to promote the expression of plasmid DNA and the authors asserted a 1,000-fold safety margin of this combination in case of IM administration.

### Cytotoxic Promotion

Poloxamer can interact with multidrug resistant cancer cells resulting in chemosensitisation of cancer cells. The pharmacological target(s) of this cytotoxicity remain(s) unclear. This anticancer action includes complex mechanism involving fluidisation of the cellular membrane, ATP depletion, inhibition of drug efflux and reduction in GSH/GST detoxification activity. Preliminary studies with animals showed that the cytotoxic activity of antineoplastic agents is increased by two to three orders of magnitude (doxorubicin and daunorubicin). The promotion of other families of antineoplastic agents is still unknown (79,128). Phase I clinical trials are in progress with formulations combining Pluronic® mixture (0.25% Poloxamer 181/2% Poloxamer 407) and doxorubicin. This system formulated in an isotonic buffered saline solution (SP1049C) is composed of mixed micelles (22–27 nm) incorporating doxorubicin. Plasma pharmacokinetics was prolonged with the Pluronic® formulation compared to conventional doxorubicin (Half-life 50 versus 30 h, respectively).

### TOXICOLOGICAL DATA

Recent observations contradict previous works asserting optimal tolerance of Poloxamer 407 whatever the site of administration used (14,78). Actually, parenteral administra-

tion of Poloxamer 407 leads to serious alterations of lipid metabolism and on renal filtration though carried out with animals using high dosage after intra-peritoneal (IP) administration. Few are the studies that have determined Poloxamer 407 concentration in physiological fluids. Authors used a colorimetric assay based on formation of a Poloxamer 407-cobalt thiocyanate assay to determine Poloxamer 407 in plasma and tissue homogenates in rat. Furthermore, a preferential uptake of Poloxamer 407 in hepatic tissue compared to renal tissue was detected and may explain alterations in lipid metabolism (14,78). An elimination half-life based on urinary excretion data was estimated to 20.9 h (36). Data on pharmacokinetic profile especially in humans remain unspecified. Such parameters like plasma concentrations, tissular distribution, metabolism or elimination may offer relevant information on potential risks related to Poloxamer 407 administration.

### Lipid Metabolism Alteration

Recent papers have stressed the potential action of Poloxamer 407 on lipid metabolism (130–141). IP administration of Poloxamer 407 induces significant hypertriglyceridemia and hypercholesterolemia. Poloxamer 407 interferes with catalytic activity of 3-OH-3 methylglutaryl Coenzyme A reductase, which represents the key-step of cholesterol biosynthesis. Poloxamer 407 alters the level of heparin releasable and intracellular lipoprotein lipase, as well. The necessary Poloxamer 407 dose to produce a hyperlipidemic effect is commonly 0.5–1 g/kg IP. Nevertheless, Blonder *et al.* (139) retrieved in New-Zealand rabbits that received therapeutically relevant doses of Poloxamer 407 (137.5 mg/kg body weight) significantly increased serum triglyceride and cholesterol with a maximum increase 2 h after the subcutaneous injection. This effect was dose-dependent and did not appear with lower Poloxamer 407 doses. Experimental models using animals (mouse, rat, rabbit) were intensively developed either to screen antilipidic drugs or to mimic artherogenic models (130–141). Further studies are necessary to clarify the impact of this metabolic alteration, which remains highly debated. Long term (1 year) administration of Poloxamer 407 to mice affected neither hepatic total cholesterol concentration nor alanine/aspartate activity. Poloxamer 407 administration did not result in either morbidity or mortality when compared to control mice (133,142).

### Alteration of Renal Filtration Capacity

Li *et al.* suggested that Poloxamer 407 might alter the filtration capacity of the kidney (129). Abe *et al.* (79) reported severe renal toxicity of Poloxamer 407 in rabbits and mice (LD50 in mice between 1.7 g and 5.0 g/kg body weight). Previous works performed with an inulin/Poloxamer 407 gel, IM administrated, showed a 60% decrease in the apparent plasma clearance of inulin by altering glomerular filtration (143,144). Nevertheless, according to Pec *et al.* (36), Poloxamer 407 being injected intra-muscularly or subcutaneously in humans would not interfere with the renal elimination of a protein cleared predominately by the kidney.

### CONCLUSION

Poloxamer 407 has been presented as an universal thermogelling vehicle for various administrations considering its local inertia and prolongation of drug residence. Though introduced more than fifty years ago, Poloxamer 407 still arouses the interest according to the numerous works that are conducted with this copolymer in very various scientific fields. In particular ophthalmic, topical and injectable preparations have been fully developed and present the advantages of promoting stabilization and water dissolution of many pharmacological drugs. In fact, most of the poloxamer 407 formulations were restricted by a drastic *in vivo* dilution leading to the loss of thermogelation. Numerous interactions between the copolymer and other components of the formulation (i.e., pharmacological drug) or additives may result in drastic alterations and need to be carefully supervised with appropriate *in vitro* or *in vivo* studies. New trends suggest combining Poloxamer 407 with other copolymers (e.g., thickening agents, other types of poloxamers...). Modified poloxamer may lead to industrial development by optimising gelling characteristics with very low polymer concentrations. Incorporation of liposomes or nanoparticles in poloxamer gel offers interesting perspectives, as well. In addition, in case of parenteral administration, if on the one hand recent studies have highlighted possible perturbation of lipidic profile or renal filtration, on the other hand other studies present Poloxamer 407 as promoting antineoplastic action by means of immuno-modulation. Nevertheless, further studies are still necessary to better understand Poloxamer 407 properties and human clinical trials presently in progress will probably offer more precise responses on its benefit-to-risk-ratio.

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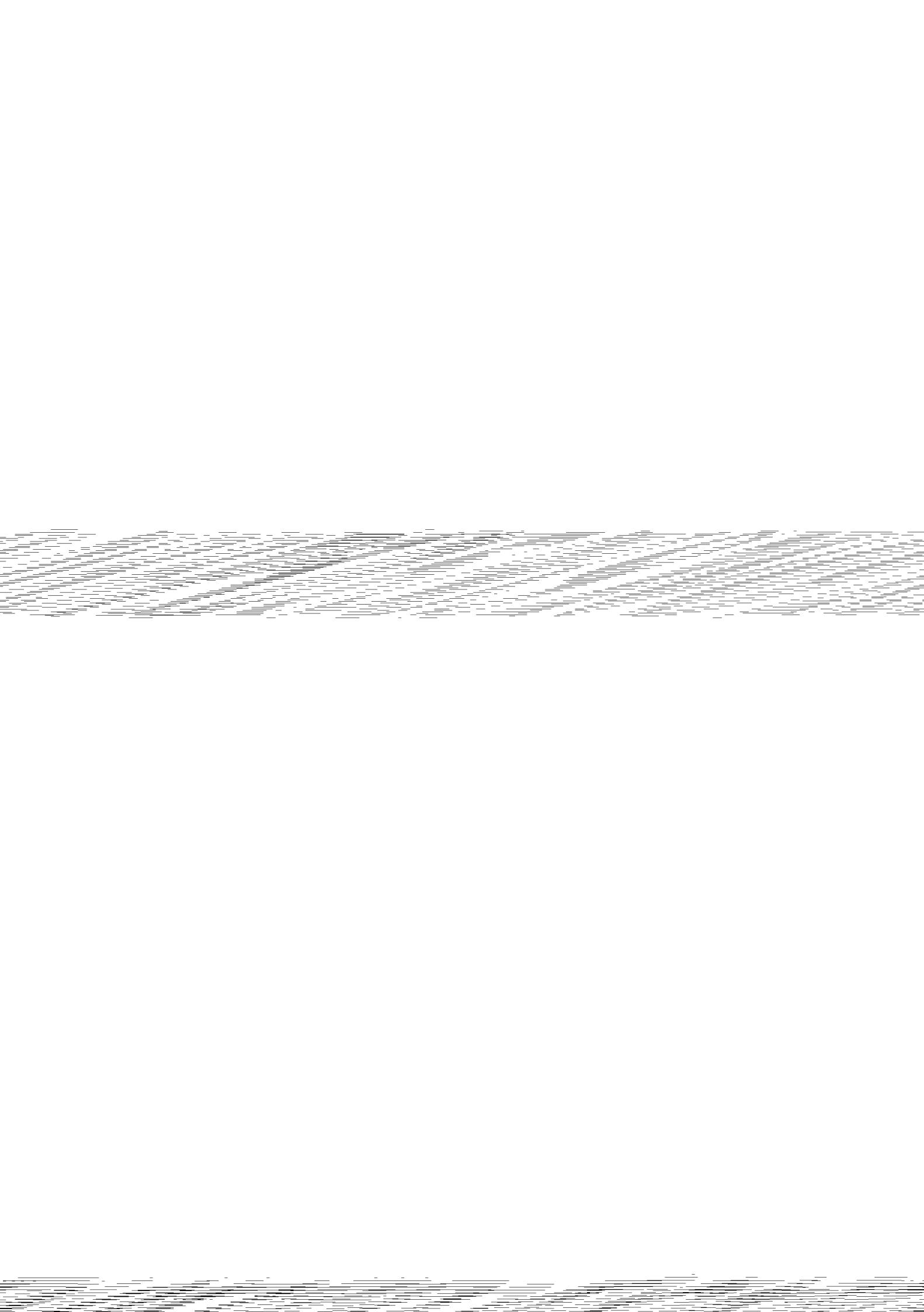
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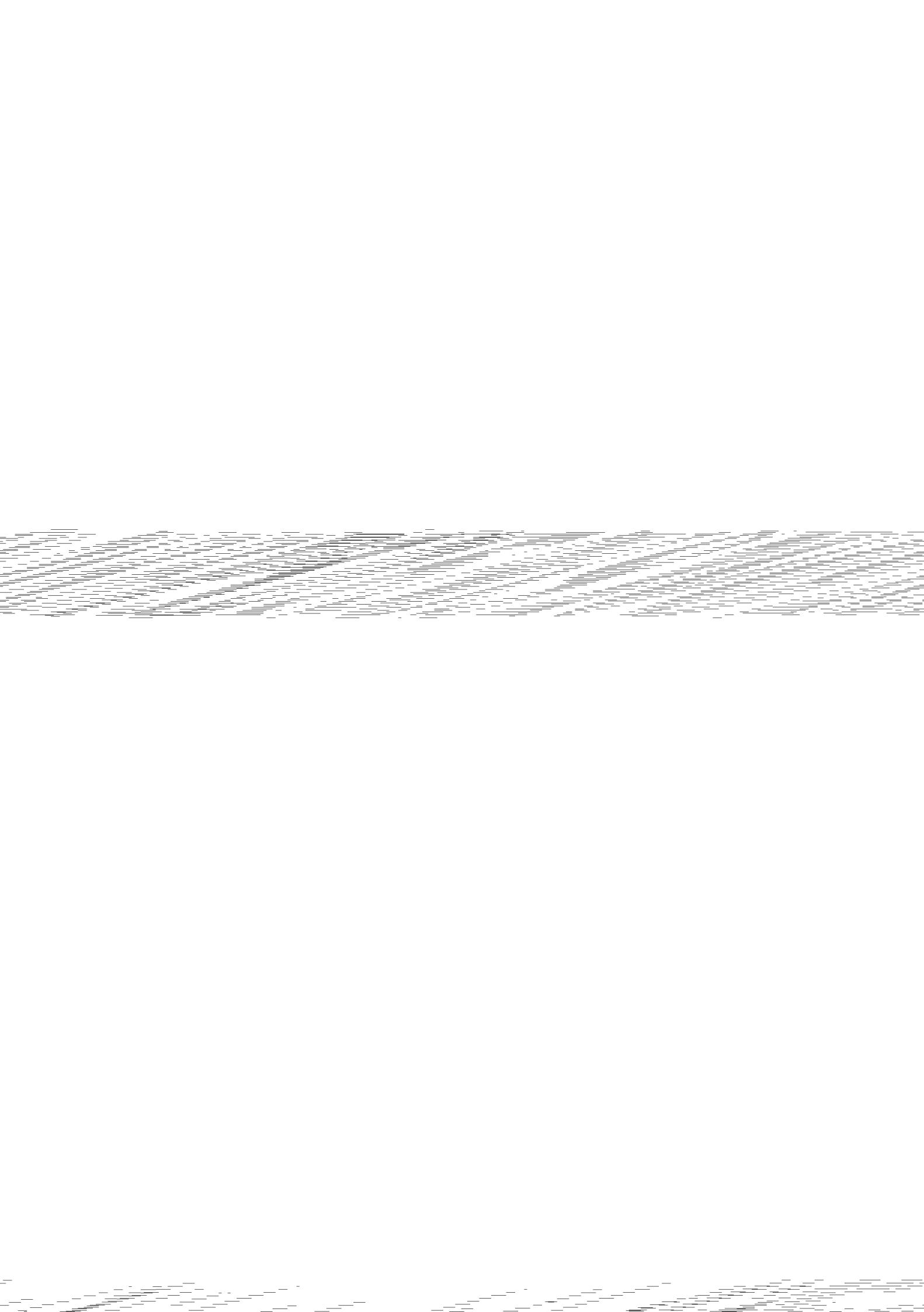
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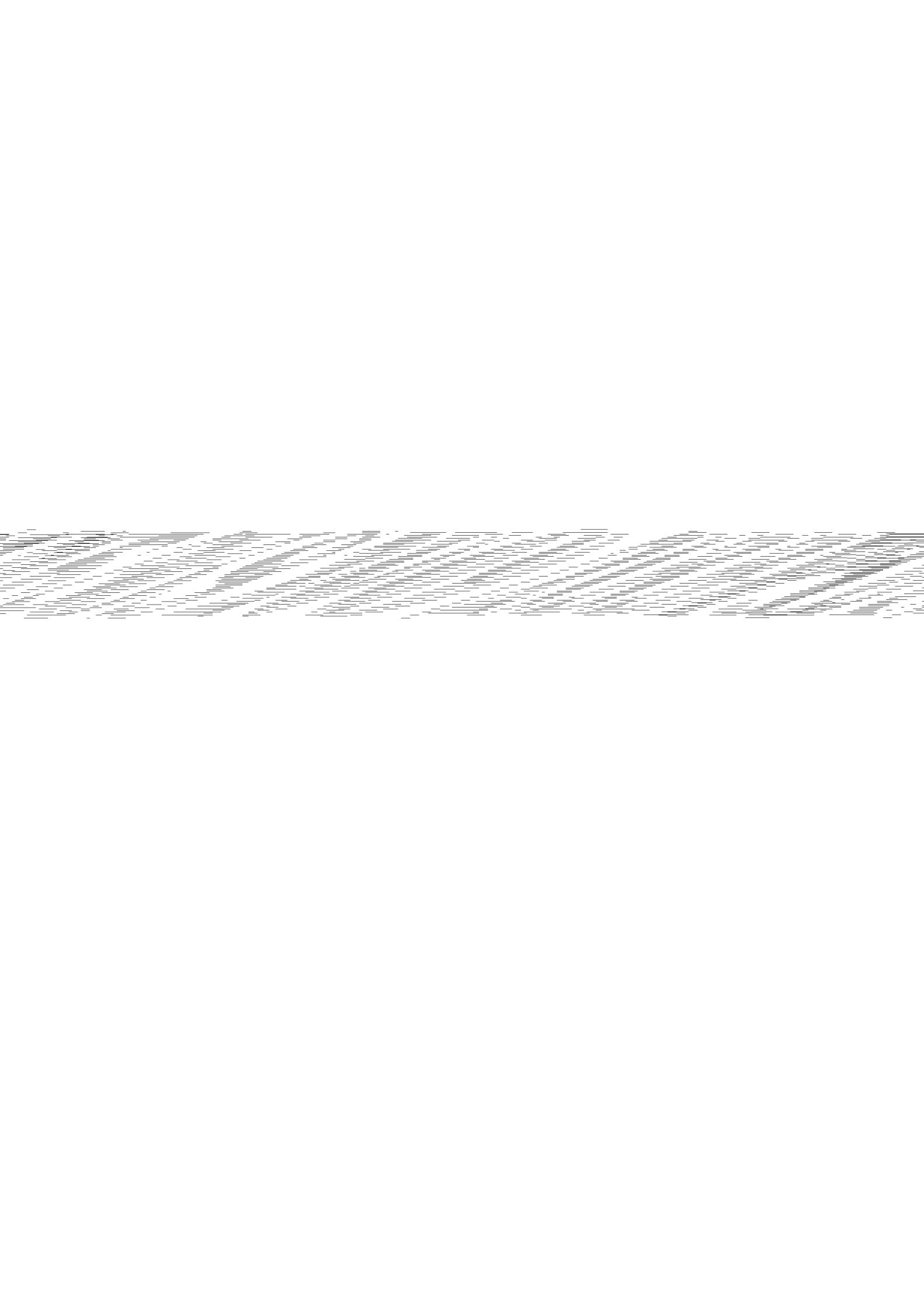
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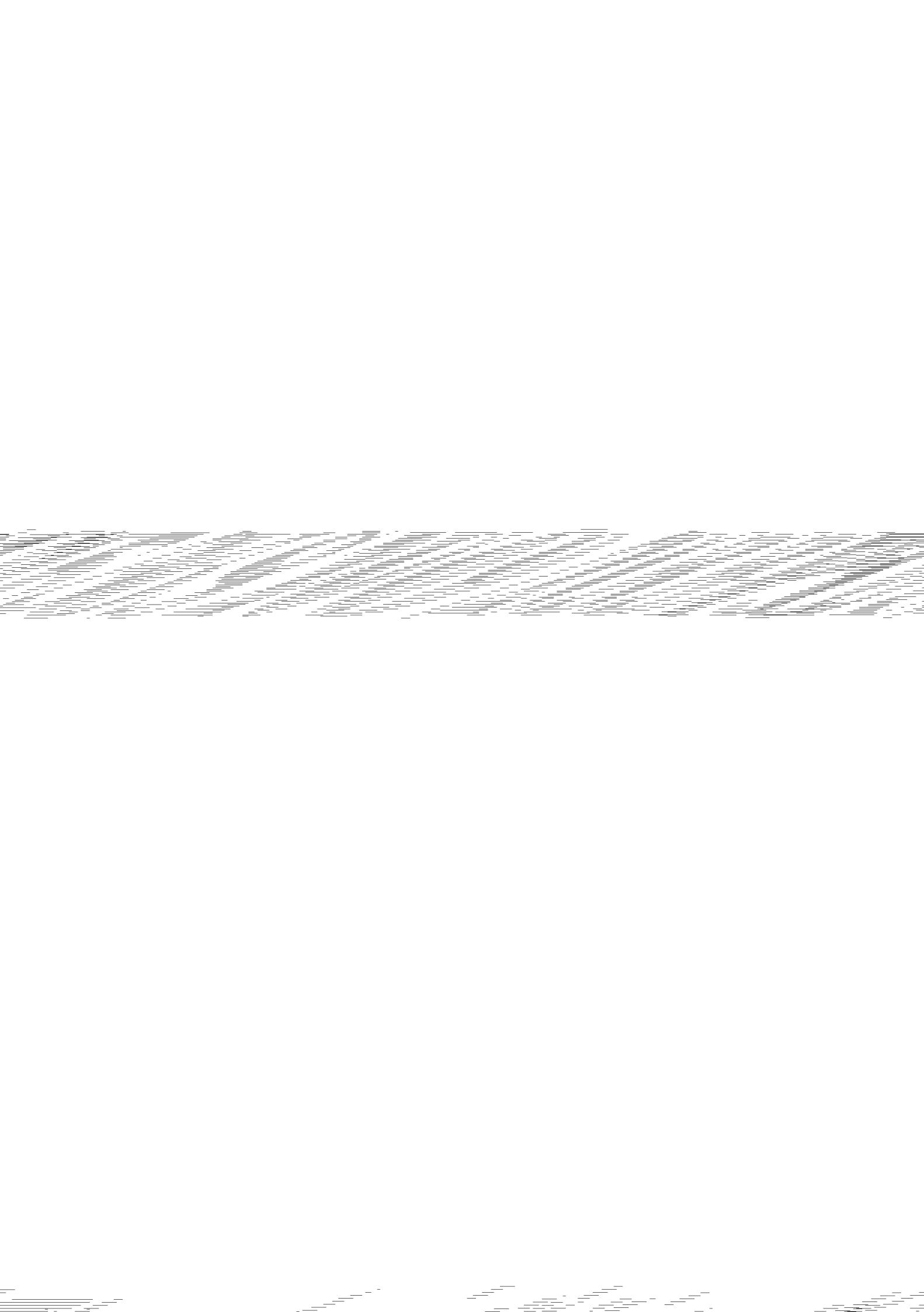
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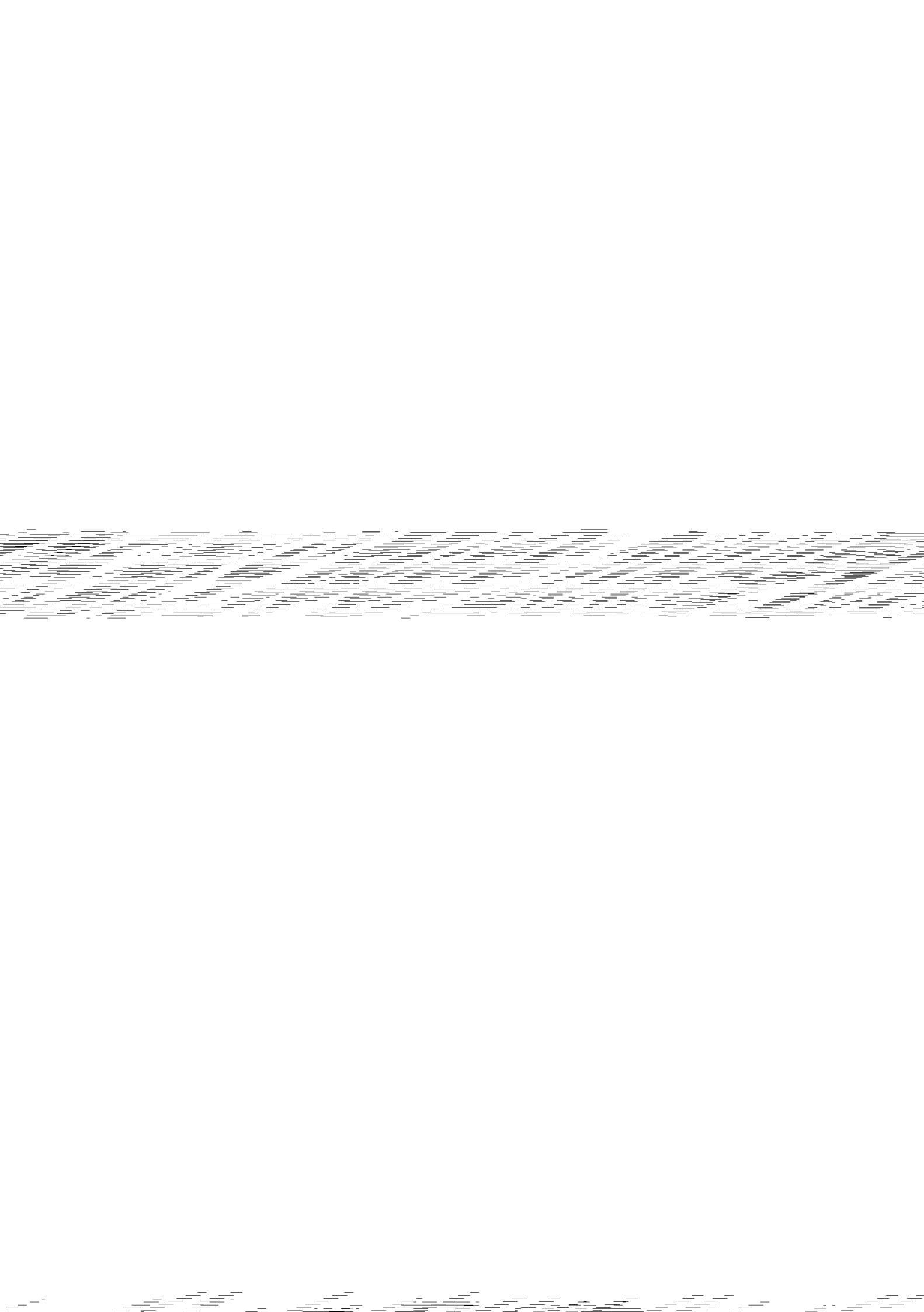


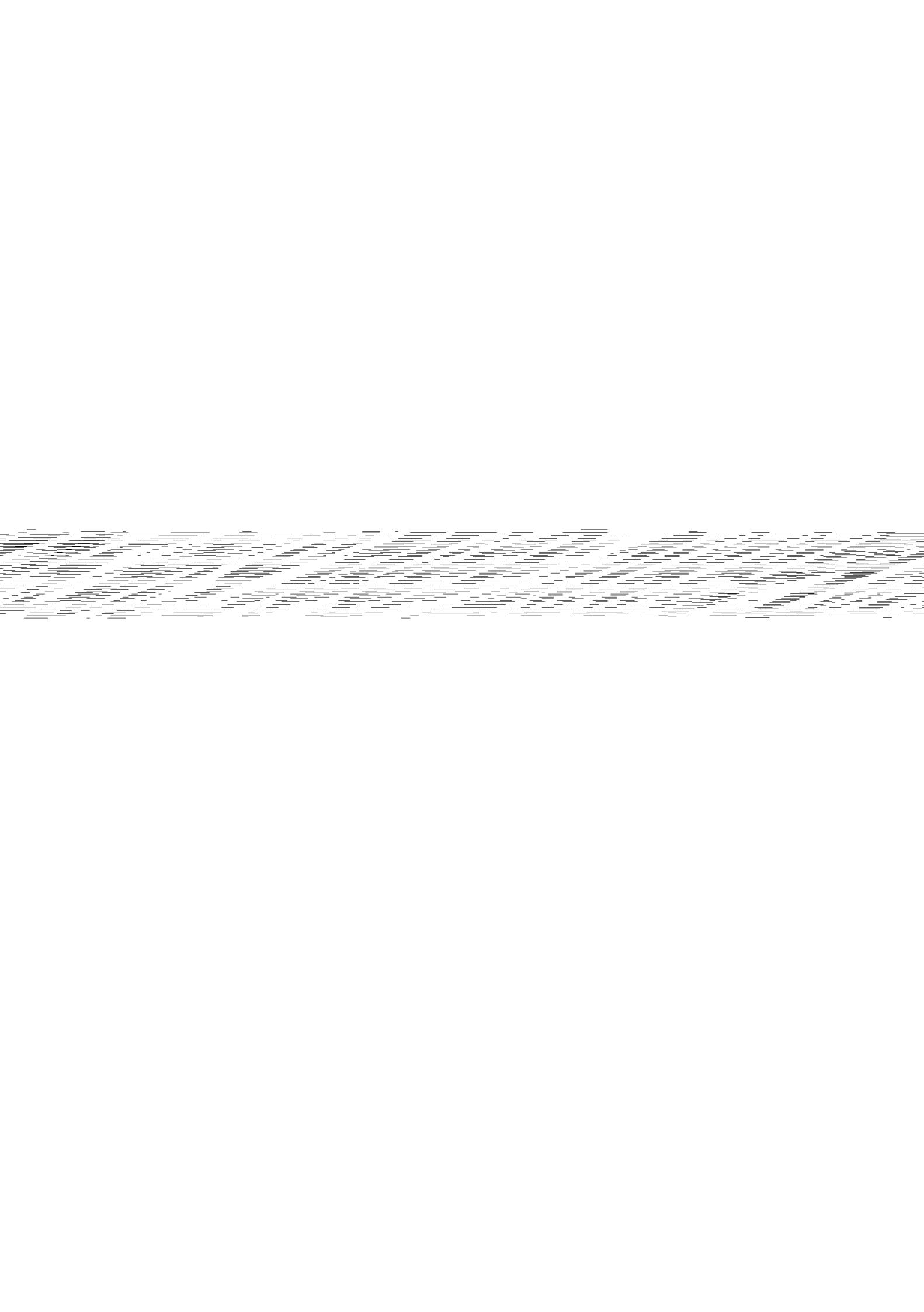


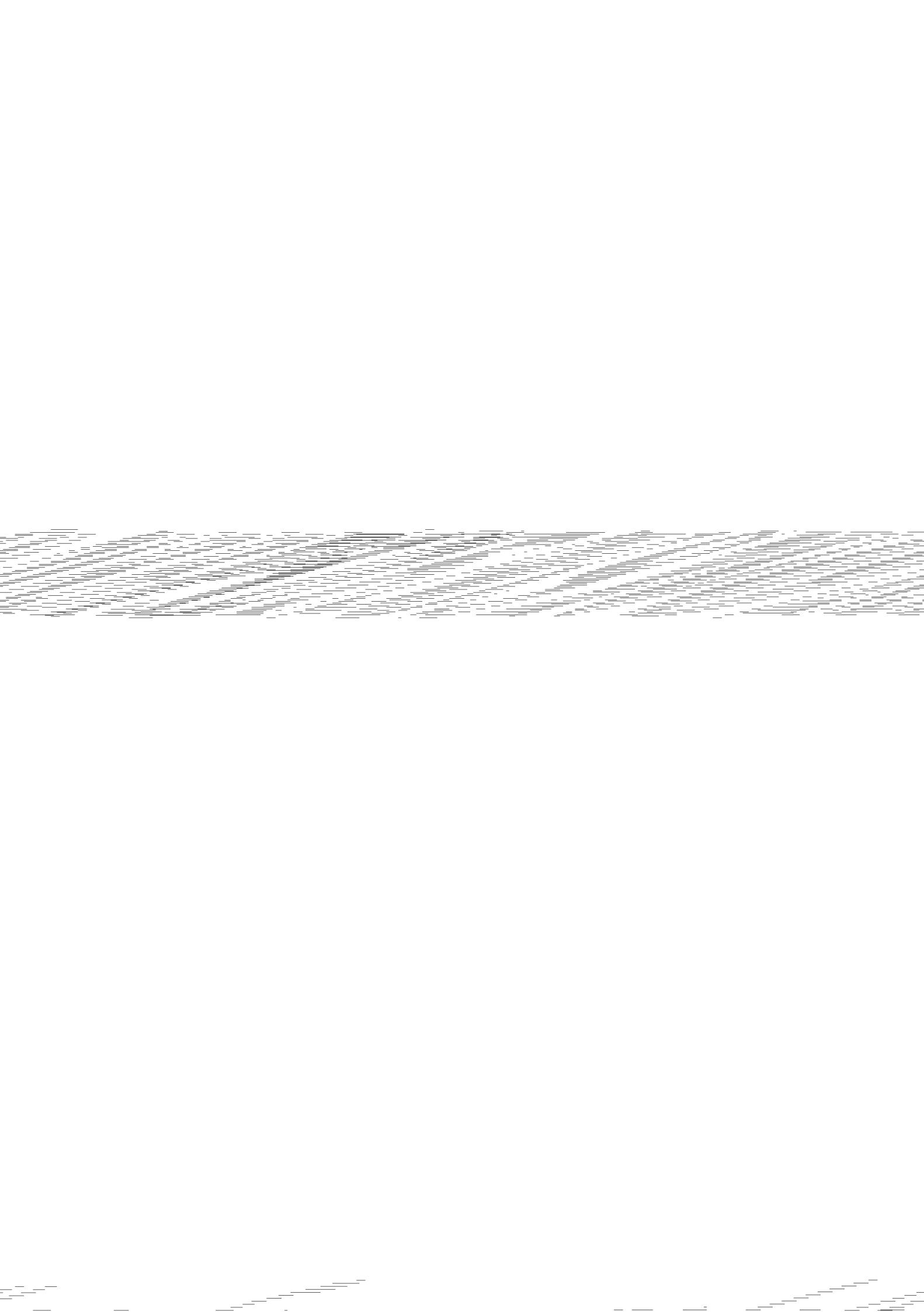


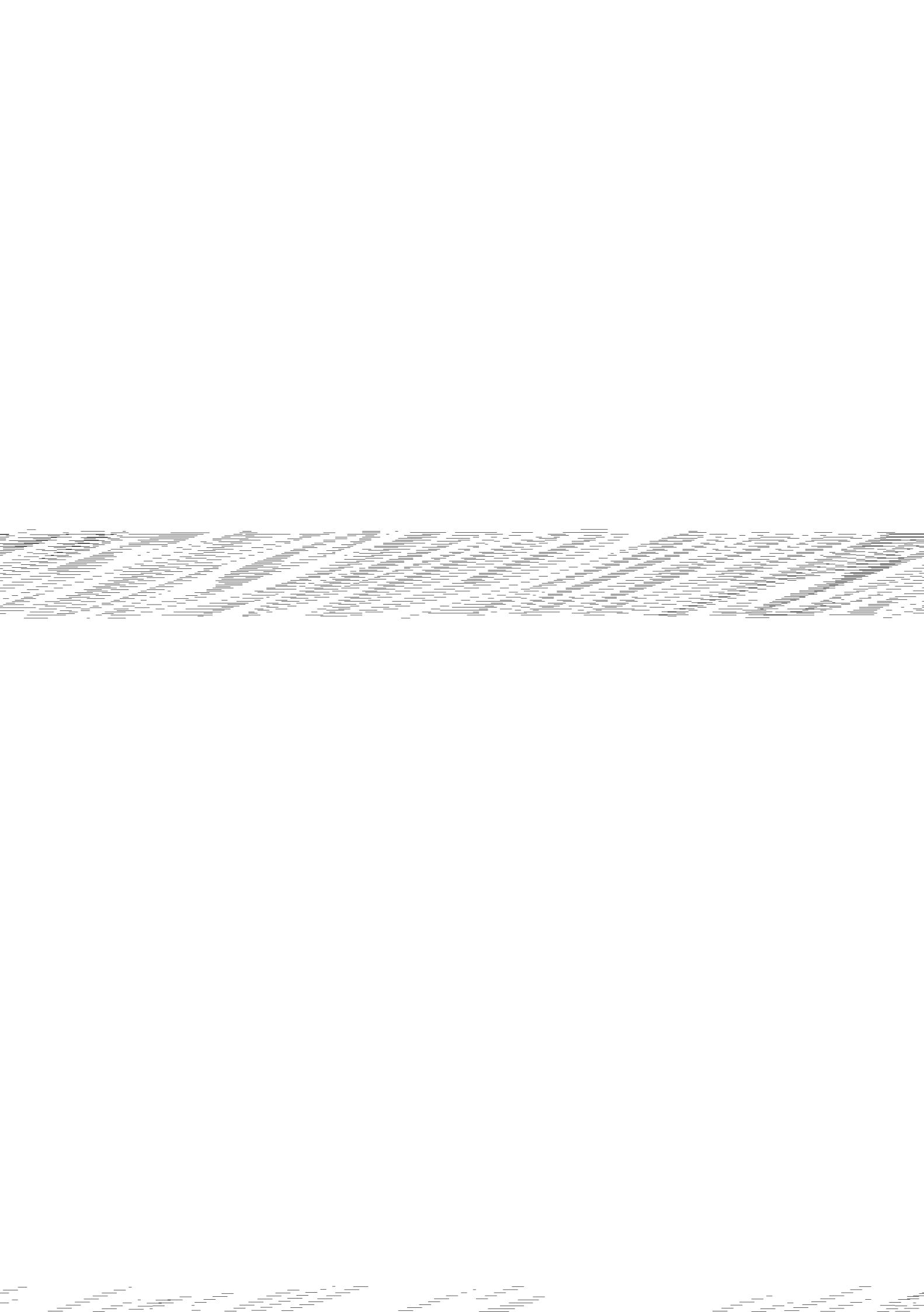


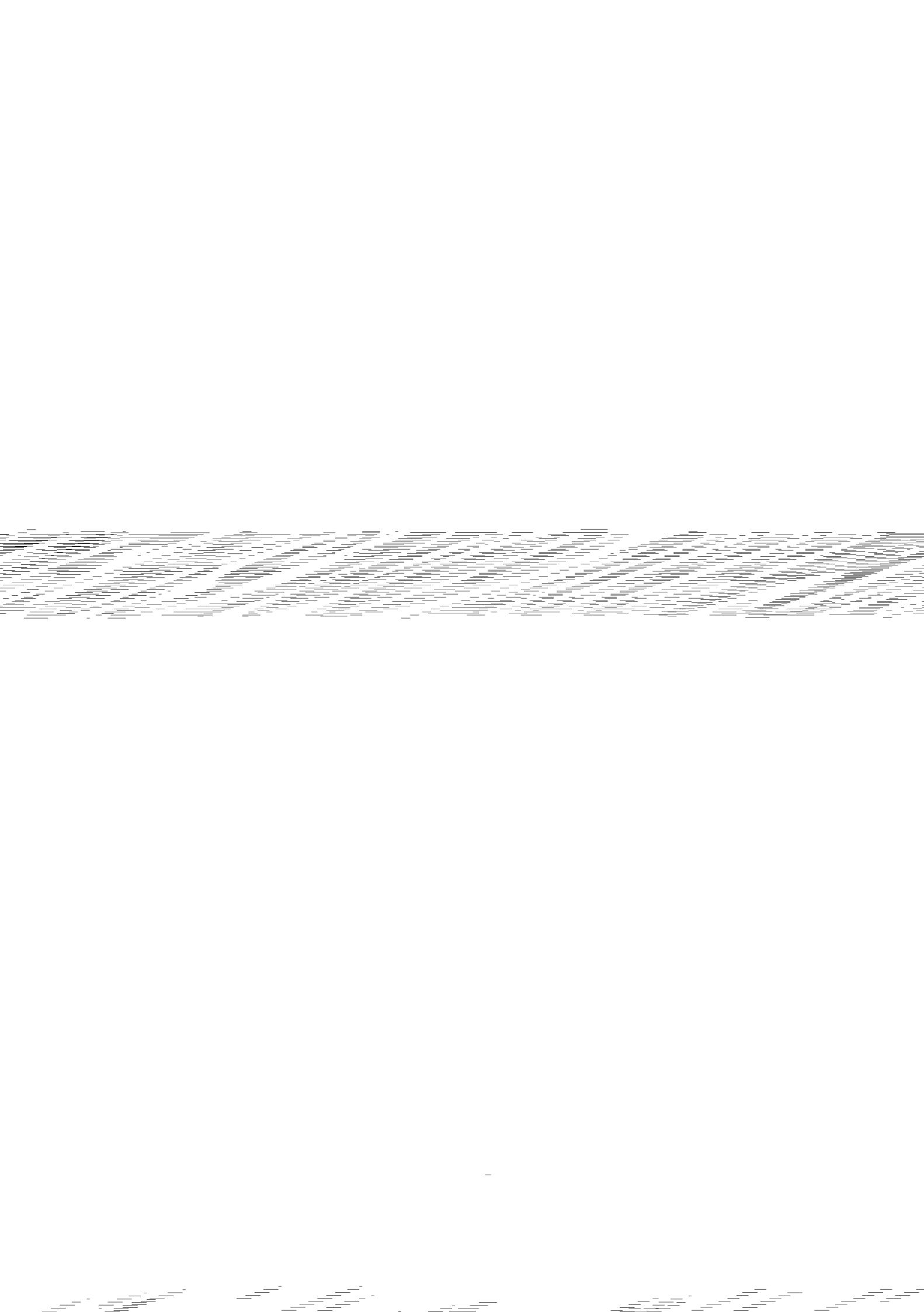


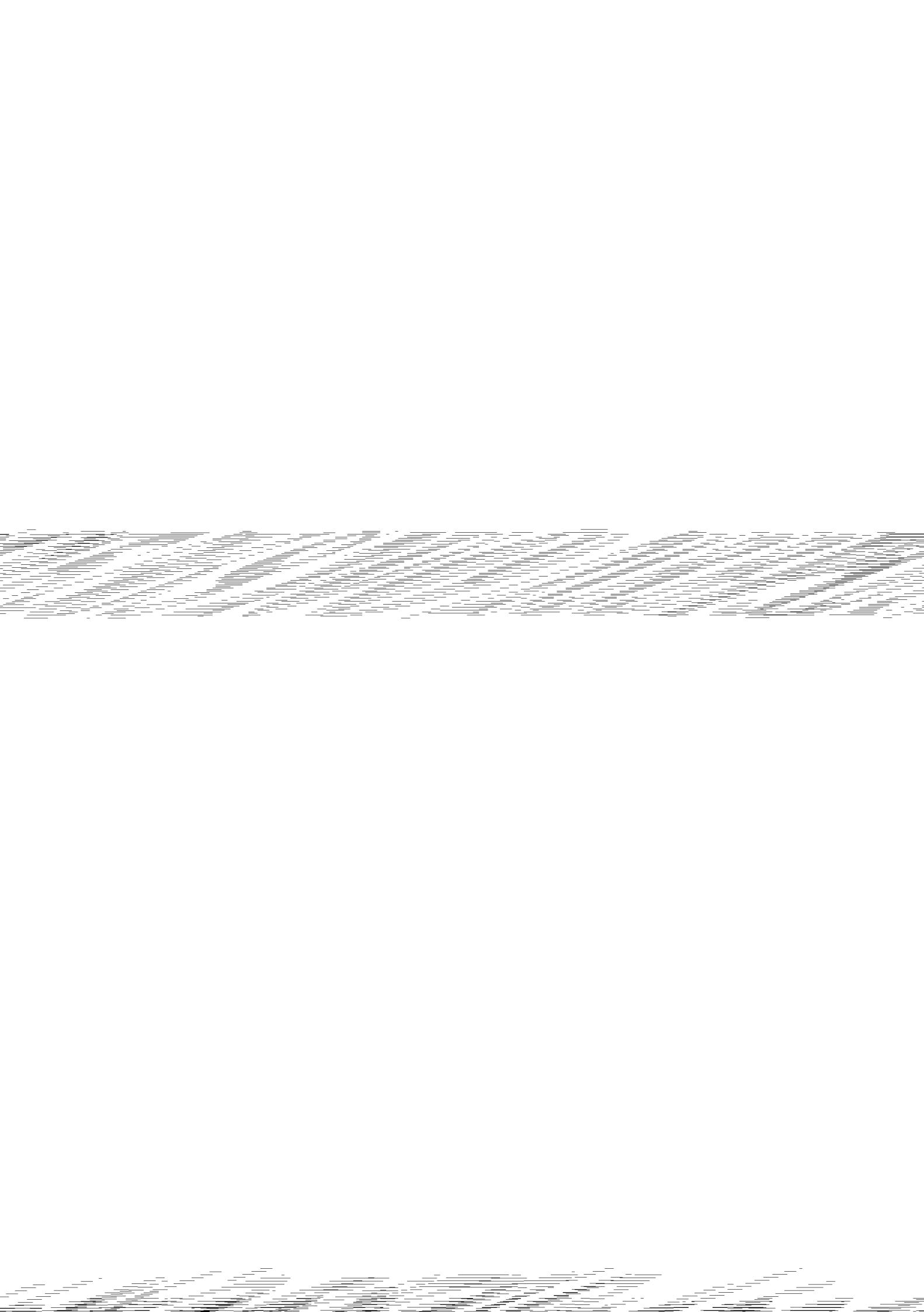


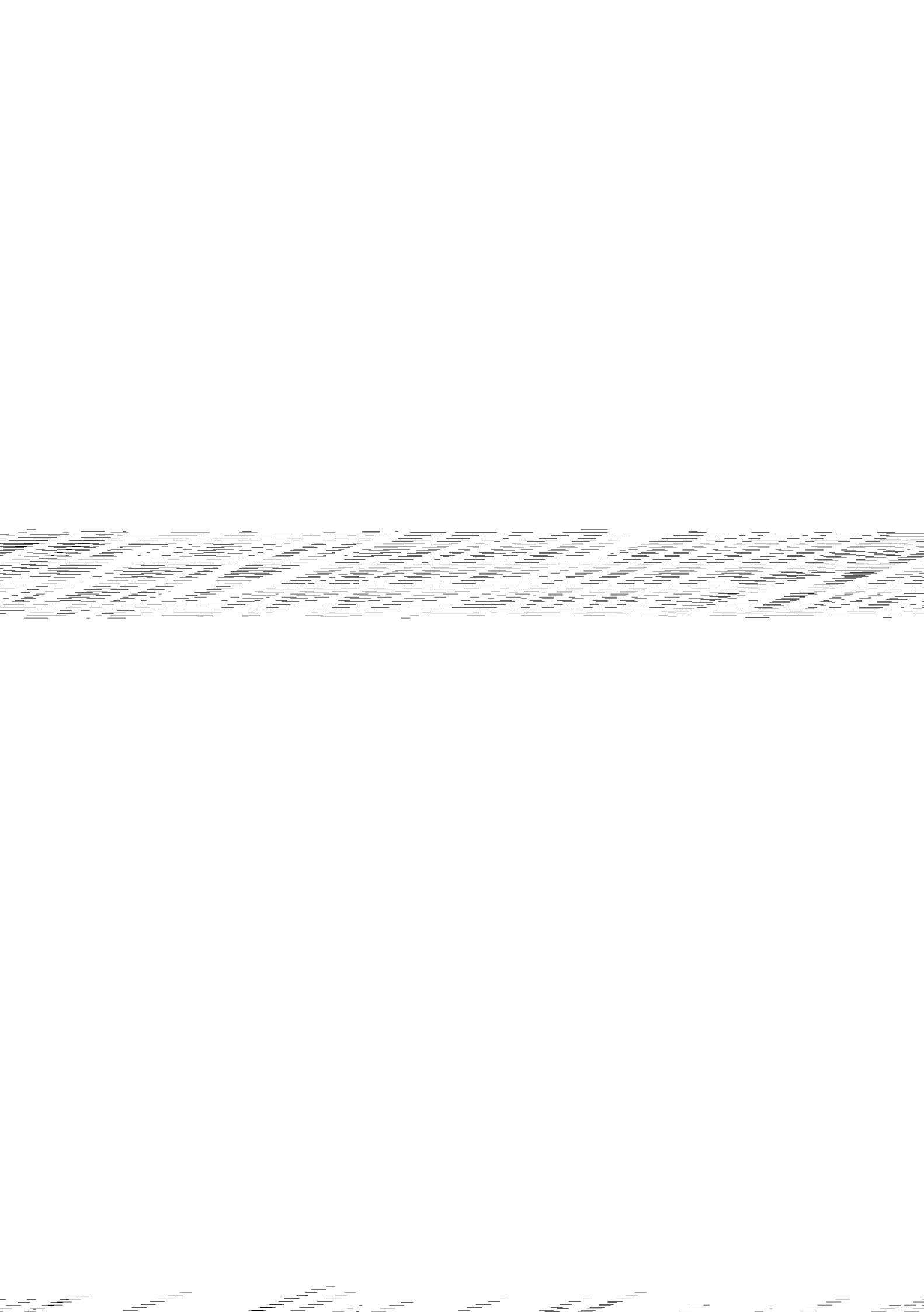


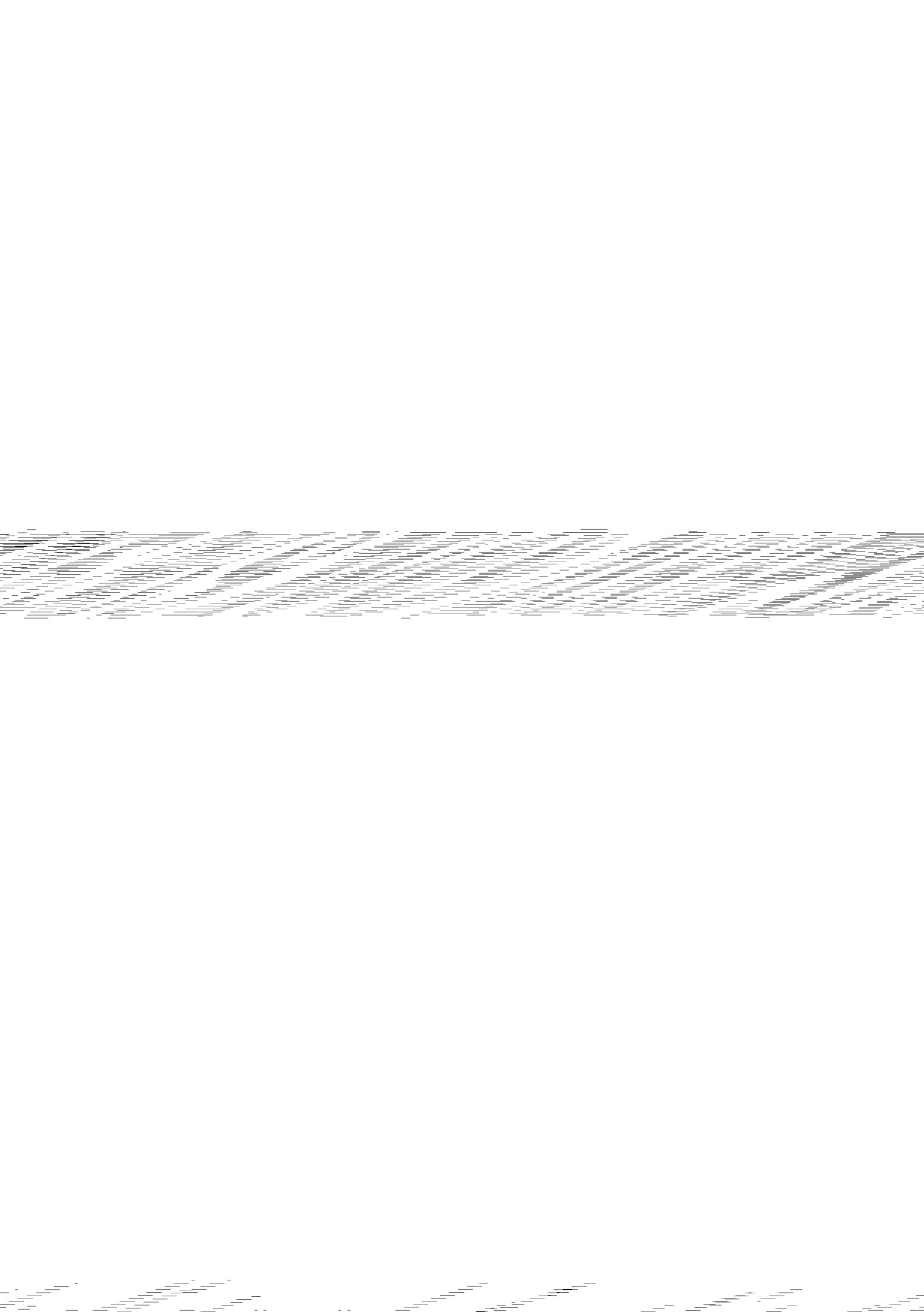


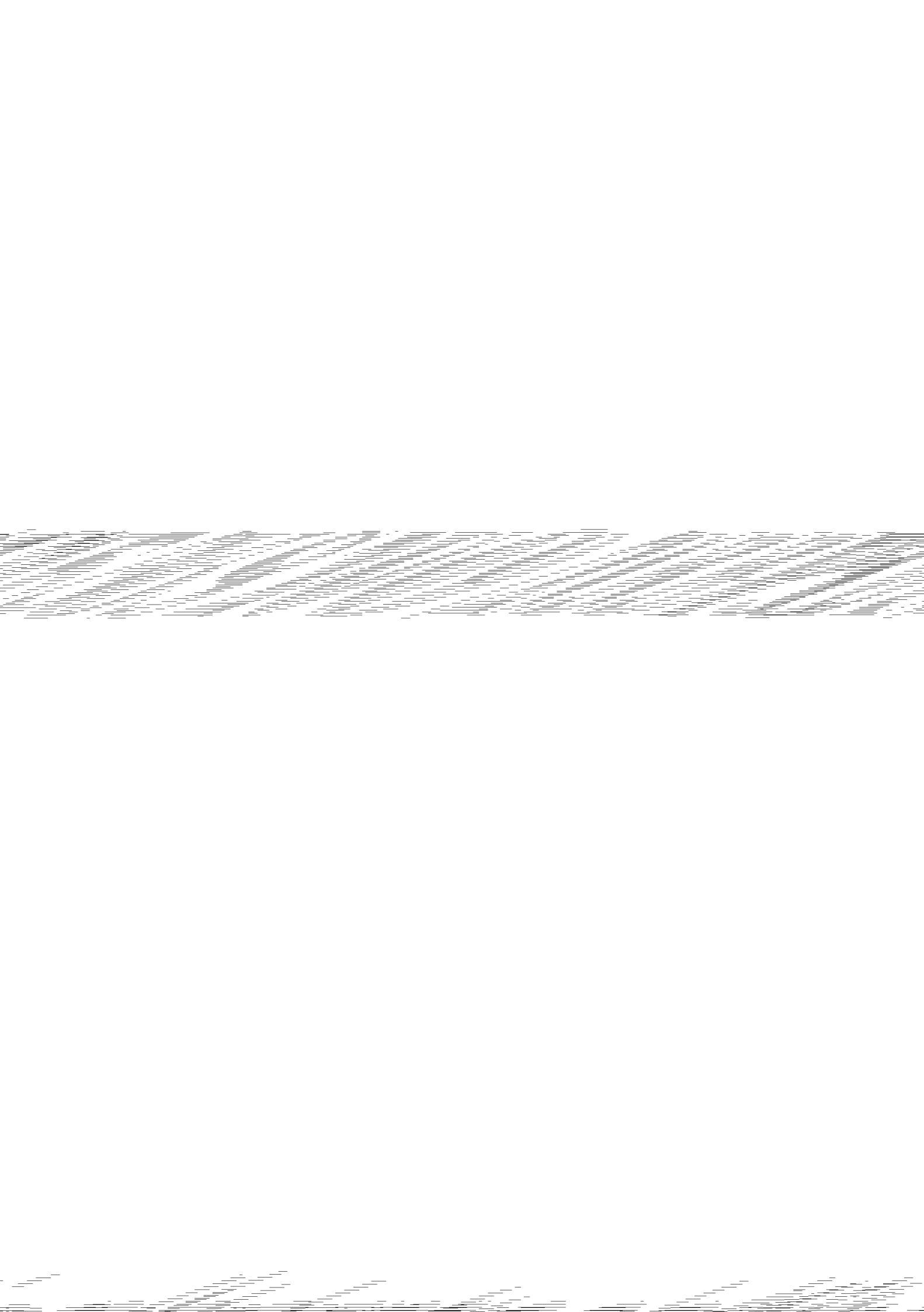


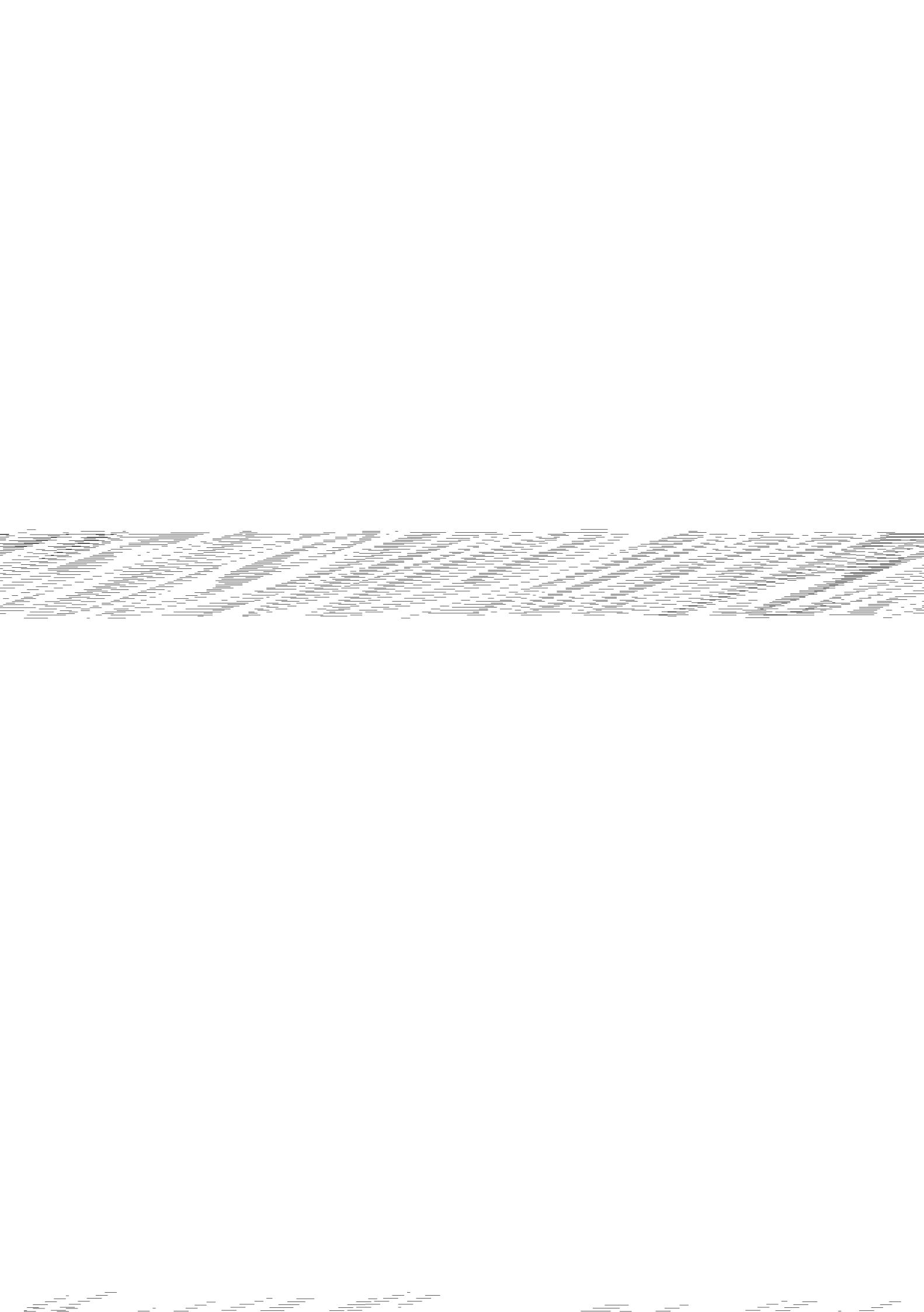


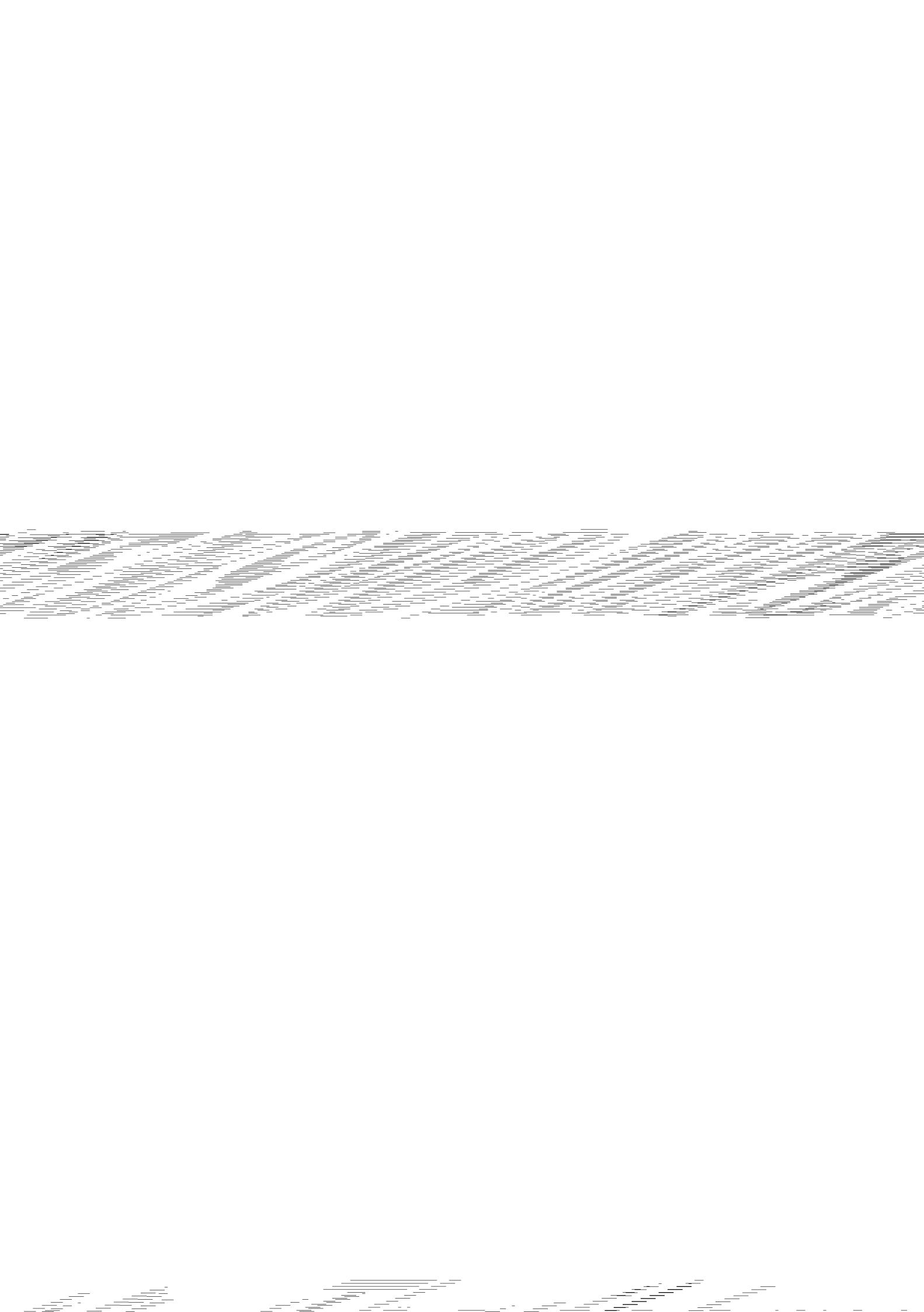


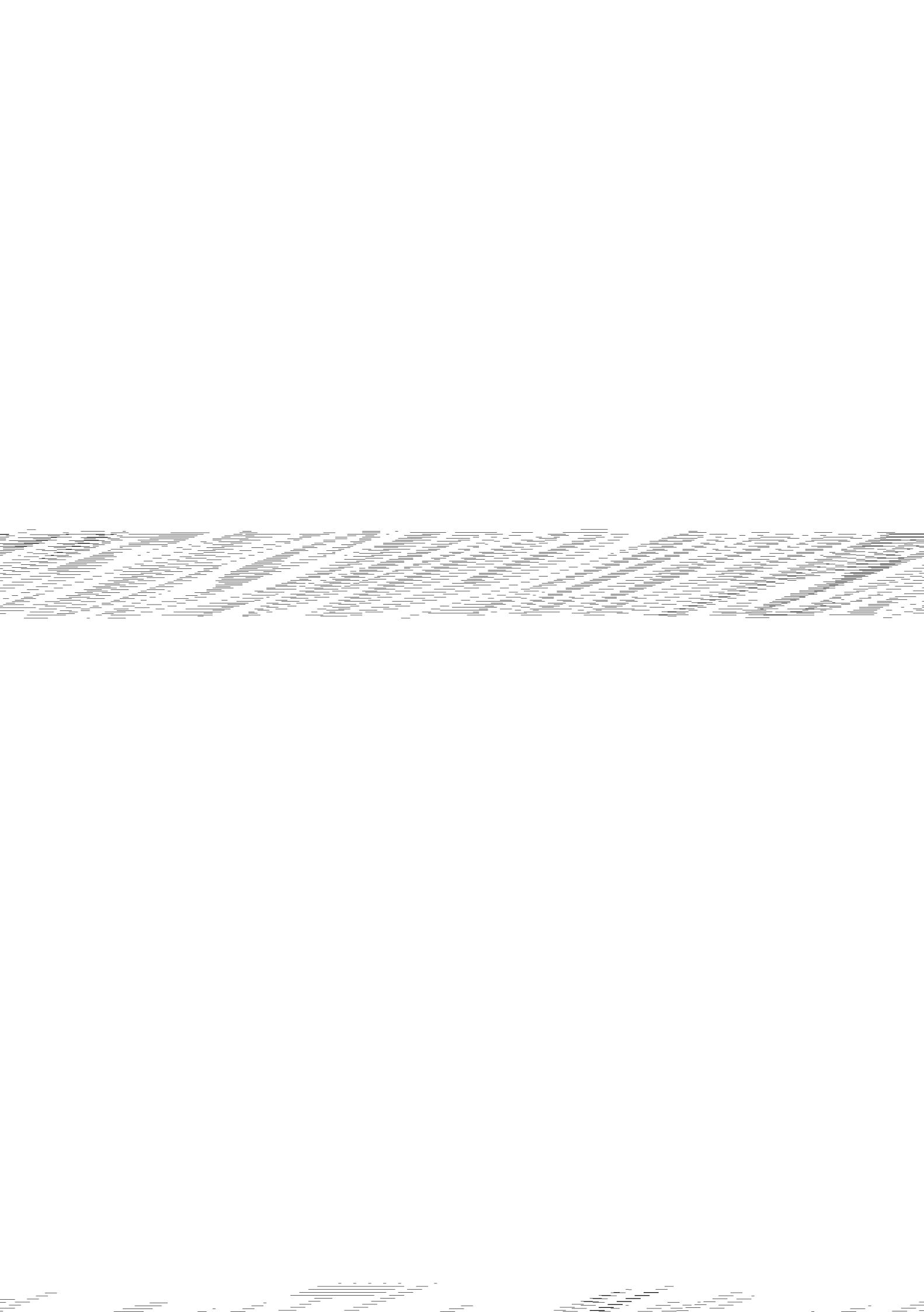


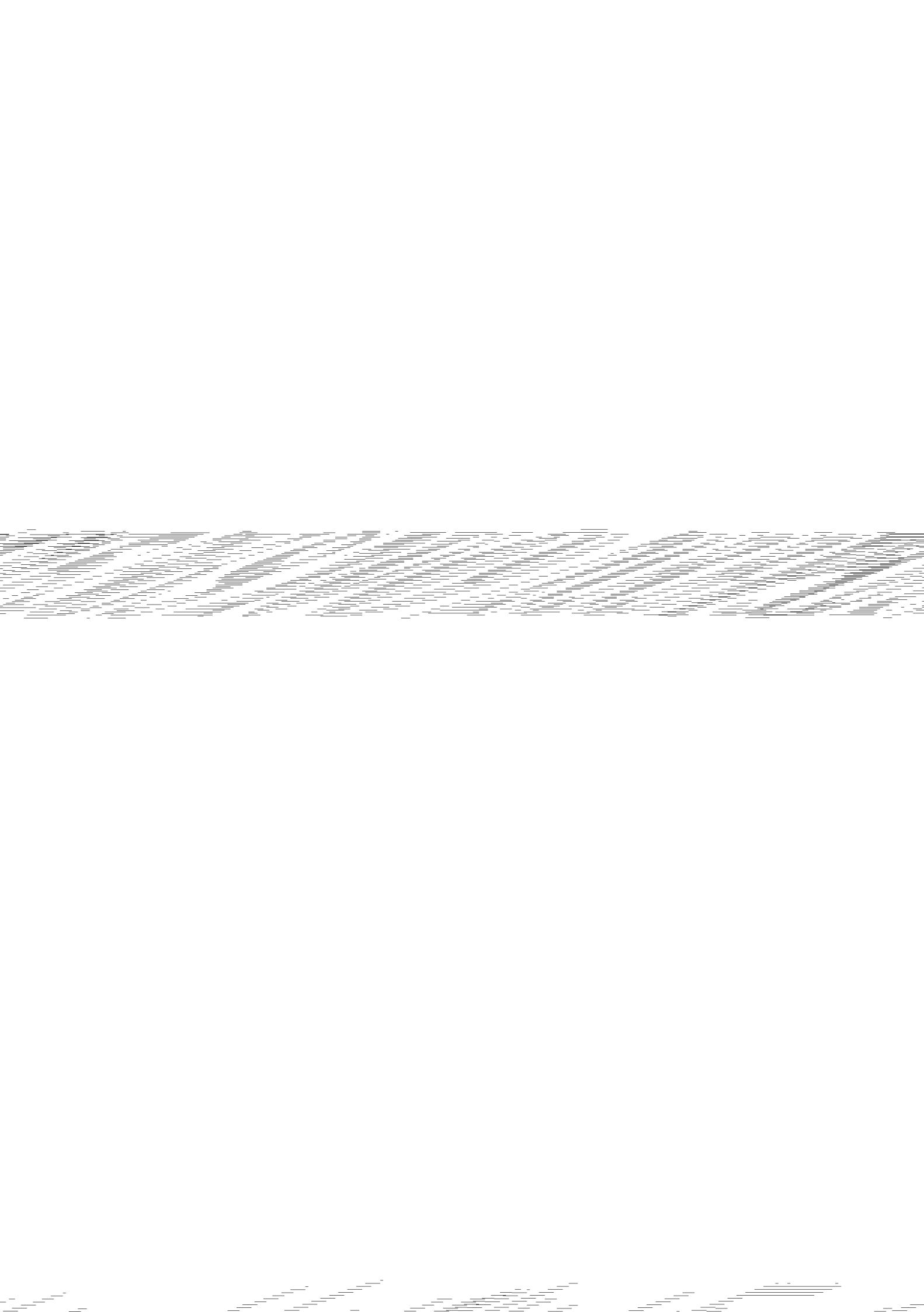


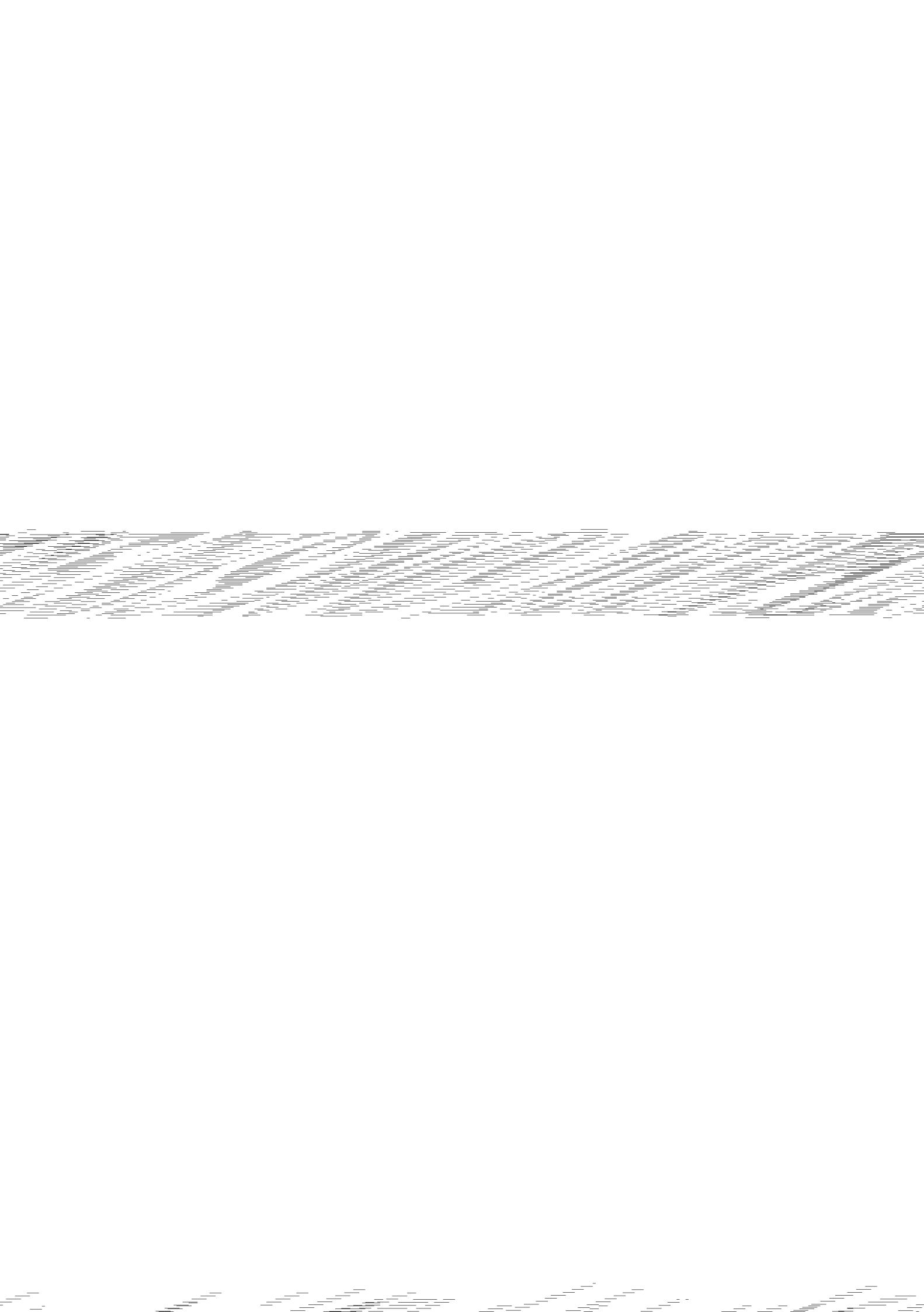


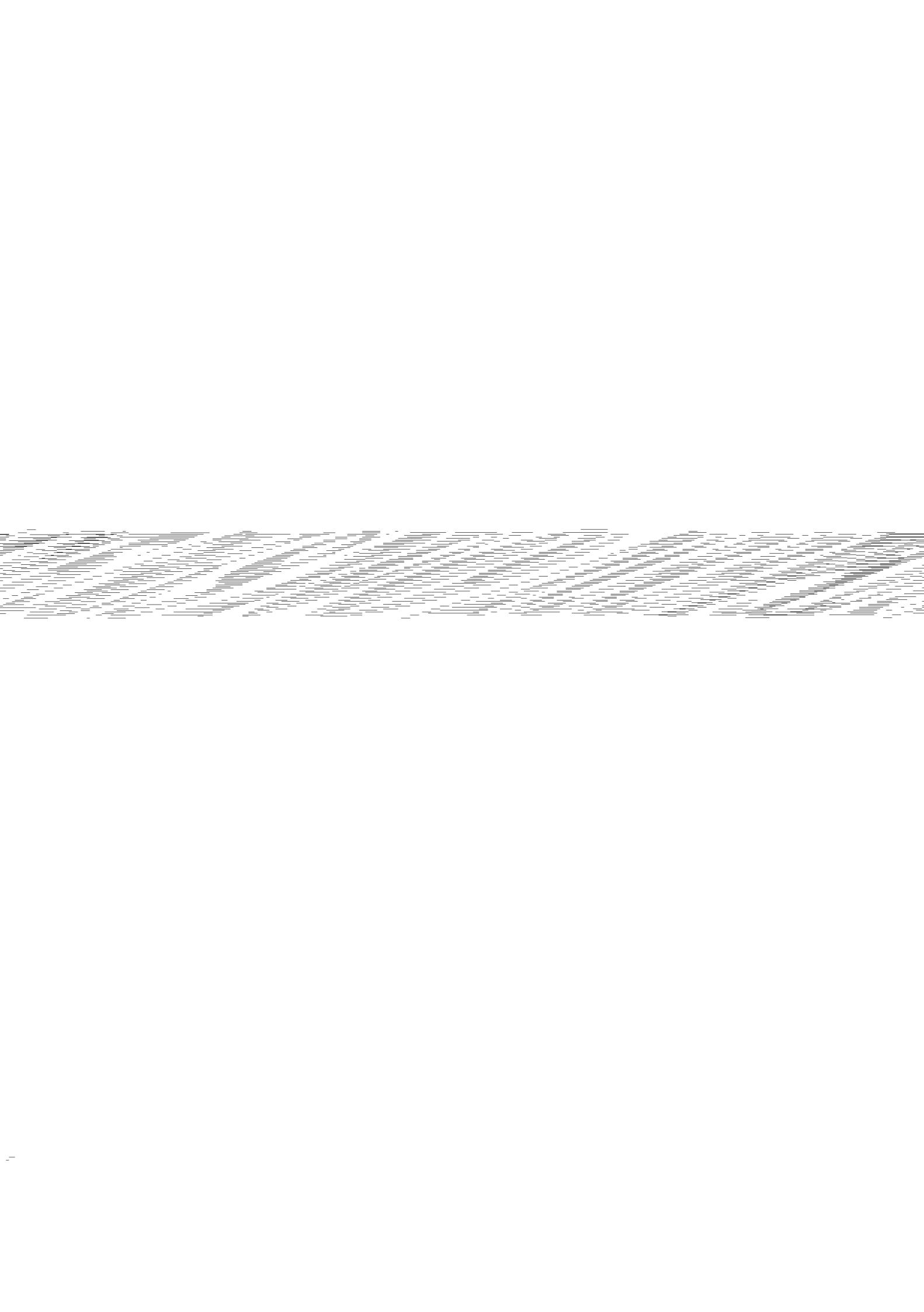


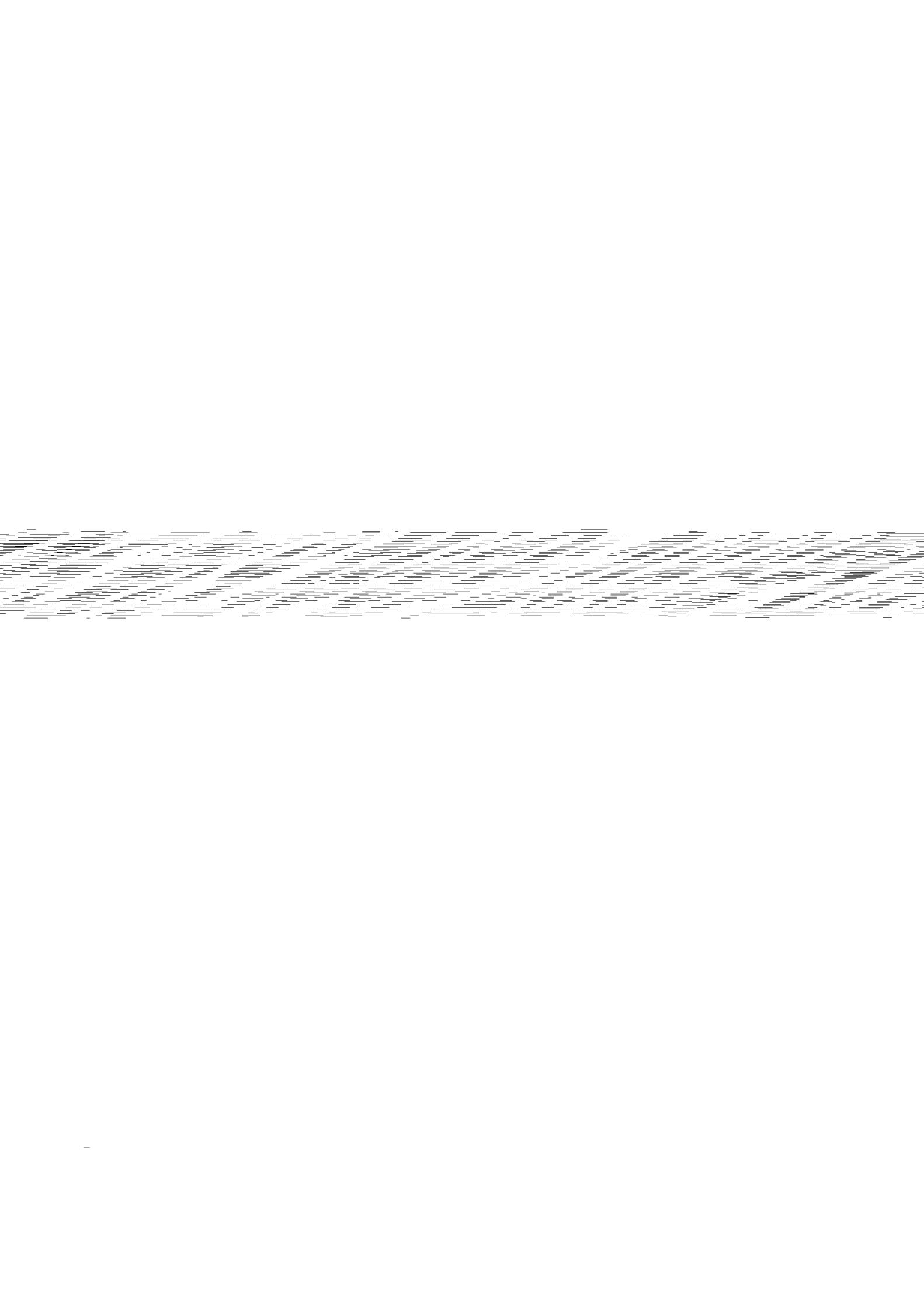


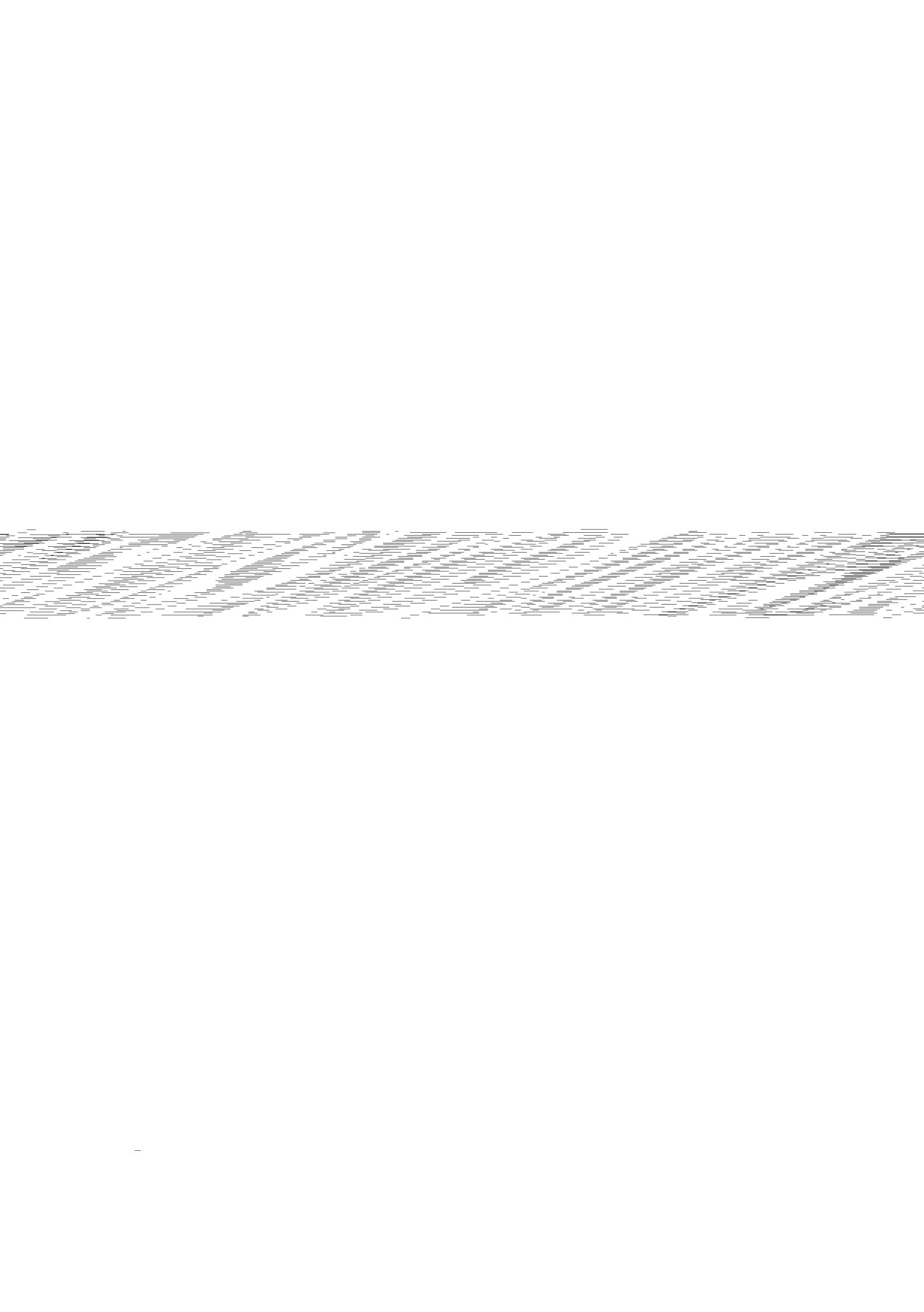


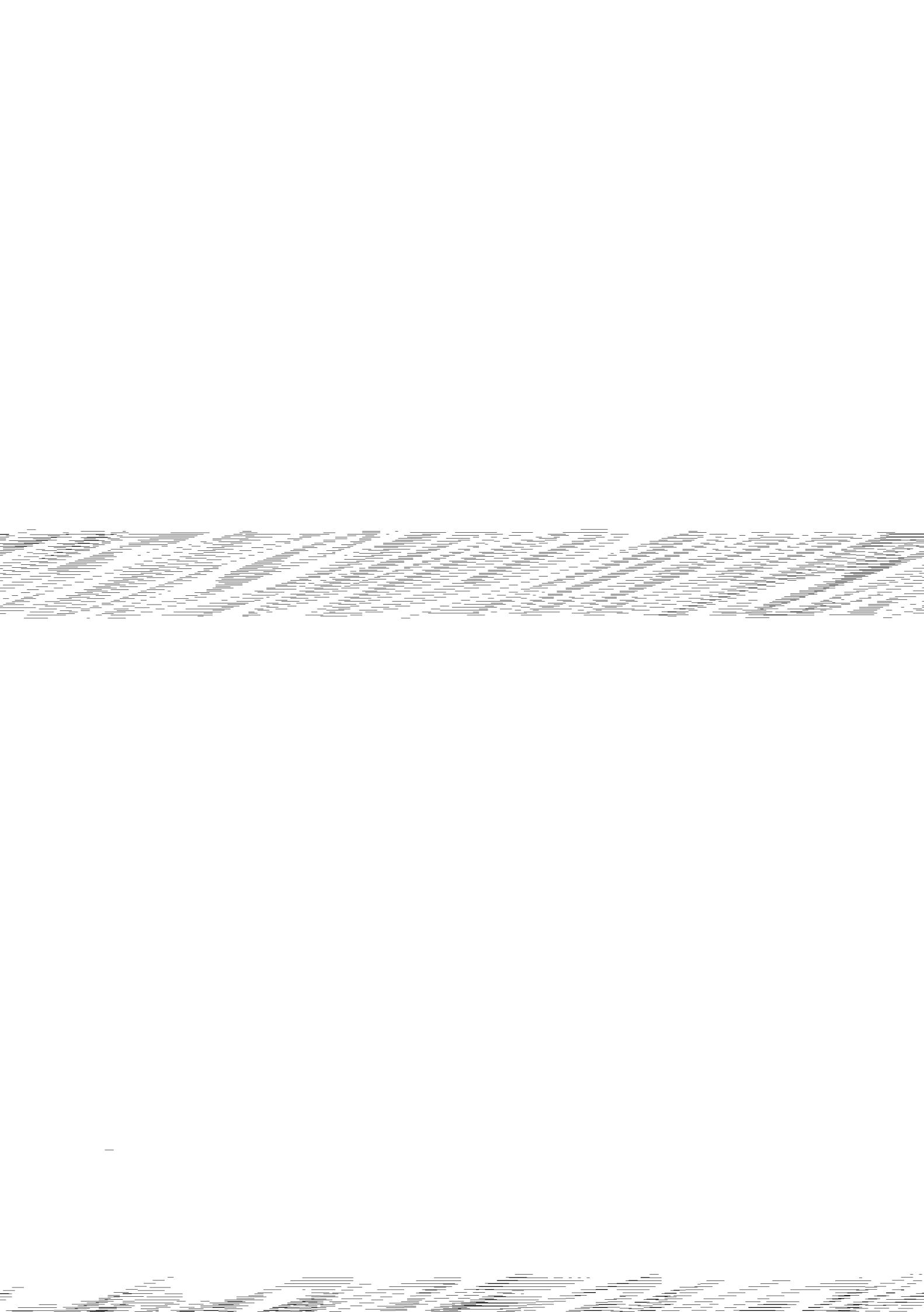


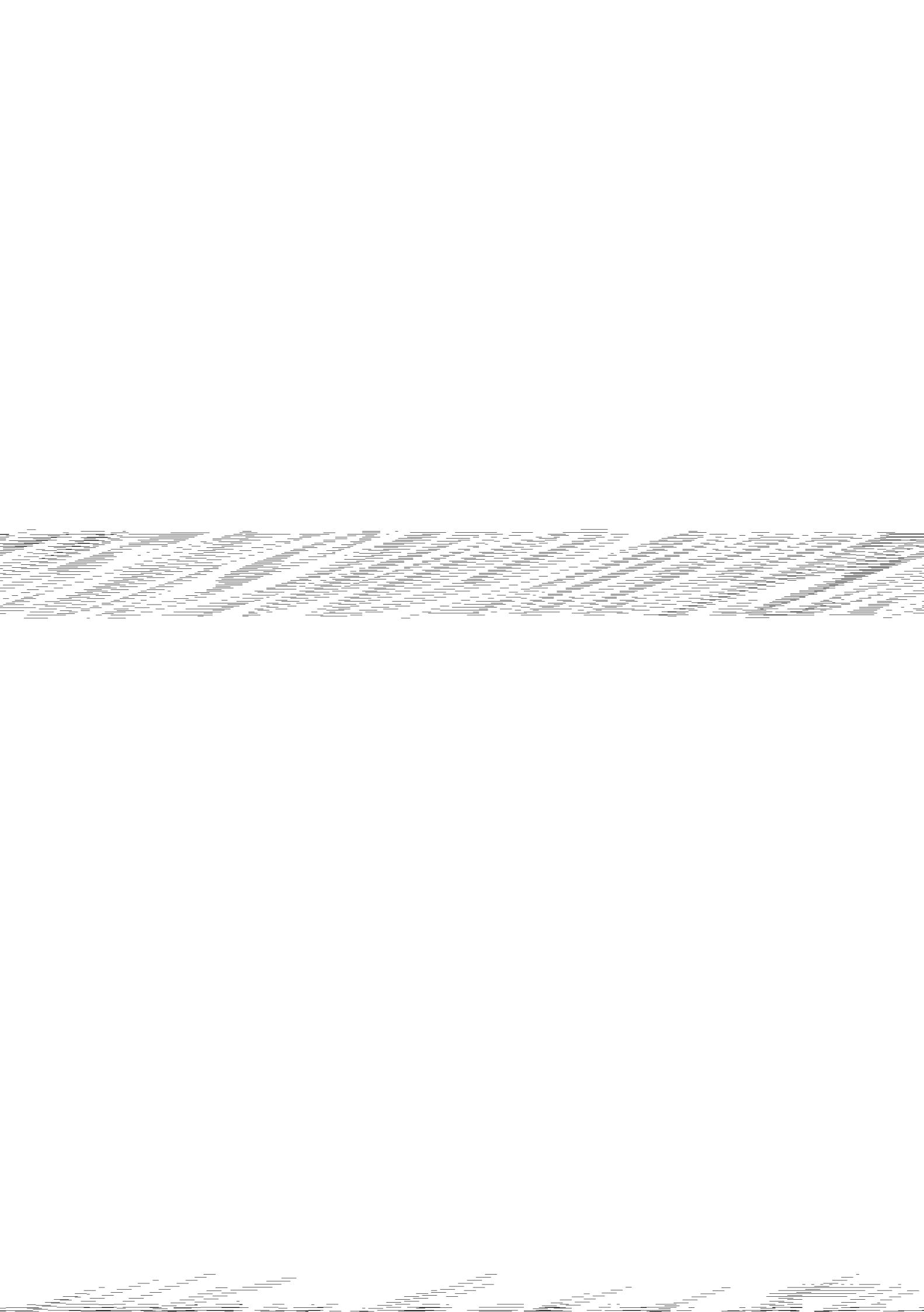


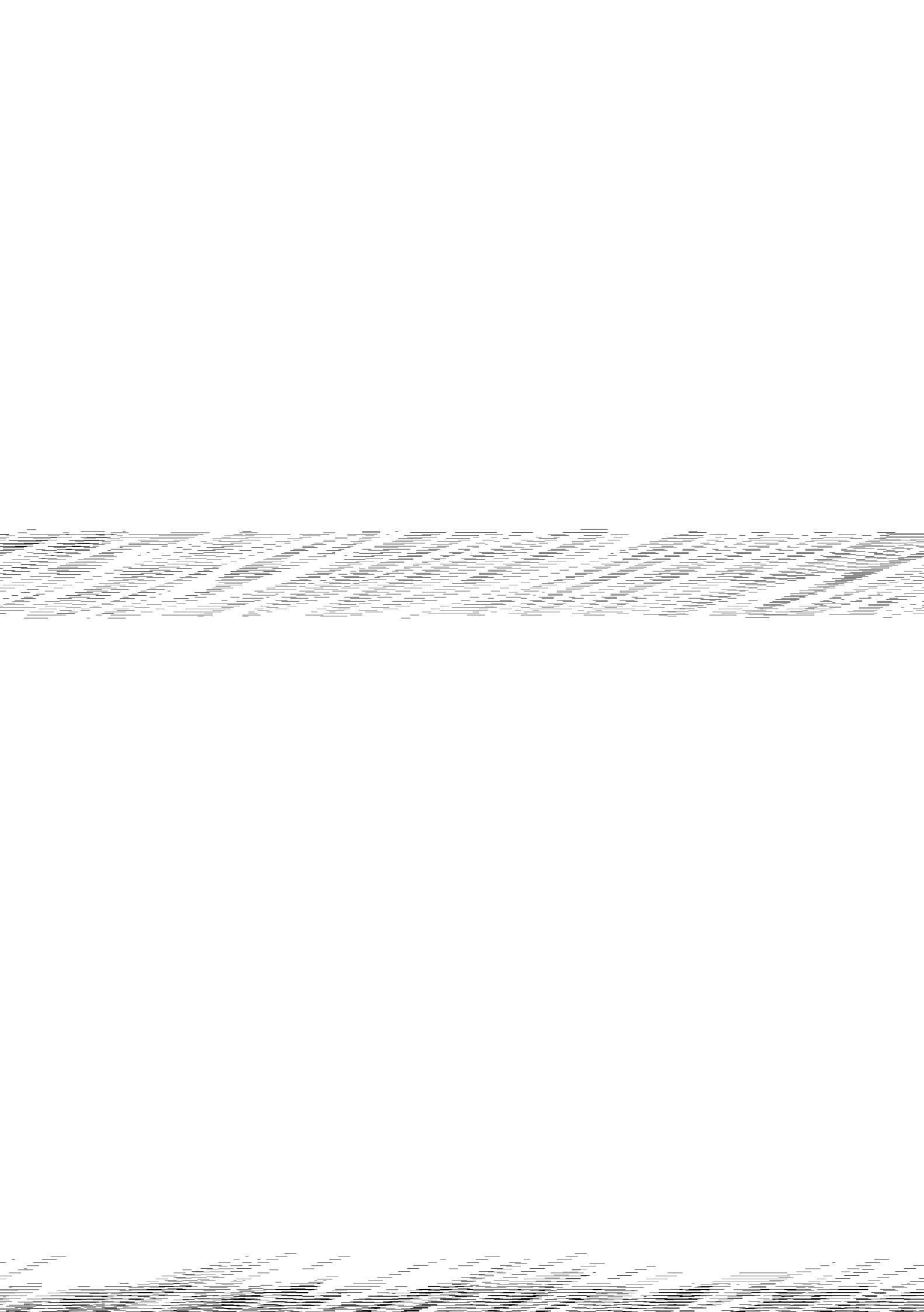


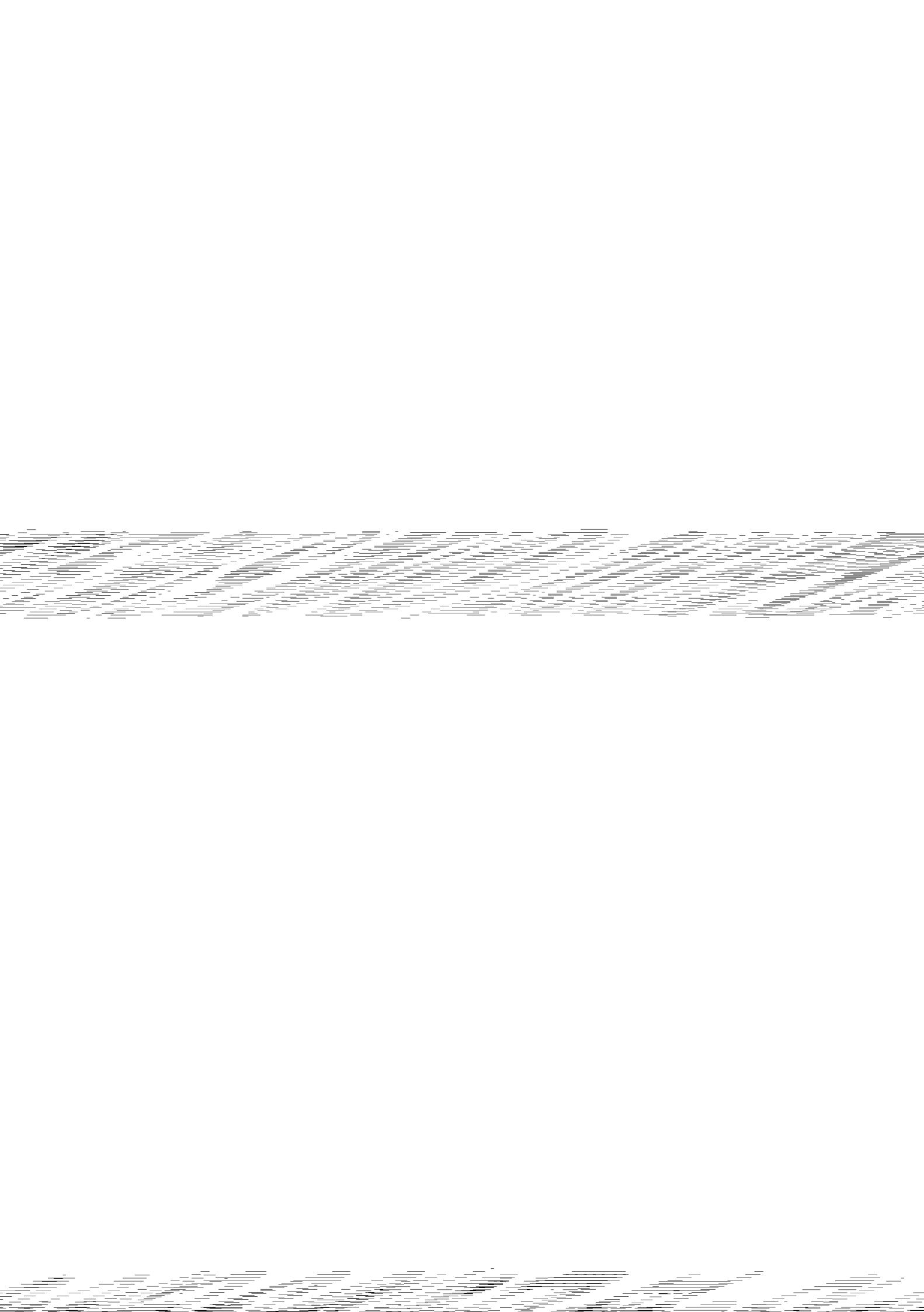


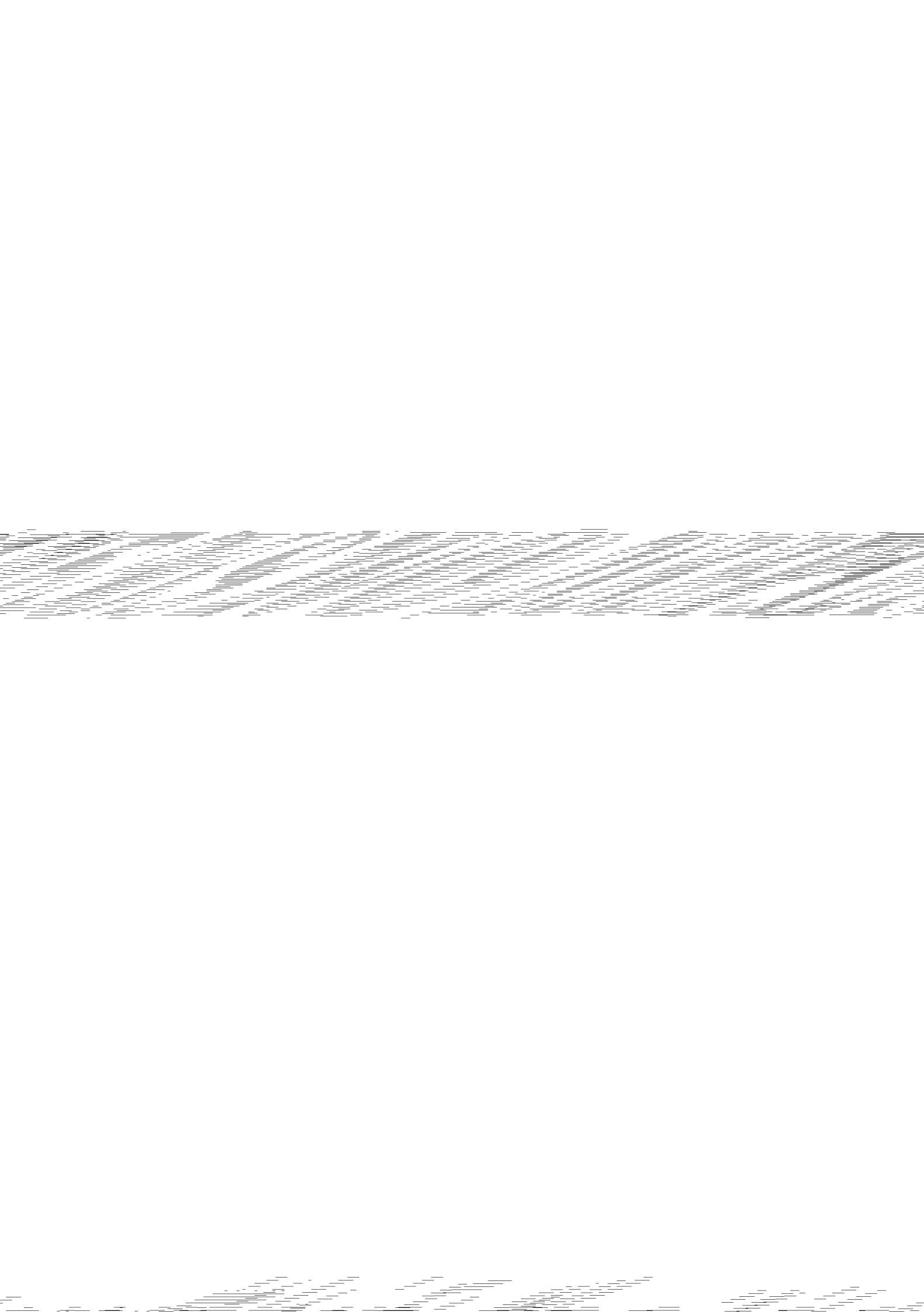


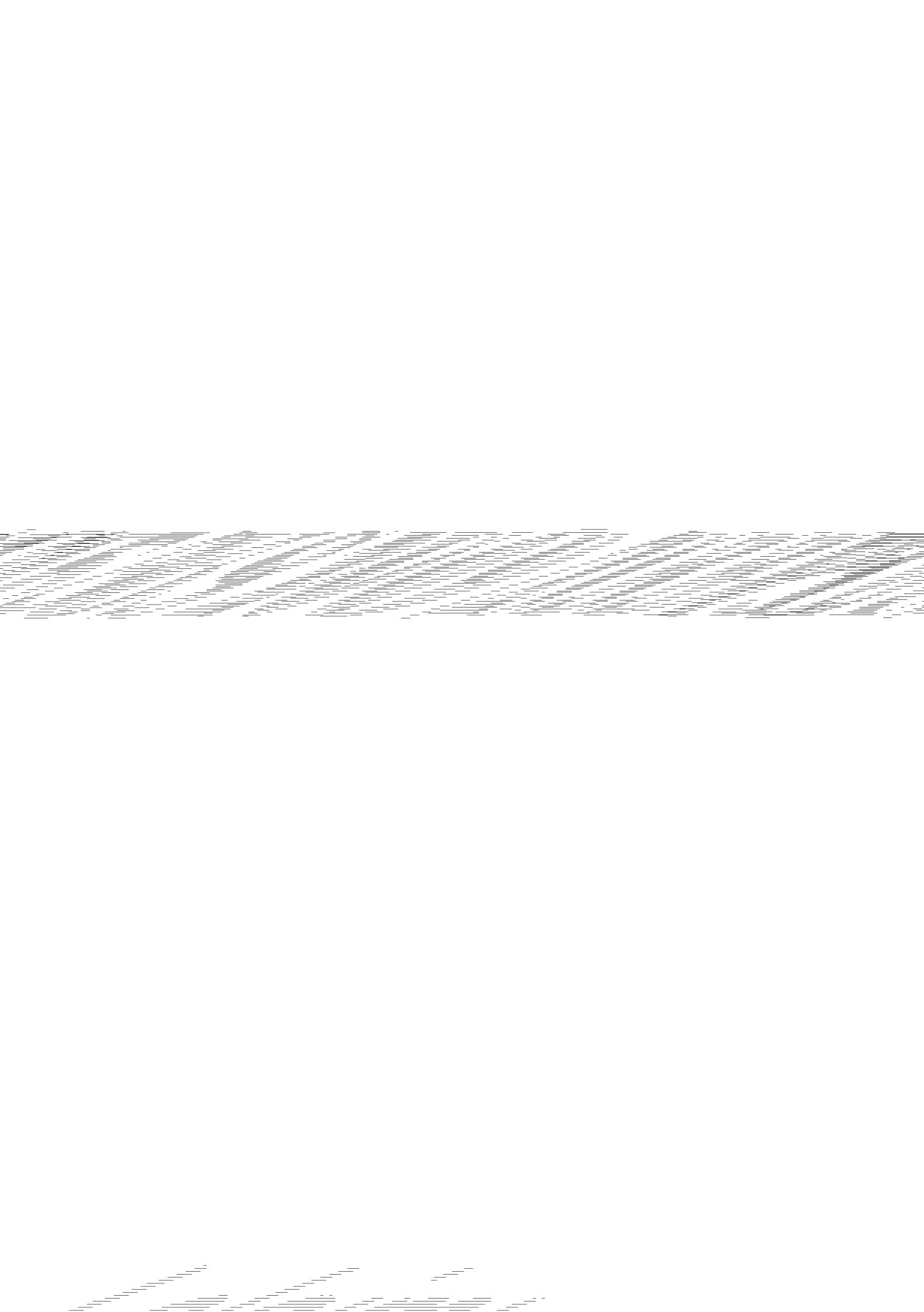


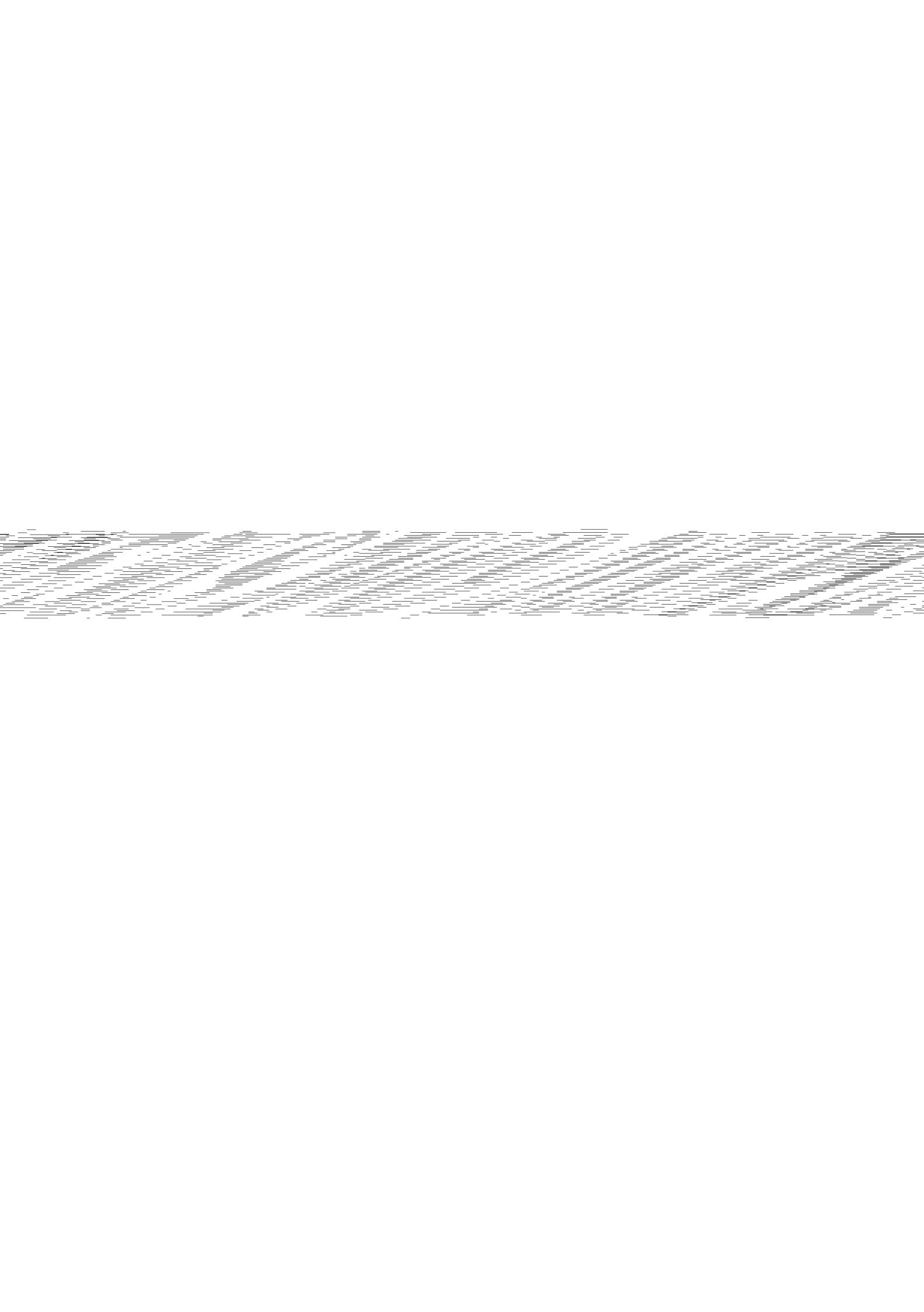


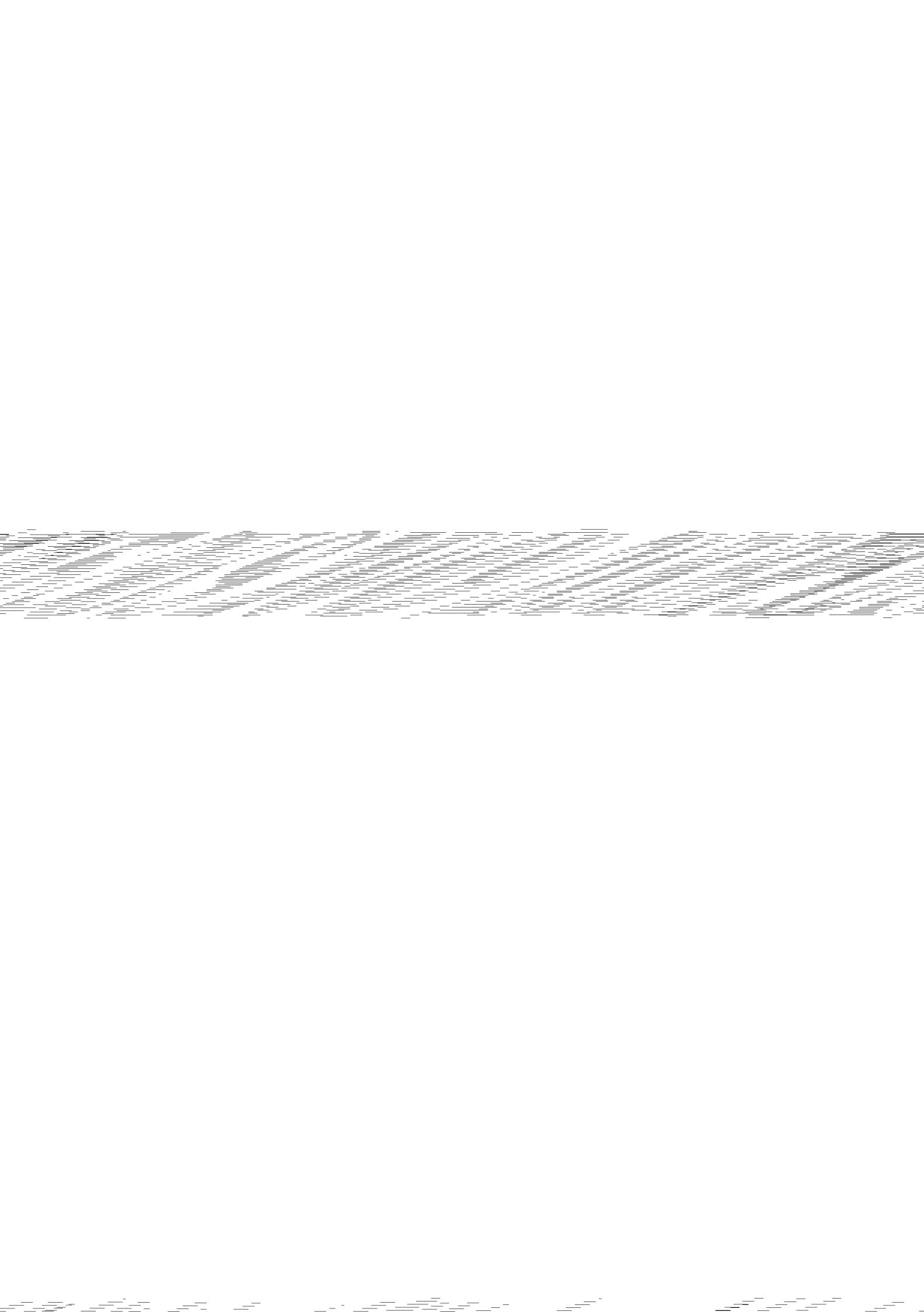


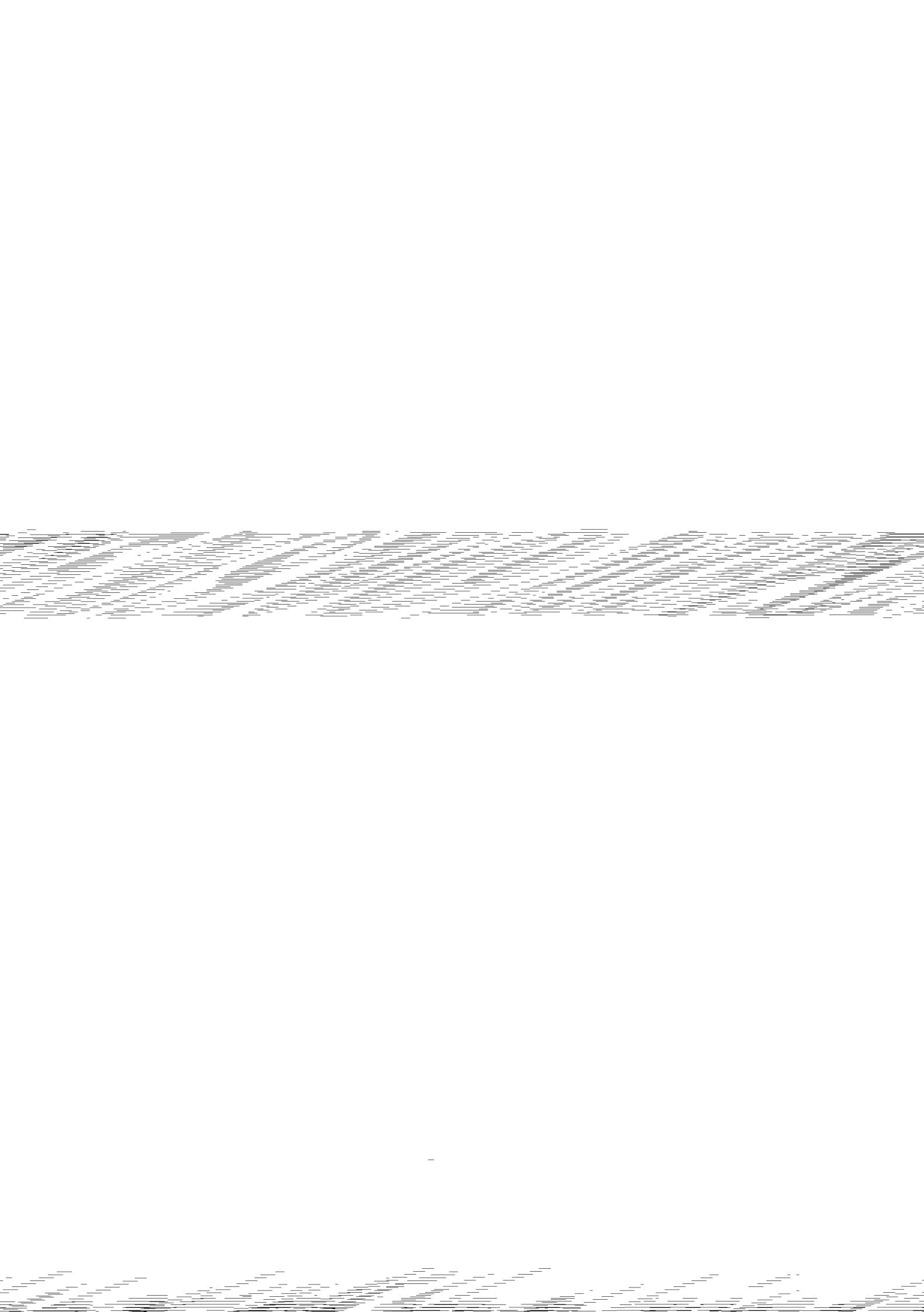


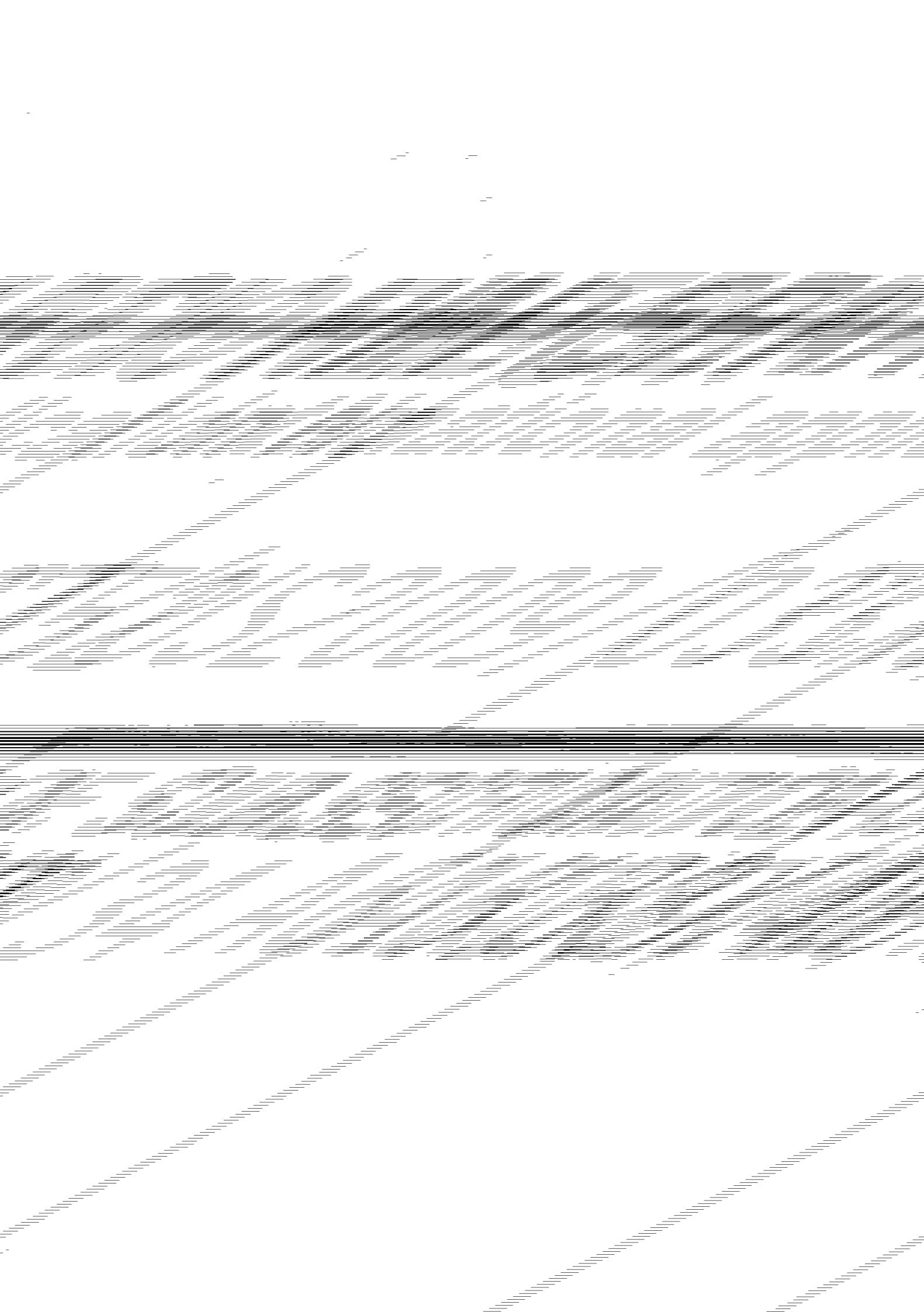


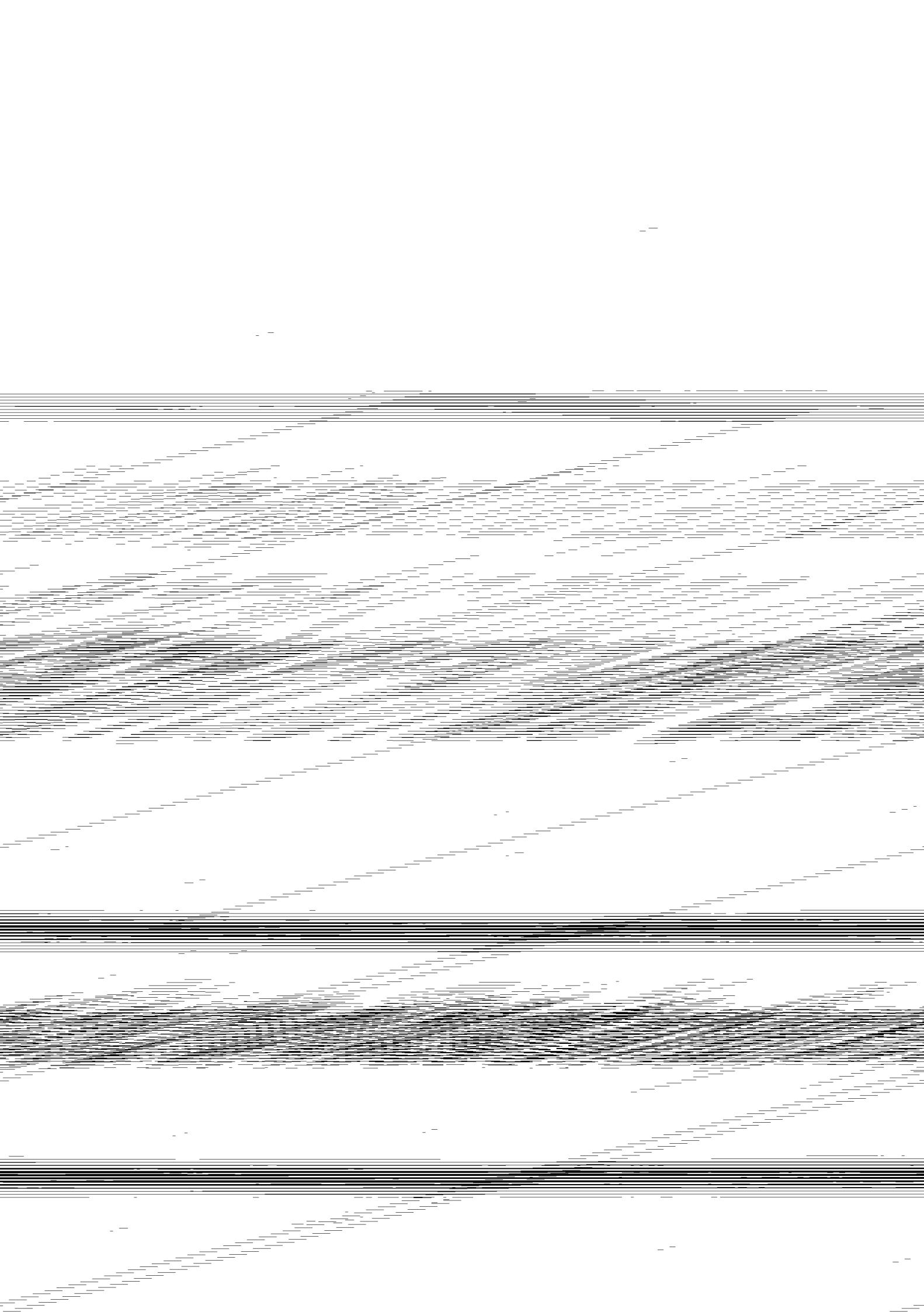






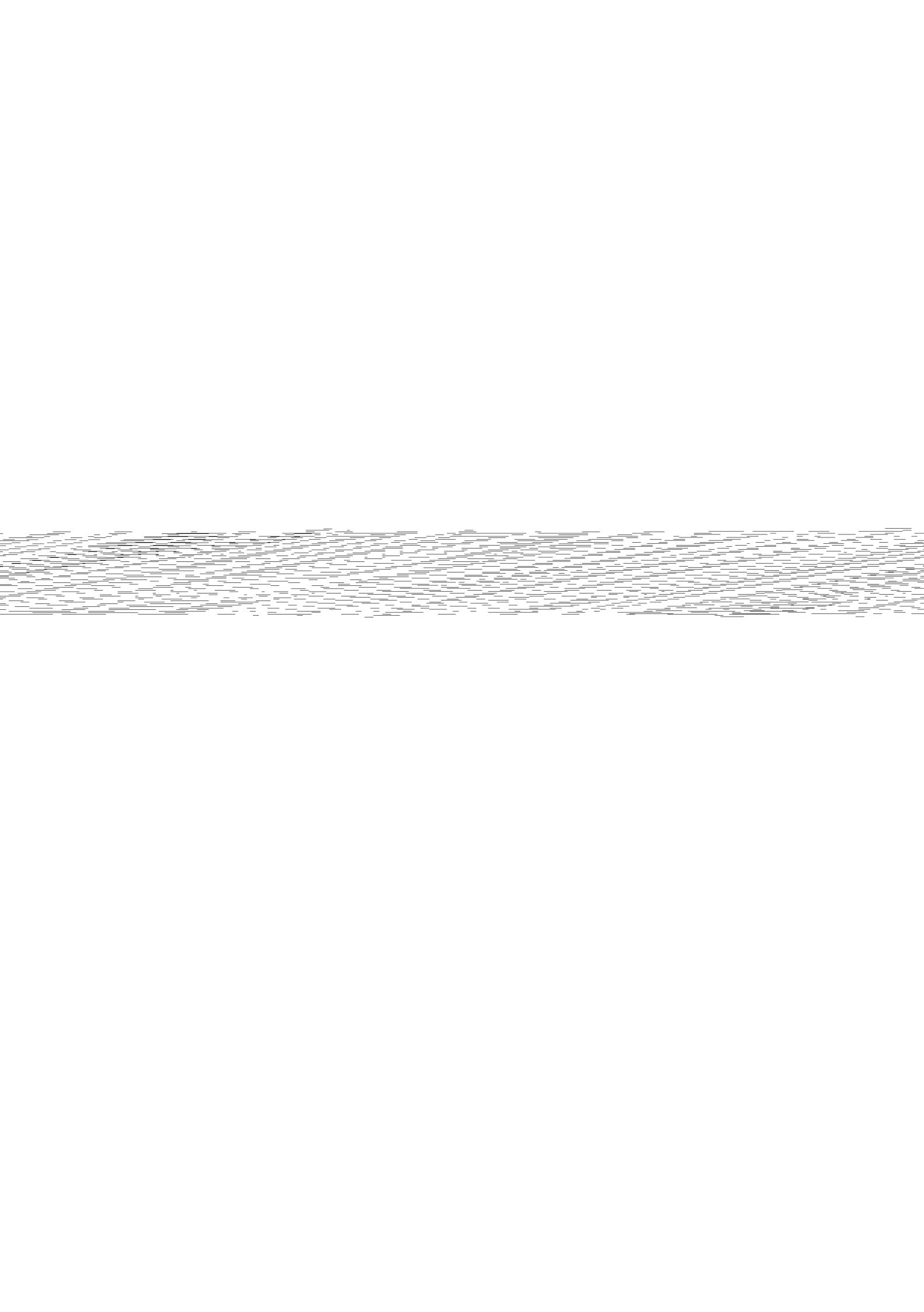


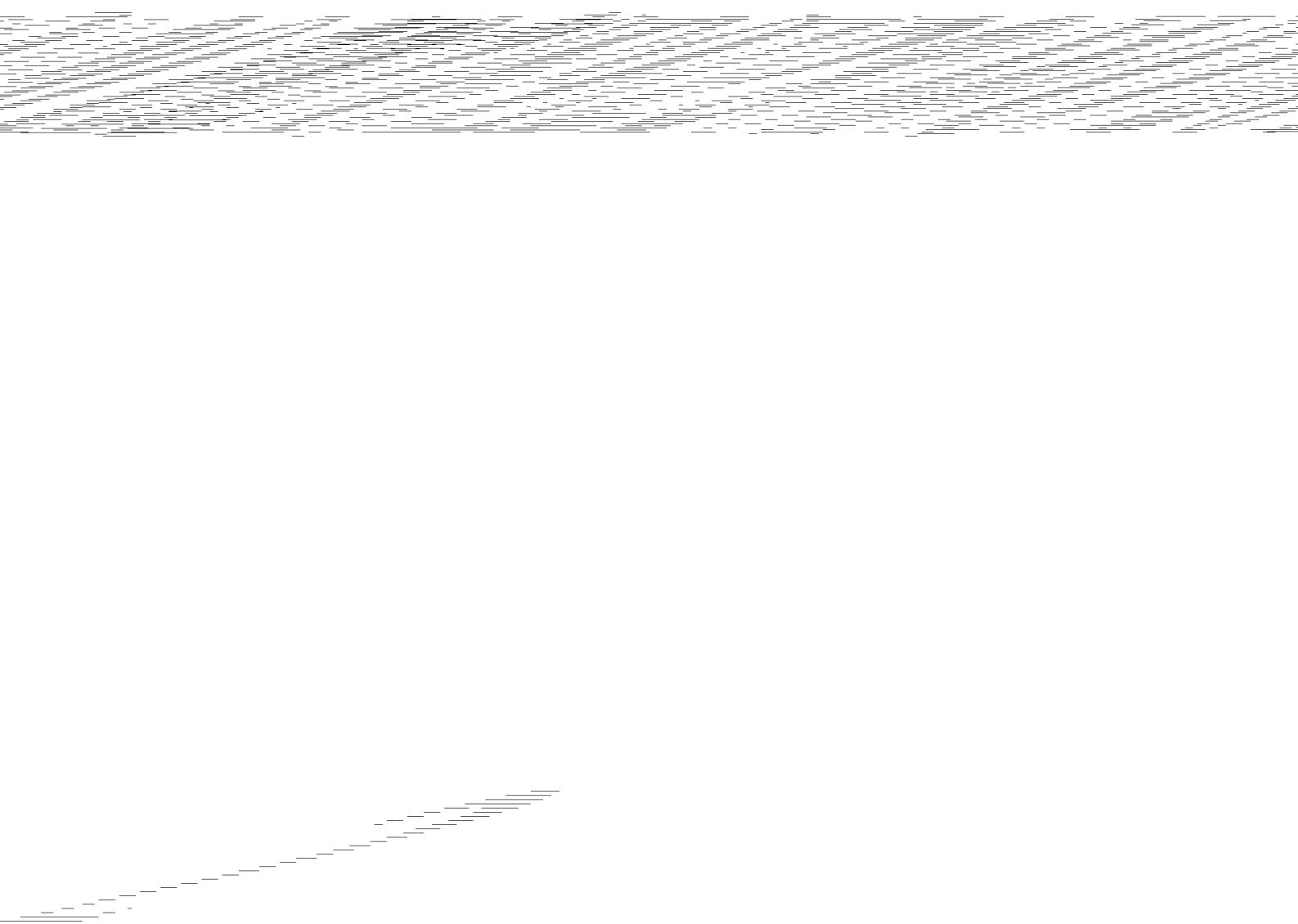


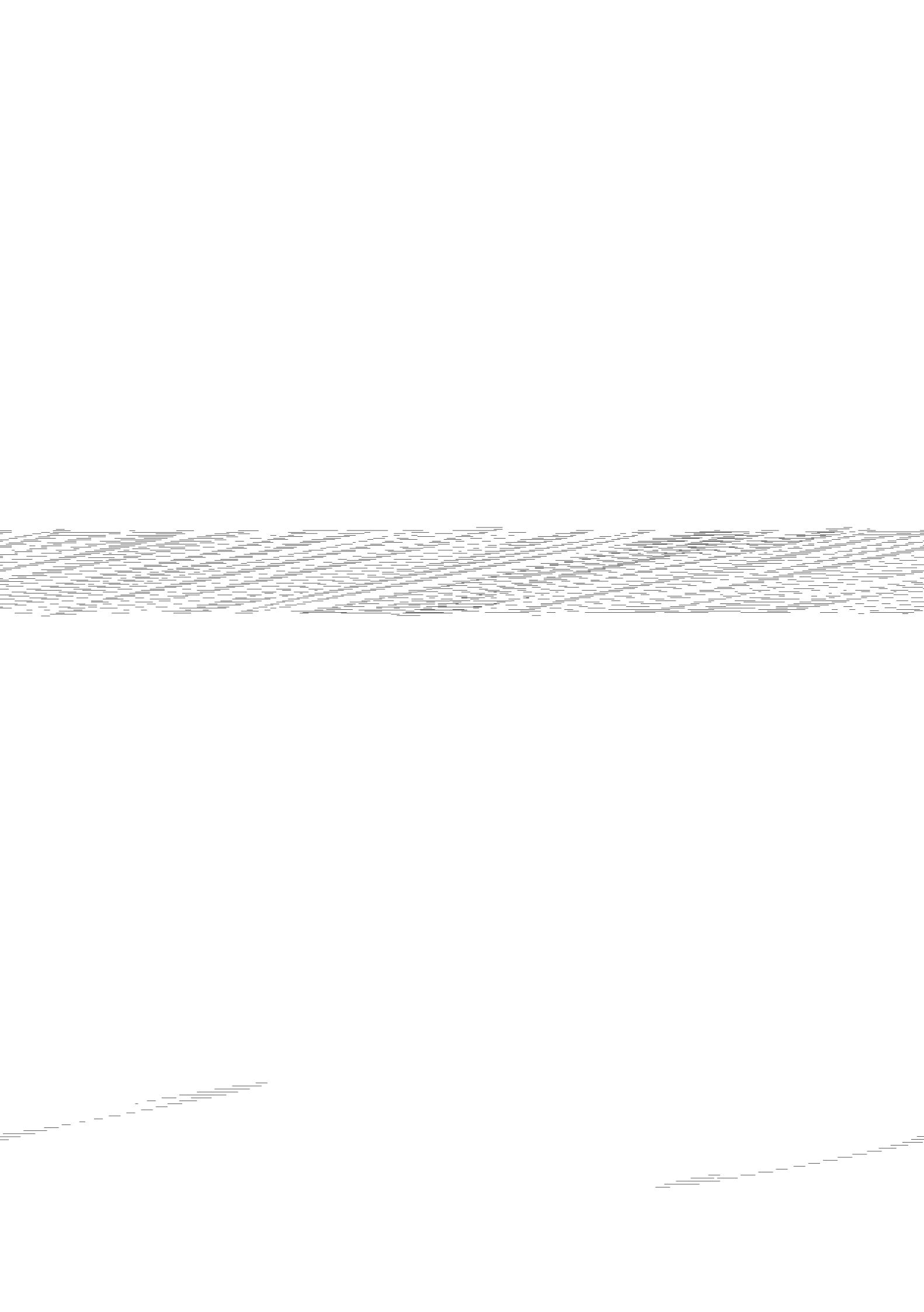


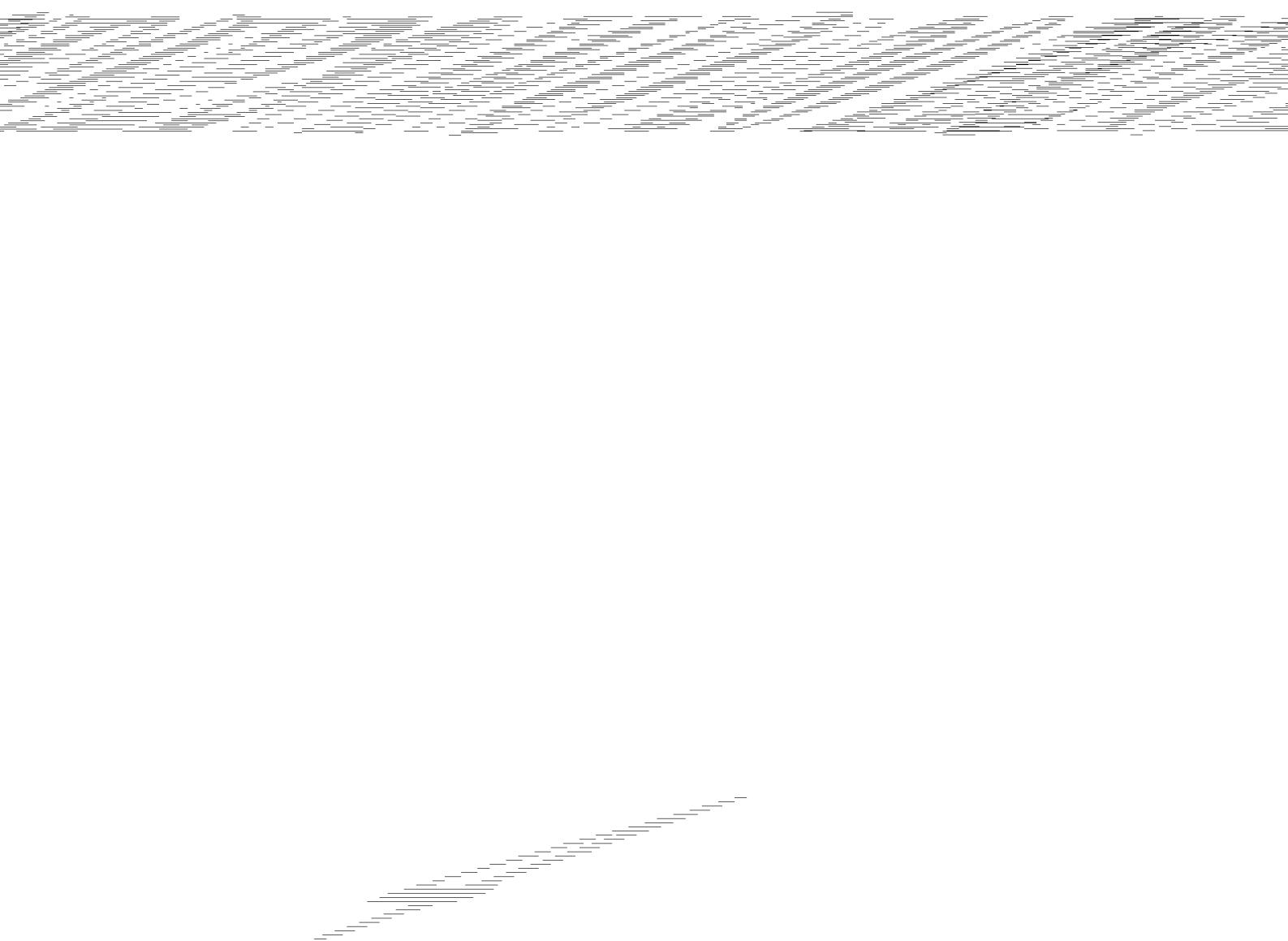


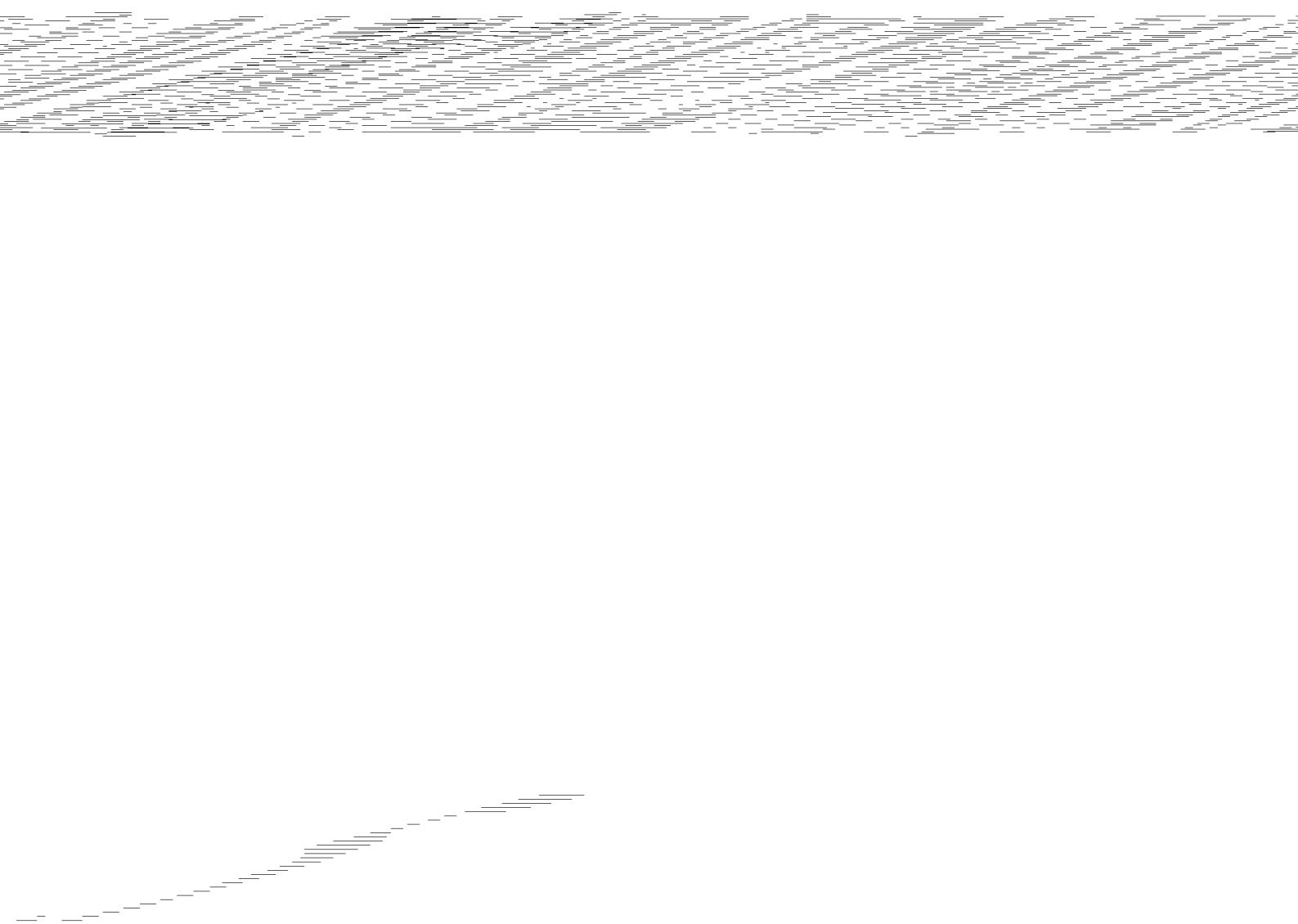










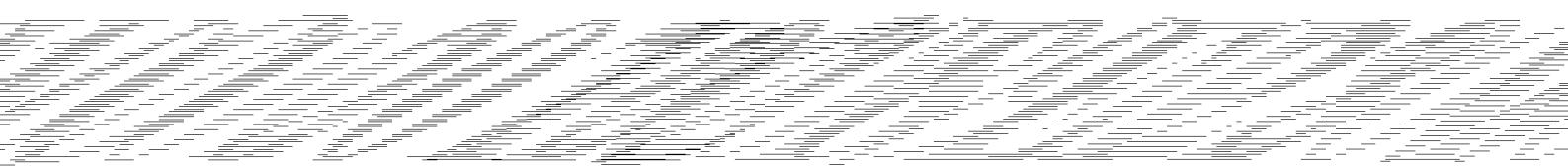


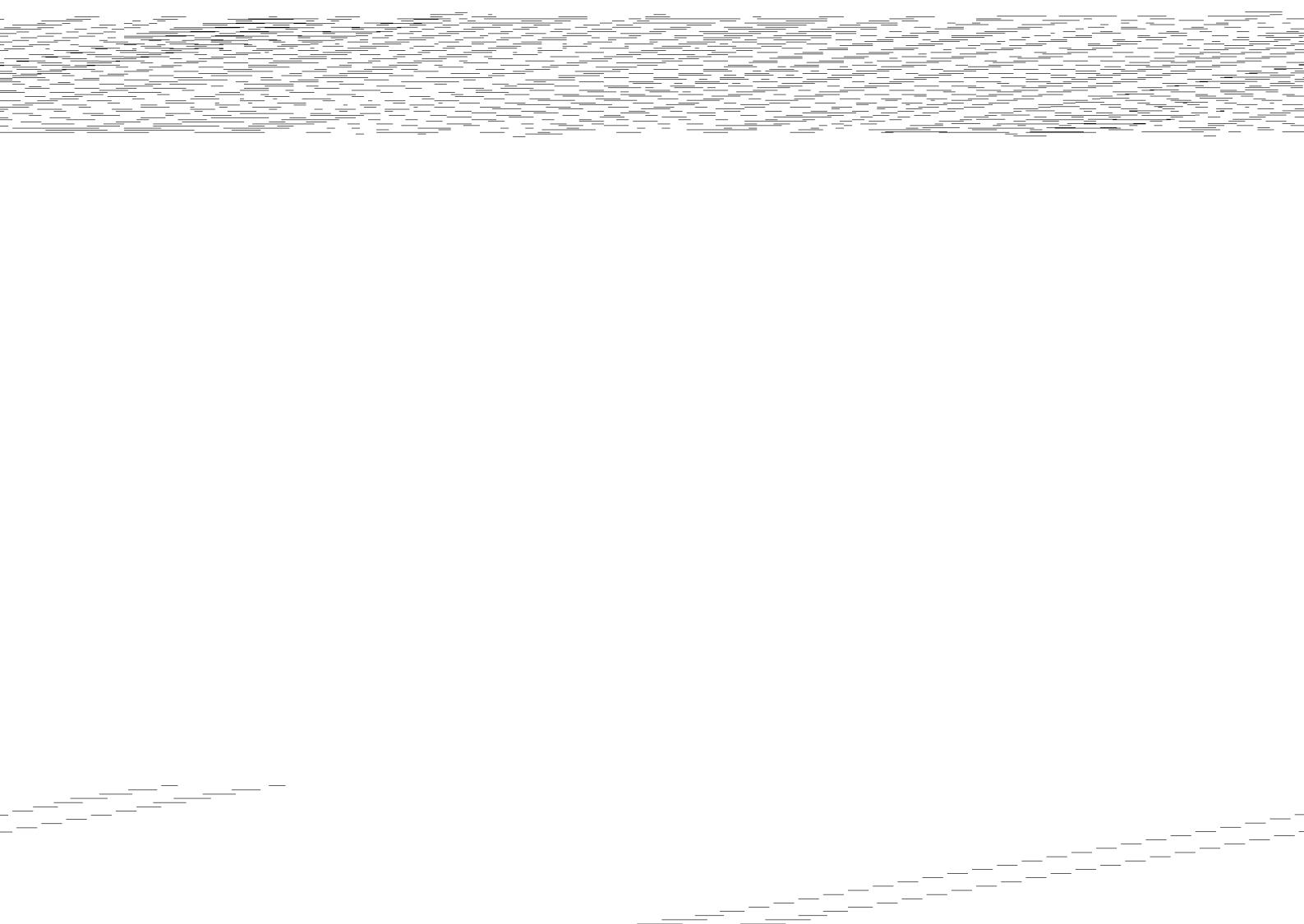


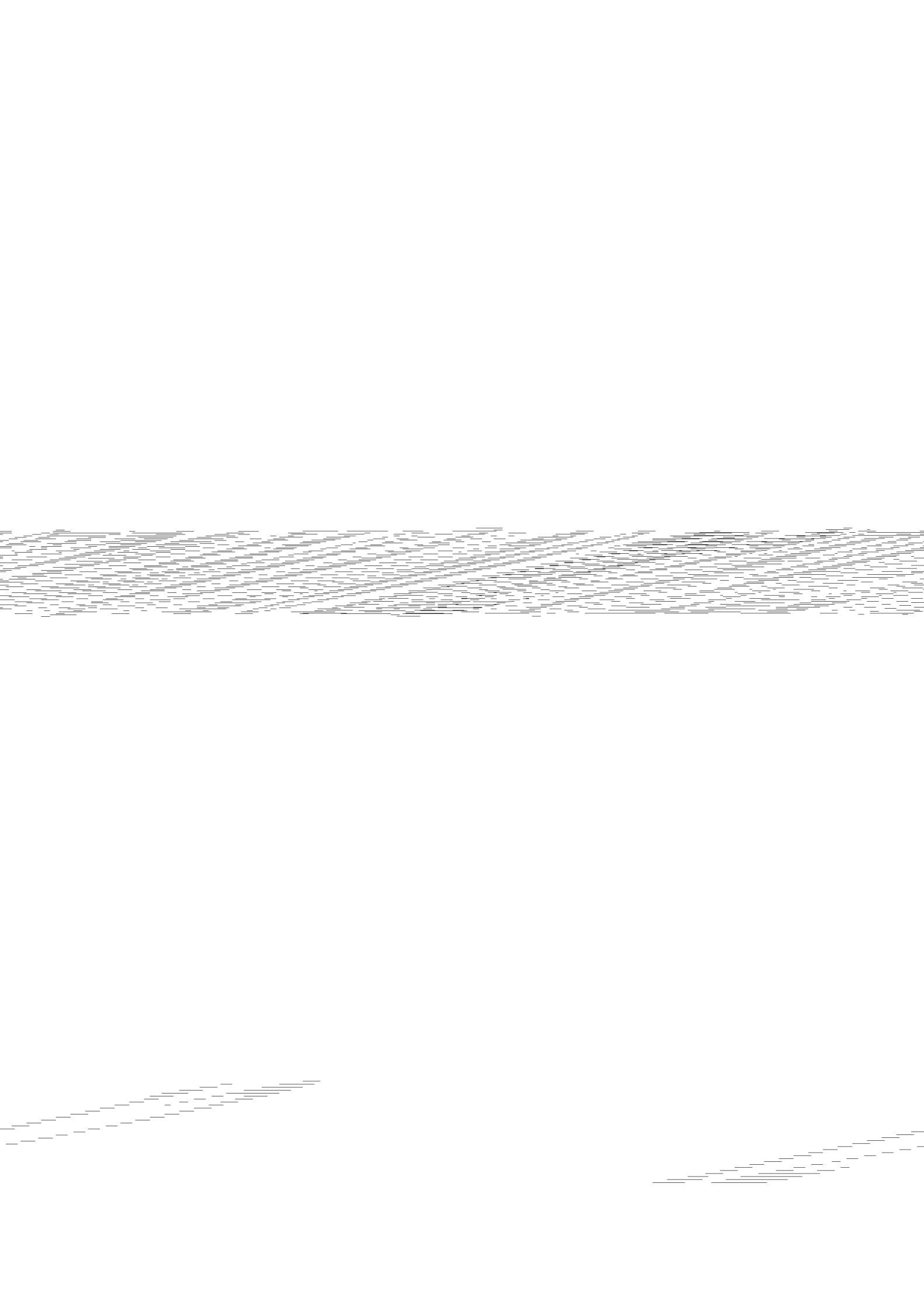




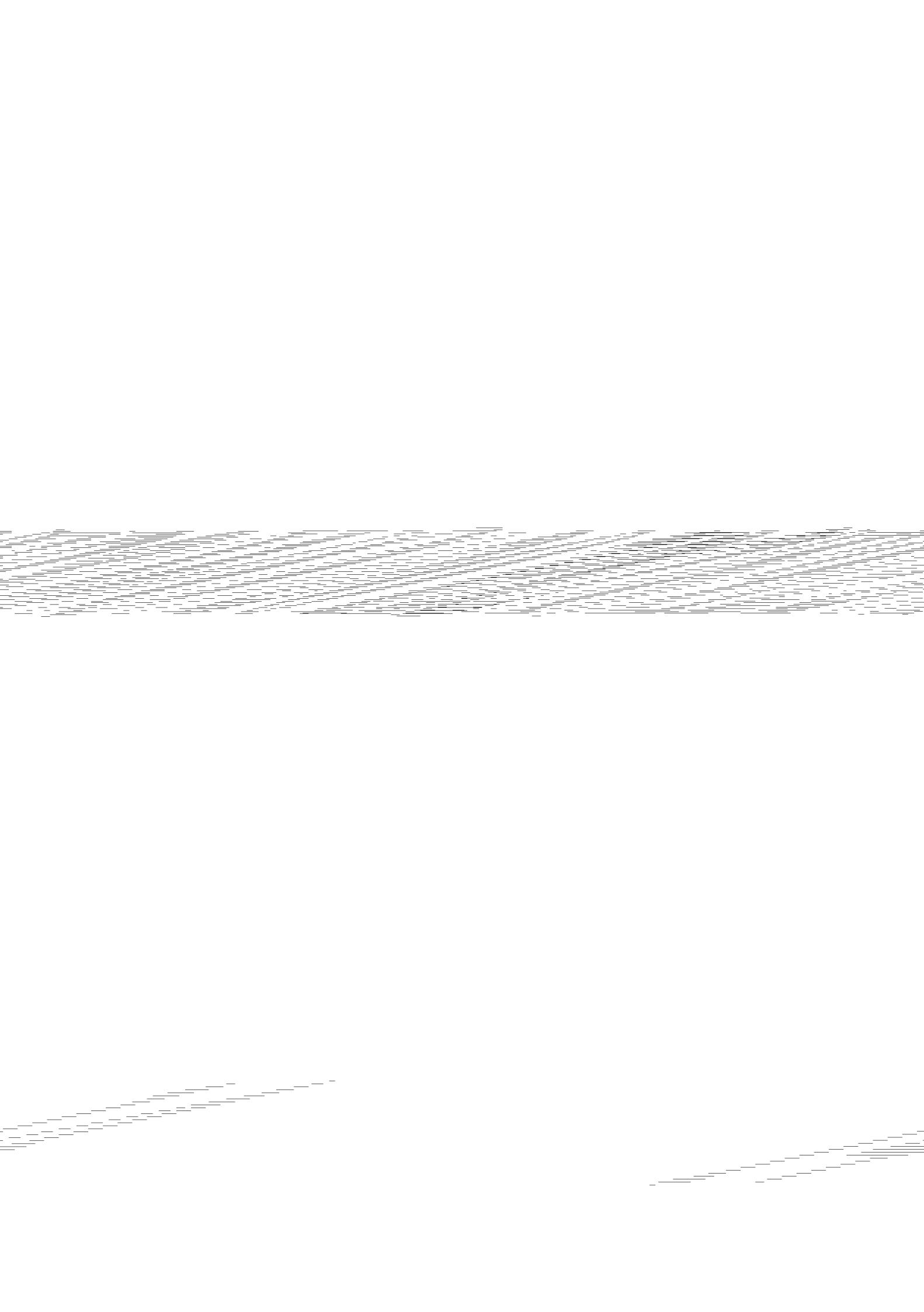




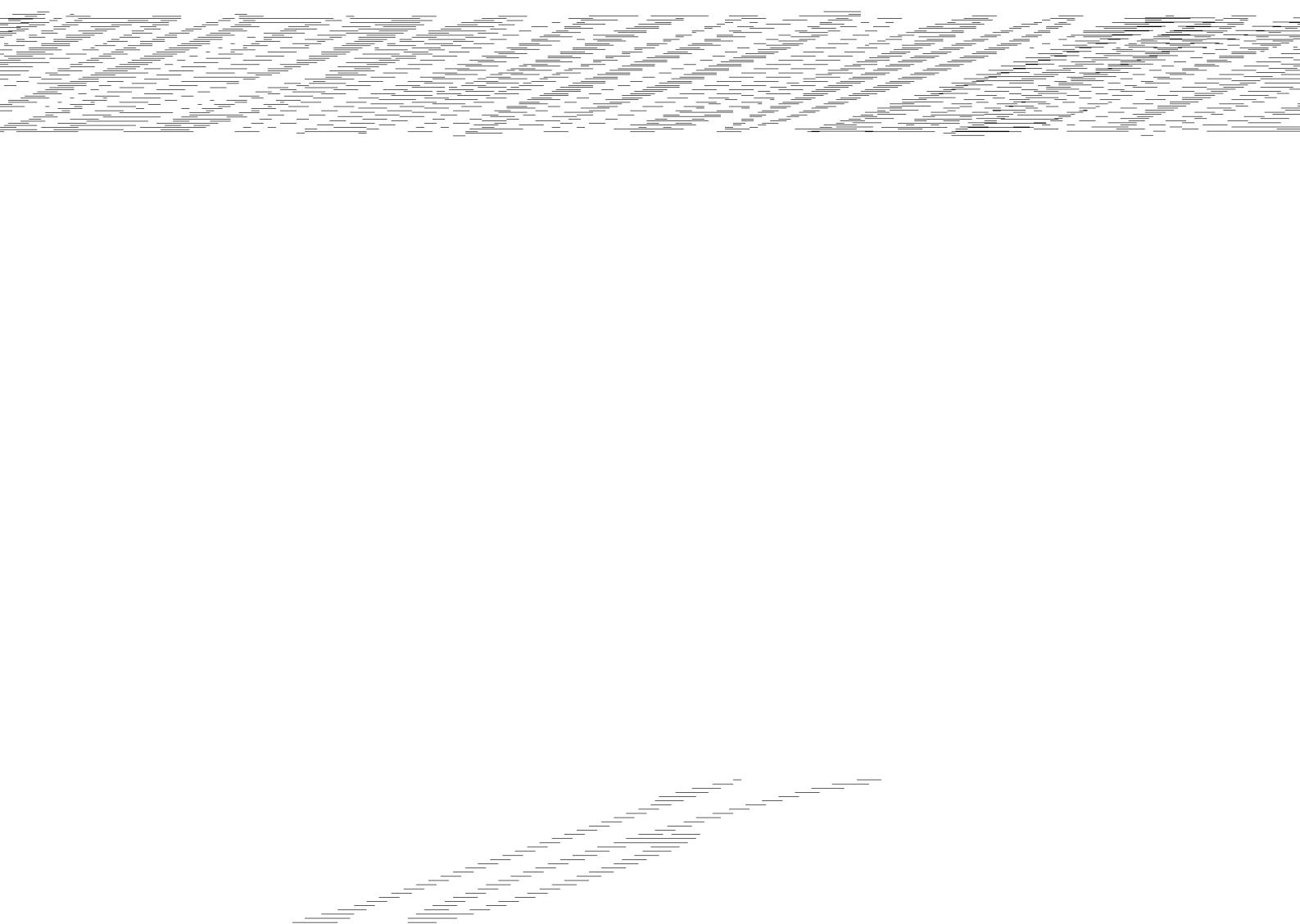


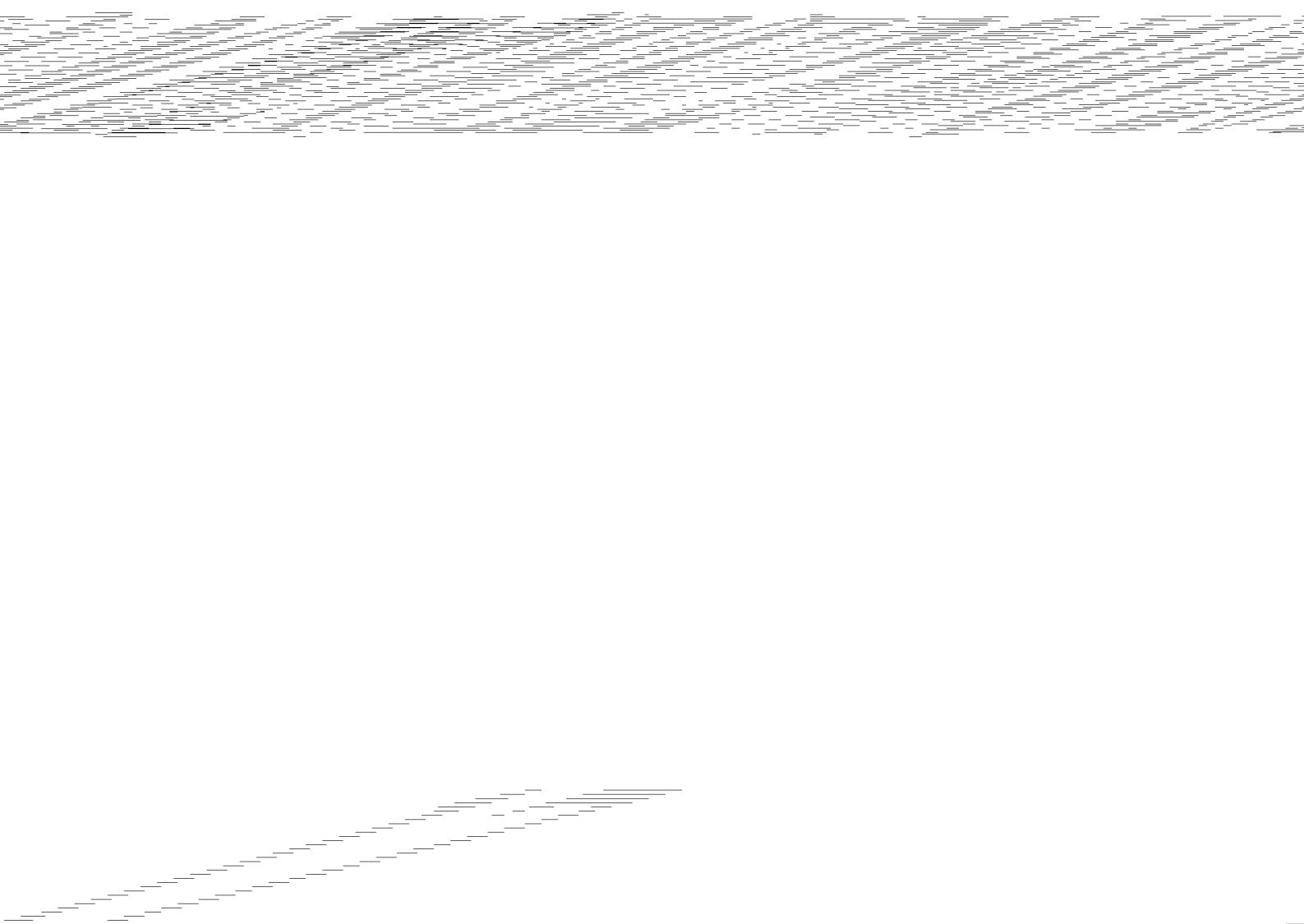




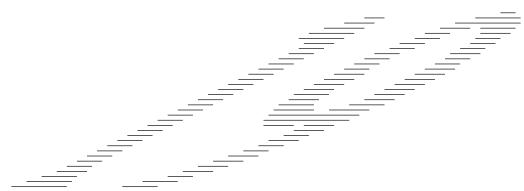






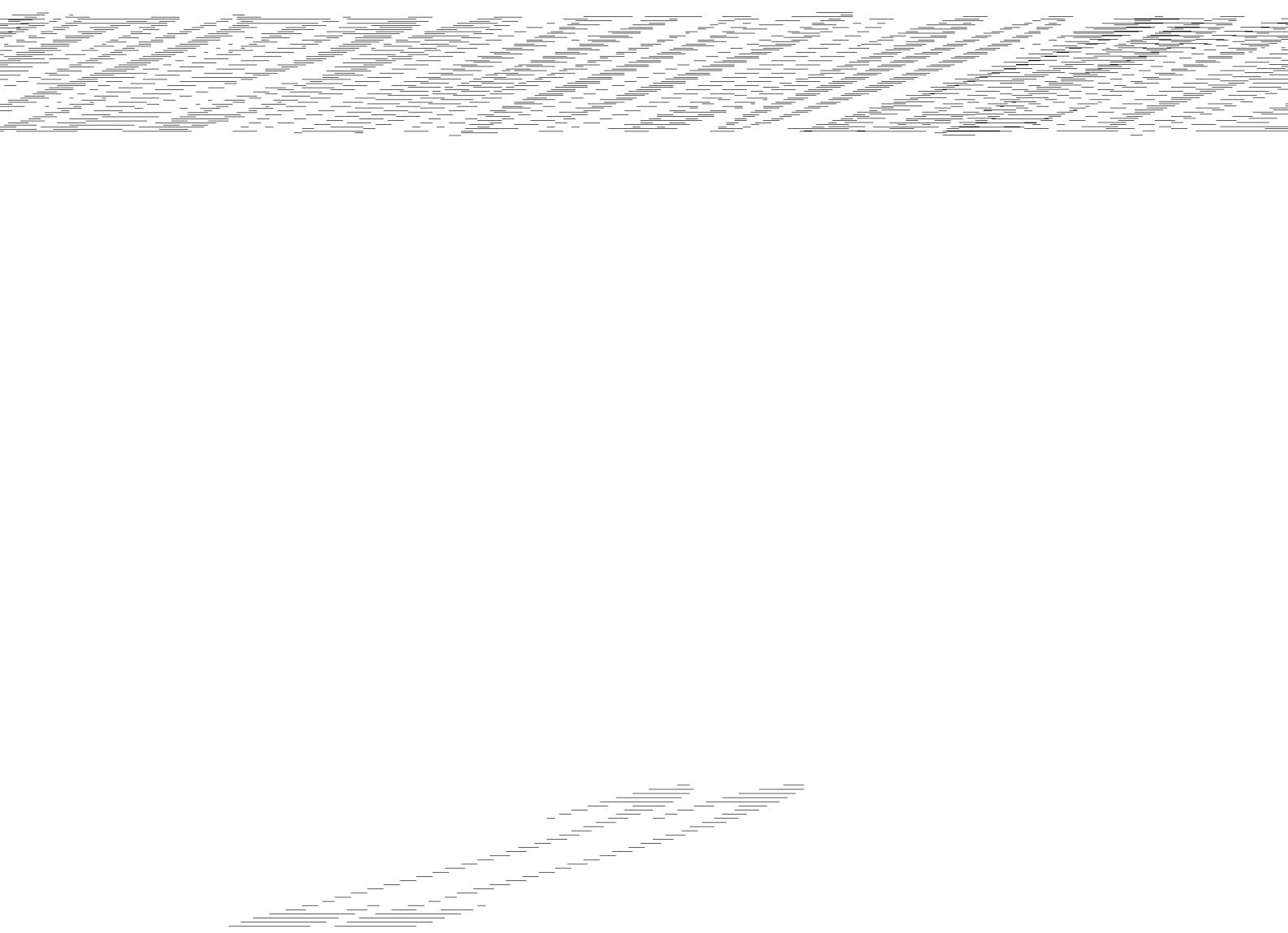


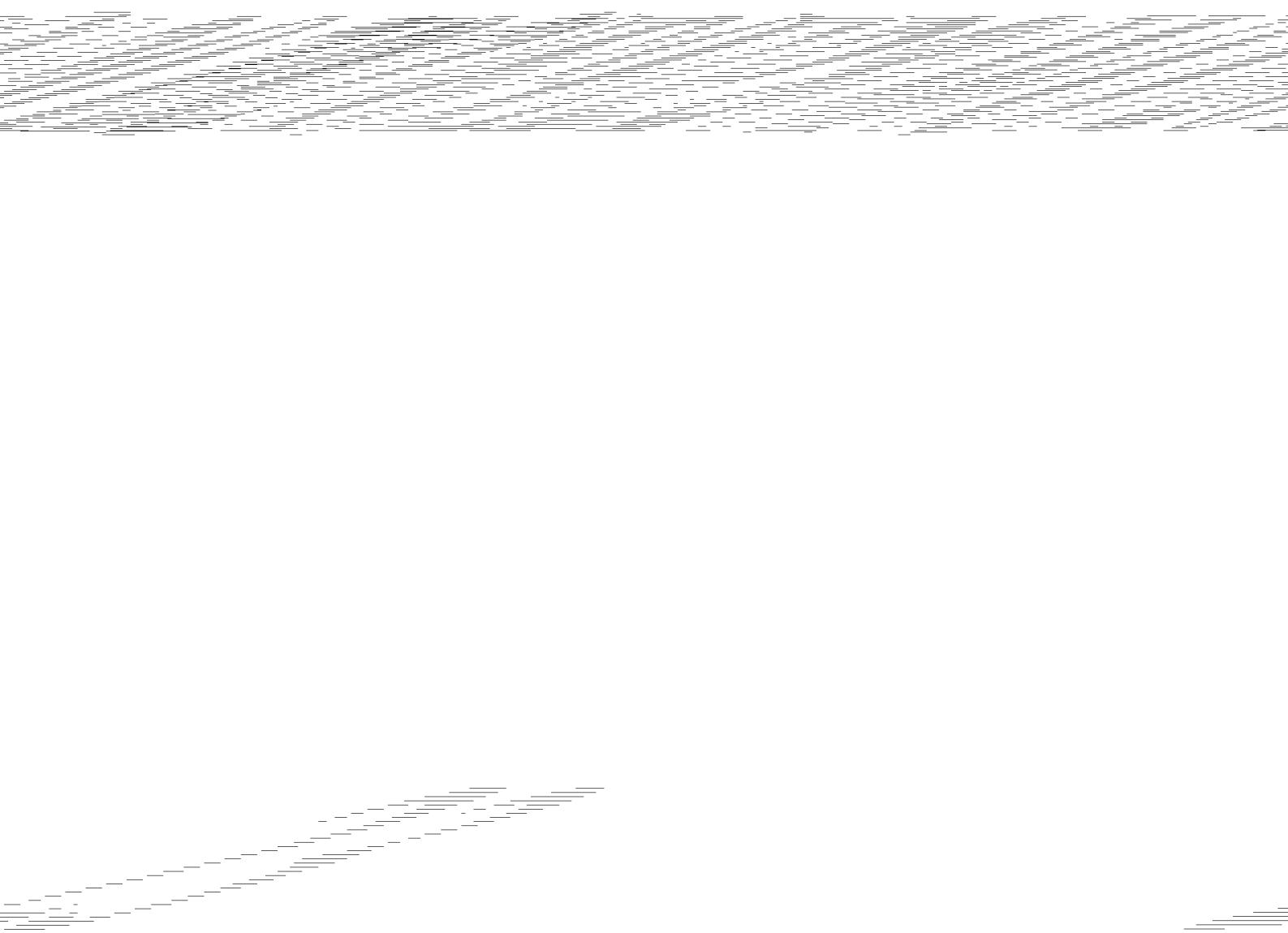










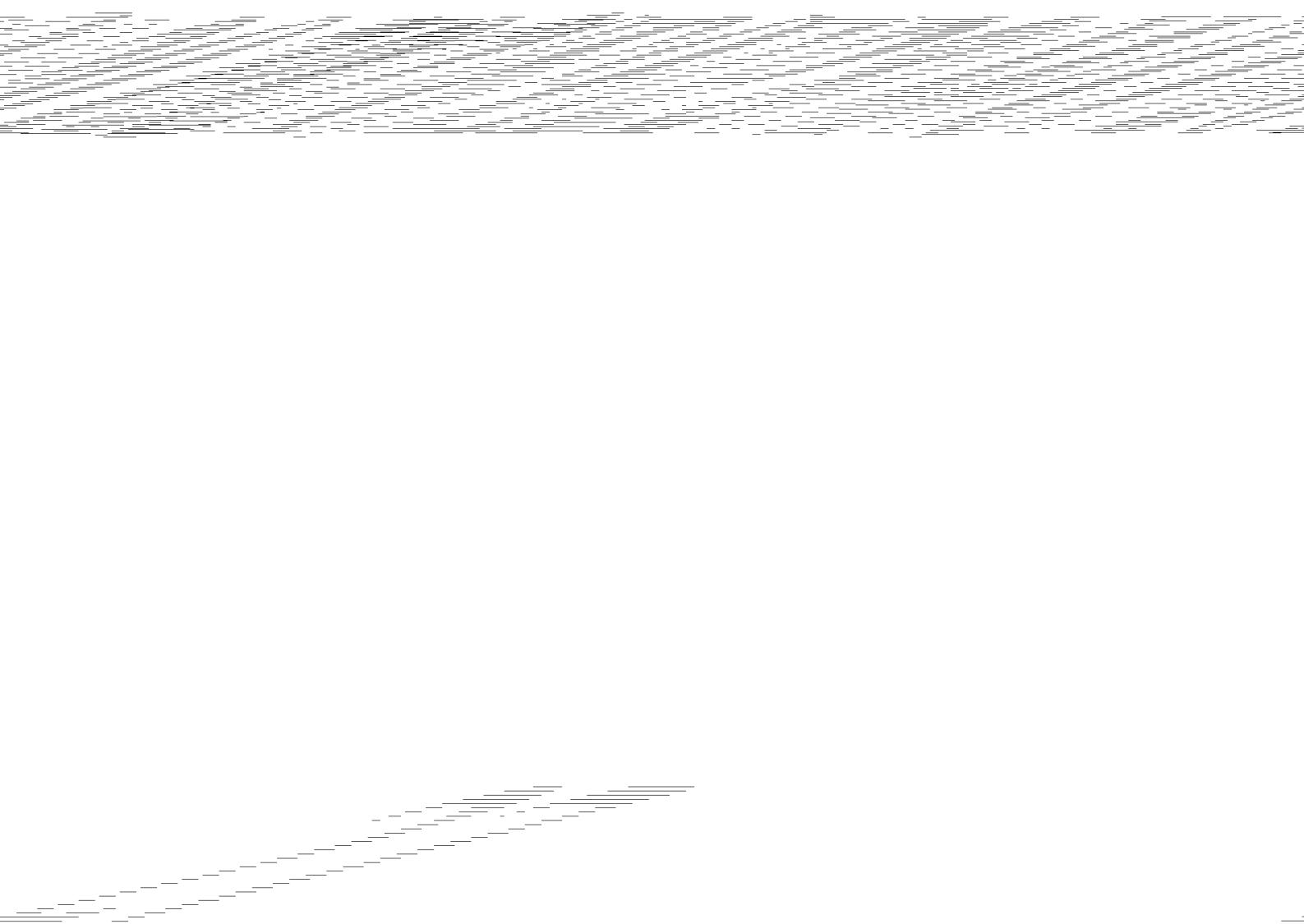


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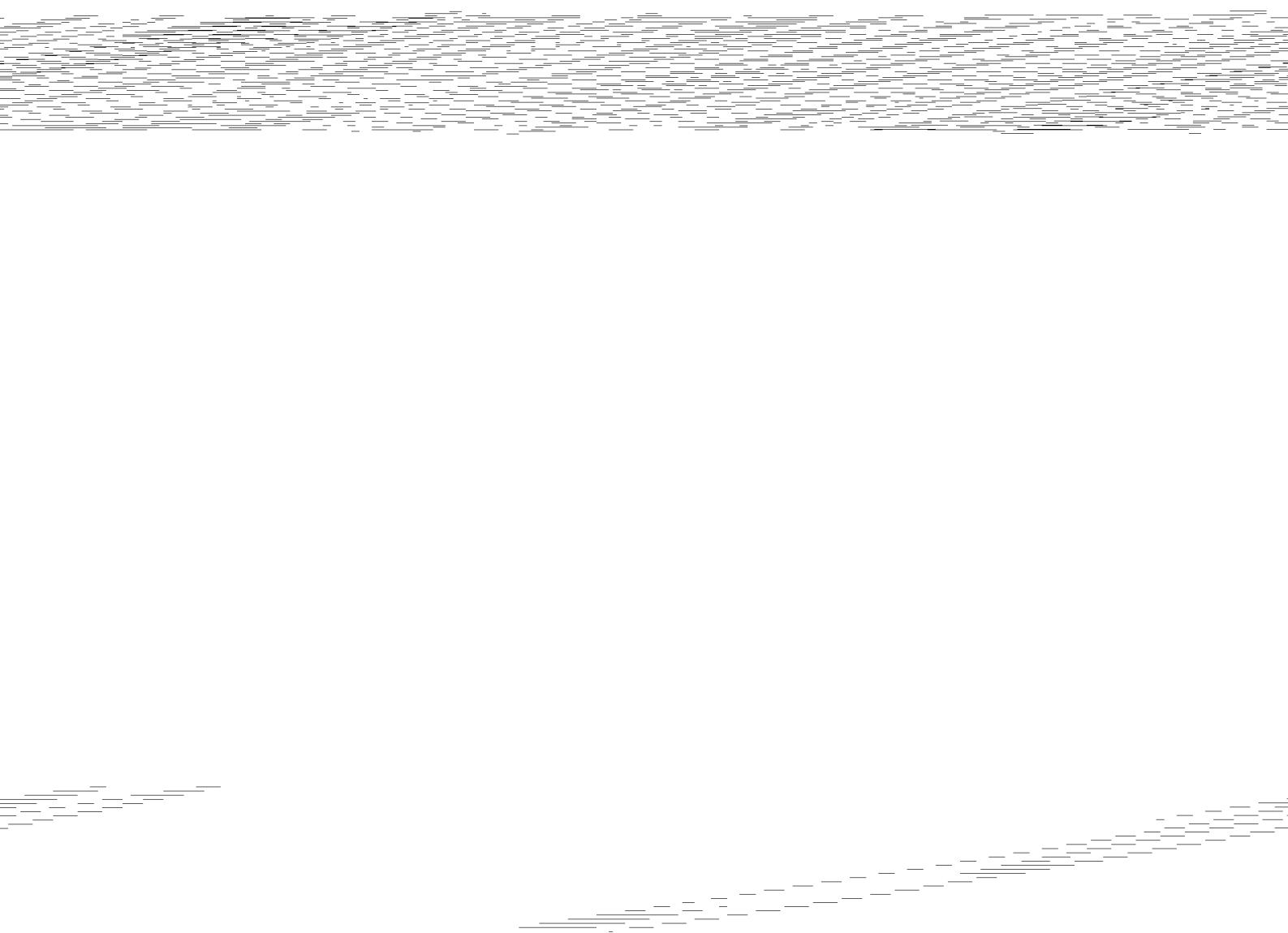
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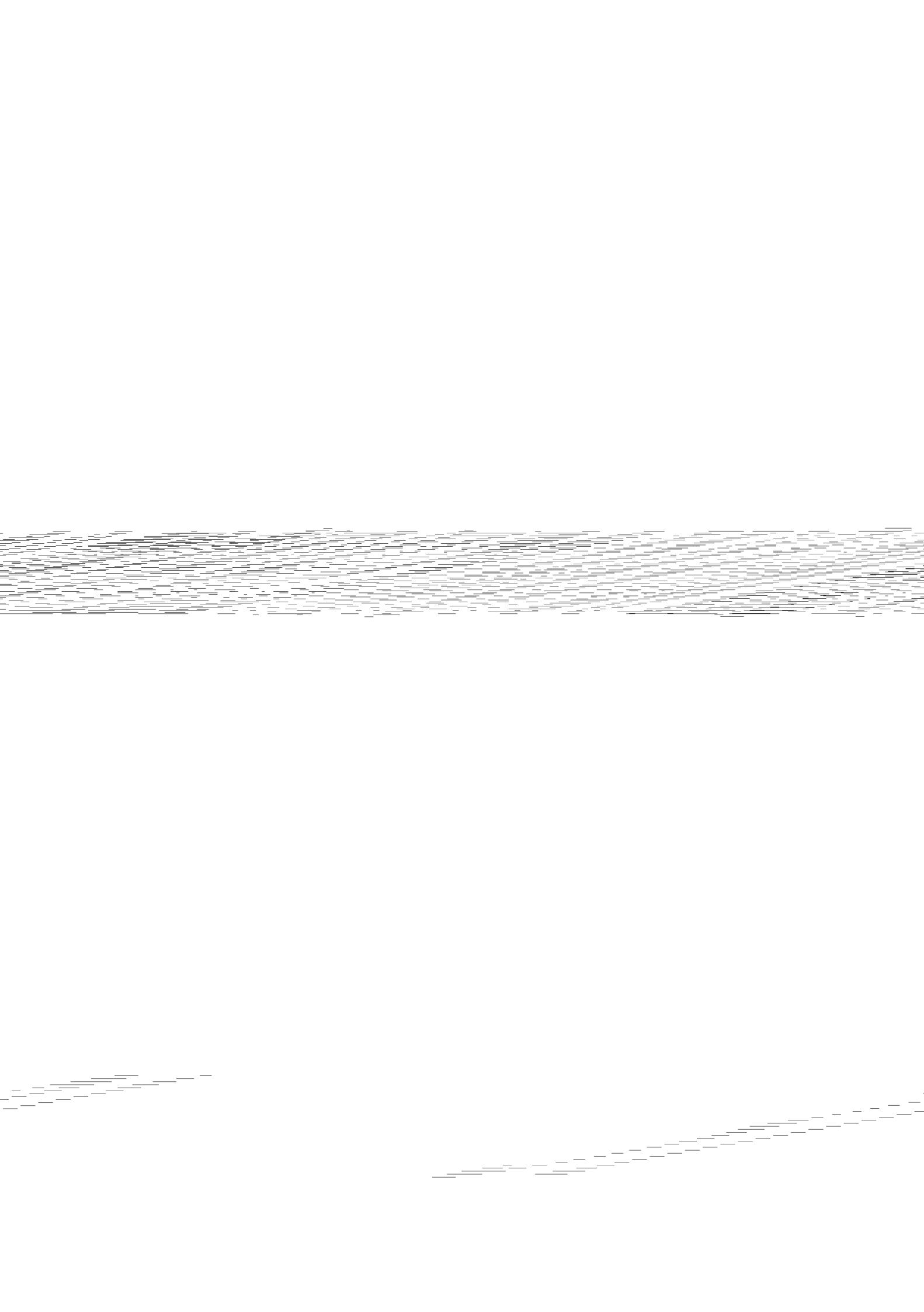


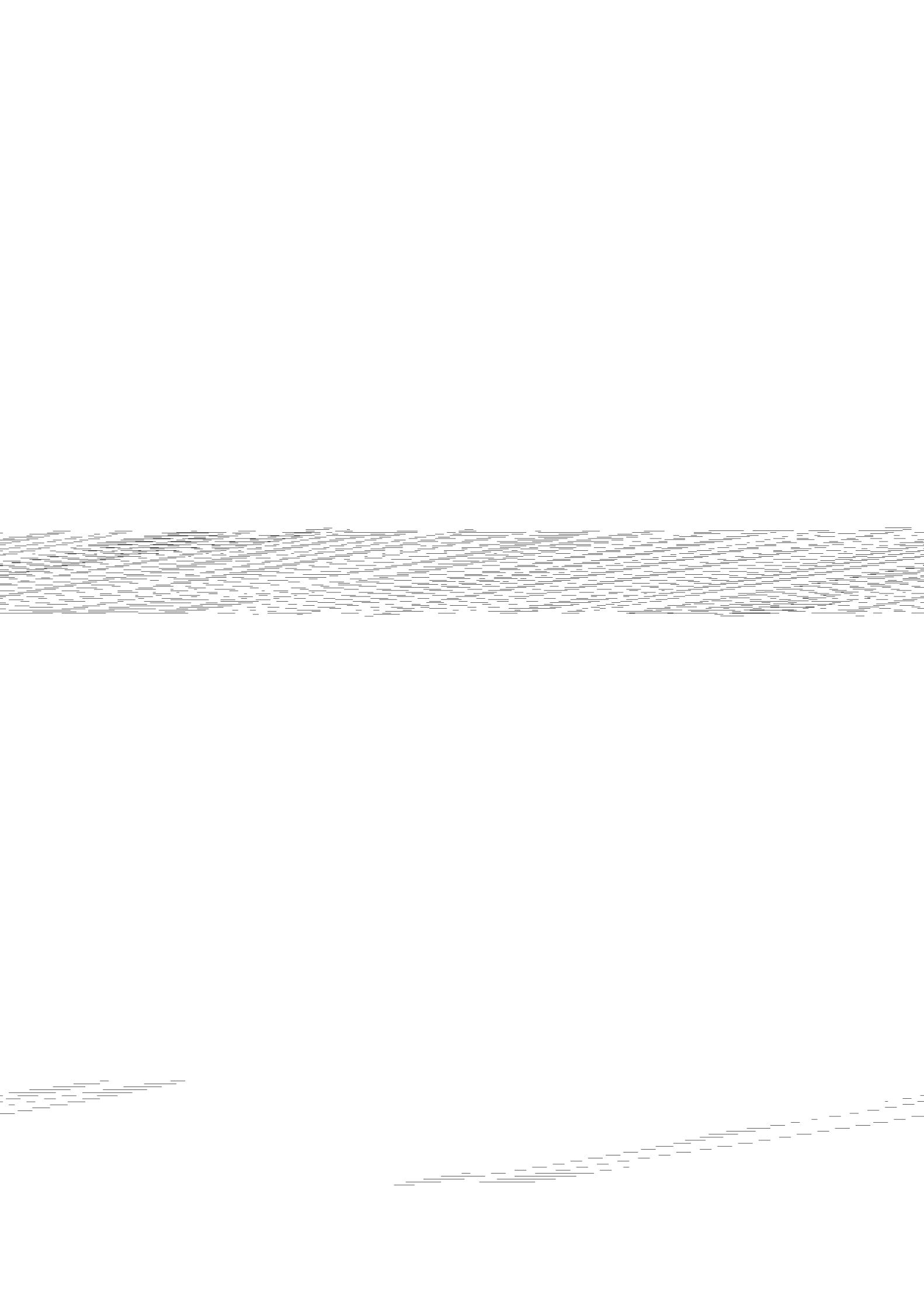


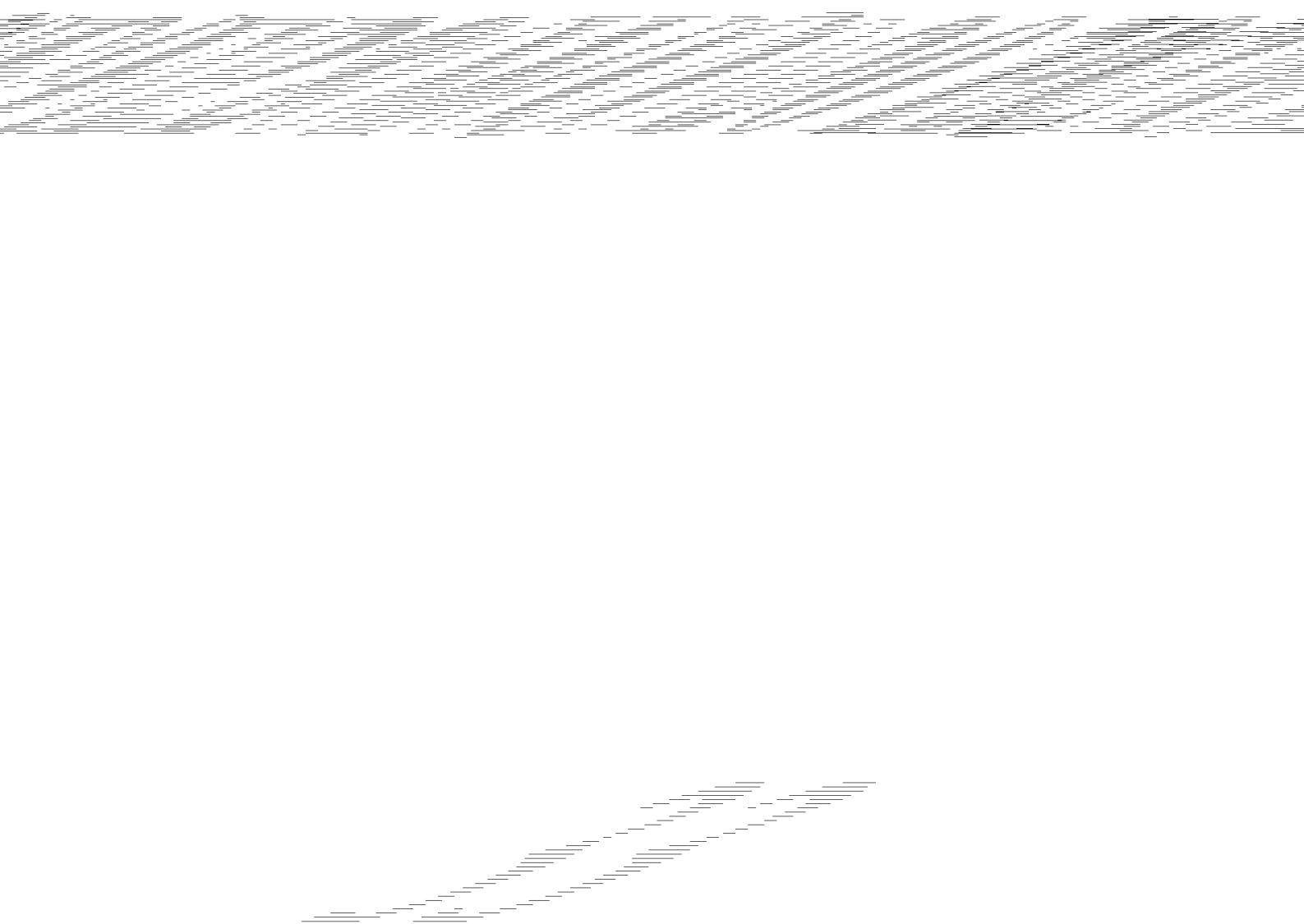






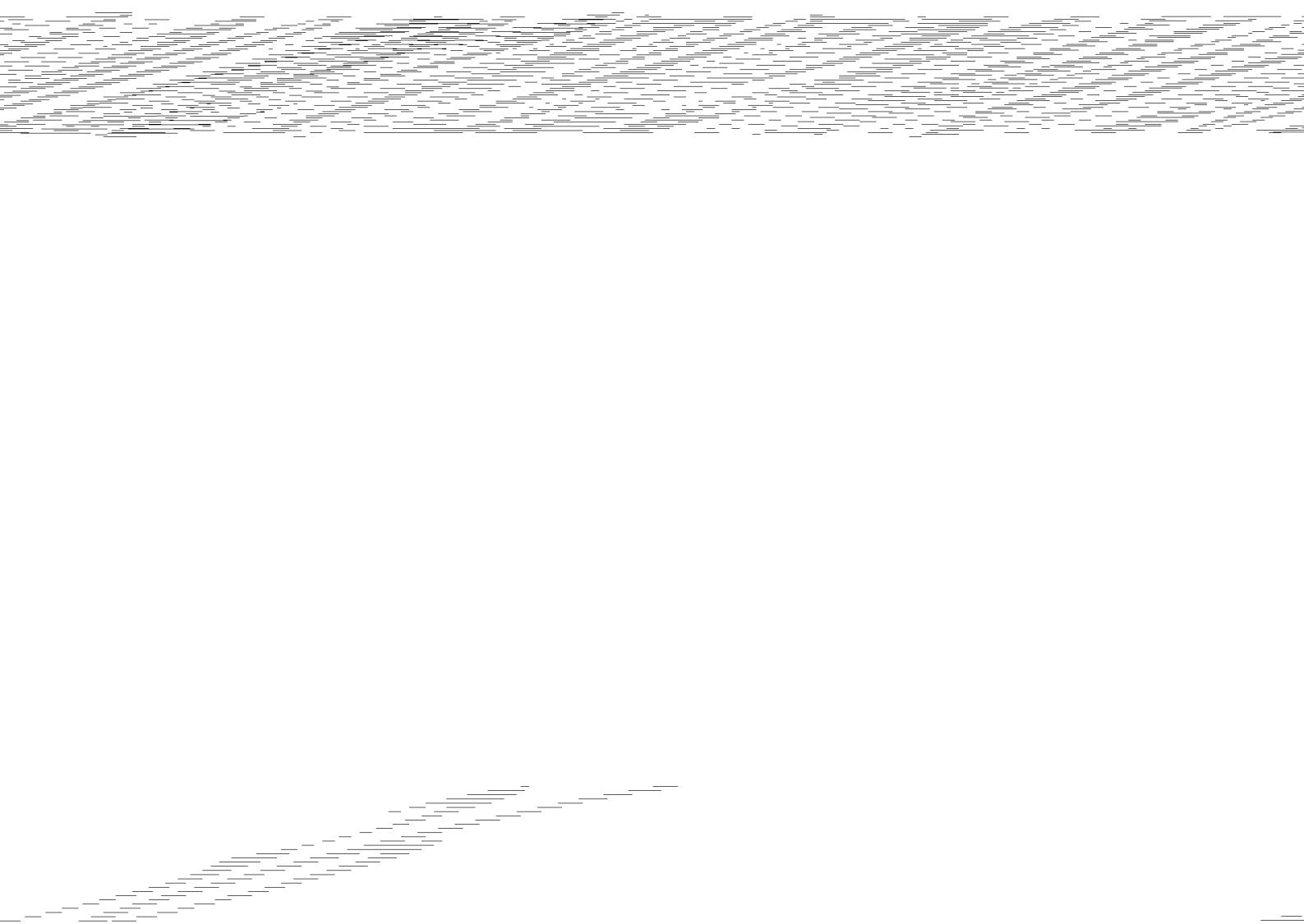


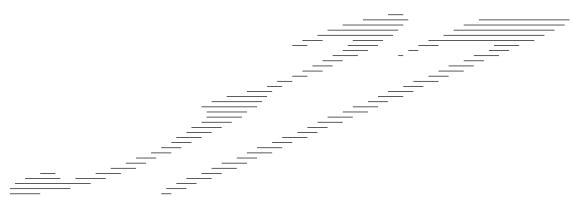




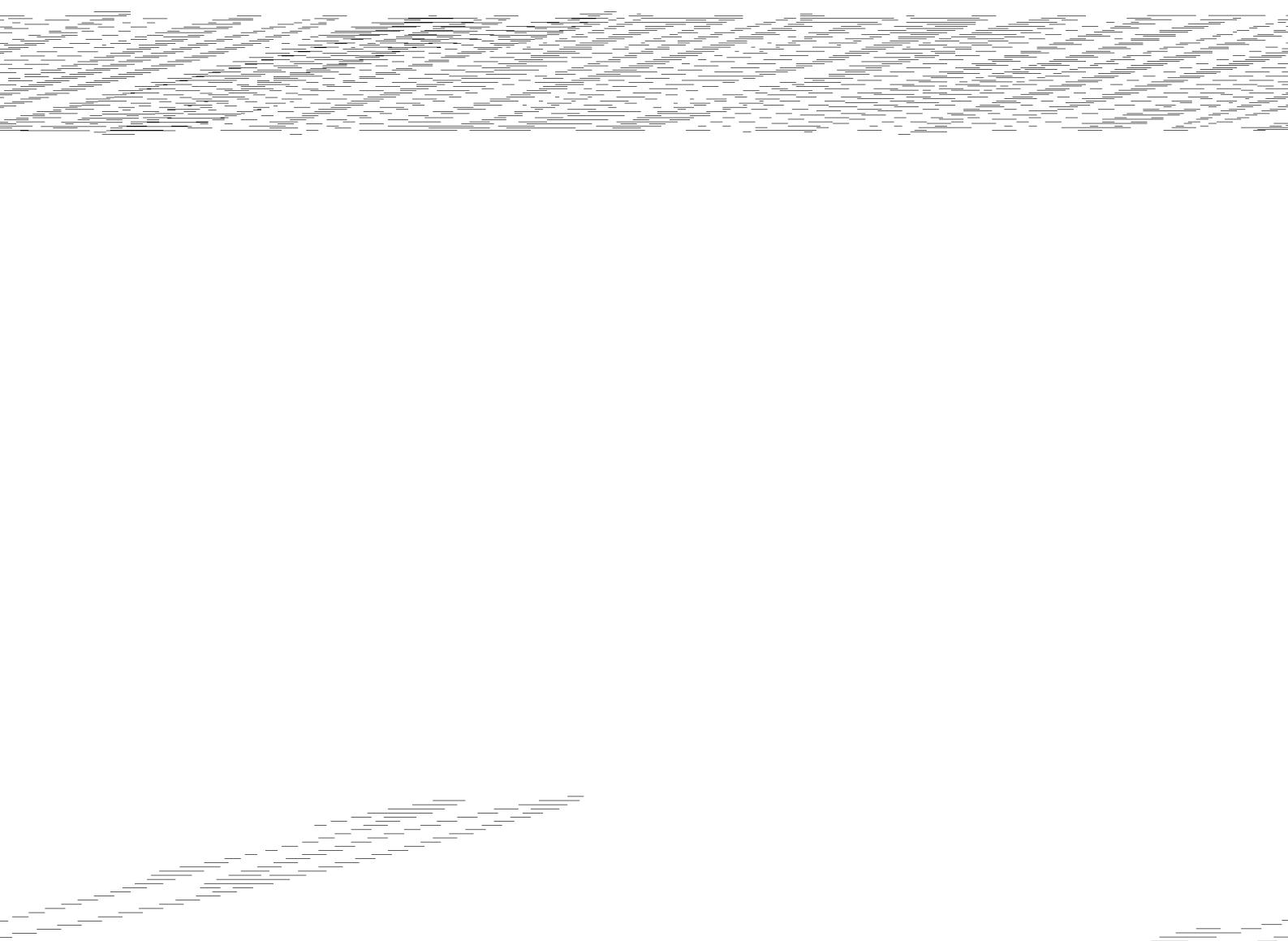




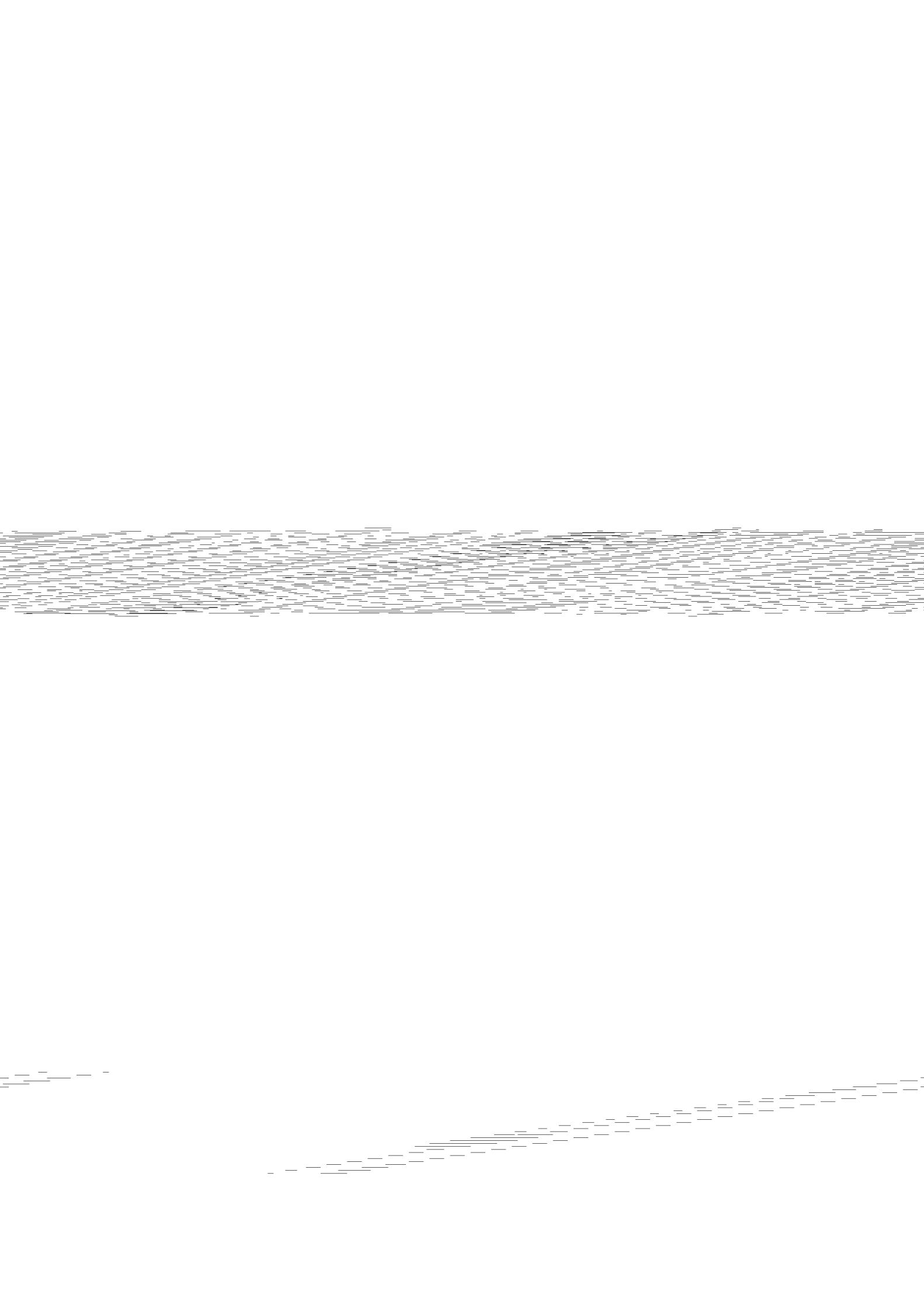


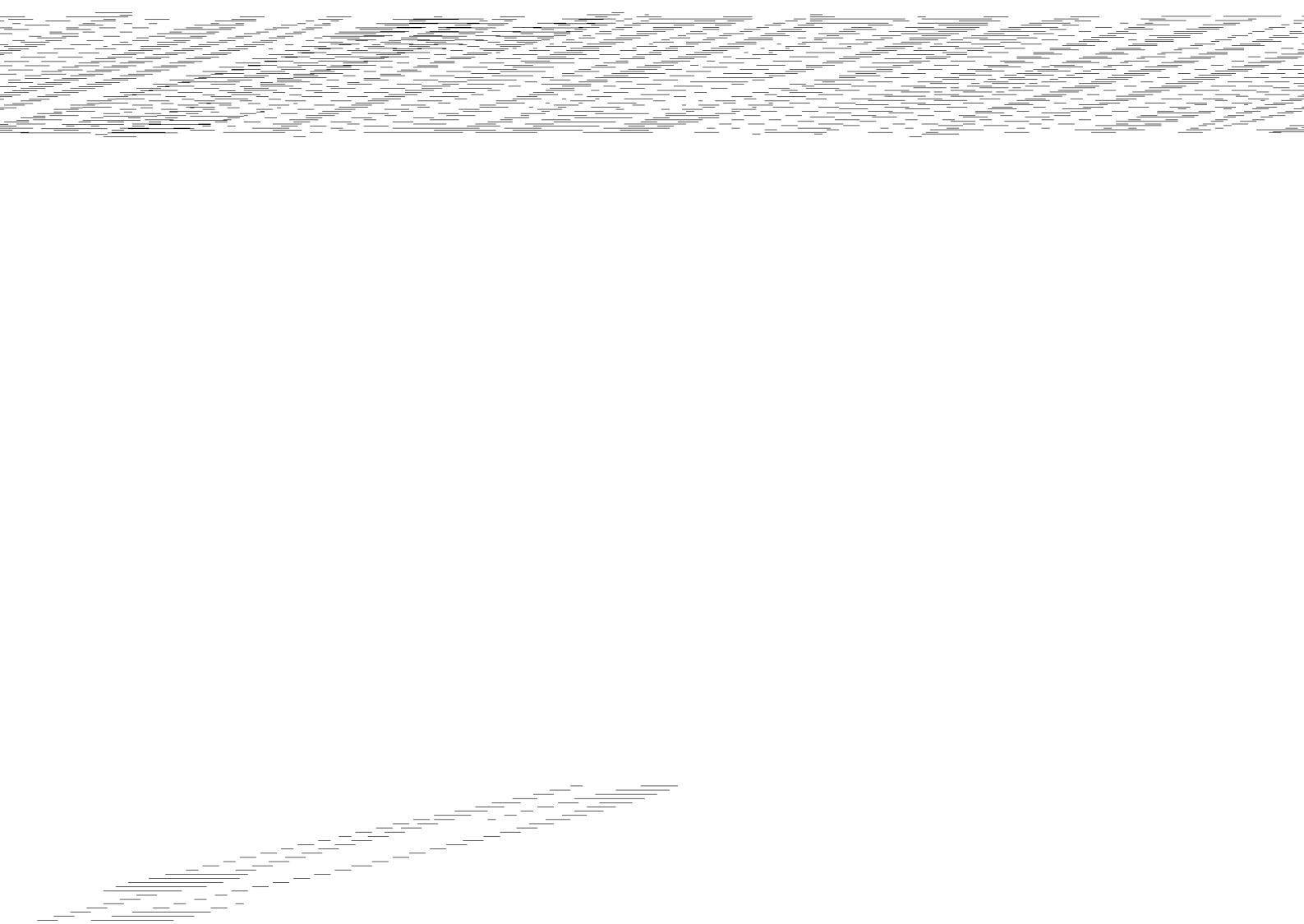


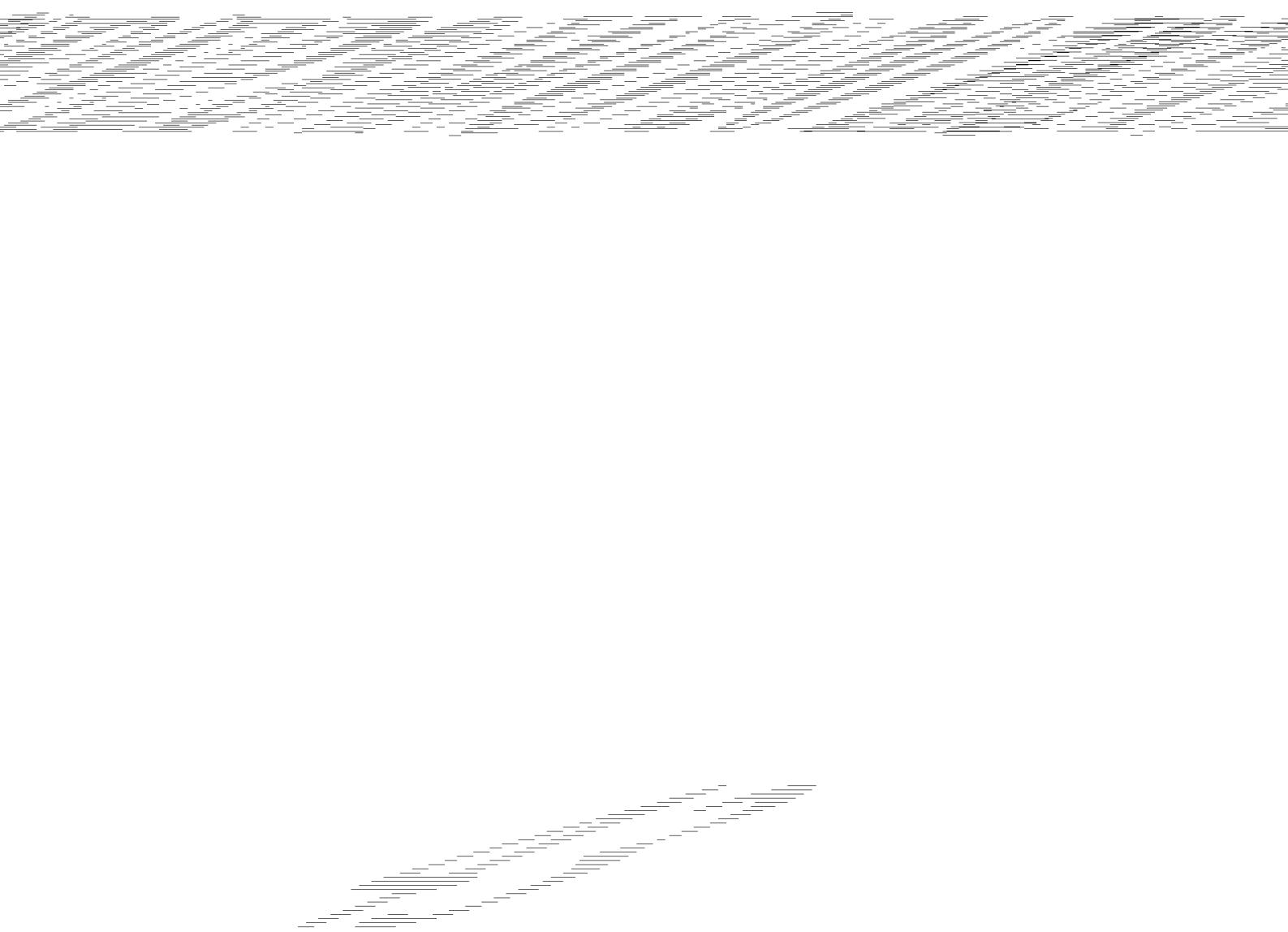


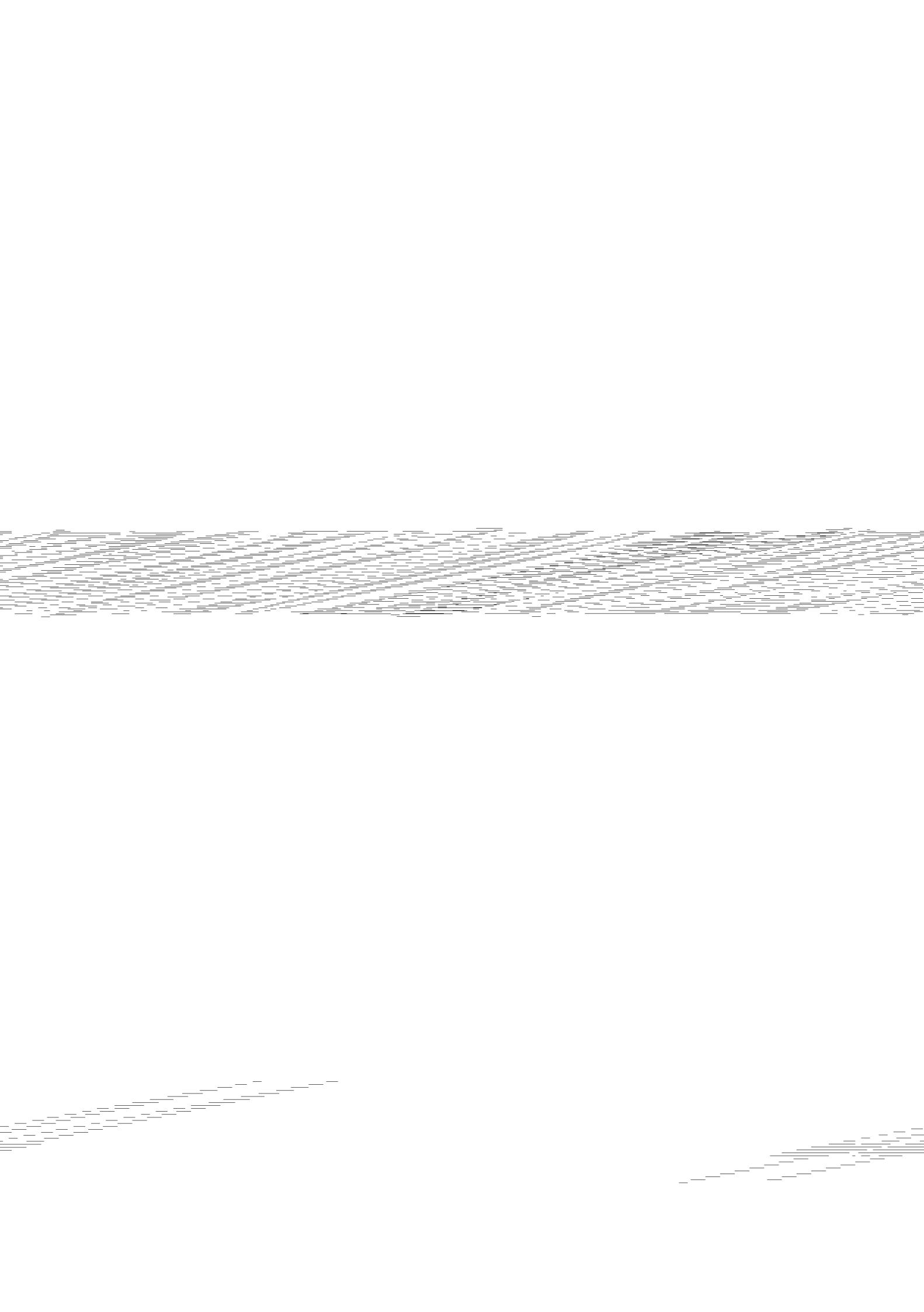












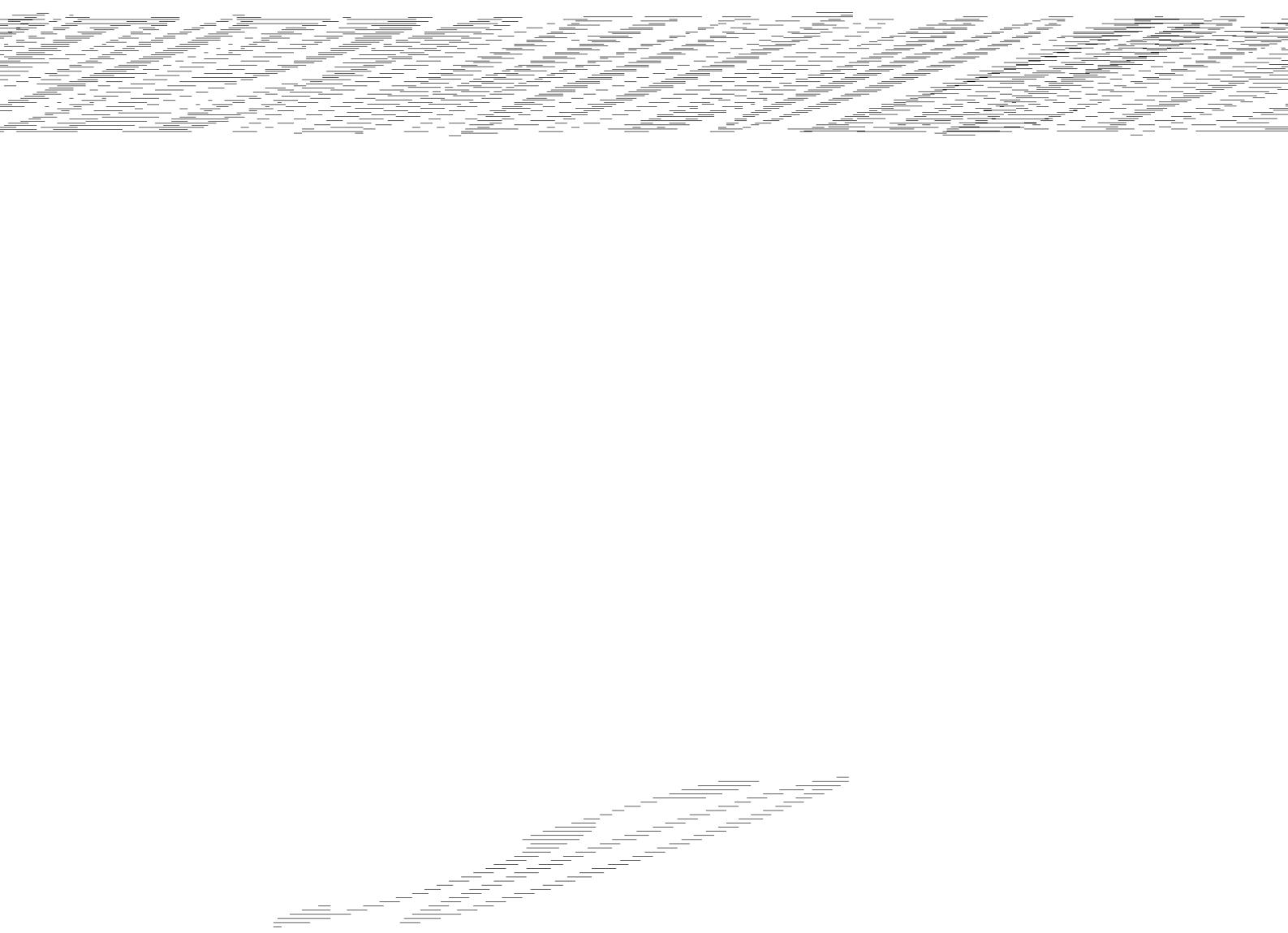


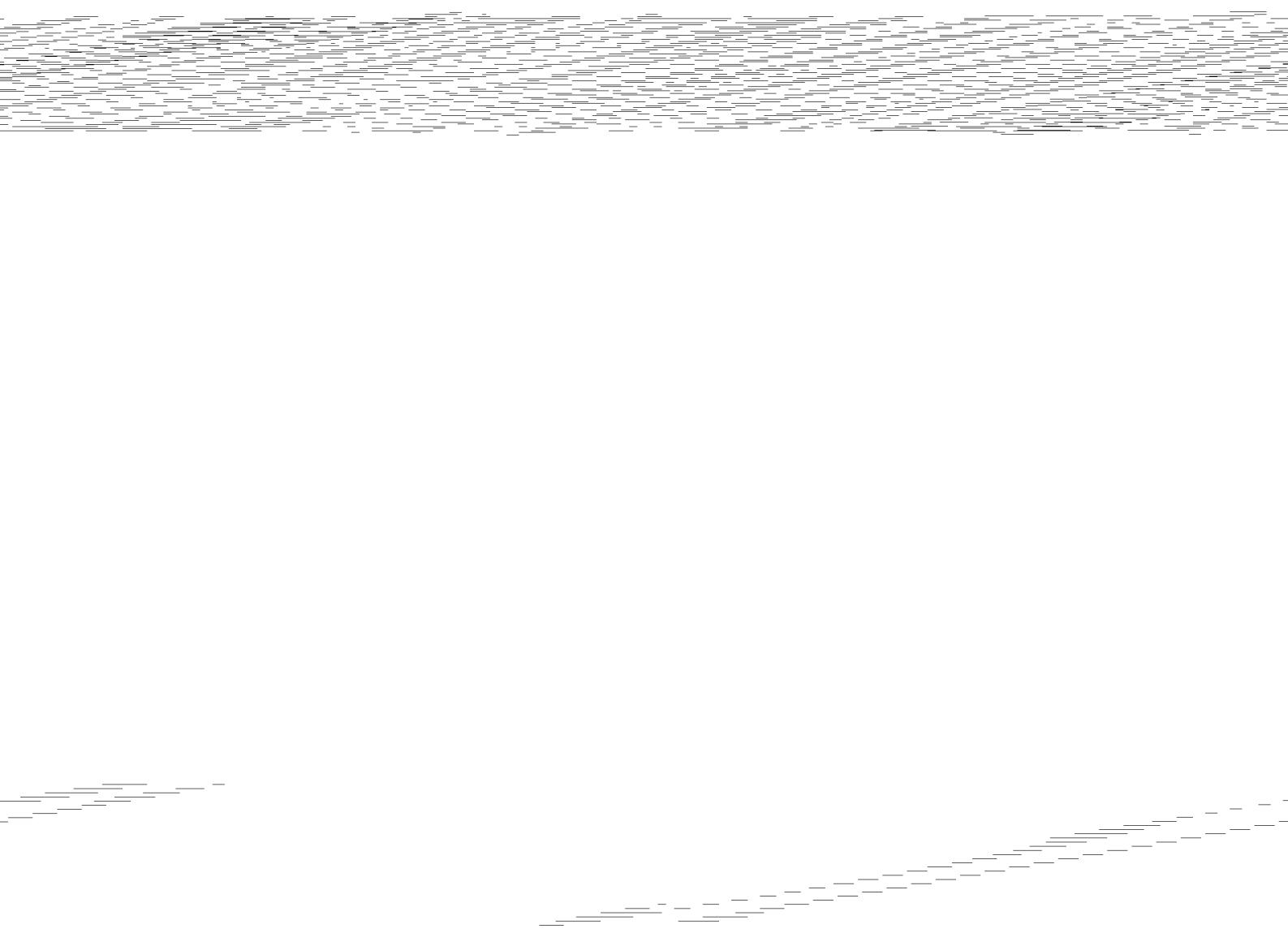


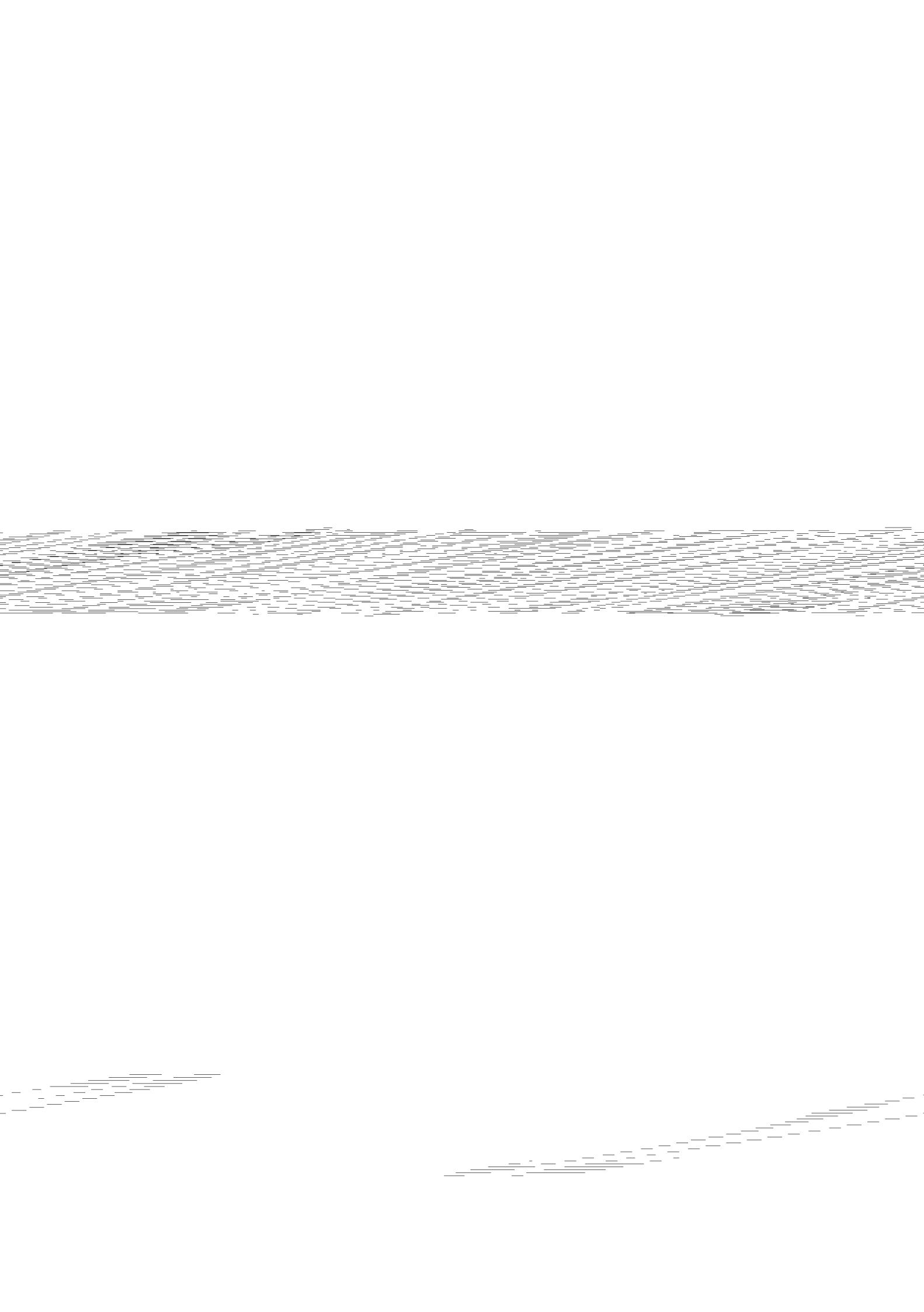


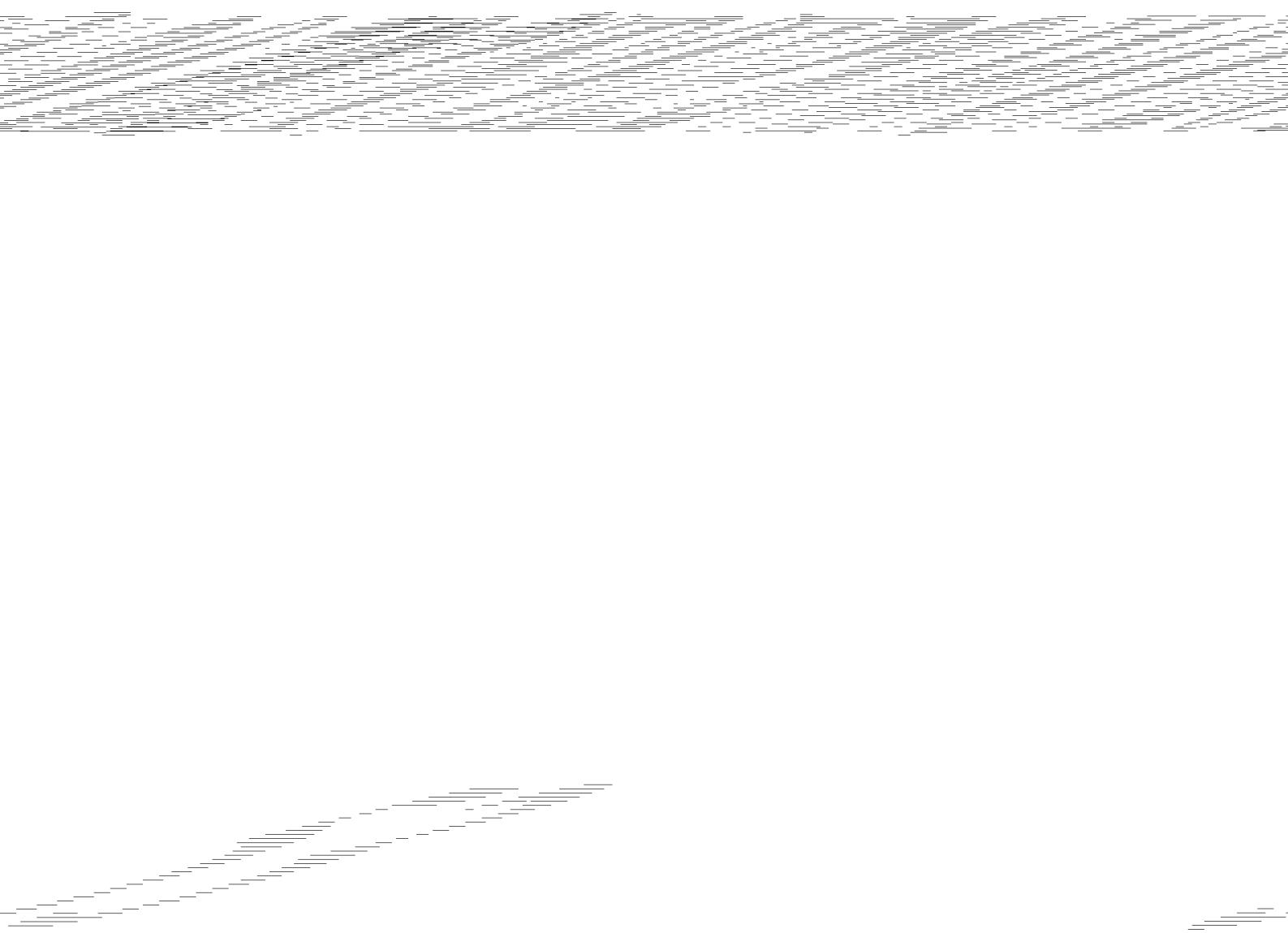


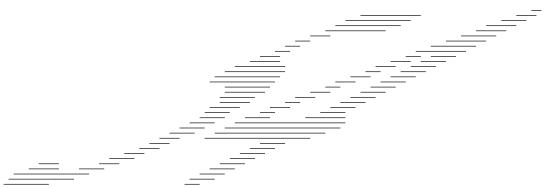
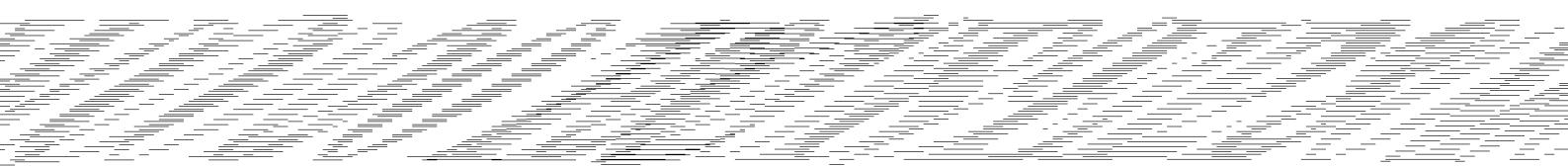


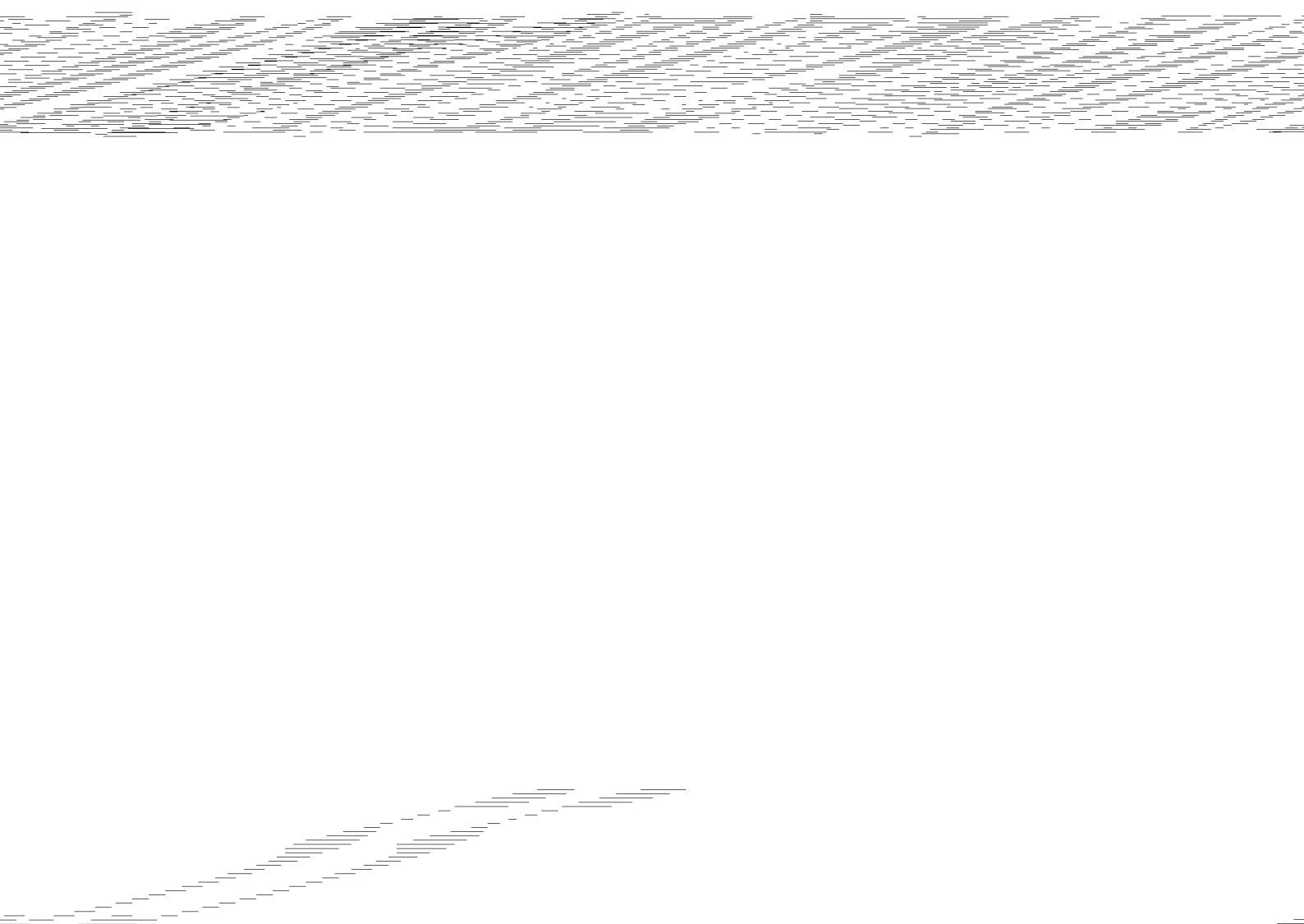




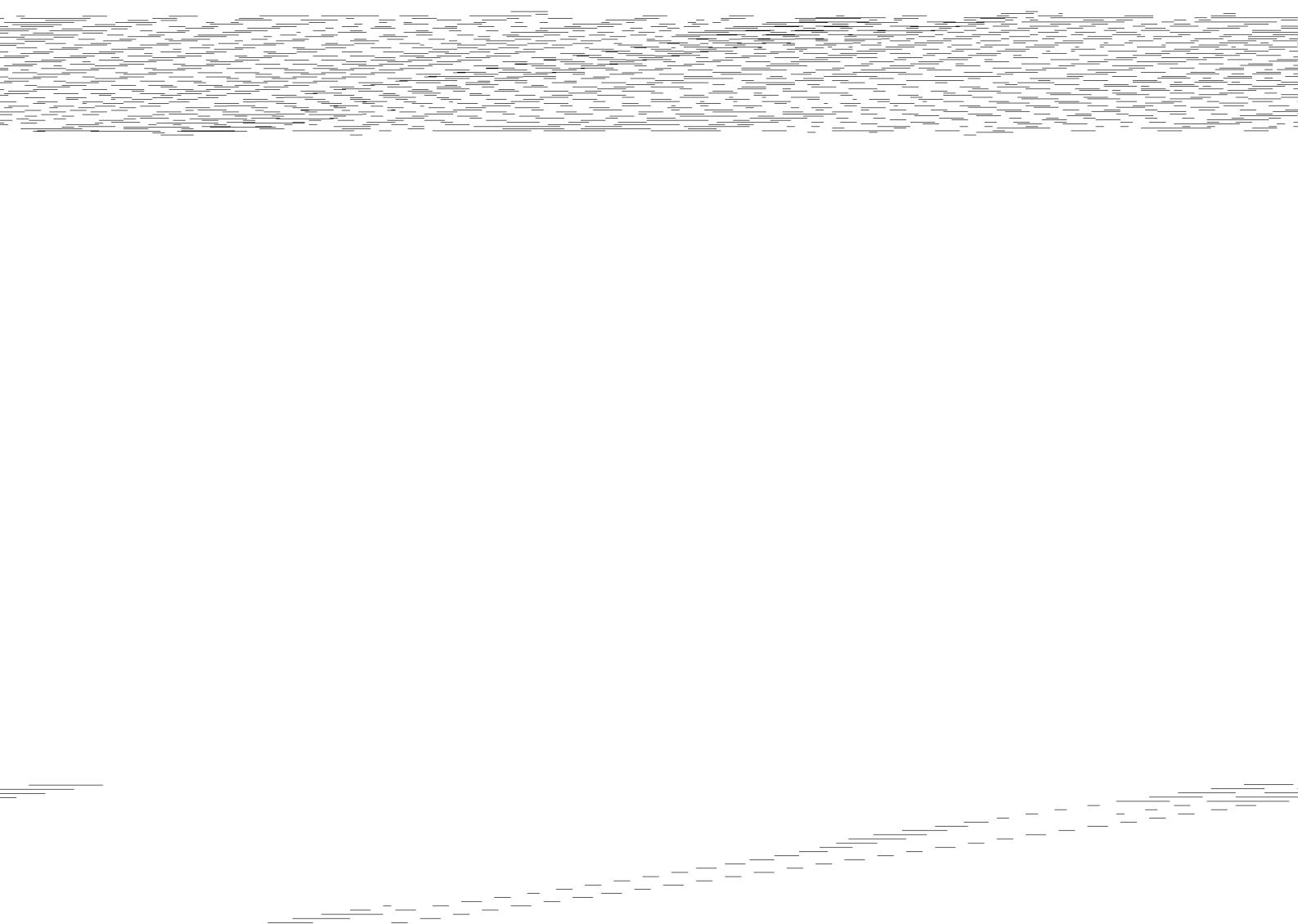




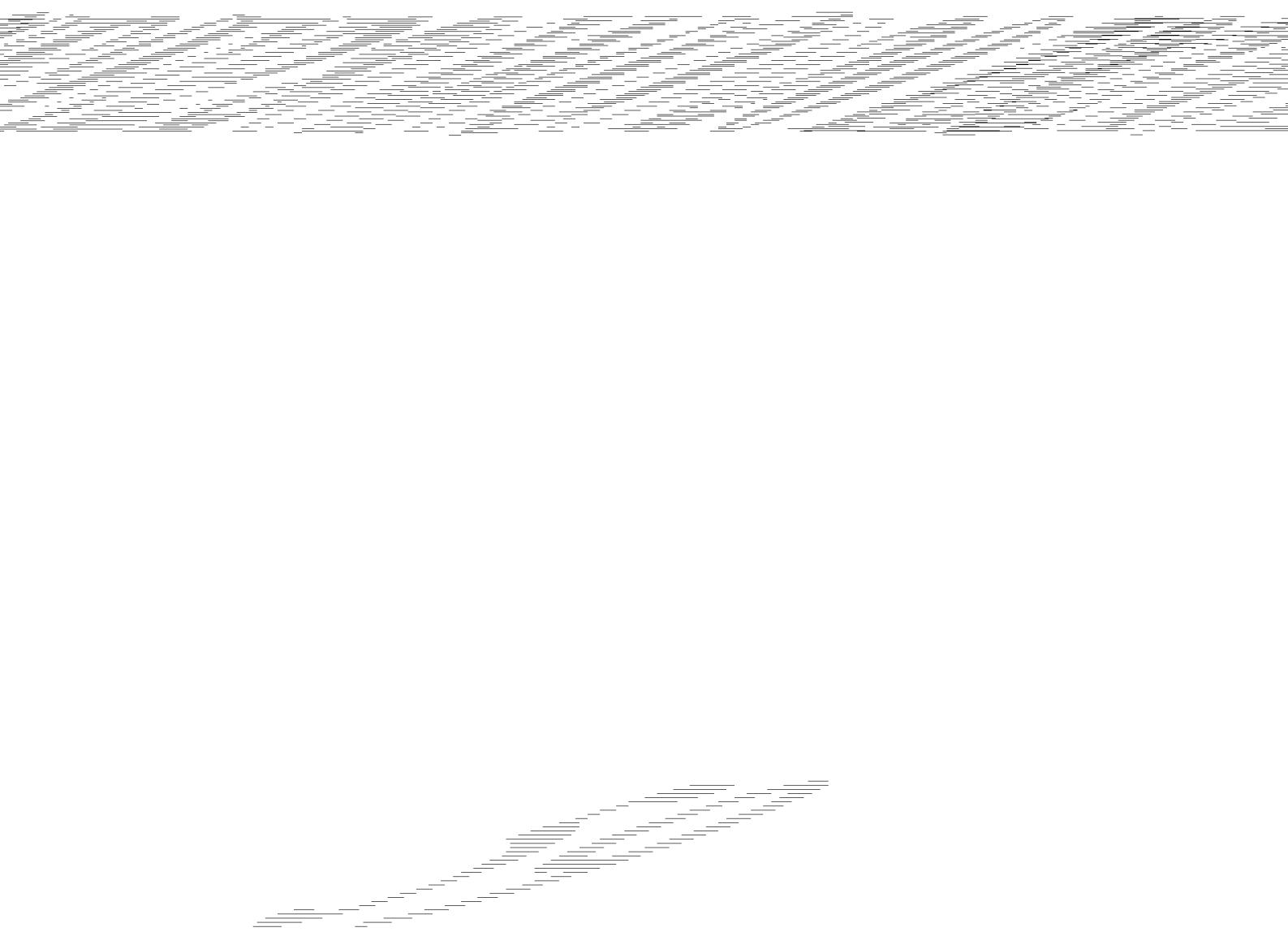




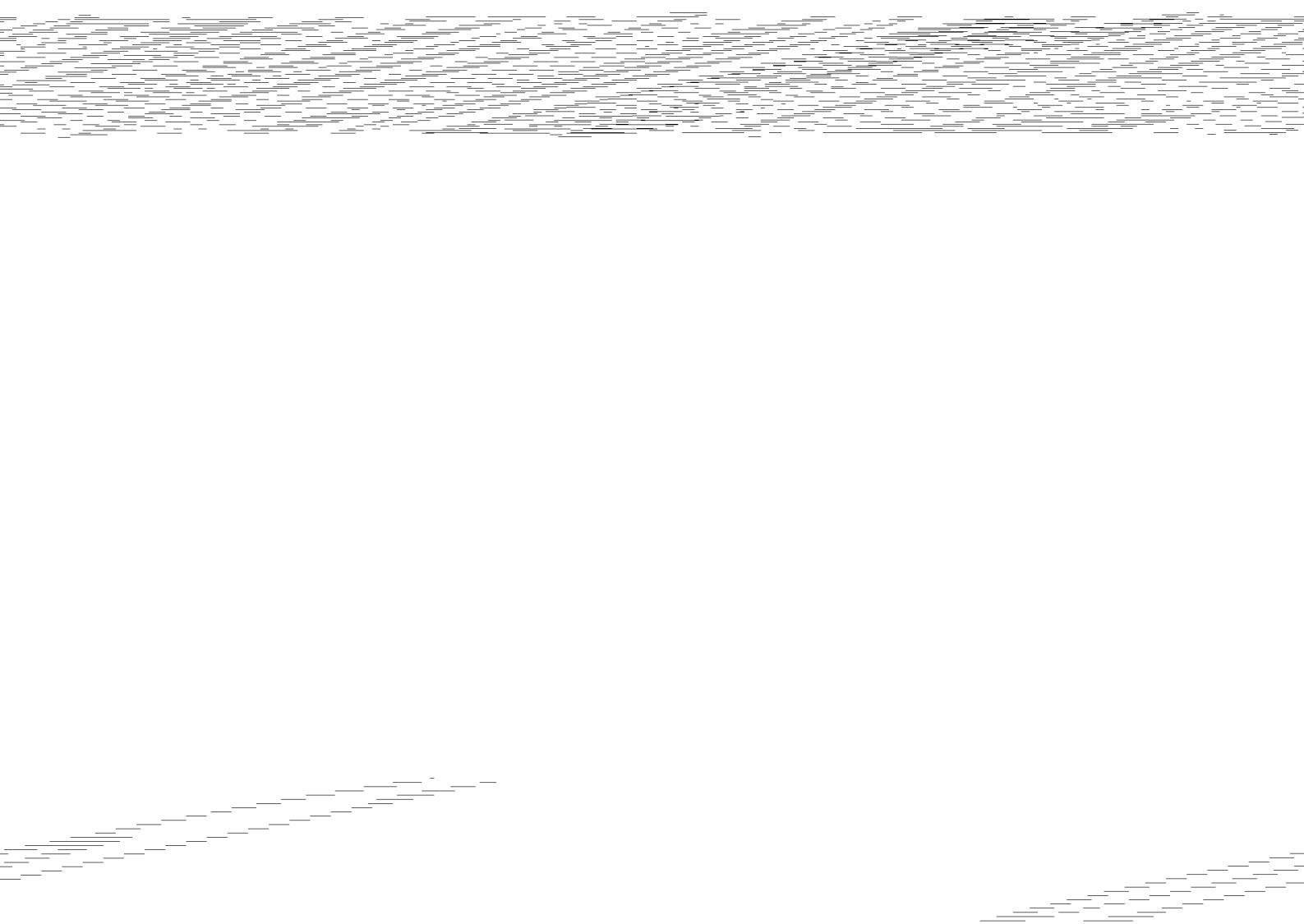


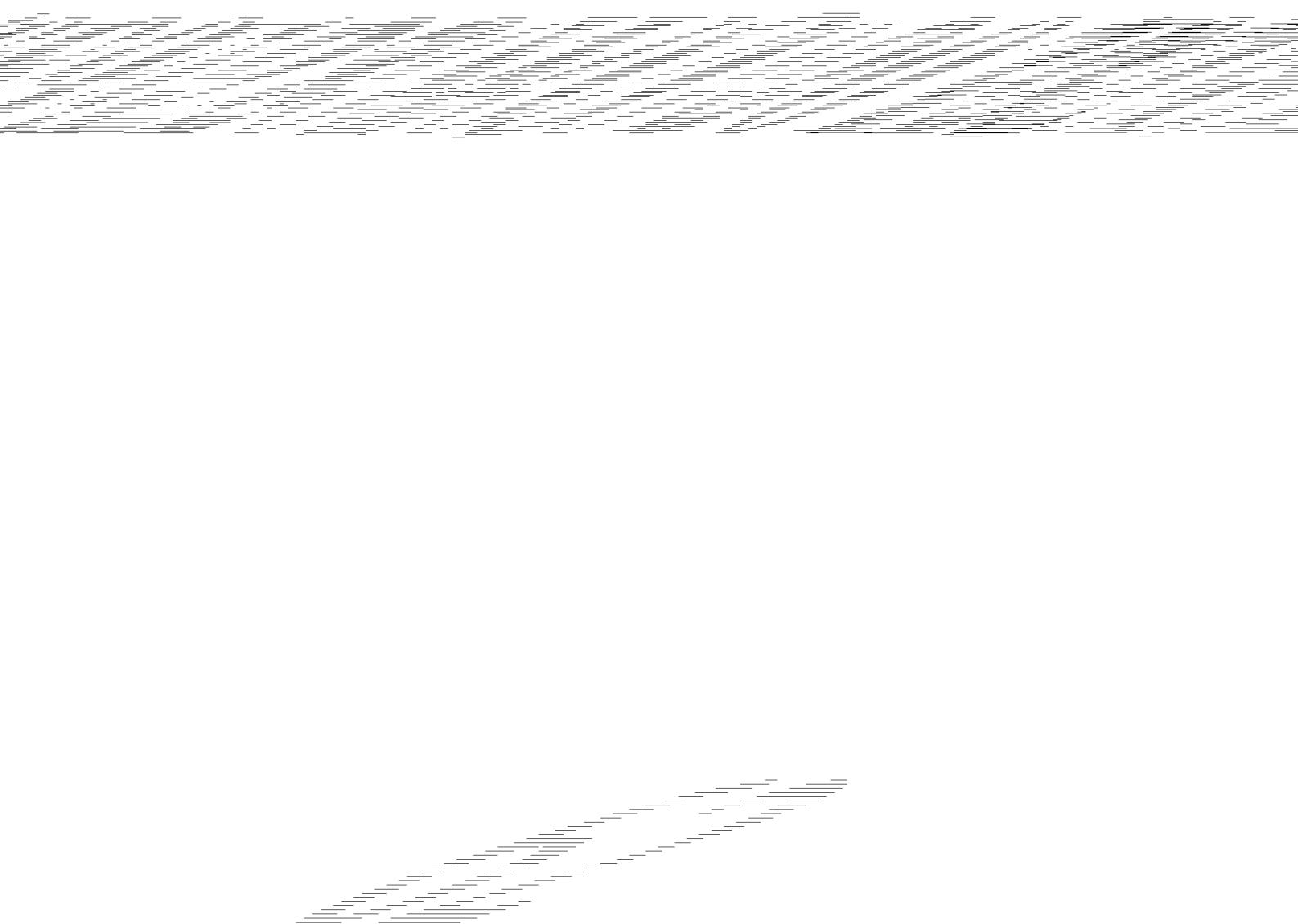


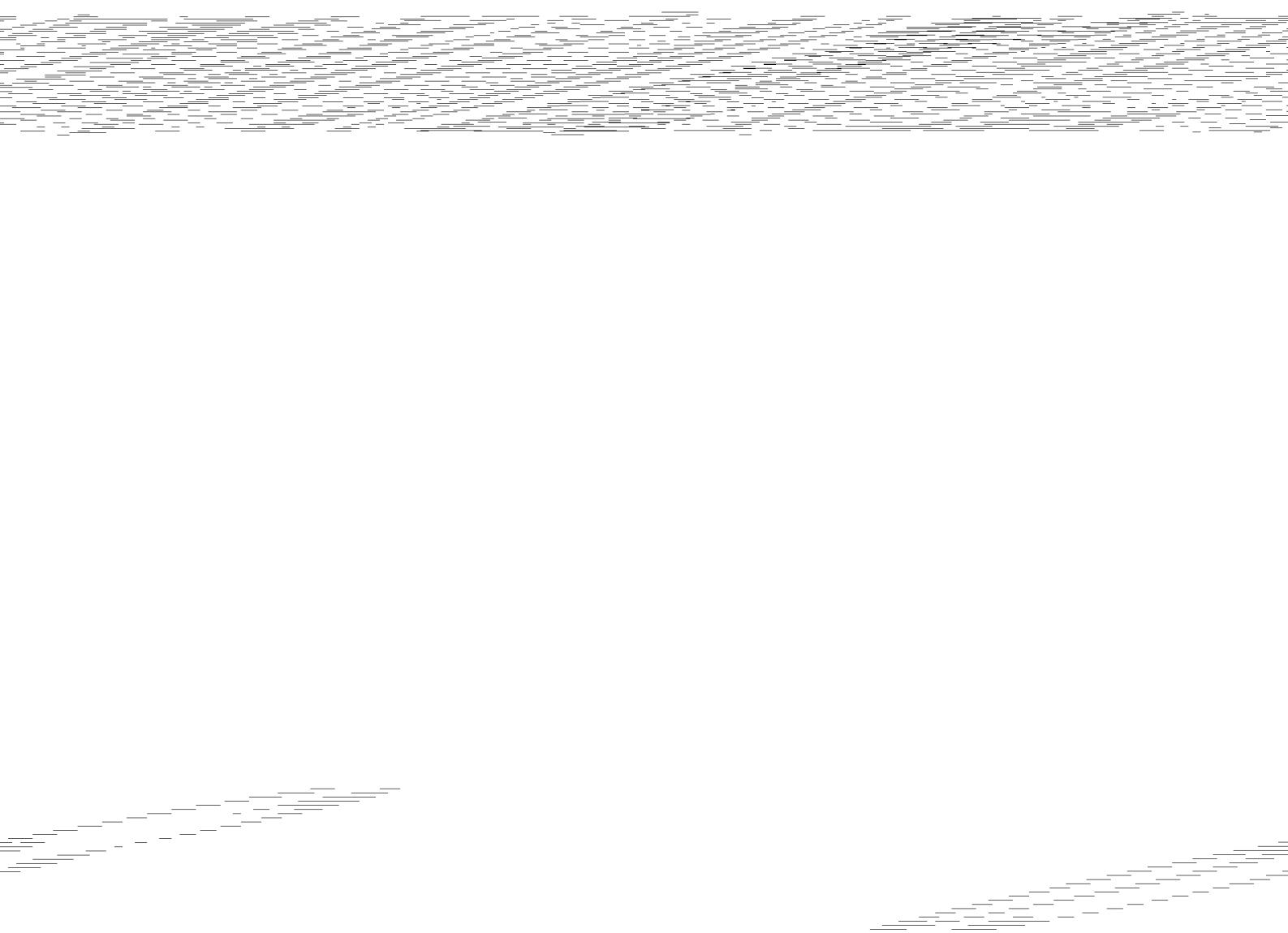


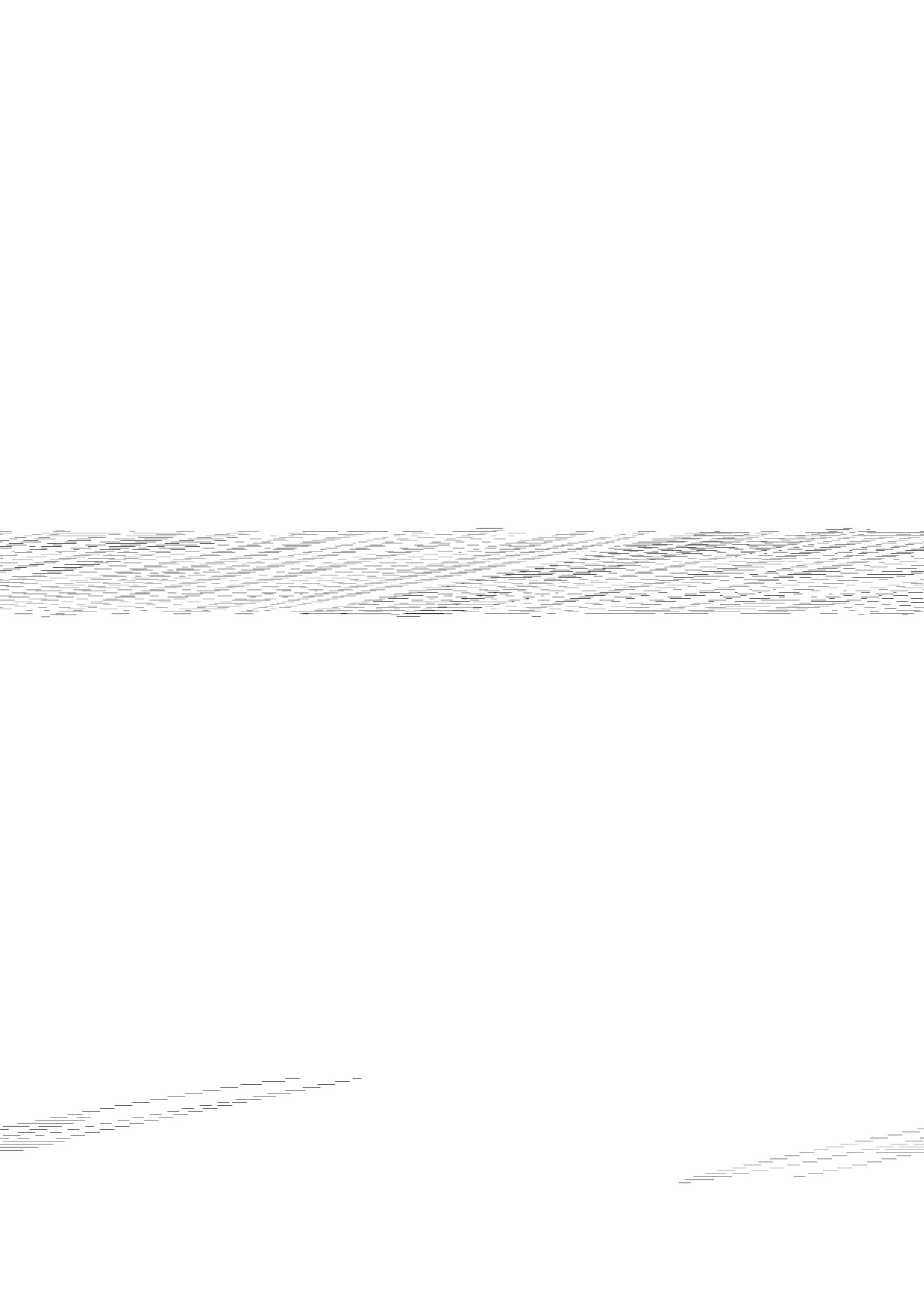




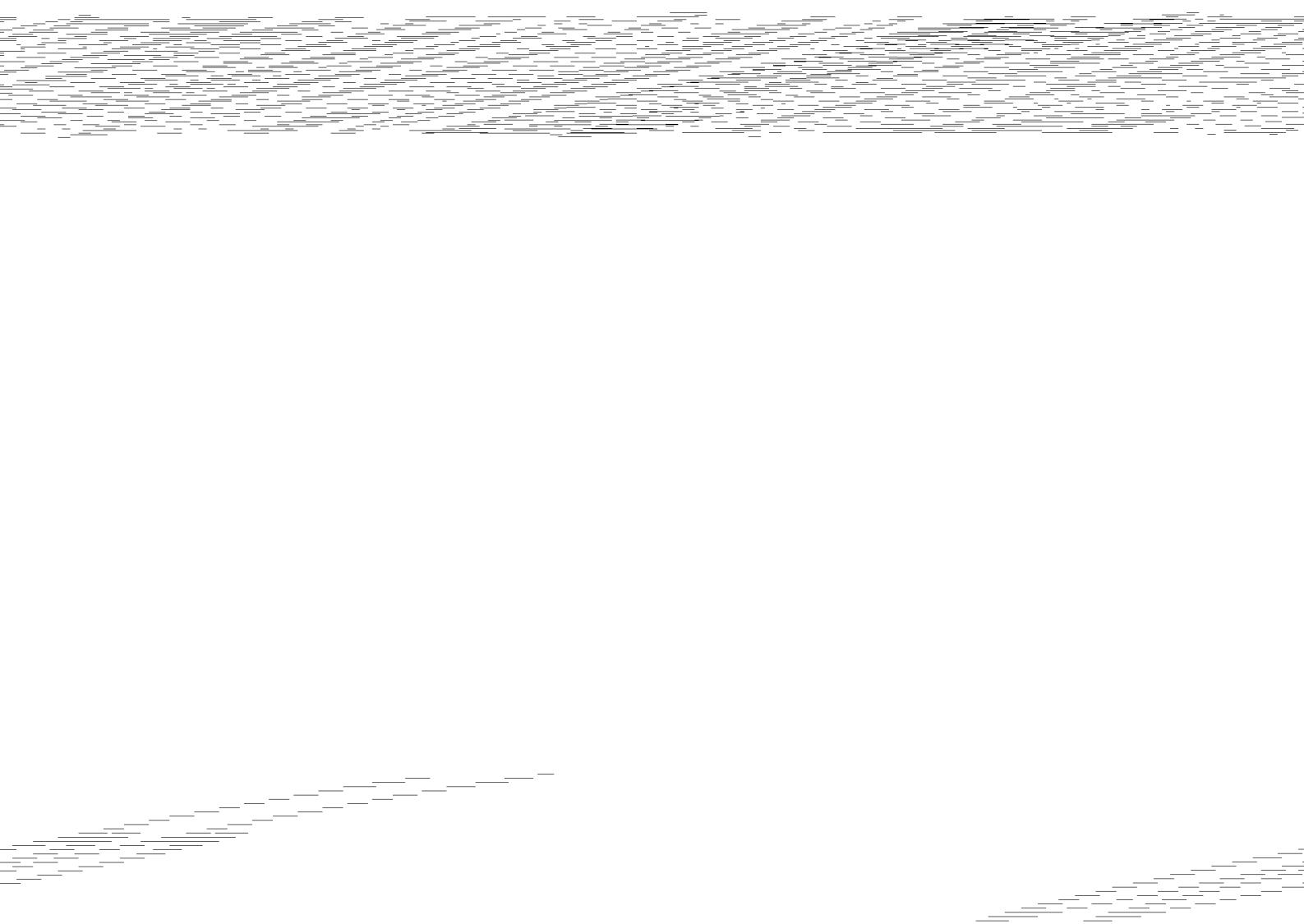












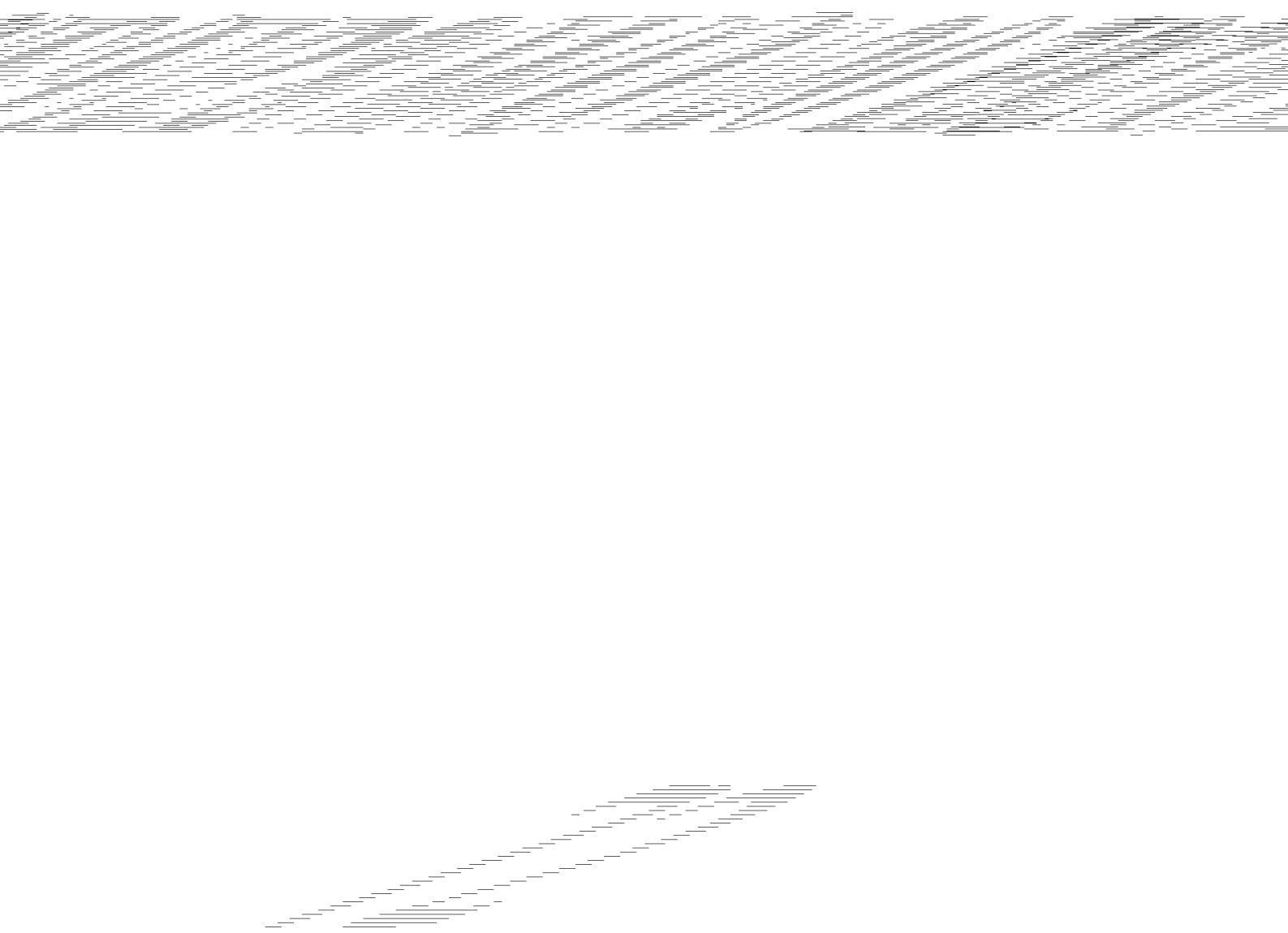


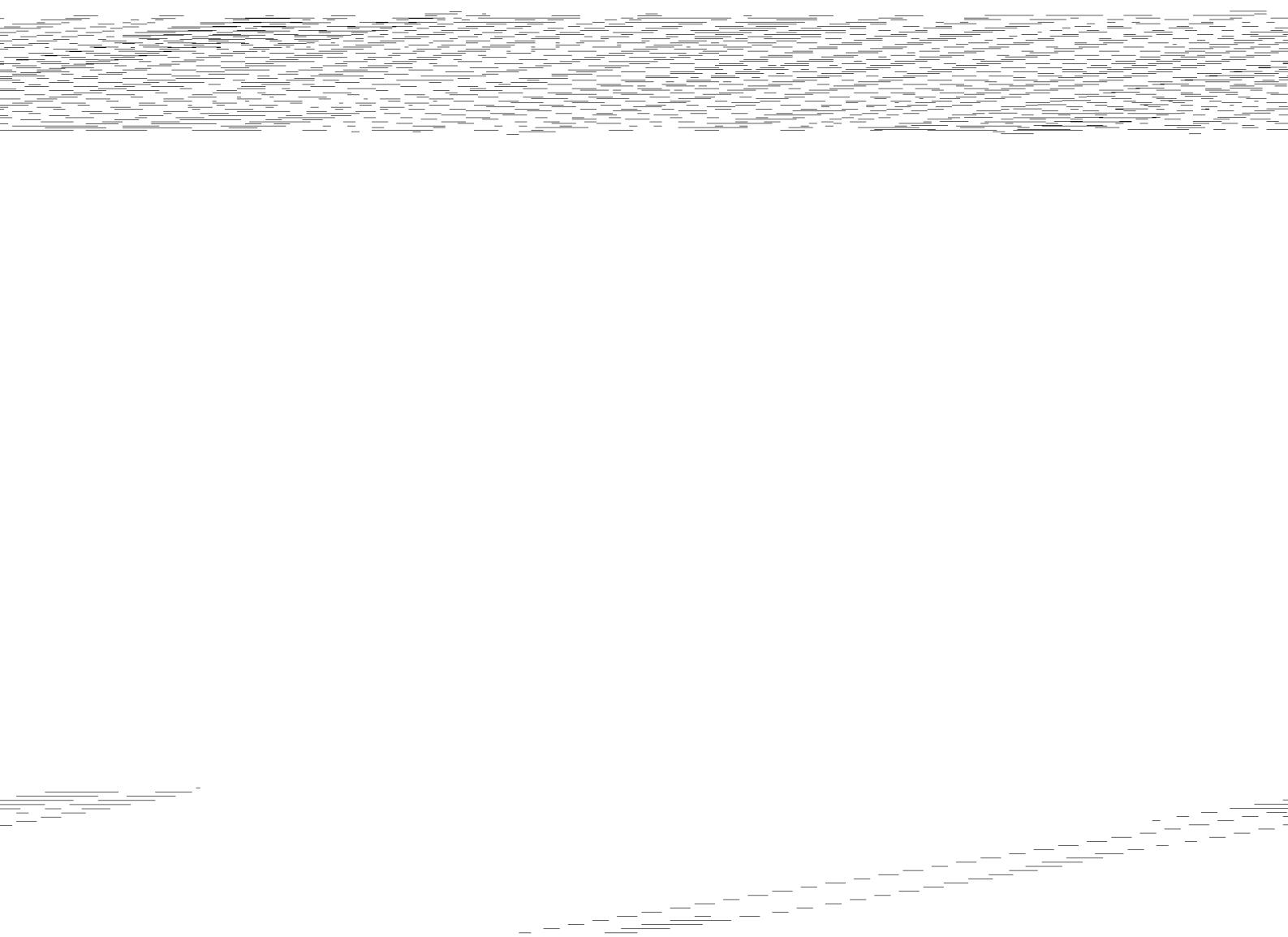


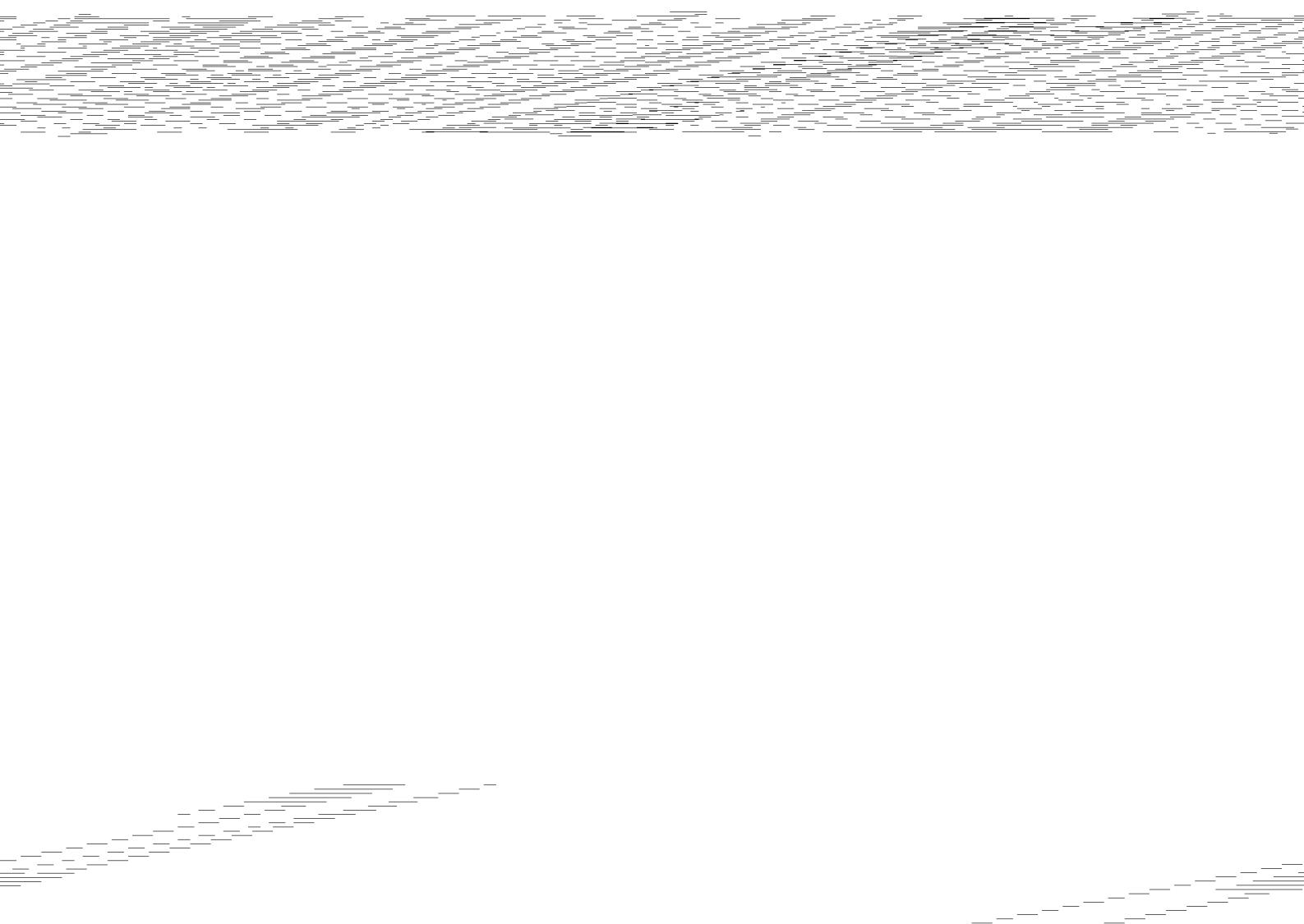
















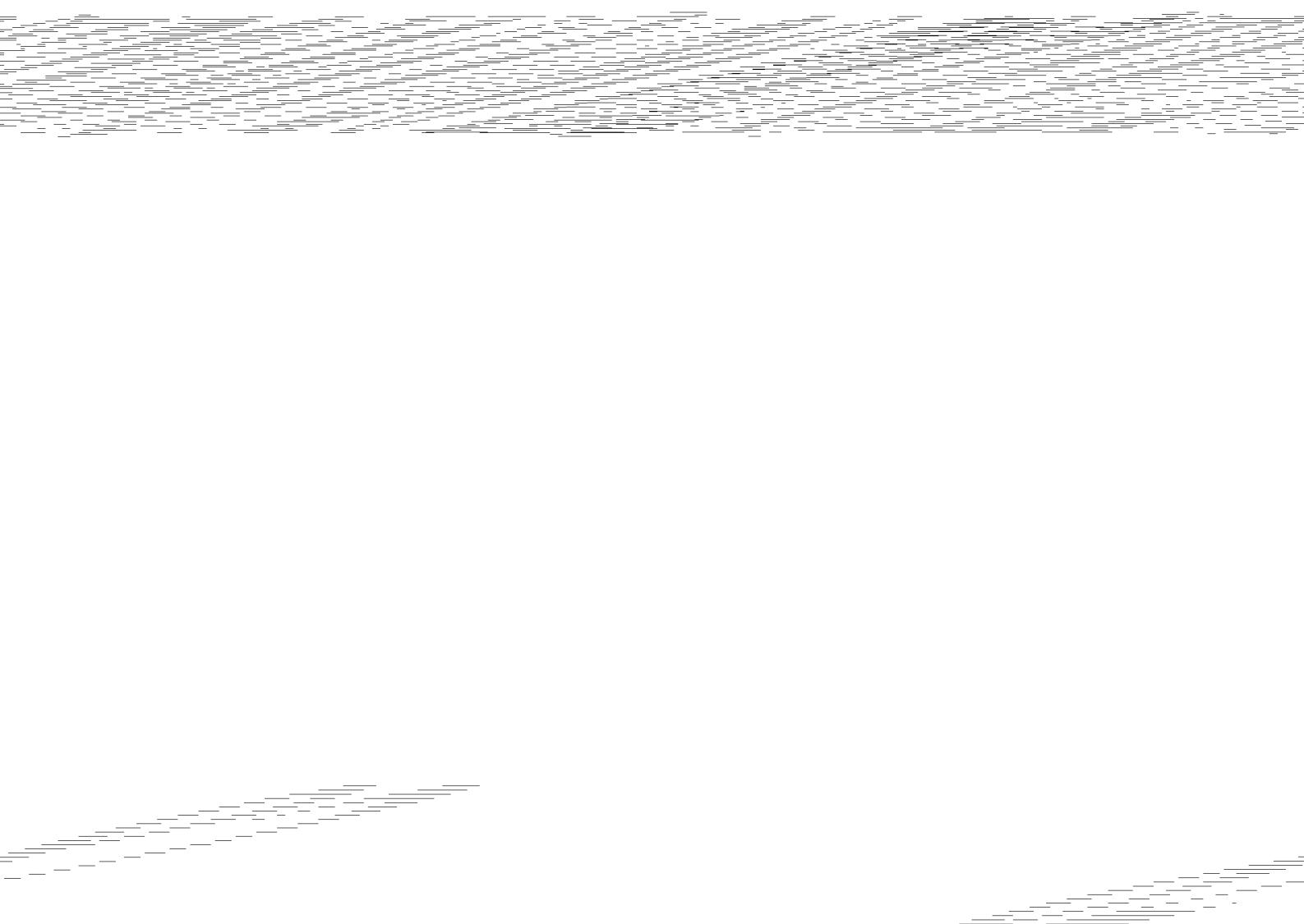




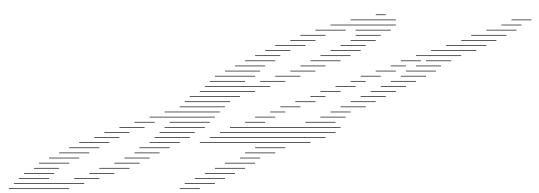
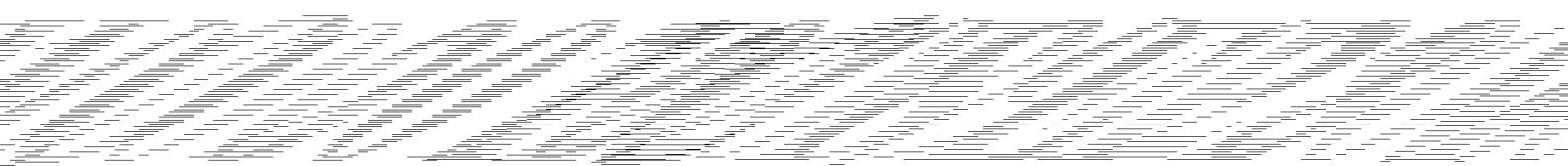


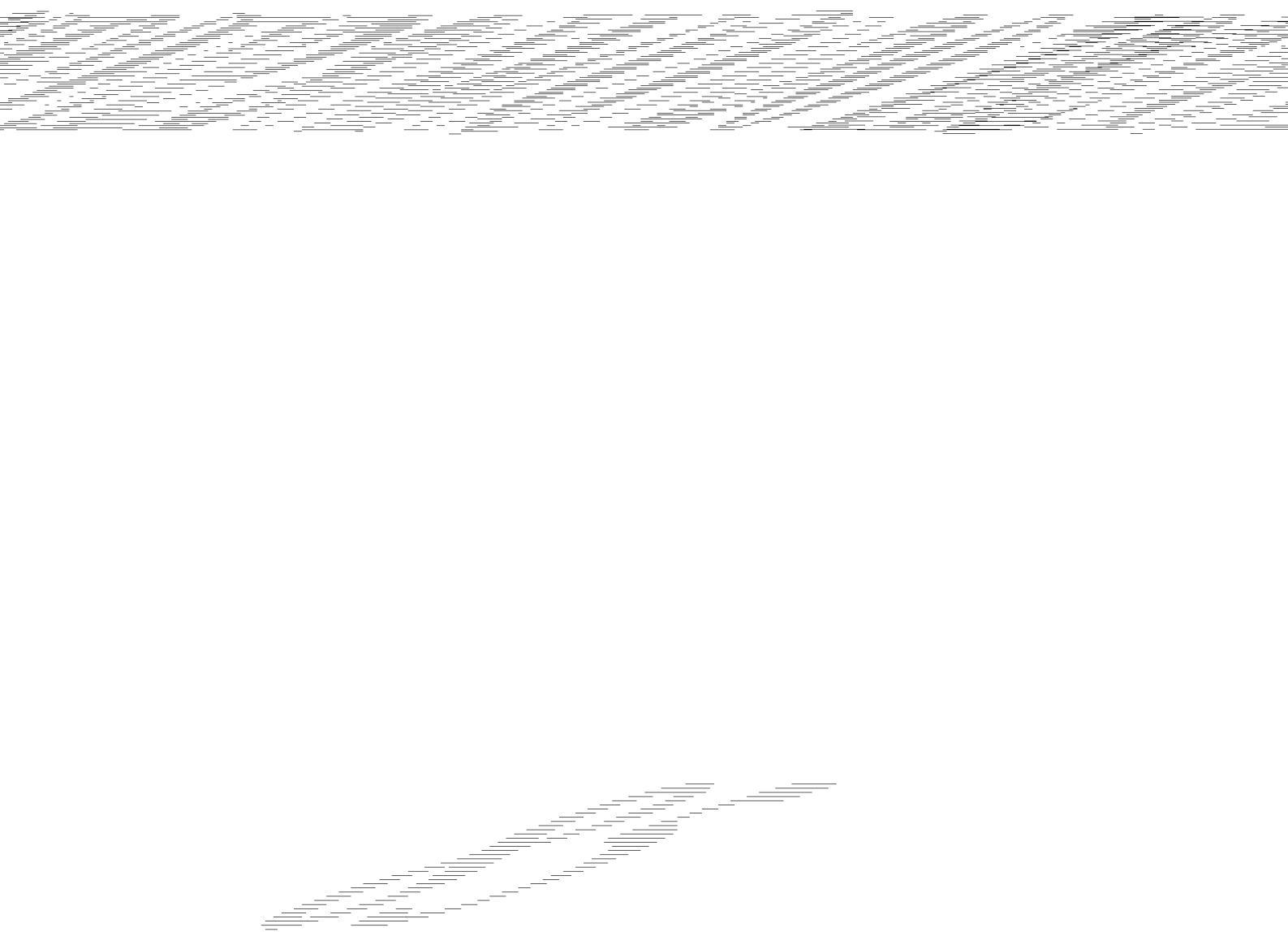






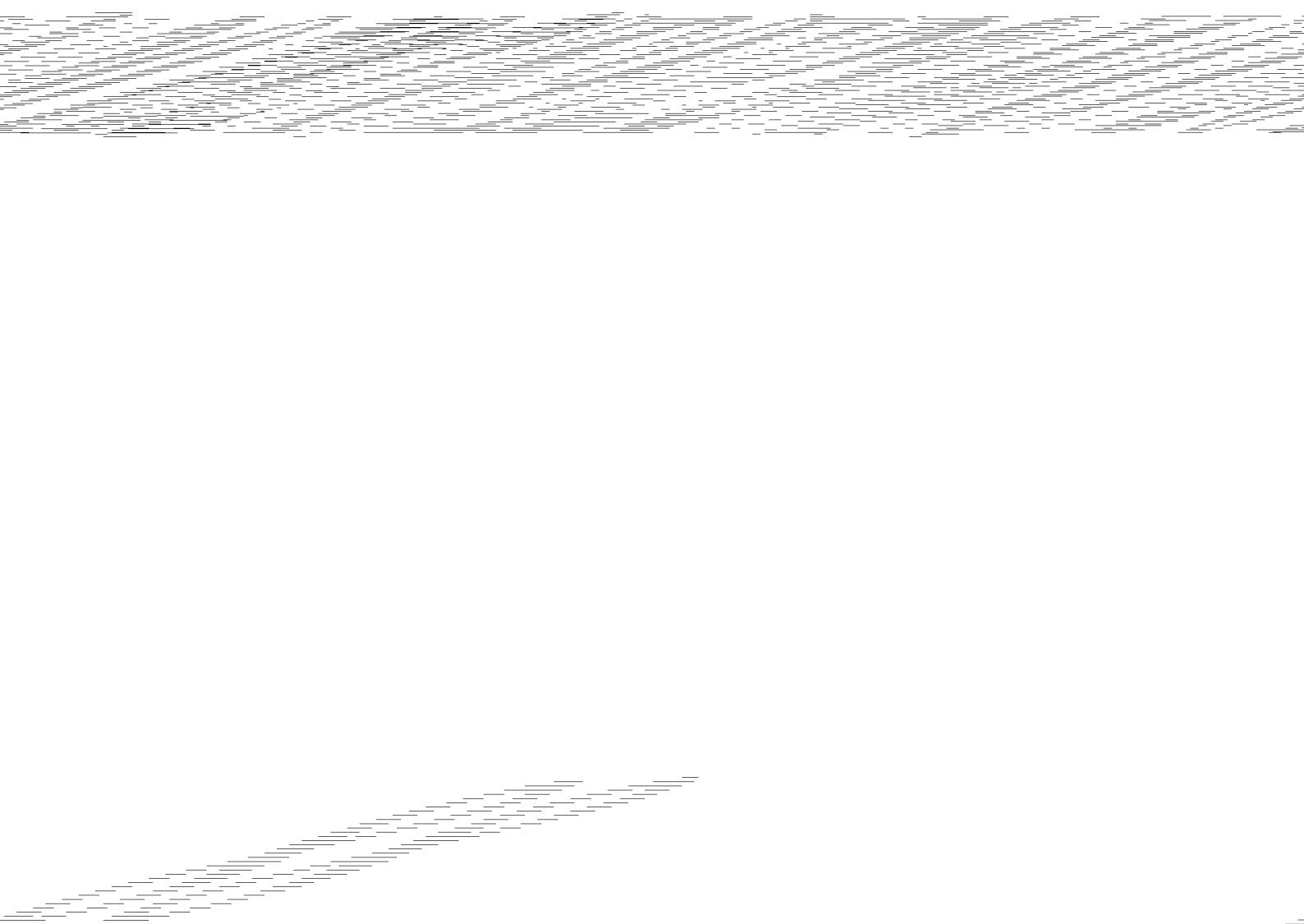


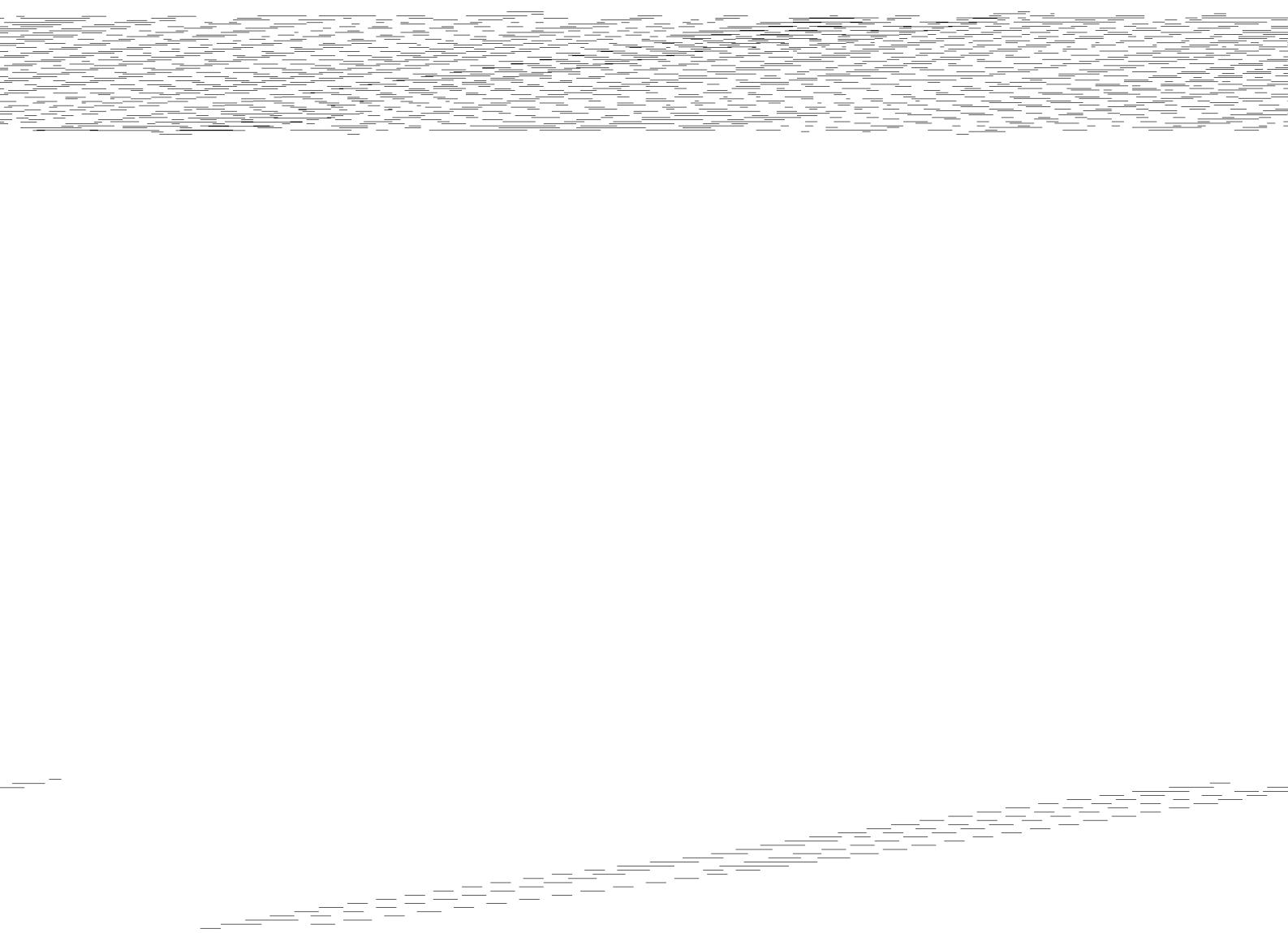










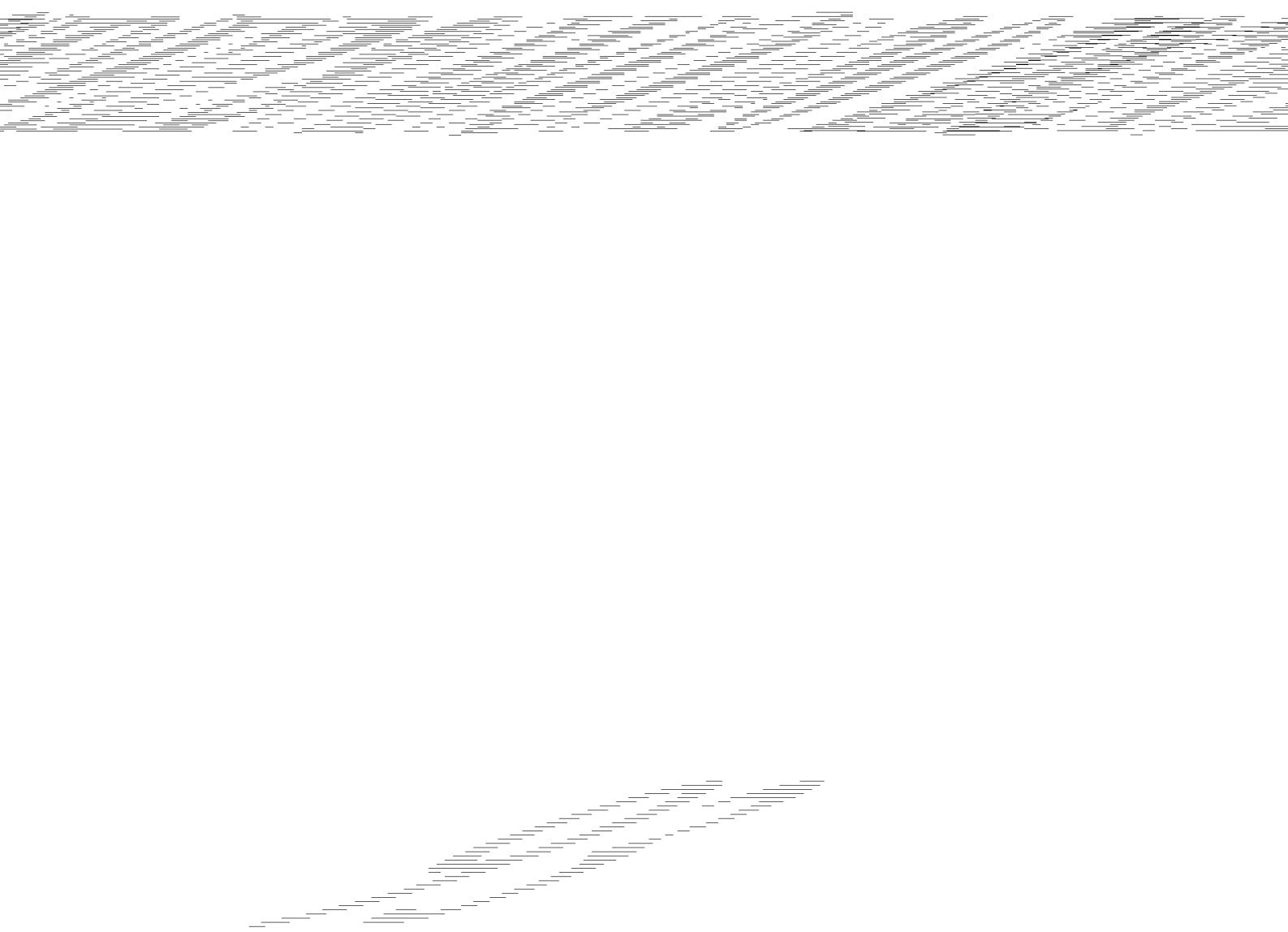


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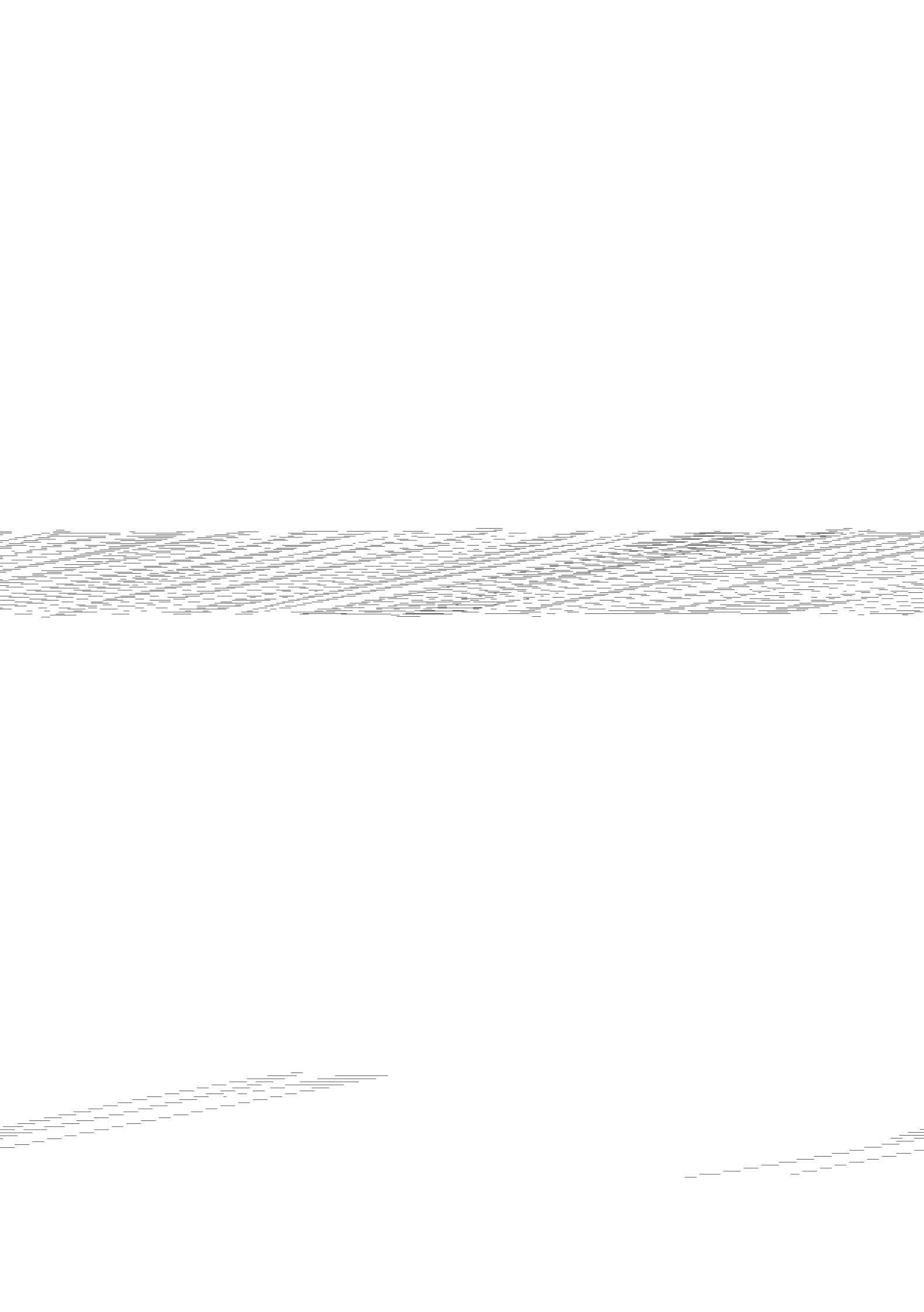




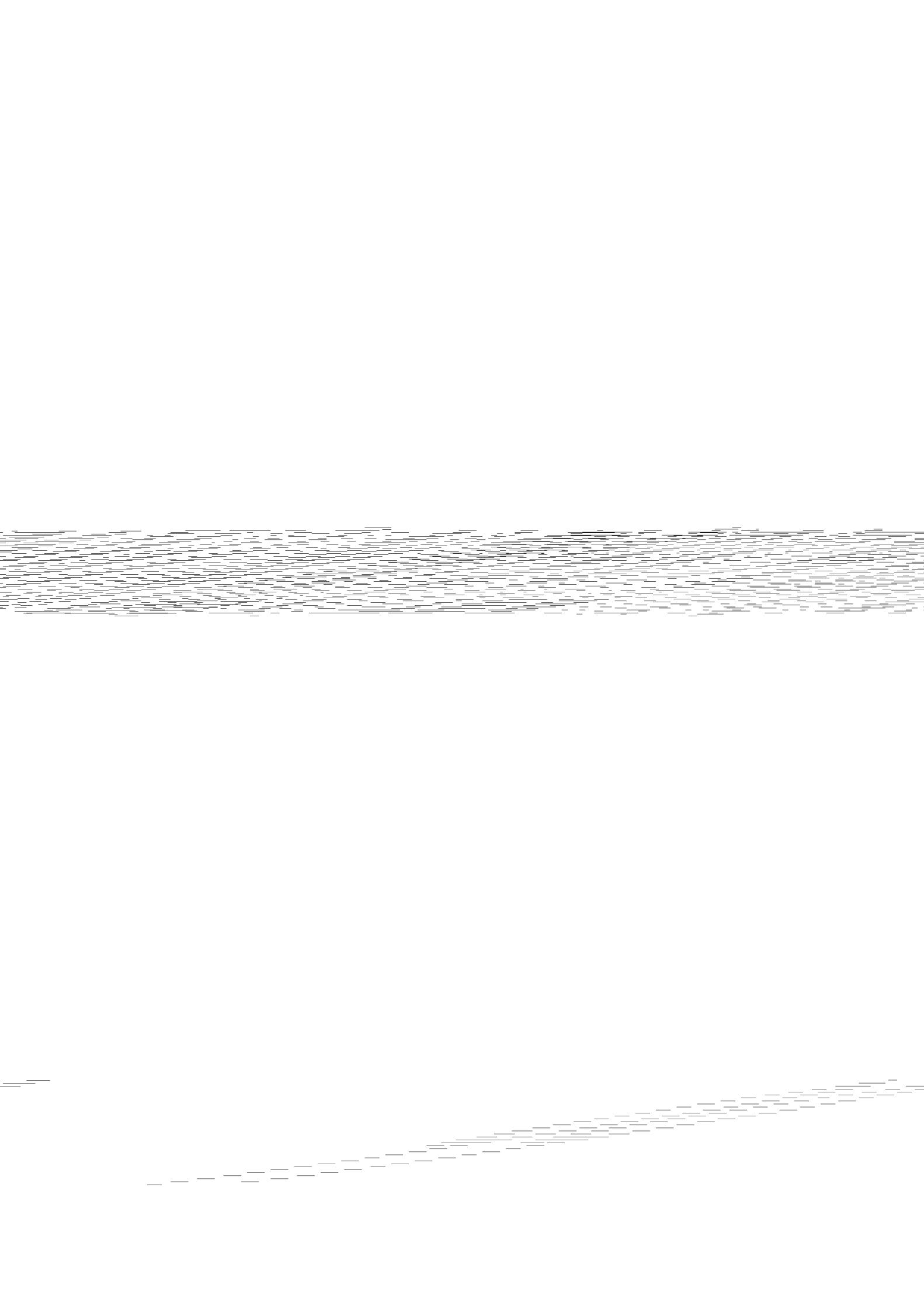






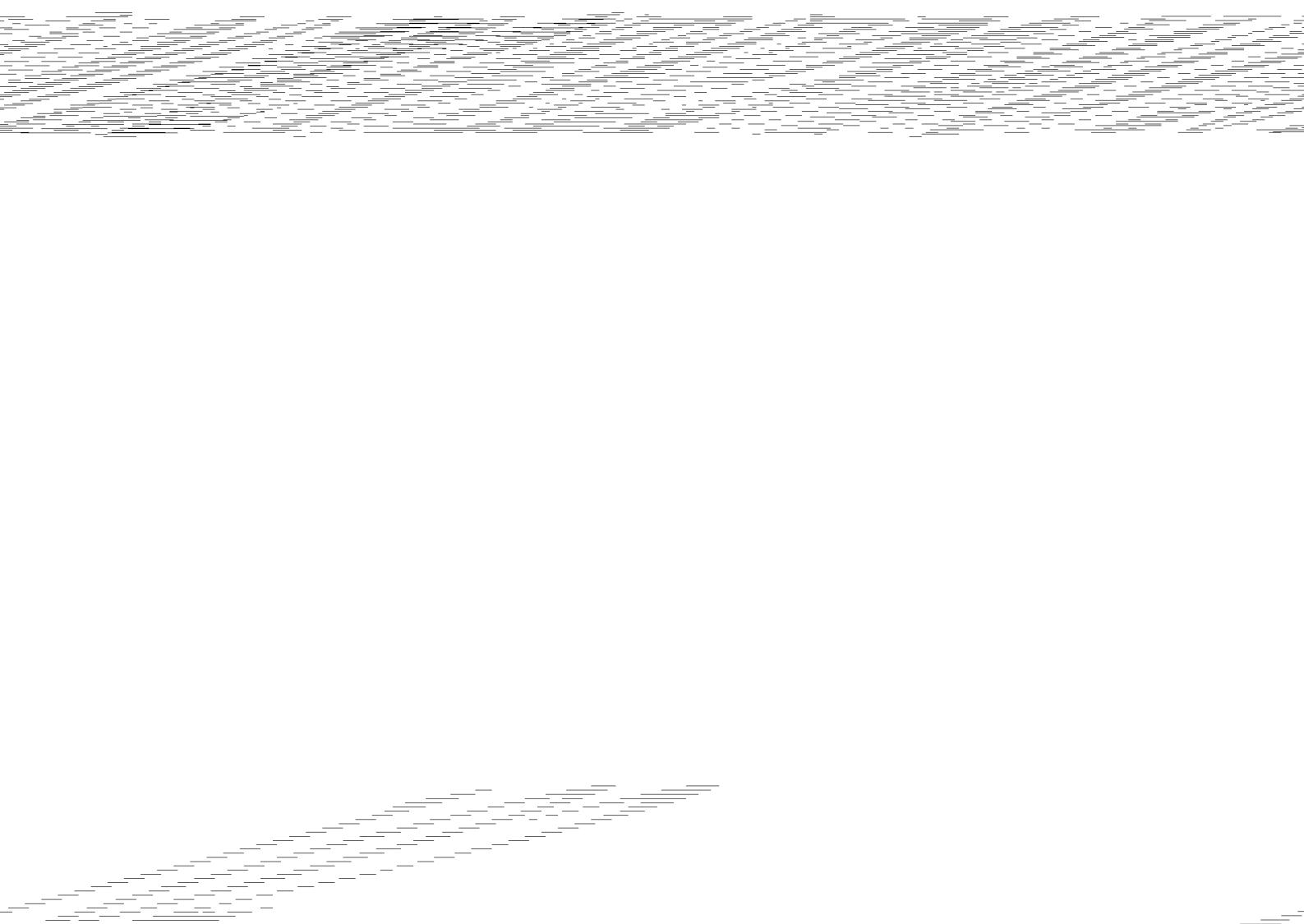




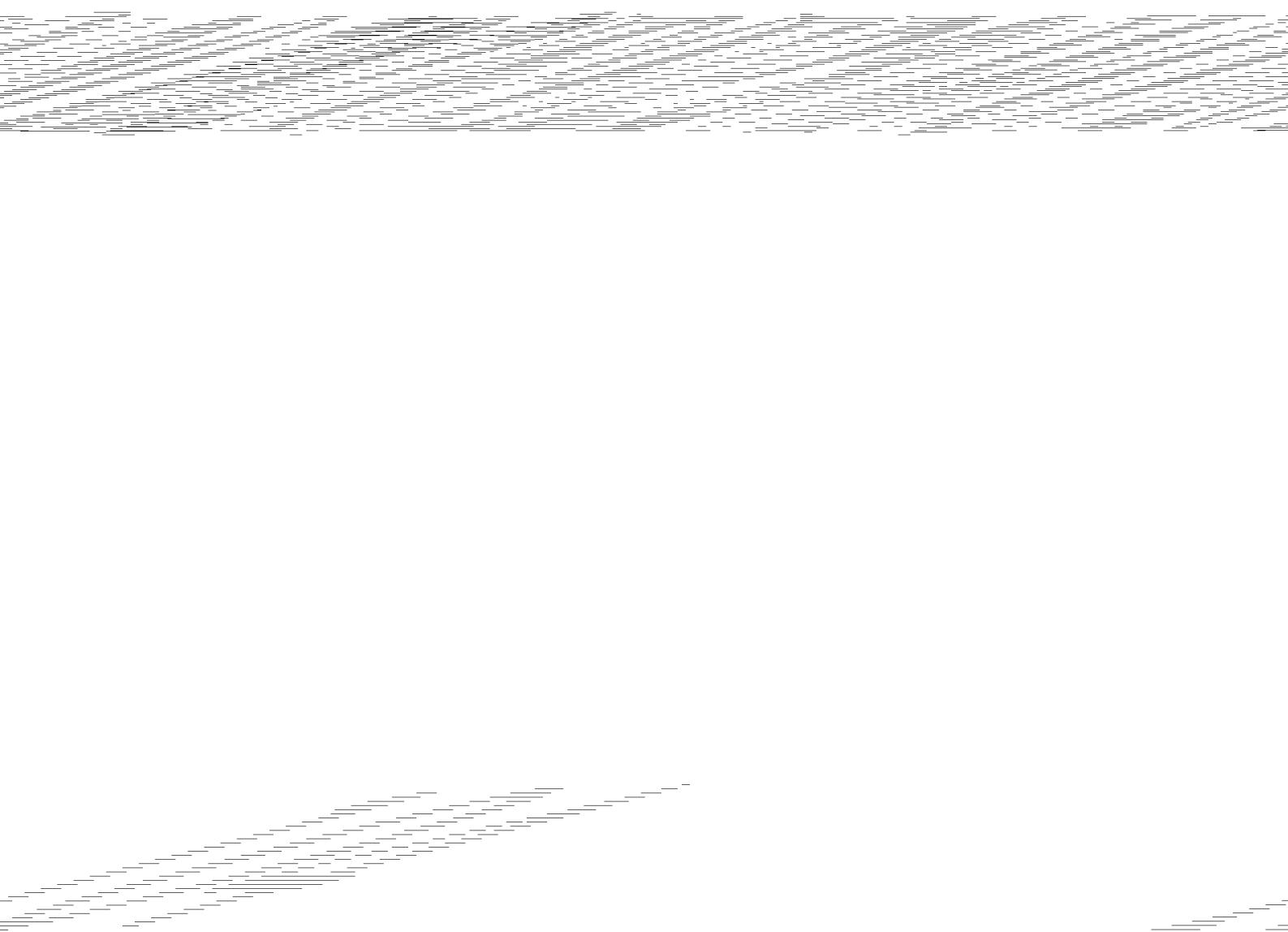












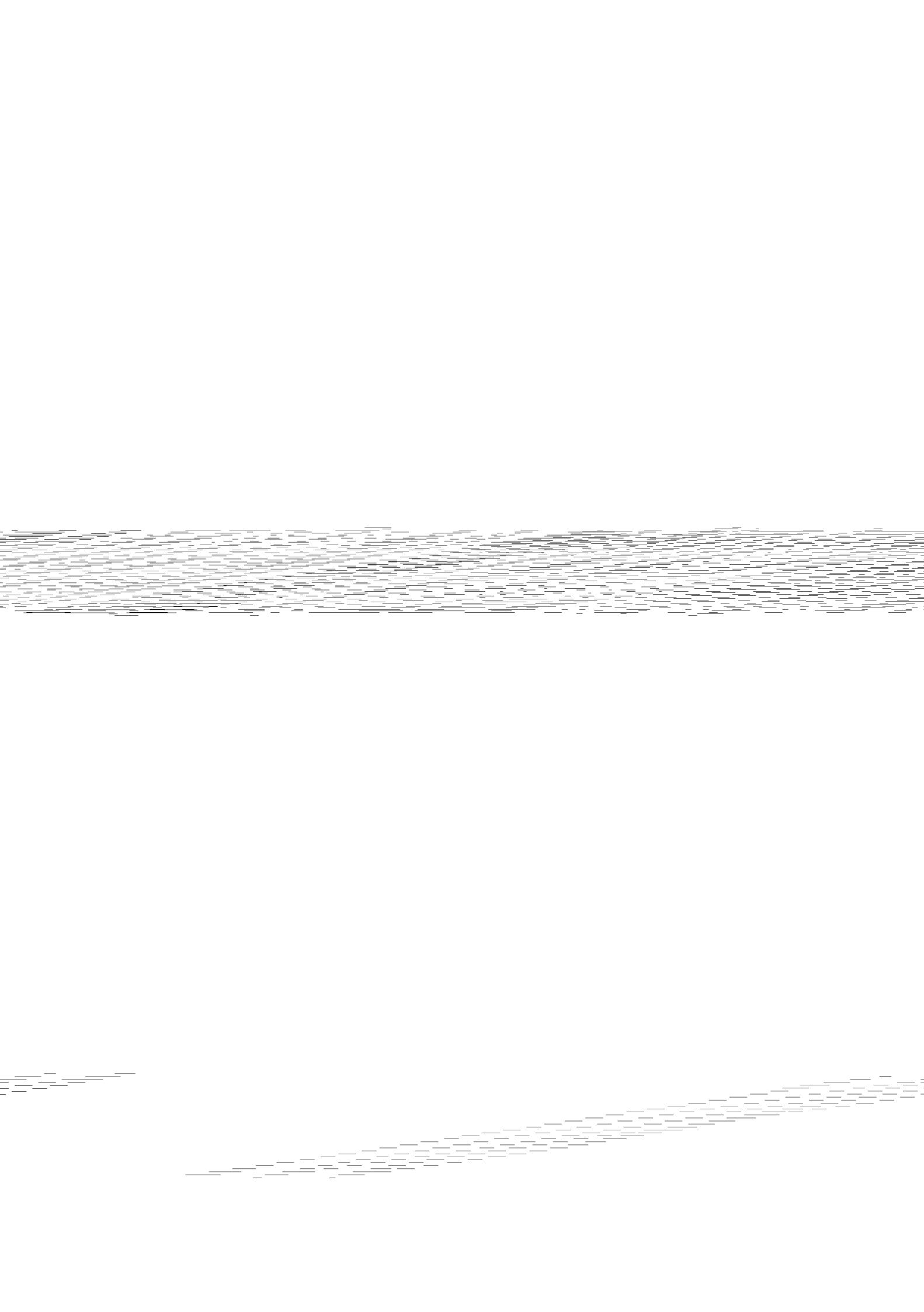


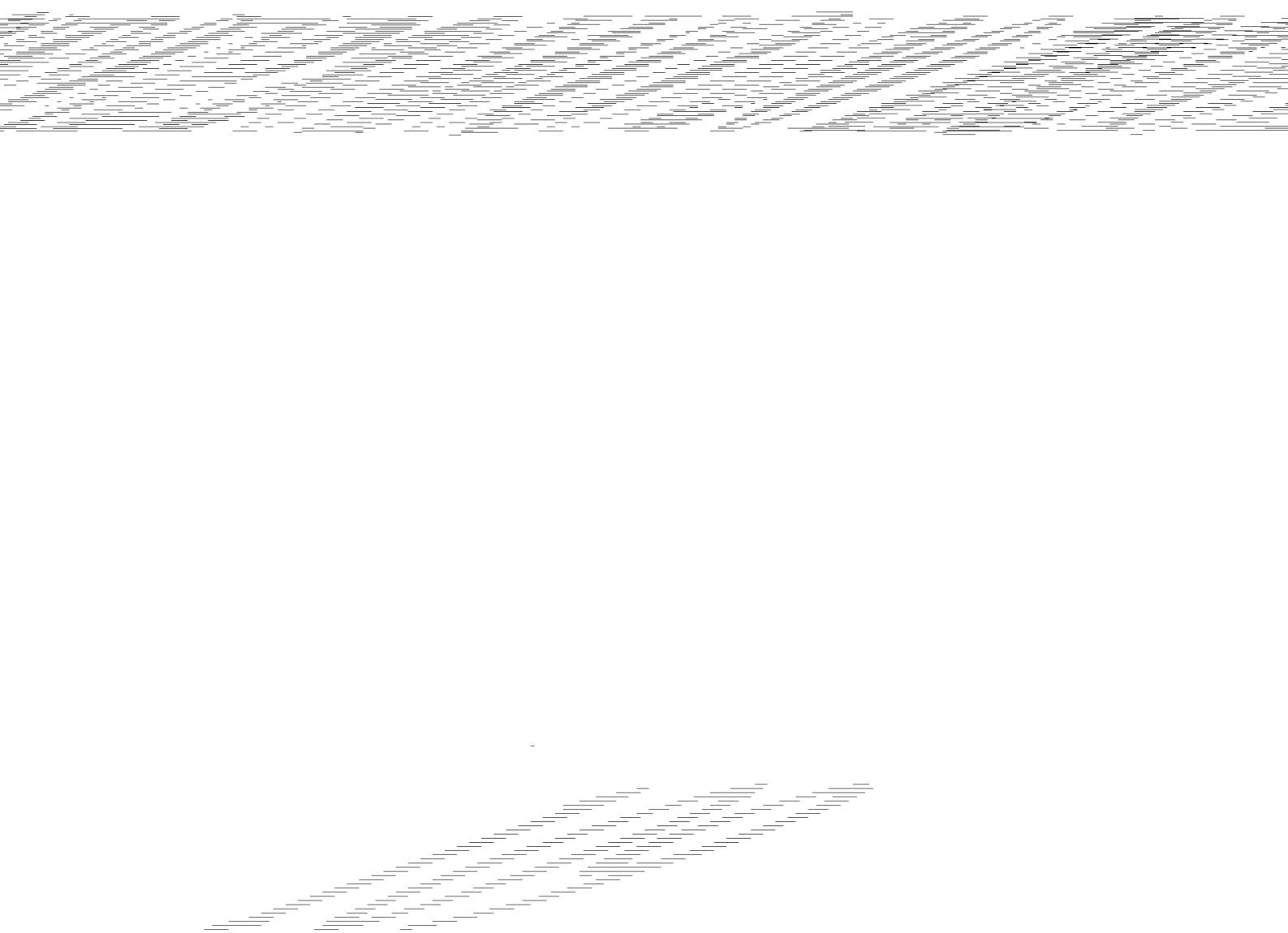
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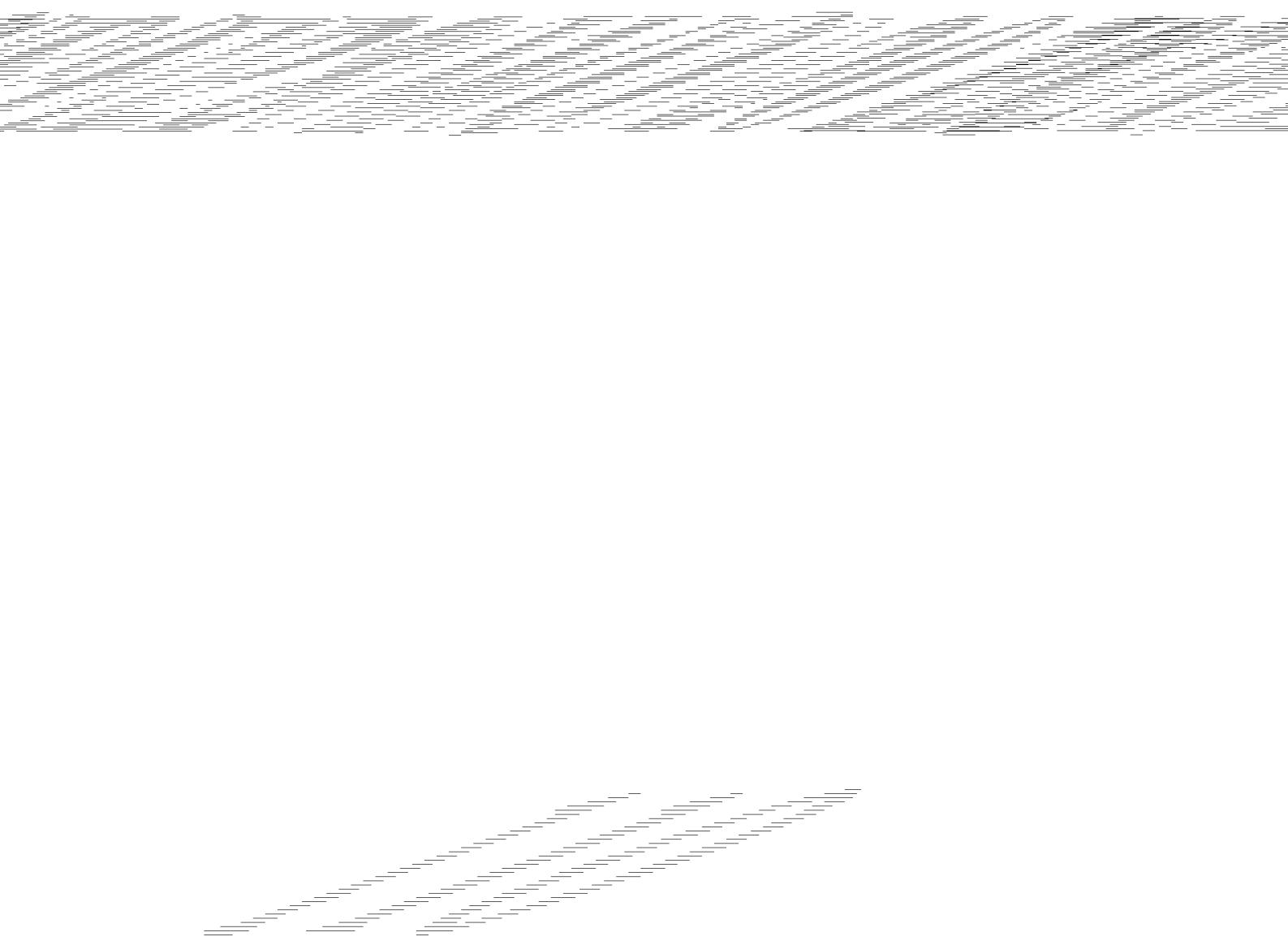
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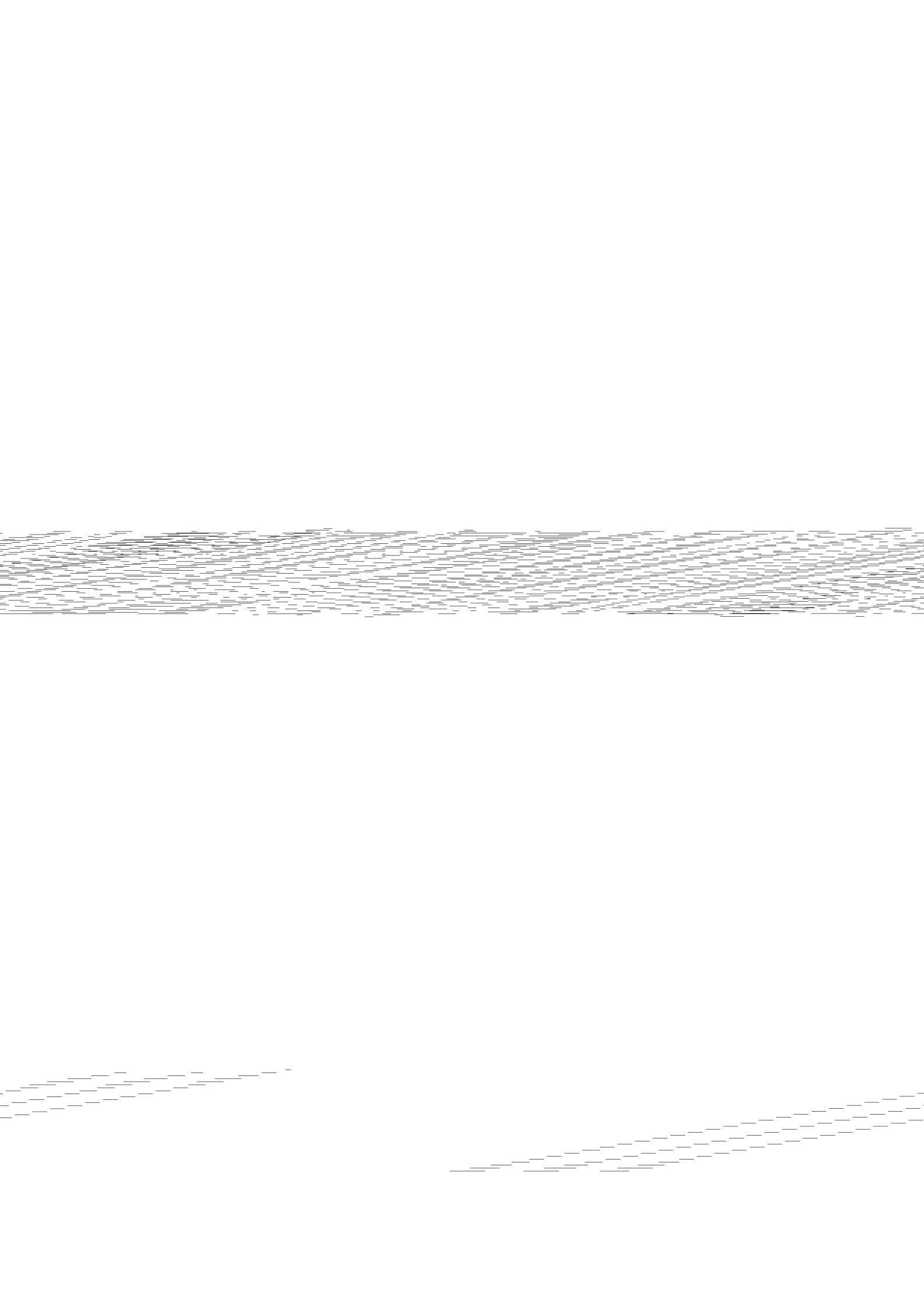
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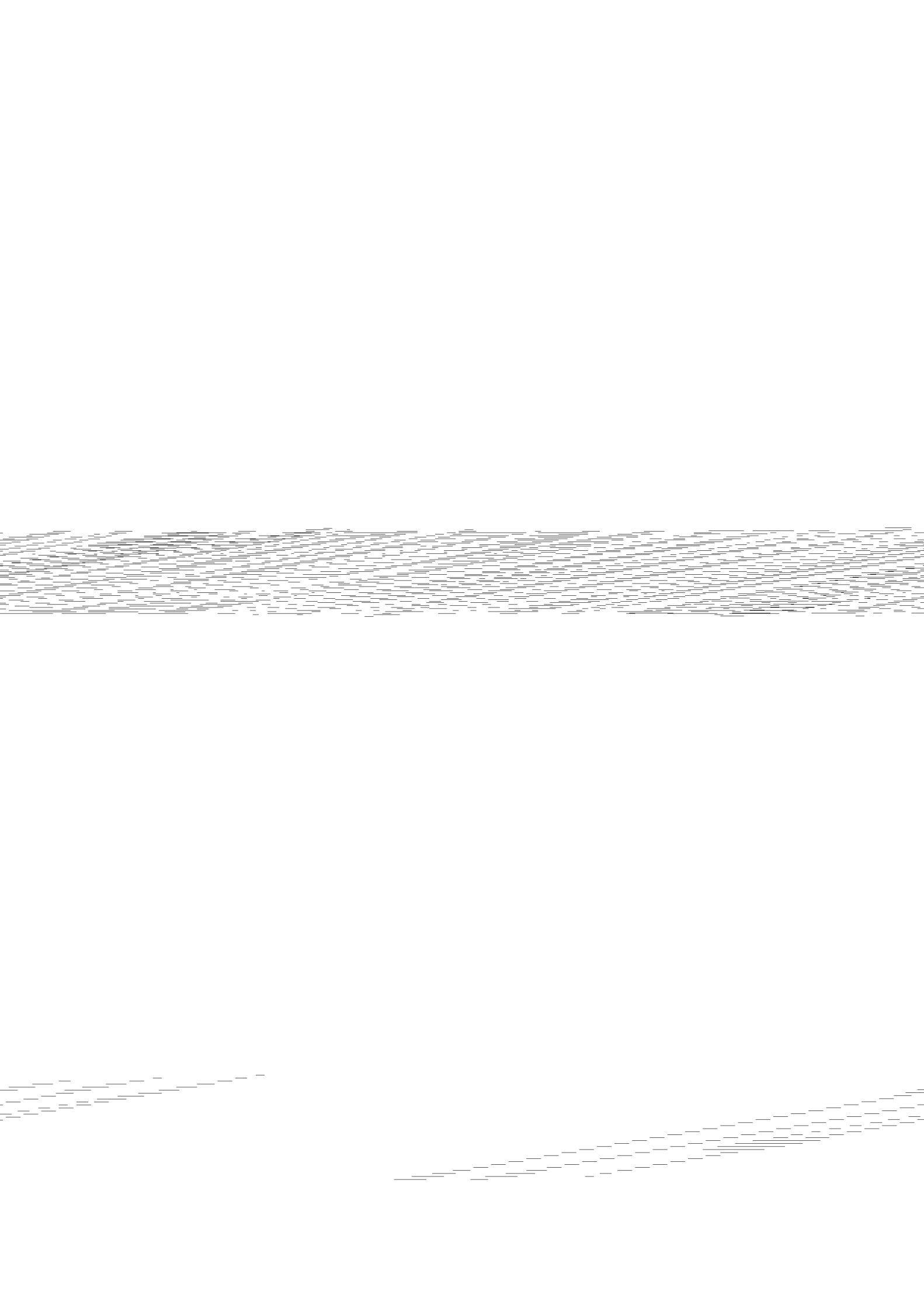




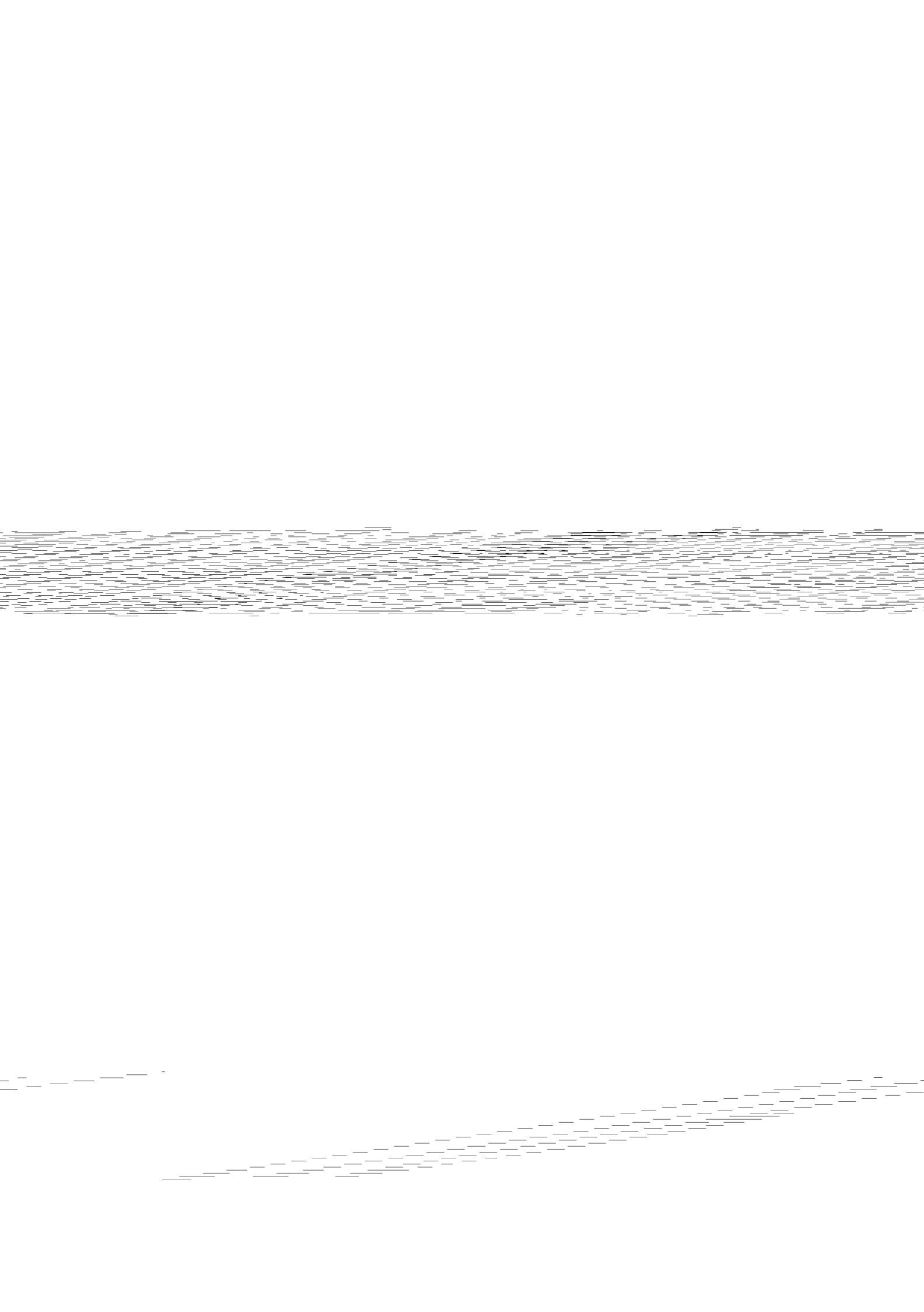


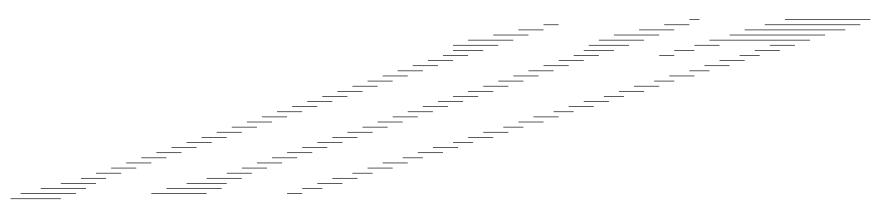
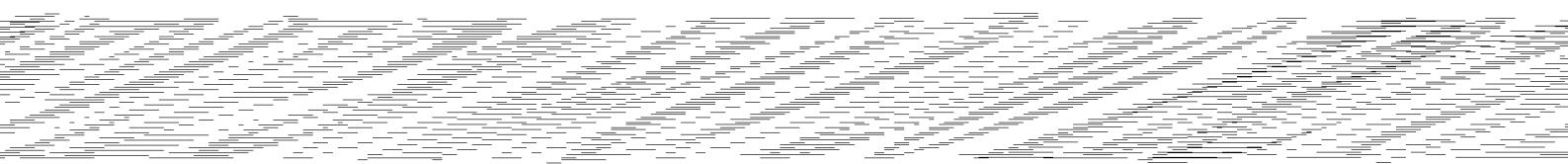














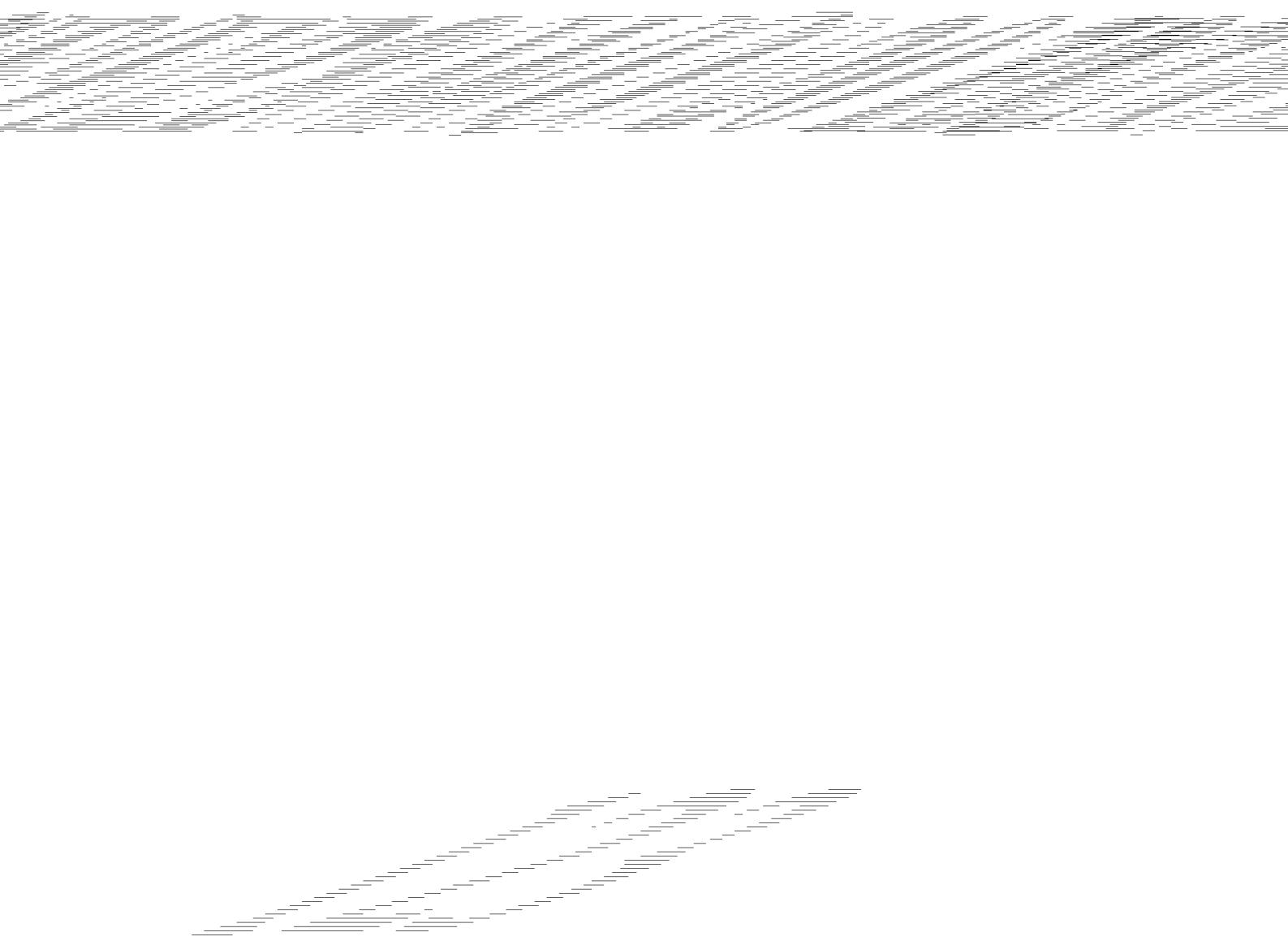


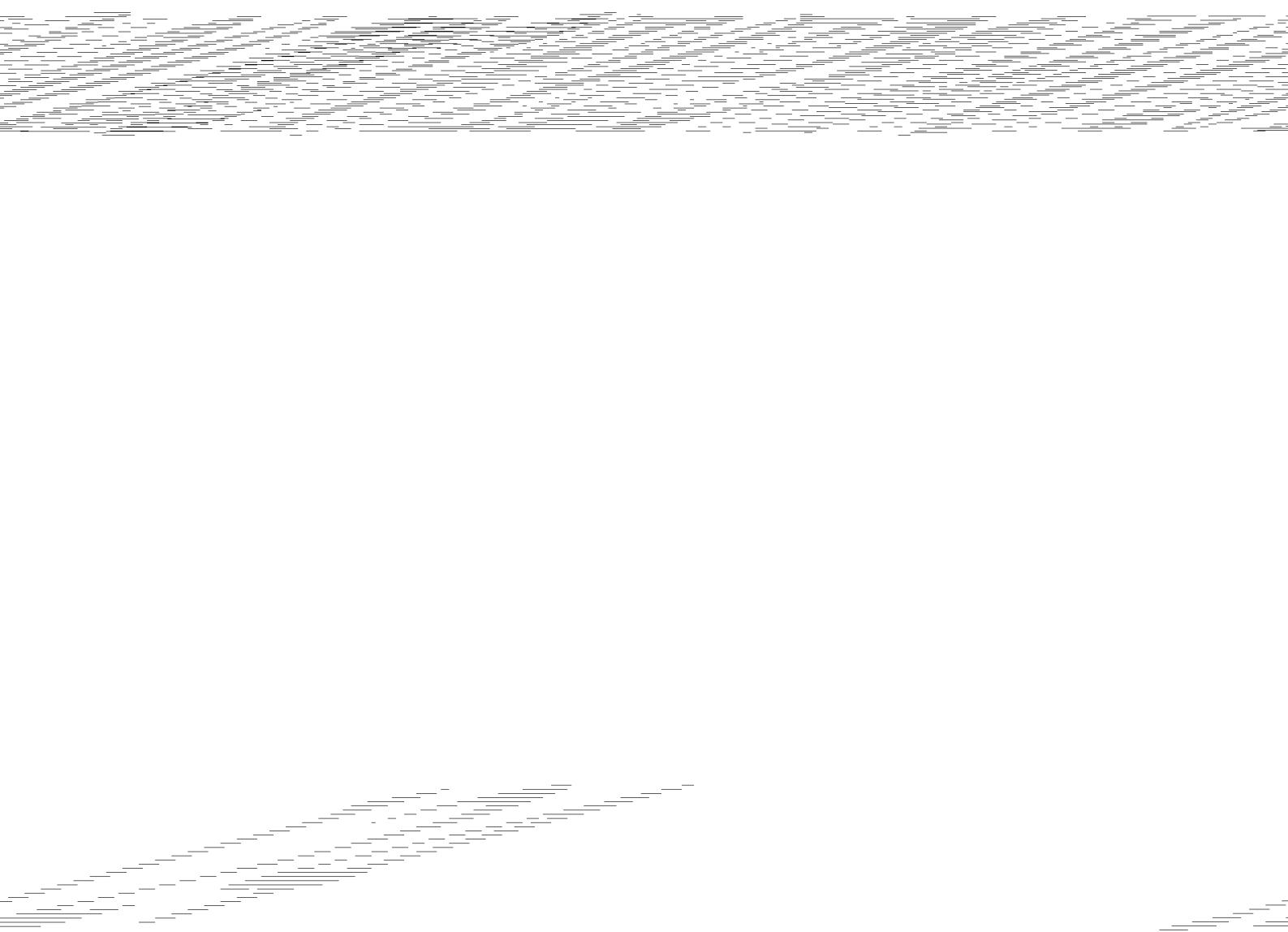
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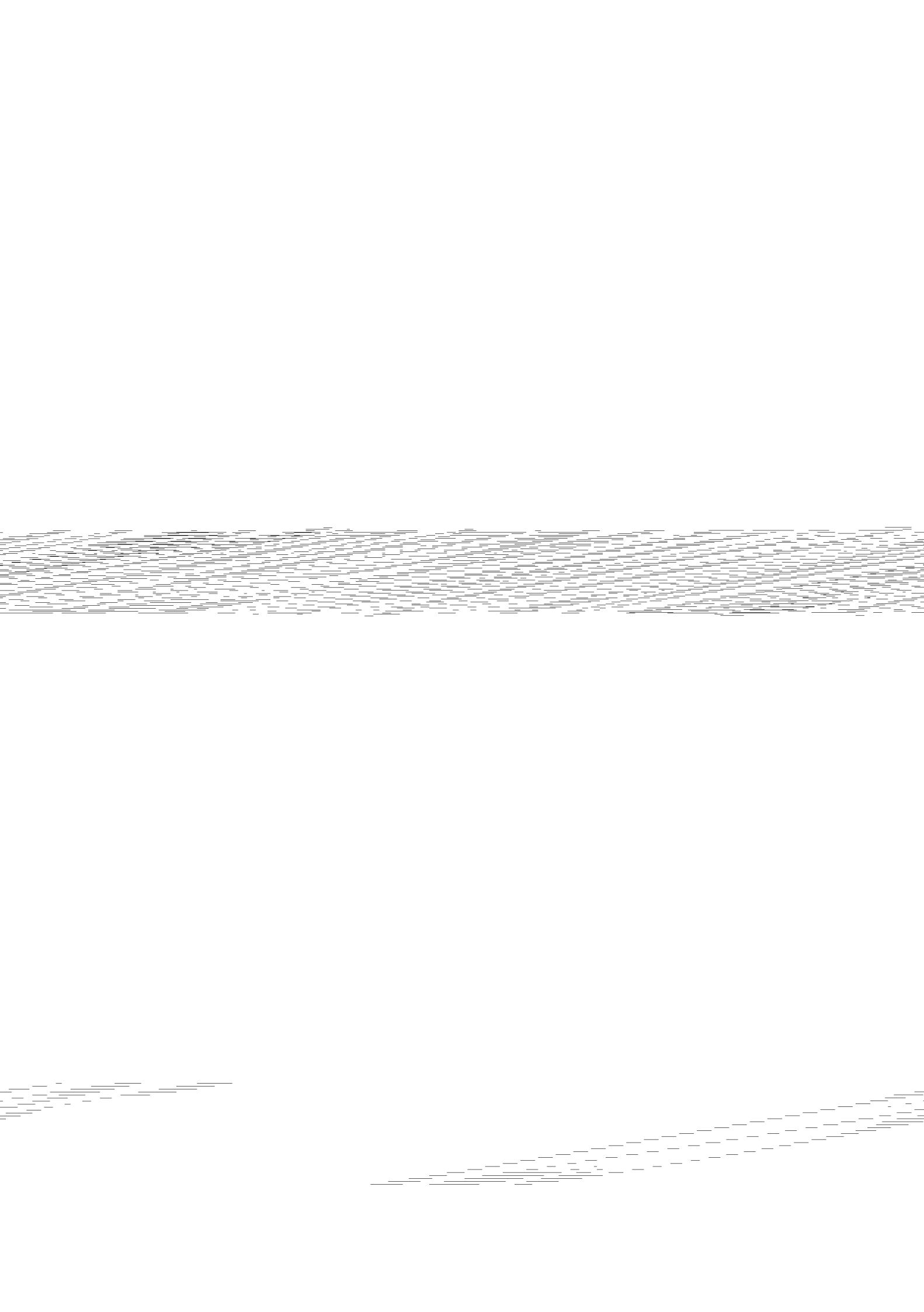
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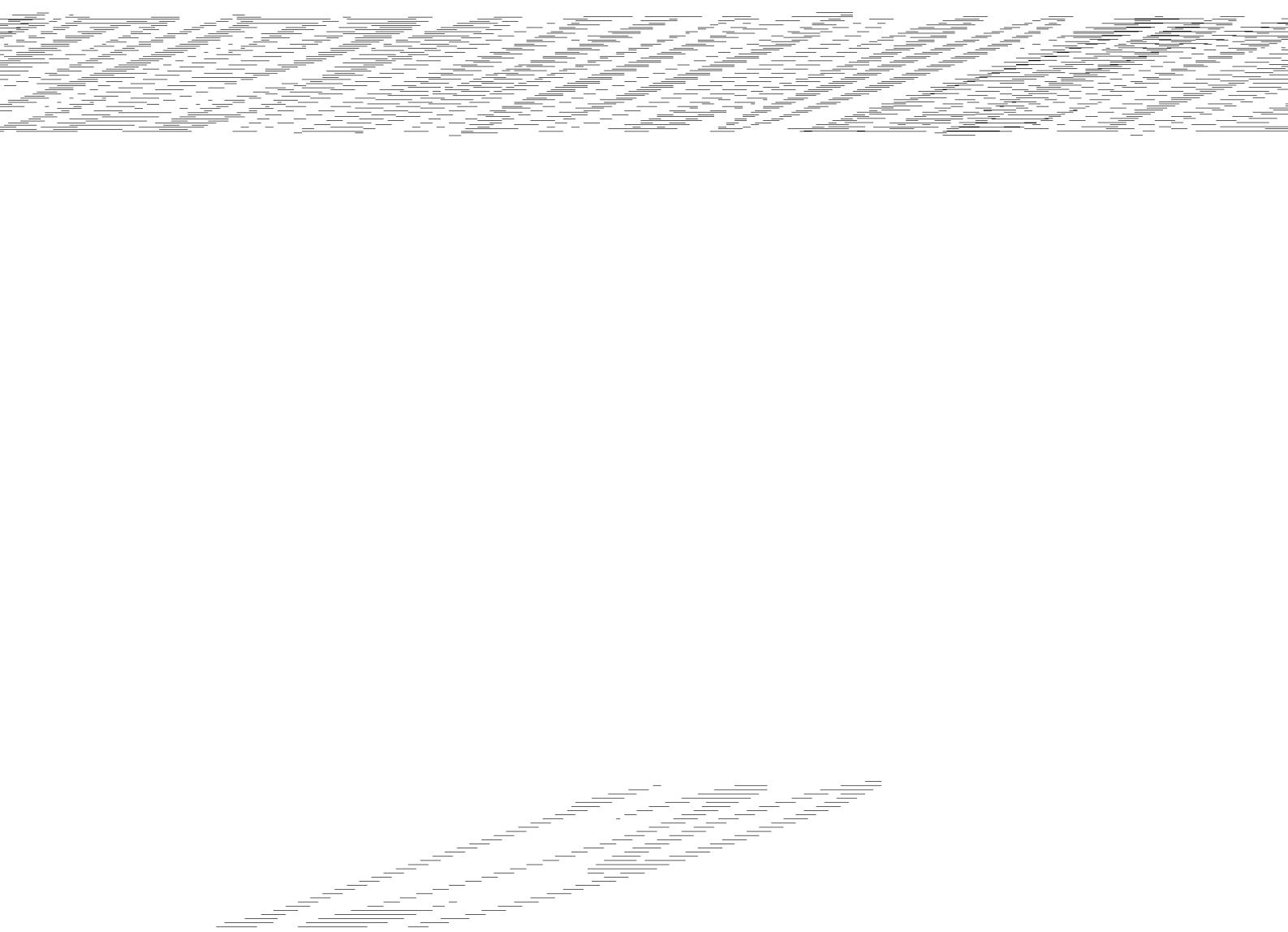


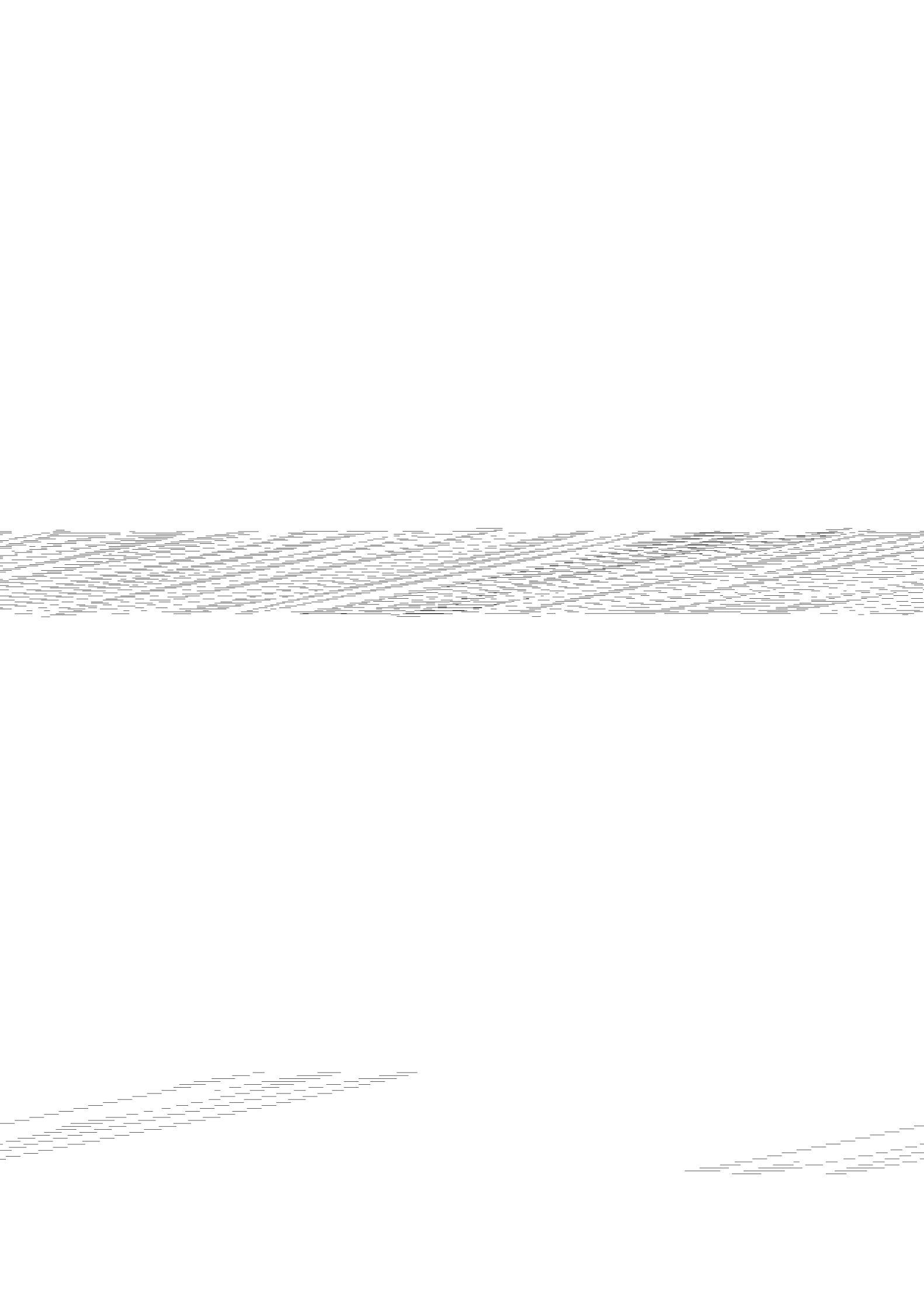


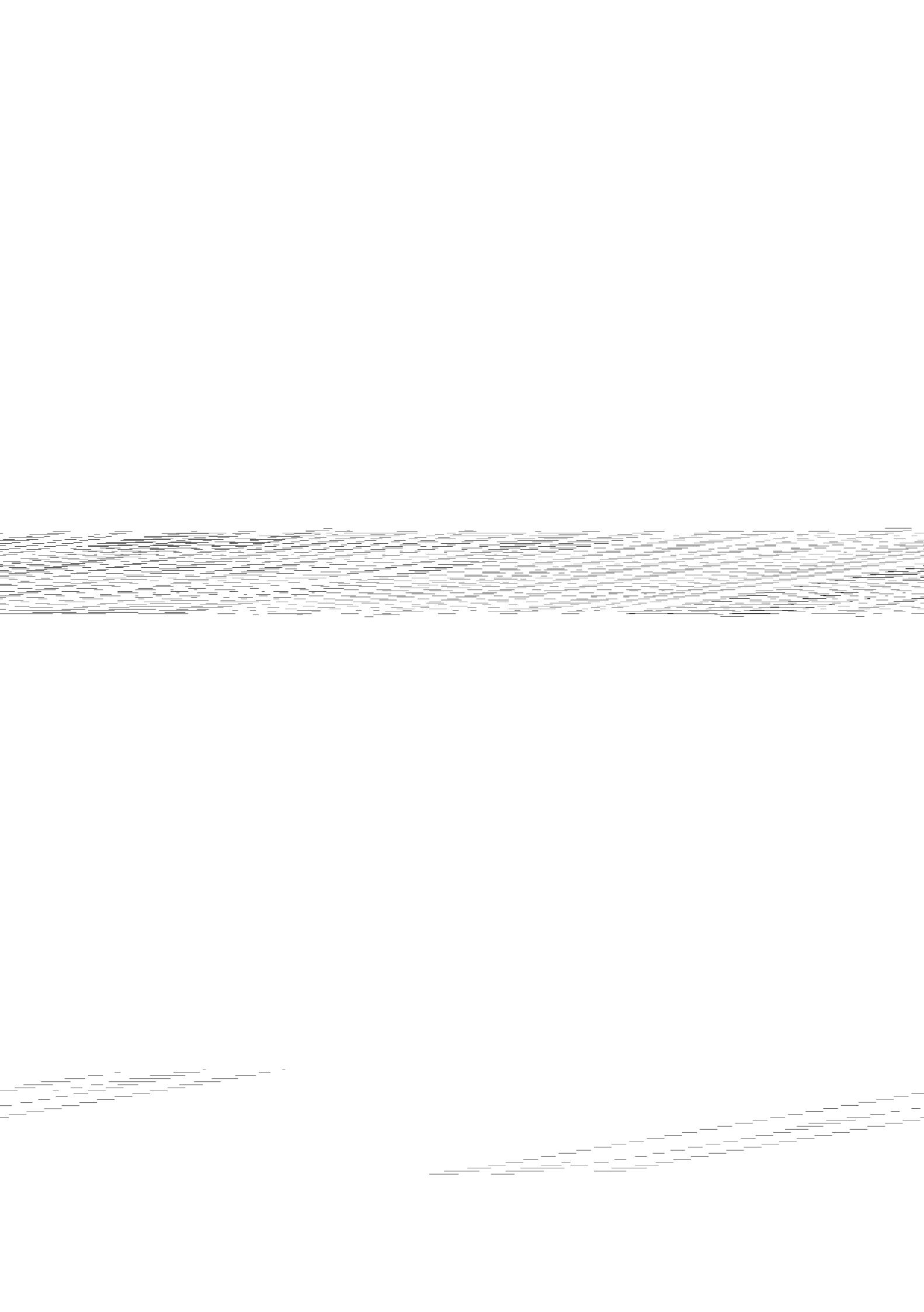






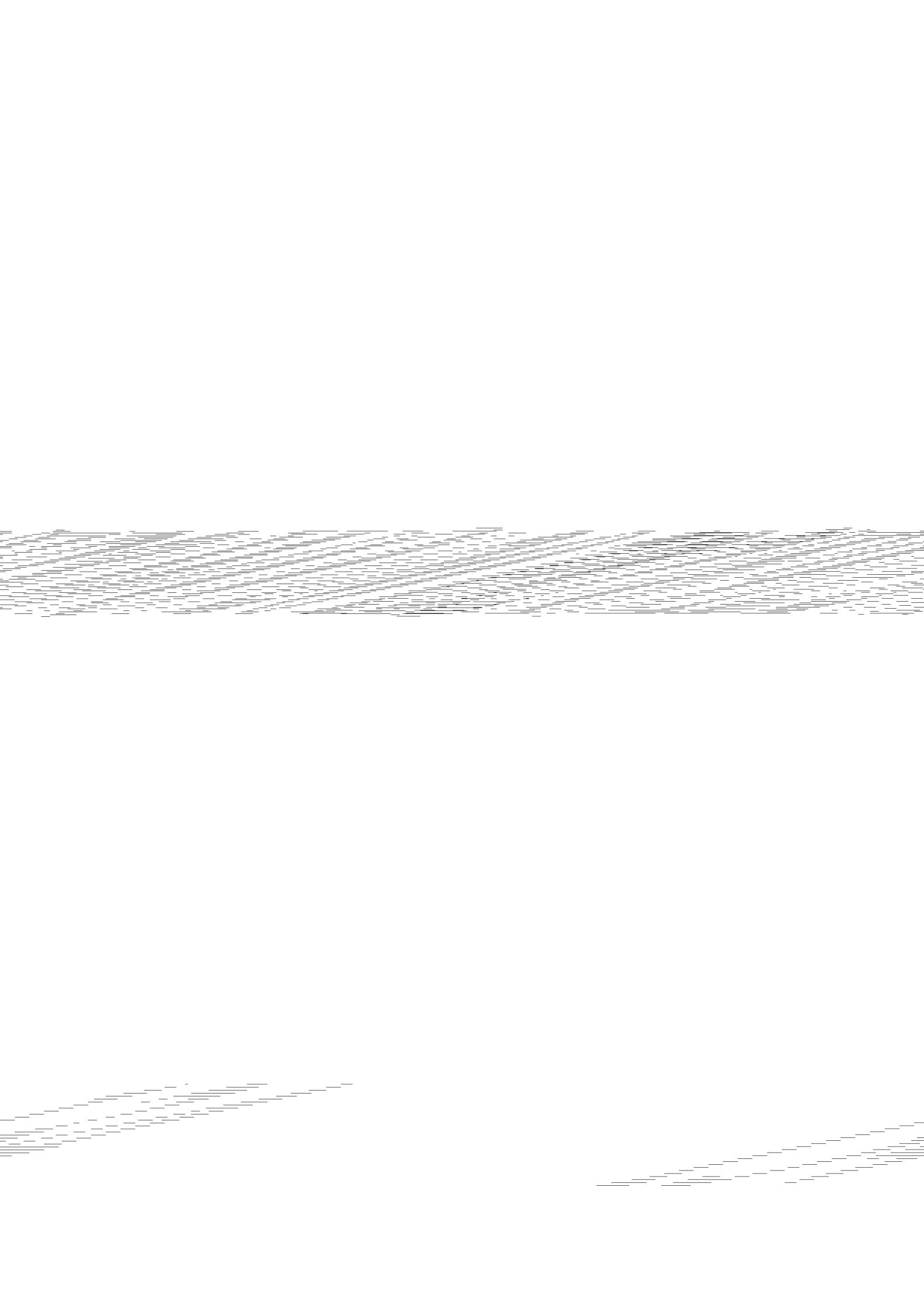


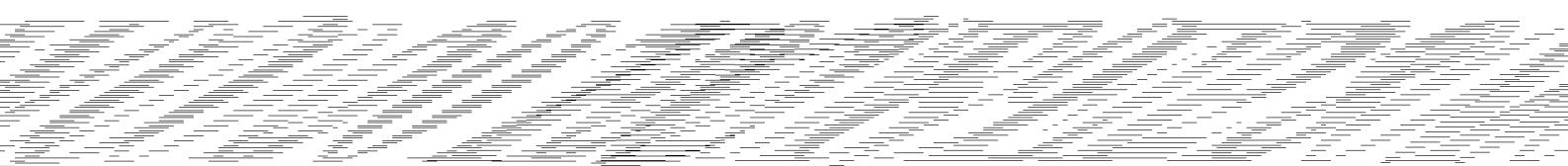




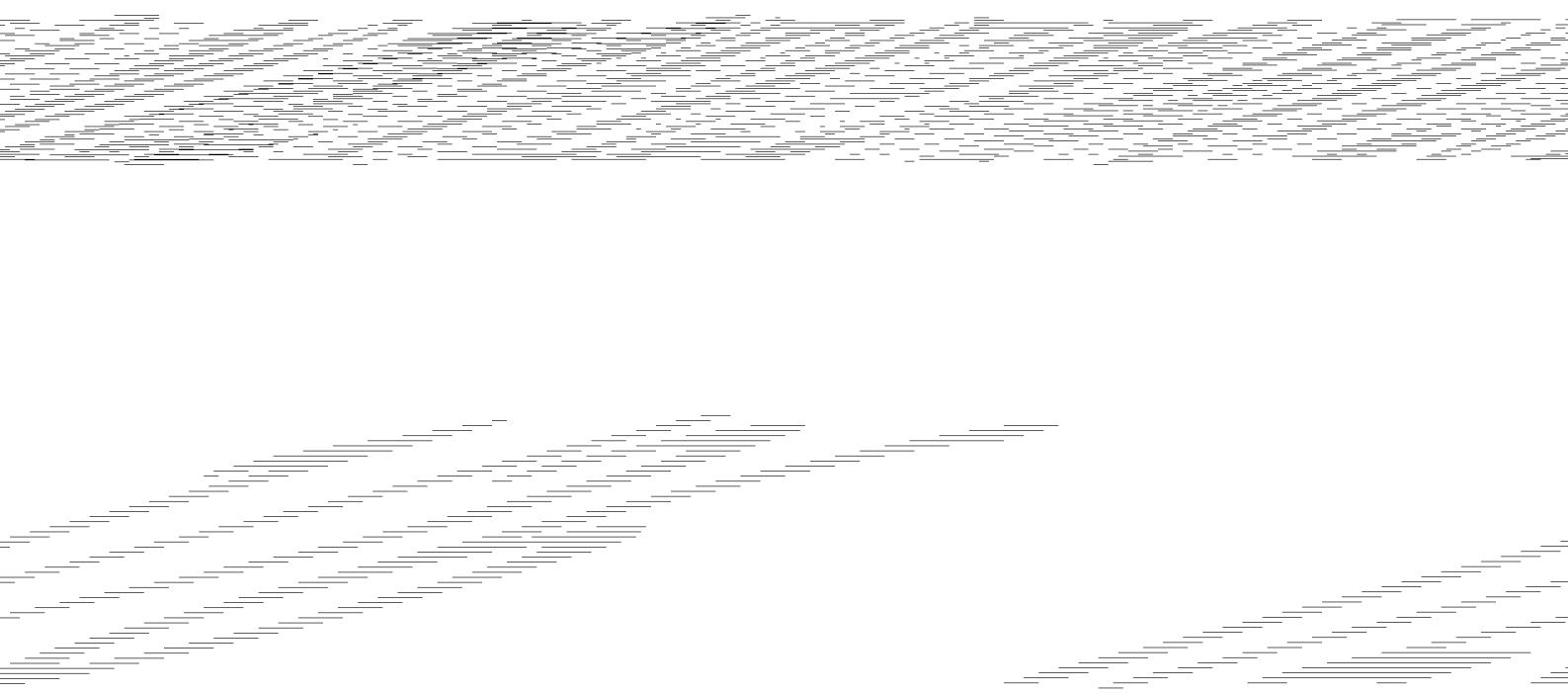


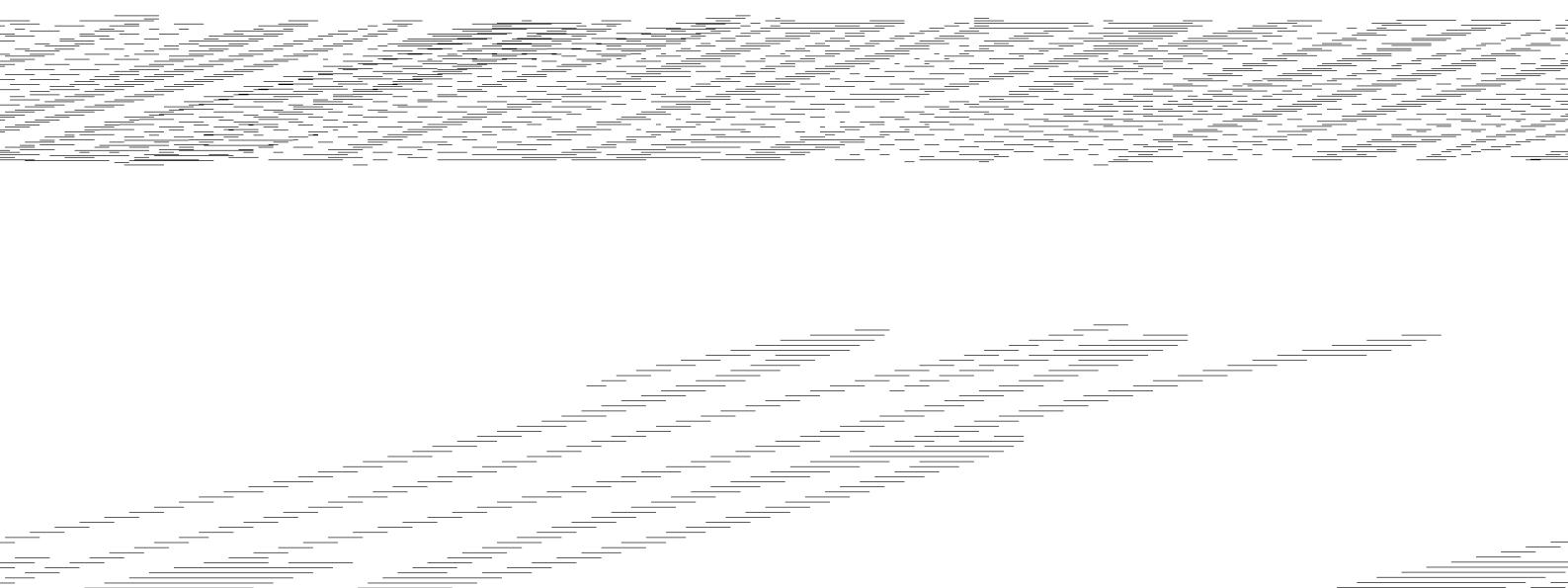


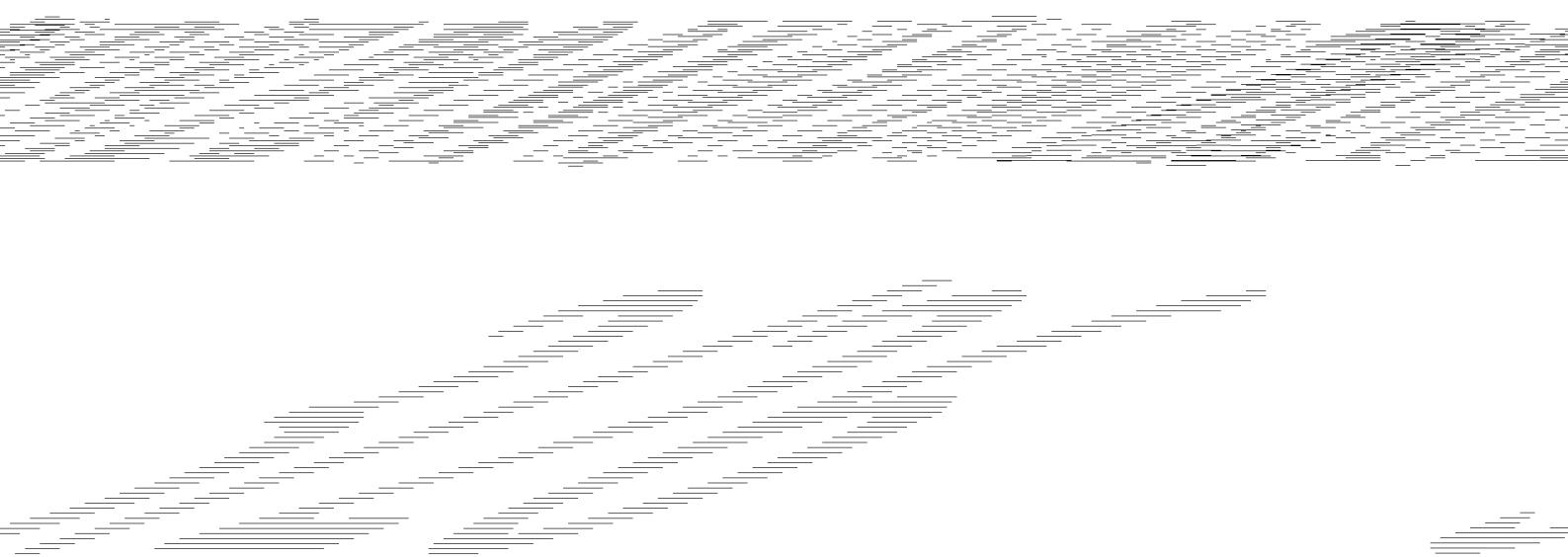


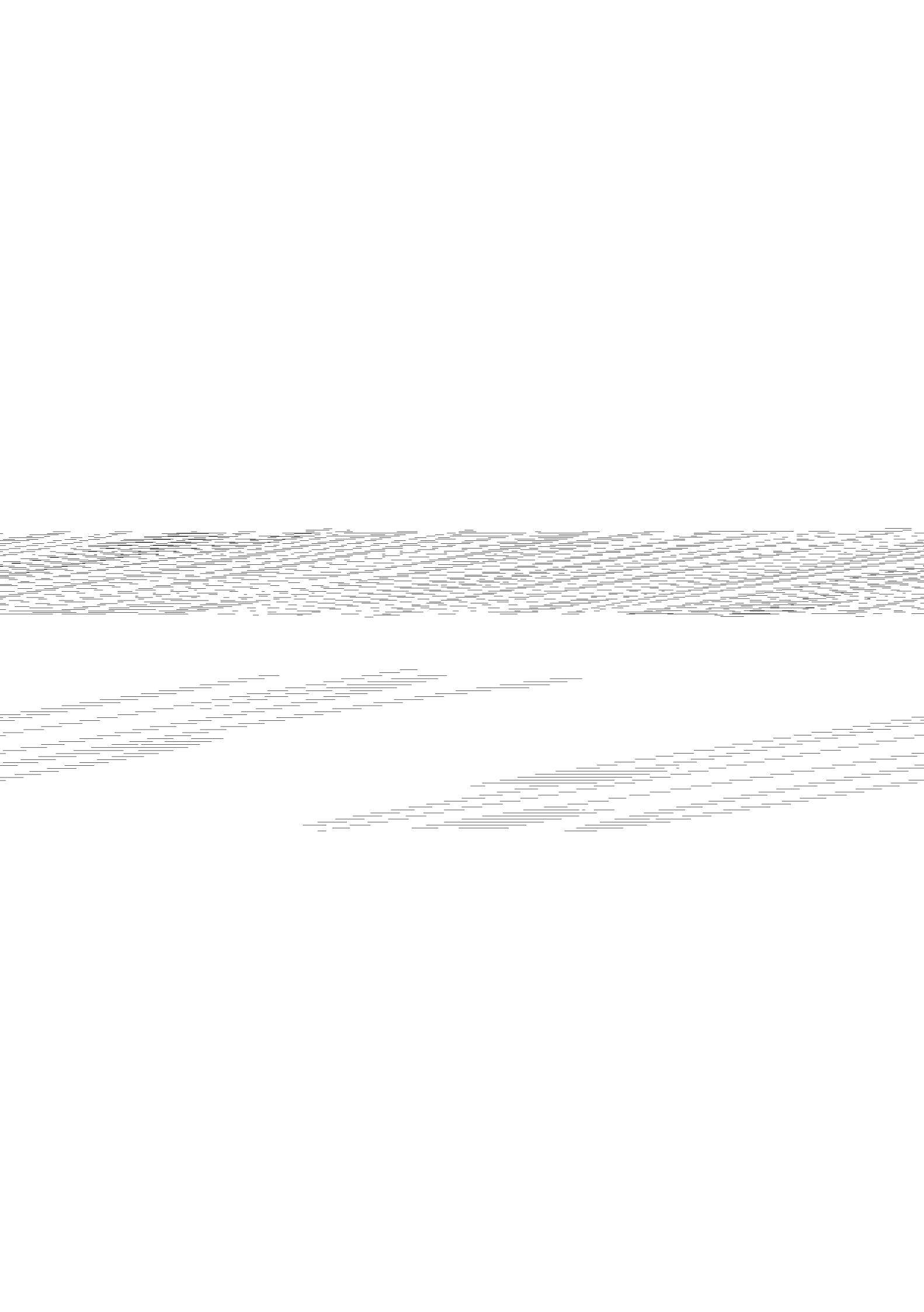




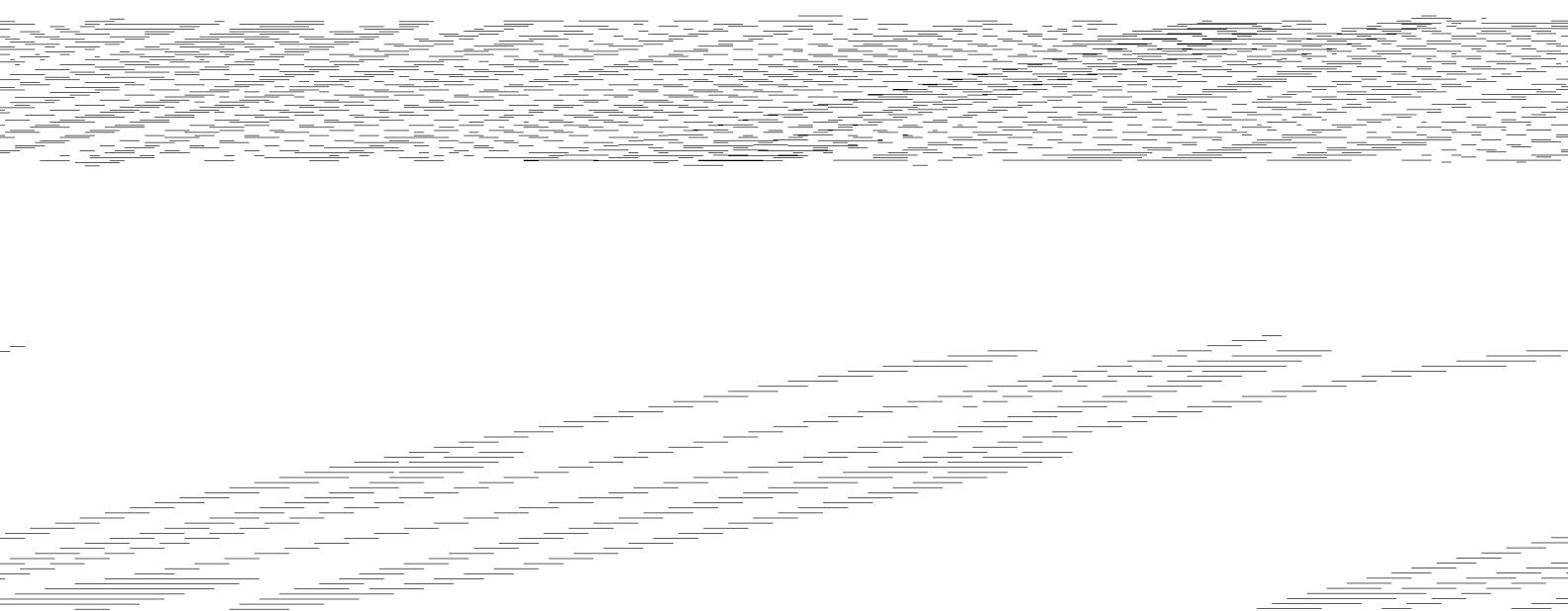


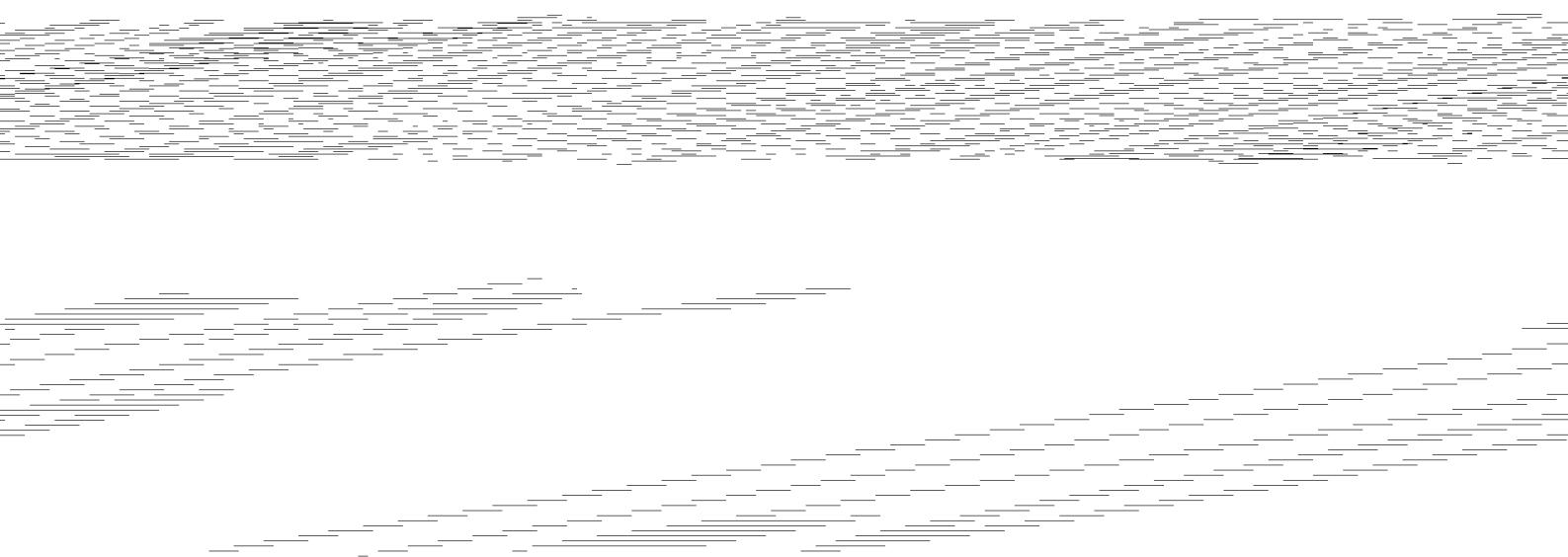


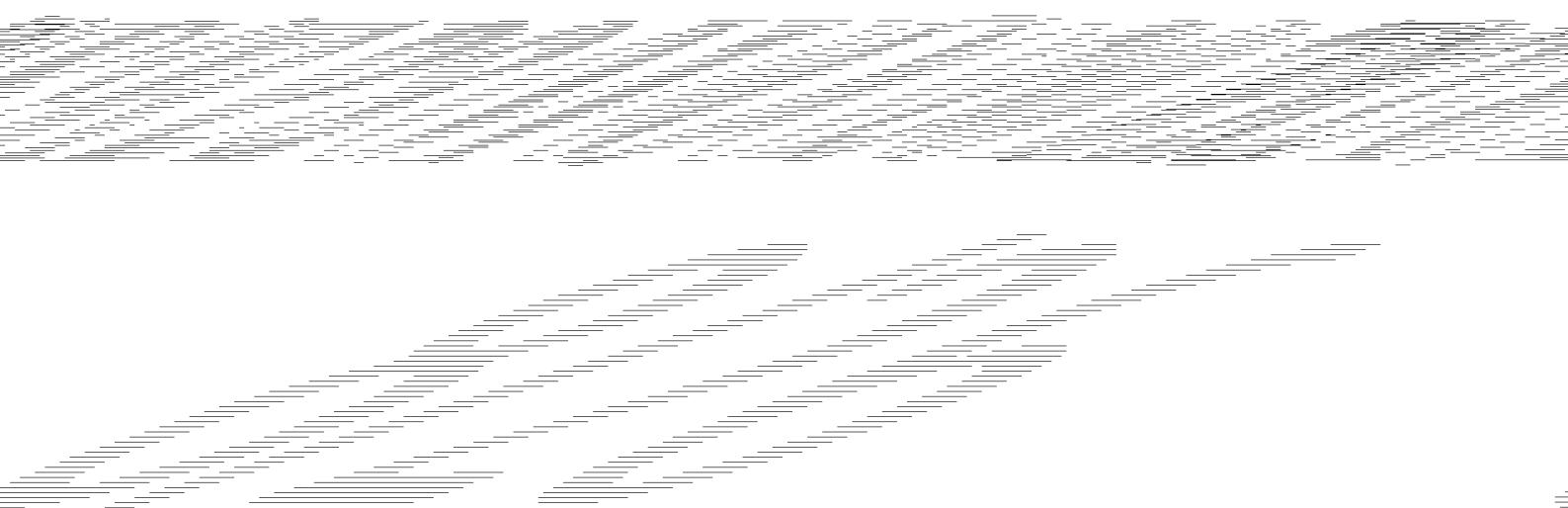


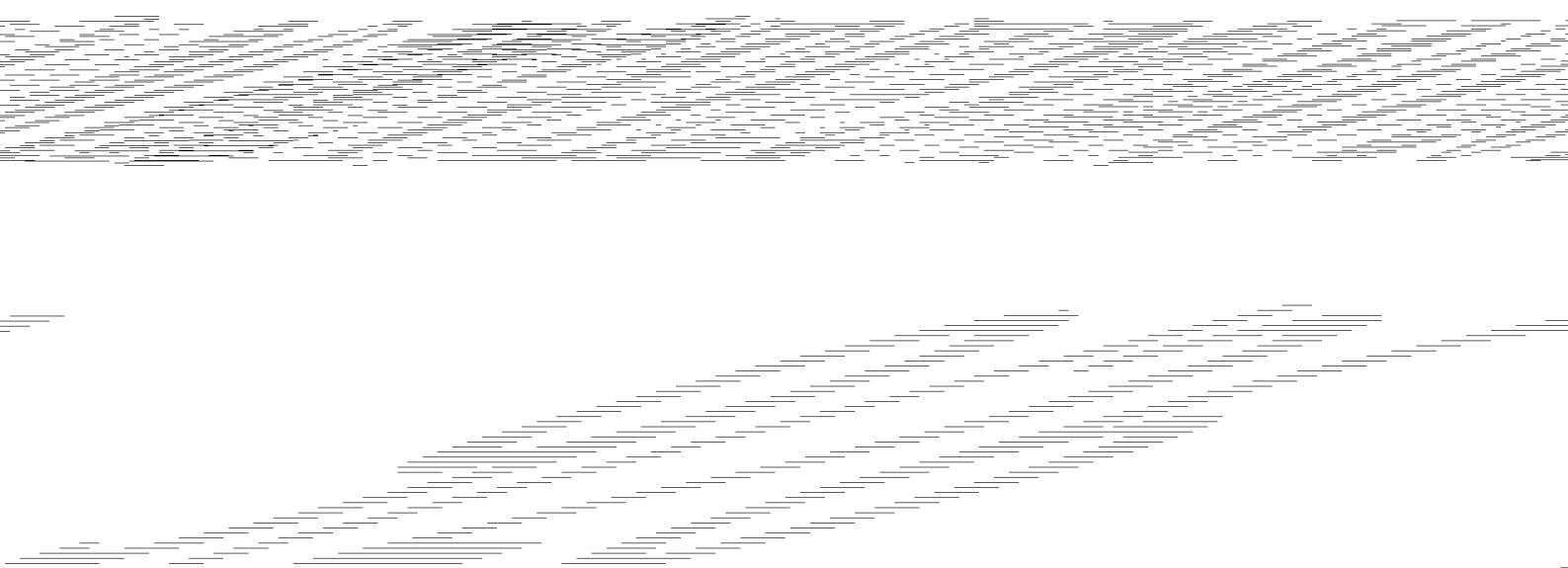








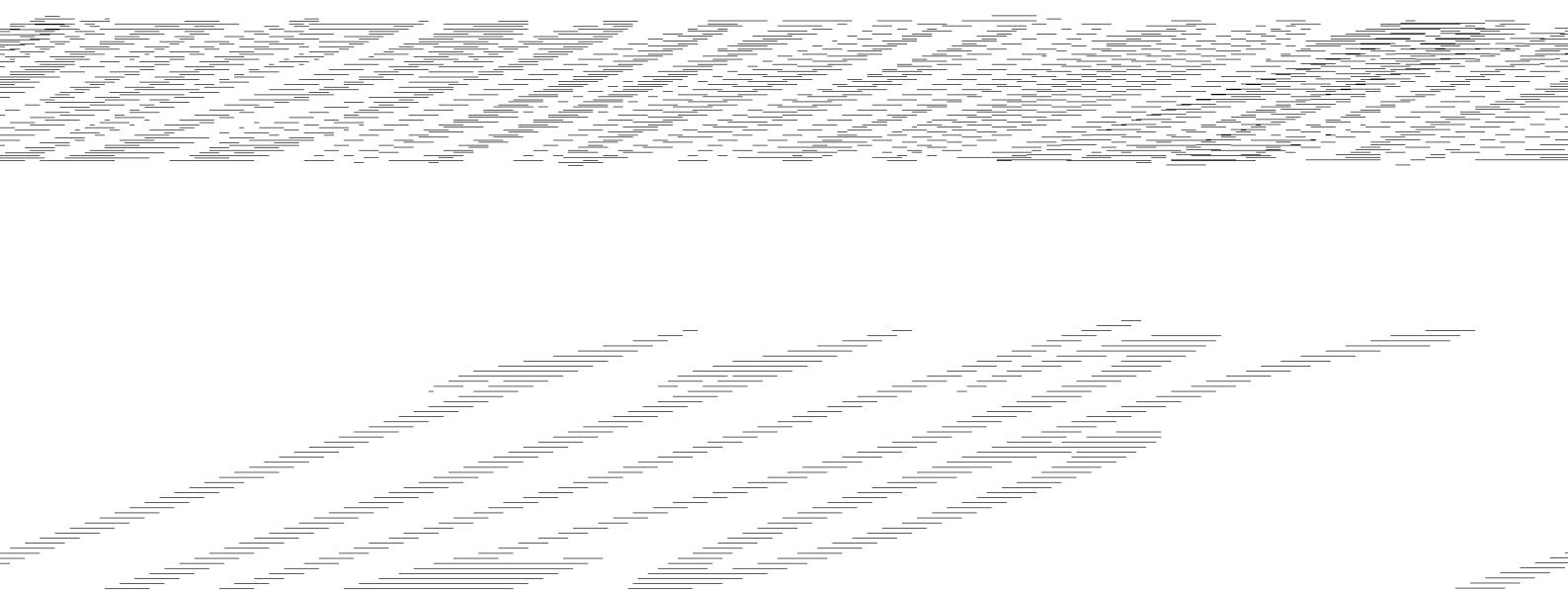


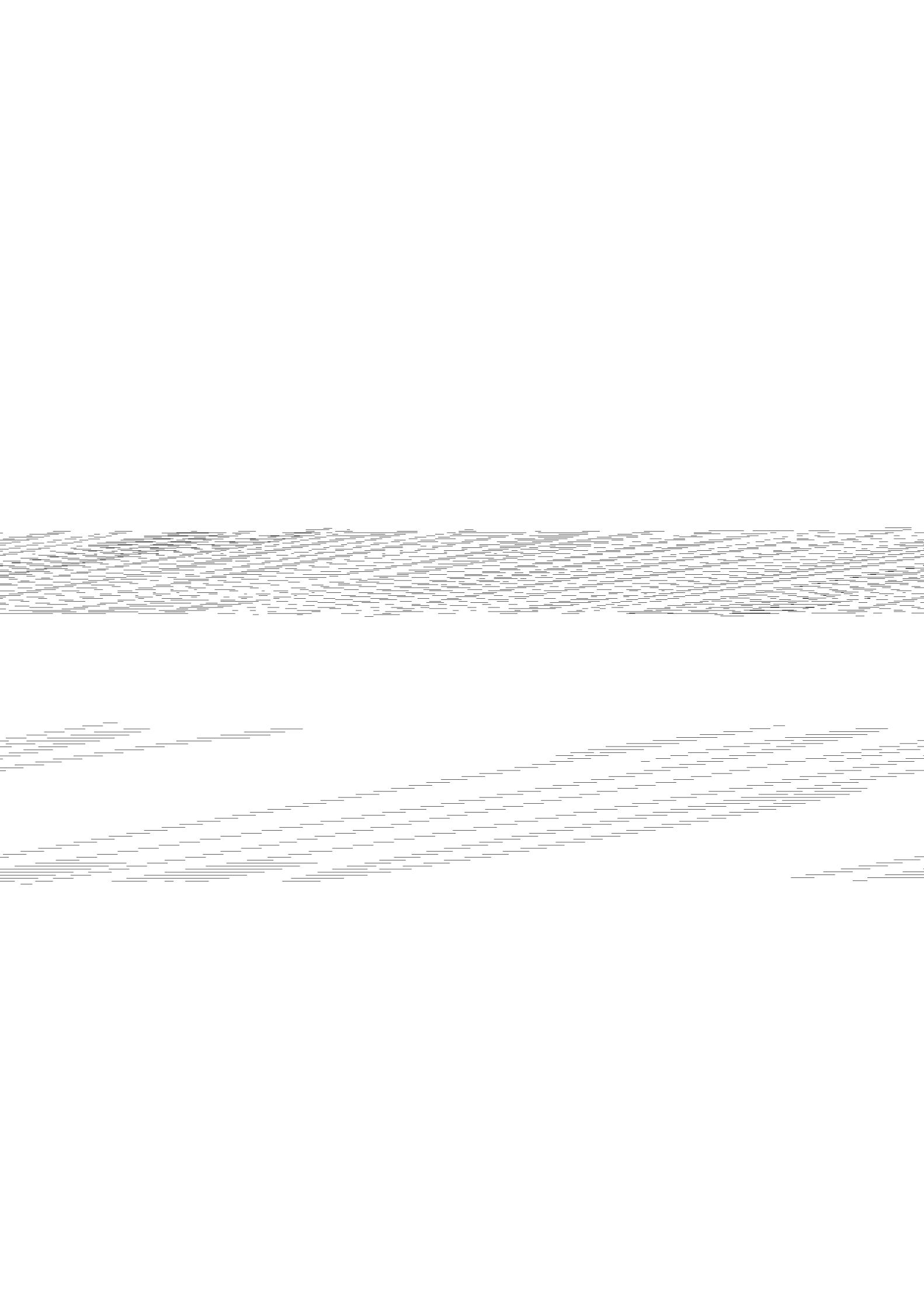


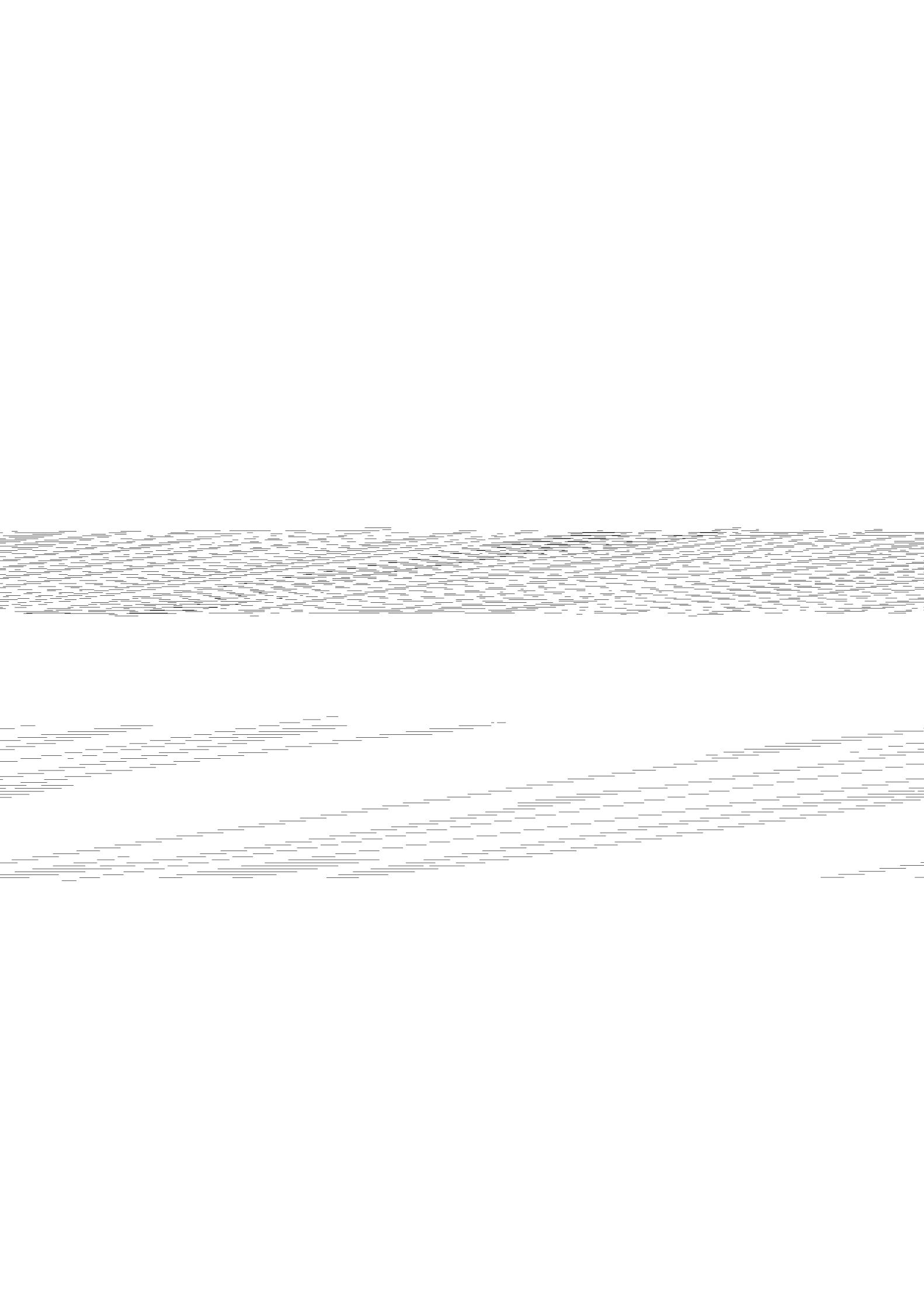
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